Laboratory studies on zinc tolerance in stigeoclonium tenue

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LABORATORY STUDIES ON ZINC TOLERANCE

IN STIGEOCLONIUM TENUE

by

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SUMMARY

The tolerance of Stigeoclonium tenue to zinc under simulated laboratory conditions was investigated using populations of the alga collected from 20 different sites in the counties of Durham and Cumberland area. The alga was found to be resistant to zinc and the resistance appears to depend on genetic and environmental factors.

TABLE OF CONTENTS

INTRODUCTION

Heavy metal contamination of fresh water is a process occuring naturally in some areas of the world. But what effect such contamination has on the geological time scale is unclear. However, today the amount of heavy metals in fresh water is often increased greatly as a result of mining and industrial waste and it is clear that such pollution has sometimes marked biological effect and considerable economic interest.

Of the various heavy metals that find their way into fresh water, zinc, although an essential micro-nutrient, may in larger concentration prevent the growth of many algae (Whitton 1970). McLean (1972) reported that Stigeoclonium tenue, a green filamentous alga, was not tolerant to the presence of zinc in the environment. The author refers to the work of William and Mount (1965) in which they found that they could not include this alga as occuring amongst the species tolerant to zinc.

It was decided to investigate the effects of zinc on Stigeoclonium tenue, as zinc is one of the metals leaching from the mine workings in the area selected for the present study. In particular a study was planned to determine the extent to which strains of this alga from zinc polluted steams showed resistance to zinc in comparison with strains from unpolluted streams.

1.1 Sources of Zinc

Zinc in commercially important amount is found in many parts of the world, such as U.S.A., Germany, U.K., Poland and Belgium, where it occurs as zinc spar or calamine $ZnCo_3$, Zinc Blend ZnS, Willemite Zn_2SO_4 and in other minerals. The earth's crust contains about 0.004% zinc (Rice 1961), and it is the primary source of zinc in water. Today the waste from factories engaged in zinc plating, glavanizing, rubber and rayon production (Bradshaw 1970) has added to the already increasing amounts coming from mining activity. It is interesting to note that zinc is still leaching from mine dumps 35 years after the mine ceased to function (Jones 1958). Today radioaative zinc with a half life of 245 days has contaminated the aquatic environment from radiocative fallout, from atomic fission and from atomic reactors where water is used as a coolant (Bachmann 1963).

1.2 Occurance of zinc in freshwater

In Japan, Morita (1955) found zinc concentration in lakes to range from 1.3 to 18.8 mg l^{-1}. In the industrial areas, Morita found higher levels. For the River Kama-na-Sawa, a river contaminated by mine waste the figure was 8.2 mg l^{-1}. Trehane (1962) gives a value of 187.5 mg l^{-1} for one of the rivers in Cardiganshire. Buchmann (1961) states that for most U.S., rivers, zinc levels range from 5 to 10 mg l^{-1}. Thus there seem to be a wide range of concentration of zinc in fresh water.

1.3 Effect of zinc in water

Zinc reacts with water in the following manner:-

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

Barton and Bethke (1960) formulated the apparent solubility product of zinc hydroxide as follows:-

$$\left[Zn^{++}\right] . (aOH)^{-2} = K' = 2 \times 10^{-17} \text{ where}$$

.(aOH^-) is the activity of hydroxyl ion.

Zinc once in water may remain available for the living organisms or as pointed out by Mortimer (1941) may get locked up in the sediments or absorbed to or co-precipated with other compounds.

1.4 P^H

P^H of the water is an important factor. The softer the water the more will metals go into solution and thus become available for absorption (Jones 1958). Alloways (1969) pointed out that by increasing the P^H there will be an increase in sorption of trace elements on soil particles and allow heavy metals to form complexes with organic matter more readily thereby reducing the amount in solution.

1.5 Use of zinc for living organisms

Zinc forms an essential component of the protoplasm associated with enzymes which regulate cellular metabolism. Here distinction is made between metal-enzyme, in which zinc is non specifically bound and which can be easily dissociated and the metao-enzyme, in which zinc is specifically bound in the matrix of proteins and is not easily separated (Hoch and Vallee 1958).

1.6 Uptake of zinc by aquatic organisms

There appear to be two main types of mechanisms operating in the uptake of zinc particulate materials in lakes, namely ion exchange or absorption reaction and biological utilization through synthesis of zinc containing enzymes. The degree of absorption will depend upon the kind and the amount of absorbing materials, chemical composition and the total concentration of zinc in the medium. Karin and Islam (1956) has shown that river-borne solids have the property of ion exchange and also the work of Mertimer (1941) and of Ohle (1955) support similar view. Thus ion exchange could play an important role in the cycle of zinc in water.

1.7 Description of Stigeoclonium tenue

Stigeoclonium tenue is a green, filamentous branched alga included in the Family *Chaetophoraceae.* It has a prostrate thallus which fixes the plant to the substrate from which an erect branched system is developed. The cells that form the erect portion has a parietal chloroplast with one or more pyrenoids.

Most of the branches ends in 'bluntly pointed setiferous cells'. The cells of the erect portion are cylindrical in shape and in some species such as Stigeoclonium subsecundum, Stigeoclonium tenue and Stigeoclonium pascheri there is a constriction at the partition wall. In some cases as the cells become more mature they assume a spherical shape with thick cell wall.

The erect portion is usually poorly developed in comparison to the basal system from which it grows. The apex is 'blunt to start with and become pointed or sometimes terminated with hairs' (Butcher (1932).

2. MATERIAL AND METHODS

2.1 WATER CHEMISTRY TECHNIQUES

2.1.1 Preparation

200 ml 'Pyrex' bottles, polythene bottles and 2 Sinta funnels, porosity size 2, were soaked in 10% Hcl over night. These were washed several times in demineralised distilled water to minimise traces of heavy metals. For the collection of water, care was taken in choosing the right conditions and time in particular where the sites received sewage discharge as this affects the water chemistry of the sites.

2.1.2 Collection and storage

Water for both cation and phosphate analysis was filtered at the site and collected in pyrex and polythene bottles. On return to the laboratory they were analysed and as some of the analysis was not completed, the water was stored in a deep freezer and analysed the next day.

2.1.3 P^H

P^H was measured in the laboratory using a P^H metre 7024 (Electronic instruments Ltd., Surrey, England).

2.1.4 Cation analysis

Analysis for Na, Mg, Ca, Zn, and Fe was carried out in a Perkin Elmer 403 Atomic Absorption Spectrophotometer. When analysing for Ca and Mg, 0.68% lanthanum chloride was added. In all cases except for Ca analysis which needed a slightly yellow richer reducing flame, an air acetylene lean blue oxidising flame was used. Measurements of Na, Ca and Mg were made on a digital read-out while a Perkin Elmer 165 Recorder was used to record Zn and Fe.

2.1.5 Orthophosphate analysis

Analysis for orthophosphates was carried out by the method suggested by Mackereth (1963) using n-hexanol extraction. Calibration curves were drawn with known standards and the results were read against distilled water at 730 μm in a 40 mm cell.

2.2 MEDIA

Two culture media were used in the experiment: Bold's Bsal Medium (Cox and Bold 1963); the No.10 of Chu (1942).

2.2.1 Bold's Basal Medium Stocks

The following stocks were made.

1.	$NaNO_3$	1.0 g	dissolved in 400 ml of distilled water
2.	$CaCl_2$	1.0 g	"
3.	$MgSO_4$	3.0 g	"
4.	K_2HPO_4	3.0 g	"
5.	KH_2PO_4	7.0 g	"
6.	NaCl	1.0 g	"

2.2.2 "AC" Micronutrients

"AC" Micronutrients was made according to Kratz and Myres (1955).

H_3Bo_3	2.86 g	dissolved in 1 litre of distilled water
$MnCl_2$	1.81 g	"
$ZnSO_4$	287.6 g	"
$CuSO_4$	249.69 g	"
$CoSO_4$	245.006 g	"
Na_2MoO_4	241.95 g	"

1 ml of this concentrated solution was diluted to 1 litre to obtain a stock solution. 0.25 ml of this stock solution was added to 1 litre of culture medium, which thereby produced a concentration of 0.016 mg of zinc ions/litre in the basic culture medium.

2.2.3 Fe III E.D.T.A.

Iron was added in the form of Fe III E.D.T.A. (ethylenediamine tetra-acetic acid). To make up the medium the following procedure may be adapted. 26.1 g of E.D.T.A., is dissolved in 268.0 ml of KOH. To, this 24.9 g of $FeSO_4$ $7H_2O$ and diluted to one litre. This is aerated over night to produce a stable ferric compound. One ml of this is added to one litre of distilled water using 31.7 g of Na E.D.T.A. For the experiments, however, 12.7 g of Na E.D.T.A., was mixed with 9.7 g of $FeCl_3$ $6H_2O$ which gives a final concentration of 2 g l^{-1} Fe III.

2.2.4 Making up Bold's Basal Medium

10 ml of $NaNO_3$, $CaCl_2$, $MgSO_4$, K_2HPO_4 and NaCl stock was added to 1 litre with 0.25 ml each of E.D.T.A. and 'AC' Micronutrients to make up 1 litre of Bold's Basal Medium.

2.2.5 Chu 10 (modified)

The following stocks were made.

Stock 1	KH_2PO_4	15.6 g	dissolved in one litre of distilled water.
Stock 2	$MgSO_4$ $7H_2O$	25.0 g	"
Stock 3	$Ca(NO_3)_2$	40.0 g	"
Stock 4	$NaHCO_3$	15.0 g	"
Stock 5	Na_2SiO_3	43.5 g	"

From the above stock solutions:

1.0 ml of $MgSO_4 . 7H_2O$

1.0 ml of $Ca(NO_3)_2$

1.0 ml of $NaHCO_3$

0.25 ml of Na_2SiO_3

0.5 ml of KH_2PO_4 (Autoclaved separately and added last)

Were added to 1 litre of distilled water with 0.25 ml each of the stock of Fe III E.D.T.A. and "AC" Micronutrients.

2.2.6 Zinc concentration in the medium for zinc toxicity tests

4.399 g of $ZnSO_4 . 7H_2O$ dissolved in a litre of distilled water provided a stock of 1 g zinc per litre. Dilutions were made from this stock in Chu 10 and Bold's Basal Medium to give a range of concentration from 0.1 to 20 mg l^{-1} for zinc toxicity tests.

2.2.7 Media for experiments with varying concentrations of phosphate (in Chu 10 Medium)

The following stocks were made:

Stock 1 0.5032 g of $NaHPO_4$ was dissolved in 1 litre of distilled water

Stock 2 8.546 g of KCl "

To have a different concentration of PO_4P in the culture medium, Chu 10 was made up with $NaHPO_4$ stock instead of KH_2PO_4. To have a concentration of 2 mg l^{-1} PO_4P, 20 ml of $NaHPO_4$ stock and 0.5 ml of KCl stock was used with 0.25 ml each of the stock of Fe III E.D.T.A., and "AC" Micronutrients. For a 15 mg l^{-1} PO_4P, 150 ml of $NaHPO_4$ was used.

2.2.8 Media for control experiments (Sodium)

With $NaHPO_4$ in the medium there was 1.48 mg of Na in the medium with 2 mg l^{-1} PO_4P and 11.6 mg of Na in the medium with 15 mg l^{-1} PO_4P. Therefore a control was set up by making a culture medium with NaCl. For this purpose two stocks were made.

Stock 1 2.643 g of NaCl dissolved in 1 litre of distilled water

Stock 2 2.399 g of NaCl dissolved in 100 ml of distilled water.

Control media withlow phosphates was made with 1 ml of stock 1 in Chu 10 while for the high phosphate medium 1 ml of stock 2 in Chu 10 was used. Both media had 0.25 ml each of the stock of Fe III E.D.T.A. and "AC" Micronutrients.

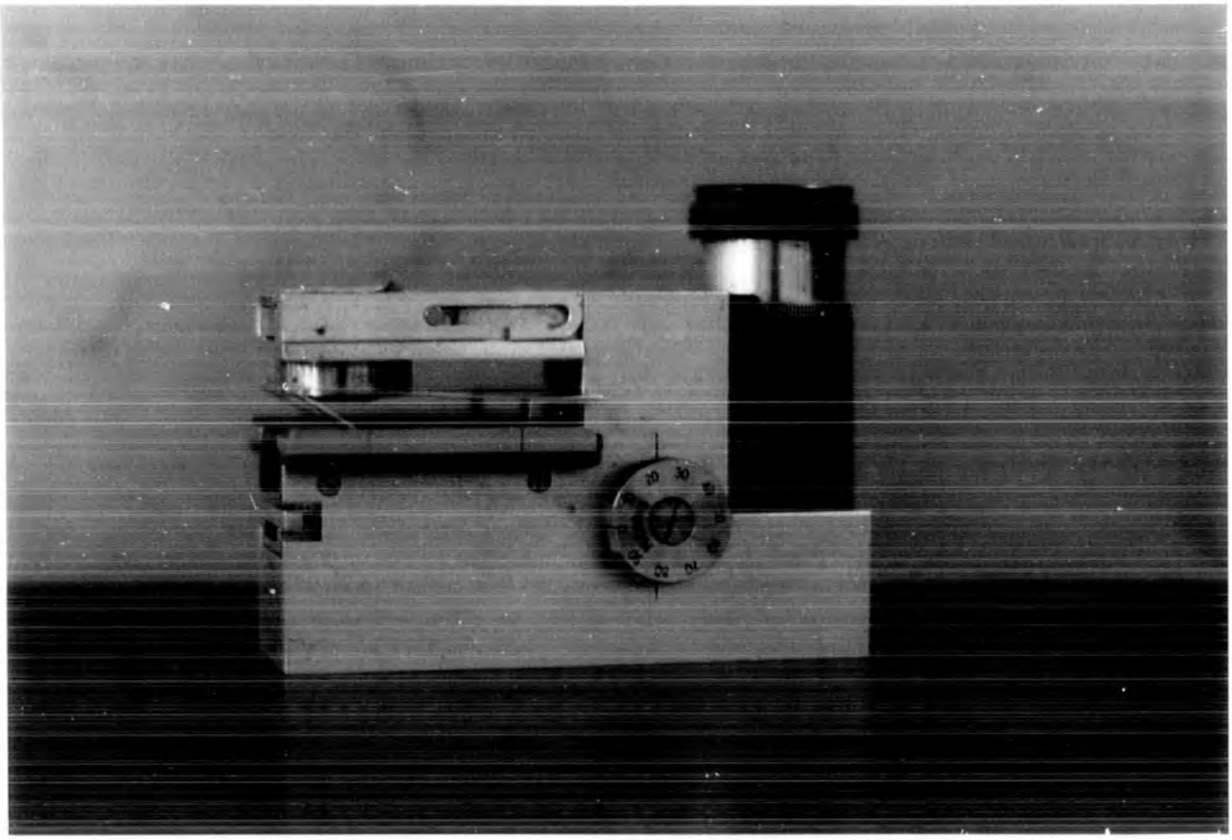


Fig. 3 Cooke McArthur field microscope

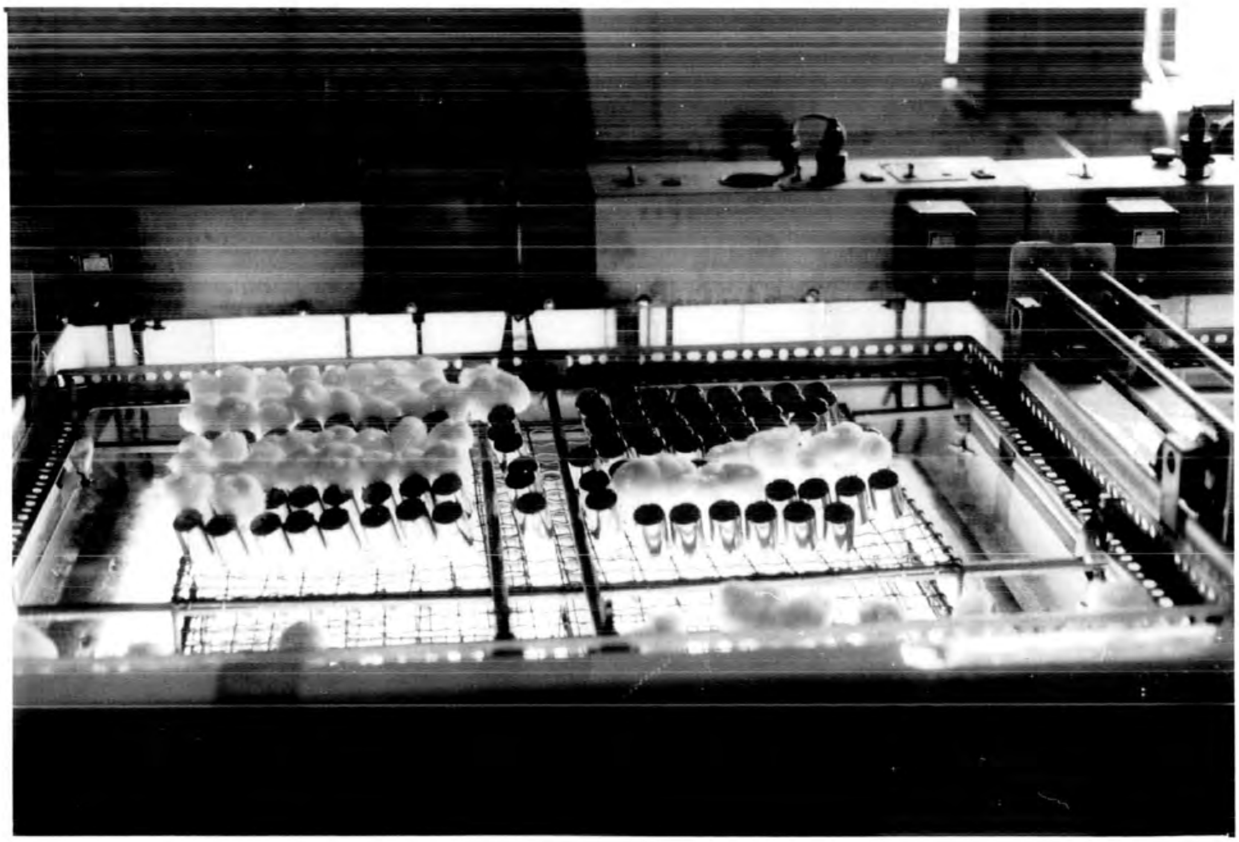


Fig. 4 Shake tank

2.3 EXPERIMENTAL CONDITIONS

2.3.1 Culture Tanks

The experiments were carried out in culture tanks in the laboratory referred to here as 'shake tanks'. These stimulate movement of the culture medium thus providing a semi-natural environment for the alga. The shake tanks were maintained at 18°C and 4000 lux continuous illumination. The light was provided by warm white fluorescent tubes and the intensity of illumination was measured with an EEL portable photoelectric photometer (fig. 4).

2.3.2 Collection and storage of Stigeoclonium tenue

The alga was identified in the field with a Cooke McArthur field microscope (fig. 3). In the laboratory, the alga was washed several times in tap water to remove its epiphytes and other debris. Then it was kept in the culture medium in the shake tanks for 24 hours before inoculation to allow the alga to get acclimatized to the culture conditions.

2.3.3. Culture method

Each experiment was performed 4 times with fresh material from the field. These were kept in the culture medium for 24 hours to get acclimatized to the experimental conditions before being introduced into boiling tubes containing zinc levels ranging from 0.1 to 20.0 mg l^{-1}. For each experiment 40 to 45 boiling tubes containing 10 ml of the medium with known concentration of zinc were used. Two replicates of each concentration was used for each experiment. In each tube, bits of Stigeoclonium tenue, approximately 5 mm in length were introduced, taking care to prevent contamination with any foreign matter. These tubes were then kept in the shake tanks. Every 24 hours the tubes were moved to minimise any effect of slight variations in light intensity in the tanks. In order to provide a control alga, 2 to 4 separate tubes with Stigeoclonium tenue in culture medium, free of any zinc above the basal micronutrient level, were maintained under the same experimental conditions.

At first zinc toxicity tests were conducted with Bold's Basal Medium were it was found that the alga was able to tolerate comparatively higher levels of zinc in the medium. This was perhaps due to the higher nutrient level in this medium. So the experiments were conducted in Chu's No.10 solution which is a more dilute medium suitable for many algae from fairly oligotrophic habitats.

2.3.4 Scoring

The alga was checked visually for growth on days 2/3/4/5/6/7 and 8. Those that showed signs of contamination with other algal species were discarded. The condition of the alga was recorded on a three point scale, viz.,

1 - 'grows well' - that is the highest concentration of zinc in the medium that did not produce any lag in growth.

2 - 'No growth' - that is the highest concentration of zinc in the medium in which no growth was visible.

3 - 'dead' - that is the lowest concentration at which the alga died.

The growth of the alga was compared with those growing in the culture medium free of any zinc above the basal micronutrient level.

In order to establish the reliability of the scoring techniques, samples that were scored as 'no growth' and 'dead' were reintroduced into fresh culture medium without zinc. In all such cases those that were scored as 'no growth', began to grow well within 3 days, while the omes that were scored as 'dead' did not recover even after 8 days.

2.4 GEOGRAPHY OF THE SITES

2.4.1 Description of the sites

Having carried out a preliminary survey of the occurrence of Stigeoclonium tenue in the counties of Durham and parts of Cumberland, 20 sites were selected for the study. The sites were from upland and lowland areas. Some of them received sewage effluent and also run off from nearby agricultural lands. Of the 20 sites (Table I), Lumley Park Burn (site 19), River Skerne (site 18), Sherburn Beck (site 20) and in Waskerley Beck (site 4), the water appeared heavily silted and muddy. The flow in all sites where Stigeoclonium tenue grew was fairly rapid and the substrate ranged from silt to large pebbles. In Rookhope Burn (site 1), the substrate consisted of large boulders and in Hollingside Lane (site 11), the alga was growing on pieces of bricks. Further the alga occupied areas which were free from shade.

2.4.2 Water analaysis

The water analysis for P^H, cation and phosphate determination was carried out only once. But care was taken in choosing the most appropriate time of the day in collecting water for analysis as most of the sites received sewage effluent. Water was collected on the 19th June, 1974, between 10 a.m. and 3 p.m. The cation and P^H analysis was completed on the same day and the water for phosphate analysis was stored in a deep freezer and analysed the next day.

RESULTS

The details of the experimental results obtained are given in Appendix 1 to XIII as follows:

Appendix 1	Results of 4 x 20 experiments with varying concentrations of zinc in Chü 10.
Appendix 11	Results of the scoring techniques validity.
Appendix 111	Results of the experiments to see if adaptation could be induced (populations from River Nent).
Appendix 1V	Results of the experiments to see if adaptation could be induced (populations from the Cold Stream).
Appendix V	Results of the experiments with low level (2 mg l^{-1}) PO_4P in Chü 10 for populations from the Cold Stream.
Appendix VI	Results of the experiments with low level (2 mg l^{-1}) PO_4P in Chü 10 for populations from River Nent.
Appendix VII	Results of the experiments with high level (15 mg l^{-1}) PO_4P in Chü 10 for populations from the River Nent.
Appendix VIII	Results of the experiments with high level (15 mg l^{-1}) PO_4P in Chü 10 for populations from the Cold Stream.
Appendix IX	Results of the experiments to study the effect of sodium (1.48 mg l^{-1}) in Chü 10 for the populations from the Cold Stream.
Appendix X	Results of the experiments to study the effect of Sodium (1.48 mg l^{-1}) in Chü 10 for the populations from the River Nent.
Appendix XI	Results of the experiments to study the effect of Sodium (11.6 mg l^{-1}) in Chü 10 for the populations from the Cold Stream.
Appendix XII	Results of the experiments to study the effect of Sodium (11.6 mg l^{-1}) in Chü 10 for the populations from the River Nent.
Appendix XIII	Tolerance to zinc in Chü 10 compared with Bold's Basal Medium.

For convenience, a summary of the relevant results has been tabulated as follows:

Table I	List of sites
Table II	Water analysis data
Table III	Zinc tolerance values (no growth mean values)

TABLE I

20 SITES IN WHICH STIGEOCLONIUM TENUE WAS FOUND

(Arranged in the West to East direction)

Site No	Grid Ref.	Name of the river/stream	condition of flow/substra	
1	NY 934416	Rookhope Burn	Fairly rapid	rocky
2	NY 795422	River Nent Tributary	Rapid	rocky
3	NY 768448	River Nent	Rapid	rocky
4	NY 076574	Waskerley Beck	Slow	silt & gravel
5	NZ 162332	Beech Burn	Slow (receiving sewage)	gravel
6	NZ 237432	River Deerness	Fairly rapid	gravel
7	NZ 237423	River Deerness tributary small brook from coal tip)	Slow	gravel
8	NZ 249432	River Browney	Fairly rapid	silt
9	NZ 259372	Cold Stream (receives run off from nearby farm)	Fairly rapid	silted
10	NZ 259372	Sunderland bridge sewage works	Slow	gravel
11	NZ 274408	Hollingside Lane sewage works	Slow	broken bricks
12	NZ 274408	Hollingside Lane away from the sewage works	Slow	large pebbles
13	NZ 274405	River Wear below the sewage works	Rapid	large pebbles
14	NZ 274405	River Wear below the sewage works	Fairly rapid	gravel
15	NZ 285509	North Burn at Chester-le-Street	Fairly rapid	gravel
16	NZ 286417	Old Durham Beck	Fairly rapid	gravel
17	NZ 289417	Small stream behind the school of Agriculture, Durham	Slow	silt & gravel
18	NZ 293208	River Skerne	Fairly rapid	silt & gravel
19	NZ 293509	Lumley Park Burn, near Chester-le-Street	Fairly rapid	silt & gravel
20	NZ 319419	Sherburn Beck	Slow	silt & gravel

19th June, 1974

TABLE II

WATER ANALYSIS OF THE 20 SITES

Site No.	Name of the River /Stream	p^H	Na	Mg (mg l^{-1})	Ca	Zn	Fe	PO_4^{-P}
1	Rookhope Burn	7.8	13.3	10.3	57.6	0.49	0.09	0.024
2	River Nent trib.	6.4	8.1	6.2	14.1	0.3	0.1	0.009
3	River Nent	7.8	8.5	19.7	82.1	1.21	0.29	0.31
4	Waskerley Beck	7.8	10.6	5.5	25.7	0.012	0.14	0.014
5	Beech Burn	7.5	24.8	30.0	67.3	0.002	0.325	0.372
6	River Deerness	7.3	68.2	59.2	102.0	0.002	0.29	0.22
7	River Deerness small brook from a coal tip	7.6	55.8	26.2	69.8	1.90	1.89	0.07
8	River Browney	7.4	95.3	27.6	67.1	0.05	0.2	0.52
9	Cold Stream	7.2	42.2	129.0	115.0	0.05	0.18	0.064
10	Sunderland Bridge sewage works	7.4	55.7	10.7	36.4	0.12	0.675	0.65
11	Hollingside Lane sewage works	7.6	55.2	7.7	50.5	0.18	0.675	8.00
12	Hollingside Lane artificial stream away from the sewage works	7.6	31.4	16.4	51.8	0.025	2.52	0.015
13	River Wear, above the Durham sewage works	7.8	80.5	26.3	68.2	0.04	0.09	0.67
14	River Wear, below the Durham sewage works	7.5	79.1	26.1	66.4	0.15	0.09	0.69
15	North Burn at Chester-le-Street	7.6	341.0	55.8	135.0	0.002	0.22	0.187
16	Old Durham Beck	7.8	344.0	88.5	168.0	0.042	0.12	0.19
17	Small Stream behind the school of Agriculture	7.5	82.3	29.6	79.2	0.012	0.12	0.62
18	River Skerne	7.3	388.0	49.3	109.0	0.73	5.5	0.508
19	Lumley Park Burn nr. Chester-le-Street	7.2	240.0	64.9	113.0	0.02	0.38	0.508
20	Sherburn Beck	7.4	295.0	71.8	141.0	0.002	0.12	1.28

TABLE III

ZINC TOLERANCE ('No growth' Value)

SITE NO	NAME OF STREAM	ZINC TOLERANCE (mg l^{-1}) (Mean of 4 values)
1	Rookhope Burn	6.37
2	River Nent Tributary	6.87
3	River Nent	10.5
4	Waskerley Beck	5.25
5	Beech Burn	2.43
6	River Deerness	2.62
7	River Deerness Small Stream	2.5
8	River Browney	4.37
9	Cold Stream	1.62
10	Sunderland Bridge Sewage Works	2.75
11	Hollingside Lane	4.25
12	Hollingside Lane artificial stream	3.62
13	River Wear, about sewage works	4.25
14	River Wear, below sewage works	4.62
15	North Burn	2.75
16	Durham Beck	2.37
17	Stream behind school of Agriculture	2.62
18	River Skerne	3.12
19	Lumley Park Burn	2.62
20	Sherburn Beck	3.12

DISCUSSION

The concentration of zinc in the culture medium in which 'no growth' was observed have been regarded as the most reliable values for tolerance to zinc, since death was observed at concentrations, 0.25 mg l^{-1} higher than that corresponding to 'no growth' and healthy growth was observed at concentrations proportionately less than the 'no growth' concentrations.

Except in a few cases, the range of the four values for the same population was 1.5 mg l^{-1}. It would therefore be safe to assume that the error in the values of tolerance to zinc is approximately $\pm$ 1 mg l^{-1}.

The laboratory test shows conclusively that Stigeoclonium tenue is tolerant to zinc over a wide range of concentrations of zinc. These observations are in agreement with the observations of Whitton (1970), who compared the tolerance of Stigeoclonium tenue and Cladophora glomerata to zinc.

The laboratory tests also showed that zinc tolerance of Stigeoclonium tenue depends on the nutrient level of the culture medium. The results of the experiments conducted to the test zinc tolerance in the more nutritive Bold's Basal medium indicated a greater tolerance to zinc. The populations from the River Nent (site 3) tolerated a range of 14.5 to 17.0 mg l^{-1} than in the relatively poorer Chü 10 medium in which lower tolerance (10.0 to 11.0 mg l^{-1}) were observed. This is in agreement with McLean's (1974) view that 'where suitable organic condition prevail, Stigeoclonium tenue, would show greater tolerance to zinc.'

A few tests were carried out to investigate the dependence of zinc tolerance on the phosphate levels of the culture medium. The results of these tests on two populations were as follows:

Table IV DEPENDENCE OF ZINC TOLERANCE ON PHOSPHATE

Specimen from	phosphate concentrations (mg l^{-1})		
	2	7.8	15.0
River Nent (site 3)	3.5	10.75	10.6
Cold Stream (site 9)	1.3	1.62	1.75

It is apparent that higher phosphate concentration promotes resistance to zinc. With respect to phosphate, McLean found that an 'increase in phosphorus from "low values" equivalent to x 20 dilutions of the sewage levels upto a concentration found in the normal control medium caused increase in algal growth'.

A similar experiment conducted to test the effect of sodium gave the results (Appendix IX - XII) which are summarised below in Table V.

Table V EFFECT OF SODIUM ON ZINC TOLERANCE

Specimen from	Chu 10 with 1.48 mg l^{-1} Na	Chu 10 normal culture with 8.2 mg l^{-1} Na	Chu 10 with 11.6 mg l^{-1} Na
River Nent (site 3)	8.25	10.5	7.0
Cold Stream (site 9)	1.25	1.62	1.25

Examination of the results in Table V does not reveal any marked trend in the effect of sodium. However it must be pointed out that all the zinc polluted sites studied came from upland areas with relatively low levels of sodium. It did not prove possible to find any site combining high sodium and high zinc levels. The effect of sodium needs more detailed investigation.

Klein (1957) observed that calcium and magnesium can affect the toxicity of zinc. Whitton (1970) has observed that the toxicity of metals on algae is affected by light and the presence of chelating agents. In the limited time available, it was not possible to investigate the effect of these and other variables on zinc tolerance of Stigeoclonium tenue. It is suggested that this aspect should be further investigated.

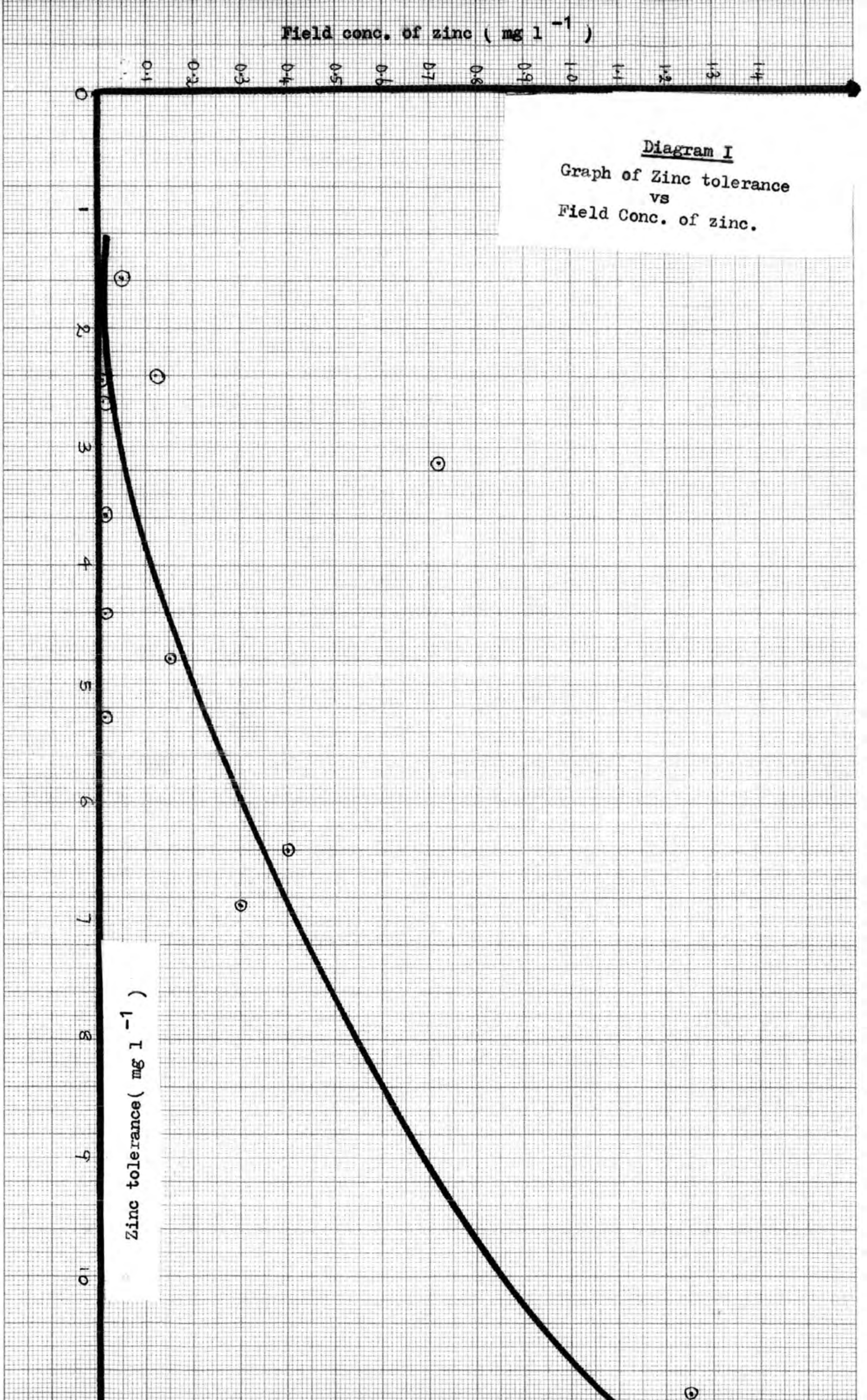


Diagram I

Graph of Zinc tolerance
vs
Field Conc. of zinc.

McLean (1974) is of the view that Stigeoclonium tenue is not tolerant to zinc. But the results of the zinc tolerance test for 20 x 4 populations obtained from 20 different sites reveal that this alga is tolerant to zinc. A tolerance range of 1.6 to 10.5 mg l^{-1} zinc was observed even though the tests were carried out under uniform controlled conditions. This is a puzzling observation. It is evident that zinc tolerance depends not only on the medium used but also on the characteristics acquired by the alga prior to the test. Such characteristics could differ due to genetic and environmental factors. Investigation of the effect of genetic factors would involve much detailed experimental work involving clonal isolates.

Environmental conditions involve several factors such as intensity of light, nature of the substratum, rate of flow of the stream, presence of other organisms and on the concentration of soluble substances in the water.

Populations used for the test were selected from sites where the conditions in respect of light and flow rate were more or less identical (vide Table I). But the analytical data obtained from water analysis of the various sites, showed considerable variation in the concentration of metallic ions and phosphate ions (Table II).

A possible explanation for the variation of zinc tolerance of Stigeoclonium tenue from site to site, is that the alga acquired adaptation to the environmental zinc concentration and thereby acquired a greater resistance to zinc toxicity. In order to verify the validity of this view, the graph of zinc tolerance vs field concentration of zinc was drawn. (Vide diagram I). The graph shows a trend which confirm this view. However the results of the experiments carried out with the highly resistant population from the River Nent (site 3) and with the poorly resistant population from the Cold Stream (site 9) did not confirm this view. In this test the two populations were subcultured for 4 and 8 weeks in the medium with a zinc concentration corresponding to the 'no growth' conditions for each population, in order to give them adequate time for acclimatization to zinc and then subjected to the usual test for zinc tolerance, as was done with the other populations. Details of these tests are given in Appendix III & IV. A summary of these results is as follows:

Table VI **EFFECT OF ADAPTATION TO ZINC**

Specimen from	normal culture	After subculture for 4 weeks	After subculture for 8 weeks
	Zinc tolerance values (mg l^{-1})		
River Nent (site 3)	10.5	10.62	10.5
Cold Stream (site 9)	1.62	1.75	1.63

No gain in zinc tolerance caused by environmental adaptability is shown. Perhaps the period (4 to 8 weeks) may have been too short for such a test. Conclusive evidence can be obtained only by prolonging the subculture period of this test.

In order to determine the effect of the other constituents on the environmental factors, sites with the same zinc pollution levels were chosen to observe whether a change in the other variable could contribute to the variation of zinc tolerance (Table VI).

Table VI **DEPENDENCE OF ZINC TOLERANCE ON FIELD CONCENTRATIONS**

Site	zinc tolerance (mg l^{-1})	Zn	Ca	Mg	Na	Fe	PO_4-P	
		Water analysis data (mg l^{-1})						
5	2.42	< 0.002	67.3	30.0	24.8	0.33	0.38	same Zn. conc.
15	2.75	< 0.002	135.0	55.8	341.0	0.22	0.19	
20	3.13	< 0.002	141.00	71.8	295.0	0.12	1.28	
4	5.5	0.012	25.7	5.5	10.6	0.14	0.014	same Zn. conc.
17	2.87	0.012	79.2	29.6	82.3	0.12	0.62	
8	4.37	0.05	67.1	27.6	95.3	0.2	0.52	same Zn. conc.
9	1.62	0.05	115.0	129.0	42.2	0.18	0.064	
13	4.25	0.04	68.2	26.3	80.5	0.09	0.69	same Zn. conc.
16	2.37	0.042	168.0	88.5	344.0	0.12	0.19	

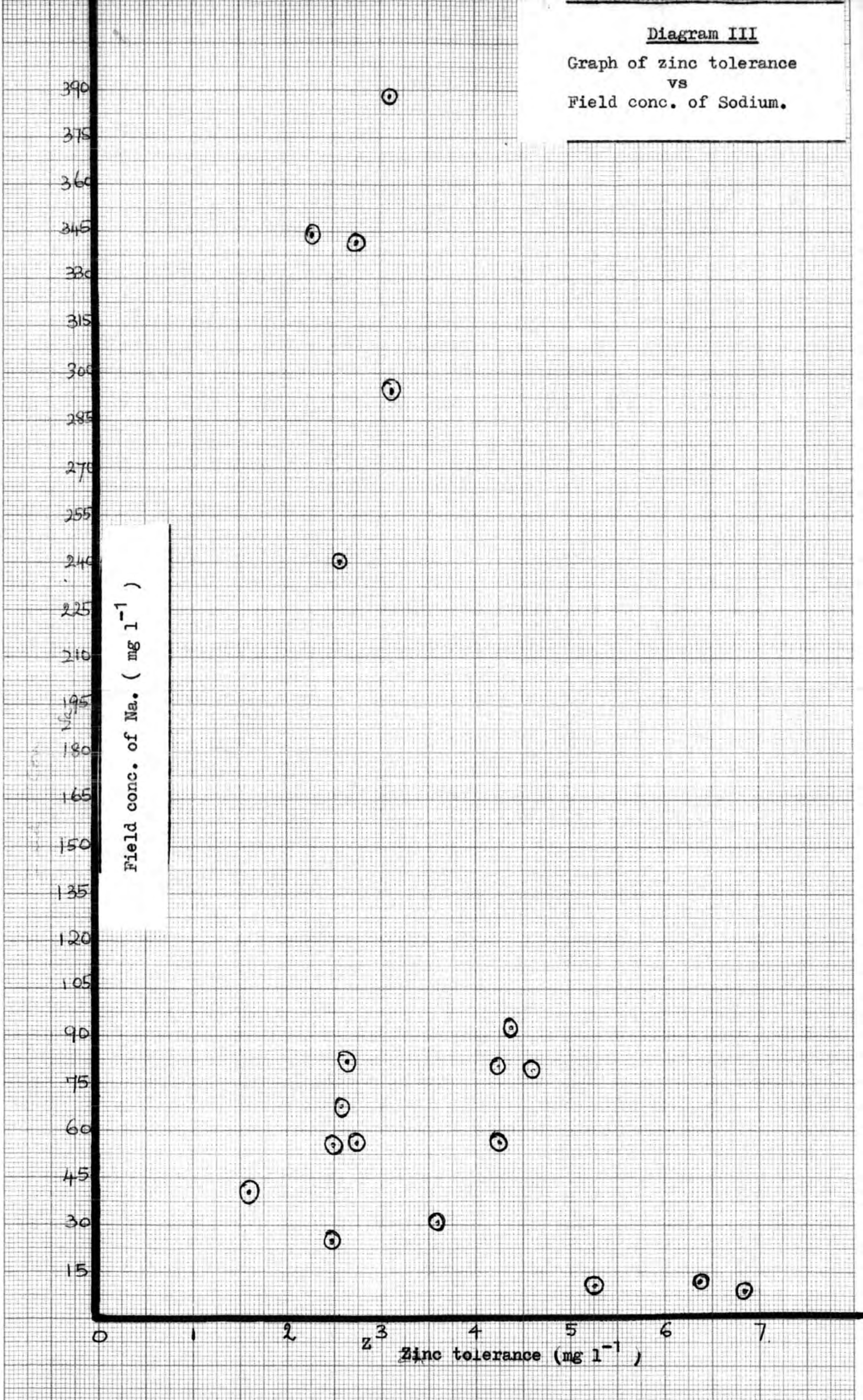
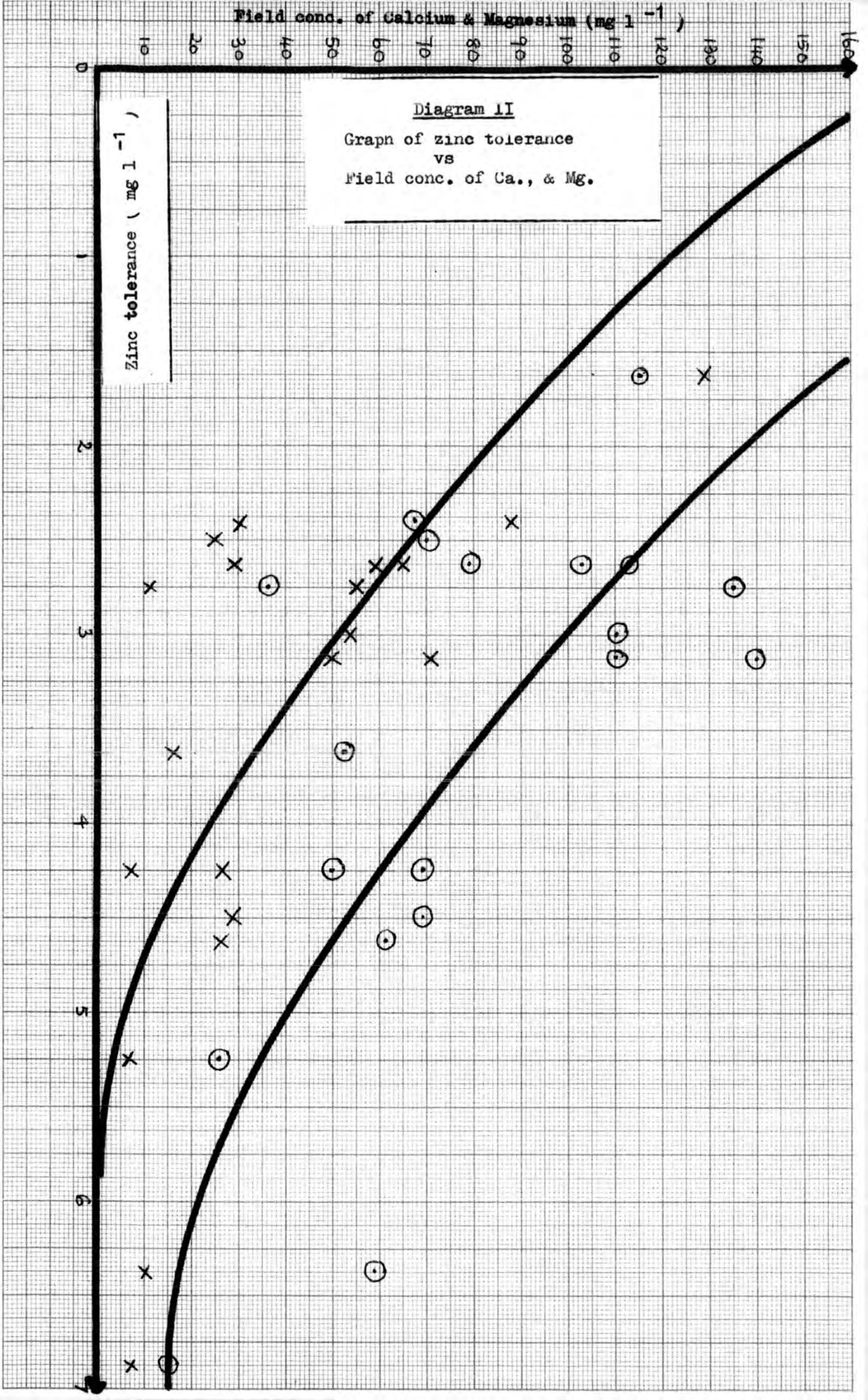


Diagram III
Graph of zinc tolerance
vs
Field conc. of Sodium.
Field conc. of Na. (mg l^{-1})
390
375
360
345
330
315
300
285
270
255
240
225
210
195
180
165
150
135
120
105
90
75
60
45
30
15
0
1
2
3
4
5
6
7
Zinc tolerance (mg l^{-1})


Field conc. of Calcium & Magnesium (mg l -1)
Diagram II
Graph of zinc tolerance
vs
Field conc. of Ca., & Mg.
Zinc tolerance (mg l -1)
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
1
2
3
4
5
6
7

The graph of zinc tolerance vs concentration of calcium and magnesium (diagram II) shows an interesting trend. The zinc tolerance of populations of alga from streams containing high calcium and magnesium contents are markedly lower. A similar observation regarding toxicity of zinc towards rainbow trout has been observed by Klein (1957), who has suggested that concentrations of sodium, potassium, calcium and magnesium are capable of antogonizing the ions of heavy metals such as lead, zinc and copper, thereby reducing the toxicity. But the graph of zinc tolerance vs concentration of sodium (Diagram III) does not reveal such a trend.

An interesting observation about the morphological changes caused by zinc is worth mentioning. Microscopic examination of branching, "hair" formation and structure of the alga was done before inoculation and 8 days after inoculation in the culture medium to observe the effects of zinc. It was observed that branching was profuse in those populations that grew very well in low concentration of zinc. As the concentration of zinc increased, there was a decrease in the number of branches and an increase in the 'hairs' and rounding up of the cell's contents into zoospores. (Fig.2).

This particular study was undertaken to determine the efect of zinc on Stigeoclonium tenue as it is one of the heavy metals that is found in varying concentrations in the area selected for this investigation. The results point to the conclusion that this alga is resistant to zinc and that strains from zinc polluted sites would have greater tolerance than strains from unpolluted streams.

These observations can have an influence on decisions regarding permissable limits for zinc effluent discharge into the aquatic medium.

Conclusions

The conclusions from this investigation can be summarized as follows:

1. Stigeoclonium tenue is tolerant to zinc.
2. Tolerance to zinc appears to depend on genetic and environmental factors.
3. Tolerance to zinc seems to be enhanced by highly nutritive medium, adaptation to zinc and low calcium and magnesium contents of the environment.

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Results of 4 x 20 experiments with varying levels of Zn in Chu 10 (modified)

Appendix I

Site No.	Grows Well Mean*	Grows Well S.D.	Grows Well Range	No Growth Mean*	No Growth S.D.	No Growth Range	Death at Mean*	Dead at S.D.	Dead at Range
1 Rookhope Burn	4.12 mgl^{-1}	0.2500	3.370 - 4.8760	6.37 mgl^{-1}	0.2500	5.6200 - 6.3700	6.625 mgl^{-1}	0.2500	5.8750 - 6.6257
2 R. Nent Tributary	2.5 mgl^{-1}	6.000	-	6.87 mgl^{-1}	0.4772	5.4387 - 8.3016	7.12 mgl^{-1}	0.4787	5.6839 - 8.5561
3 R. Nent	5.5 mgl^{-1}	0.4081	4.2757 - 6.7243	10.5 mgl^{-1}	0.4081	8.0514 - 10.5122	10.75 mgl^{-1}	0.4081	9.5257 - 11.9743
4 Waskerley Beck	2.125 mgl^{-1}	0.250	1.3750 - 2.8750	5.25 mgl^{-1}	0.2886	4.3842 - 6.1158	5.5 mgl^{-1}	0.2874	1.7244 - 6.3622
5 Beech Burn	1.62 mgl^{-1}	6.1443	1.20 - 2.04	2.43 mgl^{-1}	0.125	2.055 - 2.4372	2.65 mgl^{-1}	0.189	2.0830 - 2.6556
6 R. Deerness	1.37 mgl^{-1}	0.1442	0.9374 - 1.8026	2.62 mgl^{-1}	0.2500	1.870 - 3.3700	2.87 mgl^{-1}	0.2500	2.1200 - 3.6200
7 R. Deerness Small Stream	1.6875 mgl^{-1}	0.1248	1.3131 - 2.0619	2.5 mgl^{-1}	0.4081	1.2757 - 3.7243	2.75 mgl^{-1}	0.4081	1.5257 - 3.9743
8 R. Browney	2.12 mgl^{-1}	0.2500	1.3700 - 2.8700	4.37 mgl^{-1}	0.2500	3.6200 - 4.3775	4.625 mgl^{-1}	0.2500	3.8750 - 5.3750
9 Cold Stream	1.25 mgl^{-1}	0.000	-	1.62 mgl^{-1}	0.1442	1.1874 - 2.0526	1.81 mgl^{-1}	0.0692	1.6021 - 2.0176
10 Sunderland Bridge Sewage Works	2.0 mgl^{-1}	0.000	-	2.75 mgl^{-1}	0.8400	2.160 - 3.840	3.0 mgl^{-1}	0.840	2.16 - 3.84
11 Hollingside Lane	1.56 mgl^{-1}	0.2391	0.8427 - 2.2773	4.25 mgl^{-1}	0.2886	3.3842 - 5.1158	4.5 mgl^{-1}	0.2886	3.6342 - 5.3658
12 Hollingside Lane Small Stream	1.56 mgl^{-1}	0.1248	1.9344 - 1.1856	3.62 mgl^{-1}	0.2500	2.870 - 3.6275	3.87 mgl^{-1}	0.2500	3.1200 - 3.8775
13 R. Wear above Sewage Works	2.25 mgl^{-1}	0.2908	1.3776 - 3.1224	4.25 mgl^{-1}	0.2932	3.3704 - 5.1296	4.5 mgl^{-1}	0.2874	3.6378 - 5.3633
14 R. Wear below Sewage Works	2.06 mgl^{-1}	0.3144	1.1168 - 3.003	4.62 mgl^{-1}	0.4787	3.1839 - 6.0561	4.87 mgl^{-1}	0.4787	3.4339 - 6.3061
15 N. Burn	1.56 mgl^{-1}	0.3475	0.5175 - 2.6025	2.75 mgl^{-1}	0.2886	1.8842 - 3.6158	3.0 mgl^{-1}	0.2886	2.1342 - 3.8658
16 Durham Beck	1.62 mgl^{-1}	0.1442	1.1874 - 2.0526	2.37 mgl^{-1}	0.2500	1.620 - 3.1200	2.62 mgl^{-1}	0.2500	1.870 - 3.370
17 Stream behind the Sch. of Agric.	1.37 mgl^{-1}	0.1442	0.9374 - 1.8026	2.62 mgl^{-1}	0.2500	1.8700 - 3.3700	2.87 mgl^{-1}	0.2515	2.1155 - 3.6245
18 R. Skerne	1.56 mgl^{-1}	0.001	1.5132 - 1.6068	3.12 mgl^{-1}	0.4272	1.8384 - 4.4016	3.37 mgl^{-1}	0.2500	2.6200 - 4.1200
19 Lumley Park Burn	1.31 mgl^{-1}	0.1248	1.6844 - 9.3560	2.62 mgl^{-1}	0.4424	2.4873 - 2.7527	2.87 mgl^{-1}	0.2500	2.1200 - 3.6200
20 Sherburn Beck	1.31 mgl^{-1}	0.1248	0.9356 - 1.340	3.125 mgl^{-1}	0.2500	2.375 - 3.375	3.375 mgl^{-1}	0.2218	2.7096 - 3.3756

* Mean of 4 values

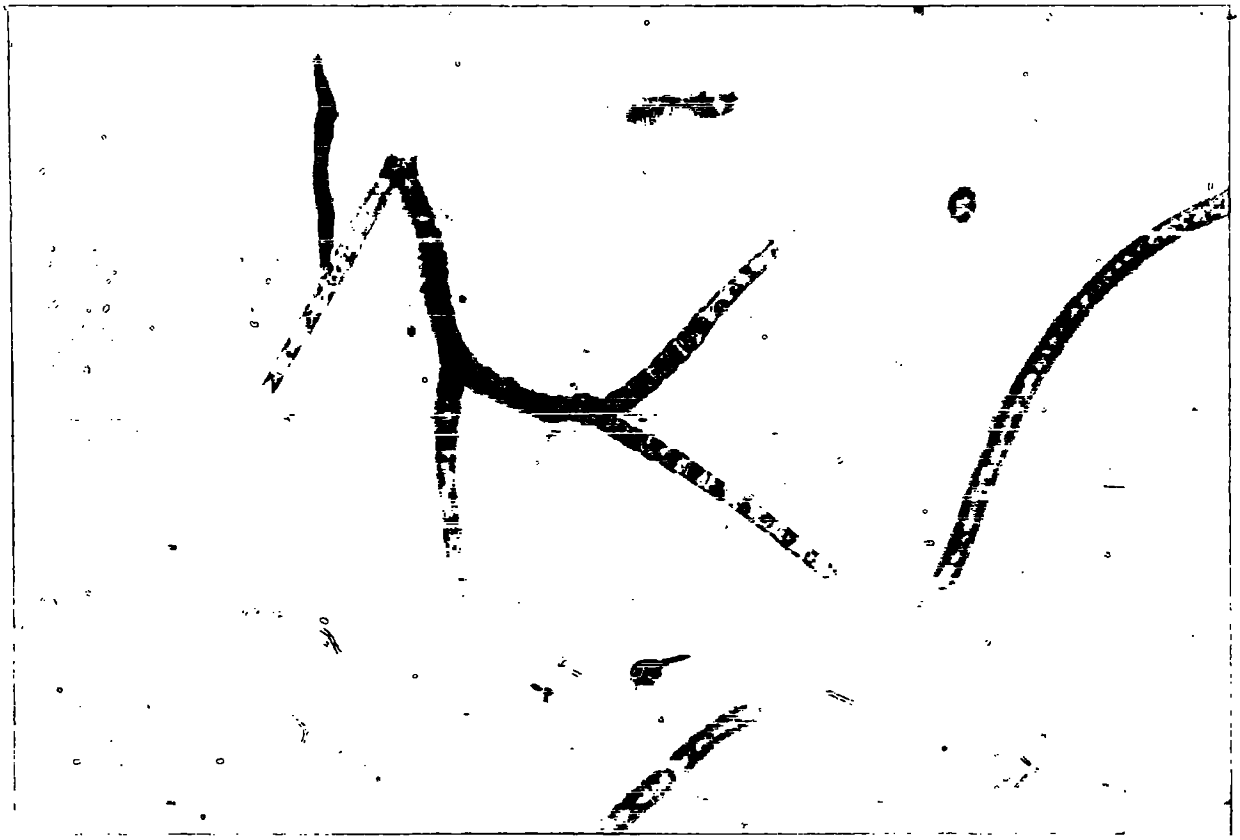

Fig. 1 Stigeoclonium tenue x 400

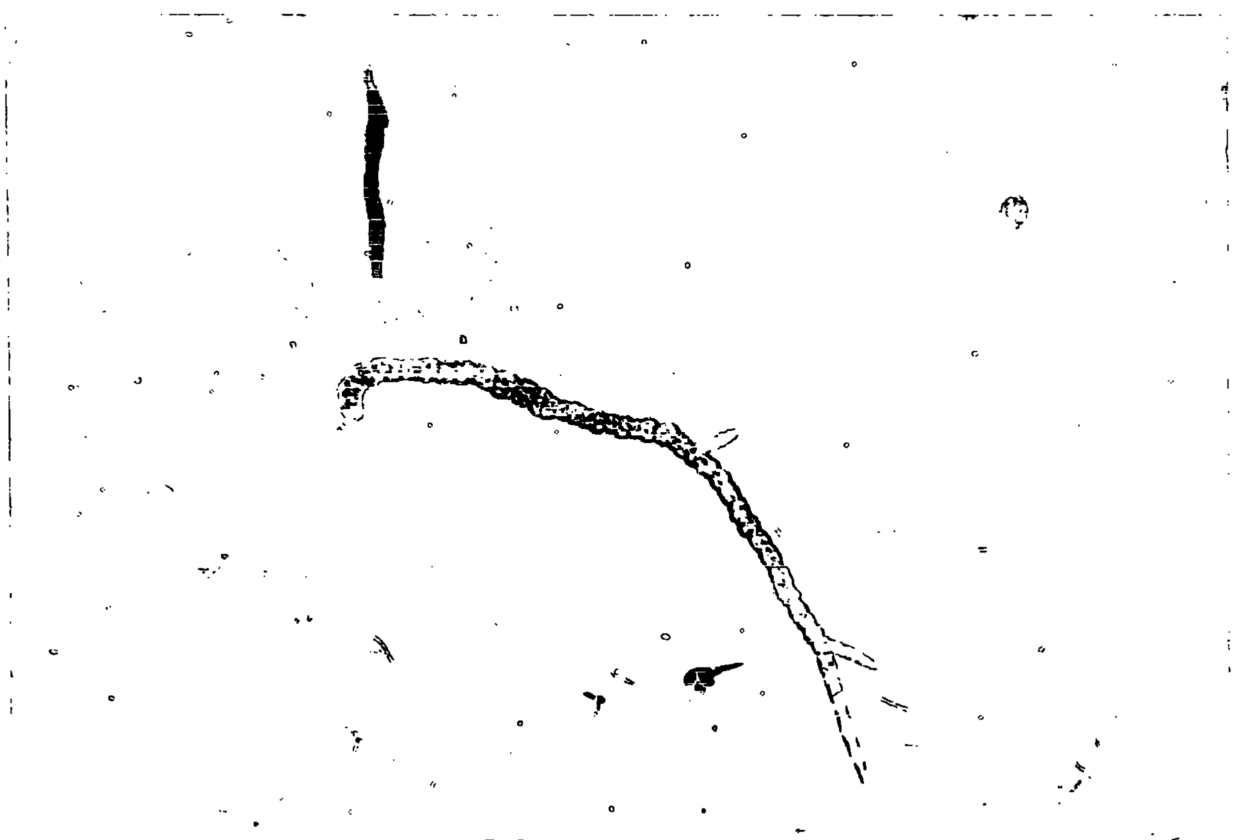

Fig. 2 Stigeoclonium tenue showing cells rounded up x 400

Appendix II

RESULTS OF 10 EXPERIMENTS WITH 'DEAD' STIGEOCLONIUM TENUE TO TEST THE VALIDITY OF SCORING TECHNIQUES

Experiment No.	with Stigeoclonium tenue from	Results after 6 days in Chu 10
1	River Nent (3)	dead
2	Hollingside Lane (11)	"
3	River Wear (13)	"
4	Sherburn Beck (20)	"
5	Lumley Park Burn (19)	"
6	River Nent tributory (2)	"
7	Cold Stream (9)	"
8	Old Durham Beck (16)	"
9	Beech Burn (5)	"
10	Rookhope Burn (1)	"

RESULTS OF 10 EXPERIMENTS WITH 'NO GROWTH' STIGEOCLONIUM TENUE TO TEST THE VALIDITY OF THE SCORING TECHNIQUES

Experiment No.	with Stigeoclonium tenue from	2 days after innoculation in Chu 10	4 days after innoculation in Chu 10
1	River Nent (3)	grows well	grows well
2	Hollingside Lane (11)	"	"
3	River Wear (13)	"	"
4	Sherburn Beck (20)	"	"
5	Lumley Park Burn (19)	"	"
6	River Nent tributory (2)	"	"
7	Cold Stream (9)	"	"
8	Durham Beck (16)	"	"
9	Beech Burn (5)	"	"
10	Rookhope Burn (1)	"	"

Appendix III

RESULTS OF 4 EXPERIMENTS WITH STIGEOCLONIUM TENUE FROM RIVER NENT (3) KEPT IN CHU 10 FOR 4 WEEKS AND TESTED FOR ZINC TOLERANCE

Experiment Nos.	1	2	3	4	Grows Well Mean	Standard Deviation
mgl^{-1} Zn	5.0	5.5	5.0	5.5	5.5	0.044

Experiment Nos.	1	2	3	4	No Growth Mean	Standard Deviation
mgl^{-1} Zn	11.0	11.0	10.5	10.0	10.62	0.063

Experiment Nos.	1	2	3	4	Death at Mean	Standard Deviation
mgl^{-1} Zn	11.25	11.25	10.75	10.25	10.87	0.062

RESULT OF 4 EXPERIMENTS WITH STIGEOCLONIUM TENUE FROM RIVER NENT (3) KEPT IN CHU 10 FOR 8 WEEKS AND TESTED FOR ZINC TOLERANCE

Experiment Nos.	1	2	3	4	Grows Well Mean	Standard Deviation
mgl^{-1} Zn	6.0	5.5	5.0	5.5	5.5	0.044

Experiment Nos.	1	2	3	4	No Growth Mean	Standard Deviation
mgl^{-1} Zn	10.5	10.5	10.0	11.0	10.5	0.044

Experiment Nos.	1	2	3	4	Death at Mean	Standard Deviation
mgl^{-1} Zn	10.75	10.75	10.25	11.25	10.75	0.044

Appendix IV

RESULTS OF 4 EXPERIMENTS WITH STIGEOCLONIUM TENUE FROM COLD STREAM (9) KEPT IN CHU 10 WITH THE HIGHEST CONCENTRATION OF ZINC IT COULD TOLERATE (1.5 mgl^{-1}) IN THE MEDIUM FOR 4 WEEKS TO TEST IF TOLERANCE COULD BE INCREASED

Experiment Nos.	1	2	3	4	Grows Well Mean	Standard Deviation
mgl^{-1} Zn	1.25	1.0	1.75	1.0	1.25	0.044

Experiment Nos.	1	2	3	4	No Growth Mean	Standard Deviation
mgl^{-1} Zn	1.75	1.5	2.5	1.25	1.75	0.070

Experiment Nos.	1	2	3	4	Death at Mean	Standard Deviation
mgl^{-1} Zn	1.87	1.25	2.75	1.5	1.84	0.083

RESULTS OF 4 EXPERIMENTS WITH STIGEOCLONIUM TENUE FROM COLD STREAM (9) KEPT IN CHU 10 WITH THE HIGHEST CONCENTRATION OF ZINC IT COULD TOLERATE (1.5 mgl^{-1}) IN THE MEDIUM FOR 8 WEEKS, TO TEST IF TOLERANCE COULD BE INCREASED

Experiment Nos.	1	2	3	4	Grows Well Mean	Standard Deviation
mgl^{-1} Zn	1.5	1.25	1.75	1.0	1.375	0.031

Experiment Nos.	1	2	3	4	No Growth Mean	Standard Deviation
mgl^{-1} Zn	1.75	1.5	2.0	1.25	1.625	0.031

Experiment Nos.	1	2	3	4	Death at Mean	Standard Deviation
mgl^{-1} Zn	1.75	1.75	2.25	1.5	1.875	0.031

Appendix V

RESULTS OF 4 EXPERIMENTS WITH STIGEOCLONIUM TENUE FROM COLD STREAM IN CHU 10 WITH 2 mgl^{-1} Po_4P

Experiment Nos.	1	2	3	4	Grows Well Mean	Standard Deviation
mgl^{-1} Zn	0.75	0.75	0.05	0.05	0.625	0.031

Experiment Nos.	1	2	3	4	No Growth Mean	Standard Deviation
mgl^{-1} Zn	1.5	1.25	1.0	1.5	1.312	0.031

Experiment Nos.	1	2	3	4	Death at Mean	Standard Deviation
mgl^{-1} Zn	1.63	1.37	1.13	1.63	1.44	0.031

Appendix VI

RESULTS OF 4 EXPERIMENTS WITH STIGEOCLONIUM TENUE FROM RIVER NENT (3) IN CHU 10 WITH 2 mgl^{-1} Po_4P

Experiment Nos.	1	2	3	4	Grows Well Mean	Standard Deviation
mgl^{-1} Zn	2.5	3.0	2.5	2.5	2.625	0.001

Experiment Nos.	1	2	3	4	No Growth Mean	Standard Deviation
mgl^{-1} Zn	4.0	4.0	3.0	3.0	3.500	0.005

Experiment Nos.	1	2	3	4	Death at Mean	Standard Deviation
mgl^{-1} Zn	4.25	4.25	3.25	3.25	3.75	0.005

Appendix VII

RESULTS OF 4 EXPERIMENTS WITH STIGEOCLONIUM TENUE FROM RIVER NENT (3) IN CHU 10 WITH 15 mgl^{-1} Po_4P

Experiment Nos.	1	2	3	4	Grows Well Mean	Standard Deviation
mgl^{-1} Zn	10.5	8.0	8.0	8.0	8.625	0.164

Experiment Nos.	1	2	3	4	No Growth Mean	Standard Deviation
mgl^{-1} Zn	12.0	12.5	8.5	9.5	10.625	0.254

Experiment Nos.	1	2	3	4	Death at Mean	Standard Deviation
mgl^{-1} Zn	12.25	12.75	8.75	9.75	10.875	0.262

Appendix VIII

RESULTS OF 4EXPERIMENTS WITH STIGEOCLONIUM TENUE FROM COLD STREAM (9) IN CHU 10 WITH 15 mgl^{-1} Po_4P

Experiment Nos.	1	2	3	4	Grows Well Mean	Standard Deviation
mgl^{-1} Zn	1.0	0.75	1.0	1.0	0.937	0.001

Experiment Nos.	1	2	3	4	No Growth Mean	Standard Deviation
mgl^{-1} Zn	1.75	1.75	1.75	1.75	1.75	0.000

Experiment Nos.	1	2	3	4	Death at Mean	Standard Deviation
mgl^{-1} Zn	1.87	1.87	1.87	1.87	1.87	0.000

Appendix IX

RESULTS OF 4 EXPERIMENTS WITH STIGEOCLONIUM TENUE FROM COLD STREAM IN CHU 10 NaCl as CONTROL (1.48 mg l^{-1} Na.)

Experiment Nos.	1	2	3	4	Grows Well Mean	Standard Deviation
mgl^{-1} Zn	1.0	1.05	1.0	1.0	1.0	0.000

Experiment Nos.	1	2	3	4	No Growth Mean	Standard Deviation
mgl^{-1} Zn	1.25	1.25	1.25	1.25	1.25	0.000

Experiment Nos.	1	2	3	4	Death at Mean	Standard Deviation
mgl^{-1} Zn	1.37	1.37	1.37	1.37	1.37	0.000

Appendix X

RESULTS OF 4 EXPERIMENTS WITH STIGEOCLONIUM TENUE FROM RIVER NENT (3) IN CHU 10 + NaCl (CONTROL) (1.48 mg l^{-1} Na.)

Experiment Nos.	1	2	3	4	Grows Well Mean	Standard Deviation
mgl^{-1} Zn	6.0	5.5	6.0	6.0	5.875	0.001

Experiment Nos.	1	2	3	4	No Growth Mean	Standard Deviation
mgl^{-1} Zn	8.0	8.5	8.0	8.5	8.25	0.001

Experiment Nos.	1	2	3	4	Death at Mean	Standard Deviation
mgl^{-1} Zn	8.25	8.75	8.25	8.15	8.5	0.004

Appendix XI

RESULTS OF 4 EXPERIMENTS WITH STIGEOCLONIUM TENUE FROM COLD STREAM (9) WITH CHU 10 + NaCl as CONTROL (11.6 mg l^{-1} Na)

Experiment Nos.	1	2	3	4	Grows Well Mean	Standard Deviation
mgl^{-1} Zn	1.0	0.75	1.0	1.0	0.937	0.000

Experiment Nos.	1	2	3	4	No Growth Mean	Standard Deviation
mgl^{-1} Zn	1.25	1.25	1.25	1.25	1.25	0.000

Experiment Nos.	1	2	3	4	Death at Mean	Standard Deviation
mgl^{-1} Zn	1.37	1.37	1.37	1.37	1.37	0.000

Appendix XII

RESULTS OF 4 EXPERIMENTS WITH STIGEOCLONIUM TENUE FROM RIVER NENT (3) IN CHU 10 WITH NaCl AS CONTROL (11.6 mg l^{-1} Na.)

Experiment Nos.	1	2	3	4	Grows Well Mean	Standard Deviation
mgl^{-1} Zn	5.0	6.5	6.5	6.0	6.0	0.008

Experiment Nos.	1	2	3	4	No Growth Mean	Standard Deviation
mgl^{-1} Zn	5.5	7.5	7.5	7.5	7.0	0.017

Experiment Nos.	1	2	3	4	Death at Mean	Standard Deviation
mgl^{-1} Zn	5.75	7.75	7.75	7.75	7.25	0.012

Appendix XIII

RESULTS OF 4 EXPERIMENTS WITH CHU 10 FOR STIGEOCLONIUM TENUE FROM [illegible] RIVER NENT

Experiment Nos.	1	2	3	4	Grows well Mean	Standard Deviation	Range
mgl^{-1} Zn	5.5	5.0	5.5	6.0	5.5	0.4081	4.2757- 6.7243

Experiment Nos.	1	2	3	4	No Growth Mean	Standard Deviation	Range
mgl^{-1} Zn	10.5	11.0	10.5	10.0	10.5	0.4081	8.0514-10.5122

Experiment Nos.	1	2	3	4	Death at Mean	Standard Deviation	Range
mgl^{-1} Zn	10.75	11.25	10.75	10.25	10.75	0.4081	9.5257-11.743

RESULTS OF 4 EXPERIMENTS WITH BOLD'S BASAL MEDIUM FOR STIGEOCLONIUM TENUE FROM RIVER NENT

Experiment Nos.	1	2	3	4	Grows Well Mean	Standard Deviation
mgl^{-1} Zn	7.5	8.0	7.75	8.5	7.937	0.060

Experiment Nos.	1	2	3	4	No Growth Mean	Standard Deviation
mgl^{-1} Zn	14.5	16.5	15.0	17.0	15.75	0.154

Experiment Nos.	1	2	3	4	Death at Mean	Standard Deviation
mgl^{-1} Zn	14.75	16.75	15.25	17.25	16.00	0.154

Tolerance compared, In Chu 10 $\longrightarrow$ 10.5 mgl^{-i} Zn

In Bold's Basal $\rightarrow$ 15.75 mgl^{-i} Zn

7-2 Stigeoclonium tenue in the Copperbelt (Zambia)

Stigeoclonium tenue was observed by the author to grow well in many of the streams in the Copperbelt region in Zambia. These streams receive mining effluent from the copper mines and also in some areas partly treated sewage. Water analysis dome by the Counties Public Health Laboratories in London for the Copperbelt Water Resaurces Survey (1971) found zinc concentrations of 0.04 and 0.11 mg l^{-1} zinc in the Rokana Water Works and the Wusakili Bridge, two sites in which this alga was seen to grow profusely now.

Ref. Copperbelt Water Resaurces Survey Final Report, Ministry of Rural Development, Dept. of Water Affairs, Zambia.

RESULTS OF 4 EXPERIMENTS ON Zn TOLERANCE (mg l^{-1})

1 - ROOKHOPE BURN

Experiment no.	Grows well	No growth	Dead at	Death at
1	4.0	6.5	7.0	6.75
2	4.0	6.0	6.5	6.25
3	4.5	6.5	7.0	6.75
4	4.0	6.5	7.0	6.75

	Grows well mean	No growth mean		Death at mean
	4.12	6.37		6.625
Standard deviation	0.2500	0.250		0.2500
Range	3.370-4.870	5.620-6.370		5.8750-6.6257

2 - R.NENT TRIBUTORY

Experiment no.	Grows well	No growth	Dead at	Death at
		(mg l^{-1})		
1	2.5	7.0	7.5	7.25
2	2.5	7.5	8.0	7.75
3	2.5	6.5	7.0	6.75
4	2.5	6.5	7.0	6.75

	Grows well mean	No growth mean	Death at mean
	2.5	6.87	7.12
Standard deviation	0	0.4772	0.4787
Range		5.4384-8.3016	5.6839-8.5561

3 - R.NENT

Experiment no.	Grows well	No growth	Dead at	Death at
		(Mg l $^{-1}$)		
1	5.5	10.5	11.0	10.75
2	5.0	11.0	11.5	11.25
3	5.5	10.5	11.0	10.75
4	6.0	10.0	10.5	10.25

	Grows well mean	No growth mean	Death at mean
	5.5	10.5	10.75
Standard deviation	0.4081	0.4081	0.4081
Range	4.2757-6.7243	8.0514-10.5122	9.5257-11.9743

4 - WASKELEY BECK

Experiment no.	Grows well	No growth	Dead at	Death at
		(Mg l^{-1})		
1	2.0	5.0	5.5	5.25
2	2.5	5.5	6.0	5.75
3	2.0	5.0	5.5	5.25
4	2.0	5.5	6.0	5.75

	Grows well mean	No growth mean		Death at mean
	2.125	5.25		5.5
Standard deviation	0.250	0.2886		0.2874
Range	1.375- 2.875	4.3842- 6.1158		1.7244- 6.3622

5 - BEECH BURN

Experiment no.	Grows well	No growth	Dead at	Death at
		($mg\ l^{-1}$)		
1	1.75	2.25	2.5	2.37
2	1.5	2.5	3.0	2.75
3	1.5	2.5	3.0	2.75
4	1.75	2.5	3.0	2.75

	Grows well mean	No growth mean		Death at mean
	1.62	2.43		2.65
Standard deviation	0.1443	0.125		0.189
Range	1.20- 2.06	2.0550- 2.4377		2.0830- 2.6556

6 - R. DEERNESS (MAIN RIVER)

(mgl^{-1})

Experiment no.	Grows well	No growth	Dead at	Death at
1	1.25	2.50	3.0	2.75
2	1.5	3.0	3.5	3.25
3	1.25	2.5	3.0	2.75
4	1.5	2.5	3.0	2.75

	Grows well mean	No growth mean	Death at mean
	1.37	2.62	2.87
Standard deviation	0.1442	0.250	0.250
Range	0.9374-1.8026	1.870-3.370	2.120-3.620

7 - R.DEERNESS (small stream from the Coal tip)

(mg l $^{-1}$)

Experiment no.	Grows well	No growth	Dead at	Death at
1	1.75	2.5	3.0	2.75
2	1.5	2.0	2.5	2.25
3	1.75	2.5	3.0	2.75
4	1.75	3.0	3.5	3.25

	Grows well mean	No growth mean	Death at mean
	1.6875	2.5	2.75
Standard deviation	0.1248	0.4081	0.4081
Range	1.3131-2.0619	1.2757-3.7243	1.5254-3.9743

8 - R.BROWNEY

Experiment no.	Grows well	No growth	Dead at	Death at
		(mg l^{-1})		
1	2.0	4.5	5.0	4.75
2	2.0	4.0	4.5	4.25
3	2.5	4.5	5.0	4.75
4	2.0	4.5	5.0	4.75

	Grows well mean	No growth mean		Death at mean
	2.12	4.37		4.625
Standard deviation	0.250	0.250		0.250
Range	1.370- 2.870	3.620- 4.3775		3.875- 5.3750

9 - COLD STREAM

Experiment no.	Grows well	No growth	Dead at	Death at
		(mg l^{-1})		
1	1.25	1.75	2.0	1.87
2	1.25	1.5	2.0	1.75
3	1.25	1.5	2.0	1.75
4	1.25	1.75	2.0	1.87

	Grows well mean	No growth mean	Death at mean
	1.25	1.62	1.81
Standard deviation	0	0.1442	0.0692
Range		1.1874-2.0526	1.6021-2.0176

10 - SUNDERLAND BRIDGE SEWAGE WORKS

Experiment no.	Grows well	No growth	Dead at	Death at
		(mg l^{-1})		
1	2.0	2.5	3.0	2.75
2	2.0	3.0	3.5	3.25
3	2.0	3.0	3.5	3.25
4	2.0	2.5	3.0	2.75

	Grows well mean	No growth mean	Death at mean
	2.0	2.75	3.0
Standard deviation	0	0.84	0.840
Range		2.16-3.84	2.16-3.84

11 - HOLLINGSIDE LANE SEWAGE WORKS

Experiment no.	Grows well	No growth ($mg\ l^{-1}$)	Dead at	Death at
1	1.5	4.5	5.0	4.75
2	1.75	4.5	5.0	4.75
3	1.75	4.0	4.5	4.25
4	1.25	4.0	4.5	4.25

	Grows well mean	No growth mean	Death at mean
	1.56	4.25	4.5
Standard deviation	0.2391	0.2886	0.2886
Range	0.8427-2.2773	3.3842-5.1158	3.6342-5.3658

12 - HOLLINGSIDE LANE STREAM AWAY FROM THE SEWAGE WORKS

Experiment no.	Grows well	No growth	Dead at	Death at
		($mg\ l^{-1}$)		
1	1.5	3.5	4.0	3.75
2	1.75	3.5	4.0	3.75
3	1.5	3.5	4.0	3.75
4	1.5	4.0	4.5	4.25

	Grows well mean	No growth mean	Death at mean
	1.56	3.62	3.87
Standard deviation	0.1248	0.250	0.250
Range	1.9344- 1.1856	2.870- 3.6275	3.1200- 3.8775

13 - R.WEAR ABOVE SEWAGE WORKS

Experiment no.	Grows well	No growth	Dead at	Death at
	($mg\ l^{-1}$)			
1	2.5	4.5	5.0	4.75
2	2.5	4.0	4.5	4.25
3	2.0	4.5	5.0	4.75
4	2.0	4.0	4.5	4.25

	Grows well mean	No growth mean	Death at mean
	2.25	4.25	4.5
Standard deviation	0.2908	0.2932	0.2874
Range	1.3776- 3.1224	3.3704- 5.1296	3.6378- 5.3622

14 - R.WEAR BELOW SEWAGE WORKS

Experiment no.	Grows well	No growth	Dead at	Death at
		(mg l $^{-1}$)		
1	2.0	4.5	5.0	4.75
2	2.5	5.0	5.5	5.25
3	2.0	5.0	5.5	5.25
4	1.75	4.0	4.5	4.25

	Grows well mean	No growth mean	Death at mean
	2.06	4.62	4.87
Standard deviation	0.3144	0.4787	0.4787
Range	1.1168- 3.0032	3.1839- 6.0561	3.4339- 6.0361

15 - NORTH BURN IN CHESTER-LE-STREET

Experiment no.	Grows well	No growth	Dead at	Death at
		($mg\ l^{-1}$)		
1	1.5	2.5	3.0	2.75
2	1.5	3.0	3.5	3.25
3	1.75	3.0	3.5	3.25
4	1.5	2.5	3.0	2.75

	Grows well mean	No growth mean		Death at mean
	1.56	2.75		3.0
Standard deviation	0.3475	0.2886		0.2886
Range	0.5175-2.6025	1.8842-3.6158		2.1342-3.8658

16 - OLD DURHAM BECK

Experiment no.	Grows well	No growth	Dead at	Death at
		(mg l^{-1})		
1	1.5	2.5	3.0	2.75
2	1.75	2.5	3.0	2.75
3	1.5	2.5	3.0	2.75
4	1.75	2.0	2.5	2.25

	Grows well mean	No growth mean	Death at mean
	1.62	2.31	2.62
Standard deviation	0.1442	0.250	0.250
Range	1.1874-2.0526	1.620-3.120	3.370-1.870

17 - SMALL STREAM BEHIND SCHOOL OF AGRICULTURE

Experiment no.	Grows well	No growth	Dead at	Death at
		(mg l^{-1})		
1	1.25	2.5	3.0	2.75
2	1.5	2.5	3.0	2.75
3	1.5	3.0	3.5	3.25
4	1.25	2.5	3.0	2.75

	Grows well mean	No growth mean	Death at mean
	1.37	2.62	2.87
Standard deviation	0.1442	0.250	0.2515
Range	0.9374-1.8026	1.820-3.370	2.1155-3.6245

18 - R.SKERNE

Experiment no.	Grows well	No growth	Dead at	Death at
	($mg\ l^{-1}$)			
1	1.5	3.0	3.5	3.25
2	1.75	3.0	3.5	3.25
3	1.5	3.5	4.0	3.75
4	1.5	3.0	3.5	3.25

	Grows well mean	No growth mean	Death at mean
	1.56	3.12	3.37
Standard deviation	0.001	0.4272	0.2500
Range	1.5132- 1.6068	1.8384- 4.4016	4.120- 2.620

19 - LUMLEY PARK BURN NEAR CHESTER-LE-STREET

Experiment no.	Grows well	No growth	Dead at	Death at
	(mg l^{-1})			
1	1.25	2.5	3.0	2.75
2	1.5	2.5	3.0	2.75
3	1.25	2.5	3.0	2.75
4	1.25	3.0	3.5	3.25

	Grows well mean	No growth mean	Death at mean
	1.31	2.62	2.87
Standard deviation	0.1248	0.04424	0.250
Range	9.356- 1.6844	2.4873- 2.7527	2.12- 3.620

20 - SHERBURN BECK

Experiment no.	Grows well	No growth	Dead at	Death at
		($mg\ l^{-1}$)		
1	1.25	3.0	3.5	3.25
2	1.5	3.5	4.0	3.75
3	1.25	3.0	3.5	3.25
4	1.25	3.0	3.5	3.25

	Grows well mean	No growth mean		Death at mean
	1.31	3.125		3.375
Standard deviation	0.1248	0.250		0.2218
Range	0.9356-1.317	2.375-3.875		2.7096-3.3756