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STUDIES ON HEAVY METAL TOXICITY AND ACCUMULATION IN THE CATCHMENT AREA OF THE DERWENT RESERVOIR

By

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This thesis is entirely the result of my own work except for the text references to publications It has not been submitted previously for any degree or diploma

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ABSTRACT

A three year long study has been carried out of the distribution of zinc, lead and cadmium within the water, sediments and submerged plants of the Derwent Reservoir and its catchment These studies have been extended to include detailed field investigations of the accumulation of heavy metals by *Lemanea fluviatilis*, an alga common in the R. Derwent Further field and laboratory investigations have been carried out of the tolerance to zinc of *Stigeoclonium tenue*.

Elevated concentrations of zinc, lead and cadmium were shown to be present in the water and sediments of the R. Derwent at its point of entry to the Derwent Reservoir. These metals were derived mainly from Bolts Burn, a polluted tributary. This latter stream was found to receive inputs of heavy metals from clearly defined sources within an active fluorspar mine. Although old mine workings were present within the catchment, these had a relatively small effect on the composition of water in Bolts Burn and the R. Derwent. Detailed investigations of the composition of stream and river water enabled various aspects of the behaviour of different fractions of metals to be described and compared in polluted and non-polluted reaches

The concentrations of zinc, lead and cadmium within the water, sediments and submerged plants of the Derwent Reservoir were found to be high when compared with data from other published studies. The pollution of the reservoir with these metals may therefore be regarded as serious. Surveys of the distribution of heavy metals in the water column were carried out at different stages in the filling cycle of the reservoir. These, together with surveys of the composition of sediments and submerged plants, enabled several of the major factors influencing the distribution of metals within the reservoir to be determined.

Studies of the accumulation of heavy metals by 47 populations of *Lemanea fluviatilis* indicated that the alga is a potentially useful 'monitor' of pollution by zinc, lead and cadmium in flowing waters. Although the enrichment ratios for these metals were not constant over a range of concentrations in the water, clear linear relationships were apparent between the concentrations of each metal in the water and in filaments of *Lemanea*. Intensive sampling from a single population in the R. Derwent demonstrated that a proportion of the zinc content of filaments was sensitive to short term fluctuations in the zinc content of the surrounding water. The results of a series of transplant experiments are also reported.

Stigeoclonium tenue was found to be abundant in several streams carrying relatively high concentrations of zinc in the water A study of populations isolated from 35 reaches demonstrated that material growing in higher concentrations of zinc in the field had an enhanced tolerance to zinc. This tolerance was stable during long term culturing, and appeared to have a genetic basis. Assays of populations from harder waters suggested that high concentrations of calcium acted to reduce the toxicity of zinc in the field. Further studies performed in the laboratory demonstrated that increases in pH and the concentrations of magnesium, calcium and phosphate all acted to reduce the toxicity of zinc to *Stigeoclonium tenue*. However, the effects of these factors on toxicity were found to differ between a zinc sensitive population and a zinc tolerant population.

ABBREVIATIONS

°C	degrees Celcius
EDTA	ethylenediaminetetra-acetic acid (disodium salt)
g	gram
h	hour
l	litre
m	metre
M	molar
mg	milligram
min	minute
ml	millilitre
mm	millimetre
µg	microgramme
µm	micrometre
n	number of measurements
nm	nanometre
NTA	nitrilotriacetic acid
O.D.	optical density
P	probability
r	Pearson correlation coefficient
s	second
s.d	standard deviation
u.v.	ultra-violet
$\bar{x}$	mean value
cond.	conductivity
filtrable	capable of passing through filter
galena	principal ore of lead (lead sulphide)
non-filtrable	incapable of passing through filter
Nuclepore	filtered through 0 2µm Nuclepore membrane filter
Sinta	filtered through no. 2 Sinta funnel
sphalerite	principal ore of zinc (zinc sulphide)
'total'	sample decanted after standing in 2 l beaker
tot. alk	total alkalinity
T.I C.	Tolerance Index Concentration

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CHAPTER 1

INTRODUCTION

1.1 GENERAL INTRODUCTION

In recent years much interest has been focused upon the pollution of fresh waters by elevated levels of metals such as zinc, lead and cadmium. This has been reflected in a large literature on different aspects of the behaviour and effects of such metals in aquatic habitats. There have been two main reasons for such interest·

i. Whilst trace amounts of some heavy metals are essential for all living systems, higher concentrations may exert toxic effects on both plants and animals. Tragic cases of human poisoning by mercury, lead and cadmium are well documented (Kiyoura, 1964; Waldron & Stöfen, 1974, Kobayashi, 1970), and occurrences such as these have led to an increased awareness of the possible consequences of discharges of heavy metals to the environment. This is evidenced by a large number of articles of an essentially descriptive nature, aimed at establishing base-line and 'pollutant' concentrations of metals in different situations. An example of the concern expressed by several workers is provided by the statement of Allan (1975): ".....whereas eutrophication may not produce lakes in which we wish to swim, lakes with toxic metal accumulations could directly affect our health. The sub-clinical manifestations of various types of heavy metal poisoning are still largely unknown or incompletely understood".

ii. Elevated concentrations of heavy metals in aquatic systems may lead to a decreased species diversity and hence an 'extreme' environment in the sense of Brock (1969). This has stimulated the interest of biologists in the effects of different metals on sensitive organisms, and the mechanisms by which tolerant organisms can withstand high concentrations of metals. The effects of one particular metal are often not defined clearly in the field, since waters affected by mining or industrial pollution frequently contain a combination of potentially toxic metals. Such studies have therefore frequently been carried out under more easily controlled physical and chemical conditions in the laboratory.

The two approaches outlined above are of necessity linked, since a knowledge of the factors influencing the effects of different metals on the biota in a particular system is necessary before establishing guide lines for the 'safe' dispersal of heavy metals within fresh waters As Wilson (1976) states. "Behaviour of, and effects caused by, metals in a river involve chemical, biological and physical processes that are markedly inter-dependent".

It is essential, therefore, that studies of pollution by toxic metals in the field should include simultaneous investigation of chemical and biological effects. The main disadvantage of such an approach is the amount of work involved in trying to consider several components of a particular system, and investigations of this kind lie mostly within the scope of interdisciplinary research teams (e.g. the recent research carried out in the 'New Lead Belt' of Missouri, see Wixson, 1977). Even comprehensive studies such as these may fail to present sufficient detail regarding certain essential considerations. For example reported analyses of water are often of limited value because they refer to a single collection and take no account of temporal variations caused by discharge, seasonal or diurnal fluctuations or changing inputs from artificial sources. Aston and Thornton (1975) discuss the use of water in monitoring waters to be abstracted for supply purposes, and state "Any programme of water sampling and analysis for the monitoring of abstracted waters must be of sufficient intensity to indicate the short and long term fluctuations which occur".

It would seem, therefore, that an intensive study of a single area subject to a high degree of contamination by toxic metals would be of value. In order to contribute significantly to present knowledge, such a study should include intensive sampling of water, sediments and biota in order to investigate the effects of different factors on the temporal and spatial variations of metals within the area chosen. Such a study would be of increased value if it included detailed investigations of the tolerance to heavy metals of selected aquatic organisms. This is an aspect that has been little investigated in the past, although Luoma (1977) states: "If one population of a species is more resistant to a toxicant than are other populations, it is direct evidence that the concentration of the toxicant in the environment is sufficient to elicit biological effects. This ... further suggests that other species may have been affected".

At the commencement of the project (September 1974) it was known that elevated concentrations of zinc, lead and cadmium were being carried into the Derwent Reservoir by its feeder river. This large reservoir is situated within the Northern Pennine Orefield, where contamination of streams and rivers by heavy metals is well documented (Say, 1977). As well as being a potential area for concern with regard to the quality of water within the reservoir, the catchment area was known to contain a wide variety of sites showing different degrees of pollution by heavy metals. The Derwent Reservoir and its catchment was therefore chosen for the anticipated study.

The following is a brief review of publications that were considered to be of relevance to the study.

1.2 SOURCES OF HEAVY METALS ENTERING THE AQUATIC ENVIRONMENT

1.21 Introduction

The terms 'heavy metals' and 'trace metals' are frequently applied to those metals to which toxic effects are attributed most frequently. 'Heavy' metals have been loosely defined as those with a density greater than five (Passow, Rothstein & Clarkson, 1961). Wilson (1976) defines 'trace metals' in natural waters as those present at a concentration of less than 1.0 mg l^{-1}. As concentrations of certain metals are frequently higher than this in waters affected by pollution, the former term will be used throughout this account. Attention is concentrated on the metals zinc, lead and cadmium, which are frequent contaminants of waters draining workings on the Northern Pennine Orefield (Say, 1977).

Sources of zinc, lead and cadmium entering the aquatic environment fall under two general headings: 'natural' and 'artificial'. Natural inputs are by definition completely unaffected by the activities of man; i.e. by erosion from undisturbed rock in the catchment area of the stream or river. Artificial inputs include elevated concentrations of heavy metals entering a watercourse directly or indirectly as a result of the disturbance of ore-bearing strata by mining, and various kinds of domestic, agricultural and industrial pollution. In practise the two kinds of input may be difficult to separate.

1.22 Natural sources

1.221 Sediments

The geology of an upstream area often has a marked effect on the trace metal content of the sediment in streams, rivers and lakes. This fact has been used as the basis for geochemical prospecting, where the heavy metal content of sediments is used as a guide to the presence of economically viable deposits of ore. For example, Carpenter, Robinson & Fagan (1971) analysed sediments from the beds of streams in two areas of Tennessee. On the basis of the results obtained, two belts of zinc and lead ore (sphalerite and galena) were located.

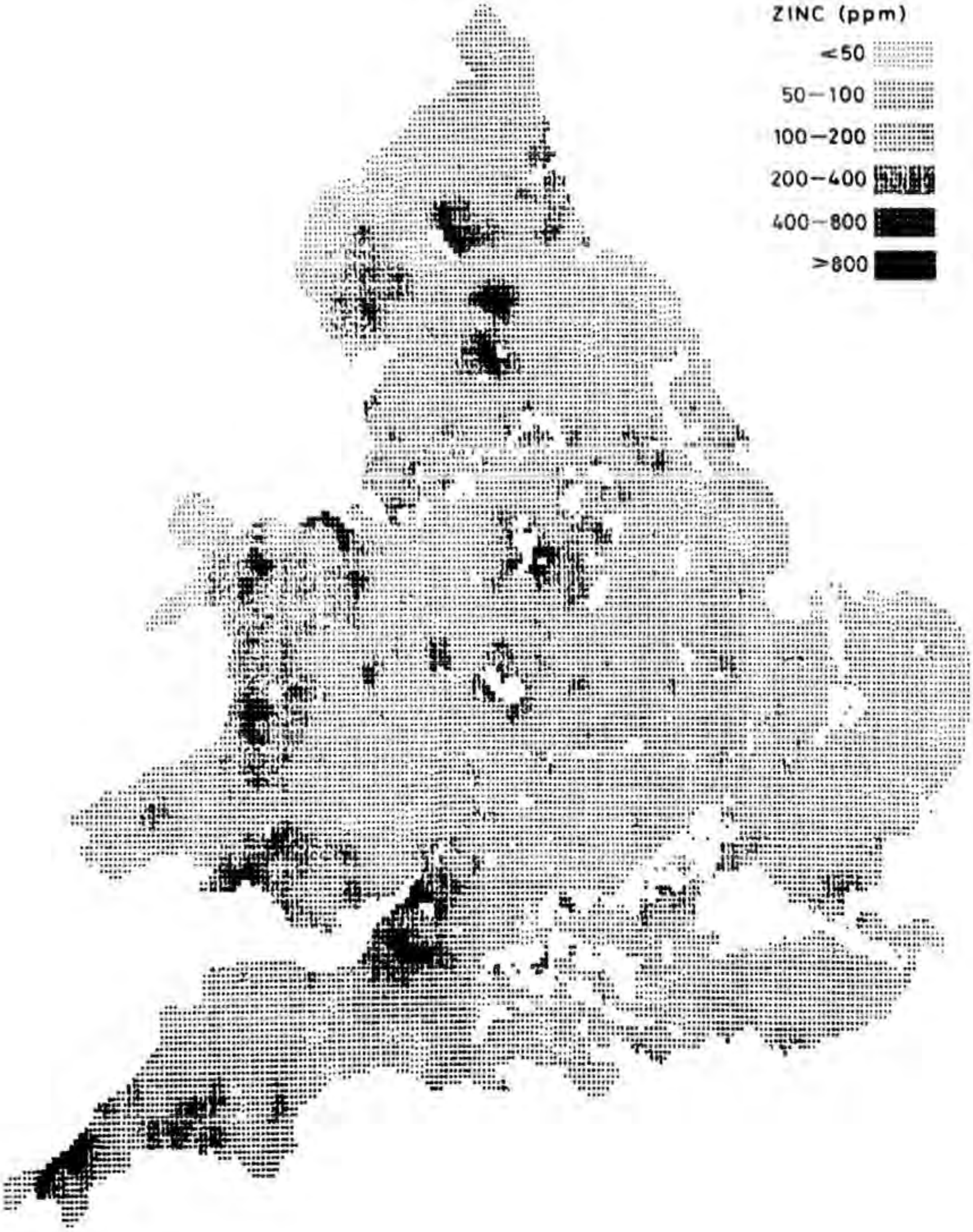
Investigations of the geochemistry of lake sediments have been employed extensively in the Canadian Shield in recent years for the location of deposits of uranium, copper and zinc. In the 'Copperbelt' region of the Shield, a single sample of lake sediment taken per 260 km^2 (10 miles^2) was found to give a general picture of the distribution of oreshoots (Allan, 1971). However, a greater density of sampling was required to locate small zones of mineralization.

In the United Kingdom, a large co-ordinated investigation of the geochemistry of sediments has been carried out by the Applied Geochemistry Research Group, Imperial College (Thornton, 1974; Thornton & Webb, 1973, 1975, 1977). Nearly 50000 samples of stream sediment were collected in 1969 from tributary drainage in England and Wales, and analysed for 30 elements including zinc, lead and cadmium. The data from this survey for zinc in England and Wales are reproduced in Fig. 1.1 (those for lead and cadmium are reproduced in Fig. 9.1). It can be seen from these figures that wide regional differences were detected in the concentrations of all three metals in stream sediments. For example, the Northern Pennine Orefield is highlighted as an area showing extensive contamination by zinc and

Fig. 1 1

Map to show the distribution of zinc in sediments collected from streams in England and Wales during 1969.

After Thornton (1974)


ZINC (ppm)
<50
50 – 100
100 – 200
200 – 400
400 – 800
>800

lead , whilst higher concentrations of cadmium are more frequent further south in the area around Reeth, Swaledale. Anomalously high concentrations of heavy metals are associated with areas of past and present mining, although broader variations in the net content of bedrock and soil can be distinguished.

It is not possible to tell from the maps to what extent the metal enrichment of stream sediments in areas of mining is a result of human disturbance, or of a general increase in the proportion of metal-rich particles weathered from the catchments. An interesting example of an occurrence of completely natural contamination by lead was documented by Låg, Hvatum & Bølviken (1969) at Kastad near Gjøvik, Norway. An area of exceptionally sparse vegetation was found within an uninhabited forested area, and subsequent analysis of the soil revealed a mean lead content of 4.7% (i.e. 47000 μg g^{-1}). Concentrations of other elements within the soil were not especially high, and the contamination was attributed to the weathering of a galena-bearing quartzite.

1.222 Waters

The examples cited above serve to illustrate that the heavy metal content of both freshwater sediments and surrounding bedrock and overburden may vary widely according to broad-scale geochemical variations and the influence of mineralization. It therefore somewhat suprising that Wedepohl (1972) states "The abundance of zinc in continental water is not expected to vary much regionally due to different rock types, mainly exposed at the surface, because of similarities in average zinc concentrations". It has long been known that streams and rivers draining from mineralized areas may contain elevated concentrations of zinc, lead and cadmium in the water because of the chemical weathering of ores such as sphalerite and galena.

It is difficult to find evidence in the literature for streams that have been contaminated for long periods by natural mineralization, as most areas have been mined for centuries (Whitton & Say, 1975). It seems likely, however, that metal-rich seepages may have occurred in such areas before mining commenced. High concentrations of heavy metals are often associated with thermal springs (White, Hem & Waring, 1963) and acid streams (Hargreaves, 1977) Examples of these have probably been present on a geological time scale.

1.23 Artificial sources

The following sections consider sources of zinc, lead and cadmium entering the freshwater environment that might be expected to have been caused by, or enhanced by, the activities of man.

1.231 Inputs from the atmosphere

Zinc, lead and cadmium in the atmosphere appear to be derived mainly from pollution by human activities. They may enter the atmosphere as fine dust blown from mine spoil etc., from chimneys associated with the smelting of ore, or from the combustion of fuels. Airbourne metals may be in particulate form as dust, or dissolved or dispersed in water droplets or ice crystals. In such forms they may be dispersed for great distances. For example, Fjerdingstad *et al.* (1974) reported levels of 20 $\mu g\ g^{-1}$ Zn and 1 $\mu g\ g^{-1}$ Cd in dried cells of *Chlamydomonas nivalis* collected from the surface of snow in East Greenland. Snow water from the collection site was reported to contain 0.9 $mg\ l^{-1}$ Zn, which could only have been derived from the atmosphere.

Wedepohl (1972), using the assumption that coal contains about 100 µg g^{-1} Zn, and that all such zinc entering the atmosphere becomes dissolved in river waters, arrived at an average concentration of 10 µg g^{-1} in such waters as a result of the burning of coal. It is clear, however, that a proportion of zinc and other metals derived from the combustion of coal will precipitate as dust and become entrapped in soil before entering drainage waters.

Although studies performed before the use of petrol became widespread have demonstrated the deposition of considerable amounts of lead near highways (e.g. Dunn & Bloxham, 1933), there is now little doubt that the internal combustion engine is a major source of atmospheric lead (Pinkerton *et al.*, 1975). Lead is added to petrol as 'anti-knock' compounds, and factories manufacturing such compounds can themselves be a major source of lead pollution (Lee, 1972).

Lead derived from exhaust fumes is mostly in the form of fine particles and may enter aquatic systems via highway runoff. Such runoff may contain a wide variety of particulate and non-particulate species of lead (Laxen & Harrison, 1977). Widely dispersed aerosols may also contribute to the lead content of rainfall For example, Hem (1972) showed that the mean value of 0.107 mg l^{-1} Pb quoted by Lazrus, Lorange & Lodge (1970) in rainfall at 32 stations in America was influenced by the combustion of fuel at two large airports.

The burning of coal and other fuels in urban-industrial complexes has been investigated by several workers as a potential source of elevated levels of heavy metals entering the aquatic environment. Thus Hallsworth & Adams (1973) demonstrated that fly-ash from power station chimneys near Nottingham contributed significantly to the zinc and lead content of local rainfall. Andren & Lindberg

(1976) attributed from 62% - 90% of the copper, mercury and lead entering the Walker Branch watershed (Tennessee) to dry atmospheric fallout, and up to 20% of the input to rainfall. Two coal fired steam plants were shown to contribute significantly to these inputs. Huff (1976) studied the relationships between atmospheric pollution, precipitation and streamwater quality near a large industrial complex in St Louis, U.S.A.. He concluded that urban-industrial sources sometimes contributed to the contaminant load of streamwater, including the levels of zinc.

Winchester & Niffong (1971) investigated possible sources of trace elements derived from atmospheric fallout (including zinc, copper, lead and nickel) entering Lake Michigan. They concluded that such metals, which were derived mostly from urban / industrial sources, contributed significantly to the pollution of water in the lake. The vertical distribution of lead in cores of sediment taken from the bottom of Lake Michigan was subsequently investigated by Robbins & Edgington (1975) and Edgington & Robbins (1976). Sections of the cores were dated using the ^{210}Pb technique, and it was shown that two significant rises in the content of stable lead in the sediment could be attributed to increases in the use of coal (*c.* 1830) and leaded gasoline (*c.* 1920).

Smelters, involved in the refining of sulphide ores of heavy metals, may be an important source of these metals to the environment via the atmosphere. Such inputs have been the subject of much study in recent years. A complex of smelters that has received especial attention is situated in the Sudbury region of Ontario, Canada, and is involved in the smelting of ores of nickel, cobalt, copper, zinc and lead. The process of smelting employed involves roasting of sulphide ores in air, and this leads to the production of large quantities of gaseous SO_2. This has caused the most visually obvious effects of atmospheric pollution in the area, with areas of deforestation caused by 'acid rain'. However Hutchinson & Whitby (1974) state that the ecological consequences of pollution by heavy metals in the area may be as great, and are merely masked by the effects of SO_2. They detected concentrations of up to 5104 $\mu g\ g^{-1}$ Ni and 2892 $\mu g\ g^{-1}$ Cu in soil and vegetation in the vicinity of a smelter in the area, and found that concentrations of these metals were elevated for distances of up to 50 km from the smelter complex. Beamish *et al.* (1975) quoted annual emissions of about 2000 tonnes of nickel and 1800 tonnes of copper to the atmosphere from smelters in the Sudbury region, and demonstrated significant pollution by these metals in the water of lakes in a nearby indian reserve.

Concentrations of zinc, lead and cadmium in the water, soil and vegetation around Sudbury are lower than those of nickel, cobalt and copper. This is because only small amounts of sphalerite and galena are smelted. Much more serious pollution by the former metals as a result of the smelting of ore has been reported from several other areas. Lagerwerff & Brower (1975) concluded that high concentrations of zinc, lead and cadmium in soils in an area of south east Kansas were probably derived from material suspended in the atmosphere when a zinc-lead smelter (now disused) was in operation. Simarlarly Thornton (1974) noted anomalously high concentrations of lead in soils near disused lead smelters in Derbyshire, and Alloway & Davies (1971) detected anomalous concentrations of lead in soils contaminated by PbO_2 released from furnaces in Cardiganshire.

Bolter *et al.* (1972) investigated the heavy metal content of several hundred samples of soil in the vicinity of a smelter in the 'New Lead Belt' area of Missouri. They concluded that high concentrations of zinc and lead (up to 635 $\mu g\ g^{-1}$ and 5220 $\mu g\ g^{-1}$ respectively) in the humus layer of soil were derived mostly from atmospheric inputs from the smelter. Concentrations of up to 11750 $\mu g\ g^{-1}$ Pb were reported by these authors in the leaves of oak and pine trees in the area.

A similar instance of pollution was investigated by Buchauer (1973) and Jordan (1975). These authors demonstrated that high levels of zinc, copper, lead and cadmium were emitted as aerosols from a zinc smelter in the Palmerton area of Pennsylvania. Buchauer reported extremely high concentrations of zinc (50000 - 80000 µg g^{-1}), lead (200 - 1100 µg g^{-1}) and cadmium (900 - 1500 µg g^{-1}) in exposed soil near the smelter. Even higher concentrations of zinc and cadmium (up to 135000 and 1750 µg g^{-1} respectively) were recorded in contaminated leaf litter.

Little & Martin (1972) surveyed concentrations of zinc, lead and cadmium in soils and vegetation around a smelting complex at Avonmouth. Elm leaves downwind of this complex were found to contain concentrations of up to 8000 µg g^{-1} Zn, 5000 µg g^{-1} Pb and 50 µg g^{-1} Cd. Elevated concentrations of the three metals were detected by these workers up to 15 km from the smelters.

An extremely serious instance of pollution by lead was reported by Djuric *et al.* (1971) in Yugoslavia (see also 1.233). Concentrations of up to 24880 µg g^{-1} Pb were measured in soil contaminated by fallout from a smelter, as compared with 0.8 - 37 µg g^{-1} in samples of soil collected from uncontaminated areas. Unwashed green 'salad' in the area contained up to 185 µg g^{-1} Pb, and hay used for feeding farm animals had a lead content of up to 430 µg g^{-1}. Increased lead burdens were demonstrated in members of the local population, and the authors concluded that it would be "little short of remarkable if evidence of lead intoxication should fail entirely to be uncovered within the population".

Kobayashi (1972) investigated the pollution of air and water by zinc, lead and cadmium emitted from the largest zinc refinery in Japan. He demonstrated a close relationship between the zinc and cadmium content of mulberry leaves and distance from the smelter, and also a marked accumulation of these metals in leafy vegetables. Kobayashi concluded that whilst pollution of this area had not taken place for as long a period as that in areas where 'Itai-Itai' disease (cadmium poisoning) had been demonstrated in humans, the extent and severity of the pollution were greater.

In the absence of dispersal by chimneys etc., finely divided dust (such as that derived from the crushing of ores) may be blown from areas of mining and seriously contaminate the surrounding area. Donovan, Feeley & Canavan (1969) demonstrated that the death of ten farm animals in fields near a lead mine in Ireland could be attributed to this phenomenon. They demonstrated concentrations of up to 16300 $\mu g\ g^{-1}$ Pb in the grass on which the animals had been grazing.

Although several of the studies described above have centered mostly on the analysis of soils and terrestrial vegetation, it seems clear that leaching by surface runoff could be expected to release significant levels of heavy metals from the contaminated areas to nearby bodies of water. The extent of such release would depend on the exchange capacity of the soil and the chemical properties of the water passing through it (see also 1.421). Rainfall rendered acidic by SO_2 emitted by smelters might be expected to be highly effective at leaching toxic metals from the soil. It has also been proposed that the high concentrations of heavy metals accumulated by leaf litter near smelters may be leached in complexed form by soluble organic acids derived from the decay of organic material (Bolter, Butz & Arseneau, 1975).

1.232 Inputs from industry

High concentrations of heavy metals may occur in industrial effluents because of the use of the metals in a wide variety of industrial processes. For example zinc is used for the manufacture of brass, pigments, paints, rubber and chemicals, and for galvanizing. Cadmium is used for electroplating and the manufacture of pigments, pesticides, alloys, chemicals and semiconductors.

Pasternak (1973) stated that "Available data on the influence of industrial pollution containing heavy metals on surface water are relatively scarse". Examination of the literature provides many examples of cases of pollution attributed to industrial sources, but few precise data on the composition of the discharges themselves. In the United Kingdom this can be explained partly by the fact that inspectors from water authorities are prevented from disclosing to the public any information on concentrations of heavy metals in industrial effluents because of prohibition under the Rivers (Prevention of Pollution) Act 1961. This means effectively that concentrations of pollutants may only be disclosed once the pollutant has been diluted in river water (Valdez, 1975).

Analyses of municipal wastewaters and sewage effluents, up to 50% of which may be derived from industrial wastes in some highly populated areas, may provide indirect evidence of the levels of heavy metals derived from industry. Oliver (1973) reported that a sample of sediment taken from the Ottawa River (Canada) downstream of a sewage plant contained elevated concentrations of metals including zinc and lead (846 $\mu g\ g^{-1}$ and 390 $\mu g\ g^{-1}$ respectively). He attributed these concentrations to local industries using the municipal sewage system to dispose of their wastes.

An example of the way in which different industrial processes may effect the concentrations of different metals in sewage sludges was provided by the work of Berrow & Webber (1972). They found that sludge from sewage plants serving towns with leather processing as a local industry tended to be enriched with chromium, which is used in the tanning process. Whilst the zinc content of most of the sludges analysed was high (around 3000 $\mu g\ g^{-1}$), two sludges with exceptionally high zinc concentrations (1% and 5%) were found to originate from towns with rayon works (employing $ZnSO_4$). High concentrations of copper, cobalt and nickel were found in sludges originating from towns with electroplating, foundry and alloy producing industries.

Barth *et al.* (1965) carried out a survey of four municipal wastewater plants receiving inputs of metals from industry on an almost continuous basis. They recorded several 'slugs' of high concentrations of zinc entering the plant as a result of releases from industry with concentrations of zinc in the wastewater reaching 9 $mg\ l^{-1}$ at times. Similar 'slugs' of zinc and lead were recorded by Oliver & Cosgrove (1974) in municipal wastewater entering a treatment plant before discharge into Lake Ontario. Concentrations of up to 70 $mg\ l^{-1}$ Zn were recorded, and the simultaneous variation of levels of zinc and lead provided strong evidence for a common industrial source.

Several investigations of the heavy metal content of sediments in rivers, lakes and estuaries have shown high concentrations that can almost certainly be attributed to direct industrial discharge, or to indirect dispersal of metals from industry via wastewater or sewerage systems. Jaffe & Walters (1975) investigated the concentrations of various metals in sediments from the Humber Estuary, and found high concentrations of titanium (up to 12000 $\mu g\ g^{-1}$) and vanadium (up to 2030 $\mu g\ g^{-1}$) that were almost certainly derived from local titanium dioxide processing plants. High concentrations of zinc in these sediments (up to 433 $\mu g\ g^{-1}$) were derived partly from a synthetic fibre plant, though a large part of the zinc and lead measured was probably derived from the discharge of crude sewage into the estuary.

Lasowski *et al.* (1976) investigated the abundance and distribution of heavy metals in the recent sediments of a system of lakes and rivers connected to the R. Rhine near Mainz, Germany. High concentrations of zinc and cadmium (up to 7841 $\mu g\ g^{-1}$ and 95 $\mu g\ g^{-1}$ respectively) were detected in river sediments downstream of a highly polluted tributary, the Schwarzbach, which drains a highly industrialized area. High concentrations of zinc, lead and cadmium (up to 2900 $\mu g\ g^{-1}$, 800 $\mu g\ g^{-1}$ and 45 $\mu g\ g^{-1}$ respectively) were detected in sediments from the R. Rhine itself by de Groot & Allersma (1975).

Helz, Huggett & Hill (1974) and Helz (1976) evaluated the relative importance of various human and natural sources of heavy metals including zinc, lead and cadmium to the northern half of the Chesapeake Bay, Maryland. In Baltimore Harbour (a subestuary of the bay) Helz concluded that direct discharge from industry was the main contributer of most of the metals that were investigated, with the exception of lead and cadmium which came mostly from municipal wastewater and atmospheric fallout. On the basis of figures provided by local industrial concerns, total annual inputs of zinc, lead and cadmium to the harbour were calculated as 470 tonnes, 76 tonnes and 1.6 tonnes respectively. It was pointed out that these figures represent only a conservative estimate because only the largest dischargers provided figures, and because "if any bias exists in these company-supplied data, it seems most likely that it would be in the direction of underestimating the discharge rate".

Loring (1976) reported elevated concentrations of zinc, copper, lead, cobalt and nickel in sediments of the Saguenay Fjord, Canada. He concluded that significant amounts of these elements in the sediments originated from local industries, which include paper production, aluminium smelting and fluorspar treatment.

Figures for elevated concentrations of heavy metals in water as a result of industrial pollution have been provided by a few authors, but actual concentrations may be expected to vary greatly over fairly short periods of time. Roberts *et al.* (1975) measured the concentrations of six metals (zinc, copper, lead, nickel, cobalt, cadmium) in an effluent in Zurich, and reported concentrations of up to 0.5 mg l^{-1} Zn. Examples of extremely high (and variable) concentrations of cadmium present in the waters of several rivers in the United Kingdom that are surrounded by highly industrialized regions were provided by Valdez (1975). Reported concentrations of this metal (in mg l^{-1}) ranged from 0.003 - 0.19 (R. Mersey, Warrington), 0.004 - 0.13 (R. Tame, Stockport) and 0.05 - 1.29 (Nant-y-Fendrod, Swansea).

1.233 Inputs from mining activities

Acid mine drainage

Highly acidic mine drainage waters occur frequently where water passes through mines (or mine spoil heaps) with a high content of FeS_2 (pyrite, marcasite or pyrrhotite). These minerals are oxidised by the action of air and water (and possibly bacteria) to produce sulphuric acid, which may lower the pH of the drainage waters to values below 3. Such conditions have been found in waters associated with some lignite, pyrite, zinc, lead, gold, silver and copper mines, but in Britain 'acid streams' are associated mostly with coal mining operations. The chemical reactions involved in the production of acid and the extensive bibliography on the occurence of highly acid streams has been reviewed by Hargreaves (1977).

Whilst the low pH of acid streams may constitute a serious pollution problem in its own right, these waters may carry very high concentrations of heavy metals in solution which may subsequently pollute other bodies of water. Hargreaves, Lloyd & Whitton (1975) investigated the chemistry and vegetation of 15 highly acidic streams in Britain and reported concentrations of zinc and lead ranging from 0.09 mg l^{-1} - 193 mg l^{-1} and 0.001 - 1.90 mg l^{-1} respectively.

Van Everdingen (1970) studied a group of acidic springs in British Columbia, Canada. The waters of these springs were found to be extremely enriched by heavy metals, and one spring with a pH of 2.5 contained up to 177 mg l^{-1} Zn. A nearby non-acidic spring (pH 8.0) contained 0.080 mg l^{-1} Zn. During periods of low flow a nearby river was found to be enriched somewhat by zinc as a result of inputs from the springs, but under normal flow conditions, extreme pollution did not take place because a proportion of the heavy metals precipitated out at the higher pH values.

Drainage from areas of base metal mining

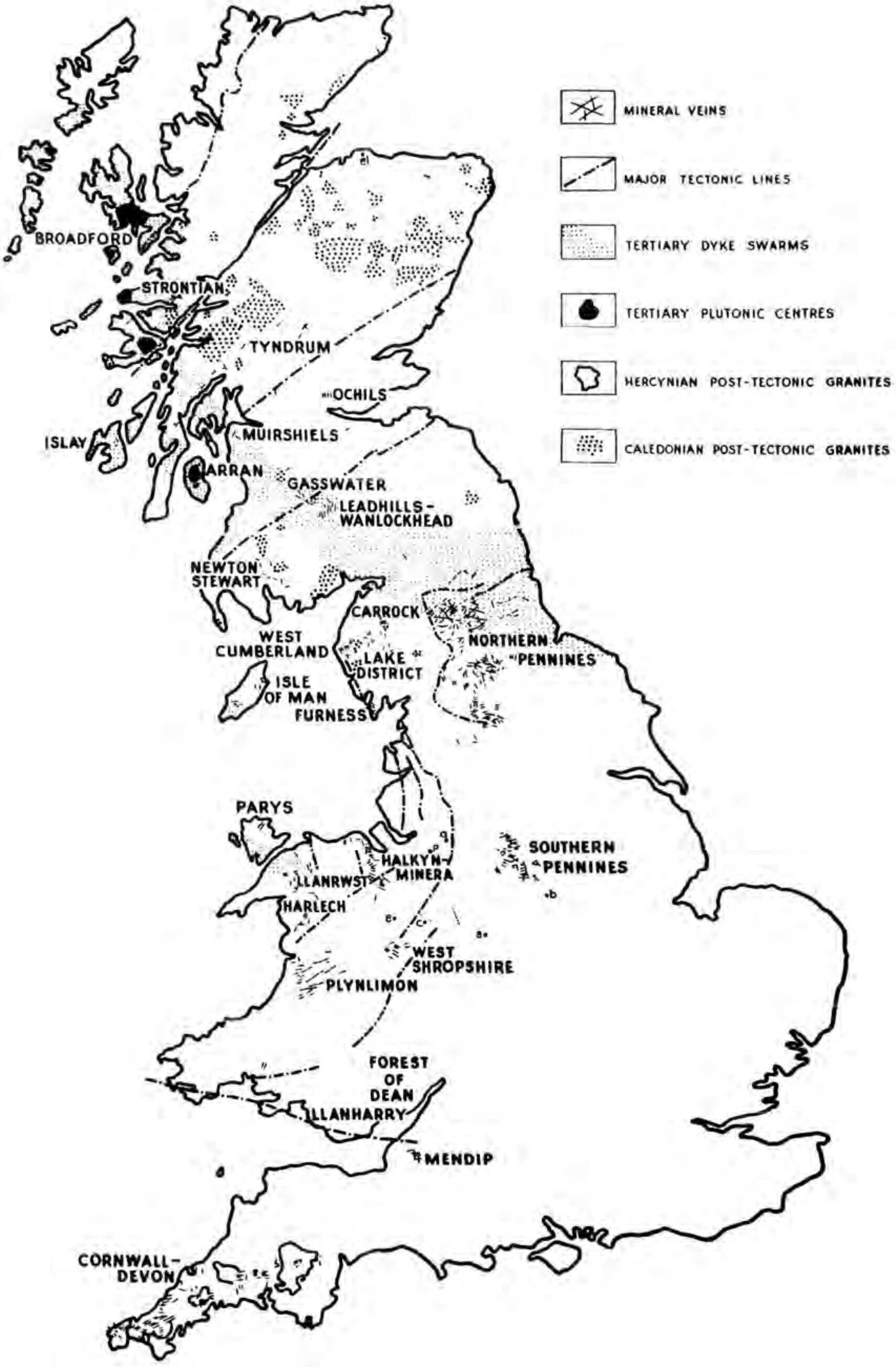
It has long been known that waters draining from the surface or underground workings of lead or zinc mines may carry elevated concentrations of heavy metals. This source of heavy metals entering the freshwater environment is of especial relevance to the present study, as many of the streams and rivers on the Northern Pennine Orefield are affected by inputs of metals derived from past or present mining activities.

The extent of contamination of the freshwater environment by metals derived from base metal mining in England and Wales is well illustrated by the results of the survey of composition of stream sediments carried out by the Applied Geochemistry Research Group (Figs 1.1, 9.1; see also 1.221). Fig. 1.2 is a map showing the location of the main mineralized areas of Britain. On comparing this with Fig. 1.1 it is clear that most of the areas with anomalously high concentrations of zinc (>800 µg g^{-1}) in stream sediments occur in areas with a high density of mineral veins. Whilst some of the zinc present in the sediment may have been weathered naturally from exposed rocks, it seems clear that contamination from past and present mining for metals such as lead, zinc and silver is responsible for most of the enrichment (Thornton & Webb, 1977).

Fig. 1.2

The main mineralized areas of Britain

(after Dunham, 1952)


MINERAL VEINS
MAJOR TECTONIC LINES
TERTIARY DYKE SWARMS
TERTIARY PLUTONIC CENTRES
HERCYNIAN POST-TECTONIC GRANITES
CALEDONIAN POST-TECTONIC GRANITES
BROADFORD
STRONTIAN
TYNDRUM
OCHILS
MUIRSHIELS
ISLAY
ARRAN
GASSWATER
LEADHILLS-WANLOCKHEAD
NEWTON STEWART
CARROCK
WEST CUMBERLAND
LAKE DISTRICT
NORTHERN PENNINES
ISLE OF MAN
FURNESS
PARYS
HALKYN-MINERA
LLANRWST
HARLECH
SOUTHERN PENNINES
WEST SHROPSHIRE
PLYNLIMON
FOREST OF DEAN
LLANHARRY
MENDIP
CORNWALL-DEVON

As well as showing areas where levels of heavy metals are enriched in stream sediments, the survey serves to highlight areas that might also have extensive enrichment of both soil and water by metals leached from dumped mine mineral or slag, deposited by aerial fallout from metal smelters (see 1.231), or draining from underground workings through adits. This is well illustrated by reference to Cardiganshire, West Wales; a region which has been studied intensively over a long period (Whitton & Say, 1975).

Ores in the Lower Palaeozoic rocks of Cardiganshire have been mined since Roman times, but most intensively from about 1750 to 1900 (Alloway & Davies, 1971). The ore was usually separated from crushed rock by water floatation, and the effluent from the dressing floors was channelled directly into nearby streams (carrying with it dissolved metal salts and suspended metal particles). The spoil, which was rich in lead and zinc ore, was often heaped onto the banks of the streams, into which it collapsed periodically. The areas affected most seriously were north Cardiganshire and Montgomeryshire, especially the rivers Ystwyth, Rheidol and Clarach. Jones (1964) gives a detailed account of the mining activities in this area.

As long ago as 1918 Griffith (1918) suggested that some 1200 ha of agricultural land in the area might be affected by mine waste. Present evidence (Alloway & Davies, 1971) suggests that this was a conservative estimate. Thornton & Webb (1975) suggest that the total area of England and Wales affected similarly may exceed 4000 km^2.

Many of the mines affecting the rivers Ystwyth and Rheidol had closed down by 1922, but serious pollution of the two rivers persisted due to the drainage of zinc and lead from the sulphides in the mine tips (McLean & Jones, 1975). In her classic account of the effects of mining on the flora and fauna of streams and rivers in west Wales Carpenter (1924) states "The presence of metallic solutions is believed to be the factor conditioning the barren quality of the streams ... The lethal process is probably accelerated in the streams themselves by the presence of small amounts of zinc accompanying the lead in solution". From this statement it can be seen that zinc was often not considered to be a major contaminant of waters in the area during the early research, although it seems clear from the work of Jones (1940) that high concentrations of the metal were probably always present in the rivers Ystwyth and Rheidol. Jones demonstrated that, even 20 years following the closure of the mines, the R. Ystwyth was still seriously polluted, and remained "the most barren of the rivers running into Cardigan Bay". Several adits draining from underground workings in the Cwm Ystwyth Mine were found by Jones to discharge water containing as much as 38 mg l^{-1} Zn into the river, and the water in the river itself carried up to 1.2 mg l^{-1} Zn These adit sources, being subterranean in origin, were diluted somewhat during periods of high flow in the river.

In a later study Treharne (1962) detected similar high concentrations of zinc in the area of the R. Rheidol. Streams derived from adits were cited as the main source of zinc entering the river. Some of these streams were extremely acidic (because of the oxidation of pyritic ores), and had concentrations of up to 187.5 mg l^{-1} Zn in the water. More recently Fuge (1972) demonstrated that even non-acidic adit waters in the area contained up to 4 mg l^{-1} Zn.

Abdullah & Royle (1972) provided a general account of concentrations of heavy metals in the water of rivers and lakes in Wales. They found that whilst concentrations in any particular river varied by as much as 60% from month to month, concentrations of zinc and other metals were always higher in some rivers (including the R. Rheidol) than in others. Since little or no industrial waste is discharged into the rivers that they investigated, Abdullah & Royle concluded that metals were leached from the top soil in mineralized areas or from old lead mines or mining waste.

Fewer published data are available concerning the extent of pollution in other areas of Britain affected by mining for non-ferrous metals. Davies (1971) gives an account of the trace metal content of soils affected by base metal mining in the west of England, especially the Tamar valley district of west Devon and east Cornwall. This area was mined intensively for copper and tin (and some lead and arsenic) during the 17th, 18th and 19th centuries. Davies demonstrated that there were no marked regional differences in the concentrations of iron, cobalt and manganese in the soil of pasture and gardens in the areas affected by mining. However, concentrations of zinc, copper, lead, cadmium and silver were markedly higher in pasture soils contaminated by mine spoil and (possibly) by the weathering of buried mineral lodes.

Brown (1977) studied the effects of mine drainage on the concentrations of copper, zinc and iron in the water, sediments and dominant invertebrate fauna of the R. Hayle, Cornwall. The catchment area of this river contains many disused mine workings, and the river still receives major inputs of heavy metals from four large adits. Concentrations of zinc in the water of the R. Hayle were found to reach 2.4 mg l^{-1} at one site, which also had the highest concentration of copper in the water (0.7 mg l^{-1}). When the results from all of the sites studied were considered together, concentrations of zinc in the water were approximately ten times greater than concentrations of copper. The situation was reversed in the sediments, where copper predominated over zinc by a factor of three. Concentrations of copper, zinc and iron were all found to increase in the river water during periods of high discharge.

An investigation of the lead content of lake water, sediments, plants, invertebrates and fish was carried out in Ullswater (English Lake District) by Welsh & Denny (1976). The elevated concentrations of metals observed (see 1.32) were attributed to drainage from several mines that had worked deposits of zinc, lead, iron and silver. The largest of these (the Greenside lead mine) was closed in 1962, but runoff from tailings and old adits within the mine were found to pass down the Red Tarn Beck to Ullswater. Water from the Eagle Crag Mine (closed in 1877) and the Hartsop Hall Mine (closed in 1942) were found to drain into the lake via the Goldrill Beck.

Donovan, Feely & Canavan (1969) investigated the extent of contamination by lead in mining areas of western Ireland. Although the death of farm animals from lead poisoning near one mine was attributed to the contamination of pasture by dust blown from spoil heaps (see 1.231), cattle were removed from pasture bordering a river polluted by inputs from the mine as a precautionary measure. Concentrations of lead in the water of this river reached 4.2 mg l^{-1} at one point.

Of the mining areas outside the British Isles that have been studied from the point of view of possible effects on surface waters, the 'New Lead Belt' of south east Missouri, U.S.A. has probably received the most attention. Although the ore body was discovered as late as the 1950's, the area has been mined intensively and became the world's largest producer of lead ore in 1970 (Gale *et al.* 1973). Alongside the development of the area as a producer of lead, a large programme of research has been carried out to assess the effects of mining on the surrounding area and to outline possible environmental problems (e.g. Wixson, 1968; Wixson & Bolter, 1972; Wixson & Jennett, 1975, Wixson & Gale, 1975; Gale, Bolter & Wixson, 1976; Wixson, 1977).

Despite close co-operation between the mining concerns and environmentalists, a certain amount of pollution of streams and rivers by heavy metals has been documented in the area. Whilst the natural chemical properties of the stream water in the 'New Lead Belt' (high values for pH and hardness) have helped to prevent problems with dissolved metals, significant amounts of zinc, lead and cadmium may be released as fine particulate material from mine milling operations The relatively high CO_2 and PO_4-P content of the mine effluents has also caused algal blooms to occur in several streams, and studies have indicated that these may trap and assimilate high concentrations of zinc, lead and cadmium

An area of the U.S.A. where problems of pollution by heavy metals derived from mining operations have apparently been less well controlled is the 'Silver Valley' area of north Idaho (Funk *et al* , 1975; Miller *et al.*, 1975). This region is centered on the valley of the Coeur d'Alene River, which has received large inputs of heavy metals and particulate material for over 80 years as a result of the mining and processing of zinc, lead, cadmium, copper, antimony and gold Although some of these inputs are known to originate from specific sources such as smelters, 'non-specific' sources are a more serious problem. These originate from large amounts of 'jig tailings' that were spread along the banks of the river before 1930. During periods of heavy runoff zinc, lead and cadmium are leached into the Coeur d'Alene River from these deposits, and these together with particulate wastes are carried downstream towards the Coeur d'Alene Lake The problem is aggravated by soil erosion caused by the denudation of vegetation by the emission of sulphur dioxide from metal smelters.

The seriousness of the pollution in this area is evidenced by the extremely high concentrations of zinc, lead and cadmium (up to 5000 $\mu g\ g^{-1}$, 6300 $\mu g\ g^{-1}$ and 100 $\mu g\ g^{-1}$ respectively) recorded in the sediments of the delta formed where the Coeur d'Alene River enters the Coeur d'Alene Lake (Maxfield *et al* , 1974a) and in the sediments of the lake itself (Maxfield *et al.*, 1974b).

In Australia, a rather similar case of pollution has been documented by Weatherley & Dawson (1973) and Weatherley (1975). The Molonglo River has been polluted for many years by the workings of a copper, lead and zinc mine situated near Canberra, which was closed in 1962. 'Slime dumps' associated with this mine were eroded by large floods in the 1940's, these carried a large quantity of mine wastes down river and deposited them on the river margins and in Lake Burley Griffin. Extensive leaching of heavy metals occurs from these deposits, and the zinc content of the lake water was found to exceed 0.4 mg l^{-1} on several occasions.

Mechanisms of mobilization of heavy metals from mine workings and mine waste

It is beyond the scope of this account to review the present knowledge of the chemical, physical and biological processes by which metals such as zinc, lead and cadmium may be mobilized from exposed ores in mine workings or tailings heaps. The mechanisms by which zinc may enter surface waters from such workings have been reviewed by Say (1977).

Although the relative amounts of different metals in the mine workings and the solubilities of their salts under the environmental conditions present will effect the absolute amounts of metals such as zinc, lead and cadmium that are leached out into mine drainage waters, the mechanisms by which the sulphide ores of these three metals may be weathered are rather similar. Sphalerite and galena occur frequently together in the oreshoots of the Northern Pennine Orefield, and cadmium sulphide is almost always present as a contaminant of sphalerite (El Shazly, Webb & Williams, 1956).

The sulphide ores of zinc, lead and cadmium may be mobilized into mine drainage waters by chemical or biological weathering. The following is brief description of these processes for sphalerite.

Chemical weathering

Chemical weathering is a process that relies on factors such as the mineral composition of the rocks, rock texture, climate and rock exposure (Krauskopf, 1967). It is generally a slow process which involves chemical processes of ionization, addition of water and carbon dioxide, hydrolysis and oxidation In the case of sphalerite, the aqueous oxidation which may release zinc ions into surface waters has been demonstrated to be an electro-chemical process (Habashi, 1976) which results in the overall reaction

$$ZnS + 2H^+ + \frac{1}{2}O_2 \longrightarrow Zn^{2+} + S + H_2O$$

Under alkaline conditions, the formation of insoluble zinc hydroxide may block such weathering.

$$Zn^{2+} + 2OH^- \longrightarrow Zn(OH)_2$$

Should the pH drop again the zinc hydroxide may re-dissolve, allowing weathering to continue

$$Zn(OH)_2 + 2H^+ \longrightarrow Zn^{2+} + 2H_2O$$

During the weathering process the sulphur produced by the above reaction is usually converted to sulphate which is then dissolved, and the zinc ion either enters solution or forms an insoluble secondary compound which is stable under surface conditions. Such secondary minerals include Smithsonite ($ZnCO_3$), formed under alkaline conditions, and hemimorphite ($Zn(Si_2O_7)(OH)_2.H_2O$) which is the least soluble zinc mineral at pH values below 6.2. Under dry conditions, smithsonite may be converted to hydrozincite (Takahashi, 1960)

$$5ZnCO_3 + 3H_2O\ (g) \rightleftharpoons 2ZnCO_3.3Zn(OH)_2 + 3CO_2\ (g)$$

Zinc is regarded as a comparatively mobile metal in the oxidation zone around lodes of sulphide ore (Krauskopf, 1967), since in such areas the common anions such as SO_4^{2-}, OH^-, CO_3^{2-} and Cl^- permit relatively large amounts to remain in solution. The weathering processes described above can be expected to be enhanced in mine spoil heaps, where relatively large surface areas of sulphide ores may be exposed.

Microbial leaching

Under certain conditions sphalerite may be oxidized by microbial activity to produce zinc sulphate.

$$ZnS + 2O_2 \longrightarrow ZnSO_4 \quad \text{(under conditions of limited aeration),}$$

or·

$$2ZnS + 4O_2 + H_2O \longrightarrow ZnSO_4 + ZnO + H_2SO_4 \quad \text{(more complete aeration).}$$

In the latter case the production of sulphuric acid will aid the mobilization of zinc ions.

Zajic (1969) has demonstrated in the laboratory that bacteria may oxidise smithsonite in the presence of elemental sulphur to release zinc ions

$$ZnCO_3 + 1\tfrac{1}{2}O_2 + H_2O + S \longrightarrow ZnSO_4 + H_2CO_3$$

Galbraith, Williams & Siems (1972) concluded that the mobilization of heavy metals by bacteria played an important part in the leaching of metals from the deposits of mine tailings around the Coeur d'Alene River. They proposed a rather complex system, whereby the action of sulphide-oxidizing and sulphur-oxidizing bacteria caused the formation of sulphuric acid, thereby increasing the concentrations of H^+ and SO_4^{2-} within the system. Metal ions such as Zn^{2+}, Pb^{2+} and Cd^{2+} then passed into solution, and the concentration of H_2S increased because of the interaction of H^+ and S^{2-}. Increased levels of H_2S created an environment suitable for the growth of sulphate-reducing bacteria which converted SO_4^{2-} to S^{2-} and simultaneously precipitated metal sulphides at a pH near 6.6. Decrease of pH with depth destroyed these sulphate- reducing bacteria, and dissolution and leaching of metals from the tailings increased . These authors presented several lines of evidence which demonstrated that microorganisms were present in the tailings, and that the processes outlined above were taking place in the field.

Weatherley & Dawson (1973) proposed that leaching of metal ions from the slimes dumps bordering the Molonglo River was due largely to the action of bacteria such as *Thiobacillus ferrooxidans*. The deposits contained pyrite, from which $FeSO_4$ was formed continually

$$2FeS + 2H_2O + 7H_2O \longrightarrow 2FeSO_4 + 2H_2SO_4$$

this was then oxidised through the action of *T ferrooxidans*

$$12FeSO_4 + 3O_2 + 6H_2O \longrightarrow 4Fe_2(SO_4)_3 + 4Fe(OH)_3$$

The ferric sulphate thus formed is an aggressive solvent of sulphide ores such as sphalerite, and reacted to release zinc from the tailings deposits

$$ZnS + 2Fe_2(SO_4)_3 + 2H_2O + 3O_2 \longrightarrow 2ZnSO_4 + 4FeSO_4 + 2H_2SO_4$$

Whether heavy metals enter the freshwater system as a result of chemical or biological weathering, the ions in solution are subject to interaction with a large number of substances. These govern the partitioning of metal species between dissolved, complexed and solid phases, and hence affect the mobility of the metals and their availability to biological systems. This aspect of the behaviour of heavy metals is of relevance to the present study, and is dealt with in 1.4.

1.3 CONCENTRATIONS OF HEAVY METALS IN THE FRESHWATER ENVIRONMENT

It is clearly of importance to attempt to establish concentrations of different heavy metals that can be termed 'background' (i.e. assumed to be of purely natural origin) or pollutant (i.e. affected by human activities) in any system being investigated. This is especially important where it seems likely that specific sources of metal enrichment from industry, mining etc. may be implicated in a particular study. Reference has already been made (1.2) to several published concentrations of heavy metals in water and sediments, and it is clear from these that very wide variations may occur. A larger number of concentrations of zinc, lead and cadmium extracted from various accounts is tabulated in 9.212 The following brief review is intended as a general guide to the ranges in concentrations of these metals that may be attributed to different natural or artificial phenomena.

1.31 Concentrations of metals in freshwaters

The difficulties encountered in comparing the many published references to concentrations of heavy metals in fresh waters have been stressed by Wilson (1976). In attempting to review such concentrations in river waters, Wilson noted that in many cases the results presented were of dubious value for a number of reasons. For example, in many cases no precautions were taken to preserve the samples; the experimental procedure was described either not at all, or incompletely; only one sample was analysed, etc.. He was therefore extremely selective in his choice of published data to be reviewed, and employed the following criteria.

i. reasonable precautions for the preservation of samples of water should have been taken,

ii. the experimental procedure should have been described fully and have reasonably low detection limits;

iii. several samples at least should have been collected at different times from the sampling site or one sample from each of many different sites should have been taken.

Despite the exclusion of a large number of accounts because of the application of these criteria, Wilson concluded that the data he selected represented reasonably well the range of concentrations of heavy metals occurring in river waters. The concentrations quoted for zinc, lead and cadmium are reproduced in Table 1.1.

Several workers have pointed out the very large variability that may occur in the concentrations of heavy metals at a particular site (e.g. Lee & Hoadley, 1967, Downing & Edwards, 1969). Relative standard deviations of between 50% and 150% are frequently reported (e.g. Abdullah & Royle, 1972, Angino, Galle & Waught, 1969, Robbins, Landstrom & Wahlgren, 1972), and in trying to establish 'background' concentrations it would seem to be a sensible precaution to consider only published concentrations of metals that rest on many analyses of water. Such a study was reported by Durum, Hem & Heidel (1971), who conducted a survey of the concentrations of selected minor elements in surface waters of the U.S.A.. Over 720 samples were passed through 0.45 μm membrane filters and analysed for As, Cd, Cr, Co, Pb, Hg and Zn. The authors reported 'normal' levels of 10 - 50 $\mu g\ l^{-1}$ Zn, 1 - 50 $\mu g\ l^{-1}$ Pb and 1 - 10 $\mu g\ l^{-1}$ Cd in the samples, and concentrations of as much as 5 $mg\ l^{-1}$ Zn, $<50\ \mu g\ l^{-1}$ Pb and 130 $\mu g\ l^{-1}$ Cd in polluted stream waters.

Further information concerning concentrations of heavy metals that could be termed 'natural' in stream waters was provided by Hem (1972). Hem reported mean concentrations of 20 $\mu g\ l^{-1}$ Zn and slightly less than 1 $\mu g\ l^{-1}$ Cd in 726 samples of water from U.S. rivers and lakes, and discussed the geochemical controls affecting these concentrations (see also 1.4). Abdullah & Royle (1972) reported that 'clean' streams in Wales typically carried around 11 $\mu g\ l^{-1}$ Zn and 0.7 $\mu g\ l^{-1}$ Pb Angino, Galle & Waught (1969) attempted to measure background concentrations and seasonal variations of minor elements in stream waters of the lower Kansas River basin, and reported a mean concentration of 60 $\mu g\ l^{-1}$ Zn. A further example of a study of concentrations

River	fraction	Zn	$\bar{x}$	Pb	$\bar{x}$	Cd	$\bar{x}$	Reference
Many in U S.A.	filtrable	10-42000	20	1-890	2	1-130	1	Durum & Hem (1972)
Feeder streams to Lake Michigan	filtrable	0.9-32	4	-	-	-	-	Robbins, Landstrom & Wahlgren (1972)
Many in U.S.A. (lead-mining area)	total	8-300	37	5-830	24	10	10	Gale, Wixson, Hardie, & Jennett (1973)
Many in U.S.A.	filtrable	3-140	31	0-70	3	0-5	0.8	Proctor, Kisvarsanyi, Garrison & Williams (1973)
Many in California	filtrable	14-?	29	0.6-?	6	0.7	0.7	Silvey (1967)
Many in Wales	filtrable	-	-	0.9-26	3	-	-	
	non-filtrable	-	-	0.3-25	4	-	-	Abdullah & Royle (1973)
Feeder streams to Cayuga Lake, New York	filtrable	-	3	-	0.9	-	0.2	Mills & Oglesby (1971)
	non-filtrable	-	9	-	2	-	0.1	
Many in Austria	total	-	-	-	-	0.3-1.9	1	Korkisch & Dimitriadis (1973)
Rhine in Germany	filtrable	24-176	60	14-43	27	1.5-4.1	2	Reichert, Haberer & Normann (1972)
Streams in U.S.A.	filtrable	-	-	0.6-14	6	0.3-45	2	Biggs, Miller & Otley (1972)

Table 1.1 Comparison of concentrations of zinc, lead and cadmium in river waters, as reported by different workers

(based on Wilson, 1976)

(all concentrations are in µg l^{-1})

in stream waters apparently unaffected by human activities was provided by Windom, Beck & Smith (1971). These authors studied concentrations of heavy metals in the waters of three rivers draining into the Atlantic Ocean in the south-east U.S.A., and reported concentrations of 0.5 - 4.9 $\mu g\ l^{-1}$ Zn.

Wedepohl (1972) concluded that a figure of 10 $\mu g\ l^{-1}$ would be a 'suitable average' concentration for continental surface waters He suggested that the value of 20 $\mu g\ l^{-1}$ Zn estimated by Turekian (1969) was a little high due to the inclusion of high values from figures given for some Russian streams by Konovalov (1959).

There seems to be a general consensus of opinion that concentrations of zinc, lead and cadmium in stream or river waters of more than about 0.1 mg l^{-1}, 0.05 mg l^{-1} and 0.001 mg l^{-1} respectively are generally the result of artificial pollution. No examples were found in the literature of freshwaters carrying concentrations higher than these in the absence of artificial inputs such as those described in 1.2.

With the exception or thermal or acidic streams, streams carrying more than about 1 mg l^{-1} Zn, 0.1 mg l^{-1} Pb or 0.005 mg l^{-1} Cd are usually restricted to areas affected by past or present mining activities. Say (1977) carried out a preliminary survey of the zinc content of streams in north-east England, and summarized the results as follows:

area	counties/ main river	no. streams sampled	streams <0.1 mg l^{-1} Zn	streams 0.1-1.0 mg l^{-1}Zn	streams >1.0 mg l^{-1} Zn
Allendale	Northumberland/ E. & W. Allen	25	8	16	1
Alston Moor	Cumbria/S Tyne & R. Nent	56	12	29	15
Derwent catchment	Northumberland/ Derwent	16	12	3	1
Teesdale	Durham/Tees	40	22	18	0
Weardale	Durham/wear	52	25	24	3

Most of the streams carrying <1.0 mg l^{-1} Zn were located on Alston Moor, a part of the Northern Pennine Orefield which is affected extensively by past and present mining. Almost all of the streams with concentrations of zinc in the water of more than 0.1 mg l^{-1} Zn were in areas affected in some way by lead mining activities.

Even in areas that have been mined extensively for heavy metals, a concentration of more than 0.5 mg l^{-1} Zn appears to be rare in larger rivers. Exceptions to this pattern in Britain include the R. Ystwyth, Cardiganshire, in which a concentration of 0.9 mg l^{-1} Zn has recently been reported (McLean & Jones, 1975). In north east England, the R. Nent is contaminated by high concentrations of zinc, ranging from more than 2 mg l^{-1} downstream of the entry of several adits in the town of Nenthead to about 1.0 mg l^{-1} at the point where it enters the R. South Tyne (Say, 1977).

The highest concentrations of zinc, lead and cadmium reported by Say (1977) in streams draining the Northern Pennine Orefield were 22.3 mg l^{-1}, 1.6 mg l^{-1} and 0.058 mg l^{-1} respectively in the water of 'Old Mine Gill' near Nenthead. Higher concentrations than these have been reported by several workers in non-acidic surface waters. For example, Jones (1940) reported a concentration of 38 mg l^{-1} Zn in the water of an adit draining underground workings in the Cwm Ystwyth Mine, Cardiganshire. Valdez (1975) reported an isolated record of a concentration of 1.29 mg l^{-1} Cd in the water of the Nant-y-Fendrod, near Swansea. The average concentration of cadmium reported in water from this river was 0.24 mg l^{-1}; these very high concentrations were derived from the refining of non-ferrous metals in the area.

The highest reported concentration of lead to come to light during the present survey of literature is 685 mg l^{-1} in the water of the Meza River in Yugoslavia (Djuric *et al.*, 1971). The lead was derived from the effluent of a smelter that had also caused widespread contamination of the surrounding countryside by atmospheric fallout. Water in the R. Meza upstream of the effluent contained 0.007 mg l^{-1} Pb; 10 km downstream of the entry of the effluent the river still carried 92 mg l^{-1} Pb.

Drainage waters in the mining district near Iglesias, south west Sardinia, are known to carry exceptionally high concentrations of zinc , lead and cadmium (B. A. Whitton & co-workers, unpublished data). Concentrations of 160 mg l^{-1} Zn, 9 8 mg l^{-1} Pb and 15.7 mg l^{-1} Cd were found by the present author to be present in the water of the main river draining the Iglésias mining area Water in a drainage channel leaving the Monteponi Mine was found to contain 5100 mg l^{-1} Zn, 31.5 mg l^{-1} Pb and 17.7 mg l^{-1} Cd (concentrations are for unfiltered water).

1.32 Concentrations of metals in freshwater sediments

Aston & Thornton (1975) state that stream sediments are "nature's closest approximation to a composite sample of the weathered products of rock and soil upstream of the sampling site". By their very nature, sediments in freshwaters might be expected to show much less short term variation in composition than the overlying water. It should therefore be easier to gain from the literature an idea of 'background' concentrations of heavy metals in freshwater sediments that can be attributed to the weathering of undisturbed soil and bedrock, and of concentrations above which 'unnatural' enrichment can be assumed to have taken place. The main difficulty that arises during such a review is that different workers have frequently used widely different techniques for the analysis of sediments This has resulted in widely different proportions of the heavy metal content of a particular sample being extracted, analysed and reported.

Whilst general agreement seems to have been reached that samples of sediment should be dried and passed through an 80 mesh ($<200\mu m$) sieve, techniques for the extraction of cations range from 'total' digestions or fusions (whereby the sediment is dissolved completely), to leaching with mild acids or chelating agents. The wide variation in results that can result from the use of different techniques has been demonstrated by Agemian & Chau (1976), and is discussed in more detail in 2.222. For the purposes of the present account, values reported for the mineral composition of sediments are discussed only if the samples have been analysed following 'total' digestion or fusion, or following digestion with one or more strong acids. Such acids usually extract a minimum of 80% of the metals present (Carmody, Pearce & Yasso, 1973, Agemian & Chau, 1976).

Allan (1975) reviewed the data on the heavy metal contents of lake sediments in Canada, and stressed that concentrations of some metals were on occasions higher in the sediments of lakes completely removed from human activity than in lakes where pollution from human sources had been implicated. An example of marked heavy metal enrichment caused by purely natural phenomena was reported by Lag, Hvatum & Bølviken (1969) who found that denudation of 100 m^2 of forest vegetation in Norway was caused by concentrations (up to 47000 µg g^{-1}) of lead in the soil that had been weathered from a subjacent body of ore.

Examination of the computer-produced maps prepared by the Applied Geochemistry Research Group (e.g. Figs 1.1, 9.1) provides an indication of the minimum concentrations of heavy metals to be expected in sediments from British streams. Over wide areas of Britain such sediments were found to contain concentrations of zinc, lead and cadmium below the lower threshold concentrations mapped (50 µg g^{-1} Zn, 40 µg g^{-1} Pb and 1.0 µg g^{-1} Cd). In mineralized areas, however, it would seem probable that these estimates of 'background' concentrations would be somewhat low even in the absence of mining activities.

Examination of published accounts of levels of metals detected in stream and lake sediments during geochemical prospecting operations gives an indication of the maximum concentrations to be expected in mineralized areas in the absence of human disturbance In lake sediments of the Canadian Shield, for example, the upper ranges of zinc recorded by Allan (1974) were <100 µg g^{-1}. With the exception of higher concentrations in the immediate vicinity of lodes of ores, Carpenter, Robinson & Fagan (1971) found that the highest concentrations of zinc and lead present in dry stream sediments in a mineralized area of north west Tennessee were about 250 µg g^{-1} and 220 µg g^{-1} respectively.

One of the few reliable methods of establishing 'background' concentrations for metals in a particular area is to study the historical pattern of deposition of metals by analysing different sections of cores of sediment taken from the bottom of a lake. Material laid down in deeper layers would pre-date that laid down whilst man was active in the catchment Kobayashi *et al.* (1976) studied a 200 m long core of sediment taken from the bottom of Lake Biwa (Japan). In samples taken at intervals from most of this core concentrations of zinc, lead and cadmium fell mostly in the ranges 100 - 150 µg g^{-1}, 20 - 35 µg g^{-1} and 0.25 - 0.60 µg g^{-1} respectively In samples taken from the surface of the sediment, however, concentrations of these metals were found to be as high as 736 µg g^{-1}, 259 µg g^{-1} and 26 µg.g^{-1} respectively. This 'remarkable' increase was attributed to human influence in recent years.

Shimp, Leland & White, 1970) studied cores of sediment taken from southern Lake Michigan, and attributed elevated concentrations of zinc, lead and cadmium in the upper few cm of the sediment to anthropogenic influence. The highest concentrations of these metals detected were 179 µg g^{-1}, 90 µg g^{-1} and 17 µg g^{-1} respectively. Further cores of sediment from Lake Michigan were investigated by Edgington & Robbins (1976) who dated different layers of sediment using the ^{210}Pb technique. Separate increases in the concentration of stable lead in the sediment were found to have taken place after about 1830 and 1920, these were attributed to the commencement of burning of coal and leaded petrol in the vicinity of the lake. The concentrations of lead recorded in deeper layers of the sediment were about 30 µg g^{-1}, concentrations were higher than 160 µg g^{-1} in the upper layers.

From examples such as these, it seems clear that the highest concentrations of zinc, lead and cadmium that could be expected to occur in freshwater sediments in the absence of human disturbance are about 300 µg g^{-1}, 300 µg g^{-1} and 3 µg g^{-1} of the total sediment. Much higher concentrations than these have been reported by several authors in freshwater sediments affected by pollution, and the following examples serve to illustrate the ranges of concentrations that have been detected in different situations

Several workers have detected concentrations of more than 500 µg g^{-1} Zn or Pb in the sediments of rivers or lakes that have been affected by pollution from industry or mining. de Groot & Allersma (1975) investigated the heavy metal content of sediments in the R. Rhine system, and reported the following concentrations (in µg g^{-1}):

	R. Rhine	R. Meuse	R Schnelt	R. Ems
zinc	2900	2500	800	1100
lead	800	600	-	-
cadmium	45	45	-	-

These concentrations were recorded in 1970, the authors reported that since then concentrations of cadmium, in particular, had increased due to further inputs from industry.

In America Romano (1976) investigated concentrations of zinc, lead and cadmium in the sediments of the Grand Calumet River, a feeder river of Lake Michigan He found a 'high degree of contamination', with mean concentrations of 1390 µg g^{-1} Zn, 545 µg g^{-1} Pb and 5.5 µg g^{-1} Cd. Oliver

(1973) reported 'background' concentrations of 84 - 86 $\mu g\ g^{-1}$ Zn and 26 - 42 $\mu g\ g^{-1}$ Pb in sediments from the Ottawa River, Canada However, concentrations of zinc and lead were found to rise to 846 $\mu g\ g^{-1}$ and 390 $\mu g\ g^{-1}$ near a sewage plant, and a concentration of 1344 $\mu g\ g^{-1}$ Pb was recorded at a point where snow (contaminated by exhaust fumes) had been dumped into the river.

Concentrations of more than about 3000 $\mu g\ g^{-1}$ Zn or Pb appear to be restricted to sediments in bodies of water that have been seriously polluted by inputs from metal mining or smelting activities. The Coeur d'Alene Lake, Idaho, has received drainage from a large metal mining area for many years (Maxfield *et al.*, 1974a, 1974b, Funk *et al.*, 1975). The resulting concentrations of zinc, lead and cadmium in the sediments (3000 - 5000 $\mu g\ g^{-1}$, 3000 - 6000 $\mu g\ g^{-1}$ and 50 - 100 $\mu g\ g^{-1}$ respectively) were described as 'very serious' by these authors.

Mackereth (1965) reported that high levels of lead were being carried into Ullswater (English Lake District) by a tributary affected by mining, and that the lead was lost rapidly to the sediments and submerged plants. These observations were followed up by Welsh & Denny (1976), who found that although little lead was present in the water of the lake, concentrations of as much as 30000 $\mu g\ g^{-1}$ Pb were measureable in sediment collected from near the entry of the polluted tributary. The highest concentrations of lead were recorded in samples taken from 10 - 20 cm below the surface of the sediment; this was attributed to the deposition of uncontaminated sediment since the closure of the Greenside Lead Mine in 1962. Despite the exceptionally high concentrations of lead reported in these samples, contamination was apparently restricted to a relatively small part of the bed of the lake.

The highest reported concentrations of zinc, lead and cadmium in sediments encountered during the present survey of the literature were reported by Skei, Price & Calvert (1972) in samples taken from the bed of the Sorfjord, Norway.

Concentrations of zinc at 16 sampling points varied from 830 - 118000 µg g^{-1} ($\bar{x}$ = 20016), concentrations of lead varied from 720 - 70000µg g^{-1} ($\bar{x}$ = 11368) and concentrations of cadmium varied from 16 - 850 µg g^{-1} ($\bar{x}$ = 121). The pollution of this fjord was due apparently to inputs from industry. Specific inputs included smelters which had been in operation for more than 50 years.

It seems probable that extremely high concentrations such as these are more widespread in freshwaters affected by mining areas than is indicated in the literature. The algology group at Durham University has for some time collected and analysed sediments from streams affected by inputs from mining. The following are examples of the higher concentrations of zinc, lead and cadmium that have been recorded·

Site	zinc	lead	cadmium
Tiefer Julius Fortunatus Stollen (Germany)	192563	1577	284
'La Mine' (France)	126980	24898	920
Dowgang Level (Northern Pennine Orefield)	170507	1119	80

(all concentrations in µg g^{-1} after HNO_3 digestion. Data held on computer file at Durham University.)

1.4 BEHAVIOUR OF HEAVY METALS IN THE FRESHWATER ENVIRONMENT

De Greef (1976) states "Once metals are in the surface water, within a short time there grows up a complicated and still not fully understood relationship between the dissolved fraction and the complex compound fraction". It is widely accepted that many factors may influence the distribution of heavy metals between different fractions in freshwaters, and that the state in which a particular metal is present may influence profoundly its transportation, sedimentation, availability to biota and toxicity.

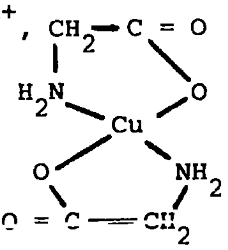
It is beyond the scope of this review to attempt to describe the large body of literature that exists concerning the chemical and physico-chemical behaviour of heavy metals in natural waters and sediments. However, a certain background knowledge of such processes is essential in any study that attempts to relate concentrations of metals in the aquatic environment to observed biological effects, and some of the more relevant studies are considered below.

1.41 Water

1 411 Speciation

Whilst accepting that the observed classifications may be the result of the interaction of many phenomena, several authors have given schemes of classification for the species of heavy metals that may be present in natural waters. Wilson (1976) reviewed such schemes, and concluded that that of Stumm & Bilinski (1973) was perhaps the most useful (Table 1.2). This scheme defines metals in freshwaters as either free ions and inorganic complexes ($<$ 10 nm), chelates and colloids (10 - 100 nm) or large colloids and precipitates ($>$100 nm) Stumm & Morgan (1970) further subdivided truly dissolved metals to include 'aquo metal ions' (i.e. a metal ion co-ordinated with water and inorganic hydroxo and poly hydroxo complexes). This speciation was dependant on pH, and could affect the adsorption of cations onto solids (including biological material).

The potential complexity of the speciation and the lack of analytical techniques capable of distinguishing between particular species have led to the development of several relatively simple methods for estimating the concentrations of metals present in a few, rather broadly defined, fractions. Whilst not being completely selective, such techniques may still provide valuable information.

Metal species	Range of diameters (µm)	Examples
Free metal ions		$Cu(H_2O)_6^{2+}$, $Fe(H_2O)_6^{3+}$
Simple radicals		UO_2^{2+}, VO_3^-
Inorganic ion-pairs and complexes		$Cu_2(OH)_2^{2+}$, $Pb(CO_3)_2^{2-}$, $CuCO_3^{o}$, $AgSH^{o}$, $CdCl^+$, $CoOH^+$, $Zn(OH)_3^-$
Organic complexes chelates and compounds	0.001	$Me-OOCR^{n+}$,  HgR_2
Metals bound to high molecular weight organic materials	0.01	Me - humic/fulvic acid polymers
Highly-dispersed colloids		FeOOH, Mn(IV)hydrous oxides
Metals sorbed on colloids	0.1	$Me.aq^{n+}$, $Me_n(OH)_y$, $MeCO_3$, etc. on clays, FeOOH, organic colloids
Precipitates, mineral particles organic particles		$CuCO_3$, $ZnSiO_3$, CdS in FeS, PbS
Metals present in live and dead biota		Metals in algae

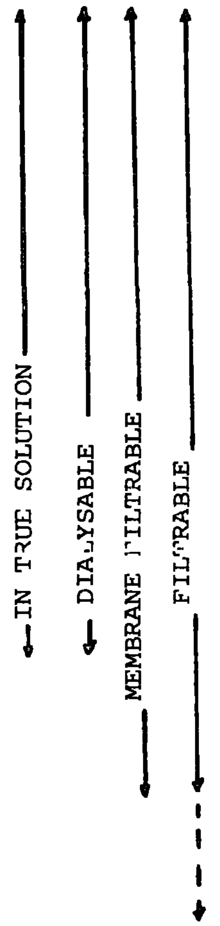


Table 1.2 Types of metal species in waters

(based on Stumm & Bilinski, 1973)

Perhac (1972) used continuous flow centrifugation to study the distribution of heavy metals between different fractions of water from two streams in Tennessee Three classes of solids were extracted from the water. coarse particulates (< 150 nm), colloidal particulates (10 -150 nm), dissolved solids Colloidal paticles were found to have the highest heavy metal content, this was typically more than ten times that of dissolved material. Nevertheless, dissolved species generally accounted for more than 95% of each metal present in the water

Gibbs (1973) used a filtration technique followed by extraction procedures and dialysis to investigate the speciation of Cr, Mn, Fe, Co, Ni and Cu in water from the R. Yukon and R. Amazon He separated five fractions of metals. in solution and organic complexes; absorbed, precipitated and co-precipitated, in organic solids, in crystalline sediments.

With the exception of filtration (considered in more detail below), several other techniques have been used to classify species of metals in water These include anodic stripping voltametry (Allen, Matson & Mancy, 1970), dialysis (Benes & Steinnes, 1974, Mayer, 1976, Hart & Davies, 1977), ion exchange (Benes, Gjessing & Steinnes, 1976) and gel filtration (Gjessing, 1970, Plumb & Lee, 1973).

Filtration is the technique employed most commonly for the operational classification of metals Whilst filtration techniques possess the advantages of convenience and speed, they may have several drawbacks:

i the filtrate may become contaminated by impurities (including metals) leached from the filter, or the filter may absorb ions or particles that are in fact smaller than the pores (Marvin, Proctor & Neal, 1972),

ii even filters with small pore sizes (e.g. 0.45 μm) may permit the passage of fine suspended particles such as clays (Kennedy, Zellweger & Jones, 1974);

iii the blockage of the filter may result in changing rates of retention of particles whilst the filtration is in progress (Hem, 1972);

iv. filtration may disturb equilibria existing between species of metals present in the original sample of water (Wilson, 1976)

In addition to the problems outlined above, different authors have used different types of filter which may differ markedly with respect to the structure of the pores, etc. (Standridge, 1976) 0.45 µm porosity membrane filters have been used most widely, although Hem (1972) pointed out that these may allow the passage of some particulate material. As the pores would tend to block with use and thus retain a greater proportion of the particles, Hem concluded that the use of these filters still provided a valid estimate of 'dissolved' species. A more detailed study of the effectiveness of different filters in removing particulate material was carried out by Kennedy, Zellweger & Jones (1974). These authors concluded that fine 'clay size' particles could pass 0.45 µm porosity filters with ease, leading to large errors in the estimation of 'dissolved' concentrations of elements including Al, Fe, Ti and Mn. They concluded that the use of 0.1 µm membrane filters reduced the passage of particulate material, without increasing the filtering time markedly. Jones, Kennedy & Zellweger (1974) compared 'dissolved' levels of Al and Fe estimated by passing stream water through 0 1 µm membrane filters with dissolved concentrations calculated from data on mineral stability and inorganic complexing. They found that the two could compare favourably, and recommended the more widespread use of 0.1 µm porosity filters

Thus although the classification into dissolved and undissolved forms of metals may seem simple, its practical application is not (Wilson, 1976). To emphasise the operational nature of the classification as normally used, the terms 'filtrable' (≡ 'dissolved') and 'non-filtrable'

(≡ 'undissolved') have been recommended by Kraynov (1968). These new terms are now used frequently, but by no means universally (Wilson, 1976).

In principle it is possible, given a total metal concentration, to calculate both the concentrations of all dissolved species of the metal and the nature of any insoluble species that are in equilibrium with the solution. To do this in practise requires a complete knowledge of all the factors affecting the behaviour of the particular metal in the system. In natural waters the results seem likely to give, at best, only an approximate indication of the true situation because of the assumptions involved and the general failure to take account of all potentially important factors (Wilson, 1976). For example, it is necessary to know the stability constants of all dissolved metal complexes and chelates that may be present as well as the solubility products of any solid compounds with which the solution may be in equilibrium. Further, such a theoretical approach assumes an equilibrium which may be seldom reached (especially in flowing water), and which may ignore other factors such as complexation by organic compounds and adsorption and co-precipitation with suspended material and precipitates (Davis & Leckie, 1977). Despite these shortcomings, such results can give a valuable insight into the chemistry of particular metals, and indicate areas where further research is needed. A few examples are therefore included below

O'Connor & Renn (1964) and O'Connor, Renn & Wintner (1964) concluded that the relative insolubility of two salts of zinc (zinc carbonate and zinc hydroxide) place an upper limit on the concentrations of zinc found in natural waters. As most natural waters contain lower concentrations of zinc than these, it seems possible that they are merely undersaturated with the metal O'Connor & Renn recognised, however, the strong evidence that other factors were acting to bind zinc to natural sediments thereby reducing the concentration in the water.

Hem (1972) studied the chemistry and occurrence of zinc and cadmium in 726 filtered samples of groundwater and surface water from the U.S A. (see also 1 3). He compared the observed concentrations with theoretical maximum concentrations derived from chemical thermodynamic equations summarized by solubility graphs These calculations suggested that the carbonate and hydroxide solubilities of zinc and cadmium were higher than the concentrations commonly found, but for 24 out of 80 analyses for which chemical equilibrium computations could be made, saturation with respect to one or both of the metals was approached closely. Hem suggested that the solubility of zinc may be limited by the silicate content of some waters, as well as by biological factors and adsorption onto sediments

Gardiner (1974) studied the complexation of cadmium in natural waters, and concluded that a large proportion of the dissolved cadmium will normally be present as the free hydrated metal ion, Cd^{2+}.aq, especially in freshwater with a pH value of about 8 or less. Thus measurement of the filtrable concentration of cadmium in such waters is likely to give a reasonable indication of the dissolved concentration of the metal

More recently computer techniques have been used to study more complex theoretical systems in which many metals are present together with all major impurities normally found in natural waters (Morel, McDuff & Morgan, 1973) These techniques, together with model experimental systems (see 1.423), provide the potential to investigate how the speciation of one metal may depend on the concentrations of others, for example through competition for ligands present in small quantities Wilson (1976) provided a comprehensive list of references to published calculations of theoretical metal speciation in natural waters

1.412 Factors influencing the behaviour of metal ions in natural waters

It is evident from the above that many factors may act to influence the speciation and behaviour of metals such as zinc, lead and cadmium in natural waters. Only a brief mention is made here of the most important of these factors, Say (1977) has provided a more comprehensive review.

pH

pH is probably the single most important factor controlling the solubility of zinc in surface waters, it also has a profound effect on the influence of other controlling factors. In a simple system (as investigated by Smith, 1973) metals may be precipitated as the hydroxide at higher pH values, e g.

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

Jurinak & Inouye (1962) investigated the solubility of zinc at various pH values for a $ZnCl_2$ - NaOH system. Significant decreases were found in the proportion of dissolved zinc above pH 8.0, and these were attributed to the formation of zinc hydroxide Zirino & Yamamoto (1972) computed a pH-dependant model (using available and estimated stability constants and individual ion activity constants) of the speciation of copper, zinc, lead and cadmium in seawater They concluded that the formation of metal hydroxides was a major factor controlling metal solubility at higher pH values, these included the natural pH of seawater (8 1)

Alkalinity

The alkalinity of water can be defined as the capacity of the water to neutralise acid (Stumm & Bilinski, 1970). This capacity is determined largely by the concentrations of HCO_3^- and CO_3^{2-} ions dissolved from rocks, and is strongly inter-related with the factors controlling pH The importance of carbonate radicals in controlling the mobility of zinc around bodies of ore has already been mentioned (1.233), and it is accepted that the formation of zinc carbonate may be an important factor controlling the solubility of zinc in surface waters. An example of the manner in which alkalinity may interact with pH to influence the speciation of zinc was documented by Ernst, Allen & Mancy (1975) who produced solubility curves for zinc at three values of alkalinity. The metal was shown to be least soluble in the solution with the highest alkalinity (10^{-3} eq l^{-1}), but all three curves were characterised by the rapid loss of zinc from solution at pH values above 7.5. Solbé (1974) considered that the solubility of Zn^{2+} is controlled mainly by the solubility of zinc carbonate, the concentrations of which is dependant on both pH and alkalinity.

Silica

Hem (1972) used thermodynamic calculations to determine the solubility of zinc silicate as a function of pH He concluded that at pH values between 7.5 and 10 the solubility of this compound was lower than that of zinc carbonate and zinc hydroxide, and may therefore impose a more important control on the solubility of zinc in some waters.

Phosphate

Compounds of phosphorus dissolved in natural waters fall into three categories, namely orthophosphates, inorganic condensed phosphates and organic phosphates. Such compounds may have a marked effect on the complexing of heavy metals, although other factors such as pH and the concentrations of other ligands may alter their effects. The precipitation of zinc phosphate at different pH values has been demonstrated by Jurinak & Inouye (1962). Say (1977) pointed out that such pecipitation might be expected to furnish a major control on the solubility of metals only in waters polluted by inputs of detergent, fertilizers etc , and might therefore not be expected to play a significant role in the oligotrophic waters of upland regions such as the Northern Pennine Orefield

Synthetic organic compounds

Although compounds such as detergents, NTA, EDTA and pesticides are known to interact with heavy metals in natural waters, it seems unlikely that they are present in the waters of upland streams and rivers

Natural organic material

Reuter & Perdue (1977) state "The role of organic ligands in metal complexing in natural waters has received little attention because of the uncertainties regarding both the abundance and nature of dissolved organic carbon compounds" However, many authors acknowledge that the interaction of heavy metals with natural dissolved organic material may exert a profound influence on many aspects of their behaviour.

It seems widely agreed that the bulk of organic material in natural waters consists of a mixture of highly oxidised and chemically stable polymeric compounds closely resembling soil humic compounds Peat is a rich source of such compounds (Rashid, 1974), and waters draining upland areas with a cover of peat are often stained yellow or brown by organic material.

Chemically, 'humic compounds' are complex substances which are considered to be formed by the reaction of amino acids with polyphenols and ammiones etc , together with biochemical transformations. Christman & Ghassemi (1966) investigated the molecular structure of colour forming organic compounds isolated from natural waters, and found conclusive evidence for the presence of phenolic nuclei in the 'colour macromolecule'. More recently Christman (1970) and Schnitzer (1971) have classified and described humic compounds. Both emphasise the importance of the fulvic acid component of these substances, together with humic acids and humins.

The importance with which interactions between heavy metals and organic material have been regarded by some authors is evident in the statement of Stevenson (1976)· "Humic substances interact with polyvalent metal ions to form complexes of varying stabilities and properties. The practical and theoretical significance of these interactions comes from the realization that practically every aspect of the chemistry of heavy metals in soils, sediments and natural waters is related in one way or another to the formation of complexes with humic substances"

Rashid (1971, 1974) has performed many studies on the complexation of heavy metals by organic compounds of natural origin. He has demonstrated that humic acids extracted from marine sediments are highly effective in adsorbing various metal ions through chelation, cation exchange and surface adsorption Such organic material was shown to bind copper more strongly than cobalt, manganese, nickel and zinc. All of these metals were found to be adsorbed by peat moss (a rich source of humic acid), but in field trials adsorption was limited to zinc, copper and iron. The binding of iron by humic material in natural waters has also been documented by Tryland & Gjessing (1975) and Shapiro (1967).

Benes, Gjessing and Steinnes (1976) reported that cobalt and zinc in natural waters seemed to be complexed by humus; this was evident in the results of both centrifugation and ion exchange experiments. They found that the proportion of ions complexed was low at pH 2, but from 40 - 50% of the ions were bound by the organic material at pH 3 - 5. The results indicated that binding was even greater at higher pH values. However, the observations may have been complicated by the hydrolysis of metal ions with subsequent adsorption onto the surfaces of the membrane filters used in the investigation

Reuter & Perdue (1977) state that organic molecules such as humic and fulvic acids participate in the formation of complexes with metals through ionizable functional groups with a range of differential acidities. The stability of metal-organic complexes formed in this manner may be higher than the corresponding inorganic complexes. These authors concluded that at an average concentration of 10 mg l^{-1} humic material in natural waters, complexation of heavy metals would be significant even in the presence of excess concentrations of other major cations. This is in accordance with the observation of Rashid & Leonard (1973) who were able to show that humic acids can prevent the precipitation of metals as insoluble sulphides or carbonates.

Ramamoorthy & Kushner (1975) used filtration studies to investigate the role of fulvic acid as a metal binding component of stream and river water They found that organic molecules with a molecular weight lower than 1400 were responsible for most of the complexing observed, and that fulvic acid was probably the major organic molecule involved. Inorganic ions such as CO_3^{2-}, HCO_3^{2-} and SO_4^{2-} were found to show no contribution to the metal binding capacity of the fulvic acid. Schnitzer (1971) attributed the binding of zinc by fulvic acid to complexation by the carbonyl, carbonxyl and hydroxyl functional groups. Ramamoorthy & Manning (1974) demonstrated that fulvic acid not only mobilised zinc rapidly as zinc fulvate, but was also capable of binding zinc phosphate complexes.

1.42 Sediments

Many of the published articles concerning the deposition of heavy metals in freshwater sediments include some discussion of the possible mechanisms by which the particular metals have entered the sediments. In many cases it seems probable that several processes are acting together, although only a few possibilities are considered by some authors. However, it seems widely accepted that the association of heavy metals with various components of sediments may act as an important control on the behaviour of metals in the overlying water. For instance, several workers have demonstrated that the sediments in lakes and reservoirs may act as 'sinks' for heavy metals carried into them by feeder streams.

1.421 Factors controlling the entry of heavy metals to sediments

With the exception of the simple precipitation of insoluble salts such as zinc carbonate or zinc hydroxide, several components of sediments have been implicated in the binding of heavy metals from the overlying water. Jenne (1968) reviewed the mechanisms controlling the distribution of several ions in water and sediments, and proposed that binding by the hydrous oxides of iron and manganese furnish the principal controls on the sedimentation of nickel, cobalt and zinc in freshwaters Because such oxides may be present as coatings on other suspended particles, Jenne proposed that they may exert chemical effects far out of proportion to their total concentrations. Sorption and desorption of metals from oxide particles and coatings were shown to be influenced by other factors including pH and the amount and strength of other complex formers present Jenne also suggested that interaction of metals with sedimentary organic material, clays and carbonates could affect sedimentation, together with the precipitation of discrete oxides or hydroxides.

Lee, Lopez & Mariani (1975) provided strong confirmation for Jenne's proposal of the importance of hydrous iron and manganese oxides, but suggested further complications in the manner in which they sediment metals They found that it was very difficult to predict the behaviour of the metal following sorption because of 'ageing' of the oxides, and because the binding process could be non-reversible and non-stoichiometric. Further, natural dissolved organic material could play a significant role in the uptake and release of metals by the oxides.

Other workers who have stressed the important role of such oxides in freshwater sediments include Jackson & Nichol (1975), who concluded that hydrous iron and manganese oxides were important factors determining the spatial distribution of heavy metals within the sediments of Canadian lakes Chester & Stoner (1975) suggested that 'excess' heavy metals in sediment from the Bristol Channel were accumulated by co-precipitation involving such oxides.

More recently Mothersill (1977) investigated factors controlling the concentrations of selected elements in the post-glacial sediments of Thunder Bay, Lake Superior. He demonstrated that the concentrations of nickel, copper, chromium and zinc showed a stronger correlation with manganese oxide than with iron oxides or organic material. Luoma (1977) investigated the biogeochemistry of zinc bound onto sediments in San Francisco Bay, and concluded that zinc in the sediments was partitioned between hydrous iron and manganese oxides and organic material. However, the role of organic material was found to be insignificant if it formed less than 1% of the sediment Zinc was found to be less available for uptake by polychaetes when the iron concentration in sediment exceeded the manganese concentration; i.e. when more zinc was partitioned into the iron oxide 'sink' by mass balance effects.

It is widely accepted that organic material in sediments, including humic material and other breakdown products of living matter, can play an important role in the binding of heavy metals. For example Gardiner (1974) found that sedimentary humic acid appeared to be the main component responsible for the binding of cadmium by river muds. Collinson & Shimp (1972) found that the accumulation of zinc, lead and cadmium in the upper layers of sediment from the Upper Peoria Lake was related directly to the organic carbon content of the sediment.

Cooper & Harris (1974) investigated the enrichment of heavy metals in the organic phase of sediment from the R Blyth, Northumberland. They suggested that the observed binding of metals to organic material was the result of ion exchange, complex formation and chelation. Hem (1972) concluded that the formation of complexes with humic acid in sediments was an important factor decreasing the mobility of heavy metals in stream waters. Gale, Bolter & Wixson (1976) observed that heavy metals carried into the Clearwater Lake from a mining area became bound by light fibrous organic debris in deeper parts of the lake

Sorption of dissolved and complexed metals onto clay minerals (often in the < 2 µm fraction of sediments) has been proposed as a mechanism by which metals may be sedimented Pita & Hyne (1975) demonstrated that sediments in reservoirs located downstream of metal mining areas tended to accumulate zinc, lead and cadmium They proposed that binding of metals from the water column by clays was the principal pathway for sedimentation. Shimp, Leland & White (1970) concluded that whilst the accumulation of metals by superficial sediments from southern Lake Michigan was related mostly to organic content, concentrations of metals in deeper layers of the sediments were correlated with the concentration of clay minerals The lesser role played by clay minerals was confirmed by Collinson & Shimp (1972).

Keulder (1975) considered the importance of the adsorption of zinc onto two clay minerals (illite and montmorillonite) during a study of the uptake of zinc by *Scenedesmus obliquus*. Montmorillonite was found to have a greater capacity for ion exchange than illite, this led to an increased number of exchange positions which aided in the uptake of zinc by exchange sites on the cell walls of the alga

Several authors have stressed that the mean size of the particles in sediments may have a marked effect on the binding of heavy metals Thus Oliver (1973) found that higher concentrations of zinc and lead were accumulated by fine grained sediments in the Ottawa River than by coarser particles. Similarly Jaffe & Walters (1975) found that sandy sediments contained lower concentrations of zinc and lead than fine-grained anoxic mud collected from the Humber estuary. Lasowski *et al.* (1976) demonstrated that the concentrations of nickel, copper, zinc and cadmium in sediments from polluted freshwater systems near Mainz (Germany) were inversely proportional to the grain size of the particular sample of sediment. It seems probable that such observations simply reflect the greater proportion of organic material, clays etc. that tend to accumulate in the finer fractions of sediments

As well as the factors that influence the binding of dissolved and complexed metal ions to sediments, it is common for discrete, unweathered particles of ore or rocks rich in heavy metals to be transported in the suspended load of streams or rivers and laid down in the sediment Sources of such material include mine milling and crushing operations. Normal weathering processes might be expected to be enhanced by the increased surface area of such particles, it therefore seems likely that they may not be inert chemically during transport or following sedimentation.

Chester & Stoner (1975) noted that a proportion of the heavy metals detected in sediment from the lower Severn estuary was present as a 'non-leachable' (by weak acid) form in mineral lattices. Loring (1976) demonstrated that from 70 - 88% of the zinc and lead present in the sediments of the Saguenay Fjord were present in the detrital fraction as discrete particles of sulphide ore Grieve & Fletcher (1976) concluded that from 80 -90% of the heavy metals (other than lead) present in the sediments of the estuary of the Fraser River were present in a non-labile form in mineral lattices

Whilst no studies of the partitioning of heavy metals were included in their accounts, it seems clear from the studies of Maxfield *et al.* (1974a, 1974b) that a proportion of the higher concentrations of zinc, lead and cadmium found in the sediments of the Coeur d'Alene Lake were present in particles derived from the crushing of ore. Similarly, a proportion of the lead content of sediments reported in part of Ullswater by Welsh & Denny (1976) probably entered the lake as discrete particles of ore when mining was active at the Greenside Lead Mine.

1.422 Release of heavy metals from sediments

The possibility that heavy metals may be released from sediments to the overlying water in rivers, lakes or reservoirs has been mentioned by several authors. However, few studies have provided conclusive evidence that such release does or does not take place. For example, Alberts *et al.* (1975) stated "The potential mobility of trace elements within and from the sediments of freshwater impoundments is of great importance in evaluating sources and sinks for these elements in the aquatic environment", but these authors provided no specific data regarding such mobility. Allan (1975) has pointed out the urgent need for research into the limnological processes resulting in the release of metals from sediments.

It is obviously common for fine-grained sediment to become re-suspended in the water for varying periods, depending on the density of the particles and the degree of turbulence If the normal concentration of dissolved and/or complexed metals in the water was governed previously by an equilibrium with the settled sediment surface, it seems probable that the increased surface area of sediment caused by re-suspension would lead to a shift in the equilibrium towards some release of metals to the water. Further, if heavy metals within the sediment had become more mobile (for example by the weathering of particles of ore), these might be expected to be released to the water during periods of turbulence

The only published study that considers specifically the release of heavy metals from re-suspended sediments is apparently that of Wakeman (1974). Wakeman compared the heavy metal content of filtered and unfiltered samples of water from the Marine Straits, San Francisco, before and after the bottom sediment was disturbed by dredging operations Filtrable concentrations of chromium, copper, lead, mercury, nickel and zinc were all found to increase significantly above background levels during the three periods of dredging that were investigated, although no quantitative data were included in Wakeman's brief account.

Release of metals from sediments might explain the observation made by Leland & Shimp (1975) that the concentrations of several metals (including zinc and lead) showed marked seasonal variation in the water of southern Lake Michigan. These authors demonstrated that higher concentrations of metals were present when the transparency of the water was low. Samples analysed for their study were passed through 0 45 µm porosity filters; the work of Kennedy, Zellweger & Jones (1974) demonstrated that some suspended material may pass through such filters (see 1 411). As heavy metals may be adsorbed onto clay minerals, it seems possible that a proportion of the metals detected by these authors might have been in the particulate phase. Romano (1976) demonstrated that that water immediately above the surface of highly polluted sediment in the Grand Calumet River (flowing into Lake Michigan) had higher zinc, lead and cadmium contents than surface water in the river. 0 45 µm porosity filters were also used in his study.

It seems unlikely that marked dissolution or desorption of heavy metals from sediments would take place under the conditions outlined above as long as the physical and chemical factors usually governing the equilibrium between sediment and water remained unchanged. Changes in such factors that might be expected to cause significant release include· changes in the redox potential of the water (Förstner & Müller, 1973), a reduction in pH or dissolved oxygen, an increase in temperature (Romano, 1976), a decrease in the hardness of the water, an increase in the concentration of complexing ligands

An example of the re-mobilisation of heavy metals from sediments to the overlying water as a result of such changes was documented by Williams, Kopp & Tarzwell (1966). These authors reported that the deepening of the Ohio River to 26 feet had resulted in the creation of a deep, sluggish channel in which organic material had become deposited During periods when the water of the river became anaerobic, the organic material became degraded and carried metals back into the river as complexes. This sequence of events, coupled with industrial discharges, led to periodic toxic effects on plankton and possibly fish.

Holmes, Slade & McLerran (1974) studied the migration and redistribution of zinc and cadmium in the sediments of the Corpus Christi Bay, Texas. In summer, concentrations of zinc and cadmium were found to be markedly higher in the upper layers of the water in the harbour (up to 0.48 mg l^{-1} and 0.08 mg l^{-1} respectively) than in water in deeper layers. This was attributed to the formation of a wedge shaped mass of anoxic water in the bottom of the harbour, in which the two metals became precipitated as sulphides Thus in summer the harbour contained a metal rich oxidised upper layer of water, a metal poor reduced layer and sediments rich in metal sulphides. In winter, an influx of oxygenated water to the harbour was found to result in the desorption of metals to the overlying water through oxidation of the sulphides. The authors concluded that "This significant transfer into the bay of the metals previously deposited on the floor is seen as a danger long after the effluent from industrial processes is stopped".

1.423 Experimental models

it is clear from the above sections that many factors, some of them not fully understood, may act to influence the concentrations of heavy metals present in different components of water-sediment systems In accepting that such systems are frequently complicated, several workers have attempted to duplicate the natural behaviour of heavy metals by constructing artificial experimental systems. The published results of a few such 'models' are considered here.

Cross, Willis & Baptist (1971) studied the distribution of radioactive and stable zinc in an experimental marine ecosystem (in a large fibreglass tank). Nine months after the addition of ^{65}Zn to the tank, they found that equilibrium had not been reached between concentrations of the isotope in filtrable and non-filtrable fractions of water. This serves to illustrate the complexity that may affect the exchange of metals between dissolved and particulate material in natural systems.

Nakhshina & Feld'man (1972) carried out a detailed experimental study on the effect of certain factors on th binding of zinc by the bottom organic layer ('ooze') in fresh waters. Following addition of from 0.05 - 1.2 mg l^{-1} Zn to the system, they found that up to 77% was bound rapidly by the organic material. 'Mud sand' (with a lower organic content) reached equilibrium with the zinc in the water at lower concentrations than the 'ooze'. pH and the concentration of calcium in the water were found to have a significant effect on the adsorption process, higher pH favoured binding to the sediment whilst high concentrations of calcium could reduce binding by as much as 25%. The authors proposed that binding of zinc by the organic component of the sediment was preceeded by the formation of intermediate polycyclic compounds

Guy & Chakrabarti (1975) studied the distribution of metal ions between soluble and particulate forms in an experimental system The system contained water, hydrous iron and manganese oxides, bentonite, solid humic acid, soluble humic and tannic acids, soluble EDTA and bicarbonate ions, together with lead, copper, cadmium, zinc and potassium ions. The strength of adsorption of metals by the different solid components was found to be in the order

$$MnO_2 > \text{humic acid} > \text{iron oxide} > \text{clay},$$

and the metals were found to be adsorbed in the order

$$Pb^{2+} = Cu^{2+} > Cd^{2+} - Zn^{2+} > K^{+}$$

It was found to be necessary to lower the pH to 4.8 before lead was released from complexes with tannic acid, as compared with a value of 3 5 before the metal was released from humic acid.

Hem (1976) measured the cation exchange capacity of a synthetic clay in freshwater. He concluded that if suspended sediment with the same properties as the clay was present in freshwater at a concentration of 10 - 1000 mg l^{-1} at pH 6 - 8, more than 90% of any lead present would be adsorbed

Davis & Leckie (1977) used an experimental system to study the effect of complexing ligands on the adsorption of copper and silver at a sediment/water interface. The system contained quartz (surface area 3.3 $m^2\ g^{-1}$) and amorphous iron oxide (surface area 182 $m^2\ g^{-1}$) in a solution of 10^{-1}M $NaNO_3$. 'Adsorption edges' for copper and silver were found to lie in the pH ranges 4 - 6 and 6 - 8 respectively. The authors concluded that the presence of complexing ligands could complicate the binding of metals onto sediments, for example when a complexing ligand was adsorbed onto clay particles with a strongly functional group directed towards the solution Interactions such as these were found to alter significantly the pH region for adsorption of metals

Lu & Chen (1977) used an experimental model to study the migration of trace metals at the interface of seawater and polluted superficial sediments. The model was used to investigate the effect of different redox conditions on the release of metals from sediments over a period of 4 - 5 months. Three main groups of metals were observed to show differing behaviour

i. those released under oxidising conditions (Cu Cd Ni, Pb, Zn),

ii. those showing no change in release with changing redox conditions (Cr, Hg),

iii. those released under more reducing conditions (Fe, Mn).

These authors also noted that equilibrium between water and sediments was reached only slowly within the system, possibly due to the presence of unknown complexing ligands

1.5 TOXICITY OF HEAVY METALS TO AQUATIC ORGANISMS

It has long been recognised that high concentrations of heavy metals such as zinc, lead and cadmium may prove toxic to aquatic plants and animals A large body of literature exists concerning the toxicity of various metals to organisms including bacteria, fungi, algae, bryophytes, vascular plants, protozoa, invertebrates and fish. The following brief review concentrates on the tolerance and adaptation to metals of aquatic plants, an aspect that has received less attention than, for example, the toxicity of heavy metals to fish. However, as some of the principles involved are common to all aquatic organisms, brief mention is made of published accounts relating to the effects of heavy metals on aquatic animals.

1.51 Animals

1.511 Introduction

The toxicity of heavy metals to animals in freshwater has been the subject of more research than has the toxicity of such metals to plants. Most of this research has concentrated on fish, which have probably received more attention than all of the other groups of aquatic organism combined (Whitton & Say, 1975).

1.512 Protozoa

Whitton & Say (1975) point out that most of the published information concerning the effects of heavy metals on protozoa relate to acid mine waters. A recent study under non-acidic conditions is that of Sartory & Lloyd (1976), who investigates the effects of selected heavy metals on 'unadapted' populations of *Vorticella convallaria* var *similis*. In culture, this organism was found to be sensitive to concentrations of 0.075 mg l^{-1} Zn and 0.0005 mg l^{-1} Pb, but grew at concentrations of <0.05 mg l^{-1} Zn and <0.0002 mg l^{-1} Pb. The authors pointed out that research is needed to determine the levels of metals above which adaptation in protozoa can occur, especially as such organisms may form an important part of the microbial communities in sewage treatment plants receiving inputs of metals. The organism used in their study was obtained from a culture collection, there was apparently no justification for the use of the term 'unadapted'.

1 513 Invertebrates

Earlier observations in the literature on the toxic effects of heavy metals to invertebrates include those made by Carpenter (1924). She noted that Welsh streams contaminated by the effluent from lead mining operations frequently showed a complete absence of groups of invertebrates including Platyhelmia, Mollusca, Trichoptera, Crustacea, Oligochaeta and Hirundinea. In some streams the only invertebrates surviving were *Tanypus nebulosus* and *Simulium latipes*. Jones (1940, 1958) found that these insects,together with some flatworms, were the only invertebrates present in the metal polluted R. Ystwyth

The crustacean *Daphnia magna* has been the subject of much research. For example Anderson (1950) studied the toxicity of 25 cations to this organism when grown in water from Lake Erie. Cadmium, lead and zinc were among the five most toxic elements, with threshold concentrations for toxicity decreasing in that order. Biesinger & Christensen (1972) carried out a further study of the toxicity of heavy metals to *D. magna*, and reported concentrations bringing about 'chronic toxicity' (i e. killing 50% of the organisms within three weeks) of 70 $\mu g\ g^{-1}$ Zn, 30 $\mu g\ g^{-1}$ Pb and 0.17 $\mu g\ g^{-1}$ Cd

Thorp & Lake (1974) carried out toxicity bioassays of cadmium on selected freshwater invertebrates, and studied the interaction of cadmium and zinc on the freshwater shrimp *Paratya tasmaniensis* The results of the bioassays on *Paratya* indicated that there might be seasonal differences in the sensitivity of the organisms to cadmium. Below a certain concentration the zinc and cadmium were shown to show a combined toxic effect that was less than additive. Above this concentration their interaction was strictly additive.

It is generally accepted that crustacea are more sensitive to elevated concentrations of heavy metals than are fish (e.g. Anderson, 1950; Arthur & Leonard, 1970). Wurtz (1962) suggested that molluscs and malacostracan crustaceans are the groups showing the least tolerance to heavy metals. In contrast, some species of insects may be relatively resistant. Thus Sprague, Elson & Saunders (1965) found that certain caddisflies and midges could tolerate concentrations of zinc and copper equivilent to 1.5 times the 'ILL' (maximum concentration permitting indefinate survival) for salmon

It has been demonstrated conclusively by some workers that certain species of invertebrates are capable of developing specially tolerant populations in environments contaminated by heavy metals. For example, Bryan & Hummerstone (1973) demonstrated that populations of the polychaete *Nereis diversicolor* from sediments with a high concentration of zinc were more resistant to zinc than were populations from uncontaminated sediments.

More recently Brown (1977) has demonstrated that the isopod *Asellus meridianus* may be present as metal-tolerant strains in rivers carrying high concentrations of copper and lead in the water In an experimental study of insects collected from the R. Hayle and R. Gannel (Cornwall), she found that copper-tolerant *A. meridianus* accumulated up to 6.8 $\mu g\ g^{-1}$ Cu after being fed a diet enriched with copper for 12 days. In contrast, non-tolerant animals showed no evidence of accumulation and died within eight days. Similarly, lead-tolerant animals accumulated up to 28 $\mu g\ g^{-1}$ Pb in four days, whereas non-tolerant organisms showed no accumulation and died within ten days. A marked difference was found in the mechanisms by which tolerant and non-tolerant animals stored copper in the hepatico-pancreas.

As with fish (see below), various factors have been proposed which may affect the toxicity of heavy metals to particular species of invertebrates For example Jones (1939b) found that relatively low concentrations of lead reduced the toxicity of copper to *Polycelis nigra*, *Gammarus pulex* and *Tubifex tubifex*. Whitley & Sikora (1970) studied the effect of pollutants including lead and nickel on the respiration rate of tubificid worms, and concluded that the effects of both metals were regulated by pH. Cairns *et al.* (1976) demonstrated that exposure of snails to sublethal concentrations of zinc in solution reduced survival time (or modified survival in other ways) during subsequent exposure to zinc

1.514 Fish

As was the case with invertebrates, the studies of of Carpenter (1924, 1925) are among the more important of the earlier accounts concerning the toxicity of heavy metals to fish. Her observations on the fauna of rivers in west Wales marked the commencement of a period of intensive study concerning the toxicity of metals to fish in the region (e g. Laurie & Jones, 1938, Jones, 1938, 1939a, 1940b). In her classic study, Carpenter (1924) observed that whilst non-polluted streams and rivers in west Wales had rich communities of fish, the entry of waste from lead mining into rivers such as the R. Rheidol and R. Ystwyth resulted in the complete elimination of the fish fauna. Carpenter attributed this to the presence of lead in the water; Jones (1940a) suggested that zinc was an important factor responsible for the observed toxic effects on fish in the R. Ystwyth.

Since these early observations, laboratory studies have developed from short term toxicity studies on fish in static systems to include a variety of more sophisticated techniques (e.g. McKee & Wolf, 1963; Edwards & Brown, 1967). Whitton & Say (1975) list some examples of experimental on the effects of heavy metals on fish, and cite reviews by Skidmore (1964), Jones (1964), Sprague (1969) and Brown (1973).

It is in such studies involving fish that most account has been taken of environmental factors that may influence the effects of a particular heavy metal. This is of especial relevance in assessing, for example, the effects of discharges of metals into waters containing fish. Edwards & Brown (1967) point out that attempts at predicting toxic effects to fish should consider information on as many of the toxicants present as possible Brown, Shurben & Shaw (1970) studied several polluted rivers in industrial regions of England, and observed that toxicity to fish in the field was always greater than that predicted by adding together the likely contributions of toxicants such as heavy metals. Other studies indicating that mixtures of heavy metals may show synergistically toxic effects include that of Anderson & Webber (1975). These authors concluded that zinc and copper were 'Supra-additive' in their toxicity to fish

Among the factors which have been mentioned as reducing the toxicity of heavy metals to fish, the one quoted the most often is the hardness of water. For instance, it seems widely accepted that zinc is almost always less toxic in hard than in soft waters (e.g. Jones, 1938; Cairns & Scheier, 1957, Lloyd, 1960; Lloyd & Herbert, 1962; Skidmore, 1964; Mount, 1966; Solbé, 1974). The influence of hardness on the solubility of zinc has already been mentioned (1.412), and it is not always clear from such studies to what extent the effects of hardness may be simply a result of the precipitation of zinc.

The influence of pH on the toxicity of heavy metals to fish seems to be less clearly understood. Mount (1966) showed that at any particular hardness zinc was least toxic to fathead minnows at pH 6.0 (the lowest value tested). Solbé (1974) summarised data of Lloyd (1960) together with data from his own studies, and concluded that combined increases in total hardness and pH led to a decrease in the toxicity of zinc to rainbow trout However, Solbé pointed out that the data were insufficient to separate the effects of pH and hardness.

In a study of the effects of copper on salmon parr in water taken from the Exploits River, Newfoundland, Wilson (1972) found that the 96 hr LC50 value could be raised from 125 µg l^{-1} Cu to more than 180 µg l^{-1} Cu by the presence of organic materials in added spent sulphite liquor.

Further complications affecting the toxicity of heavy metals to fish have been documented by some authors. For example salmon have been shown to avoid areas of a river contaminated by heavy metals by returning downstream (Sprague, Elson & Saunders, 1965). Sensitivity of particular species has also been shown to vary according to the stage of the life cycle. For example, eggs have been shown to be the stage most resistant to zinc in *Zebra danio* (Skidmore, 1965) and fathead minnows (Brungs, 1969) whereas the eggs of salmon have been shown to be more sensitive to zinc than the fry (Grande, 1966).

1.52 Plants

1 521 Introduction

It has long been noted that certain species of plants may be more resistant to elevated concentrations of toxic metals than others Although in this section emphasis is placed upon the literature concerning aquatic photosynthetic plants, brief mention is also made of other groups

1.522 Bacteria and fungi

Whitton & Say (1975) state that most reports describing bacteria and fungi in streams rich in heavy metals are concerned with acid mine drainages, where such organisms may form an important part of the microflora. However, there is a fairly comprehensive literature on the effects of heavy metals on bacteria and fungi in other habitats such as soils, and several studies have been carried out on selected species in the laboratory.

Griffiths, Hughes & Thomas (1974) investigated the occurrence in river water of bacteria resistant to heavy metals They reported that the proportion of zinc-resistant bacteria present could range from as low as 2% to as high as 30% in waters with different degrees of zinc contamination. They suggested that other chemical and physical factors could be at least as important as metal pollution in determining the development of resistant microbial communities Such factors included hardness, acidity, and the deposition of silt on the bed of the river Resistance to heavy metals was shown to be a stable, genetically determined character, and communities of bacteria were found to adapt to the toxic effects of metals with only small decreases in activity. The authors also demonstrated that bacteria associated with the roots of tolerant grasses growing on mine spoil possessed a greater resistance to metals than those associated with grasses growing on uncontaminated soil

Jordan & Lechevalier (1975) studied the effects of emissions of zinc from a smelter on the microflora of forest soils. They found that in the more contaminated soils (containing up to 80000 $\mu g\ g^{-1}$ Zn) the number of bacteria, actinomycetes and fungi was reduced greatly. This reduction in microbial growth was suggested as a reason for the observed lower rate of decomposition of leaf litter at contaminated sites All of the organisms isolated from contaminated soil were found to be tolerant to zinc, although zinc- tolerant forms were also readily isolated from uncontaminated soils The authors therefore suggested that selection for zinc-tolerant microflora might proceed rapidly following the enrichment of soils with the metal

More recently Babich & Stotzky (1977) demonstrated that several species of fungi could tolerate higher concentrations of cadmium in soil than laboratory media This difference was attributed to the effects of complexing particles (including clays) in the soil, together with the influence of pH. Babich, Bozian & Stotzky

(1977) demonstrated that the presence of clay minerals significantly reduced the toxicity of cadmium to *Aspergillus niger* and *A. fischeri*.

In a rather similar study Doelman (1977) demonstrated that the inhibition of respiration by soil microflora as a result of lead enrichment depended on the physico-chemical characteristics of the soil. Thus whilst respiration in sandy soil was inhibited by 500 $\mu g\ g^{-1}$ Pb, concentrations of up to 10000 $\mu g\ g^{-1}$ Pb had no significant effect on respiration in peaty soil. However, the microflora of peaty soils in areas affected by pollution were found to be less sensitive to lead than organisms from similar soils in unpolluted areas. Adaptation to lead in both sandy and peaty soils was found to occur within species, rather than by a shift in morphological groups.

Ashida (1965) reviewed the adaptation of fungi to metal toxicants. He adopted the terms 'training' or 'adaptation' for cases where increased resistance had been demonstrated in particular isolates, whether the resistance was the result of physiological modification of the cells or of selection for genetically tolerant strains. Ashida cited other examples of tolerant fungi that may or may not retain their tolerance in the absence of metals. Thus whilst some types were found to lose their resistance after a few 'transfers' between metal-free artificial media, others retained their resistance through frequent transfers. An example of a species that can tolerate extremely high concentrations of metals is that of *Penicillium glaucum* (Pulst, 1902), which can grow in liquid medium containing as much as 9.5% $CuSo_4$ More recently a strain of *Penicillium ochro-chloron* has been shown to be resistant to similar concentrations of copper in solution (Okamoto & Fuwa, 1974).

Ahsida (1965) listed possible mechanisms conferring resistance to metals in fungi under the following headings·

i. genetic adaptation (a) selection of spontaneously muted genes,
    (b) induction of mutation followed by selection,

ii. phenotypic adaptation (lost more quickly than (i)),

iii composite changes, e.g selection for a genotype conferring phenotypic plasticity, or for a genotype conferring some resistance and some phenotypic plasticity.

Ashida, Higashi & Kikuchi (1963) used electron microscopy to study the distribution of accumulated copper in resistant yeast cells. In the latter part of logarithmic growth resistant cells were found to produce large amounts of hydrogen sulphide, resulting in the precipitation of copper sulphide around the cell walls Hydrogen sulphide was not produced during other phases of the growth cycle, and the authors concluded that the cells possessed other characteristics conferring resistance to zinc

Chopra (1971) demonstrated that strains of *Staphylococcus aureus* that were resistant to cadmium showed markedly reduced uptake of the metal in comparison with strains lacking the gene conferring resistance. He suggested that 'non-exchangable' cadmium taken up by the resistant strain was bound to components within the cell, rather than adsorbed onto the cell surfaces.

In contrast, mercury-resistant bacteria have been shown to tolerate high concentrations of the metal by converting it to a volatile form which is then dispersed. For example Summers & Lewis (1973) demonstrated that mercury-resistant strains of *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* could convert 10^{-5}M Hg^{2+} to a form which was volatile and soluble in organic solvents.

1.523 Algae

Introduction

Whilst some species of freshwater algae are eliminated rapidly by elevated concentrations of heavy metals, others may form an important part of the microbial community in waters polluted by such metals Early published studies have highlighted the importance of algae in such situations. Subsequent field and laboratory studies have investigated various aspects of the toxicity of different metals to various species of algae, but there is still a marked paucity of literature on the subject.

The inhibitory effects of heavy metals on the growth and metabolism of algae has attracted attention over the years from the point of view of deliberate control of algal growth (Overnell, 1975), but few workers have investigated the mechanisms by which tolerant species may withstand the effects of metals in the field Thus whilst the occurrence of metal tolerant strains of particular species of bacteria and fungi is well documented (1.522), the possiblity of such adaptation by particular species of algae has received very little attention Similarly, whilst environmental factors affecting the toxicity of heavy metals to fish has been the subject of intensive research, very few workers have considered such effects for algae.

Field studies

As is the case for invertebrates and fish, the account of Carpenter (1924) presents fascinating observations on the effects of heavy metals on natural communities of algae. In her studies of the R. Rheidol, Carpenter found that the reaches downstream of the lead mines had a vegetation limited to slight coatings of brophytes and growths of the rhodophycean algae *Batrachospermum* and *Lemanea*. A later study carried out by Reese (1937) provided more detailed information on the algal vegetation of the metal polluted R Rheidol, together with that of other polluted streams in Cardiganshire. Reese reported that even 16 years after the cessation of most mining activities, there were still obvious differences in the vegetation of the R. Rheidol upstream and downstream of the Cwm Rheidol Mine. Macrophytic growths present upstream of the mine were eliminated for a length of about 100 yards, these included a spring growth of *Ulothrix* and *Stigeoclonium* and a spring and summer growth of *Lemanea* These differences were attributed to the effects of impurities brought into the river by a sidestream, rather than to drainage from the mine itself In the case of the R Melindwr, Reese concluded that silt derived from workings associated with the Goginan Lead Mine was the chief factor causing a reduction in the algal vegetation

More recently Jones (1958) carried out studies on the nearby R. Ystwyth and reported some details of the apparent effects of heavy metals on the algal vegetation For a distance of 1 mile downstream of the Cwm Ystwyth Mine the vegetation did not appear to be affected significantly by the metal content of the water. However, further downstream at Pontrhydyroes the whole appearance of the river had changed. At this point the normal flora of mosses and green algae had apparently been eliminated, and growths of *Lemanea* were abundant.

Whitton (1970b) reviewed the early literature regarding the algal flora of polluted rivers and streams in Cardiganshire. He concluded that it was hard to draw firm conclusions on the extent to which the reported changes in algal flora were due to the effects of zinc lead or silt However, it is clear from such accounts that *Lemanea* was especially tolerant to both zinc and lead in the region.

Recently Griffiths, Hughes & Thomas (1974) and McLean & Jones (1975) have reported further observations on the algal flora of streams and rivers in Cardiganshire. The findings of the former authors generally agreed with those of Reese (1937) They found that the total numbers of algae in the vicinity of disused zinc and lead workings at Frongoch and Cwm Ystwyth were considerably lower than those found in nearby unpolluted water. However, there was still a greater diversity of species of algae than of other plants or animals in the polluted reaches. The filamentous green algae *Ulothrix, Hormidium* and *Microspora* were found to be especially abundant in polluted situations, especially where streams emerged from mines. The authors concluded that such growths represented "striking manifestations of the ability of microorganisms to withstand the toxic environment". In a field study of the flora of the rivers Ystwyth and Clarach, McLean & Jones (1975) noted that both rivers still showed clear evidence of the toxicity of metals to plants They reported that species of *Hormidium*, especially *H. rivulare* Kütz, were the most tolerant algae at the most polluted stations

Whitton (1970b) pointed out that the effects of a particular metal on the algal flora of a stream or river may be easier to interpret where a single metal enters the water as the result of industrial pollution. He therefore reviewed several of the earlier studies where workers had investigated the effects of discharges of particular metals One such study was that of Butcher (1955) who sampled the algal vegetation at various points within the catchment area of the R. Trent (East Midlands) Butcher found that the effluent from a copper factory on the R Trent caused a great reduction in the flora of the river, but that growths cf algae returned four miles downstream of the effluent. Here the flora was dominated by *Chlorococcum, Achnanthes affinis* and *Stigeoclonium tenue*, instead of the normal succession of *Nitzschia palea - Cocconeis* A similar changed algal flora was observed within the R Dove following the entry of the R Churnett, and *Stigeoclonium tenue* was also found by Butcher to be present in the R. Tame downstream of a discharge of copper.

S. tenue was listed by Weimann (1952) among species of algae that were very resistant to metallic poisons in the Nordheim - Westfalen region, and Palmer (1959) quoted the alga as an 'indicator' of pollution by copper and chromium These observations are in apparent contrast to those of McLean (1974) who carried out a field study of the tolerance of *S. tenue* to metals in south and west Wales.

McLean found no growths of the alga in flowing waters polluted by high concentrations of heavy metals, and suggested that the tolerance of the alga as suggested by other workers was possibly simply the result of its tolerance and affinity for organic pollution. In the absence of the latter, high concentrations of metals such as zinc and lead would not be tolerated.

More recent studies of the effects of heavy metals on communities of freshwater algae include those of Besch, Ricard & Cantin (1972). These authors carried out an extremely detailed study of the relative tolerances of species of lotic diatoms to pollution by zinc and copper in the north-west Miramichi area of Canada *Achnanthes microcephala, Eunotia exigua, Pinnularia interrupta* t *biceps* and *Fragilaria virescens* were all found to be very resistant to very high concentrations of soluble zinc (>10 mg l^{-1}), whilst *Diatoma hiemale, Eunotia arcus, Gomphonema angustatum* and *Cymbella affinis* were apparently sensitive to concentrations of soluble zinc above about 0 1 mg l^{-1}.

Whilst the present study was in progress, Say (1977) completed a field (and laboratory) study of the microbial ecology of streams carrying high concentrations of zinc in the water. Most of the streams studied were associated with disused mine workings on the Northern Pennine Orefield. He found that even the streams most heavily polluted by zinc supported communities of algae, although the number of species present was often reduced considerably in comparison to nearby unpolluted streams. The algae showing most resistance in the field were species of *Hormidium, Ulothrix, Mougeotia* and *Microspora*. These formed abundant growths at the highest concentrations of zinc in stream waters; in most cases species of *Hormidium* (notably *H. rivulare)* were the most obvious

Laboratory studies

Many laboratory studies have been carried out on the toxicity of different heavy metals to different species of algae. A large number of these studies have been concerned with the possible uses of algae as monitors of pollution, or with the toxic effects of metal-containing algicides (Whitton & Say, 1975), and few authors have combined field and laboratory studies of toxicity. As the present study is concerned mostly with the ability of different species to tolerate different concentrations of metals in the field, only a brief summary of the more relevant articles is included here.

Several workers have used selected species of algae to assess the influence of one or more metals under controlled conditions in the laboratory. The species used, which are frequently obtained from culture collections, are therefore being used as bioassay organisms in the strict sense of the term For example Bartlett, Rabe & Funk (1974) used a modification of the 'Algal Assays Bottle Test' to assess the effects of copper, zinc and cadmium on *Selenastrum capriconutum*. Algicidal concentrations of these three metals under the experimental conditions used were 0.3 mg l^{-1}, 0.7 mg l^{-1} and 0.65 mg l^{-1} respectively. When the alga was subjected to varying concentrations of zinc in polluted stream water, it could not tolerate more than 0.5 mg l^{-1} of the metal.

Rachlin & Farran (1974) used a modification of a fish cell culturing technique to study the growth response of *Chlorella vulgaris* to selected concentrations of zinc. Their study demonstrated that a concentration of 2.4 mg l^{-1} Zn brought about a 50% reduction in growth rate within 96 hours In a rather similar study Malanchuk & Gruendling (1973) estimated the ED_{50} (median effective dose causing 50% reduction of $^{14}CO_2$ fixation) of lead for four species of algae. This concentration lay within the range 15 - 18 mg l^{-1} Pb for species of *Anabaena, Clamydomonas* and *Navicula,* but was only 5 mg l^{-1} for *Cosmarium* sp. The greater sensitivity of the desmid was attributed to its greater surface area volume ratio.

Only a few other workers have attempted to compare the sensitivity of different groups of algae to heavy metals. Overnell (1976) used the production of oxygen to measure the inhibitory effects of heavy metals on photosynthesis in seven species of marine algae Of these species, *Phaeodactylum tricornatum* and *Dunaliella tertiolecta* were found to be most sensitive to zinc, copper, cadmium and mercury. Rana & Kumar (1974b) used bioassay techniques to evaluate the toxicity of a zinc-containing effluent (from a smelter) to ten species of algae. The results suggested that *Chlorella vulgaris, Scenedesmus* sp. and *Plectonema boryanum* were relatively tolerant to zinc, whilst *Anacystis nidulans, Oscillatoria* sp. and *Nodularia spumigena* were relatively sensitive

Williams & Mount (1965) used artificial outdoor channels to study the effects of four concentrations of zinc on periphytic communities. Whilst no species were found which could be considered as 'indicators' of zinc, the number of dominant species was reduced at higher concentrations of the metal *Cladophora* was found to be especially sensitive to zinc, this is in agreement with the observation of Thomas (1962) who found that concentrations of zinc and copper proving toxic to *Cladophora* were 0.1 mg l^{-1} and 0.05 mg l^{-1} respectively Whitton & Say (1975) reviewed further studies on the toxicity of heavy metals to this alga. They noted that whilst all laboratory and some field studies indicate that *Cladophora* is one of the most sensitive of all algae to metals, other field surveys did not support this conclusion (see Whitton, 1970c) Whitton & Say (1975) also pointed out conflicting observations in the literature regarding the toxicity of heavy metals to the diatom *Nitzschia palea*

Whitton (1970a) carried out a laboratory study of the toxicity of copper, zinc and lead to 37 species of green algae. On the basis of this study, and in spite of conflicting observations outlined above, he proposed several tentative generalizations regarding species from flowing waters. *Microspora* and *Ulothrix* tended to be resistant to all three metals, whilst *Oedogonium* spp. tended to be relatively sensitive. Zygnemales were on the whole intermediate in their resistance to copper and lead, but showed a wide range of resistance to lead Whitton (1970b) suggested that it seems reasonable to assume that abundant growths of *Cladophora* indicate that the waters are not subject to repeated pollution by heavy metals in solution. On the other hand a well illuminated flowing water site with abundant growths of *Stigeoclonium tenue*, but no *Cladophora* at all, should be treated as suspect for pollution by heavy metals

It is not the purpose of the present review to consider the present knowledge of the biochemical effects of toxic or non-toxic concentrations of heavy metals. Say (1977) has reviewed such aspects for the metal zinc, and cites reviews by Vallee (1959, 1962) and Zajic (1969). Say also reviewed the methods employed by different workers to assess the toxicity of metals, and noted that in several cases the effects of a metal have been quantified by the degree of inhibition of a particular metabolic process. For algae, such process include the production of chlorophyll *a* (e.g. Hargreaves & Whitton, 1976), the rate of respiration (e.g. McBrien & Hassal, 1967) and specific photosynthetic reactions (e.g. Greenfield, 1942, Overnell, 1975, de Fillipis & Pallaghy, 1976b).

In addition to biochemical considerations, several workers have made observations on the effects of lethal or sub-lethal concentrations of heavy metals on the growth rate or morphology of different species of algae.

Thus Bartlett, Rabe & Funk (1974) noted that the most noticeable toxic effect of sub-lethal concentations of zinc, copper and cadmium on *Selenastrum capricornutum* was an extension of the lag phase of growth in culture

Hessler (1974) studied the effects of lead (added as lead nitrate) on the viability and motility of *Platymonas subcordiformis*. The most marked toxic effects were observed during logarithmic growth, when sub-lethal concentrations of lead delayed cell division and the subsequent separation of daughter cells Death of the cells was caused by 60 mg l^{-1} Pb, this concentration also affected motility by causing flagellae to be shed. When flagellae were sheared from the cells by artificial means, a concentration of 10 mg l^{-1} Pb was sufficient to prevent their regeneration. In a subsequent study Hessler (1975) investigated the possibility that high concentrations of lead might induce mutation in *P. subcordiformis*. When the alga was exposed to two mutagenic agents (ultraviolet irradiation and nitrosoguanidine), the incidence of mutations (chiefly affecting rate of growth and form of colonies) was not increased appreciably by the presence of lead chloride.

Several authors have noted that toxic effects of heavy metals on certain algae in culture may be associated with a loss of potassium from the cells. Similar observations on fungal spores by Bodnar, Villanyi & Terrenyi (1927) led to the proposal that the uptake of copper by the spores was the result of ion exchange with potassium. However, McBrien & Hassal (1965) noted that when cells of *Chlorella vulgaris* were treated with copper, more potassium was released than equivalent amounts of copper entering the cells. The authors concluded that the potassium was released because of the graded response of a barrier, normally at low permeability, to increasing concentrations of copper. This was suggested as a primary toxic effect of copper on

C. vulgaris, but not necessarily the most important since the cells were still capable of growth following the loss of large amounts of potassium More recently Overnell (1975) proposed that the loss of potassium from cells could be used as a measure of the toxicity of heavy metals to certain species of algae.

Tolerance and adaptation of algae to heavy metals

It has already been noted that both bacteria and fungi are capable of forming populations which are more tolerant to heavy metals than are other populations of the same species (see 1.522). The development of such tolerance through genetic adaptation is also well documented for terrestrial vascular plants (see 1.525). In light of this, together with the knowledge that algae are among the most successful organisms in waters polluted by heavy metals, it is suprising that there has been little research on the possibility of adaptation by species of algae to withstand heavy metals. For example, Griffiths, Hughes and Thomas (1974) reported that filamentous green algae including *Hormidium* spp. were 'strikingly' tolerant to metals in polluted waters in Cardiganshire. However, these authors presented no evidence to suggest whether the algae from such sites were metal tolerant strains of species that were normally more sensitive to heavy metals, or whether they were species that were naturally tolerant of metals. Similarly McLean (1975) noted that *Hormidium rivulare* was the sole alga growing in streams polluted by high concentrations of zinc in the Cwm Ystwyth - Dyfed area. He found that the alga could grow in laboratory culture medium at concentrations of zinc (up to 40 mg l^{-1}) far higher than could be tolerated by isolates of *Stigeoclonium* and *Spirogyra* from unpolluted streams. However, no attempt was apparently made to compare the tolerance of *H. rivulare* from polluted and unpolluted sites.

There are, however, some indications in the literature that different strains of individual species of algae may differ in tolerance to metals, and that such strains may occur naturally in environments polluted by heavy metals. Thus Kellner (1955) compared the morphology and growth of two strains of *Ankistrodesmus braunii*, and demonstrated that one strain could produce more or less normal cells and grow at higher concentrations of copper than the other Similarly Jensen, Rystad & Melsom (1974) showed that two clones of *Skelatonema costatum* showed significant intraspecific differences regarding tolerance to pollution by zinc. There was some indication that this difference was of ecological significance, as the more tolerant clone was isolated from the Sorfjord, which is heavily polluted by inputs from the metallurgical industry.

Russel & Morris (1970) investigated the ship-fouling properties of populations of the brown marine alga *Ectocarpus siliculosus*. They found evidence of intraspecific variations in response to dissolved copper which may have been associated with the habitats from which the populations were taken. Populations taken from an uncontaminated rocky shore were found to be capable of growth in concentrations of copper up to about 0.02 mg l^{-1}. In contrast, populations isolated from the hulls of two ocean-going freighters that had been treated with copper-based antifouling agents were found to be capable of growth at concentrations of copper ten times greater than this

Stokes, Hutchinson & Krauser (1973) showed that *Scenedesmus acutiformis* var *alternans* and *Chlorella fusca* isolated from lakes in the Sudbury region of Canada that were polluted by high concentrations of copper and nickel were more resistant to these metals than were laboratory strains of *Scenedesmus acumunatus* and *Chlorella vulgaris* respectively. The authors also pointed out that representatives of these genera appear to be widespread in environments rich in heavy metals. They suggested that the algae selected as laboratory strains were already more tolerant to copper than many other algae, and that strains isolated from the polluted lakes had evolved even more tolerance. Stokes (1975) documented the following characteristics in tolerant isolates of *Chlorella* and *Scenedesmus* from polluted lakes

i. The presence of tolerance is correlated with elevated concentrations of the particular metal, thus examples were found of tolerance to copper, or nickel, or both metals. The algae did not therefore show 'multiple tolerance' to several metals

ii. Tolerances were independant in that tolerance to copper and nickel in the same organism could be separated.

iii. Tolerance to copper required for its expression the presence of a certain amount of copper in the growth medium, and tolerance to copper was temporarliy 'lost' in copper-depleted cultures. Copper depleted cultures retained their tolerance to nickel, and nickel was not required in the growth medium for the expression of tolerance to nickel.

Whitton (1970a) compared the tolerance to zinc in laboratory culture of populations of *Stigeoclonium tenue* isolated from several freshwater environments, and noted apparently slightly greater resistance to the metal in a population from an effluent rich in zinc Subsequently C. Rajendran (personal communication) demonstated further examples of enhanced resistance to zinc in populations of *S. tenue* collected from zinc-polluted streams draining the Northern Pennine Orefield.

Recently de Fillipis & Pallaghy (1976c) demonstrated that a laboratory strain of *Chlorella* could develop increased resistance to zinc and mercury after a relatively brief exposure to the metals. Shortly following exposure to the metals the cells showed symptoms of metal toxicity, with sharp reductions in the rate of cell division, pigment content and export of photosynthates. After 40 days (approximately 40 series of cell divisions) the cultures had gained resistance to the metals, and had almost completely regained the rates of cell division displayed by control cultures. Resistance to zinc was found to be brought about by the development of an exclusion mechanism, whilst resistance to mercury was associated primarliy with increased volatilization of the metal by enzymatic means

Say, Diaz & Whitton (1977) have recently carried out a detailed laboratory investigation of the tolerance of species of *Hormidium* to zinc Populations of *H. rivulare* Kützë, *H. flaccidum* Kützë and *H. fluitans* (Gay) Heering were collected from flowing waters at 47 different sites, chosen to show a wide range of concentrations of zinc. At sites with higher concentrations of zinc in the water, populations of *H. rivulare* and *H. flaccidum* were found to show increased resistance to zinc which was suggested to be largely, if not entirely, the result of genetic adaptation.

In spite of observations such as these, Whitton & Say (1975) conclude that it seems probable that not all algae growing in high concentrations of heavy metals in the field have developed special tolerance to the particular metal(s) As the number of species of algae which can grow in non-polluted streams is so large, several species may exist which may posess the capacity to grow in elevated concentrations of metals 'by accident'.

Factors affecting the toxicity of heavy metals to algae

Whether or not species of algae are present in a particular environment as metal tolerant strains or forms, it seems probable that the toxic effects of individual metals may be expected to be influenced by other physical or chemical factors. Again, this aspect of the toxicity of heavy metals has received much less attention for algae than for some other groups of organisms (e.g. fish).

In the case of fish, both field and laboratory studies have shown that the concentrations of other heavy metals may influence the toxicity of any particular metal. Examples are known of antagonistic, additive and synergistic interactions (Jones, 1964). The situation with regard to algae is probably equally complicated, though less well documented. Bartlett, Rabe & Funk (1974) noted that combinations of copper, zinc and cadmium were similar in their toxicity to *Selenastrum capricornutum* to equal concentrations of zinc alone. However, combinations of copper and cadmium resulted in a greater growth rate than equal concentrations of copper, suggesting that cadmium inhibits the toxicity of copper

Hutchinson (197-) compared the toxicity of nine metals to the planktonic algae *Chlorella*, *Scenedesmus*, *Haematococcus* and *Chlamydomonas*. Among his conclusions were that copper and zinc were synergistic in their toxicity to these algae, whilst selenium antagonized the toxicity of cadmium. Pietiläinen (1975) studied the effects of lead and cadmium on natural communities of phytoplankton from brackish water. He concluded that cadmium increased the toxicity of lead when the concentration of cadmium was greater then the concentration of lead, but that the reverse was true when the concentration of lead was greater. Braek, Jensen & Mohus (1976) studied the effects of combined additions of copper and zinc ions to cultures of four common species of marine planktonic algae Synergistic toxicity by the two metals was demonstrated in the case of two species of diatoms and one dinoflagellate, but the two metals were antagonistic in their toxicity to the diatom *Phaeodactylum tricornutum*.

Although it seems clear that the toxicity of heavy metals to aquatic animals is almost always less in hard than in soft waters, few observations have been made on the effects of hardness components (such as magnesium and calcium) on the toxicity of heavy metals to algae. Braek, Jensen & Mohus (1976) noted that the toxicity of zinc to four species of algae was reduced by elevated concentrations of magnesium in the medium. They suggested that this could indicate a common route for divalent metal ions entering algal cells. In contrast Gachter (1977) found that the concentration of calcium did not appear to affect the toxicity of zinc, lead, mercury or copper to natural populations of phytoplankton from two Swiss lakes.

Among studies of vascular plants, high concentrations of phosphate in the soil have been shown to antagonise the toxicity and uptake of heavy metals (see 1.64). In the case of algae, Rana & Kumar (1974a) studied the influence of phosphate and nitrate on the toxicity of zinc to *Chlorella vulgaris* and *Plectonema boryanum*. Relatively high concentrations of phosphate, but not nitrate, were found to improve the growth of both algae and protect them against the toxicity of zinc to a certain limit. These findings are in apparent contrast to those of Greene *et al.* (1975) who reported that the toxicity of zinc to the green alga *Selenastrum capricornutum* was not affected significantly by concentrations of phosphate ranging from 0.047 mg l^{-1} to 0.930 mg l^{-1}. Rather, these authors concluded that increased ionic strength of the growth medium was the main factor reducing the toxicity of zinc to *Selenastrum*. The formation of ion pairs between zinc and ions such as Na^+, Mg^{2+} or Ca^{2+} was proposed as being the main explanation for this phenomenon. In contrast, the studies of Keulder (1975) have shown that elevated concentrations of PO_4-P, H^+, Mg^{2+} and Ca^{2+} all antagonise the uptake of ^{65}Zn by *Scenedesmus obliquus*.

In a study of a population of *Hormidium rivulare* isolated from a stream at pH 3.1 Hargreaves & Whitton (1977) demonstrated that the toxicity of zinc decreased over the range pH 3.5 - pH 7.0. In contrast Gächter (1977) observed that the toxicity of copper to a natural population of phytoplankton decreased at values over 7.4 Similarly Failla, Benedict & Weinberg (1976) have shown that decreasing the pH in stages from pH 8.2 - pH 4.8 led to increased entry of zinc to the cells of the yeast *Candida utilis* The authors concluded that this was due to an increased concentration of Zn^{2+} ions as the concentration of H^+ ions was increased.

The effects of complexing agents such as EDTA or natural organic material on the toxicity of heavy metals to algae have received more attention, especially with regard to the metal copper Steeman-Nielson & Wium-Anderson (1970) found that free copper ions were toxic to unicellular algae at concentrations normally found in natural waters. They concluded that this toxicity was not normally expressed because the copper was bound by natural organic materials. Ennis & Brogan (1961) proposed that some chelators

The effects of the artificial chelator EDTA in algal growth media have been discussed by Overnell (1976). He noted that the addition of the compound is usually necessary to prevent the precipitation of trace nutrients such as iron, but it may swamp the effects of small quantities of added heavy metals. If EDTA was omitted from the medium, however, Overnell noted that the growth of some algae could only be sustained for short periods.

Say (1977) investigated the effects of increasing concentrations of EDTA on the toxicity of zinc to a zinc sensitive population of *Hormidium rivulare*. He found that increasing the concentration of EDTA from 0 - 5 mg l^{-1} had little effect (at least in the culture medium used), but that concentrations of from 5 - 20 mg l^{-1} EDTA increased markedly the concentrations of zinc tolerated by the alga.

Say, Diaz & Whitton (1977) applied various statistical analyses to field and laboratory data on the tolerance to zinc of three species of *Hormidium*. They found that two groups of chemical factors appeared to influence the toxicity of zinc in the field. The first group, which appeared to increase the toxicity of zinc, included the heavy metals lead and cadmium. The second group, which apparently decreased the toxicity of zinc, included magnesium, calcium and various hardness factors. There was also some indication from the data that an increase in the concentration of phosphate and a decrease in pH might also decrease the toxicity of zinc to *Hormidium* spp.

Say & Whitton (1977) subsequently carried out a laboratory study of the influence of selected environmental factors on the toxicity of zinc to *Hormidium rivulare*. The toxic effects of the metal were found to be reduced by increases in the concentrations of magnesium, calcium and phosphate, and increased by rises in pH and the concentration of cadmium. In contrast, varying concentrations of sodium, chloride and sulphate ions had no detectable effects on the toxicity of zinc. At higher concentrations calcium was found to be more effective at reducing the toxicity of zinc than was magnesium, but this situation was sometimes reversed at lower concentrations. Both magnesium and phosphate were found to be more effective at reducing the toxicity of zinc to zinc-tolerant populations than to zinc-sensitive ones. The toxic effects of zinc and cadmium were found to be synergistic when the two metals were applied together. The authors concluded that the effects of all the factors investigated were sufficiently marked that they might be expected to be of considerable importance in the field.

1.524 Bryophytes

Several authors have noted that certain species of mosses or liverworts may play an important role in the communities of plants growing on soil or in water contaminated by high concentrations of metals. Thus Jones (1940) noted that the foliose liverwort *Scapania undulata* was the only macrophyte that could tolerate the elevated concentrations of zinc and lead in the water of the R. Ystwyth McLean & Jones (1975) also noted that *S. undulata* was tolerant to pollution by heavy metals, and was sometimes alone at polluted sites In contrast the moss *Fontinalis squamosa* was found by these authors to be relatively intolerant of metals in the field, and began to decay within 18 weeks of transplanting from unpolluted to polluted sites.

Say (1977) recorded the distribution of species of Bryophytes in streams on the Northern Pennine Orefield that were subject to a range of contamination by zinc. He found that the mosses *Bryum pallens*, *Pohlia nutans* and *Dichodontium pellucidum* occurred alone in streams with higher concentrations of zinc in the water. Bryophytes that were found to occur throughout the entire range of concentrations of zinc included *Scapania undulata*, *Philonotis fontana* and *Dicranella varia*. Certain other species which could apparently tolerate only low concentrations of the metal included *Hygrohypnum ochraceum*, *Brachythecium rivulare* and *Bryum pseudotriquetrum*.

Whitton & Say (1975) noted that the association of certain species of bryophytes with rocks rich in copper has been well documented (e.g. Url, 1956, Antonovics, Bradshaw & Turner, 1971). These 'copper mosses' have been used as an aid to prospecting for the metal, and there has been controversy as to whether high concentrations of copper are essential for their growth. However Shacklette (1967) has suggested that their distribution may be determined largely by the elimination of competing species.

There seems to be no evidence in the literature that species of bryophytes growing in heavy metal-rich environments may be present as metal-tolerant 'strains'. Brown & Bates (1972) could find no differences in the lead-binding capacity of plants of *Grimmia doniana* isolated from sites with a high and low degree of contamination by lead. Similarly M. Duncker (personal communication) could find no evidence for differences in the tolerance to zinc of populations of *Scapania undulata* from streams with varying concentrations of zinc in the water.

1.525 Vascular plants

There are many records in the literature of species of terrestrial angiosperms growing on soils contaminated by heavy metals, and much research has been carried out on the mechanisms by which such plants are able to tolerate particular metals. In contrast, there have been few reports of vascular plants growing in waters rich in heavy metals Besch & Roberts-Pichette (1970) studied the effects of pollution from mining on vascular plants in the north west Miramichi River system, Canada. They reported that of the species of vascular plants found in the area, submerged plants were more sensitive to metal pollution than riparian plants. Among the latter group monocotyledons were less sensitive than dicotyledons, and *Equisetum arvense* was the least sensitive of all. In the polluted Little South Tomogonops River, vascular plants were completely absent, and the bank gravels below high water mark were quite barren.

An example of a laboratory study of the toxicity of zinc to aquatic (non-lotic) angiosperms is that of Hutchinson & Czyrska (1972). These authors found that the presence of zinc markedly increased the toxicity of cadmium to *Salvinia natans* and *Lemna valdiviana*. This was true even at a concentration at which zinc itself was stimulatory. The presence of one metal was also found to increase the accumulation of the other by both plants The toxicity of cadmium to *Lemna* was found to be greater when it was in competition with *Salvinia* than when it was grown alone. In contrast, *Salvinia* was able to grow better at sublethal concentrations of cadmium in the presence of *Lemna* than its absence, and the accumulation of cadmium was reduced.

As regards terrestrial angiosperms, an extensive review of tolerance to heavy metals has been carried out by Antonovics, Bradshaw & Turner (1971). These authors compiled a list of plants that have been regarded as 'indicators' when prospecting for metals They concluded that the 'zinc violet' (*Viola calaminaria*) is the only terrestrial species that is accepted universally as an indicator of the metal zinc. Ernst (1966, 1968, 1974) has carried out a considerable amount of research on the phytosociology of communities of terrestrial plants colonizing metal-rich soils in Europe. Among his conclusions were that associations of species classified in the alliance Thlaspion calaminariae (characterised by *Thlaspi alpestri* sp *calaminare*) within the class Violetea calaminariae (characterised by *Viola calaminaria)* can be considered to be indicative of contamination of the soil by zinc.

Bradshaw and coworkers (Bradshaw, 1952, Bradshaw, McNeilly & Gregory, 1965; Gregory & Bradshaw, 1965) have carried out extensive studies of the tolerance to heavy metals of species of grasses colonizing mine spoil heaps. Of the species studied, *Agrostis tenuis* and *Festuca ovina* have been shown to be especially tolerant. Most of the species from sites rich in heavy metals have been shown to be metal tolerant genetic strains of species which have non-tolerant strains growing elsewhere. In contrast, tolerance to zinc by *Typha latifolia* apparently does not involve the evolution of tolerant races (McNaughton *et al.*, 1974). Genetical studies carried out by Gartside & McNeilly

(1974a, 1974b) have shown that the genetical control of tolerance to zinc in *Anthoxanthum odoratum* and *Agrostis tenuis* is dominant and directional with a high additive genetic variance, and is under polygenetic control Turner (1969) has emphasised the specificity of genetic tolerance in such plants, although the occurrence of several metals in a particular soil may lead to the simultaneous expression of tolerance to several metals by a single plant.

Zinc resistant *Agrostis tenuis* accumulates large quantities of zinc, and resistance is thought to be achieved by an increased ability to bind the metal to components of the cell wall (Turner, 1970; Turner & Marshall, 1971). Zinc resistant *Silene cucabilis, Rumex acetosa* and *Philonotis fontana* have been found to produce increased amounts of melate, and it has been suggested that this may complex with zinc to render it non-toxic (Mathys, 1975). Griffiths, Hughes & Thomas (1974) have suggested that the association of metal tolerant strains of bacteria with the roots of tolerant grasses may contribute to the resistance of the plants.

1.6 ACCUMULATION OF HEAVY METALS BY AQUATIC PLANTS

1.61 Introduction

The ability of submerged plants to taken up heavy metals to produce an internal concentration greater than that in the external environment has been documented in many different plants by many different authors. Studies have ranged from surveys of the mineral composition of plants in different aquatic environments to detailed investigations of the physiological mechanisms of uptake and the factors controlling them. Discussion will be limited here mostly to the former type of study (1.62), although mechanisms of uptake are mentioned briefly in 1.63.

1.62 'Monitoring' of heavy metal pollution by analysis of plants

The use of data on the accumulation of heavy metals by submerged plants has been justified in several ways. For example Adams, Cole & Massie (1973) suggested that the use of plant 'monitors' gives an integrated picture of pollution, an advantage of which is reduced cost in comparison to chemical monitoring systems. Dietz (1973) suggested that the accumulation of heavy metals by plants could be a useful method of calculating the average concentration of trace elements in the water, especially where the aqueous concentrations were at or below the detection limit.

Another possible justification for the inclusion of analyses of plants in the monitoring of pollution by heavy metals lies in the fact that any metals taken up by the plant must have been in a form available to the biota at some stage. Thus analyses of plants may provide an insight into the speciation (and potential toxicity) of metals in a particular situation. This might be difficult to achieve by chemical analyses in situations where complicated chemical and physical factors may be acting to affect the behaviour of the metals (see 1.41). Such uptake must therefore be considered to be of potential ecological significance whatever the possibility of transfer to further stages in food webs As Jenkins (1975) states: "Living biological organisms . .. continuously integrate and relate biological effects to physical and chemical measurements of the environment".

Several broad surveys have been reported of the mineral composition of different submerged species of plants. However, it is difficult to judge the significance of some of these studies because of the omission of sufficient environmental data. Where the intention of the particular study has been to relate levels of metals in the plant to levels in the water, the most usual index of accumulation has been the 'enrichment ratio' of Brooks & Rumsby (1965):

$$\text{Enrichment ratio} = \frac{\text{concentration of metal in plant}^{\text{(dry weight)}}}{\text{concentration of metal in water}}$$

This corresponds to the 'concentration ratio' quoted by some authors (e.g. Harvey & Patrick, 1967). Difficulties in comparison may arise where workers have used different measures, for example Dietz (1973) calculated 'enrichment factors' for metals based on determinations of wet weight.

The study of Dietz (1973) was concerned with the accumulation of Mn, Fe, Ni, Cu, Zn, Mg and Pb by two species of moss and four species of vascular plants in the catchment area of the R. Ruhr. He found marked differences in the amounts of metals taken up by species, and between the metal content of particular species collected from different sites. For a particular species, however, the 'enrichment factor' showed little variation between sites with different concentrations of metals in the water.

In a rather similar broad-scale study, Adams, Cole & Massie (1973) studied the mineral composition of 45 species of submerged vascular plants from different locations in Pennsylvania Whilst their study was concerned mostly with the application of plant analyses to the monitoring of nutrient pollution, they noted significant statistical variations between the concentrations of Mn, Fe, Cu and Zn in individual species sampled from different sites.

Boyd & Lawrence (1967) gave an extensive account of the mineral composition of various freshwater algae, but included little data on the environments from which the individual species were collected. Detailed studies of the accumulation of heavy metals by filamentous algae have also been carried out in the 'New Lead Belt' of Missouri (e.g. Gale *et al.*, 1973, Wixson & Gale, 1975). Keeney *et al.*, (1976) have suggested that *Cladophora glomerata*, one of the algae studied in the 'New Lead Belt', is a potentially useful monitor of concentrations of heavy metals. They found that *Cladophora* concentrated metals from the surrounding water, with a reasonably constant enrichment ratio for each metal.

Lloyd (1977) carried out a detailed study of the accumulation of heavy metals by submerged plants, including *Cladophora*, in the R. Wear system, north east England. He found marked increases in the concentrations of zinc, lead and cadmium in *Cladophora glomerata* and *Fontinalis antipyretica* on passing from upstream to downstream of the entry of an industrial effluent to the R. Wear. Clear linear relationships were found between the zinc and lead contents of *Cladophora* and the surrounding water in strictly comparable situations within the river.

Recently Empain (1976a, 1976b) has analysed the heavy metal content of several species of bryophytes from the rivers Somme and Sambre, Belgium. He studied 'profiles' of the concentrations of Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn in water and mosses at several stations along these rivers and concluded that.

i. bryophytes may 'integrate' variations in the heavy metal content of the water,

ii. they accumulate metals present in trace concentrations, thus aiding in their detection and measurement,

iii. the analysis of plants such as bryophytes provides an estimation of the total metal content of the water that is available for uptake by the biota.

The examples mentioned above serve to illustrate that several workers have used the analysis of submerged plants as an aid to the study of fresh waters polluted by heavy metals. However, it is notable that no studies have apparently been undertaken to relate the heavy metal content of a particular plant to that of the water over its entire range of occurrence in waters polluted by metals. Although it has been reported (e.g Dietz, 1973) that the enrichment ratios for certain elements in the tissues of a particular plant may be fairly constant, there are few indications in the literature of the range of concentrations of metals over which this is true for any particular species Some metals (e.g. zinc, copper) are essential for growth, and it therefore seems reasonable to assume that the relationship between the contents of the water and the plant would cease to be defined clearly at very low concentrations of the metal in the water. No studies have apparently attempted to compare the uptake of such essential metals at very low concentrations with that of metals (e.g. lead, cadmium) which have no known biological function.

Conversly, no studies of aquatic plants have apparently attempted to follow the accumulation of any metal by a particular plant from low concentrations in the water up to the highest concentrations at which the plant could survive in its natural habitat Such a study would be limited by the ecological range of the species chosen, and it would clearly be of interest to study a species with a range extending from very low to very high concentrations of metals in the water.

Whilst much work has been carried out to assess how the development of genetic tolerance to metals may affect their accumulation by terrestrial plants (e.g. Turner & Marshall, 1971, 1972), no such studies have apparently been carried out for submerged plants. This seems suprising since submerged plants are used more frequently for 'monitoring', although as pointed out in 1.523 the present knowledge of the occurrence of enhanced resistance to heavy metals by submerged plants is very limited. It seems probable that the development of genetic or physiological tolerance would complicate uptake in some way (see below) and thus affect the use of measures such as 'enrichment ratios'. A study of the accumulation of metals by a submerged plant with a range extending into high concentrations of metals, together with studies on the possible development of tolerance to the metals in the field, would provide valuable information. In view of the possible complications involved in studies of the uptake of metals by root plants (see below), the use of physiologically simple plants such as the algae in a study like that outlined above would possess obvious advantages. It has already been noted (1.523) that some species of algae show a wide range of tolerance to heavy metals in the field.

For the species noted as being especially tolerant to heavy metals in the field, only a few measurements have been made of heavy metal content. For the tolerant liverwort *Scapania undulata*, McLean & Jones (1975) presented some data on metal content in polluted streams in Cardiganshire. They noted that whilst the heavy metal content of the plant mirrored that of the water to some extent, in general *S. undulata* had a lower content of iron, manganese and lead than 'non-tolerant' bryophytes such as *Fontinalis squamosa*. Following transplanting from high to low concentrations of metals in the water, no marked change in the metal content of *S. undulata* was found to take place. More recently M Duncker (personal communication) has studied the accumulation of zinc by *S. undulata* in streams draining the Northern Pennine Orefield. She found that the concentration of zinc in populations of the plant in the field gave a good indication of the concentration in the water, but could find no conclusive evidence for or against the development of genetic tolerance to high concentrations of zinc in the field.

Lemanea was noted in 1.523 as being tolerant to high concentrations of heavy metals in the water. However, there are only a few records in the literature for concentrations of metals accumulated by this alga (Empain 1976a, Deb *et al.*, 1974, Whitton & Say, 1975). Of other species known to tolerate high concentrations of metals in the field, study of the accumulation of metals has apparently been limited to laboratory investigations of methods of uptake. Thus McLean (1975) studied the uptake of ^{65}Zn by *Hormidium rivulare* isolated from a polluted site within the Cwm Ystwyth Mine, Wales. The work of Say, Diaz & Whitton (1977) indicates that the population studied by McLean was almost certainly a specially tolerant strain. Similarly Silverberg (1975) used cytochemical techniques and X ray microanalysis to observe the ultrastructural localization of lead following accumulation by *Stigeoclonium tenue*, another species noted for its apparent tolerance to heavy metals in the field.

1.63 Mechanisms of uptake of heavy metals by submerged plants

Aquatic plants have received less attention than terrestrial angiosperms with regard to mechanisms of uptake of heavy metals, but enough information now exists in the literature to enable several broad conclusions to be drawn. In the case of physiologically simple non-rooted plants such as the algae and bryophytes, uptake of heavy metals must take place entirely from the surrounding water. There seems to be a general consensus of opinion that in many cases the uptake of metals by such plants is initially a passive process involving adsorption and/or ion exchange onto the cell wall. In some cases an 'active' mechanism may subsequently transport metal ions into the cells

In the case of aquatic bryophytes, these two processes have been demonstrated clearly by Pickering & Puia (1969). In a study of the accumulation of zinc by the aquatic moss *Fontinalis antipyretica*, a large amount of the metal was found to be taken up by rapid ion exchange/adsorption processes involving the Donnan-free-space of the cell wall. However, two other processes were also demonstrated for the uptake of zinc by the moss. A second, slightly slower, stage involved uptake into the protoplast with the outer layer acting as a barrier to diffusion. The final stage, lasting several days, consisted of an active (i.e. dependant upon metabolic energy) accumulation into the interior of the cells This phase was inhibited by metabolic inhibitors such as DNP, and was affected by temperature and light.

McLean & Jones (1975) proposed rather a different pattern of uptake of zinc by the liverwort *Scapania undulata*. They suggested that an active mechanism was involved at 'lower' concentrations of zinc in the water (0 - 10 mg l^{-1}), but that an uncontrollable passive uptake took place at concentrations of zinc over 10 mg l^{-1}. They proposed that the tolerance of *Scapania* to zinc in the field was a result of the presence of a protective system allowing the binding of accumulated zinc to 'harmless' sites within the cell.

In the case of freshwater algae, the accumulation of heavy metals by species grown in culture has probably received more study than any other aspect of the effects of such metals. Such studies have frequently involved the use of radioactive isotopes such as ^{65}Zn, although studies using isotopes have seldom been combined with work using non-radioactive metals (Whitton & Say, 1975).

There have been some apparent differences in opinion regarding the relative importance of 'passive' and 'active' uptake of metals by various species of algae, but again there now seems to be sufficient information in the literature to enable some generalizations to be made. Much of the earlier work on possible mechanisms of uptake was for seaweeds, of which only brief mention is made here.

Bachmann & Odum (1960) studied the accumulation of ^{65}Zn by marine benthic algae. Their findings indicated that uptake was directly proportional to net oxygen production, and they concluded that uptake was an active process linked to photosynthetic activity However, subsequent studies (e.g. Gutknecht, 1961, Parry & Hayward, 1973) tended to shed doubt on this interpretation. They suggested instead that the relationship between photosynthetic activity and the adsorption of zinc was a secondary effect related to fluctuations in pH.

As was noted for bryophytes, passive association of metals with adsorption / ion exchange sites on the cell surfaces plays a major part in most observed accumulation of metals by algae. For example Broda (1965) concluded that uptake of ^{65}Zn by *Chlorella fusca* was largely the result of ion exchange. The system leading to the uptake of zinc was found to contrast markedly with known 'active' systems, e.g. for the uptake of potassium, although the extent of accumulation could be similar. Broda also compared the competition of foreign ions with uptake of ^{65}Zn by *Chlorella* and by ion exchange resins. From the sequence of uptake of the different ions, he concluded that the sites involved in the accumulation of the metal were mostly carboxyl groups. These were probably carbohydrate derivatives in the 'free space' of the cell wall. However, further work also demonstrated the presence of sites with an 'anomalous afinity' for zinc.

Further research by Broda *et al*. (1967) confirmed that passive uptake was an important component of the uptake of ^{65}Zn by *Chlorella*. However, an additional active mechanism could also be detected by studies using competitors or deprivation of energy. The metabolic energy necessary for active uptake was found to be derived from energy-rich phosphates similar to ATP. The authors noted that the passive component of uptake could still be demonstrated in killed cells of *Chlorella*, similar observations were made by Cushing & Watson (1968).

More recently Matzku & Broda (1970) have presented further experimental evidence for the mechanisms of uptake of labelled zinc by *Chlorella fusca*. Two definate processes were found to be involved, the first of which was an energy- and temperature-independant binding to the cell sufaces. The ^{65}Zn taken up by this process was removed easily by washing with non-labelled zinc carrier solution. During the second process, zinc was taken up over longer periods and could not be removed completely. The uptake by this latter pathway followed saturation kinetics, and was probably controlled in part by an active pump However a third, energy-independant, path of uptake with a different temperature coefficient was also observed

Davies (1974) proposed a model for the uptake of zinc by *Phaeodactylum tricornutum* in culture. The model was rather similar to those outlined above, and consisted of three phases.

i. rapid equilibration of the concentration of ^{65}Zn adsorbed by the cell wall with the concentration in the culture medium;

ii. subsequent diffusion across the cell membrane according to Fick's diffusion law;

iii. rapid equilibration of ions reaching the inside surface of the membrane between the membrane itself, the intracellular fluid and intercellular proteins.

Rathsack & Sachert (1969) studied the uptake of copper by the giant internodal cells of *Nitella*. Again the process of accumulation was found to be initially one of ion exchange on sites in the cell wall, followed by slow permeation throughout the cell. At low concentrations of copper it was proposed that the cell wall exerted a certain protective effect by shielding the protoplasm from the toxic effects of the metal. At higher (lethal) concentrations the authors proposed that copper might react with the membrane systems of the protoplast, allowing a permeation into the vacuole.

Wixson (1977) presented an interesting discussion of the mechansims by which 'passive' uptake of metals by algal cells might play a major part in the removal of lead from apparently inert suspended particles. During a study of the accumulation of lead by filamentous algae (notably *Cladophora*) in the 'New Lead Belt', it was noted that uptake was frequently preceded by the physical entrapment of suspended mineral particles Wixson proposed that the proximity of 'abundant ionic sites' in or on the cell surfaces to the entrapped particles played a dominant role in the disassociation and solubilisation of lead, with subsequent uptake by the alga.

The extension of studies of the accumulation of heavy metals to include rooted species of aquatic plants raises the question of whether the uptake of any metals has taken place directly from the sediments in which the plants are growing. An experimental study demonstrating that uptake of 'non-essential' heavy metals may take place through the roots of angiosperms was carried out by Jones, Clement & Hopper (1973). Using perennial rye grass, these authors demonstrated that lead was taken up rapidly from solution, with subsequent transport of up to 29% of the metal from the roots to the shoots. The lead supplied to the roots during the study was in soluble form (as lead nitrate) and was not therefore directly comparable to particulate or bound heavy metals in polluted freshwater sediments. A similar study of the uptake of dissolved zinc by the roots of barley was carried out by Findenegg & Broda (1965).

Chiaudani (1969) studied the uptake of copper by plants of *Phagmites communis* growing in polluted and non-polluted sediments in six Italian lakes. He demonstrated that significant quantities of the metal were taken up by the roots and transported to the stem and shoots, leading to a linear relationship between the concentration in the sediment and in different parts of the plant. In a rather similar study Welsh & Denny (1976) studied the accumulation of lead by submerged macrophytes growing in sediments in Ullswater that had been polluted by lead They discussed the relative importance of uptake through shoots and roots, and concluded that the latter probably played a significant role. However, the authors pointed out the difficulties involved in separating the two types of uptake on the basis of field evidence alone. Denny (1972) has demonstrated that the relative importance of shoots and roots in taking up nutrients may differ markedly between different species of submerged macrophytes

Two other studies that have considered the possibility of uptake of heavy metals from sediments are those of Pet'kova & Lubyanov (1969) and Eriksson & Mortimer (1975). The former authors studied the accumulation of trace elements by aquatic macrophytes on the Ukranian Steppe, and concluded that some species could accumulate manganese and copper from the sediments through the roots. The latter authors carried out a laboratory investigation of the uptake of mercury by the rooted aquatic plants *Sagittaria latifolia* and *Scirpus cyperinus*. They found very little uptake of mercury where the concentration of the metal was high in the sediments but low in the water, and found that translocation of mercury from roots to shoots was generally insignificant.

The possibility that heavy metals accumulated from sediments by rooted plants might be released from the plants to the water has been considered by some workers. Such release has been demonstrated fairly conclusively in some cases. For example Mayes & McIntosh (1975) found that some of the zinc and cadmium accumulated by plants of *Ceratophyllum demersum* was re-released into the water of a lake at a later stage in the growth cycle of the plant Although *Ceratophyllum* is rootless, it seems probable that such release could take place from other angiosperms that had taken up metals through their roots.

Welsh & Denny (1976) suggested that rooted plants played an important and undesirable role in the recycling of lead from the sediments to the water of Ullswater. In a controlled experiment on the decay of plants they demonstrated that lead (probably taken up from sediments) was released to the water from the shoots of *Potamogeton crispus* They found that invertebrates feeding on detritus associated with decaying plant material in Ullswater had very high concentrations of lead in their flesh, and proposed that senescing and decaying shoots were responsible for the rapid release of lead into the lake.

1.64 Factors affecting the accumulation of heavy metals by plants

Whitton & Say (1977) state that "Just as toxicity of a metal may be affected by other factors in the environment, so also may accumulation be so". They suggest that the factors influencing the accumulation of metals may be expected to be similar to those affecting their toxicity, though experimental studies have been too few to make this generalization reliable. The factors quoted most often as influencing the toxicity of heavy metals include pH, magnesium, calcium, phosphate and complexing molecules (see 1.523), and examples can be found of studies in which each of these factors has been found to affect the uptake of metals by plants.

Bachmann (1963) found that the order of effectiveness of various ions in reducing the uptake of ^{65}Zn by cells of *Golenkinia pauscispina* was $H^+ > Ca^{2+} > Mg^{2+} > Na^+ > K^+$. Similarly Keulder (1975) illustrated that ions of H, P and Mg all inhibited competitively the uptake of ^{65}Zn by *Scenedesmus obliquus*.

pH

Gutknecht (1963) reported that increases in pH increased the uptake of ^{65}Zn by benthic marine algae, and decreased subsequent loss of the isotope Photosynthesis-linked changes in pH were found to affect both metabolic and non-metabolic mechanisms of uptake. Ferguson & Bubela (1974) investigated the accumulation of copper, lead and zinc from aqueous solutions by particulate algal matter, and found that uptake of all three metals was supressed by increases in pH. Lloyd (1977) noted that the enrichment ratios for several heavy metals were markedly lower in bryophytes collected from a highly acid mine drainage than in material collected from rivers at higher pH.

Mierle & Stokes (1976) studied the accumulation of copper by copper-tolerant and copper-sensitive strains of *Scenedesmus*. The initial uptake of copper was never found to reach equilibrium with the external environment at low pH values or high concentrations of calcium. Both H^+ and Ca^{2+} ions were found to reduce the electrostatic forces of negatively charged groups in the cell membranes, and H^+ ions competed with copper ions for negatively charged ligands, particularly carboxyl groups.

Magnesium and calcium

In the case of terrestrial plants, it is widely accepted that heavy metals are usually less available for uptake from limed than calcium-poor soil (e.g. Cox & Rains, 1972, John & Van Laerhoven, 1976, Francis & Rush, 1973). Fewer such studies have been carried out using other groups of plants such as algae or bryophytes. Cushing &

Rose (1970) studied the cycling of ^{65}Zn by periphyton from the Columbia River, and concluded that higher concentrations of calcium decreased the uptake of the isotope through competition for binding sites.

Kinkade & Erdman (1975) investigated the influence of Ca^{2+} and Mg^{2+} ions on the uptake and concentration of cadmium in a simulated freshwater ecosystem, including the plants *Nitella flexilis* and *Elodea canadensis*. The results indicated that the initial rate of uptake of cadmium was greater in hard than in soft water. However, the total amount of cadmium taken up by the plants over a longer period of time was much greater in soft water.

Phosphate

There have apparently been no experimental studies on the effect of phosphate on uptake of heavy metals by algae or bryophytes, although some such work has been carried out on terrestrial angiosperms. For instance Ernst (1968, 1974) has demonstrated that increased concentrations of phosphate in the soil lead to a reduction in the toxicity of zinc to *Thlaspi alpestre* spp. *calaminare*, and also to a marked change in the distribution of zinc within individual plants.

Stukenholtz *et al.* (1966) studied the interaction of phosphate with the uptake of low concentrations of zinc by *Zea mays*. They found that the depressive action of phosphate on the uptake of the metal was physiological in nature, and was expressed at the root surfaces and/or within the root cells. It was not, therefore, the result of simple chemical inactivation of zinc within the soil. Translocation of zinc within the plants was found to be inhibited by phosphate, leading to a marked reduction in the zinc content of the aerial parts Similar observations were made by Motsara (1973) who found that the uptake of ^{65}Zn by barley was inhibited at three stages in the growth cycle by the application of phosphate to the soil.

Complexing agents

It is clear that the binding of heavy metals in water by complexing agents, either natural or artificial, may complicate their uptake by plants. The most usual situation is that the metal is held too strongly by the complexing molecule to be available for binding by weaker sites on the surface of the particular plant. Further, compounds such as EDTA may physically remove metals that are held by weaker forces to the surface of a plant. Thus Matzku & Broda (1970) reported that part of the ^{65}Zn accumulated by cells of *Chlorella fusca* was removed slowly from the interior of the cells by a solution of EDTA. Cossa (1975) studied factors affecting the uptake of cadmium by the unicellular alga *Phaeodactylum tricornutum*. He observed that whilst the metal was normally taken up rapidly (according to the stage in the growth cycle), it was only accumulated weakly as cadmium-EDTA chelate. Similarly, metabolites (such as amino acids) excreted by the alga into the surrounding medium played a part in the gradual elution of cadmium from the cell surfaces (Cossa, 1976)

Other metals

An interesting example of the way in which the concentration of one metal may affect the uptake of another is provided by the work of Whitehead & Brooks (1969). These authors studied the use of aquatic bryophytes as indicators of uranium mineralization, and found that the alpha radioactivity of many species bore an *inverse* relationship to their uranium content. They suggested that this radioactivity was due largely to ^{226}Ra formed by the decay of ^{238}U, and that in a stream with high concentrations of uranium this uranium would be antagonistic to the accumulation of radium, whose complexes were much less stable. On the basis of this work, Whitton & Say (1975) pointed out the need for further work on possible antagonistic or syneristic interactions between metals being accumulated by plants.

Francis & Rush (1973) investigated the factors affecting the uptake and distribution of cadmium in plants of *Phaseolus aureus* and Japanese millet grown in nutrient solutions. The addition of zinc to the solution was found to decrease the amount of cadmium taken up by the roots. Lagerwerff & Biersdorf (1971) studied the interaction of zinc with the uptake and translocation of cadmium by radish At low concentrations, increasing concentrations of zinc inhibited the uptake of cadmium. However at higher concentrations of cadmium (100 $\mu g\ l^{-1}$), increased concentrations of zinc increased the uptake of cadmium. This was true for both roots and leaves, and the authors suggested that it indicated damage to the roots by cadmium

John (1976) investigated the relationship between cadmium in plants of oats and lettuce, and the uptake of other elements from solution. Several complicated effects were observed, for example the effect of phosporus on the uptake of zinc, or that of potassium on the uptake of calcium, could affect indirectly the uptake of cadmium. However, the addition of zinc was found to antagonise directly the uptake of cadmium through the roots.

Very few data are available on the possibility of interactions between heavy metals accumulated by algae. Broda & Findenegg (1967) investigated the competition of various ions with the uptake of ^{65}Zn by cells of *Chlorella* in culture. The following ions were found to compete with ^{65}Zn in the following order $Cd > Co > Ni, Mn > Ca > Na$. More recently Cossa (1975) studied the effect of various factors on the uptake of cadmium by *Phaeodactylum triconutum*. He noted that at several stages in the growth cycle the concentration of zinc in the cells was inversely proportional to the concentration of cadmium, indicating possible competition between the two metals.

Relationships between resistance and accumulation of heavy metals by submerged plants

In reviewing the available published data on the development of enhanced resistance to heavy metals by micro-organisms, Say (1977) noted that the development of such resistance might be expected to involve a variety of physiological processes. These may result in the exclusion of the metal from the cell, binding the metal away from sites away from the enzyme activity by components such as the cell wall, or binding by intracellular components. All of these processes may be expected to have a profound effect on the net uptake of metals by the plant. It therefore seems suprising that the possible occurrence of metal-resistant ecotypes at high concentrations of metals in the field appears to have been largely ignored during field studies of accumulation by submerged plants.

In light of the broad types of mechanisms of resistance mentioned above, it is clearly not possible to state whether a metal-tolerant strain of a particular plant might be expected to take up more or less of a particular metal than normal individuals of the same species. Examples of both situations have been documented by a few workers.

Brown & Bates (1972) reviewed the literature on the development of metal-tolerant strains of terrestrial plants, and suggested that tolerant ecotypes generally take up greater quantities of a particular metal by binding it to negatively charged groups on the cell walls Thus Turner & Marshall (1971) showed that cell walls isolated from zinc-tolerant plants of *Agrostis tenuis* accumulated greater quantities of zinc than cell walls from non- tolerant strains of the grass.

Stokes (1975) included some studies of the accumulation of metals during her work on copper- and nickel-tolerant strains of *Scenedesmus* and *Chlorella*. Tolerant cells were found to accumulate very large amounts of copper or nickel during growth, whilst non-tolerant cells accumulated much lower concentrations before death. During further studies using copper-tolerant strains of *Scenedesmus*, Mierle & Stokes (1976) noted that tolerant cells did not accumulate the metal across the plasmalemma during a two hour exposure to 0 1 mg l^{-1} Cu On the other hand, copper was transported in to the interior of non-tolerant cells where it was bound firmly (probably by sulphydryl ligands).

In contrast, some work with microorganisms has shown that the development of tolerance may involve the exclusion of the particular metal from the cell. Chopra (1971) compared the uptake of cadmium by cadmium-sensitive and cadmium-resistant strains of *Staphylococcus aureus*, and demonstrated a markedly decreased rate of uptake by resistant cells. About 40% of the cadmium taken up by the sensitive strain was found to reach a location within the cells, whilst uptake by the resistant strain was apparently limited to cadmium adsorbed adventitiously to the cell surfaces.

de Filipis & Pallaghy (1976a) have recently studied differences in the uptake of zinc and mercury by cells of *Chlorella* that had gained a 'physiological' resistance to the metals. They found that the development of resistance to zinc was accompanied by the inhibition of a temperature sensitive (probably 'active') component of uptake, and by a reduction in the number of exchange sites available in the 'free space' of the cell wall. Tolerance was therefore characterized by the development of a typical 'exclusion mechanism'.

1.7 NEED FOR FURTHER RESEARCH INTO THE EFFECTS OF HEAVY METAL POLLUTION IN FRESH WATERS

It is obvious from the above review that much work has been carried out on various aspects of the environmental and biological effects of pollution by heavy metals. The length of the review could easily have been doubled by the inclusion of related research on, for example, mercury and terrestrial angiosperms.

Despite this, it is clear that certain effects of the pollution of freshwaters by heavy metals have received little attention. It is evident from 1.2 - 1 4 that few environmental studies have investigated a single freshwater system in sufficient detail to clarify relationships between different fractions of water, sediments and submerged plants. Fewer still have combined such studies with investigations of the toxicity or accumulation of heavy metals by the organisms that can tolerate them within a particular system. It is clear, therefore, that such a study would be of use at the present time

Whilst it is widely accepted that certain terrestrial plants may only succeed in colonizing soils contaminated by heavy metals by the development of tolerant genetic strains, very little work has been carried out on the development of such tolerance by submerged plants. Similarly, several workers have suggested the use of data on the mineral composition of submerged plants in 'monitoring' pollution by heavy metals However, no comprehensive studies have been published of the composition of a single submerged species over a wide range of concentrations of heavy metals in the field. Further, the possibility that the development of genetic tolerance may affect the uptake of metals by 'monitor' species in the field has apparently not been considered. Similarly, little attention has been paid to the possible effects of other environmental factors on either the uptake of metals or their toxicity to submerged plants in freshwater systems

At the present time, therefore, a rather broad study of several aspects of the pollution of a relatively simple freshwater system by high concentrations of metals would be of value Such a study would be of further use if it concentrated on some of the aspects outlined above.

1.8 AIMS

Before the commencement of the project in September 1974, a series of exploratory surveys was carried out to select a field area for the proposed study (see chapter 3). Following this, it was decided that the broad aims of the project should be:

i. To survey the extent of contamination by zinc, lead and cadmium within the catchment area of the Derwent Reservoir. To carry out a detailed field study of the factors causing temporal and spatial variations in cancentrations and absolute levels of these metals in different fractions of stream and river water and sediments.

ii. To extend these studies to include analyses of selected submerged plants from the catchment, together with laboratory studies of the tolerance to heavy metals of a selected species if alga common in the area.

iii. To perform a similar study of the distribution of zinc, lead and cadmium within the water, sediments and submerged plants of the Derwent Reservoir itself. In light of the results of this study, to assess the extent of possible pollution together with estimates of annual additions of individual metals.

iv. To carry out a detailed field study of the accumulation of heavy metals by an alga sampled in connection with (ii) that has a wide range of tolerance to such metals in the field. To assess the influence of environmental factors and the possible development of resistance upon the use of the alga as a 'monitor' of pollution by heavy metals.

v. To carry out a detailed field and laboratory study of the resistance to zinc of a further species of alga sampled in connection with (ii). To document the extent of occurrence of enhanced resistance to zinc in this alga, and to assess the importance of certain environmental factors affecting the tolerance of zinc sensitive and zinc tolerant ecotypes.

CHAPTER 2

MATERIALS AND METHODS

2.1 DESIGNATION OF SITES FOR SAMPLING

2.11 Reaches on streams and rivers

In accordance with the computer-orientated recording system adopted at Durham University for the sampling of water, sediments and plants from flowing waters (Whitton, Diaz & Holmes, 1976), each stream or river sampled was allocated a permanent four digit stream number The name of each stream or river was usually taken from the appropriate large-scale Ordnance Survey map, but in a few cases names had to be created Such names are written with single quotation marks in this account.

The recording system allows flexibility in the choice of subunits to be used as the basis for study of a particular stream or river For example, designated 0.5 km lengths have been used as a basis for studies of macrophytic vegetation (e.g. Holmes & Whitton, 1977). For the purposes of the present project, however, all samples of water, sediment and plant material were collected from within designated 10 m reaches of the main flow of the stream. This subunit is referred to as the reach throughout this account.

Reaches were chosen to exclude any tributaries or major influences on the composition of the stream water, in a position far enough downstream of any tributaries to allow complete mixing. In each case, therefore, the volume of water entering and leaving the reach was approximately the same, and the composition of the water was comparatively uniform throughout the length of the reach. Occasionally reaches consisted of stretches of water (such as springs or adits) that entered another stream less than 10 m after emerging from the ground. In all other cases reaches were 10 m long.

Reaches were allocated a two digit number in ascending order downstream, thus the maximum number of reaches that could be designated along any one stream was 99. This was found to be sufficient even for the intensive survey carried out of a fairly large stream. Reach number 01 was only allocated to the first possible reach downstream of the source of the stream, if this was clearly defined (such as the emergence of a groundwater source or the confluence of two other streams) If the stream was more than 20 m long, the last possible reach before it terminated by entering another stream, river or lake was always numbered 99.

In allocating reach numbers along a stream, care was taken to leave numbers free for any reaches that might be sampled on a later occasion. Where a reach was to be the subject of regular study, its position was marked with a numbered wooden stake driven into the bank. Information on the location of reaches was stored in a central card index and computer file together with the following information:

i Stream number
ii Reach number
iii. Stream name
iv. Reach name
v. Map reference
vi Grid reference
vii. Map used for grid reference
viii. Tributary records · tributary of ., of ..., to sea
ix. Towns or landmarks near .., near ...
x County
xi. Altitude
xii. Width attributes (m). maximum, minimum, average

xiii Drop (m) along reach, including notes of any waterfalls

xiv Typical depth at medium flow (m)

xv Height, typical angle and vegetation on both banks

xvi. Direction of flow (N, NW, W, SW etc)

xvii Immediate land use

xviii Upstream influences upon water chemistry

xix. Substratum Wentworth broad size categories, with % breakdown of each size into types of substrata

xx. Light percentage areas of reach subject to varying degrees of shading more or less open throughout the year, moderate cover throughout the year, heavy shade throughout the year; moderate shade in summer; heavy shade in summer

2.12 Sites in the Derwent Reservoir

When water, sediments or plants were collected from the Derwent Reservoir during the first year of the project, the position of each sampling point was marked on a map of the reservoir and careful notes were made of the position of each point in relation to landmarks on the shore.

During the second year of study a more systematic series of designated sites was worked out to provide a basis for subsequent samplinq. In most cases the samples collected during the first year were referred to these sites. The reservoir was divided into a series of numbered 200 m x 200 m squares, based on the 1 km square Ordnance Survey grid system

2.2 ANALYTICAL METHODS

2.21 Composition of water

2.211 Collection, fractionation and storage

Water was collected from immediately below the surface of the stream, river or reservoir being sampled. A 2 l polythene beaker was usually used for the collection of stream water Water from various depths in the Derwent Reservoir was collected in a Ruttner bottle.

During the exploratory surveys of the composition of stream water and during the sampling of water in connection with the sampling of *Stigeoclonium* (7.2), all water for analysis was filtered in a standard manner This involved passing the water through an acid washed no. 2 Sinta funnel (quoted pore size 40 - 50 µm) and collecting 100 ml of water in an acid washed Pyrex glass bottle. Considerable care was taken to standardize the filtration technique, with a further acid washing (with 6% HCl) being carried out in the field whenever it was suspected that the pores of the sintered glass might have become contaminated.

As a preliminary investigation of the distribution of metals between various fractions, and of the effectiveness of various filter types in removing various fractions, a detailed filtration experiment was carried out in the field. The following 16 water samples were taken twice from Bolts Burn reach 99 (carrying low and high levels of suspended solids) and once from reaches 05, 07 and 25 on the R. Derwent.

i untreated water sample taken in a Pyrex bottle directly from the main flow of the reach,

ii. 'total' water sample decanted into a Pyrex bottle after five minutes standing in a 2 l polythene beaker to allow larger suspended particles to settle out,

iii. samples passed through Sinta glass funnels, numbers 0, 1, 2, 3, 4 (quoted pore sizes 200 - 250 µm, 100 - 120 µm, 40 - 50 µm, 20 - 30 µm, 5 - 10 µm respectively),

iv. samples passed through Millipore membrane filters, pore sizes 0 1 µm, 0.45 µm, 1 2 µm, 3 0 µm,

v. samples passed through Nuclepore membrane filters, pore sizes 0 2 µm, 0.4 µm, 1.0 µm, 3 0 µm,

vi. samples passed through a Whatman GF-C glass fibre paper.

The membrane filters and glass fibre paper (all 25 mm diameter) were mounted in acid washed Swinnex plastic holders. Acid washed disposable plastic syringes were used to pass water through the filters, with 30 ml of double glass-distilled water and 15 ml of stream water being passed through and discarded before the collection of 25 ml of sample Membrane filters were not acid washed before use, because of the possibility of increasing ion-exchange phenomena on the filter surfaces.

All filtered samples taken during the fractionation survey were collected in acid washed 30 ml snap-top glass vials, previously tested for ion leakage by storing 'Aristar' grade HCl of varying strength in vials for varying periods. During the fractionation survey, and on all subsequent occasions, sufficient 'Aristar' HCl (in practice two drops per 25 ml) was added to all samples for cation analysis to lower the pH below 1 0 This addition was carried out in order to minimise microbial activity, to prevent precipitation, and to increase the proportion of ionic species present. Four blank samples (double distilled water plus 'Aristar' HCl) were included with the fractionation survey and demonstrated that the addition of acid added no detectable zinc, lead or cadmium to the samples

Before the commencement of the programme of analysis of metals in stream and reservoir water, checks were made for possible interferances during atomic absorption determinations of heavy metals. serial dilutions were made of water from the four key reaches, and additions were made of relatively high concentrations of elements including Na, Mg, Fe and Al No interferances were detected, even at the highest concentrations of elements or suspended material that were encountered during the project

On the basis of the results of the fractionation survey, all subsequent collections of water included three separate samples for cation analysis, namely a 'total' sample and samples passed through a no. 2 Sinta funnel and a 0 2 µm Nuclepore membrane filter.

Throughout the project, samples for cation analysis were passed through acid washed no. 2 Sinta funnels and collected in three 300 ml heavy-duty polythene bottles One of the bottles (used for the analysis of phosphate) was impregnated with iodine to minimise bacterial growth Where laboratory pH, Eh and total alkalinity were to be measured, unfiltered water was collected in a further polythene bottle, filled underwater to avoid any change in gaseous content.

Water samples were transported back to the laboratory in an ice box or portable refrigerator, and stored in the dark at 4^{o}C. Analyses for trace cations, pH, Eh, total alkalinity and conductivity were carried out as soon as possible on return. Wherever possible anion deterimations were carried out within 48 hours of collection, but where longer delay was unavoidable the polythene bottles were stored at -20^{o}C.

2.212 Chemical analysis

Cations

All cation determinations were carried out by flame atomic absorption using a Perkin-Elmer 403 Atomic Absorption Spectrophotometer Lead and cadmium were determined using the Tm sampling boat procedure (Kahn *et al.*, 1968), which allowed lower levels to be detected than by direct aspiration. All 'total' and filtered samples for cation analysis were shaken thoroughly immediately before aspiration. The levels of 14 cations (Na, K, Mg, Ca, Zn, Cu, Mn, Fe, Al, Pb, Ni, Co, Cd, Ag) were determined on most occasions, although analyses for Ni, Co and Ag were only introduced following the first year of study.

Anions

Levels of Cl, Si and NH_4-N were determined using colourimetric procedures taken from *Standard Methods for the Examination of Water and Wastewater*, 13th edition (American Public Health Association, 1971) NO_2-N was determined by the method of Crosby (1967), NO_3-N by the method of Montgomery and Dymock (1962), PO_4-P by the hexanol extraction procedure of Mackereth (1963), and SO_4-S by titration against barium perchlorate following the removal of interfering ions by cation exchange (Colsen, 1963) Optical density measurements for the colourimetric procedures were made on a Uvispek spectrophotometer. Fluoride was measured directly using an Orion specific fluoride electrode

Other parameters

The current speed of the water in the reach being sampled was determined using an OTT current meter. Measurement was made at the fastest accessible point in the reach Where the position of this point was in doubt six separate measurements were taken and the fastest reading selected. The propellor of the current meter was positioned in approximately one third of the depth of the water at the point of measurement

Discharge was measured at the time of sampling in four reaches on Bolts Burn and the R. Derwent by means of calibrated plates installed by the Northumbrian Water Authority Where discharge could not be measured quantitatively, subjective estimates (on a 1 - 5 scale) were made of the state of flow of the stream or river at the time of sampling and over a three-day period prior to sampling.

Temperature was measured in the field using a standard laboratory thermometer, previously cross-calibrated against several similar thermometers. pH was measured in the field using a Pye Unicam portable pH meter No. 293, and in the laboratory using an E.I.L. 23A direct reading pH meter. Total alkalinity was measured as mg l^{-1} $CaCO_3$ using the potentiometric titration method recommended by the American Public Health Association (1971). Titration was carried out in the field during the final year of study. Electrical conductivity was measured using a Lock Portable Transistorised Conductivity Bridge (3CL), and optical density (240 nm, 254 nm, 420 nm) was measured directly on the Uvispek spectrophotometer in 400 mm silica cells

2.22 Composition of sediments

2 221 Collection, sieving and storage

Samples of stream and river sediment were collected from within designated 10 m sampling reaches by wading, from parts of the stream bed subject to faster current speeds. Care was taken to avoid areas of algal growth, or areas where material eroded from the bank of the reach might contribute significantly to the bottom sediment. Where sufficient areas of fine material were present, ten separate subsamples were collected and pooled together. Where this was impossible collection was occasionally limited to as few as four subsamples.

Excess water was drained from the sediment after collection, and the subsamples placed in a closeable, heat-resistant, heavy-duty paper soil sample bag for transportation back to the laboratory. No attempt was made to wash sediment samples, as it was thought that this might remove a proportion of any loosely adsorbed metals

Sediment from the bed of the Derwent Reservoir was collected by wading and aqualung diving When diving from a boat, a weighted line was lowered to the bottom and a minimum of four subsamples were collected from within a 3 m radius of the line. When working at greater depths in nil visibility, the diver held on to a 3 m 'tail' line attached to the weight. Samples were taken in acid washed polythene bottles by carefully scooping up the top 1 cm of sediment, again avoiding areas of algal growth or visible organic debris.

A series of samples of sediment was collected during the summer of 1975 from a dried mud crust that had been laid down during the previous winter at the inflow end of the reservoir. The crust, which formed a layer about 1 cm thick over rotting vegetation from the previous summer, was sampled by picking up a minimum of four small pieces at each site and removing any organic debris

All samples of sediment were dried for 48 hours at 105°C. Following this the sediment was loosened (if necessary) by gentle crushing in a pestle and mortar, and passed through an acid washed 80 mesh (<210 µm) plastic sieve as used by several other workers (e.g. Agemian & Chau, 1976 and the Applied Geochemistry Research Group, Imperial College). Hawkes and Webb (1962) showed that the <80 mesh portion of a sediment provides the greatest contrast between anomalous and background samples Sieved samples were stored in acid washed snap-top vials until analysis. Before weighing out for digestion the samples were dried for a further 48 hours (in the glass vials) and cooled in a desiccator.

2.222 Acid digestion and analysis

Many different methods have been described in the literature for the determination of the mineral composition of freshwater sediments. At the commencement of the project, however, there appeared to be little information available as to the suitability of various methods for different applications. A brief review of the more commonly used techniques has therefore been included here

In view of the analytical facilities available, only those methods which lead to the preparation of a solution of cations extracted from the sediment sample (which can then be analysed by atomic absorption spectrophotometry) were considered for use in the present project. Solution preparation methods are of two general types.

i. A certain proportion of the metals and other elements present in a sediment can be removed from the sample by leaching for a specified period at a known temperature with a relatively weak extractant such as dilute hydrochloric acid, acetic acid or a chelating agent such as EDTA. The loosely bound cations that are released by such methods are sometimes referred to as 'available' (e g. Welsh & Denny, 1976) because it is thought that they give a better estimation of ions available for uptake by plants than the higher concentrations released by stronger extraction methods. The amount of each element brought into solution depends to a considerable extent upon the type and strength of extractant chosen (Allen, 1974).

ii Several more severe extraction techniques can be employed to bring a larger proportion of the elements present in a sediment sample into solution Such methods themselves fall into two main groups

First, the sample can be fused at high temperatures (often in a platinum crucible) with a compound such as sodium carbonate, sodium hydroxide or potassium pyrosulphate. Such methods are sometimes referred to as 'total' extraction techniques, as the whole sample (including silicates) can be brought into solution. Apart from the relatively long periods taken to process samples using fusion techniques, a major disadvantage is that large concentrations of salts are introduced to the final solution. This can cause instability and lead to high instrument background readings (Agemian & Chau, 1976).

Second, the sample can be digested using one or more strong acids. Such methods provide a high degree of metal extraction, but do not dissolve silicates completely unless hydrofluoric acid is employed. They destroy organic matter, dissolve all precipitated and adsorbed metals and leach out a certain amount of the metals from the silicate lattice (Agemian & Chau, 1976). For the purposes of the present project, major advantages of such digestion techniques were the speed with which samples could be processed, and the high degree of purity of the available Analar grade concentrated acids Different workers have used different digestion techniques, for example Benoit, Cairns & Reimer (1968) used a mixture of concentrated nitric and sulphuric acids, whilst Davies (1971) used a mixture of hot nitric acid and aqua regia. Allen (1974) recommends digestion with hydrofluoric and sulphuric acids for complete sample breakdown, and with perchloric, nitric and sulphuric acids for removal of metals not incorporated in the silicate lattice.

As the main aim of the sediment surveys carried out during the project was to detect variations in heavy metal content between sites, it was not of critical importance to extract the entire metal content of sediments as long as all the samples were treated in an identical manner. As a proportion of the metals encountered were likely to be present as discrete particles of ore, however, the ideal method would be strong enough to bring these into solution.

The method chosen was an acid digestion technique using concentrated nitric acid alone. A comparison of the amounts of zinc, lead and cadmium extracted by this technique with the levels extracted by the perchloric-nitric-sulphruic acid digestion recommended by Allen (1974) showed that whilst recoveries of zinc and cadmium were very similar, higher levels of lead were extracted by concentrated nitric acid alone This was probably due to the formation of insoluble lead sulphate by the sulphuric acid in the mixed acid procedure Examination of the results of Agemian & Chau (1976) shows that for the extraction of heavy metals nitric acid digestion can compare favourably with procedures employing hydrofluoric or perchloric acids

The digestion technique involved the weighing (to four decimal places) of 50 mg of sieved, dried sediment into an acid washed 100 ml Kjeldahl flask, followed by the addition of 5 ml of Analar concentrated nitric acid. The digest was then boiled slowly for 30 minutes on an electric heating rack. At the end of this period the digest was washed into an acid washed glass centrifuge tube with double distilled water and centrifuged for five minutes at 3500 r.p.m . The supernatant was then decanted into a 50 ml volumetric flask, the precipitate resuspended in double distilled water and centrifuged for a further five minutes. The second supernatant was then added to the first and the whole made up to 50 ml after cooling to 20°C. As the concentrations of some elements (notably iron) were sometimes very high in the digest solution 25 x dilutions were made of all digests to facilitate analysis. As the background levels of various elements were found to vary between different bottles of Analar nitric acid, four blanks (processed in the same way as the digests, with the ommission of sediment) were included with each batch of digests. All digest solutions were stored at 4°C in acid washed snap-top glass vials until analysis

Sediment digest solutions were analysed for Na, Mg, Ca, Zn, Cu, Mn, Fe, Pb, Ni, Co, Cd and Ag by aspiration on the Perkin-Elmer 403 atomic absorption spectrophotometer using an acid-resistant nebulizer. The HNO_3-extractable concentration of each element (expressed as $\mu g\ g^{-1}$ dry weight) was then computed for each sediment sample.

2.23 Composition of plants

2.231 Collection, fractionation and storage

Lemanea fluviatilis

Lemanea is common in the R. Derwent, and in that river sufficient material was present for all collections of the alga to be made from within 10 m sampling reaches. Where reaches were created on other rivers during the survey of the mineral composition of *Lemanea*, the presence of sufficient material for sampling was included as one of the criteria for the designation of a 10 m reach.

Filaments were collected from the fastest accessible points in the reach by wading, except for the intensive sampling of reach 07 on the R. Derwent carried out during the spring of 1976 when sampling was restricted to a dense growth of filaments covering a 1 m^2 area of sandstone blockwork close to the shore.

For the purposes of the fractionation study of *Lemanea* filaments carried out using material from reaches 05 and 07 on the R. Derwent, 20 individual 8 cm long filaments were removed from each of the two reaches using stainless steel forceps. Each of the filaments was then washed briefly in double distilled water and carefully fractionated into four sections (measured back from the tip) 0 - 2 cm, 2 - 4 cm; 4 - 6 cm; 6 - 8 cm. The separated sections from each reach were then pooled together

Based on the results of this study, all samplings of *Lemanea* were made by collecting pooled 2 cm long filament tips (fractionated immediately after collection) and washing in river water On most occasions four separate subsamples of pooled filament tips were collected from four separate small areas within the reach, with each subsample consisting of sufficient material to comprise at least 25 mg dry weight During some of the specialised studies of metal accumulation sampling was restricted to a single sample comprising at least 50 mg dry weight.

All samples were shaken dry and transported back to the laboratory in acid washed snap-top vials in an ice box or portable refrigerator. A further brief washing was then carried out by placing the filament tips in an acid washed glass petri dish and covering with double distilled water. Unwanted filaments and invertebrates were removed at this stage. Filaments were then shaken dry, placed onto glass petri dishes, and dried for 48 hours at 105°C The dried samples were stored in acid washed snap-top vials until analysis.

Scapania undulata

Scapania forms dense mats attached to the substratum by rhizoids, from which green leafy shoots project into the surrounding water. Only completely submerged material was collected, with healthy 1 cm long shoot tips being fractionated and washed in the same way as *Lemanea* filament tips Dried filaments were ground before storage using an acid washed pestle and mortar

Nitella flexilis

Nitella was found growing in shallow water in the Derwent Reservoir, and was collected by wading. Similarly, material was collected by wading from a pool of water connected to reach 05 on the R. Tees. At each site four separate collections of the alga were made from within a small area, with each sample composed of several whole plants. At one site in the reservoir insufficient material was present to make up four subsamples, and collection was limited to a single relatively large sample

Plants were pulled up from the substratum and washed briefly in reservoir water. Great care was taken to avoid fracture of the delicate internodal cells in all stages of handling. Plants were shaken dry and transported to the laboratory in polythene bags in an ice box or portable refrigerator. The plants were then washed briefly in double distilled water, the rhizoids were removed and the plants were dried at 105°C for 48 hours. The samples were then ground and stored in the same way as samples of *Lemanea* and *Scapania*.

Glyceria fluitans

Glyceria was collected from near to the reservoir shore-line by wading. Four subsamples composed of several whole plants were collected from each site, choosing only completely submerged specimens. Leaves which were obviously dead were removed in the field, together with any loosely attached debris. The plants were washed in reservoir water before transportation to the laboratory, where they were washed in double distilled water and fractionated into the following three sections

i. 'Young' leaves, composed of short, green, actively growing leaf shoots which were usually incompletely unfurled;

ii. 'Old' leaves, composed of the characteristic long trailing underwater leaves of *Glyceria*,

iii. Stems, composed of short segments cut from the central portion of the submerged stem of the plants.

Samples were dried, ground and stored in the same way as *Nitella* plants.

2.232 Acid digestion and analysis

All stored samples of plant material were dried for a further 48 hours at 105°C and cooled in a desiccator. A maximum of 200 mg of material was then weighed out to four decimal places and placed in an acid washed 100 ml Kjeldhahl flask. Digestion in 5 ml of boiling Analar nitric acid was then carried out for 30 minutes. *Lemanea* and *Nitella* samples were found to pass completely into solution during the digestion period A small amount of insoluble residue remained after digesting *Scapania* and *Glyceria* samples and this was removed by the centrifugation technique employed with the sediment digests.

Digest solutions were made up to volume (either 25 or 50 ml depending on the weight of material digested) with double distilled water and stored in acid washed snap-top glass vials. Four blanks were included with each new batch of digests. Analyses for Na, K, Mg, Ca, Zn, Cu, Mn, Fe, Al, Pb, Ni, Co, Cd and Ag were then made by atomic absorption spectrophotometry using an acid-resistant nebulizer for aspiration.

2.3 LABORATORY STUDIES ON SELCTED SPECIES OF ALGAE

2.31 *Lemanea fluviatilis*

2.311 Factors influencing loss of zinc from filaments

Collection and preparation of alga

For the purposes of laboratory investigation of factors influencing the loss of zinc from filaments of *Lemanea*, single tufts of whole filaments (each comprising 0.2 - 0.4 g dry weight) were removed carefully from substrata within reach 07 on the R. Derwent. Tufts were taken from the faster flowing parts of the river, where silt accumulation amongst the filaments was minimal. The filaments were washed underwater and transferred underwater to 2 l polythene beakers During transportation to the laboratory the water in the beakers was maintained at a temperature close to that of the water in the reach (8°C) by placing the beakers in plastic bowls of river water In the laboratory a further brief wash was carried out using water from reach 07 that had been passed through a no. 2 Sinta funnel The tufts were then shaken dry and transferred to the flasks to be used in the experiment.

Experimental procedure

The flasks of media and solutions into which the tufts of *Lemanea* were prepared 24 hours before the commencement of the experiment, and equilibrated under the physical conditions to be used for incubation. 200 ml of each of the following solutions was placed into an acid washed 250 ml

Pyrex conical flask, with four replicate flasks of each solution

i. 'total' river water from the R. Derwent reach 05,

ii double glass distilled water;

iii. modified Chu-10 culture medium, as used for culture of *Stigeoclonium* (see 3.32),

iv. modified Chu-10 medium with EDTA chelating agent ommitted,

v solution of EDTA in double distilled water, at the same concentration (3.15 mg l^{-1}) as that in complete modified Chu-10 medium,

vi. solution of EDTA of half the above concentration,

vii. solution of 20 mg l^{-1} calcium (as $CaCl_2$) in double distilled water,

viii. solution of 20 mg l^{-1} magnesium (as $MgCl_2$) in double distilled water;

ix solution of 5 mg l^{-1} PO_4-P (as NaH_2PO_4) in double distilled water.

All of the solutions were adjusted to pH 6 6 using dilute HCl. This pH was somewhat lower than that of the R. Derwent reach 07 at the time of collection of *Lemanea* (pH 7 1)

The conical flasks were held in a shaking rack, partly submerged in a tank of water at controlled temperature (10°C) The flasks were illuminated from beneath with 6000 lx cool white fluorescent light. During the 24 hour equilibration period the flasks were closed with tight cotton wool bungs to minimise evaporation, but during the experiment they were left open to facilitate the removal of liquid.

A single washed tuft of whole *Lemanea* filaments was placed into each of the 36 flasks, which was then agitated to ensure complete submersion of the filaments and returned to the shaking rack Following this 0.5 ml aliquots of liquid were removed from each flask at regular intervals for 6 hours. The aliquots were placed into acid washed 5 ml Searle tubes which were sealed and stored in crushed ice until analysis. Zinc was determined by atomic absorption spectrophotometry within 10 hours of removal from the flasks At the end of the experimental period all *Lemanea* filaments were removed from the flasks, and the dry weight of each tuft determined

Based on the results of the zinc analyses, the time course of mean zinc loss from *Lemanea* filaments in the different solutions was calculated (as μg Zn lost per g dry weight of alga).

2.312 Tolerance to zinc of *Lemanea fluviatilis*

Although attempts to grow *Lemanea* in culture proved unsuccessful, an attempt was made in the laboratory to determine the levels of zinc causing death or partial death of whole filaments taken from selected field populations. The method used was a modification of the toxicity test used to assay the effects of zinc on *Stigeoclonium tenue*. The reason for choosing this semiquantitative procedure for assaying the effects of zinc on *Lemanea* filaments were as follows:

i. Estimates or measurements of growth were not made for *Lemanea* filaments during the assay, the effects of zinc being defined solely in terms of subjective observations of the health of individual filaments after varying periods. The semiquantitative tube assay enabled these observations to be made simultaneously on a large number of individual filaments.

ii. Although every effort was made to standardize the size of the individual filaments used in the test, the inoculum was probably subject to greater variability than would be desirable in a quantitative assay The use of boiling tubes counteracted this fault to some extent by enabling large numbers of filaments to be screened simultaneously.

iii. The use of the test enabled a certain amount of comparison to be made with the results of the toxicity testing of *Stigeoclonium tenue*.

Collection and preparation of alga

The six reaches chosen for the study of the tolerance to zinc of *Lemanea* were visited on four separate occasions for the collection of the alga On each occasion a single small stone covered in short (<5 cm) filaments was removed from the main flow of each reach and transported back to the laboratory in water from the reach of origin.

On return to the laboratory, a minimum of 40 short filaments were removed very carefully from each stone, rinsed in river water, and placed into 100 ml of modified Chu-10 growth medium in an acid washed 250 ml Pyrex conical flask. Each flask was then incubated for 24 hours under the physical conditions to be used during the toxicity test. These were· 10°C, moderate shaking; illumination with 6000 lx cool white fluorescent light. At the end of the pre-incubation period the filaments were rinsed thoroughly in further medium before transferring to the boiling tubes used for the assay.

Toxicity test

The toxicity testing procedure used for *Lemanea* filaments was based on that used for *Stigeoclonium tenue*. The main differences were a reduction in temperature (from 18°C to 10°C) and a modified method of subjective evaluation of the effects of zinc on the alga. The assay is described in detail in 2.32, and only a brief summary is given here. The sole object of the assay of *Lemanea* was to try and gain an indication of whether material from higher levels of zinc in the water could withstand higher levels of zinc under standard laboratory conditions than could material from low levels of zinc in the water

Following the pre-incubation period, filaments taken from each stone were placed into basal medium in a petri dish for the sorting of filaments to be used as innocula. The innoculum was made as uniform as possible, each consisting of a single, small, healthy filament about 4 cm long. Individual filaments fulfilling these criteria were removed carefully from the petri dish (using forceps) and placed into each of the 20 acid washed boiling tubes used in the toxicity test Each tube contained 10 ml of basal medium (see 2 32), made up to a range of zinc concentrations between 0 - 100 mg l^{-1} by the addition of $ZnSO_4$ $7H_2O$. Thus on each occasion following collection from the six reaches, six toxicity tests were carried out, each consisting of 20 tubes.

The tubes were placed (at an angle) into a metal cage within the shaking tank, so that the bottom of the tubes were partly submerged. The position of the tubes was randomized within the cage, to minimise the possible effects of differing light intensity at different parts of the shaking tank The tubes were then closed with Morton closures, and incubated for 10 days. The filament in each tube was examined on days 4, 6 and 10.

At the end of the incubation period, small aliquots of medium were removed from all tubes for the analysis of zinc by atomic absorption spectrophotometry. The initial pH of the medium in the tubes did not drift by more than 0.1 pH unit during the course of the assay, and no visible precipitation of zinc hydroxide took place in any of the tubes. Very little difference was found between the zinc content of aliquots taken from the surface of media in boiling tubes after standing, and that of shaking media.

Evaluation of toxicity test

On each occasion when the tubes were removed from the shaking tank, careful notes were made of the condition of each filament. No visible growth took place of any of the filaments during the 10 day period, and subjective observations were limited to the appearance of the filaments and the amount of growth of epiphytes Most of the observations could be summarized according to the following coded system

GG-	entire filament green and healthy,
GW-	region of white, dead cells noticeable in part of the filament (usually the tip),
WW-	filament completely white (dead).

A further suffix was added corresponding to the degree of growth of epiphytic algae, thus

GGE	filament has more than approximately 10% cover of epiphytes

2.32 *Stigeoclonium tenue*

2 321 Culture medium

The basal culture medium used for all laboratory assays of the effects of zinc on *Stigeoclonium* (and *Lemanea*) was based on the No. 10 formula of Chu (1942). The medium was modified from the original formula in particular by lowering the pH (see 2.323) and the level of phosphate. The reasons for choosing this medium were as follows

1. The medium enabled relatively rapid growth of *Stigeoclonium* to be maintained, whilst being sufficiently dilute to enable some comparison to be made with water from the reaches where alga was collected

ii. It seemed probable from a knowledge of the literature that the levels of certain chemical parameters in the culture medium (notably Ca, Mg and PO_4-P) would influence the toxicity of added zinc during toxicity tests In the original medium the levels of Ca and Mg were relatively low, and in the modified version the level of PO_4-P was also significantly reduced.

iii The results of Say (1977) had indicated that added zinc remained readily in solution in the medium for extended periods, enabling relatively high levels of the metal to be used for toxicity testing (see also 3.324). The medium contained a small amount of a chelating agent (EDTA) to further reduce the risk of precipitation of zinc and other metals

The basal medium, which was used for all assay and long term culturing purposes, was made up as follows

KH_2PO_4, 8 mg l^{-1}, $MgSO_4.7H_2O$, 25 mg l^{-1}, $Ca(NO_3)_2$, 40 mg l^{-1}, $NaHCO_3$, 8 mg l^{-1}, Na_2SiO_3, 10 mg l^{-1}, Fe (as ferric iron-EDTA chelate), 0.5 mg l^{-1}, 'C' microelements of Kratz and Myers (1955), omitting zinc, 0.25 ml l^{-1}.

The resulting concentrations of the major elements present in the basal medium were therefore (in mg l^{-1})

Na, 5.94, K, 2.25; Mg, 2 47, Ca, 9.77, Fe, 0.5; Si, 2.30, PO_4-P, 1.78, NO_3-N, 3.41, SO_4-S, 3.26.

Zinc was omitted from the microelement stock solution, and on two occasions analysis by atomic absorption spectrophotometry showed that levels of zinc in the medium were less than 0.002 mg l^{-1} Say (1977) reported a level of 0 004 mg l^{-1} Zn in the medium, probably as a result of trace levels of the element present in the Analar chemicals used to prepare the stock solutions. No difference was ever observed between cultures of *Stigeoclonium* grown in zinc-free medium and those grown in the lower levels of zinc added to the medium during toxicity testing. This indicates that the growth requirement of the alga for the element was satisfied by the trace levels present in the medium as a result of contamination.

As previously stated, a small amount of the chelating agent EDTA was added to the medium in order to avoid precipitation of zinc or any other metal during the toxicity tests. Several authors (e.g Gächter *et al.*, 1973) have shown that the presence of chelating agents in algal culture media may affect the solubility of added heavy metals (and thus possibly affect their toxicity). However, as the main purpose of the studies of the toxicity of zinc was to compare the resistance to the metal of different populations under standard laboratory conditions, it was decided to retain EDTA in the medium provided that the concentration used was always carefully standardized. Say (1977) demonstrated that increasing the level of EDTA in modified Chu-10 medium from 0 - 5 mg l^{-1} had little effect on the tolerance to zinc of an isolate of *Hormidium rivulare*.

Zinc was added to the culture medium as $ZnSO_4.7H_2O$, from a 1000 mg l^{-1} Zn stock solution in double distilled water. The results of the investigation of the effects of varying SO_4-S concentrations on the toxicity of zinc to *Stigeoclonium* (8.3) showed that increasing levels of the ion did not influence toxicity under the experimental conditions used.

During the investigations of the effects of varying chemical parameters in the medium upon the toxicity of zinc to populations of *Stigeoclonium*, the concentrations of different ions in the medium were altered by replacing their salts in stock solutions with a series of complementary salts.

These replacement procedures are described below together with the individual experimental procedures.

2.322 Physical conditions for culture

All toxicity tests were carried out in acid washed boiling tubes containing 10 ml of basal medium made up to a range of zinc concentrations All populations of *Stigeoclonium* maintained in long-term culture were cultured in 25 ml basal medium minus zinc (unless otherwise stated) in acid washed Pyrex conical flasks. All experiments were performed under controlled temperature (18°C) and light (6000 lx, cool white fluorescent light) with moderate shaking. Tubes were placed at an angle in a wire cage to increase the circulation of media during shaking, flasks were held in the shaking tank by clamping at the neck Tubes were closed with Morton closures, flasks with cotton wool bungs

2.323 Choice of pH for laboratory assays

For comparative purposes, it was hoped originally to carry out laboratory culture studies on *Stigeoclonium* at a pH value as close as possible to that of the water from which the alga was collected. However the mean field pH of the 35 collection sites (7.4) lay in the region where higher concentrations of zinc might be expected not to be in true solution (e.g. Hem, 1972), and some precipitation of $Zn(OH)_2$ might have been expected if the toxicity tests had been carried out at a pH value as high as this. An experiment was therefore carried out to investigate the effects of pH on the solubility of different levels of zinc (added as sulphate) in the basal medium. The experiment was extended to include study of the effects of calcium on zinc solubility at various pH values, and was performed simultaneously with the study of the effects of pH on the tolerance to zinc of two populations of *Stigeoclonium* (see 2 327)

Three sets of wide-necked 250 ml Pyrex conical flasks were set up, each set consisting of flasks with 100 ml of basal medium made up to a calcium level of 1 mg l^{-1}, 10 mg l^{-1} or 50 mg l^{-1} according to the procedure described in 2.327 Within each set there were 36 flasks, made up of four sets of nine flasks made up to zinc concentrations from 0 - 40 mg l^{-1}. Each set of nine flasks was adjusted to one of four pH values 6.1, 6 6, 7.1; 7.6 Adjustment was carried out (after the addition of zinc) using HCl or NaOH Thus within the experiment there were 108 flasks, containing basal media with all possible combinations of three calcium levels, nine zinc levels and four pH values.

The flasks were closed with loose cotton wool bungs (to permit gaseous exchange) and incubated for six days under the same conditions as used for the actual assays. Flasks were shaken twice daily, and the pH adjusted (if necessary) at daily intervals

At the end of the six day period each flask was shaken thoroughly and four separate 5 ml samples removed. These were:

an unfiltered sample,

a sample passed through a No. 2 Sinta funnel,

a sample passed through a 0.45 µm Millipore membrane filter;

a sample passed through a 0.2 µm Nuclepore membrane filter.

All samples were placed into acid washed Searle tubes with the addition of a drop of Aristar grade concentrated HCl to re-dissolve any precipitated zinc. The samples were then analysed for zinc using atomic absorption spectrophotometry.

The results of this experiment are considered in detail (together with the results of the assay of the effects of pH on the tolerance of *Stigeoclonium* to zinc) in 8.21. The experiment demonstrated that at pH 6.6 (at all three levels of calcium) a minimum of 92% of the highest level of zinc used (40 mg l^{-1}) was capable of passing through all of the filters. At pH values above 6 6, however, significant precipitation of $Zn(OH)_2$ took place Based on these results, all laboratory assays were carried out at pH 6.6. The pH of media in tubes and flasks was maintained by checking during the assay using a single probe pH electrode and buffering if necessary with HCl or NaOH

2.324 Resistance to zinc of *Stigeoclonium tenue* from a range of zinc levels in the field

Preparation of alga for assay

Samples of *Stigeoclonium tenue* were collected from as small an area as possible within the sampling reach such that there was still sufficient material to carry out tests It was usually possible to collect enough alga from a single clump covering about 1 cm^2 rock surface. Wherever feasible the alga was collected from the part of the reach with the fastest current speeds and with the minimum associated silt. Samples collected at one time from one small area are throughout this account referred to as one population, whether the whole sample was studied immediately or whether material from the sample was cultured for long periods.

The alga used for the standard toxicity tests was assayed within 48 hours from the time of collection in all instances except for the samples from France, Belgium and Germany. It was stored in water from the reach where it was collected until 24 hours before the test, when it was transferred to complete basal medium for the remaining 24 hours under the physical conditions to be used for the test. Samples of the alga were then removed for microscopy to check that there were no obvious algal contaminants. A small inoculum of alga was then added to each of the boiling tubes used in the assay. The inoculum was made as uniform as possible, only a few short erect filaments being present in each tube. Checks on similar aliquots of alga to those used for the tests indicated that in most cases the inoculum lay in the range 5 - 10 mg l^{-1} dry weight. Replication of several selected toxicity tests gave good reproducability using this inoculation method, which was found to be the only feasible method for inoculating *Stigeoclonium* within a short time of collection from the field.

Toxicity test

The assay used was a refinement of that described by Whitton (1970), and involved incubation of samples from each field population under standard laboratory conditions. At least 25 tubes were used for each toxicity test. As it became possible to predict the effects of zinc on a particular population, then the range of zinc concentrations used could be narrowed with a consequent increase in the accuracy of the assay. Inocula were added to the media to which any zinc had already been added, and the tubes were incubated under the standard conditions described above

Growth in the tubes was compared visually an days 2, 4 and 6, both against preserved replicates of the original inocula, and also with one tube against the others. A test was discarded if a marked increase in growth of the controls had not taken place by day 4, or if any algal contamination had become apparent. Otherwise, observations were recorded on each occasion according to the following (semi - quantitative) scale

I maximum concentration causing no inhibition of growth;

II minimum concentration causing slight inhibition,

III maximum concentration at which alga is alive;

IV minimum concentration at which alga is killed.

There was seldom any difference between the values observed for I and II on days 2, 4 and 6, in contrast to the observations on some other species made by Whitton (1970). This indicates that any slight inhibition of growth observed is due more to a reduction in the rate of exponential growth than to an increase in lag The values used for III and IV were in practice based on the observations made on day 6. Where there was any ambiguity about the results for III and IV after microscopic examination of the filaments, all filaments present in several tubes around the proposed values for III and IV were rinsed in basal medium and placed into further tubes containing 10 ml of zinc-free basal medium. Filaments were assumed to be dead if no growth had taken place in these tubes after a further 6 days culture under the conditions used for the assay.

Evaluation of toxicity test

As there were few indications from field observations as to which growth stages of *S. tenue* were the most sensitive to zinc, the data from the toxicity tests were presented in several different ways for later comparison with field concentrations of zinc. The following empirical formulae were used

just non-inhibitory = $(I.II)^{\frac{1}{2}}$

just lethal = $(III.IV)^{\frac{1}{2}}$

Tolerance Index Concentration (T.I.C.) = $(I.II.III.IV)^{\frac{1}{4}}$

2 325 Isolation of *Stigeoclonium tenue* for further study

Four different populations from each of two sites were selected for further study. These populations were from 'Hollingside Lane Artificial Stream' (0001-01), with the lowest mean field zinc concentration found (0.012 mg l^{-1}) and from Rampgill Level (0096-01), with a relatively high mean zinc concentration in the water (2.39 mg l^{-1}).

Cultures were isolated from each of the four populations from each site that had been used for the toxicity tests and maintained under similar growth conditions in 25 ml basal medium in 100 ml Pyrex conical flasks. Following the 24 hour preincubation period a single erect filament was removed from the clump of alga with forceps, washed several times in basal medium, examined for algal contaminants and placed into the flask of medium. Subculturing was carried out at frequent intervals, care being taken to avoid fresh algal contamination No attempt was made to maintain the cultures in a bacteria-free condition

2.326 Adaptation studies

Subcultures of each of the four different populations from the two reaches described in 3.325 were used in a study to ascertain whether the tolerance of the populations to zinc could be altered in any way by long-term culture in medium containing different levels of zinc. Each of the eight cultured populations were maintained for a period of six months both in zinc-free medium and in medium containing a level of zinc sufficient to cause moderate inhibition of growth. These levels of zinc were 0 75 mg l^{-1} in the case of the population from 0001-01 and 5.0 mg l^{-1} in the case of material from 0096-01. Subculturing of the 16 flasks was carried out at frequent intervals, care being taken to avoid cross-contamination. Standard assays were made at the beginning and end of the six month period

2.327 Effect of environmental factors on the tolerance to zinc of *Stigeoclonium tenue*

A single population from each of the two sites described above was used for a series of studies of the effects of various environmental factors on the tolerance of the two populations to zinc. Inocula were always taken from the same cultured population (of four) from each site. Based on the results of the original assay of the four populations from each site, the chosen population from 0001-01 is hereafter referred to as the zinc sensitive population, and the population from 0096-01 as the zinc tolerant population

The experiments outlined below all consisted of two separate series of toxicity tests carried out using inocula from the two cultured populations. Each series typically consisted of about 14 separate toxicity tests (i.e. about 340 tubes), with each test using medium with a different concentration of the chosen chemical parameter Tests were performed under the same physical conditions as those used for the standard assay. With the exception of the investigation of the effects of pH, all the experiments were performed at least twice. Results were summarized as 'just non-inhibitory', 'just lethal' and 'Tolerance Index concentration' (T.I C.). In all cases the results of the zinc toxicity tests were based on comparisons with the growth of the alga in controls with the particular level of ion under test. In the case of phosphate-free medium, the comparisons rest on observations of hair development

pH

An investigation was made of the effects of varying pH in the medium on the tolerance to zinc of the two populations. The experiment was performed at two different levels of calcium in the medium. Medium lacking calcium was prepared by replacing $Ca(NO_3)_2$ with $NaNO_3$ (to maintain the NO_3-N level). Using this medium, media containing 1 mg l^{-1} Ca and 10 mg l^{-1} Ca were prepared by the addition of $CaCl_2$. Each of these two media were then used for the preparation of eight toxicity tests, consisting of four tests using inocula from the two populations adjusted to pH 6 1, 6.6, 7.1 and 7.6. These pH values (obtained by buffering with HCl or NaOH) were chosen as being within the range at which most of the *Stigeoclonium* populations studied were found in the field The experiment therefore consisted of 16 toxicity tests, covering all combinations of four pH values and two calcium levels and repeated on two populations of the test alga. Each zinc toxicity test consisted (as with the standard assay) of at least 25 tubes of media with different zinc levels. Inocula were obtained from subcultures of the two populations that had been maintained in flasks of calcium-free/zinc-free medium at pH 6.6.

The pH of media in boiling tubes was checked at daily intervals by inserting a single probe pH electrode, and adjusted if necessary Some precipitation of zinc took place in tubes with the higher levels of the metal maintained at pH 7.1 and 7.6. The precipitate was re-suspended at daily intervals (by vigorous shaking) to ensure that the alga did not become smothered in $Zn(OH)_2$

The experiment was performed simultaneously with the investigation of the solubility of different levels of zinc at different pH values and levels of calcium in the medium (see 3 324). Using the results of this investigation, estimates of Tolerance Index Concentration were made for the pH studies based both on the total zinc in the medium and on the levels of zinc passing through filters at the various zinc concentrations.

Sulphate-S

During standard assays, the addition of zinc to the media resulted in a simultaneous increase in SO_4-S concentrations. Before attributing observed effects to the action of zinc alone, therefore, a study was made of the effects upon the toxicity of zinc of increasing concentrations of Na_2SO_4 in the growth medium, No detectable effect upon the tolerance to zinc of either population was found over the levels of SO_4-S added to the media during any of the tests. $ZnSO_4$ $7H_2O$ was therefore continued to be used as the source of zinc for laboratory assays.

NaCl

During the studies outlined below, the addition of the salts of the ions whose effects upon toxicity were being tested caused simultaneous addition of varying levels of Na or Cl A series of toxicity tests was therefore performed upon the two populations with varying levels of NaCl in the medium. No detectable effect of the salt upon zinc toxicity was found in either population over the ranges of Na or Cl concentrations added to the media during any experiments

Magnesium

Medium free of magnesium was prepared by replacing $MgSO_4$ with Na_2SO_4 to give an equilivalent SO_4-S concentration. Varying levels of magnesium in the medium were prepared (before the addition of zinc) by the addition of $MgCl_2$ Cultures to be used as inocula for the experiment were washed carefully in magnesium-free/zinc-free medium and maintained in that medium for 24 hours before the commencement of the experiment.

Calcium

Medium free of calcium was prepared by the replacement of $Ca(NO_3)_2$ with $NaNO_3$ to give an equivalent NO_3-N concentration Varying levels of calcium were added from a 1000 mg l^{-1} stock of $CaCl_2$ in double distilled water. Cultures to be used as inocula were maintained in calcium-free/zinc-free medium for 24 hours before the experiment.

Phosphate-P

Medium free of phosphate was prepared by replacing KH_2PO_4 with KCl to give an equivalent potassium concentration. Cultures were maintained in PO_4-P-free/zinc-free medium for 24 hours before inoculation. The experiment was repeated using cultures that had been preincubated for 7 days in this medium. The results of both experiments were very similar, and only the former experiment is considered in this account.

2.33 Taxonomy of *Lemanea* and *Stigeoclonium*

Both of the species of algae chosen for further study in the laboratory belong to the genera whose taxonomy is difficult. All of the populations of *Stigeoclonium* that were studied were referred to *Stigeoclonium tenue* Kütz, and no obvious morphological differences between populations were ever observed during culture under standardized conditions. However, a critical cultural study of the basal thalli was not carried out, so it is possible that some of the populations would have corresponded better to *S. pascheri* (Vischer) Cox & Bold in the study of the genus made by Cox & Bold (1966).

The taxonomy of the genus *Lemanea* is notoriously difficult. All of the populations sampled during the present study were referred to *L. fluviatilis* (L.) Ag.. Although wide variations in the morphology (colour, length of filaments, number and distribution of nodular growths, degree of branching etc.) were found between populations and within single tufts of sexual shoots, it seemed likely that these could be attributed largely to the "immense influence of certain environmental factors" on *L. fluviatilis* noted by Israelson (1944). However, no attempt was made to examine chantransial growths, to follow stages of development or to study spermatocysts by sectioning nodes. It is possible, therefore, that some forms would correspond more closely with *L. mammilosa* Kütz or *L. fusina* (proposed by Atkinson (1890) to include several forms including *L. mammilosa* and *L. rigida*). No plants were sampled during the study that appeared to correspond with *L. condensata* Israels (the only other species apart from *L. fluviatilis* recognised by Israelson (1944)).

CHAPTER 3

GENERAL BACKGROUND TO AREA OF STUDY AND EXPLORATORY SURVEYS

3.1 INTRODUCTION

At the commencement of the project several exploratory trips and surveys were made within the catchment area of the Derwent Reservoir The main objectives of these were to gain background knowledge of the physical characteristics and flora of the area of study, and an idea of the sources of heavy metals and their distribution between water, sediments and plants. It was also intended to choose species of submerged plants to be used in subsequent detailed studies of heavy metal toxicity and accumulation.

As the final choice of sites for sampling and plant materials for more detailed studies depended on a fairly detailed knowledge of the area chosen for study, this chapter contains diverse information on the physical characteristics of the area together with the results of the first surveys. A general description of the catchment area of the Derwent Reservoir is given in 3.2. A description of factors, including surface geology and mining activities, that might be expected to influence the distribution of heavy metals within the catchment area is then given in 3.3 The location of sampling reaches and the programme for the collection of samples during the exploratory surveys are described in 3 4. Following this in 3.5, 3.6 and 3.8 the results of the exploratory surveys of the composition of water, sediments and plants are presented. The results of a series of surveys of algal flora, designed to provide a general picture of the distribution of different species between different reaches, are presented in 3 7

3.2 GENERAL DESCRIPTION OF THE AREA OF STUDY

3 21 Introductory remarks

This section is concerned primarily with a description of the geographical and physical characteristics of the catchment area of the Derwent Reservoir. With the exception of published accounts of the geology of the area, the descriptions are based on personal observations and information provided by other people The Derwent Reservoir itself is considered before the R. Derwent and its tributary drainage.

3.22 Derwent Reservoir

The Derwent Reservoir is situated near Consett, Co. Durham, on the border between Co Durham and Northumberland (Fig 3 1). It was constructed between 1960 and 1966 by the Sunderland and South Shields Water Company, who own and manage the reservoir and its treatment works for the joint use of the Company and the Wear Division of the Northumbrian Water Authority (formerly the Durham County Water Board) The reservoir was formed by damming the R. Derwent near Edmundbyers, and when full to capacity (5.06×10^6 m^3) it is 5 6 km long with a surface area of 405 ha and a maximum depth of 30 m near to the dam (Fig. 3 2). Water is drawn off from points near to the dam for treatment at the Mosswood Treatment Works 4 km further down the valley of the R. Derwent. From Mosswood, water for the Wear Division of the Northumbrian Water Authority is pumped a further 3.2 km to a service reservoir at Castleside (near Consett) whence onward distribution is gravitational. The Derwent Reservoir supplies an average of 0 12 x 10^6 m^3 day^{-1} for public supply.

Recreational use of the reservoir includes a 'put and take' game fishery, with regular re-stocking with hatchery rainbow and brown trout. A large sailing club has been

Fig. 3 1

Map of the catchment area of the Derwent Reservoir

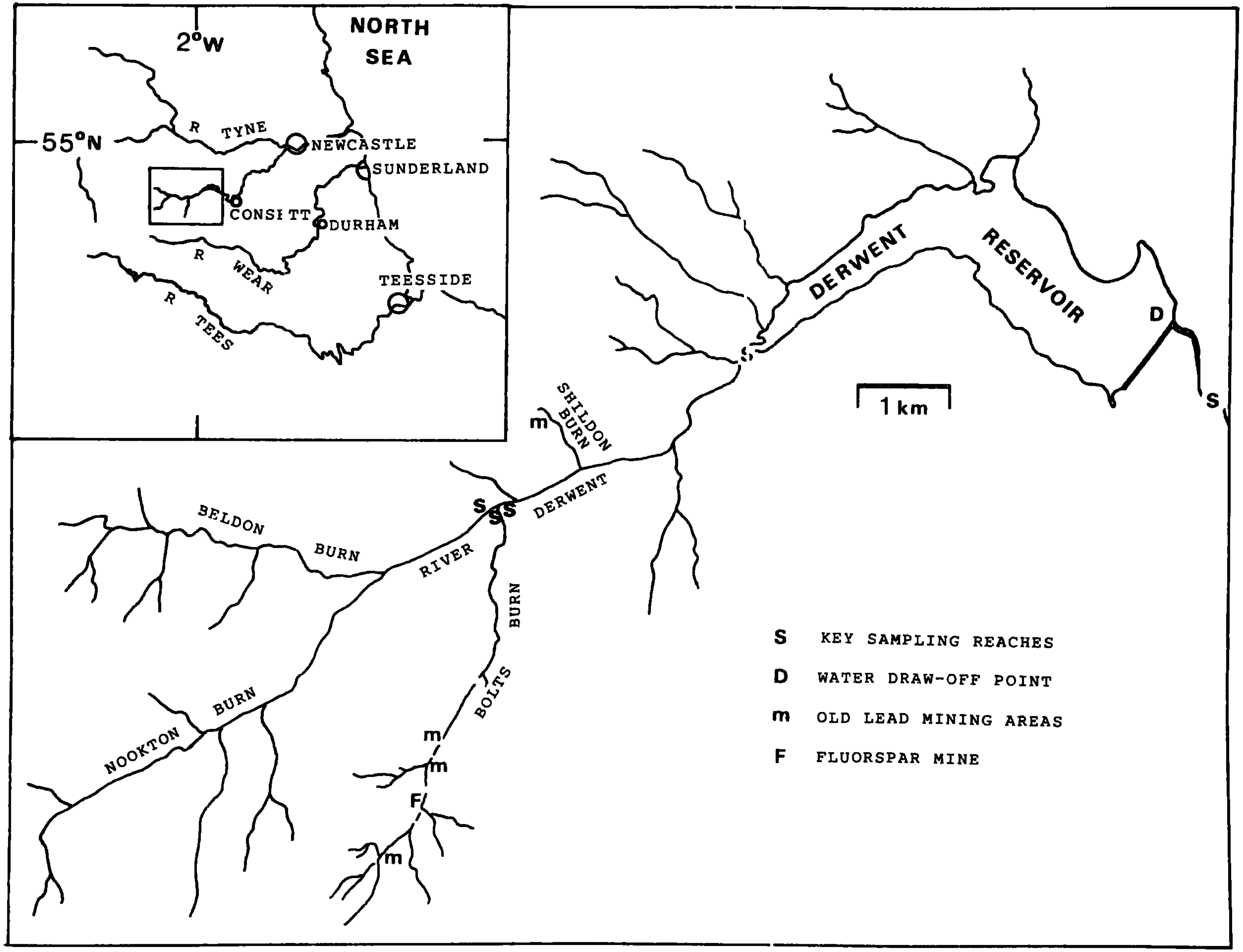
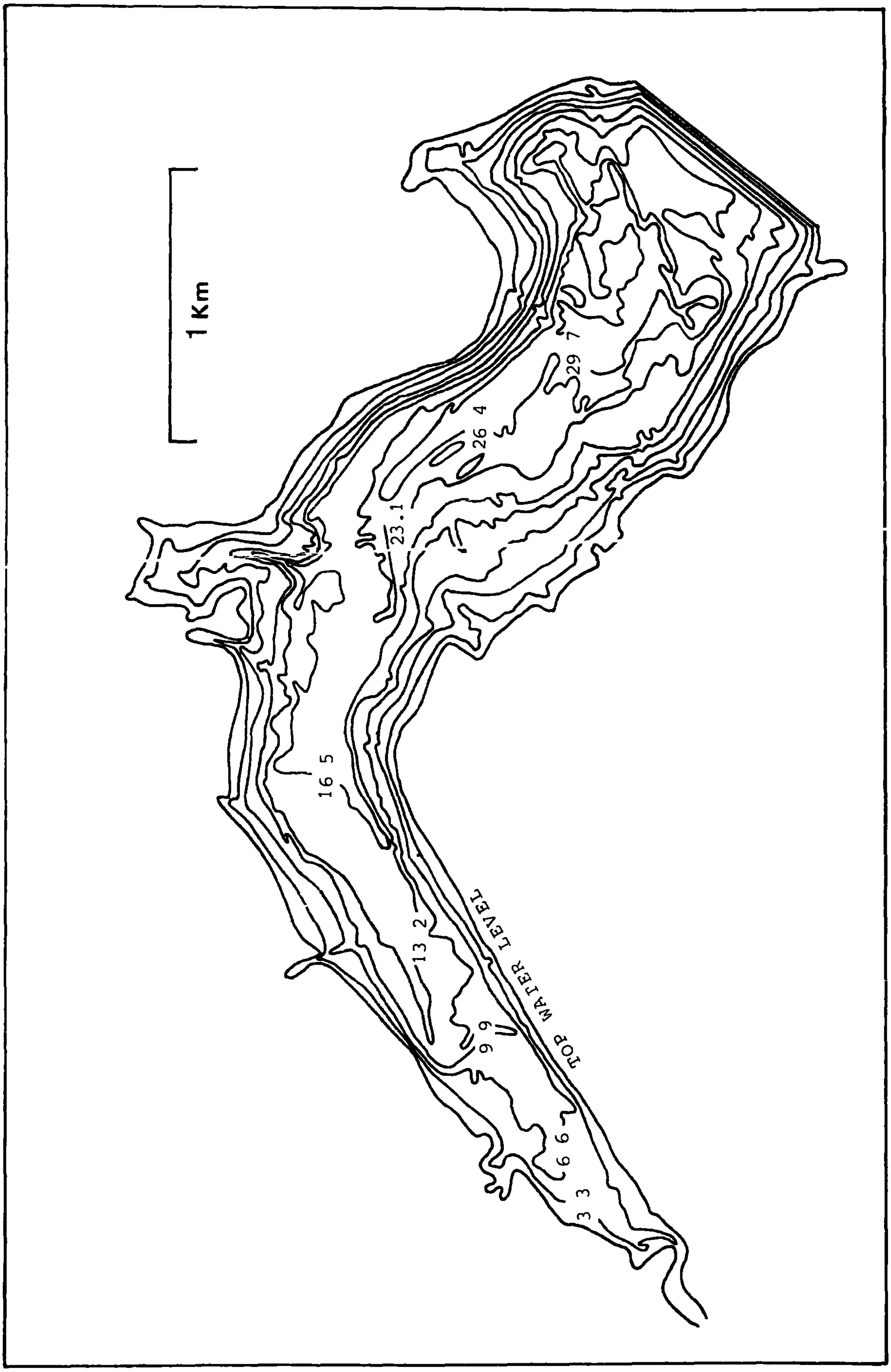

2°W
NORTH SEA
55°N
R TYNE
NEWCASTLE
SUNDERLAND
CONSETT
DURHAM
R WEAR
TEESSIDE
R TEES
DERWENT RESERVOIR
D
S
1 km
SHILDON BURN
m
DERWENT
RIVER
S
S S
BELDON BURN
BURN
BOLTS
NOOKTON BURN
m
m
F
m
S KEY SAMPLING REACHES
D WATER DRAW-OFF POINT
m OLD LEAD MINING AREAS
F FLUORSPAR MINE

Fig. 3.2

Depth contour map of the Derwent Reservoir
(all depths are in m below top water level)


1 Km
29 7
26 4
23.1
16 5
13 2
9 9
6 6
3 3
TOP WATER LEVEL

established on the north shore, and several landscaped picnic sites have been constructed around the shores for the use of visitors

A nature reserve has been established at the western end of the reservoir, below the inflow of the R Derwent The reserve comprises 60 ha of water and 82 ha of surrounding land It is managed by a committee including representatives from the Durham County Conservation Trust, the Northumberland Wildlife Trust, the Nature Conservancy Council, the Sailing Club and the Water Company with the main objective of providing a habitat attractive to birds Botanical records have also been kept of the developing plant communities within the reserve.

The level of water within the reservoir is subject to considerable fluctuations according to the incidence of rainfall, and the summer level may during dry periods fall by as much as 10 m below the overflow level. This leads to the exposure of large areas of mud around the mouth of the R Derwent in the nature reserve (Fig. 3 3). Some colonisation of this mud takes place during prolonged periods of exposure, with the development of a community of plants including *Gnaphalium uliginosum* L , *Agrostis stolonifera* L., *Juncus effusus* L , and *Juncus acutiflorus* Ehrh.

Submerged macrophytic plants are scarse within the nature reserve and elsewhere in the reservoir Species growing in shallow water include *Glyceria fluitans* (L.) R Br., *Glyceria maxima* (Hartm.) Holmberg (including a variegated form, probably a domestic introduction), *Nitella flexilis* L., *Callitriche platycarpa* Kütz, *Callitriche intermedia* Hoffm., *Hippuris vulgaris* L., *Iris pseudacorus* L , and *Polygonum amphibium* L..

Fig 3.3

Aerial photograph of the R. Derwent flowing into the western end of the Derwent Reservoir, in an area set aside as a nature reserve.

Note wide areas of exposed sediment left by the drawing down of the water level, and sparse vegetation beginning to colonise this sediment.

(reproduced with the permission of
Dr D. W. Harding)

3.23 R. Derwent catchment

The direct catchment of the Derwent Reservoir comprises 721 ha, most of it moorland, woodland and pasture around the R. Derwent and its tributaries. The R. Derwent is the largest body of flowing water feeding the reservoir, entering the nature reserve from the west

The R. Derwent is formed 5 km to the south-west of the reservoir at an altitude of *c.* 260 m by the confluence of Nookton and Beldon Burns These two bodies of water have quite large catchment areas extending over the surrounding fells, composed mainly of moorland and forest on Carboniferous sandstone Beldon Burn originates from waters draining Byerhope Moss, Quickcleugh Moss and Heatheryburn Moor. Nookton Burn is formed by streams draining Hunstanworth Moor, the north side of Redburn Common, and Nookton Fell. The height of most of the surrounding fells is approximately 490 m.

Nookton and Beldon Burns, and the R. Derwent downstream of their confluence, are shallow and fast flowing, with substrata composed mainly of sandstone boulders, cobbles and pebbles. 3.5 km downstream of the confluence of the two tributaries, Bolts Burn enters the R. Derwent from the south-west. This stream, the largest tributary to the river upstream of the reservoir, rises on Hunstanworth Moor as a series of flushes and small streams. (For the purposes of this account the source of Bolts Burn is taken as being a point immediately downstream of Burnhead Dam, which forms a small reservoir at the head of the valley)

The physical appearance of the valley of Bolts Burn has been much affected by past and present mining activities (these are described in more detail in 3 3). 3.5 km upstream of its confluence with the R. Derwent the stream flows through a large mine complex, the Whiteheaps

Mine, which is currently in operation for the mining and treatment of fluorspar. Downstream of the Whiteheaps area the stream is bordered by an extensive area of old lead workings and spoil heaps, and then for the last 1.5 km before it enters the R. Derwent the stream flows through a steep wooded valley over a series of coarse sandstone sheets and boulders.

Blanchland, a small village situated 2.5 km upstream of the reservoir, is the largest population centre through which the R. Derwent passes before it enters the reservoir. Shildon Burn, the second largest tributary entering the R. Derwent upstream of the reservoir, joins the river in Blanchland This stream rises on Blanchland Moor and flows through a wooded valley upstream of the village. Old mine workings are in evidence on both sides of the stream around Shildon village and 'Pennypie House'.

Downstream of Blanchland village the valley of the R. Derwent broadens out, and woodland and pasture border the river on both sides. Two more small tributaries (Stoney Burn and Hot Burn) enter the R. Derwent between Blanchland and the Derwent Reservoir.

3.3 FACTORS LIKELY TO INFLUENCE THE COMPOSITION OF WATER WITHIN THE AREA OF STUDY

3.31 Introductory remarks

This section consists of a brief description of some of the characteristics of the Derwent Reservoir catchment area that might be expected to exert major controls upon the composition of the water A short account of the major geological formations of the area is included, based mostly upon the published account of Dunham (1948) A detailed description of past and present mining operations within the catchment area is also included, as it was anticipated at the commencement of the project that mine workings would constitute the major source of any elevated levels of heavy metals. It was therefore felt that a fairly detailed knowledge of the location of veins of mineralization and their associated workings was necessary before the commencement of the exploratory surveys

3 32 Land use

The fell country that comprises most of the catchment of the R. Derwent is used mostly for forestry, rough grazing for sheep and grouse shooting The population density of the area is low, and there are very few inputs of treated sewage to the R. Derwent or its tributaries. A small sewage works serves the population of Blanchland, but even downstream of the village the R. Derwent carries only very low levels of inorganic phosphate and combined nitrogen in the water

Wide areas of peat occur in the catchments of Nookton and Beldon Burns, and the R. Derwent downstream of their confluence carries fairly high levels of brown humic material in the water This material remains in the water column of the Derwent Reservoir, and the transparency of the reservoir water is consequently rather low.

3.33 Rainfall

The high altitude of much of the catchment of the R. Derwent (around 500 m) might be expected to increase the frequency and duration of rainfall, as well as adding to its quantitiy (Millar, 1964). Figures provided by the Sunderland and South Shields Water Company give the annual rainfall within the catchment as 953 mm, of which 546 leaves the area as surface runoff

Daily records of rainfall (and snowfall) are made by the Water Company at a station near to the dam of the Derwent Reservoir On the basis of these records, monthly totals for the period of study have been calculated (Table 3.1). When these are compared with the annual rainfall figure quoted, it can be seen that rainfall was somewhat below average from 1974 - 1976. An interesting feature of the period chosen for the project was the very low rainfall of the winter of 1975/76, followed by further very dry weather during the 'drought' of the summer of 1976. The drought ended abruptly when 121 mm of rain fell between 9 - 12 September 1976. (The highest weekly total of rainfall previously recorded during the period of study was 53 mm.) When the monthly figures for rainfall are compared with changes in the level of water in the reservoir during the period of study (Fig. 5.2) a distinct seasonal pattern can be made out During all three years the level of water in the reservoir dropped steadily during the summer months, and increased during the winter. This could be attributed mostly to higher levels of precipitation during the winter, although increased loss of water through evaporation and transpiration no doubt decreases the amount of runoff entering the reservoir during the summer months. The water in the reservoir reached its lowest level (9 5 m below the overflow level) on 8 September 1976, and the reservoir overflowed for the first time during the period on 27 January 1977.

The R. Derwent and its tributaries are prone to sudden flash floods following heavy rains or periods of snow-melt. These were observed to be very localised on several occasions, for example, the main river could be in spate and Bolts Burn at low flow or *vice versa*.

Year	Jan	Feb	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov	Dec	Yearly total
1974	128	62	56	36	38	26	95	54	71	78	93	87	824
1975	80	26	73	49	69	46	64	90	74	24	43	25	663
1976	64	42	52	39	85	17	19	18	201	233	31	71	872
1977	134	85	84	56	47	75	18	65	11	-	-	-	-

Table 3.1 Monthly rainfall (mm) near the dam of the Derwent Reservoir during the period of study

3.34 Major geological formations

The surface rock formations in the area of study belong to the Upper Carboniferous Limestone Series (Fig. 3.4), which paradoxically consist here mainly of beds of sandstone and shale of great thickness These beds lie between the Crag Sill and Upper Felltop Limestone (Table 3 2), which outcrops as a narrow band in the upper parts of the valleys of Nookton, Beldon, Bolts and Shildon Burns. The deposits were laid down in delta channels during the Rogerley and Coalcleugh marine transgressions. All of the strata in the area are underlain by the Great Limestone and overlain by the Millstone Grit series which outcrops on higher ground, forming 'caps' to some of the fells.

3 35 Mineralization and old mining operations

The headwaters of the R. Derwent lie in the north-eastern part of the Northern Pennine Orefield, which has been described by Say (1977). Although the surface formations in the area lie mostly at considerably higher stratigraphical horizons than the beds (below the Great Limestone) in which most mineralization occurs elsewhere in the orefield, substantial oreshoots are present. Veins of mineralization extend as high as the Millstone Grit (Smith, 1923), and the Hunstanworth Veins, which proved to be the most important in the area, were probably discovered as outcrops in these beds. The development of oreshoots at such high horizons has been attributed by Dunham (1948) to the presence of the thick beds of Carboniferous Sandstone.

Lead mining in the area undoubtedly commenced in ancient times as small open-cast operations on vein outcrops. Little documentation has survived of the early history of the mines in the upper Derwent Valley, but local tradition asserts that the Healyfield Mine (downstream

Fig. 3.4

Surface geology of the R. Derwent catchment area

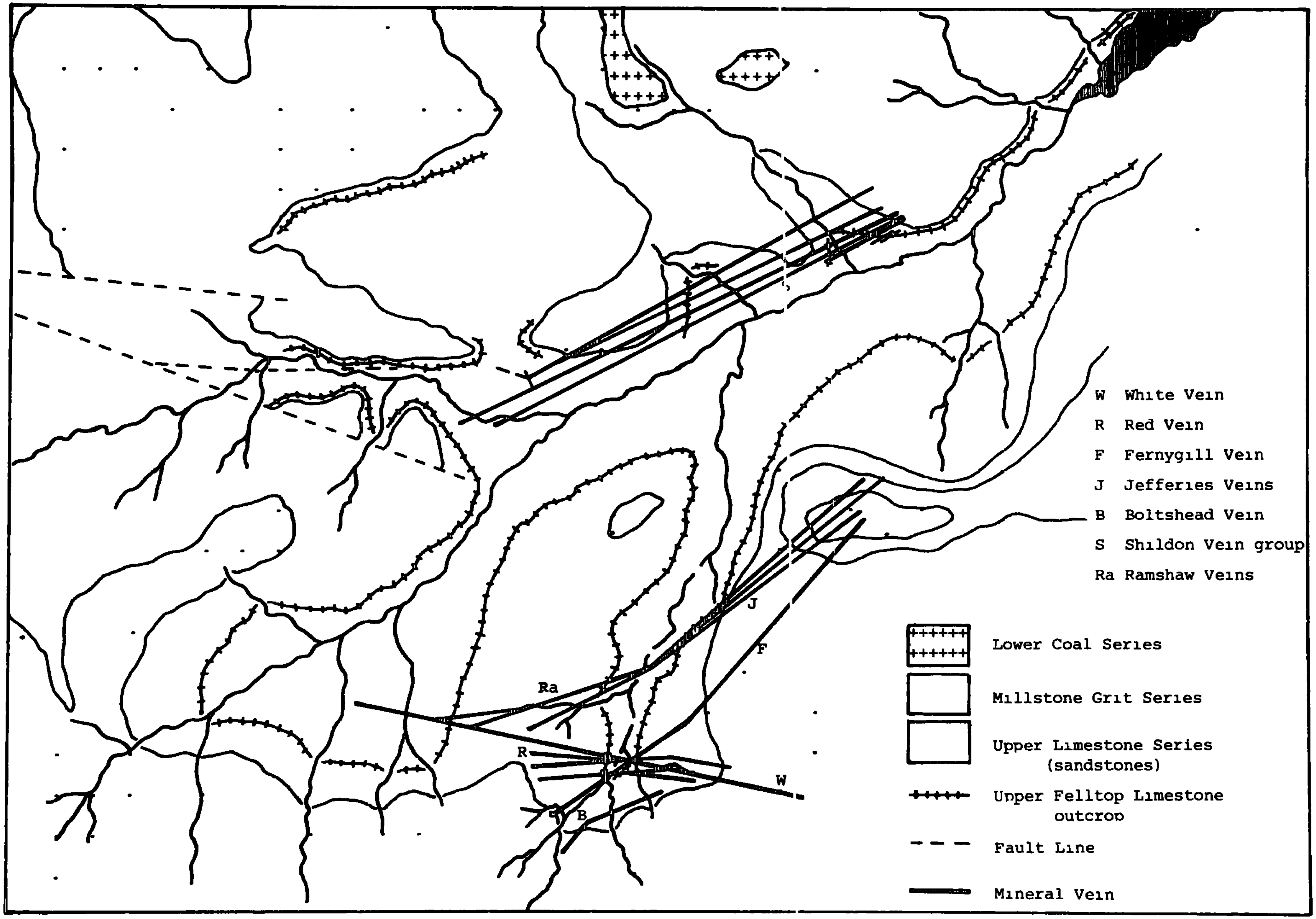

W White Vein
R Red Vein
F Fernygill Vein
J Jefferies Veins
B Boltshead Vein
S Shildon Vein group
Ra Ramshaw Veins
Lower Coal Series
Millstone Grit Series
Upper Limestone Series (sandstones)
Upper Felltop Limestone outcrop
Fault Line
Mineral Vein
Ra
R
W
J
F
B

Table 3.2

Section of strata at Shildon Mine (near Blanchland) in the R. Derwent catchment area

(after Smith, 1923)

	Thickness (m)
Sandstone, HIPPLE SILL (about 5 5 m beneath Felltop Limestone)	12.8
Shale	12.8
Sandstone, HIGH GRIT SILL	15 6
Shale, with coal and a bed of sandstone	9.0
Sandstone, LOW GRIT SILL	20.2
Shale	3.3
"Pebbles" (conglomerate?)	1.8
Shale, limestone and sandstone	2.9
Sandstone, CRAG SILL	7.9
Shale	8.4
Sandstone, PATTISONS'S SILL	12.3
Sandstone, "Whin Sill and Hazle"	12 3
Shale	12.2
Sandstone	0.9
Shale	4.1
Sandstone	1.1
Shale	9.7
Limestone, LITTLE LIMESTONE	3.9
Shale and coal	1 6
Sandstone, HIGH COAL SILL	2.1
Shale	3.7
Coal	0.9
Sandstone, LOW COAL SILL	2 4
Sandstone, "WHITE SILL"	6.6
Grey beds	0.2
Shale	1.8
Limestone, GREAT LIMESTONE	

of the Derwent Reservoir) dates back to early mediaeval times. By the end of the 16th century mining for lead and silver was the principal occupation of the scanty population in the area. The mines within the R. Derwent catchment were acquired by the London Lead Company about 1725 and worked by them until the end of the century. No records of lead production from the mines during this period have survived, in the only surviving mine report book of the company Thomas Dodd in 1806 urged that all the mines in the Derwent area should be given up "as there are no encouraging prospects in that country" The London Lead Company was nevertheless followed by several other concerns including Easterby, Hall and Company (1807 - 1810), the Derwent Mining Company (1810 - 1883) and Hunstanworth Mines Limited

The principal veins and abandoned mines which have been worked within the direct catchment of the Derwent Reservoir are described briefly below.

<u>Burntshieldhaugh Vein</u> (lead ore)

The only known workings on this vein (probably by the Beaumont Company) lie near the Devil's Water opposite Hackford Mill, 1 km north of Burntshield Haugh Farm. However the vein appears to be remarkably consistent, it is said to cross the R. Derwent near Newbiggin Hall where there are traces of old shafts in the North Bank It then extends into the Bolts Burn area where it has been worked at Jemmy's Shaft and further south-east at the Jefferies workings.

<u>Smith's or Pennypie Vein</u> (barytes)

A small vein carrying some barite, tried north of Pennypie House, 2.4 km north-west of Blanchland near Shildon Burn. The original shaft was sunk 42 m for lead, and re-opened to 8 m to extract barite in 1919.

Standalone, Fellgrove, Shildon, Old Shildon, Garden, Sluice, Gin O' The Wood and Andrew's Veins (lead ore)

These veins form a north-east trending belt about 0.8 km wide and 3.6 km long, in which they have been worked beside three of the tributaries of the R. Derwent, namely Beldon Burn, Reeding Burn and Shildon Burn. None of the veins appears to have carried particularly substantial oreshoots, and in no case was it possible to work a vein through from one valley to the next Three main mines worked these veins

i. **The Beldon Mine**

Two miles west of Baybridge (where Bolts Burn joins the R. Derwent), this mine consists of a pair of stonewalled shafts with a dump of veinstuff containing quartz, sphalerite and galena. Surface shafts suggest that workings extended for at least 500 m north-east of Beldon Burn.

ii. **The Reeding Mine**

This mine, in the valley of Reeding Burn, worked the Standalone, Fellgrove and Shildon Veins Old shafts associated with the mine follow the Shildon Vein for about 200 m south-west of the burn.

iii **The Shildon Mines**

These mines, which extend up the valley of Shildon Burn north-west of Blanchland, were probably the most productive of the three groups Even here the veins were said to be narrow. An adit started 520 m north-north-west of Blanchland Church and followed a sinuous course for over 920 m, but little other information on the area exists. The mines were last worked by the Derwent Mining Company, who carted the lead ore to Hunstanworth village (near Bolts Burn) for drossing

Ramshaw North, Middle and South Veins, Jeffereys North, Middle and South Veins (lead ore)

These veins were worked in the valley of Bolts Burn by the Ramshaw and Jeffereys Mines, the former lying south-west of Bolts Burn, the latter north-east of the stream The low lead content of the belt of veins would probably have made their exploitation impossible but for the fact that the lead ore was unusually rich in silver (0 28 g kg^{-1}) Records show that 40040 tonnes of lead concentrates (70 - 80% lead) were produced between 1845 and 1883, but total production for the two mines was probably over 100,000 tonnes. The dumps have yielded a small amount of fluorspar in recent years

3.36 Current mining operations

The only mine that is still active within the catchment area of the Derwent Reservoir is the Whiteheaps Mine near the head of Bolts Burn, at present operated by the British Steel Corporation for the production of fluorspar. This mine is described in detail because at the commencement of the project it seemed probable that it was a major source of elevated levels of heavy metals.

Mining for fluorspar

The veins worked in the Whiteheaps area are the White, Red, Shields, Company's and Fernygill Veins These form an intersecting network centered on the confluence of Bolts Burn and a small tributary, Sikehead Stream (Fig 3.4). The White Vein was the most productive of the group for lead mining, this vein continues eastwards from Whiteheaps to the disused Sikehead Mine workings at the head of Sikehead Stream. Although the vein is only sporadically mineralized with a very low lead content overall, in places it broadens out to as much as 10 m of purple fluorite and massive quartz, with narrow bands of galena that were easily taken out in narrow 'slits'. This process left large amounts of gangue in place at the completion of the old lead mining operations, and it is this material that has merited the continuation of workings for fluorspar.

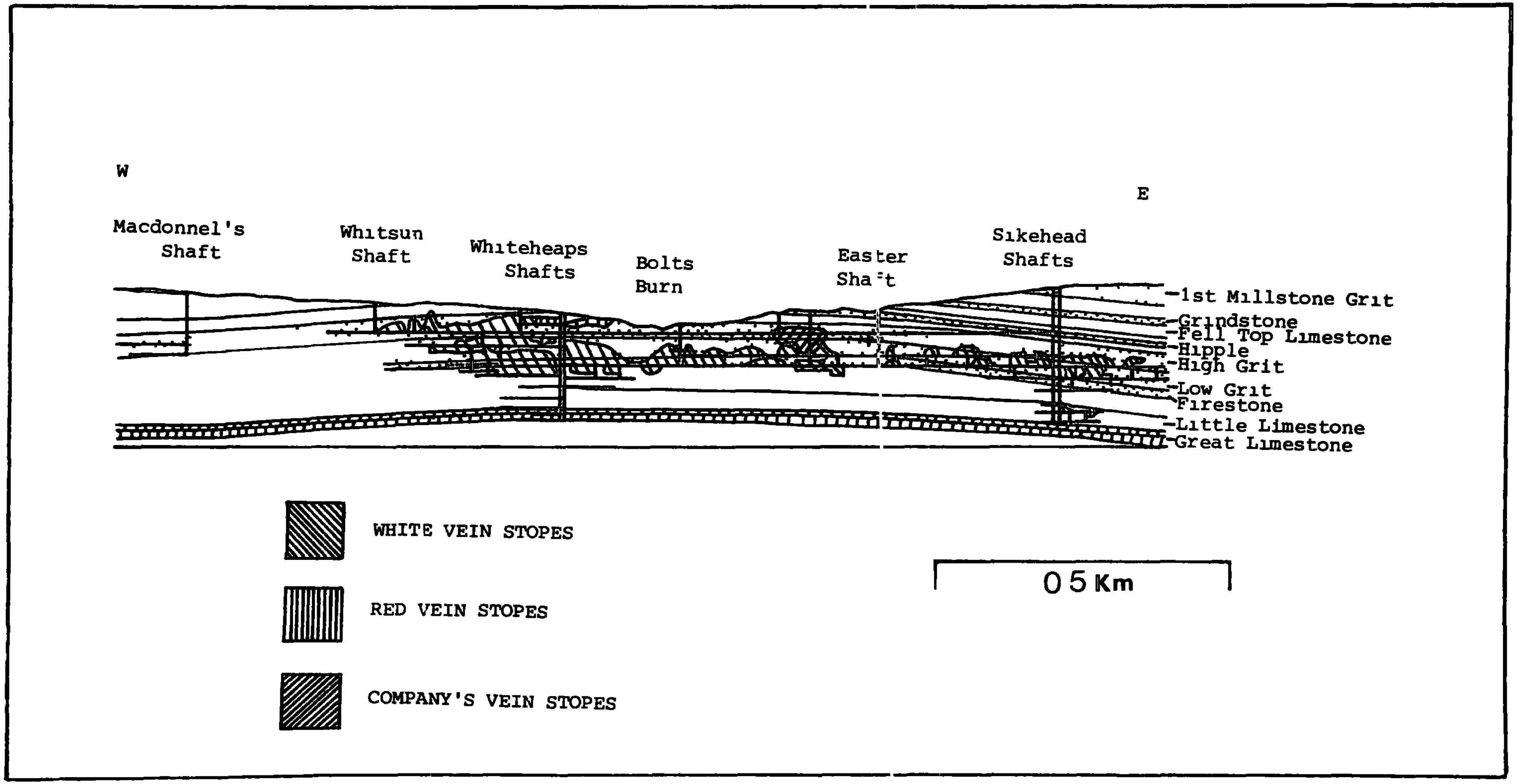
Extraction of fluorspar was commenced at Whiteheaps in 1924 by Hunstanworth Mines Limited, and was continued by that company until 1931 The present mining operations can be considered as starting in 1938 (Blanchland Fluor Mines), the company was then acquired by Colvilles Limited, who became part of the British Steel Corporation on renationalisation of the industry

Much of the gangue in the White Vein was considered too siliceous to work for fluorspar during the early operations, though the recent installation of treatment plants may have led to increased production from this vein On the Red Vein, however, which intersects the White Vein near Bolts Burn, wide bodies of high grade fluorspar were worked by the early concerns. Hunstanworth Mines Limited are said to have produced 15420 tonnes of fluorspar between 1924 and 1932, and Blanchland Fluor Mines obtained 5119 tonnes from a 3 7 m wide pillar left between two narrow 'slits' cut for lead ore in the Red Vein. The Hunstanworth Mines Ltd operations were mostly carried out above the 56 m level, however old mining records suggest that fluorspar might be present in large quantities from 73 m to as deep as 150 m.

By 1959 (Dunham, 1959) the Whiteheaps Shaft (sunk to the Great Limestone by lead miners in the 19th century) had been re-opened to a depth of 110 m, giving access to the Coal Sill Sandstones A new level had then been driven eastwards between the Red and White Veins, extending workings along the line of the section in Fig 3.5 as far as the Easter Shaft D. G. Elford (personal communication) states that workings have now been extended eastwards towards the Sikehead workings via a 1 in 4.5 incline which has now been driven to the 113 m level This should enable the Sikehead oreshoot to be worked between the

Fig 3.5

Section of strata and mine workings around the Whiteheaps and Jefferies Veins in the Bolts Burn valley (after Dunham, 1948).

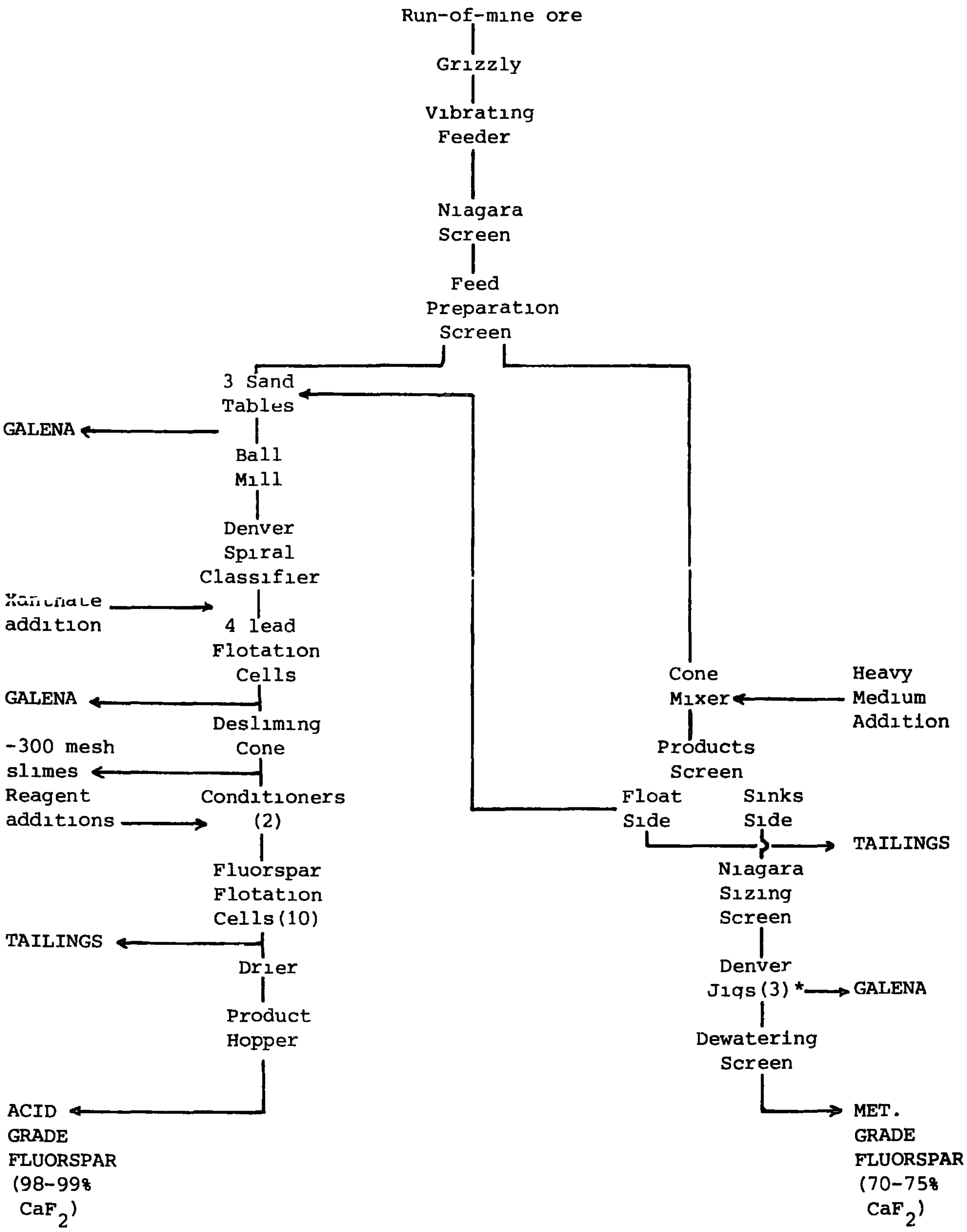

W
E
Macdonnel's Shaft
Whitsun Shaft
Whiteheaps Shafts
Bolts Burn
Easter Shaft
Sikehead Shafts
1st Millstone Grit
Grindstone
Fell Top Limestone
Hipple
High Grit
Low Grit
Firestone
Little Limestone
Great Limestone
WHITE VEIN STOPES
RED VEIN STOPES
COMPANY'S VEIN STOPES
0 5 Km

Great Limestone and the Firestone Sill, and future development could extend the incline still further to the Great Limestone. Output to date of the Whiteheaps Mine has been about 125000 tonnes of fluorspar.

Treatment of fluorspar

Apparently no fluorspar is being produced from workings at the mine at the time of preparation of this account (October 1977), although British Steel seem confident that the deepening of workings towards the Sikehead area will produce further economically viable deposits. The mine is obviously treating fluorspar on a regular basis, however, and the treatment works almost certainly deal with spar transported from other British Steel operations such as Groverake Mine in the valley of Rookhope Burn.

Fig. 3.6 is a flow diagram of the fluorspar treatment system employed at Whiteheaps. In essence the process involves screening followed by crushing and the separation of fluorspar by floatation. The galena separated during floatation is a quite valuable by-product, and is exported to Europe for smelting. Effluent from the treatment plants consists of a thick grey sludge of finely ground particulate matter (mostly fluorspar and siliceous matter with a small amount of galena) which is piped into a series of three settling ponds before being discharged into Bolts Burn. During the three year period of study the effluent was treated by adding a concentrated solution of ferrous sulphate, followed by sacks of powdered lime. These additions lead to the precipitation of a 'blanket' of ferric hydroxide, which brings down heavy metals by precipitation and adsorption, followed by settling out before the effluent was discharged. Slurry is dredged from the settling ponds at intervals and deposited onto tips within the mine perimeter



* - At present in process of replacement

Fig. 3.6 Material flows (simplified) at the Whiteheaps Mine

(based on information supplied by D. G Elford)

3 4 PROGRAMME FOR EXPLORATORY SURVEYS

Once the main physical characteristics of the area of study had been established, together with the location of areas of mining that might affect the distribution of heavy metals, a series of exploratory surveys was planned The main objectives of these surveys were

i. to locate the sources of any elevated levels of heavy metals entering the R Derwent and hence the reservoir,

ii. to provide an indication of the extent of zinc, lead and cadmium enrichment within water, sediments and plants of the reservoir and its catchment,

iii to provide a basis for the choice of reaches for subsequent more detailed surveys,

iv. to gain an idea of the distribution of species of benthic algae within the catchment, and to select species for more detailed studies of the effects of heavy metals,

v to carry out preliminary studies of the accumulation of metals by the species chosen.

The programme for sampling was as follows

3 41 Water

3.411 Collection from different reaches

During the first year of study (September 1974 to September 1975) eight surveys of the composition of stream and river water were carried out. The catchment of the R. Derwent was explored in detail during these surveys, and all major tributaries to the river were sampled at least once together with all known inputs from old and active mine workings For the purposes of these samplings nine reaches were designated on the R. Derwent to detect the possible influence of any unsuspected inputs of water (Table 3 3) The other reaches designated include eight on tributaries to the river other than Bolts Burn (Table 3 4), 21 on Bolts Burn (Table 3 5) and 14 on tributaries to Bolts Burn (Table 3.6) The locations of most of these reaches are shown in Figs 3 7 and 3 8. Sampling of water was restricted to the collection of a sample passed through a no 2 Sinta funnel, which could reasonably be expected to remove larger suspended particles, whilst allowing the passage of dissolved, colloidal and complexed metals

3.412 Mixing profile

It became clear at an early stage of the programme of exploratory surveys that large amounts of heavy metals were entering the R. Derwent from Bolts Burn. Before designating a reach downstream of the entry of the polluted tributary, the 'mixing profile' of water entering the river was investigated. A series of transects of samples of untreated water was collected at various points in the river for a distance of 125 m downstream of Bolts Burn to ensure that mixing was complete before the proposed site for reach 07. The samples of water were analysed for zinc only.

3.413 Fractionation study

Towards the end of the programme of exploratory surveys, a study was carried out to investigate the distribution of heavy metals between filtrable and non-filtrable fractions of water. It was also hoped that this study would enable filters to be chosen for use during subsequent collections of water Water from four reaches (R. Derwent reaches 05, 07 and 25, Bolts Burn reach 99) was collected and passed through 14 different filters of various types and pore sizes, together with the collection of 'total' and untreated samples Two separate fractionations

Reach	grid ref	site
01	NY 944492	R Derwent below confluence of Nookton and Beldon Burns
05	NY 957498	R Derwent above entry of Bolts Burn
07	NY 959499	R Derwent below entry of Bolts Burn
09	NY 965503	R Derwent above Blanchland, by playing field (above Shildon Burn)
10	NY 967504	R. Derwent below bridge at Blanchland (below Shildon Burn)
15	NY 975504	R Derwent above entry of Stoney Burn
20	NY 979510	R Derwent between Stoney Burn and Carrick's picnic site
25	NY 984516	R Derwent below bridge at Carrick's picnic site
30	NY 033512	R Derwent below Derwent Reservoir

Table 3.3 Position of 10m sampling reaches on the R. Derwent (stream number 0061)

Stream	reach	grid ref	
0074	99	NY 943492	Beldon Burn at confluence with R Derwent
0072	99	NY 944491	Nookton Burn at confluece with R Derwent
0071	99	NY 957498	Bolts Burn
0090	99	NY 958499	Reeding Burn at confluence with R Derwent
0124	50	NY 960510	Shildon Burn near old mine workings above Blanchland
0178	01	NY 965504	'Shildon adit' entering Shildon Burn in Blanchland
0124	99	NY 966503	Shildon Burn at confluence with R. Derwent
0110	99	NY 976504	Stoney Burn at confluence with R Derwent
0121	99	NY 983515	Hot Burn at confluence with R Derwent

Table 3 4 Position of reaches sampled on tributaries entering the R Derwent

REACH No.	GRID. REF.	SITE
20	NY 947464	Bolts Burn above Whiteheaps fluorspar Mine
27	NY 949466	Bolts Burn in mine, 5 m below entry of Sikehead Stream
28	NY 949467	Bolts Burn above Whiteheaps Adit Level
30	NY 949468	Bolts Burn below Whiteheaps Adit Level
35	NY 949471	Bolts Burn by settling ponds, above Whiteheaps effluent
37	NY 949472	Bolts Burn below entry of Whiteheaps effluent
40	NY 951474	Bolts Burn 150 m below Whiteheaps effluent
45	NY 952475	Bolts Burn above old adit in old lead mining area
47	NY 953475	Bolts Burn below old adit, above road
50	NY 953476	Bolts Burn 100 m below road
55	NY 954474	Bolts Burn 200 m above track
60	NY 955481	Bolts Burn near track, 50 m below small adit
65	NY 956484	Bolts Burn below open tip area, just below foot bridge
70	NY 957486	Bolts Burn by concrete bridge
75	NY 956487	Bolts Burn by old mine road, 90 m below tall pine tree
80	NY 956490	Bolts Burn after old road bends up to the right
85	NY 956494	Bolts Burn 5 m below rock outcrop, well into wooded part of valley
90	NY 957496	Bolts Burn before entry of calcareous flush, below open, straight stretch
92	NY 958496	Bolts Burn by cave, 45 m below entry of calcareous flush
95	NY 957497	Bolts Burn above Bay Bridge road, 50 m above fire beater shelter
99	NY 957498	Bolts Burn 5 m above its confluence with the R. Derwent

Table 3.5 Position of sampling reaches along Bolts Burn

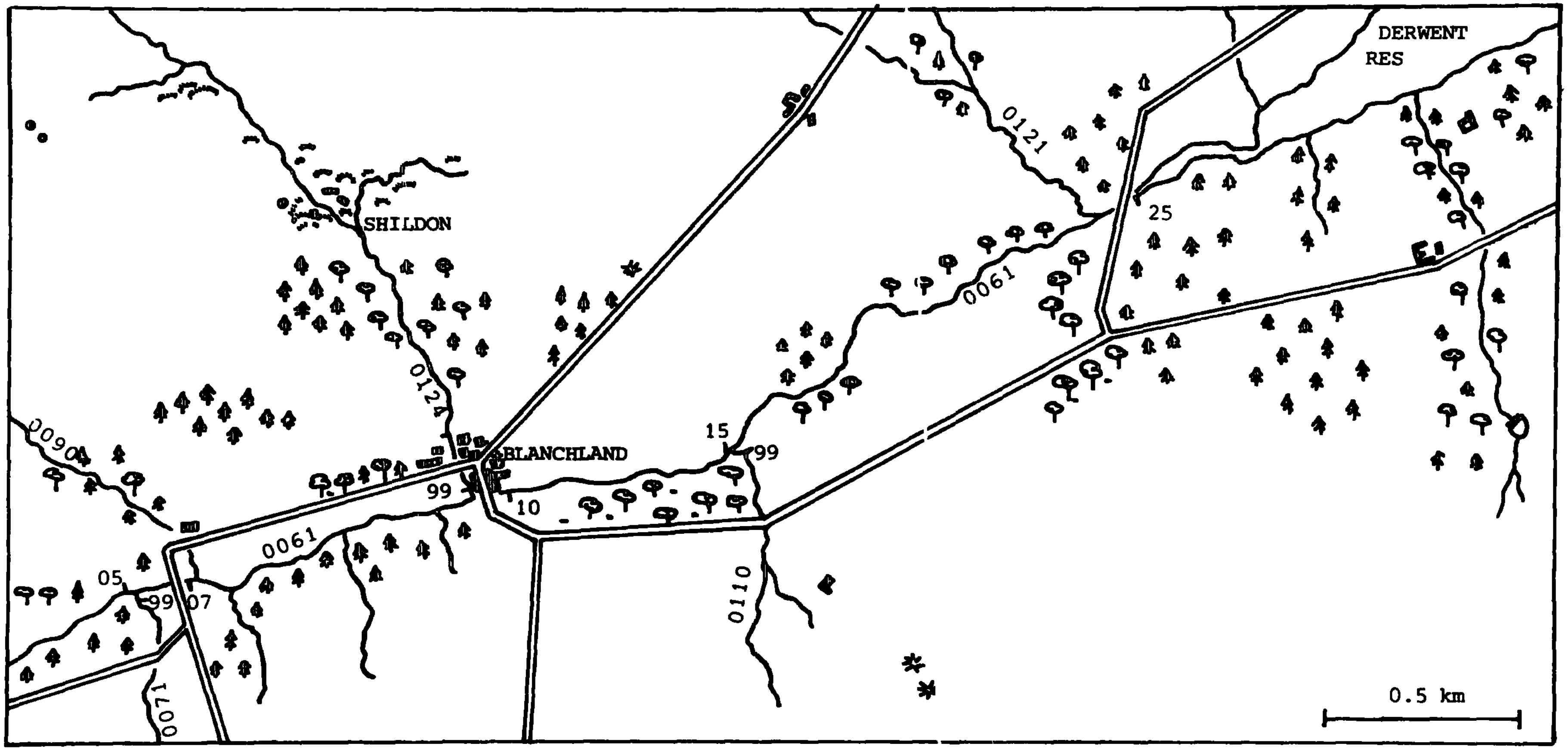
Stream	Reach	grid ref	Site
0067	99	NY 948466	Sikehead Stream entering Bolts Burn
0068	99	NY 949467	Whiteheaps Adit Level entering Bolts Burn
0069	99	NY 949471	Whiteheaps Mine Effluent
0088	99	NY 948496	'Bolts Burn Calcareous Flush'
0070	99	NY 952474	'Bolts Burn Road Adit'
0084	01	NY 955481	'Bolts Burn Track Adit'
0087	99	NY 956484	'Bolts Burn Upper Tip Tributary'
0177	99	NY 956484	'Bolts Burn Lower Tip Tributary'
0176	01	NY 956480	'Track Flush'
		NY 945462	Old Adit upstream of Whiteheaps Mine
		NY 949471	'Greenshaft Stream'
		NY 955481	Spring entering Bolts Burn at reach 60
		NY 957486	Small tributary entering Bolts Burn at reach 70
		NY 952474	Small tributary entering Bolts Burn at reach 43

Table 3 6 Position of reaches sampled on tributaries entering Bolts Burn

(stream and reach numbers have not been allocated to tributaries sampled once only during the exploratory surveys of the first year of study.)

Fig 3 7

Position of sampling reaches along the R. Derwent and its tributaries

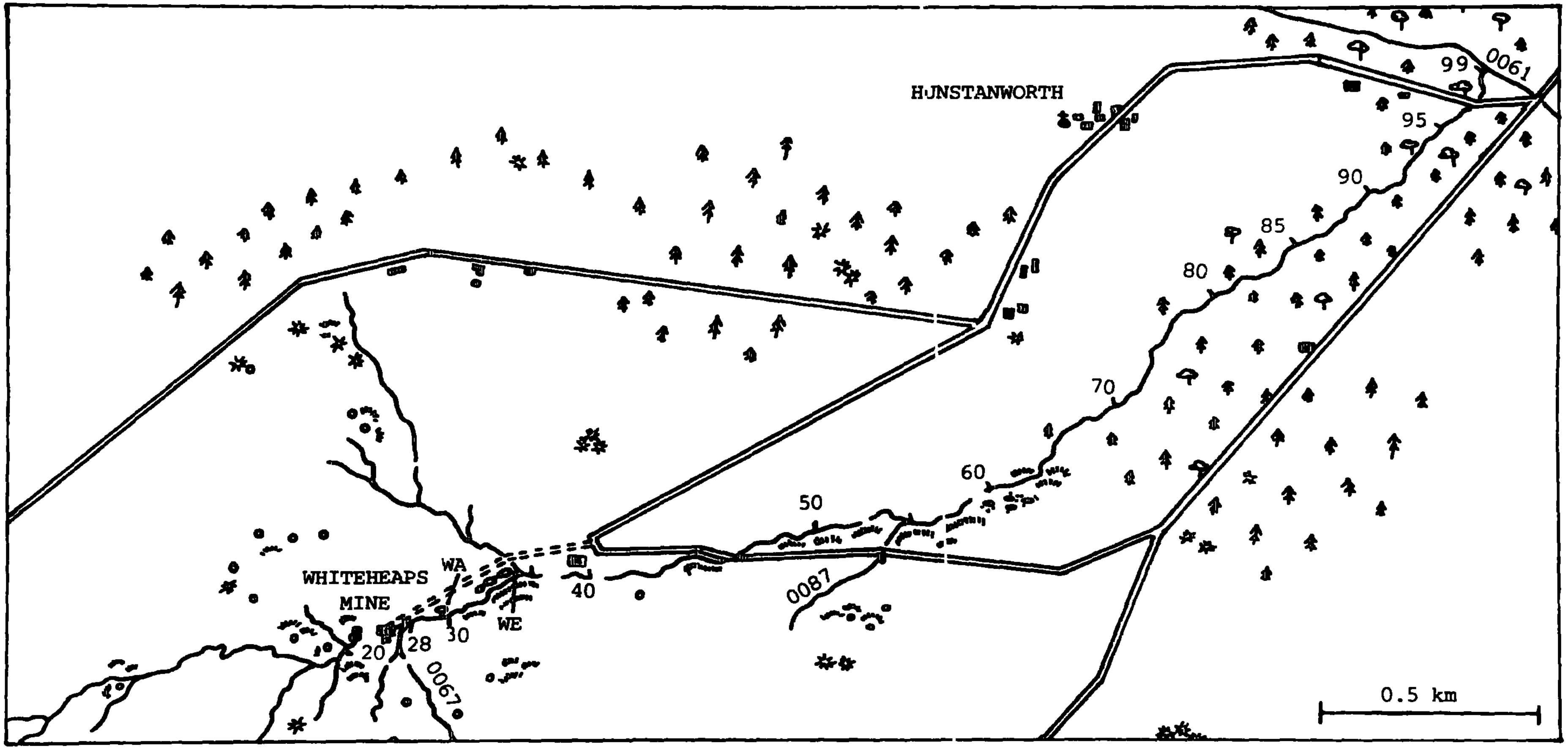


Key

0061	R. Derwent
0071	Bolts Burn
0090	Reeding Burn
0124	Shildon Burn
0110	Stoney Burn
0121	Hot Burn

Fig. 3.8

Position of sampling reaches along Bolts Burn and its tributaries



Key

0071	Bolts Burn	WA	Whiteheaps Adit
0061	R Derwent	WE	Whiteheaps Mine Effluent
0067	Sikehead Stream		
0087	'Bolts Burn Upper Tip Tributary'		

were made of water from Bolts Burn reach 99, as a marked rise in the amount of suspended solids carried by the stream water took place whilst the study was in progress.

3 414 Derwent Reservoir

On 6 May 1975 an initial transect to collect 'total' samples of water was made along the Derwent Reservoir Sampling was not extended into the nature reserve, as the nesting season for birds was in progress

3.42 Sediments

During the first year of study samples of sediment were collected from 19 reaches on the R. Derwent, Bolts Burn and selected tributaries In addition 43 samples of sediment were collected from different points (in shallow water) around the edge of the Derwent Reservoir.

All of the samples were digested using a mixed acid technique employing nitric, perchloric and sulphuric acids (Allen, 1974) The results indicated that this technique caused the precipitation of lead as the sulphate, however, and a more detailed comparison was made of the recoveries of heavy metals by this technique and by the technique employing nitric acid alone (see 2.222). Four replicate digests of sediment from reach 99 on Bolts Burn were made using each of the two techniques

3.43 Algal flora

It was not intended that the project should include a detailed investigation of the effects of heavy metals *in situ* on the communities of benthic algae growing in reaches on streams and rivers within the area of study It soon became apparent, however, that the diversity of species of algae was greatly reduced in certain reaches affected by heavy metal pollution as compared with non-polluted reaches A brief investigation was therefore planned of the number of species present at various reaches on the R Derwent and Bolts Burn. Some collections of algal flora from these reaches were made during the first year of study, but most of the collections considered in this section were made on a monthly basis from June - August 1976 As well as giving an indication of the effects of metals on algae in the field, it was intended that the collections made at the commencement of the project would provide a basis for the choice of species for further intensive studies

3 44 Plant material for analysis

During the surveys of algal flora, no species suitable for the analysis of mineral composition were found growing in Bolts Burn Within the R. Derwent only one species of alga, *Lemanea fluviatilis*, was found to be present in sufficient quantities (and in a sufficiently clean condition) to consider for sampling for analysis Before planning a detailed series of preliminary investigations of accumulation of heavy metals by the alga was made within the R. Derwent

i sampling of four replicate samples of pooled whole filaments of *Lemanea* from seven reaches along the R. Derwent (25 March 1975),

ii. as it seemed possible that younger growing tips of filaments of *Lemanea* might accumulate lower levels of metals than more basal portions of the filaments which had been in contact with the water for longer periods, individual filaments from reaches 05 and 07 on the R. Derwent were separated into 2 cm long sections which were digested separately (Based on the results of this study all subsequent collections of *Lemanea* were of pooled 2 cm long filament tips,) iii initial investigation of leaching of zinc from 2 cm filament tips after shaking for 1 hour in a known volume of double distilled water (which was used for all subsequent washings),

iv collection of pooled filament tips from several points across the R. Derwent in reach 07 (before intensive collections of *Lemanea* from this reach were confined to an area of substratum close to one bank)

3.5 EXPLORATORY SURVEYS OF COMPOSITION OF WATER

3.51 Reaches on streams and rivers

The levels of zinc, lead and cadmium in representative samples of water from 55 reaches within the catchment area of the Derwent Reservoir are given in Table 3.7. For sake of completeness, some results have been included from tributaries that were only discovered at a later stage of the project These include several small springs in the valley of Bolts Burn that only appeared following the raising of the water table during 1977

From these results it was apparent that elevated levels of zinc, lead and cadmium were being carried into the Derwent Reservoir by the R. Derwent. These metals were apparently derived mainly from two tributaries to the river, Shildon Burn and Bolts Burn, with the latter stream by far the more important

Despite the presence of some old lead workings in the valley of Bolts Burn upstream of the Whiteheaps fluorspar mine, most of the high levels of zinc, lead and cadmium carried by the stream appeared to be derived from sources within the mine. It was observed that Bolts Burn often carried very high levels of milky white suspended solids These also appeared to originate

A. TRIBUTARIES TO THE R DERWENT (EXCLUDING BOLTS BURN)

Site	Stream & Reach	Date	Zn	Pb	Cd
Beldon Burn entering the R Derwent	0074-99	19-9-74	0 002	0.002	0.0002
Nookton Burn entering the R. Derwent	0072-99	19-9-74	0.009	0.003	0.0003
Reeding Burn entering the R Derwent	0090-99	16-1-75	0 022	0.012	-
Shildon Burn near old mine workings upstream of Banchland	0124-50	6-5-75	0.021	0 038	0 0005
'Shildon Adit' entering Shildon Burn at Blanchland	0178-01	2-8-76	1.810	0.020	0.0050
Shildon Burn entering the R Derwent	0124-99	6-5-75	0 460	0.022	0.0015
Stoney Burn entering the R Derwent	0110-99	16-1-75	0.012	0.002	-
Hot Burn entering the R. Derwent	0121-99	16-1-75	0 020	0.022	-

B REACHES ON THE R. DERWENT

Downstream of the confluence of Nookton and Beldon Burns	0061-01	19-9-74	0.005	0.003	0.0003
Upstream of the entry of Bolts Burn	0061-05	19-9-74	0.002	0.004	0.0001
Downstream of the entry of Bolts Burn	0061-07	19-9-74	0.290	0.014	0.0012
Upstream of the entry of Shildon Burn	0061-09	20-2-75	0.400	-	-
Downstream of the entry of Shildon Burn	0061-10	20-2-75	0 450	-	-
Upstream of the entry of Stoney Burn	0061-15	25-3-75	0.262	0.267	0.0001
Downstream of the entry of Stoney Burn	0061-20	25-3-75	0.258	0.293	0.0001
Entering the Derwent Reservoir	0061-25	16-1-75	0 210	0 031	0 0015
Downstream of the Derwent Reservoir	0061-30	8-3-76	0.044	0 016	<0.0001

Table 3.7 Typical values for levels of heavy metals at reaches on the R. Derwent and its tributaries during exploratory surveys

(all concentrations are in mg l^{-1})

C. TRIBUTARIES TO BOLTS BURN

Site	Stream & Reach	Date	Zn	Pb	Cd
Old adit upstream of Whiteheaps Mine		6-9-74	0 120	0 090	-
Sikehead Stream entering Bolts Burn	0067-99	6-5-75	1.110	0.179	0.0023
Whiteheaps Adit Level	0068-99	6-5-75	6 800	0 020	0.0278
Whitheaps Mine Effluent	0069-99	17-10-74	1.550	2.500	0 0042
'Greenshaft Stream'		18-3-76	0.200	0 051	0.0008
'Bolts Burn Road Adit'	0070-99	20-2-75	1 210	0 130	-
Small tributary entering Bolts Burn at reach 43		24-5-77	0 046	0 080	-
'Bolts Burn Track Adit'	0084-01	11-9-75	0 510	0.625	0.0020
'Track Flush'	0176-01	3-10-76	0.181	1.325	0 0026
'Bolts Burn Upper Tip Tributary'	0087-99	20-2-75	0.300	0.013	0 0025
'Bolts Burn Lower Tip Tributary'	0177-99	16-3-76	0 319	0.010	0.0045
Spring entering Bolts Burn at reach 60		20-2-75	0.160	-	-
'Bolts Burn Calcareous Flush'	0088-99	6-5-75	<0 002	0 001	<0.0001

Table 3.7 cont. Typical values for levels of heavy metals at reaches on tributaries to Bolts Burn during exploratory surveys

(all concentrations are in mg l^{-1})

D REACHES ON BOLTS BURN

Site	Stream & Reach	Date	Zn	Pb	Cd
Bolts Burn upstream of old workings above Whiteheaps Mine	0071-05	6-9-74	0.050	0.020	-
Bolts Burn by old workings above Whiteheaps Mine	0071-08	6-9-74	0.120	0.040	-
Bolts Burn downstream of old workings above Whiteheaps Mine	0071-10	6-9-74	0.050	0.090	-
Upstream of Whiteheaps Mine	0071-20	6-9-74	0 040	0.002	0.0003
Downstream of Sikehead Stream	0071-27	3-6-76	0.156	0.022	0.0011
Upstream of Whiteheaps Adit Level	0071-28	6-5-75	1.450	0.115	0.0055
Downstream of Whiteheaps Adit Level	0071-30	6-5-75	2 430	0.033	0.0086
Upstream of Whiteheaps Mine Effluent	0071-35	6-9-74	1.590	0.090	0.0015
Downstream of Whiteheaps Mine Effluent	0071-37	6-9-74	1.540	0.020	-
150 m downstream of effluent	0071-40	17-10-75	1.540	2.500	0 0042
Upstream of 0070-99	0071-45	20-2-75	4 200	-	-
Downstream of 0070-99	0071-47	20-2-75	4 230	-	-
Bolts Burn in area of old mines	0071-50	17-10-74	0 910	0.234	0.0034
Bolts Burn upstream of 0084-01	0071-55	17-10-74	0.980	-	-
Bolts Burn downstream of 0084-01	0071-60	17-10-74	1.100	0 407	0.0050
Bolts Burn downstream of area of old mines	0071-65	20-2-75	2.500	-	-
Bolts Burn in wooded part of valley	0071-70	3-6-76	1.450	0.207	0.0055
Bolts Burn in wooded part of valley	0071-75	20-2-75	2.260	-	-
Bolts Burn in wooded part of valley	0071-80	3-6-76	1.370	0.171	0 0048
Bolts Burn in wooded part of valley	0071-85	20-2-75	2 180	-	-
Bolts Burn upstream of 0088-99	0071-90	16-3-76	1.110	1.063	0.0075
Bolts Burn downstream of 0088-99	0071-92	20-2-75	1.820	-	-
	0071-95	6-9-74	1.570	0.050	-
Bolts Burn entering the R. Derwent	0071-99	6-9-74	1.010	0.100	0 0030

Table 3.7 cont. Typical values for levels of heavy metals at reaches on Bolts Burn during exploratory surveys

(all concentrations are in mg l^{-1})

from sources within the Whiteheaps Mine. Despite several small inputs of water to Bolts Burn from old workings downstream of the Whiteheaps Mine (some of which had elevated levels of heavy metals in the water), the levels of zinc, lead and cadmium carried by the stream appeared to fall off somewhat before entering the R. Derwent.

The levels of heavy metals in the water of Bolts Burn reach 99 were apparently subject to marked fluctuation (Table 3.8). The results of the 'mixing profile' of zinc in the water of the R. Derwent confirmed that mixing of water from the polluted tributary was complete before reach 07 (see also 4 323).

3.52 Fractionation study

The results of the fractionation study carried out on water from four reaches on the R. Derwent and Bolts Burn are tabulated in Tables 3 9 - 3.13 Several generalisations can be made on the basis of these results·

i. Differences between the levels of cations in untreated and 'total' samples of water were in all cases very slight and probably attributable to analytical variability. This was found to be true even in water from Bolts Burn with a high load of suspended material (Table 3.13) This backs up subjective observations that much of the suspended load of the stream was transported easily for long distances

ii. In several cases a decrease was apparent in the proportion of various ions passing through filters was apparent as the pore size of the filter decreased (e.g. zinc passed through the range of Sinta funnels in Table 3 13). On examination of Table 3 13 it can be seen that Sinta, Nuclepore and Millipore filters all

Date	Zn	Pb	Cd
6-9-74	1.01	0.100	0.0030
19-9-74	1.48	0.033	0.0050
10-10-74	1.21	1 200	0.0045
17-10-74	0.70	0.307	0.0079
21-10-74	1.11	1 090	0 0061
16-1-75	0.95	0.145	
25-3-75	1.44	1.000	0.0046
6-5-75	1.29	0 181	0 0022

Table 3.8 Levels of zinc, lead and cadmium in the water of Bolts Burn reach 99 during exploratory surveys

(all concentrations are in mg l^{-1})

Fig. 3.9a Surface of unused 0.2 µm Nuclepore membrane filter

The pores appear as uniform 'tunnel' passing through the filter, occasionally more than 0 2 µm wide.

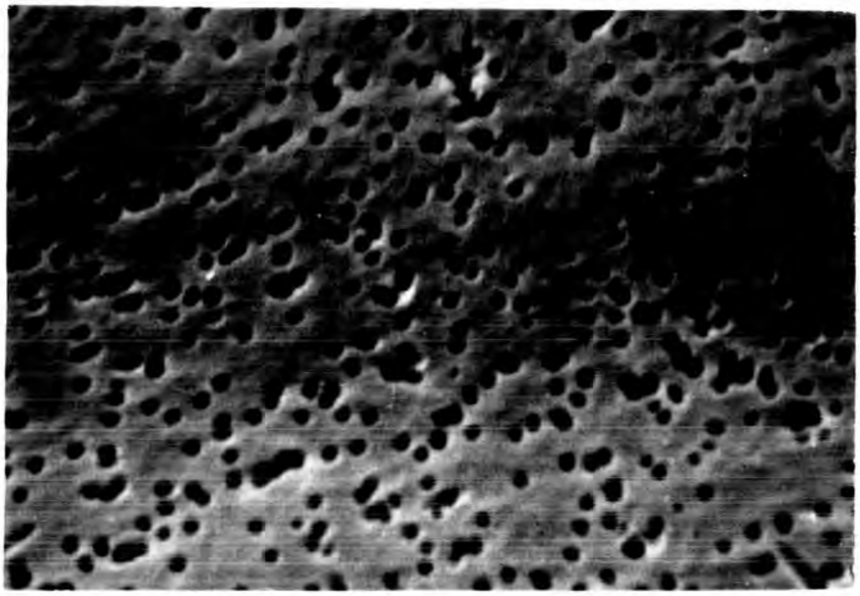
Fig. 3 9b Surface of 0 2 µm Nuclepore membrane filter after passing through 30 ml of water from Bolts Burn reach 99.

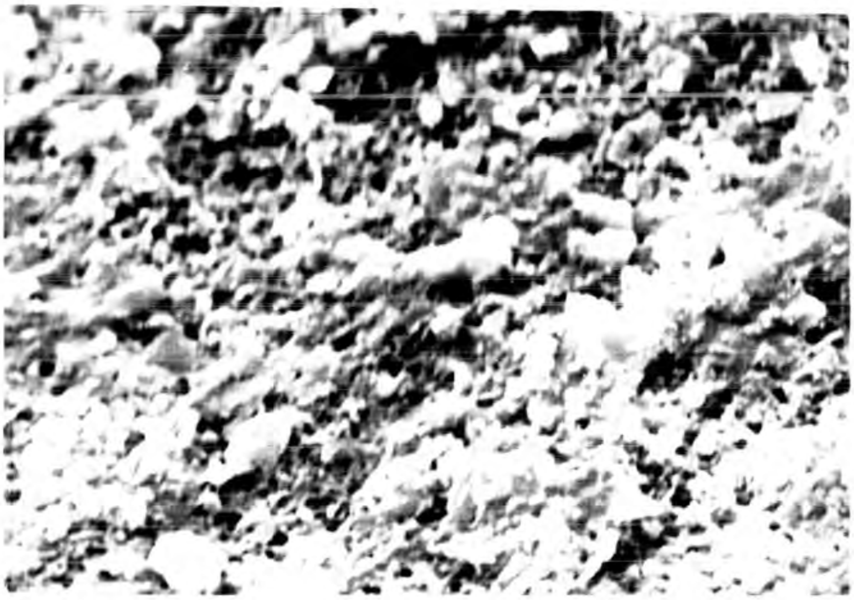
The pores are completely obscured, and the mean size of the pores must be significantly reduced.

Fig. 3 9c Surface of used no. 2 Sinta funnel.

The sintered glass has a 'sugarlump' appearance with ill defined pores, and debris clearly visible blocking some pores even after HCl washing.

Fig. 3.9 Scanning electron micrographs of the surface of two types of filter employed for the fractionation of water in the field.





filtered out a variable amount of the zinc and a large proportion of the iron, aluminium and lead present in water from Bolts Burn This was in agreement with the observation that high levels of iron, aluminium and lead were usually associated with high levels of particulate matter in the stream

iii. It is especially noticeable in Table 3.9 (fractionation of water from the R. Derwent upstream of the entry of Bolts Burn) that several of the filters caused contamination of the filtrate with various ions. Despite careful washing of the filters some contamination with sodium occurred in almost all cases, and some filters (e g. 0.45 µm Millipore filters) leached out zinc and cadmium

Based on the results of the fractionation experiment, three samples for the analysis of cations were collected on every subsequent occasion when water was sampled from reaches on streams or rivers These were

i. a 'total' water sample,

ii. a sample passed through a no 2 Sinta Funnel;

iii. a sample passed through a 0.2 µm Nuclepore membrane filter.

The Nuclepore filters were chosen as having the smallest pore size that allowed the collection of at least 25 ml of filtrate, and thus hopefully giving the best available approximation of truly dissolved levels of cations in the absence of more sophisticated techniques to determine ion speciation. Although these filters were found to cause fewer problems with contamination of the filtrate, occasional leakage of cadmium was found to take place. Estimates of the filtrability of cadmium through 0.2 µm Nuclepore filters have therefore been omitted from the rest of this account

Treatment	Na	K	Mg	Ca	Zn	Cu	Mn	Fe	Al	Pb	Ni	Co	Cd	Ag
Untreated	6.0	1.41	2.70	9.1	0.020	<0.002	0.087	0.44	0.28	<0.001	<0.01	<0.01	0.0014	<0.005
'total'	6.0	1.41	2.75	9.0	0.015	<0.002	0.087	0.44	0.28	<0.001	<0.01	<0.01	0.0018	<0.005
'sinta' no. 0	10.6	1.37	2.85	9.4	0.268	<0.002	0.080	0.47	0.28	0.002	<0.01	<0.01	0.0074	<0.005
'sinta' no. 1	11.5	1.63	2.90	9.4	0.033	<0.002	0.073	0.47	0.31	<0.001	<0.01	<0.01	0.0055	<0.005
'sinta' no. 2	10.6	1.44	2.85	8.1	0.022	<0.002	0.100	0.40	0.20	<0.001	<0.01	<0.01	0.0029	<0.005
'sinta' no. 3	13.9	1.16	2.85	9.3	0.029	0.019	0.073	0.37	0.24	<0.001	<0.01	<0.01	0.0186	<0.005
'sinta' no. 4	11.7	1.47	2.85	9.3	0.101	<0.002	0.073	0.37	0.24	<0.001	<0.01	<0.01	0.0042	<0.005
'millipore' 0.22µm	11.4	1.66	2.85	9.4	0.042	<0.002	0.087	0.36	0.25	<0.001	<0.01	<0.01	0.0520	<0.005
'millipore' 0.45µm	11.7	1.63	2.85	9.4	0.047	<0.002	0.073	0.36	0.19	<0.001	<0.01	<0.01	0.0316	<0.005
'millipore' 1.2µm	11.7	1.63	2.80	9.4	0.038	0.020	0.073	0.39	0.20	0.003	<0.01	<0.01	0.0399	<0.005
'millipore' 3.0µm	11.3	1.56	2.75	9.8	0.028	<0.002	0.067	0.37	0.22	0.002	<0.01	<0.01	0.0296	<0.005
'nuclepore' 0.2µm	8.0	1.41	2.70	9.1	0.010	<0.002	0.067	0.37	0.20	<0.001	<0.01	<0.01	0.0014	<0.005
'nuclepore' 0.4µm	18.0	1.72	3.05	10.6	0.041	0.006	0.067	0.41	0.32	<0.001	<0.01	<0.01	0.0308	<0.005
'nuclepore' 1.0µm	11.0	1.66	2.80	9.5	0.033	<0.002	0.067	0.39	0.26	<0.001	<0.01	<0.01	0.0096	<0.005
'nuclepore' 3.0µm	11.4	1.72	2.80	9.6	0.079	0.004	0.087	0.40	0.27	0.001	<0.01	<0.01	0.0648	<0.005
'GF-C'	9.3	1.63	2.75	9.4	0.089	0.010	0.127	0.93	0.34	0.002	<0.01	<0.01	0.0253	<0.005

Table 3.9 Fractionation of water from the R. Derwent upstream of Bolts Burn (reach 05)

(all concentrations are in mg l^{-1})

Treatment	Na	K	Mg	Ca	Zn	Cu	Mn	Fe	Al	Pb	Ni	Co	Cd	Ag
Untreated	7.6	1.84	3.20	10.0	0.249	<0.002	0.107	0.42	0.17	0.032	<0.01	<0.01	0.0051	<0.005
'total'	7.6	1.75	3.20	9.9	0.249	<0.002	0.107	0.44	0.16	0.030	<0.01	<0.01	0.0041	<0.005
'sinta' no. 0	10.4	1.94	3.30	12.5	0.369	0.005	0.100	0.39	0.16	<0.001	<0.01	<0.01	0.0173	<0.005
'sinta' no. 1	13.5	2.09	3.35	12.4	0.841	<0.002	0.100	0.68	0.18	<0.001	<0.01	<0.01	0.0176	<0.005
'sinta' no. 2	11.8	1.97	3.30	12.5	0.153	<0.002	0.100	0.33	0.13	<0.001	<0.01	<0.01	0.0053	<0.005
'sinta' no. 3	12.4	1.94	3.35	12.3	0.662	0.008	0.100	0.31	0.15	<0.001	<0.01	<0.01	0.0234	<0.005
'sinta' no. 4	16.0	2.25	3.45	12.5	0.544	<0.002	0.093	0.29	0.18	<0.001	<0.01	<0.01	0.0095	<0.005
'millipore' 0.22 µm	13.9	2.16	3.35	12.5	0.246	0.017	0.093	0.44	0.14	<0.001	<0.01	<0.01	0.0040	<0.005
'millipore' 0.45 µm	13.8	2.09	3.35	12.5	0.255	0.004	0.100	0.19	0.14	0.001	<0.01	<0.01	0.0098	<0.005
'millipore' 1.2 µm	14.1	5.44	3.35	13.0	0.268	0.050	0.106	0.40	0.10	<0.001	<0.01	<0.01	0.0194	<0.005
'millipore' 3.0 µm	13.5	2.13	3.30	12.4	0.274	<0.002	0.113	0.38	0.14	<0.001	<0.01	<0.01	0.0220	<0.005
'nuclepore' 0.2 µm	13.6	2.38	3.40	11.5	0.246	<0.002	0.100	0.38	0.16	<0.001	<0.01	<0.01	0.0041	<0.005
'nuclepore' 0.4 µm	12.1	2.44	3.30	12.5	0.299	<0.002	0.100	0.39	0.11	<0.001	<0.01	<0.01	0.0057	<0.005
'nuclepore' 1.0 µm	13.4	2.13	3.40	13.5	0.295	<0.002	0.100	0.80	0.18	<0.001	<0.01	<0.01	0.0060	<0.005
'nuclepore' 3.0 µm	12.8	2.19	3.45	12.4	0.268	<0.002	0.093	0.51	0.13	0.001	<0.01	<0.01	0.0035	<0.005
'GF-C'	13.6	2.13	3.30	12.6	0.271	0.051	0.100	0.42	0.63	0.001	<0.01	<0.01	0.0029	<0.005

Table 3.10 Fractionation of water from the R. Derwent downstream of Bolts Burn (reach 07)

(all concentrations are in mg l^{-1})

TREATMENT	Na	K	Mg	Ca	Zn	Cu	Mn	Fe	Al	Pb	Ni	Co	Cd	Ag
UNTREATED	7.6	2.10	3.35	12.8	0.288	0.002	0.107	0.37	0.19	0.004	0.01	0.01	0.0149	0.005
'TOTAL'	7.9	2.13	3.35	12.4	0.288	0.002	0.100	0.33	0.21	0.004	0.01	0.01	0.0152	0.005
'SINTA' no.0	10.3	2.44	3.45	12.5	0.307	0.002	0.100	0.36	0.21	0.002	0.01	0.01	0.0356	0.005
'SINTA' no.1	11.3	2.13	3.50	12.5	0.315	0.002	0.100	0.40	0.21	0.001	0.01	0.01	0.0356	0.005
'SINTA' no.2	13.7	2.09	3.45	12.5	0.262	0.002	0.093	0.80	0.24	0.001	0.01	0.01	0.0321	0.005
'SINTA' no.3	12.0	2.01	3.40	11.8	0.248	0.002	0.101	0.29	0.16	0.001	0.01	0.01	0.0218	0.005
'SINTA' no.4	13.3	2.09	3.45	12.0	0.238	0.002	0.100	0.29	0.21	0.001	0.01	0.01	0.0189	0.005
'MILLIPORE' 0.22μm	12.4	2.38	3.40	12.6	0.299	0.017	0.120	0.44	0.28	0.001	0.01	0.01	0.2270	0.005
'MILLIPORE' 0.45μm	13.8	2.31	3.50	12.8	0.268	0.002	0.100	0.30	0.28	0.001	0.01	0.01	0.0488	0.005
'MILLIPORE' 1.2μm	13.8	2.25	3.40	12.4	0.248	0.002	0.107	0.29	0.21	0.001	0.01	0.01	0.0648	0.005
'MILLIPORE' 3.0μm	13.0	2.38	3.50	12.6	0.271	0.004	0.107	0.40	0.24	0.001	0.01	0.01	0.1365	0.005
'NUCLEPORE' 0.2μm	11.0	2.02	3.25	12.6	0.257	0.002	0.107	0.51	0.21	0.001	0.01	0.01	0.0147	0.005
'NUCLEPORE' 0.4μm	9.7	2.44	3.60	12.5	0.265	0.002	0.087	0.35	0.33	0.001	0.01	0.01	0.0630	0.005
'NUCLEPORE' 1.0μm	13.4	2.34	3.40	12.5	0.290	0.002	0.101	0.41	0.24	0.001	0.01	0.01	0.0710	0.005
'NUCLEPORE' 3.0μm	13.6	2.38	3.40	12.6	0.280	0.002	0.104	0.37	0.37	0.001	0.01	0.01	0.0560	0.005
'GF-C'	13.7	2.28	3.45	12.4	0.260	0.002	0.100	0.33	0.26	0.001	0.01	0.01	0.0580	0.005

Table 3.11 Fractionation of water from the R. Derwent entering the Derwent Reservoir (reach 25)

(all concentrations are in mg l^{-1})

TREATMENT	Na	K	Mg	Ca	Zn	Cu	Mn	Fe	Al	Pb	Ni	Co	Cd	Ag
UNTREATED	18.0	4.69	6.10	30.5	1.668	0.002	0.267	0.37	0.15	0.071	0.03	0.01	0.0128	0.005
'TOTAL'	17.6	4.36	6.15	30.4	1.634	0.002	0.267	0.40	0.17	0.070	0.03	0.01	0.0136	0.005
'SINTA' no.0	23.4	4.69	6.01	28.8	1.610	0.004	0.273	0.26	0.28	0.021	0.01	0.01	0.0160	0.005
'SINTA' no.1	23.3	4.63	5.90	28.5	1.602	0.002	0.267	0.93	0.24	0.012	0.01	0.01	0.0177	0.005
'SINTA' no.2	21.4	1.34	6.55	26.9	1.122	0.002	0.280	0.06	0.13	0.004	0.01	0.01	0.0149	0.005
'SINTA' no.3	22.0	4.22	6.03	30.6	0.781	0.002	0.267	0.04	0.13	0.003	0.01	0.01	0.0166	0.005
'SINTA' no.4	23.6	4.59	6.25	29.6	0.878	0.002	0.260	0.06	0.16	0.004	0.01	0.01	0.0143	0.005
'MILLIPORE' 0.22µm	23.3	5.06	6.20	30.9	1.512	0.002	0.273	0.07	0.20	0.046	0.01	0.01	0.0358	0.005
'MILLIPORE' 0.45µm	23.3	5.13	6.45	29.6	1.541	0.002	0.273	0.06	0.22	0.051	0.01	0.01	0.0345	0.005
'MILLIPORE' 1.2 µm	21.7	4.94	6.15	30.1	1.580	0.002	0.267	0.10	0.13	0.042	0.01	0.01	0.0310	0.005
'MILLIPORE' 3.0 µm	23.1	5.03	6.20	28.8	1.610	0.002	0.280	0.10	0.19	0.072	0.01	0.01	0.03[illegible]2	0.005
'NUCLEPORE' 0.2 µm	21.6	5.06	6.25	28.1	1.502	0.002	0.267	0.07	0.31	0.027	0.01	0.01	0.0235	0.005
'NUCLEPORE' 0.4 µm	24.2	5.16	6.20	29.4	1.546	0.002	0.280	0.07	0.18	0.064	0.01	0.01	0.0406	0.005
'NUCLEPORE' 1.0 µm	24.3	5.09	6.15	28.1	1.566	0.002	0.273	0.18	0.25	0.101	0.01	0.01	0.0287	0.005
'NUCLEPORE' 3.0 µm	24.3	5.01	6.30	28.8	2.098	0.002	0.267	0.22	0.38	0.100	0.01	0.01	0.0348	0.005
'GF-C'	29.4	5.00	6.45	28.8	1.493	0.015	0.300	0.15	0.28	0.051	0.01	0.01	0.0287	0.005

Table 3.12 Fractionation of water from Bolts Burn reach 99 (low suspended solids)

(all concentrations are in mg l^{-1})

TREATMENT	Na	K	Mg	Ca	Zn	Cu	Mn	Fe	Al	Pb	Ni	Co	Cd	Ag
UNTREATED	13.6	3.84	5.00	25.0	1.463	0.006	0.240	0.94	0.72	0.120	0.01	0.01	0.0064	0.005
'TOTAL'	13.1	3.56	4.75	25.6	1.464	0.004	0.240	0.94	0.68	0.121	0.01	0.01	0.0054	0.005
'SINTA' no. 0	17.1	3.94	5.20	25.5	1.351	0.006	0.240	0.87	0.90	0.001	0.01	0.01	0.0085	0.005
'SINTA' no. 1	17.1	3.94	5.30	24.7	1.347	0.032	0.233	0.81	0.73	0.001	0.01	0.01	0.0066	0.005
'SINTA' no. 2	17.4	3.56	5.20	24.4	0.590	0.004	0.220	0.51	0.54	0.001	0.01	0.01	0.0074	0.005
'SINTA' no. 3	16.2	3.19	5.05	23.2	0.380	0.006	0.193	0.25	0.33	0.001	0.01	0.01	0.0079	0.005
'SINTA' no. 4	17.1	2.00	5.10	19.0	0.029	0.002	0.193	0.11	0.17	0.001	0.01	0.01	0.0103	0.005
'MILLIPORE' 0 22µm	17.9	2.13	5.10	24.0	1.171	0.002	0.193	0.07	0.17	0.001	0.01	0.01	0.0216	0.005
'MILLIPORE' 0.45µm	17.8	3.63	5.02	23.0	1.146	0.004	0.193	0.13	0.19	0.001	0.01	0.01	0.0075	0.005
'MILLIPORE' 1.2µm	18.3	3.50	5.10	22.6	1.171	0.002	0.200	0.29	0.29	0.001	0.01	0.01	0.0061	0.005
'MILLIPORE' 3.0µm	18.8	3.44	5.01	22.4	1.171	0.002	0.201	0 14	0.19	0.001	0.01	0.01	0.0067	0.005
'NUCLEPORE' 0.2µm	16.0	3.44	5.02	24.6	1.156	0.002	0.180	0.10	0.24	0.001	0.01	0.01	0.0061	0.005
'NUCLEPORE' 0.4µm	19.1	3.66	5.10	21.5	1.151	0.002	0.180	0.07	0.10	0.001	0.01	0.01	0.0061	0.005
'NUCLEPORE' 1.0µm	15.8	3.53	5.20	23.1	1.156	0.002	0.207	0.11	0.19	0.001	0.01	0.01	0.0081	0.005
'NUCLEPORE' 3.0µm	24.6	3.53	5.20	22.2	1.171	0.002	0.173	0.15	0.46	0.001	0.01	0.01	0.0070	0.005
'GF-C'	17.8	3.75	5.15	22.6	1.185	0.017	0.200	0.18	0.28	0.001	0.01	0.01	0.0066	0.005

Table 3.13 Fractionation of water from Bolts Burn reach 99 (high suspended solids)

(all concentrations are in mg l^{-1})

3.52 Reservoir water

Little variation was detected in the 'total' levels of zinc, lead or cadmium in samples of water taken from 22 sites within the Derwent Reservoir during the initial transect (Table 3 14). Levels of the three metals were fairly high in the water of the R Derwent at the time the transect was made, but these apparently did not penetrate as far as the boundary of the nature reserve. It therefore seemed clear that all subsequent transects to collect water would have to extend right up to the inflow point of the R. Derwent

3.6 EXPLORATORY SURVEYS OF COMPOSITION OF SEDIMENT

3 61 Reaches on streams and rivers

A wide range of levels of heavy metals was found in samples of sediment from different reaches on the R. Derwent and Bolts Burn (Table 3 15) In general the levels followed the pattern found during the exploratory surveys of the composition of water Levels of zinc, lead and cadmium were much higher in sediment from the R. Derwent downstream of the entry of Bolts Burn than in sediment from upstream of the entry of the tributary, and levels were still elevated at the point of entry of the river to the reservoir The levels of metals in sediment from Bolts Burn upstream of the Whiteheaps Mine were relatively low, but they increased markedly downstream of the mine. Levels of the three metals were also relatively high in Shildon Burn

On the occasion when the sediments were collected (5 September 1975) the bed of Bolts Burn downstream of the Whiteheaps Mine had a markedly orange appearance. This was almost certainly due to the precipitation of ferric hydroxide as a result of the addition of ferrous sulphate to the Whiteheaps Mine Effluent. This deposition probably explains the very high levels of iron detected in some of the samples from the stream. Subsequent observations indicated that these conditions were somewhat atypical.

Table 3.14

Composition of 'total' samples of water taken from a transect along the axis of the Derwent Reservoir on 6 May 1975

(samples could not be collected in nature reserve due to nesting birds)

(all levels are in mg l^{-1})

Site	Grid square	Depth	Na	K	Mg	Ca	Zn	Cu	Mn	Fe	Al	Pb	Cd
R. Derwent entering the Derwent Reservoir		0	9.40	2.20	4.60	19.3	0.105	<0.002	0.076	0.23	<0.03	0.016	<0.0008
Nature reserve boundary, 20 m from north bank		0	6.50	1.53	2.70	10.1	0.034	<0.002	0.007	0.21	0.04	0.019	<0.0001
Nature reserve boundary, same site, 5 m deep		5	6.50	1.53	2.70	10.0	0.043	<0.002	0.008	0.21	0.03	0.004	<0.0001
Centre of nature reserve boundary		0	6.50	1.53	2.60	10.0	0.030	<0.002	0.006	0.18	<0.03	0.003	<0.0001
Same site, 10 m deep		10	6.50	1.54	2.70	10.1	0.036	0.003	0.027	0.31	0.16	0.023	<0.0001
Near boundary, 20 m from south bank		0	6.50	1.55	2.70	10.2	0.028	0.002	0.009	0.21	<0.03	0.002	<0.0001
Same site, 5 m deep		5	6.60	1.59	2.60	10.0	0.038	0.002	0.027	0.67	0.40	0.046	<0.0001
Centre of reservoir, just west of east end of Low Hospital - plantation		0	6.60	1.54	2.70	10.1	0.029	0.002	0.006	0.19	0.03	0.007	<0.0001
Same site, 10 m deep		10	6.40	1.54	2.70	10.1	0.034	0.002	0.013	0.22	0.06	0.006	<0.0001
Centre of reservoir, east of Low Hospital Plantation		0	6.60	1.54	2.70	10.1	0.030	0.002	0.013	0.20	0.04	0.016	<0.0001
Same site, 10 m deep		10	6.50	1.54	2.70	10.1	0.032	0.002	0.009	0.21	<0.03	0.006	<0.0001
Centre of reservoir opposite sailing club		0	6.40	1.55	2.70	10.1	0.032	<0.002	0.011	0.19	0.04	0.017	<0.0001
Same site, 10 m deep		10	6.50	1.56	2.60	10.1	0.029	0.003	0.009	0.18	0.06	0.003	<0.0001
Centre of reservoir, just west of Hunter House		0	6.50	1.52	2.60	10.1	0.029	<0.002	0.006	0.19	0.04	0.004	<0.0001
Same site, 10 m deep		10	6.50	1.56	2.60	10.1	0.032	0.012	0.006	0.20	0.06	0.005	<0.0001
Centre of reservoir, between Pow Hill & Hunter House		0	6.50	1.51	2.60	10.2	0.029	<0.002	0.006	0.21	0.05	0.003	<0.0001
Same site, 10 m deep		10	6.40	1.54	2.60	10.3	0.034	0.005	0.008	0.21	0.06	0.009	<0.0001
Centre of reservoir, just east of Pow Hill		0	6.40	1.54	2.70	10.2	0.029	0.002	0.007	0.20	0.03	0.005	<0.0001
Same site, 10 m deep		10	6.40	1.55	2.60	10.1	0.031	0.007	0.007	0.21	0.04	0.007	<0.0001
Centre of reservoir, 100 m out from dam		0	6.40	1.53	2.70	10.1	0.030	0.002	0.003	0.18	0.05	0.004	<0.0001
Same site, 10 m deep		10	6.40	1.55	2.60	10.1	0.031	0.007	0.007	0.21	0.04	0.007	<0.0001
Same site, 15 m deep		15	6.40	1.58	2.60	10.2	0.034	0.006	0.008	0.21	0.04	0.012	<0.0001
Reservoir surface at centre of dam		0	6.40	1.59	2.70	10.0	0.035	0.006	0.006	0.20	0.05	0.004	<0.0001

Site	Stream & Reach	Date	Na	Mg	Ca	Zn	Cu	Mn	Fe	Pb	Cd	Ag
R. Derwent upstream of Bolts Burn	0061-05	5-9-75	8.0	600.0	800.0	650.0	20.0	1500.0	19750.0	270.0	<1.0	<5.0
R. Derwent downstream of Bolts Burn	0061-07	5-9-75	80.0	1400.0	1280.0	7200.0	50.0	3500.0	36250.0	5450.0	10.0	40.0
R. Derwent by Blanchland playing field	0061-09	5-9-75	10.0	600.0	680.0	2520.0	30.0	1300.0	26000.0	2100.0	8.0	10.0
R. Derwent below Blanchland Bridge	0061-10	5-9-75	8.0	1100.0	750.0	2300.0	30.0	750.0	19750.0	1100.0	7.0	<5.0
R. Derwent below bridge at Carrick's picnic site	0061-25	19-8-75	40.0	800.0	800.0	2400.0	40.0	1600.0	19750.0	1350.0	15.0	<5.0
Shildon Burn at confluence with R. Derwent	0124-99	5-9-75	170.0	1400.0	980.0	7000.0	80.0	3500.0	34750.0	2550.0	10.0	<5.0
Sikehead Stream, just inside Whiteheaps Mine	0067-98	5-9-75	170.0	550.0	1500.0	7200.0	60.0	9200.0	44750.0	5800.0	14.0	12.0
Sikehead Stream at confluence with Bolts Burn	0067-99	5-9-75	1790.0	870.0	7900.0	6600.0	690.0	410.0	14750.0	5800.0	10.0	22.0
Fine material washing towards Sikehead Stream		5-9-75	1420.0	2000.0	2500.0	40000.0	1140.0	540.0	12250.0	3650.0	18.0	20.0
Bolts Burn upstream of Whiteheaps Mine	0071-20	5-9-75	20.0	550.0	300.0	800.0	120.0	1500.0	24250.0	200.0	4.0	<5.0
Whiteheaps Adit Level	0068-99	5-9-75	580.0	950.0	3400.0	5600.0	500.0	650.0	19750.0	3000.0	7.0	11.0
Bolts Burn upstream of Whiteheaps Adit Level	0071-28	5-9-75	360.0	780.0	2800.0	26250.0	570.0	600.0	17500.0	3100.0	14.0	13.0
Bolts Burn downstream of Whiteheaps Adit Level	0071-30	5-9-75	450.0	800.0	3900.0	6200.0	715.0	700.0	19750.0	7400.0	18.0	15.0

Whiteheaps Mine Effluent	0069-99	5-9-75	1450.0	1100.0	6750.0	2800.0	395.0	590.0	49750.0	5350.0	7.0	15.0
Bolts Burn upstream of the Whiteheaps Effluent	0071-35	5-9-75	260.0	800.0	2950.0	5200.0	530.0	700.0	22250.0	3500.0	18.0	10.0
Bolts Burn downstream of the Whiteheaps Effluent	0071-37	5-9-75	390.0	800.0	3250.0	5600.0	770.0	650.0	19750.0	3800.0	10.0	10.0
Bolts Burn	0071-47	5-9-75	760.0	1400.0	9100.0	16900.0	440.0	690.0	87250.0	5500.0	26.0	15.0
Bolts Burn below track	0071-60	5-9-75	360.0	1100.0	7700.0	26500.0	350.0	1150.0	84750.0	5400.0	57.0	10.0
Bolts Burn at confluence with the R. Derwent	0071-99	5-9-75	260.0	1200.0	7550.0	17200.0	330.0	1800.0	68500.0	6150.0	45.0	13.0

Table 3.15 Mineral composition of sediments from reaches in the R. Derwent catchment during exploratory surveys

(all levels are in $\mu g\ g^{-1}$ dry weight)

3.62 Derwent Reservoir

The results of the preliminary investigation of the mineral composition of sediments from the Derwent Reservoir are presented in Table 3.16, and the results for zinc and lead are summarized in Fig. 3 10 A fairly clear trend is apparent from these results, with higher concentrations of zinc, lead and cadmium being present in sediments near to the inflow point of the R. Derwent. This pattern seemed somewhat more clear-cut for lead than for zinc, with fewer high levels of lead than high levels of zinc being present in samples from outside the nature reserve As sediments were not collected from deeper points in the reservoir, however, it seemed possible that some of the lower levels of heavy metals detected at the eastern end of the reservoir were simply the result of the erosion of material from the shore. To gain a more comprehensive picture, therefore, it was obviously necessary to extend sampling to the deep central basin of the reservoir

3.63 Comparison of digestion techniques for sediments

Significantly higher levels of lead were extracted when four replicate samples of sediment from reach 99 on Bolts Burn were digested using boiling nitric acid alone than with a mixture of nitric, perchloric and sulphuric acids (as used for the preliminary investigations described above) This was almost certainly due to the precipitation of $PbSO_4$ by the sulphuric acid. All subsequent digests of samples of sediment were therefore carried out using nitric acid alone

Site	Date	Depth	Na	Mg	Ca	Zn	Cu	Mn	Fe	Pb	Cd	Ag
Dry crust in nature reserve, south side of Derwent before entering the reservoir	8-8-75	-	300.0	1500.0	1100.0	1600.0	60.0	1420.0	24750.0	1620.0	15.0	<5.0
Dry crust on north side of river, opposite first site	8-8-75	-	550.0	1920.0	1900.0	1680.0	70.0	2400.0	34750.0	1520.0	9.0	<5.0
Dry crust on south bank of river, 200 m further east	8-8-75	-	420.0	2100.0	1750.0	2800.0	80.0	2120.0	29750.0	1280.0	7.0	<5.0
Dry crust on south bank, further in reserve, 100 m from river	8-8-75	-	450 0	2000.0	1200.0	1700.0	80.0	2300.0	36000.0	1800.0	18.0	<5.0
Dry crust further into reserve near sw corner of reservoir	8-8-75	-	170.0	2100.0	900.0	1450.0	60.0	1200.0	19750.0	1100.0	7.0	<5.0
Dry crust on north bank of river, 150 m into reserve	8-8-75	-	420.0	2000.0	1600.0	1500.0	60.0	3320 0	42250.0	1270.0	10.0	<5.0
Dry crust on north bank of river, 200 m further east	8-8-75	-	670.0	1930.0	1900.0	5000.0	80.0	1600.0	39750.0	1800.0	4.0	<5.0
Dry crust on south bank of river opposite pond in nature reserve	8-8-75	-	450.0	2500.0	2850.0	2000.0	80.0	2760.0	39750.0	1400.0	4.0	11.0

Table 3.16a Mineral composition of sediments collected from exposed sites in the Derwent Reservoir nature reserve during exploratory surveys

(all levels are in $\mu g\ g^{-1}$ dry weight)

Site	Date	Depth	Na	Mg	Ca	Zn	Cu	Mn	Fe	Pb	Cd	Ag
Centre of river channel, opposite pond in nature reserve	11-9-75	0.5	100.0	1100.0	1100.0	3600.0	30.0	2050.0	22750.0	2200.0	10.0	11.0
Centre of river mouth entering the reservoir	19-8-75	1.0	200.0	2000.0	1050.0	1400.0	60.0	670.0	34750.0	1590.0	18.0	<5.0
Centre of river mouth	15-8-75	0.5	260.0	2000 0	1300.0	1100.0	60.0	2020.0	34750.0	1280.0	10.0	<5.0
Centre of channel, 50 m east of river mouth	15-8-75	2.0	100.0	1450.0	800.0	1900.0	35.0	1400.0	26000.0	1100.0	<1.0	<5.0
Centre of reservoir, 100 east of river mouth	15-8-75	2.5	40.0	1800.0	900.0	1700.0	30.0	2010.0	34750.0	1150.0	7.0	<5.0
North bank of nature reserve, 100 m east of river mouth	8-8-75	1 0	300.0	1640.0	1050.0	1350.0	60 0	410.0	28500.0	1670.0	4.0	10.0
Centre of reservoir, opposite entry of Acton Burn	11-9-75	1.0	200.0	2250.0	1100.0	2400.0	60.0	2300.0	44750.0	1570.0	7.0	40.0
Opposite end of wood on north bank, 100 m east of Acton Burn	19-8-75	1.0	10.0	950.0	300.0	300.0	60.0	400.0	14750.0	300.0	8.0	<5.0
South bank of nature reserve, sand probably built up by wave action	8-8-75	1.0	10.0	500.0	250.0	200.0	80.0	170.0	12250.0	200.0	4.0	<5.0
Opposite end of Ruffside Plantation near north bank, 20 m out	15-8-75	1.5	80 0	1640.0	500.0	500.0	30.0	1310.0	19750.0	350.0	<1.0	<5.0
Opposite end of plantation, 50 m out from north bank	15-8-75	4.0	200 0	2400.0	900 0	1900.0	60.0	15000.0	42250.0	950.0	6.0	<5.0
Opposite end of plantation, 50 m out from south bank	15-8-75	4.0	200.0	2100.0	1200.0	1350.0	60.0	1140.0	44750.0	1000.0	7.0	<5.0
Opposite end of plantation, 30 m out from south bank	15-8-75	3.0	150.0	1900.0	1000.0	1400.0	60.0	850.0	32250.0	950.0	7.0	<5.0

Table 3.16b Mineral composition of sediments collected from submerged sites in the Derwent Reservoir during exploratory surveys

(all levels are in $\mu g\ g^{-1}$ dry weight)

Site	Date	Depth	Na	Mg	Ca	Zn	Cu	Mn	Fe	Pb	Cd	Ag
Nature reserve boundary, 20 m from south bank	15-8-75	3.0	40.0	800.0	400.0	1800.0	30.0	1450.0	14750.0	310.0	4.0	<5.0
Nature reserve boundary, centre of reservoir	15-8-75	7.0	180.0	2400.0	1100.0	1200.0	60.0	4360.0	27750.0	1090.0	<1.0	<5.0
Nature reserve boundary, 100 m out from north bank	15-8-75	4.0	80.0	1600.0	450.0	500.0	30.0	2100.0	24750.0	350.0	4.0	<5.0
North bank, 200 m west of sailing club	27-8-75	0.5	20.0	1100.0	340.0	650.0	10.0	3070.0	24750.0	310.0	8.0	<5.0
50 m east of sailing club bay on north bank, 10 m out from bank	11-9-75	3.5	20.0	1300.0	250.0	1350.0	20.0	1900.0	22250.0	270.0	<1.0	<5.0
Same site, 25 m from north bank	11-9-75	7.0	170.0	1400.0	150.0	5000.0	30.0	2200.0	26000.0	370.0	4.0	<5.0
South bank at Hunter House	6-8-75	1.0	520.0	850.0	550.0	650.0	50.0	600.0	21750.0	350.0	<1.0	<5.0
Same site, 25 m from shore	6-8-75	2.5	310.0	1400.0	650.0	6600.0	50.0	4200.0	27250.0	500.0	4.0	<5.0
Same site, 45 m from shore	6-8-75	4.0	580.0	1600.0	800.0	4000.0	50.0	4700.0	49750.0	550.0	<1.0	<5.0
Same site, 65 m from shore	6-8-75	5.0	320.0	1600.0	920.0	1200.0	50.0	4230.0	49750.0	540.0	4.0	<5.0
Same site, 85 m from shore	6-8-75	6.0	320.0	1850.0	800.0	1250.0	40.0	6400.0	52250.0	540.0	10.0	<5.0
Same site, 105 m from shore	6-8-75	7.0	1020.0	1920.0	800.0	1350.0	60.0	10270.0	62250.0	750.0	10.0	<5.0
South bank, 200 m east of Hunter House	8-8-75	1.0	420.0	1100.0	800.0	1000.0	35.0	600.0	24750.0	360.0	4.0	<5.0
50 m east of Millshield Picnic area on north bank	27-8-75	0.5	140.0	950.0	150.0	400.0	10.0	1150.0	19750.0	140.0	7.0	<5.0
South bank at Pow Hill	8-8-75	1.0	350.0	1140.0	500.0	650.0	30.0	800.0	27250.0	350.0	12.0	<5.0

Table 3.16b cont. Mineral composition of sediments collected from submerged sites in the Derwent Reservoir during exploratory surveys (all levels are in μg^{-1} dry weight)

Site	Date	Depth	Na	Mg	Ca	Zn	Cu	Mn	Fe	Pb	Cd	Ag
10 m out from Cronkley Headland, north bank	11-9-75	7.0	10.0	1200.0	190.0	200.0	30.0	1010.0	22250.0	170.0	<1.0	<5.0
20 m out from Cronkley Headland	11-9-75	3.5	8.0	1200.0	190.0	350.0	30.0	2060.0	26000.0	240.0	<1.0	<5.0
North shore, 200 m east of Millshield, below houses	27-8-75	0.5	20.0	800.0	10.0	7000.0	30.0	200.0	12250.0	4.0	<1.0	<5.0
North shore before road bends north to gated section	27-8-75	3.5	60.0	800.0	100.0	100.0	10.0	600.0	12250.0	170.0	7.0	<5.0
South bank, 50 m west of boat moorings near dam	8-8-75	1.0	1170.0	700.0	1200.0	250.0	10.0	700.0	17500.0	7.0	4.0	<5.0
South shore, near dam	8-8-75	1.0	390.0	800.0	100.0	350.0	30.0	600.0	17500.0	100.0	7.0	<5.0
North shore, near dam	19-8-75	1.0	90.0	300.0	100.0	500.0	30.0	400.0	3500.0	140.0	7.0	<5.0

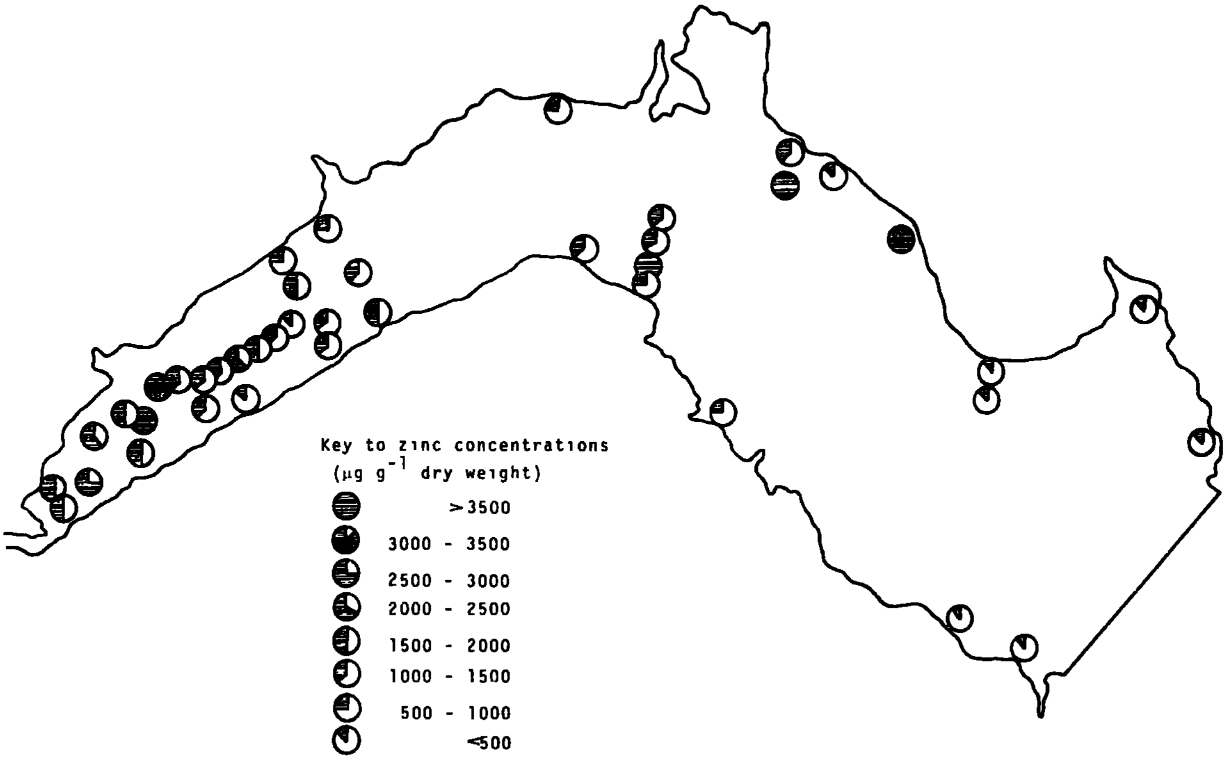
Table 3.16b cont. Mineral composition of sediments collected from submerged sites in the Derwent Reservoir during exploratory surveys

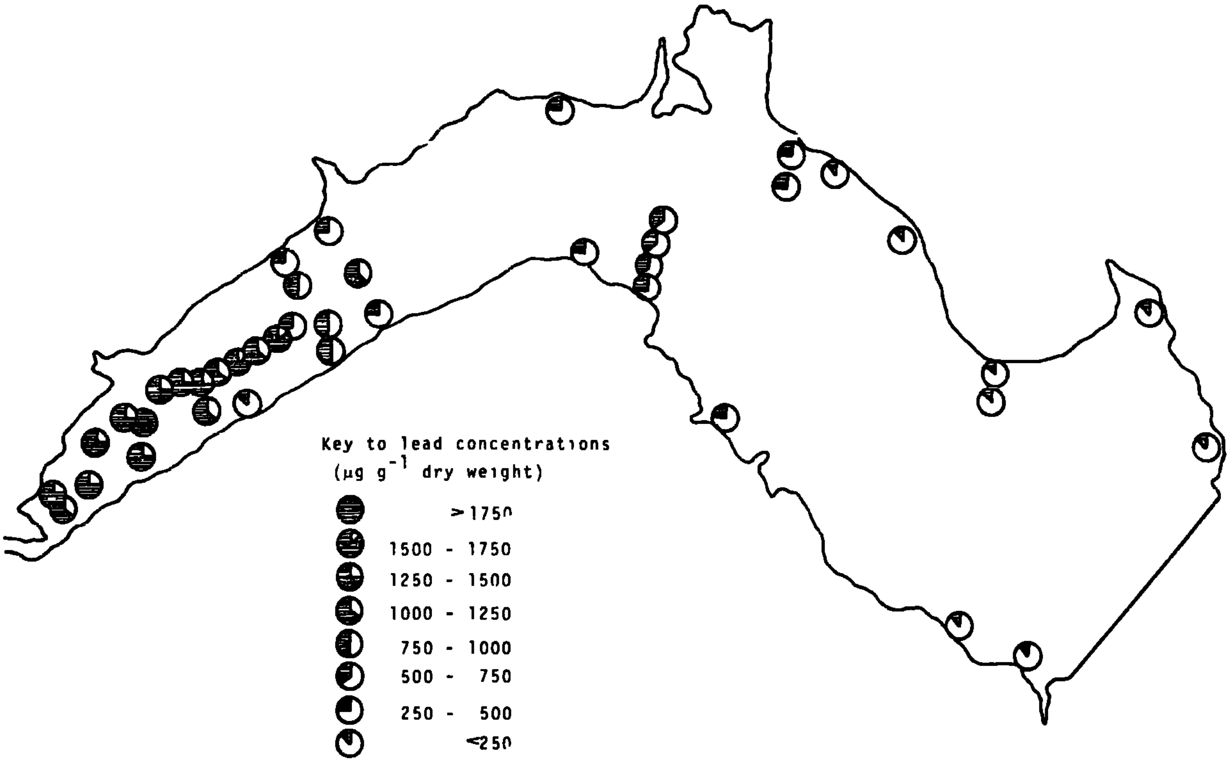
(all levels are in µg g^{-1} dry weight)

Fig. 3.10

Variations in the zinc and lead content of sediments from the Derwent Reservoir during exploratory surveys

(concentrations expressed as µg g^{-1} dry weight)


Key to zinc concentrations
(µg g^{-1} dry weight)
>3500
3000 - 3500
2500 - 3000
2000 - 2500
1500 - 2000
1000 - 1500
500 - 1000
<500


Key to lead concentrations
(µg g^{-1} dry weight)
>1750
1500 - 1750
1250 - 1500
1000 - 1250
750 - 1000
500 - 750
250 - 500
<250

3.7 EXPLORATORY SURVEYS OF ALGAL VEGETATION

The occurrence of different species as found during the exploratory surveys of the algal vegetation of Bolts Burn are listed in Table 3.17. The reaches from which the algae were collected are arranged in ascending order (i.e. passing downstream); this order corresponds to a general decrease in the concentration of heavy metals in the reaches downstream of the Whiteheaps Mine.

The most obvious feature that emerged from the surveys was a marked decrease in the number of species of algae in Bolts Burn on passing from upstream to downstream of the Whiteheaps Mine. Bolts Burn at reach 20 had a diverse algal vegetation, including numerous species of diatoms and periodic growths of *Microspora* spp A marked growth of *Ulothrix zonata* during the early spring was an annual feature of the reach during the project, and large gelatinous colonies of palmelloid *Chlamydomonas* sp. were also noted on several occasions when flows were low during the summer. A single cell of *Rhodoplax schinzii*, an alga noted only recently in streams in north east England (Holmes & Whitton, 1975), was also noted in reach 20.

Downstream of the Whiteheaps Mine at reach 40, the only live species of algae recorded in Bolts Burn were occasional cells of *Achnanthes minutissima*, *Surirella ovata* and *Amphora veneta*. Growths of algae were never visible to the naked eye in this reach, although the fine sediment on the bed of the stream became stable for extended periods during the summer of 1976. Thus the toxic action of metals appeared to play a more important role in limiting algal growth at the site than scouring by silt.

Species	Reach on Bolts Burn										
	20	27	28	35	40	50	60	70	80	90	99
Achnanthes minutissima	+	+	+	+		+	+	+	+	+	+
Mougeotia sp.	+		+								
Hormidium flaccidum		+	+			+	+	+	+	+	+
Lyngbya sp.(i)		+	+				+	+	+	+	+
Amphora veneta		+									
Microthamnion strictissimum		+	+								
Meridion circulare	+					+					
Eunotia exigua			+								
Navicula sp			+			+			+		
Mougeotia sp.	+					+				+	
Ceratoneis arcus	+					+				+	
Synedra ulna	+					+	+				
Scenedesmus obliquus						+	+	+	+	+	+
Fragilaria sp						+			+	+	+
Surirella ovata						+			+	+	
Stigeoclonium tenue	+						+	+	+	+	+
Cymbella sp.								+		+	
Oscillatoria sp.									+	+	+
Pseudanabaena catenata	+								+		
Frustula rhomboides	+								+		
Chlamydomonas sp.										+	
Chamaesiphon polymorphus											+
Navicula gracilis											+
Cosmarium subarctum	+										
Microspora amoena	+										
Cymbella ventricosa	+										
Tabellaria flocculosa	+										
Synedra rumpens	+										
Oscillatoria sp	+										
Cosmarium sp	+										
Cymbella parva	+										
Cylindrocystis brebissonii	+										
palmelloid *Chlamydomonas* sp.	+										
Gomphonema ventricosum	+										
Pinnularia subcapitata	+										
Gomphonema parvulum	+										
Ceratoneis arcus var *amphioxus*	+										
Diatoma hiemale var *mesodon*	+										
Navicula radiosa	+										
Lyngbya sp (ii)	+										
Chamaesiphon fuscus	+										
Rhodoplax schinzii	+										
Microspora sp	+										
Pinnularia viridis	+										
Oedogonium sp	+										
Ulothrix zonata	+										

Table 3.17 'Association table' of algae sampled during exploratory surveys of Bolts Burn

The number of species of algae recorded from 10 m reaches increased on passing downstream from reach 40 towards the R. Derwent. On all occasions when the stream was sampled, *Hormidium flaccidum* was found to be present downstream of about reach 50 This alga formed abundant growths at some points, and was present all the year round. *Stigeoclonium tenue* was also abundant in the stream during the summer months, but was never found upstream of reach 60.

Although *Hormidium flaccidum* extended further upstream in Bolts Burn than *Stigeoclonium tenue,* it is not possible to say whether the latter alga was more sensitive to the toxic action of metals in the stream water. Although both species could clearly tolerate both heavy metals and the scouring action of silt, their preferences for microhabitats were somewhat different. *H. flaccidum* was able to colonize rocks and rock sheets under only a moderate flow of water, where the accumulation of silt could be considerable. *S. tenue,* on the other hand, only occurred in the upper reaches in turbulent water where silt did not build up. The record for this alga from reach 60 was for a population growing beneath a small waterfall, and it seems possible that it could have survived even further upstream had suitable habitats been available. Further downstream, *S. tenue* became more abundant in relatively slow flowing water. This might have been a result of the greater dilution of suspended material in the lower reaches. In Bolts Burn reach 99, *S. tenue* was usually more abundant than *H. flaccidum* in the summer months, whilst the latter alga became more abundant as *S. tenue* died back in the winter. Of the species of algae that were recorded from reach 99, *Achnanthes minutissima* and *Lyngbya* sp. were present on all occasions when samples were collected. Both species were always associated intimately with filamentous growths of *S. tenue* or *H. flaccidum.*

Because of their abundance in Bolts Burn, *S. tenue* and *H. flaccidum* were both considered for use in the laboratory studies of tolerance to heavy metals. Before making the final choice, other reaches within the catchment were examined for the possible occurrence of the two algae. Whilst no growths of *H. flaccidum* could be found in any other reach except the R. Derwent at reach 07. *S. tenue* was found to be abundant at several other sites with elevated concentrations of heavy metals in the water. These included Shildon Burn reach 99, 'Track Flush' and the R. Derwent reach 10. Further occurrences of *S. tenue* were noted in other polluted and non-polluted streams on the Northern Pennine Orefield, and the alga was chosen for further study (see chapter 7).

3.8 EXPLORATORY SURVEYS OF COMPOSITION OF *LEMANEA FLUVIATILIS*

3.81 Variation in metal content along the R. Derwent

The results of the survey of the mineral composition of pooled whole filaments of *Lemanea* from the R. Derwent (Table 3.18) showed a rather similar trend to that found during the exploratory surveys of the composition of water and sediments Whilst little variation was found between the heavy metal content of different subsamples from the same reach, considerable variation was found when the mean contents of filaments from different reaches were compared.

The lowest mean levels of zinc, lead and cadmium (213 µg g^{-1}, 58 µg g^{-1} and 3 µg g^{-1} respectively) were found in material from reach 05, upstream of the entry of Bolts Burn. The highest levels of these metals (1223 µg g^{-1}, 581 µg g^{-1} and 121 µg g^{-1}) were found in material sampled from reach 07, downstream of Bolts Burn Some fall off in levels of metals was then apparent on passing downstream from reach 07, but the mean levels in filaments from reach 25 (where the river enters the reservoir) were still substantially higher (1042 µg g^{-1}, 372 µg g^{-1} and 52 µg g^{-1} respectively) than in material from upstream of Bolts Burn

3.82 Variation of zinc content within individual filaments

As well as the large increase in zinc content of whole filaments between reaches 05 and 07 on the R. Derwent, some increase in mean zinc content was found in successive 2 cm segments of filaments of *Lemanea* passing back from the tips (Fig. 3.11) Some of this increase may have been caused by debris and epiphytic algae attached to the more basal parts of the filaments, but the shorter time for which the younger cells in the tips of the filaments had been in contact with the surrounding water probably contributed to the differences.

Stream & reach	n		Na	K	Mg	Ca	Zn	Cu	Mn	Fe	Al	Pb	Cd
0061-05	4	$\bar{x}$	583.0	17094.0	21244.0	738.0	213.0	15.0	354.0	5516.0	1079.0	58.0	3.0
		s.d.	24.0	2423.0	1184.0	89.0	25.0	1.0	12.0	1966.0	161.0	11.0	0.2
0061-07	4	$\bar{x}$	619.0	14656.0	20600.0	850.0	1223.0	33.0	240.0	7188.0	956.0	581.0	121.0
		s.d.	48.0	479.0	986.0	54.0	33.0	5.0	29.0	234.0	124.0	45.0	12.0
0061-09	4	$\bar{x}$	558.0	16250.0	21388.0	1144.0	993.0	31.0	220.0	4084.0	623.0	402.0	79.0
		s.d.	51.0	884.0	1170.0	361.0	35.0	1.0	30.0	230.0	105.0	17.0	4.0
0061-10	4	$\bar{x}$	517.0	14844.0	20869.0	417.0	971.0	31.0	210.0	3425.0	384.0	341.0	74.0
		s.d.	47.0	1242.0	1192.0	59.0	40.0	1.0	37.0	230.0	138.0	28.0	6.0
0061-15	4	$\bar{x}$	492.0	14438.0	20613.0	413.0	969.0	23.0	188.0	4013.0	375.0	332.0	80.0
		s.d.	12.0	419.0	367.0	67.0	12.0	1.0	26.0	319.0	81.0	22.0	8.0
0061-20	4	$\bar{x}$	531.0	15917.0	20400.0	425.0	874.0	23.0	178.0	2803.0	338.0	261.0	56.0
		s.d.	60.0	312.0	507.0	20.0	25.0	1.0	11.0	281.0	25.0	24.0	8.0
0061-25	4	$\bar{x}$	513.0	11833.0	20444.0	917.0	1042.0	27.0	231.0	4375.0	638.0	372.0	52.0
		s.d	30.0	312.0	335.0	103.0	43.0	1.0	5.0	135.0	67.0	16.0	48.0

Table 3.18 Mineral composition of whole filaments of *Lemanea fluviatilis* collected from reaches on the R. Derwent during exploratory surveys

(all concentrations are in $\mu g\ g^{-1}$ dry weight)

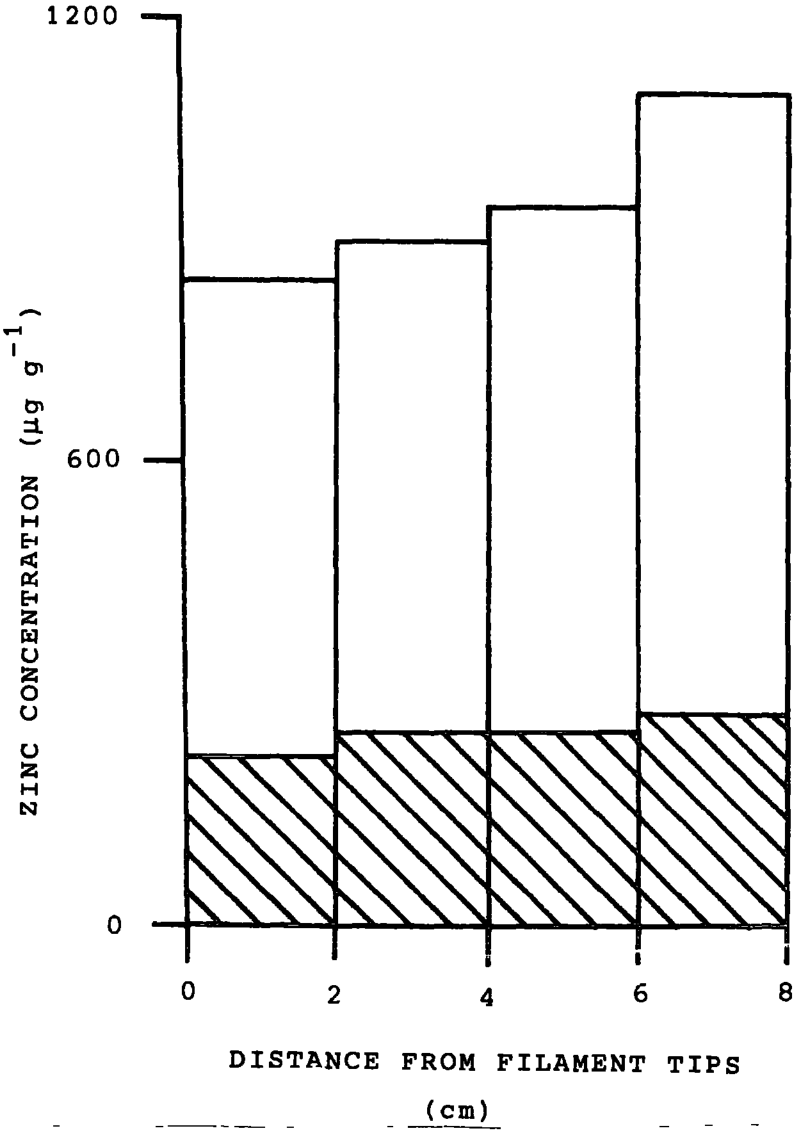


Fig. 3.11 Variation in zinc concentration along filaments of *Lemanea fluviatilis* from the R. Derwent

(shaded - R. Derwent reach 05
unshaded - R. Derwent reach 07)

3 83 Preliminary investigation of leaching of metals from filament tips

Very little zinc, lead or cadmium (<2% of the total contents) leached out when separated 2 cm long filament tips were shaken for 1 hour in double distilled water. This water was therefore continued to be used for the final wash of filament tips following collection.

3.84 Variation in metal content across reach 07 on the R. Derwent

Little variation was found in the mean contents of zinc, lead or cadmium in samples of *Lemanea* filament tips collected at different points across reach 07 on the R. Derwent For sake of comfort and safety when sampling in the dark, therefore, collection of *Lemanea* was confined to a dense area of filaments growing on an area of stone blockwork close to the shore of the reach.

CHAPTER 4

THE DISTRIBUTION OF HEAVY METALS WITHIN THE WATER, SEDIMENTS AND SUBMERGED PLANTS OF THE R DERWENT AND ITS TRIBUTARIES

4.1 INTRODUCTION

When the exploratory surveys had been completed, a more detailed study was planned to give a detailed analysis of the sources and distribution of zinc, lead and cadmium within the catchment area of the Derwent Reservoir. As a framework for this study, a series of 24 reaches on the R Derwent and its tributaries was chosen for sampling of water, sediment and plant material for analysis The reaches chosen (Table 4 1) included 11 equidistant ones along Bolts Burn (to assess changes along the stream and the influence of mine workings) and three on the R Derwent.

The results of all the surveys of the composition of water and sediments from these reaches are summarized in this chapter, together with the results of some of the analyses of submerged plants from the R Derwent. The results of intervariable correlation analyses of the chemical data from five 'key reaches' are also presented and discussed, with the aims of further elucidating factors influencing the variation of different parameters in the water. A partial budget for the levels of zinc, lead and cadmium entering and being retained by the Derwent Reservoir is presented

4.2 PROGRAMME FOR COLLECTION FROM REACHES

4 21 Water

The programme to study the composition of water from the 24 reaches on the R Derwent and its tributaries was commenced on 5 March 1976. The reaches were studied over a period of three weeks, during which there were considerable

stream no.	reach no.	grid ref.	site
0071	20	NY 947464	Bolts Burn above Whiteheaps mine
0071	27	NY 949466	Bolts Burn above Sikehead Stream
0071	28	NY 949467	Bolts Burn above Whiteheaps Adit
0071	35	NY 949471	Bolts Burn above mine effluent
0071	40	NY 951474	Bolts Burn 150 m below mine effluent
0071	50	NY 953476	Bolts Burn 100 m below road
0071	60	NY 955481	Bolts Burn below track
0071	70	NY 957486	Bolts Burn by concrete bridge
0071	80	NY 956490	Bolts Burn below bend in old mine road
0071	90	NY 957496	Bolts Burn 500 m above R. Derwent
0071	99*	NY 957498	Bolts Burn entering R Derwent
0061	05*	NY 957498	R Derwent above entry of Bolts Burn
0061	07*	NY 959499	R. Derwent below entry of Bolts Burn
0061	25*	NY 984516	R. Derwent below bridge at Carrick's picnic site
0061	30*	NY 033512	R Derwent below reservoir
0067	99	NY 949466	Sikehead Stream entering Bolts Burn
0068	01	NY 949467	Whiteheaps Adit Level
0069	99	NY 949471	mine effluent
0070	99	NY 952474	'Bolts Burn Road Adit'
0176	05	NY 956480	'Track Flush'
0084	01	NY 955481	'Bolts Burn Track Adit'
0087	99	NY 956484	'Bolts Burn Upper Tip Tributary'
0177	99	NY 956484	'Bolts Burn Lower Tip Tributary'
0124	99	NY 966503	Shildon Burn entering R. Derwent

Table 4.1 Reaches chosen for regular water sampling
(* - 'key' reach sampled more intensively)

variations in flow. Following this, four surveys of the composition of water from the reaches were carried out at monthly intervals between 1 June and 2 September 1976. Further collections were made from the reaches during the extremely wet winter of 1976/77, as it seemed possible that high flows and the raising of the water table might increase the levels of heavy metals carried into Bolts Burn by groundwater sources associated with mine workings.

Five of the 24 reaches were chosen as 'key' reaches, and were sampled at regular intervals during the second and third years of study. These were

i. R. Derwent upstream of the entry of Bolts Burn (0061-05);

ii. Bolts Burn entering the R Derwent (0071-99),

iii R. Derwent downstream of the entry of Bolts Burn (0061-07);

iv R. Derwent entering the Derwent Reservoir (0061-25),

v. R. Derwent downstream of the Derwent Reservoir (0061-30).

Stream discharge measurement plates installed and calibrated by the Northumbrian Water Authority before March 1976 enabled discharge to be measured in the five 'key' reaches upstream of the reservoir at the same time as water was collected. Except when the five reaches were sampled during surveys of all 24 selected reaches, collection of water was limited to 'total' and filtered samples for the analysis of cations The 'key' reaches were sampled under widely different flows, and on occasions coinciding with different conditions within the Whiteheaps Mine. These included samplings made at half-hourly intervals from Bolts Burn reach 99 and reach 07 on the R. Derwent during a 12 hour survey (8 March 1976), and collections at hourly intervals from these reaches during a 24 hour survey (18/19 March 1976). Samples of water from reach 25 on the R. Derwent were collected at half-hourly intervals for 12 hours on 18 March 1976

At the completion of the project reach 99 on Bolts Burn had been sampled 100 times, reach 05 on the R. Derwent had benn sampled 48 times, reach 07 101 times, reach 25 65 times and reach 30 17 times

4.22 Sediments

Sediment was sampled from 15 of the 24 reaches on 12 November 1976 The reaches chosen were all on Bolts Burn and the R. Derwent, and were sampled following heavy flooding when mixing of bottom sediment should have been more complete.

4.23 Plants

In order to study variations in the metal content of *Lemanea fluviatilis* within the R. Derwent, filaments of the alga were collected on frequent occasions from reaches 05, 07 and 25. The results of most of these samplings are presented in Chapter 6, and the detailed programme of sampling for the alga within the river is included in that chapter Presentation and discussion is limited here to variations in the mineral composition of the alga between reaches on the R. Derwent that had been sampled for water and sediment

Two species of aquatic bryophyte (*Scapania undulata*, *Hygrohypnum ochraceum*) were sampled from reaches 05 and 07 on the R. Derwent to compare their heavy metal content with that of *Lemanea*

4.3 COMPOSITION OF WATER

4 31 Water chemistry of Bolts Burn

4.311 Sources and distribution of heavy metals along Bolts Burn

The water chemistry of Bolts Burn and its tributaries is considered before that of the R. Derwent, as a knowledge of the former is necessary to understand changes in the composition of water passing along the river. Changes occuring along Bolts Burn are discussed below by considering reaches on the stream and inputs of water sequentially from the head of the valley downstream to the R. Derwent.

Bolts Burn upstream of the Whiteheaps Fluorspar Mine (0071-20)

Old mine workings (originally mining for lead and silver from the Boltshead and Fernygill Veins) were found around the head of Bolts Burn during the exploratory surveys (3.5). By the time Bolts Burn enters the Whiteheaps Mine, however, any metals derived from these workings appeared to have become diluted to a large extent by a complex of small tributaries entering the stream from the surrounding moorland. Water from reach 20 on Bolts Burn was sampled 9 times during the project, and the highest levels of zinc, lead and cadmium recorded were 0.048 mg l^{-1}, 0.080 mg l^{-1} and 0.0012 mg l^{-1} respectively (Tables 4.2, 4.3).

Sources of heavy metals entering Bolts Burn within the Whiteheaps Fluorspar Mine

(For photographs, see Fig. 4.1)

Just inside the perimeter fence of the Whiteheaps Mine Bolts Burn enters an artifical tunnel which carries it beneath a large part of the surface mine workings. A certain amount of seepage of heavy metals apparently affects the stream during this underground course, because at reach 27 (immediately after it emerges) the mean 'total' levels of zinc, lead and cadmium were found to have risen to 0.402 mg l^{-1}, 0.082 mg l^{-1} and 0.0023 mg l^{-1} respectively (n = 5 in each case).

The first clearly defined input of heavy metals to Bolts Burn occurs immediately downstream of reach 27 when Sikehead Stream (0067-99) enters the stream from the east. This small stream rises as a series of flushes and small tributaries near the old Sikehead Mine workings, but receives a significant amount of runoff from a large waste tip within the mine perimeter. The contribution of Sikehead Stream to

	STREAM	REACH			O.D.	O.D.	O.D.		pH	pH	Eh	Eh	Eh AT	TOT.	DISCHARGE		Na			K			Mg			Ca			Zn			Cu			Mn			Fe			Al			Pb			Ni			Co			Cd			Ag									
SITE	NUMBER	NUMBER	N	CURRENT $(m\ s^{-1})$	240 (nm)	254 (nm)	420 (nm)	COND.	FIELD	LAB.	FIELD	LAB.	pH 7	ALK.	(CUMECS)	TOTAL	NUCLEPORE	SINTA	TOTAL	NUCLEPORE	SINTA	TOTAL	NUCLEPORE	SINTA	TOTAL	NUCLEPORE	SINTA	TOTAL	NUCLEPORE	SINTA	TOTAL	NUCLEPORE	SINTA	TOTAL	NUCLEPORE	SINTA	TOTAL	NUCLEPORE	SINTA	TOTAL	NUCLEPORE	SINTA	TOTAL	NUCLEPORE	SINTA	TOTAL	NUCLEPORE	SINTA	TOTAL	NUCLEPORE	SINTA	TOTAL	NUCLEPORE	SINTA	TOTAL	NUCLEPORE	SINTA	F	CL	SI	PO4-P	NH4-N	NO2-N	NO3-N	SO4-S
BOLTS BURN	0071	20	9	0.927	0.078	0.070	0.021	154.0	7.4	7.1	271.1	271.3	285.8	61.7		6.0	7.2	6.5	2.01	2.06	0.93	3.36	3.72	3.48	20.0	22.4	20.9	0.021	0.020	0.009	0.002	0.002	0.002	0.070	0.051	0.050	0.20	0.06	0.09	0.09	0.05	0.05	0.015	0.005	0.006	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.0006	0.0006	0.0019	<0.005	<0.005	<0.005	0.52	11.20	3.44	0.029	0.158	0.001	0.180	8.960
BOLTS BURN	0071	27	5	0.290	0.169	0.148	0.025	302.0	7.1	7.1	255.0	252.5	258.3	66.9		9.7	10.4	9.0	2.82	2.67	2.26	4.66	4.54	4.29	28.4	26.7	25.6	0.402	0.336	0.083	0.005	0.003	0.002	0.571	0.565	0.498	0.81	0.12	0.62		0.08		0.084	0.014	0.058	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.0023	0.0020	0.0017	<0.005	<0.005	<0.005	1.25	14.85	3.18	0.052	0.162	0.002	0.405	14.700
BOLTS BURN	0071	28	8	0.740	0.158	0.211	0.129	351.5	7.0	7.0	267.5	265.0	265.3	40.8		14.4	11.0	10.4	4.10	2.84	3.14	5.33	5.36	5.23	41.2	28.5	28.3	0.773	0.455	0.317	0.023	0.006	0.003	0.762	0.837	0.812	19.68	18.77	18.42	3.43	0.14	0.14	0.996	0.014	0.042	0.01	0.01	<0.01	0.02	0.01	0.01	0.0042	0.0025	0.0049	<0.005	<0.005	<0.005	14.03	14.27	3.60	0.029	0.240	0.003	0.005	17.050
BOLTS BURN	0071	35	12	0.924	0.066	0.059	0.023	566.6	7.4	7.2	270.0	265.0	283.0	112.5		30.9	35.3	35.1	9.18	9.13	8.45	7.38	8.43	8.41	58.1	51.6	50.9	3.388	2.372	0.725	0.042	0.003	0.003	0.816	0.971	0.920	4.37	0.03	0.18	4.68	0.07	0.07	1.404	0.013	0.027	0.04	0.04	0.03	0.03	0.02	0.02	0.0119	0.0100	0.0073	<0.005	<0.005	<0.005	20.50	12.78	4.64	0.032	0.182	0.002	0.413	20.300
BOLTS BURN	0071	40	13	0.950	0.066	0.056	0.017	612.8	7.3	7.3	213.1	207.5	235.1	101.0		34.3	32.5	30.2	8.35	8.33	7.32	7.84	7.79	7.46	65.4	48.6	47.2	2.679	1.602	1.240	0.051	0.004	0.003	1.037	1.027	0.896	8.76	0.18	0.22	5.18	0.08	0.08	1.253	0.015	0.028	0.04	0.03	0.03	0.04	0.02	0.02	0.0084	0.0052	0.0045	<0.005	<0.005	<0.005	28.53	13.20	5.11	0.035	0.400	0.003	0.320	33.200
BOLTS BURN	0071	50	8	0.840	0.057	0.050	0.019	532.1	7.1	7.1	237.5	231.3	251.6	97.1		24.4	31.4	29.5	7.61	8.08	7.21	7.42	7.73	7.14	52.2	47.2	44.3	2.526	1.519	0.541	0.005	0.002	0.002	0.870	0.883	0.724	4.73	0.05	0.13	1.94	0.05	0.05	0.042	0.027	0.018	0.03	0.03	0.02	0.03	0.02	0.01	0.0075	0.0066	0.0052	<0.005	<0.005	<0.005	2.87	14.52	4.25	0.018	0.263	0.005	0.410	31.620
BOLTS BURN	0071	60	10	1.210	0.052	0.047	0.026	529.9	7.3	7.2	242.5	243.8	269.9	93.0		30.4	32.0	31.3	7.76	7.67	6.62	7.57	7.61	7.32	50.8	46.7	44.7	2.355	1.205	0.588	0.017	0.004	0.004	0.763	0.760	0.672		0.08	0.17	1.59	0.07	0.11	0.411	0.012	0.063	0.03	0.03	0.02	0.01	<0.01	0.01	0.0069	0.0052	0.0045	<0.005	<0.005	<0.005	7.59	16.52	4.17	0.029	0.214	0.004	0.362	30.850
BOLTS BURN	0071	70	6	0.900	0.048	0.040	0.006	551.8	7.4	7.4	247.5	240.0	261.3	100.9		29.5	29.6	28.3	7.62	7.30	6.45	7.18	7.25	6.84	48.1	43.0	42.0	1.665	1.054	0.335	0.016	0.004	0.004	0.630	0.587	0.534	1.75	0.05	0.33	1.33	0.07	0.28	0.378	0.020	0.062	0.03	0.02	0.02	0.02	0.01	<0.01	0.0061	0.0056	0.0044	<0.005	<0.005	<0.005	4.87	16.75	4.83	0.017	0.234	0.002	0.500	38.875
BOLTS BURN	0071	80	5	0.812	0.051	0.045	0.006	542.5	7.5	7.5	240.0	237.5	262.9	98.9		29.1	28.2	27.8	7.25	6.81	6.32	7.35	7.07	6.97	47.1	42.7	41.2	1.062	0.833	0.257	0.012	0.002	0.002	0.392	0.545	0.490	1.30	0.03	0.30	1.18	0.06	0.18	0.331	0.015	0.067	0.02	0.02	<0.01	0.01	<0.01	0.01	0.0047	0.0051	0.0031	<0.005	<0.005	<0.005	4.99	15.45	4.35	0.023	0.257	0.001	0.690	36.050
BOLTS BURN	0071	90	6	0.912	0.046	0.040	0.007	557.0	7.5	7.5	249.3	247.5	275.1	99.4		28.9	28.4	27.5	6.95	6.93	6.17	7.12	7.13	6.95	43.3	40.8	40.0	1.143	0.774	0.305	0.010	0.002	0.003	0.517	0.481	0.420	1.03	0.03	0.38	1.06	0.07	0.34	0.270	0.016	0.085	0.02	<0.01	<0.01	0.01	<0.01	<0.01	0.0049	0.0037	0.0032	<0.005	<0.005	<0.005	4.32	14.15	4.81	0.035	0.244	0.005	0.665	39.675
BOLTS BURN	0071	99	100	1.339	0.076	0.064	0.015	384.5	7.4	7.4	269.0	256.9	290.3	86.7	0.077	20.0	20.7	19.4	5.02	4.45	4.02	5.93	5.50	5.43	35.3	29.6	3.04	1.453	1.183	0.805	0.013	0.004	0.006	0.341	0.297	0.290	1.34	0.20	0.53	1.18	0.18	0.62	0.276	0.023	0.094	0.02	0.01	0.01	<0.01	<0.01	<0.01	0.0055	0.0064	0.0074	<0.005	<0.005	<0.005	5.15	13.50	5.15	0.026	0.241	0.002	0.373	20.633
R. DERWENT	0061	05	48	1.041	0.171	0.153	0.024	186.8	7.2	7.2	288.7	257.5	300.2	53.2	0.478	7.1	8.5	7.8	1.60	1.75	1.04	3.24	3.32	3.23	12.2	12.2	11.5	0.026	0.021	0.040	0.002	0.002	0.002	0.098	0.109	0.094	0.40	0.30	0.34	0.20	0.14	0.19	0.007	0.009	0.013	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.0005	0.0022	0.0023	<0.005	<0.005	<0.005	0.25	10.94	1.97	0.022	0.201	0.001	0.341	10.356
R. DERWENT	0061	07	101	1.212	0.151	0.144	0.019	256.3	7.3	7.3	267.2	267.0	304.7	62.9	0.600	9.6	10.1	9.7	2.31	2.29	2.04	3.80	3.54	3.48	16.8	15.1	15.0	0.317	0.272	0.212	0.002	0.002	0.002	0.140	0.137	0.133	0.50	0.24	0.35	0.37	0.16	0.25	0.051	0.016	0.032	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.0018	0.0053	0.0026	<0.005	<0.005	<0.005	1.14	10.27	4.03	0.015	0.238	0.001		15.112
R. DERWENT	0061	25	65	2.185	0.188	0.152	0.023	305.1	7.2	7.5	265.0	256.3	311.4	67.4	1.165	9.9	12.1	9.6	2.23	2.44	1.94	3.49	3.52	3.49	14.8	14.4	14.4	0.217	0.206		0.004	0.003	0.002	0.148	0.138	0.130	0.62	0.30	0.41	0.52	0.23	0.35	0.061	0.026	0.039	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.0018	0.0063	0.0027	<0.005	<0.005	<0.005	1.46	12.68	1.86	0.039	0.138	0.003	0.230	14.533
R. DERWENT	0061	30	16													6.8			1.55			2.75			11.7			0.064			<0.002			0.095			0.30			0.13			0.007			<0.01			<0.01			<0.0001			<0.005										
SIKEHEAD STREAM	0067	99	11	0.408	0.079	0.071	0.009	189.3	6.8	6.8	265.0	265.0	263.1	29.2		6.6	7.6	5.9	1.43	1.34	0.83	3.11	3.19	2.71	13.2	14.0	11.0	0.789	0.457	0.406	0.004	0.005	0.003	0.085	0.091	0.099	0.09	0.04	0.05	0.13	0.10	0.10	0.104	0.093	0.025	0.02	<0.01	<0.01	<0.01	<0.01	<0.01	0.0047	0.0040	0.0024	<0.005	<0.005	<0.005	1.76	8.20	2.77	0.019	0.213	<0.001	0.005	22.500
WHITEHEAPS ADIT LEVEL	0068	99	16	1.270	0.028	0.025	0.008	796.3	7.4	7.4	277.5	270.0	294.7	207.8		48.7	50.6	49.1	12.04	13.41	12.09	10.72	10.84	10.70	71.8	70.3	70.4	6.617	6.302	1.947	0.011	0.006	0.005	0.913	1.238	1.173	0.37	0.04	0.05	0.29	0.07	0.06	0.070	0.022	0.014	0.06	0.05	0.04	0.02	0.03	0.03	0.0163	0.0217	0.0158	<0.005	<0.005	<0.005	2.54	11.90	5.29	0.023	0.235	0.007	0.520	35.675
WHITEHEAPS EFFLUENT	0069	99	16	1.807	0.216	0.264	0.166	556.4	6.9	6.5	201.7	205.0	189.5	50.3		32.3	32.3	30.4	8.20	7.97	7.33	7.05	22.65	7.68	69.4	51.6	48.6	0.524	0.169	0.827	0.082	0.003	0.003	1.216	1.568	1.670	21.24	13.25	12.77	8.73	0.11	0.23	1.251	0.009	0.056	0.04	0.04	0.04	0.04	0.03	0.03	0.0037	0.0019	0.0025	0.005	<0.005	<0.005	38.10	18.20	6.98	0.011	0.466	0.016	0.390	54.225
'BOLTS BURN ROAD ADIT'	0070	99	8	0.390			0.001	147.0	6.8	6.5				7.0		6.6	7.4	6.7	1.21	1.27	1.33	3.96	4.07	3.97	15.1	14.8	14.7	1.248	1.005	1.084	0.002	0.002	0.002	1.416	1.589	1.441	0.31	0.22	0.15	0.76	0.45	0.10	0.085	0.035	0.010	0.02	0.02	0.01	<0.01	<0.01	<0.01	0.0048	0.0040	0.0033	<0.005	<0.005	<0.005		12.00	4.50	0.026	0.010	<0.001	0.850	5.100
'TRACK FLUSH'	0176	01	8		0.021	0.018	0.003	115.3	4.9	4.8	306.7	293.3	173.5	2.2		7.3	7.7	7.7	1.03	1.16	1.33	1.59	1.58	1.58	4.1	3.9	4.4	0.265	0.244	0.295	0.002	0.002	0.002	0.295	0.312	0.268	0.04	0.05	0.05	0.97	1.18	0.68	1.284	1.400	0.398	0.02	0.01	0.01	<0.01	<0.01	<0.01	0.0025	0.0035	0.0038	<0.005	<0.005	<0.005	1.26	65.2	4.89	0.021	2.205	2.001	3.895	5.487
'BOLTS BURN TRACK ADIT'	0084	01	7		0.170	0.170	0.019	194.0	4.8		320.0		192.4			7.6	7.7	7.0	1.01	1.00	1.13	1.65	1.59	1.60	6.7	6.4	6.4	0.693	0.606	0.737	0.004	<0.002	<0.002	0.243	0.247	0.259	0.38	0.15	0.14	1.34	1.26	0.99	0.905	0.793	0.254	0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.0046	0.0047	0.0049	<0.005	<0.005	<0.005	2.86	10.30	0.03	0.001	0.120	<0.001	0.360	6.700
'UPPER TIP TRIBUTARY'	0087	99	5													8.8	9.3	8.4	0.85	0.90	0.67	3.10	3.10	3.02	8.3	7.7	7.8	0.146	0.107		0.002	0.002	0.002	0.041	0.043	0.069	0.04	0.02	0.03		0.06	0.04	0.013	0.008	0.009	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.0036	0.0015	0.0012	<0.005	<0.005	<0.005	0.39							
'LOWER TIP TRIBUTARY'	0177	99	3													9.3	9.4	8.8	1.18	1.35	0.85	2.60	2.61	2.48	11.3	11.0	10.4	0.245	0.206	0.152	<0.002	<0.002	<0.002	0.025	0.025	0.052	0.07	0.06	0.06	0.06	0.06	0.07	0.022	0.014	0.016	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.0058	0.0047	0.0042	<0.005	<0.005	<0.005	0.53							
SHILDON BURN	0124	99	15	0.745	0.171	0.158	0.012	279.8	7.3	7.2	279.8	277.3	285.2	49.6		10.8	14.5	12.6	2.75	3.54	3.02	3.89	4.18	4.80	14.4	16.9	19.9	0.457	0.104	0.104	<0.002	0.005	0.003	0.118	0.128	0.090	0.27	0.74	0.12	0.13	2.63	0.08	0.023	0.089	0.012	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.0021	0.0022	0.0014	<0.005	<0.005	<0.005	0.76	13.33	3.45	0.020	0.492	0.005	0.410	16.675

Table 4.2   Mean water chemistry of intensively sampled sites in the Derwent Reservoir catchment area.

(all concentrations are in mg l^{-1})

Site	stream number	reach number	'total' Zn $\bar{x}$	'total' Zn s d	'total' Pb $\bar{x}$	'total' Pb s d	'total' Cd $\bar{x}$	'total' Cd s d
Bolts Burn	0071	20	0.021	0.018	0.015	0.025	0.0006	0.000
Bolts Burn	0071	27	0.402	0.220	0.084	0.062	0.0023	0.001
Bolts Burn	0071	28	0.773	0.368	0.996	1.320	0.0042	0.002
Bolts Burn	0071	35	3.388	1.444	1.404	2.504	0.0119	0.008
Bolts Burn	0071	40	2.679	1.190	1.253	1.669	0.0084	0.004
Bolts Burn	0071	50	2.526	1.156	0.424	0.863	0.0075	0.003
Bolts Burn	0071	60	2.355	0.882	0.411	0.819	0.0069	0.002
Bolts Burn	0071	70	1.665	0.693	0.378	0.659	0.0061	0.002
Bolts Burn	0071	80	1.062	0.233	0.331	0.615	0.0047	0.002
Bolts Burn	0071	90	1.143	0.465	0.270	0.447	0.0049	0.002
Bolts Burn	0071	99*	1.453	0.446	0.276	0.470	0.0055	0.002
R. Derwent	0061	05*	0.028	0.022	0.007	0.004	0.0005	0.001
R. Derwent	0061	07*	0.317	0.130	0.051	0.056	0.0018	0.002
R. Derwent	0061	25*	0.217	0.072	0.061	0.110	0.0018	0.005
R. Derwent	0061	30	0.064		0.007		0.0001	
Sikehead Stream	0067	99	0.789	0.186	0.104	0.046	0.0047	0.007
Whiteheaps Adit Level	0068	99	6.617	0.787	0.070	0.038	0.0163	0.010
Whiteheaps Effluent	0069	99	0.524	0.481	1.251	2.120	0.0037	0.001
'Bolts Burn Road Adit'	0070	99	1.248	0.676	0.085	0.065	0.0048	0.004
'Track Flush'	0176	01	0.265	0.116	1.284	0.378	0.0025	0.003
'Bolts Burn Track Adit'	0084	01	0.693	0.254	0.905	0.392	0.0046	0.004
'Upper Tip Tributary'	0087	99	0.146	0.091	0.013	0.008	0.0036	0.005
'Lower Tip Tributary'	0177	99	0.245	0.067	0.022	0.012	0.0058	0.005
Shildon Burn	0124	99	0.457	0.188	0.023	0.016	0.0021	0.001

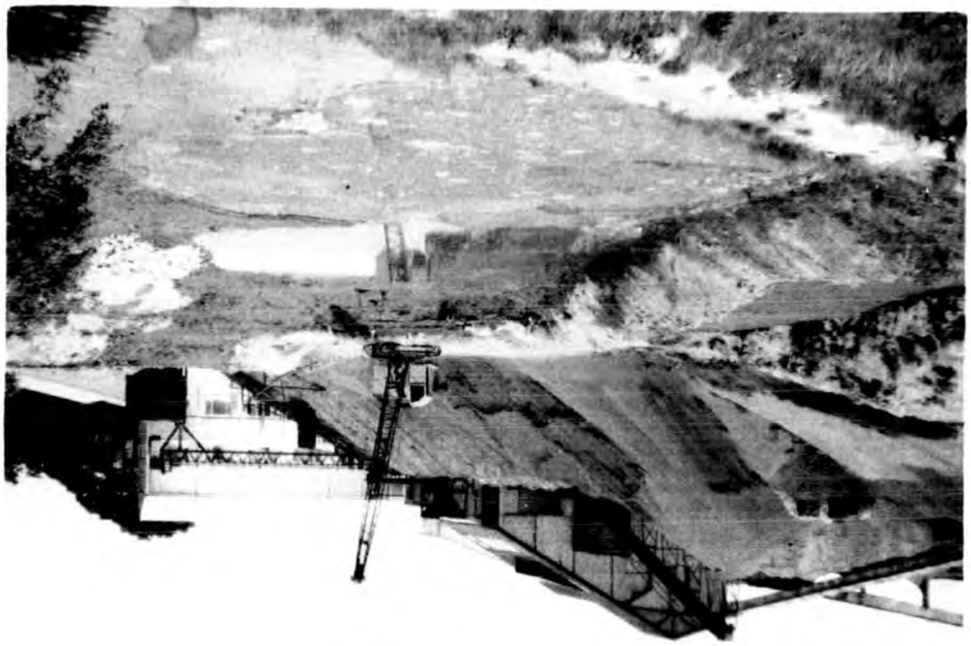
Table 4.3 Mean levels of zinc, lead and cadmium at intensively sampled sites in the Derwent Reservoir catchment area (* - 'key' reaches)

(all concentrations are in mg l^{-1})

Fig. 4 1

Surface workings at the Whiteheaps fluorspar mine.

Note fine particulate nature of the material on the tips. Bolts Burn flows underneath the wet area in the centre of the photographs.



the heavy metal content of Bolts Burn is very dependant on rainfall (it dried up during the dry summer of 1976). Mean 'total' levels of 0.789 mg l^{-1} Zn, 0.104 mg l^{-1} Pb and 0.0047 mg l^{-1} Cd (n = 11 in each case) were measured in the water of the stream.

Whiteheaps Adit Level (0068-99)
(For photographs, see Fig 4.2)

This adit discharges water into Bolts Burn 66 m downstream of the entry of Sikehead Stream, downstream of reach 28. The adit water is pumped from underground workings in the Whiteheaps Mine, and emerges from a tunnel into a small pond immediately adjacent to Bolts Burn. A 14 cm diameter pipe then carries water from the pond through an earth bank into the stream. Water from the Whiteheaps Adit Level was found to have mean 'total' levels of 6.617 mg l^{-1} Zn (n = 16), 0.070 mg l^{-1} Pb (n = 16) and 0.0163 mg l^{-1} Cd (n = 15). The levels of zinc and cadmium were the highest recorded in any tributary to Bolts Burn, and the adit was concluded to be the single most important source of these metals within the R. Derwent catchment. The high zinc content was found to be relatively stable, with an average of 95% of the 'total' zinc being capable of passing through a 0.2 µm Nuclepore membrane filter. The adit water is also characterised by high 'total' contents of sodium, manganese and iron ($\bar{x}$ = 48.7 mg l^{-1}, 71.8 mg l^{-1} and 0.913 mg l^{-1} respectively, n = 16 in each case).

During the first year of study the underground pump operating the adit was switched on manually, and switched off automatically when the water in the workings had dropped to the desired level. During the second and third years of study the operation of the pump was completely automatic. When the pump was in operation, the level of water in the pond rose and a large volume of water passed through the pipe into Bolts Burn (Fig. 4.2). A certain amount of water still flowed when the pump was switched off (Fig. 4.2d).

Fig. 4.2 Whiteheaps Adit Level

Fig. 4.2a

Pumped drainage water from undergound workings emerging from a tunnel into a small pond immediately adjacent to Bolts Burn (to the left to the photograph).

Fig. 4.2b

14 cm diameter pipe carrying water from the pond into Bolts Burn. The adit pump is not operating on this occasion, but some water still flows through the pipe.

Fig. 4.2c

Large volumes of water being discharged into Bolts Burn following raising of water level in the pond by the automatic switching-on of the adit pump.

Water from Bolts Burn reach 35 (immediately downstream of the entry of the adit) was found to be considerably enriched with zinc, with a mean 'total' level of 3.388 mg l^{-1}. The high degree of variation found in the 'total' zinc content (s.d = 1.444 mg l^{-1}) was due mainly to the operation of the mine pump.

Whiteheaps Mine Effluent (0069-99)
(For photographs, see Fig. 4.3)

The final source of heavy metal rich water to enter Bolts Burn within the Whiteheaps Mine area is the effluent from the fluorspar crushing plant This effluent discharges into the stream 350 m downstream of the Whiteheaps Adit Level, downstream of reach 35. During the three year period ferrous sulphate was added to the effluent before it was piped to the uppermost of three settling ponds, where sacks of lime were added by hand (Fig 4 3a). Although wide variations in the levels of some parameters were found in samples of water taken from the effluent discharge, certain generalizations can be made

i. The effluent was on most occasions the most important source of the high levels of particulate material that were frequently carried by Bolts Burn downstream of the Whiteheaps Mine This particulate material was found to have a high lead content (mean 'total' lead = 1.251 mg l^{-1}, mean lead concentration after Nuclepore filtration = 0.009 mg l^{-1}, n = 16) The zinc concentration of the effluent water was found to be relatively low (mean 'total' zinc = 0.524 mg l^{-1}, mean zinc concentration after Nuclepore filtration = 0 169 mg l^{-1}, n = 16) The 'total' iron content ($\bar{x}$ = 21 24 mg l^{-1}, n = 15) and fluoride content ($\bar{x}$ = 38.1 mg l^{-1}, n = 12) in the water were also found to be relatively high.

ii. The composition of the effluent discharge appeared to be very dependant upon weather conditions, presumably because the settlement of particles and binding of

Fig. 4.3a

Untreated effluent from the fluor treatment plants being discharged into the uppermost of three settling ponds. Ferrous sulphate has been added to the effluent, and sacks of lime stored in the hut are tipped into this pond (note lime spilled by pipe). Bolts Burn flows behind the hut in this photograph.

Fig. 4.3b

Effluent (under low flow) passing from the second to the third of the settling ponds Bolts Burn is to the left of the photograph, and the effluent outflow is to the left of the camera position.

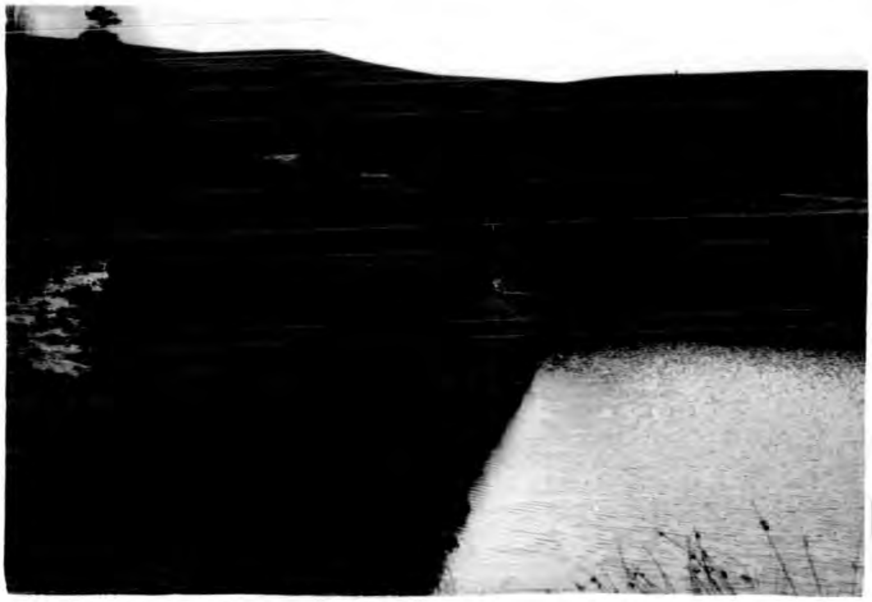
Fig 4.3c

Small amount of water passing into Bolts Burn from the effluent outflow when the fluorspar treatment plant is not operating (photograph taken on a Sunday).

Fig. 4.3d

Effluent outflow discharging large amounts of water (with high levels of particulate matter) into Bolts Burn.

Fig 4.3 Whiteheaps Mine Effluent and its settling ponds



metals by the ferrous sulphate/lime mixture took place effectively during warm weather. When working effectively the three settling ponds were orange, and the silt covering the bed of Bolts Burn downstream of the Whiteheaps effluent was in summer characteristically also orange During cold, wet weather when settlement was not as effective, the ponds and effluent discharge were often milky in appearance Under these conditions high levels of suspended solids carrying high levels of iron, aluminium and lead were discharged into Bolts Burn An extreme example of this was provided by an untreated sample of water taken from the effluent discharge on 18 March 1976, in connection with the 24 hour survey. This sample contained a large amount of particulate material, and had iron, aluminium and lead contents of 38 75 mg l^{-1}, 55 1 mg l^{-1} and 8.65 mg l^{-1} respectively.

iii The timing of discharge from the effluent could be predicted with some accuracy. Flow usually started at around 1000 on Monday mornings and was then related to shift work on two fluorspar crushing plants within the mine. One of these plants operated continuously from Monday to Friday, the other operated from about 0800 to 1600 daily.

Surface runoff

During the exploratory surveys of the composition of water, it was noticed that on several occasions the water of Bolts Burn carried high levels of suspended material upstream of the entry of the Whiteheaps Mine Effluent. This material was derived from surface runoff within the mine area, which could on occasions be a more important source of particulate lead than the mine effluent.

Many of the waste tips within the mine area are composed of very fine material (some of it dredged from the effluent settling ponds), and during wet weather large amounts of particulate material could be washed into Bolts Burn making the stream completely opaque well upstream of the effluent. Most of this particulate material was found to enter the stream between reaches 27 and 35, as was illustrated by the results of a series of water samples taken on 16 March 1976. On this occasion the effects of runoff caused the 'total' lead content of water in Bolts Burn to increase from 0.028 mg l^{-1} to 6.55 mg l^{-1} between these two reaches (the effluent actually had a diluting effect on the lead content of the stream water on this occasion).

Distribution of heavy metals in the water of Bolts Burn downstream of the Whiteheaps Mine

(For photographs, see Fig. 4.4)

At reach 40 (immediately downstream of the Whiteheaps Mine) Bolts Burn was found to carry the highest levels of 'total' zinc, lead and cadmium found at any point on the stream. Most of the zinc and cadmium carried by the stream water were derived from the Whiteheaps Adit Level, and most lead was derived in pariculate form by discharge from the mine effluent or by surface runoff from tips. As all these inputs to the stream could occur completely independantly of each other, it was found that the stream at this point could be carrying high 'total' levels of lead and low 'total' levels of zinc, or *vice versa*. Water sampled from reach 40 was found to have mean 'total' levels of 2.679 mg l^{-1} Zn (n = 13), 1.253 mg l^{-1} Pb (n = 12), and 0.0047 mg l^{-1} Cd (n = 11)

After leaving the Whiteheaps Mine, Bolts Burn flows for a further 3.5 km before entering the R. Derwent. A large number of old mine workings and tips (mostly associated with the old Ramshaw and Jefferey's Mines) are in evidence on both sides of the stream downstream of the Whiteheaps area (Fig. 4.4), and a determined effort was made to find and

Fig 4.4a

Bolts Burn around reach 46

Note dumps of coarse tailings derived from old lead and silver mining operations, and old adit ('Bolts Burn Road Adit') emerging to the left of the stream.

Fig. 4.4b

Bolts Burn around reach 87, in the lower part of the valley

Here the stream flows over sheets of Carboniferous sandstone in a thickly wooded valley, with no old mine workings.

Fig. 4.4 Bolts Burn downstream of the Whiteheaps Mine.

sample all sources of water entering Bolts Burn from these workings. A few such sources were discovered following the selection of the 24 reaches for intensive sampling, but none was found to have a significant enough effect upon the composition of water in Bolts Burn to merit regular sampling.

Regular sampling of the seven equidistant sampling reaches on Bolts Burn downstream of the Whiteheaps Mine was carried out to assess the effects of tributaries associated with the old workings. All springs or surface tributaries in the lower part of the Bolts Burn valley were sampled at least once during the project.

Only three sources of water were found in the valley downstream of the Whiteheaps Mine that might on occasions carry more zinc or lead than Bolts Burn at their entry. These were all derived from old adits, and were all found to be too small to cause a measurable increase in the level of any metal in Bolts Burn. 'Bolts Burn Road Adit' (0070-99) and 'Bolts Burn Track Adit' (0084-01) both carried fairly high levels of 'total' zinc in the water (1.248 mg l^{-1} and 0.693 mg l^{-1} respectively), but on most occasions these levels were lower than the level in Bolts Burn water at their points of entry (reaches 45 and 60). 'Track Flush' (0176-01) was found to have an interesting water chemistry, with a low pH ($\bar{x}$ = 4.9) and a high 'total' lead level ($\bar{x}$ = 1.284 mg l^{-1}, n = 8) combined with a low 'total' zinc level ($\bar{x}$ = 0.265 mg l^{-1}, n = 8). The tributary was too small to have a significant effect on the lead content of Bolts Burn, however, and on most occasions it sank into the ground before reaching the stream.

Two fairly large tributaries derived from surface runoff around the old mine workings enter Bolts Burn near reach 65. These are the upper and lower 'Tip Tributaries' (0087-99 and 0177-99), which were sampled five and three times respectively. They were found to carry slightly elevated levels of 'total' zinc in the water (from 0.1 - 0.3 mg l^{-1}), but their effect on the water of Bolts Burn was always one of dilution Both tributaries dried up during the summer of 1976.

In the thickly wooded lower part of the Bolts Burn valley (downstream of about reach 70), Bolts Burn flows swiftly over a series of gullies and waterfalls on coarse Carboniferous sandstone (Fig 4 4c) When high levels of suspended material were being discharged by the Whiteheaps Mine Effluent the stream could be completely opaque downstream to the R. Derwent In the lower part of the valley it was especially noticeable that the fine solids carried by the stream stayed in suspension very readily During summer low flow conditions a fairly thick crust of gelatinous silt was seen to collect along the bed of the stream, but this was quickly scoured during higher flows. It seems therefore that almost all of the suspended material derived from inputs within the Whiteheaps Mine sooner or later must enter the R. Derwent.

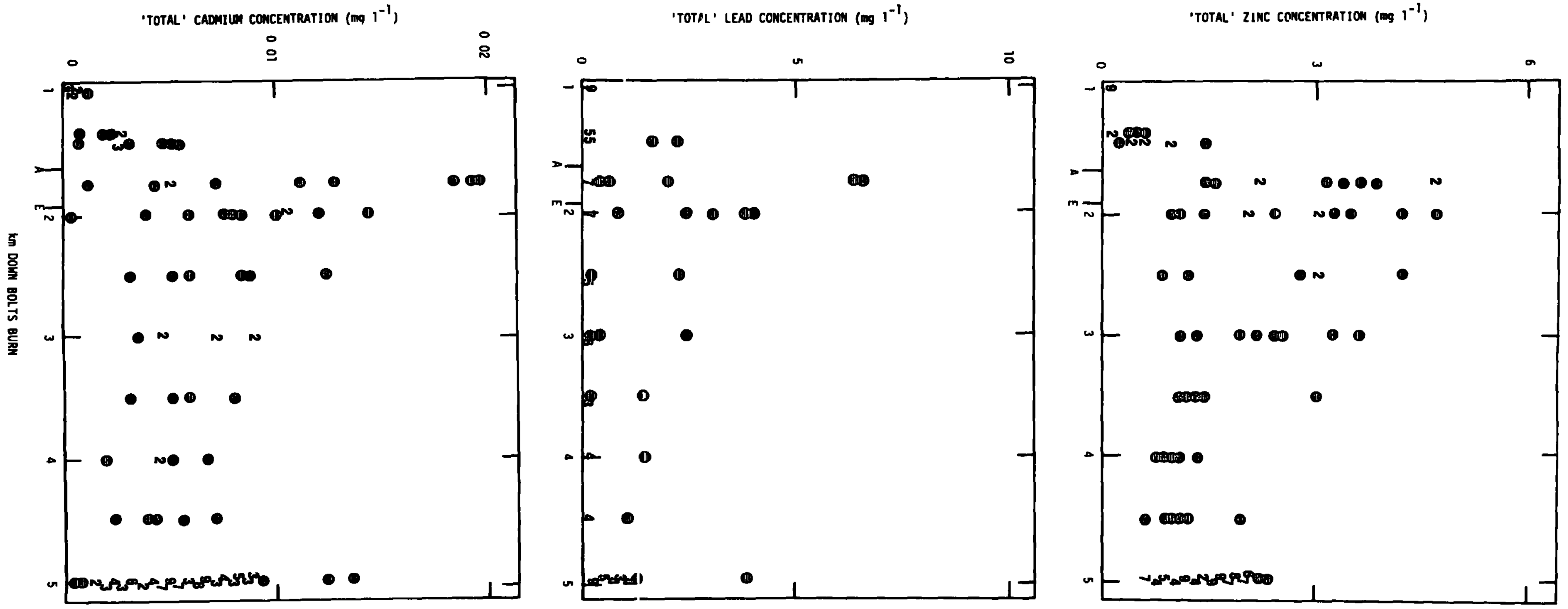
One of the more important conclusions of all the surveys of water chemistry carried out on Bolts Burn downstream of the Whiteheaps Mine was that no inputs of heavy metals large enough to have anything but a minor effect on the composition of the stream water entered Bolts Burn between the fluorspar mine and the R Derwent. Perhaps the most clearcut evidence for this conclusion lies in the fact that during no sampling survey was anything but a steady falloff in the 'total' concentration of zinc, lead or cadmium found on passing downstream from the entry of the Whiteheaps Mine Effluent

In Figs 4 5a - 4.5c all records of 'total' values for zinc, lead and cadmium are plotted against distance from the source of Bolts Burn. Although individual surveys cannot be picked out and some reaches were sampled more frequently than others, the sources of the three metals and the subsequent falloff in their levels can be made out quite clearly. The highest 'total' levels of zinc and cadmium were

Fig. 4.5

Scatter diagrams plotting all records for 'total' zinc, lead and cadmium concentration at different points along Bolts Burn

(A - entry of Whiteheaps Adit Level
E - entry of Whiteheaps Mine Effluent)


'TOTAL' CADMIUM CONCENTRATION (mg l^{-1})
0
0 01
0 02
'TOTAL' LEAD CONCENTRATION (mg l^{-1})
0
5
10
'TOTAL' ZINC CONCENTRATION (mg l^{-1})
0
3
6
km DOWN BOLTS BURN
1
A
E
2
3
4
5

recorded immediately downstream of the Whiteheaps Adit Level, and on most occasions the highest 'total' levels of lead were recorded in Bolts Burn immediately downstream of the Whiteheaps Effluent. The exceptions to this pattern were several very high 'total' lead levels (<6 mg l^{-1}) recorded upstream of the effluent as a result of surface runoff within the mine area.

4.312 Bolts Burn entering the R. Derwent (0071-99)

(For photographs, see Fig. 4.6)

At the point where Bolts Burn enters the R. Derwent, Bolts Burn was found to be seriously polluted by elevated levels of particulate and non-particulate zinc, lead and cadmium despite the fall-out and dilution of the stream water taking place between the Whiteheaps Mine and the river. The mean levels of 'total' zinc, lead and cadmium found in water from the reach were 1.453 mg l^{-1} (n = 100), 0.276 mg l^{-1} (n = 96) and 0.0055 mg l^{-1} (n = 94) respectively.

It was visually very obvious that the composition of the water of Bolts Burn reach 99 was subject to wide short-term fluctuations. On occasions the stream could be almost clear (Fig. 4.6a), while on other occasions it discharged a completely opaque stream of milky white or orange suspended solids into the R. Derwent (Figs 4.6b, 4.6c). Based on the results of the 100 samplings of water from the reach, an attempt was made to establish the limits of variability of the levels of heavy metals and other chemical parameters in the water from the reach, and the underlying controls on these variations.

If the inputs to Bolts Burn of zinc, lead and cadmium from the Whiteheaps Mine were operating in an independent manner as investigations had indicated, then the composition of water in Bolts Burn reach 99 should have been found to reflect these inputs. This would enable conclusions to be drawn on discharges within the Whiteheaps Mine on the basis of a single sampling of water. High levels of zinc and cadmium

Fig. 4.6a

Bolts Burn and the R. Derwent at low flow, with Bolts Burn carrying very low levels of suspended solids.

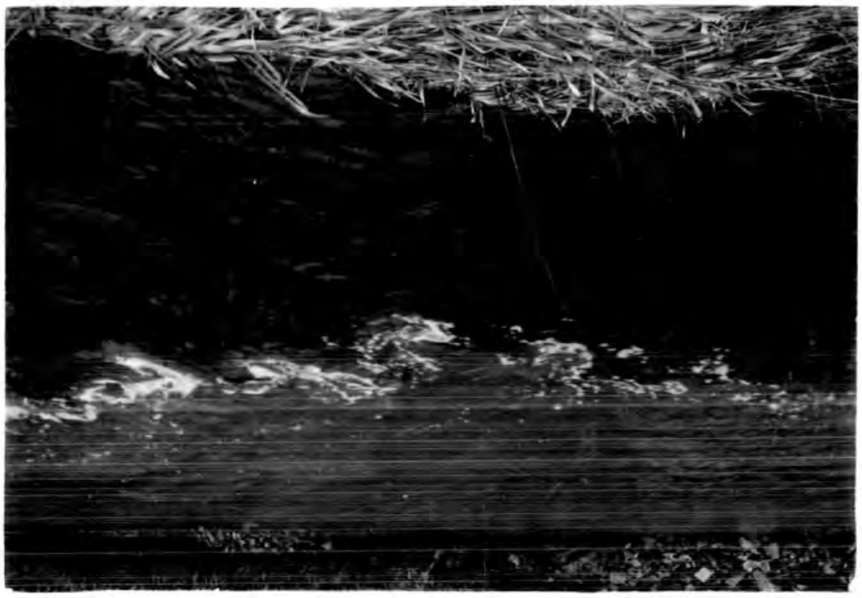
Fig. 4.6b

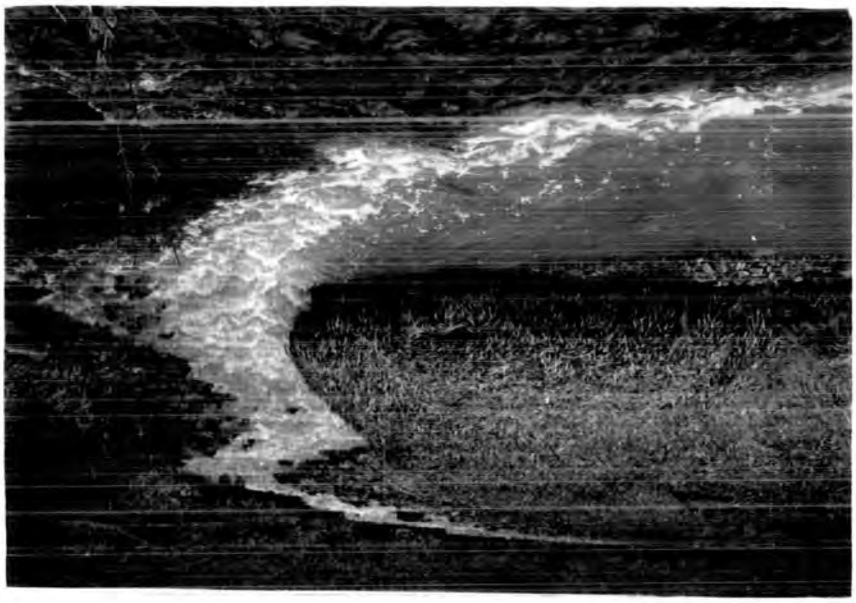
Bolts Burn and the R. Derwent at fairly high flow, with Bolts Burn carrying very high levels of suspended material derived from discharge from the Whiteheaps effluent and from surface runoff within the Whiteheaps Mine.

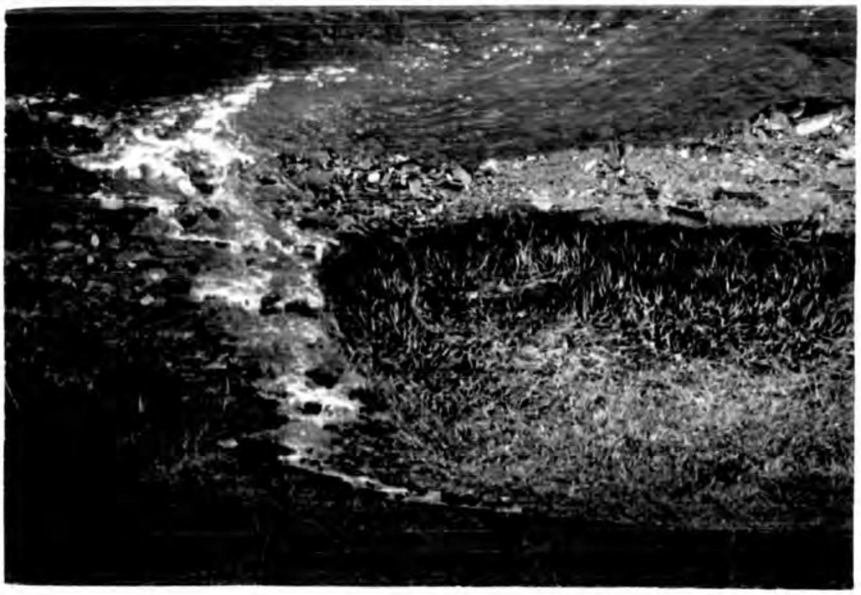
Fig. 4.6c

'Ribbon' of suspended solids clinging to the right-hand bank of the R. Derwent following the entry of Bolts Burn

Fig. 4.6 Bolts Burn entering the R. Derwent at reach 99.







derived from the Whiteheaps Adit Level should have occurred independently of high levels of lead and particulate material derived from the mine effluent and from surface runoff. Other levels characteristic of discharge from the adit or the effluent should have been found to show a positive statisical correlation with zinc or lead, and certain other parameters found in comparable concentrations in water from the adit or the effluent should have been found to show less clearly defined patterns of variation. Intervariable correlation analysis of the water chemistry data from reach 99 on Bolts Burn should therefore have demonstrated the association of certain ions into two separate 'blocks', showing co-ordinated variation caused by inputs from the adit and the effluent.

Examination of Fig. 4.7 and Table 4.4 shows that there is in fact no significant correlation between 'total' zinc and lead levels in the water of reach 99, as would be expected if inputs of the metals to the stream were independent of each other. On the other hand, a significant positive correlation ($r = + 0.6210$, $P = <0.001$) was found between variations in 'total' zinc and cadmium levels in the water. This is in agreement with the observation that higher levels of cadmium in Bolts Burn appeared to be derived from the Whiteheaps Adit Level. The levels of two other elements, sodium and magnesium, showed significant positive correlations with levels of zinc and cadmium. Both of these elements were found in higher concentrations in water from the Whiteheaps Adit Level than in water from the mine effluent.

Close relationships were found between variations in 'total' lead levels and variations in the 'total' levels of copper, iron, aluminium and fluoride in the water of Bolts Burn reach 99 (Fig. 4.8). These elements were all found in higher concentrations in water from the effluent than in water from the adit, and were all associated with suspended particulate material in the effluent discharge

	DISCHARGE	Na	K	Mg	Ca	Zn	Cu	Mn	Fe	Al	Pb	Ni	Co	Cd	Ag
Na	-0.5561**														
K	-0.5504**	0.8680**													
Mg	-0.5082**	0.2624**	0.8635**												
Ca	-0.5425**	0.7926**	0.8341**	0.7941**											
Zn	-0.3750*	0.3241**	0.2803*	0.2686*	0.1915										
Cu	-0.0049	-0.3277**	-0.1858	-0.4032**	0.0659	-0.1513									
Mn	-0.3311	0.3004*	0.3270**	0.2207	0.3041*	0.3381**	0.0316								
Fe	-0.0623	-0.4166**	-0.3140**	-0.5215**	-0.0880	0.0104	0.6699**	0.2227							
Al	-0.0716	-0.3574**	-0.2122	-0.3966**	0.1113	-0.1772	0.8638**	0.1270	0.7853**						
Pb	0.0537	-0.4490**	-0.2698*	-0.4830**	-0.1893	-0.0927	0.5635**	0.2775*	0.8241**	0.7257**					
Ni	-0.1162	-0.1156	-0.0349	-0.0159	0.0739	0.0096	0.1130	-0.0787	0.1773	0.1667	0.0758				
Co	-0.1716	0.0585	0.1123	0.1276	0.1109	-0.2632*	0.0066	-0.1435	-0.0812	-0.0168	-0.0628	-0.0405			
Cd	-0.2635	0.1790	0.2019	0.0673	0.0314	0.6427**	-0.0224	0.2611*	0.1404	-0.0464	0.0608	0.0370	-0.1658		
Ag	-0.0336	-0.3404**	-0.1325	-0.2911*	-0.1810	0.0062	0.2046	0.3046*	0.5367**	0.7750**	0.0005	0.1750	0.1750	0.1259	
F	-0.0191	-0.1736	-0.0809	-0.2500	0.2980*	-0.1925	0.8482**	0.0743	0.6985**	0.0039**	0.7833**	0.0427	-0.0960	-0.0731	-0.0522

Table 4.4 Intervariable correlation matrix showing the degree of correlation between variations in 'total' levels of 14 metals, river discharge and fluoride in Bolts Burn entering the R. Derwent (reach 99)

* - P = <0.01 ** - P = <0.001

Fig. 4.7

Scatter diagrams to show the degree of correlation between variations in 'total' zinc levels (plotted on the bottom axis in each case) and variations in the 'total' levels of other chemical parameters in Bolts Burn reach 99

All values are in mg l^{-1}

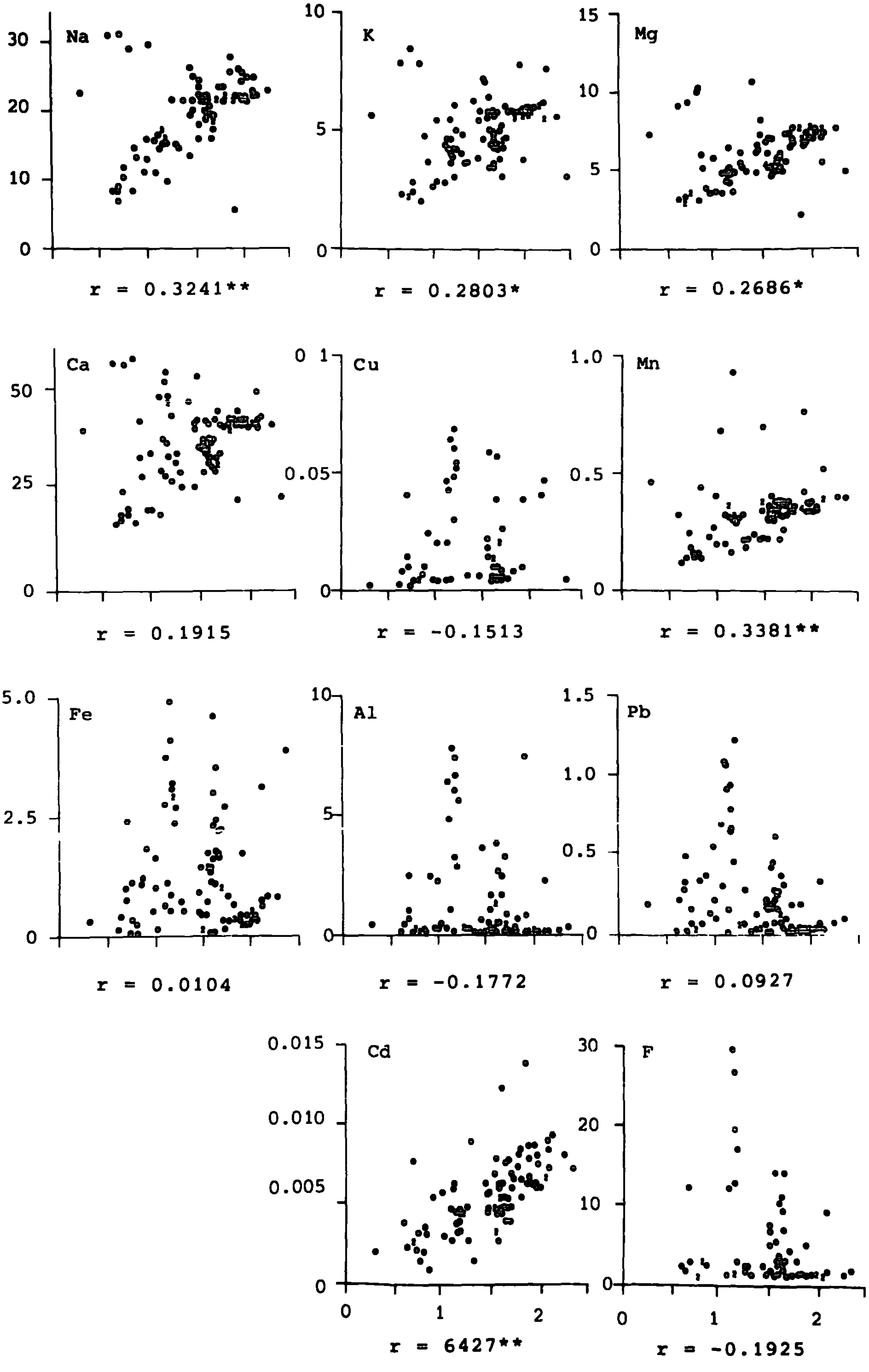
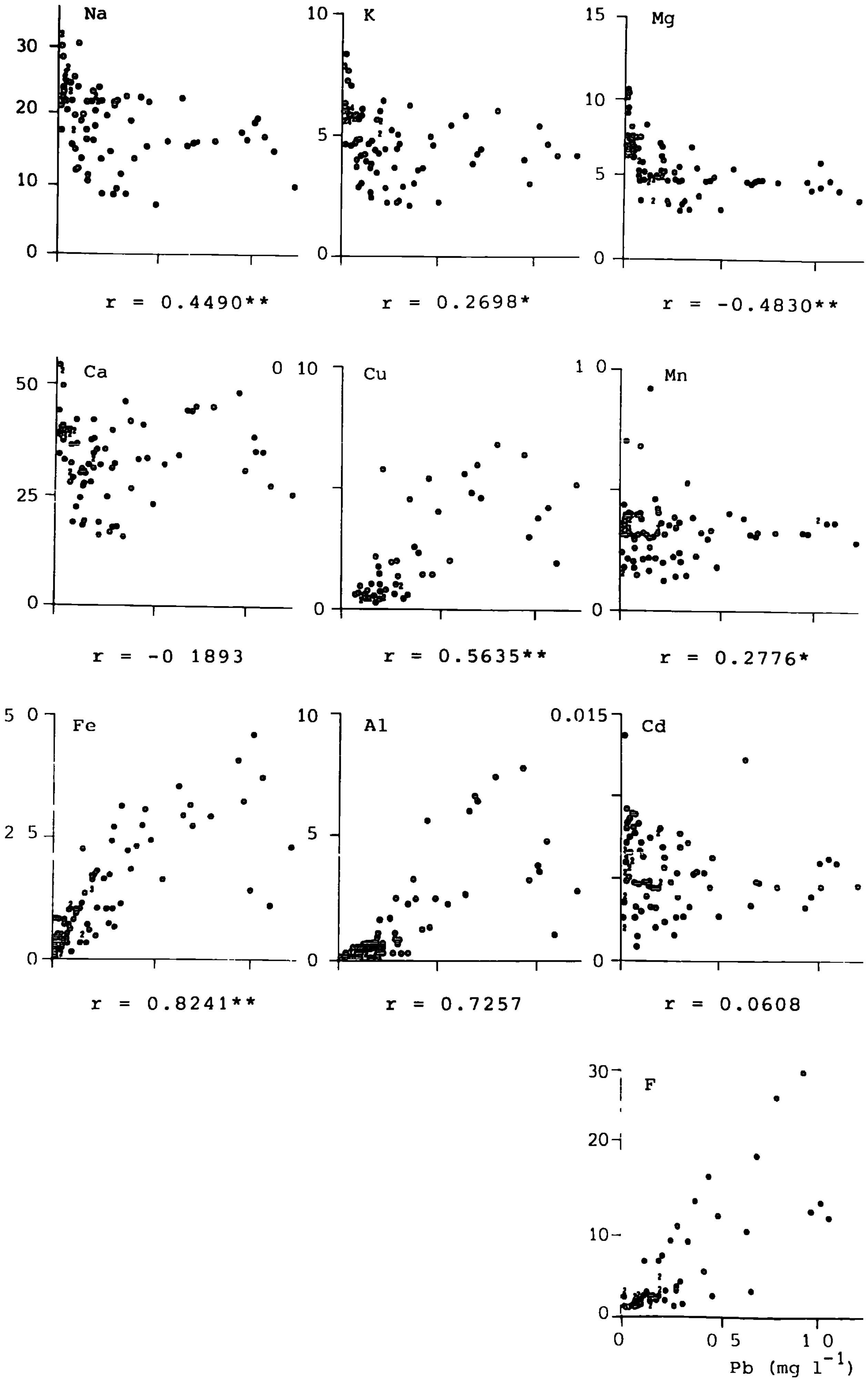

Na
30
20
10
0
r = 0.3241**
K
10
5
0
r = 0.2803*
Mg
15
10
5
0
r = 0.2686*
Ca
50
25
0
r = 0.1915
Cu
0 1
0.05
0
r = -0.1513
Mn
1.0
0.5
0
r = 0.3381**
Fe
5.0
2.5
0
r = 0.0104
Al
10
5
0
r = -0.1772
Pb
1.5
1.0
0.5
0
r = 0.0927
Cd
0.015
0.010
0.005
0
0
1
2
r = 6427**
F
30
20
10
0
0
1
2
r = -0.1925

Fig. 4.8

Scatter diagrams to show the degree of correlation between variations in 'total' lead levels (plotted on the bottom axis in each case) and variations in the 'total' levels of other chemical parameters in Bolts Burn reach 99

All values are in mg l^{-1}



r = 0.4490**

r = 0.2698*

r = -0.4830**

r = -0 1893

r = 0.5635**

r = 0.2776*

r = 0.8241**

r = 0.7257

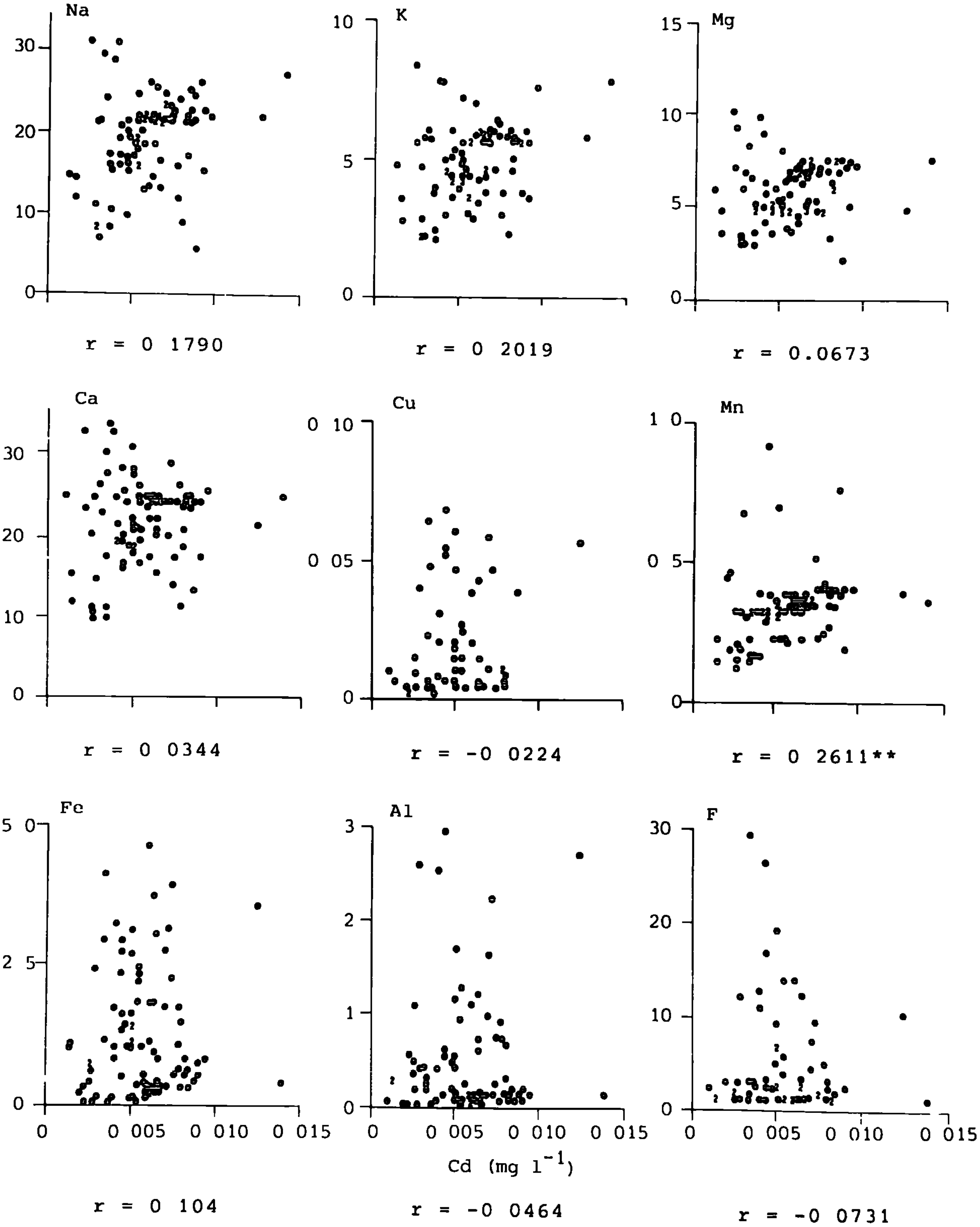
r = 0.0608

r = 0.7833**

Fig. 4.9

Scatter diagrams to show the degree of correlation between variations in 'total' cadmium levels (plotted on the bottom axis in each case) and variations in the 'total' levels of other chemical parameters in Bolts Burn reach 99.

All values are in mg l^{-1}



r = 0 1790

r = 0 2019

r = 0.0673

r = 0 0344

r = -0 0224

r = 0 2611**

r = 0 104

r = -0 0464

r = -0 0731

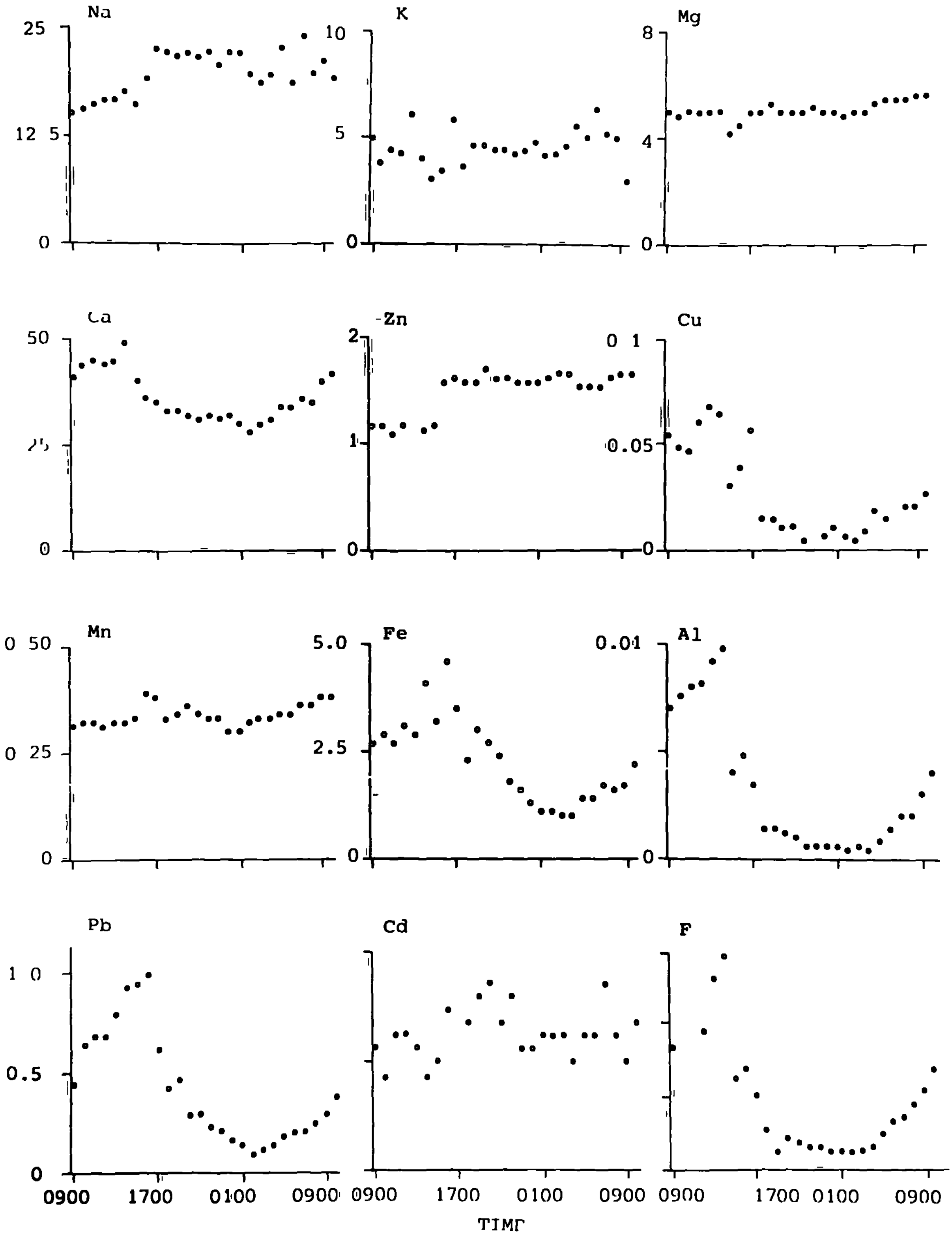
In water from reach 99, no more than 50% of the mean 'total' level of any of these elements was found to pass through 0 2 µm Nuclepore filters (compared with 86% of the mean 'total' zinc) It is interesting to note that at the higher 'total' concentrations of copper, iron, aluminium and lead the relationships between variations in the levels of the elements become less linear (the scattered points in the Cu/Pb, Fe/Pb and Al/Pb plots in Fig 4.8 'fan out'). This is probably because the highest concentrations of these elements in the water of the reach occur as suspended particles derived from surface runoff within the Whiteheaps area, with less clearly defined mineral composition than particles derived from the effluent discharge.

The effects of the separate inputs from the adit and from the effluent were clearly illustrated by the results of the 12 and 24 hour surveys carried out on 8 March and 18/19 March 1976. Although little variation was detected in the 'total' zinc or lead content of the water in reach 99 during the 12 hour survey, the zinc concentration remained high (1.5 - 2 2 mg l^{-1}) throughout the period whilst the lead concentration remained low (<0.1 mg l^{-1}). Information provided by the staff of the Whiteheaps Mine confirmed that whilst the adit had been flowing throughout the 12 hour period, the effluent had only started to flow slowly by the end of the day.

Wide variations in the concentrations of elements associated with both the adit and the effluent were detected in the water of Bolts Burn reach 99 during the 24 hour survey (Fig 4 10). At the commencement of the survey (0900) the stream water was very milky in appearance as a result of heavy discharges of suspended solids from the effluent Although no significant surface runoff was taking place within the mine area during the survey, the quality of the effluent was apparently affected by the cold weather conditions. A 'total' lead level of 8.65 mg l^{-1} was measured in a sample taken from the effluent discharge at

Fig. 4.10

Scatter diagrams showing variations in the 'total' levels of 11 cations and fluoride in Bolts Burn reach 99 during the 24 hour sampling survey of 18 - 19 March 1976


Na
25
12 5
0
K
10
5
0
Mg
8
4
0
Ca
50
25
0
Zn
2
1
0
Cu
0 1
0.05
0
Mn
0 50
0 25
0
Fe
5.0
2.5
0
Al
0.01
0
Pb
1 0
0.5
0
Cd
F
0900
1700
0100
0900
0900
1700
0100
0900
0900
1700
0100
0900
TIME

0930. The suspended solid content of the water in Bolts Burn reach 99 increased steadily throughout the day until about 1600, when a rapid decrease began to take place. The water was almost clear by midnight, and it remained clear until about 0700 when a steady increase in suspended load again began to take place

The results of analysis of 'total' water samples taken from reach 99 at hourly intervals showed a close relationship between the levels of suspended solids and the levels of the 'block' of elements associated with discharge from the effluent. Levels of copper, iron, aluminium, lead and fluoride in the water all rose to peaks at about 1600, dropped off sharply as discharge from the effluent ceased, and began to increase again as work began in the treatment plants early next morning.

Elements associated with discharge from the Whiteheaps Adit Level showed a strikingly different pattern of variation in the water of reach 99 during the 24 hour survey. Samples were taken from the adit at 0930, 1230 and 1530 on 18 March, and it is known that the adit pump was not in operation at 0930, but had switched on by 1230 This resulted in a sharp increase in 'total' zinc levels in the water of Bolts Burn reach 99 between 1500 and 1600, together with increases in 'total' levels of the associated elements sodium, magnesium and cadmium. As can be seen from Fig. 4.10 these increased levels remained steady throughout the night A trip to the Whiteheaps Mine at 1000 next morning confirmed that the mine pump was still in operation

Further evidence that discharge from the Whiteheaps Adit Level was the principal source of the zinc carried by Bolts Burn at reach 99 is presented in Fig. 4.11, which is a histogram plotting the number of records of 'total' zinc within a particular range against 'total' zinc concentration.

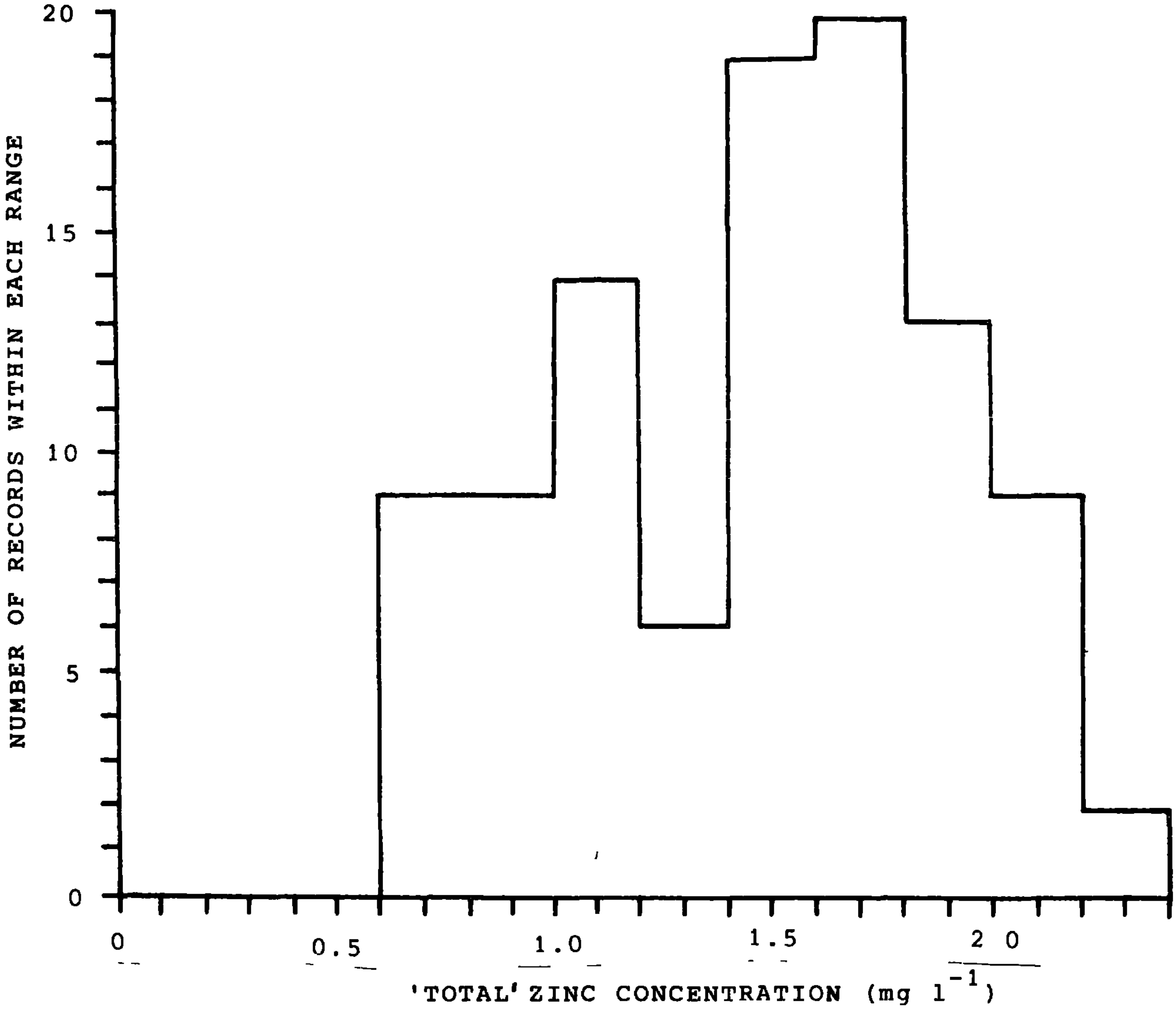


Fig 4.11 Histogram plotting frequency of different 'total' zinc concentration in Bolts Burn reach 99

The histogram has a double peaked form, with the majority of the 'total' zinc records falling into either a lower concentration range (0 6 - 1 2 mg l^{-1}) or a higher range (1 4 - 2 2 mg l^{-1}). This pattern is almost certainly caused by the switching on and off of the adit pump, despite the randomizing effect of variations in stream discharge it is still apparent 3.5 km downstream of the adit.

If the assumption is made that the 'total' zinc records in the 0.6 - 1 2 mg l^{-1} range were recorded when the adit pump was switched off and that the adit was flowing when the 'total' zinc levels fell into the 1.4 - 2 2 mg l^{-1} range, then the Whiteheaps Adit Level was flowing on approximately 66% of the occasions when water from Bolts Burn reach 99 was sampled.

No clear seasonal pattern was found for the frequency of occurrence of different 'total' levels of zinc in the water of Bolts Burn reach 99 (Fig. 4 12a), whilst the highest 'total' levels of lead were recorded during the winter and spring months (Fig. 4 12b). These results are in accordance with the observations that discharge from the Whiteheaps Adit Level appeared to be completely unaffected by changes in season or weather, whilst the quality of the Whiteheaps Mine Effluent discharge appeared to be adversely affected by wet or cold weather Surface runoff from the Whiteheaps Mine area also contributed to the levels of particulate lead carried by the water of reach 99 during wet weather.

4 32 Water chemistry of the R Derwent

4 321 Sources of heavy metals apart from Bolts Burn

Old mine workings are present in several of the side valleys to the R. Derwent valley, however only one tributary apart from Bolts Burn was found to carry elevated levels of zinc on entering the river In Shildon Burn (0124-99) the 'total' zinc content of the water ($\bar{x} = 0.457$, n = 14) was often slightly higher than that of the R. Derwent at its point of entry The absolute contribution to the tributary was however only slight, because of its small size.

Although old mine workings (associated with the old Shildon Mine) are in evidence around the upper reaches of Shildon Burn, the main source of the zinc carried by the stream water was found to be 'Shildon Adit' (0178-01) which discharges into the stream just upstream of Blanchland

Fig. 4.12

Scatter diagrams plotting all 'total' zinc and lead levels recorded in Bolts Burn reach 99 against the months in which the samples were taken.

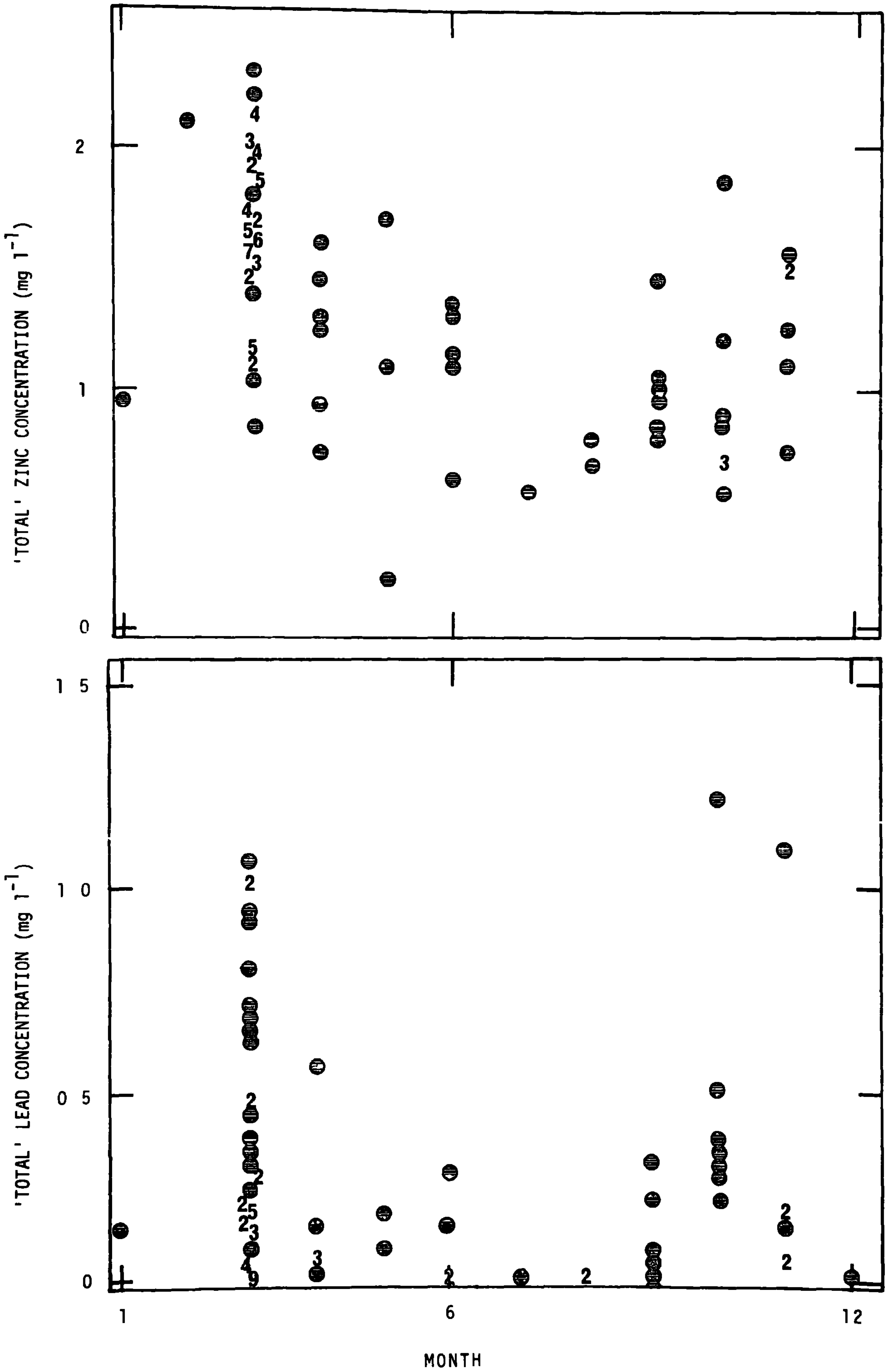
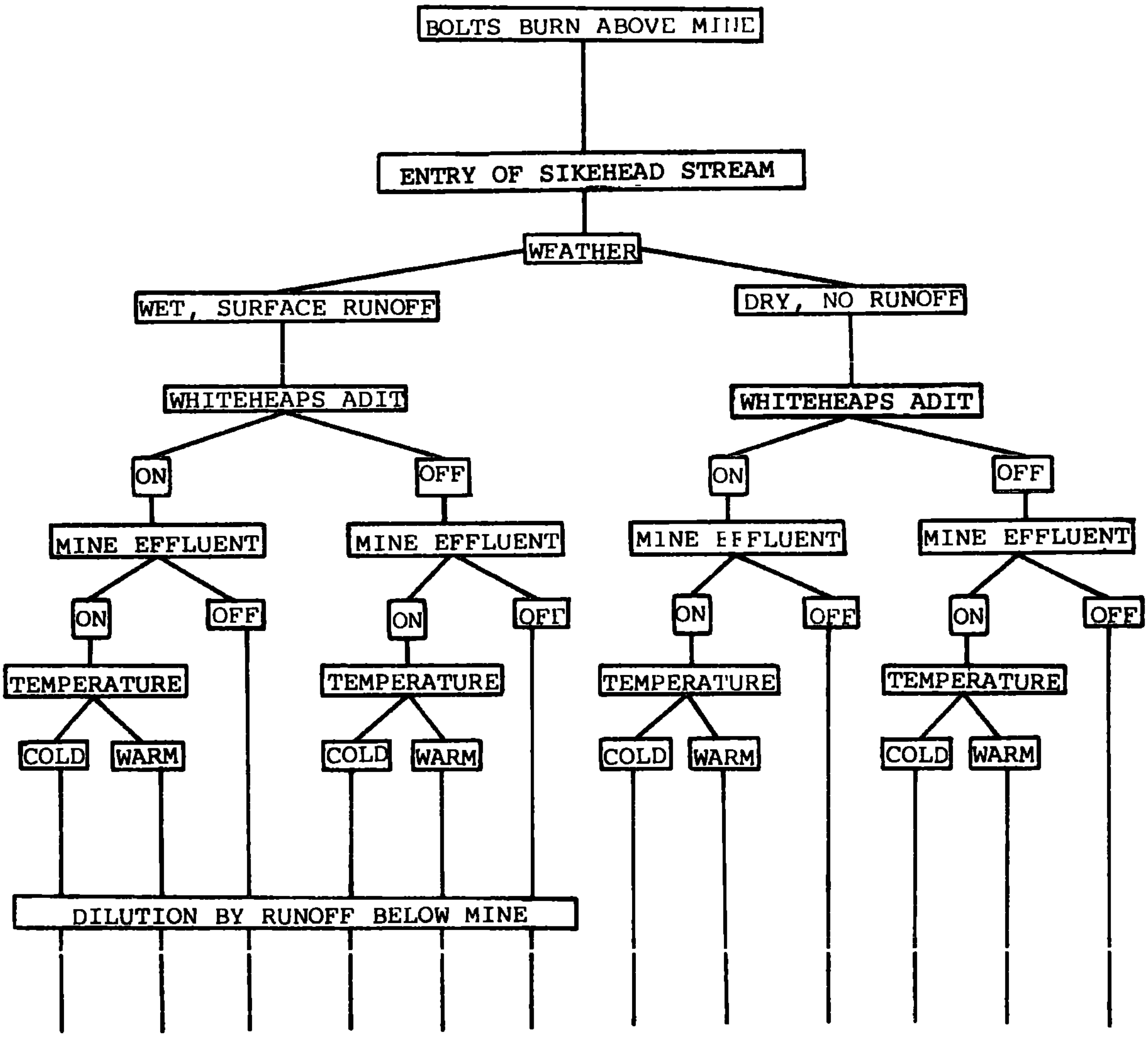

'TOTAL' ZINC CONCENTRATION (mg l^{-1})
0
1
2
'TOTAL' LEAD CONCENTRATION (mg l^{-1})
0
0 5
1 0
1 5
1
6
12
MONTH

FIG 4.13 DIAGRAM TO SHOW THE EFFECT OF FACTORS WITHIN THE WHITEHEAPS MINE ON THE CHEMISTRY OF BOLTS BURN REACH 99



DISSOLVED ZINC	H	H	H	M	M	M	VH	VH	VH	M	L	L
PARTICULATE ZINC	H	H	H	H	H	H	M	L	L	M	L	L
DISSOLVED LEAD	H	M	M	H	M	M	M	L	L	M	L	L
PARTICULATE LEAD	VH	VH	H	VH	VH	H	H	M	L	H	M	L
DISSOLVED CADMIUM	H	H	H	M	M	M	H	H	H	L	L	L
PARTICULATE CADMIUM	H	H	M	H	H	H	L	L	L	M	L	L

VH = VERY HIGH

H = HIGH

M = MEDIUM

L = LOW

4.322 R. Derwent upstream of the entry of Bolts Burn (0061-05)

The R Derwent at reach 05 was sampled under a wide variety of flow conditions during the three year period. On every occasion the 'total' zinc, lead and cadmium content of the water in the reach was found to be very low ($\bar{x}$ = 0.026 mg l^{-1}, 0.007 mg l^{-1}, 0.0005 mg l^{-1} respectively, n = 48 in each case) It can therefore be concluded that the old mine workings that had been located around the headwaters of the stream during the exploratory surveys never had a significant effect on the metal content of the river upstream of the entry of Bolts Burn. It can be seen from column 1 of Table 4.5 that variations in the 'total' concentrations of several elements show highly significant ($P = < 0\ 001$) positive or negative correlations with river discharge 'Total' zinc, manganese, iron, lead and cadmium concentrations all show significant positive correlations with discharge, i e. increases in the concentrations of these elements directly related to increases in flow Manganese and iron are elements characteristic of suspended clay minerals (Kennedy, Zellweger & Jones, 1974), and increases in the levels of these elements during higher flows probably simply reflect increases in the suspended load of the river water.

In direct contrast, 'total' concentrations of magnesium and calcium in the water of the R. Derwent reach 05 show significant negative correlations with river discharge. Concentrations of these elements therefore decreased during higher flows, probably because their sources in the upper catchment of the river are groundwater inputs (there is little exposed limestone) which maintain relatively stable discharge and are diluted more than usual during periods of high flow on the river.

The pattern of correlations between discharge and 'total' cation concentrations in the water of Bolts Burn reach 99 and the R. Derwent reach 05 is strikingly different In the water of reach 05 the three metals zinc, lead and cadmium are strongly intercorrelated and appear to be associated

	DISCHARGE	Na	K	Mg	Ca	Zn	Cu	Mn	Fe	Al	Pb	Ni	Co	Cd	Ag
Na	-0 2229														
K	-0 4021	0 8566**													
Mg	-0 5896**	0 2864	0 6215**												
Ca	-0 6482**	0 2093	0 6351**	0 9583**											
Zn	0 6224**	0 0084	-0 1891	-0 4647**	-0 5338**										
Cu	0 1605	-0 0655	-0 0697	-0 0424	-0 0343	0 3308									
Mn	0 6979**	-0 1311	-0 3316	-0 4910**	-0 5575	0 8078**	0 3505*								
Fe	0 7767**	-0 0882	-0 2684	-0 6767**	-0 7017**	0 6464**	0 0874	0 6161**							
Al	0 7071**	-0 1425	-0 2192	-0 4455*	-0 4738**	0 5508**	0 2716	0 6370**	0 8438**						
Pb	0 5253**	-0 0649	-0 1900	-0 4086*	-0 4568**	0 7720**	0 2941	0 5976**	0 6361**	0 6260**					
Ni	0 0689	-0 0109	0 0398	0 0848	0 1346	-0 1186	0 3372	-0 0994	-0 1768	-0 1009	-0 0272				
Co	0 2540	0 0222	-0 0435	0 0820	-0 1517	0 4498**	0 0441	0 5151**	0 2495	0 2129	0 2265	-0 0541			
Cd	0 1851	0.1392	-0 1383	-0 2932	-0 3375	0 4092*	-0 0823	0 3465	0 2879	0 2286	0 3952*	-0 0767	0 2486		
Ag	-0 0936	0 0020	0 0017	0 0015	0 0014	0 0019	0 0014	0 0008	0 0022	0 0007	0 0005	0 0020	0 0005	0 0003	
F	-0 4054	0 1745	0 4685	0 8186**	0 8726**	-0 3950	-0 0203	-0 4124	-0 5805*	-0.4962*	-0 3602	0 0006	-0 1149	-0 3888	

Table 4.5 Intervariable correlation matrix showing the degree of correlation between variations in 'total' levels of 14 metals, river discharge and fluoride in the R. Derwent upstream of Bolts Burn (reach 05)

* - P = <0.01 ** - P = <0.001

with particulate material (possibly including clay minerals), derived from surface runoff. In the water of the polluted tributary, however, no positive correlation was found between stream discharge and the levels of any element. In the water of Bolts Burn the inputs from the sources associated with the Whiteheaps Mine presumably 'swamped' any correlation that might be expected between discharge and elements derived from runoff of particulate clays etc.. Unlike reach 05 on the R. Derwent, 'total' zinc and lead levels in the water of Bolts Burn showed no correlation with each other, because of their separate sources within the Whiteheaps Mine.

A 'block' of six elements (Na, K, Mg, Ca, Zn, Mg) showed significant negative correlations with discharge in Bolts Burn reach 99. These elements were associated with relatively constant flows from the Whiteheaps Adit Levels, and were diluted by high flows in the stream. Levels of zinc and manganese thus show a completely opposite relationship with discharge in the R. Derwent reach 05 and in Bolts Burn reach 99.

The fact that 'total' heavy metal concentrations in the water of the R. Derwent upstream of the entry of Bolts Burn were found to increase in direct proportion to river discharge is evidence against the existence of any undetected groundwater sources of high levels of heavy metals (e.g. adits) in the upper R. Derwent catchment. Water from such sources would tend to become diluted out during higher river flows, leading to negative correlations between levels of heavy metals and discharge in the water of the river at reach 05. Although some of the particulate matter with which the metals appeared to be associated in the river water was probably washed from old mine tips around Nookton and Beldon Burns, it is worth stressing again that even under conditions of high flow only very low levels of 'total' zinc, lead and cadmium were found in the river upstream of the entry of Bolts Burn during flood conditions.

4.323 R. Derwent downstream of the entry of Bolts Burn (0061-07)

When Bolts Burn was carrying high levels of suspended solids because of inputs from the Whiteheaps Mine Effluent and / or inputs from surface runoff within the Whiteheaps area, its effect on the R. Derwent downstream of its entry could be visually quite dramatic. A milky white 'ribbon' of suspended solids followed the right hand bank of the river for a short distance below the entry of the tributary (Fig. 4.6d), and then mixed with the river water as it passed through two pools and two fast-flowing stretches Water samples were taken at various points in the river to investigate the 'mixing profile' of zinc in the river downstream of the tributary on 5 March 1976. The results (Fig. 4.14), confirmed that mixing was complete before reach 07 on the R. Derwent.

The entry of Bolts Burn was found to have a profound influence on the water chemistry of the river at reach 07. Water from the reach was sampled 101 times at this point, and on every occasion a marked increase in the 'total' concentrations of zinc, lead and cadmium were found in comparison with the water of reach 05 (Tables 4 2, 4.3). The mean recorded levels were 0.317 mg l^{-1} Zn, 0.051 mg l^{-1} Pb and 0.0018 mg l^{-1} Cd, representing mean increases of x 12.2, x 7.3 and x 3.6 when compared with the mean 'total' levels recorded in reach 05.

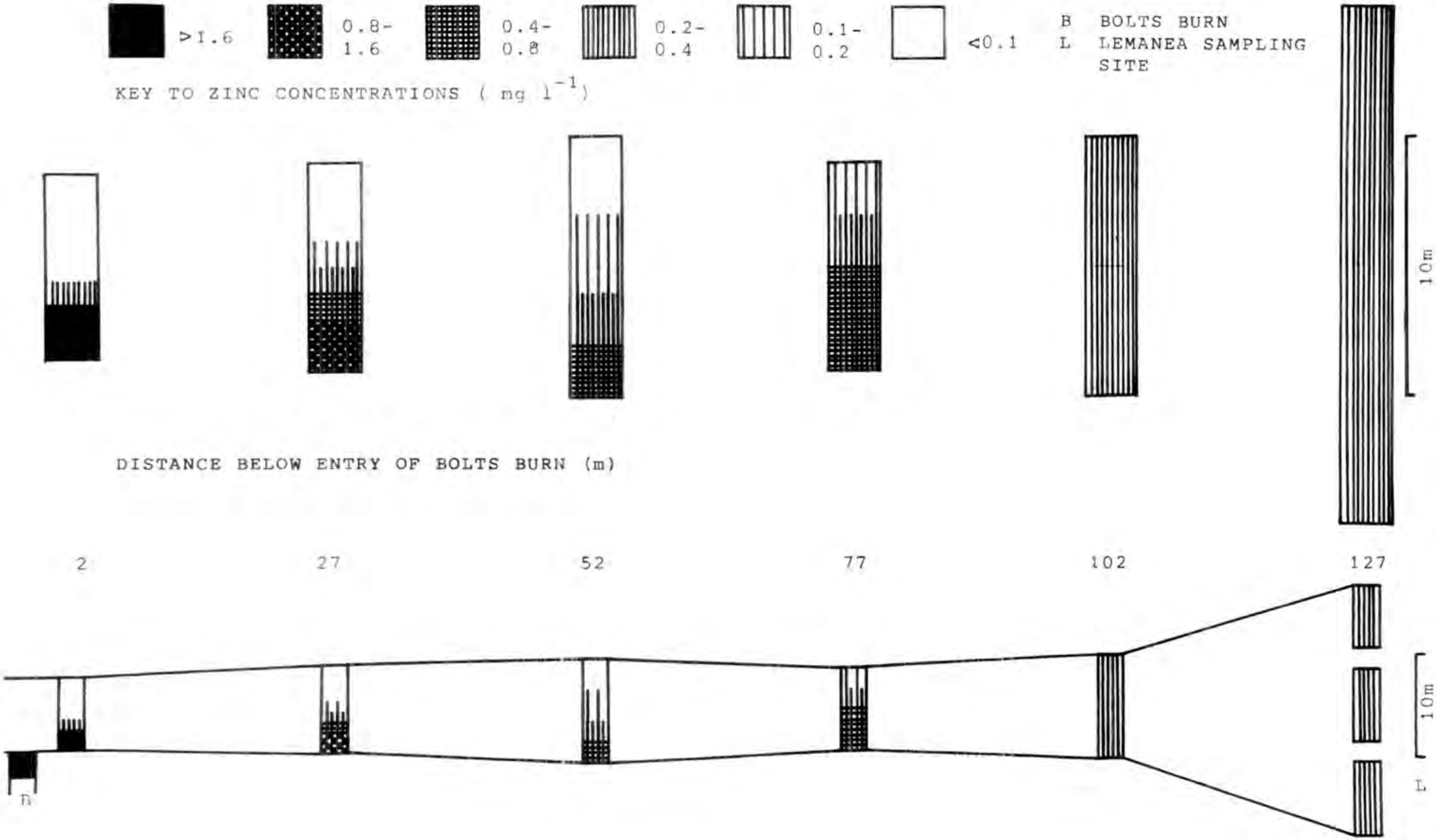
Statistical analysis of the variations of discharge and chemical parameters within the water of reach 07 was carried out in the same manner as for the data from reach 05 on the river and reach 99 on Bolts Burn (Table 4.6). The results showed that variations in 'total' levels of zinc, lead and cadmium in the water followed those found in Bolts Burn closely. 'Total' levels of zinc were found to vary independently of 'total' levels of lead, and the same two 'blocks' of elements associated with inputs from the Whiteheaps Adit Level and the Whiteheaps Mine Effluent were found to show co-ordinated independent variation. The

	DISCHARGE	Na	K	Mg	Ca	Zn	Cu	Mn	Fe	Al	Pb	Ni	Co	Cd	Ag
Na	-0 6211**														
K	-0 5379**	0 8005**													
Mg	-0 6251**	0 7598**	0 8936**												
Ca	-0 6110**	0 7783**	0 9083**	0 9778**											
Zn	-0 3378	0 3428**	0.4259**	0 4910**	0 4471**										
Cu	0 4517*	-0 1928	-0 0931	-0 1953	-0 1388	-0 2976*									
Mn	0 3113	-0 1237	-0 2159	-0 3119**	-0 2918*	-0 1910	0 0463								
Fe	0 4443*	-0 3575**	-0 4538**	-0 6185**	-0 5390**	-0 4494**	0 4286**	0 4153**							
Al	0 4375*	-0 1644	-0 2963*	-0 5260**	-0 4396**	-0 4675**	0 5077**	0 3185**	0 8199**						
Pb	0 5178*	-0 2491*	-0 1569	-0 3328**	-0 2378*	-0 3496**	0 5806**	0 1632	0 5274**	0 6351**					
Ni	0 3588	-0 1574	0 0481	0 1136	0 1156	0 0745	-0 0226	0 1235	-0 1361	-0 1802	-0 0739				
Co	-0 0458	0 1489	0 2379	0 2626*	0 2757*	-0 1074	0 2547*	0 0548	-0 0842	-0 0288	0 1227	0 1005			
Cd	-0 1895	-0 0211	-0 1464	-0 1254	-0 1070	0 0450	-0 0171	-0 0492	0 0363	-0 0112	-0 0893	-0 0562	-0 1243		
Ag	0 0005	0 0027	-0 0005	0 0016	0 0010	0 0012		0 0004				0 0003	0 0013	0 0009	
F	-0 0596	0 1045	0 1144	-0 0270	0 0440	-0 1876	0 4950**	0 0721	0 4004**	0 5768**	0.5591**	-0 1058	-0 0050	-0 0102	

Table 4.6 Intervariable correlation matrix showing the degree of correlation between variations in 'total' levels of 14 metals , river discharge and fluoride in the R. Derwent downstream of Bolts Burn (reach 07)

* - P = <0.01 ** - P = <0.001

Fig. 4.14 'Mixing profile' of zinc in the R. Derwent downstream of the entry of Bolts Burn.


>1.6
0.8-1.6
0.4-0.8
0.2-0.4
0.1-0.2
<0.1
B BOLTS BURN
L LEMANEA SAMPLING SITE
KEY TO ZINC CONCENTRATIONS (mg l^{-1})
DISTANCE BELOW ENTRY OF BOLTS BURN (m)
10m
2
27
52
77
102
127
10m
B
L

composition of the river water in reach 07 followed the composition of the water in Bolts Burn closely during the 24 hour survey, with an increase in zinc levels caused by the operation of the adit pump, and a variation of lead levels throughout the 24 hour period associated with working hours within the Whiteheaps fluorspar mine.

4.324 R. Derwent entering the Derwent Reservoir (0061-25)

The R. Derwent was sampled for water on 65 occasions at its entry point into the Derwent Reservoir, including samples taken at half-hourly intervals during a 12 hour period on 18 March 1976. Mean 'total' levels of zinc, lead and cadmium within water from the reach (0.217 mg l^{-1}, 0.061 mg l^{-1} and 0.0018 mg l^{-1} respectively) were found to be only slightly lower than the mean levels found in reach 07 (3.5 km further upstream). Because the levels of all three metals were comparatively high in the water of the reach throughout the 12 hour survey, these mean levels may be a slight over-estimate of the long-term picture.

The extremes of chemical variation found in the water of the R. Derwent at reach 07 were found to 'dampen out' somewhat by reach 25. Nevertheless marked fluctuations in 'total' lead levels were found during the 12 hour survey, and the river entering the reservoir was observed to be very cloudy on occasions following heavy release of solids from the Whiteheaps Mine.

Although some metals sedimented in the R. Derwent between reaches 07 and 25, most of the fine silt deposited along the river during low flow conditions was scoured into the reservoir during high flows. A high degree of positive correlation was found between variations in river discharge and variations in 'total' levels of 'particulate' elements such as iron, aluminium and lead (Table 4.7). This is in contrast to the behaviour of these elements in reach 07, and is probably the result of settling-out of suspended material along the river followed by re-suspension during higher flows.

	DISCHARGE	Na	K	Mg	Ca	Zn	Cu	Mn	Fe	Al	Pb	Ni	Co	Cd	Ag
Na	-0 4233*														
K	-0 1846	0 8487**													
Mg	-0 4313*	0 8142**	0 9040**												
Ca	-0 3552*	0 8349**	0 9237**	0 9851**											
Zn	0 5430**	-0 5689**	-0 4354**	-0 5352**	-0 5330**										
Cu	0 6697**	-0 1247	-0 0790	-0 2182	-0 2082	0 2291									
Mn	0 7620**	-0 4498**	-0 3453*	-0 5511**	-0 5449**	0 5231**	0 3400*								
Fe	0 7707**	-0 3405*	-0 2015	-0 4809*	-0 4260**	0 5301**	0 4343**	0 7020**							
Al	0 7217**	-0 2617	-0 1457	-0 4384**	-0 3715*	0 4324**	0 4255**	0 6739**	0 9104**						
Pb	0 8302**	-0 3096*	-0 0526	-0 2966*	-0 2112	0 5412**	0 4184**	0 5389**	0 7786**	0 7906**					
Ni	0 2901	-0 0906	0 1051	-0 0640	-0 0364	0 1485	0 0180	0 4180**	0 4224**	0 4417**	0 3098				
Co	-0 1136	0 3631*	0 4959**	0 4491**	0.4540**	-0 2957	-0 1081	-0 1217	-0 0844	-0 0457	-0 0801	0 2011			
Cd	0 0749	-0 0140	-0 0414	-0.1041	-0 1052	0 1756	0 2507	0 0577	0 0731	0 0035	0 0318	-0 0875	-0 0902		
Ag	0 0003		-0 0006					0 0011	0 0010				0 0006		
F	-0 0545	0 1492	-0 0324	-0 0652	-0 0295	-0 0046	-0 0349	0 0703	0.0653	0 0289	0 1559	-0 1995	-0.0674	-0 0740	0 0006

Table 4.7 Intervariable correlation matrix showing the degree of correlation between variations in 'total' levels of 14 metals , river discharge and fluoride in the R. Derwent entering the Derwent Reservoir (reach 25)

* - P = <0.01 ** - P = <0.001

An interesting feature of the pattern of cross-correlations shown in Table 4.7 is the high degree of positive correlation between 'total' zinc levels, discharge, and levels of the elements associated with particulate matter. This is in complete contrast to the behaviour of the metal in the water of reach 07, and is probably explained by binding of zinc by some component of particulate matter in the river between the entry of Bolts Burn and the reservoir. A high proportion (95%) of the mean 'total' zinc level in the water of reach 25 was found to be capable of passing through 0 2 μm Nuclepore filters, indicating that if significant binding of the element had taken place then the size range of the particles was very small.

The water of the R Derwent frequently carries high levels of yellow humic material (derived from peat in the catchment), and it seemed possible that the apparent sedimentation of zinc along the river was caused by a loose association of organically complexed zinc (largely capable of passing through 0.2 μm filters) with outwardly-directed functional groups on the sediment surface.

An opportunity to study a phenomenon that might have been caused by the binding of cations by organic material in the water of the R. Derwent presented itself on 16 September 1976. Water was collected from reaches within the R. Derwent catchment under high flow conditions following an extended drought. On return to the laboratory it was found that the pH of some of the samples had decreased markedly following collection, by as much as 1 pH unit in some cases. It seemed possible that this lowering in pH values was the result of the release of protons from exchange sites on organic molecules that had only been present in the water for short periods, following exchange with cations in the water.

To investigate this phenomenon further, a collection of unfiltered water was made from four 'key' reaches (still under high flow conditions) on 17 September 1976. pH was measured at intervals following collection, and samples were passed through 0.2 µm Nuclepore filters to investigate possible changes in the filtrability of zinc in the water.

The results of this experiment are presented in Table 4.8. From this table it can be seen that by 24 hours after collection a marked decrease in the pH of all of the samples had taken place. The pH of water from reach 05 on the R. Derwent decreased by 0.6 units (from 7.1 to 6.5) during the period, whilst that of water from Bolts Burn reach 99 and reaches 07 and 25 on the river decreased by 0.8 - 0.9 units. These observations are in accordance with the hypothesis cations entering the river from Bolts Burn were exchanging with protons on exchange sites on organic molecules or particles. It is apparent from Table 4.8 that little change was found throughout the period in the percentage of zinc in the water from the R. Derwent capable of passing through a 0.2 µm filter (similar results were recorded for calcium and magnesium). It seems possible that even if these ions were bound onto organic functional groups during laboratory storage, the complexes were still capable of passing through the filters

4.325 R. Derwent downstream of the Derwent Reservoir (0061-30)

The R. Derwent downstream of the Derwent Reservoir was sampled 16 times between 8 and 23 March 1976, and once on 18 March 1977. During the 1976 samplings wide flucuations took place in the flow of the R Derwent entering the reservoir, but these were not reflected in reach 30 as the reservoir was not overflowing On 18 March 1977 the reservoir was overflowing, and flows in the reach had been fairly high for some time.

The mean 'total' zinc, lead and cadmium levels recorded in water from the reach during the samplings of March 1976 were 0.064 mg l^{-1}, 0.007 mg l^{-1} and <0.0001 mg l^{-1}

pH:

Site \ time after collection (h):	0	3	6	9	12	24
R. Derwent reach 05	7.1	6.6	6.6	6.6	6.4	6.5
R. Derwent reach 07	7.2	6.7	6.6	6.6	6.4	6.5
R. Derwent reach 25	7.3	6.7	6.5	6.4	6.4	6.4
Bolts Burn reach 99	7.3	6.7	6.6	6.5	6.3	6.4

% zinc passing
through 0.2 µm
Nuclepore filter:

Site \ time after collection (h):	0	3	6	9	12	24
R. Derwent reach 05	100	97	100	100	100	100
R. Derwent reach 07	100	91	91	100	91	100
R. Derwent reach 25	97	100	100	94	96	100
Bolts Burn reach 99	98	81	84	81	84	84

Table 4.8 Effect of storage on pH and filtrability of zinc in samples collected under flood conditions in the catchment area

DATE	Na	K	Mg	Ca	Zn	Cu	Mn	Fe	Al	Pb	Ni	Co	Cd	Ag
8-3-76	7.7	1.48	2.70	11.5	0.044	< 0.002	0.080	0.32	0.13	0.016	< 0.01	< 0.01	< 0.0001	< 0.005
9-3-76	8.4	1.72	2.70	11.9	0.081	"	0.087	0.29	0.15	0.060	"	"	"	"
10-3-76	7.7	1.46	2.80	11.3	0.059	"	0.086	0.30	0.10	0.010	"	"	"	"
11-3-76	7.5	1.46	2.75	11.5	0.052	"	0.093	0.33	0.29	0.010	"	"	"	"
12-3-76	7.6	1.48	2.70	11.7	0.059	"	0.080	0.33	0.08	0.010	"	"	"	"
13-3-76	7.8	1.46	2.75	12.0	0.081	"	0.101	0.29	0.11	0.010	"	"	"	"
14-3-76	7.8	1.58	2.75	11.0	0.059	"	0.100	0.29	0.06	0.015	"	"	"	"
15-3-76	7.6	1.52	2.70	11.5	0.074	"	0.087	0.27	0.01	0.008	"	"	"	"
16-3-76	7.6	1.54	2.75	11.5	0.067	"	0.087	0.30	0.08	0.010	"	"	"	"
17-3-76	8.2	1.60	2.95	11.9	0.044	"	0.102	0.29	0.10	0.025	"	"	"	"
18-3-76	7.9	1.50	2.65	11.8	0.067	"	0.087	0.26	0.13	0.013	"	"	"	"
19-3-76	7.6	1.60	2.65	11.9	0.067	"	0.087	0.29	0.11	0.020	"	"	"	"
20-3-76	8.1	1.54	2.85	12.0	0.067	"	0.167	0.57	0.31	0.015	"	"	"	"
21-3-76	8.1	1.62	2.75	11.5	0.074	"	0.093	0.22	0.18	0.010	"	"	"	"
22-3-76	7.8	1.56	2.75	11.5	0.067	"	0.087	0.26	0.13	0.020	"	"	"	"
23-3-76	7.7	1.62	2.75	11.9	0.067	"	0.093	0.24	0.13	0.015	"	"	"	"
18-3-77	7.9	1.78	3.10	12.5	0.075	"	0.088	0.27	0.14	0.011	"	"	"	"

Table 4.8 cont Compiled 'total' water chemistry of the R. Derwent below the Derwent Reservoir (reach 30)

respectively. It is therefore clear that during this period only a very small proportion of the heavy metals carried by the river at reach 25 had penetrated as far as the compensation water outlet The sample taken on 18 March 1977 contained somewhat higher 'total' metal levels (0 075 mg l^{-1} Zn, 0.011 mg l^{-1} Pb and 0.0001 mg l^{-1} Cd)

Although no such instances were detected during the project, it was demonstrated conclusively by Mr R. W. Hunter (personal communication) that following major flooding on the R. Derwent (as happened during autumn of 1976) 'streaming' of water along the bottom of the reservoir could on occasions lead to elevated metal levels in the water of reach 30 This phenomenon is discussed in more detail in Chapter 5

4.33 Quantification of heavy metal inputs to the Derwent Reservoir

One of the objectives of including measurements of stream and river discharge in the surveys of water chemistry carried out within the catchment area of the R. Derwent was to attempt to estimate the absolute amounts of heavy metals entering the R. Derwent from Bolts Burn, together with the absolute additions of metals to the reservoir itself.

Estimates of the absolute amounts of zinc, lead and cadmium carried by Bolts Burn reach 99 and by the R. Derwent at reaches 05, 07 and 25 are given in Table 4 9. These levels were calculated using the means of all 'total' levels of the three metals and the means of all recordings of discharge.

Fig. 4.15a

Discharge measurement plate installed in Bolts Burn by the Northumbrian Water Authority.

Fig. 4.15b

Discharge measurement plate installed in the R. Derwent at its entry to the Derwent Reservoir (reach 25)

Fig. 4.15 Discharge measurement plates.

Site	Zn	Pb	Cd
Bolts Burn reach 99	0.1119	0.0213	0.0004
R Derwent reach 05	0.0124	0.0033	0.0002
R. Derwent reach 07	0.1902	0.0306	0 0011
R. Derwent reach 25	0.2528	0 0711	0.0021

Table 4.9 Estimates of absolute levels of zinc, lead and cadmium carried by Bolts Burn and the R. Derwent (g s^{-1})

On examination of these results, it can be seen that the figures for the absolute levels of metals carried by Bolts Burn at reach 99 seem erroneously low (the increase in the metal content of the river from upstream to downstream of the entry of Bolts Burn is greater than the estimated input from the stream). This is almost certainly because the measurement stake in Bolts Burn was incorrectly calibrated Further, the absolute levels of metals carried by the R. Derwent entering the reservoir appear to be slightly higher than in the river immediately downstream of Bolts Burn. Whilst some such increase would be expected because of inputs of metals from Shildon Burn, part of the difference can probably be explained by the error in the calibration of the plate in Bolts Burn leading to an under-estimation of discharge in reach 07 on the river.

The mean 'total' levels of zinc, lead and cadmium recorded in the water of the R. Derwent at reach 30 were much lower than the mean levels in the river at reach 25, with mean decreases of 70 5%, 88 5% and 97.2% respectively. Assuming that the water extracted for treatment from the eastern end of the reservoir had a similar composition to that being released into reach 30, these percentages also indicate the proportion of the three metals being accumulated within the reservoir

element	total annual addition (kg)	level of water below top water level (m)	0	3.3	6.6	9.9
		area of reservoir bed exposed (m^2)	4050000	3470445	2903040	2373705
Zn	5619.7		1.388	1.619	1.936	2.367
Pb	1983.6		0.490	0 572	0.683	0.836
Cd	63.1		0.016	0.018	0.022	0.027

Table 4.10 Estimates of annual additions to the Derwent Reservoir of zinc, lead and cadmium

(g $year^{-1} m^{-2}$ reservoir bottom)

Estimates were made of the mean annual additions of zinc, lead and cadmium to the Derwent Reservoir during the period of study (Table 4.10) These estimates were based on the calculated absolute levels of these metals in the water of the river at reach 25, and the retention rates detailed above They must be regarded as only approximate, (and in any case might be expected to vary markedly from year to year). The assumption of even deposition over the bed of the reservoir is obviously an over-simplification. Large floods (carrying fine particulate matter re-suspended from the bed of the river) were under-represented during the period of study as compared with the proportion of the reservoir water that they provide. The effects of this under-representation would have been countered to a small extent by the occasional washout of metals by 'streaming' of water through the reservoir (5.2)

The estimates are for non-particulate and suspended particulate heavy metals only, and take no account of coarse bed-load sediments being carried into the reservoir during the higher spates. Some of the coarser material derived from the Whiteheaps Mine Effluent and from surface runoff within the Whiteheaps Mine area may find its way into the reservoir in this manner.

4.4 COMPOSITION OF SEDIMENTS

The results of the survey of composition of sediments taken from reaches on Bolts Burn and the R. Derwent are pesented in Table 4.11 From this table it can be seen that a wide range of HNO_3- extractable heavy metal levels were found, for example zinc levels ranged from 235 $\mu g\ g^{-1}$ (dry weight) in sediment from reach 05 on the R. Derwent to 4850 $\mu g\ g^{-1}$ in sediment from Bolts Burn downstream of the Whiteheaps Adit Level (reach 35). The distribution of heavy metals in the stream and river sediments was

Table 4.11

The mineral composition of sediments from Bolts Burn and the R. Derwent.

THE MINERAL COMPOSITION OF SEDIMENT, FROM BOLTS BURN AND THE R DERWENT

STREAM NAME	STREAM NO	REACH	NA	MG	CA	ZN	CU	MN	FE	PB	NI	CO	CD	AG
BOLTS BURN	0071	20	680 0	1160 0	2350 0	430 0	21.0	890 0	2100	220 0	30 0	10 0	5 0	<5 0
BOLTS BURN	0071	27	2700 0	1700.0	76000 0	2180 0	570 0	580 0	14700 0	3200 0	60 0	20 0	20 0	5 0
BOLTS BURN	0071	28	1320.0	1311 0	107350 0	1919.0	478 0	787 0	17135 0	5056 0	47 0	37 0	13 0	5 0
BOLTS BURN	0071	35	970 0	1450.0	37900.0	4850 0	310.0	7100.0	2400	15000 0	80 0	70 0	29 0	15 0
BOLTS BURN	0071	40	980 0	1400.0	60000 0	2400.0	380 0	1660.0	23000 0	7700 0	60 0	20 0	18 0	5 0
BOLTS BURN	0071	50	668 0	1163 0	60562 0	1822.0	106.0	1521 0	20349 0	6880 0	39 0	48 0	14 0	5 0
BOLTS BURN	0071	60	910 0	1100 0	38900 0	2660.0	119.0	1640.0	21000 0	7800 0	60 0	50 0	20 0	5 0
BOLTS BURN	0071	70	960 0	1250.0	90000 0	3520.0	102.0	2210 0	30000 0	17500 0	50 0	30 0	16.0	10 0
BOLTS BURN	0071	80	980.0	1150.0	52500 0	2860 0	129 0	1860.0	25000 0	14200 0	50 0	30 0	18 0	10 0
BOLTS BURN	0071	90	1020 0	1200 0	47000 0	2520.0	120 0	1640 0	25000 0	11500 0	60 0	40 0	20 0	5 0
BOLTS BURN	0071	99	1098 0	1127.0	40780 0	3000.0	100.0	1084 0	25490 0	10980 0	49 0	29 0	16 0	5 0
R DERWENT	0061	05	735.0	657 0	1049 0	235 0	17 0	647 0	17160 0	69 0	29 0	29 0	5 0	<5 0
R DERWENT	0061	07	680 0	930 0	13200.0	1050.0	48.0	950 0	19100 0	2700 0	60 0	30 0	18 0	5 0
R. DERWENT	0061	25	1250.0	730 0	19000 0	1870.0	67.0	940 0	19100 0	4200 0	40 0	20 0	15 0	5 0
R DERWENT	0061	30	610 0	900.0	11000.0	1460.0	30.0	7000.0	24000 0	950 0	40 0	20 0	12 0	<5 0

ALL CONCENTRATIONS ARE IN PARTS PER MILLION DRY WEIGHT

0 05 G OF DRIED 80 MESH MATERIAL USED IN ALL DETERMINATIONS

found to show a large measure of agreement with the distribution patterns found during the surveys of composition of water, with higher levels of zinc, lead and cadmium in the reaches affected by inputs of metals.

The lowest levels of all three metals were found in reach 20 on Bolts Burn and reach 05 on the R. Derwent This is further evidence that old mine workings do not have any significant effect on heavy metal levels, even on the levels in coarse bed-load sediments. Large increases in the metal content of sediments were found between reaches 05 and 07 on the R. Derwent as a result of metal inputs from Bolts Burn, and very large increases were found in the metal content of sediments within Bolts Burn as a result of inputs from the Whiteheaps Adit Level, Whiteheaps Mine Effluent and (presumably) surface runoff within the Whiteheaps area.

The levels of zinc, lead and cadmium in the sediments of Bolts Burn and the R. Derwent downstream of the entry of the tributary apparently showed wide variation during the three year period, although the patterns of distribution were probably more stable Much higher levels of zinc, lead and cadmium were found in the sediment from some reaches during the exploratory survey of the first year of study (3 6) This was probably because precipitated ferric hydroxide from the Whiteheaps Effluent discharge formed a large proportion of the sediments of Bolts Burn when the samples were taken. The ability of such precipitates to accumulate heavy metals is well documented (e.g Jenne, 1968)

Levels of 2300 $\mu g\ g^{-1}$ Zn, 2357 $\mu g\ g^{-1}$ Pb and 4 $\mu g\ g^{-1}$ Cd were recorded in sediment from Bolts Burn reach 97 during a survey carried out by the Applied Geochemistry Research Group in 1969 (I. Thornton, personal communication). These levels compare with levels of 3000 $\mu g\ g^{-1}$, 10980 $\mu g\ g^{-1}$ and 16 $\mu g\ g^{-1}$ recorded from reach 99 during the present study (using a weaker extraction technique).

Variations in HNO_3- extractable zinc . lead ratios are plotted against distance down Bolts Burn and the R. Derwent in Fig. 4.16 It can be seen from this diagram that a large build-up in the proportion of lead in sediment from Bolts Burn was found in the upper reaches of the stream downstream of inputs from the Whiteheaps Mine. Downstream of about reach 70 the zinc lead ratio increased steadily towards reach 99 A sharp decrease in zinc : lead ratio took place between reach 05 and reach 07 on the R Derwent as a result of inputs from Bolts Burn. this was followed by an increase downstream to reach 25 and a marked increase between reaches 25 and 30 (upstream and downstream of the reservoir).

These changes in the proportions of lead and zinc in the stream and river sediments are probably caused by the greater association of lead with heavier suspended particles that settle out more quickly than zinc-rich particles on passing downstream from the Whiteheaps Mine (see also 5.3).

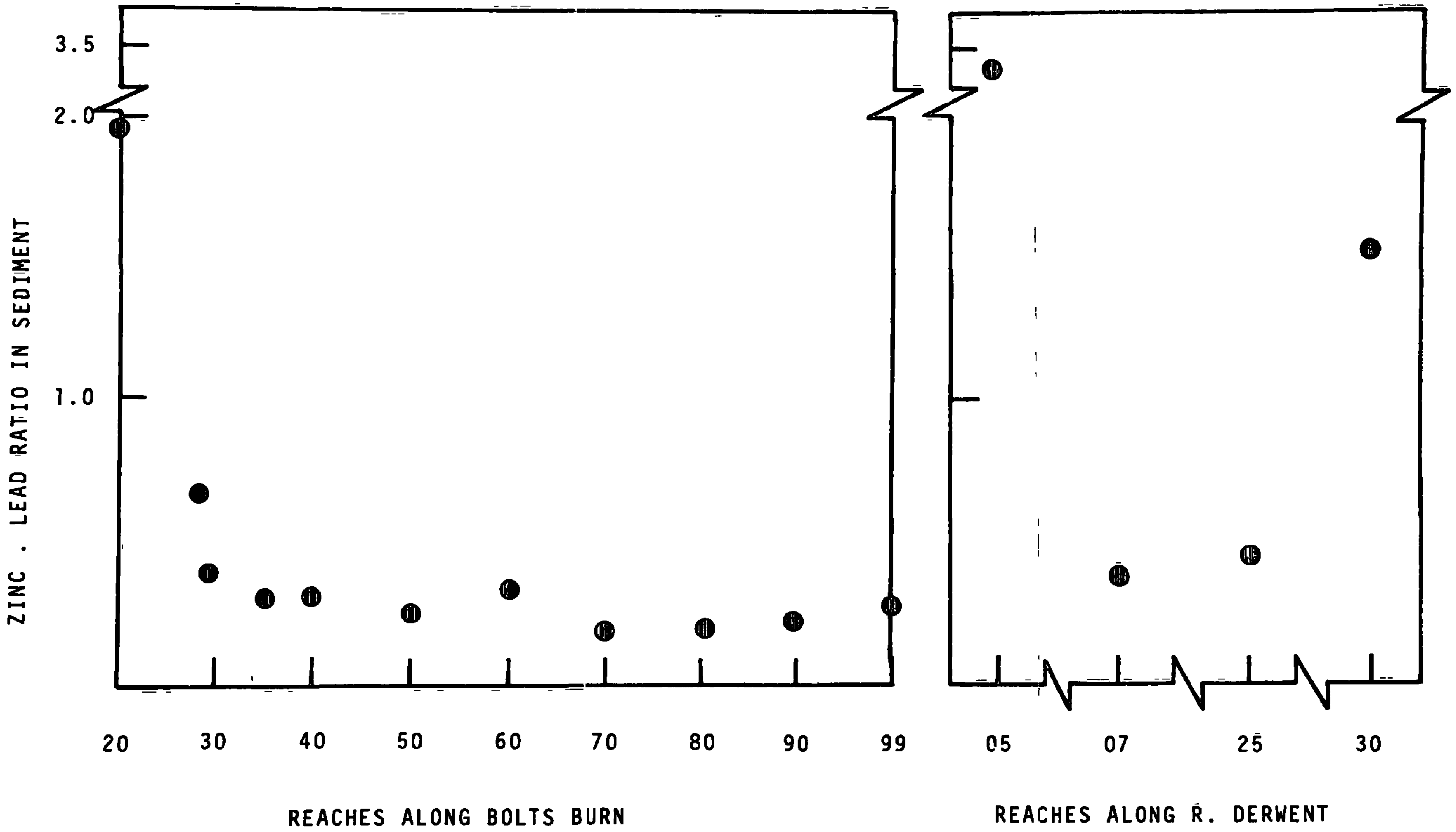
4.5 COMPOSITION OF RIVER PLANTS

4.51 *Lemanea fluviatilis*

Throughout the three year period a series of investigations was carried out of various aspects of the accumulation of heavy metals from river water by *Lemanea fluviatilis*. Most of the results of these studies are presented in Chapter 6, but the results of some of the analyses of the alga are presented here Presentation in this section is limited to a description of changes in the zinc, lead and cadmium contents of the plant within the R. Derwent, and the results are discussed in relation to the changes found in the heavy metal content of the river water and sediments and two other species of submerged plants.

Fig. 4.16

Changes in the zinc · lead ratio of sediments along Bolts Burn and the R. Derwent


3.5
2.0
1.0
ZINC . LEAD RATIO IN SEDIMENT
20
30
40
50
60
70
80
90
99
REACHES ALONG BOLTS BURN
05
07
25
30
REACHES ALONG R. DERWENT

Samples of *Lemanea* filament tips from reaches 05, 07 and 25 were collected during the spring of 1976 in connection with the sampling of water from these reaches. Material from reach 30 was collected on 18 March 1977 from two plants that had grown up in the reach following increased winter flows caused by the reservoir overflowing. Where five replicate samples of filament tips were collected on any one occasion from reaches 05, 07 or 25 (6.3), the mean mineral composition of the replicates was treated as a single sample in the calculation of the results presented in Table 4.12.

The mean zinc, lead and cadmium contents of *Lemanea* filament tips was found to mirror closely those found within the water and sediments of the R. Derwent (Table 4.12). A large increase in the concentrations of all three metals was found in the filaments between reach 05 and reach 07 as a result of inputs from Bolts Burn. This was followed by some decrease in concentration between reaches 07 and 25, and between reach 25 and reach 30.

4.52 *Scapania undulata*

The mean mineral composition of 1 cm long *Scapania* branch tips from the R. Derwent reaches 05 and 07 is presented in Table 4.13. As with *Lemanea*, a large increase in the zinc, lead and cadmium contents of the plant were found on passing from upstream to downstream of the entry of Bolts Burn.

4.53 *Hygrohypnum ochraceum*

Similar increases in the zinc, lead and cadmium contents of 1 cm long *Hygrohypnum* branch tips were found between reaches 05 and 07 on the R. Derwent (Table 4 14)

		Na		K		Mg		Ca		Zn		Cu	
Reach no.	n	$\bar{x}$	s.d.	$\bar{x}$	s.d.	$\bar{x}$	s.d.	$\bar{x}$	s.d.	$\bar{x}$	s.d.	$\bar{x}$	s.d.
05	6	4121.5	1916.8	17919.9	2175.5	18256.5	3592.7	457.0	110.5	351.9	184.7	43.5	43.8
07	59	2674.3	2067.2	16008.6	3597.5	12657.2	2366.5	325.6	176.6	916.5	192.9	43.7	24.3
25	5	2281.6	1012.6	14798.7	4142.6	10887.7	3384.9	260.5	38.0	723.4	137.7	19.1	4.9
30	2	4276.0		23080.0		16163.0		364.0		387.9		14.9	

	Mn		Fe		Al		Pb		Ni		Co		Cd	
Reach no	$\bar{x}$	s.d.	$\bar{x}$	s.d.	$\bar{x}$	s.d.	$\bar{x}$	s.d.	$\bar{x}$	s.d.	$\bar{x}$	s.d.	$\bar{x}$	s.d.
05	130.7	31.4	2057.7	896.6	639.6	437.1	78.1	53.4	73.0	33.8	74.9	40.0	37.7	14.5
07	146.3	63.9	3648.8	1217.0	588.3	253.5	196.5	71.0	57.6	35.4	43.0	29.3	66.1	34.7
25	91.1	15.1	1044.4	384.7	230.6	94.9	110.6	33.9	47.8	16.1	32.4	10.6	26.6	6.3
30	112.5		1974.0		224.0		67.7		15.0		15.0		18.0	

Table 4.12 Mean mineral composition of *Lemanea* filament tips from the R. Derwent

($\mu g\ g^{-1}$)

Reach No.	Na	K	Mg	Ca	Zn	Cu	Mn	Fe	Al	Pb	Ni	Co	Cd
05	3237.4	10024.3	1598.1	1567.2	771.1	25.8	171.6	21559.5	7269.4	208.3	95.2	89.5	28.1
07	2533 1	10945.3	1343.1	2226.9	2992.9	47.8	267 1	32543.4	6657.8	2387.3	75.7	92.2	30.0

Table 4.13 The mean mineral composition of *Scapania undulata* from the R. Derwent

Reach No.	Na	K	Mg	Ca	Zn	Cu	Mn	Fe	Al	Pb	Ni	Co	Cd
05					334.0	26.0	441.0	13064.0	4527.0	160.0	21.0	7.0	20.0
07					1438.0	27.0	384.0	10651.0	315.0	993.0		103.0	

Table 4.14 The mean mineral composition of *Hygrohypnum ochraceum* from the R. Derwent

$(\mu g\ g^{-1})$

CHAPTER 5

DISTRIBUTION OF HEAVY METALS WITHIN WATER, SEDIMENTS AND SUBMERGED PLANTS OF THE DERWENT RESERVOIR

5.1 INTRODUCTION

The study of the composition of R Derwent water (4 324, 4.325) indicated that on most occasions high proportions of the zinc, lead and cadmium entering the Derwent Reservoir were being deposited before reaching the compensation water outlet. Occasional exceptions to this pattern had been found following flooding on the R. Derwent, when elevated levels of zinc were recorded (by the Sunderland and South Shields Water Company) in the compensation water and in water drawn off for treatment (R. W. Hunter, personal communication) In order to clarify these observations and to investigate factors influencing the distribution of metals within the sediments and plants of the reservoir, a series of detailed investigations were carried out. The results of all of these investigations are considered together in this chapter.

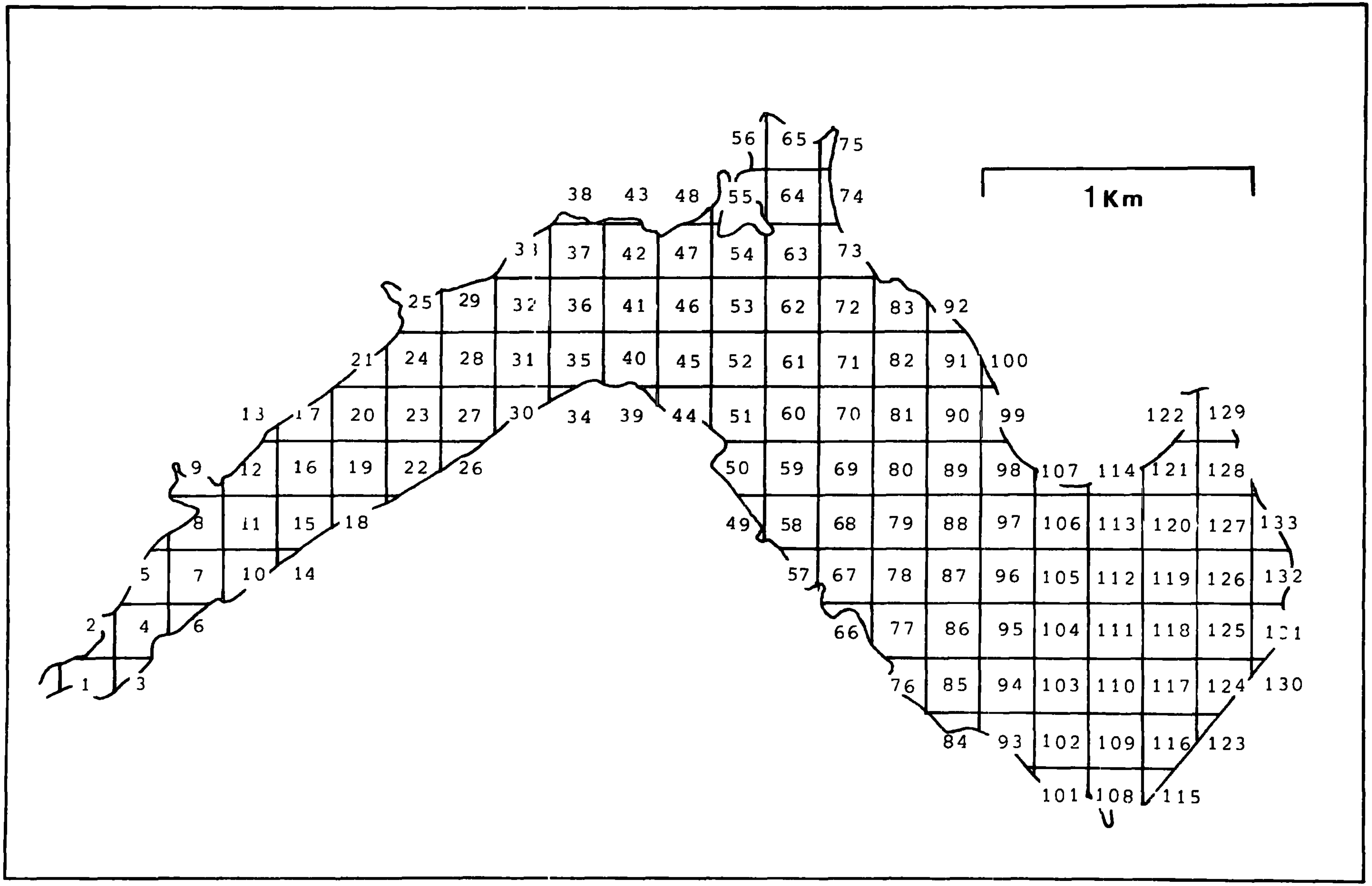
Wherever possible samples were taken from points as near as possible to the centre of designated 200 x 200 m squares (Fig. 5 1), which were based on the 1 km square Ordnance Survey grid system

5.2 COMPOSITION OF WATER

As the deposition of metals might have been expected to have been influenced by the hydrodynamic properties of the river water after it had entered the reservoir, five separate transects to collect water were made along the full length of the reservoir at different stages in the filling cycle. The results of two of these transects have been omitted

Fig. 5.1

Position of sampling grid squares in the Derwent Reservoir


1Km

from this account, as they were made under conditions very similar to those under which transect A (see below) was made. Fig. 5.2 shows the timing of the other three transects in relation to changes in the level of water in the reservoir, and Tables 5.1 - 5.3 show the distributions of 'total' zinc in the water samples taken:

A after a long period with no large inputs of water and a typical summer drop of reservoir level (11 September 1975),

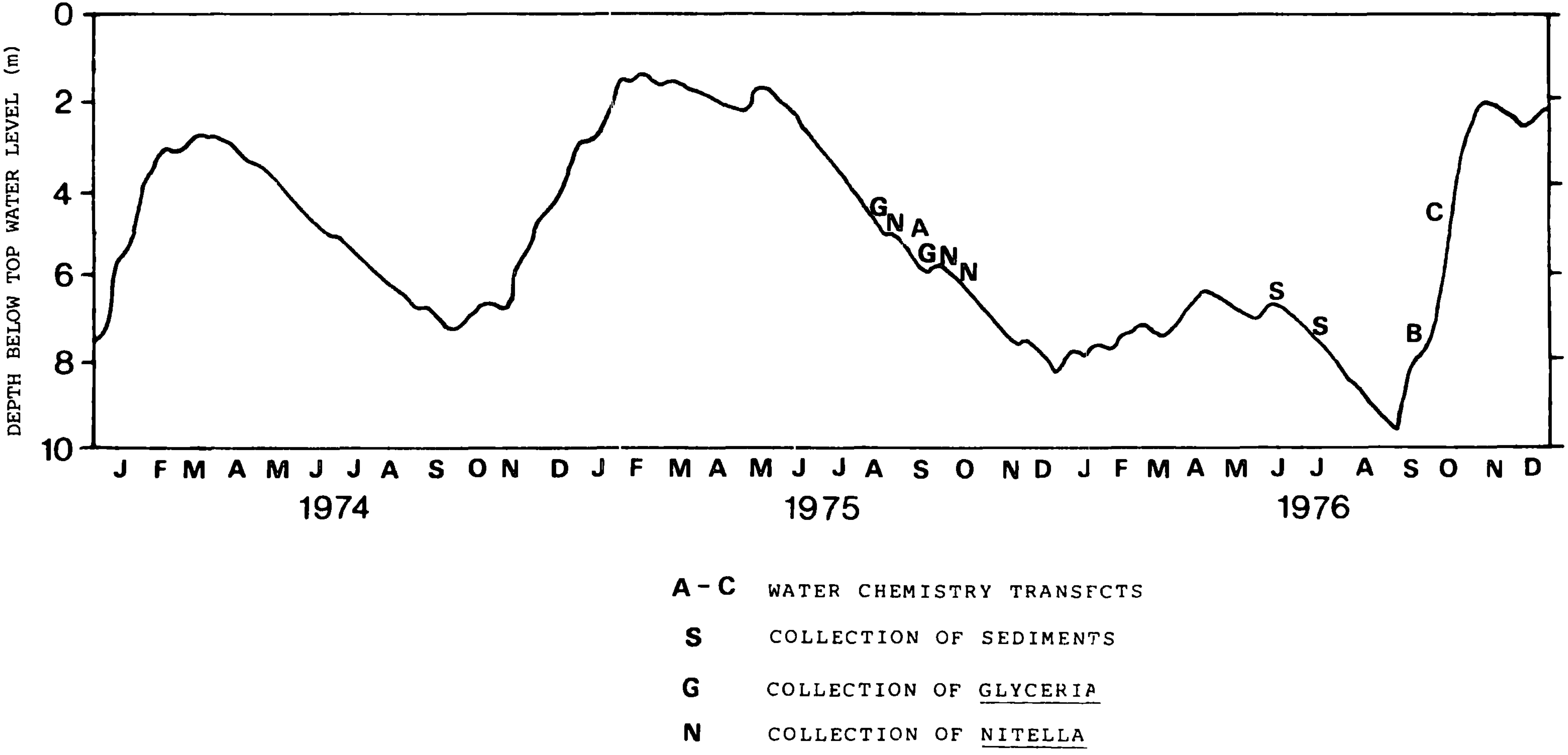
B. immediately after the heavy flooding following the severe drought of the summer of 1976 (14 September 1976),

C. after a prolonged period of filling and turbulence (8 October 1976).

After a long calm period (Transect A), the 'total' zinc levels over most of the reservoir were found to be relatively low (0.025 - 0.030 mg l^{-1}). About 0.7 km from the mouth of the R. Derwent, however, a marked increase in zinc concentrations was found, with 'total' levels rising to 0.270 mg l^{-1}. An interesting feature of this transect is that the highest 'total' zinc concentration was measured in a sample taken from a point a short distance away from the river inflow point, and not in the river itself at reach 25. This observation is probably explained by a drop in zinc concentration in the water of the R. Derwent following the switching off of the Whiteheaps Adit Level pump, as flows had been stable in the river for some time prior to the collection of samples.

Fig. 5.2

Changes in the water level of the Derwent Reservoir throughout the three year sampling period.


DEPTH BELOW TOP WATER LEVEL (m)
0
2
4
6
8
10
J F M A M J J A S O N D
J F M A M J J A S O N D
J F M A M J J A S O N D
1974
1975
1976
G N A G N N
S S
B C
A – C WATER CHEMISTRY TRANSECTS
S COLLECTION OF SEDIMENTS
G COLLECTION OF GLYCERIA
N COLLECTION OF NITELLA

Grid square	depth	Na	K	Mg	Ca	Zn	Cu	Al	Pb	Cd	F
124	0	5.7	1.90	2.80	8.5	0.033	0.003	0.03	0.004	<0.0001	0.76
111	0	5.5	1.90	2.90	10.1	0.025	0.002	<0.03	0.004	<0.0001	0.76
111	15	5.8	1.86	2.75	9.8	0.025	<0.002	<0.03	0.003	<0.0001	0.76
105	0	5.5	1.80	2.90	11.2	0.025	<0.002	<0.03	0.003	<0.0001	0.76
88	0	5.7	1.80	2.80	11.4	0.025	<0.002	<0.03	0.007	<0.0001	0.72
61	0	5.5	1.85	2.90	9.5	0.025	<0.002	0.03	0.004	<0.0001	0.70
46	0	5.7	1.80	2.90	8.9	0.025	<0.002	<0.03	0.005	<0.0001	0.73
46	15	5.6	1.83	2.85	10.1	0.025	<0.002	0.03	0.004	<0.0001	0.72
41	0	5.6	1.85	2.90	10.4	0.023	<0.002	0.04	0.004	<0.0001	0.71
28	0	5.6	1.90	2.80	11.9	0.025	<0.002	<0.03	0.004	<0.0001	0.66
23	0	5.5	1.90	3.00	10.5	0.025	0.004	<0.03	0.004	<0.0001	0.69
23	10	5.6	1.85	2.90	10.6	0.025	<0.002	<0.03	0.004	<0.0001	0.68
23	0	5.5	1.90	2.90	8.9	0.025	<0.002	<0.03	0.004	<0.0001	0.70
19	0	5.8	1.85	2.90	11.8	0.023	<0.002	<0.03	0.004	<0.0001	0.70
19	0	5.8	1.90	3.10	9.5	0.033	<0.002	0.03	0.008	<0.0001	0.72
19	3	5.8	1.85	3.00	10.0	0.030	<0.002	<0.03	0.009	<0.0001	0.70
16	0	6.0	2.00	3.20	11.1	0.028	<0.002	0.03	0.004	<0.0001	0.70
15	0	6.0	2.00	3.10	13.0	0.040	<0.002	0.03	0.004	<0.0001	0.73
11	0	6.3	2.10	3.50	10.7	0.060	<0.002	0.06	0.004	<0.0001	0.76
11	2	6.1	2.10	3.40	10.8	0.062	<0.002	0.05	0.004	<0.0001	0.74
11	0	7.1	2.40	4.00	13.6	0.084	0.002	0.04	0.005	<0.0001	0.77
7	0	9.6	3.40	5.50	19.5	0.270	<0.002	0.03	0.015	0.0003	0.88
7	0	9.3	3.25	5.20	25.2	0.146	0.002	0.06	0.012	0.0001	0.91
0061-25	-	8.6	3.10	5.00	21.5	0.092	<0.002	0.03	0.010	0.0001	0.82

Table 5.1 'Total' levels of selected chemical parameters in samples of water taken during a transect along the axis of the Derwent Reservoir on 11 September 1975

(= Transect A)

(all concentrations are in mg l^{-1})

Grid square	depth	treatment	Na	K	Mg	Ca	Zn	Cu	Mn	Fe	Al	Pb	Ni	Co	Cd	Ag
124	0	tot	7.9	1.77	2.99	9.3	0.020	0.002	0.109	0.33	0.26	0.012	0.01	0.01	0.0001	0.005
124	0	nuc	8.1	1.73	2.93	10.0	0.029	0.002	0.076	0.07	0.04	0.005	0.01	0.01	0.0009	0.005
118	0	tot	8.3	2.06	2.99	11.2	0.059	0.002	0.102	0.33	0.24	0.013	0.01	0.01	0.0001	0.005
118	0	nuc	8.5	1.81	2.93	12.1	0.048	0.002	0.071	0.08	0.04	0.005	0.01	0.01	0.0007	0.005
118	17	tot	7.0	2.23	2.62	9.3	0.167	0.002	0.370	2.33	2.23	0.191	0.01	0.01	0.0016	0.005
118	17	nuc	7.1	1.85	2.45	9.8	0.112	0.002	0.314	0.12	0.04	0.029	0.01	0.01	0.0010	0.005
111	0	tot	8.4	1.77	2.99	12.3	0.091	0.002	0.095	0.33	0.27	0.015	0.01	0.01	0.0001	0.005
111	0	nuc	8.3	1.81	2.99	11.9	0.074	0.002	0.066	0.04	0.03	0.006	0.01	0.01	0.0012	0.005
105	0	tot	8.3	1.81	3.00	12.1	0.020	0.002	0.077	0.33	0.34	0.014	0.01	0.01	0.0002	0.005
105	0	nuc	8.6	1.81	2.93	11.9	0.021	0.002	0.042	0.04	0.03	0.007	0.01	0.01	0.0023	0.005
96	0	tot	8.4	1.98	2.90	11.9	0.018	0.002	0.079	0.34	0.33	0.014	0.01	0.01	0.0001	0.005
96	0	nuc	8.3	1.85	2.90	11.9	0.018	0.002	0.069	0.06	0.03	0.008	0.01	0.01	0.0013	0.005
88	0	tot	8.3	1.98	3.01	12.1	0.014	0.002	0.076	0.34	0.28	0.014	0.01	0.01	0.0014	0.005
88	0	nuc	8.7	1.89	2.87	12.1	0.020	0.002	0.069	0.07	0.03	0.007	0.01	0.01	0.0008	0.005
88	14	tot	7.3	2.36	2.90	10.7	0.130	0.008	0.294	1.60	1.45	0.157	0.01	0.01	0.0006	0.005
88	14	nuc	7.5	1.85	2.51	10.2	0.102	0.002	0.253	0.09	0.07	0.026	0.01	0.01	0.0009	0.005
80	0	tot	8.3	1.81	2.90	12.3	0.015	0.002	0.080	0.31	0.30	0.017	0.01	0.01	0.0001	0.005
80	0	nuc	8.4	1.81	2.87	11.6	0.021	0.002	0.046	0.04	0.03	0.015	0.01	0.01	0.0002	0.005
70	0	tot	8.3	2.32	2.82	11.9	0.015	0.002	0.082	0.39	0.31	0.017	0.01	0.01	0.0001	0.005
70	0	nuc	8.4	1.73	2.82	11.4	0.047	0.002	0.043	0.03	0.03	0.008	0.01	0.01	0.0001	0.005

Table 5.2 Levels of selected chemical parameters in samples of water taken during a transect along the axis of the Derwent Reservoir on 14 September 1976

(= Transect B)

Grid square	depth	treat-ment	Na	K	Mg	Ca	Zn	Cu	Mn	Fe	Al	Pb	Ni	Co	Cd	Ag
61	0	tot	8.5	1.77	2.90	11.9	0.015	0.002	0.076	0.04	0.27	0.017	0.01	0.01	0.0001	0.005
61	0	nuc	8.3	1.77	2.79	11.6	0.014	0.002	0.042	0.06	0.03	0.007	0.01	0.01	0.0001	0.005
61	10	tot	8.2	1.98	2.85	11.6	0.062	0.002	0.171	0.59	0.47	0.033	0.01	0.01	0.0002	0.005
61	10	nuc	8.6	2 06	2.85	11.2	0.056	0.002	0.156	0.14	0 03	0.011	0.01	0.01	0.0002	0.005
53	0	tot	8.1	2 11	2.87	12.6	0.021	0.002	0.121	0.35	0.32	0.015	0.01	0.01	0.0001	0 005
53	0	nuc	8.5	1 98	2.82	11.6	0.032	0.002	0 087	0 18	0.50	0.008	0.01	0.01	0.0001	0.005
46	0	tot	8.9	1.94	2.87	10.5	0.024	0 002	0 120	0.40	0.37	0 015	0.01	0.01	0.0001	0.005
46	0	nuc	8.5	2.02	2.82	11.4	0.015	0.002	0 076	0.06	0.05	0.007	0.01	0.01	0.0001	0.005
41	0	tot	9.4	1.68	2.87	11.9	0.024	0.002	0.122	0.39	0.30	0.015	0 01	0 01	0.0001	0.005
41	0	nuc	8.3	1.56	2.79	11 4	0.018	0 002	0.078	0.07	0.03	0.007	0 01	0.01	0.0001	0.005
41	5	tot	8.2	1.89	2.93	11.6	0.024	0.002	1.352	0.44	0.37	0.017	0 01	0.01	0.0004	0.005
41	5	nuc	8 2	2.48	2.82	11.4	0.017	0.002	0.091	0.06	0.07	0.007	0 01	0 01	0.0003	0.005
36	0	tot	8.9	2.06	2.82	11.6	0.048	0 002	0.129	0 43	0.39	0.016	0.01	0.01	0.0002	0 005
36	0	nuc	8.6	1.77	2.82	11.6	0.011	0 002	0.096	0.04	0.10	0.011	0.01	0.01	0.0002	0.005
31	0	tot	8.4	1.81	2.87	11.6	0.041	0.002	0 132	0.36	0.31	0.017	0.01	0.01	0.0001	0.005
31	0	nuc	8.2	1.77	2.85	11.6	0.023	0.002	0 102	0.05	0.03	0.006	0.01	0.01	0.0001	0 005
28	0	tot	8.3	1.81	2.90	10.9	0.023	0.002	0.137	0.46	0.29	0.020	0.01	0.01	0 0001	0.005
28	0	nuc	8.6	1.81	2.73	10.2	0.023	0.002	0.112	0.08	0.03	0.007	0.01	0.01	0.0002	0.005
28	3	tot	8.8	1.89	2.90	11.6	0.115	0.004	0.206	0.71	0.50	0.036	0.01	0.01	0.0007	0.005
28	3	nuc	8.4	1.77	2.82	11.6	0.076	0.002	0.168	0.10	0.09	0.011	0.01	0.01	0.0006	0.005

Table 5.2 cont. Levels of selected chemical parameters in samples of water taken during a transect along the axis of the Derwent Reservoir on 14 September 1976

(= Transect B)

23	0	tot	8.5	2.06	2.93	12 6	0.064	0.002	0.201	0.96	0.43	0 032	0.01	0.01	0.0002	0.005
23	0	nuc	5.1	1.14	1 41	6.3	0.035	0.002	0.069	0.12	0.03	0.020	0.01	0.01	0.0009	0.005
19	0	tot	8.2	1.77	2.79	11.6	0.056	0.002	0.187	0.56	0.43	0.028	0.01	0.01	0.0003	0.005
19	0	nuc	8.4	1.68	2.79	11.4	0.038	0.002	0.157	0.06	0.04	0.010	0.01	0.01	0.0005	0.005
16	0	tot	7.2	1 68	2.79	9 8	0.279	0.002	0.352	0.65	0.45	0.056	0.01	0.01	0.0012	0.005
16	0	nuc	7.9	1.64	2.56	8.1	0.248	0.002	0.306	0.27	0.13	0 031	0.01	0.01	0.0014	0.005
0061-25	-	tot	6.0	1.73	2.23	7.2	0.353	0.014	0.468	1.44	1.12	0 237	0.01	0.01	0.0026	0.005
0061-25	-	nuc	5.7	1.68	1.92	4.7	0.311	0.014	0.357	0.24	0.26	0 078	0.01	0.01	0.0016	0.005

Table 5.2 cont. Levels of selected chemical parameters in samples of water taken during a transect along the axis of the Derwent Reservoir on 14 September 1976

(= Transect B)

(all concentrations are in mg l^{-1})

Grid square	depth	treatment	Na	K	Mg	Ca	Zn	Cu	Mn	Fe	Al	Pb	Ni	Co	Cd	Ag
124	0	tot	8.0	2.13	3.00	11.7	0.072	0.010	0.165	0.27	0.20	0.019	0.01	0.01	0.0002	0.005
124	0	nuc	8.0	2.04	3.00	11.6	0.072	0.005	0.141	0.11	0.04	0.008	0.01	0.01	0.0013	0.005
124	17	tot	7.9	2.09	3.00	11.3	0.059	0.002	0.162	0.29	0.24	0.012	0.01	0.01	0.0002	0.005
124	17	nuc	8.0	2.30	2.95	11.4	0.060	0.010	0.151	0.13	0.07	0.007	0.01	0.01	0.0022	0.005
118	0	tot	7.7	2.13	3.00	11.5	0.061	0.003	0.173	0.28	0.29	0.014	0.01	0.01	0.0012	0.005
118	0	nuc	8.1	2.13	3.00	11.6	0.061	0.007	0.148	0.09	0.03	0.007	0.01	0.01	0.0015	0.005
111	0	tot	7.4	2.13	3.16	11.9	0.059	0.002	0.162	0.25	0.21	0.013	0.01	0.01	0.0006	0.005
111	0	nuc	8.2	1.91	3.00	12.0	0.057	0.009	0.168	0.09	0.04	0.004	0.01	0.01	0.0024	0.005
105	0	tot	7.5	1.87	3.05	12.3	0.061	0.006	0.173	0.29	0.26	0.012	0.01	0.01	0.0012	0.005
105	0	nuc	9.2	1.91	3.11	11.8	0.060	0.007	0.151	0.08	0.07	0.008	0.01	0.01	0.0014	0.005
96	0	tot	7.9	1.83	3.00	11.6	0.061	0.007	0.180	0.27	0.23	0.013	0.01	0.01	0.0002	0.005
96	0	nuc	8.9	2.04	2.89	11.4	0.060	0.006	0.141	0.09	0.03	0.006	0.01	0.01	0.0012	0.005
96	15	tot	8.0	1.78	2.95	11.8	0.053	0.007	0.169	0.28	0.25	0.014	0.01	0.01	0.0004	0.005
96	15	nuc	9.3	1.83	3.00	11.8	0.053	0.007	0.141	0.09	0.07	0.007	0.01	0.01	0.0012	0.005
88	0	tot	7.9	1.87	2.95	11.9	0.064	0.007	0.173	0.30	0.27	0.015	0.01	0.01	0.0014	0.005
88	0	nuc	9.6	1.96	2.89	11.7	0.064	0.007	0.140	0.09	0.03	0.008	0.01	0.01	0.0015	0.005
80	0	tot	8.0	2.00	2.89	11.8	0.064	0.003	0.169	0.29	0.21	0.012	0.01	0.01	0.0008	0.005
80	0	nuc	8.8	1.87	2.89	11.3	0.062	0.006	0.134	0.10	0.03	0.005	0.01	0.01	0.0004	0.005
70	0	tot	7.8	1.83	2.95	12.0	0.072	0.004	0.176	0.29	0.24	0.013	0.01	0.01	0.0006	0.005
70	0	nuc	9.3	1.87	3.00	11.8	0.072	0.007	0.134	0.12	0.07	0.008	0.01	0.01	0.0008	0.005

Table 5.3 Levels of selected chemical parameters in samples of water taken during a transect along the axis of the Derwent Reservoir on 8 October 1976

(= Transect C)

Grid square	depth	treat-ment	Na	K	Mg	Ca	Zn	Cu	Mn	Fe	Al	Pb	Ni	Co	Cd	Ag
70	15	tot	7.6	1.78	3.05	12.5	0.069	0.002	0 173	0.31	0.26	0.023	0.01	0.01	0.0004	0.005
70	15	nuc	9.6	1.91	2.95	11.7	0.070	0 007	0.141	0.09	0.08	0.006	0.01	0.01	0.0005	0.005
61	0	tot	7.8	1.70	2.95	12.2	0.061	0.002	0.165	0.29	0.24	0.014	0.01	0.01	0.0001	0.005
61	0	nuc	9.2	1.87	2.89	11.8	0.060	0.006	0.134	0.08	0.03	0.006	0.01	0.01	0.0001	0.005
53	0	tot	8.0	1.83	2.84	12.3	0.069	0.002	0.165	0.29	0.22	0.014	0.01	0.01	0.0001	0.005
53	0	nuc	7.9	1.83	2.84	11.5	0.061	0 007	0.141	0.08	0.03	0.005	0.01	0.01	0.0001	0.005
46	0	tot	7.7	1.87	2.89	12.2	0.069	0 005	0.176	0.26	0.20	0.012	0.01	0.01	0.0001	0.005
46	0	nuc	9.2	1.83	2.89	11.7	0.069	0.007	0.141	0.10	0.04	0.006	0 01	0.01	0.0001	0.005
46	0	tot	7.8	1.74	2 89	11.9	0.075	0 003	0.194	0.33	0.27	0.013	0 01	0.01	0.0015	0.005
46	0	nuc	9.0	1.78	2.89	11.4	0.069	0.006	0.169	0.11	0.04	0.006	0 01	0.01	0.0010	0.005
41	0	tot	8.2	1.78	2.89	11.4	0.072	0 006	0.176	0.28	0.20	0.016	0 01	0.01	0.0004	0.005
41	0	nuc	8.4	1.78	2.95	12.0	0.072	0 005	0.151	0.10	0.03	0.008	0.01	0.01	0.0003	0.005
36	0	tot	7.6	1.70	2.95	11.7	0.069	0.005	0.162	0.27	0.23	0.014	0 01	0.01	0.0001	0.005
36	0	nuc	9.4	1.78	3.00	11.7	0.069	0.006	0.158	0.11	0.03	0.005	0 01	0.01	0.0004	0.005
31	0	tot	8.0	1.74	2.95	11.8	0.080	0.005	0.176	0.27	0.25	0.018	0.01	0.01	0 0004	0.005
31	0	nuc	9.2	1.83	2.89	11.5	0.072	0.007	0 173	0.09	0.04	0.006	0.01	0.01	0 0007	0.005
31	6	tot	7.7	1.74	2.89	11.8	0.077	0.005	0.197	0.30	0.19	0.013	0 01	0.01	0.0005	0.005
31	6	nuc	9.5	1.74	2.84	11.3	0.069	0.006	0.183	0.11	0.10	0.013	0.01	0.01	0.0005	0.005
28	0	tot	7.6	1.83	2.95	11.8	0.072	0.007	0.201	0.31	0.24	0.018	0.01	0.01	0 0006	0.005
28	0	nuc	10.3	1.74	2.95	11.8	0.072	0.005	0.176	0.09	0.13	0.008	0.01	0.01	0.0010	0.005

Table 5.3 cont. Levels of selected chemical parameters in samples of water taken during a transect along the axis of the Derwent Reservoir on 8 October 1976

(= Transect C)

Grid square	depth	treat-ment	Na	K	Mg	Ca	Zn	Cu	Mn	Fe	Al	Pb	Ni	Co	Cd	Ag
23	0	tot	7.7	1.74	2.84	11.4	0.080	0.002	0.201	0.34	0.25	0.019	0.01	0.01	0.0006	0.005
23	0	nuc	9.1	1.78	2.84	11.4	0.072	0.007	0.176	0.12	0.07	0.012	0.01	0.01	0.0008	0.005
19	0	tot	7.9	1.70	2.89	11.8	0.096	0.003	0.194	0.30	0.24	0.022	0.01	0.01	0.0007	0.005
19	0	nuc	7.7	1.74	2.84	11.4	0.117	0.005	0.194	0.20	0.08	0.017	0.01	0.01	0.0009	0.005
19	2	tot	8.1	1.74	2.84	11.4	0.107	0.006	0.211	0.31	0.25	0.026	0.01	0.01	0.0010	0.005
19	2	nuc	9.0	1.74	2.89	11.4	0.091	0.006	0.190	0.13	0.03	0.020	0.01	0.01	0.0005	0.005
16	0	tot	7.4	1.61	3.05	11.8	0.237	0 004	0.165	0.37	0.32	0.066	0 01	0.01	0.0012	0.005
16	0	nuc	8.5	1.61	3.05	11.8	0.227	0.007	0.141	0.25	0.15	0.047	0 01	0.01	0.0013	0.005
0061-25	-	tot	8.2	1.57	3.05	12.3	0.267	0.008	0.148	0.42	0.36	0.084	0.01	0.01	0.0012	0.005
0061-25	-	nuc	8.2	1.65	2.04	11.5	0.237	0.007	0.134	0.18	0.11	0.029	0 01	0.01	0.0013	0 005

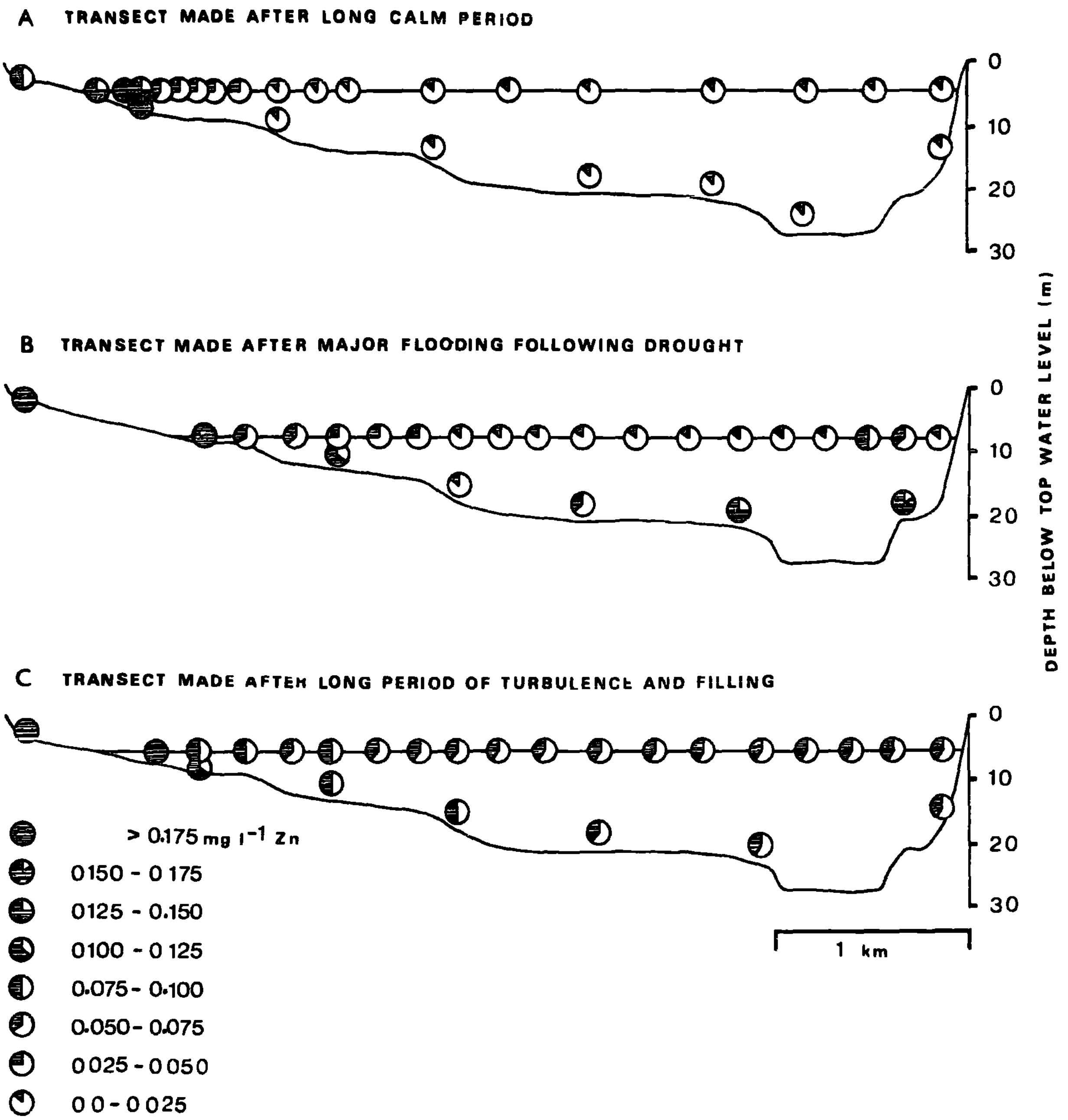
Table 5.3 cont. Levels of selected chemical parameters in samples of water taken during a transect along the axis of the Derwent Reservoir on 8 October 1976

(= Transect C)

(all concentrations are in mg l^{-1})

Fig. 5.3

Variations in the 'total' zinc content of water from sites in the Derwent Reservoir at various stages in the filling cycle.

 A TRANSECT MADE AFTER LONG CALM PERIOD

B TRANSECT MADE AFTER MAJOR FLOODING FOLLOWING DROUGHT

C TRANSECT MADE AFTER LONG PERIOD OF TURBULENCE AND FILLING

A more complicated pattern of distribution of different 'total' levels of zinc was found within the reservoir following a period of heavy flooding on the R. Derwent (Transect B) Higher levels of 'total' zinc were found in samples taken from several points 2 m over the bottom of the reservoir, and an upwelling of higher levels was detected near to the dam. These results are consistent with the 'streaming' of colder flood water along the reservoir bottom, followed by turbulent mixing near to the dam wall. For a short period following the floods on the river the composition of the compensation water was very similar to that of the inflowing R. Derwent (R W. Hunter, personal communication)

Samples of water collected following prolonged filling and turbulence (Transect C) demonstrated that mixing of water had resulted in substanially higher 'total' zinc levels throughout the reservoir (0 050 - 0 075 mg l^{-1}) than had been found during transect A. Samples of water collected during transect C were passed through 0 2 µm Nuclepore filters It was found that a minimum of 90% of the 'total' zinc level present in all of the samples was capable of passing through the filters, and was therefore presumably not present as particulate material re-suspended by turbulence.

A 'total' zinc concentration of 0 075 mg l^{-1} was recorded in water from reach 30 on the R. Derwent on 18 March 1977. The reservoir had been overflowing for several months prior to the collection of this sample, but no large floods had occurred on the R Derwent for some time. It therefore seems probable that zinc concentrations throughout the reservoir remained comparatively high during the winter and early spring of 1976/77.

The water chemistry of 'total' samples from the reservoir at site 42 collected on a daily basis from 8 - 23 March 1976 is presented in Table 5.4. The collections of water were made at a point where moving river water penetrating beyond the nature reserve might have been expected to meet the shore Elevated 'total' zinc and lead concentrations (0.289 mg l^{-1} and 0.030 mg l^{-1} respectively) were detected in the sample of water taken on 23 March following fairly high flows on the R. Derwent This indicated that higher levels of zinc could penetrate for fairly large distances into the reservoir even when major flooding had not occurred on the R. Derwent.

5.3 COMPOSITION OF SEDIMENTS

A comprehensive survey of the mineral composition of superficial sediments from the Derwent Reservoir was made during June 1976. This period was chosen because the reservoir had been very calm for a long period, and the sediment in shallow water around the entry point of the R. Derwent had become settled. Eight dried samples of sediment that had been collected during July 1975 from a dried crust of mud overlying rotting vegetation around the river entry point were digested together with the samples taken in 1976. The sediment collected in 1975 consisted of material laid down during a single winter and had not been covered by water during the dry winter of 1975/76. It was digested together with the 1976 sediment as it would have been exposed for a year if it had been sampled in 1976.

The mean levels of HNO_3-extractable zinc, lead and cadmium in all samples of sediment were 1035 $\mu g\ g^{-1}$ (dry weight), 827 $\mu g\ g^{-1}$ and 13 $\mu g\ g^{-1}$ respectively (n = 94 in each case). The highest levels of zinc and lead were concentrated in the sediments of the nature reserve (Table 5.5, Fig. 5.4), and the levels of both elements tended to decrease on passing away from the entry of

DATE	Na	K	Mg	Ca	Zn	Cu	Mn	Fe	Al	Pb	Ni	Co	Cd	Ag
8-3-76	7.9	1.66	2.80	11.9	0.037	< 0.002	0.101	0.29	0.20	0.033	< 0.01	< 0.01	< 0.0001	< 0.005
9-3-76	7.7	1.50	2.70	11.9	0.044	"	0.087	0.29	0.08	0.007	"	"	"	"
10-3-76	7.8	1.48	2.85	12.6	0.067	"	0.202	0.89	0.36	0.022	"	"	"	"
11-3-76	7.9	1.46	2.80	11.8	0.062	"	0.106	0.33	0.15	0.008	"	"	"	"
12-3-76	8.0	1.62	3.05	13.0	0.067	"	0.173	0.42	0.13	0.015	"	"	"	"
13-3-76	8.7	1.80	3.30	14.3	0.059	"	0.253	1.02	0.22	0.008	"	"	"	"
14-3-76	8.4	1.58	2.85	13.5	0.044	"	0.240	0.65	0.14	0.010	"	"	"	"
15-3-76	8.7	1.56	3.30	14.2	0.052	"	0.220	0.49	0.18	0.010	"	"	"	"
16-3-76	8.7	2.02	3.55	16.7	0.059	"	0.402	1.59	0.40	0.013	"	"	"	"
17-3-76	8.4	1.58	3.45	16.5	0.030	"	0.25	0.45	0.13	0.015	"	"	"	"
18-3-76	7.8	1.54	2.95	13.2	0.037	"	0.160	0.29	0.03	0.013	"	"	"	"
19-3-76	8.6	1.96	3.10	13.3	0.022	"	0.207	0.53	0.18	0.010	"	"	"	"
20-3-76	7.9	1.54	2.80	11.9	0.030	"	0.101	0.26	0.18	0.015	"	"	"	"
21-3-76	7.3	1.48	2.85	12.1	0.022	"	0.227	0.83	0.38	0.015	"	"	"	"
22-3-76	8.3	1.68	3.55	15.3	0.067	"	0.302	0.62	0.17	0.013	"	"	"	"
23-3-76	10.7	2.40	3.75	16.5	0.289	"	0.133	0.35	0.18	0.030	"	"	"	"

Table 5.4 Compiled water chemistry of the Derwent Reservoir near the sailing club 8 - 23 March 1976

(all concentrations are in mg l^{-1})

Table 5.5

The mineral composition of sediments from the Derwent Reservoir.

(for location of grid squares, see Fig. 3 3)

TABLE THE MINERAL COMPOSITION OF SEDIMENTS FROM THE ERWENT RESERVOIR (ALL CONCENTRATIONS IN PARTS PER MILLION DRY WEIGHT)

SITES ABOVE WATER LEVEL AT TIME OF SAMPLING

GRID NO	DISTANCE (m)	DEPTH (m)	THL DEPTH	NA	MG	CA	Z	CU	MN	FE	B	NI	CO	CD	AG
001	0 20	0 0	3 0	1500 0	900 0	800 0	2400 3	0 0	1600 0	19750 0	1650 0	60 0	40 0	1 0	0
004	0 44	0 0	5 0	1400 0	1500 0	1100 0	1600 0	60 0	1420 0	247 C 0	11 0 0	0 0	50 C	18 0	5 0
005	0 58	0 0	6 0	1600 0	1920 0	1900 0	1680 0	70 0	2400 0	34750 0	1920 0	50 0	30 0	12 0	5 0
007	0 72	0 0	5 5	1700 0	1100 0	1100 0	3600 0	30 0	2050 0	22 G 0	2200 0	60 0	50 0	20 0	5 0
008	0 88	0 0	5 5	1500 0	1930 0	1900 0	5000 0	80 0	1600 0	39750 0	1600 0	50 0	0 0	0	5 0
010	0 80	0 0	6 5	1600 0	1940 0	1460 0	1640 0	50 0	1790 0	35750 0	1560 C	40	0 0	0	5 0
011	1 07	0 0	6 5	1400 0	2000 0	1050 0	700 0	60 0	670 0	3475 0	1 9 0	5	50 0	18 0	5 0
012	1 14	0 0	4 0	1500 0	2000 0	1600 0	1500 0	50 0	20 0	2 50 0	2 0 G	80 0	30 0	10 0	5 0

SUBMERGED SITES

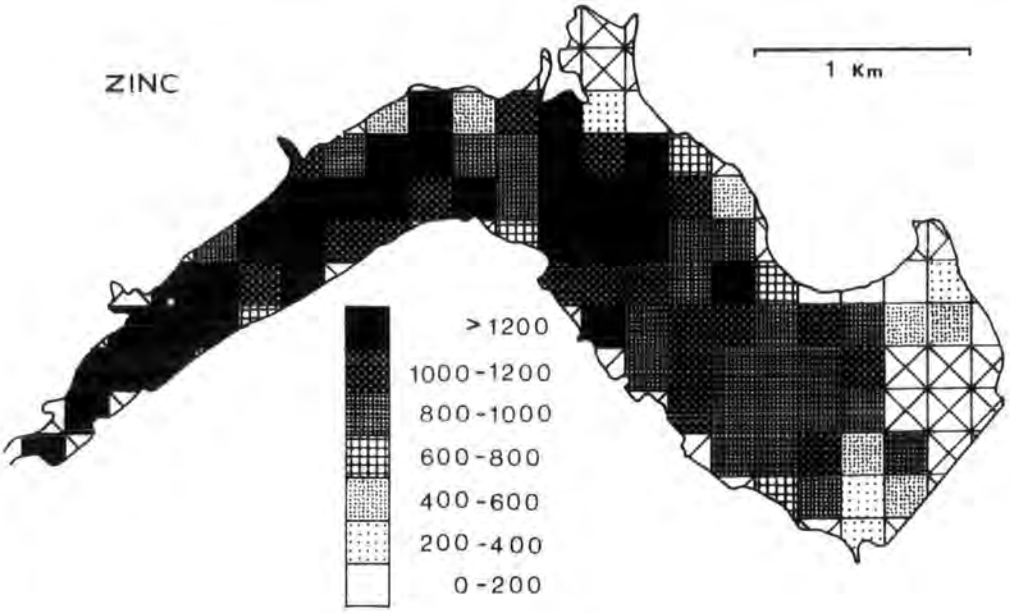
GRID NO	DISTANCE (m)	DEPTH (m)	THL DEPTH	NA	MG	CA	ZN	CU	MN	FE	PB	NI	CO	CD	AG
015	1 18	0 5	7 7	1300 0	2160 0	4390 0	1214 0	80 0	1560 0	2600 0	1658 0	60 0	20 0	15 0	7 0
016	1 29	0 9	8 1	800 0	1130 0	1030 0	429 0	48 0	1921 0	20100 0	6 7 0	50 0	60 0	11 0	5 0
017	1 2		1	1600 0	090 0	2300 0	9 3 0	88 0	2 93 0	40000 0	1017 0	50 0	60 0	14 0	5 0
018	1 3	1 2	8 4	1800 0	2290 0	2460 0	794 0	102 0	1763 0	39600 0	6 4 0	50 0	50 0	12 C	5 0
019	1 7	1 8	9 0	888 0	1584 0	3082 0	1022 0	73 0	1688 0	37633 0	1574 2	48	67 0	1 0	13 0
020	1 56	3 8	10 2	900 0	2710	34	78	101	865 0	45800 0	1700 0	50 0	40 0	15 0	7 C
021	1 50	2	9 6	1400 0	2210 0	3280 0	1222 0	95 G	3732 0	43500 0	1658 0	60 0	40 0	1 0	7 0
022	1 62	5 5	12 7	1600 0	2140 0	3490 0	1444 0	102 0	1965 0	44900 0	1867 0	80 0	60 0	17 0	5 0
023	1 73	4 8	11 8	2200 0	22 0 0	3500 0	1503 0	106 0	2456 0	55100 0	1800 0	70 0	7 0	12 0	5 0
024	1 86	5 5	12 7	1500 0	2120 0	2410 0	1294 0	106 0	2075 0	53000 0	1478 0	70 0	60 0	18 0	5
025	1 99	3 0	10 2	1700 0	2050 0	1700 0	1135 0	92 0	6324 0	56700 0	915 0	60 0	80 0	13 0	5 0
027	1 91	2 1	9 3	2100 0	1720 0	9700 0	1008 0	56 0	3742 0	39600 0	1150 0	40 0	20 0	6 0	5 0
028	2 01	5 5	12 7	1000 0	2120 0	2460 0	1325 0	109 0	2671 0	56800 0	1400 0	60 0	40 0	71 0	4 0
	1	5 5	12 7	1200 3	17 0 0	1410 0	8 1 0	77 0	9423 0	42000 0	571 0	60 0	80 0	14 0	5 0
030	2 07	3 7	10 9	480 0	1 80 0	2610 0	1065 0	80 0	4167 C	38300 0	1292 0	50 0	50 0	18 0	9 0
31	2 18	5	12 7	900 0	2070 0	2220 0	1310 0	107 0	6685 0	51960 0	1259 0	70 0	70 0	15 0	5 0
032		0		1700 0	1930 0	1320 0	1206 0	95 0	6503 9	59000 0	854 0	60 0	70 0	16 C	5 0
033	2 42	1 5	8 7	1301 0	1050 0	809 0	420 0	55 0	1764 0	30590 0	271 0	47 0	85 0	12 C	5 C
035	2 6	3 0	9 8	1800 0	960 0	940 0	476 0	57 0	491 0	14700 0	333 0	30 0	30 3	7 0	5 0
036	2 44	7 6	14 4	1500 0	1780 0	1430 0	1214 0	101 0	1938 0	46500 0	1058 0	70 0	50 0	16 0	5 0
037	2 58	7 6	14 4	1800 0	1930 0	1480 0	1222 0	84 0	1162 0	46600 0	750 0	40 0	40 0	8 0	5 0
040	2 52	5 5	12 3	1000 0	1810 0	1600 0	1284 0	82 0	4236 0	55100 0	898 0	50 0	40 0	9 0	5 0
041	2 61	7 6	14 4	1900 0	1470 0	1950 0	602 0	79 0	1189 0	38680 0	2417 0	70 0	40 0	16 0	7 0
042	2 74	4 6	11 4	2000 0	990 0	440 0	556 0	62 0	1089 0	41 00 0	404 0	40 0	30 0	14 0	5 0
044	2 82	1 8	8 6	400 0	1120 0	2680 0	770 0	73 0	832 0	25500 0	675 0	50 0	20 0	10 0	<5 0
045	2 72	6 0	15 0	275 0	1376 0	2752 0	939 0	65 0	2258 0	35413 0	2172 6	55 0	55 0	13 0	5 0
046	2 80	6 1	1 9	800 0	1660 0	3490 0	873 0	83 0	2880 0	44600 0	2021 0	70 0	40 0	14 0	5 0
047	2 50	6	11 4	1200 0	1650 0	1020 0	1183 0	85 0	6465 0	55700 0	687 6	80 0	40 0	20 0	5 0
0 0	3 10	1 8	8 4	1500 0	1610 0	1570 0	1087 0	83 0	1673 0	48700 0	633 0	60 0	10 0	13 0	<5 0
051	2 98	17 2	18 8	1810	2038 0	1448 0	1308 0	90 0	2515 0	52381 0	786 0	76 0	48 0	16 0	<5 0
052	2 92	5 1	15 9	1500 0	1960 0	1850 0	1452 0	82 0	6700 0	56700 0	948 0	70 0	40 0	6 0	< 0
053	3 01	6	11 4	1500 0	1850 0	1770 0	1266 0	75	3 88 0	551 0 0	787 0	60 0	40 9	10	0
	3 0		11 4	1700 0	2160 0	1700 0	1325 0	89 0	8275 0	49400 0	786 0	80 0	50 0	18 0	<5 0
058	3 36	3 7	10 3	1700 0	1910 0	1120 0	1208 0	88 3	2024 0	58000 0	654 0	80 0	60 0	15 0	5 0
059	3 25	4 6	11 2	2300 0	1840 0	1330 0	1111 0	82 0	1441 0	436C0 0	579 0	70 0	50 0	15 0	5 0
060	3 16	7 6	14 7	1058 0	2030 0	1248 0	1274 0	88 0	4604 0	49704 0	721 0	67 0	29 0	14 0	<5 0
061	3 12	10 7	17 3	1300 0	2000 0	1280 0	1310 0	65 0	5891 0	54400 0	821 0	58 0	60 0	0	<5 0
062	3 31	6 1	1 7	1600 0	2090 0	1270 0	1 0	5 0	6819 0	46200 0	825 4	90 0	80 0	14 0	<5 0
0 3	3 27	2 4	9 0	1500 0	1380 0	550 0	284 0	6 0	1867 C	19100 0	15 0	80 0	80 0	11 0	<5 0
067	3 66	3 7	10 3	1400 0	1880 0	810 0	1087 0	67 0	1801 0	4300 0	600 0	80 C	60 0	11 0	5 0
068	3 52	6 1	12 7	1527 0	2128 0	1947 0	1022 0	63 0	1226 0	48569 0	588 7	48 0	38 0	6 0	5 0
069	3 42	9 1	15 7	1800 0	1840 0	760 0	1151 0	89 C	0	0400 0	858 0	70 0	60 0	11 0	5 0
070	3 34	6 1	12 7	1509 0	2170 0	1500 0	1318 0	80 0	9315 0	55600 0	882 3	85 0	66 0	16 0	5 0
071	3 42	13 7	20 3	1600 0					5 3	48100 0	756 0	80 C	60 0	12 0	<5 0
072	3 41	7 8	14 2	1300 0	2070 0	950 0	1214 0	90 0	2280 0	52900 0	46 0	70 0	40 0	12 0	<5 0
073	3 41	1 8	8 4	800 0	650 0	200 0	177 0	43 0	630 0	15000 0	38 2	50 0	10 0	8 0	<5 0
077	3 82	5 5	12 1	1500 0	1880 0	700 0	1143 0	87 0	1049 0	47400 0	650 0	80 0	40 0	20 0	<5 0
078	3 81	12 2	18 8	1500 0	1910 0	860 0	1119 0	90 0	1833 0	54300 0	858 0	80 0	70 0	14 0	<5 0
079	2 71	15 2	21 8	1400 0	1930 0	800 0	1198 0	88 0	5202 0	51000 0	671 0	70 0	50 0	14 0	5 0
080	3 56	18 8	23 4	800 0	990 0	310 0	157 0	43 0	888 0	23300 0	276 0	50	50 0	6 0	5 0
081	3	5	21 8	1800 0	1700 0	1180 0	976 0	87 0	2232 0	43500 0	925 0	60 0	50 0	11 0	5 0
082	3 52	9 1	15 7	1700 0	2050 0	1090 0	1151 0	88 0	10321 0	47300 0	625 0	70 0	80 0	16 0	5 0
083	3 61	6 1	12 7	1437 0	3609 0	738 0	608 0	65 0	4260 0	35824 0	335 0	48 0	38 0	12 0	5 0
08	4 22	4 6	11 2	1200 0	2090 0	950 0	897 0	70 0	12903 0	55400 0	558 0	80 0	50 0	9 0	5 0
086	4 01	12 2	18 8	500 0	2270 0	1640 0	83 0	69 0	810 0	50300 0	638 0	70 0	20 0	7 0	5 0
087	3 96	15 2	21 8	1000 0	2010 0	720 0	865 0	78 0	2251 0	30200 0	400 0	68 0	50 0	16 0	5 0
088	3 8	1 2	18 8	1000 0	2010 0	650 0	1112 0		1	50200 0	617 0	80 0	50 0	14 0	5 0
089	3 70	20 1	26 7	1300 0	1960 0	1010 0	1246 0	82 0	5543 0	50100 0	808 0	80 0	50 0	15 0	5 0
090	3 74	18 3	24 8	1500 0	1880 0	1040 0	897 0	74 0	568 0	42600 0	548 0	60 0	40 0	19 0	5 0
091	3 72	6 1	12 7	1600 0	1270 0	670 0	508 0	53 C	2672 0	38 00 0	404 0	80 0	30 0	18 0	5 0
093	50	5 7	10 3	1500 0	1520 0	600 0	667 0	62 0	2238 0	42300 0	313 0	50 0	30 0	15 0	0
094	36	13 7	20 1	1000 0	2250 0	1150 0	928 0	93	11280 0	47303 0	604 0	70 0	5 0	1 0	5 0
095	4 24	18 3	2	800 0	1910 0	810 0	881 0	80 0	3282 0	60 0	650 0	2C 0	50	15 0	
096	14	15 8	26	132 0	2000 0	87 0	8 0	75 0	2840 0	32559 0	594 0	88 0	36 0	14 3	0
097	4 08	19 6	26 4	1500 0	2050 0	2100 0	1103 0	90 0	1721 0	5600 0	1775 0	0	60	15 0	5 0
098	4 00			1500 0	1660 0	980 C	14	78 0	384 0	40900 0	542 0	8C 0	60 C	17 0	5 0
102	4 64	12 2	18 8	1300 0	2300 0	960 0	984 0	86 0	8460 0	6000 0	33 0	70 0	40	19 0	0
103	4 52	18 3	24 9	2300 0	1771 0	810 0	1073 0	78 0	3952 0	48286 0	504 0	57 0	57 C	21 0	5 0
104	4 40	19 8	26 4	1900 0	1840 0	730 0	689 0	70 0	4989 0	43500 0	508 0	50 0	50 0	12 0	5 0
105	4 5	21 3	27 8	1085 0	2351 0	796 0	883 0	73 0	3392 0	45479 0	584 0	72 0	63 0	5 0	5 0
106	4 24	16 8	23 4	1500 0	1880 0	920 0	1087 0	73 0	4567 0	43000 0	529 0	70 0	30 0	16 0	5 0
107	4 28	4 0	10 8	1300 0	440 0	70 0	75 0	43 0	271 0	8000 0	44 0	30 0	10 0	13 0	5 0
108	4 82	6 1	12 7	1000 0	880 0	210 0	30 0	42 0	217 0	14200 0	71 0	30 0	30 0	8 0	5 0
109	4 81	12 2	18 8	1300 0	890 0	303 0	246 0	46 0	795 0	21800 0	150 0	50 0	30 0	8 0	<5 0
110	68	18 3	24 9	1062 0	1467 0	289 0	408 0	6 0	1862 0	52915 0	217 0	48 0	39 0	10 0	5 0
111	4 58	21 3	27 9	1200 0	1770 0	590 0	810 0	76 0	3271 0	43100 0	433 G	60 0	40 0	13 0	5 0
112	4 50	18 3	24 8	1300 0	2100 0	940 0	1032	74 0	8049 0	48800 0	471 0	80 0	40 0	9 0	<5 0
113	4 42	18 3	24 8	1300 0	2010 0	670 0	897 0	77 0	4100 0	49000 0	575 0	80 0	70 0	8 0	<5 0
114	4 37	3 0	9 55	1177 0	451 0	49 0	93 0	47 0	334 0	1C 90 0	43 0	20 0	39 0	10 0	<5 0
116	4 97	9 4	16 0	1500 0	2070 0	490 0	421 0	41 0	4482 3	27803 0	179 0	48 0	60 0	13 0	<5 6
117	0	15 0	23 0	910 0	2200 0	1290 0	890 0	40 0	2570 0	43 00 0	83 0	70 0	40 0	17 0	<5 0
120	4 62	7 8	14 2	1700 0	940 0	330 0	439 0	50 0	1529 0	16800 0	271 0	40 0	40 0	11 0	<5 0
121	4 56	1 5	8 1	1400 0	350 0	80 0	48 0	34 0	87 0	8100 0	10 0	20 0	20 0	8 C	<5 0
127	4 81	18 3	24 8	1500 0	2160 0	2050 0	523 0	62 0	2097 0	38200 0	279	30 0	30 0	7 0	> 0
178	4 78	8 4	13 0	926 0	935 0	232 0	253 0	30 0	1042 0	22037 0	128 0	27 0	19 3	1 0	< 0
133	4 97	2 7	8 3	2010 0	852 0	183 0	95 0	4 0	376 0	18365 0	161 0	39 0	9 0	9 C	<5 0

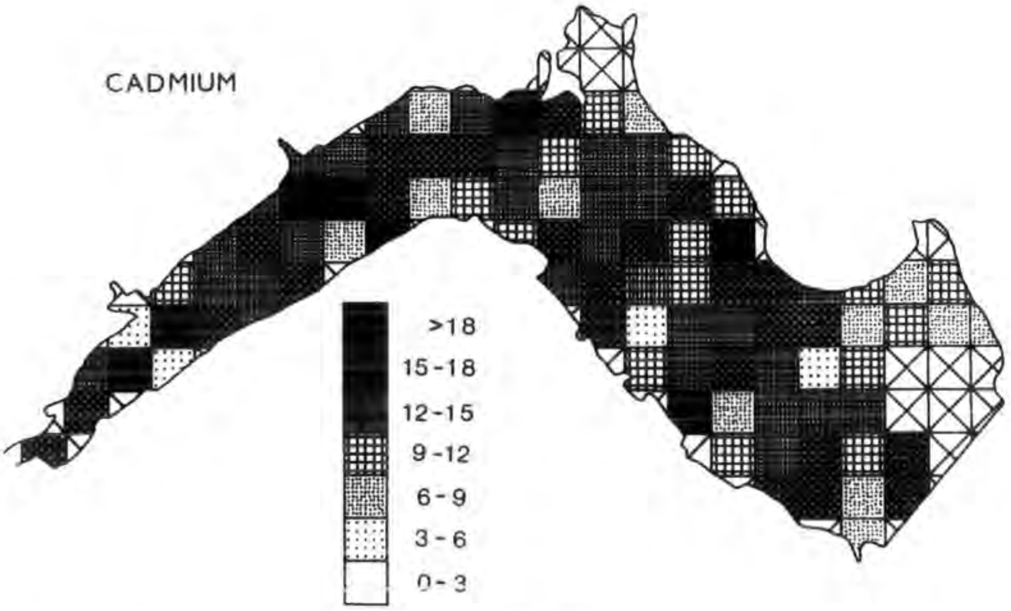
Fig. 5.4

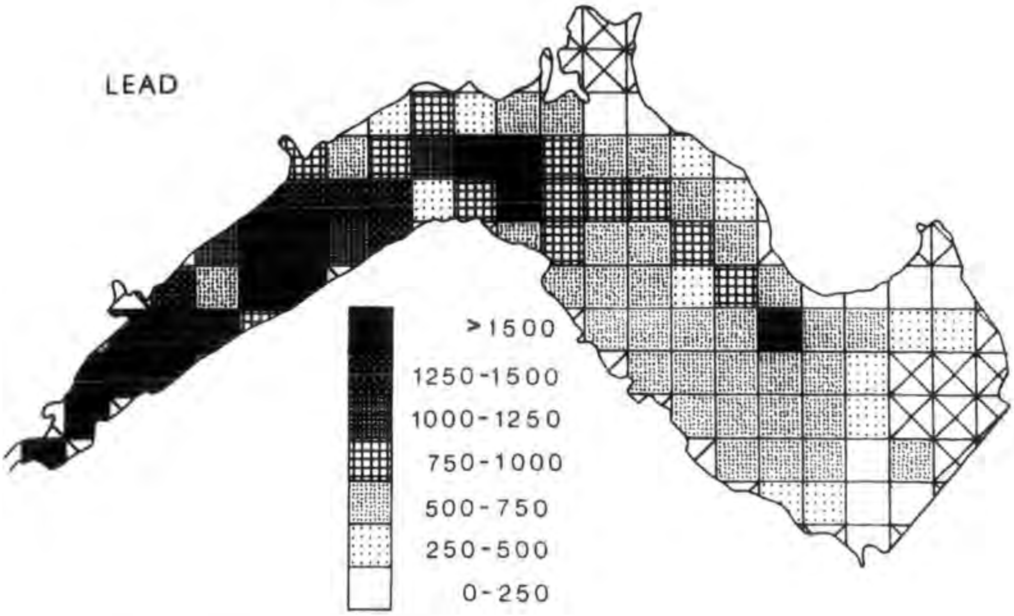
Variations in the zinc, cadmium and lead content of sediments from the Derwent Reservoir.

(concentrations expressed as $\mu g\ g^{-1}$ dry weight)

Note higher lead levels following the original course of the R. Derwent.


ZINC


CADMIUM


LEAD

☒ SQUARE NOT SAMPLED

the R. Derwent. Significant (P = <0.001) negative correlations were found between the levels of calcium, zinc and lead in the sediments and the distance of the sampling points from the river mouth (Table 5 6)

Differences in the distribution of zinc and lead, similar to those noted during the composition of stream and river sediments, were found within the sediments of the reservoir. Levels of lead were found to fall off more quickly than levels of zinc on passing away from the river mouth, with a consequent increase in lead : zinc ratios (Fig. 5.5). This was probably due to the greater association of lead with suspended particles which had settled out from the water column more quickly than zinc (a higher proportion of which was probably in a non-particulate form). An interesting feature of Fig. 5.4 is the 'line' of higher lead levels found in a series of samples of sediment following the course of the original bed of the R. Derwent for some way beyond the nature reserve. It is possible that these samples consisted in part of material laid down in the river before the construction of the reservoir. It seems more likely, however, that occasional 'streaming' of river water along the reservoir bottom had 'shunted' heavier lead-rich suspended particles and/or coarse sediment along the original bed of the river.

The elements which showed significant correlations with levels of zinc, lead and cadmium in the sediments (Table 5.6) gave some indication of the possible pathways by which the metals might have entered the sediments Elements showing significant positive correlations (P = <0.001) with variations in lead concentrations were calcium (a major component of powdered fluorspar) and silver (a major component of the galena associated with the fluorspar mined at the Whiteheaps Mine). Binding of zinc onto sedimentary clay minerals (as described by Pita & Hyne, 1975) may represent a pathway for the sedimentation of zinc, however zinc, copper, nickel and cobalt were all

Table 5.6

Intervariable correlation matrix showing the degree of correlation between concentrations of 12 elements, depth and distance from the entry of the R. Derwent for sediments from the Derwent Reservoir

(DIST = distance from the mouth of the river ; DEPTH = depth at time of sampling; TWLDEPTH = depth below top water level)

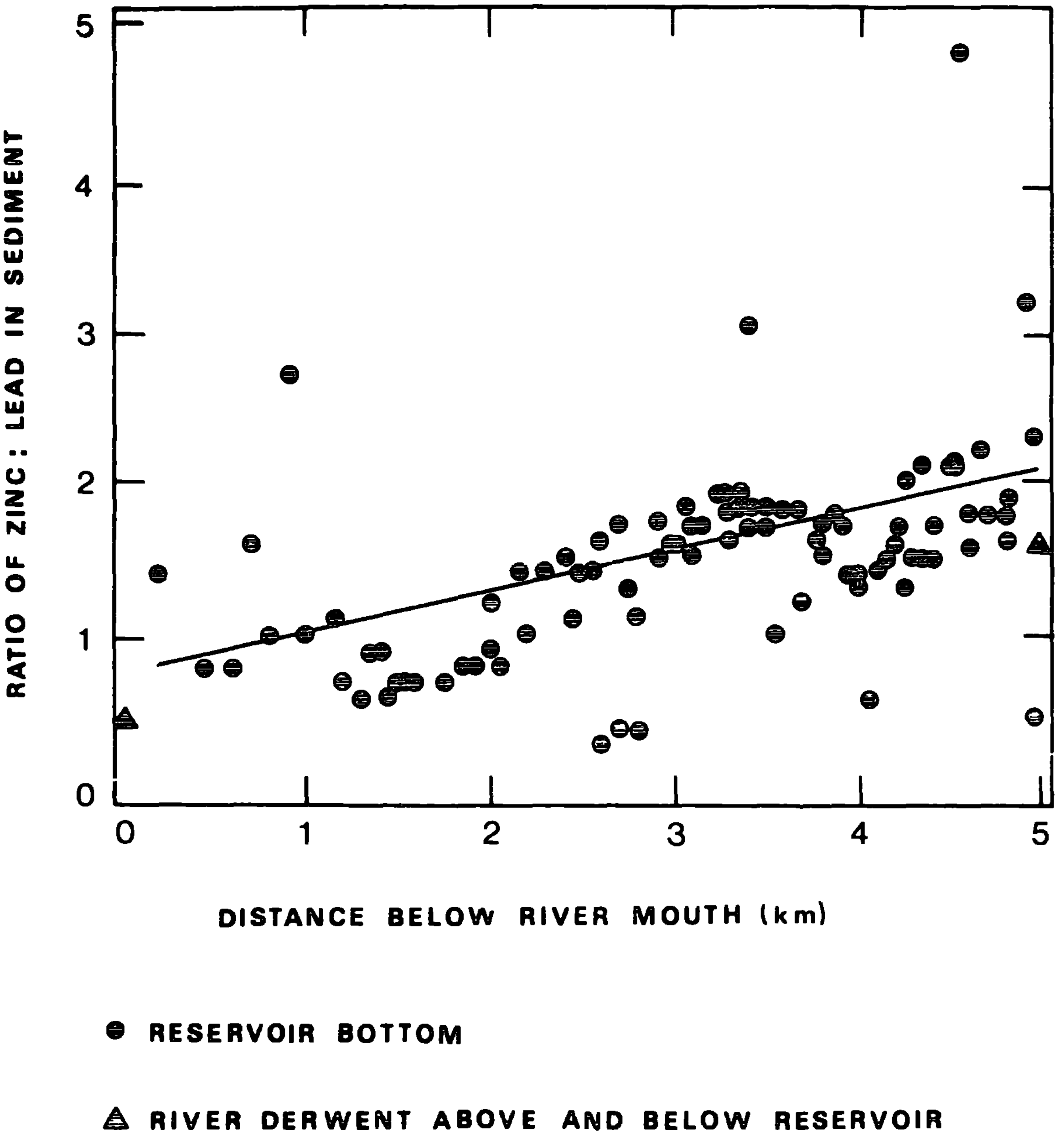
	DIST	DEPTH	TWLDEPTH	Na	Mg	Ca	Zn	Cu	Mn	Fe	Pb	Ni	Co	Cd
DEPTH	0.6440**													
TWLDEPTH	0 6516**	0.9956**												
Na	-0 0817	-0.0788	-0.0875											
Mg	-0 1223	0 2930*	0 3085*	0 1037										
Ca	-0 4871**	-0 2643*	-0 2353	0.0592	0 3422**									
Zn	-0.5829**	-0 2160	-0 2499*	0.1595	0.3646**	0 2391								
Cu	-0 0239	0 1813	0 2150	-0.0665	0.4584**	0 1542	0 1073							
Mn	0 1133	0 2158	0 2222	0 0069	0 4977**	0 0008	0 1054	0 1592						
Fe	-0 0432	0 3025*	0 3205**	0 1161	0 8438	0 2461*	0 3199*	0 3988**	0.5238**					
Pb	-0 7468**	-0 3109	-0 3185**	0 0134	0.3078*	0 5676**	0 6333**	0 1324	-0 0563	0 2136				
Ni	0 0138	0 3091*	0 3091*	0 0412	0 5519*	0 0285	0 2729*	0 3592**	0 4741**	0 6995**	0 2074			
Co	-0 1379	0 1402	0.1529	0.1539	0 3968**	-0 0334	0 1607	0 1731	0.3470**	0 4103**	0 1736	0 4133**		
Cd	-0 1587	0.0256	0 0302	0.0812	0 2097	-0.0107	-0 1495	0 2882*	0 2269	0 2536	0 2696*	0 4240**	0 2695*	
Ag	-0 3355**	-0 2008	-0 1726	-0.1996	0 0377	0 3653	0 0159	0 0356	0.0577	-0 0597	0 4156**	-0 0907	0 0904	0 1412

* - P =<0 01   ** - P =<0 001

Fig. 5.5

Scatter diagram showing the increase of zinc.lead ratios in the Derwent Reservoir sediments with increasing distance from the river entry point

$r = +0.4875$, $P = <0.001$


RATIO OF ZINC : LEAD IN SEDIMENT
0
1
2
3
4
5
0
1
2
3
4
5
DISTANCE BELOW RIVER MOUTH (km)
● RESERVOIR BOTTOM
▲ RIVER DERWENT ABOVE AND BELOW RESERVOIR

found to be significantly correlated with levels of iron in the sediments This suggests that binding by hydrous iron oxides (e.g. Jenne, 1968, Lee, Lopez & Mariani, 1975) and/or co-precipitation with iron (Jackson & Nichol, 1975) may play a significant role in the sedimentation of these metals. No significant correlation was found between variations of zinc levels and manganese levels in the sediments. This suggests that if adsorption onto hydrous oxides is a significant pathway for zinc sedimentation, then hydrous oxides play a more important part than manganese oxide. (The mean manganese content of the reservoir sediments (1866 µg g^{-1}) was much lower than the mean iron content (31250 µg g^{-1}).) Large amounts of yellow organic material are present in the waters of the R. Derwent and the reservoir, and it is possible that complexation by organic molecules represents an intermediate stage in the sedimentation of zinc (e.g Davis & Leckie, 1977).

No obvious pattern of distribution was found for HNO_3-extractable cadmium in the reservoir sediments, although the metal was clearly being accumulated. Cadmium levels showed positive correlations with variations in levels of copper (P = <0 01) iron (P = <0.01), lead (P = <0 01) and nickel (P = <0.001), but not levels of zinc. It seems likely that the main processes suggested for the sedimentation of zinc and lead both play a role in the sedimentation of cadmium. However Gardiner (1974) suggested that humic material was the main component of river muds responsible for cadmium adsorption, and it is possible that the distribution of organic components within the reservoir sediment was an overriding influence contributing to the observed distribution of the metal. (No analyses were made of the organic carbon content of sediment samples.)

5.4 COMPOSITION OF PLANTS

5.41 *Nitella flexilis*

Following the choice of *Nitella* for studies of uptake of metals within the reservoir by a non-rooted submerged plant, the alga was sampled from six sites within the reservoir between 15 August and 10 October 1975. Four replicate subsamples (each composed of several plants) were collected from five sites within the western half of the reservoir, and a single sample of pooled whole plants was collected from a site near to the dam. 'Total' water samples for the analysis of cations were also collected at each of the sites.

The mean mineral composition of *Nitella* from the sites within the reservoir is given in Table 5.7. The concentrations of zinc, lead and cadmium found in samples of the plant from the various sites are shown diagramatically in Fig. 5.6. The variations in the heavy metal content of *Nitella* plants (sampled after a long calm period within the reservoir) showed a high measure of agreement with the levels of metals found during the transect to sample water (section 5.2, Transect A) made around the time the plants were collected. The lowest level of zinc (356 µg g^{-1} dry weight) was found in material from near the south shore near the dam, and a clear transition of concentrations was then apparent on passing towards the western end of the reservoir. The highest mean zinc concentration (1811 µg g^{-1}, n = 4) was found in material from just below the river mouth. Similar distribution patterns were found for the concentrations of lead and cadmium in the plants, although levels of lead in *Nitella* fell off more quickly on passing away from the river mouth than did levels of zinc or cadmium (as was found in the sediments).

			Na		K		Mg		Ca		Zn		Cu		Mn	
Reach no	Site	n	$\bar{x}$	*s.d.*	$\bar{x}$	*s.d.*	$\bar{x}$	*s.d.*	$\bar{x}$	*s.d.*	$\bar{x}$	*s.d.*	$\bar{x}$	*s.d.*	$\bar{x}$	*s.d.*
11-9-75	011	4	3676.0	106.0	25500.0	500.0	6808.0	183.0	4368.0	57.0	1811.0	5.5	20.0	0.5	13605.0	3146.0
15-8-75	016	4	3776.0	106.0	10771.0	342.0	5865.0	277.0	4589.0	105.4	1113.0	34.5	22.0	3.0	3133.0	148.0
19-8-75	020	4	3188.0	311.0	17094.0	495.0	5956.0	205.0	5319.0	132.0	860.0	45.0	15.0	2.4	3606.0	330.0
27-8-75	042	4	2288.0	28.0	19406.0	1504.0	5894.0	145.0	5208.0	62.0	948.0	72.0	13.7	0.2	5931.0	1331.0
10-10-75	045	4	2938.0	238.0	22313.0	313.0	6525.0	25.0	5713.0	587.0	557.0	4.6	15.4	0.4	1738.0	12.5
11-9-75	109	1	2555.0		20000.0		8111.0		4444.0		356.0		24.4		1389.0	

			Fe		Al		Pb		Ni		Co		Cd		Ag	
Reach no.	Site	n	$\bar{x}$	*s.d*	$\bar{x}$	*s.d.*	$\bar{x}$	*s.d*	$\bar{x}$	*s.d.*	$\bar{x}$	*s.d.*	$\bar{x}$	*s.d*	$\bar{x}$	*s.d.*
11-9-75	011	4	12628.0	128.0	2286.0	10.5	1061.0	10.5	43.0	6.0	27.0	5.2	22.3	0.3	1.3	
15-8-75	016	4	7968.0	372.0	1747.0	123.0	433.0	10.7	34.0	4.0	39.0	2.4	15.0	0.7	1.3	
19-8-75	020	4	4417.0	724.0	2175.0	731.0	165.0	42.3	43.0	9.7	27.0	7.0	17.7	2.9	1.3	
27-8-75	042	4	4266.0	485.0	1453.0	268.0	72.5	0.0	25.0	0.0	22.0	2.0	8.1	1.0	1.3	
10-10-75	045	4	10125.0	125.0	925.0	25.0	120.0	0.0	41.0	8.0	30.0	6.5	80.0	0.0	1.3	
11-9-75	109	1	7222.0		1667.0		178.0		40.0		34.0		14.4		1.3	

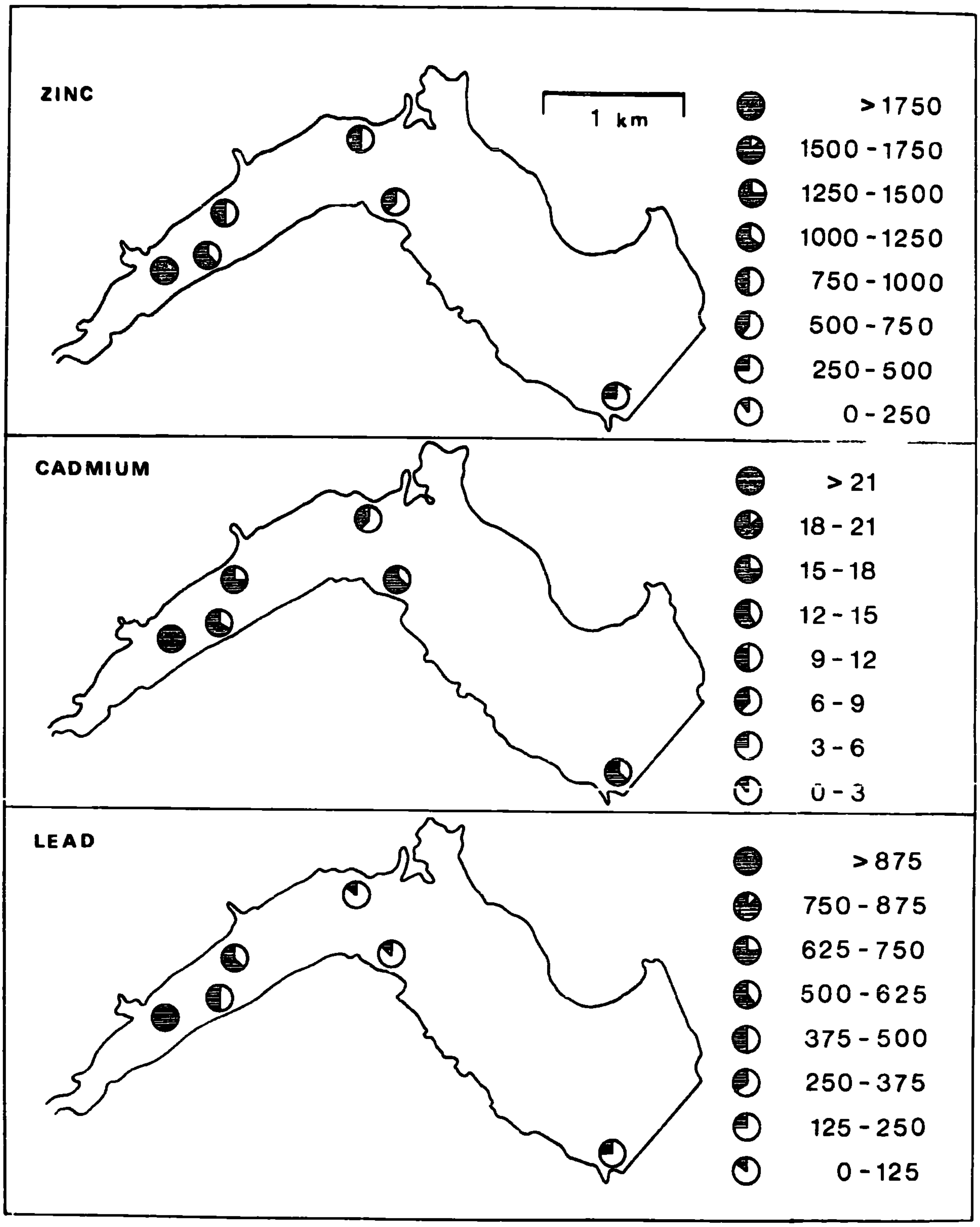
Table 5.7 The mineral composition of *Nitella flexilis* from the Derwent Reservoir

All concentrations are in µg g^{-1} dry weight

Fig. 5.6

Variations in the zinc, cadmium and lead content of whole *Nitella flexilis* plants from the Derwent Reservoir

(concentrations expressed as μg g^{-1} dry weight, mean of four replicates at each site)


ZINC
1 km
>1750
1500 - 1750
1250 - 1500
1000 - 1250
750 - 1000
500 - 750
250 - 500
0 - 250
CADMIUM
> 21
18 - 21
15 - 18
12 - 15
9 - 12
6 - 9
3 - 6
0 - 3
LEAD
>875
750 - 875
625 - 750
500 - 625
375 - 500
250 - 375
125 - 250
0 - 125

In order to compare the levels of heavy metals found in *Nitella* from the Derwent Reservoir with levels in material from a site known to be completely unaffected by metal pollution, pooled whole plants were collected from the R. Tees (0009-05) on 24 June 1977. The concentrations of zinc, lead and cadmium found in this material were 470 µg g^{-1}, 37 µg g^{-1} and 8 µg g^{-1} respectively These levels are lower than any found in material from the Derwent Reservoir, with the exception of the lowest level of zinc (356 µg g^{-1})

5.42 Glyceria fluitans

General increases in the levels of zinc, lead and cadmium in the 'young' leaves, 'old' leaves and stems of *Glyceria fluitans* were found on passing from the eastern end of the reservoir towards the river entry (Table 5.8, Figs 5 7, 5.8), although the trends were not as clear-cut as had been found in plants of *Nitella*. Within individual samples of *Glyceria* plants, 'old' leaves tended to have higher contents of zinc, lead and cadmium than did 'young' leaves or stems. In general the levels of all three metals tended to be somewhat lower in all three fractions of *Glyceria* plants than in whole *Nitella* plants in cases where the two species were samples together. There were therefore no clear indications from the data that plants of *Glyceria* (which possesses true roots) had taken up significant levels of metals from the sediments in which it was growing

An intervariable correlation matrix (Table 5.9) was computed in order to investigate the relationships between levels of zinc, lead and cadmium within the water, sediments and plants from sites where *Nitella* and *Glyceria* were collected. 'Total' levels of the three metals in the reservoir water were found to be negatively correlated ($P = < 0.001$) with distance from the entry of the R. Derwent. Levels of the metals in the sediments of the collection

Table 5 8

The mineral composition of *Glyceria fluitans* from the Derwent Reservoir

All concentrations are in µg g^{-1} dry weight

For position of sampling sites see Fig. 5 1

TABLE THE MINERAL COMPOSITION OF GLYCERIA FLUITANS FROM THE DERWENT RESERVOIR

DATE	SITE	FRACTION	NA		K		MG		CA		ZN		CU		MN		FE		AL		PB		NI		CO		CI		AC	
			MEAN	S D	MEAN	D	MEAN	S D	MEAN	S D	MEAN	S D	MEAN	S D	MEAN	D	MEAN	S D	MEAN	S D	MEAN	D	MEAN	S D	MEAN	S D	MEAN	S D	MEAN	S D
08 8 75	011	YOUNG LEAVES	2184.0	343.0	29051.0	1408.0	2387.0	133.0	1322.0	72.0	364.0	20.0	59.0	4.0	1981.0	232.0	2549.0	179.0	1092.0	100.0	191.0	12.0	16.0	5.0	13.0	9.0	7.8	7.1	1	
		OLD LEAVES	4690.0	369.0	29581.0	1721.0	2724.0	127.0	1999.0	19.0	692.0	54.0	59.0	5.7	3024.0	97.0	4789.0	261.0	1785.0	214.0	238.0	11.0	10.0	5	8.0	6.0	4.1	1.6	1.5	
		STEMS	5028.0	593.0	51964.0	2526.0	1665.0	112.0	777.0	101.0	1160.0	103.0	108.0	8.5	1915.0	121.0	1701.0	132.0	505.0	76.0	103.0	21.0	2.5		0	1.1	0	1.6	1	
09 8 75	020	YOUNG LEAVES	872.0	65.0	20894.0	266.0	1589.0	33.0	878.0	48.0	208.0	64.0	46.0	0.8	598.0	15.4	975.0	50.0	415.0	15.0	33.0	5.0	8.0	2.5	2.5		3.9	0.6	1.5	
		OLD LEAVES	1585.0	26.0	20282.0	559.0	1658.0	45.0	1748.0	138.0	295.0	7.0	37.0	1.2	1065.0	6.0	1163.0	24.0	581.0	11.0	50.0	1.9	2.5		2		2.0	0	1	
		STEMS	1805.0	379.0	45456.0	1498.0	1141.0	212.0	550.0	114.0	140.0	46.0	33.3	7.2	597.0	6.6	711.0	21.0	259.0	77.0	28.0	2.6	15.0	9.0	10.0	1.0	2.0	0.6	1.3	
09 8 75	016	YOUNG LEAVE	1158.0	94.0	17604.0	714.0	1540.0	67.0	550.0	315.0	775.0	480.0	61.0	3.7	1006.0	53.0	1691.0	50.0	649.0	54.0	76.0	2.2	11.0	4.2	2.5		3.9	2.0	1.5	
		OLD LEAVES	1899.0	58.0	12534.0	2027.0	1274.0	7.0	866.0	157.0	431.0	13.0	56.0	9.8	2083.0	21.0	2853.0	92.0	964.0	17.0	105.0	5.8	<2.5		2.5		2.4	1.1	1.5	
		STEMS	2436.0	141.0	28328.0	1852.0	1475.0	65.0	202.0	72.0	473.0	50.0	85.0	0.7	1334.0	71.0	1353.0	97.0	539.0	152.0	75.0	7.2	2.5		4.6	0.4	6	1.4	1.5	
08 8 75	040	YOUNG LEAVES	1053.0	31.0	22838.0	397.0	1455.0	45.0	931.0	93.0	109.0	0.9	18.2	0.9	357.0	10.0	1387.0	12.0	353.0	35.0	32.0	9	2.5		2.5		2.0	0.4	1.3	
		OLD LEAVES	2281.0	33.0	17500.0	04.0	1751.0	33.0	1350.0	40.0	159.0	1.0	21.0	0.2	462.0	6.3	1819.0	36.0	488.0	8.8	46.0	2	2.5		2		1.4	0	1.5	
		STEMS	1658.0	24.0	44375.0	1008.0	1163.0	22.0	683.0	42.0	86.0	1.3	16.0	0.3	161.0	2.2	619.0	11.0	234.0	21.0	19.0	2	18.0	10.0	12.0	2.7	1	0.5	1.5	
28 8 75	042	YOUNG LEAVES	706.0	21.0	12981.0	454.0	1344.0	33.0	1000.0	83.0	93.0	4.8	20.0	1.6	183.0	0.3	88.0	0.0	216.0	54.0	14.4	7	2		2		0.8	0.1	1.5	
		OLD LEAVES	1375.0	18.0	15656.0	162.0	1738.0	52.0	1744.0	78.0	149.0	2.0	2.0	1.0	503.0	5.6	1258.0	13.0	479.0	10.0	26.0	2	7.8	2.2	9.0	1.0	0.3	0.4	1.5	
		STEMS	1638.0	57.0	35458.0	386.0	1313.0	45.0	492.0	51.0	100.0	5.0	19.3	0.3	294.0	5.8	532.0	52.0	303.0	6.0	15.0	1	24.0	6.1	10.0	2.5	0.1	0.1	1.5	
8 8 75	051	YOUNG LEAVES	825.0	47.0	23625.0	871.0	1519.0	219.0	1159.0	174.0	117.0	23.0	18.0	0.6	252.0	21.0	2019.0	239.0	281.0	69.0	29.0	6.5	2.5		2.5		1.9	0.5	1.5	
		OLD LEAVES	1844.0	248.0	18938.0	590.0	1469.0	133.0	992.0	94.0	173.0	22.0	19.0	0.7	850.0	58.0	1571.0	830.0	424.0	168.0	40.0	15.0	12.0	4.3	12.0	1.2	2.1	0.4	1.5	
		STEMS	1706.0	134.0	43875.0	3511.0	1125.0	75.0	413.0	57.0	105.0	16.0	16.0	0.6	210.0	15.0	472.0	159.0	165.0	13.0	25.0	5.6	9.4	2.2	6.8	1.8	1	0.4	1.5	
28 8 75	072	YOUNG LEAVES	1213.0	64.0	14736.0	1222.0	1351.0	137.0	382.0	44.0	372.0	19.0	4.0	3.7	295.0	25.0	987.0	152.0	479.0	107.0	3.0	7.8	<2.5		2.5		7.4	3.3	1.5	
		O D LEAVES	2744.0	70.0	12377.0	749.0	2563.0	65.0	1055.0	8.0	355.0	103.0	31.0	2.0	1062.0	39.0	3619.0	131.0	1521.0	79.0	4.0	1.4	2.5		<2.5		6.0	0.2	1.3	
		STEMS	2539.0	97.0	34625.0	81.0	1436.0	17.0	310.0	40.0	168.0	6.0	74.0	1.7	675.0	34.5	792.0	34.0	358.0	20.0	3.0	8.6	2.5		4.8	1.0	0	0.4	1.5	
08 8 75	058	YOUNG LEAVES	938.0	38.0	23719.0	23.0	1481.0	21.0	1544.0	33.0	105.0	2.1	1.0	1.4	191.0	7.0	77.0	23.0	188.0	13.0	2.0	2.1	2.5		2.5		1.5	0.6	1.5	
		OLD LEAVES	1970.0	59.0	20765.0	227.0	1405.0	12.0	1215.0	21.0	137.0	2.1	17.0	0.5	443.0	17.0	1192.0	38.0	259.0	6.1	40.7	3.6	21.0	6	9	0.9	7.5	0.6	1.5	
		STEMS	9556.0	37.0	47417.0	717.0	1100.0	31.0	563.0	22.0	89.0	2.7	14.0	0.0	151.0	2.1	313.0	0.0	131.0	22.0	13.0	1.3	5.0	3.1	2		2.0	0.6	1.3	
08 8 75	094	YOUNG LEAVES	1246.0	49.0	23448.0	143.0	1550.0	16.0	1224.0	18.0	125.0	2.0	19.0	0.6	219.0	3.7	863.0	25.0	207.0	6.7	20.0	3.7	2.5		2.5		6	0.5	1.3	
		OLD LEAVES	3123.0	45.0	17180.0	464.0	1727.0	22.0	1219.0	72.0	198.0	9.9	26.1	0.8	748.0	6.0	1193.0	65.0	362.0	11.0	28.9	5.5	21.0	10.0	12.0	6.5	1.7	1.7	1.5	
		STEMS	2757.0	143.0	43260.0	218.0	1246.0	63.0	569.0	12.0	127.0	10.0	19.0	0.7	318.0	13.0	1007.0	62.0	210.0	11.0	12.0	2.4	<2.5		2		2.5	0.4	1.5	
08 8 75	109	YOUNG LEAVES	825.0	54.0	35125.0	1327.0	1075.0	74.0	1081.0	271.0	493.0	312.0	180.0	49.0	104.0	16.0	738.0	77.0	181.0	21.0	71.0	31.0	<2.5		2.5		1.5	0	1.3	
		OLD LEAVES	1062.0	115.0	19632.0	561.0	1697.0	14.0	1132.0	27.0	1238.0	187.0	188.0	107.0	1479.0	37.0	1584.0	78.1	460.0	17.0	102.0	45.0	16.0	3.7	21.0	4.2	7.6	0	1.5	
		STEMS	2981.0	33.0	42750.0	61.0	1281.0	45.0	542.0	31.0	119.0	0.9	12.0	0.7	241.0	5.0	560.0	41.0	225.0	25.0	16.0	6.2	10.0	6	4		2.1	0	1.5	

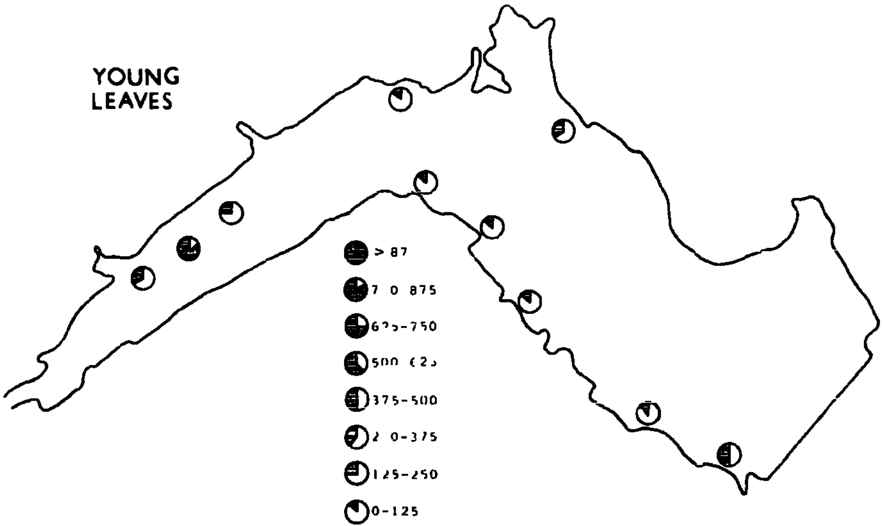
ALL CONCENTRATIONS ARE IN PARTS PER MILLION DRY WEIGHT

0.2 G OF DRIED MATERIAL USED IN ALL DETERMINATIONS

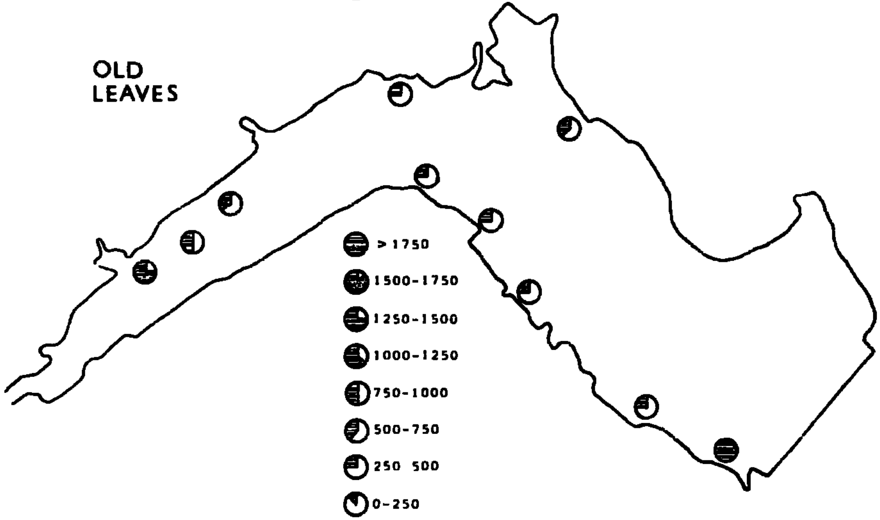
Fig. 5.7

Variations in the zinc content of different fractions of *Glyceria fluitans* plants from the Derwent Reservoir.

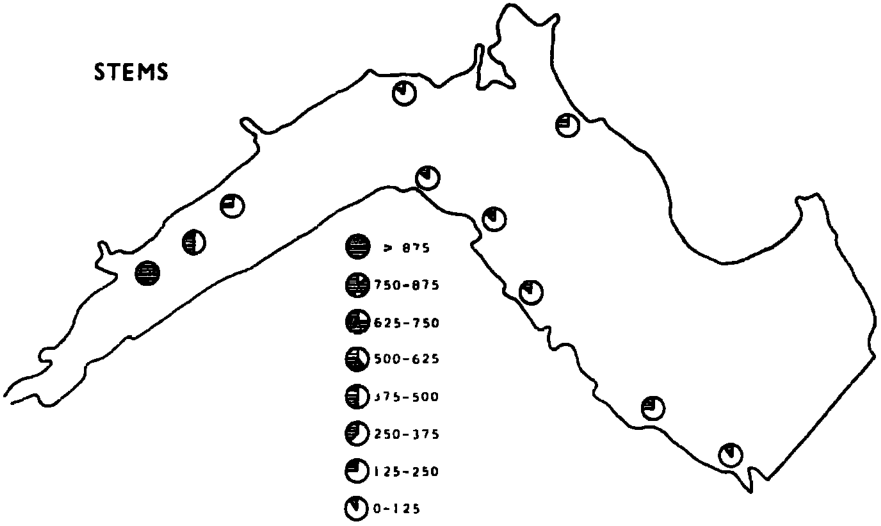
(concentrations expressed as $\mu g\ g^{-1}$ dry weight, mean of four replicates from each site)



YOUNG LEAVES



OLD LEAVES

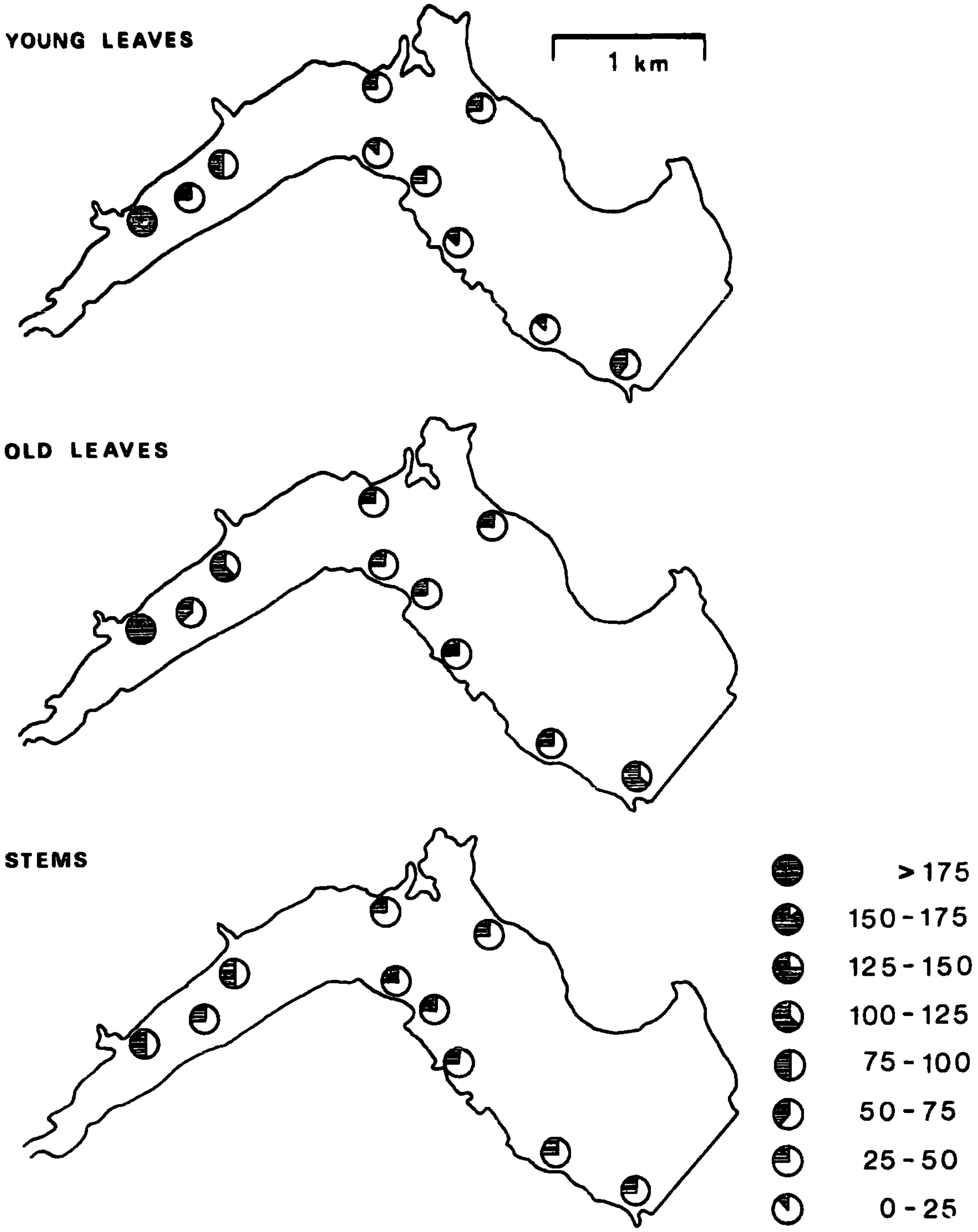


STEMS

Fig. 5.8

Variations in the lead content of different fractions of *Glyceria fluitans* plants from the Derwent Reservoir.

(concentrations expressed as µg g^{-1} dry weight, mean of four replicates at each site)


YOUNG LEAVES

OLD LEAVES

STEMS

sites were less strongly negatively correlated with distance from the mouth of the river, possibly because material washed from the banks of the reservoir contributed to the sediment at some of the sites. Weak positive correlations ($P = >0.001$) were found between 'total' levels of zinc, lead and cadmium in the water and the levels of the metals in *Nitella*. Significant positive correlations ($P = <0.001$) were found between the lead contents of all three fractions of *Glyceria* plants and the 'total' lead content of the water, but no such relationship was found for zinc or cadmium.

No significant positive correlations were found between the levels of zinc, lead or cadmium in *Nitella* plants and the levels of the metals in sediments at the collection sites. A positive correlation ($P = 0.066$) was found between the lead content of *Glyceria* stems and that of the sediment, and weaker positive correlations were found between the levels of lead in 'young' and 'old' leaves and that of the sediment. This could be considered as evidence that uptake of lead from the sediments by the roots of *Glyceria* was contributing to the levels of the metal in the plants. No significant positive correlations were found between the levels of zinc or cadmium in any fraction of *Glyceria* plants and the levels in the sediment at the collection sites.

CHAPTER 6

ACCUMULATION OF HEAVY METALS BY FIELD POPULATIONS OF *LEMANEA FLUVIATILIS*

6.1 INTRODUCTION

During the studies reported in 3.44 and 4.51, it was established that the heavy metal content of filaments of *Lemanea fluviatilis* reflected differences in the metal content of the water along the R. Derwent. During the second and third years of the project further studies were carried out of the accumulation of heavy metals by populations of *Lemanea* in the field. Some of these studies were carried out within the catchment area of the R. Derwent, but for comparative purposes sampling was extended to a range of reaches in England and Wales.

The results of a broad survey of the levels of metals accumulated by a range of field populations are reported in 6.2. The results of a study of factors influencing variation in the levels of metals accumulated by a single population in the field are presented in 6.3, and the results of a series of transplant experiments are presented in 6.4. Whilst it was not intended that the study should include detailed laboratory ivestigations of the uptake of metals by *Lemanea,* some short-term laboratory investigations were carried out on material from one reach. The results of these studies are reported in 6.5 The results of laboratory assay of the tolerance to zinc of six populations of *Lemanea* are presented in 6.6.

6.2 HEAVY METAL CONTENT OF *LEMANEA* GROWING IN RIVERS CARRYING VARYING LEVELS OF METALS IN THE WATER

6.21 Location of sampling reaches and programme for collection of *Lemanea*

Samples of *Lemanea* were collected once from each of 47 reaches on rivers in England and Wales during the spring of 1977 (Figs 6.1 , 6.2; Table 6 1). The reaches sampled were selected to show as wide a variation as possible of the levels of heavy metals in the water, and of the levels of other chemical parameters that might be expected to influence the uptake of metals. All of the rivers with the higher levels of zinc, lead or cadmium in the water were located in areas affected by past or present mining. Some of the sites in Wales were located on the basis of the work of Morgan (1970) and from information supplied by A. K Jones. Sites in Derbyshire and Swaledale were located by study of the geological maps of Thornton (1974) and by information supplied by I. Thornton (personal communication). Some of the river sites in Northern England which might have been expected to carry low levels of heavy metals in the water were located from the account of Holmes (1975).

Wherever possible four subsamples of pooled 2 cm long filament tips were collected from within each reach, together with water for analysis. Reaches in North and West Wales were sampled before reaches in Northern England, as it was hoped to collect material from all reaches at a similar stage in the growth cycle of the alga.

Figs 6.1 and 6.2

Maps to show the position of sites sampled for *Lemanea* in north-east England and in other areas of England and Wales.

Black dots represent individual sites, numbers refer to the number of sites sampled in a particular region.

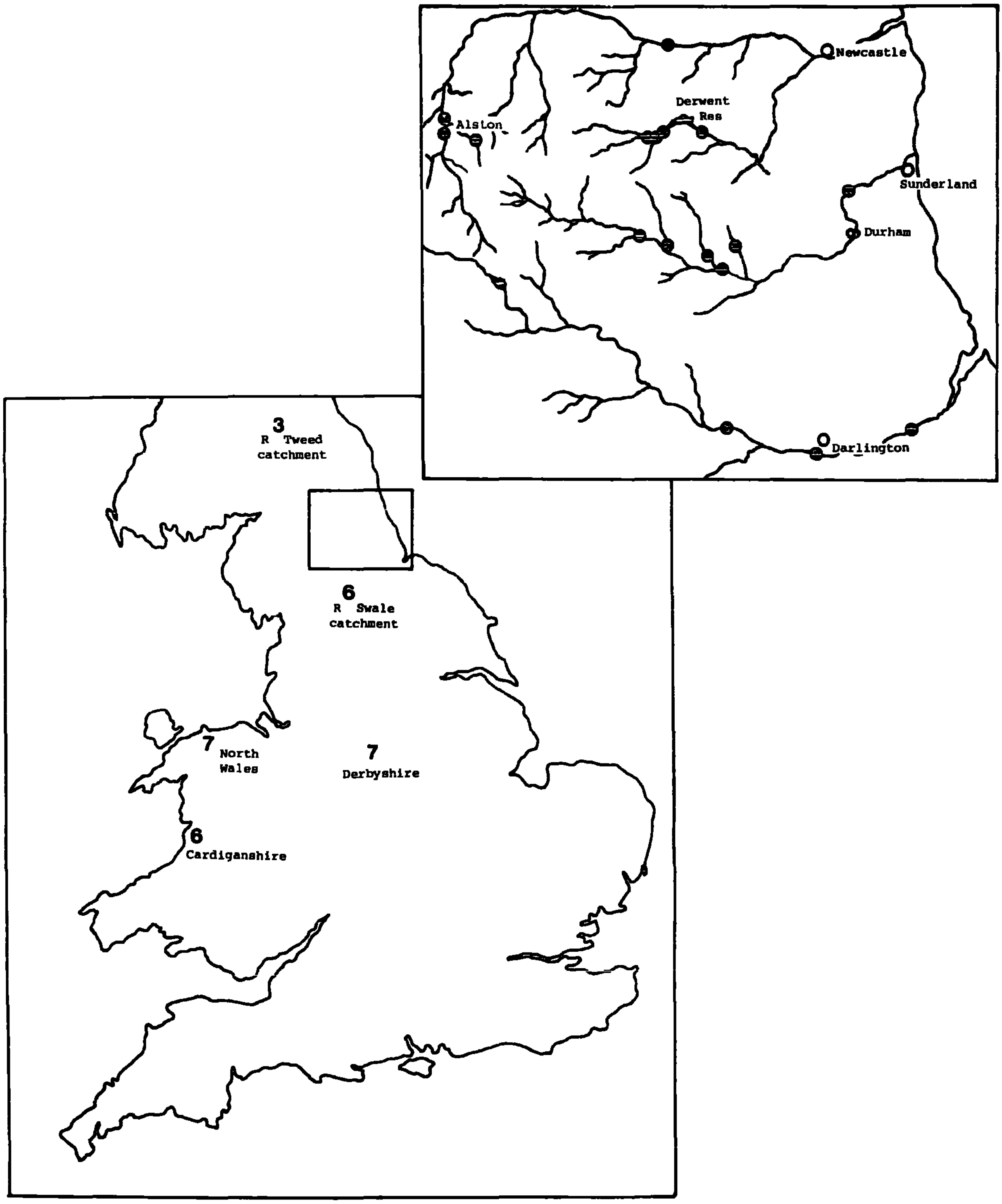

Newcastle
Derwent
Res
Alston
Sunderland
Durham
Darlington
3
R Tweed
catchment
6
R Swale
catchment
7
North
Wales
7
Derbyshire
6
Cardiganshire

Table 6.1

Location of reaches sampled for *Lemanea fluviatilis*

Stream number	Reach number	Grid ref	Site
0007	22	NZ 103383	Houselop Beck
0008	02	NY 859394	R Wear at Wearhead
0008	20	NY 987391	R Wear at Stanhope
0008	75	NZ 296473	R Wear at Finchale Priory
0009	05	NZ 814299	R Tees below Cow Green Reservoir
0009	40	NY 996232	R Tees at Eggleston
0009	50	NZ 066149	R Tees at Barnard Castle
0009	65	NZ 270124	R Tees at Blackwell Bridge
0010	04	NY 994637	R Tyne at Corbridge
0011	14	NT 994637	R Tweed at Peebles
0012	45	NY 953386	Rookhope Burn at Eastgate
0046	90	NT 487316	Ettrick Water
0048	40	NY 748467	R. Nent at Foreshield
0055	30	NY 716467	R South Tyne upstream of R Nent
0055	31	NY 716469	R South Tyne downstream of R. Nent
0056	90	NT 438278	Yarrow Water
0061	05	NY 957498	R Derwent upstream of Bolts Burn
0061	07	NY 959499	R Derwent downstream of Bolts Burn
0061	25	NY 984516	R Derwent entering the Derwent Reservoir
0061	30	NY 033512	R Derwent downstream of Derwent Reservoir
0123	80	NZ 077375	Waskerley Beck at Wolsingham
0166	15	SD 950978	R. Swale at Gunnerside
0166	25	NY 006983	R Swale at Healough
0166	40	NZ 176009	R Swale at Richmond
0189	30	SN 728782	R Rheidol downstream of Cwm Rheidol Mine
0190	20	SN 827755	R Ystwyth at Blaencwm
0190	30	SN 810744	R. Ystwyth upstream of Cwm Ystwyth Mine
0190	40	SN 799743	R Ystwyth downstream of Cwm Ystwyth Mine
0190	50	SN 743727	R Ystwyth at Pontrhydygroes
0190	60	SN 715721	R Ystwyth near Grogwynion Mine
0191	60	SH 800612	R Conway upstream of Llanrwst Mine
0191	65	SH 794623	R. Conway downstream of Llanrwst Mine
0192	30	SH 758620	Afon Craffnant upper
0192	70	SH 772631	Afon Craffnant lower
0193	90	SH 791567	Afon Llugwy at Betws y Coed
0194	80	SH 774663	Afon Ddu
0195	80	SH 776686	Afon Dulyn
0196	90	SE 043993	Arkle Beck at Reeth
0197	90	SE 056981	Cogden Gill near Grinton
0198	95	SE 015987	Barney Beck at Healough
0199	60	SK 234807	R Derwent (Derbyshire) at Cromford station
0199	50	SK 271620	R Derwent at Darley Dale
0199	20	SK 234807	R Derwent at Hathersage
0200	95	SK 256657	R Wye at Rowsley
0201	97	SK 205826	R Noe below Shatton Bridge
0202	20	SK 153833	Peakshole water Downstream of Castleton
0204	60	SK 174812	Bradwell Brook at Bradwell
3011	60	58262529	R Lot (France) downstream of Riou Mort
3011			R. Lot (France) further downstream from Riou Mort

The programme for collection was as follows:

8-9 March	R. Rhiedol and R. Ystwyth, West Wales,
10 March	rivers in North Wales,
18-25 March	rivers in North-East England,
5 April	R. Swale catchment and R. Tees;
6 April	R. Nent and R. South Tyne, North-East England,
12 April	rivers in Derbyshire,
15 April	R. Tweed catchment

Material collected by P. J. Say from two reaches on the R. Lot in Southern France was included in the study.

6.22 Field observations

Lemanea was found in all of the larger rivers examined during the study, and was present, and often abundant, in rivers carrying elevated of levels of heavy metals in the water. The highest 'total' zinc level in which the alga was found growing was 1.16 mg l^{-1} in the R. Nent (0048-40). Water from this reach also had the highest 'total' cadmium content (0.0039 mg l^{-1}) found during the study. Growth of *Lemanea* at this site was restricted to a single very small tuft of short filaments (<2 cm long) attached to a concrete substrate in very fast flowing shallow (<5 cm deep) water. No further growths of *Lemanea* were found on similar substrata nearby, and this population formed the only known record of the alga from the R. Nent. Long tufts of *Lemanea* were found to be very common in the R. Ystwyth, West Wales (0190-40; Fig. 6.3), attached to boulders and rocks sheets in fast flowing water carrying 'total' levels of zinc, lead and cadmium of 0.540 mg l^{-1}, 0.116 mg l^{-1} and 0.0016 mg l^{-1} respectively. Long tufts of *Lemanea* were

Fig 6.3 Sampling sites for *Lemanea fluviatilis* in Wales

Fig. 6.3a

R. Ystwyth downstream of Cwmystwyth Mine, West Wales.

(0190-40)

Fig. 6.3b

Afon Craffnant, North Wales.

(0192-30)

Fig 6.4

Sandstone boulder covered in filaments (sexual shoots) of *Lemanea fluviatilis*

(R. Ystwyth, West Wales, 0190-30)

also abundant in Barney Beck, Swaledale (0198-95) which had the highest 'total' lead level (0.130 mg l^{-1}) found at any of the sites. The 'total' cadmium content of the water in this reach was also high (0.0023 mg l^{-1}), but the 'total' zinc content was relatively low (0.198 mg l^{-1}).

6.23 Heavy metal content of filaments

Whilst little variation was found between the zinc, lead and cadmium contents of different subsamples of 2 cm filament tips of *Lemanea* collected at the same time from the same reach, wide variations were found between the mean levels of these three metals in material from different reaches Mean levels of zinc in *Lemanea* filament tips ranged from 107 µg g^{-1} to 2702 µg g^{-1} ($\bar{x}$ = 546 µg g^{-1}); mean levels of lead ranged from 10 µg g^{-1} to 1347 µg g^{-1} ($\bar{x}$ = 146 µg g^{-1}) and mean levels of cadmium ranged from 2 µg g^{-1} to 342 µg g^{-1} ($\bar{x}$ = 28 µg g^{-1}).

When the mean levels of zinc, lead or cadmium in 2 cm *Lemanea* filament tips was plotted against the level of each metal in the water of the reach from which the filaments were collected, clear relationships were apparent (Figs 6.5 - 6.9). Higher mean levels of all three metals in *Lemanea* filaments were found in reaches where the levels were high in the water. Although the mean levels in *Lemanea* filaments were positively inter-correlated, as were the levels of the three metals in the water (Table 6.3), there was a strong tendency for differences in the relative concentrations of the three metals in the water of some reaches to be reflected in the metal content of *Lemanea* filaments. This is well illustrated by considering four reaches in detail

1. Afon Craffnant, Wales (0192-70)

   The highest mean level of zinc in *Lemanea* filament tips (2702 µg g^{-1}) was found in material from this river, which had a relatively high 'total' level of zinc in the water (0.34 mg l^{-1}).

Table 6.2a

Levels of cations in samples of water collected together with filaments of *Lemanea fluviatilis*

(all concentrations are in mg l^{-1})

Stream & Reach	pH	Na tot	K tot	Mg tot	Ca tot	Zn tot	Zn nuc	Cu tot	Mn tot	Fe tot	Al tot	Ph tot	Pb nuc	Ni tot	Co tot	Cd tot	Ag tot
0189-30	6 5	5 8	0 36	1 37	3 60	0 192	0.200	0 005	0 042	0 09	0 16	0 021	0 025	0 005	0 005	0 0012	0 0025
0190-20	6 5	3 9	0 24	0 91	1 37	0 012	0 018	0 004	0 105	0 10	0 14	0 006	0 010	0 005	0 005	0 00002	0 0025
0190-30	6 6	4 0	0 24	0 97	1 76	0 049	0 040	0 002	0 075	0 05	0 10	0 003	0 006	0 005	0 005	0 0003	0 0025
0190-40	6 6	4 0	0 26	1 08	2 04	0 540	0 530	0 002	0 076	0 07	0 11	0 116	0 104	0 005	0 005	0 0016	0 0025
0190-50	6 8	4 7	0 31	1 25	2 74	0 270	0 270	0 004	0 052	0 05	0 07	0 031	0 024	0 005	0 005	0 0010	0 0025
0190-60	6 8	5 3	0 34	1 44	3 55	0 440	0 420	0 002	0 044	0 05	0 09	0 049	0 038	0 005	0 005	0 0015	0 0025
0191-60	7 2	5 8	0 52	1 33	4 85	0 065	0 062	0 002	0 023	0 09	0 015	0 005	0 010	0 005	0 005	0 0004	0 0025
0191-65	7 2	5 3	0 46	1 24	4 02	0 038	0 032	0 002	0 021	0 10	0 06	0 003	0 006	0 005	0 005	0 0001	0 0025
0192-70	7 0	5 1	0 43	1 26	3 94	0 340	0 330	0 002	0 012	0 05	0 07	0 013	0 019	0 005	0 005	0 0019	0 0025
0192-30	7 0	4 6	0 41	1 07	2 94	0 021	0 021	0 001	0 009	0 05	0 015	0 004	0 004	0 005	0 005	0 0010	0 0025
0193-90	7 2	4 2	0 26	0 81	2 48	0 079	0 078	0 002	0 033	0 07	0 05	0 003	0 002	0 005	0 005	0 0003	0 0025
0194-80	7 2	4 9	0 35	1 19	4 17	0 008	0 005	0 002	0 005	0 05	0 015	0 003	0 003	0 005	0 005	0 0002	0 0025
0195-80	7 1	6 0	0 43	1 83	8 62	0 007	0 004	0 002	0 001	0 02	0 015	0 006	0 004	0 005	0 005	0 00005	0 0025
0061-30	7 2	7 9	1 78	3 10	12 50	0 075	0 062	0 001	0 088	0 27	0 14	0 011	0 012	0 005	0 005	0 00005	0 0025
0123-80	7 2	7 8	1 51	3 10	16 60	0 012	0 013	0 002	0 025	0 10	0 14	0 003	0 007	0 005	0 005	0 00005	0 0025
0007-22	7 1	22 1	1 83	5 10	13 70	0 022	0 019	0 001	0 073	0 39	0 14	0 007	0 006	0 005	0 005	0 0003	0 0025
0008-20	7 5	11 3	1 86	3 02	26 20	0 111	0 048	0 001	0 092	0 25	0 18	0 027	0 010	0 005	0 005	0 0004	0 0025
0012-45	6 9	7 7	1 85	3 23	20 90	0 320	0 240	0 007	0 310	0 56	0 24	0 082	0 043	0 005	0 005	0 0013	0 0025
0008-02	7 0	4 2	1 18	1 86	12 30	0 059	0 047	0 001	0 065	0 35	0 33	0 016	0 009	0 005	0 005	0 0003	0 0025
0009-50	7 4	5 2	1 12	2 18	16 00	0 041	0 046	0 002	0 032	0 29	0 14	0 015	0 011	0 005	0 005	0 0003	0 0025
0196-90	7 4	5 6	1 12	3 47	26 70	0 059	0 046	0 002	0 022	0 26	0 10	0 010	0 008	0 005	0 005	0 0005	0 0025
0166	7 3	5 4	1 09	2 67	24 60	0 074	0 068	0 002	0 024	0 16	0 11	0 016	0 031	0 005	0 005	0 0007	0 0025
0197-90	7 3	6 0	0 92	4 00	34 80	0 148	0 110	0 002	0 002	0 02	0 015	0 008	0 007	0 005	0 005	0 0007	0 0025
0166-25	7 4	5 1	1 04	1 96	20 90	0 071	0 069	0 002	0 034	0 27	0 11	0 009	0 012	0 005	0 005	0 0007	0 0025
0166-15	7 3	4 7	1 09	1 72	15 50	0 042	0 040	0 002	0 029	0 22	0 12	0 003	0 003	0 005	0 005	0 0013	0 0025
0195-95	7 3	4 5	0 92	1 75	20 90	0 198	0 152	0 002	0 022	0 13	0.17	0 130	0 067	0 005	0 005	0 0023	0 0025
0166-40	7 4	6 3	1 18	3 72	33 70	0 066	0 048	0 002	0 024	0 14	0 15	0 015	0 010	0 005	0 005	0 0007	0 0025
0055-30	7 4	4 8	1 22	2 44	23 60	0 083	0 082	0 001	0 031	0 56	0 21	0 006	0 026	0 005	0 005	0 00005	0 0025
0055-31	7 3	5 9	1 36	2 81	24 00	0 290	0 260	0 001	0 028	0 20	0 09	0 021	0 012	0 005	0 005	0 0015	0 0025
0048-40	7 2	8 7	1 95	4 74	25 70	1 160	1 050	0 001	0 104	0 49	0 19	0 045	0 057	0 005	0 005	0 0039	0 0025
0199-60	7 1	15 5	1 80	8 42	74 30	0 060	0 055	0 001	0 034	0 19	0 11	0 024	0 026	0 005	0 005	0 0013	0 0025
0199-50	7 0	14 3	1 95	7 58	72 70	0 091	0 073	0 001	0 049	0 20	0 14	0 018	0 009	0 005	0 005	0 0014	0 0025
0200-95	7 1	11 5	1 86	7 22	96 80	0 035	0 036	0 002	0 022	0 19	0 07	0 010	0 010	0 005	0 005	0 0001	0 0025
0204-60	7 0	9 6	1 39	2 77	90 40	0 057	0 054	0 001	0 002	0 16	0 05	0 013	0 006	0 005	0 005	0 0009	0 0025
0202-20	7 2	17 5	1 48	3 72	78 60	0 102	0 080	0 001	0 201	0 23	0 28	0 011	0 004	0 005	0 005	0 0014	0.0025
0201-97	7 3	14 0	1 48	4 32	62 70	0 040	0 033	0 001	0 095	0 23	0 08	0 007	0 005	0 005	0 005	0 0008	0 0025
0199-20	7 1	12.3	1 78	4 74	35 80	0 032	0 033	0 001	0 066	0 25	0 11	0 005	0 002	0 005	0 005	0 0004	0 0025
0008-75	7 6	48 0	1 64	3 21	30 60	0 029	0 023	0 001	0 142	0 23	0 14	0 005	0 004	0 005	0 005	0 0004	0 0025
0009-40		7 2	1 51	5 70	36 00	0 029	0 016	0 009	0 029	0 07	0 21	0 0005	0 001	0 009	0 005	0 0001	0 0025
0009-65		54 2	4 40	19 20	67 00	0 032	0 032	0 019	0 021	0 03	0 11	0 001	0 001	0 019	0 030	0 0007	0 0025
0010-04		6 4	1 36	3 51	20 90	0 023	0 022	0 005	0 007	0 17	0 07	0 003	0 002	0 020	0 010	0 0005	0 0025
0046-90		4 1	0 80	2 63	9 10	0 014	0 014	0 004	0 007	0 06	0 015	0 001	0 0005	0 005	0 005	0 0005	0 0025
0056-90		4 [illegible]	1 15	2 21	5 90	0 005	0 005	0 002	0 003	0 03	0 03	0 0005	0 0005	0 030	0 020	0 0005	0 0025
0011-14		6 0	0 95	3 58	11 80	0 007	0 006	0 002	0 001	0 02	0 015	0 0005	0 0005	0 020	0 020	0 0005	0 0025
3011-60	7 4	5 0	1 80	5 50	12 50	1 100	0 600	0 005	0 150	0 45		0 012			0 010	0 0089	
3011	7 7	10 0	1 10	5 80	11 00	0 400	0 150	0 005	0 100	0 40							
0061-05	7 2	7 1	1 60	3 24	12 20	0 026	0 021	0 002	0 098	0 40	0 20	0 007	0 009	0 005	0 005	0 0005	0 0025
0061-07	7 3	9 6	2 31	3 80	16 80	0 317	0 272	0 002	0 140	0 50	0 37	0 051	0 016	0 005	0 005	0 0018	0 0025
0061-25	7 3	9 9	2 23	3 49	14.80	0 2[illegible]	0 206	0 004	0 148	0 62	0 52	0 061	0 026	0 005	0 005	0 0018	0 0025

Table 6.2b

Mean mineral composition of 2 cm long filament tips of *Lemanea fluviatilis* collected from a range of reaches in England and Wales

($\mu g\ g^{-1}$)

Stream & reach	n	Na	K	Mg	Ca	Zn	Cu	Mn	Fe	Al	Pb	Ni	Co	Cd	Ag
0189-30	4	1334 0	7816 0	16548 0	296 0	1257 0	75 0	104.0	3185 0	22273 0	419 0	29 0	2 0	33 0	<1 0
0190-20	4	1398 0	22357 0	3414.0	104 0	107 0	24 0	135 0	904 0	616 0	14 0	2 0	2 0	6 0	<1 0
0190-30	4	1269 0	22075 0	9434 0	112 0	152 0	29 0	212 0	720 0	988 0	12 0	6 0	2 0	10 0	<1.0
0190-40	4	993 0	26009 0	3108 0	93 0	976 0	82 0	146 0	1350.0	2014 0	831 0	10 0	1 0	9 0	<2.0
0190-50	4	1649 0	52556 0	5303 0	130 0	735 0	58 0	171 0	474 0	1162 0	360 0	6 0	2 0	7 0	<1 0
0190-60	4	2062 0	56183 0	4939 0	182 0	891 0	59 0	128 0	602 0	675 0	513 0	12 0	2 0	11 0	<1 0
0191-60	4	2322 0	20972 0	21489 0	527 0	419 0	37 0	105 0	475 0	174 0	21 0	3 0	3 0	13 0	<2 0
0191-65	4	1631 0	21695 0	20952 0	588 0	397 0	26 0	145 0	566 0	193 0	15 0	3 0	3 0	15 0	<2 0
0192-70	4	1411 0	18912 0	17608 0	728 0	2702 0	31 0	63 0	981 0	486 0	151 0	16 0	2 0	12 0	<1 0
0192-30	4	1890 0	21055 0	27524 0	436 0	259 0	25 0	67 0	330 0	87 0	29 0	2 0	2 0	8 0	<1 0
0193-90	4	1776 0	19843 0	21540 0	474 0	1102 0	26 0	179 0	1485 0	307 0	56 0	18 0	2 0	10 0	<1 0
0194-80	4	1776 0	20990 0	27786 0	469 0	221 0	19 0	69 0	432 0	134 0	17 0	2 0	2 0	10 0	<1 0
0195-80	3	2351 0	20178 0	24127 0	828 0	139 0	28 0	28 0	391 0	170 0	18 0	3 0	3 0	10 0	<2 0
0061-30	2	1639 0	22396 0	16162 0	360 0	347 0	11 0	109 0	1878 0	140 0	61 0	6 0	6 0	18 0	<3 0
0123-80	4	715 0	17894 0	21200 0	576 0	242 0	17 0	98 0	538 0	122 0	17 0	2 0	2 0	8 2	<1 0
0007-22	4	665 0	19667 0	21504 0	456 0	254 0	48.0	111 0	985 0	136 0	17 0	2 0	2 0	9 0	<1 0
0008-20	4	789 0	44675 0	5357 0	480 0	360 0	21 0	82 0	1203 0	780 0	115 0	2 0	2 0	8 0	<1 0
0012-45	4	786 0	18368 0	2541 0	368 0	679 0	32 0	126 0	3787 0	1126 0	424 0	23 0	2 0	13 0	<1 0
0008-02	4	665 0	13313 0	20063 0	371 0	277 0	15 0	104 0	1835 0	186 0	98 0	2 0	2 0	7 0	<1 0
0009-50	4	368 0	12035 0	15137 0	265 0	133 0	14 0	42 0	363 0	34 0	31 0	2 0	2 0	6 0	<1 0
0196-90	4	325 0	38850 0	13947 0	431 0	240 0	14 0	43 0	873 0	198 0	63 0	2 0	2 0	9 0	<1 0
0166	4	257 0	22392 0	10611 0	369 0	255 0	9 0	46 0	437 0	45 0	45 0	1 0	1 0	9 0	<1 0
0197-90	4	1314 0	130312 0	4208 0	509 0	814 0	16 0	19 0	1033 0	509 0	281 0	3 0	3 0	23 0	<1 0
0166-25	4	116 0	20713 0	16863 0	338 0	289 0	11 0	57 0	689 0	70 0	30 0	2 0	2 0	7 0	<1 0
0166-15	4	237 0	20989 0	19223 0	352 0	448 0	14 0	74 0	2220 0	116 0	43 0	3 0	3 0	9 0	<1 0
0195-95	4	587 0	52877 0	2169 0	434 0	584 0	18 0	45 0	1324 0	936 0	1347 0	2 0	2 0	19 0	<1 0
0166-40	4	316 0	28141 0	18291 0	389 0	214 0	11 0	33 0	252 0	46 0	29 0	2 0	2 0	8 0	<1 0
0055-30	4	797 0	25362 0	23534 0	447 0	395 0	16 0	64 0	731 0	60 0	78 0	2 0	2 0	6 0	<1 0
0055-31	3	396 0	18235 0	17784 0	498 0	1180 0	14 0	58 0	973 0	92 0	103 0	8 0	2 0	15 0	<1 0
0048-40	1	5921 0	2500 0	1535 0	439 0	2294 0	48 0	35 0	3816 0	548 0	360 0	22 0	11 0	342 0	5 0
0199-60	4	792 0	48522 0	678 0	601 0	255 0	36 0	62 0	720 0	558 0	64 0	2 0	2 0	16 0	<1 0
0199-50	4	647 0	46629 0	1587 0	506 0	238 0	23.0	57 0	856 0	507 0	98 0	1 0	1 0	13 0	<1 0
0200-95	4	291.0	48324 0	1923 0	1921 0	228 0	18 0	31 0	365 0	7 0	26 0	2 0	2 0	12 0	<1 0
0204-60	4	647 0	48333 0	2541 0	513 0	218 0	32 0	23 0	312 0	581 0	113 0	2 0	2 0	14 0	<1 0
0202-20	4	249 0	43093.0	1997 0	736 0	377 0	23 0	87 0	4654 0	456 0	372 0	2 0	2 0	11 0	<1 0
0201-97	4	224 0	50410 0	1997 0	897 0	417 0	21 0	142 0	7806 0	313 0	171 0	2 0	2 0	13 0	<1 0
0199-20	4	271 0	21726 0	17085 0	659 0	212 0	18 0	88 0	1231 0	106 0	39 0	1 0	1 0	5 0	<1 0
0008-75	4	281 0	46986 0	2527 0	528 0	278 0	28 0	104 0	2285 0	546 0	75 0	2 0	2 0	10 0	<1 0
0009-40	4	65.0	15804 0	19422 0	380 0	177 0	18 0	36 0	365 0	106 0	30 0	2 0	2 0	10 0	<1 0
0009-65	1	8 0	18519 0	20926 0	463 0	110.0	23 0	42 0	154 0	355 0	52 0	8 0	8 0	40 0	<4 0
0010-04	4	439 0	20041 0	22022 0	646 0	334 0	89 0	67 0	300 0	240 0	22 0	3 0	3 0	13 0	<1 0
0046-90	4	130 0	10619 0	22362 0	486 0	201 0	41 0	58 0	643 0	114 0	10 0	1 0	1 0	20 0	<1 0
0056-90	4	333 0	10263 0	24799 0	459 0	253 0	34 0	39 0	171 0	139 0	14 0	1 0	1 0	6 0	<1 0
0011-14	4	200 0	14103 0	25268 0	590 0	153 0	40 0	49 0	23 0	164 0	19 0	2 0	2 0	7 0	<1 0
3011-60	4	287 0	15868 0	14021 0	317 0	1249 0	34 0	57 0	367 0	57 0	12 0	3 0	1 0	169 0	<1 0
3011	1	742 0	15646 0	17445 0	989 0	1687 0	38 0	129 0	962 0	275 0	56 0	8 0	1 0	175 0	<1 0
0061-05	4	4122 0	17920 0	18257 0	457 0	352 0	44 0	131 0	2058 0	640 0	78 0	73 0	75 0	38.0	<1 0
0061-07	4	2674 0	16009 0	12657 0	326 0	917 0	44 0	146 0	3649 0	588 0	197 0	58 0	43 0	66 0	<1 0
0061-25	4	2282 0	14799 0	10888 0	261 0	723 0	19 0	90 0	1044 0	231 0	111 0	48 0	32 0	27.0	<1 0

Fig. 6.5

Scatter diagram to show the relationship between the mean zinc content of 2 cm long filament tips of *Lemanea fluviatilis* and 'total' levels of zinc and calcium in the water of the reach of origin.

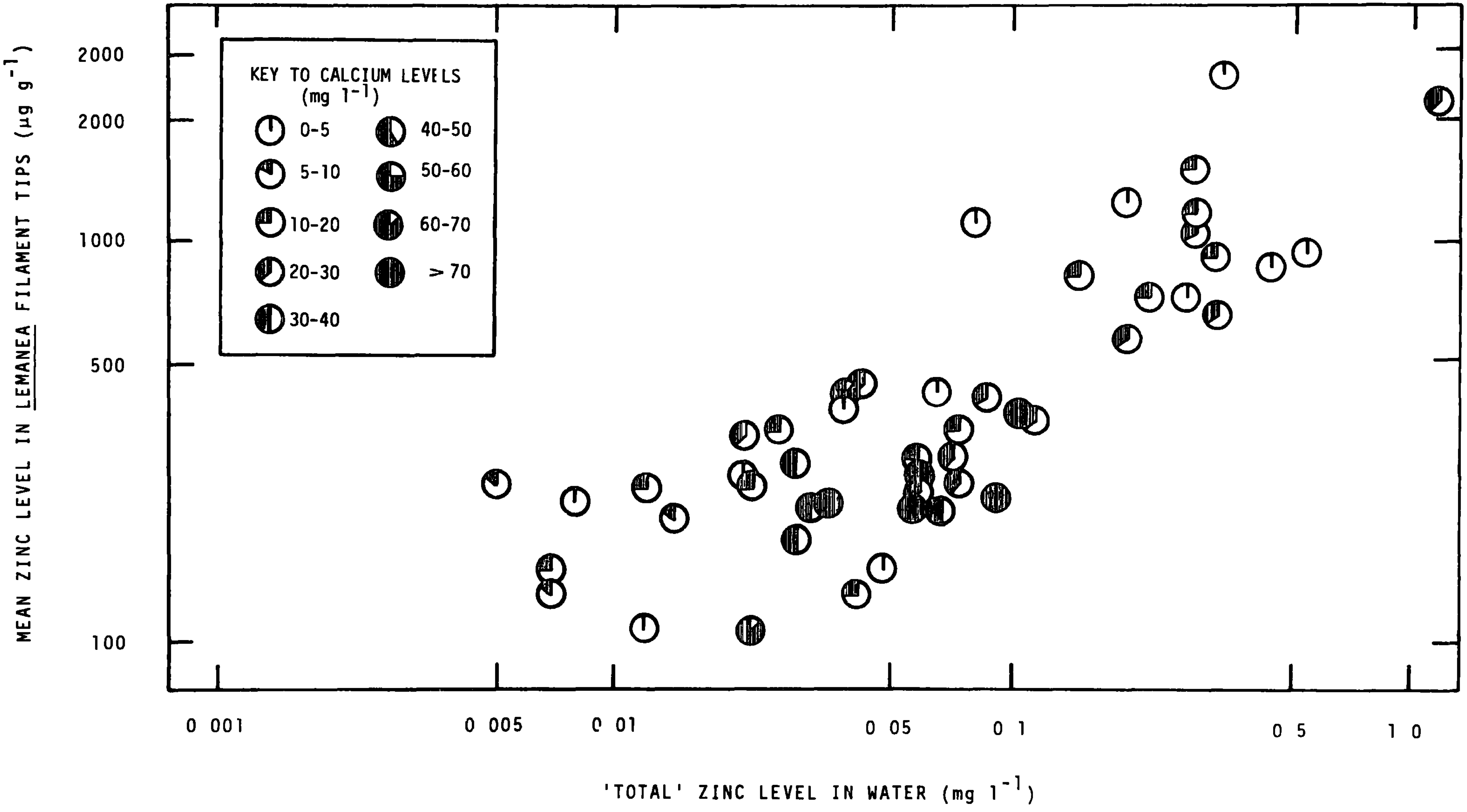

KEY TO CALCIUM LEVELS
(mg l-1)
0-5
5-10
10-20
20-30
30-40
40-50
50-60
60-70
> 70
MEAN ZINC LEVEL IN LEMANEA FILAMENT TIPS (µg g-1)
2000
2000
1000
500
100
0 001
0 005
0 01
0 05
0 1
0 5
1 0
'TOTAL' ZINC LEVEL IN WATER (mg l-1)

Fig. 6.6

Scatter diagram to show the relationship between the mean zinc content of 2 cm long filament tips of *Lemanea fluviatilis* and levels of zinc and calcium in the water of the reach of origin after Nuclepore filtration.

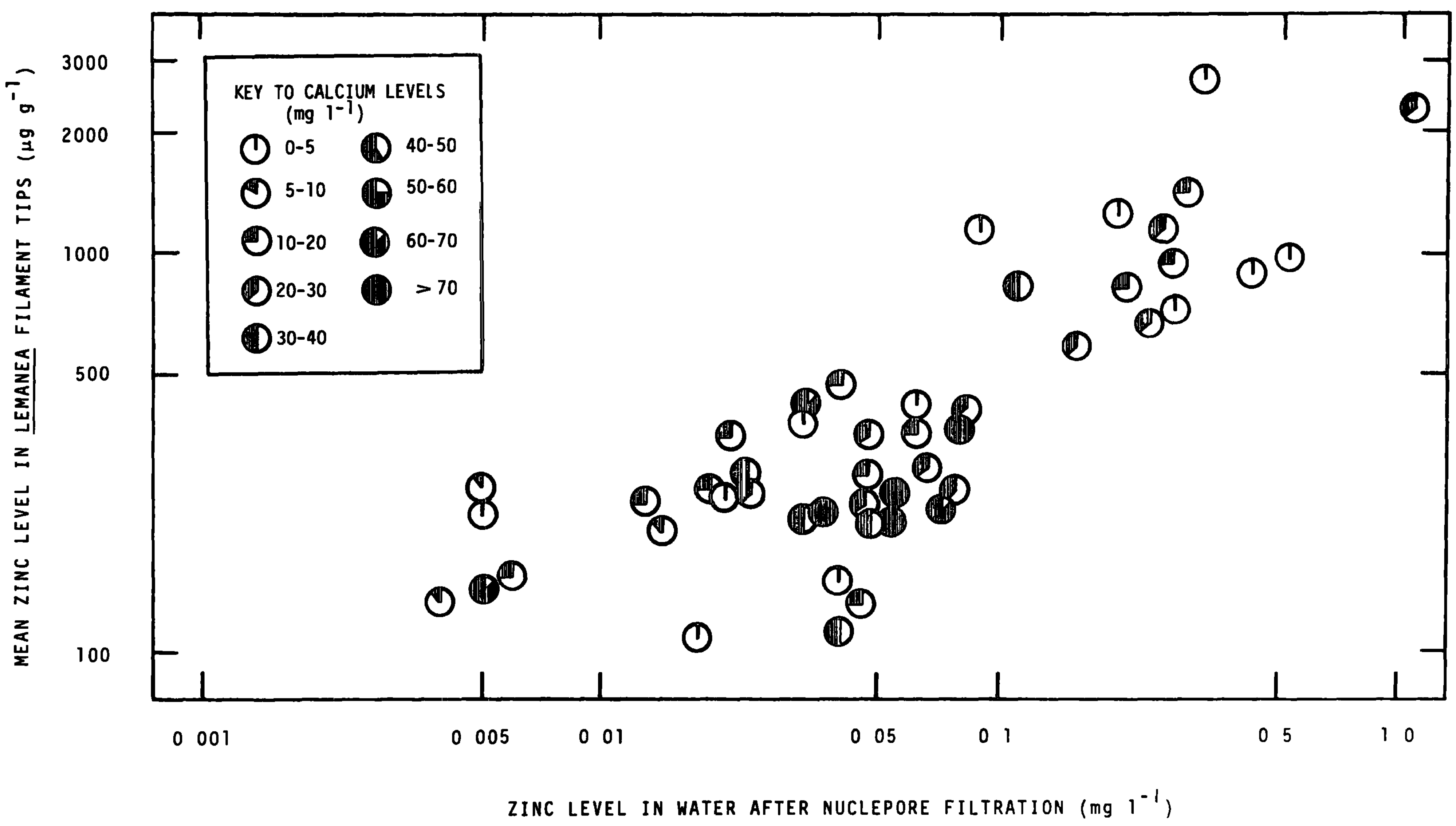

KEY TO CALCIUM LEVELS (mg 1^{-1})
0-5
5-10
10-20
20-30
30-40
40-50
50-60
60-70
> 70
3000
2000
1000
500
100
0 001
0 005
0 01
0 05
0 1
0 5
1 0
MEAN ZINC LEVEL IN LEMANEA FILAMENT TIPS (µg g^{-1})
ZINC LEVEL IN WATER AFTER NUCLEPORE FILTRATION (mg 1^{-1})

Fig. 6.7

Scatter diagram to show the relationship between the mean lead content of 2 cm long filament tips of *Lemanea fluviatilis* and 'total' levels of lead and calcium in the water of the reach of origin.

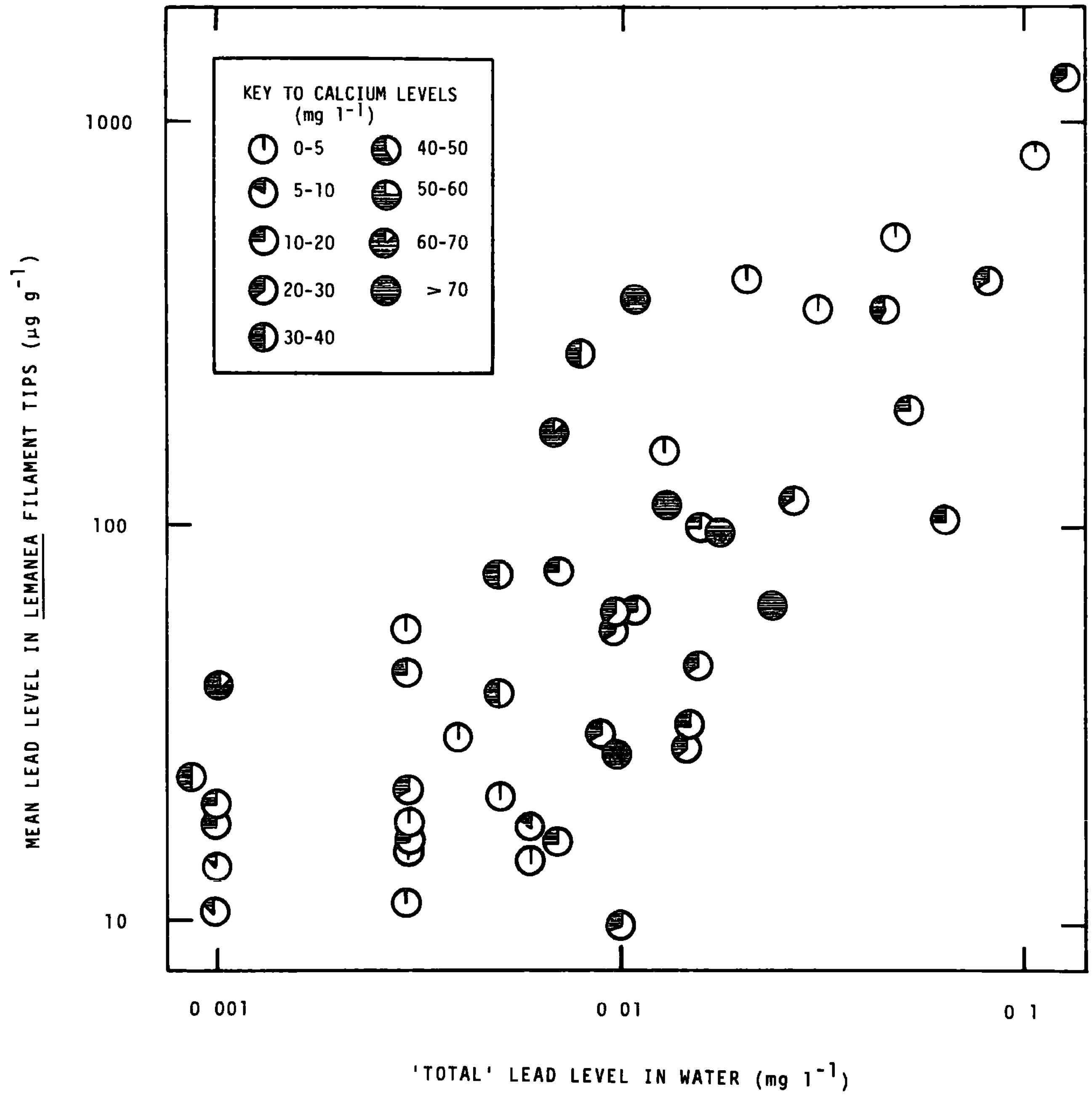

KEY TO CALCIUM LEVELS
(mg l-1)
0-5
5-10
10-20
20-30
30-40
40-50
50-60
60-70
> 70
1000
100
10
0 001
0 01
0 1
MEAN LEAD LEVEL IN LEMANEA FILAMENT TIPS (µg g-1)
'TOTAL' LEAD LEVEL IN WATER (mg l-1)

Fig. 6.8

Scatter diagram to show the relationship between the mean lead content of 2 cm long filament tips of *Lemanea fluviatilis* and levels of lead and calcium in the water of the reach of origin after Nuclepore filtration.

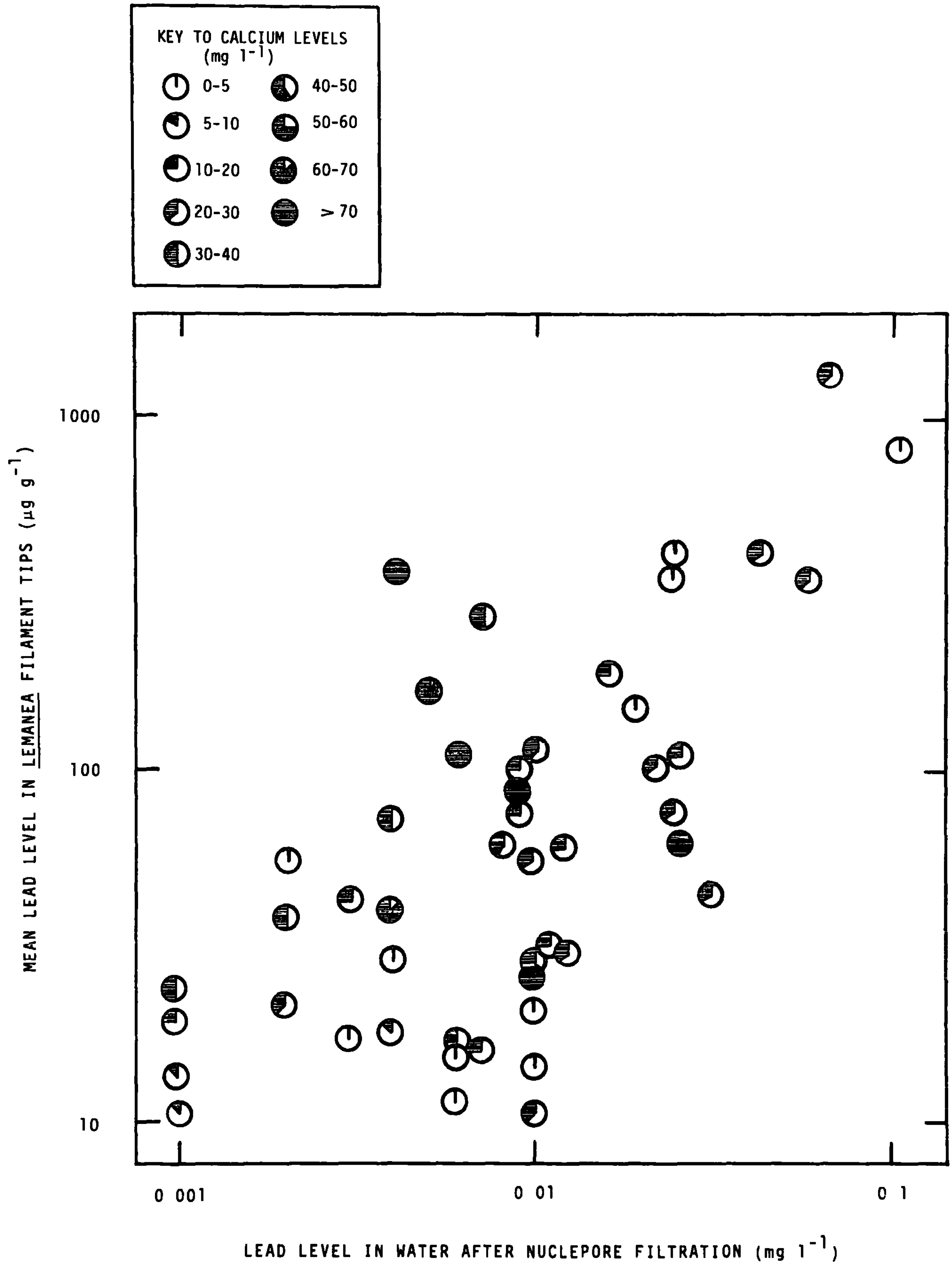
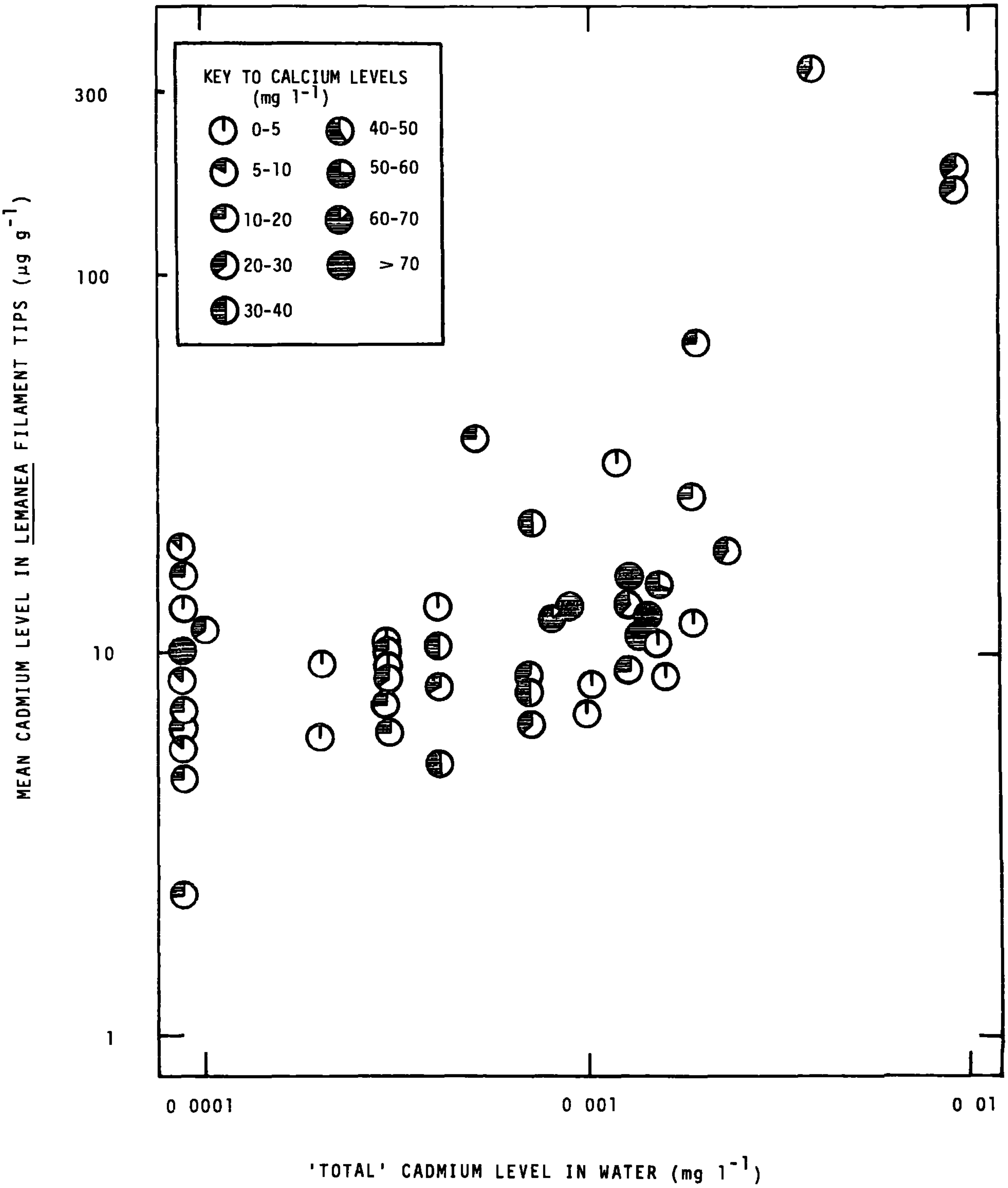

KEY TO CALCIUM LEVELS
(mg l⁻¹)
0-5
5-10
10-20
20-30
30-40
40-50
50-60
60-70
> 70
1000
100
10
0 001
0 01
0 1
MEAN LEAD LEVEL IN LEMANEA FILAMENT TIPS (µg g⁻¹)
LEAD LEVEL IN WATER AFTER NUCLEPORE FILTRATION (mg l⁻¹)

Fig. 6.9

Scatter diagram to show the relationship between the mean cadmium content of 2 cm long filament tips of *Lemanea fluviatilis* and 'total' levels of cadmium and calcium in water of the reach of origin.


KEY TO CALCIUM LEVELS
(mg l⁻¹)
0-5
5-10
10-20
20-30
30-40
40-50
50-60
60-70
> 70
300
100
10
1
0 0001
0 001
0 01
MEAN CADMIUM LEVEL IN LEMANEA FILAMENT TIPS (µg g⁻¹)
'TOTAL' CADMIUM LEVEL IN WATER (mg l⁻¹)

However, the mean levels of lead and cadmium in filaments from this reach (151 µg g^{-1} and 12 µg g^{-1} respectively) were low in comparison to material from several other sites, as were the 'total' levels of lead and cadmium in the water (0.019 mg l^{-1} and 0.0024 mg l^{-1} respectively).

ii. Barney Beck, Swaledale (0198-95)

The highest mean level of lead in *Lemanea* filament tips (1347 µg g^{-1}) was recorded in material from this stream, which also had the highest 'total' level of lead in the water (0.13 mg l^{-1}). However the zinc and cadmium contents of water ('total' level = 0.198 mg l^{-1} and 0.0023 mg l^{-1} respectively) and *Lemanea* filament tips ($\bar{x}$ = 214 µg g^{-1} and 8 µg g^{-1} respectively) were low in comparison to several other sites.

iii. R. Lot, Southern France (3011-60)

Water from the R. Lot (downstream of the entry of a polluted tributary, the Riou Morte) had the highest 'total' cadmium (0.0089 mg l^{-1}) recorded during the survey. This was reflected in the mean cadmium content of *Lemanea* filament tips from the river, which were found to contain 189 µg g^{-1} Cd. The levels of zinc in both water and *Lemanea* from the reach were also high (0.29 mg l^{-1} and 1248 mg l^{-1} respectively), but the 'total' level in the water (0.012 mg l^{-1}) and the mean level of lead in *Lemanea* were low in comparison to several other sites.

iv. R. Nent, North-East England (0048-40)

Water from the R. Nent (where a single small population of *Lemanea* was sampled) had the highest 'total' level of zinc (1.16 mg l^{-1}) recorded during the survey, together with high 'total'

Table 6.3

Intervariable correlation matrix to show the relationship between levels of 14 elements in filament tips of *Lemanea fluviatilis* and water from the reaches of origin

(* - P = <0.01 ** - P = <0.001)

	Na-Lem	K-Lem	Mg-Lem	Ca-Lem	Zn-Lem	Cu-Lem	Mn-Lem	Fe-Lem	Al-Lem	Pb-Lem	Ni-Lem	Co-Lem	Cd-Lem	Ag-Lem
Na-tot	-0 1955	0 1017	-0 1616	0 1604	-0 1645	-0 0535	-0.0626	0 1253	-0 0505	-0 0993	-0 0392	0 0226	-0.0175	0 0865
K-tot	-0 1128	-0 0250	-0 1349	0 1881	-0 1179	-0 1942	-0 2843	0 1519	-0 1838	-0 1466	0 1963	0 2790	0 2361	0 0237
Mg-tot	-0 2090	0 0608	-0 0915	0.2880	-0 1122	-0 0978	-0 3088	-0 0398	-0 1143	-0 1591	-0 0239	0.0564	0 2119	0 0836
Ca-tot	-0 3135	0 3960*	-0 4839**	0 5358**	-0 2454	-0 2528	-0 4079*	0 1841	-0 1248	-0 0415	-0 1927	-0.0839	-0 0361	-0.0906
Zn-tot	0 4205*	-0 0845	-0.3099	-0 1670	0 7387**	0 3226	0 0169	0 1844	-0 0576	0 3419*	0 2188	0 0397	0 8200**	0 2820
Cu-tot	-0 1990	-0 1199	0 1361	-0 0946	-0 0372	0 0840	-0 0977	-0 1485	0 1139	-0 0185	0 0654	0 0134	0 0845	0 1112
Mn-tot	0 1403	0 0349	-0 4538**	-0 1338	0 1731	0 0555	0 4085*	0 5785*	0 0052	0 1827	0 3873*	0 2528	0 2673	-0 0949
Fe-tot	0 2254	-0 1814	-0 1380	-0 0261	0 2056	-0 0931	0 0954	0 3659*	-0 1050	-0 0232	0 4574**	0 4128*	0.4226*	-0 1002
Al-tot	0 1670	-0 1640	-0 2364	-0 2291	0 0986	-0 1021	0 1657	0 3499*	0 0597	0 1682	0 5460**	0 4663**	0 1794	-0 0903
Pb-tot	0 1734	0 1706	-0.4975**	-0 2598	0 3092	0 2695	0 1365	0 1965	0 0931	0 8738**	0 2710	0 1001	0 1404	0 0085
Ni-tot	-0 2365	-0 2113	0.3481*	0 0484	-0 1745	0 2339	-0 2517	-0 2450	-0 0639	-0 1622	-0 1176	-0 0604	-0 0416	0 0116
Co-tot	-0 2364	-0 1875	0 2982	0 0208	-0 1479	0 0889	-0 2418	-0.2310	-0 0567	-0 1431	-0 0849	-0 0302	0 0517	0 1108
Cd-tot	0 1471	-0 0616	-0.2352	-0 1624	0 5399**	0 1696	-0 1186	0 1155	0 0341	0 2409	0 1367	0 0406	0 6918**	0 1528
Ag-tot	0 0004	0.0008	0 0005	0 0005	0 0003	0.0005	0 0008	0 0006	0 0001	0 0	0 0	0 0002	0 0004	0 0003

levels of lead and cadmium (0.045 mg l^{-1} and 0 0039 mg l^{-1} respectively). The sample of *Lemanea* from the reach provided an example of high contents of all three metals within filaments of the alga, with levels of 2293 µg g^{-1} Zn, 359 µg g^{-1} Pb and 342 µg g^{-1} Cd (the highest level found during the survey) being recorded.

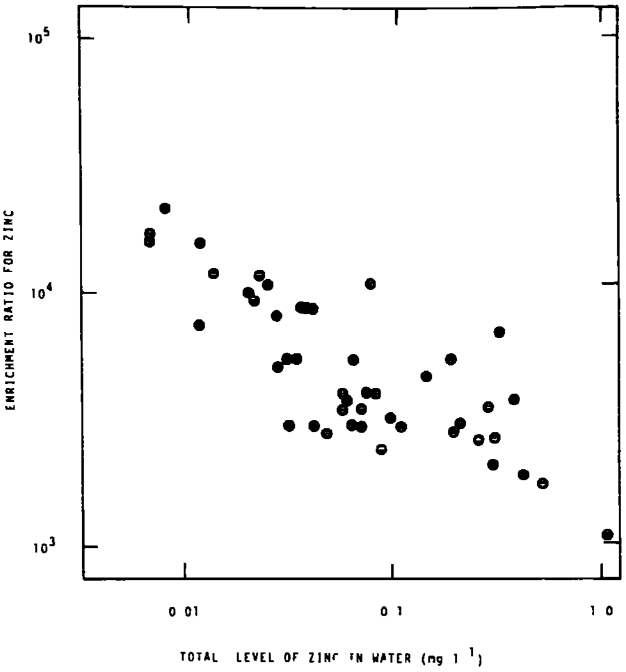
Significant positive correlations were found between 'total' levels of zinc, lead and cadmium in the water, and the mean level of the particular metal in *Lemanea* filament tips (Table 6.3). There was an increase in the linearity of the relationship in the case of zinc when levels were plotted after Nuclepore filtration (Fig. 6.6), but no such increase in linearity was observed in the case of lead (Fig. 6.8). (Estimates of the filtrability of cadmium have been omitted because of occasional contamination of the filtrate by Nuclepore filters.)

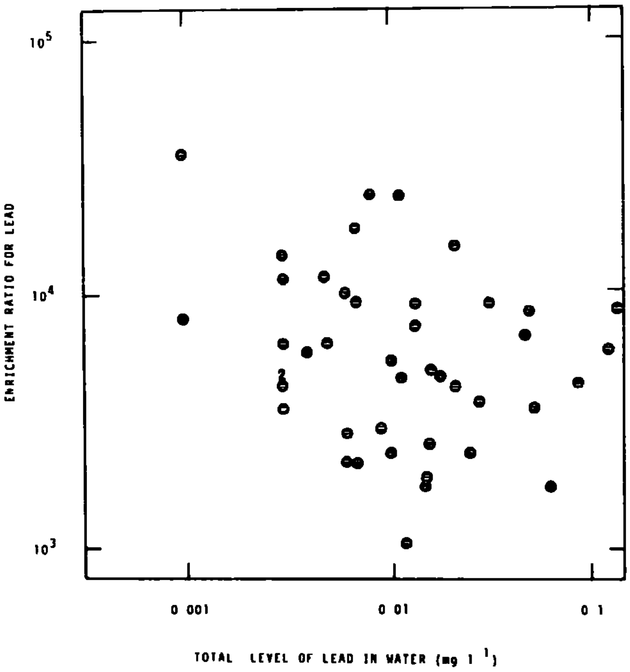
There were no clear indications that environmental factors were acting to influence the concentrations of metals accumulated by *Lemanea*. At concentrations of zinc in the water lower than about 0.02 mg l^{-1}, a slight discontinuity was apparent in the linearity of the relationship between concentrations of zinc in the water and in *Lemanea* (Figs 6.5, 6.6). Scattering of the points in figs 6.7-6.9 was too great to ascertain whether such a discontinuity was present for lead or cadmium. There was some slight indication (Figs 6.5, 6.6) that higher concentrations of zinc were taken up by filaments of *Lemanea* at lower concentrations of calcium in the water. However, there was no such indication for lead or cadmium (Figs 6.7 - 6.9).

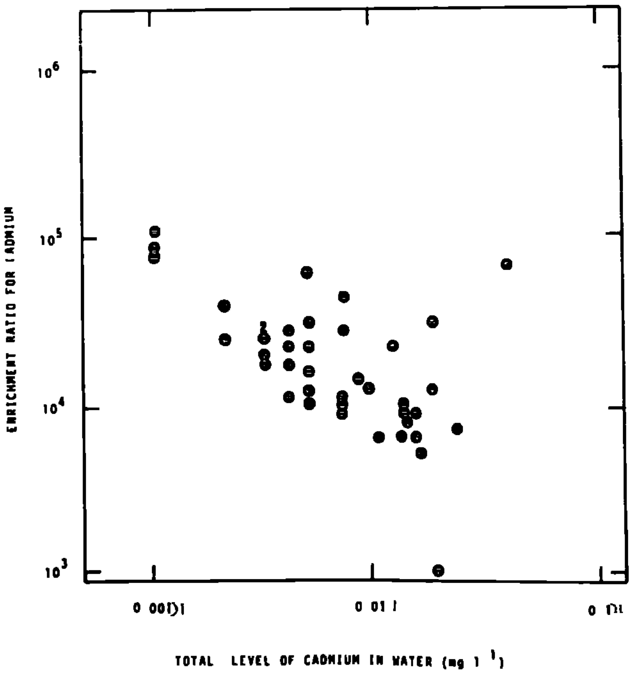
Fig. 6.10

Scatter diagrams to show the relationship between the enrichment ratios for zinc, lead and cadmium in 2 cm long filament tips of *Lemanea fluviatilis* and the 'total' level of each metal in the water of the reach of origin

(enrichment ratio = mean concentration of metal in dried plant 'total' concentration of metal in water)


10^5
10^4
10^3
ENRICHMENT RATIO FOR ZINC
0 01
0 1
1 0
TOTAL LEVEL OF ZINC IN WATER (mg l ¹)


10^5
10^4
10^3
ENRICHMENT RATIO FOR LEAD
2
0 001
0 01
0 1
TOTAL LEVEL OF LEAD IN WATER (mg l ¹)


10^6
10^5
10^4
10^3
ENRICHMENT RATIO FOR CADMIUM
2
0 0001
0 01
0 1
TOTAL LEVEL OF CADMIUM IN WATER (mg l ¹)

When the enrichment ratio for each metal was plotted against the 'total' concentration of the metal in the water, clear negative linear relationships were apparent (Fig 6.10). This was clearer for zinc and cadmium than for lead where a fairly wide scattering of points was observed. For each of the metals the decrease in enrichment ratios appeared to be constant over the whole range of concentrations encountered in the field. The mean enrichment ratios (calculated using 'total' concentrations of metals in the water) were 197 for zinc, 11581 for lead and 44762 for cadmium.

6.3 VARIATION IN THE HEAVY METAL CONTENT OF *LEMANEA* FILAMENTS WITHIN A SINGLE REACH

The results of the survey of the mineral composition of *Lemanea* from different rivers gave no indication of the sensitivity with which the metal content of the alga reflected variations in the metal content of the water at a particular site. A study was therefore planned to ascertain to what extent changes in the levels of zinc, lead and cadmium in the water of a single reach were reflected in the content of filaments of *Lemanea* growing within the reach.

6.31 Programme for collection of *Lemanea*

The reach chosen for the study was on the R. Derwent downstream of the entry of Bolts Burn (0061-07). The water in this reach was known to be subject to widely fluctuating levels of zinc, lead and cadmium (see 4.323), with levels of zinc and cadmium varying independantly of levels of lead A large population of *Lemanea* was present within the reach during the spring and early summer.

59 separate samplings of *Lemanea* filament tips were made from within the reach from 5 March - 1 July 1976. These samplings were made on every occasion when water was taken from the reach for analysis, and included collections made at hourly intervals throughout the 12 and 24 hour surveys of 8 and 18/19 March.

All samples of *Lemanea* were taken from a thick 'carpet' of the alga covering a 1 m x 1 m area of sandstone blockwork near to the south bank of the reach. (This area was more easily sampled during the 12 and 24 hour surveys.) A transect to sample *Lemanea* across the reach (3.84) had shown that the mineral composition of the alga from this area was representative of that of material from other areas within the reach.

6.32 Heavy metal content of filaments

The mean zinc content (of five replicate subsamples) of filament tips collected from the reach at hourly intervals during the 12 hour survey is shown in Fig. 6.11. No marked pattern of variation was detected throughout the period, although the mean level of zinc (from 950 - 1250 $\mu g\ g^{-1}$) remained higher than the mean level recorded during all samplings of material from the reach (916 $\mu g\ g^{-1}$, see 4.51). The Whiteheaps Adit Level was flowing throughout the 12 hour period (see 4.312), and the 'total' zinc content of the water in the reach remained high (>0 48 $mg\ l^{-1}$). No noticeable variations in the mean lead or cadmium contents of the alga were detected during the 12 hour survey

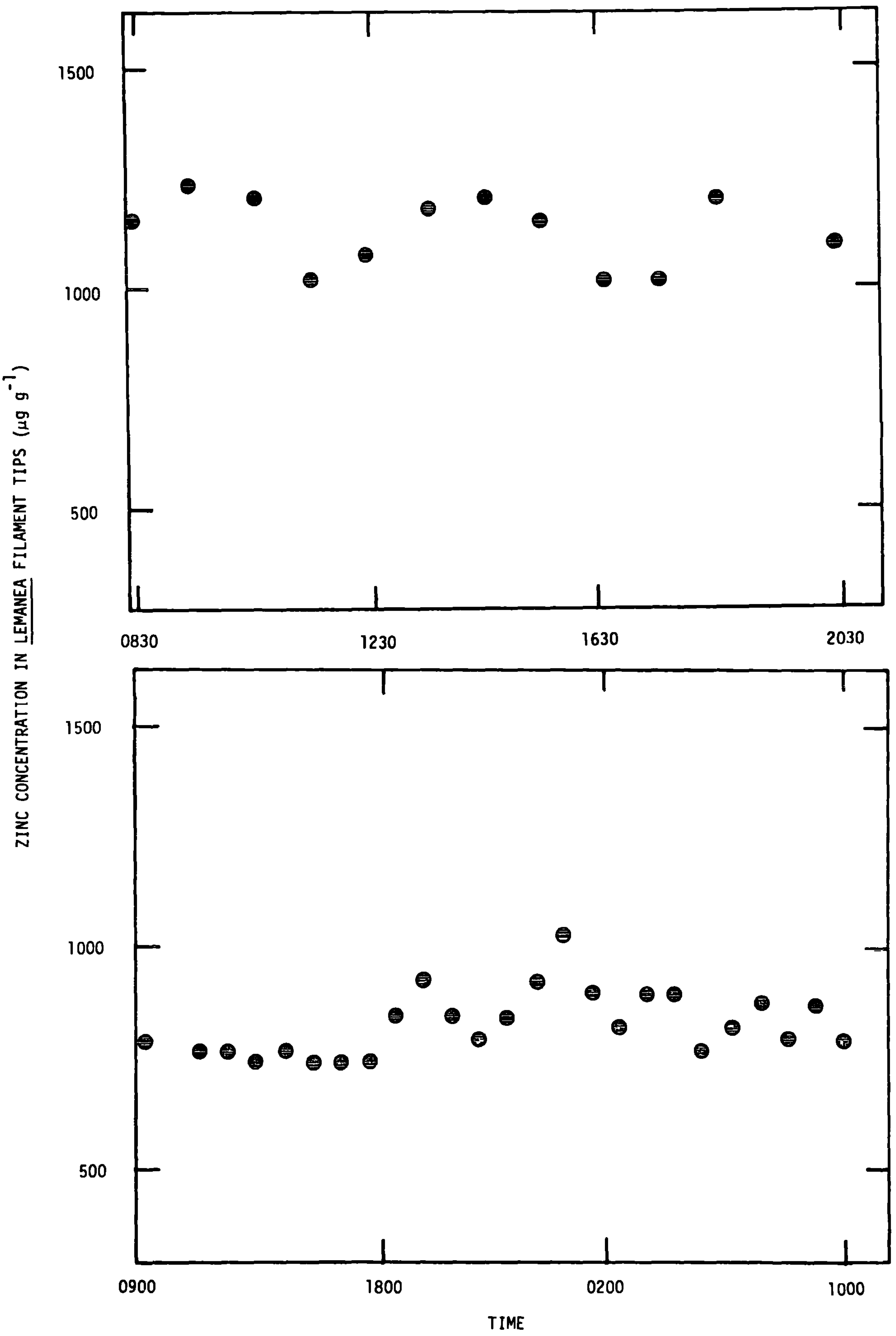
Variations in the zinc content of *Lemanea* filament tips during the 24 hour survey (Fig. 6.12) provided further evidence that the zinc content of the alga was sensitive to short-term variations in the levels of the metal in the water of the reach. A marked rise in zinc

Fig. 6.11

Variations in the mean zinc content of 2 cm long filament tips of *Lemanea fluviatilis* from the R. Derwent downstream of Bolts Burn (0061-07) during the 12 hour survey of 8 March 1976

Fig. 6.12

Variations in the zinc content of 2 cm long filament tips of *Lemanea fluviatilis* from the R. Derwent downstream of Bolts Burn (0061-07) during the 24 hour survey of 18-19 March 1976.


ZINC CONCENTRATION IN LEMANEA FILAMENT TIPS (μg g^{-1})
1500
1000
500
0830
1230
1630
2030
1500
1000
500
0900
1800
0200
1000
TIME

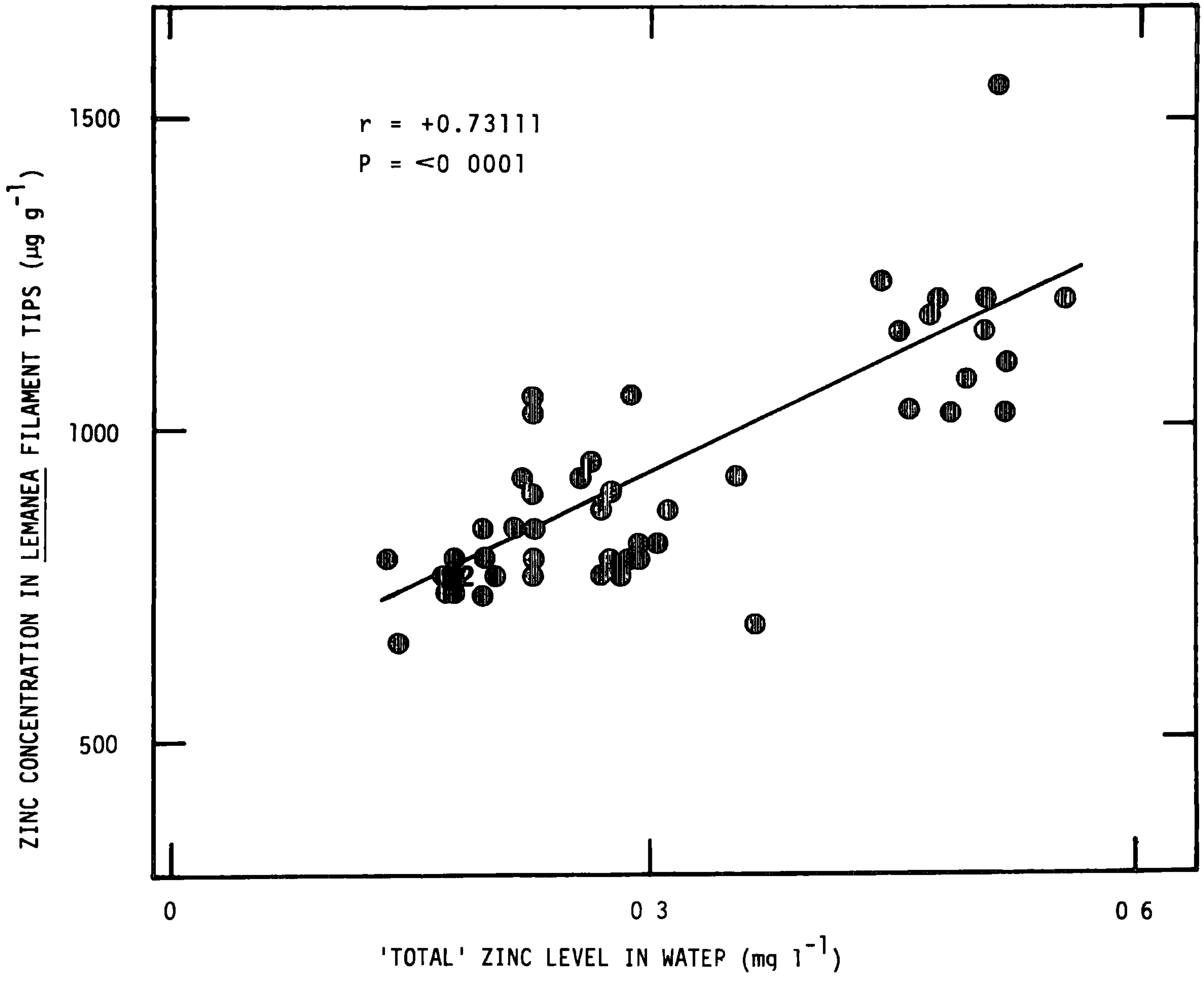
content took place between 1700 h and 1800 h, about an hour after a sharp rise had been detected in the 'total' zinc content of the water in Bolts Burn (see 4.312). Following this increase the zinc content of the alga (like that of the water) remained elevated (from 850 - 1000 $\mu g\ g^{-1}$) for the remainder of the 24 hour period. Because of dilution by a small increase in river discharge, the 'total' zinc content of the water in the reach during the latter part of the 24 hour survey (from 0.25 - 0.30 $mg\ l^{-1}$) was somewhat lower than that found during the 12 hour survey. This dilution appears to have been reflected in the zinc content of *Lemanea* filaments, for despite the rise in levels within the alga the higher concentrations recorded were lower than those recorded in samples collected during the 12 hour survey. As had been observed during the former survey, the lead and cadmium contents of *Lemanea* filaments remained fairly stable throughout the 24 hour period, giving no indication of being affected by the wide variations that took place in the 'total' content of the metals in the water.

When all values from the reach were considered together, a strong significant correlation ($r = +0.7576$, $P = <0\ 0001$) was found between the 'total' zinc content of the water in the reach and the zinc content of *Lemanea* filament tips (Fig. 6.13). A similar correlation was found between levels of zinc in the water after Nuclepore filtration and the levels of zinc in the plant. A significant positive correlation was also found between variations in the zinc content of water in Bolts Burn and variations in the zinc content of *Lemanea* filaments in the R Derwent reach 07.

The scattered points in Fig. 6.13 fall into two groups, in a rather similar manner to the levels of zinc in the water in Bolts Burn reach 99 (see 4.312). This separation of the zinc levels into two groups was probably caused by the switching on and off of the Whiteheaps Adit Level , the results suggest that when the zinc content of

Fig. 6.13

Scatter diagram to show the relationship between variations in the mean zinc content of 2 cm long filament tips of *Lemanea fluviatilis* in the R. Derwent downstream of Bolts Burn (0061-07) and variations in the 'total' zinc content of the water.


r = +0.73111
P = <0 0001
1500
1000
500
ZINC CONCENTRATION IN LEMANEA FILAMENT TIPS (µg g-1)
0
0 3
0 6
'TOTAL' ZINC LEVEL IN WATER (mg l-1)
2

Lemanea filament tips was more than 1000 µg g^{-1} the adit could be assumed to be switched on and *vice versa*.

6.4 TRANSPLANT EXPERIMENTS

The sensitivity with which changes in the levels of heavy metals in the water were reflected in the content of *Lemanea* filaments would have been detetmined directly by the speeds of uptake and subsequent release of the metals by the alga. In order to investigate the kinetics of these separate processes in the field, boulders covered in *Lemanea* filaments were transplanted between reaches with different levels of heavy metals in the water. Samples of filament tips were then removed from the boulders at regular intervals for analysis, to determine the levels of metals gained or lost over different periods.

It was known (see 7.5) that in some of the rivers from which *Lemanea* had been collected (e.g. Rookhope Burn, R. Nent, R. South Tyne, R. Derwent) *Stigeoclonium tenue* was present as zinc tolerant populations. It seemed possible that genetic adaptation by *Lemanea* to high levels of metals might alter the rates of uptake of zinc and other metals. Transplant experiments were therefore carried out to investigate possible differences in uptake kinetics between populations (from high and low zinc sites) after placing into reaches with high levels of metals in the water.

6.41 Transplants carried out in the R. Derwent

During April 1976 single boulders covered in *Lemanea* were exchanged between reaches 05 and 07 on the R. Derwent (i.e upstream and downstream of the entry of Bolts Burn) with subsequent sampling of pooled filament tips at half hourly intervals. The results indicated that some changes in levels of zinc in the filaments were very rapid, and the experiment was therefore repeated in a more critical manner (four replicate boulders, more intensive sampling) in April 1977. Only the latter survey is considered in this account.

In both transplants, a rapid initial change in the mean zinc content of filament tips took place (Fig. 6.14, Table 6.4) Material from reach 05 gained a large amount of zinc in the first few hours following transplanting into reach 07, and a corresponding rapid loss of zinc took place from the material transplanted from reach 07 into reach 05. Loss of zinc from the material from reach 07 levelled off in a more or less exponential fashion following transplanting into reach 05 (i.e. when the data were plotted on a logarithmic scale (Fig. 6.14) zinc loss followed a roughly linear pattern). The mean level of zinc gained by material transplanted from reach 05 to reach 07 showed less tendency to level off towards the end of the experiment.

By the end of the experiment (1200 hours after the boulders were transplanted) the mean level of zinc in the material transplanted from reach 05 to reach 07 had risen from 232 $\mu g\ g^{-1}$ to 1414 $\mu g\ g^{-1}$, and the mean level in material transplanted from reach 07 to reach 05 had fallen from 800 $\mu g\ g^{-1}$ to 281 $\mu g\ g^{-1}$. These levels compare with mean levels of 352 $\mu g\ g^{-1}$ in material growing in reach 05 (n = 6) and 917 $\mu g\ g^{-1}$ in material growing in reach 07 (n = 59).

The filaments of *Lemanea* on all eight boulders transplanted between reach 05 and reach 07 remained healthy throughout the experiment, but became smothered with epiphytic diatoms by the time the final samples of filaments were taken. It seems possible that the accuracy of the determinations of mean levels of zinc made at 1200 hours following the transplanting was affected somewhat by epiphytes that were not removed completely by washing.

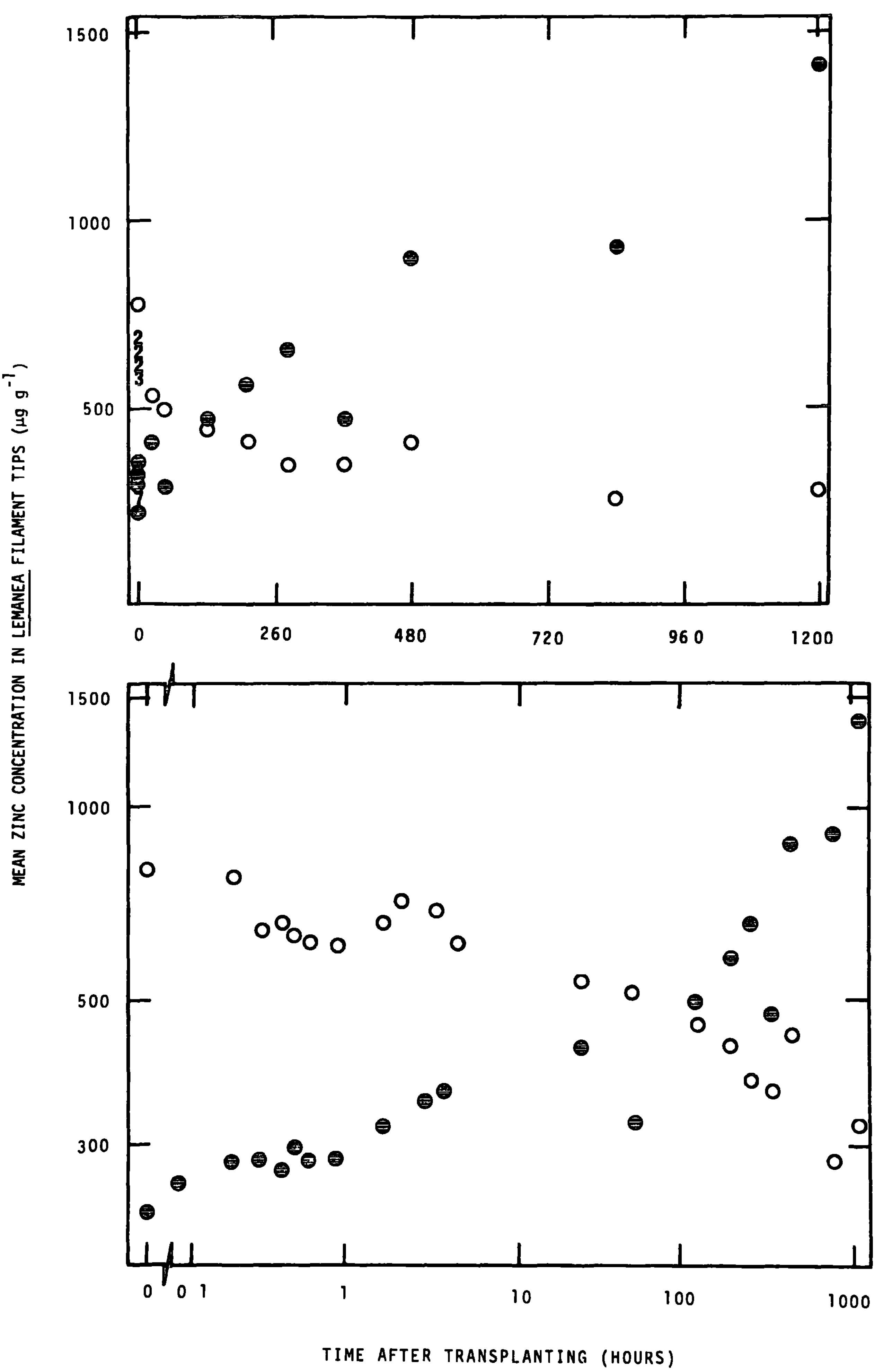
Fig. 6.14

Changes in the mean zinc content (n = 4) of 2 cm long filament tips of *Lemanea fluviatilis* in the R. Derwent following transplanting from upstream to downstream of Bolts Burn and *vice versa*.

● boulders transplanted from 0061-05 to 0061-07

○ boulders transplanted from 0061-07 to 0061-05

(data are plotted on both arithmetic and logarithmic scales)


MEAN ZINC CONCENTRATION IN LEMANEA FILAMENT TIPS (µg g^{-1})
1500
1000
500
2
2
2
3
0
260
480
720
960
1200
1500
1000
500
300
0
0 1
1
10
100
1000
TIME AFTER TRANSPLANTING (HOURS)

Time after transplant (hours)	n	Zn		Pb		Cd	
		$\bar{x}$	*s.d.*	$\bar{x}$	*s.d.*	$\bar{x}$	*s.d.*
0	4	231.8	13.9	25.7	20.7	5.0	8.2
0.8	4	261.9	14.6	41.3	21.6	1.2	1.6
0.16	4	281.5	21.1	39.9	14.9	0.9	1.3
0.25	4	282.5	5.0	32.9	25.0	2.4	1.4
0.33	4	275.9	8.8	88.6	29.6	1.8	1.3
0.42	4	296.6	8.6	59.6	50.0	1.8	1.1
0.5	4	280.3	10.9	77.3	42.0	1.8	2.7
0.75	4	285.0	30.5	38.4	12.2	7.3	6.2
1	4	296.9	6.5	68.6	46.3	1.8	3.0
1.5	4	322.6	50.4	69.0	45.9	0.4	0.3
2.5	4	349.2	12.1	70.6	47.3	3.2	4.2
3.5	4	371.4	31.8	70.1	20.8	0.7	0.6
24	4	437.2	36.3	148.5	48.0	13.3	8.5
48	4	303.1	11.8	115.3	6.9	17.8	11.4
120	4	501.0	16.4	143.4	26.6	36.4	12.9
192	4	595.1	54.6	171.2	53.1	53.2	10.9
264	4	687.9	97.4	168.7	18.7	61.2	7.2
360	4	486.9	22.5	285.7	12.3	62.9	15.8
480	2	899.9	34.1	283.3	20.8	125.0	11.2
840	3	934.7	196.6	258.0	83.6	101.6	36.8
1200	4	1413.8	106.5	219.5	60.0	63.4	16.3

Table 6.4a Changes in the mean zinc, lead and cadmium contents of filament tips of *Lemanea fluviatilis* following transplanting from the R. Derwent upstream of Bolts Burn (0061-05) into the R. Derwent downstream of Bolts Burn (0061-07)

(all concentrations are in $\mu g\ g^{-1}$)

Time after transplant (hours)	n	Zn		Pb		Cd	
		$\bar{x}$	*s.d.*	$\bar{x}$	*s.d.*	$\bar{x}$	*s.d.*
0	4	800 3	150.8	329.2	70.7	14.5	3.9
0 8	4	795 4	174.3	349 3	91.1	21.6	15.5
0.16	4	649 4	152.7	264.3	87.2	9.6	5.0
0.25	3	686 6	147.7	314.2	112 7	29.0	26.8
0.33	4	650.4	136.5	305.2	65.3	13.3	18.8
0.42	4	629.6	141.5	270.3	54.9	11.4	4.5
0.5	4	625.8	82.1	243.4	31.1	12.4	1.8
0.75	4	692.0	98.7	304.5	53.1	12.4	5.8
1	4	674.9	130.5	314.9	105.5	28.3	30.3
1.5	4	725.6	45.8	333 7	90.1	15.2	11.7
2.5	4	581.5	95.8	247.1	55.5	17.9	12.6
3.5	4	613.2	79.9	294.6	73.3	10.2	10.1
24	4	554.1	76.1	209.7	95.4	12.2	13.1
48	4	527.1	81.3	193.1	124.0	5.8	5 3
120	4	466.4	41.3	108.8	30.5	5.1	2.3
192	4	431.8	46.0	108.4	22.5	3.4	2.4
264	4	386 0	39.2	92.4	21.7	4.8	4.0
360	3	384.9	22.5	76 5	21.6	1.5	1.2
480	4	447.5	57.7	47.4	17.1	2.0	3.6
840	4	281.8	28.7	25.7	13.8	0.3	0.2
1200	4	320.1	57.1	26.6	25.8	1.0	1.4

Table 6.4b Changes in the mean zinc, lead and cadmium contents of filament tips of *Lemanea fluviatilis* following transplanting from the R. Derwent downstream of Bolts Burn (0061-07) into the R. Derwent upstream of Bolts Burn (0061-05)

(all concentrations are in $\mu g\ g^{-1}$)

6.42 Transplants into Bolts Burn

At the same time as boulders covered with filaments of *Lemanea* were exchanged between reaches 05 and 07 on the R. Derwent, a single boulder from each of the two reaches was placed into Bolts Burn reach 99 (It was not possible to find sufficient suitable boulders to increase the replication.) Samples of pooled filament tips were removed subsequently at regular intervals from the two boulders.

The material transplanted into Bolts Burn from reach 05 on the R. Derwent survived for the full 1200 hours of the experiment, the only noticeable morphological change being a slight lightening of the green colour of the filaments. Material from reach 07 survived for 840 hours, but throughout the experiment filaments were scoured from the surface of the boulder by the high load of suspended material in the water of Bolts Burn. By 1200 hours all filaments had been removed from the boulder by scouring (and sampling) No noticeable growth took place in filaments from either reach following transplanting into Bolts Burn.

The zinc content of filament tips from both reaches on the R. Derwent showed similar patterns of increase during the experiment (Fig. 6.15, Table 6.5). Levels of zinc rose sharply during the first few hours following transplanting, and then began to level off Marked rises in the lead and cadmium contents of *Lemanea* filaments also took place during the experiment, but by 720 hours after the boulders were transplanted filaments from reach 05 had taken up more lead, but less cadmium, than filaments from reach 07.

6.43 Transplants into the R. Nent

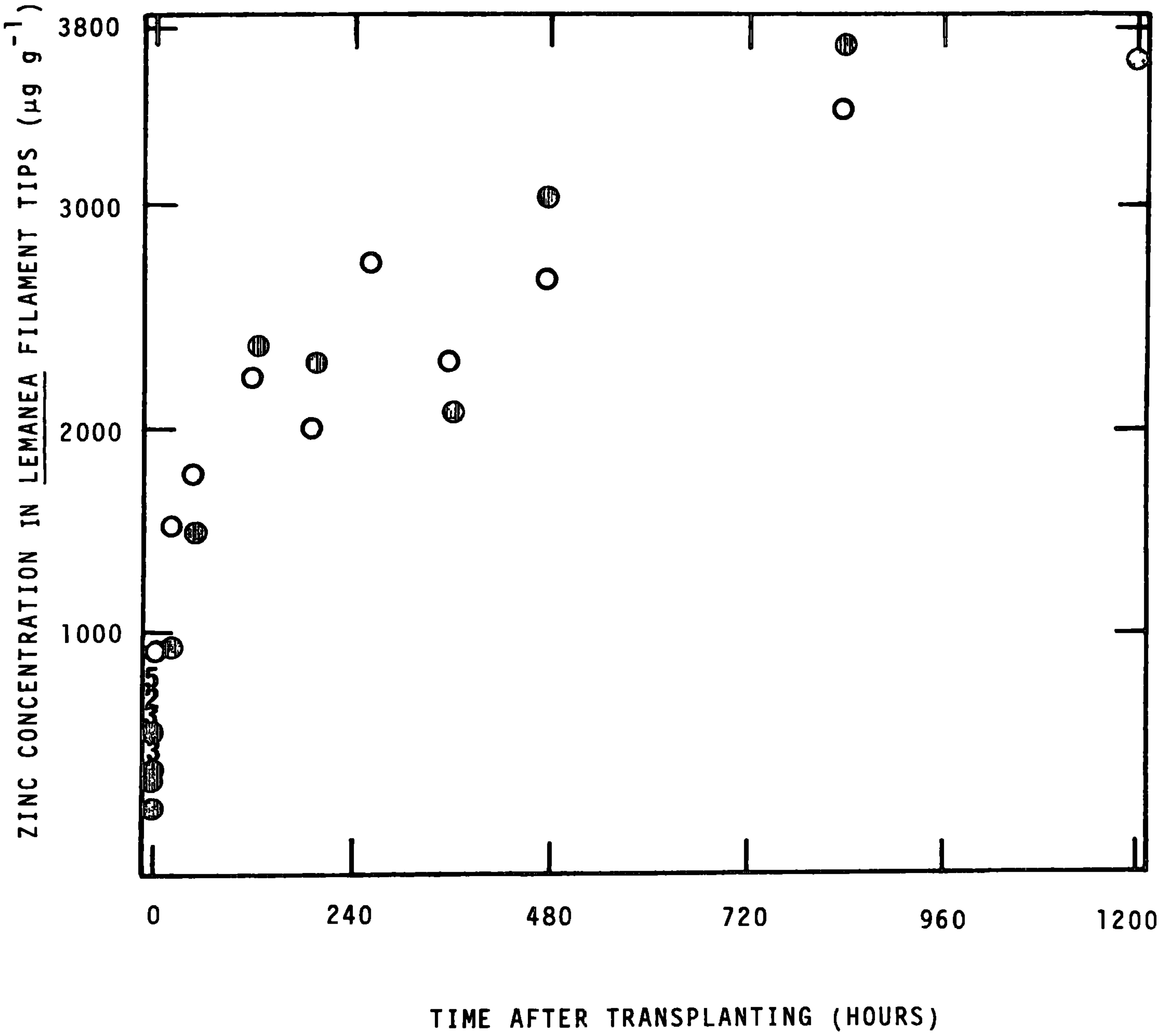
During April 1977 single boulders covered in *Lemanea* were removed from five of the reaches from which the alga had been sampled during the broad survey of mineral

Fig. 6.15

Changes in the zinc content of 2 cm long filament tips of *Lemanea fluviatilis* from the R. Derwent upstream and downstream of Bolts Burn following transplanting into Bolts Burn reach 99.

● boulder transplanted from 0061-05 to 0071-99

○ boulder transplanted from 0061-07 to 0071-99


ZINC CONCENTRATION IN LEMANEA FILAMENT TIPS (µg g-1)
3800
3000
2000
1000
0
240
480
720
960
1200
TIME AFTER TRANSPLANTING (HOURS)

Time after transplanting (hours)	Zn	Pb	Cd
0	257.2	37.9	8.4
0.08	470.4	103.1	15.5
0.16	429.3	58.1	10.7
0.25	491.3	115.6	16.6
0.33	262.9	46.4	12.9
0.42	545.3	177.7	15.9
0.5	581.0	107.0	21.4
0.75	620.5	190.9	17.9
1	700.8	204.0	16.2
1.5	602.6	256.4	19.9
2	724.9	353.2	25.1
3	772.4	337.4	33.7
24	1050.7	456.5	134.4
48	1585.0	353.0	335.7
120	2406.3	425.9	457.8
192	2337.8	405.5	413.4
264	2516.9	336.3	492.8
360	2108.3	803.4	525.6
480	3069.7	1224.9	481.7
840	3738.4	675.8	365.4
1200	3687.7	775.9	273.3

Table 6.5a Changes in the zinc, lead and cadmium contents of filament tips of *Lemanea fluviatilis* following transplanting from R. Derwent upstream of Bolts Burn (0061-05) into Bolts Burn (0071-99)

(all concentrations are in $\mu g\ g^{-1}$)

Time after transplanting (hours)	Zn	Pb	Cd
0	833.3	289.5	23.2
0.08	868.4	415.8	35.3
0.16	806.0	253.7	55.2
0.25	732.9	264.7	15.9
0.33	736.2	313.4	17.5
0.42	875.0	375.0	38.2
0.5	665.9	279.0	17.9
0.75	841.1	359.8	39.3
1	866.1	370.1	31.9
1.5	892.9	378.2	42.0
2	853.7	-	51.2
3	943.7	471.9	69.0
24	1545.3	590.5	325.1
48	1822.9	473.1	457.9
120	2236.7	699.3	304.4
192	1987.7	422.1	383.1
264	2789.3	511.4	644.1
360	2332.4	1180.8	718.7
480	2720.2	1286.7	294.0
840	3732.6	1630.7	196.8
1200	-	-	-

Table 6.5b Changes in the zinc, lead and cadmium contents of filament tips of *Lemanea fluviatilis* following transplanting from the R. Derwent downstream of Bolts Burn (0061-07) into Bolts Burn (0071-99)

(all concentrations are in $\mu g\ g^{-1}$)

composition, transported in water from the reach of origin, and placed into the R. Nent at Alston (0048-99). The reaches from which the boulders were taken were chosen as examples of reaches where *Lemanea* was common at both high and low levels of zinc in the water. They were:

R. Derwent upstream of Bolts Burn (0061-05), 'total' Zn = 0.026 mg l^{-1};

R. Derwent downstream of Bolts Burn (0061-05), 'total' Zn = 0.317 mg l^{-1};

R South Tyne upstream of R. Nent (0055-30), 'total' Zn = 0.083 mg l^{-1},

R. South Tyne downstream of R. Nent (0055-31), 'total' Zn = 0.290 mg l^{-1};

Rookhope Burn at Eastgate (0012-45), 'total' Zn = 0.320 mg l^{-1}.

The R. Nent was chosen as the site for the transplant experiment because it combined high current speeds, low levels of particulate material (unlike Bolts Burn) and high 'total' levels of zinc, lead and cadmium ($\bar{x}$ = 0.904 mg l^{-1}, 0.015 mg l^{-1} and 0.0040 mg l^{-1} respectively). As the levels of zinc (and cadmium) in the water were considerably higher than in any of the reaches from which the alga was transplanted, it was hoped that the amounts of zinc taken up following transplanting would be sufficienly great to illustrate possible differences in rates of uptake between the different populations. Following transplanting, samples of pooled filament tips were removed from each of the boulders at regular intervals for 1368 hours.

Filaments of *Lemanea* were still alive on all of the boulders at the completion of the experiment, although in the final two weeks they became smothered in epiphytic algae (especially *Stigeoclonium tenue* and *Mougeotia* spp.). No visible growth of filaments took place on any of the boulders, although the only material that looked somewhat unhealthy at the end of the experiment was that transplanted from Rookhope Burn.

Changes in the zinc, lead and cadmium contents of filaments throughout the experiment are presented in Table 6 6 and Figs 6.16 - 6.18. Although the initial levels of the three metals in filament tips differed between material from different reaches, subsequent patterns of uptake of metals were rather similar

Zinc

In all cases uptake of zinc was initially rapid, and levelled off for a short time at about 250 - 300 hours following transplanting (Fig. 6.16). Following this the zinc content of filament tips on all of the boulders increased again and was apparently still increasing slightly at the end of the experiment. The was some indication that uptake of zinc was slightly more rapid in material from 0061-05 than in material from 0061-07, but the patterns of uptake of zinc in filaments from 0055-30 and 0055-31 were very similar. Material from 0012-45 had taken up more zinc by the end of the experiment than material from the four other reaches, with an exceptionally large 'jump' in the zinc content between about 300 - 500 hours following transplanting

Lead

The lead content of filament tips from all of the boulders showed a more complicated pattern of change throughout the experiment (Fig. 6.17). In material from 0061-05, 0061-07 and 0055-30 the lead content increased rapidly during the first few days following transplanting, decreased markedly between about 120 hours and 600 hours, and then increased again towards the end of the experiment. In the case of material from 0055-31 and 0012-45 this pattern also occurred, but with an initial sharp drop in lead content at the commencement of the experiment.

Cadmium

The patterns of uptake of cadmium by filament tips on the five different boulders were again rather similar throughout the experiment (Fig. 6.18). In all cases a sharp

Time after transplanting (hours)	Zn	Pb	Cd
0	232.3	52.8	10.0
0.5	459.4	36.4	5.9
1	527.0	32.4	9.2
1.5	603.7	46.4	11.2
2	612.4	32.4	8.6
2.5	756.8	62.0	20.5
3	615.4	50.9	11.9
3.5	711.8	66.0	13.2
4	688.9	94.4	15.0
24	911.2	130.9	25.1
48	981.1	133.1	11.0
72	1164.4	153.0	20.3
168	1320.8	192.1	25.0
264	1252.0	109.5	51.3
336	2135.6	127.9	44.9
504	2082.5	-	45.3
672	3418.0	136.7	85.9
1008	3727.7	127.6	56.1
1368	5016.2	284.1	73.9

Table 6.6 a Changes in the zinc, lead and cadmium contents of filaments of *Lemanea fluviatilis* following transplanting from the R. Derwent upstream of Bolts Burn (0061-05) into the R. Nent (0048-99)

(all concentrations are in $\mu g\ g^{-1}$)

Time after transplanting (hours)	Zn	Pb	Cd
0	763.0	216.2	36.6
0.5	826.2	226.5	15.2
1	896.8	186.1	19.9
1.5	1000.0	210.0	31.7
2	1018.1	192.3	25.6
2.5	1151.3	254.9	23.0
3	1121.5	196.3	24.3
3.5	1100.0	240.0	33.0
4	1119.4	251.9	39.2
24	1149.4	247.1	25.0
48	1399.3	279.9	21.2
72	1500.5	271.7	29.9
168	1594.5	274.2	57.0
264	1551.2	213.9	48.8
336	1994.1	192.7	50.6
504	2190.3	84.8	68.2
672	2825.2	111.9	43.7
1008	3389.2	116.6	49.1
1368	3561.5	270.0	55.4

Table 6.6 b Changes in the zinc, lead and cadmium contents of filaments of *Lemanea fluviatilis* following transplanting from the R. Derwent downstream of Bolts Burn (0061-07) into the R. Nent (0048-99)

(all concentrations are in $\mu g\ g^{-1}$)

Time after transplanting (hours)	Zn	Pb	Cd
0	365.4	49.8	13.7
0.5	481.0	57.1	8.8
1	496.7	77.7	10.4
1.5	538.6	84.3	9.8
2	513.2	86.7	9.7
2.5	558.7	88.4	8.8
3	551.0	32.9	14.0
3.5	571.5	90.9	8.5
4	631.1	89.0	17.8
24	675.2	104.5	20.5
48	874.5	90.9	19.7
72	1165.6	111.8	27.8
168	1197.4	92.1	39.8
264	1138.1	89.1	24.8
336	1876.1	61.9	46.9
504	2326.9	42.3	51.9
672	3283.9	42.4	59.3
1008	4306.4	104.0	62.7
1368	4314.5	290.0	47.0

Table 6.6 c Changes in the zinc, lead and cadmium contents of filaments of *Lemanea fluviatilis* following transplanting from the R. South Tyne upstream of the R. Nent (0055-30) into the R. Nent (0048-99)

(all concentrations are in $\mu g\ g^{-1}$)

Time after transplanting (hours)	Zn	Pb	Cd
0	985.6	71.8	28.1
0.5	1100.5	70.6	20.8
1	1203.7	95.6	26.0
1 5	1231.6	67.4	19.3
2	1211.7	86.5	21.0
2.5	1235.4	87.8	23.1
3	1309.2	65.5	19.0
3.5	1284.9	65.4	20.7
4	1290.5	85.2	24.3
24	1262.3	75.5	25.7
48	1559.5	107.1	20.2
72	900.5	91.0	16.5
168	1582.5	124.5	35.6
264	1574.8	64.7	31.8
336	2115.0	112.1	27.3
504	2373.4	75.9	39.1
672	2907.0	29.1	90 1
1008	4113.6	113.6	56.2
1318	4420.4	325.0	70.4

Table 6.6 d Changes in the zinc, lead and cadmium contents of filaments of *Lemanea fluviatilis* following transplanting from the R. South Tyne downstream of the R. Nent (0055-31) into the R. Nent (0048-99)

(all concentrations are in $\mu g\ g^{-1}$)

Time after transplanting (hours)	Zn	Pb	Cd
0	923.2	254.8	17.4
0.5	998.2	272.2	15.4
1	1079.4	299.4	10.8
1.5	1170.9	308.8	9.1
2	1076.2	301.3	13.2
2.5	1200.0	265.0	20.0
3	1170.4	249.6	16.4
3.5	1131.1	278.4	21.5
4	1138.7	293.5	20.7
24	1235.9	233.1	45.2
48	1332.4	254.1	21.6
72	1565.0	309.0	28.1
168	1768.9	357.7	30.3
264	1852.1	239.4	43.7
336	1738.9	238.9	36.4
504	3846.2	248.5	46.4
672	5377.8	226.7	40.4
1008	-	-	-
1368	5069.0	293.1	79.3

Table 6.6 e Changes in the zinc, lead and cadmium contents of filaments of *Lemanea fluviatilis* following transplanting from Rookhope Burn at Eastgate (0012-45) into the R. Nent (0048-99)

(all concentrations are in $\mu g\ g^{-1}$)

Fig. 6.16

Changes in the zinc content of 2 cm long filament tips of *Lemanea fluviatilis* from five reaches in North-East England following transplanting into the R. Nent at Alston ('total' zinc level 0.904 mg l^{-1})

Key to reaches of origin:

0061-05	R. Derwent upstream of Bolts Burn
0061-07	R. Derwent downstream of Bolts Burn
0055-30	R. South Tyne upstream of R. Nent
0055-31	R. South Tyne downstream of R. Nent
0012-45	Rookhope Burn at Eastgate

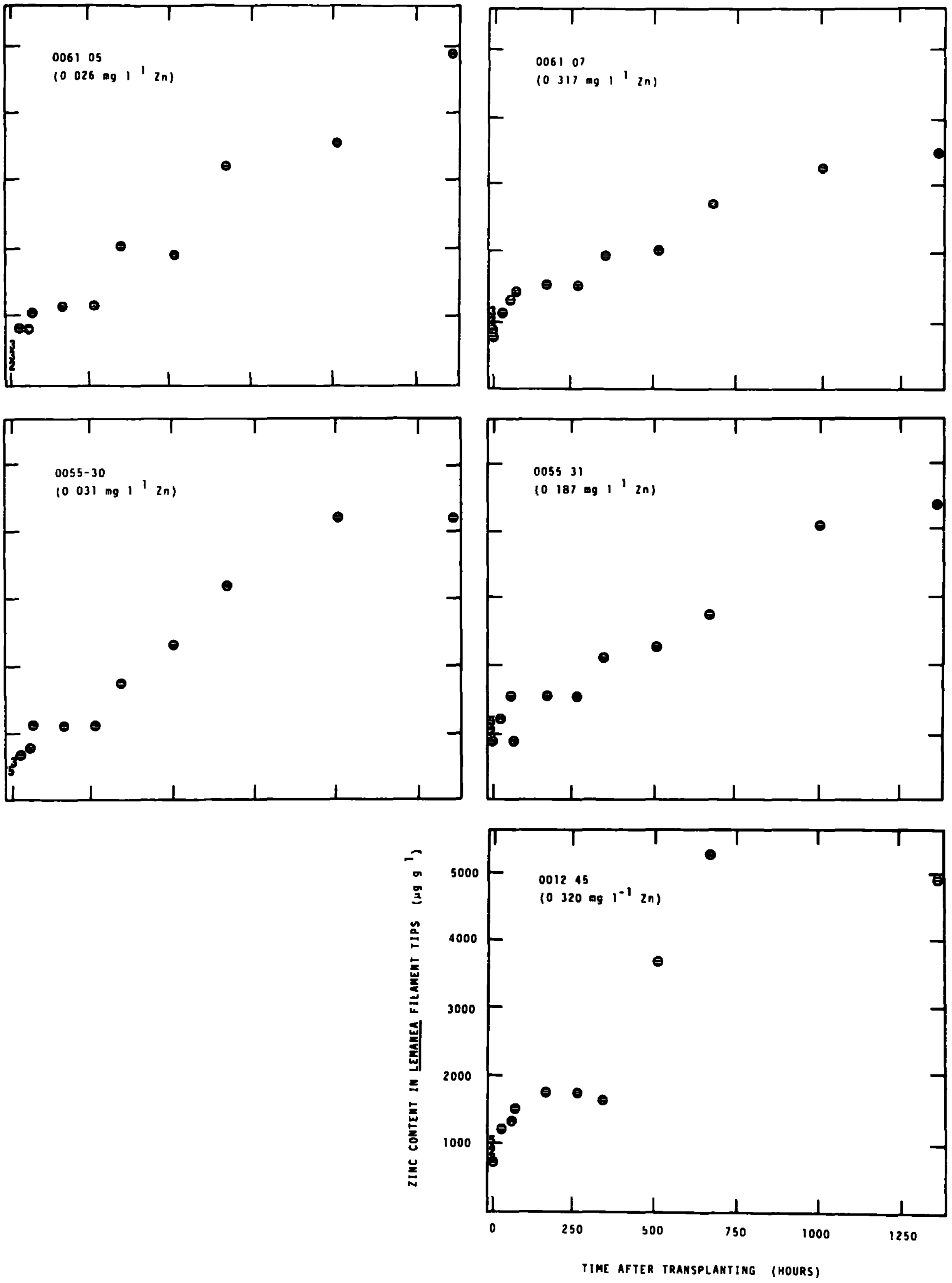

0061 05
(0 026 mg l ¹ Zn)
0061 07
(0 317 mg l ¹ Zn)
0055-30
(0 031 mg l ¹ Zn)
0055 31
(0 187 mg l ¹ Zn)
0012 45
(0 320 mg l⁻¹ Zn)
ZINC CONTENT IN LEMANEA FILAMENT TIPS (µg g ¹)
5000
4000
3000
2000
1000
0
250
500
750
1000
1250
TIME AFTER TRANSPLANTING (HOURS)

Fig. 6.17

Changes in the lead content of 2 cm long filament tips of *Lemanea fluviatilis* from five reaches in North-East England following transplanting into the R. Nent at Alston ('total' lead level 0.015 mg l^{-1})

Key to reaches of origin

0061-05	R. Derwent upstream of Bolts Burn
0061-07	R. Derwent downstream of Bolts Burn
0055-30	R. South Tyne upstream of R. Nent
0055-31	R. South Tyne downstream of R. Nent
0012-45	Rookhope Burn at Eastgate

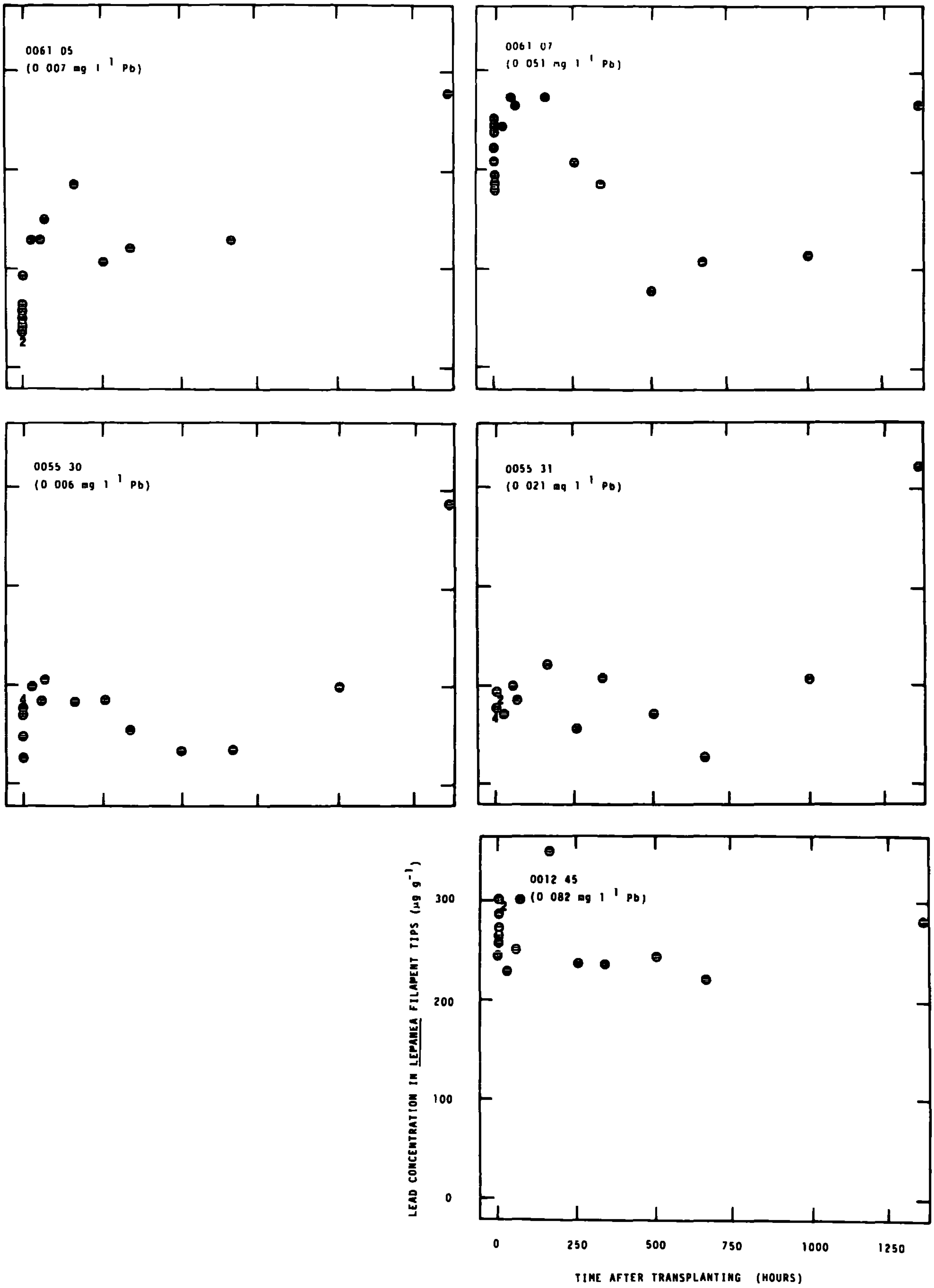
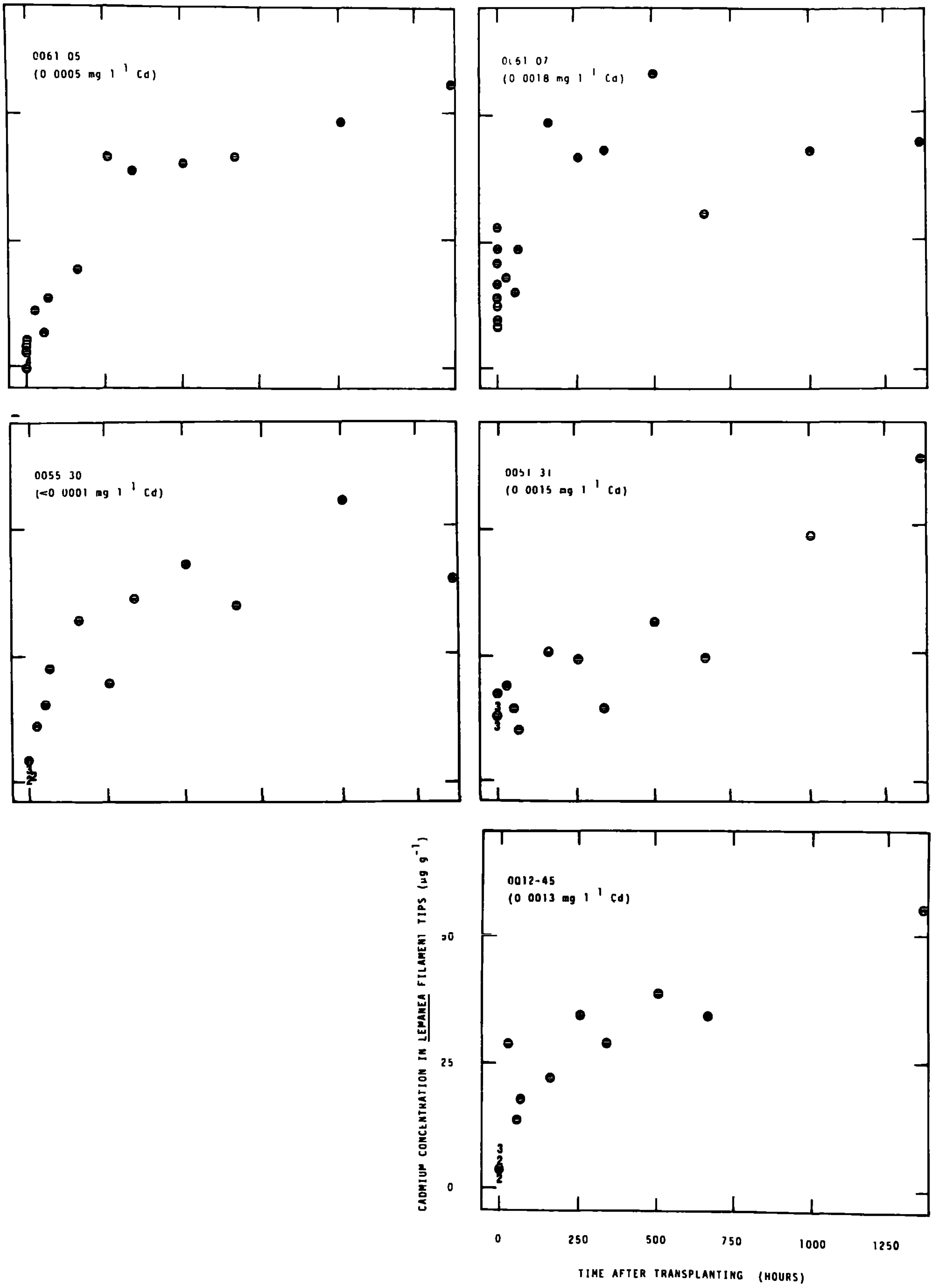

0061 05
(0 007 mg l⁻¹ Pb)
2
0061 07
(0 051 mg l⁻¹ Pb)
0055 30
(0 006 mg l⁻¹ Pb)
4
0055 31
(0 021 mg l⁻¹ Pb)
4
0012 45
(0 082 mg l⁻¹ Pb)
2
LEAD CONCENTRATION IN LEPANEA FILAMENT TIPS (µg g⁻¹)
300
200
100
0
0
250
500
750
1000
1250
TIME AFTER TRANSPLANTING (HOURS)

Fig. 6.18

Changes in the cadmium content of 2 cm long filament tips of *Lemanea fluviatilis* from five reaches in North-East England following transplanting into the R. Nent at Alston ('total' cadmium level 0.0040 mg l^{-1})

Key to reaches of origin:

0061-05	R. Derwent upstream of Bolts Burn
0061-07	R. Derwent downstream of Bolts Burn
0055-30	R. South Tyne upstream of R. Nent
0055-31	R. South Tyne downstream of R. Nent
0012-45	Rookhope Burn at Eastgate

0061 05
(0 0005 mg l -1 Cd)
0061 07
(0 0018 mg l -1 Cd)
0055 30
(<0 0001 mg l -1 Cd)
0051 31
(0 0015 mg l -1 Cd)
0012-45
(0 0013 mg l -1 Cd)
CADMIUM CONCENTRATION IN LEMANEA FILAMENT TIPS (µg g -1)
50
25
0
0
250
500
750
1000
1250
TIME AFTER TRANSPLANTING (HOURS)
2
3

increase in the levels of cadmium during the first few hours following transplanting was followed by a gradual levelling off of levels towards the end of the experiment. Levels of cadmium were rather similar in filaments from all of the boulders at the end of the experiment.

6.5 LABORATORY INVESTIGATION OF FACTORS INFLUENCING LOSS OF ZINC FROM *LEMANEA* FILAMENTS

It was apparent from the results of the field studies reported in 6.3 and 6.4 that a proportion of the zinc accumulated by filaments of *Lemanea* was subject to rapid change when the level of zinc in the surrounding water changed It seemed probable that the levels of various chemical parameters in the water could influence the speed of such changes. An experiment was therefore planned to investigate the influence of selected parameters on the release of zinc that had been accumulated by filaments of *Lemanea* growing at a high level of zinc in the field.

The material used in the experiment was taken from reach 07 on the R. Derwent. Single tufts of whole filaments were removed from the reach and placed into flasks containing four replicates of ten different solutions (see 2.311). The flasks were incubated under standard physical conditions in a shaking tank for 24 hours, and small aliquots of the solutions were removed at regular intervals to follow the time course of any loss of zinc.

The patterns of mean loss of zinc during the 24 hour period differed markedly according to the chemical composition of the particular solution (Fig. 6 19). The patterns found in the different solutions are considered below

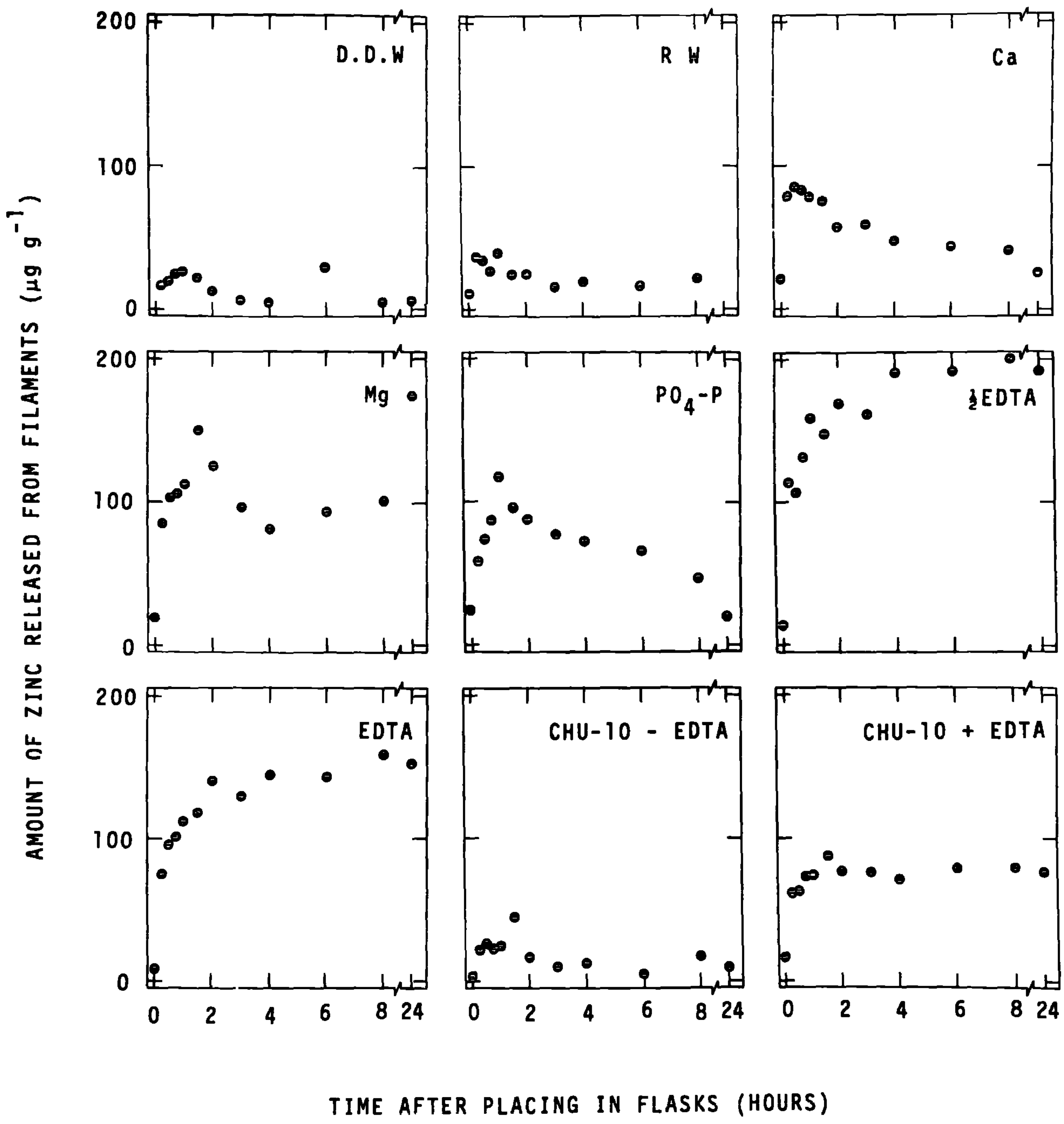
Fig. 6.19

Diagrams to show loss of zinc from whole filaments of *Lemanea* from reach 07 on the R. Derwent following placing into different solutions.

(mean of four replicates in each case)

key to solutions used:

D.D.W.	double distilled water
R.W.	water from R. Derwent reach 05
Ca	solution of 20 mg l^{-1} Ca as $CaCl_2$
Mg	solution of 20 mg l^{-1} Mg as $MgCl_2$
PO_4-P	solution of 5 mg l^{-1} P as KH_2PO_4
½EDTA	solution of 1 575 mg l^{-1} EDTA* disodium salt see p
EDTA	solution of 3.15 mg l^{-1} EDTA* disodium salt see p
CHU-10 - EDTA	Chu 10 medium with EDTA omitted
CHU-10 + EDTA	complete Chu-10 medium



AMOUNT OF ZINC RELEASED FROM FILAMENTS (μg g^{-1})
200
100
0
D.D.W
R W
Ca
Mg
PO$_4$-P
½EDTA
EDTA
CHU-10 - EDTA
CHU-10 + EDTA
0
2
4
6
8
24
TIME AFTER PLACING IN FLASKS (HOURS)

Double distilled water

Only a small amount of zinc was released into the flasks of double distilled during the course of the experiment This confirmed the findings of the initial leaching experiment that had been carried out before double distilled water was used to wash filaments (see 3.83). Loss increased to about 26 µg g^{-1} (dry weight) by the end of the first hour following placing into the flasks. Following this some of the zinc that had been released appeared to be taken up again by the filaments.

River water

The pattern of mean loss of zinc into river water (from the R. Derwent reach 05) was rather similar to that observed in double distilled water. The initial release of zinc into the water was somewhat greater and more rapid (increasing to 36 µg g^{-1} in the first 15 minutes) but after one hour some of the zinc released had apparently been re-absorbed

Magnesium

Loss of zinc from filaments of *Lemanea* into a 20 mg l^{-1} solution of magnesium followed a more complicated pattern. A very rapid release of up to 150 µg g^{-1} zinc within the first 1½ hours was followed by a rapid re-absorption (to 80 µg g^{-1}) by four hours. During the remainder of the experiment some further release of zinc took place.

Calcium

Levels of zinc released into a solution of 20 mg l^{-1} calcium were not as high as those released into the solution of magnesium of equivalent concentration, although a very rapid release of up to 86 µg g^{-1} Zn took place during the first half hour. This was followed by a steady re-absorption (down to 25 µg g^{-1}) during the remainder of the experiment.

Phosphate-P

The pattern of release of zinc into a 5 mg l^{-1} PO_4-P solution was similar to that found in the solutions of magnesium and calcium, although the 'peaked' form of the time course for the release of zinc was even more distinct.

Chu-10 medium omitting EDTA* disodium salt - see abbreviations

Little zinc was released during the 24 hour period into Chu-10 basal medium when the chelating agent EDTA was omitted The time course and magnitude of release of zinc was similar to that found in double distilled water and river water.

Chu-10 medium with EDTA* disodium salt - see abbreviations

Much more zinc was released from filaments of *Lemanea* into complete Chu-10 medium than into medium with EDTA omitted The pattern of release was different to that found in any of the solutions considered previously, with an initial rapid release of about 75 µg g^{-1} Zn followed by a levelling off in levels of zinc in the medium. There appeared to be no re-absorption of zinc that had been released

1.575 mg l^{-1} solution of EDTA* disodium salt - see abbreviations

The highest levels of zinc released during the experiment were detected in the solution of 1.575mg l^{-1} EDTA in double distilled water (i.e. half the strength normally used in Chu-10 medium). Release of zinc was initially rapid, and stabilised at around 190 µg g^{-1} after about four hours. Again there was no evidence of re-accumulation of zinc by the filaments.

3 15 mg l^{-1} solution of EDTA* disodium salt - see abbreviations

The pattern of release of zinc into a 3.15 mg l^{-1} solution of EDTA was similar to that found in a 1.575 mg l^{-1} solution of the chelating agent, but the absolute levels of zinc released were in fact slightly lower.

The mean zinc content of whole *Lemanea* filaments collected from 0061-07 at the same time as those used in the experiment was 960 µg g^{-1}. Therefore the highest level of zinc released from filaments during the experiment (190 µg g^{-1}) represented less than 20% of the total content of the filaments.

6.6 LABORATORY ASSAY OF TOLERANCE TO ZINC OF DIFFERENT POPULATIONS OF *LEMANEA*

In an attempt to ascertain whether populations of *Lemanea* growing at higher levels of zinc in the field were present as tolerant strains (as was demonstrated for *Stigeoclonium tenue*, see 7.5), a limited series of laboratory assays was planned to compare the tolerance to zinc of material from high and low levels of zinc in the water.

It was intended originally to carry out toxicity tests on filaments of *Lemanea* that had been grown in unialgal culture Several attempts were made during the three year period to grow the alga in flask culture under a wide variety of physical and chemical conditions, but despite careful washing and the choice of the cleanest material available, the filaments invariably became smothered in epiphytic algae before noticeable growth took place

Laboratory assay was therefore limited to short-term experiments on filaments soon after collection from the field. The toxicity test used (a modification of that used for *Stigeoclonium tenue*) has been described in 2.312.

Whole filaments of *Lemanea* were collected from six reaches in North-East England. Three of the reaches (0061-05, 0055-30, 0009-40) carried low 'total' levels of zinc in the water (<0.03 mg l^{-1}), whilst the other three (0061-07, 0055-31, 0012-45) had elevated 'total' levels of

Site	stream no.	reach no.	'total' zinc	level of zinc in medium (mg l^{-1})																				
				0	0.5	1.0	1.5	2.0	3.0	5.0	7.5	10	15	20	25	30	40	50	60	70	80	90	100	
R. Derwent	0061	05	0.026	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GW-	GG-	GG-	GW-	GW-	GW-	GW-	GW-	GW-	(DAY 6)
				GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GW-	GW-	GW-	GW-	GW-	GW-	WW-	WW-	WW-	WW-	WW-	WW-	(DAY 10)
R. Derwent	0061	05	"	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GW-	GW-	GW-	GW-	GW-	GW-	WW-	WW-	WW-	(DAY 6)
				GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GW-	GW-	GW-	GW-	GW-	WW-	WW-	WW-	WW-	WW-	(DAY 10)
R. Derwent	0061	05	"	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GW-	GW-	GW-	GW-	GW-	WW-	WW-	WW-	WW-	WW-	(DAY 6)
				GG-	GG-	GG-	GG-	GG-	GG-	GWE	GWE	GG-	GG-	GW-	GW-	GW-	GW-	GW-	WW-	WW-	WW-	WW-	WW-	(DAY 10)
R. Derwent	0061	05	"	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GW-	GW-	GW-	GW-	WW-	WW-	WW-	WW-	WW-	(DAY 6)
				GGE	GGE	GGE	GGE	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GW-	GW-	GW-	GW-	WW-	WW-	WW-	WW-	WW-	(DAY 10)
R. Derwent	0061	07	0.317	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GW-	GW-	GW-	(DAY 6)
				GGE	GGE	GGE	GGE	GW-	GW-	GW-	GW-	GW-	GW-	GW-	GW-	GW-	GW-	GW-	GW-	WW-	WW-	WW-	WW-	(DAY 10)
R. Derwent	0061	07	"	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GW-	GW-	GW-	(DAY 6)
				GW-	GW-	GW-	GW-	GW-	GW-	GW-	GW-	GW-	GW-	GW-	GW-	GW-	GW-	GW-	GW-	WW-	WW-	WW-	WW-	(DAY 10)
R. Derwent	0061	07	"	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GW-	GW-	GW-	(DAY 6)
				GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GW-	GW-	GW-	GW-	GW-	GW-	GW-	WW-	WW-	WW-	(DAY 10)
R Derwent	0061	07	"	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GW-	GW-	GW-	(DAY 6)
				GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GW-	GW-	WW-	WW-	WW-	(DAY 10)

Tables 6.7 and 6.8 Results of laboratory toxicity tests performed upon filaments of *Lemanea* from reaches 05 and 07 on the R. Derwent

Site	stream no.	reach no.	'total' zinc	level of zinc in medium (mg l^{-1})																				
				0	0.5	1.0	1.5	2.0	3.0	5.0	7.5	10	15	20	25	30	40	50	60	70	80	90	100	
R South Tyne	0055	30	0.031	GGE	GGE	GG-	GW-	GG-	GGE	GG-	GG-	GG-	GG-	GGE	GG-	GG-	GG-	GWE	GW-	GGE	GG-	GW-	GWE	(DAY 6)
				GGE	GGE	GG-	GW-	GG-	GG-	GW-	GG-	GG-	GG-	GWE	GW-	GW-	GW-	GWE	GW-	GWE	GW-	GW-	GW-	(DAY 10)
R. South Tyne	0055	30	"	GG-	GW-	GWE	GW-	GG-	GG-	GG-	GGE	GG-	GWE	GW-	GW-	GW-	GW-	GWE	GW-	GW-	GW-	GW-	GGE	(DAY 6)
				GGE	GW-	GGE	GW-	GG-	GG-	GG-	GGE	GG-	GW-	GW-	GG-	GW-	GW-	GW-	GW-	GW-	GW-	GW-	GW-	(DAY 10)
R. South Tyne	0055	30	"	GGE	GW-	GGE	GW-	GG-	GG-	GGE	GG-	GG-	GW-	GG-	GG-	GWE	GWE	GWE	GW-	GW-	GW-	GW-	GW-	(DAY 6)
				GWE	GW-	GW-	GW-	GG-	GG-	GG-	GG-	GG-	GW-	GG-	GG-	GW-	GW-	GWE	GW-	GW-	GW-	GW-	GW-	(DAY 10)
R. South Tyne	0055	30	"	GGE	GW-	GG-	GG-	GW-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GW-	GW-	GW-	GW-	GW-	GW-	GGE	(DAY 6)
				GWE	GW-	GG-	GG-	GW-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GW-	GW-	GW-	GW-	GW-	GW-	GW-	(DAY 10)
R South Tyne	0055	31	0.187	GG-	GG-	GG-	GW-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GW-	GW-	GW-	GW-	GG-	GG-	GW-	(DAY 6)
				GG-	GGE	GG-	GW-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GW-	GW-	GW-	GW-	GW-	GG-	GG-	GW-	(DAY 10)
R. South Tyne	0055	31	"	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GW-	GW-	GW-	GW-	GW-	GW-	GG-	GG-	(DAY 6)
				GGE	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GW-	GW-	GW-	GW-	GW-	GW-	GW-	GW-	(DAY 10)
R. South Tyne	0055	31	"	GW-	GW-	GW-	GW-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GW-	GG-	GG-	GW-	GW-	GW-	GW-	GG-	GW-	(DAY 6)
				GW-	GW-	GW-	GW-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GW-	GG-	GG-	GW-	GW-	GW-	GW-	GG-	GW-	(DAY 10)
R. South Tyne	0055	31	"	GG-	GG-	GG-	GW-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GW-	GG-	GW-	GW-	GW-	GW-	GG-	GG-	GG-	(DAY 6)
				GG-	GGE	GG-	GW-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GW-	GG-	GW-	GW-	GW-	GW-	GG-	GG-	GG-	(DAY 10)

Tables 6.9 and 6.10 Results of laboratory toxicity tests performed upon filaments of *Lemanea* from reaches 30 and 31 on the R. South Tyne

Site	stream no.	reach no.	'total' zinc	level of zinc in medium (mg l^{-1})																				
				0	0.5	1.0	1.5	2.0	3.0	5.0	7.5	10	15	20	25	30	40	50	60	70	80	90	100	
Rookhope Burn	0012	45	0.320	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GW-	GG-	GW-	GG-	GG-	(DAY 6)
				GG-	GG-	GW-	GG-	GG-	GG-	GG-	GW-	GG-	GW-	GG-	GW-	GG-	GW-	GW-	WW-	GW-	GW-	WW-	WW-	(DAY 10)
Rookhope Burn	0012	45	"	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GW-	GG-	GG-	GG-	GG-	GG-	GW-	GG-	GG-	(DAY 6)
				GGE	GGE	GW-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GW-	GG-	GG-	WW-	GW-	GW-	WW-	GW-	GW-	(DAY 10)
Rookhope Burn	0012	45	"	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GW-	GG-	GG-	GG-	GG-	GG-	GW-	(DAY 6)
				GGE	GGE	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GW-	GW-	GW-	GG-	GW-	WW-	WW-	(DAY 10)
Rookhope Burn	0012	45	"	GGE	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	(DAY 6)
				GGE	GGE	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GW-	GG-	GW-	GG-	GW-	GW-	GG-	GW-	WW-	(DAY 10)
R. Tees	0009	40	0.029	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GW-	GG-	GG-	GG-	GG-	GG-	GW-	GW-	GW-	GW-	GW-	GW-	GW-	(DAY 6)
				GGE	GGE	GGE	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GW-	WW-	GW-	WW-	GW-	WW-	GW-	(DAY 10)
R. Tees	0009	40	"	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GW-	GG-	GG-	GG-	GW-	GW-	GW-	GG-	GG-	(DAY 6)
				GGE	GGE	GGE	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GW-	GW-	GG-	GW-	GW-	GW-	WW-	WW-	WW-	WW-	(DAY 10)
R. Tees	0009	40	"	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GW-	GG-	GG-	GG-	GG-	GG-	GG-	GW-	WW-	GW-	GW-	(DAY 6)
				GGE	GGE	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	WW-	GW-	WW-	GW-	WW-	WW-	GW-	WW-	(DAY 10)
R. Tees	0009	40	"	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GW-	GW-	GW-	GG-	GW-	GG-	(DAY 6)
				GGE	GGE	GGE	GG-	GG-	GG-	GG-	GG-	GG-	GG-	GW-	GW-	GW-	GW-	GG-	GW-	GW-	WW-	GW-	GW-	(DAY 10)

Tables 6.11 and 6.12 Results of laboratory toxicity tests performed upon filaments of *Lemanea* from reach 45 on Rookhope Burn and reach 40 on the R. Tees

(For explanation of symbols, see 2.312, page 141) zinc in the water (>0 2 mg l^{-1}). The six reaches were visited on four separate occasions during April 1977 for the collection of *Lemanea*. On each occasion a small stone covered in relatively short filaments (<5 cm) was removed from each reach for use in the laboratory assay.

No visible growth of any of the filaments took place during the incubation period, and it was therefore impossible to gain an idea of levels of zinc that might inhibit growth (equivalent to the 'just non-inhibitory' concentration estimated for populations of *Stigeoclonium*) Some idea could be gained, however, of the approximate concentrations at which most filaments were partly killed (the tip of the filament usually turned white) or completely killed (the whole filament turned white) by the end of the incubation period These levels are summarized in Tables 6 7 - 6.12 and below

reach	'total' zinc	approximate level of zinc at which most filaments partly killed	approximate level of zinc at which most filaments killed
0061-05	0.026	25 - 30	60
0061-07	0.317	-*	80
0055-30	0.083	30 - 40	>100
0055-31	0.290	40 - 50	>100
0012-45	0 320	50 - 60	90 - 100
0009-40	0.029	30 - 40	70 - 80

(* - impossible to interpret from data)

CHAPTER 7

RESISTANCE TO ZINC OF *STIGEOCLONIUM TENUE* IN THE FIELD AND THE LABORATORY

7.1 INTRODUCTION

During the exploratory surveys of the algal flora of streams in the catchment area of the R. Derwent (3.7), *Stigeoclonium tenue* was found to be abundant in several reaches with both low and high levels of zinc in the water. It was also known from the results of exploratory surveys that this alga was present in other streams in North-East England with widely varying levels of zinc in the water.

Based on these observations, the studies reported in this chapter were planned both to give a general account of the behaviour of *S. tenue* in zinc rich environments, and to ascertain to what extent the apparent tolerance of the alga to zinc was the result of genetic or environmental adaptation.

At the completion of the survey to locate reaches that supported growths of *S. tenue* (3.7), 31 reaches on streams in North-East England were selected for further study (Table 7.1). The reaches were chosen to show as wide a range as possible in levels of zinc and other chemical parameters in the water. With the exception of two reaches on the R. Nent (0048-20 and 0048-99), the reaches were all on different streams

7.2 PROGRAMME FOR COLLECTION OF WATER AND ALGAE

Samples of water were collected from each of the sites (together with sampling of other reaches for the long-term chemical and floristic studies in progress at Durham University) and analysed for 31 different physical and chemical parameters. Based on the results of this study, a reduced analytical programme involving six chemical parameters was chosen for the subsequent samplings. Water and algal samples were then collected on four separate occasions during April - June 1975 from each of the reaches, when the streams were at low flow

Samples of water and *Stigeoclonium* were collected once from four further reaches in Europe (two in Germany, one in France, one in Belgium) by B. A. Whitton and Co-workers. Water from these reaches was analysed for the full range of physical and chemical parameters.

7.3 TAXONOMY

All of the populations studied were referred to *Stigeoclonium tenue* Kütz. However, a critical cultural study of the basal thalli was not carried out, so it is possible that some of the populations would have corresponded better with *S. pascheri* (Vischer) Cox *et* Bold in the study of the genus made by Cox and Bold (1966).

7 4 FIELD OBSERVATIONS

All of the higher levels of zinc at which *S. tenue* was found were in streams affected by drainage from surface or underground mine workings. These included four reaches (0061-10, 0071-99, 0124-99, 0176-01) within the catchment area of the Derwent Reservoir. In most cases mining activities had ceased in the catchment area of the particular stream, but in a few cases mining (for fluorspar) was still going on. In some cases (e g 0012-40, 0071-99) it seems possible that levels of heavy metals in the water had risen slightly in recent years.

The highest level of zinc in the water at which *Stigeoclonium tenue* was found was 20.0 mg l^{-1} (after Sinta funnel filtration) in the discharge of the Rammelsberg Stollen (4014-01) in the Harz Mountains, Germany. This population was present in a predominantly basal form not

stream & reach	site	grid ref	field pH		Na		Mg		Ca		Cu		Fe		Pb		Cd		PO_4-P	
			n	x̄	n	x̄	n	x̄	n	x̄	n	x̄	n	x̄	n	x̄	n	x̄	n	x̄
0012-40	Rookhope Burn	NY 942421	4	7 7	4	2 68	4	5 88	4	33 2	2	0 007	4	0 32	2	0 053	1	0 0010	4	0 014
0096-01	Ramsgill Le c'	NY 781434	4	7 6	4	4 53	4	22 35	4	76 4	2	<0 002	4	0 13	2	0 003	1	0 0060	4	0 015
0048-20	R Nent below Nenthead	NY 767448	4	8 0	4	2 98	4	10 25	4	47 1	2	0 002	4	0 44	2	0 046	1	0 0056	4	0 016
0173-80	Waskerley Beck	NZ 077375	4	7 8	4	1 75	4	3 80	4	22 9	1	<0 002	4	0 62	1	0 002	1	<0 0001	4	0 012
0149-20	Beech Burn	NZ 164333	4	7 8	4	6 60	4	31 70	4	88 0	1	0 016	4	0 17	1	<0 004	1	0 0004	4	0 115
0005-43	R Deerness	NZ 226422	4	7 8	4	9 03	4	37 85	4	77 3	2	0 005	4	0 24	2	0 008	1	0 0020	4	0 139
0022-05	Red Burn	NZ 225420	4	7 5	4	7 06	4	38 3	4	71 6	2	0 009	4	0 22	2	0 006	1	0 0400	4	0 038
0014-40	R Browney	NZ 222454	4	7 6	4	4 59	4	16 49	4	44 2	2	0 003	4	0 31	2	0 006	1	0 0010	4	0 170
0147-99	Nickynack Gill	NZ 262374	4	7 5	4	8 90	4	51 00	4	93 0	1	0 002	4	0 15	1	0 008	1	0 0003	4	0 146
0143-01	Sunderland Bridge Sewage outfall	NZ 264377	4	7 8	4	23 50	4	15 95	4	97 8	1	0 007	4	0 39	1	0 009	1	<0 0001	4	0 817
0142-50	Hollingside stream	NZ 274401	4	7 4	4	17 95	4	9 85	4	50 5	1	0 097	4	0 42	1	0 006	1	0 0003	4	4 711
0143-50	'Cemetary stream	NZ 273400	4	7 5	4	7 03	4	18 90	4	63 1	1	0 010	4	0 32	1	0 006	1	0 0002	4	1 094
0001-01	Hollingside Lane Artificial stream	NZ 275408	4	7 7	4	1 45	4	2 55	4	22 5	1	0 002	4	0 06	1	0 008	1	<0 0001	4	0 016
0013-11	Kilhope Burn	NY 809432	4	4 5	4	0 67	4	1 73	4	7 1	3	0 002	4	0 96	3	0 072	1	0 0005	4	0 007
0023-99	Cong Burn	NZ 267516	4	7 9	4	9 99	4	32 98	4	110 2	1	0 002	4	0 40	1	0 003	1	0 0001	4	0 388
0141-80	Old Durham Beck	NZ 294414	4	7 6	4	10 50	4	38 35	4	120 2	1	0 010	4	0 26	1	0 007	1	0 0002	4	0 444
0124-99	Shildon Burn	NY 966503	4	7 4	4	2 30	4	3 55	4	13 2	1	0 002	4	0 29	1	0 051	1	0 0200	4	0 011
0025-50	R Skerne	NZ 291207	4	7 6	4	13 18	4	39 55	4	115 8	1	0 011	4	0 55	1	0 007	1	0.0004	4	0 903
0146-99	Lumley Park Burn	NZ 288513	4	7 5	4	16 83	4	38 80	4	114 9	1	0 005	4	0 27	1	0 002	1	0 0003	4	0 556
0144-60	Sherburn Beck	NZ 319418	4	7 5	4	3 50	4	24 38	4	103 3	1	0 006	4	0 13	1	0 002	1	0 0010	4	0 556
0111-01	Redburn Flush	NY 931431	4	7 6	4	10 99	4	42 20	4	217 1	2	0 012	4	0 [illegible]	2	0 057	1	0 0140	4	0 547
0112-80	Redburn Stream	NY 931431	4	7 8	4	8 31	4	14 80	4	84 0	2	0 007	4	0 24	2	0 072	1	0 0010	4	0 007
0048-99	R Nent at Alston	NY 716467	4	8 0	4	2 90	4	9 73	4	57 0	2	0 004	4	0 20	2	0 015	1	0 0040	4	0 026
0055-31	R South Tyne	NY 716469	4	8 1	4	1 37	4	4 79	4	40 6	2	0 004	4	0 11	2	0 009	1	0 0020	4	0 0[illegible]4
0102-15	Garrigill Burn	NY 792425	4	7 4	4	1 89	4	6 30	4	35 6	2	0 003	4	0 25	2	0 362	1	0 0390	4	0 012
0061-10	R Derwent	NY 767502	4	7 6	4	2 14	4	4 43	4	19 9	1	0 002	4	0 41	1	0 023	1	0 0010	4	0 009
0103-08	Brown Gill	NY 764423	4	6 3	4	1 14	4	1 38	4	6 0	1	0 003	4	0 90	1	0 306	1	0 0020	4	0 018
0081-16	R East Allen	NY 858448	4	6 8	4	2 57	4	6 18	4	36 7	2	0 004	4	0 44	2	0 015	1	0 0004	4	0 040
0093-01	Gillgill Burn	NY 795440	4	6 0	4	1 94	4	4 68	4	18 3	2	0 004	4	0 25	2	0 497	1	0 0430	4	0 016
0071-99	Bolts Burn	NY 958499	4	7 5	4	6 13	4	7 23	4	36 6	2	0 011	4	0 79	2	0 278	1	<0 0001	4	0 010
4001-60	R Sülz	25839/ 56444	1	7 5	1	4 00	1	8 20	1	54 3	1	0 009	1	0 20	1	0 005	1	0 0020	1	-
4014-01	Rammelsberg Stollen	35978/ 57519	1	7 6	1	4 40	1	29 20	1	180 0	1	0 073	1	0 02	1	0 001	1	0 0820	1	0 005
1002-50	La Gueule near Hergenrath	25003/ 586186	1	8 3	1	3 30	1	21 40	1	80 0	1	0 006	1	0 21	1	0 003	1	0 0020	1	0 236
3006-50	'Le Pont Plan Fossé'	5959/ 53186	1	7 8	1	7 50	1	11 20	1	57 8	1	0 007	1	1 00	1	0 107	1	0 0102	1	0 830
0176-05	'Track Flush'	NY 956480	4	4 9	8	1 03	8	1 59	8	4 1	8	0 002	7	0 04	8	1 284	8	0 0030	3	0 0[illegible]1

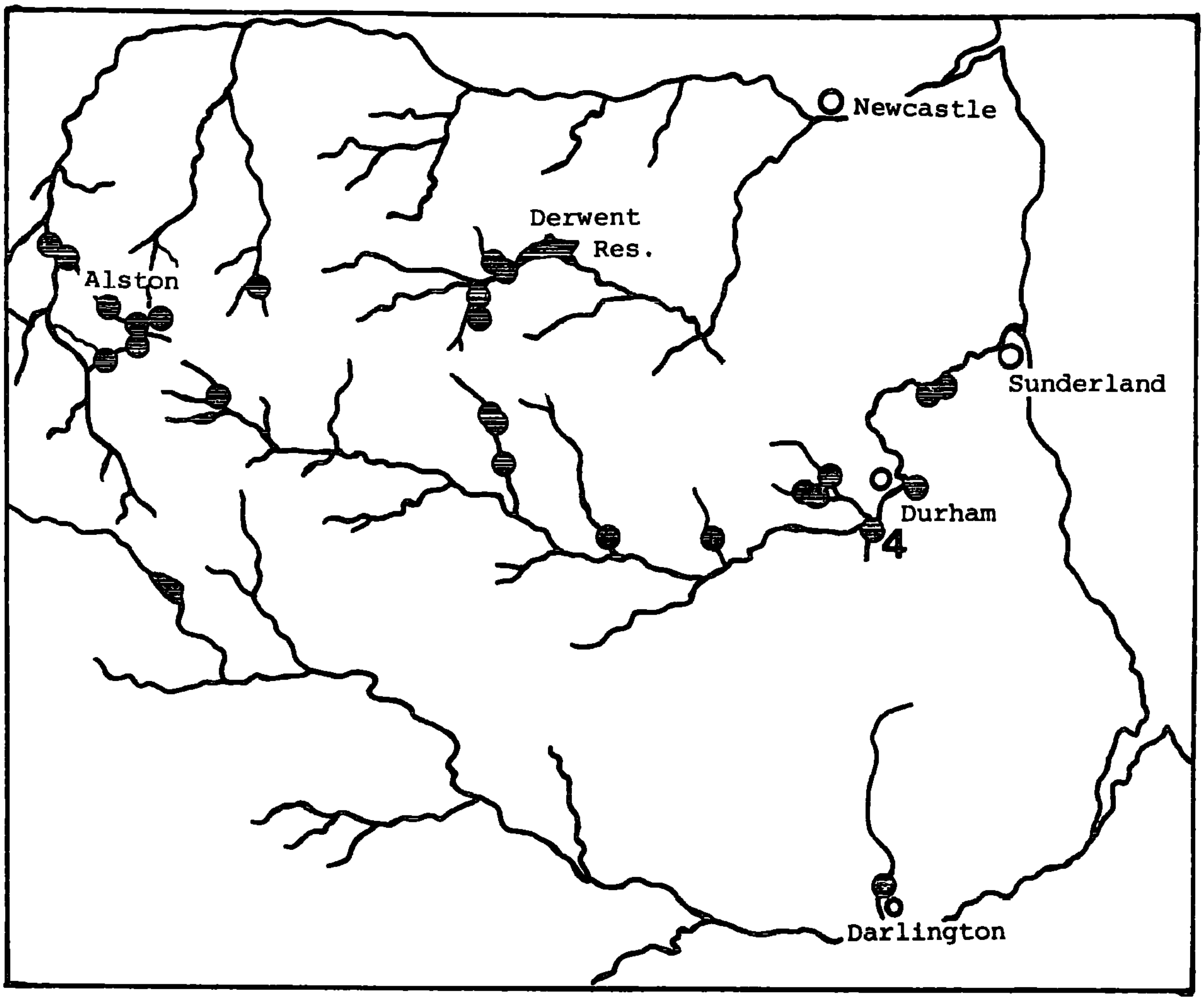
Table 7 1 Location of, and mean concentrations of selected chemical parameters in the water of sites sampled for *Stigeoclonium tenue*

(all concentrations are in mg l^{-1})

Fig. 7.1

Map to show the location of reaches in North-East England from which *Stigeoclonium tenue* was collected

1 cm = 5 km


Newcastle
Derwent
Res.
Alston
Sunderland
Durham
4
Darlington

corresponding closely to any text figure of *Stigeoclonium* (B. A. Whitton, personal communication). In culture, however, branched filaments developed, resembling those of the other populations Obvious branched filamentous growths were present at all the other sites. The highest level of zinc at which the alga was found from England was 7.1 mg l^{-1} in Gillgill Burn (0093-01). Two other reaches in the Gillgill Burn catchment were known to carry more than 20.0 mg l^{-1} Zn in the water, but detailed study of these reaches by Say (1977) never showed the presence of *Stigeoclonium*.

S. tenue was found to be abundant not only at some of the sites with low concentrations of zinc, but also at some of the higher levels of the metal. At some examples of both low and high levels of zinc in the water it was abundant throughout both spring and summer. In some of the low zinc streams this occurred even where the PO_4-P content of the water was quite low.

In the R Nent at Alston (0048-99) *S. tenue* was the dominant alga during both the spring and summer of 1975 and 1976, as had been the case for (at least) the previous six years (B. A. Whitton, personal communication). This pattern was not continued into 1977, when only sparse growths of the alga grew up in the reach. At only one site (Rampgill Level, 0096-01) was *S. tenue* observed to be the dominant alga throughout the year, with erect growths predominating at all times. This adit arises from a subterranean source and has relatively small annual temperature fluctuation. At two other sites ('Track Flush', 0176-01, 'Hollingside Artifical Stream', 0001-01) *S. tenue* was observed to occur intermittently during the winter months. The former stream arises from an adit and has a relatively small temperature fluctuation, and the latter maintained a relatively high winter temperature as it was in part artificially fed.

7.5 LABORATORY ASSAYS

From the results of the toxicity tests, it soon became apparent that higher levels of zinc were needed to inhibit the growth of some populations of the alga more than others. Higher levels of zinc were also needed to cause death of these populations by the end of the six-day culture period. In contrast, repetition of the toxicity test on the same population gave rather similar results (e.g. Table 7.2). Long-term subculturing of two different populations in zinc-free medium and in medium containing a level of zinc sufficient to cause moderate inhibition of growth did not lead to any detectable change in response over the period (Table 7.2). A rather similar study on a population from the R. Nent (0048-20) gave a similar result (C. Rajendran, personal communication). Athough no attempt was made to assess the number of cell generations occurring within the six-month culture period, these results would seem to indicate that the differences in reponse to zinc by different populations of *S. tenue* were the result of genetic adaptation by the alga. No significant correlation was found between variations in the T.I.C. among populations from one site collected on different days, and variations in the levels of zinc in the water collected on the same days.

Mean levels of laboratory tolerance in populations from sites with the higher levels of zinc varied between populations from different sites, and were clearly related to the mean levels of zinc in the field. Of the three methods of evaluating toxicity descibed above, the T.I.C. gave the highest significant positive correlation coefficient ($r = 0.91$, $P = < 0.001$) with the mean field zinc levels. When mean T.I.C. levels were plotted against the mean levels of zinc at the collection sites (Fig 7.2), however, there was some indication that the lineararity of the relationship between the two variables was subject to variation caused by other factors. A discontinuity in the otherwise linear relationship was

		Field zinc level		I	II	Just non-inhibitory		III	IV	Just lethal		T.I.C.	
Site	Pretreatment	$\bar{x}$	s.d.	$\bar{x}$	$\bar{x}$	$\bar{x}$	s.d.	$\bar{x}$	$\bar{x}$	$\bar{x}$	s.d.	$\bar{x}$	s.d.
0001-01	Fresh material	0.012	0.005	0.69	0.94	0.80	0.38	2.00	2.31	2.15	0.76	1.31	0.54
	Cultured 6 months at <0.002 mg l^{-1} Zn			0.63	0.88	0.74	0.13	2.06	2.50	2.27	0.33	1.28	0.08
	Cultured 6 months at 0.75 mg l^{-1} Zn			0.50	0.75	0.61	0.00	2.13	2.50	2.30	0.36	1.18	0.09
0096-01	Fresh material	2.390	0.513	2.00	2.38	2.18	0.41	10.75	13.50	12.14	2.24	5.10	0.54
	Cultured 6 months at <0.002 mg l^{-1} Zn			2.13	2.88	2.36	0.22	10.63	13.13	11.81	1.09	5.28	0.49
	Cultured 6 months at 5.0 mg l^{-1} Zn			2.06	2.50	2.27	0.31	11.25	13.75	12.44	1.26	5.30	0.48

Table 7.2 Results of toxicity tests performed upon representative non-adapted and adapted populations of *Stigeoclonium tenue*, after culture at various zinc levels

Fig. 7.2

Scatter diagram to show the relationship between mean values of Tolerance Index Concentration (T I.C.) for *Stigeoclonium tenue* and mean concentrations of zinc and calcium in water from the reaches of origin.

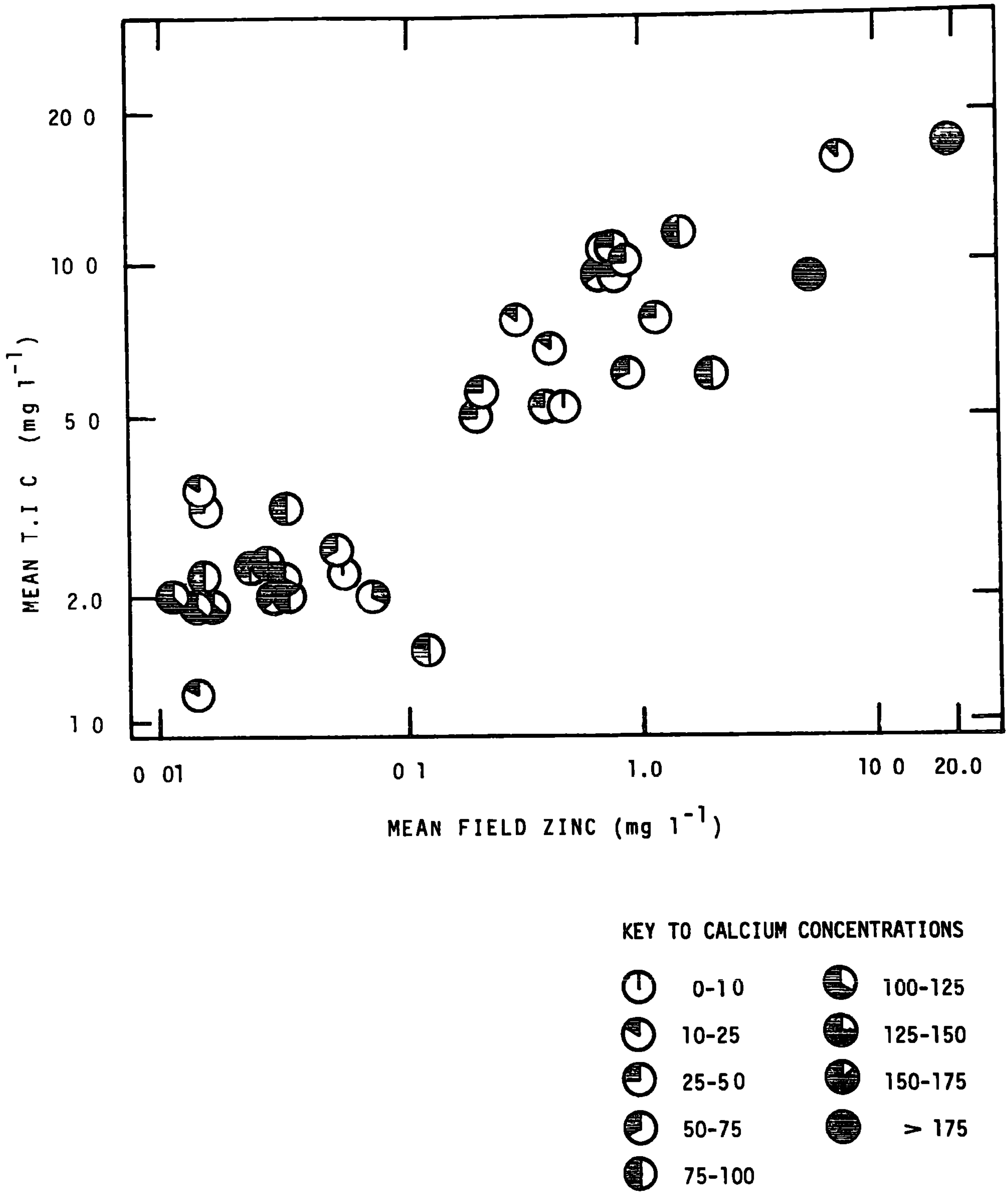

20 0
10 0
5 0
2.0
1 0
MEAN T.I C (mg l⁻¹)
0 01
0 1
1.0
10 0
20.0
MEAN FIELD ZINC (mg l⁻¹)
KEY TO CALCIUM CONCENTRATIONS
0-10
10-25
25-50
50-75
75-100
100-125
125-150
150-175
> 175

Table 7 3 Mean field zinc levels of *Stigeoclonium tenue* sampling sites, together with mean results of toxicity tests (maximum zinc levels collected during further surveys, but not included in estimate of mean levels of zinc are marked *)

Stream and reach no	field Zn $(mg\ l^{-1})$				just non-inhibitory			just lethal			T I C.		
	n	$\bar{x}$	s d	max	n	$\bar{x}$	s d	n	$\bar{x}$	s.d	n	$\bar{x}$	s d
0012-40	4	0 459	0 204	0 640	4	4 10	0 25	4	6 62	0 25	4	5 21	0 21
0096-01	4	2 390	0 513	3 080	4	2 18	0 41	4	12 14	2 24	4	5 10	0 54
0048-20	4	1 100	0 636	2 000	4	5 42	0 51	4	11 00	0 29	4	7 71	0 37
0123-80	4	0 013	0 018	0 040	4	2 11	0 25	4	5 49	0 29	4	3 40	0 26
0149-20	4	0 030	0 020	0 051	4	1 61	0 15	4	2 65	0 18	4	2 06	0 08
0005-43	• 4	0 012	0 006	0 020	4	1 39	0 20	4	2 86	0 25	4	1 99	0 22
0022-05	4	0 027	0 015	0 050	4	1 65	0 16	4	2 74	0 41	4	2 13	0 25
0014-40	4	0 014	0 011	0 030	4	2 08	0 25	4	4 62	0 25	4	3 09	0 23
0147-99	4	0 115	0 048	0 161	4	1 23	0 00	4	1 87	0 00	4	1 51	0 00
0148-01	4	0 023	0 006	0 030	4	1 94	0 00	4	2 99	0 29	4	2 40	0 12
0142-50	4	0 048	0 019	0 070	4	1 54	0 24	4	4 49	0 29	4	2 63	0 25
0143-50	4	0 025	0 030	0 071	4	1 54	0 13	4	3 87	0 25	4	2 44	0 10
0001-01	4	0 012	0 005	0 020	4	0 80	0 38	4	2 15	0 76	4	1 31	0 54
0013-11	4	0 054	0 020	0 980*	4	1 56	0 32	4	3 24	0 82	4	2 20	0 24
0023-99	4	0 014	0 007	0 020	4	1 54	0 13	4	2 99	0 29	4	2 14	0 17
0141-80	4	0 010	0 009	0 020	4	1 61	0 15	4	2 61	0 25	4	2 04	0 09
0124-99	4	0 394	0 079	0 460	4	1 84	0 29	4	24 87	2 90	4	6 74	0 77
0025-50	4	0 030	0 016	0 050	4	1 54	0 13	4	3 37	0 25	4	2 28	0 10
0146-99	4	0 015	0 011	0 030	4	1 29	0 13	4	2 75	0 25	4	1 88	0 10
0144-60	4	0 068	0 051	0 124	4	1 29	0 13	4	3 25	0 25	4	2 05	0 18
0111-01	4	5.067	0 653	5 500	4	4 97	2 49	4	17 71	7 18	4	9 36	4 19
0112-80	4	1 430	0 525	2 180	4	5 75	2 18	4	23 62	2 51	4	11 44	1 83
0048-99	4	0 904	0 490	1 400*	4	2 61	0 48	4	13.62	4 40	4	5 88	1 06
0055-31	4	0 187	0 113	0 320	4	2 05	0 21	4	11 45	2 12	4	4 81	0 30
0102-15	4	0 767	0 238	1 190*	4	3 67	0 58	4	31 15	7 53	4	10 68	2 06
0061-10	4	0 284	0 190	0 500	4	2 42	0 48	4	26 12	10 36	4	7 88	2 24
0103-08	4	0 720	0 014	1 120*	4	3 10	0 42	4	37 42	0 00	4	10 75	0 73
0081-16	4	0 215	0 105	0 290	4	1 49	0 87	4	19 56	6 94	4	5 32	2 48
0093-01	4	7 092	2 295	13 700*	4	8 66	0 00	4	31.15	4 80	4	16 39	1 25
0071-99	4	0 881	0 450	2 100*	4	5 30	0 95	4	19 84	2 91	4	10 15	0 17
4001-60	1	0 770	-	-	4	3 65	0 87	4	24 94	8 18	4	9 53	2 71
4014-01	1	20 000	-	-	4	6 63	2 00	4	48 07	5 80	4	17 81	3 74
1002-50	1	0 029	-	-	4	2 16	1 87	4	4 62	4 00	4	3 16	2 74
3006-50	1	0 710	-	-	4	3 41	0 29	4	27 00	13 93	4	9 24	2 43
0176-05	4	0 265	0 116	0 510	4	1 37	0 00	4	9 92	1 26	4	3 68	0 23

apparent at levels of zinc lower than about 0.2 mg l^{-1}, (despite a fairly large amount of variability in the mean T.I.C. values recorded from the lower levels of zinc). There was also some evidence that for a particular level of zinc in the water, the T.I.C. was lower at higher levels of calcium (Fig. 7.1)

7 6 MICROSCOPY

The populations from 0001-01 and 0096-01 that were used in the study of the stability of tolerance after long-term culture (hereafter referred to as the zinc sensitive and zinc tolerant populations) were also used for more detailed microscopic observation The behaviour of both populations in response to zinc was similar, although the absolute levels of zinc bringing about a particular response was higher in the zinc tolerant than in the zinc sensitive population. At the end of the six-day incubation period, long branched 'erect' filaments predominated in the controls. With increasing zinc levels above the just non-inhibitory level, the proportion of these long erect filaments decreased until almost all growth occurred as twisted 'basal' filaments composed of swollen cells with a few short branches. As the zinc concentrations approached the just-lethal level, growth of the original inoculum ceased, and an increasing proportion of the cells were white (dead). At the lower levels of zinc some release of zoospores occurred from the erect filaments of both populations, followed by settling and germination on the walls of the tubes The maximum levels at which settling of zoospores was observed were rather similar to the T.I.C.; a similar result was found with the one other instance (0048-99) where zoospore release occurred at all the lower concentrations of zinc during a toxicity test,

CHAPTER 8

ENVIRONMENTAL FACTORS AFFECTING THE TOXICITY OF ZINC TO *STIGEOCLONIUM TENUE*

8 1 INTRODUCTION

The data presented in 7.4 suggested that high levels of calcium and/or magnesium in stream waters reduced the toxicity of zinc to zinc tolerant populations of *Stigeoclonium tenue*. It also seemed probable from a knowledge of the literature (see 1.523) that the toxicity of zinc in the field and the laboratory might be reduced by the concentrations of other substances present (such as phosphate) In order to establish clearly what influences, if any, various factors had upon the toxicity of zinc in the laboratory, a series of experiments was performed upon cultured populations of *S. tenue*.

In order to investigate whether any observed effects differed between populations showing varying genetic tolerance to zinc, all of the experiments reported in this chapter were performed simultaneously on cultured material from a zinc sensitive population (0001-01) and a zinc tolerant one (0096-01).

8.2 pH

8.21 Effect of pH on solubility of zinc in the culture medium

The results of the experiment to investigate the extent of precipitation of various levels of zinc in the culture medium at four different pH values and three different calcium concentrations are presented in Tables 8.1a - 8.1c. All three filtration techniques employed in the experiment (see 2.323) gave similar results.

Zn:	0.25			0.5			1.0			2.5			5.0			10.0			20.0			30.0			40.0		
pH	M	N	S	M	N	S	M	N	S	M	N	S	M	N	S	M	N	S	M	N	S	M	N	S	M	N	S
6.1	93	100	100	100	96	96	100	95	100	100	93	92	98	98	85	96	96	100	98	99	83	100	100	100	100	100	100
6.6	93	95	95	100	89	89	98	100	88	100	91	91	96	96	86	97	96	98	97	97	90	94	94	94	97	99	100
7.1	100	93	100	100	60	60	80	55	55	100	85	95	81	79	53	71	72	79	82	78	55	63	71	64	46	43	42
7.6	83	83	83	96	91	85	100	41	82	88	89	88	58	42	72	89	89	86	76	74	37	73	70	72	49	51	51

Key to filters M - 0.45 µm Millipore membrane filter, N - 0.2 µm Nuclepore membrane filter; S - no. 2 Sinta funnel.

Table 8.1a Effect of pH on filtrability of zinc in modified Chu-10 medium containing 1.0 mg l^{-1} Ca

(Data expressed as percentage passing through filter)

Zn	0 25			0.5			1.0			2.5			5.0			10.0			20.0			30.0			40.0		
pH	M	N	S	M	N	S	M	N	S	M	N	S	M	N	S	M	N	S	M	N	S	M	N	S	M	N	S
6.1	100	100	100	100	100	80	89	100	100	96	96	93	98	98	100	100	100	100	100	100	100	100	100	87	98	98	97
6.6	100	89	89	100	94	100	100	100	98	88	88	96	96	96	78	96	96	94	99	99	100	98	92	94	99	99	100
7.1	100	100	100	74	74	74	90	80	100	71	60	40	58	58	54	53	51	45	65	63	69	70	63	63	60	63	60
7.6	85	85	100	81	81	75	69	77	100	74	78	67	65	60	44	32	34	38	33	30	30	18	27	19	35	33	32

Key to filters : M - 0.45 µm Millipore membrane filter; N - 0.2 µm Nuclepore membrane filter,
S - no. 2 Sinta Funnel

Table 8.1b Effect of pH on filtrability of zinc in modified Chu-10 medium containing 10 mg l^{-1} Ca

(Data expressed as percentage passing through filter)

Zn	0.25			0.5			1.0			2.5			5.0			10.0			20.0			30.0			40.0		
pH	M	N	S	M	N	S	M	N	S	M	N	S	M	N	S	M	N	S	M	N	S	M	N	S	M	N	S
6.1	100	100	100	100	100	100	100	98	100	100	100	100	100	100	100	100	100	98	94	100	96	100	100	100	97	99	95
6.6	100	100	100	100	100	100	100	100	100	89	89	96	90	90	61	97	95	82	94	100	90	100	97	88	100	95	92
7.1	83	83	83	92	92	88	92	85	77	100	100	80	100	94	70	87	87	72	71	71	70	86	84	83	65	66	54
7.6	100	100	100	60	60	50	77	77	62	67	67	88	61	61	43	38	35	35	42	42	45	47	47	36	53	53	52

Key to filters **M - 0.45 µm Millipore membrane filter, N - 0.2 µm Nuclepore membrane filter;**
S - no. 2 Sinta funnel

Table 8.1c **Effect of pH on filtrability of zinc in modified Chu-10 medium containing 50 mg l^{-1} Ca**

(Data expressed as percentage passing through filter)

Using the results of these tests, the estimates of Tolerance Index Concentration reported in 8.22 were made using both the total levels of zinc in the culture medium and the levels capable of passing through filters.

8.22 Effect of pH on toxicity of zinc

The influence of pH on the toxicity of zinc in the presence of two different concentrations of calcium is shown in Table 8.2. It can be seen that the only detectable influence of pH on the zinc sensitive population was a slight decrease in the toxicity of zinc between pH 7 1 and pH 7 6. In contrast there was an obvious influence of pH in decreasing zinc toxicity to the zinc resistant population as indicated by the values of T.I.C. based upon total zinc This was apparent even if the values of T.I.C. were calculated using levels of zinc capable of passing through a filter, rather than total zinc, when the tests were carried out in the presence of 1 mg l^{-1} Ca, but not 10 mg l^{-1} Ca.

8.3 SULPHATE-S

No detectable change in the toxicity of zinc to either the zinc sensitive or the zinc tolerant population was found when the SO_4-S concentration in the growth medium was increased from 0 mg l^{-1} to 200 mg l^{-1}.

8.4 NaCl

No detectable effect upon the toxicity of zinc to either population was found when the Cl concentration (as NaCl) was increased from 0 mg l^{-1} to 200 mg l^{-1}

8.5 MAGNESIUM

The influence of increasing magnesium ion concentrations in the medium (from 0 - 200 mg l^{-1}) on the toxicity of zinc to the two populations is shown in Fig 8.1. It can be

		Zinc sensitive		Zinc tolerant	
Ca	pH	T.I.C. 'total'	T.I.C. filtrable	T.I.C. 'total'	T.I.C. filtrable
1	6.1	0.63	0.63	3.39	3.39
	6.6	0.63	0.63	3.39	3.39
	7.1	0.63	0.63	8.80	6.78
	7.6	0.73	0.73	11.00	7.16
10	6.1	0.92	0.92	7.83	7.83
	6.6	0.92	0.92	7.83	7.50
	7.1	0.92	0.92	12.40	7.50
	7.6	1.00	1.00	14.10	7.60

Table 8.2 Effect of pH on toxicity of zinc to a zinc sensitive and a zinc tolerant population of *Stigeoclonium tenue*.

Tolerance Index Concentration (T.I.C.) is estimated both for the 'total' zinc and the level of zinc after passing through a filter. (All concentrations in mg l^{-1})

Fig. 8.1

Influence of magnesium on the toxicity of zinc to a zinc sensitive and a zinc tolerant population of *Stigeoclonium tenue.*

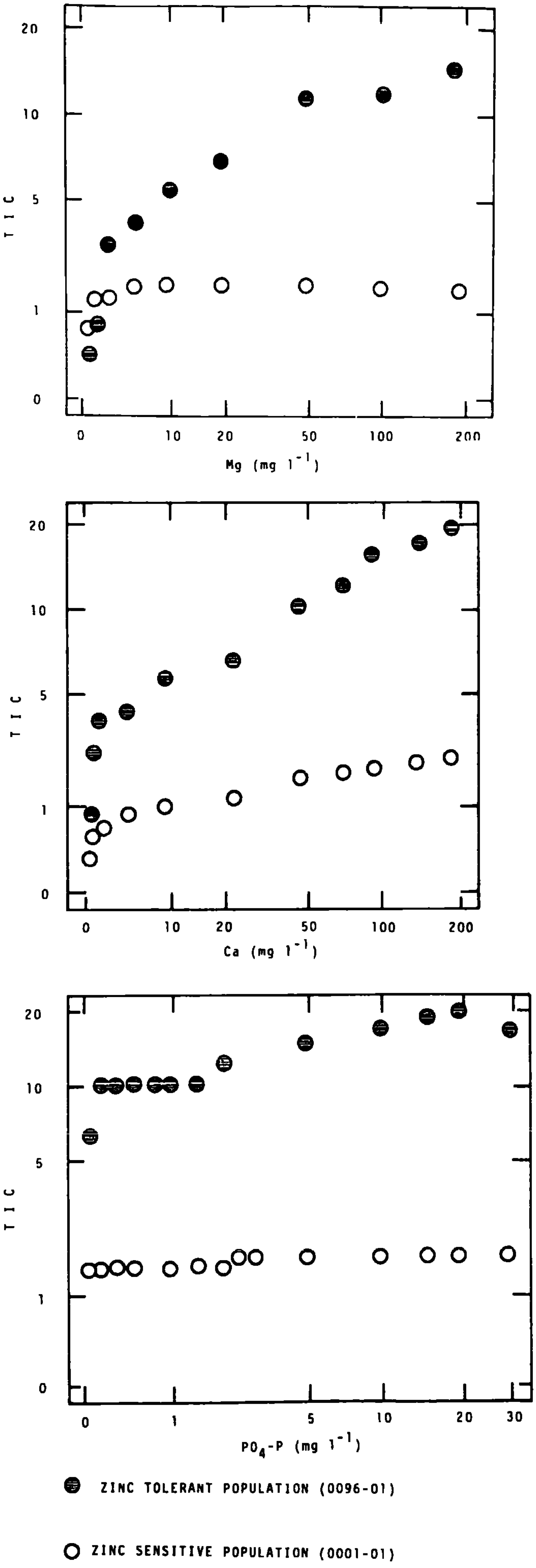
Fig. 8 2

Influence of calcium on the toxicity of zinc to a zinc sensitive and a zinc tolerant population of *Stigeoclonium tenue.*

Fig. 8.3

Influence of phosphate on the toxicity of zinc to a zinc sensitive and a zinc tolerant population of *Stigeoclonium tenue.*

(T.I.C. = Tolerance Index Concentration in mg l^{-1})


20
10
5
TIC
1
0
0
10
20
50
100
200
Mg (mg l⁻¹)
20
10
5
TIC
1
0
0
10
20
50
100
200
Ca (mg l⁻¹)
20
10
5
TIC
1
0
0
1
5
10
20
30
PO₄-P (mg l⁻¹)
● ZINC TOLERANT POPULATION (0096-01)
○ ZINC SENSITIVE POPULATION (0001-01)

seen that, as was found with pH, the responses of the two populations differed. In the case of the zinc sensitive population, a slight increase in tolerance of the alga was found as the magnesium concentration was increased from 0 - 1 mg l^{-1}, but little change took place in concentrations of magnesium higher than this. With the zinc tolerant population, the alga was actually slightly more sensitive to zinc than the zinc sensitive one in magnesium-free medium (in which a marked inhibition of growth was found in both populations). At concentrations of zinc from 1 - 200 mg l^{-1}, however, the tolerance of the population to zinc increased, with some indication of a levelling-off in response above about 50 mg l^{-1}. With both populations, growth of the alga was slightly inhibited in the magnesium-free control as compared with the other controls, and increasing inhibition of growth was observed in the controls at magnesium concentrations above 50 mg l^{-1}.

8.6 CALCIUM

The influence of increasing calcium ion concentrations (from 0 - 200 mg l^{-1}) on the tolerance to zinc of the two populations is shown in Fig. 8.2. The response of both populations was very similar to that found with magnesium, except that the toxicity of zinc to the zinc sensitive population decreased slightly throughout the range of calcium concentrations. Further, there was no evidence for a levelling-off of response in the case of the zinc tolerant population. No effect upon growth of differing calcium concentrations was evident in the zinc-free controls in the case of the zinc sensitive population, but a slight inhibition of growth was observed with the zinc tolerant population in calcium-free medium. Zoospores were released from erect filaments in several of the lower levels of zinc in the experiment on the zinc sensitive population The maximum concentrations of zinc at which these were observed to settle and germinate on the walls of the tubes were again rather similar to the T.I.C. values recorded, and showed the same pattern of slight increase with increasing calcium concentrations (Table 8.3).

Ca	Max. Zn permitting settling	T.I.C.
0	0.5	0.31
1	0 75	0.63
2	0.75	0.70
5	1.0	0.92
10	1.25	1.0
25	1.25	1.17
50	1.25	1.54
75	1.25	1.64
100	1.5	1.71
150	1 5	1.94
200	1.5	2.01

(All concentrations in mg l^{-1})

Table 8.3 Comparison for a zinc sensitive population of *Stigeoclonium tenue* of the effect of calcium on the T.I.C. with that on the maximum level of zinc permitting zoospores to settle

8.7 PHOSPHATE-P

The effect of phosphate on the toxicity of zinc again differed for the two populations (Fig. 8.3). Increasing PO_4-P concentrations had little, if any, effect on the toxicity of zinc to the zinc sensitive population. At concentrations of less than 1.5 mg l^{-1}. different levels of phosphate had little effect upon the toxicity of zinc to the zinc tolerant population, but at higher levels than this some decrease in toxicity was observed. A slight increase in toxicity took place with this population at the highest level of PO_4-P tested (30 mg l^{-1})

8.8 MICROSCOPY

In connection with investigations of the effects of magnesium, calcium and phosphate, microscopic inspection of the inoculum was carried out at the end of the incubation period. At levels of zinc near the T.I.C. the response of the alga was at all times similar, with a moderate reduction in the erect as opposed to the basal parts of the thallus. With increasing zinc concentrations, basal growth increasingly predominated over erect under all test conditions. The only other marked morphological response was the production of numerous long hairs in the tubes of medium deficient in phosphate, whether or not zinc was present in the medium. Although phosphate reduced the toxicity of zinc to the zinc tolerant population, an increased level of zinc in the medium did not permit the development of hairs at a higher level of phosphate. This behaviour contrasts with the antagonistic influence of calcium on the inhibitory effects of zinc on zoospore release and settling, in the one test where zoospores were formed throughout all the lower levels of zinc (Table 8.3).

CHAPTER 9

DISCUSSION

9.1 INTRODUCTION

In 1.1 and 1.7, it was suggested that a relatively broad study of some of the effects of pollution by heavy metals in a freshwater system would be of value. The exploratory surveys described in chapter 3 established that the catchment area of the Derwent Reservoir provided a suitable area for such a study, and the results of subsequent investigations centered on the catchment have been reported in chapters 4 - 8.

The results of the three year long study demonstrated that elevated concentrations of zinc, lead and cadmium were present within the water, sediments and submerged plants of the Derwent Reservoir and its catchment. The sources, extent and possible biological effects of this pollution are evaluated in 9.2.

The studies carried out within the catchment area included investigations of the accumulation of heavy metals by selected species of submerged plants (4.5,5.4). These studies provided valuable additional data on the distribution of metals and their availability to the biota, and are discussed in 9.23. In particular, *Lemanea fluviatilis* was found to be a species that was useful for 'monitoring' the distribution of different concentrations of zinc, lead and cadmium within the R. Derwent. Studies of the accumulation of heavy metals by this alga were therefore extended to include sampling from other rivers, transplant experiments and some laboratory investigations. The results of these studies are evaluated in 9.3.

During the exploratory surveys of the algal vegetation in the catchment area of the Derwent Reservoir, it was noted that *Stigeoclonium tenue* was present in reaches with a wide variety of concentrations of zinc in the water. A programme of field and laboratory investigations was therefore undertaken to study different aspects of the tolerance of *S. tenue* to zinc. The results demonstrated that populations of the alga growing in higher concentrations of zinc in the field were adapted forms, showing an enhanced tolerance to zinc that probably had a genetic basis. These studies, together with laboratory investigations of environmental factors that might reduce the toxicity of zinc in the field, are discussed in 9 4

9.2 CONTAMINATION OF THE DERWENT RESERVOIR AND ITS CATCHMENT BY HEAVY METALS

9.21 Composition of water

9.211 Introductory remarks

It was pointed out in 1.1 and 1.7 that few published studies have attempted to describe more than a few aspects of the behaviour of different fractions of heavy metals within a freshwater system. The studies reported in chapters 4 and 5 were carried out in an attempt to investigate in detail the distribution of zinc, lead and cadmium within the water , sediments and submerged plants of the Derwent Reservoir and its catchment. It was hoped that these studies would reveal the sources and extent of contamination by these metals, and clarify some of the factors causing spatial and temporal variations in their concentrations in different fractions.

In the following sections, concentrations of heavy metals in samples collected from the catchment are compared with published figures for other areas. Discussion is also included of the results of the programme of intensive monitoring of discharge and the composition of water at the 'key' reaches.

9.212 Catchment

It became clear from the results of the exploratory surveys (3 5) that elevated concentrations of zinc, lead and cadmium were being carried into the Derwent Reservoir by the R. Derwent. These metals were derived from two tributaries to the river, Bolts Burn and Shildon Burn, with the former stream by far the more important. The exploratory surveys showed that the heavy metal content of Bolts Burn and the R. Derwent were subject to marked fluctuation, and that an active fluorspar mine was probably the major source of the metals.

It was pointed out in 1.31 that several papers have reported large variations in concentrations when a series of samples of water from a particular site were analysed. However, the various 'natural' (hydrogeochemical) and 'artificial' factors which might influence such variation have received little study. It became clear from the results presented in 3.5 that the catchment area of the R. Derwent provided a suitable situation for such a study. Within a single small area were reaches that were almost completely unaffected by human influence, together with reaches showing a marked degree of contamination by heavy metals from well defined 'artificial' sources. In light of this, an intensive programme of sampling and analysis of water was carried out, the results of which have been reported in 4.3. The following discussion is presented in the same order as the results in that section.

Bolts Burn

Bolts Burn was shown to be contaminated extensively by zinc, lead and cadmium between the Whiteheaps Fluorspar Mine and the entry of the stream to the R. Derwent. Examination of the data presented in 4.3 allows the sources of these three metals to the stream to be distinguished clearly.

Disused mine workings bordering Bolts Burn upstream of the Whiteheaps Mine were found to have little effect on the heavy metal content of the stream water. Thus only very low 'total' concentrations of zinc, lead and cadmium were present in the stream even at high discharge or when the water table was high. Similarly, no inputs of heavy metals from old mine workings downstream of the Whiteheaps Mine were found to cause a detectable increase in the concentration of zinc, lead or cadmium in Bolts Burn. Attention was therefore focused upon the contribution of heavy metals from sources within the mine itself.

Most of the zinc, lead and cadmium carried by the stream were found to be derived in 'non-particulate' form (i.e. capable of passing through 0.2 µm Nuclepore filters or no 2 Sinta funnels) from the Whiteheaps Adit Level Although this adit discharged water that had been pumped from workings that were being exploited actively for fluorspar, the composition of the water remained relatively stable throughout the project This composition was rather similar to that of other old adits draining workings on the Northern Pennine Orefield (Table 9.1). It seems probable that the zinc and cadmium content of the adit water were the result of the passage of water over underground bodies of sphalerite (ZnS); a mineral contaminated frequently by cadmium (as CdS). The high calcium content of the water provides evidence for the contribution of deep subterranean sources of water, as substantial beds of limestone (such as the Great Limestone) are present only at great depths below the valley of Bolts Burn (Fig. 3.5).

In spite of the high zinc and cadmium content of the water, the Whiteheaps Adit Level was found to carry relatively low concentrations of lead into Bolts Burn. In contrast, water discharged from the Whiteheaps Mine Effluent was found to carry high concentrations of lead on most occasions, together with relatively low concentrations of zinc and cadmium. Other marked differences

Adit	n	Na	K	Mg	Ca	Zn	Cu	Mn	Fe	Al	Pb	Cd
Whiteheaps Adit Level	16	48.7	12.04	10.72	71.8	6.617	0.011	0.913	0.30	0.29	0.070	0 0163
Rampgill Level	4	8.2	4.4	24.2	78.3	2.67	0.002	0.141	0.13	0.03	0.027	0.005
'Scale Burn Level'	4	14.6	3.5	12.8	58.4	3.76	0.005	0.213	0.31	0.05	0.106	0 005
Dowgang Level	4	9.2	6.5	29.4	104.6	6.70	0.003	0.192	0.03	0.03	0.019	0.005
Tailrace Level	4	10.0	7.0	18.4	95.2	8.20	0.098	4.59	0 30	0.28	0.100	0.017

Table 9.1 Comparison of the composition of water discharged by the Whiteheaps Adit Level with that of water discharged by four other adits on the Northern Pennine Orefield

Data taken from Say (1977)

All concentrations are in mg l^{-1}

between the composition of the water of the adit and the effluent include the high levels of suspended solids carried by the effluent, and its extremely variable nature. The results summarized in Tables 4.2 and 4.3 demonstrate that most of the lead derived from the effluent was in particulate ('non-filtrable') form. Whilst no investigations were carried out of the composition of particles filtered from the effluent, it seems probable that a high proportion of the lead was present as particles of galena (PbS) that had escaped the settlement process. The fluorspar mined at Whiteheaps is associated intimately with galena in the Red/White/Company's Vein complex (3.35), and galena is still a valuable by-product of current mining operations (Fig. 3.6). Further indirect evidence for the presence of galena in the effluent discharge lies in its relatively high silver content (Table 4.3). Silver was a valuable component of the galena extracted from veins around Bolts Burn by the old mining concerns; indeed some of the veins were once famous for their silver content (3.35).

From the results discussed above, it is interesting to note that high concentrations of zinc and lead were found *not* to 'co-occur' in the water of either the adit or the effluent. Sphalerite is known to occur separately from galena in veins in the area (Dunham, 1948) In contrast, cadmium showed a clear tendency to be associated with zinc in the water of the adit The former metal has been described as "the unwanted step-child of zinc" (Valdez, 1975) because of its association with sphalerite.

The results of analysis of water from various points within the Whiteheaps Mine demonstrated that surface runoff during wet weather was a further important source of heavy metals to Bolts Burn. However, this source was much more difficult to quantify. Sikehead Stream, which carried a certain amount of zinc in the water, could be classified as 'surface runoff'. However, the main effect of surface was to carry particulate material from tips into Bolts Burn. This material was rather similar in composition to that carried by the Whiteheaps Effluent, and could at times be a more important source of lead to the stream.

The three sources outlined above were all subject to marked short term fluctuations in volume which were reflected in the composition of water in Bolts Burn. The Whiteheaps Adit Level was operated by an automatic pump, depending on the level of water below ground. The flow of the effluent depended upon activity within the fluorspar treatment plants, and the contribution of surface runoff depended upon the weather. In addition, the 'quality' of the effluent deteriorated markedly during cold, wet weather. This appeared to be due mostly to a decrease in the efficiency of the iron flocculation process, together with turbulence or freezing within the settling ponds.

These variations were found to be reflected clearly in the composition of the water of Bolts Burn entering the R. Derwent (reach 99). This was especially well illustrated by the results of the 24 hour survey (Fig. 4 10) and by the results of intervariable correlation analyses (Table 4.4). The 'total' zinc and cadmium concentrations in the water were found to be subject to marked short term 'co-ordinated' fluctuations due to the operation of the adit. In contrast, the 'total' lead concentration showed equally marked short term fluctuations that were independant of those of zinc or cadmium. Higher concentrations of lead were shown to be associated with particulate material derived from the effluent or surface runoff.

The general pattern that emerges from the results of the surveys of the composition of water in Bolts Burn is one of a stream affected to an overwhemimg extent by pollution with metals from 'artificial' discharges Some seasonal variations in concentration were noted for lead, these were related mostly to the effects of surface runoff within the mine and the quality of the mine effluent. No marked seasonal variations were noted for zinc or cadmium.

Discharge was the only other 'natural' parameter included in the intensive study of Bolts Burn at reach 99 which might have been expected to influence the concentrations of heavy metals in the water. Wilson (1976) has pointed out the marked lack of published studies on the effects of discharge on the concentrations of heavy metals in river waters, and cited work by Hellmann (1970) to demonstrate that such effects may be complicated. For any one element, discharge may affect the concentration in running water by:

i. increasing the absolute level dissolved from the substratum,

ii. increasing the load of suspended material, including particulate or adsorbed metals;

iii. diluting out metals derived from sources not affected by drainage.

In most cases metals derived as a result of (i) will be diluted to a variable extent by increased discharge in a similar manner to (iii).

Table 4.4 shows that for no element was a significant positive correlation found between 'total' concentration and discharge in the water of Bolts Burn reach 99. Thus there was no evidence that the concentration of any element associated with particulate material increased with discharge. This is perhaps a little suprising, since it was known that surface runoff carried large amounts of particulate iron, aluminium and lead into the stream from tips within the Whiteheaps Mine. It seems probable that such effects were complicated to such an extent by the effects of discharge from the effluent that the effects of runoff were 'swamped'. Further, the dilution of the relatively constant flow of the effluent by higher discharges might be expected to counteract the effects of inputs of particulate material from runoff.

In contrast, the 'total' concentrations of five elements (Na, K, Mg, Ca and Zn) were found to show significant *negative* correlations with discharge in the water of reach 99. All of these elements have been shown to be associated with inputs from the adit (4.311). The adit had a relatively constant flow when the pump was operating, and the correlations were probably simply the result of the dilution at higher discharges.

R. Derwent

From the results reported in 4.322, it can be seen that very low 'total' concentrations of zinc, lead and cadmium were detected on every occasion when water was sampled from the R. Derwent upstream of the entry of Bolts Burn (reach 05). In contrast, concentrations of the three metals were always much higher in the water of the river downstream of the entry of the tributary (reach 07).

With the exception of possible discharges from a few farms, no inputs from human activities were known to affect the upper reaches of the R. Derwent. Some old mine workings exist around Nookton and Beldon Burns, but the results of exploratory surveys and of the intensive sampling of water from reach 05 demonstrated that these never had a detectable effect on the composition of water in the reach. The R. Derwent upstream of the entry of Bolts Burn therefore provided an example of a reach where 'natural' factors might be expected to be almost the only ones affecting the composition of the water. In light of this, it is interesting to note that the pattern of correlations observed between discharge and the concentrations of cations in the water of the reach (Table 4.5) was completely different to that observed in the water of Bolts Burn reach 99. These differences have already been described in 4.322.

Hughes & Edwards (1977) investigated the relationships between river flow and concentrations of sodium, potassium, magnesium and calcium in the water of the R. Cynon, South Wales All of these elments were found to show negative correlations with flow in the lower reaches of the river, but this was true only for sodium and magnesium in the upper reaches The authors stressed that the results were difficult to interpret because of the complex geology of the catchment and the pumping of water from marine coal measures. However, the results are somewhat similar to those of the present study, where concentrations of sodium, potassium magnesium and calcium all showed some degree of negative correlation with discharge in the R. Derwent reach 05. Hughes & Edwards compared their results with other data published by Edwards (1973) and Likens *et al.* (1967). Despite obvious differences related to geology etc., the results were similar in that concentrations of magnesium showed the most marked inverse relationship with discharge, whilst calcium and discharge were frequently uncorrelated. During the present study concentrations of both magnesium and calcium showed highly significant negative correlations with discharge in reach 05 on the R. Derwent. This may be a result of the small amount of limestone exposed in the catchment, or of the input of magnesium and calcium from relatively constant underground sources draining subterranean limestone strata.

In 4.322, it was suggested that the significant positive correlations between discharge and 'total' concentrations of manganese, iron and aluminium in the water of reach 05 were the result of increased suspended load during higher discharges. If this was the case, then the data collected from the reach provided an opportunity to study the efficiency of 0.2 μm Nuclepore filters in removing suspended material from the river water. Kennedy, Zellweger & Jones (1974) compared the intercorrelations of Al, Fe, Mn and Ti in stream water filtered through membrane filters of 0.1 μm and 0.45 μm porosity These intercorrelations were found to be less strong following passage through the former filters, suggesting that the 0 1 μm porosity filters were removing a greater proportion of suspended material than the 0.45 μm porosity filters.

An attempt was made to use a similar comparison of correlation coefficients to evaluate the effectiveness of the filters used in the present study in removing elements associated with suspended material from the water of reach 05 on the R. Derwent. The intercorrelations between discharge and concentrations of manganese, iron and aluminium were compared for 'total' and filtered samples of water. The results gave no indication that the relationships between the four elements were affected markedly by filtering. However, the 0.2 μm filters appeared to be generally more effective than no. 2 Sinta funnels in removing Al, Fe and Mn from the water in the reach (Table 4.2).

In the water of the R. Derwent downstream of the entry of Bolts Burn, a completely different pattern of correlations was found between discharge and the concentrations of different elements (4.323). Several marked similarities were observed to the pattern found for Bolts Burn reach 99. I or example concentrations of Na, K, Mg and Ca were negatively correlated with discharge. However, some elements associated with suspended material (e.g. iron, aluminium) were positively correlated with discharge in a similar manner to the R. Derwent at reach 05. Variations in the composition of water in the river downstream of Bolts Burn therefore showed some characteristics that were common to both Bolts Burn itself and the river upstream of its entry.

It is perhaps unforunate tnat no experiments were carried out in the field to estimate the 'time of travel' of heavy metals in the R. Derwent between the entry of Bolts Burn and its entry to the Derwent Reservoir. The use of the reservoir for supply and recreational purposes would probably preclude the addition of substances such as fluorcein to the river, although alternatives such as LiCl or NaCl might have been practicable. Whatever the residence time of elevated concentrations of metals within the R. Derwent, it seems clear from 4.323 that some association of zinc and lead with some component of sediment took place in the river between Bolts Burn and the reservoir. The main evidence for this conclusion lies in the high significant positive correlations between discharge and 'total' concentrations of zinc and lead in the water of the R. Derwent at reach 25. It is interesting to note,however, that most of the zinc ($\bar{x}$ = 95%) was still capable of passing through 0.2 µm Nuclepore filters. No obvious explanation is apparent for this apparent contrast in the behaviour of zinc. It was suggested tentatively in 4 324 that zinc possibly complexed by organic material, might be bound loosely by sediment and removed by subsequent higher flows. If such a sequence of events did take place, some release of loosely bound zinc from sediments within the reservoir might be expected to take place during periods of turbulence. (see also 9.213)

A further interesting feature of the pattern of correlations presented in Table 4.7 is that, unlike zinc and lead, concentrations of cadmium in the water of the river at reach 25 were not correlated significantly with discharge. Thus by the time the river entered the reservoir, the behaviour of cadmium appeared to have 'dissassociated' itself from that of zinc. This might have been due to differences between the association of zinc and cadmium with solid phases within the river. Hem (1972) suggested that cadmium may be stable (and difficult to remove) at concentrations greater than 10 $\mu g\ l^{-1}$ in natural waters. However Gardiner (1974) observed that humic acid in sediments played a significant role in binding cadmium from river waters.

From the results in 4.325, it can be seen that only a very small proportion of the load of heavy metals carried by the R. Derwent at reach 25 penetrated through the reservoir to the compensation water outlet. It is clear, therefore, that the reservoir was acting as a 'sink' for metals in the sense of several authors (e.g. Pita & Hyne, 1975; Gale, Bolter & Wixson, 1976). This aspect is discussed further in 9.222.

Comparison with other studies of rivers and streams contaminated by heavy metals

The preceeding sections have made use of rather vague terms such as 'low' or 'high' concentrations. In 1.3 an attempt was made to establish concentrations of heavy metals in water that could reliably be termed 'background' or 'pollutant'. The following section presents a brief comparison of the concentrations of zinc, lead and cadmium detected in the water of the R. Derwent basin with other published data.

It was reported in 1.3 that several workers had attempted to establish 'background' concentrations of heavy metals that might be expected in freshwaters in the absence of pollution. Despite the difficulties encounted in making such generalizations, the published data indicate that a typical 'clean' stream might be expected to carry about 0.010 mg l^{-1} Zn, 0.001 mg l^{-1} Pb and 0.0001 mg l^{-1} Cd in the water at low flow. Based on these estimates, the concentrations of the three metals in the water of Bolts Burn upstream of the Whiteheaps Mine and the R. Derwent upstream of Bolts Burn (about 0.025 mg l^{-1} Zn, 0.010 mg l^{-1} Pb and 0.0005 mg l^{-1} Cd) seem slightly high, but certainly not remarkable. It must be noted that these figures are based on mean 'total' concentrations that include collections made at high discharge, and are therefore almost certainly overestimates for non-particulate metals The mean concentrations of zinc and lead in the water of both reaches were lower after passing through 0.2 µm Nuclepore filters (Table 4 2).

By comparison with these lower concentrations, it is clear that both Bolts Burn downstream of the Whiteheaps Mine and the R. Derwent downstream of Bolts Burn were contaminated to a high degree by zinc, lead and cadmium. Concentrations of the metals in the water of Bolts Burn at reach 99 were usually around 1.5 mg l^{-1} Zn, 0.3 mg l^{-1} Pb and 0.006 mg l^{-1} Cd. Water in the R. Derwent downstream of the entry of Bolts Burn usually carried around 0.4 mg l^{-1} Zn, 0.06 mg l^{-1} Pb and 0.002 mg l^{-1} Cd. The highest concentrations of heavy metals detected within the catchment area of the R. Derwent were found in the discharges from the Whiteheaps Mine. Thus the water of the Whiteheaps Adit Level usually carried 'total' concentrations of about 6.5 mg l^{-1} Zn and 0.015 mg l^{-1} Cd, whilst the discharge of the mine effluent could contain more than 2.0 mg l^{-1} Pb (mostly in particulate form)

The representative concentrations described above are compared with some of the higher concentrations of metals reported by other workers in Table 9.2. From results such as the ones tabulated, it seems reasonable to class Bolts Burn as a stream that is polluted markedly by high concentrations of zinc, lead and cadmium in the

Table 9.2

Comparison of concentrations of zinc, lead and cadmium found in water in some reaches during the present study with some concentrations reported by other workers

All concentrations are in mg l^{-1}

Author(s)	Year	Site	treatment	Zn	Pb	Cd
Alloway	1967	R Ystwyth above Cwm Ystwyth Mine	unfiltered	n d	n d	-
		R Ystwyth below Cwm Ystwyth Mine	unfiltered	1 35	-	-
		R Ystwyth at Llanfarian	unfiltered	0 43	-	-
Jones	1958	R Ystwyth at Llanfarian	unfiltered	0 6	-	-
McLean & Jones	1975	R Ystwyth above Cwm Ystwyth Mine	unfiltered	n d	n d	0 024
		R Ystwyth below Cwm Ystwyth Mine	unfiltered	0 88	0 032	0 074
		R Ystwyth at Llanfarian	unfiltered	0 59	n d	n d
Present study (see 6 2)	1977	R Ystwyth above Cwm Ystwyth Mine	'total'	0 049	0 003	0 0003
		R Ystwyth below Cwm Ystwyth Mine	'total'	0 540	0 116	0 0016
		R Ystwyth near Lanfarian	'total'	0 440	0 049	0 0015
Say	1977	'Old Mine Gill'	no 2 Sinta	22 3	1 60	0 058
		Gillgill Burn reach 01	no 2 Sinta	6 70	0 561	0 042
		Gillgill Burn reach 95	no 2 Sinta	1 29	0 071	0 006
Present study (see 4 3)	1977	Bolts Burn reach 20	'total'	$\bar{x}$ = 0 021	0 015	0 0006
		Bolts Burn reach 40	'total'	$\bar{x}$ = 2 679	1 253	0 0084
		Bolts Burn reach 99	'total'	$\bar{x}$ = 1 453	0 276	0 0055
Say	1977	R Nent near source	no 2 Sinta	0 08	0 012	-
		R Nent downstream of adits	no 2 Sinta	1 07	0 035	0 004
		R Nent at Nenthead	no 2 Sinta	2 70	0 038	0 009
		R Nent at Alston	no 2 Sinta	1 22	0 021	0 004
Present study (see 6 2, 7 5)	1977	R Nent downstream of Nenthead	'total'	1 100	0 046	0 0056
		R Nent at Foreshield	'total'	1 160	0 045	0 0039
		R Nent at Alston	'total'	0 904	0 015	0 0040
Present study	1977	R South Tyne upstream of R Nent	'total'	0 031	0 006	<0 0001
		R South Tyne downstream of R Nent	'total'	0 187	0 021	0 0015
Present study		R Derwent upstream of Bolts Burn	'total'	0 026	0 007	0 0005
		R Derwent downstream of Bolts Burn	'total'	0 317	0 051	0 0018
		R Derwent upstream of Derwent Reservoir	'total'	0 217	0 061	0 0018
		R Derwent downstream of Derwent Reservoir	'total'	0 064	0 007	<0 0001
Abdullah & Royle	1972	R Twymyn (Wales)	unfiltered	0 3 - 0 6	-	-
Abdullah & Royle		R Rheidol (Wales)	unfiltered	0 050-0 130	0 001-0 006	0 001
Abdullah & Royle		R Ystwyth (Wales)	unfiltered	0 250-0 270	0 001-0 002	0 001-0 003
Angino Galle & Waugh	1969	Streams in lower Kansas River Basin	1 2 µm filters	0 6, $\bar{x}$ =0 06	-	-
Beamish *et al*	1969	63 lakes in Whitefish Indian Reserve	0 45 µm filters	$\bar{x}$ = 0 001	$\bar{x}$= 0 001	$\bar{x}$ = 0 001
Donovan Feeley & Canavan	1969	River downstream of leadmine (Ireland)	unfiltered	-	4 20	-
Djuric *et al*	1971	Meza River, Yugoslavia upstream of mine	unfiltered	-	0 001-0 020	-
		Meza River, Yugoslavia downstream of mine	unfiltered	-	685 0	- -
Gale *et al*	1973	8 stations on streams in 'New Lead Belt'	unfiltered	<0 010-0 280	0 002-0 830	<0 010
Hem	1972	Surface waters and groundwaters in U S A	0 45µm filter	<0 010-1 200	-	<0 001-0 090
Huff	1976	Streams near Industrial complex	unfiltered	<0 1	-	-
Jennett, Callier & Foil	1976	Crooked Creek, U S A	unfiltered	1 62-4 90 $\bar{x}$ = 3 28	0 06-1 16 $\bar{x}$=1 10	0 12-1 57 $\bar{x}$=1 45
			0 45µm filters	1 60-6 80 $\bar{x}$ = 5 20	0 04-0 60 $\bar{x}$-0 60	0 12-1 54 $\bar{x}$=1 42
Perhac	1974	Tennessee River	unfiltered	max 0 226	max 0 01	max 0 002
Romano	1976	Grand Calumet River U S A	unfiltered	0 055-0 080	0 014-0 029	0 0008-0 0013
Valdez	1975	R Mersey, Warrington	?	-	-	0 003-0 190 $\bar{x}$ = 0 020
		R Cole, Coleshill	?	-	-	n d -0 230 $\bar{x}$ =0 030
		Nant-y-Fendrod Swansea	?	-	-	0 050-1 290 $\bar{x}$ = 0 240
Windom, Beck & Tarzwell	1971	3 rivers in south east U S A	0 45µm filters	0 70-0 54	-	-
Jones	1940	R Ystwyth above Cwm Ystwyth Mine	unfiltered	n d	n d	-
		R Ystwyth below Cwm Ystwyth Mine	unfiltered	0 9	-	-
		R Ystwyth at Llanfarian	unfiltered	0 8	trace	-

water. Similarly, the R. Derwent could be termed moderately polluted downstream of the entry of Bolts Burn.

As was stated in 1.3 Say (1977) has documented the occurrence of flowing waters contaminated by zinc in the Northern Pennine Orefield. He noted that Bolts Burn was the only stream in the catchment of the R. Derwent with a concentration of more than 1.0 mg l^{-1} Zn in the water, whilst three reaches in the area carried from 0.1 - 1.0 mg l^{-1} Zn. This compared with 15 streams in the former category, and 29 streams in the latter category, on the Alston Moor region of the Northern Pennine Orefield. Say concluded that the relative paucity of streams contaminated by zinc in the catchment area of the R. Derwent was a result of the position of the area at the outer limits of the main lead and zinc bearing strata.

When data for adit waters and acidic drainage are excluded, it appears that West Wales is the area where contamination of streams by heavy metals has been documented most extensively. It was noted in 1.3 that the rivers Ystwyth and Rheidol have a long history of contamination by mining, and data from the two rivers are included in Table 9.2. As well as carrying higher concentrations of zinc in the water than were found in the R. Derwent, it was clear from the visit made to the area that the discharge of the two rivers, and hence their absolute load of metals, was greater

From the available published data, it appears that the R. Nent downstream of Nenthead carries absolute levels of zinc that are comparable to those carried by the rivers Ystwyth and Rheidol. Data from this river, which was sampled for *Lemanea* and *Stigeoclonium*, are also included in Table 9.2. Comparison of the composition of water in the R. Nent with that in the rivers Ystwyth and Rheidol demonstrates the persistant nature of contamination by zinc as suggested by Jones (1958), since the rivers all carry high concentrations of the metal at least 40 years after the cessation of mining in their valleys.

The concentrations of zinc in the R. Nent are rather similar to those recorded in Bolts Burn, although the former river is larger Both carry concentrations of 2 - 4 mg l^{-1} Zn immediately downstream of the entry of adits discharging the metal, and both carry around 1.0 mg l^{-1} Zn at their point of entry to larger rivers. The high cadmium content of the water in both Bolts Burn and the R. Nent may be attributed to the passage of subterranean waters over sphalerite contaminated by cadmium before reaching the surface through adits. The most obvious difference in the composition of the water in the two streams lies in the much higher concentrations of lead carried by Bolts Burn. This high lead content is almost certainly maintained solely by the activities of the Whiteheaps Mine, and it is of interest that no other streams on the Northern Pennine Orefield are known to carry similar concentrations of lead and particulate material as a result of inputs from disused workings.

Because of its larger size, the absolute levels of zinc and cadmium carried by the R. Nent are clearly greater than those carried by Bolts Burn. However the magnitude of the increase in the metal content of the R. South Tyne caused by the entry of the R. Nent is similar to that caused in the R. Derwent by the entry of Bolts Burn (Table 9.2). This may be explained by the greater size of the R. South Tyne.

It is notable that the concentrations of zinc in waters draining old tips and workings in the valley of Bolts Burn appear to be low in comparison to such drainage waters in other areas of the Northern Pennine Orefield (e.g. Alston Moor). This seemed suprising at first, since old workings form an extensive complex around Bolts Burn downstream of the Whiteheaps Mine (see Fig. 4 4). The low zinc content of the water may be due in part to the low sphalerite content of the coarse tailings around Bolts Burn, and in part to the high pH of surface waters in the area. With the exception of

'Track Flush' (mean pH = 4.9), values lower than pH 7.0 were seldom recorded in waters within the catchment area. Examples of the low level of solution from tips were provided by the zinc content of the two largest tributaries to Bolts Burn, the upper and lower 'Tip Tributaries' Both of these streams percolate through extensive heaps of coarse tailings, from which they receive some direct drainage, before they enter Bolts Burn. Concentrations of more than 0.3 mg l^{-1} Zn were very seldom recorded in either tributary, and they always had a diluting effect on the main stream. Similar conditions appear to exist elsewhere in the catchment. For example, the upper part of Shildon Burn is surrounded by coarse tailings, but the stream carries only very low concentrations of zinc ($<$ 0 1 mg l^{-1}) before the entry of 'Shildon Adit'.

9 213 Derwent Reservoir

The transects to sample water along the axis of the Derwent Reservoir (5.2, Fig. 5 3) demonstrated that hydrological factors may exert a major influence on the distribution of heavy metals within the water column. It seems probable that 'streaming' of river water along the axis of the reservoir may on occasions carry higher concentrations of heavy metals as far as the draw-off points for water for treatment R.W Hunter (personal communication) has recorded occasional elevated concentrations of zinc ($>$ 0.1 mg l^{-1}) in water entering the Mosswood Works for treatment. Although some of these occurrences may have resulted from the shifting of sediment from within pipes, elevated concentrations of zinc were noted on several occasions 1 - 3 days after major flooding on the R Derwent On some of these occasions the composition of the compensation water provided strong evidence for the 'streaming' of water from the R Derwent (see 4 325, 5.2)

The concentration of 0 29 mg l^{-1} Zn recorded in a sample of water collected from the reservoir at square 42 on 23 March 1976 may have been caused by the penetration of moving water beyong the nature reserve following moderately high discharges in the R Derwent It seems likely, therefore, that elevated concentrations of heavy metals may be extracted in water for use in the sailing club more frequently than penetrate as far as the main draw-off points A concentration of 1 0 mg l^{-1} Zn was recorded in water in the sailing club storage tank before the commencement of the project in 1974 From the results of the present project it seems very unlikely that concentrations as high as this could result from the 'streaming' of river water The tank is galvanized, and the deposit of sediment within the tank might have been disturbed when the sample was taken.

Despite the apparent association of zinc with some component of particulate material during transport along the R. Derwent (see above), elevated concentrations of the metal were found throughout the water column of the reservoir following turbulent filling (Fig 5.3). These elevated concentrations were still apparent several months following the turbulance, as evidenced by the concentration of zinc in the water of the R. Derwent downstream of the reservoir (5.2) A proportion of the zinc carried into the reservoir was therefore present in a form that could remain in the water column for extended periods. It seems unlikely that a large proportion of this zinc was in true solution, as the pH of the water in the R Derwent and the reservoir is relatively high (7 2 - 7 6). Dissolved humic material in the water may therefore play a significant role in maintaining the metal in solution (see 1 412).

Possible mechanisms for the release of heavy metals from the sediments to the overlying water have been reviewed in 1 422. No direct evidence was found during the project for the occurrence of such release on a significant scale within the Derwent Reservoir Similarly, the elevated concentrations of zinc present in the water column following flooding did not appear to be associated with material re-suspended from sediments (see below) They could therefore have resulted either from the entry of a large volume of water from the R Derwent or from the re-solution (or complexing by organic material) of zinc from the sediments. The upwelling of zinc detected in the water close to the dam wall on 14 September 1976 (Fig. 5.3) provides some evidence for the former hypothesis.

Leland & Shimp (1975) noted that the filtrable heavy metal content of water in southern Lake Michigan increased as the transparency of the water decreased. No markedly elevated levels of particulate material were noted in samples of water taken at different depths from the western end of the reservoir following flooding on the R. Derwent. It seems probable that some re-suspension of sediment takes place from time to time (especially within the nature reserve), but no studies were carried out of the possible release of metals from such sediment. Such studies would be hampered by difficulties in distinguishing between high concentrations of suspended solids derived from the Whiteheaps Mine and material re-suspended from the bed of the reservoir

It would be interesting to study the extent of weathering of finely divided particles of sphalerite or galena (derived from the crushing of fluorspar) following sedimentation within the reservoir It was noted in 4 311 that suspended material was carried for long distances relatively swiftly by Bolts Burn and the R. Derwent, and it seems unlikely that particles of sulphideore reach equilibrium with their external environment during transport downstream from the Whiteheaps Mine.

9 22 Composition of sediment

9 221 Catchment area of the R. Derwent

The results of the surveys of the mineral composition of stream and river sediments provided strong confirmation of the sources of different metals that were located by the surveys of the composition of water High HNO_3-extractable concentrations of zinc, lead and cadmium were measured in sediments downstream of the Whiteheaps Mine, and some fall-off was noted on passing downstream towards the Derwent Reservoir It was noted in 1.32 that total concentrations of zinc or lead of more than about 300 µg g^{-1} appear to be restricted to areas affected by pollution. In light of this, the zinc content of sediment in Bolts Burn upstream of the Whiteheaps Mine (430 µg g^{-1}) does seem to have been affected somewhat by the old lead workings at the head of the valley. Downstream of the Whiteheaps Mine, however, the concentrations of zinc and lead were found to be much higher than this Neither metal was present at a concentration of less than 2000 µg g^{-1} between reach 27 and reach 99. Similarly, whilst the concentrations of zinc and lead in sediment from the R Derwent upstream of Bolts Burn (235 µg g^{-1} and 69 µg g^{-1} respectively) showed no evidence of marked contamination, they rose sharply downstream of the entry of the tributary and were still over 1000 µg g^{-1} at reach 25

Markedly higher concentrations (up to 26500 µg g^{-1} Zn and 5500 µg g^{-1} Pb) were measured in sediments collected from Bolts Burn during the exploratory surveys (3 15). The sediment in the stream downstream of the Whiteheaps Mine had a markedly orange appearance during the survey, and it sees probable that hydrous iron oxides derived from the settling ponds were binding high concentrations of heavy metals to the bed of Bolts Burn This may be an event that occurs annually, as the bed of the stream was also very orange during the summers of 1976 and 1977. The concentrations recorded during the main survey of sediment composition (carried out following heavy flooding in autumn 1976) may therefore represent minimum concentrations These data further suggest that a 'slug' of particulate material with high iron and heavy metal contents may move downstream to the reservoir during floods following low summer rainfall

Allan (1975) has warned of the need to take 'natural' anomalous concentrations of heavy metals into account before attributing the contamination of sediments to 'artificial' pollution (see 1 32). However, the concentrations of zinc, lead and cadmium in Bolts Burn downstream of the Whiteheaps Mine and in the R Derwent downstream of Bolts Burn are much higher than might be expected to result from undisturbed areas of mineralization. Further, the fall-off in concentrations of metals in sediments found on passing downstream from the Whiteheaps Mine demonstrate that the inputs from the mine are proportionally more important than those from old workings further downstream

Whilst the concentrations of zinc and lead in sediment from the R Derwent downstream of the reservoir (1460 $\mu g\ g^{-1}$ and 950 $\mu g\ g^{-1}$ respectively) were somewhat lower than the concentrations found in the river upstream of the reservoir, it is clear that the sediment was still enriched substantially by these metals at this point These results pose the question of whether the metals were the result of dissolved or suspended material carried through the reservoir to the compensation water, or whether they simply reflect matals laid down in the bed of the river before the reservoir was built. The marked increase in zinc . lead ratios found on passing from upstream to downstream of the reservoir (Fig. 5.5) might be considered as evidence to support the former hypothesis, although it seems probable that coarser sediment has remained in the reach for long periods It would be of interest to investigate the heavy metal content of any deposits of sediment (laid down by floods) that might still be present on the banks of the R Derwent downstream of the reservoir.

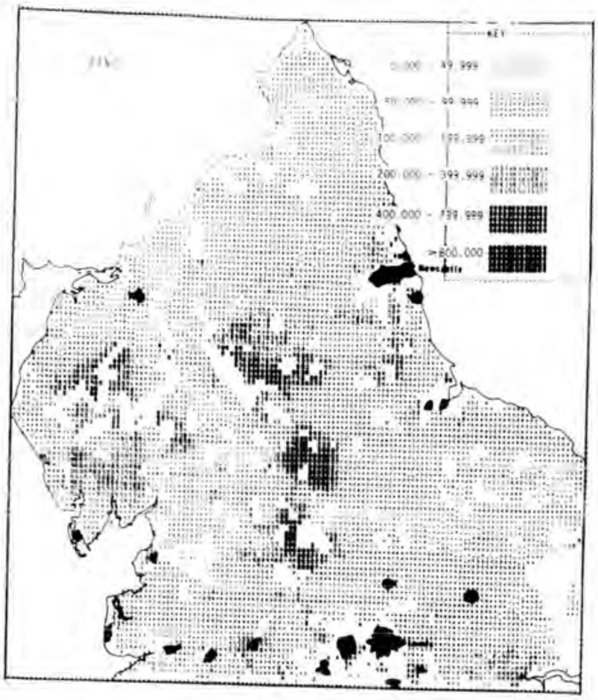
Figs 1 1 and 9.1 show the distribution of zinc, lead and cadmium in stream sediments from England and Wales, as determined by the Applied Geochemistry Research Group (see 1 221). It is clear from these figures that the concentrations of all three of these metals in the sediments

Fig 9 1

Concentrations of zinc, lead and cadmium in sediments collected from streams in Northern England during 1969.

Reproduced from line-printer maps produced by the Applied Geochemistry Research Group, Imperial College.

Data provided by Dr I Thornton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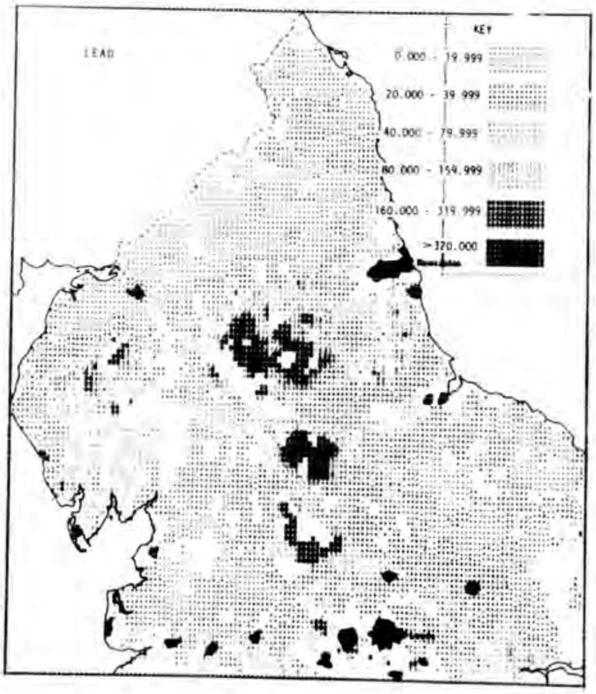
50.000 - 99.999
100.000 - 199.999
200.000 - 399.999
400.000 - 799.999
>800.000
Newcastle
Leeds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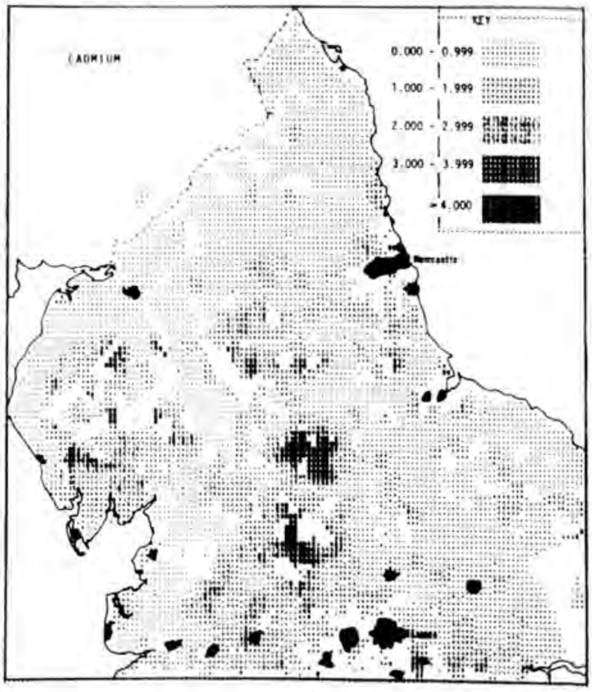
80.000 - 159.999
160.000 - 319.999
>320.000
Newcastle
Leeds


CADMIUM
KEY
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1.000 - 1.999
2.000 - 2.999
3.000 - 3.999
>4.000
Newcastle
Leeds

of Bolts Burn and the R. Derwent fall well into the higher ranges mapped ($>$ 800 $\mu g\ g^{-1}$ Zn, $>$ 320 $\mu g\ g^{-1}$ Pb and $>$ 4 $\mu g\ g^{-1}$ Cd) This is true despite the fact that the extraction techniques employed at Imperial College were stronger than those used in the present project The significance of the anomalous concentrations of metals found during the nationwide survey are discussed by Thornton & Webb (1973, 1975) in relation to possible environmental effects. Some further comparisons of the concentrations of zinc, lead and cadmium found during the present study with those reported from other areas are presented in Table 9.3

9 222 Derwent Reservoir

The results of the surveys of the composition of sediments in the Derwent Reservoir (3 6, 5 3) demonstrated that extensive contamination by heavy metals extended well into the reservoir from the point of entry of the R Derwent Whilst concentrations of zinc and lead were both very high in the sediments of the nature reserve, concentrations of lead decreased more rapidly than those of zinc on passing away from the mouth of the river These results are similar to the observations made during the transects to sample water, and indicated that a greater proportion of lead was associated with heavier particles derived from the crushing of ore.

No clear cut pattern was observed for the distribution of cadmium within the sediments of the reservoir, although the metal was clearly present in high concentrations. Thus, as had been observed in the water of the R Derwent entering the reservoir, zinc and cadmium appeared to have become 'disassociated' in their behaviour. The lack of correlation between concentrations of zinc and cadmium within the sediments (Table 5 6) provides further evidence against the deposition of large quantities of discrete particles of sphalerite. By similar reasoning, the significant positive correlation between HNO_3-extractable concentrations of lead and silver provides evidence for the deposition of discrete particles of galena.

Workers	Year	Site	Sample preparation	Zn	Pb	Cd
Agemian & Chau	1976	Lake Ontario	=	218	70	4 0
			+	290	100	40
Allan	1971		=	50-166	-	-
Aston & Thornton	1975	R Fowey (Wales)	+	31-370	34-384	-
		R Red (Wales)	+	69-2800	10-1052	-
		R Carnon (Wales)	+	150-1000	48-1440	-
		R Gannel (Wales)	+	150-7000	43-4410	-
Butterworth, Lester & Nickless	1972	Severn Estuary	+	420-590	130-200	1 6-4 7
Carmody, Pierce & Yasso	1973	New York Bight	=	16-1500	7-370	-
Chester & Stoner	1975	Bristol Channel (unpolluted)	+	80-110	tr-20	-
	1975	Bristol Channel (polluted)	+	128-20016	86-11367	-
Collinson & Shimp	1972	Upper Peoria Lake	+	185-667	52-204	2-13
de Groot & Allersma	1975	R Rhine	+	2900	800	<5
		R Meuse	+	2500	600	<5
Edgington & Robbins	1976	Lake Michigan	=	-	20-220	-
Förstner	1977	74 Lakes	+	45-220	8-75	0 10-1 20
Förstner & Müller	1973	Lake Constance	?	185	30	-
		R Rhine	?	520	155	<
		R Rhine	?	1239	369	1
		Biesboch (Netherlands)	?	3900	850	-
Gale, Bolter & Wixson	1976	Clearwater Lake	=	10-84	<3-<60	-
Grieve & Fletcher	1976	Frazer River Delta	+	53-77	5-11	-
Holmes, Slade & McLerran	1974	Corpus Christi Bay	=	<49-<200	-	-
Jaffe & Walters	1975	Humber Estuary	+	61-433	38-221	-
Kobayashi *et al*	1976	Lake Biwa, Japan	+	98-736	18-259	0 22-26 0
Loring	1976	Saguenay Fjord	+	43-145	14-66	-
Maxfield *et al*	1974a	Coeur d Alene Delta	=	3200-4700	3000-6300	16-75
	b	Coeur d'Alene Lake	=	< 200-c 5000	200-c 3500	<5-c 90
Mothersill	1977	Thunder Bay, Lake Superior	+	226	-	-
Oliver	1973	Ottawa River	-	84	26	-
		Rideau River	-	86	42	-
Pita & Hyne	1974	Reservoirs in U S A	+	46-273	36-55	-
Rehwoldt, Karimann-Teherani & Altmann	1975	Canals in Vienna	+	36-42	-	-
Romapo	1976	Grand Calumet River	=	1390	545	5 5
Schoettle & Friedman	1976	Lake George, New York	?	95-290	-	-
Shimp, Leland & White	1970	Lake Michigan	+	42-179	18-90	5- 7
Skei *et al*	1972	Sorfjord, Norway	+	830-118000	720-70000	16-350
Present study	1977	0071-20	=	430	220	5 0
		0071-99	=	3000	10980	16 0
		0061-05	=	235	69	5 0
		0061-07	=	1050	2700	18 0
		Derwent Reservoir, range	=	48-5000	10-2200	4 0-21 0
		Derwent Reservoir, mean	=	1035	824	1

Table 9 2 Comparison of concentrations of zinc, lead and cadmium found in sediments during the present study with concentrations reported by some other workers

Extraction techniques used by other workers are classified as - stronger (+), similar (=) or weaker (-) than the digestion used during the present study All concentrations are given in μg g^{-1} dried sediment

No information is available regarding the absolute rates of deposition of sediment within the reservoir. There is no quantitative basis, therefore, on which to establish the proportions of different metals entering the sediments by deposition of particles or by binding to existing deposits (mechanisms of binding have been reviewed in 1.421). Subjective observations indicated that rates of deposition of sediment were greater at the western end of the reservoir, where a crust of mud over 1 cm thick was laid down over a wide area during the winter of 1974/75 If fresh sediment is deposited only slowly in the eastern part of the reservoir, it seems possible that exchange sites available for the binding of metals might become saturated This might then lead to a decrease in the amounts of metals bound by the sediments, and a subsequent increase in the concentrations remaining in the water column.

Fractionated cores of sediment from the bed of the reservoir could not be used to establish 'background' concentrations in the catchment as suggested by Allan (1975), as mining operations pre-date the construction of the reservoir. However, it would be of interest to collect cores at different points to clarify spatial and temporal variations in the rates of deposition of metal-rich sediment and in the binding of metals by existing deposits

The higher concentrations of lead in the sediments of the nature reserve are much lower than the concentrations of up to 30000 $\mu g\ g^{-1}$ reported in the sediments of part of Ullswater by Welsh & Denny (1976). However, these exceptionally high concentrations were present only directly below the entry of a polluted stream, and were not representative of the bed of Ullswater as a whole The pollution of the Derwent Reservoir is clearly not as great as that of the Sorfjord, Norway, where concentrations of up to 118000 $\mu g\ g^{-1}$ Zn, 70000 $\mu g\ g^{-1}$ Pb and 850 $\mu g\ g^{-1}$ Cd were found in sediments by Skei, Price & Calvert (1972).

The two instances cited above seem to represent exceptionally severe pollution (Table 9 3) There is clear evidence from the literature that the concentrations of heavy metals within the sediments of the Derwent Reservoir are high enough to constitute a serious pollution problem As was found for Bolts Burn and the R. Derwent, the mean concentrations of zinc, lead and cadmium within the sediments of the reservoir (1035 $\mu g\ g^{-1}$, 824 $\mu g\ g^{-1}$ and 13 $\mu g\ g^{-1}$ respectively) were well within the higher ranges mapped during the survey carried out by the Applied Geochemistry Research Group (Figs 1.1, 9 1). Some of the concentrations of zinc and lead ($>$1600 $\mu g\ g^{-1}$) found within the nature reserve were more than twice the upper threshold concentrations mapped during the survey

Some of the higher concentrations recorded are comparable with those reported in wide areas of the sediment of the Coeur d'Alene Lake, Idaho by Maxfield *et al*. (1974a, 1974b), who descibed the pollution of sediments as 'very serious'. The concentrations of zinc and lead in the sediment from around the mouth of the river also compare with those reported from the R Rhine by de Groot & Allersma (1975) These authors reported that pollution from industrial sources had resulted in concentrations of up to 2900 $\mu g\ g^{-1}$ Zn, 800 $\mu g\ g^{-1}$ Pb and 45 $\mu g\ g^{-1}$ Cd. Golterman (1975) discussed the significance of de Groot & Allersma's findings and stated "Unless pollution of this kind is ended, the risk of diseases like Itai-Itai' occurring, perhaps at a subclinical level, is being increased" It must be noted, however, that the concentrations of cadmium found in the sediments of the Derwent Reservoir ($\bar{x}$ = 13 $\mu g\ g^{-1}$, max = 21 $\mu g\ g^{-1}$) are less than half those recorded by de Groot & Allersma It is perhaps fortunate, therefore, that the cadmium content of sphalerite from the Northern Pennine Orefield is generally lower than that present in the veins around Reeth, Swaledale (Fig 9.1)

9 23 Composition of Plants

9 231 Catchment area of the R Derwent

Because of the extremely reduced vegetation of Bolts Burn (see 3.7), it was impossible to find a species of submerged plant that was suitable for studies of the accumulation of heavy metals. However, the use of *Lemanea fluviatilis* as a monitor of the distribution and variation of heavy metals within the R Derwent proved to be a valuable supplement to data on the composition of water The results for *Lemanea* from the R Derwent are discussed together with the data from other rivers in 9.3

Two species of bryophytes (*Hygrohypnum ochraceum, Scapania undulata*) were also sampled from the R Derwent at reaches 05 and 07 As was the case with *Lemanea*, large increases in the concentrations of zinc, lead and cadmium were found in the shoots of these plants on passing from upstream to downstream of Bolts Burn. The magnitudes of these increases are compared for the three species in Table 9 4.

From this table, it is clear that wide differences were found between the enrichment ratios for zinc, lead and cadmium in the three species studied In both reaches, the two species of bryophytes took up higher concentrations of zinc, lead and cadmium than either whole filaments of *Lemanea* or 2 cm long filament tips. Similarly, the magnitude of the increases in heavy metal content between reach 05 and reach 07 was greater for all three metals in the two species of bryophyte. The observations may indicate basic differences in the capacity of the different species to bind metals, or they may be an artifact of the greater tendancy of the (leafy) bryophytes to trap particulate metals which may not be completely removed during washing In a situation such as the R Derwent where large amounts of particulate material may be present, there are obvious advantages in the use in monitoring of species (such as *Lemanea*) which trap all to a lesser extent.

Reach		0061-05				0061-07			
		n	Zn	Pb	Cd	n	Zn	Pb	Cd
Hygrohypnum ochraceum	conc.	1	334	160	20	1	1438	993	-
	E.R.tot		12846	22857	40000		4536	19471	-
	E R.nuc		15905	17778	-		5287	62063	-
Scapania undulata	conc.	4	771	208	28	4	2992	2387	30
	E.R.tot		29654	29714	56000		9438	46804	16667
	E.R.nuc		36714	23111	-		11000	149188	-
Lemanea (whole filaments)	conc.	4	213	58	30	4	1223	581	121
	E.R tot		8192	8286	60000		3858	11392	67222
	E.R.nuc		10143	6444	-		4496	36313	-
Lemanea (2cm filament tips)	conc.	6	1352	78	38	59	917	197	66
	E.R.tot		13538	11143	76000		2893	3863	36667
	E.R.nuc		16762	8667	-		3371	12313	-

Table 9.4 Comparison of concentrations of zinc, lead and cadmium in plants sampled from the R. Derwent upstream and downstream of the entry of Bolts Burn.

Concentrations are expressed as µg g^{-1} dry weight

E.R.tot = enrichment ratio for 'total' content of water

E.R.nuc = enrichment ratio for Nuclepore filtered water

From the results discussed below (9.32), it is clear that changes in the heavy metal content of filament tips of *Lemanea* at different stations along the R Derwent provide a valuable supplement to data on changes in the composition of the water. The increase in the zinc content of the plant between reaches 05 and 07 on the river (Table 9 4) may be an under-estimate in comparison to the proportional increase in the zinc content of the water. This is because a proportion of the zinc content of the alga in the unpolluted reach may be taken up actively from the surrounding water for the needs of the plant, whatever the concentration in the water The concentrations of zinc measured in the alga in reahces 07 and 25 on the river fall into the region in which a good linear relationship was found between the zinc content of *Lemanea* and the surrounding water As factors (such as hardness) which might be expected to influence uptake remain fairly constant along the river, it is suggested that the difference in the metal content of *Lemanea* from reaches 07 and 25 provides a good indication of the changes taking place in the R Derwent downstream of the entry of Bolts Burn.

Despite the fact that the galena mined from the Ramshaw and Jefferies Veins in the valley of Bolts Burn was noted for its silver content, no silver was ever detected in any of the samples of *Lemanea* taken from the R. Derwent downstream of the entry of Bolts Burn (Deb *et al.*, 1974, detected silver in filaments of *Lemanea australis* and suggested the presence of economically viable deposits of the metal in the area of study)

9 232 Derwent Reservoir

High concentrations of zinc, lead and cadmium were also found to have been accumulated by the rooted and non-rooted submerged plants that were sampled from within the Derwent Reservoir It can be concluded, therefore, that substantial amounts of these metals were present within the reservoir in a form available for uptake by plants This observation is especially interesting in the case of lead, as the metal entered the reservoir in a predominantly particulate form (4.324). These results serve to highlight the value of the inclusion of analyses of biota in studies of the partition of metals within freshwater systems, they are also rather similar to suggestions made by Wixson (1977) Wixson suggested that discrete particles of lead ore may become entrapped by the filaments of algae such as *Cladophora* and that subsequent interaction with a variety of strongly negative functional groups on the surface of the cells may play a significant role in disassociation of lead from the particles Once the metal became disassociated, Wixson suggested that it was bound to the exterior and the interior of the cells by processes similar to those reviewed in 1 63. If such accumulation of metals from particles is taking place to any great extent within the R. Derwent and the reservoir, then non-rooted plants, as well as rooted species (see below), could play a significant part in the exchange of metals between particulate and non-particulate fractions

Comparisons of the concentrations of heavy metals found within submerged plants during the present study with other published data is difficult, since few anaylses have been found in the literature for species studied during the project. It was noted in 9.231 that the enrichment ratios for zinc lead and cadmium differed between different species within the R Derwent, and other authors have noted such inter-specific differences (e.g Dietz, 1973, Adams, Cole & Massie, 1973)

The highest concentrations of zinc, lead and cadmium found in whole plants of *Nitella flexilis* from the reservoir were 1811 $\mu g\ g^{-1}$, 1061 $\mu g\ g^{-1}$ and 22 $\mu g\ g^{-1}$ respectively The lowest concentration of zinc found within the plant was 356 $\mu g\ g^{-1}$ in material collected from a point near to the dam This compares with a concentration of 470 $\mu g\ g^{-1}$ in material from the R Tees, and 240 $\mu g\ g^{-1}$ in material from a unknown source analysed by Boyd & Lawrence (1967)

The highest concentrations of zinc, lead and cadmium found in the leaves of the rooted grass *Glyceria fluitans* from the reservoir were 1238 μg g^{-1}, 103 μg g^{-1} and 8 μg g^{-1} respectively The concentrations in this plant were thus generally lower than those found in *Nitella*, despite the possiblity of uptake of metals through the roots. This might be explained by a greater number of sites on cells of *Nitella* that are available to accumulate nutrients from the surrounding water

The highest concentration of lead reported in the shoots of the rooted submerged angiosperm *Potamogeton crispus* from Ullswater by Welsh & Denny was 38 μg g^{-1} Although this was somewhat lower than the highest concentration found in *Glyceria* during the present study, comparison of results from the two bodies of water is difficult Higher concentrations of lead may be present in the water of the Derwent Reservoir than in the water of Ullswater, together with greater concentrations of suspended particles and organic material

Despite the lower concentrations of zinc, lead and cadmium that were found in plants of *Glyceria* than in plants of *Nitella*, there was some evidence (5.42) that the rooted grass took up some of its heavy metal content from the sediment in which it was growing Although such uptake is apparently quite possible (see 1.63), it is difficult to establish its importance within the Derwent Reservoir on the basis of the present results alone The main reason for this difficulty is that the concentrations of metals within both water and sediments were positively correlated The possiblity of uptake from deposits of fine sediment on the surface of the leaves of *Glyceria* (as suggested tentatively by Wixson, 1977) complicates the situation further

No studies were included of the heavy metal content of rooted terrestrial plants colonizing the exposed mud in the nature reserve during periods of low water (see Fig 3 3) Uptake through roots would be the only pathway by which these plants could take up metals into their aerial parts

The main effects of species of *Nitella* which accumulate metals largely, if not entirely, from the water would be to slow down the rate of deposition of metals to the sediments. It is also possible that metals released following the rotting of plants of *Nitella* might be complexed to dissolved organic material that had a long time of residence in the water Rooted species such as *Glyceria*, which may be able to take up metals from sediments, may release some (previously sedimented) metals to the water. Such release has been demonstrated in field experiments by Mayes & McIntosh (1975) for *Ceratophyllum demersum*. Metals accumulated by plants growing on exposed mud around the mouth of the river may be either expected to be released when these plants decompose following the filling of the reservoir, or to become buried and incorporated in the sediments whilst still bound to the plant tissue.

No experimental studies were performed on the effects of environmental factors on the uptake of heavy metals by submerged plants. However, it seems probable from the review of the literature (1 64) that the low concentrations of calcium and magnesium within the water and sediments of the reservoir might be expected to enhance the accumulation of metals Any change in management policy which might lead to an increase in the hardness of the water (e.g. transfer of water from other areas) might be expected to decrease the uptake of metals by submerged plants

Any increase in the quantity of submerged plant growths in the future would increase the importance of any interactions between the metal content of plants, water and sediment. This might lead to the release of increased levels of metals to the water by the processes suggested above Such increases in growth could be caused by a spread (or introduction) of strains or species that were tolerant to metals, or by any changes in other environmental factors that would favor the growth of existing populations Such changes could be brought about by changes in management policy, and include decreases in the fluctuations in the level of water, increases in the concentrations of essential nutrients or increase in the transparency of the water.

In their study of the cycling of lead in Ullswater, Welsh & Denny found that trout and invertebrates were carrying elevated concentrations of lead in their flesh. They concluded that the remobilization of lead from sediments by rooted submerged plants "plays a key role in the deleterious cycling of metals in the lake which could give rise to faunistic abnormalities".

In order to present a more comprehensive picture of the distribution of metals within the reservoir, it would clearly be of interest to study the metal content of other biota. Such studies were not included since the passage of metals through food webs has been shown to be complicated (Kneip & Lauer, 1973, Hutchinson *et al* , 1976), and an elaborate survey would be required to obtain reliable data. Sampling of fish would probably give a general indication of whether metals were entering the food web in appreciable quantities. However, the regular re-stocking with hatchery fish would make a critical study difficult.

9 24 Tolerance of submerged plants to heavy metals

9 241 R. Derwent catchment

No species of readily cultured algae were found to be present in both reaches 05 and 07 on the R. Derwent (see 3 7) This emphasises the toxic effects of metals derived from Bolts Burn, but meant also that no cultural studies could be carried out to compare the tolerance to heavy metals of populations of a single species from both reaches. However, several populations of *Stigeoclonium tenue* were isolated from reaches within the catchment of the R. Derwent and used in studies of tolerance reported in 7.5. Populations of *S. tenue* isolated from Bolts Burn (0071-99), Shildon Burn (0124-99), the R. Derwent (0061-10) and 'Track Flush' (0176-01) were all found to be tolerant strains, capable of growth at higher concentrations of zinc than populations from sites with low concentrations of zinc in the water Similarly, the tolerance to zinc of populations of *Hormidium flaccidum* growing in the lower reaches of Bolts Burn was found to be greater than that of populations growing in lower concentrations of zinc In a rather similar manner to the observations on the accumulation of lead by plants within the Derwent Reservoir, the above observations serve to illustrate that whatever the chemical form of zinc in polluted reaches within the catchment, the metal was able to exert toxic effects to the biota.

9 242 Derwent Reservoir

It was noted in 3.2 that growths of submerged macrophytic plants were scarse within the Derwent Reservoir during the period of study. There was no direct evidence, however, that this scarsity was due to the toxic action of heavy metals. The low concentrations of nutrients in the water, together with the low transparency and rapid fluctuations in the level of the water, may all act to limit the development of communities of plants.

No experimental studies were carried out of the toxicity of heavy metals to any species of submerged plant from the reservoir. There was therefore no direct evidence that any species were present as a metal tolerant ecotype. However, the concentrations of zinc usually present in the water of the nature reserve (0.1 - 0.2 mg l^{-1}) were within the range in which *Stigeoclonium tenue* and species of *Hormidium* appeared to show genetic adaptation to the metal (7 5, Say, Diaz & Whitton, 1977). It is therefore possible that the toxic effects of zinc within the nature reserve may be acting to prevent the development of normal communities of algae and higher plants. Whilst diving around the mouth of the river, it was noted that wide areas of water were shallow enough to permit good light penetration to the bottom. However, colonization of such areas by submerged plants was limited to sparse, 'patchy' growths of *Nitella, Glyceria* and *Callitriche*. As higher concentrations of heavy metals are rarely carried as far as the eastern end of the reservoir, it seems probable that 'unadapted' species of plants may become established there. During the spring of 1976, abundant growths of *Ulothrix zonata* were observed to grow up attached to the wall of the dam. This alga was also present in the R. Derwent upstream of Bolts Burn during the same period, but at no time was it recorded from the river downstream of the entry of the tributary. Similarly, *U. zonata* could not be found growing at higher concentrations of zinc within the nature reserve. Toxicity tests were performed upon populations of the alga from the dam wall and from the river at reach 05, and both were found to be very sensitive to zinc The 'streaming' of water along the reservoir might from time to time carry toxic concentrations of heavy metals to such non-adapted communities of plants that had become established at the eastern end of the reservoir.

It seems likely from a knowledge of the literature that concentrations of zinc and lead within the sediments of the nature reserve were high enough to exert toxic effects on plants. The concentrations of zinc in such sediments approach those described by Bradshaw (1970) in soil on a zinc mine where genetic adaptation to high concentrations of the metal was demonstrated in *Anthoxanthum odoratum*

As the reservoir has been in existence for only a relatively short period, it seems possible that certain areas may be open to colonization by more tolerant species or strains in the future. Of the species that regularly colonize exposed metal-rich mud within the nature reserve during periods of low water, *Agrostis stolonifera* is known to be capable of genetic adaptation to high concentrations of heavy metals (Smith & Bradshaw, 1970). Similarly, *Juncus effusus* and *J. acutiflorus* have been observed growing on metal-rich soil elsewhere in the Northern Pennine Orefield.

9.3 ACCUMULATION OF HEAVY METALS BY *LEMANEA FLUVIATILIS*

9 31 Observations in the field

It was noted in 1.523 that *Lemanea* has been reported to be one of the more successful species of algae in larger rivers polluted by heavy metals. Thus as long ago as 1924 Carpenter observed that *Lemanea* was a member of the reduced flora present in the R Rheidol downstream of the Cwn Rheidol Mine. Similarly Jones (1958) documented the presence of abundant growths of the alga in the zinc polluted R. Ystwyth. Observations made in the field whilst *Lemanea* was being collected for analysis (6 22) provided strong confirmation of the ability of *Lemanea* to colonize rivers polluted by elevated concentrations of zinc, lead and cadmium in the water. A.K Jones (personal communication) indicated that the alga was still abundant in the rivers Rheidol and Ystwyth, and reaches on both rivers were included in the survey

Lemanea is a plant that is usually confined to larger rivers, where fast flowing water passes continuously over stable substrata such as boulders, rock sheets, concrete or wood. As was stated in 1.3, it is rare for larger rivers such as these to carry very high concentrations of heavy metals in the water because of the effects of dilution *Lemanea* was found to be present in the highest concentrations of zinc, lead and cadmium in all of the larger rivers that were sampled It was not possible, therefore, to establish whether its growth was ever limited by the effects of metals in the field.

Only one very small population of *Lemanea* was found growing in the R Nent at Foreshield (0048-40), which had the highest zinc content of any of the reaches sampled for the alga (1.16 mg l^{-1}). However, it was uncertain whether the toxicity of zinc was acting to limit further colonization of the R. Nent by *Lemanea* Careful exploration revealed no further growths downstream of reach 40, where lower concentrations of zinc were present in otherwise similar conditions. As noted in 7 4, the R. Nent usually becomes colonized extensively by *Stigeoclonium tenue* during the spring and summer

9.32 Heavy metal content of *Lemanea* from a wide range of concentrations of metals in the field

It is clear from Figs 6.5 - 6.9 that higher concentrations of zinc, lead and cadmium in river water passing over filaments of *Lemanea* were reflected in higher concentrations in the filaments themselves. It is also clear from 6.23 that the concentration of a particular metal in 2 cm long filament tips was not affected markedly by the concentration of other metals. Thus the concentration of zinc could be relatively low in material with a high lead content (e.g. 0198-95), or material with a high zinc content could have a relatively low lead content (e.g. 0048-40) depending on the composition of the water.

There was some evidence that the concentrations of zinc in filament tips correlated better with the filtrable concentration of the metal in the water of different rivers than with the non-filtrable concentration This may be interpreted as evidence that the fraction of zinc capable of passing through 0.2 µm membrane filter corresponds more closely to the fraction available for uptake by *Lemanea* However, no such difference was noted for filtrable fractions of lead in the water This is perhaps suprising, since the studies reported in 4 3 indicated that lead showed a greater tendency than zinc to associate with suspended material in the water of the R. Derwent. Generalizations such as these should be treated with caution, however, since only a single collection of water was made from many of the sites. These collections were all made at comparable (i.e. low) discharge, but the factors that may cause fluctuations in the concentrations of metals in river waters have been stressed in 1.412.

No attempt was made to study differences in the relationship between the metal content of *Lemanea* and that of the water at the time the alga was collected, as compared with the relationship between the content of the alga and the mean content of the water over several collections. However there was evidence (see below in 9 33) that short term variations in zinc content (but not lead content) tended to 'buffer' the concentration in the plant against changes in the external concentration. This would indicate that, for zinc at least, the content of filaments correlates well with the content of a single sample of water collected at the same time as the filaments.

There was some indication, that environmental, and possibly physiological, factors were acting to influence the concentrations of heavy metals accumulated by *Lemanea* at several sites. At concentrations of zinc lower than about 0.02 mg l^{-1}, a discontinuity was apparent in the otherwise fairly linear relationship between concentrations of the metal in water and *Lemanea* (Figs 6.5, 6 6). A similar discontinuity was apparent for cadmium at 'total' concentrations lower than about 0 0003 mg l^{-1} (Fig. 6.9). The scattering of the points in Figs 6.7 and 6 8 was too great to ascertain whether such a discontinuity was present in the case of lead. It seems possible that the apparently proportionally greater accumulation of zinc at low concentrations in the water may represent the 'base level' of the metal which is accumulated for metabolic use whatever the concentration in the water Such an explanation is very doubtful in the case of cadmium, however, since essentiality has not been shown for this metal. Another possible explanation for the phenomena is that the filaments possess a certain number of sites (e.g. cation exchange or adsorption sites) which have such an affinity for the metals that they are bound whatever the external concentration. At higher concentrations in the water, further sites are 'forced' to bind zinc, perhaps in preference to other cations

There was a slight indication (Figs 6.5, 6 6) that proportionally higher concentrations were taken up from waters with a low calcium content than from similar concentrations of zinc in harder waters. There was no indication that uptake of lead or cadmium was reduced by high concentrations of calcium in the water. One possible way to investigate the effects of an environmental factor such as calcium on the accumulation of metals would be to study the degree of correlation between the concentration of the factor and the enrichment ratio for a particular metal. Such tests were not carried out, however, since the enrichment ratios for zinc, lead and cadmium were found to vary markedly according to the concentration of the metal in the water (see below)

In 1 62, it was noted that the use of enrichment ratios (or similar measures) has played an important role in studies to 'monitor' pollution through the analysis of submerged plants The success of such studies has been taken to depend on the ratio remaining relatively constant for a particular species over a range of concentrations of the particular metal. From the results already discussed in 9 231, there was some indication that the enrichment ratios for zinc, lead and cadmium in three species did *not* remain constant at different external concentrations of the metals in the external environment. The results for *Lemanea* provide further strong evidence that enrichment ratios may vary markedly with varying concentrations of metals in the water. For zinc, lead and cadmium, a clear inverse linear relationship was found between the enrichment ratio and the 'total' concentration of each metal in the water. This was apparent throughout the whole range of concentrations studied in the field, and there was no evidence for a 'levelling-off' of heavy metal content (causing a sudden decrease in enrichment ratios) at higher concentrations in the water.

It was suggested tentatively above that the higher the concentration of a metal in the water, the greater the number of exchange sites that are 'forced' to bind the metal in order to produce an equilibrium with the surrounding water. This suggested model is in agreement with the observation that enrichment ratios decreased at higher concentrations of metals in the water, provided that a wide range of complexing groups is available with a wide range of affinities for the metals It is possible that saturation of all the available sites might occur at even higher concentrations in the water, leading to a 'levelling-off' in the plant-water relationship.

The results discussed above emphasise that sufficient background work should be carried out before using any species of plant to monitor pollution by heavy metals in freshwaters. Despite the changes in enrichment ratios that were noted for *Lemanea* from different concentrations of metals, the plant still appears to possess great potential as such a 'monitor' However, evaluation of data on the metal content of the plant should rest upon comparisons with background data presented in a similar manner to those in Figs 6.5 - 6.9, rather than upon enrichment ratios alone The relationships between concentrations of metals in filaments and water do appear to be simple enough to permit the use of measures such as regression equations to describe them, and it might be possible to compensate for the observed changes in enrichment ratios by the use of 'correction factors'.

Every effort was made to sample *Lemanea* from different rivers at a comparable stage in the growth cycle However, no attempt was made to measure or estimate rates of growth at or prior to the time of sampling. It is therefore possible that the heavy metal content of some filaments or tips of filaments had not had time to reach equilibrium with the external environment following a period of relatively rapid growth However, subjective observations indicated that rates of growth of the sexual shoots were relatively slow following an initial 'burst' in the early spring. Very little growth took place in material transplanted between reaches (see 9.34), and it seems likely that rapid growth is much less of a complicating factor in studies of metal content than it may be for other species (e.g *Cladophora*)

9.33 Variation in the heavy metal content of a single population of *Lemanea* in the field

Before adopting analyses of submerged plants in studies of pollution by heavy metals, it is clearly important to know the period over which the metal content of the plant chosen has been accumulated from the surrounding water. An opportunity to study this period for *Lemanea* was provided by the presence of a large population growing in reach 07 of the R. Derwent, where rapid fluctuations in the concentrations of different metals were known to occur (9 212).

The results presented in 6 32 demonstrated that the zinc content of filament tips of *Lemanea* reflected short term fluctuations in the zinc content of the water in the reach with a suprising degree of sensitivity. Such short term variation in metal content has not apparently been demonstrated for any other aquatic plant. It is probably the result of a sensitive equilibrium between the zinc content of the water and the level of saturation of cation exchange or adsorption sites on the surfaces of the cells (see 1.63). Indirect evidence that passive accumulation (i.e. not involving metabolic energy) was involved lies in the rapid response of the zinc content of the filaments to a change in the zinc content of the water (Fig 6.13) at a temperature of only *c.* 4°C. Experiments carried out in the laboratory (see 9.35) have shown that the 'loosely bound' fraction of zinc in *Lemanea* can be removed very rapidly by EDTA, but not by distilled water This suggests that binding of zinc to a component of river water (possibly organic material) may play an essential role in the maintenance of the equilibrium between the zinc content of the plant and that of the water.

It is generally agreed (see 1.63) that the binding of zinc by many plants involves a passive ion exchange / adsorption process involving sites on the surfaces of the cells Some evidence has been found in the literature to suggest that a proportion of the zinc bound in this manner could be held loosely enough to be influenced by short term variations in the external concentration of the metal Pickering & Puia (1969) observed that whilst distilled water removed little of the zinc bound by *Fontinalis antipyretica*, a solution containing calcium ions removed up to 20% of the zinc in a short period This figure agrees well with the findings of the present study, the results of laboratory experiments (6.5) indicated that about 20% of the zinc accumulated by *Lemanea* in reach 07 on the R. Derwent was bound relatively loosely. Similarly, Harvey & Patrick (1967) found that three species of green algae desorbed ^{65}Zn rapidly after being transferred to non-radioactive growth medium.

These observations serve to illustrate that there are probably marked inter-specific differences in the strength with which metal ions are held after being accumulated by aquatic plants. It is possible, therefore, that the short term variations found in the zinc content of *Lemanea* in the R. Derwent may not take place in other plants growing in the river. It is also possible that such variation may not take place in populations of *Lemanea* growing in other rivers subject to 'bursts' of zinc. The situation in the R. Derwent may be a response to a unique combination of chemical conditions in the water.

9 34 Transplant experiments

The series of transplant experiments reported in 6.4 were carried out for two main reasons. Firstly, it was hoped to gain further information on the speed with which the metal content of the filaments could change. Secondly, it was hoped that the behaviour of the filaments following transplanting into high concentrations of metals would indicate whether adaptation to metals existed in populations from polluted rivers. This in turn might be expected to complicate the use of *Lemanea* as a monitor at high concentrations of metals in the field. These two separate objectives are discussed below

9.341 Changes in rates of uptake and loss of metals following transplanting

When four boulders covered in filaments of *Lemanea* were exchanged between reaches 05 and 07 on the R. Derwent, rapid changes in their zinc content took place within a short period of time (Fig 6.14). This provides further evidence that a proportion of the zinc content of the alga was held only loosely. Similarly, rapid increases in zinc content were observed when filaments were transplanted into water with a high zinc content (Figs 6.15, 6.16)

Following these initial rapid changes, the rates of increase or decrease in zinc content levelled off somewhat. However, the filaments took long periods (up to six weeks) to reach equilibrium with respect to zinc content. It was not possible to determine whether this slower phase of uptake or loss was the result of slower exchange with the surrounding water, or an artefact caused by the growth of the filaments

9.342 Differences in kinetics of uptake of metals between populations

From the results of the transplant experiments, it was not possible to demonstrate whether populations of *Lemanea* growing in rivers with high concentrations of heavy metals in the water were specially tolerant forms. However it is clear from the results of the transplants that if such adaptation does exist, it has no marked effects on the concentrations of zinc, lead or cadmium accumulated by the filaments

On examination of Fig. 6.14, it can be seen that the zinc content of *Lemanea* transplanted from reach 05 to reach 07 on the R Derwent approached 1000 $\mu g\ g^{-1}$ after about 850 hours. This concentration is similar to that in filaments native to the reach; the higher zinc content of the transplanted material at 1200 h was almost certainly the result of epiphytic algae and entrapped particles that were not removed completely by washing.

When material from several reaches was transplanted into water with a relatively high metal content, no marked differences in subsequent patterns of uptake were noted between different populations (Figs 6.15 - 6.18). Similarly, populations transplanted from low concentrations of metals survived for as long as populations from high concentrations. In the case of material transplanted from five reaches into the R. Nent, rather discontinuous patterns of change were noted for zinc, lead and cadmium content (Figs 6 15 - 6.18) Samples of water were not collected at the same time as samples of filaments from the tranplanted boulders, but it seems likely that these changes were caused by discharge-related variations in the metal content of the water. However, all five populations showed 'co-ordinated' variation in zinc, lead and cadmium content throughout the period.

The use of similar transplants using tolerant species of bryophytes has been suggested by McLean & Jones (1975) and Benson-Evans & Williams (1976). From the results of the transplant experiments, it seems clear that boulders covered in *Lemanea* could be placed into reaches where pollution by metals was suspected, and sampled for metal content after a suitable period (e.g six weeks). The concentrations of metals that were accumulated at equilibrium by the filaments transplanted into Bolts Burn and the R. Nent were very similar to those that might have been expected (Figs 6.5 - 6 9) had natural populations existed in the two reaches.

9.35 Observations in the laboratory

9.351 Factors influencing loss of zinc from filaments

From the results presented in 6.5, it is clear that the composition of the surrounding medium had a marked effect on the amount of zinc lost by filaments of *Lemanea* from reach 07 on the R. Derwent. Whilst almost no zinc was desorbed into double distilled water or river water, up to 20% of the total zinc content of the filaments was lost rapidly into solutions of EDTA. As it seems unlikely that organic material in river water would ever have as strong a capacity to complex zinc as EDTA, this percentage probably represents the maximum proportion of the zinc content of the alga that might be expected to take part in 'short term' variation within the R. Derwent. The fact that at least 80% of the zinc content of the alga is 'inaccessible' to EDTA indicates that this proportion was bound firmly by the cells. It could either be bound within the cytoplasm or on exchange sites on the cell surfaces with a very strong affinity for the metal This proportion of the zinc content of the filaments might be expected to reflect the concentration of the metal in the water over an extended period prior to sampling

It is interesting to note that lower amounts of zinc were released into Chu-10 growth medium containing a similar concentration of EDTA. This is probably the result of the saturation of some of the complexing sites on the EDTA molecules by other ions in the medium.

Although the river water (from reach 05 on the R Derwent) used in the experiment had a high content of yellow organic material, only small amounts of zinc were released into it during the experiment This may mean that the available exchange sites associated with organic material in the river became saturated rapidly, this would not occur in the river where large volumes of dilute organic material passed continuously over the population of *Lemanea*.

A more complicated pattern of release of zinc was observed when filaments were placed into solutions containing ions of calcium, magnesium or phosphate. Whilst initial release into each of these solutions was rapid, the zinc was apparently re-accumulated by the filaments after a period of about one hour. Although the pH of the media in all of the flasks remained reasonably constant during the experiment, it is possible that localised rises in pH amongst the filaments (caused by photosynthesis) resulted in precipitation of zinc and/or favoured re-association with complexing sites in the cell walls. Another possible explanation is that the test ions themselves were accumulated by the filaments, resulting in a subsequent decrease in their effectiveness in removing zinc from the filaments or competing for uptake sites

9 352 Toxicity tests

No conclusive results emerged from the toxicity tests performed upon filaments of *Lemanea* from different reaches (6 6, Tables 6.7 - 6.12) There was therefore no evidence for or against the existance of genetic tolerance to zinc in this alga. The assay used is clearly not suited to use with the relatively large filaments, which showed no sign of growth during the period of incubation. More reliable results might have been obtained using stones covered in *Lemanea* in constant flow channels, although it would obviously be impossible to imitate the torrent conditions favoured by the plant in the field B.A. Whitton (personal communication) has cultured sexual shoots of *Lemanea* in the past, and carpospores of *Lemanea tortulosa* have been germinated in culture by Rosenberg (1935). Further efforts using a wider variety of media and conditions of incubation might therefore have resulted in growing cultures that could have been used in toxicity tests.

9.4 TOXICITY OF ZINC TO *STIGEOCLONIUM TENUE*

9.41 Resistance to zinc of *Stigeoclonium tenue* in the field and the laboratory

9.411 Field observations

It is evident that *Stigeoclonium tenue* may be found growing in flowing waters carrying a wide range of (filtrable) concentrations of zinc. The highest concentration of zinc at which the alga was found was 20.0 mg l^{-1} in the discharge of the Rammelsberg Stollen (an adit draining mine workings in the Harz Mountains, Germany). *S. tenue* may be incapable of colonizing stream sites with even higher concentrations of zinc in the water The only filamentous green algae which have been repcrted for such sites are *Hormidium rivulare, H. flaccidum, Ulothrix moniliformis* and *Mougeotia* (? 2 spp.) (Say, 1977, Say, Diaz & Whitton, 1977)

S. tenue was found to dominate the vegetation in some streams with moderately high concentrations of zinc (> 1.0 mg l^{-1}) in the water (e.g. 0071-99, 0048-99, 0096-01, 0102-15). There was also some evidence that this period of dominance extended for a longer period each year in such streams than in streams carrying lower concentrations of zinc in the water. In fact this alga may be considered one of the more successful species in streams polluted by zinc

9.412 Laboratory observations

The results of the laboratory assays (7.5) demonstrated conclusively that populations of *S. tenue* growing in streams carrying high concentrations of zinc in the water are adapted forms, capable of withstanding higher concentrations of zinc than populations from unpolluted streams (Table 7.3). It was shown that the level of resistance to zinc of a zinc sensitive and zinc tolerant population did not change during long term subculturing at low and high concentrations of zinc in the medium (Table 7.2). This is interpreted as evidence for a genetic basis for the resistance of populations from higher concentrations of zinc.

From the data summarized in Fig. 7.2, it is difficult to state the exact concentration of zinc in the field above which populations become adapted to the metal. In any case, this threshold would be expected to vary according to the influence of other environmental factors upon toxicity (see 9.42). However, it does seem reasonable to conclude that the threshold concentration for the presence of tolerance lies within the 'discontinuity' noted in Fig. 7 2 at around 0.2 mg l^{-1} Zn. However, the toxicity test did appear to be rather insensitive to differences in the resistance to zinc of populations isolated from relatively low concentrations of zinc. It is uncertain, therefore, whether adaptation to the metal might be present at even lower concentrations than 0.2 mg l^{-1} in the field.

The available data (Table 7.3) show that for at least six sites, concentrations of zinc may occur in the water that are higher than any found when samples of water were collected at the same time as samples of *S. tenue*. In Kilhope Burn (0013-11), where the maximum concentration of zinc is much higher than any found during the period of study, the T.I C. corresponds to the concentration of zinc found during that period rather than to the maximum concentration known for the site. This concentration is known to rise during high flows because of runoff from spoil heaps, and *S tenue* was observed to have been killed in the stream by some unknown factor later in 1975. It therefore seems probable that the growth of the alga in this reach on Kilhope Burn depends on inoculation from unpolluted sites further upstream.

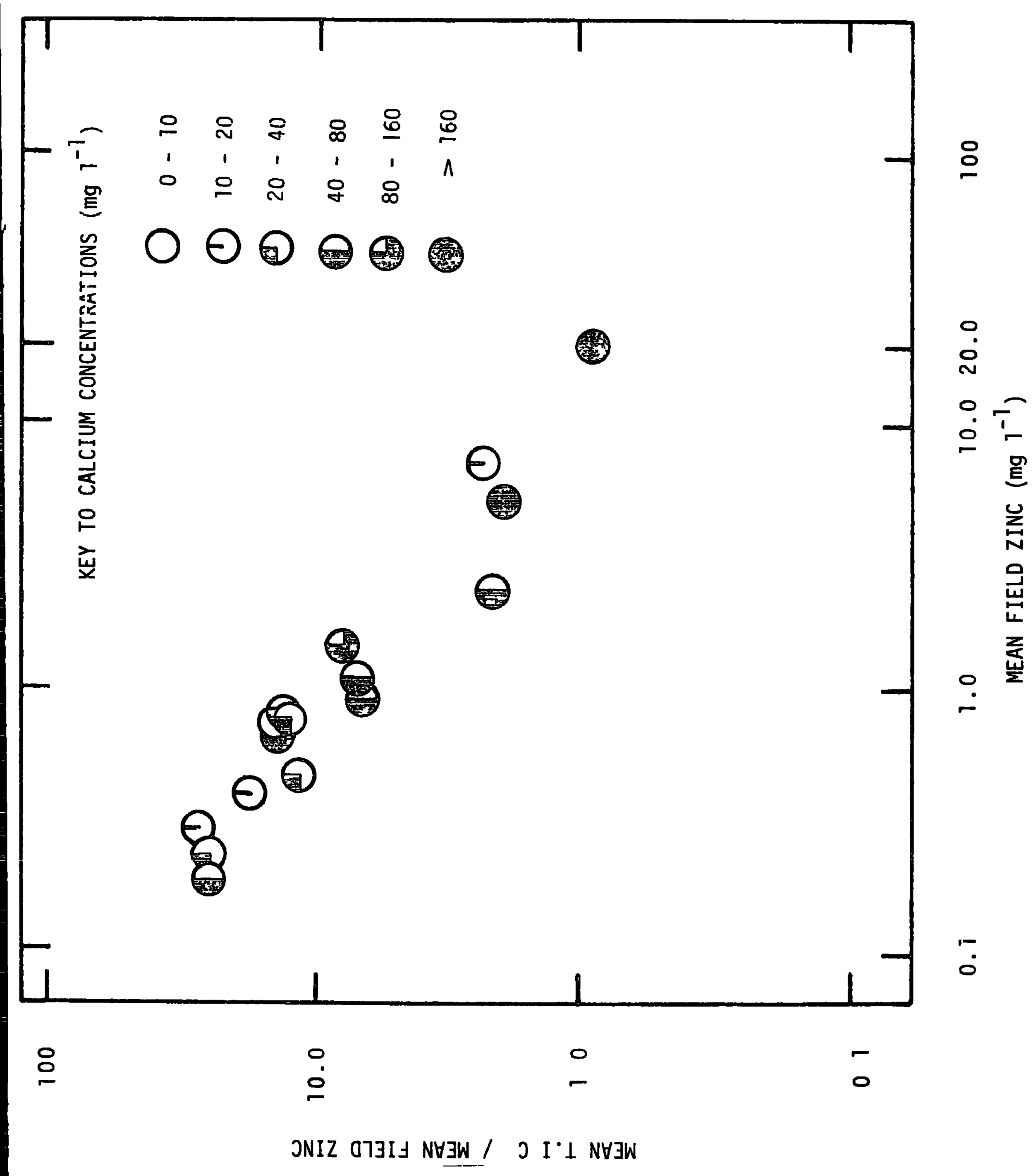
As mentioned in 1.523, McLean (1974) suggested that the resistance of *S. tenue* to heavy metals could be related to the affinity of the alga to organically polluted conditions. Although this may be an important factor in the survival of the species at some sites, the data presented in Table 7.3 show that neither organic pollution, nor even simply the presence of high concentrations of phosphate, are essential for the success of the species in streams polluted by elevated concentrations of zinc.

The data in Fig. 9.2 show that, under the conditions of culture that were used, *S. tenue* is usually much more tolerant to zinc in the laboratory than in the field. The greater the concentration of zinc in the field, however, the nearer were the results for T.I.C. found to correspond to the concentrations of zinc in the field. It would seem from the data summarized in Table 7.3 that the values for T I.C. were lower in material collected from streams with high concentrations of calcium in the water than in material from streams with similar concentrations of zinc in soft water. Based on the present data alone, however, this conclusion should be treated with caution as there was a significant tendancy for waters containing high concentrations of zinc to contain high concentrations of calcium (Fig. 9.2).

Fig. 9.2

Relationship between the ratio, mean T.I.C (tolerance index concentration) mean field concentration of filtrable zinc at sites sampled for *Stigeoclonium tenue*.

For clarity, only those populations clearly adapted to tolerate the higher concentrations of zinc are shown. Filtrable concentrations of calcium at the sites are indicated by the degree of shading of the circles.


KEY TO CALCIUM CONCENTRATIONS (mg l^{-1})
0 - 10
10 - 20
20 - 40
40 - 80
80 - 160
> 160
MEAN FIELD ZINC (mg l^{-1})
0.1
1.0
10.0
20.0
100
MEAN T.I C / MEAN FIELD ZINC
0 1
1 0
10.0
100

If data from waters rich in calcium (and magnesium) are excluded, even the concentrations of zinc that were 'just non-inhibitory' in the laboratory were always much higher than the mean concentration of zinc in the water of the reach of origin. It is obviously difficult to compare laboratory conditions with longer term exposure to zinc in the field, and only a few of the factors that might influence the toxicity of zinc in the field are discussed below,

Tolerance to zinc might be lower during a particular phase in the life cycle. In the laboratory, increased concentrations of zinc brought about a reduction in the erect portion of the thallus in comparison to the basal part, but only at the site with the highest concentration of zinc was the alga more or less restricted to a basal form in the field Observations made in the laboratory showed that neither zoospore production nor germination were stages that were especially sensitive to zinc, although it seems possible that the settling stage may be more critical in the field due to local concentration of zinc at the interface between the water and the substratum.

If tolerance in the laboratory is related to maximum, rather than to mean, concentrations of zinc in the field, then the absolute values for T.I C. approach concentrations in the water more closely Chemical factors which might decrease the toxicity of zinc in the laboratory, or increase toxicity in the field, would have a similar effect. The inclusion of a chelating agent in the growth medium was no doubt responsible in part for the lack of sensitivity of tests performed upon populations from lower concentrations of zinc in the field

The pH of the growth medium was lower than that of the water at most of the collection sites (with the exception of 'Track Flush', pH = 4.9). However, no clear indication could be drawn from the literature (see 1.523) of how changes in the pH might be expected to influence toxicity. Further, zinc may not be the only toxic metal affecting the survival of the alga at many of the sites. The filtrable concentrations of some other heavy metals (notably lead and cadmium) tended to be higher in the water at sites with high concentrations of zinc, although their absolute concentrations were very low at most sites. Say, Diaz & Whitton (1977) demonstrated conclusively that zinc and cadmium exerted synergistic toxicity to populations of *Hormidium rivulare*. However data for the tolerance to zinc of *S. tenue* from 'Track Flush' indicated that the presence of high concentrations of lead in the water did not increase markedly the toxicity of zinc in the field (Tables 7.1, 7.3)

9.42 Environmental factors reducing the toxicity of zinc to *Stigeoclonium tenue* in the laboratory

It was shown in 8.1 - 8 7 that the influence of environmental factors antagonistic to the toxic effects of zinc differed between a zinc sensitive and a zinc tolerant population This shows the importance of knowing about the environment from which a test organism is taken before using it in laboratory assays. It was pointed out in 1.523 that many such studies make use of organisms taken from culture collections, an example is the work of Trotter & Hendricks (1976a, 1976b) who used cultures of *Stigeoclonium* growing on glass slides to assess the toxicity of effluents containing chlorine.

Rises in pH and the concentrations of magnesium, calcium or phosphate in the medium all had a marked effect in reducing the toxicity of zinc to a zinc tolerant population of *S tenue*. However, under the conditions used only calcium had a marked effect in reducing the toxicity of the metal to a zinc sensitive population The observation that a rise in pH over the range pH 6.6 - pH 7.6 led to a decrease in the toxicity of zinc contrasts with the work of Mount (1966) with fathead minnows. Similarly Say & Whitton (1977) demonstrated that rises in pH from pH 3 to pH 8 led to an increase in the toxicity of zinc to a zinc sensitive and a zinc tolerant population of *Hormidium rivulare,* and Hargreaves & Whitton (1976b) found similar effects for a population of *H. rivulare* isolated from a stream at pH 3.1. Despite these contrasts with other published results, the filtration experiments descibed in 8.2 suggest that the effects of pH on the toxicity of zinc were not an artefact caused by the precipitation of the metal at higher pH values. However, this does not preclude the 'protection' of filaments at higher pH by the precipitation of a 'blanket' of zinc hydroxide on the surfaces of the cells.

As the populations of *Thlaspi alpestre* ssp. *calaminare* studied by Ernst (1968, 1974) were collected from soil with a high zinc content, it is uncertain whether phosphate ever plays a special role in antagonising the toxicity of zinc to terrestrial plants normally growing in low zinc environments However, it is well established that high concentrations of phosphate may induce zinc deficiency in various crops (e g Burlison & Page, 1967, Stukenholtz *et al.,* 1966; Motsara, 1973). The results of the present study are in accordance with those of Rana & Kumar (1974a) who found that phosphate reduced the toxicity of zinc to *Chlorella vulgaris* and *Plectonema boryanum* The culture of *C. vulgaris* used by these authors was isolated from an effluent of unknown zinc content, whilst the culture of *P. boryanum* was obtained from a culture collection.

Comparison of the results of the toxicity tests with observations on the growth of *S tenue* in a medium free of zinc, but with a similar concentration of test ion, show that magnesium, calcium and phosphate had a quite different influence on antagonism than on growth. The difference is most obvious for magnesium, where raising the concentration from 50 mg l^{-1} to 200 mg l^{-1} brought about an increased antagonism to the toxicity of zinc, whilst at the same time causing a marked reduction in total growth. In the one test where zoospores were formed in all the lower concentrations of zinc, the influence of calcium in antagonising zinc was rather similar for both the settling of zoospores and also for toxicity to growth as a whole (as measured by T I C.). This agrees with the observations made in 7.5, where the concentrations of zinc just permitting the settling of zoospores of two different strains corresponded well with the Tolerance Index Concentration.

Greene *et al.* (1975) suggested that the protection of *Selanastrum capricornutum* from the toxic effects of zinc by ions of Na, Mg, Ca and P was due largely to increases in ionic strength They further suggested that the formation of 'ion pairs', between zinc and such ions might lower the availability of zinc to the alga The results presented in 8.3 - 8.7 tend to contradict this proposal for *S. tenue*, as some ions (e.g. Na^+, Cl^-, SO_4^{2-}) had no detectable effects on toxicity even at very high concentrations.

9 5 CONCLUDING REMARKS

During the present study it was shown that the water and sediments of the R. Derwent contained elevated concentrations of zinc, lead and cadmium These concentrations were high in comparison with data published for most other freshwater systems, and the pollution of the river may be considered to be serious (see 9.212). Enrichment by zinc, lead and cadmium was shown to extend well into the Derwent Reservoir itself, with contamination of water and sediments being especially severe in an area set aside as a nature reserve Despite the large size of the reservoir, 'streaming' of flood water from the R Derwent could on occasions carry elevated concentrations of heavy metals as far as the area where water is drawn off for treatment.

No studies were carried out of the toxicity of heavy metals to plants growing within the reservoir. However, it seems likely that the concentrations of one or more heavy metals in the water and/or sediments of the nature reserve were high enough to limit colonization by some species or strains of submerged plants. The R. Derwent has probably been contaminated to some degree by heavy metals for many years, and it seems probable that the communities of plants within the river have become adapted to the toxic effects of metals. However, it seems possible that the relatively young contaminated environment of the reservoir is still open to 'invasion' by some metal tolerant species or strains of aquatic plants.

The results of intensive sampling of water from four key reaches in the catchment have been presented in Chapter 4. Markedly different patterns of variation in, and interrelationships between, concentrations of heavy metals and other elements were found at these four reaches. These differences were attributed mostly to the effects of discharge and inputs from the Whiteheaps fluorspar Mine. The results of these studies emphasise the possible complexity of factors that may influence concentrations of heavy metals and other chemical parameters at any point on a particular stream or river, as noted by Wilson (1976) and Hughes & Edwards (1977)

Say (1977) noted that with the exception of Bolts Burn, the catchment of the R. Derwent contained fewer streams contaminated by zinc than did other areas of the Northern Pennine Orefield. He attributed this to the position of the catchment at the edge of the main ore-bearing area of the orefield. It is clear from the results of the present study that the high concentrations of zinc, lead and cadmium noted in Bolts Burn and the R. Derwent were maintained almost solely by the effects of discharges from the Whiteheaps fluorspar Mine. From the variations in concentrations that were encountered during the three year period, it seems reasonable to assume that if all inputs of heavy metals from the Whiteheaps Mine were removed, then the concentrations of zinc carried by the water of Bolts Burn entering the R. Derwent would never rise above about 0.4 mg l^{-1} (roughly one-third of the mean concentration recorded during the project). Even more marked decreases might be expected in the case of lead, leading to the virtual elimination of the pollution problem after the water of Bolts Burn was diluted by the R. Derwent At the time of preparation of this account, it seems probable that changes in the method of treatment of the Whiteheaps Mine Effluent will result in significant decreases in the levels of zinc, lead and cadmium entering Bolts Burn

From the results presented in chapter 6 and discussed in 9 3, it is clear that *Lemanea fluviatilis* is potentially a very useful plant for 'monitoring' pollution by heavy metals in flowing waters. It is suggested that the results of the present project provide sufficient background data to enable useful information to be gained by simply sampling pooled 2 cm long tips of filaments of *Lemanea* from populations in the field. One criticism of the use of *Lemanea* in monitoring lies in its relatively short season of growth However this, together with the relatively slow growth of the sexual shoots, would enable reliable estimates to be made of the period of time for which filaments had been present in the river being examined. Such estimates could be made less easily for species like *Cladophora,* which grow much more rapidly. Another possible drawback to the use of *Lemanea* is that its occurrence in rivers is more restricted than other species used for monitoring (e.g. *Cladophora, Eurhynchium)*. However, it is clear from 6.4 that accurate results may be obtained by transplanting boulders covered in *Lemanea* into flowing waters where natural populations do not occur, and sampling for metal content after a suitable period (5 - 6 weeks). Other advantages in the use of *Lemanea* for monitoring include the fact that the filaments usually grow in comparable (i e fast flowing) sites within different rivers, where they show very little tendancy to trap silt

The results presented in 6 4 demonstrated conclusively that the zinc content of *Lemanea* in the R. Derwent reach 07 was subject to marked short term fluctuations in response to fluctuations in the content of the water This did not appear to be the case for lead or cadmium, and it is uncertain to what extent the fluctuations in zinc content were affected by other environmental factors (e g the organic content of the water). However, the kinetics of uptake of zinc, lead and cadmium by material from five reaches after transplanting into a high concentration of zinc appeared to be affected in some way by changes in the heavy metal content of the water following transplanting (Figs 6.16 - 6.18). It is unforunate that samples of river water for analysis were not collected at the same time as samples of *Lemanea* during the course of the transplant experiment.

No conclusive evidence was found during the present study to demonstrate whether or not populations of *L. fluviatilis* growing in higher concentrations of zinc in the field were forms that were adapted in any way to withstand the toxic effects of the metal. However, it is clear that if such adaptation does exist, it does not complicate the use of the plant as a monitor of contamination by heavy metals.

In contrast, conclusive evidence was found that populations of *Stigeoclonium tenue* growing at higher concentrations of zinc in the field were adapted forms that could withstand higher concentrations of zinc than could populations from non-polluted sites The level of tolerance shown by a particular population was shown to be a stable character that was probably determined genetically.

However, observations made in the field indicated that the level of tolerance needed for the alga to survive at a particular concentration of zinc may be affected markedly by other environmental factors such as the concentration of calcium. The results of assays carried out in the laboratory confirmed that the range of variations in concentrations of calcium and magnesium that were found in the water at several of the sites could be expected to influence markedly the toxicity of zinc to any particular strain. Say, Diaz & Whitton (1977) provided strong evidence that zinc and cadmium were synergistic in their toxicity to *Hormidium rivulare*. No investigations were carried out of such possible synergism to *S. tenue* However, the results for a population isolated from a site with a relatively high ratio of lead . zinc concentrations in the water indicated that synerism between these two metals does not occur to a high degree. Because of the probable influence of such environmental factors the 'threshold' concentration of zinc in the water above which adaptation to zinc occurs in *S. tenue* probably differs between sites. However, the results of the present project indicate that this threshold occurs commonly in the region 0.1 - 0.3 mg l^{-1} Zn.

SUMMARY

A three year study has been carried out of the sources and distribution of the heavy metals zinc, lead and cadmium within the water, sediments and submerged plants of the Derwent Reservoir and its catchment. This has been extended to include a survey of accumulation of heavy metals by populations of *Lemanea fluviatilis* growing in a number of rivers in England and Wales Further field and laboratory studies have been carried out on the resistance to zinc of *Stigeoclonium tenue*

Data from several surveys of the composition of water demonstrated that relatively high concentrations of zinc, lead and cadmium were being carried into the Derwent Reservoir by the R. Derwent. The mean (unfiltered) concentrations of these metals in the water of the river entering the reservoir (based on 65 samples) were 0.217 mg l^{-1} Zn, 0.061 mg l^{-1} Pb and 0 0018 mg l^{-1} Cd. Markedly higher concentrations were noted on several occasions

Surveys of the composition of water, sediments and submerged plants within the catchment of the R. Derwent showed that almost all of the heavy metals carried by the river were derived from only two tributaries. Of these, Bolts Burn, entering the river 3.5 km upstream of the reservoir, was by far the more important. This large tributary was sampled for water 100 times at its point of entry to the R. Derwent, with mean (unfiltered) concentrations of 1.45 mg l^{-1} Zn, 0.28 mg l^{-1} Pb and 0.0060 mg l^{-1} Cd being recorded.

On every occasion when the R. Derwent was sampled for water, only very low concentrations of zinc, lead and cadmium were found upstream of the entry of Bolts Burn ($\bar{x}$ = 0.026 mg l^{-1}, 0 007 mg l^{-1} and 0 0005 mg l^{-1} respectively, n = 48). Large increases in the concentrations

of these metals were found downstream of the entry of the tributary, with a subsequent gradual fall-off in concentrations on passing downstream towards the reservoir. This pattern was still apparent when the river was in flood or when the water table was high.

Surveys of the composition of water within the valley of Bolts Burn demonstrated that almost all of the heavy metals carried by the stream were derived from three clearly defined sources within an active fluorspar mine. These were the Whiteheaps Adit Level, effluent from a fluorspar treatment plant, surface runoff from tips within the mine area. Most zinc and cadmium were found to enter Bolts Burn in non-particulate form from the adit Most lead entered the stream as discrete particles from the effluent discharge or in surface runoff Inputs from the three sources were found to be completely independant of each other, this led to marked short term, independant fluctuations in the concentrations of zinc, lead and cadmium within the water of Bolts Burn downstream of the fluorspar mine. 100 separate samples of water were collected from Bolts Burn at its point of entry to the R. Derwent, and variations in the concentrations and interrelationships between different elements were shown to be attributable directly to changes in inputs from the three sources as described above. The only marked effect of increased discharge upon the heavy metal content of water in the reach was to dilute out elements derived mostly from the Whiteheaps Adit Level In contrast, variations in the concentrations of various elements in the water of the R. Derwent upstream of Bolts Burn were found to be related to a large extent to variations in discharge. Some of these elements (e g Mn, Fe, Al) were associated with suspended material that increased during higher discharges. Others (e.g. Mg, Ca) were diluted out on such occasions

Substantial build-ups of concentrations of zinc, lead and cadmium were noted in water, sediments and submerged plants at the inflow end of the reservoir in an area set aside as a nature reserve Here the concentrations of metals present in the water were similar to those recorded in the R. Derwent, and concentrations of up to 2000 µg g^{-1} Zn, 1500 µg g^{-1} Pb and 15 µg g^{-1} Cd were recorded in sediments (after digestion with HNO_3) Concentrations of zinc in sediments were found to fall off more slowly than those of lead on passing away from the mouth of the river, and high concentrations of the former metal (around 750 µg g^{-1}) were still present in sediment near the dam. Similar decreases were noted in the heavy metal content of rootless and rooted submerged plants on passing away from the mouth of the river.

The distribution of heavy metals within the water column of the reservoir was shown to be affected considerably by hydrological factors Water with an elevated zinc content was shown to 'stream' along the full length of the reservoir following high spates on the R. Derwent, and these higher concentrations almost certainly entered the water drawn off for treatment on occasions. Increased concentrations of zinc were detected throughout the reservoir following a prolonged period of filling and turbulence

The concentrations of zinc, lead and cadmium in sediments at the inflow end of the reservoir were high in comparison with most other published studies of the composition of lake sediments. Comparable concentrations reported in the literature include those of Maxfield *et al.* (1974b) in the sediments of the Coeur d'Alene Lake, Idaho. In that lake the pollution by heavy metals was described as 'very serious' The concentrations of zinc in sediments within the nature reserve also approached those described by Bradshaw (1970) in soil on a mine where adaptation to zinc was demonstrated in *Anthoxanthum odoratum* The low calcium content of the water and sediments within the reservoir makes it seem unlikely that calcium plays any significant role in reducing the toxicity of heavy metals (as shown for *Stigeoclonium tenue*), and it seems possible that the toxic action of metals is limiting colonization by some submerged species

Of the two most common submerged macrophytes within the reservoir, *Nitella flexilis* probably accumulated all of its metal content from the water. However, it seems possible that *Glyceria fluitans* took up some metals from the sediment in which it was rooted. It is notable that whilst fractionation studies demonstrated that a large proportion of the lead that entered the Derwent Reservoir was in particulate form, high concentrations of this metal (up to 1060 $\mu g\ g^{-1}$) were taken up by plants of *Nitella flexilis* within the nature reserve.

On the basis of the results of regular sampling of water from the R. Derwent together with measurements of discharge, estimates were made of the annual additions of zinc, lead and cadmium to the reservoir It was calculated that the average amounts of these metals being retained per m^2 of reservoir bottom (when full) per year were : zinc, 1.4g, lead, 0.5g, cadmium, 0 016g.

Studies of the accumulation of heavy metals by *Lemanea fluviatilis* provided valuble supplementary data on the distribution of metals within the R. Derwent. As well as reflecting changes in concentrations of metals along the river, the zinc content of the plant was found to be sensitive to short term fluctuations in the zinc content of the water. The lead and cadmium content of filaments did not show such short term fluctuations.

Further collections of *Lemanea* from 47 sites in England and Wales confirmed that the alga is a potentially useful 'monitor' of pollution by zinc, lead and cadmium in flowing waters. The results emphasise the need to carry out comprehensive background studies before using any species of submerged plant as such a 'monitor', since the enrichment ratios for zinc, lead and cadmium were found to decrease markedly with increased concentrations of each metal in the water. However, clear linear relationships were found between the concentration of each metal in the water and in filaments of *Lemanea*. There was a slight indication from the data that high concentrations of calcium in the water caused a reduction in the concentration of zinc taken up by filaments at a particular site, but no such indications were found for lead or cadmium.

When filaments of *Lemanea* were exchanged between reaches with high and low concentrations of zinc in the water, the subsequent rates of uptake or loss of zinc confirmed that a proportion of the content of the metal in the plant was bound only loosely. About 20% of the zinc content of material from the R. Derwent downstream of Bolts Burn was removed rapidly by a solution of EDTA, but not by double distilled water as used for washing filaments.

When filaments of *Lemanea* from several reaches were transplanted into reaches with high concentrations of zinc in the water, no differences in patterns of uptake of zinc, lead or cadmium were observed between filaments from unpolluted and polluted reaches. Rather, the heavy metal contents of all of the populations reached equilibrium at a concentration very similar to that which might have been expected had natural populations been present in the reach into which they were transplanted There was therefore no evidence that populations of *Lemanea* growing in higher concentrations of zinc in the field were forms with an enhanced resistance to the metal. Even if such enhanced tolerance to zinc does exist in *Lemanea*, it does not appear to affect the use of the alga for 'monitoring'

Field and laboratory studies were carried out on the tolerance to zinc of *Stigeoclonium tenue*, an alga common within Bolts Burn and the R. Derwent 35 sites in England, France, Belgium and Germany were included, the majority of them being chosen to represent examples of pollution by zinc resulting from past or present mining activities. *S. tenue* was sometimes abundant in waters polluted by zinc, and was found at sites with concentrations of up to 20 mg l^{-1} of (filtrable) zinc in the water.

The results of toxicity tests performed in the laboratory demonstrated that material growing in higher concentrations of zinc in the field had a greater resistance to the metal than material growing in lower concentrations of zinc The level of tolerance remained unchanged in both a zinc sensitive and a zinc tolerant population after six months of culturing at different concentrations of zinc in the medium It was therefore suggested that the enhanced tolerance was the result of genetic adaptation. The results of the assays of different populations suggested that the 'threshold' concentration of zinc above which adaptation occurred was about 0.2 mg l^{-1}. Assays of populations taken from high concentrations of calcium in the field demonstrated that these were less tolerant of a particular concentration of zinc than were populations from waters with low concentrations of calcium.

Concentrations of zinc that brought about a marked reduction in total growth during assay had a relatively greater effect on the erect part of the thallus than on the basal system. Nevertheless in the field *S. tenue* was restricted to a basal growth form only at the site with the highest concentration of zinc.

Investigations were carried out of the effects of pH, magnesium, calcium and phosphate on the toxicity of zinc to *S. tenue* The effects of these factors on toxicity were found to differ between a zinc sensitive and a zinc tolerant population. With the zinc sensitive population a rise in pH, magnesium or calcium brought about a slight reduction in toxicity, the effect being most pronounced with calcium. Rises in all four parameters brought about a reduction in the toxicity of zinc to a zinc tolerant population, the reduction being marked in the cases of magnesium, calcium and phosphate.

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RESISTANCE TO ZINC OF *STIGEOCLONIUM TENUE* IN THE FIELD AND THE LABORATORY

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Field and laboratory studies have been carried out on the tolerance to zinc of *Stigeoclonium tenue* growing in flowing waters. Thirty-four different sites in England, France and Germany were included, the majority of them being chosen to represent examples of zinc pollution resulting from past or present mining activities. *S. tenue* was sometimes abundant in zinc polluted waters, and was found at sites with up to 20.0 mg l^{-1} of zinc capable of passing through a filter. At sites with field mean zinc levels of about 0.2 mg l^{-1} and above, populations show increased resistance to zinc in comparison with populations from sites with lower zinc levels, this increased resistance being largely, if not entirely, the result of genetic adaptation. Assays of populations from sites with high calcium levels suggest that these are less tolerant of a particular level of zinc than are populations from sites with low calcium levels. Levels of zinc bringing about a marked reduction in total growth during assay have a relatively greater effect on the erect part of the thallus than on the basal system. Nevertheless in the field *S. tenue* was restricted to a basal growth form only at the site with the highest level of zinc.

The occurrence of *Stigeoclonium tenue* Kutz. in streams polluted by heavy metals has been reported by many authors (e.g. Weimann, 1952; Palmer, 1959; Fjerdingstad, 1965; Whitton, 1970). There is some evidence that populations of this alga from sites with high zinc levels are more tolerant to zinc than those from sites with low zinc levels (Whitton, 1970; Whitton & Say, 1975). However, as a result of a field survey in South and West Wales, McLean (1974) suggested that the tolerance of *S. tenue* to zinc (and lead) is possibly simply the result of its tolerance and affinity for organic pollution. In the absence of the latter (high levels of) zinc would not be tolerated. The present survey was planned both specifically to clarify these conflicting observations in the literature and also to give a general account of the behaviour of *S. tenue* in zinc rich environments.

MATERIALS AND METHODS

LOCATION OF SITES

Materials of *Stigeoclonium tenue* were collected from 34 stream (or river) sites, 30 in North-East England, 2 in France and 2 in Germany (Table 1), the sites being chosen to show as wide a range as possible in levels of zinc in the water. With a single exception, the sites were all on different streams. All the higher levels of zinc were from streams in regions with a long history of mining. In most cases mining activity had ceased in the catchment area of the particular stream, but in some cases mine operations were still going on, and with a few streams the levels of zinc have probably risen slightly in recent years.

TAXONOMY

All the populations studied were referred to *Stigeoclonium tenue* Kutz. However, a critical cultural study of basal thalli was not carried out, so it is possible that some populations would correspond better with *S. pascheri* (Vischer) Cox & Bold in the study of the genus made by Cox & Bold (1966).

SAMPLING PROGRAMME

Samples of water were collected from each of the sites and analysed for 31 different physical and chemical parameters Based on the results of this survey, a reduced analytical programme involving six chemical parameters was chosen for the present study Water and algal samples were then collected on four separate occasions during April–June 1975 from each of the English sites and once from each of the French and German sites during July–September 1975 Some of the sites sampled are the subject of long-term chemical and floristic studies by the authors, and certain data from these are also included

WATER ANALYSIS

All the samples for the present study were collected during periods of low or medium flow, when the water chemistries of the streams might be expected to have been relatively stable for some days prior to the sampling Apart from the in situ measurement of pH, all the analyses for the reduced sampling programme were made on water filtered through a No 2 SINTA glass funnel Considerable care was taken to standardize the filtration technique, this including a wash of the funnel with HCl whenever there was any likelihood of zinc contamination of the pores of the sintered glass Samples for cation analysis were stored in HCl-washed PYREX bottles, while samples for phosphate analysis were stored in heavy duty iodized polythene bottles In most cases the samples were kept until analysis at as low a temperature as possible without actually freezing them but if delay was inevitable, the samples for phosphate analysis were stored at −20°C

TABLE I Location and mean water chemistries of *Stigeoclonium tenue* sampling sites Sites 1–30 sampled four times, sites 31–34 only once (All concentrations in mg l^{-1})

Site no	Site	Grid ref	Field pH	K	Mg	Ca	Cu	Fe	Pb	Cd	PO_4-P
1	Rookhope Burn	NY 942421	7 7	2 68	5 88	33 2	0 007	0 32	0 053	0 0010	0 014
2	Rampgill Level	NY 781434	7 6	4 53	22 35	76 4	<0 002	0 13	0 003	0 0060	0 015
3	R Nent below Nenthead	NY 767448	8 0	2 98	10 25	47 2	0 002	0 44	0 046	0 0056	0 016
4	Waskerley Beck	NZ 077375	7 8	1 75	3 80	22 9	<0 002	0 62	0 002	<0 0001	0 012
5	Beech Burn	NZ 164333	7 8	6 80	31 70	88 0	0 016	0 17	0 004	0 0004	0 115
6	R Deerness	NZ 226422	7 8	9 03	37 85	77 3	0 005	0 24	0 008	0 0020	0 139
7	Red Burn	NZ 225420	7 5	7 06	38 3	71 6	0 009	0 22	0 006	0 0400	0 038
8	R Browney	NZ 222454	7 6	4 59	16 19	44 2	0 003	0 31	0 006	0 0010	0 170
9	Nickynack Gill	NZ 262374	7 5	8 90	51 00	93 0	0 002	0 15	0 008	0 0003	0 146
10	Sunderland Bridge Sewage outfall	NZ 264377	7 8	23 50	15 95	97 8	0 007	0 39	0 009	<0 0001	0 817
11	Hollingside stream	NZ 274401	7 4	17 95	9 85	50 5	0 097	0 42	0 006	0 0003	4 711
12	Cemetery stream	NZ 273400	7 5	7 03	18 90	63 1	0 010	0 32	0 006	0 0002	1 094
13	Hollingside Lane Artificial stream	NZ 275408	7 7	1 45	2 55	22 5	0 002	0 06	0 008	<0 0001	0 016
14	Kilhope Burn	NY 809432	4 5	0 67	1 73	7 1	0 002	0 96	0 072	0 0005	0 007
15	Cong Burn	NZ 267516	7 9	9 99	32 98	110 2	0 002	0 40	0 003	0 0001	0 388
16	Old Durham Beck	NZ 294414	7 6	10 50	38 35	120 2	0 010	0 26	0 007	0 0002	0 444
17	Shildon Burn	NY 966503	7 4	2 30	3 55	13 2	0 002	0 29	0 051	0 0200	0 011
18	R Skerne	NZ 291207	7 6	13 18	39 55	115 8	0 011	0 55	0 007	0 0004	0 903
19	Lumley Park Burn	NZ 288513	7 5	16 83	38 80	114 9	0 005	0 27	0 002	0 0003	0 556
20	Sherburn Beck	NZ 319418	7 5	3 50	34 38	103 3	0 006	0 13	0 002	0 0010	0 556
21	Redburn Flush	NY 931431	7 6	10 99	42 20	217 1	0 012	0 05	0 057	0 0140	0 547
22	Redburn Stream	NY 931431	7 8	8 31	14 80	84 0	0 007	0 24	0 072	0 0010	0 007
23	R Nent at Alston	NY 716467	8 0	2 90	9 73	57 0	0 004	0 20	0 015	0 0040	0 026
24	R South Tyne	NY 716469	8 1	1 37	4 79	40 6	0 004	0 11	0 009	0 0020	0 024
25	Garrigill Burn	NY 792425	7 4	1 89	6 38	35 6	0 003	0 25	0 362	0 0390	0 012
26	R Derwent	NY 767502	7 6	2 14	4 43	19 9	0 002	0 41	0 023	0 0010	0 009
27	Brown Gill	NY 764423	6 3	1 14	1 38	6 0	0 003	0 90	0 306	0 0020	0 018
28	R East Allen	NY 858448	6 8	2 57	6 18	36 7	0 004	0 44	0 015	0 0004	0 040
29	Gillgill Burn	NY 795440	6 0	1 94	4 68	18 3	0 004	0 25	0 497	0 0430	0 016
30	Bolts Burn	NY 958499	7 5	6 13	7 23	36 6	0 011	0 79	0 278	<0 0001	0 010
31	R Sulz	25839/56444	7 5	4 00	8 10	54 3	0 009	0 20	0 005	0 0020	—
32	Rammelsberg Stollen	35978/57519	7 6	4 40	29 20	180 0	0 073	0 02	0 001	0 0920	0 005
33	La Gueule near Hergenrath	25003/586186	8 3	3 30	21 40	80 0	0 006	0 21	0 003	0 0020	0 236
34	Le Pont Péan Fosse	5959/53186	7 8	7 50	11 20	57 8	0 007	1 00	0 107	0 0102	0 830

The comprehensive water analysis for the initial survey included the following temperature, O D at three different wavelengths, conductivity, pH, Eh total alkalinity, Na, K, Mg, Ca, Zn, Cu, Mn Fe Al, Pb Ni, Co, Cd, Ag, Al, Si, PO_4–P NH_4–N, NO_2–N, NO_3–N, Cl, SO_4–S, F The data from these were used to select a reduced number of parameters which both varied markedly among the sites, and which from a knowledge of the literature (e g Ernst, 1974 Whitton & Say 1975) it seemed possible that they might influence zinc toxicity Data on levels of zinc are included in Table III the other results for this reduced analytical programme are given in Table I Of the parameters in the reduced programme pH was measured using a PYE UNICAM meter model 293 cations using a PERKIN–ELMER 403 atomic absorption spectrophotometer, and PO_4–P using the n-hexanol extraction method of Mackereth (1963)

CULTURE MEDIUM

The medium used for laboratory assays was based on the No 10 formula of Chu (1942), but modified in particular by lowering the pH and the level of phosphate A version of the medium free of added zinc was used for routine culturing and experimental controls although analysis of this medium showed levels of zinc < 0 002 mg l^{-1}, there was no detectable difference between cultures grown in this medium and cultures in the lower concentrations of added zinc This indicates that if *S tenue* has any growth requirement for this element, it is satisfied by very low levels of it The basal medium was made up as follows KH_2PO_4, 8 mg l^{-1}, $MgSO_4$ $7H_2O$ 25 mg l^{-1}, $Ca(NO_3)_2$ 40 mg l^{-1} $NaHCO_3$, 8 mg l^{-1} Na_2SiO_3 10 mg l^{-1} Fe 0 5 mg l^{-1} (as ferric iron—ethylenediaminetetra-acetate chelate) C microelements of Kratz & Myers (1955) omitting Zn 0 25 ml l^{-1} This medium, with a pH of 6 6 and the presence of a chelating agent was adopted in order to avoid significant precipitation of zinc or any other metal during the toxicity tests The medium is 0 9 pH units below the mean field pH values of the sites studied Filtration of samples of media with added zinc (as $ZnSO_4$ $7H_2O$) through 0 22 μm NUCLEPORE filters has shown that at even the highest concentrations used (40 mg l^{-1} Zn) in the tests involving standard basal medium, at least 88% zinc was present in the filtrate (P J Say personal communication) The highest levels of added zinc led to a reduction in pH of only about 0 1 units (Checks on pH during culture showed no detectable shift from initial values) The concentrations of the major elements present in basal medium are (in mg l^{-1}) Na 5 94 K 2 25 Mg 2 47 Ca, 9 77, Fe, 0 5, Si, 2 30 PO_4–P, 1 78, NO_3–N 3 41 SO_4–S 3 26

PREPARATION OF ALGA FOR ASSAY

Samples of *Stigeoclonium tenue* were collected from as small an area as possible such that there was still sufficient material to carry out tests It was usually possible to collect enough alga from a single clump covering about 1 cm^2 rock surface Wherever feasible the alga was collected from the part of the site with the fastest current speeds and with the minimum associated silt Samples collected at one time from one small area are throughout this account referred to as one population, whether the material was studied immediately or subcultured for long periods

The alga used for the standard toxicity tests was assayed within 48 h from the time of collection in all instances except for the samples from France and Germany It was stored in water from the stream of collection until 24 h before the test when it was transferred to basal medium at 18°C, 6,000 lx (cool white fluorescent light) with moderate shaking, for the remaining 24 h Samples of the alga were then removed for microscopy to check that there were no obvious algal contaminants A small inoculum of alga was added to each of the boiling tubes used in the assay these containing 10 ml medium The inoculum was made as uniform as possible only a few short filaments being present in each tube Checks on similar aliquots of alga to those used for the tests indicated that in most cases the inoculum lay in the range 5–10 mg l^{-1} dry weight

TOXICITY TEST

The assay used was a refinement of that described by Whitton (1970), and involved incubation of samples of each field population under standard laboratory conditions At least 25 tubes were used for each test As it became possible to predict the effects of zinc on a particular population, then the range of zinc concentrations used could be narrowed, and the accuracy of the assay was then increased Inocula were added to media to which any zinc had already been added, and the tubes were then incubated under similar conditions to those used during the pre-incubation period 18°C, 6,000 lx, with moderate shaking, the tubes being placed in a rack at an angle

Growth in the tubes was compared visually on days 2, 4 and 6, both against preserved replicates of the original inocula, and also with each tube one against the other If, by day 4 a marked increase had not taken place in the controls, or if any algal contamination had become apparent, the test was discarded Otherwise, observations were recorded on each occasion according to the following (semi-quantitative) scale

I maximum concentration causing no inhibition,
II minimum concentration causing slight inhibition,
III maximum concentration at which alga is alive,
IV minimum concentration at which alga is killed

There was seldom any difference between the values observed for I and II on days 2, 4 or 6, in contrast to observations on some other species studied by Whitton (1970) This indicates that any slight inhibition of growth observed is due more to a reduction in rate of exponential growth than in increase in a lag The values used for III and IV for the assay were in practice based on the observations recorded for day 6 Wherever there was any ambiguity about the results for III and IV, filaments were removed both for microscopic inspection and for re-incubation in basal medium

EVALUATION OF TOXICITY TEST

As there were few indications from field observations as to which growth stages of *S tenue* are the most sensitive to zinc the data from the toxicity tests are presented in several different ways for later comparison with field concentrations of zinc The following empirical formulae are used

just non-inhibitory $= (\mathrm{I} \quad \mathrm{II})^{\frac{1}{2}}$
just lethal $= (\mathrm{III} \quad \mathrm{IV})^{\frac{1}{2}}$
Tolerance Index Concentration (T I C) $= (\mathrm{I} \quad \mathrm{II} \quad \mathrm{III} \quad \mathrm{IV})^{\frac{1}{4}}$

ADAPTATION STUDIES

Four different populations from each of two sites were used for further study to determine whether any changes in tolerance could be detected after long periods of growth in medium containing various zinc concentrations These populations were from site 13 with the lowest zinc concentration found (0 012 mg l^{-1}) and site 2, with a relatively high zinc concentration (2 39 mg l^{-1}) The cultures were maintained under similar growth conditions to those used for the standard tests, with the exception that incubation took place in 100 ml conical flasks with 25 ml medium Each of the eight populations was maintained for 6 months both in a medium lacking any zinc and in one found during an initial assay to cause moderate inhibition of growth for the particular population Sub culturing was carried out at frequent intervals, care being taken to avoid fresh contamination Standard assays were made at the beginning and end of the 6-month period

RESULTS

FIELD OBSERVATIONS

The highest zinc level at which *Stigeoclonium tenue* was found was at 20 0 mg l^{-1} at site 32 in the Harz Mountains, Germany This population was present in a predominantly basal form not corresponding closely to any text figure of *Stigeoclonium* In culture, however, branched filaments developed resembling those of the other populations Obvious branched filamentous growths of the alga were present at all the other sites The highest zinc level at which the alga was found from England was 7 1 mg l^{-1} Two other sites carrying concentrations of zinc higher than 20 0 mg l^{-1}, although the subject of detailed study, have never shown the presence of *Stigeoclonium*

S tenue was found to be abundant not only at some of the sites with low zinc concentrations, but also at some of the higher ones At some examples of both low and high zinc sites it was abundant throughout both spring and summer In some of the high zinc streams this occurred even where the PO_4–P content was quite low, in apparent contrast to the behaviour of *S tenue* observed in low zinc rivers by McLean & Benson-Evans (1974), where the alga remained conspicuous in summer only at those sites with a high organic content. At site 23 *S. tenue* has been the dominant alga in both spring and summer for (at least) the last 7 years. At only site 2 has *S. tenue* been observed to be the dominant alga throughout the year, with erect growths predominating at all times. This stream arises from a subterranean source and has a relatively small annual temperature fluctuation.

ASSAYS

From the results of the toxicity tests, it soon became apparent that higher levels of zinc were needed to inhibit the growth of some populations of the alga more than others, and that these populations also required higher concentrations of zinc to cause death by the end of the 6-day culture period. In contrast many tests have shown that repeat experiments on the same population give rather similar results (e.g. Table II). Long-term subculturing of two different populations in the presence of low and "high" zinc concentrations did not lead to any change in response over the period (Table II). A rather similar study on a population from site 3 has also given a similar result (C. Rajendran personal communication). These results indicate that differences in response to zinc by different populations in short-term tests are a reflection of genetic and not environmental adaptation.

TABLE II. Results of toxicity tests performed upon representative non-adapted and adapted populations of *Stigeoclonium tenue* after culture at various zinc levels. (All concentrations in mg l^{-1})

Site no.	Pretreatment	Field zinc level $\bar{x}$	Field zinc level s.d.	I $\bar{x}$	II $\bar{x}$	Just non inhibitory $\bar{x}$	Just non inhibitory s.d.	III $\bar{x}$	IV $\bar{x}$	Just lethal $\bar{x}$	Just lethal s.d.	T.I.C. $\bar{x}$	T.I.C. s.d.
13	Fresh material	0.012	0.005	0.69	0.94	0.80	0.38	2.00	2.31	2.15	0.76	1.31	0.54
	Cultured 6 months at 0 mg l^{-1} Zn			0.63	0.88	0.74	0.13	2.06	2.50	2.27	0.33	1.25	0.08
	Cultured 6 months at 0.75 mg l^{-1} Zn			0.50	0.75	0.61	0.00	2.13	2.50	2.30	0.36	1.18	0.09
2	Fresh material	2.390	0.513	2.00	2.38	2.18	0.41	10.75	13.50	12.14	2.24	5.10	0.54
	Cultured 6 months at 0 mg l^{-1} Zn			2.13	2.88	2.56	0.22	10.65	13.13	11.81	1.09	5.28	0.49
	Cultured 6 months at 5.0 mg l^{-1} Zn			2.06	2.50	2.27	0.31	11.25	13.75	12.44	1.26	5.50	0.48

The results of the toxicity tests performed on *S. tenue* from all thirty-four sites are given in Table III. Levels of laboratory tolerance are clearly related to the mean field zinc levels (Table IV). However, no significant correlation could be found between variations in the T.I.C. among populations from one site collected on different days, and variations in the water chemistry collected on the same days. A scatter diagram of the results for the T.I.C. studies (Fig. 1) suggests that at mean field zinc levels of about 0.2 mg l^{-1} and above the alga is more resistant to zinc than from sites with lower mean field zinc levels.

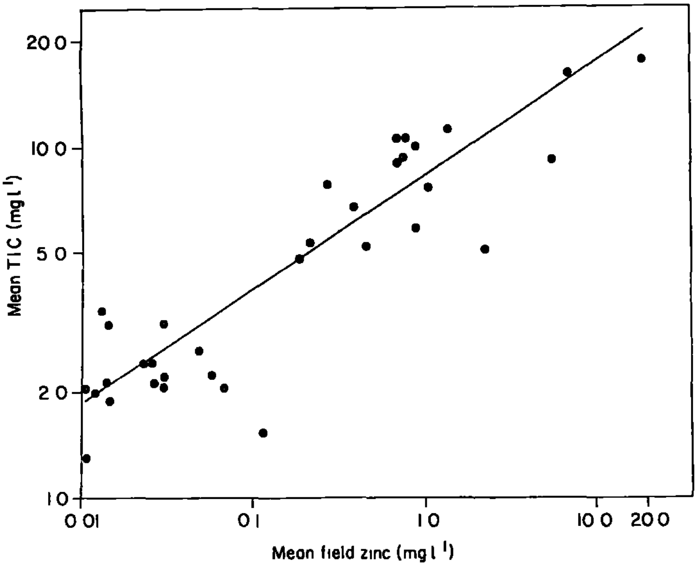

FIG 1 Correlation of logarithm of mean values of Tolerance Index Concentration and logarithm of mean concentrations of field zinc for *Stigeoclonium tenue* Regression equation is $= 0\,33x+0\,90$ correlation coefficient $= 0\,91$

Of the three methods of evaluating toxicity described above the T I C gave the highest correlation coefficient with the mean field zinc level (Table V) With all three methods, however, the absolute values indicated in the laboratory were almost always higher than the mean field zinc levels (Table III, Fig 2)

MICROSCOPY

The populations from sites 2 and 13 used in the study of adaptation were also used for more detailed microscopic observation The behaviour of both populations in response to zinc was similar, although the absolute levels of zinc bringing about a particular response was higher in the adapted than the non-adapted population At the end of the 6-day incubation period, long branched erect filaments predominated in the controls With increasing zinc levels above the just non-inhibitory level, the proportion of these long erect filaments decreased until almost all growth occurred as twisted "basal" filaments composed of swollen cells with a few very short branches As these concentrations approached the just lethal level, growth of the original inoculum ceased, and a greater proportion of the cells were white (dead) At the lower zinc levels some release of zoospores occurred from the erect filaments of both populations, followed by settling and germination on the walls of the tubes The maximum levels at which settling of zoospores was observed were rather similar to the mean T I C a similar result was found with the one other instance (site 23) where zoospore release occurred at all the lower zinc concentrations (Table IV)

TABLE III Mean and maximum field zinc levels at *Stigeoclonium tenue* sampling sites, together with mean results of toxicity tests (Maximum zinc levels collected during further surveys but not included in estimate of mean levels of zinc are marked *) All toxicity tests carried out four times using four different populations for sites 1–30 but the same population repeated four times for sites 31–34 (All concentrations in mg l^{-1})

Site no	Field Zn $\bar{x}$	Field Zn s d	Field Zn max	Just non-inhibitory $\bar{x}$	Just non-inhibitory s d	Just lethal $\bar{x}$	Just lethal s d	T I C $\bar{x}$	T I C s d
1	0 459	0 204	0 640	4 10	0 25	6 62	0 25	5 21	0 21
2	2 390	0 513	3 080	2 18	0 41	12 14	2 24	5 10	0 54
3	1 100	0 636	2 000	5 42	0 51	11 00	0 29	7 71	0 37
4	0 013	0 018	0 040	2 11	0 25	5 49	0 29	3 40	0 26
5	0 030	0 020	0 051	1 61	0 15	2 65	0 18	2 06	0 08
6	0 012	0 006	0 020	1 39	0 20	2 86	0 25	1 99	0 22
7	0 027	0 015	0 050	1 65	0 16	2 74	0 41	2 13	0 25
8	0 014	0 011	0 030	2 08	0 25	4 62	0 25	3 09	0 23
9	0 115	0 048	0 161	1 23	0 00	1 87	0 00	1 51	0 00
10	0 023	0 006	0 030	1 94	0 00	2 99	0 29	2 40	0 12
11	0 048	0 019	0 070	1 54	0 24	4 49	0 29	2 63	0 25
12	0 025	0 030	0 071	1 54	0 13	3 87	0 25	2 44	0 10
13	0 012	0 005	0 020	0 80	0 38	2 15	0 76	1 31	0 54
14	0 054	0 020	0 980*	1 56	0 32	3 24	0 82	2 20	0 24
15	0 014	0 007	0 020	1 54	0 13	2 99	0 29	2 14	0 17
16	0 010	0 009	0 020	1 61	0 15	2 61	0 25	2 04	0 09
17	0 394	0 079	0 460	1 84	0 29	24 87	2 90	6 74	0 77
18	0 030	0 016	0 050	1 54	0 13	3 37	0 25	2 28	0 10
19	0 015	0 011	0 030	1 29	0 13	2 75	0 25	1 88	0 10
20	0 068	0 051	0 124	1 29	0 13	3 25	0 25	2 05	0 18
21	5 067	0 653	5 500	4 97	2 49	17 71	7 18	9 36	4 19
22	1 430	0 525	2 180	5 75	2 18	23 62	2 51	11 44	1 83
23	0 904	0 490	1 400*	2 61	0 48	13 62	4 40	5 88	1 06
24	0 187	0 113	0 320	2 05	0 21	11 45	2 12	4 81	0 30
25	0 767	0 238	1 190*	3 67	0 58	31 15	7 53	10 68	2 06
26	0 284	0 190	0 500	2 42	0 48	26 12	10 36	7 88	2 24
27	0 720	0 014	1 120*	3 10	0 42	37 42	0 00	10 75	0 73
28	0 215	0 105	0 290	1 49	0 87	19 56	6 94	5 32	2 48
29	7 092	2 295	13 700*	8 66	0 00	31 15	4 80	16 39	1 25
30	0 881	0 450	2 100*	5 30	0 95	19 84	2 91	10 15	0 17
31	0 770	—	—	3 65	0 87	24 94	8 18	9 53	2 71
32	20 000	—	—	6 63	2 00	48 07	5 80	17 81	3 74
33	0 029	—	—	2 16	1 87	4 62	4 00	3 16	2 74
34	0 710	—	—	3 41	0 29	27 00	13 93	9 24	2 43

TABLE IV List of T I C values for the three examples noted where zoospore release and settling was observed in every tube up to a particular zinc concentration (All concentrations in mg l^{-1})

Site	T I C	Maximum concentration at which zoospores settled
2	5 10	6 0
13	1 31	1 25
23	5 88	6 0

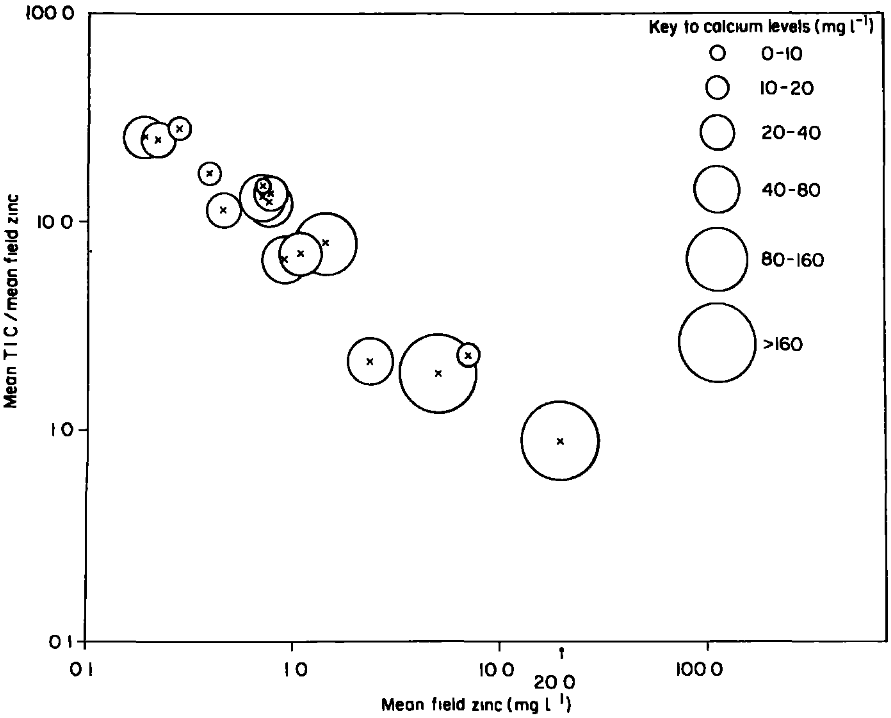

FIG 2 Relationship between the ratio, mean T I C (Tolerance Index Concentration) mean field level of non-filterable zinc, and the mean field level of non-filterable zinc For clarity, only those populations clearly adapted to tolerate the higher zinc levels are shown Open circles are used to indicate the levels of calcium at the various sites (Data plotted on logarithmic scale)

DISCUSSION

It is evident that *Stigeoclonium tenue* may be found at sites with a wide range of zinc concentrations in the water, reaching up to 20 0 mg l^{-1} in the Harz Mountains It may be incapable of colonizing those few stream sites which occur with even higher concentrations of zinc, the only filamentous green algae recorded so far from these being *Hormidium rivulare*, *H flaccidum*, *Ulothrix moniliformis* and *Mougeotia* (? 2 spp) (P J Say & B A Whitton, unpublished data) *Stigeoclonium tenue* sometimes dominates the stream flora at sites with moderately high levels of zinc, and there is some evidence that this period of dominance extends for a longer period each year than in streams lacking zinc pollution, but which are otherwise rather similar In fact this species may be considered one of the more successful algae in the presence of zinc pollution

The results of the toxicity tests show conclusively that populations of *S tenue* growing in zinc polluted streams are adapted forms, able to withstand higher zinc concentrations than populations from unpolluted streams (Table V) Most, if not all, of this adaptation is genetic The data summarized in Fig 1 suggest that there may be a "threshold" level of about 0 2 mg l^{-1} Zn, above which adaptation to zinc takes place However the toxicity tests are rather insensitive to differences between populations from relatively low levels of zinc, so it is uncertain whether adaptation to different zinc levels might be detected at even lower levels than 0 2 mg l^{-1} Zn

The available data (Table II) show that for at least six sites zinc levels may sometimes occur in the water higher than any found in the samples collected over the period for which algal populations were removed for the tests In the site (14) where the maximum zinc level is very much higher than any found during the study period, the T I C corresponds to the zinc levels found during that period, rather than to the maximum known for the site The composition of the water at the site is known to vary markedly according to flow conditions and *S tenue* was observed to have been killed here later in 1975 by some unknown factor It therefore seems probable that growth of *S tenue* at this site depends on inoculation from upstream unpolluted sites

TABLE V Correlation coefficients for zinc toxicity tests
r = correlation coefficient *P* = significance level

	Log just non-inhibitory	Log just lethal	Log T I C
Log mean field zinc	r = +0 85 *P* = < 0 001	r = +0 87 *P* = < 0 001	r = +0 91 *P* = < 0 001
	Mean T I C /mean zinc ratio—all populations	Mean T I C /mean zinc ratio—adapted populations only	
Mean field calcium	r = −0 44 *P* = < 0 005	r = −0 73 *P* = < 0 001	

As mentioned earlier, McLean (1974) suggested that the resistance of *S tenue* to heavy metals could be related to the affinity of the alga to organically polluted conditions Although this may well be an important factor in the survival of the species at some sites, the present data show that neither organic pollution, nor even simply the presence of high phosphate levels (see Table I), are essential for the success of the species at sites carrying very high levels of zinc

The data in Fig 2 show that under the conditions used the alga is usually much more tolerant to zinc in the laboratory than in the field but that the greater the field zinc level the nearer the laboratory results correspond to observations from the field It would seem from the data summarized in Table V that for a particular level of field zinc, the higher the field calcium level the lower the value of the T I C Based on the present data alone, however, this conclusion should be treated with caution as there is a significant tendency for the sites with higher zinc levels also to have higher calcium levels (Fig 2) If data from calcium (and magnesium) rich sites are excluded, even the levels of zinc just non-inhibitory in the laboratory are always much higher than the mean field zinc levels at the site from which the alga was taken It is obviously difficult to compare culture conditions with long-term exposure to zinc in the field, and only a few of the many obvious factors which might play a role are discussed here

Tolerance to zinc might be lower during a particular phase in the life cycle In the laboratory, increasing zinc levels bring about a reduction in the erect part of the thallus in comparison with the basal part, but at only the site with the highest level of zinc was the alga more or less restricted to a basal growth in the field Laboratory observations show that neither zoospore production nor germination is an especially sensitive stage, although it is possible that the settling stage may be more critical in the field due to local concentration of zinc at the rock-water interface, especially if a thin bacterial film is also present

If laboratory tolerance is related to maximum, rather than mean, field zinc level, the absolute values become slightly closer Several chemical factors which reduce the toxicity of zinc in the laboratory or increase its toxicity in the field would have a similar effect The presence of a chelating agent in the growth medium is no doubt in part responsible for the lack of sensitivity of tests made with populations from the lower field zinc levels The pH used in laboratory tests is lower than the mean value from which the populations were taken In most streams and rivers zinc will not be the only toxic agent influencing the survival of the alga The field levels of several other heavy metals show a marked correlation with zinc, especially lead and cadmium, although the absolute levels of these in the (non-filtrable fraction of the) water are quite low (Table I)

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ENVIRONMENTAL FACTORS REDUCING THE TOXICITY OF ZINC TO *STIGEOCLONIUM TENUE*

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The influence of pH over the range 6 1 to 7 6 magnesium calcium and phosphate on the toxicity of zinc to *Stigeoclonium tenue* has been shown to differ in a zinc tolerant population as compared with a zinc sensitive one With the zinc sensitive population a rise in pH from 7 1 to 7 6 and rises in magnesium and calcium bring about a slight reduction in the toxicity of zinc, the effect being most pronounced with calcium Rises in all four parameters bring about a reduction in the toxicity of zinc to the zinc tolerant population the reduction being marked in the cases of magnesium calcium and phosphate

Various field and laboratory observations have shown that the influence of zinc may be reduced by the concentrations of other substances present Most of the data are however for aquatic animals Among the factors which have been mentioned as reducing the toxicity of zinc, the one quoted the most often is the hardness of the water For instance it seems widely accepted that zinc is almost always less toxic to fish in hard than in soft waters (Lloyd 1960 Skidmore 1964 Mount 1966) At any particular hardness an increase in pH over the range 6 0 to 8 0 leads to an increase in the toxicity of zinc to fathead minnows (Mount, 1966) Some of the other factors which reduce the toxicity of zinc to fish such as high levels of dissolved oxygen seem unlikely to have much relevance for the study of stream algae Among plant studies, the further factor which seems to have been shown most clearly to antagonize zinc is the level of phosphate Some of the data to support this are indirect but it has been shown experimentally for *Thlaspi alpestre* L ssp *calaminare* (Lej) Markgr that increased phosphate levels lead to a reduction in zinc toxicity and also to a marked difference in the distribution of zinc inside the plant (Ernst 1968 1974) There have apparently been no experimental studies of the factors reducing zinc toxicity to algae, but Keulder (1975) has shown that hydrogen magnesium copper and phosphate ions all antagonize the uptake of ^{65}Zn by *Scenedesmus obliquus* (Turp) Kruger

In a previous account of the field tolerance of *Stigeoclonium tenue* Kutz to zinc (Harding & Whitton 1976), the data suggested that high levels of calcium and/or magnesium in stream waters may reduce the toxicity of any zinc present The data did not give any indication that phosphate influences zinc toxicity The present study was undertaken to establish clearly what influences, if any magnesium, calcium and phosphate have on the toxicity of zinc to *S tenue*

MATERIALS AND METHODS

Populations of *Stigeoclonium tenue* Kutz were obtained from Hollingside Artificial Stream , Co Durham, a site carrying low mean zinc levels (0 012 mg l^{-1} Zn, after passage through a sintered glass funnel), and Rampgill Level, an adit draining old lead workings at Nenthead Cumbria, and carrying relatively high mean zinc levels (2 39 mg l^{-1} Zn) It has been shown (Harding & Whitton, 1976) that the latter population has a much higher tolerance to zinc in the laboratory than has the former, and it seems clear that these differences in tolerance are genetic The two populations are termed the zinc sensitive and the zinc tolerant ones, respectively, throughout the rest of this account

The general methods used in the study were similar to those used by Harding & Whitton (1976), including composition of basal medium, growth conditions and design of toxicity tests The two populations were maintained as unialgal cultures in basal medium, subculturing being carried out at weekly intervals The effects of various additions to the basal medium were tested during 6-day growth tests, with the results summarized as just non-inhibitory just lethal and tolerance index concentration (T I C), (Harding & Whitton 1976) Each experiment involved about fourteen simultaneous zinc toxicity tests i e about 340 tubes All experiments were repeated at least twice, and gave similar results on each occasion

The factors which were studied for their possible influence on the toxicity of zinc were pH, magnesium calcium and phosphate Media used for the tests on pH were adjusted to the correct value with either HCl or NaOH The required levels of the three ions were obtained by addition of $MgCl_2$, $CaCl_2$ and NaH_2PO_4, respectively A medium lacking Mg was obtained by replacing $MgSO_4$ with Na_2SO_4, one lacking Ca by replacing $Ca(NO_3)_2$ with $NaNO_3$, and one lacking PO_4-P by replacing KH_2PO_4 with KCl With the exception of the media used in the pH tests, all were adjusted to an initial pH of 6 6 with HCl or NaOH, and did not drift more than 0 1 pH unit during any experiment As varying concentrations of Na , Cl^- and SO_4^{2-} ions were of necessity, added to the culture tubes while setting up the experiments tests were carried out to ascertain whether NaCl or Na_2SO_4 could in any way affect tolerance No detectable effect of either of these was found (over the ranges of the ions used as controls in the toxicity tests) Precipitation of zinc during the course of experiments at pH 6 6 never exceeded 12% of the amount added, so any influence of this on the tolerance index concentration was small The magnesium and calcium levels were checked at the end of the tests in the tubes with the highest initial levels, and the change was negligible in all cases However in the experiments on pH, when the higher levels of zinc were added to media at pH 7 1 and 7 6 some zinc was precipitated The extent of this precipitation was studied by filtering aerated solutions through HCl-washed Millipore 0 45 μm filters, 0 2 μm Nuclepore filters and a No 2 Sinta funnel All three filtration techniques gave similar results Using the results of these tests estimates of tolerance index concentration were made for the pH studies based both on the total zinc and the filtrable zinc present in test solutions (see Table I)

A few modifications to the methods of Harding & Whitton (1976) were made to the toxicity tests Algal materials were incubated in a medium free of the test ion for 1 day before setting up the experiment, and in the case of phosphate a further experiment was carried out where the alga had been incubated for 7 days in a phosphate-free medium prior to inoculation In all cases the alga was washed thoroughly before being transferred into medium deficient in a particular ion In the series of controls with similar ranges of magnesium, calcium and phosphate to those used with the toxicity tests, it was found as might be expected, that a marked reduction in growth occurred in the absence of these ions The slight growth that did occur with media free of either magnesium or calcium was presumably a result of the fact that the pre-incubation period was insufficient to starve the alga completely of these elements In the absence of added phosphate growth was restricted to the development of long, narrow pointed hairs These hairs were still developing even after starving the alga for 7 days, and the inocula used for toxicity tests following this treatment included many such hairs With both populations magnesium caused a moderate reduction in growth at 100–200 mg l^{-1} in comparison with growth over the range 1–50 mg l^{-1} Phosphate caused a slight inhibition at the highest concentration tested (30 mg l^{-1} PO_4-P) with the zinc sensitive population, but not the zinc tolerant population Calcium had no effect on either population at even the highest concentration tested, 200 mg l^{-1} Ca

The results of the zinc toxicity tests are based on comparisons with the growth of the alga in controls with the particular level of ion under test In the case of phosphate-free medium, the comparisons rest on observations of hair development

RESULTS

The influence of pH on the toxicity of zinc in the presence of two different calcium concentrations is shown in Table I It can be seen that the only detectable influence of pH on the zinc sensitive population is a slight decrease in toxicity between pH 7 1 and 7 6 In contrast there is an obvious influence of pH in decreasing zinc toxicity to the resistant strain as indicated by the values of T I C based on total zinc This is apparent even if the values of T I C are calculated using zinc passing through a filter rather than total zinc when the tests are carried out in the presence of 1 mg l^{-1} Ca but not 10 mg l^{-1} Ca

TABLE I Effect of pH on toxicity of zinc to a zinc sensitive and a zinc tolerant population of *Stigeoclonium tenue* Tolerance Index Concentration (T I C) is estimated both for the total zinc and the filtrable zinc present (All concentration in mg l^{-1})

		Zinc sensitive		Zinc tolerant	
Ca	pH	T I C total	T I C filtrable	T I C total	T I C filtrable
1	6 1	0 63	0 63	3 39	3 39
	6 6	0 63	0 63	3 39	3 39
	7 1	0 63	0 63	8 80	6 78
	7 6	0 73	0 73	11 00	7 16
10	6 1	0 92	0 92	7 83	7 83
	6 6	0 92	0 92	7 83	7 50
	7 1	0 92	0 92	12 40	7 50
	7 6	1 00	1 00	14 10	7 60

The effect of varying magnesium calcium and phosphate concentrations on the toxicity of zinc to *Stigeoclonium tenue* is shown in Fig 1 It can be seen that the responses of the zinc sensitive and zinc tolerant populations again differ Phosphate had little if any effect on the toxicity of zinc to the zinc sensitive population but in all other cases the test ion had some influence on zinc toxicity In the case of the zinc tolerant population there was a marked reduction in toxicity with all three test ions the T I C rising up to the highest level tested for both magnesium and calcium but showing a slight reduction at the highest concentrations of phosphate The tests illustrated in Fig 1 for the influence of phosphate are those for the material pre-incubated for only one day in phosphate-free medium The results for material pre-incubated for seven days were however very similar

Microscopic inspection showed that at levels of zinc near the T I C the response was at all times similar with a moderate reduction in the erect as opposed to the basal part of the thallus With increasing zinc concentrations basal growth increasingly predominated over erect growth under all test conditions The only other marked morphological response was the production of numerous long hairs in the samples deficient in phosphate, whether or not zinc was present in the medium Although phosphate antagonized the toxic action of zinc to the zinc tolerant population an increased level of zinc in the medium did not permit the development of hairs at a higher level of phosphate This behaviour contrasts with the antagonistic influence of calcium on the inhibitory effects of zinc on zoospore release and settling in the one test where zoospores were formed throughout all the lower zinc concentrations (Table II)

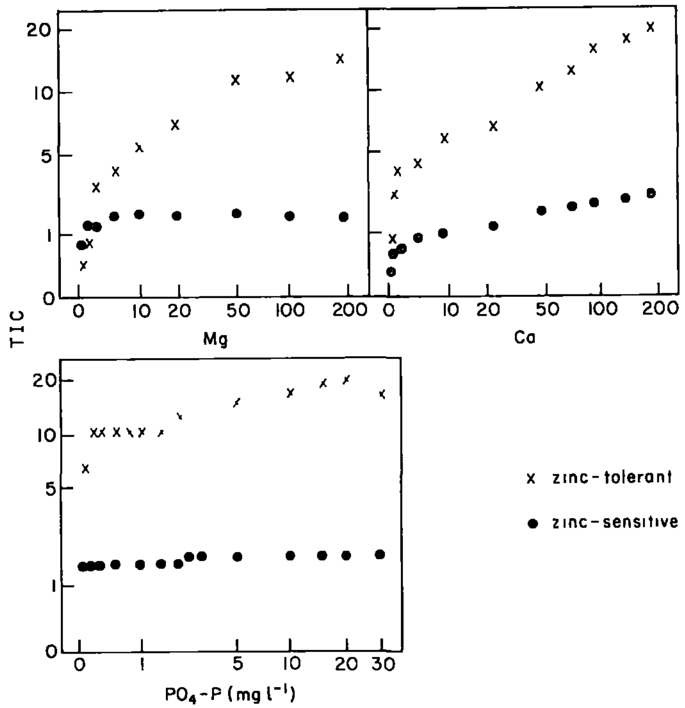

FIG. 1 Influence of Mg, Ca and PO$_4$-P on the toxicity of zinc to a zinc-sensitive and a zinc-tolerant population of *Stigeoclonium tenue* (T.I.C. = tolerance index concentrations in mg l^{-1}.)

TABLE II Comparison for a zinc sensitive population of *Stigeoclonium tenue* of the effect of calcium on the T.I.C. with that on the maximum level of zinc permitting zoospores to settle (All concentrations in mg l^{-1}.)

Ca	Max. Zn permitting settling	T.I.C.
0	0.5	0.31
1	0.75	0.63
2	0.75	0.70
5	1.0	0.92
10	1.25	1.0
25	1.25	1.17
50	1.25	1.54
75	1.25	1.64
100	1.5	1.71
150	1.5	1.94
200	1.5	2.01

DISCUSSION

The present results for *Stigeoclonium tenue* where the influence of agents antagonistic to zinc differ according to which population is tested show the importance of knowing about the environment from which a test organism is taken before using it in laboratory assays Rises in pH magnesium calcium and phosphate all had a marked effect in reducing the toxicity of zinc to a zinc tolerant population of *S tenue* but under the conditions studied, only calcium had a marked effect with a zinc sensitive population The observation that a rise in pH over the range pH 6 6 to 7 6 led to a decrease in toxicity of zinc contrasts with the results of Mount (1960) for fathead minnows However the filtration experiments described above suggest that this effect with *S tenue* is not an artifact resulting from the solubility of zinc decreasing over this pH range

As the population of *Thlaspi alpestre* ssp *calaminare* used by Ernst (1968 1974) came from a high zinc site, it is uncertain whether phosphate ever plays a special role in antagonizing zinc toxicity for plants normally growing in a low zinc environment It is however well established that high levels of phosphate may induce zinc deficiency in various crops (Burleson & Page, 1967)

Comparison of the results of the toxicity tests with observations on growth of the alga in a medium free of zinc, but with a similar level of test ion show that magnesium, calcium and phosphate have a quite different influence on antagonism than on growth The difference is most obvious with magnesium where raising the concentration in the medium from 50 to 200 mg l^{-1} brings about an increased antagonism to zinc toxicity while at the same time causing a marked inhibition in total growth In the one test where zoospores were formed at all the lower zinc concentrations, the influence of calcium in antagonizing zinc was rather similar for both the settling of zoospores and also for toxicity to growth as a whole as measured by the tolerance index concentration This agrees with the observations made previously (Harding & Whitton 1976), where the levels of zinc just permitting settling of zoospores from three different strains corresponded well with the tolerance index concentration

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