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HEAVY METALS AND AQUATIC BRYOPHYTES: ACCUMULATION AND THEIR USE AS MONITORS.

by

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This thesis results entirely from my own work and has not previously been offered in candidature for any other degree or diploma

M.G. Kelly

October 1986

Water is the essence of earth
Plants are the essence of water
Chandagya Upanishad
circa 600 - 400 B.C.

ABSTRACT

An experimental study was made of accumulation and loss of heavy metals by the aquatic moss Rhynchostegium riparioides and of the processes involved. The information gained were used to assess the effectiveness of this species as a monitor.

Growth of Rhynchostegium continued throughout the year with peaks in spring and autumn. There were positive correlations between growth and water and air temperatures. Strong differences in growth rates in the four streams were not related to nutrient or heavy metal concentrations.

Various patterns of mesh bag were tested as containers for transplanted Rhynchostegium to be used as a monitor. No significant differences in accumulation by moss were found between boulders or bags, or in accumulation with different patterns of bags. Accumulation was reduced slightly at the centre of bags packed with large quantities of moss.

The physiology of Zn accumulation was also studied. A large part of accumulation (> 70%) in the early stages (< 12 h) was in a form readily exchanged for competing cations such as Ca and Ni; over longer time periods there was significant accumulation into a more tightly bound compartment. There was no evidence that uptake into this latter compartment was under the direct control of the plant's metabolism.

There was differential accumulation of Cr(III) and Cr(VI) in laboratory experiments; during a case study it was possible to "predict" the speciation of Cr in the water by the concentrations accumulated by the moss.

These results confirm that bryophytes are useful as monitors of heavy metal pollution in a wide range of circumstances. A range of such applications are outlined, along with recommendations for standard methods for using moss bags.

Abbreviations

t	time
s	second
min	minute
h	hour
d	day
wk	week
yr	year
µm	micrometre
mm	millimetre
cm	centimetre
dm	decimetre
m	metre
km	kilometre
µg	microgram
mg	milligram
g	gram
kg	kilogram
t	tonne
ml	millilitre
l	litre
meq	milliequivalent
µmol	micromole
mmol	millimole
µM	micromolar
M	molar
N	normal
°C	degrees Celsius
kPa	kiloPascal
µS	microSiemen
CCCP	carbonyl cyanide-m-chlorophenylhydrazone
DCMU	3-(3,4-dichlorophenyl)-1,1-dimethylurea
DNP	2,4-dinitrophenol
EDTA	ethylenediaminetetra-acetic acid (disodium salt)
FRP	filtrable reactive phosphate
HA	humic acids
HEPES	N-2-hydroxypiperazine-N'-2-ethanesulphonic acid
n	number of measurements
df	degrees of freedom
$\bar{x}$	mean
SD	standard deviation
p	probability
r	correlation coefficient
F	variance ratio
N.S.	not significant
AAS	atomic absorption spectrophotometry
AFS	apparant free space
ANOVA	analysis of variance

DFS	Donnan free space
PAR	photosynthetically active radiation
Q_{10}	temperature quotient
STW	sewage treatment works

cond.	conductivity
curr.	current speed
temp.	temperature

Conventions

N.S.	$p < 0.05$
*	$p > 0.05 \leqslant 0.01$
**	$p > 0.01 \leqslant 0.001$
***	$p < 0.001$

Amblystegium	abbreviated name for *Amblystegium riparium*.
Fontinalis	abbreviated name for *Fontinalis antipyretica*.
Rhynchostegium	abbreviated name for *Rhynchostegium riparioides*.

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1. INTRODUCTION

1.1 GENERAL INTRODUCTION

The toxic effects of heavy metals have been known for a long time. The deleterious effects of lead on herbage around smelt mills of County Durham was described by Calvert (1884) who went on to say: "It is a source of regret that where manufacturing industries are carried on there are usually emanations of smoke and gas".

The extent of the problems caused by heavy metals has since spread from localized areas where ores were mined and smelted to include regions of most developed parts of the world. They are now second only to organic pollution as a source of pollution in British rivers (Hargreaves, 1981) and their increase in importance has been mirrored by growing public concern.

Techniques for measuring concentrations of heavy metals are becoming increasingly refined and include sophisticated techniques such as atomic absorption spectrophotometry (AAS), inductivly coupled plasma emission spectrophotometry, direct potentiometry and anodic stripping voltametry. There is also considerable interest in the development of methods to assess the impact of heavy metals on the biota, both for its inherent interest to ecologists and as an additional monitoring technique. At one level this may be an "intermediate technology" (*sensu* Schumacher, 1973) approach to monitoring concentrations, whilst at another it may provide information additional to straightforward chemical analyses.

This thesis is concerned with studies on the uptake of heavy metals by aquatic mosses and with using this information to develop methods for using these as monitors of heavy metal pollution and, in particular, intermittent or episodic heavy metal pollution.

The term "heavy metal" has been retained throughout despite criticisms (Nieboer & Richardson, 1980), which point out that the term has no rigid definition and is often extended to include metalloids (such as selenium) and lighter metals (such as aluminium and barium) with similar connotations of toxicity. Say & Whitton (1981) argue that whilst the term continues to arouse public interest and scientific discussion then it has a place, albeit not in a strictly biological or chemical context. The elements considered in this study; chromium, zinc, cadmium and lead, all fit the "classical" definition of a heavy metal by having a density greater than five.

The remainder of this introduction gives a brief overview of the sources, environmental chemistry and biology of heavy metals in freshwater systems. It deals mainly with zinc, the principal element in this study, but contains some references to other metals. This is followed by a more detailed consideration of the aims and scopes of biological monitoring and the correct choice of organisms for particular circumstances.

1.2 HEAVY METALS IN FRESHWATERS

1.21 Sources of heavy metals in freshwaters

The production and consumption of heavy metals have shown a rapid increase in this century (Nriagu, 1979), accompanied by increases in the total emissions to the environment (Rühling & Tyler, 1968; Livett et al., 1979). The problem may be considered in two parts: problems associated with the "production" of heavy metals, e.g. mining and smelting and problems associated with their use.

The principal ore-producing countries of the world are U.S.A., Canada, U.S.S.R., Australia and South Africa (Govett, 1976). Much of the remainder of the resources are concentrated in a small number of Asian, African and Latin American countries; for example 70% of the world's known copper reserves are concentrated in Chile, Peru, U.S.A., U.S.S.R., Zaire and Zambia and the principal tin deposits are located in S-E. Asia and Bolivia (Govett, 1976). U.K. deposits of heavy metals (principally lead, copper, tin, arsenic and associated "gangue" minerals) are confined to upland regions of N. England, N. Wales and S-W. England and have been exploited since Roman times (Raistrick & Jennings, 1965). Many of these seams have been exhausted or rendered uneconomic by cheap imports from abroad. Streams and rivers in these regions, however, often still contain elevated concentrations of heavy metals (Webb, 1978).

The present problems associated with heavy metals are coupled with an increasing diversity of uses. Zinc, for example, was traditionally used for galvanizing steel; more recent uses include manufacture of such widespread commodities as batteries and detergents. The production, use and eventual disposal of these products are all responsible for the release of heavy metals into the environment. It is estimated that 19.6×10^6 t of lead have been released to the environment by anthropogenic causes (Nriagu, 1979). 50% of all anthropogenic emissions of lead at the present time are derived from combustion of petroleum-based fuels (Moore & Ramamoorthy, 1984), although recent legislation in U.S.A. limiting lead additives in petroleum has caused a significant decline in lead transported by the Mississippi River which, with tributaries, drains 40% of the U.S.A. (Trefry et al., 1985).

1.22 Environmental chemistry of heavy metals in freshwaters

1.221 Concentrations in freshwaters

The ubiquity of many heavy metals (1.21) makes reliable estimates of background concentrations (i.e. uninfluenced by anthropogenic input) very difficult to measure, due both to the problems of defining uncontaminated regions and to the problems of contamination during sample collection and analysis (Martin et al., 1980). "Clean" stream waters in N. Wales contained 0.011 mg l^{-1} Zn, 0.00041 mg l^{-1} Cd and 0.0007 mg l^{-1} Pb (Abdullah & Royle, 1972). Shiller and Boyle (1985) measured dissolved (= 0.4 µm filtrable) concentrations of zinc of between 0.00002 and 0.0018 mg l^{-1} in rivers draining relatively undisturbed regions of the world. Their measurements within the U.S.A. ranged from 0.000071 mg l^{-1} to 0.0156 mg l^{-1}; however heavily polluted reaches may contain much higher concentrations (Whitton, 1980). Ranges of several orders of magnitude have also been shown for other heavy metals (e.g. Laxen, 1984b).

1.222 Chemical behaviour

Even the highest concentrations of heavy metals in natural waters are, in chemical terms, very dilute. This has important implications for their behaviour with respect to other ions, ligands etc.. Silver, for example, is readily precipitated by chloride ions (Cotton & Wilkinson, 1972). The relative concentrations of these two ions in natural waters makes chloride a major factor in determining the speciation of dissolved silver (Whitlow & Rice, 1985).

The solubility of the common metal complexes in natural waters is well known (Chemical Rubber Company, 1985). Of the major anions, heavy metal salts with phosphates, silicates and carbonates tend to be insoluble in cold water, whilst nitrates and sulphates are rather more soluble. The single most important factor controlling the speciation of metal ions, however, is pH. Solubility of zinc decreases as pH rises, up to pH 9.5. The other two common species in natural waters, $ZnCO_3$ and $Zn(OH)_2$, cannot control zinc solubility unless the concentration of dissolved carbon dioxide is very high (Hem, 1972). Williemite (zinc silicate) is less soluble than these but the generally low concentrations of dissolved silica preclude this from acting as a significant control on solubility (Hem, 1972). The second major effect of pH is to affect adsorption of metals onto particulates. An increase in pH increases the amount of zinc adsorbed (Shiller & Boyle, 1985).

Cadmium, very close to zinc in Group IIb has a fairly similar aquatic chemistry (Hem, 1972); however lead (Group IVb) is less soluble and readily forms precipitates with oxides, carbonates and phosphates depending upon redox conditions and alkalinity. Lead is a polyvalent cation with one oxidation state stable in natural waters (Pb(II)); chromium is also polyvalent, with two oxidation states frequent in natural waters (Cr(III) and Cr(VI)). Cr(VI) is usually found as chromate (CrO_4^-) and behaves as an anion. These two forms have very different chemical and biological behaviour (Cranston & Murray, 1980; Moore & Ramamoorthy, 1984).

1.223 Adsorption and transport of heavy metals

The aquatic chemistry of heavy metals is further adjusted by the type and availability of ligands and surfaces on which adsorption can occur. The processes involved include exchange adsorption onto charged sites (O'Connor & Renn, 1964), complexation by organic compounds such as humic and fulvic acids (Tipping et al., 1983) and adsorption and co-precipitation onto manganese and iron oxides (Lion et al., 1982; Laxen, 1984a). The proportions adsorbed vary with the metal and the concentration; the mean ratio of 0.22 µm filtrable metal to "total" metal in a survey of 105 sites in N. England was 0.88 for zinc, 0.81 for cadmium and 0.61 for lead (calculated from data in Wehr & Whitton, 1983a). The total amount of metals that may be transported in this way is considerable; the Susquehanna

River, S-E. U.S.A. transports 88.4 t yr^{-1} chromium, 45.7 t yr^{-1} silver and 3048.2 t yr^{-1} nickel (Turekian & Scott, 1967) and the Grand Calmut River discharges 84 t yr^{-1} zinc, 1.2 t yr^{-1} cadmium and 24 t yr^{-1} lead into L. Michigan (Romano, 1976). The total amounts of metals transported are greatest at high flows (Bradley & Lewin, 1982).

1.3 HEAVY METALS AND THE AQUATIC BIOTA

1.31 Introduction

This section is a brief review of the biology of heavy metals in freshwater biota. The first part (1.32) reviews the effects of heavy metals at the organismic and community levels, their movement through food chains and the health implications where man is the top consumer. This is followed by a review of the current knowledge of the physiology of metal accumulation by aquatic plants (1.33). References to terrestrial and marine systems will be included where relevant.

It is useful at this point to define two terms that will recur throughout the thesis: "uptake" and "accumulation". The plant physiologist defines uptake as the processes by which a plant takes up solutes prior to their assimilation. "Accumulation" has a less clear definition but generally refers to the whole suite of processes by which an organism concentrates ions and other compounds from its environment. The term "bioaccumulation" is often used instead of "accumulation"; however the prefix "bio" may lay too much stress on active biological processes, whereas passive processes are often more important (1.33). The term "exchange" refers to reversible adsorptive processes at the cell wall rather than to specific exchange by ion-carrier mechanisms.

1.32 Effect of heavy metals on aquatic biota

1.321 Differential tolerance and toxicity

Examples of between- and within-species variation in tolerance of organisms to heavy metals are well documented (Antonovics *et al.*, 1971). As the total concentration of zinc in the environment increases so the total number of species decreases (Whitton & Diaz, 1980). This decrease, however, is not confined to individual taxa; all larger taxa contain a few species tolerant to elevated concentrations of zinc (Whitton, 1980). It is generally assumed that tolerance to heavy metals is a trait acquired by natural selection (Bradshaw & McNeilly, 1981) and indeed populations of the filamentous green algae *Stigeoclonium tenue* and *Hormidium* spp. from streams with elevated zinc concentrations assayed for tolerance showed genetic adaptations to high (> 0.2 mg l^{-1}) concentrations of zinc (Harding & Whitton, 1976; Say *et al.*, 1977). Shehata & Whitton (1982) induced zinc tolerance in the blue-green alga *Anacystis nidulans* by repeatedly sub-culturing into higher concentrations of zinc. Similar effects of increased metal concentrations have also been observed in field populations of aquatic invertebrates (Armitage, 1979; Armitage & Blackburn, 1985; LaPoint *et al.*, 1984).

There is also good documentation for differential toxicity depending upon water chemistry at particular sites. The studies on *Hormidium* described above (Say *et al.*, 1977) were extended to include the effects of various other chemical factors in the media (Say & Whitton, 1977). Magnesium, calcium and phosphate all reduced significantly the toxicity of zinc, whilst a rise in pH had the reverse effect. The effect of various natural complexing agents such as humic, fulvic and phenolic acids was to reduce the toxicity of zinc to *Elodea nuttallii* (van der Werff, 1984) and increased concentrations of humic acids reduced the toxicity of cadmium and copper to Daphnia spp. (Winner, 1984). These latter two studies show the apparent amelioration of toxicity to be due to speciation of ions outside the organism and this is an opinion of a large number of workers at the present time (Langston & Bryan, 1984; Laegreid et al., 1984).

1.322 Movement of heavy metals through freshwater ecosystems

The relative insolubility of heavy metals (1.222) means that sediments are often an important sink for heavy metals (Harding & Whitton, 1978). They may be subsequently released from the sediments by biogeochemical cycling (Hamilton-Taylor et al., 1984), by turbulence (Everard & Denny, 1985a) or by uptake by rooted macrophytes (Welsh & Denny, 1976; 1980). Primary producers have been shown to be important factors in the recycling of metals (Hamilton-Taylor et al., 1984; Baccini, 1985). High concentrations of heavy metals have been measured in a wide range of primary producers: planktonic algae (Lund, 1957), filamentous riverine algae (Harding & Whitton, 1981) and aquatic bryophytes (Wehr & Whitton, 1983a), some of which form important food sources for aquatic invertebrates (Cummins, 1975) and, in turn, for fish. Some "bioconcentration" of metals occurs along the food chain (Everard & Denny, 1984); however there is also evidence for direct absorption of metals across gills (Voyer et al., 1975) or into eggs (Davies et al., 1976). Muscle tissue, the main human food from fish, contains relatively low concentrations of heavy metals (Phillips, 1980) and the risks to human health are low except where fish form a large part of the diet.

1.33 Physiology of heavy metal uptake in plants

1.331 Heavy metals as "essential" nutrients

There is good evidence that zinc, copper and possibly cobalt are essential in small quantities for the growth of all higher plants (Mengel & Kirkby, 1982; Salisbury & Ross, 1978).

1.332 Mechanism of cation uptake

In contrast to major nutrients, detailed studies on the physiology of uptake of minor nutrients and heavy metals are relatively scarce. It is worth considering briefly the processes by which major nutrients are accumulated and from these to speculate on mechanisms of uptake that may be relevant to heavy metals. A number of mechanisms for cation accumulation have been identified:

i. adsorption onto cell walls. This is not strictly "uptake" (sensu 1.31) as the ions do not cross the plasmalemma; adsorption may, in turn, create a potential gradient (ii and iii below) into the cell by increasing the concentration of cations and the osmotic pressure in the region adjacent to the plasmalemma (Lüttge & Higinbotham, 1979);

ii. diffusion down concentration gradients;

iii. diffusion down electrochemical gradients. The interior of cells is usually strongly electronegative and this potential may be maintained by an ion pump actively extruding cations (especially hydrogen). This is fuelled by adenosine triphosphate (ATP) (Poole, 1978);

iv. cations may be "actively" transported into cells by "carrier" molecules which bind to the ion, diffuse through the membrane and release the ion on the other side (Lüttge & Higinbotham, 1979).

The nature of transport of any particular ion in a given circumstance is deduced by applying equations such as the Nernst equation to the measured concentrations inside and outside the cell and to the measured potential (Lüttge & Higinbotham, 1979); however there are few estimates of the concentrations of heavy metals in the cytoplasm with which these methods may be used. What is clear, is that the requirement of cells for the "essential" heavy metals is low (Salisbury & Ross, 1978) and that required is subsequently bound into enzymes. Zinc is essential for, in particular, carbonic anhydrase, found in chloroplasts (Mengel & Kirkby, 1982) and superoxide dismutase (Hewitt, 1983). These two enzymes account for approximately 17% of cellular zinc in spinach and parsley. Copper is a constituent of plastocyanin, a constituent of the electron transport chain between photosystems I and II (Mengel & Kirkby, 1982), superoxide dismutase and cytochromes a and a_3. These three enzymes generally account for nearly all cellular copper (Hewitt, 1983). Under normal conditions, therefore, the concentrations of "free" zinc and copper will be very low. Under ~~metal-stressed~~ high metal conditions, production of low molecular weight proteins such as ~~metallothionins~~ metallothioneins (Rauser & Curvetto, 1980) and other complexing agents (Mathys, 1977; Grill *et al*., 1985) may be induced; these again act to decrease the concentration in the cytoplasm.

Low cytoplasmic concentrations, coupled with the electronegativity of cells may act to make conditions generally favourable for the "passive" accumulation of heavy metals by aquatic plants. As there is an expenditure of energy in the maintenance of these conditions, there may be an apparent reliance of uptake on metabolism. Similarly, Parry and Hayward (1973), in a study of zinc uptake by the volvocale *Dunaliella tertiolecta* concluded that some experiments that demonstrated "active" transport may in fact be artefacts of pH changes in the media due to metabolism.

In studies dealing with heavy metals there has been much emphasis upon the role of the cell wall in the preliminary stages of uptake. This is not always a view held by conventional plant physiologists, however Mengel and Kirkby (1982) noted that the calcium content of plants is causally connected with their cation exchange capacity. Calcium is a useful analogue of heavy metals in many ways; it is a minor nutrient which is generally excluded from the cytoplasm (Marmé, 1983) and it is also divalent with a strong tendency to adsorb. Cations such as potassium, whilst of more interest to plant physiologists, are less likely to adsorb onto the cell wall.

An extension to this hypothesis is provided by Peterson (1969) who showed that the concentration of zinc in the cell walls of Agrostis tenuis increased as the plants tolerance to zinc increased. Similar results were obtained by Turner and Marshall (1972), indicating that the cell walls of these plants may be physiologically adapted as a tolerance mechanism. Exclusion mechanisms have also been found in some algae (Foster, 1977).

If these theories regarding the role of the cell wall are correct then they may, in turn, help to explain the role of calcium in mediating metal accumulation. Wehr and Whitton (1983a) found calcium concentrations in plants and water to be a major factor controlling metal accumulation by Rhynchostegium; such a finding may relate to competition at cell wall exchange sites.

Ritchie and Larkum (1984) used efflux-type experiments with radioactive tracers to study the size of the cell wall compartment in Enteromorpha intestinalis. These indicated that in this species the cell wall compartment was large and exchange occurred over a longer time period than might have been predicted. This was an interesting result; many previous workers had assumed that cell wall exchange was a rapid process, lasting a matter of a few minutes and in some species such as Sphagnum (Clymo, 1963) this was shown to be so. The relevance of Ritchie and Larkum's result is that it enables processes extending over much longer periods to be interpreted in terms of cell-wall exchange, although not all of this is cation-exchange *per se*.

There is good agreement that the preliminary stages of metal uptake are due to exchange adsorption (Pickering & Puia, 1969; Jastrow & Koeppe, 1980; Mengel & Kirkby, 1982); however there is disagreement as to whether or not subsequent accumulation is "active" or "passive". Cutler and Rains (1974) interpret the effects of temperature, anaerobic conditions and metabolic inhibitors as affecting the sequestration of cadmium subsequent to diffusion across the plasmalemma. The term "metabolically controlled" may be a suitable compromise between the apparently favourable conditions for passive uptake (see above) and the metabolic processes which are associated with this.

1.4 HEAVY METALS AND BRYOPHYTES

1.41 Introduction

A large proportion of studies on heavy metals and bryophytes are concerned with terrestrial species; it is worthwhile, therefore, to consider briefly these studies before making a more detailed analysis of existing literature on heavy metals and aquatic bryophtyes.

1.42 Heavy metals and terrestrial bryophytes

There has been considerable interest in the accumulation of heavy metals by bryophytes and their effect. Cryptogams were frequently found to be conspicuous elements of the flora on mineral veins (Shacklette, 1965; Purvis & James, 1985). One genus of moss, *Mielichhoferia*, the so-called "copper moss" was confined to copper-rich substrates (Hartman, 1969), although subsequent results have indicated that it is probably high concentrations of sulphur compounds rather than copper itself which determines the distribution (Wilkins, 1977). There has also been much interest in the responses of cryptogams to air pollution. Both lichens (Hawksworth & Rose, 1976) and bryophytes were used to monitor gaseous pollution (Gilbert, 1968; Syratt & Wanstall, 1969; LeBlanc & Rao, 1974) and subsequently also airborne heavy metal pollution (Goodman et al., 1977; Rao et al., 1977). The studies of these two contrasting sources of pollution have been unified by recent theories concerning the mechanism of accumulation of heavy metals (1.433).

1.43 Aquatic bryophytes and heavy metals

1.431 Accumulation studies

Several studies have documented heavy metal accumulation by aquatic bryophytes. Again, these encompass both biogeochemical prospecting and pollution monitoring. Composite samples of up to five species of aquatic bryophyte and peat samples suspended in the river in mesh bags were both reliable indicators of uranium mineralization in the catchment, whilst samples of stream water were unreliable (Whitehead & Brooks, 1969). Streams draining lead-zinc mines in W. Wales were the subject of studies by MacLean and Jones (1975), Burton and Peterson (1979) and Jones et al. (1985). Concentrations of zinc and lead in water were high (up to 27.5 mg l^{-1} zinc and 1.7 mg l^{-1} lead). Concentrations of zinc in bryophytes were up to 1950 µg g^{-1} in Scapania undulata (MacLean & Jones, 1975) and 7023 µg g^{-1} in Philonotis fontana (Burton & Peterson, 1979) and of lead up to 14825 µg g^{-1} in Scapania undulata (MacLean & Jones, 1975) and 15940 µg g^{-1} in Jungermannia gracillima (as Solenostoma gracillima; Burton & Peterson, 1979). Higher concentrations were found in Rhynchostegium riparioides in the R. Etherow, N. England, (Say et al., 1981) and by Empain (1976) and

Mouvet (1985) working on industrially polluted rivers in France. Say *et al*. (1981) attempted to correlate concentrations found in mosses with concentrations in water and in sediments and whilst correlations between mosses and water were generally significant, correlations between mosses and sediment were either insignificant or only slightly significant. Subsequent research has extended the above work to develop databases on the accumulation of a range of heavy metals by *Scapania undulata* (Whitton *et al*., 1982), *Fontinalis antipyretica* (Say & Whitton, 1983) and *Rhynchostegium riparioides* (Wehr & Whitton, 1983a). Decreased accumulation at low pH's has been demonstrated in ~~acid-stressed~~ acidic streams in Scotland (Caines *et al*., 1985).

1.432 Toxicity of heavy metals to aquatic bryophytes

There are relatively few studies on metal toxicity to aquatic bryophtes. *Rhynchostegium riparioides*, *Scapania undulata* and, to a lesser extent, *Fontinalis antipyretica* have been recorded over a wide range of aquatic metal concentrations (1.431), although populations in heavily polluted reaches may be genetically adapted to these environments. Field transplants of *Fontinalis squamosa* and *Scapania undulata* from unpolluted to polluted reaches of the R. Ystwyth caused degradation of the chlorophyll pigments (measured as chlorophyll : phaeophytin ratio) after exposure for ten weeks (MacLean & Jones, 1975). Faster changes were observed in laboratory experiments using three species of *Fontinalis*, *Rhynchostegium riparioides* and *Amblystegium fluviatile* (Glime & Keen, 1984). *Rhynchostegium* was the most tolerant, followed by *Amblystegium* and the *Fontinalis* species.

The critical concentrations are relatively high when bryophytes are compared with other groups of plants; Weise *et al*. (1985) measured reductions in the natural rates of photosynthesis in <u>Fontinalis antipyretica</u> only above 1.0 mg l^{-1} zinc and Sommer and Winkler (1982) noted reductions in photosynthesis in <u>F</u>. <u>antipyretica</u> at similar concentrations of cadmium and copper. Calcium had an ameliorating effect on toxicity (Sommer & Winkler, 1982).

1.433 Mechanism of uptake of heavy metals by bryophytes

A large cation exchange capacity has been widely quoted as a major factor controlling heavy metal accumulation by bryophytes (Rühling & Tyler, 1970; Richardson, 1981). There is increasing evidence, however, that a significant part of the heavy metal measured in plants may be in other compartments: trapped as particulates in and between leaves; discrete precipitates within the cell wall and inside the cell.

The problem of particulate trapping is fundamental to studies on heavy metal uptake. How many "results" are merely artefacts of inadequate washing of samples? Mats of terrestrial bryophytes have particularly effective morphologies for particulate trapping' with large surface area : volume ratios (Brown, 1984). Methods for "detecting" particulate contamination include measurement of ash weight as a proportion of the dry weight; Shacklette (1965) used ash weights of greater than 10% as indicative of contamination. An alternative is measurement of the ratio of iron and titanium, high concentrations of titanium indicating particulate contamination (Mitchell, 1960; Richardson, 1981). Such "contamination" may, however, be the first stage of true uptake.

Observations of bryophyte cell walls from metal-enriched environments using transmission electron microscopy has revealed the presence of electron dense deposits in the cell wall. These have been subsequently confirmed to be metal-rich deposits using electron microprobe techniques

(Mouvet, 1984; Satake & Miyasaka, 1984). Satake et al. (1983) confirmed particles in Jungermannia vulcanicola from mercury-enriched streams to be sulphides of mercury, although the reason why these are deposited remains unclear.

The importance of cation-exchange in bryophytes has been mentioned above (1.332). Clymo's (1963) investigation also showed bryophytes to have high concentrations of unesterified polyuronic acids, the most likely sites of ion-exchange in the cell walls, compared with higher plants. This emphasis upon extracellular accumulation by subsequent workers has tended to play down the role of intracellular uptake of heavy metals. Different metabolic effects have been investigated; light had no influence on metal accumulation by bryophytes. Neither zinc accumulation by Fontinalis antipyretica (Pickering & Puia, 1969) or cadmium uptake by Rhytidiadelphus squarrosus (Brown & Beckett, 1985) were affected, although cadmium uptake by the lichen Peltigera (Beckett & Brown, 1984) was reduced in the dark and by dark pretreatment. Increased temperature caused increased zinc uptake by Fontinalis antipyretica (Pickering & Puia, 1969).

1.5 BIOLOGICAL MONITORS OF HEAVY METAL POLLUTION

1.51 Definitions and scope of biological monitoring

The term "biological monitoring" has been applied to a large number of situations where a biological response is used to determine the effect of changing environmental conditions (Herricks & Schaeffer, 1985). A few workers have attempted to formalize terminology. There is, for example, a distinction between "indicators" which are essentially qualitative and "monitors" which are more quantitative (Bonde, 1977; Whitton, 1984). A third major group are test organisms which are used primarily in the laboratory to evaluate and predict possible environmental effects.

Herricks and Schaeffer (1985) define the field-orientated and laboratory-orientated techniques as bioassessement and bioassays respectively. Biological assessment may be further subdivided (Mason, 1981) into surveys (to define patterns of variation in space); surveillance (the repeated measurement of variables in order to detect trends) and monitoring.

Another fundamental division of biological monitoring techniques is into methods which supplement chemical and physical analyses and methods which may replace conventional analyses. This division is less clear cut and depends in part upon the levels of technology available.

Biological approaches to monitoring the environment are not new. Cairns (1984) lists historical examples of biological monitoring which include the king's wine taster and canaries in coal mines, to detect anthropogenically-introduced toxins and naturally occurring carbon monoxide respectively. A large proportion of pollution is biological in origin and "biological" methods have been the traditional approach. Examples of such techniques include the analysis of water for pathogens, dating from the work of Koch in the last century (Bonde, 1977), biochemical oxygen demand and the saprobic system of Kolkwitz and Marsson (1908, 1909), a biotic index based on associations of particular taxonomic groups (Wilhm, 1975).

The majority of field-orientated biological monitoring techniques in use are based upon community structure. The effects of organic pollution in U.K. rivers are assessed using a variety of indices, the Trent Biotic Index (Woodiwiss, 1964) and the Chandler Biotic Index (Chandler, 1970; Hargreaves et al., 1979) being the two most common (Mason, 1981). The different categories of organisms reflect differing tolerance to organic pollution, from Plectoptera (least tolerant) to chironomid larvae (most tolerant). Other forms of pollution, such as heavy metals, may give distorted results (Hellawell, 1978). Diversity indices, reflecting a more general "level" of environmental stress, are better suited to these situations (Hellawell, 1978; Mason, 1981) and a number of these have also been developed (Armitage et al., 1983). Biological methods for detecting other forms of pollution, although common in the scientific literature, have had less impact on Water Authority biologists.

1.52 Biological monitors of heavy metals

1.521 Introduction

The remainder of this section will consider biological monitoring of heavy metals with reference to freshwaters but, again, including other references where relevant. As commented above (1.51) these methods have not been so readily adopted as methods dealing with organic pollution. In order to adopt these biological techniques as "standard" methods alongside conventional chemical analyses and the few biological methods that have been widely accepted, genuine advantages must be demonstrated. The time, the cost and the level of technical (including taxonomic) ability required are all important considerations.

1.522 Invertebrates

Despite reservations as to the applicability of biotic indices in monitoring heavy metal pollution (Hellawell, 1977; Mason, 1981), Solbé (1977) found both the Trent and Chandler biotic indices to be suitable for monitoring zinc pollution in Willow Brook, Northamptonshire. Other workers have used diversity indices (Cairns, 1979) or have performed more detailed studies concentrating on the relative tolerances of particular taxonomic groups (Armitage, 1980; Armitage & Blackburn, 1985; Norris et al., 1982). The nature of this type of study limits its practical use to individuals familiar with invertebrate taxonomy at the family level or below.

Several studies, both marine (Brooks & Rumsby, 1965) and freshwater (Burrows & Whitton, 1984; Everard & Denny, 1984) have reported the ability of invertebrates to accumulate heavy metals. Routine use of invertebrates in this way, however, is limited by the need to allow them a period to vent their gut contents (Say *et al.*, 1986). Whilst this may be a constraint, there are some significant advantages of certain types of invertebrate-based accumulation studies exemplified by Bryan and Hummerstone's (1977) study in the Looe estuary, Cornwall This made use of organisms from different "niches" to indicate silver and lead pollution in different chemical fractions of the environment.

1.523 Plants

Increasing environmental stress is often associated with decreased species richness (Whittaker, 1975); this has been observed with increased concentrations of heavy metals in the environment (Whitton & Diaz, 1980; Say & Whitton, 1980). Such a technique is not, however, an exclusive monitor for heavy metals and a more practical approach is to observe changes in selected items of the biota. Whitton (1984), for example, notes that both *Stigeoclonium tenue* and *Cladophora glomerata* are common in eutrophic rivers in temperate regions; the latter, however, is very intolerant to heavy metals and the presence of *Stigeoclonium* alone is frequently associated with elevated concentrations of heavy metals.

Whilst there are few studies like that of Foster (1982) with detailed analysis of plant community structure, there have been several attempts to quantify colonization of artificial substrates by algae. Deniseger *et al.* (1986) report a recent example of this using glass microscope slides as the substratum. Bacillariophyta were dominant above and below a lead, zinc and copper mine but Chlorophyta (*Mougeotia* and *Ulothrix*) were virtually absent at the downstream site; an interesting observation in the light of Patterson and Whitton (1981). Species diversity, species evenness and a dissimilarity index were also calculated to compare the sites; these showed strong differences between sites during the summer. Diversity of algal species has been reported to be lowest closest to the source of pollution (Austin & Deniseger, 1985).

A second approach is to use plants as accumulators and there is a very large literature on this. There are several advantages of plants over animals and sediments and in some instances they may be more practicable than straightforward water samples (Whitton et al., 1981). Amongst these advantages are their high levels of accumulation, their ease of collection, storage and transport compared with water samples, possible indication of intermittent pollution events and indication of the fraction of a metal likely to affect biota. Plants are less mobile than invertebrates and do not require additional time to vent gut contents. Accumulation of heavy metals by virtually every group of aquatic plant has been reported; these include algae (Keeney et al., 1976; Johnson et al., 1978; Harding & Whitton, 1981), bryophytes (MacLean & Jones, 1975; Burton & Peterson, 1979; Say et al., 1981), pteridophytes (Paschal & McNamara, 1975) and angiosperms (Heisey and Damman, 1982; Fayed & Abd-El-Shafy, 1985; Staves & Knaus, 1985). Lower plants have an obvious advantage in that all minerals may be assumed to derive from the water, whilst in rooted higher plants there is evidence (Denny, 1972) that minerals may derive from both the substrate and the water and may be translocated within the plant. These features may themselves be used in a more sophisticated monitoring system; Welsh & Denny (1980) noted that whilst copper was translocated from the root to the shoot, lead was not. Entry of lead into the shoot was shown to be the result of stirring of the sediments under turbulent conditions (Everard & Denny, 1985a; 1985b).

It is pertinent to end this section with some examples of situations where plants have played a positive role in aquatic monitoring. Two of these studies concern aquatic mosses and the third deals with estuarine *Enteromorpha*. Say and Whitton (1983) collected samples of water and *Fontinalis antipyretica* from below an intermittent zinc discharge on the R. Wear, near Frosterley. The concentration of aquatic zinc was low and showed little variation for some 50 km below the effluent. The concentration of zinc in 2-cm tips of *Fontinalis* was much higher near the source of the pollution and decreased gradually downstream. The high concentrations in the moss presumably reflect periods when the zinc concentration in the water was much higher. A similar type of study was conducted by Mouvet (1985) using *Cinclidotus danubicus* to detect discharges of copper at four sites along the R. Bienne, France. The use of biological monitors in estuaries is equally effective. Fluctuating water levels and chemical composition makes conventional water samples difficult to interpret, however Harding (1980) used analyses of *Enteromorpha* to give an integrated record of pollution in the Mersey estuary.

1.524 Use of moss-bags

The use of mesh bags for experimental transplants is not new. They have been used widely in decomposition studies (Sharma & Goel, 1986) and in studies of organic pollution (Standing Committee of Analysts, 1983). In studies of air pollution they were packed with *Sphagnum* which was teased out to increase the surface area and suspended at known heights above the ground for long periods of time (Goodman *et al*., 1977). Whether this constitutes a truly biological method is debatable as the *Sphagnum* was oven-dried and washed prior to use. It was, however, a novel technique and quite widely used.

Similarly, their use in aquatic monitoring is not new. Mention has already been made of Whitehead and Brooks' (1969) study and it is probable that several water management bodies in different parts of the world are using or have used similar techniques. The principle advantage is in being able to use bryophyte monitoring techniques that have already been developed (e.g. Wehr & Whitton, 1983a; Wehr et al., 1983) in situations where there are no indigenous populations of bryophytes. At other sites, information regarding the nature of pollution may be better collected using transplanted rather than in situ mosses. Another major advantage is in being able to fully standardize all aspects of technique, from collection of the moss, through the length of exposure and the conditions of exposure in the river, to digestion and analysis.

1.6 GROWTH OF AQUATIC BRYOPHYTES

The growth rate of aquatic bryophytes has been the subject of relatively few studies given their importance in nutrient cycling (Meyer, 1979), as substrata for invertebrates (Glime & Clemons, 1972) and in tufa-formation (Pentecost, 1981). Of more particular interest to this study is the ability of populations of Rhynchostegium to persist throughout the year (Wehr & Whitton, 1983b) and to remain available for monitoring purposes.

Before discussing the different methods of measuring growth it is first necessary to consider the ecological forms of terrestrial and aquatic species. Gimmingham & Birse (1957) discuss the different growth forms of bryophytes. Rhynchostegium (as Eurhynchium riparioides) is classified as a smooth mat with its shoot system prostrate and adpressed to the rock, offering little resistance to the water. This species along with others such as *Fontinalis antipyretica* and *Amblystegium riparium* is also often found as "wefts" attached to the "trailing edge" of boulders. A lush bryoflora is more likely to develop on boulders least subject to movement under high flow and which are consequently more difficult to remove for measurements.

A number of techniques have been used in studies of growth rates of terrestrial bryophytes. Clymo (1970) suggests that close agreement between two or more different methods is the best evidence of accuracy and the more diverse the methods the better their mutual support. Few of the studies subsequent to Clymo (1970) do this, although the reason is as likely to be sampling difficulties as negligence. Russell (1984) reviewed the methods that had been used successfully and applied these to a variety of species in the sub-Antarctic. Of eight techniques that were tried, none were satisfactory for all of the 16 species under consideration. Four of the techniques were suited only to bryophtyes with vertically growing growth forms and one, photography, was suitable only for saxicolous species forming radially expanding colonies on rock surfaces. The most consistently ~~sucessful~~ successful method was the "cranked-wire" technique of Clymo (1970), in which a piece of wire shaped like a car starting handle is pushed into a cushion parallel to the stems. Growth is measured from the amount of wire still projecting above the surface. Again, this is a technique suited only to vertically growing species. Well-defined annual segments may be used directly (Longton, 1970; Baker, 1972) but relatively few species show such variation. The only method really suited to flowing-water situations is "tags" of thread tied around the tip a known distance from the tip.

"Tags" have been used in a number of studies (Overbach & Happach, 1956; Tallis, 1959; Benson-Evens & Brough, 1966) but the method does have some significant drawbacks. Tags may interfere with external capillary action in ectohydric species (Russell, 1984) but this is unlikely to be a problem in aquatic habitats. Rieley et al. (1979) noted that applying the tags was liable to disturb the normal growth of the moss, but the tags are relatively easy to attach in the open mats and wefts typical of Rhynchostegium and the water current very quickly settles the moss into its previous positions. The charge that the method is tedious and time-consuming (Tallis, 1959; Russell, 1984) has no answer.

1.7 AIM

This study follows earlier projects funded by the European Economic Community (contract 074-74-1 ENV.UK) and by the Department of Environment (contract DGR/480/571 "Bryophytes for Monitoring River Water Quality"). These studies concentrated upon developing methods for processing and analyzing aquatic bryophytes and establishing broad relationships between metal concentrations in the water and in a number of species. The present project continued this work and concentrated on developing methods for using moss-bags to monitor intermittent heavy metal pollution.

The species chosen for the study was Rhynchostegium riparioides (synonyms Eurhynchium riparioides, Platyhipnidium riparioides). This has several advantages over other common aquatic plants (Whitton et al., 1981), in particular it is probably the most common aquatic bryophyte species in the country (N.T.H. Holmes, unpublished). It also accumulates heavy metals to higher concentrations than several other common species of aquatic bryophytes (1.431, Say et al., 1981; Wehr & Whitton, 1983b), is robust, perennial and relatively easy to identify. In addition, a brief comparison was made with another common species, Fontinalis antipyretica.

The review of the literature revealed a number of areas, both fundamental and applied, where further study would be valuable. Therefore the specific aims of the this study were as follows:

i. to study the effects of mesh-bags on metal accumulation by Rhynchostegium and to compare this with simultaneous uptake by populations on boulders;

ii. to investigate the physiological processes involved in metal accumulation and the cellular location of metals. This was to concentrate on the role of the cell-wall in metal accumulation;

iii. to make an in-depth study of a site of intermittent heavy metal pollution and to use aquatic bryophytes to monitor this. Particular practical problems associated with deploying moss-bags were to be investigated;

iv. to study the growth rate of Rhynchostegium in a number of streams over a 12-month period and to compare the effects of different concentrations of heavy metals.

2. METHODS

2.1 ANALYTICAL METHODS

2.11 Routine laboratory procedures

All laboratory glassware, with the exception of snap-cap vials, was made of borosilicate ("Pyrex") glass. Volumetric glassware used for atomic absorption spectrophotometry (AAS) was of "A" grade. Snap-cap vials, made from soda glass, were used for all digest samples and for some water samples. Previous workers in Durham (e.g. Wehr, 1983) found no problems of contamination arising from these tubes. Plastic apparatus was chosen following recommendations of Batley and Gardner (1977) and was made of either polypropylene or polyethylene. Water samples were collected into either 30 ml snap-cap vials (see above), or 60 ml polypropylene bottles ("Azlon"). Moss samples were stored in 250 ml bottles made from high-density polyethylene.

Two grades of water were used: single-distilled water was used for most general laboratory purposes and for rinsing of glassware and deionized water was used for analytical purposes, for media and for final rinsing of analytical glassware (see below). Two sorts of deionized water were used:
To December 1984; double-distilled water passed through a Houseman model C ion-exchange column.
From January 1985; "Milli-Q" reagent grade water system (Millipore Corp.).
Both are termed "deionized" water throughout.

Inorganic metal salts (with the exception of $Na_2SiO_3.5H_2O$) were of "Analar" grade. All were obtained through British Drug Houses (BDH), Poole, Dorset, as were most other reagents with the exception of N-2-hydroxyethylpiperazine-N'-2-ethanesulphonic acid (HEPES), hydroxylamine hydrochloride (both from Sigma) and humic acid (from Aldrich). Standards for AAS were BDH "Spectrosol" 1000 mg l^{-1} standard solutions prepared in 1 N acid. Working standards were prepared from these for each batch of analyses. HNO_3 for analytical purposes was of atomic absorption grade (Fisons), other acids were "Analar" grade.

The following procedure was adopted for washing apparatus; very dirty vessels were given a preliminary scrub in tap water plus detergent and rinsed in tap water. All apparatus was given a preliminary rinse in distilled water, immersed in 4% HNO_3 for a minimum of 30 minutes (Laxen & Harrison, 1981), rinsed six times in distilled water and set to dry. Apparatus used in the collection and analysis of anion samples was rinsed using the above protocol except that 10% H_2SO_4 was used in place of HNO_3. Analytical glassware was given an additional two rinses in deionized water prior to drying.

2.12 Routine atomic absorption spectrophotometry procedure

All analyses of metals were performed on a Perkin-Elmer (PE) model 5000 Atomic Absorption Spectrophotometer with a PE model 5000 Automatic Burner Control Unit. Low level determinations were performed on the above with a graphite furnace (PE model HGA 500) and autosampler (PE model AS 40) replacing the burner unit.

Most determinations were performed using an air-C_2H_2 flame. For analyses of Mg, Ca, Fe, Cd and Pb, standard solutions contained the same molarity of acid as the sample solutions. No pretreatment of samples was required, except for K, Mg and Ca. For K, an addition of 15000 mg l^{-1} NaCl to a 4 ml aliquot of sample (final concentration = 10% v/v) was made, to suppress ionization interferences (Perkin-Elmer Corporation, 1982) and for Mg and Ca an addition of 7% $LaCl_3$ was made (final concentration = 0.51% v/v) to suppress chemical interferences from Al, Be, P, Si, Ti, Va and Zi (Perkin-Elmer Corporation, 1982). A deuterium-arc lamp was used to correct for background absorption (light scattering by particles and molecular absorption of light by molecules in the flame) for all determinations on the furnace and for analysis of Cd and Pb using the flame.

The detection limit fluctuated slightly between batches, depending upon the machine set-up but typical detection limits are given in Table 2.1. Analytical quality control followed guidelines given by Cheeseman and Wilson (1978); preliminary studies provided estimates of random errors and these data were used to construct tables giving values of each variable ± 1 S.D. (67% confidence interval) and ± 1.98 S.D. (98% confidence interval) (Table 2.2).

Table 2.1 Detection limits, or lowest measured concentrations (in mg l^{-1}) for AAS.

	Graphite furnace	flame
Na	-	0.005
K	-	0.05
Mg	-	< 0.20
Ca	-	< 0.20
Cr	< 0.002	0.010
Mn	< 0.001	0.005
Fe	0.005	0.03
Zn	< 0.001	0.003
Cd	< 0.0005	0.003
Pb	< 0.0010	0.02

Table 2.2 Random errors associated with determination of metals in water samples (mg l^{-1}) by flame A.A.S..

metal	concentration	+ 1 SD	+ 1.96 SD
Na	0.200	0.007	0.014
	2.000	0.015	0.029
K	2.000	0.072	0.141
	10.00	0.108	0.212
Mg	0.200	0.005	0.010
	2.00	0.02	0.04
	10.00	0.08	0.16
Ca	0.200	0.013	0.026
	2.00	0.02	0.04
	10.00	0.08	0.16
Mn	0.20	0.06	0.12
	1.00	0.09	0.18
Fe	0.050	0.002	0.004
	0.200	0.006	0.012
	1.00	0.025	0.049
Zn	0.0500	0.0016	0.0032
	0.200	0.002	0.004
	1.00	0.01	0.02
Cd	0.050	0.001	0.002
	0.200	0.002	0.004
	1.00	0.01	0.02
Pb	0.200	0.005	0.010
	1.000	0.007	0.014
	4.000	0.067	0.013

2.13 Collection and analysis of water

Water was collected from rivers and streams in a 2-litre polypropylene beaker, which had been rinsed several times in the water before the sample was taken. Two fractions of water were collected. "Total" water is water decanted from the beaker after being allowed to stand for approximately five mins to allow larger particles (> 20 µm diameter; Black, 1965) to settle out. "Filtrable" water is water that was passed first through a 0.22 µm Nuclepore filter (Sterilin). The water was collected from the beaker using a 20 ml polypropylene syringe (Faber Sanitas) and forced through a Swinnex filter holder (Millipore Corp.) containing the filter, acid washed as described in 2.11. The first few drops through the filter were shaken in the sample bottle and discarded and approximately 25 ml of sample collected. For several sites the filter became clogged and was replaced. Again, the first few drops through were discarded. 0.4 µm Nuclepore filters were used for one experiment (7.25) to facilitate collection of a large volume (250 ml) of stream water. Water samples were stored in an ice box until return to the laboratory, where 2 drops of HNO_3 were added to each bottle. Samples were stored at 4 °C until analysis.

Samples that were to be used for anion analysis were filtered through a GF/C filter into an H_2SO_4-washed, iodized polyethylene bottle and stored at 4 °C until analysis.

The methods used to measure physico-chemical variables are summarized below:

Light was measured using either a Skye Instruments sensor and meter or a Macam Q101 sensor and meter. Both had "cosine corrected" radiation sensors designed to measure photosynthetically active radiation (PAR). The Macam sensor was not waterproof so readings were taken at water level but not below.

Temperature was measured in situ using a mercury thermometer or a WTW thermistor attached to a WTW model LF 91 meter.

Current speed was measured on a calibrated Ott current meter. For most experiments the impeller was placed in the fastest current in the reach, about 10 cm below the surface and perpendicular to the direction of flow (Patterson, 1983). During the growth experiments and where moss-bags were being used, the impeller was placed about 50 cm downstream of the sample.

Conductivity was recorded either using an Electronic Switchgear model MC mark V conductivity meter or, in situ, using a WTW model LF 91 conductivity meter.

pH was recorded using an Orion model 407A meter with an Orion model 91-05 combination electrode, or using a WTW model PH 91 meter and Ingold combination electrode.

Alkalinity was measured by a potentiometric titration against 0.02 M H_2SO_4 (American Public Health Association, 1981).

Dissolved oxygen was measured in situ using a WTW model OXI 91 meter and WTW electrode model EO 90. The electrode required a steady current of 15 cm s^{-1} past the membrane and, if the stream did not provide this, then the electrode was stirred until it gave a steady reading.

1

2.14 Analysis of anion samples

PO_4-P (as Filtrable Reactive Phosphate (FRP)) was determined colorimetrically according to the method of Murphy and Riley (1962), modified by Eisenreich et al. (1975). Cl^-, NO_3-N and SO_4-S were analyzed using a Laboratory Data Control (LDC) ion chromatograph with a Chrompack SPH 125 autosampler and an LDC ConductoMonitor III conductivity meter attached to a Kipp and Zonen BD40 chart recorder. This is a high-pressure liquid chromatograph with a 25 cm Hamilton X-100 ion-exchange column packed with PRP X-100 resin (Hamilton). A 2-cm guard column containing the same resin was used to protect the separation column by removing potentially poisonous substances. The eluent was 4 mM P-hydroxybenzoic acid at pH 8.5 with a flow rate of 2 mm min^{-1} and a pump pressure of about 1.59×10^4 kPa. Peak heights were recorded at full-scale deflections of between 1 and 10 $\mu S\ cm^{-1}$. Concentrations were calculated from peak heights.

2.15 Collection and analysis of moss samples

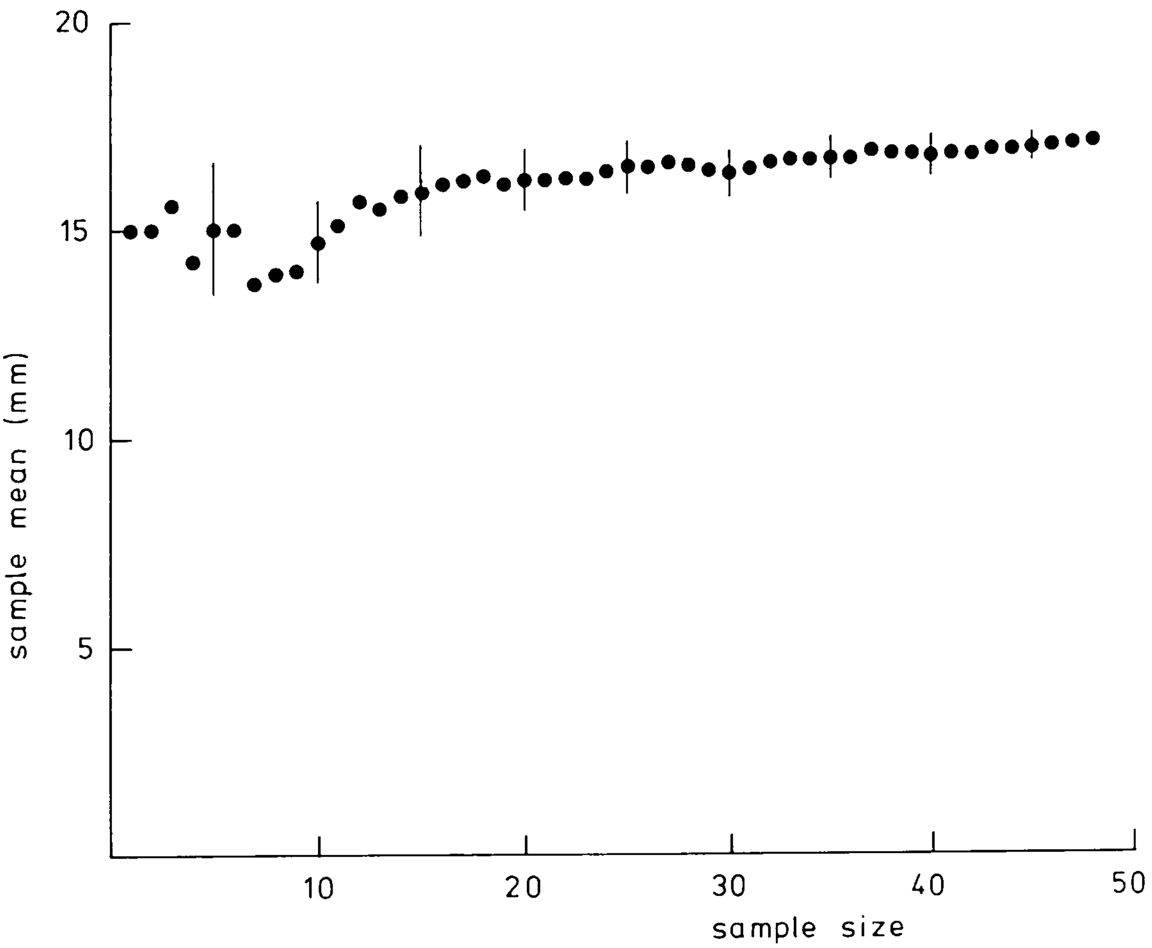
The procedure for collecting moss was adapted to suit the final use: to provide samples directly for analysis, or to provide material for transplant experiments or laboratory use. For samples that were to be analyzed directly (i.e. with no further experimentation), moss was collected from at least five separate locations within a reach (Wehr & Whitton, 1983a). For material that was to be experimented upon further, larger collections were usually required and, in order to preserve existing populations, moss was collected from just above and below, as well as within the designated reach (Wehr, 1983). It was picked off boulders, rinsed several times in the stream water to remove loosely attached sediment, invertebrates and algae, and placed in sample bottles. Moss was stored in an ice box until return to the laboratory and thereafter in a refrigerator until required. All samples for analysis were processed within 48 h by rinsing in distilled water and then transferring shoots to a petri dish where 2-cm tips were removed and rinsed several times in deionized water. These were then blotted and put into HNO_3-washed snap-cap vials. Five replicate sub-samples of each sample were taken, each containing 25-30 tips (Wehr & Whitton, 1983a). These were dried at 105 °C before being weighed and transferred to boiling tubes with 5 ml of 2 M HNO_3, placed on a Tecam "dri-block" at 120 °C for 45 mins and allowed to cool. The samples were centrifuged to separate the clear solution from the slurry. The clear solution was poured into a 25 ml volumetric flask and the slurry was recentrifuged with deionized water. The clear solution from this was added to the volumetric flask and made up to volume. The samples were stored in their original vials until analysis.

2.2 GROWTH MEASUREMENTS

Measurements of growth rate were performed on populations of Rhynchostegium attached to large, but not immovable, boulders to reduce the probability of the boulder being washed away during periods of high flow, yet still enable the boulders to be removed for measurements. After October 1985 two boulders were marked at each site to further reduce the probability of all data for one month being lost.

Growth rate was measured by the attachment of thread ("tags") to individual shoots at a known distance from the tip and measuring the shoot extension after one month. Of the eight techniques reviewed by Russell (1984), only the attachment of tags was really suited to the growth form of Rhynchostegium (1.6). A preliminary experiment in 0310-90 tested the effect of increasing sample size on the sample mean (Fig. 2.1). From these data a sample size of 35 shoots was chosen.

Fig. 2.1 The effect of sample size on mean and 95% confidence intervals in measurements of growth rate of <u>Rhynchostegium</u> in 0310-90.


20
15
10
5
sample mean (mm)
10
20
30
40
50
sample size

Boulders were removed from the water, placed in an enamel tray and tags of thread cut and looped over shoots, 10 mm from the tip. Black and white thread was used in alternate months to avoid confusion with the previous month's growth. 35 shoots were labelled per site and the boulder returned to the water in the same position as before. The sites were visited at approximately one month intervals. At each visit boulders were removed as before, the length from the thread to the tip was measured for each shoot and a fresh piece of thread looped on 10 mm from the tip. The total number of labelled shoots was counted and fresh shoots were labelled to bring the total up to 35 again. The boulder was replaced as before, water samples collected and physico-chemical variables measured.

The rate of growth was calculated as:

$$\text{growth rate (mm wk}^{-1}\text{)} = (L_n - L_o) \times (7/n)$$

where; n = number of days since previous sample

L_o = length of shoot at start (= 10 mm)

L_n = length of shoot at time = n

Occasions where the length of the shoot was less than 10 mm were also recorded, hence the final figure refers to "net", not "total" growth.

2.3 FIELD EXPERIMENTS

2.31 Designation of sampling sites

All sample sites were allocated a unique six figure code in accordance with standard practice in Durham (Whitton *et al.*, 1976). The first four figures refer to the stream and the last two to a particular 10 m stretch, termed a "reach". No significance is attached to stream numbers, they serve solely to enable records to be stored on a computer. The reach number allows any 10 m reach to be relocated at future dates and is kept, along with details of landmarks, grid references etc.. Reach 01 is reserved for the source and 99 for the reach immediately above a confluence. A fuller description of the system may be found in Holmes and Whitton (1981).

2.32 Experimental regime

Moss for field experiments was collected (2.15) and transferred to the experimental site in beakers enclosed in an ice box. Where moss attached to boulders was required then medium-sized boulders with a good cover of moss were chosen. In both instances moss was kept moist but not immersed in stream water during transport.

Stakes to support moss-bags were placed approximately mid-stream and hammered securely into the stream bed. Transplanted boulders were placed alongside the stakes. Moss was placed in moss-bags (for quantities, see Chapter 5), which were looped over stakes using stout string. The necks of the bags were kept loose to facilitate the removal of samples. A maximum of three bags was attached to each stake.

Sample intervals were spaced according to experience. An initial phase of rapid uptake, followed by a longer period of slow accumulation was expected, so the sample intervals were closely spaced (30-min intervals) for the first four hours and subsequently longer time intervals were used. At each sampling occasion, small amounts of moss were withdrawn from the bags and pooled to give a sample which was stored in a polyethylene bottle for transport to the laboratory and treatment as described in 2.15. Physico-chemical measurements and water samples were collected at hourly intervals for the first four hours and subsequently each time a moss sample was taken.

2.4 LABORATORY EXPERIMENTS

2.41 Experimental regime and media

As far as possible, all experiments were performed on a single population of Rhynchostegium (from 0310-90). This is a low-zinc site and for experiments where moss from a zinc-enriched site was required this was collected from 0288-70. The tips were prepared as described in 2.15 and stored moist, in the dark at 4 °C until used.

The nutrient medium for laboratory studies was a modification of that described by Chu (1942), known in Durham as Chu 10E. The medium was buffered with 2.5 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulphonic acid (HEPES) and adjusted to pH 7 using 2 M NaOH. Iron was chelated with 3.15 mM ethylenediaminetetra-acetic acid (EDTA). Physico-chemical parameters and the concentrations of elements are listed in Table 2.3.

The medium was prepared at least 12 h in advance of each experiment and left to equilibrate to the correct temperature. Metal stocks were added immediately before the moss was introduced. Ten tips (dry weight = ± 10 mg) were added to 200 ml of medium in a 250 ml Ehlenmeyer flask and placed in a shaker tank (Gallenkamp) set usually at 15 °C and with 100 µmol photon m^{-2} s^{-1} continuous subsurface illumination. Preliminary experiments had shown that provided the flasks were shaken, the medium remained saturated with respect to dissolved gases under these conditions.

Table 2.3 Concentrations of elements (mg l^{-1}, before pH adjustment) and other physico-chemical parameters in Chu 10E.

N	6.84	Na	4.53
Mg	2.47	Si	1.44
P	0.89	S	3.27
K	1.12	Cl	1.13
Ca	9.78	Mn	0.012
Fe	0.50	Co	0.002
Cu	0.005	Zn	0.013
Mo	0.003	B	0.125
HEPES	2.5 mM	EDTA	3.15 mM

pH 7.0

conductivity 144 µS cm^{-1}

total alkalinity 2.5 meq l^{-1}

At the end of the incubation period tips were washed in deionized water before being either dried and digested (2.15) or further treated.

2.42 Elution methods

2.421 Background

A method which allows rapid compartmentalization of ions within a plant is, obviously, highly desirable. Several methods exist for the precise compartmentalization of ions, yet these are generally too time consuming (e.g. autoradiography) or involve the use of techniques that it is not possible to adapt to large numbers of field populations (e.g. tracer -flux analysis). For the purposes of this study two compartments were of interest (1.332):

a. the "cell wall", "exchangeable", or "Donnan Free Space" (DFS) fraction and

b. the "intracellular" fraction.

The principle requirement of a method, therefore, was that it allowed rapid differentiation between these two fractions. Several workers have used either competing ions (Rühling & Tyler, 1970; Brown & Buck, 1979) or chelating agents such as EDTA (Ernst & van der Werff, 1978; Shehata & Whitton, 1982) to remove the exchangeable component. The principle of both is to extract the exchangeable component without damaging the plasmalemma in the process. This precludes the use of traditional soil eluents such as acetic acid (Agricultural Development and Advisory Service, 1981).

The sequential elution method of Brown and Buck (1979) was used as a framework around which other methods were tested. The full treatment extracts four separate components, of which only the exchangeable and total intracellular were of interest. The following modifications were therefore made: only a single rinse in deionized water was performed at the start and step 3 (steeping in boiling, deionized water) was omitted. The eluents were: 20 mM (= 1000 mg l^{-1}) $NiCl_2$ (Brown & Buck, 1979), 0.138 mM (= 40 mg l^{-1}) EDTA (Shehata, 1982; Shehata & Whitton, 1982) and 0.75 M HCl (Brown & Slingsby, 1972). Preliminary experiments to check for membrane damage, to consider the effect of increased exposure times and to examine their effect on Zn removal from isolated cell walls were performed, prior to the selection of a method.

2.422 Measurement of membrane damage

In order to assess the methods outlined above a simple "probe" for checking membrane integrity was used. This was based upon the analysis of the concentration of K released by the treatments. K is known to be selectively accumulated by plant cells and to be located predominately inside the cell (Flowers & Lauchli, 1983). Release of K, therefore, implies damage to the membrane (Brown & Slingsby, 1972; Puckett _et_ _al_., 1977) and a breakdown in the metabolic processes which maintain the Na^+/K^+ pumps in the membrane. Puckett _et_ _al_. (1977), for example, noted a good correlation between the total K loss from _Umbilicaria_ _muhlenbergii_ and _Cladonia_ _rangiferina_ and reduction in ^{14}C fixation.

The extent of K leakage was assessed qualitatively by comparing the K concentration washed out of the moss by the various treatments. An initial wash in deionized water displaced K in the "Apparent Free Space" (AFS) and served as a control. The moss was then washed in the experimental solutions and in deionized water again. If there was no damage to the membrane, then the subsequent washes would be expected to contain low concentrations of K, whereas if the membrane was damaged, then K would continue to leak out. A K concentration significantly higher than in the initial solution indicates some damage to the membrane.

2.423 Routine procedure for separating exchangeable fraction

$NiCl_2$ was selected as the most appropriate eluting agent for zinc (see 6.32). A routine procedure was derived, as follows;
tips were given a preliminary rinse in deionized water (1 h), followed by two rinses in 20 mM $NiCl_2$ (1 h each); the solutions were pooled, made up to volume and analyzed; finally the tips were rinsed in deionized water (1 h), dried, weighed and digested (2.15).

2.43 Preparation of cell walls

The method used to prepare cell walls was adapted from Ritchie and Larkum (1982). Cell contents were extracted using Triton X-100 and methanol to yield a tip which retained its gross morphology, yet was largely free from cell organelles. This they described as a "crude cell wall preparation", to distinguish it from the purer preparations based upon homogenized tissue which have been devised (e.g. Demarty et al., 1978). For the purposes of this study the benefits that would have been gained from the latter technique were outweighed by that of retention of the shape and form of the living tissue.

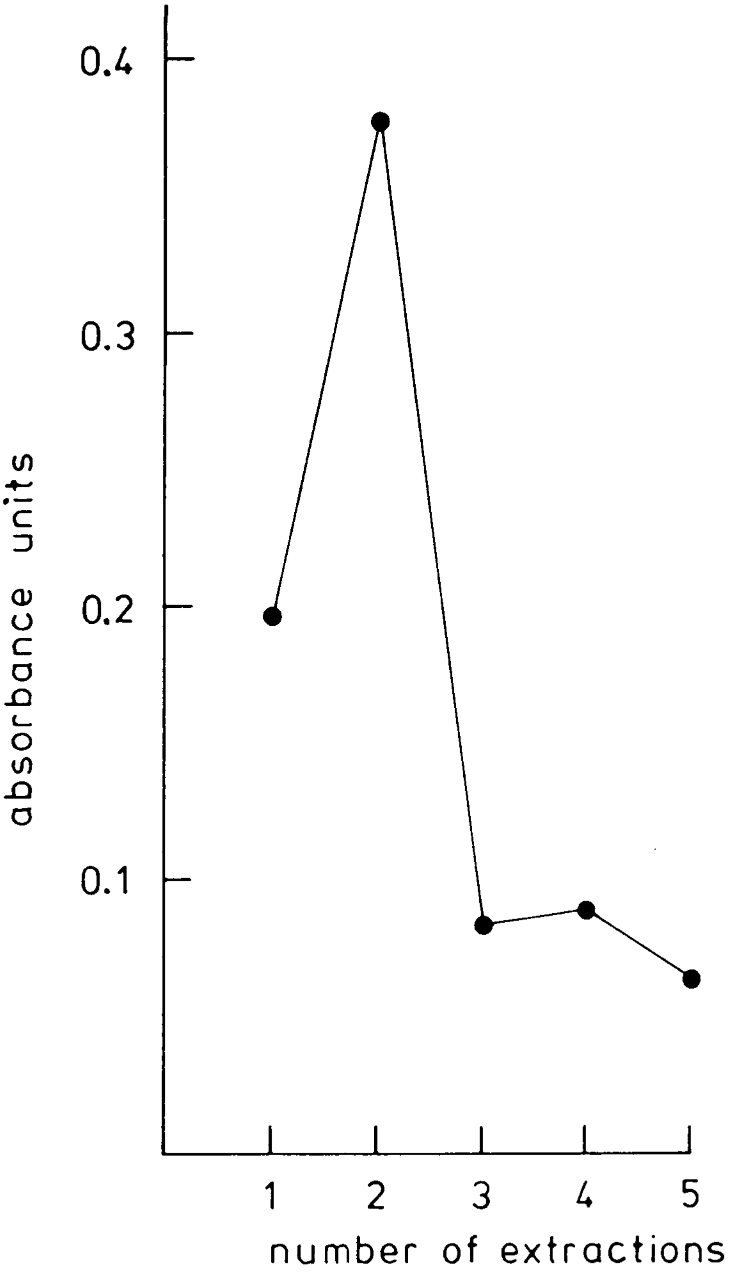
Tips that were used in the preparation of cell walls were given a preliminary wash in 0.1 M hydroxylamine in 0.01 M HNO_3, to remove encrusting manganese and iron oxides (Gupta & Chen, 1975) and were then shaken alternately in 0.5% Triton X-100 and 90% methanol (1 h each) until all pigmentation was removed. The concentration of chlorophyll (measured as $chlorophyll_a$ by the method of Marker et al., 1980) in successive methanol extractions is given in Fig. 2.2. In one experiment an additional eluent, 0.2 M ammonium oxalate in 0.2 M oxalic acid (Tipping et al., 1985), was included to selectively solubilize Fe oxides.

In order to demonstrate the cation exchange behaviour of the cell walls, the crude cell wall preparations were converted to the H^+ form by four washes, each of five minutes duration followed by one wash overnight, in 0.1 M HNO_3 (Wainwright & Beckett, 1975). This was followed by successive washes in deionized water. A preliminary experiment showed at least eight washes to be required until no further drop in pH, caused by H^+ ions being washed out of the AFS, was observed.

2.5 ELECTRON MICROSCOPY METHODS

Individual leaves of Rhynchostegium were fixed in a solution of 2.5% glutaraldehyde, 1.5% formaldehyde and 0.05 M cacodylate buffer at pH 7.0 for 2.5 h, postfixed in 1% osmium tetroxide and rinsed in two 10 minute

Figure 2.2 Concentration of chlorophyll in successive extractions of *Rhynchostegium* during cell-wall preparation


0.4
0.3
0.2
0.1
absorbance units
1
2
3
4
5
number of extractions

changes of deionized water. They were then passed through a dehydration series consisting of two 10 min changes in 10%, 20%, 25%, 30%, 50% and 75% ethanol, three 10 min changes in 100% ethanol and 24 h in a 50:50 mix of 100% ethanol and Spurr's low viscosity resin (Spurr, 1969). After two 24 h changes in resin the sample was polymerized at 80% °C overnight. Sections 80 - 250 nm in thickness were cut using a diamond knife. The sections were stained with uranyl acetate and lead citrate, mounted on copper grids and viewed using a Philips EM 400 transmission electron microscope.

2.6 STATISTICS AND COMPUTING

2.61 Hardware and software

Computing facilities consisted of the Northumbrian Universities Multiple Access Computer (NUMAC) and a microcomputer. The NUMAC machine consisted of an IBM 360/370 mainframe (replaced by an Ahmdahl 470/V8 in September 1985) running under the Michigan Terminal System (MTS). The microcomputer was a Research Machines (RM) Nimbus, capable of connecting to NUMAC, but more frequently operated independently. This used the MS-DOS operating system.

Most data were analyzed on the mainframe, using the Michigan Interactive Data Analysis Software (MIDAS) package (Fox & Guire, 1976) or the MINITAB package (Ryan et al., 1982). Certain procedures not available in these (e.g. some forms of analysis of variance (ANOVA)) were performed on the RM Nimbus using the "Multiplan" spreadsheet package (Microsoft, 1982).

This thesis was prepared using the "Word" word processing package (Microsoft, 1983) on the RM Nimbus with a Toshiba P1351 printer.

2.62 Preliminary analysis of data

Raw data was entered into datafiles and transformed within MIDAS or Multiplan to give metal concentrations as µg g^{-1} dry weight. Descriptive measures were obtained for each sample (= five sub-samples in field experiments and four in laboratory experiments) and sample means were abstracted and used to create new datafiles arranged by treatment.

Coefficients of variation within samples were generally < 15%. In some instances, however, they rose to 50% and even 100%. In such instances, the individual sub-samples were examined and very often one sub-sample was obviously responsible for the bulk of the variation. There is no universally accepted procedure for dealing with maverick values but in this work a single value was removed from a sample mean only if by doing so the coefficient of variation was reduced by 50% or more.

The preliminary analysis of data involved simple tests to satisfy that the data fitted a normal distribution and, where appropriate, transformations were applied. MIDAS was used to compute $\log_e$, $\log_{10}$ and square-root values for each case and to produce coefficients of skewness and kurtosis, histograms and correlation matrices for each variable. These were compared and the most appropriate transformation chosen.

2.63 Statistical analysis of data

The majority of the data could be analyzed by relatively straightforward bivariate statistics, e.g. product-moment correlation coefficients, ANOVA and "Student" t-tests, all available within the MIDAS package. Regressions were compared using a form of ANOVA (Parker, 1979; Mead & Curnow, 1983) calculated within the Multiplan spreadsheet (Microsoft, 1982). The residual mean squares arising from each individual regression were compared using a "2-tailed" F-test and, if the difference was not significant, then an ANOVA, comparing the sum of the two individual regressions with a single regression composed of the two sets of data combined, was performed.

Non-parametric tests were calculated within MIDAS; the tests used were Spearman's Rank Correlation, Kendall's Tau coefficient and Kendall's coefficient of concordance (Statistical Research Laboratory, 1976; Elliott, 1977).

3. STUDY SITES

3.1 INTRODUCTION

This chapter introduces the areas and streams where field experiments were performed and material for laboratory experiments was collected. Sites were chosen for a number of reasons; nearness to Durham, availability of healthy Rhynchostegium and presence or absence of heavy metals and other environmental features were the main ones. The study was well located in that the Northern Pennine Orefield, the largest orefield in Britain and once the most important lead mining region in the world, is within 30 km of Durham (Fig. 3.1), providing a wide range of sites. Some studies, however, were performed on lowland sites closer to Durham and for the case study (Chapter 8), a site at Bolton, N-W. England provided a stimulating problem.

The general geography and geology characteristic of the region is outlined first, under four headings; Alston Moor and Weardale represent the Northern Pennine Orefield, sites closer to Durham are located on the Durham Coalfield and the case study site is the Croal Valley. This account is based upon Geological Survey Memoirs (Dunham, 1949; Smith & Francis, 1967; Tonks et al., 1931) along with other material. The quality of the water is recorded as River Chemical Class (National Water Council, 1981; see Toms, 1975, for descriptions of the four classes). A summary of environmental data at sites in N-E. England is presented next, followed by a more detailed consideration of the sites used for the growth studies (Chapter 4) and the case study (Chapter 8).

Fig. 3.1 Map of N-E. England showing location of sampling regions.

1 = 0005, R. Deerness;

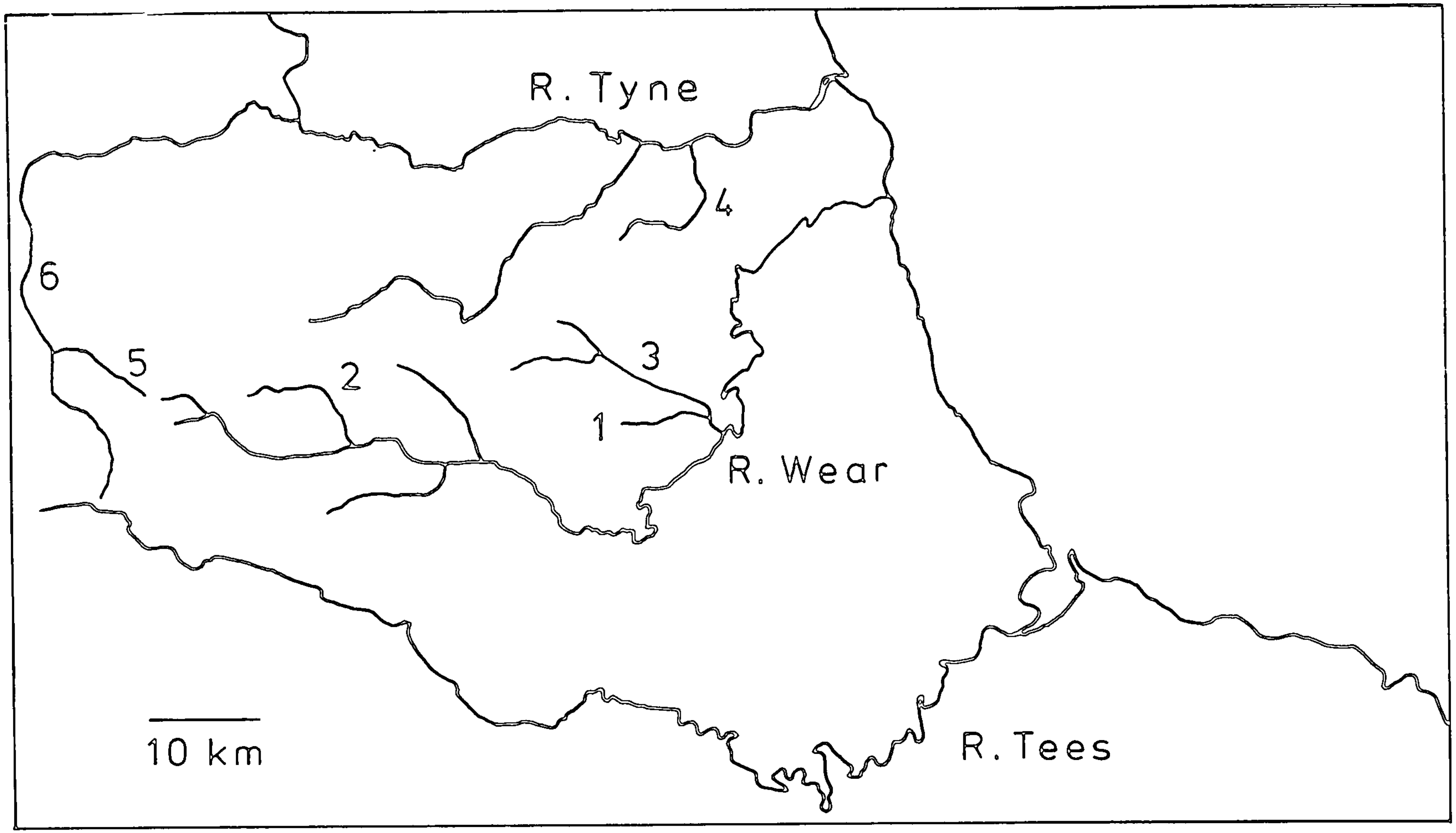
2 = 0012, Rookhope Burn;

3 = 0014, R. Browney;

4 = 0024, R. Team;

5 = 0048, R. Nent;

6 = 0055, R. South Tyne.


R. Tyne
4
6
3
5
2
1
R. Wear
10 km
R. Tees

3.2 GEOGRAPHICAL LOCATION AND PHYSICAL CHARACTERISTICS

3.21 The Northern Pennine Orefield

3.211 Introduction and geological background

The Northern Pennine Orefield extends over a large area of Northern England, from the Tyne Valley south to the Craven district of Yorkshire and is divided into two parts by the Stainmore Gap between Barnard Castle and Brough. The present study is concerned only with the northern part of the orefield. This may be visualized as a plateau uplifted along the Pennine fault at its western margins, tilted to the east (Taylor et al., 1971) and dissected by three main rivers; the Tyne, Wear and Tees and their tributaries. It represents the highest land in England outside of the Lake District, extending up to 893 m at the summit of Cross Fell. As much of the geology of the region is common to both Alston Moor and Weardale it seems pertinent to include a few general comments here.

The greater part of the upland region is underlain by the Lower Carboniferous Series (Johnson, 1981). The Carboniferous rocks in turn rest upon a basement of Lower Palazeoic rocks into which the Weardale Granite was intruded during the Devonian Period (Dunham, 1981). At the end of the Carboniferous Period these were uplifted to form the Alston Block and at the same time volcanic activity in the region caused the Great Whin Sill of quartz dolerite to be intruded into the Carboniferous strata and, subsequently, for mineralization to occur. The whole area was given its eastwards tilt in the Tertiary Period (Johnson, 1981).

The Lower Carboniferous Limestone Series are composed of alternating limestones, shales, sandstones and thin coal seams laid down in a wide delta, the relative proportions of each changing in a N-S direction. The Upper Carboniferous Great Limestone was deposited on top of this, followed by an alternating series of limestones and other strata. The limestone is gradually depleted and by the top of the sequence a sandstone known as Millstone Grit (related, but not identical to, the Millstone Grit of Yorkshire and Lancashire) dominates (Johnson, 1981).

3.212 Alston Moor

The Alston Moor area covers the R. South Tyne valley south from Alston along with its tributary valleys and is nearly all of an altitude above 300 m (Dunham, 1949). The western edges are drained by the R. Eden but the majority of the region is drained by the R. South Tyne (0055) which rises at Tynehead at the foot of Cross Fell and flows first west and then north to Hexham and the confluence with the R. North Tyne. The principal settlements are at Alston, Nenthead and Garrigill.

The area was formerly a very productive lead mining area, especially in the Nenthead region and was extensively worked by the London Lead Company. There are abundant mineral veins and artefacts of this mining in the region including a large number of spoil heaps and many adits and shafts. Several streams in the area drain through these and are consequently enriched in heavy metals (Say, 1977). The R. Nent (0048), which collects these along with the drainage waters from several levels, also contains high concentrations (Armitage, 1979).

0091 and 0288 are both small right-bank tributaries of 0048, approximately 1 km apart; 0288 flows past the entrance to drainage levels and several spoil heaps and surface workings and is consequently metal-enriched; 0091 has relatively low concentrations of heavy metals. 0102 is a right-bank tributary of 0055 containing elevated concentrations of heavy metals. 0055, despite numerous tributaries such as 0102 which are metal- enriched, contains only slightly elevated concentrations of heavy metals until its confluence with 0048 at Alston (Wehr, 1983).

Table 3.1 Sampling sites in Alston Moor (blank = not sampled).

stream-reach	name	grid ref.	altitude (m)	river chemical class
0048-80	R. Nent	NY 739469	320	2
0055-30	R. South Tyne	NY 716468	280	1A
0091-05	Foreshield Burn	NY 751469	330	
0102-85	Garrigill Burn	NY 743419	350	
0288-70	Blagill Burn	NY 739471	320	
0288-90	Blagill Burn	NY 739473	330	

3.213 Weardale

The R. Wear starts at Wearhead by the confluence of Killhope Burn and Burnhope Burn and flows for 107 km to join the North Sea at Wearmouth. Weardale, the upland part of the catchment, extends down as far as Wolsingham, 20 km from Wearhead. A large area of Weardale is mineralized and there were many large mines in the past, both in the main valley and in its tributaries.

0012 is an important left bank tributary of the Wear with a catchment which includes Rookhope, probably the most important lead-mining settlement in Weardale (Raistrick & Jennings, 1965); consequently it contains relatively high concentrations of heavy metals. 0012-28 is in the upper part of the valley and 0012-45 is close to its confluence with the R. Wear. 0310 is a right-bank tributary of 0012, about 2 km long. 0309 is a right bank tributary of the Wear which collects water from Chapel Fell and from Greenhaws Hush (a "hush" is a type of surface working) and flows through the hamlet of Daddryshield before its confluence. The reach used in the study (0309-80) is 100 m below the hamlet of Daddryshield.

Table 3.2 Sampling sites in Weardale (blank = not sampled).

stream-reach	name	grid ref.	altitude (m)	river chemical class
0012-28	Rookhope Burn	NY 919428	340	3
0012-45	Rookhope Burn	NY 953387	230	2
0309-80	Daddryshield Burn	NY 895380	290	
0310-90	"Race Fell Burn"	NY 918427	350	

3.22 Durham Coalfield

The final division of the Upper Carboniferous is known as the "Coal Measures". A cycle of shale, sandstone and coal seams was repeated in a broad coastal delta covered by a luxuriant swamp vegetation (Johnson, 1981). This was uplifted and tilted at the end of the Carboniferous period (3.211) such that the present coal seams extend eastwards into the North Sea. The only other major deposit in the region is the Permian Reef of Magnesium Limestone (dolomite) (Smith & Francis, 1967). The physiography of the region is very different to the North Pennine Orefield; lower, with a gentler relief and a fertile cover of boulder clay left by the last glaciation. Consequently it is densely populated, farmed and exploited for industry. These all have important implications for the ecology of surface waters.

The main rivers in the region are the Wear and the Tyne. 0014 is a left-bank tributary of the Wear rising near Consett and flowing approximately 30 km to a confluence 7.5 km above Durham City. 0014-40 is approximately 10 km above this confluence and 1 km below a Sewage Treatment Works (STW) at Langley Park. 0005 is a right-bank tributary of 0014 which flows through a valley once intensively exploited for coal. In the past it contained high concentrations of suspended solids as a result of mining activities which have now ceased. The confluence with 0014 is 2 km above the confluence between 0014 and the R. Wear.

The other study site on the Durham Coalfield was a right-bank tributary of the R. Tyne. 0024 rises near Stanley and flows for 27 km to the Tyne. There are a large number of effluents entering along its length (Wehr _et al_., 1981) including some heavy metal effluents. The reaches used (0024-20 and 0024-22) are both located close to Causey Arch, 2 km downstream of a heavy metal input from a battery factory as well as effluents from a STW and, in the past, pumped groundwater from a colliery. There are both diurnal (Fig. 3.2) and longer term (Table 3.4) fluctuations in the concentration of zinc in the water.

Table 3.3 Sampling sites in the Durham Coalfield.

stream-reach	name	grid ref.	altitude (m)	river chemical class
0005-44	R. Deerness	NZ 226421	80	1B
0014-40	R. Browney	NZ 222455	90	1B
0024-20	R. Team	NZ 202560	130	3
0024-22	R. Team	NZ 203560	130	3

Fig. 3.2 Changes in zinc concentration in 0024-22 on 25-08-83 between 0900 and 2200.

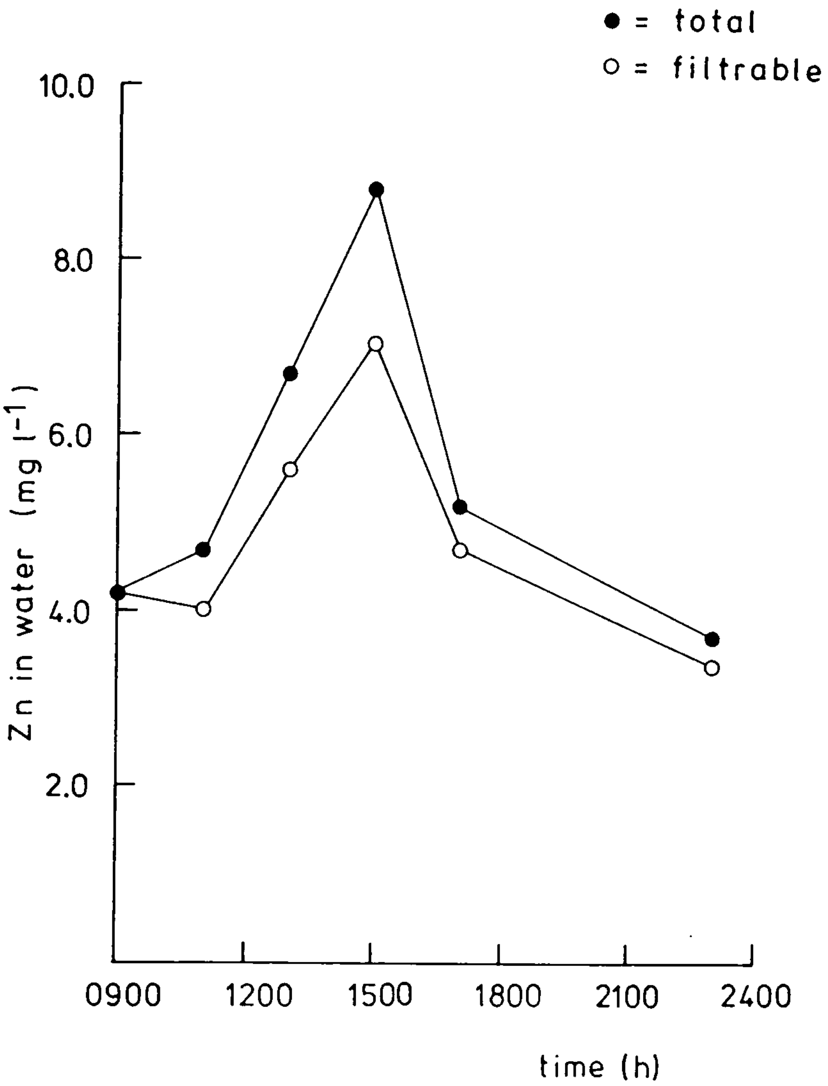

● = total
○ = filtrable
10.0
8.0
6.0
4.0
2.0
Zn in water (mg l^{-1})
0900
1200
1500
1800
2100
2400
time (h)

Table 3.4 Zinc concentrations (mg l-1) measured in R. Team between 1980 and 1986.

date	reach	$Zn_{filtrable}$	reference
5.80	20	0.28	Wehr _et_ _al_. (1981)
21.5.81	20	0.30	Wehr & Whitton (1983a)
5.81 - 6.82	20	0.19 - 6.90	Wehr & Whitton (1983b)
25.8.83	22	3.40 - 7.07	this thesis (Fig. 3.2)
1.6.84	22	1.36 - 1.86	this thesis (Table 5.7)
6.8.84	20	4.34	this thesis (Fig. 6.13)
8.8.84	20	3.09	this thesis (Fig. 6.13)
10.8.84	20	2.75	this thesis (Fig. 6.13)
12.8.84	20	1.65	this thesis (Fig. 6.13)
14.8.84	20	1.22	this thesis (Fig. 6.13)
16.8.84	20	1.30	this thesis (Fig. 6.13)
11.6.86	22	0.87	this thesis (Table 7.4)
24.7.86	20	0.34	this thesis (Table 3.8)

3.23 Croal Valley

The Croal Valley lies in an area of N-W. England that has been the focus of intense industrial activity. The river collects water from a number of tributaries which rise on the W. Pennine moors and flows through Bolton to a confluence with the R. Irwell, itself a tributary of the Mersey. The area is situated on the Upper Carboniferous Coal Measures, overlain by a deep deposit of boulder clay. The Croal Valley itself has deposits of delta gravels, sands and silts (Tonks _et_ _al_., 1931). General features of the Mersey catchment are given by Holland and Harding (1984).

Three sites were used; two on the R. Croal (0267) and one (0378-90) on a small right-bank tributary, Blackshaw Brook (Table 3.5).

Table 3.5 Sampling sites in Croal Valley (blank = not sampled).

stream-reach	name	grid ref.	altitude (m)	river chemical class
0267-80	R. Croal	SD 736075	60	2
0267-90	R. Croal	SD 749066	50	2
0378-90	Blackshaw Brook	SD 744075	50	

3.3 ENVIRONMENTAL BACKGROUND TO SITES IN N-E. ENGLAND

3.31 Introduction

A wide range of water chemistries were encountered during the study; these are summarized in this section, along with details of macrophytes found in the study reaches.

3.32 Water Chemistry

The availability of suitable substrates for colonization in riffle sections meant that most of the reaches studied had moderately fast current speeds (Table 3.6); the turbulence in these areas acted to keep them close to saturation with respect to dissolved oxygen, even in reaches with considerable inputs of organic pollution (e.g. 0014-40, 0024-20; 0024-22; see Table 3.3). Typically pH values were > 7.0 (exception = 0012-28), indicating the pH preference of the species recorded in previous studies (Merry et al., 1981; Wehr, 1983; Wehr & Whitton, 1983a). During a more intensive study of four of these reaches (0091-05; 0288-70; 0309-80; 0310-90; see Chapter 4) these values fluctuated by up to ± 0.3 pH units.

On the basis of anion and cation analyses, the study sites in N-E. England resolve into two distinct groups (Table 3.7; Table 3.8): Durham Coalfield (FRP > 100 µg g^{-1}; NO_3-N > 10 mg l^{-1}; Na > 40 mg l^{-1}; K > 10 mg l^{-1}) and the Northern Pennines (FRP < 30 µg g^{-1}; NO_3-N < 2 mg l^{-1}; Na < 10 mg l^{-1}; K < 10 mg l^{-1}). These reflect both differences in bedrock geology and the different intensities of land use and population etc. (3.2). Those streams in the Northern Pennines with relatively high concentrations of nutrients (0012-45; 0288-70; 0288-90) are associated with local concentrations of population or more intensive agricultural activity.

There is a wide range of concentrations of hardness components; concentrations of Ca range from 17.6 mg l^{-1} (0310-90) to 85 mg l^{-1} (0005-44). Most are, however, over 40 mg l^{-1} and there is no clear distinction between upland and lowland streams.

The highest concentration of zinc measured during the survey was 2.05 mg l^{-1} (0012-28) although concentrations of up to 7.07 mg l^{-1} have been measured in 0024-22 (Table 3.4). Concentrations in the metal-enriched streams of the Northern Pennines were generally less than 0.5 mg l^{-1} with two exceptions: 0012-28 (Zn_{total} (Zn_t) = 2.06 mg l^{-1}; $Zn_{filtrable}$ (Zn_f) = 2.05 mg l^{-1}) and 0048-80 (Zn_t = 1.39 mg l^{-1}; Zn_f = 0.475 mg l^{-1}). There is a large difference in the ratios of Zn_t to Zn_f between these streams; the mean ratio was 0.82 (SD = 0.25).

Table 3.6 Physico-chemical variables in study reaches in N-E. England.

stream -reach	date	current speed ($m\ s^{-1}$)	cond. ($\mu S\ cm^{-1}$)	pH	total alkalinity ($meq\ l^{-1}$)	dissolved oxygen (% sat)	dissolved oxygen ($mg\ l^{-1}$)
0005-44	24.7.86	0.502	1397	7.7	10.0	108	11.5
0012-28	26.5.86	0.316	159	6.5	1.2	97	10.3
0012-45	21.6.84	0.280	285	7.8	4.7	121	13.1
0014-40	24.7.86	0.609	674	7.8	4.5	86	9.1
0024-20	9.8.84	0.719	740	7.1	2.0	98	10.3
0024-22	11.6.86	0.435	718	7.7	3.3	95	9.9
0048-80	15.8.84	0.083	491	8.0	5.9	106	10.3
0055-30	24.7.86	0.433	288	8.1	3.7	107	10.5
0091-05	13.6.86	0.483	234	7.7	4.3	110	11.7
0102-85	11.6.86	0.345	124	7.5	1.8	103	11.3
0288-70	13.6.86	0.381	280	8.1	4.5	101	11.3
0288-90	7.8.86	0.789	211	7.6	2.7	104	10.9
0309-80	13.6.86	0.635	360	7.8	6.5	104	11.0
0310-90	13.6.86	0.479	136	7.4	2.2	102	10.4

Table 3.7 Anion chemistry in study reaches in N-E. England on 24-7-1986 (AM = Alston Moor; DC = Durham Coalfield; WD = Weardale).

stream-reach	area	FRP ($\mu g\ l^{-1}$)	NO_3-N ($mg\ l^{-1}$)	SO_4-S ($mg\ l^{-1}$)	Cl^- ($mg\ l^{-1}$)
0005-44	DC	101	148	190	66.7
0012-28	WD	3.9	0.5	173	9.4
0012-45	WD	23.7	0.6	76	8.8
0014-40	DC	1215	10.7	124	62.2
0024-20	DC	3654	55.3	86	71.1
0024-25	DC	3683	44.8	67	62.2
0048-80	AM	3.6	0.8	113	5.7
0055-30	AM	6.1	0.4	19	6.2
0091-05	AM	9.3	0.1	2	0.6
0102-85	AM	8.7	0.1	9	0.9
0288-70	AM	12.6	1.1	68	6.8
0288-90	AM	10.6	0.1	55	7.2
0309-80	WD	7.6	0.3	10	6.5

Table 3.8 Metal chemistry (mg l^{-1}) in study reaches in N-E. England on 24.7.86 (stm-rch = stream-reach code; AM = Alston Moor; WD = Weardale; DC = Durham Coalfield).

stm-rch	area	Na	K	Mg	Ca	Mn	Fe	Zn	Cd	Pb
0005-44	DC	137.0	20.0	58.5	103.0	0.349	0.039	<0.007	0.00010	<0.0001
0012-28	WD	7.96	4.79	11.7	59.9	1.06	<0.02	2.06	0.00162	0.0097
0012-45	WD	8.47	4.76	9.00	59.2	0.111	<0.02	0.330	0.00710	0.0016
0014-40	DC	42.9	9.45	20.55	5.0	0.056	0.78	<0.007	0.00023	0.0005
0024-20	DC	48.0	10.50	8.28	42.6	0.542	0.135	0.343	0.00095	0.0023
0024-22	DC	42.6	10.15	9.00	46.0	0.527	0.125	0.323	0.00058	0.0015
0048-80	AM	9.12	3.32	11.4	58.8	0.044	0.073	0.475	0.00414	0.0028
0055-30	AM	3.94	1.09	4.38	43.2	<0.008	<0.02	0.067	0.00019	0.0022
0091-05	AM	8.56	2.05	6.72	42.5	<0.008	0.035	0.010	0.00009	0.0032
0102-85	AM	5.34	2.10	7.92	54.5	<0.008	<0.02	0.427	0.00480	0.0124
0288-70	AM	4.64	1.84	6.22	36.5	0.022	0.152	0.239	0.00071	0.0110
0288-90	AM	7.70	3.29	8.52	54.8	<0.008	0.035	0.229	0.00079	0.0016
0309-80	WD	6.99	4.35	12.0	70.3	<0.008	<0.02	0.200	0.00070	0.0065
0310-90	WD	4.48	0.90	2.22	17.6	0.008	0.02	0.010	0.00010	0.0004

3.33 Macrophytes

Sixteen macrophytes (sensu Holmes & Whitton, 1977) were encountered during this study (Table 3.9). The selection of reaches was weighted towards those with populations of *Rhynchostegium*; twelve out of fourteen reaches contained this species. The next most abundant species were *Fontinalis antipyretica* (three reaches), *Lemanea fluviatilis* (four reaches), *Cladophora glomerata*, *Stigeoclonium tenue* and *Amblystegium riparium* (three reaches each). The richest aquatic bryophyte floras were found in upland streams such as 0055-30, 0091-05 and 0310-90; some species (e.g. *Hygrohypnum* spp., *Cinclidotus fontinaloides* and *Racomitrium aciculare*) were found only here. The latter two species were characteristic of the upper parts of boulders subject to only periodic immersion. *Amblystegium riparium* was confined to lowland sites, reflecting its observed predilection for relatively nutrient rich waters (Whitton *et al*., 1981; Holland & Harding, 1984). *Cladophora glomerata* is also characteristic of eutrophic waters but is intolerant of heavy metals (Whitton, 1970); it is found at the two sites on Durham Coalfield with low concentrations of heavy metals (0005-44 and 0012-40) but also at 0091-05 in Alston Moor which is relatively nutrient poor. Two aquatic vascular plants were observed; *Equisetum arvense* at two sites in the Northern Pennines and *Ranunculus penicillatus* at 0014-40. *Potamogeton pectinatus* was observed in 0024 approximately 1 km upstream of 0024-20.

Table 3.9 Macrophytes present in study reaches in N-E. England (Lf = *Lemanea fluviatilis*; Ba = *Batrachospermum* sp.; Cg = *Cladophora glomerata*; Mo = *Mougeotia* sp.; Bp = *Bryum pseudotriquetrum*; Cf = *Cinclidotus fontinaloides*; Ra = *Racomitrium aciculare*; Rr = *Rhynchostegium riparioides*; Ar = *Amblystegium riparium*; Af = *A. fluviatile*; Fa = *Fontinalis antipyretica*; Ho = *Hygrohypnum ochraceum*; Hl = *H. luridum*; Ea = *Equisetum arvense*; Rp = *Ranunculus penicillatus* var penicillatus).

stream-reach	Lf	Ba	Cg	St	Mo	Bp	Cf	Ra	Rr	Ar	Af	Fa	Ho	Hl	Ea	Rp
0005-44	1		1						1	1		1				
0012-28					1											
0012-45	1								1							
0014-40			1						1			1				1
0024-20				1					1	1						
0024-22				1					1	1		1				
0048-80					1											
0055-30	1						1		1			1	1			
0091-05		1	1	1			1	1	1		1				1	
0102-85									1							
0288-70									1							
0288-90									1							
0309-80	1								1							
0310-90						1			1			1		1	1	

3.4 DETAILS OF SITES USED FOR GROWTH STUDY

3.41 Introduction

Four sites in the Northern Pennines were chosen for the growth study such that all could be sampled in one day. Criteria used to select sites included a good growth of *Rhynchostegium* attached to movable boulders (in practice the size of boulders was determined ultimately by the flow regime within the stream). All sites were in upland areas; no lowland sites in the immediate vicinity were able to fulfil the above criteria; and they included sites with and without metal contamination and with different nutrient regimes.

3.42 Environment of streams

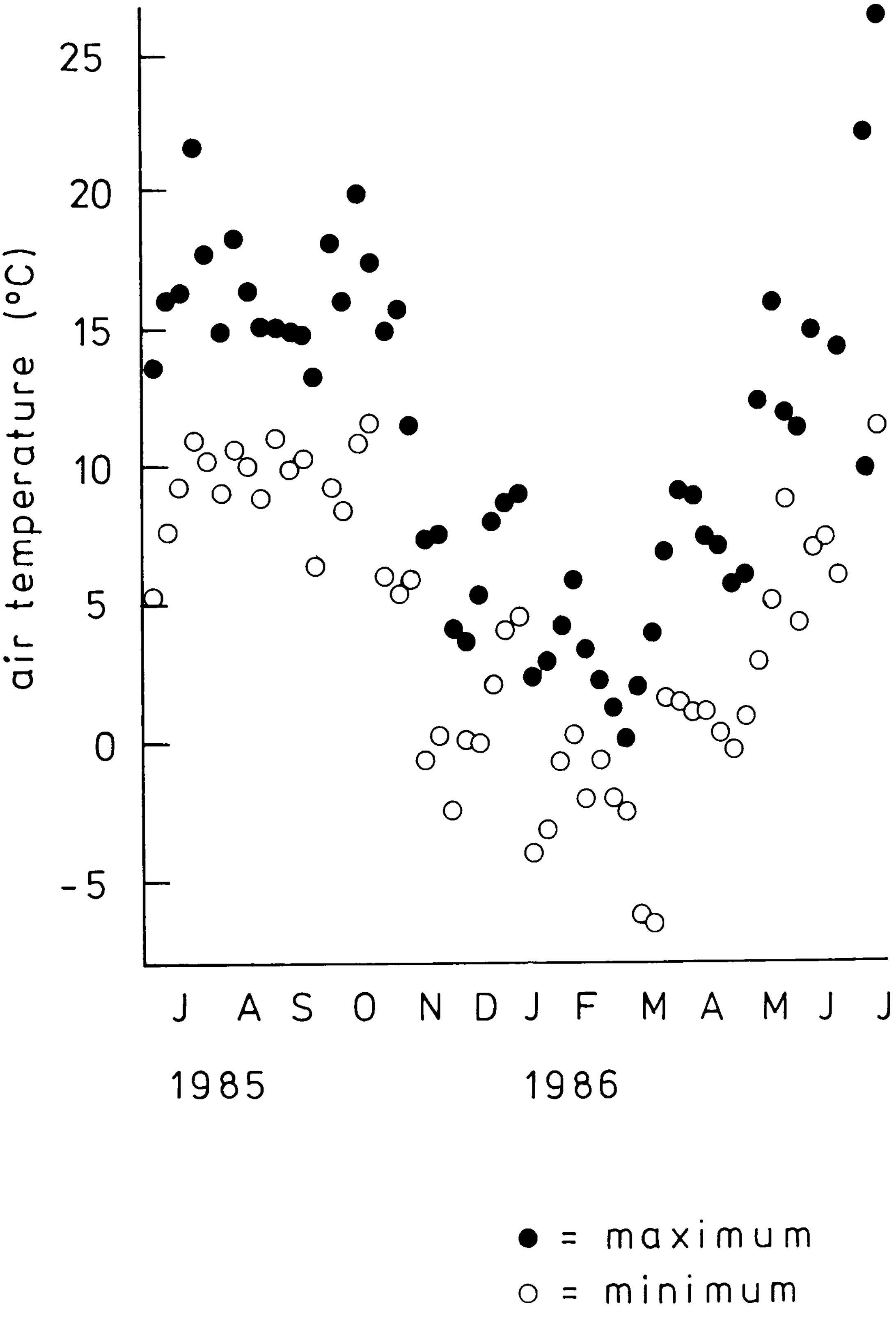
3.421 Introduction

It was not possible to collect all of the environmental data from the immediate locality of the study sites. Meteorological and discharge data were supplied by the Northumbrian Water Authority (N.W.A.) from a meteorological station at Burnhope Reservoir in Weardale (NY 846393, 357 m) and a flow-gauging station at Harwood Beck in Upper Teesdale (NY 849309, 390 m). These sites are both within 20 km of the field sites and are of a similar altitude (Table 3.1; Table 3.2). These data are presented to give a broad indication of seasonal changes in the environment during the study period.

3.422 Air temperature

Maximum and minimum air temperatures collected from Burnhope Reservoir are plotted as weekly means (Fig. 3.3). These indicate the relatively cool climate experienced at these altitudes; only three weeks had a weekly mean maximum temperature of 20 °C or more, whilst the minimum

Fig. 3.3 Weekly mean maximum and minimum temperatures at Burnhope Reservoir in 1985-86.


air temperature (°C)
25
20
15
10
5
0
-5
J
A
S
O
N
D
J
F
M
A
M
J
J
1985
1986
● = maximum
○ = minimum

temperature was at or below 0 °C for fourteen weeks. The coldest month was February and the warmest was July.

3.423 Precipitation

Precipitation was collected in a rain gauge, 12.5 cm in diameter and 30 cm above the ground at Burnhope Reservoir, at 0900 each morning. Monthly means for 1984-85 and 1985-86 are presented as a histogram (Fig. 3.4). The maximum precipitation in 1985-86 fell during the winter months; the wettest month was January 1986 and the driest was October 1985. Note also the particularly wet August in 1985 compared with data for 1984.

3.424 Flow

Flow data from Harwood Beck are presented as a histogram (Fig. 3.5) to give an indication of likely variations in the reaches under consideration. There is a significant correlation (r = 0.905 ***) between mean flow and mean rainfall; maximum flows were recorded during winter but, as in 3.423, the value recorded for August 1985 was particularly high.

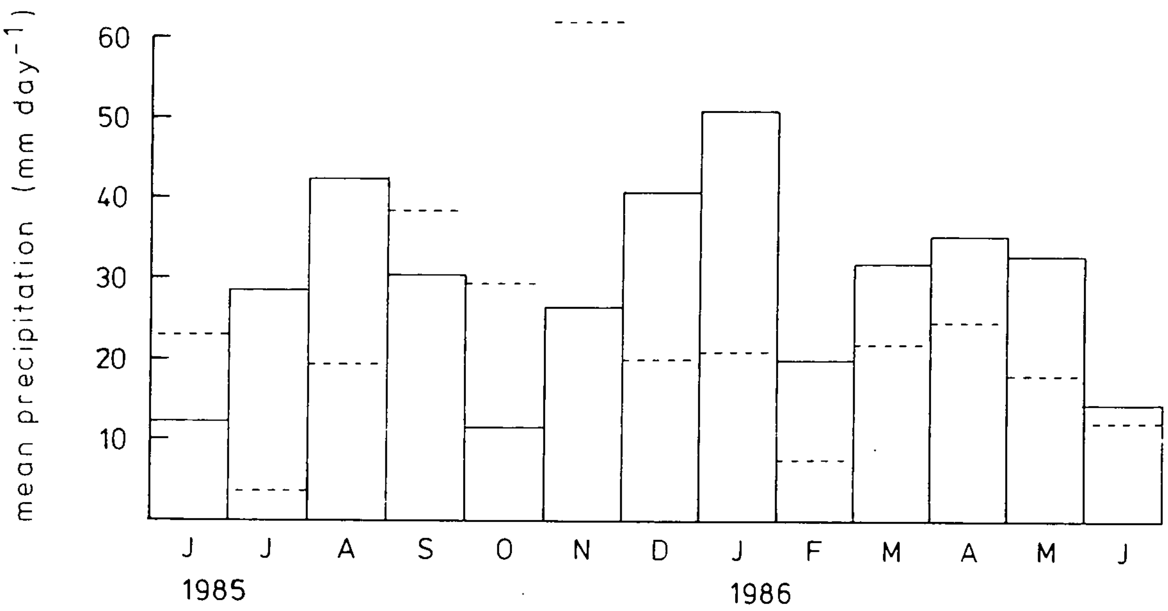
3.43 Details of study sites

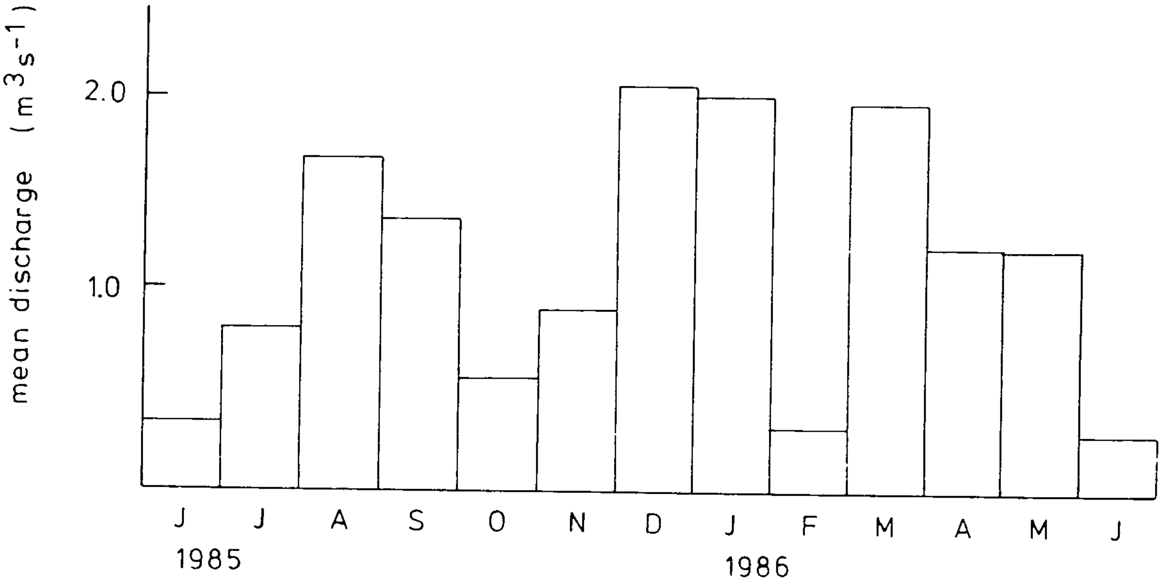
3.431 Foreshield Burn, reach 0091-05

This site drains open moorland and some improved pasture and contains relatively low concentrations of nutrients (Table 3.7) and heavy metals. The reach was heavily shaded by Acer pseudoplatanus and Sorbus aucuparia (Fig. 3.6). Rhynchostegium was fairly abundant, providing about 30% cover, and was the dominant macrophyte although several others were observed (Table 3.9). It was found on cobbles and boulders between 200 and 400 cm in diameter.

Fig. 3.4 Monthly mean precipitation at Burnhope Reservoir in 1984-85 and 1985-86. Solid line = 1985-86; dashed line = 1984-85.

Fig. 3.5 Monthly mean discharge at Harwood Beck in 1985-86.


mean precipitation (mm day^{-1})
60
50
40
30
20
10
J
J
A
S
O
N
D
J
F
M
A
M
J
1985
1986


mean discharge (m^{3}s^{-1})
2.0
1.0
J
J
A
S
O
N
D
J
F
M
A
M
J
1985
1986

3.432 Blagill Burn, reach 0288-70

0288-70 is situated at the foot of a steep wooded bank which caused heavy shading (see Table 4.3), again by Acer and Sorbus (Fig. 3.7). It was immediately upstream of a disused adit. Distribution of Rhynchostegium was patchy; on a small (about 2 m high) waterfall and on an artificial weir, both below the study reach, cover was between 80% and 90%; however in other parts it was virtually non-existent. In 0288-70 it had a cover of between 10% and 20% on cobbles and boulders between 150 and 350 cm in diameter. It was the only macrophyte in the reach (Table 3.9).

3.433 Daddryshield Burn, reach 0309-80

This reach was partially shaded by Acer, Sorbus and Betula pubescens; however the part where the boulders were situated was shaded only by herbaceous vegetation and by the bank (Fig. 3.8). The reach consisted of a series of small (about 20 cm high) waterfalls composed of large boulders (size = 250 to 600 cm in diameter) and bedrock blocks with cobbles and pebbles between. The waterfalls were covered with Rhynchostegium and Lemanea fluviatilis in approximately equal abundance.

3.434 "Race Fell Burn", reach 0310-90

0310-90 drains a Pinus plantation and open moorland before joining 0012. Although there are several mines in the area, the stream itself does not have elevated concentrations of heavy metals. The reach is 2 m above and 8 m below a small (about 1 m high) waterfall and is unshaded except by herbaceous vegetation (Fig. 3.9). It has a very dense cover of Rhynchostegium all year round (Wehr & Whitton, 1983b), particularly on the waterfall and on bedrock, cobbles and boulders between 150 and 500 cm in diameter. The plants are particularly robust, with large

Fig. 3.6 Foreshield Burn, reach 0091-05.

Fig. 3.7 Blagill Burn, reach 0288-70.

Fig. 3.8 Daddryshield Burn, reach 0309-80.

Fig. 3.9 "Race Fell Burn", reach 0310-90.

leaves and shoots of above average length and weight (Wehr, 1983). Other macrophytes found in the reach include Fontinalis antipyretica, Bryum pseudotriquetrum and Hygrohypnum luridum (Table 3.9).

3.5 DETAILS OF SITES USED FOR CASE STUDY

3.51 Introduction

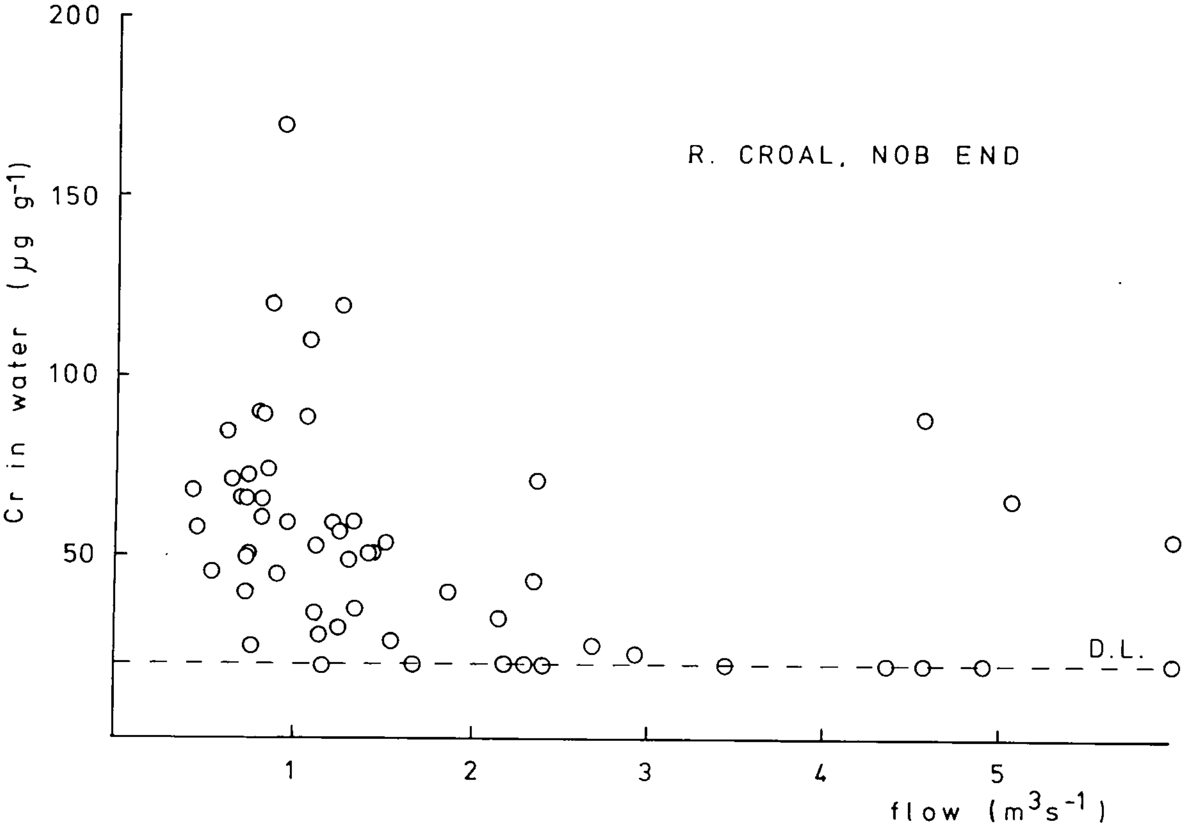
The Hall Chemical Works smelted chromate in Bolton between 1880 and 1968. The spoil heap resulting from these activities was subsequently landscaped and revegetated (Gemmell, 1972; 1973; 1974). Before revegetation there was considerable leaching of chromium into the R. Croal (3 - 5 t yr^{-1}), with pronounced effects on the biota (Breeze, 1973). Afterwards, approximately half of the rainfall on the site was returned to the atmosphere as evapotranspiration (Gemmell, 1977) and two settling tanks collected much of the remaining drainage. The situation has improved as a result of this but the R. Croal still receives significant inputs of chromium (unpublished computer records of North West Water Authority (N.W.W.A.)) and these are investigated in this study.

3.52 Environmental Background

3.521 Relationship between flow and chromium concentrations

The variation in chromium concentrations in the Croal below the Hall Chemical Works was studied using data supplied by the N.W.W.A.. The maximum concentration recorded between 1982 and 1984 was 0.215 mg l^{-1}. 56% of records for this period were below 0.053 mg l-1 (lowest quartile of range) and only 7% were above 0.108 mg l^{-1} (upper two quartiles of range). The greatest variation in the chromium concentration occurred at periods of low flow (Fig. 3.10) which tended to occur during July and August; this period was chosen for the study.

Fig. 3.10 Relationship between mean daily flow and total chromium in R. Croal between January 1982 and December 1984.


R. CROAL, NOB END
Cr in water (μg g-1)
200
150
100
50
D.L.
1
2
3
4
5
flow (m3s-1)

3.522 Description of study sites

Three sites were chosen for study (Table 3.10). Two were on the Croal, above (0267-80) and below (0267-85) the Hall Chemical Works. 0267-80 was a fast-flowing canalized stretch of the river (Fig. 3.11) and 0267-85 was a riffle approximately 2 km downstream (Fig. 3.12). Both sites had abundant growths of *Cladophora glomerata*; other macrophytes at the two sites were *Fontinalis antipyretica* and *Ranunculus fluitans* at 0267-80 and *Amblystegium riparium*, traces of *Fontinalis antipyretica*, *Ranunculus fluitans* and *Rorippa nasturtium-aquaticum* at 0267-85. The third site (0378-90) was on a small tributary along the N-W. edge of the spoil heap (Fig. 3.13), immediately before the stream entered a culvert (Fig. 3.14). The water at this site was significantly harder than at the other sites (Table 3.10) reflecting the local effects of the spoil (Gemmell, 1977). In addition, it had an unstable substrate of fine sand and silt, which supported visually-obvious growths of the blue-green alga *Plectonema* but no macrophytes.

Table 3.10 Physical and chemical variables at case study sites on 1-7-85

site	current speed $(m\ s^{-1})$	cond. $(\mu S\ cm^{-1})$	pH	total alkalinity $(meq\ l^{-1})$	Ca $(mg\ l^{-1})$	Cr $(mg\ l^{-1})$
0267-80	0.84	368	7.6	3.8	35.5	0.043
0267-85	0.38	393	7.5	4.1	36.3	<0.017
0378-90	0.36	652	8.6	7.4	75.3	0.504

Fig. 3.11 R. Croal at Darcy Lever, reach 0267-80.

Fig. 3.12 R. Croal at Nob End, reach 0267-90.

Fig. 3.13 Blackshaw Brook, reach 0378-90.

Fig. 3.14 Blackshaw Brook, reach 0378-90, showing N-W. edge of spoil heap.

Some additional water chemistry data for the period of the study was obtained from the N.W.W.A., from a sampling point about 1 km downstream of 0267-85. This included biochemical oxygen demand (B.O.D.), chemical oxygen demand (C.O.D.) and anion analyses not made during the study (Table 3.11). Values for B.O.D. and C.O.D. reflect the organically-polluted state of the river; values for the nutrient analyses are typical of a moderately eutrophic river.

Table 3.11 Water chemistry in R. Croal, 13-6-85 to 20-7-85 (all values, except pH, as mg l^{-1}).

	n	min	max	mean	SD
pH	13	7.3	7.5		
B.O.D.	13	2.2	24.0	10.5	6.8
C.O.D.	13	21.0	141.0	65.4	38.0
NH_4-N	13	0.20	2.85	1.65	0.77
NO_3-N	13	0.40	1.75	0.82	0.36
NO_2-N	13	0.08	0.24	0.14	0.05
Cl^-	13	17.0	44.0	26.8	8.1
FRP	13	<0.05	0.30		

4. GROWTH OF RHYNCHOSTEGIUM

4.1 INTRODUCTION

This chapter presents results of a seasonal survey of the rate of growth of Rhynchostegium at four sites in the Northern Pennines. This provides a fundamental background against which seasonal variations in the availability of moss for monitoring purposes and its subsequent accumulation of metals may be interpreted. Details of these sites, along with some meteorological and environmental data are given in Chapter 3. Sites were sampled at monthly intervals between July 1985 and June 1986. Due to a reduced growth rate in winter months, temperature was measured but no growth measurements were made in February 1986.

Two preliminary laboratory experiments are described first (4.2). This is followed by a description of the environmental background to the four streams in section 4.3 and growth rates at the four sites in section 4.4. In section 4.46, results from the four sites are compared and correlations with environmental variables made. Finally, estimates of annual dry matter production at the four sites are made (4.5, 4.6).

4.2 PRELIMINARY EXPERIMENTS

4.21 Introduction

Two brief laboratory experiments are described to test an initial assumption regarding growth of shoots - that there is a short region of apical elongation below which there is a linear relationship between length and dry weight.

4.22 Shoot extension at apex

The zone of elongation of shoot tips of Rhynchostegium from 0310-90 was determined using a low-power binocular microscope.

Leaves were cut off from the apex in three planes (Fig. 4.1); these are closely bunched to form a cluster at the tip and in a zone of elongation extending 3 mm back from the tip. Below this leaves are spaced equidistantly.

4.23 Relationship between mass and shoot length

A second preliminary experiment was performed to relate increases in length to corresponding increases in shoot mass.

Shoots collected from each of the four reaches were cut into a variety of lengths, from 0.5 cm to 3.0 cm and samples consisting of known numbers of each length dried and weighed.

There were significant correlations between mass and length for all four sites (Fig. 4.2, Table 4.1), with regression coefficients of between 0.87 and 1.65. Three of the populations had slopes of > 1.0 and positive intercepts; this indicates that mass increases faster than length along the stem and that the apical 0.5 cm in these populations has a slightly higher dry weight than the region below.

4.3 ENVIRONMENT OF STREAMS

4.31 Water Temperature

Variations in monthly spot temperature readings are closely correlated (Table 4.2), with an average range between the four sites of 1.9 °C (Fig. 4.3). These differences are correlated with the altitude of the streams; 0309-80 is the lowest and on 10 out of 12 occasions was the warmest whilst 0310-90 is the highest and generally the coolest.

Fig. 4.1 Shoot of *Rhynchostegium* from 0310-90, showing zone of apical elongation. Scale bar = 1 mm.

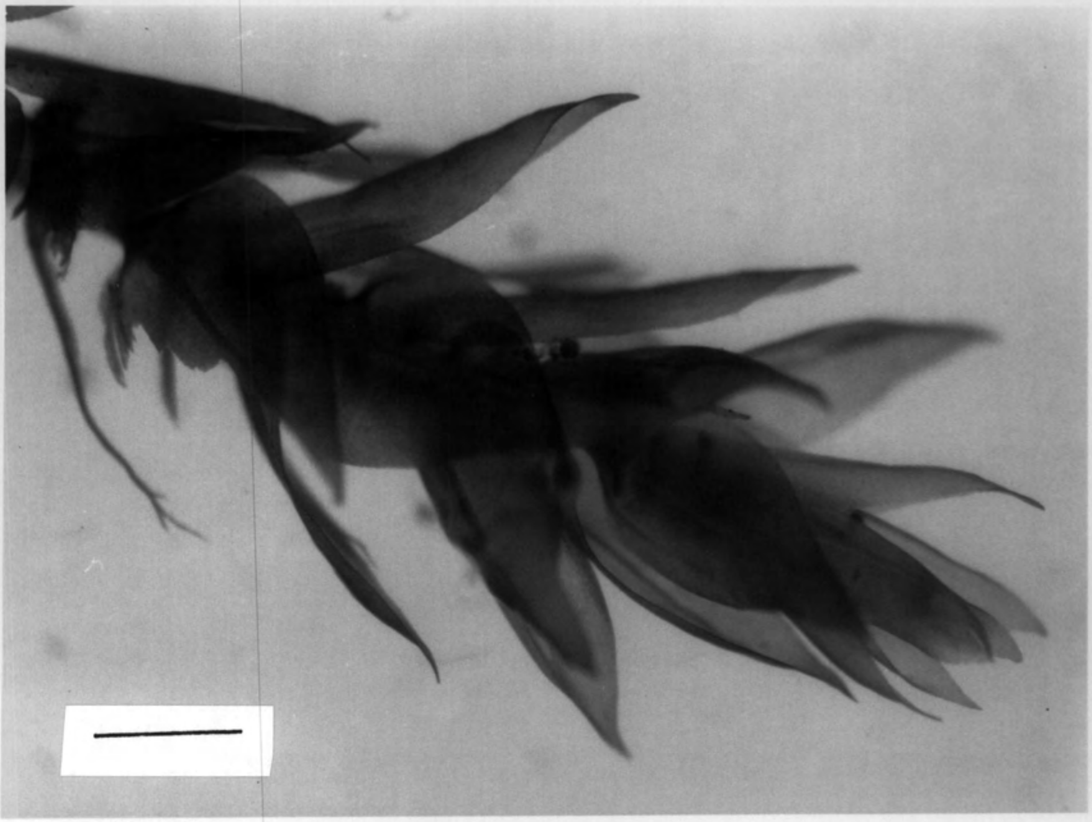
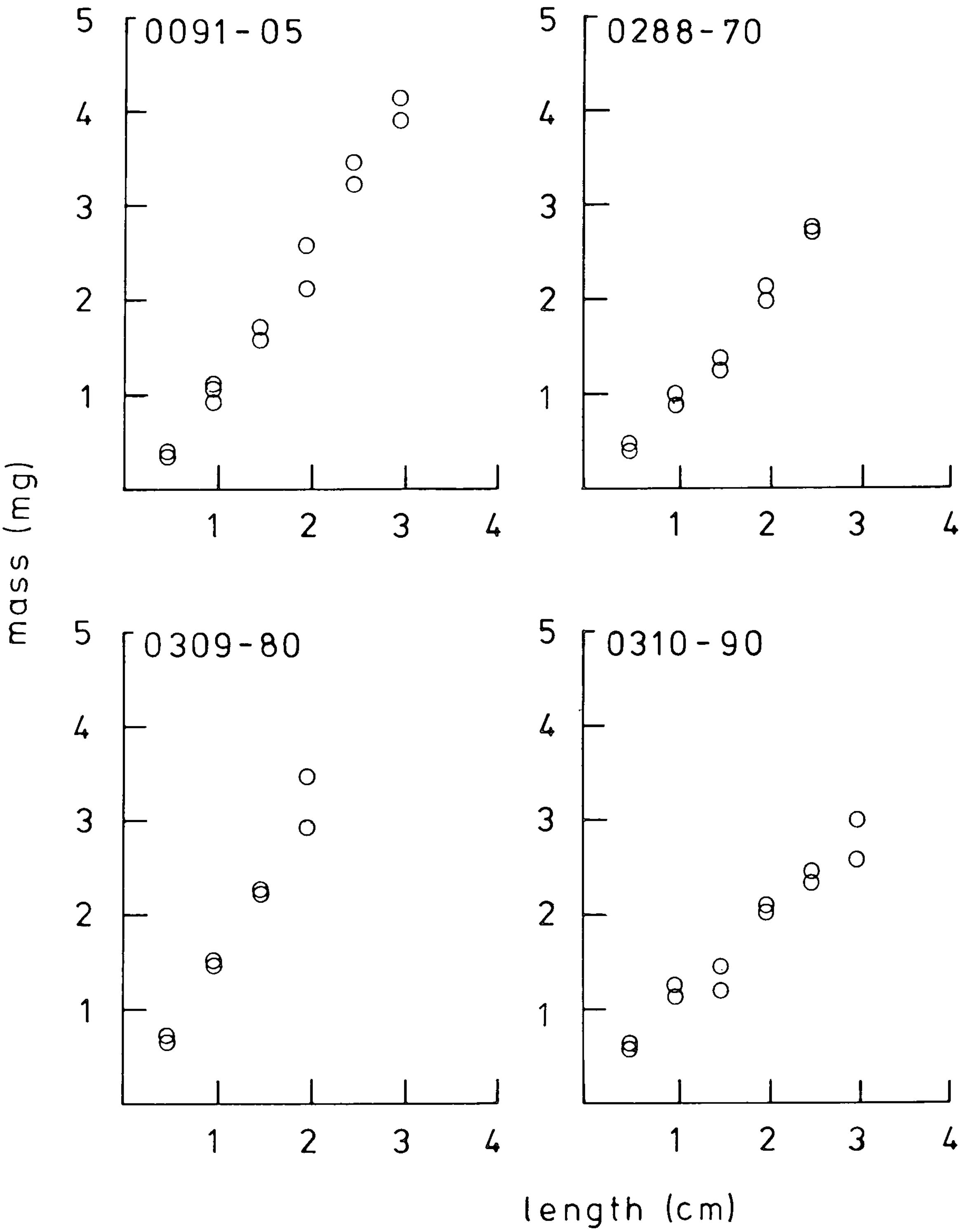


Fig. 4.2 Relationship between shoot mass and length of *Rhynchostegium* in four reaches in N-E. England used in growth study.


0091-05
0288-70
0309-80
0310-90
mass (mg)
length (cm)
1
2
3
4
5

Spot temperature readings also correlated closely to mean monthly maximum and minimum temperatures collected at Burnhope Reservoir (Table 4.2).

Table 4.1 Correlations and regressions between shoot mass and length for four populations of Rhynchostegium.

stream-reach	regression equation	correlation coefficient	significance
0091-05	$y = 1.45\ x - 0.43$	0.993	***
0288-70	$y = 1.14\ x - 0.21$	0.991	***
0309-80	$y = 1.65\ x - 0.14$	0.988	***
0310-90	$y = 0.87\ x - 0.20$	0.979	***

4.32 Light

It was not possible to collect light flux data throughout the 12 month period; however a set of figures for comparative purposes was collected on 13.6.86. These give an indication of the degree of shading experienced at each of the four sites by comparing photon flux at water level with photon flux in a nearby unshaded area.

Readings were collected at the two positions at each site over the course of approximately 1 h. These were very variable as the weather alternated between bright and overcast. Typical values (Table 4.3) show attenuation of light to be greatest at 0091-05 (81%), followed by 0288-70 (70%), 0309-80 (26%) and 0310-90 (21%). There were few trees or shrubs at 0309-80 and none at 0310-90 and the shading at these sites probably represents shading by the stream banks.

Fig. 4.3 Seasonal variations in spot temperature measurements in four reaches in N-E. England.

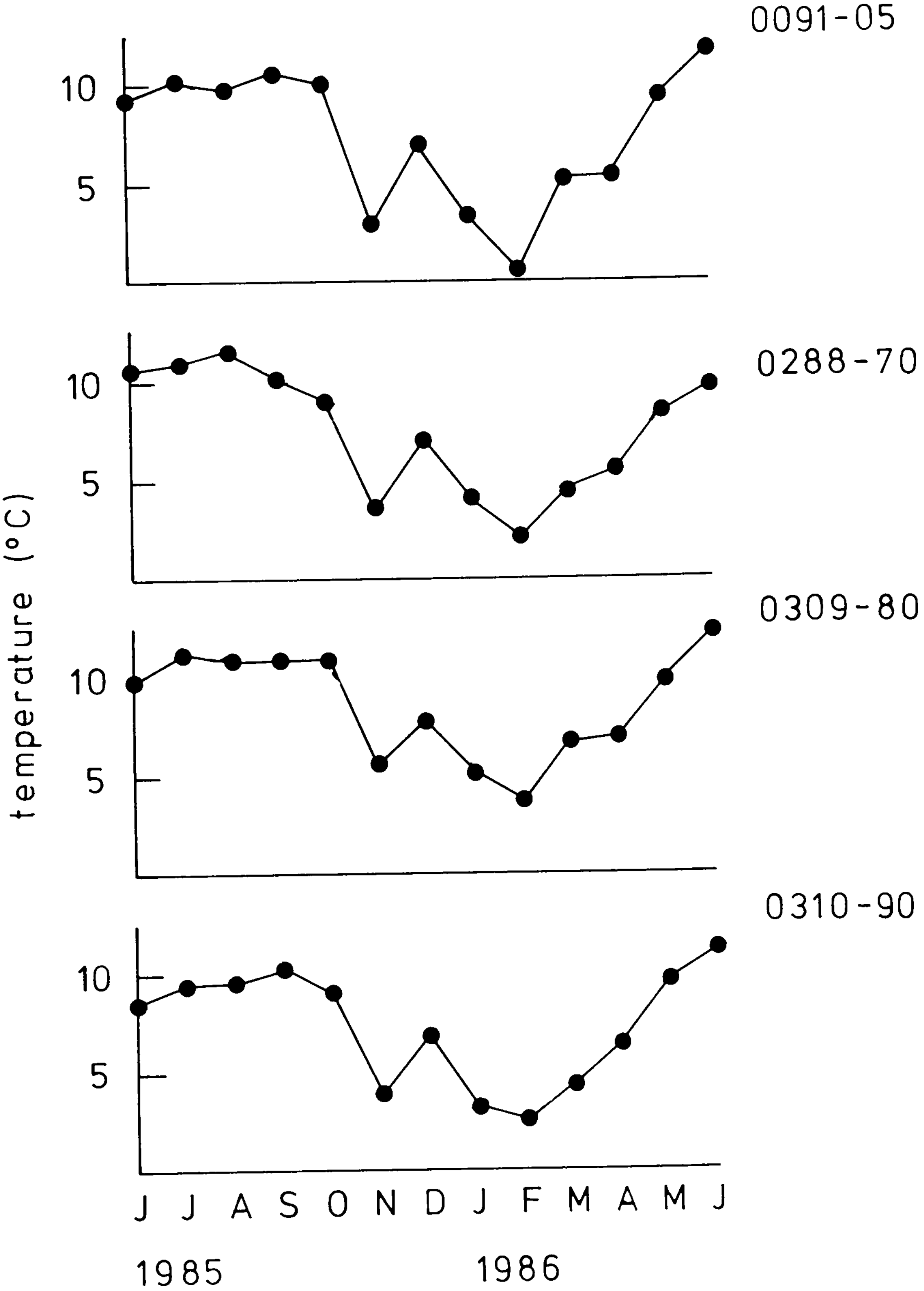

0091-05
0288-70
0309-80
0310-90
10
5
temperature (°C)
J
J
A
S
O
N
D
J
F
M
A
M
J
1985
1986

Table 4.2 Correlations between spot water temperature readings in study reaches and monthly mean maximum (air_{max}) and minimum (air_{min}) air temperatures at Burnhope Reservoir.

air_{max}	1.000					
air_{min}	0.960***	1.000				
$water_{0091-05}$	0.961***	0.946***	1.000			
$water_{0288-70}$	0.941***	0.961***	0.947***	1.000		
$water_{0309-80}$	0.981***	0.969***	0.992***	0.949***	1.000	
$water_{0310-90}$	0.942***	0.949***	0.927***	0.971***	0.973***	1.000
	air_{max}	air_{min}	$water_{0091}$	$water_{0288}$	$water_{0309}$	$water_{0310}$

Table 4.3 Typical values for photon flux density at each site (μmol photon m^{-2} s^{-1}) used in growth study.

site	time	photon flux		% attenuation
		water level	control	
0091-05	1100 - 1200	240 ± 118	1300 ± 680	81
0288-70	1000 - 1100	85 ± 23	284 ± 73	70
0309-80	1400 - 1500	776 ± 400	1054 ± 32	26
0310-90	1500 - 1600	724 ± 145	918 ± 121	21

4.33 Water Chemistry

Several aspects of water chemistry measured over the 12 month period reflect the contributions of different catchment geologies to the streams and, in particular, the influence of the Carboniferous Limestone. Water chemistry is summarized in Section 3.32 and in Table 4.4.

All of the streams showed large ranges of pH measurements. A pH of 6.9 was measured in 0288-70 in August 1985 and one of 8.4 in 0309-80 in June 1985. These were the extremes recorded; all of the means were in the range 7.6 to 7.8. The pH in 0091-05 tended to be the highest, followed by 0309-80, 0288-70 and 0310-90. Total alkalinity was highest in 0309-80 and least in 0310-90. Total alkalinities at 0091-05 and 0288-70 were very similar.

Conductivity and the concentration of hardness components (Mg and Ca) all reflect the influence of background geology; the "hardest" water was at 0309-80 and the "softest" at 0310-90. 0091-05 and 0288-70 were, again, very similar (Table 3.6; Table 3.8).

0288-70 generally contained higher concentrations of nutrient elements than other streams and 0091-05 contained lower concentrations (Table 4.4).

0310-90 had a high concentration of FRP (30.7 mg l^{-1}) compared with other streams (Table 3.7).

The concentrations of both Mn and Fe showed considerable variability, however concentrations of both were higher in 0091-05 and 0288-70 than in 0309-80 and 0310-90 (Table 4.4).

0288-70 and 0309-80 were chosen to represent Zn-enriched streams and 0091-05 and 0310-90 to represent low-Zn streams. The mean concentrations of Zn in the Zn-enriched steams were 0.230 mg l^{-1} (0288-70) and 0.216 mg l^{-1} (0309-80). Concentrations in the low-Zn streams were an order of magnitude lower. The demarcation between enriched and unenriched sites for Cd and Pb was less clear (Table 3.8); the range of mean Cd concentrations was from 0.0010 mg l^{-1} to 0.0015 mg l^{-1} and the range of mean Pb concentrations was between 0.0028 mg l^{-1} and 0.0139 mg l^{-1}.

4.4 GROWTH OF MOSSES IN STREAMS

4.41 Introduction

Although the proportion of streams and rivers in which Rhynchostegium is found is high, the microhabitat at any site which it may occupy is quite specific; all populations were attached to a stable substrata, bedrock in 0310-90, waterfalls at 0310-90 and 0288-70 and boulders at 0091-05 and 0309-80.

During the course of the study several boulders were lost, presumably due to high flows and these were replaced by larger boulders. On boulders, Rhynchostegium was found mainly on the "trailing edge" and on the top of the boulder. That on the trailing edge was often in a large weft whilst moss on the top grew in an intricate mat pattern which appeared to be an effective sediment trap. At 0310-90 this process led, during the course of

Table 4.4 Ranking of environmental variables measured in four reaches in N-E. England during growth study.

variable	rank
physico-chemical variables	
current speed	0310-90 > 0309-80 = 0288-70 > 0091-05
conductivity	0309-80 > 0288-70 > 0091-05 > 0310-90
pH	0091-05 > 0309-80 > 0288-70 > 0310-90
total alkalinity	0309-80 > 0091-05 > 0288-70 > 0310-90
oxygen saturation	0309-80 > 0091-05 > 0288-70 > 0310-90
anions	
FRP	0310-90 > 0288-70 > 0091-05 > 0309-80
NO_3-N	0288-70 > 0310-90 = 0309-80 > 0091-05
SO_4-S	0288-70 > 0309-80 > 0310-90 > 0091-05
Cl^-	0288-70 > 0310-90 > 0309-80 > 0091-05
cations	
$Na_{filtrable}$	0309-80 > 0310-90 > 0091-05 > 0288-70
$K_{filtrable}$	0309-80 > 0288-70 > 0091-05 > 0310-90
$Mg_{filtrable}$	0309-80 > 0288-70 > 0091-05 > 0310-90
$Ca_{filtrable}$	0309-80 > 0288-70 > 0091-05 > 0310-90
$Mn_{filtrable}$	0288-70 > 0091-05 > 0309-80 > 0310-90
$Fe_{filtrable}$	0091-05 > 0288-70 > 0310-90 > 0309-80
$Zn_{filtrable}$	0288-70 > 0309-80 > 0091-05 > 0310-90
$Cd_{filtrable}$	0288-70 > 0309-80 > 0091-05 > 0310-90
$Pb_{filtrable}$	0288-70 > 0309-80 > 0310-90 > 0091-05

the study, to the establishment of a sedge and of the acrocarpous moss Bryum pseudotriquetrum on the boulder.

4.42 Foreshield Burn, reach 0091-05

Boulders in this reach proved relatively unstable; they were recovered from the streams on just nine out of twelve occasions. The rate of growth of moss was quite variable; the rate in July and August being of the same order as rates for November to March. The maximum rate of growth of Rhynchostegium was observed in June 1986 (Fig. 4.4); smaller peaks were measured in September and December. The low rate of growth was not significantly correlated with temperature (Fig. 4.5; $r = 0.652$).

4.43 Blagill Burn, reach 0288-70

The boulders were recovered from this reach on all but one occasion (January 1986). The growth rate in July 1985 was very rapid (1.40 mm wk^{-1}); however this rate subsequently declined and remained at approximately 1.0 mm wk^{-1} until November 1985 (Fig. 4.6). There was still some growth during the winter months (0.31 mm $week^{-1}$ in December 1985 and 0.38 mm week in February/March 1986). By spring the growth rate was increasing again and the maximum growth rate during this study was observed in June 1986 (1.94 mm wk^{-1}).

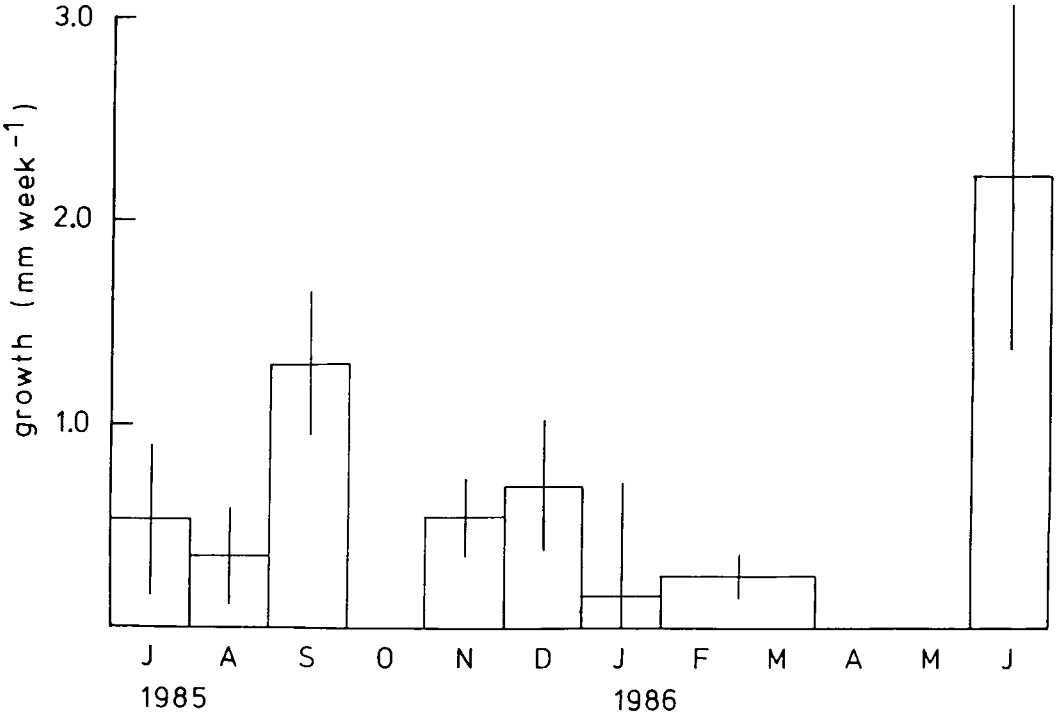
There was no significant relationship ($r = 0.609$) when growth rate was considered as a function of temperature (Fig. 4.7).

4.44 Daddryshield Burn, reach 0309-80

This was the lowest (Table 3.2) and the warmest (Fig. 4.3) of the four streams and the growth rate of Rhynchostegium was correspondingly faster than in the other streams (Fig. 4.8). Boulders were recovered on all but one

Fig. 4.4 Seasonal changes in growth rate of _Rhynchostegium_ in 0091-05.

Fig. 4.5 Relationship between growth rate of _Rhynchostegium_ and water temperature in 0091-05.


3.0
2.0
1.0
growth (mm week^{-1})
J
A
S
O
N
D
J
F
M
A
M
J
1985
1986

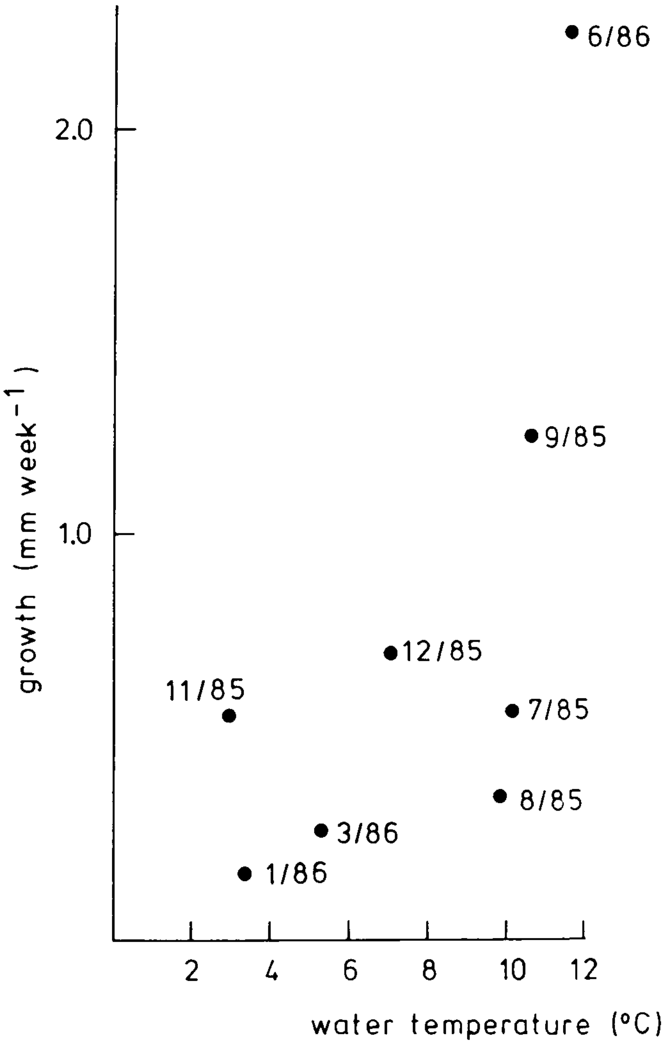
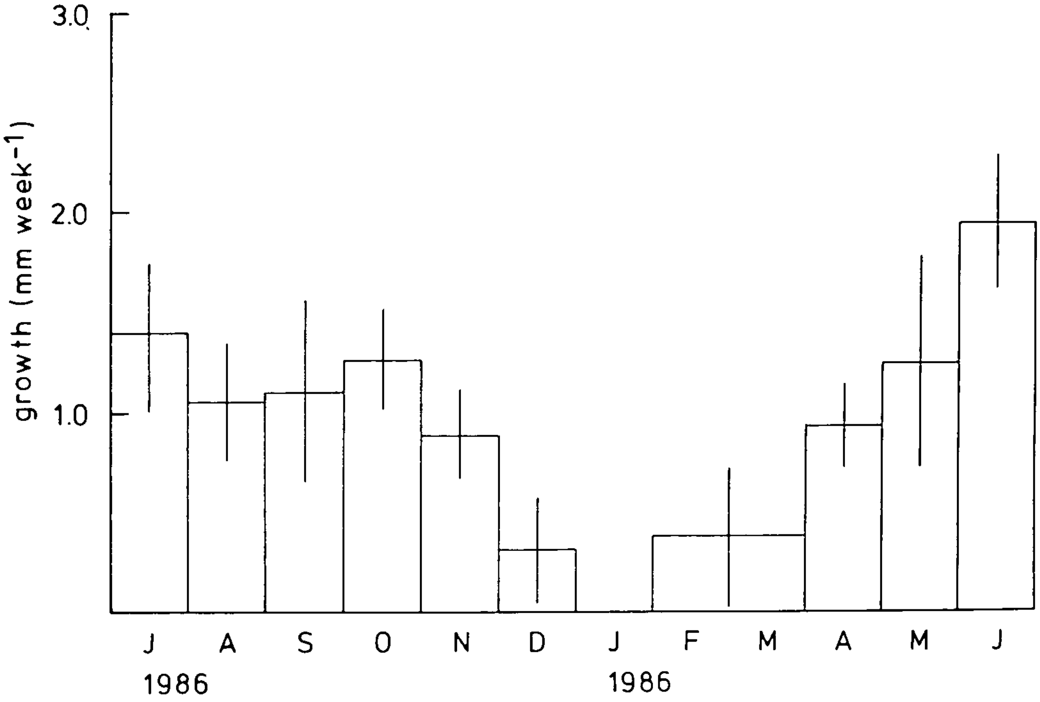
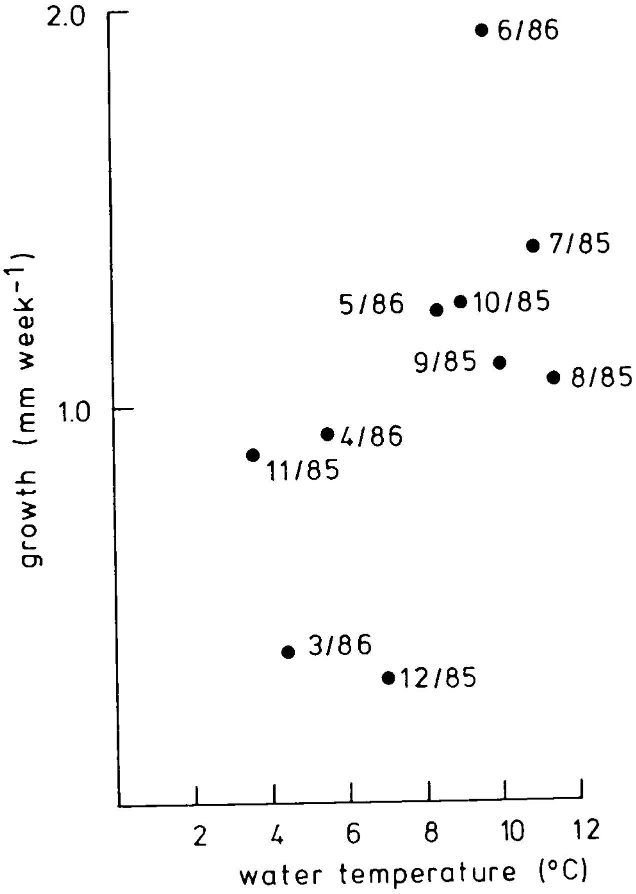

6/86
9/85
12/85
11/85
7/85
8/85
3/86
1/86
2.0
1.0
growth (mm week^{-1})
2
4
6
8
10
12
water temperature (°C)

Fig. 4.6 Seasonal changes in growth rate of *Rhynchostegium* in 0288-70.

Fig. 4.7 Relationship between growth rate of *Rhynchostegium* and water temperature in 0288-70.


3.0
2.0
1.0
growth (mm week^{-1})
J
A
S
O
N
D
J
F
M
A
M
J
1986
1986


2.0
1.0
growth (mm week^{-1})
6/86
7/85
5/86
10/85
9/85
8/85
4/86
11/85
3/86
12/85
2
4
6
8
10
12
water temperature (°C)

one occasion; however in March 1986 only one labelled tip was found. The maximum rate of growth was observed in July 1985 (2.31 mm wk^{-1}) and there was a gradual decrease in the rate until December (0.40 mm wk^{-1}). The rate picked up again in the Spring and by June 1986 was 2.15 mm wk^{-1}. The rate of growth was significantly correlated with water temperature (r = 0.842 **; Fig. 4.9) and indicates relatively rapid growth at warm water temperatures in the Summer and slower growth in the Winter.

4.45 "Race Fell Burn", reach 0310-90

0310-90 is both the highest and the most exposed of the four streams. This is reflected both in the lower temperatures (4.31) and in the relatively slow rates of growth (Fig. 4.10). The rate of growth was highest in June 1986 (1.44 mm $week^{-1}$) and the growth rate in the winter months was very slow (0.12 mm wk^{-1} in February/March). The stream does, however, appear to have a smaller range of discharges; Rhynchostegium is found attached to relatively small boulders and on no occasion was the boulder lost due to high flows.

This stream differed from the others in that there was a well developed peak of growth in Autumn, to a maximum of 1.2 mm wk^{-1}; otherwise there was no clear relationship (r = 0.521) between water temperature and growth rate (Fig. 4.11).

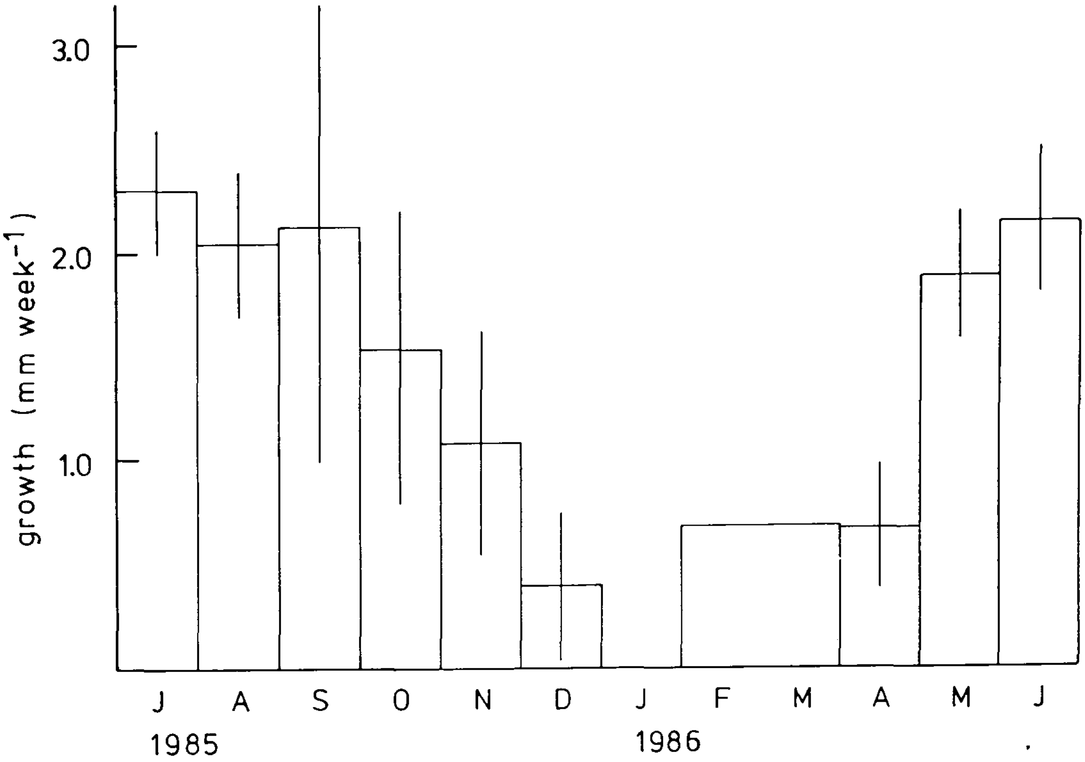
4.46 Synthesis of data

Analysis of data was performed in two parts; analysis of the relationship between growth rate and environmental data for each stream, followed by analysis of the combined data from the four reaches.

Data collected from different reaches were compared by both parametric and non-parametric tests. Two sorts of parametric tests were performed;

Fig. 4.8 Seasonal changes in growth rate of <u>Rhynchostegium</u> in 0309-80.

Fig. 4.9 Relationship between growth rate of <u>Rhynchostegium</u> and water temperature in 0309-80.


3.0
2.0
1.0
growth (mm week^{-1})
J
A
S
O
N
D
J
F
M
A
M
J
1985
1986

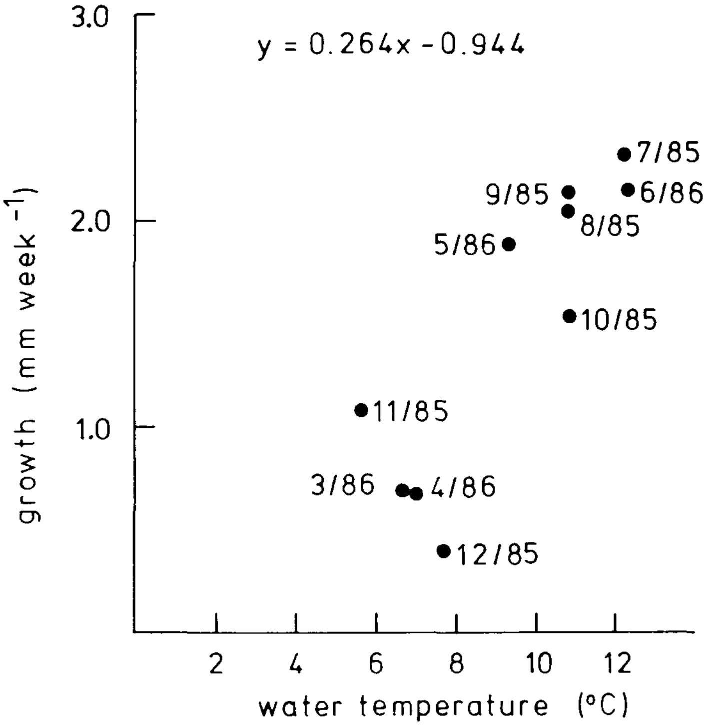
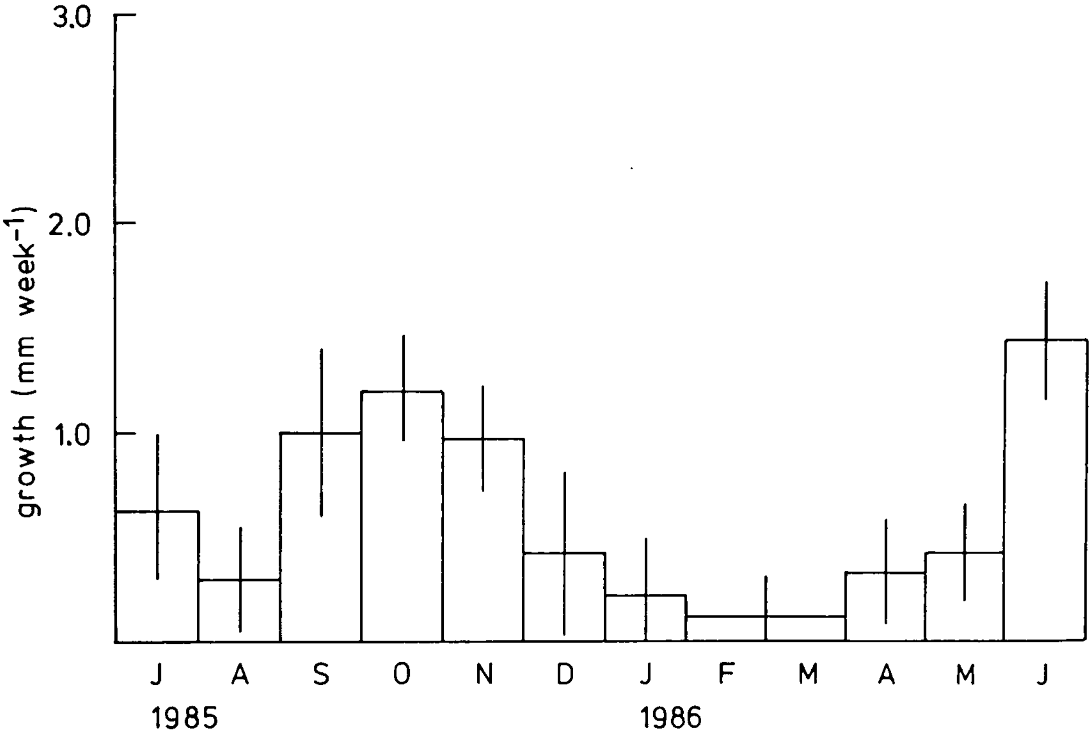
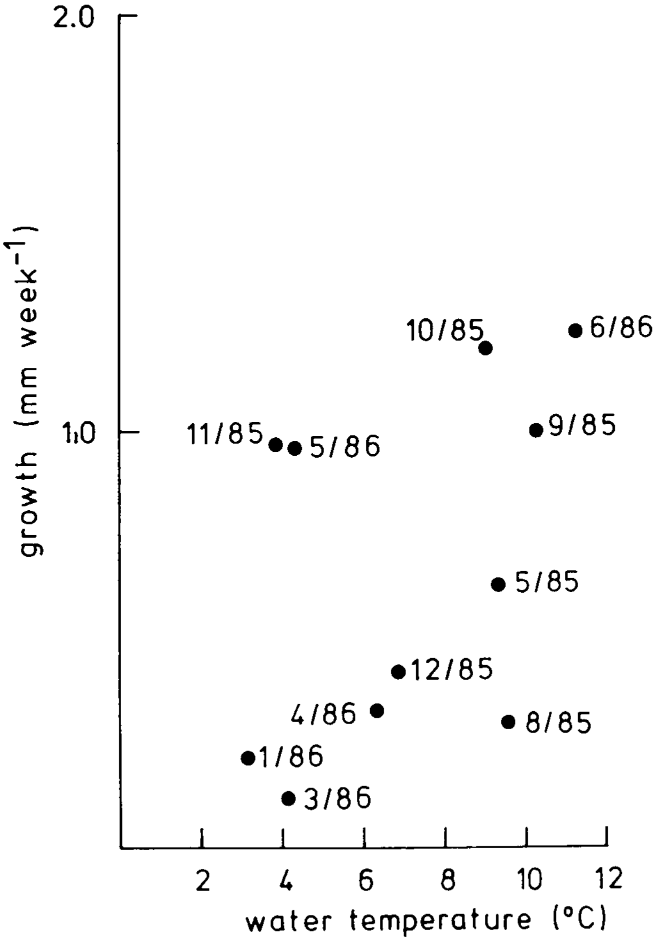

3.0
2.0
1.0
y = 0.264x - 0.944
7/85
9/85
6/86
8/85
5/86
10/85
11/85
3/86
4/86
12/85
growth (mm week^{-1})
2
4
6
8
10
12
water temperature (°C)

Fig. 4.10 Seasonal changes in growth rate of *Rhynchostegium* in 0310-90.

Fig. 4.11 Relationship between growth rate of *Rhynchostegium* and water temperature in 0310-90.


3.0
2.0
1.0
growth (mm week^{-1})
J
A
S
O
N
D
J
F
M
A
M
J
1985
1986


2.0
1.0
growth (mm week^{-1})
10/85
6/86
11/85
5/86
9/85
5/85
12/85
4/86
8/85
1/86
3/86
2
4
6
8
10
12
water temperature (°C)

product-moment correlation coefficients and pairwise t-tests between each pair of variables. The rate of growth was correlated significantly between three pairs of streams; 0091-05 and 0310-90, 0288-70 and 0309-80 and 0288-70 and 0310-90 (Table 4.5); these indicate strong linear relationships between the rate of growth in these pairs of streams and no such relationship between other pairs of streams. There were strong differences between monthly rates of growth between several pairs of streams. These were shown by the pairwise t-tests. Three out of six comparisons were significant at $p > 0.05$ (Table 4.6); these were between 0091-05 and 0310-90, 0288-70 and 0309-80, and 0288-70 and 0310-90. Differences in the monthly rates of growth between 0091-05 and 0288-70, between 0091-05 and 0309-80 and between 0309-80 and 0310-90 were not significant. The same pairs of streams showed significant relationships in each case; however it is unlikely that pairs of streams which do not have significant linear correlations will have resovable differences in monthly rates of growth.

Table 4.5 Correlation coefficients between growth rate of <u>Rhynchostegium</u> in four streams.

0091-05	1.000			
0288-70	0.736	1.000		
0309-80	0.426	0.853*	1.000	
0310-90	0.866*	0.772*	0.480	1.000
	0091-05	0288-70	0309-80	0310-90

Table 4.6 Comparisons of growth rate of *Rhynchostegium* in four streams using pairwise t-tests.

0091-05	1.000			
0288-70	-0.889	1.000		
0309-80	-2.318	-3.237*	1.000	
0310-90	0.964*	3.012*	3.921	1.000
	0091-05	0288-70	0309-80	0310-90

A variety of non-parametric tests were performed; Spearman's rank correlation (Rho) and Kendall's Tau-B both gave very similar results with significant differences between 0091-05 and 0310-90, 0288-70 and 0309-80 and 0288-70 and 0310-90 (Table 4.7; Table 4.8), as for the parametric tests. Kendall's coefficient of concordance, a measure of agreement calculated between all sampling sites gave a value of 0.754; a significant value which indicated that there was some degree of similarity between the four sites.

Table 4.7 Spearman's rank correlation (Rho) calculated between growth rate of *Rhynchostegium* at four sites in growth study.

0091-05	1.000			
0288-70	0.536	1.000		
0309-80	0.429	0.842*	1.000	
0310-90	0.881*	0.754*	0.503	1.000
	0091-05	0288-70	0309-80	0310-90

Table 4.8 Kendall's Tau-B coefficient calculated between growth rate of Rhynchostegium at four sites in growth study.

0091-05	1.000			
0288-70	0.536	1.000		
0309-80	0.429	0.842^{*}	1.000	
0310-90	0.714^{*}	0.600^{*}	0.503	1.000
	0091-05	**0288-70**	**0309-80**	**0310-90**

The possible effect of seasonal changes was also investigated. The growth rate at one site, 0310-90 was correlated with mean rainfall (negative), at two sites with mean discharge (negative), at two with maximum air temperature (positive) and at one, 0309-80, with minimum air temperature (negative) (Table 4.9).

Table 4.9 Correlations between environmental variables and growth rate of Rhynchostegium in four streams.

stream-reach	**0091-05**	**0288-70**	**0309-80**	**0310-90**
mean rainfall	-0.741	-0.734	-0.366	-0.817^{*}
mean discharge	-0.671	-0.910^{**}	-0.641	-0.850^{**}
air temp.$_{max}$	0.572	0.864^{*}	0.929^{**}	0.452
air temp.$_{min}$	0.383	0.725	0.922^{**}	0.288

Time-series analysis using a cross correlation was applied to the data. The test was whether in any population there was evidence of a constant delay between changes in the environment and the growth rate of moss. In each, cross-correlations between variables were calculated using increasing monthly lags from zero to three months (Table 4.10). Overall, correlations tended to decrease with increasing lags. 0310-90 showed a one month lag in response to air and water temperatures; however only one of these (water temperature) was significant and none of the other streams showed a similar lag.

Only one site had a significant relationship between the rate of moss growth and water temperature (0309-80, see 4.44); however when data from all sites were pooled then there were significant relationships between growth rate and water temperature (r = 0.627 ***, Fig. 4.12), between growth rate and mean maximum air temperature (r = 0.513 **, Fig. 4.13) and between growth rate and mean minimum air temperature (r = 0.507 ***, Fig. 4.14). Using the significant regressions to extrapolate back, then it is possible to calculate an approximate minimum temperature below which the moss does not grow; This corresponds to a water temperature of 1.7 °C, a weekly mean maximum air temperature of -1.6 °C and a weekly minimum air temperature of -5.6 °C; however no allowance is made for the effect of light on growth rate in this.

4.5 Annual growth rates

Estimates were also made of total growth over the 12 month period. Approximate rates for the months where no data were collected were estimated from water temperature and Fig. 4.12. Three such replacements were necessary for 0091-05 and one each for 0288-70 and 0309-80. Increases in length over the year ranged from 33.4 mm in 0310-90 (uncorrected) to 77.6 mm in 0309-80 (uncorrected) or 73.3 (corrected) (Table 4.11). The total increase in 0309-80 was more than double that in 0310-90.

The regression equations calculated in 4.23 (Table 4.1) were used to convert these values from increases in length per unit time to increases in

Table 4.10 Time-series analysis of relationships between growth rate of *Rhynchostegium* and environmental variables.

variable	lag (months)			
	0	1	2	3
0091-05				
rainfall	0.741	0.300	0.120	-0.030
flow	-0.671	0.218	0.051	0.183
max. air temp.	-0.572	0.311	0.006	-0.318
min. air temp.	0.383	0.348	0.104	-0.542
water temp.	0.664	0.269	0.090	-0.637
0288-70				
rainfall	-0.734	0.242	0.174	-0.282
flow	0.910**	-0.101	0.022	-0.420
max. air temp.	0.864*	0.383	0.009	-0.169
min. air temp.	0.725	0.289	-0.026	-0.301
water temp.	0.548	0.273	-0.022	-0.350
0309-80				
rainfall	-0.366	0.226	0.065	-0.693
flow	0.641	0.022	0.184	0.543
max. air temp.	0.929**	0.736*	0.468	-0.030
min. air temp.	0.922*	0.660*	0.212	-0.205
water temp.	0.859*	0.700*	0.248	-0.187
0310-90				
rainfall	-0.817*	0.033	0.276	-0.080
flow	-0.850*	-0.126	0.166	0.070
max. air temp.	0.452	0.553	0.434	0.303
min. air temp.	0.288	0.536	0.528	0.222
water temp.	0.482	0.683*	0.560	0.176

Fig. 4.12 Relationship between growth rate of Rhynchostegium, measured in four reaches in N-E. England over 12 months, and spot water temperature measurements: summed data.

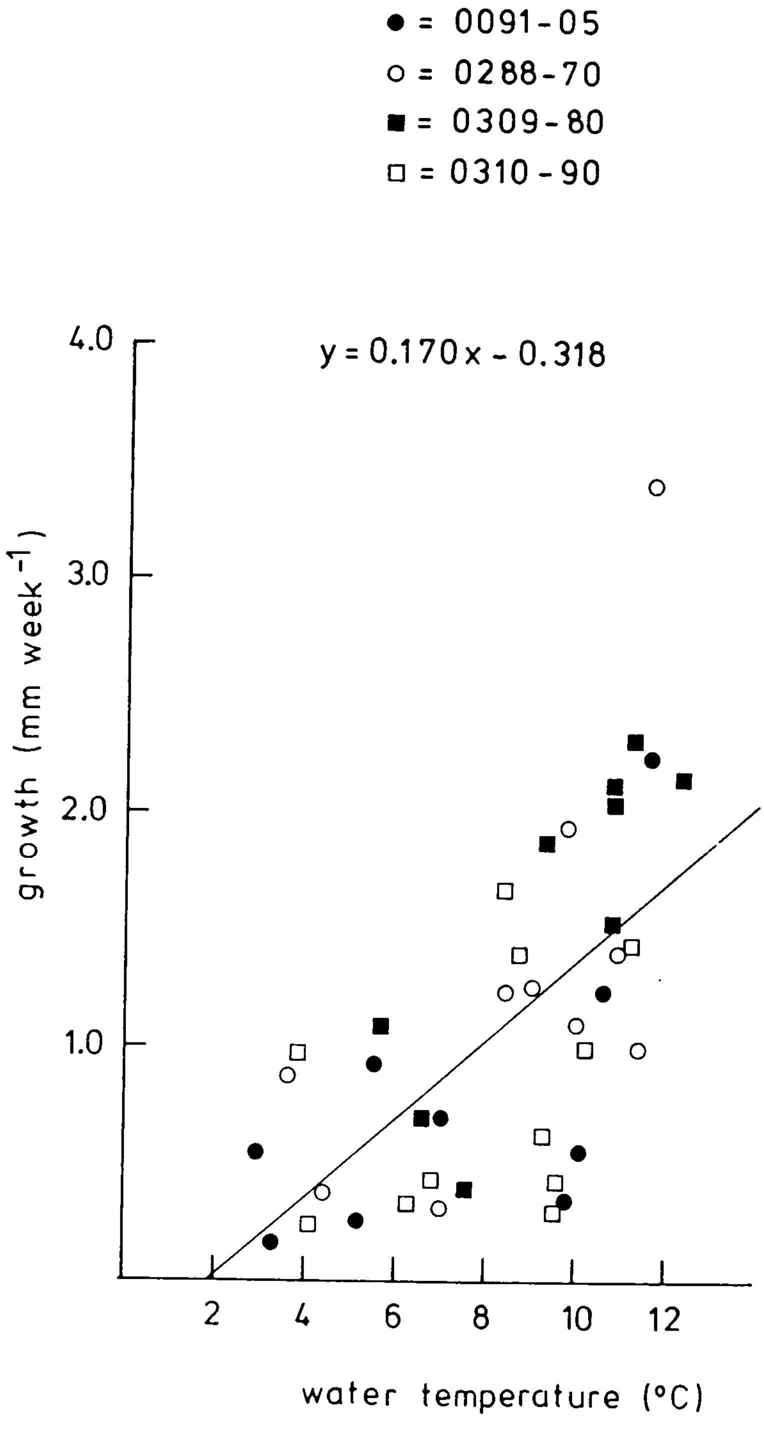
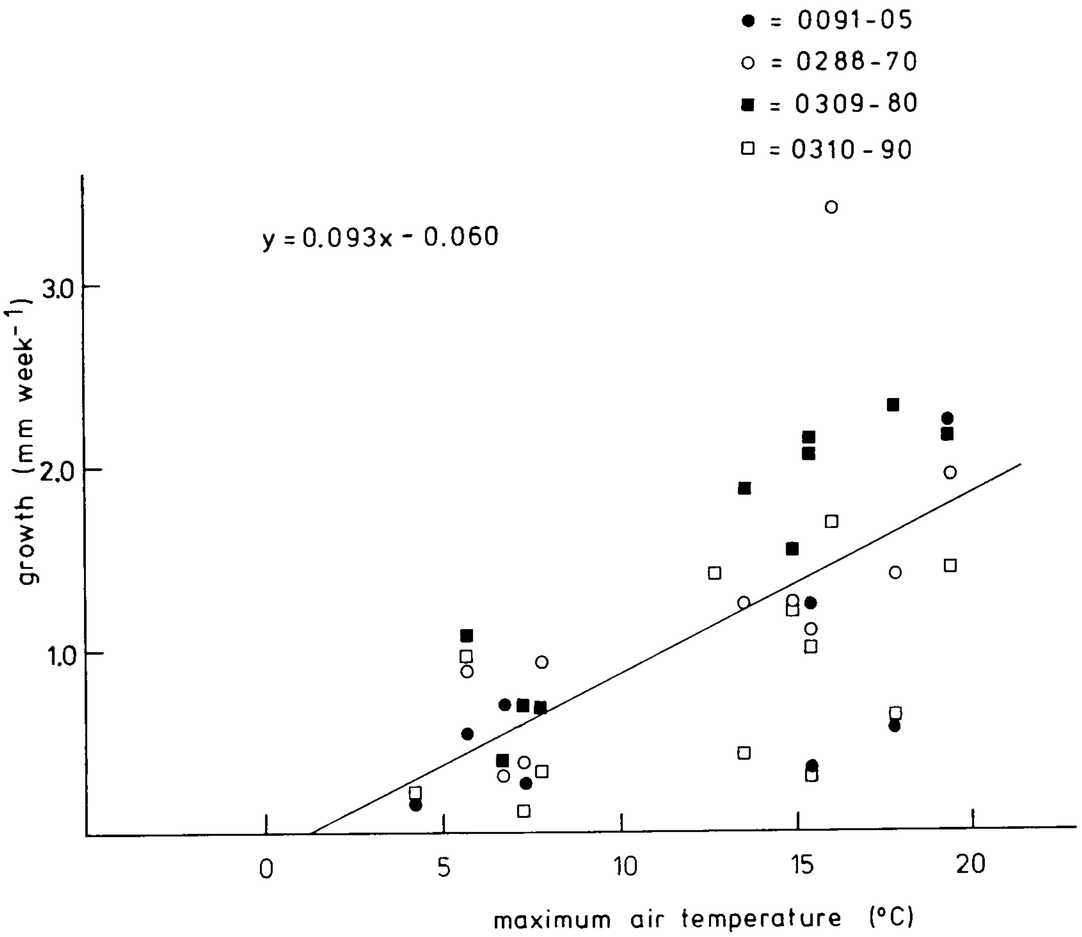
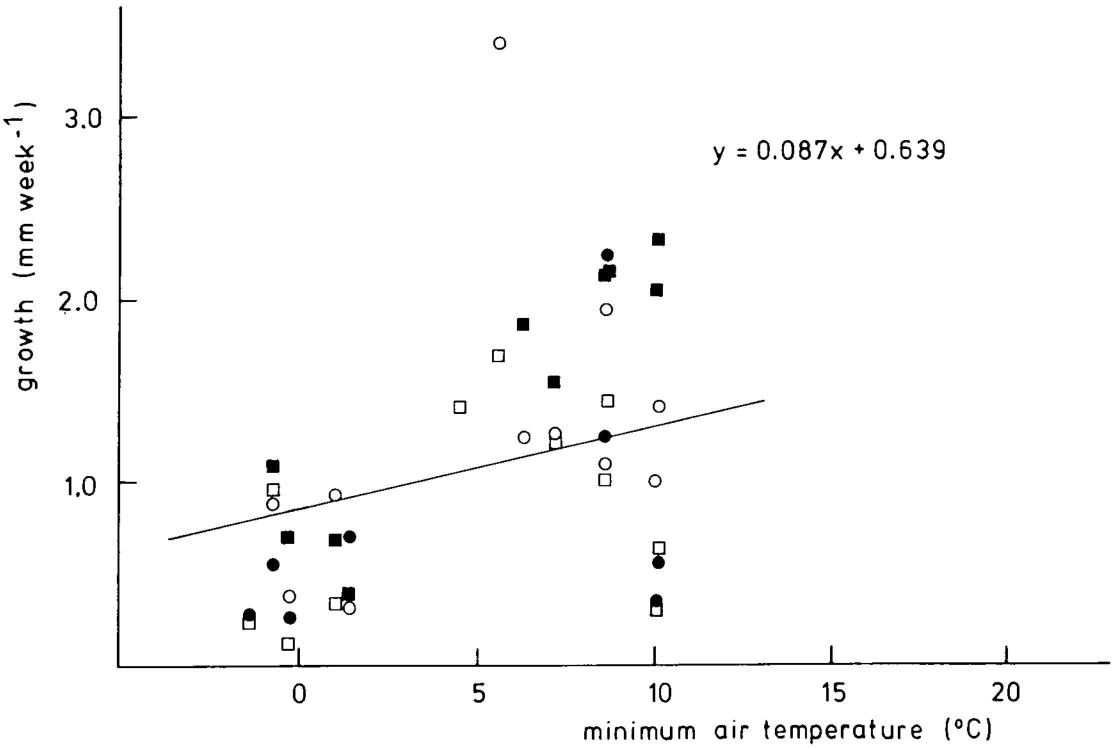

● = 0091-05
○ = 0288-70
■ = 0309-80
□ = 0310-90
y = 0.170x - 0.318
growth (mm week⁻¹)
4.0
3.0
2.0
1.0
2
4
6
8
10
12
water temperature (°C)

Fig. 4.13 Relationship between growth rate of <u>Rhynchostegium</u>, measured in four reaches in N-E. England over 12 months, and mean maximum air temperature: summed data.

Fig. 4.14 Relationship between growth rate of Rhynchostegium, measured in four reaches in N-E. England over 12 months, and mean maximum air temperature.


● = 0091-05
○ = 0288-70
■ = 0309-80
□ = 0310-90
y = 0.093x - 0.060
growth (mm week^{-1})
3.0
2.0
1.0
0
5
10
15
20
maximum air temperature (°C)


y = 0.087x + 0.639
growth (mm week^{-1})
3.0
2.0
1.0
0
5
10
15
20
minimum air temperature (°C)

mass (Table 4.11). Increases in mass of between 29.2 mg (0310-90) and 120.8 mg (corrected value, 0309-80) per shoot per year were recorded.

Table 4.11 Increase in length and mass of Rhynchostegium over 12 months, calculated from mean monthly growth rate with and without correction for missing data.

	0091-05	0288-70	0309-80	0310-90
length (mm yr^{-1})				
uncorrected	39.5	54.3	77.6	33.4
corrected	43.3	51.3	73.3	-
mass (mg yr^{-1})				
uncorrected	56.9	61.7	127.9	29.2
corrected	62.4	58.2	120.8	-

4.6 Shoot density and annual production of dry matter

Measurements of shoot density were made using a quadrat of area 6.7 cm^{-2}. This was placed on patches of Rhynchostegium on boulders and the number of tips was counted. From these it was possible to estimate annual dry matter production by multiplying by the increase in mass per shoot per year (corrected values; Table 4.11). Spatial density ranged from 4.4 shoots cm^{-2} (0288-70) to 6.4 shoots cm^{-2} (0310-90) (Table 4.12); the greatest density was associated with the slowest growth rate (0310-90) but comparison with other sites showed no clear relationship between growth rate and shoot density. Dry matter production is expressed as g dm^{-2} yr^{-1} (Table 4.12) and showed the series 0310-90 < 0288-70 < 0091-05 < 0309-80. The rate in 0309-80 was three times faster than the rate in 0310-90.

Table 4.12 Spatial densities and annual rates of dry matter production of *Rhynchostegium* shoots on boulders..

stream-reach	spatial density (shoots cm^{-2})		dry-matter production ($g\ dm^{-2}\ yr^{-1}$)
	mean	SD	
0091-05	5.8	0.94	36.2
0288-70	4.4	0.26	25.6
0309-80	4.8	0.29	58.0
0310-90	6.4	0.31	18.7

5. DEVELOPMENT OF METHOD FOR USE OF MOSS-BAGS

5.1 INTRODUCTION

Although moss-bags have been used in the past for monitoring heavy metal pollution in freshwaters (1.524), results from these studies have rarely reached the literature. Individual workers have developed methods according to specific needs and circumstances and there has been no attempt to produce a standard method. This chapter is concerned with the development of such a method.

The experiments all involved manipulations of moss between streams of known water chemistries. Rhynchostegium was used for all but one of the experiments; in the one exception Fontinalis antipyretica was used. Interest was focussed on the metal Zn, although analyses of Cd and Pb were also made, where relevant. The principle question asked was what are the effects, in terms of rates of accumulation etc., of transferring moss from boulders to mesh bags? Experiments are concerned with the physical environment within bags, the rate of uptake by moss in bags compared with that on boulders and the effects of bag style and packing on final concentrations of heavy metals.

5.2 TYPES OF BAGS

Three types of bag were tested. The materials were chosen so as to be readily available to future workers and were all obtained from high-street shops. Features of their design are listed in Table 5.1.

Types I and III were assembled from the fabric; nylon monofilament fishing line (breaking strain = 5.4 kg) was used to sew pattern I and stout sewing thread for pattern III. Type II was obtained made-up and is a bag of the type in which bulbs, fruit and vegetables and tennis balls etc. are often sold.

Table 5.1 Mesh bags used in transplant experiments.

pattern	fabric	size (cm)	colour
I	garden netting, sewn together with nylon monofilament	15 x 15	green
II	manufactured nylon mesh bag	30 x 30	orange
III	muslin, sewn up with stout thread	15 x 15	white

5.21 Effect upon physical environment within bag

If moss-bags are to be used for extended periods then it is of interest to examine the effects of the bag upon the physical environment around the moss. Experiments were conducted on the effect of each type of bag on water current and light penetration.

The effect of the bags on current speed was tested using an artificial stream tank and current meter (2.13). The bag was held across the width of the tank and the current speed through a double layer of fabric was measured 50 cm downstream of the bag (Table 5.2). Pattern I had little effect upon the stream current whilst pattern III, due to its close weave, was a considerable obstacle. Pattern II fell between patterns I and III.

Light penetration through the bags was also measured. Pattern I caused the least attenuation and patterns II and III both removed a third of incident light.

Table 5.2 Effect of mesh-bags on physical environment of moss.

Pattern	mesh size (meshes cm^{-1})	current speed ($cm\ s^{-1}$) before	after	% diff.	light flux (% attenuation)
I	0.9	0.246	0.209	15	13.3
II	0.6	0.213	0.086	49	33.3
III	0.07	0.225	0.001	99.5	33.3

5.3 EXPERIMENTS ON METAL ACCUMULATION WITHIN MESH BAGS

5.31 Comparison of accumulation by Rhynchostegium on boulders and in bags.

Uptake of Zn, Cd and Pb by Rhynchostegium on boulders and in mesh bags (pattern I, 5.2) was followed, using a population from 0310-90 transplanted into 0012-45. Environmental variables in the two streams are given in Table 5.3.

Uptake of all three metals continued throughout the experiment (Fig. 5.1). Two phases of uptake of Zn and Pb were apparent; an initial, rapid phase lasting approximately 6 h, followed by a period of slower accumulation. The demarcation into two phases was not apparent for Cd, however the concentrations, and therefore the fluxes, are two and three orders of magnitude respectively lower than for Zn and Pb.

Table 5.3 Selected environmental variables in 0012-45 during transplant experiment (metals as mg l^{-1} for filtrable fraction; blanks = missing values).

date	time	curr. ($m\ s^{-1}$)	temp. (°C)	pH	Ca	Zn	Pb
19.6.84	0950	0.294	15.5	7.6	43.0	0.149	0.0181
"	1050	0.372	16.0	7.7	42.7	0.139	0.0135
"	1150	0.350	17.0	7.8	43.6	0.116	0.0183
"	1250	0.280	17.5	7.8	43.6	0.100	0.0171
"	1350	0.315	18.0	7.8	43.3	0.101	0.0142
"	1450	0.339	18.5	7.8	43.1	0.110	0.0107
"	1550	0.375	18.5	7.8	42.4	0.112	0.0097
"	1750	0.366	19.0	7.8	49.3	0.095	0.0167
"	2150	0.282	18.0	7.9	42.9	0.088	0.0087
20.6.84	1010	0.326	15.0	7.8	43.9	0.143	0.0207
21.6.84	0950	0.319	13.0	7.8			
23.6.84	1200	0.219	11.0	7.7	56.4	0.148	0.0084

Samples of in situ Rhynchostegium were also collected during the study for comparison with transplanted moss (Table 5.4). At the end of the experiment (t = 101 h), the concentration of Zn in the transplanted moss was approximately equal to the concentration in the in situ moss, the concentration of Cd was slightly below, and the concentration of Pb was significantly below that in the in situ moss.

Fig. 5.1 Accumulation of Zn, Cd and Pb by Rhynchostegium on boulders and bags (pattern I, 5.2) transplanted into 0012-45 on 20/6/1984.

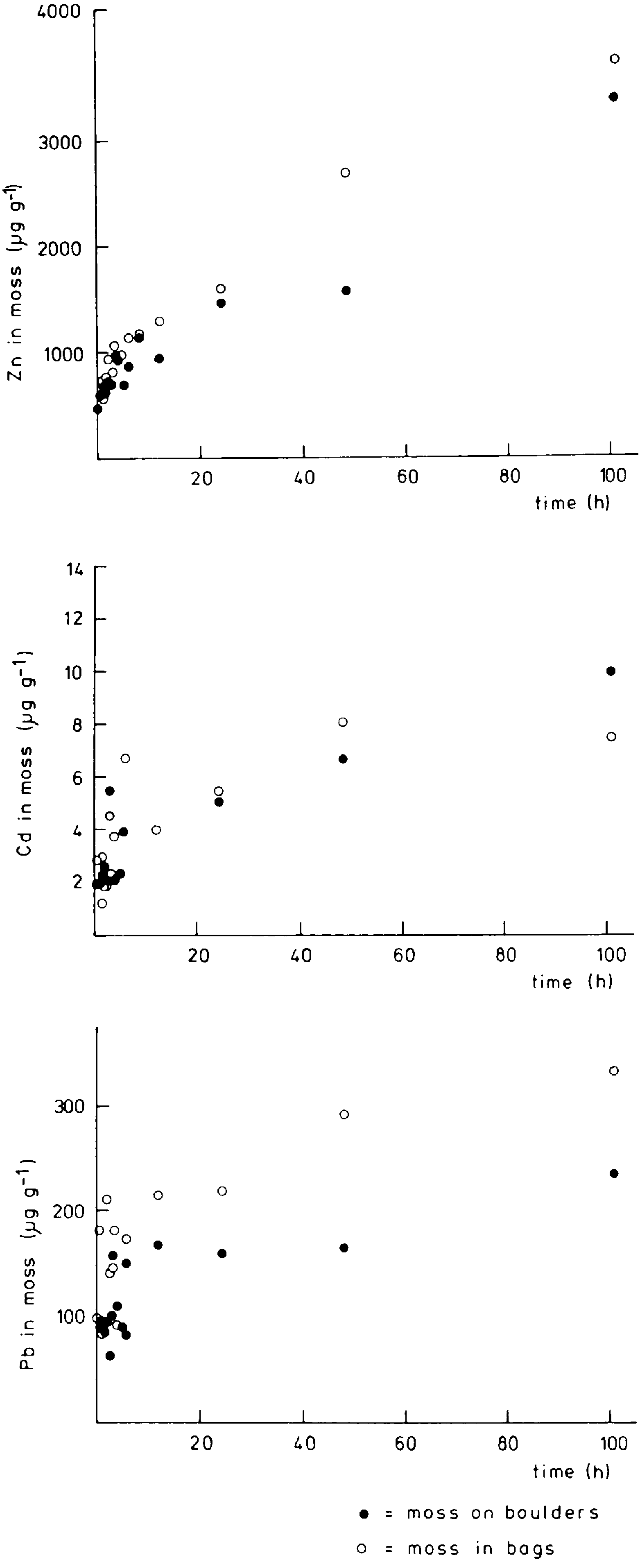

4000
3000
2000
1000
Zn in moss (μg g^{-1})
20
40
60
80
100
time (h)
14
12
10
8
6
4
2
Cd in moss (μg g^{-1})
20
40
60
80
100
time (h)
300
200
100
Pb in moss (μg g^{-1})
20
40
60
80
100
time (h)
● = moss on boulders
○ = moss in bags

Table 5.4 Comparison of concentrations of metals in <u>in situ</u> moss and in moss transplanted into 0012-45 on boulders and in bags (metal accumulation in µg g^{-1}, figures in brackets = 95% confidence limits).

		transplanted moss		<u>in situ</u>
Time (h)	metal	bag	boulder	moss
4.0	Zn	875.8	939.7	2851.4
		(31.0)	(49.8)	(879.0)
	Cd	3.80	2.34	10.38
		(0.53)	(0.31)	(2.09)
	Pb	94.0	108.0	426.0
		(9.8)	(11.4)	(255.0)
101.0	Zn	3775.3	3385.9	3537.2
		(234.4)	(587.7)	(556.6)
	Cd	7.49	9.97	11.40
		(0.67)	(1.38)	(2.06)
	Pb	333.0	235.0	462.0
		(35.5)	(101.3)	(113.0)

Four sets of regression equations were calculated; on all sample means for each treatment, both with and without a natural logarithm transformation, and on the samples < 6.0 h ("rapid phase") and > 6.0 h ("slow phase"). Regressions calculated for all samples, both with and without $\log_e$ transformation, were significant for Zn, Cd and Pb (Table 5.5). The rapid phase of Zn accumulation by moss in bags was significant whilst that on boulders was not. Both treatments were highly significant for the slow phase. For Cd uptake the initial 6 h is significant for

Table 5.5 Regression equations and significance levels for Zn, Cd and Pb accumulation during transplant experiment in 0012-45 (degrees of freedom and, therefore, levels of significance vary between treatments); see 5.31.

	bags v time	boulders v time
ZINC		
all data, normal	y = 31.03 x + 835.88 ** r = 0.983 **	y = 25.32 x + 712.36 ** r = 0.974 **
all data, $\log_e$	y = 522 x + 436.31 ** r = 0.881 **	y = 402 x + 426.25 ** r = 0.823 **
< 6.0 h	y = 78.05 x + 668.40 ** r = 0.816 **	y = 47.92 x + 641.15 N.S. r = 0.577 N.S.
> 6.0 h	y = 28.94 x + 977.74 ** r = 0.988 **	y = 24.91 x + 737.97 ** r = 0.976 **
CADMIUM		
all data, normal	y = 0.057 x + 3.028 ** r = 0.722 **	y = 0.074 x + 2.868 ** r = 0.879 **
all data, $\log_e$	y = 1.237 x + 1.852 ** r = 0.815 **	y = 1.352 x + 1.763 ** r = 0.844 **
< 6.0 h	y = 0.65 x + 1.026 * r = 0.697 *	y = 0.41 x + 1.787 N.S. r = 0.505 N.S.
> 6.0 h	y = 0.027 x + 5.206 N.S. r = 0.558 N.S.	y = 0.068 x + 3.234 ** r = 0.998 **
LEAD		
all data, normal	y = 2.017 x + 154.41 ** r = 0.828 **	y = 1.424 x + 99.81 ** r = 0.832 **
all data, $\log_e$	y = 36.25 x + 125.47 ** r = 778 **	y = 26.78 x + 77.33 ** r = 0.818 **
< 6.0 h, normal	y = 2.27 x + 146.29 N.S. r = 0.097 N.S.	y = 1.32 x + 93.08 N.S. r = 0.092 N.S.
> 6.0 h, normal	y = 1.59 x + 185.23 * r = 0.948 **	y = 1.19 x + 116.04 N.S. r = 0.849 *

"bags" yet not significant for "boulders". The slow phase is the opposite, not significant for "bags" and highly significant for "boulders". For Pb uptake, neither "bags" or "boulders" are significant for the rapid phase and only "bags" are significant for the slow phase. There is considerable "noise" in the initial phases of uptake of all three metals.

The significant regressions were compared (bag v boulder) using ANOVA (Table 5.6). Of the six comparisons only one, Zn with no transformation, was significant where moss in bags appeared to accumulate Zn at a significantly higher rate than moss on boulders. This ANOVA was performed, however, on the sample means only. If the 95% confidence limits for each point (Fig. 5.1) are also taken into account, then the variation within the samples appears to be greater than the variation between the two treatments.

Table 5.6 Analysis of variance between treatments (boulders v bags) during transplant experiment

	Zn	Cd	Pb
no transformation	6.23*	0.631	1.402
$\log_e$ transformation	1.397	0.088	0.822

5.32 Comparison of different patterns of bags

The effect of different meshes on accumulation of Zn by Rhynchostegium in moss-bags was tested by transplanting a population from 0310-90 to 0024-22. Three patterns of bag were used (5.2), each containing sufficient moss for one sample to be taken for analysis. Additional samples of moss were attached to the stakes with lengths of string. These acted as controls. Environmental variables were measured throughout the course of the experiment (Table 5.7).

Table 5.7 Selected environmental variables during moss-bag transplant experiment in 0024-22 (metals in mg l^{-1} for filtrable fraction; blanks = missing values).

site	date	time	curr. (m s-1)	temp. (°C)	pH	Ca	Zn
0310-90	1.6.84	0900		10.0	5.9	20.1	0.03
0024-22	1.6.84	1120		12.0	7.0	56.1	1.56
"	"	1220	0.291	12.3	7.0	60.1	1.86
"	"	1320	0.232	13.0	7.0	58.6	1.81
"	"	1420	0.267	13.5	7.1	58.6	1.50
"	"	1520	0.261	13.8	7.0	67.2	1.47
"	"	1620	0.268	14.0	7.0	74.0	1.61
"	"	1720	0.238	13.8	7.0	73.9	1.71
"	"	1930	0.215	13.0	7.0	72.3	1.81
"	"	2320		11.0	6.9	67.8	1.65
"	2.6.84	1120	0.274	13.5	6.9	60.1	1.36
"	3.6.84	1120		13.0	6.8	65.0	1.67
"	4.6.84	1950	0.245	13.0	6.6	49.0	1.80

Unlike the previous experiment, the uptake curves appeared to reach saturation after 4 - 6 h (Fig. 5.2), and the "rapid" phase of uptake lasted for 5 h. This was based upon a visual examination of the uptake curves and is slightly different to that used in 5.31. The regressions based upon all of the samples were all significant; the $\log_e$ transformed data had both higher levels of significance in the regression and higher correlation coefficients. All of the regressions calculated for the rapid phase were

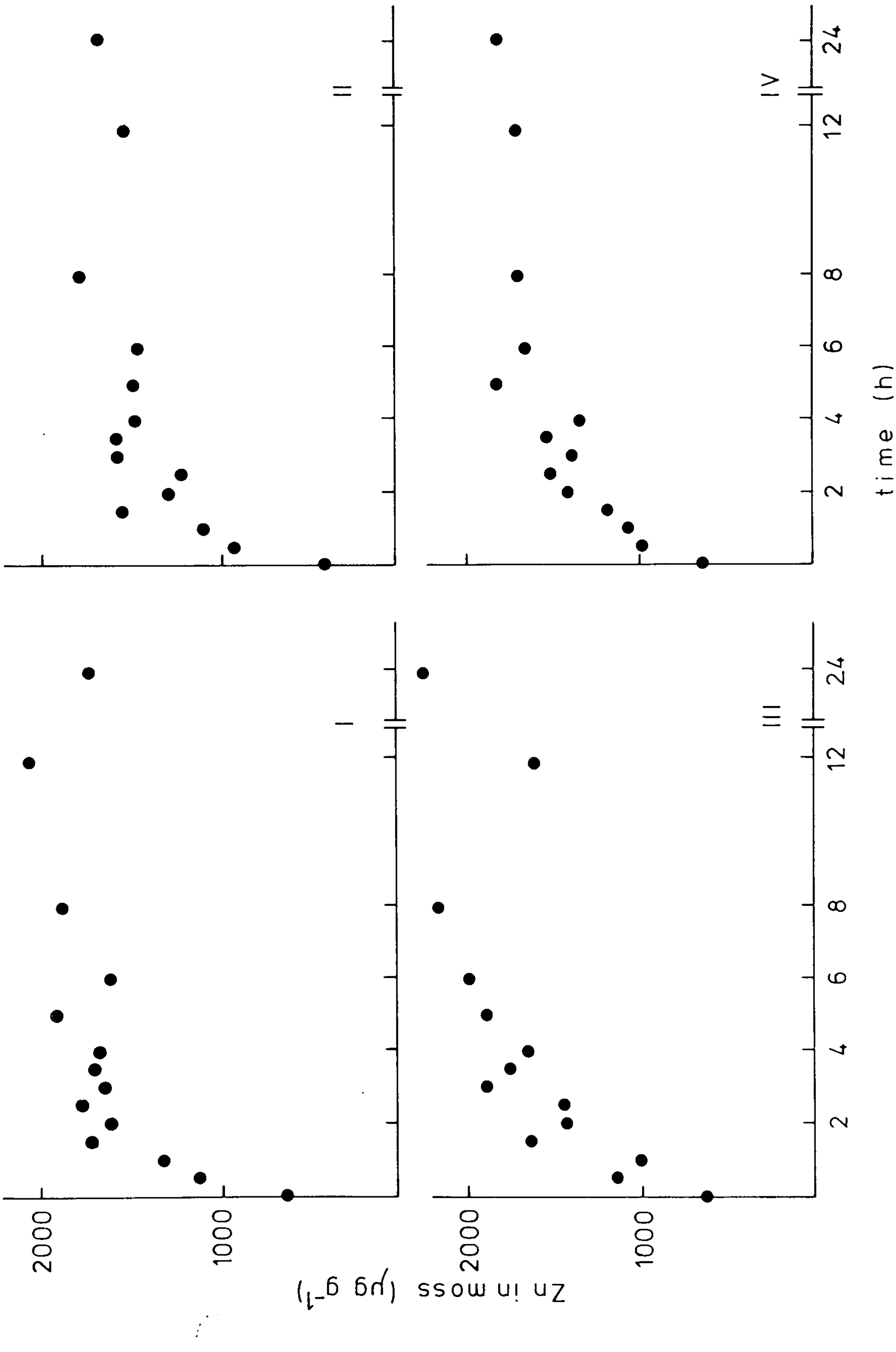
Fig. 5.2 Accumulation of Zn by Rhynchostegium transplanted into 0024-22 in three patterns of mesh bag. on 1/6/1984.

i = control

ii = pattern I

iii = pattern II

iv = pattern III.


I
II
III
IV
2000
1000
Zn in moss (µg g^{-1})
2
4
6
8
12
24
time (h)

also highly significant and highly correlated; however two of the regressions for the slow phase were not significant. These were between time and pattern II bags and time and pattern III bags.

ANOVA was performed between all pairs of significant regressions (Table 5.8); however none of the comparisons were significant. In all but one instance the value of F was less than 1.0, indicating that residual variation exceeded the variation of the combined regressions.

Table 5.8 Analysis of variance between accumulation of Zn in different patterns of mesh bag during transplant experiment in 0024-22.

	control	pattern I	pattern II
1. all data, no transformation			
pattern I	0.378		
pattern II	0.171	0.003	
pattern III	0.652	0.077	0.075
2. all data, loge transformation			
pattern I	0.544		
pattern II	0.002	0.360	
pattern III	0.381	0.00004	0.289
3. < 5.0 h, no transformation			
pattern I	0.021		
pattern II	0.463		
pattern III	0.372	0.557	0.028
4. > 5.0 h, no transformation			
pattern I	0.512		
pattern II	0.968	0.319	
pattern III	1.846	-	-

5.33 Variation in Zn accumulation between replicate bags.

The within- and between-bag variation was tested on data collected during a transplant experiment between 0005-44 and 0024-22. Five replicate bags (pattern II, Table 5.1) of moss were suspended in the river and at each sampling occasion a sample was collected from each.

During the experiment the concentration of Zn in 0024-22 was very high (x (± SD) = 6.34 (± 1.20) mg l^{-1}, n = 6) and this is reflected in the high concentrations measured in both transplanted (Table 5.9) and *in situ* *Rhynchostegium* (29764 µg g^{-1}).

Table 5.9 Concentrations of Zn in water and in *Rhynchostegium* during transplant experiment in 0024-22.

Time from start (h)	Zn in water (mg l-1)	Zn in moss (µg g-1)
2	4.04	5274
4	5.65	7815
6	7.07	9874
8	4.75	10796
12	3.40	10328

ANOVA performed on the data (Table 5.10) showed significant variation between samples of the *in situ* population and between bags at t = 2 h and t = 6 h. There were not significant amounts of variation between replicate bags at other times.

Table 5.10 Analysis of variance within and between replicate bags of moss transplanted into 0024-22.

source	degrees of freedom	sum of squares	mean square	F
t = 0 h				
between	4	1.24×10^{4}	3.09×10^{3}	2.448
within	18	2.27×10^{4}	1.26×10^{3}	N.S.
t = 2 h				
between	4	3.07×10^{9}	7.67×10^{8}	13.84
within	18	9.97×10^{8}	5.54×10^{7}	**
t = 4 h				
between	4	3.87×10^{8}	9.67×10^{7}	1.30
within	19	1.42×10^{9}	7.47×10^{8}	N.S.
t = 6 h				
between	4	3.21×10^{9}	8.03×10^{8}	12.07
within	18	1.20×10^{9}	6.65×10^{7}	**
t = 8 h				
between	4	3.16×10^{9}	7.89×10^{8}	0.52
within	20	3.34×10^{10}	1.51×10^{9}	N.S.
t = 12 h				
between	3	2.46×10^{9}	8.19×10^{8}	0.72
within	15	1.71×10^{10}	1.14×10^{9}	N.S.
in situ				
between	3	3.07×10^{11}	1.02×10^{11}	5.03
within	14	2.85×10^{11}	2.04×10^{10}	*

5.34 Homogeneity of metal accumulation within large packed bags.

The effect of bag packing on the rate of metal accumulation was tested on a population from 0055-30 transplanted to 0048-80 in large moss-bags (pattern II, 5.2) and left for four days. After four days the bags were carefully removed from the river and samples were taken from the centre and the edge. Physico-chemical variables and cation samples were also collected (Table 5.11).

Table 5.11 Selected environmental variables in 0055-30 and 0048-80 during moss-bag transplant experiment (metal concentrations in mg l^{-1} for filtrable fraction).

reach	date	time	current speed ($m\ s^{-1}$)	pH	Ca	Zn
0055-30	10.8.84	1340	0.679	7.6	25.8	0.075
0048-80	15.8.84	1345	0.166	8.0	65.4	1.62

The two treatments were not significantly different for any of the three metals (Table 5.12). For Zn and Pb the samples from the edge contained higher concentrations than samples from the centre, whilst for Cd the opposite was true. The 95% confidence limits for Cd, it should be noticed, are very large (Fig. 5.3).

Fig. 5.3 Accumulation of Zn, Cd and Pb by Rhynchostegium at the centre and edge of moss-bags transplanted into 0048-44. Vertical bars = 95% confidence limits. (16|8|84)

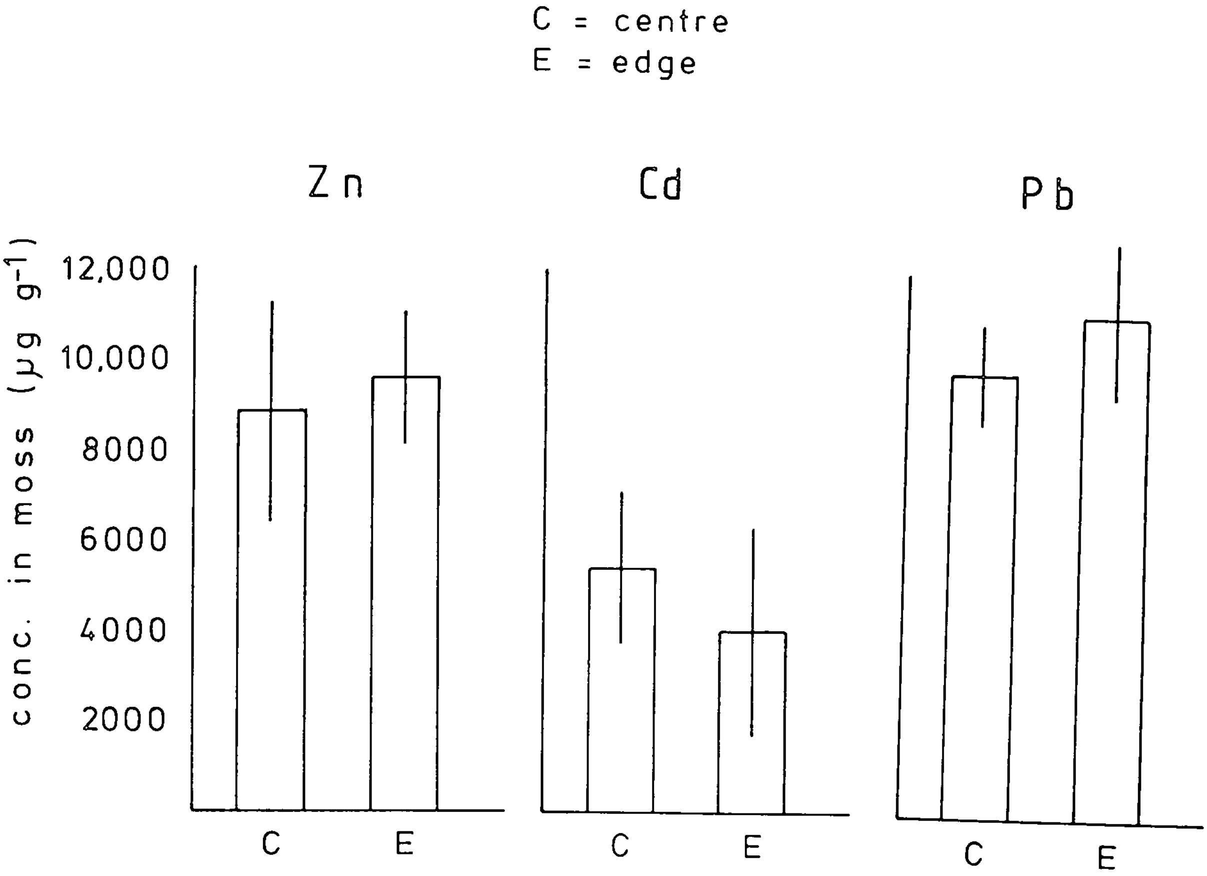

C = centre
E = edge
Zn
Cd
Pb
conc. in moss (μg g-1)
12,000
10,000
8000
6000
4000
2000
C
E
C
E
C
E

Table 5.12 Comparison of metal accumulation at "centre" and "edge" of moss-bags using "Student" t-test.

	centre	edge	"Student" t	significance
Zn	20032	21318	0.23	N.S.
Cd	1.16	0.84	1.10	N.S.
Pb	2182	2347	0.33	N.S.

5.35 Accumulation of Zn by Fontinalis on boulders and in bags

An experiment similar to 5.31 was performed using Fontinalis in order to demonstrate common features of uptake by the two species. This involved a transplant between 0014-40 and 0024-22 using boulders and mesh bags (pattern II, 5.2).

Uptake both by moss on boulders and in bags appeared to reach a plateau after about 24 h (Fig. 5.4). Samples collected over the entire period were well correlated with $\log_e$ time (Table 5.13). There was no clear demarcation into a "rapid" and "slow" phase. Two linear sections were chosen for further analysis; < 4.0 h and > 12.0 h and comparisons were made between boulders and bags for each of these and the whole data. No comparison was significant (Table 5.14).

Fig. 5.4 Accumulation of Zn by <u>Fontinalis</u> transplanted into 0024-22 in boulders and bags. Vertical bars = 95% confidence intervals. (13/12/1983)

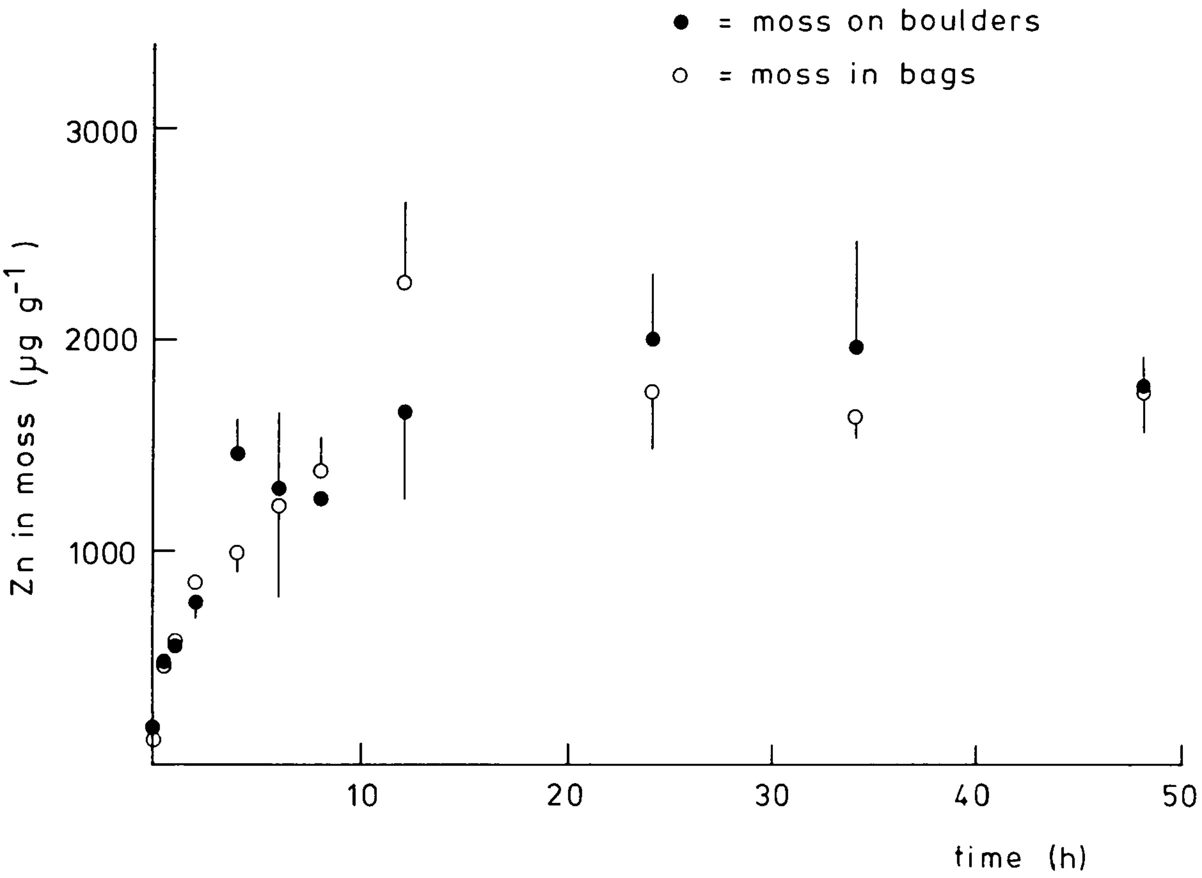

● = moss on boulders
○ = moss in bags
Zn in moss (μg g^{-1})
3000
2000
1000
10
20
30
40
50
time (h)

Table 5.13 Regressions and correlations between Zn concentrations in Fontinalis and time during transplant experiment between 0014-40 and 0024-22.

treatment	regression equation	F	correlation coefficient
all data, no transformation			
boulder	y = 26.7 x + 961	10.55*	0.754**
bag	y = 23.0 x + 979	5.47*	0.637*
all data, ln transformation			
boulder	y = 365 x + 679	73.89**	0.950***
bag	y = 333 x + 687	25.14**	0.871***
< 4.0 h, no transformation			
boulder	y = 312 x + 212	109.13**	0.986**
bag	y = 189 x + 338	18.80**	0.928**
> 12.0 h, no transformation			
boulder	y = 2.25 x + 1796	0.10 N.S.	0.214 N.S.
bag	y = 14.0 x + 2261	2.33 N.S.	-0.734 N.S.

Table 5.14 Analysis of variance between significant regressions for data collected during Fontinalis transplant experiment (5.35).

treatment	F	significance
all data, no transformation	0.082	N.S.
all data, $\log_e$ transformation	0.083	N.S.
< 4.0 h, no transformation	5.45	N.S.
> 12.0 h, no transformation	-	

6. EXPERIMENTAL STUDIES ON METAL ACCUMULATION AND LOSS

6.1 INTRODUCTION

Experimental studies on metal accumulation and loss were performed both in the laboratory and the field. These provide a background for studies on practical aspects of monitoring techniques (Chapters 5 and 8). Field experiments involved transplants between streams of known water chemistry to examine rates of uptake and loss under natural conditions.

The experiments reported here concern the mechanism of metal accumulation. They deal first with accumulation of heavy metals over an extended period of time (6.2) and then proceed to consider the role of the cell wall and of metabolism in this process (6.3, 6,4). Finally the rates at which heavy metals are lost and the factors which affect this are considered (6.5). Experiments were focussed on Zn, although a few results for Cd and Pb are included.

6.2 ACCUMULATION OF HEAVY METALS OVER THREE WEEKS

The time course of heavy metal accumulation by Rhynchostegium was investigated by transplanting moss to a metal-enriched stream and sampling over a 23-d period.

Moss was collected from 0091-05 and transplanted to 0288-90 in mesh bags (5.2, pattern II). Samples of moss were collected from both transplanted and indigenous populations at 0, 1, 3, 7, 11, 17 and 23 d from the start of the experiment. Water samples were collected and physico-chemical variables measured on each sampling occasion (Table 6.1).

Table 6.1 Selected environmental variables during long-term (= 23 d) transplant experiment (metal concentrations in mg l^{-1} for the filtrable fraction; blanks = not measured).

stream -reach	date	temp. (^oC)	pH	Ca	Zn	Cd	Pb
0091-05	23.7	12	7.6	50.8	0.036	0.0002	0.0011
0288-90	23.7	12	8.2	61.6	0.273	0.0008	0.0022
0288-90	24.7	12	8.2	50.4	0.268	0.0013	0.0070
0288-90	26.7	12	8.2	61.4	0.250	0.0018	0.0040
0288-90	30.7	13	8.2	60.6	0.276	0.0064	0.0027
0288-90	3.8	13	7.6	26.4	0.155	0.0014	0.0054
0288-90	9.8	11	7.7	43.6	0.337	0.0017	0.0057
0288-90	15.8	12	7.7	63.8	0.296		

The mean concentration of Zn in 0288-90 was almost an order of magnitude greater than in 0091-05 (Table 6.1); the minimum Zn concentration in 0288-90 (3.8.84) coincided with a spate. Concentrations of Cd and Pb in 0288-90 were not so high compared with 0091-05; Cd in 0288-90 ranged from 0.0008 mg l^{-1} to 0.0064 mg l^{-1} compared with 0.0002 in 0091-05 and Pb concentrations in 0288-90 ranged from 0.0022 mg l^{-1} to 0.0070 mg l^{-1} compared with 0.0011 mg l^{-1} in 0091-05. The maximum concentrations of Zn, Cd and Pb all occurred on different days (Table 6.1).

Zn accumulated throughout the experiment (Fig. 6.1). The final concentration was significantly greater than the concentration measured in _in situ_ moss. The accumulation of Cd appears to be complete after seven days (Fig. 6.1) when the concentration in the transplanted moss was still significantly lower than the concentration in _in situ_ moss. Accumulation of Pb was also complete by the end of the experiment (Fig. 6.1) and the concentration was approximately the same as that within the _in situ_ moss. There were considerable variations in the concentration of Zn, Cd and Pb in the _in situ_ moss; none of these, however, correlated with the corresponding concentrations in water samples (Table 6.2). After 4-d these changes in Zn and Pb concentrations in _in situ_ moss were mirrored by changes in the concentrations in transplanted moss; there were not enough data, however, for a valid statistical comparison of these.

Table 6.2 Linear correlations between concentrations of Zn, Cd and Pb in water samples and _in situ_ moss over 23 d.

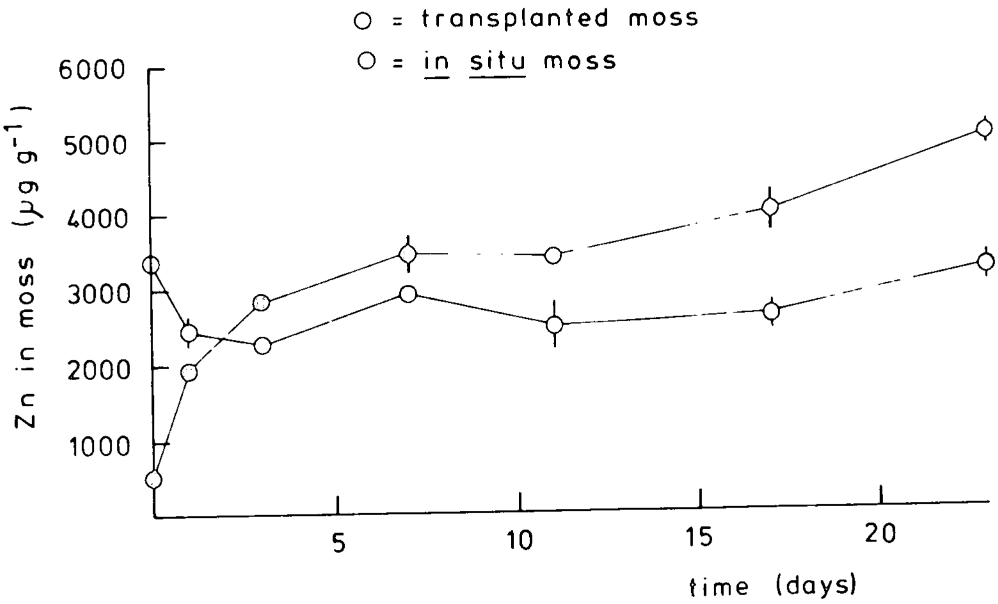
metal	correlation coefficient	
Zn	0.055	N.S.
Cd	0.500	N.S.
Pb	0.067	N.S.

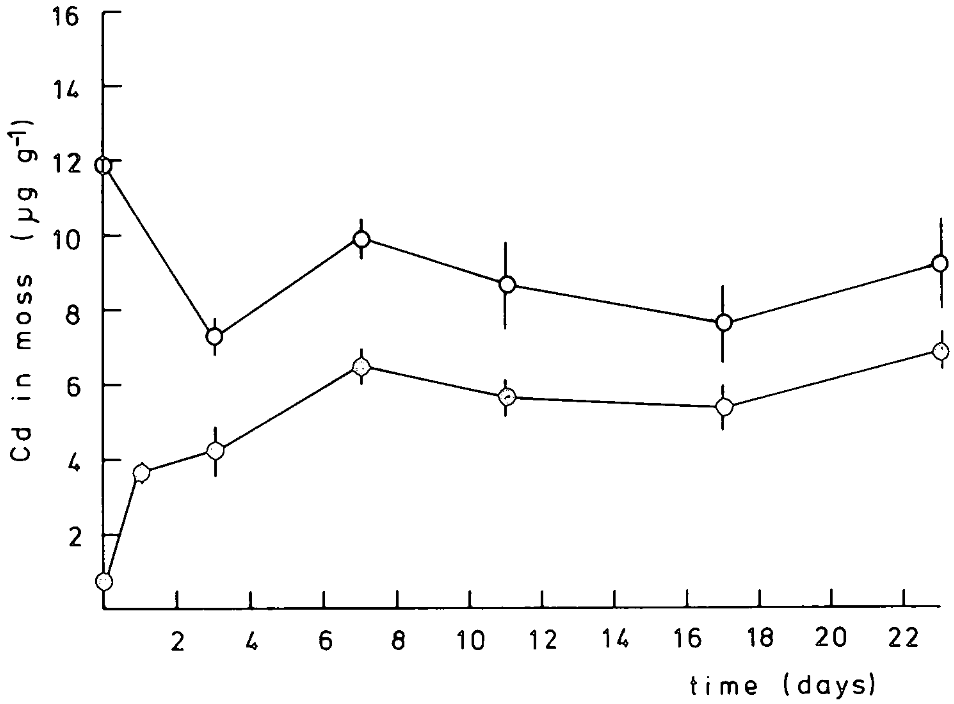
6.3 ROLE OF CELL WALL EXCHANGE IN ZINC ACCUMULATION

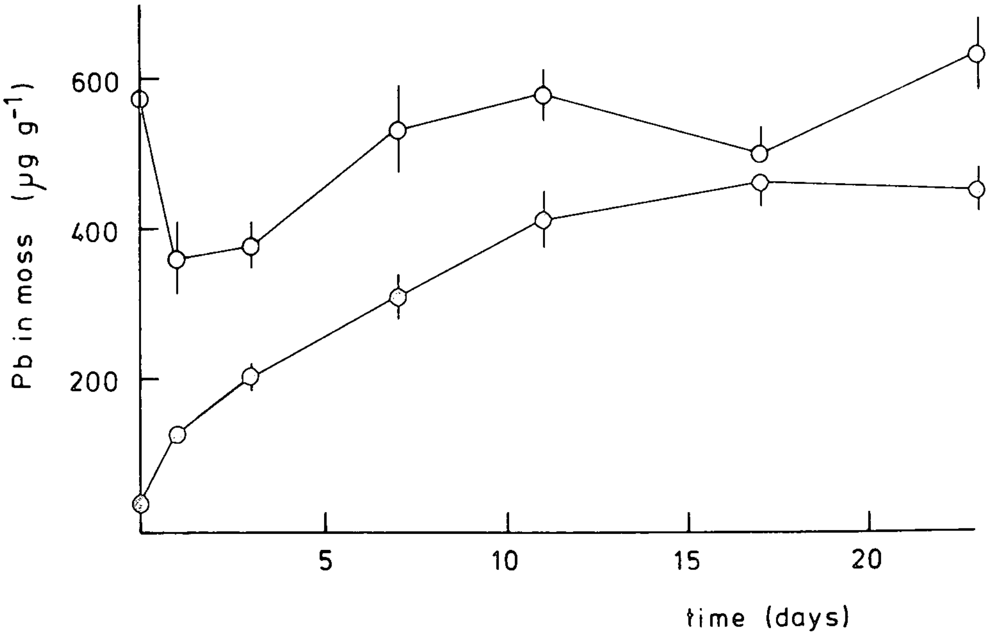
6.31 Introduction

The time course of accumulation of metals into moss samples both over short term (> 48 h, chapter 5) and longer term (> 23 d, 6.2) experiments, indicates that accumulation continues for longer than would be expected if ion-exchange processes alone were involved. The role of ion-exchange processes relative to other processes was investigated by removing

Fig. 6.1 Accumulation of Zn, Cd and Pb by Rhynchostegium transplanted from 0091-05 to 0288-90 in pattern II moss-bags during August 1984. Vertical bars = 95% confidence intervals.


○ = transplanted moss
○ = in situ moss
6000
5000
4000
3000
2000
1000
Zn in moss (µg g^{-1})
5
10
15
20
time (days)


16
14
12
10
8
6
4
2
Cd in moss (µg g^{-1})
2
4
6
8
10
12
14
16
18
20
22
time (days)


600
400
200
Pb in moss (µg g^{-1})
5
10
15
20
time (days)

ions adsorbed onto the cell wall and analyzing these and those remaining in the plant separately.

6.32 Methodology

6.321 Comparison of eluents

The ability of three eluents (2.421, 2.422) to remove K and Zn from <u>Rhynchostegium</u> was tested on three populations: 0310-90, collected and fractionated with no further treatment, 0310-90 that had been shaken in 1 mg l^{-1} Zn for 1 h and 0288-90, collected and fractionated with no further treatment (Table 6.3).

82% - 90% of K in the plant was recovered by eluting with HCl whilst only 9% - 25% was eluted by $NiCl_2$ and EDTA (Table 6.3). There is less agreement between $NiCl_2$ and EDTA for the percentage of Zn recovered, although the proportions recovered by HCl, which damages the membranes, are in excess of each.

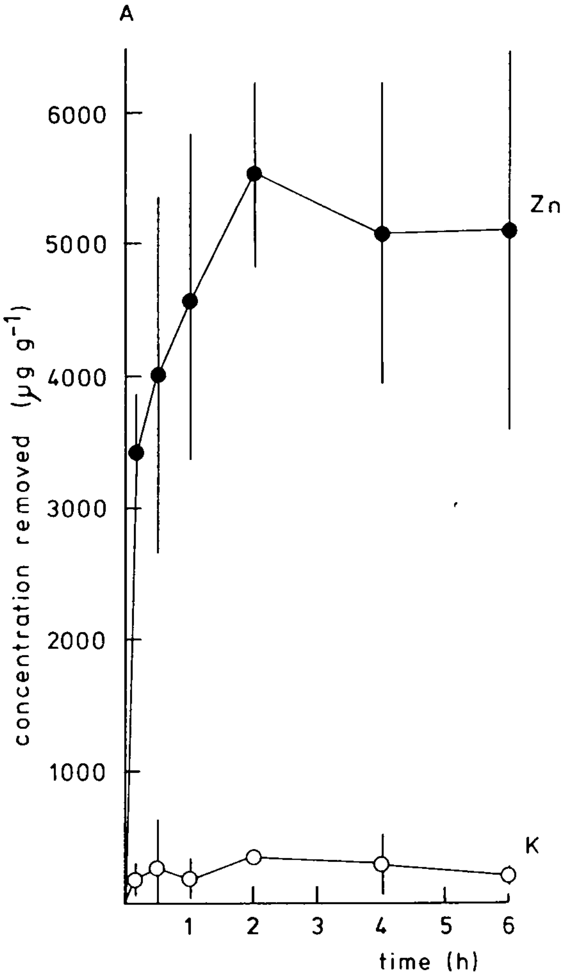
6.322 Effect of increasing exposure times

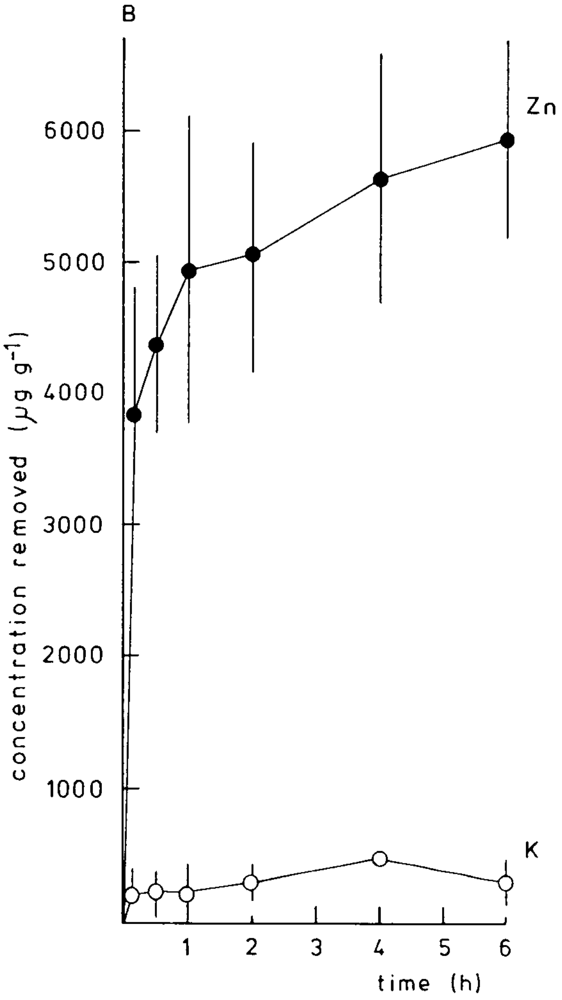
The effect of increasing exposure times to $NiCl_2$ and EDTA was tested on <u>Rhynchostegium</u> from 0310-90 that had previously been exposed to 1 mg l^{-1} Zn (Fig. 6.2). Elution was initially rapid; 67% of the final concentration of Zn removed by $NiCl_2$ and 65% of that removed by EDTA was removed in the first ten minutes. The rate subsequently decreased and after 2 h, 108% and 85% respectively, had been removed, both within the 95% confidence intervals of the final value. There was little variation in the concentration of K removed throughout the experiment.

Table 6.3 Concentrations of K and Zn (as percent of total) in various fractions after treatment with three eluents (A & D = deionized water washes; B & C = washes in eluent; E = residual digest; see 6.321 for further details).

eluent	metal	A	B + C	D	E
$NiCl_2$					
0310-90	K	1.43	11.00	5.74	81.82
	Zn	1.78	45.11	2.44	50.67
0310-90 + Zn	K	8.06	24.95	5.99	61.00
	Zn	0.80	84.32	0.90	13.98
0288-90	K	3.40	9.23	6.22	81.15
	Zn	1.56	53.97	0.55	43.92
EDTA					
0310-90	K	1.86	9.25	1.46	87.43
	Zn	2.60	75.14	3.47	18.79
0310-90 + Zn	K	7.50	23.24	2.36	66.90
	Zn	0.55	65.17	0.90	33.38
0288-90	K	6.27	10.78	1.54	81.41
	Zn	1.06	82.66	0.58	15.69
HCl					
0310-90	K	1.81	92.10	0.77	5.31
	Zn	2.09	82.66	1.12	14.12
0310-90 + Zn	K	6.85	82.51	1.70	8.93
	Zn	0.56	96.23	0.31	2.90
0288-90	K	2.40	93.50	0.70	3.40
	Zn	0.72	94.54	0.31	4.43

Fig. 6.2 Effect of increasing exposure time on elution of K and Zn from *Rhynchostegium* by $NiCl_2$ (top) and EDTA (bottom). Vertical bars = 95% confidence limits.


A
6000
5000
4000
3000
2000
1000
concentration removed (µg g^{-1})
Zn
K
1
2
3
4
5
6
time (h)


B
6000
5000
4000
3000
2000
1000
concentration removed (µg g^{-1})
Zn
K
1
2
3
4
5
6
time (h)

6.323 Removal of Zn from isolated cell walls

In order to remove ambiguity about the effect of $NiCl_2$ and EDTA on whole plants, their ability to remove Zn from isolated cell walls (2.43) was also tested. The cell walls were shaken in 10 mg l^{-1} Zn for 1 h, rinsed several times and washed in eluent for three 1 h periods. $NiCl_2$ removed 95.7% of Zn after one wash (Table 6.4) and 99.4% after three washes. EDTA was less successful, only 88.4% was removed after three washes. Following this experiment and those reported in 6.321 and 6.322, $NiCl_2$ was selected as the most appropriate eluent for subsequent experiments.

Table 6.4 Removal of Zn (as percentage of total) from isolated cell walls by $NiCl_2$ and EDTA (see 6.323 for further details).

	eluent	
elution	EDTA	$NiCl_2$
1	75.7	95.7
2	8.4	3.2
3	4.3	0.6
residual	11.6	0.6

6.33 Accumulation of Zn over 12 h

The cellular location of accumulated Zn was investigated in an experiment performed in the laboratory using $NiCl_2$ as an eluent (6.323). Rhynchostegium from 0310-90 was exposed to 1.0 mg l^{-1} Zn and samples were collected over a 12-h period. Tips were rinsed in deionized water and shaken in $NiCl_2$ for two periods of 1 h before being dried, weighed and digested. Both the $NiCl_2$ fraction and the residual digest were analyzed for Zn.

At the start of the experiment, 29% of the Zn was removed by $NiCl_2$ and 71% was in the residual digest (Fig. 6.3). The Zn that had accumulated in the moss samples was largely removed by $NiCl_2$. This exchangeable fraction accounted for 77.5% (SD = 2.6%) of the total throughout the 12-h study. 2% was removed by the preliminary wash in deionized water and 23% remained in the residual fraction. The total amount of exchangeable Zn increased throughout the experiment and there was also a small increase in the amount of Zn in the residual fraction.

A second method of presenting these data was to use a double-reciprocal plot adapted from the Lineweaver-Burke plot of enzyme kinetics. The equation describing uptake is therefore assumed to be:

$$\frac{1}{[Zn_t]} = \frac{K + t}{[Zn_{max}]\, t} \qquad \text{................1}$$

where: $[Zn_t]$ = Zn accumulated at time t

$[Zn_{max}]$ = maximum possible concentration of Zn

K = time required for half maximum accumulation

t = time.

This converts to a straight line form:

$$\frac{1}{[Zn_t]} = \frac{K}{[Zn_{max}]\, t} + \frac{t}{[Zn_{max}]\, t} \qquad \text{.....2}$$

$$\frac{1}{[Zn_t]} = \frac{K}{[Zn_{max}]}\,\frac{1}{t} + \frac{1}{[Zn_{max}]} \qquad .....3$$

Conversion of the data in this way makes it possible to estimate the time for half maximum accumulation to occur (K) and the maximum possible accumulation (Zn_{max}) as the intercept on the y axis and the slope multiplied by the intercept, respectively. Accumulation by both exchangeable and residual fractions fit these equations well within the time of the experiment (Fig. 6.4) and the following constants were calculated:

i. exchangeable fraction

$[Zn_{max}]$ = 4348 µg g^{-1} K = 1.0 h

ii. residual fraction

$[Zn_{max}]$ = 1052 µg g^{-1} K = 1.6 h

6.34 Accumulation of Zn over 14 d

The cellular location of accumulated Zn was also studied over a longer time period using the same approach as 6.33. Samples were collected over a period of 14 d.

There was little change in the concentration of Zn over between 24-h and 14-d (Fig. 6.5); however the proportion that was removed by $NiCl_2$ decreased and the residual proportion increased.

There was also an exponential decrease in the concentration of Zn in solution (Table 6.5), which was inversely correlated (r = -0.973 ***) with the total Zn accumulated by the tips. The concentration of Zn in control flasks with no tips added was not significantly different at t = 14-d from the concentration at the start (Table 6.5). Data were again tested against

~~the concentration at the start (Table 6.5). Data were again tested against~~ a double-reciprocal plot; however only the residual fraction was significantly correlated (Fig. 6.6) and the following constants were calculated:

Zn_{max} = 2778 ug g^{-1} K = 0.81 d = 19 h

Table 6.5 Concentration of Zn (± SD) remaining in solution during 14 d batch culture experiment (14* = control solution at 14-d).

time (d)	Zn in media (mg l^{-1})
0	1.086 (0.077)
1	0.306 (0.043)
2	0.230 (0.022)
3	0.198 (0.074)
4	0.182 (0.015)
6	0.148 (0.033)
8	0.129 (0.019)
14	0.074 (0.021)
14*	1.024 (0.023)

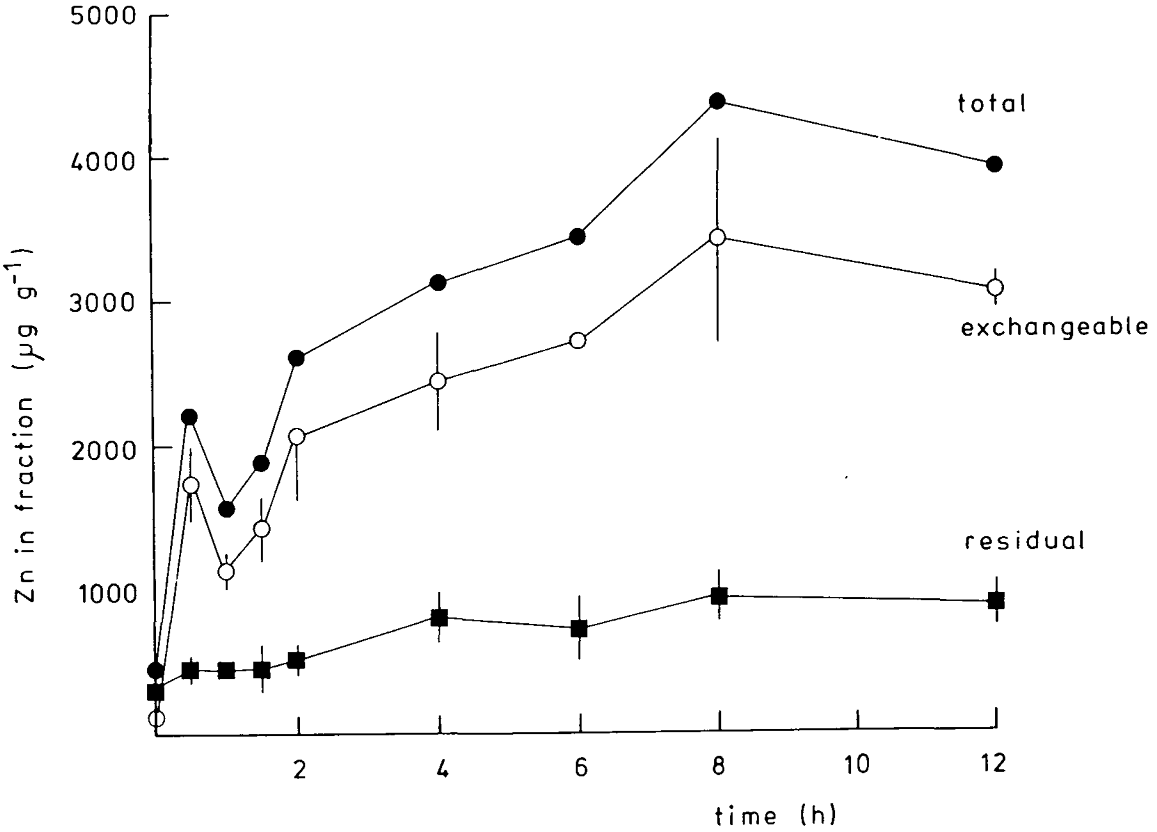
6.4 THE ROLE OF METABOLISM IN ZN ACCUMULATION

6.41 Introduction

The previous section (6.3) demonstrated that there was significant accumulation over periods of several hours into a compartment that was not elutable using a competing cation. The question posed by these results is by what mechanism is this fraction accumulated? Is it a purely passive process, or is it mediated by energy-requiring processes? These questions

Fig. 6.3 Accumulation of Zn in exchangeable and residual fractions of *Rhynchostegium* over 12 h at 15 °C in laboratory batch culture. Vertical bars = 95% confidence intervals.

Fig. 6.4 Double-reciprocal plot of data presented in Fig. 6.3.


5000
4000
3000
2000
1000
Zn in fraction (μg g⁻¹)
2
4
6
8
10
12
time (h)
total
exchangeable
residual

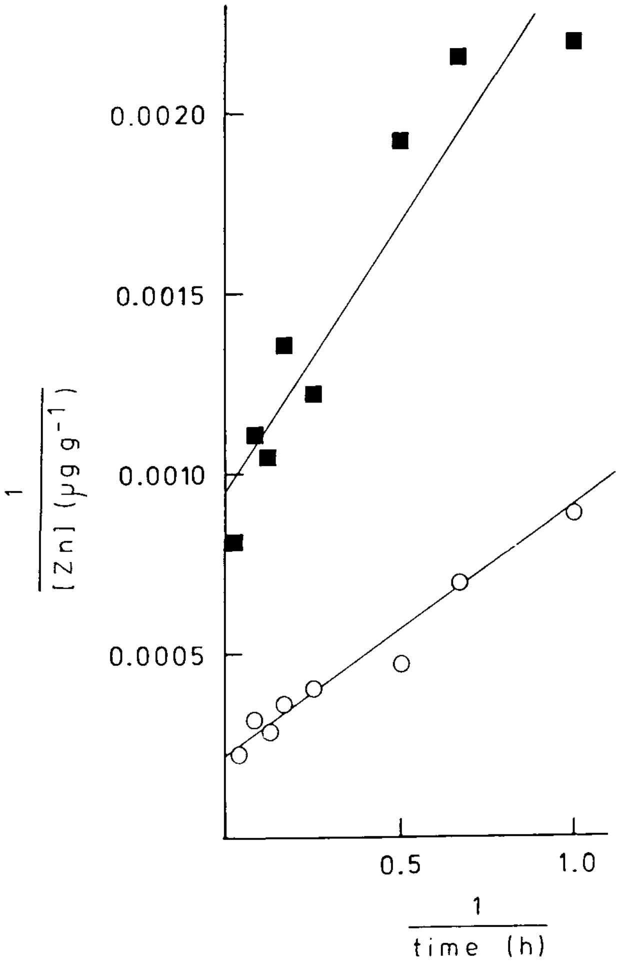
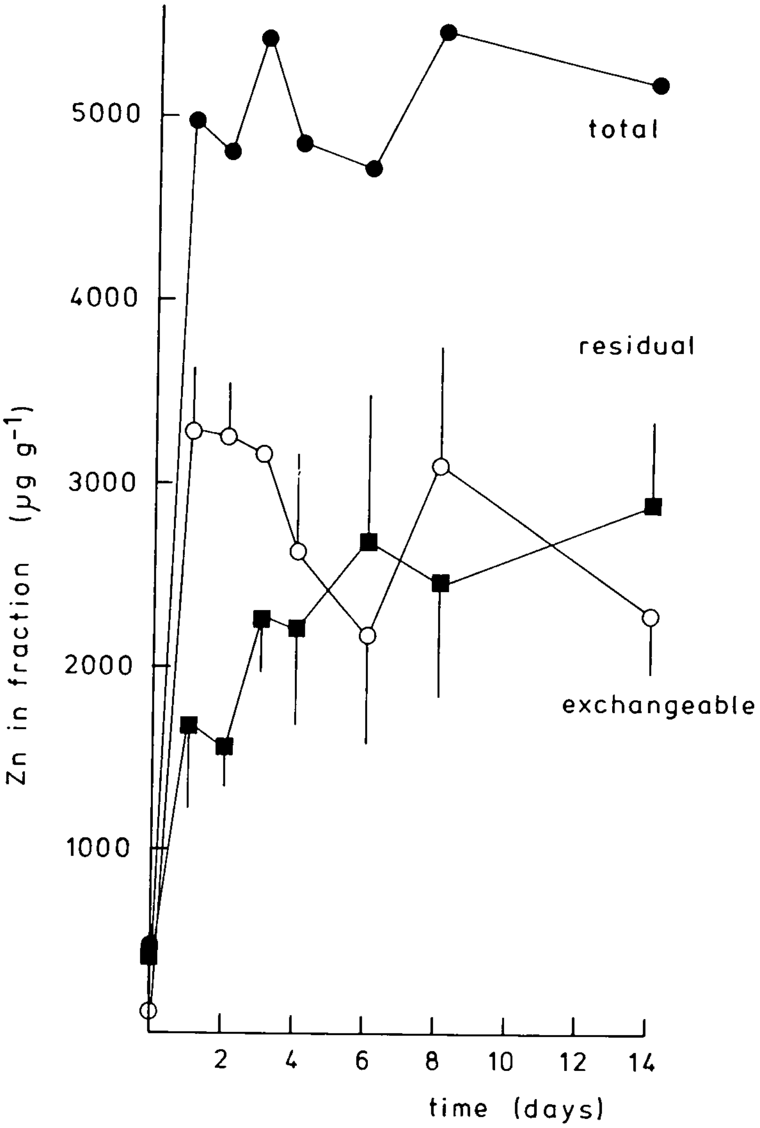
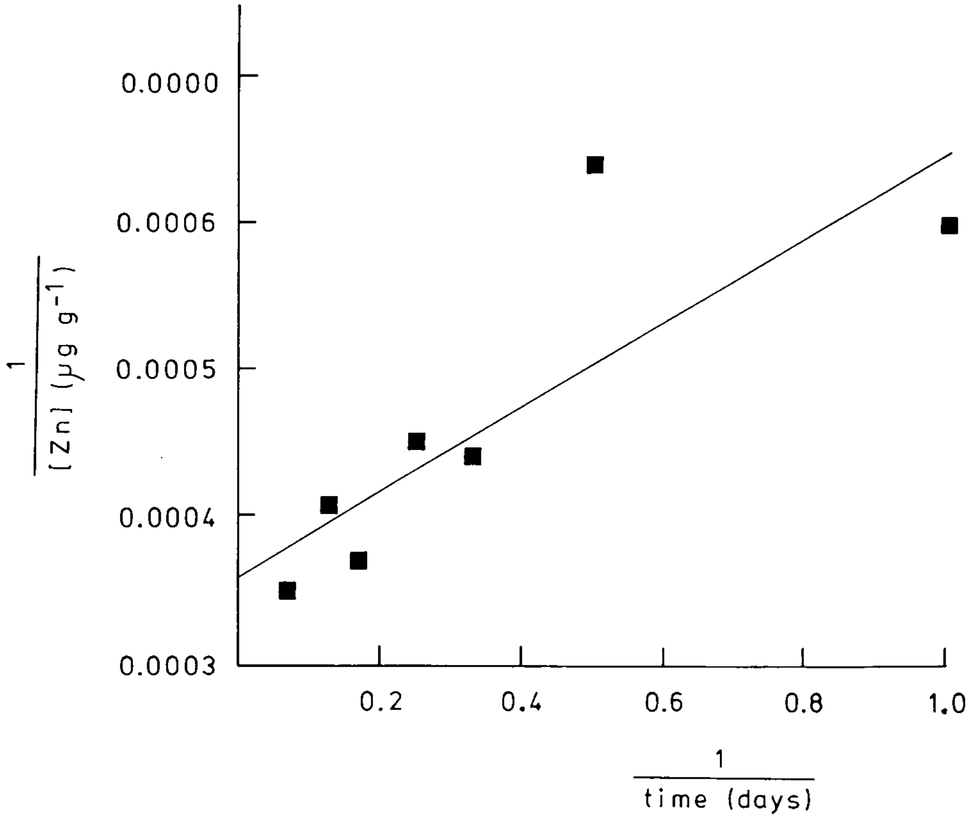

0.0020
0.0015
0.0010
0.0005
1 / [Zn] (μg g⁻¹)
0.5
1.0
1 / time (h)

Fig. 6.5 Accumulation of Zn in exchangeable and residual fractions of Rhynchostegium over 14 d at 15 °C in laboratory batch culture. Vertical bars = 95% confidence limits.

Fig. 6.6 Double reciprocal plot of Zn accumulation in residual fraction over 14 d.


5000
4000
3000
2000
1000
Zn in fraction (µg g^{-1})
total
residual
exchangeable
2
4
6
8
10
12
14
time (days)


0.0000
0.0006
0.0005
0.0004
0.0003
$\frac{1}{[Zn]\ (\mu g\ g^{-1})}$
0.2
0.4
0.6
0.8
1.0
$\frac{1}{\text{time (days)}}$

are considered in this section by comparing metal accumulation by tips in different metabolic states; under different light regimes, at different temperatures and in the presence and absence of various metabolic inhibitors.

6.42 Effect of light

6.421 In laboratory

The influence of light was tested by comparing Zn accumulation by tips exposed to full light in incubation tanks (= 100 μmol photon m^{-2} s^{-1}) with accumulation by tips in flasks darkened with aluminium foil and two layers of black polyethylene. The light flux in the latter treatment was zero.

Tips were preincubated in Chu 10E at pH 7.0 (2.41) for 7 d prior to the experiment to precondition them to the laboratory environment. They were then placed in flasks as described above and harvested (four flasks per treatment) after 1 h, 24 h and 168 h (= 7 d).

Accumulation of Zn followed similar kinetics to previous experiments (Fig. 6.7, see also 5.3, 6.2), with a concentration of approximately 4000 μg g^{-1} after 1 h, 10000 μg g^{-1} after 24 h and 12000 μg g^{-1} after 168 h. There was no significant difference between the light and dark treatments at any of the three sample times (Table 6.6).

Table 6.6 Comparison, using "Student" t-test, of Zn accumulation by Rhynchostegium incubated in laboratory batch culture at 15 °C in light and dark.

	Zn in moss (µg g^{-1})			
time (h)	light	dark	"Student" t	
1	4305	4208	0.445	N.S.
24	10561	10568	0.019	N.S.
168	11599	13026	1.390	N.S.

6.422 In field

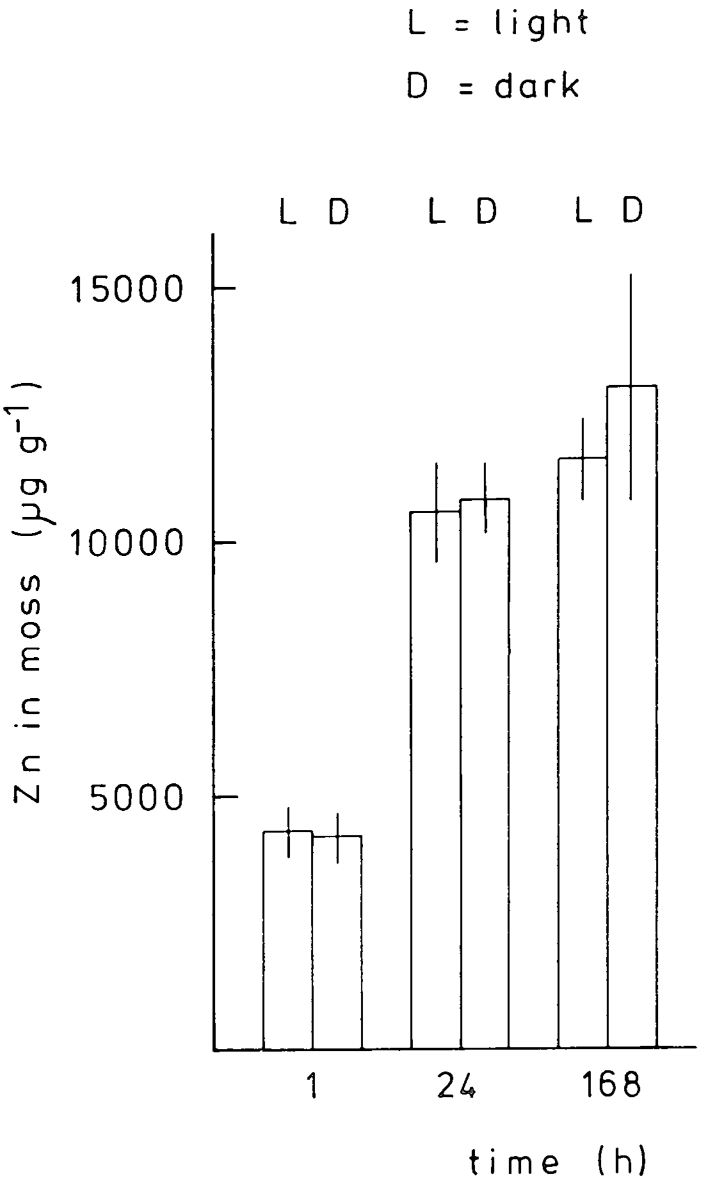
A second experiment to investigate the effect of light was conducted in the field. Moss was transplanted between 0310-90 and 0288-90. At 0288-90 an area of the stream of approximately 1.5 m x 1.5 m was covered with "fertilizer"-type bags (black or white with black interiors) constructed from heavy duty polyethylene. These were supported on a framework of tent poles (Fig. 6.9).

Moss was placed in 0288-90 in moss-bags (5.2, pattern II), either inside the darkened area or outside. Samples of each were collected over an 8-h period. Water samples were also collected and physico-chemical variables measured. Values of some physico-chemical variables and of light-flux are given in Table 6.7. Light flux within the darkened area was consistently 0 µmol photon m^{-2} s^{-1} (one reading of 1 µmol photon m^{-2} s^{-1}). Outside, the flux ranged from 120 µmol photon m^{-2} s^{-1} (overcast) to 1200 µmol photon m^{-2} s^{-1} (sunny), the maximum being recorded at 1240.

During the course of the experiment, moss in the darkened area consistently accumulated more Zn than moss in the light (Fig. 6.8). The differences were significant at all times except t = 1 and t = 4 (Table 6.8).

Fig. 6.7 Accumulation of Zn by Rhynchostegium incubated in laboratory at 15 °C in light and dark. Vertical bars = 95% confidence limits.

Fig. 6.8 Accumulation of Zn by Rhynchostegium transplanted into 0288-90 in light and dark. Vertical bars = 95% confidence limits. (1|4|86)


L = light
D = dark
L D L D L D
15000
10000
5000
Zn in moss (μg g^{-1})
1
24
168
time (h)

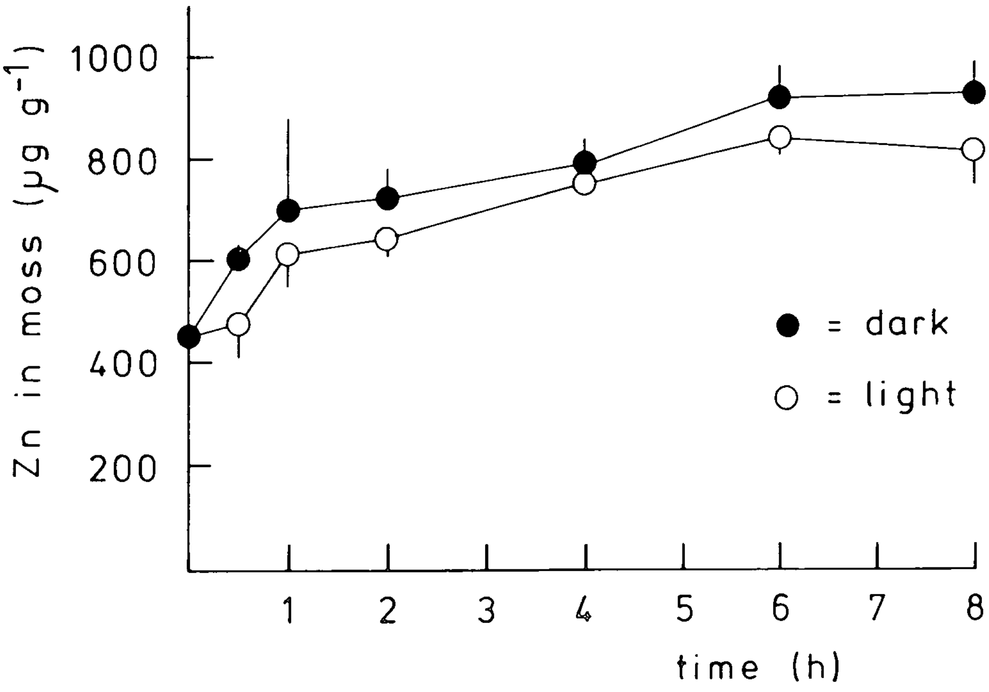

1000
800
600
400
200
Zn in moss (μg g^{-1})
● = dark
○ = light
1
2
3
4
5
6
7
8
time (h)

Fig. 6.9 Apparatus assembled in 0288-90 for field comparison of Zn accumulation in light and dark.

Table 6.7 Selected environmental variables in 0288-90 during field comparison of Zn uptake in light and dark.

time	light flux (µmol photon m^{-2} s^{-1})	temp (^{o}C)	pH	Ca (mg l^{-1})	Zn (mg l^{-1})
1040	125	5.1	7.3	30.7	0.192
1140	166	5.3	7.3	27.5	0.187
1240	1200	6.0	7.4	28.9	0.179
1440	157	6.5	7.4	27.7	0.176
1640	170	6.3	7.4	27.3	0.182
1840	120	6.3	7.4	27.2	0.181

6.423 Effect of dark preincubation on Zn uptake

A second transplant experiment was performed in the field to compare the effects of dark and light pretreatments on subsequent rates of Zn accumulation. Rhynchostegium from 0310-90 was preincubated in the dark and light at 0310-90 for 48 h (Fig. 6.9) and then transferred to 0288-90 for a further 24 h.

There was no significant difference between any of the treatments after the preincubation period in 0310-90 (F = 1.68, N.S.). Transfer to 0288-90 resulted in Zn accumulation by the moss in all four treatments (Table 6.9) although there were several significant differences between treatments (F = 44.21 ***). The sample preincubated in the dark accumulated significantly more when transplanted into the light but there was no significant difference in final Zn concentrations in moss preincubated in the light (Table 6.10).

Table 6.8 Effect of light on Zn accumulation by Rhynchostegium transplanted into 0288-90 (accumulation was greater in the dark on all sampling occasions).

time (h)	"Student" t	
0.5	4.786	**
1	1.377	N.S.
2	3.400	**
4	2.265	N.S.
6	3.497	**
8	4.436	**

Table 6.9 Zn accumulation by Rhynchostegium transplanted into 0288-90 in light and dark.

pre-treatment	post-treatment	mean	95% confidence limits
dark	dark	1123	1154 - 1092
	light	1367	1420 - 1314
light	dark	1417	1500 - 1334
	light	1486	1568 - 1404

Table 6.10 "Student" t-tests between treatments in light/dark transplant experiment in 0288-90; See table 6.9.

dark - dark	1.00			
dark - light	11.05***	1.00		
light - dark	9.16***	1.38	1.00	
light - light	11.54***	3.37**	1.64	1.00
	dark - dark	dark - light	light - dark	light - light

6.43 Effect of temperature

The effect of temperature was investigated by comparing accumulation of Zn in the laboratory at two temperatures; 10 °C and 25 °C. Otherwise parameters were as before (2.41) and tips were again equilibrated at 15 °C for seven days prior to the experiment.

There was significantly greater accumulation at 25 °C after 1 h, 24 h and 168 h (Table 6.11). The difference was 35% after 1 h, 24% after 24 h and 25% after 168 h. These were adjusted to give the predicted Q_{10} values (the effect of a 10 °C rise in temperature, assuming the effect of temperature to be linear). These adjusted values were 23% after 1 h, 16% after 24 h and 16.5% after 168 h, giving Q_{10} values of 1.23, 1.16 and 1.16 respectively.

6.44 Effect of metabolic inhibitors

6.441 Methodology

A preliminary laboratory experiment compared the effect of a range of metabolic inhibitors on K efflux from tips of <u>Rhynchostegium</u> (2.422). Details of the six inhibitors are given in Table 6.12.

Table 6.11 Effect of temperature on Zn accumulation by *Rhynchostegium* in laboratory batch culture; comparison of sample means.

	Zn in moss (µg g^{-1})			
time	10 °C	25 °C	"Student" t	
1 h	3578	4838	7.056	***
24 h	7553	9404	7.970	***
168 h	8915	11119	7.740	***

Three fractions were collected: after 1 h rinses in each of deionized water, the metabolic inhibitor and deionized water again. Each fraction was subsequently analyzed for K and the tips were dried and weighed.

The concentrations of K released by the preliminary wash in deionized water ranged from 43.4 µg g^{-1} to 100.4 µg g^{-1} (Table 6.13). All of the inhibitors released some K, ranging from 159.1 µg g^{-1} (HH) to 8696 µg g^{-1} (DCMU) and significant K was also detected in the second rinse in deionized water. The ranking of the inhibitors was: control < HH < NaN_3 < CCCP = CH < DNP < DCMU.

6.442 Laboratory experiment

Two metabolic inhibitors were used in this experiment: 2,4-DNP and DCMU. Both of these caused significant K leakage in the previous experiment (6.441) which suggested that membrane transport systems were strongly affected by the inhibitor (2.422). The inhibitors were chosen for this and because there is a relatively large literature on the biochemical effects of each. Samples were preincubated in the inhibitor at the concentrations used in 6.441 for 90 min prior to the experiment, rinsed and transferred straight to the experimental media.

Table 6.12 Metabolic inhibitors tested.

name & abbreviation	molecular weight	working conc.	pH	reference
2,4-dinitrophenol (DNP)	184.1	10^{-4} M	4.1	Dawson *et al*. (1969) Nieboer *et al*.(1976)
hydroxylamine hydrochloride (HH)	69.5	10^{-4} M	2.0	Heber *et al*. (1985)
carbonyl cyanide-m-chlorophenylhydrazone (CCCP)	204.6	10^{-4} M	5.6	Heber *et al*. (1985)
cycloheximide (CH)	281.4	5×10^{-5} M	7.1	Badour (1978)
sodium azide (NaN_3)	65.0	10^{-4} M	7.2	Dawson *et al*. (1969) Nieboer *et al*. (1976)
3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU)	233.1	10^{-4} M	6.3	Heber *et al*. (1985)

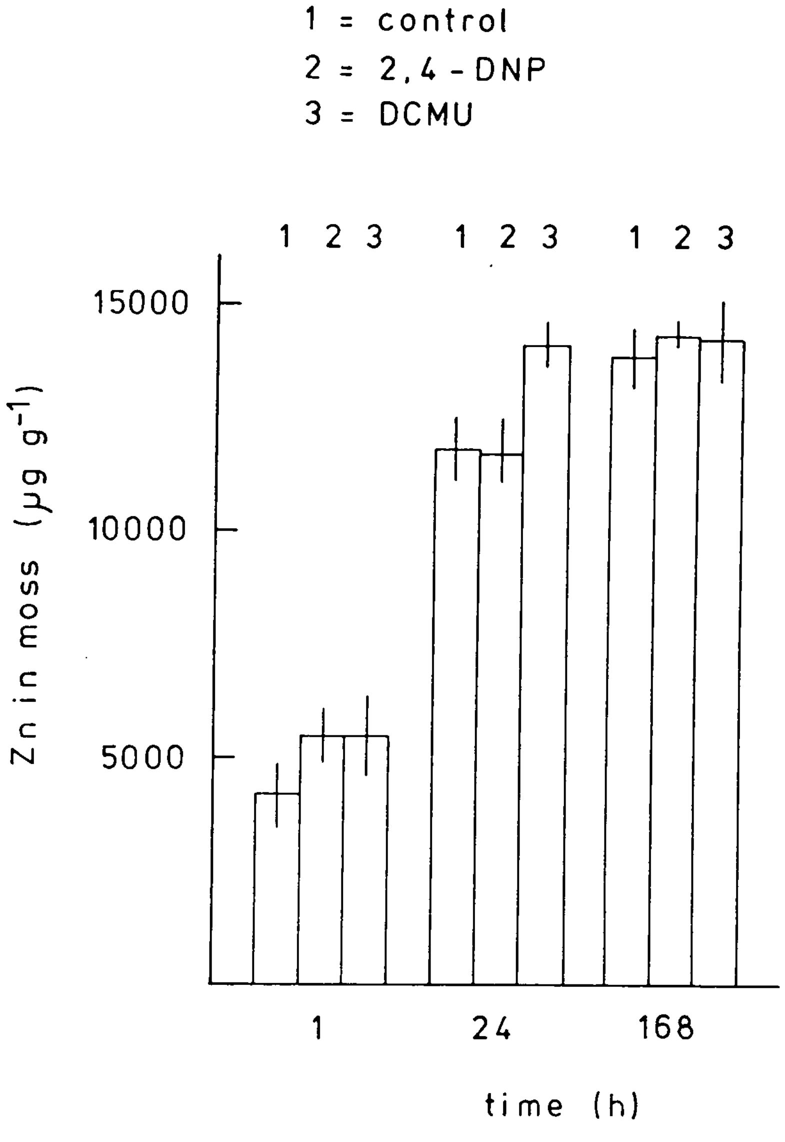
Table 6.13 Concentrations of K released by treatment of Rhynchostegium for 1 h at 15 °C with different metabolic inhibitors.

	K in fraction (µg g^{-1})		
treatment	deionized water	metabolic inhibitor	deionized water
control	54.1	45.8	57.6
DNP	96.9	2148	829.5
HH	69.2	159.1	82.8
CCCP	58.6	680.8	514.0
CH	43.4	743.0	389.0
NaN_3	63.1	369.3	131.2
DCMU	100.4	8696	1784.3

The effect of the inhibitors on the growth of the moss was apparent after 168 h. At this time "control" tips showed signs of fresh growth (bright green shoots appearing from the original tips); DNP-treated moss looked little different to the start and the DCMU-treated moss was a brown colour.

There were several significant differences in Zn accumulation between treatments (Fig. 6.10). These were at t = 1 h between control and DNP and between control and DCMU and at t = 24 h between control and DCMU and between DNP and DCMU. There were no significant differences at t = 168 h (Table 6.14).

Fig. 6.10 Accumulation of Zn by Rhynchostegium at 15 °C in laboratory batch culture after pretreatment with metabolic inhibitors. Vertical bars = 95% confidence limits.


1 = control
2 = 2,4 - DNP
3 = DCMU
1 2 3 1 2 3 1 2 3
15000
10000
5000
Zn in moss (μg g-1)
1
24
168
time (h)

6.5 LOSS OF HEAVY METALS

6.51 Introduction

The successful monitoring of intermittent heavy metal pollution relies upon the ability of Rhynchostegium to both accumulate heavy metals and to retain elevated concentrations of these after the "pulse" of pollution has passed downstream. Experiments were carried out to investigate the factors which affect the rate at which heavy metals are lost from Rhynchostegium.

Preliminary experiments are described which characterize these processes: an experimental transplant from a Zn-enriched stream into a low-Zn stream (6.52), a series of samples of Rhynchostegium collected from a

Table 6.14 Effect of 1 h pretreatment with metabolic inhibitors on Zn accumulation by Rhynchostegium at 15 °C in laboratory batch culture; comparison of sample means (C = control).

time (h)	treatments	"Student" t	
1 h	C v DNP	4.732	**
	C v DCMU	3.770	**
	DNP v DCMU	0.000	N.S.
24 h	C v DNP	0.218	N.S.
	C v DCMU	8.688	***
	DNP v DCMU	8.455	***
168 h	C v DNP	2.354	N.S.
	C v DCMU	1.129	N.S.
	DNP v DCMU	0.307	N.S.

stream in which the concentration of Zn in the water was slowly decreasing (6.53) and the effect of increasing lengths of exposure to Zn on the subsequent rates of loss (6.54). In addition a laboratory experiment was performed to investigate the effect of various washing treatments on metal loss (6.55).

6.52 Rate of loss of Zn

An experiment was designed in order to investigate the loss of Zn from moss following a transplant between 0288-90, a stream with elevated concentrations of Zn, and 0091-05.

Rhynchostegium was transplanted both on boulders and in moss-bags (5.2, pattern II) and samples were collected from these and from in situ moss.

Loss of Zn from moss was evident over a longer period than uptake (Fig. 6.11), the majority occurring within the first four days of the experiment. The rate of loss subsequently decreased and after 23 d the concentration in the transplanted moss remained above that in the in situ Rhynchostegium.

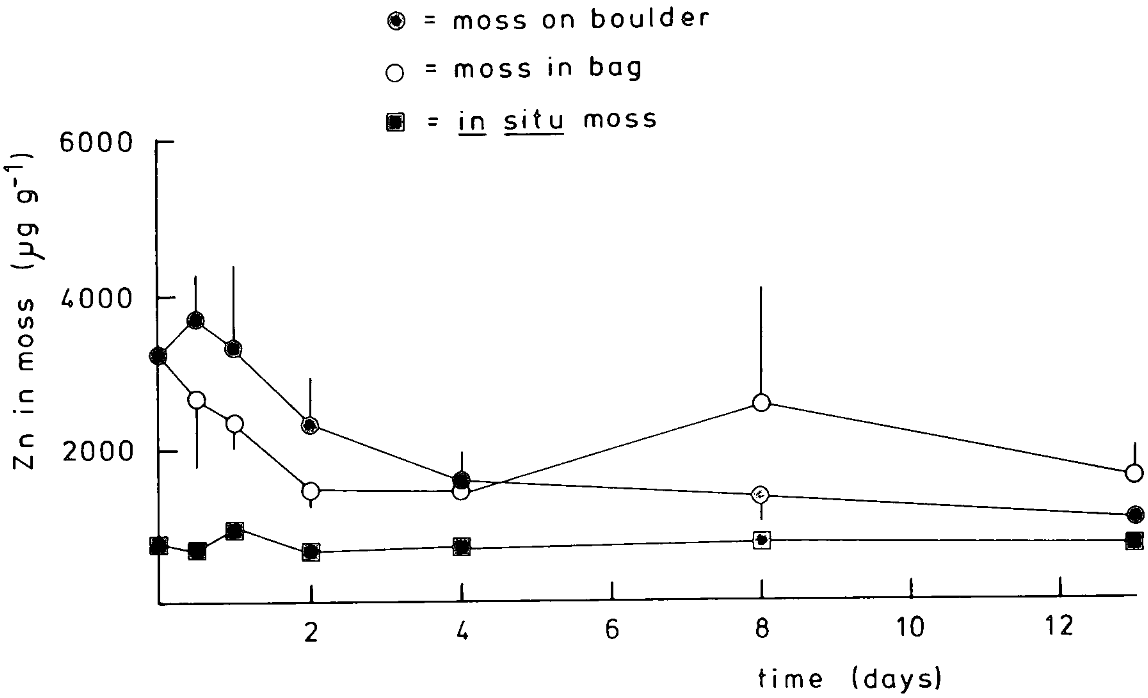
6.53 Loss of Zn under field conditions

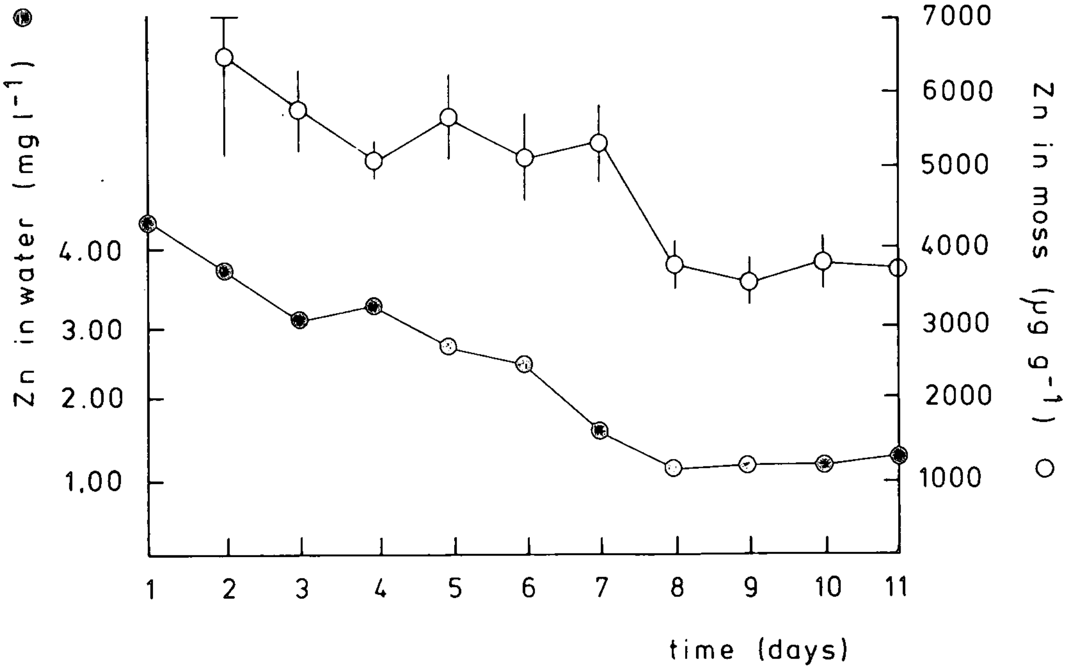
An experiment was designed in order to investigate the effect of a changing Zn concentration in water on the concentration of Zn in in situ Rhynchostegium. Samples of water and of Rhynchostegium were collected over a period of 10 d from 0024-20.

The concentration of Zn in the water declined from 3.70 mg l^{-1} to 1.30 mg l^{-1} during the course of the experiment. This was accompanied by loss of Zn from Rhynchostegium (Fig. 6.12). There was a significant correlation (r = 0.89 **) when the concentrations in the moss and in the water were compared; however a time-series analysis, using cross-correlations showed

Fig. 6.11 Loss of Zn from Rhynchostegium transplanted from 0288-90 to 0091-05. Vertical bars = 95% confidence limits. (September 1983)

Fig. 6.12 Concentrations of Zn in water and Rhynchostegium in 0024-20, 07-08-84 to 16-08-84. Vertical bars = 95% confidence limits.


● = moss on boulder
○ = moss in bag
■ = in situ moss
Zn in moss (μg g^{-1})
6000
4000
2000
2
4
6
8
10
12
time (days)


Zn in water (mg l^{-1}) ●
4.00
3.00
2.00
1.00
7000
6000
5000
4000
3000
2000
1000
Zn in moss (μg g^{-1}) ○
1
2
3
4
5
6
7
8
9
10
11
time (days)

the strongest correlation to be associated with a lag period of one day (Table 6.15).

Table 6.15 Cross-correlations computed between Zn accumulation by Rhynchostegium and aqueous Zn in 0024-20 over 11 d for three increasing daily lags (maximum positive correlation is underlined).

lag (d)	0	1	2	3
n	10	10	9	8
r	0.890	<u>0.976</u>	0.879	0.754

6.54 Effect of length of exposure to Zn

The effects of increasing length of exposure to Zn was tested by a field transplant in which Rhynchostegium from 0310-90 was transplanted into 0012-28, approximately 30 m away, for different lengths of time before being returned to 0310-90 along with Rhynchostegium from a nearby metal-enriched stream, 0309-80. Samples of moss and water were collected over a period of 48 h.

The total Zn accumulated was dependent upon the length of its exposure, as expected from previous experiments; hence Rhynchostegium with a short period of exposure (1 h) accumulated 1130 µg g^{-1} Zn, after 4 h it contained 1660 µg g^{-1} and after 24 h it contained 2373 µg g^{-1} Zn.

The rate of loss was dependent upon the length of the original exposure, Zn was lost very rapidly (i.e. within 2 h) from moss which had been exposed for short periods (Fig. 6.13). That which had had 24 h exposure retained Zn for longer periods; the concentration was still significantly above background after 12 h in 0310-90. The population from 0309-80 lost Zn over the entire experimental period.

The effectiveness of Rhynchostegium as a monitor depends upon being able to determine enriched concentrations a number of hours after exposure. The length of this period was examined by performing "Student" t-tests between the concentration at t = 48 h, assumed to be the background, and the concentration at times previous to this. If the criterion of a significant difference represents a quantifiable effect then a 1 h exposure to Zn is detectable only for about 1 h after being returned to a low-Zn environment (Table 6.16). Exposures of 4 h and 24 h were detectable for longer periods of time, between 12 h and 24 h.

Table 6.16 Significance of differences (calculated by "Student" t-test) between concentration of Zn in Rhynchostegium at t = 48 h and at times previous to this.

time	-1	-4	-24	0309
0.0	0.0000	0.0000	0.0000	0.0000
0.5	0.0027	0.0000	0.0000	0.0000
1.0	0.0129	0.0000	0.0000	0.0000
2.0	0.6454	0.0000	0.0000	0.0000
4.0	0.0044	0.0001	0.0000	0.0000
6.0	0.8191	0.0001	0.0000	0.0000
8.0	0.3211	0.0000	0.0112	0.0000
12.0	0.9024	0.0000	0.0085	0.0003
24.0	0.6360	0.0000	0.2779	0.0005
48.0	1.0000	1.0000	1.0000	1.0000

6.55 Effect of external medium

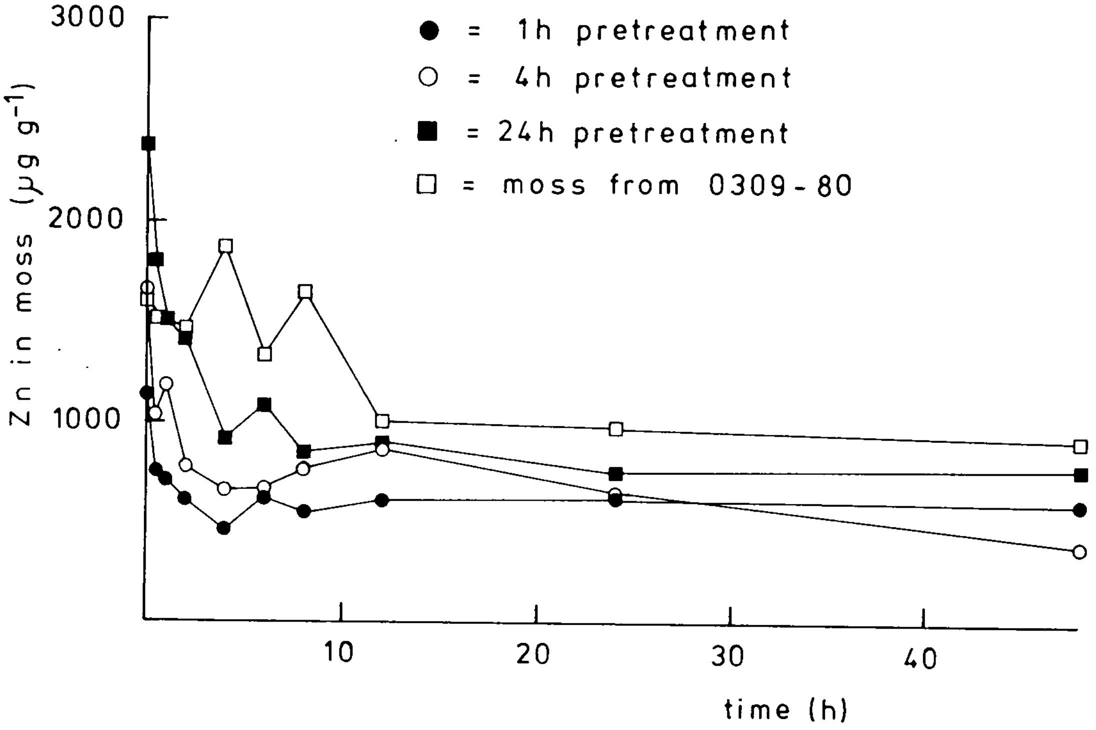
The effect of the external medium on the loss of Zn from Rhynchostegium was tested in a laboratory batch culture experiment. The following media were used: Chu 10E + 3.15 mg l^{-1} EDTA; Chu 10E - EDTA; 3.15 mg l^{-1} EDTA; 20 mg l^{-1} Ca; 5 mg l^{-1} PO_4-P and deionized water. All were buffered at pH 7.0 using HEPES. Two populations of Rhynchostegium were used; a population from 0288-90, a metal enriched stream and a population from 0310-90, an uncontaminated stream, which had been exposed to 1 mg l^{-1} Zn for 1 h prior to the experiment. Shoots were incubated in the experimental media for 24 h.

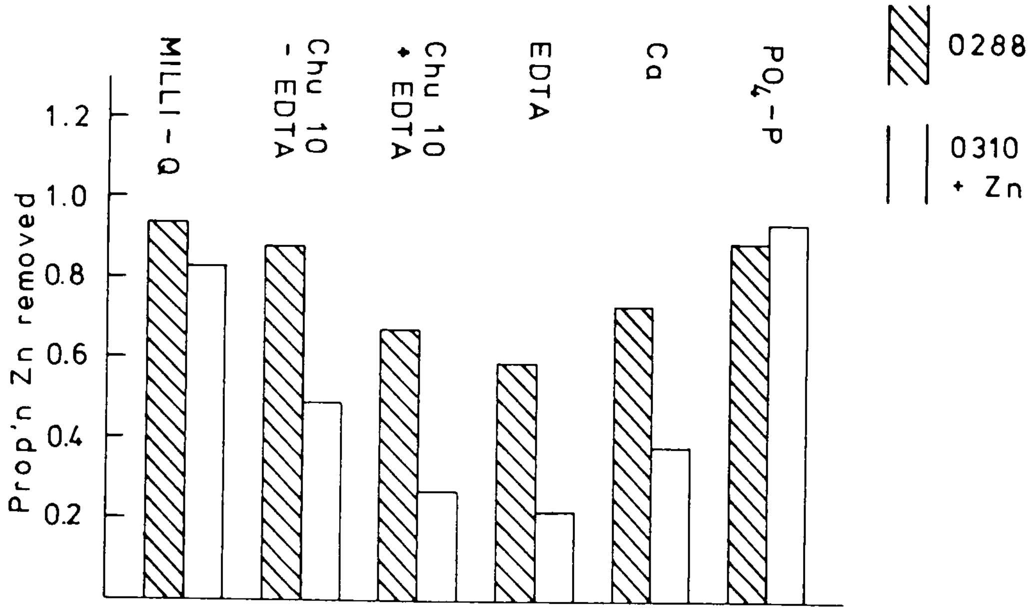
Zn was more easily removed from the population from 0310-90 that had been exposed briefly to Zn, than from the population from 0288-90 (Fig. 6.14). The general order of loss from the two populations was similar; for the population from 0310-90, the treatment with PO_4-P released the least Zn, followed by deionized water, Chu 10E -EDTA, Ca, Chu 10E + EDTA and EDTA. The order of loss for Rhynchostegium from 0288-90 was the same, except that deionized water removed slightly less Zn than PO_4-P.

The greatest difference between the two populations was observed in the Chu 10E + EDTA (difference = 0.40), followed by Chu 10E - EDTA (0.39), Ca (0.35), EDTA (0.31), deionized water (0.11) and PO_4-P (-0.05).

Fig. 6.13 Loss of Zn from *Rhynchostegium* in 0310-90 after different lengths of exposure to Zn in 0012-28 and 0309-80. (25|5|86 - 26|5|86)

Fig. 6.14 Loss of Zn from two populations of *Rhynchostegium* incubated at 15 °C in a range of media. See text for further details.


● = 1h pretreatment
○ = 4h pretreatment
■ = 24h pretreatment
□ = moss from 0309-80
Zn in moss (μg g^{-1})
3000
2000
1000
10
20
30
40
time (h)


MILLI-Q
Chu 10 - EDTA
Chu 10 + EDTA
EDTA
Ca
PO_4-P
▨ 0288
□ 0310 + Zn
Prop'n Zn removed
1.2
1.0
0.8
0.6
0.4
0.2

7. ZINC ACCUMULATION BY ISOLATED CELL WALLS

7.1 INTRODUCTION

This chapter reports a series of laboratory experiments carried out in an attempt to examine the possible contribution of the cell wall in Zn accumulation by Rhynchostegium. Previous chapters (5 and 6), noted rapid uptake into a phase which was readily exchangeable; this was interpreted as representing ion-exchange onto the cell wall (6.3). As this phase was clearly large and played an important role in the early stages of uptake, a more detailed investigation into its properties was carried out.

Preliminary experiments are concerned with the structure and gross morphology of the cell wall (7.2). These are followed by a description of the ionic composition of the cell wall in different media and natural waters and the kinetics of exchange of Zn in the presence of other ions (7.3, 7.4). Finally, the role of Ca in mediating Zn accumulation is investigated (7.5).

7.2 PRELIMINARY STUDIES

7.21 Ultrastructure of cell walls

Material from 0310-90 was prepared for electron microscopy to examine the fine structure of the cell wall of Rhynchostegium.

The cell has a very thick cell wall (Fig. 7.1), accounting for one third of its total diameter. Two distinct areas of the cell wall are visible: a lighter coloured region comprising the primary and secondary cell walls, whilst darker, wedge-shaped areas (labelled "U" on Fig. 7.1) appear to be extensions of the middle lamella. These areas may be composed of pectic materials. No pattern that corresponded to these areas was visible in surface views of the cell under a light microscope when a stain specific for pectins, ruthenium red, was used to stain unfixed cells.

7.22 Relative mass of cell walls

In order to complement the above visual examination, the mass of the cell wall relative to the mass of the whole tip was estimated at the start of the study, using material from 0310-90.

Duplicate samples of fresh tips (from 5 to 50 tips per sample) were blotted and dried at 50 °C for 1 h and weighed. One set of tips was dried at 105 °C for 24 h and reweighed and the second set extracted (2.43), dried at 50 °C for 1 h and weighed, then dried at 105 °C and weighed. The first 1 h drying period removed water from surfaces and the subsequent weight is referred to as "fresh weight".

There were significant linear correlations between fresh weights and dry weights for all combinations of whole tips and cell walls. The dry weights of both whole tips and cell walls were 0.93 of their fresh weight (SD = 0.04 and 0.03 respectively). The fresh weight of the cell walls was 0.77 (SD = 0.12) of the fresh weight of tips and their dry weight was 0.72 (SD = 0.12) of the fresh weight of tips.

From these data it was possible to estimate the relative mass of the cell wall:

(f.wt = fresh weight and d.wt = dry weight; subscripts t and w refer to whole 2-cm tips and cell wall preparations respectively.)

if $\quad d.wt_w = 0.93\ f.wt_w$

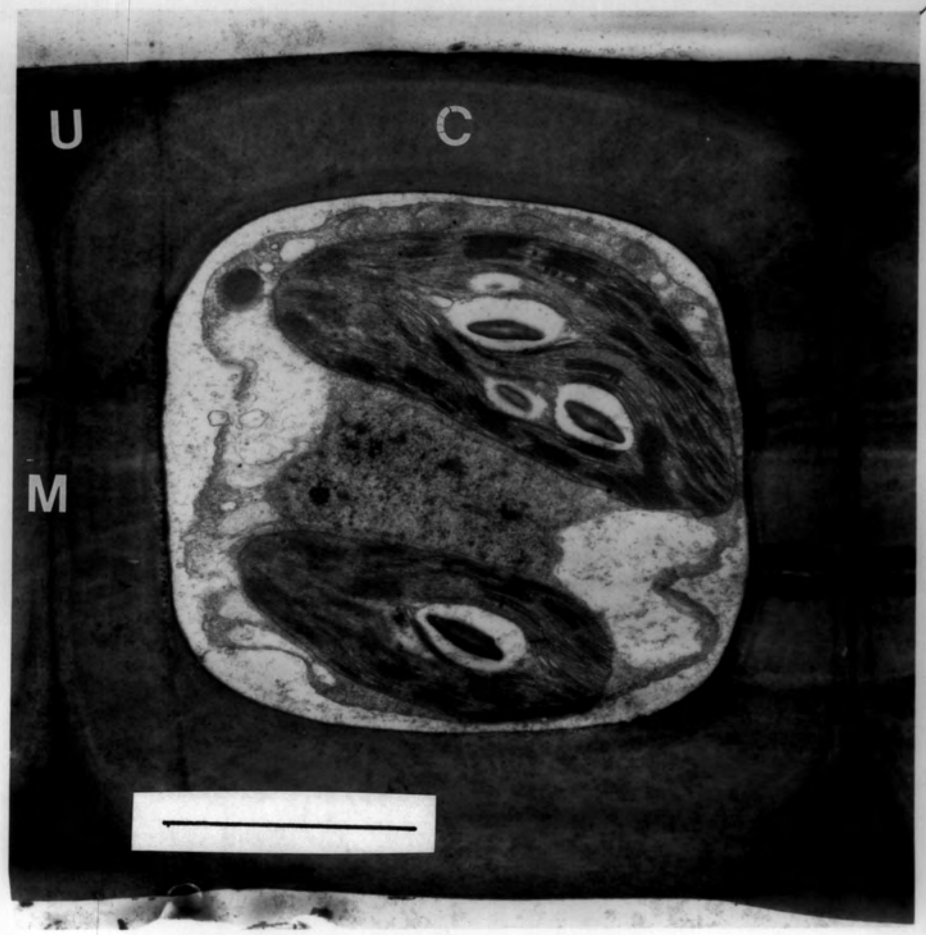
and $\quad f.wt_w = 0.77\ f.wt_t$

then $\quad d.wt_w = 0.93 \times 0.77\ f.wt_t$

$\quad d.wt_w = 0.72\ f.wt_t$

Fig. 7.1 Transverse section of a leaf cell of Rhynchostegium, x10000.

C = cell wall; M = middle lamella; U = unknown (see 7.21). Scale bar = 1µm


U
C
M

$$\begin{aligned}\text{if}\quad & d.wt_t = 0.93\ f.wt_t\\ \text{then}\quad & f.wt_t = (d.wt_w\ /\ 0.72) = (d.wt_t\ /\ 0.93)\\ & d.wt_w = (0.72\ /\ 0.93)\ d.wt_t\\ & d.wt_w = 0.77\ d.wt_t\end{aligned}$$

In the sample of moss collected from 0310-90 the cell wall constituted 77% of the dry weight.

7.23 Ionic composition of cell walls: laboratory experiment

The next experiment examined the concentrations of cations in cell walls and compared the protonated state with walls at equilibrium with medium. In order to obtain cell walls at equilibrium with media, samples of 25 tips were shaken in 50 ml of medium for 1 h. A Chu 10E medium was used with the following modifications; with and without 3.15 mg l^{-1} EDTA and with and without 20 mg l^{-1} humic acid (HA).

There were increases in the concentrations of several of the ions (Table 7.1); Ca was consistently the most abundant ion, accounting for 0.55 (mean for all treatments; SD = 0.02) of the total ions in the wall at the end of the experiment. Other major contributors were Na ($\bar{x} = 0.12$, SD = 0.01) and Mg ($\bar{x} = 0.10$, SD = 0.005). Fe accounted for 0.21 (SD = 0.01) of the total; however there were higher concentrations of this in control samples (0.82 in 0288 and 0.81 in 0310) indicating that Fe was actually lost during the incubation.

There were significant differences between the concentrations in the walls of the two populations for several ions (Table 7.2); the concentration of Zn in the two populations was significantly different for all four treatments and K and Mn showed significant differences between populations for three of the four treatments.

Table 7.1 Concentrations of metals (in μg g^{-1}) in cell walls of <u>Rhynchostegium</u> before and after equilibration in various media, see 7.23.

sample	treatment	Na	K	Mg	Ca	Mn	Fe	Zn	total
0288-90	control	368	192	113	155	26	4138	38	5030
		(144)	(21)	(6)	(56)	(4)	(132)	(1)	
0310-90	control	424	166	90	139	12	3857	98	4786
		(148)	(17)	(2)	(27)	(12)	(155)	(12)	
0288-90	+EDTA-HA	2016	241	1511	8886	15	3446	104	16115
		(251)	(14)	(84)	(616)	(2)	(473)	(20)	
0310-90	+EDTA-HA	1978	398	1604	9201	32	3409	46	16668
		(643)	(112)	(123)	(662)	(5)	(401)	(4)	
0288-90	-EDTA-HA	1886	256	1515	9052	16	3160	84	15969
		(423)	(51)	(64)	(506)	(2)	(667)	(22)	
0310-90	-EDTA-HA	2271	299	1531	8607	33	3324	47	16112
		(463)	(31)	(87)	(487)	(10)	(397)	(6)	
0288-90	+EDTA+HA	2318	260	1447	8302	15	3665	450	16457
		(334)	(31)	(27)	(199)	(2)	(450)	(11)	
0310-90	+EDTA+HA	1830	348	1548	9060	29	3404	57	16276
		(247)	(55)	(63)	(1034)	(12)	(557)	(8)	
0288-90	-EDTA+HA	1827	172	1424	8223	13	2973	102	14734
		(136)	(24)	(41)	(261)	(2)	(737)	(17)	
0310-90	-EDTA+HA	1847	290	1611	9059	26	3524	46	16403
		(201)	(32)	(64)	(336)	(17)	(295)	(6)	

Table 7.2 Comparison, using "Student" t-tests, of metal concentrations in cell walls of <u>Rhynchostegium</u> from 0288-90 and 0310-90 before and after equilibration in various media.

treatment	Na	K	Mg	Ca	Mn	Fe	Zn
controls	0.537	1.879	7.381	0.502	2.170	2.769	10.364
			***			*	***
+EDTA-HA	0.110	2.781	1.239	0.698	5.947	0.120	5.560
		*			***		***
-EDTA-HA	1.228	1.433	0.303	1.270	3.250	0.421	3.237
					*		*
+EDTA+HA	2.349	2.781	2.939	1.439	2.490	0.729	5.318
			*		*		**
+EDTA-HA	0.152	5.965	4.932	3.926	1.578	1.387	6.233
		***	**	**			***

ANOVA was used to compare treatments (Table 7.3). There were significant differences within the population from 0288-90 for K and Ca but no significant differences between treatments for populations from 0310-90. The various treatments were also compared using "Student" t-tests (Table 7.3); six out of 84 treatments were significant; all of these occurred within the population from 0288-90. Control samples from the two reaches had significant differences between Mg, Fe and Zn (Table 7.2).

7.24 Nature of Mn and Fe in cell wall

A brief experiment was conducted to investigate the nature of Mn and Fe in cell walls in 7.23. Tips were prepared as usual (2.43), omitting the initial rinse in hydroxylamine hydrochloride and were protonated (stage 1). They were then treated in hydroxylamine hydrochloride (1 h; stage 2) and in 0.2 M ammonium oxalate/ 0.2 M oxalic acid (4 h; stage 3), in the dark at room temperature. Samples were taken after each stage and analyzed for Mn, Fe and Zn (Fig. 7.2).

At the start there was significantly more Mn in the cell walls from 0288-90 compared with 0310-90 ("Student" t = 7.15 ***). Nearly all was removed after the wash in hydroxylamine. The population from 0310-90 contained significantly more Fe at the start ("Student" t = 14.44 ***). Fe was removed at each stage; however there was still significantly more Fe in 0310-90 after treatment with both hydroxylamine ("Student" t = 6.32 **) and oxalate ("Student" t = 11.54 ***). The concentration of Zn was significantly different between populations only after the oxalate treatment ("Student" t = 3.57 *); however some Zn was removed by each.

Table 7.3 Effect of EDTA and humic acids on adsorption of metals by <u>Rhynchostegium</u> cell walls; comparisons using ANOVA and "Student" t-tests.

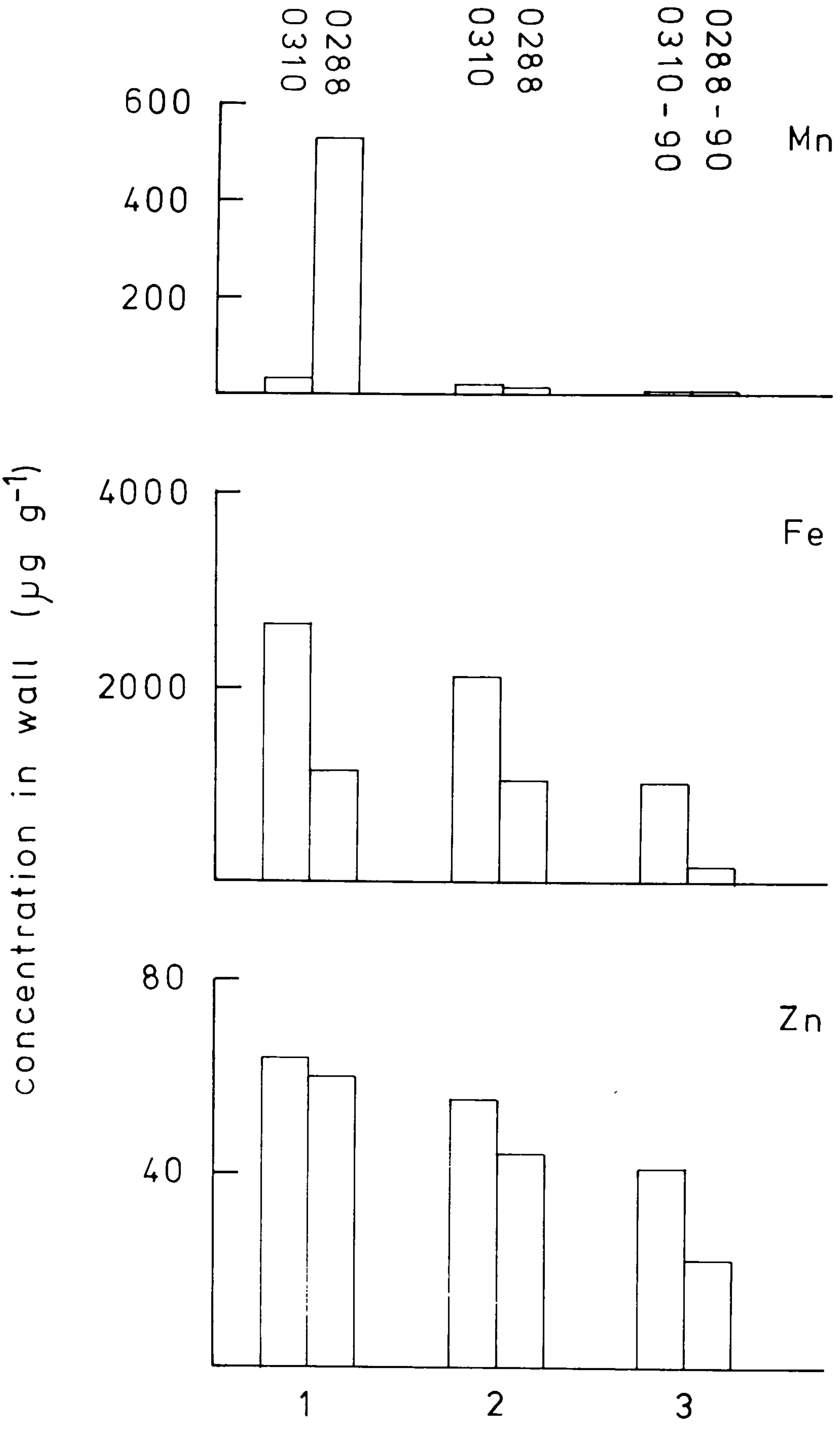
site	comparison	Na	K	Mg	Ca	Mn	Fe	Zn
0288-90	ANOVA	2.051	6.223 **	2.483	3.705 *	1.832	1.056	1.063
	"Student" t-tests							
	+EDTA-HA v -EDTA-HA	0.527	0.563	0.059	0.418	0.579	0.699	1.383
	+EDTA-HA v +EDTA+HA	1.445	1.125	1.469	1.803	0.151	0.668	0.887
	+EDTA-HA v -EDTA+HA	1.312	5.038 **	1.877	1.982	1.501	1.080	0.192
	-EDTA-HA v +EDTA+HA	1.602	0.142	1.953	2.760 *	0.876	1.253	0.845
	-EDTA-HA v -EDTA+HA	0.260	2.982 *	2.390	2.915 *	2.409	0.377	1.316
	+EDTA+HA v -EDTA+HA	2.713 *	4.515 **	0.931	0.484	1.644	1.602	0.771
0310-90	ANOVA	0.914	2.266	0.833	0.578	0.274	0.151	2.576
	"Student" t-tests							
	+EDTA-HA v -EDTA-HA	0.740	1.704	0.967	1.448	0.175	0.301	0.244
	+EDTA-HA v +EDTA+HA	0.429	0.794	0.811	0.230	0.385	0.016	2.168
	+EDTA-HA v -EDTA+HA	0.388	1.856	0.107	0.383	0.672	0.460	0.047
	-EDTA-HA v +EDTA+HA	1.683	1.558	0.311	0.794	0.445	0.232	1.845
	-EDTA-HA v -EDTA+HA	1.681	0.398	1.485	1.530	0.701	0.806	0.252
	+EDTA+HA v -EDTA+HA	0.109	1.833	1.408	0.016	0.335	0.381	2.015

Fig. 7.2 Removal of Mn, Fe and Zn from cell walls of Rhynchostegium.

1 = control;

2 = after treatment with hydroxylamine hydrochloride;

3 = after treatment with ammonium oxalate / oxalic acid.


0310
0288
0310
0288
0310-90
0288-90
Mn
600
400
200
Fe
4000
2000
Zn
80
40
concentration in wall (μg g^{-1})
1
2
3

7.25 Ionic composition of cell walls: field experiment

The concentrations of cations adsorbed from a variety of river and stream waters were compared using similar methodology to that above. Water samples collected from streams with a variety of background chemistries (Table 7.4), filtered through 0.45 µm Nuclepore filters and stored in an ice-box until approximately 2 h prior to use when they were brought up to 15 °C. Protonated cell walls were equilibrated in these waters for 1 h.

Table 7.4 Selected environmental data for study sites used in 7.24 (metal concentrations as mg l^{-1} for filtrable fraction).

site	conductivity ($\mu S\ cm^{-1}$)	pH	total alkalinity ($meq\ l^{-1}$)	Mg	Ca	Zn
0024-20	718	7.7	3.3	13.70	56.9	0.870
0091-05	159	7.4	3.0	2.75	21.7	0.017
0102-85	124	7.5	3.2	2.14	14.8	0.393
0288-80	214	7.5	3.2	3.88	27.3	0.191
0309-80	284	7.7	5.4	6.06	38.5	0.153
0310-90	122	7.2	1.8	1.89	14.4	0.019

Adsorption followed a similar pattern to 7.23 (Table 7.5); Ca accounted for the largest proportion ($\bar{x}$ = 0.61; SD = 0.03), followed by Mg ($\bar{x}$ = 0.06; SD = 0.005), Na ($\bar{x}$ = 0.04; SD = 0.01) and K ($\bar{x}$ = 0.02; SD = 0.005). The proportions of Mg and K in walls are therefore elevated with respect to the concentrations adsorbed from media (7.23). Fe again accounted for a large proportion ($\bar{x}$ = 0.25; SD = 0.03) although this is little different to controls (Table 7.1). The concentration of Zn adsorbed was quite variable.

Correlations between concentrations in cell walls and aquatic variables were computed for all of the metals (Table 7.6). There were correlations between most ions in the cell walls and their corresponding ion in solution. The exceptions were K and Fe; no variables correlated with either. Fe in the cell wall was not correlated with the concentrations of any other metals in the cell walls; otherwise concentrations of most metals in the cell walls were correlated with several others. Some examples of these correlations are presented as graphs; Zn in cell walls plotted against Zn in water shows a very close relationship at all values (Fig. 7.3); Ca in the cell wall and Ca in the water and Ca in water and Zn in cell walls were also significantly correlated (Fig. 7.4; Fig. 7.5).

7.26 Kinetics of exchange of Zn^{2+} for H^+

This was measured in two ways: with and without the use of an ionic strength adjuster. Samples of 25 protonated tips were dropped into 50 ml of 50 mg l^{-1} Zn in a beaker. pH was measured at intervals until it showed no further change. Samples were stirred continuously between measurements. Exchange was rapid (Fig. 7.6), 95% taking place within four minutes. A similar curve was obtained when 0.1 M $NaNO_3$ was added to the Zn solution as an ionic strength adjuster (Fig. 7.7). Tips from the first experiment were analyzed for their Zn content; the mean of four replicates was 12839 (SD = 601) µg g^{-1}. The expected concentration was calculated as follows:

pH at start	= 4.89	$[H^+] = 1.29 \times 10^{-5}$ M
pH after 15 minutes	= 3.77	$[H^+] = 1.70 \times 10^{-4}$ M
change in pH	= 1.12	$[H+] = 1.57 \times 10^{-4}$ M

Table 7.5 Metal composition of cell walls of <u>Rhynchostegium</u> (in µg g^{-1}), equilibrated in different natural water samples for 1 h at 15 °C. See section 7.25.

population	site	Na	K	Mg	Ca	Mn	Fe	Zn	total
0288-90	0024-20	964	384	1350	10242	98	4130	480	17648
		(282)	(34)	(55)	(434)	(5)	(300)	(48)	
0310-90	0024-20	729	272	1347	9960	80	3300	460	16148
		(130)	(63)	(31)	(226)	(1)	(92)	(9)	
0288-90	0091-05	746	400	983	10007	18	4176	58	16388
		(122)	(169)	(41)	(247)	(5)	(424)	(6)	
0310-90	0091-05	711	164	934	9674	12	3759	84	15338
		(99)	(44)	(41)	(300)	(1)	(418)	(12)	
0288-90	0102-85	584	368	922	8075	45	4214	302	14510
		(168)	(65)	(70)	(882)	(11)	(377)	(23)	
0310-90	0102-85	587	216	882	7876	13	3743	310	13627
		(296)	(32)	(43)	(602)	(4)	(241)	(20)	
0288-90	0288-90	579	337	1014	10055	31	4298	175	16489
		(182)	(48)	(7)	(269)	(14)	(222)	(4)	
0310-90	0288-90	606	268	997	10041	12	3662	194	15780
		(169)	(73)	(25)	(351)	(2)	(216)	(37)	
0288-90	0309-80	530	346	1170	11031	26	4210	156	17469
		(48)	(43)	(19)	(87)	(8)	(279)	(8)	
0310-90	0309-80	595	182	1124	10771	12	3495	180	16359
		(152)	(9)	(35)	(382)	(0.5)	(279)	(11)	
0288-90	0310-90	561	437	923	8740	33	4263	52	15009
		(47)	(98)	(30)	(533)	(11)	(239)	(5)	
0310-90	0310-90	579	226	832	7920	10	3521	71	13159
		(92)	(23)	(24)	(230)	(0.5)	(387)	(14)	

Table 7.6 Correlations between water chemistry variables and concentrations of metals adsorbed onto cell walls; data for populations from 0288-90 and 0310-90 combined. Aq = aqueous metals; cw = metals in cell wall.

variable	Na_{cw}	K_{cw}	Mg_{cw}	Ca_{cw}	Mn_{cw}	Fe_{cw}	Zn_{cw}
conductivity	0.704 *	0.096	0.946 **	0.468	0.868 **	0.793	0.793 **
pH	0.313	0.102	0.816 **	0.655 *	0.477	0.144	0.691 *
total alkalinity	0.021	0.168	0.592 *	0.904 **	0.002	0.096	0.036
Na_{aq}	0.753 **	0.145	0.842 **	0.250	0.917 **	0.243	0.816 **
K_{aq}	0.699 *	0.106	0.933 **	0.424	0.882 **	0.251	0.810 **
Mg_{aq}	0.675 *	0.078	0.964 **	0.513	0.845 **	0.251	0.785 **
Ca_{aq}	0.598 *	0.028	0.987 **	0.693 *	0.730 **	0.238	0.683 *
Mn_{aq}	0.754 **	0.148	0.830 **	0.229	0.920 **	0.238	0.829 **
Fe_{aq}	0.428	0.030	0.151	0.083	0.007	0.114	0.060
Zn_{aq}	0.605 *	0.107	0.752 **	0.092	0.884 **	0.195	0.983 **

1.57×10^{-4} mols released into 50 ml solution

therefore $1.57 \times 10^{-4} / 1000 = y / 50$

$y = 7.85 \times 10^{-6}$ mols H^+

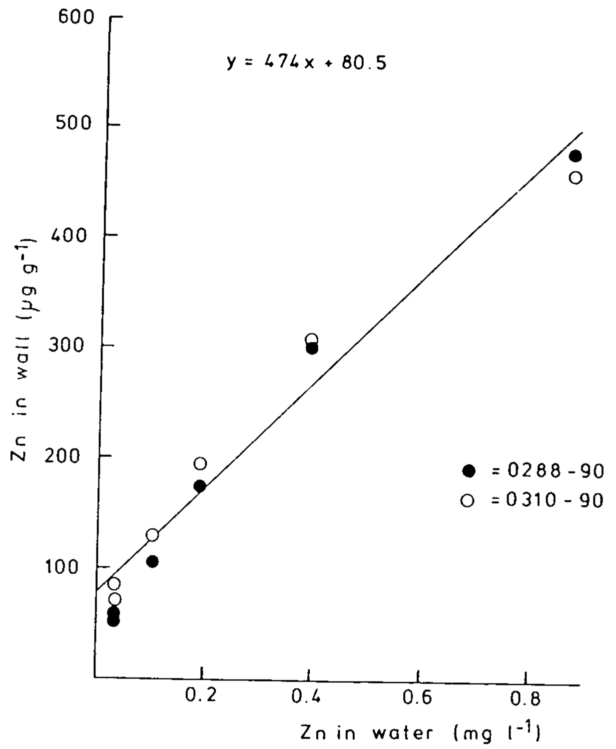
$= 7.85 \times 10^{-3}$ mmol H^+ released by 25.27 mg cell wall

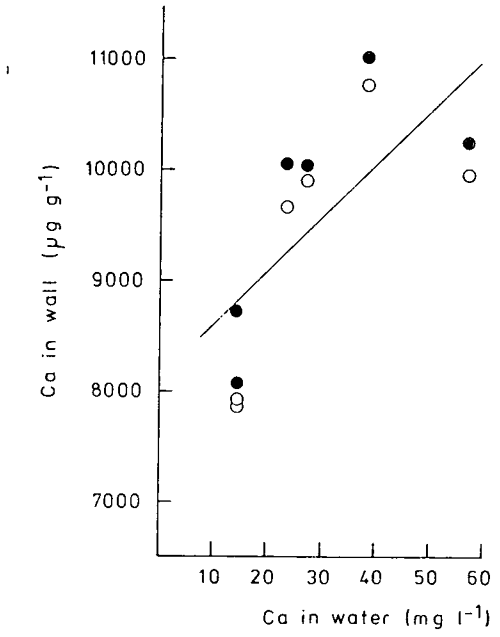
therefore $7.85 \times 10^{-3} / 25.27 = z / 1000$

Fig. 7.3 Relationship between Zn in solution and Zn in cell wall of *Rhynchostegium*. Cell walls suspended in natural water samples for 1 h at 15 °C.

Fig. 7.4 Relationship between Ca in solution and Ca in cell wall of *Rhynchostegium*. Cell walls suspended in natural water samples for 1 h at 15 °C.

Fig. 7.5 Relationship between Ca in solution and Zn in cell wall of *Rhynchostegium*. Cell walls suspended in natural water samples for 1 h at 15 °C.


y = 474x + 80.5
● = 0288-90
○ = 0310-90
Zn in wall (µg g^{-1})
600
500
400
300
200
100
0.2
0.4
0.6
0.8
Zn in water (mg l^{-1})


Ca in wall (µg g^{-1})
11000
10000
9000
8000
7000
10
20
30
40
50
60
Ca in water (mg l^{-1})

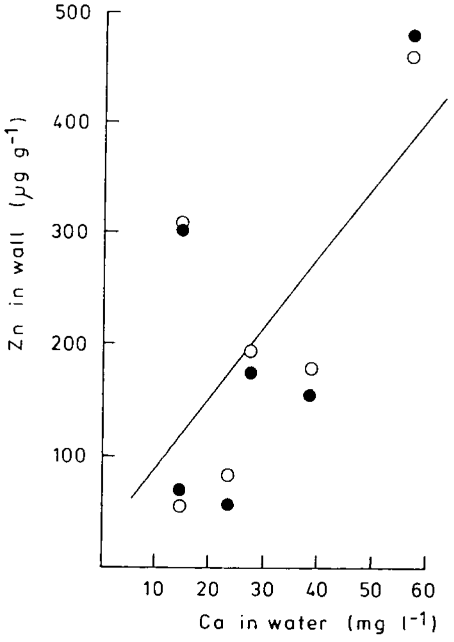
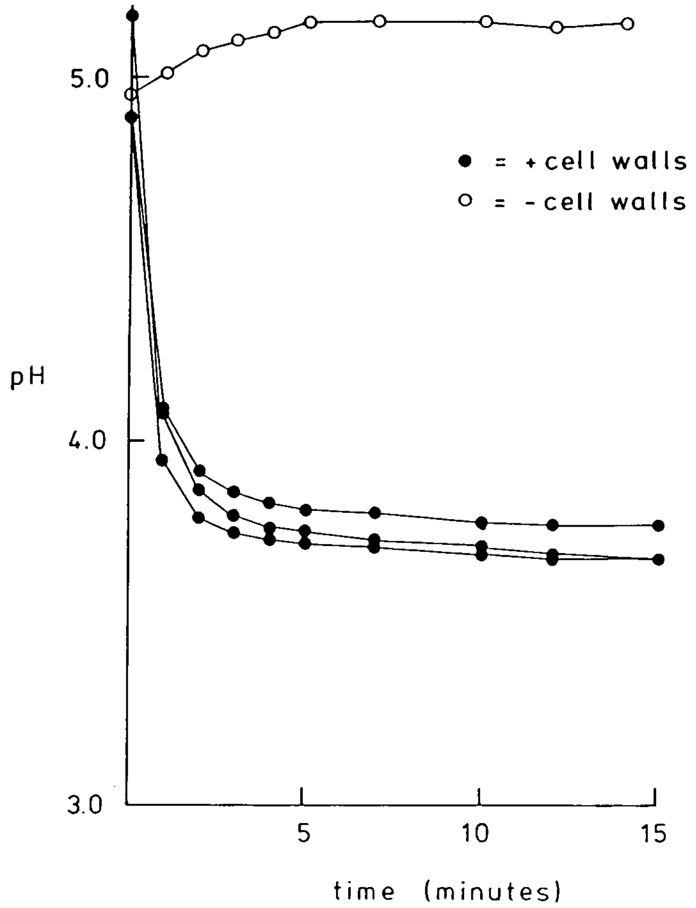
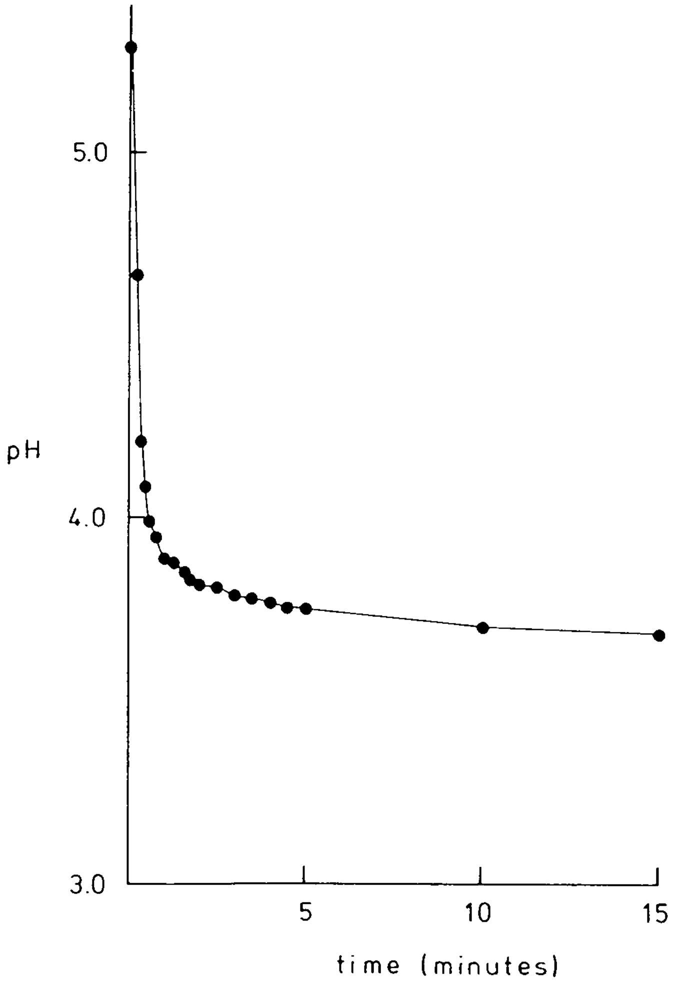

Zn in wall (µg g^{-1})
500
400
300
200
100
10
20
30
40
50
60
Ca in water (mg l^{-1})

Fig. 7.6 Efflux of H^+ from cell walls of Rhynchostegium incubated in 50 mg l^{-1} Zn.

Fig. 7.7 Efflux of H^+ from cell walls of Rhynchostegium incubated in 50 mg l^{-1} Zn + 0.1 M $NaNO_3$.


5.0
● = + cell walls
○ = - cell walls
pH
4.0
3.0
5
10
15
time (minutes)


5.0
pH
4.0
3.0
5
10
15
time (minutes)

solve for z to give mmols H^+ released per gram cell wall

$$z = 0.310 \text{ mmols g}^{-1}$$

assume 2 H^+ are replaced by 1 Zn^{2+}

if 0.310 mmols g^{-1} H^+ released

then 0.310 / 2 = 0.155 mmols g^{-1} Zn are adsorbed

1 mmol Zn = 0.0654 g; therefore 0.155 mmol = 0.01014 g = 10.14 mg g^{-1}.

The mean expected concentration in the four replicates was 13000 (SD = 1700) µg g^{-1}; this was not significantly different from that actually measured in the tips.

7.3 STOICHIOMETRY OF EXCHANGE OF ZN FOR DIFFERENT CATIONS

7.31 Exchange of Zn^{2+} for H^+

Exchange of Zn^{2+} for H^+ was tested over a range of Zn concentrations, from 0 to 100 mg l^{-1}, using the same experimental conditions as above (7.26) with no ionic strength adjustor ~~with~~ and a 1 h incubation time.

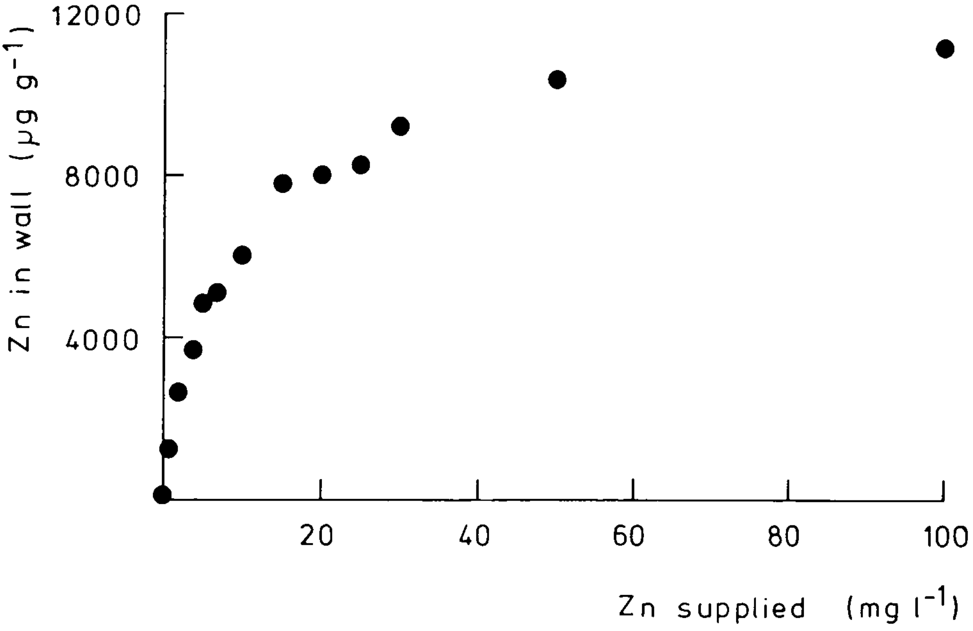
Adsorption followed saturation kinetics, typical of a Langmuir isotherm (Fig. 7.8). The rate of adsorption started to decline above about 5 mg l^{-1} Zn in solution. There was a linear relationship between Zn adsorption and the change in pH observed in the solution over the range 0 to 25 mg l^{-1} Zn (Fig. 7.9; r = 0.979 ***).

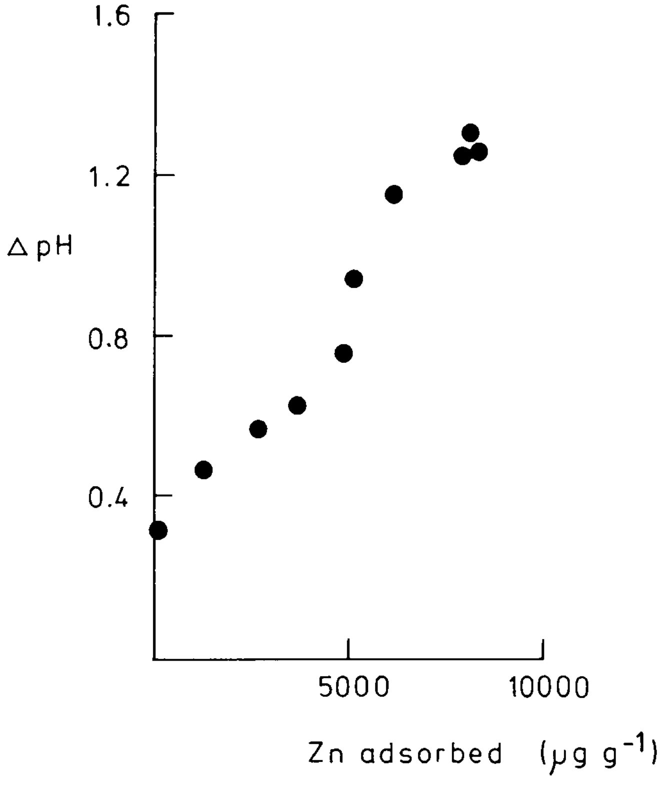
7.32 Exchange of Zn^{2+} for K^+ and Ca^{2+}

The above experiment was repeated using K^+ and Ca^{2+} instead of H^+. Cell walls were equilibrated in 0.1 M solutions of KCl or $Ca(NO_3)_2$. Zn

Fig. 7.8 Relationship between Zn in medium and Zn adsorbed onto protonated cell walls of Rhynchostegium at 15 °C in laboratory batch culture.

Fig. 7.9 Effect of increasing concentrations of Zn^{2+} on H^+ release by cell walls of Rhynchostegium.


12000
8000
4000
Zn in wall (μg g^{-1})
20
40
60
80
100
Zn supplied (mg l^{-1})


1.6
1.2
0.8
0.4
Δ pH
5000
10000
Zn adsorbed (μg g^{-1})

was supplied in 2.5 mM HEPES at pH 7.0. Adsorption of Zn onto both K-form and Ca-form cell walls showed the same kinetics as 7.31 (Fig. 7.10); however the concentrations adsorbed were approximately double. The data were tested against both Langmuir and Freundlich isotherms; Zn adsorption against both forms deviated from linearity when plotted on a Freundlich isotherm but fitted a Langmuir isotherm well (Fig. 7.11; note that this and the remainder of this section uses µmol g^{-1} rather than µg g^{-1}). This mode of representation is formally identical to the Lineweaver-Burke equation of enzyme kinetics (see Appendix 2). Applying the interpretation of the Michaelis-Menton equation to this it becomes possible to estimate the equilibrium concentration of Zn and the dissociation constant as the intercept and slope respectively;

K-form:

slope = -126.9; intercept = 413.7 µmol g^{-1}

Ca-form:

slope = -225.7; intercept = 457.2 µmol g^{-1}

Exchange of Zn for both cations had slopes of < 1.0 and negative intercepts (Fig. 7.12), indicating some release of each as soon as the tips were suspended in a dilute solution. Lines drawn on Fig. 7.12 with slopes of 2.0, 1.0 and 0.5 indicate the expected curves if, respectively, two ions are released for every Zn ion adsorbed; one is released for every one ion adsorbed; or one is released for every two ions adsorbed.

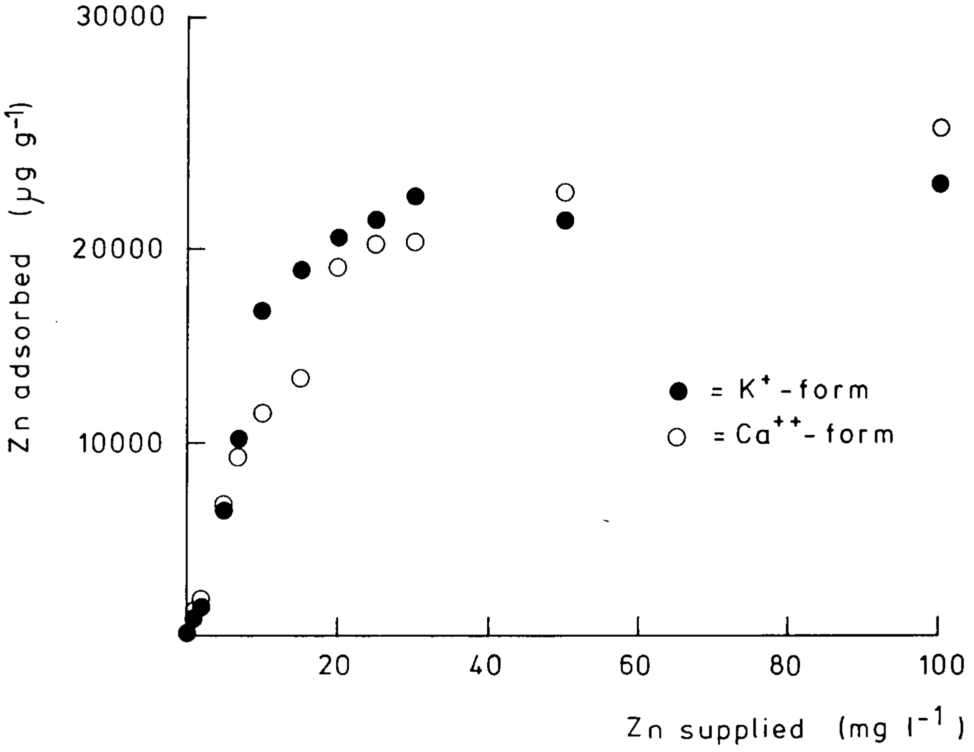
7.4 ACCUMULATION OF Zn BY CELL WALLS

7.41 Accumulation over 6 h

This experiment tests the assumption made in previous experiments that exchange processes were completed within 1 h. Accumulation of Zn by

Fig. 7.10 Relationship between Zn in medium and Zn adsorbed onto cell walls of Rhynchostegium in K- and Ca-forms at 15 °C in laboratory batch culture.

Fig. 7.11 Double-reciprocal plot of data presented in Fig. 7.10.


30000
20000
10000
Zn adsorbed (μg g^{-1})
20
40
60
80
100
Zn supplied (mg l^{-1})
● = K$^+$-form
○ = Ca^{++}-form

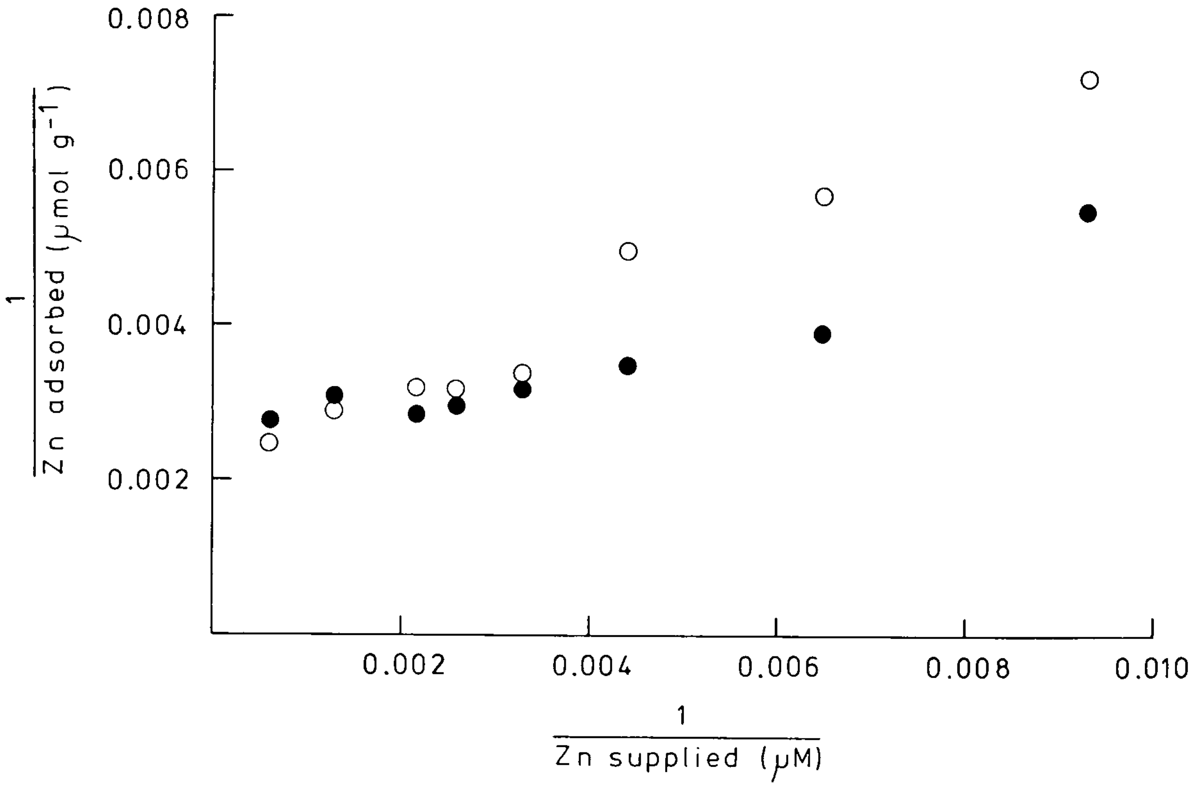
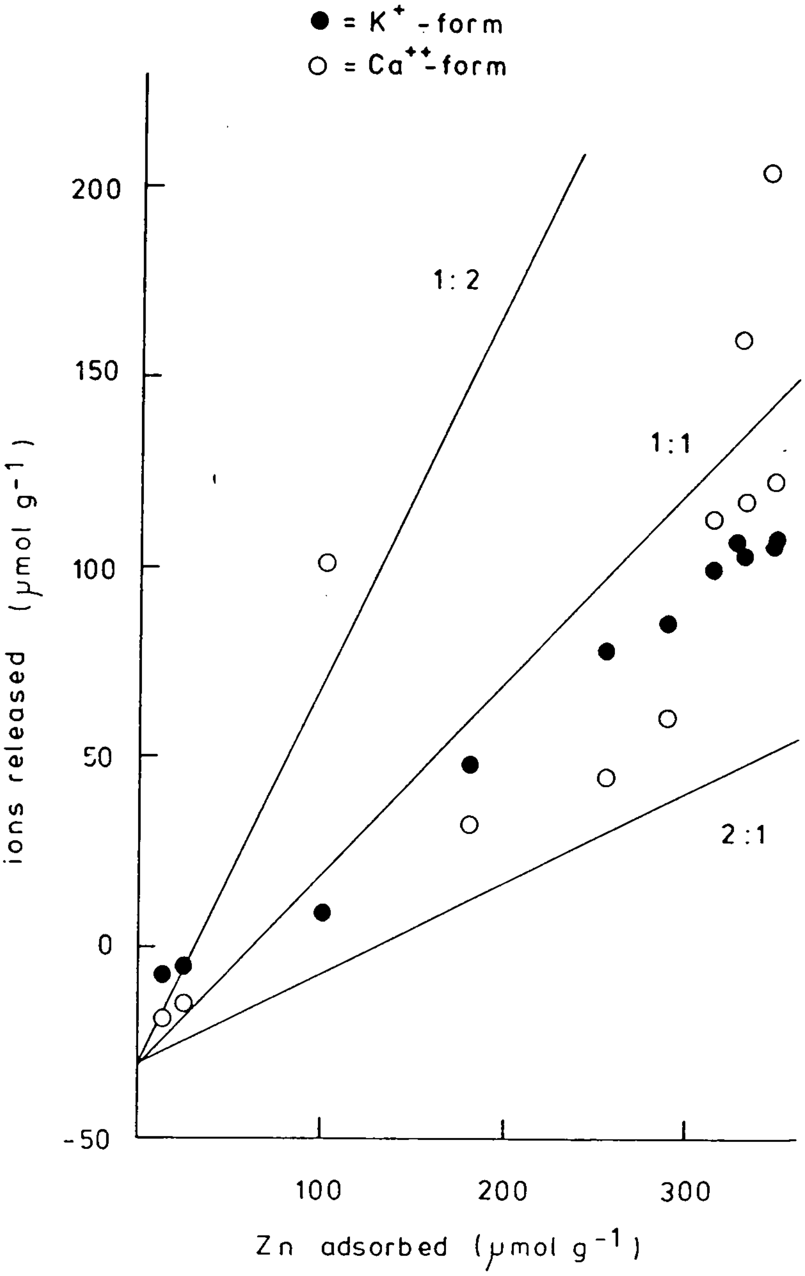

0.008
0.006
0.004
0.002
1 / Zn adsorbed (μmol g^{-1})
0.002
0.004
0.006
0.008
0.010
1 / Zn supplied (μM)

Fig. 7.12 Release of K and Ca following incubation in different concentrations of Zn at 15 °C in laboratory batch culture. Solid lines indicate different ratios of exchange of K or Ca for Zn.


● = K⁺ -form
○ = Ca⁺⁺-form
ions released (μmol g⁻¹)
200
150
100
50
0
-50
1:2
1:1
2:1
100
200
300
Zn adsorbed (μmol g⁻¹)

protonated cell walls was followed over 6 h. All other conditions were as before.

85% of uptake after 6 h was completed within the first 15 minutes (Fig. 7.13), 96% after 1 h and the remainder within 2 h.

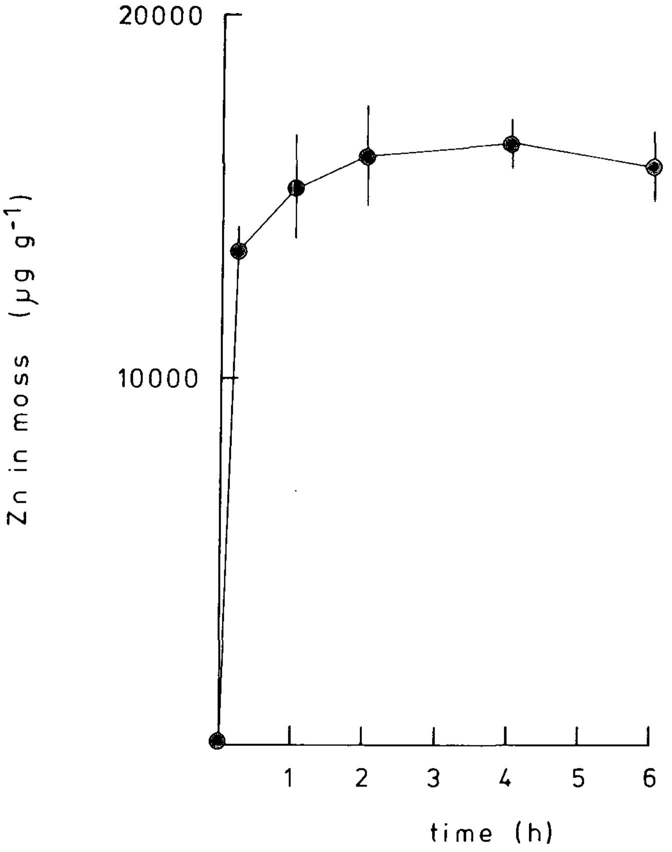
7.42 Accumulation by whole tips and by cell walls

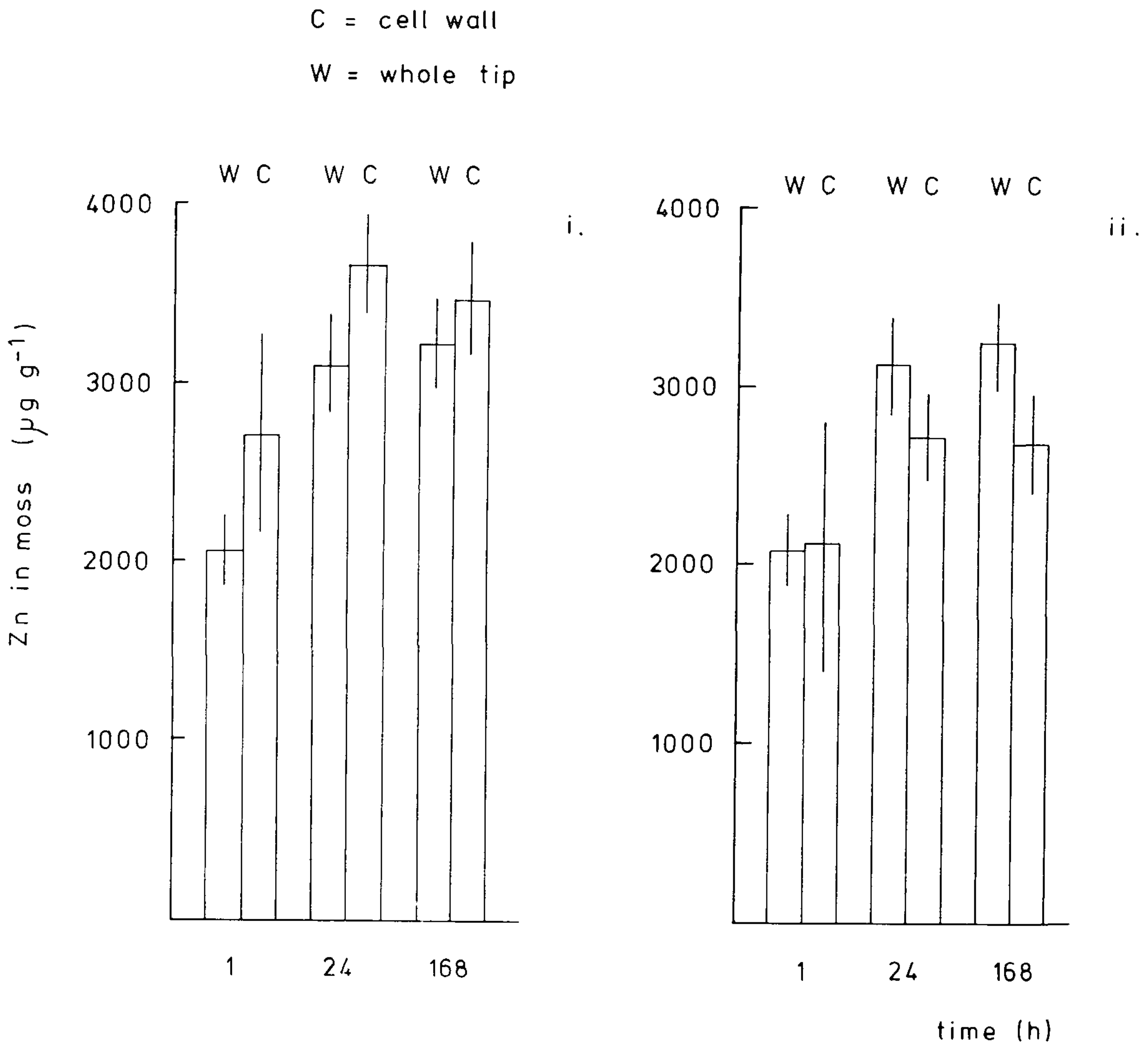
Previous experiments all used concentrations of Zn in excess of concentrations found in natural conditions and have used cell walls in a protonated state that would be unusual under natural conditions. Accumulation of Zn at a more realistic concentration was compared in protonated cell walls and in whole tips in order to relate observations made under laboratory conditions to whole shoots.

Ten tips per treatment were incubated in 200 ml medium plus 1.0 mg l^{-1} Zn and samples of cell walls and whole tips were harvested after 1 h, 24 h and 168 h (= 7 d). Accumulation by cell walls was calculated before (Fig. 7.14 i) and after (Fig. 7.14 ii) results had been corrected to the concentration of zinc as a proportion of the concentration in the whole tip. This assumed that cell walls accounted for 77% of the total dry weight (7.22). There was no significant difference between corrected values for whole tips and cell walls after 1 h and 24 h but at 168 h the whole plant accumulated significantly more Zn than the cell walls (Fig. 7.14 ii). Accumulation by cell walls at 24 h was significantly higher than at 1 h ($p = > 0.05$) but accumulation at 168 h was not significantly different from accumulation at either 1 h or 24 h. Accumulation by whole tips at both 24 h and 168 h was significantly higher than at 1 h.

Fig. 7.13 Zn accumulation by cell walls of Rhynchostegium incubated in 50 mg l^{-1} Zn at 15 °C in laboratory batch culture. Vertical bars = 95% confidence limits.

Fig. 7.14 Zn accumulation by whole tips and cell walls of Rhynchostegium at 15 °C in laboratory batch culture. i. = uncorrected data; ii. = corrected data, see 7.42 for further details. Vertical bars = 95% confidence limits.


Zn in moss ($\mu g\ g^{-1}$)
20000
10000
1
2
3
4
5
6
time (h)


C = cell wall
W = whole tip
W C
W C
W C
i.
ii.
Zn in moss ($\mu g\ g^{-1}$)
4000
3000
2000
1000
1
24
168
time (h)

7.5 ROLE OF CALCIUM IN CONTROLLING ZINC ADSORPTION

7.51 Accumulation of Zn by cell walls equilibrated in Ca solutions

The previous experiments used cell walls that had been fully protonated. In natural waters, where Ca and Mg are the dominant cations these will occupy a large proportion of exchange sites and may play a role in determining the concentration of Zn that is adsorbed.

Cell walls were first protonated and then equilibrated in solutions of $Ca(NO_3)_2$ at a range of concentrations. These were rinsed and suspended in 50 ml of 10 mg l^{-1} Zn buffered at pH 7.0 for 1 h. Samples were analyzed for both Ca and Zn (Fig. 7.15).

The mean concentration of Zn was 14622 µg g^{-1} and the 95% confidence limits were 15046 µg g^{-1} and 14198 µg g^{-1}. There was little variation in the concentration of Zn adsorbed at any equilibrium concentration of Ca. Three samples exceeded these limits and four were below; however there was no systematic trend. The concentration of Ca adsorbed increased up to about 15 mg l^{-1} Ca supplied.

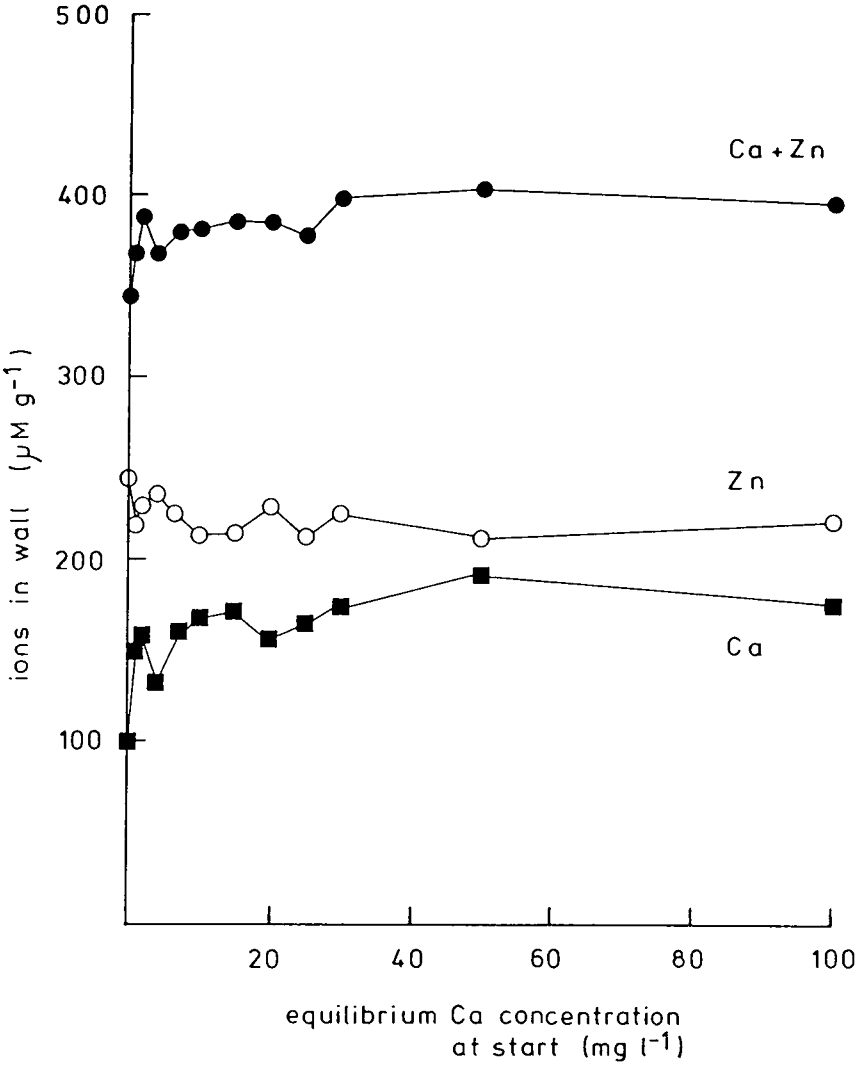
7.52 Competition for exchange sites between Zn and Ca

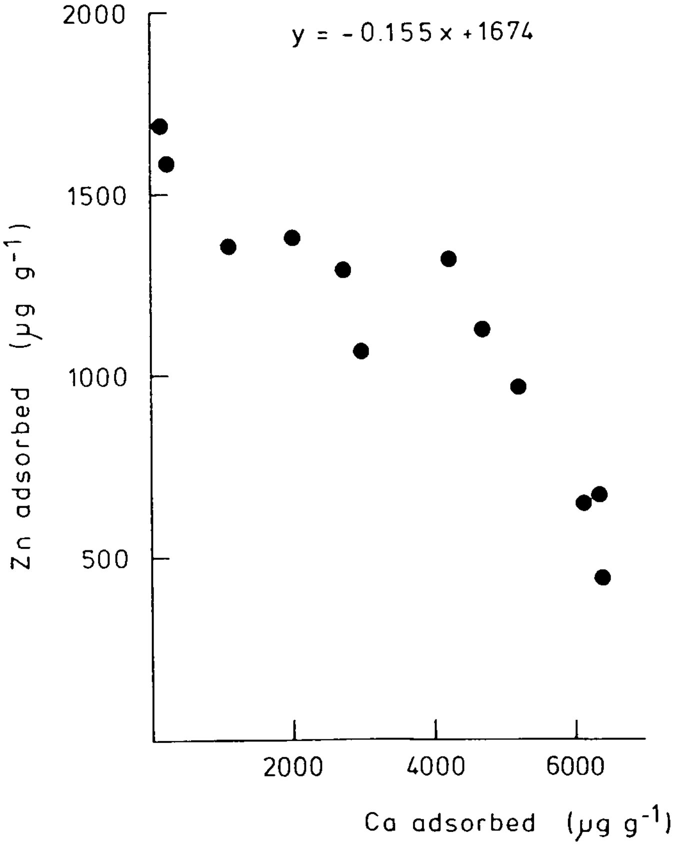
Previous experiments examined adsorption from Zn solutions onto cell walls equilibrated to different ions. In this experiment the cell walls were protonated as before and then supplied with solutions of two ions: a single concentration of Zn and a range of Ca concentrations.

Ca adsorption fitted the Langmuir isotherm (r = 0.995 ***). The highest concentrations of Zn were adsorbed at low concentrations of Ca in solution (Fig. 7.16) and there was a significant linear correlation between Ca adsorption and Zn adsorption, with a slope of -0.155 (r = 0.920 ***).

Fig. 7.15 Interactions between adsorbed Ca and Zn: effect of increasing concentrations of Ca in cell wall on subsequent Zn adsorption. Samples incubated at 15 °C under standard batch culture conditions.

Fig. 7.16 Interactions between adsorbed Ca and Zn: effect of increasing concentrations of Ca in medium on subsequent Zn adsorption. Samples incubated at 15 °C under standard batch culture conditions.


500
400
300
200
100
ions in wall (μM g^{-1})
Ca + Zn
Zn
Ca
20
40
60
80
100
equilibrium Ca concentration at start (mg l^{-1})


2000
1500
1000
500
y = -0.155x + 1674
Zn adsorbed (μg g^{-1})
2000
4000
6000
Ca adsorbed (μg g^{-1})

8. CASE STUDY

8.1 INTRODUCTION

Previous chapters have demonstrated those features which affect the accumulation of metals by aquatic bryophytes, particularly with reference to the use of moss-bags (Chapter 5). In this chapter aquatic bryophytes will be used in a study of a real situation with intermittent heavy metal pollution. Both indigenous and transplanted mosses will be studied as monitors of Cr pollution in the R. Croal (0267), N-W. England. Their effectiveness, compared with conventional water sampling techniques, will be considered.

8.2 OUTLINE OF STUDY

The study was conducted in three parts:

i. preliminary transplant of *Rhynchostegium* below the Hall Chemical Works to investigate its properties of metal accumulation in 0267-90,

ii. weekly visits, over a six week period, in which samples of *in situ* and transplanted moss and water samples were collected from three sites,

iii. experiments in the laboratory to confirm the speciation of Cr in 0267.

8.3 FIELD STUDIES

8.31 Uptake of Cr by Rhynchostegium in 0267-90

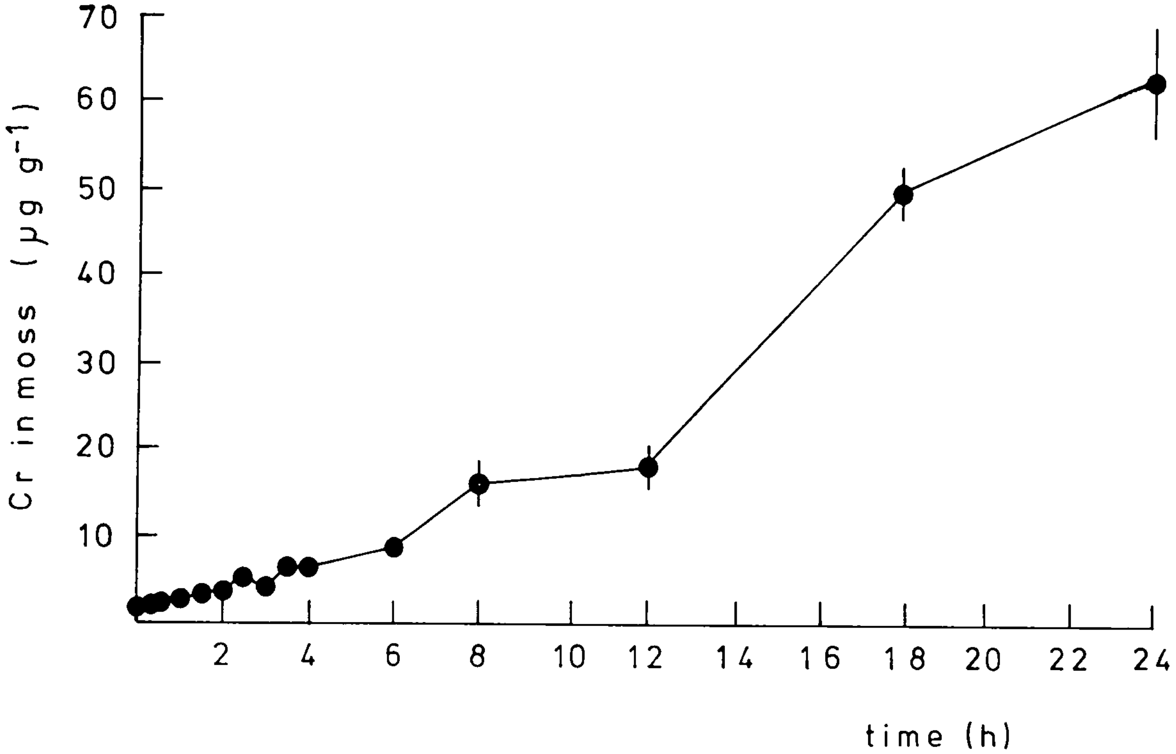
Uptake of Cr by *Rhynchostegium* in the Croal was investigated by transplanting a population from 0310-90 into 0267-90 using moss-bags. Environmental variables were measured during the course of the experiment (Table 8.1).

The concentration of Cr in the moss at the start of the experiment was 3.5 µg g^{-1}. Accumulation continued throughout the experiment (Fig. 8.1), with no evidence of an asymptote being approached. Accumulation was slow for the first 12 h; the concentration ratio (concentration in moss (µg g^{-1})/ concentration in water (mg l^{-1})) at this point was 286. After this, accumulation was more rapid and the concentration ratio at 24-h was 1260. The concentration of Cr in Rhynchostegium showed significant correlations with conductivity (r = 0.934 **), total alkalinity (0.963 **) and dissolved oxygen (-0.962 **).

Table 8.1 Environmental variables during transplant experiment in 0267-90.

time from start (h)	temp. (°C)	pH	cond. (µS cm^{-1})	Ca (mg l^{-1})	Cr (mg l^{-1})
0	14.0	7.6	405	33.9	0.037
1	14.3	7.3	397	25.9	0.033
2	14.5	7.4	388	30.3	0.028
3	14.9	7.5	381	36.3	0.035
4	15.3	7.6	378	31.1	0.041
5	15.6	7.5	381	38.4	0.046
6	16.0	7.6	379	38.7	0.049
8	16.3	7.5	382	37.2	0.051
10	16.3	7.5	388	37.3	0.047
12	16.4	7.5	393	38.1	0.056
18	15.6	7.3	423	39.5	0.040
24	15.6	7.5	421	39.6	0.049

Fig. 8.1 Accumulation of Cr by Rhynchostegium transplanted into 0267-90 in June 1985. Vertical bars = 95% confidence limits.


Cr in moss (μg g^{-1})
70
60
50
40
30
20
10
2
4
6
8
10
12
14
16
18
20
22
24
time (h)

8.32 Cr in moss over six-week period

8.321 Indigenous moss

Samples of the most abundant indigenous species at 0267-80 and 0267-90 were collected over a six-week period. These species were Fontinalis antipyretica at 0267-80 and Amblystegium riparium at 0267-90.

Aqueous Cr was detectable at 0267-90 using flame AAS, on four of the six occasions. The maximum concentration recorded was 0.047 mg l^{-1}. No Cr was detected at 0267-80 (Fig. 8.2).

Despite low Cr concentrations in the water, Cr was detected in Fontinalis on three of the six sampling occasions. The maximum concentration recorded was 50 µg g^{-1}. Cr was detected in in situ Amblystegium on all occasions at 0267-90. This included one occasion when no Cr was detectable in water samples and once when it was at detection limit (0.020 mg l^{-1}). The concentrations ranged from 130 µg g^{-1} to 298 µg g^{-1}. There was no direct correlation with the concentrations measured in the water. The concentrations measured on the occasions when Cr was at or below detection limit in the water were both higher than the highest concentration measured at 0267-80. On 25th July, one of the two dates when no Cr was measured in the water, the concentration in Amblystegium was the highest recorded at any point during the study.

8.322 Transplanted moss

Moss-bags were put out on all occasions but were only recovered on three occasions at 0267-80, and not at all at 0267-90. At 0267-80, Cr was detected in transplanted Rhynchostegium on each of these occasions (Table 8.2). Concentrations ranged from 43.3 µg g^{-1} to 35.8 µg g^{-1}; these concentrations were slightly lower than corresponding concentrations in indigenous Fontinalis but do show significant accumulation of Cr compared with the concentration in 0310-90 (1.9 µg g^{-1}; SD = 0.2).

Table 8.2 Concentration of Cr in water and in mosses at 0267-80.

date	concentration in water (mg l^{-1})	concentration in moss (µg g^{-1}) Fontinalis (*in situ*)	Rhynchostegium (transplanted)
25.7.85	< 0.010	50.0 (SD = 4.7)	43.3 (SD = 2.8)
1.8.85	< 0.010	44.0 (SD = 5.4)	35.8 (SD = 2.9)
8.8.85	0.021	< 25.0 (SD = 1.2)	37.0 (SD = 6.4)

There was no indigenous moss in Blackshaw Brook; however the moss-bags were recovered on all occasions. On each occasion, however, these bags had been covered by the shifting sand and silt substratum. Consequently, although the concentrations of Cr in the water were very high, the concentrations in the transplanted moss were low (Fig. 8.3).

8.323 Comparison with N.W.W.A. data

The N.W.W.A. collected a routine water sample on 25.7.85 at 1115; this was approximately 1 h prior to the collection of the moss sample with the highest Cr content found during the study. Water samples collected with the moss sample had a low concentration of Cr (< 0.020 mg l^{-1}); however 1 h earlier the N.W.W.A. sample contained 0.030 mg l^{-1} Cr. The difference between the two samples is small (0.010 mg l^{-1}) and it is not possible to state conclusively whether this elevated Cr concentration was responsible for the high concentration in Amblystegium riparium.

Fig. 8.2 Concentrations of Cr in water and mosses in 0267-80 and 0267-90 during six-week study period in July and August 1985. Vertical bars = 95% confidence limits.

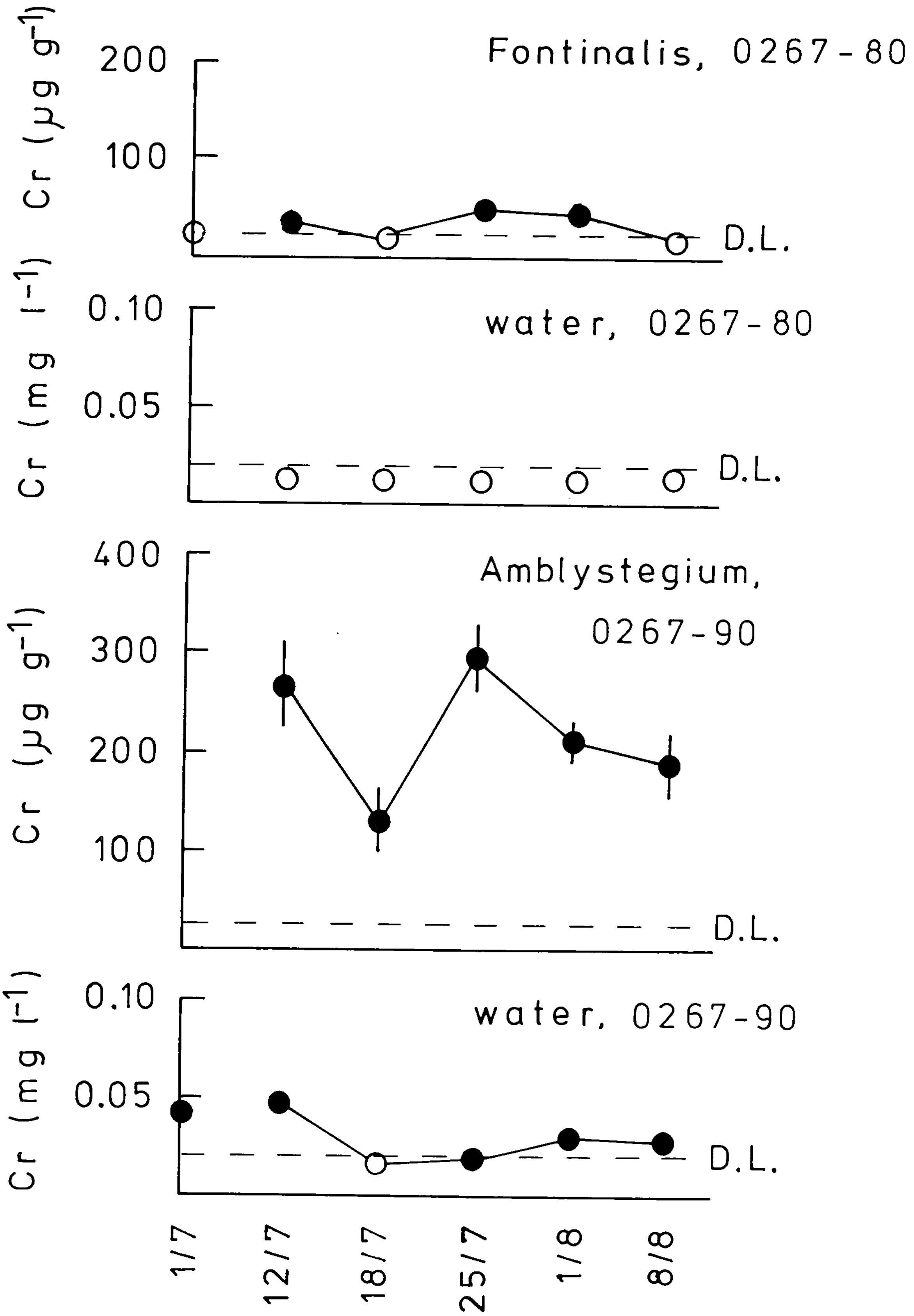
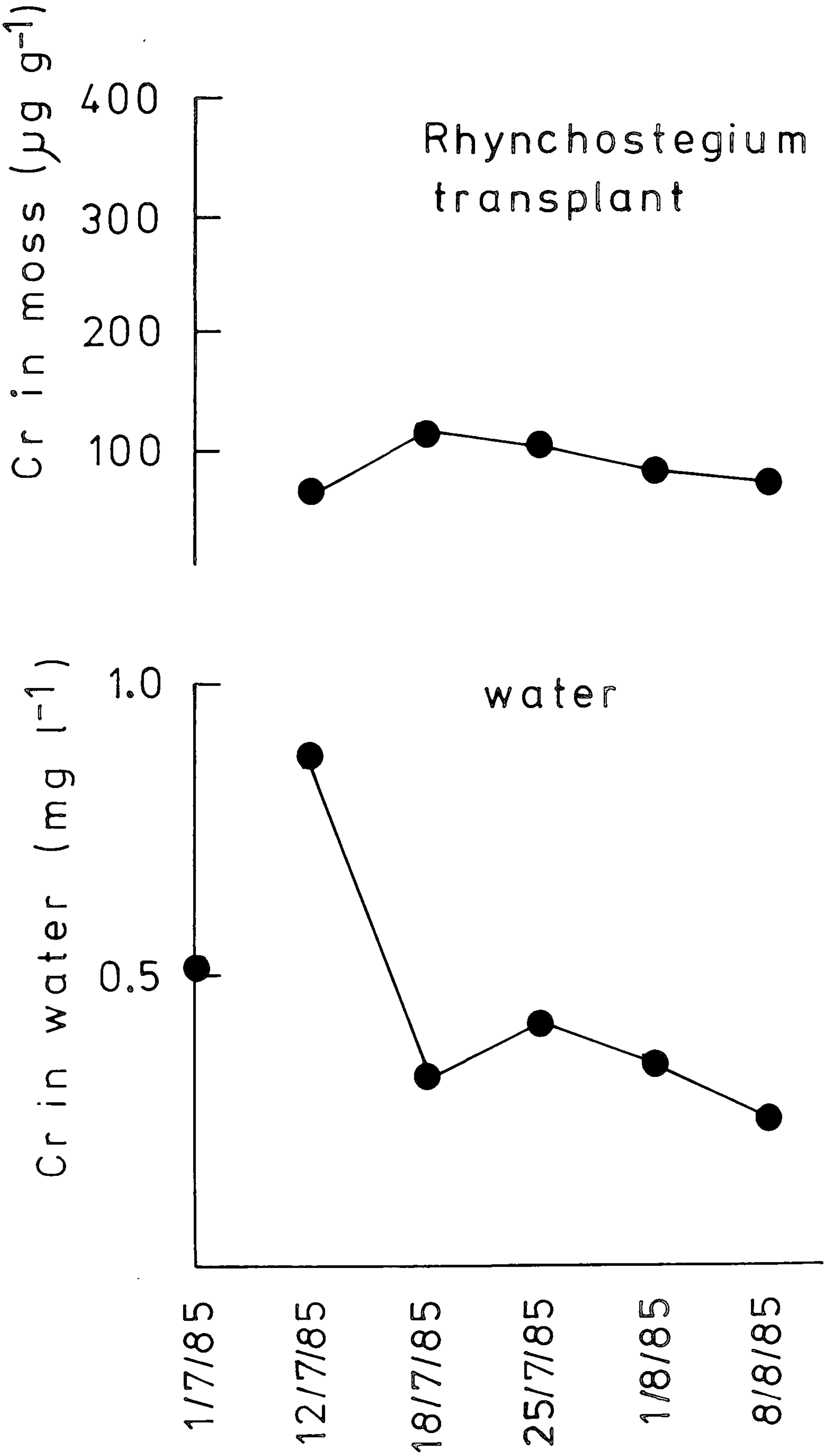

Fontinalis, 0267-80
Cr (μg g^{-1})
200
100
D.L.
water, 0267-80
Cr (mg l^{-1})
0.10
0.05
D.L.
Amblystegium, 0267-90
Cr (μg g^{-1})
400
300
200
100
D.L.
water, 0267-90
Cr (mg l^{-1})
0.10
0.05
D.L.
1/7
12/7
18/7
25/7
1/8
8/8

Fig. 8.3 Concentrations of Cr in water and in transplanted moss in 0378-90 during six-week study period in July and August 1985.


Cr in moss (μg g^{-1})
400
300
200
100
Rhynchostegium
transplant
Cr in water (mg l^{-1})
1.0
0.5
water
1/7/85
12/7/85
18/7/85
25/7/85
1/8/85
8/8/85

8.4 LABORATORY EXPERIMENTS

8.41 Introduction

The various oxidation states of Cr are known to behave biologically in very different ways. In addition to the field experiments, therefore, some preliminary experiments were conducted in the laboratory to compare accumulation by Rhynchostegium of the two forms of Cr most commonly found in nature, Cr(III) and Cr(VI).

8.42 Initial uptake rates in the laboratory

Accumulation of Cr from 10 mg l^{-1} solutions of Cr(III) (as $CrK(SO_4)_2$) and Cr(VI) (as $KCrO_4$) was followed over a 24-h period.

Accumulation of both forms of Cr continued throughout the experiment. There was a difference of approximately two orders of magnitude between the concentrations of the two forms (Fig. 8.4). After 24 h the moss in Cr(III) had accumulated 2600 µg g^{-1} of Cr, whilst that in Cr(VI) had accumulated about 20 µg g^{-1}. Over the first 6 h the slopes (= Cr in moss / time) were 3.8 for Cr(III) ($r = 0.930$ ***) and 0.20 for Cr(VI) ($r = 0.810$ **).

8.43 Uptake of Cr at different aqueous Cr concentrations

The results in 8.42 show a large difference in the concentrations of the two forms of Cr accumulated by Rhynchostegium over 24 h. As it was unknown how this relationship continued, either below 1 mg l^{-1} (i.e. concentrations expected in the field), or above, an experiment was conducted using concentrations of Cr(III) and Cr(VI) from 0.01 - 10.0 mg l^{-1}.

Rhynchostegium was incubated in media containing a known concentration of Cr(III) or Cr(VI) for 24 h. There were four replicates of each treatment.

As in 8.42, higher concentrations of Cr were found in the moss incubated in Cr(III) than in that incubated in Cr(VI), throughout the concentration range studied. When considered on a $\log_{10}/\log_{10}$ basis (Fig. 8.5) the relationship between the concentrations of metal in medium and in moss was linear for both chemical forms.

8.5 DISCUSSION

8.51 Introduction

The case study represents a relatively self-contained unit of results and it is therefore relevant to include some discussion of these here. Further comments of a more general nature are presented in the main discussion (9.4).

8.52 Comparison of laboratory and field results

Part of the object of the study was to investigate differential accumulation of Cr(III) and Cr(VI) in the laboratory and, from this, predict the chemical speciation of Cr in the Croal. In the laboratory there was a marked difference in the concentrations of the two forms of Cr accumulated by Rhynchostegium (8.4). Cr(III) was accumulated to concentrations approximately one order of magnitude greater than Cr(VI) (Fig. 8.5); this greater accumulation of Cr(III) meant that relatively low aqueous concentrations (< 0.01 mg l^{-1}) were readily detected without use of the graphite furnace or preconcentration techniques.

The concentrations of aqueous Cr during the field study were relatively low but it was readily accumulated by Rhynchostegium to a final concentration of 63 μg g^{-1} (Fig. 8.1). This is in the same order of magnitude, but still significantly lower than the concentrations measured

Fig. 8.4 Accumulation of Cr(III) and Cr(VI) by Rhynchostegium over 24 h at 15 °C in laboratory batch culture.

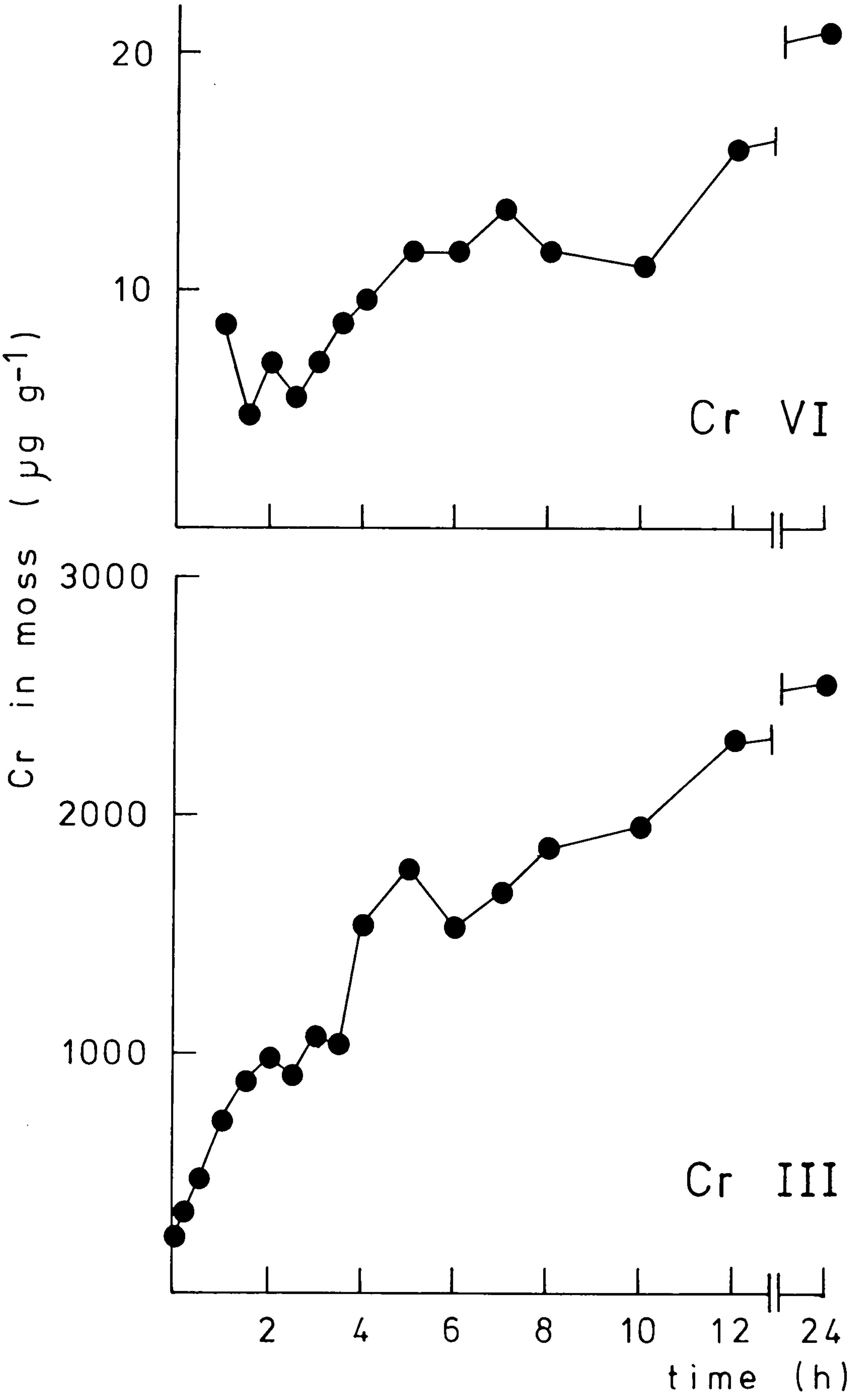
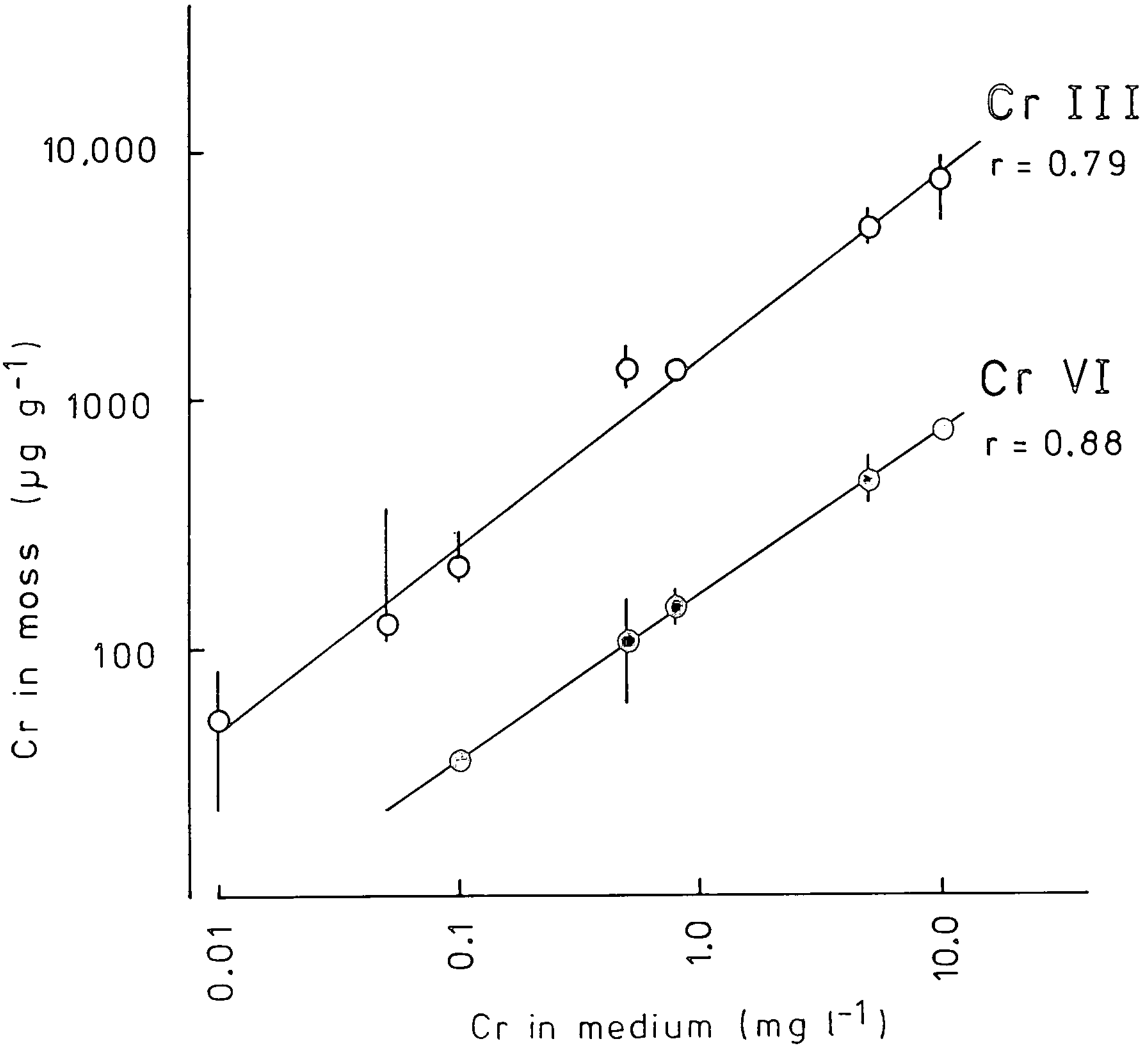

20
10
Cr VI
3000
2000
1000
Cr III
Cr in moss (µg g⁻¹)
2
4
6
8
10
12
24
time (h)

Fig. 8.5 Accumulation of Cr(III) and Cr(VI) by Rhynchostegium at different aqueous concentrations of Cr at 15 °C in laboratory batch culture. Vertical bars = 95% confidence limits.


Cr III
r = 0.79
Cr VI
r = 0.88
10,000
1000
100
Cr in moss (µg g^{-1})
0.01
0.1
1.0
10.0
Cr in medium (mg l^{-1})

in the laboratory at similar aqueous concentrations of Cr(III) (Fig. 8.5). There were, however, considerable differences between conditions in the laboratory and in the field. These include lower temperatures and higher calcium and nutrient concentrations in the field (Table 3.10; Table 3.11). The field results support a hypothesis that the predominant form of Cr in the Croal is Cr(III), although the nature of Cr in the original waste was Cr(VI) (Gemmell, 1973). This is partly explained by the mechanism of reclamation used, which included addition of $FeSO_4$ to reduce Cr(VI) to the less toxic Cr(III) form (Gemmell, 1974; 1977). Both forms are frequently encountered in natural waters (Cranston & Murray, 1980) and oxidation from Cr(III) back to Cr(VI) is slow (Cotton & Wilkinson, 1972; Earley & Cannon, 1965).

8.53 Comparison of aquatic and accumulated concentrations

The relationship between aqueous concentrations and concentrations in aquatic bryophytes has not been established for Cr in the same way as for other metals (Whitton <u>et</u> <u>al</u>., 1982; Say & Whitton, 1983; Wehr & Whitton, 1983a). Such work gives an indication of the accumulation expected in a given aqueous concentration of metal. When plants are used as monitors of intermittent pollution then such relationships do not hold and a bivariate scatter plot of aqueous Cr v Cr in moss during this study shows no relationship. This is more likely to be a reflection on the fluctuating concentrations in the river, rather than a fundamental difference between Cr and other metals.

There is evidence of slight Cr contamination at 0267-80 on two occasions, both missed by flame AAS (Fig. 8.2). Such pulses as these may have originated from one of several storm sewer overflows upstream of the site (D.J. Holland, pers. comm.) and it would have been possible at the time to use in situ aquatic bryophytes to trace the source of the pollution, as has been shown previously (Say & Whitton, 1983; Mouvet, 1984).

0267-90 is downstream of the Hall Chemical Works; however it was not always possible to detect Cr in the water. On two occasions the concentration of Cr was below the detection limit for flame AAS; the concentrations recorded in the moss on both occasions were significantly higher than in moss from 0267-80 (Fig. 8.2). This difference cannot be explained in terms of different accumulation rates by the two species. Fontinalis accumulated more Zn per unit mass than Amblystegium (Wehr, 1983; Wehr & Whitton, 1983b) and if the Cr is in the form of cationic Cr(III) then it is reasonable to presume that this holds true also for Cr. The concentration of Cr on 25th July was significantly higher than on any other occasion (Fig. 8.2) and this is evidence of a period of high concentrations of Cr prior to the sample being collected. This is the only reason to account for the higher Cr concentrations recorded in the moss. The water sample collected by the N.W.W.A. one hour previous to these samples did contain an elevated concentration of Cr (0.030 mg l^{-1}) but this was probably not high enough to account for all of the acccumulation observed in Amblystegium (8.323).

More quantitative conclusions could have been drawn, however, if there were more data on the relationship between aqueous and accumulated Cr. Such data is more difficult to collect due to the lower concentrations of Cr encountered and to the scarcity of sites of Cr pollution. Wehr & Whitton (1983a), for example, detected Cr in only 27 out of 105 "total" water samples and it was excluded from their subsequent analysis. These data were analyzed during the present study and there was a significant positive relationship between $\log_{10}$ Cr in water (Cr_{aq}) and $\log_{10}$ Cr in 2-cm tips of Rhynchostegium (Cr_{tip}) with the following parameters:

$$\log_{10} Cr_{tip} = 1.131 (\log_{10} Cr_{aq}) + 3.034$$

$$F = 25.20 \;{***} \qquad r = 0.782 \;{***}$$

Calculation of predicted values is necessarily crude because the data in Wehr and Whitton's (1983a) study concerns Rhynchostegium only. At the Cr_{aq} = 0.020 mg l^{-1} the expected concentration in Rhynchostegium is 13 µg g^{-1} which is an order of magnitude lower than concentrations measured in Amblystegium in 0267-90 but corresponds to the lowest concentrations measured in Fontinalis at 0267-80. An increase in Cr_{aq} of 0.010 mg l^{-1} to 0.030 mg l^{-1} causes an increase in the concentration in tips to 20.5 µg g^{-1}, still an order of magnitude lower than concentrations in Amblystegium. It is unlikely that the aqueous concentrations measured by N.W.W.A. (8.323) would have been responsible for the high concentrations in the moss (298 µg g^{-1}) and there had probably been a period of higher concentrations prior to collection of the samples. The aqueous concentrations predicted by the above equation to give such a concentration in Rhynchostegium is approximately 0.300 mg l^{-1}, double the highest concentration measured by Wehr and Whitton (1983a) but in the range that may be expected at periods of low flow in 0267 (Fig. 3.10). The concentrations measured in Fontinalis and transplanted Rhynchostegium at 0267-80 indicate periods with aqueous concentrations of 0.030 mg l^{-1} to 0.070 mg l^{-1}.

9. DISCUSSION

9.1 INTRODUCTION

This chapter discusses fundamental and applied aspects of the results and will relate these to the use of aquatic mosses as monitors of heavy metal pollution.

9.2 GROWTH EXPERIMENTS

9.21 Introduction

The successful use of a moss for monitoring purposes requires considerable information on its growth biology (1.6). The present study has gone some way to provide this for Rhynchostegium. Some aspects of this will be discussed here.

9.22 Seasonality in growth rate of Rhynchostegium

Although there were differences between the growth rate in the four streams studied (chapter 4) it is possible to make a few comments. Peak growth in all streams occurred in late spring and early summer, although in three of the four streams there was a second peak in October (4.4) which coincided with the formation of capsules (Smith, 1978; Watson, 1982; Wehr & Whitton, 1983b). The temperatures at this time were still relatively warm but becoming cooler (Fig. 4.2); this is therefore more than just a temperature effect and the coincidence of gametophore formation and this increased growth rate may indicate that there is some phenological control within the plant similar to that in Fontinalis dalecarlica (Glime, 1984).

Time-series analysis, using cross-correlations (Table 4.10), showed little evidence of a lag between a variable and its effect on the rate of growth except in 0310-90 where the three temperature variables all had a lag of one month in their effect.

The effect of temperature itself is interesting. Observations were made at a range of temperatures, from 3.1 °C to 12.3 °C (no measurements were made in February when temperatures had dropped to 0.6 °C in 0091-05) and some growth was observed at each occasion. There was a significant relationship between growth rate and water temperature within this range (Fig. 4.12) and extrapolation of the regression indicates that growth would cease altogether at a water temperature of 1.7 °C and a mean weekly maximum air temperature of -1.7 °C. Such a hypothesis pays no regard to seasonal variations in light quality which would also be at a minimum during the winter months. Minimum temperatures for growth of Rhynchostegium are low compared with those required for growth of Cladophora glomerata (Whitton, 1967; Cambridge et al., 1984) but is not unusual for an aquatic bryophyte; Priddle (1980) noted respiration in both Calliergon sarmentosum and Drepanocladus sp. at 1.2 °C and back-extrapolation of his results indicates that Drepanocladus still had the theoretical capacity to respire at 0 °C. Similarly, Fontinalis duriaei was cold-adapted to temperatures of between 0 and 1 °C (Fornwall & Glime, 1982). There was no evidence of a temperature ceiling during this study (Fig. 4.13); maximum growth rates have been observed for aquatic bryophytes at temperatures well above those found in this study (Sanford, 1979; Glime, 1982; Maberley, 1985a; 1985b).

The ability of Rhynchostegium to survive and grow at low temperatures may explain its persistence through winter months. In a previous study (Wehr, 1983; Wehr & Whitton, 1983b) estimates were made of relative abundance (sensu Holmes & Whitton, 1977; 1981) of aquatic mosses at seven sites in N-E. England. At all sites Rhynchostegium showed less seasonality than a number of other aquatic bryophytes (including Amblystegium riparium and *Fontinalis antipyretica*) and macrophytic algae (*Cladophora glomerata*, *Stigeoclonium tenue* and *Lemanea fluviatilis*).

9.23 Differences between streams

There were several differences in monthly growth rates of *Rhynchostegium* between streams (4.46). The growth rate was fastest in 0309-80 (4.44); this was both the lowest (Table 3.2) and the warmest (Fig. 4.3) of the four streams and, with 0310-90, the least shaded (Table 4.3). These factors may have contributed to the relatively rapid rate of growth; however the concentrations of nitrate and phosphate were relatively low compared with other streams (Table 4.4). The slowest rate of growth was found in 0310-90 (4.45) which was the highest (Table 3.2), coolest (Fig. 4.3) and most exposed stream, although it had higher concentrations of nutrient anions than 0309-80 (Table 4.4). In 0091-05 a low growth rate was associated with low concentrations of nutrients (4.42). The lowest concentrations of these were measured in 0091-05, associated with a fairly low rate of growth (4.42).

A further factor which may cause differential growth rates between streams is desiccation. The depth of water at the sites varied considerably and some populations spent more time out of the water than others. *Rhynchostegium* has been shown to be relatively tolerant to emersion (Penuèlas, 1984) and this may explain the co-incidence of low growth rates with low flows in the summer.

9.24 Comparison with other species

The rate of growth of *Rhynchostegium* is much slower than recorded in a number of other aquatic plants. The maximum rate of 2.31 mm wk^{-1} recorded during this study (4.44) is very low compared with growth rates of aquatic angiosperms such as *Hydrilla verticillata* (Barko, 1982), *Elodea nuttallii*

(Kunii, 1984), *Ranunculus fluitans* (Eichenberger & Weilenmann, 1982) and *Potamogeton pectinatus* (Howard-Williams, 1978) and algae such as *Cladophora* (Whitton, 1967; Cambridge *et al.*, 1984) and *Enteromorpha flexuosa* (Allen, 1970). The rate is, however, comparable with those for a variety of other bryophytes. There are problems in making direct comparisons with these records partly because a large number of such records are based on single annual means rather than the more detailed study conducted here. Such figures have to be adjusted before a comparison may be made. Clymo (1970) notes large differences in the rate of growth of *Sphagnum* between lowland and upland sites in Great Britain and slow rates have been recorded for *Racomitrium lanuginosum* at upland sites (Tallis, 1959), *Polytrichum* (Longton, 1970) and *Dicranum* (as *Chorisodontium*; Baker, 1972) in Antarctica and *Sphagnum* in Alaska (Luken, 1985); these rates were all below 0.5 mm wk^{-1} (corrected for length of growing season). Rates were higher (but still averaged below 1 mm wk^{-1} over the whole year) for three species at lowland sites in Wales (Benson-Evans & Brough, 1966). The rate of growth of Rhynchostegium may be slow compared with other aquatic plants but it is of the same order as for a range of other bryophytes.

Other measurements of growth rates of aquatic bryophytes include that of Marsden (1979) who measured the growth rate of *Scapania undulata* to be 0.35 mm wk^{-1}; a low figure compared with these results, perhaps surprisingly so as Marsden's study was conducted in the summer. An explanation may lie in the differences between growth forms. *S. undulata* grows in closely packed "turfs" or "cushions" (*sensu* Gimmingham & Birse, 1957) which may cause considerable self-shading (Harper, 1977); advantages in this type of growth form may relate to its ability to resist abrasion and scour. Growth rates of *Fontinalis dalecarlica* (Johnson, 1978) and *F. hypnoides* (Glime, 1982) in the field were two to three times the maximum observed here and the growth rate of *Amblystegium riparium* in the laboratory was also considerably faster (Sanford, 1979). Semi-aquatic populations of Sphagnum cuspidatum showed growth rates of up to 3.5 mm wk^{-1} both in the laboratory (at 15 °C) and in the field (Press *et al*., 1986). A tentative hypothesis concerning the differences lies in the "niches" of these different species in streams. Several aquatic bryophytes have well defined "niches" in streams (Tutin, 1949; Glime, 1970; Slack & Glime, 1985) which may relate to the physiological limits of each (Penuèlas, 1984). One such may be boundary layer resistances to aquatic CO_2 diffusion (Jenkins & Proctor, 1985). Streamlined morphology enables Fontinalis to maximise this, whilst mat-forming species such as Rhynchostegium are, perhaps, restricted to more turbulent areas. This in turn may increase the losses due to abrasion and scour (Conboy & Glime, 1971) and the length of time that they are not immersed (9.22).

9.25 Effect of heavy metals on rate of growth of Rhynchostegium

During the study there were no signs of adverse effects of heavy metals in either of the streams which had elevated concentrations. One of these, 0309-80, actually had the fastest growth rate of the four streams (4.44). This raises questions regarding adaptations and tolerances to heavy metals. Rhynchostegium was described as a species of intermediate tolerance to zinc by Whitton and Diaz (1980) and its occurrence over a wide range of zinc concentrations has been noted. Work by N.G.W. Fenton (Durham, unpublished data) showed Rhynchostegium from a low-zinc stream (0310-90) to be naturally tolerant of concentrations of zinc well in excess of those experienced here. Glime & Keen (1984) showed it to be more tolerant to copper than three species of Fontinalis, a species on which there has been some work on toxicity (Sommler and Winkler, 1982; Weise *et al*., 1985). These workers showed Fontinalis to continue to photosynthesize at relatively high concentrations of heavy metals compared with filamentous algae such as Cladophora (Whitton, 1970), Stigeoclonium (Harding & Whitton, 1976) and Hormidium (Say et al., 1977); however the latter two species were capable of developing tolerances relatively rapidly. It is unlikely that Rhynchostegium requires a genetic adaptation to survive at these concentrations.

9.3 MECHANISM OF ACCUMULATION OF ZINC

9.31 Accumulation and loss by whole tips

Several aquatic plants have been shown to accumulate heavy metals over long periods of time (Marsden, 1979; Harding & Whitton, 1981; Bond et al., 1985; Everard & Denny, 1985b) and existing data for zinc accumulation by Rhynchostegium implies that a plateau was reached within 12 h (Wehr, 1983). However this study showed that accumulation of zinc, cadmium and lead continued over a period of several days (6.2). These conflicting results require some explanation: samples of in situ moss were collected and analyzed for their metal content over the experimental period. Changes in concentrations of zinc and cadmium in the in situ moss are mirrored by fluctuations in the concentrations in transplanted moss from about day six onwards (6.2). Their accumulation period lasts for about six days and subsequent changes reflect changes in dissolved zinc and cadmium (Table 6.1). The data for lead does not follow such a pattern; the rate of accumulation is slower and continues over a period of 16 days (Fig. 6.1). Zinc and lead accumulation by transplanted Scapania undulata showed an initial phase of accumulation of a similar duration to that of Rhynchostegium (6.2) (Marsden, 1979); these were followed by changes in concentrations in transplanted moss which reflected changes in concentrations in in situ material.

A second point of interest in the results from the long-term transplant experiments is that the final concentration factor of zinc in transplanted moss is significantly higher than the concentration in in situ moss (Fig 6.1) and vice versa for cadmium and lead. This may reflect varietal differences between populations; Wehr (1983) found similar marked differences with respect to zinc accumulation. Wehr (1983) found varietal differences between populations in a transplant involving five populations between low-zinc streams and 0048-90. The differences in 6.2 are more likely to reflect these inter-population differences than the development of an tolerance mechanism and this conclusion is substantiated by the study on zinc tolerance in Rhynchostegium referred to above (9.24)

The extended period of accumulation implies that there are more processes involved in accumulation than simply adsorption onto cell wall exchange sites. Much emphasis has been placed on the role of exchange adsorption in metal accumulation by bryophytes (Rühling & Tyler, 1970; Brown & Bates, 1972; Brown & House, 1978), although recently workers have interpreted these results in terms of other processes as well (Brown, 1984). The apparent location of zinc during accumulation indicated the important role of exchange-adsorption (Fig 6.5) but a significant proportion was accounted for by the residual fraction. Throughout the first 12 h, the exchangeable fraction accounted for 77.5% of accumulated zinc (Fig. 6.3) which compares with 80% of zinc found in the cell wall of Fontinalis antipyretica by differential centrifugation (Burton & Peterson, 1979), and 93% of accumulated copper extracted from the cell wall of Solenostoma crenulatum (Brown & House, 1978). Results from analyses of roots of higher plants are similar (Turner, 1970). The residual 24.5% of zinc that was not removed by $NiCl_2$ is assumed to represent intracellular uptake. Brown (1982) and co-workers were able to release much of this by rupturing the plasmalemma using dilute acids. The potassium released at this stage indicated that the metals originated from inside the cell (2.422) rather than from discrete precipitates outside the cell which have been observed for some species (Silverberg, 1975; Sharpe & Denny, 1976; Satake & Miyasaka, 1984; Mouvet, 1984) although these have been observed to form within a minute of contact with metal salts, albeit at high concentrations (Sharpe & Denny, 1976).

Over a 14-d exposure period the proportion of extracellular zinc decreased and the residual proportion increased (Fig. 6.5). This may be an artefact of the batch culture conditions (see 7:25) since there was an exponential decrease in the concentration of zinc in flasks (Table 6.5) which would lead to new equilibria being established between the media and the exchange sites. Less than 50% of zinc is located in the exchangeable fraction by the end of the exposure period (Fig. 6.5). Such a high proportion in the latter provides strong evidence that metal accumulation by bryophytes over extended periods of time may be accounted for by intracellular uptake.

Moreover, a comparison of accumulation by whole tips and by cell walls (7.42) provides support for this hypothesis. Accumulation by both was very similar after 1 h (as in Fig. 6.5), whilst after 24 h and 168 h whole tips constituted a significantly larger fraction (Fig. 7.14). The difference here is not so pronounced as in Fig. 6.5 but the cell wall represents a "model" system and consequently will have more exchange sites per unit mass compared with whole plants.

The mechanism by which this intracellular fraction was accumulated was investigated during the study. The results show (6.421) no effect of light on zinc accumulation in the laboratory but the results of this experiment were in contrast with a field experiment (6.422) in ~~where~~ which there was decreased zinc accumulation in the light (Fig. 6.8). The former gives the more typical results compared with data for accumulation of heavy metals into algae (Gutnecht, 1961; Skowronski, 1984b), bryophytes (Pickering & Puia, 1969; Brown & Beckett, 1985) and lichens (Beckett & Brown, 1984) and for the influx of major ions into mosses (Sinclair, 1968). Dark preincubation led to decreased accumulation of zinc (Table 6.9); this effect was greatest when the moss was transplanted into the zinc-enriched stream in the dark (6.423). These three experiments give apparently conflicting results which suggest that the state of the moss prior to the experiments may have been different in each case.

There were significant effects of temperature at all three sample times (Table 6.11); corresponding temperature quotient (Q_{10}) values were 1.23 after 1h and 1.16 after 24 h and 168 h (6.43). Lüttge and Higinbotham (1979) quote Q_{10} values of between 1.2 and 1.6 as being characteristic of thermal agitation and 2 to 3 as being typical of membrane diffusion. The values found here are therefore low even if the process is assumed to be passive; however they compare favourably with values for zinc accumulation by Fontinalis antipyretica (Q_{10} = 1.2; Pickering & Puia, 1969) and for nickel accumulation by the lichen Umbilicaria muhlenbergii (Q_{10} = 1.1; Nieboer et al., 1976; both values calculated from data in these papers) and for accumulation of major ions such as phosphate into root tissues (Clarkson, 1974). High Q_{10} values alone do not necessarily imply that metabolic energy is involved since any process with an appreciable energy barrier to surmount may give similar results (Nobel, 1974; Bowling, 1976); these may include passive diffusion across a membrane. The results shown in 6.43 did not separate the effects of exchange adsorption and the intracellular absorption and a large exchangeable componant may reduce the overall Q_{10} value. Even if the present Q_{10} values are doubled they are still below values quoted by Lüttge and Higinbotham (1979) as being typical of truly "active" uptake.

The results from other studies using metabolic inhibitors are far from consistent and tend to reinforce the difficulties in interpretation which must be overcome if inhibitors are to be used effectively (Webb, 1963; Bowling, 1976). In this study inhibitors were used to separate the effects of oxidative- and photo- phosphorylation (6.442). There was no consistent difference between treatments (Fig. 6.10); significant differences were observed only where samples treated with the inhibitor had accumulated more zinc than the controls and this may indicate "leaky" membranes. Neither inhibitor caused reduced accumulation. Other workers have reported a decrease in overall membrane potential in bryophytes using azide, 2,4-DNP and ammonium chloride. These all affect oxidative phosphorylation which may imply that respiration rather than photosynthesis, was the source of energy (Contardi & Davies, 1978); however other equally valid interpretations of these results are possible because of the questionable specificity of the inhibitors. DNP has been observed to cause a reduction in accumulation of zinc by Fontinalis antipyretica over long periods of time (Pickering & Puia, 1969) and to reduce cadmium accumulation by the green alga Stichococcus bacillaris (Skowronski, 1984a). Skowronski (1984a) also reported reduced accumulation in the presence of CCCP, an uncoupler of photophosphorylation and Felle and Bentrup (1977) noted a reduced potential difference in the liverwort Riccia fluitans in the presence of CCCP.

It is only possible to speculate on the probable mechanism of intracellular zinc uptake by Rhynchostegium; there are still some fundamental questions that need to be resolved. Lüttge and Higinbotham (1979) discuss examples of processes such as enzymic reactions which may reduce intracellular concentrations of ions and thus enhance diffusion. Such processes may therefore appear to be "active" as changes in metabolism affect rates of passive diffusion. The formal proof that uptake is active requires a calculation of the Nernst potential for zinc which, in turn, requires measurement of the intracellular zinc concentration and the membrane potential. The intracellular role of zinc is as structural components in several enzyme systems, notably carbonic anhydrase. Under normal conditions zinc which does enter cells would be largely bound into these proteins and therefore it is reasonable to assume that the intracellular concentration of zinc is very low and that there may be a concentration gradient into the cell. In addition it is well established (Nobel, 1974; Lüttge & Higinbotham, 1979) that the interiors of cells are usually strongly electronegative, which would also cause diffusion into the cell down an electrochemical gradient (Sharpe & Denny, 1976).

Consideration of the rate of loss of metals from Rhynchostegium may provide further information on localization. Moss from metal-enriched streams lost zinc at a slower rate than mosses that had only had a brief exposure to zinc (6.54; 6.55; see also Wehr, 1983). This tends to confirm the assumption that a large proportion of zinc is localized in a compartment of the cell from where it is not easily lost. Those factors which enhanced zinc loss (e.g. EDTA and calcium) were expected to remove cations from exchange sites and had the most pronounced effect on the population with only a brief exposure (= 1 h) to zinc (6.54). A gradual decrease in ambient zinc concentrations resulted in a closely-matched loss of zinc from in situ Rhynchostegium (6.53) indicating slow equilibration of Rhynchostegium to changes in its environment. Similar changes were observed during the long term transplant (6.2) and are reflected in the broad correlations between metal concentrations in bryophytes and in water (Whitton et al., 1982; Say & Whitton, 1983; Wehr & Whitton, 1983a). The overall impression, therefore, is of an equilibrium between water and both extra- and intracellular compartments. This hypothesis accounts both for short-term fluctuations represented by changes in the exchangeable compartment and longer-term gains and losses from the intracellular compartment.

9.32 Role of the cell wall

The cell wall of Rhynchostegium is substantial (7.21), accounting for some two thirds of the total dry weight of the cell. There is obviously great potential for exchange adsorption onto a cell wall of this size; in the absence of competing ions in excess of 11000 µg g-1 of zinc may be adsorbed (7.26, 7.31). Zinc is normally a minor component of the ionic balance of freshwaters (1.221) and the dominant cations are usually magnesium and calcium, which account for the greatest proportion of adsorbed metals in the range of waters tested here (7.23 and 7.25). There were significant correlations between concentrations in water and in cell walls for a number of ions and there were also several cross-correlations; the concentration of zinc, for example, was positively correlated with the concentration of calcium in water (Table 7.6). This is a surprising finding; other workers have found a strong negative influence of aqueous calcium on zinc accumulation by whole 2-cm tips of Rhynchostegium (Wehr & Whitton, 1983a). The experiment was, however, based on a relatively small number of samples and this discrepancy may be due to the type of stream visited; there was also a positive correlation between dissolved concentrations of zinc and calcium, the ore-veins being intruded into the Carboniferous limestone (3.211). The laboratory experiment using a single zinc concentration and a range of calcium concentrations showed the more likely effect of aqueous calcium on zinc adsorption is that there is a strongly negative relationship (Fig. 7.15).

The effect of calcium on zinc accumulation by Rhynchostegium was also investigated by Wehr (1983) and Wehr and Whitton (1983a). Wehr's experiment was of a similar design to 7.52; accumulation of a single zinc concentration was tested over a range of calcium concentrations. The effect was rapid and almost linear over the range 0 - 20 mg l^{-1} calcium; at higher concentrations the effect decreased. A similar result was found in this study (7.52) when zinc adsorption was plotted against the calcium concentration in the media (Fig. 7.16) indicating that the "effect" observed by Wehr (1983) may have been largely due to competition at the cell wall; this is in keeping with the generally apoplastic location of calcium in plants (Marmé, 1983; Demarty et al., 1984). No similar effects were observed when cell walls were equilibrated to various concentrations before being supplied with zinc; indeed there was little variation in the concentrations of either calcium or zinc over the entire range tested (Fig. 7.15). The most probable explanation is that new equilibria between the cell wall and the media were set up when equilibrated cell walls were introduced to the hypotonic media. In the study by Wehr and Whitton (1983a), multivariate statistics were used to investigate the factors affecting metal accumulation by Rhynchostegium and dissolved calcium had a strong negative influence on zinc accumulation. It was not possible to make a direct comparison of the regression coefficients as Wehr and Whitton's was calculated using $\log_{10}$-transformed data; however it is likely that a large component of these effects may relate to competition at the cell wall. Similar effects have been observed for a number of other species such as the chlorococcale Golenkinia (Bachmann, 1963), Lemanea (Harding & Whitton, 1981) and Hylocomium splendens (Rühling & Tyler, 1970) but not for Scapania undulata (Whitton et al., 1982) because there were problems in this study in separating the effects of pH and aqueous calcium. Competitive inhibition of intracellular cadmium uptake by magnesium and calcium was also observed in Rhytidiadelphyus squarrosus (Brown & Beckett, 1985); this was interpreted as indicating that a relatively non-specific uptake system was involved for this metal.

Other environmental factors tested during the present study were phosphate, EDTA and humic acid. Phosphate was tested only in one experiment (6.55) and had little effect on metal loss after either a long- or a short-term exposure to zinc. Wehr (1983) also found phosphate to have little effect on zinc accumulation although it has been noted to have an ameliorating effect on zinc toxicity to Hormidium rivulare (Say & Whitton, 1977), Anacystis nidulans (Shehata & Whitton, 1982) and zinc-tolerant strains of Stigeoclonium tenue (Harding & Whitton, 1977). It has been suggested that factors in the environment which affect accumulation may also affect toxicity (Whitton & Say, 1975); this does not appear to be the case for phosphate on zinc accumulation over the ranges experienced in nature (Wehr, 1983).

EDTA did have a significant effect on loss of zinc (6.55) but not in the study involving adsorption onto cell walls (7.23) although the concentrations used were similar in each case. In 7.23 only six of the differences were statistically significant (Table 7.3); four of these were between solutions with and without EDTA. It is possible that the high concentrations of iron in the cell wall masked the effects of EDTA. Cell wall preparations were given a preliminary wash in hydroxylamine hydrochloride (2.43) to remove iron and manganese oxides (Gupta & Chen, 1975) but a recent publication (Tipping et al., 1985) has shown that this may not have solubilized all the iron deposits; these in turn may cause additional adsorption and co-precipitation of heavy metals in their own right (Laxen, 1984a; 1984c) and in addition may adsorb humic substances (Tipping, 1981a; 1981b) which would further increase the number of potential binding sites (Tipping et al., 1983). Treatment with oxalate (Tipping et al., 1985) removed approximately half of the iron which remained after treatment with hydroxylamine (7.24). This, in turn, had an effect on the concentration of zinc remaining in the plant (Fig. 7.2).

Results in 6.55 and Wehr (1983) indicate that the presence or absence of low concentrations of EDTA may have a significant effect upon zinc accumulation.

Several of the above comments concerning EDTA apply also to humic acids. Again, no conclusive statement may be made from the results presented here (7.23); several other workers have noted a decline in the accumulation of heavy metals (van der Werff, 1984) or in their toxicity to both plants (Laegreid et al., 1984) and animals (Winner, 1984) and it is likely that these are the result of reactions outside cells (Langston & Bryan, 1984). The high affinity of iron oxides for humic materials (Tipping 1981a; 1981b) may have masked the expected effect (7.23, 7.24).

9.33 Growth and heavy metal accumulation and loss

In addition to the physiological processes involved in heavy metal accumulation and loss, each process may also be influenced by growth, respectively exposing new sites for metal adsorption or absorption or "diluting" existing metal concentrations. It is possible to test both of these views from data in chapters 4 and 6. The conclusion in 9.2 was that Rhynchostegium is relatively slow-growing compared with other freshwater algae and macrophytes; average rates were about 1 mm week^{-1} rising to 2 mm week^{-1} at peak periods (9.23). The rates of loss of heavy metals were dependent upon the length of initial exposure; in material from metal-enriched streams the process could take several days (6.51; Marsden, 1979; Wehr, 1983). Growth of Rhynchostegium in such a short length of time would be very low. 1 mm of shoot would represent 5% of total mass (4.23); if 40% of accumulation after 23 d occurs within 24 h then the remaining 60% would be accounted for by 2 - 3 mm of growth or 10% - 15% of the final mass.

9.4 USE OF AQUATIC BRYOPHYTES AS MONITORS

9.41 Moss-bag methodology

The results in chapter 5 showed the accumulation of heavy metals by Rhynchostegium in moss-bags to proceed at the same rate as by moss on boulders (5.31) and to be relatively unaffected by the pattern of the bags (5.32). These are encouraging features if a standard method for using moss-bags is to be proposed. Some features of the results, however, require further comment.

It was noted in 5.31 that the initial stages of accumulation of all metals was "noisy". Four of the six regressions based on the rapid phase were not significant and this was seen in bags (zinc and cadmium) and for both boulders and bags (lead). In 5.32 there was no such initial "noise" (Fig. 5.2).

The kinetics of accumulation of zinc were also very different between the two experiments. 5.31 fitted the straightforward linear function ($y = m x + c$) closer than the $\log_e$-normal function ($y = \log_e x + c$; Table 5.6)), whilst the accumulation curve in 5.32 fitted the $\log_e$-normal function better than the "normal". Similarly, when the curves were arbitrarily split into "rapid" and "slow" sections the division fell at 6 h for 5.31 and 5 h for 5.32. An apparent saturation was observed in 5.32 but not in 5.31. When the regression equations for this initial phase are compared then the initial slopes in 5.32 are 2.5 times more rapid than in 5.31.

The streams in which these experiments were conducted were very different: 0024-22 (5.32) is a lowland stream, very eutrophic and with hard water, whilst water in 0012-45 (5.31) is less hard and less eutrophic (Table 3.7; Table 3.8). There are considerable differences in catchments and in types and concentrations of metal inputs. Both experiments had fundamentally similar designs and similar quantities of moss were used in each bag. That the differences from 5.32 were observed in both treatments in 5.31 implies that it is a feature of the transplant and not of the moss-bag and may be related to differences in water chemistry.

Although the results in 5.34 are not conclusive they do illustrate a trend and it is worthwhile to urge caution on the subject of how much moss to pack into a bag. If there is reduced accumulation at the centre of the bag then presumably this is due to reduced percolation of water through the contents of the bag and it is surprising not to see an effect when type III bags (5.22) are considered. Why there should be a difference between percolation through moss and not through muslin is not clear. It is suggested that the contents of a bag should be restricted to 4 - 5 samples (± 100 g wet weight). Fewer bags are required than if each bag contained enough only for one sample yet the quantity of moss in each bag does not approach the volume of moss used in 5.34.

Everard (1983) adopted the practice of workers on air pollution (Goodman et al., 1977) and teased out the moss in the bags to form circular discs with increased surface area : volume ratios. This may be a sensible precaution in lakes or in slow-flowing rivers and streams; however in rivers or streams the effect of the current is both to percolate water through the bag and to aggregate the moss into a streamlined form.

The technique used here for fixing bags in rivers was to attach them to stakes (2.32). Suspended bags have been used successfully by other workers both in lakes (Denny, 1981; Everard & Denny, 1985b) and in rivers (I.G. Burrows, unpublished); however in rivers they are susceptible to vandalism and to high flows. The technique is most successful when the bag can be securely attached well out of reach of the bank and it may be possible to use other supporting structures such as fences, if these cross streams. The techniques used by Mouvet (1984), Prigg (1977) and E. Mycock (pers. comm.) involve anchoring the moss-bags securely to the stream bed using stones or concrete blocks. It is the author's experience that such blocks have to be quite substantial to prevent their removal during high flows. It is possible that the size of the block required could be predicted from the emperical relationship governing the size of the boulder moved by a particular flow rate. A stake which is securely hammered into the stream bed can resist very high flows although in the process it may trap suspended material; it is probably more secure under such conditions than a boulder but it is far more conspicuous to passers-by. The most widespread problem associated with transplants is vandalism. This appears to be a fairly universal problem associated with the use of unattended field equipment and is exacerpated by use of materials which attract attention, reaches which are close to habitation and by experiments during school holidays. Efforts may be made to minimise these problems; pattern II bags were made from a bright orange material which made them conspicuous whilst pattern I moss-bags were coloured green (Table 5.1). No data were collected on the relative qualities of each with respect to vandalism but the darker colour is likely to attract fewer casual passers-by. Anglers will take a greater interest in the stream and are likely to spot any object, particularly one which is liable to snag a line; they will also be equipped with waders etc. to remove them. Anglers are also likely to have an above-average interest in water quality and may be enthusiastic if they know why the bags have been placed there.

The main difference in the way in which the moss is exposed to water between boulders and bags is that in the latter method the moss lies closer to the bed of the stream and is therefore closer to its natural position. Results in 5.31 show that there is no significant difference in accumulation rates between moss in these two positions.

A final consideration if the method is to be proposed for routine monitoring purposes, is to determine the point at which the transplanted moss is suitable for use as a monitor. This would be at the point when fluctuations in the concentration in the water will be reflected by changes in the concentration in the moss. This is obviously not during the rapid phase; however once the rate of accumulation has slowed down then it is possible that a change in the concentration of metal in the water may be detected by a change in the concentration in the moss. The rapid phase lasted for approximately 6 h (chapter 5) and accumulation of zinc and cadmium continued at a slow rate over five or six days and accumulation of lead continued over a longer period of time (6.2). At the end of this period moss responded to changes in aqueous metal concentrations in the same way as indigenous populations (9.31). This may be a long time if a routine survey using moss-bags is envisaged; however in situations where two sites (e.g. above and below a discharge) are being compared then accumulation over a shorter period of time (e.g. 24 h) may be sufficient.

In 6.2 the differences in final concentrations of zinc accumulated were ascribed to varietal differences (9.31). This is itself a potential source of confusion; in 6.2 the difference at t = 23 d was significant at $p > 0.05$ and if significant differences are to be taken as evidence of metal contamination, then efforts must be made to overcome such varietal differences. Significant differences between populations were also found by Wehr (1983). The simplest method is to collect all Rhynchostegium from a single source and to use this in all transplant experiments.

9.42 Experience of case study

Part of the preceding discussion is illustrated by the experience of the case study (chapter 8). On several occasions stakes and bags were lost, probably due to interference by passers-by (8.322); however this study demonstrated the feasability of using aquatic bryophytes in conjunction with straightforward water samples for monitoring intermittent pollution, especially when the concentrations in the water approach the detection limit for a particular method (8.321). Interpretation at three levels was possible. Concentrations at 0267-80 indicated the presence of an input between the two reaches (Fig. 8.2); an extension of this to include sites upstream of 0267-80 would have been useful in detecting sources of the low chromium concentrations measured in plants in these reaches. The second approach used the computer database to predict aquatic concentrations (8.53). The latter approach was hampered by lack of a suitably sized database for each of the three species which might have allowed multivariate statistics to be used and a more accurate prediction to be made. Finally, by including laboratory experiments it was possible to predict the chemical species of chromium in the river (8.4, 8.52).

9.43 Aquatic bryophytes as monitors

Both indigenous and transplanted populations of Rhynchostegium are suitable for use as monitors. With indigenous populations there may be problems associated with varietal differences (9.31); Rhynchostegium is frequently observed with capsules (Smith, 1978) and it has been suggested that these play a significant role in spore dispersal (Wehr, 1983). There is a possibility of varietal differences in populations along a river or stream compared with populations of Fontinalis antipyretica, for example, which only rarely produces spores (Smith, 1978) and whose dispersal is probably largely effected by vegetative fragments carried downstream (Glime et al., 1979). There are no isoenzyme or similar studies on aquatic bryophytes to confirm this. Any such differences may be a function of the distance downstream between populations which, in the case of many monitoring studies is likely to be quite small.

There are a number of ways in which these bryophytes may be used; in the terminology of Goltermann et al. (1978) these may divided into different "levels" depending upon the needs and the facilities of different water monitoring bodies. The first "level" is as a record of a suspected pollution source by a concerned member of the public. A scenario is envisaged where a member of an angling club observes a fish kill and contacts the water authority. A sample of moss collected at the time may subsequently be analyzed if heavy metals are suspected. A second "level" would apply to a suspected pollution source known to a Water Authority; a preliminary field assessment of the site would be followed by a planned series of transplants or samples and their subsequent analysis. Such studies were carried out by Harding (1979; 1980) in Wincham Brook, Cheshire. It may be necessary at this stage to take duplicate or triplicate samples in the manner of "Formal Samples" (Toms, 1975), which are available for independent analysis. The final "level" is an extension of the second level, feedback from which may lead to additional samples or a field or laboratory experiment to confirm the chemical species (e.g. 8.4) or the accumulation or loss properties of a particular population. It is at this stage that a knowledge of the localization of the metal (chapters 6 & 7) may play a significant role.

The previous paragraph presupposes that the study is to be carried out in an area where facilities etc. are available; this may not be the case in remote or third world regions. Whitton et al. (1981) list ten advantages of plant samples over water samples; these include their ease of collection, storage and transport which may make them potentially useful for Environmental Impact Assessments and pilot studies in these regions. Effective use of such techniques would require an understanding of the relationship between dissolved and accumulated concentrations of heavy metals for the species in question; this is presently known for only a few species (Whitton et al., 1982; Say & Whitton, 1983; Wehr & Whitton, 1983a) and there may be enough data in the literature to establish relationships for a few more. The value of the data may be enhanced by a knowledge of pH and aquatic calcium concentration although it may be possible to substitute conductivity for the latter. In such cases the mosses are being used simply as "concentrators" of aquatic metals and it may be possible to devise simple methods for the extraction and subsequent colorimetric analysis which would represent a genuine "Intermediate Technology" approach to water quality monitoring.

Some studies have ignored the taxonomy of bryophyte samples and instead analyzed aggregates comprising several species (Whitehead & Brooks, 1969; Prigg, 1977). This is a further potential simplification of the method which may have some value in particular circumstances; differential accumulation by different species (Burton & Peterson, 1979; Say et al., 1981; Wehr & Whitton, 1983b) means that non-homogeneous samples may give variable results.

An example of the use of bryophytes to supplement analysis of water samples is given in chapter 8; data on chromium accumulation by Rhynchostegium transplanted into 0267-90 (8.31) were compared with similar data collected in the laboratory (8.42) to give some indication of the speciation of the chromium; in this study it was in the form of Cr(III)(8.52, 9.42). A second use is to investigate rates of accumulation and loss by a particular population in a particular reach. This may follow a sample of indigenous moss which contained elevated concentrations of heavy metals, posing the questions how long ago did the episode occur and what was its concentration? It may not be possible to answer these accurately; an estimate of the dissolved metal concentration corresponding to the concentration in the plant is possible. Results in 6.54 indicate that the rate of loss was dependent upon the length of time of exposure to the metal which may not be known; however the approximate time of the event may be and concentrations estimated by back-extrapolation from Fig. 6.13

for different lengths of exposure give a range of values indicating either rapid loss from a brief exposure to a high concentration or slower loss from longer exposure to lower concentrations.

Everard and Denny (1985b) used a "level III" approach to investigate fluxes of lead in Ullswater. This fundamental study required a different approach to straightforward monitoring studies. It was not possible, for example, simply to relate metal concentrations in moss to metal concentrations in water; however they were able to show the role of turbulence in influencing metal accumulation.

9.5 CONCLUDING REMARKS

The range of applications of aquatic bryophytes for monitoring heavy metal pollution is potentially wide (9.43) and may be both fundamental and applied in nature. The accumulation of heavy metals by Rhynchostegium is rapid (chapters 5 & 6) and is uncomplicated by interactions with other components of the food chain and movement (compared with accumulation by animals) or with accumulation through roots (compared with higher plants). Rhynchostegium is also relatively perennial (chapter 4). Whilst the lack of a suitable substratum may be a limitation to colonization by Rhynchostegium under natural conditions, the use of moss-bags extends the possible range of situations where it may be used. Moss-bags are cheap to deploy and the basic technique (chapter 5) may be adapted to suit a wide range of local circumstances (9.41, 9.43). The bivariate models of metal accumulation, comparing metals in streamwater with metals in mosses is a useful starting point for case studies of intermittent heavy metal pollution.

These relationships are maintained by a range of physiological phenomena (9.31; 9.32). Intracellular accumulation is as important as exchange-adsorption (6.3) and is fundamental to the retention of heavy metals after intermittent pollution events (6.55). Knowledge of these processes is still limited by the lack of a fundamental physiological examination of heavy metal uptake in these species (9.31); however these will contribute more to fundamental botany than to applied monitoring where knowledge of the heavy metal accumulation properties of various species will provide a suitable framework for a wide range of applications.

SUMMARY

1. A study, combining both field and laboratory experiments, was made of heavy metal accumulation and loss by aquatic mosses with particular reference to their suitablity for use as *in situ* or transplanted monitors.

2. The growth rate of *Rhynchostegium riparioides* was studied at four sites in the Northern Pennines using cotton tags to measure shoot extension. Growth continued throughout the 12-month period with maximum growth rate in the spring and a smaller peak in the autumn. Growth rate was related strongly to water temperature and maximum and minimum air temperatures, of a number of environmental variables measured. There were also differences between growth rates in different streams, apparently not related to concentrations of nutrients or heavy metals; it is suggested that these may relate to differences in the length of time emersed.

3. Field experiments were used to evaluate mesh-bags as containers for transplanted aquatic mosses. There were no significant differences between accumulation of heavy metals by moss on boulders or in bags or between accumulation of zinc by moss in three different patterns of bag. There were some significant differences between replicate bags of *Rhynchostegium* over a 12-h period and there was also slightly reduced accumulation at the centre of bags packed with large quantities of moss. The technique was, however, generally robust and applicable to a range of situations.

4. An experiment using Fontinalis antipyretica in place of Rhynchostegium was also performed. There was no significant difference in accumulation of zinc between Fontinalis on boulders or in bags. Use of this species may extend the range of application of moss-bags.
5. An eluting agent, to remove zinc from exchange sites, was used to study intracellular locations of zinc. After preliminary experiments $NiCl_2$ was chosen in preference to EDTA. This removed about 70% of zinc accumulated during the first 12-h. This proportion decreased over time and after 14 d less than 50% of zinc was located in the exchangeable fraction; however this may, in part, be an artefact of batch culture conditions. Accumulation by both exchangeable and residual fractions fitted saturation kinetics over the first 12 h; thereafter only accumulation by the residual fraction did.
6. The possible role of metabolic activity was tested indirectly by manipulating the plant's environment. Results of studies on the effect of temperature and metabolic inhibitors indicated no direct control by metabolism on zinc accumulation by Rhynchostegium. Studies on the effect of light gave ambiguous results.
7. The substantial cell wall of Rhynchostegium was capable of adsorbing large quantities of ions (> 12000 $\mu g\ g^{-1}$ zinc from a single-salt solution); calcium was the dominant ion adsorbed from natural waters and this had a strong influence on zinc adsorption. A further fraction was bound more tightly by the oxide deposit on the surface of the cell.
8. Exchange of zinc for different ions was rapid and reached 90% completion within three minutes. On a molar basis exchange of zinc for single ion solutions of H^+, K^+ and Ca^{2+} was in the ratio of less than 1 : 1. There were no significant differences between exchange of zinc for monovalent or divalent cations.

9. Three species of aquatic moss were used as monitors of intermittent chromium pollution in the R. Croal, N-W. England, above and below the site of a disused smelter. There was evidence of slight contamination in the river above the smelter; however concentrations of chromium in moss below the smelter were considerably higher. There was no relationship between chromium concentrations in water and in moss which supports other evidence of periodic fluctuations in the concentration of chromium in the river.
10. Laboratory studies showed differential accumulation of Cr(III) and Cr(VI) by Rhynchostegium in laboratory batch culture. These results were applied to the case study of chromium pollution and confirmed the presence of Cr(III) in water.
11. A standard method for using aquatic bryophytes to monitor heavy metal pollution is suggested. This involves transplanting moss in moss-bags above and below a suspected discharge. These are left for a predetermined length of time before being collected and analyzed according to standard methods. An elevated concentration of metal in the moss below the discharge is an indication that there has been a release of heavy metals during the period of exposure.
12. It is argued strongly that aquatic bryophytes are suitable as monitors for a wide range of heavy metal pollution problems. From the existing framework of knowledge, monitoring techniques may be adapted for many situations and at a variety of "levels" of sophistication. These include situations of intermittent heavy metal pollution where accumulation of heavy metals into a compartment which is not rapidly exchangeable may provide a record of the event for some time after it has occurred.

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APPENDIX 1

METHOD FOR USING MOSS-BAGS TO MONITOR HEAVY METAL POLLUTION

INTRODUCTION

This method is for comparison of metal concentrations in samples of _Rhynchostegium_ _riparioides_ or _Fontinalis_ _antipyretica_ from above and below a discharge. It is particularly useful if the discharge is suspected to be intermittent and thus may be missed if water analyses alone are performed. The method involves transplanting moss into the reach. If there is a large population of moss in the reach then a transplant may be unnecessary and _in situ_ moss may be used instead. It is based upon work performed in Durham, from 1979 to 1986. Further details of metal accumulation by _Rhynchostegium_ may be found in this thesis, Wehr (1983) and Wehr & Whitton (1983a; 1983b) and by _Fontinalis_ _antipyretica_ in Say & Whitton (1983). A comparison of methods for processing mosses is reported in Wehr _et_ _al_. (1983) and a study on methods for using moss-bags in this thesis.

PRINCIPLE OF METHOD

Aquatic mosses are transplanted into reaches above and below a suspected discharge. After a standard period of immersion they are removed, digested in nitric acid and their metal concentration determined.

APPARATUS

field
acid-washed container for collection of moss
ice-box
stakes
moss-bags
twine
club hammer
polyethylene bottle to transfer moss to laboratory
laboratory
petri dishes
crystallising dishes
forceps
scalpel
drying oven, set at 105 °C
heating block
boiling tubes
bench-top centrifuge
25 ml volumetric flasks
atomic absorption spectrophotometer
reagents
deionized water
2 M HNO_3 (atomic absorption grade)
standard solutions for atomic absorption spectrophotometry

METHOD

1 Collection of moss

1.1 The reach from which moss is collected should be free from any possibility of contamination by heavy metals.

1.2 The reach is defined with reference to one or more easily relocatable landmarks
1.3 The population of Rhynchostegium at the reach should not be significantly reduced by removing moss for transplanting. The moss should be available throughout the year.
1.4 Collection of moss is restricted to plants which are fully submerged and, as much as possible, located within the area of maximum current velocity. Plants are collected from at least five separate locations within the reach.
1.5 The moss is rinsed and shaken several times in stream water to remove associated sediments, invertebrates and entangled filamentous algae. Excess water is squeezed from the plants, which are stored in a polyethylene beaker rinsed several times in stream water.
1.6 Mosses are stored in an ice-box and are transferred immediately to the monitoring site.

2 Use of moss-bags
2.1 Approximately 15 - 20 g (wet weight) of moss is put into each bag. This provides about twice as many 2-cm tips as is required for one sample for analysis. One or two samples may be retained for independent analysis at this stage.
2.2 Steel stakes are positioned above and below the discharge, away from the bank, in an area with a fast current and are driven into the stream bed. The bags are attached to these by twine so that they remain submerged.
2.3 A suggested sampling program is to analyze samples from each reach every fortnight, unless there is a suspected pollution incident, when a sample should be taken as soon as possible and at intervals of approximately 12 h after this.
2.4 The sample is removed from the bag, washed several times in stream water, squeezed free of excess moisture and placed in a container. It is transferred to the laboratory in an ice-box.

3 Laboratory treatment
3.1 If samples cannot be treated immediately then they are stored in a refrigerator overnight and treated the following morning.
3.2 The moss is rinsed thoroughly in a stream of distilled water to remove obvious attached sediment and is transferred to deionized water where 2-cm tips are removed. This usually includes the main axis and several branches.
3.3 Approximately 125 tips are taken to give a final dry weight of about 200 mg. These are washed two to three times in deionized water and divided into five sub-samples which are placed in acid-washed vials and dried to a constant weight. It is suggested that additional samples for independent analysis are stored at this stage.
3.4 Dried sub-samples are digested in 5 ml of 2 M HNO_3 at about 120 °C for 45 minutes and allowed to cool. Sub-samples are centrifuged to separate the clear solution from the slurry and the clear solution is poured into a 25 ml volumetric flask. The slurry is re-centrifuged with deionized water and the supernatant is added to the volumetric flask and made up to volume.
3.5 Samples are analyzed by atomic absorption spectrophotometry against acid-matched blanks and standards. The results are converted from mass of metal per unit volume to mass of metal per unit mass as follows:

$$\text{conc. in sample } (\mu g\ g^{-1}) = \frac{\text{conc. in digest } (\text{mg l}^{-1}) \times 25 \text{ ml}}{\text{mass of sample (mg)}} \times 1000$$

3.6 The mean, standard deviation and 95% confidence limits are calculated for each sample and the samples from above and below the discharge compared.

COST PER SAMPLE

These costs are calculated assumming only very basic laboratory facilities are available. Costs are based upon prices in October 1986, exclusive of V.A.T. at 15%.

1. Capital items to equip for process

	cost
centrifuge	£300
heating block	£290
automatic pipette	£ 70
moss-bags (1)	.05 p
stakes (2)	.50 p
	£660.00

2. Recurrent items

acid (AAS grade)	.02 p
staff (3)	£ 12.00
replacement of capital items	.10 p
overheads (electricity, gas)	.30p
	£ 12.42

3. Analysis

AAS (at commercial rates)	£ 20.00

NOTES

1. Mesh bags were made from a garden mesh from a local nursary at a cost of 40p m^{-1} for a 2 m wide roll. Nylon monofilament fishing line was used to sew this into bags of approximately 15 x 20 cm (5.2). If it is assumed that 20 bags may be made from 1 m of mesh then the cost per bag is negligable. Bags may be re-used.
2. Stakes were made from mild steel and cost 50 p each.
3. Labour is assumed to cost £3.85 h^{-1}.

APPENDIX 2

RELATIONSHIP BETWEEN LANGMUIR ISOTHERM AND MICHAELIS-MENTON EQUATION

The Michaelis-Menton equation of enzyme kinetics and the Lanmuir isotherm of physical chemistry both relate reactions to the number of available reaction sites and have the same basic form.

The Michaelis-Menton equation is usually written as:

$$\frac{1}{V} = \frac{Km}{V_{max}} \frac{1}{S} + \frac{1}{V_{max}} \qquad (1)$$

where:

Km = Michaelis-Menton constant
S = substrate concentration
V = velocity
V_{max} = maximum velocity

and the Langmuir isotherm as:

$$\frac{1}{x/m} = \frac{1}{C} \frac{1}{kb} + \frac{1}{b} \qquad (2)$$

where:

x/m = amount adsorbed per unit mass
C = equilibrium concentration of adsorbate
b = adsorption maximum
k = term relating to bonding energy.

When they are used to examine Zn uptake then 1/Zn may replace 1/V or 1/(x/m) and Zn_{max} is used to describe the maximum value of Zn (= Vmax or b). Then:

$$\frac{1}{kbC} + \frac{1}{Zn_{max}} = \frac{1}{Zn_{max}} \frac{1}{C} + \frac{1}{Zn_{max}}$$

$$\frac{1}{kb} = \frac{Km}{Zn_{max}}$$

let $b = Zn_{max}$

$$\frac{1}{kZn_{max}} = \frac{Km}{Zn_{max}}$$

$$\frac{1}{k} = Km$$