IN VITRO CULTURE STUDIES OF ENVIRONMENTAL FACTORS AND THE EFFECTS OF COPPER ON A FOULING ALGA, *CLADOPHORA GLOMERATA* (L) KÜTZING, WHICH OCCURS IN SOUTH AFRICAN FRESHWATER SYSTEMS.

by

Reneé Joy Le Roux

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PREFACE

The experimental work described in this thesis was conducted in the Botany Department, University of Cape Town, Cape Town, from May 1995 to April 1997, under the supervision of Associate Professor J.J. Bolton.

These studies represent original work by the author and have not been submitted in any other form to another University. Where data or work by others were used, it has been duly acknowledged in the text.

Reneé Joy Le Roux

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ABSTRACT

The filamentous green macroalga, *Cladophora glomerata* (L.) Kütz., is problematic as a fouling organism in eutrophic freshwater systems in South Africa. A general consensus among scientific researchers is that *C. glomerata* thrives under high light conditions, temperatures between 15°C and 25°C, high inorganic nutrient concentrations, alkaline waters and low heavy metal concentrations. Although extensive research on the biology of *C. glomerata* has been conducted in other regions of the world no scientific work has been done in South Africa in the past. At present the common strategies employed to control macroalgal blooms in South Africa are to apply a dosage of copper sulphate, preceded by sulphuric acid where waters are alkaline, manual removal and draining the canals. For the purpose of this thesis the influence of specific environmental factors on the growth of *C. glomerata* and the effectiveness of copper sulphate as an algicide were investigated in the laboratory.

The growth of *C. glomerata* was vigorous in tap water enriched with 14 mg N l^{-1} and 2 mg P l^{-1}. The effects of light intensity, ranging from 0-150 μmol photons m^{-2} s^{-1}, temperature, from 10°C to 35°C, and photoperiods, namely a long-day (16:8 light-dark cycle) and short-day (8:16 light-dark cycle) were investigated on the growth of *C. glomerata*. Maximum growth rates at light saturation point (G_{max}) were optimum at 25°C, with slightly reduced growth at 15°C, 20°C and 30°C. The *C. glomerata* filaments died at 35°C and at 10°C the filaments remained healthy but no growth occurred irrespective of light intensity and photoperiod. G_{max} was approximately three times higher at the long-day photoperiod compared to the short-day photoperiod at temperatures between 20°C and 30°C and double at 15°C. Light saturation points shifted with temperature, reaching their maximum at 25°C at both long-day (126 μmol photons m^{-2} s^{-1}) and short-day (148 μmol photons m^{-2} s^{-1}) photoperiods. This study suggests that the seasonal growth of *C. glomerata* in South African freshwater sytems should be possible from mid-spring to mid autumn when water temperatures are above 10°C. Optimum growth should occur during summer when daylengths are longer and light intensities are higher.

Accumulative Cu uptake, Cu uptake rates and photosynthesis were determined by exposing *C. glomerata* tufts to a medium Cu concentration range from 0-10 mg Cu l^{-1} at a solution pH of either 8, 7 or 6 for algal incubation time periods of either 30, 60, 120 or 240 minutes. Accumulative Cu uptake and uptake rates increased with increasing medium Cu concentrations and decreasing solution pH levels at all algal incubation time periods. Accumulative uptake increased with time until it reached a saturation point at all medium Cu concentrations after one hour at solution pH 8 and two hours at solution pH 7 and 6. Copper uptake rates were highest within the first 30 minutes of algal incubation at all pH levels and medium Cu concentrations. Although photosynthetic inhibition, measured as O_2 evolution one hour and 24 hours after algal exposure to Cu, was affected by increasing medium Cu concentration and decreasing solution pH in some cases, it was inconsistent with an increase in algal incubation time in the Cu. This could be attributed to algal density since the uptake distribution and amount into the algal cells may not have been constant throughout the algal tuft, thereby affecting some cells whilst others remained healthy. The growth rates of *C. glomerata* were determined over five days when *C. glomerata* filaments were grown in tap water supplemented with N and P at pH 8, after they were exposed to a medium Cu concentration range from 0-20 mg Cu l^{-1} at solution pH levels of either 8, 7 or 6 for 30 minutes. Growth rates were only adversely affected when exposed to solution pH levels of 7 and 6 at medium Cu concentrations of 20 mg l^{-1} and above 6 mg l^{-1} respectively with no reduction in growth occurring at solution pH 8 at all Cu concentrations. It was evident using transmission electron microscopy that Cu does enter the cell and cause thylakoid membrance disruption, and the longer the exposure to Cu the greater the extent of damage. It was concluded that $CuSO_4$ is an effective algicide, and reducing the water pH does enhance the algicidal effects. The minimum solution Cu concentration required to effectively reduce algal blooms will depend on the water chemistry, algal density and water flow parameters. Other potential algal growth control methods which should be considered are manipulating the amount of light reaching the canals and the use of biological control methods such as the addition of decomposing barley straw, fungal pathogens and grazers.

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CHAPTER 1

THE ECOLOGICAL STATUS AND MANAGEMENT OF FRESHWATER ECOSYSTEMS IN SOUTH AFRICA, AND THE ROLE OF ALGAE

1.1. GENERAL INTRODUCTION

The phenomenon of cultural eutrophication occurs worldwide, especially in regions where the human population density is high. Cultural eutrophication of a water body has been characterized by a high productivity rate caused by the excessive input of nutrients as a result of effluent that contains detergents, human and animal wastes, agricultural runoff contaminated by fertilizers and industrial discharges (Palmer, 1980). The most conspicuous manifestation of eutrophication is the selective growth of phytoplankton, filamentous weedy algae and aquatic macrophytes. Algae are the main primary producers in water impoundments and therefore play an integral and beneficial part in the ecosystem (Lembi and O' Neal, 1988). However, large algal blooms can be regarded as being detrimental since they block waterwork systems, lower the O_2 concentration in the water upon decay and reduce the species diversity of the water bodies. Moreover, dense growths of algae are aesthetically unpleasing as they hinder recreational activities and often the decomposition of the algae leads to undesirable odours (Palmer, 1980).

Much of South Africa is semi-arid and water is a scarce resource, especially in areas with a growing urban population. Water usage by industrial, agricultural and domestic sectors has been projected to rise by 3% from 1990 to the year 2000 (Rawhani, 1991). Many areas receive erratic rainfall which exerts pressure on the already overexploited water resources (Davies *et al.*, 1995). Further exacerbating the problem, eutrophication is considered as one of the most serious threats to water quality in South Africa (Toerien *et al*, 1975).

Few reports of problematic macroalgal growths in water impoundments were officially recorded in South Africa prior to the 1970's. This can be attributed either to the absence of "pest" algal blooms or merely the lack of ecological research work conducted (Joska and Bolton, 1996). Toerien (1975) reported noxious growths of filamentous algae in irrigation canals and water impoundments in the Transvaal (Gauteng) and Orange Free State (Free State) Provinces. A recent survey undertaken by Joska and Bolton (1996), indicated that the problems of excessive filamentous algal weed growth occurring in water systems in the Gauteng-Free State region as well as the Cape river systems are increasing. These algal blooms are resulting in unnecessary financial expenditure in rising costs for water purification, loss of livestock and reduced potable water supplies (Grobler and Silbauer, 1984). In recent years much attention has been focused on this problem and solutions to combat the causal as well as the resultant factors have been implemented. Since the main causal factor of eutrophication is nutrient loading, it would be ideal to limit nutrient inputs.

In the past in South Africa, the Department of Water Affairs and Forestry controlled water pollution from point sources by requiring effluent to conform to general and special effluent standards. Despite the vigorous attempts at enforcing the uniform effluent standard approach, the quality of the water resources has continued to deteriorate, since the implementation of this approach often depended on technological, economical and sociopolitical issues (van der Merwe and Grobler, 1990). Recently, the Department of Water Affairs and Forestry has perceived the predicament and resorted to a new approach to water pollution control. A report by van der Merwe and Grobler (1990), outlined the new approach to water pollution control which encompasses two objectives. Firstly, the emissions of non-hazardous wastes will have to adhere to site-specific effluent standards which would depend upon the specific water quality requirements of receiving waters. Secondly, the input of hazardous effluent (substances detrimental to humans and the environment) should be limited or prevented on the whole either by reducing source supply or by recycling to minimize wastes. In the Republic of South Africa pollution control is unfortunately a long-term solution due to the lack of skills, technology, policies and strategies needed to execute the "new approach". The obvious deficiency of knowledge regarding the quality of surface and ground water resources, as well the water

quality in effluent, motivated the Department of Water Affairs and Forestry to commission a series of monitoring programmes.

A National Monitoring System will provide a means for observing and assessing water quality changes nationally. In the past, water quality monitoring actions were exclusively focused on physical and chemical measurements. Recently, it has come to the attention of water quality managers that additional complementary techniques should be implemented to enhance the assessment and management of aquatic systems. For this reason, biomonitoring or biological monitoring programmes have been introduced. Biomonitoring of aquatic ecosystems refers to the systematic use of biological responses to assess changes in the environment with the intention of applying this information in water quality-control programmes (Mathews *et al.*, 1982). The South African biomonitoring programme of riverine ecosystems was developed based on a three-tiered hierarchical classification scheme. This included, in descending order, a bioregional classification (where rivers within this region were similar in physical and biotic components), sub-regional classification (based on patterns of river zonation within bioregions) and river types (variation between rivers within a sub-region). Within all three levels, the biological responses of the ecosystems will be determined by examining changes in distribution patterns of fish, macroinvertebrates and riparian vegetation (Eekhout *et al.*, 1996). The assessment of water quality in many countries in Europe is additionally monitored with the use of algae, predominantly diatoms (Dell'Uomo, 1995; Hofmann, 1995; Kelly *et al.*, 1995; Prygiel and Coste, 1995; Sabateur *et al.*, 1995) and to a lesser extent macroalgae (Pipp and Rott, 1995). The use of algae as potential biomonitors in South African aquatic ecosystems was however neglected, probably as a result of the limited availability of freshwater algae expertise in South Africa.

The Catchment Monitoring System will provide information required to manage water quality in specific catchments. The principal aims will be to identify water quality problems and assess the effectiveness of management strategies being employed in specific catchment areas. Once a more comprehensive understanding of the status of the water systems is obtained a Compliance Monitoring System will be developed to ensure and enforce that the effluent emission standards are met. A Project Monitoring System will be implemented if any

discrepancies arise in specific water quality projects which are not addressed by the above three monitoring systems.

A preliminary questionaire sent to water engineers and heads of Irrigation Boards and Municipalities, investigated the extent of problematic macroalgal growth in potable water resources in South Africa. Fourty-two percent of the respondents claimed that they experienced problems with algal growth in their water supplies (Joska and Bolton, 1994 a). A project was initiated at the University of Cape Town in the Department of Botany with the primary aims of identifying, assessing and monitoring the major problematic macroalgal species occurring in irrigation canals as well as investigating the effectiveness of present management strategies employed. A preliminary study by Joska and Bolton (1994 a), identified the major problematic, fouling alga occurring in the Crocodile and Kalkfontein Irrigation Schemes (Northern Cape and Free State Provinces) to be the Chlorophyte, *Cladophora glomerata* (L.) Kütz. Additional problematic genera occurring in irrigation systems country-wide include the Chlorophytes, *Oedogonium*, *Rhizoclonium*, *Spirogyra* and the Charophyte, *Nitella*.

At present, in South Africa excessive macroalgal growths are controlled predominantly by manual removal, draining of canals and the application of copper sulphate. In some water schemes, for example, the Kalkfontein and Hartebeespoort, the addition of $CuSO_4$ is often preceded by an initial dosing with sulphuric acid (Joska and Bolton, 1994 a). For the purpose of this M.Sc project, I focused exclusively on the fouling alga *Cladophora glomerata*, which is a problem in the Kalkfontein irrigation canal system. Initially, a literature review on the biology of *C. glomerata* was compiled. Subsequently a series of laboratory experiments were conducted to determine the influence which various environmental factors, such as light, temperature and photoperiod, have on *Cladophora glomerata*. Furthermore detailed *in vitro* studies were performed with the main aim of investigating the effectiveness of $CuSO_4$, applied in various ways, in controlling excessive growth of *C. glomerata* in irrigation canals. Finally, assessments of presently applied and potential management strategies used in controlling *C. glomerata* growth were made.

CHAPTER 2

LITERATURE REVIEW ON THE BIOLOGY OF *CLADOPHORA GLOMERATA* (L) KÜTZ.

2.1 TAXONOMIC POSITION, DISTRIBUTION AND STRUCTURE

The taxonomic class Cladophorophyceae belongs to the division Chlorophyta which refers to the green algae. Within this class only one order, the Cladophorales, exists which comprises *ca* 32 genera, divided into *ca* 420 species of which most are marine. The order, Cladophorales, is characterised by siphonocladous thalli (uniseriate filaments which can be branched or unbranched), the cell wall comprising highly crystalline cellulose, multinucleate cells with numerous irregular or angular reticulate chloroplasts and reproducing sexually by means of biflagellate gametes or quadriflagellate meiospores, asexually by means biflagellate or quadriflagellate zoospores or vegetatively (Van den Hoek *et al.*, 1995).

The genus *Cladophora*, within the order Cladophorales, was erected in 1843 by Kützing who transferred many species from the Linnean genus *Conferva*. The genus *Cladophora* can be identified by branched uniseriate filaments with multinucleate cells containing a reticulation of several angular, discoid chloroplasts, with the thalli being attached to rocky substrata or free-floating algal masses (Van den Hoek, 1963; Van den Hoek *et al.*, 1995). The distribution of marine *Cladophora* is widespread extending from temperate to the tropical seas but virtually absent from polar waters (Van den Hoek *et al.*, 1995). Van den Hoek (1963) recorded 27 marine *Cladophora* species and varieties and 10 freshwater species. A more recent updating by Van den Hoek *et al.* (1995), reduced the number of marine and freshwater species occurring in Europe to 25 and 9 respectively, whilst 31 marine species are described from the coasts of North East America and 18 in South Africa (Seagrief, 1984). Bakker *et al.* (1995) affirm that marine *Cladophora* consists of many species. Their findings indicated that distinct internal transcribed spacer (DNA sequencing) types were displayed by the marine species with little variation within

types. However Marks and Cummings (1996), based on DNA sequencing, suggest that freshwater *Cladophora* may comprise very few or possibly one genetic species which is ecologically and morphologically variable.

At present only three of the *ca* 10 freshwater species have been documented to occur in South Africa, namely *Cladophora fracta* (Müll. ex Vahl) Kütz, *Cladophora rivularis* (L.) nov. comb. and *Cladophora glomerata* (Joska and Bolton, 1994 b). *C. glomerata* has a cosmopolitan distribution and has been recorded in the Great Lakes of North America (Bellis, 1968 a and b; Herbst, 1969; Lowe *et al.*, 1982; Jackson and Hamdy, 1982), other freshwater bodies in the U.S.A. (Fitzgerald, 1968; Dodds, 1991a; Sheath and Cole, 1992; Bergey *et al.*, 1995), United Kingdom (Whitton, 1967; Wharfe *et al.*, 1984; Shamsudin and Sleigh, 1995), Australia (Freeman, 1986), India (Khanum, 1982), Argentina (Parodi and Caceres, 1991) and South Africa (Joska and Bolton, 1994 a). The habitats in which *C. glomerata* has been found are widespread extending from ultraoligotrophic waters to extremely eutrophic conditions (Dodds, 1991 a; Dodds and Gudder, 1992; Cambra and Aboal, 1992). Sheath and Cole (1992) indicated that *C. glomerata* is distributed throughout a wide range of habitats in North America. These include the biomes of the boreal forests, western coniferous forests, eastern hemlock-hardwood forests, deciduous forests, coastal plains, tropical forests and even in the desert chaparral.

Taxonomic differentiation of species based on morphological characteristics is often complicated by the plasticity of the thallus with prevailing environmental conditions (Van den Hoek, 1963; Van den Hoek *et al.*, 1995). In general, *C. glomerata* has been described as a plant characterized by a distinct acropetal organization associated with dominating apical growth to one with an irregular organization dominated by intercalary growth, depending entirely on genetic and environmental factors (Van den Hoek, 1963). The primary feature which distinguishes *C. glomerata* morphologically from other freshwater *Cladophora* species is the degree of branching. For example, *C. basiramosa* Schmidle and *C. rivularis*, show no branching of the filaments compared to *C. glomerata* where branching is remarkably frequent along the entire length of the filament (Van den Hoek, 1963). Branch insertion in *C. glomerata* varies markedly from being oblique to horizontal and occasionally lateral. When growth is predominantly apical, the branching pattern appears to be pseudodichotomous (Van den Hoek, 1963).

C. glomerata thalli are usually attached to a substrate by uniseriate branched rhizoidal growths (Van den Hoek *et al.*, 1995) but may become detached from their basal segments to form dense floating masses of long and poorly branched filaments (Van den Hoek, 1963). In *C. fracta*, rhizoidal growths are seldom produced and thus the whole plant is free-floating whilst in *Cladophora aegagropila* (L.) Rabenhorst, rhizoidal outgrowths are produced from either apical or basal sections of the cell or filament (Van den Hoek, 1963). In Lake Doré, Ontario, Kindle (1934) observed that filaments of *C. aegagropila* intertwined irregularly and formed free-floating ball-like structures.

Ultrastructurally, the cell wall of *C. glomerata* consists of alternate microfibrillar and amorphous layers (Moore and Traquair, 1976), covered by a well-defined proteinaceous cuticle (McDonald and Pickett-Heaps, 1976). Internal to the cell wall lies a very thin stratum of cytoplasm which contains microtubules orientated parallel to the longitudinal axis of the cell. Each multinucleate cell contains many reticulate, bilenticular chloroplasts dispersed throughout the cytoplasm as well as numerous pyrenoids (Van den Hoek, 1963; Graham, 1982).

2.2 REPRODUCTION

Reproduction is either sexual or asexual in *C. glomerata* (Van den Hoek, 1963; Shyam, 1980). Documentation of sexual reproduction in *C. glomerata* is rare (Marks and Cummings, 1996). Shyam (1980) demonstrated that the species *C. callicoma* (Kütz), considered to be closely related or a variety of *C. glomerata* (Van den Hoek, 1963), collected from freshwater bodies in India exhibited a definite alternation of generations. Sexual reproduction in *C. callicoma* occurs by the release of gametes from gametangia which subsequently swim vigorously until fusion. Gamete fusion is exclusively heterogamous (fusing with different mating types) and isogamous (gametes from different mating types are identical in form and size). Furthermore, gamete fusion never occurred between gametes of the same plant. Shortly after fusion karyogamy (fusion of the nuclei) occurs and the resulting zygote germinates immediately giving rise to the diploid sporophyte generation. The gametophyte and sporophyte plants are

isomorphic with the only discernible difference being the release of quadriflagellate zoospores by the latter.

In *C. glomerata* a lack of alternating generations and a rather high ploidy level was reported by Van den Hoek, (1963) and Shyam, (1980). However, the study by Sinha and Noor (1967) indicated that the form *C. glomerata* var. *fasciculata f. fasciculata* displayed an alternation of haploid and diploid generations. Despite, a few rare reports indicating sexual reproduction, the quantity of research studies demonstrating a lack of sexual reproduction and a predominantly asexual behaviour is overwhelming. Asexual reproduction occurs by the release of biflagellate or quadriflagellate zoospores usually from the apical cells of the main axis (Van den Hoek, 1963; Shyam, 1980). The shape of the sporangia or gametangia is influenced by environmental factors but generally they are cylindrical to club-shaped. Swarming of zoospores commonly occurs after an abrupt change in environmental conditions. Once the zoospores have settled they surround themselves with a firm wall and germinate immediately. Shortly after, rhizoids are formed and the plantlets are capable of growing into long, branched filaments. Under unfavourable environmental conditions zoospores do not swarm but surround themselves by a thick cell wall and remain dormant, termed akinetes, until conditions are favourable. Vegetative reproduction has been commonly reported to occur in *C. glomerata* (Van den Hoek, 1963; Shyam, 1980). Van den Hoek (1963) demonstrated that when apical cell fragments were placed into culture virtually all cells released zoospores. In comparison, when basal cell fragments were placed into culture, most grew into long filaments with sporulation occurring in the apical cells.

2.3. ECOPHYSIOLOGY

Whitton (1970 a), specified a number of environmental and ecological factors which promote the dominance of *C. glomerata*. These include high light conditions, temperatures between 15°C and 25°C, alkaline waters (pH usually above 7.5), exceptionally low concentrations of heavy metal ions, high inorganic nutrients especially N and P and high water velocity. He also noted that *C. glomerata* shows a high resistance to grazing and inter-specific competition. In this chapter the responses of *C. glomerata* to light, temperature, pH and heavy metal ions have not

been dealt with in detail since they form the body of this thesis and consequently these topics will be discussed in subsequent chapters. However the remaining factors which affect the growth of *C. glomerata* have been reviewed.

2.3.1. MACRONUTRIENTS

a. Phosphorus and Nitrogen

Whitton (1970 a) and Dodds and Gudder (1992) have comprehensively reviewed the response of *C. glomerata* to phosphorus and nitrogen. Planas *et al.* (1996) repeatedly observed a stimulation of *C. glomerata* growth when exposed to high P levels. In experiments by Bolas and Lund (1974) *C. glomerata*, collected from the River Stour, was placed in a medium containing river water and a medium composed of borehole water with and without supplemented PO_4. Growth of *C. glomerata* was good in the media with added PO_4 and in the river water medium, whereas growth in the borehole medium without added PO_4 was poor. The river and borehole waters were very similar in chemical composition except that the river water contained PO_4 whereas a complete absence of PO_4 was found in the borehole water, which could explain why poor growth of *C. glomerata* occurred in the latter. Similarly in a field experiment conducted in the River Arrow, United Kingdom, an area above an effluent discharge contained a lower P concentration and supported poor *C. glomerata* growth compared to a site below the effluent discharge. When P concentration was adjusted at the upstream site so that it equalled the downstream site P level, no significant difference in *C. glomerata* growth occurred between the two sites (Pitcairn and Hawkes, 1973). In a study by Neil and Jackson (1982) P pumped into a water mass at the Rathfon Point, Lake Erie, at 0.35 kg P day^{-1} for 95 days to a depth of 0.5 m, resulted in 90% of the area being covered with *C. glomerata* filaments which was previously devoid of this alga. These studies suggest that P concentration is indeed a limiting factor for *C. glomerata* growth but at what ambient water concentrations and internal tissue contents?

Sawyer (1947) predicted that a critical total P concentration above 0.01 mg P l^{-1} would support prolific *C. glomerata* blooms in lakes. Pitcairn and Hawkes (1973) noted that Midland rivers, in the United Kingdom, containing less than 1 mg P l^{-1} exhibited modest amounts of *C. glomerata*. In laboratory experiments conducted by Pitcairn and Hawkes (1973) growth of *C. glomerata*, collected from the River Blythe (U.K.), was good in 1 mg P l^{-1} and only increased

measurements of *Cladophora* suggested a critical P limiting value of 0.16% of dry weight in a river in southern Ontario.

These studies indicate slightly different ambient water values of P required to stimulate or limit growth as well as diverging critical growth-limiting tissue P concentrations. It must however be emphasized that the ambient water P concentration required for growth would vary according to water chemistry and water flow. Water chemistry such as water hardness, alkalinity and the concentration of organic compounds influence the concentration and biological availability of soluble reactive P in the water (Pitcairn and Hawkes, 1973). The uptake of soluble reactive P is affected by water flow patterns. High water velocities reduce the thickness of the boundary layer around the algal thallus and hence uptake rates are increased. The higher the uptake rates, the lower P concentrations in the ambient water required to limit growth and vice-versa (Raven, 1992). Planas *et al.* (1996) considered P uptake rates between 0.298 and 2.949 mg g dry weight^{-1} h^{-1} as indicative of internal P limitation in *C. glomerata* collected from the North and South Basins of Windermere (England).

Wong *et al.* (1979) claimed that internal P concentrations correlated strongly with external ambient water concentrations. However, Lohman and Prescu (1992) found that as soluble reactive phosphorus levels in the water declined, internal tissue P concentrations increased indicating that *C. glomerata* has the ability to store P. The ability of *C. glomerata* tissue to store P was shown by Fitzgerald (1968). P-limited *C. glomerata* tissue had relatively high alkaline phosphatase activity of 500 units mg^{-1}, compared to an algal tissue sample from Lake Mendota which was not P-limited (90 units mg^{-1}). The storage capacity of P in algal tissue would explain why different critical yield limiting concentrations of P were reported for *C. glomerata* even though tissue samples were extracted from the same area as reported above by Auer and Canale (1982 a and b).

Lastly, defining of limiting concentrations of P in the surrounding medium may be complicated by the relationship between the requirements for N and P by *C. glomerata* (Dodds and Gudder, 1992). Pitcairn and Hawkes (1973) investigated the interaction between N and P in the laboratory. *C. glomerata* from the River Blythe was exposed to P concentrations of 0.5, 1.5 and

3 mg P l^{-1}. At each P concentration, N was added yielding a concentration range of 1.5, 3.5, 5.5 and 7.5 mg N l^{-1}. Growth was optimal at 1.5 mg P l^{-1}, limited at 0.5 mg P l^{-1} and inhibited at 3 mg P l^{-1}. The addition of N did not alter the latter pattern at the three lower N concentrations, however at 7.5 mg N l^{-1} growth was enhanced at the low P level and vastly reduced at 1.5 and 3 mg P l^{-1}. These results from the laboratory experiments corresponded to field patterns, which showed that critical P limiting levels varied with N levels (Pitcairn and Hawkes, 1973).

Like P, N is an essential macronutrient for the growth of *C. glomerata* (Whitton, 1970 a) however *C. glomerata* is capable of growing in waters where the N supply limits primary production (Dodds and Gudder, 1992). Ohle (1953 cited in Herbst, 1969) proposed that in both oligotrophic and eutrophic waters the demand for N by *C. glomerata* can be satisfied under natural conditions. This claim was supported by the observation that despite NO_3-N concentrations of less than 50 μg l^{-1} recorded in the Manawatu River, New Zealand (Freeman, 1986) and about 300 μg l^{-1} in Lake Erie (Lorenz and Herdendorf, 1982) *C. glomerata* growth was neither limited nor saturated. Unlike the huge ambient N range which supports *C. glomerata* growth, Merezhko *et al.* (1992) showed that the vital activity of *Cladophora fracta* occurring in irrigation canals in Ukraine was severely depressed at 100 μg l^{-1} and above and death of the alga occured within 2-3 days.

Tissue N concentration in *C. glomerata* was revealed to closely track ambient N levels (Lohman and Prescu, 1992). Gerloff and Fitzgerald (1976) stated that the critical N level for optimal growth of *C. glomerata* is 1.1% of dry weight. Likewise Wong and Clark (1976) suggested that *C. glomerata* growing in the Southern Ontario region was limited by critical N concentrations of 1.2-1.5% of dry weight. Many research studies indicate that N tissue concentrations exceed the critical limiting concentration throughout the growing season. Neil and Jackson (1982) indicated that total N values in *C. glomerata* tissue collected from Lake Erie were generally above 1.8% of dry weight and were therefore not N-limited. Likewise, *Cladophora glomerata* collected from the Manawatu River had total tissue N concentrations of more than 2% of the dry weight throughout the study period (Freeman, 1986). In a recent survey by Planas *et al.* (1996) it was demonstrated that tissue N levels in *C. glomerata* were entirely above the critical value of 1.1% of dry weight in the North Basin of Windermere and that only 20% of the samples

were below critical levels in the South Basin. By contrast, in a study by Lohman and Prescu (1992) *C. glomerata* was observed to be N limited most of the time throughout the year. Tissue N was predominantly between 0.78% and 1.80% of dry weight with intermittent increases between 2.81 and 3.4% (highest dissolved inorganic N measured).

In summary, the natural supply of ambient N, generally does not limit *C. glomerata* growth, however the natural supply of P does even though higher critical internal tissue N concentrations were found to limit *C. glomerata* growth compared to P. These observations indicate that *C. glomerata* growth is more sensitive to P than to N which may lead to the assumption that an increase in P levels in the water is the primary cause of increased problematic *C. glomerata* blooms.

b. Silicon

Moore and Traquair (1976) demonstrated that the growth of *C. glomerata* was stimulated when cultured in soil water extracts containing Si. At a concentration of *ca* 1 mg l^{-1}, growth enhancement was evident and higher concentrations promoted growth of *C. glomerata* to a greater extent. This infers that Si is significant for the growth of this alga. Confirmation of the Si requirement for *C. glomerata* growth is shown by the fact that germanium inhibits the growth. Germanium is a specific inhibitor of silicic acid metabolism (Werner, 1967 cited in Moore and Traquair, 1976). Only organisms that require Si are inhibited by GeO_2 at concentrations less than 75 mg l^{-1}. At 75 mg l^{-1} GeO_2 severely inhibited the growth of *C. glomerata* with reduced growth inhibition at 25 mg l^{-1} GeO_2. Filaments exposed to these concentrations were abnormal compared to filaments from control cultures. At 75 mg l^{-1} GeO_2, cell division ceased and increase in mass was attributed to extreme thickening of cell walls. The exposure of *C. glomerata* cells to 25 mg l^{-1} GeO_2 resulted in an interruption of cross-walls between cells (Moore and Traquair, 1976). The above study has conclusively established that GeO_2 is extremely toxic to *C. glomerata.*

How does Si enhance growth in *C. glomerata*? The ultrastructure of walls from cells grown in medium with 5 mg l^{-1} Si consisted of alternate microfibrillar and amorphous layers. The outer portion of the wall, about ⅓ of the total thickness, was more electron-dense than the inner

portion indicating an accumulation of Si. Cells grown in medium without added Si lacked the outer thickened dense layer (Moore and Traquair, 1976). It is therefore evident that Si is a functional component of the cell wall in *C. glomerata*.

c. Calcium and Magnesium

Bellis (1968 a) reported the importance of Ca and Mg for the growth of *C. glomerata*. In a laboratory study *C. glomerata* filaments died within 4 days when exposed to a Ca-free medium. Minimum water concentrations of Ca and Mg required to support growth were 1.2 mg l^{-1} and 0.7 mg l^{-1} respectively. Optimum growth occurred at Ca levels of 6.4 mg l^{-1} and Mg levels of 1.7 mg l^{-1}. Concentrations of Ca as high as 64.0 mg l^{-1} were not inhibitory but stimulated branching and sporulation. This high Ca tolerance could explain why *C. glomerata* thrives in alkalinic hard waters.

2.3.2. MICRONUTRIENTS

Other nutrients which have been reported to be essential for *C. glomerata* growth include vitamin B_1 (Moore and Traquair, 1976; Hoffman and Graham, 1984), vitamin B_{12} (Hoffman and Graham, 1984) and trace quantities of Zn and Cu (Whitton, 1970 b). Vitamins B_1 (thiamine) and B_{12} were shown to improve dry weight production and they were essential for the induction of zoosporogenesis.

2.4. ECOLOGY

2.4.1. EPIPHYTIC AND GRAZER INTERACTIONS

Under favourable environmental conditions the cellulose wall structure of *C. glomerata* (Lowe *et al.*, 1982) as well as the absence of a mucilage layer (Chudyba, 1965) provide a suitable substrate for the attachment of a variety of epiphytic organisms. Literature reports indicate that the taxonomic composition of epiphytes is fairly uniform (Whitton, 1970 a). The epiphytic communities which colonize the filaments of *C. glomerata* are often structurally complex with populations of bacteria, prostrate diatoms, diatoms on pads and stacks and multicellular filamentous green algae. This diversity and complexity accommodate epiphytic grazing by a

variety of heterotrophs which include protozoa, rotifers, microcrustaceans, insects and larval fish. (Lowe *et al.*, 1982)

A survey undertaken by Chudyba (1965) in the River Skawa, Poland, established that common epiphytic genera inhabiting *C. glomerata* filaments were mainly *Chamaesiphon* (Cyanobacteria), and the diatoms *Meridion*, *Diatoma*, *Synedra*, *Cocconeis*, *Amphora*, *Epithemia*, *Eunotia*, *Rhoicosphenia*, *Cymbella* and *Gomphonema* (division Bacillariophyta). A later survey by Lowe *et al.* (1982) in Lake Michigan, revealed that *Cladophora glomerata* tufts were often inundated with similar epiphytes consisting of the diatoms, *Cocconeis pediculus* Ehr., *Diatoma vulgare* Bory, *Gomphonema olivaceum* (Lyngb.) Kütz and *Rhoicosphenia curvata* (Kütz) Grun. and the filamentous green alga, *Ulothrix*. Dodds (1991 b) reported that *Cladophora glomerata* collected from the rivers in the Montana region, hosted epiphytes predominantly belonging to the genera, *Epithemia* (diatom) and *Nostoc* (cyanophyte). Shumsudin and Sleigh (1995) observed that seasonal growths of *Cladophora glomerata* which occur in the chalk streams of the lowlands of Southern England, hosted different epiphytes with varied substrate. The most common epiphytes included, diatoms namely *Diatoma vulgare* Bory, *Rhoicosphenia curvata* Kütz. Grun., *Meridion circulare* Ag. and *Nitzschia palea* Kutz., cyanophytes namely *Phormidium incrustatum* Gom. and *Chamaesiphon* Gert. and the filamentous alga, *Scenedesmus quadricauda* Breb. (Chlorophyta).

Bergey *et al.*, (1995) investigated the distribution of epiphytes along the length of *Cladophora glomerata* filaments occurring in the Eel River, South Fork (California). Distinct epiphyte distribution patterns were observed in various water velocity regimes. *Cocconeis pediculus* dominated filament apices in medium and fast flowing waters. *Epithemia turgida* (Ehr.) Kütz and *Epithemia sorex* (Ehr.) Kütz dominated on filament bases at all velocities and on filament apices only when velocity was slow. Steinman and McIntire (1986) alleged that epiphyte colonization is higher in slow velocities than in fast velocities. Bergey *et al.* (1995) therefore attributed the brown colour of *Cladophora glomerata* tufts in slow waters to the dense encrustation by diatoms. Luttenton and Rada (1986) similarly observed a transition of epiphytes along *Cladophora glomerata* filaments. *Cocconeis pediculus* was sparse on apices, abundant on mid-filaments and intermixed with other diatoms near the base in slow flowing waters.

Bergey *et al.* (1995) proposed three mechanisms which could possibly explain the epiphyte distribution pattern on *Cladophora glomerata* filaments. Firstly, the distribution could be attributed to the preference of epiphytes to colonize certain areas along the growing filament. This speculation emerges as rather unlikely since *Cocconeis pediculus* was abundant on young growing apices as well as old or sporulated apices.

Secondly, the epiphytic distribution pattern could be ascribed to grazing patterns by heterotrophs. Bergey *et al.* (1995) observed that *Cocconeis pediculus* was most abundant on filament apices in fast flowing waters, usually a location where grazer densities are reduced because of fast velocities. Since *Cocconeis* is a taxon which is claimed to be grazer-resistant it would be able to withstand high grazing-pressure which is usually concentrated near the basal parts of the filaments where water flow is slower. Therefore, it seems unlikely that the dominance of *Cocconeis pediculus* on filament apices, where it is prone to dislodgement if poorly attached, was influenced by grazing-pressure.

A research experiment by Dodds (1991 b) in the Madison, Gallatin and Jefferson Rivers, Montana, showed the interactive effects of nutrients on *Cladophora glomerata* and its accompanying epiphytes. No competition was evident when nutrients were abundant, however when N was limiting, competition was apparent. Similarly, Fitzgerald (1969) reported that successful competition between the host, *Cladophora glomerata*, and its epiphytes only occurred when N availabilty was limiting. In Lake Wisconsin, Madison, no decrease in epiphytes occurred when limiting or surplus P cultures were compared, however in all cultures where available N was a limiting factor there appeared to be an antagonistic action between the alga and epiphytes. In Lake Mendota the presence of epiphytes on *Cladophora glomerata* appeared to be associated with periods when the alga had surplus available N. In Monona Bay nearly all samples with surplus N were heavily coated with epiphytes, mainly diatoms. When N limited, *Cladophora glomerata* filaments appeared epiphyte-free and the filaments were green and healthy. Fitzgerald (1969) observed that under low N levels, the cell walls of *Cladophora glomerata* tend to increase their production of mucilage. The mucilage layer on the cell wall facilitates the dislodgement of epiphytes, since it is continuously dissolving in the surrounding water. Although, epiphytes may compete with the host for N under limiting conditions, some

species appear to be less sensitive than others. Epiphytes on *Cladophora glomerata* in the Madison River, appeared to be less sensitive to NO_3 levels than the host (Dodds, 1991 b). However, these epiphytic genera, *Nostoc* and *Epithemia* are cyanobacterial and cyanobacterial-containing organisms respectively and may therefore be capable of fixing atmospheric N_2 (Bahls and Weber, 1988). From the above discussion it could be deduced that non N_2 -fixing epiphytes populating *C. glomerata* compete for N with their host and that the presence of dense growths of these epiphytes could be a useful preliminary indication of the presence of surplus available N in the environment.

The third speculation by Bergey *et al.* (1995), asserts that epiphyte distribution on the *Cladophora glomerata* filament is influenced by nutrient limitations especially N. The non N_2-fixing diatom, *Cocconeis pediculus* would occur on the apices where N availability is higher since nutrient acquisition is promoted by fast water velocities (Raven, 1992). *Epithemia spp.* on the other hand, which shelter N_2-fixing bacteria, dominate filament bases where nutrients are more limiting due to slower water velocities (Bergey *et al.*, 1995).

Patrick *et al.* (1983) postulates that *C. glomerata* offers a poor food source to herbivorous fauna and therefore suggests that grazer resistance may be an important aspect of the community ecology. Occasional reports (Gregor and Deacon, 1988) do exist where grazing has occurred on *C. glomerata.* However in a study by Dodds (1991 b) no significant difference in apical growth of *C. glomerata* was observed at any density of *Brachycentrus occidentalis* (an invertebrate). However, the invertebrate, *Brachycentrus occidentalis,* seemed to have removed a significant proportion of the epiphytes at its highest density. Bergey (1995) showed that the aquatic caterpillar *Petrophila confusalis* in the Eel River, South Fork, Northern California, selectively grazed on large or stalked diatoms leaving the small prostate forms attached to the substrate. This selective grazing of epiphytes was not however correlated with taxonomic groups but rather with algal morphology since the grazing ability was closely related to the mechanical efficiency of the grazer's mouthparts in algal removal and handling.

Dodds (1991 b) maintained that positive interactions (mutualism) between epiphyte grazers and grazer-resistant macrophytes do occur. For example, the grazer benefits the resistant

macrophyte by removing epiphytes which may compete for light or nutrients and which may increase the risk of dislodgement by increasing drag. The macrophyte in return benefits the grazer by providing habitat for epiphytes. Competition for light between the epiphyte and *C. glomerata* and epiphytic shading will only be detrimental under low light conditions especially below *ca* 20 μmols photons.m^{-2}.s^{-1} which has been observed as limiting to support *C. glomerata* growth (Lorenz *et al.*, 1991). In a study by Graham *et al.* (1982), maximum net photosynthesis of *C. glomerata* in the Great Lakes occurred at light intensities between 300-600 μmol photons m^{-2} s^{-1}, with higher light intensities being photoinhibitory. At such high light levels epiphytic shading would benefit the alga by reducing the light reaching the thallus, especially considering the high light intensity at the water surface of *ca* 1500 μmol photons m^{-2} s^{-1} which can occur on clear, bright summer days (Dodds, 1991 b).

2.4.2. WATER FLOW REGIMES

C. glomerata is able to withstand the shear stress caused by water motion in the benthic regions of rivers. The thallus is tough but flexible and allows flow to occur through and around it (Dodds, 1991 c). In general, increased water velocity enhances *C. glomerata* growth due to increased nutrient acquisition and different flow regimes affect branching patterns (Whitton 1970 a; Dodds and Gudder, 1992). Van den Hoek (1963) noted that the thallus was streamlined and branching more pronounced in high water flows. Likewise, Parodi and Caceres (1991) observed an increase in branching frequency with increased water velocity in *C. glomerata* collected from rivers occurring in the vicinity of Buenos Aires (Argentina). Bergey *et al.* (1995) examined the architecture of *C. glomerata* in relation to water velocity in the Eel River, N. California. Contrary to previous findings, no differences in branching patterns were observed in slow, medium and fast flowing waters, however the architecture of the young *C. glomerata* tufts was influenced greatly by changes in water velocity regimes. When an abrupt increase in water velocity occurred fragmentation of *C. glomerata* was the greatest. This observation was attributed to the presence of denser epiphytic loads on *C. glomerata* in slow water velocities . Therefore when an abrupt increase in water velocity occurred, filament drag was increased which tended to increase fragmentation.

CHAPTER 3

PRELIMINARY CULTURE INVESTIGATIONS ON *CLADOPHORA GLOMERATA*

3.1. INTRODUCTION

In culture studies, unialgal and where possible, axenic cultures are usually employed in order to determine the effects of particular environmental factors on the target species. In addition, if feasible, cultures of one particular strain or population are conventionally preferred in order to eliminate any genetic or ecotypic variation. In this chapter the attempts at obtaining and maintaining sufficient unialgal material of *C. glomerata* to perform laboratory experiments are described. The impacts of several culture media on the growth of *C. glomerata* were also determined.

3.2 METHODS

3.2.1 COLLECTION AND STOCK CULTURES

In May 1995, tufts of algae identified as *C. glomerata* were collected from the Kalkfontein irrigation canal systems in the Free State region, South Africa. The algal materials were cleaned and separated from visible vegetative epiphytes and debris, and placed into 1-l glass jars containing aerated fresh tap water. The *C. glomerata* stock materials were stored in growth rooms maintained at 15°C at a light intensity, measured as photosynthetically active radiation using a Skye SKP 200 light meter, of *ca* 60 μmol photons m^{-2} s^{-1} supplied by cool white fluorescent tubes (Osram L30W/20) for a 16 hour day length.

3.2.2. CULTURE MEDIA

All culture media were sterilised in a stainless steel Memmert water bath for 2 hours at *ca* 90°C one day prior to use. Synthetic media were manipulated to yield required pH levels by using either dilute HCl or a 1M NaOH solution. All media pH levels were measured with a calibrated Crison pH/mV meter, Model 506.

a. Soil Water Extract Medium (described by Starr, 1964)

One cm^3 of clay-loam soil, collected outside the Botany Department, was boiled in one litre of distilled water in a 1-l conical glass flask covered with aluminium foil. The soil-water mixture was steamed consecutively three times for a period of 1½ hours at 100°C in a water bath. Between each steam event the soil-water mixture was allowed to cool to room temperature for 24 hours. Subsequently the soil-water mixture was paper filtered and the resultant soil extract was autoclaved at 120°C for 30 minutes. The final medium had pH 7.5.

b. Woods Hole Medium (after Stein, 1973)

The preparations of full strength Woods Hole medium and its stock solution components are described in Table 3.1. Distilled water was used to make up all the chemical stock solutions as well as the Woods Hole medium. The half strength Woods Hole medium was prepared by dividing the concentrations in the full strength by two. Final media pH for both the full and half strength Woods Hole was about 8.

c. Tap Water Medium

A single sample of tap water was collected from the sink tap in the laboratory. The measured pH of the tap water ranged between 8.5 and 9. The tap water was allowed to stand for two days prior to use to allow chlorine gas to disapate. The chemical composition of the tap water provided by the Cape Town's City Engineer Department is presented in Table 3.2.

d. Bolds Basal Medium (after Nichols and Bold, 1965)

The preparations of the Bolds Basal medium and its chemical components are presented in Table 3.3. Chemical stock solutions and the Bolds Basal medium were made up with distilled water. The pH of the final Bold Basal medium was 6.

Table 3.1. Components of the synthetic growth medium, Woods Hole (after Stein, 1973)

Component nutrients	Stock solution	Dilution (ml l^{-1}) Full strength
Macronutrients:		
$CaCl_2.2H_2O$	36.76 g l^{-1}	1
$MgSO_4.7H_2O$	36.97	1
$NaHCO_3$	12.60	1
K_2HPO_4	8.71	1
$NaNO_3$	85.01	1
$Na_2SiO_3.9H_2O$	28.42	1
Micronutrients:		
Na_2.EDTA	4.36 g l^{-1}	1
$FeCl_3.6H_2O$	3.15	1
$CuSO_4.5H_2O$	0.01	1
$ZnSO_4.7H_2O$	0.02	1
$CoCl_2.6H_2O$	0.01	1
$MnCl_2.4H_2O$	0.18	1
$Na_2MoO_4.2H_2O$	0.006	1
Vitamins:		
Thiamine.HCL		0.1 mg.l^{-1}
Biotin		0.5 μg.l^{-1}
Cyanocobalamin		0.5 μg.l^{-1}
Buffer:		
Tris (hydroxymethyl-aminomethane)	50 g/200ml	2

Table 3.2. Analytical composition of tap water derived from the Steenbras Dam Supply for the period 1991-93. (obtained from the Cape Town's City Engineer's Department).

Nutrient composition	Concentration Range ($mg.l^{-1}$)
Minerals:	
Cl	17-27
SO_4	12-18
Ca	12-20
Mg	1.1-2.4
Na	9-13
K	0.3-0.8
Trace Metals:	
Al	0.10-0.45
Fe	0.006-0.119
Mn	0.003-0.043
Nitrogen:	
N (Ammoniacal)	0.01-0.03
N (albuminoid)	0.01-0.08
Other inorganics:	
Si	0.6-1.4
P	0.001-0.058
F	0.01-0.04

Table 3.3. Components of the synthetic growth medium, Bolds Basal. (after, Nichols and Bold, 1965)

Component Nutrients	Stock solution	Dilution
Macronutrients:		
$NaNo_3$	10 g $400ml^{-1}$	10 ml 940 $ml.^{-1}$
$CaCl_2.H_2O$	1	10
$MgSO_4.7H_2O$	3	10
K_2HPO_4	3	10
KH_2PO_4	7	10
NaCl	1	10
EDTA:		
EDTA	50 g l^{-1}	1 ml l^{-1}
KOH	31	1
Iron:		
$FeSO_4.7H_2O$	4.98 g l^{-1}	1
H_2SO_4	1.0 ml l^{-1}	1
Boron:		
H_3BO_3	11.42 g l^{-1}	1
Micronutrients:		
$ZnSO_4.7H_2O$	8.82 g l^{-1}	1
$MnCl_2.4H_2O$	1.44	1
MoO_3	0.71	1
$CuSO_4.5H_2O$	1.57	1
$Co(NO_3)_2.6H_2O$	0.49	1

a total of 20 experimental flasks. Flasks were cultured at 25°C ± 2°C, at 60 μmol photons m^{-2} s^{-1} at a photoperiod of 16:8 light/dark cycle for 4 days. At the end of the fourth day the algal segments were remeasured and the healthy-looking segments placed into fresh media for further growth observations.

All dissecting and handling implements used were sterilised by repeatedly passing them through an alcohol flame. All glassware was sterilized by soaking in detergent, acid washed with dilute hydrochloric acid, rinsed three times with distilled water and oven dried at 60°C.

3.3. RESULTS AND DISCUSSION

The initial method applied in order to obtain unialgal cultures proved unsuccessful although numerous attempts were made. The *C. glomerata* tufts cultured in both the full strength Woods Hole medium and the Soil Water Extract medium were infested with a filamentous green alga after 1 week. Chudyba (1965) and Lowe *et al.* (1982) have shown that the cell wall structure of *C. glomerata* is suitable for hosting a range of epiphytes. The epiphyte, *Stigeoclonium tenue* (Agardh) Kütz, appeared to generate from small vegetative cells which were attached to the cell wall of *C. glomerata.*

Hoffman and Graham (1984) suggest that zoosporogenesis in *C. glomerata* is significantly induced by a short day photoperiod of 8 hours. A temperature range between 15°C and 20°C promotes zoospore formation and a low light intensity of less than 60 μmol photons m^{-2} s^{-1} was shown to play a less important role. When sporulation occurs the apical cells are left empty (Van den Hoek, 1963). Despite exposing *C. glomerata* filaments to environmental conditions which have been reported to stimulate zoosporogenesis no evidence of zoospores, indicated either by empty cells or as spore release or growth of sporelings in the dishes, was observed. The only growths observed, under the microscope at the 400 X magnification on the glass slides which were placed into the replica dishes, were diatoms. Furthermore the *C. glomerata* filaments usually died within two weeks after they were placed into culture.

The third attempt involved agitated cultures, which was based on the claim by Van den Hoek (1963) that *C. glomerata* thrives in fast flowing waters and the fact that the plants were collected from canals with water flow of about 1 m sec^{-1} (Joska, pers. comm.). In addition, Steinman and McIntire (1986) state that epiphytes colonize slower in high water velocities than in slower water movements. In spite of these claims, the agitated algal segments grew very slowly. The dissected algal segments appeared green and epiphyte free for 1 week after being placed into culture although upon further examinations (2 and 3 weeks old cultures), they became inundated with epiphytes or bleached and subsequently died. Although the *C. glomerata* filaments seemed clean initially when viewed under the compound microscope at 100 X magnification, it was probable that epiphyte cells persisted which germinated in time.

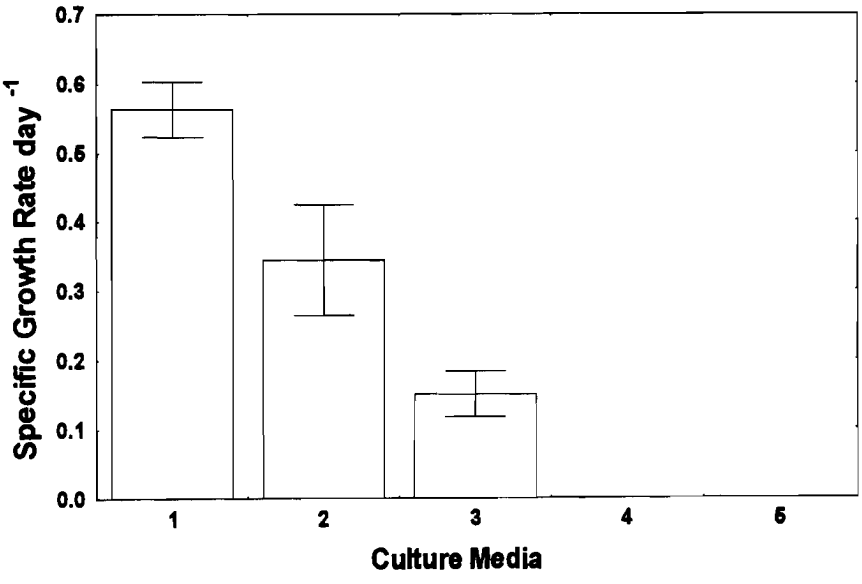
From the above observations it was eventually decided that freshly collected *C. glomerata* materials would be used for the experiments. Laboratory experiments would be conducted over short time intervals, not exceeding 5 days, to eliminate the possible encroachment by epiphytes.

The final investigation was to resolve in which medium *C. glomerata* grows optimally. Specific growth rates were computed using:

$$r = [\ln (L_z/L_0)]/(t_z-t_0) \quad \text{.. equation 3.1}$$

where r = specific growth rate (SGR), L_z and L_0 represent the average filament lengths of the 20 segments in each test tube, at t_z = day z and t_0 = day 0, respectively (Rueness and Tananger, 1984). The results are plotted in Fig. 3.1.

A statistically significant difference exists ($F=94.616$, $p \leq 0.05$) using a One-Way Anova on Statgraphics v 5.0, between the SGRs of *C. glomerata* cultured in different media (Fig. 3.1). The highest SGR of 0.56 day^{-1} was by the filaments growing in the tap water. The SGRs of *C. glomerata* filaments cultured in full strength Woods Hole and soil water extract medium were 60% (0.34 day^{-1}) and 27% (0.15 day^{-1}) respectively, of the SGR achieved by the filaments grown in tap water. No algal growth was observed in the Bolds Basal medium although the filaments remained green and healthy. In the ½ strength Woods Hole medium virtually all the filaments died.



LEGEND

Medium 1= Tap water
2= Full strength Woods Hole
3= Soil water extract
4= Bolds Basal
5= Half strength Woods Hole

Fig. 3.1. The specific growth rates of *C. glomerata* filaments exposed to different culture media for a period of 4 days. Means are plotted ± SE (vertical lines), n = 4

The poor growth or death of the filaments cultured in the Bolds Basal, Woods Hole (full and half strength) and soil water extract compared to the filament growth in the tap water could be attributed to nutrient limitations such as P (Bolus and Lund, 1974; Neil and Jackson, 1982 and Planas *et al.*, 1996), Si (Moore and Traquair, 1976) or vitamins B_1 and B_{12} (Hoffman and Graham, 1984) which are essential for *C. glomerata* growth, or low media pH (Whitton 1970 a).

glomerata in Wong and Wainwright's (1994) study was probably attributed to the fact that they supplemented the medium with vitamins and Si. However, in their report no mention was made of the medium pH.

Although growth was evident in the full strength Woods Hole medium and soil water extract in this study, growth was optimal in the tap water. The only other reported laboratory study which had used tap water as a growth culture medium for *C. glomerata* was Gibson *et al.* (1990). Many laboratory studies have however, cultured *C. glomerata* in natural waters collected from where the alga was found (Bolas and Lund, 1974; Betzer and Kott, 1969; Simpson and Eaton, 1986; Lester *et al.*, 1988). For the purpose of this project it would have been impractical to transport enough water, for all the laboratory experiments, from the Kalkfontein canal due to the immense distance of the laboratory from the canals (620 kilometers in a straight line). Further experiments could have been conducted in order to determine why the *C. glomerata* filaments died in the half strength Woods Hole medium, to investigate the growth response of *C. glomerata* in various other synthetic media, modified Woods Hole or Bolds Basal media, and to invesitgate the influence of NP enrichment of tapwater. However, due to time constraints, the fact that these investigations were not part of the main objectives of the project and the healthy growth obtained in tap water, it was decided that tap water enriched with N and P would be utilised as the basic culture medium in subsequent experiments.

Although growth of *C. glomerata* was effectively achieved in tap water with no N or P supplementation at 25°C at a light intensity of 60 μmol photons m^{-2} s^{-1} set at a 16 hour day length (Fig. 3.1), specific growth rates at different light, temperature and photoperiod regimes may be enhanced to a greater extent. For this reason, N and P were added to the tap water to ensure that their concentrations far exceeded the critical limiting concentrations suggested by Pitcairn and Hawkes (1973). So when the effects of different factors on the growth of *C. glomerata* were investigated, the remote possibility of N and P limitations occurring was diminished. Furthermore, highly eutrophied water bodies where *C. glomerata* growth is stimulated are characterised by high N and P concentrations. Therefore concentrations as high as 14 mg N l^{-1} and 2 mg P l^{-1} were added, as $NaNO_3$ and K_2PO_4 respectively, to the tap water in order to simulate eutrophic conditions. Gibson *et al.*(1990) similarly supplemented their aged

tap water growth medium with 2 mg P l^{-1} but with a slightly lower N concentration of 4 mg l^{-1}. However, a N:P ratio of 14:2 is a more realistic ratio in relation to metabolic needs than is Gibbon et al.'s 4:2.

LEGEND

Medium 1= Tap water
2= Full strength Woods Hole
3= Soil water extract
4= Bolds Basal
5= Half strength Woods Hole

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The poor growth or death of the filaments cultured in the Bolds Basal, Woods Hole (full and half strength) and soil water extract compared to the filament growth in the tap water could be attributed to nutrient limitations such as P (Bolus and Lund, 1974; Neil and Jackson, 1982 and Planas *et al.*, 1996), Si (Moore and Traquair, 1976) or vitamins B_1 and B_{12} (Hoffman and Graham, 1984) which are essential for *C. glomerata* growth, or low media pH (Whitton 1970 a).

CHAPTER 4

THE EFFECTS OF LIGHT, TEMPERATURE AND PHOTOPERIOD ON *CLADOPHORA GLOMERATA*

4.1 GENERAL INTRODUCTION

Environmental factors including temperature and irradiance play a fundamental role in regulating the growth of algae. Both factors impact growth by executing their effects on the primary growth response of photosynthesis. Temperature also influences the secondary growth processes of respiration and nutrient uptake (O'Neal and Lembi, 1995). Raven and Geider (1988) assert that temperature is perhaps the most widely measured environmental variable that affects algal growth. The rate of growth at sub-optimal low or high temperatures is essentially limited or inhibited by some specific temperature-sensitive process. In addition the specific growth rate at limiting photon flux densities appears to be relatively temperature sensitive.

4.1.1. ENVIRONMENTAL FACTORS AND *C. GLOMERATA*

Dodds and Gudder (1992) state that in nature different species of the genus *Cladophora* are adapted to high or low light habitats. A general consensus amongst researchers is that *C. glomerata* thrives under high light conditions (Whitton, 1970 a). Field studies by Sand-Jensen *et al.* (1989) in the River Suså, Denmark showed that high *Cladophora* biomass only occurred during the summer months when light availability was high. Bolus and Lund (1974) similarly noticed that *C. glomerata* collected from the Great Stour River, England, preferred well illuminated areas, although exposure to full sunlight appeared to be harmful to the alga. On the other hand a study by Neel (1968) illustrated that summer growths of *C. glomerata* in a Kentucky stream, United States of America, were restricted to shady areas possibly indicating that the alga was adapted to low light conditions.

Literature reports generally indicate a temperature tolerance range from 10°C to 30°C for *C. glomerata* growth (Bellis and McLarty, 1967; Graham *et al.*, 1982) although some deviating

accounts have been observed. Waern (1952) observed *C. glomerata* plants at a lower temperature limit of 7°C on bare surfaces in the Oregrund Archipelago. Chudyba (1965) recorded plants of *C. glomerata* in the River Skawa, Poland, in river water temperatures as low as 9°C during autumn. Joska (pers. comm.) reported that water temperatures of 6°C still supported plants of *C. glomerata* in the Kalkfontein irrigation canals, South Africa. Whitton (1970 c, cited in Whitton 1970 a) found that a *Cladophora* sp. possibly *C. glomerata* occurring in tropical pools exhibited a maximum temperature tolerance of 37°C during the growing season of the alga. Similarly *C. glomerata* plants in water temperatures well over 30°C have also been observed by Chudyba (1965).

Studies indicating deviating temperature and light limiting ranges for *C. glomerata* in the field may be either attributed to taxonomic problems in species identification, genetic variability, producing different strains in the species or alternatively phenotypic variation. Marks and Cummings (1996) indicated that freshwater *Cladophora* samples collected from different localities in the United States of America as well as from South Africa showed very similar nucleotide sequences of the internal transcribed spacer regions (DNA sequencing). They concluded that freshwater *Cladophora* is genetically similar irrespective of geographical locality and may even comprise of one or very few species which are ecologically and morphologically different.

Limiting temperature and light ranges for growth have an important influence on the seasonal distribution patterns of *C. glomerata*. Thurman and Kuehne (1952) working in Texas, United States of America, considered the alga to be winter dominant because they observed maxima in March and November (spring and autumn respectively). They concluded that the disappearance in summer indicated a lack of tolerance of water temperatures of higher than 24°C. Similarly Whitton (1970 a) reported that significant reductions in standing crops of *C. glomerata* have been observed in field studies in temperate regions during the hottest months. However field observations by Bellis and McLarty (1967) showed that maximum production of *C. glomerata* in the south-western region of Lake Ontario occurred in summer through two short periods of intensive vegetative growth. The tendency for the interval between the first and second periods appeared to increase in successively more southern locations. Herbst (1969)

likewise noticed a two-cycle growth pattern in the Great Lake region. Rapid growth began during the May-June period and by late June or early July filaments were 12-15 cms in length. The plants remained this size until the end of July or early August when renewed growth of the filaments or germination of summer zoospores initiated the second cycle, the time dependent upon local ecological factors of light and temperature. Growth continued rapidly until October after which only sparse patches were found. A more recent study by Mantai *et al.* (1982) illustrated a similar seasonal pattern of *C. glomerata* from two sites along the eastern shores of Lake Erie namely, Point Gratiot and Niagara. A dramatic increase in standing crop during early June was observed which was abruptly ended by a sharp decrease in mid-July. It was concluded that temperature was probably the limiting factor for *C. glomerata* growth since N and P levels were well above the critical cellular concentrations of 1.1% and 0.06% respectively.

Herbst (1969) concurred that the first growth peak of *C. glomerata* was a function of water temperature. In the Great Lakes water temperatures generally dropped below 15°C until June and consequently metabolic rates were slow before then. As water temperature rose metabolic activity increased. Likewise, Lorenz and Herdendorf (1982) attributed the bimodal growth pattern observed in the infralittoral zone of Lake Erie as the result of several environmental factors influencing the energetics of *Cladophora*. As water temperatures rose above 10°C new *Cladophora* growths appeared predominantly from small filaments which had overwintered. Maximum growth occurred in the late spring or early summer as water temperatures rose from 10°C to 23°C. Although incident light and photoperiod were close to optimum levels, a decline in standing crops occured when water temperatures reached 23°C to 26°C thereby demonstrating an upper temperature limit. They concluded that high temperatures of more than 26°C may have increased respiration to rates that were greater than the gross production which resulted in a negative energy balance. As a consequence of this temperature-induced negative energy balance, growth ceased, the condition of the cells deteriorated as evidenced by a reduction in tissue nutrients and the cells weakened with increased susceptibility to detachment. Blum (1957) however maintained that the mid-season decline of *C. glomerata* in the Saline River, Michigan, was probably not due to high temperature but to very low night temperatures which inhibited cellular activities. Lorenz and Herdendorf (1982) suggested additional factors which may have induced summer die-off. For example, an increase in the cell wall thickness

could have impeded nutrient diffusion and light penetration. Another possible factor was slow water movement which affected *Cladophora* growth during the summer in Lake Erie. Periods of calm decreased water movement past the cells resulting in a weak diffusional gradient. Furthermore less filament movement as a result of low flow rates may have produced self-shading.

From the latter discussion it is apparent that the growth and hence the seasonal distribution of *C. glomerata* are influenced primarily by temperature and to a lesser extent by light availability. Although extensive research has been undertaken on *C. glomerata* especially in the northern hemisphere including the Great Lakes Region, very little research has been conducted in South Africa. A series of investigations were therefore initiated which primarily determined the effects of temperature, light and photoperiod on the growth responses of South African *C. glomerata*.

4.2. MATERIALS AND METHODS

4.2.1. COLLECTION AND STOCK CULTURES

Laboratory studies were conducted at the University of Cape Town, Botany Department. Fresh material of *C. glomerata* was collected at monthly intervals from the Kalkfontein irrigation canal. Algal tufts collected from floating masses in the canals were lightly squeezed to remove excess water and inserted into plastic bags which were transferred to a cooler bag and transported to the laboratory within 24 hours. Subsequently algal materials were cleaned of macroscopic epiphytes and debris by thorough rinsing in tap water and placed into culture. Stock cultures were maintained at a water temperature of 15°C ± 1°C in 1-l glass jars containing fresh tap water which was changed weekly. Aeration was supplied by using unfiltered air bubbled through aeration stones. A light intensity of 60 μmol photons m^{-2} s^{-1} was provided by cool white fluorescent tubes (Osram L30W/20) set on a photoperiod of 16:8 light-dark cycle. For experimental purposes algal materials used were maintained in the laboratory for at least one week but never more than one month. All glassware utilised was soaked in detergent, acid washed with dilute HCl, rinsed three times with distilled water and oven dried at 60°C.

4.2.2. EXPERIMENTAL SET-UP

All the growth experiments were conducted in a growth chamber with an air temperature maintained at 10°C. An overhead light bank fitted with three fluorescent tubes (GEC Alsthom 65W) generating cool white light was used as the sole source of irradiance. Light intensity was measured in μmol photon m^{-2} s^{-1} as photosynthetically active radiation (PAR) using a Skye SKP 200 light meter. Light intensities of 0, 25, 45, 75, 100 and 150 μmol photons m^{-2} s^{-1} were obtained by manipulating the distance of the flasks away from the light source as well as shielding the experimental flasks with nylon shade cloth. The above values presented are an indication of light intensities measured externally to the flasks but at the same level of the algal incubation. Temperatures examined were 10°C, 15°C, 20°C, 25°C, 30°C and 35°C. Water temperature was manipulated by placing the flasks into perspex water baths (47cm x 25cm x 13 cm) which were fitted with Tempette Junior TE-8J (Techne) thermostats. The experimental flasks rested on white material sheets which were elevated to approximately ½ the height within the water baths. The water levels in the water baths were always maintained above the flasks 100-ml mark to ensure that the algal filaments were always exposed to the selected experimental temperature. The required temperature for each experiment was monitored for at least three days prior to the experimental run in order to eliminate any fluctuations. Photoperiods namely a long-day, 16:8 light-dark cycle and a short-day, 8:16 light-dark cycle were simulated by connecting an automatic time switch to the light source. The entire set-up was surrounded by two layers of shade cloth and black plastic bags to prevent any outside light from penetrating the systems.

4.2.3. EXPERIMENTAL PROCEDURES

C. glomerata segments of 10 mm each were cut under water in a glass culture dish lid, under which a grid block consisting of squares with dimensions of 1 mm by 1 mm each was taped, using a Kyowa Optical Stereomicroscope Model SD-2PL. Segments were cut from unbranched filaments in order to obtain uniform filament lengths and morphology. Since the apices were normally branched the segments were cut from the intercalary regions. It must be noted that although apical growth in *C. glomerata* is dominant and excluding the apices may have resulted

in reduced activity, *C. glomerata* is also capable of diffuse growth. The dissected segments were placed into 100-ml Erlenmeyer flasks, with each one containing 50 ml of aged tap water (refer Table 3.2 for chemical components) supplemented with 14 mg N l^{-1} and 2 mg P l^{-1} as $NaNO_3$ and K_2HPO_4 respectively. These N and P concentrations which were added far exceeded the minimum limiting values for *C. glomerata* growth as suggested by Pitcairn and Hawkes (1973). For all experiments, the tap water was collected in May 1996 and stored in 20-L plastic containers maintained at 20°C in the dark. By allowing the tap water to stand for at least two weeks before utilisation permitted the dissipation of any chlorine gas. The N and P enriched tap water medium was sterilised by steaming in a Memmert stainless steel water bath for 2 hours at about 90°C and cooled to room temperature overnight. The pH, measured with a Crison pH/mV meter (Model 506), naturally ranged between 8-8.5 for all the experiments.

Twelve experiments were conducted each over a 5 day period, at one of the 6 specified temperatures at either a long-day or short-day photoperiod at all the 6 manipulated light intensities. Individual experiments comprised 4 replicate flasks, containing twenty *C. glomerata* filaments each, exposed to the 6 light intensities thereby yielding a total of 24 experimental flasks. The positions of the flasks within treatments were unfortunately not randomised daily since it was difficult to rebalance the flasks in the water bath. No aeration was supplied since bubbling itself may have affected *C. glomerata* growth and it was virtually impossible to obtain a constant rate in all the flasks. However the mouths of the flasks were loosely covered with polythene, to allow spontaneous diffusion of ambient air into the water medium. At the termination of the experiment the algal filament lengths were remeasured by applying the same grid-technique used previously. Where branches formed during the experiments, they were measured using the 1 mm^3 square grids.

4.3. COMPUTATION AND STATISTICAL ANALYSES

The raw data measurements of the filament lengths were transformed to specific growth rates using the same formula as equation 3.1., that is

$$r = [\ln (L_z/L_0)]/(t_z-t_0) \qquad \text{..equation 3.1}$$

where r = specific growth rate (SGR), L_z and L_0 represent the average filament lengths of the 20 segments in each test tube, at t_z = day z and t_0 = day 0, respectively (Rueness and Tananger, 1984)

Specific growth rate data were fitted to a Hyberbolic Tangent Function after Jassby and Platt (1976), which models the Photosynthetic-Irradiance curve. The original formula:

$$P = P_{max} \tanh (I/I_k) \qquad \text{.. equation 4.1}$$

was manipulated and photosynthetic parameters represented by **P** were replaced by SGR parameters, **G**, yielding the formula:

$$G = G_{max} \tanh (I/I_k) \qquad \text{.. equation 4.2.}$$

where **G** = estimated SGR, G_{max} = light saturated SGR, **tanh** = hyberbolic tangent function, **I** = measured Irradiance (light intensity) and I_k = half-saturation light point.

A user-specified regression (equation 4.3) was computed by applying a modified version of equation 4.2.

$$G = (G_{max} * (\tanh^{**}(I/I_k) - (1/\tanh)^{**}(I/I_k)) / (\tanh)^{**}(I/I_k) + (1/\tanh)^{**}(I/I_k)))$$

.. equation 4.3

where tanh = 2.718281828

The Rosenbrock and Quasi-Newton estimation method was employed and the regression was fitted with a 2-D function and observed values. Light saturation points at G_{max} were calculated by multiplying the half-saturation light point (I_k) by 2.

All statistical analyses were executed on a software computer package, Statistica v 5.0 using STA-NLV (Non-linear estimations) or Anova/Manova bases. Computed r-values of the non-linear regression lines were generated by the STA-NLV package. Statistical significance were obtained by applying either an Analysis of Variance (F-ratios) or Kruskall-Wallis test (H). Tests for normality were performed using parametric probability plots and homoscedacity was determined by the Cochran's (Chi^2) or Bartlett's tests (B). Statistical significances were denoted where p was ≤ 0.05.

4.4. RESULTS

Algal specific growth rates were primarily affected by temperature (Fig. 4.1 a and b). At 10°C, the lowest temperature examined, although the algal filaments remained a healthy green colour no growth occurred irrespective of light intensity and photoperiod. At 35°C, the maximum temperature investigated, all the algal filaments turned white and died within 5 days. SGRs were similar for temperatures between 20°C and 30°C at most light intensities, and at both photoperiods (Fig. 4.1 a and b), however they were significantly (H=9.56) reduced at 15°C, long-day (Fig. 4.1 a). SGRs at 15°C, long-day photoperiod, were decreased by approximately 40% at light intensities of 25, 45 and 75 μmol photons m^{-2} s^{-1} and by 65% at the two higher irradiance levels of 100 and 150 μmol photons m^{-2} s^{-1} compared to the SGRs at the optimal temperature. At the short-day photoperiod, SGRs at 15°C were similar to the SGRs at 20°C, 25°C and 30°C at light intensities from 0-75 μmol photons m^{-2} s^{-1} but at higher light intensities SGRs were reduced by about 30-35%.

Estimated maximum specific growth rates (G_{max}) at light saturation point were extrapolated from the non-linear regression curves and presented in Table 4.1. G_{max} increased with an increase in temperature from 10°C (no growth observed) to 25°C where the highest values, 4.29 day^{-1}

(long-day) and 1.36 day^{-1} (short-day) were recorded and reduced by 20% and 15% at 30°C at long-day and short-day photoperiods respectively. At the long-day photoperiod, G_{max} was significantly lower at 15°C, being 40% of G_{max} at 25°C. At a short-day photoperiod, G_{max} at 15°C, was significantly reduced to 68% of G_{max} at 25°C.

Table 4.1. Growth parameters estimated from the Growth-light Response Curves modelled to equation 4.2. $G = G_{max} \tanh (I/I_k)$ after Jassby and Platt (1976).

Temperature	**G_{max} =** maximum SGR day^{-1} at light saturation			**I_k =** ½-saturation light point (μmol photons m^{-2} s^{-1})		**Light saturation at G_{max}** (μmol photons m^{-2} s^{-1})	
	Photoperiod (light:dark hours per per day)						
	16:8	8:16	ratio	16:8	8:16	16:8	8:16
10°C	0	0	-	0	0	0	0
15°C	1.60	0.93	1.72	27.55	27.81	55.10	55.62
20°C	4.05	1.17	3.48	50.62	18.06	101.24	36.12
25°C	4.29	1.36	3.15	62.86	73.97	125.72	147.94
30°C	3.37	1.14	2.96	44.02	55.17	88.04	110.34
35°C	died	died	died	died	died	died	died

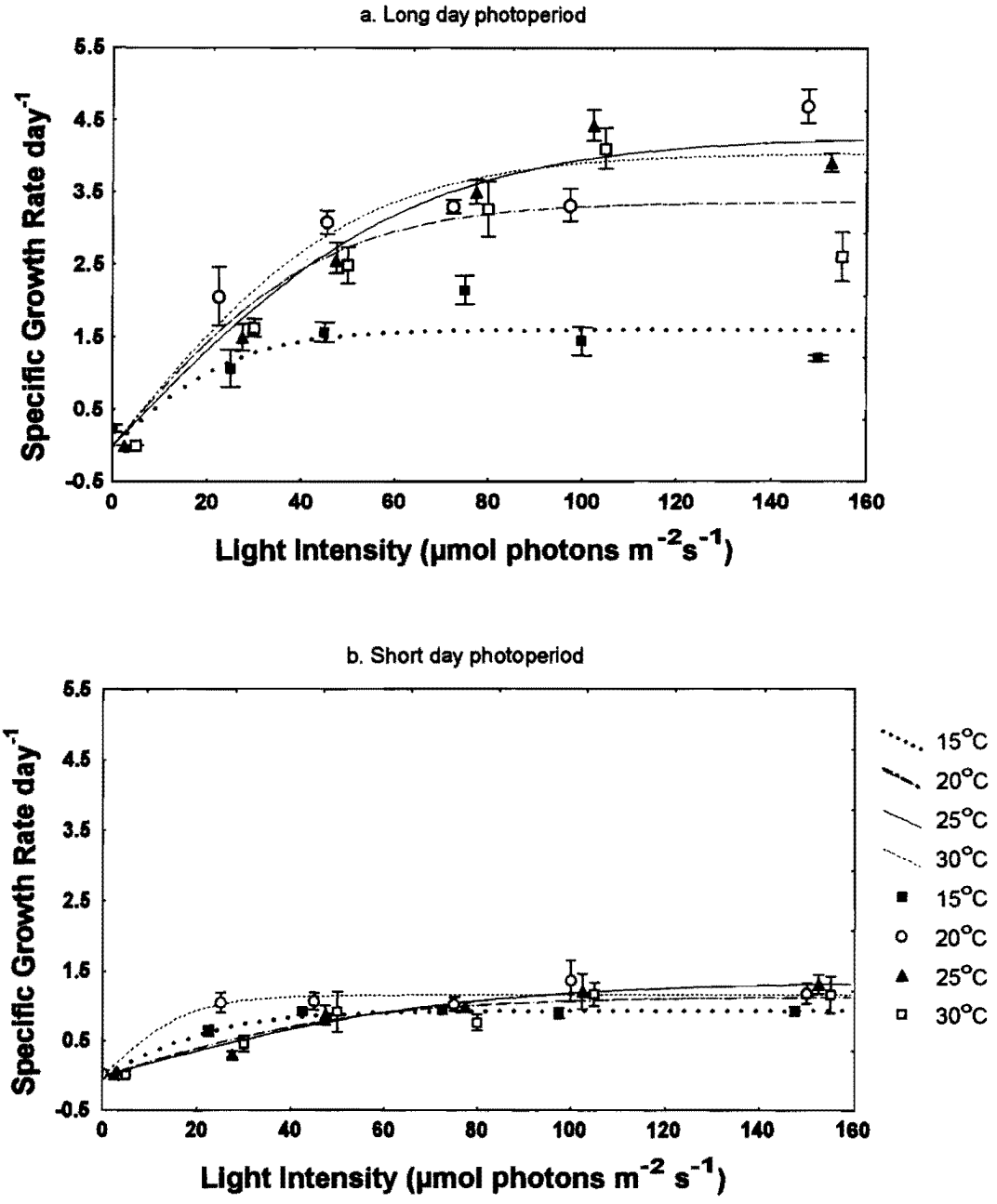


Figure 4.1 a and b. The effects of light intensity and temperature on the specific growth rates of *C. glomerata* at two photoperiods. a. Long-day (16:8 light-dark cycle) and b. Short-day (8:16 light-dark cycle). Means (n=4) are plotted with SE (vertical lines) and fitted with non-linear regression lines (line graphs).

There was a direct correlation between the specific growth rate and light intensity as shown by the correlation coefficient value (r) presented in Table 4.2. at all temperatures and both photoperiods. That is, at each temperature at both photoperiods, the specific growth rate increased from 0 μmol photons m^{-2} s^{-1} to a point where it reached a saturation point after which no further increase in growth occurred with further increase in light intensity (Figs. 4.2 a, b, c and d).

Table 4.2. The correlation coefficient values of the non-linear regression curves of the specific growth rate against irradiance at each temperature at both photoperiods.

	r value	
Temperature	Photoperiod (light:dark hours per day)	
	16:8	8:16
10°C	-	-
15°C	0.76	0.95
20°C	0.92	0.82
25°C	0.97	0.88
30°C	0.88	0.75
35°C	-	-

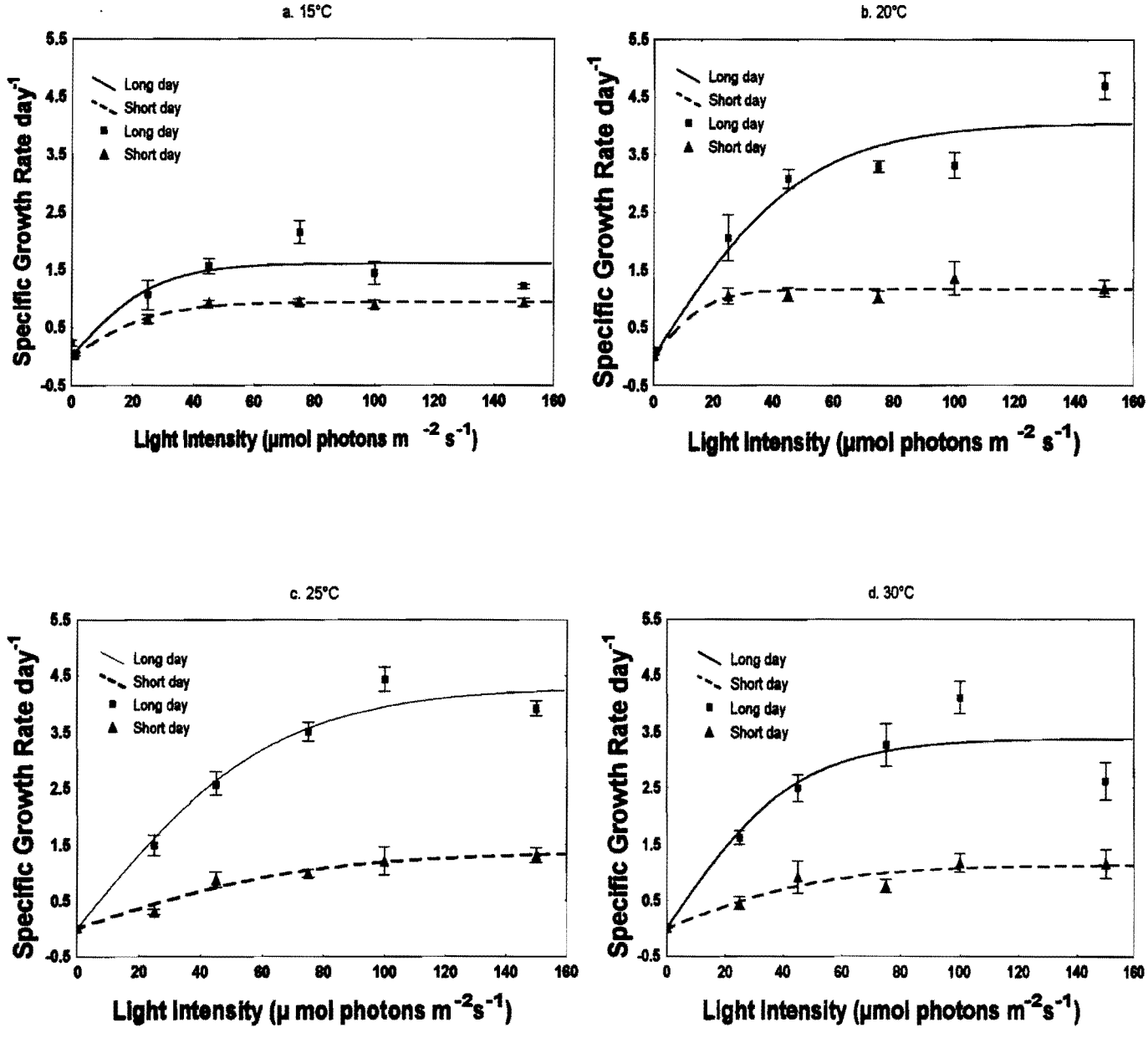


Figure 4.2 a,b,c and d. The effects of light intensity and photoperiod on the specific growth rates of *C. glomerata* at different temperatures. a. 15 °C, b. 20°C, c. 25°C and d. 30°C. Symbols represent the data means (n=4) plotted with ± SE (vertical lines). The line graphs are the fitted non-linear regression curves.

In general, maximum SGRs (G_{max}) of the algal filaments exposed to long-day and short-day photoperiods exhibited a tendency to shift their light saturation points with temperature. At a long-day photoperiod, light saturation points increased proportionately from 55 μmol photons m^{-2} s^{-1} at 15°C to 125 μmol photons m^{-2} s^{-1} at 25°C where the highest estimation was predicted, with a decline to 88 μmol photons m^{-2} s^{-1} at 30°C (Table 4.1). At the short-day photoperiod, the light saturation points increased from 55 μmol photons m^{-2} s^{-1} at 15°C to 147 μmol photons m^{-2} s^{-1} at 25°C with a reduction to 112 μmol photons m^{-2} s^{-1} at 30°C. The light saturation point of 36 μmol photons m^{-2} s^{-1} at 20°C (short-day) however was much lower than at all the other temperatures (Table 4.1). Light saturation points were similar at both photoperiods at 15°C, however the light saturation point at 20°C, short-day, was 64% lower compared to the long-day light saturation point at 20°C. At 25°C and 30°C, the light saturation points at the long-day photoperiods were both ± 20 μmol photons m^{-2} s^{-1} less than the light saturation points at the short-day light cycle (Table 4.1).

Photoperiod also exhibited a significant (H=59.94) effect on the SGRs of *C. glomerata*. The algal SGRs were noticeably higher at a long-day photoperiod (16:8 light-dark cycle) compared to the short-day photoperiod (8:16 light-dark cycle) at all the temperatures and light intensities which sustained growth (Fig. 4.2.). Ratios of long-day:short-day G_{max} values are presented in Table 4.1. G_{max} estimates were higher at a long-day at temperatures of 15°C, 20°C, 25°C and 30°C, compared to the short-day light cycle. The magnitude of the ratios was greatest at 20°C and 30°C, with G_{max} being *ca.* 3 times more at the long-day relative to G_{max} at the short-day photoperiod.

4.5. DISCUSSION

Wong *et al.* (1978) state that temperature plays an important role in *C. glomerata* dominance especially when nutrients are readily available. In this study, at both the long- and short-day photoperiods (16:8 or 8:16 light/dark cycles) the maximum SGR (G_{max}) of *C. glomerata* at light saturation point was optimal between 20°C and 25°C, slightly suppressed at 15°C and 30°C, with 35°C being lethal and 10°C allowing survival, but not growth (Table 4.1 and Figs. 4.1 a and b). Similar temperature growth effects were reported by Whitton (1967) who collected *C. glomerata* from the River Wear in England, Bellis (1968 b) who cultured *C. glomerata* derived from vegetative plants growing attached to stones in Medway Creek, Lake Ontario, Hoffman and Graham (1984) who harvested *C. glomerata* from Lakes Mendota, Madison and Wisconsin (United States of America) and Robinson and Hawkes (1986) who worked on unialgal cultures of *C. glomerata.* Optimum growth for *C. glomerata* was reported to be between 20°C and 25°C whilst temperatures below 10°C and above 30°C were limiting boundaries of detectable growth.

Slightly lower optimum and maximum limiting temperatures for growth were described by Storr and Sweeney (1971) who cultured *C. glomerata,* at a temperature range of 14.5°C to 26°C at a light intensity of 800 foot candles for 30 days, collected from the Oswego region of Lake Ontario and Wong *et al.* (1978) when correlating the effects of water temperature and seasonal pattern of *C. glomerata* in seven river systems in southwestern Ontario. In both studies optimum temperature for growth was reported to be 18°C however Wong *et al.* (1978) noticed a lower upper temperature tolerance of 23.5°C compared to Storr and Sweeney (1971) who observed reduced growth at 26°C.

Likewise, different temperature responses of *C. glomerata* photosynthesis have been reported. In a study by Graham *et al.* (1982) photosynthesis measured as O_2 evolution was optimum between 13°C and 17° for *C. glomerata* which was collected during spring time from Lake Huron, Harbor Beach, Michigan. *Cladophora glomerata* collected from the upper layers of the water in the Leeds and Liverpool Canals, Merseyside, and subsequently cultured in the laboratory displayed optimum photosynthesis at a slightly higher temperature of *ca* 20°C

(Simpson and Eaton, 1986). Adams and Stone (1973) reported yet a higher water temperature range, between 19°C and 24°C, for optimal net photosynthetic activity of *C. glomerata* collected from Green Bay, Lake Michigan. More recent laboratory studies by Lester *et al.* (1988) using *C. glomerata* plant materials collected from the same region (Green Bay, Lake Michigan) indicated still higher optimal temperatures for net photosynthesis, from 28°C to 31°C, which varied with season.

These diverging findings of *C. glomerata's* response to temperature by various researchers could be attributed to many factors. Inconsistent culture conditions for example, algal age, photoperiod, light intensity and nutrient medium used may inherently affect the alga's growth response to temperature. Moreover, the different temperature regimes of *C. glomerata* reported may likely be a factor in selecting for ecotypic sub-specific variation.

The seasonal distribution of *C. glomerata* in the Kalkfontein irrigation canal scheme, from where the materials were collected for this laboratory study, coincides with the optimum and limiting temperature ranges for growth as reported in the Results 4.3. Joska (pers. comm.) observed that in the Kalkfontein area, the water temperatures in winter drops below 10°C which coincided with reduced or negligible biomass of *C. glomerata* occurring in the irrigation canals. In summer, the water temperatures generally rise above 20°C but seldom above 25°C. Accordingly, prolific *C. glomerata* growth was continuous throughout the summer in the Kalkfontein scheme, unlike the bimodal growth patterns observed by Herbst (1969), Bellis and McLarty (1967) and Mantai *et al.* (1982) in the Great Lake region as discussed in the Introduction 4.1.1.

The influence of light intensity in controlling *C. glomerata's* seasonal dominance has received secondary importance compared to temperature (Graham *et al.*, 1982). Maximum SGR of *Cladophora glomerata*, collected from the Kalkfontein irrigation canals in this study, occurred with a light saturation point of 125 μmol photons m^{-2} s^{-1} at a temperature of 25°C with a long day length of 16 hours (Table 4.1). Similarly Hoffman and Graham (1984) reported that isolates of *C. glomerata* from Lakes Mendota, Madison and Wisconsin yielded maximum dry weight production at 125 μmol photons m^{-2} s^{-1} at 25°C. However higher light intensities required to

support optimum growth at water temperatures of 15° and 20°C compared to this study were reported by other researchers. At 15°C, irrespective of day length, the maximum SGR of *C. glomerata* in this study was saturated at a low light intensity of 55 μmol photons m^{-2} s^{-1} (Table 4.1) compared to *C. glomerata* collected from streams in the Spessart Mountains, Germany, where growth increased up too 100 μmol photons m^{-2} s^{-1} with no saturation evident at 15°C (Leukart and Hanelt, 1995). Lorenz *et al.* (1991) reported that *C. glomerata* collected from Lake Erie and subsequently cultured at 15°C at a 14 hour day length, demonstrated yet a higher optimum light intensity of 168 μmol photons m^{-2} s^{-1} for growth. Compared to this study where maximum SGR for *C. glomerata* at 20°C at a 16 hour day length was at 100 μmol photons m^{-2} s^{-1}, Robinson and Hawkes (1986) and Whitton (1967) both described somewhat higher maximum light intensities, of 6000 lx (± 120 μmol photons m^{-2} s^{-1}) and 150 μmol photons m^{-2} s^{-1} respectively, for *C. glomerata* growth when exposed to continuous illumination at 20°C.

Minimum light levels which restrict *C. glomerata* growth were not temperature dependent in this study. Specific growth rates were only significantly impeded in total darkness, 0 μmol photons m^{-2} s^{-1} with 25 μmol photons m^{-2} s^{-1}, the next incremental light level, supporting some growth from 15°C to 30°C at both photoperiods examined (Fig. 4.1 a and b). Higher minimum light requirements for *C. glomerata* growth in Lake Erie were observed by Lorenz and Herdendorf (1982). The maximum depth of *C. glomerata* colonization in their study coincided with light intensities above 50 μmol photons m^{-2} s^{-1} which indicated that values of less than 50 μmol photons m^{-2} s^{-1} were limiting to growth at water temperatures of 15°C. Indeed it was predicted in this study by the Jassby and Platt (1976) equation that maximum SGRs at 15°C for both short- and long-day photoperiods occurred at light saturation points around 55 μmol photons m^{-2} s^{-1} (Table 4.1), although in the studies by Graham *et al.* (1982) and Lorenz *et al.* (1991) slightly lower minimum light levels of less than 30 μmol photons m^{-2} s^{-1} were reported to be limiting to growth at 15°C.

The different maximum and minimum light levels reported for *C. glomerata* growth and photosynthesis in various studies at similar temperature ranges could be attributed to many factors. Firstly, different environmental conditions under which stock cultures were maintained could affect light requirements especially when algal growth is not monitored over long time

periods. Algal stock cultures in this study were maintained at a light intensity of 60 μmol photons m^{-2} s^{-1} set on a 16 hour day length. The optimum light intensity for growth at 15°C however was much lower in this study, 55 μmol photons m^{-2} s^{-1}, compared to the optimum light intensity for growth, of 100 μmol photons m^{-2} s^{-1}, reported by Leukart and Hanelt (1995) despite their stock cultures being maintained under white light at a much lower light intensity of 10-20 μmol photons m^{-2} s^{-1} . The second factor which may likely influence optimum light requirements for growth or photosynthesis is photoperiod. In this study the light saturation points at maximum SGR were higher at a short day length (8 hours) compared to the long-day length (16 hours) at all temperatures except at 20°C (Table 4.1). Likewise Hoffman and Grahams' (1984) study showed that dry weight production at a 8:16 light-dark cycle was greatest at 250 μmol photons m^{-2} s^{-1} whereas under long-days (16:8 light-dark cycle) greatest production occurred at 125 μmol photons m^{-2} s^{-1}. However, the optimum light intensity for *C. glomerata* growth of 150 μmol photons m^{-2} s^{-1} reported by Whitton (1967) was higher than the the optimum light intensity reported by Robinson and Hawkes (1986), of 120 μmol photons m^{-2} s^{-1}, despite consistent photoperiod conditions of continuous irradiance at 20°C.

Thirdly, the higher light requirements reported by other researchers compared to this study may be an artefact of whether algal clumps were used rather than algal filaments which were dissected in this study. The use of algal clumps may lead to self-shading thereby shifting the light requirements to higher levels. However in the study by Leukart and Hanelt (1995) where algal filament tips of *C. glomerata* were cultured at 15°C, a higher optimum light intensity for growth (100 μmol photons m^{-2} s^{-1}) was measured compared to this study (55 μmol photons m^{-2} s^{-1}) at 15°C. The last factor which is likely to explain the different light requirements for *C. glomerata* growth despite similar culture conditions is ecotypic sub-specific variation.

According to findings in this laboratory study and field observations by Joska (pers. comm.), light intensity appeared to play a less significant role in the seasonal distribution patterns of *C. glomerata* in the Kalkfontein canal system compared to temperature. *In vivo* measurements of light intensity in water fluctuate depending on time of the day, water turbidity, weather conditions and water depth. Joska (pers. comm.) found that light intensities at the water surface of the Kalkfontein irrigation canals generally ranged between 1000 and 1500 μmol photons m^{-2}

s^{-1} in the summer, spring and autumn months and between 600 and 800 μmol photons m^{-2} s^{-1} during winter. She further observed that the percentage of total light loss at 25 cm below the water surface for the period of January 1995 to July 1997 was usually less than 90%. These measurements indicate that light intensities below the water surface where *C. glomerata* is found, were generally higher than the minimum light requirements to support optimum growth at water temperatures between 15°C and 30°C which were reported in the laboratory experiments (Table 4.1.).

The optimum irradiance level where maximum SGR occurred, expressed by the light saturation points in this study, shifted with changes in temperature (Table 4.1). At sub-optimal temperatures for *C. glomerata* SGR, illustrated to be 15°C, 20°C and 30°C, at a 16:8 hour light-dark cycle the light saturation points were lower than at the optimum temperature of 25°C. The short-day photoperiod (8:16 light-dark cycle) showed a similar pattern except at 20°C where the light saturation point was drastically reduced to 36 μmol photons m^{-2} s^{-1} (Table 4.1). The low light saturation point of 36 μmol photons m^{-2} s^{-1} predicted could be a function of the possible onset of reproductive activity. In a study by Hoffman and Graham (1984) zoosporogenesis in *C. glomerata* was significantly induced by a short-day photoperiod of 8 hours at a temperature range between 15°C and 20°C with light intensity of less than 60 μmol photons m^{-2} s^{-1} playing a less important role. Unfortunately, in this study, reproductive activity by means of either zoospores, zoosporangia or sporelings was not seen after the five day period. Furthermore, it was difficult to ascertain whether empty cells were a function of zoospore release or merely injury from cutting. The dubious low light saturation point at 20°C at a short-day photoperiod may however be a statistical artefact. Since specific growth rates were similarly low at all light intensities (Fig. 4.2 b.) examined, a shift in one data point may generate a slightly different non-linear regression curve which would completely change the computed light saturation point.

Shifts in the light saturation points for *C. glomerata* photosynthesis were likewise recorded as water temperatures changed from sub-optimum to optimum. Leukart and Hanelt (1995) reported that photosynthesis of laboratory-grown *C. glomerata* at 15°C at various light intensities set on a light cycle of 14 hours was saturated at 60-67 μmol photons m^{-2} s^{-1}. A higher

photosynthetic saturation point of 380 μmol photons m^{-2} s^{-1} for *C. glomerata* collected from canals in the Leeds and Liverpool Canal, Merseyside, was recorded when optimum water temperatures for photosynthesis was 20°C (Simpson and Eaton, 1986). The study by Lester *et al.* (1988) reported that optimum net photosynthesis in *C. glomerata* collected from Green Bay, Lake Michigan, occurred at water temperatures between 28°C and 30°C which coincided with a much higher light saturation range from 345-1125 μmol photons m^{-2} s^{-1}. However *C. glomerata* collected from Lake Huron, Harbor Beach Michigan, was shown by Graham *et al.* (1982) to photosynthesize optimally between light intensities as high as 300-600 μmol photons m^{-2} s^{-1} despite lower water temperatures, ranging between 13°C and 17°C.

The general shift in light saturation points with temperature can be construed as a function of resource allocation between photosynthesis and downstream reactions (Raven and Geider, 1988). At sub-optimal low or high temperatures compared to at optimal temperatures, temperature-sensitive processes, for example, respiration would require a greater allocation of resources in order to maintain the same level of cellular activity. This means that the allocation of resources to pigments and polypeptides related to light-harvesting systems is reduced at sub-optimal temperatures which indirectly results in lower light saturation points exhibited by the alga at maximum growth rates. Conversely, Leukart and Hanelt (1995) maintain that at optimum temperatures for growth or photosynthesis the content and activity of Rubisco (the first carboxylating enzyme in the C_3 photosynthetic pathway) are high thereby increasing the photosynthetic capacity. Thus an increase in the density of the photosynthetic units would shift the saturation point to higher irradiance levels.

Kelly *et al.* (1983) assert that photosynthetic efficiency is a function of light availability (that is, duration for which the light is available) and algae often respond to changes in light conditions by adjusting the concentrations of photosynthetic pigments, nucleic acids and other cellular components which ultimately affect specific growth rates (Schlesinger and Shuter, 1981). At short day lengths the alga will have higher concentrations of photosynthetic pigments in order to optimise the use of available light compared to long- day photoperiods. Consequently the light saturation points for algae exposed to short day lengths will be higher than for algae grown at longer day lengths. This pattern was clearly demonstrated in this study (Table 4.1) where light

saturation points at G_{max} were higher at a short-day photoperiod compared to the longer day photoperiod between 15°C and 30°C, except at 20°C (for reasons explained in earlier text). The increase in resource allocation to photosynthetic apparatus under short- day lengths accompanies a negative growth effect which is explicitly shown in Figs. 4.2 (a- d). where the specific growth rates of *C. glomerata* were lower at a 8:16 light-dark cycle compared to the longer day length of 16 hours at all temperatures which sustained growth. The growth of *C. glomerata*, collected from the Oswego region of Lake Ontario, comparably improved from a 12 hour day to a 14 hour day when exposed to a temperature range of 14.5°C to 26°C at a light intensity of 800 foot candles for 30 days (Storr and Sweeney, 1971). Robinson and Hawkes (1986) likewise demonstrated that the growth rate of unialgal cultures of *C. glomerata* increased progressively as the duration was increased up to a maximum of 24 h light d^{-1}. Hoffman and Graham (1984) claim that the observed pattern of photoperiod on vegetative growth is an indirect consequence of reproductive activity. In their study it was noticed that zoosporogenesis in *C. glomerata* was predominantly promoted by short-day photoperiods (8:16 light-dark cycles), at a temperature range between 15°C and 20°C. It was further observed that algal filament growth was drastically reduced in cultures which underwent sporulation. However, reduced filament length at 20°C, short-day photoperiod was not observed in this study (Table 4.1) indicating that reproductive activity could not have occured.

The seasonal distribution pattern of *C. glomerata* in the Kalkfontein canal may also be affected by photoperiod. Day lengths (data received from the South African Weather Bureau in Pretoria) during the winter season in the Kalkfontein canal region are much shorter (10 ±1 hours of daylight) than during the summer season (15 ±1 hours of daylight). As reported in this laboratory study the maximum SGRs of *C. glomerata* at light saturation point (Table 4.1.) were reduced at short-day photoperiods compared to long- day photoperiods at all temperatures examined.

CHAPTER 5

THE EFFECTS OF THE HEAVY METAL, COPPER, ON THE GROWTH OF *CLADOPHORA GLOMERATA*

5.1. INTRODUCTION

Trace amounts of heavy metals are essential for algal metabolic processes and growth, however high concentrations have often been reported to have toxic effects on cells, mainly as a result of their ability to denature protein molecules (Gadd and Griffiths, 1978). The sensitivity of *Cladophora glomerata* to heavy metals has been exploited in the control of nuisance algal blooms in freshwater ecosystems since the beginning of the 20th century (Cedeno-Maldonado and Swader, 1972). Whitton (1970b) illustrated that of 37 species of Chlorophyta studied from flowing waters, *C. glomerata* was 36th in order of resistance, that is extremely sensitive, to Zn; 32nd to Cu^{2+} and 37th to Pb. In a laboratory investigation by McHardy and George (1990), toxic effects in *C. glomerata* cells, grown in a Chu #10 solution with a Zn concentration range from 0-4 mg l^{-1} and at a pH of 7.2 for one week, were first noted when the alga was exposed to 0.4 mg Zn l^{-1}. In these cells abnormalities occurred in the cytoplasm which were not evident in the cells of the control treatment. The *Cladophora* cells which were exposed to Zn concentration of 4.0 mg l^{-1} were observed to be completely dead. Thomas (1962 cited in Whitton, 1970 a) reported slightly lower toxic levels of heavy metals in the regions of *ca* 0.1 mg Zn l^{-1} and *ca* 0.05 mg Cu l^{-1} for *Cladophora*. Leland and Carter (1984) indicated still lower Cu concentrations of 5 μg l^{-1} which essentially inhibited the colonization of *Spirogyra spp.* and *Cladophora spp.* for a limited time period in the Convict Creek, California. Algal densities comparable to the control did not appear until 12 weeks after dosing.

Although many case studies explicitly demonstrate the sensitivity of *Cladophora* to heavy metals thus indicating an effective control measure, Eipper (1959) maintained that *Cladophora* is not exceptionally sensitive to heavy metals, since adequate control was not effectively attained at

a concentration of 1 mg Cu l^{-1}. Observations by Bellis (1968) similarly showed good growth of *C. glomerata* despite it being exposed to a culture medium containing 2 mg Zn l^{-1}. In the study by Betzer and Kott (1969) filaments of a *Cladophora sp.* collected from the Saline channel (Israel) and cultured in various concentrations of Cu^{2+}, were only adversely affected at concentrations as high as 2-4 mg Cu l^{-1} when exposed to the Cu for 4 days. These findings could be an indication that some *C. glomerata* strains have developed tolerance mechanisms against heavy metals as demonstrated by Hillebrand and De Vries (1986). In their study it was demonstrated that different strains of *C. glomerata* varied in their tolerance level to copper. Within a copper concentration range of 0.002-0.58 mg l^{-1} the three strains reacted differently with one being extremely sensitive whilst one was very tolerant. Furthermore, it was detected that strains collected in the same place exhibited different tolerance levels. The growth rate of the most tolerant strain was decreased by 10% from 0.002 to 0.58 mg Cu l^{-1} whereas the growth rate declined by 50% in the sensitive strain of *C. glomerata* when exposed to the same Cu concentration range. The study clearly indicated that both tolerant and sensitive strains of *C. glomerata* exist in nature. Similarly isolates of *Chlorella vulgaris* Beijerinck from the River Hayle, England, displayed non-tolerance as well as tolerance when exposed to a Cu concentration range of 0.05- 0.3 mg l^{-1}. At a Cu concentration of *ca* 0.05 mg l^{-1} the growth rate of the non-tolerant strain was decreased with 0.30 mg Cu l^{-1} being completely inhibitory. The tolerant strain however was not adversely affected below 0.10 mg Cu l^{-1} (Foster, 1977). Whitton (1970a) however observed growth sensitivity of 20 different populations of *C. glomerata* exposed to Zn, Cu and Pb with no significant detectable differences between any of them despite the fact that one of the algal populations was extracted from a Cu polluted stream.

Although algae may have evolved to mitigate the effects of heavy metals, many researchers have ascertained that environmental conditions have an underlying influence on the biological toxicity of heavy metals. Gadd and Griffiths (1978) have maintained that there are three basic abiotic factors which need to be assessed before the biological toxicity of the metal can be established. These factors include firstly, the prospect of environmental constituents binding the heavy metal ions. Stokes (1983) maintained that the biological toxicity of heavy metals is attributed mainly to the concentration of free ionic metals rather than to the total copper concentration. In aquatic habitats, heavy metals can be bound and removed from the water by organic sediments, which

effectively reduce the total metal ion concentration in solution and consequently biological toxicity. In the study by Walker and Colwell (1974), bacteria mainly *Pseudomonas* spp. collected from Colgate Creek in the Baltimore Harbour were capable of degrading petroleum oil despite it being enriched with a high Hg content. The high Hg-resistance of *Pseudomonas* had been partially credited to the binding capacity of mercury with specific organic complexes in the oil thereby reducing the free ionic Hg concentration. Likewise other organic compounds for example citrate, cysteine, glutamate and EDTA (Ethylene-diamine-tetra acetic acid) can have profound effects on biological responses when included in growth media (Gadd and Griffiths, 1978). These compounds act as chelators, that is, the complex formed by a ligand (a Lewis base attached to a metal atom in a complex) that can bond with two or more atoms to a metal atom (Ebbing and Wrighton, 1990). Many laboratory studies have demonstrated the amelioratary effect of chelators on copper toxicity. MacLeod *et al.* (1967) showed that metabolic injury to the bacterial cells of *Aerobacter aerogens* was evident when exposed to a diluent of distilled water and Cu^{2+}. However, the toxic effects of Cu^{2+} on *Aerobacter aerogens* were reduced or prevented by the addition of yeast extract or cysteine to the diluent. Similarly, in a study by Zimmerman (1966) toxic effects of copper to the bacteria, *Serratia marcescens*, were relieved by the addition of copper-chelating agents which sequestered the free Cu^{2+} ions. In the study by Stokes and Hutchinson (1975) a relationship between the toxicity of copper to laboratory cultures of *Scenedesmus* (chlorophyte) and the complexation of copper by the synthetic chelator, EDTA was revealed. The toxic effect of copper was masked in the presence of EDTA up to a point where the copper concentration exceeded the binding capacity of the EDTA. At this point, copper uptake into algal cells increased dramatically and cell division was inhibited. McKnight *et al.* (1983) therefore proposed that when investigating the minimum toxic concentration of a metal, the optimal strategy would be to first add enough copper to titrate the ligand. The concentration of copper required would vary with ligands since synthetic chelators such as EDTA and triethanolamine appear to bind copper more strongly than natural ligands such as humic and fulvic acids. Accordingly the cupric ion activity with synthetic ligands will be lower than with natural ligands for similar copper and ligand concentrations.

The capacity for ligands to bind Cu^{2+} ions could explain the results obtained by numerous laboratory researchers e.g. Eipper (1959), Bellis (1968 a) and Kott and Betzer (1969) who found good *C. glomerata* growth despite relatively high heavy metal concentrations. In comparison, in media without complexing agents, toxicity may be pronounced. Steeman Nielsen and Wium-Andersen (1970) revealed that a copper concentration as low as 5 $\mu g\ l^{-1}$ was toxic to cells of the unicellular green alga, *Chlorella pyrenoidosa* (= *C. fusca* Sh. et Kr., Strain 211-8b), when EDTA and citric acid was omitted from the growth medium.

Despite the substantial body of evidence supporting the proposal that free cupric ions are the main determinants of biological toxicity, there are also indications that biological activity is not directly related to the concentration of ionic Cu. Florence *et al.* (1983) indicated that total growth inhibition of the marine diatom, *Nitzschia closterium* Ehr., was obtained by the addition of lipid-soluble Cu complexes with a 5×10^{-8} M 8-quinolinol synthetic ligand. They attributed the toxicity of the lipid-soluble Cu complexes to their ability to catalyse the intercellular formation of highly destructive hydroxyl free radicals obtained from molecular O_2. Tubbing *et al.* (1993) demonstrated that minor additions of total Cu in the range of 0.05-0.16 μM already affected functional and structural aspects of bacterial and phytoplankton communities in spite of rather high concentrations of EDTA, suspended matter and dissolved organic carbon. Likewise Tubbing *et al.* (1994) showed that chelex-bound Cu induced biological toxicity in *Selenastrum capricornutum* (Reinsch.) which was cultured in Cu and EDTA enriched synthetic medium or River Rhine water. These studies indicate that complexed Cu contributes to Cu toxicity which may have significant consequences, since in freshwater systems the percentage of organically bound Cu may be very high and the fraction of free Cu is usually less than 10% (Tubbing *et al.*, 1994).

Secondly, the toxicity of heavy metal ions to algae can be remarkably masked by the presence of other ions. Anions such as PO_4^{2-}, SO_4^{2-} and CO_3^{2-} and cations such as Mg^{2+}, Ca^{2+} and Fe^{2+} or Fe^{3+} are able to reduce heavy metal toxicity by precipitation, depending on their concentrations and the pH of the solution (Gadd and Griffiths, 1978). In the study by Abelson and Aldous (1950) the toxic effects of Ni, Co, Cd, Zn and Mn to many bacterial species including *Escherichia coli*, were reduced in media with a high Mg^{2+} content compared to media

containing the same metal concentrations but a low Mg^{2+} content. Steeman-Nielsen *et al.* (1969) demonstrated that the presence of high Fe in a medium had a detoxifying effect on copper to the alga *Chlorella pyrenoidosa*. This has been attributed to the prospect that the Cu ions were adsorbed to the negatively charged sides of ferric hydroxide. Similarly, Cu toxicity to *Nitzchia closterium* was reduced by adsorption of Cu^{2+} on Mn^{3+} and Fe^{2+} hydroxide coatings around the cell, with Mn^{3+} being more effective than Fe^{2+}, possibly due to its ability to scavenge the toxic superoxide free-radical (Stauber and Florence, 1985). The masking effect of the Ca^{2+} ion was illustrated by Rai and Raizada (1985), who found that the uptake of Cu^{2+} and Pb^{2+} by *Nostoc muscorum* (cyanobacterium) was reduced by 10^{-3}M Ca^{2+}, with Ca^{2+} diminishing the effects of the heavy metals studied. A more recent investigation by Issa *et al.* (1995) likewise showed that when the green alga, *Kirchneriella lunaris* (Kirchner) Mobius, was incubated in the presence and absence of Ca, the growth rate was inhibited by all heavy metals applied including Cd^{2+}, Co^{2+}, $Mn^{2+}$and Ni^{2+}. However, the effect of heavy metal inhibition on the growth rate was more pronounced in cultures containing heavy metals alone with higher growth rates observed in Ca-containing cultures.

Thirdly, the availability of heavy metals to biological organisms is considerably affected by the pH in an aquatic environment (Gadd and Griffiths, 1978) assuming that free ionic metals ions are the main determinants of biological toxicity. In the model by Lin and Benjamin (1992) the relationship between solution pH and the concentration of metal ion particles has been conceptualized. The fate of metal ions, e.g. Cu^{2+} is governed by adsorption/desorption processes which occur at the interface between the aqueous solution and cationic oxides and hydroxides. The O_2 donor group (either OH or O^-) on the surface can react with cations in solution as expressed by the equation

$$\mathbf{SOH + M^z \rightleftharpoons SOM^{(z-1)} + H^+}$$

where SOH donates a singly protonated surface oxide site, and M represents a cationic adsorbate

The pH of a solution is determined by the, -[logarithm of the H^+ concentration], therefore by increasing the concentration of H^+ the solution pH will decrease (Ebbing and Wrighton, 1990).

In the above model, when an increase in the solution pH (low H^+ concentration) occurs the forward reaction commences which results in an increase in the sorption of the cationic metals, e.g. Cu^{2+}. Eventually, a point will be reached with time when the concentration of H^+ increases sufficiently to initiate the reverse reaction. The reverse reaction would accordingly be favoured with decreasing solution pH resulting in an increase in free cationic metals. Lin and Benjamin (1992), acknowledge that the presence of complexing ligands may alter the adsorption characteristics of metals substantially. Organic complexing ligands are often strong adsorbates themselves, which complicates the interpretation of results when they are added to systems containing metals and oxide sorbents.

Winner (1986) investigated the interactive effects of water hardness on the chronic toxicity of Cd to *Daphnia pulex* (a freshwater animal). Water hardness is indirectly related to pH (Ca^{2+} or Mg^{2+} concentrations affect the H^+ concentration in the solution through compound complexation, Ebbing and Wrighton, 1990), with an increase in water hardness resulting in an increase in pH and vice versa. The survivorship of the daphnids, exposed to 5 ug Cd l^{-1} for 20 days, increased with an increase in water hardness from 58-115 mg l^{-1}. Very few studies (Macfie, 1994) have focused on the relationship between pH and Cu^{2+} availability and its effects on algae with no investigations found pertaining to *C. glomerata*. However in general, McKnight *et al.* (1983), suggested that for Cu-sensitive algae, low dosages of $CuSO_4$ (0.01ppm) should be effective in controlling algal growth regardless of water pH and organic complexing content. In the case of a copper tolerant alga, excluding alkaline water, control should be possible with dosages of about 1 ppm. In alkaline water, precipitation of $CuCO_3$ and hydroxides prevents cupric ion activities (ie, toxicity) sufficiently high to limit the growth of a Cu-tolerant nuisance alga. The effective dosage of an alga with intermediate Cu sensitivity exhibits the most dependence on water chemistry. In acidic waters, low dosages of Cu ($\leq$ 0.10 ppm) should be effective. In water with a relatively high organic content, enough Cu must be added to titrate the humic complexing agents ($\pm$ 0.10 ppm Cu) before a toxic effect will be obtained. Likewise dosages greater than 0.10 ppm are required in alkaline waters because of Cu precipitation.

In some water schemes in South Africa, for example the Kalkfontein and Hartebeespoort dams, the addition of copper sulphate is preceded by an initial dose of sulphuric acid in order to reduce the pH (Joska and Bolton, 1994 a). Despite the extensive application of this method to control macroalgal growth in irrigation canals in South Africa, no scientific research relating to the correct dosages required has been conducted prior to this project. In addition, the use of $CuSO_4$ and certainly the addition of sulphuric acid to natural water bodies are environmentally unacceptable and prohibited in many countries (Joska and Bolton, 1994 a). Irrigation canals systems however are not considered as "natural" water bodies, but the water extracted from these systems invariably find their way back to the natural environment through the natural water cycle.

The main objective in this aspect of the project was to determine the correct application of $CuSO_4$ as an algicide whereby maximum growth control is obtained at the minimum Cu dosage and correct pH which is toxic to *C. glomerata*. Subsequently, pertinent questions needed to be asked which include:

1. How do the solution pH, medium Cu concentration and time of algal incubation in the Cu affect the uptake of Cu by *C. glomerata*?
2. How is the biological activity, measured either as photosynthesis or growth, of *C. glomerata* affected by Cu uptake?
3. Are the effects of Cu on growth and photosythesis permanent or temporary? and
4. What effect does Cu have on the intracellular structure of the *C. glomerata* cell?

5.2. MATERIALS AND METHODS

5.2.1. COLLECTION AND STOCK CULTURES

C. glomerata was collected from the Kalkfontein irrigation canal scheme (Free State) in December 1996, January and February 1997. *C. glomerata* tufts squeezed of excess water were transported in plastic bags to the Botany Department (University of Cape Town) within 24 hours. Stock culture materials cleaned of macroscopic debris were placed into 1-l glass jars containing aerated tap water at 20°C at a light intensity of *ca* 60 μmol photons m^{-2} s^{-1} supplied by cool white fluorescent tubes (Osram L30W/20) set on a 16:8 light-dark cycle. Stock cultures

were maintained for at least 2 weeks prior to experimental procedures but never exceeding laboratory age of 1 month. For all the experiments, unless otherwise stated, the basic culture medium used was aged tap water (2 weeks) supplemented with 14 mg N l^{-1} and 2 mg P l^{-1} as $NaNO_3$ and K_2PO_4 respectively, which will be referred to as the enriched tap water medium in subsequent text. Culture media were pre-heated for 2 hours at about 90°C in a Memmert stainless steel water bath and cooled to room temperature overnight before each experiment was conducted. All solution pH levels were maintained by adding 1ml of a 2 M Tris (hydroxymethyl)-aminomethane buffer per litre of the enriched tap water medium. The buffer solution was manipulated to yield required pH levels by adding drops of either dilute HCl or a 1 M NaOH solution. All solution pH measurements were processed using a calibrated Beckman ϕ 40 pH meter. The source of Cu, excluding the natural supply of Cu in the tap water, was derived from a 0.03 mM $CuSO_4$ stock solution. Aliquots were added to the enriched tap water medium prior to each experimental run to yield the final required Cu concentrations in mg l^{-1}. All glassware utilised was soaked in detergent, acid washed with dilute HCl, rinsed three times with distilled water and oven dried at 60°C.

5.2.2 PHOTOSYNTHETIC EXPERIMENTS

a. Preliminary investigations

According to Littler (1979), algal thallus weight should not exceed 0.03 g dry weight l^{-1} in order to minimize the impact of bottle volume to algal weight ratio. A desiccation experiment to determine the appropriate wet weight required to conduct the photosynthetic experiments was performed. *Cladophora glomerata* tufts of *ca* 0.5 g after blotting on tissue paper were each placed into three replicate foil dishes and oven dried at 50°C for 30, 60, 120 and 240 minutes and for 2 days. Three replicate foil dishes without alga were likewise oven dried for the various time periods in order to serve as controls thus eliminating possible external factors. The replicate foil dishes with alga and without alga were reweighed after each time period and water contents were calculated as percentage of total moisture lost over the specified time periods which are presented in Table 5.1.

Water loss from *C. glomerata* thalli was 43% of the wet weight after 30 minutes and increased to 92.4% of the wet weight after 120 minutes after which no further water loss took place with an increase in time (Table 5.1). From these results, the approximate wet weight required for the photosynthetic experiments was calculated using the formula:

[[wet weight saturation (%) - maximum thallus desiccation (%)] * wet weight used]/ 100%

..**equation 5.1**

When substituting values in the formula, [[100%-92.4%]*0.5 g]/100%], a value of 0.038 g dry weight is obtained which approximates to the recommended thallus weight of 0.03 g dry weight l^{-1} of Littler (1979). It was subsequently decided that the feasible wet weight per *C. glomerata* tuft should be *ca* 0.5 g for each bottle.

Table 5.1. The desiccation of *C. glomerata* thalli with time.

Sample	Initial wet weight (g)	Dry thallus weight (g) with time					Maximum Desiccation (%)
		Time (hours)					
		0.5	1	2	3	48	
A	0.47	0.27	0.14	0.03	0.03	0.03	93.6
B	0.50	0.27	0.11	0.05	0.05	0.05	90.0
C	0.49	0.20	0.09	0.03	0.03	0.03	93.6
average	0.49	0.25	0.34	0.04	0.04	0.04	92.4

b. Photosynthetic experimental procedures

C. glomerata was acclimatized for 3 days prior to each experiment. All experiments were conducted in a perspex water bath, dimensions 60 cm by 35 cm by 20 cm, fitted with a B.Braun Melsungen AG thermomix 1460 connected to a Fryka-Kaltetechnik (DLK 300) cooling unit. Water temperature was maintained at 25 ± 1 °C (within the optimum temperature range for *C. glomerata* photosynthesis reported by Adams and Stone 1973; Lester *et al.*, 1988) with continuous illumination supplied by Osram Vialox 66 NAV-E 400W mercury vapour bulbs at 300 μmol photons m^{-2} s^{-1}, the estimated light saturation level for photosynthesis by Graham *et al.*, 1982; Simpson and Eaton, 1986; Lester *et al.*, 1988.

In total twelve photosynthetic experiments were conducted with a matrix of three medium pH levels of either 6, 7 or 8 and four exposure times to the Cu varying for either 30, 60, 120 or 240 minutes. For the individual experiments Cu concentrations of 0, 2, 6 and 10 mg Cu l^{-1} were employed. Algal tufts of *ca* 0.5 g wet weight each were placed into 100-ml Erlenmeyer flasks filled with 100 ml of the sterilised enriched tap water medium supplemented with Cu, with each Cu concentration having three replicate flasks. After algal exposure to Cu for the various time periods and at each pH level, *C. glomerata* tufts were directly transferred, after shaking off excess Cu supplemented medium, to the enriched tap water medium without Cu addition at a pH of 8.

c. Photosynthetic measurements

Photosynthesis was measured using a YSI Model 50B, dissolved oxygen meter calibrated to 100% air saturation. The O_2 probe covered with a thin membrane permeable to gases determines the amount of O_2 in the solution in mg l^{-1}. For each photosynthetic measurement a total of twenty-seven 310-ml Pyrex glass bottles fitted with airtight screw lids were filled with the enriched tap water medium (0 mg Cu l^{-1} and pH 8) bubbled for 5 minutes with liquid N_2 in order to reduce the initial O_2 content. Bottles were gently lowered at a 45° angle into the bucket containing the medium until completely filled and subsequently closed whilst submersed. Care was taken so that air bubbles in the bottles were limited. Three replicate bottles at each Cu concentration with *C. glomerata* tufts were exposed to light in order to determine net photosynthesis. Respiration was determined by inserting the algal tufts into three replicate

bottles, at each Cu concentration, covered with two layers of heavy duty aluminium foil. Pyrex glass bottles filled with the enriched tap water medium without alga were included with each experiment to measure the initial O_2 concentration of the medium prior to incubation and another measurement, serving as a control, taken 1 hour after incubation in order to eliminate microbe activity and external parameters. The photosynthetic measurements were taken 1 hour after algal transfer to the Pyrex glass bottles (the minimum time required to observe a detectable change in the O_2 probe reading) which were incubated in the water bath. Each measurement was taken after the probe was stabilized (about 30 seconds) with continuous manual agitation of the probe. The photosynthetic measurements for each experiment were taken 1 hour and 24 hours after algal incubation in the Cu. Between measurements the algal materials were placed into 100-ml Erlenmeyer flasks containing 100 ml of the enriched tap water medium (pH 8).

5.2.3. COPPER UPTAKE EXPERIMENTS

Atomic absorption spectrophotometry analysis was performed by Cameron Chemical Consultants in order to determine the uptake of Cu by *C. glomerata*. Samples of the enriched tap water medium containing the different Cu concentrations after exposure to *C. glomerata* tufts for 30, 60, 120 and 240 minutes at pH levels of 6, 7 and 8 were collected. Samples of the enriched tap water containing the different Cu concentrations without alga were also collected which served as controls in order to eliminate the possible uptake of Cu by bacteria although the media were sterilised prior to experimental run. Tap water was collected to determine the natural supply of Cu in tap water thereby serving as a blank. All samples were frozen at -18°C until analyzed. Samples were defrosted by allowing them to thaw at room temperatures for a 24 hour period and subsequently analyzed for total copper using a Varian AA 375 atomic absorption spectrophotometer at a λ of 324,8 nm. A standard curve was used to extrapolate sample Cu concentrations in mg l^{-1}.

5.2.4. GROWTH EXPERIMENTS

Intercalary segments of *C. glomerata*, 10 mm each, were cut under water in a glass culture dish lid under which a grid block consisting of squares with dimensions of 1 mm by 1 mm each was taped. A Kyowa Optical Stereomicroscope Model SD-2PL was used. Ten segments of *C.*

glomerata were placed into 100-ml Ehrlenmeyer flasks each containing 100 ml of enriched tap water. Cu was added as $CuSO_4$ yielding final medium concentrations of 0, 2, 6, 10 and 20 mg Cu l^{-1} with each Cu concentration adjusted to pH levels of 6, 7 and 8. In total there were 15 combinations with 3 replicates each giving a total of 45 experimental flasks. Experimental flasks containing the *C. glomerata* segments were exposed to the Cu at the 3 pH levels for a period of 30 minutes at *ca* 60 μmol photons m^{-2} s^{-1} at *ca* 25°C after which they were transferred to 100-ml Ehrlenmeyer flasks each containing 100 ml of enriched tap water without added Cu adjusted to a pH level of 8. Growth was monitored 1, 2, 3 and 5 days after Cu exposure using the same grid technique applied at the start of the experiment. The growth medium in each flask was replaced daily.

5.2.5. TRANSMISSION ELECTRON MICROSCOPY

Ten, 5 mm segments of *C. glomerata* were incubated in 100-ml Erlenmeyer flasks with each containing 100 ml of the enriched tap water medium supplemented with 10 mg Cu l^{-1} or no added Cu at pH levels of 8 and 6. The flasks were incubated for 30 minutes and for 3 hours under continuous cool white light at 60 μmol photons m^{-2} s^{-1} at a temperature of *ca* 25°C. The labelled segments were subsequently placed into 20 ml of 0.1 M PO_4 buffer (Appendix 5.1.) with 2% gluteraldehyde overnight (18 hours) at 4°C. Segments were rinsed twice with 2 ml PO_4 buffer and placed into 1 ml osmium tetroxide for 30 minutes followed by rinses with 2 ml PO_4 buffer and 2 ml distilled water. Algal segments were dehydrated gradually by soaking in 0.5 ml of 30%, 50%, 70% , 80% , 90% and 95% ethanol for 10 minutes each, two 10 minutes periods in 100% ethanol and finally in 1 ml of 100 % acetone for two 10 minute periods. Segments were suspended in a Spurr's low viscosity resin for 3 days and subsequently embedded into rubber mats and placed into the oven at 60°C for 16 hours. Resin boats were sectioned at about 95 nm using a diamond knife attached to a Leica Reichert Ultracut S ultramicrotome and placed onto Cu grids. Sections were stained in uranyl acetate for 10 minutes, rinsed with distilled water and stained in lead citrate for 5 minutes. The sections were viewed under a Zeiss EM 109 Transmission Electron Microscope and photographed.

5.3. DATA TRANSFORMATION AND STATISTICAL ANALYSIS

5.3.1. PHOTOSYNTHETIC EXPERIMENTS

Net photosynthesis = O_2 concentration in Light bottle - Initial O_2 concentration of the medium ..**equation 5.2.**

Respiration = Initial O_2 concentration-O_2 concentration in Dark bottle ..**equation 5.3.**

All concentration values (mg O l^{-1}) were converted to mg O_2 .g algal dry^{-1} weight. hr^{-1} using the formula:

O_2 concentration (mg O_2 l^{-1}) / volume (L) * incubation time (h) * algal dry weight (g) ..**equation 5.4.**

with volume = 0.31 L and incubation time = 1 hour

Gross Photosynthesis (mg O_2 g algal dry $weight^{-1}$ hr^{-1}) = (Net Photosynthesis + Respiration)- control .. **equation 5.5.**

For comparison, the measured photosynthetic values obtained from equation 5.5 were converted to percentages with the control treatments 0 mg Cu l^{-1}, at each pH level and incubation time in the Cu, representing the maximum photosynthesis exhibited (100%).

5.3.2. COPPER UPTAKE EXPERIMENTS

The Cu absorbed by the alga was determined indirectly by subtracting the remaining Cu concentration in the medium which was exposed to the alga from the Cu concentration determined in the solution without algal exposure.

The resulting Cu concentrations values were converted to mg Cu g algal dry weight^{-1} using the formula:

Cu concentration (mg l^{-1})* volume / dry weight (g) ..**equation 5.6**

where volume = 0.31 L.

Copper uptake rates by *C. glomerata* expressed in mg Cu g algal dry weight^{-1} h^{-1} were calculated by dividing equation 5.6. by the incubation time of the alga in Cu.
It is acknowledged that this indirect estimation of Cu in the alga assumes that Cu only existed either dissolved in the water or associated with algal tissue and ignores Cu adsorption on to walls of culture vessels and Cu precipitation. Unfortunately, it was not possible to measure the amount of Cu in the alga tissue itself since the same clumps of *C. glomerata* were used for the recovery experiments. Cu precipitation was however indirectly estimated by exposing *C. glomerata* to different solution pH.

5.3.3. GROWTH EXPERIMENTS

Raw measurement data were transformed to growth rates using a slightly modified version of equation 3.1 (Rueness and Tananger, 1984) :

$$\mu = [\ln (L_z/L_0)]/(t_z-t_0) \quad \text{.. equation 5.7}$$

where μ = specific growth rate (SGR), L_z and L_0 represent the average filament lengths of the 20 segments in each flask, at t_z = day z and t_0 = day 0, respectively.

All data analyses were computed on a software computer package, Statistica v 5.0 using Anova/Manova bases. Statistical significances were obtained by applying One-way or Two-way Analysis of Variances tests (F-ratios). Tests for normality were performed using parametric probability plots and homoscedacity was determined by the Cochran's (Chi^2 test) or Bartlett's tests (B). Statistical significances were denoted where p was ≤ 0.05 (indicated by).

5.4. RESULTS

5.4.1. PHOTOSYNTHESIS

In general algal photosynthesis at 2, 6 and 10 mg Cu l^{-1} was observed to be lower than the control treatments (0 mg Cu l^{-1}) at all pH levels and incubation times in Cu and therefore was expressed as a percentage of the control. Photosynthesis measured 1 hour and 24 hours after algal exposure to Cu (which will be referred to as P_1 and P_{24} respectively in subsequent text) for 30, 60, 120 and 240 minutes at media pH levels of 8, 7 and 6 are represented in Figures 5.1 to 5.4. The short-term effect of Cu on photosynthesis is indicated by P_1 and the extent of photosynthetic recovery is represented by P_{24}

P_1 was significantly affected by Cu concentration after algal incubation in Cu for 30 minutes (Fig. 5.1 a-c) at pH 7 (F=15.87) and pH 6 (F=15.71) but not at pH 8 (F=1.98). P_1 and P_{24} were not significantly different from each other at all Cu concentrations at pH 8 (F=0.16), pH 7 (F=0.03) and pH 6 (F=0.005) after 30 minutes of algal incubation in the Cu indicating no photosynthetic recovery. However, P_1 (F=4.4) and P_{24} (F=4.58) were more reduced at all Cu concentrations at pH 6 compared to pH levels of 8 and 7.

After 60 minutes of algal exposure to Cu (Fig. 5.2 a-c), P_1 was significantly affected by Cu concentration at pH 8 (F=11.62) and pH 7 (F=4.58) and pH 6 (F=107.67). There was no significant change between P_1 and P24at all Cu concentrations at pH 8 (F=0.003) but P_1 and P_{24} were statistically different at pH 7 (F=13.02) and pH 6 (F=40.07). P_1 at pH 7 increased slightly from *ca* 70% at 2 mg Cu l^{-1} and 6 mg Cu l^{-1} and from 58% at 10 mg Cu l^{-1} to more than 90% as indicated by P_{24} in Fig. 5.2 b. At solution pH 6, P_{24} at Cu concentrations of 6 mg Cu l^{-1} and below increased from *ca* 40% to *ca* 80% indicating some degree of recovery, although at 10 mg Cu l^{-1} photosynthesis remained depressed (Fig. 5.2 c). P_1 was significantly (F=34.13) reduced at pH 6 at all Cu concentrations compared to at pH 7 and pH 8. At solution pH 6, P_1 was dramatically reduced, being less than 40% of the control compared to the reductions at pH 7 and 8 which ranged between 60% and 80% at Cu concentrations above 2 mg Cu l^{-1} (Fig. 5.2 a-c).

After 120 minutes of algal incubation in Cu (Fig. 5.3 a-c), P_1 was significantly different with an increase in Cu concentration at pH 7 (F=14.31) and pH 6 (F=20.66) but not at pH 8 (F=4.36). No significant variation occurred between P_{24} and P_1 at pH 8 (F=0.02) and pH 7 (F=3.0) but P_{24} was observed to be distinctly higher than P_1 at pH 6 (F=17.04) at all Cu concentrations. At 2, 6 and 10 mg Cu l^{-1}, P_{24} increased by approximately 30% compared to P_1 (Fig. 5.3 c). No significant relationship was demonstrated between pH and photosynthetic inhibition at all Cu concentration at P_1 (F=0.08) and P_{24} (F= 1.76) with algal exposure to Cu for 120 minutes.

After 240 minutes of algal incubation in Cu (Fig. 5.4 a-c), P_1 decreased significantly with an increase in Cu concentration at pH 8 (F=43.02), pH 7 (F=15.68) and pH 6 (F=89.78). P_{24} was statistically similar to P_1 at pH 7 (F=3.34) at Cu concentrations of 2 and 6 mg Cu l^{-1} but P_{24} was slightly increased by 20% compared to P_1 at 10 mg Cu l^{-1} (Fig. 5.4 b). At both pH 8 (F= 15.25) and pH 6 (F= 94.92), P_{24} was significantly higher than P_1 at all Cu concentrations (Figs. 5.4 a and c). At pH 8, P_{24} increased by 15%, 45% and 20% compared to P_1 at 2, 6 and 10 mg Cu l^{-1} respectively. At pH 6, P_{24} increased by 15%, 70% and 50% at 2, 6 and 10 mg Cu l^{-1} respectively (Fig. 5.4 c). There was no significant effect of pH on P_{24} at all Cu concentrations (F=2.72), however P_1 was significantly (F=4.76) reduced with a decrease in pH at 10 mg Cu l^{-1}. P_1, when *C. glomerata* was incubated in 10 mg Cu l^{-1}, was reduced to less than 50% at pH 8, 40% at pH 7 and below 30% at pH 6 compared to the control treatments.

The time of algal incubation in Cu only significantly affected P_1 at solution pH 8 (F=4.04), solution pH 6 (F=3.49) and P_{24} at solution pH 6 (F=8.91) at all Cu concentrations. No significant effects of algal incubation time in the Cu were observed on P_1 at solution pH 7 (F=2.46), on P_{24} at solution pH 8 (F=2.15) and at solution pH 7 (F=1.97) at all Cu concentrations. A statistically significant effect of algal incubation time in the Cu with increasing medium Cu concentrations was only evident on P_1 at solution pH 6 (F=4.53).

5.4.2. COPPER UPTAKE

a. The effects of pH on accumulative Cu uptake and uptake rates by *C. glomerata*

The control treatments, 0 mg Cu l^{-1}, exhibited no Cu uptake at all pH levels and incubation time periods, which was expected. However, the accumulative uptake of Cu by the algal cells increased significantly with a decrease in pH, from 8 to 6, at Cu concentrations of 2 mg l^{-1} (F=5.46), 6 mg l^{-1} (F=8.69) and 10 mg l^{-1} (F=17.36) at all algal incubation times in the Cu as shown in Fig. 5.5 a-c respectively. Uptake rates were not significantly different between the pH levels at the various algal incubation times at Cu concentrations of 2 mg l^{-1} (F=1.70, Fig. 5.6 a) and 6 mg l^{-1} (F=1.4751, Fig. 5.6 b). However they were significantly different at a Cu concentration of 10 mg l^{-1} (F= 7.31, Fig. 5.6 c). Uptake rates by *C. glomerata* incubated in 10 mg Cu l^{-1} (Fig. 5.6 c.) at a medium pH of 6 were higher than at both pH levels of 7 and 8 with a medium pH of 8 yielding the lowest uptake rates at all incubation times. Uptake rates were approximately 3, 2, 5 and 6 times higher at a solution pH 6 at incubation times of 30, 60, 120 and 240 minutes respectively compared to uptake rates at a solution pH of 8.

b. The effects of *C. glomerata* incubation time in Cu on accumulative Cu uptake and uptake rates

Accumulative Cu uptake was not significantly different (F= 1.13) with an increase in algal incubation time in the Cu at all Cu concentrations at a pH of 8 (Fig. 5.7 a). Although, at the two lower pH levels of 7 (Fig. 5.7 b) and 6 (Fig. 5.7 c), accumulative Cu uptake increased dramatically within the first 2 hours after which uptake saturation was reached at Cu concentrations of 2, 6 and 10 mg Cu l^{-1}. As illustrated in Figure 5.8 a-c the uptake rates of Cu in *C. glomerata* were significantly decreased with an increase in incubation time at all Cu concentrations at pH 8 (F=6.61), pH 7 (F=13.03) and pH 6 (F=14.43). Uptake rates were highest after algal incubation for 30 minutes in the Cu and declined sharply after 1 hour with gradual uptake gradients with further increases in incubation times.

c. The effects of Cu concentration on the accumulative Cu uptake and uptake rates by *C. glomerata*

Accumulative Cu uptake and uptake rates increased significantly with Cu concentrations from 0-10 mg l^{-1} after algal incubation in Cu for 30 (F=9.87), 60 (F=11.14), 120 (F=32,12) and 240 (F=33.06) minutes at all pH levels (Fig. 5.7 a-c and Fig. 5.8 a-c). The maximum Cu uptake rates for each Cu concentration at the three levels are presented in Table 5.2. At solution pH 8, the maximum Cu uptake rates by *C. glomerata* increased with medium concentration from 0-2 mg Cu l^{-1} afterwhich the rates stabilized at 2 mg Cu g algal dry weight^{-1} h^{-1} when exposed to 6 and 10 mg Cu l^{-1}. Maximum uptake rates at solution pH 6 and 7, however continued to increase linearly with an increase in medium Cu concentration.

Table 5.2. The maximum Cu uptake rates of *C. glomerata* when exposed to various medium Cu concentrations at three pH levels.

Media Cu Concentration (mg Cu l^{-1})	Maximum Uptake Rates (mg Cu algal g dry weight^{-1} h^{-1})		
	pH levels		
	8	7	6
0	0.0	0.0	0.0
2	0.9	1.8	1.8
6	2.0	3.0	3.5
10	2.0	4.5	6.7

values are averages (n=6)

5.4.3. SPECIFIC GROWTH RATES

Specific growth rate inhibition of *C. glomerata* after exposure to Cu for 30 minutes at concentrations of 0, 2, 6, 10 and 20 mg Cu l^{-1} at pH levels of 6, 7 and 8 was observed to increase significantly with a decrease in pH levels at Cu concentrations above 2 mg Cu l^{-1}. (F=

9.54) after a 5 day observational period (Fig. 5.9). At Cu concentrations of 0 mg Cu l^{-1} (the control treatment) and 2 mg Cu l^{-1}, it is evident that pH has no significant effect on the SGRs (Fig. 5.9). When comparing the recovery of the filaments with time, the SGRs of *C. glomerata* after exposure to medium Cu concentrations of 0-20 mg l^{-1} for 30 minutes at pH 8 (Fig. 5.10 a) were not significantly different at each time period observed (F= 0.56) irrespective of medium Cu concentration. At pH 7 (Fig. 5.10 b), *C. glomerata* SGRs were similar at Cu concentrations between 0-10 mg Cu l^{-1} at each time period. However they were significantly (F=5.037) reduced at 20 mg Cu l^{-1} for the first three days with a slight recovery after day 5 but not to the same extent as the lower concentrations. A significant effect of pH and Cu (F=11.41) was observed on the SGRs of *C. glomerata* with time at pH 6 after exposure to Cu for 30 minutes. Similar positive SGRs at 0 and 2 mg Cu l^{-1} were observed, although no growth of the filaments occurred within the first 3 days after Cu exposure to 6 mg Cu l^{-1}. A slight recovery was observed after day 3 with the SGR being 15% of the SGRs displayed by the filaments exposed to 0 and 2 mg Cu l^{-1}. The filaments exposed to 10 and 20 mg Cu l^{-1} lost their chlorophyll after day 1 and died.

5.4.4. THE EFFECTS OF COPPER ON THE INTRACELLULAR STRUCTURE

The micrographs generated from Transmission Electron Microscopy are displayed in Plates 5.1 a-d. *C. glomerata* cells exposed to 0 mg Cu l^{-1} (controls) at medium pH 8 (Plate 5.1 a) and pH 6 (Plate 5.1 b) for 180 minutes illustrated no disruption of the thylakoid membranes of the reticulate chloroplasts. Thylakoid membranes were closely and tightly packed together and arranged parallel to one another. The algal cells exposed to 10 mg Cu l^{-1} at a medium pH of 6 for 30 minutes (Plate 5.1 c) and 3 hours (Plate 5.1 d), showed evidence of chloroplast disruptions. The thylakoid membranes of the chloroplasts in Plate 5.1 c, were noted to be loosely bound and strewn throughout the cytoplasm of the cell. In Plate 5.1 d, the "normal" structure of the chloroplast was lost and the thylakoid membranes appeared to be coagulated in dense blotches.

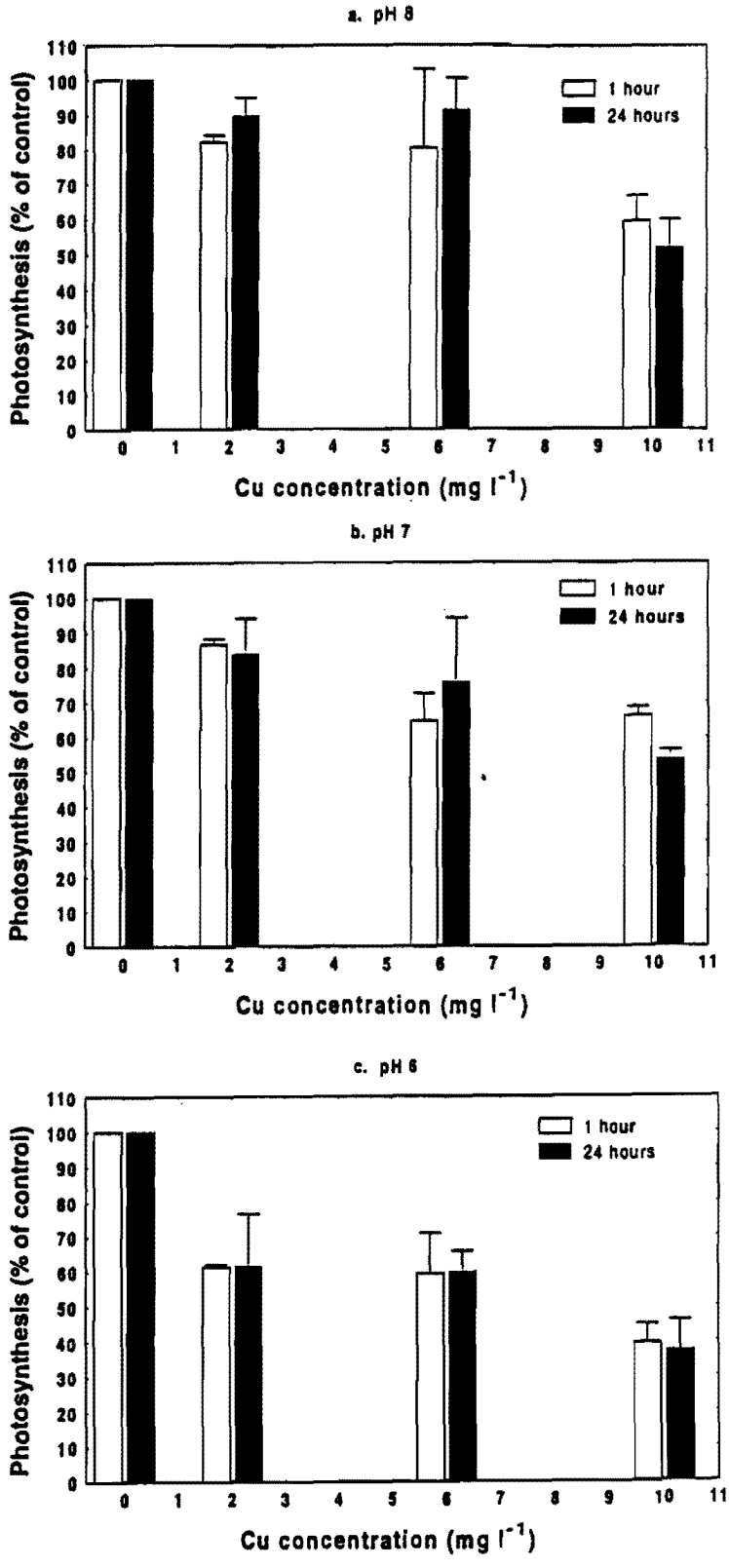


Figure 5.1 a-c. The photosynthetic activity of *C. glomerata* when exposed to different medium Cu concentrations for 30 minutes at three pH levels. a. pH 8, b. pH 7 and c. pH 6. Data plotted are means (n=3) with ± S.E. (vertical lines). Photosynthesis was presented as a percentage of the control treatments (0 mg Cu l-1) which were assumed to photosynthesize maximally at each pH level.

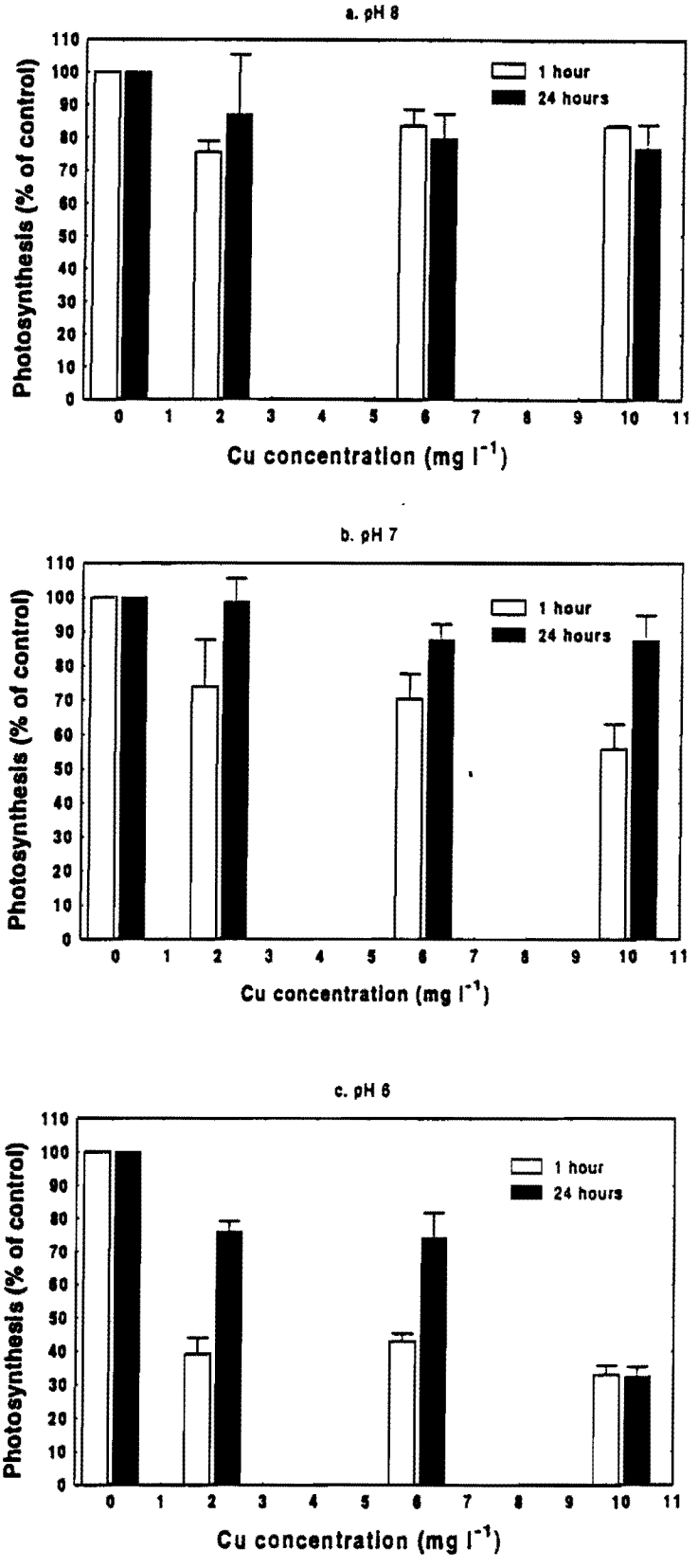


Figure 5.2 a-c. The photosynthetic activity of *C. glomerata* when exposed to different medium Cu concentrations for 60 minutes at three pH levels. a. pH 8, b. pH 7 and c. pH 6. Data plotted are means (n=3) with ± S.E. (vertical lines). Photosynthesis was presented as a percentage of the control treatments (0 mg Cu l-1) which were assumed to photosynthesize maximally at each pH level.

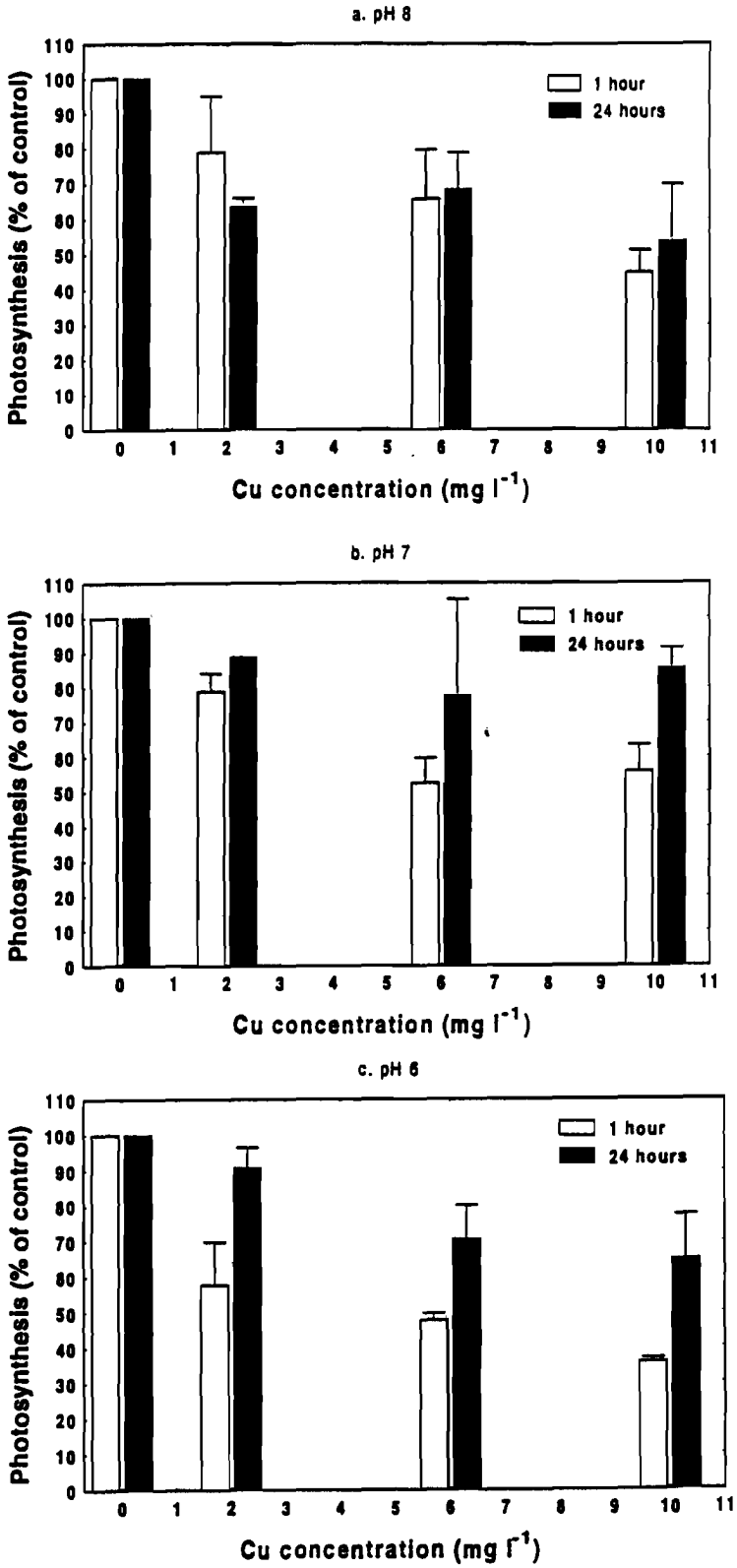


Figure 5.3 a-c. The photosynthetic activity of *C. glomerata* when exposed to different medium Cu concentrations for 120 minutes at three pH levels. a. pH 8, b. pH 7 and c. pH 6. Data plotted are means (n=3) with ± S.E. (vertical lines). Photosynthesis was presented as a percentage of the control treatments (0 mg Cu l-1) which were assumed to photosynthesize maximally at each pH level.

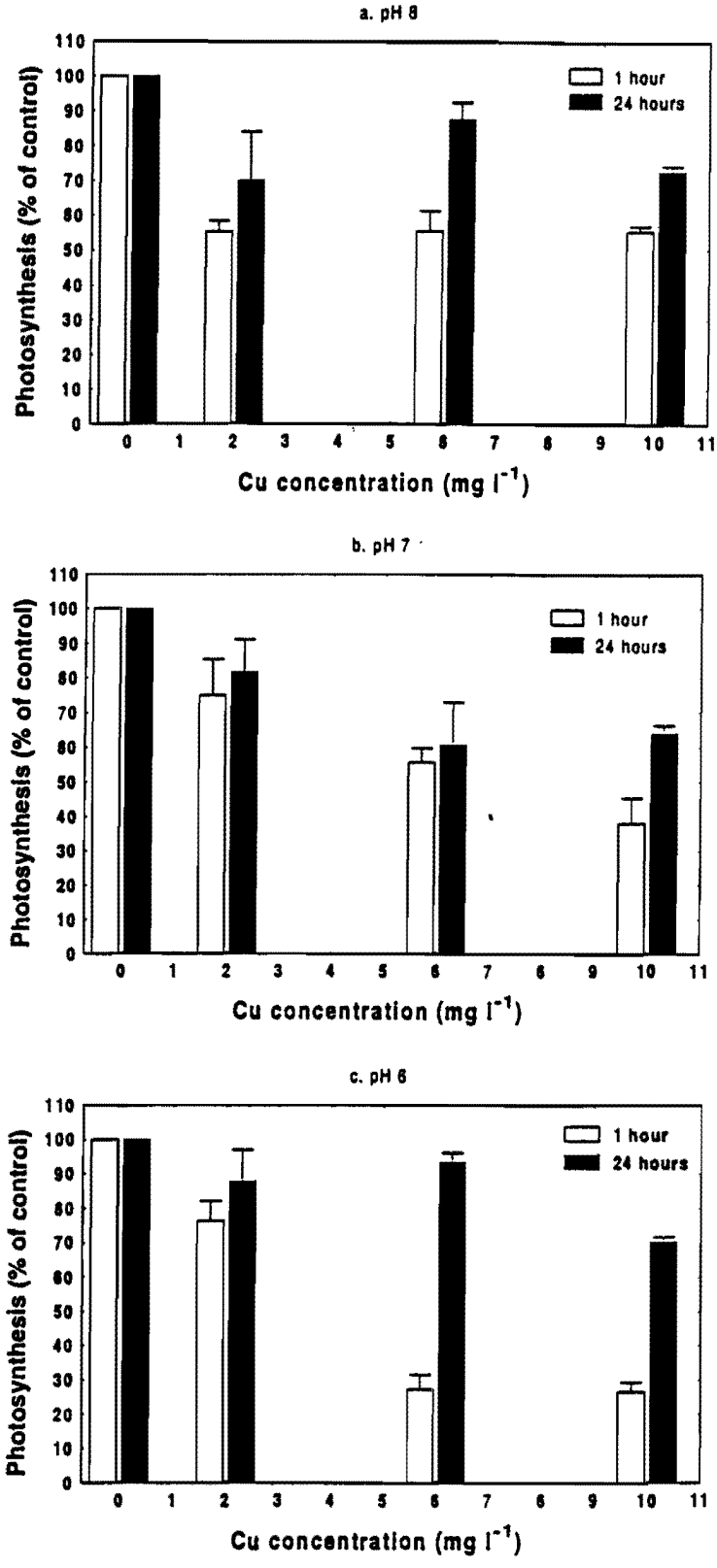


Figure 5.4 a-c. The photosynthetic activity of *C. glomerata* when exposed to different medium Cu concentrations for 240 minutes at three pH levels. a. pH 8, b. pH 7 and c. pH 6. Data plotted are means (n=3) with ± S.E. (vertical lines). Photosynthesis was presented as a percentage of the control treatments (0 mg Cu l-1) which were assumed to photosynthesize maximally at each pH level.

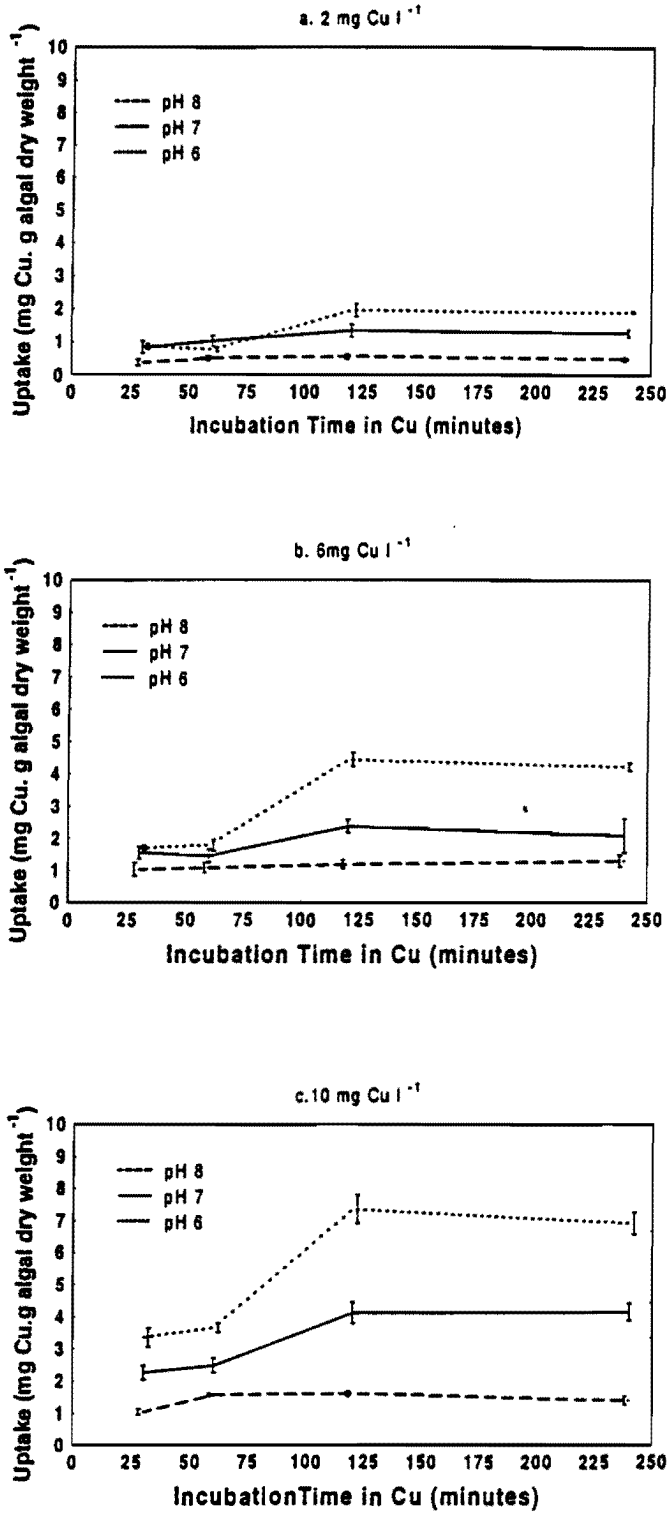


Figure 5.5 a-c. Accumulative Cu uptake by *C. glomerata* with an increase in incubation time at different solution pH levels. *C. glomerata* was exposed to different medium Cu concentrations of a. 2 mg Cu l^{-1}, b. 6 mg Cu l^{-1} and c. 10 mg Cu l^{-1}. Data are means (n=6) with ± S.E. (vertical lines).

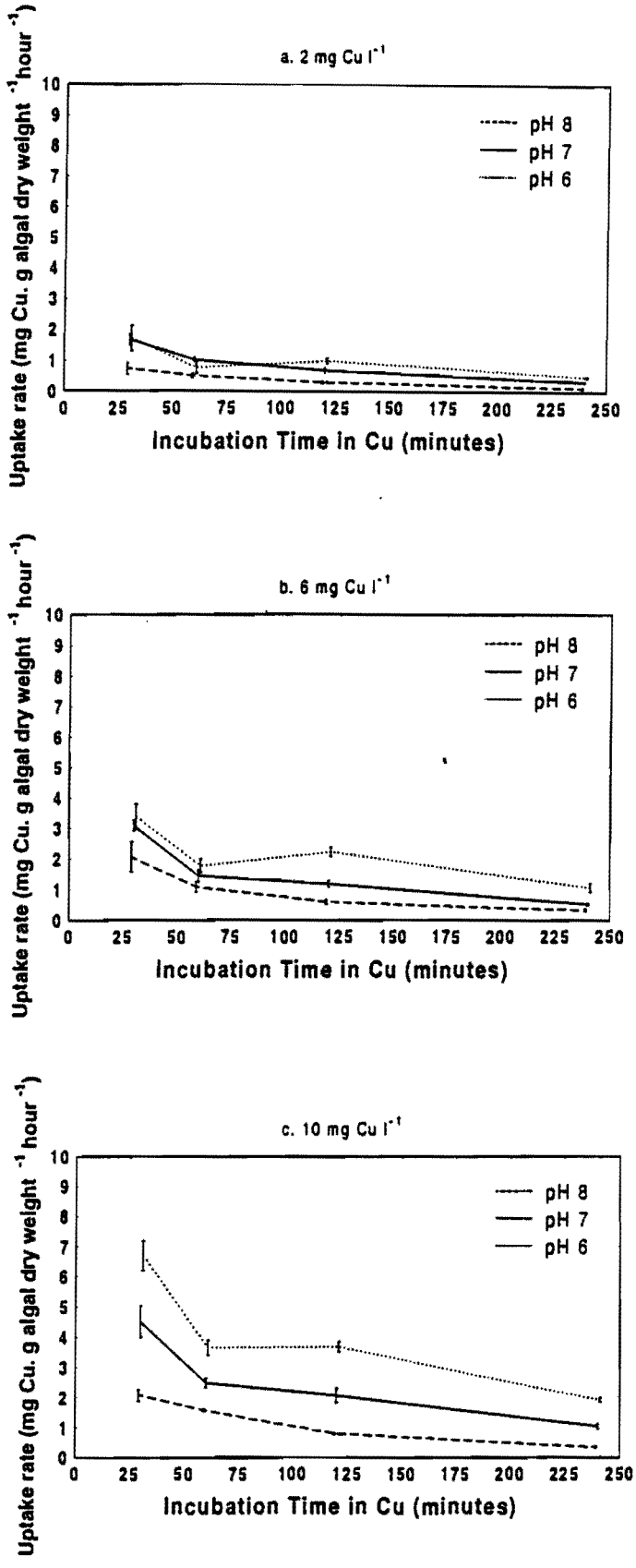


Figure 5.6 a-c. Cu uptake rates by *C. glomerata* with an increase in incubation time at different solution pH levels. *C. glomerata* was exposed to different medium Cu concentrations of a. 2 mg Cu l^{-1}, b. 6 mg Cu l^{-1} and c. 10 mg Cu l^{-1}. Data plotted are means (n=6) with ± S.E. (vertical lines).

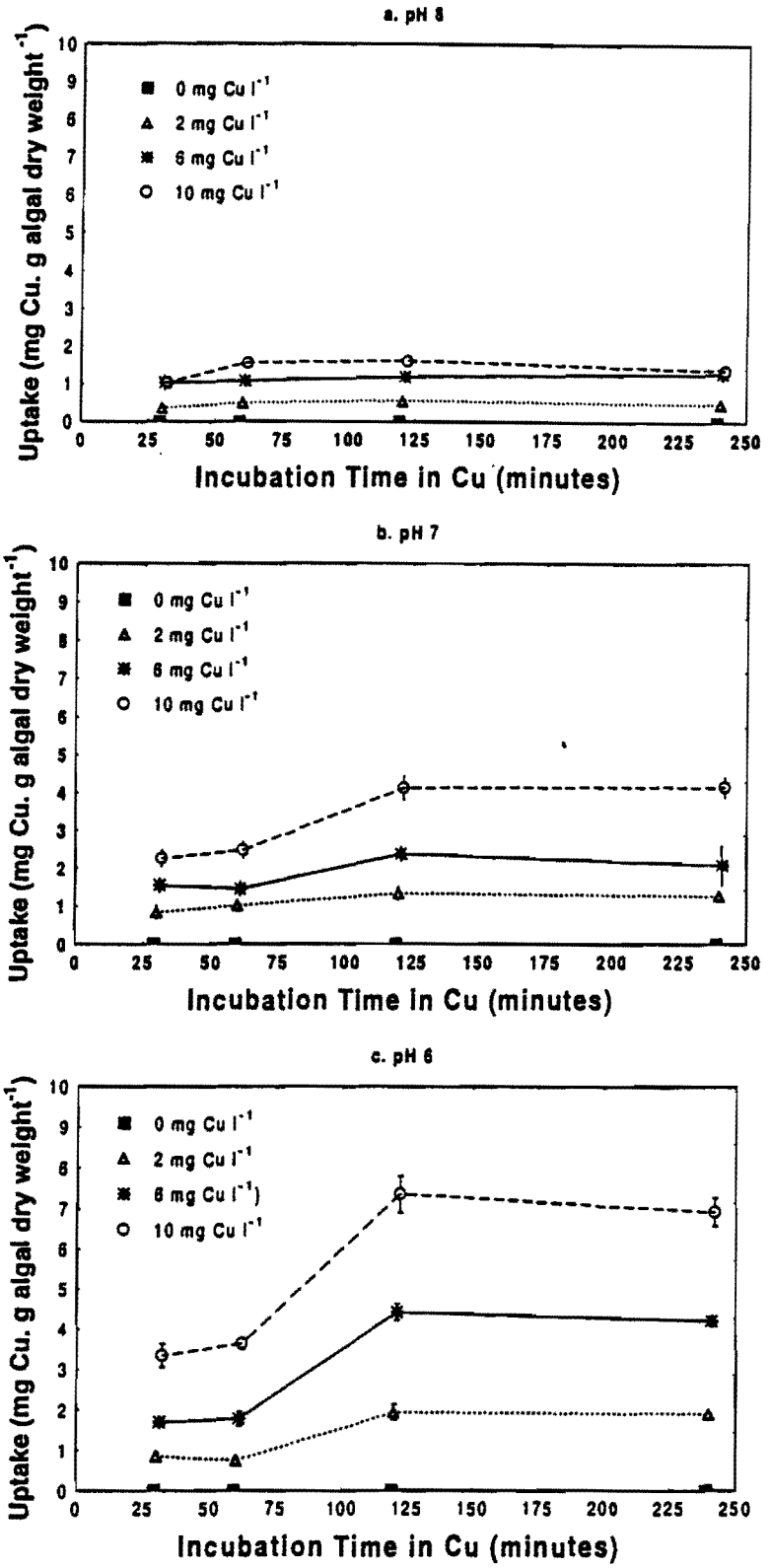


Figure 5.7 a-c. Accumulative Cu uptake by *C. glomerata* with an increase in incubation time at different medium Cu concentrations. *C. glomerata* was exposed to solution pH levels of a. pH 8, b. pH 7 and c. pH 6. Data plotted are means (n=6) with ± S.E. (vertical lines).

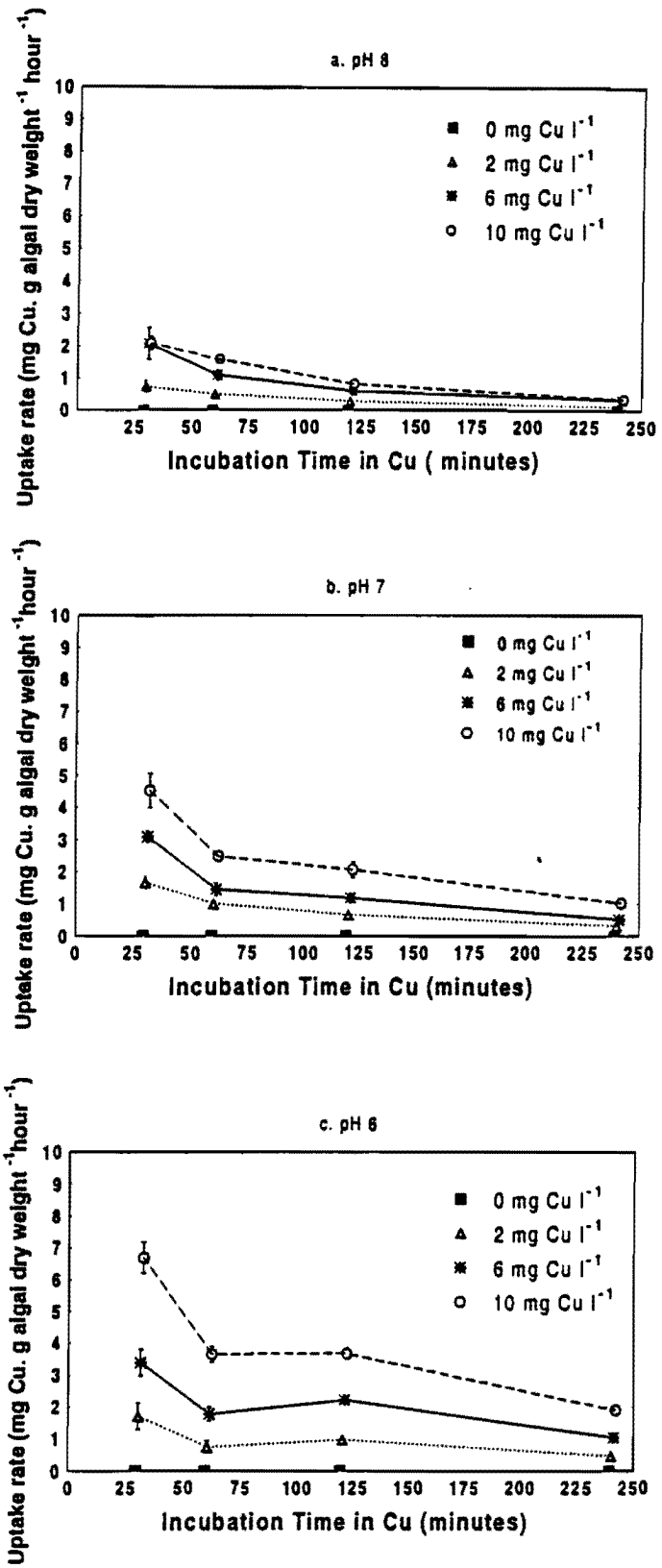


Figure 5.8 a-c. Cu uptake rates by *C. glomerata* with an increase in incubation time at different medium Cu concentrations. *C. glomerata* was exposed to solution pH levels of a. pH 8, b. pH 7 and c. pH 6. Data plotted are means (n=6) with ± S.E. (vertical lines).

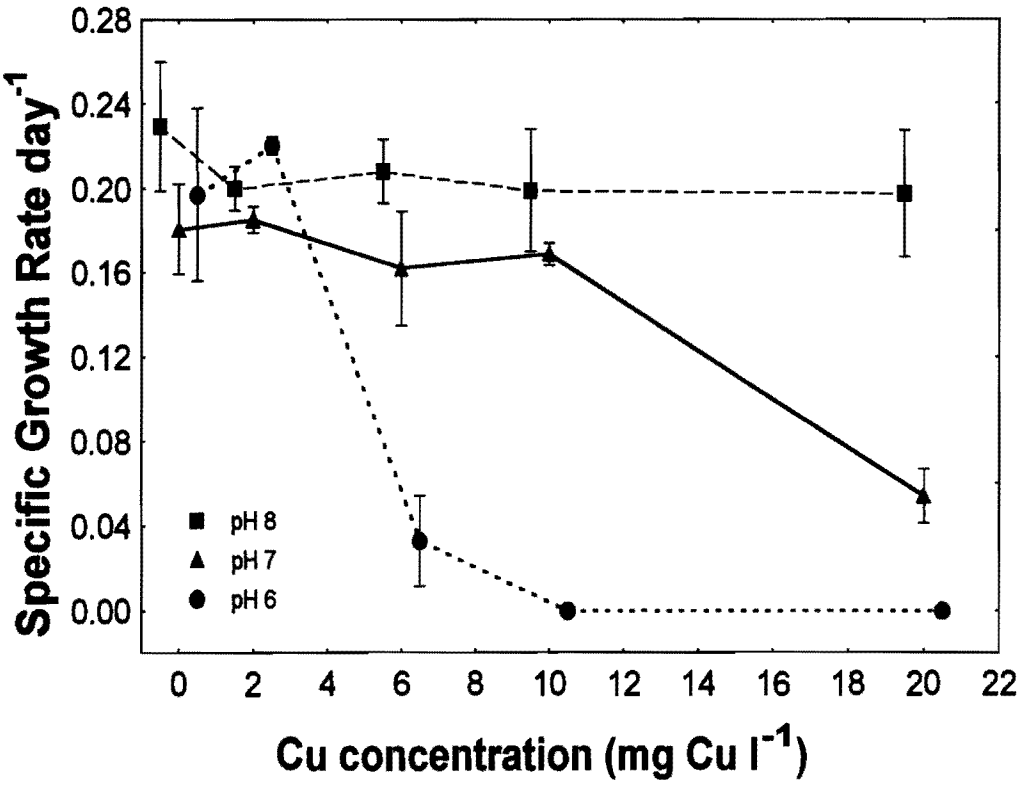


Figure 5.9. The specific growth rate of *C. glomerata* after 5 days, when incubated in different medium Cu concentrations at three solution pH levels for 30 minutes. Data plotted are means (n=3) with ± S.E. (vertical lines).

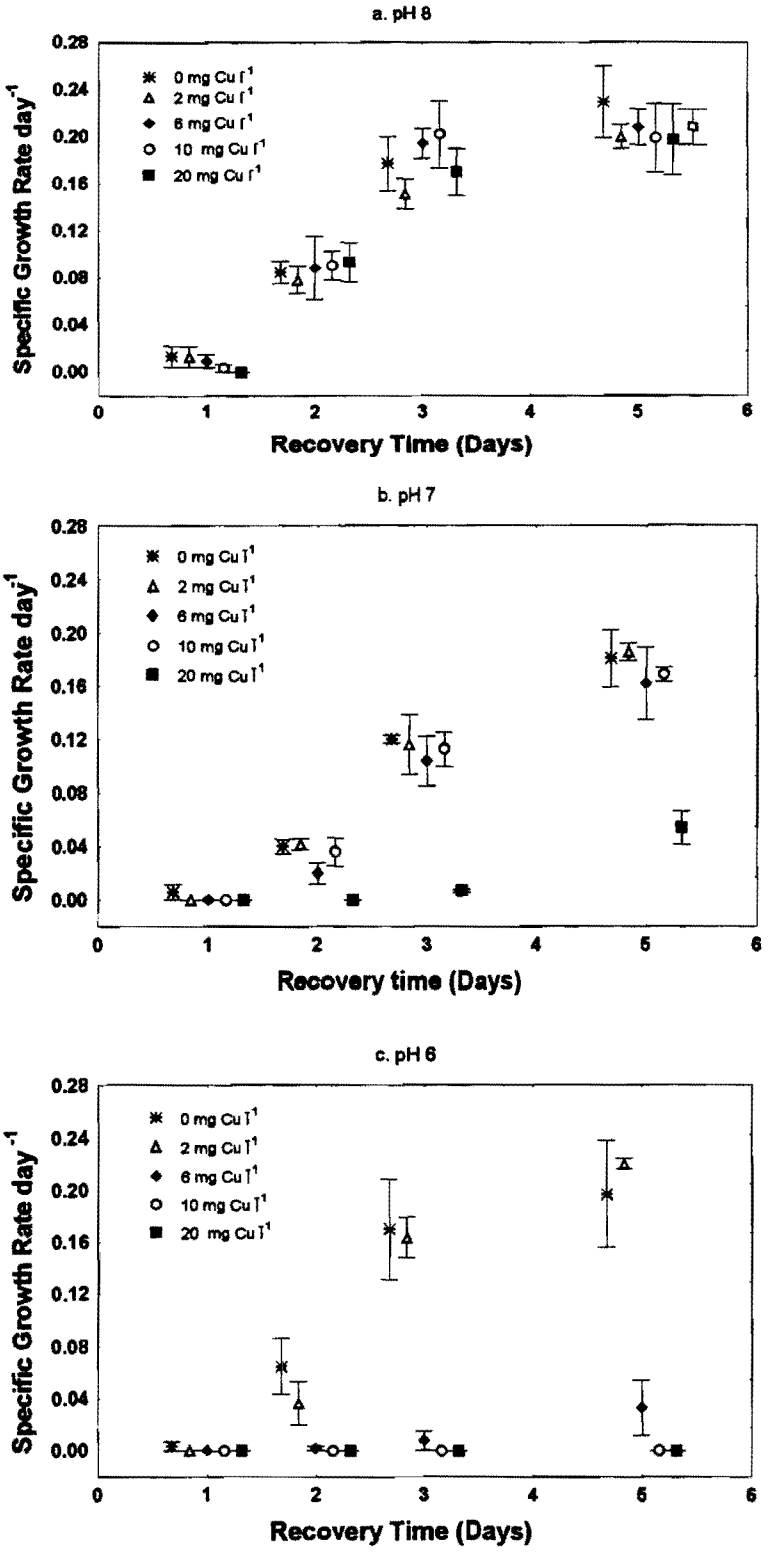


Figure 5.10 a-c. The specific growth rate of *C. glomerata* with time after incubation in different medium Cu concentrations at three solution pH levels for 30 minutes. a. pH 8, b. pH 7 and c. pH 6. Data plotted are means (n=3) with ± S.E. (vertical lines).

a.

b.

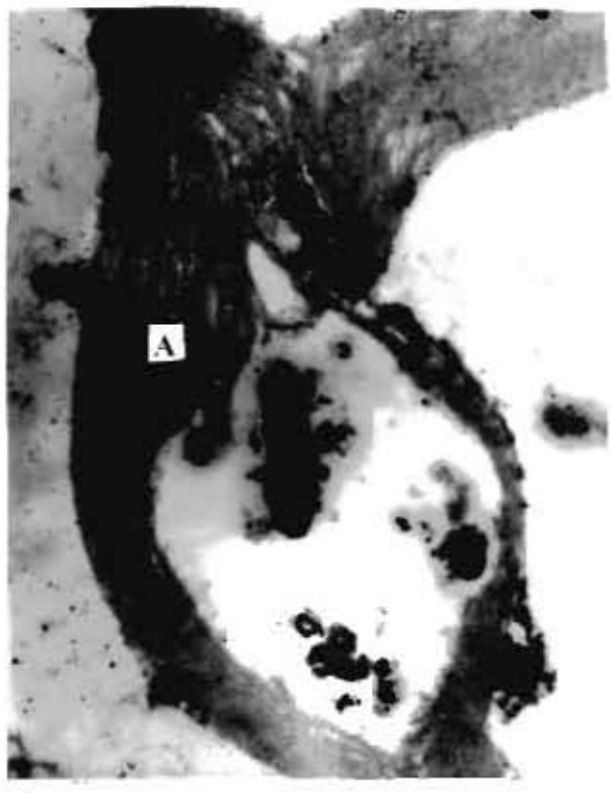
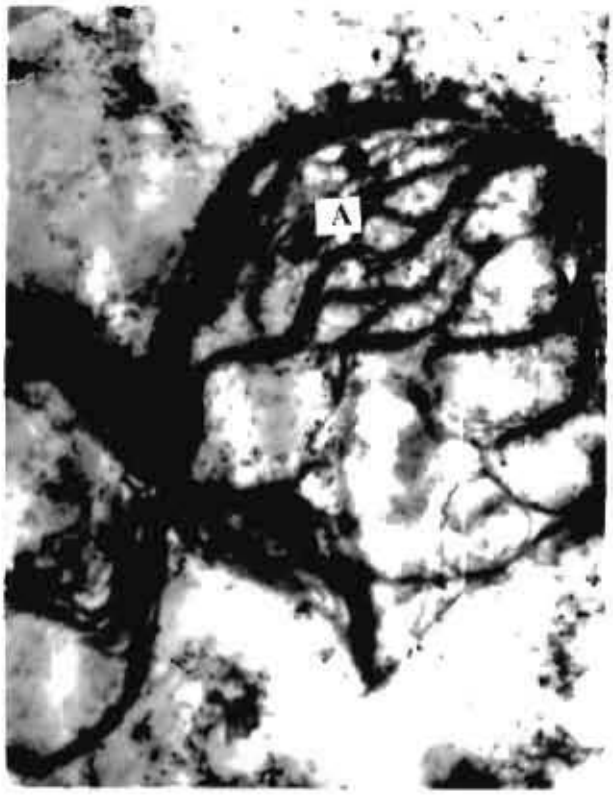


Plate 5.1. a, b. Transmission Electron Micrographs showing the structure of the thylakoid membranes (**A**) in the chloroplast of a *C. glomerata* cell after incubation in a 0 mg Cu l-1 at solution pH 8 for 180 minutes. b. 0 mg Cu l-1 at solution pH 6 for 180 minutes. Magnification: 12 000X and Scale 1cm=0.83μ

c.



d.

Plate 5.1. cont. c and d. Transmission Electron Micrographs showing the structure of the thylakoid membranes (**A**) in the chloroplast of a *C. glomerata* cell after incubation in c.10 mg Cu l -1 at solution pH 6 for 30 minutes and d. 10 mg Cu l-1 at solution pH 6 for 180 minutes. Magnification: 12 000X and Scale: 1cm=0.83μ

5.5. DISCUSSION

Accumulative Cu uptake is a measure of the quantity of Cu taken up by the algal cells and the uptake rate indicates the speed at which Cu is taken up by the algal cells. Both these parameters and hence heavy metal toxicity to *C. glomerata* may be dependent on the Cu^{2+} concentration in the medium which is indirectly dependent on medium pH (Gadd and Griffiths, 1978), algal incubation time in the Cu, the nutritional status of the alga (Reuter *et al.*, 1981) and the alga's ability to mitigate heavy metal toxicity (Macfie *et al.*, 1994).

Stiff (1971), reported that inorganic alkaline substances bind Cu^{2+} in neutral and high pH ranges and decreasing the pH liberates cupric ion from these complexes. Thus, it was postulated that the observed differences in copper toxicity in test waters of different hardness is due to the degree of Cu^{2+} binding with inorganic anions (Miller and MacKay, 1980). Accordingly, at a pH below neutral, metals exist as free ionic cations, but at a pH above neutral, the ionic cations bind to inorganic substances and precipitate as insoluble hydroxides or oxides (Gadd and Griffiths, 1978 and McKnight *et al.*, 1983). Since many heavy metal hydroxides are insoluble, it could be inferred that the toxicity of heavy metals to algae is greatest at low pH and declines as pH increases assuming that free ionic Cu is the main determinant of algal toxicity as suggested by Stokes (1983).

By applying the above rationale it may be assumed that Cu uptake would increase with decreasing solution pH. In this study accumulative Cu uptake increased with a decrease in pH at all Cu concentrations and at all incubation times in Cu (Fig. 5.5 a-c). In accordance biological activity in the form of algal photosynthesis, measured 1 hour after algal exposure to Cu, was observed to be increasingly impeded by lowering the pH level from 8 to 6 as revealed in the experiments where *C. glomerata* was exposed to Cu for 30, 60 and 240 minutes (Figs. 5.1, 5.2 and 5.4 a-c). Likewise, it was shown that SGRs of *C. glomerata* were reduced to a greater extent with decreasing pH levels over the 5 day observational period (Figs. 5.10 a-c).
The effect of pH on Cu^{2+} uptake may however be masked by low copper concentrations. In the study by Macfie *et al.* (1994) cultures of *Chlamydomonas reinhardtii* (Ehr.) exposed to 0-20 μM Cu at pH levels of 5 and 7 for 5 days demonstrated that the algal cell wall provided some protection from potentially toxic concentrations of Cu. It was however noticed that the cell wall

only provided protection at low Cu concentrations with algal cell densities reduced to similar amounts at higher Cu concentrations. Macfie *et al.* (1994) suggested that at high cationic concentrations the metal binding sites on the cell wall may become saturated with a further lack of protection of the cell membrane. The notable increase in uptake rates by *C. glomerata* with decreasing pH levels at a Cu concentration of 10 mg Cu l^{-1} (Figs. 5.6 c) and not at 2 and 6 mg Cu l^{-1} (Figs. 5.6 a and b) in this study may be partially attributed to the above phenomenon. At solution pH 8, the uptake rates are expected to be lower than at pH 7 and 6 at all Cu concentrations due to the binding capacity of Cu^{2+} to inorganic substances (Figs. 5.7 a-c). At 2 and 6 mg Cu l-1 at pH 6 and 7 the concentration of Cu^{2+} ions could probably be sufficient to bind to the cell wall and reduce the uptake rate, but not high enough, as 10 mg Cu l^{-1} would be, to saturate all the metal binding sites. The effect of pH at low Cu concentrations may also be masked in the presence of ligands (Gadd and Griffiths, 1978). Although no synthetic ligands were added to the culture medium in this study, naturally occurring chelators may have been present in the tap water which may explain why the uptake rates obtained were only affected by pH at Cu concentrations of 10 mg Cu l^{-1} (Fig. 5.6 c). This relationship is illustrated by the SGRs of *C. glomerata* after 5 days when exposed to Cu for 30 minutes at pH 6, 7 and 8. Specific growth rates were only affected by pH levels at Cu concentrations above 2 mg Cu l^{-1} (Fig. 5.9).

The concentration of Cu in the medium from 0-10 mg l^{-1} had significant effects on the uptake of Cu into the algal cells at all three pH levels (Figs. 5.7 and 5.8 a-c). A solution Cu concentration of 6 mg Cu l^{-1} was needed before maximum uptake rates were stabilized at a pH of 8, and at a solution pH of 7 and 6 the maximum uptake rates progressively increased with an increase in medium Cu concentration (Table 5.2). Similarly, in a study by McHardy and George (1990), the uptake of Zn by *C. glomerata* increased with increasing concentrations in the test solutions when exposed to solution Zn concentrations from 0.15 - 4.0 mg l^{-1} with solution pH ranging between 7.2 and 8.4. Uptake was proportional to concentration of test media up to a Zn concentration of 0.4 mg l^{-1}. At concentrations between 1.0 and 4.0 mg.l^{-1} Zn, the uptake rate stabilized.

Whitton *et al.* (1981) state that many aquatic macroalgal species have the ability to take up heavy metals from water, producing an internal concentration greater than the surroundings. The maximum amount of Cu accumulated by *C. glomerata* examined in this study was 7 mg Cu g algal dry weight^{-1} when exposed to a medium Cu concentration of 10 mg Cu l^{-1}, pH 6 and for an incubation time in the Cu of 2 hours (Fig. 5.7c). In a field study by McHardy and George (1985) the uptake of heavy metals by *C. glomerata*, isolated from an urban river which received a supply of polluted urban runoff, was examined. Heavy metals were present in higher concentrations in the algal tissue, in the range of 8.08 -16.6 μg g^{-1}, than in the water itself. Trollope and Evans (1976) recorded concentrations of copper, 50-60 μg g^{-1}, in *Cladophora* algal tissue collected from freshwater sites in the Swansea Valley. The diverse levels of Cu accumulated in the tissue of *C. glomerata* may indicate a sensitive response to change in ambient levels (Oertel, 1991).

The increase in medium Cu concentrations from 0-10 mg Cu l^{-1} affected the biological activity of *C. glomerata* through photosynthesis and growth. Photosynthesis, measured 1 hour after algal exposure to Cu, was reduced with an increase in Cu concentration from 0-10 mg Cu l^{-1} when *C. glomerata* was exposed to Cu for 30 to 240 minutes as shown in Figs. 5.1- 5.4 (b and c). Similarly, the SGRs of *C. glomerata* were reduced significantly with an increase in concentration above 2 mg Cu l^{-1} at pH 6 and 7 with a Cu concentration of more than 6 mg l^{-1} completely killing the alga at a solution pH of 6 (Fig. 5.9). Previous studies investigating the minimum Cu concentration required to reduce growth in *C. glomerata* reported much lower values, usually less than 1 mg Cu l^{-1} [Leland and Carter (1984), McHardy and George (1990) and Thomas (1962)] however Betzer and Kott (1969) indicated a much higher concentration of 2-4 mg Cu l^{-1} before effective control was obtained.

The different minimum Cu concentrations obtained by various researchers in determining effective growth control of *C. glomerata* may partially be attributed to the chemical status of the medium as discussed in the Introduction 5.1. For this reason McKnight *et al.* (1983) suggested that a general procedure in determining the minimum effective $CuSO_4$ dosages would be to calculate the cupric ion activity as a function of total copper added from the relevant physico-chemical characteristics of the aquatic body and compare that with the cupric ion

uptake rate was after 30 minutes of algal incubation in the Cu at pH 7 and 6, accumulative uptake saturation was reached after 2 hours of algal incubation in Cu. The question of "Why does accumulative Cu uptake saturate?" may be ascribed to Cu^{2+} depletion in the solution. The depletion of Cu^{2+} ions could explain why saturation occurs faster at pH 8 than at pH 6 and 7. However, uptake saturation cannot be attributed to total Cu^{2+} depletion since uptake rates had not equalled zero even after 240 minutes of algal incubation in the Cu at all Cu concentrations and pH levels (Figs. 5.8 a-c). Uptake saturation may however be a factor of the algal's inability to absorb any more Cu^{2+} ions due to a saturation of the internal cell concentration. Although the internal cell concentration may have been saturated, Cu was still taken up but at much reduced rates (Figs. 5.8 a-c) This was probably a consequence of the replacement of the Cu^{2+} which was utilised by the alga in order to maintain algal physiology (Hillebrand and De Vries, 1986).

The maximum uptake rates of Cu by *C. glomerata* were attained within the first 30 minutes of algal incubation in the Cu at all Cu concentrations and pH levels (Figs. 5.6 a-c). Vymazal (1984), similarly demonstrated that the periphytic filamentous algae, *C. glomerata* and *Oedogonium rivulare* (Le Clerc.) Link, exhibited rapid uptake of Cu. Maximum Cu uptake by *C. glomerata* appeared during the first hour of exposure with approximately 89.9% of total copper (36 $\mu g\ l^{-1}$) removal within this period. McHardy and George (1990) however reported that the uptake of Zn by *C. glomerata* was dramatic in the first 10 minutes. The deviating uptake rates of heavy metals reported above may not only be consequences of different chemical compositions of the test medium but also the algal vigour. For example, Oertel (1991), illustrated that young filaments of *C. glomerata* collected from the littoral zone of the River Danube played a more dominant role in metal uptake than old ones. The nutritional status of the alga likewise could affect Cu uptake. Reuter *et al.* (1981), reported that copper uptake is dependent on silicon nutrition, with more copper being assimilated by silicon limited cells. Indirectly, this observation could be related to the finding by Macfie *et al.* (1994) who suggested that the cell wall plays a role in ameliorating the effect of possibly toxic concentrations of heavy metal ions. Since Moore and Traquair (1976) claimed that Si nutrition is important for cell wall formation in *C. glomerata*, it would follow that the less developed the cell wall when the algal cells are Si limited the higher the uptake of heavy metal ions.

From the above discussion, it is apparent that Cu uptake is governed by solution pH, medium Cu concentration and time of algal incubation in the Cu. It is also evident that an increase in Cu uptake causes an increase in growth reduction and photosynthetic inhibition. The question which is now asked is " Are the effects of Cu on algal growth and photosynthesis permanent or not?"

Although recovery of photosynthesis, that is 24 hours after algal exposure to Cu, was observed in some cases, no consistent patterns were observed with pH, Cu concentration and algal incubation time in Cu (Figs. 5.1-5.4 a-c). As explained previously this could be an artefact of some algal cells being affected by the Cu whilst those which were not, were still capable of photosynthesising. However, the SGRs of *C. glomerata* after exposure to Cu concentrations above 10 mg Cu l^{-1} at pH 6 indicated that the effects of Cu on the algal cells were irreversible since complete death occurred within the first day, indicating complete toxicity (Fig. 5.10a). The SGRs of the algal cells exposed to 6 mg Cu l^{-1} at a pH 6 (Fig. 5.10 c) and 20 mg Cu l^{-1} at pH 7 (Fig. 5.10 b), showed some signs of recovery after day 3. The recovery of the SGRs could indicate that the concentration of Cu in the cell is not sufficient to cause extensive and permanent damage. For example, SGRs of the algal cells, exposed to a medium pH 8, were similar irrespective of medium Cu concentration over the 5 days (Fig. 5.10 a). This observation probably indicates that the low Cu uptake amount of less than or equal to 1 mg Cu. g algal dry $weight^{-1}$, at solution pH 8 and algal incubation time in the Cu for 30 minutes (Fig. 5.7 a), was insufficient to cause irrepairable damage. In this study the amount of Cu in the algal tissue increased with an increase in the algal incubation time in the Cu at a solution pH of 6 from 3.5 mg Cu. g algal dry $weight^{-1}$ after 30 minutes of algal incubation in the Cu to *ca* 7 mg Cu. g algal dry $weight^{-1}$ after a 2 hour algal incubation time in the Cu (Fig. 5.7 c). In accordance greater thylakoid damage occurred after 3 hours compared to 30 minutes of algal incubation in 10 mg Cu l^{-1} at pH 6 as illustrated in Plates 5.1 c and d respectively.

The last question which needs to be discussed is " What are the physiological effects of Cu in an algal cell?" The cupric ion has been shown to be an inhibitor of photosynthesis in algal cells (Gross *et al.*, 1970). Initially it was elucidated that Cu has a direct effect on the light reactions of photosynthesis (Macdowall, 1949). A study by Haberman (1969) showed that Cu was inhibiting the dark reaction and played no role in altering the reactions associated with the

photoacts (the structures involved in the light reaction of photosynthesis). Cedeno-Maldonado and Swader (1972), using isolated chloroplast from *Spinacia oleracea L.* (spinach), exposed to a $CuSO_4$ concentration range of 0-60 μM, indicated that Cu^{2+} inhibited uncoupled photosynthetic electron transport to NADP at very low concentrations. Exposure of the chloroplast to the inhibitor for short periods of time in the dark reduced the degree of inhibition while exposure for short periods to the light had opposite effects. Likewise Luderitz *et al.* (1989), reported that the the photosynthesis of the cyanophytes, *Oscillatoria redeki* Van Goor and *Aphanizomenon gracile* Lemmermann, was more susceptible and sensitive to Cu toxicity effects during the light phase than the dark indicating a connection between Cu action and light-driven physiological processes. Therefore, it appears that light is essential for the interaction of Cu^{2+} with the electron transport mechanism of the chloroplast which supports Gross *et al.*'s, 1970 initial claim.

Steeman Nielsen and Wium Andersen (1970) observed that the influence of Cu on photosynthesis may be through different mechanisms. In *Chlorella pyrenoidosa* (a chlorophyte) Cu did not penetrate the cell immediately but rather influenced the alga by blocking mechanisms in the cell membrane in such a way that no division took place. Declined photosynthetic rates only occurred some hours after Cu application due primarily to the accumulation of photosynthetic products which secondarily blocked photosynthesis. In *Nitzschia palea* Kütz. (a diatom) on the other hand, a relatively high decrease in photosynthesis was observed after 20 minutes of application which was similar to the results obtained in Figs. 5.1-5.4, a-c) where photosynthesis was already reduced after 1 hour of algal exposure to Cu. Steeman Nielsen and Wium Andersen (1970) maintained that Cu probably penetrated into the cell causing the membranes to become leaky and the cells to lose considerable amounts of organic matter. Similar cellular damages were encountered when *C. glomerata* cells were exposed to 10 mg Cu l^{-1}. In the transmission electron micrographs, the thylakoid membranes of the chloroplast lost their structure due to the Cu, with the membranes being either loosely bound (Plate 5.1 c) or coagulated into dense groups (Plate 5.1 d) throughout the cytoplasm compared to the normal chloroplast structure of the control treatments (Plates 5.1 a and b).

Copper toxicity may also disrupt other physiological processes. Stauber and Florence (1987) proposed that Cu may interrupt cell membrane function. The Cu ions are likely to interfere with cell permeability or the binding of essential metals. Subsequent to Cu transport into the cytosol, the ions may react with free thiols thereby disrupting enzyme active sites and cell division. Davies (1976) suggested that Cu and Hg might prevent the production of methionine which appears necessary for cell division. It has also been proposed that metals inhibit phytoplankton cell division by binding reactive thiols on the tubulin molecule, which is important in spindle formation during mitosis. Foster (1977) revealed that *Chlorella vulgaris* exposed to high concentrations of Cu (0.3 $mg.l^{-1}$) exhibited cell abnormalities. These included increased cell size and a decrease in yield of cell number. These observations were possibly a manifestation of the fact that heavy metals may specifically inhibit cell division in algae. That is, dividing cells failed to separate, resulting in multicellular aggregates. Copper may manifest its toxicity by reducing cell defence mechanisms against H_2O_2, which has been shown to be very toxic to algal growth, by firstly, inhibiting the dissociating enzyme catalase, probably by displacing Fe from the enzyme active centre and secondly having superoxide dismutase activity which leads to the production of more H_2O_2 from the superoxide radical. Cu may also play a role in catalysing the decomposition of H_2O_2 to produce hydroxyl radicals which may accelerate oxidative deterioration of membrane lipids (Stauber and Florenece, 1987).

CHAPTER 6

AN ASSESSMENT OF APPLIED OR POTENTIAL MANAGEMENT STRATEGIES USED IN CONTROLLING *C. GLOMERATA* GROWTH IN IRRIGATION CANALS IN SOUTH AFRICA

6.1. INTRODUCTION

The control of problematic growths of filamentous macroalga in eutrophic freshwater bodies can and does incur great financial costs in South Africa (Joska and Bolton, 1994 a). Although the solution of reducing nutrient inputs into these systems would be ideal, it can only be achieved through improving methods of sewage and industrial effluent treatments. However,it is implausible to limit or prevent nutrient input from agricultural run-off especially since many agricultural lands are concentrated around major water schemes in South Africa. In these cases supplementary methods would need to be employed for the eradication of problematic macroalgae. Growth of *C. glomerata* can be controlled either by means of manual removal, draining the canals, chemical substances, biological methods or by the simulation of unfavourable environmental conditions. These methods, however usually alleviate the problem temporarily. In this chapter, the various methods applied at present to control filamentous macroalgal growth in South Africa will be briefly assessed and the viability of potential control methods will be examined.

6.2. PRESENT METHODS EMPLOYED

6.2.1. THE USE OF COPPER SULPHATE AS AN ALGICIDE

The use of $CuSO_4$ as an effective algicide and the predosing of sulphuric acid in alkaline water in order to enhance the algicidal effects have been scientifically confirmed in this study (Chapter 5). The minimum Cu concentration required to obtain effective control will depend greatly on

the water chemistry in the canals, algal mat density and the application method. In many of the irrigation canal systems $CuSO_4$ is either discarded into the canals as a once-off instant event or by filtration using perforated bags (Joska pers. comm.). The residence time of the Cu in the water and its availability to the alga is usually for short time periods depending on the water flow rate in the canals. Consequently, when water flow is fast, the use of perforated bags would be more effective and when water flow is slow or static, an instantaneous application of $CuSO_4$ would suffice. Since maximum uptake rates occurred within the first 30 minutes after algal exposure to Cu (Figs. 5.6 and 5.8) it may be assumed that these application methods should be successful. As a basic guideline, it is recommended that an application of a minimum of 6 mg Cu l^{-1} at a solution pH 6 for an incubation period of less than 30 minutes will obtain effective control of *C. glomerata* growth at low algal densities. However increased water pH would require higher minimum Cu concentration and dense algal mats would probably require repeated dosages of $CuSO_4$ to achieve effective growth control. Minimum Cu concentrations required to obtain effective algal growth control, which are suggested by this study, greatly exceed the Cu concentration, 0.8 mg l^{-1}, applied to irrigation canals when the water pH is reduced to 6 (Du Plessis, 1992 a and b).

6.2.2. MANUAL REMOVAL

Although manual removal of macroalgae by means of raking may be more expensive than the use of $CuSO_4$ (estimated to cost four times more than the use of $CuSO_4$ in the Hartebeespoort irrigation canals Bruwer, 1991) it can be perceived as being socio-economically beneficial to South Africa. It provides additional employment opportunities, which are much needed in this country, and it partially eliminates the problem of filamentous macroalgal blooms. Manual removal of *C. glomerata* has been successful in some small irrigation schemes but it has often be impeded in areas which are inaccessible as well as by climatic factors such as excessive rainfall (Joska and Bolton, 1994 a). Barrett and Newman (1993) further claim that this control method is short-term since regrowth of fragments, which are left behind after raking, is rapid.

6.2.3. DRAINING THE CANALS

Usher and Blinn (1990) suggested that fluctuations in flow regimes could have drastic effects on the biomass of *C. glomerata* in the laboratory. Recently, Blinn *et al.* (1995) observed that a fluctuation in water discharge in the field reduced the distribution and standing biomass of *C. glomerata*. At Lees Ferry on the Colorado River near Arizona, it was noted that repeated 12 hour exposures for a period of five days vastly reduced the biomass. Furthermore, a decline in biomass was more pronounced with day-time compared to night-time exposures and with longer time periods. This strategy has been utilised in many irrigation canal systems in South Africa where the canals have been dried up for lengthy time periods in an attempt to eliminate nuisance algal blooms (Joska, pers. comm.). This strategy however is of a temporary nature since McNaught (1964, cited in Hoffman and Graham, 1984) observed that rising water levels initiated growth in akinetes (resting zoospores) of *C. glomerata* which were previously dry on the upper levels of the substratum.

6.3 POTENTIAL CONTROL METHODS

6.3.1. MANIPULATING ENVIRONMENTAL FACTORS

The growth responses of *C. glomerata* to various environmental factors, including temperature, light intensity and photoperiod, may not be true reflections of field responses, but do provide some indication of optimum conditions required to support growth. On the other hand laboratory studies could also yield an indication of unfavourable growth conditions which could be simulated in the field to reduce prolific nuisance growths of *C. glomerata*. It would be implausible to manipulate temperature and photoperiod conditions *in vivo*, but the amount of light reaching water surfaces could be controlled. In an experiment by Demal and Fortin (1987), luxuriant growths of *C. glomerata* in the Avon River, a tributary of the Tames River, southwestern Ontario was shown to be impeded by shading certain areas with cloth. *Cladophora glomerata* growth, measured as dry weight, was reduced by 70% compared to algal growth in a control unshaded site, which received approximately 73% (i.e. 1300-3600 μmol photons m^{-2} s^{-1}) more light than the shaded areas which received between 480 and 1332 μmol photons m^{-2} s^{-1}. In a detailed study by Dawson and Kern-Hansen (1979) in the area of mid-Jutland, Denmark,

the biomass of the submerged aquatic macrophytes, *Ranunculus peltatus* (Schank), *Elodea canadensis* (Rich.) and *Potamogeton crispus* (L.) were vastly reduced by artificially shading the streams. The biomass of these macrophytes were also reduced in naturally shaded areas compared to open areas however the differences varied with seasonal and annual light fluctuations.

Although the scope for planting bankside trees may not be a feasible option in South Africa, the response of *C. glomerata* growth to light intensity may be exploited by water system managers to mediate deleterious effects of growths. If placements of water work systems are placed at a depth below that which minimizes *C. glomerata* colonization, intake fouling may be minimized (Lorenz *et al.*, 1991). The concept of blocking light from water bodies has a few shortcomings. Firstly, light levels fluctuate seasonally as well as with water turbidity. In field observations by Lester *et al.* (1988) and Lorenz and Herdendorf (1982) *C. glomerata* colonization depth and vertical distribution in Lake Erie varied with location depending on turbidity, sediment load in the water and the local degree of light attenuation. In the Zuikerbosch canal at the Rand Water Supply Station, South Africa, huge growths of *Cladophora* sp. were observed in 1983 when water turbidity levels were low. Subsequently, the biomass of *Cladophora* sp. seemed to fluctuate according to water turbidity levels, being depressed at high turbidity and enhanced at low turbidity (Steynberg, pers. comm. cited in Joska and Bolton, 1994 a). Secondly, in South Africa, the use of shade cloth does not seem to be readily accepted because of the high costs involved (Joska and Bolton, 1994 a). Thirdly, manipulating the light availability in canals and rivers may have detrimental effects on other aquatic organisms (Dawson and Kern-Hansen, 1979). Furthermore, in this study (chapter 4), *C. glomerata* growth was only reduced at low light intensities, below 25 μmol photons m^{-2} s^{-1}, and negligible growth was only obtained at 0 μmol photons m^{-2} s^{-1} irrespective of temperature and photoperiod conditions examined. These low light intensities required to impede *C. glomerata* growth suggest that virtually all incoming light would need to be obstructed from the canals and that water work systems should be placed at water depths below that which obtain light intensities sufficient to support growth. Since Joska (pers. comm.) observed that the light intensity penetrating the water column at and above 25 cms below the water surface exceeded maximum light requirements to support optimum growth throughout the year, it would advisable if water work systems are placed at water depths

more than 25 cm below the water surface.

6.3.2. BIOLOGICAL METHODS

a. The use of decomposed barley straw as an algicide

Water extracts containing decomposed barley straw residues have recently been shown to have antialgal activities towards green algae and cyanophytes. Welch *et al.* (1990) demonstrated that the presence of rotting barley straw in a section of the Chesterfield canal (United Kingdom) significantly reduced the amount of *C. glomerata* during the second season after the initial application. In a laboratory study by Gibson *et al.* (1990) the presence of rotting barley straw in water inhibited the growth of planktonic and filamentous algae. The inhibitory effect was most effective after a six month period at 20°C. When the straw was autoclaved all the inhibitory effects were lost which may suggest that bacterial populations within the straw were the main determinants of the inhibitory action or the possible compound active against algae breaks down with heating. Likewise Newman and Barrett (1993) reported that growth of the blue-green alga, *Microcystis aeruginosa* Kütz, was inhibited by the presence of decomposing barley straw in the water. Regrowth of the cells exposed to inhibitory concentrations were however similar to the control, when transferred to a barley straw-free medium.

The inhibition of algal growth by decomposed barley straw can possibly be attributed to several factors. In the report by Welch *et al.* (1990) nutrient competition between the alga and microflora, increased algal grazing by invertebrate animals associated with the straw and possible algicidal effects by pesticides used on barley straw were regarded as highly unlikely in producing the antialgal growth effects. Newman and Barrett (1993) suggested that the antialgal effects of decomposing barley straw may be credited to the production of antibiotics by the fungal flora and the release of straw cell wall components modified during microbial decomposition. However they subsequently implied that phenolic compounds released from decomposed cell wall components and other aromatic compounds from incomplete decomposition of lignin would be present in the barley water extract at lower concentrations than those reported by Dedonder and Van Sumere, (1971 cited in Newman and Barrett, 1993) to be toxic to algal growth. Indeed in the study by Welch *et al.* (1990) the maximum

concentration of any biologically active compound released by decomposed barley straw was suggested to be about 37 $\mu g\ l^{-1}$.

Pillinger *et al.* (1994) however maintained that since only a low dose of decomposing straw is required in natural waters for algal control, the inhibitor must be active at exceedingly low concentrations. In the research investigation by Pillinger *et al.* (1994), the inhibitory effects of decomposed barley straw on the growth of the green alga, *Chlorella vulgaris* Beijerinck, and *Microcystis aeruginosa* (cynaophyte) were attributed to the presence of tannins, a plant phenolic compound. In their study a tannin extract derived from oak leaves and the authentic tannin (1,2 naphthoquinone) significantly reduced algal growth compared to the controls at concentrations of 20 $mg\ l^{-1}$ and 25 $\mu g\ l^{-1}$ respectively. The study further indicated that aeration of the barley straw as well as solution pH above 7 were necessary conditions to activate the antialgal activity of tannic acid. This suggested that oxidizable phenolic groups were the main determinants of the antialgal activity.

The application of decomposed barley straw in South African water systems has only been occasional over the past two years. In a preliminary study at the University of Cape Town (unpublished data) the growth rates of *C. glomerata* filaments were similar irrespective whether they were exposed to autoclaved and unautoclaved media of either decomposed barley straw water extract (15 g of barley straw per 20 litres of aged water, adapted from Pillinger *et al.*, 1994) or aged tap water enriched with 14 mg $N\ l^{-1}$ and 2 mg $P\ l^{-1}$ for 5 days at a light intensity of 100 μmol photons $m^{-2}\ s^{-1}$ at 25°C with 16 hours day light. However more scientific experimental work needs to be conducted in South Africa to determine which environmental conditions, *in vitro* and *in vivo*, will be required to effectively decay the barley straw and subsequently inhibit algal growth. The effects of different solution pH, aeration and different barley straw concentrations will also need to be assessed. Although Welch *et al.* (1990) claimed that few adverse environmental effects may be associated with the use of barley straw and indeed it may be perceived as enhancing invertebrate and vertebrate populations, freshwater systems would need to be monitored continuously since long-term unforeseen effects may be possible.

b. Fungal Pathogens

Bott and Rogenmuser (1980) found that a fungus, *Acremonium kiliense* Grutz, possessed pathogenic activity against *C. glomerata* without being parasitic. Cells exposed to the fungal pathogen became unhealthy and chlorotic compared to the control cells. It was observed that a water-soluble substance produced by *A. kiliense* was responsible for the cells health degeneration therefore growth inhibition. At present investigations regarding the use of potential fungal pathogens to control fouling blooms of algal growth are being conducted in South Africa. M. Morris (pers. comm.) suggested that Oomycetes (commonly referred to as water molds) may have possible pathogenic activity towards *C. glomerata.*

c. Grazers

Another possible biological control for *C. glomerata* growth may be the introduction of herbivorous animals. Although Patrick *et al.* (1983) postulated that *C. glomerata* renders a poor food source and Dodds (1991 b) indicated that the invertebrate, *Brachycentrus occidentalis*, rather grazed on the epiphyte community, rather than *on C. glomerata* itself, a few occasional reports (Gregor and Deacon, 1988) do exist where grazing has occurred on *C. glomerata.* However, the introduction of exotic animal species into water bodies may have other unforeseen detrimental ecological consequences. Furthermore, although the introduction of the fish, *Ctenophyngodon idella* (grass carp), was initially successfull in controlling algal growth in some South African water bodies, the fish density was eventually reduced as a result of the local human population fishing them out (D. Davidson, pers. comm. cited in Joska and Bolton, 1994 a).

APPENDIX 5.1

Preparation of the alkaline phosphate buffer (after Smith, 1980)

Dissolve 6.93 g of Na_2HPO_4 and 20.65 g NaOH separately in distilled water (total dissolving volume is not to exceed 1.0 L). Mix the two resulting solutions together and make up to 1.0 L. The Na_2HPO_4 will not dissolve readily so it requires more water than the NaOH. The final buffer pH exceeds 8.

REFERENCES

Abelson, P.H. and Aldous. E. 1950: Ion antagonisms in microorganisms: interference of normal magnesium metabolism by Ni, Co, Cd, Zn and Mn. *J. Bacteriol.* 60:401-413.

Adams, M.S. and Stone, W. 1973: Field studies on photosynthesis of Cladophora glomerata (Chlorophyta) in Green Bay, Lake Michigan. *Ecology* 54(4):853-862.

Auer, M.T. and Canale, R.P. 1982 a: Ecological studies and mathematical modelling of *Cladophora* in Lake Huron: 2. Phosphorus uptake kinetics. *J. Great Lakes Res.* 8(1):84-92.

Auer, M.T. and Canale, R.P. 1982 b: Ecological studies and mathematical modelling of *Cladophora* in Lake Huron. 3. The dependence of growth rates on internal phosphorus pool size. *J. Great Lakes Res.* 8(1):93-99.

Bahls, L.L. and Weber, E.E. 1988: Ecology and distribution in Montana of *Epithemia sorex* Kütz., a common nitrogen-fixing diatom. *Proc. Mont. Acad. Sci.* 48:15-20.

Bakker, F.T., Olsen, J.L. and Stam, W.T. 1995: Evolution of nuclear rDNA ITS sequences in the *Cladophora albida/sericea* clade (Chlorophyta). *J. Mol. Evol.* 40:640-651.

Barrett, P.R.F. and Newman, J.R. 1993: Paper 41: The control of algae with barley straw. *PIRA Conference Proceedings: Straw-A valuable material.*

Bellis, V.J. 1968 a: Unialgal cultures of *Cladophora glomerata* (L) Kütz. II. Response to calcium-magnesium ratio and pH of the medium. *Proc. 11th Conf. Great Lakes Res.* 11-15.

Bellis, V.J. 1968 b: Unialgal cultures of *Cladophora glomerata* (L.) Kütz. 1. Response to temperature. *J. Phycol.* 4:19-23.

Bellis, V.J. and McLarty, D.A. 1967: Ecology of *Cladophora glomerata* (L) Kütz. in Southern Ontario. *J. Phycol.* 3:57-63.

Bergey, E.A. 1995: Local effects of a sedentary grazer on stream algae. *Freshwater Biology* 33:401-409.

Bergey, E.A., Boettiger, C.A. and Resh, V.H. 1995: Effects of water velocity on the architecture and epiphytes of *Cladophora glomerata* (Chlorophyta). *J. Phycol.* 31:264-271.

Betzer, N. and Kott, Y. 1969. Effects of halogens on algae II. *Cladophora sp. Water Research* 3:257-264.

Blinn, D.W., Shannon, J.P., Stevens, L.E. and Carder, J.P. 1995: Consequences of fluctuating discharge for lotic communities. *J. N. Am. Benthol. Soc.* 14(2):233-248.

Blum, J.L. 1957: An ecological study of the algae of the Saline River, Michigan. *Hydrobiologia* 9:361-408.

Bolus, P.M. and Lund, J.W.G. 1974: Some factors affecting the growth of *Cladophora glomerata* in the Kentish Stour. *Water Treatment and Examination*, 23:25-51.

Bott, T.L. and Rogenmuser, K. 1980: Fungal pathogen of *Cladophora glomerata* (Chlorophyta). *Applied and Environmental Microbiology*, 40 (5):977-980.

Bruwer, C. 1991: *Chemical control of benthic algae in Hartebeespoort irrigation canals.* (Brochure) Department of Water Affairs, Private Bag X313, Pretoria.

Cambra, J. and Abaol, M. 1992: Filamentous green algae of Spain: Distribution and ecology. *Limnetica* 8:213-220.

Cedeno-Maldonado, A. and Swader, J.A. 1972: The Cupric Ion as an Inhibitor of Photosynthetic Electron Transport in Isolated Chloroplasts. *Plant Physiol.* 50:698-701.

Chudyba, H. 1965: *Cladophora glomerata* and accompanying algae in the Skawa river. *Acta Hydrobiol.* 7 (1):93-126.

Davies, A.G. 1976: An assessment of the basis of mercury tolerance in *Danaliella tertiolecta*. *J. Marine Biol. Ass. UK.* 56:39.57.

Davies, B.R., O'Keeffe, J.H. and Snaddon, C.D. 1995: River and Stream Ecosystems in Southern Africa: Predictably Unpredictable. (Chap. 18). In: Cushing, C.E., Cummins, K.W. and Mishall, G.W. (eds). *River and Stream Ecosystems. Ecosystems of the World.* Elsevier, New York. pp 537-599.

Dawson, F.H. and Kern-Hansen, U. 1979: The effects of natural and artificial shade on the macrophytes of lowland streams and the use of shade as a management technique. *Int. Revue ges. Hydrobiol.* 64(4):437-455.

Dedonder, A. and Van Sumere, C.F. 1971. cited in Newman, J.R. and Barrett, P.R.F. 1993: Control of *Microcystis aeroginosa* by Decomposing Barley Straw. *J. Aquat. Plant Manage.* 31:203-206.

Dell'Uoma, A. 1995: Assessment of water quality of an Apennine river as a pilot study for diatom-based monitoring of Italian watercourses. In: Whitton, B.A. and Rott, E. (eds). Use of Algae for Monitoring Rivers II. *Proceedings of an International Symposium.* Innsbruck, Austria. Rott, Austria. pp 65-72.

Demal, L. and Fortin, M. 1987: Shading with bank side trees to control *Cladophora. Revue canadienne des ressources hydrinques* 12(4):43-51.

Dodds, W.K. 1991 a: Factors associated with dominance of the filamentous green alga *Cladophora glomerata. Wat. Res.* 25(11):1325-1332.

Dodds, W.K. 1991 b: Community interactions between the filamentous alga *Cladophora glomerata* (L) Kuetzing, its epiphytes, and epiphyte grazers. *Oecologia* 85:572-580.

Dodds, W.K. 1991 c: Micro-environmental characteristics of filamentous algal communities in flowing freshwaters. *Freshwater Biology* 25:199-209.

Dodds, W.K. and Gudder, D.A. 1992: The ecology of *Cladophora. J. of Phycol.* 28:415-427.

Du Plessis, B.J. 1992 a: *'n Ligginspesifieke handleiding vir die chemiese dosering van probleemalge in besproeiingkanaal-stelsels: Hartebeespoort-Staatswaterskema.* Report No. N A210/09/DIQ/0192. Department of Water Affairs and Forestry, Pretoria. 35 pp.

Du Plessis, B.J. 1992 b: *'n Liggingspesifieke handleiding vir die chemiese dosering van prbleemalge in besproeiings-kanaalstelsels: Oranje-Rietrivier-Staatswaterskema.* Report No. N C510/00/DIQ/0592. Department of Water Affairs and Forestry. Pretoria. 43 pp.

Ebbing, D.D. and Wrighton, M.S. 1990: *General Chemistry* (3rd ed). Houghton Mifflin Co. Boston. pp937.

Eekhout, S., Brown, C.A. and King, J.M. 1996: *National Biomonitoring Programme for Riverine Ecosystems. Technical considerations and protocol for the selection of reference and monitoring sites.* NBP Report Series No 3. Institute for Water Quality Studies Department of Water Affairs and Forestry. Pretoria, South Africa. 68pp.

Eipper, A.W. 1959: Effects of five herbicides on farm ponds plants and fish in New York. *Fish and Game J.* 6:45-56.

Fitzgerald, G.P. 1968: Detection of limiting or surplus nitrogen in algae and aquatic weeds. *J. Phycol.* 4:121-126.

Fitzgerald, G.P. 1969: Some Factors In The Competition Or Antagonism Among Bacteria, Algae, And Aquatic Weeds. *J.Phycol.* 5:351-359.

Florence, T.M, Lumsden, B.G. and Fardy, J.J. 1983: Evaluation of some physico-chemical techniques for the determination of the fraction of dissolved copper toxic to the marine diatom *Nitzschia closterium. Analytica Chim. Acta.* 151:281-295.

Foster, P.L. 1977: Copper exclusion as a mechanism of heavy metal tolerance in a green alga. *Nature* 269:322-323.

Freeman, M.C. 1986: The role of nitrogen and phosphorus in the development of *Cladophora glomerata* (L.) Kutzing in the Manawatu River, New Zealand. *Hydrobiologia* 131:23-30.

Gadd, G.M. and Griffiths, A.J. 1978. Microorganisms and Heavy Metal Toxicity. *Microbial Ecology* 4:303-317.

Gerloff, G.C. and Fitzgerald, G.P. 1976: The nutrition of Great Lakes *Cladophora. U.S. Environmental Protection Agency, Office of Research and Development, Duluth, Minnesota. Report No. EPA-600/3-76-044.*

Gibson, M.T., Welch, I.M., Barrett, P.R.F. and Ridge, I. 1990: Barley straw as an inhibitor of algal growth II. laboratory studies. *Journal of Applied Phycology*. 2: 241-248.

Graham, L.E. 1982: Cytology, ultrastructure, taxonomy, and phylogenetic relationships of Great Lakes filamentous algae. *J. Great Lakes Res.* 8(1):3-9

Graham, J.M., Auer, M.T., Canale, R.P. and Hoffman, J.P. 1982: Ecological studies and mathematical modelling of *Cladophora* in Lake Huron: Part 4. Photosynthesis and respiration as a function of light and temperature. *J. of Great Lakes Res.* 8:100-111.

Greger, P.D. and Deacon, J.E. 1988: Food Partitioning among fishes of the Virgin River. *Copeia* 1988(2):314-323.

Grobler, D.C. and Silbauer, M.J. 1984: Impact of eutrophication control measures on the trophic status of South African impoundments. *Water Research Commission Report, Pretoria, ISBN 0908356277.* 146pp.

Gross, R.E., Pugno, P. and Dugger, W.M. 1970: Observations on the mechanisms of copper damage in *Chlorella. Plant Physiol.* 46:183-185.

Haberman, H.M. 1969: Reversal of copper inhibition in chloroplast reactions by manganese. *Plant Physiol.* 44:331-336.

Hawkes, H.A. 1964: Effects of domestic and industrial discharges on the ecology of riffles in Midland streams. In: *International Conference on Water Pollution Research*, London, Sept. 1962, pp293-317. Pergamon Press, Oxford.

Herbst, R.P. 1969: Ecological Factors and the Distribution of *Cladophora glomerata* in the Great Lakes. *The American Midland Naturalist* 82(1):91-98.

Hillebrand, H and De Vries, P.J.R. 1986: In: *Algal Biofouling*. Evans. L.V and Hoagland, K.D. (eds) Chap. 12. Elsevier, Amsterdam.

Hoffman, G. 1995: Recent developments in the use of benthic diatoms for monitoring eutrophication and organic pollution in Germany and Austria. In: Whitton, B.A. and Rott, E. (eds). Use of Algae for Monitoring Rivers II. *Proceedings of an International Symposium*. Innsbruck, Austria. Rott, Austria. pp 73-79.

Hoffman, J.P. and Graham, L.E. 1984: Effects of selected physiochemical factors on growth and zoosporogenesis of *Cladophora glomerata* (Chlorophyta). *J. Phycol.* 20:1-7.

Issa, A.A, Abdel-Basset, R. and Adams, M.S. 1995: Abolition of Heavy Metal Toxicity on *Kirchneriella lunaris* (Chlorophyta) by Calcium. *Annals of Botany* 75:189-192.

Jackson, M.B. and Hamdy, Y.S. 1982: Projected *Cladophora* Growth In Southern Georgian Bay In Response To Proposed Municipal Sewage Treatment Plant Discharges To The Mary Ward Shoals. *J. Great Lakes Res.* 8(1):153-163.

Jassby, A.D. and Platt, T. 1976: Mathematical formulation of the relationship between photosynthesis and light for phytoplankton. *Limnol. Oceanogr.* 21:540-547.

Joska, M.A.P. and Bolton, J.J. 1994 a: *Final report for the Water Research Commission Project: A preliminary investigation of algal weeds in inland waters 1992-1993*. Doc. 4/94. 63pp.

Joska, M.A.P. and Bolton,J.J. 1994 b: *Guide to Common Filamentous Freshwater Macroalgae in South Africa*. Apendix 1 to the Final Report for the Water Research Commission Project No. K5/426. pp 1-36.

Joska, M.A. and Bolton, J.J. 1996: Filamentous freshwater macroalgae in South Africa - a literature review and perspective on the development and control of weed problems. *Hydrobiologia* 340:295-300.

Kelly, M.G., Thyssen, N and Moesland, B. 1983: Light and the annual variation of oxygen- and carbon-based measurements of productivity in a macrophyte-dominated river. *Limnol. Oceanogr.* 28(3):503-515.

Kelly, M.G., Whitton, B.A. and Lewis, A. 1995: Use of diatoms to monitor eutrophication in United Kingdom rivers. In: Whitton, B.A. and Rott, E. (eds). Use of Algae for Monitoring Rivers II. *Proceedings of an International Symposium*. Innsbruck, Austria. Rott, Austria. pp 79-87.

Khanum, A. 1982: An ecological study on freshwater algal mats. *Bot. Bull. Academia Sinica* 23:89-104.

Kindle, E.M. 1934: Concerning "Lake balls", "*Cladophora* balls" and "Coal balls." *American Midland Naturalist* 15:752-760.

Kützing, F.T. 1843: *Phycologia generalis*. Kostens des Verfassers. Leipzig.

Leland, H.V and Carter, J.L. 1984: Effects of copper on species composition of periphyton in a Sierra Nevada, California stream. *Freshwater Biology* 14:281-292.

Lembi, C.A. and O'Neal S.W. 1988: Algae as weeds:economic impact, ecology, and management alternatives. (Chap 18) In: *Algae and Human Affairs*. Lembi, C.A. and Waaland, J.R. (eds). Cambridge University Press, pp 455-481.

Lester, W.W., Adams, M.S. and Farmer, A.M. 1988: Effects of light and temperature on photosynthesis of the nuisance alga *Cladophora glomerata* (L.) Kütz from Green Bay, Lake Michigan. *New Phytol.* 109:53-58.

Leukart, P. and Hanelt, D. 1995: Light requirements for photosynthesis and growth in several macroalgae from a small soft-water stream in the Spessart Mountains, Germany. *Phycologia* 34(6):528-532.

Lin, C. and Benjamin, M. 1992: The effects of Strongly Complexing Ligands on the Adsorptive Partitioning of Metal ions. *Water Research* 26(4):397-407.

Littler, M.M. 1979: The Effects of Bottle Volume, Thallus Weight, Oxygen Saturation Levels, and Water Movement on Apparent Photosynthetic Rates in Marine Algae. *Aquatic Botany* 7:21-34.

Lohman, K. and Priscu, J.C. 1992: Physiological indicators of nutrient deficiency in *Cladophora* (Chlorophyta) in the Clark Fork of the Columbia River, Montana. *J. Phycol.* 28:443-448.

Lorenz, R.C. and Herdendorf, C.E. 1982: Growth dynamics of *Cladophora glomerata* in western Lake Erie in relation to some environmental factors. *J. Great Lakes Res.* 8(1):42-53.

Lorenz, R.C., Monaco, M.E. and Herdendorf, C.E. 1991: Minimum light requirements for substrate colonization by *Cladophora glomerata. J. Great Lakes Res.* 17(4):536-542.

Lowe, R.L., Rosen, B.H and Kingston, J.C. 1982: A comparison of epiphytes on *Bangia atropurpurea* (Rhodophyta) and *Cladophora glomerata* (Chlorophyta) from Northern Lake Michigan. *J. Great Lakes Res.* 8(1):164-168.

Luderitz, V., Nicklisch, A. and Roloff, B. 1989: Copper toxicity for blue-green algae with reference to their physiological status. *Arch. Hydrobiol. Suppl.* 82(4):449-459.

Luttenton, M.R. and Rada, R.G. 1986: Effects of disturbance on epiphytic community architecture. *J.Phycol.* 22:320-326.

MacDowall, F.D. 1949: The effect of some inhibitors of photosynthesis upon the chemical reduction of a dye by isolated chloroplasts. *Plant Physiol.* 24:464-480.

Macfie, S.M., Tarmohamed, Y. and Welbourn, P.M. 1994: Effects of Cadium, Cobalt, Copper and Nickel on Growth of the Green Alga, *Chlamydomonas reinhardtii*: The Influences of the Cell Wall and pH. *Arch. Environ. Contam. Toxicol.* 27:454-458.

MacLeod, R.A., Kuo, S.C. and Gelinas, R. 1967: Metabolic injury to bacteria. II. Metabolic injury induced by distilled water or copper in the plating diluent. *J. Bacteriol.* 93:961-969.

Mantai, K.E., Garwood, P.E. and Peglowski, L.E. 1982: Environmental factors controlling physiological changes in *Cladophora* in Lake Erie. *J. Great Lakes Res.* 8(1):61-65.

Marks, J.C and Cummings, M.P. 1996: DNA sequence variations in the ribosomal internal transcribed spacer region of freshwater *Cladophora* species (Chlorophyta). *J. Phycol.* 32:1035-1042.

Matthews, R.A., Buikema, A.L. Jr., Cairns, J. Jr. and Rodgers, J.H. Jr. 1982: Biological monitoring. Part II A. Receiving system functional methods, relationships and indices. *Water Research* 16:129-139.

McDonald, K.L. and Pickett-Heaps, J. D. 1976: Ultrastructure and differentiation in *Cladophora glomerata*. 1. Cell division. *Amer. J. Bot.* 6395):592-601.

McHardy, B. and George, J. 1985: The uptake of Selected Heavy Metals by the Green Alga, *Cladophora glomerata. Symposia Biologica Hungarica* 29.

McHardy, B and George, J. 1990: Bioaccumulation and Toxicity of zinc in the Green Alga, *Cladophora glomerata. Environmental Pollution* 66: 55-66.

McKnight, D., Chisholm, S. and Harleman, D. 1983: $CuSO_4$ Treatment of Nuisance Algal Blooms in Drinking Water Reservoirs. *Environmental Management* 7(4):311-320.

McLarty. D.A. 1961: Observations on the nature and control of excessive growth of *Cladophora sp.* in Lake Ontario and Lake Erie. *Research Report R621 of the Ontario Water Resources Commission 36pp.*

McNaught, M.E 1964: cited in Hoffman, J.P. and Graham, L.E. 1984: Effects of selected physiochemical factors on growth and zoosporogenesis of *Cladophora glomerata* (Chlorophyta). *J. Phycol.* 20:1-7.

Merezhko, A.I., Kharchenko, T.A., Pasichnyy, A.P., Velichko, I.M., Piontkovskiy, A.Z. and Sasonov, V.V. 1992: Elimination of nuisance algae from irrigation systems. *Hydrobiological Journal.* 28(1):89-95.

Miller, J.g. and MacKay, W.C. 1980: The Effects of Hardness, Alkalinity and pH of Test Water on the Toxicity of Copper to Rainbow Trout (*Salmo Gairdner*). *Water Research* 14:129-133.

Moore, L.F. and Traquair, J.A. 1976: Silicon, a required nutrient for *Cladophora glomerata* (L) Kütz. (Chlorophyta). *Planta (Berl)* 128-179-182.

Neel, J.K. 1968: Seasonal succession of benthic algae and their macro-invertebrate residents in a headwater limestone stream. *J. Wat. Pollut. Control Fed.* 40(2), R10-R30.

Neil, J.H. and Jackson, M.B. 1982: Monitoring *Cladophora* growth conditions and the effect of phosphorus additions at a shoreline site in Northeastern Lake Erie. *J. Great Lakes Res.* 8(1):30:34.

Newman, J.R. and Barrett, P.R.F. 1993: Control of *Microcystis aeroginosa* by Decomposing Barley Straw. *J. Aquat. Plant Manage.* 31:203-206.

Nichols, H.W. and Bold, H.C. 1965: *Trichosarcina polymorpha* gen. et sp. nov. J. Phycol. 1:34-38.

Oertel, N. 1991: Heavy-metal Accumulation in *Cladophora glomerata (L) Kutz* in the River Danube. *Ambio* 20(6):264-268.

Ohle, W. 1953: cited in Herbst, R.P. 1969: Ecological Factors and the Distribution of *Cladophora glomerata* in the Great Lakes. *The American Midland Naturalist* 82(1):91-98.

O'Neal, S.W. and Lembi, C.A. 1995: Temperature and irradiance effects on growth of *Pithophora oedogonia* (Chlorophyceae) and *Spirogyra* sp. (Charophyceae). *J. Phycol.* 31:720-726.

Palmer, C.M. 1980: *Algae and Water Pollution.* Castle House Publication Ltd. England.

Parodi, E.R. and Cáceres, E.J. 1991: Variation in number of apical ramifications and vegetative cell length in freshwater populations of *Cladophora* (Ulvophyceae, Chlorophyta). *J. Phycol.* 27:628-633.

Patrick, R., Rhyne, C.F., Richardson, R.W.(III), Larson, R.A., Bott, T.T. and Rogenmuser, K. 1983: The potential for biological controls of *Cladophora glomerata. EPA 600/3-83-065.*

Pillenger, J.M., Cooper, J.A. and Ridge, I. 1994: Role of phenolic compounds in the antialgal activity of barley straw. Journal of Chemical Ecology, 20(7):1557-1569.

Pipp, E. and Rott, E. 1995: Recent developments in the use of benthic algae (excluding diatoms) for monitoring rivers in Austria and Germany. In: Whitton, B.A. and Rott, E. (eds). Use of Algae for Monitoring Rivers II. *Proceedings of an International Symposium.* Innsbruck, Austria. Rott, Austria. pp 160-165.

Pitcairn, C.E.R. and Hawkes, H.A. 1973: The role of phosphorus in the growth of *Cladophora. Water Res.* 7:159-171.

Planas, D., Maberly, S.C. and Parker, J.E. 1996: Phosphorus and nitrogen relationships of *Cladophora glomerata* in two lake basins of different trophic status. *Freshwater Biology* 35:609-622.

Prygiel, J. and Coste, M. 1995: Recent trends in monitoring French rivers using algae, especially diatoms. In: Whitton, B.A. and Rott, E. (eds). Use of Algae for Monitoring Rivers II. *Proceedings of an International Symposium*. Innsbruck, Austria. Rott, Austria. pp 87-97.

Rai, L.C. and Raizada, M. 1985: Effect of nickel and silver ions on survival, growth, carbon fixation and nitrogenase activity in *Nostoc muscorum* : regulation of toxicity by EDTA and Calcium. *J. of General and Applied Microbiology* 31:329-337.

Raven, J.A. 1992: How benthic macroalgae cope with flowing freshwater resource acquisition and retention. *J. Phycol.* 28:133-146.

Raven, J.A. and Geider, R.J. 1988: Temperature and algal growth. *New. Phycol.* 110:441-461.

Rawhani, S. 1991: To irrigate or not to irrigate: the South African dilemma. In: *Extended abstracts of the South African Irrigation Symposium*, Durban 1991:19-20.

Reuter, J.G., Chisholm, S.W. and Morel, F.M. 1981: The Effect of copper toxicity on silicic acid uptake and growth in *Thalassiosira pseudonana* (Bacillariophyceae). *J.Phycol.* 17:270-278.

Robinson, P.K. and Hawkes, H.A. 1986: Studies on the growth of Cladophora glomerata in laboratory continuous-flow culture. *Br. Phycol. J.* 21:437-444.

Rueness, J and Tananger, T. 1984: Growth in culture of four red algae from Norway with potential for mariculture. *Hydrobiologia* 116/117:303-307.

Sabater, S., Guasch, H., Picon, A., Romani, A.M. and Munoz, I. 1995: Using diatom communities to monitor water quality in a river after the implementation of a sanitation plan (River Ter, Spain). In: Whitton, B.A. and Rott, E. (eds). Use of Algae for Monitoring Rivers II. *Proceedings of an International Symposium*. Innsbruck, Austria. Rott, Austria. pp 97-106.

Sand-Jensen, K., Jeppesen, E., Nielsen, K., Van der Bijl, L., Hjermind, L., Nielsen, L.W. and Iversen, T.M. 1989: Growth of macrophytes and ecosystem consequences in a lowland Danish stream. *Freshwater Biology* 22:15-32.

Sawyer, C.N. 1947: Fertilization of lakes by agricultural and urban drainage. *J. New Eng. Water Wks Ass.* 1:109

Schlesinger, D.A. and Shuter, B.J. 1981: Patterns of growth and cell composition of freshwater algae in light-limited continuous cultures. *J. Phycol.* 17:250-256.

Siegrief, S.C. 1984: *A Catalogue of South African Green, Brown and Red Marine Algae*. Memoirs of the Botanical Survey of South Africa No. 47. pp 16-17.

Simpson, P.S and Eaton, J.W. 1986: Comparative studies Of the photosynthesis of the submerged macrophyte *Elodea canadensis* and the filamentous algae *Cladophora glomerata* and *Spirogyra* sp. *Aquatic Botany* 24:1-12.

Sheath, R.G. and Cole, K.M. 1992: Biogeography of Stream Macroalgae in North America. *J. Phycol.* 28:448-460

Shumsudin, L. and Sleigh, M.A. 1995: Seasonal changes in composition and biomass of epiphytic algae on the macrophyte *Ranunculus penicillatus* in a chalk stream, with estimates of production, and observations on the epiphytes of *Cladophora glomerata*.

Hydrobiologia 306:85-95.

Shyham, R. 1980: On the life-cycle, cytology and taxonomy of *Cladophora callicoma* from India. *Amer. J. Bot.* 67(5):619-624.

Sinha, J.P. and Noor, M.N. 1967: Chromosome numbers in some members of Chlorophyceae of Chotanagia (India). *Phykos* 6:106-110.

Smith, V.R. 1980: A phenol-hypochlorite determination of ammonium-nitrogen in Kjeldahl digests of plant tissue. *Comm. Soil Sci. Pl. Anal.* 11:67-94.

Starr, R.C. 1964: The culture collection of algae at Indiana University. *Amer. J. Bot.* 51:1013-1044.

Stauber, J.L. and Florence, T.M. 1985: The influence of iron on copper toxicity to the marine diatom, *Nitzschia closterium* (Ehrenberg.) W. Smith. *Aquat. Toxic.* 6:297-305

Stauber, J.L. and Florence, T.M. 1987: Mechanism of toxicity of ionic copper and copper complexes to algae. *Marine Biology* 94:511-519.

Steemann Nielsen, E., Kamp-Nielsen, L. and Wium Andersen, S. 1969: The Effect of Deleterious Concentrations of Copper on the Photosynthesis of *Chlorella pyrenoidosa. Physiologia Plantarum* 22:1121-1133.

Steeman Nielsen, E. and Wium-Andersen, S. 1970: Copper ions as poison in the sea and in freshwater. *Marine Biology* 6:93-97.

Stein, J.R. 1973: *Handbook of Phycological Methods. Culture Methods And Growth Measurements.* Cambridge University Press. London. pp 16-17.

Steinman, A.D. and McIntire, C.D. 1986: Effects of current velocity and light energy on the

structure of periphyton assemblages in laboratory streams. *J. Phycol.* 22:352-361.

Stiff, M.J. 1971: The Chemical States of Copper in Polluted Fresh Water and a Scheme of Analysis to Differentiate Them. *Water Research* 5:585-599.

Stokes, P.M. 1983: Responses of freshwater algae to metals. (chap 3). In: Round, D.J and Chapman, F.E. (eds). *Progress in Phycological Research.* Elsevier Science Publishers, B.V. pp 88-112.

Stokes, P.M and Hutchinson, T.C;1975: Copper Toxicity to Phytoplankton as affected by organic liquids, other cations and inherent tolerance of algae to copper. In: *Toxicity to Biota of Metal Forms in Natural Waters.* Andrew, R.W., Hodson, P.V. and Kanasewich, D.E. (eds), pp 159-185. Proc. Workshop Duluth Minnesota, Int. Joint. Commission.

Storr, J.F. and Sweeney, R.A. 1971: Development of a theoretical seasonal growth response curve of *Cladophora* glomerata to temperature and photoperiod. Proc. 14th Conf. Great Lakes Res. 119-127.

Thomas, 1962 (german), cited in Whitton, B.A 1970 a: Review Paper. Biology of *Cladophora* in Freshwaters. *Water Research* 4:457-476.

Thomas, M.L. 1988: Photosynthesis and Respiration of aquatic macro-flora using the light and dark bottle oxygen method and dissolved oxygen analyser. Chapter 7 In: Lobban, C.S., Chapman, D.J. and Kremer, B.P. (eds): *Experimental Phycology.* Cambridge University Press, Cambridge. PP 64-77.

Thurman, M.H. and Kuehne, R.A. 1952: An ecological study of *Cladophora glomerata* (Chlorophyceae) near Dallas. Field Lab. 20:26-28.

Toerien, D.F. 1975: A review of eutrophication and guidelines for its control in South Africa. *C.S.I.R. Special Report. Wat. 48.* Pretoria. 110pp.

Toerien, D.F., Hayman, C.L. and Bruwer, M.J. 1975: A preliminary trophic status classification of some South African impoundments. *Water S.A.* 1:15-23.

Trollope, D.R. and Evans, B. 1976: Concentrations of copper, iron, lead, nickel and zinc in freshwater algal blooms. *Environ. Pollution* 11:109-116.

Tubbing, D.M.S., Cleven, R.F., Iqbal, M., Van Meent, D. and Verweij, W. 1994: The Contribution of Complexed Copper to the Metabolic inhibition of Algae and Bacteria in Synthetic Media and River water. *Water Research* 28(1):37-44.

Tubbing, D.M.J., Santhagens, L.R., Admiraal, W. and van Beelen, P. 1993: Biological and Chemical aspects of differences in sensitivity of natural populations of aquatic bacterial communities exposed to copper. *Envir. Toxicol. Water Qual.* 8:191-205.

Usher, H.D. and Blinn, D.W. 1990: Influence of various exposure periods on the biomass and chlorophyll A of *Cladophora glomerata* (Chlorophyta). *J. Phycol.* 26:244-249.

van der Merwe, W. and Grobler, D.C. 1990: Water quality management in the R.S.A: Preparing for the future. *Water S.A.* 16 (1) 49-53.

Van den Hoek, C. 1963: *Revision of the European species of Cladophora.* E.J. Brill Co., Leiden. 248pp.

Van den Hoek, C., Mann, D.G. and Jahns, H.M. 1995: *Algae: An introduction to phycology.* Press Syndicate of the University of Cambridge. Cambridge. Chap 23, pp 408-418.

Vymazal, J. 1984: Short-term uptake of heavy metals by periphyton algae. *Hydrobiologia* 119:171-179.

Waern, M. 1952: Rocky-shore algae in the Oregrund Archipelago. *Acta Phytogeogr. Suec.* 30:1-298.

Walker, J.D. and Colwell, R.R. 1974: Mercury-Resistant Bacteria and Petroleum Degradation. *Applied Microbiology* 27(1):285-287.

Welch, I.M., Barrett, P.R.F., Gibson, M.T and Ridge, I. 1990: Barley straw as an inhibitor of algal growth 1.studies in the Chesterfield Canal. *Journal of Applied Phycology*. 2:231-239.

Welch, E.B., Horner, R.R. and Patmont, L.R. 1989: Prediction of nuisance periphytic biomass: a management approach. *Wat. Res.* 4:401-405.

Werner, D. 1967. cited in Moore, L.F. and Traquair, J.A. 1976: Silicon, a Required Nutrient for *Cladophora glomerata* (L) Kütz. (Chlorophyta). *Planta (Berl)* 128-179-182.

Wharfe, J.R., Taylor, K.S. and Montgomery, H.A.C. 1984: The growth of *Cladophora glomerata* in a river receiving sewage effluent. *Water Res.* 18(8):971-979.

Whitton, B.A. 1967: Studies on the growth of riverian *Cladophora* in culture. *Archiv für Mikrobiologie*. 58:21-29.

Whitton, B.A. 1970 a: Review paper. Biology of *Cladophora* in freshwaters. *Water Research* 4:457-476.

Whitton, B.A. 1970 b: Toxicity of Zinc, Copper and Lead to Chlorophyta from Flowing Waters. *Arch. Mikrobiol.* 72:353-360.

Whitton, B.A. 1970 c. cited in Whitton, B.A. 1970 a: Review paper. Biology of *Cladophora* in freshwaters. *Water Research* 4:457-476.

Winner, R.W. 1986: Interactive effects of water hardness and humic acid on the chronic toxicity of Cadmium to *Daphnia pulex. Aquatic Toxicol.* 8:281-293

Wong, S.L. and Clark, B. 1976: Field determination of the critical nutrient concentration for *Cladophora* in streams. *J. Fish. Res. Board Can.* 33:85-92.

Wong, S.L., Clark, B., Kirby, M. and Kosciuw, R.F. 1978: Water temperature fluctuations and seasonal periodicity of *Cladophora* and *Potamageton* in shallow rivers. *J. Fish. Res. Bd. Cananda* 35:866-870.

Wong, S.L., Clark, B. and Kosciuw, R.F. 1979: An examination of the effects of nutrients on the water quality of shallow rivers. *Hydrobiologia* 63:231-239.

Wong, S.L. and Wainwright, 1994: Interpretation of Ultrastructural Changes in *Cladophora glomerata* Resulting from Hyamine Toxicity. *Bull. Environ. Contam. Toxicol.* 52:325-332.

Zimmerman, L. 1966: Toxicity of copper and ascorbic acid to *Serratia marcescens. J. Bactriol.* 1537-1542.