SOME EFFECTS OF ADVERSE CONDITIONS

ON TWO TERRESTRIAL ALGAE

IN RELATION TO

CHINA CLAY SUBSTRATES

by

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Abstract

Aspects of the ecology and physiology of Zygogonium ericetorum and Hormidium flaccidum on china clay wastes are investigated and consideration is given to the suitability of china clay sandtips as a substrate for plant growth. Particular attention is paid to the sequence of plant colonisation and the role of the respective algae in soil formation. Effects of light, temperature, humidity and herbivores are considered.

Effects of the city Plymouth as an air pollution source, on the algal growth and colonisation of the Dartmoor china clay tips are discussed. In the laboratory, sulphur dioxide solutions of 20, 30 and 50ppm, at low pH, gave rise to an alteration in the growth pattern of H. flaccidum cultures resulting from irregular cell division and a marked increase in cell size. Electron microscopy studies of such cells showed the presence of vesicles within the chloroplast, probably indicative of an impairement of photosynthetic activity.

A structure resembling a "quasi-crystalline lamellar lattice" is described from electron micrographs of untreated material of Z. ericetorum.

The presence on Z. ericetorum of a fungal pathogen of the genus Chloridium is reported.

Note on nomenclature

Nomenclature of plants belonging to the British flora follows Clapham, A.R., Tutin, T.G. and Warburg, E.F. (1962) 'Flora of the British Isles', 2nd edition and James, P. W. (1965) 'A new checklist of British lichens', Lichenologist 3, 95- 153.

Following common practice, Calluna vulgaris (L) Hull (heather) is referred to simply as Calluna.

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1: Introduction

The china clay industry is an important one in Southwest England, an area which is otherwise largely devoted to tourism and farming. The china clay product is in high demand on the world market as an inert filler and the principal supplies come from Devon and Cornwall, and Georgia, U.S.A. Unfortunately, the extraction of the mineral yields large quantities of waste material dumped in the form of silica spoil tips and mica dams and, as at present only a very limited quantity of these waste products can be used, the area occupied by the china clay industry in the Southwest is extensive and increasing. The natural colonisation of the tips is a lengthy process and does not appear to have been studied previously.

The most important pioneer species is a filamentous green alga, Zygogonium ericetorum which is frequently associated with another filamentous green, Hormidium flaccidum. These form extensive mats over the surface of china clay waste and thus it would appear likely that they play an important part in the stabilisation of clay wastes and in soil genesis. Z. ericetorum is known to be a coloniser of bare acid soil on moorland but its possible significance on china clay waste has not previously been investigated.

The china clay workings west of St. Austell, Cornwall, are situated in a rural area more than 50 Km distant from the nearest large town. The second main workings at Lee Moor, Dartmoor, are situated 9 Km. to the northeast of Plymouth, a city of approximately 250,000. The conurbation is a significant pollutant source and its importance as such is likely to be greatly increased with the building of a proposed large powerstation at the mouth of the River Tamar. It is conceivable that an increase in the concentration of atmospheric pollutants in the area could have a damaging effect on the growth of terrestrial algae on china clay wastes at Lee Moor and that this could seriously affect the natural plant colonisation sequence on china clay waste.

The thesis is divided into three sections. The first section is concerned with the china clay industry in the Southwest, the extent of the waste problem and the general colonisation sequence with particular reference to the Drakelands Corner area at Lee Moor. The second section is concerned with the biology of the two important pioneer algae, Z. ericetorum and H.flaccidum, and the growth on china clay wastes. Being an unfavourable substance for algal colonisation, particular attention is paid to such adverse factors as dehydration,erosion and the effects of animals. The final section considers an additional adverse environmental factor namely air pollution or, more specifically, the effects of sulphur dioxide. Both effects in the field and in the laboratory, including ultrastructural changes, have been investigated.

SECTION I

CHINA CLAY IN SOUTHWEST ENGLAND

Geology, extraction and aspects of the ecology of silica sand spoil heaps.

2: Literature Review

China clay a decomposition product of granite, is widely distributed throughout the world; however, its commercial extraction is restricted to comparitively few places as deposits are frequently mixed with impurities and badly stained. In Britain, china clay is of very localised occurence and large deposits are found only in Cornwall and south Devon. However, due to the high quality, these deposits together with those in Georgia, United States, yield the bulk of the china clay used throughout the world today. Hence it may be classed as one of the most important mineral resources in this country.

Kaolin was named and used by the Chinese from the beginning of the Tang Dynasty (A.D.618) but its products were not introduced into Europe until the 16th century. The discovery of china clay in this country is credited to William Cookworthy who first found a small deposit of the mineral at Tregonning Hill, West Cornwall, in 1748 and subsequently set up china clay factories in Plymouth and Bristol. Cookworthy soon found a better supply of china clay about 6.5 Km. southwest of St. Austell in the Hensbarrow area, and this is now the chief producing area and in 1971 produced about 80 per cent of the British output. Most of the remaining 20 per cent is accounted for by the southwest Dartmoor area (Perkins, 1972). Clay mining began here in the 1830's when a large area of Lee Moor was opened. Although extraction was originally the responsibility of many small companies, today English Clays Lovering Pochin and Company (E.C.L.P.) control 90 per cent of the British output.

The process of kaolinisation has affected the granite formations of southern Dartmoor, the southern half of Bodmin Moor, the whole of Hensbarrow, the northern half of Carnmenellis and a small area of the Land's End massif (Fig 1). In this process, pressures created by disturbances in the earths interior millions of years ago forced hot acid gases and fluids up through the granite masses breaking down the felspar constituent of the granite to give a soft white powder termed Kaolinite ($Al_2O_3 2SiO_2 2H_2O$). The quartz and mica constituents of the granite remained unchanged. Because of this mode of formation, the china clay deposits are extremely deep and take the form of elongated funnel shapes.

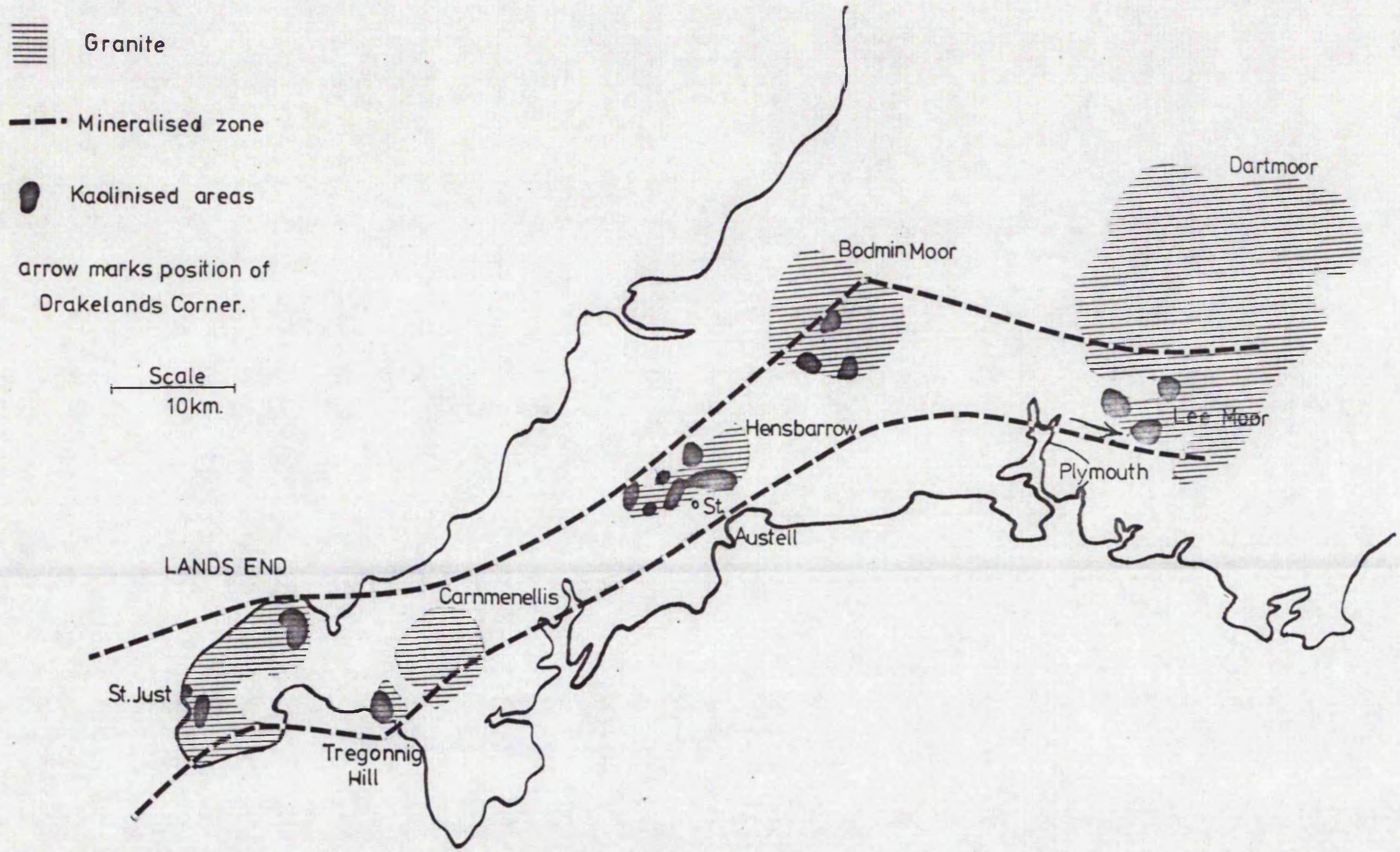

Fig 1 Map of Southwest peninsula

The extraction or "winning" process involves separating the Kaolin from the quartz and mica components, and the moorlands provide plenty of fresh, clear water for the traditional method of washing-out the deposits. Today, the "winning" of clay from the slopes is achieved by hydrostatic mining using high pressure jets of water to disintegrate the matrix. The crystals of Kaolinite take the form of hexagonal plates and it is due to this sheet-like structure,minute particle size, relative freedom from impurities, good quality colour and chemical inertness in addition to its cheapness, that china clay is so important in many industries.

The impact of china clay workings on the environment is unique amongst extractive industries. Old china clay workings have been largely left derelict and even with modern technical advances, restoration of the waste tips presents certain difficulties. The land covered by clayworkings in the Southwest is equivalent to an area of over 100 Km. and the extrapolation of the intended build-up of sand output implies rather more than a doubling in size of the existing wastetip area at Lee Moor by the end of the century, and possibly more than a quadrupling in area at St. Austell (Blunden 1975).

For every tonne of clay extracted, there are 4 to 5 tonnes of quartz, 1 tonne of micaceous residue, 1 tonne of rock and 1 tonne of overburden (ECC, 1973a). These proportions vary from area to area and from pit to pit, as well as with improving extraction techniques. In the Lee Moor area, the deposits have a higher mica content (Perkins, 1972). The tonnages of material involved are large and in the Lee Moor area alone over 8,000 tonnes of clay are extracted per week. It appears that the worlds' china clay markets will continue to grow at an increasing rate. This means increasingly large amounts of waste material in the form of quartz sand and mica slurry residues. Deposits are extremely deep and none have been exhausted so that companies are reluctant to "sterilise" the deep reserves by backfilling, even in disused pits. The mica, which is dumped in lagoons, is less of a problem than the silica which is piled into heaps. The combination of deep unrestored pits and high, mainly conical wastetips has produced the unique and characteristic china clay landscape. (Fig 2)

Fig. 2 China clay excavations at Hensbarrow, St. Austell area, showing conveyer along which coarse sand is taken to be stockpiled as large tips, as seen in background.

Fig. 3 Old china clay workings at Drakelands Corner, Lee Moor area, showing micaceous area in foreground, dominated by _Juncus_ and _Salix_ spp., and sand tips behind, supporting _Calluna vulgaris_ and _Ulex europaeus_. Distance A-B across excavated area equals approximately 80M.

Because of the cost involved in transport, there is only a limited and mainly local market for waste quartz sand (ECC, 1973b). A plant producing calcium silicate bricks has been in operation since 1966 at Lee Moor and now uses over 2000 tonnes of waste sand per week and faces a growing demand for this product. However, with 46,000 tonnes of sand accumulating a week at Lee Moor alone, this is only a small outlet (ECC,1973b). Future plans in the Lee Moor area involve large scale landscaping of worked sites and provision of flooded pits for reservoirs (ECC, 1973c).

The waste products of the china clay industry present an inhospitable substrate for colonisation and the silica waste generally remains bare of plant life "fortens of years" according to Perkins (1972). Until recently, the sand was dumped as conical tips. Fresh material was continually being added from the top which meant that it was impossible for vegetation to become established whilst tipping was in progress (ECC, 1973a). Nevertheless, the sand waste, being coarse gravel, adjusts itself to a stable slope as it is dumped whilst itsextremely porous nature ensures that there is good internal drainage (Perkins, 1972). Thus, once left, the tips are relatively stable. The new method of tipping is in terraced heaps with 24M lifts (ECC, 1973a).

Goodman (1974) has described the most important substrate factors that inhibit plant growth on china clay wastetips as the extreme degree of slope, wind erosion, low nutrient status and sheet and gully erosion. He lists instability, water stress, extremes of surface temperature and absence of soil organisms as also being relevant although of less signifance, and notes the absence of potentially toxic elements. At some earlier dumping sites, sand was mixed with overburden (top soil) and this must have assisted the colonisation of plants (ECC, 1973a). The overburden could vary in depth from one metre to 14 or 16 metres or, exceptionally, more than this (Cook, 1880).

The only detailed published work on the colonisation of china clay waste material appears to be that of Barnes and Stanbury (1951) and they were concerned with plant distribution on mica slurries. They noted that as the dams dried out, a weft of the alga Zygogonium ericetorum developedover the surface and that this stablised the surface to some extent. This was followed by Hypnum schrebei*, Spergularia rubra and Salix atrocinerea seedlings which exhibited a random distribution. Finally, they noted the appearance of Juncus effusus and Agrostis tenuis which persisted, and composed the bulk of the vegetation in well-colonised sites. Bradshaw etal (1975) describe old waste tips as becoming slowly colonised by calcifuge grasses and shrubs and mention Ulex spp., Lupinus arboreus, Salix caprea and Rhododendron ponticum. They suggest that acid oakwood is the climax.

Bradshaw etal (1975) have investigated the possibility of establishing a vegetation cover over waste sand tips. They found that the substrate, although lacking in toxic metals and containing sufficient micronutrients, was highly deficient is macronutrients and that these, if supplied artifically, were leached. Similar properties are reported by May etal (1973) for china clay wastes from Georgia and South Carolina in America. It was found possible to establish a vegetation cover by seeding with a mixture of grass seeds and clover seeds inoculated with Rhizobium spp.

* = Pleurozium schreberi

2: Methods of Investigation

The study area

A study area was chosen to investigate the problems of plant colonisation on china clay waste. The locality selected was to the south of the present Lee Moor workings on Dartmoor (Fig. 1 and 45) on an area of moorland dominated by the old workings of Hemerdon and Smallhanger (Grid. Ref. SX5860). The Hemerdon/Smallhanger group of waste tips, where most of the present work was carried out, will be referred to as the Drakelands Corner area, this being the nearest hamlet (Fig 4). It represents the most extensive area of old, undisturbed workings of open access close to Plymouth.

The Drakelands Corner area is approximately 198M. above sealevel. To the north, the Dartmoor granite rises to 492M, whilst to the south the ground drops gradually to the sea. Plymouth lies 9 Km. to the southwest.

The Hemerdon workings were opened in 1855 and the adjacent Smallhanger Workings in 1861. Both continued in operation until the 1920's when they were closed and subsequently left derelict. Just prior to closure, a great deal of waste sand was sold in the building boom at that time and consequently it is difficult to accurately date any of the remaining tips. Very few large tips remain and most of the mounds are only a few metres in height. Figs 3 and 5 show the Drakelnds Corner area and the extent of exposed china clay waste. The remainder of the area consists of moorland with Calluna vulgaris predominating. The area does not appear to have been subjected to burning so that area of heath of different ages are present. Examples of pioneer, building, mature and degenerated heath, as described by Gimingham (1972), are all present. The vegetation throughout the area is similar to that of much of the southern moor on Dartmoor but the large areas of flooded ground permit a richer variety of acid bog species to become established.

Today, the area is heavily grazed, predominantly by sheep but also by cattle and ponies. Rabbits occur in some numbers and are probably attracted by the clay waste as a suitable substrate for burrowing. Human activities take the form of horse riding and motor-cycle scrambling over the rough land whilst the pits are used extensively as dumping areas for old cars, bedsteads and

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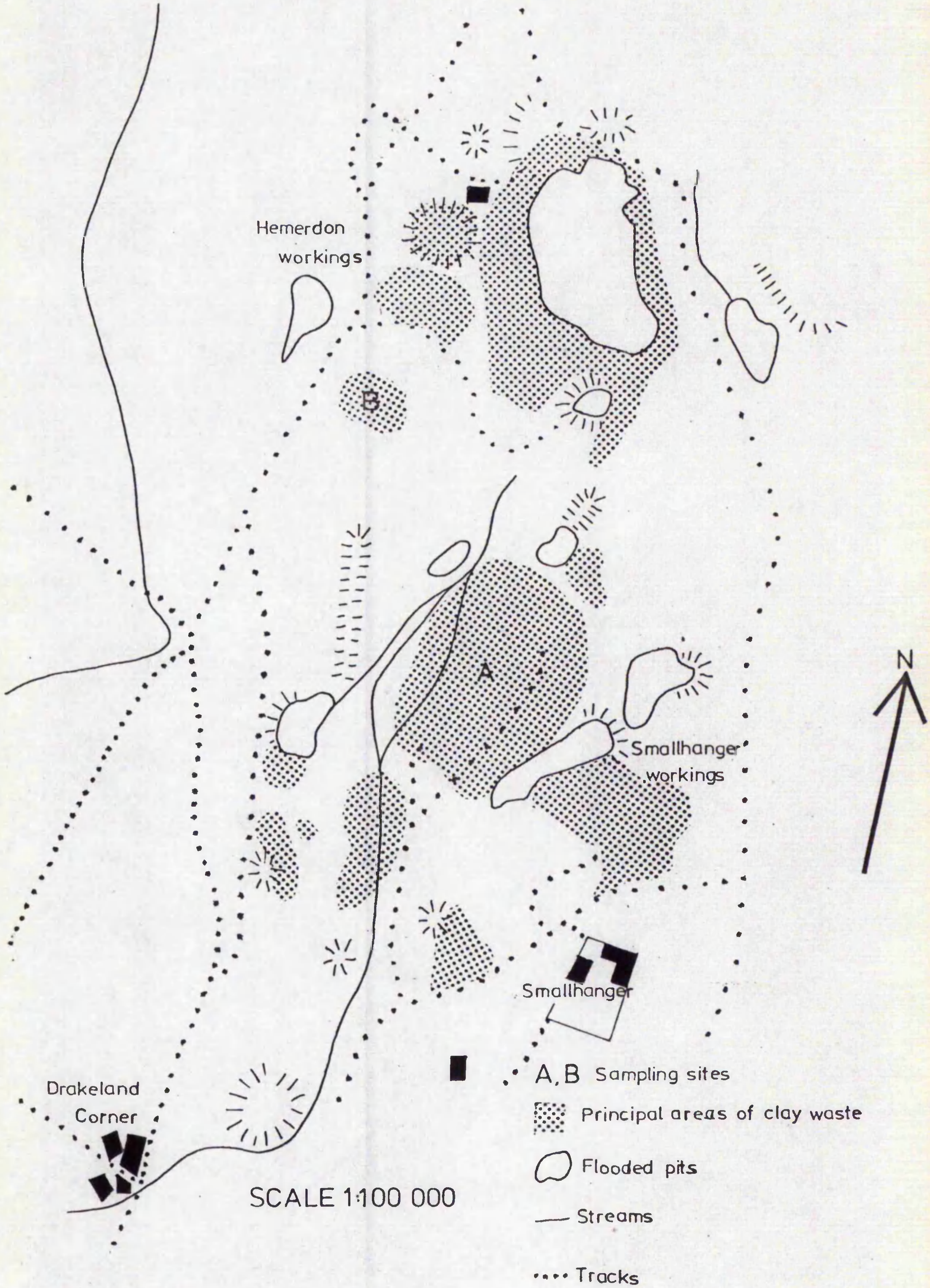
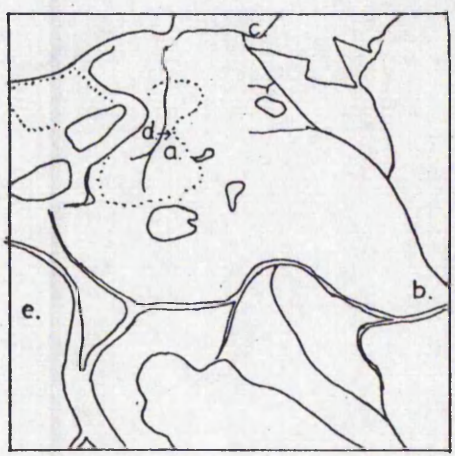


Fig. 4 Map of Drakelands Corner area.

Fig. 5 Aerial photograph of Drakelands Corner area looking south.
(photo S. Johnson)



Key.

a, b	Sites of vegetation charts, as in fig. 4.
c.	Drakeland Corner hamlet.
d	Viewpoint from which fig. 3 was taken.
e.	Active clay workings.

other unwanted articles. These activities tend to occur mainly around the north and west parts.

Initial work involved collating information on the succesional sequence of plants on old china clay waste. Barnes and Stanbury (1951) looked at the developing plant communities on mica dams but there are no similar reports for sand tips. An obvious criticism of studying colonisation patterns is that it is not possible to examine the progressive development of vegetation in one area over a sufficient period of years. There appears to be no reports of the time taken for sand tips to become naturally vegetated; Perkins (1972) simply states that the process takes "tens of years". In the present study, it has been necessary to take the approach of selecting sites which appear to show progressive seral stages. Attempts to gain some idea of the age of tips by dendrochronology of *Calluna vulgaris* stems proved unsatisfactory as it was not usually possible to obtain main stem material from the oldest plants. The oldest stems examined showed a maximum of fifteen annual rings.

Soil analyses

Soil profiles were exposed in a number of sites to the depth of the unaltered china clay substrate which varied from a few millimetres to 60cm. below the surface. From these profiles it was possible to reconstruct the gradual build-up of something approaching a soil structure from the silica waste material although it was not possible to take into account the time factor involved.

The techniques used throughout in recording edaphic factors were as outlined below, except where otherwise mentioned in the text.

Collection and treatment of soil samples

All soil samples were collected by first clearing away the ground vegetation and the top few millimetres of semi-decayed humus, where applicable, and then taking a sample from the next few centimetres. Small, similarly-sized samples were taken from six points within the site area and mixed well, the samples being from a homogeneous part of the area, both horizontally and vertically. Soil samples were analysed as soon as was practicable after collection. Any large lumps were broken up and the samples were air or oven-dried, as appropriate. For chemical estimations, samples were first passed through a 2mm. sieve to remove gravel and larger sized particles.

Mechanical analysis

The relative proportions of gravel, fine and coarse sand, silt and clay were determined by sieving to remove the larger particles and then separating the silt from the clay by differential settlement in water.

Organic matter content

The dry ashing method was used, igniting a known weight of oven-dry soil at 600°C for two hours or until constant weight was obtained.

Soil Water

(i) Field water content. The percentage loss in weight of fresh soil after heating at 105°C to constant weight.

(ii) Field capacity. The total water holding capacity of a soil obtained by standing a Gooch crucible containing a known weight of air-dry soil with its base in distilled water for 24 hours and then determining the water content of the soil.

(iii) Hygroscopic water content. The percentage loss in weight of air-dried soil heating at 105°C to constant weight.

Soil acidity

Determined electrometrically with a Beckmann Electromate pH metre using equal volumes of soil and water.

In order to determine the buffering capacity of waste sand, 14cm. diameter funnels were plugged with glass wool and filled with freshly collected sand to a depth of 5 cm. Then, 25ml. aliquots of distilled water and of water samples with pH valves adjusted with hydrochloric acid or sodium hydroxide were eluted twice through the soil column and the resultant pH value measured.

Inorganic nutrients

(i) Total acid soluble salts. Known weights of dry ashed soil were placed in 250ml. beakers with 10ml. of N. hydrochloric acid and allowed to stand for about one hour with occasional stirring. After adjusting the total volumes to 50ml. with distilled water, the mixture were filtered through weighed and dried filterpapers (grade 52). After washing, the ashsamples were dried to constant weight at 105°C and the weight of acid soluble salts was estimated.

(ii) Total changeable metallic cations. Using Brown's method (1943), 2.5g. of oven-dry soil were mixed with 25ml. N. acetic acid (pH 2.31) in a 50ml. conical flask, the flask was stoppered and the mixture allowed to stand for one hour with occasional shaking. After this time, the pH was determined as accurately as possible. The amount of exchangeable metallic cations was determined by applying the following equation

m.e. exchangeable cations per 100g. soil = (observed pH − 2.31) x 22

This was applicable up to 10 m.e. exchangeable metallic cations per 100g. soil.

Vegetation analyses

The vegetation of old china clay workings was investigated using quadrats and mapping techniques. A series of small sand tips in close proximity to each other and exhibiting varying degrees of vegetation cover was selected. Sixty point quadrats, using a frame holding six pins, were used to investigate cover at each site. Frequency was recorded on the Domin Scale in thirty-two random 10cm^2. quadrats. In addition, the more complex community characterised by Calluna in its degenerate phase was investigated using $\frac{1}{4}$m^2. random quadrats (200 samples), this size being found to be the minimum area suitable for sampling this particular community.

Two contrasting sites were selected for detailed mapping. Both these sites supported Calluna as the dominant species but at one site, the Calluna was in its "building" phase whilst at the second site it was in the "degenerate" phase. These phases, as described by Gimingham (1972), are characteristic of heath land where the bulk of Calluna plants within one area tend to be of a similar age. The building and degenerate phases give the two most characteristic vascular plant communities found on old sand-tips in the Drakelands Corner area. Stands which appeared to be representative of these two communities were selected and the position of 50M^2 quadrats within these stands was plotted using random numbers. The location of these two sites, A and B, are shown in fig. 4. Each 5m^2 quadrat was divided into $\frac{1}{2}$m^2 areas with string and the vegetation was charted within each $\frac{1}{2}$m^2 using a frame of that size subdivided into 10cm^2 units. Soil samples were taken for pH and organic content analysis at marked intervals within the charted areas.

Site A, characterised by vigorously growing Calluna, was on a slope and profiles were constructed at $\frac{1}{2}m^2$ intervals using half-metre rules.

Site B was on flat ground. It was intended to record microtopography using a point quadrat frame but the dense growth of Calluna and Ulex gallii bushes prevented accurate readings of the soil level from being obtained.

Aerial photographs of old china clay workings at Lee Moor were taken in April 1976 using a Kodak Ectachrome infra-red film in order to see whether this method could be used to differentiate between seral stages on the spoil tips.

Results of the various vegetation analyses have been presented as percentage cover tables, frequency diagrams and vegetation charts. In addition, results of quadrat investigations in the vicinity of site B have been used to prepare a simple vegetation analysis of the degenerated phase Calluna community after the method of Hopkins (1957).

4: Results

Dumped china clay waste at Drakelands Corner takes several forms, namely :

(i) Sandtips, consisting of silica waste containing scattered granite rocks. The particles are predominantly sand (2.0 - 0.2 mm diameter) and larger, and therefore the substrate is porous.

(ii) Micaceous residues, containing higher proportions of fine sand (0.2mm - 0.02 mm. diameter) and smaller flat particles . These are deposited in so-called mica dams which are close to the water table so that they are generally damp.

(iii) Overburden, consisting predominantly of large granite rocks.

Most of the present work is concerned with the sand tips.

Analyses of china clay waste are given in tables 1 to 4 from which it can be seen that the acidity ranges from 3.9 to 5.7 in the samples tested, with the majority of the samples around 4.3. The surrounding undisturbed moorland soils have a pH value of around 4.4.

From table 3 it can be seen that the bulk of waste sand material consists of gravel and coarse sand-sized particles and therefore is likely to be free-draining. The sand is almost pure silica and its mineral content is very low. This is reflected in the low cation exchange capacities recorded in table 4. As Bradshaw et al (1975) have shown, all major plant nutrients are in short supply in both sand and mica substrates.

The organic content of the soil ranges from 0.3 to 13.2 per cent (tables 1 and 4) but would exceed this in areas where the Calluna cover has been long established. As the vegetation cover increases this is associated with an increase in organic content of the soil. Soil profiles (Fig. 6) shows that whilst there is only a thin algal cover over the sand tips, no differentiation of the substrate into horizons is evident. With the establishment of the first Calluna plants, the top few centimetres of sand become discoloured as organic matter becomes incorporated. As the vegetation cover develops, the degree of discolouration and the soil depth increases. Eventually, as the rate of leaf

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Table 1. pH and organic contents of china clay waste from sites at Drakelands Corner.

Sample	pH	% organic matter
Bare, exposed silica waste	4.2	0.4
Silica waste with a thin cover of *Z. ericetorum* only	4.3	2.2
Silica waste with an extensive cover of *Z. ericetorum*: Site 1	4.0	7.0
Site 2	4.8	3.0
Silica waste with *Calluna* cover	3.9	13.2
Mica slurry	5.1	3.4
Surrounding moorland soil	4.4	-

Table 2. Buffering capacity of silica waste.

(Change in pH of water after double percolation through 5cm Sand Column)

Initial pH	Final pH
4.5	4.9
5.8	4.8
6.6	5.2

Table 3. Particle size analysis of silica waste.

Fraction	Particle diameter	Composition % air dry sand
Gravel	>2.0	34
Coarse sand	2.0 - 0.2	48
Fine sand	0.2 - 0.02	2
Silt	0.02 - 0.002	8
Clay	<0.002	8

Table4Analyses of soil samples from three areas of a single tip

Site	pH	Humus content	Mechanical analysis % Gravel	Coarse sand	Fine sand	Silt	Clay
Slope supporting established *Calluna*.	4.8	3%	34	48	2	8	8
Eroding non-vegetated slope	5.7	2.4%	25	58	3	8	5
Flat micaceous area around base of tip	5.1	3.4%	15	37	20	14	14

Water Content % Field capacity	Hygroscopic water content	Total acid soluble salts	Exchangeable metallic cations
27	0.8	2.2%	5.1 me/100g. soil
32	0.4	1.1%	3.3me/100g. soil
40	0.6	3.1%	3.1me/100g. soil

Fig. 6 Diagrammatic soil profiles of vegetated china clay waste substrates. I

A. South-facing slope of sandtip with thin _Z.ericetorum_ and occasional _Calluna_ and _Festuca_ spp. plants.

B. Flat crown of sand tip with luxuriant _Z.ericetorum_ cover and scattered _Calluna_ plants.

C. South-facing slope of sand tip with well-established _Calluna_ cover.

D. Flat micaceous area with luxuriant _Z. ericetorum_ cover.

E. Flat micaceous area with luxuriant _Z. ericetorum_ cover. Water-table close to surface.

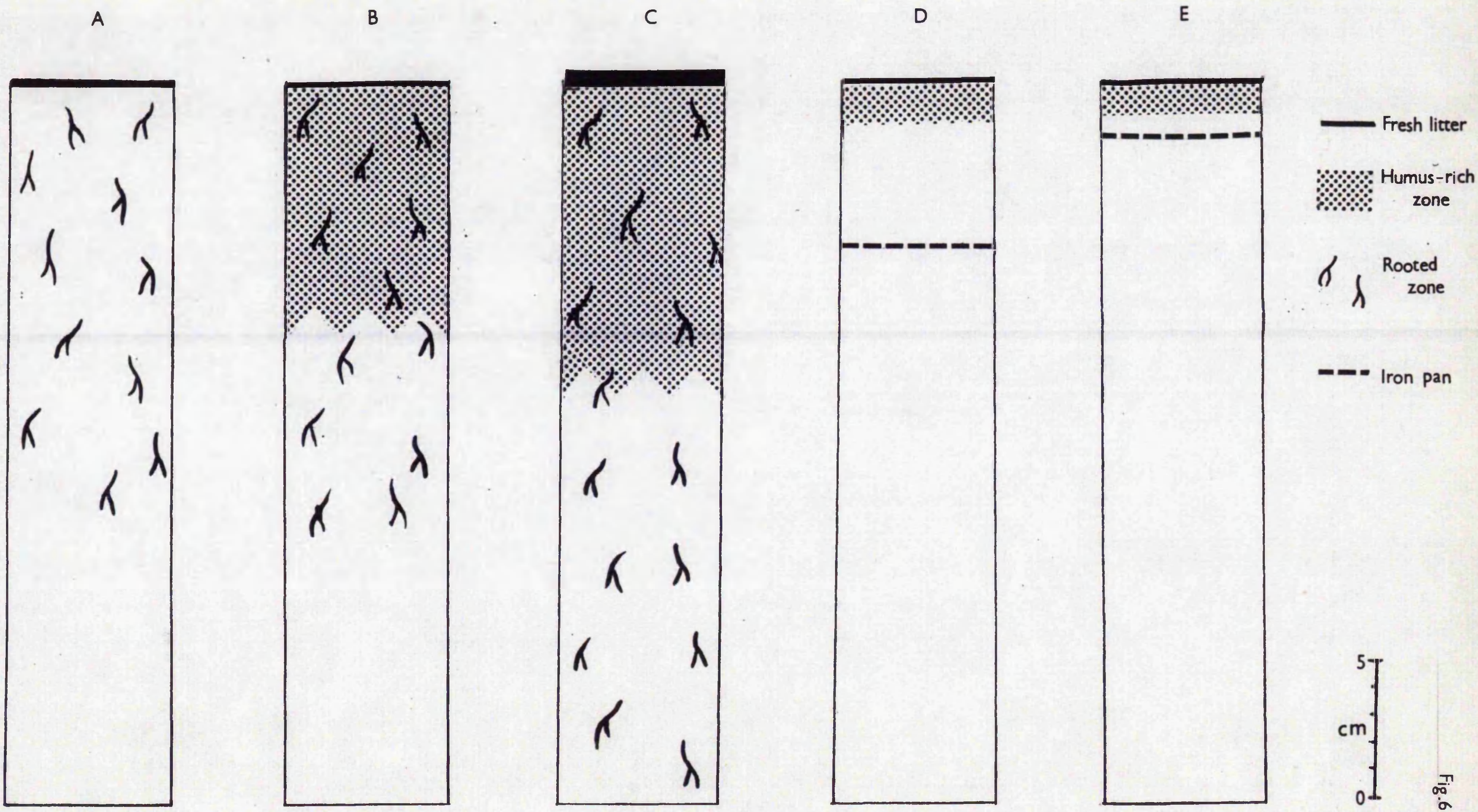
A
B
C
D
E
Fresh litter
Humus-rich zone
Rooted zone
Iron pan
5
cm
0

Fig.6

litter deposition exceeds that of decay, a mor soil becomes established over the sand. Beneath this is generally a greyer zone of eluviation of humus into the substrate. The profile presents a podsol-type appearance. As these young soils have developed directly on waste material, no attempt has been made to equate the observed horizons with those recorded for podsol profiles.

Fig. 7 shows a profile of an eroding tip which supports a well-established Calluna heath vegetation. The plant roots extended well below the top humified layers and this is characteristic of all the profiles examined. In general, the shorter much-branched roots are confined to the upper few centimetres and it is likely that the deeper roots, where these are active, serve principally to take up water which rapidly drains from the surface layers and to anchor the plants in the loose substrate.

Poorly drained sand and mica areas near the base of tips show an iron pan close to the surface (Fig 6). These areas generally support only small, lowgrowing plants with the noteable exception of bushes of Salix spp.

A number of profiles showed the presence of a second, strongly humified layer beneath the surface (Figs 8 and 9). These profiles were characterised by a thin mor layer at the surface and below this, a layer of sand. Beneath this was a second, much thicker mor layer containing the remains of Calluna stems. Both mor layers had significantly higher organic matter contents than the intervening sand. At first it was suspected that the lower layer represented the original soil level and that sand was dumped over it during mining operations. If this had been the case then it would imply that in the area, the china clay occurred naturally only 16 to 20 cm. below the surface whereas, as Cook (1880) points out, the overburden is rarely less than one metre in depth and is usually considerably more than this. Assuming that, in fact, the china clay had been dumped here, it would take a great many years for 16 to 20 cm. of humus to build-up over dumped sand. Consequently, the most probable explanation is that this area has seen sequential dumping of sand, overburden

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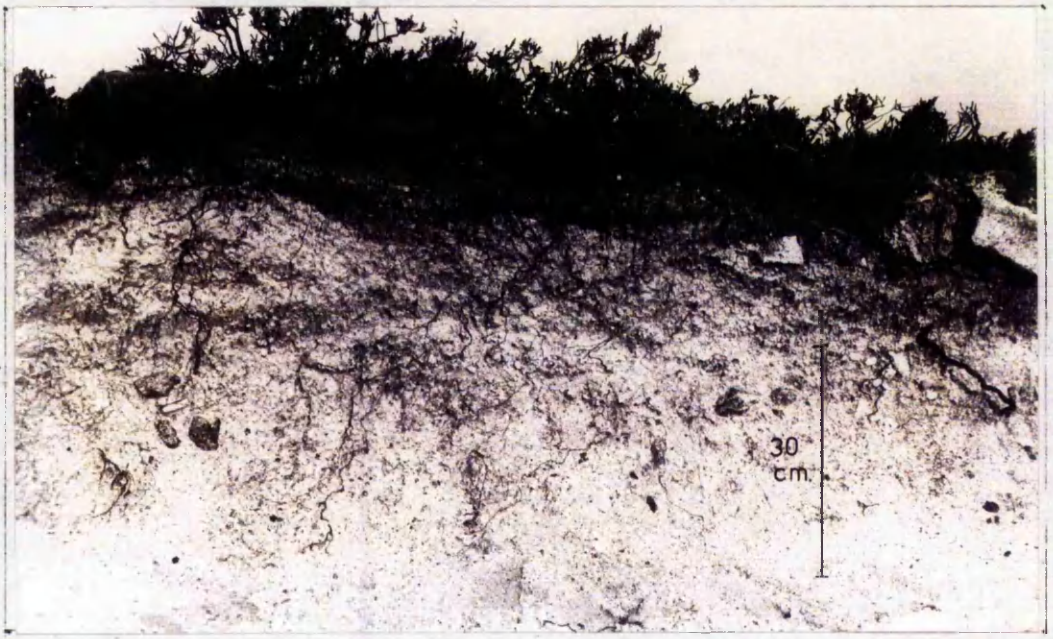

Fig. 7 Exposed profile of waste sand supporting Calluna cover in its building phase. Note depth of roots
Drakelands Corner July '75.

Fig. 8 Exposed profile of waste sand showing second strongly humified layer beneath surface. Soil supports Calluna vulgaris, Festuca ovina and Pteridium aquilinum.
Drakelands Corner July '75.

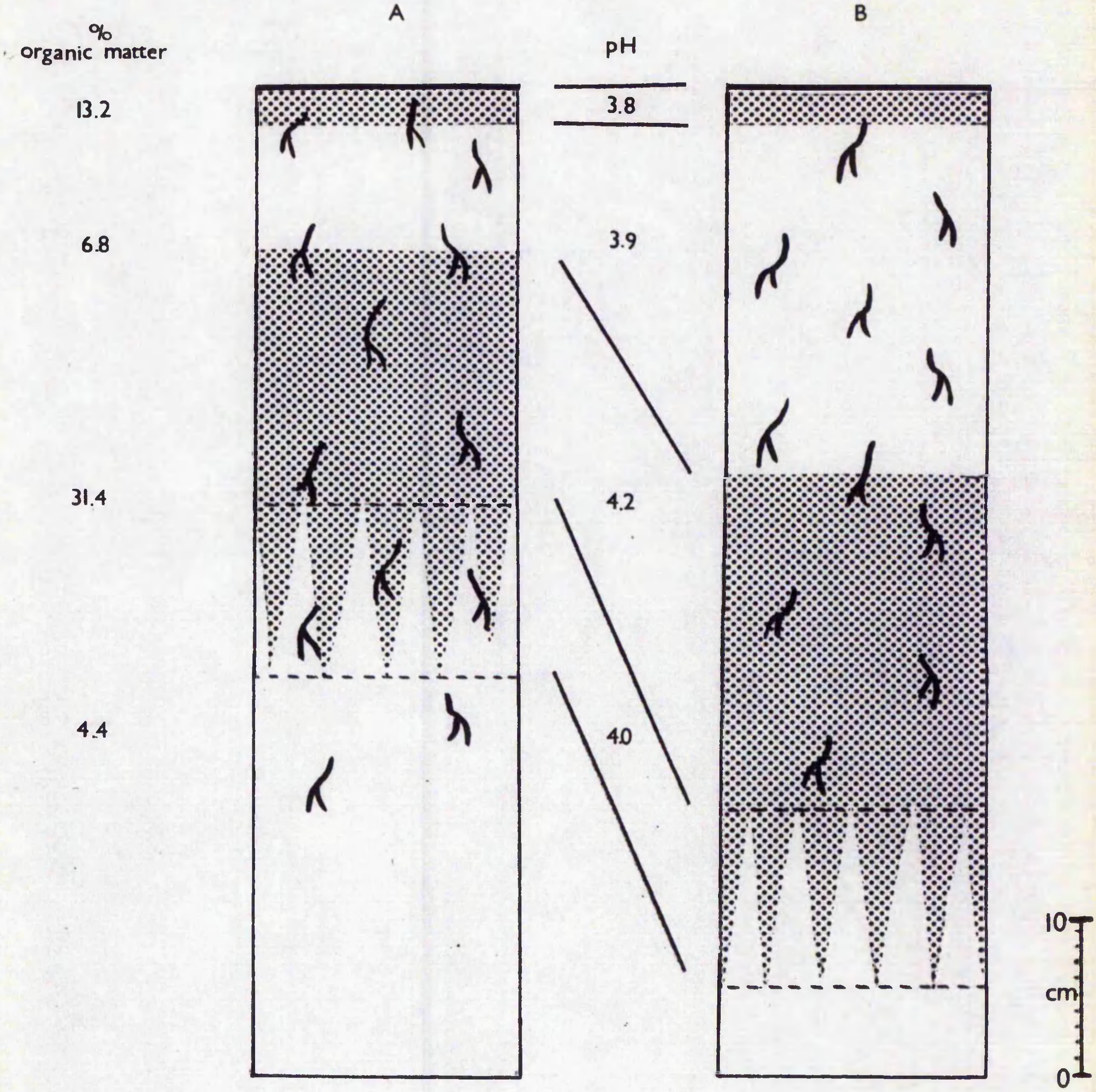

Fig. 9 Diagrammatic soil profiles of vegetated china clay waste substrates II.
Key as in Fig. 6

A. and B. Flat area of sand with well-established Calluna and Festuca spp. cover.

and then further sandwaste before being left.

Because of the free-draining nature of the sand, it would be expected that plants growing on it would be subject to water stress for much of the year. In fact, the china clay producing areas are all situated in the Southwest on moorland at least 180M. above sealevel. These are consequently areas where rainfall is high and potential evaporation low. In an average year, water would be deficient in the surface layers for only a short period during the summer months.

The vegetation data has been presented in tabular form (table 6) as percentage cover of species as recorded by percentage hits using point quadrats, and also in the form of frequency diagrams (Fig. 10) from random quadrats.

From the results, it can be seen that very few species are involved in the colonisation sequence. Table 5 lists the principal species occurring on old sandtips at Drakelands Corner. Zygogonium ericetorum is present before any vascular plants appear and persists except locally where established Calluna produces abundant litter on the surface of the sand. Annual and short-lived perennial plants (Spergularia rubra, Poa pratensis, Aira praecox, Sagina subulata and Rumex acetosella) appear in the early stages but are subsequently eliminated in the seral progression. The most complex community to become established is that characterised by degenerate Calluna plants and this is one of the communities which has been charted in detail.

The two vegetation charts illustrate representative stands of two principal communities on sand tips (Figs 12 and 15). These are:

Site A. Open community characterised by Calluna in its building phase.

Site B. More or less closed community characterised by Calluna in its degenerate phase.

The first site was on a south-east facing slope with two drainage gullies running from top to bottom (Fig 11). Because of the high porosity of the sand, most rain passes through the heaps but during heavy rainfall there can be surface run-off leading to erosion and gully formation on the slopes. Perkin (1972) describes china clay wastetips as showing classic examples

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Table 5 Principal species recorded growing on sand tips at Drakelands Corner

Vascular Plants	Angiosperms	Sagina subulata
		Spergularia rubra
		Ulex europaeus
		Ulex gallii
		Potentilla erecta
		Calluna vulgaris
		Erica cinerea
		Erica tetralix
		Rumex acetosella
		Salix spp.
		Agrostis setacea
		Agrostis tenuis
		Anthoxanthum odoratum
		Holcus lanatus
		Poa pratensis
		Festuca ovina
	Gymnosperms	Pinus sylvestris
	Pteridophytes	Pteridium aquilinum

Bryophyta	Polytrichum piliferum	Lichens	Cetraria glauca*
	Dicranum scoparium		Cladonia coccifera
	Hypnum cupressiforme		Cladonia impexa*
	Hypnum cupressiforme var. ericetorum		Cladonia uncialis
	Diplophyllum albicans		Cornicularia aculeata
			Hypogymnia physodes*
			Usnea subfloridana*

Algae Chlorophyta	Zygogonium ericetorum
	Hormidium flaccidum

* Largely confined to old heather stems

Fig.10 Frequency diagrams based on analysis of sites using 32 random $10 cm^2$ quadrats.

(300 × ¼M quadrats at site 4)

Site 1 W/SW facing slope. Largely bare ground with scattered herbaceous species and young Calluna bushes.

Site 2 S facing slope. Extensive growth of Zygogonium ericetorum and scattered established Calluna bushes.

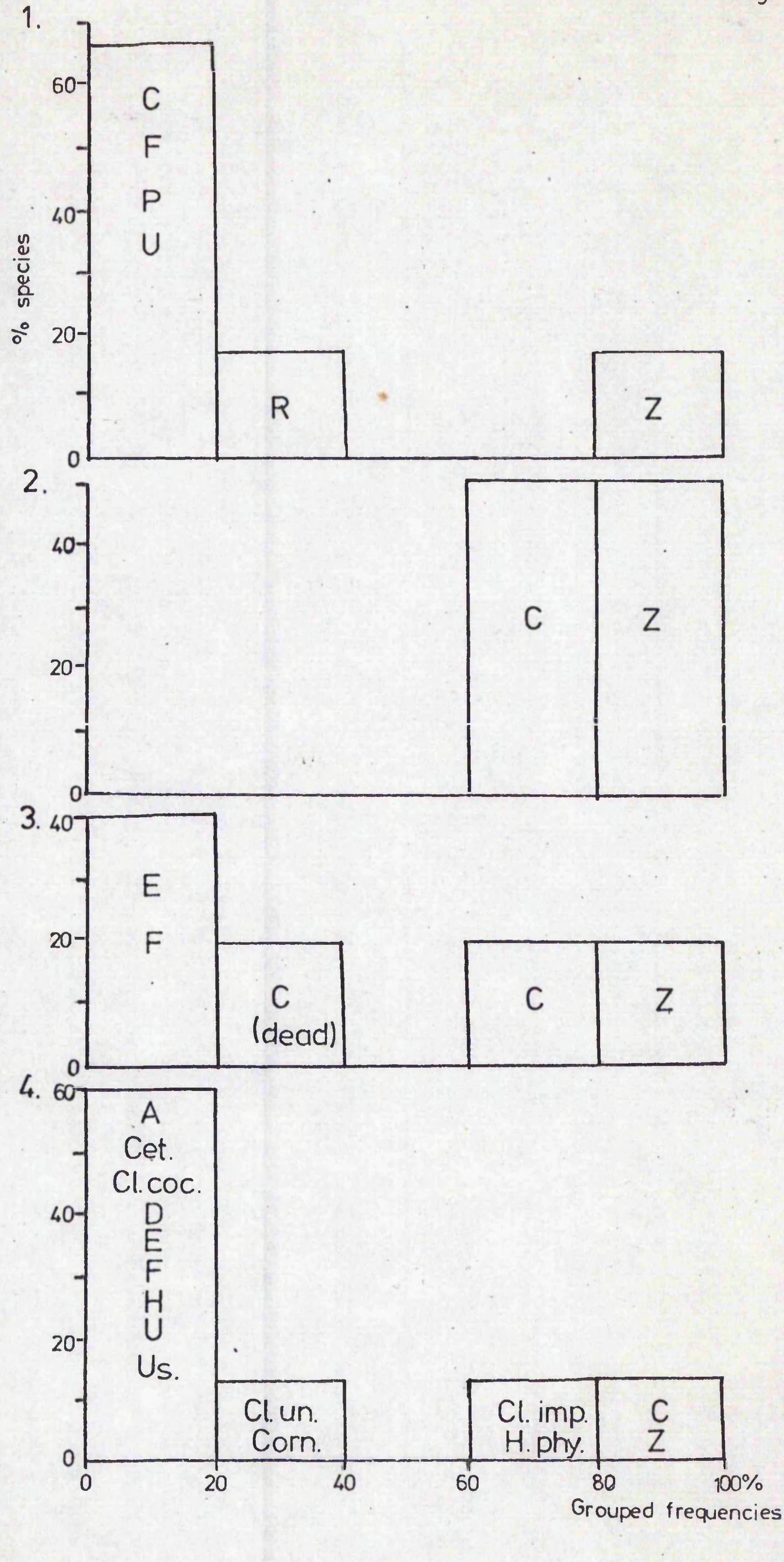
Site 3 SW facing slope. Scattered dead and regenerating young Calluna bushes.

Site 4 Flat. Dominated by degenerate Calluna bushes.

The species recorded within each frequency class are indicated by the following abbreviations:

A	Agrostis tenuis	F	Festuca ovina
C	Calluna vulgaris	H	Hypnum cupressiforme var. ericetorum
Cet	Cetraria glauca	H.phy	Hypogymnia physodes
Cl.coc	Cladonia coccifera	P	Polytrichum piliferum
Cl.imp	Cladonia impexa	R	Rumex acetosella
Cl.un	Cladonia uncialis	U	Ulex europaeus
Corn.	Cornicularia aculeata	Us.	Usnea subfloridana
D	Dicranum scoparium	Z	Zygogonium ericetorum
E	Erica cinerea		

Fig. 10



1.
2.
3.
4.
% species
C F P U
R
Z
C
Z
E F
C (dead)
C
Z
A Cet. Cl.coc. D E F H U Us.
Cl.un. Corn.
Cl. imp. H. phy.
C Z
0
20
40
60
80
100%
Grouped frequencies

Table 6 Percentage cover of species on sand tips

Expressed as% hits using 102 point quadrats

For details of sites refer to Fig. 10

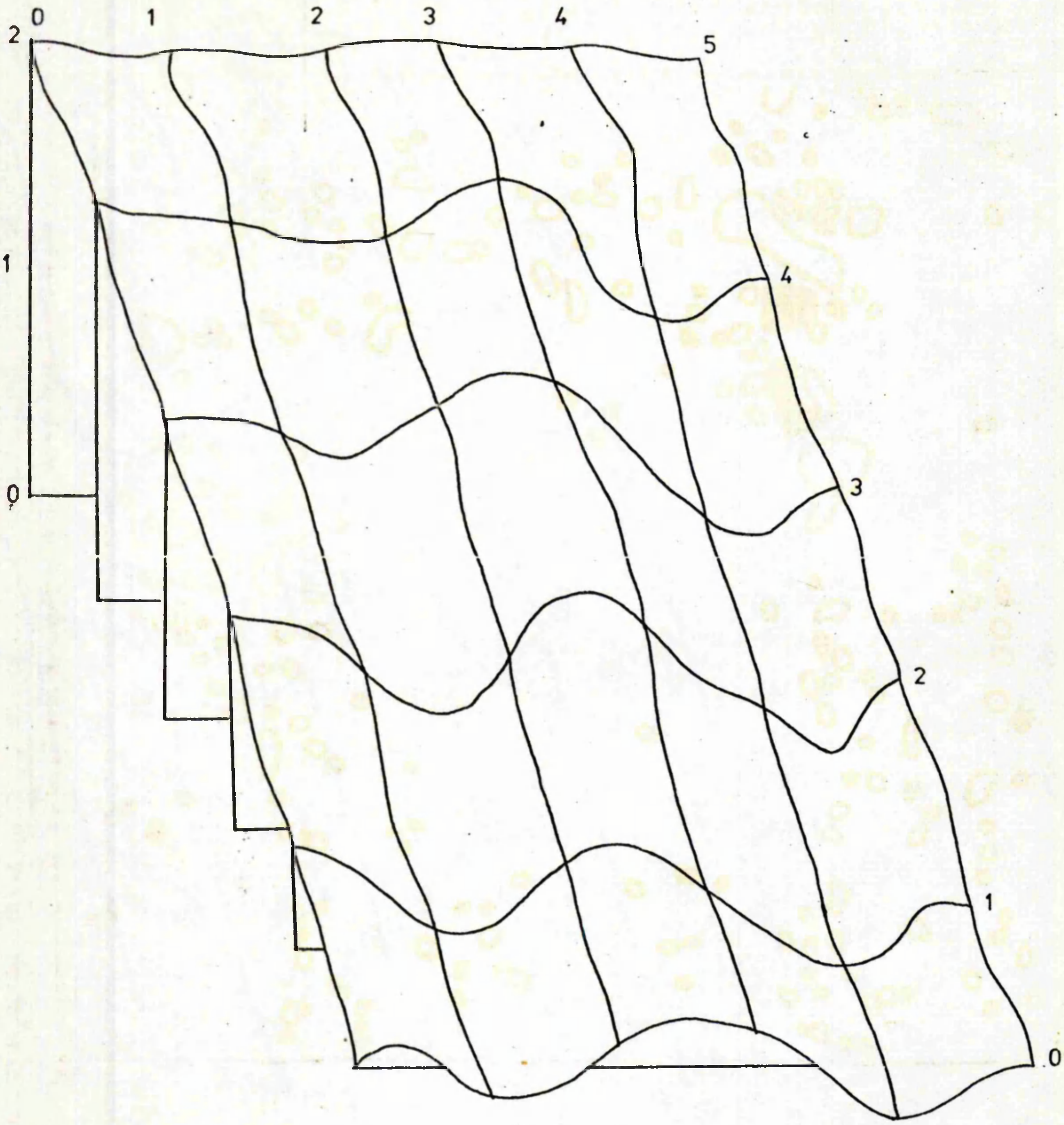
Species	Sites			
	1	2	3	4
Calluna vulgaris	4	25	18	67
Calluna vulgaris(dead)			5	
Festuca ovina	5	-	2	6
Agrostis tenuis	-	-	-	2
Erica cinerea	-	-	-	7
Ulex europaeus	-	-	-	6
Rumex acetosella	3	-	-	-
Polytrichum piliferum	1	-	-	-
Dicranum scoparium	-	-	-	3
Hypnum cupressiforme	-	-	-	5
Cladonia coccifera	-	-	-	3
Cladonia impexa	-	-	-	35
Cladonia uncialis	-	-	-	9
Cornicularia aculeata	-	-	-	10
Hypogymnia physodes	-	-	-	23
Usnea subfloridana	-	-	-	2
Cetraria glauca	-	-	-	2
Zygogonium ericetorum	77	99	96	90

of rainwater gullying and furrowing. These gullies can be up to one metre deep but are usually considerably less although in the area under consideration they averaged about half a metre in depth. A similar site to the charted one is shown in fig 13.

The community at site A was an open one and only three species were commonly frequent. Soil analyses (table 7) show that the sand was uniformly acid (around pH 4.3) with a low organic content (around 2.6%). Even beneath *Calluna* bushes the organic levels were low (3.6%). Most of the plants were confined to drainage channels and the more shaded north-facing sides of the gullies. Plants were almost completely absent from the south-facing sides of the gullies and from the sheeptrack that was present along the top of the mapped area. Those plants on the gully ridges were small with poorly developed root systems. This pattern of *Calluna* on sandtips is striking and may be seen in fig 13 which illustrates a number of gullies and a sheeptrack. The gullies only receive rainwater during heavy or prolonged precipiation. *Agrostis tenuis* and *Festuca ovina* grow more luxuriantly in the gullies than on the ridges where they occur as scattered individuals.

The second Site B, supported a more complex community and was characterised by *Calluna* in its degenerate phase. The site was flat and composed of rock overburden mixed with sand. The pH of the soil was around 4.0 but the organic matter content was considerably higher than at site A (Table 7). A representative stand of this community is illustrated in fig 14. Vegetation cover was extensive but the *Calluna* bushes showed a strong tendency for the central parts to die leaving a gap which widened as further branches died back whilst the outer ring of branches remained green. Gimingham (1972) states that this process occurs in *Calluna* bushes at an age between 25 and 30 years. The extent of dead *Calluna* is illustrated in fig 15. Both *Calluna* and *Ulex* spp. remained small due to heavy grazing pressure in this area. Another difference between this area and site A was the abundance of lichens. Lichens were present in the first area but only represented by a few scattered specimens whereas in the present

contd. p. 46

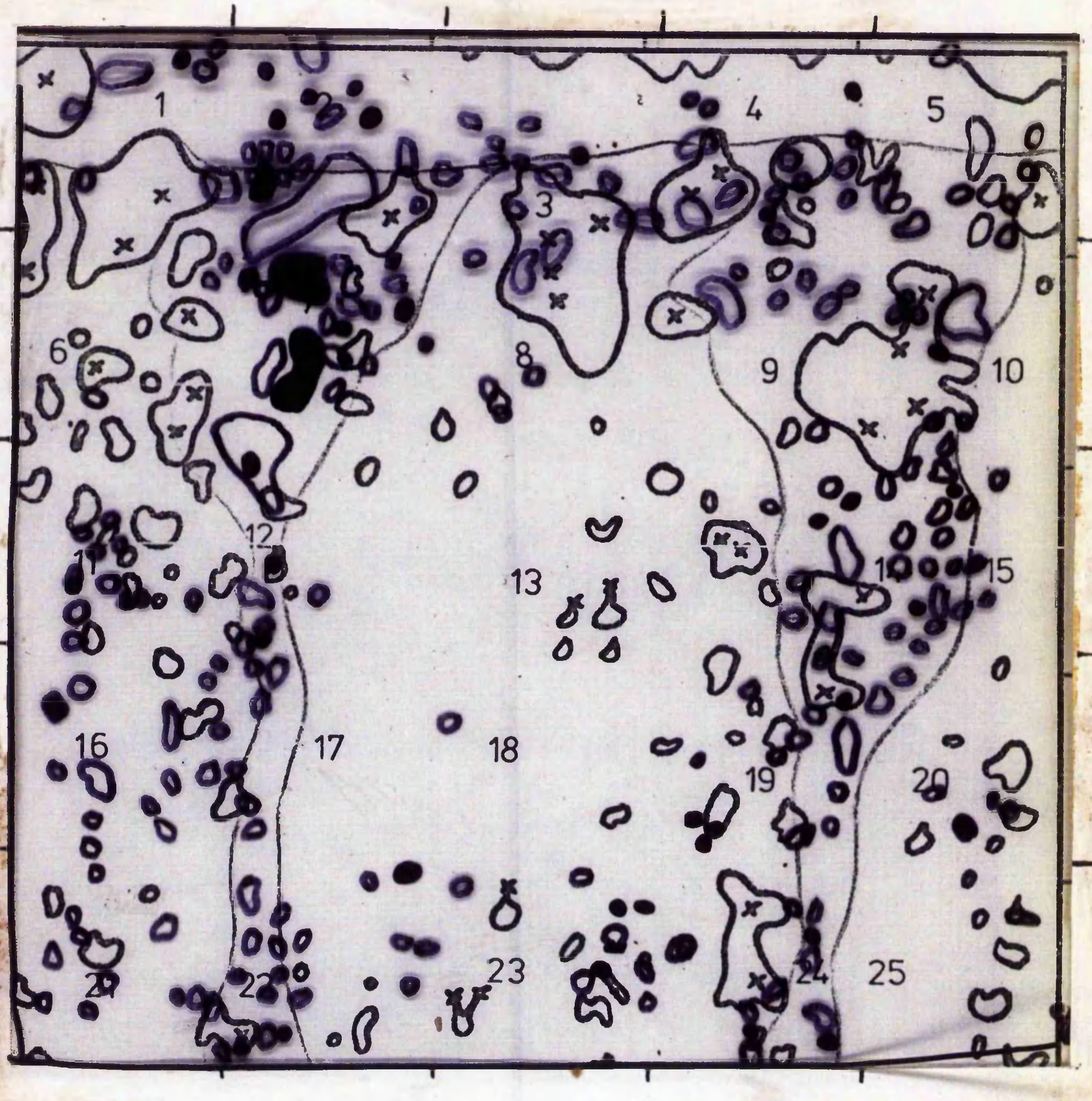


Vertical exaggeration X 2

Scale: 2cm. to 1M.

Fig.11 Elevation of site A.

Fig.12a

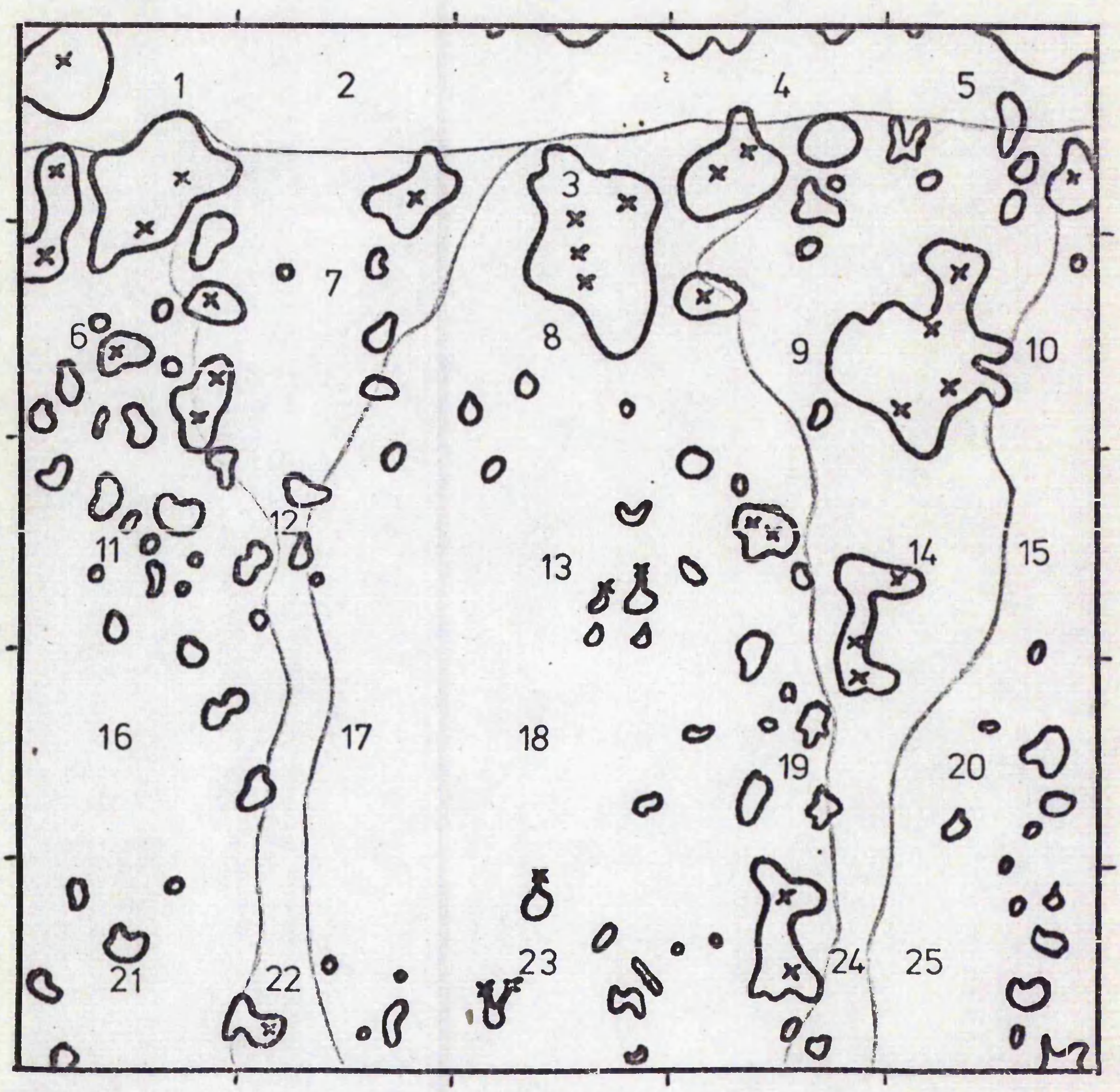


Festuca ovina

Agrostis tenuis

SCALE

1M



(x) Calluna vulgaris   X Position of main stems in larger bushes.

1-25 Position of soil samples

SCALE

1M

Fig. 12,12a Vegetation chart of site A.

Fig. 13 Silica sand slopes showing distribution of Z.ericetorum (grey tone) and Calluna bushes down gullies. Juncus effusus in damp area at base. Arrow marks position of sheep track. Drakelands Corner July '75 Height of slopes approximately 3M.

Fig. 14 Old china clay area dominated by Calluna in its 'degenerate' phase with open areas between bushes bare apart from Z.ericetorum cover. Drakelands Corner July '75

Foreground 2 M. across.

Fig. 15a

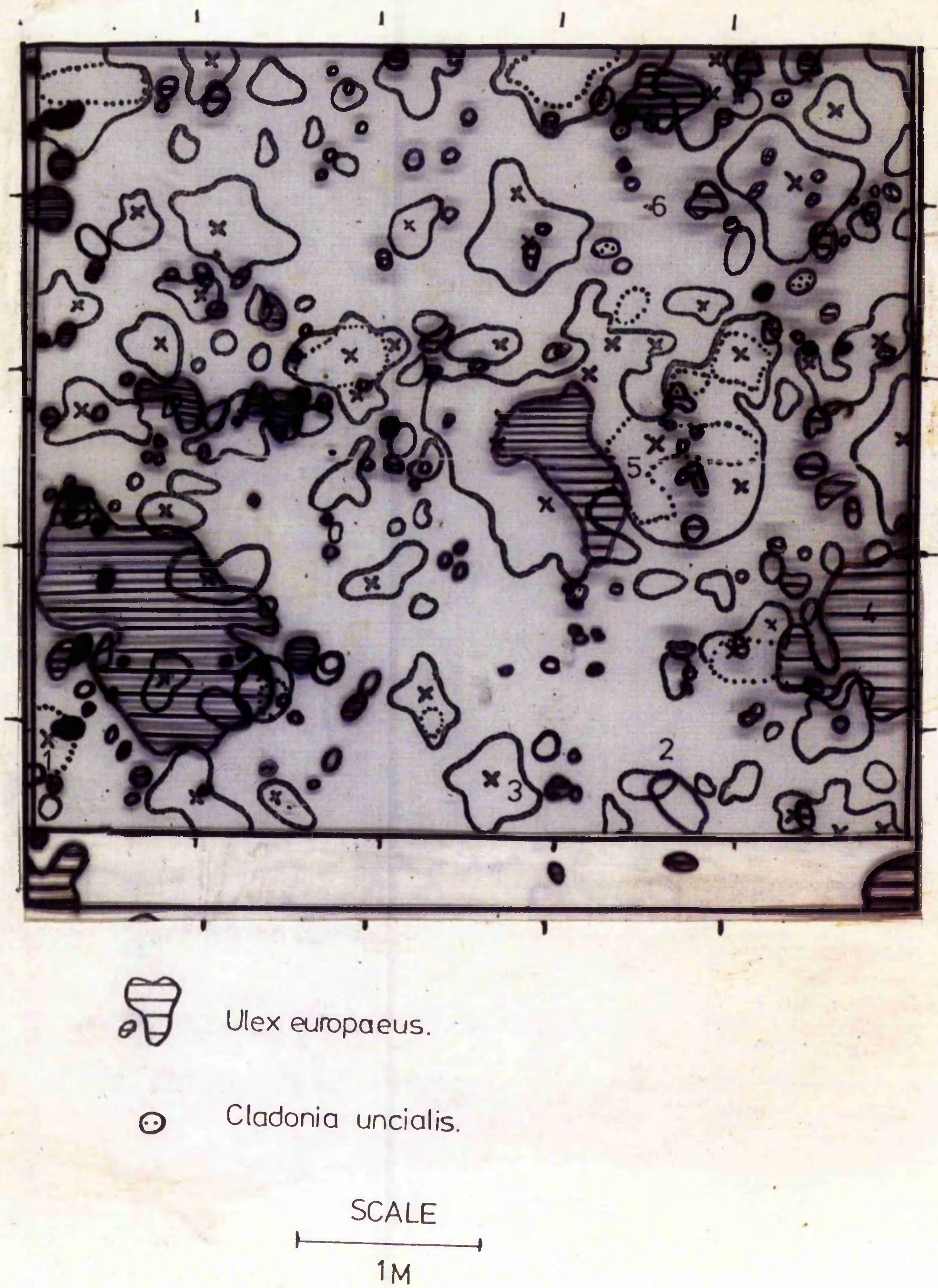
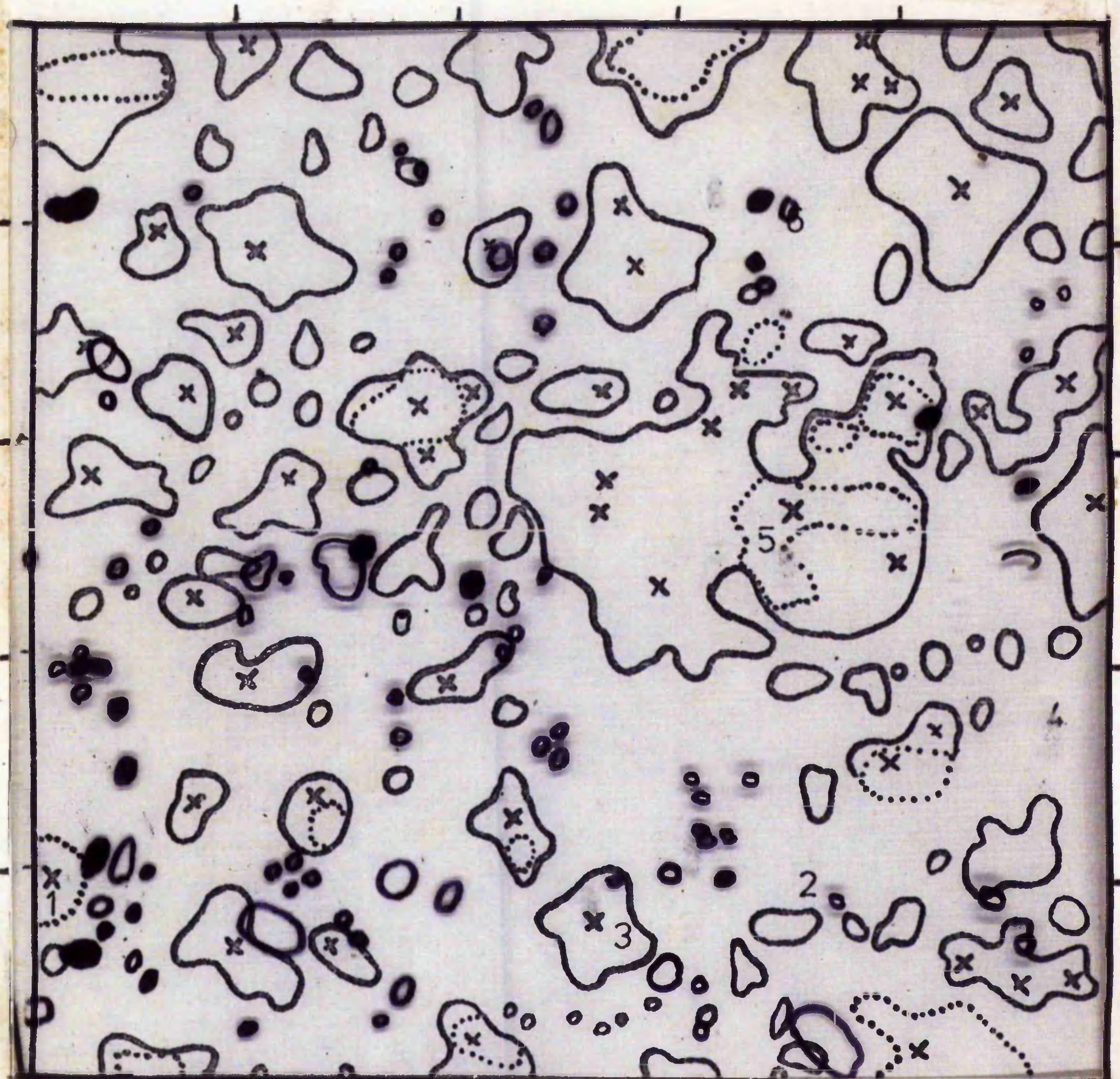


Fig. 15b



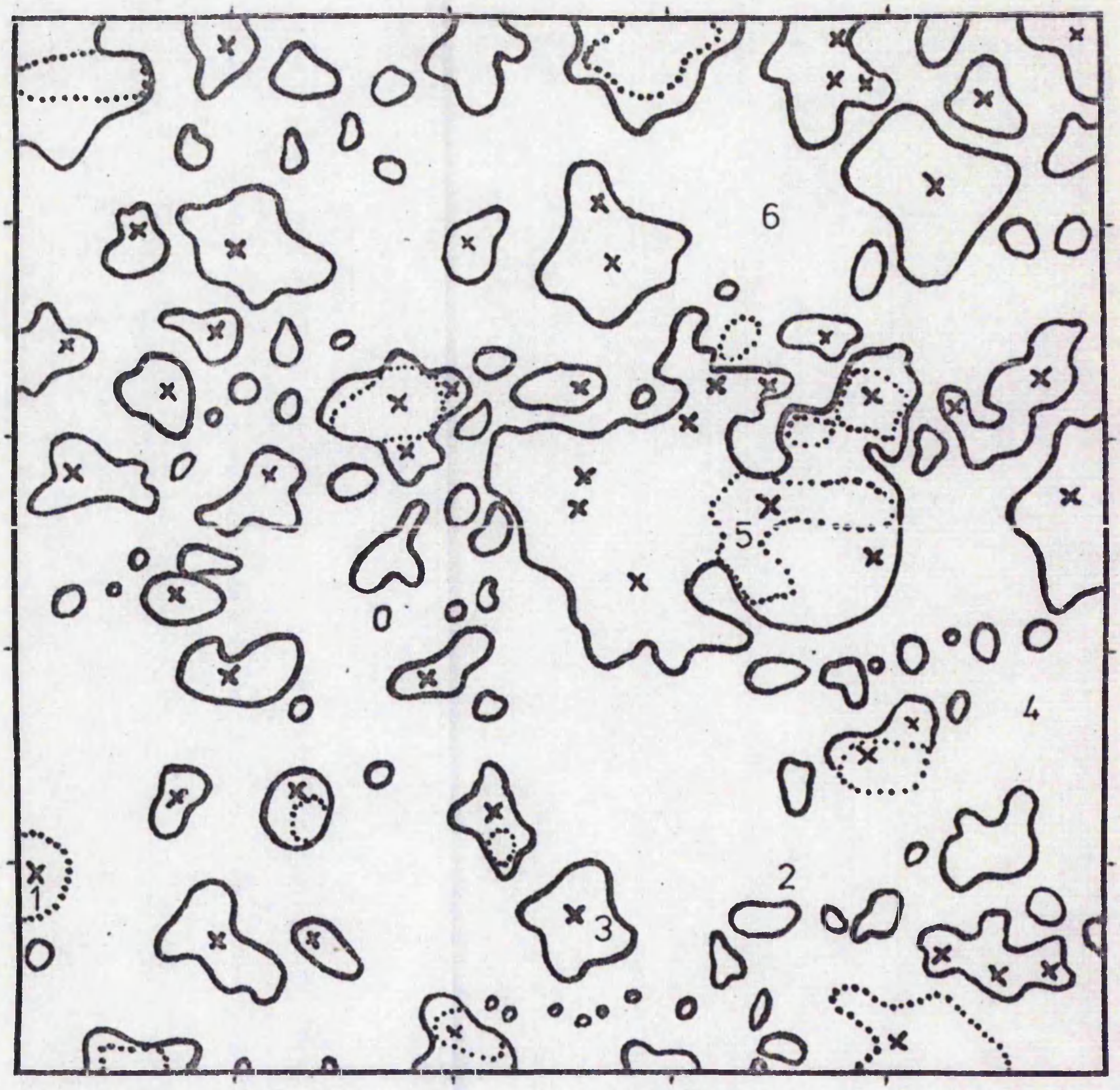
Festuca ovina.

Agrostis tenuis.

1-6 Position of soil samples.

SCALE

1M



(X) Calluna vulgaris — X Position of main stems in larger bushes. Dotted lines denote dead areas.

SCALE
1M

1-6 Position of soil samples

Fig 15,15a,15b Vegetation chart of site B.

Table 7 pH and organic matter of soil samples collected at sites A and B

(Sampling sites marked on figs. 12 and 15)

Site A			Site B		
Soil sampling site	pH	% organic matter	Soil sampling site	pH	% organic matter
1	4.4	2.2	1	3.8	33
2	5.2	2.9	2	4.0	7
3	4.0	3.6	3	4.0	17
4	4.4	3.1	4	4.1	16
5	4.4	3.1	5	4.0	34
6	4.2	2.9	6	4.0	7
7	5.0	3.2			
8	4.3	3.5			
9	4.2	3.1			
10	4.1	2.5			
11	4.2	2.5			
12	5.0	3.1			
13	4.3	3.3			
14	4.2	3.0			
15	4.1	2.8			
16	4.1	2.5			
17	4.3	1.3			
18	4.2	3.2			
19	4.0	3.3			
20	4.1	0.5			
21	4.0	2.9			
22	4.2	1.3			
23	4.1	3.2			
24	4.2	2.8			
25	4.2	0.4			

area several species had significant cover values (table 6). The characteristic lichens were several Cladonia species and Hypogymnia physodes, although only Cladonia uncialis has been included on the vegetation chart. Both Cladonia impexa and Hypogymnia physodes occur on most of the dead and prostrate Calluna bushes, with C. impexa spreading to the surrounding soil. Because of the close association of these lichens with Calluna and their widespread occurence on this plant, they have not been indicated on the vegetation charts. Calluna heath does not form a continous sward on china clay; bushes generally occur isolated from one another. The intervening spaces are frequently unoccupied by flowering plants and this means that Zygogonium ericetorum is not eliminated from the community but persists as a mat-like cover over the ground between plants.

From general observations, it was apparent that the open spaces between Calluna and Ulex gallii bushes were frequently characterised by the presence of Cladonia uncialis and Dicranum scoparium. In order to detect any mosaic patterns in the distribution of the main species of the degenerate Calluna community, the results of 300 x $\frac{1}{4}M^2$ quadrats were analysed for associations between the seven principal component species after the method used by Hopkins (1957). From the results, the following diagram was constructed.

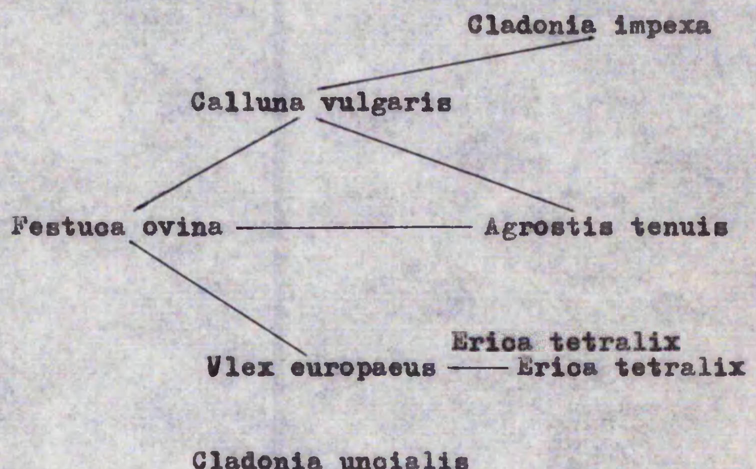


Fig 16 Associations between the principal species of degenerate Calluna community.

Harris and Cooke (1969), van Genderen (1974) and others, recognise false colour or infra-red film in aerial photography to be the most appropriate film for remote sensing for vegetation stress analysis. As mesophyll tissue is an extremely good reflector of IR radiation, the leaves of growing plants give a bright pink or red signal whilst there is a loss of IR reflectance in plants not actively growing or in vegetation under stress. IR aerial photographs of old china clay tips at Lee Moor were taken in order to see whether any differences could be picked up between newly colonised and well-vegetated areas. Fig. 17 illustrates one such area but a great deal of the subtle colour changes have been lost in making a print from the original colour transparency. However, fig. 17 shows that non-vegetated spoil gave a bright blue signal whilst vegetated areas showed a general background of pinkish-green with black clumps of Ulex spp. Colour differentiation of different vegetated areas was not distinct. This may have been because the vegetation was not actively growing at this time of year (early April) or because the photographs were taken at too great a height. The most striking feature on the false colour photographs was the bright pink signal given by fields and gardens of the village of Wotter where the cultivated vegetation was actively growing. Runoff from the fields shows as a pink trail where plants have been able to grow more vigorously in the enriched soil. Other pink patches indicate flushes of grass associated with damp patches.

To determine whether the colonisation sequence as seen at Drakelands Corner was typical of old china clay mining areas in the Southwest, visits were made to other areas in Devon and Cornwall (Fig. 1).

Very similar plant communities were seen at two other sites on Dartmoor, namely old tips at Whiteworks in the middle of the southern moor (Grid Ref. SX 648670) and dumped waste by the River Plym at Cadover Bridge (Grid. Ref. SX143717).

Disused workings on the west side of Bodmin Moor (Grid. Ref.SX143717) consisted of three or four steeply conical tips and a flooded pit. The tips supported the characteristic species

Fig. 17 I.R. photograph of aerial view of mining village of Wotter, Lee Moor, (Grid Ref. SX5662) with associated china clay works and arable land.
April '76 (Photo S. Johnson)

Fig. 18 China clay waste colonised by Z. ericetorum, Calluna vulgaris and Rhododendron ponticum. Carthew, St. Austell area July '75

found at Drakelands Corner and it was noted that Calluna was more numerous on the tips than on the surrounding well-grazed moorlands. This may have been a reflection of reduced grazing pressure due to the steeply-sloping sides on the tip. Rumex acetosella and Polytrichum piliferum were frequent on the more disturbed areas of the tips.

Two areas of the extensive Hensbarrow excavations near St. Austell were examined. Waste tips at Gomm near Carthew (Grid. Ref. SX006553), which were approximately fifty years old, were covered with what appeared to be "climax" vegetation. This consisted of Rhododendron ponticum (up to 4.5M high) together with smaller amounts of Salix spp. and Sorbus aucuparia. These shrubs formed a dense cover over the tips eliminating any ground flora. More recent tips in the area supported a mixture of Calluna vulgaris and young Rhododendron ponticum plants (Fig. 18). Both these species appeared to become established around drainage channels as noted for Calluna at Drakelands Corner. R. ponticum seedlings appeared in places where there was little apart from Zygogonium ericetorum on the sand. R. ponticum is seen on many of the tips in the Hensbarrow area but is extremely scarce at the Drakelands Corner area although grown in gardens close by.

At the second Hensbarrow site, Trevalour Downs (Grid. Ref. SX9658), Rhododendron ponticum was absent. It is likely that many of the tips here were dumped with overburden as they supported an almost complete flowering plant cover with a large proportion of grasses. These tips had only a few relatively bare areas where Zygogonium ericetorum was able to survive.

A summary of the stages in colonisation of china clay waste tips in the Southwest of England is given in table 8.

Table 8 Schematic colonisation sequence of china clay sand tips.
From observation and vegetation analyses.

(a) refers to annual species
species underlined persist through succeeding seral stages

Bare sand waste

↓

Zygogonium ericetorum — cover varies from sparse to ± continuous

↓

Spergularia rubra(a) Festuca ovina
Sagina subulata Poa annua (a)
Rumex acetosella Aira praecox(a)
Polytrichum piliferum

Variety of small herbaceous specie

↓

Calluna vulgaris
Ulex spp.
Erica spp.
Agrostis tenius

Shrubby species with Calluna vulgaris predominating.
Bare areas between clumps with extensive algal cover

↓

Pinus sylvestris
Salix spp.
Rhododendron ponticum
Quercus spp.

Salix spp. in damper places
Rhododendron ponticum may be dominant in some areas.

erosion by man, animals, weather, erosion.

5: Algal Colonisation Sequence

Algae, in particular *Zygogonium ericetorum*, are primary colonisers of china clay substrate. The algal mats consist almost exclusively of *Z. ericetorum* and extend over open, undisturbed areas of china clay waste. This cover may often go unnoticed since it does not have a distinctive colour when dry, but when wet it appears dull purple in colour and, in places where the alga is growing luxuriantly, it is possible to lift from the substrate pieces with an area of several square centimetres. Although the algal filaments are microscopic, multiple strands may be seen macroscopically by slowly tearing the removed pieces of algal crust.

Once an alga has become established on an area of soil, it frequently alters the microhabitat such that conditions become favourable for other algae to establish themselves. In order to investigate whether any other algae grow with *Z. ericetorum* on china clay waste, samples of sand were collected and incubated in the laboratory. The method used was that described by Round (1965) which makes use of the fact that soil placed in petri-dishes and moistened with distilled water develops a rich algal flora on its surface and that many, if not all of the actively growing soil species will adhere to coverslips placed on the soil surface. Small surface sand samples were collected from a number of sites that appeared to show different seral stages. Surface soil to a depth of approximately 5mm. was placed in a sterile glass petri-dish using the lid of the dish together with a sheet of thin plastic to remove the sample and transfer it, in a relatively undisturbed condition, to the bottom half of the dish. Heat sterilised coverslips were placed at random onto the surface of the soil and the soil was moistened with distilled water. The plates were kept at 23°C and illuminated at 4300–5300 lux through a twelve-hour day. At intervals over a period of one month, coverslips were removed rapidly, using a "flipping" action, and each was examined microscopically for a twenty minute period. This was to ensure that each was examined thoroughly for a comparable time. Algae were recorded on a presence or absence basis. Although some indication

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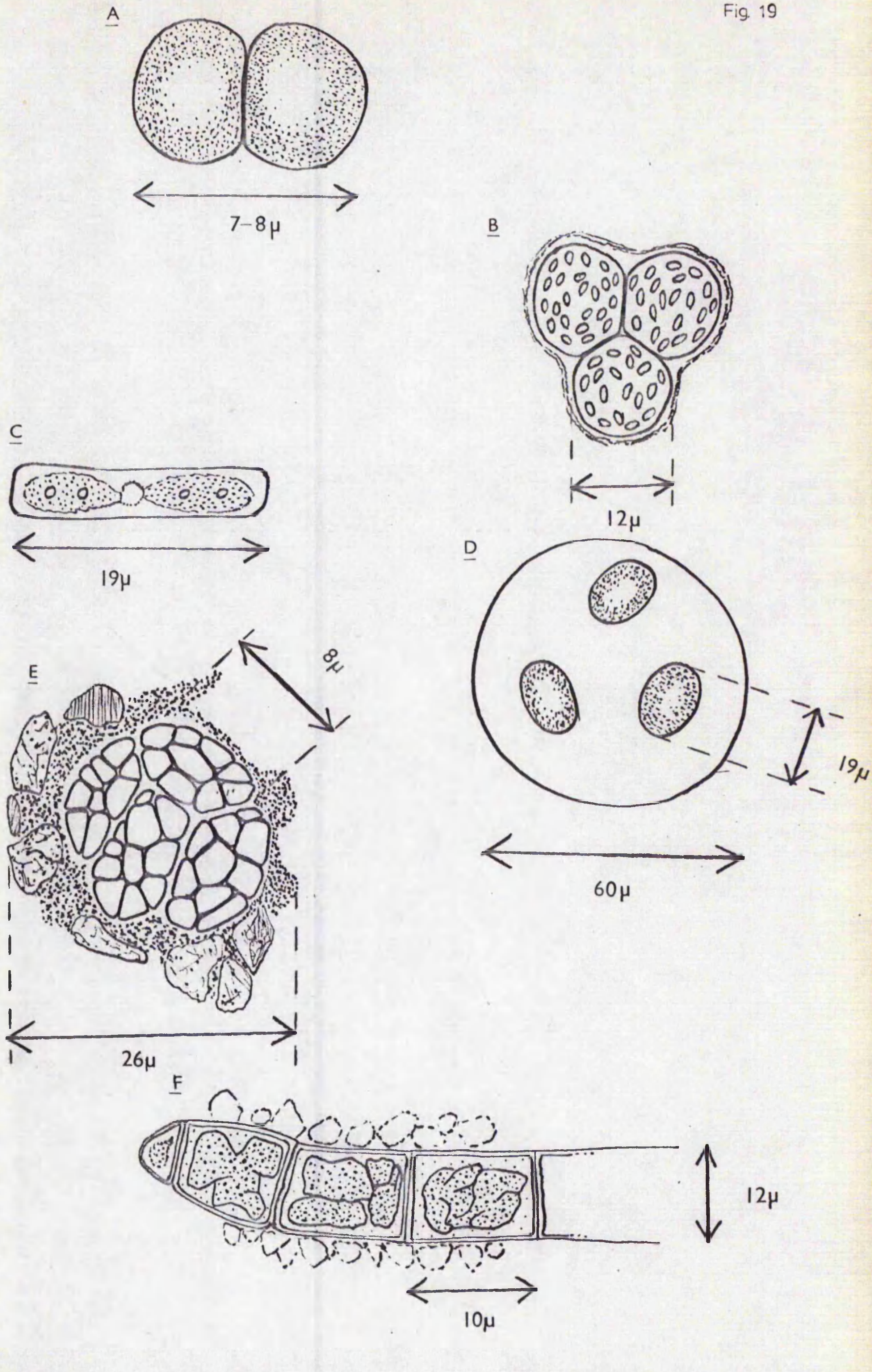
Fig. 19 Algae found in silica sand samples from Drakelands Corner. (Not to scale)

A	Order: Chlorococcales.	Cells in twos, occasionally fours. Parietal chloroplast.
B	Order: Chlorococcales	Oocystis. Cells in twos, threes or fours. Many discoid chloroplasts.
C	Order: Desmidiales	Saccoderm desmid. Two chloroplasts sepated by clear area. Several pyrenoids.
D	Colonial green	Up to 24 cells (usually less) embedded in mucilage. Ovoid cells with parietal chloroplast.
E	Green alga	Soil particles adhering to colonies.
F	Filamentous green	Unbranched filaments. Soil particles adhering to cellwalls.

Fig. 19



of their abundance was recorded, criteria of dominance based upon number of individuals or area coverage were not applicable to this method. Round (1965) states that in general the dominant species appear in the first few days and remain dominant on the soil for some months. In order to see whether any seasonal differences in algal species could be detected, the above experiment was carried out in July 1974 and subsequently repeated in April 1975.

In addition, 7cm^2 pots of silica sand with Z. ericetorum growing on the surface were kept in a cold green house with frequent watering for six months after which the algal flora was investigated.

Results

The results of the investigations of the algal flora on waste sand are given in fig. 19 and tables 9, 10 and 11. In the case of the first series (July 1974), surface samples and samples from 5mm beneath the surface were compared from sites in close proximity to each other but showing different seral stages. In the second series (April 1975), north and south facing slopes of the same tip were compared. Where a particular alga was recorded only once it has not been included in the results; similarly a full list of the rich algal flora recorded from sites where a Calluna cover was established has not been attempted. Illustrations of the principal types that were not fully identified have been included (fig. 19). In general, the same taxa of algae were collected in both series. Table 11 is a summary of the principal algae occuring on waste sand as compiled from the results of these experiments. Zygogonium ericetorum is the first alga to appear and the only other species which may contribute significantly to the algal mat is Hormidium flaccidum.

After six months in greenhouse conditions, pots of waste sand with Z. ericetorum mats were found to have been overgrown by other algae and mosses in many instances. Although in all cases, Z. ericetorum was still present, between the months of August and February the algal flora was found to have altered significantly in many of the pots. Those pots containing Z. ericetorum on peaty soil still supported a luxuriant growth of the alga. Table 12 lists the principal changes in vegetation cover after six months.

Table 9 Algae recorded from silica sand samples, Drakelands Corner - 1

Samples collected by July 1974

Samples from the following sites:

1. Bare sand. Macroscopically devoid of algal cover. Top 5mm
2a. ± bare sand. Sparse cover Z. ericetorum. Top 5mm
 b. As above. Sample from below surface.
3a. Sand with Z. ericetorum cover. Top 5mm.
 b. As above. Sample from below surface
4a. Sand with extensive Z. ericetorum cover plus Cladonia spp. and Polytrichum piliferum. Top 5mm.
 b. As above. Sample from below surface
5a. Sand and litter with Z. ericetorum and Calluna cover. Top 5mm
 b. As above. Sample from below surface.

Key

No cross	Occasional. Less than 5 individuals/colonies seen.
+	Infrequent
++	Common
+++	Abundant

Table 10 Algae recorded from silica sand samples, Drakelands Corner - II (overleaf)

Samples collected April 1975

Samples from the following sites:

1. Bare sand. South-facing eroded slope
2. Bare sand. North-facing stable slope
3. Z. ericetorum cover. South-facing slope
4. Z. ericetorum cover. North-facing slope
5. Z. ericetorum and Calluna cover. South-facing slope.
6. Z. ericetorum and Calluna cover. North-facing slope.

Key

No cross	Occasional. Less than 5 individuals/Colonies seen
+	Infrequent
+ +	Common
+ + +	Abundant

Table 9

Sample	Algae recorded following incubation		
	After 4 days	After 11 days	After 30 days
1.	Zygogonium ericetorum	Zygogonium ericetorum	Zygogonium ericetorum
		Vaucheria sp.	Alga A
2a.	Zygogonium ericetorum	Zygogonium ericetorum	Zygogonium ericetorum ++
	Hormidium flaccidum		Hormidium flaccidum
2b.	Zygogonium ericetorum	Zygogonium ericetorum	Zygogonium ericetorum ++
	Hormidium flaccidum	Hormidium flaccidum	Hormidium flaccidum ++
			Alga C
3a.	Zygogonium flaccidum +	Zygogonium ericetorum +	Zygogonium ericetorum ++
			Hormidium flaccidum +
3b.			Zygogonium ericetorum ++
			Blue-green alga
			Alga B
4a.	Zygogonium ericetorum +	Zygogonium ericetorum +	Zygogonium ericetorum +++
	Hormidium flaccidum		Hormidium flaccidum ++
	Alga A	Alga A	Alga A ++
			Alga B
4b.		Zygogonium ericetorum	Zygogonium ericetorum ++
	Hormidium flaccidum		
			Alga B
5a.	Zygogonium ericetorum	Zygogonium ericetorum +	Zygogonium ericetorum +++
		Alga B	Hormidium flaccidum ++
			Pennate diatoms ++
			Alga D
5b.	-	Zygogonium ericetorum	Zygogonium ericetorum ++

Details of algae are given in fig 19

Table 10

Sample	Algae recorded following incubation		
	After 24 days	After 32 days	After 35 days
1.	-	-	Zygogonium ericetorum
2.	-	-	-
3.	Zygogonium ericetorum +++	Zygogonium ericetorum +++	Zygogonium ericetorum +++
	Hormidium flaccidum+	Hormidium flaccidum++	Hormidium flaccidum ++
		Gleocystis/Palmella	Gleocystis/Palmella
	Alga C +		Alga C +
	Alga F ++	Alga F ++	Alga F ++
4.	Zygogonium ericetorum ++	Zygogonium ericetorum +	Zygogonium ericetorum+
	Gleocystis/Palmella +	Gleocystis/Palmella +	Gleocystis/Pamella +
	Vaucheria sp. +		Vaucheria sp +
			Alga B
5.	Zygogonium ericetorum ++	Zygogonium ericetorum +++	Rich flora with previously listed species predominating.
	Hormidium flaccidum+	Hormidium flaccidum+	
	Gleocystis/Palmella++	Gleocystis/Palmella+++	
	Alga A ++	Vaucheria sp +	
	Alga B ++	Alga B +++	
	Alga C ++		
	Alga D +		
6.	Zygogonium ericetorum ++	Zygogonium ericetorum ++	Rich flora with
	Gleocystis/Palmella++	Gleocystis/Palmella +	previously listed
	Alga A +	Dactylococcus sp. +++	
	Alga B ++	Alga A+++	
		Alga D ++	
		Alga E +	

Details of algae are given in fig 19

Table 11 Algal colonisation of silica sand. Summary of tables 9 and 10

Substrate	Algae recorded: Frequent	Algae recorded: Occasional
More or less bare sand	Zygogonium ericetorum (sparse)	Chlorococcales type (alga A) Vaucheria sp.
Sand with *Z. ericetorum* cover	Zygogonium ericetorum Hormidium flaccidum Oocystis (alga B) Saccoderm desmids (alga C) Gleocystis/Palmella	Vaucheria sp. Filamentous green (alga F)
Climax *Calluna* cover	Zygogonium ericetorum Hormidium flaccidum Oocystis (alga B) Gleocystis/Palmella Chlorococcales type(algaA) Colonial green (alga D)	Vaucheria sp. Saccoderm desmids (alga C) Pennate diatoms Desmococcus/Apatococcus(algaE) Dactylococcus sp.

Table 12 Change in cover values of Zygogonium ericetorum grown in $7cm^2$ pots in a cold greenhouse over a 6month period (August 1973 – February 1974).

Key +++ Abundant. Cover half soil surface

++ Frequent. Cover $\frac{1}{8}$ – $\frac{1}{2}$ soil surface

+ Uncommon Cover less than $\frac{1}{8}$ soil surface

Initially all pots contained Z. ericetorum covering at least $\frac{3}{4}$ soil surface

Pot number	Substrate	Zygogonium ericetorum	Hormidium flaccidum	Other filamentous algae	Mosses Proto-nema	Mosses Game-tophytes
1	Silica sand	+	–	+++	+	++
2	"	++	+	–	++	+++
3	"	++	–	++	–	+
4	"	++	+	++	–	+++
5	"	–	++	–	++	+
6	"	+++	–	–	+	–
7	"	+++	+	–	+	–
8	"	++	–	–	++	–
9	"	++	++	+	–	–
10	"	++	+	–	–	–
11	"	+	+++	+	+	–
12	"	+	+	–	+	–
13	"	++	–	+	++	+
14	Peaty soil	+++	++	++	–	++
15	"	+++	–	–	++	–
16	"	+++	+	–	–	–
17	"	+++	–	–	–	–

6: Discussion

Soil is an organised, natural body resulting from the weathering of parent rock near the earth's surface with the incorporation of organic matter. Mining spoil, on the other hand, is the parent material that will eventually become soil and the process of soil genesis may take decades. In the china clay industry, both silica sand and micaceous residues are waste products that show many of the characteristics exhibited by mining spoils in general.

(i) pH. Both silica sand and mica have a low pH; that of the tips in the Drakelands Corner area is around 4.3. The sand shows a certain buffering capacity but this must be of only limited degree as reflected in the low cation exchange capacities (around 3 meq/100 g.) recorded. The C.E.C. of productive soils frequently exceeds 100m.eq./100g. soil.

(ii) Drainage. Both silica sand and mica are open free-draining materials but as micaceous wastes tend to be deposited in flooded mica dams, water loss here is less of a problem. Silica sand consists predominantly of gravel and coarse sand-sized particles. Being a porous substrate from which the colloidal minerals have been removed in the china clay extraction process, rain percolates rapidly through the sand and only small amounts of water are absorbed. The hygroscopic water content of this waste is very low (around 0.5% water).

(iii) Erosion. The rate of erosion of spoil material is closely dependent upon the infiltration capacity of water. This is related to the length and steepness of the slope, the sand: clay ratio and the extent of crusting and compaction in the surface layers. Sand tips are subjected to both sheet and gully erosion and the latter is very marked on old waste tips. Erosion is dealt with more fully in Section 11.

(iv) Nutrients. The china clay industry is situated in an area of high rainfall and low evapotranspiration which suggests that the sandtips are prone to leaching of nutrients. The sand is almost pure silica and its mineral content is therefore very low. Bradshaw <u>et al</u> (1975) showed that china clay spoil contained sufficient micronutrients but was highly deficient in macronutrients. If macronutrients, in particular ammonium nitrogen and nitrates, were supplied artificially they were rapidly leached.

(v) Toxic elements. According to Bradshaw et al (1975), china clay waste differs from many mining spoils in that it does not appear to contain toxic levels of metals.

The properties of china clay waste are reflected in the plants that eventually colonise it. These are all low nutrient, acid requiring species.

At first, only certain micro-organisms are able to survive on the sandtips. These are easily transported with dust and remain viable for a long time in the dry state. With the advent of wet weather, they are able to grow on the spoil surface. Of these micro-organisms, the filamentous algae are important in the colonisation sequence as they are able to bind and consolidate the sand particles because of their ability to grow in the form of a dense network. On china clay waste, this role is occupied almost exclusively by a single species, Zygogonium ericetorum, although Hormidium flaccidum also occurs locally and the two often grow together once the Z. ericetorum mat is established. Once an algal mat has become established over an area of spoil, the sand is less liable to erosion and conditions become favourable for soil formation.

The initial impact of vegetation growth on the spoil is to provide a surface cover, the accumulation of litter and the translocation of nutrients. Nutrients are likely to be only slowly released from dead organic matter as the acid spoil conditions will reduce bacterial activity. There is likely to be a loss of considerable amounts of nutrients in runoff water and by erosion. These effects are likely to be particularly critical in the early stages when the sand tips supported only a cover of Z. ericetorum. A factor in explaining the observation that the first rooted plants often become established in depressions and gullies may be that it is in those areas that runoff water is channelled. The concentration of Calluna plants in gullies on sand spoil slopes is most marked in situations where the runoff water is coming from Calluna heath as opposed to bare silica sand. However, the benefits of slightly higher levels of water and nutrients are offset to some extent by the greater likelihood of erosion.

Vascular plants, which have the capacity for biological sorting of mineral salts and enriching the soil with organic matter, play the main role in soil formation although algae are important in the initial phase of the process. An incipient humus horizon with a brownish colour forms at the surface of the soil. A profile description of well vegetated sites shows that the effect of vegetation and weathering eventually extend to a depth of at least 15cm. Good drainage of spoil to some extent favours the development of extensive, deep-rooting systems.

Following the establishment of an algal cover and the appearance of the first annual and small herbaceous perenial plants on the sandtips, the colonisation sequence becomes dominated by the development of Calluna vulgaris. The surrounding heathland which supplies the potential colonists is of the type described by Gimingham (1972) as Calluna - Ulex galli/europaeus heath and is characteristic of lowland heaths in Western Britain. Later seral trends have been obscured by grazing.

The stages in the Calluna life cycle and the associated cryptogams are similar to those described by Gimingham (1972), Coppins and Shimwell (1971), and others for unburned heath. However, these workers found that Zygogonium ericetorum was restricted to the pioneer phase in the Calluna development and did not reappear at a later stage. On china clay spoil tips, even during the building phase of the Calluna, a continous closed community is not established and plants from preceeding seral stages, noteably Z. ericetorum, are not eliminated from the community. Fritsch and Salisbury (1915) have shown that the average rate of growth of Calluna is more rapid where peat is deep than where the latter is shallow, and it is likely that the natural growth of woody plants on sandtips is slow because of the lack of nutrients, in particular nitrogen and phophorus. This, and the competition between individuals for available water and nutrients are likely to be important factors in the spatial distribution of Calluna plants.

Old china clay tips in the Drakelands Corner area show a pattern of plant communities at different seral stages large areas support vegetation of similar age. In the community characterised by Calluna in its degenerate phase, two ~~sig~~ scales of pattern may be observed. The large scale of aggregation of Calluna bushes is due to the existence of a network of sheeptracks outlining the clumps. Individual Calluna bushes tend to be spatially isolated from others of the same species and the ground between them generally remains uncolonised apart from a cover of Z. ericetorum. This phenomenon has been observed elsewhere. Gimingham (1972) records that intervening spaces between Calluna plants on dry soil in the South of England may remain bare or partly lichen covered for many years. Roff (1964) observed partial suppression of grasses and other angiosperms in a ring around larger Calluna bushes in the Breckland extending to 50cm beyond the periphery of the Calluna canopy. He was able to show that this effect was associated with the presence in the soil of living Calluna roots. At Drakelands Corner, the only plants apart from Z.ericetorum growing on these bare areas are Cladonia uncialis and Dicranum scoparium. Neither of these plants grow closely associated with Calluna. The Calluna plants themselves exhibit a morphological development which involves degeneration in the centre of the patch occupied by the individual, so that a "gap phase" is formed. Most of the other plants in the community are associated with the Calluna bushes, in particular the mosses and lichens. Generally, the gap is recolonised by Calluna seedlings thus completing the cycle, or by other flowering plants, but in the area under consideration no widespread recolonisation was evident. However, it is likely that a community is able to remain in this condition for some years before recolonisation occurs.

SECTION 11

ZYGOGONIUM ERICETORUM AND HORMIDIUM FLACCIDUM

ON CHINA CLAY WASTE

AND EFFECTS OF ENVIRONMENTAL

FACTORS.

7: Literature Review

Terrestrial algae have been less thoroughly investigated than aquatic algae and consequently much remains to be discovered concerning this large and quite important category of algae. Fritsch (1922c) distinguished two distinct algal communities in terrestrial sites. He described a community living between the soil particles beneath the surface and composed of microscopic forms, and a community of forms living on or above the soil surface and including types which form considerable expanses readily visible to the naked eye. Members of the latter group are most subject to environmental extremes.

Surface algae can modify the soil in which they are growing and Shields and Darrel (1964), in their review of the literature concerning soil algae, describe improved infiltration, decreased runoff and the formation of a stable substrate for seed germination as the most important of these modifications. Any possible competition effects these algae may show are likely to be outweighed by their role in reducing leaching and in maintaining a reserve supply of mineral elements and organic matter in a semi-available form. Blue-green algae may assume a significant role in increasing soil nitrogen, particularly in semi-desert environments.

There are many reports of soil algae constituting the pioneer stage of plant succession on substrates poor in nutrients. Booth (1941) describes how blue-green algae form a complete algal layer over hundreds of acres of badly eroded land in the south-central United States and how this cover may last for many years until vascular plants are able to become well-established. Gayel and Shtina (1974) emphasise the importance of algae in the initial phases of soil formation on the sands of the steppe, semi-desert and desert areas of the North Caspian region. In these areas, filamentous algae (in particular Hormidium spp., Phormidium spp., Schizothrix spp., and Plectonema spp.) are important in fixing the moving surface of sands, bare of vegetation. Experimental evidence of Forest, Willson and England (1959) suggests that there are no specific pioneer terrestrial algae but that colonisation is accomplished by the commonest algae in the surrounding areas that are able to survive the adverse conditions. Fritsch (1922c) points out that in temperate areas, Chlorophyceae rather than Cyanophyceae are important in colonising bare areas.

An important pioneer alga in this country is Zygogonium ericetorum (Kutz)Hansg., a filamentous green alga classified with the Zygnematales. There has been much dispute over the taxonomic position of this alga within the order. The species was first described by Kutzing in 1843, and he proposed the genus Zygogonium as distinct from Zygnema on the basis of the location of the zygospore in the conjugation tube between the two gametangia, a feature which subsequent workers have shown not to be constant. De Bary's grounds for a genus Zygogonium, put forward in 1858, were based on the assumption that the conjugating cells first formed "progametangia" which afterwards fused to form a zygospore. West and Starkey (1915), in their study of the cytology of Z.ericetorum, examined de Bary's material and came to the conclusion that it was showing abnormal conjugation. They proposed that there were insignificant grounds for separating the species from the genus Zygnema and that consequently the genus Zygogonium should be discarded. The only distinguishing character they are able to confirm was a secondary one, namely the presence of a single dumb-bell shaped chloroplast in each cell rather than two separate chloroplasts as shown by Zygnema species. Transeau (1951) however, lists 14 species referable to the genus Zygogonium and gives seven characteristics of the genus. He considers that this demonstrates the justification of its separation from the genus Zygnema, and Zygogonium as a genus is still upheld today.

Transeau (1951) states that Zygogonium ericetorum has been reported from all continents and is the only species of the genus described from Europe. There can be little doubt that it is a variable species and Czurda (1932) suggests that the forms mentioned in the literature can develop, under certain conditions, from various species. Gau (1934) disputes this and maintains that each form is referable to a different species.

The classic habitat for Z. ericetorum is damp ground and peaty water. Hosiaisluoma (1975) describes the alga as being a characteristic dominant of muddy peat areas of raised bogs throughout northern and central Europe. In America, a species of Zygogonium probably referable to the present one, is reported by Lynn and Brook (1969) to be a conspicuous feature of many thermal areas of the Yellowstone Park where the soil is acidic. Peterson (1935) classifies Z. ericetorum amongst the "hydroterrestrial algae", that is those growing on soil which is always damp or wet due to the ground water level being close to the surface. Clearly, this is not always the case for Fritsch (1916) has described a morphologically distinct extreme terrestrial form from bare areas on Hindhead Common. According to Peterson (1935), it is a characteristic species of acid soils varying in pH from 3.7 to 5.2; Lynn and Brook (1969) found that the optimum pH range of their Zygogonium species ranged from 1.0 to 5.0.

Zygogonium ericetorum is a well-adapted species for a terrestrial or semi-terrestrial environment. It has strongly thickened and mucilaginous walls and whole filaments can exist for long periods with the cells in the akinete stage, as described by Fritsch (1916). The cells contain abundant fat globules especially during the winter months and Peterson (1935) has suggested that these serve the same function of frost resistance in algae as in more advanced plants. Collyer and Fogg (1955) believe that in some classes of algae, including the Chlorophyceae, water deficiency of available nitrogen are the important factors in leading to an accumulation of lipids.

A characteristic feature of material from many sites is the presence of a purple pigment in the cellsap. This has been extracted by Alston (1958) who describes it as an iron-tannin complex. Fritsch (1916) believes that the pigment is at least in part a protection against strong illumination.

Another adaption is the extremely concentrated cellsap and the rapidity with which moisture is absorbed from the atmosphere. Fritsch (1922c) has shown that some cells remain unplasmolysed when Z. ericetorum filaments are mounted in a 10 per cent solution of sea salt, a concentration amounting to 15 atmospheres. He uses this to explain the occurrence of this alga together with Rhizoclonium riparium above the tideline in the south of France. Fritsch concluded that resistance to dessication, high temperature and high salt concentration were all interlinked factors. McLean (1967) found that in the green alga Spongichloris typica, desiccation resistance and heat resistance appeared to be two separate phenomena both of which were ephemeral. There was an indication that increased desiccation resistance was favoured by low concentrations of nutrients in the medium whilst heat resistance was favoured by high concentrations of nutrients. In Spongiochloris typica these phenomena were not related to an accumulation of lipids.

Zygogonium ericetorum has frequently been referred to as a coloniser of bare areas. Fritsch (1922c) remarks how it often produces extensive wefts covering many square metres of ground, particularly on sandy soil. Lynne and Brock (1969) report the presence of extensive Zygogonium mats up to 300 m^2 and 6 cm. deep from thermal areas of the Yellowstone National Park where a distinctive community of other algae and invertebrates has been developed living within the mats. In this country, Z. ericetorum is locally a characteristic species on burnt heathland soils, as described by Fritsch and Salisbury (1915). Three main stages in the development of heath after burning are recognised namely the pioneer stage, building stage and mature/degenerate stage, these phases being associated with the morphological development of Calluna vulgaris. Coppins and Shimwell (1971) found Z. ericetorum was restricted to the pioneer phase in the East Yorkshire heath types they explained. West and West (1897) suggest that interlacing wefts of the alga have high absorptive properties thereby regulating to some extent the moisture content of the surface soil and permitting the more successful establishment of other plants. In peat hollows on moorland, the black muddy ground is frequently colonised by Z. ericetorum but here, Moore and Bellamy (1974) suggest that the thin mat presents a barrier to colonisation by other plants, as the peaty water becomes saturated with oxygen as a result of the alga's photosynthesis so that corrosive oxidation of the peat is promoted. Fritsch and Salisbury (1915) report no difference in cover of the alga throughout the year but John (1942) states that although present throughout the year it becomes more conspicuous in the spring.

Hormidium is a genus of filamentous green algae classified as members of the Ulotrichales. The generic name has been used in the past to cover a wide range of filamentous algal types. Hormidium flaccidum and H. nitens are separated by rather dubious cultural characteristics, the latter forming a silky growth at the surface of liquid cultures. Lund (1947) doubts whether this is a distinct species from H. flaccidum. However, H. flaccidum is recognised to be widespread and morphologically quite variable in soils and it is likely that there are a number of physiological races. Mattox (1971) studied eight different isolates from wet and dry habitats and found that in slowly ageing cultures, three types of resistant cells were produced, all of which survived dessication and in a few isolate could withstand 100°C for one hour.

Certain reports refer to both Zygogonium ericetorum and Hormidium flaccidum growing together on soils. Fritsch (1916) noted a situation on Hindhead Common where H. flaccidum was overgrowing the Z. ericetorum mat in some areas. Fritsch and Salisbury (1915) refer to both species being encountered, although somewhat locally, on burnt heathland. John (1942) collected material of Z. ericetorum, which had filaments of H. flaccidum growing amongst it, from Epping Forest and Oxshott Heath. Lund (1947) has found that the algal flora of very acid soils (pH 3.7 - 4.8) consists of comparatively few species, almost all members of the Chlorophyceae. Because the algal flora remains very restricted in species even after enrichment of these acid soils, she assumes that the amount of competition between species naturally occurring on these soils is comparatively slight.

Hormidium flaccidum tends to be characteristic of rather heavy, base-deficient soils although Lund (1947) records it from soils varying in pH from 4.4 to 7.6. Piercy (1917) describes how, on certain soils as grasses die during dry summers their place is taken by H. flaccidum which grows rapidly in the succeeding wet periods to form a dense mat. The following spring however, grass seedlings crowd out the growth of the alga. H. flaccidum is very drought resistant; as it dries, longitudinal folds develop along the filament indicating that the cellwall adapts its contours to the shrunken protoplast. Because of the close contact between cellwall and protoplasm, a rapid uptake of any moisture is ensured. Fritsch (1922) has demonstrated that it is able to take up almost twice as much water as air-dry Z. ericetorum. He has also shown that H. flaccidum has survived dessication in the soil for 36 months.

8: Zygogonium ericetorum and Hormidium flaccidum - Morphology

Zygogonium ericetorum (Kutz) Hansg.

The material of Zygogonium ericetorum from the Drakelands Corner sandtips was comparable with the so-called "extreme terrestrial form" described by Fritsch (1916). The most prominent features of the mature cells (Fig 21) were the presence of a purple vacuolar pigment and the large "dumb-bell" shaped chloroplast. Cell size was very variable but mature cells from sandtip areas were from 15 to 33 µ in length and approximately 22 µ in width. The shorter cells contained a single chloroplast with a single pyrenoid, the longer cells contained two chloroplasts joined by a relatively narrow bridge, and two pyrenoids. The cells contained, numerous fat bodies arranged in a dense peripheral layer beneath the walls. These were particularly abundant in material collected during the winter months to the extent that the chloroplasts were masked by them (Fig 20). The ultrastructure of this alga is described in Section 111, page 178.

A characteristic feature of the cell contents of Z. ericetorum was the presence in the sap of a purple pigment. The absorption spectrum of an aqueous extract of this pigment was found to be similar to that reported by Alston (1958) for this species. He described the pigment as an iron-tannin complex. It was noted that in dense mats of the alga at Drakelands Corner, only those filaments at the surface were deep purple whilst those beneath were macroscopically green although some purple colouration was visible in these cells on microscopic examination.

The cellwalls in the Drakelands Corner material were up to 2.5 µ in width. Many of the cells appeared to be permanently in the akinete condition. In this condition, the protoplast becomes rounded-off and full of fat bodies and produces a new wall around itself and inside the existing thick cellwall (Fig 20). Often, a small cell containing deep purple pigment but little protoplasm was noted situated adjacent to an akinete. This appeared to be a pigment cell as described by Fritsch (1916) who suggested that it was formed by the extrusion of purple sap from a developing akinete into an adjacent newly formed cell.

The growth of filaments is by the active division of akinetes during periods of favourable environmental conditions followed

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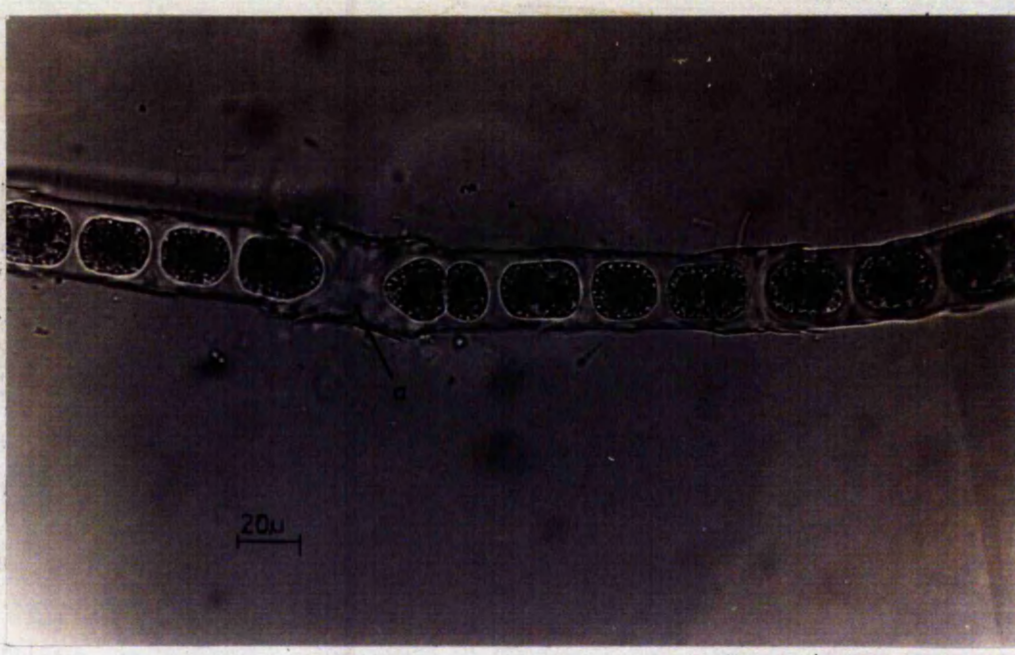

Fig. 20 Filament of Zygogonium ericetorum from silica sand spoil.
Note thick cellwalls, short cells packed with oil globules
and thickened collar (a).

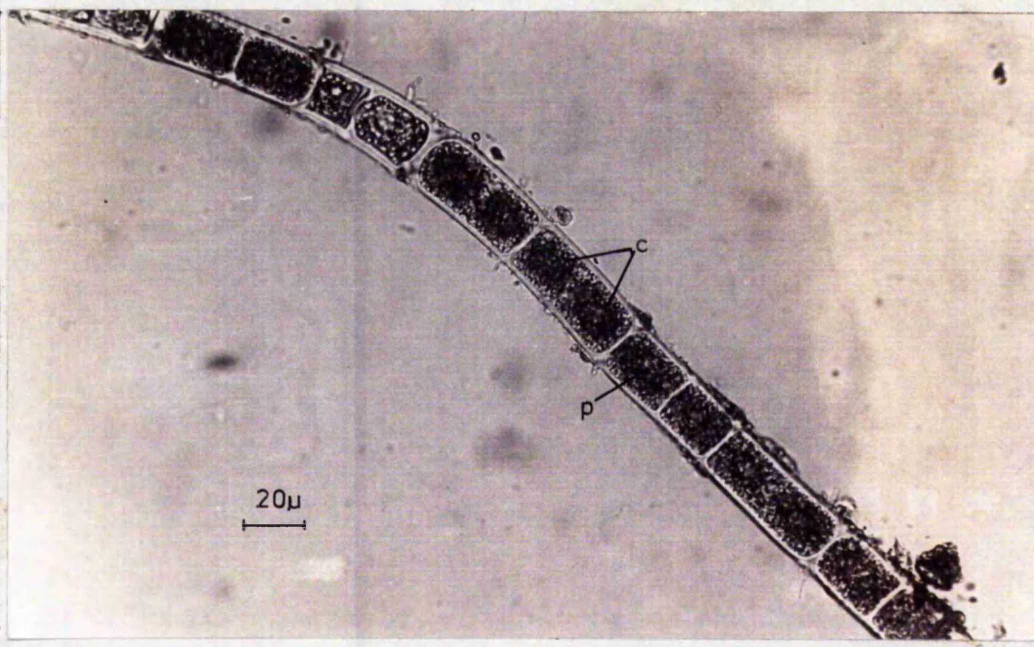

Fig. 21 Filament of Zygogonium ericetorum from damp mica area.
Note actively dividing, thin-walled cells, double chloroplast (c)
each with a pyrenoid (p).

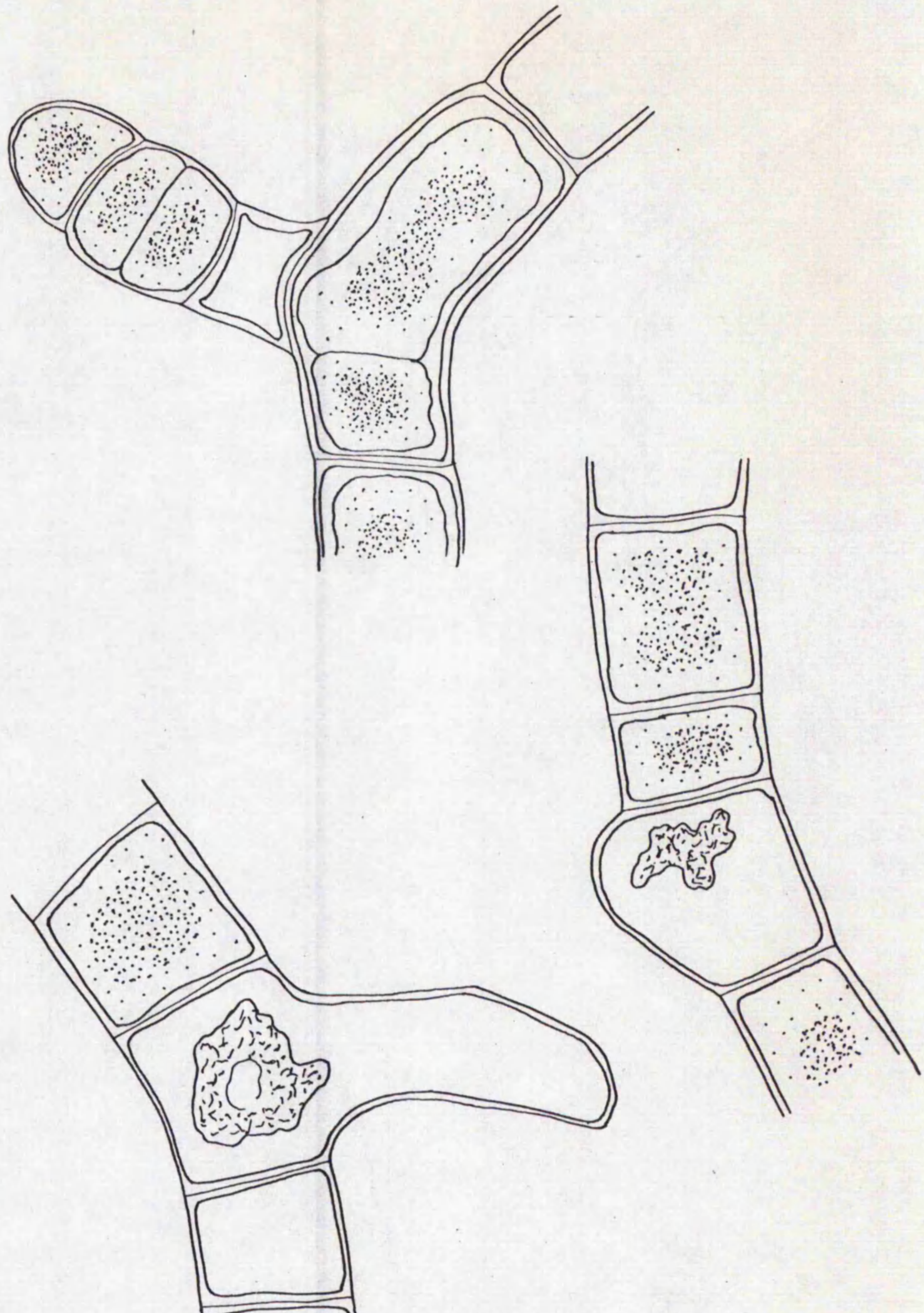

Fig.22 H.P. Diagrams of Z. ericetorum showing side branches.

by the rounding-off of these protoplasts and their formation into new akinetes in adverse conditions. Because the formation of each akinete requires the production of a new wall inside the existing one, this leads to an increasing thickness in the cellwalls and this is particularly noticeable in the crosswalls. Examination of a filament under the microscope shows the presence of strongly thickened collar-like crosswalls at intervals (Figs 20 and 23), the interval between two such strongly thickened walls representing the products of division of a single akinetes. Hence, by examination it has been possible to learn something of the past activity of cells within a filament.

Filaments were found to be up to 1cm. or more in length and unbranched, although short branches were infrequently noted. A collection from one damp micaceous area yielded a large proportion of filaments with side branches (Fig. 22), and subsequent collections showed that these could also be demonstrated in a small proportion of filaments from material collected in other damp mica areas but were extremely rare in material from sandtips. Side branches ranged from a simple swelling on one side of a cell to branches of two or three cells. These have been referred to as rhizoids by Fritsch (1916), and West (1916) mentions that occasionally branches consisting of ten or even fifteen cells have been recorded.

Increase in the number of filaments appears to be by fragmentation with dead cells constituting a potential rupture point in the filament. Filaments were frequently noted where large numbers of the cells had died and filaments were also seen in which isolated and apparently healthy akinetes were scattered amongst the predominantly dead cells. The latter was especially noted in material growing in dry conditions at the end of the summer.

Sexual reproduction has only rarely been reported for Z. ericetorum and there are conflicting reports as to the exact nature of the process in ths species. However, it seems apparent that conjugation is basically scalariform, as in the genus Spirogyra. No evidence of sexual reproduction was found in any of the Drakelands Corner material examined. However, amongst a collection of material from a moorland site on millstone grit at Cown Edge, Derbyshire (Grid Ref. SK018918) a number of filaments showing early stages of conjugation were observed. These filaments showed protuberances from many of the cells (Fig.23),

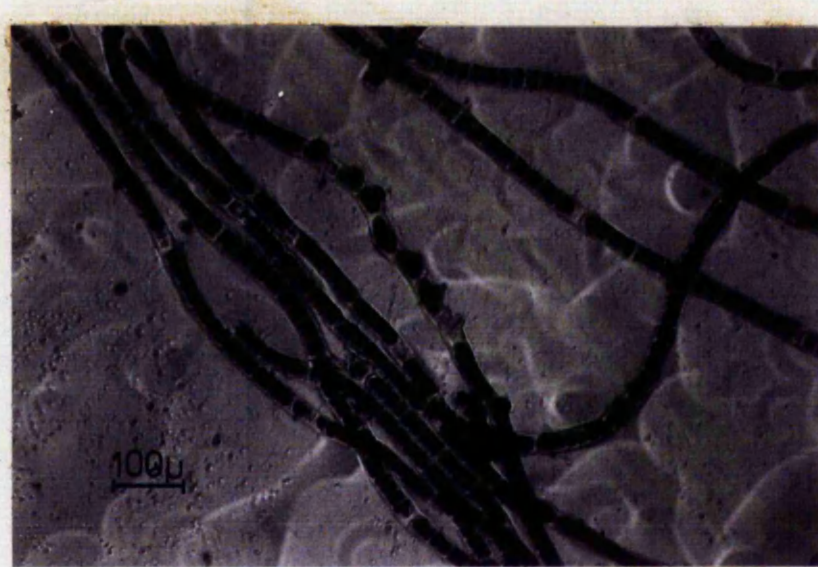

Fig. 23 Filaments of Z. ericetorum showing possible early conjugation.
Cown Edge, Derbyshire. Oct. '75
Stained lactophenol / cotton blue

Fig. 24 Dead filaments of Z. ericetorum attacked by Chloridium sp. with conidia (c).
Drakelands Corner, Devon

similar to those described by Alston (1958) and Hosiaisluoma (1975) in young conjugating material of this species. No actual conjugation was seen in any of the material examined.

<u>Occurrence of a fungal pathogen on Z. ericetorum</u>

Material of <u>Z. ericetorum</u> collected from Drakelands Corner and elsewhere frequently contained a fungus associated with the filaments and within the actual cells. In certain small areas almost every filament showed at least a few infected cells whilst in nearby areas it was absent. The fungus appeared to be parasitic on the algal cells. In the early stages of infection, the hyphae commonly enveloped the algal cellwall in the region of a crosswall (Fig 25), subsequently penetrating the cellwall and eventually filling the cell with hyphae. From this infection point, hyphae extended to adjacent cells by passing through the crosswalls or along the outside of the filaments (Fig.24). Both dead and living cells were attacked in this manner.

The fungal hyphae were swollen and septate with thick brownwalls. Occasionally, a few detatched spherical conidia were noted in the material examined (Fig. 24) and on one occasion, a structure resembling a short chain of conidia associated with the hyphae was found (Fig. 27). No other reproductive stages were observed at any time.

During electron microscopy studies of <u>Z. ericetorum</u>, the fungus was observed in some of the material. Fig. 26 shows three hyphae on the outer surface of a filament. This material was fixed and embedded as outlined on page 173. It can be seen that the hyphae are thick-walled and appressed to the algal cell. It is not known whether the smaller structures (a) are associated with the fungus or belong to another organism. Similar small bodies were observed in the cellwall and on the inner side of the wall, in the absence of large fungal hyphae, in other material. These may possibly have been small Chytrids.

Material was sent to the Commonwealth Mycological Institute for identification and a report was received from Dr. Sutton in which he was able to link the mycelium to a single fertile conidiophore belonging to <u>Chloridium</u> LK ex Fr. which was not referable to any of the common taxa present in the herbarium at the Institute. <u>Chloridium</u> is an imperfect fungus belonging to the Dematiaceous Hyphomycetes. According to Ellis (1971), the genus is characterised

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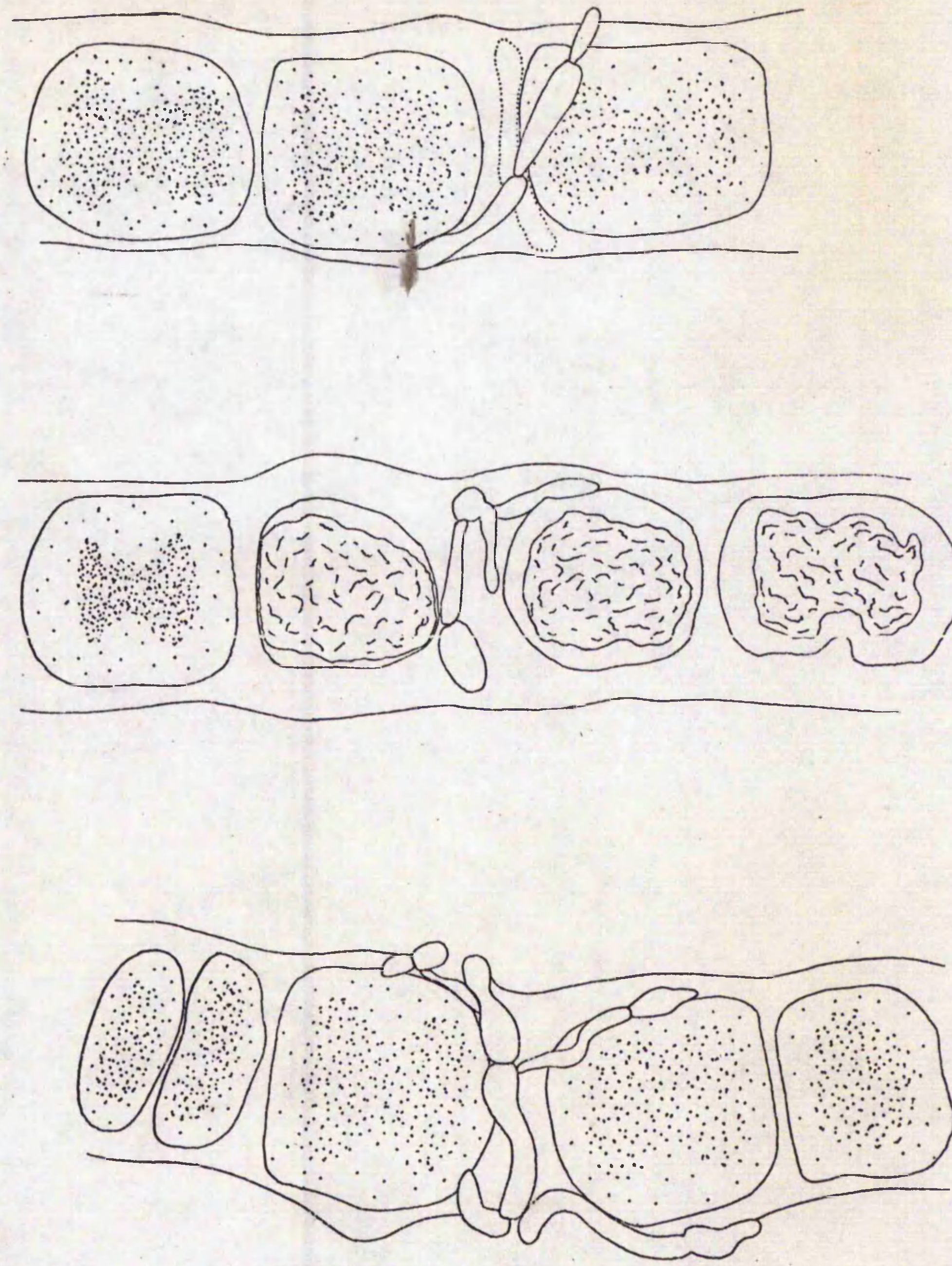


Fig. 25 Camera lucida drawings of early infection stages of Z. ericetorum by Chloridium sp. ×700

Fig. 26 Section of part of a filament of Z. ericetorum in the region of a crosswall showing 3 hyphae appressed to the cellwall

(a) small fungal body.

Specimen glutaraldehyde fixed ; permanganate post-fixed.

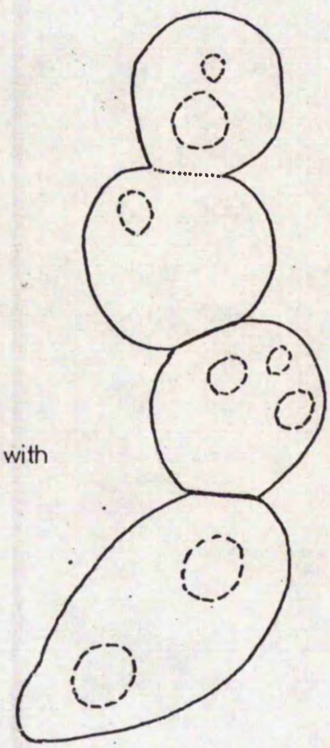


Fig. 27 H.P. diagram of ?conidia associated with Chloridium sp.

by having conidia which are aggregated in slimy masses and often form long columns at the ends of smooth, brown, unbranched conidiophones.

The Chloridium sp. was subsequently found in material from the following sites:

Gomm Tip, Carthew, Cornwall 185M. Old chinaclay tip (SX006553)
Gunnislake Clitters, Cornwall 30M. Old copper/arsenic mine tip (SX422724)
Axe Edge Moor, Derbyshire 460M. Wet heath (SK 024710).

No fungal pathogens have, to date, been reported on the genus Zygogonium but a number have been recorded from aquatic members of the Zygnematales. Sparrow (1960) lists a total of 125 species of aquatic Phycomycetes as parasites, saprophytes or epiphytes on Mougeotia spp, Spirogyra spp. and Zygnema spp., many of which resulted in death of infected filaments.

Hormidium flaccidum (Kutz) Fott - Morphology

The identification of the alga isolated from china clay at Drakelands Corner was confirmed as belonging to this species by Lund of the Freshwater Biological Association (personal communication). The characteristics of this isolate are outlined below.

The filaments were unbranched, frequently of a considerable length, and consisted of a single row of cells (Fig. 28). Their width averaged 5μ and the length of the cells ranged from one-and-a-half to twice their width. Each cell contained a single, band-shaped parietal chloroplast which extended for almost the entire length of the cell and approximately two-thirds of the circumference. Each chloroplast contained a single pyrenoid. Generally, one or two large vacuoles were present in the cell and fat bodies were frequently seen. Rarely, cells became abnormally wide and subsequently divided by a longitudinal wall; two or three adjacent cells were sometimes seen to behave in this manner (Fig 29) and this gave the appearance of occasional swellings along the length of a filament. Filaments were occasionally noted in material growing in liquid culture where this phenomenon had developed to the extent that two parallel filaments, separate from one another except at either end, were produced (Fig. 29 a).

The ultrastructure of H. flaccidum is described in section 111, page 175.

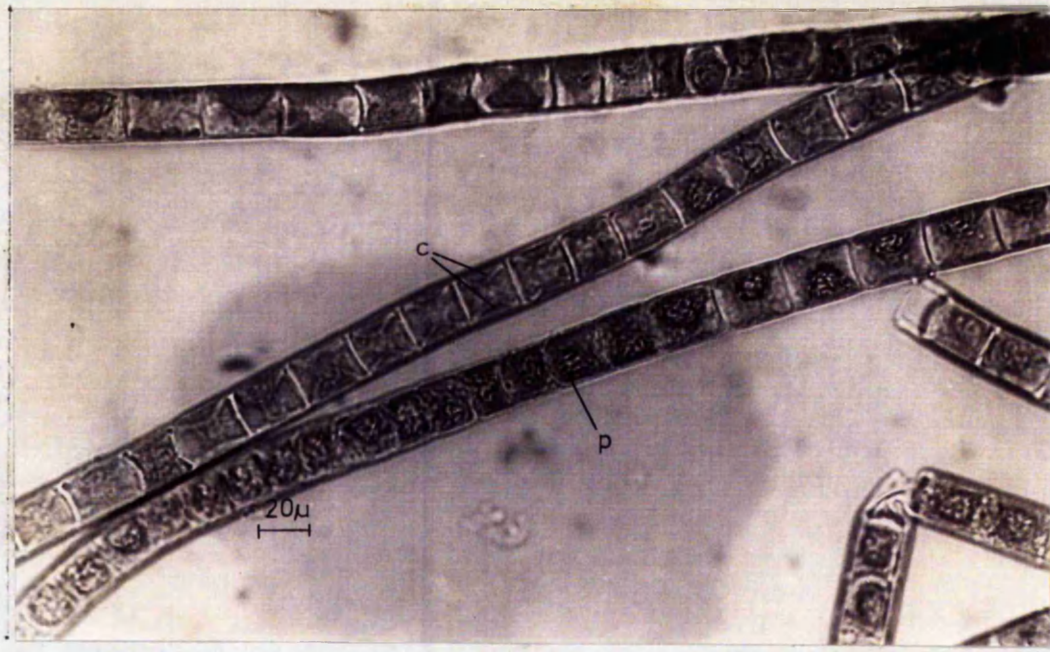
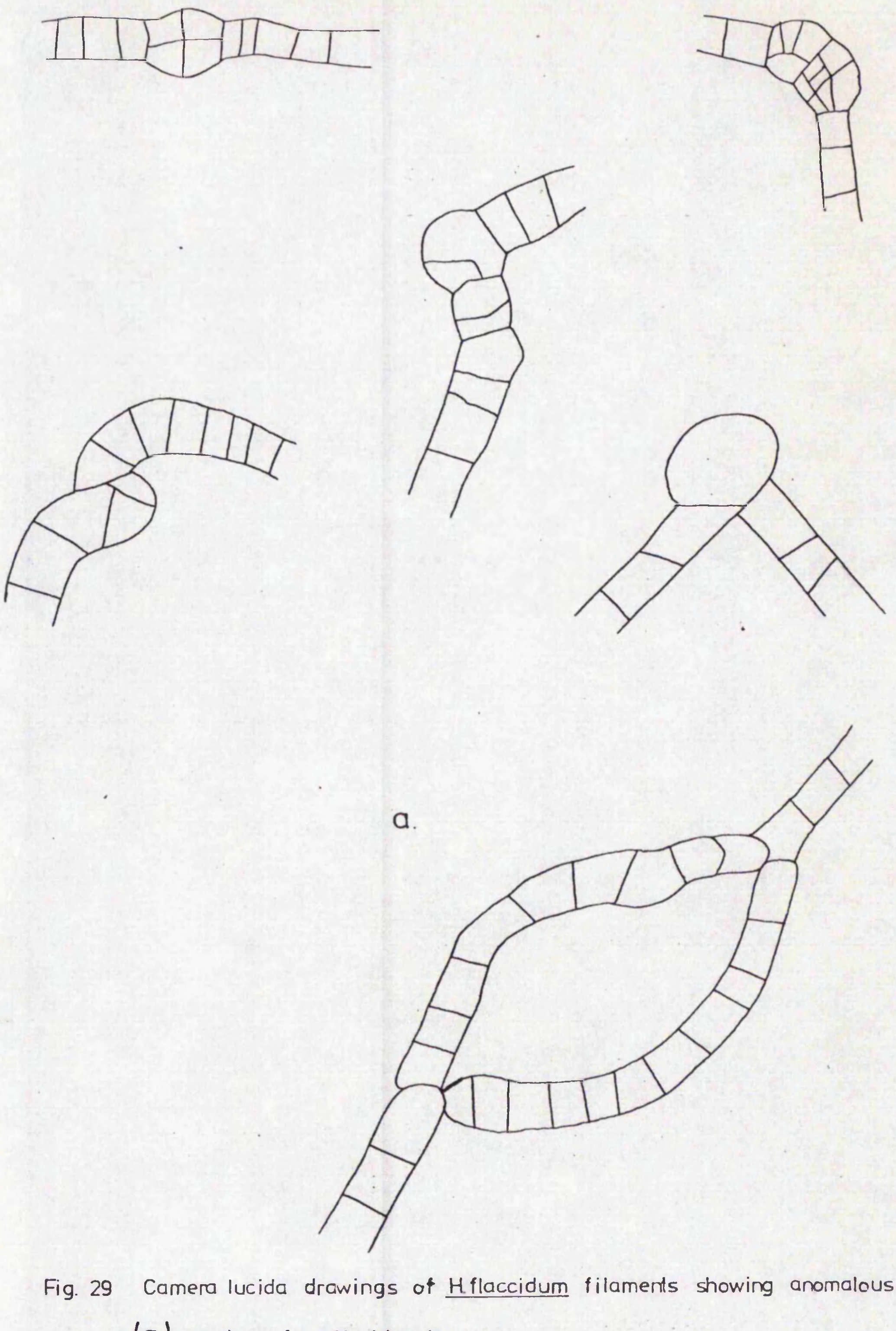


Fig. 28 Filaments of Hormidium flaccidum from silica sand spoil.
Note parietal chloroplast (c) and pyrenoid (p).



a.

Fig. 29 Camera lucida drawings of H.flaccidum filaments showing anomalous growth.

(a) specimen from liquid culture

Growth of filaments on Knop's, agar was followed by transferring short lengths of cells onto fresh media and watching their growth by microscopic examination. It was found that, when grown at 23°C and illuminated at 4300 to 5300 lux through a twelve hour day, a doubling in length of a filament was achieved within 35 hours from transfer. The filaments did not extend in a straight line but adopted a characteristic sinuously-curving pattern.

9: **Colonisation rates of *Zygogonium ericetorum* on china clay waste.**

Previous work has shown that *Z. ericetorum* is a pioneer species on china clay waste and that it can occur with *Hormidium flaccidum*. A series of field experiments were set up in order to gain some idea of the rate of colonisation of *Z. ericetorum* over china clay substrates. For these experiments, permanent $\frac{1}{4}M^2$. quadrats were marked out in suitable areas and the principal algal cover was plotted at regular intervals throughout a one year period. A variety of situations were chosen so that different aspects, degrees of slope and substrates could be compared.

The position of the quadrats was marked by wooden pegs, gently hammered flush with the ground level at each corner, taking care to disturb the microhabitat as little as possible. Recording of the area covered by algae within the quadrat was achieved by laying a $\frac{1}{4}M^2$ gridded frame divided into 25 squares over the area and mapping the position of the algal cover onto graph paper. A disadvantage of this method is that in situations where only a thin cover of the alga is present, this is easily overlooked particularly when the ground is dry. In fact it was only possible to mark the positions of the most obvious patches. The area of these patches, as recorded on graph paper, was determined in the laboratory.

Fourteen quadrats were set out at sites where the algal cover was sparse or at the edge of algal patches, so that the natural increase of existing mat could be charted. At a further thirteen sites, the algal mat together with top soil to a depth of 1cm. was removed from the $\frac{1}{4}M^2$. area and replaced by heat-sterilised quartz sand.

The quadrats were marked out in April 1975. The area, covered by the algae was recorded initially and at monthly intervals from this time until August after which I was obliged to move to Nottingham and and was only able to revisit the sites on a further two occasions. The results are presented together with meterological data for Plymouth (Mount Batten) and Lee Moor for the relevant period.

Results of the permanent quadrat observations have been presented in table form (table 13) and graphically (Figs. 30 and 31). The first fourteen quadrats were marked out in areas where there was an already existing cover and change of percentage area covered is recorded (Fig. 30). In the remaining thirteen quadrats,

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Table 13 Rates of colonisation of *Zygogonium ericetorum* mats on di

Quadrat number	Original area covered 29/4	Change in area covered						Site Descrip		
		30/5	25/6	29/7	26/8	11/12	30/3	Aspect	Degree of slope	Su st
1	35%		21%	6%	5%	3%	21%		Flat	Sa
2	29%		18%	13%	17%	6%	8%		Flat	Sa
3	61%		40%	25%	34%	17%			Flat	Sa
4	16%			21%	7%				5°	Sa
5	15%		28%	38%	41%	61%	+	NW	55°	San
6	20%		28%	39%	54%	54%	+	NW	50°	San
7	14%		16%	29%	48%	+	-	NW	55°	San
8	11%		10%	13%	23%	33%	-	NW	60°	San
9	17%		33%	40%	+	55%	45%	SW	35°	San
10	43%		47%	47%	74%	70%	33%	SW	35°	Sand
11	20%		20%	30%	64%	62%	31%	SW	25°	Sand
12	75%		84%	72%	71%	83%	28%	NW	32°	Sand
13	15%	14%	23%	22%	21%	-	-	NE	70–90°	Sa
14	33%	19%	29%	33%	35%	43%	-	NE	75–90°	Sa
15	0		11%	24%	37%	36%	83%	S/SE	15°	Sa
16	0		1%	13%	23%	34%	80%	SW	15°	Sa
17	0		12%	18%	25%	46%	90%	SW	15°	Sa
18	0		9%	11%	26%	38%	88%	SE	10°	Sa
19	0		4%	14%	18%	42%	71%		2°	S
20	0	19%	24%	29%	64%	87%	94%		Flat	M
21	0	35%	33%	52%	84%	100%	100%		Flat	M
22	0	10%	17%	33%	82%	97%	100%		Flat	M
23	0	9%	20%	36%	76%	94%	100%		Flat	M
24	0	12%	20%	51%	85%	96%	100%		6°	M
25	0	5%	10%	+	+	+	+	NW	33°	S
26	0	6%	10%	+	+	+	+	NW	36°	S
27	0	6%	14%	+	+	+	+	NW	36°	S

+ continous sparse cover

- site lost by erosion

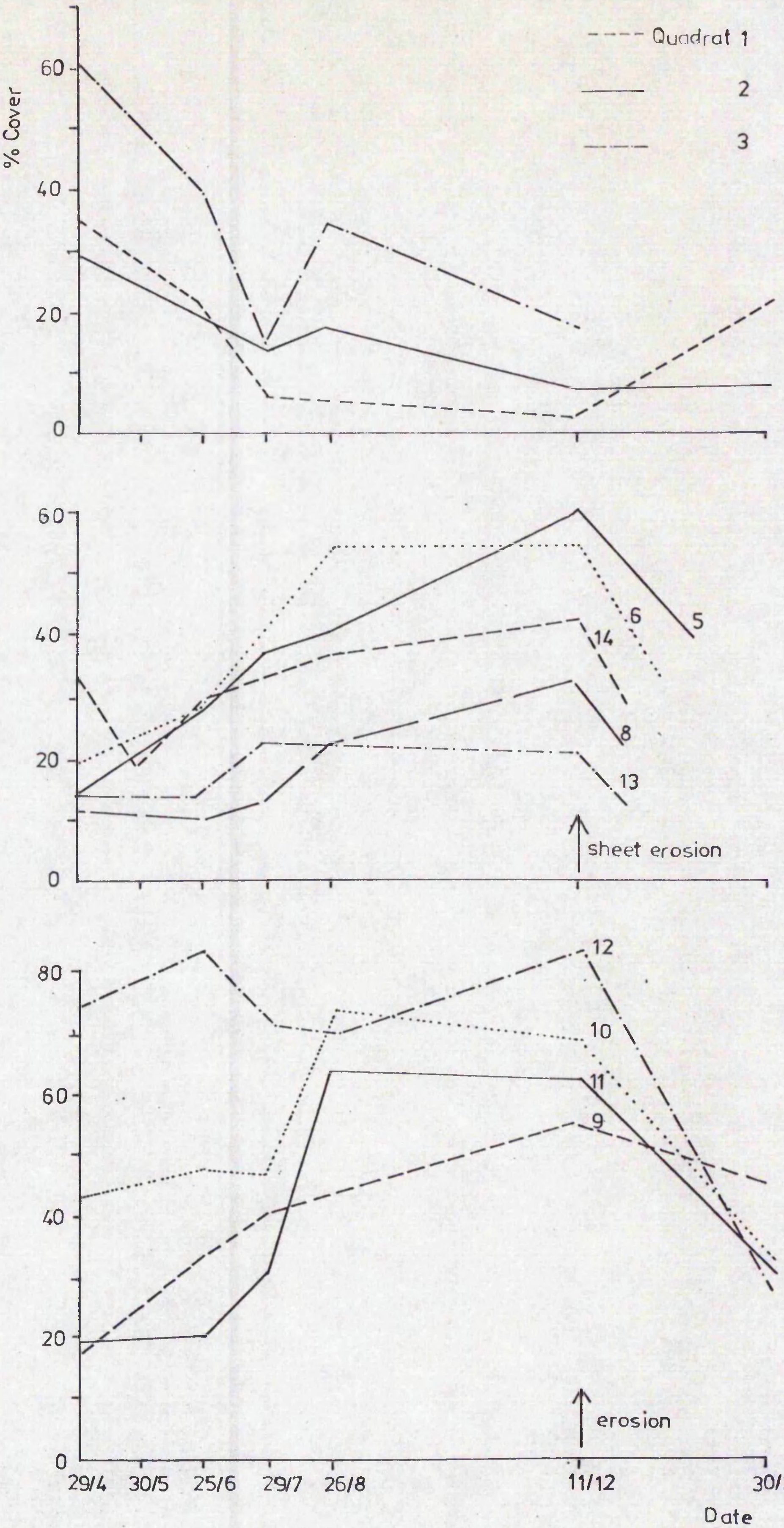
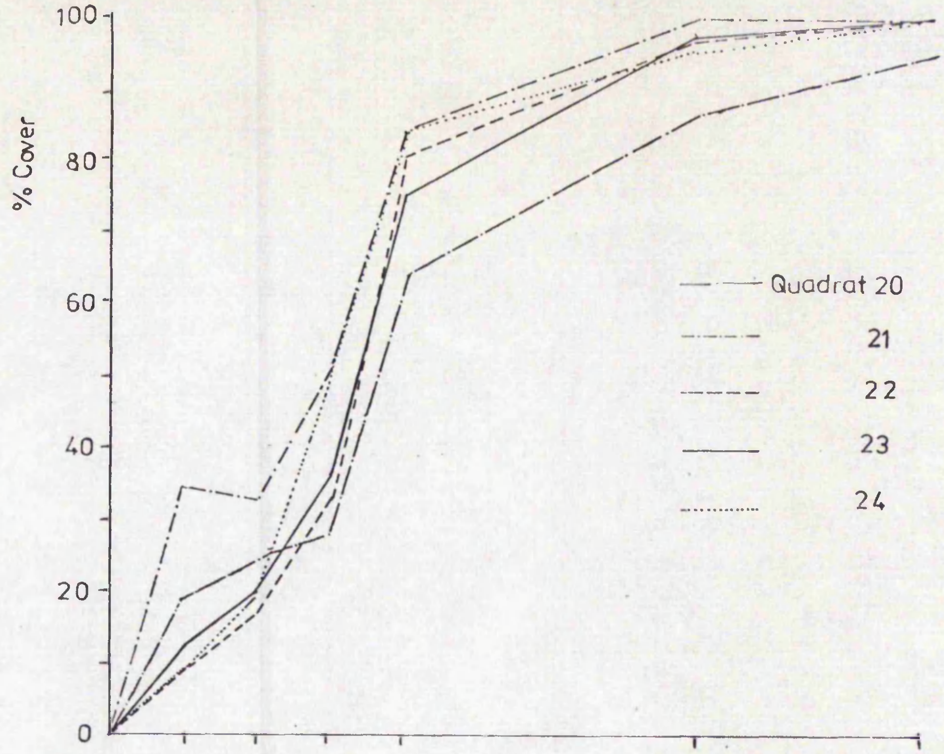


Fig. 30 Change in existing cover of Z. ericetorum in $1/_2 M^2$ quadrats (Nos.1 - 14) over a twelve month period.



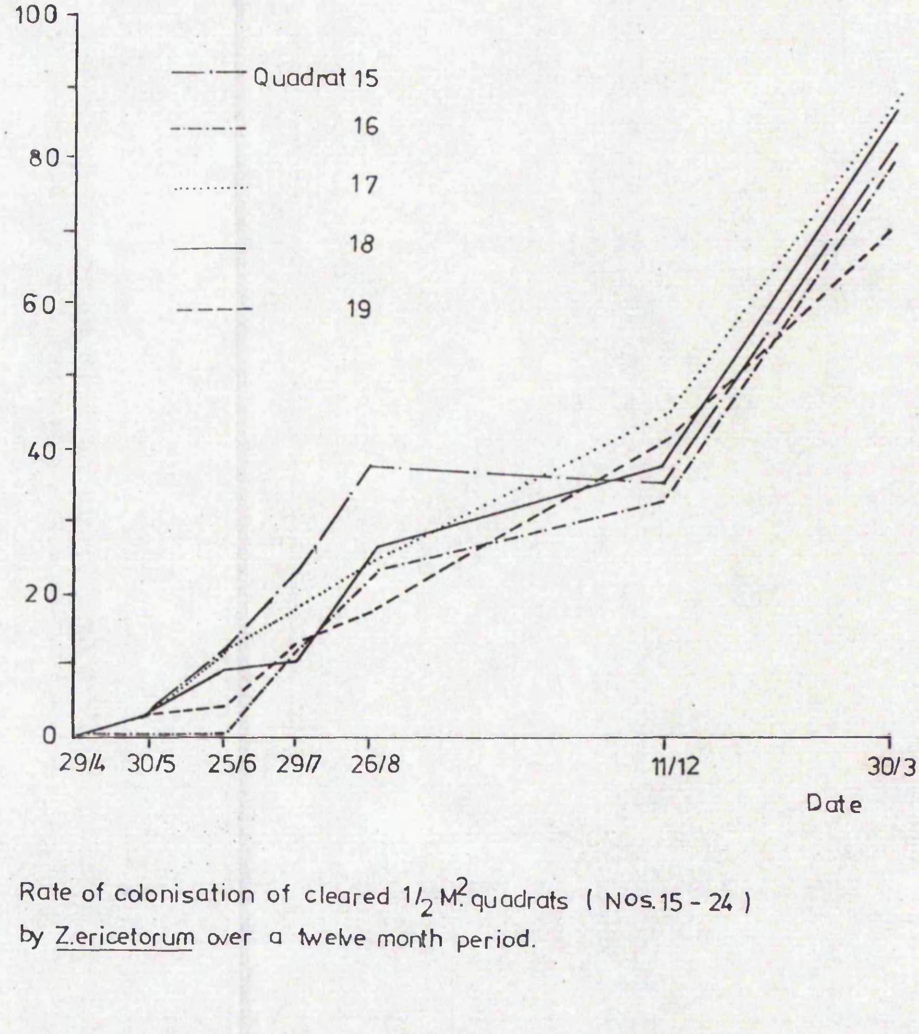


Fig. 31 Rate of colonisation of cleared $1/_2$ M^2 quadrats (Nos. 15 - 24) by Z.ericetorum over a twelve month period.

the initial algal cover was cleared so that percentage increase in area has been recorded (Fig. 31).

The sites covered ranged from flat, heavily compacted areas regularly trampled by livestock (quadrats 1, 2, 3, 4) to steep slopes where sheet erosion was a common phenomenon (quadrats 5, 6, 7, 8, 11, 13, 14). Most of the sites were on silica sand but there were also a few on flat, micaceous waste (quadrats 20-24).

Rainfall figures for the period concerned and the preceeding eleven months have been plotted in Fig 32. These are figures for Lee Moor House (Grid Ref. SX 573629) as supplied by the South West Water Authority. Lee Moor House lies 2½Km. to the Northwest of the Drakelands Corner area at a height of 230M. Potential evapotranspiration has been calculated from average data for the Devon mean county height (151 metres) corrected to the Drakelands Corner height (198 metres) using the Ministry of Agriculture, Fisheries and Foods Tech. Bull. No. 16 (1967). Potential evapotranspiration does not vary greatly from year to year. Temperature readings were not available for Lee Moor House and Fig. 33 shows the monthly means together with the extreme high and low temperatures for Plymouth (Mount Batten Grid Ref. SX 485533).

The results of the monthly observations on the algal cover show that in suitable areas the rate of colonisation by Z. ericetorum can be quite rapid. Spread into new areas appears to be largely by algal filaments being washed down from surrounding areas so that the rate of cover is most rapid where there is a good population of the alga in the vicinity and during periods of heavy rainfall. Rate of increase appears to be greatest between September and April, which is also a period of high rainfall. On well-drained sand heaps (quadrats 25,26 and 27) the algal cover remains sparse but in situations where the ground remains moist a more extensive cover is able to establish itself. The most rapid rate of colonisation was recorded in flat micaceous areas (quadrats 20-24) where the ground was almost permanently moist. In well-drained, compacted areas the algal cover appears to be reduced during the summer months (quadrats 1, 2, 3). In such areas where trampling by livestock is also of importance, the Z. ericetorum

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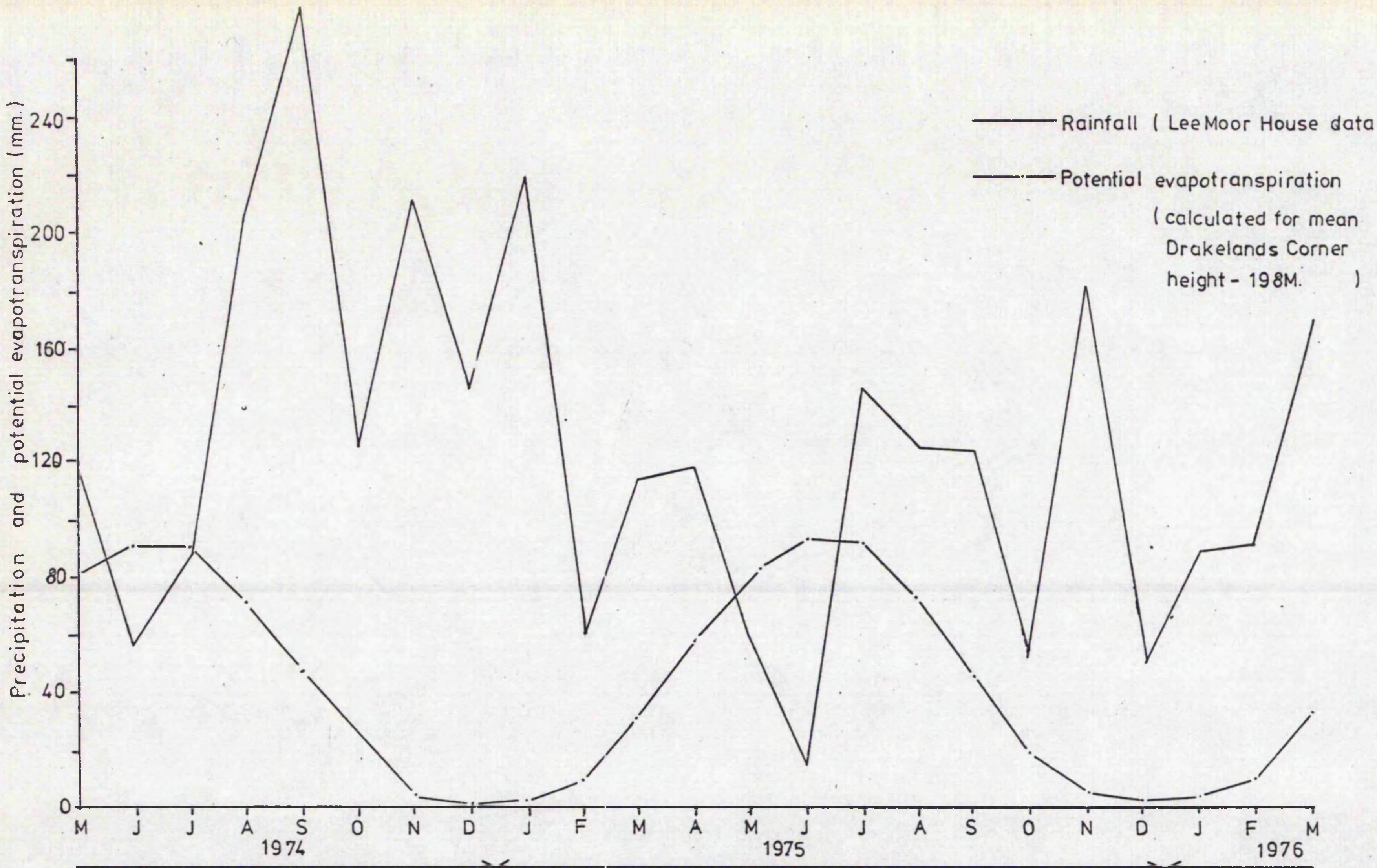

Fig.32 Rainfall and potential evapotranspiration at Lee Moor, Devon from May'74 to March '76.

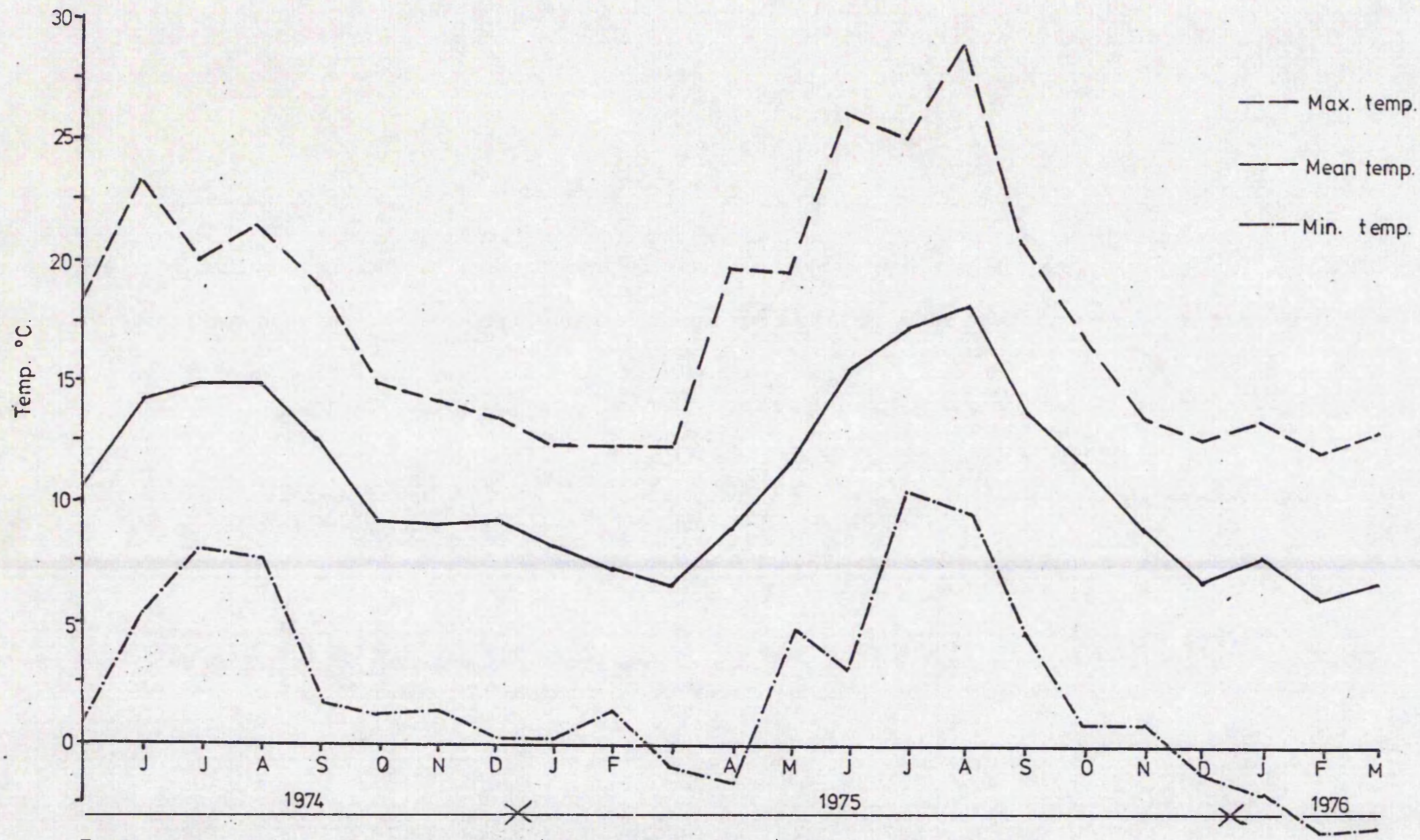

Fig. 33 Meantemperature and extremes recorded at Mount Batten, Plymouth from May '74 to March '76.

Cover is frequently lost in dry weather except in the slots or depressions of old hoofmarks. It may be that such microhabitats retain moisture for longer periods that the surrounding sand. Fig. 32 shows that it is only for a few weeks during the summer that evapotranspiration exceeds rainfall and the surface layers of the soil are liable to dry. However, the summer of 1975 was exceptionally hot and dry so that the results may be somewhat misleading when applied to a typical summer pattern.

Another feature which was shown to be of significance regarding the rate of algal colonisation was the extent of sheet erosion occuring on the slopes. It is generally believed that silica sand heaps, once dumped, are relatively stable but whilst this may be true for the complete tip, the present results show that a significant amount of surface sliding of material occurs. This occurs particularly during the winter months, presumably because of heavier rainfall. Regarding Z. ericetorum, fresh material is continually being brought down onto newly exposed areas but a continous cover of the alga is never established for long.

Investigation into possible allelopathic effects between algal cover and flowering plants.

It has been noted that only rarely does a continous sward of flowering plants become established on china clay waste. Although a continous algal cover frequently grows over the surface, flowering plants generally occur isolated from one another. Some experiments were carried out to investigate whether Zygogonium ericetorum or Hormidium flaccidum produced extracellular products which could interfere with the germination of flowering plants in the area.

Chemical antogonism between plant species is probably an underrated process from the ecological point of view; it is conceivable that many plants produce exudates that can interfere with the growth of other individuals in the immediate vicinity under the right conditions. Moore (1976) has summarised the topic to date with regard to flowering plants but there are also instances of lower plants producing inhibitory substances. Pyatt (1968), for instance, demonstrated the presence of a substance inhibitory to the germination of a number of grasses, in an aqueous extract of Peltigera canina. In this instance, the lichen extract from a heavily polluted area close to a steelworks was found to lack this inhibitory power.

Method

Seeds of *Festuca ovina*, *Aira praecox*, *Spergularia rubra*, *Rumex acetosella* and *Calluna vulgaris* were used. With the exception of *Calluna vulgaris* (obtained from Suttons Seeds Ltd) all the seeds were collected from the Drakelands Corner area.

The algal extracts were prepared by homogenising 100g (wet weight) of cleaned algal material and 300 mls. of preboiled rainwater from the area, in a Silverson mixed emulsifier for several short periods (to minimise temperature increase) totalling fifteen minutes. The mixture was left to filter overnight. The following day, the extract was divided into two portions, one of which was boiled. Seeds were given the following treatments:-

(i) Rainwater	(ii) Rainwater + 100ppm IAA
(iii) Algal extract	(iv) Algal extract + 100ppm IAA
(v) Boiled algal extract	(vi) Boiled algal extract + 100ppm IAA

Rainwater and all solutions containing *Z. ericetorum* extract had pH values of 5.4 ± 0.1.

Seeds were plated out on filter paper moistened daily with 4ml. aliquots of the appropriate solution. Petri-dishes of 9cm. diameter were used to hold 25 seeds and four replicates of each were set up. The dishes were kept at 23°C and subjected to 12 hour photoperiods. Numbers of germinating seeds were counted at intervals up to two weeks.

Results

No significant differences at the 0.001 level were detected in the germination rates with any of the species looked at. Table 14 gives the results of experiments using *Z. ericetorum* extract treatments (i) and (iii). Similar trends were shown with the other treatments and using *H. flaccidum* extracts. Enhanced germination levels were obtained in treatments (ii), (iv) and (vi) due to the addition of IAA.

Table 14 Effects of aqueous extracts of Z. ericetorum on seed germination.

Species	Treatment	% germination 5days	6 days	7 days	9 days	10 days	13days	15 days
Festuca ovina	Rainwater		11	15	32			
	Algal extract		9	12	31			
Aira praecox	Rainwater	12	31	45	55			
	Algal extract	11	21	26	54			
Spergularia rubra	Rainwater	10	17	36	52	82		
	Algal extract	14	22	32	43	77		
Rumex acetosella	Rainwater	9	14	15		26		
	Algal extract	14	16	20		23		
Calluna vulgaris	Rainwater	0	0	0	2		6	8
	Algal extract	0	0	0	4		10	16

10: Environmental factors affecting the growth of Zygogonium ericetorum and Hormidium flaccidum.

Although Hormidium flaccidum is considered, much of this section concerns Z. ericetorum as this was the species constituting the overall bulk of the algal mats on china clay waste.

1. Light

Z. ericetorum grows in very exposed situations so that it is unlikely that high light intensities encountered in the field are a limiting factor in its distribution. Material from well-illuminated situations is often a darker purple colour than that from more sheltered situations or well-shaded sites. However, this is not always the case and the most strongly pigmented material is that growing on flat, damp micaceous residues irrespective of shading. Where thick mats of Z. ericetorum occur, the purple colouration is generally restricted to the surface layers. It has been suggested by Fritsch (1916) and others that the pigment plays a photoprotective role against strong illumination. (see also p.116)

2. Temperature

Growing directly on the surface of silica sand Z. ericetorum is subjected to a wide range of temperature extremes throughout the year. Being of a dark purplish colour, the alga is liable to absorb more heat than the surrounding white china clay waste. On a hot, sunny day in July, temperatures of up to 38°C were recorded with a soil thermometer at the surface of an extensive algal mat whereas the maximum surface temperature of an adjacent area of bare silica sand was 33°C. At the end of the hot summer of 1975, a large proportion of Z. ericetorum examined consisted of dead cells, in particular material at the surface of the mats. This was probably due as much to the dry conditions as to high temperatures.

Similar remarks apply to H. flaccidum regarding the temperature extremes encountered by it. Piercy (1917) states that where it grows with grass, it is able to tolerate dessication for a longer period than the latter and with the advent of wet weather is able to grow over the bare areas formed by dead grass.

Laboratory experiments showed that six hours at 50°C was sufficient to kill all cells of Z.ericetorum whether the material was dry or previously moistened with distilled water. The same treatment was also lethal for H. flaccidum.

Fig. 34 Z. ericetorum washed onto bare micaceous area.
Drakelands Corner March '75
Coin 27mm. diam.

Fig.35 Z. ericetorum washed down face of a cutting from vegetation above.
Drakelands Corner March '75

3. Humidity

Zygogonium ericetorum has for long been recognised as an alga capable of surviving in comparitively adverse conditions, and silica sand must represent one of its most extremes habitats. Because the china clay deposits are all situated in the Southwest of England, in areas with comparitively high rainfall, the habitat is possibly less extreme than well-drained substrates further east. As Fig. 32 shows, the calculated evapotranspiration only exceeds rainfall for a short period during the summer. This period may be more extensive that is shown as evapotranspiration is calculated for normal agricultural soils which loose water less rapidly than sand.

Results of the permanent quadrat observations have shown that water flooding over the tip surface is important in bringing about the dissemination of algal material. In permanently damp low-lying mica areas this process can bring about recovery of the algal cover in a cleared area within four or five months during the summer (Fig. 31) and probably less time in the winter. In such areas which are subject to periodic flooding it is often possible to see where new algal material has been washed onto previously bare micaceous areas and has accumulated around any permanent objects such as stones and small grass plants (Fig.34).

Another illustration of the spread of Z. ericetorum by water drainage is to be seen wherever china clay waste has been exposed beneath a vegetated area as a result of a man-made cutting or by natural erosion. Fig 35 shows a cutting exposing silica sand over which a closed community of Calluna vulgaris, Ulex gallii and Festuca ovina has become established. It is apparent that Z. ericetorum has been washed down the bare slope from the peaty soil above. It is interesting that in this particular community there are few open areas where the alga can grow, so that it is confined to a thin though widespread cover around the bases of the existing plants. When, however, it is spread to the exposed claywaste surface there is no competition from other plants and Z. ericetorum is able to grow more luxuriantly.

Although Z. ericetorum is able to tolerate the comparitively arid conditions of china clay tips, it is clearly better adapted to grow in damper situations. The mica areas support a more luxuriant deep purple growth of the alga than do the sandtips. Fig. 13 illustrates part of an excavated area where the bottom of the pit is more or less permanently damp and consists of a large proportion of micaceous residues whilst the pit sides are predominantly silica sand and progressively better drained with increasing distance from the pit bottom. The algal cover in this fig. is evident as a grey discolouration of the china clay waste and it can be seen that the cover is most extensive at the bottom of the pit and the lower area of the sides. These are the areas where the water content of the substrate is relatively high.

It was apparent that material from these wetter areas was growing more actively than material from higher up the slopes. A similar site to that shown in Fig. 13 was selected in order to investigate whether any differences were apparent in material from different areas of such a slope. Sampling points were selected at intervals along a transect laid down the slope from the top, where Calluna heath was established, to the base where Z. ericetorum was growing immersed in water. At each point a representative sample of the alga was collected for microscopic examination. Thirtyrandom cells were scored from each sample and records kept of various parameters. Results are given in table 15 from which it can be seen that Z. ericetorum varies quite considerably from different sites. Figs. 20 and 21 show filaments from submerged material and material growing on well-drained silica sand, both at the same magnification. Both appear to be referable to Z. ericetorum.

Bearing in mind the spread of the alga by rainwater running down slopes, it is likely that material is regularly washed from the natural population occurring beneath Calluna heath at the top of the pit. It is possible that the same filament may be gradually washed further down the slope and consequently moves from a well-drained substrate to a progressively damper one. If this is the case, then it is likely that as its environment alters, so too does the morphology of the cells. As the results of the above experiment show (Table 15), Z. ericetorum collected from damp areas has longer cells with thinner cellwalls than does material from drier sites.

Table 15 Morphology of Z. ericetorum at different siteson a sand heap

Site	Average filament width	Average cell length	Average cell-wall thickness	% dead cells
A	24 μ	17 μ (14.5-24)	2.5 μ	20
B	19 μ	24 μ (22-36)	2.5 μ	50
C	24 μ	24 μ (17-36)	2.5 μ	25
D	17 μ	23 μ (24-31)	1 μ	70*
E	17 μ	32 μ (24-41)	1 μ	33
F	17.5 μ	43 μ (24-93.5)	1.5 μ	10
G	19 μ	43 μ (26-72)	1 μ	5

Cell measurements are averages of 30 random cells (to nearest 0.5 μ) excepting % dead cells which are estimates.
Extreme measurements are included in brackets. * heavy parasitism by Chloridium sp.

Site Details

A. Beneath Calluna heath. Different locality to other sites
B. Flat area at top of slope
C. 3M from top. Dry slope
D. 6M from top. Fairly dry slope
E. 9M from top Damp slope
F. 10M from top. Very damp area
G. 10.5M from top. Permanently waterlogged area.

In order to investigate whether it is possible for the thick-walled terrestrial form of Z. ericetorum to change morphologically so as to resemble the aquatic form, a simple laboratory experiment was instigated. Blocks of silica sand supporting a cover of Z. ericetorum (terrestrial form) were cut out from a sandtip at Drakelands Corner. The size of these blocks was 20 x 30 x 8cm such that they covered the bottom of a 13 litre perspex tank. In removing the blocks, because of the loose nature of the sand, it was necessary to spray the sides with a resin, Unisol 51, which has the property of forming a non-toxic skin over the soil and helps to hold the soil particles together. Each of the blocks was carefully placed on a bed of gravel in a perspex tank and the sides of the tank were darkened with black polythene to the level of the soil surface. Each tank was covered with a glass sheet. Four tanks were set up and treated as follows :

Tank 1. Left dry. No subsequent additions of water

Tank 2. Left dry, but sprayed once a week with approximately 10 mls. of distilled water using a hand sprayer.

Tank 3. Approximately 500mls of distilled water added to keep the sand moist.

Tank 4. Approximately 1.5l. of distilled water added so that there was standing water at the soil surface.

The tanks were kept in a growth cabinet at 12°C. illuminated at 6460 to 8600 lux through a 14 hour day. At intervals up to 39 weeks, algal material was removed from various areas of each tank for microscopic examination. It was necessary to terminate the experiment after 39 weeks. Material in the dry tank had shown little or no growth and in fact consisted of dead cells and cells in the akinete stage, whilst conditions in the tank containing standing water were becoming stagnant.

Results of this experiment are given in table 16 and fig.36. Within three weeks, the algae in the two well-watered tanks had assumed a different macroscopic appearance from those in the other two tanks. In the well-watered conditions, the algae appeared to be growing actively and bundles of filaments were showing the sinuous growth habit characteristic of many filamentous algae

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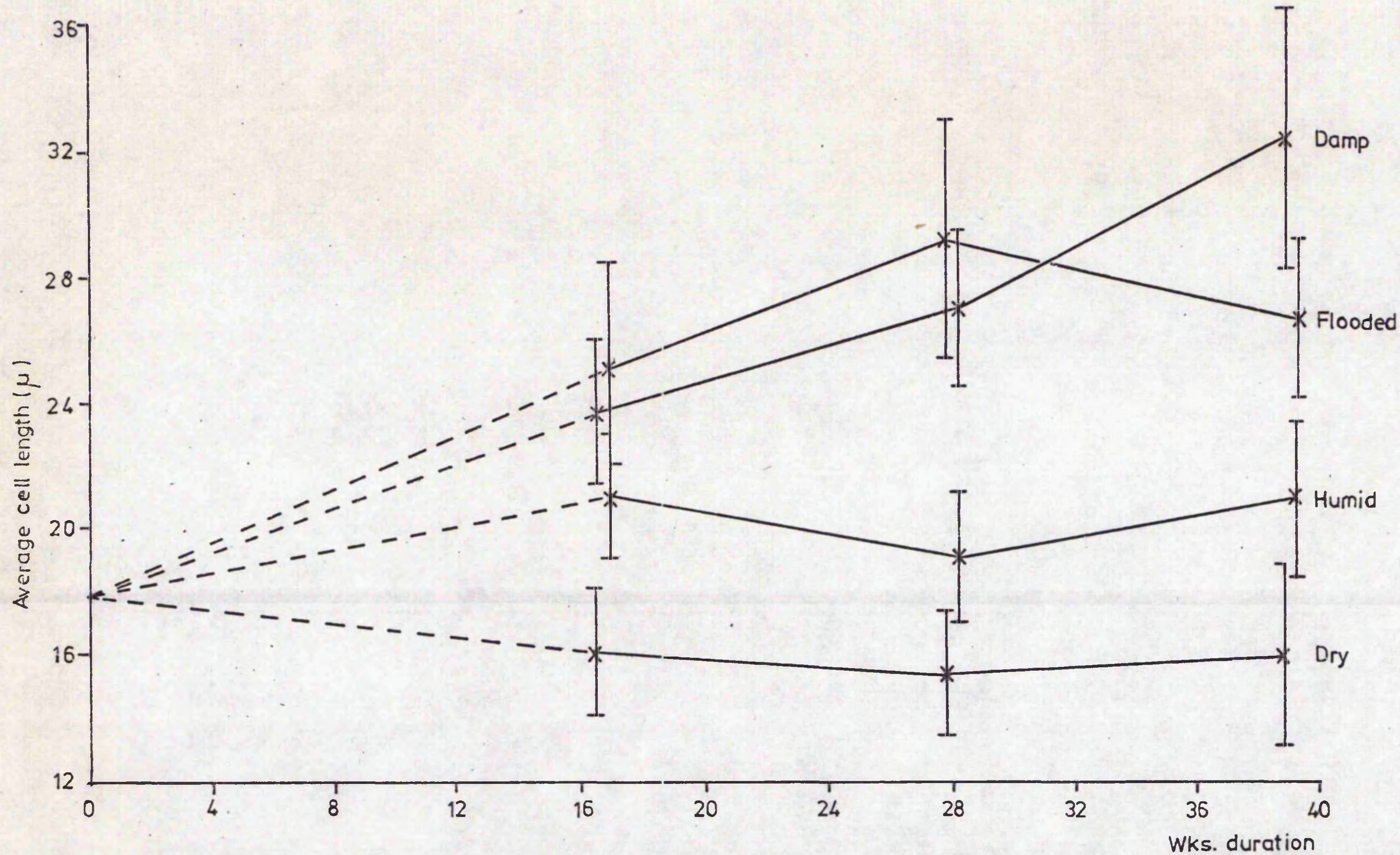

Fig. 36 Change in cell length of _Z. ericetorum_ material when grown under different water conditions.

Means and standard deviations plotted.

Table 16 Change in Z. ericetorum morphology when grown under different water regimes.

Conditions	Time (weeks)	Cell packets*	Average cell length (μ)	Range (μ)	S (μ)	Cellwall thickness (μ)
Dry	10weeks	1-2cells				
	17	1-2	16	13-18.5	2	4
	28	1-2	15.5	11-20.5	2	3
	39	1-2	16	11-22	3	3
Humid	10weeks	1-2				
	17	1-2	21	17-22	2	4
	28	(1)-2	19	15-24	2	3
	39	(1)-2	21	15-26	2.5	3
Damp	10weeks	2-4				
	17	(2)-4	24	20.5-28	2	2
	28	4	27	22-33	2.5	2
	39	4(6-8)	32	26-39	4	1.5
Flooded	10weeks	4				
	17	4-6	25	20.5-31.5	3	2
	28	4-6(-8)	29	22-37	4	1.5
	39	4-6(-8)	27	22-31.5	2.5	1.5

Measurements to nearest 0.5 μ

* Refers to interval between two thick cros walls("collars") representing the products of division of a single akinete.

growing in moist but not submerged conditions. The material in standing water had adopted an outstretched "slime-like" appearance over the substrate. Cell measurements at this stage were similar under all four conditions which suggests that the differences in macroscopic appearance were due solely to different rates of cells division. Subsequent measurements showed a trend towards the four treatments splitting into four morphologically distinct populations (Fig, 36), but even after 39 weeks, due to large standard deviations, the four populations were not all distinct fom one another. It is worth mentioning here the large inhert variably between cells in the same filament which is discussed in the appendix. Nevertheless,the results do suggest that under optimum conditions it is likely that the terrestrial form of Z. ericetorum will divide actively such that the products of division have longer cells with thinner cellwalls. This is further substantiated in the observation that filaments are occasionally met with in material of the terrestrial form where a few consecutive cells can be as much as two or three times the usual length of these cells and have considerably thinner cellwalls.

On sandtips, Z. ericetorum tends to grow best in more sheltered microhabitats. In areas subjected to occasional trampling by sheep and horses the alga is frequently confined to old slots during dry periods. This was noted during the summer months in a number of permanent quadrats. Any depression in the sand appears to support a better growth of the alga than do small hummocks but although this may be macroscopically clear it is difficult to quantify the relative amounts of alga between one area and another. The thickness of the algal cover in such areas ranges from just a single filament to one millimetre thickness of filaments. In such situations the bulk of the organic matter at the substrate surface consists of Z. ericetorum filaments. In an attempt to quantify its luxuriance of growth, measurements were compared of the organic content of the surface soil plus alga in different samples. Suitable sites were selected and the microtopography was assessed using a point quadrat frame to ascertain the relative positions of each point. At each point, a soil plus alga sample 1 cm deep was collected using a number 16

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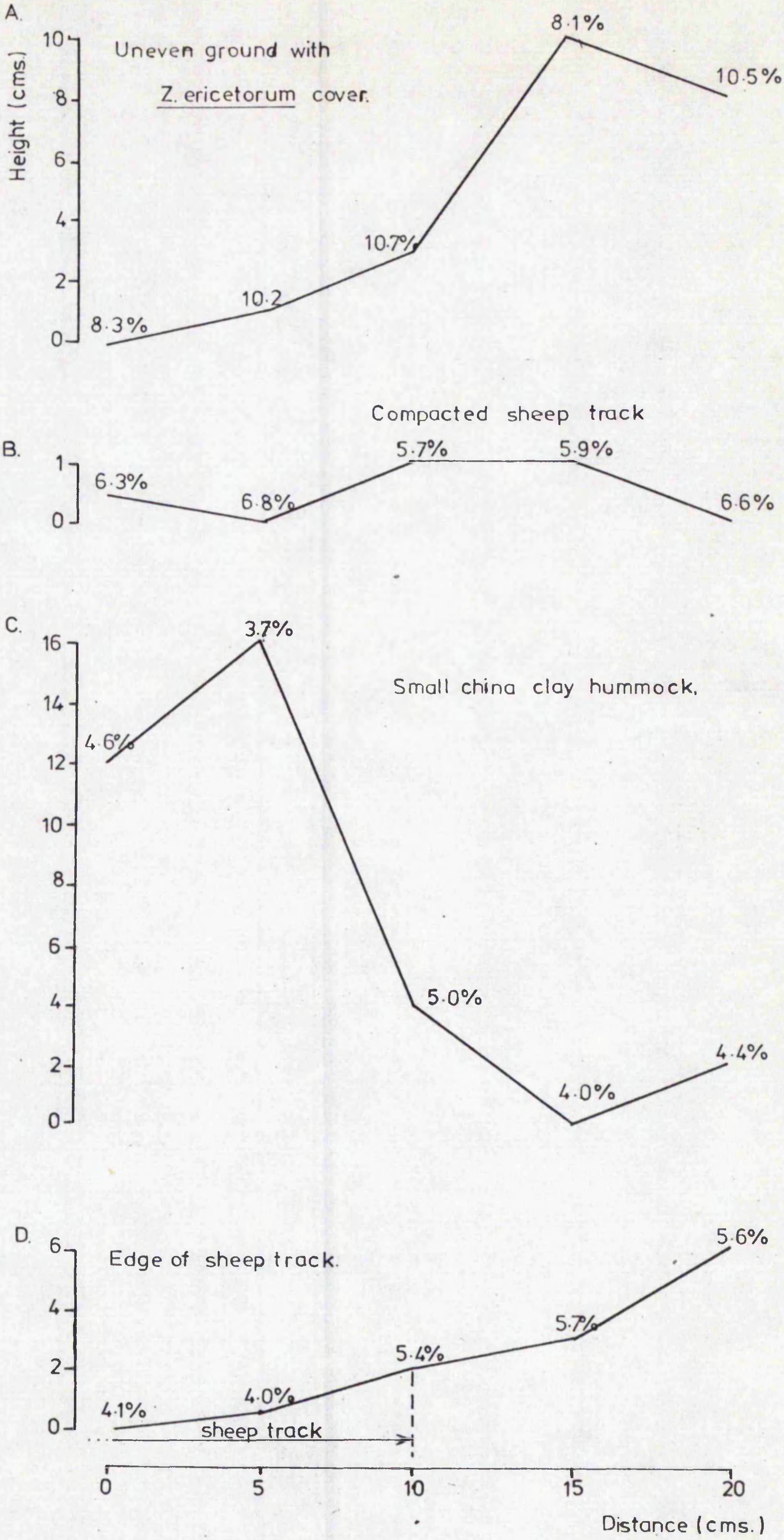

Fig.37 %Organic content and microtopography of china clay waste areas.

Table 17 Field water contents for sand samples from bare sites and adjacent sites covered by Z. ericetorum mat.

Site	Description	Field water constant
A1	Flat, algal-covered area	2.2%
A2	Nearby bare sheep track. Flat.Compacted	8.6%
B1	Algal-covered slope	16.85%
B2	Nearby bare slope	7.95%
C1	Algal-covered slope close to rabbit burrow	3.1%
C2	Bare slope close to rabbit burrow	3.55%
D1	Algal-covered flat area	8.25%
D2	Nearby bare flat area	6.25%
E1	Algal-covered slope	11.3%
E2	Nearby bare slope	4.65%

Table 18 Moisture contents of air-dry algae

Sample	Air-dry weight	Dry weight (100°C)	Moisture lost	Moisture lost as % dry wt.	Weight after exposure to air	Permanent loss	Permanent loss as % total loss
A.	1.977g	1.890g	0.086g	4.6%	1.952 g	0.025g	28.6%
5g. wet wt Z. ericetorum B.	2.098g	2.016g	0.082g	4.1%	2.078g	0.020g	24.6%
C.	1.551g	1.482g	0.069g	4.6%	1.537g	0.014g	20.5%
0.5g. D. H. flaccidum	0.079g	0.073g	0.007g	9.5%	0.078g	0.002g	26.0%

corkborer (diameter 2.5cm). In the laboratory organic contents of these samples were assessed and the results are shown in fig. 37. The correlation between algal cover and organic content was not absolute although there was a tendency for slightly higher organic contents to be recorded from small depressions. However, the obvious difference between algal cover in old sheep slots or any irregular depressions, and the almost complete lack of algae immediately surrounding these areas, although visible to the naked eye, was not fully brought out by these results.

In places where an algal cover has become established over the surface of the soil, this may have the effect of reducing waterloss from the soil. To investigate whether this was the case, a number of sand samples were collected from the site with a good algal cover and adjacent sites with no apparent cover. Collections were made during a warm, dry period in July and 20g. surface samples were used to estimate the field water contents. From the results given in table 17, it may be seen that higher soil water contents were not consistent with the presence of an algal cover although in general, in situations where the water content was not higher in the sample beneath the algal mat then the two samples showed comparable waterlevels. This is not the case however, with the sheeptrack samples where the water content of the track was significantly higher than an adjacent algal-covered area.

When subjected to drought, the cells of Z. ericetorum do not alter significantly. The filaments show some transverse contractions and irregular longitudinal folds are seen along the walls but in most cells the protoplast shows little or no contraction. With H. flaccidum, the longitudinal folding of the walls is much more pronounced in the dry state but as the cells contract, the protoplast appears to remain in close contact with the walls. It is likely that for these cells to survive periods of drought they must retain a proportion of water whilst in the air-dry condition. Using field material of Z. ericetorum and H. flaccidum, drying experiments were conducted to find out the percentage of water retained by the air-dry algae. Collections of 5g. (wet weight) of Z. ericetorum were used but only 0·5g. of uncontaminated

H. flaccidum could be obtained. Pieces of algal mat were allowed to reach air-dry condition in the laboratory and were then heated at 100°C to constant weight. This material was then left exposed to the air for several days in order to establish the percentage of moisture that could be reabsorbed from the atmosphere. Each sample was run simultaneously. Results are given in table 18. The Z. ericetorum material lost approximately 45% of its water on heating and the H. flaccidum as much as 9.5% . In each case about one quarter of this moisture was permanently lost, this presumably representing that part retained by living protoplasts and irreplaceable after their death.

As a result of the hot, dry summer of 1975 much of the Z. ericetorum material from sand tips which was examined towards the end of summer consisted of a large proportion of dead cells. During the summer some flat areas on silica sand were observed where there had clearly been sufficient water during the winter months for a luxuriant growth to become established but which were now dry. Fig. 38 illustrates such an area and shows the distinctive mottled pattern of the algal mat where the dark purple colour of the algal mat has become increasingly broken up by grey areas. As table 19 shows, these grey areas correspond to filaments consisting largely of dead cells. The shrivelled cell contents of these cells will not reabsorb water even after long periods of immersion. Hence it appears that during periods of drought, algal mats in such areas gradually die off. Closer examination later in the summer showed the presence of white areas in addition to the purple and grey zones. Examination of the cells from each of the zones showed that the purple zone correspond to that of healthy filaments, in the grey zone the cells were predominantly dead but with some viable akinetes , whilst the white zone consisted of many dead filaments together with some which appeared almost totally unaffected. This zone was intermediate as regards condition of the filaments between the purple and grey zones. Analyses of the field water contents for the algal mats and underlying soil from each of these zones did not bear out the assumption that these filaments were dying as a result of drying-out (Table 20) but field water contents vary considerably and fluctuations in the water content are

contd. p.109

Fig. 38 Mat of Z.ericetorum drying out and showing mottled appearance.
Still waterlogged in top right-hand corner
Drakelands Corner July '75
Coin 27mm. diam.

Fig.39 Dried pieces of Z.ericetorum mat on bare areas between Calluna bushes.
Drakelands Corner July '75
$1/_2$ M. rule scale

Table 19. Percentages of living cells in 25 filaments of Z. ericetorum from a grey zone and 25 filaments from a purple zone on clay waste (see text).

(i) Grey zone

% cells appearing viable	0; 3; 0; 0; 0; 30; 6; 0; 13; 0; 38; 0; 0; 0; 55; 0; 43; 0; 0; 0; 0; 0; 7; 21; 4.
Total no. cells/ filament (to nearest 10)	250; 700; 340; 50; 300; 360; 550; 200; 280; 100; 50; 360; 350; 220 ; 85; 50; 88; 80; 150; 250; 150; 80; 60; 225; 280.

$\bar{x}$ % viable cells = 8.8; s = 15.5

(ii) Purple zone

% cells appearing viable	94; 95; 30; 55; 92; 64; 78; 89; 77; 97; 77; 95; 99; 98; 85; 99; 90; 84; 98; 87; 45; 97; 99; 69; 64.
Total no. cells/ filament (to nearest 10)	165; 230; 460; 210; 335; 165; 170; 130; 265; 120; 65; 55; 300; 120; 135; 100; 50; 135; 120; 130; 170; 120; 225; 125; 110.

$\bar{x}$ % viable cells = 82.3; s = 18.2

quite likely after such an area has started to dry out, not a great deal of emphasis can be placed on these results.

Table 20 pH and field water contents from zones of *Z. ericetorum* mat.

Zone		pH	Field water content
Purple zone	Algal mat	4.3	13.4%
	Underlying Soil	4.0	4.6%
Grey zone	Algal mat	4.6	30.7%
	Underlying Soil	4.2	8.3%
Discoloured zone	Algal mat	4.5	12.9%
	Underlying Soil	4.4	21.6%

As the algal mats dry they become detached from the substrate and begin to crack so that during periods of drought, loose brittle patches of *Z. ericetorum* occur scattered on the ground (Fig. 39). These mats consist predominantly of dead cells at the surface but below the surface of the mats are a large proportion of cells in the akinete stage. With the advent of wet weather, because of their mucilaginous cellwalls, the filaments absorb water rapidly and the patches adhere to the ground.

4. pH

Zygogonium ericetorum occurs on moorland and on china clay waste in the Drakelands Corner area but in these situations the pH of the substrate on which it is growing is generally between 4 and 5. The alga is reported to be of widespread occurrence on acid moorland soils and table 21 lists some localities away from china clay areas where it was found growing luxuriantly. From the pH values of these soils it may be seen that *Z. ericetorum* appears to be restricted to acid soils although not necessarily to moorland.

As a result of gravel excavations along the Trent Valley in Nottinghamshire, some areas of sand and gravel have been left and become colonised by plants. As this substrate consists largely of gravel and coarse sand and lacks significant levels of toxic metals, it was suspected that these areas may show similarities to china clay sand tips,

Table 21 Localities for Zygogonium ericetorum together with pH values of the Soil.

Locality	Grid Ref.	Site	Soil type	pH
Blackaton Cross, Devon	SX574627	Drainage Channel	Peat	4.5
Gunnislake Clitters, Cornwall	SX422724	Spoil tip of old Copper mine	Sandy	5.0
Birklands, Notts.	SK635675	Open area Sherwood Forest	Loamy	4.2
Wollaton Hall, Notts.	SK530390	Bareground beneath Fagus	Loamy	4.1
Axe Edge Moor, Derbys.	SK024710	Boggy moorland	Waterlogged peat	4.0
Goyt Valley, Derbys	SK018719	Moorland	Peat	4.3
Cown Edge, Derbys.	SK018918	Moorland	Millstone grit	4.4
Raven's Low, Derbys.	SK017725	Boggy moorland	Waterlogged peat	3.8
Hallam Moors, Derbys.	SK234871	Moorland	Peat	4.4

and a number of them were visited. It was found that filamentous algae and Bryophytes (principally Ceratodon purpureus and Bryum argenteum) were indeed pioneer plants and occurred principally around the bases of small tips where drainage ensured a better water supply. The filamentous algae included Hormidium flaccidum in some quantity but Z.ericetorum was absent. The pH of this sandwaste averaged 6.0 - 6.2.

Hormidium flaccidum formed a dense green mat over a small area of moorland in the Goyt Valley, Derbyshire where elsewhere Z. ericetorum was common. The soil at this site was loamy with a pH of 5.6.

These field observations suggest that Z. ericetorum is restricted to acid soils whilst H. flaccidum is less restricted by soil pH and may be at an ecological advantage on soils of higher pH. The sites at which Z. ericetorum was recorded included moorland, woodland, parkland and spoil waste of a copper and arsenic mine in the Tamar Valley. At this last site it was one of the few plants growing over the tip waste, the others being Bryophytes. In the Drakelands Corner area, Z. ericetorum was recorded from the ground layer of Calluna heath, from china clay waste, from trackways, and temporarily open areas in Calluna heath produced by rotting cow dung. These observations seem to suggest that within the limitations of an acid soil, Z. ericetorum will grow on any open areas where competition has been minimised. Its primary habitat appears to be the closed community on moorland soil where it exists but is unable to grow abundantly.

Table 22 pH tolerance of Z. ericetorum and H. flaccidum in buffers

(a) Zygogonium ericetorum

pH	Buffer used	% dead cells (averages of 3 replicates)	
		After 28 days	
3	McIvaine's phosphate	25; 30; 25	
4.3		5; 5; 5	
4.8		60; 75; 75	
6.0		75; 95; 100	
7.0		100; 100; 100	
4.6	Citrate	15; 40	After 50 days
5.0		45; 35; 40	75; 75; 60
5.5		60; 60; 60	75; 95; 95
6.0		90; 95; 95	100; 100; 100
5.2	Sorensen's phosphate	100; 75; 75	
5.5		95; 95; 70	
6.0		95; 75; 60	
7.0		65; 50; 60	
7.8		100; 90; 100	

(b) Hormidium flaccidum

pH	Buffer used	% dead cells
5.2	Sorensen's	5; 10; 5
5.5	Citrate	15; 2; 5
5.6	Sorensen's	2; 2; 5
6.4	Sorensen's	15; 5; 30
7.0	McIvaine's	50; 30; 30
7.0	Sorensen's	20; 15; 25
9.5	Czarda's	25; 20; 30
11.0	Czarda's	100; 100; 100

Material of Z. ericetorum and H. flaccidum from Drakelands Corner was grown in buffers at a range of pH values in order to establish the pH tolerance of these species under laboratory conditions. Several different buffers were used in order to distinguish between the effects of the chemical constituents of the buffers and the effects of different pH values. Material was grown in 20ml. aliquots of buffer in medical flats at 23°C and illuminated at 4300-5300 lux through a 12 hour day. Initially the algae were grown in buffered Knop's medium but it was found that this encouraged bacterial and fungal contamination to the extent that it was impossible to score the cells. In subsequent experiments the material was maintained in buffers without the addition of culture media. The buffers used were McIlvaine's phosphate, citrate, Sorensen's phosphate and Csarda's phosphate (see appendix). Because the algae did not grow well in these buffers, scoring was by assessing the numbers of dead cells in the different treatments. Dead cells were assessed after 28 days as detailed in appendix.

The results in table 22 are averaged cellcounts of five separate samples examined from each medical flat and results from each of the three flats per treatment are presented. The pH values of the buffers were checked at the start and termination of the experiment and were found not to have altered. From the results, it can be seen that Z. ericetorum shows a tendency to die off under the conditions of the experiment at pH values higher than 5. The threshold level would appear to vary with the buffer used and Sorensen's buffer, in particular, did not kill all the cells even at high pH values. At these levels, the surviving cells were all in the akinete stage. H. flaccidum on the other hand, was only killed off significantly at higher pH values and even at pH 9.5. only about one quarter of the cells were killed.

5. Erosion

Results from the observations of permanent quadrats have indicated that erosion is an important process in controlling the spread of Z. ericetorum over sandheaps. Both sheet and gully erosion are operative. As mentioned previously, vascular plants tend to become established in the gullies but where sheet erosion occurs, the algal cover together with any seedlings are soon lost. Sheet erosion appears to be most evident during the

Fig.40 Small sand heap (approx. 2·5M. high) showing well-vegetated and sparsely-vegetated slopes. Ulex europaeus bushes around base.
Drakelands Corner

Fig. 41 Actively eroding slope of sand heap undercut by the action of sheep.
Drakelands Corner
Height 2M.

winter months when rainfall is heaviest. Areas of surface sand up to one metre square and held together by algal filaments can be washed lower down a slope or accumulate at the bottom of a tip. It is interesting that the bulk of the tips at Drakelands Corner supporting Calluna heath, show one side which is actively eroding. Fig 40 shows a typical small tip about 2.5M. in height in which one side (on the right in the fig.) is much more sparsely vegetated than the opposite side. Fig 41 shows the side of an actively eroding tip where one large piece of top soil has recently become detatched. In this case, a sheep track passes along this side and the passage of these animals, together with their habit of sheltering here, has accelerated the rate of erosion.

A number of tips of Drakelands Corner were examined in order to see whether there was any constancy in the aspects of the tips that showed most erosion. All the larger tips in the area which carried a Calluna cover were investigated. These amounted to twenty-three tips. Table 23 lists the aspects of eroded slopes and shows that there may be a tendency for south, southwest and west facing slopes to be more prone to erosion. These are the sides that receive the predominant winds and rain and are also the first to dry out during sunny weather. However, tips occur in which north or east are the only ones to show marked erosion. It is likely that a more important factor is the stacking of the tips as they are dumped. The steepest slope is the one least likely to become vegetated or to develop only a thin algal cover and therefore is less stable and more liable to erosion.

Table 23 Aspects of eroded slopes of old sandtips at Drakelands Corner.

Aspect	No. of tips
N	1
NE	0
E	1
SE	1
S	5
SW	8
W	6
NW	1
	Total 23 tips

Fig. 42 Portion of a sheep track across silica sand showing narrow band of disturbed substrate. Drakelands Corner
1/2 M. rule scale.

Fig. 43 Open area of heathland used as a defaecating area for rabbits and overgrown with Z. ericetorum. Drakelands Corner
Distance A-B equals approximately 1.5 M.

Apart from the agents of wind and rain, animals are locally important in eroding the thin algal cover over china clay waste. Rabbits give rise to bare areas of sand by their burrowing, but probably of greater importance in the Drakelands Corner area are sheep. Because sheep keep to well-defined and narrow tracks, the extent of the eroded area is small, but as large numbers of sheep are kept on the moor the area is covered with narrow tracks (Fig. 5) Due to the continual use of these tracks, the sand is loose and frequently disturbed in many areas but the algal cover extends right up to the edge of these tracks (Fig.42) which may be only 15 to 20 cms in width. Because of this, the algal-free tracks stand out white against the purple-grey of Z. ericetorum over the ground.

In some areas, the substrate becomes heavily compacted due to the passage of animals so that the sand is less disturbed and in wet weather, Z. ericetorum extends from the sides of the tracks and forms a patchy cover over the path. In dry weather, the alga tends to die away except in any fairly permanent depressions along the track such as old sheep slots. This was the situation in the permanent quadrats numbers 1, 2 and 3 in the colonisation experiment (Table 13) and in one of the sites where algal cover was assessed as described in the humidity section (Fig. 37 D).

In micaceous areas, the ground becomes very churned up so that if a track is heavily used, recolonisation by algae is not possible.

6. Nitrogenous runoff

Openings in a closed moorland community may be produced where horse or cattle dung is deposited or where rabbits defaecate regularly. Generally, vegetation is restricted in these immediate areas but it was observed at Drakelands Corner that in such situations, Z. ericetorum forms a dense cover over the otherwise bare area (Fig. 43). Its growth does not appear to be inhibited by the rotting dung, in fact it appears to growmore luxuriantly than in surrounding areas although this may simply be a reflection of the lack of competition by other plants. One feature consistently noted was that Z. ericetorum growing in dung-enriched areas lacked the purple pigment characteristic of this alga. No purple colouration of the cells was visible under microscopic examination and the pigment could not be detected spectrophotometrically in an aqueous extract.

Table 24 Cell measurements of *Z. ericetorum* from green and purple zones on mica substrates.

Purple zone			Green zone		
Cell length					
n	$\bar{x}$	S	n	$\bar{x}$	S
50	21.7 µ	2.4 µ	50	38.3 µ	6.1 µ
Cell width (width of filament)					
n	$\bar{x}$	S	n	$\bar{x}$	S
50	21 µ	0.5 µ	50	1.7 µ	0.5 µ

Measurements taken from the largest cell of each of 50 filaments

Table 25 Soil and algal mat analyses from green and purple zones on mica substrate

	Purple zone		Green zone	
Site 1	pH	Field water constant	pH	Field water constant
Algal mat	4.5	12.1%	4.8	3.8%
Underlying soil	4.3	2.4%	4.6	0.9%
Site 2				
Algal mat	7.3	10.8%	7.2	12.2%
Underlyingsoil	7.1	7.3%	7.35	7.7%

Site 1 Flat mica area with large green zone around rabbit dung.

Site 2 Flat mica area with small purple patch surrounded by green zone around rabbit dung.

In fact such areas are conspicuous by their bright green colouration.

In mica areas where there is an established deep purple cover of Z. ericetorum bright green patches occur immediately around areas where dung has been deposited and green trails corresponding to run-off channels radiate outwards from these depositions. These green areas were far more extensive during the winter months than during the summer and this may be due to the heavier rainfall leaching nutrients from the dung over a wider area. On examination, both green and purple areas were seen to be composed of Z. ericetorum. Collections were made from both zones of one such area and cell measurements taken of representative samples. Results of these measurements are given in table 24. It can be seen that both cell length and cell width are significantly increased in the material growing in the green zone. Table 25 lists pH values and field water contents of the algal mats and underlying soil of these two zones and also from a second site consisting of bright green Z. ericetorum surrounding a small purple patch. In this site it can be seen that the pH is uniformly higher in both purple and green zones which suggests that the lack of pigmentation may not be due solely to a change in pH.

Whilst there is some evidence that dung may stimulate growth of Z. ericetorum, urine on the other hand can be lethal. Grey patches appear on the algal mat where animals have been urinating and this was simulated by pouring freshly collected sheeps' urine over an undisturbed area colonised by the alga. Urine in which the urea had broken down, did not have this effect.

Laboratory experiments were carried out to ascertain the concentrations of urine necessary to bring about death of the algal cells and, as sheep were the predominant large animal of the area, their urine was used. A supply was obtained at Cattedown abattoir, Plymouth, from freshly slaughtered animals that had been grazed on lowland pastures in the area. The urine had a pH value of 9.3. Before use, it was centrifuged to remove fatty material. In the experiments, the following material was used:

(i) Discs of <u>Z. ericetorum</u> mat from a micaceous area, cut out using a number 18 cork borer (4 cm diameter)

(ii) <u>H. flaccidum</u> culture isolate on Knop's agar

(iii) <u>H. flaccidum</u> growing on moist, sterile silica sand under laboratory conditions.

The <u>H. flaccidum</u> was grown in petri-dishes; discs of <u>Z. ericetorum</u> were arranged either three to a petri-dish or eight to a 13 litre sealed perspex tank and on a bed of sterilised sand. Algal material was treated with 2 ml aliquots of the appropriate test solution daily. For each treatment, petri-dishes were set up in triplicate. Fresh sheep's urine was used serially diluted with distilled water and dilutions of B.D.H. urea were also used. The highest concentration of urea utilised was 2.57g./100ml; this is equivalent to 1.2% nitrogen, the average level of nitrogen in sheeps urine as recorded by Sears and Newbold (1942). During the course of the experiments, thealgae were maintained at 23°C and illuminated at 4300-5300 lux through a 12 hour day.

In the first experiment, algal material was treated for 0,1, 2, 3, 4 or 5 days. In each instance, the urine treatments when completed were followed by treatments with 2mls. of distilled water on successive days. After eight days, the algae were examined microscopically. The <u>Z. ericetorum</u> material was found to be dead in all treatments excluding the controls. All the cells were dead and filaments from both the top and bottom of the algal mats were similarly moribund. The <u>H. flaccidum</u> material was still green although the cells were strongly plasmolysed. After eleven days, the treatments were noticeably darker green in colour than the controls and within twenty-eight days all treatments, apart from the controls, were dead. Hence it is evident that a single application of 2mls. of undiluted sheeps urine is sufficient to bring about a 100 per cent kill of cells of both <u>Z. ericetorum</u> and <u>H. flaccidum</u> under the conditions of the experiment. The unnatural conditions within a petri-dish, namely a small enclosed air space and the accumulation of the urine within it, may help to account for the results of this experiment.

A second experiment was set up to partly overcome these problems by using 13 litre perspex tanks rather than petri-dishes and placing the algal discs on sand to permit drainage from them. This experiment was carried out using Z. ericetorum only. The discs were each given single 2ml applications followed on successive days by distilled water and the treatments were dilutions of urine down to a thirty-two fold dilution. Each treatment was carried out in a separate tank. After 3, 6 and 12 days, material was examined, and percentages of dead cells estimated. Table 26 gives the results of this experiment from which it can be seen that Z. ericetorum was killed even by 16-fold dilutions.

It was found that if discs treated with single applications of undiluted urine were kept in the same tank as the control discs, after six days both controls and urine treatments were dead and the tank smelt strongly of ammonia. However, if the experiment reported above using serial dilutions was repeated with decomposed urine no significant difference could be detected between treatments and controls within twelve days although the tanks at the end of this time smelt strongly of ammonia andthe pH of the urine used had not altered. It appears that it is a component of fresh urine which is lethal to the alga and this component is still toxic after dilution. This may explain why, in wet weather the dead grey patches of Z. ericetorum noted in the field are more extensive than in dry weather.

An experiment was carried out using dilutions of B.D.H. urea on Z. ericetorum and H. flaccidum in petri-dishes and the results are presented in table 27. It may be seen that concentrations of urea down to at least 0.64g./100ml. will kill both algae at about the same level as a dilution of sheeps urine of 1 in 16 . If it is assumed that sheeps urine contains 1.2% nitrogen, as reported by Sears and Newbold (1942), then urea is seen to show similar levels of toxicity to the urine used.

Table 26 Dilutions of sheep urine required to kill mats of Z. ericetorum

Treatment	Period of observation (% dead cells)		
	3 days	6 days	12 days
Urine	100%	100%	100%
Urine/2	100%	100%	100%
Urine/4	36%	100%	100%
Urine/8	45%	80%	100%
Urine/16	20%	40%	95%
Urine/32	15%	20%	50%
Control	5%	2%	2%

Table 27 Effect of single treatments of urea solutions

Dilution urea	Period of observation (% dead cells)					
	Z. ericetorum	H. flaccidum on Knop's agar		H. flaccidum on silica sand		
	10 days	10 days	24 days	24 days	16days	24 day
2.5g./1	100%	90%	100%	100%	100%	100%
1.28g./1	100%	80%	100%	20%	100%	100%
0.64g/1	100%	10%	100%	3%	cells plasmolysed	100%
0.32g/1	15%	2%	cells plasmolysed	5%	" "	cells plasmo -lysed
Control	5%	2%	2%	2%	5%	5%

11: Discussion

It has been shown that soil algae constitute the initial successional stage on china clay spoil. Clearly, they are better suited for this role than rooted plants as they require less water, being better able to resist dessication and less liable to displacement. The algal cover may last for many years until flowering plants are able to form an abundant ground cover. In the Drakelands Corner area, the sand heaps do not develop a continous cover of flowering plants and the algal cover is able to persist between the rooted plants. However, as none of these tips are more than one hundred years old, and most are younger than this, ~~it may be that~~ insufficient time has elapsed for the climax vegetation of the area to develop.

Forest, Willson and England (1959), from their observations of algal establishment on eroded prairie soils, have come to the conclusion that there are no specific pioneer algae. They maintain that colonisation is accomplished by the commonest algae in the surrounding undisturbed soil. In this country, Zygogonium ericetorum is a frequent pioneer species. On china clay waste it develops to the almost complete exclusion of other species. Established mats may have Hormidium flaccidum filaments growing amongst them and locally becoming dominant, but very few other algae are found. Lund (1947) says that the algal flora of highly acid substrates (pH 3.7 - 4.8), although numerically large, is restricted to a small number of species. The porous nature of silica sand means that these species are likely to be further reduced as cells which are smaller than the pore size of the substrate are liable to be washed into the sand during periods of heavy rainfall.

Z. ericetorum appears to be little affected by the chemistry of the underlying substrate provided it is acid. In the Drakelands Corner area, it will grow wherever patches of ground remain bare long enough for it to become established. H. flaccidum will also grow under these conditions but seems to be at a competitive advantage on heavier and less acid soils.

In order for a pioneer species to grow successfully on sand spoil it must be relatively resistant to dessication and both Z. ericetorum and H. flaccidum meet this requirement. Because of the tolerance of these algae to extremes of heat and cold, temperature looses significance as a factor in determining distribution. Their growth is more closely related to available nutrients and moisture.

It has been shown that oven-dried material of Z. ericetorum and H. flaccidum will increase in weight if left exposed to the air. This is due to the absorption of moisture from the atmosphere and must be a purely physical process. In the case of Z. ericetorum, the greater part of the thick wall is mucilaginous so that the dry filaments swell considerably on wetting. Fritsch (1922a) demonstrated that air-dry material of Zygogonium ericetorum and Prasiola crispa (Hormidium form) fluctuate in weight with the degree of humidity in the air to a greater extent than does non-living material. The absorption of moisture from the atmosphere, which is likely to occur particularly at night and during daybreak, may well allow growth to continue even during long periods of drought. In situations with an adequate water supply, cell division in Z. ericetorum occurs more frequently and the cells are more elongate than when growing on drier sites. For this reason, the micaceous areas of old china clay workings become covered with a luxuriant growth of the algae. During dry spells, the algae growing on sand spoil are subject to extreme dessication and some cells die. After prolonged dry periods, dark greyish patches of dead filaments are evident on Z. ericetorum mats. Other filaments consist of dead cells and resistant akinetes. It has been suggested by Fritsch (1916) that the accumulation of lipid droplets within the akinetes is associated with dessication. McLean (1967) has shown in the green alga Spongiochloris typica in culture, high dessication resistance is favoured by low nutrient levels in the medium and it may be that the low nutrient levels of sand spoil are a factor in the dessication resistance of Z. ericetorum and H. flaccidum on this substrate.

As Fritsch (1922c) and Booth (1941) have pointed out, in many situations soil losses are greatly reduced as a result of algal growth binding the surface particles into a less easily eroded layer which is also effective in breaking the force of falling water. During soil formation on sandtips, one of the most important aspects of the activity of the algae is their participation in the fixation of the substrate surface. The tips are prone to sheet and gully erosion by rainwater and to erosion by digging and trampling. The degree of erosion of a spoil area is related to the degree of slope and several categories may tentatively be recognised on china clay waste from the permanent quadrat observations.

(1) Slopes level or nearly level (0-5° approximately). Water erosion is slight. In damp areas close to the watertable, bare areas are soon covered with a luxuriant growth of Z. ericetorum. In well-drained areas, spread of the alga is restricted to periods of high rainfall and during dry weather much of the algal material dies.

(2) Slopes undulating or rolling (5-16° approximately). Runoff slow to medium. Bare areas become covered with a fairly luxuriant growth of Z. ericetorum.

(3) Slopes moderately steep or hilly (16-36° approximately). Runoff medium to rapid. Sheet and gully erosion develops where a vegetation cover is lacking. Z. ericetorum cover sparse to moderate.

(4) Steeply sloping or very hilly (36°+). Runoff very rapid. Slopes highly erodible. Algal cover generally thin and frequently removed by sheet erosion.

The observation that old spoil tips show one side which is poorly vegetated and eroding has been made by Greenwood (1963) on conical pit heaps in County Durham. He found that the poorly vegetated southwest aspect lost solids as a result of rainwater runoff and strong wind. It is significant that it was predominantly the south, west and southwest aspects of silica sandtips at Drakelands Corner that were the least vegetated.

The rate of colonisation of Z. ericetorum onto a bare area appears to be largely governed by the availability of water. Provided that there is a good population of the alga in the vicinity to be washed onto the bare area and that there is sufficient available moisture to allow growth of this fresh material, then an algal cover can be established within a few months. On tips where little or no algal material is present, it can take many years for an algal cover to develop. A luxuriant cover is never established over well-drained sand spoil.

If the algal cover becomes broken for any reason, the bare patch formed does not generally constitute a point from which further erosion will occur. A cover of Z. ericetorum will, for instance, grow right up to the edge of a sheeptrack where the passage of animals prevents further growth.

Soil algae are important in providing a continually renewable source of humus and on sandtips may be the only such source until a flowering plant cover is established. As previously mentioned, the Z. ericetorum cover ranged from sparse on well-drained sites to luxuriant in continously damp areas. This increased luxuriance may be due to higher levels of available water, higher levels of nutrients or to a combination of these two factors. Certainly, Z. ericetorum filaments growing within the influence of deposited dung have longer, thinner-walled cells, suggestive of enhanced growth, than do filaments outside this zone of influence. Urine on the other hand is fatal to the alga. Sears and Newbold (1942) found that 70 per cent of the nitrogen and 80per cent of the potassium returned to the soil by sheep excrements was contained in the urine. They also demonstrated that the urinary nitrogen and potassium were in a readily available form for assimilation by plants whereas the full utilisation of these nutrients from faeces could only be realised after prolonged action by soil micro-organisms. Their field observations showed that sheep urine caused rapid grass growth whilst dung caused a slower but more sustained growth mainly of clover. Watkin (1957) confirmed the observations that urine gave rise to enhanced growth of grasses but was able to demonstrate only a poor response to even large quantities of dung. It may be that a rapid release of large quantities of nutrients caused by the deposition of urine proves toxic to Z. ericetorum and H. flaccidum, whilst their slow, long-term release from dung is beneficial. The high pH (9.3) of urine is unlikely to be an important factor as decomposed urine of the same pH was not fatal to either alga under laboratory conditions. It has been demonstrated that urea shows a similar toxic effect to sheep urine so that it may be the urea itself which is toxic. Urea is rapidly converted to carbon dioxide and ammonia which are soon lost from the soil and this may explain why decomposed urine appears to lack the toxicity effect.

In artificial culture, nitrogen is usually supplied to algae in the form of ammonia salts or nitrates. Round (1965) points out that most algae can assimilate either but evidence suggest that the ammonium salt is preferred. Nitrogen supplied in the form of organic compounds is first deaminated. Round describes Hormidium sp. as an alga with an excessive capacity for deamination resulting in the release of ammonia into the medium. Zygnema sp. however has a much lower capacity which may be a limiting factor in nitrogen assimilation. Collyer and Fogg (1955) suggest that the accumulation of lipid droplets within algal cells may be a response to nitrogen deficiency.

A feature of Zygogonium ericetorum growing within the influence of deposited dung is that the cells are lacking in the purple pigment characteristic of this species. The function, if any, of this pigment is not known. Fritsch (1916) believed that it plays a photoprotective role as the most deeply pigment filaments are those growing where the illumination is greatest. Alston (1958) showed that this pigment is an iron tannin complex which suggests that it would be formed where ferric ions are freely available in the surrounding water. This would be likely in acid bogs and in the Drakelands Corner area, material with the deepest pigmentation was that growing on waterlogged micaceous waste. Allen and Alston (1959) report on the development of a similar pigment in Spirogyra pratensis under culture conditions and conclude that the ability of the alga to synthesise such a pigment is dependent upon its ability to tolerate an ecological niche in which iron is available in sufficient quantity to interact with the precurser. Pearsall (1950) states that a red-brown or red-black colour is characteristic of many plants in upland flushes and is probably associated with a deficiency in available nitrogenous substances. It is interesting that the pigment is lacking in material of Z. ericetorum growing in a situation where levels of nitrogen are not likely to be limiting.

The algal mats on china clay waste form a distinct micro-environment. Algal mats of Z. ericetorum consist almost exclusively of this species together with small amounts of Hormidium flaccidum. Other plants are only occasionally found with the exception of the Dematiaceous Hyphomycete, Chloridium sp. It is likely that the algal biomass provides food for a number of animals. In sorting field material, large numbers of Nematodes are found many of which contain partly digested Z. ericetorum in their guts. Also frequently found are lavae of craneflies (Tipulidae) which appear to be algophagous. Lynne and Brock (1969) record a Zygogonium species as being a food source for the brinefly, Ephydra bruesi, in the Yellowstone National Park.

In situations where the growth of Z. ericetorum is luxuriant, West and West (1897) have suggested that the high water-holding capacity of the mats permit seedlings to develop which could not do so on bare soil. In the Drakelands Corner area, Calluna seedlings are sometimes seen growing on the algal mat. No allelopathic effects were demonstrated for Z. ericetorum and H. flaccidum in the limited experiments carried out and it may be that any possible competition effects between the algae and flowering plants are outweighed by their role in reducing leaching and in maintaining a reserve supply of mineral elements and organic matter in a semi-available form.

The main value of algal organic matter is that it immediately enters the food chains where it is consumed by heterotrophic micro-organisms and algophagous animals. In doing so, the algal mass is renewed. Being a constant factor that acts for many years, the algae prepare the unstable, sandy substrate, deficient in organic matter, for other plants to establish themselves and continue the formation of soils.

SECTION 111

EFFECTS OF SULPHUR DIOXIDE ON

ZYGOGONIUM ERICETORUM AND *HORMIDIUM FLACCIDUM*

12: Literature Review

Air and water have long been considered infinite resources to be used by man as needed, but more recently it has been realised that these resources are finite and that some control is necessary over the products we discharge. For a long time, there has been concern over air pollution in urban areas. As early as 1273, during the reign of Edward 1, the first smoke abatement law was passed in England in response to a popular belief that food cooked over buning coals could cause illness and even death. In 1306, concern over air pollution was so great that a royal proclamation was issued, prohibiting the burningof coal in London. During the period of the Industrial Revolution, air pollution became a significant hazard in many areas. Because particulate air pollution is an obvious and visible form of pollution, great strides have been made to control it, and recently concentrations of smoke emissions have decreased considerably.

However, this has not been the case with "invisible air pollutants" whose concentrations have continued to increase and whose effects are often manifest over wide areas. Probably the most important of these is sulphur dioxide although it is seldom released into the air in isolation but with varying concentrations of other pollutants. Sulphur dioxide is liberated into the air by processes involving the combustion of fossil fuels and certain industrial processes involving sulphur compounds or impurities. An estimate of the quantity of sulphur dioxide released into the atmosphere on a global basis as a result of mans activity has been given by Rasmussen et al (1974) as 130 x 10^9Kg. $SO_2 yr^{-1}$. There are also natural sources of the gas, the chief of these being volcanic activity, and although quantities released naturally are extremely difficult to quantify, Rasmussen et al consider the concentrations to be rather less than those from man-made sources. An important natural, secondary source of sulphur dioxide is from the oxidation of hydrogen sulphide released from decaying vegetation by bacterial action.

Ground level concentrations of sulphur dioxide can vary from 1.05vpm in extremely polluted areas to 0.007 vpm or less in remote, rural areas with background levels of 0.04 to 0.15vphm, according to the National Society for Clean Air (1971). In this country, levels above 0.2vpm are rarely recorded now, but although levels in urban areas are tending to decline, those in rural areas are gradually increasing (Ferry, Baddeley and Hawksworth, 1973). This is due in part to the discharge of pollutants at higher levels above the ground thereby ensuring a more effective means of dispersal. The National Society for Clean Air (1971) have pointed out that the highest concentrations of sulphur dioxide today tend to occur in the industrial north and the Greater London areas.

Sulphur dioxide is a heavy, colourless gas with an acrid smell. It is very soluble in water (one volume of water dissolves 45 volumes of the gas at 15°C.), and chemically very reactive either oxidising to sulphate or photochemically reacting with other atmospheric contaminants. Sulphur dioxide is removed from the atmosphere by various means involving water or other compounds. In solution, it exists in a number of ionic states which are in equilibrium depending on the pH of the solution. Hales and Sutter (1973) have assumed that dissolution proceeds according to the following scheme:

$$SO_2\,gas + H_2O \rightleftharpoons SO_2\,aq + H_2O$$

$$SO_2\,aq + H_2O \rightleftharpoons H^+ + HSO_3^-$$

$$HSO_3^- + H_2O \rightleftharpoons H_3O^+ + SO_3^{2-}$$

Where $SO_2\,aq$ is undissociated SO_2 (i.e. Sulphurous acid). Puckett et al (1973) concluded that free sulphurous acid (H_2SO_3 uncharged) is present at low pH, the bisulphite ion (HSO_3^-) predominates at intermediate pH values and the sulphite ion (SO_3^{2-}) is formed at high pH although this may be a simplification of an as yet, imperfectly understood situation.

Great difficulties are experienced in equating levels of sulphur dioxide in solution with those in air. The problem arises because the gas is extremely soluble and will continually dissolve in thin films of water, building up to a concentration much greater than that in the immediately surrounding air. Most experimental solubility data is not appropriate to the comparitively low levels of atmospheric sulphur dioxide encountered in the field. Saunders (1970) suggested that 0.035vpm sulphur dioxide in air would give rise to a solution of 35ppm in water. Hales and Sutter (1973) have shown that 0.04 ppm in air could result in a concentration as great as 408 ppm in triple distilled water at 25°C.

Rasmussen et al (1975) estimate the lifetime of a molecule of sulphur dioxide to range from between 20 minutes and seven days. They consider the major sinks for atmospheric sulphur dioxide as being precipitation scavenging, chemical conversion and absorption by soil, water, stone and plants.

(1) Precipitation scavenging. This is by "rainout", namely the absorption of gas and sulphate particles in the cloud, and "washout", the removal of these sulphur compounds from the cloud level by precipitation. Hutcheson and Hall (1974) have shown that the rate of "washout" is dependent upon the background acidity of the rain, the height of the pollutant source and the rate of rainfall. Meetham (1950) estimates that only 17 per cent of atmospheric sulphur dioxide is removed by this method.

(2) Chemical Conversion. The most important atmospheric reaction involved here is the photochemical oxidation of sulphur dioxide which occurs in heavily polluted atmospheres.

(3) Absorption by soil, water, stone and plants. This is the fate of sulphur compounds arriving by precipitation scavenging. The oceans are assumed to be an important sink and soils are capable of absorbing significant amounts of sulphur dioxide. Smith, Bremner and Tabatabai (1973) report that soils of high water content are particularly effective at removing the gas. It is likely that sulphur gases adsorbed by soils will be oxidised to sulphate and subsequently removed by leaching or by plant uptake. It has been shown by many workers that areas which have suffered heavy pollution for long periods show increased acidity and sulphate contents of the soil and some of these reports are reviewed by Webster (1967).

Vegetation covers the greater part of the land surface of the globe and probably accounts for much of the sulphur dioxide absorbed. A.C. Hill (1971), examining uptake of a number of air pollutants by an alfalfa canopy showed that sulphur dioxide was removed efficiently by the upper portion of the canopy as well as by the immediate subsurface vegetation. Wind velocity above the plants, height of the canopy, and light intensity all affected the pollutant removal rate and there was a relationship between absorption rate and solubility. An average uptake rate of 85µl. sulphur dioxide/min./M^2 from air containing 5vphm sulphur dioxide was obtained.

An additional important sink is dry deposition as described by Meetham (1950). This involves the absorption of aerosols and subsequent deposition on the ground, but the process is not fully understood. Meetham suggests that up to 80 per cent of sulphur dioxide can be removed by this method. Shepherd (1974) has shown that the rate of dry deposition is normally limited by surface resistance but is independent of wind speed.

Historically, there have been a great many reports of effects of sulphur dioxide on flowering plants recorded over the past eight decades. Early work concentrated upon fairly dramatic effects produced by high concentrations of the gas but more recent work has focused on the effects of much lower, more realistic, concentrations. Literature relating to effects of atmospheric pollutants on flowering plants has been adequately reviewed by a number of authors including Katz (1949), Thomas (1951) and Webster (1967). The study of adverse effects of sulphur dioxide as an air pollutant is complicated by the fact that some plants growing on soils deficient in sulphur are able to utilise low levels of the gas absorbed from the atmosphere and this results in growth enhancement. This has been reported by various workers including Fried (1948) and Cowling, Jones and Lockyer (1973). The ability of a plant to utilise this sulphur effectively without damage is dependent upon the rate of absorption of sulphur dioxide and the rate of production of sulphites, which are believed to be toxic. Thomas <u>et al</u> (1950) considered that the sap of plants showed a limited capacity for buffering the absorbed sulphur dioxide thereby reducing its toxicity. It has been suggested by workers, including Syratt and Wanstall (1969) and deCormis (1969), that tolerant plants are able to store excess sulphur in the form of sulphates. Katz (1949) noted that the sulphur content of leaves increased substantially with duration of exposure and under natural conditions, reflected the degree of sulphur dioxide pollution to which they had been subjected. Many workers subsequently investigated sulphur accumulation by plants as a means of assessing the degree of pollution in an area (e.g. Guderian, 1970 and Pyatt 1973). Although it is an approach that is frequently used, as Laaksovirta and Silvola (1975) point out, the sulphur contents of plants can vary in unpolluted areas and sulphur, being an essential element in the metabolism of plants is obtained from a number of sources.

Some investigators have studied synergistic effects and found that the effects of two pollutants are not necessarily additive. Synergistic inhibition of apparent photosynthetic rates has been shown for sulphur dioxide and nitrogen dioxide at low concentrations using alfalfa by White et al (1974), and Bull and Mansfield (in press) have demonstrated a similar synergistic inhibition for dark respiration in Vicia faba. White et al (1974) report decreased injury threshold concentrations due to combinations of ozone and sulphur dioxide.

Bennett et al (1974) propose that the enhancement of plant growth caused by low levels of air pollutants, as recorded by Cowling, Jones and Lockyer (1973), is only seen when compared with growth of controls in clean, filtered air. They argue that "clean air" is an untenable standard, however it is defined, for many areas and, because low concentrations of pollutants have been present in the air for a long period of time, many plants have become adapted and are at a disadvantage when grown in clean air. Pollution is an environmental change and as such might be expected to cause evolutionary changes in populations. This is now well documented for heavy metal tolerance in a number of species and has been reviewed by Antonovics et al (1971). Bell and Mudd (in press) suggest that air pollutant resistant ecotypes can also arise. They have shown that native, wild Lolium perenne from a polluted rural area of the South Pennines is more resistant to coal smoke pollution in the field and to sulphur dioxide fumigations in chamber experiments than is a commercial strain of the same grass. Taylor and Murdy (1975), studying populations of Geranium carolinianum at sites at varying distances from a coal-fired powerstation, have demonstrated that populations remote from the pollution source were more sensitive, in terms of mean leaf area damaged, than were populations growing in the vicinity of the powerstation. The powerstation in this instance had been operating for 31 years which suggests a rapid selection of pollution tolerant populations in this annual plant.

Little work has been carried out on effects of air pollutants on plant communities. Most of the literature consists of species lists from areas of increasing atmospheric pollutions but little seems to be known of the competitive ability of two or more species or of changes in successional patterns brought about by alterations in the air quality. Both direct effects of the pollutants on the vegetation and indirect effects of pollutants on the soil structure and nitrification ecosystems are likely to be operative. Preliminary work of Hajdúk and Růzícka (1968) suggests that indirect effects via the soil microflora predominate. They demonstrated that fumigations of soil by sulphur dioxide resulted in a significant decrease in the number of fungi although an increase in the numbers of Actinomycetes was recorded.

The biochemical effects of sulphur dioxide arise from its ability to act either as an oxidising agent or a reducing agent. Generally, it is more toxic to non-flowering plants than to higher plants and this may in part be explained by the fact that higher plants generally develop a cuticle over their aerial organs which reduces waterloss and the rate of absorption from the atmosphere. Sulphur dioxide is more toxic to such plants when their stomata are open and Thomas (1951) was one of the first to show this. Non-flowering plants however, are soon exposed to the effects of gaseous pollutants by their ability to absorb moisture over their whole surface.

The extreme sensitivity of lichens to industrialisation has long been recognised. In 1859, Grindon noted the disapperance of many species from industrial South Lancashire. Thecausative factors for such declines have been in dispute. Horwood (1907) noted that lichens grew most actively in winter which is when pollution levels are greatest but Rydzak (1969) and others suggested that the relatively drier climate around cities was responsible for the disapperance of lichens. Although lichens are affected by the relative humidity of the atmosphere, their scarcity over wide areas around cities, particularly where the humidity is high suggests that dryness is of only secondary importance. Indeed, it appears that in the wetter, Atlantic parts of Europe, the areas of "lichen deserts" (regions around a pollution source from which lichens are excluded) are larger than those of more continental climates (Ahmadjian and Hale, 1973). Today, there is much evidence that sulphur dioxide is the overriding cause of the destruction of lichen vegetation (Ferry, Baddeley and Hawksworth, 1973) and the distribution of lichens around urban and industrial centres has been mapped by many workers including Brodo (1966), Laundon (1967), Skye (1968) and Pyatt (1970). Sulphur dioxide is also recognised to be the principal factor involved in the disappearance of epiphytic Bryophytes around cities and industrial areas as shown by workers including Gilbert (1968, 1970) and Syratt and Wanstall (1969). There appears to be no similar work involving free-living algae.

Experiments on the toxicity of sulphur dioxide to lichen thalli suggest that it is the algal partner which is more susceptible. Rao and Le Blanc (1965) exposed lichen thalli to 5vpm sulphur dioxide for 24 hours at varying humidities and were able to demonstrate bleaching of the chlorophyll, permanent plasmolysis and the formation of brown spots on the chloroplasts of the algal cells. Subsequent analysis showed that chlorophyll a had been degraded to phaeophytin a and that this breakdown was greatest at higher humidities. However, the concentrations they used were much higher than would occur naturally in polluted areas, so their results may have little relevance to the disappearance of lichens from industrial areas. At such high concentrations, the observed pigment breakdown may have been a secondary, rather than a primary, effect. Showman (1972), in contrast, found that gaseous sulphur dioxide damage was not associated with chlorophyll breakdown. He exposed some common lichens and their isolated phycobionts to levels of 2, 4 and 6 vpm sulphur dioxide. Some of his experiments were conducted in plastic chambers, which are known to adsorb sulphur dioxide (D. J. Hill, 1971), so the effective concentrations to which the material was exposed may have been lower. However, he found that net photosynthesis and respiration were reduced. The inhibition was greater in the phycobionts than in the intact thalli.

Sulphur dioxide dissolves in waterfilms on the lichen surface before it can affect the plant and several workers have studied the effects of the gas in solution. For instance, D. J. Hill (1971) used buffered sulphate solution on three lichens of known differing pollution sensitivities, namely Usnea subfloridana the most sensitive, Hypogymnia physodes of intermediate sensitivity, and Lecanora conizaeoides the most tolerant. He was able to show that the incorporation of $H^{14}CO_3^-$ in the light was inhibited by lower concentrations of sulphite in Usnea sp. than in Lecanora sp; with Hypogymnia sp. showing intermediate sensitivity. Sulphite was toxic at pH4 and below but not at pH5 or above, at the concentrations used. As Puckett et al (1973) point out, sulphite in solution may not necessarily be the form which is active in the field and they have used buffered sulphur dioxide solutions in their experiments with a number of lichen species. They have also demonstrated the increased toxicity at low pH and tentatively suggest that it is in part due to the destruction of chlorophyll by an irreversible oxidation process. Hill's recent experiments (1974) have shown that Hypogymnia physodes is able to recover in 24 hours from almost total reduction in the ability to fix $H^{14}CO_2^-$ caused by sulphite treatment. This suggests that sulphite may be a competitive inhibitor of carbon dioxide uptake and at low concentrations subsequent recovery could occur. A similar recovery was not seen with Usnea subfloridana, a species more sensitive to atmospheric pollution. Here it was shown that the sulphite becomes bound with protein, a process likely to be damaging to plants in the long term with slow rates of protein synthesis.

The available evidence suggests that mosses are at least as sensitive to air pollutants as lichens and Gilbert (1968) has shown that protonema are generally more sensitive than gametophytes. He examined the effects of aqueous solutions of sulphate, sulphite, bisulphite and sulphurous acid on moss protonema and mature gametophytes. Unfortunately, although epiphytes are exposed to these forms of sulphur dioxide in the field, it is very difficult to equate these with gas concentrations. Nash and Nash (1974) demonstrated that protonema of Polytrichum ohioense were killed by levels of 0.2 vpm sulphur dioxide whilst mature gametophytes were resistant to levels of 2-4 vpm. These results suggest that the absence of many moss species from industrial areas is due to levels of sulphur dioxide toxic to protonema but not necessarily affecting the mature gametophyte; in other words there is a block in the reproductive cycle.

It appears that effects of sulphur dioxide on the cells are similar for mosses and the phycobionts of lichens. Syratt and Wanstall (1969) found, as Rao and Le Blanc (1965) found with lichens, that sulphur dioxide causes breakdown of chlorophyll to phaephytin in moss gametophytes. Using rather high levels of gaseous sulphur dioxide on three epiphytic Bryophytes they were able to show that 2vpm caused a breakdown of up to two-thirds of the chlorophyll at high humidities. Gilbert (1968) found that sulphite in acid conditions killed mosses more readily than sulphite under more neutral conditions. Inglis and Hill (1974) have shown that, as with lichens, uptake of $H^{14}CO_3^-$ in the light was inhibited at low pH and in the mosses studied, no recovery was noted after treatment. This suggests that these mosses may have sustained irreversible damage.

Sulphur dioxide can act either as an oxidising or a reducing agent in the cell and Malhotra and Hocking (1976) summarise some of the important metabolic effects as being direct interference with photosynthetic carbon dioxide fixation (competitive inhibition of ribulose diphosphate carboxylase by SO_3^{2-}) and direct interference with energy metabolism (inhibition of mitochondrial ATP produced by SO_3^{2-}). Many indirect effects result from the formation of sulphites and organic sulphonates with other cell constituents.

The extent to which effects of pollutants on moss protonema and on the algal component of lichens can be extrapolated to give an idea of effects on free-living terrestrial algae is uncertain. Although in fumigation experiments with lichens, visible damage is most obvious in the alga, free-living alage may respond differently. Moreover, as Hill and Ahamadjian (1972) have shown, aspects of algal physiology can change markedly when the alga is grown in culture. Field observations of Barkman et al (1968) have indicated that Prasiola crispa occurs in large industrial areas such as Rotterdam but may be absent from very heavily polluted areas, and that absence of Trentepohlia umbrina from towns may be due to low humidities. Pleurococus viridis on trees extends further into cities than even the most pollution tolerant lichens, as recorded by Hawksworth and Rose (1970). Smith (1973) found no correlation between numbers and viability of airborne algae collected and the total sulphur and hydrocarbon concentrations in the air throughout a twelve month period. In this case the average total sulphur content recorded was low throughout the year, averaging 0.016vpm. All these observations may possibly suggest that terrestrial algae are perhaps more resistant to atmospheric pollutants than those which have entered into a symbiotic association with fungi.

13: Methods available for monitoring sulphur dioxide

A major problem in the investigation of effects of sulphur dioxide on *Zygogonium ericetorum* and *Hormidium flaccidum* was that of continously and accurately monitoring levels of the pollutant. This was particularly the case with laboratory experiments and this is one of the reasons why many of these experiments were carried out using sulphur dioxide in solution prepared by dilution of a known concentration of stock solution. The following techniques were utilised for monitoring atmospheric levels of the pollutant.

1. Lead peroxide candle

Ambient sulphur dioxide concentrations in the field have often been monitored by this method (e.g. de Tur ville, 1970). A coating of gel containing lead peroxide is applied to a fabric binder on a cylindrical support and the reactive surface is exposed to the atmosphere for a period of time, generally four weeks. At the end of thistime, the degree of sulphation expressed as mg. $SO_3/100cm^2$/day is determined by a turbidimetric barium sulphate procedure. Hence, this method gives a crude assessment of the level of sulphur dioxide pollution over a comparitively long period of time in arbitary units.

2. Warren Springs sulphur dioxide sampler

Air pollution in Britain is currently recorded by standard daily smoke and sulphur dioxide sampling gauges supervised by the Ministry of Technology. The concentration of sulphur dioxide in the air is estimated by bubbling a measured sample of filtered air through a dilute solution of hydrogen peroxide in a Dreschel bottle. The sulphur dioxide is converted to sulphuric acid and the amount of acid is determined by titration with a standard alkali solution. The estimation is affected by other strong acids or alkalies in the air although these are usually assumed to be present at much lower concentrations than sulphur dioxide. Results are expressed in $\mu g/M^3$. Unfortunately, there is no generally applicable calibration for relating results using this method with those obtained using the lead peroxide method. A disadvantage of the Warren Springs sampler is that its accuracy is much reduced at levels of 0.01vpm sulphur dioxide and below, which are frequently the levels of inter est in the field in rural areas. It is also usually necessary to run the pump on mains electricity which restricts its use in rural areas.

3. Casella miniature sulphur dioxide sampler

This portable, battery-operated sampler works on the same principal as the Warren Springs sampler but gives more accurate readings over a short period of time. A known volume of filtered air is bubbled through an electrolyte containing hydrogen peroxide and the increase in acidity caused by the sulphur dioxide is measured as a change in conditivity of the electrolyte, this varying directly with the sulphur dioxide present and the sampling time. Air is sampled from a height of 25 cm above the ground, if the apparatus is operated at ground level. The method gives reasonably reliable readings for concentrations of sulphur dioxide down to 0.005vpm and has been used in the field experiments described in this section. It has the disadvantage of only indicating ambient sulphur dioxide levels at the time of operation and hence may give quite different results from a single site depending upon the day and the time of day chosen.

4. Dräger detector tubes.

Drägwerk manufacture air pollutant detector tubes which are used as a rapid means for the detection of a high levels of pollutants. The Dräger gas detector consists of a bellows pump operated by hand, which supplies 100 cm^3 of air with each stroke, and a detector tube filled with coloured crystals specific for each pollutant. A known volume of air is drawn through the detector tube and the extent of discolouration of the crystals in the tube corresponds to the relative amount of the pollutant in the air sample. This technique is rapid but only gives a crude estimate of the levels of the gas. The sulphur dioxide tube records levels dowm to 0.1vpm and is consequently of little use for measuring ambient levels of thegas in the field.

5. Bioassay method.

One approach to estimating concentrations of sulphur dioxide in rural areas, where continous monitoring devices are expensive to set up and difficult to maintain, is to deduce the degree of air pollution from effects on vegetation. Many lichens have been shown to be extremely sensitive to air pollution and it is generally accepted that they are largely affected by sulphur dioxide.

Hawksworth and Rose (1970) proposed a qualitative scale for estimating sulphur dioxide pollution in England and Wales using epiphytic lichens. They recognised ten zones based on lichen species and their frequency which showed a continumm along an air pollution gradient. These zones have been equated with mean winter values of sulphur dioxide, winter being the time when pollutant levels are likely to be highest and the lichens are most actively metabolising. Hence it is conceivable that an isolated pollution episode in the past may alter the lichen flora so that the area would be assigned to a more heavily polluted zone than levels of sulphur dioxide currently experienced in the area. The Hawksworth and Rose (1970) scheme has been used in the field investigations described in this section.

None of the methods outlined above are suitable in laboratory investigations. Continuous monitoring devices used in many laboratories today, particularly in North America, are flame photometric detectors, which determine levels of total sulphur, and infra-red gas analysers, which can record accurately very low concentrations of sulphur dioxide.

14: The Field Situation

Air Pollution in the Plymouth area

The Southwest of England is a predominantly rural area with generally low levels of air pollution. Warren Springs Laboratory suggest concentrations of sulphur dioxide of 0.01vpm or less in rural areas. The prevailing winds are from off the sea and consequently are almost free of pollutants. The inland areas are predominantly agricultural and the coastal areas, particularly the south coast, cater for the tourist industry. There are few conurbations, the principal ones being Plymouth, Exeter and Torbay and these are all situated around the southern coast.

Plymouth is the largest city in the Southwest peninsular with a population of approximately 250,000. It is a city with important dockyards and is a centre for light industry. The bulk of the city is composed of large housing estates interspersed with open spaces. Industry is primary located around the docks, on the northwest edge of the city and to the east, in and around Plymouth. Data on the air quality of the area is supplied by Warren Springs Laboratory who operate three sulphur dioxide and particulate monitoring stations within the city boundaries. In addition, a Warren Springs sulphur dioxide sampler has been operating on the Plymouth Polytechnic Campus, in the centre of the city, since January 1975. Levels of smoke and sulphur dioxide recorded at these sites are less than expected levels for large urban areas (Fig. 44) and between the years 1961 and 1971 neither pollutant showed any significant changes in concentration from year to year (Warren Springs Laboratory , 1972).

There is a single powerstation in the Plymouth area, situated at Cattedown (Fig 45) and this is the most important point source of sulphur dioxide. The original coal-fired powerstation has been in operation since 1899 and has a stack 46M. in height, but an additional and much larger unit was completed in 1960 and this is oil-fired and has two stacks one of 91M. and one of 105M. (Plymouth Power Station publicity handout, 1972). The Central Electricity Generating Board carried out a survey of sulphur dioxide and particulate levels in the vicinity of the powerstation five years prior to the opening of the oil-fired station in 1960 and during the subsequent eight years. A report of the findings was

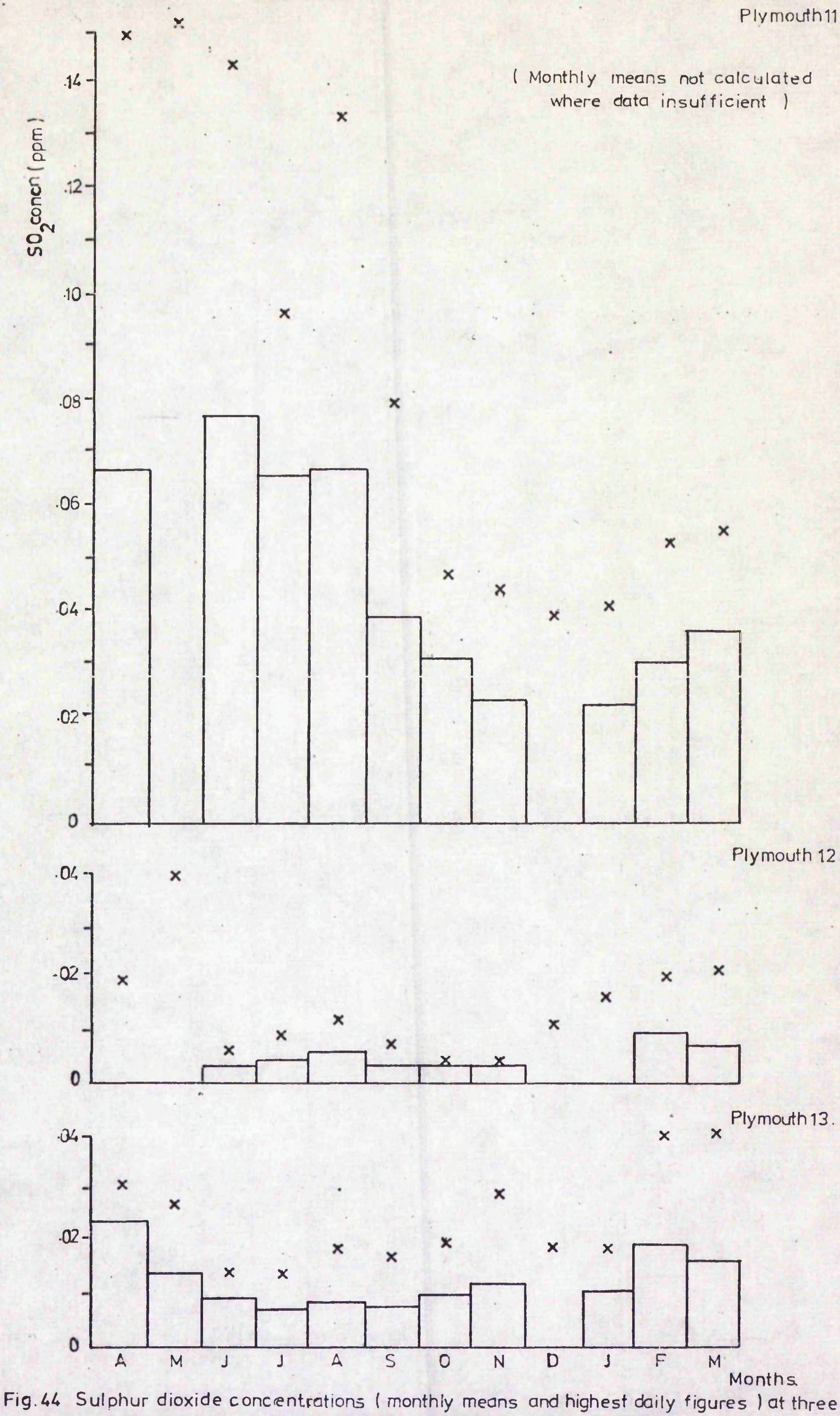

Fig. 44 Sulphur dioxide concentrations (monthly means and highest daily figures) at three monitoring stations in Plymouth between April 1974 and May 1975.

(Warren Springs data expressed in ppm) prepared by de Turville (1970). Ten sampling sites in the Plymouth area were used and monthly levels of particulates were recorded using deposit gauges and sulphur dioxide using lead dioxide candles. It was concluded that there was no significant correlation between the lead dioxide candle results and the tonnage of sulphur burnt at the powerstation and also that the levels of sulphur dioxide were not significantly lower prior to the oil-fired station becoming operational in 1960.

The Cattedown powerstation consumes approximately 168 tonnes of coal and 624 tonnes of oil per day with a mean combined sulphur content of 20 tonnes per day (Plymouth Power Station publicity handout, 1972). In 1973, the Government gave its consent to the building of a new £120 million powerstation at Insworke Point (Grid Ref. SX 435534) on the banks of the River Tamar just to the west of Plymouth. The proposed powerstation, which is to be oil-fired, will have a stack 206M. It will burn oil containing no more than three per cent sulphur and it has been estimated that it will release 426 tonnes of sulphur oxides per day (Hawksworth, 1971). The site of the powerstation is backed by the 121M. Maker Ridge to the west with Plymouth rising to a height of 90M to the east. The aerodynamics of plume dispersal from a point source over such terrain are complex (Geiger, 1961). With southwest prevailing winds, the pollutants would be carried towards Plymouth and across the southern half of the Dartmoor National Park. Hawksworth (1971, 1973) and others have expressed concern over the effects of such a major pollutant source on the lichen flora which, it is felt, could be significantly depleted over a large area of Dartmoor and the South Hams. These areas at present are subject to extremely low concentrations of sulphur dioxide and frequently support luxuriant growths of pollution-sensitive species. The area likely to be affected by significantly increased sulphur dioxide levels includes the china clay workings at Lee Moor and Drakelands Corner. Due to the present climate of economic restraint, the future of the powerstation seems uncertain and in 1976, the C.E.G.B announced that the start of the work would be deferred.

It has been shown by Gilbertson (1975) that pollution from Plymouth has a detectable effect on the acidity of rainfall over and downwind of the city. In early February 1974 he demonstrated an increase in rainfall acidity particularly to the northwest of

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Fig. 45 Plymouth and area to the east showing field trial sites.

a. Cattedown

b. Laira Bridge

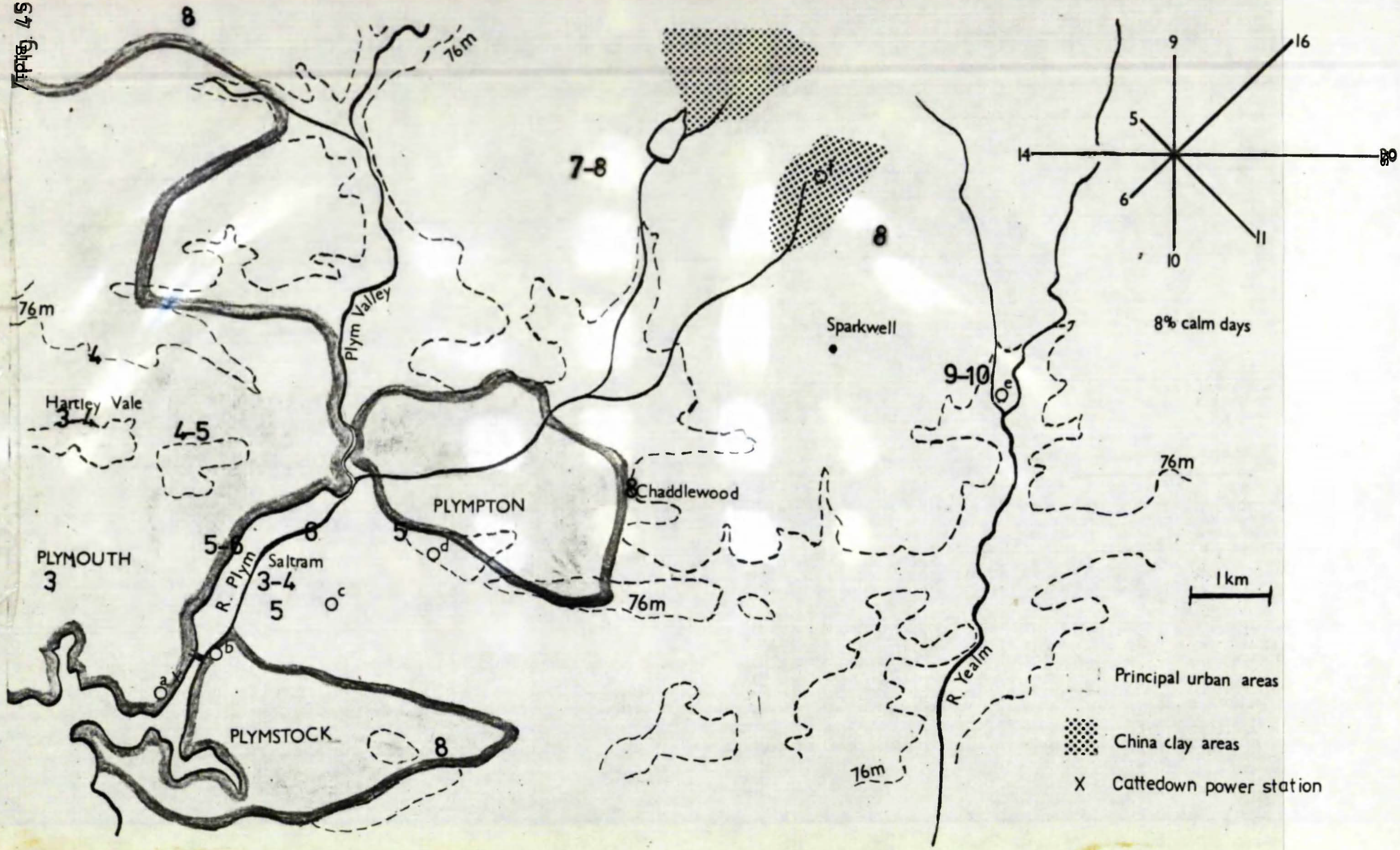
c. Chelson Meadows

d. Plympton by-pass

e. Stert Runs

Windrose for Cattedown power-station 1964-67

Overlay: Hawksworth and Rose (1970) pollution scale. Values for sites in the Plymouth region.



8
76m
7-8
9
16
5
14
6
11
10
8
Plym Valley
Sparkwell
8% calm days
76m
4
9-10
Hartley Vale
3-4
4-5
8 Chaddlewood
76m
PLYMPTON
PLYMOUTH
3
5-6
R. Plym
Saltram
3-4
5
8
5
76m
1 km
R. Yealm
Principal urban areas
China clay areas
X Cattedown power station
PLYMSTOCK
8
76m

the city up to 18Km. from the city centre, although these results were based upon a rather small number of samples. It is likely that these effects, if real, are comparatively local. Dougall (1973) showed that at Seale-Hayne Agricultural College, Newton Abbott, 41 Km to the northwest of Plymouth, levels of sulphur in the rainwater were not elevated in rainfall with southwesterly winds. However, north and north-east winds, the latter from the general direction of Exeter 20Km away, carried higher concentrations of sulphur in the precipitation.

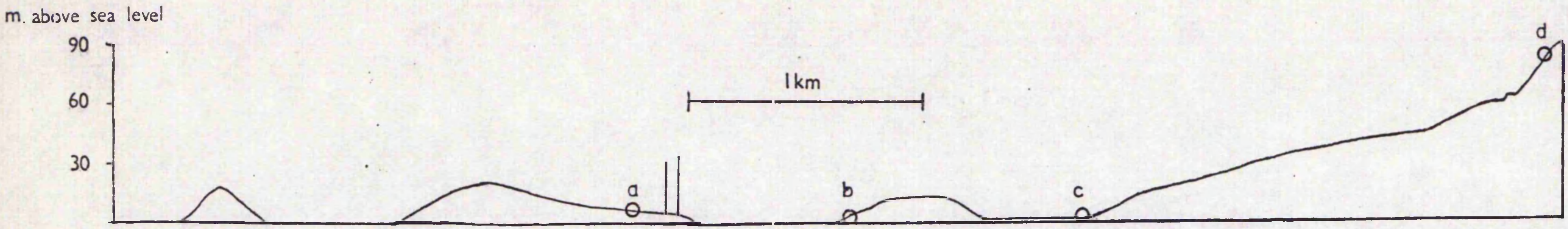
Field Trials

At present, the Cattedown powerstation is the most important point source of sulphur dioxide in the Plymouth area. It is surrounded by a number of limestone quarries. With the predominant west and southwest winds, pollutants from these sources are liable to be carried towards the lower slopes of Dartmoor. Under certain weather conditions, pollutants may be carried some distance up the deep valley formed by the River Plym (Fig 45). In order to gain an idea of the extent of sulphur dioxide pollution to the north and east of Plymouth the air pollution scale of Hawksworth and Rose (1970) was adopted.

Field trials were carried out to assess the performance of Zygogonium ericetorum when growing under such conditions of varying atmospheric pollution as found in the Plymouth area. A transect was taken in a northeasterly direction from the powerstation (Fig. 45). It was felt that because levels of sulphur dioxide were not expected to be high and the alga was likely to be fairly tolerant of air pollution, that it was unnecessary to use a large number of sites along the transect and four sites were chosen which, it was hoped, would be subject to differing degrees of air pollution. These sites were as follows:

(a) Cattedown. Shed roof in a small backgarden. High density, urban residential area. 100M west of the powerstation. This site was not of ready access so that sulphur dioxide monitoring was carried out at (b) by the Plym Estuary, 750M northwest of the powerstation. No suitable trees were present in either area to ascribe a zone on the Hawksworth and Rose (1970) pollution scale.

(c) Chelson Meadows.Old, flattened area of Plymouth rubbish tip, now open grassland. 2.4Km northeast of the powerstation. Zone 5 on



Profile of transect showing power station stacks
and sites a,b,c, and d.

Date.	Temp °C	R.H.	Wind	sites b SO$_2$ levels (ppm.)	c	d	e	Plymouth Poly. site
25/7/74	18.5	75%	Mod. SW.	·004	·005	·005	·003	
14/8	17	97%	Mod. S.	·001	·001	·003	·001	
19/9	16	85%	Light W.	·001	·002	·001	·001	
21/10	12	70%	Mod. W.	·002	·001	·005	·001	
21/11	9	90%	Calm	-	-	·009	·006	
18/12	9	78%	Mod.W/SW	·003	·003	·003	·002	
21/1/75	9.5	73%	Mod. W.	·001	·001	·002	·001	·014
26/2	12	81%	Mod. S.E.	·003	·004	·007	·002	·024
21/3	8	83%	Mod. S.E.	·001	·005	·004	·001	·025
17/4	11	98%	Mod. W.	·002	·002	-	-	0
14/5	12.5	88%	Light SW.	·001	-	·002	·000	·008
18/6	14	90%	Light S.	·004	·006	·006	·002	·007
17/7	18	84%	Light SW.	·006	·003	·008	·002	0

Date	Wind	site f SO$_2$	Plymouth Poly. site
9/2/75	Mod. E.	·005	·015
27/2	Mod. SE.	·001	·024
14/3	Light E.	·004	·030
29/4	Mod. W.	·002	·011
27/5	Mod. NE.	·007	·002
18/6	Light S.	·005	·007
31/7	Calm	·009	·012

Sites.

a Cattedown.
b Laira bridge.
c Chelson Meadows.
d Plympton by-pass.
e Stert Runs.
f Drakelands Corner.

Fig. 46 Transect and meteorological data recorded from field-trial sites.

the Hawksworth and Rose (1970) pollution scale.

(d) Plympton by-pass. Steep, grassy embankment well above the level of the traffic. 4 Km northeast of the powerstation and at a height above that of the highest stack. Zone 5 on the Hawksworth and Rose (1970) pollution scale.

(e) Stert Runs. Southwest edge of coppice with standards type woodland recently planted with conifers. 11.5Km northeast of the powerstation. Zone 10 on the Hawksworth and Rose (1970) pollution scale . This site served as a control.

At each site, three 15 x 21 x 8cm plastic trays containing silica sand and a 5 cm disc of Z. ericetorum mat from Drakelands Corner, were set out and left for a twelve month period, July 1974 to July 1975. At monthly intervals, the trays were examined and, where necessary, vegetation was cleared from around them. It was found that during the summer months, the growth of vegetation at site C was so rapid that for much of the time the trays were sheltered from the prevailing winds. It was not possible to install continous sulphur dioxide monitoring equipment at each site and, as a compromise, at each visit a Casella portable sulphur dioxide analyser was used to record concentrations over a 30 minute period and readings were also taken of temperature, relative humidity and wind direction (Fig. 46).

At the end of the twelve month period, the trays were taken back to the laboratory. Two of those at site (d) had been lost through interference. The trays were examined for algal growth and pH, field water contents, organic matter and sulphate levels of the top 1cm of silica sand were assessed (Table 29).

Sulphur accumulation experiments Estimates of the levels of sulphur in plant tissues are frequently used to assess the degree of pollution of an area. To determine whether algal mats of Z. ericetorum accumulate sulphur and can thereby be used as an indicator of sulphur dioxide pollution, 1g. (dry weight) portions of algal mat which had been killed by heating to 50°C for 5 hours, were placed in flat, nylon packets 5 cm. square (mesh 0.5mm^2) and fixed to the ground by pegs at the corners. Similar packets were made and filled with absorbent cottonwool. These packets were placed out in open areas at Drakelands Corner and also on a more heavily polluted area of moorland, Hallam Moors (Grid Ref. SK 234871) in Derbyshire. They were left in the field over the four winter months (December to March) and

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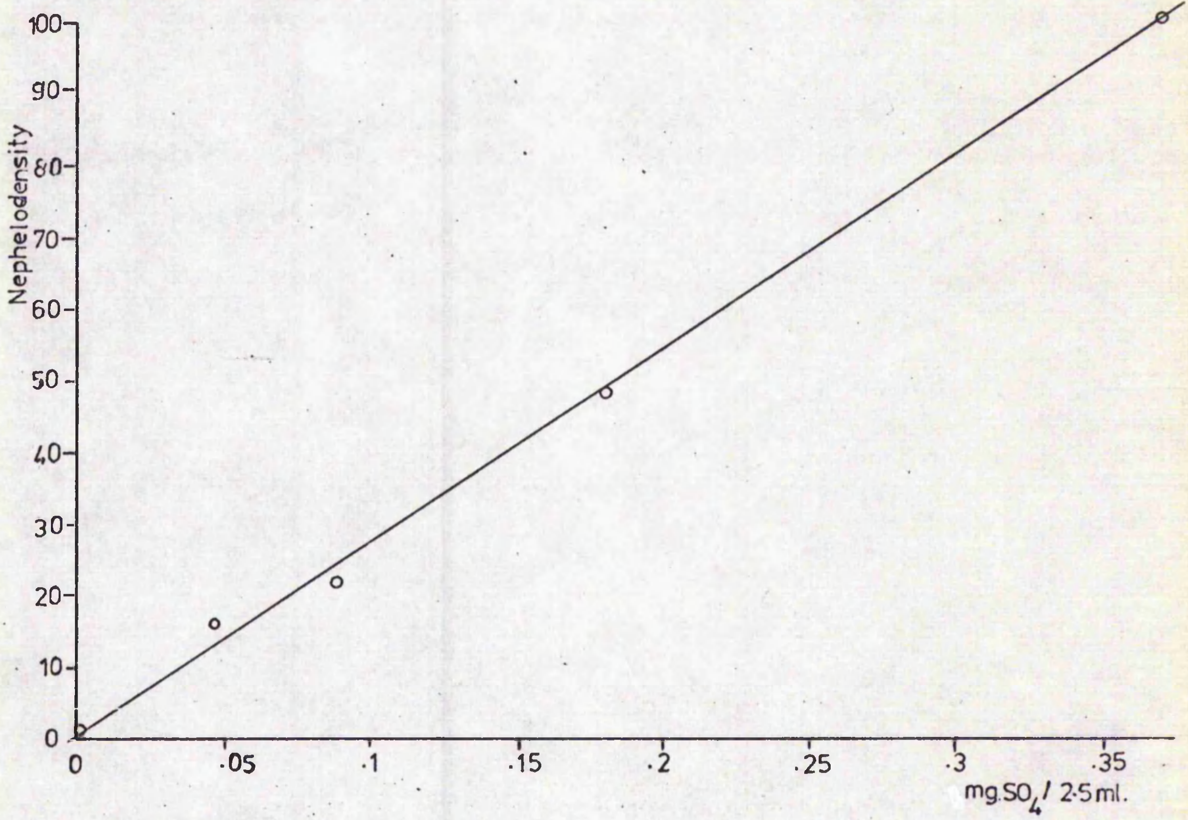


Fig. 47 Sulphate calibration curve.

Table 28 Sulphate contents of algal material and cottonwool.

Test material		Locality	$SO_4^=$ level ($\mu g SO_4^=$/ 100mg d.wt.)	
			Initial	After 4months exposure
Zygogonium ericetorium mat.	1.	Drakelands Corner	37	
	2.	do.	29	
	3.	do.		37
	4.	do.		31
Absorbent cotton wool.	1.	do.	12	
	2.	do.	5	
	3.	do.		34
	4.	do.		26
	5.	Hallam Moors		68
	6.	do.		54

[Initial readings represent background levels]

Table 29 Results of air pollution field trials
Sites: a Cattledown; c. Chelson Meadows; d. Plympton by-pass; e. Stert Runs.

Site	Tray No.	Soil analyses (Top 1cm)				Change inalgal cove		
		pH	Fieldwater content %	Organic matter %	Sulphate* O.D.	Predominantsp. Z. ericetorum	H. flaccidum	**% living cell Z. ericetorum filaments**
Initial		5.1		1.8		✓		80%
a	1.	6.5	7.2	2.2	0.05		✓	38%
	2.	5.1	1.1	2.2	0.02		✓	6%
	3.	5.7	1.7	2.2	0.02	✓		20%
c	1.	6.3	3.0	2.6	0.02	✓	✓	90%
	2.	5.8	2.2	1.4	0.04	✓		77%
	3.	6.4	4.2	2.5	0.02	-	✓	-
d.	1.	-	-	-	-	-	-	-
	2.	-	-	-	-	-	-	-
	3.	5.7	2.0	2.6	0.04	✓		0%
e	1.	5.0	8.7	2.7		-	-	-
	2.	5.2	10.6	3.0	0.00	✓		73%
	3.	4.9	6.8	1.0	0.03	✓	-	85%

* Water soluble sulphate from 12g. silica sand

then taken back to the laboratory for sulphate analysis. The method used was modified from that of Toennies and Bakay (1953).

0.5g. (dry weight) of algal material was ground in a Griffiths' tube with 10ml of 80 per cent acetone. The liquid was decanted and centrifuged at 3,000r.p.m. for 15 minutes. The supernatant was washed in a separating funnel with 15ml of 60:80 petroleum ether to remove the chlorophyll, and the lower 2-3 ml. of acetone-water stored in a stoppered vial at room temperature with a small amount of activated charcoal. After allowing to stand for 4 hours, the charcoal was removed by centrifuging and the acetone-water made up to 2.5ml with deionised water, if necessary. 0.5ml of 3 per cent hydrogen peroxide was added to the decolourised acetone water extract and shaken well. Immediately, the following were added: 2.5ml 40 per cent ethanol glycol, 0.2ml. 6N. hydrochloric acid, and 5ml of barium chloride reagent.(1.34M barium chloride in deionised water). After mixing well, each tube was allowed to stand for 15 minutes before its turbidity was measured using an EEL nephelometer head coupled to an EEL unigalvo, type 200. Good mixing was essential in order to obtain a steady reading. Zero and full-scale deflection were set on the galvanometer by using a sulphate-free blank (2.5 ml deionised water in place of the acetone-water extract) and a sulphate standard respectively. In order to draw a calibration graph (Fig. 47), sulphate standards were made up from analar ammonium sulphate in deionised water and used in place of the extract. Full-scale deflection was set using a standard of 0.5 mg. Ammonium sulphate in 2.5ml deionised water (i.e. 370 µg SO_4^{2-}). Results (Table 28) were expressed as µg SO_4^{2-}/100mg. d. wt.

A laboratory experiment was carried out to demonstrate sulphur uptake by dead and living <u>Z. ericetorum</u> mats. Algal material was placed at the bottom of a number of Dreschel bottles and subjected daily to 15 minute fumigations from a gas cylinder of 5vpm sulphur dioxide over a period of two weeks. The bottles were kept under constant light conditions. At the end of the period, sulphate estimations were carried out on the algal mats. None of the living material appeared to have been affected by the fumigations, and no differences in sulphate levels could be shown between control and test material or between living and dead mats. It was suspected that the gas cylinder was delivering levels of sulphur dioxide considerably lower than 5vpm.

Results

Fig. 45 illustrates the area to the east of Plymouth and shows the principal sources of atmospheric pollution namely the urban areas, which contribute particularly during the winter months, and the powerstation which is a point source throughout the year. The windrose shows that the winds are predominantly from the west and southwest although with a significant minority coming from the east. The Hawksworth and Rose (1970) pollution scale indicated, that the areas experiencing the highest concentrations of sulphur dioxide pollution corresponded to the urban areas although the epiphytic lichen floras suggested zones of atmospheric pollution to the east and northeast of Plymouth. Concentrations of sulphur dioxide within Plymouth city boundaries (Fig. 44) are low compared with other urban areas. The most polluted station is Plymouth 11 situated in the city centre and surrounded by commercial buildings. The other two stations are situated in residential areas with Plymouth 12 at an open suburban site receiving very little atmospheric pollution.

The sulphur dioxide readings taken at the sites of the field trials (Fig. 46) were all low with the Plymouth by-pass site (d) yielding the highest concentrations. The ridge of hills to the south of Plymouth (Fig. 45) is the first high ground northeast of the powerstation and it may be that much of the sulphur dioxide is deposited here. Geiger (1961) has discussed the shape of smoke plumes emanating from stacks and shows that the best dispersal is obtained under unstable conditions which normally prevail on bright summer afternoons. Fig 46 also records sulphur dioxide readings taken at Plymouth Polytechnic using a Warren Springs sampler on the same days as the field measurements were taken. The Polytechnic lies to the north-west of the powerstation and it is evident that the highest concentrations were recorded on days with an easterly or southeasterly wind. However, these days occurred during the winter months when levels would be expected to be higher.

Results of the field trials are given in table 29. At the Cattedown site (a) the Z. ericetorum had become largely overgrown by a luxuriant growth of a Hormidium sp. with thickened cellwalls.

The former alga was still growing well in two of the three trays at the Chelson Meadows site (c) but here the trays were sheltered during the summer months by the rapid growth of grasses around them. At the slightly more polluted Plympton by-pass site (d), the Z. ericetorum was dead in one of the trays but the other two trays had been destroyed by the activity of rabbits. At the sheltered and little polluted Stert Runs site (e), Z. ericetorum was growing luxuriantly in two of the trays whilst the remaining tray had become overgrown by mosses. These results tentatively suggest a possible correlation between growth of Z. ericetorum and levels of sulphur dioxide pollution but no such trend was detected in pH and sulphate values of the silica sand and it is not possible to interpret the results, involving a small number of samples, in terms of air pollutant effects.

The use of sulphate measurements (Fig 47 and Table 28) proved to be of limited value in assessing sulphur dioxide pollution levels using Z. ericetorum. Three samples of Z. ericetorum mats from different sites on sandtips at Drakelands Corner gave value of 13, 59 and 62 µg sulphate/100mg d. wt. The material used in the field experiments had a sulphate level of approximately 35 and this had not altered significantly after 4 months exposure in the field at Drakelands Corner. However, absorbent cotton wool placed at the same site had increased in sulphate level approximately three fold so that its final sulphate level was comparable with that of the Z. ericetorum material. Absorbent cotton wool exposed for the same period of time at the more polluted Hallam Moors in Derbyshire was found to have increased in sulphate level by approximately six times that of the initial material. It is likely that much of this sulphate was originally deposited as sulphur dioxide. Absorbent cotton wool may be a better indicator of sulphate deposition at ground level than is Z. ericetorum which appears to vary rather widely in sulphate in content in the field.

Zygogonium ericetorum was found growing in mat form in many places on the Derbyshire moors, which are more polluted than anywhere on Dartmoor. Hawksworth (1974) estimates that the winter levels of sulphur dioxide in the Peak district are within the range 0.002 to 0.03ppm. Z. ericetorum was found growing in one site corresponding to zone 2-3 (0.04-0.05 ppm sulphur dioxide winter levels) of the Hawksworth and Rose (1970) pollution scale (Cown Edge Rocks, Grid Ref. SK019920). This site was a millstone grit outcrop just east of the Greater Manchester conurbation. The alga was only growing in mat form along a sunken path in fairly sheltered situations although this may have been because of the limitation of suitable habitat. Z. ericetorum was also found growing on bare ground at Wollaton Park (Table 21) in a suburban area of Nottingham. These observations suggest that the alga is tolerant of moderate levels of air pollution.

15: Laboratory Experiments

Methods

In these experiments, the algal material was maintained at 23°C±1°C and illuminated at 4,300 to 5,300 lux by white fluorescent strip lights over a twelve hour photoperiod. All fumigations involving gaseous sulphur dioxide and short-duration immersions in sulphurous acid were carried out at the same time of day, namely as close to midday as possible. Initial experiments were carried out using gaseous sulphur dioxide but these proved unsatisfactory as it was not possible to determine the actual levels of the gas to which the plant material was subjected. Subsequent experiments involved the use of stock solutions of sulphur dioxide diluted to the desired concentration.

A. Experiments using gaseous sulphur dioxide

The following techniques were used:

(a) Sulphur dioxide was chemically generated by reacting dilute sulphuric acid with sodium metabisulphite within an air-tight container according to the equation

$$Na_2S_2O_5 + H_2SO_4 \rightleftharpoons Na_2SO_4 + 2SO_2 + H_2O.$$

Amounts of the reactants were adjusted so as to give an estimated concentration of 10vpm sulphur dioxide within each sealed 13 litre perspex tank containing the test material. The relative humidity within each tank was maintained by exposure to saturated solutions of appropriate salts. Potassium acetate, potassium nitrite and sodium carbonate were used to give relative humidities of 20, 45 and 92% respectively after the method used by Rao and Le Blanc (1965). After 24 hours exposure, the test material was removed and examined microscopically.

Treatments of 10vpm caused strong plasmolysis in at least 50 per cent of the cells of Zygogonium ericetorum and Hormidium flaccidum. Results indicated a higher percentage of plasmolysed cells in those treatments at lower humidities but this difference was not statistically significant.

An obvious criticism of this method is that an unknown quantity of the sulphur dioxide generated would have been adsorbed onto the perspex tank walls and dissolved in the saturated solutions. Although 10vpm is an excessively high level of the gas, the actual concentrations to which the material was exposed is likely to have been considerably lower than this.

(b) Sulphur dioxide from British Oxygen Company compressed gas cylinders was used.

(i) Dilution of the gas within an enclosed container. A volume of 100vpm sulphur dioxide was collected and by means of a graduated hypodemic syringe, 2 ml. were injected into a sealed 400ml glass container through the lid. The container was left in thedark in a refrigerator for a few hours for the gas to equilibriate at a concentration of 5ppm sulphur dioxide. Samples of the gas were with - drawn and injected into disposable petri-dishes containing H. flaccidum cultures so as to give theoretical concentrations of 0.25, 0.5 and 1vpm sulphur dioxide. The petri-dish lids and injection points were sealed with silicone grease. Injections of the gas were carried out daily at midday over a two-week period after which the material was examined microscopically.

(ii) Dilution of the gas by bleeding into an air stream. Using a 1000vpm sulphur dioxide supply, the gas was passed at a known rate into a stream of scrubbed air from a second cylinder at a known flow rate. Flow rates were determined by passing the gases through flow-meters. The mixed air stream was then passed into 13 litre plastic tanks coated with acid-resistant lacquer (Canning hot lacquer, medium gold) containing the test material. Fumigations of 1 and 5vpm sulphur dioxide were given for one hour each day over a two-week period.

(iii) Direct use of sulphur dioxide from cylinders containing low concentrations of the gas. Cylinders of 0.5, 1 and 5vpm sulphur dioxide were used and the gas was passed through sealed perspex tanks lined with acid-resistant lacquer and containing the experimental material. The fumigation periods lasted for 15 minutes.

Each of the above techniques had a number of disadvantages that help to explain why, in the experiments carried out no significant differences were recorded between test and control material in terms of percentages of dead cells, growth rates and chlorophyll breakdown. The principal drawback was that much of thegas had been taken up between leaving the gas cylinder and reaching the test material so that the algae were subject to very low levels of sulphur dioxide. According to BOC (personal communication), due to uptake of the gas by the cylinder walls, gas cylinders filled with concentrations of sulphur dioxide below 100vpm generally release levels of the gas which are below the stated value. These levels also vary from day to day. In cylinders containing less than 10vpm sulphur dioxide, almost all the gas disappears.

B. Experiments using sulphur dioxide in solution

As mentioned previously, the use of sulphur dioxide in the gaseous phase presents difficulties as it adsorbs readily onto surfaces. However, it is likely that the gas dissolves in water-films on the plant surface before it is able to affect the plant, and for this reason studies using solutions of sulphur dioxide may give more realistic responses in laboratory experiments. Aqueous solutions have the advantage of being easy to prepare and enable the interacting effects of pH and concentration of the toxicant to be studied. A drawback is that it is not clear to what concentration levels of sulphur dioxide may build up in waterfilms on plant surfaces although it is generally accepted that these levels can be considerably higher than those in the surrounding air.

The stock solution used in the experiments was concentrated sulphurous acid (approximately 7 per cent sulphur dioxide). This solution was assayed by the iodine-thiosulphate standardisation procedure according to Vogel (1961) in which sulphur dioxide as sulphite is oxidised by iodine to sulphuric acid and the excess iodine is back titrated with sodium thiosulphate solution. All assays gave results of the order of 5 per cent sulphur dioxide. Appropriate dilutions of the stock solution were made in either distilled water or McIlvaines buffer. Test material was treated in one of two ways:-

(a) Generally, filaments were submerged in 5ml of the appropriate solution in a covered watchglass and after an appropriate length of time according to the experiment they were scored for dead and living cells (see appendix). Each treatment was set up in triplicate and controls of the alga in the bathing solution only (distilled water or buffer) were also set up.

(b) In some experiments Hormidium flaccidum growing on Knop's agar was flooded with appropriate solutions for 15 minutes periods and then the surplus fluid was drained off. Again, these experiments were carried out in triplicate together with the controls.

Effects of sulphur dioxide which were examined were changes in growth pattern, change in ultrastructure, membrane damage, changes in pigment complement, and percentage kill of cells.

Changes in growth pattern were recorded by camera lucida. It was possible to follow the course of development of filaments growing on solid media in petri-dishes, these cultures being flooded daily with sulphur dioxide solutions.

Methods and results of electron microscopy are described under subsection (C), page 172.

An MC-1, Mark V conductivity bridge was used to determine whether sulphur dioxide in solution produced any damage to cell membranes such that leakage of the cell contents occurred. Conductivity readings were taken at short intervals over a twenty-four hour period in order to detect any changes in conductivity over this period.

Chlorophyll extractions were carried out on treated and control material. Up to 1g of weighed algal material was ground in a mortar with 10ml 80 per cent acetone together with small quantities of quartz sand and 150 mg. of sodium carbonate. The homogenate was centrifuged for 15 minutes at 10,000r.p.m. and the supernatant extracted in a separatory funnel with 10 ml 40: 60 petroleum ether. The upper petroleum ether layer containing the chlorophyll was washed twice in distilled water and concentrated under reduced pressure. The absorption spectrum of the extract was determined using a Pye Unicam spectrophotometer, SP1800.

Separation of the pigments was carried out by thin layer chromatography on plates coated with a 1mm thick layer of silica gel G developed in 60 parts 60: 80 petroleum ether, 35 parts acetone, 5 parts methanol. The separate pigments were eluted with 90 per cent acetone or with ether for spectrophotometeric characterisation but, in the experimental material, the amounts of the individual pigments were too small to be detected on the spectrophotometer. Similarly, they could not be satisfactorily detected using a Joyce Loe bl chromoscan. Pigments were eluted for characterisation after a concentrated extract of Z. ericetorum had been applied to the plates but this involved using several grams of alga and this quantity of test material was not available.

The methods used for scoring cells and assessing dead cells are given in the appendix.

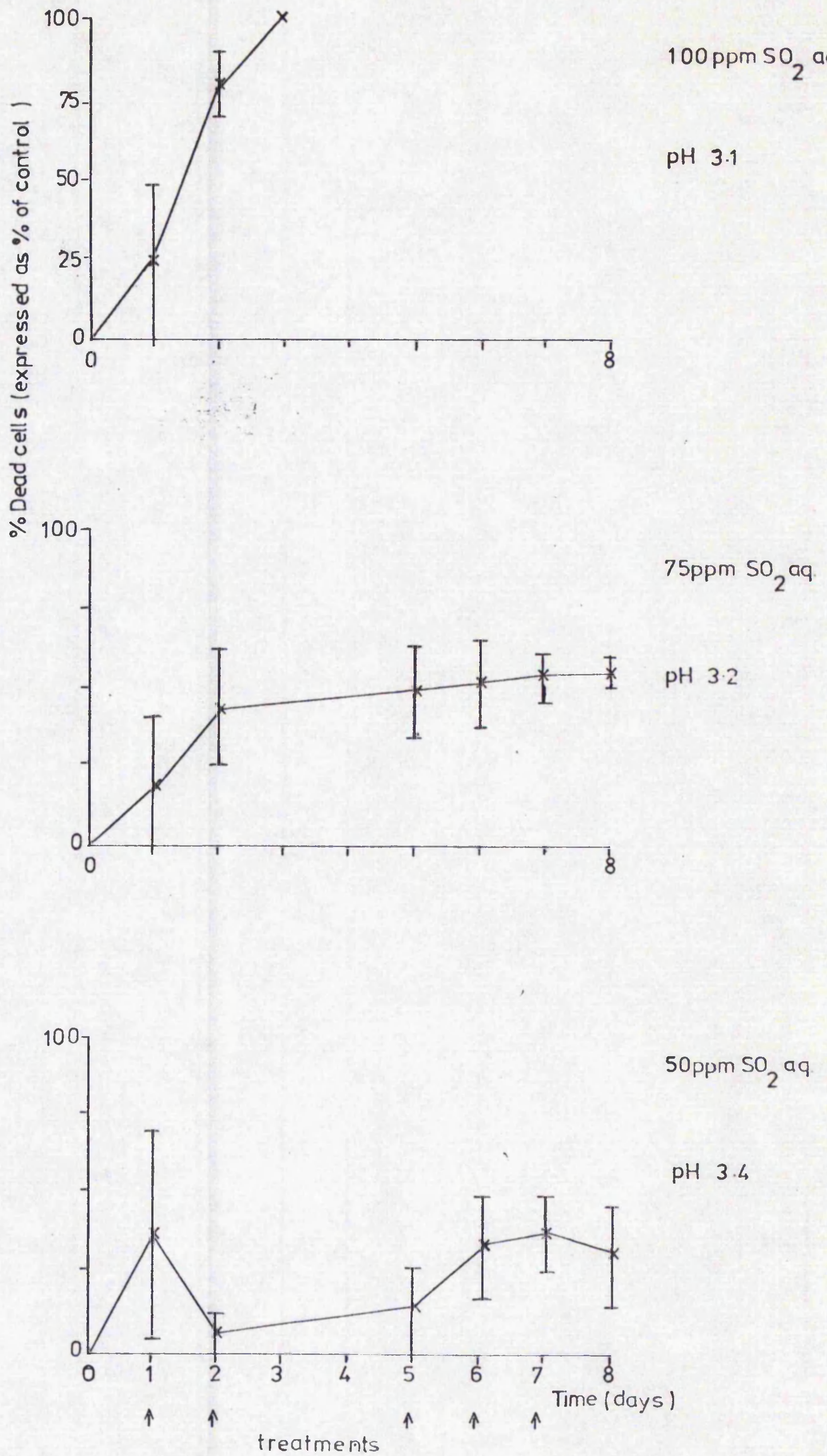


Fig. 48 Percentages of H. flaccidum cells killed by daily 15 minute treatments of sulphur dioxide solutions.
Means and standard deviations plotted.

Results

The results reported below, refer to those experiments in which the algae were exposed to sulphur dioxide in aqueous solution.

The higher concentrations of sulphur dioxide solution were found to kill all cells of Hormidium flaccidum and Zygogonium ericetorum. A single 15 minute application of 200ppm sulphur dioxide was sufficient to kill H. flaccidum growing on Knop's agar. Fig 48 shows the percentage of H. flaccidum cells killed by daily 15 minute doses of 50, 75 and 100ppm sulphur dioxide solutions. The pH of these solutions ranged from 3.4 (50ppm sulphur dioxide) to 3.0 (200ppm sulphur dioxide). Daily treatments of 100ppm sulphur dioxide solution killed all cells within three days but 75ppm had only killed approximately 50 per cent of the cells in the material examined after eight days.

Hormidium flaccidum was less tolerant when grown on an orbital shaker in flasks containing 25ml 0.5 per cent Knop's broth to which sulphur dioxide solution had been added. When cultures growing in Knop's broth were transferred to media containing 50ppm sulphur dioxide solution, the algae were dead within two days. Similar results were obtained when grown in media containing 25ppm sulphur dioxide solution. H. flaccidum cultures in Knop's broth containing 5 and 10ppm sulphur dioxide were dead within four days. Levels of 0.5 and 1ppm did not appear to be fatal to the alga and H. flaccidum was still growing after six days under these conditions. At six days, and at subsequent two-daily intervals, the levels of sulphur dioxide in these flasks were replenished by aseptically removing 2.5 ml of the culture medium and replacing with 2.5ml of sulphur dioxide solution at the appropriate dilution. This was to allow for any breakdown of the existing sulphur dioxide which may have occurred. Despite these additions, the algae continued to grow in these flasks containing 0.5 and 1ppm sulphur dioxide for 18 days after which the experiment was terminated.

As it was not possible to grow Z. ericetorum successfully under laboratory conditions, experiments using this alga were carried out by floating filaments in appropriate sulphur dioxide solutions in covered watchglasses. The solutions were made up in McIlvaine's buffer pH4 (see appendix). Levels of sulphur dioxide in solution from 10ppm to 50ppm killed all cells

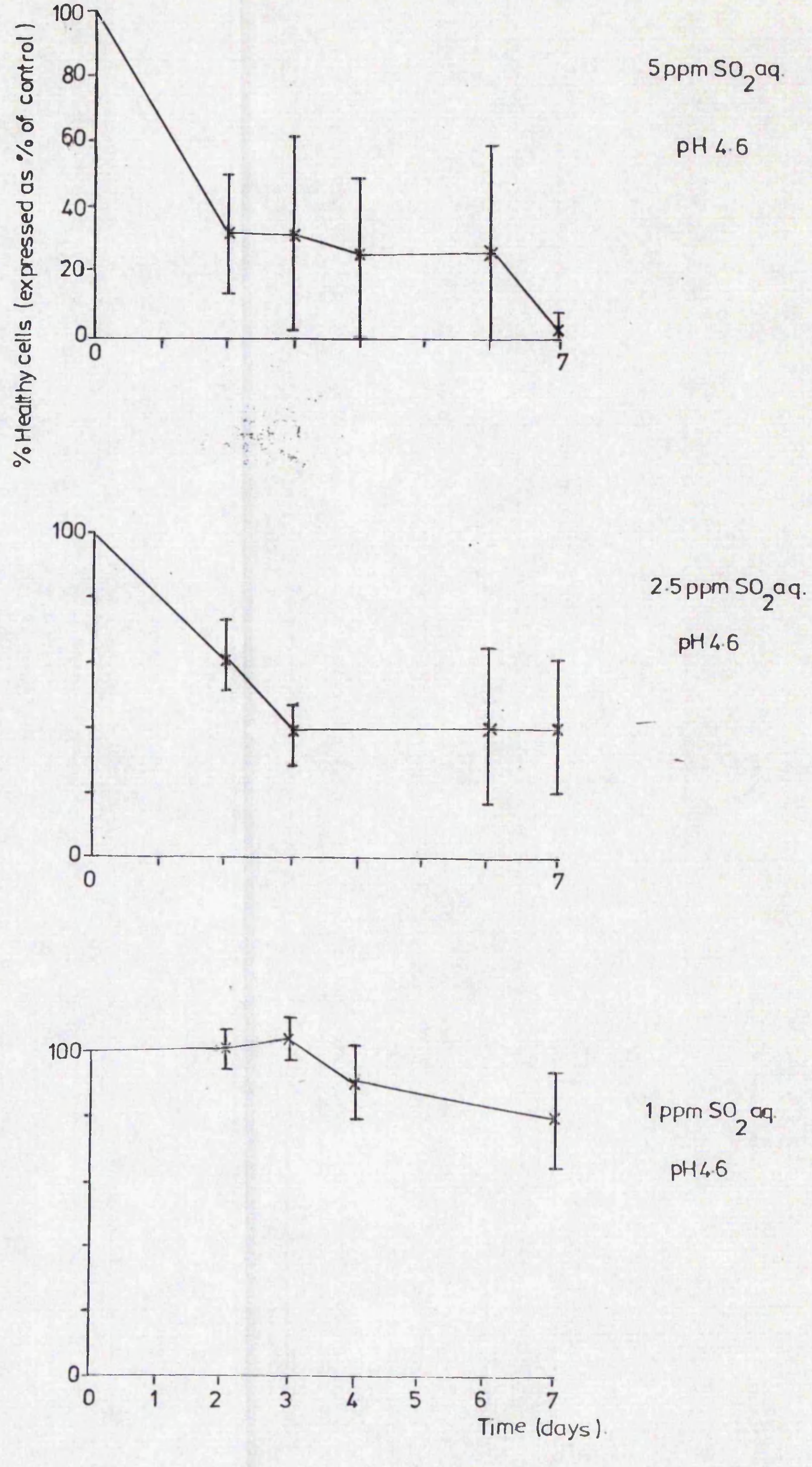


Fig. 49 Percentages of Z. ericetorum cells killed by continuous immersion in sulphur dioxide solutions.

Means and standard deviations plotted.

within two days but difficulties were experienced in accurately assessing percentages of dead cells in treatments of less than 10ppm. This problem is considered under "Scoring cells" in the appendix. The problem was partly overcome by assessing healthy (i.e. unstained and unplasmolysed) cells and the results of this experiment (Fig 49) have been expressed in these terms. Concentrations of 5ppm sulphur dioxide solution were found to be fatal to almost all cells within 7 days, but 2.5ppm killed only about 50 per cent of the cells, whilst filaments in 1ppm sulphur dioxide solution were largely not killed within 7 days with only about 20 per cent dead cells.

<u>Growth patterns</u> Alterations in morphology of <u>H. flaccidum</u> were noted when exposed to low concentrations of sulphur dioxide solution. A distinctive change in the growth pattern was recorded which was related to the level of sulphur dioxide and the pH of the solution. Because of the difficulties of growing <u>Z. ericetorum</u> in culture, it was not possible to carry out these experiments using this species.

The filaments of <u>H. flaccidum</u> normally grow in a sinuous curving fashion on solid media. Generally, crosswalls are produced perpendicular to the axis but in material affected by sulphur dioxide in solution, oblique crosswalls are produced and this gives the filaments a contorted appearance (Fig. 50).

Four-day old cultures of <u>H. flaccidum</u> on Knop's agar were daily flooded for 15 minutes over an 8 day period with sulphur dioxide solutions made up in McIlvaine's buffer and examined the following day. Table 30 shows the growth pattern of the cultures, all replicates at each treatment behaving similarly. In cultures showing the contorted growth pattern, all the newly-produced growth was of this type. In all the treatments listed in table 30, the buffer controls grew normally but at pH3 and 4 some of the filaments showed the contorted growth pattern. Fig 50 shows low power camera lucida drawings of a few typical filaments from the edge of cultures treated with 30ppm sulphur dioxide solution at different pH values. At pH3, the cultures had produced some new growth of straight filaments before dying. The combination of low pH and sulphur dioxide in solution was fatal to <u>H. flaccidum</u> in these experiments.

The contorted growth pattern of filaments could be induced to a limited extent by the application of McIlvaine's buffer at pH3

contd. p.166

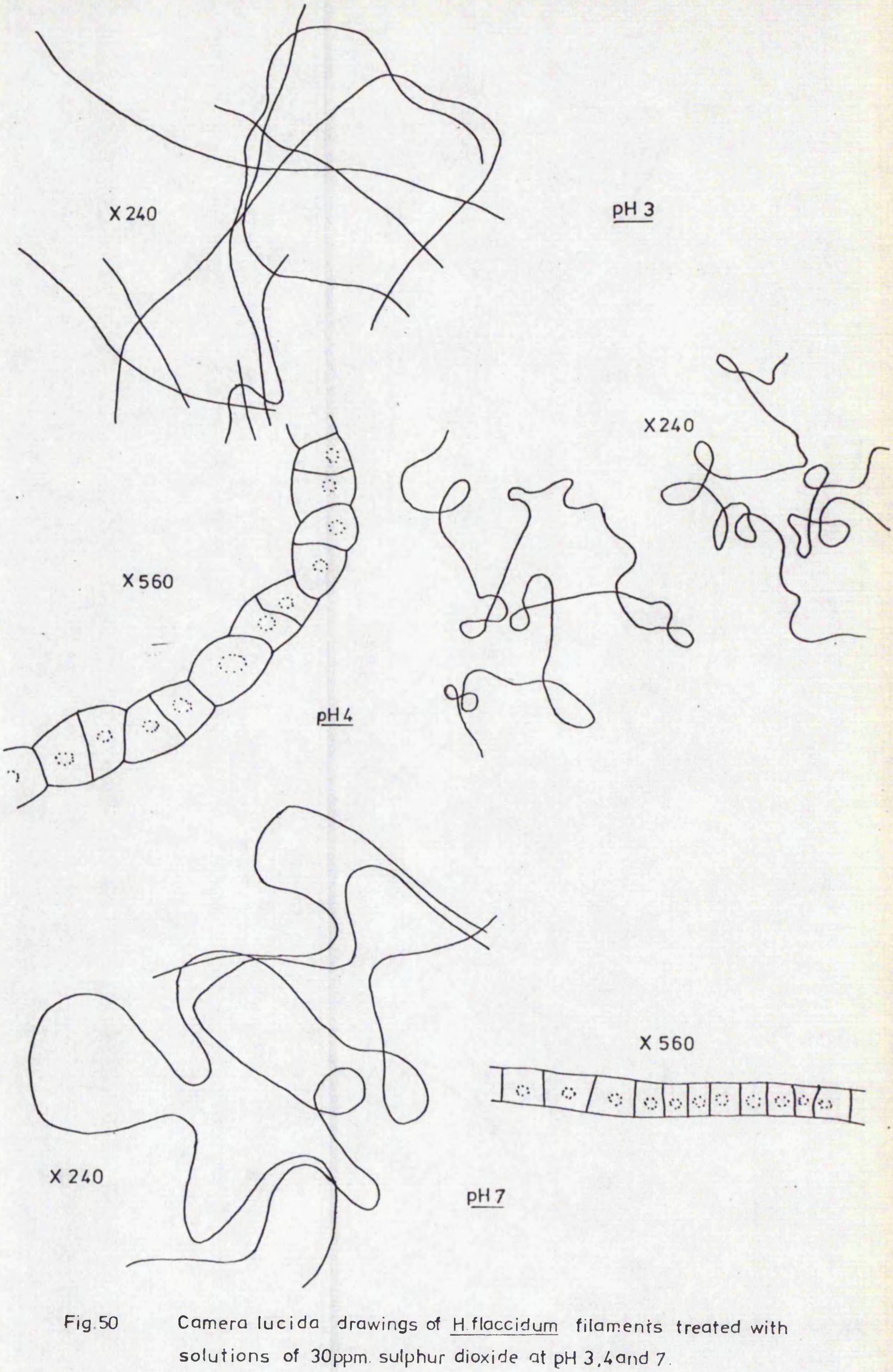


Fig. 50 Camera lucida drawings of H. flaccidum filaments treated with solutions of 30ppm. sulphur dioxide at pH 3, 4 and 7.

Table 30 Growth pattern of Hormidium flaccidum filaments after daily washings with buffered sulphur dioxide solutions over eight days.

(Results of 3 replicates and controls at each treatment.)

SO_2 conc (aq.)	pH 3	pH4	pH 5	pH 6	pH 7
50ppm	Dead	Con-torted poor growth	Con-torted	Con-torted	Normal
30ppm	Dead	Con-torted	Con-torted	Normal	Normal
20ppm	Dead	-	Con-torted	-	-
10ppm	Dead	Con-torted poor growth	Normal	Normal	Normal
1ppm	Normal	Con-torted	Normal	Normal	Normal

"Normal" growth refers to the typical sinuous growth pattern exhibited by the alga.

and 4; this effect was enhanced by low concentrations of sulphur dioxide in solution. A single application of sulphur dioxide solution for 15 minutes was found to induce thecontorted growth pattern. At concentrations up to 50ppm sulphur dioxide solution, the contorted growth pattern was produced over a wider pH range, from pH3 to pH6.

Fig 50 also shows high power camera lucida drawings of representative sections of normal and contorted filaments in which oblique crosswalls of the latter can be seen. These cells are considerably swollen. Cells of filaments from cultures treated over 8 days with 30ppm sulphur dioxide solution at several pH values were measured. At each pH value, 10 consecutive cells from each of 10 separate filaments were examined and the greatest and least width of each of these cells was recorded. Fig. 51 shows the results obtained from which it can be seen that these widths varied very little with treatments at pH3 and 7. The filaments at these treatments appeared similar to control material. At intermediate pH values the cells were measurably swollen and this was most marked in treatments at pH5 where cells were on average 4µ wider at the greatest width than at the least width.

When contorted filaments were transferred to fresh media, the subsequent growth was of the normal, sinuous type.

<u>Pigments</u> Chlorophyll extractions were carried out on each of the treatments of <u>H. flaccidum</u> with 1, 10, 30 and 50ppm sulphur dioxide solutions listed in table 30. The absorption spectra of the majority of the treatments and of all the controls was that of chlorophyll a in petroleum ether, with peaks at 431 and 662m µ. Extracts from the dead cultures treated with 10, 30 and 50ppm sulphur dioxide solutions at pH3 showed the absorption spectrum characteristic of phaeophytin a with peaks at 411 and 667m µ as also did the contorted but poorly-growing cultures treated with 10, 30 and 50ppm sulphur dioxide solutions at pH4.

Insufficient experimental material was available for chlorophyll extractions of <u>Z. ericetorum</u> but a concentrated extract of 1g. (wet weight) of field material of the alga was prepared and separated by thin layer chromatography. Fig. 52 shows the pigments which were separated together with their identity from their absorption spectra.

<u>Plasmolysis</u> Percentages of plasmolysed cells at the end of a twenty-four hour period were assessed when filaments of the two algae were

contd. p.169

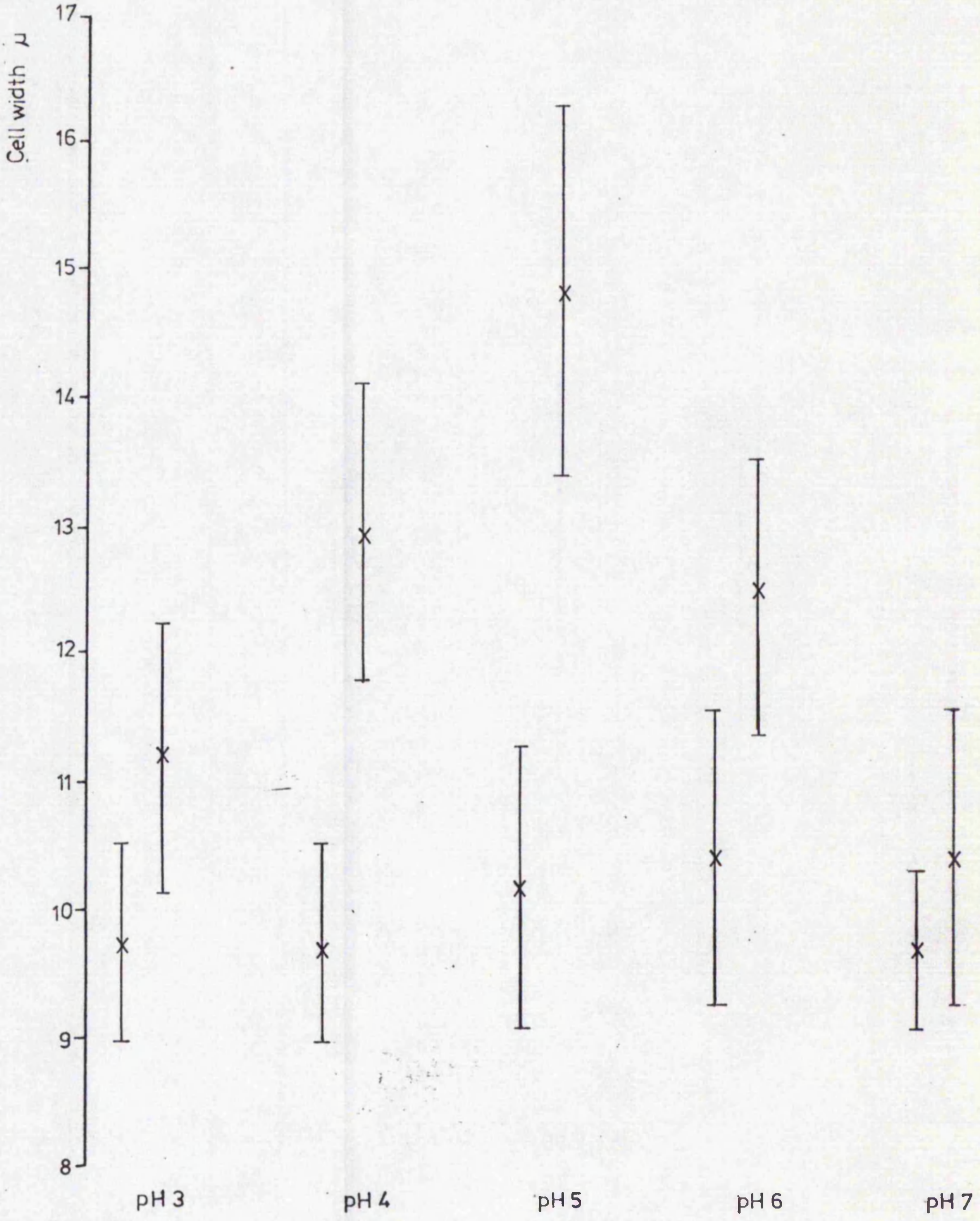
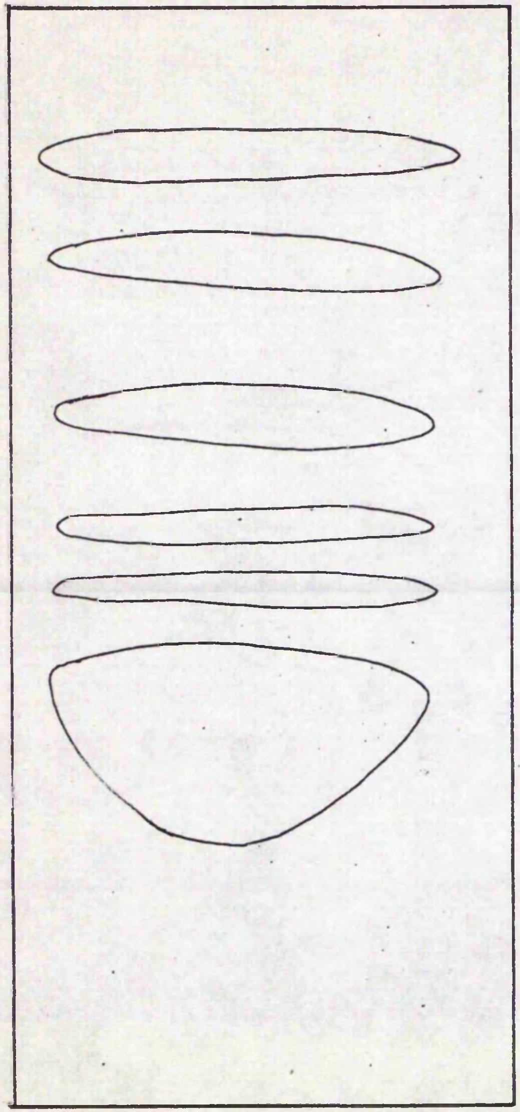


Fig. 51 Maximum and minimum cell width of H. flaccidum cells treated with solutions of 30 ppm. sulphur dioxide at pH 3,4,5,6 and 7.

Means and standard deviations plotted.



Pigment	Colour (white light)	Colour (U.V. light 254 nm	Colour (U.V. light 366 nm	R.F	Abs. peaks (in 90% acetone) nm
? Phaeophytin	Grey-green	Pink	Pink	0·86	681
Chlorophyll a	Blue-green	Crimson	Crimson	0·76	408, 685
Chlorophyll b	Apple-green	Pink	Pink	0·63	451, 680
Lutein	Deep yellow	Dark green	Dark green	0·53	460, 649
? β carotene	Yellow	-	-	0·46	410, 666
? Zeaxanthin/Violoxanthin	Pale yellow	-	-	0·30 - 0·43	420, 668

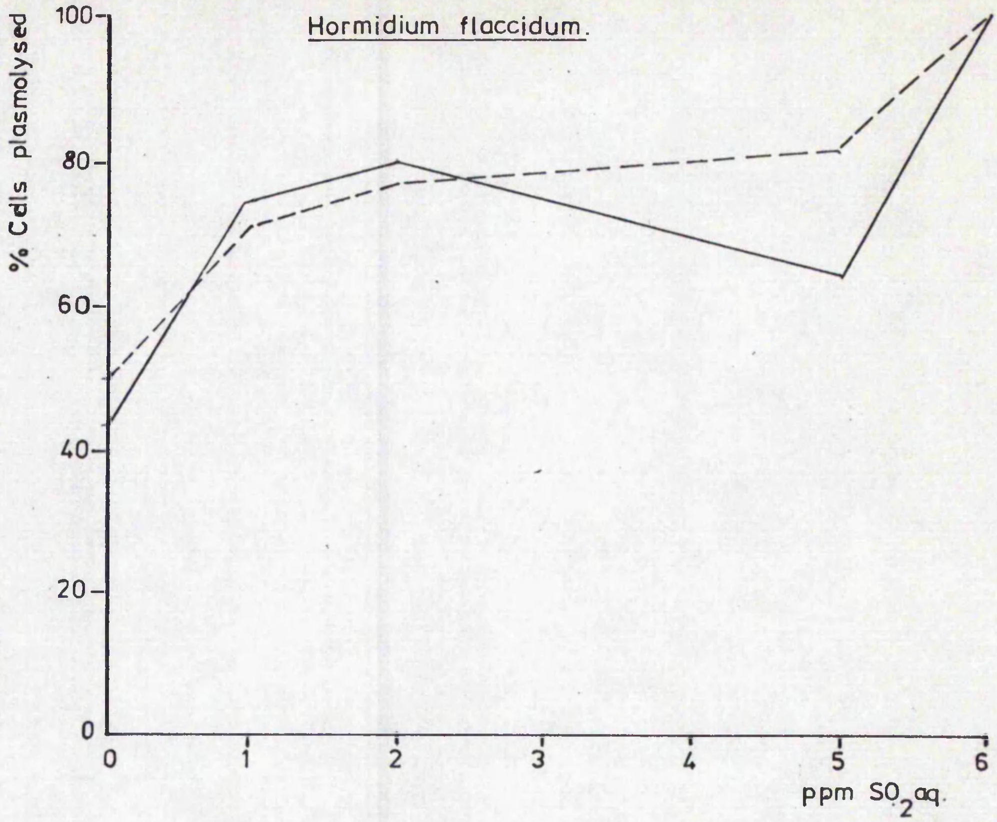
Fig. 52 Separation and characterisation of pigments of Z. ericetorum by T.L.C.

immersed in aqueous solutions of sulphur dioxide of 1, 2, 3, 4, 5 and 6ppm (Fig. 53). It was not possible to detect dying cells within the twenty-four hour period at these concentrations. The controls of deionised water were taken to represent 0ppm sulphur dioxide. A large percentage (around 50 per cent) of the cells of the H. flaccidum controls were plasmolysed but results suggest that cells of Z. ericetorum were more resistant to plasmolysis at levels up to 4ppm than were cells of H. flaccidum. Previous experiments showed that levels of 5ppm were eventually fatal to all cells of both species, whilst it is likely that they were able to tolerate levels of 1ppm sulphur dioxide solution.

Conductivity The conductivity of 10ml aliquots of sulphur dioxide solutions up to 50ppm in the presence of 0.5g (wet weight) of H. flaccidum was measured over a 24 hour period. Readings were taken at 15 minute intervals over the first 2 hours and then at subsequent convenient intervals, and the results expressed in terms of percentages of the initial readings (Fig. 54).

Treatments of 20, 30 and 50ppm sulphur dioxide solutions resulted in cells which were yellow-brown and appeared dead after 24 hours. The conductivity readings showed a similar trend in these treatments. There was an initial lowering in the conductivity over the first 4 hours in sulphur dioxide solutions both with and without the alga. At the end of the 24 hour period, the conductivity of the solutions containing the alga had risen to around the initial value whilst those of the sulphur dioxide controls had remained more or less constant. The treatments at 5ppm behaved similarly although the initial lowering of conductivity lasted for a shorter time. The treatments of alga in deionised water showed an initial increase in conductivity. The increase in conductivity recorded in the deionised water and 5ppm sulphur dioxide controls was believed to have been due to contamination.

As no rapid rise in conductivity was recorded in the solutions in the presence of H. flaccidum, these results suggest that even though the cells may have been killed by the treatments, there was no extensive leaching of the cell fluids into the solutions. In other words, if the cell membranes had been destroyed they were still able to restrict the free movement of solutes.



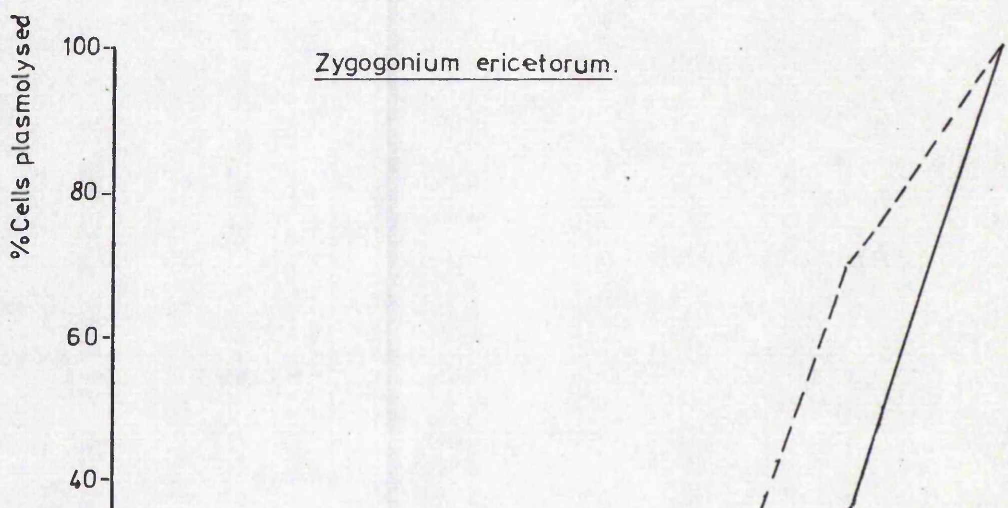


Fig.53 Percentages of cells of H. flaccidum and Z. ericetorum plasmolysed after 24 hrs. immersion in sulphur dioxide solutions.

2 replicates plotted.

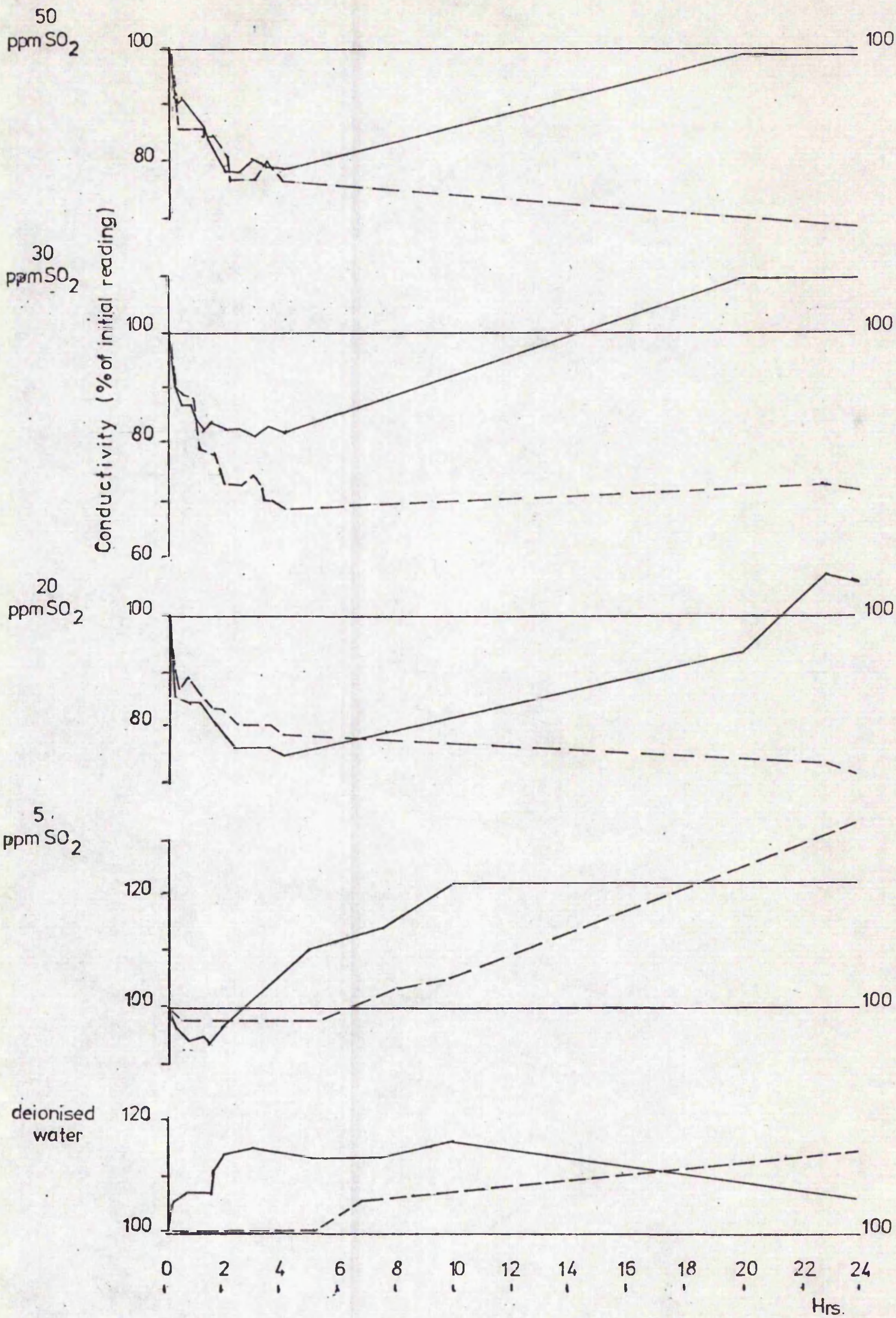


Fig.54 Conductivity of sulphur dioxide solutions + Hormidium flaccidum. Means of 3 replicates.

- - - SO_2 solution ——— SO_2 solution + H. flaccidum.

16: Electron Microscopy

Very little work has been published on the ultrastructural injury caused by air pollutants. Thomson _et al_ (1965, 1966) have looked at effects of peroxyacetyl nitrate and ozone on palisade cells of _Phaseolus vulgaris_ and have demonstrated an abundance of electron-dense granules within the chloroplast stroma and, in some cells, a disruption of the organisation of the grana within the choloroplast. Wei and Miller (1972) demonstrated somewhat similar effects together with other ultrastructural changes in _Glycine max_ mesophyll cells brought about by fumigations with 40 and 50ppb of hydrogen fluoride. Wellburn _et al_ (1972) found that short duration fumigations of up to 1ppm sulphur dioxide or 3ppm nitrogen dioxide on 25 day old _Vicia faba_ plants in the laboratory caused swelling of the thylakoids within the chloroplasts although no damage outside the chloroplasts was detected. The most recent report of effects of air pollutants on plant ultrastructure is by Malhotra (1976) who examined effects of up to 500ppm aqueous sulphur dioxide on leaves of _Pinus contorta_ var. _latifolia_. He showed swelling of the thylakoid discs and disintegration of other intrachloroplast membranes resulting in the formation of small vesicles in older tissues. There have been no reports of effects of air pollutants on the ultrastructure of nonflowering plants.

There appear to be no reports of the ultrastructure of _Hormidium flaccidum_ although Floyd _et al_ (1972), amongst others, have described _Ulothrix fimbriata_ and _Stigeoclonium helveticum_, two closely related algae in the same family. Similarly, there is no published account of the ultrastructure of _Zygogonium ericetorum_ although there are a number of reports of the closely related _Zygnema_ spp. Chardard (1967) produced the first account of the ultrastructure of a species of _Zygnema_. Oparina (1971) examined the chloroplast structure of a _Zygnema_ sp. and McLean and Pessoney (1971) looked at the akinetes produced by a _Zygnema_ sp, using light and electron microscopy. The latter authors have also reported (1970) the existence of a "quasi-crystalline lamellar lattice" in the chloroplasts of _Zygnema_ sp. in culture at the end of its log phase. This lattice consisted of a multilayered sandwich of membranes and matrix bounded by a single thylakoid membrane.

Methods

Control material and material treated with sulphur dioxide solutions were used. Control material consisted of Hormidium flaccidum growing on Knop's agar and thin-walled Zygogonium ericetorum collected from damp micaceous areas at Drakelands Corner.

Fixation of material was carried out using glutaraldehyde with osmium tetroxide as a postfixative, glutaraldehyde with potassium permanganate as a postfixative and Lufts' permanganate fixative. The two most satisfactory methods used were as follows:

1. The filaments were fixed for 1½ hours in Lufts' permanganate fixative (see appendix) at 0.5°C in a refrigerator. After fixation, the material was washed three times with the buffer (30 minutes each time) and then dehydrated through a graded series of acetone in buffer (20, 50, 70, 95, 100) with two changes in absolute acetone. The material was left in each for 30 minutes and then the cells were gradually infiltrated with Epon-Araldite. The material was placed in an oven at 48°C for 8 hours and then at 60°C for 12 hours to promote polymerisation. This method was suitable for Hormidium flaccidum but poor impregnation of Z. ericetorum cellwalls was obtained and on sectioning, only collapsed cells could be seen.

2. This method proved more satisfactory for Z. ericetorum. The filaments were fixed under vacuum for 2 hours in 6% glutaraldehyde in 0.05M sodium cacodylate buffer (see appendix) at pH5. After fixation, the material was washed twice in buffer (15 minutes each time) and then postfixed under vacuum for 1 hour in 2 per cent potassium permanganate in buffer. The material was then washed in buffer and dehydrated and embedded in resin as described above.

Sections were cut on either a Porter-Blum MT-2 ultramicrotome or a Reichert OMU2 ultramicrotome and thin sections were picked up with uncoated or carbon-coated 200-mesh grids. The sections were stained on the grids at room temperature with either Reynold's lead citrate (see appendix) for 10 minutes or with uranyl acetate solution for 1 minute followed by lead citrate solution for 1½ minutes (see appendix). The sections were examined under a Phillips 300 electron microscope at 80Kv or an AEI 802A electron microscope.

Fig.55 Median L.S. H.flaccidum cell. Electron micrograph.
c Chloroplast ; e Electron-dense bodies ; n Nucleus ; w Cellwall.

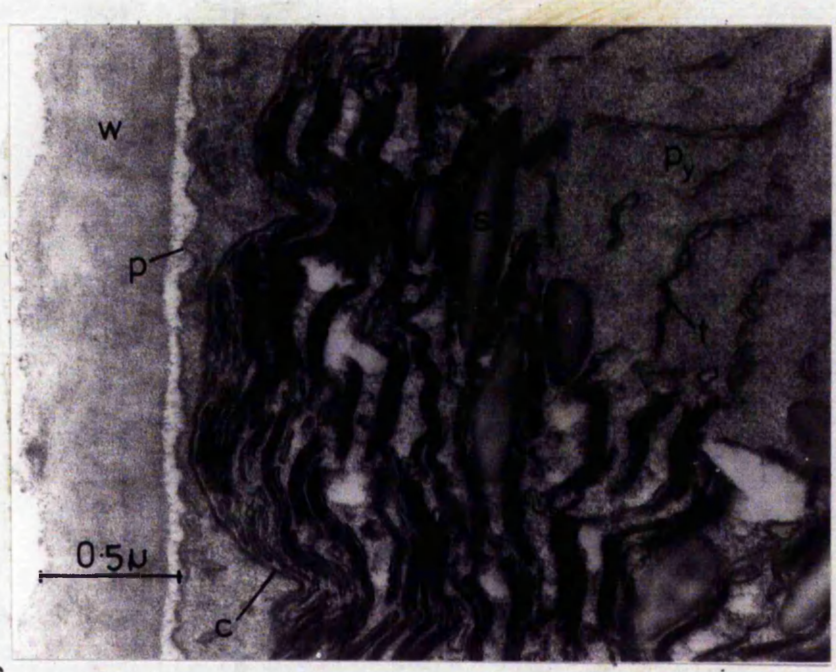


Fig.56 Section of a portion of H.flaccidum cell. Electron micrograph.
c Chloroplast ; p Plasmalemma ; py Pyrenoid ; s Starchplate ; t Thylakoid ;
w Cellwall.

Results

(i) Hormidium flaccidum

Cells averaged 8 to 14 µ in length and 5 to 6 µ in width. Fig. 55 shows a typical median longitudinal section through a cell of H. flaccidum. The cellwalls were comparitively thin (up to 0.5 µ). The nucleus occupied the centre of the cell. The cytoplasm contained many mitochondria and round or oval electron-dense bodies of the order of 0.3 to 0.6 µ in diameter. The bodies were situated in the cytoplasm and in the vacuoles and were particularly large and abundant in senescent cells. They were too deeply stained to be lipid bodies and appeared to correspond to structures which Chardard (1967) noted in Zygnema sp. and believed to be tannin globules. The chloroplast was parietal and band-shaped and was bounded by a double membrane. The thylakoids were distributed throughout the chloroplast but in many instances were arranged in short stacks or grana as in higher plants. These are generally referred to as pseudo-grana as the stacks generally consist of fewer thlakoids than grana of higher plants and have a less regular appearance due to the variable length of the thylakoids. The arrangement of the pseudo-grana was irregular and the number of thylakoids adhering together in one place normally varied from 2 to 8. Starch plates were frequently interpolated between the lamellae and particularly occurred in a sheath around the pyrenoids (Fig. 56). Each cell contained a single pyrenoid traversed by thylakoids. The chloroplast lamellae splayed out into single thylakoids at the end of the pyrenoid matrix (Fig. 56) and ran through it, often with a sinuous course. Occasional stacking of thylakoids occurred within the pyrenoid (Fig. 57). The pyrenoids in H. flaccidum were of the type described by Dodge (1973) as a single central pyrenoid with a starch-containing plastid. Fig 56 shows part of the outer edge of a cell. From left to right, the cellwall, the plasmalemma, the chloroplast limiting membrane, thylakoids stacked into pseudo-grana, the starch sheath and pyrenoid traversed by single thylakoids are apparent.

Filaments of H. flaccidum growing on Knop's agar in petri-dishes were given daily treatments of 20, 30 and 50ppm sulphur dioxide solutions in McIlvaine's buffer pH4 over a period of 16 days and then prepared for electron microscopy. All the newly-produced filaments from these treatments showed the contorted growth already described. The controls, treated with buffer solution only,

contd. p. 178

Fig.57 Median L.S. swollen cell of H. flaccidum treated with 50ppm SO_2 solution at pH 4 for 16 days. Electron micrograph.
p Pyrenoid ; v Vesicles

Fig. 58 As above showing abundant vesicles (v) in the chloroplast.

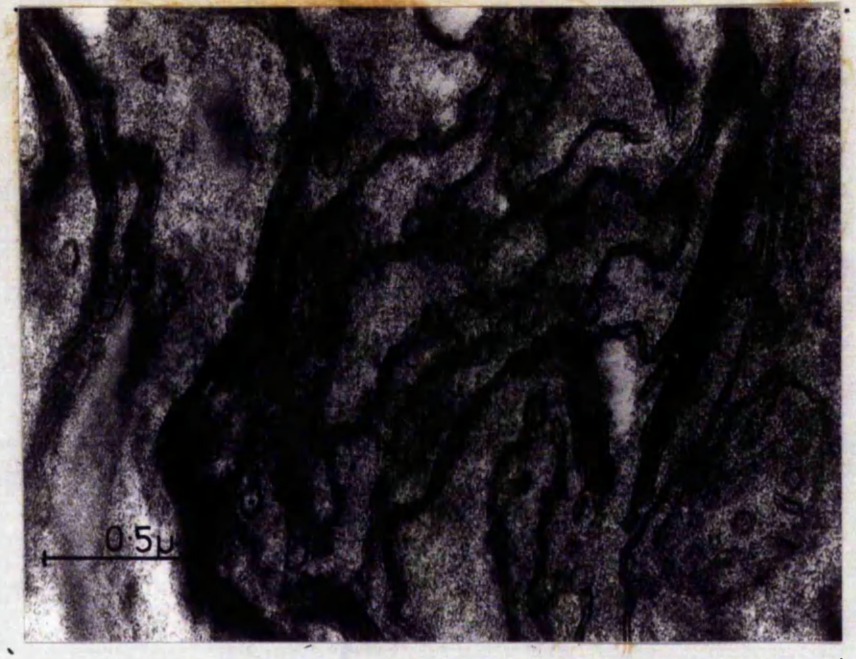


Fig. 59 Portion of chloroplast of H. flaccidum cell treated with SO_2 solution, pH 4, for 2days showing stroma and grana thylakoids and vesicles.

Fig. 60 As above.

exhibited normal growth.

Figs 57 and 58 show typical median longitudinal sections of two swollen cells. Excepting cell size, most of the features were similar in the treated cells and the controls. Mitochondria were abundant and of a similar size, the electron-dense bodies were present and so too were the nucleus and pyrenoid. The somewhat diffuse pyrenoid seen in fig 57 was not a constant feature of the cells. However, a difference was noticeable within the chloroplast, namely the apparent breakdown of the stroma lamellae resulting in the pseudo-grana appearing more distinct. This was a constant feature in almost all the cells examined and in all the treatments from 20 ppm to 50 ppm sulphur dioxide solution. It was not seen in the control cells treated with buffer only, or in untreated cells. The appearance was not the same as that of the senescing cells in both treated and control material. Chloroplasts of senescing cells showed an almost total disorganisation of lamellae with most of the thylakoids forming tubular structures and was accompanied by a considerable increase in the electron-dense bodies in the cytoplasm and vacuoles.

Figs 59 and 60 show some thylakoids from sulphur dioxide treated material, under higher magnification. The presence of small vesicles of the order of 0.06 μ in diameter andbounded by a single unit membrane may be seen. These vesicles appear to be budded-off from the stroma thylakoids whilst the grana thylakoids remain unaltered. Malhotra (1976) demonstrated the formation of numerous vesicular bodies in the chloroplasts of leaves of <u>Pinus contorta</u> var. <u>latifolia</u> when treated with 100ppm aqueous sulphur dioxide, although these vesicular bodies were considerably larger than the vesicles seen in <u>H. flaccidum</u> material. Both Malhotra (1976) and Wellburn <u>et al</u> (1972) report the swelling of thylakoids as a response to sulphur dioxide treatment. The diameter of the majority of the thylakoids in sulphur dioxide treated <u>H. flaccidum</u> was little changed from the control but the vesicles present may constitute the swollen portion of the thylakoids.

(ii) <u>Zygogonium ericetorum</u>

Cells were very variable in size ranging from 15 μ to 33 μ in length and about 22 μ in width. The cells were thickwalled (up to at least 3 μ in thickness) and the cytoplasm contained large numbers of oil droplets. These features led to difficulties in finding suitable methods of impregnation and fixation. In the sections obtained, much of the cell lumen was occupied by lipid bodies as has been demonstrated in electron micrographs of the akinetes of Zygnema sp. by McLean and Pessoney (1971) and akinetes of Spongiochloris typica by McLean (1968). Fig. 61 shows part of a longitudinal section through a cell of Z. ericetorum. The cellwalls were about 1.8 μ in thickness (see Fig. 26) although material examined was "thin-walled" and growing on a damp micaceous area. There was a single, large dumbell-shaped chloroplast with many thylakoids in pseudo-grana of up to ten thylakoids. The grana lamellae tended to be comparitively short (approximately 0.8 μ) whilst the stroma lamellae were generally longer (approximately 1.2 μ) Starch grains were interpolated amongst the lamellae and around the two pyrenoids (Fig. 62). The pyrenoids were traversed by a few, small, tubular structures which may have been thylakoids.

McLean and Pessoney (1970) have reported the presence of a large quasi-crystalline lamellar lattice within the chloroplasts of some cells of Zygnemasp. This lattice, which did not appear in the cells until cultures were at the end of the log phase of growth, resembled aligned rings and rippled lines in a regularly repeating pattern. It was believed to be initiated by one or two thylakoids sliding between two neighbouring thylakoids, these then assuming a three-dimensional configuration. In the present work, sections of some cells showed the presence of a large number of coiled thylakoids in a localised area of the chloroplast around the pyrenoids (Fig. 62). The appearance of these was very similar to the initiation and early stages of lattice formation in Zygenma sp. published by McLean and Pessoney (1970) and presents the possibility that Zygogonium ericetorum is capable of forming lattices.

In addition to control material, Z. ericetorum that had been immersed in a buffered solution of 1ppm sulphur dioxide (pH4) for seven days was examined under the electron microscope. No differences were detected in the ultrastructure. There appeared to be no change in the size of the organelles and the chloroplast structure remained unaltered.

Fig. 61 L. S. of Z. ericetorum cell showing large bilobed chloroplast and cytoplasm full of inclusions, probably lipid droplets.

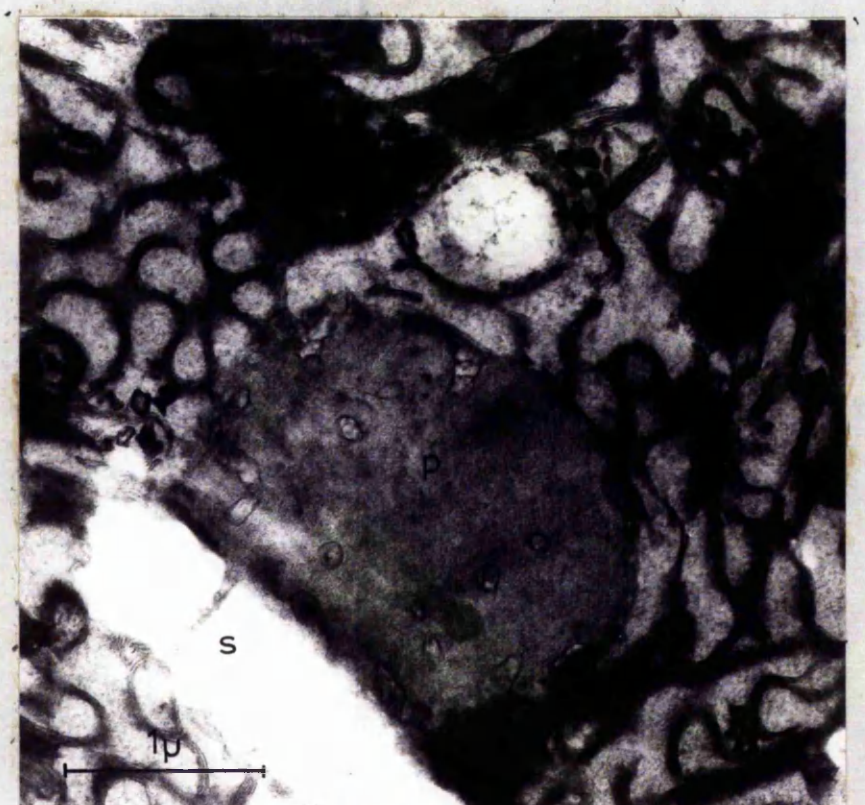


Fig. 62 Portion of chloroplast of Z. ericetorum showing pyrenoid (p) and starch-plate (s), and thylakoids in an arrangement similar to early stage of Mc Lean and Pessoney's (1970) quasi-crystalline lamellar lattice.

17. Discussion

The Plymouth area, supporting only light industry and being affected by predominantly southwest and west winds off the sea, is an area experiencing comparitively low levels of atmospheric pollution. The concentrations of sulphur dioxide and particulates recorded from the pollution monitoring sites within the city boundaries are relatively low compared with other urban areas and have shown little change from year to year over the last 14 years. Gilbertson (1975) has presented some evidence to suggest that sulphur dioxide is dispersed in a north-easterly direction from Plymouth across the lower slopes of Dartmoor. The results obtained, applying the Hawksworth and Rose (1970) pollution scale, support these conclusions but suggest that the sulphur dioxide is more rapidly diluted, its effects not extending as far as the lower slopes of Dartmoor. The isolated, short-duration sulphur dioxide readings taken at ground level using the portable meter suggest that levels of the pollutant are higher on the west slopes of Amados Hill (Plymptor by-pass site) than at the lower elevation sites closer to the city. This would indicate that sulphur dioxide from the powerstation is largely deposited on land at a similar height to the stacks.

The abundance of lichens on degenerate Callunetum at Drakelands Corner, including such pollution sensitive species as Usnea subfloridana, suggests that only low levels of sulphur dioxide occur in this area. However, as Geiger (1961) points out, the climate immediately above the ground can be substantially different from that recorded by meteorological stations and as the lichens referred to are all either terricolous or growing at ground level they are sheltered to a large extent from the prevailing winds. Gilbert (1968) showed that shelter can have a significant effect on modifying sulphur dioxide levels in urban areas. In an exposed situation he found that concentrations of the pollutant were reduced in a layer of up to 15cm over short turf.

Barkman et al (1968) report the presence of Prassiola crispa, which may be synonomous with Hormidium flaccidum, from the centre of Rotterdam and the present work records Zygogonium ericetorum from a polluted area of moorland on the western slopes of the Pennines in Derbyshire. Pleurococcus viridis is a common epiphyte of tree-trunks in urban and industrial areas and it may be that terrestrial algae as a group are comparitively pollution tolerant. Gilbert (personal communication) mentioning the greater tolerance of terricolous Bryophytes and lichens (as compared with epiphytic forms) to air pollution, suggests that Z. ericetorum may be able to exploit the more sheltered habitats close to the ground in moderately polluted areas.

There is no satisfactory method of equating concentrations of sulphur dioxide in solution with those in the air and consequently it is difficult to interpret the results of the laboratory experiments in terms of the field situation, even allowing for the very artifical conditions of the laboratory experiments. Saunders (1966) and Puckett et al (1973), on the basis of limited experimental evidence, have proposed that aqueous solutions of sulphur dioxide are equivalent to 100-fold lower concentrations in air but strongly dependent upon temperature. If this relationship is accepted, then levels of 50ppm aqueous sulphur dioxide, at which effects on Hormidium flaccidum were demonstrated, may represent realistic atmospheric levels of the pollutant in industrial areas. Zygogonium ericetorum proved less suitable experimental material but the results suggest that it shows a somewhat similar sensitivity to that of H. flaccidum. Both were killed by continous immersion in solutions of 5ppm sulphur dioxide and both were morphologically little altered by continous immersion in solutions of 1ppm. However, other workers using sulphur dioxide solutions to treat plants have demonstrated a greater tolerance in their experimental material to the pollutant. Gilbert (1968) treated cultures of a number of Bryophytes with solutions of sulphur dioxide for 48 hours and showed that even at concentrations of 600ppm, at pH 6.6 neither the gametophytes nor the protonema were killed. However, sensitive species could only survive concentrations below about 20ppm at pH 3.2. Gilbert also records the presence of a Chlorophyceae contaminant which was able to tolerate levels of 600ppm sulphur dioxide solution. In the present work a contaminant of the Chlorococcales type was noted in some cultures and was able to tolerate levels of 200ppm aqueous sulphur dioxide. Malhotra (1975) found that pine needles incubated in aqueous sulphur dioxide solutions were little affected by concentrations up to 100ppm and Puckett et al (1973) showed that some lichens could tolerate incubation for 6 hours in concentrations of 7.5 ppm sulphur dioxide solution without their carbon fixation rates being reduced.

The degree of ionisation and the ionic forms constituting sulphur dioxide solutions are strongly dependent upon pH. Puckett et al (1973) has shown that all forms of dissolved sulphur dioxide become increasingly better oxidising agents as the pH of the medium is lowered. The present results indicate that sulphur dioxide is most toxic to the algae investigated at low pH. These solutions are likely to consist predominantly of sulphurous acid and bisulphite ions, and cell membranes are likely to be more permeable to these forms than to the more highly charged sulphite ions (Hales and Sutter, 1973). A high pH reduced the effects of the pollutants and this may be because the sulphur is converted to sulphate, which is less toxic.

The algal material was subjected to two types of sulphur dioxide solution treatments namely continous immersion over a period of several days and daily, short duration immersions. The latter may be more representative of the field situation although in these experiments some of the sulphur dioxide solution was taken up into the agar medium so that thealgae were continually exposed to some sulphur dioxide. Hill (1974) has shown that in the lichen Hygogymnia physodes there is a recovery from the short term effects of sulphite on photosynthesis.

The alteration in the growth pattern of filaments of H. flaccidum caused by short, daily doses of up to 50ppm aqueous sulphur dioxide at low pH was a result of irregular divisions of the cells. Piercy (1917) has reported that filaments of this species may show bends at points where splitting of filaments is occuring and that this can lead to the filament assuming a zig-zag form. However, the contorted appearance as seen in filaments treated with sulphur dioxide solutions, is not recorded. Bleasdale (1973) points out that cell division is controlled by a balance between partially oxidised sulphur radicals, such as sulphites which inhibit cell division, and reduced sulphur radicals, sulphydryl compounds which promote cell division. Hence an imbalance of these radicals as a result of sulphur dioxide treatment may be expected to affect cell division in H. flaccidum. This alteration in growth pattern was accompanied by a swelling of the cells and an alteration in the ultrastructure of the chloroplasts. The formation of vesicles from the thylakoids in the chloroplast was comparable to the ultrastructural alterations caused by sulphur diodixe in higher plants as noted by Wellburn et al (1972) a nd

Malhotra (1976). Chlorophyll breakdown was detected in some of the cultures of contorted filaments. Even a slight degradation of chlorophyll or rearrangement of thylakoids is likely to impair metabolic process and investigations of carbon dioxide assimilation rates in the alga would prove profitable. It is unlikely that the observed changes in chloroplast ultrastructure were indicative of senescence because if contorted filaments were transferred to fresh media they continued to grow giving rise to 'normal', unswollen cells. Senescent cells in both control and sulphur dioxide treated material had a rather different appearance under the electron microscope.

All the experiments were conducted at 23°C, a considerably higher temperature than would be present in the natural habitat of the algae during the winter months when highest levels of pollution usually occur. It may be that the toxicity of sulphur dioxide is affected by temperature. Hill (1974) found that the concentration of sulphite required to affect photosynthesis in the lichen Usnea sp. was similar at 5°C and 15°C but most experiments on toxicity effects o f sulphur dioxide on lichens and Bryophytes have been carried out at temperatures of 18°C or above.

This work has considered sulphur dioxide as an air pollutant in isolation whereas, although it isthe most widespread air pollutant that can cause severe damage to plants, it seldom occurs on its own but is usually associated with generally smaller amounts of other pollutants. The biological effects of more than one pollutant simultaneously are likely to be additive or even synergistic as has been demonstrated by White et al (1974) and Bull and Mansfield (in press). Because the algae are terricolous they are most likely to be affected by pollutants reaching them by "washout", and in fact sulphur dioxide is likely to be the most important of these.

18: Discussion and Conclusions

Both Zygogonium ericetorum and Hormidium flaccidum are well adapted for a terrestrial environment. Both are able to withstand long periods of dessication without serious damage, although the combination of long dry spells and hot sunny weather may kill large numbers of cells. Despite the faster growth rate of H. flaccidum, the important species in the colonisation of china clay waste is Z. ericetorum. The former grows with Z. ericetorum in smaller amounts but becomes locally dominant where the soil is less acid.

Silica sand waste is very porous substrate and flowering plants growing on it tend to be restricted, especially in the early seral stages, to gullies and other depressions where runoff water provides a more regular supply of water and nutrients. Terrestrial algae, however, are less demanding; Z. ericetorum forms an almost continous cover over china clay waste which, in places where the substrate is particularly unstable, may be absent or present as a fine weft of filaments and is best developed in damp areas. The cover has the effect of consolidating the surface and contributing to the organic content of the substrate. However, sheet erosion occurs on the tips and on steep slopes, and during periods of heavy rainfall the surface layer of alga plus sand is frequently washed away only to be replaced by further algal material being washed onto the newly-exposed areas.

The response of both algae to environmental changes can vary from filament to filament and even from cell to cell within a filament, a feature particularly marked in Z. ericetorum. This can, in part, be explained by the different ages of cells within a filament and their different physiological states. It would be expected that resistant cells such as akinetes show a greater tolerance and this is the case with regard to response to drying and to temperature extremes. However, there remain a small percentage of cells which are tolerant of high pH and of concentrations of sheep urine and sulphur dioxide solutions which are fatal to most of the cells. This suggests that, in the field, a proportion of cells are able to survive periods of adverse environmental conditions so that recovery of the population is possible.

A significant increase in the ambient sulphur dioxide concentrations in the Lee Moor area may initially affect the algal population on the china clay waste but it is likely that recovery would follow. A destruction of the algal mat cover in such areas would seriously hinder the seral development of vegetaion. If the proposed powerstation at

Plymouth is built, it is unlikely that sulphur dioxide concentrations in the area would increase sufficiently to affect either Z. ericetorum or H. flaccidum although the lichen communities of the degenerate Callunetum may well be affected. Moreover, because of the uneven nature of the terrain, shelter would be an important factor in reducing the concentrations of pollutants over much of the area. Any effects on the algal cover would probably first be noticed on the exposed southwest facing slopes of the tips. Continuous monitoring of the algal cover when the powerstation becomes operative would be profitable and the china clay workings at St. Austell would serve as a suitable control area.

It is likely that air pollution tolerant strains of both Z. ericetorum and H. flaccidum occur in different areas, as has been recorded for higher plants. It would be interesting to compare populations of Z. ericetorum from Drakelands Corner with those from the Derbyshire moors to see whether they show a different tolerance to levels of air pollution. Populations of H. flaccidum from the centre of Plymouth may show a different response to those at Drakelands Corner.

Morphologically, material of Z. ericetorum from different areas can vary in cell size, pigmentation, the presence of side branches and cellwall thickness. It seems that these populations are all referable to the same species and that the morphology is determined by the environmental conditions under which they are growing.

The china clay industry occupies a considerable area of land in Southwest England and this area is likely to increase with extensive reserves and a continuing demand on the World Market for china clay. Although efforts are being made by ECC to find outlets for the silica sand waste and to revegetate tips, it is likely that this will primarily affect the large tips so that the natural colonisation of clay waste will continue to be of importance in some areas.

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APPENDIX

Isolation and culture techniques

Hormidium flaccidum was isolated and maintained in unialgal culture on 0.5 per cent Knop's agar slants at 23°C and illuminated at 4,300 to 5,300 lux by white fluorscent strip lights over a twelve hour photoperiod. It was also grown in 25ml aliquots of Knop's broth in 50 ml flasks on an orbital shaker and on moist sterilised silica sand in petri-dishes. Experiments using this species were conducted using either field material (where stated) or culture material on Knop's agar in petri-dishes in the majority of instances.

Despite persistent attempts, no success was achieved in culturing Zygogonium ericetorum. This was suspected to be partly due to the slow growth of this alga and partly due to its thick, mucilaginous walls which made surface sterilisation difficult. It was however, possible to maintain the alga in mixed culture on moist silica sand in petri-dishes and in liquid media. In laboratory experiments using this alga, fresh material from china clay wastes was used after as much extraneous material as possible had been removed by micromanipulation under a bionocular microscope.

Media used in attempts to isolate H. flaccidum and Z. ericetorum

Medium	H. flaccidum	Z. ericetorum
0.5% Knop's agar	+ +	-
0.05% Knop's agar	+	-
0.5% Knop's agar + 0.06% iron sequestrene	+ +	-
Steam-sterilised china clay + plain agar (50:50)	+ +	-
Steam-sterilised china clay + 0.5% Knop's agar:-		
50:50	+ +	-
50:50 + $CaCO_3$	+	-
50 50+ $(NH_4)_2SO_4$	+	-
75: 25	+ +	-
Godward's (1942) agar	Not tried	-
Czurda's (1932) agar	Not tried	-
0.5% Knop's broth	+ +	+

Key ++ good growth, + poor growth, - No growth

Media

1. Knop's agar 0.5%

Calcium nitrate	1g
Potassium dihydrogen phosphate	0.25g
Potassium nitrate	0.25g
Ferric chloride	.1% aqueous (1 drop)
Magnesium sulphate	0.25g
Distilled water	350 ml
Agar	6.3g

Dissolve each separately in a little water, mix and make up to 350ml.

2. Godward's medium (1942)

Potassium nitrate	250mg
Sodium sulphate	58mg
Magnesium sulphate. $7H_2O$	80mg
di-Potassium hydrogen orthophosphate	28mg
Calcium nitrate	20mg
Calcium carbonate	10mg
Potassium silicate	3mg
Distilled water	1l

3. Czurda's medium (1932)

Potassium nitrate	100mg
Magnesium sulphate. $7H_2O$	10mg
di-Potassium hydrogen orthophosphate	10mg
Calcium sulphate	5mg
Ferrous sulphate. $7H_2O$	5mg
Distilled water	1l

Buffers

1. McIlvaine's phosphate

pH	Solution (A ml)	Solution B (ml)
3.0	39.8	10.2
4.0	30.7	19.3
5.0	24.3	25.7
6.0	17.9	32.1
7.0	6.5	43.6

Solution A 0.1M citric acid

Solution B 0.2M di-Sodium hydrogen phosphate

2. Sorensen's phosphate

pH	Solution C (ml)	Solution D(ml)
5.29	2.5	97.5
5.59	5.0	95.0
5.91	10.0	90.0
6.98	60.0	40.0
8.04	95.0	5.0

Solution C 0.15M di-Sodium hydrogen phosphate

Solution D 0.15M potassium dihydrogen Orthophosphate

3. Citrate buffer

pH	Solution E (ml)	Solution F (ml)
3.0	82	18
4.0	59	41
5.0	35	65
6.0	11.5	88.5

Solution E 0.1M citric acid

Solution F 0.1M sodium citrate

N.B. Czarda's phosphate, see under 'Media'

Staining for dead cells

Dead and plasmolysed cells were detected by the use of stains and also by examination at daily intervals to observe progressive discolouration of cell contents. In some instances filaments which were believed to be dying as shown by the above methods were transferred to fresh media in order to see whether new growth could be stimulated and it was found that these cells invariably died.

Various stains have been tried in the past to distinguish dead and plasmolysed cells. Bobrov (1955) found thionin in weak aqueous solution, Sudan 111 and Sudan Black were all suitable for differenting between normal cells and cells damaged by smog pollution in handcut sections of Poa annua. Damaged cells were stained whilst normal cells remained unstained. Fritsch (1922b) investigated the permeability of a number of algae including Z. ericetorum and H. flaccidum to various stains in dilute aqueous solution and concluded that the cells of the two algae were permeable to different stains and that the degree of permeability varied considerably from one stain to another.

Stains used in the present investigations were 1 per cent thionin, 0.01 per cent neutral red, 0.02 per cent nile blue, 1 per cent methylene blue and 0.05 per cent toluidine blue, all in aqueous solutions. In some experiments thionin was utilised but it was found that results using this stain could be misleading as there was a relationship between the number of cells stained and the duration of staining. This was most marked when the period of immersion in the stain exceeded twenty minutes when increasingly large numbers of cells became stained. Although it was not general practice to leave material in the stain for this length of time and although all results were obtained from using at least two different stains, it was decided to select another stain as the principal one. Of these tried, neutral red gave most unsatisfactory results whilst the others all differentiated between dead and living cells, some more clearly than others. Dilute methylene blue was found to give the best results with both algae. After staining, dead cells appeared blue, plasmolysed cells were blue-green whilst unplasmolysed cells remained green. The cell types retained their identity after thirty minutes immersion.

Scoring cells

Standard methods for counting single cells under the microscope were not appropriate for counting filamentous organisms. Methods devised for estimating numbers of filamentous bacteria and blue-green algae such as that of Burham _et al_ (1973) were also unsuitable for the larger filaments of green algae.

The method used for scoring cells in the present investigation has disadvantages but proved to be the most practicable when counting large numbers of cells. To ascertain percentages of dead cells, three slide preparations were made from each replicate, random fields were viewed under the microscope and one hundred consecutive cells scored from each of five filaments.In addition, percentages of dead cells were assessed by scanning many fields of view. Generally, results of cell counts and visual estimates were comparable. When examing cultures of _H. flaccidum_ attention was paid to filaments at the periphery of the culture (i.e. the new growth produced after subculturing).

The disadvantage of the material used was the inherent variability among the filaments. The response to treatments varied not only from filament to filament but from cell to cell within one filament and was particularly marked in _Z. ericetorum_. Filaments selected from different parts of an area of a few square centimetres in the field could be very variable so that it was necessary to examine filaments representing the condition of the patch as a whole. Because of this variety in response of different cells, the standard deviations in the results of some of the experiments are large.

Electron microscopy formulae

Luft's permanganate fixative

Solution A	20ml
0.1 N hydrochloric acid	22ml
Potassium permanganate	2g
Freshly distilled water	60ml

Solution A 14.7g Sodium barbitone; 9.7g Sodium acetate.$3H_2O$; 500ml distilled water.

Sodium Cacodylate buffer

Solution A 0.2M sodium cacodylate.$3H_2O$

Solution B 0.2M hydrochloric acid

To each 100ml of solution A add 94ml of solution B. Dilute with water to 400ml.

Reynold's lead citrate stain

To make 10ml solution, dissolve 0.01 - 0.04g lead citrate in 10ml. singly distilled water in a screw-topped vial. Add 0.1ml 10N sodium hydroxide solution and shake vigorously.

Uranyl acetate/lead citrate stain

Uranyl acetate Saturated solution of 50 per cent ethanol

Lead citrate 10per cent aqueous solution. N sodium hydroxide added dropwise to remove any cloudiness.