The algal communities of colliery spoil heaps

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The Algal Communities of Colliery Spoil Heaps

by

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ABSTRACT

A survey of the soil algae present on old coal mine spoil heaps at East Holywell, Tyne and Wear (O.S. sheet NZ 37 ref. 313730) was carried out during 1979-1980. Samples were taken at monthly intervals from eight permanent quadrats. The sites chosen provided a range of spoil types, including some devoid of higher plants and others where colonization was well developed. Algae were isolated and their density quantified using a dilution plate technique.

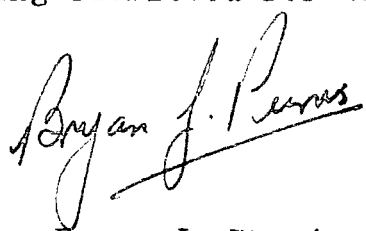
A limited flora was recorded at favourable sites on both shale (15 genera) and washeries waste (19 genera).

The density of algae in the spoil at sites which were stable, moist and had a pH of about 7 was comparable to that reported for a range of natural soils.

Seasonal fluctuations in algal density were recorded with a general increase in spring and late summer and a decrease in autumn and winter. A marked decrease in mid-summer which coincided with a period of drought was recored at several sites.

At five of the eight sites both a small number of algal species and low population densities were recorded. This was ascribed to the extreme nature of the physical and chemical environment. Evidence was however obtained that at one site the soil algae were acting as primary colonizers.

This thesis is entirely the result of my own work. It has not been accepted for any other degree, and is not being submitted for any other degree.



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List of Abbreviations

BTU	British Thermal Unit
^{o}C	degrees centigrade
cm	centimetre
d.f.	degrees of freedom
EDTA	ethylenediaminetetra-acetic acid (disodium salt)
$\sum$	sum of
g	gram
kg	kilogram
km	kilometre
l	litre
lb	pound
m	metre
mg	milligram
min	minute
ml	millilitre
mm	millimetre
um	micrometre
N	number in a sample
p	probability
s	second
$\bar{x}$	mean

CHAPTER 1

INTRODUCTION

1.1 INTRODUCTION

There are 3×10^9 tonnes of colliery spoil in Great Britain spread over an area of 15×10^3 hectares (Gutt *et al.*, 1974). While most of this derelict land resulted from the activity of the coal industry during the industrial revolution, spoil is still being produced at a rate of 5×10^7 tonnes per year. This spoil forms habitats of particular scientific interest. As Bradshaw (1970) has observed, artificial environments, like old mine sites, can give great rewards to researchers in the field of ecology because the habitats are distinctive, definable, often of known age and ecologically simple.

Soil algae are, apparently, almost ubiquitous; indeed it is rare to read in the literature of a site from which they are absent. Under favourable conditions their ecology is complicated by an abundance of species and a complex pattern of interactions. They evidently colonize and inhabit colliery spoil, for conspicuous surface growths are visible at certain times of year. At such sites their biology should be simplified by the severity of the physical and chemical conditions. Therefore it was the original aim of this project to establish the existence and describe some of the features of the algal communities of colliery spoil in N-E. England.

1.2 ECOLOGY OF PIT HEAPS

1.21 Physical Conditions

1.211 Texture

Commercially valuable coal is extracted from strata in the later Carboniferous. These rocks in N-E. England were formed in the delta of a great river which ran in a south westerly direction from a land mass to the north east (Willis, 1951). They consist of several different types, including limestone, shale, sandstone and coal in a regular sequence called the Yoredale Cyclothem. In mining operations a variety of rocks are excavated during the sinking of a shaft but the bulk of the spoil or waste material is formed during the coal extraction and is composed of the strata in the immediate neighbourhood of the coal seams. Therefore spoils may contain limestone, shale, flags, sandstone and seatearths (Doubleday, 1971). The actual composition of a particular heap depends upon the coal seams worked and there may be variation from one part of a heap to another as the operation of the mine moved from seam to seam.

Freshly tipped spoil has a range of particle sizes but typically the largest rarely exceed 30 cm. Spoil is characterised by having a very high content of "stones" which often amounts to 50 - 55% volume/volume and by having only 25 - 30% volume/volume of particles <2 mm (Rimmer, 1978). As a spoil ages the material of which it is made up weathers. The proportion of "stones" decreases and the proportion of soil forming particles increases. Richardson (1973) estimates that the weathering is generally to a depth of 10 - 20 cm after 20 years and that the proportion of particles <2 mm increases by a factor of 2.5 in a similar time. The nature of the soil forming upon the surface of a spoil heap depends partly upon the size of the soil particles involved and partly

upon the size of the pores between the particles. In a healthy fertile soil there is a wide range of pore sizes which can be grouped into two basic categories; small pores (0.002 mm to 0.02 mm) and large pores (> 0.02 mm). The smallest soil particles i.e. the clay particles, bond together to form groups called domains, which are up to 0.005 mm in diameter, and these domains may be grouped with silt particles to form microaggregates (0.005 - 1.0 mm) or with sand particles to form aggregates (1.0 - 5.0 mm) (Buckman & Brady, 1969).

The pores between domains and aggregates are the small pores or capillaries and these are chiefly responsible for the water holding capacity of a soil, while the large pores between larger particles are principally concerned with drainage and gaseous exchange. This structure depends heavily upon organic stabilizing agents to maintain it and the organic materials involved are thought to be polysaccharide gums which bond and cross-link soil particles (Swincer et al., 1969).

Raw shale has a low proportion of fine particles (< 2 mm) and is totally devoid of organic matter. Its total porosity is therefore low, at 15 - 25%, compared to an ideal agricultural soil which has 50 - 55%. Consequently it has a low water holding capacity and is susceptible to drought. Weathering leads to an increase in the < 2 mm fraction but the lack of organic content to stabilize these particles results in their downward movement through the soil profile, which further reduces the porosity. On level or gently sloping weathered shale the decrease in porosity with depth is typically from 40% at or just below the surface to 5% at 30 cm depth (Rimmer, 1978). This results in poor drainage and waterlogging in winter. The surface of colliery spoil is therefore a

a habitat of extremes with waterlogging in winter and subject to severe drought in summer. (Richardson & Greenwood, 1967) The development of a soil on the surface of this material will undoubtedly be influenced by the growth and development of the soil microflora of which the soil algae are an important part.

This picture of the physical nature of spoil material may be modified by the accidental burning of the pit heap. Colliery spoil contains a proportion of coal and other combustible material which, when high temperatures develop due to oxidation, results in spontaneous combustion. The actual ignition of the spoil occurs at very high temperatures, in excess of 800°C (Doubleday, 1971), deep within the heap. Ironically this may improve the surface environment by the removal of sulphur compounds, such as hydrogen sulphide and sulphur trioxide resulting in a consequent reduction in potential acidity. However, it does slow down the weathering so that mechanical analyses of red shale (burnt shale) show 60% of particles < 2 mm compared with 40% of such particles in unburnt shale after 50 years of weathering (Molyneux, 1963).

1.212 Stability

The loose rock fragments with their thin layer of weathered spoil form a very unstable environment, which is subject to three forms of erosion:

a. sheet erosion - in which loose material is removed from the surface by the action of wind and water;

b. gully erosion - in which deep, steep sided channels are cut down the sides of the heap by running water;

c. terrace slip - in which sparse vegetation forms a barrier to water running down the surface of the heap and behind which fine spoil accumulates forming a terrace. These terraces eventually become unstable

and slide down the slope.

The main agents of erosion are wind and water and the role of the second of these has been extensively studied. When rain falls on newly tipped spoil it easily soaks into the surface i.e. the spoil has a high infiltration capacity. The weathering of the spoil results in a reduction of the pore size of the material at the surface and a point is eventually reached when the infiltration rate is exceeded by the rainfall rate. At this point run-off occurs. The time taken for this point to be reached and the amount of run-off which will occur depends upon a number of factors, the chief of which are:

a. texture of the spoil;

b. size and shape of the heap;

c. rainfall intensity and distribution.

On flat or gently sloping surfaces the texture of the spoil and the intensity of the rainfall are the most important factors. Spoil composed of very fine particles, such as the washery waste at East Holywell, have a very low porosity and will be subject to run-off and hence erosion during light showers, while coarse shale will only be eroded when the rainfall is quite intense. As already explained the down washing of fine particles results in decreased porosity below the surface of quite coarse shale and this combined with the fact that in a heavy downpour the soil pores are reduced in size by the impact of raindrops, will mean that run-off and hence erosion will occur on all types of spoil.

On sloping surfaces the angle of the slope is of paramount importance in determining the amount of run-off and it has been shown that while slopes of 30^{o} or less are quite stable, slopes of 40^{o} are highly

unstable. Only in the case of heaps composed of very coarse spoils are slopes as steep as 40° anywhere near stable. In research on loam soils Baver (1948) showed that the length of bare slopes is an important factor in determining the amount of erosion and this was particularly so during heavy rain.

Dennington and Chadwick (1978) in a study of rainfall and run-off at three sites in Yorkshire showed that the amount of run-off was related to the amount of rainfall; between 34%and 38% of annual rainfall was lost in this way; a grass cover significantly reduced this figure. They ascribe this effect to:

a. reduction in the rate at which rainfall reaches the spoil surface;
b. increased surface evaporation;
c. effect of vegetation upon the substrate structure.

No estimate seems to have been made of the amount of material lost due to the action of wind; however several workers have commented upon its probable significance. Certainly the experience of living in a mining area suggests that large quantities of material are removed from the surface of pit heaps as dust on dry windy days.

1.213 Temperature

The surface of bare soil is always subject to a wide temperature range, but that of colliery spoil is subject to even more extreme temperatures. Spoil, as already described, is usually in heaps and the amount of radiation/unit area is proportional to the cosine of the angle between the direction of the suns rays and the perpendicular to the surface. As many pit heaps have surfaces sloping at 30° to 40° this effect is exaggerated. Ludwig and Harper (1958) have summarised the relationship between soil colour and soil temperature

and the fact that colliery spoil is usually dark in colour means that it will be a better absorber and emitter of solar radiation and therefore subject to greater daily temperature ranges. Finally because of the texture of colliery spoil, it is usually very dry in summer and hence there will be a steep temperature gradient between the surface and a few centimetre below the surface.

Richardson (1958) recorded surface temperatures on a pit heap at Ouston in County Durham and showed that on a south facing slope with an angle of 30°, the temperature range in the surface 2 - 3 cm was 8 - 48°C. In July 1953 he recorded temperatures in excess of 45°C lasting for periods between three and six hours on 16 successive days. A maximum recorded temperature of 57°C was maintained for one hour. Such temperature ranges are significant from the point of view of surface living algae. Lund (1967) pointed out that soil algae in the temperate regions do not tolerate temperatures much in excess of 30°C although desiccated algal cells are able to withstand much higher temperatures than fully hydrated ones. These observations have been supported experimentally by Holm-Hansen (1963).

1.22 Chemical Environment

The chemical environment of colliery spoil is dominated by two features. Firstly there is often a high level of acidity which may result in some elements reaching toxic concentrations. Secondly there is usually a serious nutrient deficiency. These conditions result from the geological nature of the material making up the spoil. The commonest minerals present in spoil are aluminosilicates such as illite, muscovite, and kaolinite together with quartz, felspar, hematite,

geothite and iron pyrites (Doubleday, 1971).

1.221 Acidity

Acidity is the principle growth-limiting factor in many types of colliery waste (Chadwick, 1973). The majority of shale heaps from deep coal mining in Northumberland and Durham consist of moderately to intensely acid spoil. In a survey of 44 sites in N-E. England, Doubleday (1971) found that the pH of acid tips usually falls within the range 3.0 to 5.0. However, the pH of some types of shale may be as low as 1.5 to 2.0. At the other extreme values as high as 8.7 have been recorded. Gemmell (1977) summaries the most important factors influencing the pH of colliery shale as:

a. duration of exposure of the waste;
b. presence and mineral form of iron pyrites and the abundance of aluminium hydroxide;
c. presence and abundance of acid neutralizing materials such as calcium and magnesium carbonate minerals;
d. whether combustion has occurred.

The very low pH which develops on many pit heaps is the result of the weathering of iron sulphides. This may proceed along a number of multistage pathways, but Temple and Delchamps (1953) have stressed the role of autotrophic bacteria in the process and have postulated a pathway which accounts for most of the observed facts about the process. Thiobacillus thiooxidans is prevalent in soils where pyrite is undergoing oxidation and T. ferrooxidans can be isolated from acid mine drainage. It is suggested that these organisms are involved in the following process:

a. finely divided pyrite or marcasite undergoes chemical oxidation to ferrous sulphate;

$$FeS_2 + H_2O + 3\tfrac{1}{2}O_2 \rightarrow FeSO_4 + H_2SO_4$$

b. bacterial oxidation of ferrous sulphate by T. ferrooxidans occurs in acid solution;

$$2FeSO_4 + \tfrac{1}{2}O_2 + H_2SO_4 \rightarrow Fe_2(SO_4)_3 + H_2O$$

c. ferric sulphate combines with finely divided pyrite resulting in reduction of the ferric sulphate and oxidation of the pyrite;

$$Fe_2(SO_4)_3 + FeS_2 \rightarrow 3FeSO_4 + 2S$$

d. elemental sulphur is then oxidised by T. thiooxidans and it is the sulphuric acid produced in this last step which produces the very low pH, which in turn favours the continuation of the process.

$$S + 3O_2 + 2H_2O \rightarrow 2H_2SO_4$$

The nature and mineral form of the iron pyrites in the waste seem to be important in governing the rate and degree of acidification. Studies in the U.S.A. (Carruccio, 1973) have revealed that framboidal pyrite is the most reactive form and probably the principal source of acidity in all colliery wastes. Its high reactivity is almost certainly a consequence of its small grain size and large surface area exposed for oxidation.

The combustion of colliery spoil results in the complete oxidation of pyrite. Therefore, although the

waste may have a very low initial pH, its potential acidity is absent and the pH will gradually rise as the shale weathers.

The exact level of pH will vary during a twelve month period due to climatic factors influencing the generation of acid. Baver (1927) reported that an acid soil showed a steady lowering of the pH from May to September and Williams and Chadwick (1977) reported that the pH of acid colliery spoil was lowest in summer and autumn and highest in winter and spring. If the process postulated by Delchamps and Temple is accepted, it might be expected that the generation of acid and consequent lowering of the pH would be effected by changes in temperature and availability of water.

1.222 Toxicity

A consequence of the low pH of many spoils is that a number of metals become more soluble. Williams and Chadwick (1977) reported high levels of Al, Mn, Cu, Zn and Fe in acid colliery spoil. They also noted a marked seasonal pattern in the concentrations of these elements and were able to show a significant difference between the concentrations of all these elements in winter and spring compared to summer and autumn. They ascribed these differences to changes in the rate of oxidation of iron pyrites resulting in changes in the amount of sulphuric acid in the spoil. Berg and Vogel (1973) summarise the available data on the relationship between exchangeable Al and pH and show a close relationship in which exchangeable Al increases with decreasing pH. They also report that Al is present in spoil at toxic concentrations when the pH is less than 5.5. Williams and Chadwick (1977) stress the difficulty of defining toxic concentrations of elements because the effect of particular concentrations

of particular elements upon vegetation depends to a great extent upon the other elements present. Harding (1970) reported that 0.5 mg l^{-1} Al in calcium nitrate solution reduced root length of Agrostis tenuis by 50% but 27 mg l^{-1} Al in a weak nutrient solution had little effect. However the levels of Al and Mn reported by Williams and Chadwick from their acid sites were so high that they would undoubtedly have a detrimental effect upon plant growth. In association with increased concentrations of other potentially phytotoxic elements such as Zn and Fe the overall chemical environment of acid spoil will certainly be hostile.

1.223 Nutrient Status

The low pH of much colliery spoil also affects the availability of major plant nutrients. Phosphate fixation occurs in acid conditions. Doubleday (1971) has outlined three ways in which this may occur in colliery spoil:

a. phosphate ions may combine with iron in solution to form a highly insoluble precipitate of ferric phosphate;
b. phosphates may react with labile aluminium released from clay minerals under acid conditions to form the highly insoluble aluminium phosphate;
c. phosphate may be absorbed in large quantities by amorphous ferric hydroxide which often forms as a coating over the mineral particles. This mechanism is thought to be of principal importance. The consequence of these reactions is that there is little or no phosphate available in acid colliery spoil.

The other major plant nutrients i.e. nitrogen and potassium are not affected by the pH of the spoil but are usually in short supply. Although estimations

of total nitrogen show values which compare favourably with normal soils, estimations of available nitrogen give very low values. Williams (1975) has shown that this is because much of the nitrogen is present in a fossilized form which is unavailable to plants. Nitrogen must be available as either ammonium or nitrate ions but both of these are readily lost by leaching on freely draining spoils. Nitrate is more readily leached than ammonium ions because the latter are held strongly by cation exchange capacity. In an analysis of spoil from five pit heaps in Yorkshire, values for available nitrate nitrogen of between 0.8 and 3.9 mg kg^{-1} and for available ammonium nitrogen of 2.1 to 17.1 mg kg^{-1} were recorded (Tasker and Chadwick, 1978). When compared to an agricultural loam soil these values are seen to be very low.

Potassium is not subject to rapid leaching unless cation exchange capacity is very low or the development of acidity causes a rapid release of the element from clay minerals. However not until the pH falls below 4.0 is the availability of potassium likely to become limiting. Therefore this element is only in short supply on the most acid sites.

Trace elements are usually available in excess. Doubleday (1971) showed that with the exception of Al and Mn, none are likely to be present at toxic levels.

1.23 The Flora of Pit Heaps

The physical and chemical conditions described so far present a very hostile environment. However natural colonization of colliery spoil does occur and on many heaps a climax community of woodland or scrub is achieved in a period of 80 years (Richardson, Shenton & Dicker, 1971). The process of colonization cannot begin immediately the spoil is tipped but must wait until

the conditions have ameliorated a little and the surface has been rendered receptive to plant propagules by weathering. Brierley (1956) and Greenwood (1963) both found that there were no particular pioneer species peculiar to this habitat but that colonization was achieved by plants from surrounding agricultural and waste land. An example of this fact is given by Richardson et al. (1971) in which it was reported that pit heaps in the western, upland part of Co. Durham were often colonized by Calluna vulgaris but similar sites in the central and eastern, lowland part of the county were not. Such a difference might be ascribed to the difference in altitude between the sites but it was also found that pit heaps next to isolated patches of moorland in the central and eastern regions were not only colonized by C. vulgaris but also by a range of other moorland species. Greenwood (1963) studied the flora of eight pit heaps in N-E. England in some detail. She found that certain plants were constant features of the pit heap community irrespective of its location and age. These five were Agrostis tenuis, Festuca rubra, Hieracium perpropinquum, Plantago lanceolata and Tussilago farfara. A further 11 species were common, occurring abundantly on seven out of the eight sites and only being absent from the very youngest site. They were Rubus sp., Arrhenatherum elatius, Deschampsia flexuosa, Festuca ovina, Holcus mollis, Heracleum sphondylium, Hieracium vulgatum, Hypochaeris radicata, Rumex acetosella, Pohlia nutans and Cladonia sp. These species also appear in similar lists from sites in Nottinghamshire, Derbyshire and S. Yorkshire (Brierley, 1956), in the Wigan district of S. Lancashire (Molyneux, 1963) and Durham (Pickersgill, 1971).

Greenwood (1963) observed that perennial species

with a means of vegetative propagation were especially common on pit heaps but was unable to confirm the observation of Brierley (1956) that annuals played an important part in the colonization of young heaps. Both investigators found that a high proportion of the plants at such sites had wind borne seeds but Greenwood did not confirm Brierley's correlation between the percentage of wind dispersed plants in the flora and the age of the spoil heaps. A characteristic flora does seem to develop on pit heaps in which the most important capacity of the plants involved is an ability to survive severe drought. This means that the general appearance of these sites is of rough grass with a high proportion of perrenial herbs and with mosses and lichens playing an important part on the surface of the ground.

1.3 ECOLOGY OF SOIL ALGAE

1.31 Occurrence

Soil algae occur all around the world and form a part of nearly every type of terrestrial community. This world wide distribution has now been documented in an extensive literature, which has been reviewed by Shields and Durrell (1964). In the U.K. the first investigations were carried out by Bristol (1919). She cultured algae from dried soil samples which had been collected over a number of years from the experimental plots at Rothampstead Experimental Station. In her work she demonstrated the remarkable ability of soil algae to survive drying for many years. In later work (Bristol, 1927) she cultured algae from fresh soil samples and made the first attempt to estimate their abundance. All of the soils which she studied were cultivated and the first records of algae occurring in uncultivated soils were reported by James (1935). His study however was on a very limited range of soils including two different woodland clay soils and a chalk soil. It was John (1942) who first conducted a study of a wide range of English soils and this was followed by a more limited survey of Scottish soils by Fenton (1943). The picture was completed by a detailed floristic study of most of the major British soil types by Lund (1945). In recent years quantitative studies of English soils have been made by Broady (1979)a in which a range of soils were examined and an effort made to measure the abundance of algae in each soil type.

In the U.S.A. the earliest study was conducted on Colorado soils by Robbins (1912). This was followed by investigations of the soil flora of the Missouri Botanic Garden (Moore & Karrer, 1919; Moore & Karrer, 1926). Durrell (1959) carried out an extensive survey of the

soils of Colorado and (1962) of Death Valley, California. Willson and Forest (1957) reported on the soils of the Oklahoma prairie and Bold and his co-workers have investigated a number of Texas soils (Deason & Bold, 1960; Chantanachat & Bold, 1962; Bischoff & Bold, 1963).

Soil floras have also been described from Cuba (Arce & Bold, 1958), from Australia (Tchan & Whitehouse, 1953) , from India (Mitra, 1951), from Canada (Lowe & Moyse, 1934), from the Belgian Congo Duvigneaud & Symoens, 1950) and from Central America, Jamaica and South America (Durrell, 1963).

In Europe a series of studies was conducted on the soils of Denmark (Petersen 1935) and extensive floristic studies have been carried out in the U.S.S.R. The latter are nearly all reported in Russian but they have been reviewed in English by Forest (1965).

In recent years much interest has centred on the soils of Antartica and these have been described by Broady (1979b).

In the course of these studies almost every soil type in the temperate regions of the earth and a number of tropical soils have been investigated. Few soil samples have been reported as containing no algae and it is, in the experience of workers in this field, very unusual to discover a soil which is algae free (MacEntee, 1970).

1.32 Density of soil algae

Only a few of the studies of the soil flora have attempted to measure the density of algae. Such measurements do pose considerable technical problems and no one method will provide a completely satisfactory answer. Direct observation of soil films is quite uninformative because few algae are visible and those which are cannot be identified. Lund (1945) developed

a technique of placing a sterile coverslip on the surface of moist soil and then allowing colonization of it to occur. The coverslip can be examined satisfactorily and the algae present identified but it is difficult to relate such observations to the abundance of the organisms in the soil. Cholodny (1930) used a similar technique in which microscope slides were buried in the soil and after colonization were stained and examined. Tchan (1953) suggested the use of fluorescence microscopy for the direct counting of soil algae because the chlorophyll in their cells will glow with a red fluorescence which enables them to be distinguished from soil particles. This method however has not found wide acceptance and has the disadvantage that classes of algae cannot be distinguished.

A totally different approach is to extract the pigment present in a soil sample and measure its concentration by the amount of light which it will absorb. The concentration of pigment can be taken as a measure of the algal biomass. This method has been adopted by Singh (1961) to estimate the density of blue-green algae in Indian soils and by Shubert and Starks (1978) to estimate the algal biomass in coal mine spoils. The disadvantages of this method are that it only measures biomass and no indication of species present is obtained. Furthermore its application is restricted to certain types of soils because the extraction technique collects pigments other than chlorophyll such as humic acid and chlorophyll breakdown products.

The most widely used method has been some form of dilution culture method. This approach was pioneered by Bristol (1927). She added a weighed quantity of soil to a known volume of sterile culture medium and shook the mixture for half an hour. She then took

half of this mixture and prepared serial dilutions in which the soil concentration was halved on each occasion. 15 ml of each suspension was then inoculated into sterile sand and the density of algae was estimated by counting the tubes in which no algae appeared. A simplified version of this method was used by Petersen (1935). In more recent studies by King and Ward (1977) algal density was estimated by shaking 2 g of soil with 100 ml of Bold Basal Medium (Bischoff & Bold, 1963) and then aseptically pipetting 1 ml of this suspension onto the surface of sterile agar medium. After spreading with a sterile inoculating loop the plates were incubated for three weeks and then the number of colonies which developed were counted. A very similar method was used by Broady (1979) with the additional precaution of drying the agar plate before pipetting the soil suspension so that conditions on the agar were not too wet.

All the plating and dilution techniques have the advantage that species can be enumerated but equally all are open to several criticisms. Firstly the kinds of algae which develop depends to some extent upon the cultural conditions provided. Liquid cultures will tend to encourage the growth of aquatic forms; for instance Bold has obtained Pandorina, a clearly aquatic species, from soil 30 cm below the surface of a pinewood. Pringsheim (1951) reported that when a small volume of soil was introduced into a large volume of culture medium Nostocaceae developed but when the volume of the soil added was increased the culture was dominated by diatoms. In addition changes in the composition of the culture medium can encourage and discourage various groups of algae. A second drawback of these methods is that all algae are counted equally

and no distinction is possible between resting stages and metabolically active algae. Finally, while the method works quite well for chlorophyceae and diatoms, which are largely unicellular, it does underestimate the mucilaginous and filamentous forms. Blue-green algae with their mucous sheaths are very difficult to separate by shaking or homogenisation and variable results have been obtained for filamentous algae because of variation in their tendency to fragment.

The results obtained by several workers from dilution cultures show variations in the number of algae in different soils. Bristol Roach (1927) recorded numbers of algal "germs" in the surface soil of agricultural land varying from 91 g^{-1} to 105.5×10^3 g^{-1}. Petersen (1935) obtained the results shown in Table 1.1 from surface soil in various habitats in Denmark.

TABLE 1.1 Density of algae in different types of soil (Petersen, 1935)

soil type	density (algal cells ml^{-1} of surface soil)
pasture	40×10^3
arable	10×10^3
garden loam	$200 \times 10^3 - 3 \times 10^6$
dune	200
heath	20×10^3
forest	10×10^3
clay	66×10^3

A summary of the earlier estimates obtained by various counting methods has been given by Lund (1967). Recent studies by King and Ward (1977) using the agar plating technique have recorded algal densities which varied from 54.16×10^3 cells g^{-1} in the soil of a golf course to 5.74×10^3 cells g^{-1} in woodland soil. Broady (1979)a using the same technique as King and Ward found much larger differences between sites and recorded much higher algal densities. The lowest value he reports is for soil from a colliery spoil tip where he found 1×10^3 algae cm^{-2} and the greatest number he found was 16.3×10^6 cm^{-2} from undisturbed Festuca grassland. Taking into account the different methods of expressing the results there is still a difference of one or two orders of magnitude between the findings of these two workers.

1.33 Factors affecting the distribution and abundance of soil algae

As green plants dependent upon light, soil algae are subject to the same limitations of the physical environment upon their growth and distribution, as other autotrophic organisms. They are generally found in greatest abundance at the soil surface, as would be expected by their requirement for light but it is true that they do occur down to considerable depths in the soil (Petersen, 1935). Indeed they are at times more abundant, a few centimetres below the surface (Shtina, 1959). This is now generally thought to be the result of the downward movement of cells due to water and soil animals. When such movement exceeds the rate of reproduction at the surface, the number of algae in sub-surface samples will exceed those at the surface. Alexander (1977) claims that algal cells below the surface probably exist in a dormant condition as aliens in a foreign environment.

Moisture is by far the most important factor (Shields & Drouet, 1962) affecting algal abundance in the soil. These organisms are capable of surviving for many years in air dry soil but they only produce really large populations on damp soil. Bristol (1919) showed that one species of diatom could survive for at least 73 years in the air dry state and Trainor (1970) has reported that in a soil from which he originally isolated 31 species in 16 genera after one year's storage, he was still able to isolate 11 species from nine genera after 10 years storage. Furthermore Trainor and McLean (1964) showed that when they introduced a measured amount of a Spongiochloris culture into sterile soil, they could recover 2×10^5 cells g^{-1} after one year's air dry storage. On the other hand Tchan and Whitehouse (1953) have shown that waterlogging is also unfavourable to the

soil algal flora. Lund (1967) has shown that prolonged saturation of soil, providing it does not become anaerobic, results in the development of a typical ephemeral aquatic flora. Stokes (1940) estimated that the ideal moisture content of a soil for the growth and reproduction of soil algae was between 40% and 60% of its moisture holding capacity. Therefore in the soil algae we have a group of organisms intermediate in position between the aquatic and the aerial algae, with distinctive moisture requirements and not simply a depauperate aquatic community.

On a world wide scale it is difficult to separate the effect of moisture from that of pH because most arid soils are alkaline and most permanently wet soils are acidic (Shields and Durrell 1964). However pH is the most widely investigated environmental factor affecting soil algae. Lund (1967) suggests that its importance has been overestimated because of the ease with which it can be recorded. However the picture that emerges is that most species can grow on soil which is neither too acidic nor too alkaline i.e. pH 5.5 to 8.5. There are, however, distinct differences between the groups of algae in their pH range. A typical example of this is the findings of Granhall and Henriksson (1969) who carried out a survey of Swedish soils and found no blue-green algae at sites with a pH of less than 5.0 and the greatest abundance at sites with a pH above 7.0. In fact several workers have shown that blue-green algae are more diverse and more abundant in alkaline soils. In acid soils, on the other hand Petersen (1935), John (1942) and Lund (1945) all noted the poor representation of diatoms and the dominance of Chlorophyta. At a more detailed level MacEntee, Schreckenberg and Bold (1972) have reported that palmelloid green algae are rare on acidic sites.

In a recent survey, Broady (1979a) presented data that suggest that while the chlorophycean flora is equally diverse in acidic and alkaline soils, the density of green algae is much lower at low pH sites. Stokes (1940) also showed a decrease in the density of algae with lower pH although he did not distinguish between groups of algae. Finally MacEntee (1970) studied the effect of pH on the growth and spatial pattern of soil algae on strip-mine spoils in N-E. Pennsylvania. He reported the total absence of diatoms and the very low incidence of blue-greens, both of which he associated with the low pH of the site. In addition, he found that at no site were there more than six genera and when this is compared with the 16 genera reported from a single site by Trainor (1970) it suggests that acid colliery spoil has a very restricted flora.

As described in section 1.22 pH is connected to several other chemical factors including the availability of inorganic nutrients. Lund (1945),(1946) and (1947) showed that calcareous soils which are rich in phosphorus and nitrogen have a considerably richer flora than other soils. However Shtina (1959) did not find any marked effect upon the algal flora when she added phosphorus to experimental plots. This may have been due to an already sufficient supply of phosphorus in the soil as the existing level was not measured. There is little information available on the importance of potassium however there are some data on the importance of silicate. In a habitat composed largely of siliceous particles it is surprising to find that the concentration of silicate in the soil solution can be a factor limiting the distribution of certain diatoms (Lund, 1967).

The effect of temperature fluctuations depends upon the condition of the cells and the rate at which changes occur. Air dry algae can undoubtedly survive

temperatures far outside the range they are likely to encounter in nature. Trainor and McLean (1964) showed that 2 x 10^4 cells g^{-1} survived in air dry soil that had been heated to 100°C for one hour. This compared with 2 x 10^5 cells g^{-1} which could be isolated from similar soil which had not been heated. Therefore while a large number of cells had died a considerable proportion survived such rigorous treatment. MacEntee, Schreckenberg and Bold (1972) investigated the effect of temperature extremes upon the species diversity of air dry soil from pinewoods in New York State, U.S.A. They found that storage at 0°C for one week reduced the number of algal genera in the soil from six to one and that heating above 60°C for a period of time as short as one hour eliminated virtually all the algae in the soil. In contrast they found in soil from a drought affected pasture that Nostoc, Anabaena and Bracteococcus could survive heating for six hours at 100°C while Anabaena, Phormidium and Calothrix could survive heating for two hours at 110°C. In the whole range of conditions which they investigated they found Tetracystis, Chlorococcum, Hormidium and Chlamydomonas to be especially resistant to temperature extremes. They also noted that these were the commonest algae isolated from fresh samples of the same soil. In nature the most extreme conditions in which algal communities have been examined are the takyars of Russia. Here abundant algae are found in sites where the surface temperatures reach 87°C in summer and -11.5°C in winter. It is clear from this data that while the number of algae present and the species diversity in a soil may be reduced by extreme temperatures, some of the commonest soil organisms can withstand a remarkable range of conditions.

The final factor to consider is the influence of other organisms upon the soil algae. The activities of

man undoubtedly have a profound effect upon the soil flora as numerous surveys show changes in biomass and species diversity produced by agricultural techniques. Most of the early work reported more algae in disturbed agricultural land than in undisturbed natural habitats, but the recent survey by Broady (1979a) reported the highest number of algae in undisturbed grassland sites. One human effect of particular interest is that of herbicides upon the soil flora. Cullimore and McCann (1977) and Metting and Rayburn (1979) both showed that at least some soil algae were sensitive to these compounds. In contrast the application of insecticides to rice fields has been shown to produce a distinct algal bloom (Ragu & MacRae, 1967). This effect was shown to be due to the inhibition by the insecticide of small crustaceans which normally feed upon the algae. This example highlights the possible role of heterotrophs in the regulation of soil algal populations. In isolation cultures it is common to find large numbers of soil amoebae, many of which are gorged with algal cells (author's observation) and these are probably a significant factor in the ecology of soil algae. Little work has been done in this field but Parker and Turner (1961) did study the interaction of algae with algae, algae with bacteria, algae with actinomycetes, algae with fungi and algae with protozoa in two membered cultures. They report a wide variety of interactions including cooperation, commensalism, competition, parasitism as well as direct predation.

1.34 Seasonal Variation in Soil Algae

In view of the previously described effects of soil moisture, temperature, inorganic salts and light upon soil algae it might be expected that they would show a regular seasonal periodicity. However the evidence

for this is fragmentary. Martin (1939) recorded the seasonal variation in species isolated from virgin Utah soils by sampling at three monthly intervals over a two year period. He found Chlorococcum and diatoms to be the dominant algae at all times of year and that these were associated with various blue-green algae which showed no regular periodicity. He did not give figures for the density of algae at all seasons but states that there were 8×10^5 cells g^{-1} in the soil in autumn and this number decreased during the winter and then increased again from June onwards. Petersen (1935) recorded as a general observation that soil algae were more abundant in spring and autumn and Lund (1945) recorded the seasonal variation of diatoms in a garden soil and found maxima in spring and early summer and again in the autumn. These differences corresponded to differences in rainfall and the lowest density in summer always occurred with the mid-summer drought. He recorded the lowest density of algae in February but was unable to discern any seasonal succession of species. Blue-green algae are said to show marked seasonal fluctuations (Fogg, Stewart, Fay & Walsby, 1973) and Shields, Mitchell and Drouet (1957) recorded more blue-green algae in desert soils in November than in June or July. Broady (1979) recorded the number of algae cm^{-2} in a winter wheat field over seven months. The first sample was taken immediately after ploughing in October and there was a steady increase in numbers up to May. This was followed by a sharp decrease in June which coincided with a spell of hot dry weather. In this study, however, there is colonization of a newly exposed surface as well as seasonal variation. It is therefore apparent that there has not been a study in which the numbers of algae have been recorded at regular intervals over a twelve month period at an undisturbed site.

1.4 ECOLOGICAL ROLE OF SOIL ALGAE

1.41 Colonization

The role of algae in the soil ecosystem has been investigated from a number of angles. Their role as pioneer organisms in the colonization of bare earth has been extensively studied in a number of different circumstances. Fritsch and Salisbury (1915) commented upon the appearance of various algae as the first visible colonizers of burnt heath at Hindhead Common in England. They observed Cystococcus humicola as the primary colonizer associated with Gloeocystis vesiculosa, Scenedesmus obliqeus, Trochiscia aspera and Mesotaenium violasceum. They also reported the observation of succession in the soil algal community, with these organisms being replaced by Hormidium flaccidum and Zygogonium ericetorum. Fritsch (1922) also reported succession in the colonization of rock surfaces in which the pioneers were blue-green algae such as Gloeocapsa, Gloeothece, Aphanocapsa and Nostoc, which were succeeded by filamentous blue-greens like Lyngbya, Scytonema Stigonema and Hapalosiphon. He also observed that different algae act as colonizers on different soils. So that while the sandy soils which he examined were colonized by Zygogonium, the heavier soils were colonized by Hormidium and Prasiola.

In the United States, interest has centred on the soils of the arid south western states. Forest, Willson and England (1959) removed blocks of prairie soil in Oklahoma, and after sterilization, replaced them in situ and recorded their colonization. They concluded that there were no special pioneer algae but that colonization was effected by the commonest algae in the surrounding natural prairie. They did not observe any successional sequence but noted that Chlorococcum humicola, Hantzschia amphioxus, Lyngbya aestuarii,

Navicula mutica and Pinnularia borealis were always common in both the natural prairie and the sterilized soil.

Volcanic eruptions in various parts of the world have offered unique opportunities of studying the colonization of large areas of totally sterile soil. The first such study was by Treub in (1888), when he visited the island of Krakatoa just three years after its dramatic eruption. He recorded the important role played by blue-green algae in the re-establishment of vegetation there. His observations were confirmed by other expeditions such as that of Campbell in (1909). The cinders on the island were coated in algae such as Oscillatoria species which formed a black slimy film. The jelly matrix of these organisms provided a moist substrate in which the spores and seeds of higher plants could germinate. Griggs (1933) recorded the colonization of volcanic ash at Katmai in Alaska. He reported the absence of blue-green algae on this occasion. Liverworts and green algae seemed to play a more important role. Brock (1973) challenged the widespread assumption of the pre-eminent role of blue-green algae in the first stages of colonization which was largely based upon the observations on Krakatoa. His study of Surtsey found that blue-greens were quite unimportant at this site and that mosses and associated chlorophyta were far more important. He suggested that the difference might be due to variations in temperature however he depended upon the identification of algae in fresh soil samples and this may be why he produced results at variance with those of Schwabe (1972). Schwabe found a wide variety of blue-green algae in Surtsey soil when it was examined in enrichment culture. Finally Carson and Brown (1978) studied the colonization and succession of soil algae

at Kilauea Iki in Hawaii. They also found that Chlorophyta were the principle component of the soil flora and that Chlorella species were the first and most wide spread colonizers. They only recorded blue-greens at sites where there had been some accumulation of humus. They described various patterns of colonization and succession which they ascribed to variation in nutrient status, moisture level and accumulation of organic matter.

Colliery spoil has much in common with volcanic ash. They are, because of the geological origin, both initially devoid of algae. They both have a tendency to form acidic soils, with fairly high levels of metals, and carbon dioxide and sulphur dioxide are common at both situations. Colliery spoil however has the advantage from the researchers point of view of being more common and its production is not dependent upon unpredictable volcanic events. Shubert and Starks (1978 Starks and Shubert 1978) have studied the colonization and succession of soil algae on colliery spoil in western North Dakota. Using species diversity, chlorophyll 'a' measurement and phaeophytin measurement they have shown a progressive increase in the algal flora of a spoil as it ages. They have also reported a definite successional trend which was not just a simple addition of species to an existing community but involved a complex pattern of changing species composition. They measured an improvement in soil parameters which correlated significantly with soil algal succession and this prompts several questions as to the role of soil algae when they act as an edaphic factor in the amelioration of soil condition.

1.42 Erosion Control

One consequence of the existence of a high algal density in soil is that they may promote the aggregation of soil particles (Marathe, 1972; Bailey, Mazurack & Rosowski, 1973). Marathe reported that blue-green algae, inoculated into sterile garden soil produced 35.7 to 42.8% increase in soil aggregation. This effect reaches its highest development in the arid areas of the south western states of the U.S.A. where algae form a crust over extensive areas of old fields and abandoned farm land. This crust has been described by Booth (1959), who investigated its significance in erosion control. This area of the U.S.A. was formerly oak savanna and was cleared for agriculture by burning. Bennett (1934) showed that this resulted in a massive increase in runoff with a consequent increase in erosion. Booth demonstrated that the effect of the algal crust, which has developed on this land, is to substantially reduce soil losses by binding together surface soil particles. In addition the algal crust does not slow down infiltration as might be supposed and in fact has been shown to improve it in very heavy rain. This results in a higher soil moisture content below the algal crust compared to bare soil. Fletcher and Martin (1948) showed that while a continuous algal crust does act as a barrier to possible colonizing seeds in the field, this effect is ameliorated by cracking and curling of the crust in dry conditions which allows seeds to enter. The uncurling of the crust when moisture returns, results in the retaining of the moisture in the soil and promotes the growth of seedlings below the crust.

1.43 Contribution to the nutrient budget of soil

Francé (1913) states that German farmers consider 'grüne Schimmel auf der Erde' a sure sign of abundant crops. It would seem therefore that they have learned in a purely practical way that a copious algal vegetation on the surface of the ground is associated with favourable conditions and a high yield of crops. While this cannot be considered as scientific evidence it is worthy of note because these folk lores often contain a germ of truth. Algae like all other autotrophic organisms will consume the mineral nutrients in the soil and it might be assumed that in doing so will deprive higher plants of these nutrients. However, Petersen (1935) has pointed out that the major algal growth is on or near the surface of the soil and not in the region of the plant roots. There can therefore be little or no direct competition and in fact the assimilation of these nutrients by the algae may retain nutrients which would otherwise be lost by leaching. Furthermore there is some evidence that algae may make certain nutrients more available to crop plants. Fuller and Rogers(1952) compared the uptake of radioactive labelled phosphate by barley plants from H_3PO_4 and from a manure of Palmella algal culture. They concluded that while the amount of phosphate taken up from the two sources was approximately the same, the phosphate from the degenerating algal cells was in a more readily available form.

Of more general interest is the contribution to the nitrogen content of the soil made by blue-green algae. De (1939) demonstrated conclusively that blue-green from the soil could fix atmospheric nitrogen. Earlier investigations claiming nitrogen fixation were all inconclusive because they failed to demonstrate the absence of bacterial contaminants

in the cultures used. De showed that Anabaena isolated from the soil of rice fields in India were capable of fixing atmospheric nitrogen and that this process was inhibited by the presence of nitrate nitrogen in the culture medium. Furthermore he showed that approximately 35% of the fixed nitrogen was in the external medium and not in the cells. This available nitrogen might be released by excretion from the cells or become available upon the death and decomposition of the algae. In either case the nitrogen will become available to higher plants. Fogg (1947) has pointed out that blue-green algae have a high nitrogen content of 7 to 8% dry weight and a low C:N ratio of 10:1 so that bacterial decomposition will lead to the immediate production of ammonia which is a form of nitrogen which is readily available to higher plants.

The occurrence of nitrogen fixing blue-green algae in soils is widespread (e.g. Henriksson et al, 1972; Watanabe & Yamamoto, 1971) but their exact contribution has been most thoroughly investigated in desert soils and tropical soils (MacRae & Castro, 1967). Fletcher and Martin (1948) reported the presence of Nostoc and the absence of N_2 fixing bacteria in desert soil and this was confirmed by Cameron and Fuller (1960). Shields Mitchell and Drouet (1957) measured the nitrogen content of such soils and found that algal crust contained an average of 8.2 mg l^{-1} of nitrate and nitrite nitrogen and 1639 mg l^{-1} amino nitrogen. Adjacent surface soil without an algal crust contained only 1.5 mg l^{-1} nitrate and nitrite nitrogen and 866 mg l^{-1} amino nitrogen. Thus it has been demonstrated that blue-green algae have the capacity to contribute to the soil ecosystem.

1.5 AIMS

In N-E. England the presence of large numbers of algae in colliery spoil at certain times of year is clearly shown by conspicuous surface growth. The diversity of the algal flora of colliery spoil has been the subject of studies in the U.S.A. (MacEntee, 1970; Shubert & Starks, 1978; Starks and Shubert 1978) and in England (Broady, 1979)a but the results of these studies are not completely consistent. Therefore it was the first aim of this project to identify and describe the algae making up the soil community in different types of colliery spoil.

In addition it was planned to describe seasonal changes in these algae and various environmental variables in their vicinity, as a step towards providing an "explanation" of any differences observed. The site chosen provided a suitable range of spoil types, including ones devoid of higher plants and ones where colonization was well developed.

CHAPTER 2

MATERIALS AND METHODS

2.1 MEASUREMENT OF SOIL pH

During preliminary observations on the pit heaps at East Holywell it was noted that adjacent areas showed wide variations in soil pH and therefore a systematic survey was conducted.

Samples of approximately 50 g of soil were collected at 25 m intervals on the surface of the tip. Each sample was stored in a polythene bag for transport back to the laboratory for testing. Allen *et al.* (1974) stated that prolonged storage of soil samples could lead to changes in pH. To minimise this effect testing was carried out as soon as possible, and never more than 6 hr after collection.

There are two methods available for the measurement of soil pH. The first of these is the colorimetric method which uses indicators whose colour changes with hydrogen ion activity. This method is useful for obtaining a rough indication of pH range, especially in the field, but with soils of the type found on pit heaps with a distinct colour, such methods are inaccurate.

The alternative is an electrometric method. This involves the measurement of pH using a glass electrode and a pH meter. As the pH meter used, a Beckman Chem-Mate, was a school model with limited sensitivity, a combination of these methods was employed. The approximate pH of a sample was determined using Universal indicator and the pH meter was set to that range with a citric acid/phosphate buffer. The precise pH value was then recorded with the meter. Tests with solutions of known pH showed that this method was

accurate to $\pm$ 0.2 pH units.

The method of preparing a sample for pH measurement can have important effects upon the results obtained. The normal practice is to take measurements upon a slurry of soil and water, but the ratio of soil to water has been the subject of some controversy. Over the range from sticky soil to a soil/water ratio of 1 to 5 the change may be as much as 1 pH unit (Synder, 1935; Huberty & Haas, 1940).

In the light of these considerations the following standard method was adopted for the preparation and measurement of pH. A 100 ml beaker was half filled with soil and then distilled water was added up to the 100 ml mark. The soil and water were stirred with a glass rod for 30 s and then allowed to stand for 15 min. Some of the supernatant was then removed and its approximate pH determined with universal indicator. The precise pH was then recorded using a pH meter whose electrode was always suspended in the water above the sedimented soil.

2.2 COMPILATION OF SPECIES LIST

As the flora of several pit heaps has been described in the literature, it was considered useful to compile a species list for the East Holywell site so that similarities and differences with other sites could be established. To this end all species in flower during May, June and July 1978 were collected and identified. This was supplemented during the period March 1979 to February 1980 when in order to sample from the permanent quadrats, monthly visits were made to the site. Upon each visit specimens of plants in flower and mosses in fruit were collected and identified using standard references.

2.3 MONTHLY SAMPLING OF SOIL ALGAE

2.31 Choice of Sampling Sites

Physical conditions on the surface of a pit heap vary considerably in different parts of the heap. As described in Section 1.212 the stability of the surface material is greatly influenced by the slope of the surface. The temperature is influenced by the aspect of the slope as described in Section 1.213. Therefore it was decided that all the sampling sites should be flat so that they would receive equal amounts of light and rainfall and be subject to the same degree of erosion. All sites were away from buildings and tall vegetation so that no shade was cast which might further influence physical conditions. Finally, some parts of the East Holywell site were used regularly as footpaths and therefore all sites were situated in a little used area of the tip which was as free from human disturbance as possible.

The surface of the tip varied in three distinct ways. There were two spoil types; shale which was made up of burnt rock fragments and washeries waste which was a fine black dust. The shale areas of the tip showed marked variation in pH, with some areas having a pH of 4.0 while others had a pH of between 7.0 and 8.0. Finally some areas of the tip were covered with vegetation while others were completely bare. Four pairs of sites were selected to cover these variables as shown in Table 2.1 and Figs 2.1, 2.2, 2.3 and 2.4.

TABLE 2.1 Features of the permanent quadrats

site code	spoil type	pH	vegetation
Y1	red shale	8.0	bare
Y2	red shale	7.0	bare
P1	red shale	3.8	bare
P2	red shale	4.0	bare
G1	black dust	7.0	*Tussilago farfara*
G2	black dust	7.0	*Tussilago farfara*
B1	black dust	7.0	bare
B2	black dust	7.0	bare

At each site a permanent 1 m^2 quadrat was established. This was marked by colour coded stakes at the corners of the quadrat.

FIG 2.1 Spoil surface at Y sites

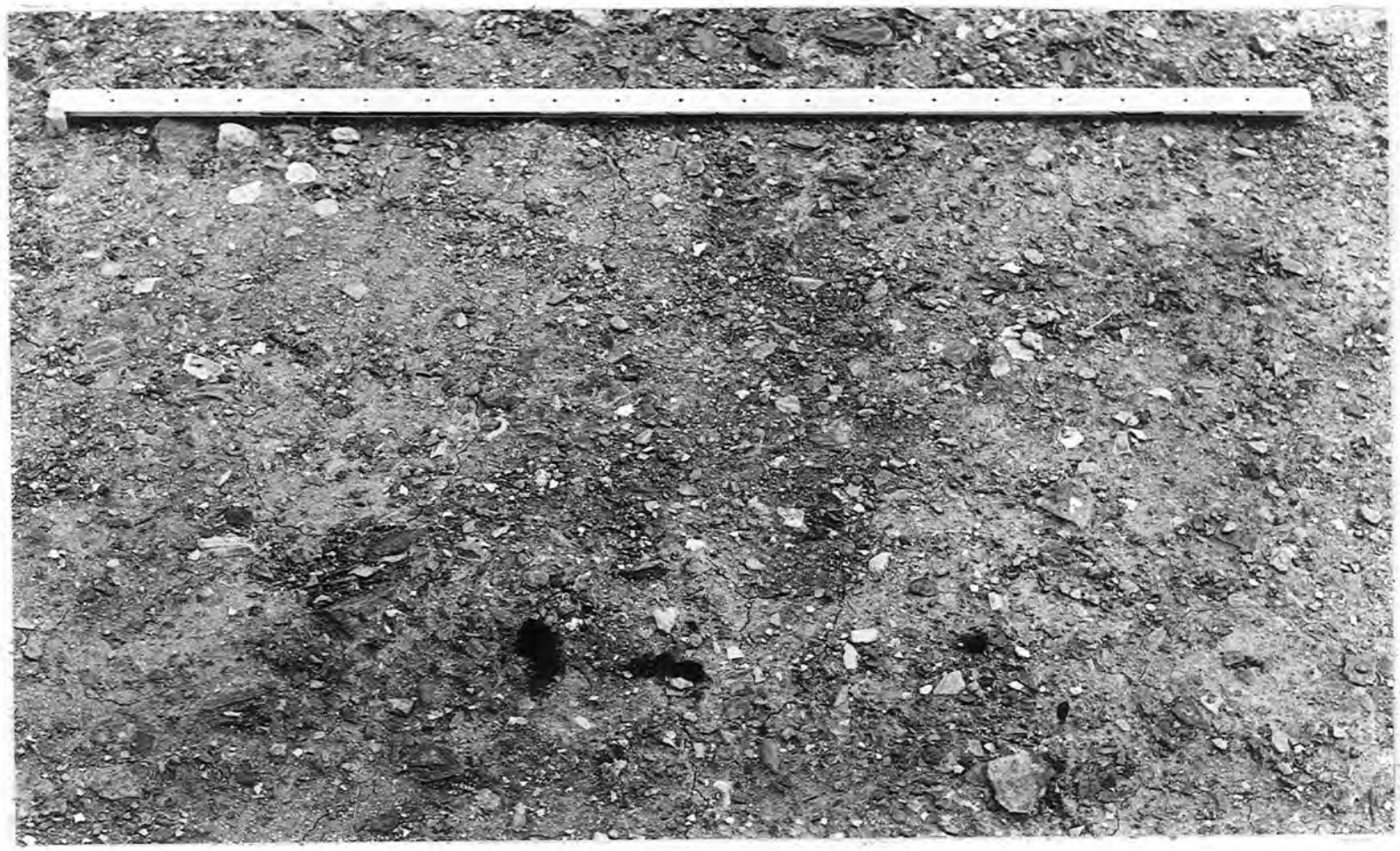


FIG 2.2 Spoil surface at P sites

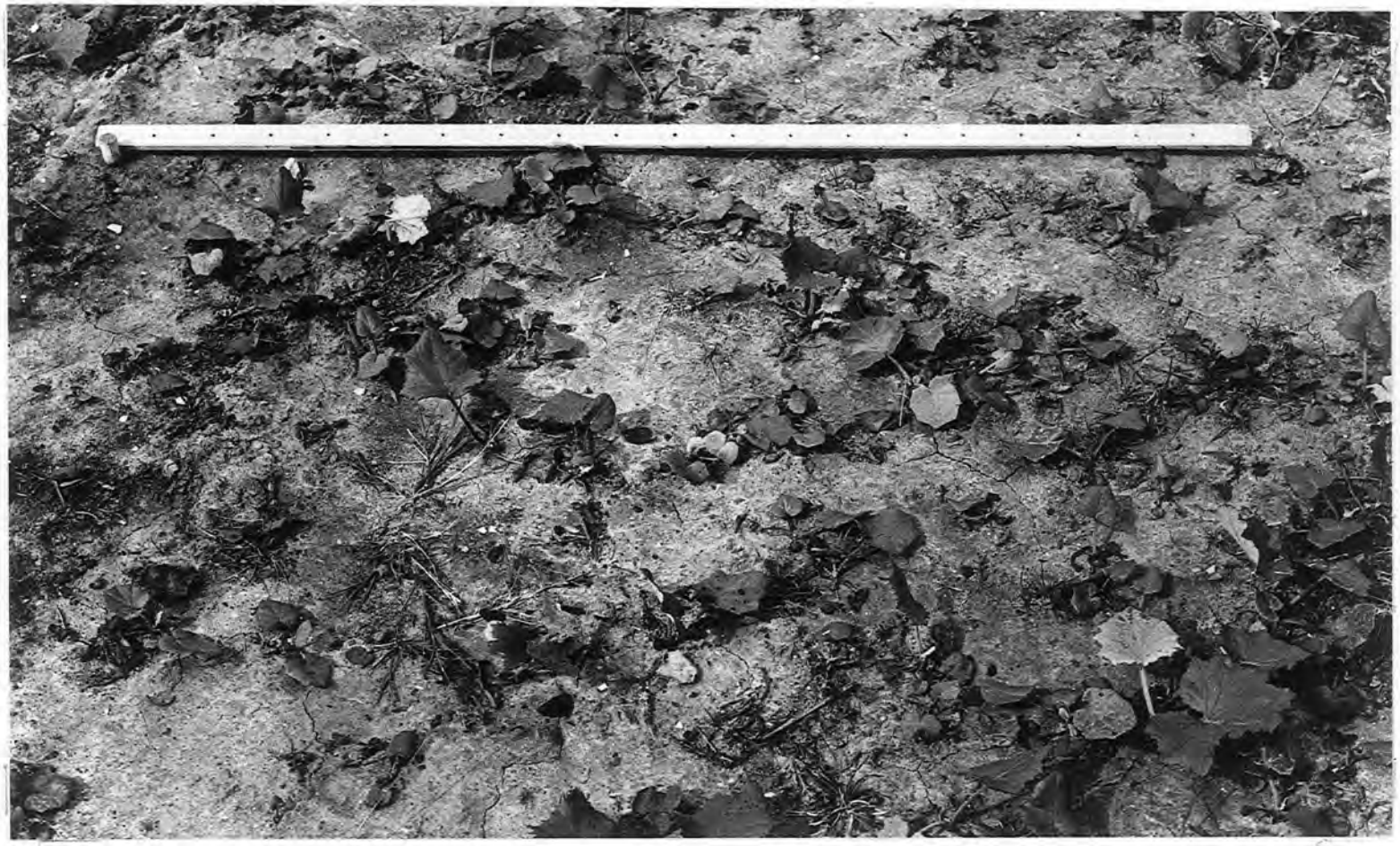


FIG 2.3 Spoil surface at G sites

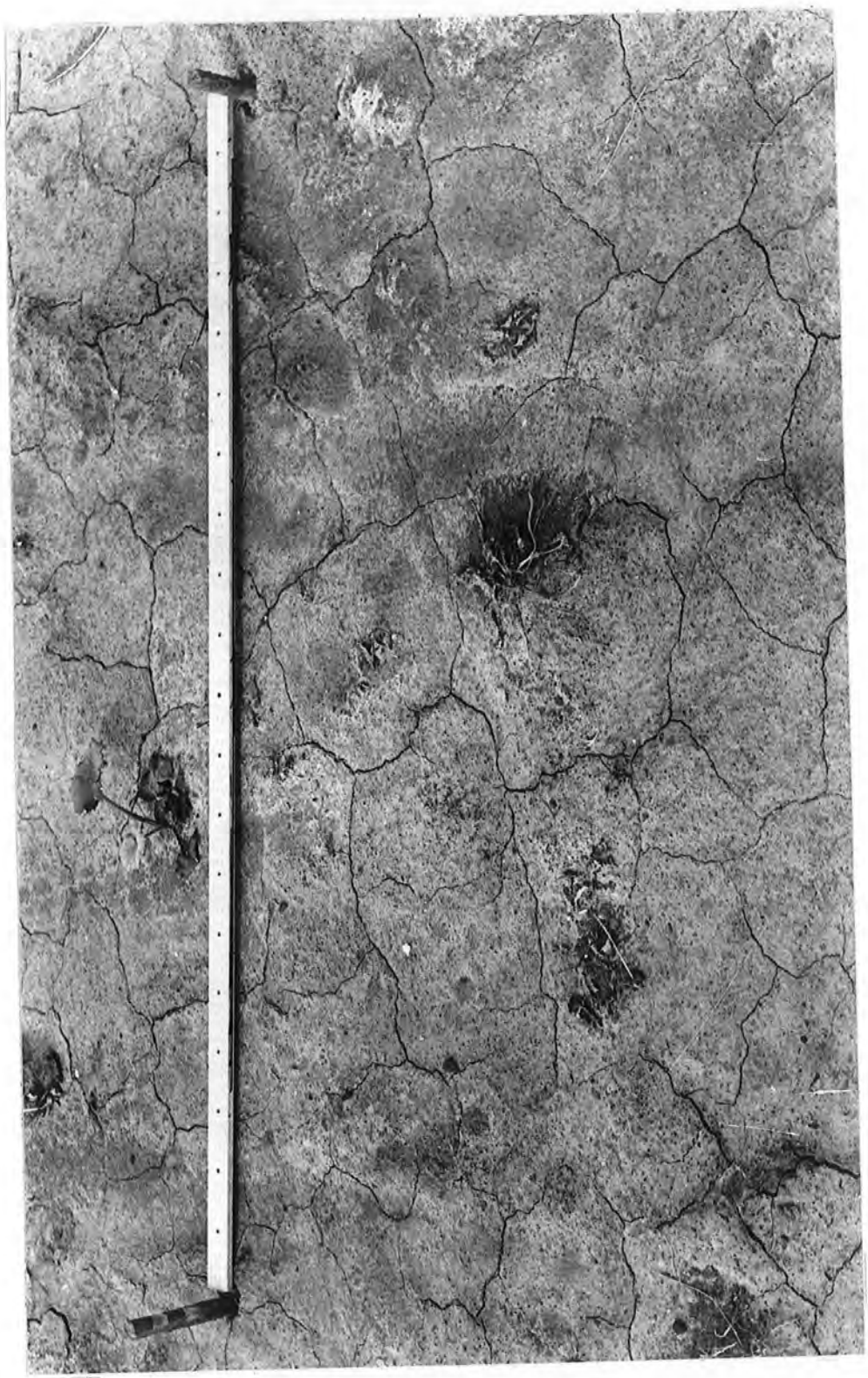


FIG 2.4 Spoil surface at B sites

2.32 Sampling Technique

Each month at each site a quadrat frame was placed on the surface of the tip to enclose the permanent quadrat. Points were selected within the quadrat by obtaining a pair of random numbers (Fisher, 1965). The first number of this pair was used to fix the east/west line by measuring that number of centimetres down the north/south side of the quadrat, starting in the north west corner. The second number was used to fix the north/south line by measuring that number of centimetres along the east/west side of the quadrat starting at the north west corner. The intersection of these two lines was the sample point. The points chosen in this way were recorded on sampling maps for each quadrat so that the same point would not be sampled twice.

At each point, specified in the way just described, a sample of spoil was collected using a cylindrical borer 1 cm in diameter. This was pushed into the spoil to a depth of 1 cm and the sample cylinder of spoil thus obtained was pushed out with a glass rod. The sample collected therefore had a volume of 0.79 ml and represented 0.79 cm^2 of tip surface area. In the event of the sampling point falling over a stone, the stone was collected and the sample taken from under the stone. At each site ten samples were collected in this way, each month for 12 months between March 1979 and February 1980. Sampling dates are shown in Table 2.2. The ten samples from each site, each month, were pooled to give the monthly sample for that site. The borer and glass rod were sterilised between sites by washing in alcohol and the collected material was transported to the laboratory in sterile specimen tubes.

TABLE 2.2 Dates samples were taken in the monthly sampling programme

March	13. 3. 79
April	19. 4. 79
May	19. 5. 79
June	21. 6. 79
July	31. 7. 79
August	20. 8. 79
September	29. 9. 79
October	27.10. 79
November	27.11. 79
December	28.12. 79
January	30. 1. 80
February	28. 2. 80

2.33 Storage of samples

The freshly collected samples were spread upon sterile filter paper in sterile petri dishes and covered with sheets of filter paper. They were then allowed to dry at room temperature until they reached a constant weight. This normally took approximately 10 days. Once air dry the samples were packed into sterile specimen tubes and stored in a cool dark cupboard until required for culturing.

In the event a considerable time elapsed between the collection of samples and culturing. In order to establish the effect of this storage upon the samples, the material collected in January 1980 was dried and cultured immediately i.e. effectively two weeks after collection. A second set of cultures were established from the same material in June 1980 when they had been stored for five months.

TABLE 2.3 Chu 10 culture medium with A/C microelements and low Mn

macronutrients		
KH_2PO_4	15.6 g l^{-1}	use 0.5 ml l^{-1}
$MgSO_4 \cdot 7H_2O$	25.0 g l^{-1}	use 1.0 ml l^{-1}
$Ca(NO_3)_2 \cdot 4H_2O$	57.6 g l^{-1}	use 1.0 ml l^{-1}
$NaHCO_3$	15.85g l^{-1}	use 1.0 ml l^{-1}
$Na_2SiO_3 \cdot 5H_2O$	43.5 g l^{-1}	use 0.25ml l^{-1}
Fe/EDTA solution containing		
Sodium EDTA	12.7 g l^{-1}	
$FeCl_3 \cdot 6H_2O$	9.7 g l^{-1}	use 0.25ml l^{-1}

micronutrients - A/C microelements, low Mn

H_3BO_3	2.86 g l^{-1}
$MnCl_2 \cdot 4H_2O$	0.181 g l^{-1}
$ZnSO_4 \cdot 7H_2O$	0.222 g l^{-1}
$CuSO_4 \cdot 5H_2O$	0.029 g l^{-1}
$CoSO_4 \cdot 7H_2O$	0.042 g l^{-1}
$Na_2Mo_4 \cdot 2H_2O$	0.027 g l^{-1}

use 0.25 ml l^{-1} of this solution

Made up to one litre with distilled water and solidified with 1.5 % agar

TABLE 2.4 Concentration of salts in Chu 10 medium

KH_2PO_4	7.8	x	10^{-3}	g l^{-1}
$MgSO_4 \cdot 7H_2O$	2.5	x	10^{-2}	g l^{-1}
$Ca(NO_3)_2 \cdot 4H_2O$	5.76	x	10^{-2}	g l^{-1}
$NaHCO_3$	1.585	x	10^{-2}	g l^{-1}
$Na_2Si_3 \cdot 5H_2O$	1.0925	x	10^{-2}	g l^{-1}
Sodium EDTA	3.175	x	10^{-3}	g l^{-1}
$FeCl_3 \cdot 6H_2O$	2.425	x	10^{-3}	g l^{-1}
H_3BO_3	7.15	x	10^{-4}	g l^{-1}
$MnCl_3 \cdot 4H_2O$	4.525	x	10^{-5}	g l^{-1}
$ZnSO_4 \cdot 7H_2O$	5.555	x	10^{-5}	g l^{-1}
$CuSO_4 \cdot 5H_2O$	7.25	x	10^{-6}	g l^{-1}
$CoSO_4 \cdot 7H_2O$	1.05	x	10^{-5}	g l^{-1}
$Na_2Mo_4 \cdot 2H_2O$	6.75	x	10^{-6}	g l^{-1}
Agar	15.0			g l^{-1}

2.4 METHOD OF EXAMINING SOIL ALGAE

A variety of methods have been adopted by past workers to examine soil algae. However, the various techniques may be grouped under two headings.

2.41 Direct microscopic examination

The examination of small quantities of soil under the microscope is not very rewarding. The separation of algal cells from the multitude of soil particles is difficult and even in samples where algal cells are abundant, they are often in a stage which does not show the features necessary for identification. Lund (1947) used a technique in which he placed microscope coverslips on the surface of moist soil and when these had been colonized by the microflora he was able to examine them under the microscope. This method has the advantage of only collecting those algae which are actively growing in the soil but has the disadvantage of selecting only motile forms or those with a motile phase in their life cycle. Tchan (1953) developed a technique of direct observation using fluorescence microscopy in which algal cells can be seperated from soil particles by the reddish fluorescence of the chlorophyll which they contain. This method also detects only those cells which are active in the soil but has the disadvantage that it is impossible to classify the cells seen.

2.42 Culture techniques

The limitations of direct observation just outlined have led most workers to adopt some form of culture technique. The least artificial form of culture is the moist plate technique of Lund (1947). In this method a quantity of soil in a petri dish is moistened with sterile water so that it is damp but not flooded.

It is then illuminated until large numbers of algae develop. This development of algae from soil may be speeded up by the addition of a nutrient medium in place of the water. Such a treatment is called an enrichment culture and is an approach favoured by many workers. In its most straightforward form an enrichment culture consists of a quantity of soil added to mineral medium in a flask and illuminated. Samples can be removed from the culture at intervals and the species present identified. This technique was first used by Bristol (1920) in her pioneering work and has also been employed by John (1942) and Shubert and Starks (1978). It is open to the objection that the aquatic conditions of the culture may encourage aquatic and semi-aquatic species and suppress euterrestrial forms hence giving a misleading impression of the soil flora. To overcome this objection the mineral medium may be solidified with 1 - 2% agar and a soil suspension spread upon its surface. This method has been used by King and Ward (1977) and Broady (1979a).

Various mineral media have been devised for the culture of algae but all include a supply of the elements potassium, magnesium, calcium, sodium and phosphate, nitrate and sulphate plus various microelements.

In the light of these considerations it was decided to culture soil from East Holywell on mineral salt agar made up with Chu 10 medium (Chu 1942) plus microelement addition with low manganese, solidified with 1.5% agar. The detailed composition of this medium is given in Tables 2.3 and 2.4. This medium has a pH of 8.0 at normal working strength and was used as such for neutral soil samples. To culture soil from acidic sites the pH of the mineral medium adjusted by the addition of appropriate quantities of

0.1N sulphuric acid until the pH was lowered to 4.0. Sulphuric acid was chosen for making this adjustment because as explained in Section 1.22 it is the presence of H_2SO_4 which produces the low pH of some colliery spoils. During the preparation of acid Chu 10 it was necessary to autoclave the acid mineral medium and the agar solution separately and mix them just before pouring the plates to avoid the hydrolysis of the agar. All media were sterilized by autoclaving for 20 min at 0.95 kg cm^{-2} which gives a temperature of 121°C. They were poured into sterile plastic petri dishes.

2.5 METHODS OF ESTIMATING ALGAL ABUNDANCE

Straightforward counting of algae present in soil samples is impossible with an ordinary microscope because of the difficulty of direct observation and can only be achieved with fluorescence microscopy as described in Section 2.6. This has resulted in the development of various indirect techniques for the estimation of algal abundance. These methods may be considered under three headings.

2.51 Pigment analysis

Using solvents it is possible to extract pigments from soil samples and to measure the concentration of pigment by the amount of light which it absorbs. If it is assumed that the pigments extracted are all from soil algae then the pigment concentration can be taken as a measure of algal abundance. This method has been used by Singh (1961) to estimate the abundance of blue-green algae in Indian soils and by Shubert and Starks (1978) to estimate algal abundance in coal mine spoils. The disadvantages of this approach are that it only measures the total algal biomass and can give no indication of the abundance of the various groups of algae. Furthermore it has been suggested that it may over-estimate the number of algae present because

pigments such as humic acid and the breakdown products of chlorophyll are extracted along with the chlorophyll (Fogg et al ., 1973).

2.52 Most probable number technique

This approach was developed by Bristol (1920) and was modified and improved by Petersen (1935). A weighed quantity of soil is added to a known volume of culture medium and tenfold serial dilutions made down to a concentration of 10^{-8}. These cultures are incubated and illuminated for a period of time and then examined for the presence of algae. The most probable number of algae in the original sample is calculated from the number of cultures at each dilution which contain algae. Cullimore and M^{c}Cann (1977) used the technique and in addition to estimating the gross algal flora, also estimated the population of individual algal genera by noting the presence and absence of each genera at each dilution. However such data is difficult to interpret because differences in the community dynamics of cultures containing innocula of different genera at different concentrations. The method in general is subject to the disadvantages common to all liquid cultures outlined in Section 2.62.

2.53 Dilution and plating techniques

The basis of this approach is that a weighed quantity of soil is suspended in a known volume of water and a quantity of this suspension is spread upon a sterile mineral agar plate. After incubation and illumination, colonies of algae develop on the surface of the agar which may be counted. If each colony develops from a single algal cell, the number of colonies represents the number of algae in the suspension. Knowing the dilution of the suspension the number of cells in the soil sample may be calculated.
This method has been used by King and Ward (1977),

Metting and Rayburn (1979) and Broady (1979a) to estimate algal abundance.

2.6 METHOD USED IN THE MONTHLY SAMPLING PROGRAMME

In the light of the points discussed in the preceeding sections and because the work was to be carried out in secondary school biology laboratory it was decided to adopt a dilution and plating technique. This had the advantage of requiring no highly specialised equipment, but would give an estimate of both total algal abundance and the abundance of specific groups of algae. Furthermore it would permit the isolation of particular algae into unialgal cultures for identification purposes.

2.61 Preparation of dilution plates

The air dried field samples were often compacted into hard lumps, especially those from sites B1 and B2, because of the amount of clay present in the sample. It was therefore decided to break up the samples by light grinding in a mortar but samples from sites P1, P2, Y1 and Y2 contained fragile shale fragments and therefore this treatment had to be kept to a minimum. A standard procedure was adopted of grinding for 30 s with a pestle covered with rubber tubing.

Samples from sites P1, P2, Y1 and Y2 also contained a high proportion of large particles $>$ 0.25 mm in diameter. Details of the distribution of particles of different sizes are given in Table 3.5. These particles would not remain suspended in water long enough to enable a representative sample to be removed from the suspension and therefore the ground air dried field samples were shaken in a set of Endecott soil sieves for 2 min and the $\leqslant$ 0.25 mm fraction collected. In order to standardize any effect of these pre-treatments upon the samples all samples were ground in a mortar

and sieved.

1 g of the $\leqslant$ 0.25 mm soil particles was then added to 100 ml of sterile distilled water and mixed in a blender for 2 min to produce an even suspension. This suspension was then diluted by the transfer of an appropriate aliquot to another tube and the addition of sterile distilled water to give dilutions of $5\ g\ l^{-1}$, $1\ g\ l^{-1}$ and $0.5\ g\ l^{-1}$. Preliminary testing indicated that this was the appropriate dilution range. When applied to various spoils it was found that there were considerable differences in the numbers of algae present and not all these dilutions were appropriate at each site. Dilutions were eventually selected which would give numbers of colonies greater than 5 and less than 500 on an agar plate. The range of dilutions used for each site are given in Table 2.5.

TABLE 2.5 Dilutions used for culturing monthly samples at each site

site	dilutions used
G1	$0.5\ g\ l^{-1}$, $1.0\ g\ l^{-1}$
G2	$0.5\ g\ l^{-1}$, $1.0\ g\ l^{-1}$
Y1	$1.0\ g\ l^{-1}$, $5.0\ g\ l^{-1}$
Y2	$10.0\ g\ l^{-1}$
B1	$10.0\ g\ l^{-1}$
B2	$10.0\ g\ l^{-1}$
P1	$10.0\ g\ l^{-1}$
P2	$10.0\ g\ l^{-1}$

Four 1 ml aliquots were pipetted from each suspension onto four replicate mineral agar plates. These cultures were then spread with a sterile inoculating loop and then incubated at room temperature

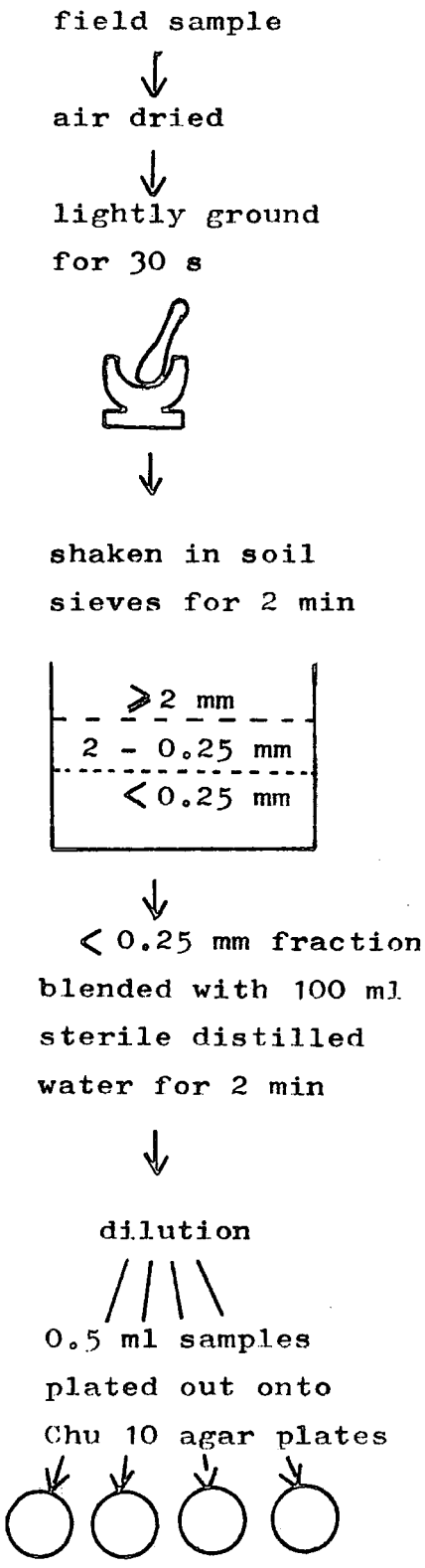
which varied between 8 and 18°C. They were illuminated by two 40 watt daylight fluorescent tubes which provided a constant light intensity of 3 Wm^{-2}. Initial results obtained using this technique showed an unacceptably high degree of variability between replicates. Re-examination of the plates showed that this was due to dense clusters of colonies on some plates. The colonies in each of these clusters were all of the same species and they were therefore probably formed by the bursting of reproductive bodies containing non-motile spores. As the colonies in a cluster were not widely dispersed it was likely that the bursting of the reproductive body occurred while the cells were suspended in the thin water film on the surface of the agar after being plated out. In order to eliminate this effect the agar plates were dried in an oven at 70°C for one hour with their lids removed and then cooled before the suspension was spread upon them. In addition the amount of inoculum was reduced to 0.5 ml. The effect of this was to reduce the amount of water on the surface of the plate and to cause that which was present to be absorbed into the surface of the agar in a time of one to six hours. The procedure summarised in Fig. 2.5 was adopted as the standard method form the preparation of dilution cultures in the monthly sampling programme.

2.62 Culture conditions for dilution plates

The dilution cultures were placed on a flat bench with a white surface beneath lighting units containing two 40 watt daylight fluorescent tubes 50 cm above the cultures giving a constant illumination of 3 Wm^{-2} at the culture surface.

It was not possible to control the temperature and therefore variation occurred between 8 and 18°C. However for most cultures most of the time the temperature

FIG 2.5 Standard method for preparation of dilution cultures



was 16°C.

Cultures from sites G1 and G2 produced algal colonies which were discernible with the naked eye after 10 days. After 28 days adjacent colonies of the faster growing algae, on these plates tended to fuse. This was especially true at the 1 g l^{-1} dilution and it made counting difficult after this time. In contrast cultures from sites Y1, Y2, B1, B2 and P1 produced much slower growing colonies which were only visible with the naked eye after 28 days. However although these colonies were small they were clearly visible with a stereomicroscope at day + 28. In order to check that all colonies on these plates had developed to a visible size by day + 28, a set of plates were left for several months and counted at day + 56 and day + 112. No increase in the number of colonies was detected and therefore all plates were counted after 28 days illumination in the monthly survey.

2.63 Counting of dilution plates

The number of algal colonies on each plate was between 5 and 500. This was ensured by the selection of appropriate dilutions. The colonies were counted under a x 8.5 stereomicroscope. To facilitate the counting of dense plates a grid of centimetre squares was marked on the lid of a plastic petri dish and the plate to be counted was placed over the grid. The number of algal colonies in each square of the grid was counted and recorded on a score sheet. The normal convention of counting colonies on the north and west boundaries of a square and ignoring those on the south and east, was adopted.

Chlorophyta and diatoms presented few problems in counting because they form discrete colonies which are approximately circular in shape. Occasionally colonies had developed in such close proximity that

they had fused by the time of counting. To avoid differences in interpretation of fused colonies on different plates it was decided to count only clearly separate plant masses and to count fused colonies as one.

Cyanophyta presented a more difficult problem because they formed irregular spreading masses which quickly covered the plate. Therefore in most cases it was only possible to count totally independent plant masses and these must often have been the product of the fusion of several colonies. The appearance of colonies of members of the Ulotrichales had a very characteristic wavy appearance on the surface of the agar. This was true of unicellular genera such as Stichococcus as well as for filamentous genera. Therefore colonies of this group were counted separately in order to follow changes in their abundance.

2.7 UNIALGAL CULTURES

Isolated observations of soil algae are unsatisfactory for reliable identification. The organism may be in a stage of its life cycle which does not show its characteristic features and there are many examples of organisms which have been described as different species and subsequently shown to be different stages of one organism. Thus it was necessary to isolate organisms into unialgal cultures and to observe them systematically in order to obtain reliable identifications. This was only possible for a limited number of the algae observed because it took such a long time. The organisms on the dilution plates for the months of September, October and November 1979 were studied in this way.

Bold (1970) has described how the morphology of a colony formed by a particular species on agar may be

used as a supplementary attribute in the identification of soil algae. Therefore dilution plates were scanned with a x 8.5 stereomicroscope and colonies showing different forms were isolated into unialgal culture. The differences between colonies used were:-

a. outline of colony - colonies with a regular outline were differentiated from those with an irregular outline;
b. texture of the colony - a distinction was made between smooth colonies, rough colonies and wavy colonies;
c. appearance of the surface of the colony - a distinction was made between colonies with a moist surface and those with a dry surface.

Once a particular type of colony had been recognised, a single isolated colony of that type was picked from the surface of the agar with a sterile mounted needle under a stereomicroscope. This colony was then dispersed in a drop of sterile distilled water on a microscope slide and a drop of this suspension was then spread on a mineral agar slope with a sterile inoculating loop. These slopes were then incubated in a culture room which had a glass wall on its north side. The temperature of this room was maintained at a constant $14^{o}C$. No artificial illumination was used but culture were only illuminated by the light from the north facing window. This work was done during May and the first two weeks of June 1980 and therefore the cultures were illuminated for approximately 18 hours per day. The cultures were examined after one and four weeks.

2.8 DETAILED STUDY OF SOIL ULOTRICHALES

Members of the Ulotrichales were frequently encountered on the dilution plates and formed an important and easily recognised element in the soil flora of two sites. Material from the dilution plates, when examined microscopically showed numerous small variations which made it difficult to assign them to particular species. Therefore 30 colonies were isolated from the dilution plates of the samples collected in September and October 1979 and were cultured in the manner described in Section 2.7.

CHAPTER 3

SITE DESCRIPTION

3.1 GEOGRAPHICAL DESCRIPTION

3.11 Location

The Fenwick Pit is at East Holywell in the county of Tyne and Wear (O.S. sheet NZ 37 map ref. 313730). The spoil heaps occupy approximately two thirds of the site of the mine which covers an area of eight hectares. The mine is one km north-west of the village of Earsdon on the flat S-E. Northumberland plain. It is bounded upon its southern border by the Briardene Burn and upon its western border by the road from Earsdon to Backworth (Fig. 3.1, p 75).

3.12 Geology

The S-E. Northumberland plain is built entirely upon coal bearing strata of Carboniferous age. Rocks of both the Upper Carboniferous and the Lower Carboniferous are present but it is the coal measures in the Upper Carboniferous which are important in terms of productivity. These strata have a maximum thickness of 610 m and are covered by sands, gravels and clays of Ice Age origin. They are composed of a series of sandstones, shales, fireclays and coals in a regular sequence called the Yoredale Cyclothem.

Mining at East Holywell has been in the Middle or Main Productive Group of these strata which includes the 14 major coal seams listed in Table 3.1.

FIG 3.1 Map of East Holywell spoil heaps

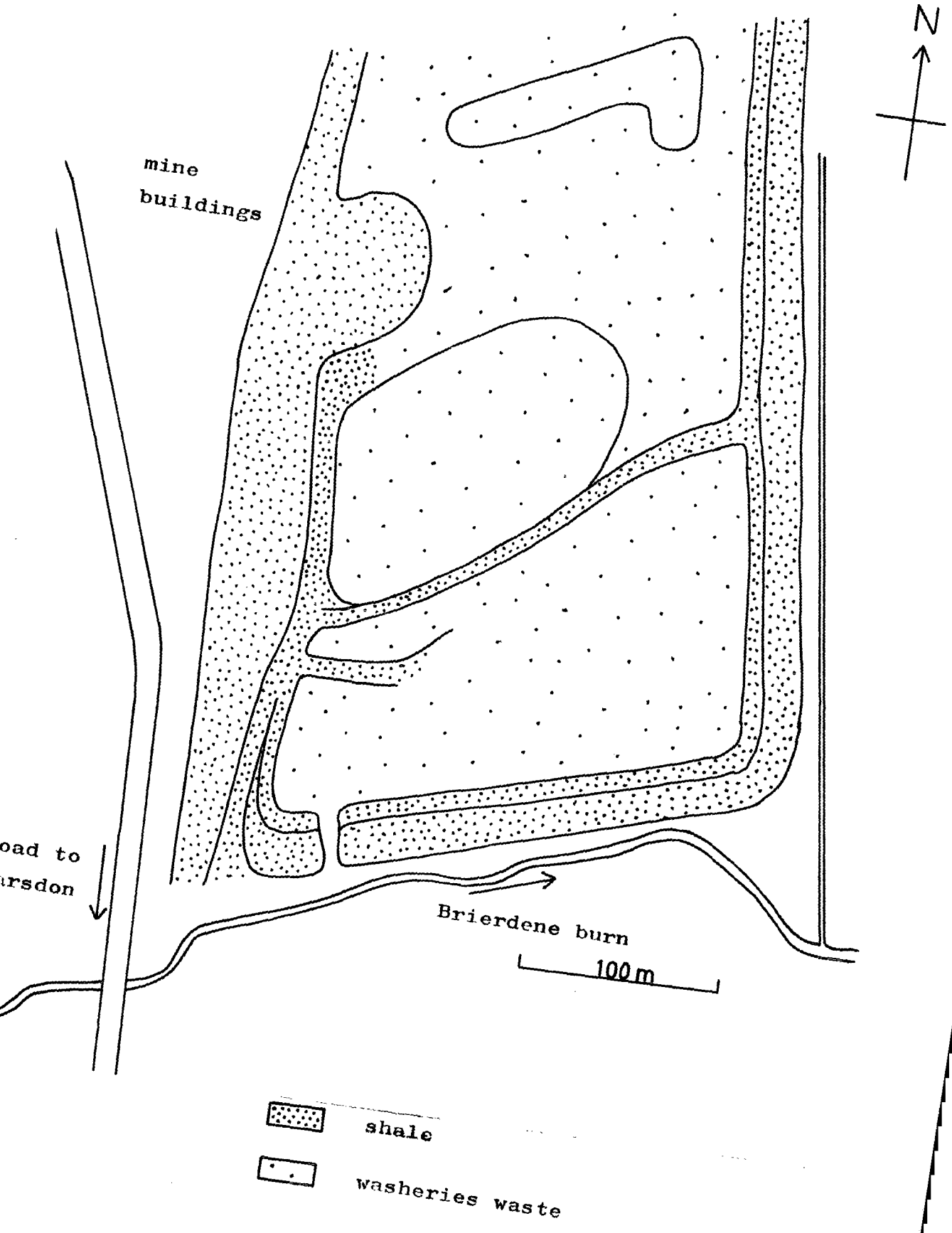


TABLE 3.1 Major coal seams in the Main Productive Group, Upper Carboniferous, S-E. Northumberland. Arranged in descending order (Trueman, 1954)

Closing Hill
Hebburn Fell
3/4 or 70 fathom
High Main
Grey
Yard
Bensham
6/4
5/4
Low Main
Plessey
Beaumont
Tilley
Top Busty
3/4
Brockwell
Victoria
Marshall Green

The names of coal seams vary in different parts of the coal field and not only has the same seam been given different names in different places but the same name has been given to different seams in different places. The names used here are those of the Backworth area of Northumberland which were in use during the working life of the Fenwick Pit (Trueman, 1954).

The seams vary in thickness throughout the coal field and therefore not every seam is worth mining at each site. They are separated by bands of sandstone,

locally called post-stone; shale, locally called metal and fireclay called seggar or seatearth. It is these materials which make up much of a pit heap.

The geology of East Holywell has an additional complication in that a major fault called the 90 fathom dyke runs through the rocks in an east-west direction, approximately half a kilometre south of the main pit shaft. Dyke is the local name for a fault and should not be confused with igneous intrusions of the same name. This fault has a throw of 122 m in the cliff at Cullercoats where it is exposed and has an even greater throw inland near the mine site. This feature produced considerable problems during the working life of the pit.

3.2 HISTORY OF THE SITE

The Fenwick Pit was opened in 1828 and was operated by the East Holywell Coal Company until the nationalisation of the mines when it passed to the ownership of the National Coal Board. The company operated three pits in this area. The first also called the Church Pit, is described as the A pit in the company records. It worked the high main seam and was followed by the B pit about which little is known. The C and D pits are the shafts at the site of the modern mine buildings, and are in fact approximately one kilometre north of the A and B pits (East Holywell Coal Company Records).

There have been pit heaps on the site since the beginning of mining operations. According to a report of the Newcastle Weekly Chronicle of 1873, the East Holywell Coal Company was sinking a new shaft. This was probably the C pit and was to mine the High and Low Main seams and the Yard seam. The same report records that the pit heap was in a very ragged

condition and was totally uncovered, from which we may infer that the original pit heap was not colonized by vegetation.

According to Mr R. Walker of 10 West View, Earsdon, a miner who worked in this and neighbouring pits for 51 years, the mine was substantially reorganised in the 1940's. The original pit heap was removed and used to fill subsidence ponds in the vicinity. This reorganisation was completed in 1947 when coal washeries were installed on the site. At that time the existing pit heap was built in a rectangular shape with a depression in the centre. The sides and rim of this heap were formed from shale on the site and the depression in the centre was used as a settling lagoon for the washeries waste. The water from the lagoon was discharged into the Brierdene Burn.

The pit heap is at present burning as evidenced by the sulphurous fumes and steam produced by the central portion. The exact date at which this process began is not known but it must have been since 1947. Various smaller heaps surround the main heap and some of these are of fine coal. Others are of fused shale and probably came from the core of the original heap. This indicates that it too was burning and therefore the shale of the sides and rim of the main heap are of burnt or red shale.

According to a report of the East Holywell Veterans Club, the mine worked eight seams during its operating life (Table 3.2). During the modern period, when the present pit heap material was brought to the surface, the mine was working the Beaumont seam. The coal from this seam was characteristically high in sulphur (Tables 3.3, p80 and 3.4, p80). It therefore seems reasonable to assume that the spoil from which

the pit heap was built had a high sulphur content.

TABLE 3.2 Coal seams mined at East Holywell
(East Holywell Veterans Club Records)

nearest to the surface	High Main seam
	Main seam
	Yard seam
	5/4 seam
	Bensham seam
	Low Main seam
	Busty seam
	3/4 seam
deepest	Beaumont seam

Miners who worked the Fenwick Pit recall that it was a dry pit until it expanded into the Old Church pit workings. They also remember that the Fenwick had a reputation of being a mine with a lot of post-stone.

In its final years the Fenwick worked the Bensham and 5/4 seams but by this time spoil was not being added to the pit heap. Instead it was being transported to other dumping sites at Backworth. The pit finally closed in 1973, and since that time the site has not been put to any other use. The present pit heap is scheduled to be reclaimed by Tyne and Wear County Council in the near future.

3.3 ENVIRONMENTAL CONDITIONS

3.31 Texture of spoil

There are two distinct types of colliery spoil at East Holywell. The sides and rim of the main heap are made of shale which is the direct product of mining

TABLE 3.3 Characteristics of coal from the Beaumont seam at East Holywell (N.C.B. Boring Book)

total sulphur	1.5 - 2.0%
ash	5.0 - 7.5%
coal rank	502 (strongly caking)
	600 (medium caking)
volatile matter	36 - 42%
calorific value	15,100 - 15,500 BTU lb^{-1}
moisture	2.0 - 4.0%
carbon	85 - 87%

TABLE 3.4 Detailed chemical composition of coal from the Beaumont seam at East Holywell (Northumberland Coalfield Seam Map)

S		1.57%
composed of		
SO_4	pyritic S	organic S
0.03%	0.98%	0.56%
CO_2		1.65%
Cl		0.19%
P		0.004%

operations (Fig. 3.2, p 82). The central portion of the main heap is made up of washeries waste, which is the dust that settles from the waste water from coal washing machines (Fig. 3.3, p83). The general distribution of these two types of waste can be seen in the map of the site (Fig. 3.1, p 75).

No detailed mechanical analysis of the spoil was made, but in the course of preparing material for culture, data was collected which gives some indication of the texture of different parts of the heap. Ten samples were collected from each of eight sites, four of which were on shale and four on washeries waste. Each sample was air dried and then lightly ground in a mortar with a rubber pestle. This procedure, necessary for the separation of soil particles, had the unfortunate effect of breaking up some of the softer shale fragments. The soil was then shaken in a set of Endecott soil sieves for 2 min. The air dry weight of the material retained in each sieve was recorded and used to determine the proportions of each particle size range at each site. The results are shown in Table 3.5, p 87 .

3.32 Surface stability

Erosion at East Holywell has been considerable. The washeries waste is covered with a network of drainage channels and the sides of the tip are cut by deep gullies. Wind erosion is also probably important. In March 1979 the spring thaw resulted in the melting of snow which had lain on the surface of the tip for about 10 weeks. The snow had acted as a trap for wind blown particles and with the melting this material was deposited as a layer over the surface of previous years vegetation. At its thickest this amounted to 5 mm of dust. This illustrated dramatically the way in which material was being added to the surface as well

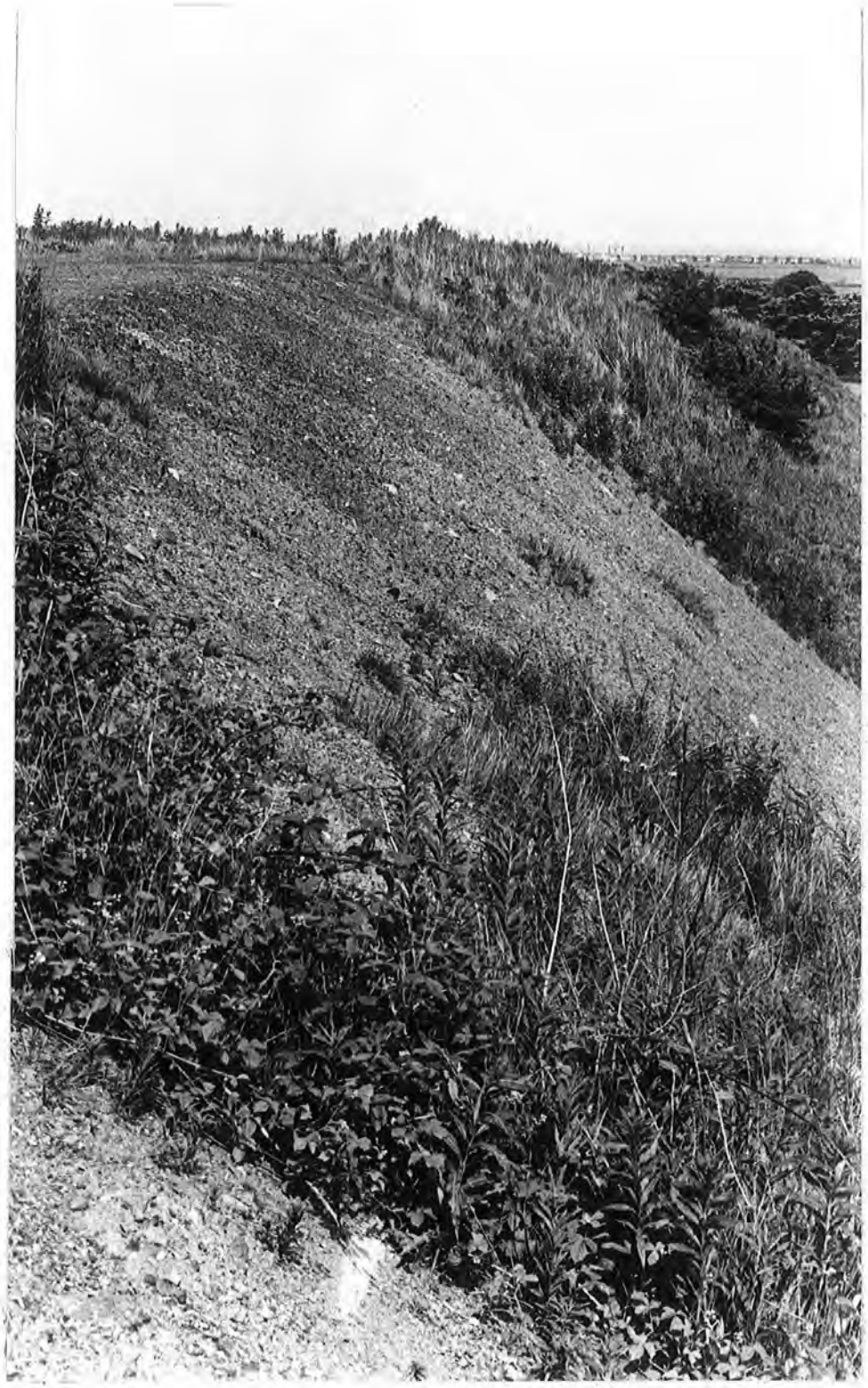


FIG. 3.2 Shale areas at East Holywell

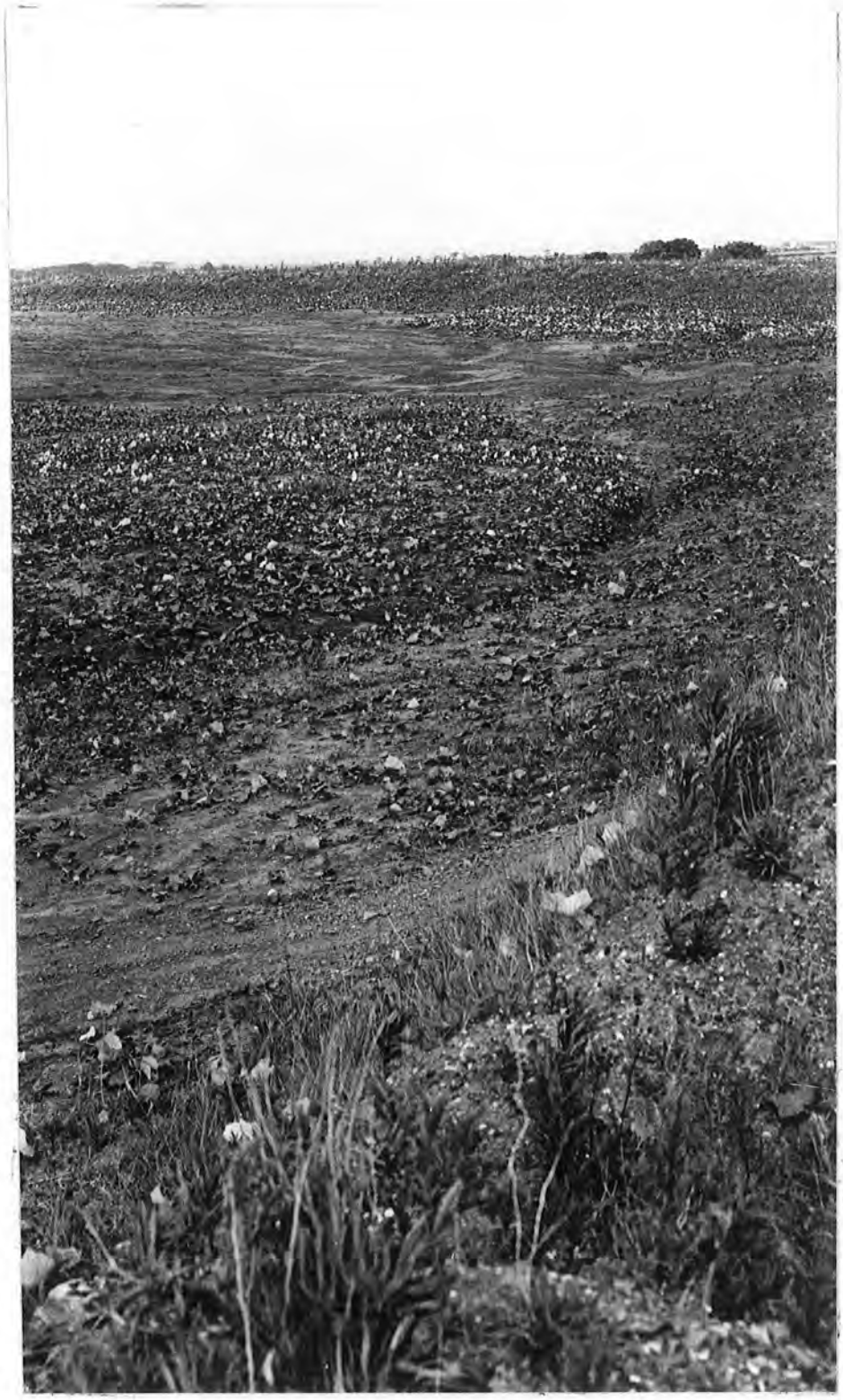


FIG. 3.3 Washeries waste area at East Holywell

TABLE 3.5 Texture of spoil

site	mean % ⩾ 2 mm	mean % coarse sand 2 mm - 0.25 mm	mean % fine sand silt and clay < 0.25 mm
Y1	21.4 ± 7.4	60.8 ± 4.3	39.0 ± 4.3
Y2	21.4 ± 7.6	57.8 ± 4.0	42.1 ± 4.0
P1	15.9 ± 4.4	59.6 ± 4.6	40.4 ± 4.6
P2	15.2 ± 3.1	61.5 ± 1.7	38.5 ± 1.7
B1	0	46.4 ± 5.7	52.6 ± 5.7
B2	0	37.0 ± 5.1	63.0 ± 5.1
G1	1.0	67.7 ± 4.6	31.0 ± 4.6
G2	0	58.3 ± 2.1	41.8 ± 2.1

as being removed by the action of the wind.

3.33 Chemical conditions

Measurement of soil pH at various sites upon the pit heap showed wide variations. Therefore a systematic survey was conducted using the methods described in Section 2.1, p 50 . The results of this survey are presented in Fig. 3.4, p 86 .

3.34 Meteorological conditions

Situated on the N-E. coast of England, East Holywell has a cool, dry, temperate climate. The period of study did have one unusual feature. Sampling began in March 1979 which was at the end of the hardest winter for many years. The site had been covered in deep snow throughout January and February and snow was still lying on the site in March. These conditions are unusual for this part of Britain.

Detailed weather measurements were not made on the site but the Coastguard station at Tynemouth, four km to the southeast records daily weather conditions and these are published by the British Meteorological Office. Fig. 3.5 shows the total rainfall and the mean maximum and mimimum temperature during the 28 days preceeding each sample date based upon this published data.

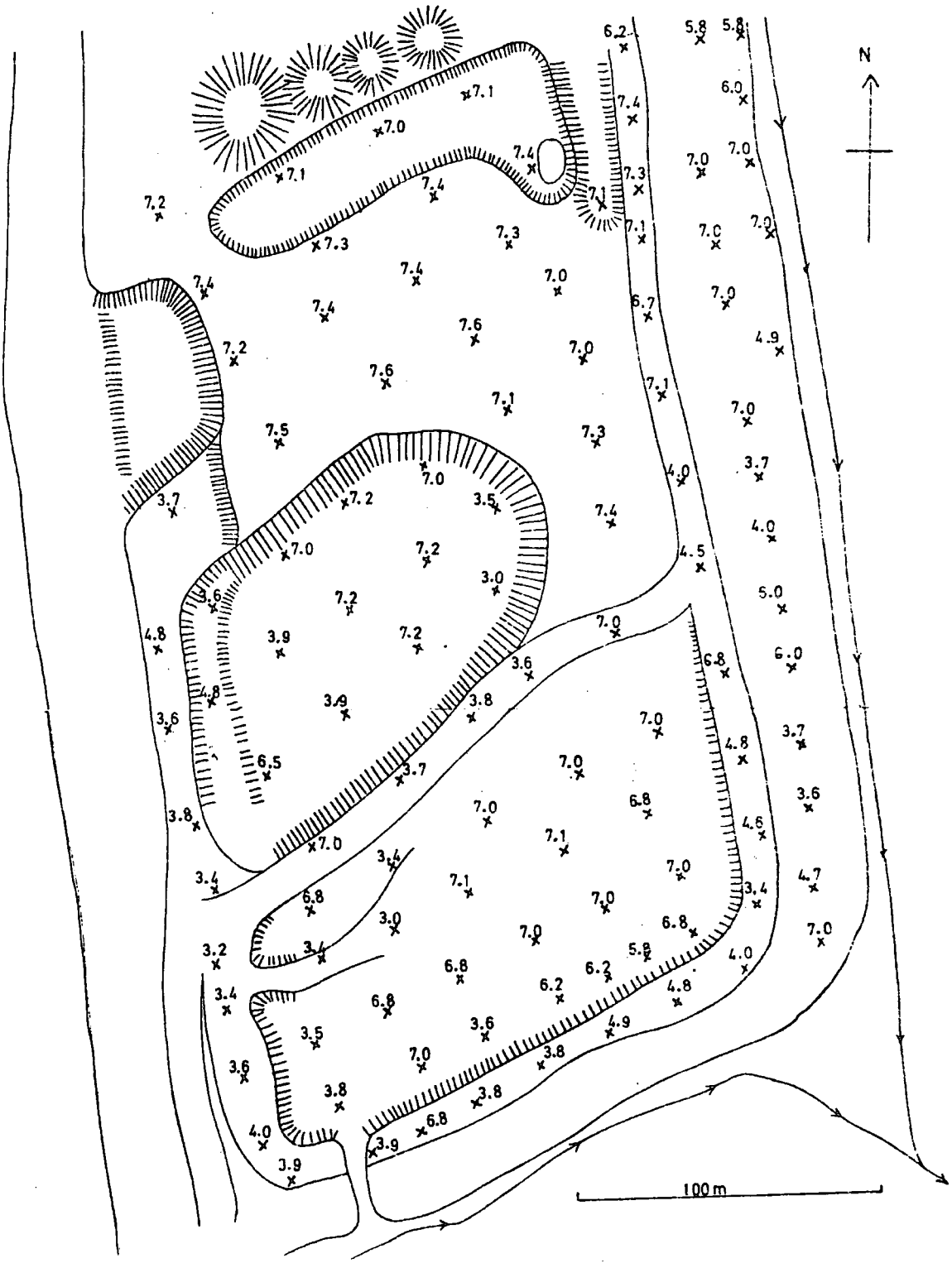


FIG 3.4 Spoil pH at East Holywell

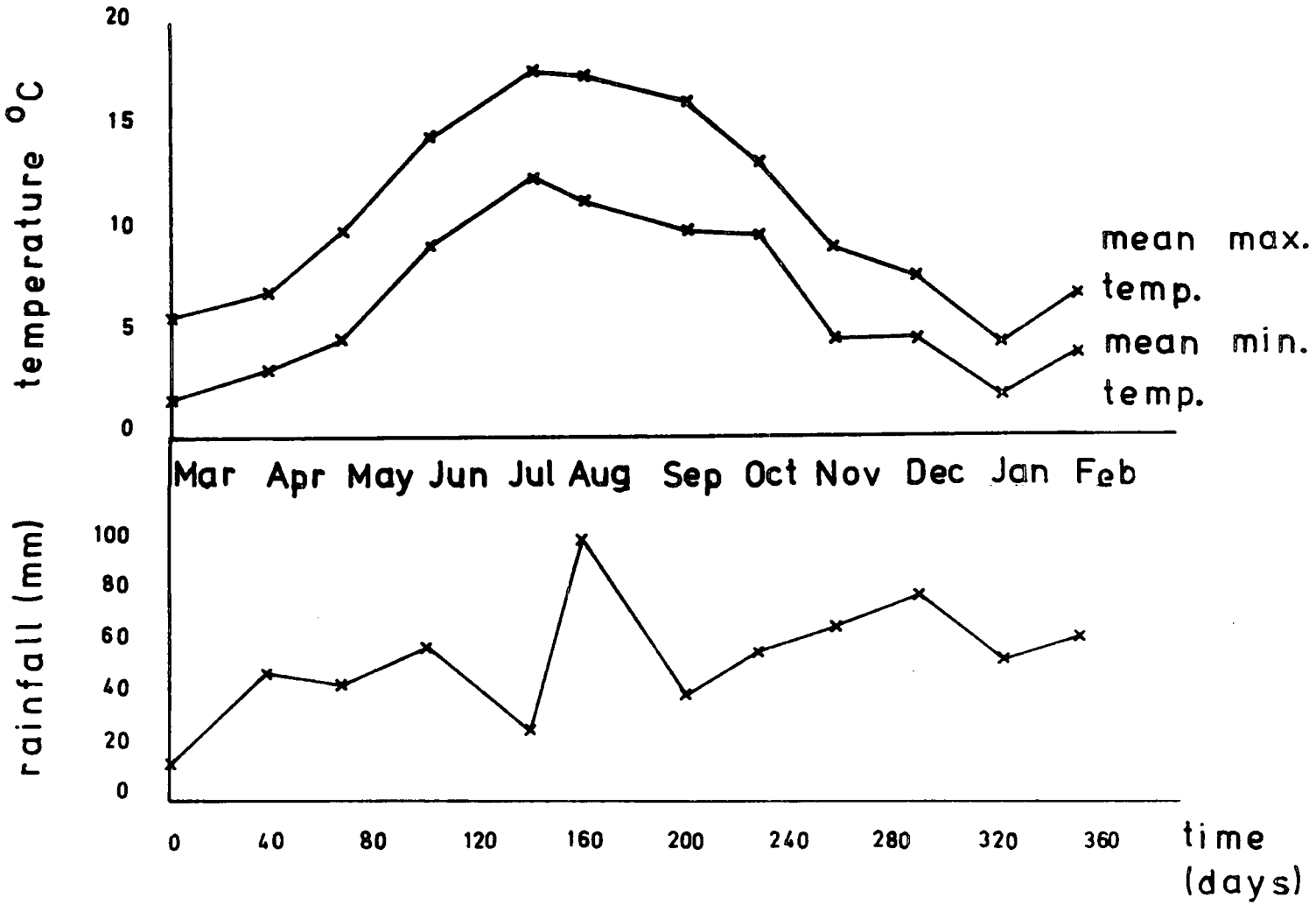


FIG. 3.5 Monthly variation in temperature and rainfall at Tynemouth, Tyne and Wear

3.4 BIOLOGICAL STATUS

The vegetation of several pit heaps has been described in the literature (Section 1.23, p 28). In order to facilitate comparison between this site and others previously described it was decided to compile a list of all the higher plant species occurring on the site. In this respect two quite separate communities can be recognised:-

a. a community on washeries waste
b. a community on shale

3.41 Washeries waste community

Although it was not possible to exactly date the end of washeries operations, the mine was in full operation until the late 1960's therefore it is unlikely that colonization of this material has been possible for more than 10 years. The area was irregularly colonized by a very limited community of which Tussilago farfara was the dominant plant and which at times occurred in pure stands. This was accompanied by the mosses Bryum argenteum and B. bicolor.

3.42 Shale community

The shale was probably brought to the surface during the 1920's and 30's. It is known that it was subject to extensive re-grading in the late 1940's and therefore the surface has been available for colonization for approximately 35 years. Shale is very heterogenous and this shows in the pattern of vegetation. Some parts of the tip have 100% cover while others are totally barren. The north and east faces of the tip have the more dense vegetation. This is a common observation on sites such as this and is usually ascribed to lower temperatures and higher moisture during the critical mid-summer period at such locations. Most areas of the shale at the tip had approximately 30% cover. The species list (Table 3.6, p89) was compiled during the summers of 1978 and1979.

TABLE 3.6 Species common in shale community at East Holywell

Acer pseudoplatanus L.
Agrostis tenuis Sibth.
Arrhenatherum elatius (L.) Beauv.exJ & C.Presl.
Capsella bursa-pastoris (L.) Medic.
Centaurea nigra L.
Chamaenerion angustifolium (L.) Scop.
Cirsium arvense (L.) Scop.
Crataegus monogyna Jacq.
Dactylis glomerata L.
Dactylorchis incarnata (L.) Vermeul.ssp.pulchella H.-Harr.f.
Deschampsia flexuosa (L.) Trin.
Festuca ovina L.
Festuca rubra L.
Fumaria officinalis L.
Heracleum sphondylium L.
Hieracium perpropinquum L.
Hieracium vulgatum L.
Lamium purpureum L.
Linaria vulgaris Mill.
Matricaria matricarioides (Less.) Porter
Papaver rhoeas L.
Plantago lanceolata L.
Ranunculus acris L.
Reseda luteola L.
Reseda lutea L.
Rubus fruticosus agg.
Rumex acetosa L.
Rumex crispus L.

Sambucus nigra L.
Senecio jacobaea L.
Senecio vulgaris L.
Sisymbrium officinale (L.) Scop.
Sonchus asper (L.) Hill
Sorbus aria agg.
Stellaria media (L.) Vill.
Taraxacum officinale Weber,sensu lato
Trifolium medium L.
Tripleurospermum maritimum (L.)Koch ssp.inodorum (L.)Hyl.ex. Vaarama
Tussilago farfara L.
Ulex europaeus L.
Urtica dioca L.
Veronica persica Poir.

Mosses

Bryum bicolor Dicks.
Bryum caespiticium Hedw.
Barbula convoluta Hedw.
Barbula fallax Hedw.
Campylopus paradoxus Wils.
Campylopus pyriformis Schultz
Ceratodon purpureus Hedw.
Eurhynchium praelongum Hedw.
Pohlia nutans Hedw.
Polytrichum piliferum Hedw.
Rhynchostegium confertum Dicks.

Lichens

Cladonia sp.

CHAPTER 4

RESULTS

4.1 ESTIMATES OF ABUNDANCE OF SOIL ALGAE

4.11 The effect of storage of spoil samples upon estimated density of soil algae

It was necessary to store the spoil samples for an average of five months before culturing (Section 2.33, p59). In order to establish the effect of this treatment upon the samples, those collected in January 1980 were cultured two weeks after collection and again after five months storage. The estimated density of algae in these samples after two weeks is shown in Table 4.1 and after five months storage in Table 4.2. Algae were isolated from all the spoil samples after two weeks storage, however few cells were present in spoil from sites Y2, P1, B1 and B2. After five months storage, samples from all sites showed a reduction in the density of algae. Samples from most sites showed a reduction of approximately 50% but those from site Y1 were more severely affected and showed a reduction of 71.5%. The percentage reduction in the mean number of algae at each site is shown in Table 4.3.

TABLE 4.3 Reduction in the mean density of algae for each site after five months storage

Site	% reduction in mean density of algae
G1	49.05%
G2	59.0%
Y1	71.5%
Y2	50.0%
P1	100.0%
B1	100.0%
B2	62.5%

TABLE 4.1 Estimated density of algae in January samples after 2 weeks storage (cells g^{-1} x 10^3)

Site	G1	G2	Y1	Y2	P1	B1	B2
Replicate /dilution							
1/0.5	276	56	-	-	-	-	-
2/0.5	114	118	-	-	-	-	-
3/0.5	128	78	-	-	-	-	-
4/0.5	128	84	-	-	-	-	-
1/1	102	90	199	-	-	-	-
2/1	82	69	174	-	-	-	-
3/1	100	40	145	-	-	-	-
4/1	119	38	152	-	-	-	-
1/5	-	-	-	-	-	-	-
2/5	-	-	-	-	-	-	-
3/5	-	-	-	-	-	-	-
4/5	-	-	-	-	-	-	-
1/10	-	-	-	0.5	0.2	0.6	0.7
2/10	-	-	-	1.4	0.1	0.1	0.2
3/10	-	-	-	1.2	0.0	0.0	0.4
4/10	-	-	-	1.3	0.0	0.0	0.3
Mean	131.1	76.5	167.5	1.1	0.075	0.175	0.4
	± 60.56	± 24.9	± 24.4	± 0.41	±0.095	± 0.20	± 0.2

TABLE 4.2 Estimated density of algae in January samples after 5 months storage (cells g^{-1} x 10^{-3})

Site	G1	G2	Y1	Y2	P1	B1	B2
Replicate /dilution							
1/0.5	74	22	-	-	-	-	-
2/0.5	66	40	-	-	-	-	-
3/0.5	64	24	-	-	-	-	-
4/0.5	78	54	-	-	-	-	-
1/1	61	27	48	-	-	-	-
2/1	65	30	54	-	-	-	-
3/1	49	34	47	-	-	-	-
4/1	71	20	57	-	-	-	-
1/5	-	-	48	-	-	-	-
2/5	-	-	38.6	-	-	-	-
3/5	-	-	39	-	-	-	-
4/5	-	-	51.6	-	-	-	-
1/10	-	-	-	1.0	0.0	0.0	0.3
2/10	-	-	-	0.1	0.0	0.0	0.2
3/10	-	-	-	0.9	0.0	0.0	0.0
4/10	-	-	-	0.2	0.0	0.0	0.1
Mean	66.8	31.4	47.9	0.55	0.0	0.0	0.15
	± 8.9	± 11.3	± 0.6	± 0.17	-	-	± 0.13

4.12 Monthly estimate of algal density

The results for each culture are presented and are identified by a code which gives the site, the number of the replicate and the dilution of spoil used; e.g. G1/1/0.5 identifies a culture of spoil from site G1 which was the first replicate and in which spoil was spread on the plate in a suspension that contained 0.5 g l^{-1} of spoil. The results of different dilutions are presented together with each density expressed as the number of viable algal units $g^{-1} \times 10^3$.

The results obtained for site G1 are presented in Table A1.1. Fig. 4.1 shows the mean monthly totals plotted with 95% confidence limits. These were computed from the data as

$$\bar{x} \pm t \times S/\sqrt{N}$$

The results for December 1979 contain only four replicate estimates, obtained at dilution 0.5 g l^{-1} because cultures prepared at a dilution of 1.0 g l^{-1} contained $>$ 500 colonies per plate and were judged too dense to count accurately.
The smallest population was recorded in March 1979 when there were $14.5 \pm 3.9 \times 10^3$ algae g^{-1}.
The largest population was in December 1979 when there were $183.5 \pm 25.1 \times 10^3$ algae g^{-1}. Fluctuations in the numbers occurred with peaks in the algal population in June 1979, September 1979 and December 1979.

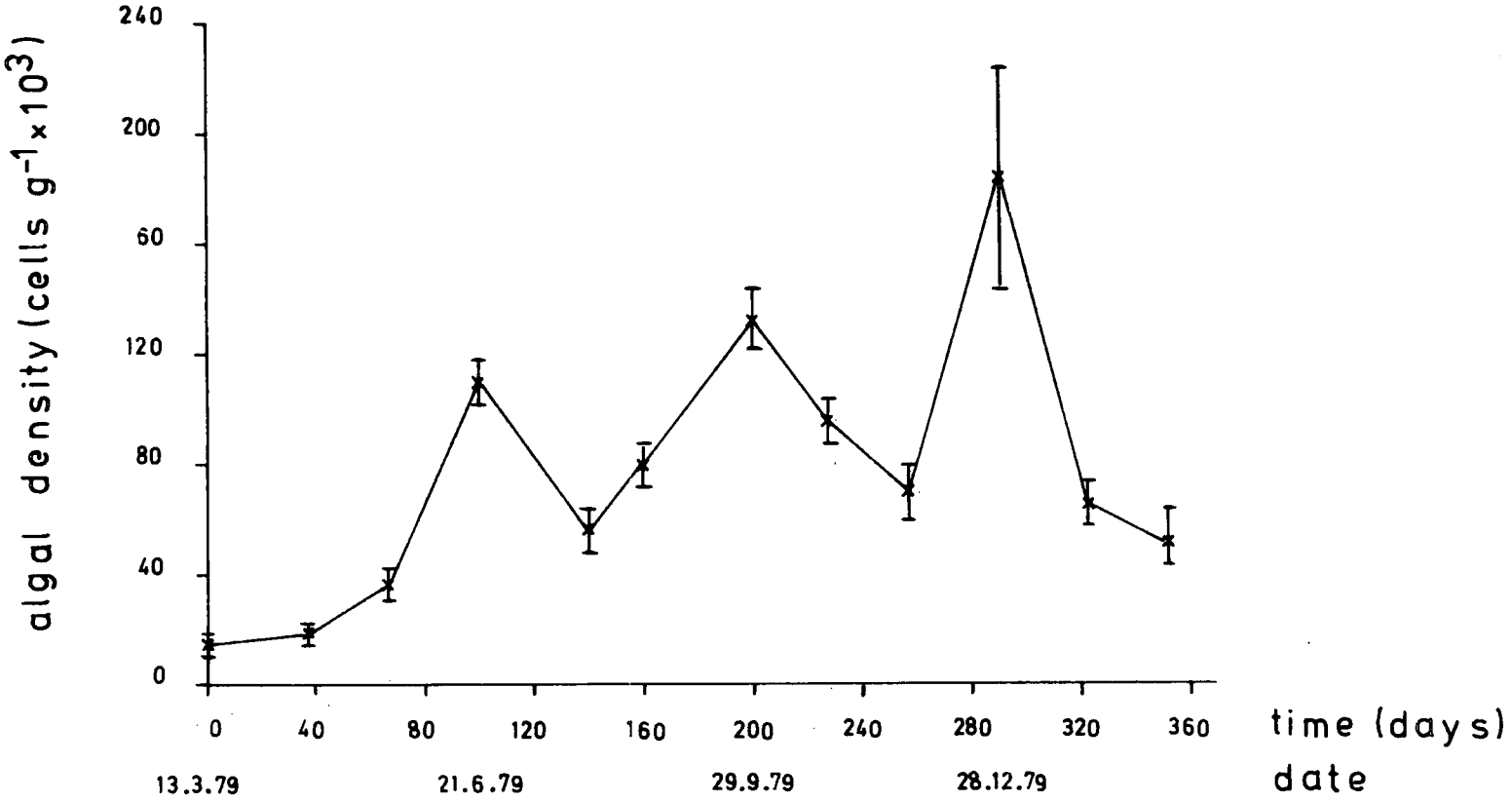


FIG. 4.1 Monthly variation in algal density at site G1

Blue-green algae were a large and important component of the soil flora at this site and were recorded separately. The results are presented in Section 4.14. The soil flora at other sites was almost exclusively composed of Chlorophyta with occasional diatom colonies. In order to obtain comparable results for this site Table A1.2 was compiled which shows the estimated monthly totals of algae other than blue-green algae. These results are displayed, plotted with 95% confidence limits in Fig. 4.2.

The results for site G2 are presented in Table A1.3 and the monthly mean number of algae, plotted with 95% confidence limits are displayed in Fig. 4.3. The range of values recorded at this site was much narrower than at G1. The lowest monthly mean was in January 1980 and was $31.4 \pm 11.3 \times 10^3$ algae g^{-1} and the highest monthly mean, in March 1979 was $80.0 \pm 13.9 \times 10^3$ algae g^{-1}. In general there was little fluctuation from month to month at this site but there was a small steady increase in numbers during the spring and summer and an equivalent decrease in numbers in the autumn and winter. No results are available for September 1979 at this site because the quadrat was severely disturbed and it was thought that it would have to be abandoned. However on visiting the site in October 1979 no further disturbance had occurred and recording was recommenced.

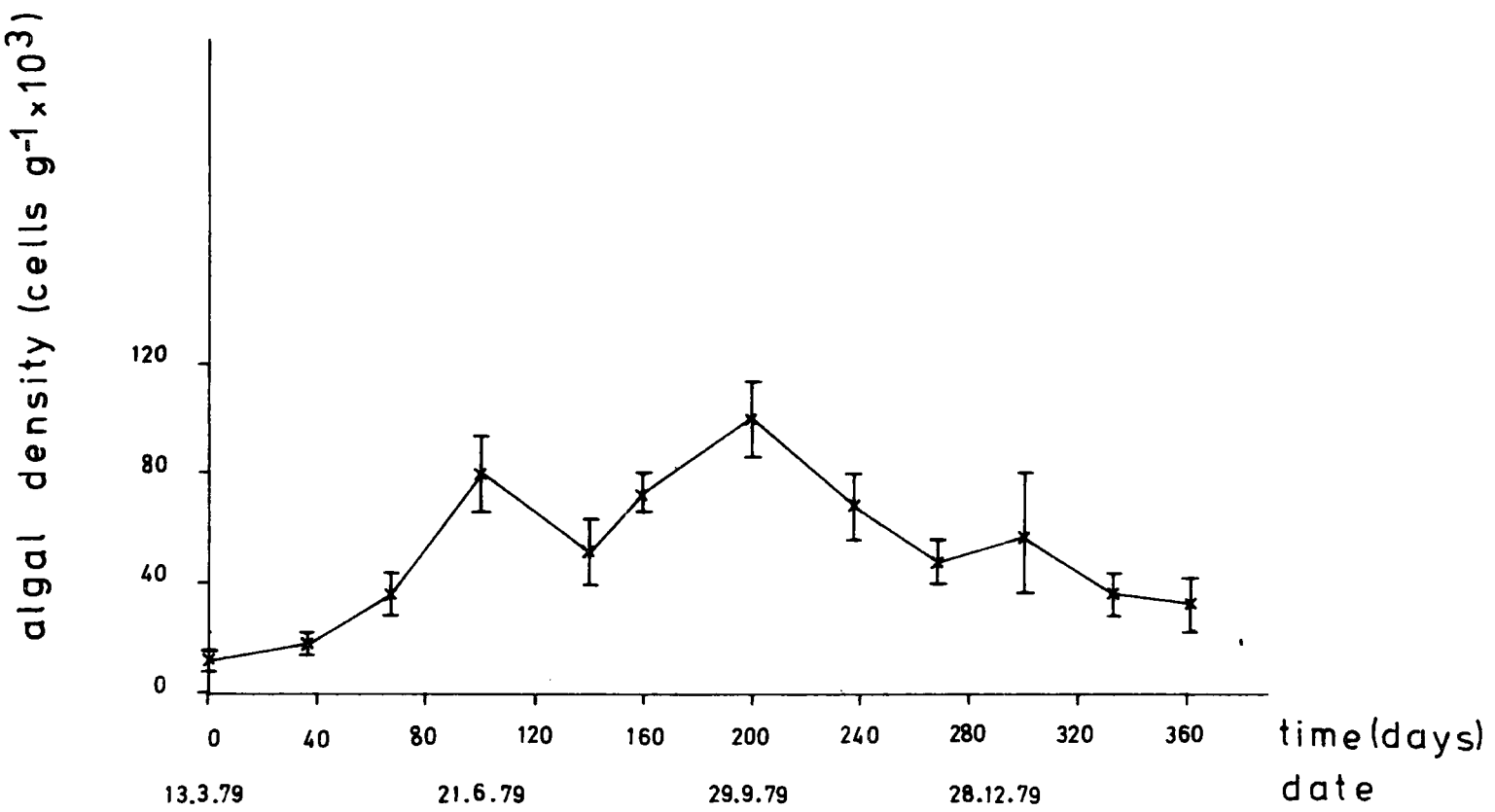


FIG. 4.2 Monthly variation in density of algae other than blue-greens at site G1

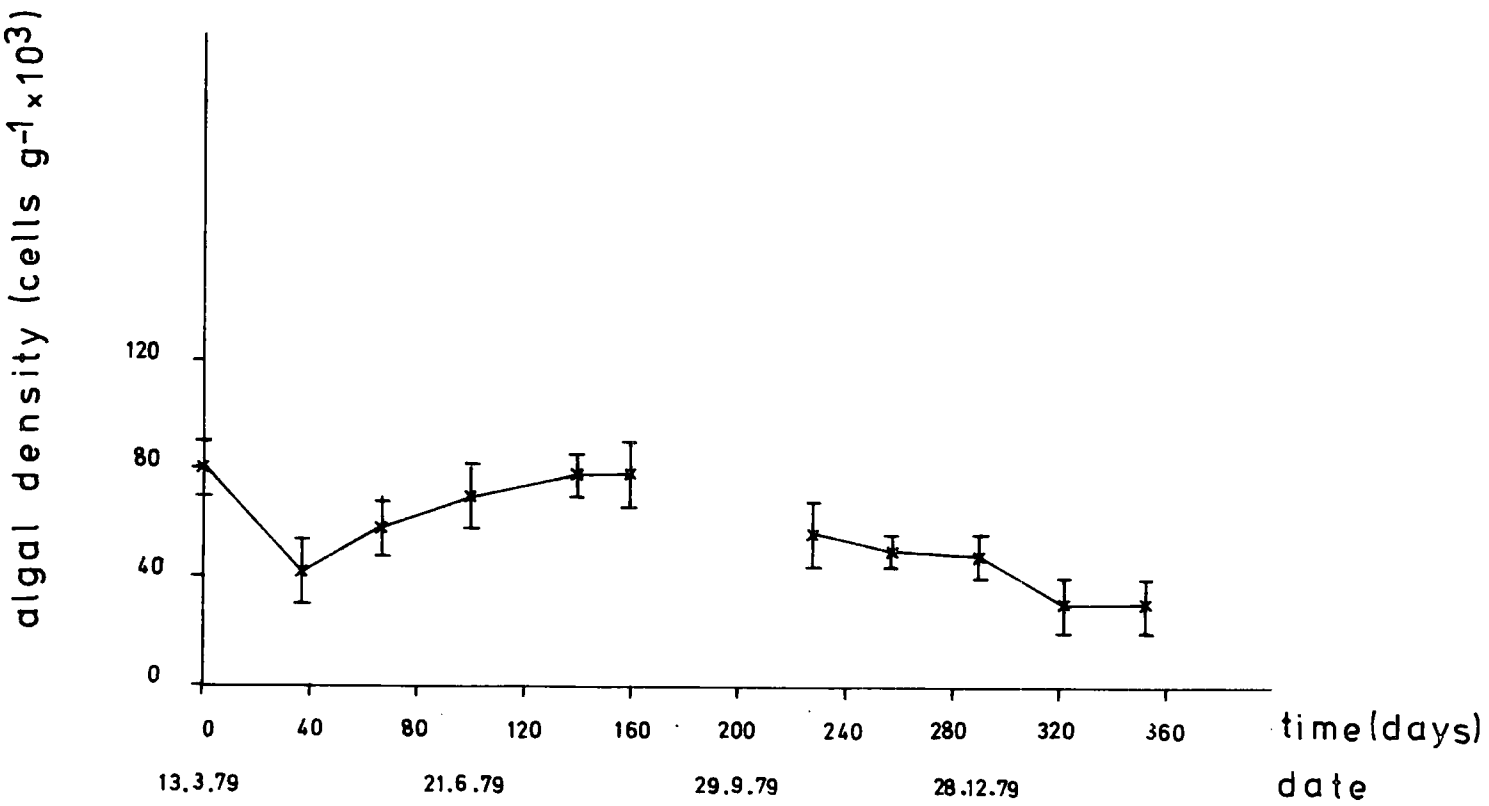


FIG. 4.3 Monthly variation in algal density at site G2

The results for site Y1 are presented in Table A1.3 and the monthly mean number of algae, plotted with 95% confidence limits, are displayed in Fig. 4.4. Only four replicate estimates are available for September 1979 because cultures prepared at the lower dilution were judged too dense to count accurately, containing as they did more than 500 colonies per plate.

This site showed the widest range of values of any site. The smallest population was recorded in March 1979 when there were $1.4 \pm 0.8 \times 10^3$ algae g^{-1} of spoil and the largest population was recorded in September 1979 when there were $164 \pm 18.2 \times 10^3$ algae g^{-1} of spoil. For much of the year the population was around 40 to 50 $\times 10^3$ algae g^{-1} with two distinct peaks in September 1979 and December 1979.

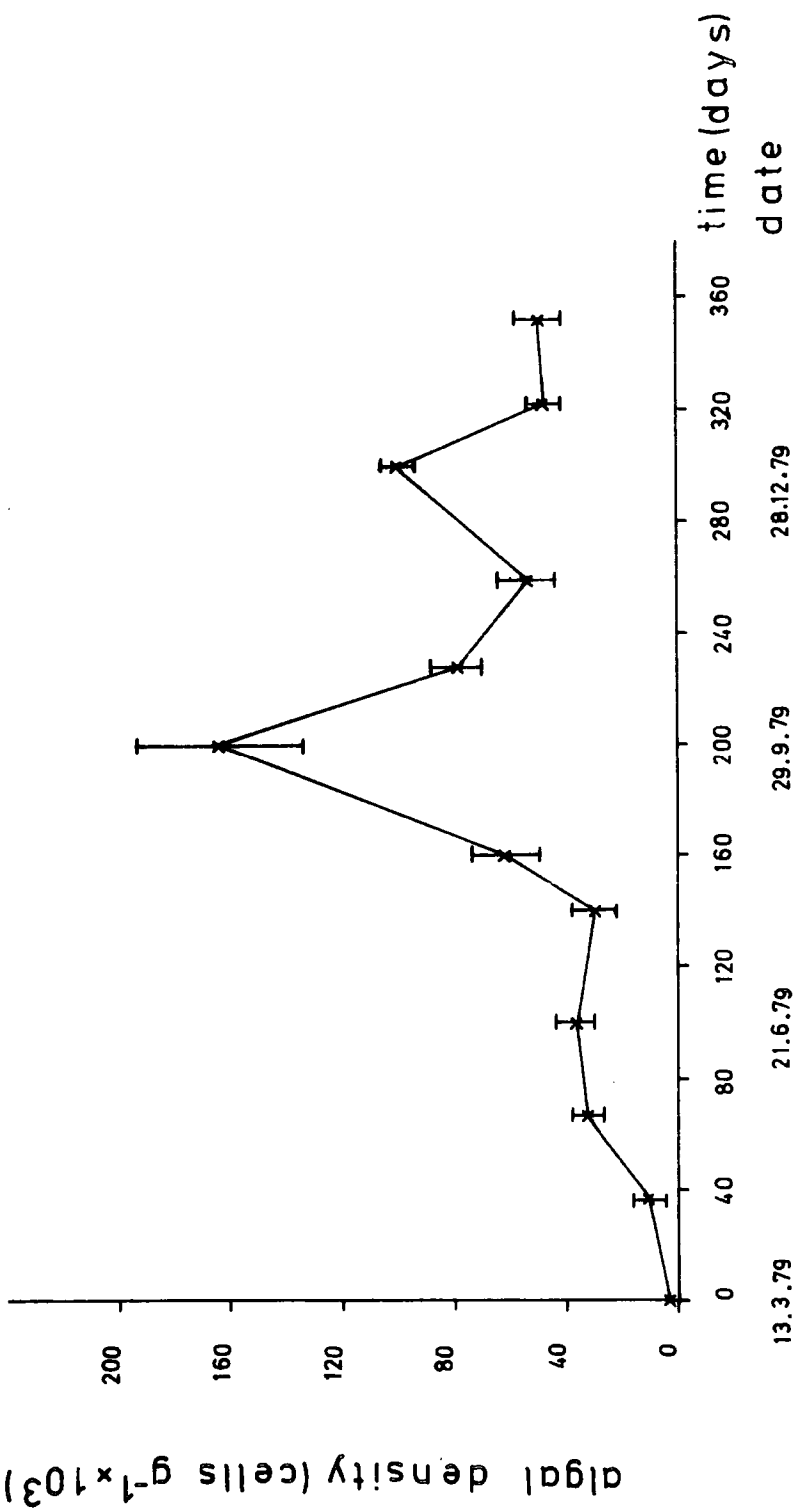


FIG. 4.4 Monthly variation in algal density at site Y1

The results for sites Y2, P1, P2, B1 and B2 are presented in Tables A1.5, A1.6, A1.7, A1.8 and A1.9 respectively. The incidence of algae in the spoil at each of these sites was considerably lower than at the preceeding sites and it was decided to adopt a minimum acceptable level of detection of five colonies per plate at any dilution. Below this level it was felt that isolated cells would have a disproportionate effect upon the population estimate. Suspensions with a concentration of more than 10 g l^{-1} were not practical because the density of spoil particles at such high concentrations made the detection of algal colonies impossible. On many occassions the spoil from these sites contained no algae at all.

Spoil from site Y2 contained some algae in every month except March 1979, but in June 79, Oct 79 and Dec 79 the numbers were so small as to be below the minimum acceptable level of detection, even when using a dilution of 10 g l^{-1}. Most algae were detected in the sample for September 1979 but even then the population only reached $1.43 \pm 0.5 \times 10^{3}$ algae g^{-1}.

Spoil from site P1 contained even fewer algae than Y2. No algae at all were isolated from the samples for March 79, October 79, November 79, December 79, January 80 or February 80. Between April 79 and September 79 algae were present but always below the minimum acceptable detection level.

Spoil from site P2 contained more algae than P1 and the figures indicate a population level similar to that at Y2. Most algae were present in August 1979 when the population reached $1.5 \pm 0.5 \times 10^3$ algae g^{-1}. No results were obtained for this site from October 79 to February 80 because the permanent quadrat was subjected to severe and continuous disturbance by motor cyclists.

Spoil from sites B1 and B2 contained such a low level of algae that the figures were below the minimum acceptable level of detection on all but one month of the year. In September 1979 the population at B1 reached $0.93 \pm 0.2 \times 10^3$ algae g^{-1} and at B2 it reached $0.55 \pm 0.19 \times 10^3$ algae g^{-1}.

The mean monthly totals of viable algal units g^{-1} of spoil at each site are presented in summary in Table 4.4.

TABLE 4.4 Summary of the mean monthly algal density at each site (cells g^{-1} x 10^3)

Month / Site	March 79	April 79	May 79	June 79	July 79	Aug 79	Sept 79	Oct 79	Nov 79	Dec 79	Jan 80	Feb 80
G1	14.5 ± 3.9	18.4 ± 3.8	35.75 ± 8.6	110.6 ± 9.6	55.5 ± 11.0	81.1 ± 9.4	132.5 ± 12.6	94.6 ± 13.2	71.25 ± 8.7	183.5 ± 25.1	66.8 ± 8.9	54.5 ± 13.5
G2	80.0 ± 13.9	41.75 ± 14.75	58.1 ± 10.4	69.9 ± 15.7	78.1 ± 8.1	77.6 ± 15.6	-	55.9 ± 12.3	49.5 ± 5.8	47.6 ± 8.7	31.4 ± 11.3	40.0 ± 9.8
Y1	1.4 ± 0.8	10.5 ± 4.8	32.0 ± 4.4	36.9 ± 8.25	29.9 ± 7.4	63.2 ± 14.5	164.0 ± 18.2	77.5 ±8.1	53.4 ±9.9	101.5 ±6.1	47.9 ± 6.6	49.5 ± 8.8
Y2	0.0 ± 0.0	0.95 ± 0.33	0.53 ±0.28	0.18 ± 0.13	0.28 ± 0.09	0.88 ± 0.29	1.43 ± 0.5	0.8 ± 0.57	0.13 ± 0.09	0.3 ± 0.14	0.55 ± 0.47	0.7 ± 0.45
P1	0.0 -	0.075 ± 0.05	0.125 ±0.09	0.075 ±0.09	0.225 ±0.17	0.15 ± 0.13	0.100 ±0.08	0.0 -	0.0 -	0.0 -	0.0 -	0.0 -
P2	0.0 -	0.73 ± 0.61	0.5 ± 0.32	0.05 ± 0.06	0.6 ± 0.20	1.5 ± 0.50	0.48 ± 0.13	- -	- -	- -	- -	- -
B1	0.0 -	0.0 -	0.25 ± 0.17	0.08 ± 0.1	0.0 -	0.28 ± 0.22	0.93 ±0.20	0.0 -	0.0 -	0.0 -	0.0 -	0.1 ±0.0
B2	0.0 -	0.0 -	0.23 ± 0.22	0.23 ± 0.32	0.15 ± 0.1	0.35 ± 0.34	0.55 ± 0.19	0.0 -	0.08 ± 0.09	0.0 -	0.15 ± 0.13	0.08 ± 0.075

4.13 Occurrence and abundance of soil Ulotrichales in spoil samples

Colonies of soil Ulotrichales were recognisable on the agar plates by their characteristic appearance. The outline of the colonies was irregular and their surface was thrown into radiating, wavy folds. These features were strongly marked in members of the filamentous genera but were also clearly detectable in unicellular forms such as Stichococcus. It was therefore possible to count the number of Ulotrichalean colonies at each site each month.

The Ulotrichales formed an important part of the soil flora. They were regularly detected at sites G1, G2 and Y1 and were isolated from sites B1 and B2 in September 1979. At site G2 some of these algae were isolated every month except March 1979. However, the numbers were always below the minimum acceptable level of detection at the dilutions used at this site. There was no marked seasonal fluctuation in the numbers although there were rather more present in July 1979.

At site G1 these algae were much more abundant and were detected every month except March and April 1979. The numbers increased in the spring with a peak in June 1979. This was followed by a decline in July followed by an annual maximum of $8.0 \pm 3.2 \times 10^3$ algae g^{-1} in September 1979. There was then a steady decline in numbers during the autumn and winter. The results for this site are presented in Table A1.10 and are displayed, plotted with 95% confidence limits in Fig. 4.5.

At site Y1, Ulotrichalean colonies were recorded in every month except March 1979. The population was generally at a low level of around 1.0×10^3 algae g^{-1} of spoil. This population level was similar to that at G2, however at the dilutions used for samples from this

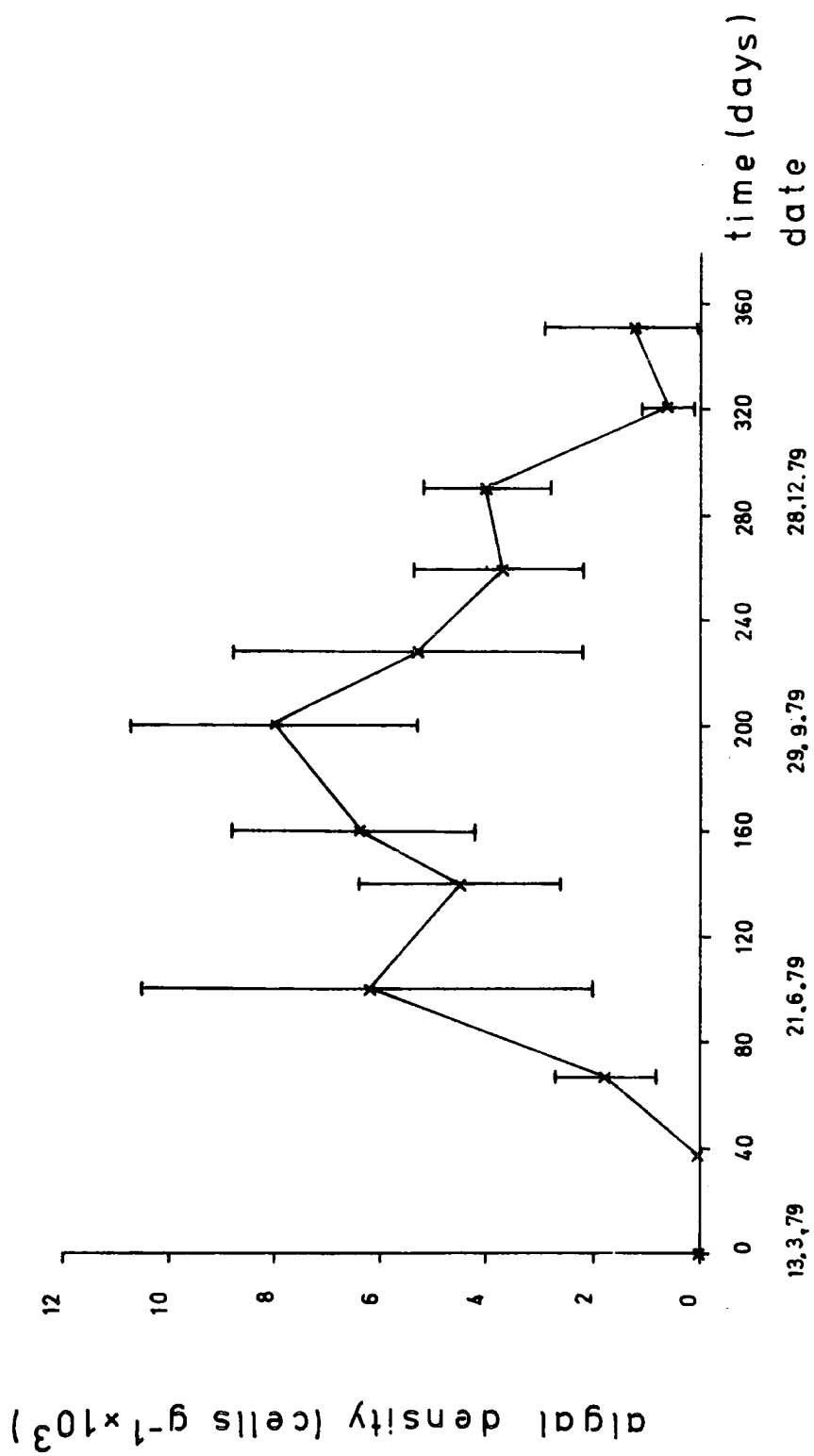


FIG. 4.5 Monthly variation in density of Ulotrichales at site G1

site, sufficient algae were isolated to get a reasonably reliable estimate of the population. There were two distinct peaks in the population in June 1979 and in August 1979, when it reached $3.63 \pm 1.52 \times 10^3$ algae g^{-1} and $6.05 \pm 1.3 \times 10^3$ algae g^{-1} respectively. The results from this site are presented in Table A1.11 and displayed, plotted with 95% confidence limits, in Fig. 4.6.

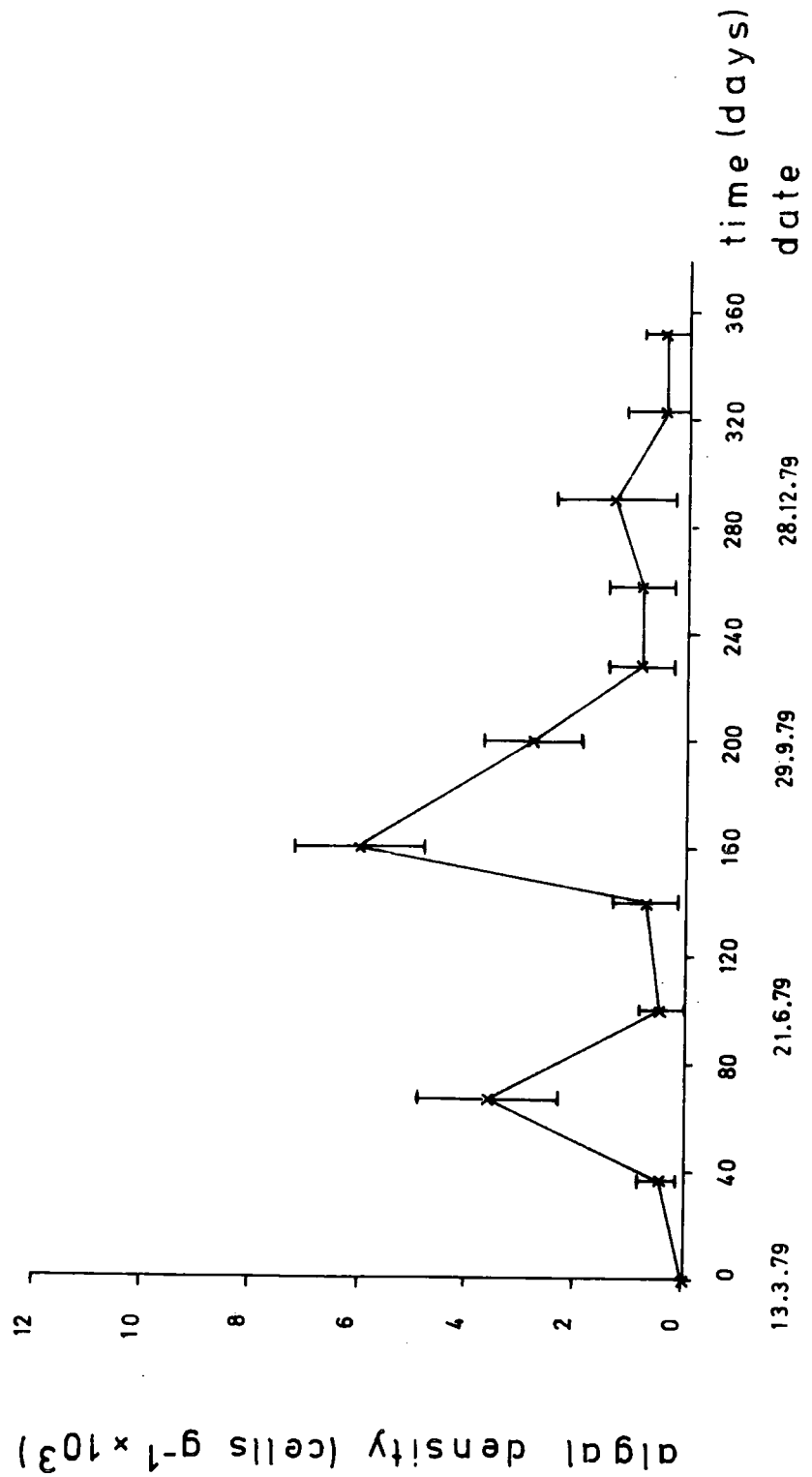


FIG. 4.6 Monthly variation in density of Ulotrichales at site Y1

4.14 Occurrence and abundance of blue-green algae in the spoil samples

Blue-green algae could be easily recognised on the surface of the agar plates by their characteristic colour and could therefore be counted separately. Isolated specimens were detected at site G2 in January 80, December 79, November 79 and May 79; at Y1, Y2, B1 and B2 in September 79 and at B2 in January 80 and B1 in March 79. However at site G1 blue-green algae were a large and important component in the soil flora. Numbers fluctuated widely from month to month, with few or no blue-greens being detected in March, April and May 1979 but dramatic peaks in the population being recorded in June 79, September 79 and December 79. The largest population recorded was in December 1979 when there were $107.5 \pm 19.55 \times 10^3$ blue-green algal units g^{-1} of spoil. The estimated numbers of blue-green algae present in the spoil each month are presented in Table A1.12 and displayed, plotted with 95% confidence limits, in Fig.4.7.

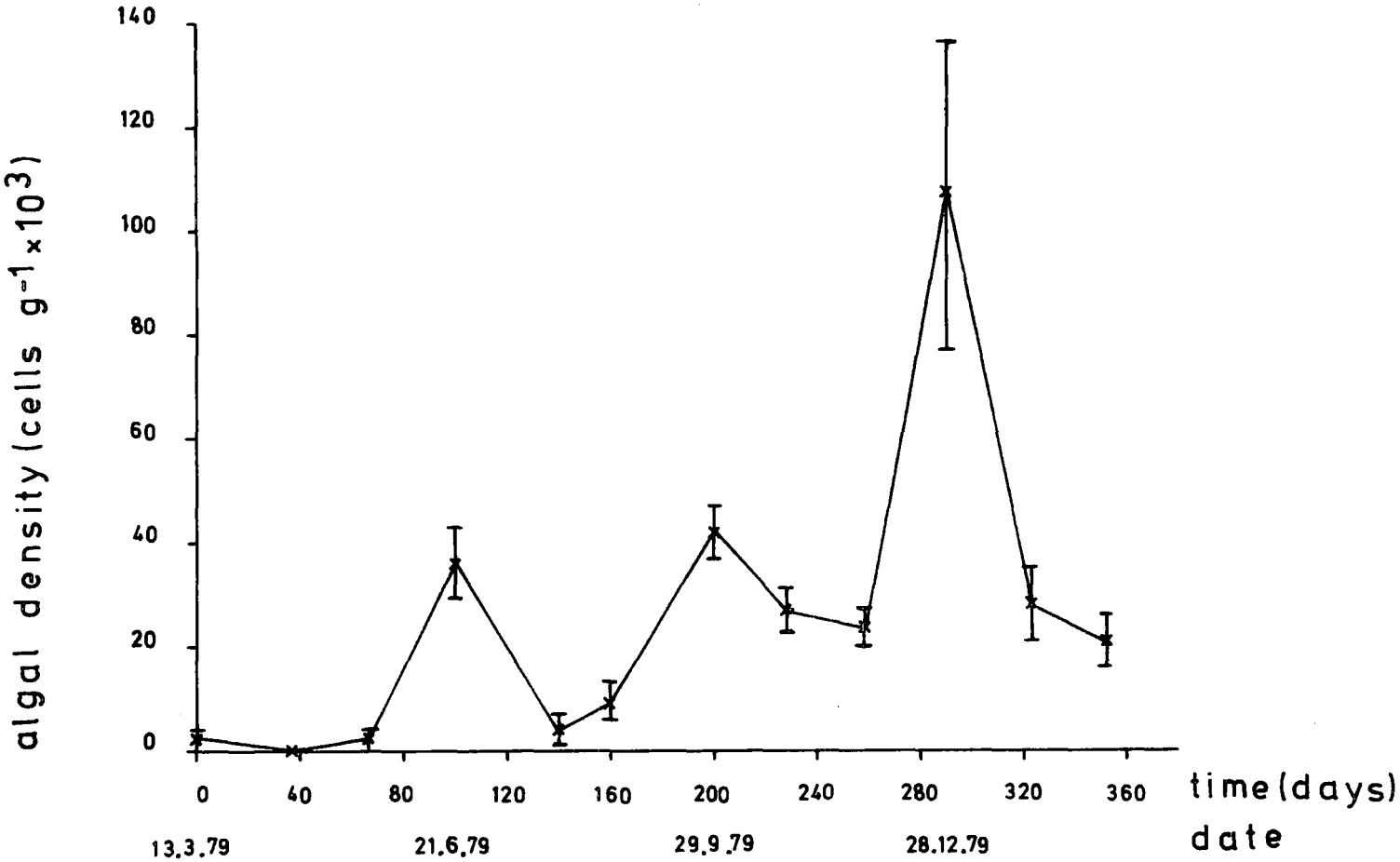


FIG. 4.7 Monthly variation in density of blue-green algae at site G1

4.2 SOIL ALGAE IDENTIFIED FROM SPOIL AT EAST HOLYWELL

In Table 4.5 is presented a list of all the soil algae isolated and identified from spoil at East Holywell. The algae isolated from sample sites on washeries waste are listed site by site in Table 4.6 and in Table 4.7 the algae isolated from shale sites are listed in a similar fashion.

Algae of 29 different species were identified from the various sites. These include representatives of 24 genera. The majority of species (22) were Chlorophyta with just three species of Cyanophyta and two species of Bacillariophyta. This flora was most diverse at sites Y1, G1 and G2 and was markedly reduced in diversity at sites Y2, P1, P2, B1 and B2 (Table 4.8, p114). Several species were identified from a range of sites but only Chlorella sp. 2 was identified from every site (Table 4.9, p115).

Several species were found equally upon shale and washeries waste while some species were restricted to one or other of these spoil types (Table 4.10, p116).

TABLE 4.5 Soil algae isolated from colliery spoil at East Holywell

Chlorophyta

Bracteacoccus sp.

Chlorococcum sp.

Chlorella sp. 1

Chlorella sp. 2

Chlorella sp. 3

Coccomyxa sp.

Lobococcus incisa Reisigl

Myrmecia sp.

Muriella terrestris B. Petersen

Pseudochlorella pyrenoidosa Lund

Spongiochloris sp.

Tetracystis sp.

Ulotrichales

Hormidium crenulatum Klebs

Hormidium flaccidum A. Braun

Hormidium sterile Deason et Bold

Stichococcus bacillaris Nageli

Stichococcus minor Nageli

Chaetophorales

Chlorosarcina sp.

Chlorosarcinopsis minor Herndon

Jaagiella alpicola Vischer

Chaetophorales R4

Chaetophorales R10

Chaetophorales R20

Chaetophorales R22

Cyanophyta

Anabaena sp.

Lyngbya sp.

Oscillatoria sp.

Bacillariophyta

Navicula sp.

Pinnularia sp.

TABLE 4.6 Soil algae identified at sites on washeries waste

SPECIES	SITES			
	G1	G2	B1	B2
CHLOROPHYTA				
Bracteacoccus sp.	X	X		
Chlorococcum sp.	X	X		
Chlorella sp. 1	X	X		
Chlorella sp. 2	X	X	X	X
Chlorella sp. 3	X	X		
Coccomyxa sp.	X	X		
Lobococcus incisa				
Myrmecia sp.	X			
Muriella terrestris	X	X		
Pseudochlorella pyrenoidosa				
Spongiochloris sp.				
Tetracystis sp.	X	X		
Hormidium crenulatum	X			
Hormidium flaccidum	X			
Hormidium sterile				
Stchococcus bacillaris	X	X		
Stichococcus minor	X			
Chlorosarcina sp.	X	X		
Chlorosarcinopsis minor				
Jaagiella alpicola	X	X		
Chaetophorales R4				
Chaetophorales R10				
Chaetophorales R20	X			
Chaetophorales R22		X		
CYANOPHYTA				
Anabaena sp.				X
Lyngbya sp.	X	X	X	X
Oscillatoria sp.	X			
BACILLARIOPHYTA				
Navicula sp.	X			X
Pinnularia sp.				

X indicates presence at a site

TABLE 4.7 Soil algae identified at sites on shale

SPECIES	SITES			
	Y1	Y2	P1	P2
CHLOROPHYTA				
Bracteacoccus sp.				
Chlorococcum sp.	X			
Chlorella sp. 1	X			
Chlorella sp. 2	X	X	X	X
Chlorella sp. 3	X	X		
Coccomyxa sp.	X	X		
Lobococcus incisa	X			
Myrmecia sp.				
Muriella terrestris				
Pseudochlorella pyrenoidosa	X	X		
Spongiochloris sp.	X			
Tetracystis sp.	X			
Hormidium crenulatum	X			
Hormidium flaccidum	X	X	X	
Hormidium sterile	X			
Stichococcus bacillaris	X			
Stichococcus minor				
Chlorosarcina sp.	X			
Chlorosarcinopsis minor	X			
Jaagiella alpicola				
Chaetophorales R4	X			
Chaetophorales R10	X			
Chaetophorales R20				
Chaetophorales R22				
CYANOPHYTA				
Anabaena sp.				
Lyngbya sp.				
Oscillatoria sp.				
BACILLARIOPHYTA				
Navicula sp.	X			
Pinnularia sp.	X			

X indicates presence at a site

TABLE 4.8 Number of species recorded at each site

Site	number of species recorded
G1	19
G2	12
B1	2
B2	4
Y1	19
Y2	5
P1	2
P2	1

TABLE 4.9 Frequency of occurrence of algal species

Identified at all sites:

Chlorella sp. 2

Identified at four sites:

Lyngbya sp.

Chlorella sp. 3

Coccomyxa sp.

Hormidium flaccidum

Identified at three sites:

Chlorococcum sp.

Chlorella sp. 1

Tetracystis sp.

Stichococcus bacillaris

Chlorosarcina sp.

Identified at two sites:

Bracteacoccus sp.

Muriella terrestris

Pseudochlorella pyrenoidosa

Jaagiella alpicola

Navicula sp.

TABLE 4.10 Algae from sites with different spoil types

Species present on shale and washeries waste	Species present only on shale	Species present only on washeries waste
Chlorella sp. 1	Lobococcus incisa	Bracteococcus sp.
Chlorella sp. 2	Pseudochorella pyrenoidosa	Myrmecia sp.
Chlorella sp. 3	Spongiochloris sp.	Muriella terrestris
Chlorococcum sp.	Hormidium sterile	Stichococcus minor
Coccomyxa sp.	Chlorosarcinopsis minor	Jaagiella alpicola
Tetracystis sp.	Chaetophorales R4	Chaetophorales R20
Hormidium flaccidum	Chaetophorales R10	Chaetophorales R22
Stichococcus bacillaris	Pinnularia sp.	Anabaena sp.
Chlorosarcina sp.		Lyngbya sp.
Navicula sp.		Oscillatoria sp.
Hormidium crenulatum		

CHAPTER 5

RESULTS - TAXONOMY

5.1 DESCRIPTIONS OF ALGAE ISOLATED FROM COLLIERY SPOIL

Algae were isolated from the dilution plates as described in Section 2.7. The following descriptions are based upon systematic observations of these unialgal cultures over periods of up to two months. Identifications were based on Bourrelly (1966), Bold & Wynne (1978) and Pickett-Heaps (1975).

5.11 Ulotrichales

Filamentous green algae of the order Ulotrichales were often isolated from sites G1, G2 and Y1 and occasionally from all other sites. The material examined in the dilution cultures was very variable and its characteristic features were often obscured by the accumulation of storage granules. This latter complication was due to the long period of incubation i.e. one month, which meant that almost all filaments were old, as most of these species grow very quickly in culture and are quite capable of making significant growth within one week. A number of isolates were made (Section 2.8) and observed systematically. They were ascribed to the following species using standard references (Bourrelly, 1966; Komarony, 1976).

Hormidium crenulatum

This species was isolated from both the red shale area of the tip and the washeries waste. The isolate examined in culture was from site G1. The filaments were 15 to 18 µm in diameter and each cell was ½ x to 1 x as long. The chloroplast was an incomplete band encircling approximately two thirds of the cell with a single pyrenoid which was normally quite distinct. The cell walls are rather thicker than in some species of Hormidium and the whole filament is covered by a thin mucilaginous sheath (Figs 5.1 & 5.2). In field material the cell wall was considerably thicker as was the mucilaginous sheath. In older filaments the cells were in groups of 15 to 20 which although still connected into a continuous filament were separated from each other by apparently empty cells with thickened walls. These seemed to be points of flexibility in the filament but were no weaker than any other point as was shown by the fact that breaks in the filament occurred quite independently of these points (Fig. 5.3). In some regions of the older filaments longitudinal divisions occurred producing short regions of biseriate filaments (Fig. 5.4) which gave rise to loop like structures (Fig. 5.5) as described by Fritsch and John (1942).

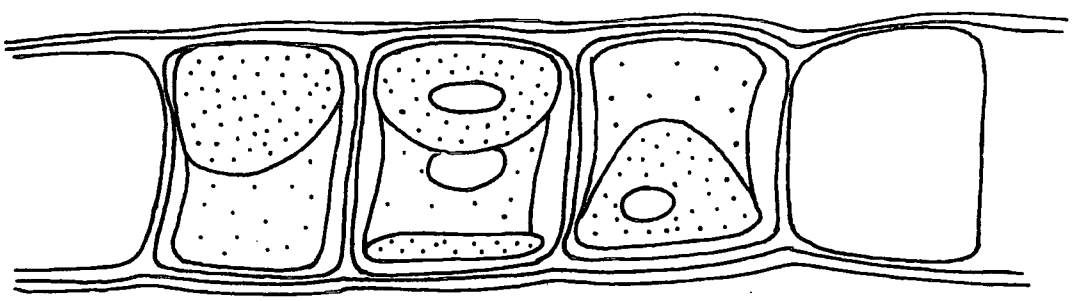
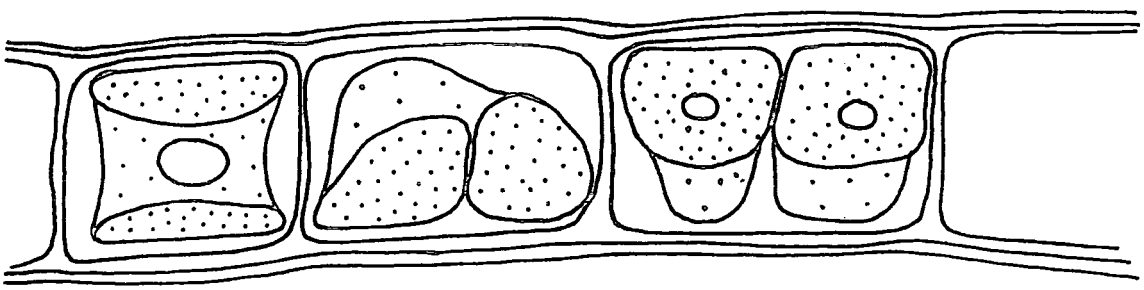


FIG 5.1 Hormidium crenulatum showing identation of cross walls



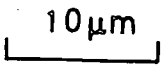


FIG 5.2 Hormidium crenulatum cells dividing

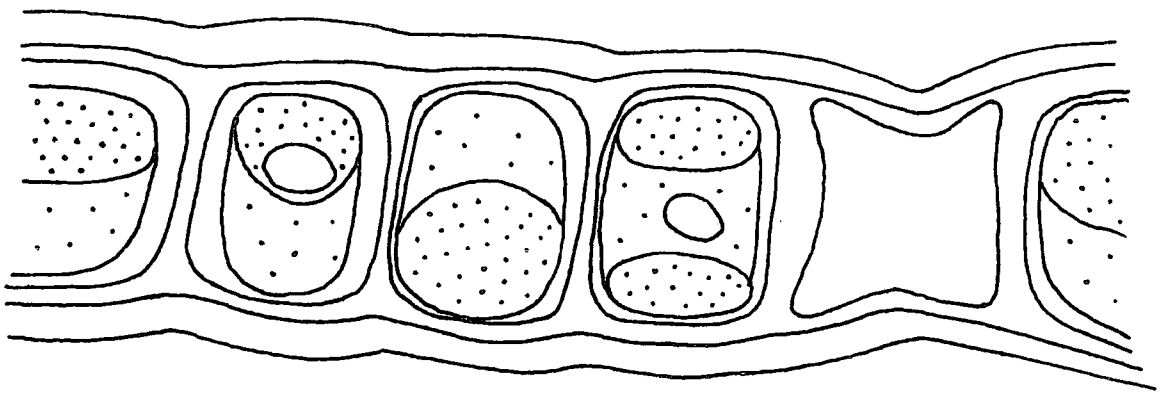
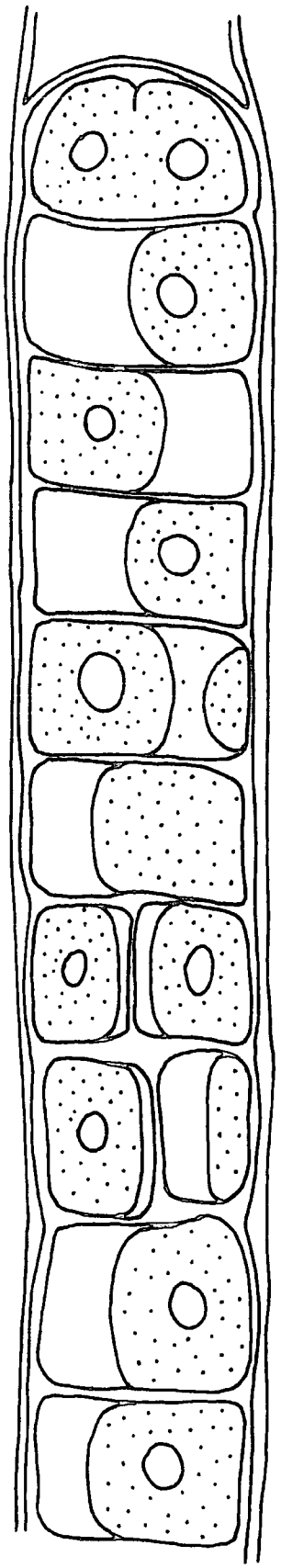


FIG 5.3 Hormidium crenulatum field material



10µm

FIG 5.4 Hormidium crenulatum filament partly biseriate from 1 week old culture

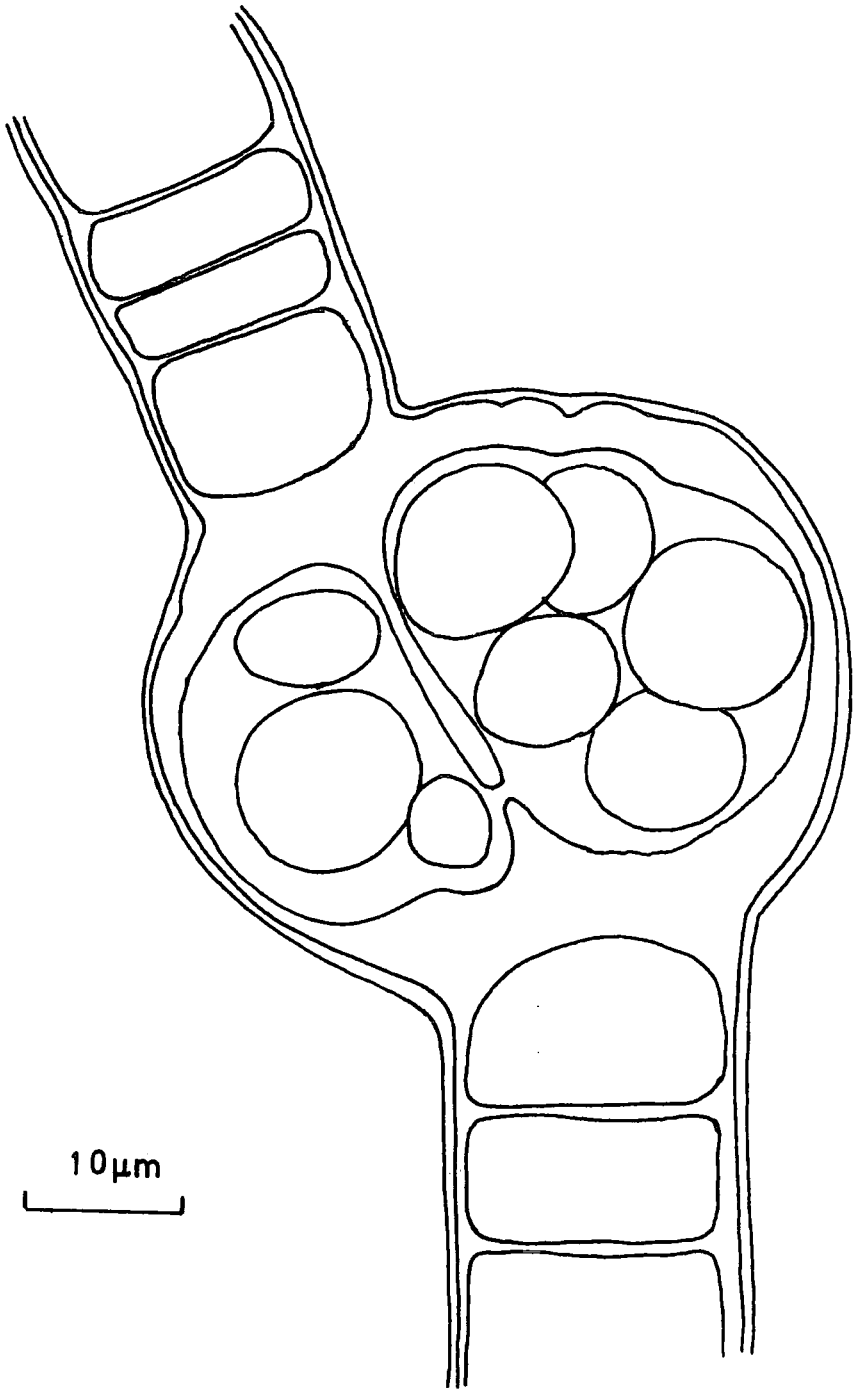


FIG 5.5 Hormidium crenulatum showing loop structure

Hormidium flaccidum

Seventeen of the algae isolated could be ascribed to this species. They consisted of simple filaments varying in diameter from 5 µm to 12 µm which could be divided in to three size categories

a. 5 - 7 µm - 12 examples Fig. 5.6
b. 7 - 9 µm - 3 examples Fig. 5.7
c. 11 - 12 µm - 2 examples Fig. 5.8

The cells were between 1 x and 2 x as long as they were broad. The chloroplast was an incomplete band that extended over half of the cell wall. It contained a single distinct pyrenoid which was surrounded by starch. The filaments were of indeterminate length and in general showed no tendency to fragment (Piercy, 1917). However it was noted that a tendency to fragment did appear in old cultures and therefore this character seems to be a consequence of the physiological state of the organism rather than an inherited taxonomic character. Mattox and Bold (1962) described the size range of this species as between 5 and 8 µm but Ramanathan (1962) describes it as between 5 and 14 µm. In the examples observed a wide range of filament diameter was observed and as no clear distinction could be made between the narrower and broader filaments it was decided to adopt the wider size limits of Ramanathan.

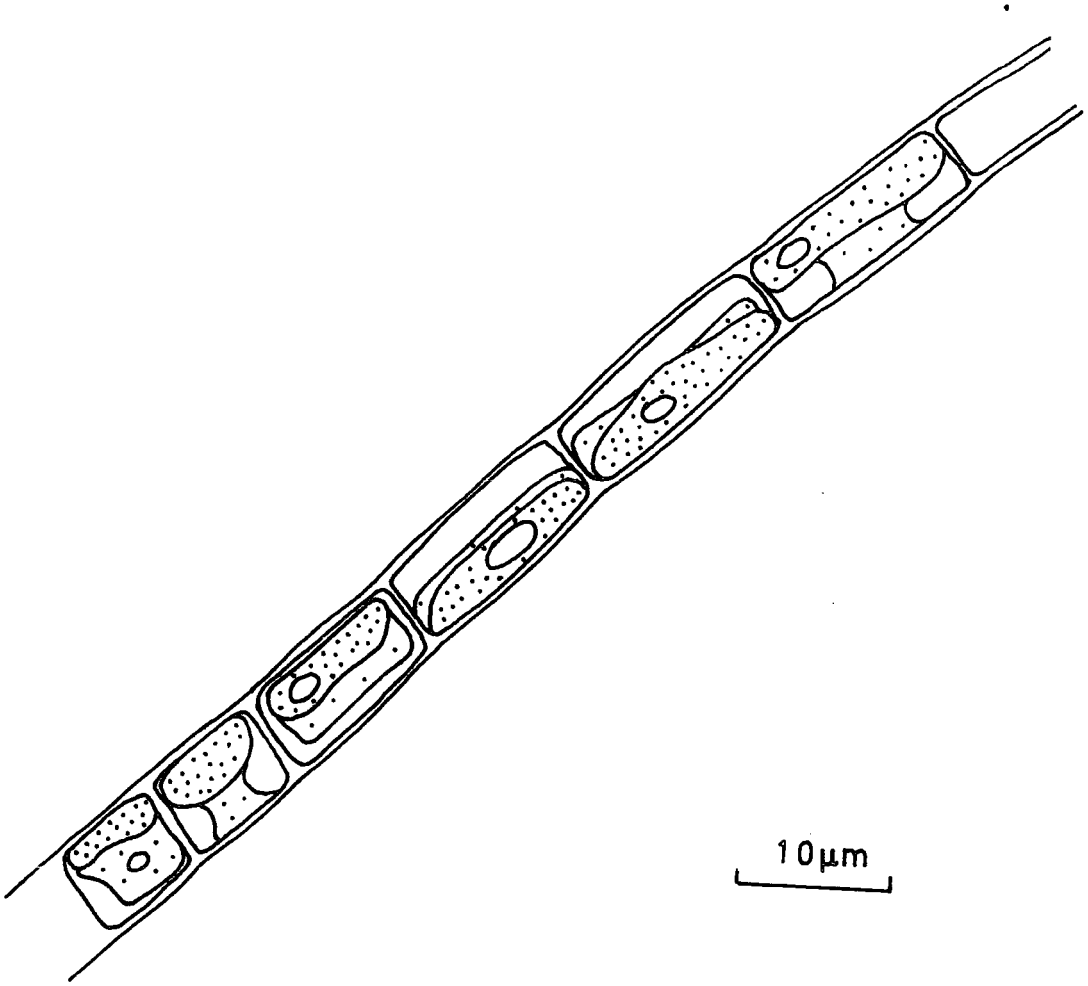


FIG 5.6 Hormidium flaccidum

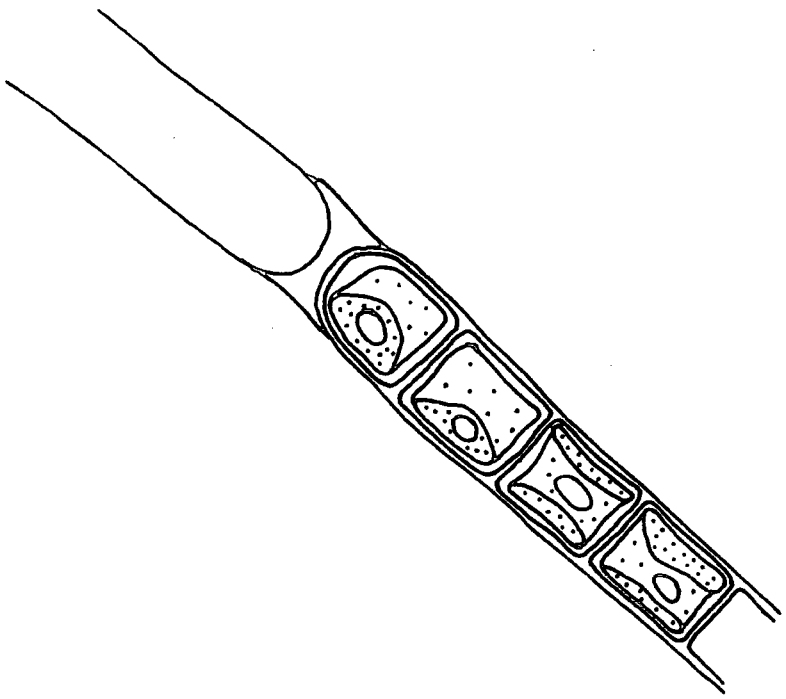


FIG 5.7 Hormidium flaccidum showing flexible joint

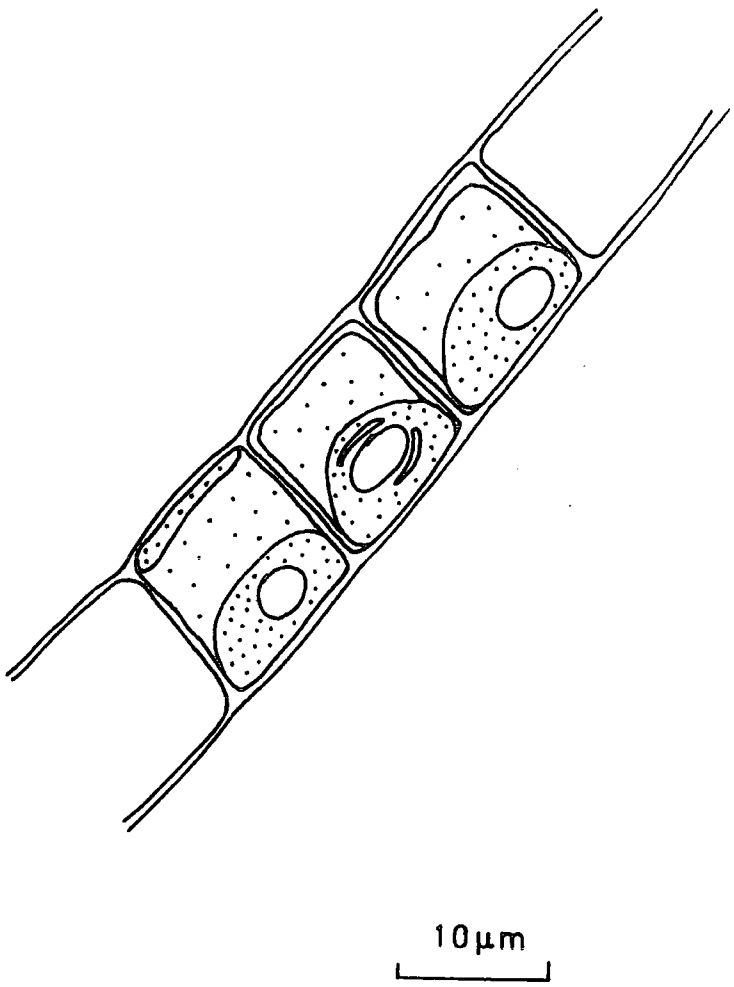


FIG 5.8 Hormidium flaccidum from 1 week old culture

Hormidium sterile (Fig. 5.9)

One isolate from site Y1 which had a colony form on agar similar to that formed by Stichococcus sp. i.e. smooth shiny surface with irregular outline suggesting filaments, was identified as Hormidium sterile. It consisted of cells 5 µm wide which were 1 x to 1½ x as long. Most cells were solitary but some were joined into short filaments of up to eight cells. The chloroplast was parietal and covered about half of the cell wall. In unstained preparations there appeared to be a very indistinct pyrenoid and when stained with iodine a pyrenoid was visible surrounded by starch. If a pyrenoid had not been present these cells would have been identified as a small species of Stichococcus but Deason and Bold (1960) described a species similar to this and named it Hormidium sterile. The cells of this isolate were somewhat shorter than those described by Bold and Deason but in view of the observations of Hayward (1974) on Stichococcus and the great variation in cell length in other members of these genera, cell length does not seem to be a very reliable character. No zoospores were observed and Deason and Bold described the species as not producing zoospores, however Cain, Mattox and Stewart (1973) reported the successful induction of zoosporogenesis.

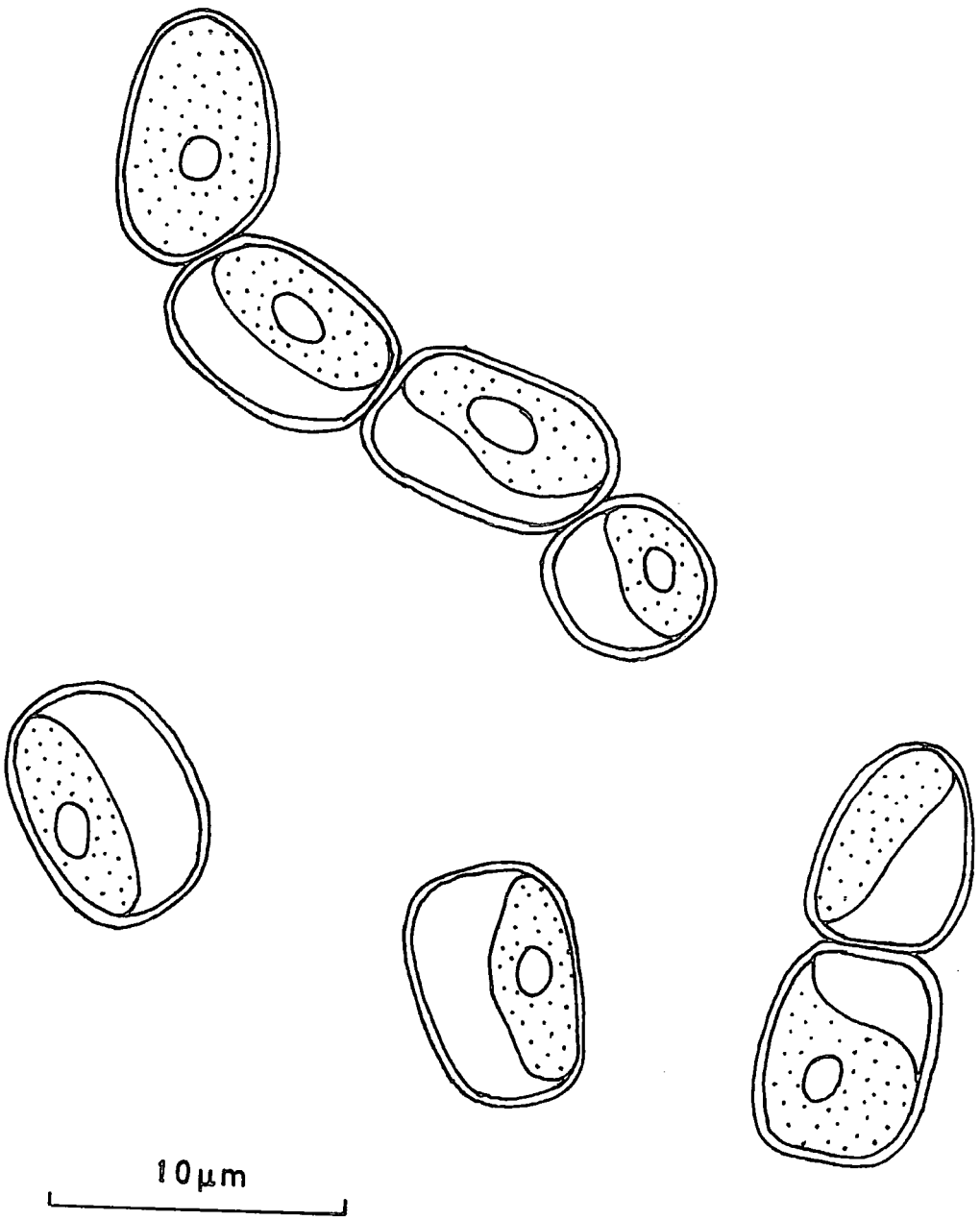


FIG 5.9 Hormidium sterile

Stichococcus bacillaris

Five algae isolated from sites G1 and Y1 were identified as belonging to this species. They all formed smooth colonies on agar which at first had a regular outline but in time developed filament like projections from the edges of the colony. The cells were 3 - 7 µm in diameter and varied from 1 x to 3 x as long as wide. They had a parietal chloroplast which was always in a median position along the side of the cell and lacked a pyrenoid. On staining with iodine young cells were shown to contain a large single starch body within the chloroplast. The chloroplasts never encircled more than half of the cell. The majority of cells were solitary (Fig. 5.10), however filaments of various lengths were observed. The longest seen was made up of 54 cells but more commonly the filaments were composed of two to eight cells. All filaments fragmented easily and the end walls were never in complete contact (Fig. 5.11). Old cells have chloroplasts which are yellow green in colour and have one or two prominent oil droplets at the ends of the cell (Fig. 5.12).

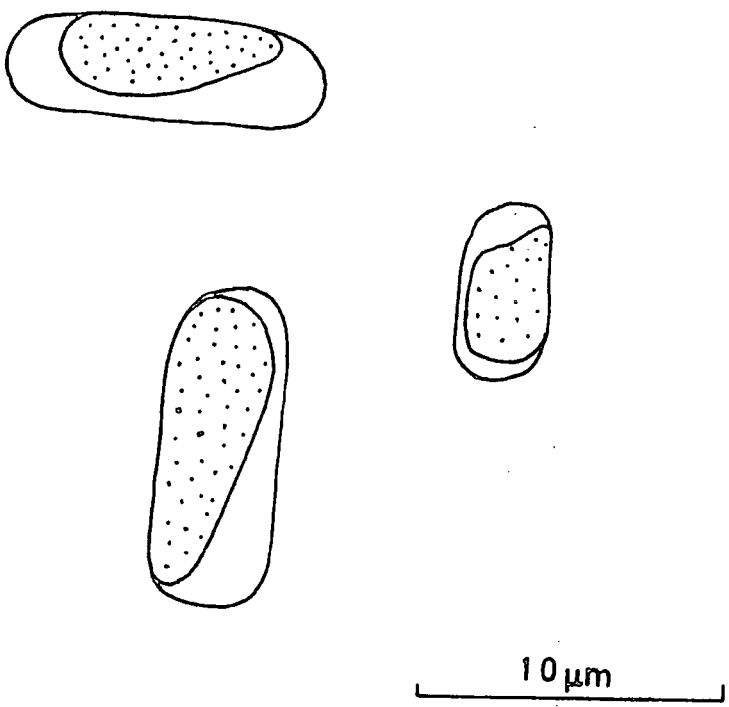


FIG 5.10 Stichococcus bacillaris solitary young cells

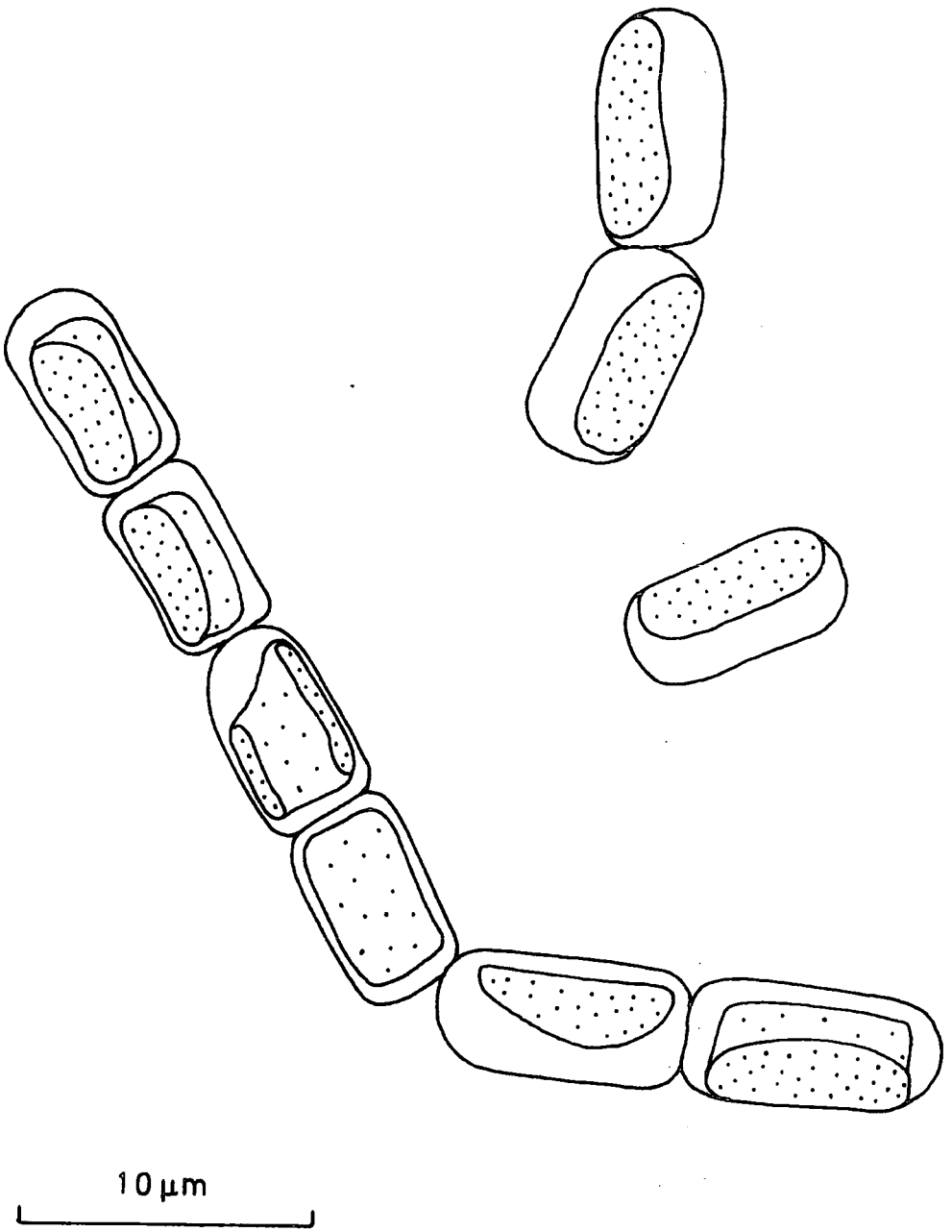


FIG 5.11 Stichococcus bacillaris young cells forming fragile filament

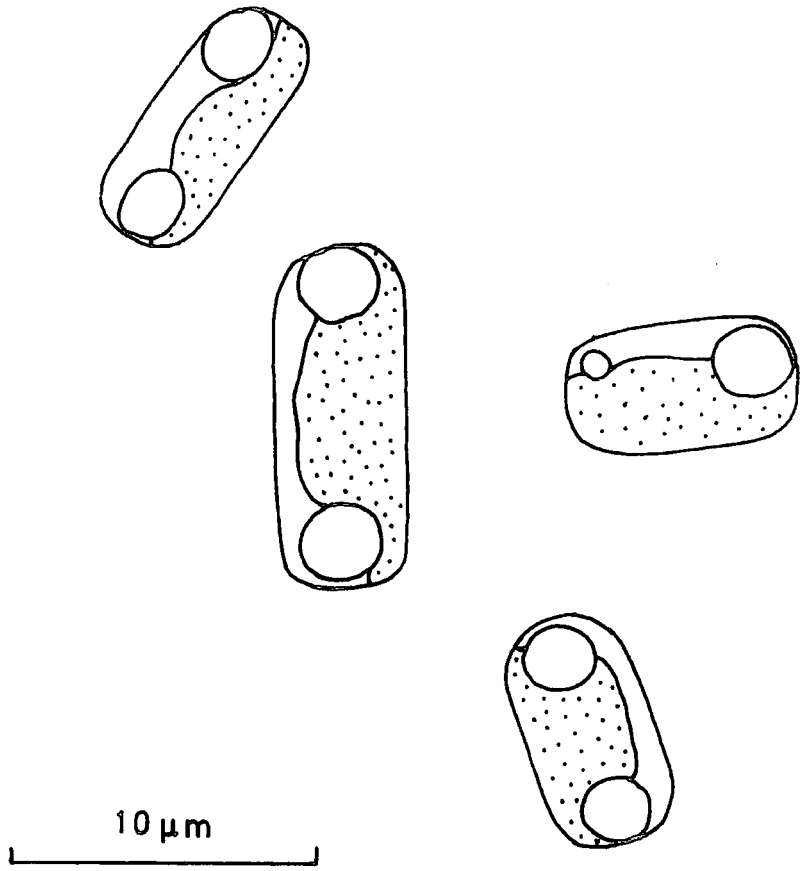


FIG 5.12 Stichococcus bacillaris old cells

6. Stichococcus minor

One isolate from site G1 was ascribed to this species. The cells were 2 µm to 2.5 µm in diameter and 3 µm to 8 µm long. They had a parietal chloroplast lacking a pyrenoid and in old cells had oil droplets at the ends of the cell. The cells were always solitary and were never seen to form filaments (Fig. 5.13). They closely resemble S. bacillaris but are consistently smaller and more rounded or elliptical then all of the isolates described as S. bacillaris. The cells described closely resemble those described by Grintzesco and Peterfi (1932).

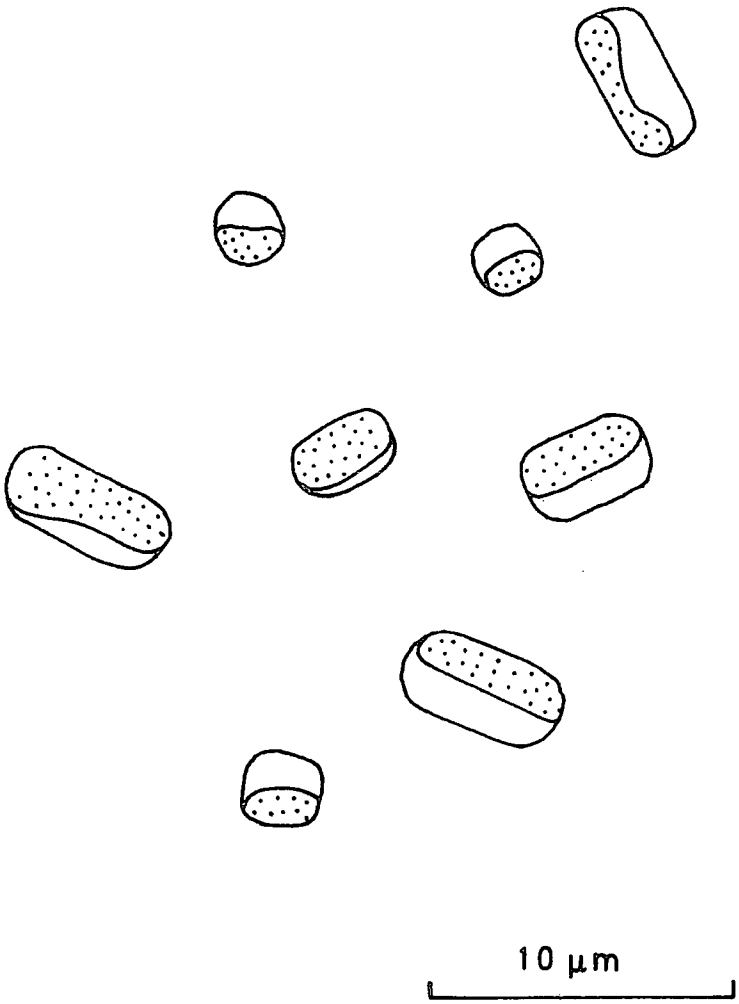


FIG 5.13 Stichococcus minor

5.12 Chaetophorales

Sites Y1, G1 and G2 all yielded isolates which were identified as members of the Chaetophorales. Many of these were species that form cubical packets of cells but several were filamentous forms.

Chlorosarcina sp.

The colonies of this alga on agar were dark green in colour with an irregular outline and a moist lustrous surface. Solitary cells were spherical and up to 12.5 µm in diameter. Most cells formed packets of two, four or eight cells in which adjacent cell walls were flattened. The chloroplast was closely appressed against the outside of the cell and contained no pyrenoid. The appearance of cells in a two day old culture is shown in Fig. 5.14 and their appearance in 23 day old culture is illustrated in Fig. 5.15.

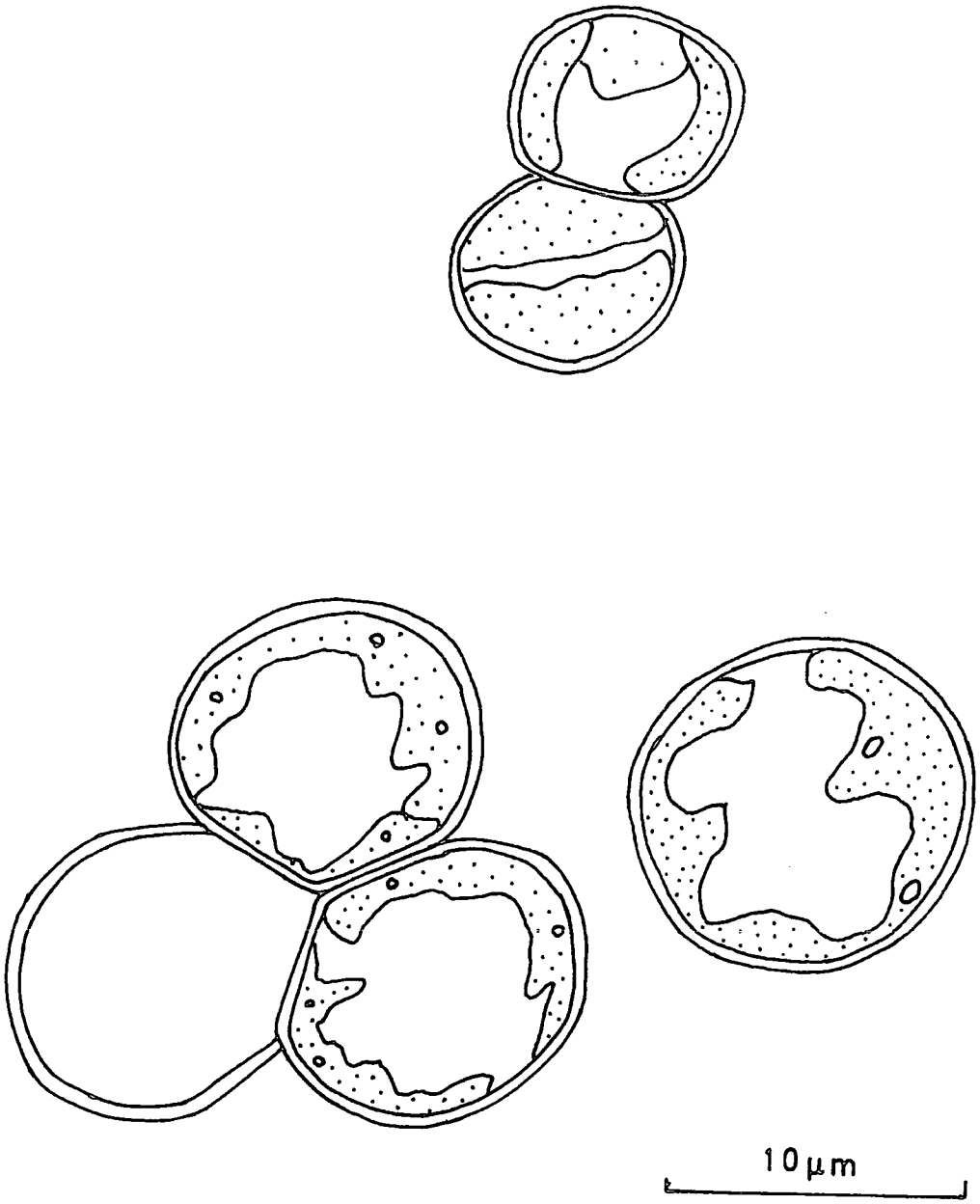


FIG 5.14 Chlorosarcina sp. cells from 2 day old culture

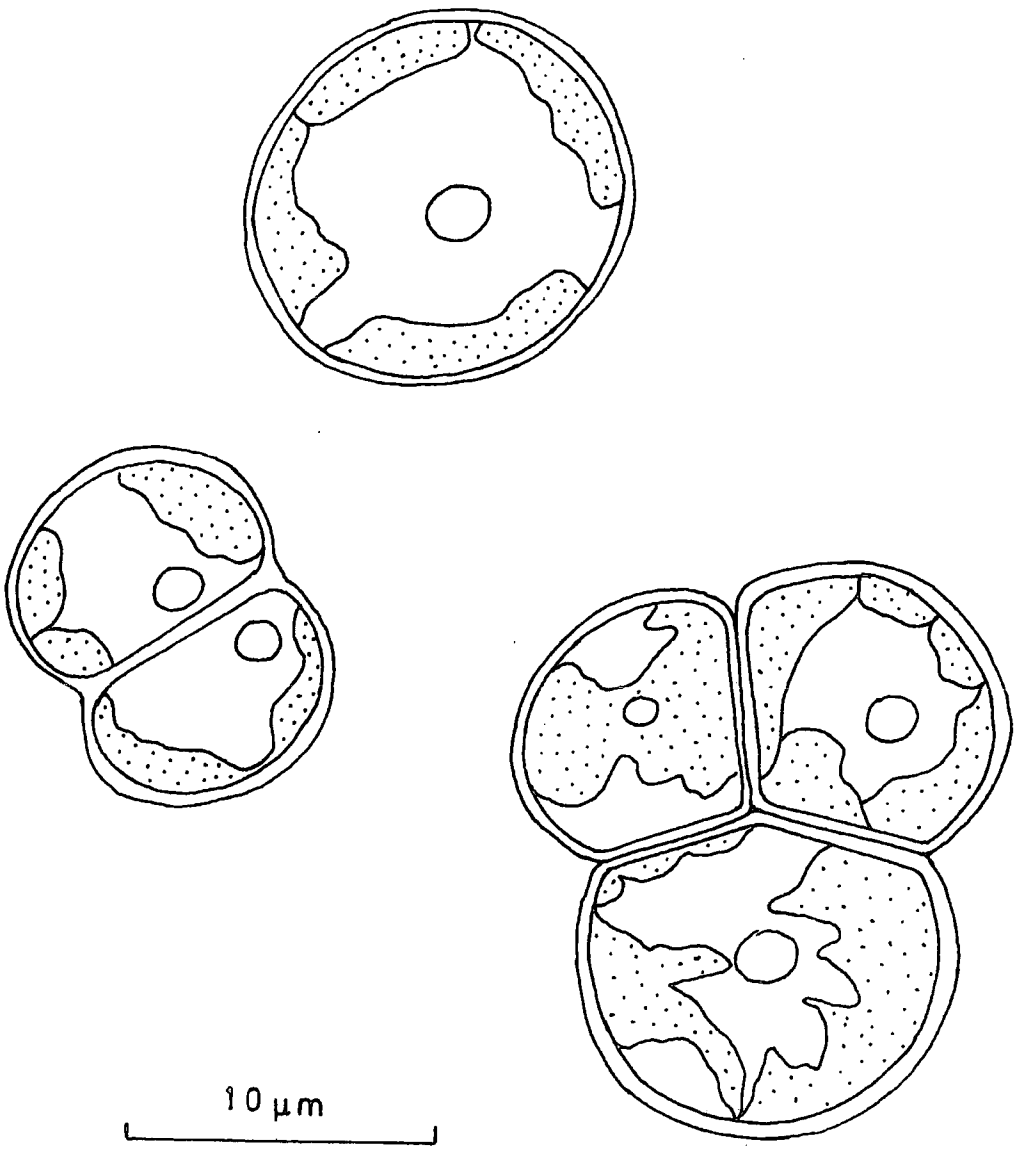


FIG 5.15 Chlorosarcina sp. cells from 23 day old culture

Chlorosarcinopsis minor

This organism was isolated from the September sample at site Y1. The appearance of the colonies on agar was similar to Chlorosarcina. Individual cells varied in diameter form 6.5 to 10.0 µm. In two day old culture (Fig. 5.16) most cells were solitary. They contained a single cup shaped chloroplast closely appressed to the wall of the cell, with a prominent single pyrenoid. In 23 day old cultures most cells formed rough cubical packets (Fig. 5.17). This organism conformed closely to the description given by Herndon (1958).

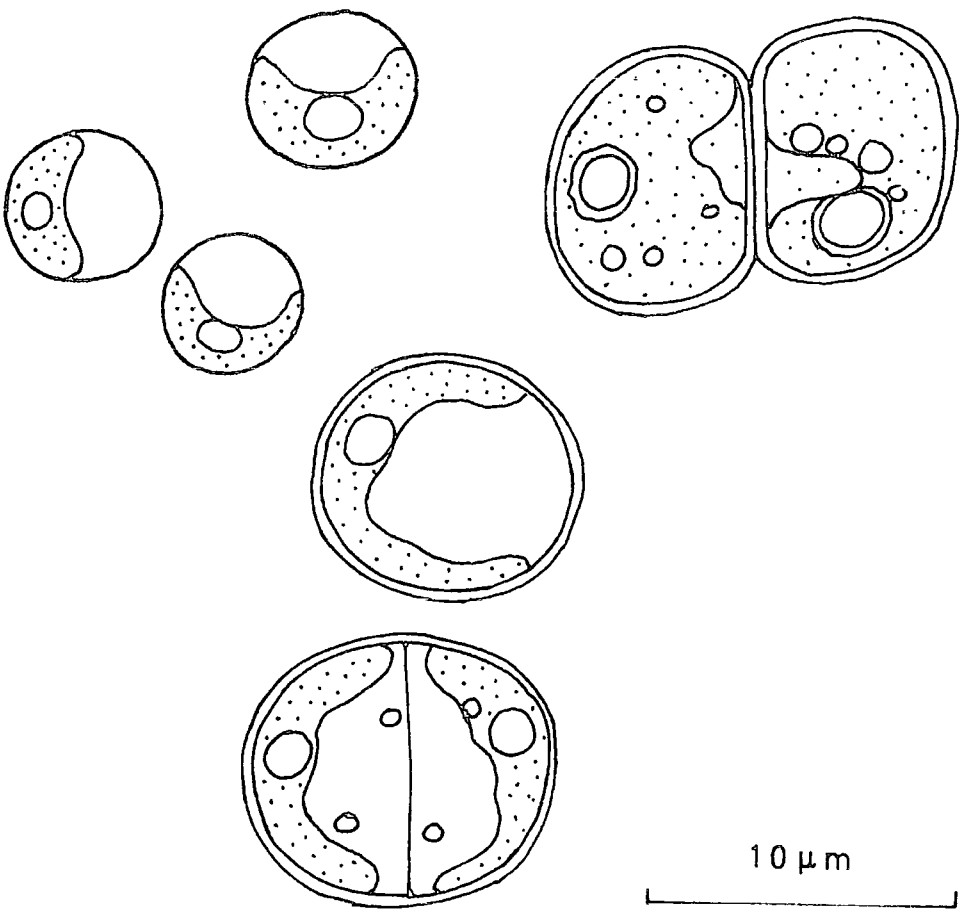


FIG 5.16 Chlorosarcinopsis minor in 2 day old culture

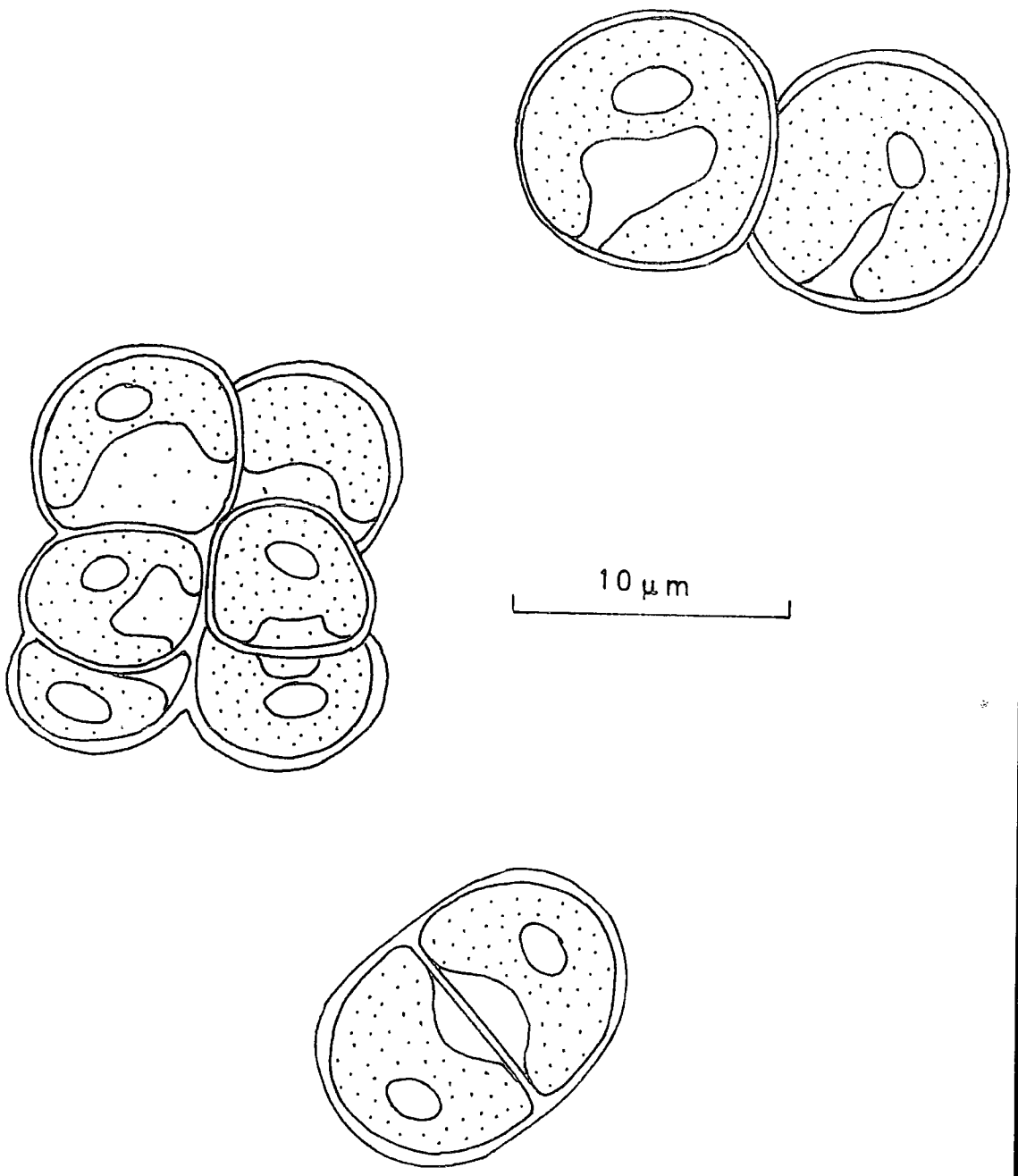


FIG 5.17 Chlorosarcinopsis minor in 22 day old culture

Jaagiella alpicola

Algae assigned to this species were isolated on several occasions from spoil at sites G1 and G2. The colonies on agar had a massive centre with radiating filaments around the edge, (Fig. 5.18). Part of the colony was pressed close to the agar or may even have been penetrating it and this part was made up of long multiseriate filaments which may be referred to as the basal system. The second part of the colony was raised above the agar as a mound of cells and may be referred to as the aerial system. This part was made up of very short multiseriate filaments or cubical packets of cells. The larger colonies had a more massive aerial system and a reduced basal system while smaller colonies always had an extensive basal system and a reduced aerial system. This suggests that the basal system develops before the aerial system. The short aerial filaments taper from a maximum width of eight cells to a minimum width of two cells (Fig. 5.19). Each cell had a single parietal cup shaped chloroplast which was very dense and contained storage granules. A pyrenoid was absent.

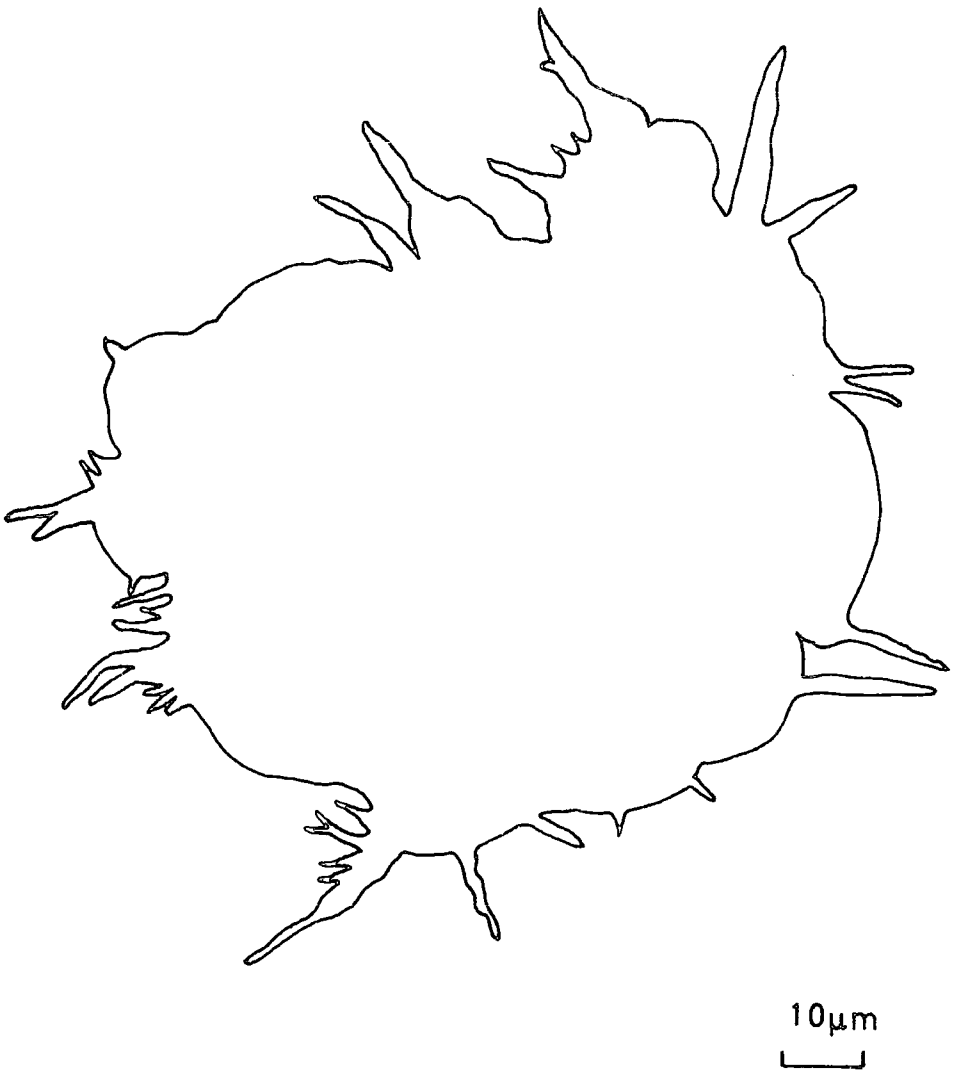


FIG 5.18 Jaagiella alpicola outline of colony on agar in 2 month old culture

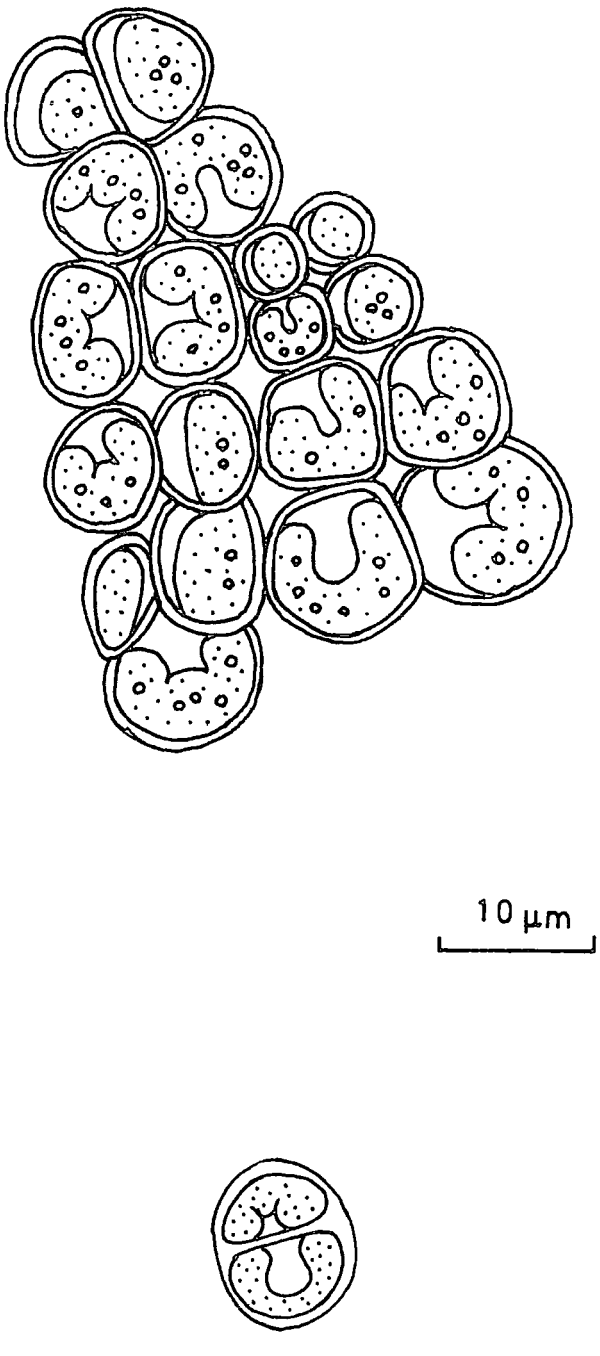


FIG 5.19 Jaagiella alpicola cells from aerial filament

The basal filaments tapered from their wide and complex base (Fig. 5.20), to a single cell at their tip (Fig. 5.21). They were usually branched with short uniseriate filaments given off at regular intervals. The cells in the older parts of the basal filaments were identical to those in the aerial filaments but the cells near the tip of the basal filaments are elongated into short cylinders with a band like chloroplasts that encircles about two thirds of the cell. In these young basal cells there sometimes appeared to be one or two pyrenoids but these are not always present and were only visible in the terminal four cells (Fig. 5.22). Zoospore production was observed on a number of occasions (Fig. 5.23).

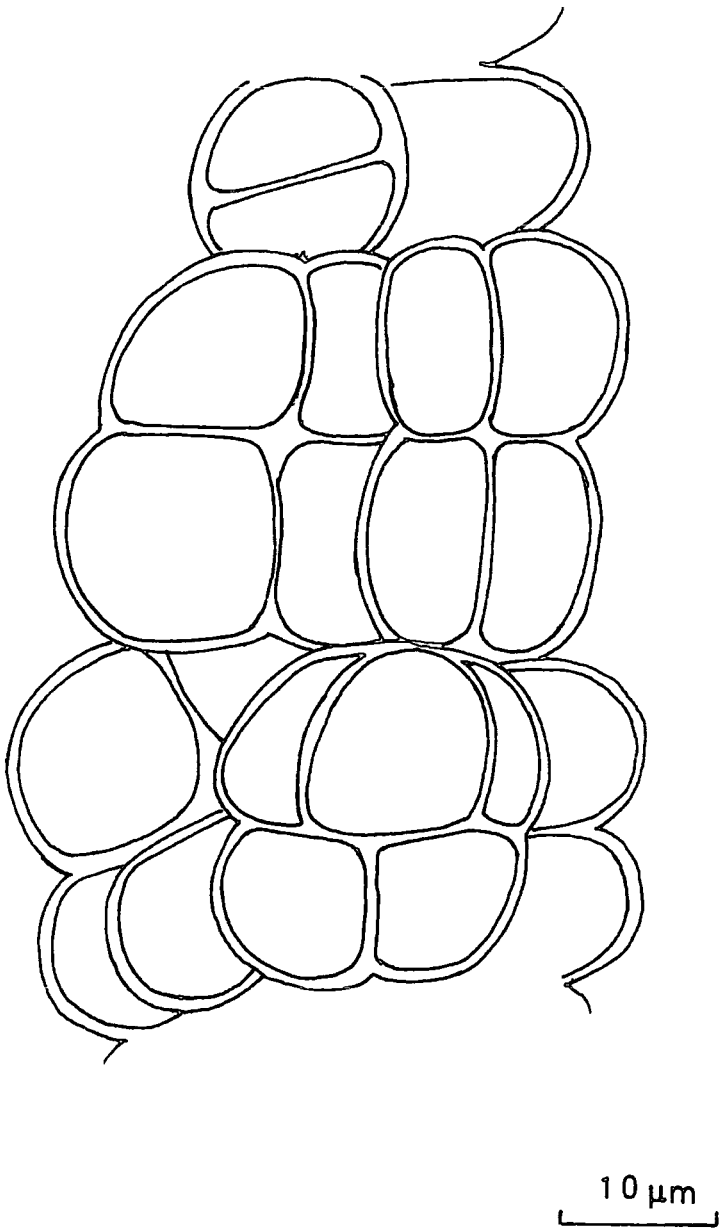


FIG 5.20 Jaagiella alpicola basal filament composed of tetrads of cells

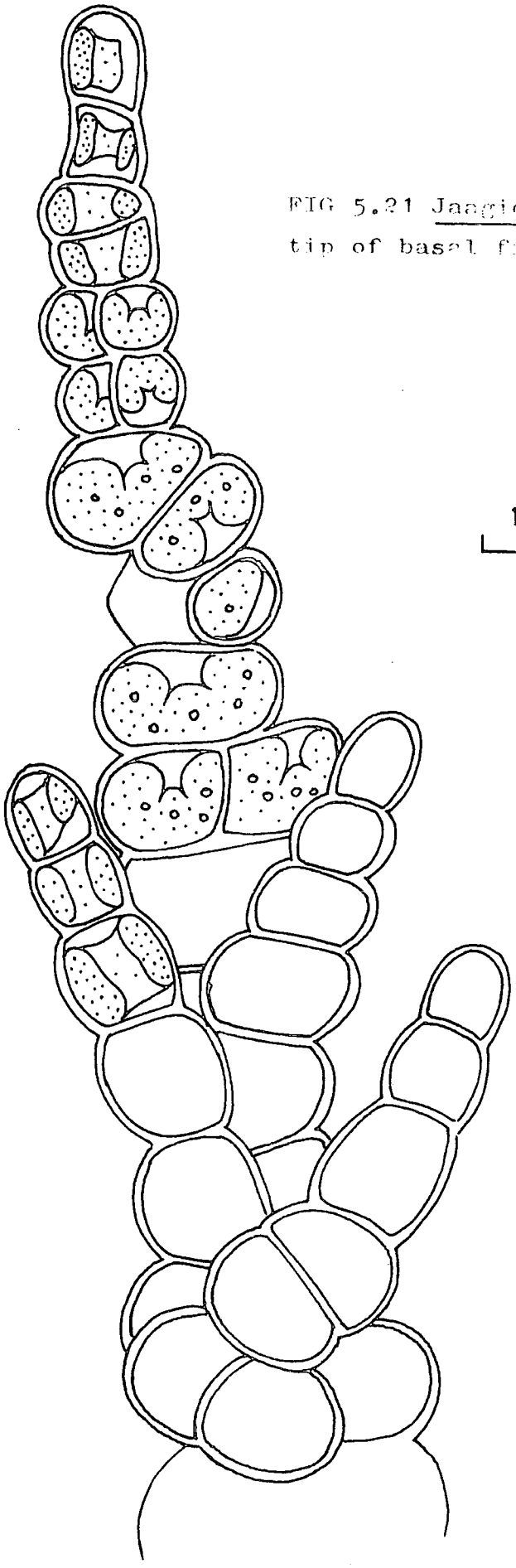
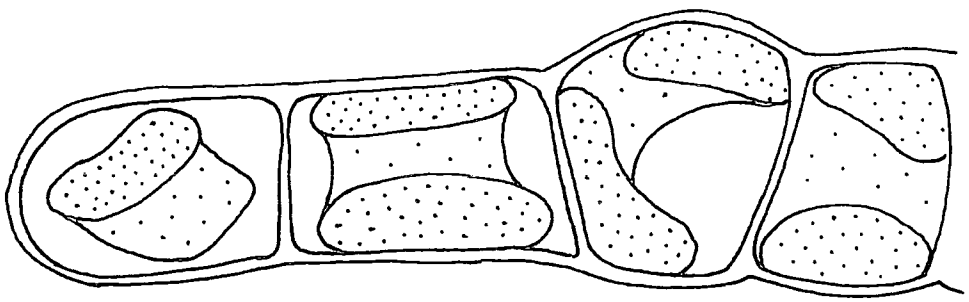
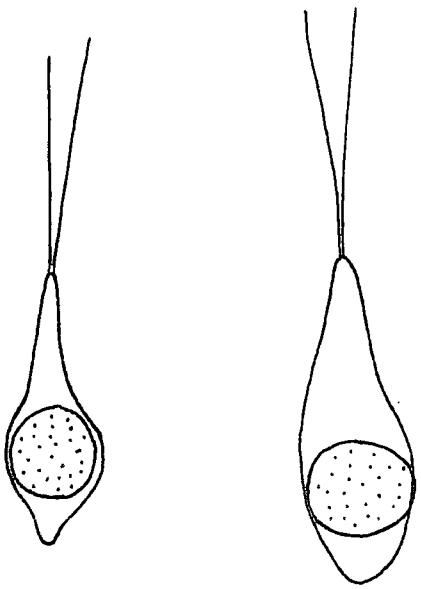


FIG 5.21 Jaagiella alpicola
tip of basal filament



10 µm

FIG 5.22 Jaagiella alpicola terminal cells of basal filament



10 µm

FIG 5.23 Zoospores of Jaagiella alpicola

Three other organisms which showed Chaetophoralean characteristics were isolated. These did not match any described species and therefore are referred to by the author's isolation code number.

Organism R4

This organism which was isolated from site Y1, produced very rough small colonies which were clearly raised above the surface of the agar. It was extremely slow growing and after 20 days in culture was made up of a few groups of two or four cells with a tendency to form short filaments (Fig. 5.24). Each cell was broadly spherical and on average 8.8 μm in diameter. In some cells the shape was modified by mutual compression on adjacent walls. The chloroplast was parietal and usually appeared divided into two lobes. Two small pyrenoids were typically present.

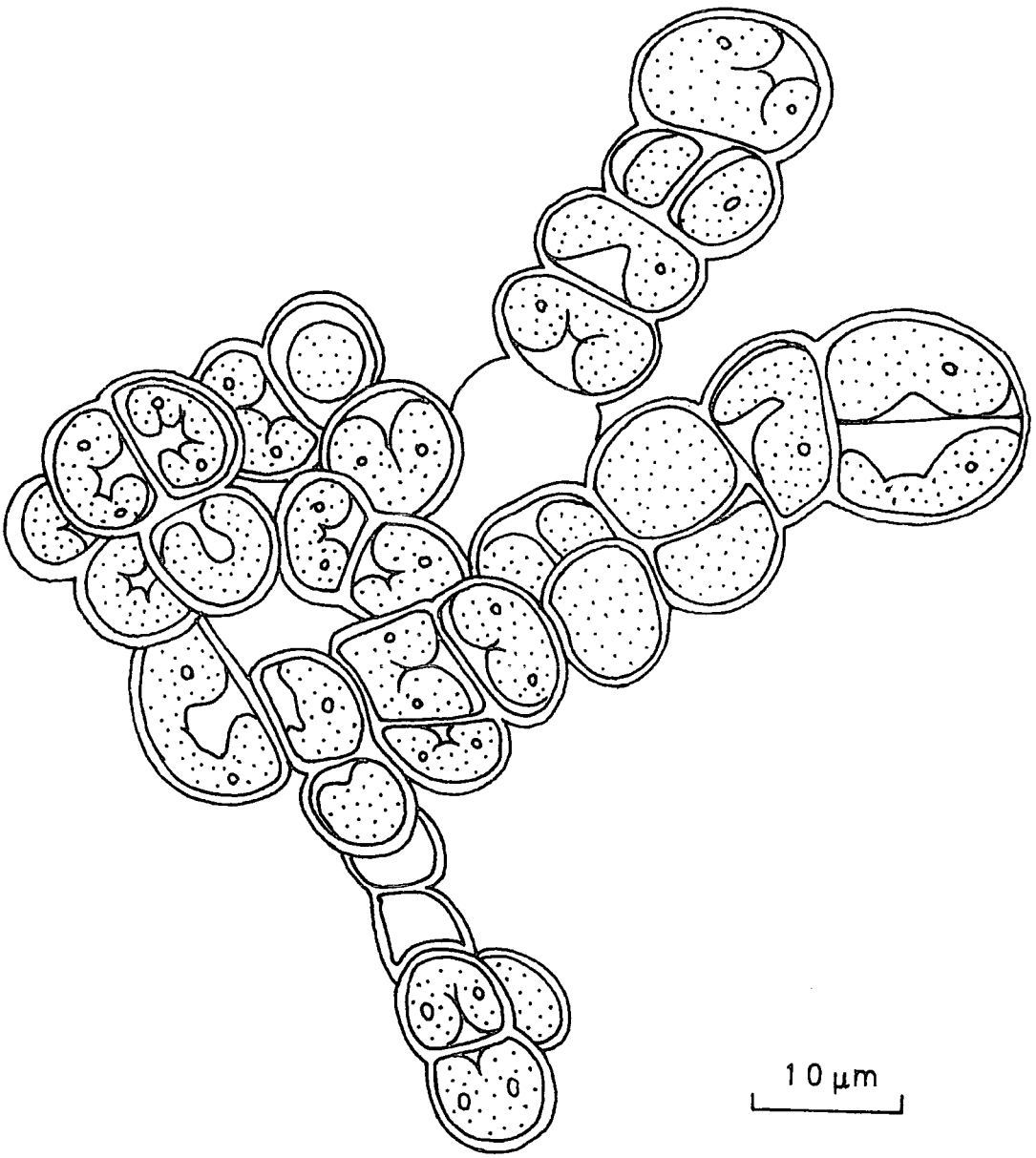


FIG 5.24 Organism R4

Organism R10

This organism was isolated on three occasions from spoil at site Y1. On agar it formed solid irregular colonies with a ridged surface that gave the appearance of solid waves. In two day old cultures the cells were spherical green unicells, 5 to 10 μm in diameter, embedded in a thin jelly matrix. Each cell had a single cup shaped chloroplast with a single prominent pyrenoid (Fig. 5.25). Many cells in this young material were observed forming zoospores. This occurred by one cell dividing into two or rarely four zoospores which each had two flagella of equal length, a parietal chloroplast and a prominent stigma. Newly emerged zoospores were pear shaped but active zoospores were more elongated (Fig. 5.26). In 23 day old cultures the cells formed irregular groups (Fig. 5.27). The edges of the plant mass showed a distinct tendency to form short filaments made up of more elongated cells (Fig. 5.28). The cells at the edge of the colony remained green but the cells in the centre of the colony were bright orange as a result of abundant haematochrome in the vacuoles of the cells. Zoospore formation was less common in older cultures and was seen only once in 23 day old material. In 46 day old material the cells formed a creeping cushion in which the centre was bright orange and the edge was green. The centre of the colony formed a pseudoparenchyma and the edge was fringed with short filaments as shown in Figs 5.29, 5.30.

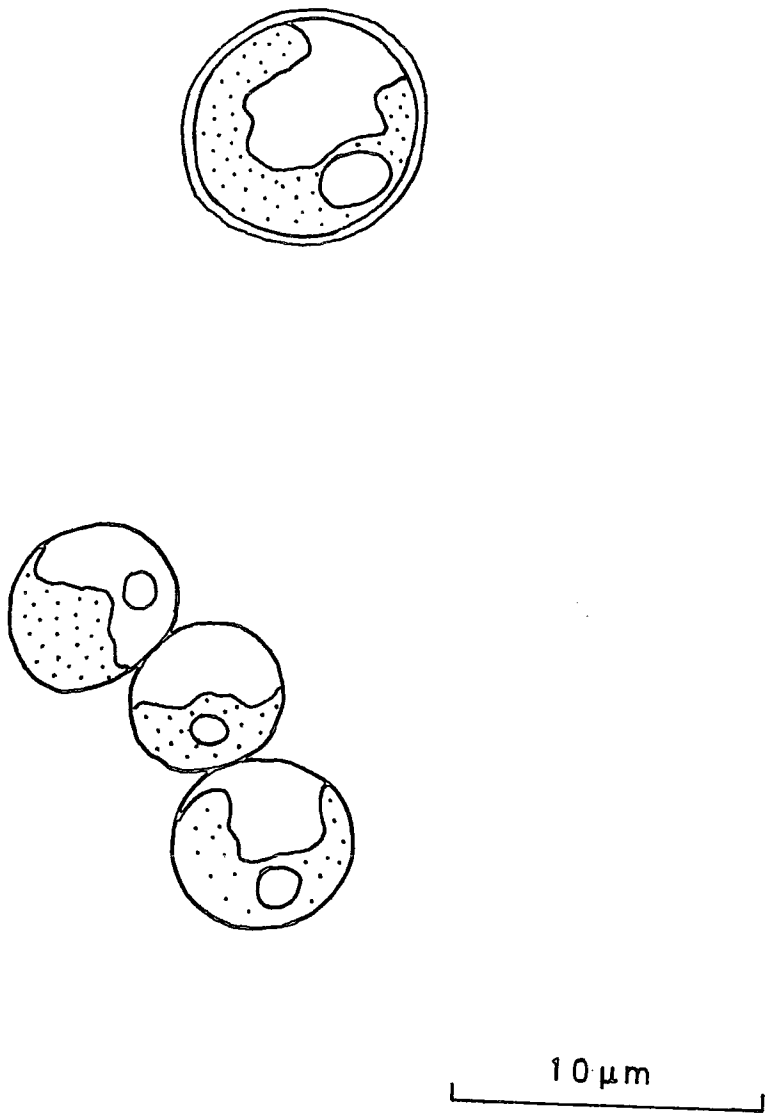


FIG 5.25 Cells of organisms R10 in 2 day old culture

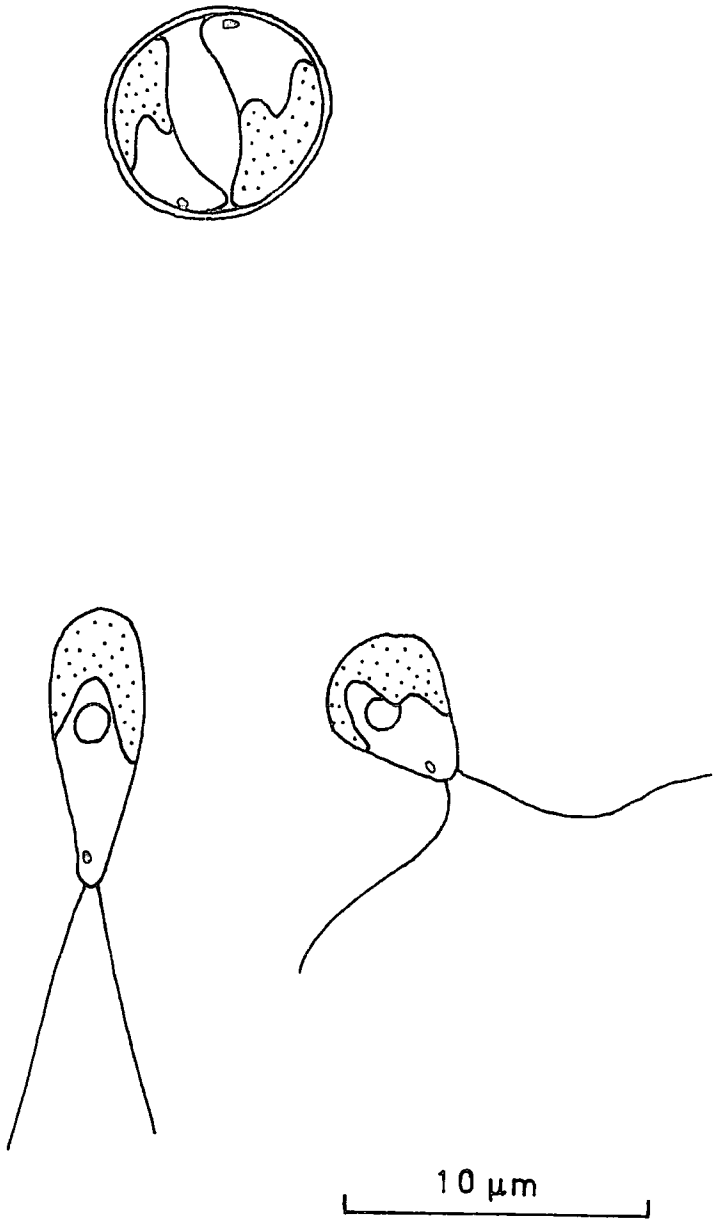


FIG 5.26 Zoospore formation in organism R10

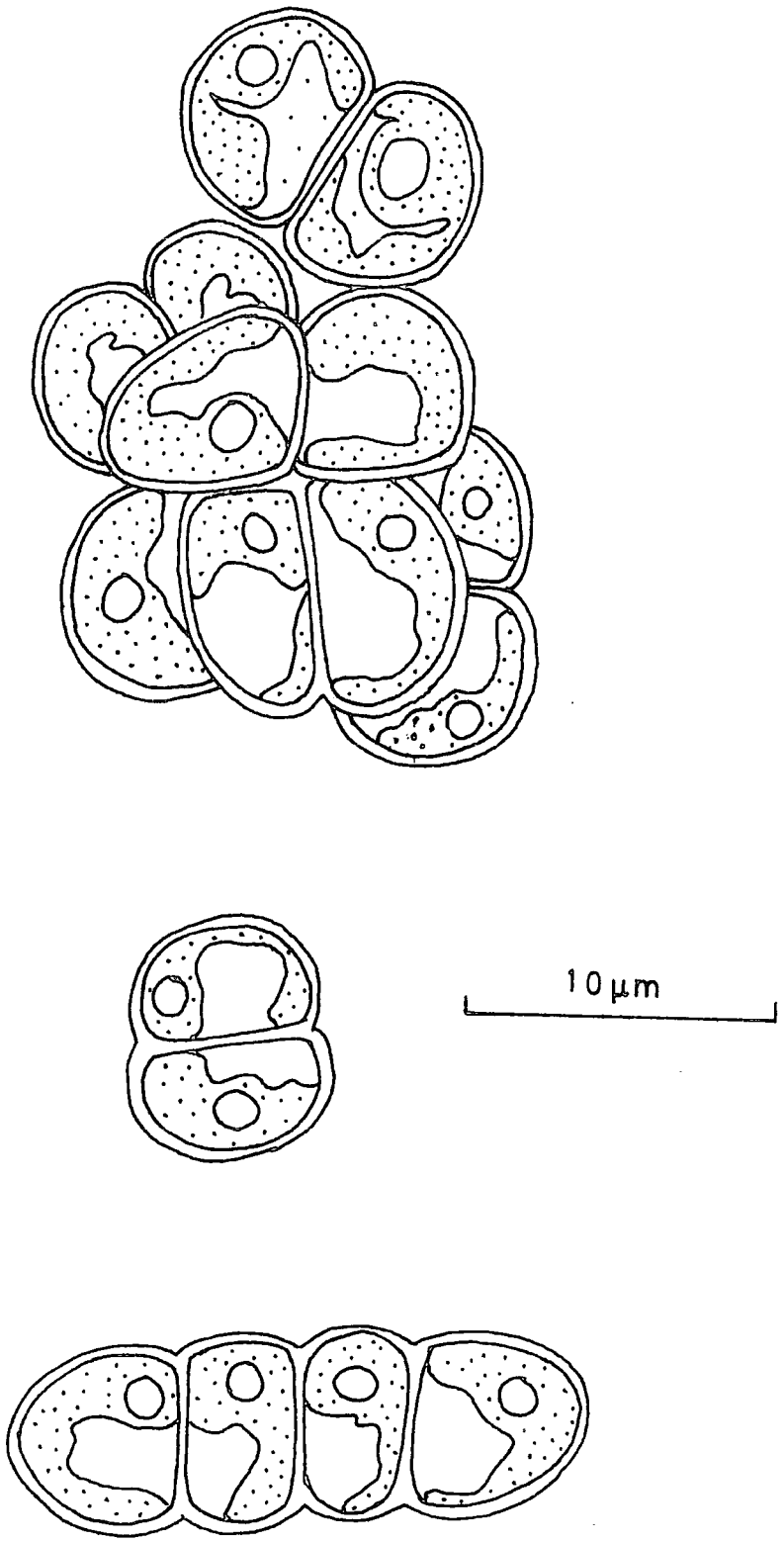


FIG 5.27 Groups of cells of organism R10 in 23 day old culture

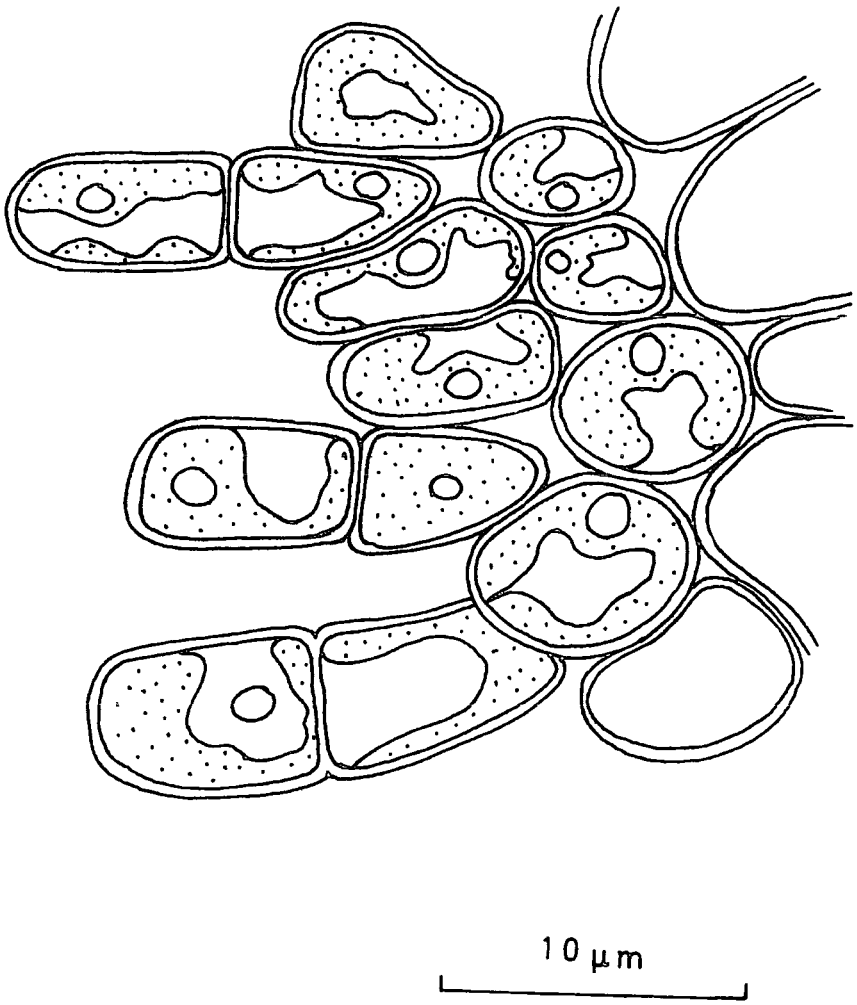


FIG 5.28 Early stages of filament formation in 23 day old culture of organism R10

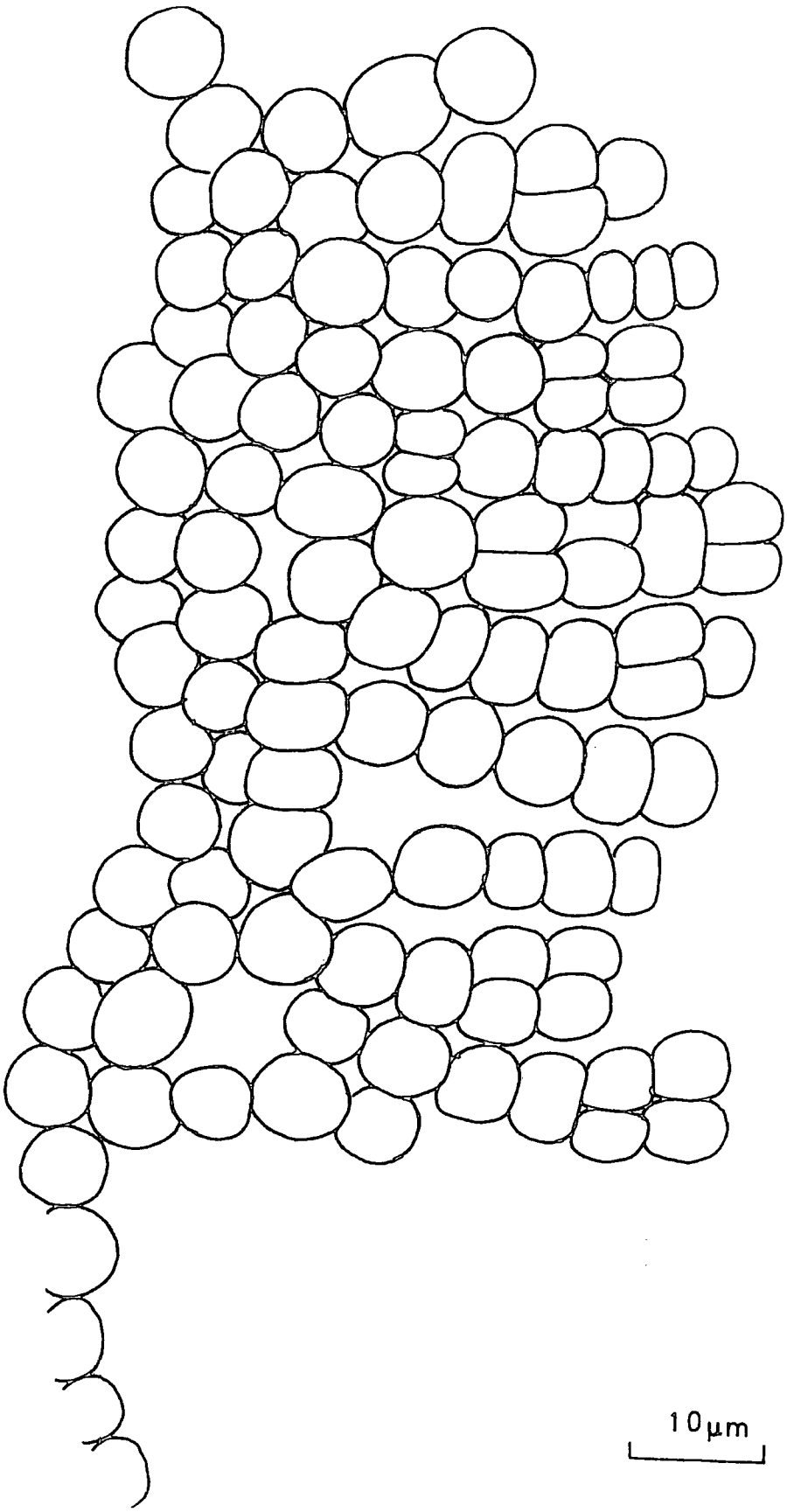


FIG 5.29 Short filaments formed at the edge of 46 day old colonies of organism B10

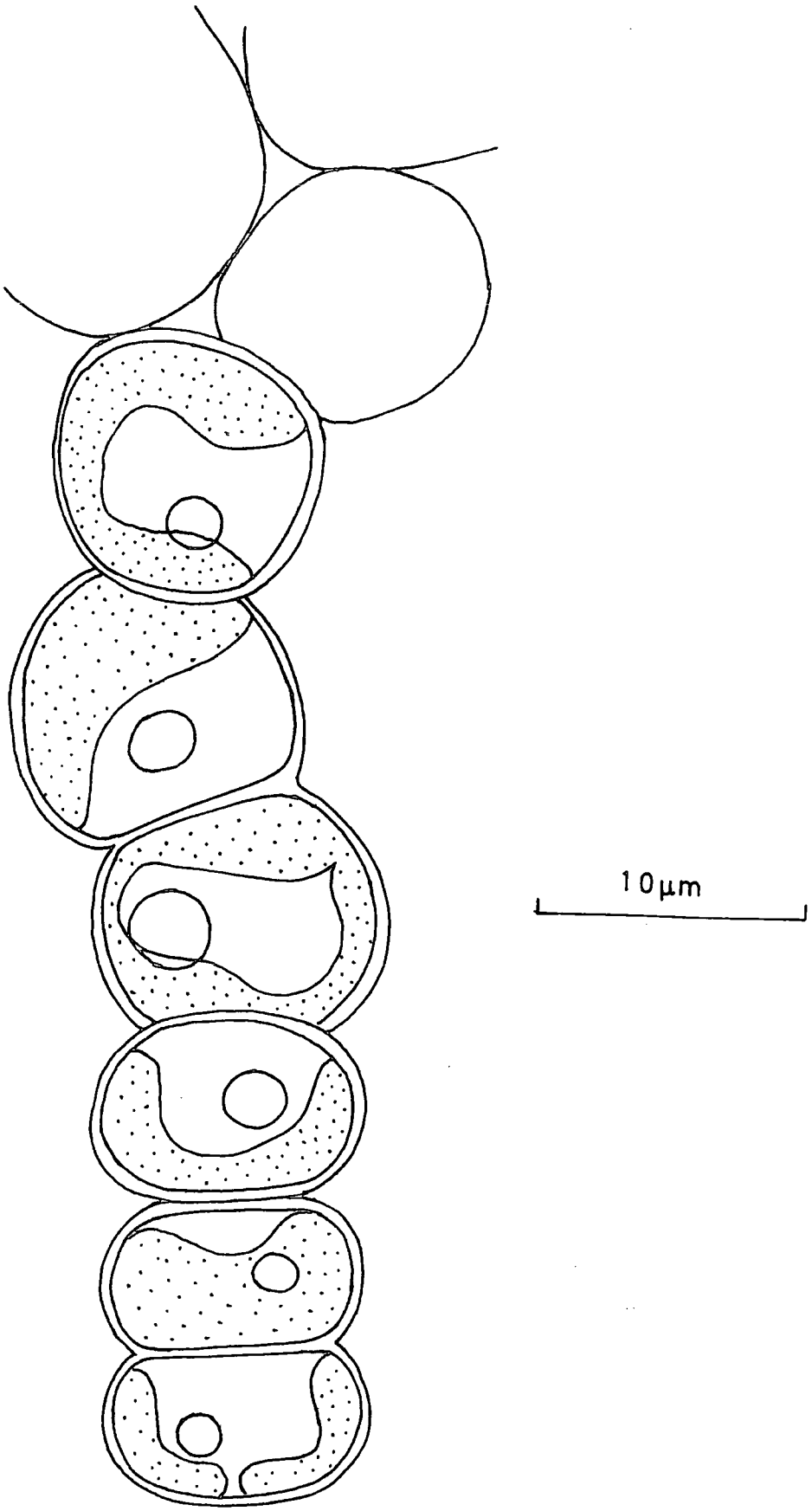


FIG 5.30 Filament in 46 day old culture of organism R10

Organism R22

This organism was isolated from site G2. The cells formed very reduced filaments which were uniseriate and branched. The branching occurred in three dimensions but in the 23 day old culture material no filament was longer than six cells. The cells were elongated, being at most 12 μm long and 5 μm wide. They had a parietal band shaped chloroplast with a clearly visible pyrenoid. Cells not forming part of a filament were spherical with an average diameter of 6.25 μm (Figs 5.31, 5.32). In 30 day old cultures most cells formed simple branched structures (Fig. 5.33). The organism was never seen to produce zoospores.

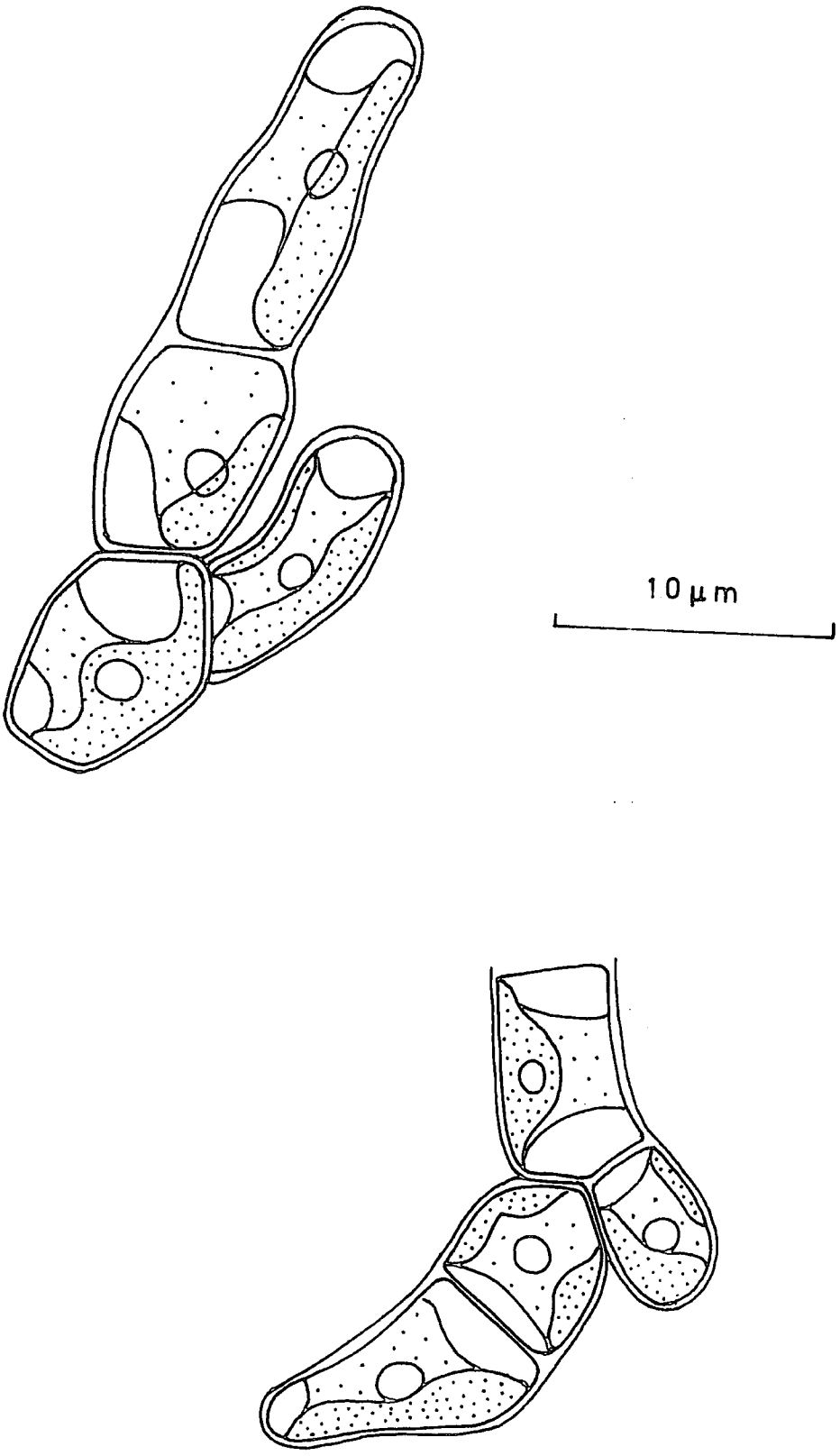


FIG 5.31 Short filaments in 23 day old culture of organism R22

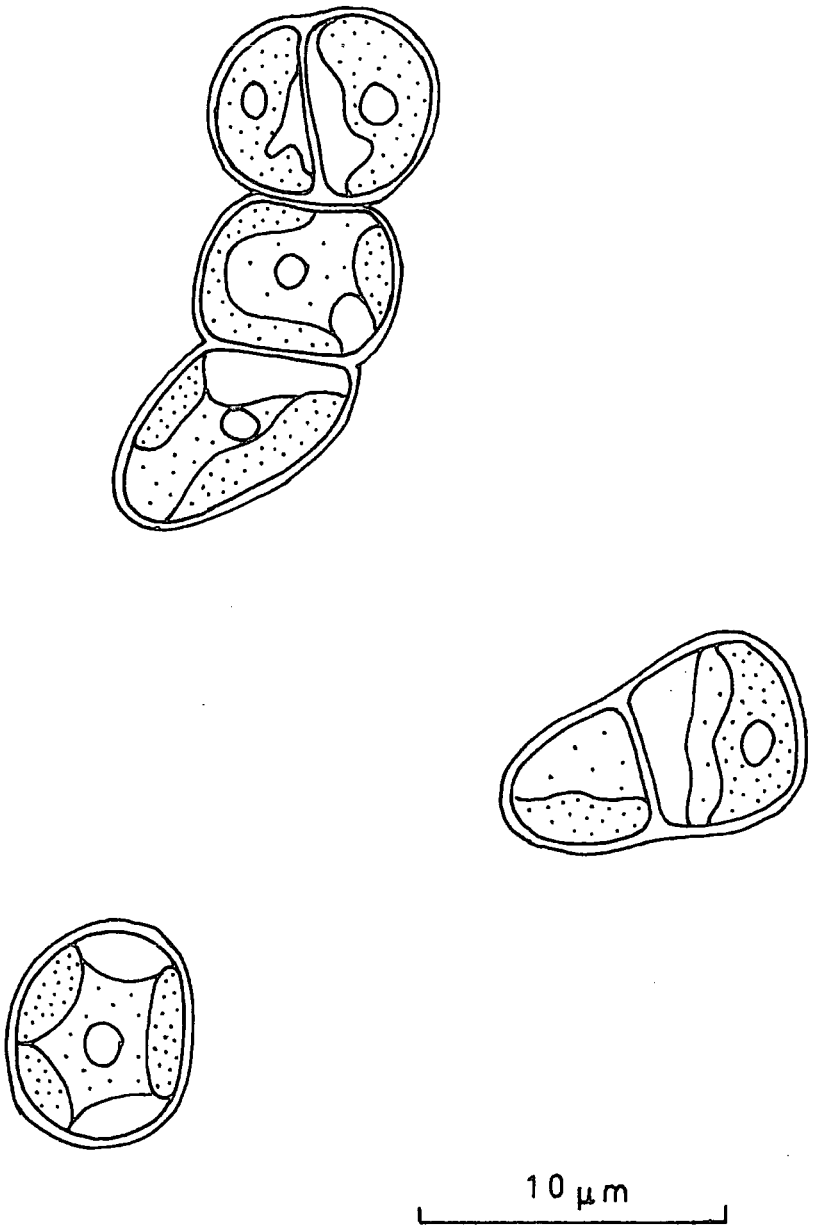


FIG 5.32 Groups of cells in 23 day old culture of organism R22

FIG 5.33 Organism R22 in 30 day old culture

5.13 Chlorococcales

The members of the Chlorococcales were by far the most common and most abundant algae at all the sites studied. Examination of field material of algae in this group was particularly confusing for they frequently did not show the characters necessary to distinguish genera. Material from 28 day dilution cultures was always so full of storage granules that few details of the cell contents could be discerned. It was therefore necessary to rely upon the appearance of colonies on agar to indicate the presence of different organisms. On this basis a number of organisms which each produced a characteristic colony type were isolated into unialgal culture and studied at regular intervals. Inevitably this approach will have resulted in some genera being overlooked because they formed a colony similar to another genus. The following descriptions are of the organisms studied in unialgal culture.

Lobococcus incisa

This organism was isolated on two separate occasions from spoil at site Y1. It was a spherical unicell with cell diameter that varied between 7 and 18 µm. Each cell contained a parietal lobed chloroplast. In small cells the chloroplast has two lobes but in larger and older cells there were three or four lobes (Fig. 5.34). There were no pyrenoids present but there was a centre of starch storage in each lobe of the chloroplast.

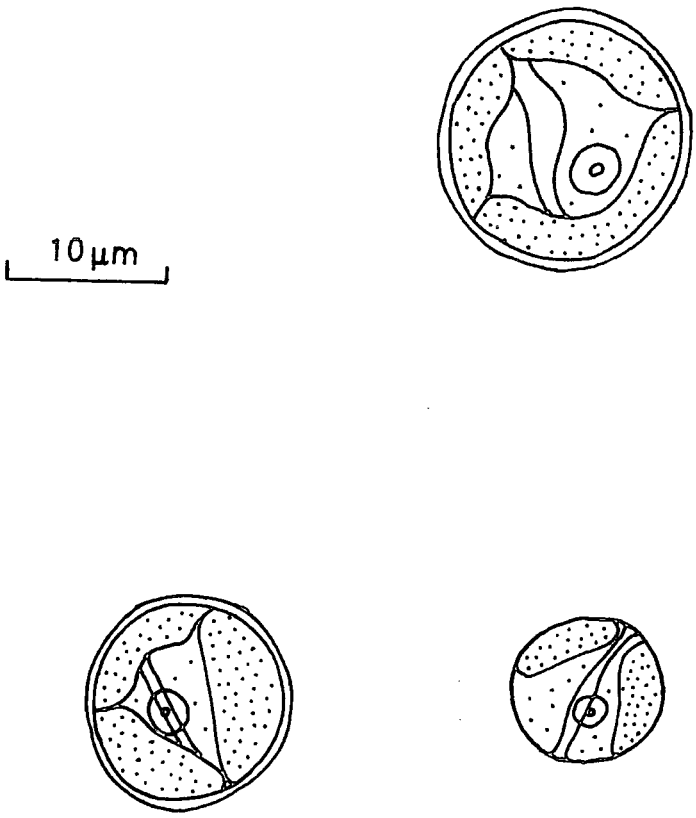
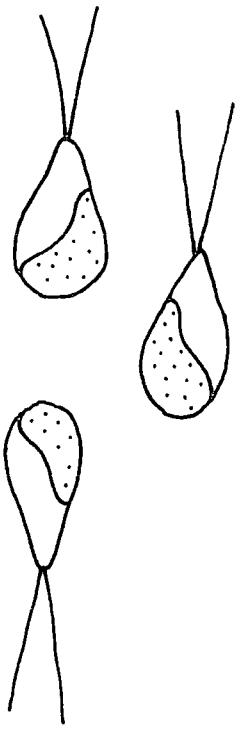
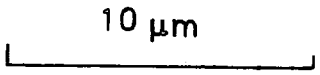


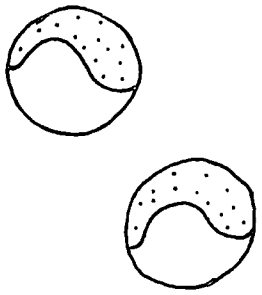
FIG 5.34 Lobococcus incisa

The cells were uninucleate and the nucleus was clearly visible in living cells. Reproduction was by zoospores and autospores. Zoospores had two flagella and were pear shaped upon release, however they rapidly rounded off and became immobile (Fig. 5.35).



a. Upon release





b. 2 min after release

FIG 5.35 Zoospores of Lobococcus incisa

Myrmecia sp.

An organism was isolated from site G1 which was assigned to this genus. It was a non-motile unicell, 13 µm in diameter. The chloroplast was parietal and lacked a pyrenoid. The cell wall had a very distinctive polar thickening which was most apparent in older cells. The cells also turned orange due to the accumulation of secondary carotenoid pigments as they aged (Fig. 5.36)

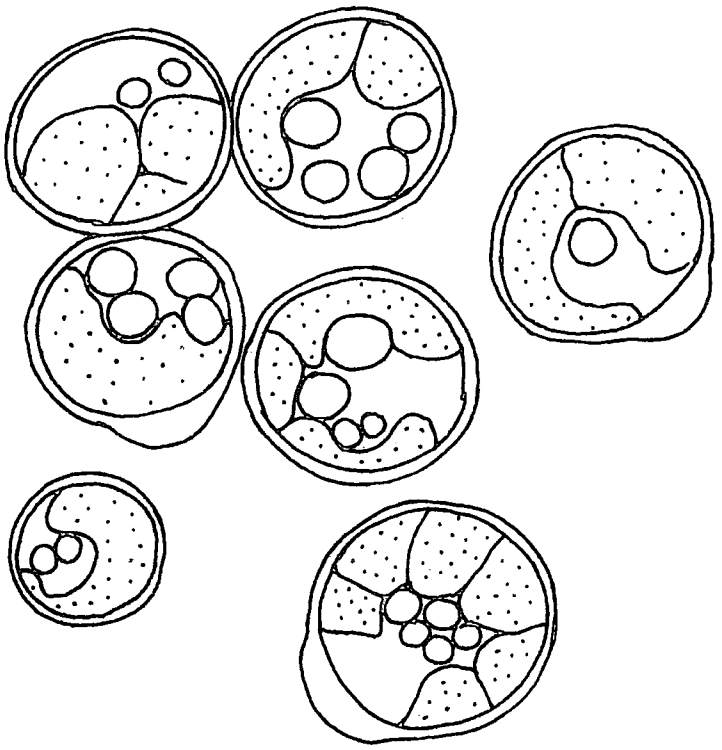


FIG 5.36 Myrmecia sp.

Muriella terrestris

This organism was repeatedly isolated from spoil at sites G1 and G2. It formed smooth circular colonies on agar. The cells ranged from 5 to 17.5 µm in diameter but most were about 9 µm in diameter. Each cell contained several parietal chloroplasts which never contained a pyrenoid. The smaller cells contained two to three chloroplasts and the larger 15 or more. Reproduction by the formation of autospores and the bursting of the mother cell wall was frequently observed but in hundreds of observations on this organism over a period of three years it was never seen to produce zoospores (Fig. 5.37).

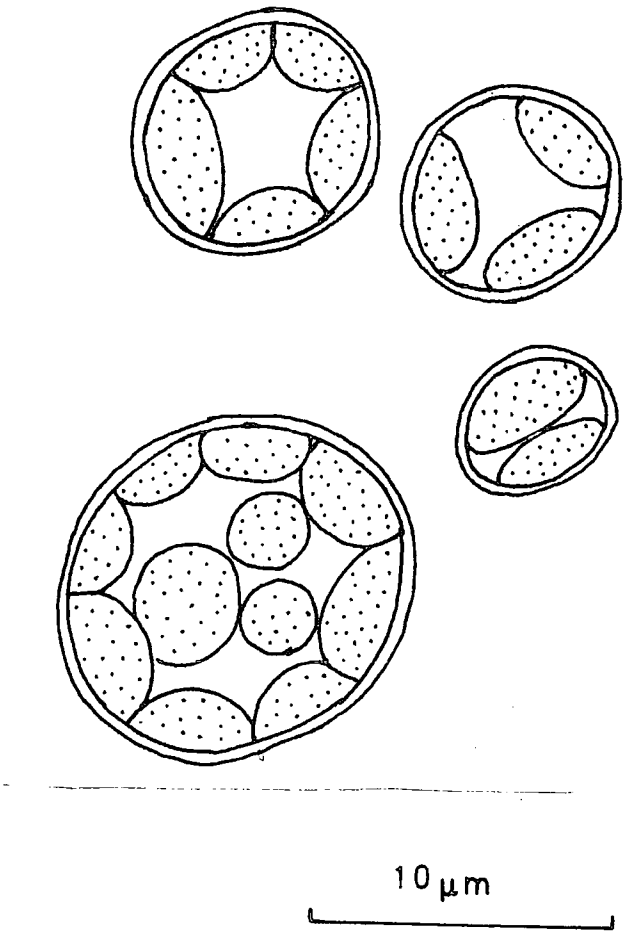


FIG 5.37 Muriella terrestris

Tetracystis sp.

Members of this genus were isolated frequently from a number of sites, but the organism studied in culture and described here was obtained from spoil at site Y1. Single cells were spherical and had a diameter of 16.5 µm. The chloroplast was parietal and cup shaped and contained a single prominent pyrenoid. The most distinctive feature however was the arrangement of cells into groups of four by the vegetative division of a single cell (Fig. 5.38). These characteristic groups were present in young and old cultures. Zoosporangia releasing zoospores were observed in five day old cultures (Figs 5.39, 5.40).

Brown and Bold (1964) have described twelve species of Tetracystis but in this study insufficient information was collected to identify the organism beyond the genus. Brown and Bold also assigned this genus to the Chaetophorales but as in all other section of this work we have preferred to follow Bourelly (1966) and place the organism with the Chlorococcales.

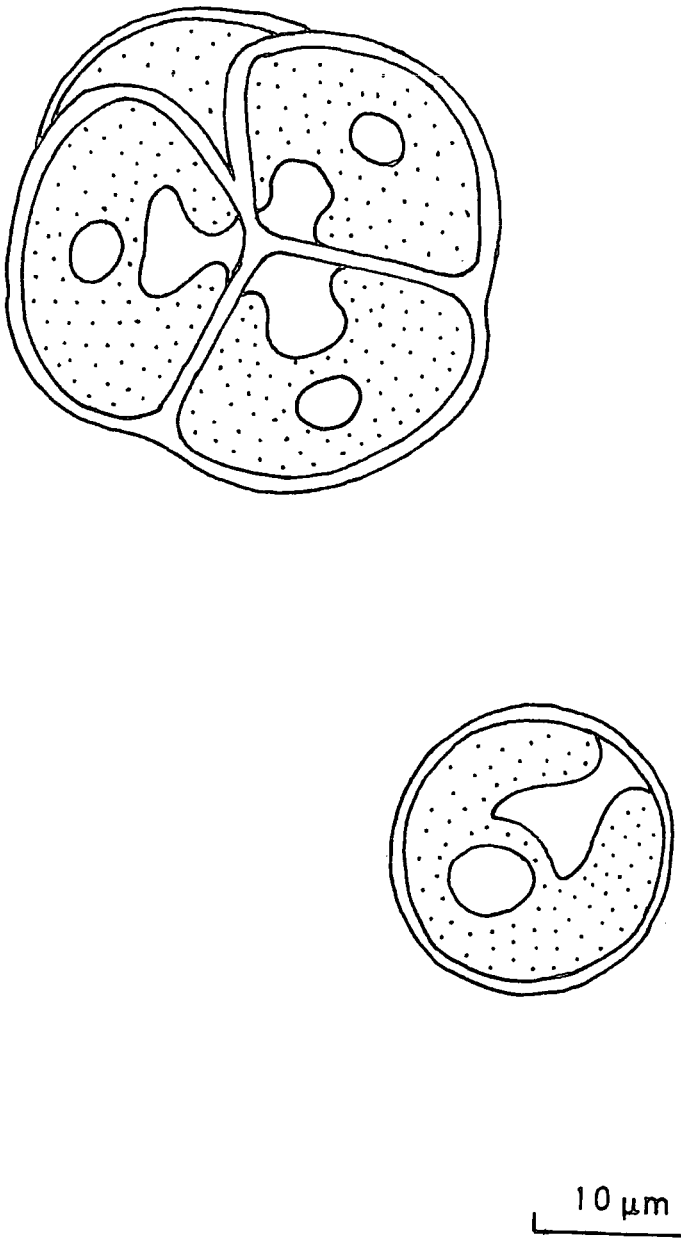


FIG 5.38 Vegetative cells of Tetracystis sp.

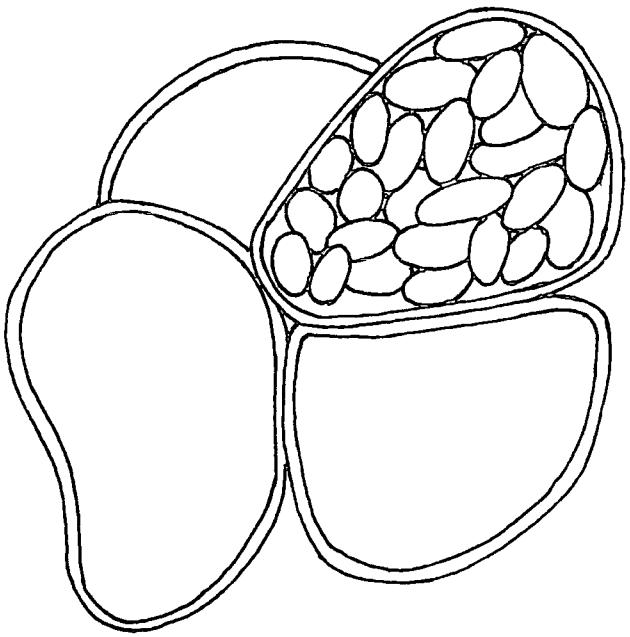


FIG 5.39 Zoosporangia of Tetracystis sp.

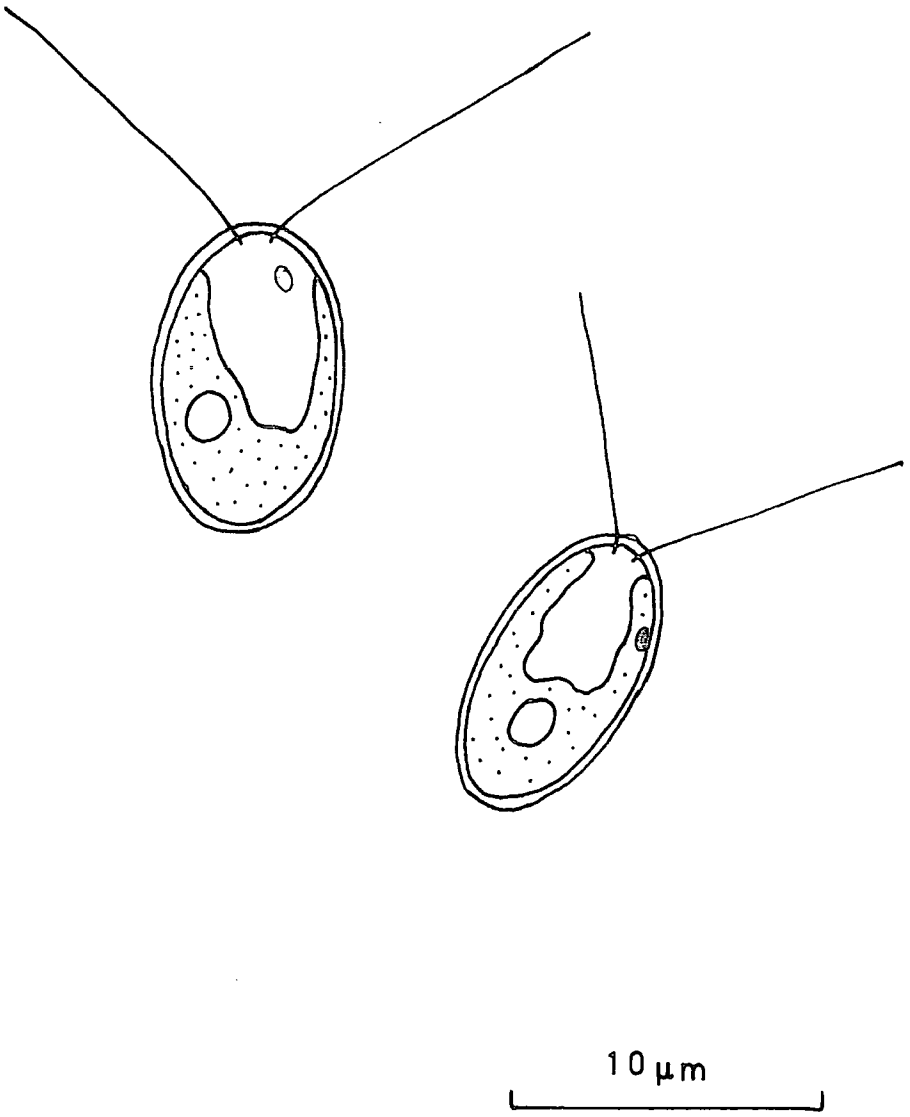


FIG 5.40 Zoospores of Tetracystis sp.

Chlorococcum sp.

Members of this genus were amongst the most common algae isolated. The organism illustrated was from spoil at site Y1. The single cells were spherical and averaged 17 to 18 μm in diameter. The chloroplast was parietal and cup shaped with a single prominent pyrenoid (Fig. 5.41). In older cells the chloroplast had gaps so that it almost had a reticulate appearance (Fig. 5.42). Cells were in groups of four or eight but were always spherical. Zoospores were formed in a vesicle as shown in Fig. 5.43.

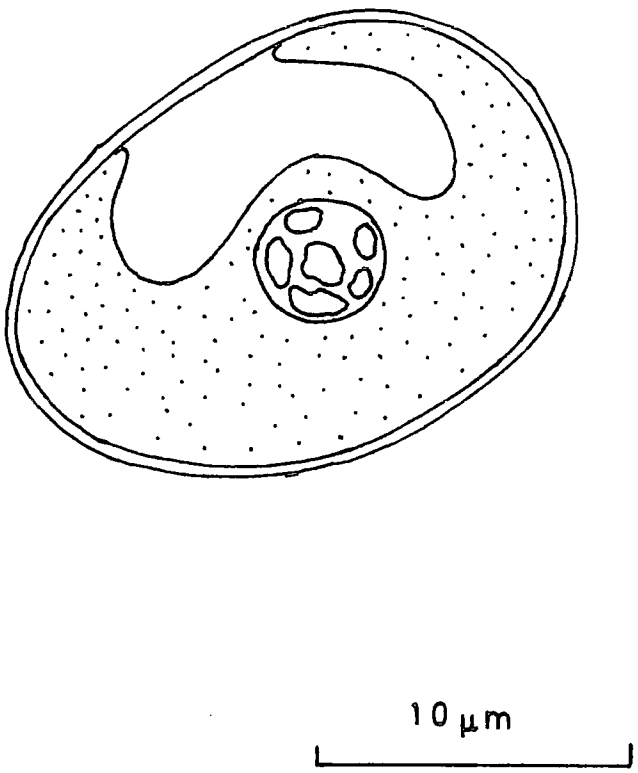


FIG 5.41 Young cell of Chlorococcum sp.

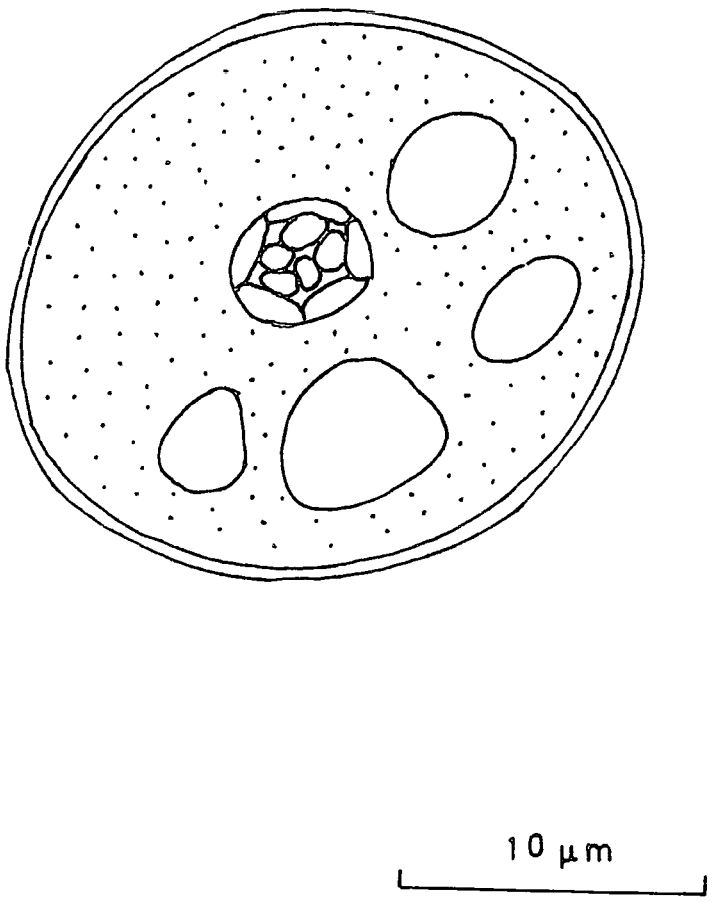


FIG 5.42 Old cell of Chlorococcum sp.

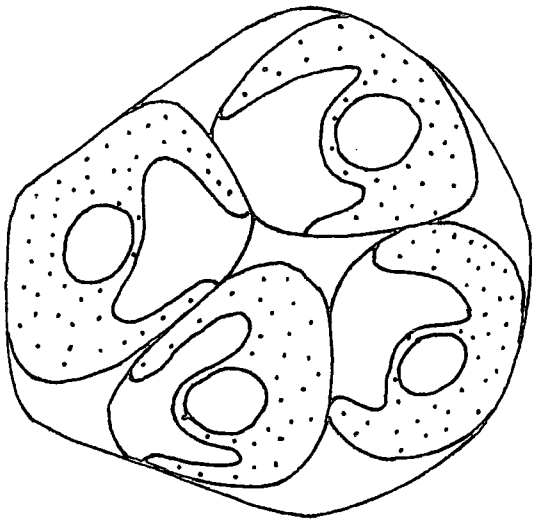


FIG 5.43 Zoosporangium of Chlorococcum sp.

Chlorella

Members of this genus were by far the most common algae found in colliery spoil. They were isolated from every site studied and were present in large numbers. There were numerous different forms present which varied in cell shape, cell size, presence of pyrenoid and form of chloroplast, however, all had the essential features of the genus i.e. they were solitary green unicells which reproduced by the production of autospores that were liberated by the rupture of the parent cell wall.

Chlorella sp.1

The smallest organism observed was an average of 2.5 μm in diameter with a few cells reaching 5 μm in diameter. Most cells contained two distinct chloroplasts which lacked a pyrenoid, however, in larger cells one chloroplast was often lobed so that it appeared to have three chloroplasts (Fig. 5.44).

Chlorella sp. 2

Most members of this genus were between 5 and 7 μm in diameter. Some had a tendency to form oval cells and contained a single pyrenoid, (Figs 5.45, 5.46). Others were always spherical and always lacked a pyrenoid (Fig. 5.47)

Chlorella sp. 3

The largest member of the group observed, was 15 μm in diameter, with a single chloroplast and no pyrenoid. The chloroplast was frequently lobed (Fig. 5.48).

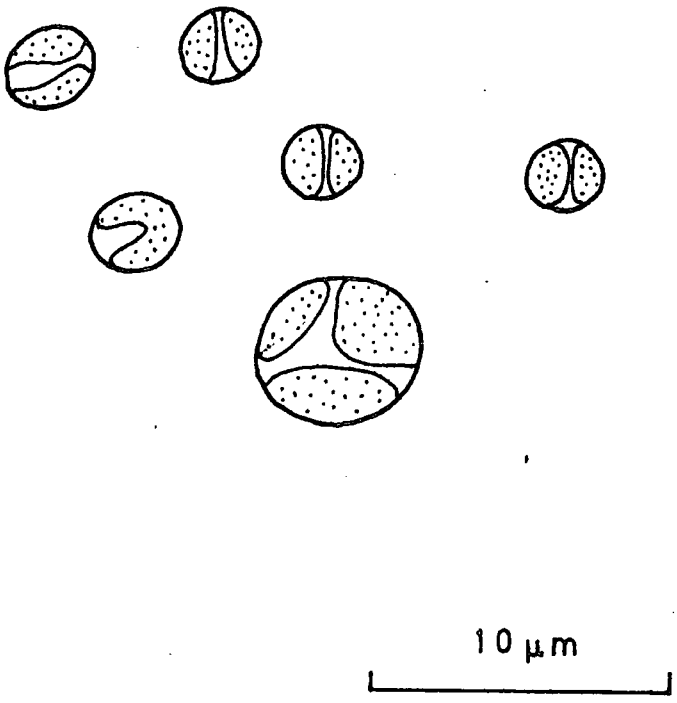


FIG 5.44 Chlorella sp. 1

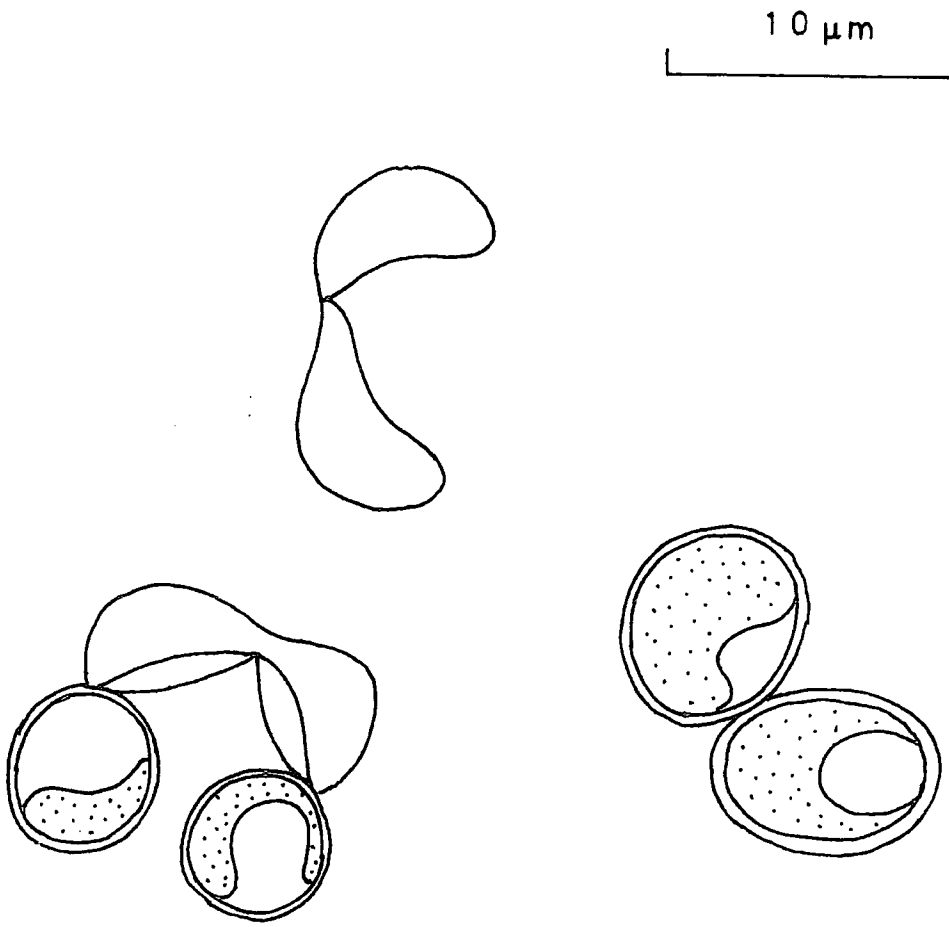
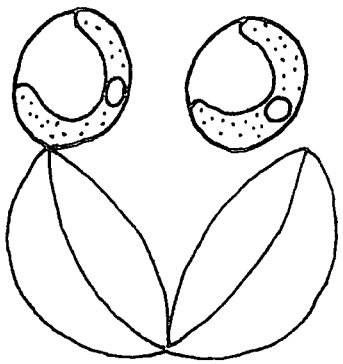


FIG 5.45 Chlorella sp. 2



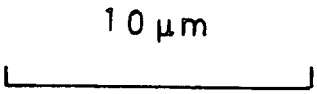


FIG 5.46 Chlorella sp. 2 release of autospores

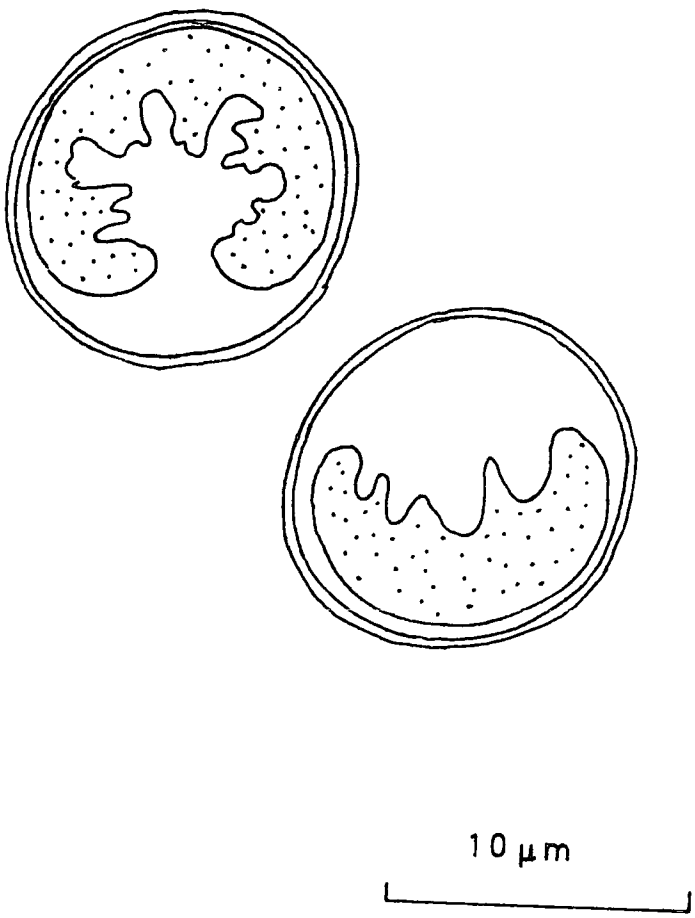


FIG 5.47 Chlorella sp. 2

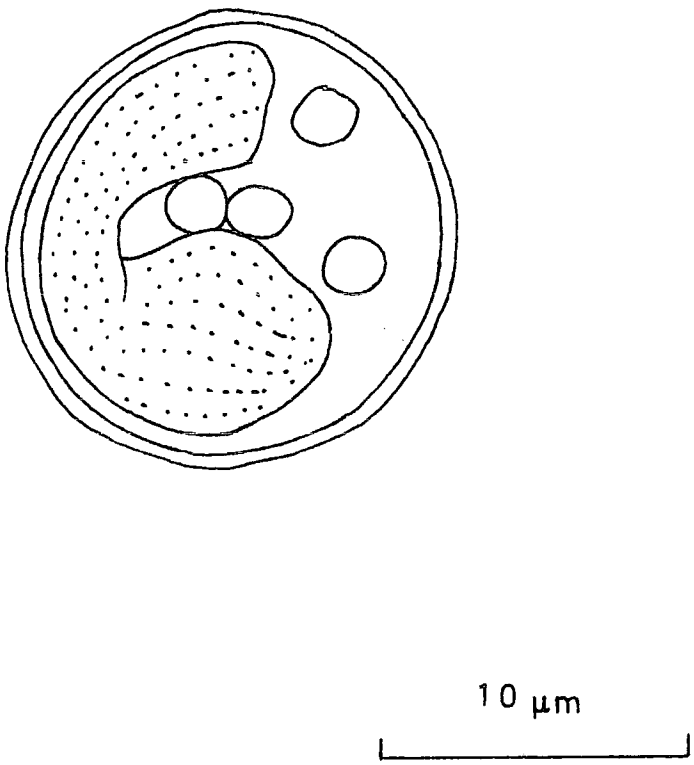


FIG 5.48 Chlorella sp. 3

Pseudochlorella pyrenoidosa

A single isolate from site Y1 which was originally identified as an oval Chlorella was subsequently shown in culture to accord with the description of Pseudochlorella pyrenoidosa. The cells were always ellipsoidal with a single band shaped chloroplast with a single prominent pyrenoid. It reached a maximum size of 12.5 µm in length and 6 µm in width. The organism was never seen to produce zoospores (Fig. 5.49).

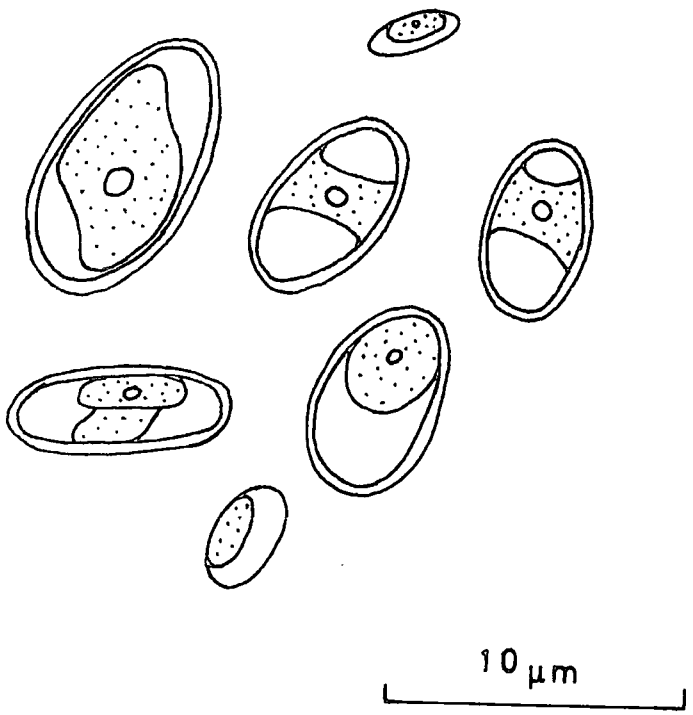


FIG 5.49 Pseudochlorella pyrenoidosa

CHAPTER 6

DISCUSSION

6.1 INTRODUCTION

The algal flora of colliery spoil at East Holywell has a number of characteristic species and the algal density shows marked seasonal variations. These phenomena may be partially explained by the prevailing levels of certain environmental variables at the site.

6.2 ALGAL FLORA OF COLLIERY SPOIL

6.21 Flora of sites Y1, G1 and G2

A number of species (11) were found equally upon shale (Y1) and washeries waste (G1 and G2) but several species were restricted to one or other spoil type (Table 4.10, p116).

This suggests that the general conditions necessary for the development of a soil algal community were fulfilled at these sites i.e. that the spoil surface was stable and not subject to severe erosion, that the moisture level in the surface layers was adequate and that the temperature never reached lethal levels. Chemically these sites were neutral and it is therefore likely that no substances reached toxic levels. The nutrient supply must have been adequate for the commoner soil algae.

The restriction of some algae to site Y1 may be due to specific nutrient requirements which are met by the shale and not by the washeries waste. Alternatively it may be due to the inability of these species to tolerate the levels of a particular physical or chemical parameter at the washeries waste sites such as shading by higher plants.

Similar explanations may well account for the

differences in flora between sites G1 and G2. However, the frequent presence of blue-green algae at site G1 and their sporadic occurrence at G2 is not readily explained. These sites are similar in their physical (Table 3.5, p 84) and chemical nature (Table 2.1, p 53) and both are colonized by Tussilago farfara. The only noticeable difference is that T. farfara at G1 is denser than at G2. This may alter the light level or the moisture level just enough to make colonization by blue-greens more likely.

All these sites are within an area of 100 m^2 and, with considerable movement of material from one part of the tip to another by wind (Section 3.32, p 81), it is unlikely that the difference between these sites is due to spatial isolation.

6.22 Flora of sites B1 and B2

At B1 the flora was reduced to two species and at B2 to just four species (Table 4.6, p 112). These sites are similar to G1 and G2 in terms of the physical texture of the spoil (Table 3.6, p 89) and pH (Table 2.1, p 53). Therefore these factors cannot be the cause of the observed differences. The major difference between B sites and G sites was that the latter were colonized by Tussilago farfara. The presence of this higher plant cover would alter the conditions at the spoil surface. In spring and summer the growth of the leaves of T. farfara shaded the surface and kept it cool and moist. In autumn, the death of T. farfara leaves supplied additional nutrients to the surface of the spoil and throughout the year the roots of T. farfara stabilized the surface. This last effect may well be the most important because in wet conditions uncolonized washeries waste is subject to severe water erosion and in dry conditions it is subject to severe wind erosion (Section 3.32, p 81).

6.23 Flora of sites P1 and P2

At P1 the flora is reduced to two species and at P2 to a single species (Table 4.6, p 112). These sites are similar to Y1 in spoil texture (Table 3.5, p 84) and none are colonized by higher plants. Therefore conditions of temperature, moisture and stability will be similar and cannot be responsible for differences between these sites. However P1 and P2 are both sites with a low spoil pH (Table 2.1, p 53). Therefore the reduced flora may be due to lower pH directly or may be due to some substances reaching toxic level in acid conditions. Alternatively, the availability of plant nutrients, especially phosphorus, may be reduced in acid conditions (Section 1.223, p 27) and this may be limiting the development of a flora.

6.24 Flora of site Y2

Site Y2 had a flora consisting of only five species. This site is similar to Y1 in pH, spoil texture and degree of higher plant colonization, therefore differences in moisture level, temperature or acidity seem unlikely to be the cause of the differences between these sites. Colliery spoil however, is very variable in its chemical composition (Section 1.22, p 23) and it may well be that the spoil at this site is deficient in essential plant nutrients or contains some substance which is toxic to soil algae. More detailed measurement of chemical parameters is necessary to explain differences such as these.

6.3 VARIATIONS IN ALGAL DENSITY IN COLLIERY SPOIL

6.31 Effect of methods employed upon estimate of algal density

6.311 Storage of samples

Samples of spoil collected each month were stored in an air dry condition (Section 2.33, p 59). This storage was necessary because of a delay in perfecting the standard culturing technique and resulted in samples being cultured five months after they had been collected. It was very likely that this treatment would alter the number of algae in the sample and therefore in order to quantify this effect, the sample for June 1980 was cultured immediately after air drying and again after five months storage. The results (Tables 4.1, 4.2 and 4.3) show that in spoil from all sites the algal density was reduced by storage. The sites which were of particular importance were G1, G2 and Y1 because these contained algae at a measurable density. These results suggest that the figures for algal density at site G1 and G2 in the main survey may be only half the number of algae which were in the spoil when it was first collected and the results for site Y1 may represent only a quarter of the algae present at collection.

6.312 Culture preparation

The culture technique and culture medium can influence the types and number of algae isolated from a spoil sample (Sections 1.32, p 32 , 2.4, p 62 , 2.5, p 64). The agar plate technique used gives conditions of moisture, temperature and light which approximate to the conditions at the soil surface. However nutrient supply on the surface of mineral salt agar is likely to be much more favourable than in the soil and certainly more generous than on the surface of colliery spoil (Section 1.223, p 27). Many of the algae which grow

to form colonies on agar plates may perhaps not be growing actively in the spoil, but present simply as spores.

In the course of preparation of dilution cultures spoil and algae were subjected to relatively vigorous handling. The grinding, sieving and blending of spoil with water (Section 2.61, p 66) may well have resulted in the destruction of some algal cells. However it may also have caused filamentous algae to fragment and reproductive bodies to rupture. Whatever effects this treatment may have had they applied equally to all samples.

In the case of blue-green algae the technique was really inappropriate to the estimation of their abundance. The majority of blue-greens isolated were filamentous forms with a thin mucilaginous sheath such as Lyngbya sp. Such algae would not be readily separated by the agitation used and may well have fragmented irregularly. Furthermore their rapid spreading growth over the surface of agar plates presented great difficulties in counting (Section 2.63, p 70). Therefore the numbers presented in Fig. 4.7, p109 can be taken only as a general indication of their abundance.

6.32 Sites with low algal density

Numerous studies have been made of the algae in soils from many parts of the world (Section 1.31, p 31). In these accounts most soil types in temperate regions and a number of tropical soils have been described. Throughout these reports it is extremely unusual to find accounts of soils which are algae free. In this survey none of the sites were completely devoid of algae for the whole year but spoil from site P1 contained no algae for six months of the year, site B1 contained no algae for seven months of the year, site B2 contained no algae for four months of the year and site Y2 contained no algae

for one month. At other times the algal density of these sites was extremely low and reached a maximum of only 1.43×10^3 cells g^{-1} at site Y2 in September 1979. At sites B1, B2 and P1 the algal density never exceeded 1.0×10^3 cells g^{-1}.

An attempt was made to measure the algal spore input from aerial plankton at these sites. The results (Appendix 2, p225) showed that P1, B1 and B2 were receiving an average of 2 cells cm^{-2} day^{-1}. Thus even the very low populations recorded at these sites were greater than can be accounted for by aerial plankton accidentally falling on the surface. Site Y2, which was on the western rim of the tip and received the full force of the prevailing westerly winds showed an aerial plankton input of 21 cells cm^{-2} day^{-1}. Although this is 10 times the rate of input at other sites it still seems insufficient to account for the number of algae in the spoil. Therefore it seems likely that some algae are growing in the spoil at these sites during the spring and summer.

The development of a substantial algal population at sites B1 and B2 may have been limited by adverse physical conditions or by a limited nutrient supply (Section 6.22, p188). Like these sites, site P1 was devoid of higher plants but was a shale site. The low level of algal density here was probably due to chemical rather than physical factors. The shale provided a stable substrate but the pH was recorded at 3.8. Several workers have noted a reduction in algal density at low pH sites, especially MacEntee (1970) who described a very restricted and depauperate flora on acid strip mine spoil in N-E. Pennsylvania, U.S.A. When it is recalled that low pH in colliery spoil may be associated with toxic levels of several metals (Section 1.222, p 26) and with restricted availability of some nutrients

(Section 1.223, p 27), it is not surprising that very few algae were present at this site. Site P2, for which only incomplete data are available had similar physical and chemical characteristics to site P1 and showed a similar pattern of variation in algal density.

6.33 Algal density on stable washeries waste

Sites G1 and G2 were on washeries waste which was stabilized by Tussilago farfara. This was the only higher plant growing on this material and was associated with a limited number of mosses (Section 3.41, p 88). Both sites had high algal densities at all times of year. However, site G1 had a number of blue-green algae in the samples. In presenting the results for this site (Fig. 4.2, p 97 & Table A1.2, p 214) it was decided to subtract the number of blue-greens from the total to give a figure for green algae and diatoms. This was done for two reasons. Firstly figures for blue-greens can only be taken as a general indication (Section 6.312, p190) and secondly this procedure gives figures which may be compared with those for site G2 at which blue-greens did not form a significant element in the flora . When sites G1 and G2 are compared, it is seen that the density of green algae and diatoms in the spoil at each site is broadly similar (Table 6.1, p194).

TABLE 6.1 Comparison of the range of algal density at sites G1 and G2

	site G1	site G2
maximum density of green algae and diatoms (cells $g^{-1} \times 10^3$)	90.1 ± 13.9	80.0 ± 13.9
minimum density of green algae and diatoms (cells $g^{-1} \times 10^3$)	12.1 ± 3.7	31.4 ± 11.3

The high algal densities throughout the year at these sites indicate that washeries waste colonized by T. farfara provided a suitable environment for algal growth. The marked contrast with B sites on uncolonized washeries waste may be due to the amelioration of the physical conditions at the surface or to nutrient enrichment by the decaying T. farfara in the autumn (Section 6.22, p188).

6.34 Algal density on bare neutral shale

Sites Y1 and Y2 were both site with a pH of between 7.0 and 8.0 and both were devoid of vegetation. They were adjacent to areas showing natural colonization and supporting floras similar to that described in Section 3.42, p88 . Y2 has already been referred to in Section 6.31 because it supported a very limited algal population. In contrast site Y1 had a high algal density throughout the year. The mean algal density for the period March 1979 to February 1980 was 58.9 x 10^3 algal cells g^{-1}.

The physical conditions of these two sites were similar and the difference is likely to be due to differences in the chemical composition of the spoil (Section 6.24, p189).

6.4 SEASONAL PERIODICITY OF ALGAE IN COLLIERY SPOIL

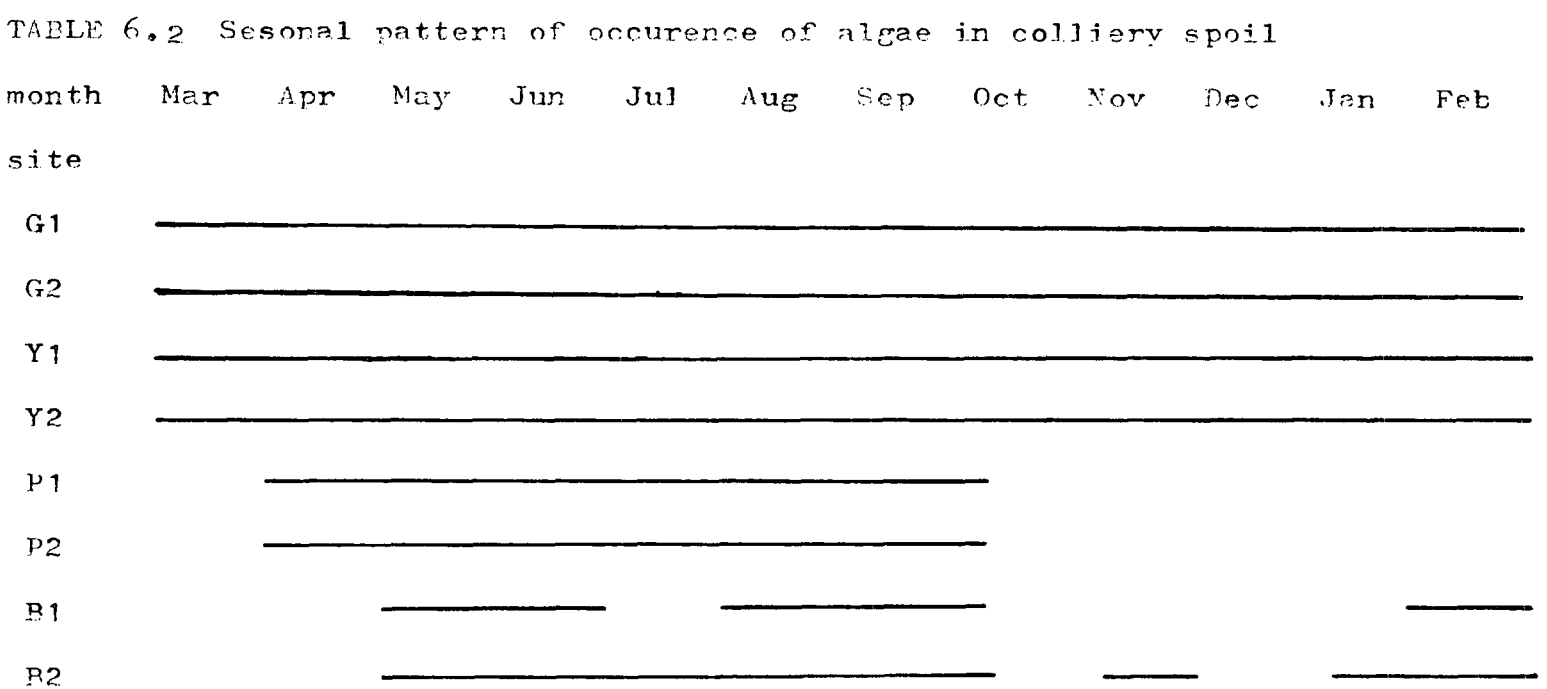
6.41 Pattern of occurrence of algae in spoil

There was a clear seasonal pattern to the isolation of algae from several sites (Table 6.2). While algae were isolated throughout the year from G1, G2, Y1 and Y2., at the other sites algae were only present at certain seasons.

Sites G1, G2, Y1 and Y2 were the sites at which most algal species were isolated and where the highest algal densities were recorded. At these sites conditions were most favourable to algal growth and seasonality was expressed in a variation in algal density (Sections 6.42, 6.43, p198).

At sites P1 and P2 algae were isolated only between April and September. These were acid sites from which few species were isolated and at which algal densities were always very low. It has been suggested (Sections 6.23,p189, 6.32, p191) that the depauperate flora and low algal density may be due to the low pH

TABLE 6.2 Sesonal pattern of occurence of algae in colliery spoil



and consequent toxic levels of metals or nutrient deficiencies. However there is evidence to suggest that spoil pH is lower during the summer (Stokes, 1940 and Baver, 1927) and measurements of mine on incubated spoil confirm that the pH of the spoil from these sites does drop at higher temperatures. April to September is of course the time when temperatures are highest (Fig. 3.3, p 83). It may therefore be that during the summer the effect of low pH is sufficient to limit the species which can survive in this spoil but not such as to totally preclude the growth of algae. In winter the combination of adverse chemical and physical conditions may be so unfavourable that no algae can survive in the spoil.

At site B1 algae were present in the spoil in a more irregular pattern. The months when algae were isolated were those in which there was a considerable rainfall during the previous 28 days and when the mean temperature was higher (Fig. 3.3, p 83). This suggests that the growth of algae at this site was only totally precluded by lack of moisture and is merely limited by instability or lack of nutrient (Section 6.22, p188). This view is supported by the absence of algae from the spoil in July 1979 when there was a short midsummer drought (Fig. 3.3, p 83).

At site B2 a pattern similar to that at B1 was recorded and as these sites are so similar it is probable that it may be explained in the same way.

6.42 Variations in algal density at site Y1

At site Y1 there was a steady increase in the algal density between March and June (Fig. 4.4, p100). A full statistical analysis was not undertaken but the difference between monthly values which were thought to be important was tested using a t test.
t was compute as

$$t = \frac{(\bar{x}_1 - \bar{x}_2) - 0}{\sqrt{\frac{\sum(x_1 - \bar{x}_1)^2 + \sum(x_2 - \bar{x}_2)^2}{N_1 + N_2 - 2}} \times \sqrt{\frac{1}{N_1} + \frac{1}{N_2}}}$$

The difference between the mean algal density in March and the mean algal density in June was significant ($t = 5.57$, d.f. = 14, $p = < 0.01$). The mean algal density showed a small decrease between June and July when there was a short summer drought. This difference was not significant ($t = 1.76$, d.f. = 14, $p > 0.1$). However it is consistent with a decrease in the algal population at other sites at this time of year (Section 6.41) and is therefore probably a real effect. The algal density at this site reached its maximum value in September 1979 and this was followed by a decline in October and November and a second peak in December.

Y1 was completely barren and therefore there was no higher plant cover to modify the conditions at the surface of the spoil. The major influences upon the surface conditions must have been the rainfall and temperature. Comparison between Fig. 4.4, p100 and Fig. 3.3, p 83 confirms that the pattern of change in algal density closely follows the pattern of weather

conditions favourable to algal growth.

6.43 Variation in algal density at G sites

If a comparison is made between the pattern of change in algal density at site G2 (Fig. 4.3, p98) and the pattern at site G1 (Fig. 4.2, p97) it can be seen that they are broadly similar. The variations are less dramatic than at site Y1 and this may be due to the presence of a cover of higher plants which may result in less variation in the conditions at the surface of the spoil. At site G2 there is a steady increase in algal density between April and July. The difference between these two months is significant ($t = 6.1$, d.f. = 14, $p < 0.001$). This is followed by a decrease between October and January.

At site G1 there was an increase in algal density between March and June followed by a decrease in July. The difference between June and July is significant ($t = 4.13$, d.f. = 14, $p < 0.002$). This once again coincides with the mid-summer drought. The algal density reaches a peak in September and then decreases until January. Therefore the general pattern at these sites is of an increase in algal density in the spring and summer and a decrease in the autumn and winter. The decrease in July at site G1 coincides with a similar decrease at site Y1 (Section 6.42, p198) and the failure to isolate algae from some sites (Section 6.41, p195). No similar decrease occurred at site G2 and there is no readily apparent explanation for this. It may be that the species present at G2 are less susceptible to drought conditions or that the spoil at this site dried out more slowly.

6.44 Seasonal variation in Ulotrichales

At sites G1 and Y1 a clear pattern of seasonal variation in the density of Ulotrichales in the spoil was recorded (Section 4.13, p104). This followed the general pattern of variation in total algal density at these sites (Sections 6.42, 6.43). Site G1 showed a steady increase between May and September and steady decrease between September and January. The uncolonized site Y1 once again showed more dramatic variations with peaks in the population in May and August. At both sites there was a drop in the density of these algae coincident with the mid-summer drought in July. At site Y1 this drop was clearly significant but at site G1 it was not statistically significant ($t = 0.9$, d.f. = 14, $p > 0.1$). The general pattern is similar to that for the total algal density at these sites.

6.45 Variation in Cyanophyta

The pattern of variation in Cyanophyta seemed to be more closely linked to the available moisture. The three peaks which occurred in the density of these algae coincided fairly closely with the three occasions when the rainfall during the preceeding 28 days was at its highest i.e. June, September and December 1979. It is perhaps not strictly accurate to call the variation in Cyanophyta seasonal because a season is defined in terms both of rainfall and temperature. However these algae do clearly respond to changes in weather conditions.

6.5 SIMILARITY OF EAST HOLYWELL TO OTHER SITES

6.51 Physical conditions

The shale at East Holywell is considerably less stony than newly tipped shale, which according to Rimmer (1978) characteristically contains only 25% - 30% of material with a particle size > 2 mm. Furthermore, four samples of shale analysed by Molyneux (1963) had "stone" and gravel fractions which ranged from 40% - 63%. However, Richardson (1973) estimated that weathering in the surface layers of tips resulted in an increase in the $\leqslant 2$ mm fraction by a factor of x 2.5 in 20 years. As this tip is thought, from historical evidence, to date from 1947, a less than 2 mm fraction in excess of 70% might be expected.

The high proportion of coarse sand in the spoil at East Holywell is also notable. Three of the samples analysed by Molyneux had less than 10% of the fine earth made up of such particles. One of his sites had up to 43% of such particles and this he associated with the fact that the material had been burnt. The heap at East Holywell is burning at present time and evidence suggests (Section 3.2, p 77) that the original heap was also burning. Therefore the shale at East Holywell appears to be similar in composition to that described from other sites which have been subject to burning and have weathered for 33 years.

Washeries waste does not seem to have been the subject of any other investigations. As a soil forming material it has very special physical properties. These stem from the total absence of > 2 mm particles and the approximately equal proportions of coarse sand and clay particles. The occasional "stones" which do occur in this material seem to have been added by the action of animals or deposited by run-off water from the surrounding rim. The results of this unusual particle

size distribution are that the material quickly becomes waterlogged because of the high proportion of clay particles. In fact even light rain produces pools on the surface of the tip. In dry conditions however, the surface does not become hard like clay but remains soft and powdery due to the high proportion of coarse sand. Both these properties reult in an unstable surface, subject to severe sheet erosion. In wet conditions a considerable amount of run-off will occur with the consequent removal of surface material and in dry conditions wind erosion will remove the powdery surface. This later effect has never been quantified but observations of mine (Section 3.32, p 81) suggest that it is of considerable importance.

The shale parts of the heap at East Holywell are also subject to erosion. The sides of the main heap are steep and where they are devoid of vegetation there is severe gully erosion. The vegetated areas show clear evidence of terrace slip. Therefore all parts of the tip with exception of the small flat areas covered with vegetation, have an unstable surface.

6.52 Chemical conditions

Seasonal variations have been reported in soil pH (Section 1.221, p 24). Baver (1927) recorded a steady drop in the pH of acid soils in Ohio, U.S.A. from April to September, followed by a rise to the original value by the spring. As measurements were taken in September 1978 at East Holywell, it is likely that the reported values are the extreme low points of the pH on acid areas at these tips.

The shale making up the sides and rim of the heap had a pH ranging from 3.2 to 7.4 and a mean pH of 5.0. The pattern, of groups of samples with radically different pH, is the result of material with very

different chemical properties from different parts of the mine being dumped next to each other. This is just what would be expected as the spoil will vary as mining operations moved through different strata.

Shale at East Holywell is typical of that found throughout the Northumberland and Durham coalfield (Section 1.221, p 24). As there is strong evidence that the shale here has been burnt it is to be expected that the pH of these sites will steadily rise due to leaching and the absence of potential acidity as a result of the combustion of the sulphur compounds. No evidence of such a change was obtained but the time scale of three years is probably too short to detect such changes.

The washeries waste is much less variable than the shale. The pH ranges from 6.2 to 7.6. This also is to be expected because this material has a common geological origin. Isolated low pH values on washeries waste were always associated with sites where the material was mixed with substantial quantities of shale.

6.53 Biological status

The shale community at East Holywell fits almost exactly the description of a typical pit heap flora in N-E. England given by Greenwood (1963) (Section 1.23, p 28). Of the five species Greenwood found on all eight of the pit heaps which she studied, all were present at East Holywell. Of the eleven species which she recorded as very common, ten were found at East Holywell.

The present state of the vegetation on this heap is consistent with natural colonization over a period of 35 years and is halfway through the succession to woodland or scrub described in Section 1.23, p 28 .

Worthy of particular note was the rich moss layer at this site. It covered the shale surface in all

places where colonization had occurred and was the most visually distinctive feature of the vegetation.

6.54 Algal flora

Several workers have reported the number of genera isolated from different colliery spoils as a measure of the diversity of their algal flora. On this basis it is possible to compare the spoil at East Holywell to other sites (Table 6.3, p205). The picture which emerges from this is that colliery spoil supports a rather restricted algal flora. The most diverse site was that in N. Dakota, U.S.A. At that site 21 genera were recorded but even this was reduced in comparison with an adjacent unmined soil which contained 33 genera. At the English sites and at the acid site in N-E. Pennsylvania, U.S.A. the flora was dominated by Chlorophyta with a few diatoms and a limited number of Cyanophyta. At only the N. Dakota site were there substantial numbers of Cyanophyta.

TABLE 6.3 Comparison of diversity of algal floras on colliery spoils

site	total genera recorded	Chlorophyta	Xanthophyta	Bacillariophyta	Cyanophyta
East Holyell shale	15	13	-	?	-
East Holywell washeries waste	19	15	-	1	3
N-E. Pennsylvania U.S.A. acid strip mine spoil (McEntee, 1970)	18	16	1	-	1
N. Dakota U.S.A. 30 year old strip mine spoil (Shubert & Starks, 1979)	21	7	1	3	10
Wardley N-E. England (Broady, 1979)		only Chlorophyta recorded			
grass covered shale	13				
sparce vegetation on shale, pH 6.2	11				
sparce vegetation on shale, pH 4.6	9				
barren shale pH 6.5	5				

When a comparison is made on a similar basis to other soils it can be seen that the algal flora of colliery spoil is much less diverse than that of woodland or arable fields and similar in diversity to that of sand dunes (Table 6.4).

TABLE 6.4 Comparison of diversity of algal flora in other soils

site	total number of genera recorded
East Holywell shale	15
East Holywell washeries waste	19
arable field (Broady, 1979)a	22
Rothampstead classical grassland (Broady, 1979)a	23
garden soil (Broady, 1979)a	21
dune (Broady, 1979)a	12
woodland (King and Ward, 1977)	46
unmined site adjacent to spoil heap (Shubert and Starks, 1978)	33

6.55 Algal density

The results for algal density at East Holywell may be compared to those obtained by Broady (1979) for colliery spoil at Wardley, N-E. England. He reported algal densities of 2.5×10^6 cells cm^{-2} on colliery shale closely covered with grass and 1.4×10^6 cells cm^{-2} on shale covered with a sparse vegetation. On barren shale he reported 1×10^3 cells cm^{-2}. At East Holywell all the shale sites were uncolonized by higher plants and three of them, Y2, P1 and P2 had algal densities similar to those of Broady's uncolonized site. However site Y1 had a maximum algal density of 164×10^3 cells g^{-1}. Allowing for the different method of expressing the results this is not very different from Broady's value for colonized sites.

The densities recorded at sites G1 and G2 (Tables A1.2 and A1.3, p 214) showed maxima of 132.5×10^3 cells g^{-1} and 78.1×10^3 cells g^{-1} respectively. When these figures and those for site Y1 are compared to those for other soils (Section 1.32, p 32) it can be seen that they are similar to those reported for a range of natural soils and rather lower than the figures for most cultivated soils.

6.6 SUMMARY

The algal flora recorded at East Holywell was similar to those described by McEntee (1970) in N-E. Pennsylvania, U.S.A. and Broady (1979a) in N-E. England but different from that described by Shubert and Starks (1978, Starks and Shubert 1979) in N. Dakota, U.S.A. (Section 6.54, p 204). Although it was reduced in diversity compared to other soils, the algal density of favourable sites was similar to that of many natural soils (Section 6.55, p 208).

The pattern of seasonal change in the occurrence and abundance of algae in colliery spoil was readily

discernable. The general increase in algae in the spoil in spring and late summer, the decrease coincident with the mid-summer drought and the general decrease in the autumn confirmed the observations of previous workers (Section 1.34, p 41).

The comparison of the results for algal diversity, algal density and seasonal variability enable some assessment to be made of the importance of various factors affecting the distribution and abundance of soil algae. Previous work has indicated that available moisture is the most important factor (Section 1.33, p 37). The following observations all seem to be due to differences in available moisture and confirm the prime importance of this factor:-

a. the contrast between B sites and G sites in algal diversity (Section 6.22, p188) and algal density (Section 6.32, p191);
b. the fluctuation in algal occurrence at site B1 (Section 6.41, p195);
c. the reduction in algal density coincident with the mid-summer drought at site B1 (Section 6.41, p195), site Y1 (Section 6.42, p198), site G1 (Section 6.43, p 199) and amongst Ulotrichales (Section 6.44, p 200);
d. the variation in the abundance of Cyanophyta (Section 6.45, p200).

Other factors seem to limit the species which may colonize a soil and the density of algae which may develop in a soil but none seem to eliminate all algae from the soil as lack of moisture does.

The effect of pH cannot usually be separated from the effect of available moisture (Section 1.33, p 37) but in this study sites Y1 and P sites had similar moisture conditions but different pH. The results confirm those of McEntee (1970) that the diversity of the soil algal flora is substantially reduced at low pH

sites and also show that algal density is reduced. The mechanism by which algal growth is limited at such acid sites is not clear and more detailed measurement of chemical parameters is needed to help clarify this point.

In England the effect of temperature (Section 1.33, p 37) seems to be limited to affecting the rate of algal growth through metabolism. At no time must the surface temperature have reached lethal levels for the highest temperature was recorded in August 1979 and at no site was there a reduction in algal density.

It became clear as a result of the comparison between G and B sites that the conditions on the surface of the soil are substantially changed by the presence of a cover of higher plants and that this directly affects the soil algal flora. In the light of these results, washeries waste would provide an opportunity for controlled experiments to examine the interactive effects of shade, moisture and nutrients on the algal flora.

Finally it is possible to comment upon the ecological role of soil algae in colliery spoil. Newly tipped colliery spoil has been likened to volcanic ash in that it begins totally devoid of plant life (Section 1.4, p 43). It was supposed that soil algae would be important in colonizing barren shale as Starkey and Shubert (1978) have reported. Broady (1979a) had cast doubt upon the possibility of this as a result of the very low algal density which he measured in barren shale at Wardley, N-E. England. The results of this work suggest that at least at one site (Y1) algae were acting as primary colonizers. However they clearly do not always fulfil that role, for on washeries waste, substantial algal populations only appear in the spoil which has already been colonized by higher plants.

The action of algae in stabilizing the surface of spoil and thus reducing erosion, reported from other

situations (Section 1.42, p 46) is not clear cut on the surface of colliery spoil. It is true that the surface of washeries waste with a high algal density did not show that intricate pattern of erosion channels visible at sites with a low algal density, but on the surface of shale no difference could be detected between sites with algae and those without.

In general it is clear that on colliery spoil many sites are near the limits of the conditions under which soil algae can survive. However on the more favourable parts of colliery spoil heaps algae do play a part at the beginning of natural colonization.

Appendix A1

DETAILED MONTHLY ESTIMATES OF ALGAL DENSITY AT EACH SITE

TABLE A1.1 Monthly estimates of algal density at site G1 (cells g^{-1} x 10^3)

Month	March 79	April 79	May 79	June 79	July 79	Aug 79	Sept 79	Oct 79	Nov 79	Dec 79	Jan 80	Feb 80
Replicate												
G1/1/0.5	16	20	44	104	52	102	138	92	82	214	74	64
G1/2/0.5	18	14	2 4	120	80	74	120	94	72	194	66	72
G1/3/0.5	12	14	44	106	48	86	134	122	76	162	64	54
G1/4/0.5	16	18	48	106	56	76	130	76	76	164	78	32
G1/1/1	16	20	34	119	53	83	128	97	67	-	61	57
G1/2/1	7	22	29	126	59	76	156	85	78	-	65	38
G1/3/1	12	24	29	105	43	77	139	93	63	-	49	64
G1/4/1	19	15	34	99	53	75	115	98	56	-	71	55
Mean	14.5	18.4	35.75	110.6	55.5	81.1	132.5	94.6	71.3	183.5	66.8	54.5
	± 3.9	± 3.8	± 8.6	± 9.6	± 11.0	± 9.4	± 12.6	± 13.2	± 8.7	± 25.1	± 8.9	± 13.5

TABLE A1.2 Monthly estimates of algal density minus blue-greens at site G1 (cells g^{-1} x 10^3)

Month	March 79	April 79	May 79	June 79	July 79	Aug 79	Sept 79	Oct 79	Nov 79	Dec 79	Jan 80	Feb 80
Replicate												
G1/1/0.5	16.0	20.0	43.0	58.0	52.0	92.0	94.0	68.0	62.0	104.0	48.0	40.0
G1/2/0.5	18.0	12.0	22.0	88.0	78.0	68.0	82.0	78.0	44.0	30.0	30.0	38.0
G1/3/0.5	10.0	14.0	44.0	82.0	46.0	74.0	84.0	88.0	48.0	36.0	36.0	34.0
G1/4/0.5	12.0	16.0	43.0	101.0	56.0	64.0	94.0	50.0	50.0	34.0	40.0	16.0
G1/1/1	11.0	20.0	34.0	88.0	46.0	70.0	80.0	68.0	46.0	66.0	34.0	44.0
G1/2/1	6.0	22.0	27.0	83.0	50.0	65.0	120.0	60.0	55.0	61.0	32.0	18.0
G1/3/1	11.0	24.0	29.0	78.0	37.0	72.0	92.0	69.0	40.0	58.0	27.0	44.0
G1/4/1	13.0	15.0	26.0	68.0	47.0	68.0	75.0	64.0	35.0	70.0	44.0	33.0
Mean	12.1	17.9	33.5	79.5	51.5	71.6	90.1	69.0	47.7	56.7	36.1	33.1
	± 3.7	± 4.2	± 8.8	± 14.9	± 12.0	± 8.9	± 13.9	± 11.5	± 7.2	± 24.5	± 7.2	± 10.9

TABLE A1.3 Monthly estimates of algal density at site G2 (cells g^{-1} x 10^3)

Month	March 79	April 79	May 79	June 79	July 79	Aug 79	Sept 79	Oct 79	Nov 79	Dec 79	Jan 80	Feb 80
Replicate												
G2/1/0.5	56	22	70	60	78	86	-	84	58	48	22	62
G2/2/0.5	62	42	48	92	92	74	-	52	56	44	40	28
G2/3/0.5	96	32	46	62	88	112	-	58	44	52	24	40
G2/4/0.5	82	24	56	42	76	74	-	44	52	32	54	34
G2/1/1	90	52	46	65	75	70	-	53	42	39	27	39
G2/2/1	86	62	63	79	73	66	-	52	48	54	30	39
G2/3/1	88	56	6 9	80	67	62	-	57	51	55	34	38
G2/4/1	80	44	6 7	79	76	77	-	47	45	57	20	40
Mean	80.0	41.75	58.1	69.9	78.1	77.6	-	55.9	49.5	47.9	31.1	40.0
	± 13.9	± 14.75	± 10.4	± 15.7	± 8.1	± 15.6	-	± 12.3	± 5.8	± 8.7	± 11.3	± 9.8

TABLE A1.4 Monthly estimates of algal density at site Y1 (cells g^{-1} x 10^3)

Month	March 79	April 79	May 79	June 79	July 79	Aug 79	Sept 79	Oct 79	Nov 79	Dec 79	Jan 79	Feb 79
Replicate												
Y1/1/1	3	15	35	21	40	71	145	87	69	106	48	65
Y1/2/1	1	19	29	41	38	56	152	77	54	95	54	45
Y1/3/1	1	13	38	49	29	95	182	66	42	91	47	62
Y1/4/1	2	10	29	36	34	51	177	77	62	104	57	46
Y1/1/5	0.8	4.6	36	31.8	29.8	50.4	-	84.6	56.2	101.6	48	42.2
Y1/2/5	0.8	6.8	26.8	42.6	25.8	60.6	-	74.4	50.6	109.6	38.6	44.8
Y1/3/5	1	8	35	36	26.2	64.6	-	86.4	38.6	104.6	39	45.8
Y1/4/5	1.4	7.8	27.8	37.8	16.8	57.2	-	67.4	54.4	100	51.6	44.8
Mean	1.1	10.6	32	36.9	30	63.2	164	77.5	53.4	101.3	47.9	49.5
	± 0.8	± 4.8	± 4.4	± 8.3	± 7.4	± 14.5	± 16.2	± 8.1	± 9.9	± 6.1	± 6.6	± 8.8

TABLE A1.5 Monthly estimates of algal density at site Y2 (cells $g^{-1} \times 10^3$)

Month	March 79	April 79	May 79	June 79	July 79	Aug 79	Sept 79	Oct 79	Nov 79	Dec 79	Jan 80	Feb 80
Replicate												
Y2 /1/10	0.0	1.2	0.7	0.2	0.4	0.5	1.8	0.3	0.2	0.3	1.0	0.6
Y2/2/10	0.0	1.2	0.4	0.3	0.2	0.9	0.9	1.6	0.2	0.2	0.1	0.7
Y2/3/10	0.0	0.5	0.8	0.0	0.2	0.9	1.1	0.5	0.0	0.2	0.9	0.2
Y 2/4/10	0.0	0.9	0.2	0.2	0.3	1.2	1.9	0.8	0.1	0.5	0.2	1.3
Mean	0.0	0.95	0.53	0.18	0.28	0.88	1.43	0.80	0.13	0.30	0.55	0.70
	-	± 0.33	± 0.28	± 0.13	± 0.09	± 0.29	± 0.50	± 0.57	± 0.09	± 0.14	± 0.47	± 0.45

TABLE A1.6 Monthly estimates of algal density at site P1 (cells g^{-1} x 10^3)

Month	March 79	April 79	May 79	June 79	July 79	Aug 79	Sept 79	Oct 79	Nov 79	Dec 79	Jan 80	Feb 80
Replicate												
P1/1/10	0.0	0.1	0.2	0.2	0.4	0.0	0.1	0.0	0.0	0.0	0.0	0.0
P1/2/10	0.0	0.1	0.1	0.1	0.2	0.2	0.1	0.0	0.0	0.0	0.0	0.0
P1/3/10	0.0	0.1	0.0	0.0	0.3	0.1	0.0	0.1	0.0	0.0	0.0	0.0
P1/4/10	0.0	0.0	0.2	0.0	0.0	0.3	0.2	0.0	0.0	0.0	0.0	0.0
Mean	0.0	0.075	0.125	0.075	0.225	0.150	0.100	0.0	0.0	0.0	0.0	0.0
	-	± 0.05	± 0.09	± 0.09	± 0.17	± 0.13	± 0.08	-	-	-	-	-

TABLE A1.7 Monthly estimates of algal density at site P2 (cells g^{-1} x 10^3)

Month	March 79	April79	May 70	June 79	July 79	Aug 79	Sept 79	Oct 79	Nov 79	Dec 79	Jan 80	Feb 80
Replicate												
P2/1 /10	0.0	0.7	0.8	0.0	0.6	0.8	0.3	-	-	-	-	-
P2/2 /10	0.0	0.3	0.4	0.1	0.8	1.7	0.6	-	-	-	-	-
P2/3/10	0.0	0.3	0.1	0.1	0.3	1.9	0.5	-	-	-	-	-
P2/4/10	0.0	1.6	0.7	0.0	0.7	1.6	0.5	-	-	-	-	-
Mean	0.0	0.73	0.5	0.05	0.6	1.5	0.48	-	-	-	-	-
	-	± 0.61	± 0.32	± 0.06	± 0.20	± 0.50	± 0.13	-	-	-	-	-

TABLE A1.8 Monthly estimates of algal density at site B1 (cells g^{-1} x 10^3)

Month	March 79	April 79	May 79	June 79	July 79	Aug 79	Sept 79	Oct 79	Nov 79	Dec 79	Jan 80	Feb 80
Replicate												
B1/1/10	0.0	0.0	0.3	0.1	0.1	0.2	1.1	0.1	0.0	0.0	0.0	0.1
B1/2/10	0.0	0.0	0.4	0.0	0.0	0.6	0.8	0.0	0.1	0.0	0.0	0.1
B1/3/10	0.1	0.0	0.0	0.0	0.0	0.2	0.7	0.1	0.1	0.0	0.0	0.1
B1/4/10	0.0	0.0	0.3	0.2	0.1	0.1	1.1	0.1	0.0	0.0	0.0	0.1
Mean	0.0	0.0	0.25	0.08	0.0	0.28	0.93	0.0	0.0	0.0	0.0	0.1
	-	-	± 0.17	± 0.1	± 0.0	± 0.22	± 0.20	-	-	-	-	± 0.0

TABLE A1.9 Monthly estimates of algal density at site B2 (cells g^{-1} x 10^3)

Month	March 79	April 79	May 79	June 79	July 79	Aug 79	Sept 79	Oct 79	Nov 79	Dec 79	Jan 80	Feb80
Replicate												
B2/1/10	0.0	0.0	0.0	0.1	0.2	0.8	0.7	0.0	0.0	0.0	0.3	0.0
B2/2/10	0.0	0.1	0.1	0.7	0.0	0.2	0.7	0.0	0.1	0.0	0.2	0.2
B2/3/10	0.0	0.0	0.3	0.1	0.2	0.0	0.5	0.1	0.2	0.0	0.0	0.1
B2/4/10	0.0	0.0	0.5	0.0	0.2	0.4	0.3	0.0	0.0	0.0	0.1	0.0
Mean	0.0	0.0	0.23	0.23	0.15	0.35	0.55	0.0	0.08	0.0	0.15	0.08
	-	-	± 0.22	± 0.32	± 0.1	± 0.34	± 0.19	-	± 0.09	-	± 0.13	± 0.075

TABLE A1.10 Monthly estimates of density of Ulotrichales at site G1 (cells g^{-1} x 10^3)

Month	March 79	April 79	May 79	June 79	July 79	Aug 79	Sept 79	Oct 79	Nov 79	Dec 79	Jan 80	Feb 80
Replicate												
G1/1/0.5	0.0	0.0	0.0	2.0	6.0	8.0	2.0	2.0	6.0	4.0	0.0	2.0
G1/2/0.5	0.0	0.0	2.0	16.0	4.0	2.0	12.0	8.0	2.0	4.0	0.0	0.0
G1/3/0.5	0.0	0.0	4.0	2.0	0.0	8.0	10.0	10.0	2.0	6.0	0.0	6.0
G1/4/0.5	0.0	0.0	0.0	4.0	6.0	10.0	10.0	0.0	4.0	2.0	2.0	0.0
G1/1/1	0.0	0.0	2.0	10.0	4.0	3.0	6.0	4.0	4.0	2.0	0.0	6.0
G1/2/1	0.0	0.0	2.0	7.0	5.0	8.0	8.0	8.0	7.0	6.0	0.0	2.0
G1/3/1	0.0	0.0	2.0	7.0	3.0	7.0	10.0	2.0	2.0	4.0	1 .0	0.0
G1/4/1	0.0	0.0	2.0	1.0	7.0	5.0	6.0	8.0	3.0	4.0	2.0	0.0
Mean	0.0	0.0	1.75	6.13	4.38	6.38	8.0	5.25	3.75	4.0	0.625	1.25
	± 0.0	± 0.0	± 1.28	± 5.1	± 2.2	± 2.8	± 3.2	± 3.69	± 1.9	± 1.5	± 0.92	± 2.1

TABLE A1.11 Monthly estimates of density of Ulotrichales at site Y1 (cells g^{-1} x 10^3)

Month	March 79	April 79	May 79	June 79	July 79	Aug 79	Sept 79	Oct 79	Nov 79	Dec 79	Jan 80	Feb 80
Replicate												
Y1/1/1	0.0	0.0	6.0	0.0	0.0	5.0	2.0	0.0	0.0	0.0	1.0	1.0
Y1/2/1	0.0	1.0	2.0	0.0	2.0	6.0	2.0	1.0	2.0	2.0	0.0	0.0
Y1/3/1	0.0	1.0	4.0	0.0	0.0	8.0	5.0	0.0	0.0	1.0	0.0	0.0
Y1/4/1	0.0	0.0	5.0	1.0	2.0	5.0	3.0	0.0	1.0	0.0	0.0	0.0
Y1/1/5	0.0	0.0	1.2	0.2	0.4	8.0	2.0	1.6	0.8	1.2	0.4	0.0
Y1/2/5	0.0	0.8	1.4	1.2	0.2	5.4	3.0	0.8	1.2	1.2	0.4	1.4
Y1/3/5	0.0	0.1	3.6	0.0	0.6	1.8	2.8	1.8	0.4	1.0	0.8	0.4
Y1/4/5	0.0	0.1	2.8	0.8	0.0	6.2	2.8	1.2	1.4	1.0	0.1	0.2
Mean	0.0	0.45	3.33	0.4	0.725	6.05	2.83	0.8	0.85	1.3	0.38	0.38
	±0.0	± 0.44	± 1.52	± 0.5	± 0.82	± 1.3	± 0.99	± 0.73	± 0.7	± 1.27	± 0.38	± 0.54

TABLE A1.12 Monthly estimates of density of blue-green algae at site G1 (cells $g^{-1} \times 10^3$)

Month	March 79	April 79	May 79	June 79	July 79	Aug 79	Sept 79	Oct 79	Nov 79	Dec 79	Jan 79	Feb 79
Replicate												
G1/1/0.5	0.0	0.0	1.0	46.0	0.0	10.0	44.0	24.0	20.0	110.0	26.0	24.0
G1/2/0.5	0.0	2.0	2.0	32.0	2.0	6.0	38.0	18.0	28.0	134.0	36.0	34.0
G1/3/0.5	2.0	0.0	0.0	24.0	2.0	12.0	50.0	34.0	28.0	90.0	28.0	20.0
G1/3/0.5	4.0	2.0	5.0	42.0	0.0	12.0	36.0	26.0	26.0	96.0	38.0	16.0
G1/1/1	5.0	0.0	0.0	31.0	7.0	13.0	48.0	29.0	21.0	-	27.0	13.0
G1/2/1	1.0	0.0	2.0	43.0	9.0	11.0	36.0	26.0	23.0	-	22.0	20.0
G1/3/1	1.0	0.0	0.0	27.0	6.0	5.0	47.0	24.0	23.0	-	22.0	20.0
G1/4/1	6.0	0.0	8.0	41.0	6.0	7.0	40.0	34.0	21.0	-	27.0	22.0
Mean	2.4	0.5	2.25	35.75	4.0	9.5	42.4	26.9	23.75	107.5	28.4	21.1
	±2.3	± 0.9	± 2.9	± 8.2	± 3.4	± 3.1	± 5.6	± 5.4	± 3.2	±19.55	± 5.7	± 6.2

Appendix A2

ESTIMATION OF ALGAL SPORE INPUT FROM AERIAL PLANKTON

Algal spores were constantly arriving on the surface of the pit heap from the aerial plankton. Sites with very low algal density may not have contained any actively growing algae and the cells isolated may have been simply those settling out of the aerial plankton.

In order to quantify this algal spore input at the surface of the tip, sterile Chu 10 agar plates were exposed at four sites for varying periods of time. These plates were then incubated under the same conditions as the monthly dilution plates. The number of algal colonies developing upon these plates were counted as a measure of algal spore input.

The measurements were taken on 22nd. March 1980 in bright cold conditions with a light westerly wind.

The results obtained are presented in Table A2.1.

From these results it was possible to calculate a crude estimate of the number of cells cm^{-2} day^{-1} arriving on the surface of the tip.

TABLE A2.1 Estimates algal spore input at low algal density sites (cells cm^{-2} day^{-1})

	number of algae arriving ($cm^{-2}day^{-1}$)			
site	P1	Y2	B1	B2
replicate				
1	2.56	17.28	2.56	1.28
2	1.6	21.76	2.88	1.28
3	1.6	24.64	4.8	1.12
mean	1.92	21.23	3.4	1.23

SUMMARY

During a survey of the soil algae present on old coal mine spoil heaps at East Holywell, Tyne and Wear, eight sites were examined. The principle characteristics of the sites were :
B1 and B2 - uncolonized washeries waste with a pH of about 7;
G1 and G2 - washeries waste with a pH of about 7 colonized by Tussilago farfara;
P1 and P2 - uncolonized shale with a pH of about 4;
Y1 and Y2 - uncolonized shale with a pH of about 7.

The following features of the algal communities were recorded.

a. A reduced soil flora consisting of 29 species from 24 genera was present. The flora was dominated by Chlorophyta (22 species) with only a few Cyanophyta (3 species) and Bacillariophyta (2 species).

b. Most species were isolated from washeries waste which had been colonized by Tussilago farfara and from shale sites with a pH of about 7.

c. 8 species were found only at shale sites, 10 only on washeries waste while 11 species were found equally on both spoil types.

d. A very depauperate flora was present at five sites (B1, B2, P1, P2 & Y2) where less than five species of algae were recored.

e. Ulotrichales were recorded occasionally from most sites, but were most abundant at sites Y1 and G1.

f. Cyanophyta were abundant at one site (G1) on washeries waste.

g. At four sites (B1, B2, P1 & P2) no algae could be recovered from the spoil at certain times of year. Furthermore, when algae were present their density was always very low and generally did not exceed 1.0×10^3 cells g^{-1}.

h. At favourable sites on both washeries waste (G1 & G2) and shale (Y1) the density of the algae in the spoil was similar to that reported for a range of natural soils and lower than that for most cultivated soils.

i. Storage of air dry samples of spoil for five months led to a 50% reduction in the density of algae in the spoil.

j. At sites where there were algae present in the spoil throughout the year (G1, G2, Y1 & Y2) there was a general increase in algal density in spring and late summer and a decrease in autumn and winter.

k. There was a decrease in algal density in mid-summer at some sites (G1 & Y1) and this coincided with a period of drought.

l. Available moisture seemed to be the most important factor limiting colonization and controlling the density of algae in the spoil.

m. The number of species in the soil algal flora was reduced substantially at low pH sites.

n. On washeries waste the presence of a cover of higher plants was associated with an increase in both the number of species and the algal density. This was probably due to the more stable surface and increased available moisture but may possibly have been due to nutrient enrichment.

o. Soil algae did not act as primary colonizers on washeries waste; this was probably due to the extreme instability of the surface.

p. Soil algae did act as primary colonizers at one neutral shale site (Y1). The absence of any colonization at other sites may well be due to the extreme chemical and physical conditions preventing permanent colonization by soil algae.

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