Some physicochemical factors associated with filamentous freshwater algal growths in the Breede river, and the derivation of a rapid test for screening their copper tolerances.

Sean Privett

Honours thesis, Department of Botany
University of Cape Town 1994
Supervisor: Prof. J.J. Bolton

Abstract

The seasonal occurrence of freshwater filamentous algae at eight sites in the Breede river and its adjacent canal systems were examined. These were related to the physicochemical conditions pertaining to those sites over a course of eight months. An excessive growth of Oedogonium sp. was linked to moderately high levels of nutrient loading together with low conductivities in canal waters during spring. The occurrence of Enteromorpha intestinalis was found to coincide with high conductivity, temperature and pH, as well as low stream flow. The three Cyanophyta taxa were associated with conditions of high temperature in March and absent during the rest of the year. Cladophora was only found at one site characterised by high pH and water flow. Two species of Spirogyra were tolerant to eutrophication, while a third species was only found in the upper stretches in cleaner waters. Stigeoclonium tenue and Melosira sp were found restricted to sites with high nutrient load. As copper sulphate is used to control problem algal growths in the canal systems, a rapid test for examining the tolerance to copper of filamentous algae was derived and tested on four species. The method proved effective in determining the relative copper tolerances of the four species examined. The composition of the growth medium utilised in the assay was found to influence the results. A decrease in pH or the removal of the chelating ligand (EDTA) increased toxicity.

Introduction

South Africa is essentially an arid country, and consequently our water resources are a particularly valuable asset which require optimal management. A world-wide increase in the occurrence of excessive growths of aquatic angiosperms and algae, as a result of eutrophication caused by human expansion, has been recognised as a potential threat to our inland waterways. These growths are aesthetically displeasing, impede water flow and inhibit the growth of other algal species which are often more palatable to river fauna (Joska and Bolton 1993a). Furthermore the control of these growths is relatively expensive, ecologically deleterious and does not provide a permanent solution to the problem.

Research into factors influencing the occurrence, abundance and control of freshwater filamentous algae, although common in many other countries, has been virtually non-existent in South Africa. This study forms part of a four year research program into freshwater filamentous algae occurring in South African waters, with particular emphasis on problem species. It is a follow up to an earlier study on filamentous algae found in the Breede River and its connected canal irrigation scheme (see Joska and Bolton 1993).

The Breede River lies in a semi-arid region of the South Western Cape, South Africa. The major crops of the region are reliant on canalised waters, particularly during the long dry summer months (Joska and Bolton 1993a). Four canal systems extract waters from the river at various points along a 100 km stretch between Worcester and Bonnievale. During the dry summer months the canals are supplemented by waters from the Kwaggaskloof Dam (Joska and Bolton 1993a). Recently complaints have been received from the management of this scheme relating to abnormal growths of filamentous algae in the canals. These have been reported to restrict water flow and block irrigation pipes (Bolton and Joska 1993). Major algal growths in other parts of the world have often been linked to unnatural levels of

physicochemical characteristics, such as high nutrients (Whitton 1970, Dodds and Gudder 1992, Ten Cate et al 1991). One of the aims of this project was thus to ascertain which, if any, physicochemical parameters can be linked to the occurrence of algal blooms in the Breede River canal system. Relationships of physicochemical parameters with the occurrence of other less abundant species was also sought.

The second component of this project relates to the explicit requirements needed for the control of problem algae in the Breede River environment. Control of excessive algal growth in South Africa has up until now taken the form of either manual removal or the application of copper sulphate. Chemical control is both more economical and less labour intensive than manual removal. Copper sulphate ($CuSO_4.5H_2O$) is the most commonly used algicide for the control of large growths of filamentous algae throughout the world (Lembi etal. 1988). In South Africa copper sulphate is the primary measure used to control algal blooms in irrigation canals (Joska and Bolton in press). Levels of copper required to kill algae vary considerably between habitats, even within a single species. As an example, the common problem alga *Cladophora glomerata* have been described as highly sensitive to copper (killed by concentrations < 0.1 mgl^{-2} within 6 days) by Whitton (1969), to very resistant and requiring 10 mgl^{-1} for four days for a complete kill (Betzer and Kott 1969- cited in Whitton 1970). These differences may be genetically related, or as a result of physicochemical variation between sites (Whitton 1970b). International literature on copper treatment is thus not necessarily compatible with local conditions and it is necessary for us to undertake our own investigations in this field. As a starting point for this research, laboratory work was carried out in order to devise a rapid assay for testing copper tolerance of our local species.

For clarity, this study has been divided into two separate, although interrelated components. The first chapter examines the ecological conditions pertaining to the growth of filamentous algae in the Breede system, with particular emphasis being placed on the conditions relating to abnormal algal growth. The second chapter concentrates on the methodological derivation of a rapid assay for testing copper tolerances of filamentous algae with the aim of integrating this into future research on copper control.

CHAPTER 1. ENVIRONMENTAL CONDITIONS INFLUENCING THE OCCURRENCE AND ABUNDANCE OF SOME BREEDE RIVER FILAMENTOUS ALGAE.

Introduction

As with all living organisms, individual algal taxa are restricted to habitats characterised by a particular suite of environmental parameters which satisfy their physiological requirements. Although the distribution and abundance of these taxa is undoubtedly influenced by a wide variety of these physicochemical factors (Dodds 1991), their occurrence can often be related to one, or a few, limiting variables. In the case of problem species, a knowledge of the conditions limiting a species abundance can have considerable value for control programmes. This study explores the association between occurrence and abundance of filamentous algal taxa and the prevailing physicochemical characteristics at three river and five canal sites in the Breede river valley over a period of seven months.

Methods

Study Site

The section of the Breede River valley investigated in this survey lies between the Brandvlei Dam in the north west and the end of the Sanddrift canal in the south east, a distance of approximately 105km. The irrigation scheme consists of four canal systems drawing water off the Breede River (Joska and Bolton 1993a). An earlier survey of 19 sites along this stretch was used to choose the optimum localities for this study (Joska and Bolton 1993a). The following eight sampling sites were selected for further investigation and include sites along the river as well as two of the canals (see fig.1):

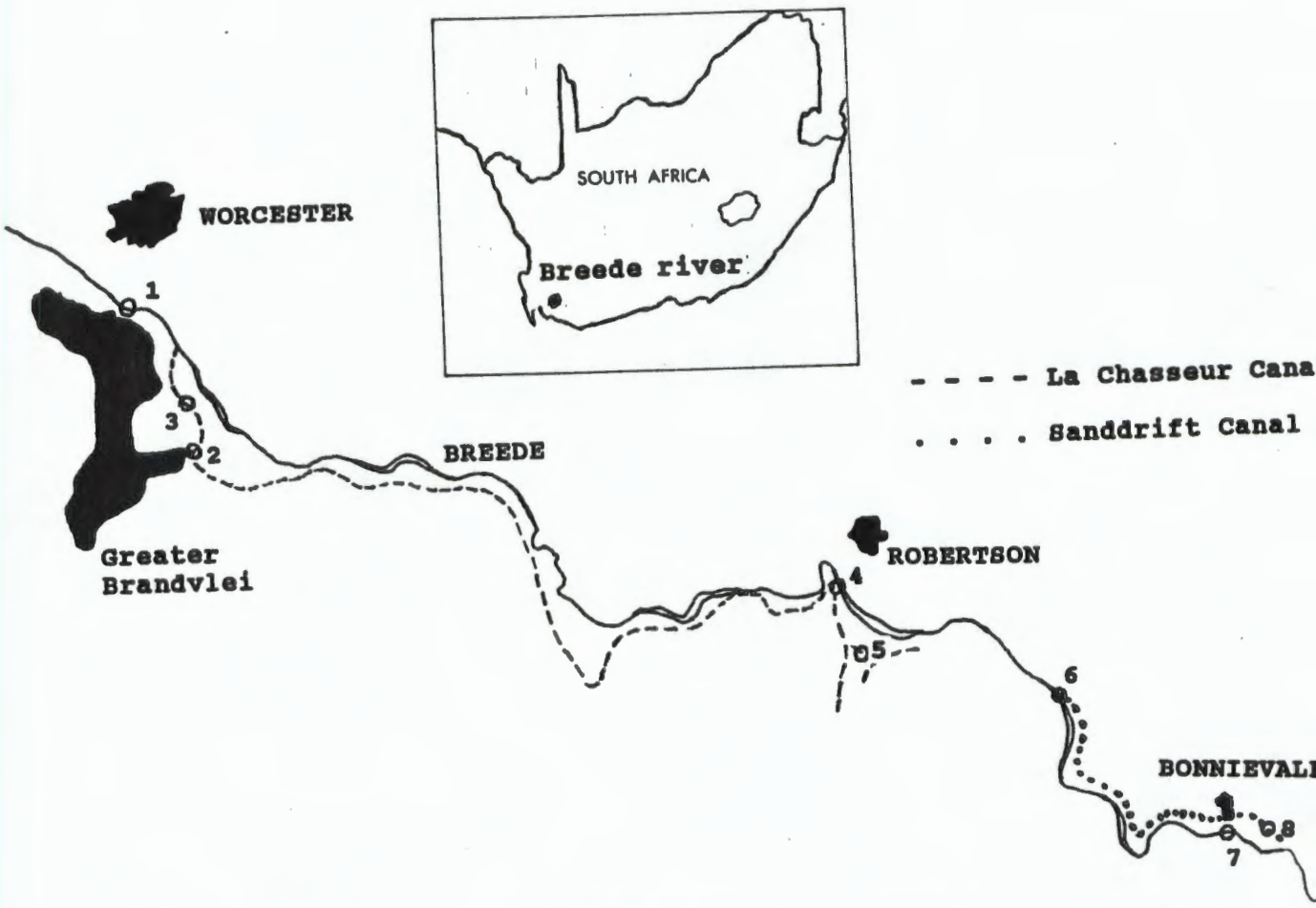

FIG.1 STUDY SITES ALONG THE BREEDE RIVER VALLY (adapted from Schreuder <u>etal.</u> 1988). (Insert locality of Breede river)

Name given in this study	Description of site
1R	Outflow of the Brandvlei dam into the Breede River at Nekkies.
2L	Kwaggashoek Dam outflow canal into the La Chasseur canal.
3L	La Chasseur canal 2.5 km from the intake, at the Riverside Road turn-off on the R43.
4R	Breede River site at Robertson Bridge picnic site.
5L	La Chasseur canal at "Keisershof Farm" where it intersects the road to Vrolikheid Nature Reserve.
6R/S	Intake of the Sanddrift canal at the sliding weir on the Breede River.
7R	Breede River at Bonnita Bridge (Bonnievale)
8S	Sanddrift canal near its end point at two water tanks on LHS of road to Drew.

Four field trips were undertaken to these sites during 1994: at the end of the summer (24 March), during autumn before the first rain (24 May), during the middle of winter after heavy rains (21 July), and in spring (7 September). The Sanddrift canal was being rebuilt at site 8 during July and could thus not be sampled. Instead sampling was undertaken a few kilometres further back where the Sanddrift canal intersects the main road at Bonnievale. Site 8 was visited again in September as that stretch of canal had been re-opened.

Environmental measurements

At each site temperature, conductivity, pH, turbidity and water speed was measured. For individual sites, measurements of these parameters were made at approximately the same time of the day during all four field trips. Temperature was measured using an alcohol thermometer in the upper 10cm of the water column. Temperatures in the canal water did not vary with depth, while river waters were all shallow sampling sites at the bank. Conductivity was measured with a YSI Model 33 SCT conductivity

meter, while pH was measured using pH paper. Water speeds were measured directly with a 10 metre tape and a floating orange. Water flow rates at the river sites (which were all at the bank of the river) proved very slow in March and were not measured thereafter. Turbidity was measured during the first field trip. However the waters at the eight sampling site all showed minimal turbidity and were consequently not measured during the following trips.

Laboratory analysis of nutrients

Laboratory analysis was used to determine levels of soluble nitrate, nitrite, ammonium and orthophosphate at each site. Water samples were collected at each site in 100ml pyrex bottles which had been acid washed and rinsed with distilled water. Three replicate samples were collected at each site. The samples were kept cold so as to inhibit bacterial activity until analysis. The March samples were analyzed in the Oceanography department U.C.T.. Nitrate was measured as nitrite after reduction through a Cadmium column, while nitrite was measured by the standard diazotation technique as described by Nyadahl (1976). Ammonium was measured by the phenolhypochlorite method and soluble phosphate by the acid molybdate method (Grasshoff *etal.* 1983). Due to logistical problems the July and September samples were analyzed using a different technique to that used in March, in the form of an Ion chromatography method in the Geochemistry department U.C.T. (DIONEX AI-450 Chromatography method). This system makes use of ionpac analytical columns which are specifically designed to resolve a large number of inorganic and acid ions and cations from a single injection of sample using an hydroxide eluent system (DIONEX Automated sampler operation Manual). Phosphate measurements in September were analyzed using the same acid molybdate method as in March.

Algal sampling and identification

At each site collections were made of algae that appeared to be different to the naked eye and their abundance recorded according

to the following periphyton abundance classification of Quinn (1991):

Class	Observation
0	not visible on hand held stones
1	visible on hand held stones
2	visible on bed covering few surfaces (<20% cover)
3	visible on bed covering many surfaces (20-50% cover)
4	visible on bed covering most surfaces (50-80% cover)
5	visible as complete cover of bed (80-100%)

Taxa were identified in the laboratory with the aid of two freshwater algae keys (Prescott 1954, Joska and Bolton 1993b). Unfortunately owing to a lack of fertile specimens no species determinations could be made, but all macroalgae (except two Cyanophyta species) were identified to the genus level. In those cases were more than one species in a genus were encountered, they where labelled differently and differences in their diagnostic features noted. Measurements of cell lengths and widths were taken and notes made on general diagnostic features.

Results

Environmental Data:
(For raw data see Appendix 1)

Water temperature (Fig.2)

Temperatures decreased from between 24 and 26 degrees in late summer to between 11.5 and 13.5 in mid winter. The autumn and spring temperatures were similar to each other and intermediate between summer and winter (14-18 C). Temperature generally varied very little between sites, although there was a trend for a slight increase in temperature downstream in July and September.

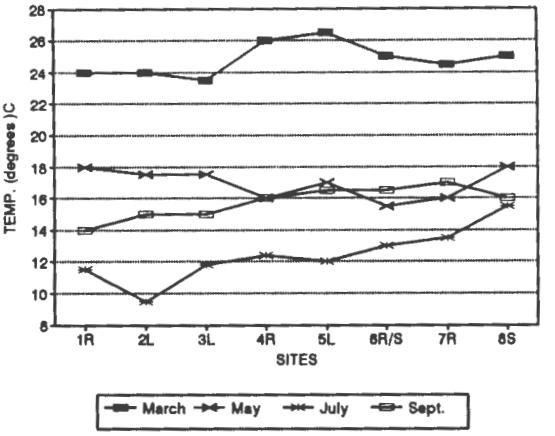

Fig. 2. Seasonal variation in temperature at the eight study sites.

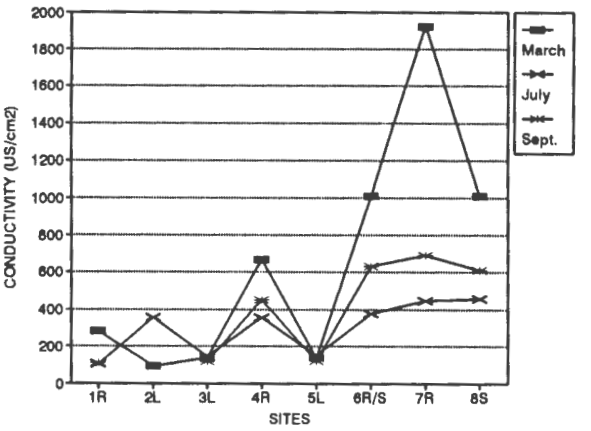

Fig. 3. Seasonal variation in conductivity at the eight study sites.

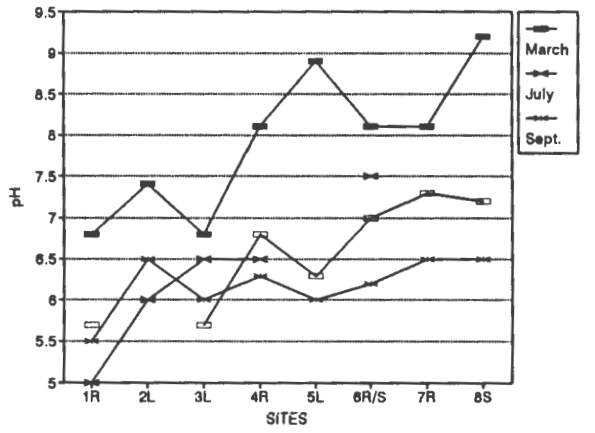

Fig. 4. Seasonal variation in pH at the eight study sites. (not all sites were sampled in May)

Conductivity (Fig.3)

Conductivity increased with distance downstream at all three sampling times. It increased on average 10 fold between sites 1 and 7. Conductivity was lowest in winter at all except the La Chasseur canal sites. With the exception of these sites conductivity was highest in March and intermediate in September. Conductivity at all sites along the La Chasseur canal was very low (remained at similar levels to the upper river site (1R)), while Sanddrift canal values were very similar to those observed in the lower river sites.

pH (Fig.4)

pH increased with distance downstream in the river and the canals, with the increase being smallest in July and greatest in May. Generally pH was highest in March, decreased until July before increasing again by September. pH showed no distinguishable pattern between the river and the two canals.

Water speed.

Water speed at the three river sites (1R, 4R and 7R) was very slow (in the order of 0.01 $m.s^{-1}$) when compared to the two canals (0.1 - 0.58 $m.s^{-1}$). Water speeds were lowest in March at all sites, and generally highest in July. In September the waters were still flowing much faster in the canals than they had in March. Water speeds decreased along the canals with distance from their source and were slightly faster in the La Chasseur canal when compared with the Sanddrift canal.

Nutrient load

The results of the March and July/September nitrate analyses are examined separately owing to the differences in analytical techniques used for measuring their concentrations. It is unfortunate that the two methods produced markedly different results for nitrate (fig 5), while the ion chromatography method was unable to detect any ammonium or nitrite owing to the

concentrations being below the methods range of detection. The results do nevertheless provide important information regarding the relative differences in concentration between sites at the various sampling times. Nutrient concentrations quoted in the discussion are those measured in March. They are similar to the data obtained from the official gauging station on the La Chasseur canal in March 1994.

Nitrate (Fig.5)

Nitrate concentrations were highest at the three river samples from Robertson to Bonnievale. Nitrate levels in the canals were generally lower than in the river.

Phosphate (Fig. 6)

There was little difference between March and September phosphate levels. In March phosphate concentrations were highest at Robertson Bridge and decreased downstream to Bonnievale. There was only a 0.06 mgl-1 variation in mean P04-P concentrations in March. In September there was even less variation in phosphate concentrations between the sites, with the river site at Bonnievale showing the highest concentration.

Nitrite (Fig.7)

Concentrations of nitrite varied by only 0.07mgl-1 between the sites. The highest concentrations occurred at the river sites at Robertson and Bonnievale. The high levels experienced at Bonnievale probably resulted from the downstream conversion of the ammonium produced at Robertson to nitrite.

Ammonium (Fig.8)

Concentrations of ammonium increased to a peak at Robertson bridge and then declined again downstream. Once again variation between sites was very small (0.2mgl-1)

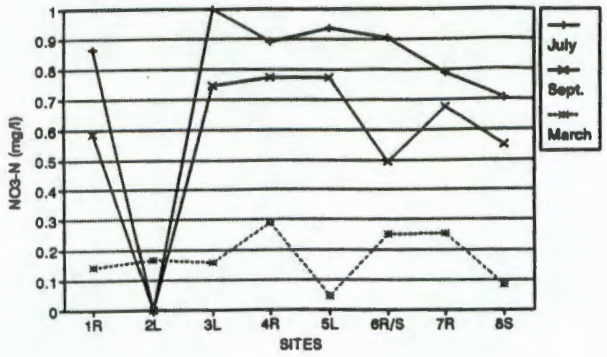

Fig. 5. Concentrations of soluble nitrate at the eight study sites at three sampling times. The dashed line represents the March samples which were analyzed using a different method to the July and September samples (solid lines). In all cases values are the mean of three water samples.

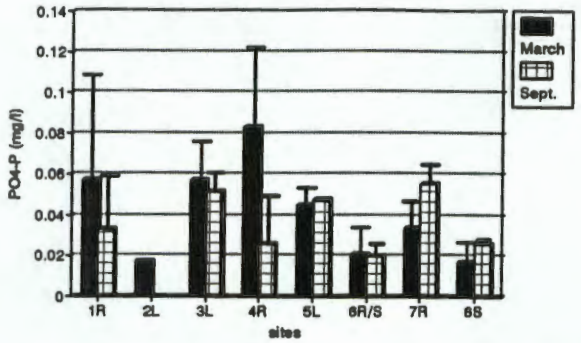

Fig. 6 The concentration of ortho-phosphate at the eight sites in March and October. No value was obtained at site 2L in October as the canal was empty. All values are the mean of three water samples and standard deviations are included.

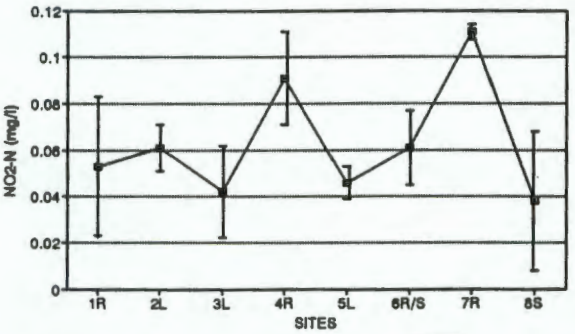

Fig. 7. The concentration of nitrite at the eight study sites in March. All concentrations are the mean of three water samples and the standard deviation is included.

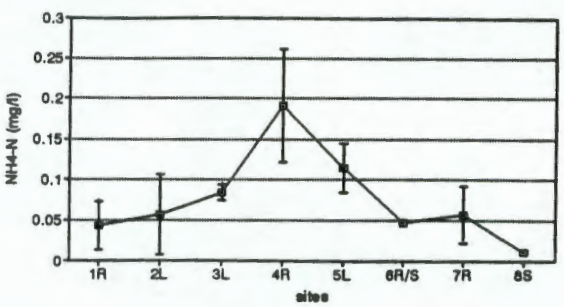

Fig. 8. The concentration of ammonium at the eight study sites in March. All values are the mean of three water samples and standard deviations are included.

Species Data:

Table 1. Description of filamentous algae species collected in this study. **(classification of taxa from Bold and Wynne 1985)**

1. DIVISION CHLOROPHYTA

A. Order: Oedogoniales

Family: Oedogiaceae

Genus: Oedogonium

Species 1:

Species Description: Some cap cells occur along the unbranched filaments. Cells light green, rectangular shape with right angle corners. Cells contain 10 pyrenoids embedded in a single large mesh-like chloroplast.

Habitat: found growing mostly unattached to branches and roots of terrestrial plants in the canals.

Cell length: 70 - 105 μm, average 79.3 μm
Cell diameter: 56 - 77.5 μm, average 68.8 μm

This species was found at site 2 in July and September, site 3 in July and September, and site 8 in July.

Species 2:

Species Description: Some cap cells visible along the unbranched filaments. Cells less green under microscope than species 1, with 8 pyrenoids. Filaments up to 2 meters long.

Habitat: Growing attached to sediment and walls of shallow, fast flowing canal.
Cell length: 84 - 108.5 μm, average 97.1 μm
Cell diameter: 56 - 59.5 μm, average 58.6 μm

This species was only found at site 5 in July and September.

B Order: Ulotrichales

Family: Chaetophoraceae

Genus: Stigeoclonium

Species: Stigeoclonium tenue

Species Description: This species is characterized by highly branched filaments with pointed, hair-like tips. Filaments cling together in gelatinous balls when removed from water. Cells are very small. Chloroplasts are parietal and in the larger cylindrical cells. There are 3 to 7 pyrenoids per chloroplast.

Habitat: Growing epiphytically to reeds.

Cell length: 8 - 20.5 μm, average 14.5 μm
Cell diameter: 6 - 15 μm, average 12 μm

This species was observed at site 4 in March, site 5 in March, site 6 in May and site 8 in March.

Genus: Cladophora

Species 1:

Species Description: Filaments highly branched, branching visible to the naked eye. Cells very large, filled with a single dark green chloroplast giving the cell a granular appearance.

Habitat: Found attached to rocks in riffles of fast flowing weir.

Cell length: 380 - 700 μm, average 504 μm
Cell diameter: 90 - 107 μm, average 98 μm

This species was found only at site 6, in March, May and September.

Genus: Rhizoclonium

Species 1:

Species Description: Filaments unbranched, individual cells filled with dark green 'lumpy' matrix, and no other structures visible. Faint layers visible near the end walls.

Habitat: Attached to the side of a concrete weir, in moderately fast flowing waters.

Cell length: 91 - 122.5 μm, average 108.5 μm
Cell diameter: 35 - 38.5 μm, average 35.75 μm

This species was only observed at site 6 in May.

C Order: Ulvales

Family: Ulvaceae

Genus: Enteromorpha

Species: Enteromorpha intestinalis

Species Description: Numerous very small cells forming uniformly green sheets of long hollow tubes, walls one cell thick.

Habitat: attached to reeds growing in slow moving waters adjacent to river bank.

Cell diameter: average 15 μm

This species was observed at site 4 in March and May, site 6 in March and May, and site 7 in March.

D order: Zygnemales

Family: Zygnemataceae

Genus: Spirogyra

Species 1

Species Description: Filaments unbranched containing two distinctly spiralled chloroplasts. Each chloroplast with numerous pyrenoids (much paler than chloroplast, which is bright green). Cells plane end. Some fertile cells at site 1 in March, zygospore Length 103 μm, width 58 μm.

Habitat: Found attached to rocks or canal wall in narrow band along the splash zone.

Cell length: 140 - 250 μm, average 220 μm
Cell diameter: 40 - 62 μm. average 49 μm

This species was observed at site 1 in March and September, site 2 in May, and site 3 in March.

Species 2:

Species description: Filaments unbranched containing five distinctly spiralled chloroplasts. Cells dark green, densely packed with chloroplasts.

Habitat: slow moving shallow waters in amongst reeds.

Cell length: 17 - 28 μm average 21 μm
Cell diameter: 4.5 - 5.5 μm average 5 μm

This species was observed at site 6 in May and site 7 in March.

Species 3:

Species description: Filaments unbranched containing 2 or 3 spiralled chloroplasts. Cells plane end.

Habitat: attached to rocks.

Cell length: 98 - 285.5 μm, average 208.25 μm
Cell diameter: 47.8 - 50.75 μm, average 49.5 μm

This species was found at sites 7 and 8 in March.

Genus: Zygnema

Species 1:

Species description: Cells contain two distinctly stellate chloroplasts, each chloroplast containing one pyrenoid. Filaments brown and dead looking to the naked eye, but light green under the microscope.

Habitat: in still waters on the floor of a non-flowing canal.

Cell length: 69 - 106 μm, average 91 μm
Cell diameter: 36 - 41 μm, average 38.5μm

This species was observed at site 2 in September.

2. DIVISION RHODOPHYTA

A. Order: Compsopogonales

Family: Erythrotrichaceae

Genus: Comsopogon

Species 1

Species description: Thallus made up of densely packed small cells around a central row of cells, branching frequently. Thallus pale pink, slippery to touch.

Habitat: Found in slow flowing waters, growing epiphytically on grass roots.

Cell dimensions: not measured.

This species was found at site 1 in March.

3. DIVISION CYANOPHYTA

A. Order: Oscillatoria

Family: Oscillatoriaceae

Genus: Oscillatoria

Species 1:

Species description: Trichome solitary with no sheath and tapering cap like structures on the end. Light blue in colour, intertwined with other species. Cells square shaped, right angle corners.

Habitat: Always found in amongst other filamentous algae, in particular *Spirogyra*, as a small proportion of total biomass.

Cell length: average 2 μm
Cell diameter: average 6 μm
This species was observed in March at sites 2, 3 and 4, and in May at site 4.

4. CHRYSOPHYTA

A. Order: Heterosiphonates

Family: Vaucheriaceae

Genus: Vaucheria

Species 1:

Species description: Large filaments with no individual cells. Occasionally branched at right angles to main filament. Filaments green in colour with a granular texture.

Habitat: Only found in amongst dominant growths of *Oedogonium* sp.1 in moderately fast flowing waters. Not found growing attached to any substrate.

Cell diameter: 84 - 119 μm, average 109.5 μm

This species was observed at site 3 in July and September.

B. Order: Raphidomonadales

Family: Bacilliarophyceae

Genus: Melosira

Species 1:

Species description: Cells shaped like capsules, with many inconspicuous disc-like golden chloroplasts situated around the periphery of the cell. This species forms a brown film in the water which has the appearance of dead filaments.

Habitat: not attached, floating in still waters.

Cell length: 29.75 - 30.5 μm, average 29.8 μm
Cell diameter: 15 - 18 μm, average 17.5 μm

This species was observed at site 8 in July and site 4 in September.

Two species of Cyanophyta (Blue-green sp1 and Blue-green sp2) were also collected, however their dimensions and diagnostic features were not noted. Blue-green sp1 was found at site 4 in March, while Blue green sp2 was found at site 8 in March.

Algal taxa at each site.

Site 1R

On the first field trip (March), water was still being released from the Brandvlei dam into the river at this site. A 10cm wide zone of *Spirogyra* sp 1 was found growing on the rocks at the dam outflow. In May the dam discharge had ceased, this zone had disappeared and only a few small tufts of *Compsopogon* could be found at this site. In July no algae was present. It was evident that the water line had risen and fallen by some 6 meters during the period since the May field trip. In September the same *Spirogyra* sp1 as had been evident in March reappeared and was again growing in a zone along the water line. The dam was still not releasing water in September.

Site 2L

In March water was being discharged into the canal from the Kwaggashoek Dam. There existed a dense 100% cover of *Spirogyra* sp1 attached to the canal walls in a 10cm zone along the splash zone. In May the water was not running and stood approx. 0.3 m deep. Some *Spirogyra* sp1 was still evident in scattered clumps attached to the sediment. The *Spirogyra* was no longer evident in the July field trip when *Oedogonuim* sp1 was collected from a wooden plank over which water still trickled. By September the canal was almost dry (2cm deep) and only a few scattered tufts of *Zygnema* and *Oedogonium* sp1 were found in the bottom sediment.

Site 3L

This site was characterized by very low algal biomass during the first three visits of the year and a marked increase by the September sampling trip. In March there was a few small filaments of *Spirogyra sp1* and *Oscillatoria* growing attached to the wall of a side canal. The site was not visited in May but in July there were a few strands of *Oedogonium* sp1 growing unattached on branches of trees in the canal. *Vaucheria* filaments were found in the *Oedogonium* and made up about 5% of

total biomass. By September there were long strands of *Oedogonium* sp1 (up to 40cm) occurring at the water level all along the canal wall. This species, although prolific on any plant debris, lying in the water was not found attached to the canal walls. *Vaucheria* was again found within the *Oedogonium* and made up about 20% of total biomass.

Site 4R

Algal biomass was generally low during all sampling visits to this site. In March and May *Enteromorpha* (probably *E.intestinalis*) was commonly found growing attached to the roots of reeds, or unattached floating in still water. The Blue green *Oscillatoria* was also found attached to the reeds in small amounts at these sampling times. Following the heavy rains no algae were found in July. While in September *Melosira* and *Stigoeclonium tenue* had colonized the reeds but no *Enteromorpha* was visible.

Site 5L

In March *Stigeoclonium tenue* was found growing attached to roots hanging into the canal. The site was not visited in May, but in July about 50% of the canal bed was covered with *Oedogonium* sp2. Filaments removed from the canal were found to be up to 2 metres long. This species of *Oedogonium* grows attached to pebbles as well as the canals concrete wall. In September, growth of this species had increased to cover approximately 80% of all surfaces. Large pieces of filaments were seen drifting in the water down the canal.

Site 6R/S

The canal itself was closed during all except the last sampling trip, however, sampling was carried out in the inflow canal on the river side of the sluice gate. The waters of this canal are homogenous with the Breede River at that point and it could thus also be included as a river sample. In March small amounts of *Enteromorpha* and *Oscillataria* were found attached to the

canal/weir wall, while a profuse population of *Cladophora* was found growing in the fast moving riffles of the weir, just below the sampling site. In May small amounts of *Rhizoclonium* and *Enteromorpha* were found growing in the shallow stagnant waters of the canal itself, while limited growth of *Oedogonium* sp2 was found in the river. No algae were found in July. A dense colony of *Cladophora* had recolonised the weir riffles in September.

Site 7R

Spirogyra sp2 and *Spirogyra* sp3 as well as *Enteromorpha* were found in small amounts in a non-flowing pool below the bridge in March. *Enteromorpha* and *Spirogyra* sp2 were again found in May. After the rains the pool disappeared and no algae were visible during the July and September trips.

Site 8S

This site was empty in July owing to it being rebuilt and contained no algae in September as it had only recently been re-opened. In March the majority of the walls as well as approximately 40% of the bottom of the canal were covered with filaments of *Spirogyra* sp3. Approximately 1% of the algae biomass was made up of *Stigeoclonium tenue* and an unidentified blue green algae (Blue green sp2). In May the canal had been closed upstream for repair and although there was some water in it, no algae were visible. In July *Melosira* was found growing in the Sanddrift canal at Bonnievale. In September the canal at site 8 was flowing again but no algae had become established.

Discussion

During the course of the year there exists a marked seasonal change in the physical conditions in the waters of the Breede River and its adjacent canals. Long dry summers result in low stream flow, high temperatures (max. 26.5°C), high conductivity (max. 1923 μSiemens.cm^{-2}), increased pH (max 9.2) and increased nutrient load. Winters in contrast produce cold conditions with high intensity rainfall events which flush the system with clean water. This results in major decreases in the afore-mentioned physicochemical parameters, as well as greater spatial uniformity of these measurements between the sites. By September, atmospheric warming and reduced flow result in a return towards the summer conditions.

With the exception of the July visit, when high rainfall had resulted in greater uniformity between all waters sampled, spatial variation in environmental conditions was considerable between sites. Temperature, conductivity and pH showed a general increase with distance downstream in the river, and in the canals, while differences existed between indices of eutrophication in canals and adjacent river sites. The lower levels of conductivity and nutrients in the canals act as evidence for eutrophication in the river environment. Levels of all four nutrients analyzed increased considerably at the Robertson Bridge site when compared with measurements upstream. This is most likely the result of treated sewage effluent being added to the river from the Robertson sewage works just downstream of this site (Joska pers. comm.). Using the data from the March sampling it is apparent that although this site shows levels of nutrient load considerably higher than others surveyed in this study, the levels recorded are not particularly high in relation to other published data. As an example, downstream of the Taupo sewage treatment site in the Waikato River, Australia, concentrations of PO_4-P increased from 0.5 to 4 mgl^{-1} (as against 0.02 to 0.08 mgl^{-1} in this study) (Quinn 1991). Immediately below a trout hatchery outflow pipe in an upper tributary of the Breede river (Du Toits Kloof Valley), Bolton and Brown (1993) reported an increase in PO_4-P concentration from 0.02 to 0.4 mgl^{-1} and in

NO_3-N from 0.2 to 2 mg^{-1} (as compared with 0.15 to 0.3 mgl^{-1} in the present study). However conductivities from the Robertson Bridge site onwards are high in relation to other published data. They equal the highest recorded by Entwisle (1989) in polluted waters of the Yarra river in Australia (1923 μSiemens.cm^{-2} as against 2160 μSiemens.cm^{-2}), and are higher than those measured in the lower reaches of the Thames in England (max. 966 μS.cm2) (John etal. 1990). Earlier measurements along the same stretch of the Breede River by Schreuder etal. (1988) and Joska and Bolton (1993) both produced maximum conductivities (approx. 1000 $\mu S.cm^{-2}$) much lower than those recorded in this study.

These seasonal and spatial differences in physicochemical properties of the water in turn have a direct impact on the filamentous algal biota at each site. The only substantial growths of filamentous algae recorded during the study were encountered at two localities along the La Chasseur canal, at sites 3L and 5L in July and September. A similar, but better developed, bloom was recorded from the River side Road site (5L) in November 1993 (Joska and Bolton 1993a). In this bloom the alga concerned was identified as *Oedogonium capillare* which formed a 30% cover on the sides and the bottom of the canal walls. Uncertainty exists as to whether the *Oedogonium* found growing at this site in 1994 was the same species, as it was growing unattached, caught by branches and roots of trees lying in the canal. This may be because the growth is in an early stage of development, having drifted from upstream, and will only become attached once spores are produced. Further research needs to be carried out into the life cycle and development of this problem species. The alga responsible for the bloom at Kaisershof (5L) was also identified as an *Oedogonium*. This species was firmly attached to all substrate including the concrete canal wall and floor. It has been recorded as a separate species in this study owing to its characteristic 6 - 8 pyrenoids, as against the 10 pyrenoids of the River side Road (3L) filaments. It is however conceivable that this division is artificial, and that the two *Oedogonium* species forming blooms under similar environmental conditions are in fact one species (possibly *O. capillare*). *Oedogonium* was the only

genus which formed excessive growths during the course of this investigation. It was found at 5 sites, but only in July and September. According to Joska and Bolton (1993) *Oedogonium* is not a common problem alga, and although it is found in the majority of South African rivers, it generally forms only a small percentage of total algal biomass. Joska and Bolton (1993) suggest that the pollution tolerance of certain *Oedogonium* species could enable them to form large growths under favourable conditions. Both sites at which excessive growths were recorded showed similar, moderately high levels of PO_4-P (0.05 mgl^{-1}) and NO_3-N (1.0 mgl^{-1}) in September. Similar levels of nutrients were found at Bonnievale Bridge (7R) were *Oedogonium* was absent, however flow rates were much lower at this site. As mentioned earlier, it is important to note that although these levels of nutrients are high for the sites investigated in this study, they do not represent excessively eutrophicated waters. The other characteristic shared by these two sites was their very low conductivity measurements (120 $\mu S.cm^{-2}$ in September). With the exception of the Brandvlei Dam outlet (1R), all the other sites showed conductivities at least 3-fold higher than those measured at these sites. It can thus be hypothesised that the rapid growth of *Oedogonium* in the La Chasseur canal is favoured in spring by moderately high levels of nitrates and phosphates in fast flowing waters in conjunction with low levels of conductivity. These findings are supported by Biggs (1990) who, with the aid of discriminant analyses, showed that Oedogonium dominated communities in New Zealand rivers are characterized by low conductivities and medium to high levels of dissolved reactive phosphorous.

Physicochemical parameters can also be linked with the occurrence of some of the other taxa found in this study. *Enteromorpha intestinalis* was observed only in March and May, and only at the three lower river sites 4R,6R,7R. These sites are characterized by high conductivity (668-1923 $\mu S.m^{-2}$) and high pH (> 6.5). Furthermore this species was only found in areas of slow water movement, attached to reeds at sites 4 and 6, and in a rock pool at site 7. Ten Cate <u>etal.</u> (1991) reported that *Enteromorpha* in Dutch freshwater ditches is favoured in eutrophicated waters with

values below 5. The pH of 5 in May is thus a further possible reason for the decline of the species at this site. *Spirogyra sp1* also occurred at site 2 where it continued to grow in the cooler waters in May with a pH of 6. Certain species of *Spirogyra* have been suggested as indicators of pollution (Biggs 1987), while other studies have described member of this genus as occurring in clean to slightly polluted waters (Entwisle 1989, Simons and van Beem 1990). Joska and Bolton (1993) emphasized the importance of determining which species in local rivers tolerate pollution and which species are indicative of good water quality. From this study it would appear the *Spirogyra sp1*, which was only found in the upper three sites, is confined to low conductivity, low eutrophicated waters, while *Spirogyra sp2* and *Spirogyra sp3* are more tolerant to pollution, as is evident from their occurrence at sites 6, 7 and 8.

Stigeoclonium tenue was found in small amounts at various sites during all except the July field trip. Although not restricted to organically polluted environments, this species is often used as an indicator of eutrophication owing to its high tolerance to pollution (McLean and Benson-Evans 1974). *S.tenue* was correlated to sites of high nutrient input from trout farms in the Molenaars River, Western Cape (Guthrie 1992). In this survey *S.tenue* was only found at sites from Robertson Bridge (site 4) and downward. As mentioned earlier, levels of all four nutrients analyzed increased considerably at the Robertson Bridge site when compared with levels upstream. Another species found in the quiet waters at Robertson Bridge (4R) was the diatom *Melosira* which is commonly found in polluted or eutrophic waters (Joska and Bolton 1993b). This species has been noted as a reliable 'indicator' species of waters with high conductivity and temperatures >17oC in New Zealand waters (Biggs 1990). It was also found in the stagnant waters of the Sanddrift canal at Bonnievale in July, where the temperature was a relatively low 16^0C, but conductivity was the highest measured at any site in winter ($460\mu S.m^{-2}$). The presence of *S.tenue* and\or *Melosira* sp. at a site in the Breede River system could therefore be used as a probable indicator of high conductivity, eutrophic waters.

CHAPTER 2. A RAPID SCREENING TEST FOR COPPER TOLERANCE IN FRESHWATER FILAMENTOUS ALGAE.

Introduction

Copper is a necessary element for all living organisms, however at increased concentrations it can prove fatal to filamentous algae. Consequently copper, in the form of copper sulphate, is the most commonly used algicide for the control of excessive growths of this group of organisms. The tolerance of filamentous algae to copper varies considerably between taxa, and to a lesser degree within taxa from different sites (Whitton 1970a). Tests into the tolerance of filamentous algal taxa to copper have all taken the form of growth or photosynthesis inhibition experiments, utilising low copper concentrations (Whitton 1970, Steeman Nielsen and Wium-Andersen 1970, Hillebrand and De Vries 1986, Bartlett etal. 1973, Takaruma etal. 1989, Singh and Chaudhary 1993). These tests require a duration in the order of 7 days to obtain enough information on the level of growth or photosynthesis inhibition that a species experiences at each concentration. While they may be effective at demonstrating differences in relative tolerance levels between isolates or taxa, they provide little practical value for setting concentrations and durations necessary for a complete kill, as is necessary in control programs.

There is thus a need to develop a more rapid test which could be utilised to determine the concentration, and duration time necessary for the control of local Breede River taxa. The technique for determining cell mortality used in this study was developed from that used by Goodman et al (1976) and is based on the observation that living plant cells plasmolyse in hypertonic solutions whereas dead cells do not. The behaviour of the cell membrane therefore gives an immediate indication as to the effectiveness of a particular copper treatment.

Copper sulphate treatment of algal blooms in the Breede river canal system is administered by the placing of hessian bags

suspended from poles into the flowing waters during periods of high algal biomass (Joska pers. comm.). This programme would appear to have little scientific basis, with minimal knowledge as to the concentration of copper being added to the waters, its duration time, or the levels of copper required to kill the local problem species. It is with the aim of investigating this last mentioned unknown that it was decided to develop this rapid test for determining the copper tolerance of problem filamentous algal species.

While developing this assay it became apparent that the chemical characteristics of the medium influences the toxicity of copper on algae. Certain chemicals with affinity towards metals act as ligands which complex or chelate metals, thereby greatly reducing their toxicity (Stokes 1983). EDTA (ethylene diamine tetra acetic acid) is the synthetic ligand utilised as the metal chelator in the growth medium used in the assay. Another parameter reported to influence the effectiveness of copper as an algicide is the pH of the solution (Bruwer 1991). The influence of these factors on the toxicity of copper to algae were therefore also investigated.

Methods

Culture techniques

Filamentous algal specimens were collected on three separate occasions from various river and canal sampling sites in the Breede river valley. These were transported to the laboratory where they were rinsed in distilled water before being transferred to growth medium. The growth medium used throughout this investigation was Woods Hole which is fully described in Stein (1973). Specimens were kept in transparent plastic bottles with 500 ml of medium which was changed once a week. They were incubated on a shaker at 55 revolutions/min, in the U.C.T Botany department phytotron. The settings for the phytotron were 15°C, relative humidity 50%, day length (16:8) and light intensity 90 - 120 $\mu E\ m^{-2}s$.

Copper concentrations

The range of copper concentrations required for the bioassay were generated by serial dilutions of a 1 molar stock solution of copper sulphate ($CuSO_4.5H_2O$) made up to a volume of 1l with glass-distilled water. The stock solution was then added to the standard medium (Woods Hole without any $CuSo_4.5H_2O$) so as to obtain the required copper concentrations of 0.1, 1.0, 2.5, 5.0, 7.5 and 10.0 mgl-1.

Derivation of the assay

A pilot study was undertaken to formulate the protocol for the bioassay. By treating algal filaments in the above 6 copper solutions for various lengths of time ranging from 10 mins to 8 hrs it was possible to ascertain the length of time required before the copper had no further influence on the cells. In this way the optimum time period for the copper treatment was established. The pilot study was also used to determine whether the impact of copper on cells may not be immediate, but only expressed some time interval after removal from the treatment. After being removed from the copper solutions and washed in distilled water, the filaments were transferred to bottles containing the standard medium. These were then kept in a shaker under the same standard phytotron conditions as noted above. Filaments were extracted and examined under the microscope at various time intervals from 10 minutes to 8 hours after removal from the copper solutions. This data could then be used to determine the length of time required, after the filaments are removed from copper solutions, before cell mortality should be estimated.

From the results of the pilot survey the following bioassay for the rapid screening of copper tolerance in filamentous algae was derived;

1. Roughly equal sized samples (approximately 250 mg) of the filamentous algae are detached with tweezers and washed in distilled water.

2. These are then transferred to one of seven 20 ml petri-dishes (one filled with distilled water as a control and the others containing the six copper solutions). Triplicate sets of the seven petri dish treatments are required for replication.
3. After one hour the filaments are removed from their copper solutions, rinsed in distilled water, and placed in 50 ml of fresh Woods Hole medium in glass bottles. These are then placed on a shaker under controlled conditions.
4. After a further hour in the phytotron, the filaments are removed from the bottles, damp dried on filter paper, and transferred to petri dishes containing 33% w/v sucrose solution (as used by Goodman et al 1976).
5. After 10 minutes, the material is placed on a microscope slide, mounted in sucrose solution with a coverslip. The slides are then examined microscopically and the numbers of plasmolysed and unplasmolysed cells counted, 50 cells being scored from 5 random fields of view.

An important tip when examining the various filaments for plasmolysis is to first examine those cells which were treated at the lowest concentration of copper (0.1 mgl^{-1}) to gain a clear indication of the form of the healthy (plasmolysed) cells. Then examine the opposite extreme (filaments treated in 10$mg.l^{-1}$) before counting the number of plasmolysed cells in the intermediate concentrations.

Running the Test

Three filamentous algal species common in the Breede river, *Spirogyra* sp1, *Stigeoclonium tenue* and *Oedogonium* sp2, as well as *Cladophora glomerata* from Kalkfontein (canal system 30 km from Kimberley in the Free State, South Africa), were tested for copper tolerance using this bioassay. Plasmolysis of healthy cells and the appearance of damaged cells of *S.tenue, Oedogonium* sp2 and *C.glomerata* are shown in plates 1 to 4. The method of analysing damage had to be adjusted slightly for *Spirogyra* as its copper-damaged cells closely resemble plasmolysed cells. However

the damaged cells of *Spirogyra* are easily differentiated from healthy cells by the contraction of their spiral chloroplasts which is clearly visible under the microscope (plate 5). *Spirogyra* was therefore not placed in the sucrose solution but examined directly for chloroplast damage. The statistical significance of the differences in copper tolerance between the four species was examined using a Type I, two-factor analysis of variance and the Tukey test for determining between which population means differences exist (Zar 1984)

Post-treatment recovery

The ability of filaments to recover from high concentrations of copper was examined. Filaments of all four species were treated over various time spans at high concentrations of copper, examined for mortality and then placed in fresh copper-free growth medium and returned to the phytotron. After one and two weeks they were re-examined to ascertain levels of recovery from the treatments. The percentage recovery records the fresh growth since treatment, as a proportion of the pre-treatment size of the filament (which remains visible as blackened cells, see plate 6). A value of 100% indicates that the filaments have reached their pre-treatment size or greater.

Influence of pH.

The influence of pH on the toxicity to filamentous algae of copper in solution was tested by running the assay at pH's of 7.5 and 6.5. The pH of the six copper sulphate solutions, the controls and the copper-free growth medium were adjusted to the required level by the addition on a stirrer of HCl and NaOH. The bioassay was then run and cell mortality measured in the normal way.

Influence of EDTA.

In order to test whether the presence of EDTA masks the toxic effect of copper, the bioassay was run using normal Woods Hole growth medium with and without EDTA. Because EDTA plays an

important role in complexing a variety of metals in the medium, its absence may have other deleterious impacts on algae. EDTA was therefore also left out of the copper-free control so as to test the possible impacts of other trace metals once the chelator was absent. All other factors which could have influenced mortality were kept constant (pH 6.5, temp 15^{0}C). Differences between the two treatments were tested for significance using the paired-sample t test (Zar 1984).

Results

Species investigated

For a description of *Spirogyra* sp1, *Oedogonium* sp2 and *Stigeoclonium tenue*, see table 1 in chapter 1.

Cladophora glomerata

Site of collection: Kalkfontein (Kimberley, Free State)
Filaments not branched, large dark green cells visible to the naked eye. Chloroplasts fill cell with dark green granular matrix.
Cell dimensions: diameter 90 - 102 μm, average 98 μm
length 610 - 745 μm, average 705 μm

Deriving the technique for the assay.

The initial detailed experiments were carried out on *Spirogyra* sp1 owing to the ease with which mortality of its cells can be observed. However later tests on the other three species were found to produce results consistent with those of *Spirogyra*. Only the *Spirogyra sp1* results will be discussed here. In the pilot tests mortality was measured using the following key;

0. CELLS IN NO WAY DAMAGED
1. CELLS DIFFERENT FROM CONTROL ALTHOUGH NO EVIDENCE FOR DEATH.
2. < 50% CELLS DEAD
3. 50% TO 99% CELLS DEAD
4. ALL THE CELLS DEAD

Fig. 9 (A to E) graphically depicts the damage (using this key) caused by the six copper concentrations for some of the exposure times between 10 mins and eight hours examined in the pilot survey. The z-axis depicts the time required after removal from copper exposure before damage is fully expressed.

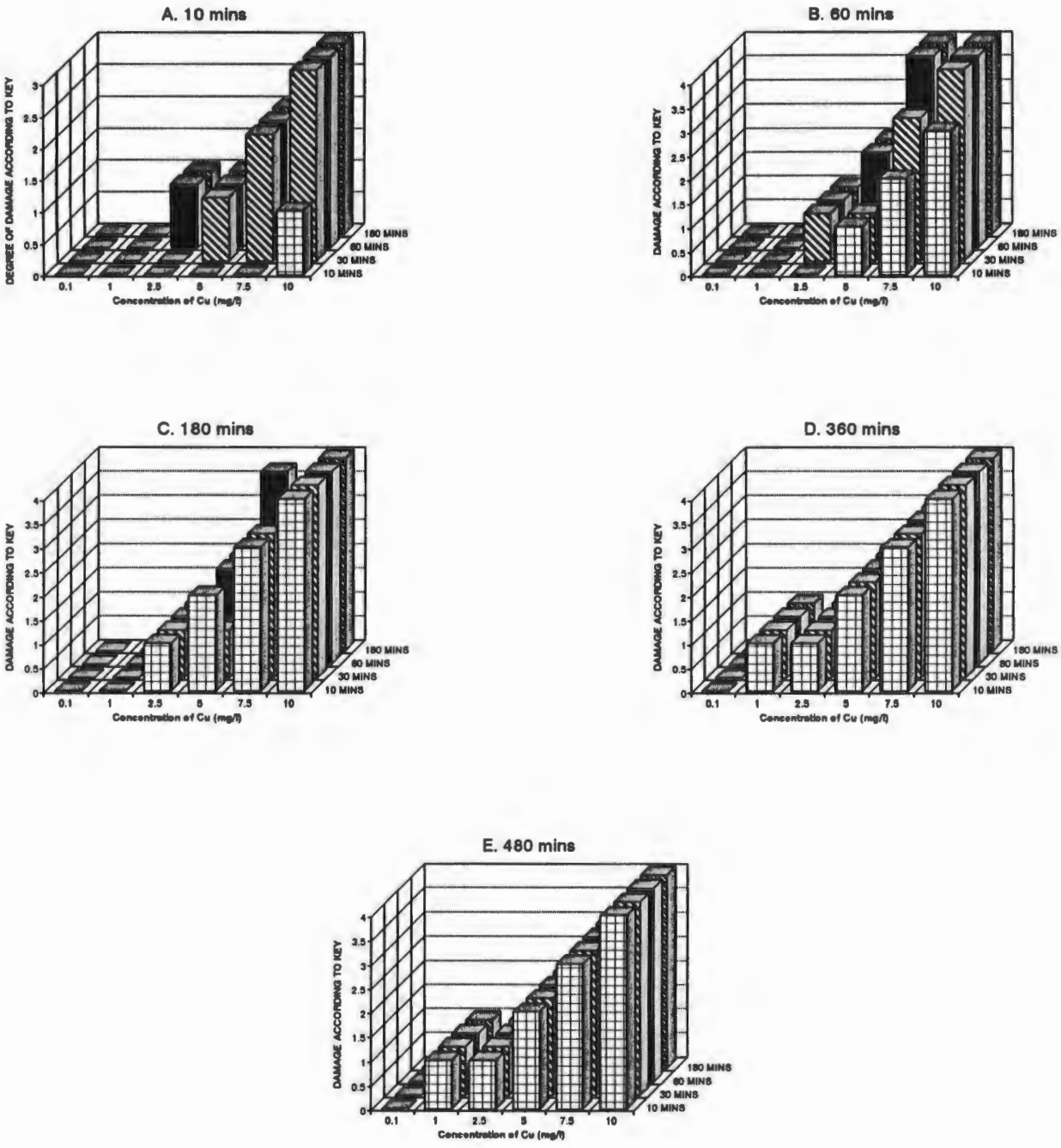

Fig. 9 Exposure of *Spirogyra* sp1 to the six copper concentrations for A) 10 mins, B) 1 Hr, C) 3 Hrs, D) 6 Hrs and E) 8 Hrs. Z-axis is time after removal from copper before damage was observed.

Table 2. The time required in the various concentrations to allow for the maximum damage of copper on *Spirogyra*.

	Concentration					
	0.1	1.0	2.5	5.0	7.5	10.0
maximum damage according to key	0	1	1	2	3	4
Time required in copper (minutes)	N/A	360	60	60	30	30

All the concentrations with the exception of 0.1 $mg.l^{-1}$ resulted in some visible degree of damage to the *Spirogyra* filaments if exposure time was long enough (fig.9 and table 10). With the exception of the 1.0 $mg.l^{-1}$ concentration, the impacts of the copper treatments were completed within the first hour of exposure. After removal from the 1 hour exposure all damage was expressed within a further 60 minutes (fig.9).

Determining the tolerance of some common species using the test.

According to analysis using multi-factor ANOVA there is a significant difference between the concentrations causing mortality in the four species (F-ratio = 9.640, significance level < 0.0001). More useful are the results of the Tukey test which indicate that *Stigeoclonium* is significantly different from the other three species, which in turn are killed at concentrations quite similar to each other (Table 3).

Table 3. Tukey multiple range analysis for determining mortality differences between species.

SPECIES	TUKEY MEAN	HOMOGENOUS GROUPS	
Spirogyra	37.91667	X	
Cladophora	44.91667	X	
Oedogonium	51.75	X	
Stigeoclonium	66.667		X

Unfortunately this form of analysis clumps the concentration data for each species when comparing mortality between species. It thus does not give any indication of differences in mortality between species at a particular concentration. This can better be observed in the format of a bar graph (fig 10).

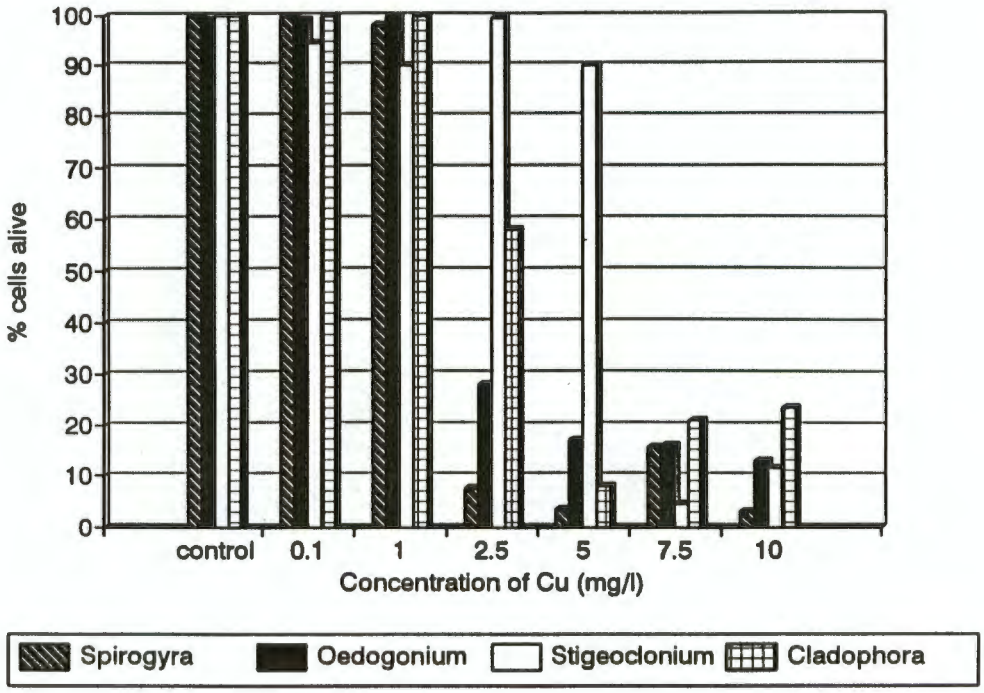

Fig. 10. Damage caused to four filamentous algae species exposed to copper for 1 Hr at the various concentrations.

There exists obvious differences in the tolerance levels of the species at each concentration. All the species are tolerant up to $1mgl^{-1}$, *Cladophora* is tolerant up to 2.5 mgl^{-1}, while *Stigeoclonium* is the most tolerant and is virtually unaffected by concentrations of copper up to 5 mgl^{-1}. None of the species are completely killed, even by the highest concentration.

Post-treatment recovery

Tables 4 and 5 as well as fig.11 demonstrate$ the ability of filamentous algae to recover from various degrees of copper poisoning within two weeks of the treatment.

Table 4. Recovery of copper treated filamentous algae after one week.

Species	Copper conc. ($mg.l^{-1}$)	time in copper (mins)	post-treatment % cells alive	% cells alive after 1 week
Spirogyra	7.5	60	1	10
	7.5	360	1	10
	7.5	480	0	0
	10.0	60	1	1
	10.0	480	0	0
Cladophora	5.0	60	8	50.5
	7.5	60	21	35
	10.0	60	20	28
Oedogonium	7.5	60	10	15
	10.0	60	11	32

After one week the filaments were able to recover from all except the eight hour treatments at high concentrations. However the maximum recovery achieved was to 50.5 % of the original filament size (see plate 6).

Table 5. Recovery of filamentous algae two weeks after treatment.

Species	Copper conc. (mgl^{-1})	time in copper (mins)	post-treatment % cells alive	% cells alive after 2 week
Spirogyra	5.0	300	9	55
	7.5	300	9	100
	10.0	300	0	100
Stigeoclonium				
	5.0	300	81.5	100
	7.5	300	7	100
	10.0	300	3.5	100

After two weeks their was a dramatic increase in the degree of recovery of all filaments examined, with most specimens recovering completely in this time (see plate 7). Table 5 demonstrates that both the most tolerant and the most sensitive species treated were able to fully recover. The recovery of the sensitive *Spirogyra sp.* is represented diagrammatically in figure 11.

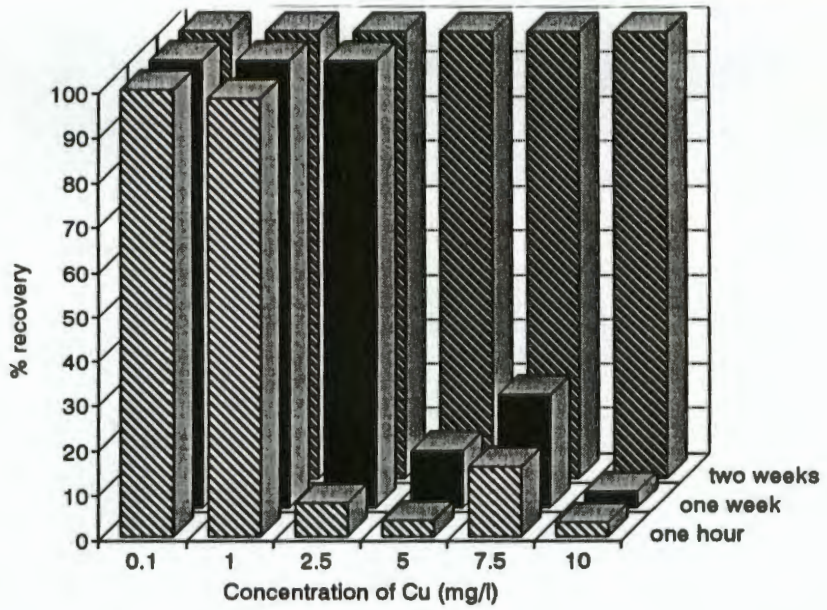

Fig. 11. The recovery over a two week interval of *Spirogyra* sp. filaments treated in the various copper concentrations for one hour. % recovery is an estimation of the number of cells which have grown since the treatment, as a percentage of the pre-treatment number. (100% indicates that the filament has regained at least its pre-treatment size).

The influence of pH.

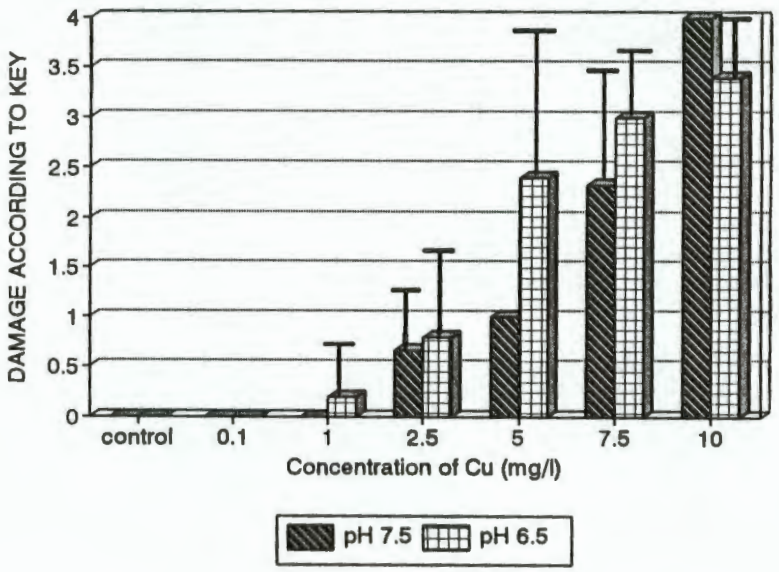

Fig. 12. Influence of two different pH values on the toxicity of copper sulphate to *Spirogyra sp.*(values are means of 3 replicates and standard deviations are included)

A decrease in pH from 7.5 to 6.5 resulted in an increase in the toxicity of copper to *Spirogyra*. However except at the 5 $mg.l^{-1}$ concentration, this increase was small. The experiment was run using damage according to the key (before the method was improved by counting damaged cells), and consequently no statistical test

could be conducted on the data. From the graph it would appear unlikely that any significant difference exists between the results from the two pH's. Time constraints prevented the test being repeated using cell counts. This will be necessary in order to gain a more accurate assessment of the impact of pH on copper toxicity towards filamentous algae.

Influence of EDTA on toxicity in culture.

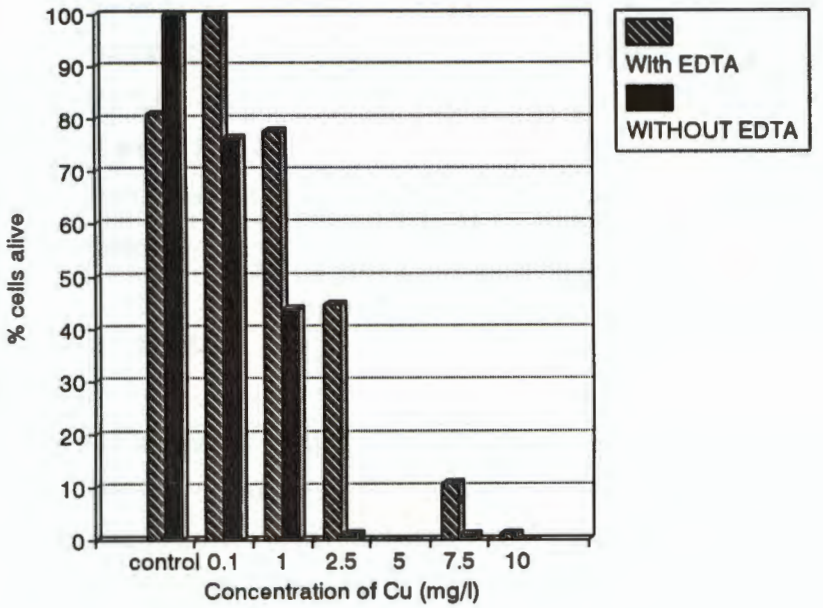

Fig. 13. Influence of EDTA on the toxicity of copper towards *Spirogyra sp. (sign. < 0.05)*

The mortality of *Spirogyra* cells increased considerably when the EDTA was removed from the copper solutions (Fig.13). This relationship was proven statistically significant using a paired t-test (significance level <0.05). The zero mortality in the control without EDTA indicates that the absence of EDTA from the medium has no short-term negative effects on the algae in copper-free medium.

compared with other published results. However the results of this rapid test do correspond well with published data on the broader generic level. Lembi et al (1988) state that *Cladophora* and *Stigeoclonium* are amongst the more tolerant genera, whilst *Spirogyra* and *Oedogonium* are more sensitive. *Stigeoclonium* is generally considered as very resistant to all heavy metals (Harding and Whitton 1976) and when compared with *Cladophora glomerata* was found to be much more tolerant to copper (Whitton 1970b). From the results obtained in this analysis it is feasible to rank the four species examined according to their tolerance to copper in artificial media. *Spirogyra* sp1 is the least tolerant to copper treatment, then follows *Oedogonium* sp2, *Cladophora*, with the most tolerant being *Stigeoclonium*.

Post-treatment recovery

Another interesting finding of this test was that for all species examined some cells survived even at the highest concentrations. Complete mortality of the most sensitive species examined (*Spirogyra sp1.*) only occurred after eight hours of exposure to the 7.5 and 10.0 $mg.l^{-1}$ concentrations. Although time until complete mortality was not determined for the other species, *Cladophora glomerata* has been reported to require a 10 mgl^{-1} concentration of copper for an exposure time of four days (Retzer

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Furthermore, filaments are not only highly resistant to copper treatments but they are also capable of rapid recovery. Filaments were found to be able to reestablish from single living cells after copper treatment (plate 5,6 and 7). While the first week after treatment is characterised by a gradual recovery, by the end of two weeks almost all of the algal material had recovered to their pre-treatment levels. As a result treatment with copper sulphate has to be repeated regularly over a short interval during the growing season. As an example, chemical control procedures in the Hartbeespoort canal system require

repetition every six weeks (Bruwer 1991), while Betzer and Kott (1969 cited in Whitton 1970)) stated that treatment in a saline channel in Israel had to be repeated at intervals of 5 - 10 days.

The influence of pH.

Bruwer (1991) reports that the algicidal potential of copper sulphate increases when pH is decreased from 8.3 to 6.5 in the Hartbeespoort irrigation canals. Conversely in acidic streams it has been reported that zinc toxicity in some species actually increases as pH increases from acid to neutral (Hargreaves and Whitton 1976). These changes in toxicity of heavy metals at different pH's could have important implications for the management of problem algal growths using algicides. To test the impact of changing pH on the toxicity of copper on species from the Breede River, the copper screening test was run at a pH of 7.5 and 6.5. The results suggest that a reduction in pH does increase the toxicity of copper towards the alga, however the significance of this relationship could not be tested and will require further investigation. According to Mervin Palmer (1980), a decrease in toxicity with increase in pH is as a result of copper sulphate precipitating as either copper carbonate or copper hydrate in alkaline waters. Furthermore the effectiveness of ligands in complexing metals is strongly pH dependent (Stokes 1983). This interaction could easily be tested by changing the pH of growth media including and excluding EDTA, and examining the impact of a particular copper concentration on algal cells. An analysis of pH data in Breede river canals indicate that although pH values do reach as high as 9.2 at one site, and are generally high in summer, the canal waters at site 3L and 5L where the major growths were encountered had pH values of 5.7 and 6.3 respectively (Fig. 4 and appendix 1). These low pH values could help explain the success of copper treatments without acidification in the Breede canals.

The influence of EDTA on the toxicity of copper in growth medium.

The toxicity of heavy metals to algae in synthetic media is governed by the chemical make-up of the medium. Fitzgerald and

Faust (1963) reported that the toxic concentrations of copper sulphate for *Chlorella* ranged from 1 to 8 $mg.l^{-1}$ depending upon the source of iron and chelating agent. The synthetic chelator in Woods Hole medium is EDTA (ethylene diamine tetra acetic acid) which according to Steeman Nielsen and Wium-Andersen (1970) can prevent any toxic impact of copper at concentrations found in nature. It was thus felt that this chelating agent could be contributing to the high concentrations of copper required to kill specimens in this study. By removing EDTA from the medium, there was a significant increase in the mortality of cells in *Spirogyra* filaments. Furthermore the zero mortality of the control (no copper or EDTA) suggests that the removal of EDTA does not have any impact on cell survival in the copper-free medium over the short term. Takaruma et al. (1989) omitted EDTA from their growth medium because it formed complexes with the added copper. Although all the tests performed on the various species in this project used medium with EDTA, the results of this experiment indicate that it may be worthwhile to exclude this component in the future. Alternatively a more realistic account of the requirements for successful copper treatment will be obtained by using canal water collected directly from the sites at which the algae occur. The inclusion of EDTA does, however, not reduce the accuracy of the earlier experiments with regards to the species comparative copper tolerances.

The rapid screening test for copper tolerance devised in this set of experiments proved effective in differentiating the copper sensitivities of four filamentous algal species. The relative speed of this assay enables the tolerance of algal species to be determined much faster than existing techniques described in the literature (see Whitton 1970). Although effective in delimiting the copper tolerances of the four species relative to each other, the methods used in these experiments may not give an accurate indication of the *in situ* tolerances of the species. If we are to gain an accurate indication of copper concentrations required to kill problem species in the field, these methods need to be adapted to better simulate the natural physical conditions of the canal environment. Nevertheless the basic protocol of the assay has been established and shown to be effective.

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APPENDIX 1

PHYSICOCHEMICAL DATA

Site No.	Temperature (degrees C)				Conductivity mS/m2)			pH				Water speed (m/s)			River/ Canal Width (m)
	March	May	July	Sept.	March	July	Sept.	March	May	July	Sept	March	July	Sept	
1R	24	18	11.5	14	284	105	110	6.8	5	5.5	5.7	/	/	/	20
2L	24	17.5	9.5	15	96	355	/	7.4	6	6.5	/	0.15	/	/	4.8
3L	23.5	17.5	11.8	15	139	148	119	6.8	6.5	6	5.7	0.35	0.53	0.52	4
4R	26	16	12.4	16	668	355	450	8.1	6.5	6.3	6.8	/	/	/	50
5L	26.5	/	12	16.5	139	155	122	8.9	/	6	6.3	0.1	0.13	0.24	0.6
6R/S	25	15.5	13	16.5	1013	375	630	8.1	7.5	6.2	7	0.4	/	0.58	5
7R	24.5	/	13.5	17	1923	445	690	8.1	/	6.5	7.3	0.016	/	/	50
8S	25	18	15.5	16	1012	460	610	9.2	7.8	6.5	7.2	0.33	/	0.45	2

/ Not measured

Site No.	Nitrate-N (mg/l) March	July*	Sept*	Nitrite-N (mg/l) March	Ammonium-N (mg/l) March	Phosphate-P (mg/l) March	Sept.
1R	.14 (0.1)	.86 (.004)	.58 (.002)	.05 (.03)	.04 (.03)	.058 (.05)	.033 (.01)
2L	.16 (.01)	0	0	.06 (.01)	.06 (.05)	.017 (.0009)	/
3L	.17 (.05)	.99 (.002)	.74 (.004)	.04 (.02)	.08 (0.01)	.058 (.02)	.052 (.01)
4R	.29 (.02)	.89 (.002)	.77 (.002)	.09 (.02)	.19 (.07)	.083 (.04)	.026 (.02)
5L	.05 (.02)	.94 (.001)	.77 (.001)	.05 (.007)	.11 (.03)	.045 (.009)	.048 (.001)
6R/S	.25 (.06)	.91 (.13)	.49 (.02)	.06 (.016)	.05 (.004)	.021 (.014)	0.02 (.005)
7R	.25 (.01)	.79 (.02)	.68 (13)	.11 (.003)	.06 (.03)	.034 (.014)	.056 (.01)
8S	.09 (.01)	.70 (.001)	.55 (.02)	.04 (.03)	.01 (.002)	.017 (.002)	.026 (.002)

*Dionix method / not measured

(Standard Deviation in brackets)

APPENDIX 2

PLATE SECTION

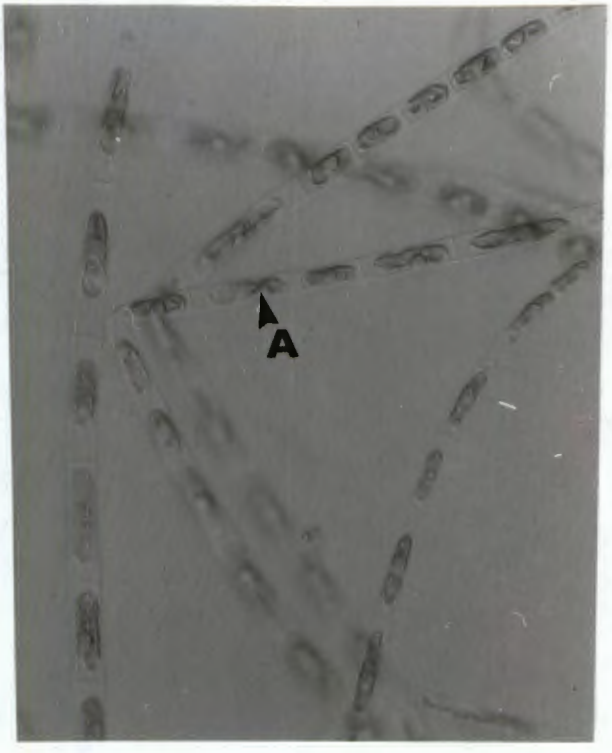

Plate 1. Undamaged cells of *S.tenue* which have plasmolysed (A) after exposure to a 33% w/v sucrose solution. (Magn. 504X)

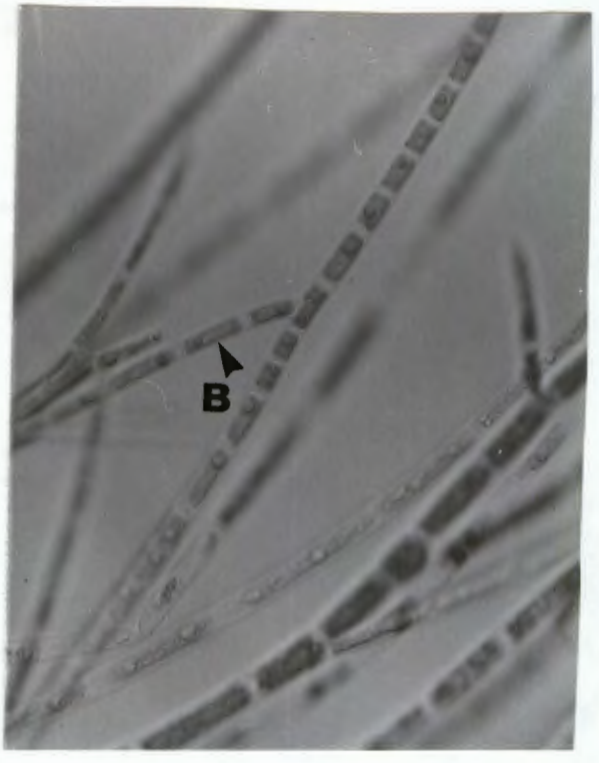

Plate 2. Copper damaged cells of *S.tenue* having remained unplasmolysed (B) after exposure to 33% w/v sucrose solution. (Magn. 504X)

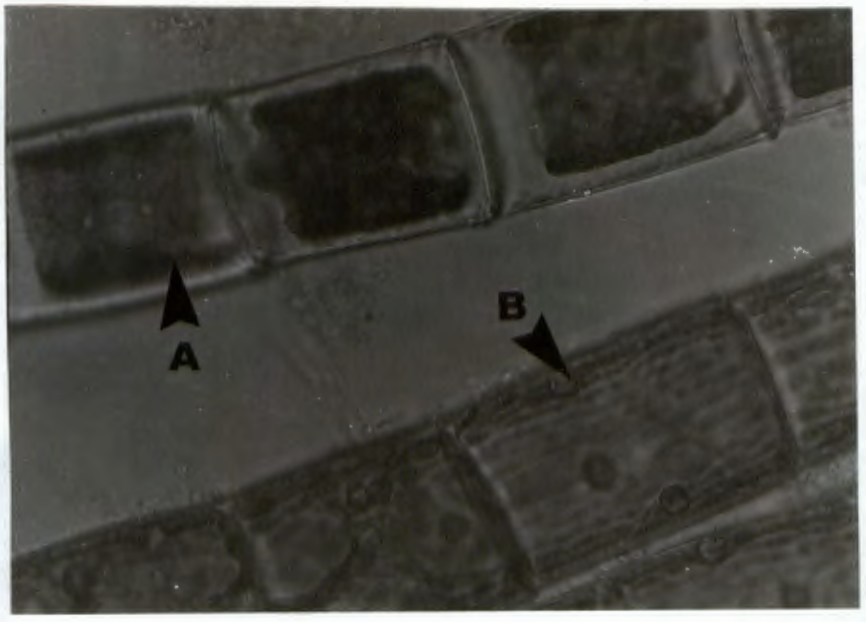

Plate 3. Undamaged *Oedogonium* sp2 filaments which have plasmolysed in a sucrose solution (A) alongside copper damaged cells which did not plasmolyse (B). (Magn. 504X)

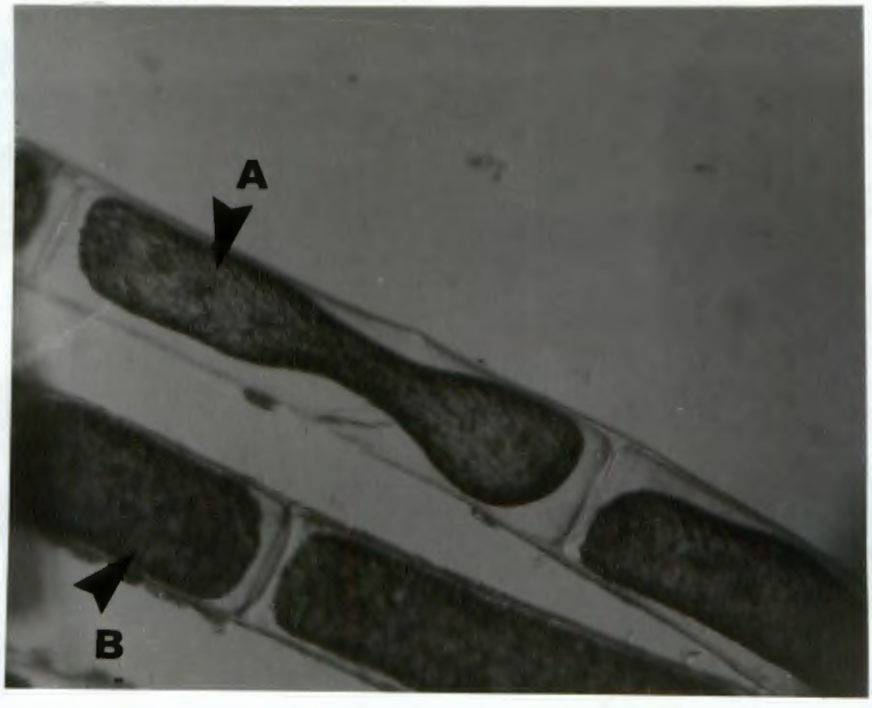

Plate 4. Healthy *C. glomerata* filaments undamaged by copper treatment which underwent plasmolysis (A) in sucrose solution, adjacent to copper damaged cells (B). (Magn 126X)

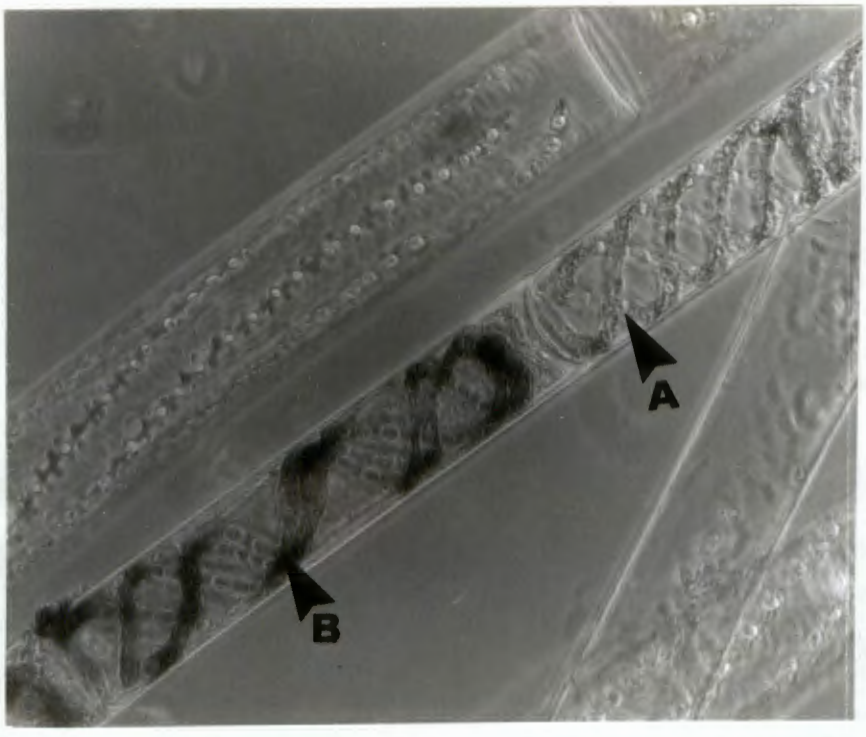

Plate 5. A healthy *Spirogyra* spl cell (A), undamaged by copper treatment, adjacent to a copper damaged cell (B) in which the blackened contracted chloroplast is clearly visible. (Magn 504)

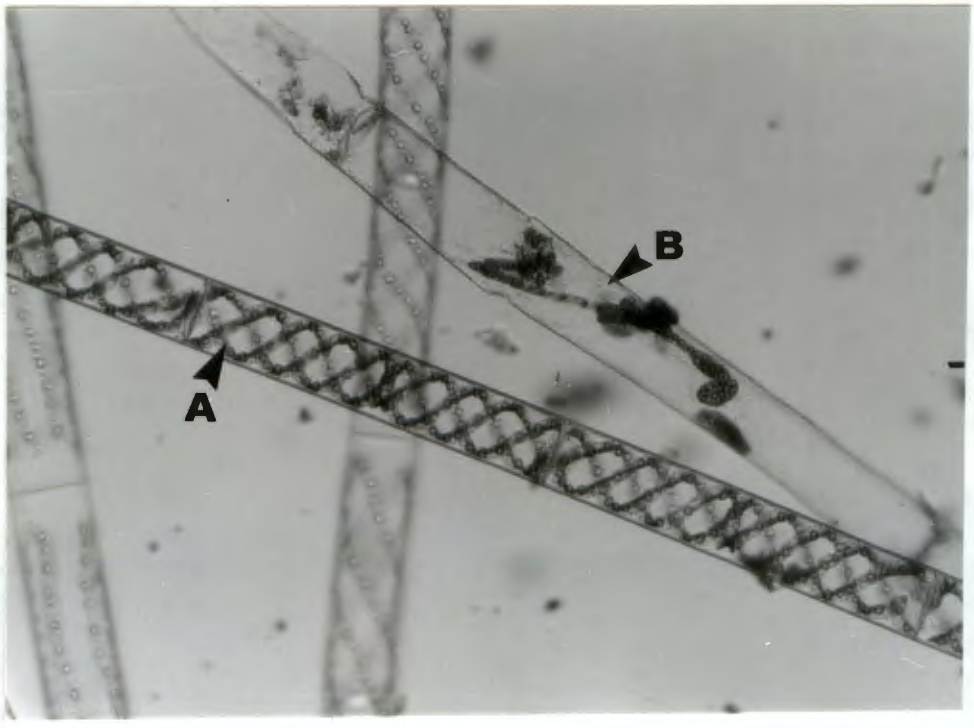

Plate 6. Filament A has recovered over a period of one week from a copper treatment of 7.5 mgl^{-1} for 6 hours. The filaments killed during the exposure to copper are still visible as nearly empty blackened cells (B). (Magn 126X).

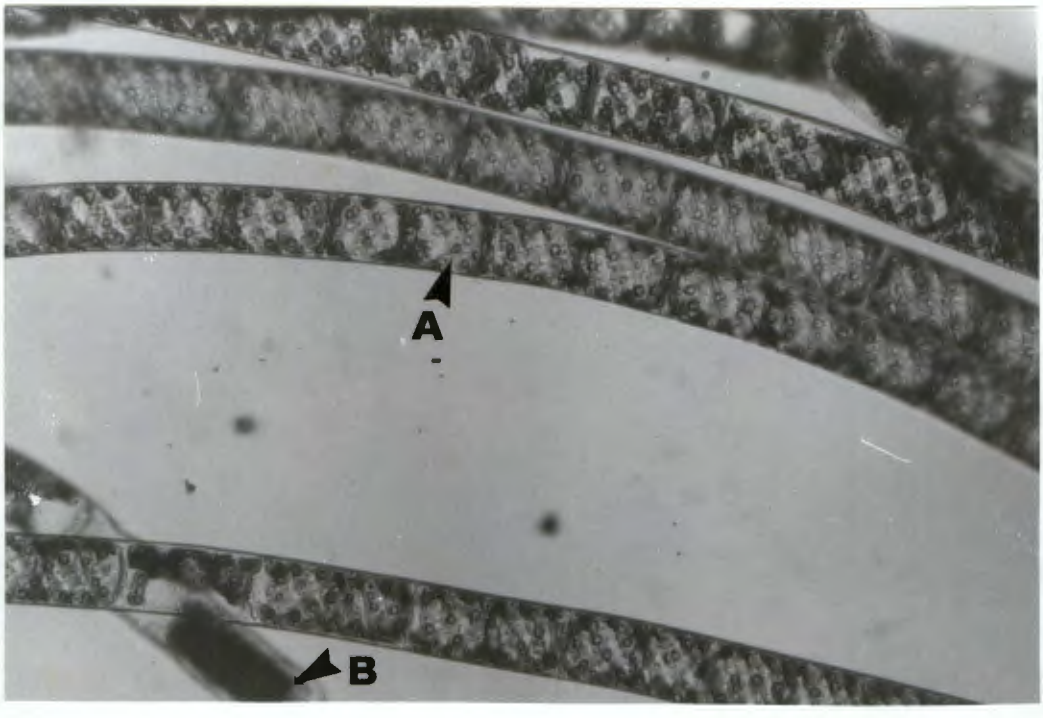

Plate 7. The recovery of *Spirogyra* sp1 filaments (A) two weeks after removal from a copper treatment of 7.5 mgl^{-1} for 6 hours. The cells killed during the exposure to copper (B) only make up a very small proportion of the filaments. (Magn. 126X)

Acknowledgments

I am most grateful to John Bolton for his supervision of this project, as well as for his assistance in improving earlier drafts of this work. The knowledge and assistance gained from Podge Joska was invaluable, and my thanks must also go to Neil Griffin for ensuring that my methodology was scientific.

This study was financed by the Foundation for Research and Development and by my parents for whom I am particularly thankful.