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SOME ASPECTS OF ZINC ACCUMULATION IN SUBMERGED PHOTOSYNTHETIC PLANTS IN A HIGH ZINC-LEVEL STREAM

by

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M.Sc. Dissertation
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ABSTRACT

During the course of the present study data was collected in relation to the accumulation of zinc in submerged plant species in an upland stream flowing through a mineralised area.

The zinc contents of seven submerged species of algae and bryophytes were determined and related to the chemistry of the water from which they were collected. Accumulation is approximately linear for a number of species within defined environmental limits of zinc.

Evidence from transplant experiments does not support the view that tolerant strains of *Scapania undulata* (L.) Dum. exist.

The use of plants as reliable monitors of elevated levels of zinc in natural waters and the inter-relationship of environmental parameters are discussed. The possibility of using transplant techniques to monitor zinc levels is further discussed.

CHAPTER 1

INTRODUCTION

1.1.1 General Comments

In recent years great concern has been shown over the effects of pollution in the sense of the introduction of harmful materials into the environment as a result of man's activities. Disasters such as that which occurred at Minamata from 1953 - 61 when organic-mercury was released in to the aquatic environment are well documented (Tucker 1972 Irukayama 1961). Less dramatic examples however can be found in any heavily industrialised society such as Britain and as the level of operations increases, so does the problem posed by elimination of the waste materials of those activities. The natural cycles operating within the environment are no longer able to cope with the rate of discharge.

Heavy metals, defined as those with a density greater than five (Passow et al.1961), such as zinc, lead and cadmium are used extensively in industrial processes. Galvanising, electroplating, the electronics industry, paper processing and paint manufacturing industries are all producers of heavy metal wastes and all have an increasing importance in the economy of industrial nations.

In sites of natural occurrence of heavy metal ores, where lodes are exposed to weathering actions, high metal levels will be produced, but mining activities may magnify the levels to problem proportions. In Britain, one such area is the Northern Pennine Orefield which, at the end of the nineteenth and beginning of the twentieth centuries was extensively mined for lead. Although lead mining is no longer carried out, the legacy of spoil heaps, with their persistent heavy metal levels (Jones 1958), and the reworking of both spoil heaps and closed mines is a present feature of the area. Today's mining activities are for the extraction of fluorspar, a non-metallic mineral extensively used in the steel and plastics industries.

There are a number of extensive studies on the effect of mine workings, notably by Carpenter (1924) and Jones $(^{1940}_{1958})$, both working on the River Ystwyth in mid-Wales. More recent work has been concerned with the use of aquatic plants as indicators of raised levels of heavy metals in water. (The work of Goodman and Roberts (1971) and Little and Martin (1974) in devising a system for monitoring levels of Zn, Pb and Cd in air encourages the search for suitable aquatic species). Whitehead and Brooks (1969) have advocated the use of aquatic bryophytes as indicators of uranium mineralisation and Lloyd (1977) has investigated the accumulation of zinc in a number of plant species growing in waters with raised levels of heavy metals. However the number of studies is limited, and it is the aim of this project to measure some of the physical, chemical and biological parameters relating to the uptake of a heavy metal, zinc, by submerged aquatic plant species growing in an area of past and present mining activity.

1.2 The Element Zinc

Zinc, as its ore, and its oxide, cadmia was used by the Romans, being smelted, together with copper ores and calamine to produce brass, but it does not seem to have been recognised as a separate metal until 1700, by Kunkel (Sherwood-Taylor 1954). It occurs in nature as two major ores, zinc blende or sphalerite, ZnS and calamine or zinc carbonate, $ZnCO_3$, from which it is smelted before purification by electrolysis.

1.3 Zinc and Water

Before entering in to a relationship with water and being released into the environment, ores of heavy metals must be oxidised to form soluble compounds. Under natural conditions this exposure to oxidising conditions occurs when geological phenomena cause the emergence of ores to the atmosphere. Man's influence can accelerate the process by means of drainage from mines and the formation of spoil heaps. Sulphide is the commonest form of heavy metals and oxidation would be expected to produce sulphate ion, SO_4^{2-}, and also intermediate products of free S and SO_2, but these occur rarely in oxidised zones (Krauskopf 1967). Conversion occurs to insoluble compounds which are stable under surface conditions - oxides, carbonates, sulphates or silicates - which are carried down to unoxidised regions of the ore deposit and precipitated by reaction with sulphide minerals. Any soluble products formed will be removed completely in run off. The importance of water in the oxidation process is not completely understood. Krauskopf (1967) suggests its role as that of providing carbonic acid to dissolve out sulphide to form ions and allow quicker reaction with oxygen, while Sato (1960) proposes the oxidation of water to hydrogen peroxide, H_2O_2, which then oxidises the sulphide. An overall equation for the oxidation of zinc sulphide might be:

$$ZnS + 2O_2 + 2HCO_3^- \rightarrow ZnCO_3 + H_2CO_3 + SO_4^{2-}$$

but it is more realistic to imagine the oxidation as a number of small steps each taking place in slightly different chemical environments and the actual pathway differing with local conditions.

This oxidation of sulphide leads to the formation of acid solutions, either by hydrolysis of the metal ion or by precipitation of the insoluble hydroxide, the degree of acidity varying with the proportion of different metal ions present in the ore bed. Where a large proportion of iron ore in the form of sulphide is present, ferric oxide is formed which is very insoluble:

$$4\,FeS_2 + 15O_2 + 8H_2O \rightarrow 2Fe_2O_3 + 8SO_4^{2-} + 16H^+$$

Mean levels of zinc in surface waters may be rather meaningless because of the variability in local conditions and also the extent of man's influence, but Wedepohl (1972) suggests a figure of 10 $\mu g\ l^{-1}$ Zn. Abdullah and Royle (1972) find an average level of zinc in streams unaffected by ore deposits of 11 $\mu g\ l^{-}$. Reported levels of Zn in natural waters however are difficult to evaluate since the method of water collection, particularly in earlier work, is often not specified nor whether it represents the total water sample or one which has been filtered to remove suspended materials. In his large scale survey in 1969 of the elemental content of stream sediments over the U.K. Thornton (1974) finds very high levels of Zn, $>$800000 p.p.m, in areas of past and present mining activity (Fig 1.1), while in an analysis of fluorspar mine waste in Weardale a level of 20 360 $\mu g\ g^{-1}$ Zn is found by Cooke et al.(1976).

1.3.1. Factors affecting the solubility of Zinc in Water

i. pH

Streams with low pH values ($<$3), generally associated with coal mining activity, are associated with high levels of heavy metals (Hargreaves et al. 1975). This can be related to the precipitation of

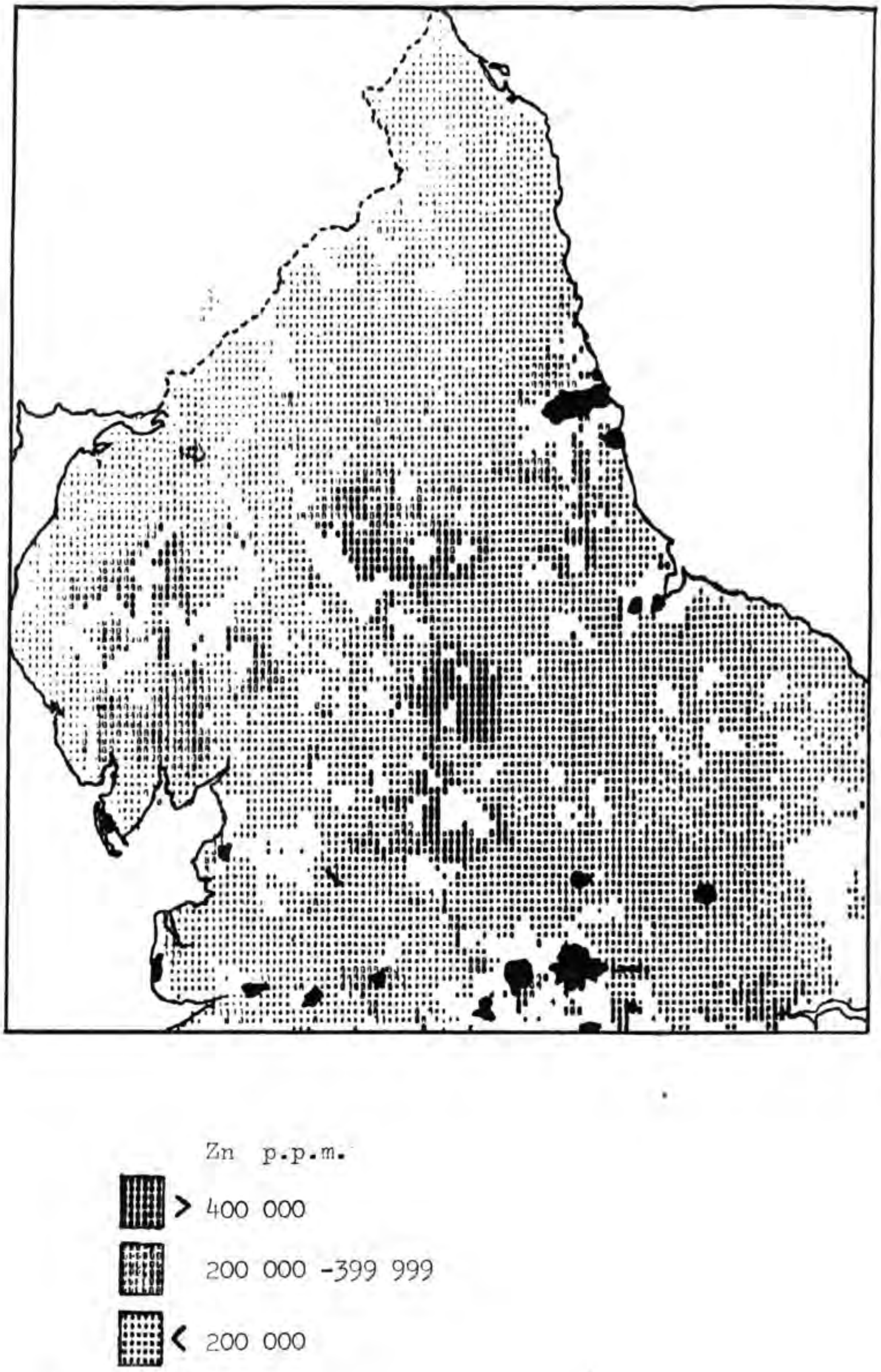


Fig. 1.1 Map showing the distribution of zinc in sediments collected from streams in Northern England during 1969

(after Thornton 1974)

insoluble hydroxides in simplified systems under conditions falling below pH7 (Bachmann 1961).

$$Zn^{2+} + H_2O \rightleftharpoons ZnOH^+ + H^+ \rightleftharpoons Zn(OH)_2 \downarrow + H^+$$

Jurniak and Inouye (1962) demonstrated the solubility of zinc in distilled water in a $ZnCl_2$ - NaOH system and show that as the pH rises above 6.8 significant decreases in the level of dissolved Zn occur. Grime and Hodgson (1969) report that many minerals become soluble as pH falls and as they then become more available for uptake, become toxic. As pH falls below pH 4.5 in soils aluminium toxicity is induced in *Rumex acetosa* and *Holcus lanatus* resulting in a catastrophic decline in their % occurrence.

In stream conditions the simple model of Jurniak and Inouye will be complicated by competition reactions occurring between Zn and other cations and this phenomenon is used in the precipitation of heavy metals in settlement ponds (P. Entwistle pers. comm.) (1.7)

ii. Complexing of Zn

The production of the hydroxide complex of zinc is also complicated by the presence of other anions In sea water Zirino and Yamamoto (1972) show that at pH 8.1 it is of greater importance than others, but as pH is lowered to pH 7.0 its importance decreases, with an increase in Chloride ($ZnCl^+$ and $ZnCl_2^o$), carbonate ($ZnCO_3^o$) and sulphate ($ZnSO_4^o$) complexes. Hem (1972) in reviewing various data on the solubility of zinc in various inorganic aqueous environments indicates a minimum value for zinc concentration at pH 9.5, while remaining in solution as the dominant species (Zn^{2+}) up to pH values a little over 7.0.

The effect of elevated phosphate levels is demonstrated by Jurniak and Inouye (1962) in their work on the solubility of zinc in distilled water, where at high levels all zinc is precipitated as zinc phosphate. The effect of increasing pH on this reaction is to decrease levels of ionic zinc (Zn^{2+}) and phosphate. In upland streams where man's activities are minimal, low levels of phosphate would be expected, but where for instance a sewage treatment plant was operating then phosphate enrichment might be expected to have an effect on zinc solubility. Rana and Kumar (1974) also propose the formation of complexes between phosphate and zinc ions at cell membranes rendering the zinc less mobile.

iii. Organic Complexes

Stumm and Morgan (1970) find that many heavy metals are found in stream and lake waters at much higher concentrations that can be explained by their theoretical solubilities and propose a classification of metal ions and complexes in natural waters. In addition to free ions they separate particles on their size in to inorganic complexes ($<$ 10nm) chelates and colloids (10 - 100nm) and large colloids and precipitates ($>$100nm). A number of substances have been proposed as ligands in forming complexes with cations and increasing their solubilisation. Perhac (1974) notes that flowing waters can carry large amounts of heavy metals in colloidal form. Many of these colloid are associated with organic acids produced by decaying plant material (humic and fulvic acids) forming complexes (Shapiro 1964). The upper reaches of upland streams, draining off blanket peat can be expected to contain high levels of humic compounds and this author has observed brown organic material in the upland reaches of the River Wear system and they have also been observed by Say (1977) in the River South Tyne catchment.

The metal complexing capacity of the humic acids of soil is noted by Randhawa and Broadbent (1965) to be pH dependent. The total zinc forming complexes with humic acids increases with rise in pH up to 8.5, but declines again at high values. Schnitzer (1971) shows that zinc can be mobilised by fulvic acids at pH levels at which it would normally be insoluble.

iv. Total Alkalinity

Ernst, Allen and Mancy (1975) produce solubility curves for zinc at different levels of alkalinity and show the lowest solubility with the highest alkalinity, but further show the overriding effect of pH, with rapid losses of Zn occurring at all alkalinities when pH rises above pH 7.5. Say (1977) finds a marked reduction in the toxicity of zinc to laboratory population of the alga Hormidium rivulare in the presence of both Ca and Mg and observes large numbers of 'particles' deposited in the mucilage attached to Hormidium rivulare at pH values

7 and 8, making the tentative suggestion that they could be localised deposits of precipitated Zn $(OH)_2$.

1.3.2. Water Flow

Levels of soluble metals in natural waters are believed to be higher during low flows (Jones 1940, 1958) when dilution by uncontaminated run off is least. Low flows occur typically during summer months. In contrast, the suggestion has been made that metals weathered to soluble for may be flushed out of old mine workings and tailings by rain falling after warm dry spells (Carpenter 1925). In a programme of sampling and analysis from the River Ystwyth in mid-Wales, Grimshaw et al.(1976) show both seasonal and short-term variations. High concentrations of zinc are observed both at times of low flow and during the initial phases of storm run off, and they point to summer convection storms in mining areas as being of particular importance to river biota. In relation to seasonal variatio they find a much greater range in bulk metal supply than in concentrations. Clearly duration of contact with sources of Zn, whether of an intermittent or continuous nature will affect accumulation in the biota.

1.4 Zinc in Plant Material

1.4.1 The role of Zinc in plant metabolism

The growth promoting effect of zinc on the mould *Aspergillus niger* was reported in 1869 by Raulin and demonstrated on maize by Mazé in 1914, but its essential plant nutrient effect was not generally accepted until the 1930's (Price 1970). Since then its essentiality in small amounts has been shown in Rhizopus nigricans (Waksman and Foster 1938), flagellates (Ondratscheck 1941), Chlorella pyrenoidosa (Myers 1951) and rats (Todd et al.1934). Higher plants require of the order of 1 μ mole zinc g^{-1} dry weight (Price 1970). Price and Quigley (1966) working on cultures of Euglena show a linear relationship between growth rate and internal zinc concentrations. Synthesis of specific cell constituents may require more zinc than normal growth and zinc is reported as a constituent of cytochrome c in Ustilago sphaerogena (Brown et al.1966) and for specific enzyme production in Escherichia coli (Torriani et al.quoted by Price 1966). Price(1970) reports that the ability of plants to remove zinc is retained down to less than 10 nM but it appears that once assimilated in to a tissue, it is virtually immobile (Millikan and Hanger 1965).

1.4.2 Zinc accumulation in aquatic plants

Accumulation is the process whereby a plant can take up ions against a gradient so that the internal concentration is greater than that in the external environment. Two approaches have been adopted by workers to the process of accumulation of heavy metals. The first attempts to relate elemental composition of plant material to that of the aquatic environment, while the second investigates the physiological mechanism of elemental uptake, frequently employing radio-nuclides as labels. One example of this second approach is the study by Pickering and Puia (1969) on the uptake of ^{65}Zn in Fontinalis antipyretica which shows three phases. The first is rapid and is explained as processes of ion-exchange and adsorption in the free-space of the cellular membrane; the second lasts some

90 minutes and is uptake through the outer layer of the protoplast, which acts as a diffusion barrier; the final phase, which can be inhibited by metabolic factors, is
O / one of active accumulation in the cell vacuale. McLean and Jones (1975), in a study of Scapania undulata find two uptake mechanisms, an active system at zinc levels of 0-10 mg 1^{-} Zn and a passive system at levels of 10-25 mg 1^{-}Zn. They propose a series of sites, possibly within the cell wall, for active uptake, which when saturated at higher levels of Zn allow the metal to swamp the cell.

In the terrestial moss, Rhytidiadelphus squarrosus Gullväg et al.(1974) show accumulation of lead within the cytoplasm and nucleus as lead inclusion bodies and Stokes (1973) finds intranuclear inclusions in copper tolerant Scenedesmus sp. when fed on Cu SO_4.

Bryan (1969) studying uptake of ^{65}Zn in Laminaria digitata finds it to be a gradual net uptake process with no evidence that exchange of zinc takes place. Because uptake is not regulated, the older part of the lamina contains more zinc than younger portions. Similarly, Lloyd (1977) finds increased levels of zinc, lead and cadmium in older material of Fontinalis antipyretica in the River Wear system, and Harding (1978) increasing levels of zinc in older sections of filaments of Lemanea fluviatilis from the River Derwent.

The relationship between levels of metals in plant material and that in their aquatic environment gives rise to a quantitative assessment of the accumulation of heavy metals by aquatic organisms and the term 'enrichment ratio' was proposed by Brooks and Rumsby (1965) in their studies of New Zealand bivalves. Other workers have used similar terms including concentration factor (Bowen 1966) and enrichment factor (Dietz 1973) but the term enrichment ratio, as defined below and used by Whitton and Say (1975) is used in this study.

$$\text{Enrichment ratio} = \frac{\text{Concentration in the organism } (\mu g\ g^{-1} \text{ dry weight}}{\text{Concentration in surrounding medium } (mg\ l^{-1})}$$

Studies of accumulation of heavy metals in terrestial plants are numerous including work by Turner (1969), Antonovics et al.(1971) on angiosperms, Brown and Bates (1972), Ward et al.(1977) on bryophytes. Preston et al. (1972), Foster (1976) show accumulation in marine algae. Less work is available on fresh water species. An analysis of 14 freshwater genera by Boyd and Lawrence (1967) gives enrichment ratios varying from 3 000 to 12 000 for the majority of elements and marked enrichment for Zn, Cu, Fe and Mn. Studies in the old lead mining area of Durham by Leeder (1972), Harding (1977) and Lloyd (1977) show a wide variation for a number of aquatic species. (See Table 1.1).

1.4.3 Submerged plants as monitors of zinc

The use of aquatic plants as monitors or indicators of heavy metals hinges on the stability of the enrichment ratios if the conditions affecting the plant and the water remain constant. Field studies by Keeney et al.(1976) and Lloyd (1977) on fresh water algae and bryophytes show considerable enrichment of zinc but there is little comparability between species and this is probably to be

TABLE 1.1 ENRICHMENT RATIOS FROM STUDIES IN THE NORTHERN PENNINE OREFIELD

AUTHOR	SPECIES	ENRICHMENT RATIO
Leeder (1972)	*Lemanea fluviatilis*	7000 - 92000
	Hygrohypnum ochraceum	15000 - 300000
	Mimulus guttatus	500 - 4000
Lloyd (1977)	*Mougeotia* sp.	1030
	Scapania undulata	1150 - 130000
	Hygrohypnum ochraceum	45000 - 120000
	Mimulus guttatus	11500 - 38500
Harding (1978)	*Lemanea fluviatilis*	3000 - 16000
	Scapania undulata	11000 - 37000
	Hygrohypnum ochraceum	5000 - 16000

TABLE 1.2 PREVIOUS REPORTS OF ALGAL AND BRYOPHYTE SPECIES OCCURRING IN STREAMS CARRYING HIGH LEVELS OF HEAVY METALS

AUTHOR	SPECIES
Carpenter (1924)	*Batrachospermum* sp., *Lemanea fluviatilis*
Reese (1937)	*Ulothrix* sp., *Stigeoclonium tenue*, *Lemanea fluviatilis*, *Mougeotia* sp., *Microspora* sp.
" (1940)	*Scapania undulata*
Whitton (1970)	*Microspora* sp., *Stigeoclonium tenue*, *Spirogyra* sp., *Ulothrix* sp.
McLean and Jones (1975)	*Hormidium rivulare*, *Scapania undulata*, *Microspora* sp., *Ulothrix* sp.
Griffiths et al. (1975)	*Ulothrix* sp., *Hormidium* sp., *Mougeotia* sp.
Say (1977)	*Hormidium rivulare*, *Mougeotia* sp., *Scapania undulata*, *Philonotis fontana*, *Dicranella varia*, *Dichodontium pellucidum*

expected since chemical conditions such as pH, species of metal present are likely to be very different. In reviewing accumulation of heavy metals by algae, Lloyd (1977) finds great variation from species to species and even within the same species of alga Harvey and Patrick (1967) point to surface area - volume relationships as being important and differences in morphology, growth rate and seasonal appearance would seem to be significant. One advantage in the use of algae as monitors is that they obtain metals only from the surrounding waters but this is not so clearly the case in bryophytes with their rhizoidal attachments to the substratum. Lloyd finds accumulation in bryophytes to higher levels than any algae or angiosperms and Empain (1976) points to the integrating effect of aquatic bryophytes in smoothing out fluctuations of concentration of heavy metals in the aquatic environment. Many species of bryophytes however do not remain permanently submerged particularly through summer droughts and this author has observed periodic inundations and exposures of a number of bryophytes during the course of the present study. Contamination by silt and growth of epiphytic species are two further problems, affecting both algae and bryophytes, experienced by this author and by Lloyd (1977).

1.5 Tolerance to Heavy Metals

In a review of heavy metal tolerances in plants Turner, (1968) reports many species as being tolerant and able to survive at concentrations of heavy metals which exclude other species. Thus, studies on lead tolerant Agrostis tenuis (Jowett 1964), and Festuca ovina (Wilkins 1957) and copper tolerant Agrostis tenuis (McNeilly and Bradshaw 1968) show the specificity of the tolerances and their high heritability Whitton and Say (1975) report Lemanea sp. and possibly Batrachospermum sp. as being highly resistant to both zinc and lead with the green algae Microspora sp. and Ulothrix sp. as being tolerant to copper, zinc and lead. Say (1977), in studying populations of Hormidium sp. growing in high concentrations of Zn, shows them to be adapted forms and for Hormidium rivulare that this adaptation may be genetically determined. Duncker (1976) in a transplant and toxicity tests of populations of the bryophyte Scapania undulata from high and low concentrations of zinc in stream water fails to support evidence for resistant strains, but her investigation was made difficult by drought affecting all field populations.

Turner (1968) in reviewing tolerance proposes two possible mechanisms, one of exclusion from the metabolic system of heavy metal ions, and one of isolation of the metal within the cell allowing normal processes to continue. He points to the role played by the cell wall in acting as a heavy metal accumulator in plants.

1.6 Species diversity in high Zinc-level streams

Extreme environments in the sense of Brock (1969) are characterised by a low species diversity and Whitton and Say (1975), in reviewing the effects of heavy metals on the flora and fauna of rivers, report the effect of Zn in reducing the number of 'dominant' species. In a survey of the flora of high zinc-level streams, Say (1977) finds that filamentous green algae, in particular Hormidium rivulare and Mougeotia sp. are the most abundant algae throughout a range of high zinc values with other algae showing more restricted distributions. Scapania undulata, Philonotis fontana and Dicranella varia were the bryophytes capable of growing throughout the range of elevated zinc values. At highest zinc levels he finds algal communities dominated by species including Hormidium, Mougeotia and the bryophytes Bryum pallens, Pohlia nutans and Dichodontium pellucidum. In a botanical survey of Rookhope Burn, Leeder (1972) finds the stream to be floristically poor with only one bryophyte, Hygrohypnum ochraceum and one alga, Lemanea fluviatilis growing in sufficient quantities for his analysis of heavy metals.

Table 1.2 shows some of the species previously reported from streams with high Zn-levels.

1.7 Background to area of study

The following discussion of geology and mineralogy of the study area is largely from Dunham (1945).

The Pennine Ridge from the English Midlands to the Scottish Borders is characterised by continuous outcrops of lower Carboniferous strata which are extensively mineralised in Derbyshire and a group of northern dales (Allendale, Alston Moor, Weardale and Teesdale). In the northern dales of the Alston Block (Fig 1.2) the majority of lead-zinc deposits are found in either the sandstone shales or limestones of the Yoredale Series underlying the Millstone Grit (Fig. 1.3). This Northern Pennine Orefield includes the River Wear Basin.

Fig. 1.2 Regional Geology of North Eastern England

(After Smith, 1923, Fig. 2.1)

———————	Base of Permian
-----------	Base of Carboniferous
∘ ∘ ∘ ∘ ∘ ∘	Major faults

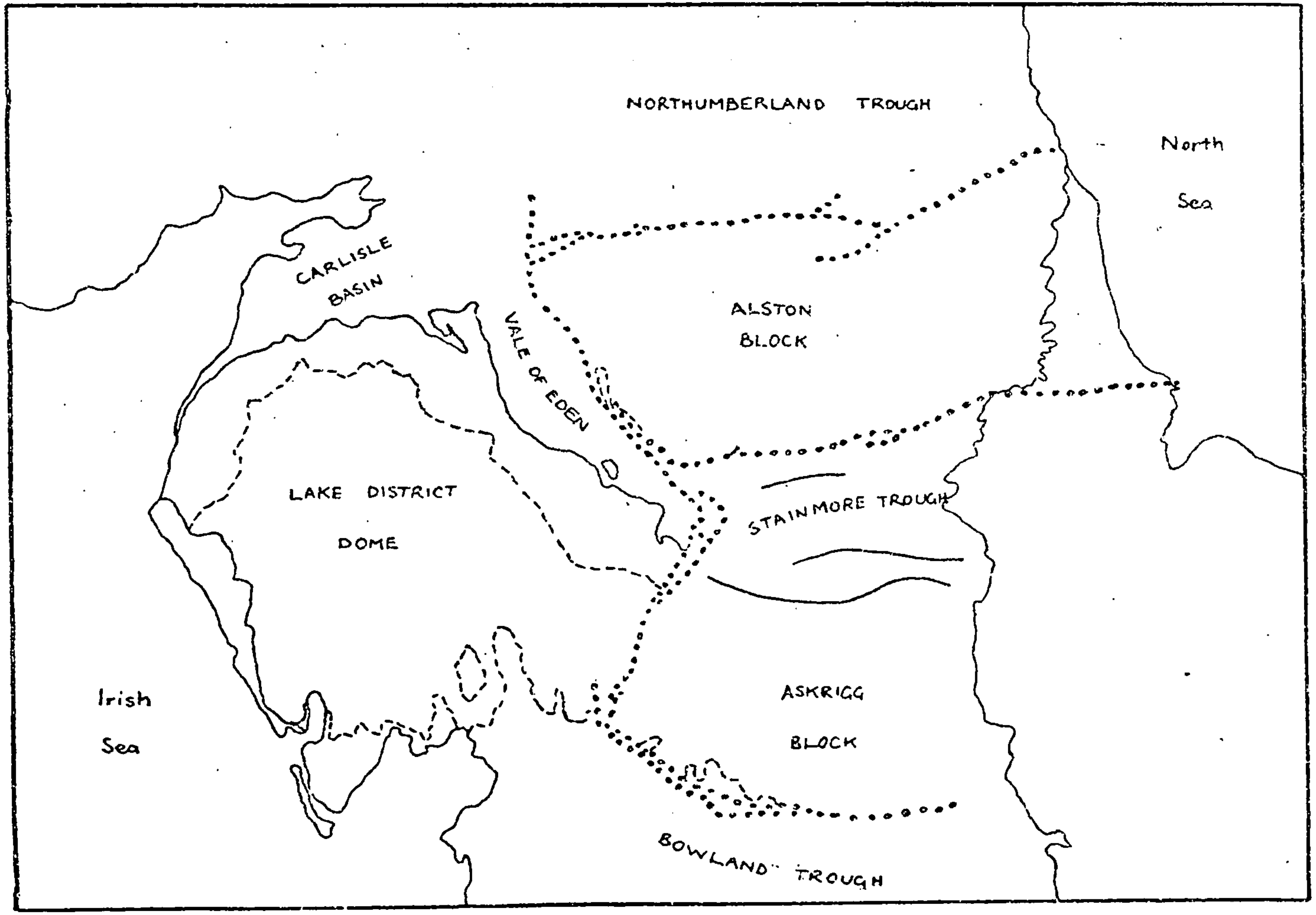
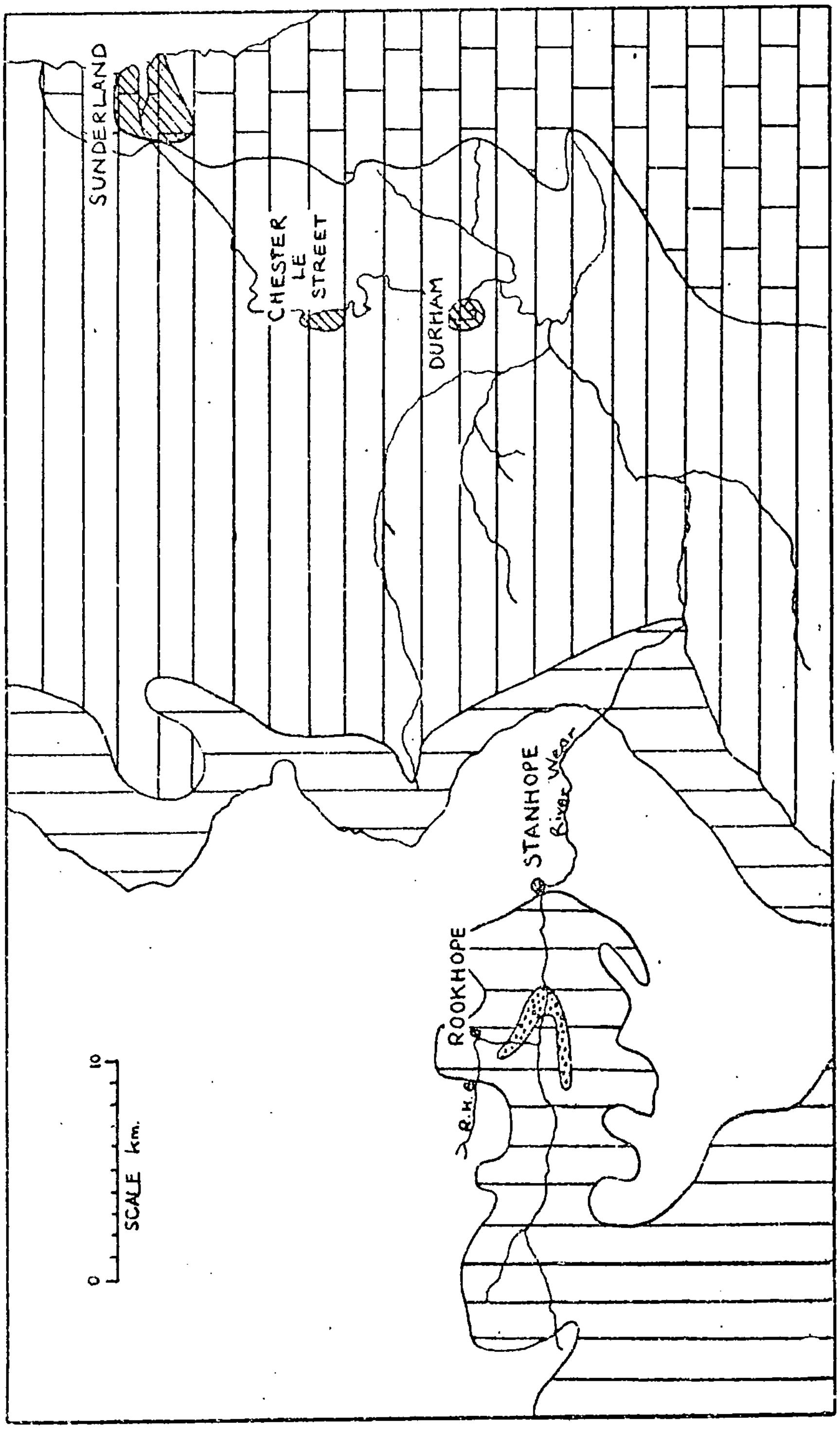

NORTHUMBERLAND TROUGH
North Sea
CARLISLE BASIN
ALSTON BLOCK
VALE OF EDEN
LAKE DISTRICT DOME
STAINMORE TROUGH
Irish Sea
ASKRIGG BLOCK
BOWLAND TROUGH

Fig. 1.3 Section map of the River Wear Basin showing the geological structure.

□	Carboniferous Limestone Series
▥	Millstone Grit
▤	Coal Measures
▦	Magnesian Limestone
░	Whin Dyke
RHB	Rookhope Burn


SUNDERLAND
CHESTER LE STREET
DURHAM
STANHOPE
River Wear
ROOKHOPE
R.H.e
SCALE km.
0
10

The metalliferous area of Weardale extends eastwards from the pass of Killhope for some 34 km to Harperley embracing in addition to the valley of the Wear, side valleys to north and south including Rookhope Valley, the area of the present study. The orefield was formerly the most productive area in England for zinc and lead concentrates and since the end of the nineteenth century is second only to Derbyshire in the production of fluorspar. Mineral veins intersect throughout the orefield with minerals occurring as bands in veins running vertically or thin horizontal sheets or flats (fig. 1.5).

The minerals of economic importance formed in the deposits, referred to as primary minerals, consist of galena (PbS), sphalerite ($Zn\,S$) and fluorite (CaF_2). Barite ($Ba\,SO_4$) and witherite ($Ba\,CO_3$) formed the basis of a thriving barytes industry at one time in Weardale, but in Rookhope Valley form, along with quartz (SiO_2), chalcopyrite ($CuFeS_2$) and calcite ($CaCO_3$), the major gangue minerals.

Rookhope Burn arises as a number of small streams flowing off the fells to the east of Allenheads Village (grid reference NY 862453). It runs east for some 7 km to Rookhope Village (grid reference NY 940427) and then south for 5 km to join the River Wear beyond Eastgate (grid reference NY 953386)

Lead mining has a long history in Rookhope Valley dating back to Norman times, but the large scale development was begun in the early eighteenth century by the Beaumont Company. The Weardale Lead Company took over the mining activities in 1883 and actively produced lead until 1931. The present mining in the Valley is for fluorspar, two old lead mines having been re-opened. Grove Rake mine

Fig. 1.4 Sketchmap of Rookhope Burn showing sites of mining activity and sampling sites of present study.

▨	Active fluorspar mine
✱	Old mining shafts
⩫	Mine tailings
●	Sampling site (1 - 6)
⟶	*Scapania* transplant site (A-C)

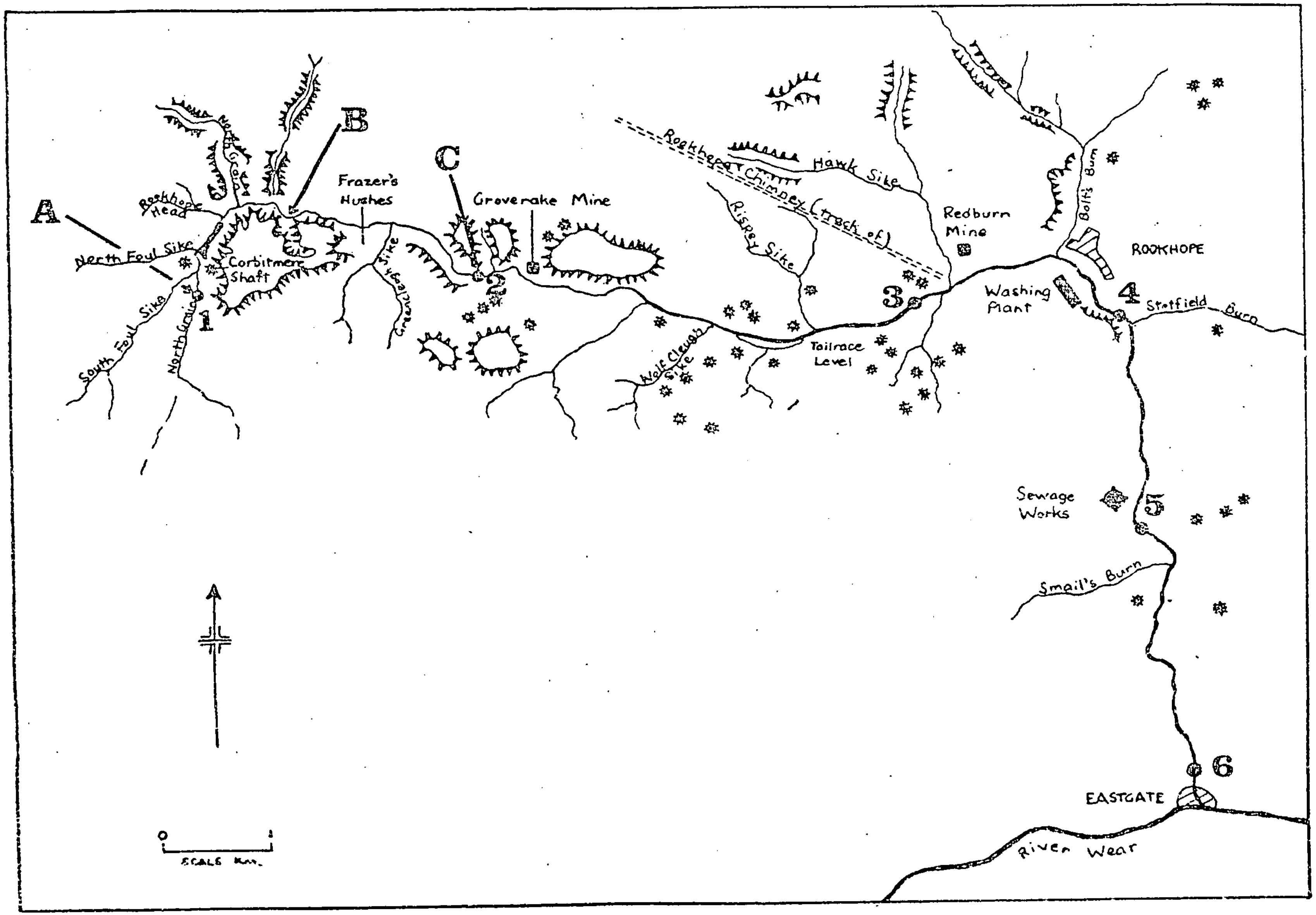
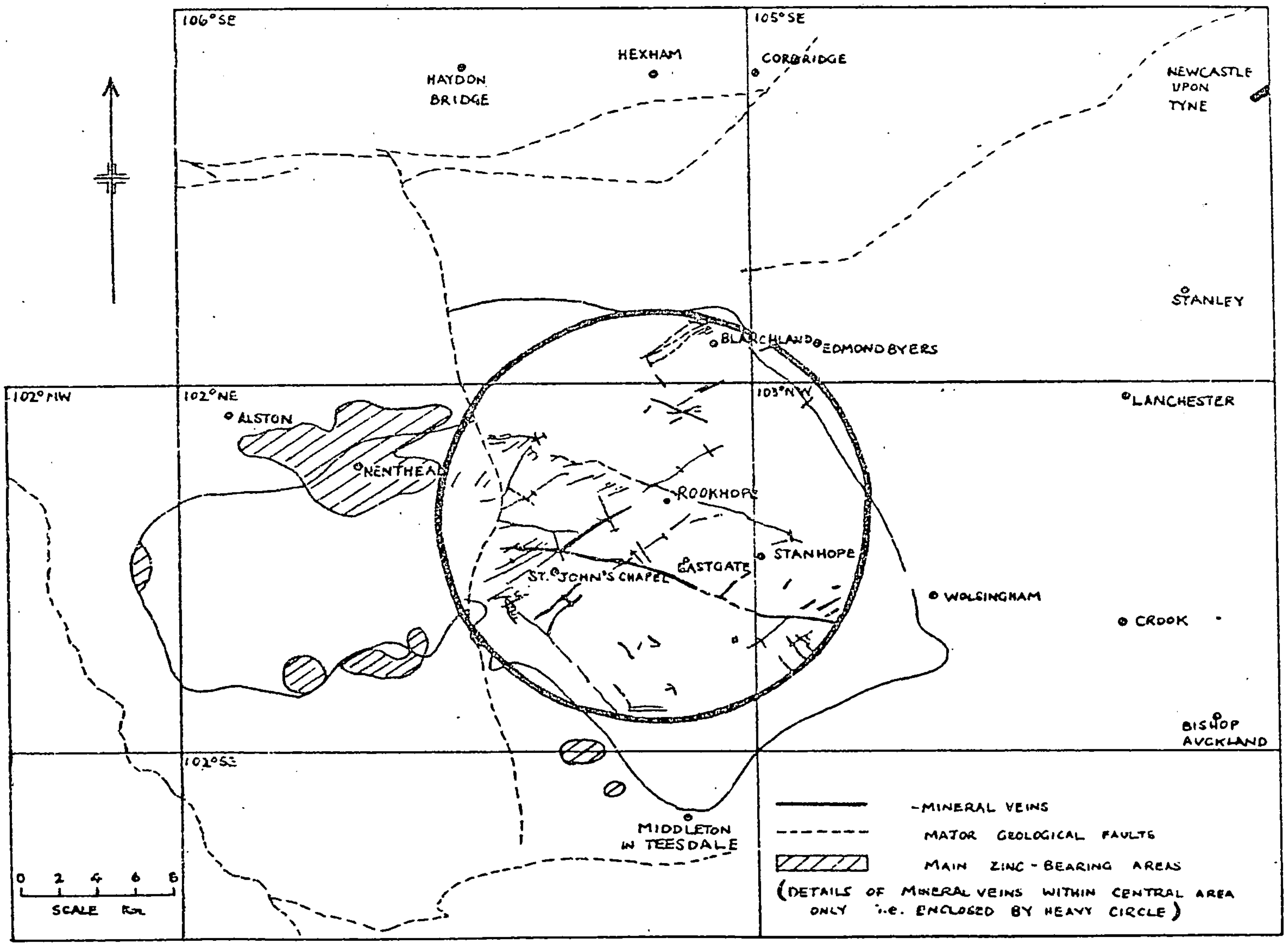

A
B
C
1
2
3
4
5
6
Rookhope Head
North Grain
North Foul Sike
South Foul Sike
North Grain
Corbitmere Shaft
Frazer's Hushes
Greencleugh Sike
Groverake Mine
Rookhope Chimney (track of)
Hawk Sike
Rispey Sike
Wolf Cleugh Sike
Tailrace Level
Redburn Mine
Bolt's Burn
ROOKHOPE
Washing Plant
Stotfield Burn
Sewage Works
Smail's Burn
EASTGATE
River Wear
0
1
SCALE Km.

Fig. 1.5 Sketch map showing the distribution of minerals in the Northern Pennine Orefield.

(After Dunham 1948)

NOTE: Mineral Veins only included in central area.


106°SE
105°SE
102°NW
102°NE
103°NW
102°SE
HAYDON BRIDGE
HEXHAM
CORBRIDGE
NEWCASTLE UPON TYNE
STANLEY
BLANCHLAND
EDMONDBYERS
LANCHESTER
ALSTON
NENTHEAD
ROOKHOPE
STANHOPE
EASTGATE
ST. JOHN'S CHAPEL
WOLSINGHAM
CROOK
BISHOP AUCKLAND
MIDDLETON IN TEESDALE
0 2 4 6 8
SCALE Km
MINERAL VEINS
MAJOR GEOLOGICAL FAULTS
MAIN ZINC-BEARING AREAS
(DETAILS OF MINERAL VEINS WITHIN CENTRAL AREA ONLY i.e. ENCLOSED BY HEAVY CIRCLE)

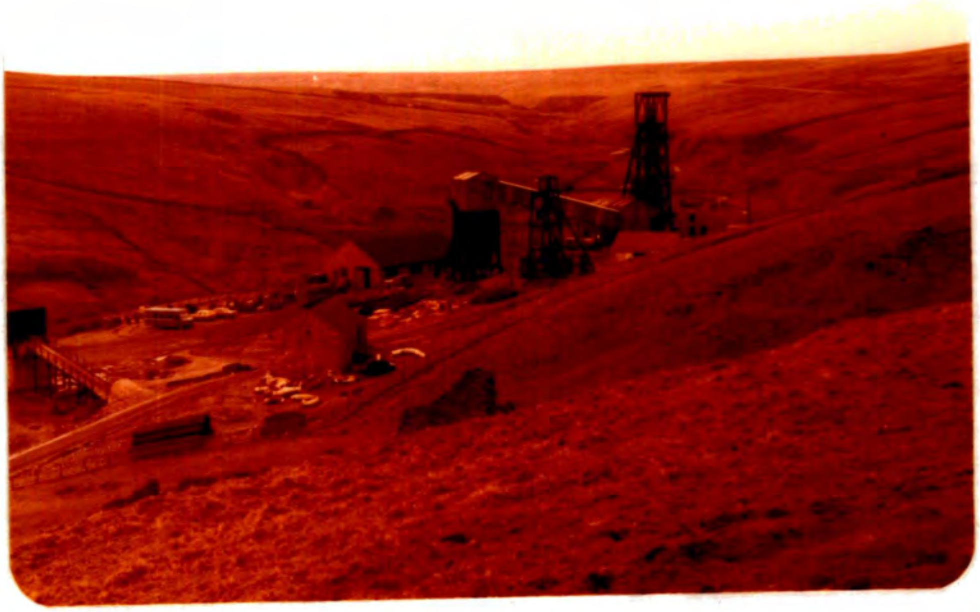
(grid reference NY 894442 Fig 1.4, 1.6) near the head of Rookhope Burn, operated by the British Steel Corporation, and Redburn Mine (grid reference NY 928431; fig 1.4) just above Rookhope Village, now operated by Swiss Aluminium Mining (U.K). This latter corporation also operates a washing plant in Rookhope Village which treats ore from the mine and processes the old lead mine tailings from around the smelter. Associated with this plant are a number of settlement pools in which ore from which fluorspar has been removed is first treated with ferrous sulphate and later lime to precipitate heavy metals before the effluent is discharged in to Rookhope Burn (P. Entwistle pers. comm). Further prospecting by Swiss Aluminium Mining (U.K) over some 200 sq. miles at present being carried out may lead to the sinking of new shaft

The earlier mining activities have left behind large heaps of waste tailings on the banks of Rookhope Burn from Corbitmere Dam (grid reference NY 877446; Fig. 1.4) down to the village of Rookhope and drainage channels and from the mines themselves. Run off from the tailings heaps and mine drainage water contribute to the waters of the stream.

The only other occupation of any significance in the valley is sheep farming on the upper fells and to improve grazing land extensive drainage has been carried out involving the cutting of deep channels through the peat to assist run off of surface waters.

Fig. 1.6 (a) Upper Rookhope showing tailings heaps.

(b) Upper Rookhope showing Grove Rake mine.



1.8 Aims of the present study

Jones (1958, 1964), and Jones and Howells (1969), in work on the River Ystwyth and Rheidol in mid- Wales show that zinc pollution is a persistent problem whether due to drainage from old mine workings or work relating to new prospecting. The catchment area of Rookhope Burn lends itself well to a study of accumulation of zinc in submerged aquatic species having effects from old mine workings and present mining activities.

The aims of the present study in this area are then:

i. to investigate the distribution of submerged algae and and bryophytes with a view to assessing their usefulness as monitors of zinc pollution.

ii. To collect botanical and hydrogeochemical data on levels of zinc in submerged algae and bryophytes in a stream receiving effluent from areas of past and present mining activity.

iii. To investigate the effect on growth of transplanting plant material from regions of low and higher zinc concentrations with a view to discovering evidence of ecotypic tolerance within a species.

CHAPTER 2

MATERIALS AND METHODS

2.1 Water Analysis

2.1.1 Collection and Storage of Water

All containers used for collection and transport of water were soaked in 10% hydrochloric acid for at least three hours and repeatedly washed in distilled water before use to ensure freedom from contamination. Water samples were collected from the main current of the stream, just below the surface.

(i) Water for cation analysis was allowed to stand for at least 10 minutes in a 2l polythene beaker to allow large suspended particles to precipitate. 'Total' samples of about 30 cm^3 were transferred to a 70 x 25 mm snap-top glass specimen tube. 30 cm^3 'nuclepore' samples were obtained by filtering water through a Swinnex - 25 filter fitted with a 25 mm diameter 'Nuclepore' polycarbonate membrane, pore size 0.2 µm. Acid-washed disposable plastic syringes were used to force water through the filters with 25 cm^3 of distilled water and 10 cm^3 of stream water being passed through and discarded initially. This size of pore provides a better separation of suspended materials from dissolved or complexed metals than filters with a larger pore size, say 0.45 µm which may allow passage of some clay-size particles (Kennedy, Zellweger and Jones 1974). Both 'total' and 'nuclepore' samples were acidified by the addition of one drop of Aristar-grade concentrated nitric acid.

(ii) Water for anion analysis was filtered through an acid-washed No. 2 'Sinta' glass funnel in to a 2l polythene beaker. Three samples of 200 cm^3 of filtered water were collected in rigid screw-top polythene bottles. Water for phosphate analysis was collected in a bottle impregnated with iodine to prevent bacterial activity prior to analysis.

Samples were kept cool in an ice-box until return to the laboratory. Cation samples were stored at 4°C and anion samples deep frozen until analysis was carried out.

A final sample of water was collected in a polythene bottle, capped underwater to exclude air, and returned to the laboratory in the ice-box for immediate determinations of pH, optical density and electrical conductivity (2.1.2, 2.1.5, 2.1.6).

Single samples of water were taken for each analysis since the coefficient of variation within site is well within acceptable statistical limits (Leeder 1972).

2.1.2 Environmental Parameters

(i) pH was measured in the field using a Pye Unicam 293 portable pH meter and in the laboratory using an E.I.L. 23A direct-reading pH meter.

(ii) Temperature was recorded in the field using a standard laboratory thermometer.

(iii) Total alkalinity, expressed as mg l^{-1} $CaCO_3$, was determined in the field using the potentiometric filtration technique recommended by the American Public Health Association (1971).

(iv) Optical density measurements at wavelengths 420 nm, 240 nm and 254 nm of 'nuclepore' filtered samples were measured on a Uvispek spectrophotometer in 4 cm cells.

(v) Electrical Conductivity was measured in μ mho cm^{-1} on an E.I.L. portable conductivity bridge, Model MC-1, MkV.

(vi) Water flow was estimated at each visit on a five point scale, 1 = low flow 5 = high flow.

2.1.3 Cation Analysis

All analysis for Cations was completed on a Perkin-Elmer 403 atomic absorption spectrophotometer. Lead and cadmium were analysed using the Tm sampling boat procedure (Kahn et al.1968) to allow low levels of these elements to be determined. Zinc and iron were analysed using a standard aspiration technique and calcium and magnesium were determined by standard aspiration technique after addition of standard amounts of lanthanum chloride (Perkin-Elmer manual 1971).

2.1.4 Anion Analysis

NH_4-N, NO_3-N, NO_2-N PO_4-P were analysed using the methods described by Stainton et al.(1977), colorimetric determinations being carried out on a Uvispek spectrophotometer in 4 cm cells at the appropriate wavelength. Calibration was by preparation of five standards in the concentration ranges of each anion.

	ANION	METHOD	DETECTION LIMITS	STANDARDS	WAVE LENGTHS
i.	NH_4-N	reaction of ammonia with phenol and hypochlorite under alkaline conditions to form indophenol blue	5-1000 μg l^{-1}	0.01,0.05,0.10,0.20 0.50 μg l^{-1}	643 nm
ii.	NO_3-N	reduction by copper-cadmium couple to nitrite, NO_2-N (See below)		0.01,0.05,0.10,0.20 0.50 μg l^{-1}	
iii.	NO_2-N	determination by formation of pink-azo dye	1-500 μg l^{-1}	0.001,0.002,0.004 0.005,0.01 μg l^{-1}	543 nm
iv.	PO_4-P	reduction by acid-molybdate and ascorbic acid to form a blue complex	5 - 250 μg l^{-1}	0.01,0.02,0.05 0.10,0.20 ug l^{-1}	885 nm

2.2 Plant Analysis

2.2.1 Collection and Storage of Plant Samples

Samples of plants to be analysed were collected in 50 x 15mm acid-washed plastic specimen tubes, four replicates for each sample from suitable areas within the site.

Scapania undulata grows in dense tufts attached to rocks by rhizoids. Healthy 1 cm tips of submerged material were collected, washed in stream water to remove large sediments and returned to the laboratory in an ice-box, where they were washed in distilled water. As a general principle washing was kept to a minimum to prevent excessive leaching out of metals, although Jupp (unpublished data) finds little leaching of zinc from *Scapania undulata* when allowed to stand in distilled water for two days - 3.4% after one day and a further 0.9% after two days.

Hygrohypnum ochraceum grows on rocks at the side of the stream and on exposed rocks in the main flow. Its pleurocarpous habit traps much silt but 1 cm tips collected and washed as for *Scapania* shoots proved to be free of silt.

Dichodontium pellucidum growing on rocks exposed at low and medium flows only shows healthy growth after submergence following heavy rain. 1 cm tips were collected and washed as for *Scapania*.

Batrachospermum sp. grows submerged in fast flowing water attached to sandstone rocks. The attachment organs were removed completely during laboratory washing and only healthy growth analysed. It proved silt free and the easiest species to prepare for digestion.

Lemanea fluviatilis specimens were always free of silt. 2 cm tips were removed and washed.

Mougeotia sp. and *Stigeoclonium tenue* filaments retain inorganic and organic debris which was removed as far as possible by dissecting out filaments on an acid-washed ceramic tile using stainless steel instruments.

After washing, samples were dried in acid-washed snap-top specimen tubes at 105^{o}C for 48 hours. The time elapsing before drying was kept as short as possible, and was never more than eight hours, to prevent loss in weight due to respiration (Baker et al.1964). After drying samples were transferred to a desiccator to cool before weighing to four decimal places.

2.2.2 Digestion and Analysis of Plant Material

(i) Digestion was carried out by transferring as much dried material as possible from each specimen tube to an acid-washed 100 cm^3 Kjeldahl flask. 5 cm^3 of Aristar-grade concentrated nitric acid was then added to the specimen tubes to wash out any dry matter adhering to the sides of the tube and any cell contents released on to the bottom of the tube, caused by the rupture of cells during the drying process. In most cases about five minutes was long enough to loosen any dried plant remains and the nitric acid was transferred to the Kjeldahl flask. Two washings of the tubes with the minimum of distilled water completed the transfer of material and the tubes were then dried at 105°C, cooled in a desiccator and re-weighed to four decimal places to determine the dry weight of plant material. Digestion was carried out by boiling for 30 minutes, when the diges was transferred to a 25 cm^3 volumetric flask and made up to volume with distilled water, transferred to acid-washed snap-top specimen tubes and stored at 4°C until analysis. At each session of acid digestion two blanks of nitric acid were included.

(ii) Analysis for zinc was carried out by atomic absorption spectrophotometry using an acid resistant nebuliser for aspiration. All samples required further dilution before accurate determinations could be made.

2.3 Choice of sites and sampling programme

Table 2.1 is a list of the sites sampled, numbered 1 - 6 on fig. 1.4. The stream and reach numbers refer to the classification system held in the Botany Department, Durham University. Each reach number refers to a specific stretch of the stream being 'that part of the main current of a stream typically with a length of exactly 10m and never exceeding this length, with approximately the same quantity of water leaving it as entering it'. (Say 1978).

Sites were selected on the basis of water samples collected on reconnaisances early in May 1978.

Site 1 on South Grain, represents an upland area free of any mining influences. (Fig. 2.1a).

Site 2 is situated below old mine tailings. (Fig. 2.1b)

Site 3 is below Grove Rake mine and old mine adits.(Fig. 2.2a)

Site 4 below the fluorspar washing plant and settlement pools and Redburn Mine.(Fig. 2.2b)

Site 5 below the sewage treatment plant. (Fig. 2.3)

Site 6 above the entry of Rookhope Burn in to the River Wear.

Two other criteria were used in deciding on sites:

(i) they should allow sampling of the six sites to take place during a single day

(ii) they should coincide with, or relate closely to data previously collected by workers from the Botany Department Durham University, and available on computer file.

More detailed site descriptions are given in Appendix A.

Samples of water and plant material were taken on six occasions between May and July 1978: 18th May, 1st June, 15th June, 27th June, 7th July, 17th July.

TABLE 2.1 LIST OF SAMPLING SITES IN ROOKHOPE BURN CATCHMENT

SITE NUMBER	STREAM NUMBER	REACH NUMBER	STREAM NAME	SITE REFERENCE	GRID REFERENCE	MAP REFERENCE	km DOWNSTREAM
1	0219	75	South Grain	200m above entry of South Foul Sike	NY 877442	54°47 02°10	0.5
2	0012	15	Rookhope Burn	300m above Grove Rake Mine	NY 893443	54°47 02°09	8.1
3	0012	30	Rookhope Burn	Immediately below road bridge	NY 924430	54°46 02°06	5.1
4	0012	38	Rookhope Burn	Below fluorspar washing plant	NY 941423	54°46 02°05	3.1
5	0012	41	Rookhope Burn	Above foot bridge	NY 944416	54°45 02°04	3.9
6	0012	46	Rookhope Burn	Eastgate, below road bridge	NY 953384	54°44 02°04	0.6

TABLE 2.2 LIST OF TRANSPLANT SITES IN UPPER ROOKHOPE VALLEY

SITE	STREAM NUMBER	REACH NUMBER	STREAM NAME	SITE DESCRIPTION	GRID REFERENCE
A	0219	75	South Grain	200m above entry of South Foul Sike	NY 877442
B	0012	-	Rookhope Burn	100m below entry of Shorngate Sike	NY 881447
C	0012	15	Rookhope Burn	300m above Grove Rake Mine	NY 893443

SOUTH GRAIN

Fig. 2.1 (a) South Grain at site one.

(b) Upper Rookhope Burn looking downstream towards site two. Grove Rake Mine can be seen in the distance.

Fig. 2.2 (a) Rookhope Burn looking downstream from Site three.

(b) Rookhope Burn looking upstream from Site four. The fluorspar Washing Plant can be seen in the background.

Fig. 2.3

Rookhope Burn looking downstream from Site four. The banks can be seen to be well covered with vegetation in this lower reach.

2.4 Floristic Survey

During the course of the sampling programme a brief floristic survey was carried out to give some idea of the range of submerged and emergent plant species present in the stream.

2.5 Transplant Experiment

2.5.1 Marking and transplanting of *Scapania undulata*

Three sites designated A, B and C (Table 2-2) were chosen in upper Rookhope where populations of *Scapania undulata* were growing in obviously healthy condition. Early analyses had shown these sites to have marked differences in levels of Zn in plant tissue. At each of the three sites, three rocks were selected from beneath the surface of the stream in the main current which had good growths of *Scapania* attached to them. Two shoots on each stone were marked 5 mm behind the growing point by tying lengths of red thread around the shoot. Rocks were marked with their site of origin with red paint. One rock was left at its site of origin and the other two transported in a bucket containing stream water to the other two sites. Rocks were replaced in the main current of the stream with the *Scapania* shoots submerged. (Fig. 2-4). Samples of *Scapania* from each rock were placed in previously labelled plastic bags and returned to the laboratory in an ice-box for analysis.

2.5.2 Estimation of chlorophyll a

The method used for chlorophyll extraction was that of Lorenzen (1967) previously employed on *Scapania* by Duncker (1976). Chlorophyll a was extracted in 95% methanol in sealed McCartney bottles at 70°C in a covered water bath for 20 minutes. Ten shoots of *Scapania* were broken up in to small

Fig. 2.4

Scapania transplant in position in upper Rookhope Burn.

(Following the taking of this photograph the rock was transferred to a point in the stream where it was fully submerged.

fragments, but not ground and a second extraction with 95% methanol proved necessary to complete the removal of chlorophyll. Extracts were filtered through 24 mm Whatman G/FC 9 glass microfibre papers and made up to 10 cm^3. The extracts were analysed on a Perkin-Elmer 402 scanning spectrophotometer in 4 cm cells. Absorbance was read between 590 nm and 750 nm at the position of maximum absorbance (λ max $\sim$ 665 nm). Each sample was then acidified with one drop of 1N sulphuric acid, shaken for ten seconds and the absorbance re-read.

Calculation was by application of the equations devised by Marker (1972) and the results expressed as the ratio pheophytin a / chlorophyll a + pheophytin a.

CHAPTER 3 - RESULTS

3.1 Water Analysis

The results of the cation analyses completed for the six sample dates from May to July are shown in Appendix B. 'Total' and 'nuclepore' samples are included and a student's 't' test carried out for each site. Weak significant differences ($p<0.10$) are found for zinc at sites one and four and for cadmium at site two. Iron shows weakly significant differences (p 0.10) at three sites, two, five and six (see 3.1.3). In following sections 'nuclepore' water samples are referred to except where otherwise stated.

3.1.1 Heavy metal cations

Table 3.1 shows the mean levels of zinc, lead and cadmium occurring at the six sampling sites. Means of 'nuclepore' samples are shown on a logarithmic plot (Fig. 3.1) where it can be seen that zinc increases by two orders of magnitude from site two to site three (0.039 mg l^{-1} to 1.678 mg l^{-1}). A smaller increase between the same sites is also observed for cadmium (0.0012 mg l^{-1} to 0.0022 mg l^{-1}). The maximum value for lead however occurs at site four (0.093 mg l^{-1}). When means of all sample sites at each collection (Table 3.2) are displayed graphically (Fig. 3.2) concentrations of zinc in the stream can be seen to be an order of magnitude greater than lead at each collection and two orders of magnitude greater than cadmium, with cadmium showing the least fluctuation over time.

3.1.2 Levels of major cations

Table 3.3 shows the mean levels of calcium, magnesium and iron occurring at the six sites. Means of 'nuclepore' samples are

TABLE 3-1 MEAN LEVELS OF Zn, Pb AND Cd IN WATER AT COLLECTION SITES IN ROOKHOPE BURN CATCHMENT (mg l^{-1} ELEMENT: sem = 1 STANDARD ERROR OF MEAN)

Site	Zn				Pb				Cd			
	'tot'		'nuc'		'tot'		'nuc'		'tot'		'nuc'	
	$\bar{x}$	sem	$\bar{x}$	sem	$\bar{x}$	sem	$\bar{x}$	sem	$\bar{x}$	sem	$\bar{x}$	sem
1	0.049	0.010	0.026	0.003	0.011	0.001	0.016	0.003	0.0014	0.0002	0.0011	0.0001
2	0.048	0.006	0.039	0.007	0.034	0.001	0.031	0.003	0.0014	0.0001	0.0012	0.0001
3	1.908	0.198	1.678	0.237	0.078	0.010	0.032	0.008	0.0025	0.0001	0.0022	0.0002
4	0.865	0.118	0.575	0.096	1.136	2.410	0.093	0.023	0.0040	0.0026	0.0031	0.0021
5	0.578	0.063	0.460	0.063	0.401	0.256	0.062	0.008	0.0018	0.0002	0.0015	0.0001
6	0.326	0.076	0.228	0.037	0.151	0.062	0.051	0.008	0.0014	0.0001	0.0013	0.0001

TABLE 3-2 MEAN LEVELS OF Zn, Pb AND Cd IN 'NUCLEPORE' WATER SAMPLES (mg l^{-1}) AND WATER FLOW (0-5 SCALE) IN ROOKHOPE BURN CATCHMENT ON SIX SAMPLING DATES

Sample Date	Zn	Pb	Cd	Water Flow
18.5.78	0.446	0.048	0.00125	4
1.6.78	0.375	0.040	0.00138	3
15.6.78	0.705	0.028	0.00142	2
27.6.78	0.498	-	0.00140	4
7.7.78	0.491	0.057	0.00180	5
17.7.78	0.529	0.065	0.00175	4

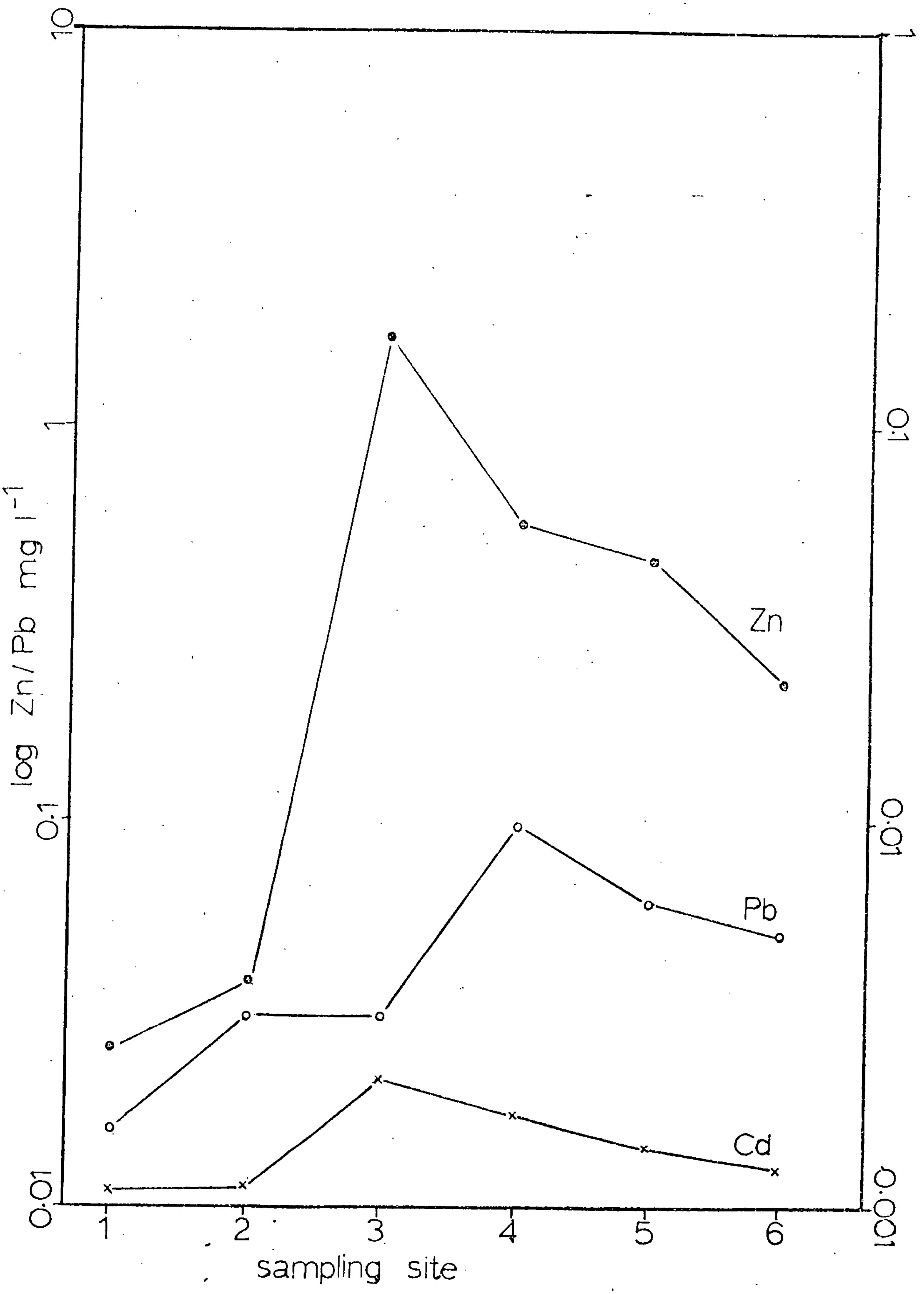


Fig. 3.1 Variation in mean levels of Zn, Pb and Cd in 'nuclepore' water samples at sampling sites.

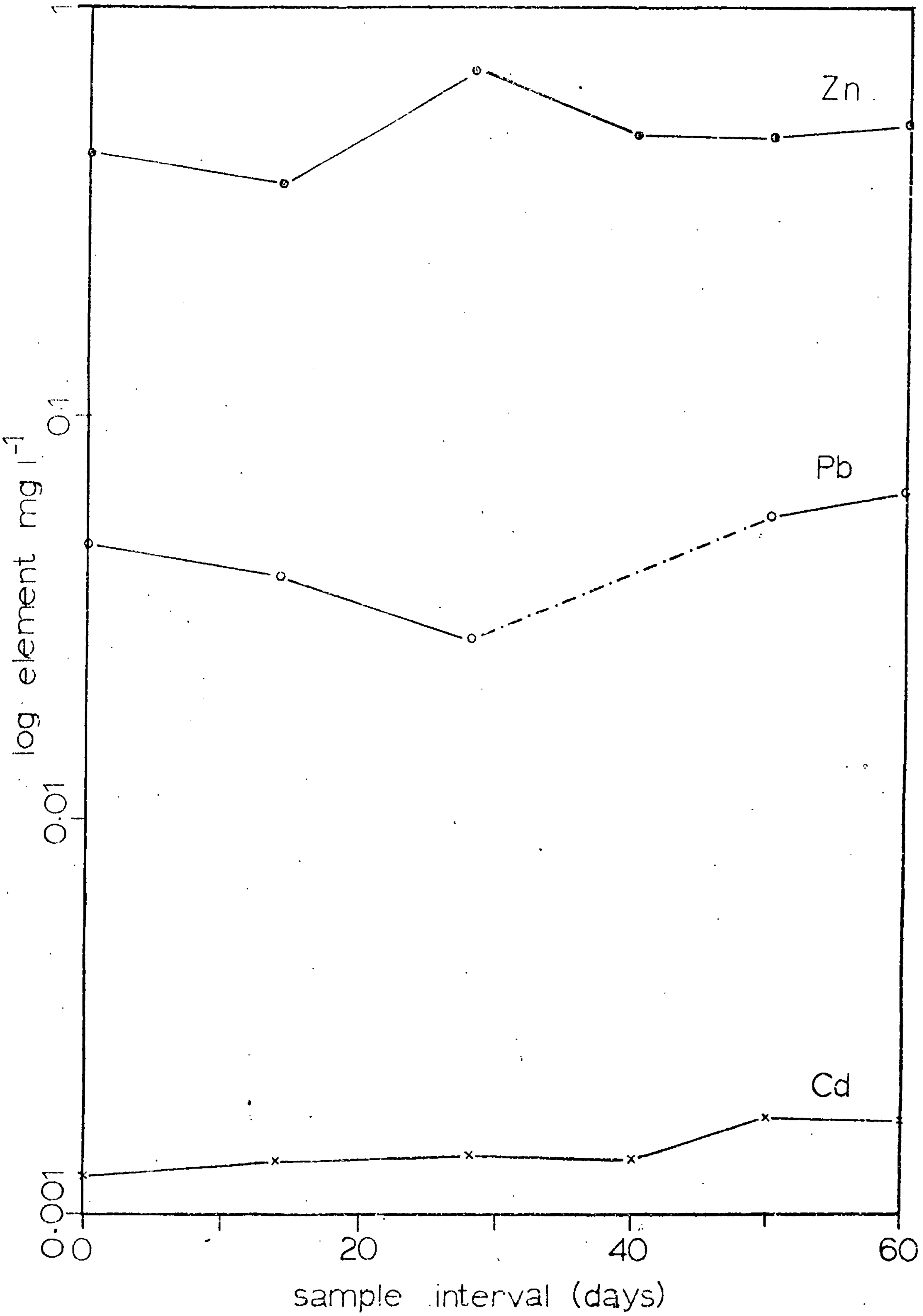


Fig. 3.2 Variation in mean levels, of Zn, Pb and Cd in 'nuclepore' water samples over sampling programme

shown on a logarithmic plot (Fig. 3.3). Levels of calcium

line missing?

and magnesium show similar profiles, marked increases occurring between sites one and three. The major sources of these two elements are the limestone rocks through which drainage waters run in the upper catchment area. Calcium rises from 5.24 mg l^{-1} to 7.6 mg l^{-1} between sites one and three but then falls to 6.8 mg l^{-1} at site six. This difference between the two elements between sites four and six may be related to the influence of the washing plant in releasing calcium in to the water (1.7; 3.1.3).

39.3

43.98

Mg

Iron shows a different profile with highest levels at site one of 1.32 mg l$^{=1}$ falling sharply to site three to reach a level of 0.31 mg l^{-1}, then more slowly to record a minimum level at site six of 0.21 mg l^{-1}. This would suggest highest levels of non-filtrable iron occurring in the slightly acid waters draining off the peat in the upper reaches of the catchment. Iron forms a significant part of the dry matter of multicellular plants (100 μg g^{-1} dry matter; Stout 1971) and would be expected to be released in greater concentrations than a trace element such as zinc (Zn μg g^{-1} dry matter) by decaying vegetation.

Means of all sample sites at each collection (Table 3.4; Fig. 3.4) again shows the similarity in the behaviour of calcium and magnesium with an increase in both during the first 28 days followed by a fall at 40 days and a subsequent rise in the final stages of the survey. Estimates of water flow (3.3, Table 3.2, Fig. 3.8) indicate a fall in discharge during the first period of the survey and the relationship of flow to levels of cations is included in Chapter 4:

TABLE 3-3

MEAN LEVELS OF Ca, Mg AND Fe IN WATER AT COLLECTION SITES IN ROOKHOPE BURN CATCHMENT (mg l^{-1} ELEMENT : sem = 1 STANDARD ERROR OF MEAN)

Site	Ca				Mg				Fe			
	'tot'		'nuc'		'tot'		'nuc'		'tot'		'nuc'	
	$\bar{x}$	sem	$\bar{x}$	sem	$\bar{x}$	sem	$\bar{x}$	sem	$\bar{x}$	sem	$\bar{x}$	sem
1	4.85	0.47	5.24	0.64	1.30	0.09	1.41	0.10	1.68	0.19	1.32	0.13
2	18.83	2.71	17.82	2.56	3.63	0.46	3.58	0.45	0.90	0.08	0.70	0.07
3	43.27	5.71	39.37	6.16	8.43	1.12	7.63	1.13	0.59	0.15	0.31	0.07
4	47.38	8.33	42.67	4.59	7.77	0.94	7.45	0.81	2.52	1.95	0.30	0.06
5	43.13	4.69	41.93	4.37	7.47	0.81	7.30	0.78	0.62	0.12	0.29	0.09
6	45.55	4.33	43.98	3.97	6.92	0.65	6.80	0.65	0.59	0.19	0.21	0.06

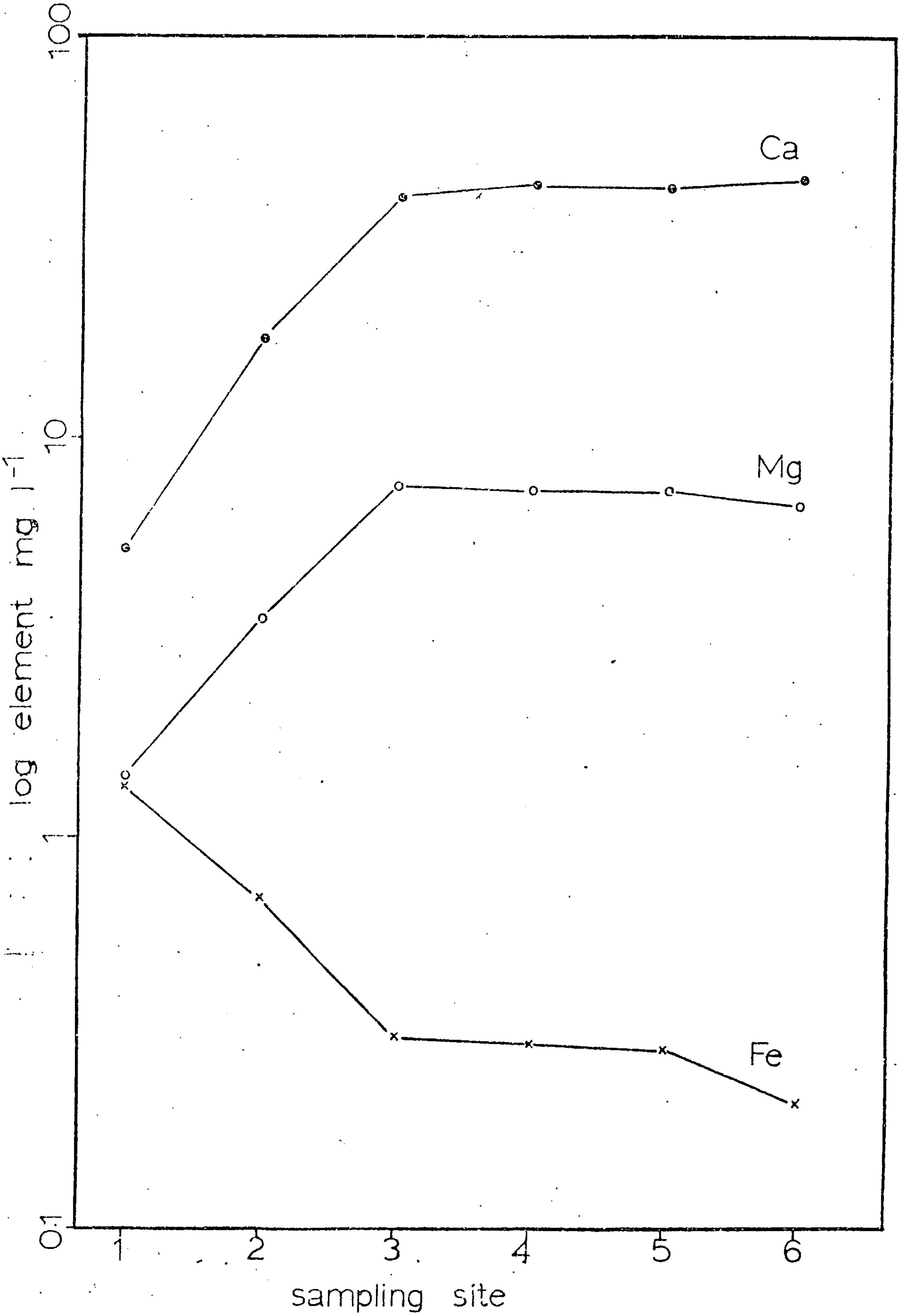


Fig. 3.3 Variation in mean levels of Ca, Mg and Fe in water at sampling sites.

TABLE 3-4 MEAN LEVELS OF Ca, Mg AND Fe IN WATER ON SIX SAMPLING DATES IN ROOKHOPE BURN CATCHMENT (mgl^{-1}, NUCLEPORE SAMPLES).

Sample Date	Ca	Mg	Fe
18/5/78	21.40	3.81	0.54
1/6/78	30.83	5.73	0.40
15/6/78	42.28	7.62	0.44
27/6/78	25.34	4.80	0.63
7/7/78	28.88	4.78	0.58
17/7/78	42.28	7.43	0.54

TABLE 3-5 MEAN LEVELS OF Fe IN 'TOTAL' AND 'NUCLEPORE' SAMPLES AT COLLECTION SITES ($mg\ l^{-1}$)

Sampling Site	'Total'	'Nuclepore'
1	1.68	1.32
2	0.90	0.70
3.	0.59	0.31
4	2.52	0.30
5	0.62	0.29
6	0.59	0.21

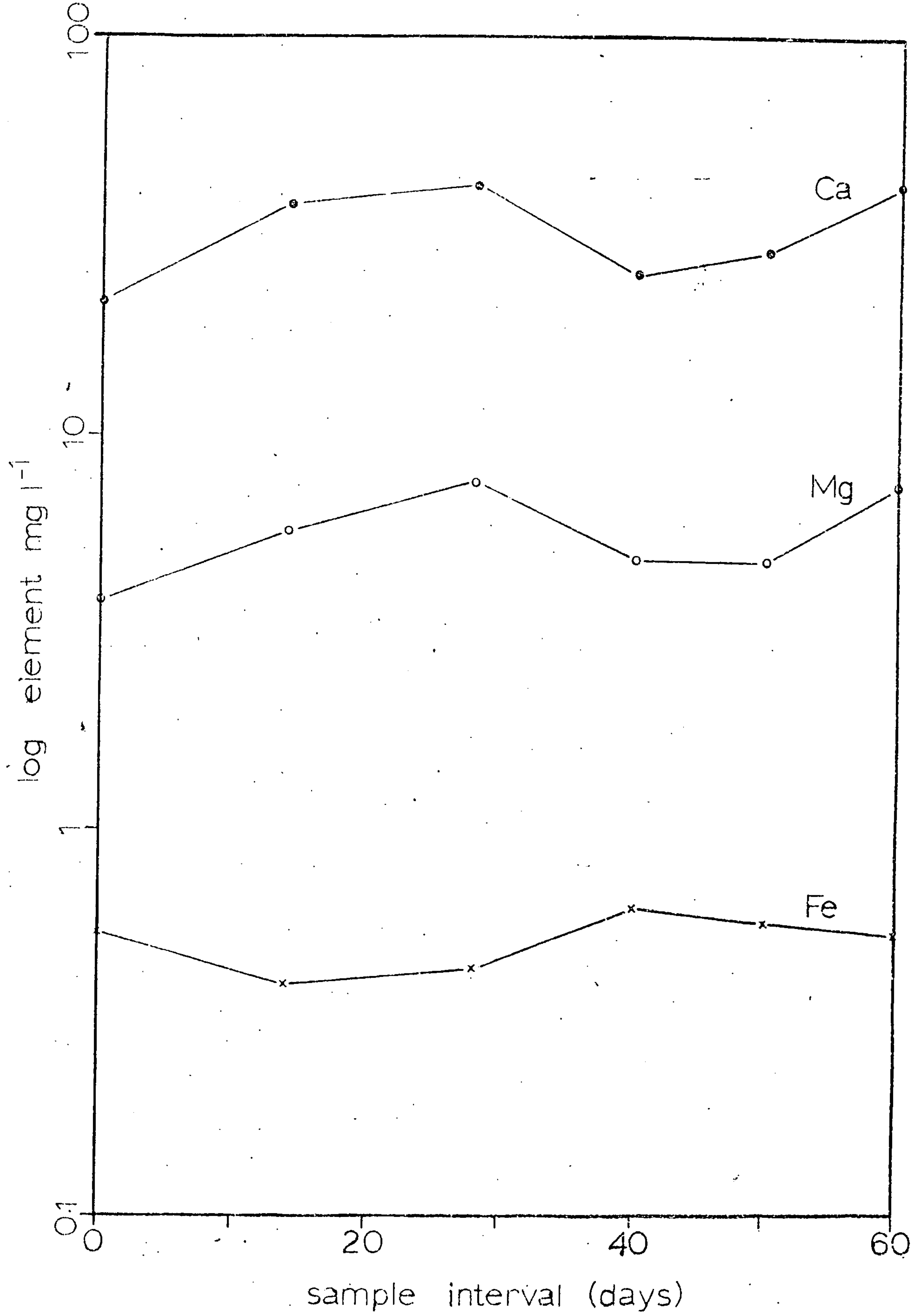


Fig. 3.4 Variation in mean levels of Ca, Mg and Fe in water over sampling programme.

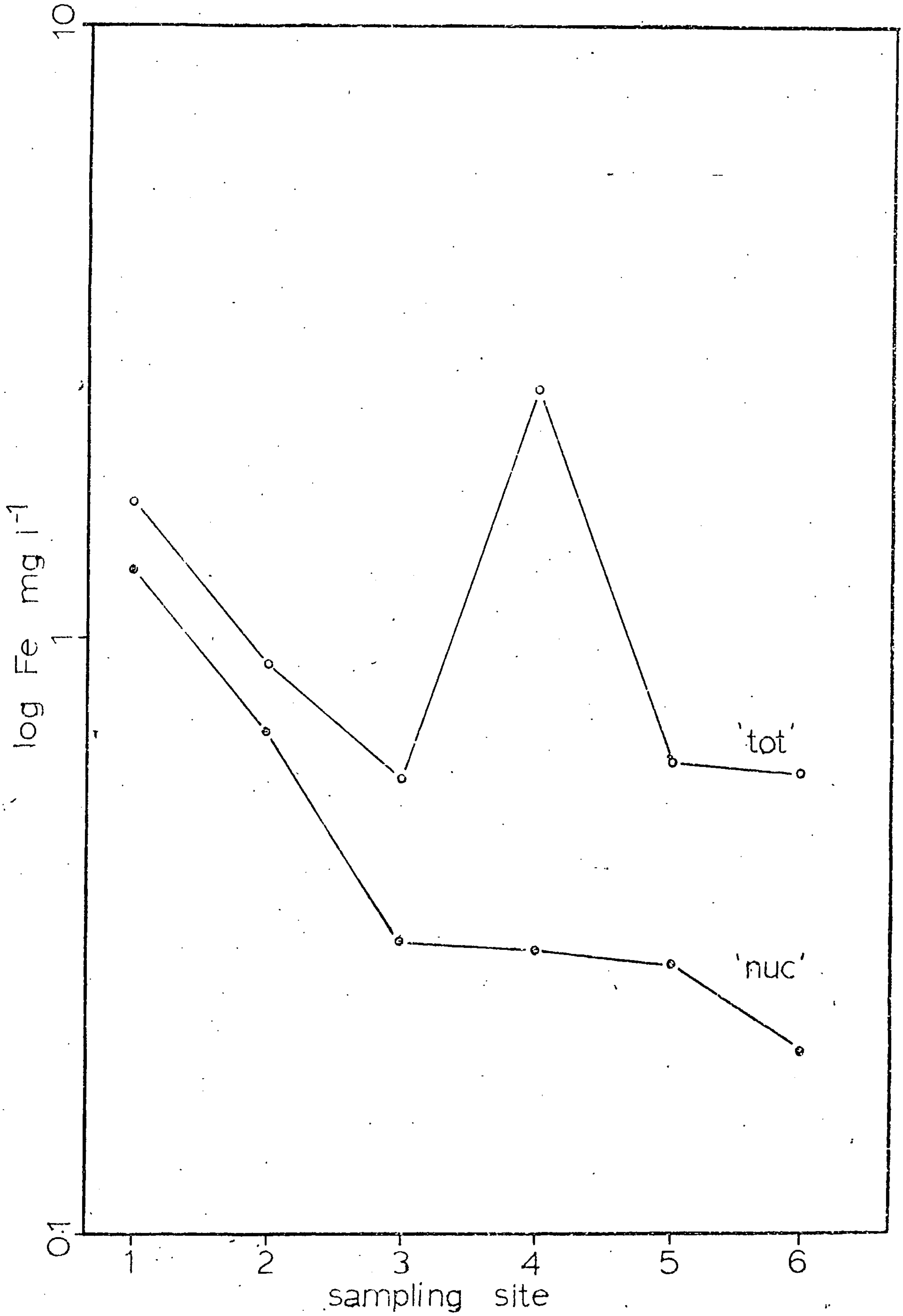


Fig. 3.5 Variation in mean levels of Fe in 'total' and 'nuclepore' water samples.

3.1.3 The relationship between 'total' and 'nuclepore' samples

Although no very significant differences are observed between 'total' and 'nuclepore' samples over the period of the survey (3.1) consideration of the mean levels of iron at the six sites (Table 3.5; Fig. 3.5) shows a distinction in the levels of the element at site four where the 'total' sample has a mean concentration of 2.52 mg l^{-1} ($5^2 = 22.8$) while the 'nuclepore' sample has a concentration of 0.30 mg l^{-1} ($5^2 = 0.02$). Closer examination of individual samples (Appendix B) reveals this to be largely a reflection of the samples collected on 17th July 1978 when 'total' zinc at site four was 12.25 mg l^{-1} while 'nuclepore' zinc was 0.24 mg l^{-1}. These samples were collected below the washing plant at a time when the settlement pools were discharging into the stream which became visibly laden with colloidal and suspended material. Examination of the levels of other cations at this date and site also reveals large differences for zinc ('total' 1.38 mg l^{-1}; 'nuclepore' 0.22 mg l^{-1}), lead ('total' 6.16 mg l^{-1}; 'nuclepore' 0.184 mg l^{-1}) and calcium ('total' 84.0 mg l^{-1}; 'nuclepore' 55.5 mg l^{-1}). The process of adding ferrous sulphate and lime to the settlement pools (1.7) will clearly account for the elevated levels of iron and calcium, but since the process is intended to precipitate heavy metals it does not account for the raised levels of filtrable zinc and lead.

3.1.4 Anion Analysis

Table 3.6, Fig. 3.6 show the mean levels of NO_3-N, NH_4-N and PO_4-P occurring at the six sites over the sampling period. NH_4-N and NO_3-N can be seen to be at similar concentrations in stream water but with contrasting levels at individual sites.

NH_4-N shows its maximum value (0.244 mg l^{-1}) at site one which is supplied by drainage waters rich in decomposition products of peat from the upper catchment. Levels further downstream are lower with a minimum of 0.144 mg l^{-1} occurring at site four.

The profile for NO_3-N shows an increase from a minimum value at site one of 0.098 mg l^{-1} to 0.174 mg l^{-1} at site three. Site four is lower, which can be related to the lower productivity above the reach. Sites five and six show the highest concentrations with a maximum of 0.331 mg l^{-1} which can be related to the presence of the sewage plant above site five and increased agricultural activity in the lower reaches.

A decline in levels of PO_4-P over the first four sites from a maximum at site two of 0.17 mg l^{-1} to 0.009 mg l^{-1} at site four is followed by an increased concentration at sites five and six to a maximum level of 0.019 mg l^{-1} related to the effects of the sewage treatment plant.

An observed increase in productivity of the stream at sites five and six can be related in part to the raised levels of PO_4-P and NO_3-N at these sites.

Table 3.7, Fig. 3.7 shows mean levels of anions at all sample sites at each collection. The profiles for NO_3-N and PO_4-P are broadly similar and can be related to water flow through the course of the study (3.3). Overall levels of PO_4-P at each collection are an order of magnitude lower than NO_3-N and NH_4-N but the proportional changes occurring are similar, there being a threefold difference between maximum and minimum levels of NO_3-N (0.098 mg l^{-1} to 0.331 mg l^{-1}), a two-fold difference in

TABLE 3-6 MEAN LEVELS OF ANIONS IN WATER AT COLLECTION SITES IN ROOKHOPE BURN CATCHMENT (mg l^{-1} ANION; sem = 1 STANDARD ERROR)

Sampling Site	NO_3-N		NH_4-N		PO_4-P	
	$\bar{x}$	sem	$\bar{x}$	sem	$\bar{x}$	sem
1	0.098	0.024	0.244	0.042	0.016	0.003
2	0.135	0.057	0.153	0.016	0.017	0.005
3	0.174	0.037	0.171	0.020	0.015	0.002
4	0.141	0.033	0.144	0.012	0.009	0.002
5	0.252	0.033	0.163	0.012	0.017	0.001
6	0.331	0.065	0.150	0.008	0.019	0.003

TABLE 3-7 MEAN LEVELS OF ANIONS IN WATER ON SIX SAMPLING DATES IN ROOKHOPE BURN CATCHMENT (mg l^{-1})

Sample Date	NO_3-N	NH_4-N	PO_4-P
18.5.78	0.154	0.161	0.0179
1.6.78	0.106	0.112	0.0116
15.6.78	0.150	0.193	0.0160
27.6.78	0.319	0.157	0.0214
7.7.78	0.219	0.188	0.0172
17.7.78	0.182	0.235	0.0095

TABLE 3-8 MEAN pH, TEMPERATURE, TOTAL ALKALINITY, ELECTRICAL CONDUCTIVITY AND OPTICAL DENSITY OF WATER AT SAMPLING SITES IN ROOKHOPE BURN CATCHMENT

Sampling Site	pH	Temperature (°C)	Total Alkalinity (mgl^{-1} $CaCO_3$)	Electrical Conductivity (µ mho cm^{-1} x 10^2)	Optical Density (1cm path)		
					420nm	254nm	240nm
1	6.9	10.1	7.0	0.7	0.068	0.530	0.564
2	7.2	13.2	37.0	1.6	0.024	0.326	0.428
3	7.5	15.3	55.6	3.7	0.011	0.105	0.186
4	8.0	15.1	72.1	3.5	0.008	0.083	0.091
5	7.9	15.5	68.6	3.9	0.006	0.091	0.100
6	8.1	13.8	84.3	3.8	0.010	0.097	0.103

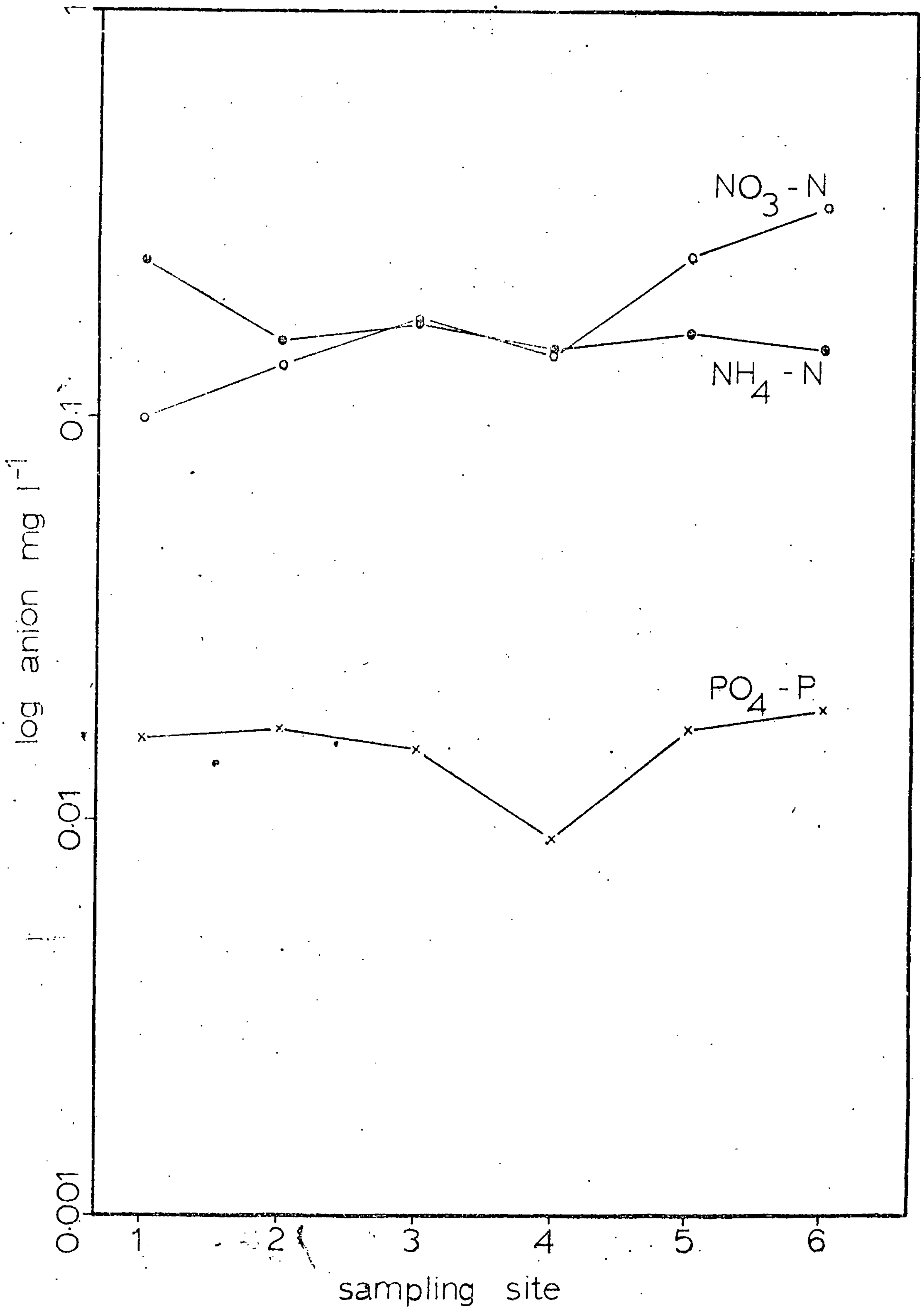


Fig. 3.6 Variation in levels of anions in water at sampling sites.

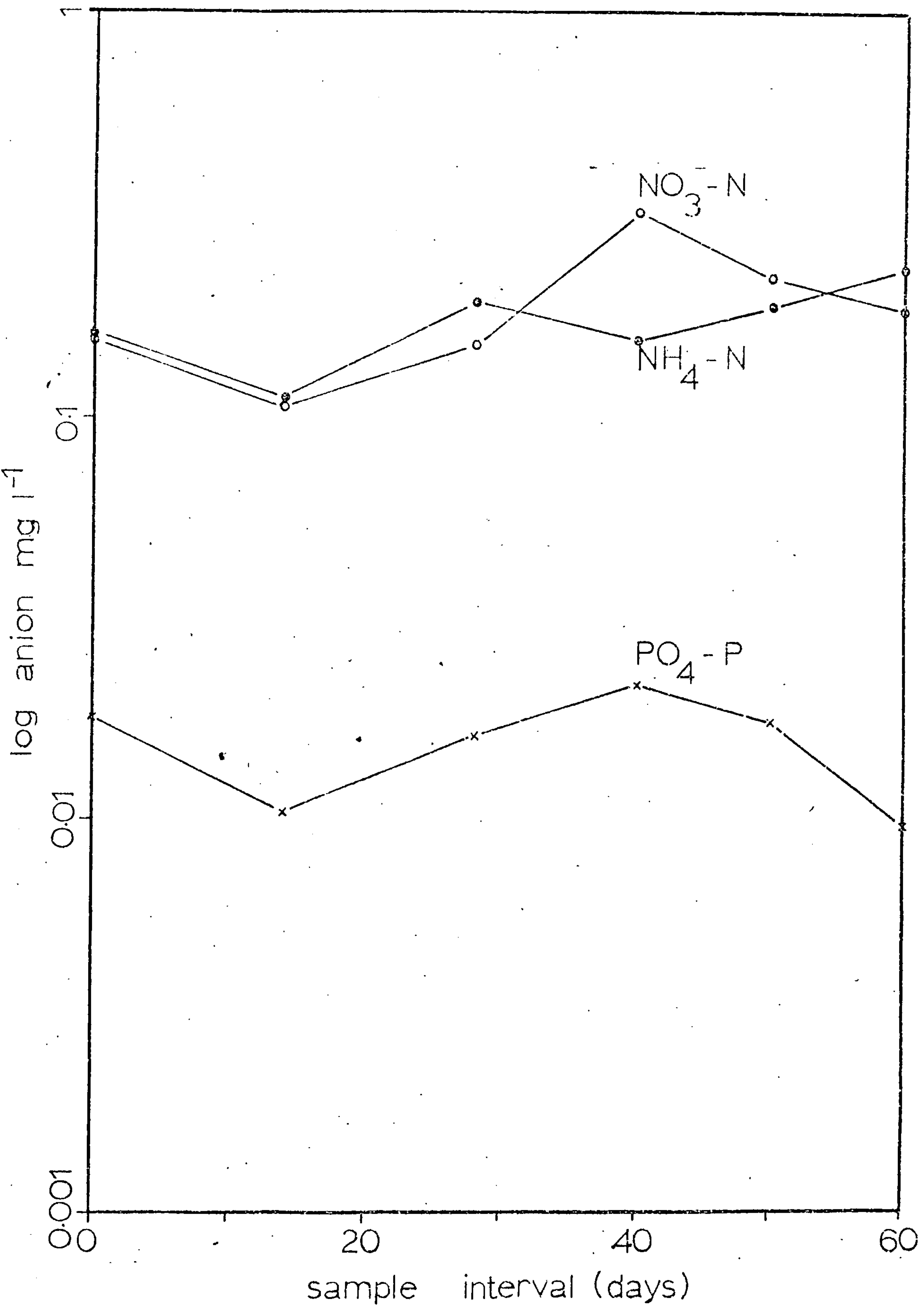


Fig. 3.7 Variation in levels of anions in water over sampling programme.

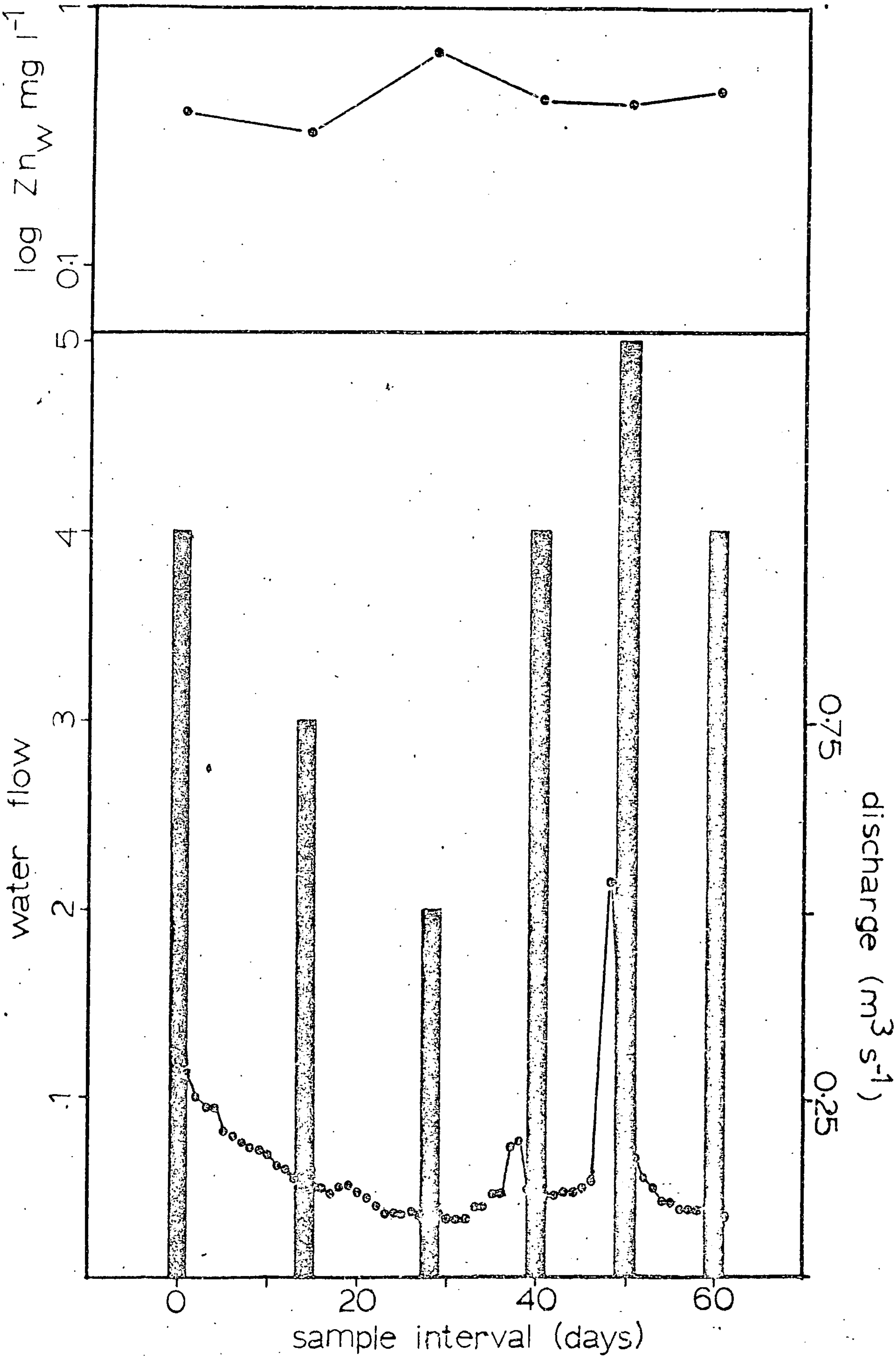


Fig. 3.8 Variation in Zn in water, water flow and discharge over sampling programme.

(discharge ●)

PO_4-P (0.009 mg l^{-1} to 0.019 mg l^{-1}) and only just less than two-fold for NH_4-N (0.144 mg l^{-1} to 0.244 mg l^{-1}).

3.2 Water Flow

Estimates of water flow for the sampling dates are included in Table 3.2 and are shown as a histogram in Fig. 3.8. During the first three collections flows in the stream fell from a medium-high flow (4) to a medium low flow (2) as a result of a continuous dry spell lasting beyond 15th July 1978 (28 days). At the time of the fourth sample (40 days) flow had risen to medium-high (4) following heavy overnight rain and sample five (50 days) was a high flow (5) following eight days of prolonged rain. Intermittent rain between days 50 and 60 led to a medium-high flow (4) at the final sample date (60 days). The relationship between flow and levels of cations and anions will be discussed in the final chapter.

Late in the project discharge levels from the Northumbrian Water Authority monitoring station at Eastgate became available (Appendix D) and these are referred to in the discussion. Mean levels of water flow (m^3s^{-1}) are included on Fig. 3.8.

3.3 Environmental parameters

Table 3.8 shows mean values of pH, temperature, total alkalinity, electrical conductivity and optical density at each site during the sampling programme. Fig. 3.9 shows variation in mean values of pH, total alkalinity and electrical conductivity. Both alkalinity and conductivity show maximum values downstream indicating an increase in the total of dissolved substances in the water as subsidiary streams join Rookhope Burn. At the first site pH is 6.9 indicating the presence of slightly acid waters running off the peat of the upper fells, but any pronounced effect is probably ameliorated by water running over limestone rocks (3.1.2).

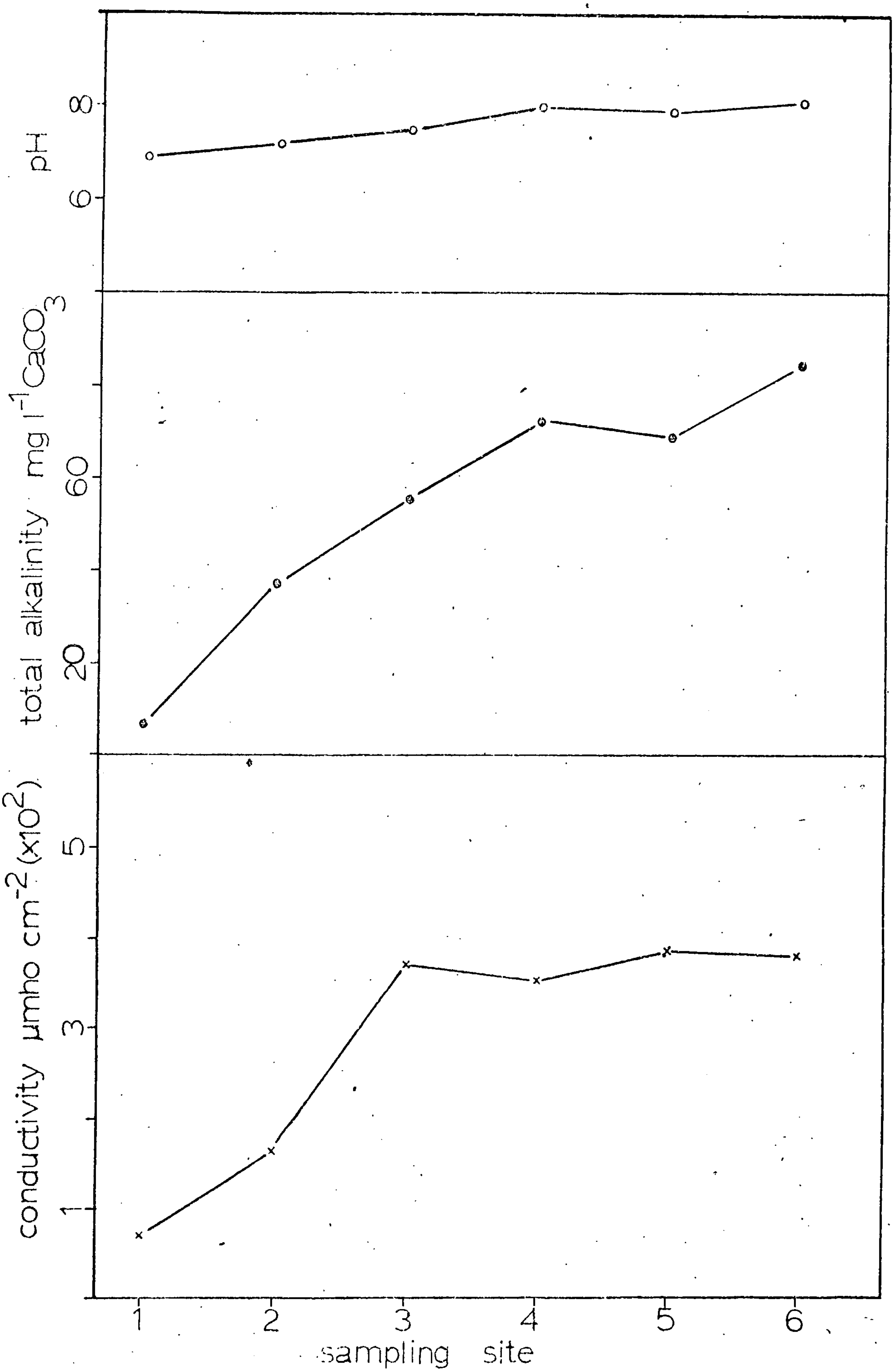


Fig. 3.9 Variation in pH alkalinity and electrical conductivity at sampling sites.

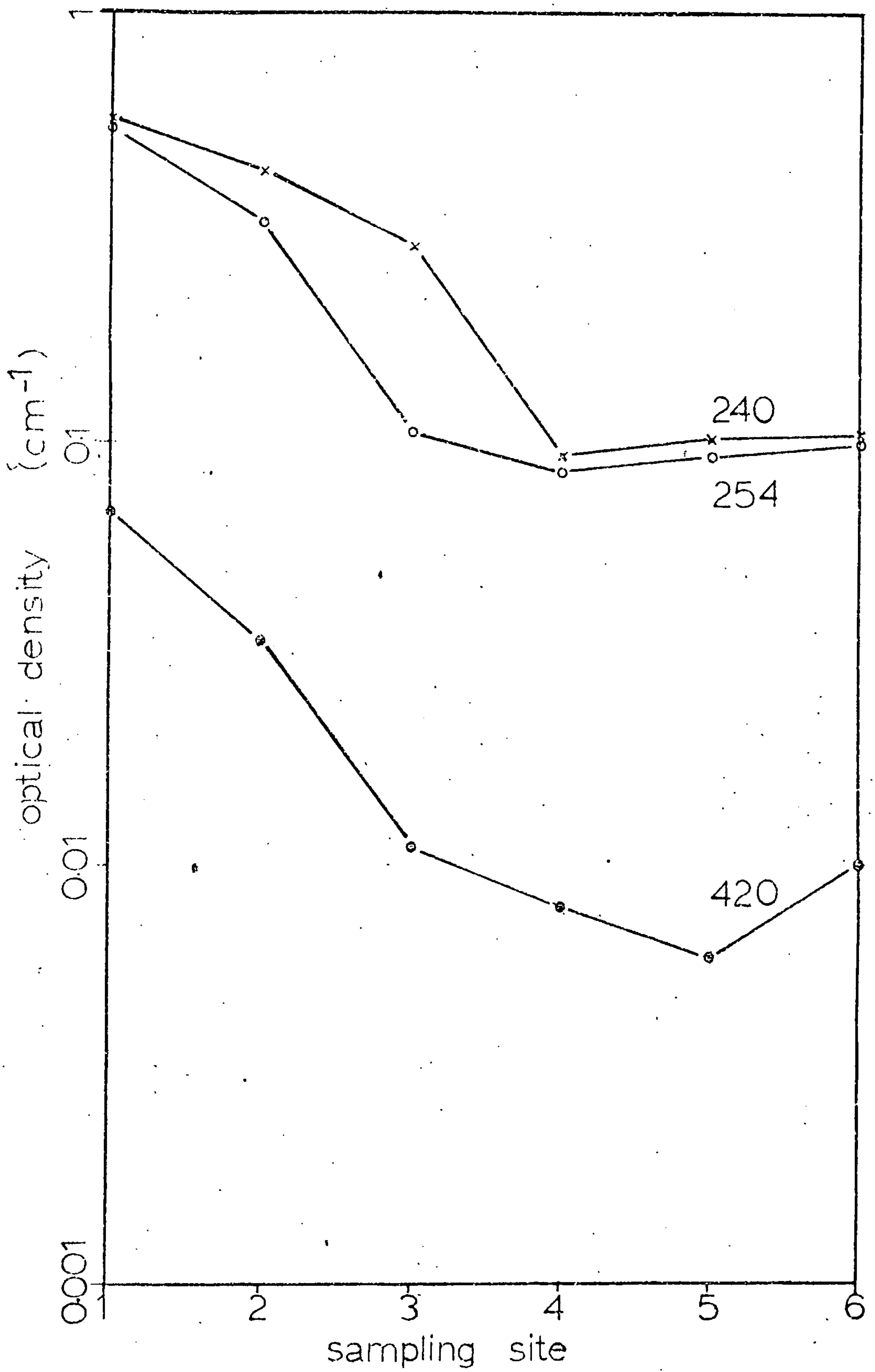


Fig. 3.10 Variation in optical density at sampling sites.

Variation in optical density (Fig 3.10) shows maximum levels in water running off peat in the uppermost reach on South Grain. The level 0.068 for a 1 cm path length at 420 nm indicates the presence in the water of humic materials which is supported by optical densities of 0.530 and 0.564 at 254 and 240 nm respectively indicating high levels of organic materials generally. In lower reaches where influent water flows over less peaty material lower levels are observed. Site six shows a rise which can be related to the greater productivity of the stream and surrounding vegetation and consequent increase in decomposition products at lower reaches of the stream.

Temperature shows an increase from sites one to five which is related to both altitudinal effects and to the rate of flow in the stream, in the lower reaches flow being slower and the stream wider allowing a greater heating effect from solar radiation. The lower temperature at site six can be related to the shading by the trees growing on the banks of the stream in this reach.

3.4 Plant analysis

3.4.1 Concentration of zinc in species sampled

The results of the plant analyses are shown for the species sampled in Tables 3.9, i-v, together with the level of zinc in water samples collected at the time. Means are calculated from four replicates except where otherwise noted. Enrichment ratios are included for each sample. Where samples were collected from two sites Student's 't' tests have been performed on levels of zinc in plant and in water and are included in the table. Means ± 1 standard error of zinc in plants and water are recorded for each site.

TABLE 3-9 (i) - (v) LEVELS OF Zn IN 'NUCLEPORE' WATER SAMPLES (Zn_w) AND PLANT MATERIAL (Zn_p) AND ACCUMULATION RATIOS FOR SPECIES SAMPLED ($\bar{x}$ = MEAN ± 1 sem)

(i) Zinc accumulation in Scapania undulata (1 cm tips)

Site	Stream Number	Reach Number	Stream Name	Zn_w mg l^{-1}	† Zn_p µg g^{-1}	Enrichment Ratio
1	0219	75	South Grain	0.022	69	3136
				0.020	67	3350
				0.033	75	2272
				0.019	54	2842
				0.039	71	1821
				0.024	67	2792
			$\bar{x}$ =	0.026 ± 0.003	67.17 ± 2.9	
2	0012	15	Rookhope Burn	0.045	369	8200
				0.028	354	12643
				0.032	678	21188
				0.053	369	6962
				0.062	233	3758
				0.103	359	3485
			$\bar{x}$ =	0.039 ± 0.007	393.67 ± 61	
3	0012	30	Rookhope Burn	1.30	* 7295	5612
				2.60	* 4840	1862
				1.28	* 564	441

't' - test. Sites 1 and 2 Zn_p $t = 5.37$, $p < 0.001$

Zn_w $t = 1.707$, N.S.

---

† Zn_p = mean of 4 replicates except * spot samples only.

(ii) ZINC ACCUMULATION IN HYGROHYPNUM OCHRACEUM (1 cm TIPS)

Site	Stream Number	Reach Number	Stream Name	Zn_w mg l^{-1}	†Zn_p µg g^{-1}	Enrichment Ratio
2	0012	- 15	Rookhope Burn	0.045	342	7600
				0.028	369	12179
				0.032	274	8563
				0.053	315	5943
				0.062	287	4629
				0.103	460	4466
			$\bar{x}$ =	0.039 ± 0.007	341.17 ± 27.70	
5	0012	- 41	Rookhope Burn	0.46	9912	21548
				0.26	4584	17631
				0.37	6049	16349
				0.72	6859	9526
				0.52	9310	17904
				0.43	6244	14521
			$\bar{x}$ =	0.46 ± 0.063	7159 ± 836.57	

't' - test. Sites 2 and 5 Zn_p $t = 8.146$; $p < 0.001$

Zn_w $t = 6.642$; $p < 0.001$

(iii) ZINC ACCUMULATION IN DICHODONTIUM PELLUCIDUM (1 cm TIPS)

3	0012	- 30	Rookhope Burn	1.37	24624	17974
				2.22	9850	4437

† Zn_p = mean of 4 replicates

(iv) ZINC ACCUMULATION IN LEMANEA FLUVIATILIS (2 cm TIPS)

Site	Stream Number	Reach Number	Stream Name	Zn_w 'nuc' mg l^{-1}	†Zn_p µg l^{-1}	Enrichment Ratio
6	0012	- 46	Rookhope Burn	0.191	1793	9387
				0.191	2647	13859
				0.34	2353	6921
				0.34	1303	3832
				0.178	2046	11494
			$\bar{x}$ =	0.248 ± 0.034	2028.4 ± 211.14	

(v) ZINC ACCUMULATION IN THREE ALGAE (WHOLE SPECIMENS)

Species	Site	Stream Number	Reach Number	Stream Name	Zn_w 'nuc' mg l^{-1}	†Zn_p µg g^{-1}	Enrichment ratio
Batrachospernum sp.	2	0012	- 15	Rookhope Burn	0.045	2464	54756
					0.028	805	28750
					0.032	863	26969
					0.053	460	8679
					0.062	287	4629
					0.103	315	3058
				$\bar{x}$ =	0.054±0.011	865.67 ± 334.71	
Stigeoclonium tenue	4	0012	- 38	Rookhope Burn	0.66	6313	9565
Mougeotia sp.	4	0012	- 38	Rookhope Burn	0.94	1363	1450
					0.51	13689	26841
	5		- 41		0.37	8818	23832
					0.72	1148	1594
	6		- 46		0.191	7832	41005

---

† Zn_p = mean of 4 replicates.

(i) *Scapania undulata*

At the two sites where *Scapania* was growing profusely significantly lower mean levels (t - 5.37; $p < 0.001$) of zinc are found in plant material at the site of lower zinc levels in water, even though differences in water are not significant (t = 1.707). This is discussed in relation to the existence of different strains of the bryophyte in the final chapter (4.2).

The spot samples from site three although showing higher concentrations of zinc in plant material (564 μg g^{-1} to 7295 μg g^{-1} dry weight) show lower enrichment ratios than samples from site two but it must be remembered that samples were not replicated and were subject to only periodic inundation at high flows. It was also noticed that the growth of *Scapania* from site three was less robust than at sites one and two and 1 cm tips certainly included some dead material. Little reliance can therefore be based on these samples.

(ii) *Hygrohypnum ochraceum*

Samples of the bryophyte obtained from sites two and five show a significant difference (t = 8.146; $p < 0.001$) in concentration of zinc in tissue. Levels at site five are very high having a mean value of 7159.67 ± 836.57 μg g^{-1} dry weight while at site two much lower levels are recorded, mean zinc concentration being 341.17 ± 27.70 μg g^{-1} dry weight. When considering enrichment ratios, those at site two are seen to be significantly lower (t = 4.185; $p < 0.01$), 7397 ± 1331 at site two compared with 16246 ± 1642 at site five. However the standard error of each is high and not too much should be read in to the difference.

(iii) *Dichodontium pellucidum*

Samples of the bryophyte were obtained from site three on only two collections when flow was medium-high, plant material only being fully submerged on one of these occasions (50 days) when a concentration of 24264 µg g^{-1} dry weight was recorded. On the second occasion (60 days) the bryophyte was growing as an emergent species and a much lower level (9850 µg g^{-1} dry weight) was recorded, even though the level of zinc in water was much higher (2.22 mg l^{-1} compared to 1.37 mg l^{-1}).

(iv) *Lemanea fluviatilis*

Samples of the alga were collected from site six on five occasions. At 14 days the alga was overgrown with epiphytic species and proved impossible to clean. A mean concentration of 2028.4 ± 211.14 µg g^{-1} dry weight was recorded with a range of enrichment ratios from 3832 to 13859.

(v) Whole specimens of the three algae *Batrachospermum* sp., *Stigeoclonium tenue* and *Mougeotia* sp. show a wide range of concentrations of zinc. *Batrachospermum* has a mean level of 868.67 ± 334.71 µg g^{-1} dry weight with a range of 287 - 2464 µg g^{-1} dry weight. Such a variability, with high concentrations of zinc in plant tissue (2464 µg g^{-1} dry weight) at relatively low levels of zinc in water (0.045 mg l^{-1}) and low concentration of zinc (315 µg g^{-1} dry weight) at high levels in water (0.103 mg l suggests that the use of this species is somewhat unreliable in reflecting levels in water which may be related to the large amounts of mucilage present around the filaments affecting uptake of ions from the water. *Stigeoclonium tenue* was present at only one collection at site four with a high concentration of 6313 µg g^{-1} dry weight.

Mougeotia sp., like *Stigeoclonium*, was a transient species occurring at three sites intermittently. It occurred in shallows out of the main flow of the stream and was found to accumulate high concentrations of zinc, 1148 µg g^{-1} dry weight to 13689 µg g^{-1} dry weight.

3.4.2 Relationships between species and sites

Fig. 3.11 is a scatter diagram of the concentrations of zinc in plant tissue and level of zinc in water for the species collected. A roughly linear relationship is seen to exist between the two from 0.02 mg l^{-1} to about 0.5 mg l^{-1} of zinc in water. The species included in this range are all permanent submerged members of the stream community, *Scapania undulata*, *Hygrohypnum ochraceum*, *Lemanea fluviatilis* and *Batrachospermum* sp. *Mougeotia* sp. seems to be somewhat variable. At lower levels (0.191 - 0.51 mg l^{-1} zinc) it conforms to the linear relationship, but at higher levels (0.72 and 0.94 mg l^{-1} zinc) lower concentrations in plant material are recorded. A similar situation exists in the specimens of *Scapania undulata*, *Dichodontium pellucidum* and *Stigeoclonium tenue* sampled at high levels and there is a suggestion of a plateau occurring in the relationship. This

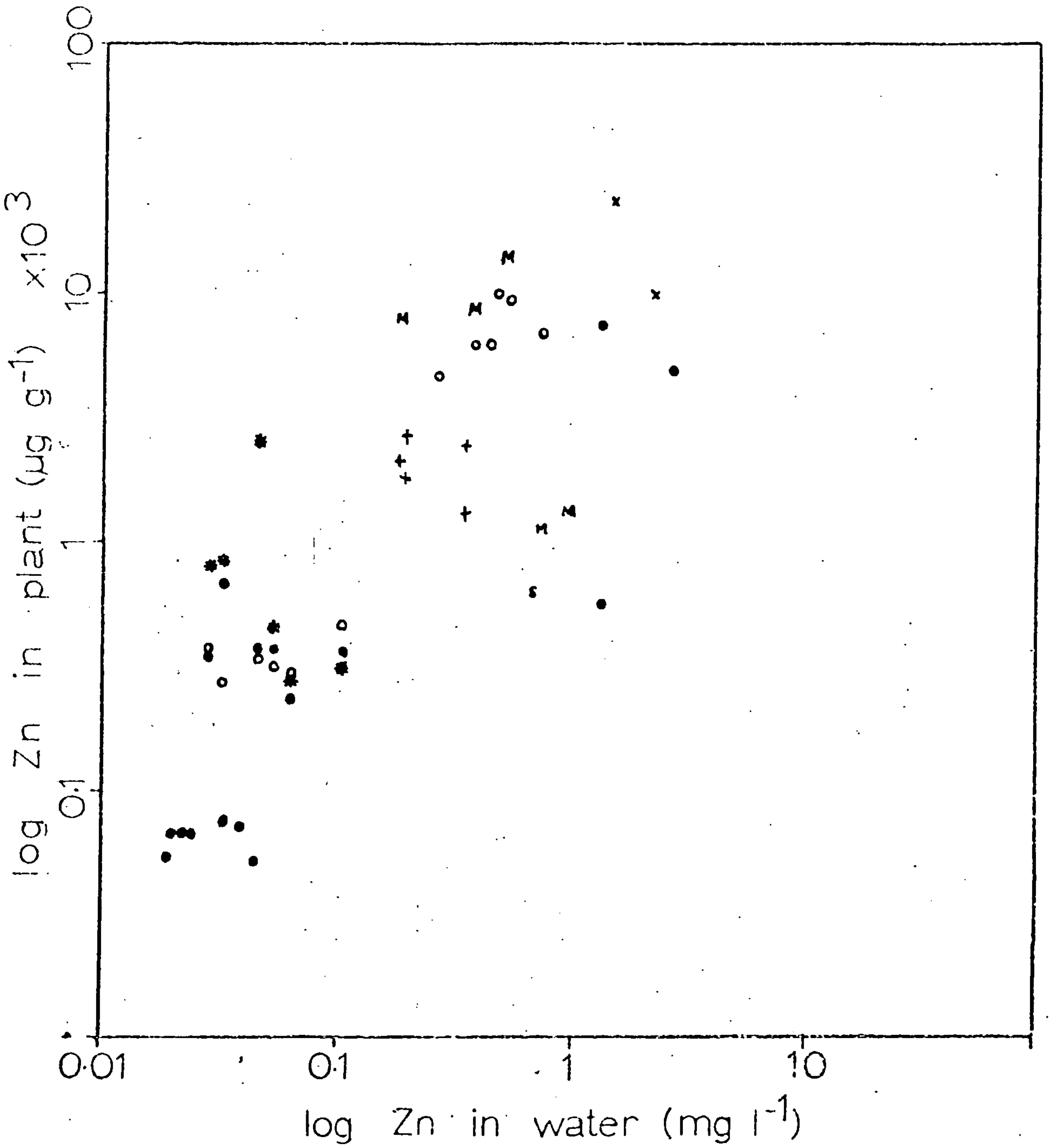


Fig. 3.11 Scatter diagram to show relationship between zinc content of plant material and 'nuclepore' level of zinc for species sampled.

Key

● *Scapania undulata*

○ *Hygrohypnum ochraceum*

✱ *Batrachospermum* sp.

\+ *Lemanea fluviatilis*

× *Dichodontium pellucidum*

M *Mougeotia* sp.

s *Stigeoclonium tenue*

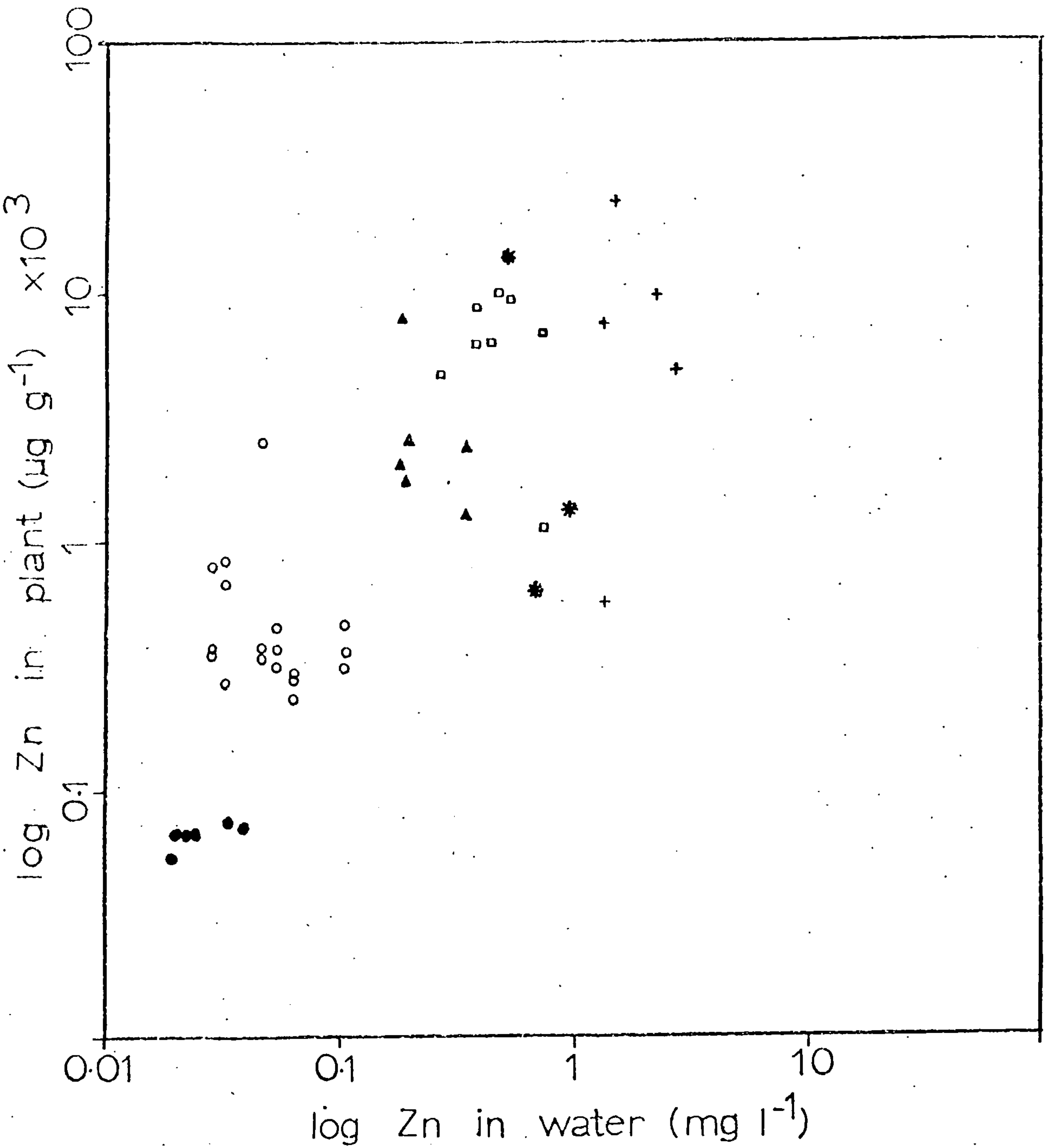


Fig. 3.12. Scatter diagram to show relationship between zinc content of plant material and 'nuclepore' level of zinc for sites sampled.

Key

● Site 1

○ " 2

+ " 3

✱ " 4

□ " 5

▲ " 6

may be related to uptake mechanisms for cations reaching a saturation level in plant tissue.

When levels of zinc in plants and water are plotted according to the sites from which they were collected in that stream (Fig. 3.12) a clustering can be seen to exist at sites one, two, five and six at which lower levels of zinc are present in water.

At sites with higher levels of zinc (three and four) a greater variability is seen to exist.

Fig. 3.13 is a scatter diagram of the enrichment ratios plotted against levels of zinc in water at the collection sites for the species sampled. If a linear relationship exists for uptake of zinc at different levels of zinc in the surrounding medium then a horizontal trend would be expected. Although variability can be seen to be great this is in general true for levels of zinc up to about 0.5 mg l^{-1} zinc in water. At higher levels a downward trend in the distribution can be observed indicating that at these elevated levels less zinc is being accumulated.

On transformed data ?

Linear regressions were calculated for two groups of plant species. For all species sampled the regression equation is $y = 4400\,x + 1595$, $r = +\,0.524$. When only Scapania undulata, Hygrohypnum ochraceum and Lemanea fluviatilis are considered, omitting data for transient algae and bryophytes subject to only periodic inundations the regression equation is $y = 13887\,x + 303$ $r = +\,0.893$. The increased correlation coefficient suggests that the three species forming permanent submerged populations will be more reliable in reflecting levels of zinc in water.

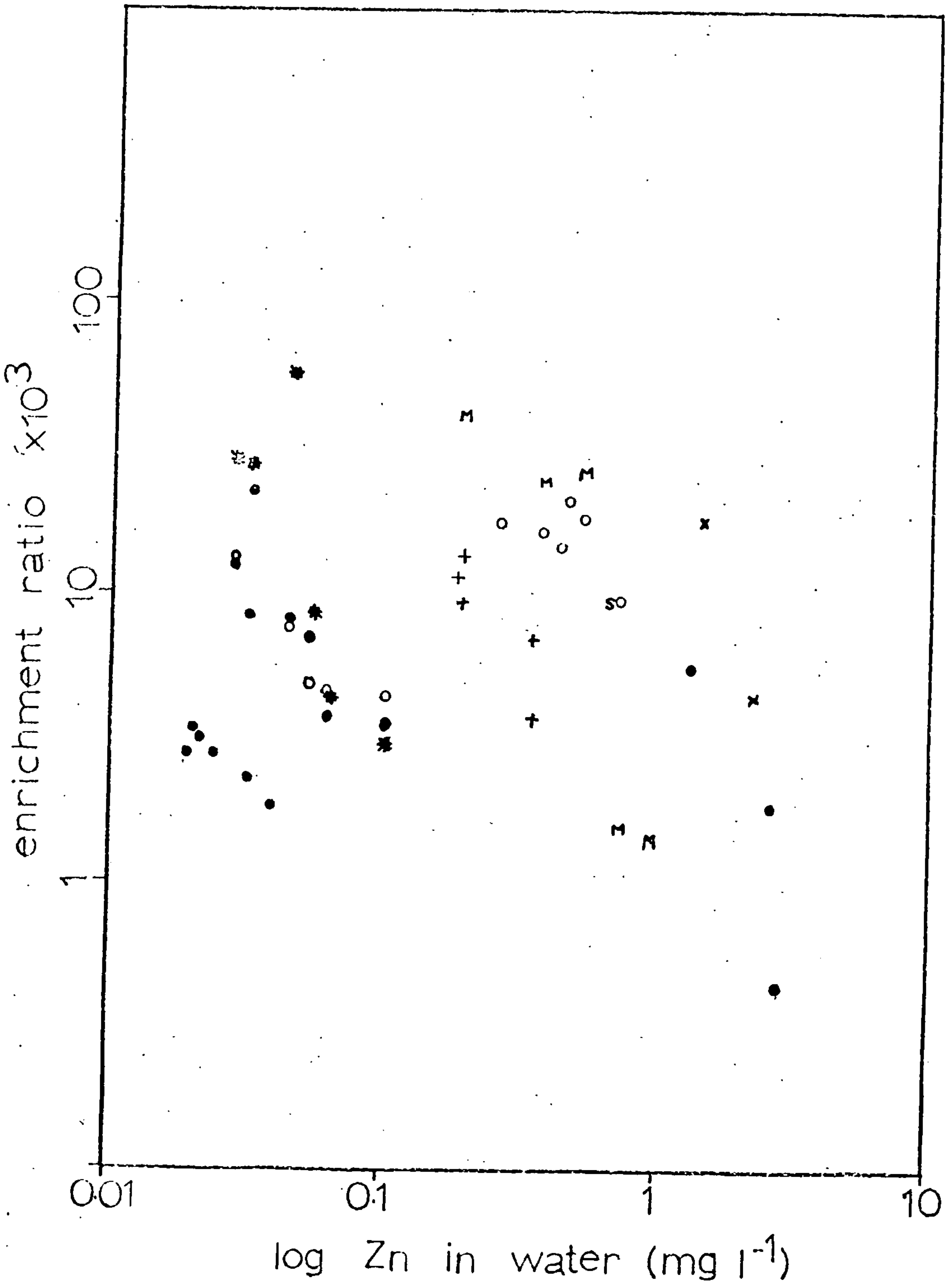


Fig. 3.13 Scatter diagram to show relationship between enrichment ratios and 'nuclepore' level of zinc for species sampled.

Key

●	*Scapania undulata*
○	*Hygrohypnum ochraceum*
✱	*Batrachospermum* sp.
+	*Lemanea fluviatilis*
×	*Dichodontium pellucidum*
M	*Mougeotia* sp.
S	*Stigeoclonium tenue*

3.5 Floristic survey

Table 3.10 shows the species found growing in Rookhope Burn and its tributary, South Grain, during the months of May, June and July 1978. Not all species were growing profusely and so were not available for heavy metal analysis. There are no species which have not previously been reported from streams with elevated levels of heavy metals although Lloyd (1977) reports Fontinalis antipyretica as being absent from Rookhope Burn. Most plants have a restricted distribution certainly of areas where they grow profusely.

(i) Bryophytes

Scapania undulata, although showing the widest distribution occurring at four of the six sites, only grew well in South Grain and upper Rookhope Burn. Isolated populations were found at sites three and four. In sites one and two it forms dense carpets of submerged and emergent growth attached to sandstone rocks on the bed of the stream. Hygrohypnum ochraceum occurs at three sites, but profusely at only sites two and five. Most populations are not continuously submerged being attached to sandstone boulders in mid stream. Dichodontium pellucidum grows as patches attached to silt wedged between rocks. It is a small form of the moss, 1 - 1.5 cm tall and is exposed to the air for much of its life. In medium-high flows it is submerged and was only sampled for heavy metals analysis at those times. Hygrohypnum luridum, Fontinalis antipyretica and Hygroamblystegium fluviatile were observed as isolated populations in reaches five and six.

(ii) Algae

Lemanea fluviatilis grows submerged, attached to limestone blocks in site six. Growth is dense with occasional overgrowth of epiphytic species. Batrachospermum sp. grows well in site two

TABLE 3.10 RESULTS OF FLORISTIC SURVEY OF SUBMERGED PLANTS IN THE STUDY AREA

BRYOPHYTES		Site					
		1	2	3	4	5	6
*	*Scapania undulata* (L.) Dum.	✓✓	✓✓	✓		✓	
*	*Hygrohypnum ochraceum* (Turn. ex Wils) Loeske.		✓✓			✓✓	✓
	H. *luridum* (Hedw.) Jenn.					✓	
*	*Dichodontium pellucidum* (Hedw.)Schp.			✓			
	Fontinalis antipyretica Hedw.					✓	
	Hygroamblystegium fluviatile (Hedw.) Loeske.					✓	✓
ALGAE							
*	*Batrachospermum* sp.		✓✓				
*	*Lemanea fluviatilis* (L.) Ag.						✓✓
*	*Stigeoclonium tenue* Kützt.				✓✓		
*	*Mougeotia* sp.			✓		✓✓	✓
	Ulothrix sp.			✓			✓
	Spirogyra sp.					✓	✓
	Hormidium sp.					✓	✓
	Microspora sp.					✓	✓

---

* Used in plant analyses

✓✓ growing profusely

✓ present

attached to sandstone rocks and was continuously submerged during the course of the sampling programme. *Stigeoclonium tenue* and *Mougeotia* sp. both show periods of dense growth, *Stigeoclonium* growing attached to sandstone boulders in site four at one collection and *Mougeotia* growing in shallows subject to periodic inundations with water from the main current. The remaining algae, *Ulothrix* sp.,*Spirogyra* sp., *Hormidium* sp. and *Microspora* sp. grow in communities dominated bt *Mougeotia* sp.

3.6 Transplant experiment

(i) The results of measurement of growth of shoots of *Scapania undulata* are presented in Table 3-11 as means of the new growth of ten shoots (nm) and percentage increase in length. A chi-squared test was carried out ($X^2 = 40.04$; $p<0.001$) indicating a significant difference in the growth of transplanted shoots. Growth is greatest (3.90 nm; 78%) in control shoots at site C and least in shoots transplanted from site B to site A. (1.00 nm; 20%). Differences in the other sites were less marked. The amount of branching occurring during the experiment (* = number of shoots showing branching) was also greatest in control site C. No branching was observed in any of the shoots originating from Site A.

(ii) Chlorophyll a/pheophytin a ratios are shown in Table 3.12 expressed at pheophytin a/chlorophyll a + pheophytin a. Student's 't' statistics for pairs of ratios on different dates is 0.080 (N.S.) suggesting no difference exists due to the transplant treatment. However, it is noticeable that shoots from site A generally contain a greater proportion of chlorophyll a than the shoots from sites B and C and that this difference is maintained after transplantation. *Scapania* growing in the uppermost regions is noticeably more robust and healthy with less sediment attached to it

TABLE 3 -11 GROWTH OF MARKED SHOOTS OF SCAPANIA UNDULATA AT TRANSPLANT SITES

		RECIPIENT SITE					
		A		B		C	
		MM	%	MM	%	MM	% / Initial
DONOR SITE	A	2.60	52	2.89	57.8	2.92	58.4
	B	1.00	20*	2.13	42.6*	3.20	64.0*
	C	2.40	48*	2.78	55.6**	3.90	78.0***

* = number of shoots showing branching

$\chi^2 = 40.04; \ p < 0.001$

TABLE 3 - 12 CHLOROPHYLL a/PHEOPHYTIN a RATIO IN MARKED SHOOTS OF SCAPANIA UNDULATA EXPRESSED AS THE RATIO PHEOPHYTIN a/ CHLOROPHYLL a + PHEOPHYTIN a

			RECIPIENT SITE		
			A	B	C
DONOR SITE	A	19/6/78	0.157	0.192	0.138
		17/7/78	0.160	0.086	0.164
	B	19/6/78	0.073	0.427	0.678
		17/7/78	0.121	0.613	0.535
	C	19/6/78	0.590	0.495	0.331
		17/7/78	0.525	0.430	0.327

t = 0.080, NS ? valid

dates.

TABLE 3-13 LEVEL OF Zn ($\mu g\ g^{-1}$ DRY WEIGHT) IN SCAPANIA UNDULATA AND IN WATER AT TRANSPLANT SITES ($mg\ l^{-1}$ 'nuc' sample)

i. SCAPANIA UNDULATA

			RECIPIENT SITES		
			A	B	C
DONOR SITE	A	19/6/78	69.76	73.68	72.94
		17/7/78	58.45	198.64	125.32
	B	14/6/78	586.27	491.82	519.11
		17/7/78	386.64	315.16	527.66
	C	19/6/78	639.63	728.35	696.21
		17/7/78	423.58	698.22	582.77

t = 0.43, N.S.

II. WATER

	A	B	C
19/6/78	0.012	0.035	0.034
17/7/78	0.024	0.039	0.040

than at the lower sites.

(iii) Concentrations of zinc in plants (ug g^{-1} dry weight) and levels in water (mg l^{-1}) for controls and transplants are displayed in Table 3.13. Student's 't' statistic for concentration of zinc in Scapania shoots shows no significant difference (t = 0.43) due to the transplant treatment but in general it can be noted that Scapania transplanted from low to higher zinc levels in water show an increase in zinc concentration (e.g. transplant from A → B; 73.68 → 198.64 µg g^{-1} dry weight) and transplants to sites with lower zinc levels show a decrease in zinc concentration (e.g. transplant from B → A; 586.27 → 386.64 µg g^{-1} dry weight).

The most interesting feature however is that despite the fact that levels of zinc in water at all three sites differ very little, Scapania from site A has consistently lower levels of zinc than either of the other two sites, even after transplation for 37 days of the experiment.

3.7 Correlation analysis

An intervariable correlation analysis was completed for the mean levels of 16 variables in water during the sampling programme presented in Table 3-14. While correlation analysis with a small number of samples (n = 6) is not statistically very meaningful it serves to indicate which factors may be interrelated and which opposed. It does not suggest caused relationships and a more elaborate programme of sampling and more sophisticated statistical analysis such as principal component analyses would be required. The results of the analysis are presented in the form of a matrix in Table 3-15.

TABLE 3-14 Mean levels of chemical and physical variables in water during the sampling programme.

(n = 6 except 'nuc' Pb where n=5)

Sample Site	'nuc' Zn	'nuc' Pb	'nuc' Cd	'nuc' Ca	'nuc' Mg	'nuc' Fe	NO_3-N	NA_4-N	PO_4-P	pH	Alkalinity	OD 420	OD 254	OD 240	Conductivity	Temperature
1	0.047	0.016	0.0011	5.24	1.41	1.32	0.098	0.244	0.016	6.83	6.99	0.068	0.530	0.564	0.70	9.7
2	0.054	0.031	0.0012	17.82	3.58	0.70	0.135	0.153	0.017	7.17	37.00	0.024	0.326	0.428	1.631	12.6
3	1.68	0.032	0.0023	39.37	7.63	0.31	0.174	0.171	0.015	7.48	55.58	0.011	0.105	0.186	3.708	14.4
4	0.58	0.093	0.0018	42.67	7.45	0.30	0.141	0.144	0.009	8.05	72.13	0.008	0.083	0.091	3.542	14.7
5	0.46	0.062	0.0015	41.93	7.30	0.29	0.252	0.163	0.017	7.83	68.58	0.006	0.091	0.100	3.875	15.0
6	0.23	0.051	0.0013	43.98	6.80	0.21	0.331	0.150	0.019	8.03	84.33	0.010	0.097	0.103	3.825	13.4

TABLE 3-15 Correlation matrix for mean levels of chemical and physical variables in water

$^{*}r > 0.811$, $p < 0.05$; $^{**}r > 0.917$, $p < 0.01$; $^{***}r > 0.974$, $p < 0.001$

	Zn	Pb	Cd	Ca	Mg	Fe	NO_3-N	NH_4-N	PO_4-P	pH	Alkalinity	OD_{420}	OD_{254}	OD_{240}	Conductivity	Temperature
Zn	1	.062	.954**	.487	.623	-.468	.010	-.185	-.283	.204	.270	-.436	-.521	-.425	.544	.537
Pb		1	.342	.728	.693	-.668	.268	-.609	-.620	.858*	.742	-.678	-.719	-.782	.661	.722
Cd			1	.617	.744	-.590	-.004	-.358	-.508	.393	.416	-.573	-.655	-.575	.645	.685
Ca				1	.981***	-.972**	.692	-.767	-.181	.943**	.965**	-.925**	-.991***	-.995***	.993***	.916*
Mg					1	-.961**	.577	-.746	-.256	.876*	.904*	-.929**	-.989***	-.969**	.985***	.952**
Fe						1	-.684	.875*	.105	-.901*	-.960**	.982***	.988***	.954**	-.963**	-.939**
NO_3-N							1	.487	.523	.692	.778	-.595	-.639	-.683	.705	.469
NH_4-N								1	.155	-.779	-.836*	.924**	.819*	.756	-.720	-.828*
PO_4-P									1	-.240	-.082	.138	.201	.215	-.122	-.282
pH										1	.974***	-.853*	-.916*	-.963**	.902*	.819*
Alkalinity											1	-.915*	-.950**	-.965**	.938**	.849*
OD_{420}												1	.963**	.911*	-.913*	-.966**
OD_{254}													1	.981***	-.985***	-.956**
OD_{240}														1	-.982***	-.912*
Conductivity															1	.915
Temperature																1

Zinc shows a significant positive correlation with cadmium ($p < 0.01$) but a weak correlation with lead. This will be related to the sources of these metals in the catchment (4.1). Although not statistically significant, quite large negative correlations are found between all three heavy metals and all measures of optical density, estimating organic material in the water, and this will be related to chemical chelation speciation of the cations (4.1.4). Negative correlations are seen between the heavy metals, lead and cadmium, and iron which will be discussed in relation to the adsorptive effect of hydrated iron oxides. Significant negative correlations of iron with calcium ($r = -0.972$, $p < 0.01$) and magnesium ($r = -0.961$, p 0.01). / verb

Calcium and magnesium show a strong positive correlation with each other ($r = 0.981$, $p < 0.001$) which can be related to their co-occurrence in limestone rocks (4.1.2) and as would be expected they show positive correlations with pH (Ca, $r = 0.943$, p 0.01; Mg, $r = 0.876$, $p < 0.05$) and total alkalinity (Ca, $r = 0.965$, p 0.001; Mg, $r = 0.904$ $p < 0.05$). In both cases calcium shows the stronger correlation and appears to be making the greater contribution being present in greater concentrations in water (Table 3-3, 3-4). Strong negative correlations are seen with all measures of optical density which will be related to the formation of organic complexes. (4.1.4). A strong positive correlation ($p < 0.001$) shows with electrical conductivity indicating the contribution made by these cations to the total solute concentration of the water.

Iron and NH_4-N are positively correlated ($p < 0.05$) which is related to the common source of these factors. Negative correlations of iron with pH ($p < 0.05$) and total alkalinity ($p < 0.01$) can be related to precipitation effects of increased alkalinity and water hardness. (4.1.5). Strong positive correlations are seen with all measures of optical density ($p < 0.01$) which can be related to the chelating effects of humic materials.

PO_4-P and NO_3-N show a positive correlation with each other (N.S) and PO_4-P is negatively correlated with NH_4-N (N.S).

Strong significant negative correlations are observed between both pH ($p < 0.05$) and total alkalinity ($p < 0.05$) and all measures of optical density indicating the acid soft nature of the water draining off the peat in the upper catchment.

Positive correlation with both calcium ($p < 0.05$) and magnesium ($p \; 0.01$) and temperature can probably be related to solubility effects but the strong negative correlation with iron ($p < 0.01$) and measures of optical density are probably explained by altitudinal effects and the rate of flow of the stream along its length.

CHAPTER 4

DISCUSSION

The present study is subject to the difficulties of interpretation inherent in all short-term, limited scale sampling programmes but raises some interesting points regarding uptake of a heavy metal by plant species and these will be discussed in relation to the use of plants as monitors of levels of zinc in the aquatic environment.

4.1 Water Chemistry

4.1.1 Sources of heavy metals in Rookhope Burn

? The low background levels of heavy metals recorded at site one in the present study are higher than those occurring in upland streams in non-mining areas in the United Kingdom which are in the range 0.002 to 0.01 mg l^{-1} (Whitton and Say 1975). Leeder (1972) quotes levels in the River Tweed in a non-mineralised region of 0.002 to 0.006 mg l^{-1} in the months of April to June 1976. Major increases in zinc and cadmium occur above site three in the present study (3.1.1) suggesting that the run-off from old mine tailings in the upper reaches of Rookhope Burn are contributing little to levels in water and the main sources would appear to be the active fluorspar mines and drainage from the mine adits. Say (1977) quotes figures from Tailrace level (NY 917428) of zinc in water over the year 1972 - 75 varying from 7.6 to 9.0 mg l^{-1} and from data held on the computer file at the Botany department, Durham University, a figure of 6.15 mg l^{-1} of zinc is recorded for Viaduct Flush (NY 925430). Both of these sources are adits draining old mine workings and must contribute significantly to levels in the main stream even after dilution. The high correlation between zinc and cadmium found in the present study (3.8) suggest a similar source for cadmium, Say (1978) reporting a level of 0.017 mg l^{-1} from Tailrace Level. This agrees with the findings of Leeder (1972) who suggests that the washing plant contributes little to raised levels of zinc in Rookhope Burn.

Maximum levels of lead are found below site four (3.1.1) where the major influence will be the activities at the washing plant. The highest level of lead recorded in 'nuclepore' water samples in the stream during the survey was on the final sampling date (3.1.3). During this time effluent was being discharged from the settlement pools and clearly they are the major contributors to elevated lead levels in the stream.

Interference effects between lead and cadmium are found by Say (1977) to increase zinc toxicity in species of <u>Hormidium</u> during laboratory toxicity tests and the co-occurrence of these two elements at elevated levels of zinc at site four may well account for the absence of any permanent flora at this site (3.6).

Levels of all heavy metals fall below site four suggesting loss of metals from water by precipitation, adsorption and dilution. The differences recorded in levels of zinc in 'total' and 'nuclepore' samples (3.1.3) although not statistically significant will have an effect on accumulation since soluble forms are taken up to higher levels than are colloidal or particulate forms.

4.1.2 The role of major cations and the speciation of heavy metal cations

The high levels of cations of calcium and magnesium recorded in the lower reaches of Rookhope Burn (3.1.2) indicate the hard calcareous and alkaline nature of the water. The antagonistic effect of calcium on heavy metal cations reported by Jones (1958) may afford some protection to plant material from the effects of zinc accumulation. Say and Whitton (1977) find that increased concentrations of both calcium and magnesium reduce the toxicity of zinc to laboratory populations of Hormidium sp. and propose a hypothesis of calcium competing with zinc for uptake sites in the plant.

The role of iron in reducing the pool of heavy metals available to the plant may be significant. Iron oxides and hydrous oxides are increasingly precipitated in hard alkaline waters and at the pH values observed in this study the adsorptive properties of metal hydroxide on to sediments is high. Furstenau (1970) reports maximal adsorption of iron species on to quartz at pH 2-3. This increase in precipitation of hydrous oxides of iron with their strong cation exchange mechanisms (Williams et al 1974) will remove hydrolyzable metal by adsorption. Increase in pH will enhance this effect.

Jenne (1968) proposes that the hydrous oxides of iron and manganese furnish the principal control on the fixation of zinc in freshwater sidements. In the lower reaches of the stream where the effluent from the settlement pools exerts its influence (1.7, 3.1.3), then the high levels of colloidal and particulate iron present will certainly play a role in removing heavy metals from solution.

4.1.3 The role of anions in zinc accumulation

The low levels of anions (Table 3-6) are characteristic of upland streams. The increase in NO_3-N and PO_4-P below sites five and six are probably related to the effects of the sewage treatment plant and an open drain at Eastgate together with run off from agricultural land. The preence of PO_4-P is reported to reduce the toxicity of zinc to plants (Say et al 1977), but the major effect of the anions is likely to be one of reduced productivity of the stream waters.

4.1.4 The role of organic complexes

Amongst soluble species of metals the role of complexing and chelating agents appears important. Bolter and Butz (1976) report natural organic acids from decaying leaf litter increase the solubility of heavy metal compounds by an increased complex formation in the presence of soluble organic acid or fulvic acid and suggest that increasing pH increases the formation of complexes with fulvic acid. The high levels of humic materials seen in the upper reaches in the present study (Table 3-8) will increase the pool of available metals by forming organic complexes. Complexing by humic acids is reported to reach a maximum at pH 8.5 (Randhawa and Broadbent 1965), and Bondarenko (1972) reports calcium humates as being most stable at neutral or alkaline pH values. At the pH values reported in the present study humic materials will play a role in the upper reaches in solubilising zinc. The role of fulvic acids is complicated by the report by Bondarenko (1972) of calcium fulvates being more stable or low pH values.

4.1.5 pH and temperature effects

pH and temperature will have effects on the solubilities of metals, and ph on the adsorptive properties of oxides and hydrous oxides (4.1.2). Increase in pH and decrease in temperature result in reduced solubilities of metals and increase in pH is reported by Bachmann (1961) to increase uptake of ^{65}Zn in laboratory studies of freshwater algae. Along the length of the stream increase in pH is accompanied by increase in temperature, but effects such as the increasing water hardness and total alkalinity are probably more significant.

4.1.6 Levels of flow in the stream

Flow clearly plays a part in controlling levels of dissolved substances in aquatic environments. In general, levels of metals are highest at lowest flows, agreeing with the findings of Grimshaw et al (1976). They attribute this inverse relationship to a 'dilution' effect with a negative relationship between discharge and concentrations of zinc. The relationship is however confused by 'flushing', occurring when storm run off follows heavy summer precipitation. During dry summer months, at elevated temperatures, oxidation of mineral ores can be presumed to occur resulting in raised concentrations in sources of heavy metals. The abrupt leaching of these metals at such times will lead to high metal concentrations in water related to high discharge. Clearly the present study goes no way towards providing information on this aspect and a longitudinal study such as that by Grimshaw et al is required.

The relation between anions and discharge shows maximum levels of anions occurring at maximum flows relating to the different origins of these substances.

4.2 Plant Material

Of the plant species sampled during the present study, three seem to present themselves as candidates for further consideration for use as monitors of zinc levels in water polluted by mine drainage. Scapania undulata, Hygrohypnum ochraceum and Lemanea fluviatilis show relatively stable enrichment ratios, at least within certain environmental limits. They all occur as permanent members of the submerged populations of the stream, are relatively robust plants and do not present great problems in preparation for analysis. Both S. undulata and H. ochraceum have a fairly broad distribution along the stream and work by Harding (1978)has shown the potential of L. fluviatilis for transplantation to areas of elevated zinc levels in upland streams for analysis of zinc content after a suitable exposure time. Lemanea samples with a zinc content of 213 µg g^{-1} dry weight were transplanted to a polluted site where samples showed concentrations greater than 1000 µg g^{-1} dry weight. Subsequent analysis of these transplanted samples revealed levels above 1000 µg g^{-1} dry weight. Corresponding decreases were observed in reciprocal transplants. Duncker (1976) demonstrated that transplants of Scapania undulata left in sites for eight weeks show similar increases. The limited transplantation experiment carried out in the present study shows a similar trend of increase at sites of higher zinc levels in water but perhaps a longer equilibration time is needed for a more significant result.

In general it can be said that increased levels of zinc in water are correlated with increased concentrations in plant material although the variability is great. S. undulata shows relatively high contents of zinc at sites with low levels of zinc in water e.g. site two, mean zinc concentration 393.67 $\mu g\ g^{-1}$ dry weight with 0.039 $mg\ l^{-1}$ in water compared with site one, mean zinc concentration 67.17 $\mu g\ g^{-1}$ dry weight with 0.026 $mg\ l^{-1}$ in water. McLean and Jones (1975) in uptake studies of 65 Zn in Scapania show a general resistance to heavy metals and it is unfortunate that replicated samples for the bryophyte at higher zinc levels are not available from the present study. The absence of Scapania at such sites may be related to the preference of the species for soft acidic waters. Say (1977) finds Scapania in waters with 10.55 $mg\ l^{-1}$ $Ca\ Co_3$ and pH 3.8 - 6.8 growing attached to a sandstone or shale substrate. The lower reaches of Rookhope Burn have a pH range of 7.9 - 8.1, a total alkalinity of 68.6 - 84.3 $mg\ l^{-1}$ $Ca\ Co_3$ and limestone blocks forming the substratum. However at the collection with the highest zinc level (site three, 15th June 1978, $Zn_W = 2.6\ mg\ l^{-1}$) the concentration of zinc in Scapania (4840 $ug\ g^{-1}$ dry weight) is not the highest content recorded suggesting some measure of regulation of zinc accumulation. This is supported by the lower enrichment ratios for Scapania at site three (441 - 5612) compared to site two (3485 - 21188). Although the transplant studies reported here, nor those of Duncker (1976) do not support the view that different strains of Scapania exist it is worth noting again that populations of the bryophyte growing in water containing very similar levels of zinc are markedly different in zinc concentration despite the presence at the uppermost site of humic materials able to complex with zinc ions, and lower levels of calcium and magnesium.

4.3 Relationship to other studies

A number of other studies of zinc accumulation in the mineralised area of Weardale have included the three species Scapania undulata, Hygrohypnum ochraceum and Lemanea fluviatilis. Results of analyses of zinc in plant and water are presented in Table 4-1 from the work of Leeder (1972), Duncker (1976), Lloyd (1977) and Harding (1978). Despite differences in the methodology of water collection, plant sampling and plant digestion procedure discussed below, some general features do emerge.

The anomalous results obtained for Scapania undulata from site one on South Grain are similar to those obtained by other workers on North Grain sike, another tributary of Rookhope Burn. Concentrations of zinc in plants are an order of magnitude lower in these two streams compared to concentrations in Rookhope Burn.

Concentrations of zinc in Hygrohypnum ochraceum at sites with low levels of zinc (0.1 mg l^{-1}) water reported in the present study range from 274 ug g^{-1} dry weight to 369 ug g^{-1} dry weight. Harding reports a concentration of 334 ug g^{-1} dry weight in samples from the River Derwent at a water level of 0.021 mg l^{-1}.

At sites with water levels greater than 0.20 mg l^{-1} plant content ranges from 4584 ug g^{-1} dry weight to 9912 ug g^{-1} dry weight in the present study. Reports from other workers are in the range 1438 ug g^{-1} dry weight to 16480 ug g^{-1} dry weight.

The results for Lemanea fluviatilis from the present study range from 1308 ug g^{-1} dry weight to 2647 ug g^{-1} dry weight with zinc level in water greater than 0.1 mg l^{-1} in all samples. Samples from other waters at levels greater than 0.1 mg l^{-1} range from 721 ug g^{-1} dry weight to 2890 ug g^{-1} dry weight.

Results then are of a similar order of magnitude to other workers but there is a great variability from one study to another making comparability difficult. Enrichment ratios in Scapania undulata ranging

TABLE 4-1 LEVELS OF Zn IN PLANTS (Zn_p, µg g l^{-1} dry weight) AND WATER (Zn_w, mg l^{-1}) IN STUDIES IN THE MINERALISED AREA OF WEARDALE

SPECIES	AUTHOR	STREAM NAME	Zn_w mg l^{-1}	Zn_p µg g^{-1}	Enrichment ratio	
Scapania undulata	Duncker (1976)	North Grain Sike	0.031	129	4161	
			0.126	452	3587	
	Lloyd (1977)	North Grain Sike	0.131	150	1145	
			0.015	266	17733	
		Rookhope Burn	0.100	2450	24500	
			0.030	3890	129667	
	Harding (1978)	R.Derwent	0.021	771	36714	
			0.272	2992	11000	
Hygrohypnum ochraceum	Leeder (1972)	Rookhope Burn	0.221	5256	23783	
			0.149	6730	45168	
			0.077	4109	53363	
	Lloyd (1977)	Rookhope Burn	0.030	3600	120000	
			0.100	4530	45300	
			0.520	3960	7615	
			0.330	16480	49939	
			0.310	3500	11290	
			0.240	11790	49125	
	Harding (1978)	R.Derwent	0.021	334	15905	
			0.272	1438	5286	
Lemanea fluviatilis	Leeder (1972)	Rookhope Burn	0.077	1197	15545	
			0.149	1514	10161	
	Lloyd (1977)	Rookhope Burn	0.240	2890	12042	(old tissue)
			0.310	1310	4226	(young tissue)
	Harding (1978)	R.Derwent	0.021	351	16714	
			0.272	916	3368	
			0.206	723	3510	

from 1145 to 129667 and in Hygrohypnum ochraceum from 4466 to 120000 make generalisations difficult. The major source of difference particularly in the older studies is probably in the sampling of plant material. In the present study 1 cm tips of the bryophytes and 2 cm tips of the alga were removed. Other workers have used different standards or specified samples as being of 'healthy growth'. Lloyd (1977) compares zinc concentrations in 0.5 cm sections of Hygrohypnum ochraceum and shows increasing concentration passing both from the tip (tip: 369±9 µg g^{-1} dry weight, 0.5-1 cm: 848±9 µg g^{-1} dry weight; 1.5-2.0 cm: 1857± 78 µg g^{-1} dry weight).

Other problems arise from the water sampling technique used. The filtered samples from early studies is equivalent to the 'total' sample of the present study (i.e. filtered through a 'sinta' glass funnel). It has been shown in the present study that 'total' levels of zinc can be significantly higher than 'nuclepore' samples at some collections (3.1.3) and the effect of this will be to reduce enrichment ratios.

A third source of difference is in the digestion procedure used. Leeder (1972) uses a muffle furnace at 500°C to prepare samples, a technique which is reported by Zak (1966) to lead to low values in the determination of metals. Lloyd (1977) in comparing a number of digestion processes finds lower levels of zinc, lead and cadmium in dry ashing methods when compared to wet digestion. Any reduction in the apparent zinc content of plant material will again reduce the magnitude of the enrichment ratio.

4.4 Plants as monitors of zinc in natural waters

It seems unlikely from the results of the present study that plants can be used to monitor zinc at higher levels in water since this requires both stable enrichment ratios and a low variability which are not observed in the species studied. Reference to Fig. 3.11 and 3.12 shows a plateau in accumulation in those species growing at elevated levels of zinc. Most hypotheses of uptake discuss binding sites for metals (Pickering and Puia 1969, McLean and Jones 1975, Say and Whitton 1977). These must be finite and, once saturated, uptake must be inhibited. In addition Dietz (1973) shows many bryophytes adsorbing manganese and iron externally. This is a passive process and as such will lead to erroneous results for accumulation since it is not under the control of the plant. If similar external adsorption occurs of zinc then there must be doubts about the use of bryophytes as monitors. The large amount of mucilage secreted by algae such as Batrachospermum sp. may also play a role in taking up heavy metals externally.

Changes in levels of zinc in water may be rapid such as will occur following 'flushing' (4.1.6) and plant species should be able to respond to these sudden increases. They must also be capable of integrating fluctuations in the normal levels of zinc so as to reflect a general level of the metal.

The source of zinc to a species used for monitoring levels in water should be the aquatic phase, of the environment. Rooted angiosperms such as Mimulus guttatus reported in a high zinc-level stream (Leeder 1972, Lloyd 1977) can be assumed to be absorbing at least in part from the substratum. Even the absorptive function of the rhizoidal system of bryophytes may result in uptake of the plants' metal intake from sediments.

If sampling is to take place throughout the annual cycle of a stream, plants need to be permanent members of the stream community and occur in sufficient abundance to allow repeated sampling. The transient algae observed in the present study are unsuitable for monitoring procedures. Similarly emergent species, or those subject to periodic inundations such as Dichodontium pellucidum are also unsuitable.

The sampling procedure is required to be easily performed by non-specialists, and the three species sampled consistently, *Scapania undulata*, *Hygrohypnum ochraceum* and *Lemanea fluviatilis*, all proved satisfactory on those grounds.

The technique of transplanting to areas of raised zinc-levels must be limited by the natural distribution of the species and interpretation of accumulation must be related to the other physical and chemical parameters of the stream. More information is required in relation to the speciation of metals in natural waters and the interaction with complexing and chelating molecules, synergism and antagonism of other cations.

The existence of tolerant strains has been shown in a number of aquatic species (1.5) but the work of Duncker (1976) and the present study (3.6) fail to provide evidence that this is the case in *Scapania undulata*. If tolerant strains do exist then laboratory studies are required to distinguish them from non-tolerant forms and accumulation studies carried out such as those of Duncker on *Scapania* and Harding (1978) on *Lemanea fluviatilis*.

The limited data available from the transplant experiment of the present study regarding growth suggests that more studies of growth rates for tolerant and non-tolerant strains are required. It may be that tolerance is characterised by a lower relative growth rate (R_{max} of Grime and Hunt 1975) enabling species to survive in conditions of heavy metal stress. Comparability of 1 cm or 2 cm tips of plant material referred to in 4.3 may be rendered invalid by marked differences in growth rates of different strains and further, whether the high contents of older tissues (4.3) is a function of time of exposure to high zinc levels or some factor such as general metabolic activity which could be related to the age of the tissue.

From the results of the present study and consideration of the results of other authors a strategy can be envisaged of employing a range of bryophytes and algae such as *Scapania undulata*, *Hygrohypnum ochraceum* and *Lemanea fluviatilis* as monitors of zinc levels in water. Uptake needs to be determined in field and laboratory conditions using a standardised methodology of permanent populations and transplant specimens, and accumulation ratios so determined interpreted in relation to chemical and physical parameters of the water. At highest levels of zinc where a plateau of accumulation occurs it could be used to indicate a saturating level of the metal in water.

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SUMMARY

(i) Data has been collected relating to the accumulation of zinc in seven species of bryophytes and algae growing submerged in an upland stream flowing through an area of past and present mining activity.

(ii) Six sampling sites were established in the catchment of Rookhope Burn, a tributary of the River Wear.

(iii) Levels of Zn, Pb, Cd, Ca, Mg, Fe, NO_3-N, NH_4-N and PO_4-P in water were determined at 10-14 day intervals over a 60 day sampling programme. The environmental variables of water measured at each site were pH, temperature, total alkalinity, electrical conductivity and optical density. Water flow was estimated at each collection.

(iv) Growth, chlorophyll a concentration and zinc content of transplanted shoots of Scapania undulata (L.) Dum. were determined at sites with differing levels of zinc in water.

(v) Zinc and cadmium levels in water show a positive correlation ($r = 0.954$, $p < 0.01$)

(vi) Levels of zinc in water show a linear relationship with the zinc content of plant tissues up to 0.5 mg l^{-1} zinc in 'nuclepore' water samples. Above this level a plateau is observed.

Enrichment ratios for the seven species sampled are determined and for the three species _Scapania undulata_ _Hygrohypnum ochraceum_ and _Lemanea fluviatilis_ are found to be relatively stable up to 0.5 mg l^{-1} zinc in water.

(vii) No evidence is found from the transplant study for the existence of different strains of _Scapania undulata._

(viii) Accumulation is briefly discussed in relation to the levels of cations and anions determined and in relation to the environmental measures.

APPENDIX A

SITE DESCRIPTIONS IN ROOKHOPE BURN CATCHMENT

SITE 1 SOUTH GRAIN

Stream Number: 0219 Reach Number: 75

Site Description: 200m above entry of South Foul Sike.

Grid Ref: NY 877442 Map Ref: 54°47′ 02° 10′

Width: 0.5 - 1.3m Depth: 0.1 - 0.5m Fall: 1m

Substrate: Sandstone rocks and gravel.

Submerged flora: *Scapania undulata* growing densely on exposed and submerged rocks

Upstream features: Extensive drainage on upper fells.

SITE 2 ROOKHOPE BURN

Stream Number: 0012 Reach Number: 15

Site Description: 300m above Grove Rake mine.

Grid Ref: NY 894443 Map Ref: 54°47′ 02°09′

Width: 1 - 2.5m Depth: 0.10 - 0.25m Fall: 0.5m

Substrate: Sandstone rocks and gravel.

Submerged flora: *Scapania undulata* growing densely on exposed and submerged rocks in rapids. *Hygrohypnum ochraceum* submerged and emergent on sandstone rocks. *Batrachospermum* sp. continuously submerged, attached to sandstone rocks.

Upstream features: old lead mine tailings.

SITE 3 ROOKHOPE BURN

Stream Number: 0012 Reach Number: 30

Site Description: immediately below road bridge

Grid Ref: NY 924430 Map Ref: 54°46′ 02°06′

Substrate: Sandstone rocks, heavy deposits of sediments.

Submerged flora: Very restricted growth. Small populations of *Scapania undulata* and *Dichodontium pellucidum* growing as emergent species except at high flows. Growth of *Mougeotia* sp. in shallows at low flows.

Upstream features: Adits from old lead mine workings, Grove Rake mine, Redburn mine.

SITE 4 ROOKHOPE BURN

Stream Number: 0012 Reach Number: 38

Site Description: Below fluorspar washing plant

Grid Ref: NY 941423 Map Ref: 54°46′ 02°05′

Width: 3-5m Depth: 0.25 - 0.40m Fall: 0.25m

Substrate: Sandstone rocks with heavy precipitates of iron oxides

Submerged flora: *Stigesclonium tenue* attached to rocks as a transient species. *Hygrohypnum ochraceum* as stunted growth on exposed rocks only. *Mougeotia* sp. in shallows at lowest flows.

Upstream features: Fluorspar washing plant.

SITE 5 ROOKHOPE BURN

Stream Number: 0012 Reach Number: 41

Site Description: Above foot bridge

Grid Ref: NY 944416 Map Ref: 54°45′ 02°04′

Width: 3-5m Depth: 0.25 - 0.30m Fall: 0.15m

Substrate: Limestone blocks, sandstone rocks and gravel.

Submerged flora: *Hygrohypnum ochraceum* submerged and emergent. Small populations of *Hygrohypnum luridum*, *Fontinalis antipyretica* and *Hygroamblystegium fluviatile*

Upstream features: Sewage treatment works.

SITE 6 ROOKHOPE BURN

Stream Number: 0012 Reach Number: 46

Site Description: Eastgate, below road bridge

Grid Ref: NY 953386 Map Ref: 54°44′ 02°04′

Width: 5-7m Depth: 0.25 - 0.30m Fall: 0.15m

Substrate: Limestone blocks and gravel.

Submerged flora: Dominated by *Lemanea fluviatilis*, continuously submerged, attached to limestone blocks. Populations of *Hygrohypnum ochraceum* as emergent species.

Upstream features: Caravan park, open drain from Eastgate village, farmland. Banks of stream extensively covered by trees giving considerable shade.

APPENDIX B-1 TABLES (1) - (VI LEVELS OF CATIONS AT SIX SAMPLE SITES ON COLLECTION DATE (DAYS)

('tot' = total samples, 'nuc' = nuclepore samples; all in mg l^{-1})

(i) Site 1

Collection	Zn		Pb		Cd		Ca		Mg		Fe	
(Days)	'tot'	'nuc'	'tot'	'nuc'	'tot'	'nuc'	'tot'	'nuc'	'tot'	'nuc'	'tot'	'nuc'
1	0.047	0.022	0.009	0.007	0.0010	0.0010	2.87	2.91	0.911	1.046	1.20	1.07
14	0.22	0.020	0.009	0.008	0.0013	0.0010	4.79	4.68	1.20	1.19	1.70	1.29
28	0.094	0.033	0.025	0.020	0.0010	0.0010	6.32	5.99	1.42	1.71	2.24	1.70
40	0.042	0.019	0.008	-	0.0013	0.0011	4.54	4.52	1.36	1.39	1.55	1.05
50	0.052	0.039	0.013	0.014	0.0020	0.0013	4.95	7.45	1.50	1.60	1.20	1.08
60	0.034	0.024	0.009	0.029	0.0017	0.0013	5.60	5.90	1.40	1.50	2.20	1.75
$\bar{x}$	0.049	0.026	0.011	0.016	0.0014	0.0011	4.85	5.24	1.298	1.405	1.68	1.32
sem	0.010	0.003	0.001	0.003	0.002	0.0001	0.47	0.64	0.088	0.103	0.19	0.13
(ii) Site 2												
1	0.051	0.045	0.035	0.029	0.0019	0.0015	10.6	10.5	2.3	2.3	1.00	0.90
14	0.042	0.028	0.032	0.029	0.0014	0.0013	19.7	19.3	4.2	3.9	0.70	0.56
28	0.034	0.032	0.039	0.025	0.0010	0.0011	25.2	24.5	4.8	4.9	0.67	0.53
40	0.036	0.053	0.025	-	0.0012	0.0010	13.1	13.2	2.8	2.8	1.23	0.82
50	0.076	0.062	0.047	0.045	0.0014	0.0011	16.9	13.9	2.8	2.8	0.94	0.84
60	0.040	0.013	0.025	0.029	0.0016	0.0013	27.5	25.5	4.9	4.8	0.86	0.56
$\bar{x}$	0.048	0.039	0.034	0.031	0.0014	0.0012	18.83	17.82	3.63	3.58	0.90	0.70
sem	0.006	0.007	0.001	0.003	0.0001	0.0001	2.71	2.56	0.46	0.45	0.08	0.07

(iii) Site 3

Collection	Zn		Pb		Cd		Ca		Mg		Fe	
(Days)	'tot'	'nuc'	'tot'	'nuc'	'tot'	'nuc'	'tot'	'nuc'	'tot'	'nuc'	'tot'	'nuc'
1	1.43	1.30	0.057	0.053	0.0024	0.0019	28.4	27.6	5.6	5.4	0.56	0.37
14	2.08	1.30	0.037	0.033	0.0028	0.0026	49.8	27.2	10.1	5.8	0.32	0.21
28	2.75	2.60	0.045	0.009	0.0025	0.0023	59.5	58.2	11.2	11.4	0.36	0.04
40	1.67	1.28	0.198	-	0.0022	0.0017	30.7	29.4	6.0	6.0	1.32	0.54
50	1.52	1.37	0.078	0.047	0.0023	0.0019	33.7	35.0	6.3	6.2	0.58	0.38
60	2.00	2.22	0.054	0.016	0.0029	0.0028	57.5	58.8	11.4	11.0	0.38	0.34
$\bar{x}$	1.91	1.68	0.078	0.032	0.0025	0.0022	43.3	39.4	8.4	7.6	0.59	0.31
sem	0.19	0.24	0.01	0.008	0.0001	0.0002	5.7	6.2	1.12	1.13	0.15	0.07

(iv) Site 4

Collection (Days)	Zn 'tot'	Zn 'nuc'	Pb 'tot'	Pb 'nuc'	Cd 'tot'	Cd 'nuc'	Ca 'tot'	Ca 'nuc'	Mg 'tot'	Mg 'nuc'	Fe 'tot'	Fe 'nuc'
1	0.66	0.66	0.064	0.059	0.0013	0.0010	27.5	27.6	5.1	4.9	0.42	0.45
14	0.62	0.51	0.082	0.077	0.0014	0.0013	47.0	45.7	8.7	8.3	0.27	0.07
28	0.95	0.94	0.068	0.060	0.0021	0.0016	54.8	54.3	10.0	9.6	0.22	0.21
40	0.91	0.52	0.176	-	0.0019	0.0016	33.5	33.4	6.1	6.2	1.08	0.49
50	0.67	0.60	0.268	0.086	0.0040	0.0031	37.5	39.5	6.1	6.1	0.90	0.36
60	1.38	0.22	6.160	0.184	0.0026	0.0021	84.0	55.5	10.6	9.6	12.25	0.24
$\bar{x}$	0.87	0.58	1.136	0.093	0.0022	0.0018	47.4	42.7	7.8	7.5	2.52	0.30
sem	0.12	0.09	0.410	0.023	0.0004	0.0003	8.3	4.6	0.90	0.81	1.95	0.06

(v) Site 5

Collection (Days)	Zn 'tot'	Zn 'nuc'	Pb 'tot'	Pb 'nuc'	Cd 'tot'	Cd 'nuc'	Ca 'tot'	Ca 'nuc'	Mg 'tot'	Mg 'nuc'	Fe 'tot'	Fe 'nuc
1	0.55	0.46	0.154	0.077	0.0013	0.0011	28.9	28.3	4.9	4.7	0.57	0.22
14	0.19	0.26	0.058	0.052	0.0013	0.0011	46.1	41.9	8.3	7.9	0.48	0.12
28	0.64	0.37	0.136	0.032	0.0016	0.0013	56.6	54.2	9.4	9.4	0.63	0.12
40	0.83	0.72	0.196	-	0.0019	0.0017	34.5	34.0	6.2	6.3	1.10	0.71
50	0.60	0.52	0.184	0.084	0.0026	0.0019	37.1	38.5	6.2	6.1	0.76	0.36
60	0.46	0.43	1.680	0.066	0.0021	0.0016	55.6	54.7	9.8	9.4	0.20	0.18
$\bar{x}$	0.58	0.46	0.401	0.062	0.0018	0.0015	43.1	41.9	7.5	7.3	0.62	0.29
sem	0.06	0.06	0.256	0.008	0.0002	0.0001	4.7	4.4	0.8	0.8	0.12	0.09

(vi) Site 6

Collection (Days)	Zn 'tot'	Zn 'nuc'	Pb 'tot'	Pb 'nuc'	Cd 'tot'	Cd 'nuc'	Ca 'tot'	Ca 'nuc'	Mg 'tot'	Mg 'nuc'	Fe 'tot'	Fe 'nuc
1	0.260	0.191	0.082	0.063	0.0010	0.0010	32.8	31.5	4.6	4.5	0.47	0.22
14	0.182	0.129	0.042	0.041	0.0011	0.0010	51.5	46.2	7.6	7.3	0.34	0.13
28	0.203	0.191	0.048	0.022	0.0014	0.0012	55.8	56.5	8.5	8.7	0.30	0.06
40	0.660	0.340	0.204	-	0.0014	0.0013	36.9	37.5	6.2	6.1	1.45	0.18
50	0.430	0.340	0.132	0.066	0.0020	0.0017	38.9	38.9	6.0	5.9	0.72	0.46
60	0.220	0.178	0.400	0.063	0.0015	0.0014	57.4	53.3	8.6	8.3	0.24	0.18
$\bar{x}$	0.326	0.228	0.151	0.051	0.0014	0.0013	45.6	44.0	6.9	6.8	0.59	0.21
sem	0.076	0.037	0.062	0.008	0.0001	0.0001	4.3	4.0	0.6	0.6	0.19	0.06

B - 2 Results of Student's 't' test of pairs of 'total' and 'nuclepore' samples (n = 6)

('t' test for Pb missing; 'nuclepore'sample 27th June 1978, day 40 , not available for analysis)

SAMPLE SITE		Zn	Cd	Ca	Mg	Fe
1	't'	2.105	1.342	0.490	0.790	1.564
	p	< 0.10	NS	NS	NS	NS
2	't'	0.793	2.121	0.273	0.077	1.881
	p	NS	< 0.10	NS	NS	< 0.10
3	't'	0.745	1.342	0.463	0.503	1.69
	p	NS	NS	NS	NS	NS
4	't'	1.933	1.000	0.495	0.205	1.138
	p	< 0.10	NS	NS	NS	NS
5	't'	1.323	1.342	0.186	0.221	2.200
	p	NS	NS	NS	NS	< 0.10
6	't'	1.162	0.707	0.272	0.118	1.907
	p	NS	NS	NS	NS	< 0.10

APPENDIX C I - VI

Levels of anions at 6 sampling sites on 6 sample dates (mg l^{-1})

i Sample date 18/5/78

Site	NO_3-N	NH_4-N	PO_4-P
1	0.047	0.150	0.027
2	0.180	0.140	0.005
3	0.094	0.150	0.015
4	0.114	0.120	0.011
5	0.201	0.145	0.018
6	0.288	0.140	0.032

ii Sample date 1/6/78

1	0.066	0.135	0.012
2	0.030	0.090	0.013
3	0.118	0.097	0.019
4	0.114	0.100	0.004
5	0.194	0.128	0.010
6	0.115	0.120	0.013

iii Sample date 15/6/78

1	0.053	0.245	0.011
2	0.035	0.165	0.022
3	0.138	0.200	0.019
4	0.108	0.170	0.010
5	0.276	0.195	0.019
6	0.289	0.180	0.016

iv Sample date 27/6/78

Site	NO_3-N	NH_4-N	PO_4-P
1	0.184	0.200	0.023
2	0.393	0.135	0.039
3	0.296	0.166	0.019
4	0.286	0.135	0.011
5	0.166	0.164	0.018
6	0.587	0.141	0.019

v Sample date 7/7/78

1	0.163	0.337	0.018
2	0.140	0.164	0.016
3	0.277	0.167	0.015
4	0.155	0.155	0.014
5	0.299	0.155	0.021
6	0.279	0.150	0.019

vi Sample date 17/7/78

1	0.072	0.395	0.006
2	0.029	0.225	0.009
3	0.119	0.245	0.006
4	0.066	0.185	0.005
5	0.377	0.190	0.017
6	0.427	0.170	0.016

APPENDIX D (i) - (iii)

Water records from Eastgate monitoring station on Rookhope Burn May to July, 1978 including mean daily flow $m^3 s^{-1}$ (cumecs)

>= collection date.

(Data kindly supplied by Northumbrian Water Authority).

(i) may '78

	DISCHARGE				STAGE						
	MEAN DAILY FLOW		HIGHEST	LOWEST	HIGHEST	TIME		LOWEST	TIME		MEAN
DAY	TCMD	CUMECS	CUMECS	CUMECS							
1	61.1	0.708	0.788	0.660	351	1	0	334	9	0	340
2	47.9	0.554	0.697	0.458	339	9	0	285	8	0	312
3	41.8	0.484	1.170	0.389	395	8	15	256	23	15	281
4	58.7	0.680	1.133	0.520	391	9	0	308	22	45	333
5	158.9	1.839	3.825	0.647	598	3	0	332	14	30	440
6	111.6	1.291	2.511	0.853	511	9	0	359	8	0	404
7	57.4	0.664	0.853	0.560	359	9	0	316	7	45	333
8	42.0	0.486	0.554	0.451	315	9	0	282	8	45	295
9	33.7	0.391	0.449	0.367	281	9	0	246	21	30	256
10	30.2	0.350	0.371	0.340	248	9	0	234	21	30	236
11	31.0	0.359	0.378	0.342	251	1	0	233	13	0	242
12	35.1	0.406	0.533	0.336	311	4	30	232	16	30	261
13	49.0	0.567	0.613	0.530	326	21	30	310	9	0	317
14	50.5	0.584	0.680	0.515	329	20	45	307	12	15	320
15	59.7	0.691	0.997	0.515	376	20	30	307	15	15	335
16	44.0	0.509	0.586	0.451	321	9	0	282	7	15	302
17	32.8	0.379	0.451	0.347	282	9	0	237	8	30	251
> 18	26.5	0.306	0.347	0.286	237	9	0	209	20	45	218
19	23.1	0.268	0.291	0.254	211	9	30	193	21	45	200
20	21.4	0.248	0.262	0.239	197	9	30	185	23	30	190
21	20.6	0.238	0.242	0.236	187	12	15	184	19	45	185
22	20.6	0.238	0.244	0.236	188	10	30	184	23	30	185
23	18.6	0.215	0.242	0.206	187	9	30	168	20	30	173
24	17.4	0.202	0.213	0.197	172	0	30	163	1	15	164
25	16.0	0.185	0.200	0.175	165	11	30	151	18	0	156
26	15.6	0.181	0.186	0.177	157	9	15	152	20	20	154
27	14.8	0.171	0.188	0.166	158	9	0	146	17	0	149
28	14.5	0.168	0.180	0.165	154	6	45	145	18	30	147
29	13.5	0.157	0.165	0.154	145	9	0	139	15	0	140
30	13.3	0.154	0.160	0.151	142	12	0	137	8	15	139
31	12.2	0.142	0.151	0.136	137	9	0	128	19	45	131

(ii) june '78

	DISCHARGE				STAGE						
	MEAN DAILY FLOW		HIGHEST	LOWEST	HIGHEST	TIME		LOWEST	TIME		MEAN
DAY	TEMP	CUMECS	CUMECS	CUMECS							
> 1	11.[illegible]	0.132	0.146	0.1[illegible]	134	10	30	118	[illegible]	[illegible]	125
2	11.1	0.123	0.136	0.127	[illegible]	20	15	122	15	45	[illegible]
3	11.1	0.128	[illegible]	0.127	124	[illegible]	[illegible]	[illegible]	23	30	123
4	10.5	0.121	0.127	[illegible]	122	9	0	115	21	0	118
5	11.0	0.127	0.135	0.121	127	1	45	118	18	0	122
6	11.0	0.128	0.132	0.127	125	[illegible]	[illegible]	122	13	15	122
7	11.0	0.128	0.133	0.125	126	16	45	121	10	30	123
8	10.6	0.122	0.127	0.117	122	1	30	116	18	45	117
9	9.7	0.112	0.125	0.102	121	9	0	106	21	45	112
10	9.4	0.109	0.114	0.10[illegible]	114	14	0	106	9	0	110
11	9.0	0.104	0.107	0.101	109	9	0	105	15	[illegible]	107
12	9.2	0.106	0.108	0.102	110	11	0	106	16	45	108
13	9.0	0.104	0.108	0.102	110	8	15	106	17	15	107
14	9.2	0.107	0.114	0.10[illegible]	114	10	15	104	16	[illegible]	109
> 15	9.0	0.105	0.117	0.100	[illegible]	10	0	104	18	0	107
16	8.5	0.098	0.1[illegible]	0.092	108	16	30	[illegible]	19	0	103
17	8.0	0.092	0.096	[illegible]	105	10	45	97	18	15	99
18	7.7	0.089	0.092	0.087	[illegible]	9	0	95	18	15	97
19	7.6	0.089	0.091	0.084	98	9	0	93	2	0	96
20	7.8	0.090	0.095	0.084	101	0	15	93	19	0	97
21	9.0	0.105	0.116	0.092	112	23	0	99	13	30	107
22	8.9	0.103	0.113	0.097	113	13	15	102	20	30	106
23	10.1	0.117	0.154	0.104	139	8	15	107	9	15	115
24	10.2	0.118	0.156	0.102	140	9	0	106	2	45	116
25	13.7	0.182	0.333	0.101	231	8	30	105	16	30	150
26	17.6	0.203	0.316	0.1[illegible]	223	9	0	106	6	45	165
> 27	10.8	0.125	0.140	0.119	130	10	45	117	18	45	121
28	10.2	0.118	0.125	0.114	121	11	0	114	18	15	117
29	10.1	0.117	0.119	0.115	117	10	15	113	18	15	115
30	10.6	0.122	0.128	0.116	123	20	30	115	9	0	119

(iii) july '78

	MEAN DAILY FLOW	DISCHARGE			STAGE						
			HIGHEST	LOWEST	HIGHEST	TIME		LOWEST	TIME		MEAN
DAY	[illegible]	CUMECS	CUMECS	CUMECS							
1	10.2	0.115	0.124	0.113	120	12	0	113	20	45	116
2	10.5	0.122	0.144	0.114	133	7	30	114	3	30	119
3	11.6	0.134	0.144	0.130	133	9	0	124	12	30	126
4	46.5	0.538	1.183	0.132	391	9	15	125	9	0	266
5	33.4	0.387	0.575	0.262	319	9	0	197	8	45	253
6	18.7	0.217	0.208	0.191	190	9	0	160	8	15	174
> 7	14.2	0.165	0.182	0.156	155	9	0	140	5	13	145
8	12.2	0.141	0.158	0.127	141	9	45	122	7	0	131
9	10.7	0.123	0.128	0.116	123	13	15	115	6	30	120
10	9.8	0.113	0.117	0.105	116	11	0	108	18	30	113
11	9.7	0.112	0.125	0.095	121	4	15	101	20	15	112
12	7.8	0.090	0.115	0.080	115	9	0	94	16	45	97
13	[illegible]	0.069	0.102	0.083	106	5	0	92	20	30	97
14	7.5	0.086	0.090	0.081	97	7	45	91	15	0	95
15	8.1	0.094	0.104	0.084	107	19	45	93	4	45	100
16	7.2	0.084	0.087	0.080	95	9	0	90	12	30	93
> 17	7.2	0.083	0.092	0.077	99	21	0	88	23	0	92
18	7.6	0.088	0.098	0.079	103	5	30	89	2	0	96
19	7.1	0.082	0.085	0.080	94	9	0	90	20	15	92
20	7.7	0.090	0.101	0.081	105	5	0	91	15	15	97
21	7.8	0.090	0.101	0.080	105	9	0	90	23	15	98
22	7.8	0.091	0.100	0.085	104	8	45	94	9	0	98
23	8.1	0.094	0.100	0.090	104	9	0	97	6	45	100
24	7.5	0.087	0.100	0.080	105	21	0	90	0	15	95
25	7.6	0.088	0.102	0.081	106	8	45	91	15	0	96
26	9.2	0.107	0.114	0.097	114	16	0	102	8	0	104
27	7.9	0.092	0.097	0.087	102	9	0	95	5	45	99
28	7.5	0.087	0.101	0.081	105	18	15	91	1	15	95
29	7.0	0.081	0.084	0.080	93	20	30	90	24	0	91
30	10.3	0.119	0.125	0.081	125	10	0	91	9	0	117
31	9.8	0.108	0.125	0.102	125	12	15	106	15	30	110