ASPECTS OF THE ACID TOLERANCE OF ALGAE FROM THE DURHAM AREA

by

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ABSTRACT

Algal samples were collected from a diverse range of aquatic habitats in the Durham area, with pH values ranging from 3.2 - 9.2, and their acid tolerance in culture was investigated. The pH ranges of occurrence of species in the field were tabulated and their ability to survive in culture at pH 3.3 recorded. The results reveal that some species are restricted to low pH environments among the samples taken, whereas other acid tolerant species can also be found at normal or high pH sites.

Acid tolerance was found not to be specific to any particular algal division, the only common division not represented among acid tolerant species being the Cyanophyta. This is in agreement with results of investigations in America.

Comparison of species found to be acid tolerant from environments other than minewater drainages, with the flora of an acid minewater drainage at Brandon, suggests that pH is probably the major factor determining the flora of the latter, rather than pH-independent factors characteristic of minewater.

Evidence was found showing that tolerance of low pH conditions is a characteristic of particular species to a considerable extent, and samples of these species taken from quite alkaline environments were found to survive at low pH in culture. However, there is also some evidence for the occurrence of adaptation within a species and this may be important.

No clear patterns emerged from a floral comparison of the Brandon Acid Streams catchment area and the surrounding countryside.

Some experiments on transport of algae by air were carried out, but limited data were collected. It appears that the Acid Stream species are not common among the algae in the air a short distance from the Stream.

CONTENTS

INDEX TO MAPS

INDEX TO TABLES

1 INTRODUCTION

1 INTRODUCTION

1.1 Distribution and dispersal of algae.

The factors controlling the distribution of algae are many and complex and, although algal ecology has been studied for over fifty years, the difficulties involved in determining the relative importance of each of these factors have greatly impeded advancement of understanding in this field.

In an early paper, STRÖM (1924) reports that many algae are cosmopolitan in occurrence, although geographical localization is noticeable in some cases, especially for some desmids. Since then, a great deal of research into the classification of particular floral communities typical of particular environments has been carried out (BUTCHER, 1933; BLUM, 1956). The field is reviewed by LUND (1965). More recent work, based mainly on laboratory culture experiments, on the effects of many environmental factors including calcium concentrations, nitrogen availability, pH and temperature, is presented in a comprehensive review by MOSS (1972, 1973a, b, c). He compares the effects of these factors on eutrophic and oligotrophic algae. Elucidation of the rôles of these diverse environmental factors is, and will be, of ever-increasing importance in the field of water management.

Although great steps forward have been made in the above field, very little research has been carried out into the factors influencing and controlling the dispersal of algae. These dispersal mechanisms may well be of great importance in explaining their distribution patterns, but often no obvious patterns emerge from studies of the colonisation of new water bodies (TALLING, 1951). Only over the last fifteen years has progress been made in this field.

A paper on the topic in general was published by MAGUIRE (1963) but most of the available data are concerned with the rôles of specific animals in the dispersal of algae. Studies on waterfowl have shown these to be capable of carrying viable algae internally for considerable periods (PROCTOR, 1959; SCHLICHTING, 1960) although ATKINSON (1970), in a specific study of a planktonic diatom *Asterionella formosa*, finds no evidence for internal transport of viable cells of this species.

A considerable amount of work done in Texas and North Carolina has shown that certain insects are capable of carrying viable algal cells for considerable distances (REVILL *et al*, 1967), and MILLIGER & SCHLICHTING (1968) demonstrate that transport of algae in the gut of an aquatic beetle may be exceedingly important. Little research appears to have been carried out on the dispersal of algae solely by wind currents. SCHLICHTING (1964) reviews the problem and JAWORSKI & LUND (1970) studied *Asterionella formosa* to try to gain some information on dispersal of planktonic algae which have been notably absent from many of the studies. The field is still very young and many problems await solution.

1.2 Extreme aquatic environments.

BROCK (1969) defines an extreme environment as one from which whole taxonomic groups of organisms are absent. It is important to remember that environments which might be considered extreme by man are not necessarily extreme in this sense, e.g. the Arctic. In studying many natural environments florally, the complexity of environmental factors mentioned above, and the diversity of the flora, make development of a scientific model of the system extremely difficult. Extreme environments can often be florally described completely without too much difficulty, and their study can give information of use in elucidating the complex interaction patterns in more normal ecosystems. In addition, an extreme aquatic environment provides a habitat in the field vastly different from surrounding waters but usually similar to other such extreme habitats in other parts of the world. Comparison of such systems can give valuable information on factors determining algal colonisation. The field is reviewed by WHITTON (1972).

In addition to investigations of a general nature, extreme environments often justify study where they cause particular pollution problems. This is especially true in the case of acid streams caused by strip mining in America (PARSONS, 1952). This thesis is particularly concerned with extreme acid environments of this type. Detailed studies of the flora of such streams in America have been carried out (LACKEY, 1938, 1939; BENNETT, 1969), although no data have as yet been published concerning similar streams in Britain. Other sources of acid water include peat mires where the pH can approach a value of 2.0 or less, and volcanic sources such as acid thermal springs (which are often dominated by *Cyanidium caldarium*), and volcanic lakes. Little data are available on the flora of the latter, although some work has been done in Japan (UENO, 1938) where lakes of acidity of both volcanic and humic origin occur (UENO, 1958).

Problems associated with studies of acid environments are that these are often associated with high heavy metal levels, especially iron and aluminium in the case of minewater.

About 12 photosynthetic organisms are commonly found growing at pH = 3.0 or below, as well as a large number of bacteria and fungi, some of which will survive at pH values as low as zero.

1.3 Aims of Study.

The main aim of this study is to investigate some aspects of the tolerance of algae in the Durham area to low pH conditions, and to relate the results to the flora of a very acid stream at Brandon, thus perhaps elucidating the major factors governing its flora. Acid tolerances of algae in culture are compared with their observed distribution over the sampling sites used, in order to determine whether the results show any evidence for the formation of ecotypes adapted to low pH conditions. Comparisons are made between the flora of the Acid Stream and that of its catchment.

Finally, the results of some investigations carried out into the aerial transport of acid tolerant algae are considered in an attempt to clarify further any patterns that emerge from the study.

2 DESCRIPTION OF THE AREA AND SITE SELECTION

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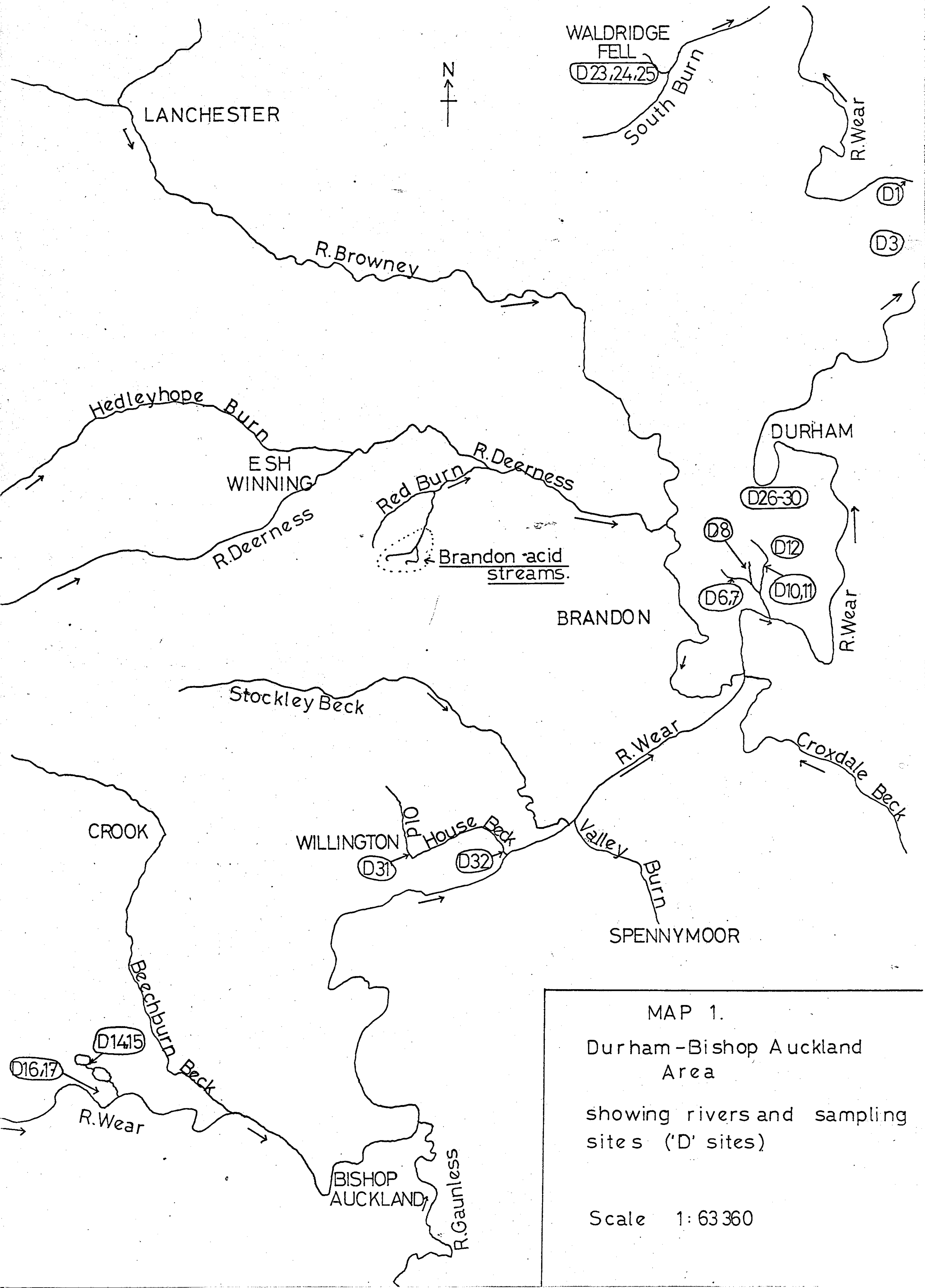
2.1 Durham Area Study.

The area studied for this part of the project was a part of the River Wear catchment between Witten-le-Wear and Durham, and continuing north to Waldridge Fell, near Chester-le-Street.

The River Wear is a relatively fast-flowing river subject to rapid rises and falls at any time of the year, and is moderately hard and eutrophic by the time it reaches Durham. The area is mainly arable farmland or urban on a substratum of coal measures, sandstones and shales, with some peaty moorland areas. The upper part of the River Wear flows through old lead mining areas and thus receives a small load of heavy metals which may have some slight effects on the flora. Drainage from old coal workings also affects many of the watercourses in the area. Several studies of the algal flora of the area have been made in the past (GRIFFITHS, 1936; DEWDNEY (Ed.) 1970) especially of the River Wear (BUTCHER, 1932; PEABODY & WHITTON, 1968; WHITTON & BUCKMASTER, 1970).

For the initial experiment to study the effects of pH on the occurrence of algae in the area and their acid tolerance, samples were collected from sites with a wide range of pH. 32 sites were selected, the main criterion being to achieve as wide a pH range as possible whilst also including a wide range of conditions with respect to eutrophy, physical conditions, substrate type, hardness etc., to reduce the influence of these parameters on the overall results. Sites chosen ranged from a pool on bare peat at pH = 3.2 to a lake outflow stream in an old gravel pit area at pH = 9.2; from pools and small streams to the River Wear; and from unpolluted moorland waters to sewage works effluent. About half of the sites were flowing, and half standing water. The sites were numbered D1 - D32. Positions are shown on Map 1.

The number of sites that could be studied was limited by available time and culture space. For this reason 21 sites were



MAP 1.

Durham-Bishop Auckland Area

showing rivers and sampling sites ('D' sites)

Scale 1: 63360

selected from the original 32 after preliminary microscopical examination to give the widest range of floral types. Samples were then put into culture as described in section 3.2 at low pH and site pH, and the acid tolerances of the various species investigated. Results from the acid stream catchment survey (see next section) and floral lists for the Brandon Acid Streams (pers. comm. HARGREAVES, 1973) were also included in the general results.

2.2 Brandon Acid Streams.

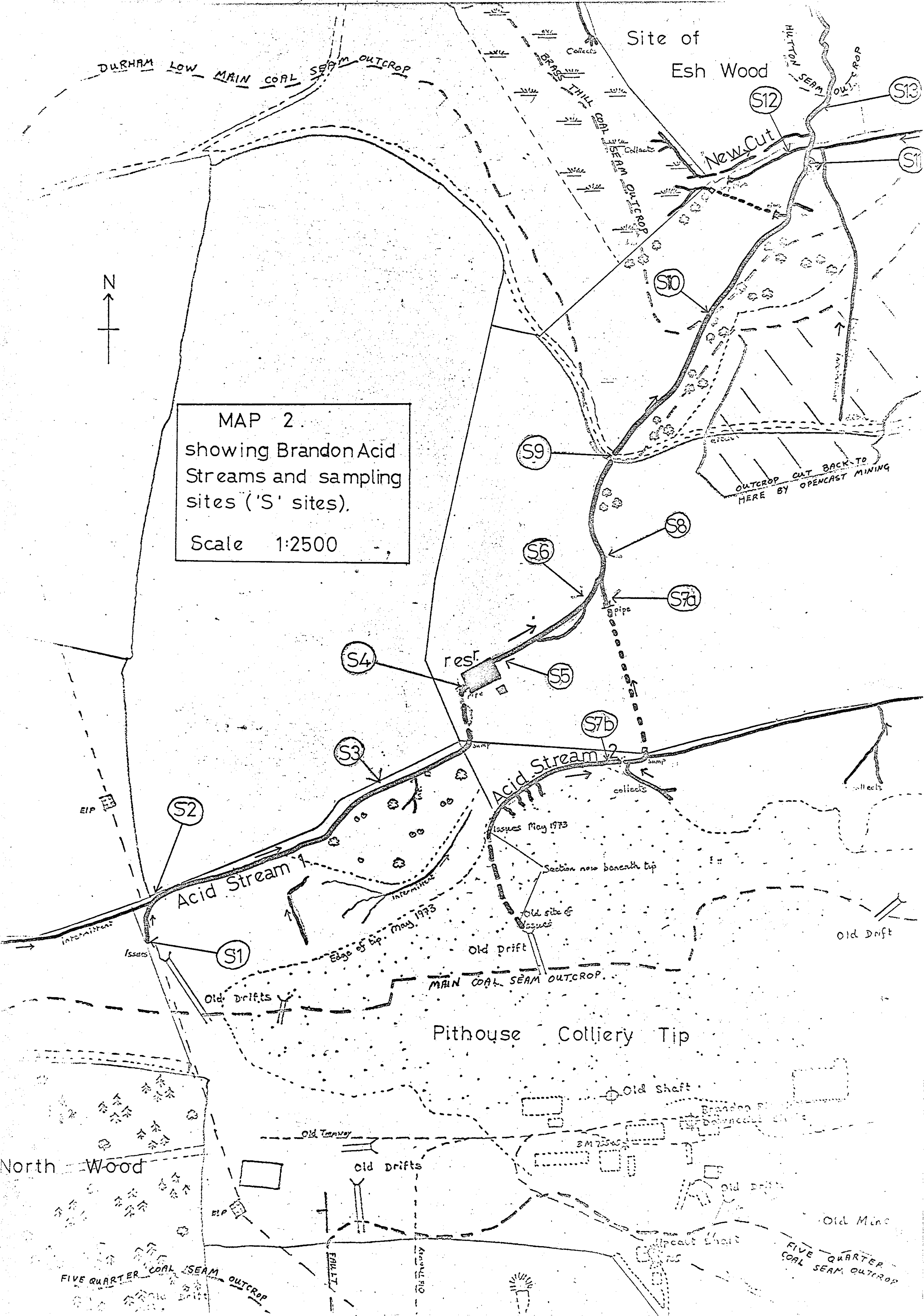
(i) In the second part of the experimental work a series of floral samples were taken in the catchment of two very acid streams near the site of Brandon Pithouse Colliery (see Map 1 for position in relation to Durham area). The valley of the acid streams leads down to Red Burn and ultimately to the River Deerness. The whole area has been extensively mined for several centuries by means of small adit mines into the various seams outcropping down the valley sides. Shaft mining in the area has been carried out since at least as long ago as 1838 (DURHAM DIOCESAN RECORDS, 1838) so it is likely that most of these drifts have been disused, except for drainage purposes, for over a century, and most are not traceable on the ground.

The main shaft of the modern Pithouse Colliery was sunk in 1926 down to Tilley Seam level, and the modern workings would interconnect with the old drifts in the Five Quarter and Main seams. The colliery was finally closed in 1966 leaving a spoil heap 800 m. across (ROBINSON, 1971). The acid streams both spring from near the base of this tip and both appear to run from old drifts in the Main seam outcrop - see Map 2. Acid Stream 1 flows from an earthenware pipe near the site of one of these drifts whereas Acid Stream 2 seeps from the base of the tip as shown. However, the latter appears to originate from the old drift now covered by the tip, rather than from seepage through it, since the tip has been shown to have no water table of its own, and creep movements consistent with seepage along its base have been recorded. Acid Stream 1 has been shown on approximately its present course on maps since 1897.

Acid Stream 1 leaves its source with a pH of 2.6 and a remarkably constant flow of around 0.3 $l.s^{-1}$, although this is drastically increased during rainy weather a few metres downstream by drainage from the tip. Flowing along the present base of the tip, the stream passes through a reservoir (built early this century for an unknown purpose) to its confluence with Acid Stream 2. It then continues towards the site of Esh Wood, a plantation of some antiquity, at whose boundary the pH is raised to 6.4 due to dilution by other streams. In 1970 contractors started levelling the tip and in 1971 dredged out the stream to prevent flooding of farmland, thus the present flora of the stream is the result of 2 years' colonization and growth. Since October 1972 regular sampling of the stream, both chemical and floral, has been carried out at Durham (pers. comm. HARGREAVES, 1973) and the sites used have been marked on Map 2. pH values corresponding to these sites are shown in Table I (numbered S1 - S13).

Acid Stream 2 has a source pH of around 3.0 and retains this until its confluence with a surface water stream, as shown, where the pH rises and hydrated iron (III) oxides are precipitated. It then passes through a pipe to join Stream 1. Two of the sampling sites listed are on Stream 2.

The cause of the acid contamination of the water is the oxidation of iron pyrites (FeS_2) leading to production of soluble acidic compounds - mainly sulphuric acid. Acid mine-water appears to be specifically associated with coal seams containing or adjacent to deposits of pyrites,


MAP 2.
showing Brandon Acid Streams and sampling sites ('S' sites).
Scale 1:2500
N
DURHAM LOW MAIN COAL SEAM OUTCROP
BRASS THILL COAL SEAM OUTCROP
HUTTON SEAM OUTCROP
Site of Esh Wood
New Cut
OUTCROP CUT BACK TO HERE BY OPENCAST MINING
Acid Stream 1
Acid Stream 2
resr
Issues
intermittent
Issues May 1973
Section now beneath tip
Old site of issues
Edge of tip May 1973
Old Drift
Old Drifts
MAIN COAL SEAM OUTCROP
Pithouse Colliery Tip
Old Shaft
Old Tramway
North Wood
FIVE QUARTER COAL SEAM OUTCROP
Old Mine
FAULT
S1
S2
S3
S4
S5
S6
S7a
S7b
S8
S9
S10
S12
S13
collects
sump
pipe
EIP

especially in cases where there has been a fire in the workings. At Pithouse the Main seam has very low sulphur content so the pyrites is presumably present in adjacent strata. Formation of acid can arise either by atmospheric oxidation of pyrites or the action of sulphur oxidizing and iron oxidizing bacteria. Little is known concerning the relative importance of these two mechanisms.

Atmospheric oxidation can basically be considered as occurring by the following steps (JOSEPH, 1953):

$$2FeS_2 + 3O_2 + 2H_2O \rightarrow 2FeSO_4 + H_2SO_4$$

also $$4FeSO_4 + 2H_2SO_4 \rightarrow 2Fe_2(SO_4)_3 + 2H_2O$$

and $$Fe_2(SO_4)_3 + 4H_2O \rightarrow 2Fe(OH)_3 + 3H_2SO_4$$

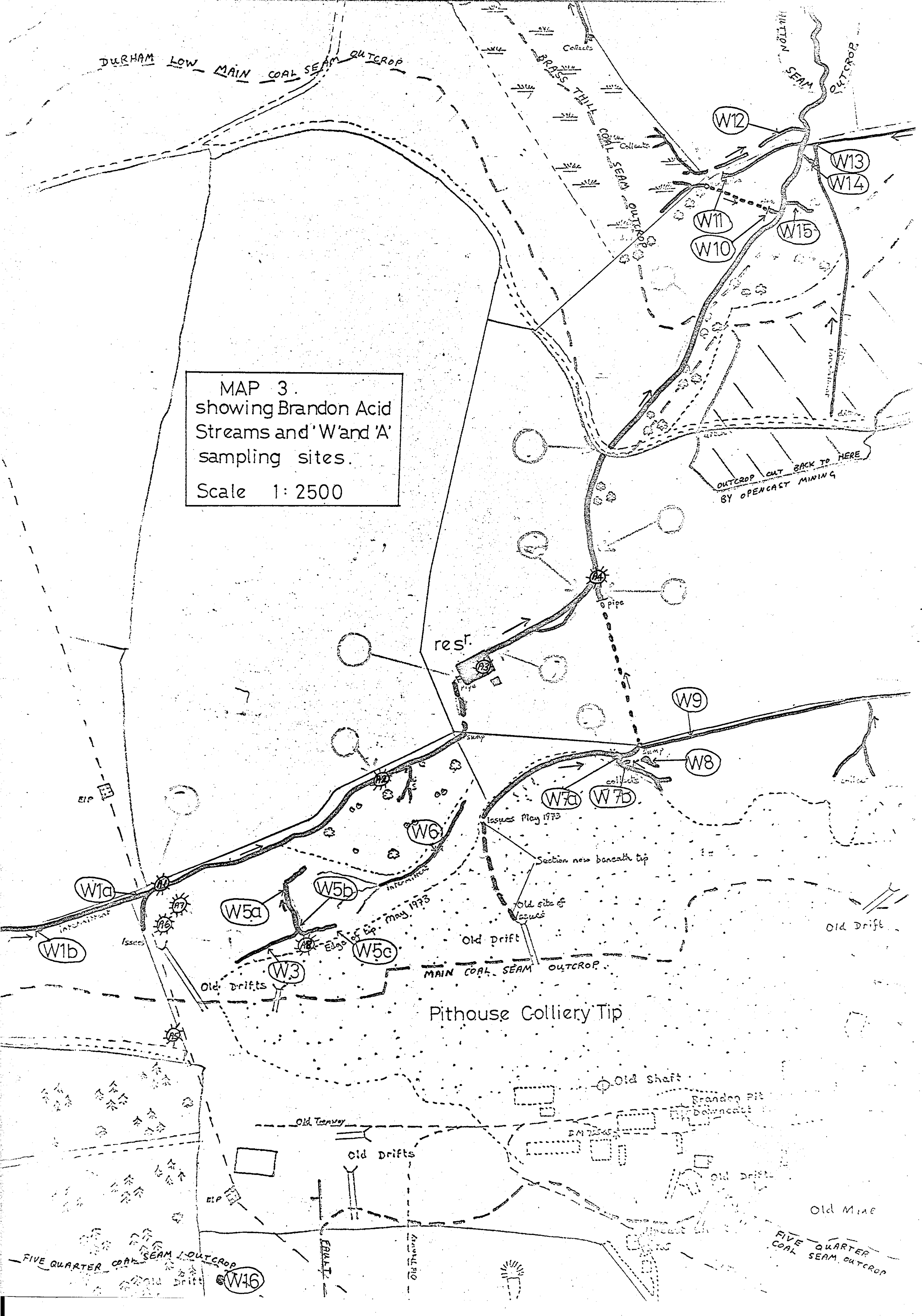
although the actual mechanisms of reaction under the conditions may be complex and are little understood. The second two steps are dependent on concentrations and pH values. Most of the iron in the upper few metres of the acid streams will be in the iron (II) state. At higher pH the iron is precipitated - at pH values greater than about 3.3 in most stream waters.

Bacterial oxidation appears to be mainly due to three organisms: _Ferrobacillus ferrooxidans_, _Thiobacillus thiooxidans_ and _Ferrobacillus sulfooxidans_ (BAKER & WILSHIRE, 1970). These are believed to be responsible for a considerable proportion of the acid production.

(ii) In order to try to determine whether any important patterns exist in the relation of the flora of the acid streams with that of their catchment, a detailed study of other flowing water in the catchment was carried out. Algal samples were taken from 20 sites in the area including all flowing water in the area as well as one or two standing water and intermittently flowing sites. These sites were numbered W1 - W16 and are fully described in Tables I and II. Their locations are shown on Map 3. Where several sites were chosen on the same stream all were given the same number with suffixes a, b, c..... etc. starting from the lower end. Samples were put into culture as described later and examined after 3 weeks. The whole survey was repeated 7 weeks later.

2.3 Air-flora study.

In studying factors controlling the distribution and spread of algae in an area it is obviously relevant to investigate the mechanisms of dispersal of species. Although a certain amount of work has been done (see Introduction), this field is still little understood. In an attempt to obtain some information as to the acid tolerant algae present in the air around the catchment, sets of plastic beakers containing acidified growth medium were left out at selected sites, 4 directly above the Acid Stream, and 4 elsewhere in the area. The sites were numbered A1 - A8 and are marked on Map 3. Thus it was hoped that observation of growth in these beakers would give some information as to the mobility of species in the area. It would have been preferable to repeat the experiment at a large number of sites, but this was not possible due to lack of time.


MAP 3.
showing Brandon Acid
Streams and 'W' and 'A'
sampling sites.
Scale 1: 2500
DURHAM LOW MAIN COAL SEAM OUTCROP
BRASS THILL COAL SEAM OUTCROP
HUTTON SEAM OUTCROP
OUTCROP CUT BACK TO HERE BY OPENCAST MINING
MAIN COAL SEAM OUTCROP
FIVE QUARTER COAL SEAM OUTCROP
Pithouse Colliery Tip
W1a
W1b
W3
W5a
W5b
W5c
W6
W7a
W7b
W8
W9
W10
W11
W12
W13
W14
W15
W16
A1
A2
A3
A4
A5
A6
A7
A8
res^r.
pipe
sump
Issues
intermittent
Edge of tip May 1973
Issues May 1973
Section now beneath tip
Old site of issues
Old Drift
Old Drifts
Old Shaft
Brandon Pit
Old Tramway
FAULT
Old Mine
ELP

3 METHODS

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3.1 Collection of Samples.

(i) Algal samples were collected using 20 ml. sterile plastic blood-sample tubes. For rocky substrates growth from an area of 1 cm^2 was scraped up and collected along with samples of any visible filamentous growth in the vicinity and 10 ml. of water from the site. For mud and clay substrates the top few millimetres of mud were collected over the same area. In any cases where a large diversity in substrate or growth was apparent at a site, samples from all types were taken. In repeating samples at a later date care was taken to repeat the original sample in as much detail as possible. Samples were briefly examined and then cultured within 24 hours of collection.

(ii) Air-flora samples were collected by leaving out acid-washed plastic 250 ml. beakers each containing about 30 ml. of modified Chu 10 D* medium for two-week periods. Six beakers were left at each site (site nos. A1 to A8). Two of each set contained growth medium at pH=2.6, two at pH=5.5 (adjustment of pH being carried out by addition of 2.5 M sulphuric acid), as well as two containing nutrient agar at pH=5.5. One of each pair of beakers was covered by 1 mm^2 mesh black muslin. Where dilution occurred due to the heavy rain experienced during the sampling period, nutrients and acid were added immediately after collection of the beakers to restore Chu 10 D nutrient concentrations and original pH values before incubation in a 12°C culture room for three weeks.

3.2 Culture Methods.

The culture medium used throughout the project was a modification of Chu's 10 D medium (CHU, 1942)*. This was chosen as experience of other workers has shown it to be a very suitable medium for mixed cultures, being high enough in nutrient levels for most species to survive but not so high as to encourage over-rapid development of dominance by eutrophic organisms. Soil extract was added to facilitate growth of motile organisms having been first rendered free of viable algae by repeated boiling and cooling. Cultures

* See Appendix I.

of 'D' and 'W' samples were carried out in 20 ml. of medium in 25 mm. boiling tubes in a shake-tank at 15°C with a light intensity at the base of the tubes of 5.0 klux. Air-flora samples were cultured in 100 ml. conical flasks in a 12°C room on a white tray in a light intensity of 3.0 klux. All culture vessels, storage bottles and pipettes were washed with detergent followed by 2 M hydrochloric acid, then rinsed in distilled water and dried at 110°C before use on every occasion. Culture tubes were sealed with Morton Closures. For each batch of medium made up, samples were kept in tubes, under the conditions described, as blanks to confirm the algal sterility of the medium and glassware. Examination after two weeks showed only bacterial and a little fungal growth.

The 'D' samples were cultured at pH=3.0 ; pH=3.5 and pH of the site of origin measured at the time of sampling. In addition, a mixed inoculum was prepared from all the samples and cultured at a range of pH values from 2.6 - 4.0. The 'W' samples were cultured in three series:-

Series X - pH=3.0.
Series Y - pH= that of the acid stream 1) below confluence for 'W' samples on tributaries, 2) at the nearest point for other 'W' samples.
Series Z - pH= that of the site of origin at the time of sampling.

'A' samples were cultured as already described.

Samples were shaken and allowed to settle and filaments broken up where necessary. The inoculum was then taken by sterile dropper from the surface of the sediment and the surface of the liquid, care being taken to include fragments of any filamentous species present. The amount of liquid included in each inoculum was insufficient significantly to alter the pH of the prepared culture medium. Cultures were grown for a period of two to three weeks in each case, after which time they were examined microscopically and the pH measured. During the two or three days taken to examine a set of cultures, the tubes were stored in a 5°C culture room which appeared to be effective at slowing down any changes in pH or floral composition which were occurring. One of the major problems encountered in culturing the samples was the change in pH brought about by growth of the organisms. Cultures at an initial pH of 3.5 and above were found to be fairly unstable with respect to pH which usually rose to 6 or 7. Cultures at pH=3.0 and pH=2.6 were found to be very stable. This is further discussed in later sections.

3.3 pH measurement.

Measurements of site pH were carried out in the field using a Pye Model 293 portable pH meter, with an E_o7 electrode. This was also used for measurement of pH of cultures. The latter were carried out by withdrawing 2 ml. of medium for the measurement using a sterile pipette thus avoiding any carry-over of organisms. In making up culture media an EIL Model 23A direct reading pH meter was used after careful cleaning of the electrodes. Lack of algal growth in the blanks confirmed that no algal contamination had been introduced from this source.

3.4 Microscopical examination.

Examination of the cultures was carried out by preparing six 22 x 50 mm. slides from each culture using a sterile dropping pipette. Care was taken to include material from the tube walls and liquid surface, as well as from centre and bottom of the tube. Each slide was then scanned three times longitudinally under a X 10 objective, followed by five scans using a X40 objective. Lack of time available limited the amount of detailed investigation on difficult species that could be carried out using higher magnification and oil immersion techniques. Each living species present was recorded, as well as its condition, if this was particularly unhealthy. Problems arose in some cases in deciding whether an alga was living or not, especially in the case of diatoms. These were recorded as living if any significant amount of cellular matter was present.

Lack of time precluded any growing up of unialgal cultures for identification purposes, thus it was not possible to identify some of the palmelloid green cells present. Some other small species were not identified, especially small diatoms. Taxonomic problems are further discussed in Appendix II.

4 RESULTS

The data collected are set out in the following pages in tabulated form. Firstly some details of the sites used are summarized to give an indication of the range of environments encompassed by the study. The results of the floral analyses of the cultures are then given and some comparisons drawn. Finally results of the air-flora study are mentioned.

4.1 Details of sites.

Table I summarizes details of the various sites used in the project. Of the 'D' sites, only the 21 selected for the study have been included. The exact locations of the sites are shown in maps 1 - 3.

Notes to Table I.

Date:- For 'D' samples this is the date of collection of the sample.
'W' samples were collected on 2 occasions - 30.5.73 and 20.7.73.
Samples from 'S' sites have been collected at regular intervals since October 1972 (pers. comm. HARGREAVES).
'A' samples A1 - A4 were collected on 2 occasions - 26.6.73 and 3.8.73.
A5 - A8 were collected on 13.8.73.

pH:- For 'D' samples value is pH at time of collection.
For 'W' samples value is mean of values from 2 samplings.
For 'S' samples value given is mean of all readings taken under normal conditions (i.e. not flood or freezing conditions).
Except for W9 and W10, pH values for 'W' and 'S' sites show a high degree of constancy.

Flowing/Still:- F - represents water always flowing.
IF - represents water intermittently flowing.
S - represents standing water.

Flow:- 0 - represents no flow.

1 - represents an estimated normal flow of less than 0.01 cumec. ($10\ l.s^{-1}$.)

2 - represents an estimated normal flow from 0.01 - 2.5 cumec.

3 - represents an estimated normal flow greater than 2.5 cumec.

Shaded/ Open:- S - represents heavy shade.

L - represents light shade.

0 - represents unshaded, open.

'Yes' in the column marked 'heavy algal growth' represents readily visible algal growth.

'W' Samples:- the following are tributaries of acid stream 1 (i.e. water flows directly from the site into the acid stream) - W1a, W1b, W5a, W5b, W5c, W10, W12, W13, W14, W15.

The following are tributaries of acid stream 2 - W7a, W7b.

The following were abandoned due to excessive growth of bracken by the second sampling - W2, W4.

'A' Sites:- Site A1 is directly above site S2.

Site A2 do. S3.

Site A3 do. reservoir.

Site A4 is directly above confluence of acid streams.

Sites A5 - A8 are not directly above the acid stream.

Table 1. Site descriptions.

Site no.	O.S. map ref.	date	pH	flowing/ still	flow	substrate	shaded/ open	surrounding vegn. type	heavy algal grth	notes
D1	NZ297472	11/5/73	8.3	F	3	sandstone	L	open woods	Yes	R.Wear, Finchale.
D3	NZ 289457	11/5/73	7.6	S	0	mud	O	rough grass	No	Ditch at Brasside.
D6	NZ 270402	11/5/73	6.9	F	1	concrete	L	decid.woods	Yes	F.E. from S.D.W.
D7	NZ 270402	11/5/73	7.2	F	1	mud	L	" "	Yes	20m. below D6, stream
D8	NZ 271403	11/5/73	6.4	IF	0-1	peaty	S	coniferwood	No	Slow v.shaded stream
D10	NZ 274403	11/5/73	-	-	0	soil	L	grass,road	Yes	By stream below SDW.
D11	NZ 274403	11/5/73	-	-	0	stone	L	" "	"	" " "
D12	NZ 274404	11/5/73	8.0	S	0	enamel	O	grassland	No	Old bath in field.
D14	NZ 159315	11/5/73	9.2	F	1	gravel	O	grass,bush	Yes	Outflow from lake.
D15	"	"	"	"	"	"	"	"	"	" " "
D16	NZ 163313	11/5/73	8.9	S	0	gravel	O	grass,bush	Yes	Pool in old road.
D17	NZ 163313	"	"	"	"	"	"	"	"	" " " "
D23	NZ 255492	12/5/73	4.8	IF	0-1	grass	S	grass,bush	No	Pool on Waldridge Fell
D24	NZ 254493	12/5/73	3.9	S	0	peat bog	O	grass,sedge	No	" " " "
D25	NZ 253494	12/5/73	3.2	S	0	bare peat	O	" "	No	Peat pool, " "
D26	NZ 275415	12/5/73	6.2	S	0	wood tub	O	none near	Yes	Tub near laboratory.
D27	"	"	6.3	"	"	" "	"	"	"	" " "
D29	"	"	6.5	"	"	" "	"	"	"	" " "
D30	"	"	6.6	"	"	old sink	"	"	"	Sink " "
D31	NZ 213354	13/5/73	6.8	F	2	pebbles	O	grassland	Yes	Old House Beck
D32	NZ 230355	13/5/73	7.3	F	2	pebbles	O	grassland	Yes	Old Ho. Beck, Page Bank
W1a	NZ 210405		4.7	IF	0-1	clay	L	grassland	No	Grassy ditch, above con
W1b	NZ 211405		4.6	IF	0-1	clay	S	bushes	No	Same ditch 70m. upstr'm
W3	NZ 212405		7.0	IF	0-1	clay	O	grass,sedge	No	Stream at base of tip
W5a	NZ 213405		7.5	F	1	clay	L	grass	No	Slow moving stream
W5b	NZ 213405		7.5	F	1	clay	O	none	No	Fast section on tip.
W5c	NZ 213405		7.5	IF	0-1	clay	O	none	Yes	'Source' of W5 stream.
W6	NZ 214406		7.7	IF	0-1	clay	L	woodland	Yes	Ruts in old track.
W7a	NZ 216406		8.0	F	1	stony	O	rough grass	Yes	Trib. of acid str. 2.
W7b	NZ 216406		8.1	F?		clay	O	" "	Yes	Backwater of W7a.
W8	NZ 216406		8.4	S	0	clay	O	rough grass	No	Pool near W7.
W9	NZ 217406		7.7	IF	0-1	clay	L	grass,barley	No	Ditch along field.
W10	NZ 216409		6.7	F	1	concrete	L	grassland	Yes	Pipe 1 outfall.
W11	NZ 216410		7.2	S	0	sandstone	O	grassland	Yes	Stone trough.
W12	NZ 216410		7.2	F	1	peaty	L	cleared wood	No	New Cut above conf.
W13	NZ 216410		6.7	IF	0-1	concrete	L	grassland	No	Lined ditch.
W14	NZ 216410		7.0	F	1	mud	O	grassy bog	No	Pipe 2 outfall.
W15	NZ 216409		6.9	IF	0-1	clay	L	grass,sedge	No	Boggy stream.
W16	NZ 213403		6.5	S	0	clay	O	grassland	No	Pools in subsidence.

Site no	O.S. map ref.	date	pH	flowing / still	flow	substrate	shaded / open	surrounding vegn. type	heavy algal grth	notes
S1	NZ212405		2.6	F	1	clay	L	grass, bush	Yes	Source of acid stream
S2	NZ 212405		2.6	F	1	clay	L	grass, bush	Yes	Below W1a
S3	NZ 213405		2.6	F	1	pebbles	S	wood	No	
S4	NZ 214406		2.6	F	1	iron pipe	O	barley	Yes	Reservoir in flow
S5	NZ 214406		2.6	F	1	concrete	O	barley	Yes	Reservoir outflow
S6	NZ 214406		2.7	F	1	pebbles	L	grass	Yes	Above conf. of acid streams
S7a	NZ 214406		5.2	F	1	clay	L	grass	No	Acid stream 2
S7b	NZ 214405		3.1	F	1	clay	O	rough grass	Yes	Acid stream 2
S8	NZ 214406		3.8	F	1	pebbles	L	wood	Yes	Below conf. of acid stream
S9	NZ 215408		3.8	F	1	rocky	L	few trees	No	At road bridge
S10	NZ 215409		3.3	F	1	mud	L	few trees	Yes	Near dead tree
S11	NZ 216410		3.8	F	1	mud	O	boggy grass	No	Esh Wood boundary
S12	NZ 216410		6.7	F	1	mud	O	boggy grass	No	Pipe 3 outfall
S13	NZ 216411		6.4	F	1	mud	O	cleared wood	Yes	Below New Cut
A1	NZ 212405						L	grass, bush		½ m. above stream
A2	NZ 213405						S	wood		do.
A3	NZ 214406						O	none near		½ m. above reservoir
A4	NZ 214406						L	wood		½ m. above stream
A5	NZ 212404						O	grass		near North Wood B'dary
A6	NZ 212405						O	rough grass		3 m. east of S1
A7	NZ 213404						L	bushes		10 m. south of S2
A8	NZ 213405						O	none		3 m. south of W5b

Table II shows sites in order of pH. Samples D10 and D11, D14 and D15, D16 and D17, D26 and D27, D29 and D30, were cultured together, so giving 16 sets of 'D' cultures in addition to the mixed cultures at pH 2.6 - 4.0. 'S' sites 1 - 5 have been grouped together as flora, pH and most other chemical factors are fairly constant.

TABLE II

Table of Sites in order of pH

Site No.		pH
S1-5	Acid stream 1 down to reservoir	2.6
S6	Acid stream 1 above confluence of Acid Streams	2.7
S7b	Acid stream 2 above main tributary	3.1
D25	Waldridge Fell	3.2
S10	Acid Stream 1	3.3
S8-9	Acid Stream 1	3.8
S11	Acid Stream 1 Esh Wood boundary	3.8
W1b	Ditch, tributary of Acid Stream 1	4.6
W1a	Ditch, tributary of Acid Stream 1	4.7
D23	Waldridge Fell	4.8
S7a	Acid Stream 2 above confluence of Acid Streams	5.2
D26/27	Tubs near laboratory	6.3
D8	Conifer wood stream	6.4
S13	Acid Stream 1 below New Cut	6.4
W16	Pools in field near North Wood	6.5
D29/30	Tubs near laboratory	6.6
W13	Lined ditch near Esh Wood	6.7
S12	Pipe 3 outfall	6.7
W10	Pipe 1 outfall	6.7 ø
D31	Old House Beck (A.690 road bridge)	6.8
D6	High School Sewage Works final effluent	6.9
W15	Boggy stream, tributary of Acid Stream 1	6.9
W3	Stream at base of tip	7.0
W14	Pipe 2 outfall	7.0
D10/11	Edge of stream below Hollingside Lane S.D.W.	-
D7	Stream below D6 effluent	7.2
W11	Stone trough near pipe 3	7.2
W12	New Cut above confluence	7.2
D32	Old House Beck, Page Bank	7.3
W5a, b & c	Stream from tip	7.5
D3	Ditch, Brasside	7.6
W6	Ruts near wood	7.7
W9	Ditch along field	7.7 ø
D12	Old bath in field	8.0
W7a	Main tributary of Acid Stream 2	8.0
W7b	Backwater of main tributary of Acid Stream 2	8.1
D1	River Wear, Finchale	8.3
W8	Pool near W7	8.4
D16/17	Pools in old gravel road, Witton-le-Wear	8.9
D14/15	Outflow stream from small lake, Witton-le-Wear	9.2

pH values given are mean values as in Table 1

ø W9 and W10 were found to have very variable pH values.

4.2 Culture dates and pH values.

'D' cultures were set up individually at pH values of pH = 3.0, pH = 3.5 and pH = site pH as listed in Table II, in addition to cultures of a mixed inoculum at pH values of pH = 2.6, 2.8, 3.0, 3.2, 3.4, 3.6, 3.8, 4.0. These were incubated from 13.5.73 to 2.6.73.

'W' cultures were set up as described in 3 series. Initial pH values of series Y and Z are given in Table III below.

TABLE III.

Site no.	pH value series Y	pH value Series Z. 31.5.73 sample.	pH value Series Z. 21.7.73 sample.
W1a	2.6	4.8	4.6
W1b	2.6	4.8	4.4
W3	2.6	6.9	7.1
W5a	2.6	7.2	7.8
W5b	2.6	7.2	7.8
W5c	2.6	7.2	7.8
W6	2.6	7.5	7.9
W7a	5.2	8.0	8.0
W7b	5.2	8.0	8.2
W8	3.8	8.3	8.5
W9	3.8	7.2	8.3
W10	3.8	7.1	6.2
W11	3.8	7.5	6.9
W12	3.8	7.1	7.3
W13	3.8	6.4	7.0
W14	3.8	6.8	6.9
W15	3.8	6.9	6.9
W16	2.6	6.3	6.7

Cultures were examined after approximately 3 weeks incubation starting on 21.6.73 and 12.8.73 respectively.

It was found that cultures at an initial pH of 3.5 or greater tended towards a pH value of around 7.0, whereas pH values of cultures at pH = 2.6 remained constant, and values for cultures at initial pH = 3.0 only rose to a maximum of pH = 3.3.

4.3 Floral analysis of cultures.

(i) Algae found alive on examination of cultures are tabulated in Table IV. Results from both sets of 'W' samples have been combined, as have results from sites W5a, W5b, W5c. Algae surviving in any of the cultures are listed, as this indicates presence of the alga at the site in some form, even if not actively growing. Those surviving only in cultures at pH = 3.0 or less are distinguished by marking their presence with a circle rather than a cross.

Results for 'S' sites are a combination of results from all floral samples taken since October, 1972 (pers. comm. HARGREAVES). All these species have been shown to be capable of survival in the medium used.

Notes to Table IV.

Chlorophyceae.

Many of the palmelloid green forms present could not be identified, as well as some of the smaller motile and non-motile forms. Therefore it is quite possible that some cells listed as non-motile greens may be of the same species as some of the motile forms. (M = motile, NM = non-motile).

Measurements given for filamentous species are the mean widths of the filaments. For non-filamentous species, either measurements in two directions are given or, for spherical species, the diameter is listed. For Oedogonium spp the diameter of the oogonia is given prefixed by o- (in um).

The algae listed under Hormidium/Ulothrix 9µm may belong to one or several species.

Mougeotia has been listed under 3 different sizes but this may not correspond to 3 species. Lack of time prevented study of the reproductive stages for identification.

Chrysophyceae.

The Chrysophyte found in acid water on Waldridge Fell was provisionally identified as Chrysocapsa sp (pers. comm. HIBBERD) and is very similar to the organism found in Brandon Acid Stream, so these have been listed together although they may not be the same species.

Bacillariophyceae.

Measurements given are mean cell lengths along the largest axis.

Musci.

At least two different species of moss protonema were observed.

(ii) Mixed inoculum cultures.

Results of the floral analysis of these cultures are given in Table V. The same notes apply as for Table IV, and the results are seen to correlate well with those in the latter Table. ~~The increase in the number of species listed with increasing pH is shown in the graph following~~.

(iii) 'Association Table'.

From Table IV it can be seen that some species were only found in cultures from low pH sites, whereas other species, although found at low pH, appeared to occur ubiquitously. A few species are confined to the upper half of the pH scale. Representative species of these types have been selected from the results and arranged in the form of an 'Association Table' (SHIMWELL, 1971), ~~but with sites in order of pH rather than geographical location.~~

TABLE IV. Summary of algae found in all Cultures.

Site	S1-5	S6	S7b	D25	S10	S8/9	S11	W1b	W1a	D23	S7a	D26/27	D8	S13	W16	D29/30	W13	S12	W10	D31
pH	2.6	2.7	3.1	3.2	3.3	3.8	3.8	4.6	4.7	4.8	5.2	6.3	6.4	6.4	6.5	6.6	6.7	6.7	6.7	6.8
Cyanophyceae:-																				
Chamaesiphon sp.																				
Oscillatoria spp												X				X	X		X	
Lyngbya spp.												X				X	X		X	
Anabaena spp.																X				
Pseudanabaena catenata												X			X					
Nostoc spp.												X			X	X				
Calothrix sp.																X				
Chlorophyceae:-																				
Chlamydomonas sp.(?)6µm	X	X			X	X			X		X	X		X	X	X	X	X	X	
green NM 6µm								O	O			O			X		X			
Chlamydomonas sp. 6x3µm								O	O				X		X	X			X	X
green NM 6x3µm	X	X				X						X	X				O		O	
green NM 6x3µm									O							X				
Chlamydomonas sp. 9µm						X	X					X			X					
do. 10x 8µm								O							X				X	O
green M&NM 10-15µm				X				X	X	X		X	X			X	X		X	X
green NM 15-30µm				X					X			O			X					
green M 3 µm																				
green NM 3 µm								O	O				O		O		X		X	O
[illegible] colonial green 6 µm									X			O					X		X	
Characium sp. 7µm						X		X												
[illegible]dron sp.																				
[illegible]botrys sp.																				
[illegible]us sp.																				

Site	D6	W15	W3	W14	D10/11	D7	W11	W12	D32	W5abc	D3	W6	W9	D12	W7a	W7b	D1	W8	D16/17	D14/15
pH	6.9	6.9	7.0	7.0	-	7.2	7.2	7.2	7.3	7.5	7.6	7.7	7.7	8.0	8.0	8.1	8.3	8.4	8.9	9.2
Cyanophyceae:-																				
Chamaesiphon sp.									X					X						X
Oscillatoria spp	X	X	X	X					X	X	X						X			
Lyngbya spp.	X	X	X	X	X				X	X	X						X			
Anabaena spp.	X				X				X		X	X	X		X	X	X	X	X	
Pseudanabaena catenata		X		X	X	X	X				X				X	X	X		X	X
Nostoc spp.				X									X		X	X		X	X	
Calothrix sp.									X											
Chlorophyceae:-																				
Chlamydomonas sp.(?)6µm	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	X
green NM 6µm		X	O		X		X	X	X	X	X	O	O	O	X	X		X	X	X
Chlamydomonas sp. 6x3µm				X	X		O	X				X	X		O					
green NM 6x3µm				O	O			X		O		O	O	O			X	O		X
green NM 6x3µm										X	O								O	O
Chlamydomonas sp. 9µm		X	O	X	X	O	X			O							O			
do. 10x 8µm	O								O	O		O	X	O						
green M&NM 10-15µm	X		X	X	X	X	X		X	X	X	X	X	X	X	O	X	X	X	X
green NM 15-30µm		X						X	O	O				O		X		O		
green M 3 µm									O	X	X				X	X				X
green NM 3 µm		O	X	X	X	X	X	X	X		X		X	X	O	X	X	X	X	
[illegible] colonial green 6 µm	X	X		X		X	O	X	X		X		X	X	X	X	X	X		
Characium sp. 7µm				O	X															
[illegible]dron sp.						X													X	
[illegible]botrys sp.	X													X			X			
[illegible]us sp.					X				X							X	X		X	X

TABLE IV (contd.)

Site	pH	Scenedesmus arcuatus	S. quadricauda	Scenedesmus spp.	Hormidium/Ulothrix 9 µm	Ulothrix subtilissima	U. moniliformis	Hormidium rivulare	Stichococcus bacillaris	S. minutus	Microspora sp.10-15µm	Microspora sp.10 µm	Stigeoclonium tenue	Microthamnion strictissimum	Protococcus viridis	Aphanochaete repens	Gongrosira sp.	Oedogonium sp.4-5µm	" 6 µm	" 10µm(c-30)	" 12µm(c-45)	" 16µm(c-38)	" 20 µm	" 25-30µm(c-45)	Mougeotia sp. 9-10 µm	" 22-25 µm	" 30 µm
D14/15	9.2			X					O		X		X			X	X	X				X			X		
D16/17	8.9		X					X	O	O			X			X	X	X			X			X	X		
W8	8.4				X	X		X	X					X	X										X		
D1	8.3	X	X	X				X					X		X		X					X					
W7b	8.1			X				X	O				X							X						X	X
W7a	8.0												X														X
D12	8.0		X	X					X						X												
W9	7.7					X	O	X	X	O															X		
W6	7.7						X	X	X	X															X		
D3	7.6		X		O		X	X	X	O																	
W5abc	7.5							X	X	X																	
D32	7.3		X				X	X		O	X		X	X	X				X		X		X				
W12	7.2																										
W11	7.2					X		X	X	O			X														
D7	7.2	X	X	X	O		X	X	X	X			X														
D10/11	-		X		X		X	X	X	O			X			X		X				X		X			
W14	7.0							X	X	X			X														
W3	7.0				X		O	O	X	O																	
W15	6.9							X		O			X														
D6	6.9		X					X	O				X	X													
D31	6.8						X	X	O	O				X													
W10	6.7						X	X	X	X			X														
S12	6.7													X													
W13	6.7												X		X												
D29/30	6.6										X								X								
W16	6.5							X	X	O															X		
S13	6.4													X													
D8	6.4														X												
D26/27	6.3				O			X	O	O	X	X	X	X					X		X				X		
S7a	5.2																										
D23	4.8							X						X											X		
W1a	4.7				O			X	X					X											X		
W1b	4.6				X		X	X	O	O				O											X		
S11	3.8							X																			
S8/9	3.8							X	X																		
S10	3.3							X																			
D25	3.2							X																			
S7b	3.1							X																			
S6	2.7							X	X																		
S1-5	2.6							X	X																		

TABLE IV (contd.)

Site	S1–5	S6	S7b	D25	S10	S8/9	S11	W1b	W1a	D23	S7a	D26/27	D8	S13	W16	D29/30	W13	S12	W10	D31	D6	W15	W3	W14	D10/11	D7	W11	W12	D32	W5abc	D3	W6	W9	D12	W7a	W7b	D1	W8	D16/17	D14/15
pH	2.6	2.7	3.1	3.2	3.3	3.8	3.8	4.6	4.7	4.8	5.2	6.3	6.4	6.4	6.5	6.6	6.7	6.7	6.7	6.8	6.9	6.9	7.0	7.0	–	7.2	7.2	7.2	7.3	7.5	7.6	7.7	7.7	8.0	8.0	8.1	8.3	8.4	8.9	9.2
Zygnema sp.40-45µm																																	X						X	X
Spirogyra spp.																																							X	X
Cylindrocystis sp.								X							X										X	O							X					X		
Closterium acerosum								X							X																		X							
Closterium spp.												X																												
Roya obtusa								X	X	X		X																												
Cosmarium sp.30µm												X							X														X			X	X		X	X
Cosmarium spp.																X																	X							
Cosmarium sp. 18µm																X																								
Euastrum pectinatum																X																								
Cladophora glomerata																																					X			X
Rhizoclonium hieroglyphicum																																							X	X
Chrysophyceae:-																																								
Synura petersenii								X																																
Chrysocapsa sp./ BAS Chrysophyte	X	X	X	X	X	X	X			X		O																												
Hydrurus sp.?								O?	X?	X		X?										O																		
Xanthophyceae:-																																								
Vaucheria spp.																X									X				X								X			
Bacillariophyceae																																								
Melosira sp.																																				X	X			
Ceratoneis sp.																																					X			
Diatoma vulgare																																			X	X	X			
Fragilaria sp.																																								X

TABLE IV. (contd.)

Site	S1-5	S6	S7b	D25	S10	S8/9	S11	W1b	W1a	D23	S7a	D26/27	D8	S13	W16	D29/30	W13	S12	W10	D31	D6	W15	W3	W14	D10/11	D7	W11	W12	D32	W5abc	D3	W6	W9	D12	W7a	W7b	D1	W8	D16/17	D14/15
pH	2.6	2.7	3.1	3.2	3.3	3.8	3.8	4.6	4.7	4.8	5.2	6.3	6.4	6.4	6.5	6.6	6.7	6.7	6.7	6.8	6.9	6.9	7.0	7.0	–	7.2	7.2	7.2	7.3	7.5	7.6	7.7	7.7	8.0	8.0	8.1	8.3	8.4	8.9	9.2
Meridion circulare																									X															
Opephora sp.																								X	X	X											X	X		
Synedra ulna																X			X								X	X							X		X		X	
Synedra (epiphytic)												X																									X		X	X
Eunotia exigua	X	X	X		X	X	X	O	O	X	X			X																										
Achnanthes sp.																			X		X			X	X					X					X	X		X	X	
Cocconeis sp.																								X																X
Rhoicosphenia curvata																X								X													X			X
Amphipleura sp.															X									X																
Amphiprora paludosa																																			X					
Caloneis (silicula?)																																							X	
Cymatopleura sp.																								X																
Cymbella sp.																																			X	X	X		X	
Gomphonema sp.																		X																			X			X
Gyrosigma sp.																														X										
Nitzschia acicularis																							X							X									X	
N. ovalis	X	X				X											X		X																	X		X		
N. palea			X		X	X	X	X			X				X				X					X			X		X		X		X		X			X		
Pinnularia sp. 20-25 µm																	X		X					X	X	X			X	X			X		X			X	X	
" 30-35 µm									X						X	X					X												X					X		
" 45 µm																						X															X			
P. acoricola 15 µm	X																	X																		X				
P. viridis 120 µm																			X			X														X				
Cryptophyceae:-																																								
Cryptomonas sp.	X	X			X	X	X				X																													

TABLE IV (contd.)

Site	S1–5	S6	S7b	D25	S10	S8/9	S11	W1b	W1a	D23	S7a	D26/27	D8	S13	W16	D29/30	W13	S12	W10	D31
pH	2.6	2.7	3.1	3.2	3.3	3.8	3.8	4.6	4.7	4.8	5.2	6.3	6.4	6.4	6.5	6.6	6.7	6.7	6.7	6.8
Euglenophyceae:-																				
Euglena mutabilis	X	X		X	X	X		O						X	O					
Euglena (viridis?)															O					
Euglena (gracilis?)																				
Trachelomonas spp.								X											X	
Musci:-																				
Moss protonema	X	X				X		O	O						O					X

Site	D6	W15	W3	W14	D10/11	D7	W11	W12	D32	W5abc	D3	W6	W9	D12	W7a	W7b	D1	W8	D16/17	D14/15
pH	6.9	6.9	7.0	7.0	–	7.2	7.2	7.2	7.3	7.5	7.6	7.7	7.7	8.0	8.0	8.1	8.3	8.4	8.9	9.2
Euglenophyceae:-																				
Euglena mutabilis		X			X	X				X										
Euglena (viridis?)																				
Euglena (gracilis?)															X					
Trachelomonas spp.	X													X						
Musci:-																				
Moss protonema							O		X	O	X								X	

Table V.

Floral results for survival in cultures of mixed inoculum.

Initial pH	2.6	2.8	3.0	3.2	3.4	3.6	3.8	4.0
pH after 3 weeks	2.6	2.8	3.1	3.3	3.5	4.2	5.0	5.7
Chrysocapsa sp./BAS sp.	X	X	X	X	X	X	X	X
Eunotia exigua	X	X	X	X	X	X	X	X
Euglena mutabilis	X	X	X	X	X	X	X	X
Hormidium rivulare	X	X	X	X	X	X	X	
10-15µ greens. M & NM	X	X	X	X	X	X	X	X
Stichococcus bacillaris	X		X	X	X	X	X	X
6µ green - tight colony	X	X	X	X	X		X	X
6µ non-motile green	X	X	X	X		X	X	X
6µ Chlamydomonas sp.(p)	X	X	X	X				X
Ulothrix moniliformis	X	X	X		X		X	
6x3µ green NM		X		X	X			
15-30µ green spores	X						X	X
6x3µ Chlamydomonas sp.			X		X	X	X	X
Pinnularia sp. 20µ			X	X	X	X		
Ulothrix/Hormidium sp.			X	X		X	X	
Microthamnion strictissimum			X		X	X		X
Cylindrocystis sp.				X	X	X		
Mougeotia sp. 9µ				X	X	X	X	X
Nitzschia palea					X	X	X	X
Characium sp. 7µ					X			X
Stigeoclonium tenue						X	X	X
Closterium acerosum						X	X	X
Oedogonium sp.4-5µ (o15)						X	X	X
Oedogonium sp. 45µ						X	X	
Mougeotia sp. 21µ						X		X
Nitzschia sigmoideae						X		
Trachelomonas sp.							X	X
Cosmarium sp. 30µ							X	X
Closterium sp.							X	X
Protococcus viridis							X	X
Oedogonium sp. 30µ(o 45)							X	X
Microspora sp. 12µ							X	X
Scenedesmus quadricauda							X	X
Vaucheria sp.							X	
Oscillatoria spp.								X
Lyngbya spp.								X
Anabaena sp.								X
Pseudanabaena catenata								X
Nostoc sp.								X
Calothrix sp.								X

TABLE VI.

'Association Table'.

Site	S1-5	S6	S7b	D25	S10	S8/9	S11	W1b	W1a	D23	S7a	D26/27	D8	S13	W16	D29/30	W13	S12	W10	D31	D6	W15	W3	W14	D10/11	D7	W11	W12	D32	W5abc	D3	W6	W9	D12	W7a	W7b	D1	W8	D16/17	D14/15
pH	2.6	2.7	3.1	3.2	3.3	3.8	3.8	4.6	4.7	4.8	5.2	6.3	6.4	6.4	6.5	6.6	6.7	6.7	6.7	6.8	6.9	6.9	7.0	7.0	-	7.2	7.2	7.2	7.3	7.5	7.6	7.7	7.7	8.0	8.0	8.1	8.3	8.4	8.9	9.2
hrysococcus sp./ BAS Chrysophyte	X	X	X	X	X	X	X			X		X																												
ryptomonas sp.	X	X			X	X	X				X																													
unotia exigua	X	X	X		X	X	X	X	X	X	X			X																										
oya obtusa								X	X	X		X																												
uglena mutabilis	X	X		X	X	X		X						X	X							X			X	X				X										
ormidium rivulare	X	X	X	X	X	X	X	X	X	X		X			X				X	X	X	X	X	X	X	X	X		X	X	X	X	X			X	X	X	X	
tichococcus bacillaris	X	X				X		X	X			X			X				X	X	X		X	X	X	X	X			X	X	X	X	X		X		X	X	X
itzschia ovalis	X	X				X											X		X																	X		X		
hlamydomonas sp.(?)6µm	X	X			X	X			X		X	X		X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	X
itzschia palea			X		X	X	X	X			X				X				X					X			X		X		X		X		X			X		
icrothamnion strictissimum								X	X	X		X		X				X		X	X								X									X		
tichococcus minutus								X				X			X				X	X		X	X	X	X	X	X		X	X	X	X	X						X	
ougeotia sp. 9-10µm								X	X	X		X			X																	X	X					X	X	X
lothrix moniliformis								X											X	X			X		X	X			X		X	X	X							
ight colonial green 6 µm									X			X					X		X		X	X		X		X	X	X	X		X		X	X	X	X	X	X		
scillatoria spp.												X				X	X		X		X	X	X	X					X	X	X						X			
yngbya spp.												X				X	X		X		X	X	X	X	X				X	X	X						X			
ostoc sp.												X			X	X								X									X		X	X		X	X	
seudanabaena catenata												X			X							X		X	X	X	X				X				X	X	X		X	X
nabaena spp.																X					X				X				X		X	X	X		X	X	X	X	X	
tigeoclonium tenue												X					X		X		X	X		X	X	X	X		X						X	X	X		X	X
rotococcus viridis													X				X												X					X			X	X		
cenedesmus quadricauda																					X				X	X			X		X			X			X		X	
Opephora sp.																								X	X	X											X	X		
nkistrodesmus spp.																									X				X							X	X		X	X

TABLE VII. Survival of Algae at a pH of 3.3 or below.

Site	S1-5	S6	S7b	D25	S10	S8/9	S11	W1b	W1a	D23	S7a	D26/27	D8	S13	W16	D29/30	W13	S12	W10	D31	D6	W15	W3	W14	D10/11	D7	W11	W12	D32	W5abc	D3	W6	W9	D12	W7a	W7b	D1	W8	D16/17	D14/15
pH	2.6	2.7	3.1	3.2	3.3	3.8	3.8	4.6	4.7	4.8	5.2	6.3	6.4	6.4	6.5	6.6	6.7	6.7	6.7	6.8	6.9	6.9	7.0	7.0	-	7.2	7.2	7.2	7.3	7.5	7.6	7.7	7.7	8.0	8.0	8.1	8.3	8.4	8.9	9.2
Chrysocapsa sp./ BAS Chrysophyte	X	X	X	X	X					X		X																												
Cryptomonas sp.	X	X			X																																			
Eunotia exigua	X	X	X		X			X	X																															
Euglena mutabilis	X	X			X			X							X							X								X										
Hormidium rivulare	X	X	X	X	X			X	X	X		X			X				X	X	X	X	X	X	X	X	X		X	X	X	X	X			X		X	X	
Stichococcus bacillaris	X	X						X	X			X			X					X	X		X	X	X	X	X			X	X	X		X		X		X	X	X
Nitzschia ovalis	X	X																																						
Chlamydomonas sp.(?)6µm	X	X			X										X	X						X	X					X	X					X		X				
green NM 6x3µm	X	X													X		X		X					X	X		X	X		X		X	X	X				X		
Moss protonema	X	X						X	X						X											X	X			X										
Nitzschia palea			X		X														X					X						X					X					
green M&NM 10-15µm				X						X			X			X	X		X	X	X		X	X		X	X	X		X	X	X	X			X	X			X
green NM 15-30µm				X				X	X			X			X					X									X	X				X				X		
green NM 6µm								X	X			X					X					X	X	X	X		X			X	X	X	X	X				X	X	X
green NM 3µm								X	X				X		X		X		X	X		X	X	X		X	X	X		X	X				X	X		X	X	
Chlamydomonas sp.6x3µm								X	X							X								X			X								X		X			
Stichococcus minutus								X				X			X				X	X		X	X		X	X	X		X	X	X		X						X	
Hydrurus ?sp.								X	X													X																		
Hormidium/Ulothrix 9µm									X			X											X		X	X					X									
tight colonial green 6µm									X			X															X						X							
Ulothrix moniliformis								X											X				X			X					X	X	X							
Chlamydomonas sp.10x9µm								X												X	X								X	X		X		X						
" 9µm																							X			X				X							X			
[illegible]cium sp.																								X														X		
[illegible]									X																					X	X								X	X
[illegible]								X	X																															

(iv) Survival at low pH.

Algae which survived in individual site cultures at initial pH of 3.0 or less are tabulated in Table VII. Since the maximum pH reached by any of these cultures was 3.3 these organisms can survive in culture at or below this value. Certain 'S' sites are omitted as incubation of cultures of samples from these sites at low pH was not carried out. The organisms are listed in basically the same order as in Table VI.

The following were also found but are not listed in the Table:

W5abc	Pinnularia sp.	45µm.	W7a	Cosmarium sp.	30µm.
S1-5	Pinnularia acoricola	15µm.	D7	Cylindrocystis sp.	

W16 Euglena (viridis?)

In order to discover whether acid tolerance of a species was related to the pH of the site at which it was found, the variances of the distributions of pH values of sites at which the species had been shown to occur (Table IV), and of those from which the species survived at low pH (Table VII), were compared. The variance of the distribution of pH values was calculated in each case from:

$$S^2 = \frac{\sum_n (pH_n)^2}{N} - (\overline{pH_n})^2$$

where N = number of sites

pH_n = pH value of site n.

For each species the larger variance was then divided by the smaller to give 'F', the variance ratio.

The calculation was carried out for all species found to occur in at least 6 cultures in both cases, and results are given below. Algae of doubtful taxonomic position or unidentified have been omitted.

TABLE VIII

Species	N_1	N_2	s_1^2	s_2^2	F
Ulothrix moniliformis	7	10	1.029	0.734	1.401
Hormidium rivulare	27	28	3.323	3.354	1.009
Stichococcus bacillaris	21	23	2.965	2.745	1.080
S. minutus	15	17	0.768	0.704	1.091
Chrysocapsa sp/BAS Chrysophyte	7	7	1.565	1.565	1.000
Eunotia exigua	6	7	0.717	0.821	1.146
Nitzschia palea	6	12	3.903	2.943	1.326
Euglena mutabilis	7	10	3.728	3.807	1.021

N_1 = number of sites from which species survived in culture at pH 3.3 (omitting S8/9, S11, S7a, S13, S12)

N_2 = number of sites from which species survived in culture at any pH (omitting S8/9, S11, S7a, S13, S12)

s_1^2 = variance of distribution of pH values of sites from which species survived in culture at pH 3.3 (omitting S8/9, S11, S7a, S13, S12)

s_2^2 = variance of distribution of pH values of sites from which species survived in culture at any pH (omitting S8/9, S11, S7a, S13, S12)

The values of F obtained from the data were compared with values from a table of 25% points of the 'F' distribution (MERRINGTON & THOMPSON, 1943). This corresponds to a probability level in this case of 50% since by dividing the larger variance by the smaller, differences in both directions have been considered (BAILEY, 1959). The results from the data were compared with the tabulated values of F corresponding to $f_1 = N_1 - 1$ degrees of freedom in the numerator and $f_2 = N_2 - 1$ degrees of freedom in the denominator. Variances were compared, rather than means, since most sites have pH values around the centre of the range.

Using this method, any significant differences in the acid tolerance of samples dependent on original site pH should show up as a difference in the variances of the distributions of the pH values. At the 50% level none of the 'F' values obtained exceed the tabulated values, so showing no significant difference in the two distributions at this level. Thus, from the results obtained, there is no evidence of adaptation of particular algal cells within a species to tolerate low pH conditions.

(v) Floral Comparison of Acid Stream 1 and its Catchment.

No species were found to survive in cultures of 'W' samples at pH = 2.6 (W1a, W1b, W3, W5a, W5b, W5c, W6 cultures of series Y) that did not also occur in Acid Stream 1 nearby. (Series Y cultures for other 'W' sites could not be used for this comparison due to changes of culture pH.) Most of the Acid Stream species were found in these cultures although the Chrysophyte and *Cryptomonas* sp. were notable by their absence.

(vi) Floral Comparison of the Acid Stream Catchment and the 'Durham area'.

The occurrence of some of the species comprising the flora of Acid Stream 1 in the catchment was compared with their occurrence in the 'D' samples from Table IV. Few of the acid stream species were found to occur in more than a small number of 'W' or 'D' sites. *Homidium rivulare* was found to occur in 11 out of 15 'D' sites and 13 out of 16 'W' sites listed, showing no apparent difference in distribution with respect to pH in the 2 areas. Similarly *Stichococcus bacillaris* and *Chlamydomonas* sp.(p?) 6μm were present in most sites of both series although absent from 'D' sites at pH = 3.2 and 4.8. However, these differences can hardly be regarded as significant. ~~This is discussed in Section 5.2.~~

(vii) Floral analysis of 'Air-Flora' study Cultures.

Species found alive in the floral analysis of the 'air-flora' cultures are shown in Table IX.

Samples from sites A1 - A4 were collected on two occasions, 29.6.73 and 13.8.73, and the results have been combined. Samples from sites A5 - A8 were collected on 13.8.73 only, and results for A5 at pH = 5.5, and A7, are missing, as the collecting vessels were damaged by animals or vandals in these cases.

Results for open beakers and those covered with muslin, were not significantly different, so these have been combined in each case.

TABLE IX

Floral Analysis of Cultures from Air-Flora Experiment.

Site	A1	A1	A2	A2	A3	A3	A4	A4	A5	A6	A6	A8	A8
pH of medium	2.6	5.5	2.6	5.5	2.6	5.5	2.6	5.5	2.6	2.6	5.5	2.6	5.5
Chlamydomonas sp.(p) 6 µm	X	X		X				X					
green NM 6 µm			X	X	X	X	X	X	X				
Chlamydomonas sp.6x3 µm	X	X	X	X	X	X	X	X	X	X			
green NM6x3 µm	X	X	X	X	X	X						X	X
Chlamydomonas sp.10x8µm					X	X		X			X		
green M&NM 10-15 µm		X	X	X	X		X	X	X			X	X
green NM 15-30 µm	X	X	X			X							
green M 3 µm			X						X				
green NM 3 µm			X	X	X	X	X		X		X		
tight colonial green 6 µm			X	X	X	X	X	X				X	
Characium sp.	X	X											
Ulothrix sp. 7-8 µm		X			X								
U. moniliformis		X	X	X	X	X	X	X					
Hormidium rivulare		X	X	X	X	X	X	X					
Stichococcus bacillaris	X	X	X	X	X	X	X	X					
S. minutus			X	X	X		X		X	X		X	X
Protococcus viridis	X		X	X	X	X	X	X	X	X			X
Mougeotia sp. 10 µm		X	X										
BAS Chrysophyte		X											
Eunotia exigua	X	X		X	X	X		X					
Nitzschia ovalis		X		X									
Pinnularia sp. 20 µm				X		X							
Pinnularia sp. 35 µm		X		X							X		
P. acoricola		X											
Euglena mutabilis	X	X	X	X	X		X	X					
Euglena sp. 10 µm						X							
Moss protonema	X	X	X	X		X	X	X			X		X

5 DISCUSSION

5 DISCUSSION

Firstly the limitations of the experimental methods used are discussed and within the restrictions imposed by these limitations, the results are then considered and conclusions drawn.

5.1 Discussion of Experimental Methods.

All the foregoing data are based on examination of organisms in culture, so care must be taken in applying the results to algae in the field. In examination of cultures various factors prevented identification of all species present. Some smaller diatoms and green algae were disregarded, and many green species could not be identified although these are mostly listed descriptively. Further discussion of taxonomic problems is given in Appendix II.

All samples were cultured at field pH values under the same conditions as the acid cultures. Thus comparisons carried out between survival of a species at low pH and at field pH were based on the same conditions except for presence of sulphuric acid, and differences in composition of the algal population. Few organisms were found which did not survive in the medium used.

The major problem encountered in culturing organisms at low pH was the change in pH brought about by their growth in cultures at initial pH values above pH = 3.0. Other workers have used buffers to stabilize pH but it was thought that this would introduce other factors affecting growth, and for a comparison with the Brandon Acid Streams it was considered best to use only sulphuric acid. Cultures at lower pH were extremely stable. Practical difficulties precluded regular adjustment of pH. Therefore, results from cultures at pH = 3.5 or above were combined with field pH results.

Competition may also have occurred in cultures to some extent - evidence for this being that a few species were only recorded in culture at pH = 3.0 or below, but not in cultures at higher pH, although other evidence indicates that these species are not obligate acidotrophs. (These are marked with a circle on Table IV). However, this will be noticed not to be widespread except in the case of some unidentified green algae where the forms recorded at low pH may be non-motile stages of other species recorded at higher pH. Ulothrix/Hormidium sp. and Stichococcus minutus appeared to fare particularly badly, especially where large growths of blue-green algae or Stigeoclonium tenue occurred. Observations from all cultures have therefore been included in Table IV to minimise errors. Formation of microhabitats at different pH from the main body of a culture is another possible source of error (YONGUE & CAIRNS, 1971), and this was minimised by growth in a shake-tank.

Limitations are imposed on all the results in that the data were collected only at one time of one year, and site pH values were only measured once or twice. The experiments need to be repeated over several years to obtain reliable data. Also the data were based on presence and absence of living cells only.

5.2 Acid tolerance of algae.

The results set out in Tables IV and VI indicate the pH ranges of occurrence of species from the sampling sites used. It is interesting to note that few appear to be obligate acidotrophs on the basis of these data. Those that do appear to be limited to low pH sites only include Chrysocapsa sp./ BAS Chrysophyte, Cryptomonas sp., Eunotia exigua and Roya obtusa, the latter showing a very narrow range of occurrence. Other species occur over a larger range of pH but are less common at higher pH., e.g. Euglena mutabilis. Many species, however, appear to be ubiquitous in occurrence, e.g. Hormidium rivulare, Stichococcus bacillaris, and Chlamydomonas sp.(p), and able to tolerate very low pH conditions.

However, it must be remembered that these data are based entirely on survival in culture and give no indication of cover in the field, or of growth and competitive ability. For instance, although in the results Euglena mutabilis does not appear to be specific to acid flowing waters, it is in fact one of the most reliable indicator organisms for this type of environment, often growing profusely and forming a green coating obscuring the stream bed. It has been found in all acid streams so far studied in Britain (pers. comm. HARGREAVES, 1973) and is reported to occur similarly in the U.S.A. (LACKEY, 1938).

In general, studies carried out in the U.S.A. show similar results (LACKEY, 1939; BENNETT, 1969) for acid tolerant species. MOSS (1973) investigating the effects of pH on various species in culture gives results which correlate well with those given above for most species appearing in most cases. He reports Eunotia sp. and Euglena gracilis as being the most tolerant of acid conditions of the species he has studied. Under his classification, most acid tolerant species would be regarded as oligotrophs.

From the results it is obvious that a high degree of acid tolerance does not seem tobe specific to any particular division of algae. Members of the Chlorophyta, Chrysophyta, Bacillariophyta, Cryptophyta and Euglenophyta have been found in the field at sites of pH below 3.0. Divisions not represented are in general fairly rare or have relatively few freshwater representatives. The only common freshwater division not reported from acid waters is the division Cyanophyta. In these results, Oscillatoria spp., Lyngbya spp., Pseudanabaena catenata and Nostoc spp. are all absent from sites of pH below 6.3, and Anabaena spp. are absent from sites below pH = 6.6. All occur widely at all pH values above these up to pH = 8.3.

This absence is discussed by BROCK (1973) who ascribes the inability of members of this division to survive in acid waters, to the fact of their being procaryotic algae and suggests that acid environments were possibly of great importance in allowing the evolution of eucaryotic organisms. It is interesting to consider why procaryotic algae are unable to survive while many species of bacteria are able to thrive in acid waters (JOSEPH, 1953). Brock suggests the possibility of using mild artificial acidification of reservoirs in order to prevent Cyanophycean blooms, and improvement of nitrogen fixation by liming to increase soil water pH in the cultivation of certain crops. MOSS (1973) reports minimum pH values for growth of between 6.2 and 6.9 for a *Gloeotrichia* sp. and a *Gloeocapsa* sp. which is in agreement with the above observations.

From the results a very high degree of correlation is apparent between species surviving at pH = 2.6 in the cultures of a mixed inoculation prepared from the 'D' samples (Table V) and the flora of the Brandon Acid Stream for sites S1 - 5 as represented in Table IV. None of the samples used in preparation of the mixed inoculum were from minewater drainages. Most of the positively identified species listed in Table VII as surviving at low pH occur in the Acid Streams at the same pH, and several of the unidentified organisms may also occur. Taking into account the result (Section 4.3 (v)) that all algae from the catchment of the Stream which survived in culture at pH = 2.6 are found in the upper part of the Stream, these results throughout suggest that pH and associated factors are the main controlling influences in determining the flora of Brandon Acid Streams, as opposed to non-pH-linked factors characteristic of minewater.

5.3 Relation between acid tolerance and field pH.

If Table VII is examined it will be seen that many algae surviving in culture at pH $\leqslant 3.3$ were found in samples from environments of normal pH in the field, although the number may fall off slightly towards the extreme upper end of the pH range. From 'F' values listed in Table VIII, no significant differences between the two distributions compared are apparent even at 50% probability level, for the eight species considered. The only species surviving at low pH solely when taken from low pH environments in the field are those whose occurrence is confined to these acid sites, i.e. Eunotia exigua, Cryptomonas sp., Chrysocapsa sp./BAS Chrysophyte, and Roya obtusa. No cultures of these organisms at higher pH were attempted. Thus there is no evidence here for the formation within a species of ecotypes adapted to tolerate acid conditions. However, these data are based simply on survival in culture, and may not be representative of behaviour in the field.

If such adaptation did occur then it would be expected, assuming that the organisms are readily transported short distances, that members of an acid tolerant species within the vicinity of an acid stream, i.e. in its catchment, would be more acid tolerant than the same species from elsewhere. Comparison of the number of 'W' sites from which algal spp. found in the Acid Stream survived, with the number of such 'D' sites, shows in most cases that the number of such 'W' sites is the larger, although in no cases is the difference significant. However, it is worth noting that Euglena mutabilis from the two 'D' sites at which it was found, did not survive low pH, whereas cells of the same species did survive when taken from all 4 catchment sites at which it occurred. Thus, although no significant evidence for ecotype formation was obtained, further study is necessary.

The evidence presented above suggests that acid tolerance is typical of a species to a great extent, although the behaviour of Euglena *mutabilis* suggests that adaptation to acid environments may also be a controlling factor.

5.4 Air-Flora Experiment.

Little can be concluded from the air-flora experiments conducted since only a limited number of collecting vessels could be set out, and of these, a considerable proportion at sites away from the Acid Streams were damaged by animals or vandals. From the results collected it is seen that no algae were found in the cultures that did not occur in the catchment, except one *Ulothrix* sp. However, this is not surprising as the catchment does not appear to have a substantially different flora from that of the surrounding area. Very few Acid Stream species were found in the cultures at short distances away from the Stream, although many of these species appeared in the beakers 0.5 m. above the Stream - some possibly due to splashing during flood periods in the case of Site A1.

One can conclude from these data that the Acid Stream species are not very abundant in the air a few metres away from the Stream.

Remarkably little difference was observed between collections at pH = 2.6 and those at pH = 5.5, but no blue-green algae were found in any samples, (an expected result). The absence of difference between results for beakers covered with muslin and those left open suggests that contact between insects and the growth medium was not an important factor in the colonisation of the cultures. Further investigations would prove most interesting.

5.5 Recommendations for further research.

Further work of the type described above but on a quantitative basis is needed to elucidate the occurrence and extent of formation within a species of ecotypes especially tolerant to low pH environments.

Repeating the experiments carried out but using cultures at a range of pH values below pH = 3.0 might show ecotypic variation in the lower pH limit for survival within species.

A great deal more work using a large number of sampling sites to investigate fully the air-flora of the Acid Stream catchment, might yield some very enlightening results.

6 SUMMARY

6 SUMMARY

Algal samples were collected from habitats with a wide range of pH values and their tolerance to low pH was investigated. The observed pH ranges of occurrence of species in the field are tabulated, and the ability of the species to survive at low pH, recorded.

The results indicate that some species are apparently confined to sites of low pH environments, whereas other tolerant species also occur in normal or high pH environments. It appears from the results that pH is probably the major factor determining the flora of the Brandon Acid Streams. Evidence found suggests that tolerance of low pH conditions is to some extent characteristic of the species concerned, irrespective of the environment from which it has been taken. However, there is also evidence suggesting the occurrence of adaptation within a species.

Some experiments on transport of algae by air were carried out, but limited data were collected.

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APPENDIX 1

The medium used throughout for cultures was basically Chu's 10D medium modified by the addition of microelements and soil extract. The medium contained the following in a litre of distilled water:

KH_2PO_4	7.80 mg.	H_3BO_3	715 µg.
$MgSO_4.7H_2O$	25.0 mg.	$MnCl_2.4H_2O$	45.0 µg.
$Ca(NO_3)_2$	40.0 mg.	$ZnSO_4.7H_2O$	55.6 µg.
$NaHCO_3$	15.8 mg.	$CuSO_4.5H_2O$	20.0 µg.
Na_2SiO_3	10.9 mg.	$CoSO_4.7H_2O$	20.5 µg.
E.D.T.A. Na salt	4.20 mg.	$Na_2MoO_4.2H_2O$	68.5 µg.
$FeCl_3.6H_2O$	1.21 mg.		

This gives concentrations of the various elements of:

$(PO_4)P$	1.80 $mg.l^{-1}$.	Fe	250 $\mu g.l^{-1}$.
Mg	2.56 $mg.l^{-1}$.	B	127 $\mu g.l^{-1}$.
Ca	950 $\mu g.\ l^{-1}$.	Mn	12.7 $\mu g.l^{-1}$.
$(NO_3)N$	6.85 $mg.l^{-1}$.	Zn	12.5 $\mu g.l^{-1}$.
C	2.25 $mg.l^{-1}$.	Cu	51.2 $\mu g.l^{-1}$.
Si	2.46 $mg.l^{-1}$.	Co	43.0 $\mu g.l^{-1}$.
		Mo	27.2 $\mu g.l^{-1}$.

1 ml. of soil extract was also added to every litre of medium. This was prepared by repeated boiling and cooling of 25 cm^3. of soil with 100 ml. of water, followed by centrifuging to clear the liquid.

APPENDIX 2

Taxonomic problems.

As mentioned, the detailed study of unialgal cultures in order to investigate reproductive processes was not possible, so many species could not be identified, e.g. Mougeotia spp., Zygnema spp., Spirogyra spp., Oedogonium spp., Vaucheria spp.

However, the major area of difficulty was the identification of some green algae. Only motile forms of Chlamydomonas observed are listed under this generic name although many of the palmelloid green algae with pyrenoids may be of this genus. Eye spots were not apparent but often the adverse environment in the cultures caused excessive accumulation of storage products, masking many morphological characteristics. Thus it was not possible to assign such algae a generic name with any certainty. The 6 µm non-motile greens listed may be Chlamydomonas sp.(p) or Chlorella sp.(p) and the larger non-motile greens may also include Chlorococcum spp.

The smaller green algae observed may in some cases be forms of Stichococcus sp. affected by the adverse conditions, and the smaller motile biflagellate greens, perhaps including some algae listed as Chlamydomonas sp. may be Zoospores of Hormidium sp. Therefore it should be borne in mind that some of the descriptive classifications may encompass more than one species, and conversely that several of these organisms may be of the same species in some cases.

An especially interesting problem arises in the identification of Ulotrichales found, particularly in acid cultures. This is complicated by the morphological changes caused by the acid environment,added to which the generic classification of this order is under a certain amount of dispute. The minor nature of readily observable differences between the genera Hormidium and Ulothrix and the wide range of morphology within each genus, makes assignment to a genus difficult. RAMANATHAN (1964) distinguishes them on the basis of differentiation of the base and apex of a filament which is present in Ulothrix but absent in Hormidium, as well as Hormidium having, in general, shorter filaments. BOURELLY (1966) also points out that Hormidium produces mainly biflagellate zoospores in contrast to Ulothrix. A certain amount of confusion also exists in distinguishing Hormidium and Stichococcus and FOREST (1954) includes Ulothrix, Hormidium and Stichococcus in the same genus Ulothrix. Most workers seem to distinguish between Hormidium and Stichococcus by including all spp. with a pyrenoid and motile spores in the genus Hormidium. Also Stichococcus is not usually found in the form of filaments of more than a few cells, although in the cultures observed here, filaments of up to 40 cells have been noted.

The confusion is also added to by the fact that Hormidium Lindley is an orchid genus and Fott proposes the name Chlorhormidium which is used by Bourelly.

The species listed in these results have been identified using Ramanathan's keys, giving the acid tolerant Hormidium species found as Hormidium rivulare Kütz. (Syn. Ulothrix rivulare Kütz; Stichococcus rivulare (Kütz.) Gay). The species is very similar to Hormidium fluitans but does not break up so easily.

The common Stichococcus species observed was listed as Stichococcus bacillaris Naegli, although the long filaments fit the description for Hormidium pseudostichococcus Heering. Stichococcus minutus listed is distinguished from the very similar Stichococcus chlorelloides by its slightly terminally shifted chloroplast.