ROLE OF TRACE ELEMENTS ON THE GROWTH AND PHYSIOLOGY OF SELECTED MICROALGAE

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CERTIFICATE

This is to certify that the thesis entitled **"ROLE OF TRACE ELEMENTS ON THE GROWTH AND PHYSIOLOGY OF SELECTED MICROALGAE"** is the bonafide record of the work carried out by *Mrs. S. Srisudha* under my guidance and supervision in the CMFRI and that no part thereof has been presented for any other Degree.

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DECLARATION

I hereby declare that this thesis entitled **"ROLE OF TRACE ELEMENTS ON THE GROWTH AND PHYSIOLOGY OF SELECTED MICROALGAE"** has not previously formed the basis of the award of any degree, diploma, associateship, fellowship or other similar titles or recognition.

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PREFACE

In 1912, Gabriel Bertrand, regarded as the father of trace element research promulgated a theory, now known as the Bertrand's law "A plant cannot live with a deficiency while an excess is toxic".

The past three decades have seen a remarkable advance in the application and appreciation of the significant role played by trace metals in the health and productivity of plants. The effort has been spread widely, from the ecological point of view at one extreme, down to the molecular view point and encouragingly some highly successful attempts have been made to deal with the intimate details of the actions of metal ions both essential and toxic.

Pioneer work on the nutritional significance of trace elements began in about 1928 and flourished throughout the 30's and later years. The recent spectacular rise in interest in trace elements in marine products has followed after a few catastrophic episodes such as the Minamata and Nigata Bay incidents, occurrence of itai-itai disease caused respectively by mercury and cadmium poisoning. Consequently there has been a concerted effort by marine scientists to identify, quantify and study the impact of these pollutants on the marine ecosystem.

In view of the great attention drawn by biologists, nutritionists and technologists in utilisation of algae as one of the possibilities

to bridge the inadequacy and scarcity of proteins and their use as live food in aquacultural practices, the present investigation of the influence of the three trace metals copper, manganese and zinc in enhancing the growth and physiological activities of *Isochrysis galbana* Parke and *Synechocystis salina* Wislouch has been undertaken. These studies are also of significance in setting criteria and standards for water quality management by suggesting threshold values for the three metals, Cu, Mn and Zn, beyond which they become toxic affecting the ecosystem.

The research work for the thesis was started after the completion of six months course programme in Mariculture. During the research tenure, the candidate got familiarised with various analytical techniques connected with the fields of marine biology and oceanography. For certain specialised techniques, the candidate made use of the facilities available at Water Steam Chemistry Laboratory, IGCAR, Kalpakkam for conducting metal speciation studies using pulse polarographic analyser. In connection with transmission electron microscopic investigations, the candidate was helped by Central Plantation Crops Research Institute, Kayamkulam for understanding the impact of metals at the intracellular level. Most of the other facilities were developed by the candidate on her own initiation with the guidance of scientists, technical staff and fellow scholars of CMFRI.

It is hoped that the results and conclusions drawn from these investigations will be useful in the development of mass cultures of algae as live food in hatchery systems as well as in the pollution control of estuarine and nearshore environments.

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CHAPTER I

1. INTRODUCTION

Microalgae acquire nutrients from their environment in order to sustain their growth and division. The classification of nutrients is made on the basis of their quantitative requirements into two groups: (a) Macronutrients (e.g. Nitrate, Phosphate, Silicate). (b) Micronutrients or trace or oligoelements (e.g. Cu, Co, Mn, Mo, Zn).

The term 'trace element' is rather loosely used in the current literature to designate the elements which occur in small concentrations in natural biologic systems. Thus for practical purposes, other terms such as 'trace metals', 'trace inorganics', 'heavy metals', 'microelements' and 'micronutrients' are treated as synonymous with the term trace elements.

An understanding of the mechanism of biological and chemical interactions among trace metals and planktonic organisms is the key to elucidate the role of trace metals in the ecology of the oceans and the role of the organisms in the geochemistry of metals.

Many of the trace elements are normal constituents of marine organisms and are essential for their metabolism. However, at higher concentrations these elements become toxic. The response of marine life to increasing concentrations of trace elements in seawater according to Perkins (1974) is oligodynamic i.e., stimulatory at low doses and toxic at higher levels.

Most of the trace elements are found in living organisms in low concentration. While some of these are known to have definite functional roles, others are accumulated without any apparent reason. Apart from hydrogen, oxygen and carbon, about fifteen elements namely calcium, copper, chlorine, fluorine, iodine, iron, magnesium, manganese, molybdenum, nitrogen, phosphorus, potassium, sodium, sulphur and zinc are known to have specific roles in animal and plant nutrition. Essential elements generally exist in combination with organic molecules either as metallo-proteins or as metaprotein complexes.

According to Arnon (1950), an element is considered essential for an organism when the organism can neither grow nor complete its life cycle in its absence or it cannot be replaced by any other element and has a direct influence on the metabolism of the organism.

In natural concentrations, trace elements constitute the prosthetic group of enzymes or function as enzyme activators. At elevated concentrations they act as inactivators of enzyme systems and as protein precipitants. While there are conflicting opinions on the exact mechanism of toxic action of individual elements, it is generally recognised that the toxicity of metals is related to their electronegativities.

According to the severity of toxic action, Bowen (1966) has classified metals into: (i) very toxic - effects seen at concentrations below 1 ppm (ii) moderately toxic effects - appear at concentrations between 1 and 100 ppm. (iii) scarcely toxic - effects rarely appear except in the absence of a related essential element.

Some of the other possible modes of toxic action of metals as outlined by Aberg (1948) are (i) as antimetabolites (ii) as substances forming stable precipitates or chelates or catalysing the decomposition of essential metabolites (iii) combining with cell membrane and affecting its permeability (iv) structural replacement of electrochemically important elements.

The availability of trace metals as essential micronutrients to microalgae and their toxicity to all planktonic organisms are dependent upon their chemical speciation in the water. Chemical speciation in the marine environment consists of distinguishing the chemical forms of an element in solution, colloidal and particulate phases. For eg. the availability of zinc and iron and the toxicity of zinc, copper and cadmium are controlled by their free ionic activities in aquatic systems.

Organisms play an important role in increasing trace metal solubilities by releasing complexing agents in the medium, or, on the contrary, they may enhance the incorporation of metals into particles and thus foster metal sedimentation in marine environment.

In order to properly evaluate the effects of trace metals to the aquatic ecosystem, one must have some measurement of the action of these substances to the important components of this ecosystem. Research with simple unicellular algal plankters is not only of the direct interest to phycologists, but it also contributes in a broader sense to our knowledge of the importance of trace elements in nutrition and as

bioindicators of pollution. The transfer of these substances to other environmental compartments and their potential in removing toxic substances from the ecosystem have gained great concern and recognition.

Each of the trace metals either singly or in combination along with the major seasonality of environmental parameters can affect the biota as a whole and the food chain in particular.

The idea of employing algae for studying the combined effects of several metal ions is important because algae are primary producers of aquatic ecosystems and in natural waters, metal ions nearly always occur in combination and not in isolation leading to the phenomenon of synergism and antagonism.

In the aquatic ecosystem especially in the estuarine and nearshore regions the quantity of trace metal availability is dependent on several factors. Apart from the existence of these elements in natural conditions, there is considerable input from land run off, especially if there is a discharge point from man made sources. The threshold levels at which these trace metals form essential nutrients enhancing the growth can also pose problems of toxicity, when there is a subtle increase in concentration resulting in impairment of growth kinetics, physiological activity as well as intracellular changes.

The natural distribution of trace elements in seawater has been compared and discussed by Fabricand *et al.*,(1962) and Schutz and Turekian

(1965). The great deal of variability in the concentrations of metals in natural waters can be attributed to the differences in rainfall, in the amounts of suspended material, weathering of rocks and human contamination. The concentration of metals found in seawater, open oceans and rivers has been reviewed by Pytkowicz and Kester (1971), Riley and Chester (1971), Preston *et al.*,(1972) and Abdullah *et al.*,(1972).

The work within the country centred around only on the magnitude of the trace metals in dissolved and particulate form in Indian waters. It has been reported from West Coast of India by Sreekumaran *et al.*,(1968), Central West Coast of India by Sankaranarayanan and Reddy (1973), Vellar estuary by Jegatheesan and Venugoplan (1973), Venugopalan and Ramadhas (1975), Bay of Bengal by Chalapati Rao and Satyanarayana Rao (1974), Braganca and Sanzgiry (1980), Rajendran *et al.*,(1982), Goa waters by Zingde *et al.*, (1976), Cochin backwaters by Sankaranarayanan and Rosamma Stephen (1978), Rajendran and Kurian (1986) and Laccadive Sea by Sanzgiry *et al.*,(1979).

Inspite of the importance of algae in aquatic food chains, relatively little attention has been focused upon them. Several reviews by Whitton (1970), Rice *et al.*, (1973), Leland and Luoma (1977), Gadd and Griffiths (1978), Davies (1978, 1983), Leland, Luoma and Fielden (1979), Sorentino (1979) and Rai *et al.*, (1981) have been published recently. The reviews are mainly based on toxicological investigations with certain heavy metal species and they furnish meagre information about the significance of trace elements in algal nutrition. Further more, none of these reviews

places adequate emphasis on the role of environmental factors in modifying trace metal toxicity, on their synergistic and antagonistic interactions and their impact on the life cycle of algae.

The main objective of the study was primarily to determine the magnitude of selected trace elements, the concentrations of which would possibly accelerate growth resulting in larger biomass and sustained period of exponential phase for economically viable harvest.

The study on the effect of three trace elements namely Cu, Mn and Zn on two species of algae, *Isochrysis galbana* Parke and *Synechocystis salina* Wislouch under different conditions of salinity, pH and temperature involves several combinations for each metal, from which the relative set of conditions has been adduced. The scheme of the experiments was statistically designed for interpretation of data and factors were assessed and graded according to relative importance. The methodology adopted for data interpretation is analysis of variance by split-plot design method.

The thesis has been divided into five chapters. The introductory chapter explains the relevance of the research work undertaken. Chapter II gives a review on the work pertaining to the above mentioned three trace elements in relation to nutrition as well as on the toxic aspects about which there is an abundance of literature. Chapter III presents a detailed description of the material and specialised methods followed for the study. The results and conclusions of the various experiments on effect of metals on growth and other physiological activities are discussed in Chapters IV and V.

CHAPTER II

2. REVIEW OF LITERATURE

As the present experimental study deals with the role of copper, manganese and zinc in regulating growth of *I. galbana* and *S. salina*, greater stress has been laid on the above three metals in the literature review.

2.1. Copper:

Copper was first demonstrated to be an essential element for plant growth in 1931, (Sommer, 1931). Since then it has been shown to act as an important factor in several biochemical processes. In photosynthesis, it is the main constituent of plastocyanin which affects the electron transport from cytochrome to the photocatalyst P 700 in the photosystem I (Markley *et al.*, 1975; Gregory, 1977) as well as a cofactor for several enzymes.

Trace amounts of copper are essential for metabolic processes of algae (Spencer, 1957; Manahan and Smith, 1973; O'kelley, 1974 and Sorentino, 1979). Higher concentrations are toxic and for many years copper sulphate has been used as an algicide to prevent undesirable algal blooms. The biological importance of copper in the sea has been discussed by Lewis and Cane (1982). There have been occasional reports of copper limitation in natural waters but the evidence is not conclusive.

The introduction of the Controlled Ecosystem Pollution Experiment (CEPEX) programme involving the employment of natural communities of

algae and other associated organisms constitutes a major advancement in water pollution biology (Menzel and Case, 1977). Thomas and Seibert (1977) had studied the ecological characteristics and envisaged that copper pollution in the ocean can cause a profound decrease in species diversity eventually leading to the dominance by resistant forms. Hollibaugh *et al.*, (1980) made similar observations of shift in species composition when natural assemblages were exposed to toxic levels of metals. Harrison, Eppley and Renger (1977) had investigated nitrogen metabolism of planktonic algae under the influence of copper in controlled ecosystem enclosures. Whittaker *et al.*, (1978) studied the effects of copper sulphate on the suppression of *Aphanizomenon flos-aquae* blooms in shallow, eutrophic prairie lakes of South Western Manitoba. Subba Rao (1981) had accounted for the variability of trace metal distribution together with differential growth response of phytoplankton to trace metal concentrations. Wolter *et al.*, (1984) had highlighted the influence of low concentrations of copper (radioisotope Cu-64) on phytoplankton of natural water samples.

The toxicological effects of copper have been studied by various investigators (McBrien and Hassall, 1967; Kanazawa and Kanazawa, 1969; Steemann Nielsen *et al.*, 1969; Steemann Nielsen and Wium-Anderson,1970, 1971; Cedeno-Maldonado *et al.*, 1972, 1974; Parry, 1972; Button and Hostetter, 1977; Overnell, 1975, 1976; Goering *et al.*, 1977; Kuwabara, 1981; Aliotla and Pollio, 1982; Rachlin *et al.*, 1983; Schenck, 1984; Leland and Kuwabara, 1984; Bednarz and Dratnal, 1985; Kanakavalli Susarla, 1987 and Hall *et al.*, 1989a,b). Much of the existing evidence suggest that

at low activities, copper primarily reacts with the surface of a cell. As a result, membrane related activites such as cell division and permeability as indicated by the release of cellular potassium and reduced packed cell volume are often affected (Hassall, 1963; McBrien and Hassall, 1965; Steemann Nielsen *et al.*, 1969; Overnell, 1975; Riisgard, 1979 and Riisgard *et al.*, 1980). Inability of the cells to divide resulting in enlargement has been observed by Erickson *et al.*, (1970), Bentley-Mowat and Reid (1977) and Morel *et al.*(1978). The reversal of these surface effects by the action of chelators has been noticed by Erickson (1972) and Anderson and Morel (1978).

Considerable variability in sensitivity to copper was evident among certain species of marine diatoms, dinoflagellates, chlorophycean members etc. causing adverse effects on their growth, survival and development. These observations were documented by several workers (Saward *et al.*, 1975; Rosko and Rachlin, 1975, 1977; Davey, 1976; Jensen *et al.*,1976; Sharon and Bellinger, 1976; Sunda and Guillard, 1976; Pace *et al.*,1977; Thomas *et al.*, 1977, 1980; Saifullah, 1978; Gnassia-Barelli *et al.*, 1978, 1982; and Davies and Sleep, 1980). Brand *et al.*(1986) had studied the action of copper on the reduction of reproduction rates in marine phytoplankton and compared the sensitivity of different algal groups.

The dependence of copper toxicity on the basis of its ionic activity (Sunda and Guillard, 1976 and Gavis *et al.*,1981) and modification with increasing complexation by organic ligands (Zhou and Wangerskey,

1965; Sunda and Lewis, 1978; McKnight and Morel, 1979; Morel *et al.*, 1979; Fisher and Frood, 1980; Richland and Wood, 1983; Anderson *et al.*,1984 and Florence and Stauber, 1986) or by binding to sulfhydryl or other functional groups (Eichorn, 1974; Fisher and Jones, 1981) have been well correlated by the above investigators. The ability of algae to ameliorate copper toxicity by excretion of complexing materials have been demonstrated by Vanden Berg *et al.*, (1979).

Recognising the importance of toxicant resistant populations of aquatic species in plant toxicology, relatively few field studies of metal tolerance have been conducted. Populations of phytoplankton *Scenedesmus acutiformis* and *Chlorella fusca* from metal contaminated areas near Sudbury, Ontario were found to be more resistant to the toxic effects of copper than populations of these species from uncontaminated areas (Stokes *et al.*,1973a,b and Stokes and Hutchinson, 1976).

The mechanism of tolerance brought about by cellular exclusion i.e., by chelation of free metal ions in solution (Davey *et al.*,1973; Swallow *et al.*,1978; Jackson and Morgan, 1968; Fisher, 1981; Fisher and Fabris, 1982 and Hawkins and Griffiths, 1982a,b) and by intracellular detoxification has been reported by Silverberg, Stokes and Ferstenberg (1976).

Numerous intrinsic and extrinsic factors are responsible for modifying the copper accumulation rates as well as growth and survival

of algae. Thus variations in thermal regimes markedly affected these parameters in *Coccochloris elebans*, *Dunaliella tertiolecta* and *Skeletonema costatum* and a negative correlation has been obtained for the same species with decreasing salinity of the medium (Mandelli, 1969). The role of salinity in decreasing copper toxicity has also been experimentally demonstrated by Eisler and Gardner (1973).

The influence of pH on the toxicity of copper and growth rate of algae have been substantiated by the following workers (Steemann Nielsen and Kamp-Nielsen, 1970; Gachter, 1976; Hargreaves and Whitton, 1976a,b; Sunda and Guillard, 1976 and Michnowicz and Weaks, 1984). Similarly, several other factors are also known to affect the physiological and biochemical processes and on their response of algae to metals (Bryan, 1976).

2.2. Manganese:

Manganous ions are the principal dissolved species in natural waters and they exist almost exclusively as insoluble MnO_2 in aerated waters (Ahrland, 1975). Bender *et al.*, (1977) had detected a low concentration of manganese in open oceans i.e., approx. 2×10^{-9} mol l^{-1}. Stimulation of growth in natural populations by added manganese has been reported for several lakes (Goldman, 1972 and Shapiro and Glass, 1975), inshore waters of Plymouth Sound (Harvey, 1947).

The initial report of manganese requirement for algae was made by Hopkins in 1930a,b. Later on the essentiality of this metal for algal

growth was also evident from the investigations of Harvey (1947), Walker (1954), Pirson and Bergmann (1955) and Eyster *et al.*, (1956, 1958). O'Kelley (1974) had stated that the chlorotic appearance of some algal cells is due to manganese deficiency. Weissner (1962) and O'Kelley(1974) had substantiated the role of manganese in the reactions of some enzymes in the Kreb's cycle and other metabolic processes.

The most studied effect of manganese on algal metabolism is its impact on the oxygen evolving system of photosynthesis (Vernon, 1962; Cheniae and Martin, 1968; Cheniae and Martin, 1969). Teichler-Zalden (1969) have emphasised the necessity of manganese to carry out photo - synthetic reactions involving photosystem II in *Anacystis* and *Chlamydomonas*. But Homann (1967) had demonstrated photosystem I mediated phosphorylation to be more sensitive to manganese deficiency in *Scenedesmus* and *Ankistrodesmus*. Tanner *et al.*, (1960) had shown the importance of this metal in glycollate synthesis. Noro (1978) had stated that manganese is an essential element for the normal growth of *Dunaliella tertiolecta* but adverse effects were documented when concentration in the growth medium was less than 0.1 mg Mn/1. Constantopoulos (1970) highlighted that in *Euglena gracilis*, growth was strongly dependent upon manganese and the galactosyl glyceride content was lower in Mn-deficient autotrophic cells than in normal autotrophic cells.

Higher concentrations inhibit algal growth as this element in the anionic form was found to be toxic to *Microcystis* at a level of

2 mgl^{-1} (Velichko, 1968). Hayward (1969) correlated the amount of metal incorporation into the cells of *Phaeodactylum tricornutum* with higher concentrations of manganese. Rosko and Rachlin (1975) had determined the concentration of this metal which caused 50% inhibition of growth in *Nitzschia closterium* after a 96 hr. exposure. Ecotoxicological aspects of metal ions such as Mn on the photosynthesis of microplankton and nannoplankton in the Zuari estuary, Goa had been discussed by Rajendran *et al.*,(1978). The capacity of *Scenedesmus* to store manganese in their cells and utilise it during favourable conditions had been observed by Jahnke and Soulen (1978). Sanders (1978) had indicated the enrichment of estuarine phytoplankton by the addition of dissolved manganese. Christensen *et al.*,(1979) had reported a depression in growth rate and 50% reduction of cell volume at a concentration of 31 and 50 mgl^{-1} of Mn in *Selenastrum capricornutum* and *Chlorella stigmatophora* respectively.

Manganese was found to be a negative fertility factor in centric diatom *Ditylum brightwellii*, in that auxospores and sperms were formed preferentially in Mn-free medium (Steele, 1965). Brand *et al.*, (1983) had also measured the limitation of reproduction rates in neritic and oceanic species of phytoplankton.

The regulation of cellular manganese and its transport rates in *Chlamydomonas* sp using model chelate buffer systems have been discussed by Sunda and Huntsman (1985). Stauber and Florence (1985) concluded that manganese plays an effective role as a protective agent

against copper toxicity in *Nitzschia closterium*. Information on the role of various environmental factors on the availability and toxicity of manganese to algae has not received much attention so far.

2.3. Zinc:

The earliest demonstration of a zinc requirement in algae was in *Stichococcus bacillaris* (Eilers, 1926). The essentially of zinc has been shown in many other species. Thereafter it is assumed to be universally required by algae.

Zinc is an important micronutrient for growth and metabolism of various algae (Foster and Demson, 1940 and O'Kelley, 1968) and much work has been done on its metabolism especially in *Euglena* (O'Kelley, 1974). Walker (1954) had stated that 4.5 mg of Zn is required for the growth of *Chlorella* sp. Zinc plays a vital role in maintaining the integrity of ribosomes. It had been shown by Praske and Plocke (1971) that under conditions of zinc deficiency in *Euglena* the ribosomes disappeared, but returned when zinc was added. In autotrophic cultures, a linear relationship between specific growth rate and internal zinc concentration of cells has been established by Price and Quigley (1966). Zinc is also known to form stable complexes with DNA and RNA and thereby maintain their stability.

Higher concentrations of zinc inhibit the growth of various algae (Rana and Kumar, 1974 and Whitton, 1970). Passow *et al.*,(1961) have

observed that higher concentrations of zinc affect the permeability of the plasma membrane leading to the leakage of electrolytes. The kinetics of zinc uptake by the diatom *Phaeodactylum tricornutum* was explained by Davies (1973) in terms of rapid absorption of metal on to cell membrane followed by diffusion controlled uptake and binding to proteins within the cell. The inhibition of photosynthesis in coastal waters and carbon fixation as a function of zinc uptake have also been confirmed by Davies and Sleep (1979a,b).

The relationship between radioactive zinc uptake and population growth patterns of *Microcystis littoralis*, *Chlorella vulgaris* and in two species of marine chlorophycean flagellates has been analysed by Nair *et al.*, (1973) and Nair and Mulay (1979). The tolerance level of three algal species and *Anacystis nidulans* to zinc has been detected by Jensen *et al*,(1974) and Shehata and Whitton (1982). Resistance of *Stigeoclonium tenue* to zinc has been studied by Harding *et al.*, (1976).

Rajendran *et al.*, (1978) had investigated the effect of metal ions such as zinc on the photosynthesis of microplankton and nannoplankton in the Zuari estuary, Goa. Anderson *et al.*,(1978) found the zinc ion activity in contrast to the total zinc ion concentration was responsible for limiting the growth rate of *Thalassiosira weissflogii*. The growth response of the green alga *Chlorella vulgaris* (Rachlin and Farran, 1974), diatom *Nitzschia closterium* (Rosko and Rachlin, 1975), *Chlorella saccharophila* and *Navicula incerta* to selected concentrations

of zinc which reduced the population growth by 50% after 96 hrs of exposure was estimated by Rachlin *et al.*, (1982, 1983).

The complexation of zinc by metabolites excreted from the marine diatoms and the influence of dissolved organic compounds on toxicity have been highlighted by Fisher and Frood (1980), Fisher and Fabris (1982), Imber and Robinson (1983) and Imber *et al.*, (1985). Canterford and Canterford (1980) have expressed the correlation between toxicity and metal speciation in *Ditylum brightwellii*. Canterford *et al.*, (1978), Fahim *et al.*, (1981), Fisher *et al.*, (1984) and Fisher (1985) have assessed the accumulation rates and toxicity of zinc in marine phytoplankters.

Effect of zinc on the growth characteristics, photosynthesis and pigment composition has been studied by De Filippis and Pallaghy (1976a,b), Rai and Kumar (1980), Rai *et al.*, (1981a,b), Les and Walker (1984) and Kanakavalli Susarla (1987). The influence of low concentrations of zinc in *Prorocentrum micans* (Rabsch *et al.*, 1984) and in phytoplankton of natural water samples has been reported by Wolter *et al.*, (1984).

The role of environmental factors such as temperature and pH affecting the uptake of ^{65}Zn in relationship to the metabolism of *Dunaliella tertiolecta* has been examined by Parry and Hayward (1973). Michnowicz and Weaks (1984) and Harrison *et al.*, (1986) have also established the effect of pH on the toxicity of zinc to *Selenastrum capricornutum* and *Chlamydomonas variabilis*.

The survey of the above literature indicates that more research and view points are necessary for a better understanding of the impact of environmental factors in modifying the rate of zinc uptake and its effect on algae.

2.4. Interaction of metals (Cu, Mn and Zn):

While the action of single metal ion on individual algal species is clearly worth studying, there is a great need also to study how far combinations of metal ions affect the physiological, biochemical and ecological processes of various organisms. There are several reports on particular organisms in relation to the occurrence of trace elements in combination (Whitton, 1970; Hutchinson, 1973; Gopal, Rana and Kumar, 1975 and Henriksen and Wright, 1978) which suggest that trace metal species are never found in isolation in aquatic ecosystem.

Most studies of trace metal interactions on algae have involved a mixture of copper with one other metal, eg. Cd, Hg, Ni, Zn and Mn etc. The effects exerted on algae depend upon the type of metal used and the specific algal organisms exposed. Much of the information pertaining to the interaction of trace metals namely Cu, Mn and Zn represents cases of either synergism or antagonism.

The combined effects of copper and zinc have been determined by Braek *et al.*,(1976) in four species of diatoms and also in *Asterionella japonica* and *Scenedesmus quardicauda* by Fisher *et al.*,(1981) and

Peterson, (1982). The interactions of copper and manganese have been studied in a dinoflagellate, and various other species of diatoms by Anderson and Morel (1978), Sunda *et al.*, (1981), Sunda and Huntsman, (1983) and Stauber and Florence (1985).

CHAPTER III

3. MATERIALS AND METHODS

The materials for these investigations are two unicellular algae *Isochrysis galbana* Parke (golden yellow flagellate) and *Synechocystis salina* Wislouch (blue green alga) used as live food in aquacultural practices. Both the strains are from those maintained in CMFRI laboratory collection.

Isochrysis galbana belongs to the family Isochrysidaceae under the order Isochrysidales and class Haptophyceae. *Synechocystis salina* belongs to the family Chroococcaceae under the order Chroococcales and class Cyanophyceae.

3.1. Culture conditions:

The flagellate and the blue green alga were grown in enriched seawater as batch cultures. Prior to preparation of the culture medium, the seawater collected from offshore was allowed to age in carbuoys. Further seawater was filtered through sartorius filter paper; nutrients were then added and autoclaved. The cool sterilised medium was transferred to sterile culture flasks. The cultures were not bacteria free.

Various nutrient solutions like Miquel's medium (Miquel, 1890), Erdschreiber solution (Schreiber, 1925) and Walne's medium (Walne, 1974) are generally used to culture the microalgae. As the present study

involves the determination of role of trace metals and their uptake, it was decided to select a medium which was devoid of trace metals. Hence Miquel's medium was adopted for all the trace metal experiments.

The composition of the medium is given below:

Miquel A

Potassium nitrate	-	20.2 gm
Distilled water	-	100 ml

Miquel B

Sodium phosphate	-	4 gm
Calcium chloride	-	4 gm
Ferric chloride	-	2 gm
Concentrated HCl	-	2 ml
Distilled water	-	98 ml

To each litre of filtered seawater 0.55 ml of A and 0.5 ml of B are added.

The salinity of the seawater used to prepare the medium was 30-35‰ for the culture of *I. galbana* whereas *S. salina* was grown at 15-20‰ salinity.

The stock and experimental cultures were maintained in 500 ml of the medium in one litre flasks (corning) plugged with sterilised cotton. They were maintained in logarithmic phase. An aliquot of the culture

was replaced with fresh medium every seven to ten days. All stock and test cultures were illuminated with day light flourescent tubes. The light: dark period within the chamber was 10 : 14 hrs with a mean light intensity of 34.61 x 10^{15} quanta x cm^{-2} x sec^{-1}. The ambient temperature ranged from 24°C to 35°C.

Aeration was not provided to the cultures. Instead the culture were shaken manually to give three to four rotations every now and then to keep them in uniform suspension. Settling was not noticed for a month, but later on developed a tendency to settle down.

An automatic temperature control system was fabricated for conducting experiments under different temperatures (25°C, 30°C, 35°C, 40°C) simultaneously. Four tanks of 100 litre capacity were filled with water and heated by an immersion heater. The heater of each tank was connected to a Jumo thermometer through a specially designed thermostat unit which had the provision to give connections to all the four systems together. The illumination was provided with fluorescent tubes.

3.2. Growth measurements:

3.2.1. Measurement of cell concentration:

The cells were fixed in Lugol's iodine solution and counted with a calibrated haemocytometer.

3.2.2. Determination of photosynthetic activity by ^{14}C technique:

In the ^{14}C method, the incorporation of the tracer in the organic matter of the planktonic algae was used as a measure of the production.

Cultures were diluted ten times and taken in 60 ml bottles. Dilution was necessary to prevent shading effect and blocking in the millipore filters during filtration. The ^{14}C was added in the form of sodium bicarbonate at pH 9.0. The isotope was supplied by BARC, Bombay. One ml of the hot solution with an activity of 5 μCi was diluted five times. To each sample 1 μCi (ca. 2.22×10^6 dpm) was added. Dark bottle controls were also maintained simultaneously. Incubation was done for a period of two to four hours under the same light intensity as the stock culture. All samples had replicates.

The activity of the sample was determined with the aid of a Liquid Scintillation Counter (LSS 20) having an efficiency of 84%. The preparation of the sample for liquid scintillation counting was done in the following way: To the filter paper containing the sample, one ml of acetone was added to dissolve the tissue and the filter paper. To this a 15 ml scintillation fluid was added. The different components of the scintillation fluid are:

POPOP	-	7 gms
Naphthalene	-	100 gms
Dioxane	-	1000 ml

The scintillation vials with the required sample were fed into the counter to determine the activity.

The rate of production was expressed as mgC/litre/hr.

$$\text{Rate of production} = \frac{\text{net activity (net cpm) x carbon-dioxide}}{\text{added activity (added cpm) x hrs of incubation}}$$

where the net activity was determined and added activity was computed by the biological method of Steemann Nielsen (1 965).

3.2.3. Determination of quantitative variation of algal pigments by spectrophotometry:

The quantity of pigments is also used as an index of physiological activity. The concentration of chlorophylls and carotenoids was estimated by spectrophotometric analysis of acetone extracts (Strickland, 1963 and Strickland and Parsons, 1972).

A known volume of the culture was filtered through Millipore HA filters of pore size 0.45 μ. One to two drops of Magnesium carbonate were added to the samples while filtering to prevent acidification. The pigments were extracted by adding 10 ml of 90% acetone to each filter. The extraction was carried out at low temperature for 20 hrs. The extracts were centrifuged and the extinction of the clear solution was measured by ECIL spectrophotometer.

3.2.4. Thin layer chromatography:

Quantitative chromatographic method helps in the determination of microgram quantities of chlorophylls and carotenoids in planktonic marine algae.

Experimental cultures of *I. galbana* and *S. salina* grown under different concentrations of Cu, Mn and Zn were considered for chromatographic studies.

Preparation of pigment extract:

The algae from 50-100 ml of the culture were harvested by centrifuging at 6000 rpm for 10 minutes, and the supernatant was discarded. Pigments were readily released from the cells by extracting with small volumes of 90% acetone. After several such extractions the cell residue was colourless. Pigments were immediately transferred to diethyl ether, by adding an equal volume of ether to the combined acetone extracts and shaking with a volume of 10% NaCl solution at least 10 times that of the acetone extract. The pigments migrated to the ether layer, and acetone and water soluble impurities were removed in the aqueous phase. This step was carried out immediately for maintaining the stability of chlorophylls and carotenoids in the samples. The ether layer was concentrated for chromatography under a stream of nitrogen. Condensed water was removed from the concentrated ether extract by centrifugation and

the sample was then made completely anhydrous by the addition of a few crystals of sodium chloride.

Preparation of TLC plates:

The most important factor in the preparation of the plate lies in the quality of the adsorbent. Adsorbents should be innocuous and should cause no damage or chemical change of any kind to the pigments. As silica-gel is known to degrade chlorophylls, it was decided to use cellulose as the adsorbent.

Chromatographic plates (20 cm x 20 cm) were prepared by homogenizing a slurry of the cellulose in distilled water (30 g of cellulose to 160 ml of water) for 1 min at medium speed in a blender. Four plates were coated with layers 0.25mm thick from this quantity of cellulose. The slurry was spread onto chromatographic plates with a standard spreader, air-dried for 30 min and then dried at 95°C for 1 hour. The plates were cooled at room temperature, any condensation removed from the back, and then stored in a glass desiccator over silica-gel. The plates were reactivated by heating at 90°C for 30 min before use.

The degree of resolution of the pigments depends on the composition of the solvents and the state of hydration of the cellulose. Solvent mixtures which gave good results were:
First dimension - n-propanol : light petroleum (60°C-80°C) = 2.5 : 97.5(V/V)
Second dimension - light petroleum (60°C-80°C) : chloroform : acetone = 70 : 30 : 0.5 (V/V/V).

The ether pigment extract was further evaporated in a small glass tube (4cm long x 1cm wide) under a gentle stream of nitrogen. This concentrate was applied to the chromatogram with a fine 10 µl pipette to obtain a small (2-3-mm diameter) concentrated origin spot for optimal resolution. Along with the sample, standard chlorophyll a was also applied. Duplicate of samples were also run simultaneously.

The chromatographic tanks lined with filter paper were equilibrated for a few minutes with 200 ml of the appropriate solvent mixture before the plates were inserted. Development in the first dimension took about 20 min, after which the plates were air-dried in absolute darkness for several minutes and then run in the second dimension for 15 min. After development, the plates were withdrawn from the tank, examined under UV light for location of chlorophylls and other products. The chromatograms were traced out immediately, the solvent front and colour of the pigment spots were noted. The R_F values were calculated for both the dimensions by

$$R_F = \frac{\text{Distance travelled from the origin to the pigment spot}}{\text{Distance travelled by the solvent front from the origin}}$$

Chlorophyll 'a' was identified based on spot colour and R_F of the standard. The other pigments were identified with the help of the guidelines given by Jeffrey (1968, 1981).

For quantitative analysis, the pigment spots were scraped off the plates onto a butter paper with a small stainless steel spatula, placed in

centrifuge tubes and eluted with about 5.0 ml of acetone (chlorophyll a), 3.0 ml of ethanol (carotenoids and xanthophyll) and 3.0 ml of methanol (chlorophyll c). After about 5 min, the cellulose powder was removed by centrifugation, the pigment solution was adjusted to a precise volume, and the extinction measured in a Beckmann spectrophotometer. Extinction coefficients used for the calculation of pigment concentrations were as given by Jeffrey (1968, 1974), Jeffrey *et al.* (1975).

3.3. Analytical Techniques:

Apart from the standard methods applied for chemical analysis of seawater for various parameters, certain sophisticated techniques were applied in view of the high accuracy and sensitivity that are required for trace metal analysis.

Trace metals:

All sample containers and glasswares were washed with detergent, rinsed with tap water, distilled water and soaked in nitric acid overnight. They were washed again in double distilled water in order to reduce the degree of contamination.

All stock solutions of the three metals-copper, manganese and zinc were prepared fresh before the test run. The dilution for each concentration of each metal was done with the culture medium. The inoculum for all the experiments was taken from the stock cultures during the exponential phase.

Modern instrumental methods like Atomic Absorption Spectrophotometry (AAS), Differential Pulse Anodic Stripping Voltammetry (DPASV) are sensitive techniques for the determination of trace metals in seawater and in marine organisms.

3.3.1. Atomic Absorption Spectrophotometry:

Many of the elements exist in seawater at concentration below their detection limits by AAS, hence they can be readily concentrated by extraction into a suitable solvent which results in enhanced sensitivity.

The seawater collected from offshore was filtered through a 0.45 μ Millipore filter paper. In this filtered seawater the pH was adjusted between 4 and 5 with hydrochloric acid as a precautionary measure for reducing the danger of absorption or precipitation of colloidal material on the walls of the container.

Samples of seawater (750 ml) were placed in 1000 ml separating funnel and 35 ml of methylisobutyl ketone (MIBK) was added followed by 7 ml of 1% Ammonium pyrollidine dithiocarbamate (APDC) solution. (The 1% aqueous solution of APDC was prepared fresh and purified by shaking with an equal volume of MIBK, separating the phases and filtering the lower aqueous phase). The samples were then equilibriated for 30 min on a mechanical shaker. The phases were separated and the lower aqueous phase was utilised for further determination of the concentration of copper, manganese and zinc by AAS. Blank correction was also done simultaneously.

The following procedure was adopted for monitoring the uptake of Cu, Mn, Zn by *I. galbana* and *S. salina* . Analysis was conducted by AAS at an interval of two days until the eighth day had reached.

Cultures of the two microalgae grown under different concentrations of Cu, Mn and Zn ranging from 0.010 ppm to 0.150 ppm were selected in replicates. Suitable volume of aliquot was centrifuged for fifteen minutes at 7,000 rpm in order to separate the algae from the supernatant. Supernatant from each treatment was added to the measuring cylinder. Algal pellets were washed with distilled water, centrifuged and washings added to cylinders containing respective supernatants. This step was repeated again. The supernatants and washings were made to a known volume. Samples of supernatant were collected into acid washed vials and later on analysed in AAS.

The algal pellets were transferred to weighed acid washed test tubes and dried to constant weight at 90°C. Dried alga was weighed and covered with two ml of nitric acid and the tubes were left overnight at room temperature until the vigorous action ceased. Tubes were set in a sand bath for two hours or until the liquid cleared. It was further diluted to ten ml with distilled water and analysed in AAS for determination of metal concentration. This experiment demonstrated the uptake of metal in algae with reference to the increasing concentration of the metal in the medium and time duration.

3.3.2. Polarography:

Substance specificity and general methodological properties with respect to sensitivity and accuracy make advanced modes of polarography and voltammetry a powerful and convenient approach to study at trace levels the speciation of metals which are important from the view point of marine ecochemistry.

A preliminary study on the determination of copper complexes in *I. galbana* and *S. salina* was conducted using Model 174A Polarographic analyser attached to a X-Y recorder BD 90. The above system is connected to the analytical cell. The cell contains three electrodes which are immersed in the solution to be analysed. The working electrode is the electrode where the reaction of interest occurs which is usually a hanging mercury drop electrode (HMDE). The reference electrode provides a stable potential with which the potential of the working electrode is compared. The counter electrode is a conductive material that is chemically inert, made of platinum. The voltammogram or polarogram is the result of electron transfer between the electrode surface and a species in solution. The current potential plot reflects the changes that are taking place in the concentration of a species in solution.

Standardisation of various polarograph techniques with seawater samples indicated differential pulse anodic stripping voltammetry to be the most suitable one.

Stripping voltammetry is a two step technique in which the first step consists of the electrolytic deposition of a chemical species onto an inert electrode surface at a constant potential. The second step consists of the application of a voltage scan to the electrode which causes an electrolytic dissolution or stripping of the various species back into solution at characteristic potentials.

Cultures of *I. galbana* grown in 0.200 ppm Cu under different light period of 16 hrs and 60 hrs were used as test samples for the above experiment. But *S. salina* was grown only under the same light period.

Digestion with nitric acid was conducted to release the metal ions from the cells. Polarograms were traced out for acid digested cells of the two species and also for their respective supernatants.

Fine micropipettes were used for standard addition of the metal after washing the pipette tips with acid and water. Deionised and double distilled water was used for washing and in preparing reagents. The general procedure followed for stripping voltammetry is presented below.

The samples were taken into the analytical cell. The electrodes were immersed into the solution. Deaeration was given with stirring. The solution was purged for 10 min with purified nitrogen gas to eliminate oxygen interference. A new mercury drop was generated on hanging mercury drop electrode. The used mercury drop was dislodged to form a new drop. The deposition was carried out for 15 min at -0.4 V with

stirring of the solution. A pulse amplitude of 50 mV and current sensitivity of 5 μA were used. Droptime was 1 sec. After deposition, the sample was equilibriated for 30 sec and was scanned in a positive direction at a rate of 10 mV/sec. The scanning range spans the potential region where the chemical species of interest are electrolysed back into solution. Peak area was calculated as the product of peak height and width at half height.

The concentration of copper in the original sample was calculated using the following equation of Peterson and Wong (1981).

$$C_u = \frac{i_1 \, vC_s}{(i_2 - i_1)V}$$

where i_1 = sample peak height

i_2 = standard addition peak height

v = volume of standard solution added

V = volume of original sample

C_s = concentration of standard solution

C_u = concentration of original sample

3.3.3. Electron microscopy:

Transmission electron microscope studies were conducted with *I. galbana* and *S. salina* to assess the effect of Cu on their intracellular organisation. The procedure for TEM studies was adopted from Hayat (1973).

Cells were concentrated by centrifugation and fixed overnight in 3% glutaraldehyde in 0.1 M phosphate buffer. The cells were rinsed in the buffer prior to fixation. Further they were embedded in agar. A 2%(W/V) aqueous agar solution was prepared by melting the agar in a bath of

boiling water and keeping it in a liquid state at 60°C until use. Small drops of agar were placed on a preheated (60°C) microscopic slides. The concentrated cells were dropped on the agar and mixed with it. The agar embedded specimens were then allowed to solidify either at room temperature or in the refrigerator. Blocks were cut from the solidified agar and handled subsequently as tissue blocks.

Agar blocks were post-fixed in 1% Osmium tetroxide for 5-6 hrs. Further they were washed in buffer and presoaked in aqueous uranyl acetate. Dehydration was carried out in ethanol series starting from 25%, 50%, 75%, 95% and 100% and finally transferred to 100% acetone. Embedding was done in Spurr resin and the molds were allowed to dry in oven.

These specimen molds were trimmed and semi-thin and ultra thin sections were cut using LKB Bromma 8800 Ultratome. The semi-thin sections were stained in methylene blue azure II and basic fuchsin, rinsed in distilled water, dried and mounted for observation. The ultra thin sections after staining with uranyl acetate and lead citrate were observed in Carl Zeiss TEM for structural details.

Negative staining technique was adopted to study the fine structure of *I. galbana*. In this method, a drop of the culture suspension was applied to Formvar coated carbon grids, stained with 0.5% ammonium molybdate, dried and observed in TEM.

The impact of selected environmental factors such as salinity, pH and temperature on the growth and physiology of *I. galbana* and *S. salina* under different concentrations of Cu, Mn and Zn was also studied.

CHAPTER IV

4. RESULTS AND DISCUSSION

4.1. Distribution of trace metals in seawater:

The water collected from Cochin nearshore waters utilised for trace metal experiments was analysed by AAS to detect the concentration of copper, manganese and zinc at different depths i.e. 8m-25m. Concentrations ranged from 12.2 - 25.6ppb for copper, 1.15-2.25 ppb for manganese and 6.0-38.0 ppb for zinc (Table 1).

Table 1. Distribution of dissolved copper, manganese and zinc in Cochin nearshore waters at different depths.

Metal concentration (ppb)	8m	12m	25m
Copper	15.30	12.20	25.60
Manganese	1.15	1.60	2.25
Zinc	18.00	38.00	6.00

Distribution of dissolved copper increases with depth. The concentration of manganese was found to be very low in relation to the other two metals though a slight increase was observed at 25m. Zinc concentration was the highest of all the three metals and moreover it was appreciably concentrated at 12m. Dissolved zinc decreased in deeper layers.

The concentration of dissolved copper found at the surface (12.2 - 15.3ppb) in the present study more or less falls within the range (13.3 µg/l) as reported by Sanzgiry *et al.*, (1979) in the Laccadive Sea. Increase of dissolved copper from the surface to deeper layers as observed by Chalapati Rao and Satyanarayana Rao (1974), Sanzgiry *et al.*, (1979) and Rajendran *et al.*,(1982) in the waters of Bay of Bengal and Laccadive Sea was not applicable to Cochin nearshore waters. Zingde *et al.*, (1976) noted lower values of copper (4.5 ppb) and zinc (18.7ppb) and an elevated concentration of manganese (45.2 ppb) in Goa waters. Values of zinc and manganese are in accordance with the report of Rajendran *et al.*, (1982) in Western Bay of Bengal. Their findings that zinc is higher at surface than at deeper layers and manganese concentration increased with depth in the inshore waters are in agreement with the observations made in respect of Cochin waters.

In comparison to the present study, higher values of copper (0.10 - 1.20 ppm) and zinc (0.69 - 4.0 ppm) were observed by Rajendran and Kurian (1986) in Cochin Backwaters. Sankaranarayanan and Rosamma Stephen (1978) have estimated only the particulate concentration of the three trace metals Cu, Mn and Zn in Cochin Backwaters. Occurrence of significant concentrations of copper and zinc may be attributed to the discharge of industrial pollutants and due to harbour activity.

In the absence of regular monitoring of data and seasonal variation studies, it is difficult to assess the nature and magnitude of changes that

have taken place in the concentration of copper, manganese and zinc respectively.

4.2. Effect of trace metals on the growth and physiology of algae with reference to the environmental factors - salinity, pH and temperature:

4.2.1. Copper:

Isochrysis galbana.

Under the three treatments of copper employed, the flagellate was found to grow rapidly at higher salinities. The decline in cell concentration, chlorophyll a,c and production was noticed from the sixteenth day whereas the carotenoid content alone decreased from the eighteenth day.

Maximum cell concentration was recorded from the sixth to the eighth day in 0.05 ppm, 0.10 and 0.15 ppm Cu about 42%, 63% and 14% respectively higher than the control. The biomass was greater in 0.10 ppm than in the other two treatments during all phases of growth except on the sixth day wherein the cell activity was higher in 0.05 ppm Cu (Fig. 1).

The amount of chlorophyll a,c and carotenoids was less in the lag phase but later on increased steadily and reached its maximum between the twelveth and sixteenth day for all the three concentrations of copper. The concentrations of chlorophyll a,c per unit number of cells were found to be higher in 0.05 ppm and 0.10 ppm Cu (Figs. 2,3). Carotenoid content showed a wide fluctuation in the three salinities, higher at 35 ppt in early

FIG. 1. *Isochrysis galbana*

Effect of copper on biomass in different salinities (S).

S_1 - 15 ppt

S_2 - 25 ppt

S_3 - 35 ppt

Copper Concentrations

 - 0.050 ppm

 - 0.100 ppm

 - 0.150 ppm

 - Control

FIG·1

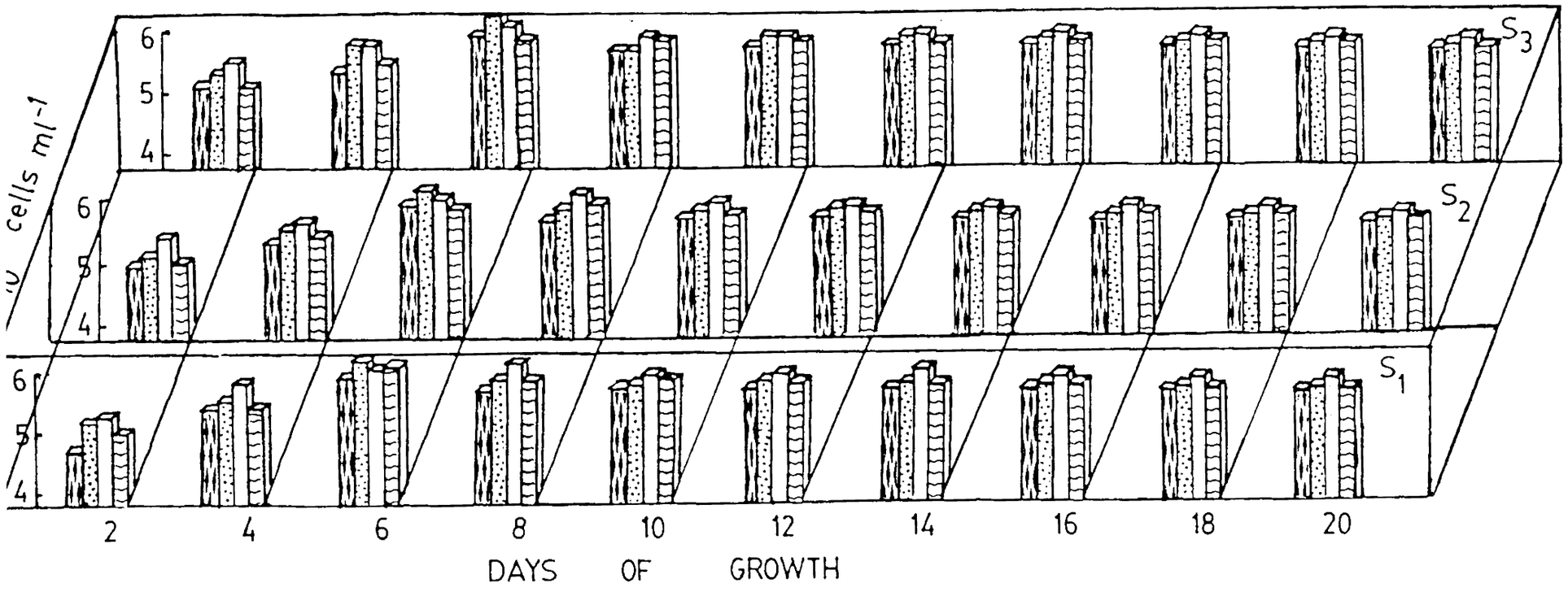


FIG. 2. *Isochrysis galbana*

Effect of copper on the chlorophyll 'a' content in different salinities (S).

S_1 - 15 ppt

S_2 - 25 ppt

S_3 - 35 ppt

Copper concentrations

 - 0.050 ppm

 - 0.100 ppm

 - 0.150 ppm

 - Control

FIG. 2

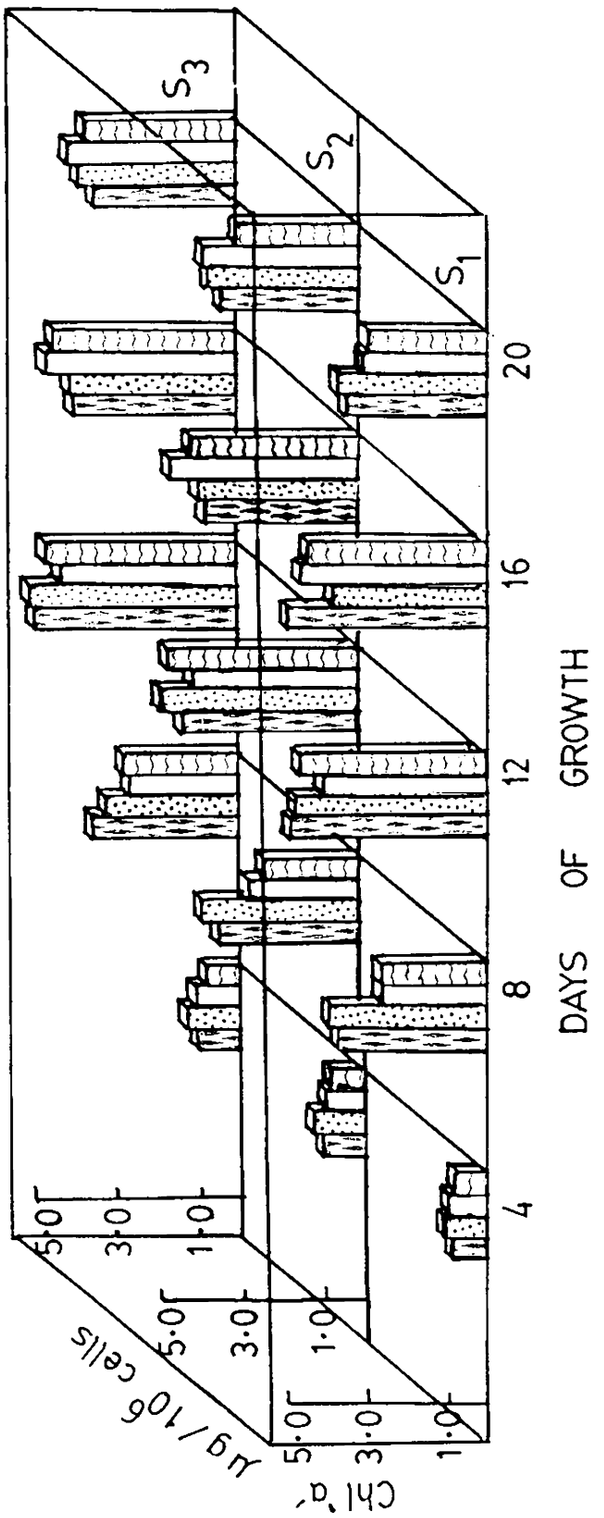

S3
S2
S1
5·0
3·0
1·0
5·0
3·0
1·0
5·0
3·0
1·0
μg/10⁶ cells
Chl'a'
4
8
12
16
20
DAYS OF GROWTH

FIG. 3 *Isochrysis galbana*

Effect of copper on the chlorophyll 'c' content in different salinities (S).

S_1 - 15 ppt

S_2 - 25 ppt

S_3 - 35 ppt

Copper concentrations

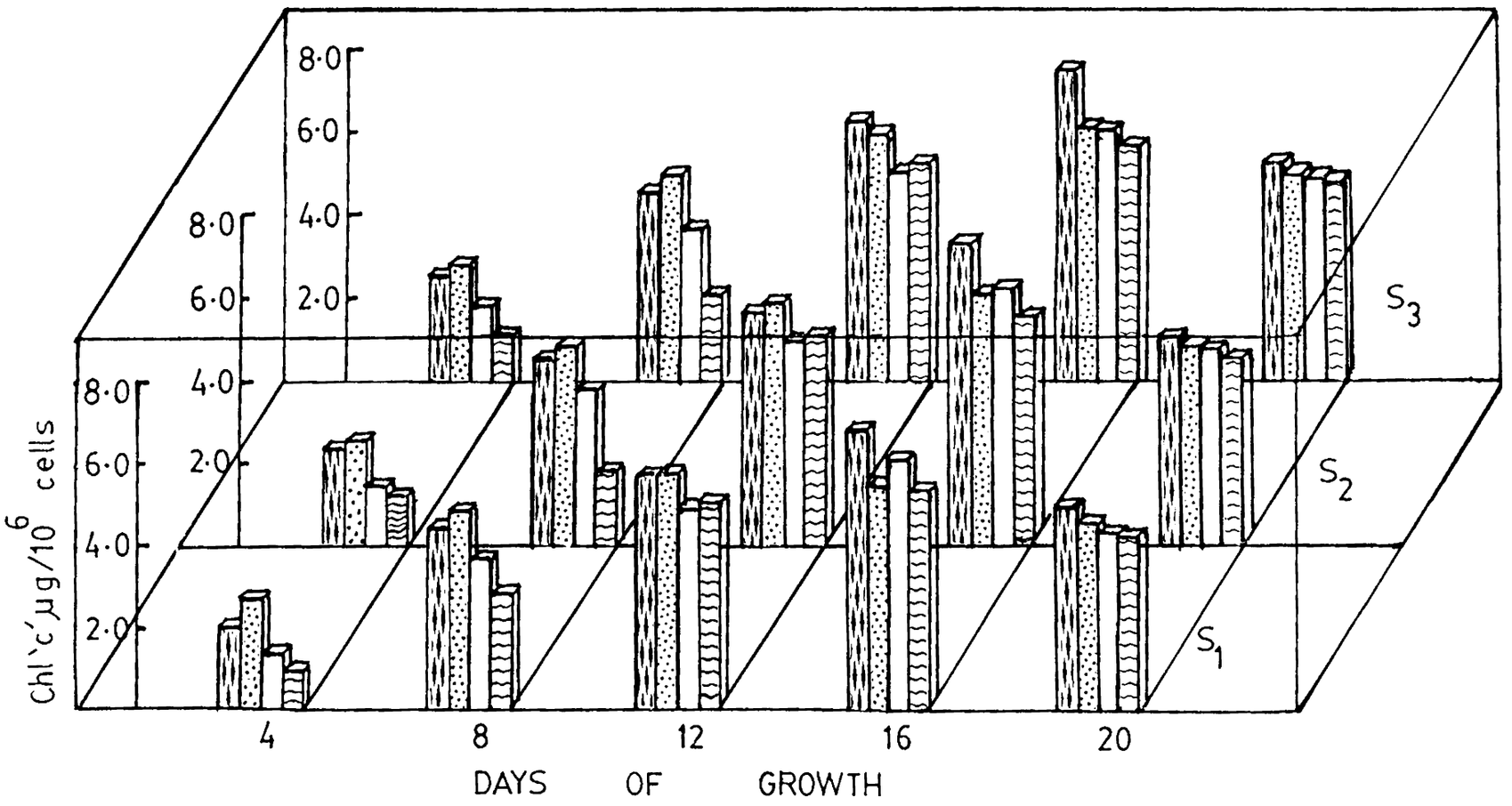
 - 0.050 ppm

 - 0.100 ppm

 - 0.150 ppm

 - Control

FIG·3


8·0
6·0
4·0
2·0
8·0
6·0
4·0
2·0
8·0
6·0
4·0
2·0
Chl 'c' µg/10^6 cells
S3
S2
S1
4
8
12
16
20
DAYS OF GROWTH

phase and greater at 25 ppt during later stages of growth (Fig.4). Maximum quantity of chlorophyll a was seen in 0.10 ppm Cu, whereas chlorophyll c and carotenoids were higher in 0.05 ppm Cu. In 0.15 ppm Cu treatment, the proportion of chlorophyll a,c and carotenoids were found to be lower than control between twelve to fourteen days. The quantity of chlorophyll a was comparatively lower than that of chlorophyll c and carotenoids.

The rate of production increased from 15 ppt to 35 ppt. Only in 0.05 ppm Cu, the flagellate exhibited higher metabolic activity with the maximum rate of 7.68 mg C/l/hr. The other two treatments indicated lower values than that of control during all phases of growth (Fig. 5).

The growth rate of the flagellate was stimulated at pH 6.0 than at pH 7.0 or at pH 8.0. The amount of pigments and the rate of production declined from the sixteenth day onwards, whereas a constant variation in cell concentration was not detected.

The cell content reached its maximum on the fourth day in 0.05 ppm Cu at pH 6.0 about twice that of control. Comparatively the cell concentration was lower than control in 0.10 ppm and 0.15 ppm Cu till the twelveth day. At all pH levels from 6.0 to 8.0 and in the three selected copper treatments, biomass decreased from eighth to the twelveth day and subsequently showed a marginal increase from the sixteenth day (Fig. 6).

FIG. 4. *Isochrysis galbana*

Effect of copper on the carotenoid content in different salinities (S).

S_1 - 15 ppt

S_2 - 25 ppt

S_3 - 35 ppt

Copper concentrations

 - 0.050 ppm

 - 0.100 ppm

 - 0.150 ppm

 - Control

FIG. 4

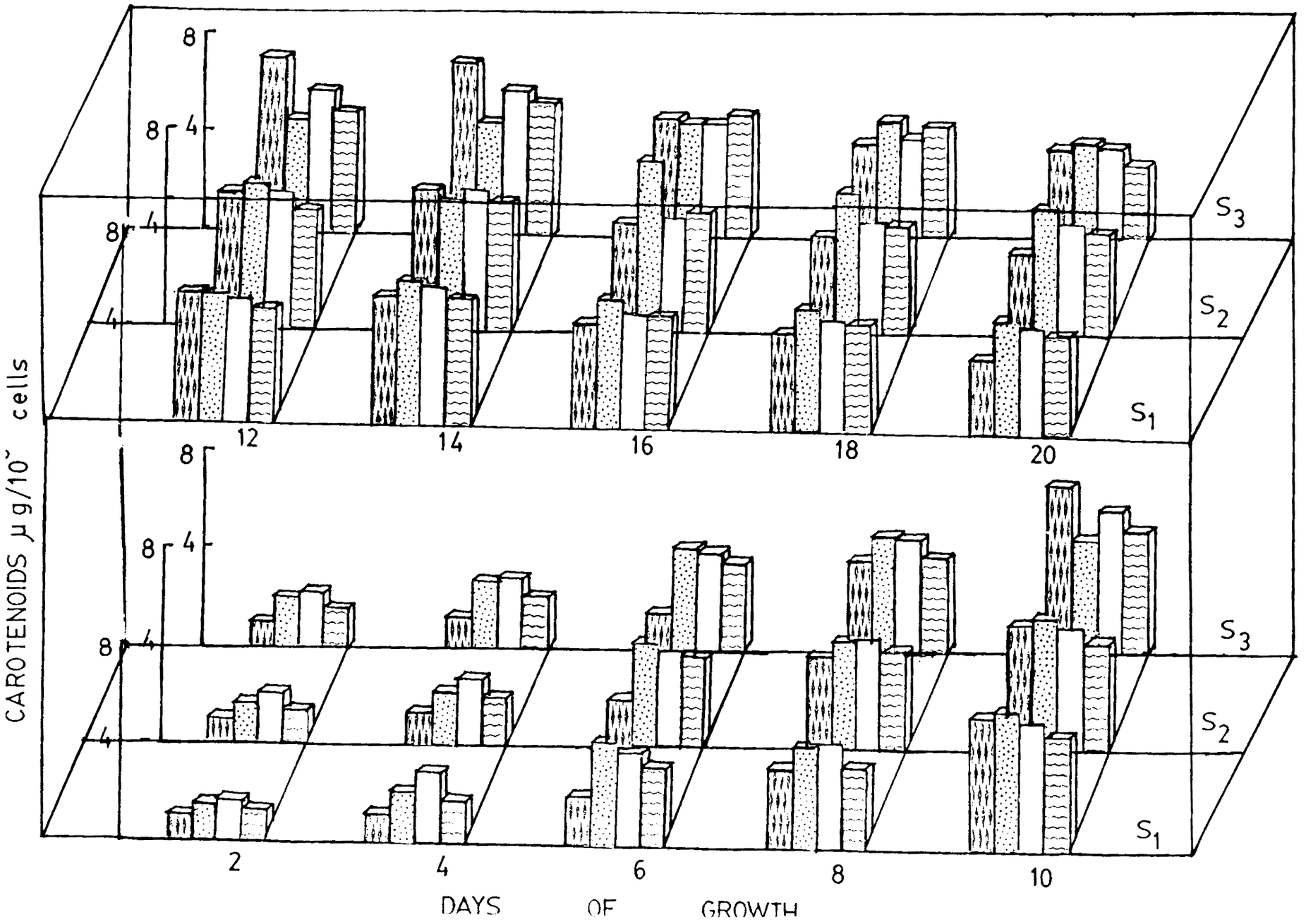

CAROTENOIDS µg/10˜ cells
8
8 4
8
4
12
14
16
18
20
S3
S2
S1
2
4
6
8
10
DAYS OF GROWTH

FIG. 5. *Isochrysis galbana*

Effect of copper on the rate of carbon production in different salinities (S).

S_1 - 15 ppt

S_2 - 25 ppt

S_3 - 35 ppt

Copper concentrations

- 0.050 ppm
- 0.100 ppm
- 0.150 ppm
- Control

FIG. 5

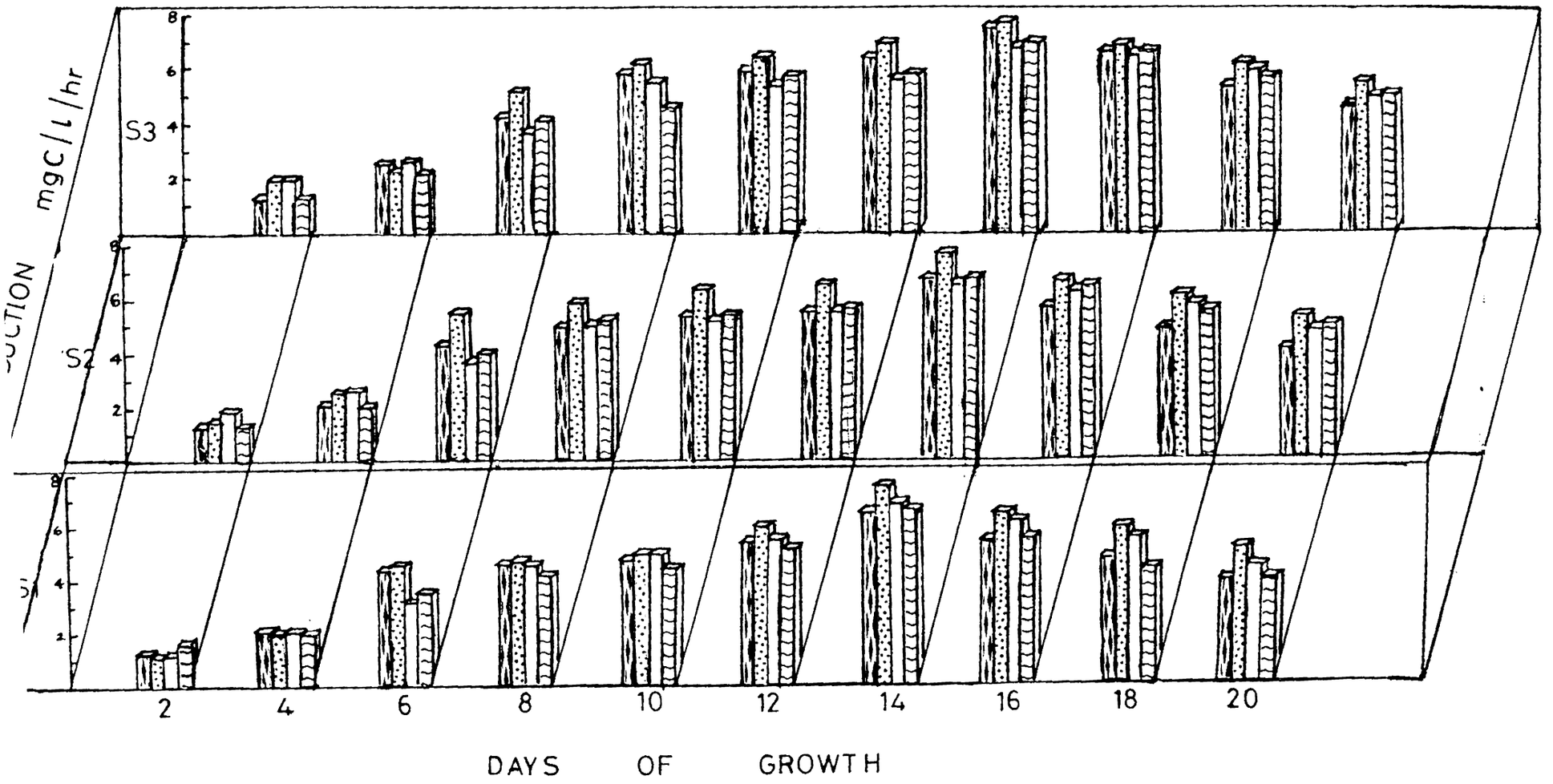


FIG. 6. *Isochrysis galbana*

Effect of copper on biomass at different pH.

Copper concentrations

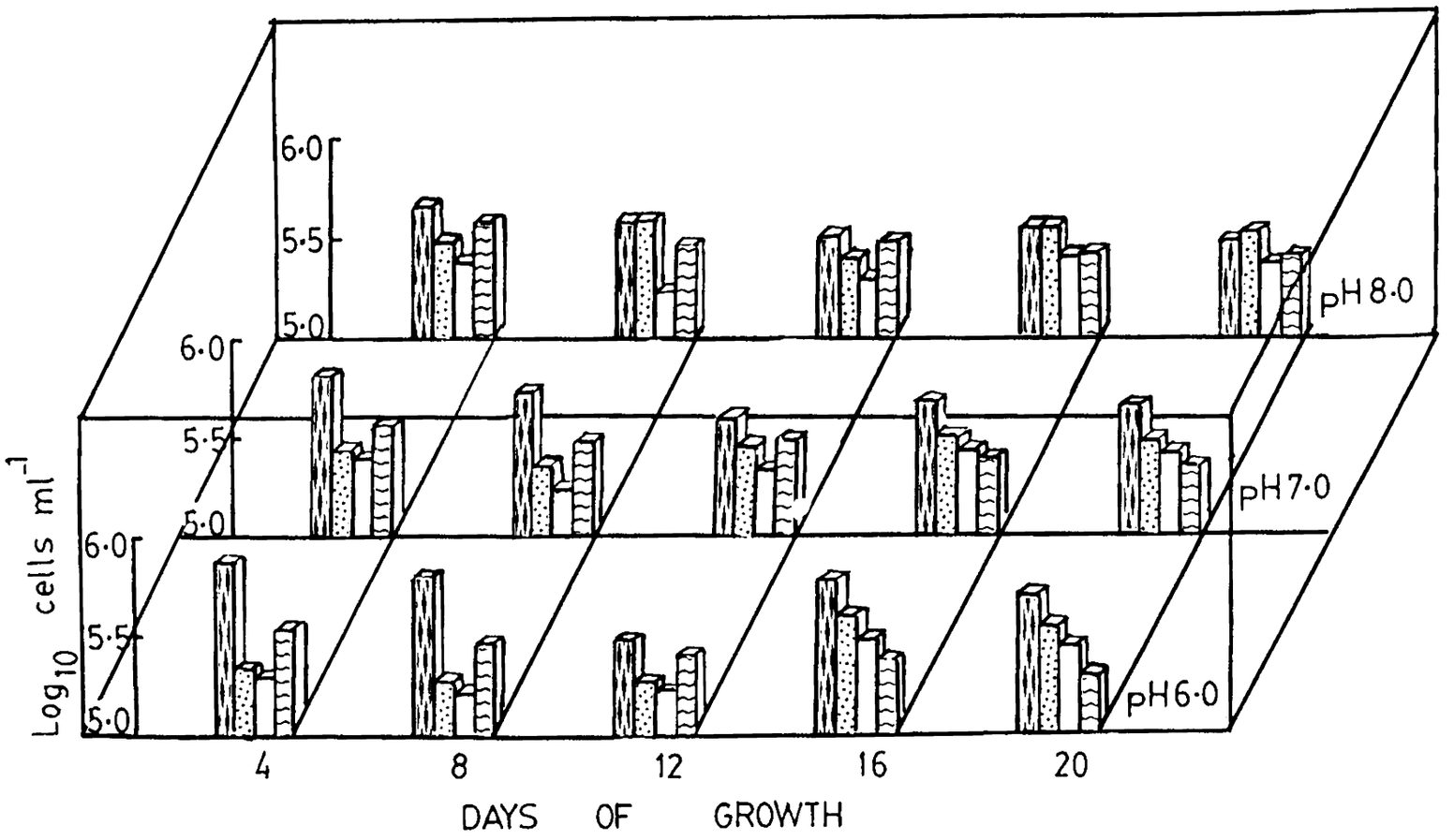
 - 0.050 ppm

 - 0.100 ppm

 - 0.150 ppm

 - Control

FIG. 6


6·0
5·5
5·0
pH 8·0
pH 7·0
pH 6·0
Log10 cells ml-1
4
8
12
16
20
DAYS OF GROWTH

The peak value for chlorophyll a was observed on the eighth day in 0.10 ppm Cu, for chlorophyll c and for carotenoids, it was noticed on the twelveth day in 0.05 ppm Cu at pH 6.0. The quantity of total pigments declined from pH 6.0 to pH 8.0 in all the three copper treatments during the logarithmic phase but the effect was reversed on the approach of stationary phase (Figs. 7,8 B).

Rate of produc ion was maximum at pH 6.0 on the eighth day showing an increase of 14% of control. The effect of pH on the metabolic activity of the flagellate was not significant (Fig. 8 A).

The effect of temperature on the growth rate of the alga was measured in terms of rate of production. For the three selected copper treatments 0.05 ppm, 0.10 ppm and 0.15 ppm, the carbon production increased from 20°C to 30°C, thereafter a slow decline was noticed at 35°C and 40°C. Maximum value was recorded at 30°C in 0.05 ppm Cu about 6% higher than that of control (Table 2).

Synechocystis salina.

Lower salinities 15-25 ppt were found to be more favourable for the growth of the blue green alga. Cell concentration remained higher at 25 ppt during the early phase and greater at 15 ppt towards the end of the exponential phase. In all the three salinities, biomass was greater in 0.02 ppm Cu than in 0.05 ppm and 0.07 ppm Cu. Maximum

FIG. 7. *Isochrysis galbana*

Effect of copper on the chlorophyll 'a' and 'c' content at different pH.

A. Chlorophyll 'c'

B. Chlorophyll 'a'

Copper concentrations

 - 0.050 ppm

 - 0.100 ppm

 - 0.150 ppm

 - Control

FIG. 7

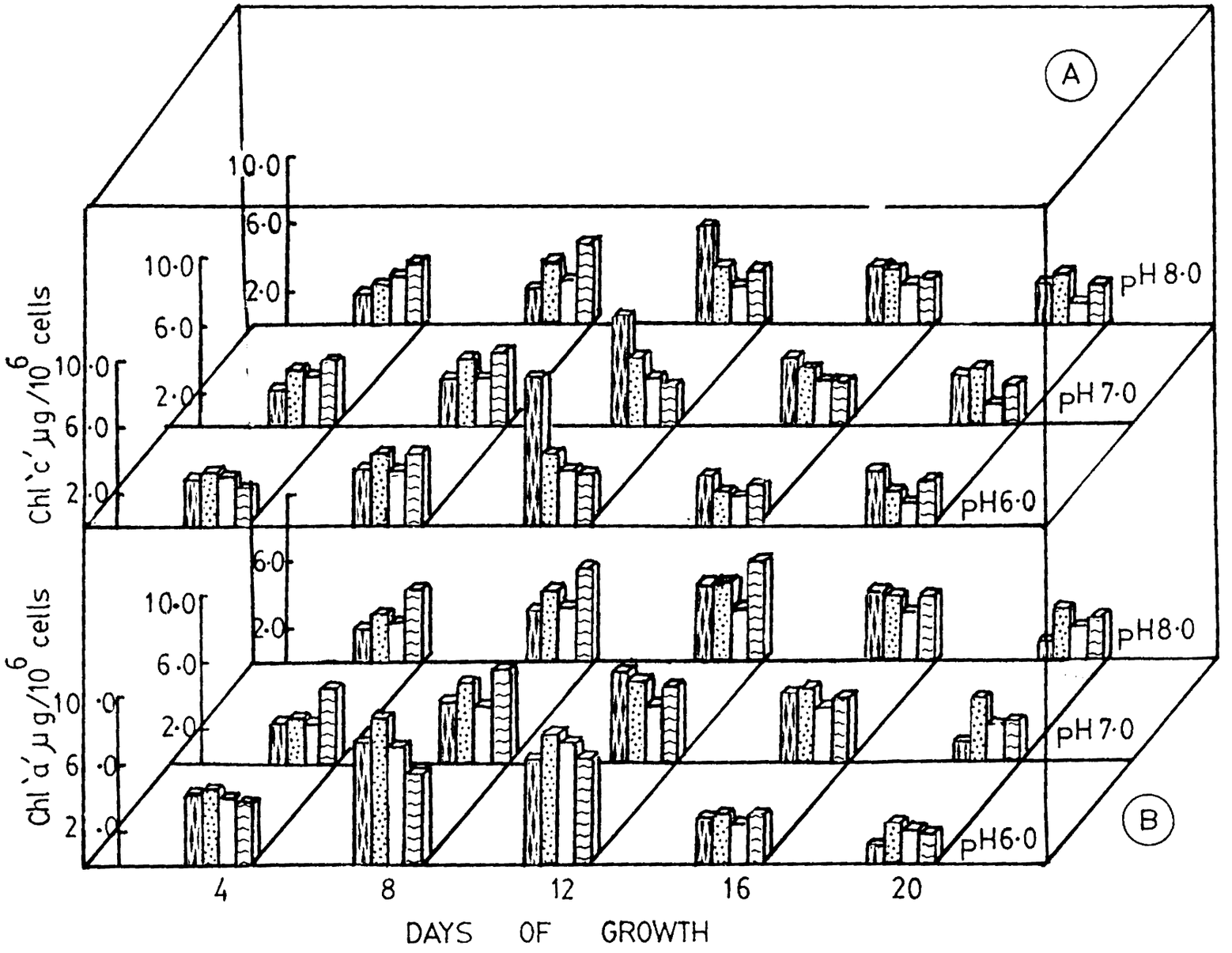

A
B
pH 8·0
pH 7·0
pH6·0
10·0
6·0
2·0
Chl 'c' µg/10^6 cells
Chl 'a' µg/10^6 cells
4
8
12
16
20
DAYS OF GROWTH

FIG. 8. *Isochrysis galbana*

Effect of copper on the carotenoid content and on the rate of carbon production at different pH.

A. Production

B. Carotenoid

Copper concentrations

 - 0.050 ppm

 - 0.100 ppm

 - 0.150 ppm

 - Control

FIG . 8

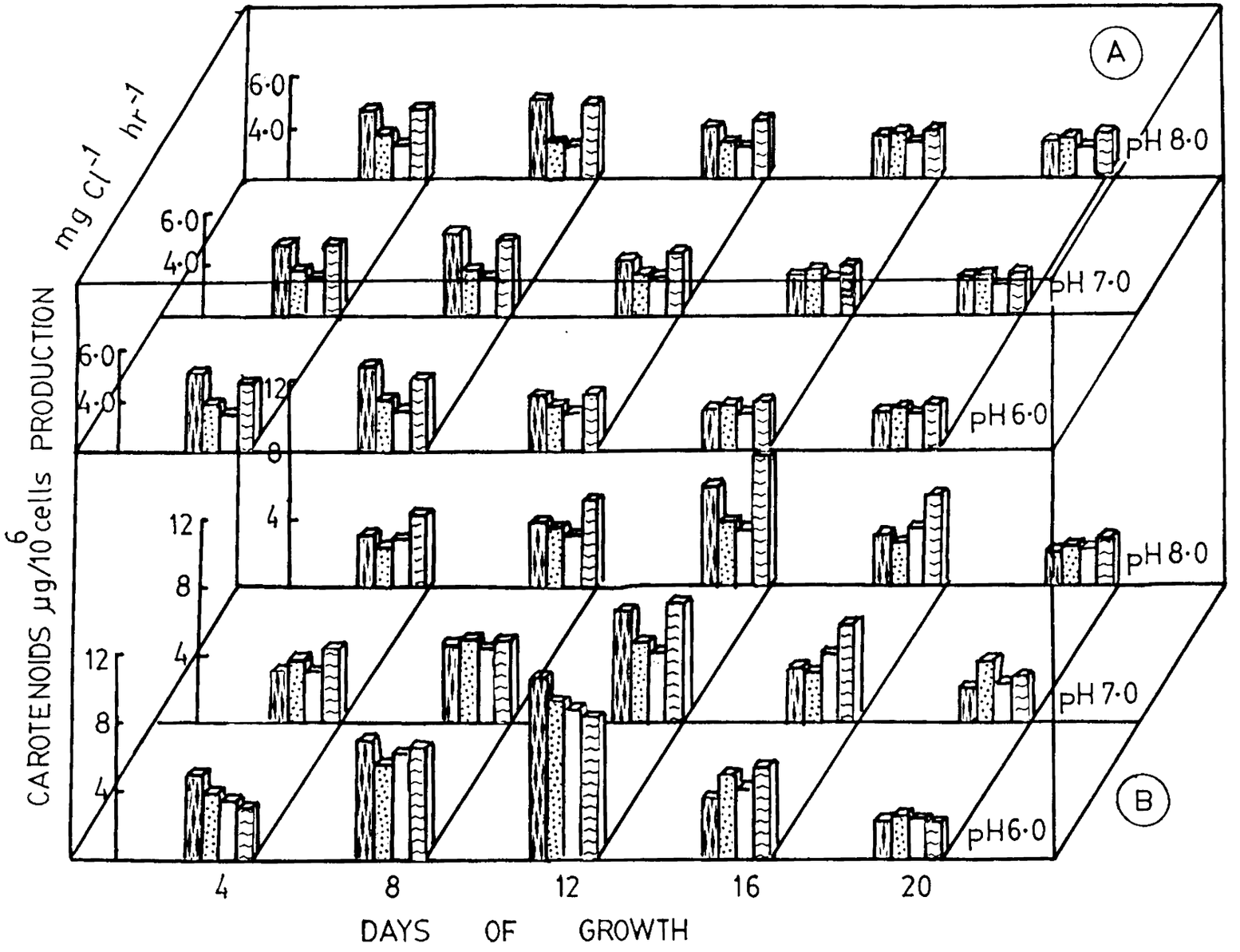

CAROTENOIDS µg/10^6 cells PRODUCTION mg C/l^-1 hr^-1
6·0
4·0
12
8
4
pH 8·0
pH 7·0
pH6·0
A
B
4
8
12
16
20
DAYS OF GROWTH

Table 2. Effect of Cu, Mn and Zn on the rate of carbon production (mgC/l/hr) at different temperatures in *I. galbana*.

Metal Concn (ppm)	20°C	25°C	30°C	35°C	40°C
Cu					
0.050	0.981	1.42	7.24	5.60	0.714
0.100	0.840	1.08	7.06	5.12	0.670
0.150	0.812	0.94	6.40	4.46	0.502
Mn					
0.050	1.08	1.90	7.30	5.42	0.738
0.100	1.24	2.32	7.50	5.70	0.761
0.150	1.32	2.24	7.62	5.96	0.810
Zn					
0.050	0.974	1.56	7.10	5.76	0.748
0.100	0.962	1.68	7.32	5.82	0.761
0.150	0.928	1.02	6.98	5.06	0.620
Con	0.958	1.10	6.84	4.62	0.520

cell concentration was attained on the sixteenth day at 15 ppt in 0.02 ppm Cu (Fig. 9).

A wide variation in the concentration of chlorophyll a and non-chlorophyllous pigments was recognised throughout the life cycle in relation to various salinities and copper concentrations. On the sixth day, the amount of chlorophyll a and non-chlorophyllous pigments per unit number of cells reached their maximum level of 11.24 µg and 9.0 µg in 0.15 ppm and 0.10 ppm Cu respectively. Consequently, a constant decline in the total quantity of pigments was observed even though there was an increase in cell concentration (Table 3).

With an increase in copper concentration, the rate of production was higher at 35 ppt but during the later stages of growth carbon production was more at lower salinities. Production recorded a maximum of 8 mg C/l/hr in 0.15 ppm Cu at 35 ppt on the sixth day about six times than that of control. On the approach of stationary phase, production values were lower than those of control in 0.07 ppm and 0.15 ppm Cu (Table 3).

Alkaline pH range above 7.0 was more suitable for the growth of *S. salina*. Lower copper concentrations of 0.02 ppm and 0.05 ppm accelerated the growth rate of the alga in comparison to 0.07 ppm Cu, wherein the activity of the blue green alga was lower than that of control.

FIG. 9. *Synechocystis salina*

Effect of copper on the biomass in different salinities (S).

S_1 - 15 ppt

S_2 - 25 ppt

S_3 - 35 ppt.

Copper concentrations

 - 0.020 ppm

 - 0.100 ppm

 - 0.050 ppm

 - 0.150 ppm

 - 0.070 ppm

 - Control

FIG. 9

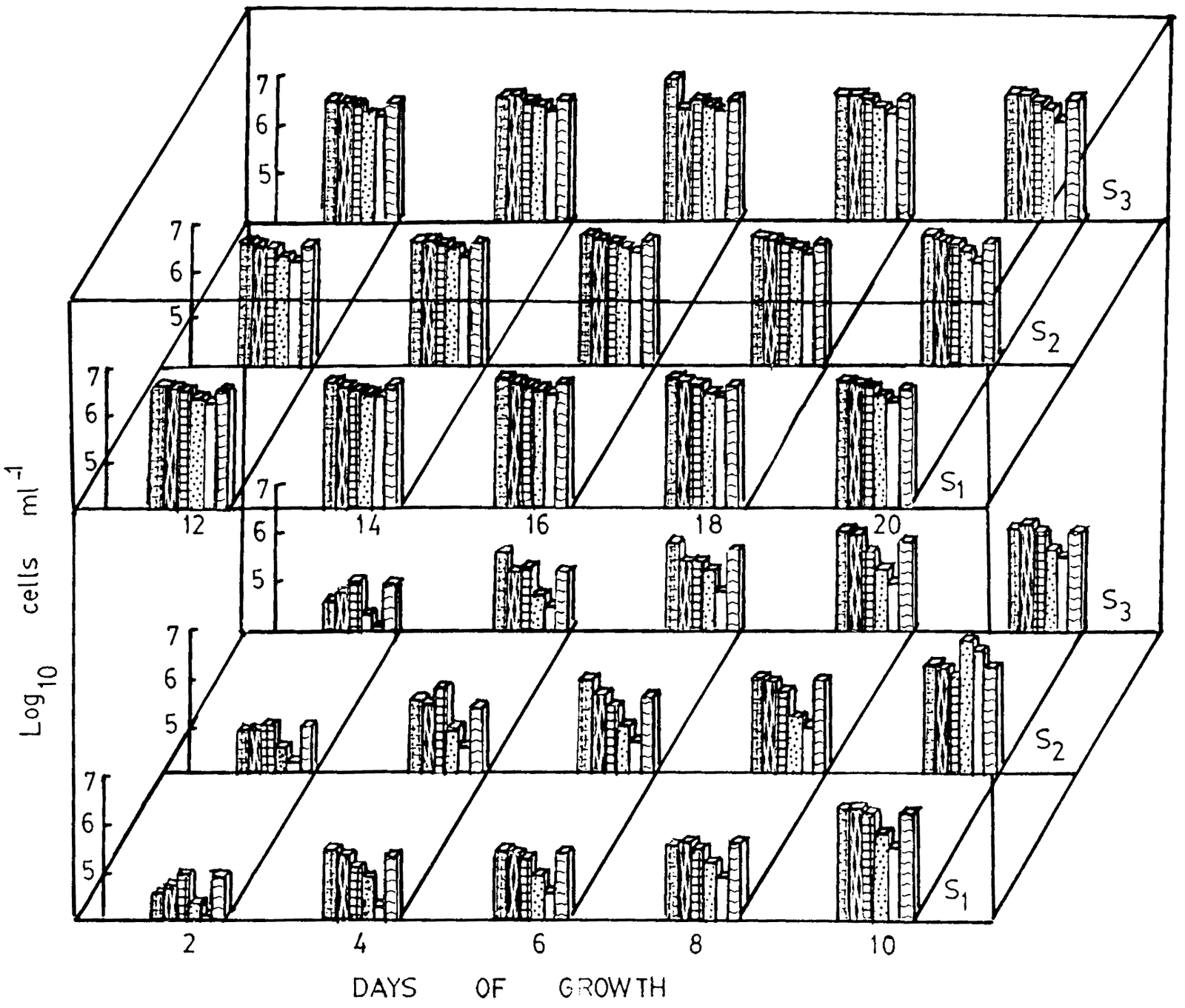

Log10 cells ml-1
7
6
5
S3
S2
S1
12
14
16
18
20
2
4
6
8
10
DAYS OF GROWTH

Table 3. Effect of copper on the physiological activity of *S. salina* in different salinities.

		15ppt				25ppt				35ppt			
Days of growth	Cu. Concn. (ppm)	Cell Concn/ ml	Chl 'a' µg/10^6 cells	Non-chl. Pig. µg/ 10^6 cells	Prodn. mgC/ l/hr.	Cell Concn/ ml.	Chl. 'a' µg/10^6 cells	Non-chl. Pig. µg/ 10^6 cells	Prodn. mgC/ l/hr.	Cell Concn/ ml.	Chl. 'a' µg/10^6 cells	Non-chl Pig. µg/ 10^6 cells	Prodn. mgC/l/ hr.
2	0.020	38,000	0.480	0.512	0.324	80,000	0.491	0.520	0.646	75,000	0.463	0.496	0.632
	0.050	55,000	0.491	0.486	0.508	70,500	0.452	0.510	0.610	81,000	0.546	0.581	0.614
	0.070	95,000	0.610	0.596	0.662	1,10,000	0.591	0.586	0.728	1,00,000	0.591	0.520	0.720
	0.100	20,000	0.410	0.578	0.298	40,000	0.622	0.604	0.556	39,500	0.602	0.586	0.542
	0.150	10,000	0.564	0.670	0.254	20,500	0.648	0.565	0.512	19,500	0.588	0.621	0.508
	CON	90,000	0.541	0.601	0.604	1,00,000	0.583	0.620	0.623	90,500	0.592	0.590	0.604
4	0.020	2,60,000	1.60	2.00	1.38	3,50,000	1.98	2.06	2.24	4,10,000	1.17	7.50	2.88
	0.050	2,50,000	1.64	1.08	1.20	3,00,000	1.55	1.87	1.42	1,60,000	1.32	1.70	1.04
	0.070	1,20,000	3.04	2.33	1.02	1,90,000	2.24	2.63	1.16	1,90,000	1.42	1.44	1.12
	0.100	80,000	3.49	2.75	0.84	90,000	5.19	4.67	0.91	50,000	2.07	5.00	0.73
	0.150	20,000	4.02	3.86	0.56	40,000	5.86	1.35	0.59	30,000	2.40	5.33	0.57
	CON	2,50,000	2.62	2.63	1.18	2,40,000	3.70	2.83	1.21	1,65,000	5.18	5.30	0.98
6	0.020	2,80,000	4.51	4.07	1.62	8,80,000	1.27	1.68	4.50	6,90,000	1.01	1.88	3.84
	0.050	2,70,000	2.13	1.74	1.54	4,30,000	3.60	1.77	2.92	2,60,000	2.76	3.56	2.16
	0.070	2,30,000	1.76	1.91	1.46	2,60,000	5.62	1.58	1.66	2,50,000	1.57	2.67	1.48
	0.100	90,000	3.49	4.56	0.92	1,10,000	5.12	7.40	1.20	1,60,000	5.03	6.00	1.12
	0.150	40,000	8.05	9.00	0.61	50,000	11.20	8.60	0.63	55,000	7.18	8.00	0.62
	CON	2,60,000	1.78	2.46	1.50	4,20,000	1.57	1.35	2.86	4,60,000	1.39	1.84	2.74
8	0.020	4,30,000	2.24	2.06	2.30	10,50,000	0.92	1.01	4.78	9,50,000	0.972	1.48	4.70
	0.050	4,20,000	1.08	0.98	2.26	8,60,000	1.13	0.96	3.94	9,00,000	1.550	1.91	3.96
	0.070	3,10,000	0.93	0.83	1.98	4,80,000	1.85	0.95	2.86	4,40,000	0.987	1.17	2.72
	0.100	1,50,000	1.88	1.91	1.24	1,70,000	2.12	2.48	1.38	1,50,000	2.360	3.24	1.22
	0.150	80,000	3.06	3.83	0.94	1,00,000	3.86	3.30	0.95	90,500	3.030	2.87	0.94
	CON	4,10,000	0.96	1.24	2.60	8,80,000	1.08	0.98	3.90	6,60,000	0.975	0.91	3.34
10	0.020	20,50,.000	1.81	1.34	4.62	18,50,000	0.832	0.916	4.94	13,00,000	0.840	0.950	4.90
	0.050	19,50,000	0.94	0.90	4.40	15,50,000	0.920	0.810	4.36	14,50,000	0.938	0.962	4.28
	0.070	15,50,000	0.86	0.85	3.84	11,50,000	0.948	0.776	3.60	10,50,000	0.854	0.810	3.54
	0.100	6,50,000	0.99	0.91	2.46	5,50,000	1.170	1.380	2.28	4,30,000	0.990	1.060	2.10
	0.150	3,20,000	1.17	1.45	1.65	3,50,000	1.220	1.430	1.70	3,10,000	1.120	0.9 1	1.66
	CON	17,50,000	0.91	1.02	3.96	14,50,000	0.952	0.936	3.98	11,50,00	0.902	0.977	3.84

		15 ppt				25 ppt				35 ppt			
Days of growth	Cu. Concn. (ppm)	Cell Concn/ ml	Chl 'a' $\mu g/10^6$ cells	Non-chl. Pig. $\mu g/10^6$ cells	Prodn. mgC/ l/hr.	Cell Concn/ ml	Chl 'a' $\mu g/10^6$ cells	Non-Chl. Pig. $\mu g/10^6$ cells	Prodn. mgC/ l/hr.	Cell Concn/ ml	Chl 'a' $\mu g/10^6$ cells	Non-chl. Pig. $\mu g/10^6$ cells	Prodn. mgC/ l/hr.
12	0.020	38,50,000	0.771	0.802	4.74	37,50,000	0.740	0.828	4.98	35,50,000	0.784	0.723	4.60
	0.050	35,50,000	0.640	0.698	4.56	36,00,000	0.636	0.623	4.42	33,00,000	0.655	0.646	4.46
	0.070	31,50,000	0.615	0.642	3.88	30,50,000	0.617	0.631	3.64	28,50,000	0.605	0.613	3.62
	0.100	21,50,000	0.704	0.736	2.60	19,50,000	0.728	0.716	2.40	16,50,000	0.716	0.694	2.56
	0.150	18,00,000	0.760	0.641	1.78	16,50,000	0.761	0.639	1.80	15,50,000	0.780	0.646	1.72
	CON	32,50,000	0.612	0.683	4.22	30,50,000	0.662	0.681	4.10	29,50,000	0.642	0.670	4.18
14	0.020	44,50,000	0.454	0.498	4.52	43,50,000	0.412	0.502	4.46	41,50,000	0.436	0.490	4.40
	0.050	43,00,000	0.430	0.407	4.34	40,50,000	0.449	0.474	4.28	39,50,000	0.457	0.442	4.16
	0.070	36,50,000	0.398	0.309	3.64	33,50,000	0.418	0.415	3.52	31,50,000	0.413	0.431	3.42
	0.100	28,50,000	0.490	0.508	2.46	26,50,000	0.477	0.486	2.40	24,00,000	0.451	0.480	2.31
	0.150	23,00,000	0.512	0493	1.54	21,50,000	0.502	0.498	1.46	19,25,000	0.477	0.462	1.34
	CON	38,50,000	0.436	0.488	4.10	36,50,000	0.431	0.491	3.98	34,00,000	0.412	0.439	3.86
16	0.020	50,50,000	0.206	0.230	4.20	48,50,000	0.244	0.212	4.14	49,50,000	0.215	0.198	4.12
	0.050	45,75,000	0.231	0.280	4.16	43,50,000	0.219	0.288	3.94	43,00,000	0.207	0.249	3.90
	0.070	39,80,000	0.201	0.202	3.42	36,00,000	0.205	0.200	3.20	33,50,000	0.194	0.200	3.02
	0.100	29,50,000	0.276	0.249	2.22	26,00,000	0.265	0.232	2.10	25,50,000	0.261	0.239	2.12
	0.150	24,00,000	0.309	0.336	1.36	23,50,000	0.316	0.302	1.14	20,50,000	0.303	0.280	0.99
	CON	41,00,000	0.206	0.230	3.96	37,50,000	0.205	0.247	3.72	33,50,000	0.219	0.239	3.50
18	0.020	48,50,000	0.194	0.205	3.86	45,75,000	0.190	0.202	3.74	43,00,000	0.175	0.194	3.52
	0.050	44,50,000	0.188	0.191	3.40	41,00,000	0.172	0.187	3.28	39,50,000	0.181	0.177	3.16
	0.070	38,50,000	0.180	0.194	3.12	34,00,000	0.169	0.174	3.10	31,50,000	0.172	0.152	2.96
	1.100	26,50,000	0.205	0.191	2.04	24,00,000	0.204	0.198	1.96	21,50,000	0.196	0.190	1.84
	0.150	20,50,000	0.212	0.209	1.30	19,25,000	0.250	0.261	1.24	16,50,000	0.200	0.209	1.12
	CON	37,00,000	0.194	0.200	3.38	35,50,000	0.196	0.218	3.30	33,00,000	0.198	0.188	3.20
20	0.020	44,50,000	0.169	0.194	2.40	42,75,000	0.157	0.148	2.36	40,50,000	0.148	0.118	2.28
	0.050	42,00,000	0.154	0.181	2.26	40,50,000	0.131	0.128	2.20	38,00,000	0.134	0.122	2.14
	0.070	35,50,000	0.137	0.154	1.96	33,00,000	0.119	0.138	1.84	29,50,000	0.092	0.116	1.62
	0.100	23,00,000	0.175	0.188	1.64	21,50,000	0.150	0.173	1.60	19,50,000	0.128	0.154	1.46
	0.150	15,50,000	0.189	0.172	0.98	13,00,000	0.161	0.152	0.97	11,50,000	0.149	0.160	0.96
	CON	34,00,000	0.144	0.177	2.16	31,50,000	0.111	0.121	2.06	30,50,000	0.135	0.131	2.00

In all the three treated concentrations of copper, biomass increased from pH 6.0 to pH 8.0. Cell content was maximum on the sixteenth day in 0.02 ppm Cu at pH 8.0 about 20% higher than that of control (Fig.10 B).

At all pH levels, the quantity of chlorophyll a and non-chlorophyllous pigments increased till the twelveth day, thereafter decreased till the end of the stationary phase. Chlorophyll a and non-chlorophyllous pigments reached their peak level on the twelveth day in 0.02 ppm Cu, about 22% and 10% higher than those of control (Fig. 11).

Carbon production recorded its highest value for the three employed copper concentrations on the twelveth day. In 0.02 ppm and 0.05 ppm Cu, the rate of production was greater than that of control during all phases of growth (Fig.10 A).

Low concentration of copper 0.02 ppm stimulated the growth rate of the blue green alga. Carbon fixation rate increases from 20°C to 25°C and thereafter exhibited a decline towards higher temperatures. Carbon production was maximum at 25°C in 0.02 ppm about 28% higher than that of control. At different temperatures when the blue green alga was treated with 0.07 ppm Cu, the production rate was lower than that of control (Table 4).

Copper speciation studies:

By the application of anodic stripping voltammetry, sample peak heights were determined from the polarograms of *I. galbana* and

FIG. 10. *Synechocystis salina*

Effect of copper on biomass and rate of carbon production at different pH.

A - Production

B - Biomass

Copper concentrations

 - 0.020 ppm

 - 0.050 ppm

 - 0.070 ppm

 - Control

FIG.10

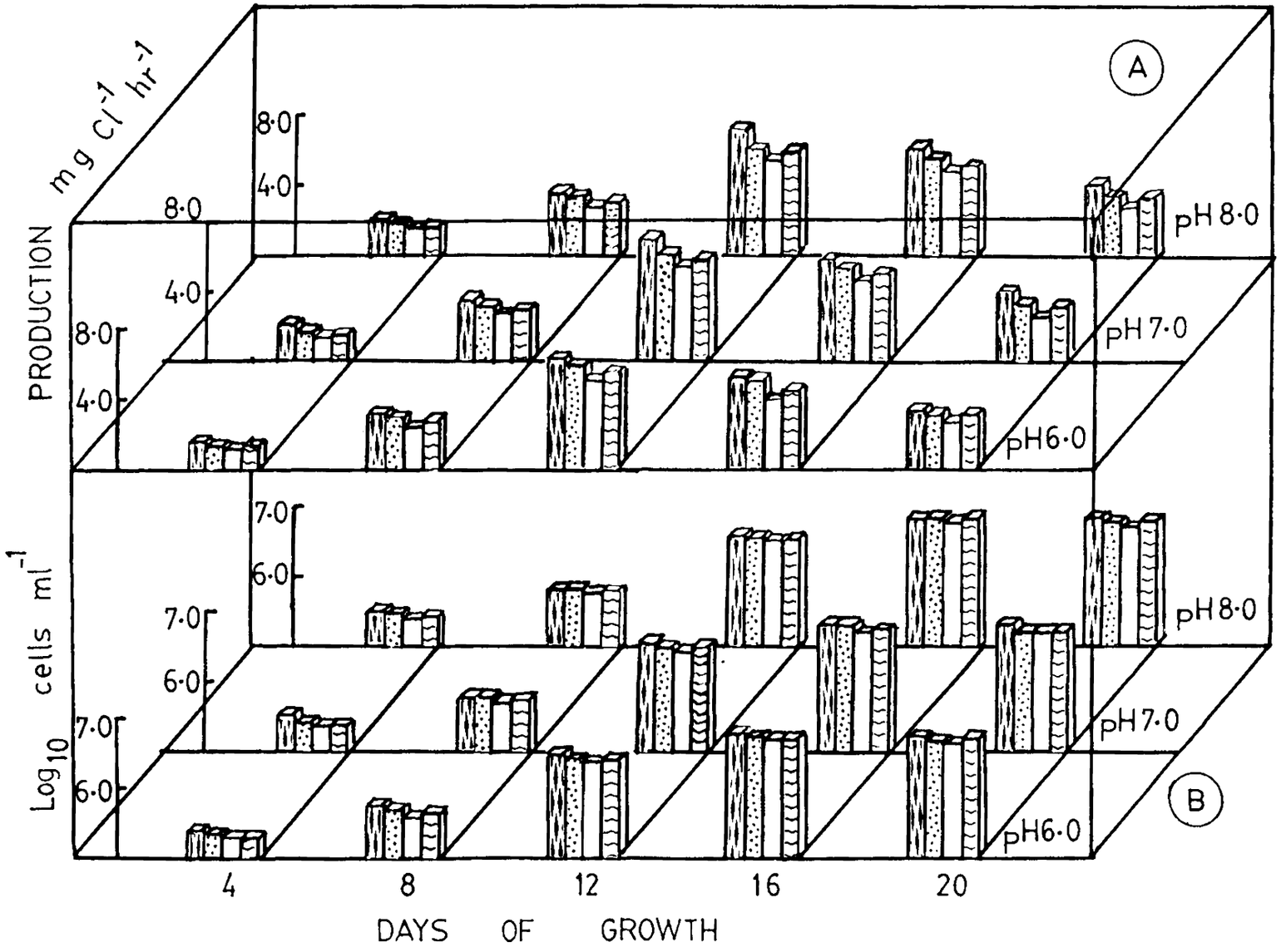

A
B
PRODUCTION mg C l⁻¹ hr⁻¹
Log10 cells ml⁻¹
8·0
4·0
7·0
6·0
pH 8·0
pH 7·0
pH6·0
4
8
12
16
20
DAYS OF GROWTH

FIG. 11. *Synechocystis salina*

Effect of copper on the chlorophyll 'a' content and non-chlorophyllous pigments at different pH.

A - Non- chlorophyllous pigments

B - Chlorophyll 'a'

Copper concentrations

 - 0.020 ppm

 - 0.050 ppm

 - 0.070 ppm

 - Control

FIG. 11

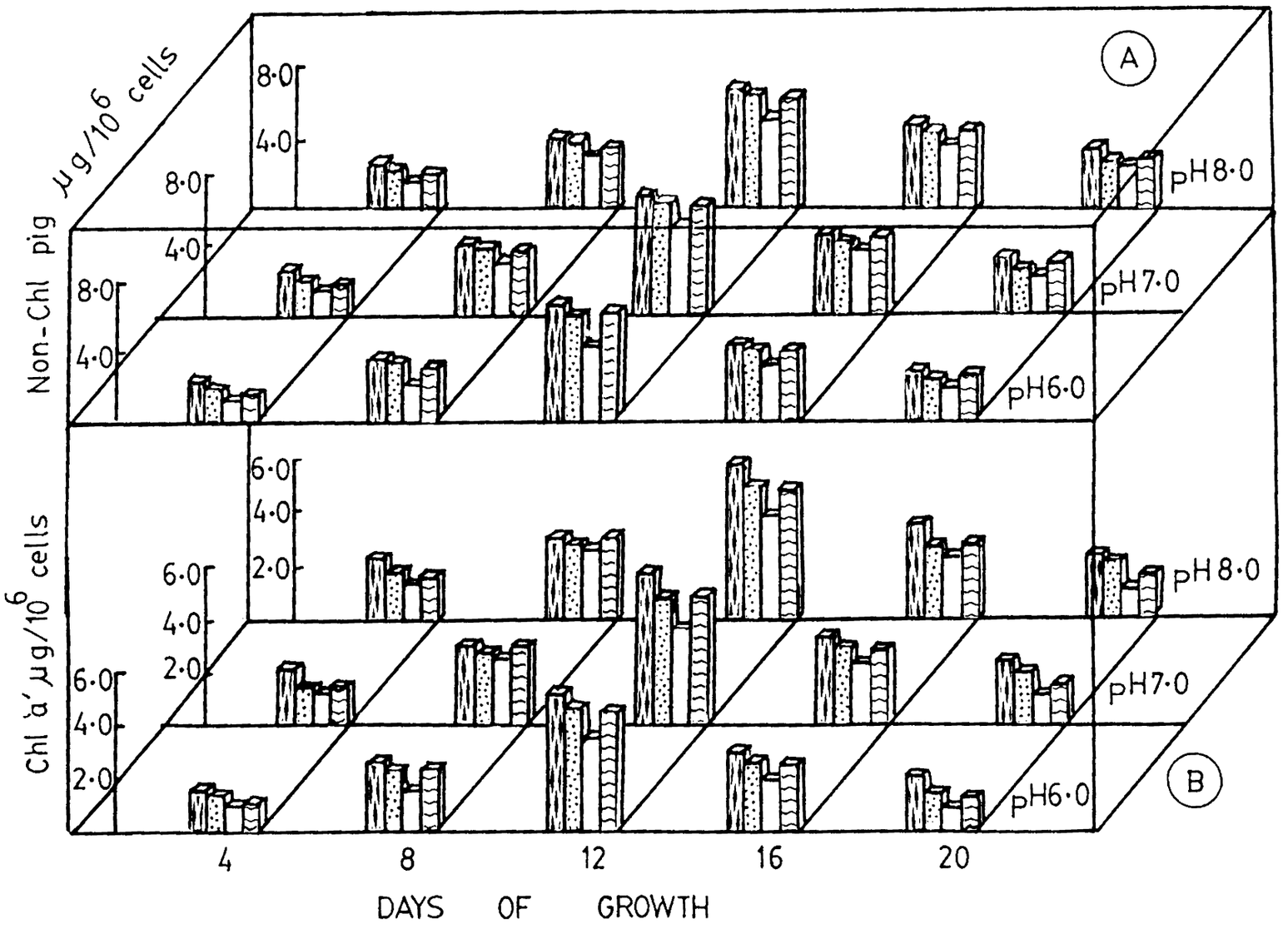

A
B
Non-Chl pig µg/10⁶ cells
Chl 'a' µg/10⁶ cells
8·0
4·0
6·0
2·0
pH8·0
pH7·0
pH6·0
4
8
12
16
20
DAYS OF GROWTH

Table 4. Effect of Cu, Mn and Zn on the rate of carbon production (mgC/l/hr) at different temperatures in *S. salina*.

Metal Concn(ppm)	20°C	25°C	30°C	35°C	40°C
Cu					
0.050	3.84	14.40	6.22	5.14	3.40
0.100	3.16	12.26	6.01	4.94	2.95
0.150	2.52	08.32	5.60	4.32	2.20
Mn					
0.050	2.91	11.85	6.11	5.26	3.24
0.100	3.62	12.60	6.72	5.30	3.56
0.150	4.14	13.16	7.08	5.82	3.60
Zn					
0.020	3.76	11.94	5.96	4.98	3.04
0.050	4.02	13.36	6.54	5.42	3.28
0.070	3.40	10.25	5.18	4.80	2.30
Con	2.64	11.24	5.80	4.89	2.25

S. salina. Sample peak height for the cells of the flagellate exposed to 16 hours light period was 1.5 cm and in the supernatant 6.7 cm, whereas the cells grown in continuous light for 48 hrs showed a peak height of 2.4 cm and the supernatant 1.5 cm (Fig. 12).

On exposure to a shorter photoperiod, the flagellate exhibited a greater peak height in the supernatant than in the cells indicating that the accumulation of copper in the cells was relatively less. Instead on exposure to a longer photoperiod, peak height was greater in the cells than in the supernatant which is a clear evidence to show that with greater exposure time, larger amount of copper ions gets incorporated into the cells (Fig. 13).

The calculated concentration of copper in the original sample was 0.26 ppm in the culture grown under continuous 16 hrs light period and 0.052 ppm in the culture grown under 48 hrs light period which was comparatively low to prove that some of the copper ions had undergone complexation accounting for its low concentration.

In *S. salina,* sample peak height in the acid digested cells was 4.2 cm and in the supernatant 0.2 cm (Fig. 13). In this strain with the addition of 0.200 ppm Cu, there was quick adsorption of ions onto the cells in a short duration than observed in the flagellate. The calculated copper concentration of the original sample was 0.187 ppm indicating that remaining amount of ionic copper would have formed

FIG. 12.

Voltammetric determination with DPASV at the HMDE of complexation capacity of *I. galbana* with copper. Anodic stripping peak heights are indicated in the Voltammogram.

X calib - 100 mV/cm; Y calib - 500 mV/cm

A - Cells exposed to 16 hrs light period

B - Supernatant of the above.

C - Cells exposed to 48 hrs light period.

D - Supernatant of the above.

FIG - 12

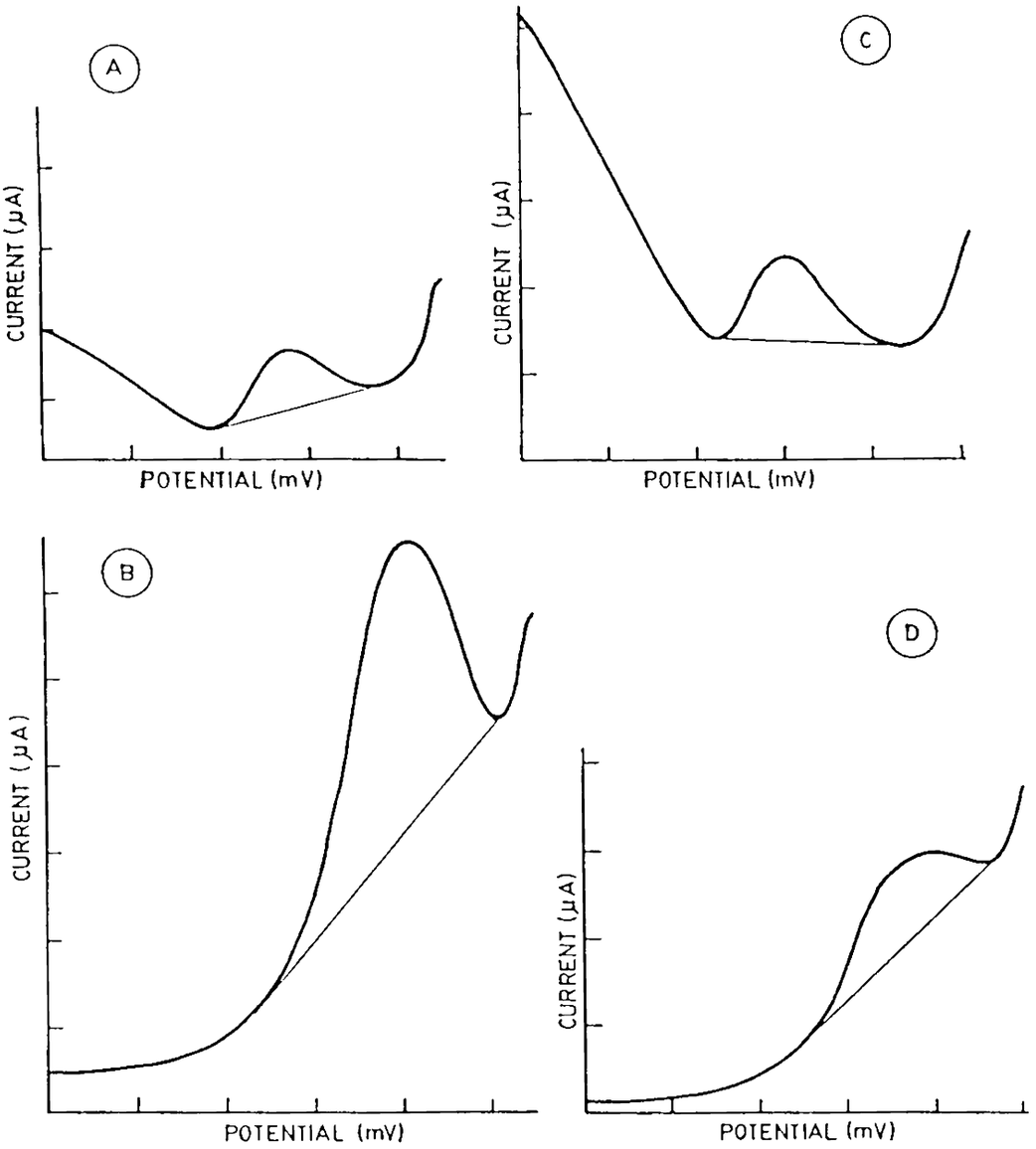

A
CURRENT (μA)
POTENTIAL (mV)
C
CURRENT (μA)
POTENTIAL (mV)
B
CURRENT (μA)
POTENTIAL (mV)
D
CURRENT (μA)
POTENTIAL (mV)

FIG. 13.

Voltammetric determination with DPASV at the HMDE of complexation capacity of *S. salina* and the culture medium with copper.

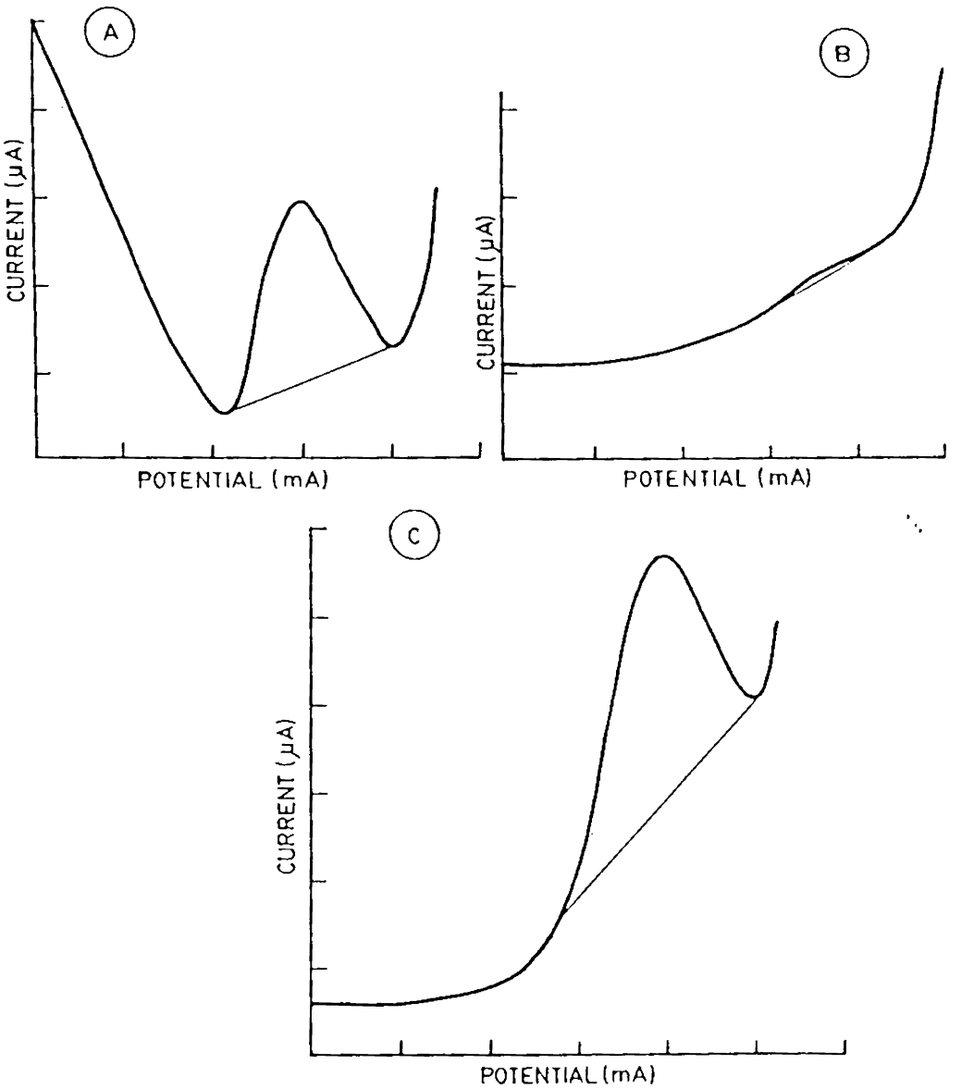
X calib - 100 mV/cm; Y calib - 500 mV/cm.

A - Cells

B - Supernantant

C - Culture medium with 0.200 ppm Cu.

FIG-13


A
CURRENT (μA)
POTENTIAL (mA)
B
CURRENT (μA)
POTENTIAL (mA)
C
CURRENT (μA)
POTENTIAL (mA)

complexes with other complexing agents and would have got incorporated into the particles of the medium.

Effect of copper on the ultrastructural organisation of *I. galbana* and *S. salina:*

To understand the effect of copper at the intracellular level, it was necessary to study the structure of control cells in detail for comparison with the metal treated ones.

General structure of *I. galbana:*

A golden yellow flagellate with an active movement. Freshly mounted live cells appear slightly elongated and compressed. Each cell carries two equal flagella and a reduced knob like haptonema in between the flagella. Flagella are visible as two stiff hair like structures but during the process of specimen preparation one of the flagella was lost (Plate:I). The cells spin rapidly and show a gliding movement. Microanatomy of the flagellum and the flagellar base was not analysed.

Ultra thin sections of a cell display the following details: A single golden brown saucer shaped parietal chloroplast per cell with a pyrenoid embedded at the basal region. Chloroplast is bounded by a double membrane and contains thylakoid lamellae. A single nucleus which is roughly spherical in shape lies posterior to the large golgi body and is often flattened against the face of the chloroplast. Golgibody

Plate I: *Isochrysis galbana*

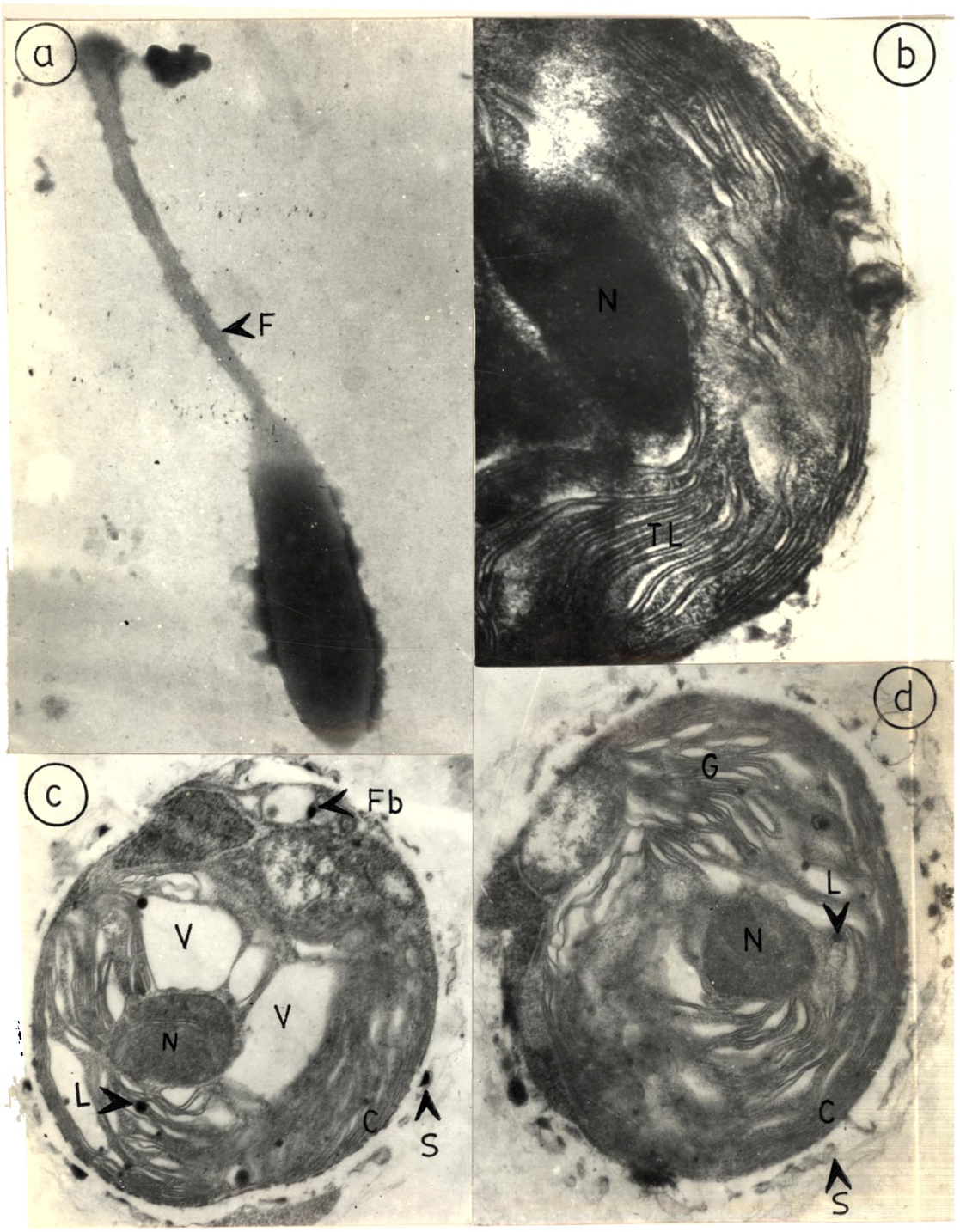
a) Electron micrograph of a whole mount control cell showing only one flagellum (F) (X 12,000).

b) Electron micrograph showing section of a control cell with a parietal saucer shaped chloroplast containing thylakoid lamellae (TL) and a nucleus (N) flattened against the face of the chloroplast. (X 20,000).

c) Electron micrograph of longitudinal section of a Cu-treated cell showing the flagellar base (Fb), enlarged vacuoles (V), lipid globules (L), nucleus (N) and chloroplast (C). Cell is covered by scales(S) (X 12,000).

d) Electron micrograph of longitudinal section of a control cell showing chloroplast (C), nucleus (N), lipid globule (L), golgibodies (G) and scales (S) (X 12,000).

PLATE -I



lies close to the flagellar basal bodies and haptonemal base (Plate:II). Tubular cristae representing the mitochondria was not clearly visible in the sections taken. Lipid globules are distributed throughout the cell and their number increases with ageing. Cells are covered over by unmineralised scales (Plate:I).

When the cells of the flagellate were treated with a higher dosage of copper, intracellular disorganisation was not noticed in comparison to control. The only difference that could be detected was the presence of enlarged size vacuoles and more number of lipid globules (Plate:I).

General structure of *S. salina:*

Small spherical cells of diameter 3 μ with bluish green colour. Sometimes the cells occur in two's exhibiting the division stage clearly. Each cell is surrounded by a gelatinous sheath and enclosed by typical wall layers. The cell envelope is made up of an inner most cytoplasmic membrane, electron dense layer, an electron transparent layer and a thin medium dense layer with a wrinkled appearance. Electron micrographs of negatively stained preparations indicated the presence of radial mucilaginous filaments projecting from the cell's surface (Plate:III, IV).

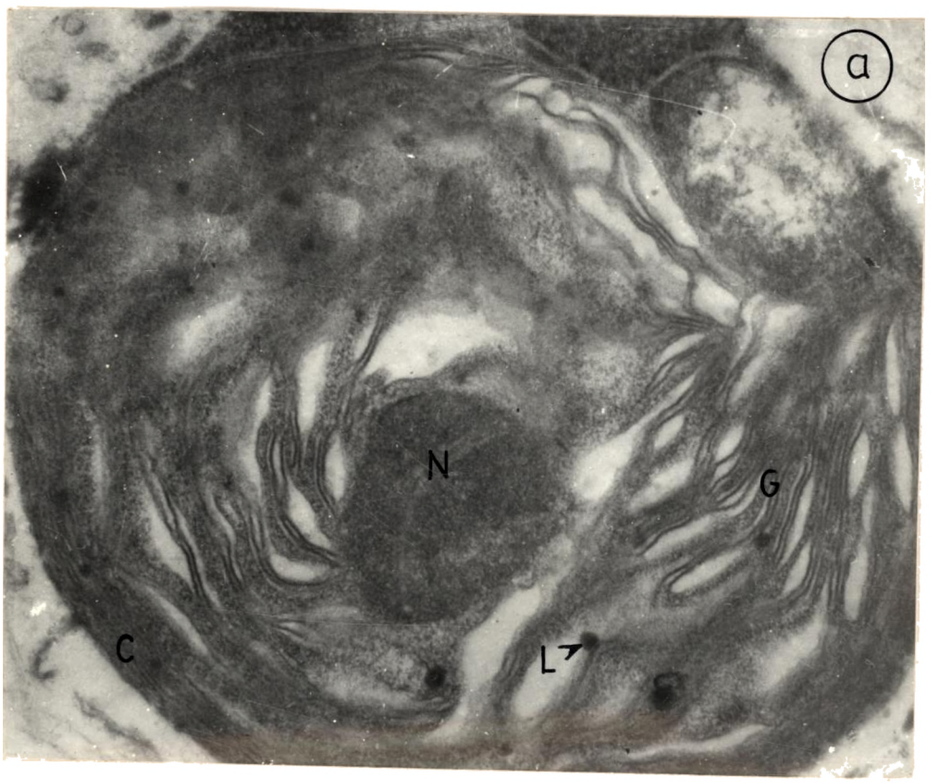
Within each cell, the photosynthetic lamellae or thylakoids are arranged peripherally in four to five concentric layers running

Plate II:

a) Electron micrograph showing the golgi structure (G) in *I. galbana*. Nucleus (N), Chloroplast (C) and lipid globules (L) are also visible in the section (X 12,000).

b) Electron micrograph indicating a portion of the dilations of the cisternae (Ci) in the golgi apparatus of *I. galbana* (X 12,000).

PLATE - II



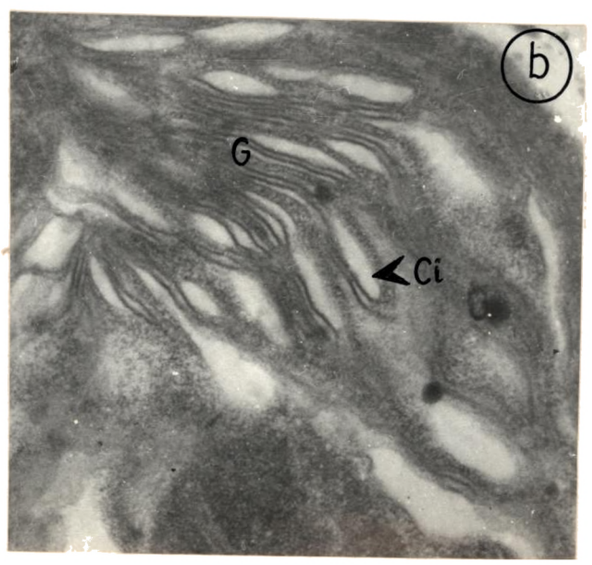
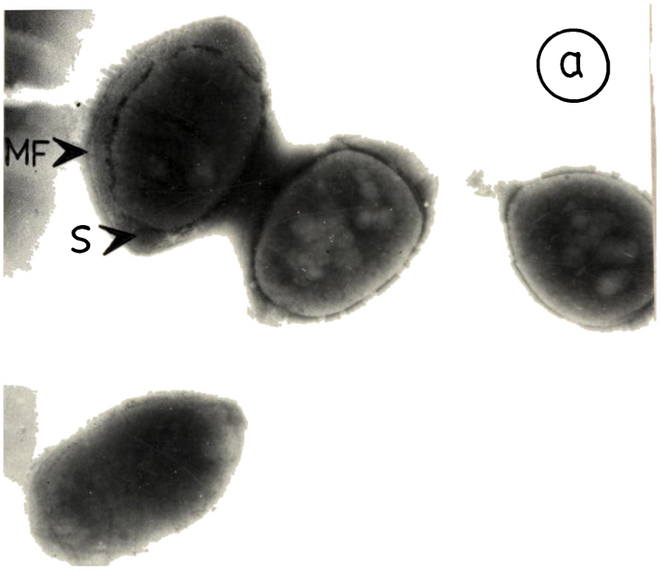


Plate III:

a) Electron micrograph of negatively stained preparation of a control *S. salina* cell indicating the projecting mucilaginous filaments (MF) from the cell surface. Mucilaginous sheath (S) is also visible. Note that the cell occurs in two's exhibiting the division stage clearly (X 12,000).

b) Electron micrograph of a mature control cell of *S. salina* showing the accumulation of cyanophycean granules (C) and lipid inclusions (L). (X 20,000).

PLATE - III



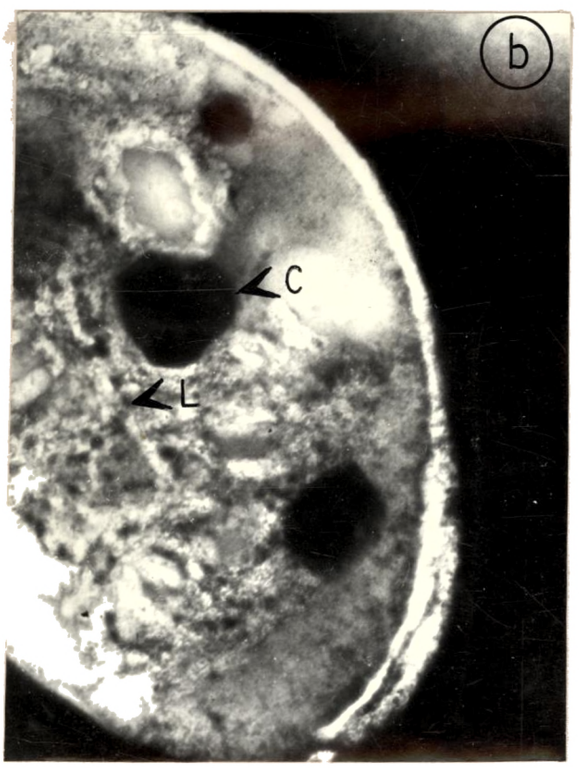


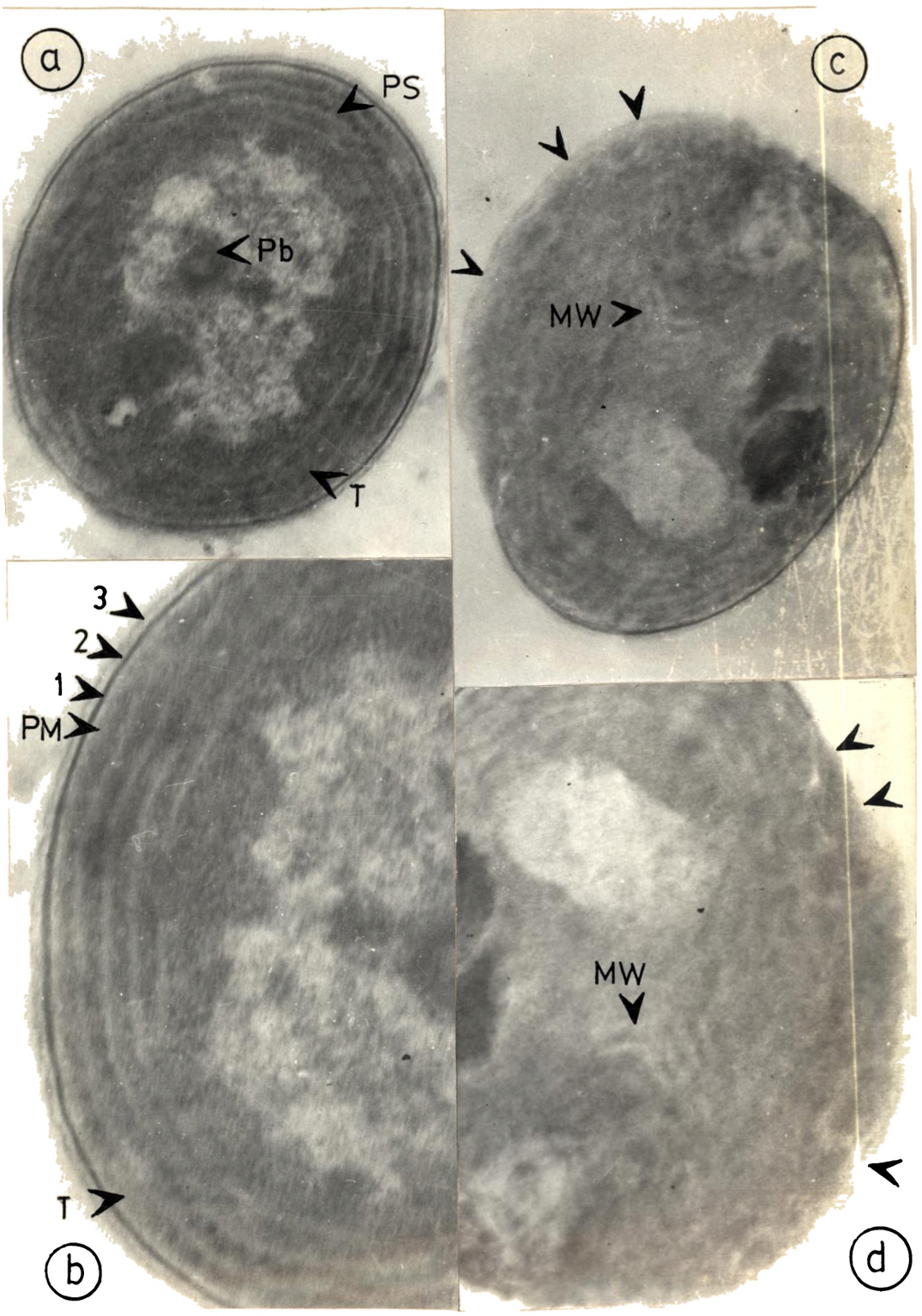
Plate IV: *Synechocystis salina*

a) Electron micrograph of a control cell showing the multi-layered cell wall, thylakoids (T), phycobilisomes (PS) and polyhedral bodies (Pb). (X 20,000).

b) Electron micrograph of a portion of a control cell showing the cell wall layers which are numbered 1 to 3 starting adjacent to the plasma membrane (PM). (X 20,000).

c) and d). Electron micrograph of a Cu - treated cell showing the presence of membrane whorls (MW) and the solid arrows indicating the discontinuity of cell envelope and photosynthetic lamellae. (X 20,000).

PLATE - IV



parallel to the envelope layer. On the outer surface of thylakoids, regular rows of electron dense structures are closely attached. These granules carrying the light harvesting phycobiliproteins are known as phycobilisomes (Plate: IV).

Cyanophycean granules are irregular in shape and larger in size especially in older cells. Apart from this, are seen the polyhedral and the polyphosphate bodies. Small structured granules representing the lipid inclusions are found scattered among the thylakoids (Plate:III).

This blue green alga when treated with a higher dosage of copper exhibited significant detoriation in the cell's internal organisation. Presence of membrane whorls in metal exposed cells and disorganisation of cell envelope and photosynthetic lamellae were noticed at certain regions of the cell (Plate:IV). Polyhedral bodies increased in size with copper exposure and also there was an overall reduction in cell size. No lipid inclusions were observed in metal treated cells.

Discussion:

Trace amounts of copper are essential for the metabolic processes of algae. Essentiality of copper as a micronutrient has been confirmed by a number of investigators (Green *et al.*, 1939; Walker, 1953; O'Kelley, 1974; Sorentino, 1979). Different types of phytoplankton vary in their ability to withstand the toxic effects of the metal. Major trend among species in their resistance to copper toxicity has been a phylogenetic one,

with cyanophycean members being the most sensitive, diatoms, green algae, the least sensitive, coccolithophores and dinoflagellates intermediate in sensitivity (Mandelli, 1969; Erickson *et al.*, 1970; Brand, Sunda and Guillard, 1986).

There is mounting evidence that copper can be toxic to phytoplankton at its natural concentration in unpolluted waters. With regards to the two species considered for the present study, the coccoid cyanophycean member *Synechocystis salina* is found to be more sensitive to copper than the haptophycean member *Isochrysis galbana*. Copper concentrations namely, 0.05 ppm and 0.10 ppm were more suitable for the growth of the flagellate but concentrations above 0.10 ppm were inhibitory to the blue green algae. Concentration of copper as low as 1 ppb was reported to be toxic to phytoplankton (Steemann Nielsen *et al.*, 1969; Erickson, 1972; Kanakavalli Susarla, 1987). Higher concentrations of copper greater than 0.15 ppm impaired the growth rate and resulted in biomass reduction. Whitton (1970) has listed several macro and micro algae to be tolerant to 1.5-2.0 mg/l Cu and has shown that 0.02 ppm to 0.10 ppm Cu has a stimulatory effect. Concentration of 10-50 µg/l Cu has little or no effect upon growth rate in *Coccolithus huxleyi* and *Phaeodactylum tricornutum* (Bernhard and Zattera, 1970). Indication of good growth at 0.04 ppm Cu in *Scenedesmus* (Stokes *et al.*, 1973a,b) is also true in the case of *S. salina*. Gnassia-Barelli (1978) has shown that the toxicity threshold in *Cricosphaera elongata* reaches only when the concentration is 0.05-0.10 µg Cu l^{-1}.

Foster (1977) has indicated that the tolerant strain of *Chlorella vulgaris* was not affected by 0.100 ppm Cu and the culture survived even at 1.00 ppm Cu.

Sensitivity to copper varies at different stages of growth and it also depends upon the biochemical make up of the cell. At a higher concentration of copper, 0.15 ppm, the quantity of pigments and the rate of carbon fixation were found to be lower than those of control. Loss of mobility and reduction in photosynthetic rate were also reported by Anderson and Morel (1978) in *Gonyaulax tamarensis*. Saward *et al.*,(1975) have noticed a depression in both standing crop of phytoplankton and rate of photosynthesis/unit chlorophyll a in 0.03 to 0.10 ppm Cu.

Prolongation of the lag period in *I. galbana* and *S. salina* in 0.15 ppm Cu may simply reflect the biochemical differences between the cells of the lag and exponential phases. Copper could block the initial division temporarily, thus extending the phase, but may not have any effect on the divisions during exponential phase (Morel *et al.*, 1978).

Copper is known to affect cell separation in *S. salina* during the early growth phase resulting in formation of multicellular aggregates which persist until the late log phase. Similar effect has also been noticed by Foster (1977) in *Chlorella vulgaris*. Kanazawa and Kanazawa (1969) have attributed the toxic effect of copper on cellular division due to events related to cell membrane rather than with those occurring inside the cell. Transmembranous movement of ions is usually influenced

to a large extent by the difference in pH between the outside and inside of the cell. Greater tolerance of *I. galbana* to copper can be accounted by the organism's capacity to block the entrance of cations to the cell. This may be accomplished either through binding of the metal to the cytoplasmic membranes or to the extracellular polysaccharides or by changing the chemical form in which copper is present, so as to render it toxicologically inactive.

Preliminary investigations using the polarographic analyser revealed a pattern of uptake of copper similar to that reported for a range of metals in other algae (Mandelli, 1969; Button and Hostetter, 1977; Anderson and Morel, 1978), namely an initial rapid uptake and thereafter a slower rate of uptake. Though the surface area of *S. salina* cells is comparatively smaller than that of *I. galbana* but on a cell to cell basis, prolific cell number in *S. salina* may account for the greater adsorption of copper ions on the cell surface on short term exposure. Cells of *S. salina* sorb approximately three times more copper than the cells of *I. galbana* resulting in a greater peak height. The other reason can be most of the copper remained bound to the cell wall and probably did not enter the protoplasm in *I. galbana* whereas most of the copper sorbed by *S. salina* could have been associated with plasmalemma or entered into the cytoplasm inhibiting metabolically active sites.

Dense cultures of *I. galbana* and *S. salina* on the approach of stationary phase seemed to overcome the inhibitory act of copper, an

effect believed to be partly brought about by exudates which complexed the copper ions (Steemann Nielsen and Wium-Andersen, 1971; Erickson, 1972). Higher tendency towards copper complexation exhibited by *S. salina* can inactivate numerous enzymes through displacement of the activating metal or through binding to sulfhydryl or other functional groups (Eichorn, 1974).

Substantial evidences indicate that the biological availability of copper can be reduced by phytoplankton exudates. The early work of Fogg and Westlake (1955) demonstrated that the presence of copper complexing extracellular polypeptides produced by *Anabaena cylindrica* reduced the toxicity of copper to this blue green alga. Steemann Nielsen *et al.*, (1969) and Steemann Nielsen and Wium-Andersen (1971) proposed that the exocellular slime coat of *Chlorella pyrenoidosa* and organic matter excreted by *Nitzschia palea* is effective in reducing copper toxicity. More definitive studies (Sunda, 1975; Sunda and Guillard, 1976; Gnassia-Barelli *et al.*, 1978; Mcknight and Morel, 1980) have demonstrated that cultures of *Thalassiosira psuedonana, Cricosphaera elongata, Skeletonema costatum, Anabaena flos-aquae, Anabaena cylindrica* excrete substances into the media that are capable of complexing copper. On the other hand Swallow *et al.*, (1978) found no measurable copper complexation in culture filtrates of seven of the eight species studied. The preceding discussion that algae possess the capacity to produce extracellular chelators which complex and thereby detoxify the action of copper is also applicable to *I. galbana* and *S. salina*.

The effects of copper ions and their accessibility to algae depend upon the external factors like salinity, pH and temperature. In 0.05 ppm and 0.10 ppm copper treatments, stimulation of growth in *I. galbana* was noted at higher salinities at an acidic pH range of 6.0 and at a temperature of 30°C. In case of *S. salina* enhancement of growth was pronounced in 0.02 ppm and 0.05 ppm copper treatments in lower salinities, at alkaline pH level above 7.0 and at a temperature of 25°C.

Mandelli (1969) found a negative correlation of salinity with the log of the ratio of copper uptake and phytoplanktonic biomass, whereas a direct relation between salinity and reduction of copper toxicity to *Cladophora* has been reported by Betzer and Kott (1969). Forstner and Prosi (1979) suggested that an increase in salinity of an aquatic system leads to competition between adsorbed heavy metals and dissolved cations, latter partially replacing the heavy metals thereby decreasing their bio-availability. These facts are in proof of the presence of greater biomass, higher concentration of pigments and carbon production in *I. galbana* at higher salinity of 35 ppt. Reduction in the toxicity of Cu to *S. salina* in saline waters is probably due to the alkaline pH medium.

In *I. galbana*, an increase in pH towards the alkaline level has been found to increase the toxicity of copper which is in confirmity with the reports of Steemann Nielsen and Kamp-Nielsen, (1970), Gachter, (1976), Hargreaves and Whitton, (1976a,b) in *Chlorella*, natural populations of algae and *Hormidium rivulare*. Peterson and Healey (1985) have

also demonstrated that the toxicity of cupric ion increased with pH in *Scenedesmus quadricauda*.

There are evidences to support the fact that copper exerts more toxic effect in acidic conditions on *S. salina* and that the toxicity declines at alkaline pH. This is because at acidic pH, metals exist in free ionic forms, whereas at alkaline pH they tend to precipitate as insoluble carbonates, phosphates, sulphides, oxides or hydroxides (Forstner and Prosi 1979). Michnonwicz and Weaks (1984) have demonstrated that adjustment of pH to higher values had a marked effect in ameliorating the pH shock to the cells of *Selenastrum capricornutum*.

Kanazawa and Kanazawa (1969) have shown the inhibitory effect of copper was considerably stronger in *Chlorella pyrenoidosa* at pH 6.3 than at pH values 7.1 and 5.1. Peterson and Patrick Healey (1985) have also demonstrated that copper toxicity increased by 3 to 48 times between pH 5 and 6, where Cu^{2+} dominates the dissolved pool, thereafter decreased between pH 7 and 8 with a shift to uncharged inorganic complex domination. The increase and decrease in toxicity of copper to *I.galbana* and *S. salina* at acidic or alkaline pH might be as a result of increased or decreased bioavailability of cupric ion under the selected pH conditions.

Very little is known about the impact of temperature in increasing or decreasing the toxic effect of copper to algae. Maximum rate of carbon production was observed between 25°C-30°C in *S. salina* and *I. galbana*.

A reduction in the rate of photosynthesis at higher temperatures above 40°C may be due to an increase in the viscosity of protoplasm, denaturation of proteins or nutritional starvation (Heilbrunn, 1952; Hutner *et al.*, 1957).

The purpose of electron microscope studies was to document and quantify cellular changes brought about by short term exposure to copper and to use the quantification of these changes as a means of focusing attention on the key structural changes induced by heavy metal toxicity. No apparent effect on the structural integrity of *I. galbana* was observed in comparison to *S. salina*. Enlarged size of the vacuoles in copper treated *I. galbana* cells can be compared with the increased vacuolation in the cytoplasm of *Scenedesmus* (Silverberg *et al.*, 1976). An increase in the number of lipid inclusions in *I. galbana* may indicate a cellular mechanism of detoxification, because the affinity of metals for potential sulfur binding sites is an established fact (Rachlin *et al.*, 1984).

The structural details of *I. galbana* and *S. salina* were identified based on the reports of Hibberd, 1980, Lang, 1968; Gantt and Conti, 1969, Schulz-Baldes and Lewin, 1976 and Lounotmaa *et al.*, 1980.

Significant reduction in the cell size of *S. salina* has been also reported by Rachlin *et al.*, (1982, 1984) on exposure of *Plectonema boryanum* to Co, Ni or Cd and not with copper. Presence of membrane whorls in metal exposed cells could be a manifestation of toxicity or they may represent a cellular detoxification mechanism. Polyhedral bodies contain

the key enzyme of Calvin cycle. An increase in their size may cause coalescence of polyhedral bodies with these enzymes, making the enzymes less available to the cell and thereby interfering with the cell's ability to fix carbon-dioxide. Reduction in the quantity of cellular lipid material in copper treated cells of this alga has been also confirmed by Rachlin *et al.*, 1982 in *Plectonema boryanum*.

4.2.2. Manganese:

Isochrysis galbana.

Higher concentrations of maganese did not have an appreciable effect on the growth of the flagellate. At 0.05 ppm concentration the activity of this alga is accelerated to a greater extent, where, in all the three salinities, the cell content and the rate of photosynthetic activity were found to be higher than those of control. But this observation was not recognised in the other two concentrations of manganese namely 0.10 ppm and 0.15 ppm respectively.

From the lag phase till the end of the growth cycle, biomass increased from 15 ppt to 25 ppt and then decreased at 35 ppt salinity. The flagellate exhibited highest cell number on the twelveth day about 42% higher of control in 0.05 ppm Mn. Low cell content in 0.10 ppm Mn and 0 15 ppm Mn may account for the less concentration of total pigments and carbon production than those of control (Fig.14).

FIG. 14 *Isochrysis galbana*

Effect of manganese on biomass in different salinities(S).

S_1 - 15 ppt

S_2 - 25 ppt

S_3 - 35 ppt.

Manganese concentrations

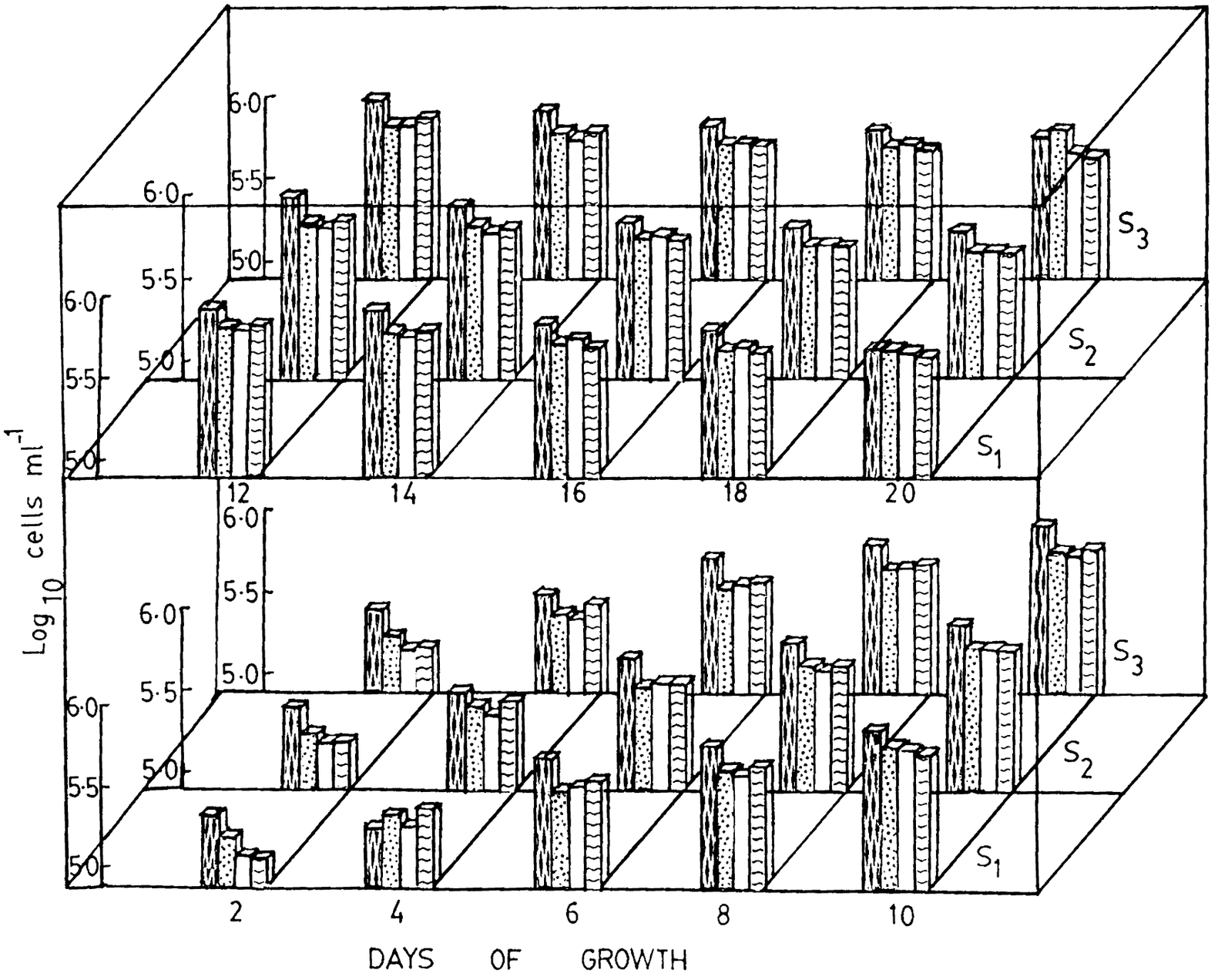
 - 0.050 ppm

 - 0.100 ppm

 - 0.150 ppm

 - Control

FIG. 14


Log10 cells ml-1
6·0
5·5
5·0
S3
S2
S1
12
14
16
18
20
2
4
6
8
10
DAYS OF GROWTH

The amount of chlorophyll a,c and carotenoids in all the three manganese treatments showed an increase till the fourteenth day and thereafter decreased till the twentieth day. In relation to salinity, the total quantity of pigments increased from 15 ppt to 25 ppt and then declined at 35 ppt. Maximum values of chlorophyll a,c and carotenoids were recorded in 0.05 ppm Mn about 8-9% higher than those of control (Figs. 15, 16, 17). The proportion of carotenoids was found to be greater than that of control. Rate of carbon production increased from 15 ppt to 25 ppt and then reduced at 35 ppt. Till the twelveth day, in all the three selected concentrations of manganese, there was a constant increase in the carbon fixation rate per hour, which subsequently declined towards the end of the growth phase. Highest rate of production was observed in 0.05 ppm Mn at 25 ppt on the twelveth day exhibiting an increase of 12% of control. Marginal lower values of carbon content were reported in 0.10 ppm and 0.15 ppm Mn (Fig. 18).

The overall physiological activity and growth rate of the flagellate were enhanced at pH 8.0 in all the three selected manganese treatments.

Biomass, total quantity of pigments and carbon content increased from pH 6.0 to pH 8.0. Maximum cell content was recorded on the twelveth day at pH 8.0 about 15%, 47% and 41% respectively higher than those of control in 0.05 ppm, 0.10 ppm and 0.15 ppm Mn (Fig.19B).

FIG. 15. *Isochrysis galbana*

Effect of manganese on the chlorophyll 'a' content in different salinities (S).

S_1 - 15 ppt

S_2 - 25 ppt

S_3 - 35 ppt

Manganese concentrations

 - 0.050 ppm

 - 0.100 ppm

 - 0.150 ppm

 - Control.

FIG.15

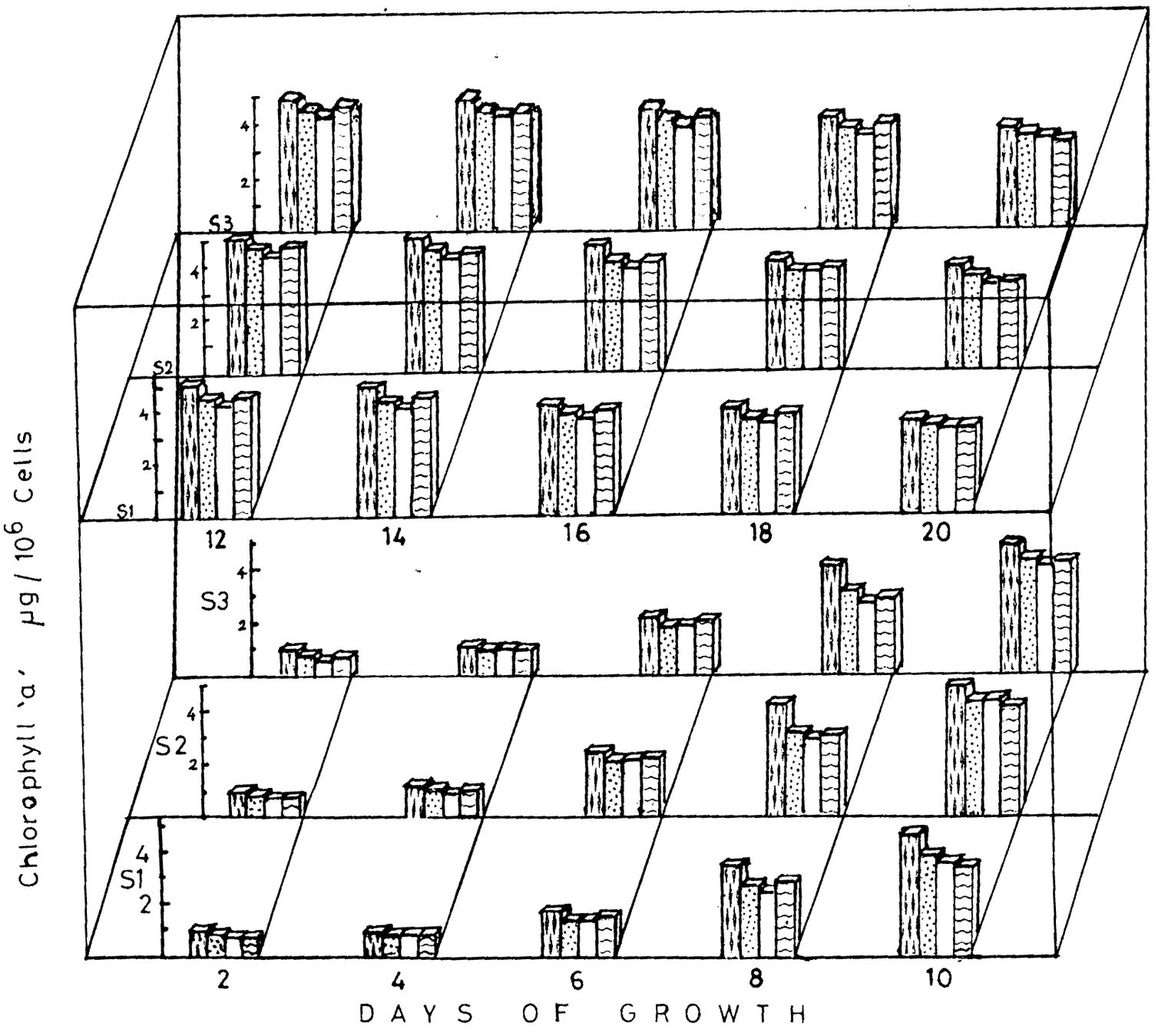

Chlorophyll 'a' µg / 10⁶ Cells
S3
S2
S1
4
2
12
14
16
18
20
2
4
6
8
10
DAYS OF GROWTH

FIG. 16. *Isochrysis galbana*

Effect of manganese on the chlorophyll 'c' content in different salinities (S).

S_1 - 15 ppt

S_2 - 25 ppt

S_3 - 35 ppt

Manganese concentrations

 - 0.050 ppm

 - 0.100 ppm

 - 0.150 ppm

 - Control

FIG.16

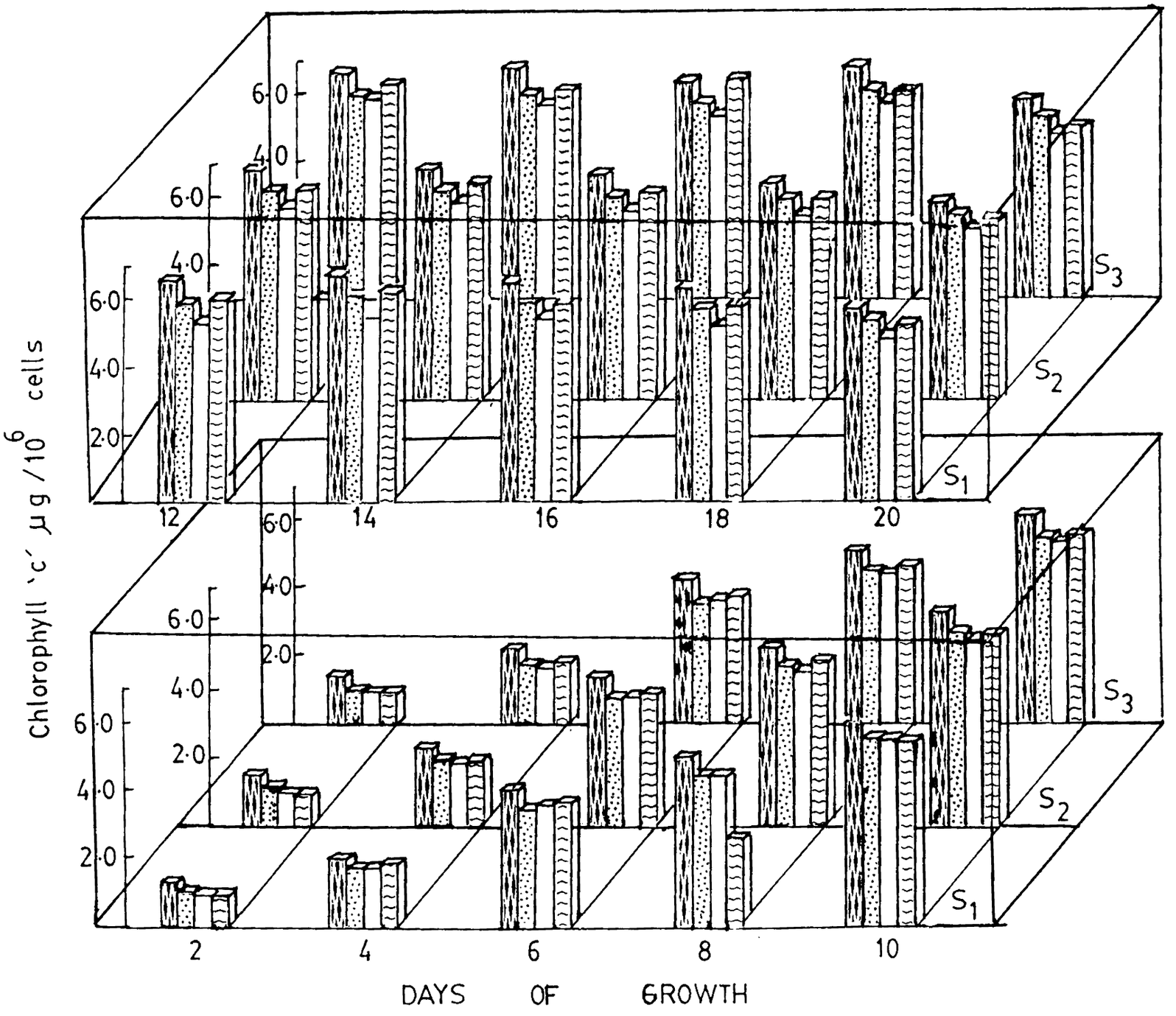

Chlorophyll 'c' µg /10^6 cells
6·0
4·0
2·0
12
14
16
18
20
S3
S2
S1
2
4
6
8
10
DAYS OF GROWTH

FIG. 17 *Isochrysis galbana*

Effect of manganese on the carotenoid content in different salinities (S).

S_1 - 15 ppt

S_2 - 25 ppt

S_3 - 35 ppt

Manganese concentrations

 - 0.050 ppm

 - 0.100 ppm

 - 0.150 ppm

 - Control

FIG.17

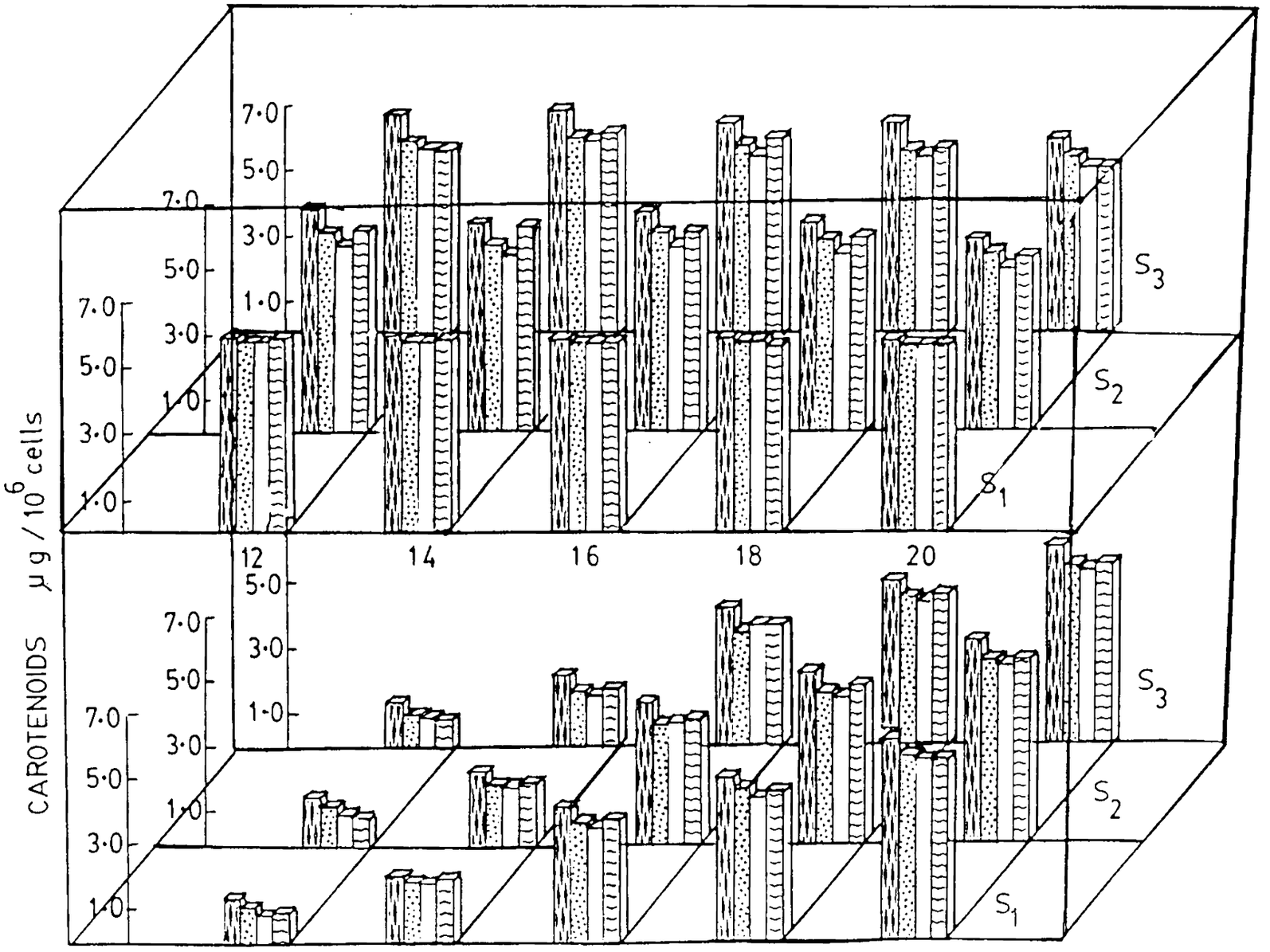


FIG. 18 *Isochrysis galbana*

Effect of manganese on the rate of carbon production in different salinities (S).

S_1 - 15 ppt

S_2 - 25 ppt

S_3 - 35 ppt

Manganese concentrations

 - 0.050 ppm

 - 0.100 ppm

 - 0.150 ppm

 - Control

FIG.18

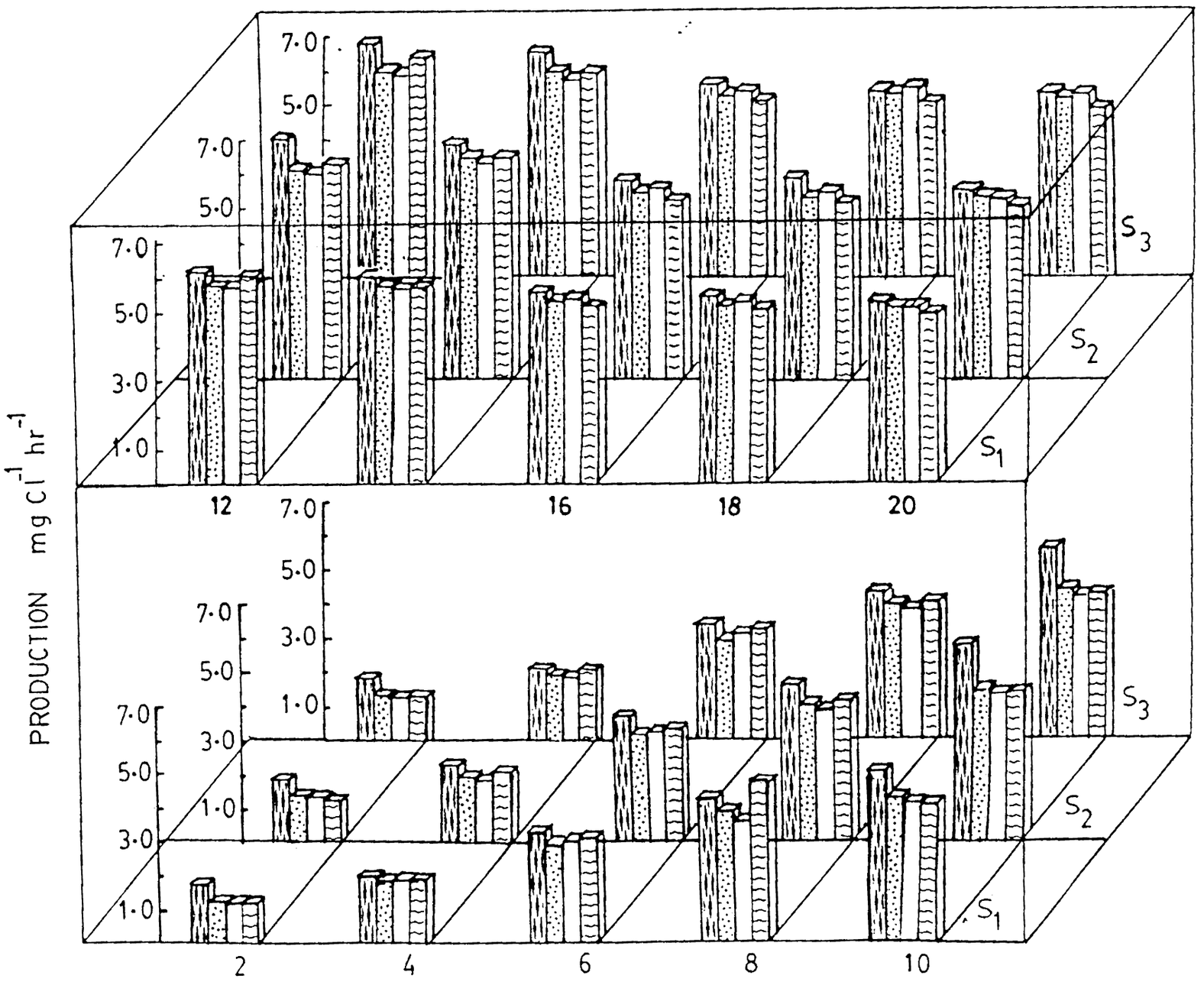


FIG. 19 *Isochrysis galbana*

Effect of manganese on biomass and rate of carbon production at different pH.

A - Production

B - Biomass

Manganese concentrations

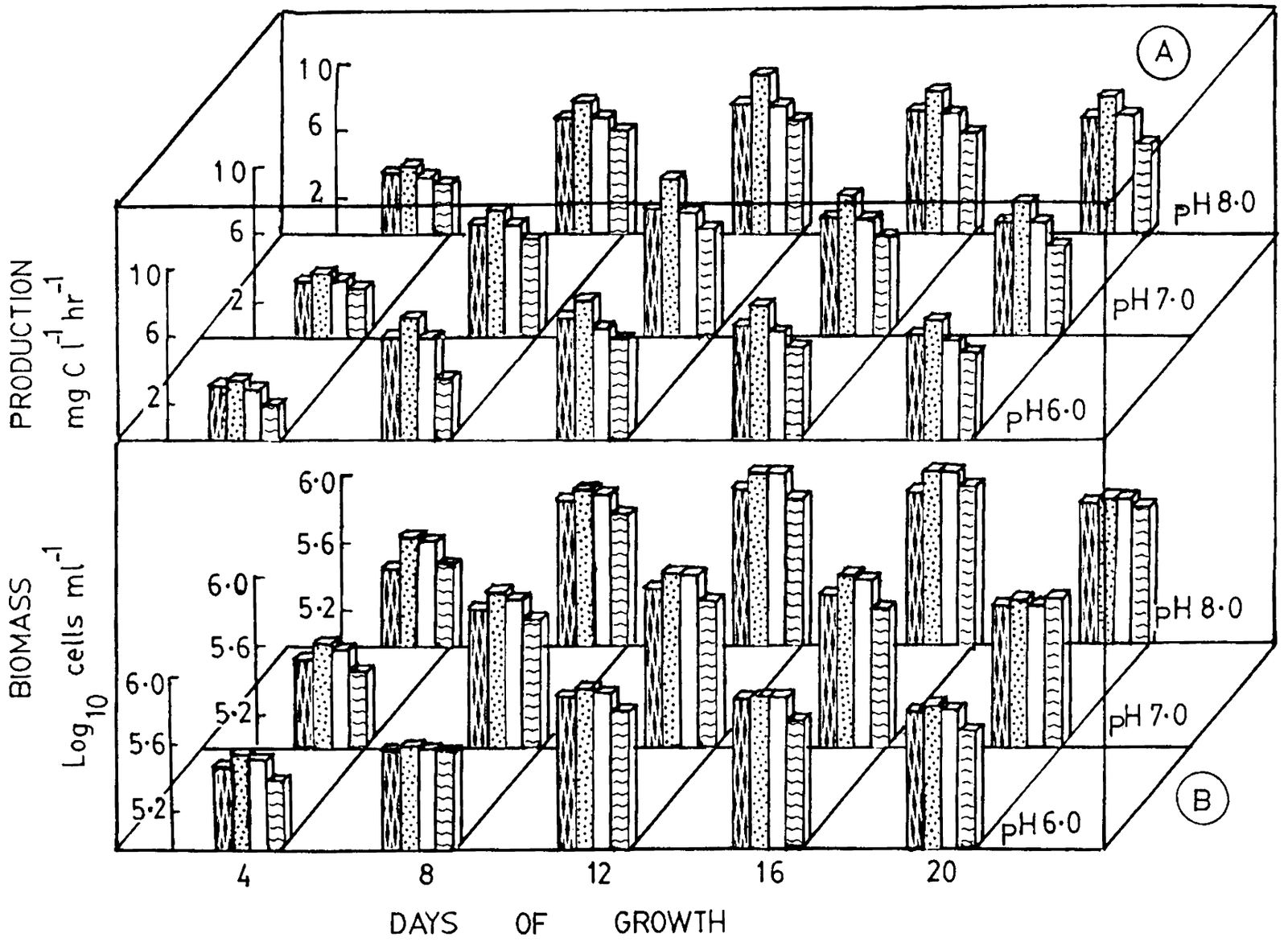
 - 0.050 ppm

 - 0.100 ppm

 - 0.150 ppm

 - Control

FIG.19


A
B
PRODUCTION
mg C l⁻¹ hr⁻¹
BIOMASS
Log10 cells ml⁻¹
pH 8·0
pH 7·0
pH 6·0
10
6
2
6·0
5·6
5·2
4
8
12
16
20
DAYS OF GROWTH

The proportion of chlorophyll a,c and carotenoids did not indicate much variation among each other. Chlorophyll a,c and carotenoids reached their peak level at pH 8.0 about 15%, 23% and 17% higher than that of control in 0.10 ppm Mn (Figs. 20, 21).

The rate of carbon production was maximum on the twelveth day registering an increase of 16%, 42% and 12% higher than control in 0.05 ppm, 0.10 ppm and 0.15 ppm Mn. Comparatively, the metabolic activity of the alga was higher at pH 8.0 in 0.10 ppm Mn than observed in the other two manganese treatments (Fig. 19A).

As reported in the case of copper, a similar trend was observed on the effect of manganese on the growth rate of the flagellate at different temperatures. Highest value of carbon production was recorded at 30°C in 0.15 ppm Mn and a declining tendency was noticed at higher temperatures of 35°C and 40°C (Table 2).

Synchocystis salina.

Lower salinity of 15 ppt was found to be more favourable for the growth of the blue green alga. For all the employed manganese treatments ranging from 0.02 ppm to 0.15 ppm, the cell content was higher with increasing concentration of manganese till the tenth day, thereafter a reverse trend was followed from the twelveth to the twentieth day. Though the biomass showed a constant hike, the quantity of total pigments reduced drastically on the sixth day itself

FIG. 20 *Isochrysis galbana*

Effect of manganese on the chlorophyll 'a' and Chlorophyll 'c' content at different pH.

A - Chlorophyll 'c'

B - Chlorophyll 'a'

Manganese concentrations

 - 0.050 ppm

 - 0.100 ppm

 - 0.150 ppm

 - Control

FIG. 20

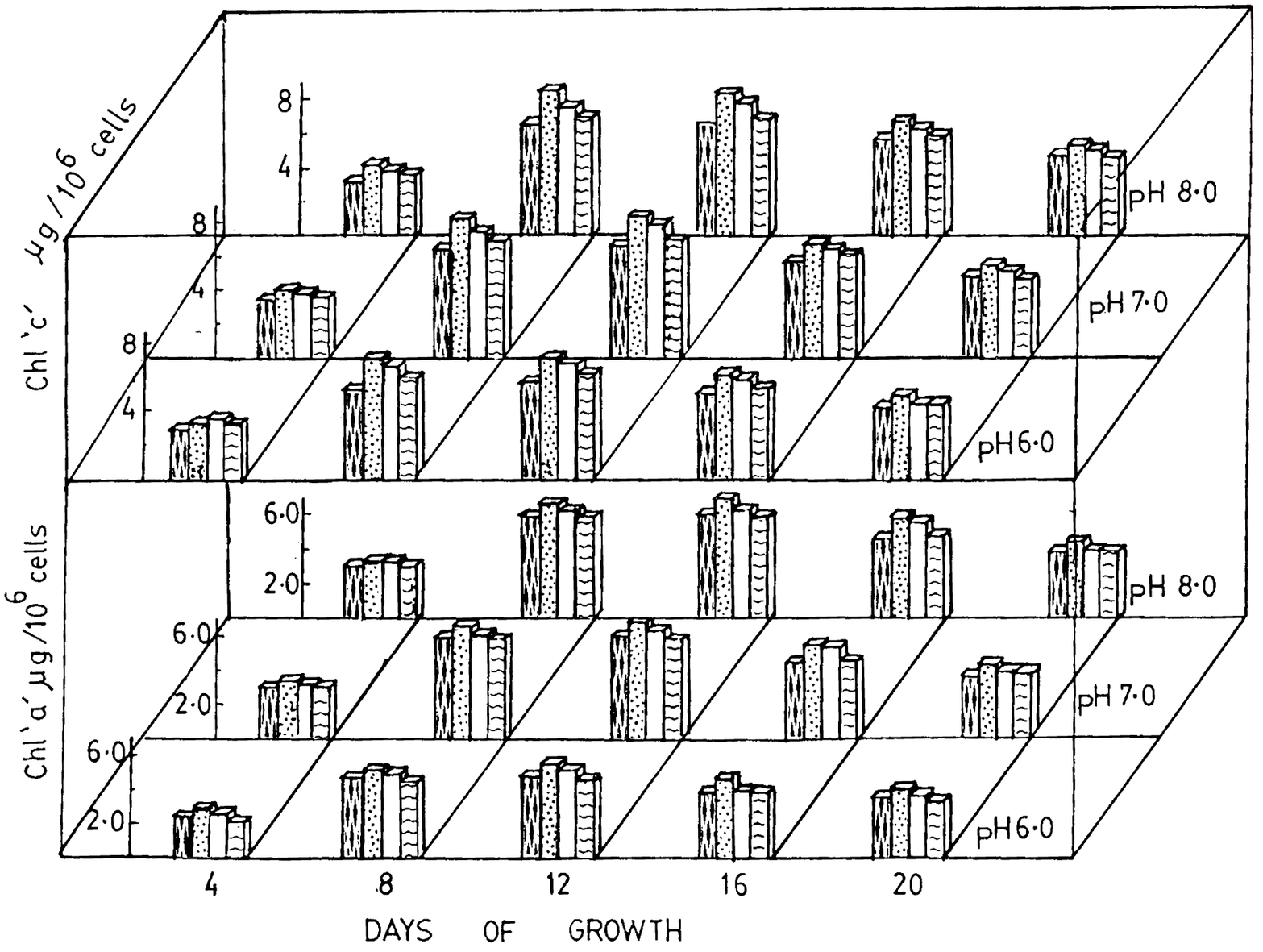

Chl 'c' µg/10^6 cells
8
4
8
4
8
4
pH 8·0
pH 7·0
pH6·0
Chl 'a' µg/10^6 cells
6·0
2·0
6·0
2·0
6·0
2·0
pH 8·0
pH 7·0
pH6·0
4
8
12
16
20
DAYS OF GROWTH

FIG. 21. *Isochrysis galbana*

Effect of manganese on the carotenoid content at different pH.

Manganese concentrations

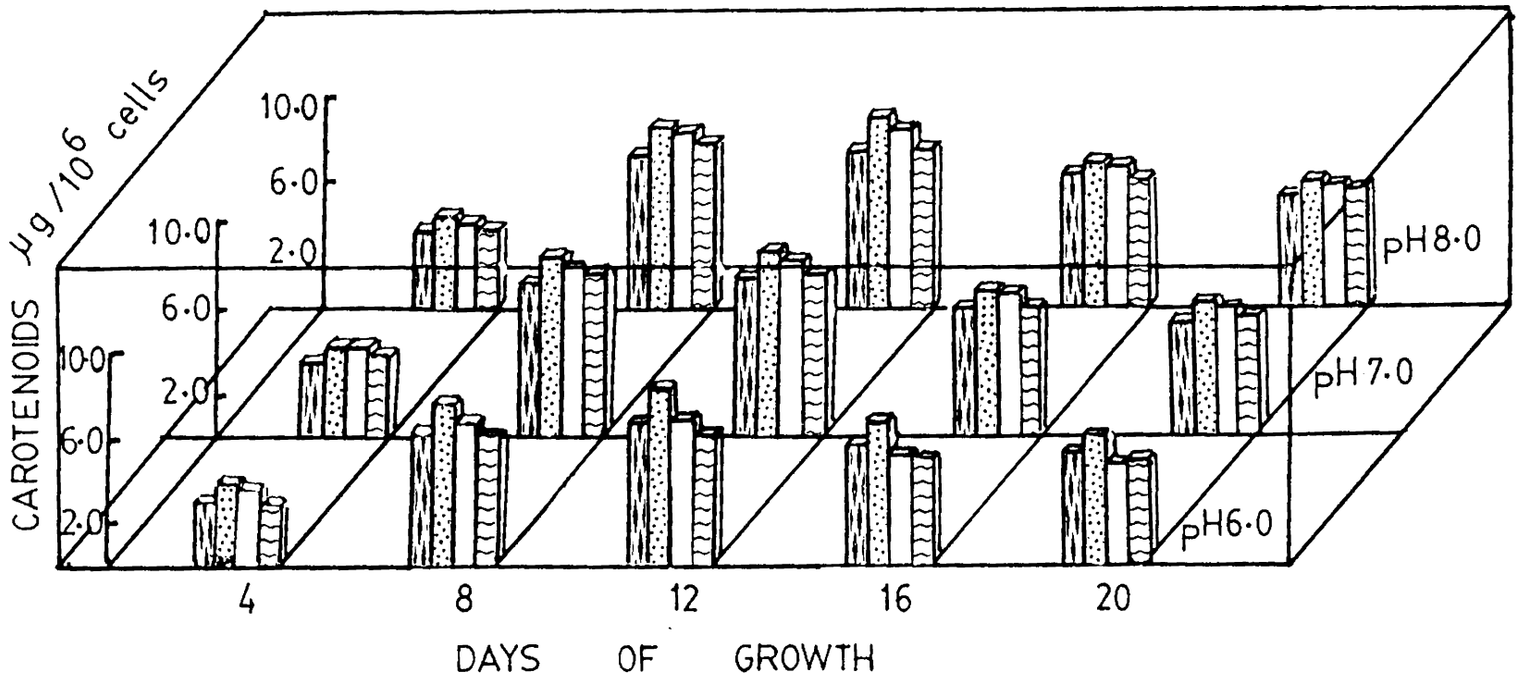
 - 0.050 ppm

 - 0.100 ppm

 - 0.150 ppm

 - Control.

FIG. 21


CAROTENOIDS µg/10⁶ cells
10·0
6·0
2·0
10·0
6·0
2·0
10·0
6·0
2·0
pH 8·0
pH 7·0
pH 6·0
4
8
12
16
20
DAYS OF GROWTH

Biomass increased from 15 ppt to 35 ppt during the early phase of growth but later on exhibited greater cell number at 15 ppt salinity. The blue green alga attained maximum cell concentration on the eighteenth day at 15 ppt about 34% higher of control in 0.02 ppm Mn (Fig. 22).

The chlorophyll 'a' and non-chlorophyllous pigment content reached their maximum on the fourth day in the concentrations ranging from 0.02 ppm to 0.15 ppm Mn at 15 ppt which were substantially higher than those of control (Table 5)

Greater production rate was observed between 15 ppt to 25 ppt and declined further at 35 ppt salinity. Production was maximum on the fourteenth day at 15 ppt in all the five selected manganese treatments registering higher values than those of control (Table 5).

Higher concentrations of manganese were found to be more favourable in enhancing the growth rate of the blue green alga at pH 8.0. However significant variation in cell concentration, ratio of total chlorophyll pigments and carbon production between pH 6.0 to pH 8.0 was not observed. Unlike 0.05 ppm Mn, 0.10 ppm and 0.15 ppm Mn were more suitable for the growth of this alga.

Biomass increased from pH 6.0 to pH 8 0 and showed a five fold hike between the eighth and the twelveth day. Maximum cell content was

FIG. 22. *Synechocystis salina*

Effect of manganese on biomass in different salinities (S).

S_1 - 15 ppt

S_2 - 25 ppt

S_3 - 35 ppt.

Manganese concentrations

 - 0.020 ppm

 - 0.050 ppm

 - 0.070 ppm

 - 0.100 ppm

 - 0.150 ppm

 - Control

FIG·22

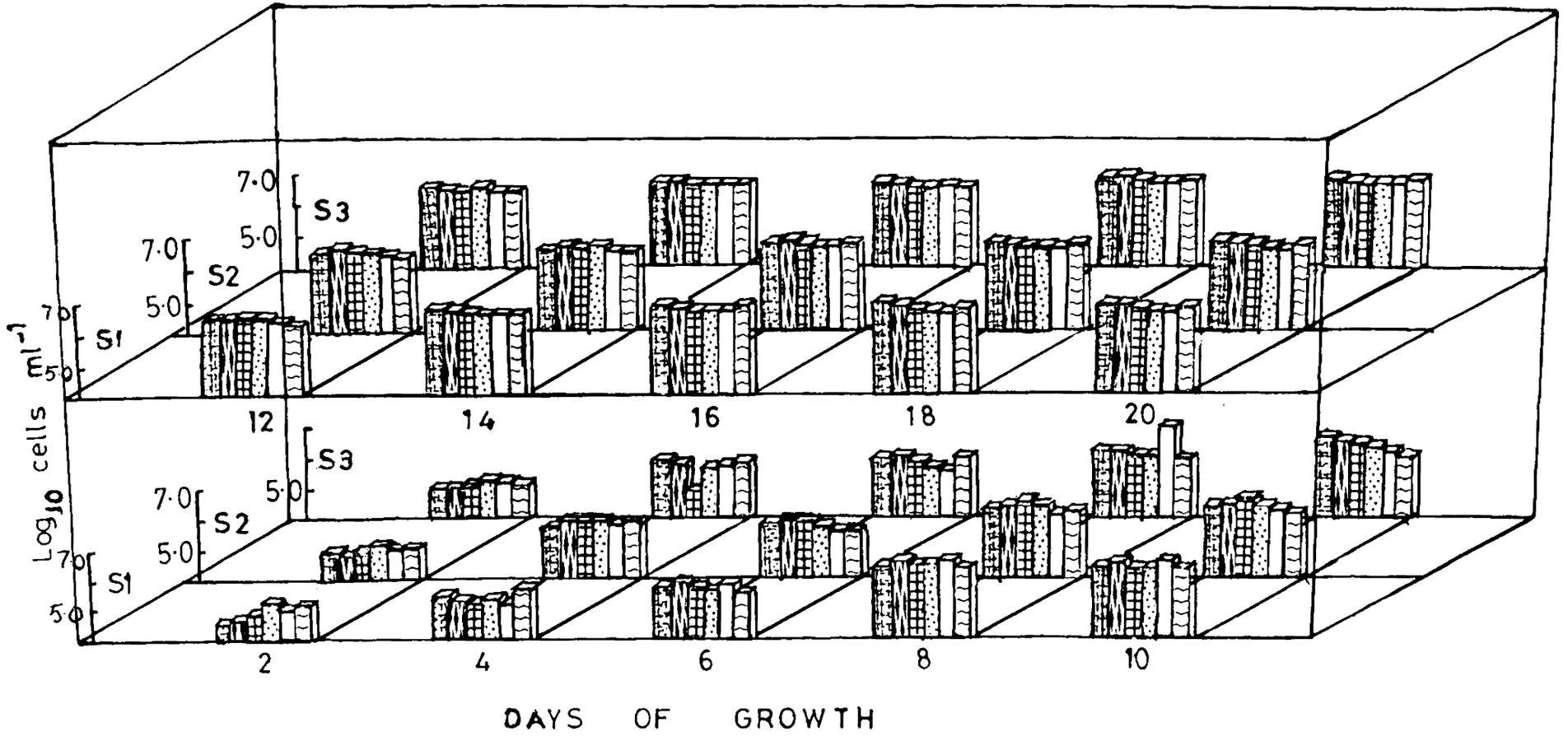

7·0
S3
5·0
7·0
S2
5·0
7
S1
5
Log10 cells ml-1
12
14
16
18
20
S3
5·0
7·0
S2
5·0
7
S1
5
2
4
6
8
10
DAYS OF GROWTH

Table 5. Effect of manganese on the physiological activity of *S. salina* in different salinities.

		15ppt				25ppt				35ppt			
Days of growth	Mn Concn (ppm)	Cell Concn/ ml	Chl 'a' $\mu g/10^6$ cells	Non-chl. Pig. $\mu g/10^6$ cells	Prodn. mgC/l/ hr.	Cell Concn/ ml	Chl.'a' $\mu g/10^6$ cells	Non-chl. Pig. $\mu g/10^6$ cells	Prodn. mgC/l/ hr.	Cell Concn/ ml	Chl.'a' $\mu g/10^6$ cells	Non-chl. Pig. $\mu g/10^6$ cells	Prodn. mgC/l/ hr.
2	0.020	40,000	0.612	0.512	0.422	55,000	0.543	0.442	0.850	1,00,000	0.480	0.412	0.724
	0.050	50,000	0.720	0.630	0.504	70,000	0.488	0.408	1.100	1,10,000	0.432	0.388	0.650
	0.070	90,000	0.614	0.681	0.680	1,00,000	0.432	0.392	1.320	1,50,000	0.468	0.406	0.796
	0.100	1,70,000	0.810	0.742	0.984	1,50,000	0.323	0.263	0.980	1,75,000	0.402	0.312	0.974
	0.150	1,10,000	0.890	0.770	0.740	1,30,000	0.302	0.216	0.756	1,75,000	0.496	0.411	0.812
	CON	1,60,000	0.772	0.610	0.896	1,50,000	0.416	0.348	1.400	1,30,000	0.410	0.403	1.200
4	0.020	2,70,000	4.60	3.52	0.986	5,20,000	2.60	1.810	1.220	6,00,000	2.11	1.97	1.180
	0.050	2,30,000	4.36	4.17	0.874	10,50,000	1.76	1.686	1.380	5,20,000	1.63	2.04	1.220
	0.070	1,70,000	4.04	4.59	0.880	7,80,000	1.32	0.897	1.300	3,70,000	1.86	1.78	0.986
	0.100	2,10,000	4.51	3.24	0.742	6,30,000	1.27	0.635	0.996	3,30,000	2.49	2.58	0.970
	0.150	1,50,000	5.20	4.00	0.730	5,70,000	1.99	1.930	0.912	3,65,000	3.04	2.96	0.864
	CON	4,80,000	1.89	1.54	0.980	6,50,000	1.47	1.600	1.64	6,00,000	1.53	2.07	0.010
6	0.020	4,80,000	0.147	0.144	1.440	5,30,000	1.340	2.180	1.66	7,35,000	1.27	0.72	1.72
	0.050	6,80,000	0.188	0.191	1.56	7,10,000	0.651	0.988	1.72	8,50,000	0.93	0.57	1.84
	0.070	4,90,000	0.181	0.102	1.48	5,90,000	0.923	1.220	1.54	5,50,000	1.25	0.90	1.50
	0.100	4,30,000	0.146	0.140	1.32	4,50,000	0.770	0.911	1.40	4,30,000	2.04	1.26	1.36
	0.150	3,70,000	1.850	1.680	1.08	3,10,000	1.170	2.450	1.10	3,50,000	1.99	2.80	1.20
	CON	3,10,000	0.644	1.030	0.97	3,20,000	1.260	1.690	0.98	7,30,000	1.46	1.43	1.18
8	0.020	20,50,000	0.312	0.205	2.92	17,50,000	0.169	0.263	2.84	13,00,000	0.847	0.308	2.32
	0.050	28,00,000	0.259	0.157	3.26	22,50,000	0.187	0.209	3.10	13,00,000	0.860	0.385	2.40
	0.070	26,90,000	0.286	0.134	2.84	26,00,000	0.134	0.123	2.80	11,50,000	0.737	0.313	2.16
	0.100	23,00,000	0.312	0.148	2.60	21,80,000	0.275	0.216	2.56	10,75,000	1.080	0.381	1.94
	0.150	30,75,000	0.201	0.101	2.70	9,90,000	0.452	0.323	1.65	9,00,000	0.998	0.411	1.42
	CON	21,00,000	0.248	0.167	2.80	15,50,000	0.274	0.271	2.40	8,50,000	1.270	0.576	2.01
10	0,020	19,50,000	0.335	0.377	2.80	20,50,000	0.139	0.254	2.88	33,00,000	0.303	0.449	3.10
	0.050	26,00,000	0.194	0.200	3.21	23,00,000	0.192	0.313	3.20	28,50,000	0.286	0.323	3.42
	0.070	20,75,000	0.280	0.207	2.76	43,50,000	0.261	0.202	2.96	21,50,000	0.381	0.242	3.06
	0.100	19,25,000	0.207	0.249	2.54	20,75,000	0.265	0.231	2.62	16,50,000	0.320	0.315	2.60
	0.150	36,50,000	0.175	0.175	2.78	18,50,000	0.240	0.238	1.70	13,00,000	0.296	0.262	1.61
	CON	19,50,000	0.143	0.217	2.70	14,50,000	0.222	0.290	2.44	10,50,000	0.264	0.257	2.30

Table 5. Contd.....

Days of growth	Mn Concn. (ppm)	15ppt Cell Concn/ ml	15ppt Chl 'a' µg/10^6 cells	15ppt Non-chl. Pig. µg /10^6 cells	15ppt Prodn. mgC/l/ hr.	25ppt Cell Concn/ ml	25ppt Chl 'a' µg/10^6 cells	25ppt Non-chl. Pig. µg/ 10^6 cells	25ppt Prodn. mgC/l/ hr.	35ppt Cell Concn/ ml	35ppt Chl 'a' µg / 10^6 cells	35ppt Non-chl. Pig. µg/ 10^6 cells	35ppt Prodn. mgC/ l/hr.
12	0.020	39,50,000	0.312	0.137	5.82	33,00,000	0.244	0.164	5.76	31,00,000	0.406	0.232	5.70
	0.050	35,50,000	0.255	0.146	5.30	39,00,000	0.261	0.154	5.42	32,00,000	0.348	0.209	5.31
	0.070	38,50,000	0.175	0.109	5.48	36,00,000	0.331	0.175	5.40	28,50,000	0.305	0.218	5.20
	0.100	31,50,000	0.316	0.181	5.04	30,50,000	0.276	0.230	5.00	29,50,000	0.302	0.203	4.92
	0.150	29,00,000	0.180	0.110	4.90	24,00,000	0.301	0.204	4.82	22,50,000	0.377	0.284	4.76
	CON	20,50,000	0.247	0.163	4.72	23,00,000	0.243	0.118	4.76	23,00,000	0.219	0.239	4.70
14	0.020	48,50,000	0.284	0.123	6.10	44,50,000	0.206	0.154	6.04	42,00,000	0.312	0.202	6.00
	0.050	45,75,000	0.205	0.139	5.98	46,00,000	0.204	0.120	5.66	41,50,000	0.280	0.194	5.54
	0.070	44,50,000	0.151	0.103	5.60	43,50,000	0.231	0.156	5.52	35,00,000	0.249	0.202	5.32
	0.100	43,00,000	0.261	0.194	5.24	40,50,000	0.198	0.200	5.06	33,00,000	0.194	0.188	4.84
	0.150	39,80,000	0.175	0.108	5.02	36,00,000	0.255	0.191	4.84	31,50,000	0.265	0.207	4.70
	CON	39,50,000	0.262	0.188	4.98	33,50,000	0.206	0.131	4.90	32,00,000	0.198	0.191	4.82
16	0.020	56,75,000	0.137	0.087	5.72	51,50,000	0.214	0.087	5.66	34,00,000	0.190	0.412	5.10
	0.050	50,50,000	0.137	0.103	5.40	49,50,000	0.172	0.125	5.32	30,00,000	0.151	0.493	4.98
	0.070	41,00,000	0.169	0.239	5.22	39,90,000	0.261	0.288	5.16	26,50,000	0.250	0.574	4.84
	0.100	39,90,000	0.130	0.088	4.90	36,00,000	0.188	0.206	4.84	25,50,000	0.136	0.486	4.72
	0.150	40,50,000	0.150	0.094	4.54	37,00,000	0.177	0.144	4.42	28,50,000	0.198	0.309	4.40
	CON	49,00,000	0.107	0.069	4.62	43,50,000	0.144	0.133	4.50	30,50,000	0.154	0.336	4.42
18	0.020	63,50,000	0.131	0.239	5.48	53,00,000	0.107	0.166	5.32	56,00,000	0.093	0.109	5.38
	0.050	53,50,000	0.093	0.368	5.26	50,00,000	0.093	0.104	5.10	57,00,000	0.103	0.196	5.20
	0.070	49,50,000	0.111	0.121	4.85	49,80,000	0.049	0.177	4.70	51,00,000	0.148	0.173	4.76
	0.100	44,50,000	0.150	0.202	4.64	43,50,000	0.119	0.138	4.56	50,00,000	0.115	0.108	4.62
	0.150	39,00,000	0.180	0.190	4.32	36,80,000	0.191	0.200	4.20	48,50,000	0.209	0.181	4.30
	CON	47,50,000	0.067	0.107	4.40	88,50,000	0.134	0.115	4.42	52,50,000	0.131	0.152	4.52
20	0.020	61,00,000	0.148	0.118	4.84	59,00,000	0.157	0.122	4.80	57,50,000	0.092	0.125	4.76
	0.050	59,50,000	0.060	0.109	4.61	56,50,000	0.072	0.104	4.54	54,50,000	0.073	0.136	4.40
	0.070	54,00,000	0.115	0.152	4.20	53,00,000	0.120	0.147	4.16	49,00,000	0.099	0.206	4.02
	0.100	44,50,000	0.128	0.202	4.02	47,50,000	0.056	0.194	4.20	45,50,000	0.149	0.255	4.16
	0.150	43,50,000	0.084	0.232	3.96	43,50,000	0.129	0.149	4.02	42,00,000	0.196	0.133	4.00
	CON	52,00,000	0.083	0.154	4.18	53,00,000	0.150	0.146	4.24	51,70,000	0.135	0.135	4.18

observed on the sixteenth day at 35 ppt salinity about 8% and 13% higher of control in 0 10 ppm and 0.15 ppm Mn (Fig. 23B).

The amount of chlorophyll a, non-chlorophyllous pigments and carbon production increased from pH 6.0 to pH 8.0 recording its maximum on the twelveth day about 19%, 7% and 14% higher than that of control in 0.15 ppm Mn (Figs. 23A, 24).

Higher concentrations of manganese accelerated the growth rate of *S salina*. Production was maximum at 25°C, subsequently showed a decline towards higher temperatures. Peak level of production was recorded in 0.15 ppm Mn at 25°C about 17% higher than that of control (Table 4).

Discussion:

Essentiality of manganese for algal growth was evident from the investigations of Harvey (1947), Walker (1954) and Eyster *et al.*, (1956, 1958).

The results obtained in the present study have shown that higher concentrations of manganese stimulated the growth of *I. galbana* and *S. salina*. The blue green alga had a greater tolerance to manganese than the flagellate. The role of Mn in the reaction of some enzymes in the Kreb's cycle and other metabolic processes has been substantiated by O'Kelley (1974).

FIG. 23 *Synechocystis salina*

Effect of manganese on biomass and rate of carbon production at different pH.

A - Production

B - Biomass

Manganese concentrations

 - 0.050 ppm

 - 0.100 ppm

 - 0.150 ppm

 - Control

FIG. 23

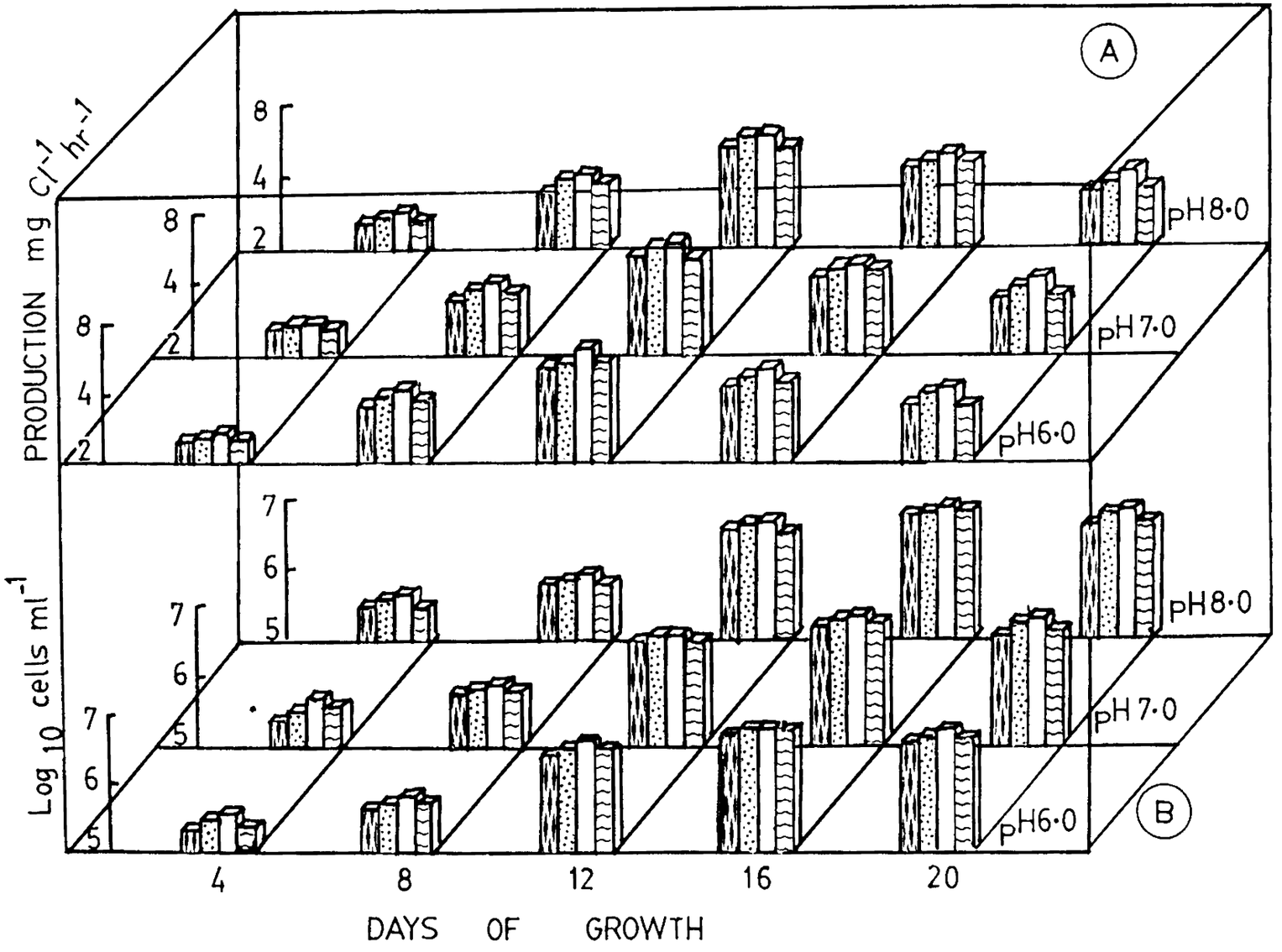

A
B
PRODUCTION mg C/-1 hr-1
Log 10 cells ml-1
pH8·0
pH7·0
pH6·0
8
4
2
7
6
5
4
8
12
16
20
DAYS OF GROWTH

FIG. 24. *Synechocystis salina*

Effect of manganese on the chlorophyll 'a' content and non-chlorophyllous pigments at different pH.

A - Non-chlorophyllous pigments

B - Chlorophyll 'a'

Manganese concentrations

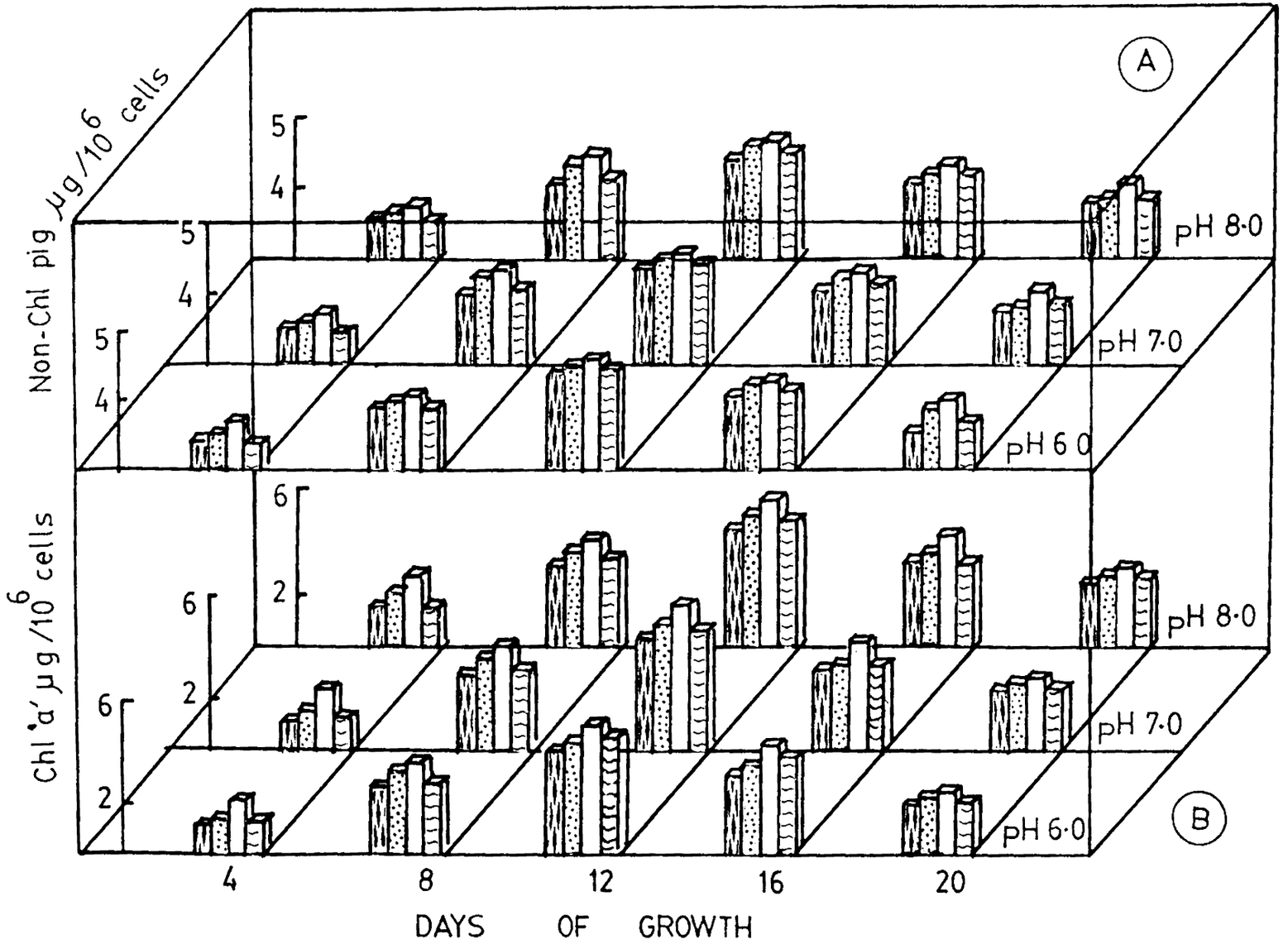
 - 0.050 ppm

 - 0.100 ppm

 - 0.150 ppm

 - Control

FIG.24


A
B
Non-Chl pig µg/10^6 cells
Chl 'a' µg/10^6 cells
pH 8·0
pH 7·0
pH 6·0
5
4
6
2
4
8
12
16
20
DAYS OF GROWTH

The most studied effect of manganese on algal metabolism is its impact on the oxygen evolving system of photosynthesis (Vernon, 1962; Cheniae and Martin, 1968, 1969). It has been observed in the present study that in both the species, the amount of total pigments and the rate of carbon production were far higher than those of control indicating the significance of manganese to carry out photosynthetic reactions. Such an emphasis on the necessity of manganese has been demonstrated in *Anacystis* and *Chlamydomonas* by Teichler - Zalden (1969).

Many phycologists have shown that fairly higher concentrations of manganese inhibited the growth of various algal species. Velichko(1968) has found that 0.2 ppm Mn was toxic to *Microcystis*. Rosko and Rachlin (1975) detected a depression in the growth rate of *Nitzschia closterium* by 50% in 25.7 ppm Mn, whereas Christensen *et al.*,(1979) noticed a suppression in growth rate and 50% reduction in cell volume at a concentration of 31 and 50 mg l^{-1} in *Selenastrum capricornutum* and *Chlorella stigmatophora* respectively. But Noro (1978) has observed an adverse effect on *Dunaliella tertiolecta* when the concentration of manganese was less than 0.10 ppm. Brand *et al*, (1983) have also reported that the marine phytoplankton reproductive rates were limited by manganese ion activity of $10^{-10}M$ to $10^{-11}M$.

Rajendran *et al.*,(1978) have estimated that even a low level of manganese 8 $\mu g/l$ increased the rate of carbon assimilation of micro and nannoplankton of Zuari estuary, Goa. In the present investigation,

concentrations greater than 0.05 ppm Mn were found to enhance the growth rate of *I galbana* and *S. salina*. Sunda *et al.*, (1981) have indicated that addition of as little as 10^{-9M} $MnCl_2$ stimulated the growth of *Chaetoceros socialis* over that of control, whereas 10^{-6M} to 10^{-7M} $MnCl_2$ gave maximum stimulation of growth in a natural community of phytoplankton.

Greater tolerance of the blue green alga to manganese can be attributed to the development of alternate metabolic pathways or by reducing the permeability of the cell to manganese. Production of extracellular metabolites with chelating potential can also be released into the medium or may remain attached to the cell wall and thereby counteract the toxicity of manganese (Steemann Nielsen and Kamp-Nielsen, 1970; Wikfors and Ukeles, 1982)

The role of environmental factors influencing the availability and toxicity of manganese to algae has not received much attention so far. In the present investigation, it has been found that higher concentrations of manganese greater than 0.10 ppm, a salinity of 25 ppt at pH 8.0 and a temperature of 30°C were favourable for the growth of *I galbana* whereas under similar concentrations of manganese a salinity of 15 ppt at pH 8.0 and a temperature of 25°C enhanced the growth rate of *S salina*.

In *I. galbana*, change of pH to alkaline level in the medium accelerated the stimulatory effect of manganese on biomass, photsynthetic

pigments and carbon production. An inverse relationship was observed between the toxicity of manganese and pH in *I galbana* and *S. salina* i.e. the toxicity of manganese declined at higher pH levels. This may be due to the fact that the hydrogen ion may alter the availability of manganese indirectly by affecting metal speciation resulting in an increase of both organic and inorganic complexes at higher pH and directly by competing with metals for cellular binding sites and thereby providing protection for the cell from toxicity (Peterson and Healey, 1984, 1985).

Stauber and Florence (1985) have shown that manganese (II) in seawater is oxidised very slowly to MnO_2. In the presence of algae, manganese (II) may be oxidised by oxygen at the cell surface to Mn(III). The precipitation potential of Mn is known to differ with pH and this may account for the decreased bioavailability of manganese to *I. galbana* and *S. salina* at higher pH levels. Further studies on manganese speciation in seawater in the presence of algae would give a clear understanding on the action of manganese at different pH levels.

An increase in the toxicity of manganese to *I. galbana* and *S. salina* at higher temperatures can be explained by the enhanced respiratory activities of the two algae (Forstner and Wittmann, 1979). It can be also possible that a multitude of factors might jointly interact to affect the toxicity in a complex manner

4.2.3. Zinc :

Isochrysis galbana

Lower concentrations of zinc namely 0.01 and 0.05 ppm were more favourable for the growth of the flagellate at a salinity of 25 ppt than at 15 ppt or 35 ppt.

Cell activity increased from 15 ppt to 25 ppt and declined at 35 ppt. Biomass was maximum on the twelveth day exhibiting an increase of 86% and 26% over that of control in 0.01 ppm and 0.05 ppm Zn. However in 0.10 ppm and 0.15 ppm, the cell content was found to be lower than that of control during the entire life cycle (Fig. 25).

Similarly the quantity of chlorophyll a,c, carotenoids and the rate of carbon production increased till the twelveth day and thereafter a decline was noticed. Maximum values of chlorophyll a,c, carotenoids and carbon content were recorded on the twelveth day about 32%, 23%, 24% and 17% higher than that of control in 0.01 ppm Zn in 25 ppt. The amount of pigments and the rate of production were lower than those of control in 0.10 ppm and 0.15 ppm Zn.(Figs. 26, 27, 28, 29).

Growth of the flagellate was stimulated at higher pH levels. Biomass and the photosynthetic activity of the alga were higher than control in 0.05 ppm and 0.10 ppm throughout the life cycle. Cell concentration reached its maximum on the twelveth day in 0.05 ppm about 21%

FIG. 25. *Isochrysis galbana*

Effect of zinc on biomass in different salinities (S).

S_1 - 15 ppt

S_2 - 25 ppt

S_3 - 35 ppt

Zinc concentrations

 - 0.010 ppm

 - 0.050 ppm

 - 0.100 ppm

 - 0.150 ppm

 - Control

FIG. 25

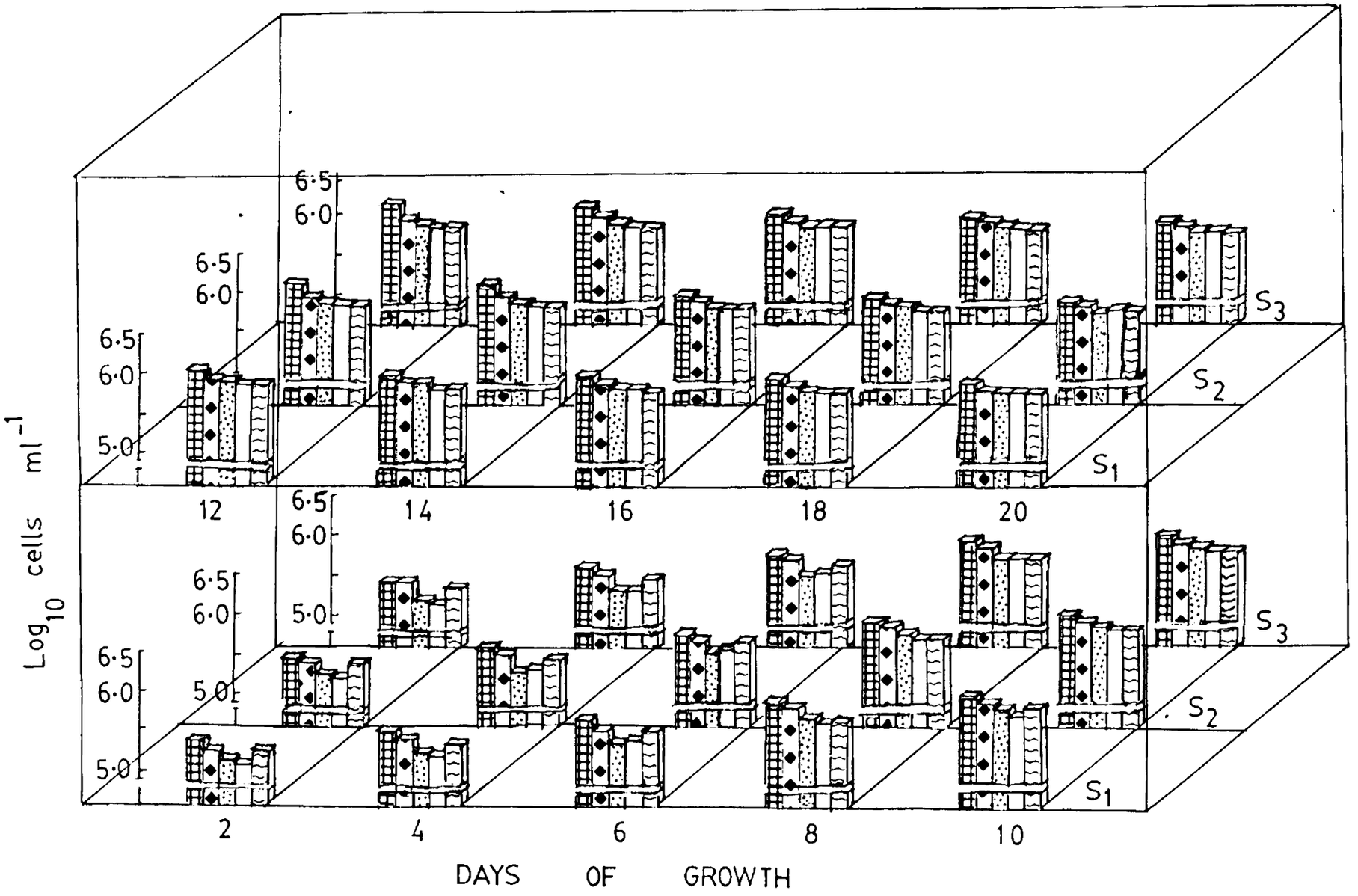

6·5
6·0
5·0
S3
S2
S1
12
14
16
18
20
2
4
6
8
10
Log10 cells ml-1
DAYS OF GROWTH

FIG. 26. *Isochrysis galbana*

Effect of zinc on the chlorophyll 'a' content in different salinities (S).

S_1 - 15 ppt

S_2 - 25 ppt

S_3 - 35 ppt

Zinc concentrations

 - 0.010 ppm

 - 0.050 ppm

 - 0.100 ppm

 - 0.150 ppm

 - Control

Fig. 26

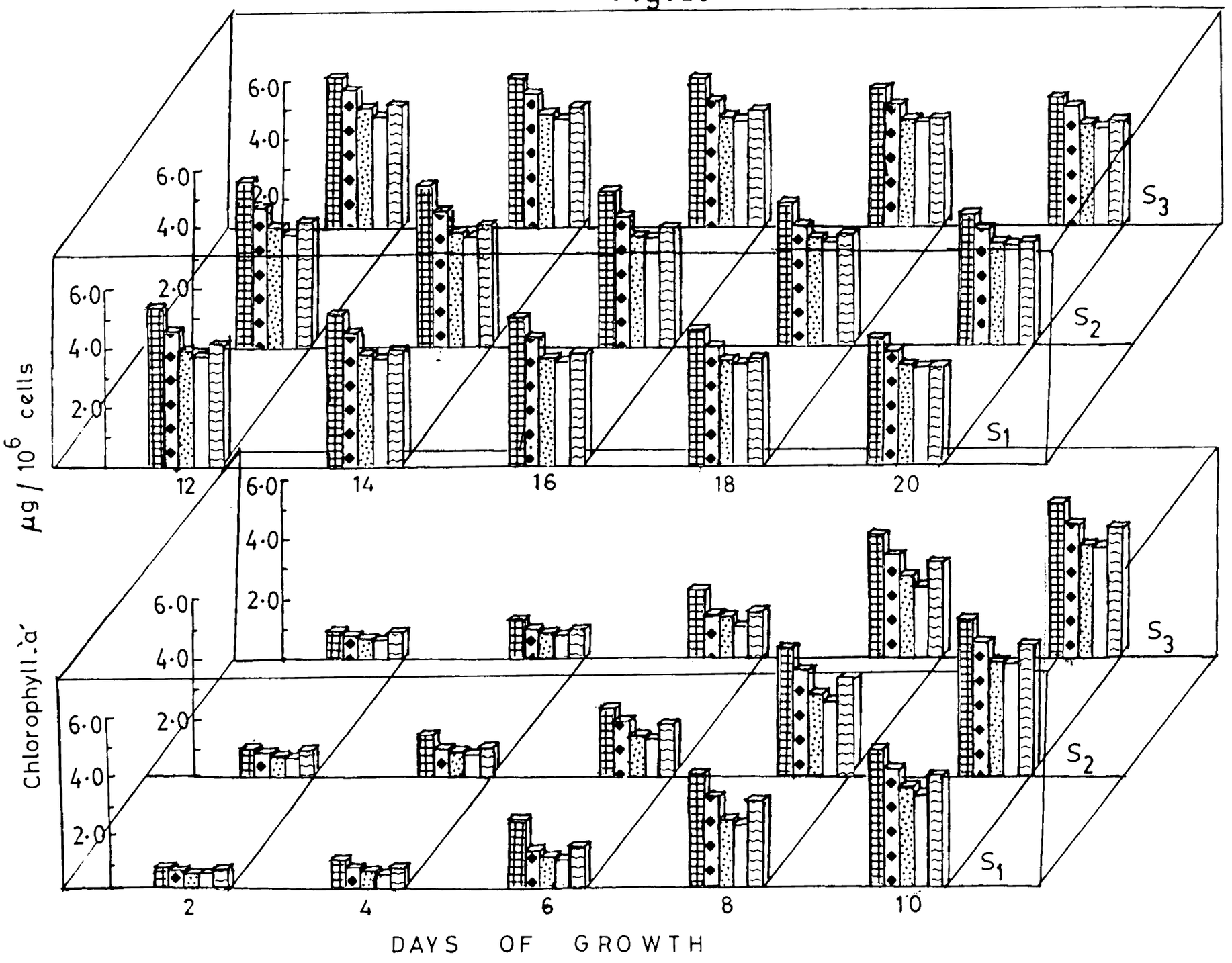

Chlorophyll 'a'
µg / 10^6 cells
6·0
4·0
2·0
S1
S2
S3
12
14
16
18
20
2
4
6
8
10
DAYS OF GROWTH

FIG. 27 *Isochrysis galbana*

Effect of zinc on the chlorophyll 'c' content in different salinities (S).

S_1 - 15 ppt

S_2 - 25 ppt

S_3 - 35 ppt

Zinc concentrations

 - 0.010 ppm

 - 0.050 ppm

 - 0.100 ppm

 - 0.150 ppm

 - Control

FIG·27

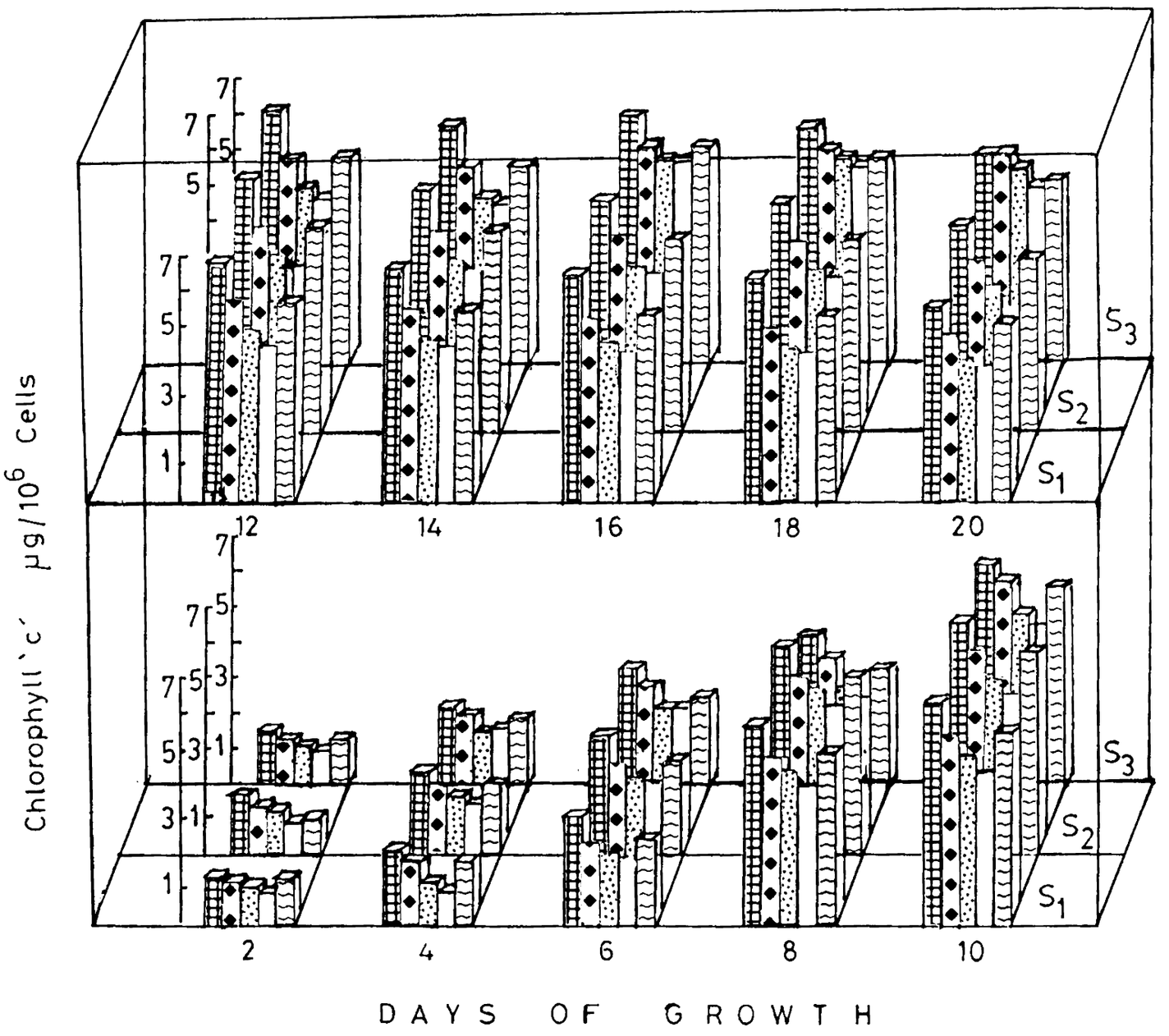

Chlorophyll 'c' µg/10^6 Cells
7
7
5
5
7
5
3
1
12
14
16
18
20
S3
S2
S1
7
7
5
3
7
5
3
1
5
3
1
3
1
1
2
4
6
8
10
S3
S2
S1
DAYS OF GROWTH

FIG. 28. *Isochrysis galbana*

Effect of zinc on the carotenoid content in different salinities (S).

S_1 - 15 ppt

S_2 - 25 ppt

S_3 - 35 ppt

Zinc concentrations

 - 0.010 ppm

 - 0.050 ppm

 - 0.100 ppm

 - 0.150 ppm

 - Control

FIG. 28

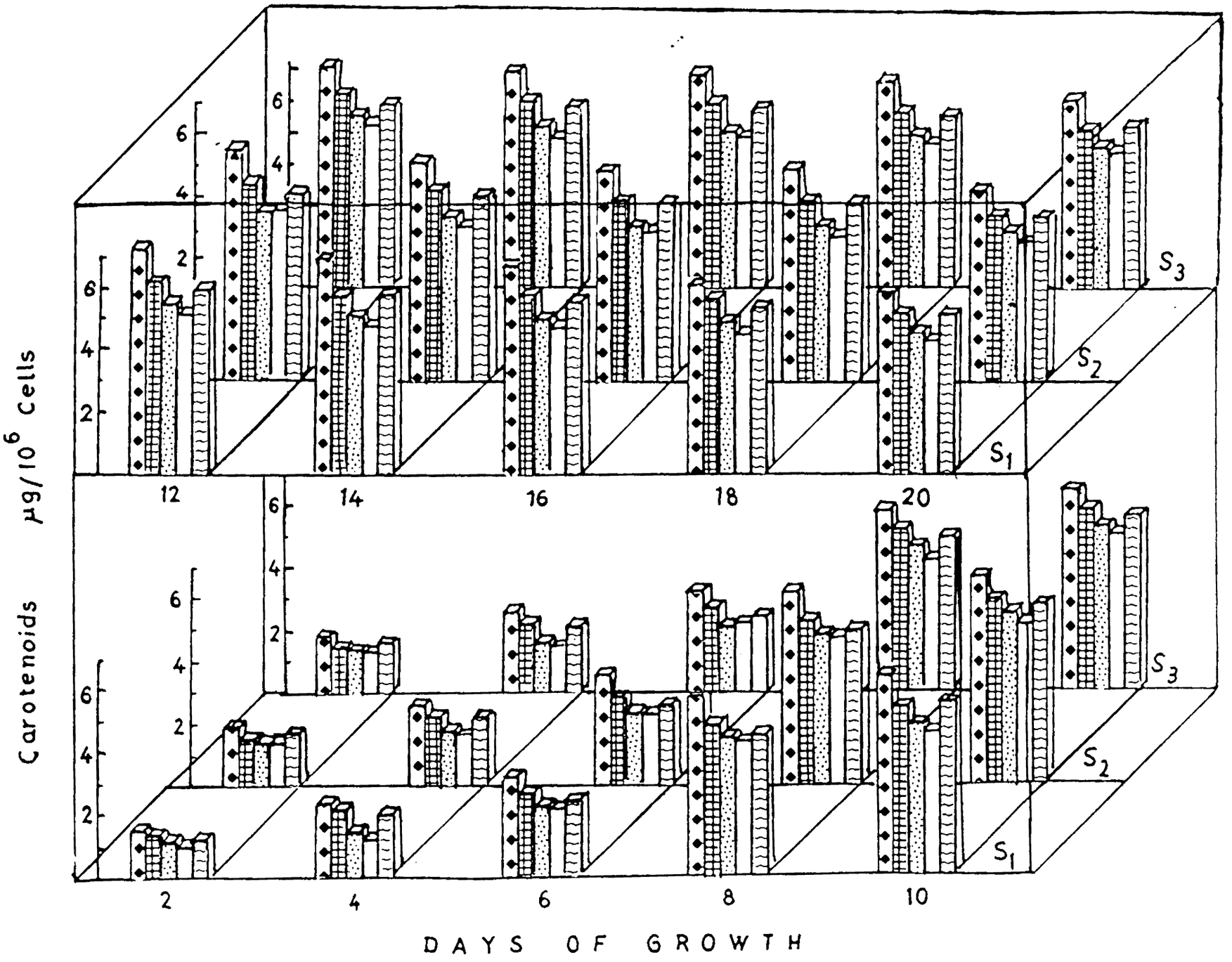

Carotenoids µg/10^6 Cells
DAYS OF GROWTH
S1
S2
S3
2
4
6
8
10
12
14
16
18
20

FIG. 29 *Isochrysis galbana*

Effect of zinc on the rate of carbon production in different salinities (S).

S_1 - 15 ppt

S_2 - 25 ppt

S_3 - 35 ppt

Zinc concentrations

 - 0.010 ppm

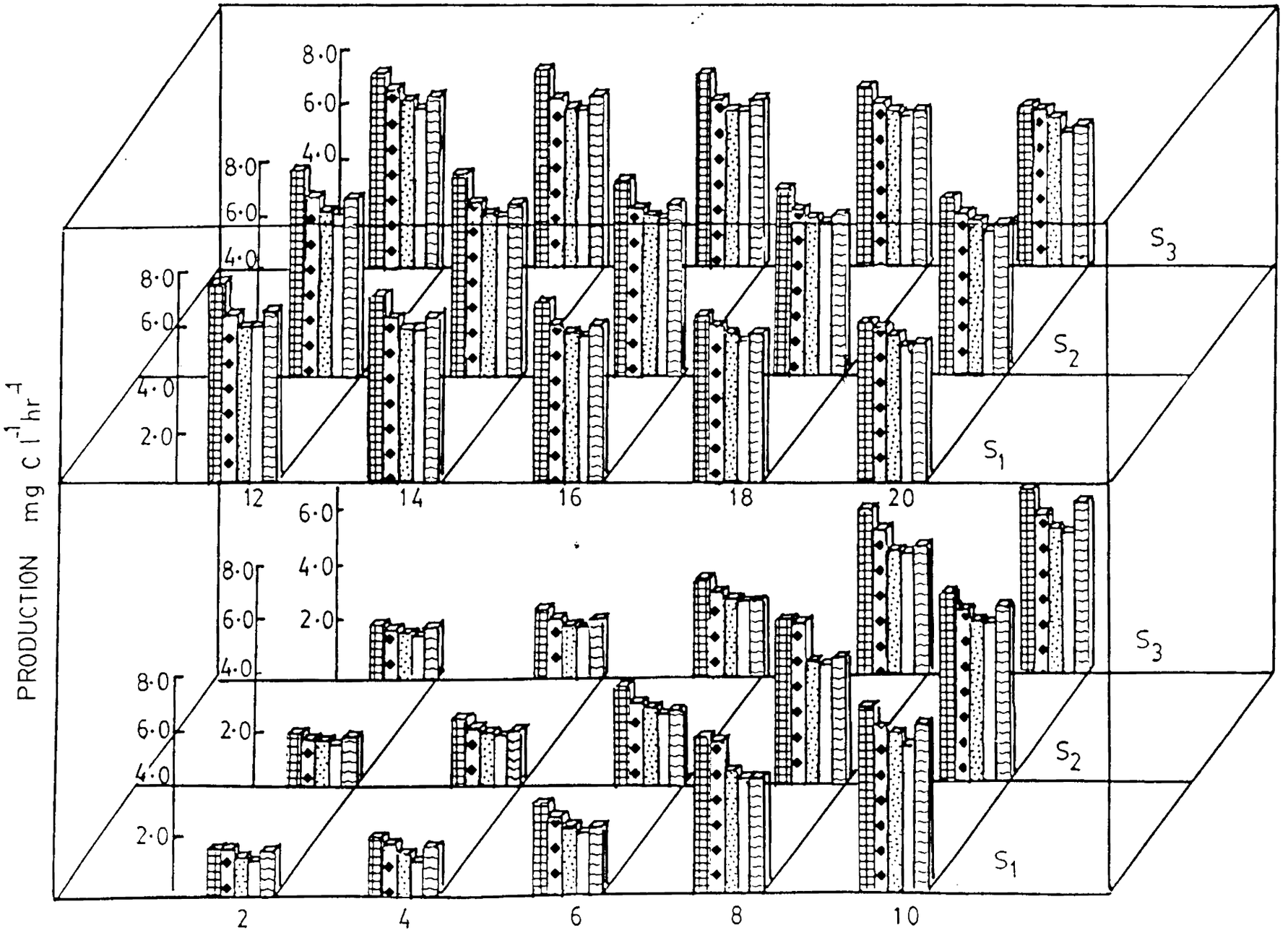
 - 0.050 ppm

 - 0.100 ppm

 - 0.150 ppm

 - Control

FIG. 29



higher than that of control (Fig. 30B). The proportion of carotenoids was found to be higher than that of chlorophyll a and c The concentration of total pigments and the rate of production were 33% and 43% higher than those of control at pH 8.0 (Figs. 30A, 31, 32) Only in 0.15 ppm Zn, the physiological activity of the alga was low in relation to control.

The effect of temperature on the rate of carbon production in 0.05 ppm, 0.10 ppm and 0.15 ppm Zn increased from 20°C to 30°C, showing the maximum level at 30°C, subsequently exhibited a constant decline towards a higher temperature of 40°C (Table 2).

Synechocystis salina

During all phases of growth, cell content and photosynthetic activity of the blue green alga increased from 15 ppt to 25 ppt and declined towards 35 ppt salinity. 0.02 ppm and 0.05 ppm Zn were more favourable for the growth of *S. salina*. Between the sixth and the eighth day there was three to four fold increase in the cell concentration in all the three selected zinc concentrations and at all salinities. Maximum cell concentration was attained in 0.05 ppm Zn at 25 ppt salinity about 35% higher than that of control (Fig. 33B).

The concentration of chlorophyll a and non-cholorophyllous pigments reached their maximum level on the eighth day at 25 ppt salinity about 24% and 18% higher than that of control in 0.05 ppm (Figs. 34, 35). The rate of carbon production increased till the eighth day and subsequently

FIG. 30 *Isochrysis galbana*

Effect of zinc on biomass and rate of carbon production at different pH.

A. - Production

B. - Biomass

Zinc concentrations

 - 0.050 ppm

 - 0.100 ppm

 - 0.150 ppm

 - Control

FIG.30

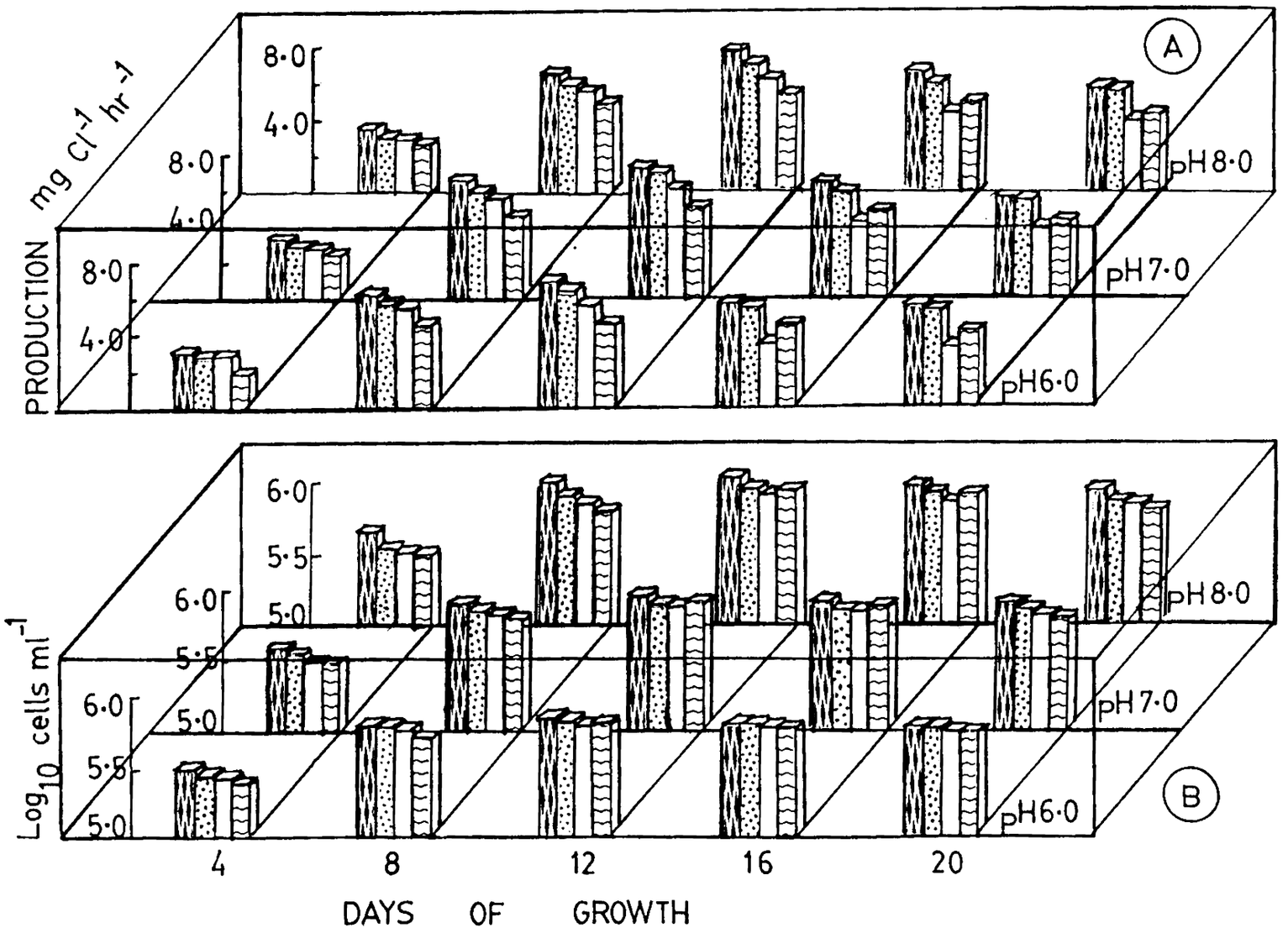

A
B
PRODUCTION mg C l-1 hr-1
Log10 cells ml-1
8·0
4·0
6·0
5·5
5·0
pH8·0
pH7·0
pH6·0
4
8
12
16
20
DAYS OF GROWTH

FIG. 31. *Isochrysis galbana*

Effect of zinc on the chlorophyll 'a' content at different pH.

Zinc concentrations

 - 0.050 ppm

 - 0.100 ppm

 - 0.150 ppm

 - Control

FIG. 31

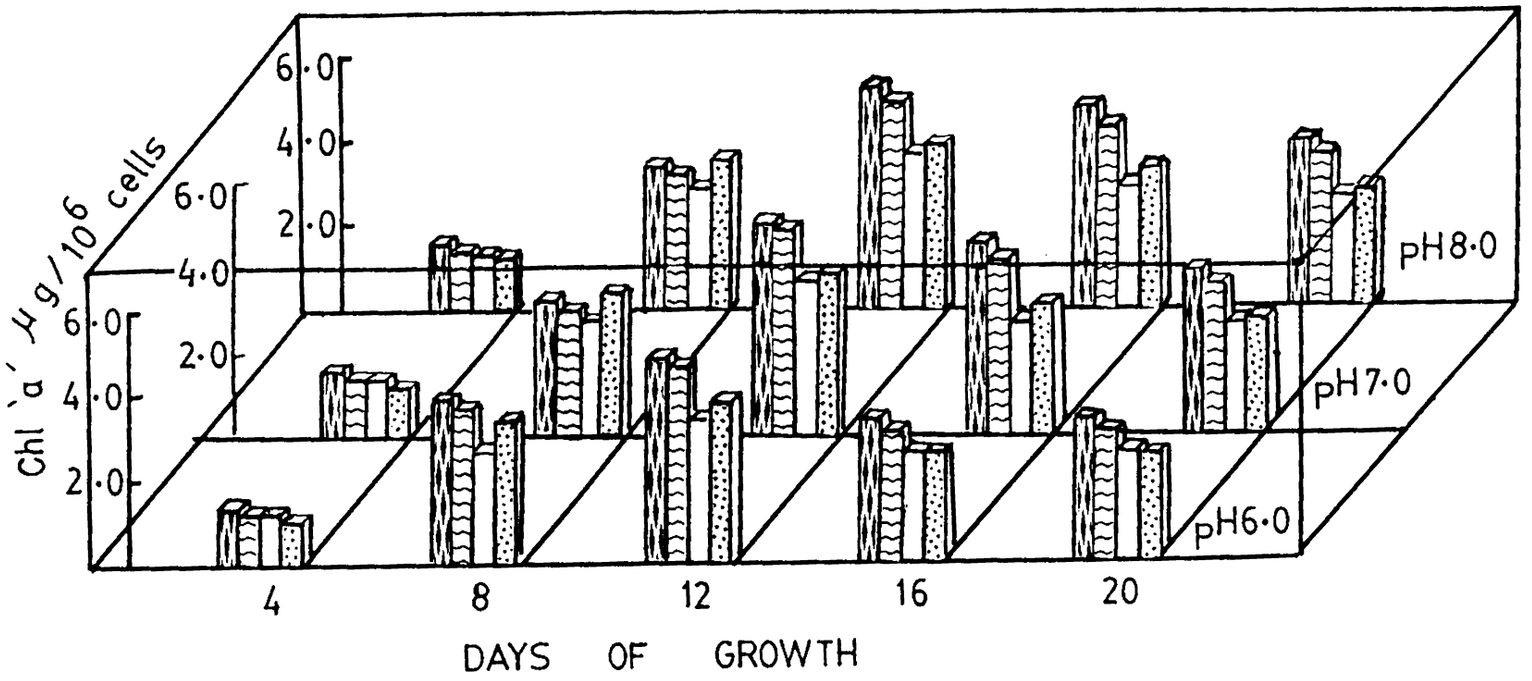

Chl 'a' μg/10⁶ cells
6·0
4·0
2·0
6·0
4·0
2·0
6·0
4·0
2·0
pH8·0
pH7·0
pH6·0
4
8
12
16
20
DAYS OF GROWTH

FIG. 32. *Isochrysis galbana*

Effect of zinc on the chlorophyll 'c' and carotenoid content at different pH.

A - Carotenoid

B - Chlorophyll 'c'

Zinc concentrations

 - 0.050 ppm

 - 0.100 ppm

 - 0.150 ppm

 - Control.

FIG. 32

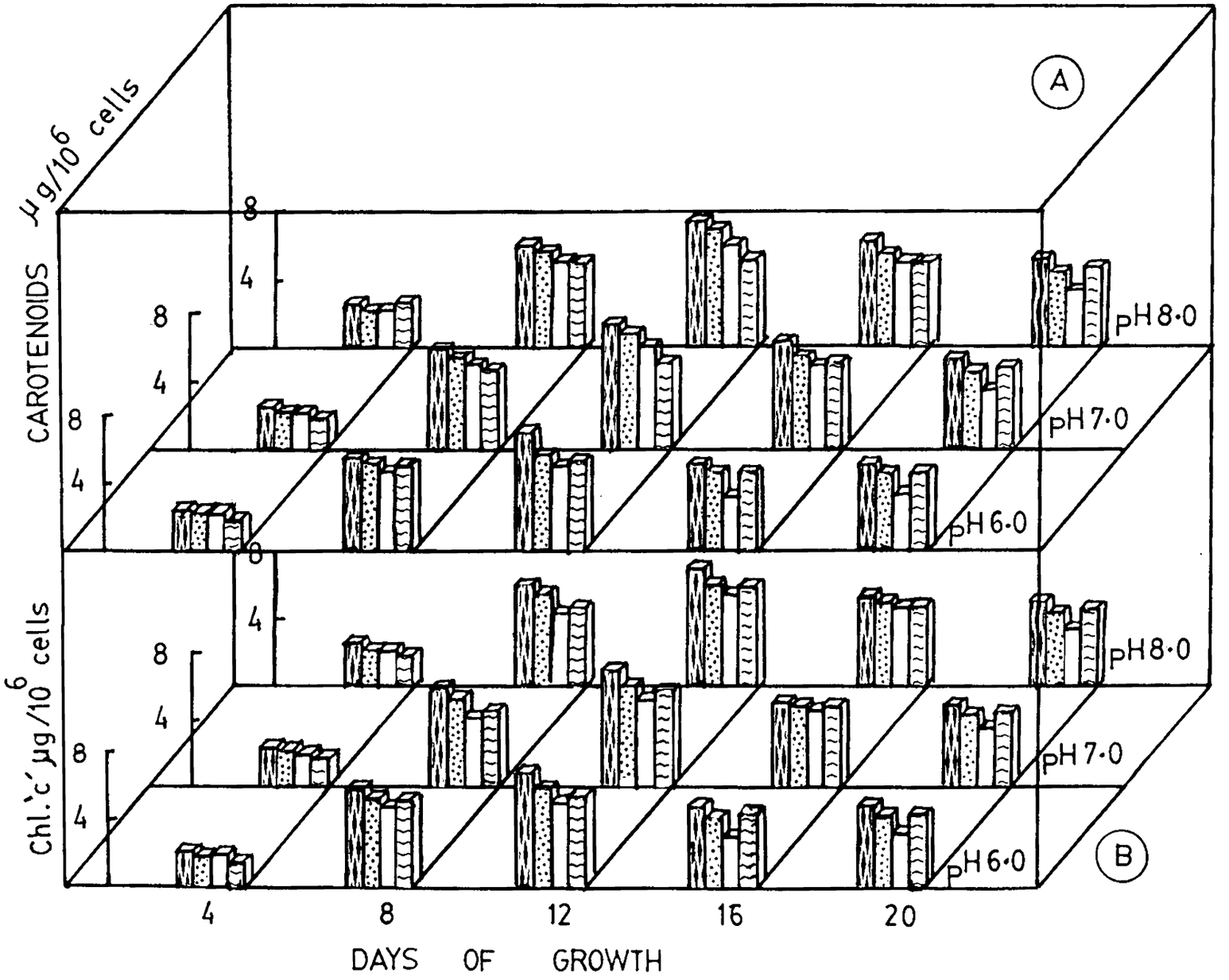

CAROTENOIDS µg/10⁶ cells
Chl.'c' µg/10⁶ cells
A
B
pH 8·0
pH 7·0
pH 6·0
8
4
4
8
12
16
20
DAYS OF GROWTH

FIG. 33. *Synechocystis salina.*

Effect of zinc on biomass and rate of carbon production in different salinities (S).

S_1 - 15 ppt

S_2 - 25 ppt

S_3 - 35 ppt

A - Production

B - Biomass

Zinc concentrations

 - 0.020 ppm

 - 0.050 ppm

 - 0.070 ppm

 - Control.

FIG·33

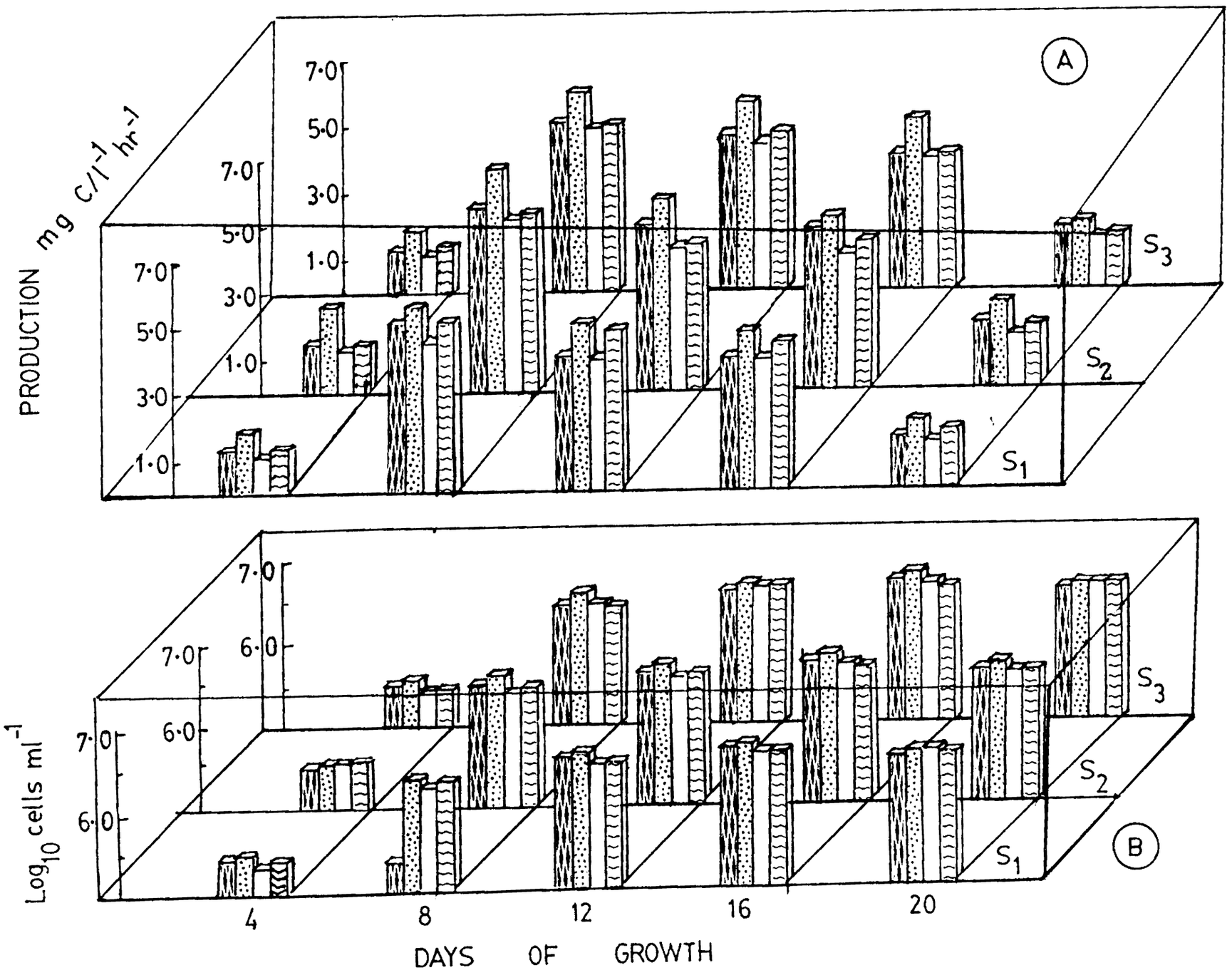

A
B
PRODUCTION mg C/l⁻¹ hr⁻¹
Log10 cells ml⁻¹
7·0
5·0
3·0
1·0
6·0
S3
S2
S1
4
8
12
16
20
DAYS OF GROWTH

FIG. 34. *Synechocystis salina*

Effect of zinc on the chlorophyll 'a' content in different salinities(S).

S_1 - 15 ppt

S_2 - 25 ppt

S_3 - 35 ppt.

Zinc concentrations

 - 0.020 ppm

 - 0.050 ppm

 - 0.070 ppm

 - Control

FIG·34

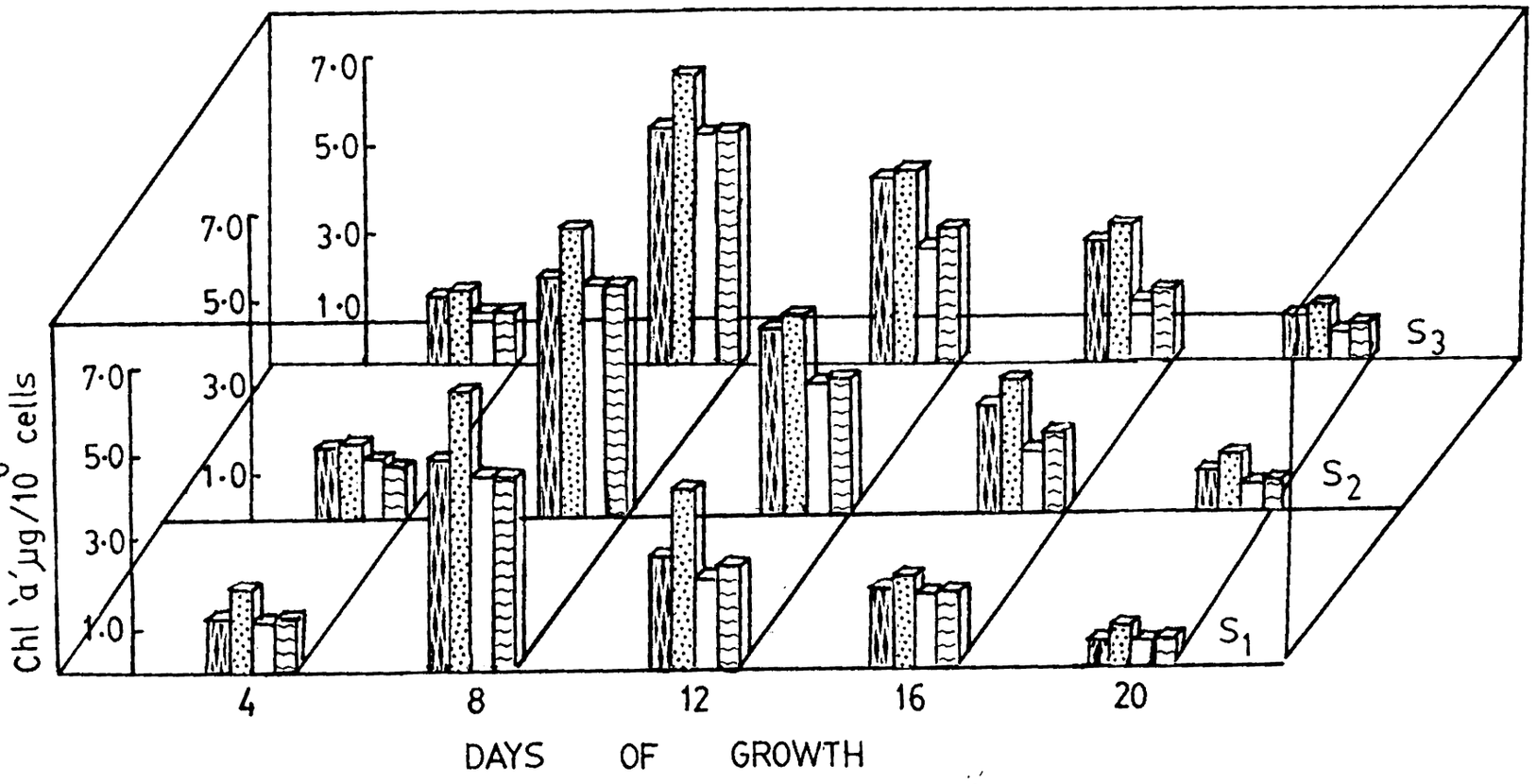

7·0
5·0
3·0
1·0
7·0
5·0
3·0
1·0
7·0
5·0
3·0
1·0
S3
S2
S1
Chl 'a' µg/10^6 cells
4
8
12
16
20
DAYS OF GROWTH

FIG. 35. *Synechocystis salina*

Effect of zinc on the content of non-chlorophyllous pigments in different salinities (S).

S_1 - 15 ppt

S_2 - 25 ppt

S_3 - 35 ppt

Zinc concentrations

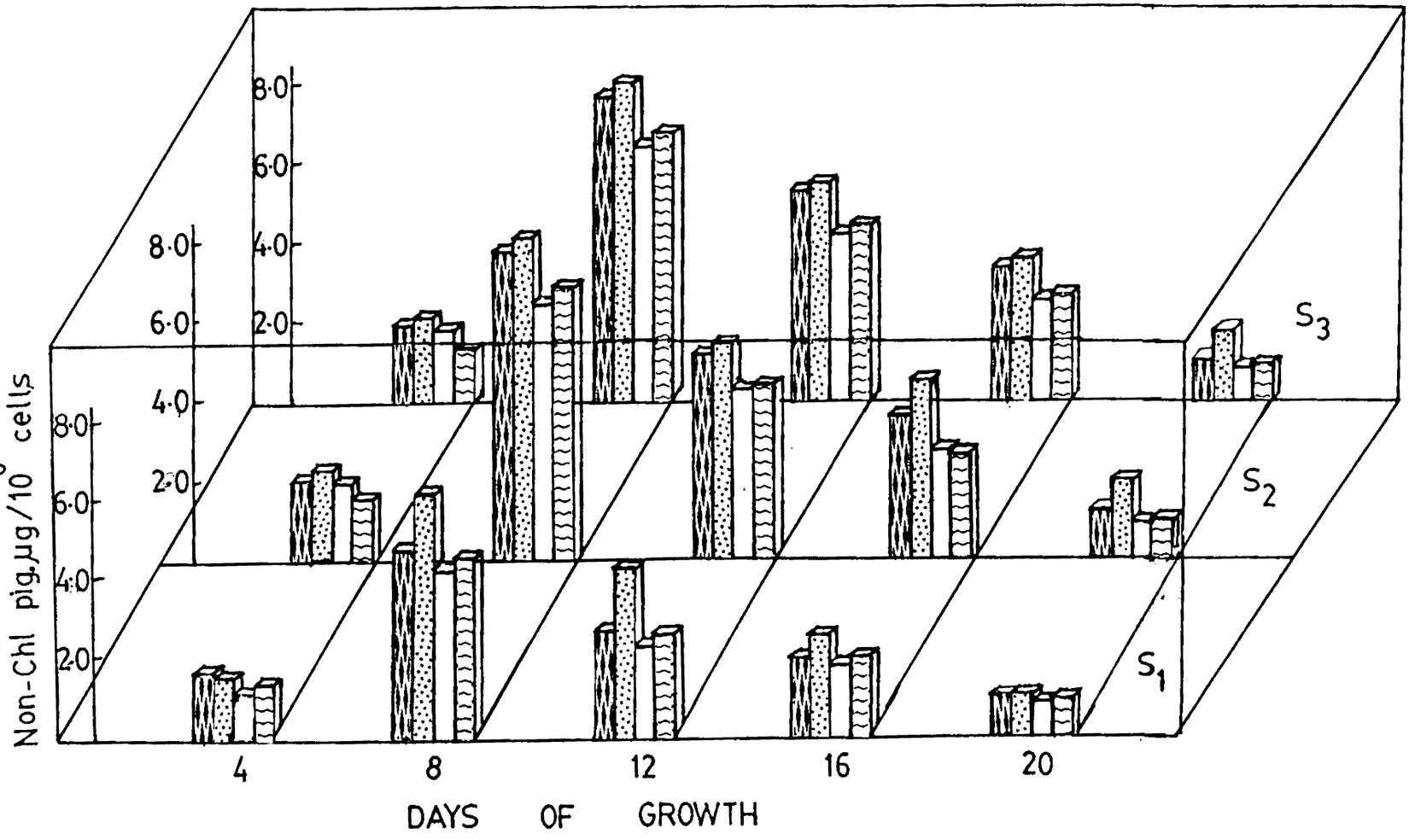
 - 0.020 ppm

 - 0.050 ppm

 - 0.070 ppm

 - Control

FIG. 35


Non-Chl pig.µg/10^6 cells
8·0
6·0
4·0
2·0
S3
S2
S1
4
8
12
16
20
DAYS OF GROWTH

a decline was noticed on the approach of stationary phase. Highest value of 6.81 mg C/l/hr was recorded on the eighth day at 25 ppt salinity in 0.05 ppm Zn (Fig. 33A).

A reduction in the growth rate of the blue green alga was observed in 0.07 ppm Zn in relation to control.

The growth rate and metabolic activity of *S. salina* were enhanced to a larger extent at higher pH levels in all the three selected zinc concentrations. Biomass increased from the fourth day to the sixteenth day, thereafter declined on the twentieth day. Cell concentration increased five to six times between the eighth day and the twelveth day. Biomass was maximum on the sixteenth day in 0.05 ppm at pH 8.0 (Fig. 36B).

The amount of total pigments and the rate of carbon production increased from pH 6.0 to 8.0 in 0.02 ppm, 0.05 ppm and 0.07 ppm Zn. Maximum values of chlorophyll a, non-chlorophyllous pigments and carbon production were recorded on the twelveth day at pH 8.0 with about 23%, 29% and 31% higher values than those of control in 0.05 ppm Zn (Figs. 36A, 37).

At different temperatures, 0.05 ppm Zn was more favourable in enhancing the production rate of *S. salina* in relation to control. Production was maximum at 25°C and declined thereafter with an increase in temperature. In 0.07 ppm Zn treatment, the carbon production was lower than that of control (Table 4).

FIG. 36. *Synechocystis salina*

Effect of zinc on biomass and rate of carbon production at different pH.

A - Production

B - Biomass

Zinc concentrations

 - 0.020 ppm

 - 0.050 ppm

 - 0.070 ppm

 - Control

FIG.36

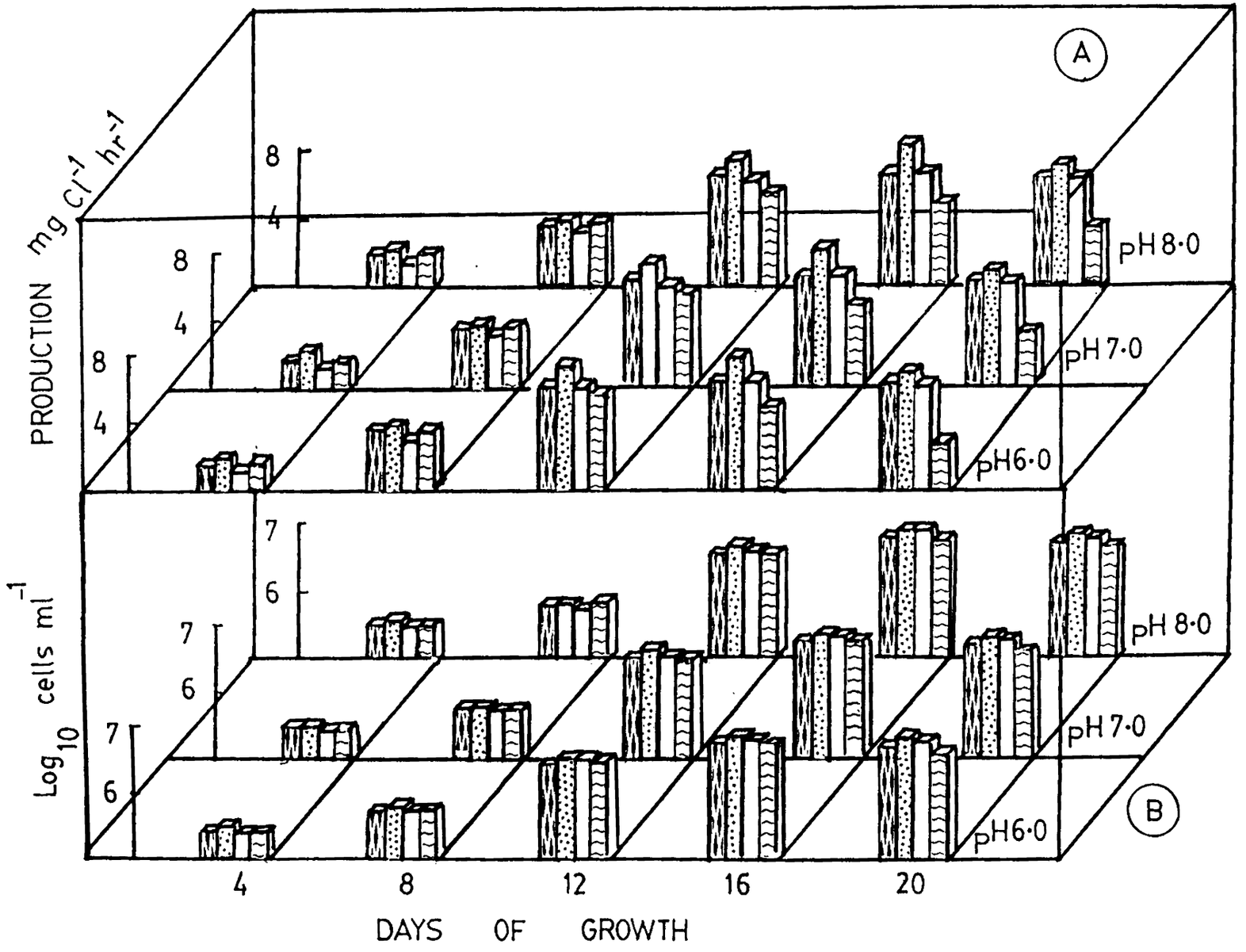

A
B
PRODUCTION mg C l⁻¹ hr⁻¹
Log10 cells ml⁻¹
pH 8·0
pH 7·0
pH6·0
8
4
7
6
4
8
12
16
20
DAYS OF GROWTH

FIG. 37. *Synechocystis salina*

Effect of zinc on the chlorophyll 'a' content and non-chlorophyllous pigments at different pH.

A - Non-chlorophyllous pigments

B - Chlorophyll 'a'

Zinc concentrations

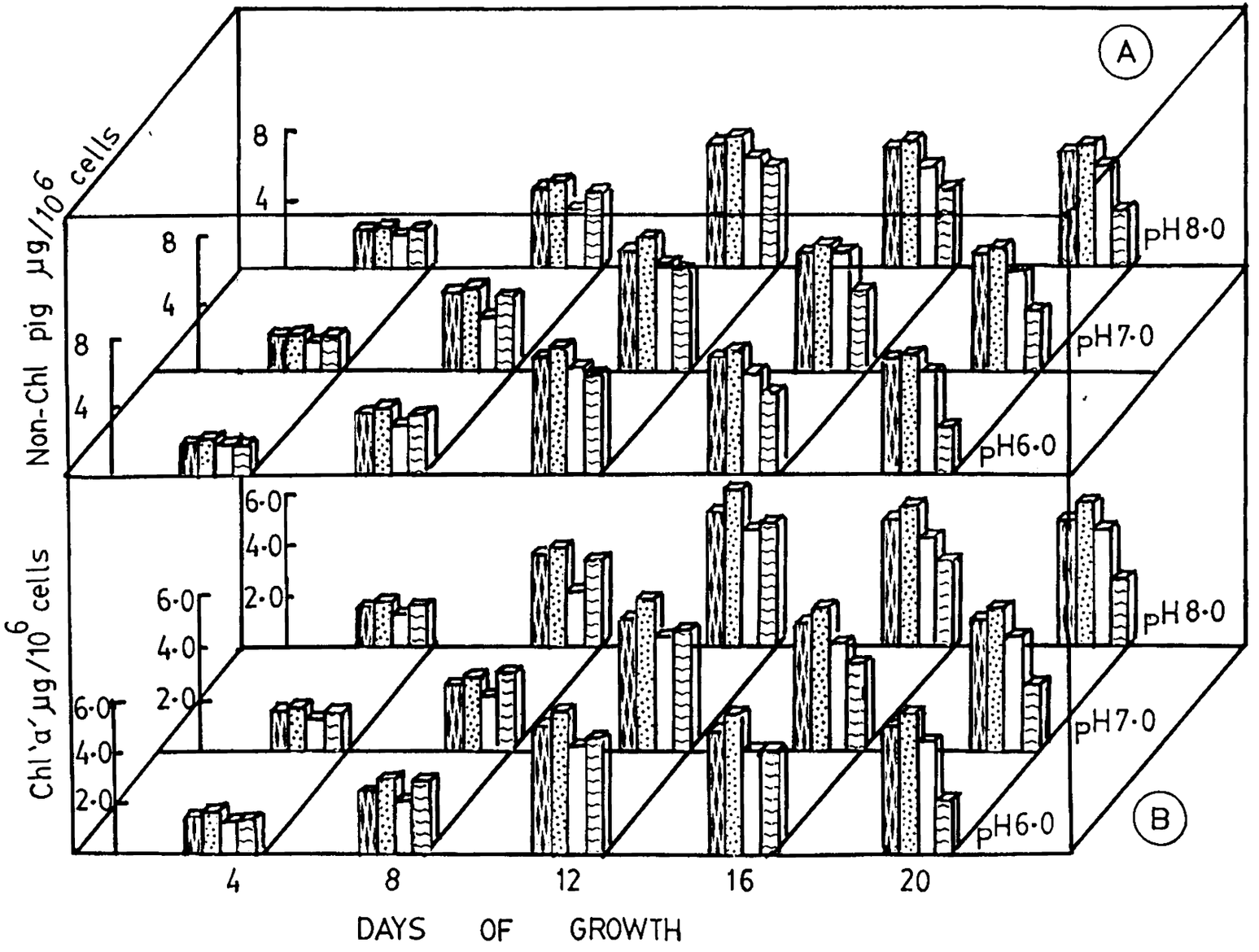
 - 0.020 ppm

 - 0.050 ppm

 - 0.070 ppm

 - Control

FIG. 37


A
B
Non-Chl pig µg/10⁶ cells
Chl 'a' µg/10⁶ cells
8
4
6·0
4·0
2·0
pH 8·0
pH 7·0
pH 6·0
4
8
12
16
20
DAYS OF GROWTH

Discussion :

Zinc is an important micronutrient for the growth and metabolism of various algae and much work has been done on its metabolism especially in *Euglena* (O'Kelley, 1974). Jensen *et al.*,(1974) observed that though zinc is an essential element, it becomes more toxic at higher levels and different species vary widely in their sensitivity to zinc toxicity.

Tolerance capacity of *I. galbana* and *S. salina* to zinc did not differ appreciably. It was found that 0.01 ppm to 0.05 ppm Zn was stimulatory to *I. galbana* and 0.02 ppm to 0.05 ppm Zn accelerated the growth and photosynthetic activity of *S. salina*. Inhibition of growth in the two species occurred in treatments above 0.10 ppm Zn. But reports of previous workers have indicated that a still higher level of zinc (0.271 ppm to 7.10 ppm) caused a 50% reduction in cell division in various species of marine diatoms and chlorophytes (Rachlin and Farran 1974; Rosko and Rachlin, 1975; Rachlin *et al.*, 1982, 1983).

An initial growth lag in *S. salina* reflecting a reduction in the metal ion activity through passive binding to dead cells has been also noted by Bartlett *et al.*(1974) in *Selenastrum capricornutum*.

Dense cultures of *I. galbana* and *S. salina* are able to resist the toxic action of zinc and the cells begin to grow eventually. This can be accounted by the fact that the exudation of waste products from the

senescent cultures which complex the medium will thus not only compete for and thereby reduce the amount of metal taken up but will also decrease the rate of incorporation into the plant cells, both processes providing a degree of protection against the toxic effect of the metal (Davies, 1973).

Fisher and Fabris (1982) have also established that the metal complexing capacities of exudates differed in the filtrates of cells in log and senescent phases, suggesting a difference in exudate composition. Moreover the evidence that the senescent cells complexed only zinc can be one of the possible reasons for overcoming the toxic action of zinc by the senescent cultures of *I. galbana* and *S. salina*.

In the present study, the number of days for division of *I. galbana* and *S. salina* were found to increase with increasing zinc concentration. Such a correlation is in confirmation with the earlier observation made in *Chlorella saccharophila* by Rachlin *et al.*, (1982) and in *Navicula incerta* by Rachlin *et al.*, (1983).

On exposure to a higher concentration of 0.10 ppm Zn, *I. galbana* and *S. salina* showed reduction in growth which is a reasonable determinant of toxic effect. De Filippis *et al.*, (1981) proposed that the ability of metals to inhibit NADP - oxidoreductase, lowering the cells's NADPH could manifest itself in lowered growth rates. The degree of response of the alga is dependent on the amount of metal which has not been bound in the

cell's detoxifying mechanisms (Rothstein, 1959 and Simkiss, 1979).

Davies (1978) suggested that the cellular surface of phytoplankton consisted of a mosaic of cationic and anionic exchange sites, and that initial uptake of heavy metals was related to simple ion exchange where positively charged metal ions displace cations present on the cell surface. The amount of heavy metal bound to the cell surface at equilibrium is determined by both the affinities of the binding sites for the cations and their free ion concentration in solution. As the zinc concentrations employed are metabolised by the cells of *I. galbana* and *S. salina*, it is further speculated that once inside the cell, the zinc ions initially become compartmentalized into the cell's polyphosphate bodies and other organelles in an attempt to reduce the toxic burden of the metal, if it exceeds 0.10 ppm (cf. Jensen *et al.*, 1982).

An increase in cell number, higher production of pigments and an increase in the rate of carbon fixation over that of control in zinc treatments of 0.01 ppm and 0.05 ppm in *I. galbana* and 0.02 to 0.05 ppm in *S. salina* has been also observed by Kanakavalli Susarla (1987) in *Scenedesmus bijugatus*. But Davies and Sleep (1979b) have found 0.01 - 0.015 ppm Zn as the minimal concentration causing detectable inhibition of carbon fixation i.e. less than 90% of control values. Inspite of an increase in toxicity with dosage, Fisher *et al.*, (1981) have demonstrated

that the cells of *Asterionella japonica* did not lose their ability to photosynthesise, but their carbon fixation rates exceeded those of control and the chlorophyll content was higher in zinc treated cells.

Short term studies on the uptake and accumulation of zinc in *I. galbana* and *S. salina* indicated that the amount of zinc taken up by the cells increased with increasing external metal concentration. A linear relationship between metal taken up and external metal concentration was also observed in *Laminaria digitata* and in *Ditylum brightwellii* (Bryan, 1969 and Canterford *et al.*, 1978).

The role of environmental factors such as salinity, pH and temperature on modifying the toxicity of zinc to algae has received little attention by phycologists. In the present studies, a salinity of 25 ppt at higher pH levels above 7.0 and a temperature of 25°C - 30°C were more suitable for the growth of the flagellate and the blue green alga. Styron *et al.*, (1976) in his studies on uptake of ^{65}Zn by six genera of marine phytoplankton under a factorial array of temperature and salinity combinations noticed not only the pattern of metal uptake varied widely for different organisms but there was no consistency between the uptake rates.

Parry and Hayward (1973) have reported that the uptake of ^{65}Zn in *Dunaliella tertiolecta* was temperature and pH dependent and was

indirectly linked with metabolism. The ^{65}Zn associated into living cells has shown to be firmly bound and it has been suggested that the binding sites are proteinaceous compounds. It was also found that pH changes in the medium caused changes in the rate and amount of ^{65}Zn uptake. At pH 9.0, cells took up 50% more ^{65}Zn than at pH 7.0. Zinc uptake markedly increased at pH values higher than 9.0. Similar observations were evident in *I. galbana* and *S. salina* wherein the enhancement of growth and greater photosynthetic activity occurred at alkaline pH levels above 7.0. But Michnowicz and Weaks (1984) have noticed depression of growth at all pH levels in *Selenastrum capricornutum* in 0.200 ppm Zn.

Preliminary findings of Patrick (1971) and Carins *et al.*, (1978) are confusing about the influence of temperature in regulating the metabolic activity of algae. Patrick (1971) found that toxicity of zinc to *Nitzschia linearis* increased with increasing temperature from 22°C to 30°C, whereas Cairns *et al.*, (1978) found contradictory effects of zinc on different algae. For *Cyclotella menghiniana*, zinc toxicity increased with increase in temperature as observed in the present study in *I. galbana* and *S. salina*, but for *Scenedesmus quadricauda* zinc toxicity decreased. The mechanism governing the increased toxicity of zinc at high temperatures may be due to enhanced respiratory activities. Other processes that may affect toxicity are adsorption and desorption of zinc ions by an increase or a decrease in temperature.

4.3. Interaction of two metals on *L. galbala* and *S. salina:*

4.3.1. Copper and Manganese:

In both the species the cell concentration increased till the eighth day and decreased on the fourteenth day. A mixture of 0.05 ppm Cu and 0.07 ppm Mn was favourable for the growth of the flagellate during the early phase and 0.15 ppm Cu and 0.005 ppm Mn was more suitable for the algal growth towards the end of the exponential phase. The latter concentration of copper and manganese enhanced the total pigment content of the blue green alga to a larger extent. Decline in the concentration of pigments was noticed after the second day in the flagellate and after the fourth day in the blue green alga. A distinctive feature exhibited in both the species was a significant hike in the quantity of total pigments on the fourteenth day. This may be attributed to the excretion of extracellular products as the culture ages thereby decreasing the toxic effect of the two metals. An addition of a low concentration of manganese can account for nullifying the toxic effect of the higher dosage of copper 0.15 ppm which, when treated alone would be harmful (Tables 6,7).

4.3.2. Copper and Zinc:

Cell concentration increased till the eighth day and then declined on the fourteenth day. Higher concentrations of copper 0.10 ppm and zinc 0.15 ppm enhanced the total pigment content of both algae. Concentration of chlorophyll a and non-chlorophyllous pigments in the blue green alga was at its maximum level on the fourth day, thereafter

Table 6. Interaction of two metals on *I. galbana*.

METAL		SECOND DAY			FOURTH DAY			EIGHTH DAY			FOURTEENTH DAY		
Concn(ppm)		Cell Concn/ ml	Chl'a' $\mu g/10^6$ cells	Carot. $\mu g/10^6$ cells	Cell Concn/ ml	Chl 'a' $\mu g/10^6$ cells	Carot. $\mu g/10^6$ cells	Cell Concn/ ml	Chl 'a' $\mu g/10^6$ cells	Carot. $\mu g/10^6$ cells	Cell Concn/ ml	Chl 'a' $\mu g/10^6$ cells	Carot. $\mu g/10^6$ cells
Cu + Mn	0.005 + 0.020	25,000	2.32	3.00	7,40,000	1.31	1.52	16,30,000	1.60	1.18	8,50,000	1.84	2.35
	0.050 + 0.070	20,000	9.94	5.00	7,00,000	2.29	1.25	6,50,000	1.14	0.69	5,20,000	1.73	1.06
	0.100 + 0.150	30,000	7.63	5.00	2,00,000	1.23	2.45	2,30,000	2.20	1.30	1,60,000	4.76	2.34
	0.150 + 0.005	45,000	5.09	3.33	80,000	3.44	5.00	1,00,000	6.72	3.50	70,000	6.24	1.71
Cu + Zn	0.005 + 0.020	50,000	6.89	6.00	1,60,000	1.28	2.75	2,00,000	13.13	5.50	1,20,000	9.35	10.42
	0.070 + 0.100	40,000	7.71	7.00	1,80,000	1.59	1.67	2,50,000	2.36	8.00	1,80,000	6.79	3.89
	0.100 + 0.150	40,000	9.43	13.13	1,50,000	1.99	1.67	2,60,000	1.10	1.38	2,00,000	3.27	1.88
	0.150 + 0.005	60,000	5.66	6.25	1,20,000	2.87	1.42	1,70,000	3.02	1.58	1,50,000	2.84	3.83
Zn + Mn	0.005 + 0.020	35,000	10.48	17.14	4,05,000	1.48	3.46	6,70,000	1.54	1.79	4,50,000	3.42	2.56
	0.070 + 0.100	50,000	8.98	12.00	2,00,000	5.53	5.95	3,00,000	2.35	9.61	2,50,000	8.97	9.60
	0.100 + 0.150	30,000	14.17	24.17	3,60,000	3.70	3.56	5,20,000	1.64	5.29	3,60,000	4.28	3.33
	0.150 + 0.005	30,000	15.20	23.30	2,40,000	3.50	4.17	3,10,000	2.27	8.06	2,00,000	8.50	13.00

Table 7. Interaction of two metals on *S. salina*.

METAL		SECOND DAY			FOURTH DAY			EIGHTH DAY			FOURTEENTH DAY		
Concn.(ppm)		Cell concn./ ml	Chl 'a' $\mu g/10^6$ cells	Non-chl. Pig. $\mu g/10^6$ cells	Cell Concn/ ml	Chl 'a' $\mu g/10^6$ cells	Non-chl. Pig. $\mu g/10^6$ cells	Cell Concn/ ml	Chl 'a' $\mu g/10^6$ cells	Non-chl. Pig. $\mu g/10^6$ cells	Cell Concn/ ml	Chl 'a' $\mu g/10^6$ cells	Non-chl. Pig. $\mu g/10^6$ cells
Cu + Mn	0.005 + 0.020	40,000	2.40	2.32	8,00,000	5.12	4.70	35,00,000	4.80	5.00	30,00,000	5.10	6.02
	0.050 + 0.070	45,000	3.20	3.00	8,50,000	6.50	6.01	40,00,000	6.12	5.96	35,00,000	6.43	5.90
	0.100 + 0.150	35,000	2.16	2.00	3,00,000	4.48	4.10	20,00,000	4.30	4.42	18,00,000	4.88	4.92
	0.150 + 0.005	50,000	3.32	4.02	2,75,000	6.80	8.16	15,00,000	6.50	8.10	12,00,000	7.10	8.66
Cu + Zn	0.005 + 0.020	55,000	3.30	4.42	2,50,000	6.78	8.92	15,05,000	6.92	9.90	15,00,000	7.22	9.10
	0.070 + 0.100	45,000	4.30	5.51	2,00,000	8.50	10.20	10,00,000	8.98	10.85	7,00,000	8.40	9.86
	0.100 + 0.150	50,000	4.62	5.70	3,10,000	9.40	12.40	20,50,000	10.12	12.06	16,00,000	9.62	11.63
	0.150 + 0.005	65,000	4.16	5.62	5,00,000	8.24	11.30	25,00,000	8.50	11.52	20,00,000	8.82	10.25
Zn + Mn	0.005 + 0.020	45,000	4.51	5.18	4,50,000	8.10	10.26	23,00,000	7.62	9.40	20,00,000	7.80	9.61
	0.070 + 0.100	50,000	4.66	5.46	5,00,000	9.80	12.30	25,00,000	10.28	11.50	22,00,000	10.20	11.26
	0.100 + 0.150	40,000	4.31	5.01	4,00,000	8.60	10.02	20,00,000	7.72	9.60	15,00,000	7.62	9.40
	0.150 + 0.005	60,000	5.38	6.14	5,50,000	10.56	13.20	30,00,000	9.40	12.10	25,00,000	9.01	11.32
CONTROL		55,000	2.13	1.74	4,30,000	3.49	4.02	20,50,000	5.03	6.00	15,00,000	4.51	5.62

showed a slight increase on the eighth day. In this species, the proportion of non-chlorophyllous pigments was far higher than that of chlorophyll a. In the case of the flagellate, a lower concentration of copper and zinc namely 0.005 ppm and 0.02 ppm was more effective in increasing the pigment content. Remarkable hike in the concentration of chlorophyll a and carotenoids was also observed in all the combinations on the fourteenth day. Combination of a lower concentration of copper along with zinc was more suitable in stimulating the photosynthetic activity of the flagellate (Tables6, 7).

4.3.3. Zinc and Manganese:

As mentioned in the above two cases, biomass increased till the eighth day and then declined on the fourteenth day in both the species. The combination of 0.15 ppm Zn and 0.005 ppm Mn was more suitable in enhancing the pigment content of the blue green alga on the fourth day and on the second day in the flagellate. But in *L. galbana* from the fourth day onwards a drastic reduction in the concentration of chlorophyll a and carotenoids was observed. However on the fourteenth day, in the two following combinations 0.07 ppm Zn and 0.10 ppm Mn; 0.15 ppm Zn and 0.005 ppm Mn, chlorophyll a increased nearly four times (Tables 6,7).

From the above instances, it is possible to understand the synergistic and antagonistic behaviour of the metal ions when they are combined together.

4.4. Interaction of three metals on *L. galbana* and *S. salina:*

4.4.1. Copper and Manganese and Zinc:

Identification of a particular set of combination as the best cannot be specified as there is a modification in the action of the trace metals with the age of the culture and interference from environmental factors.

In the flagellate, 0.02 ppm Cu + 0.15 ppm Mn + 0.005 ppm Zn accelerated the amount of carotenoids (18.25 $\mu g/10^6$ cells) to a great extent and 0.100 ppm Cu + 0.150 ppm Mn + 0.005 ppm Zn enhanced the quantity of chlorophyll a (11.17 $\mu g/10^6$ cells) to a larger extent. Both the recorded values were far higher than that of control. (Table 8).

After the second day the quantity of total pigments declined though there was a marginal increase on the fourteenth day.

In the case of *S. salina* on the second day, the proportion of non-chlorophyllous pigments was larger than that of chlorophyll a except in 0.150 ppm Cu + 0.005 ppm Mn + 0.020 ppm Zn where the chlorophyll a content was more than the non-chlorophyllous pigments. This combination was found to be favourable in increasing the photosynthetic activity of the blue green alga (Table. 9).

Table 8. Interaction of three metals on *I. galbana*.

METAL	SECOND DAY			FOURTH DAY			EIGHTH DAY			FOURTEENTH DAY		
Concn(ppm)	Cell Concn/ ml	Chl 'a' $\mu g/10^6$ cells	Carot $\mu g/10^6$ cells	Cell Concn/ ml	Chl 'a' $\mu g/10^6$ cells	Carot $\mu g/10^6$ cells	Cell Concn/ ml	Chl 'a' $\mu g/10^6$ cells	Carot $\mu g/10^6$ cells	Cell Concn/ ml	Chl 'a' $\mu g/10^6$ cells	Carot $\mu g/10^6$ cells
0.005 + 0.020 + 0.050	7,00,000	2.37	6.07	8,20,000	0.68	0.97	13,80,000	0.33	2.90	7,00,000	2.58	1.21
0.020 + 0.050 + 0.070	8,00,000	1.65	18.25	7,80,000	0.21	0.57	16,30,000	1.25	1.13	9,50,000	1.86	2.47
Cu + Mn 0.050 + 0.070 + 0.100	9,00,000	1.72	8.89	9,50,000	4.20	0.94	20,50,000	0.37	1.02	10,50,000	1.54	2.14
+ Zn 0.070 + 0.100 + 0.150	9,00,000	0.49	2.77	3,60,000	0.15	1.25	5,70,000	0.56	1.58	4,40,000	1.56	3.07
0.100 + 0.150 + 0.005	11,00,000	3.00	2.73	2,80,0 00	1.98	2.14	3,00,000	1.48	2.00	2,30,000	5.64	4.78
0.150 + 0.005 + 0.020	2,00,000	11.17	5.00	2,60,000	2.64	4.38	2,50,000	2.68	3.60	2,00,000	0.91	1.33
CONTROL	7,00.000	5.70	3.25	6,00,000	0.88	0.92	14,50,000	0.59	0.58	7,70,000	0.34	5.84

Table 9. Interaction of three metals on *S. salina*.

METAL		SECOND DAY			FOURTH DAY			EIGHTH DAY			FOURTEENTH DAY		
Concn (ppm)		Cell Concn/ ml	Chl 'a' $\mu g/10^6$ cells	Non-Chl. Pig. $\mu g/10^6$ cells	Cell Concn/ ml	Chl 'a' $\mu g/10^6$ cells	Non-Chl. Pig. $\mu g/10^6$ cells	Cell Concn/ ml	Chl 'a' $\mu g/10^6$ cells	Non-Chl. Pig. $\mu g/10^6$ cells	Cell Concn/ cell	Chl'a' Pig μg/ cells	Non-Chl Pig. $\mu g/10^6$ cells
	0.005 + 0.020 + 0.050	6,00,000	3.04	6.86	10,00,000	1.60	2.80	20,00,000	2.40	3.00	30,00,000	3.04	5.10
	0.020 + 0.050 + 0.070	8,00,000	3.70	7.52	15,00,000	1.88	1.96	30,00,000	1.96	2.50	40,00,000	3.32	4.62
Cu + Mn	0.050 + 0.070 + 0.100	10,00,000	4.16	8.62	18,00,000	2.42	3.24	40,00,000	2.60	3.86	50,00,000	4.10	5.20
+ Zn	0.070 + 0.100 + 0.150	10,50,000	3.28	4.96	12,00,000	1.70	2.60	25,00,000	1.91	2.40	35,00,000	2.24	2.04
	0.100 + 0.150 + 0.005	9,00,000	2.24	3.20	10,50,000	1.24	2.32	21,00,000	1.52	2.36	25,00,000	1.28	4.42
	0.150 + 0.005 + 0.020	5,00,000	6.36	5.31	7,00,000	1.08	2.50	15,00,000	1.20	2.66	20,00,000	0.98	4.52
	CONTROL	6,00,000	4.20	5.60	9,00,000	1.20	1.84	15,00,000	0.96	1.02	30,00,000	0.84	0.95

Discussion:

Trace metal interactions have not been studied extensively in phytoplankton. Only few reports have been found comparing the effects of toxic metals added individually with their effects when combined. The effects exerted on algae depend on the type of metal used and on the specific algal organisms exposed.

Most phytoplankton studies have examined the interactions of a nontoxic metal with a toxic one. The results obtained in the present study showed that low levels of zinc (0.005 ppm) enhanced copper inhibition of growth in *I. galbana*. Such a kind of synergistic behaviour was noticed in *Amphidinium carteri, Thalassiosira pseudonana, Skeletonema costatum* by Braek *et al.*, (1976). However much more frequently toxic and nontoxic metals have been found to interact competitively, so that the latter decrease toxic metal uptake or inhibition. In *I. galbana* and *S. salina*, a higher level of zinc (0.150 ppm) was found to relieve copper (0.100 ppm) inhibition of growth thereby enhancing the pigment content considerably. The antagonistic action of zinc on copper was also observed in *Phaeodactylum tricornutum* by Break *et al.*, (1976). Further evidence that copper and zinc compete for the same active sites comes from a study reported by Price and Quigley (1966) in which the growth of *Euglena gracilis* in zinc limited cultures was markedly higher in media lacking added copper than when copper was present.

Cells of *I. galbana* and *S. salina* in the presence of manganese were much more tolerant to copper than cells cultured in the absence of manganese. Only very small amounts of manganese (0.005 ppm) are needed to protect against copper toxicity which when supplied alone would have inhibited the growth of both the species. Increasing the manganese concentration to 0.150 ppm gave no additional protection thereby indicating that the extracellular binding sites must have been saturated with manganese at 0.005 ppm level. This mechanism by which manganese alleviates the toxicity of copper in *I. galbana* and *S. salina* has been confirmed in *Nitzschia closterium* by Stauber and Florence (1985). They also proposed that manganese adsorbs copper very effectively on the membrane surface and prevents its penetration into the cells. In the presence of algae, copper ions had a greater affinity for manganese in seawater which may partially account for the relative effectiveness of manganese as a protective agent.

Sunda *et al.*, (1981) found that addition of 10^{-7}M $MnCl_2$ totally reversed the toxicity of copper ($3x10^{-8}$M) to *Chaetoceros socialis* obtained from sea water depths. They proposed a mechanism involving cupric ion competition for manganese activated sites within the algal cell i.e. copper induces manganese deficiency which can be overcome by increased manganese concentration. The present study does not indicate the reversal of copper toxicity by manganese. These results support the finding of Stauber and Florence (1985) in *Nitzschia closterium*. Anderson and Morel (1978) also reported that manganese did not reverse the toxicity of copper to flagellar

motility in *Gonyaulax tamarensis*. Huntsman and Sunda (1980) therefore, concluded that copper, manganese interactions occur within the cell at specific loci, rather than at the membrane surface. Although the site of copper and manganese competition has not been identified, evidence suggests that it may be associated with the chloroplast. Habermann (1969) observed that both the Hill and Mehler reactions in isolated chloroplasts exhibit manganese reversible copper inhibition and hypothesised that copper and manganese compete for the same enzymatic sites.

Regarding the interaction of zinc and manganese, it has been found that a high concentration of zinc (0.150 ppm) and a low concentration of manganese (0.005 ppm) stimulated the growth and pigment content of the flagellate and the blue green alga. Treatment of 0.150 ppm Zn alone to the two algal species resulted in inhibition of growth. This antagonistic action of manganese on zinc suggests that manganese and zinc ions may compete for the same binding sites and thereby prevent the incorporation of zinc into the algal cells accounting for the detoxification.

Experiments on the interaction of all the three metals copper, manganese and zinc indicated that a low concentration of manganese and zinc (0.005 ppm + 0.020 ppm) along with a higher concentration of copper (0.150 ppm) enhanced the growth of *S. salina* whereas in the case of

I. galbana a definite set of combinations could not be recognised. A mixture of 0.020 ppm Cu + 0.050 ppm Mn + 0.005 ppm Zn accelerated the amount of carotenoids to a greater extent and a mixture of 0.100 ppm Cu + 0.150 ppm Mn + 0.005 ppm Zn increased the quantity of chlorophyll a per unit number of cells far higher than that of control.

Due to the lack of previous evidences on the interaction of two metals namely zinc and manganese, three metals namely copper, manganese and zinc, it has not been possible to substantiate the results for further discussion.

4.5. Chromatographic separation of pigments in *I. galbana* and *S. salina:*

On chromatographic separation, the pigments of the flagellate could be resolved into five fractions: light green - chlorophyll 'c', amber yellow - neofucoxanthin, orange-fucoxanthin, yellow-diadinoxanthin and blue green - chlorophyll a. In similar concentrations of copper, manganese and zinc, wide disparity was noticed in the R_F values (Table. 10).

Analysis of absorption spectra ranging from 420 nm to 465 nm for neofucoxanthin, fucoxanthin and diadinoxanthin indicated that there was no marked variation in the absorption maximum (450 nm) for the first two pigments in various concentrations of copper, manganese and zinc except in 0.050 ppm Zn level, the absorption maxima was at 455 nm (Figs. 38, 39, 40). In the case of diadinoxanthin, there are two peak

Table 10. R_F values of chlorophyll and carotenoids from *I. galbana* separated in two solvent systems on cellulose thin layer plates.

I - First Dimension II - Second dimension

Sp.	Manganese (ppm)								Zinc (ppm)								Copper (ppm)								Control	
No.	0.010		0.050		0.100		0.150		0.010		0.050		0.100		0.150		0.010		0.050		0.100		0.150			
	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II
1	0.29	0.06	0.37	0.08	0.26	0.07	0.32	0.07	0.40	0.05	0.38	0.07	0.39	0.08	0.41	0.07	0.27	0.00	0.31	0.00	0.25	0.00	0.27	0.06	0.38	0.08
2	0.68	0.24	0.76	0.35	0.61	0.25	0.69	0.25	0.71	0.25	0.77	0.39	0.75	0.30	0.78	0.25	0.59	0.24	0.66	0.35	0.65	0.36	0.66	0.31	0.67	0.30
3	0.73	0.51	0.74	0.62	0.67	0.50	0.71	0.49	0.76	0.52	0.79	0.66	0.75	0.42	0.78	0.40	0.66	0.55	0.72	0.66	0.69	0.66	0.69	0.56	0.70	0.48
4	0.77	0.80	0.78	0.86	0.74	0.74	0.76	0.75	0.81	0.77	0.80	0.83	0.81	0.81	0.80	0.82	0.77	0.86	0.80	0.90	0.81	0.86	0.78	0.85	0.81	0.60
5	0.92	0.08	0.94	0.84	0.90	0.72	0.93	0.81	0.96	0.89	0.93	0.81	0.93	0.83	0.97	0.82	0.93	0.73	0.95	0.90	0.95	0.83	0.94	0.72	1.00	0.72

Sp. No. - Spot Number

Sp. no. - 1 - Chlorophyll 'c' 3 - Fucoxanthin 5 - Chloropyll 'a'

2 - Neofucoxanthin 4 - Diadinoxanthin

FIG. 38.

Variation in the absorption spectra of neofucoxanthin (A), fucoxanthin (B) and diadinoxanthin (C) in *I. galbana* in different concentrations of copper.

FIG. 38 ABSORPTION SPECTRA

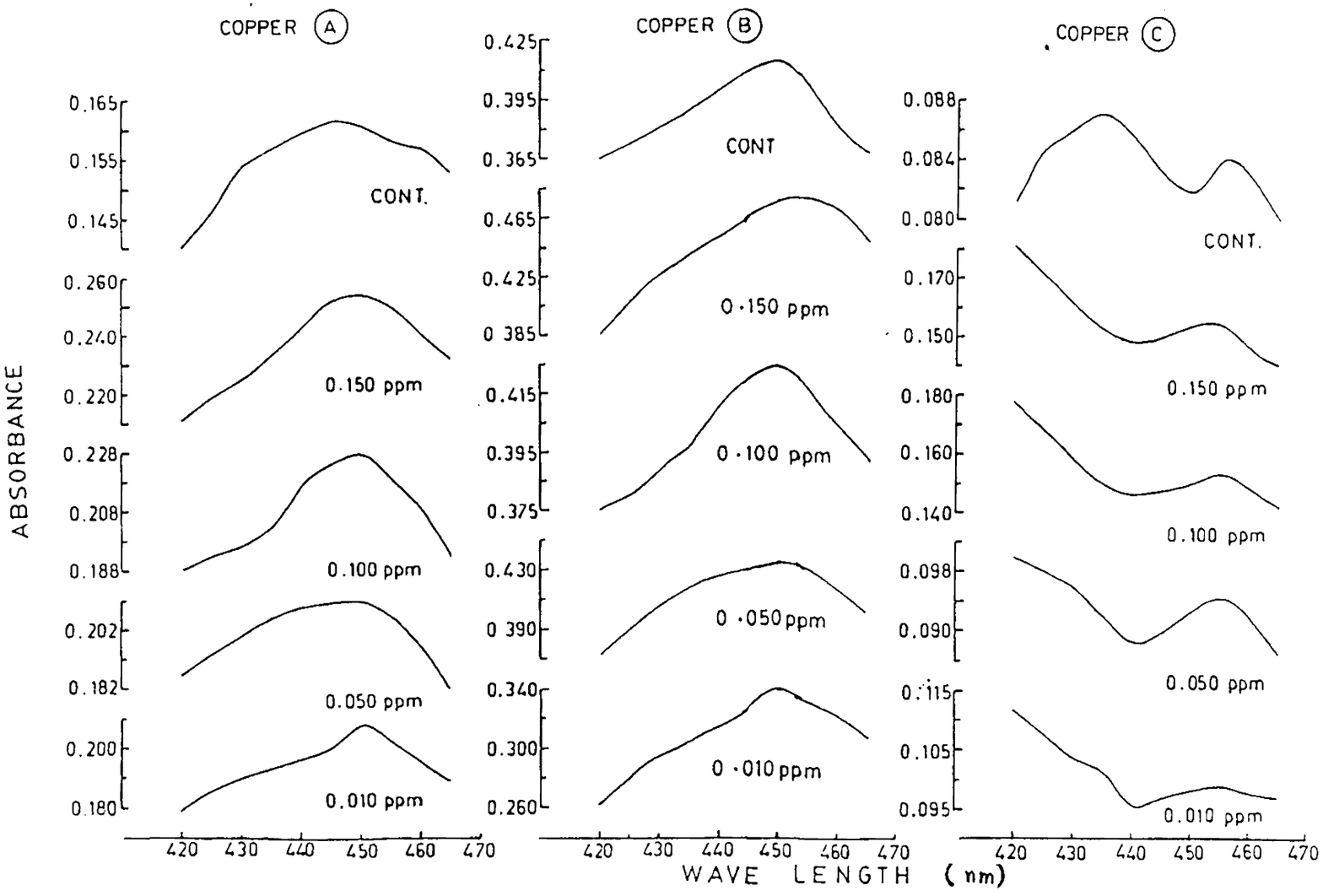


FIG. 39.

Variation in the absorption spectra of neofucoxanthin (A), fucoxanthin (B) and diadinoxanthin (C) in *I. galbana* in different concentrations of manganese.

FIG·39 ABSORPTION SPECTRA

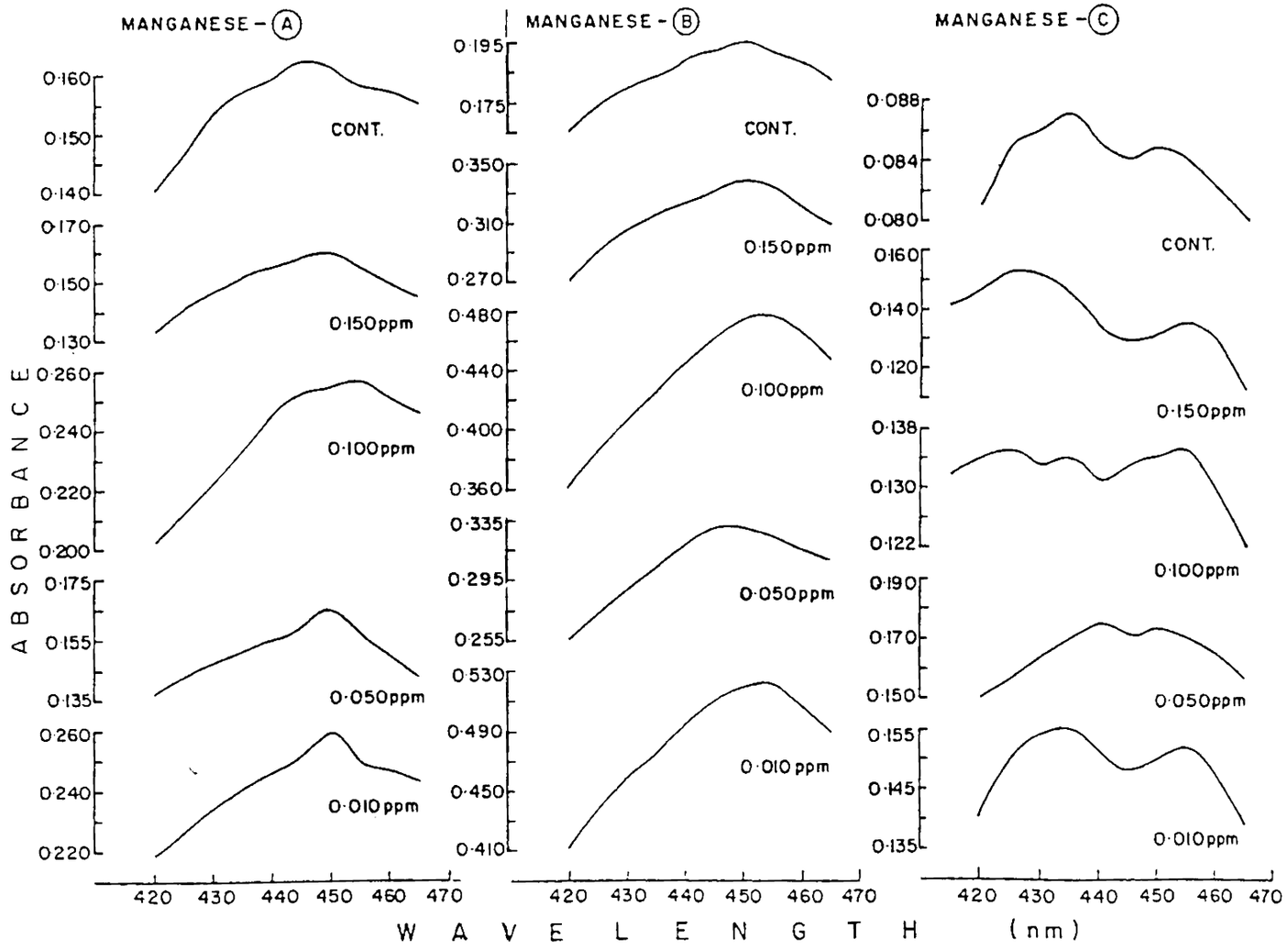
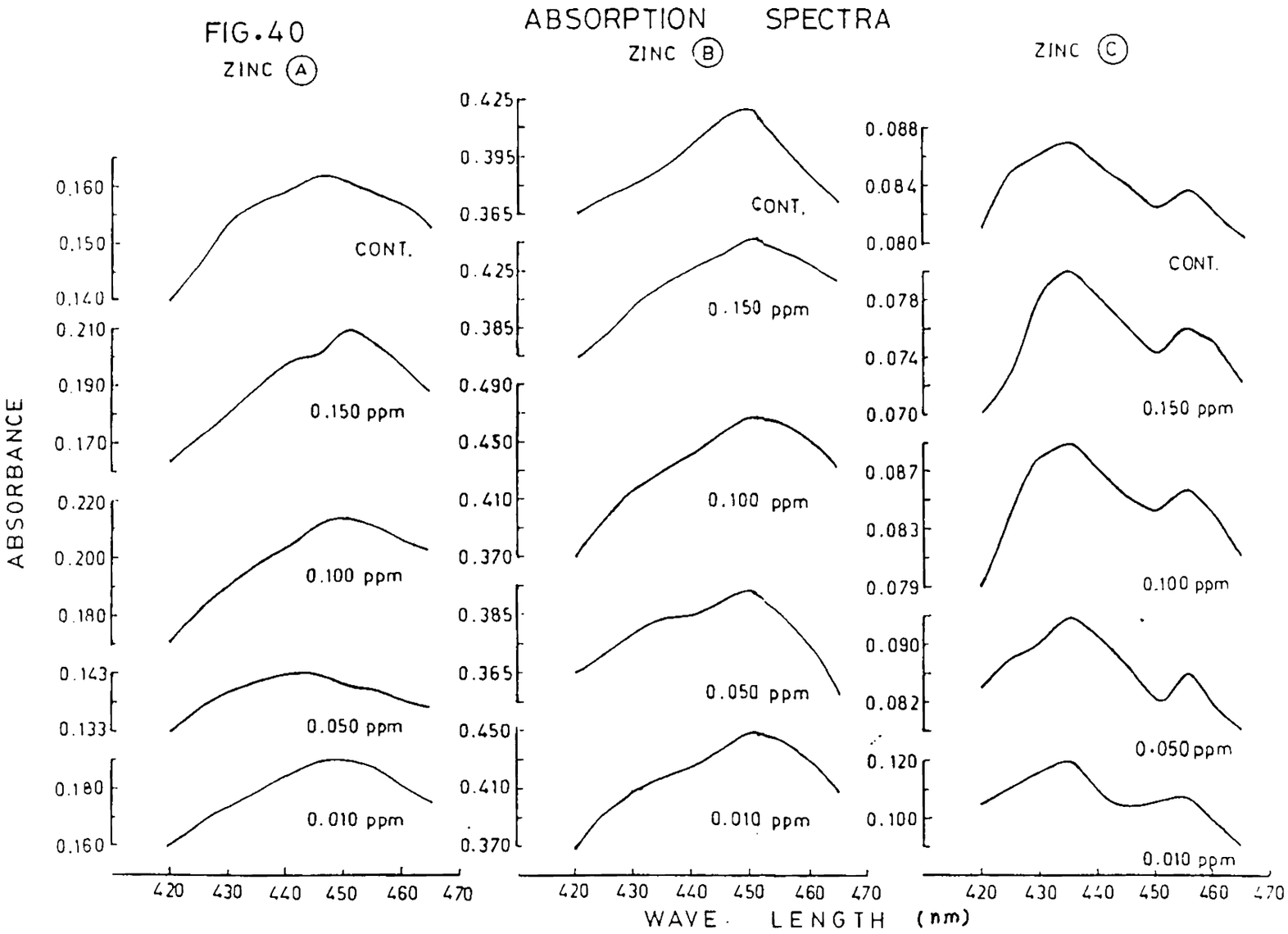


FIG. 40.

Variation in the absorption spectra of neofucoxanthin (A), fucoxanthin (B) and diadinoxanthin (C) in *I. galbana* in different concentrations of zinc.

FIG.40


ABSORPTION SPECTRA
ZINC (A)
ZINC (B)
ZINC (C)
ABSORBANCE
CONT.
0.150 ppm
0.100 ppm
0.050 ppm
0.010 ppm
0.160
0.150
0.140
0.210
0.190
0.170
0.220
0.200
0.180
0.143
0.133
0.180
0.160
0.425
0.395
0.365
0.425
0.385
0.490
0.450
0.410
0.370
0.385
0.365
0.450
0.410
0.370
0.088
0.084
0.080
0.078
0.074
0.070
0.087
0.083
0.079
0.090
0.082
0.120
0.100
420 430 440 450 460 470
WAVE LENGTH (nm)

levels first at 420 nm for copper, 425 - 440 nm for manganese, 435 nm for zinc and a second peak ranging between 450 - 455 nm for all the three metals. In control, the absorption maxima for neofucoxanthin and fucoxanthin was at 450 nm and for diadinoxanthin, the first peak at 435 nm and the second peak at 455 nm.

In *S. salina* five fractions were resolved with three unidentified green pigments which may represent the phycobilins, blue green chlorophyll a and an orange carotene. As in the case of the flagellate, there was a wide variation in the R_F values in similar concentrations of copper, manganese and zinc (Table. 11). Since the blue green alga was more sensitive to copper, even at a lower concentration the pigment separation into distinct components was not discreet.

Chromatograms of *I. galbana* and *S. salina* are represented in Plate V and VI.

4.6. Uptake of Cu, Mn and Zn by *I. galbana* and *S. salina* as determined by AAS.

Results indicate that uptake of the three metal ions increases as the level in the medium is increased. The weight of the metal per milligram dry weight of algae increased with increase of metal in the medium and generally decreased with the age of the culture (Table 12). This observation is in confirmation with the findings of Stokes (1975)

Table 11. R_F values of chlorophyll 'a' and non-chlorophyllous pigments from *S. salina* separated in two solvent systems on cellulose thin layer plates.

I - First dimension II - Second dimension

Sp no.	Manganese (ppm)								Zinc (ppm)								Copper (ppm)		Control	
	0.010		0.050		0.100		0.150		0.010		0.050		0.100		0.150		0.010			
	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II
1	0.86	0.70	0.69	0.95	0.68	0.73	0.60	0.82	0.58	0.85	0.67	0.78	0.71	0.86	0.81	0.65	0.70	0.80	0.62	0.81
2	0.75	0.80	0.87	0.90	0.83	0.73	0.83	0.87	0.82	0.85	0.91	0.84	0.80	0.85	0.86	0.80	0.83	0.88	0.85	0.85
3	0.99	0.70	0.87	0.67	0.83	0.65	0.88	0.70	0.91	0.79	0.87	0.79	0.95	0.72	0.92	0.76	0.90	0.81	0.91	0.75
4	0.91	0.89	0.88	0.80	0.95	0.90	0.82	0.91	0.91	0.94	0.93	0.94	0.90	0.97	0.96	0.86	0.92	0.93	0.92	0.96
5	0.93	0.87	0.98	0.87	0.99	0.78	0.94	0.90	0.95	0.81	0.96	0.91	0.95	0.83	0.99	0.81	0.98	0.85	0.96	0.85

Sp. no. Spot number

Sp. no. 1. Chlorophyll 'a' 3 - unknown 5. unknown

2. unknown 4 - Carotene

Plate V:

Two-dimensional chromatograms of pigments in *I. galbana* separated on cellulose thin layer plates.

Spot numbers:

1. Chlorophyll 'c'
2. Neofucoxanthin
3. Fucoxanthin
4. Diadinoxanthin
5. Chlorophyll 'a'

PLATE - V

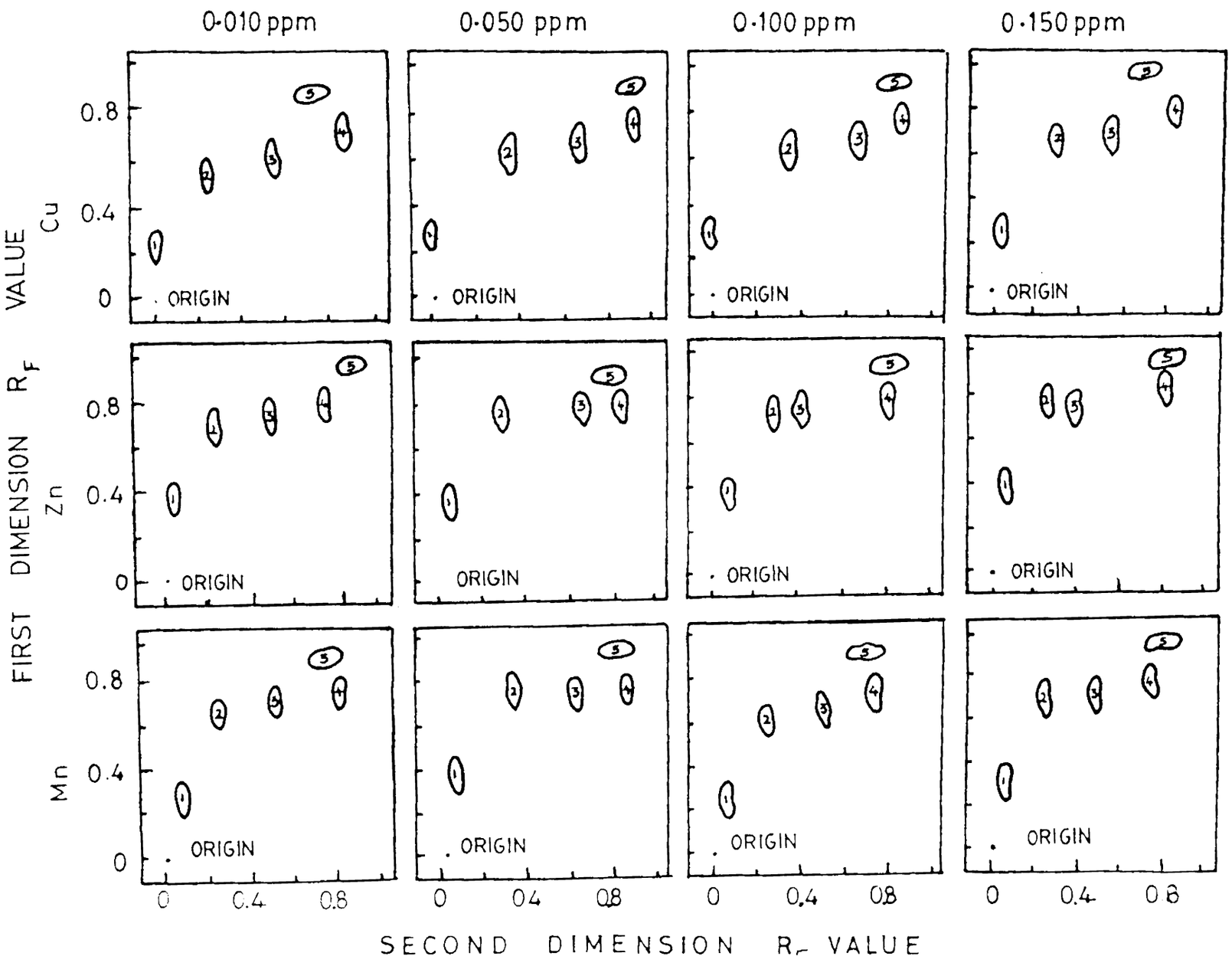

0·010 ppm
0·050 ppm
0·100 ppm
0·150 ppm
FIRST DIMENSION R_F VALUE
Cu
Zn
Mn
ORIGIN
0
0.4
0.8
SECOND DIMENSION R_F VALUE

Plate VI:

Two-dimensional chromatograms of pigments in *S. salina* separated on cellulose thin layer plates.

Spot numbers:

1. chlorophyll 'a'
2. unknown
3. unknown
4. carotene
5. unknown

PLATE-VI

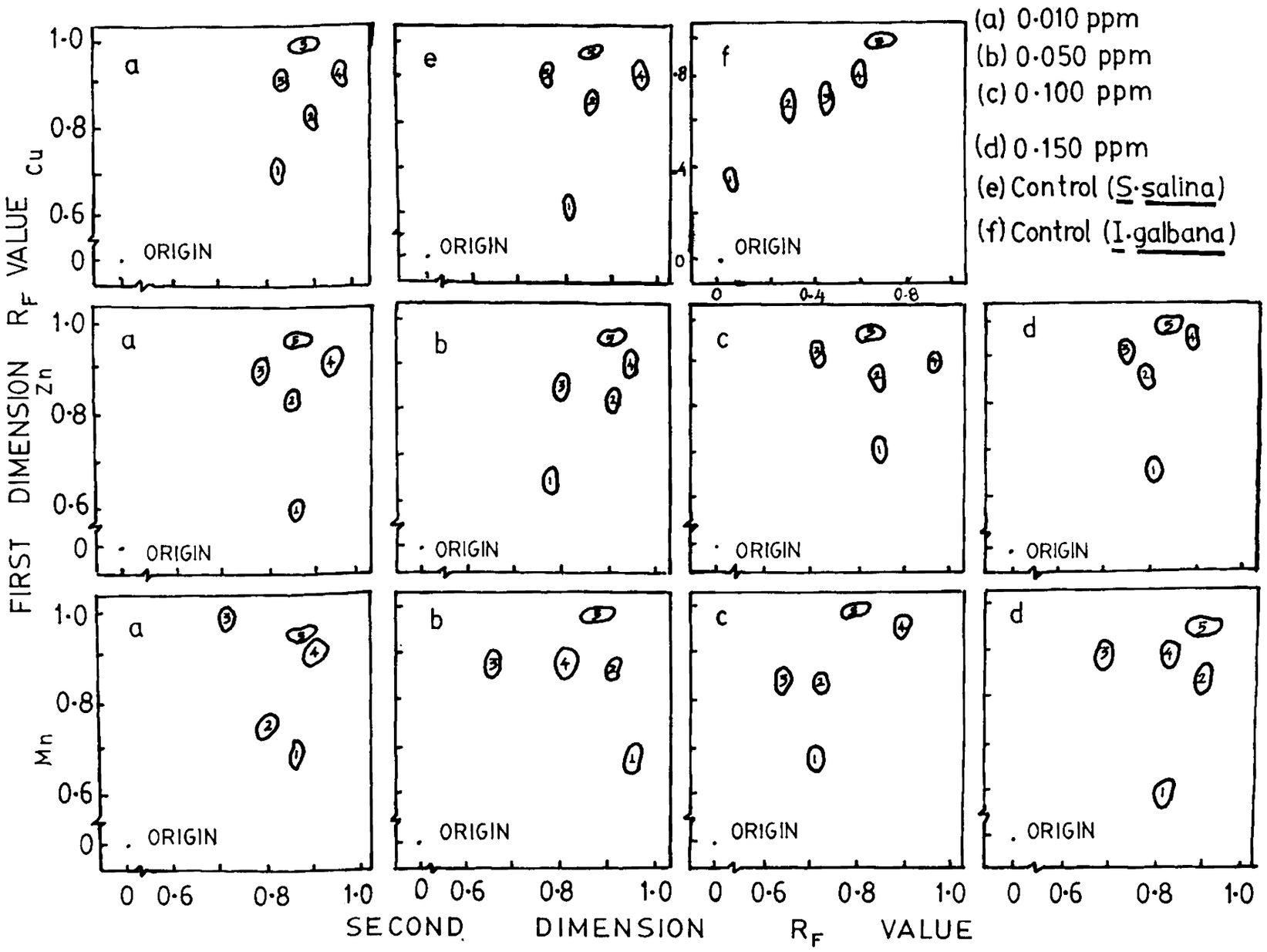

(a) 0·010 ppm
(b) 0·050 ppm
(c) 0·100 ppm
(d) 0·150 ppm
(e) Control (S. salina)
(f) Control (I. galbana)
FIRST DIMENSION R_F VALUE
SECOND DIMENSION R_F VALUE
Cu
Zn
Mn
ORIGIN
0
0·4
0·6
0·8
1·0

Table 12. Uptake of Cu, Mn and Zn by *I. galbana* (I.g) and *S. salina* (S.s) as determined by AAS.

Metal concn (ppm)	μg metal per mg dry weight algae					
	Second day		Fourth day		Eighth day	
	I.g	S.s	I.g	S.s	I.g	S.s
Cu						
0.010	04.61	05.44	04.00	05.12	04.01	04.61
0.050	10.90	10.60	08.10	09.63	05.00	05.80
0.100	13.30	14.17	09.32	10.10	05.44	06.26
0.150	19.80	20.30	12.55	13.30	07.66	08.70
Mn						
0.010	05.26	06.12	04.17	04.65	04.40	04.88
0.050	09.78	11.90	08.03	09.64	05.01	06.80
0.100	10.70	13.26	09.78	10.62	05.20	07.44
0.150	17.66	18.70	11.64	12.30	06.78	08.02
Zn						
0.010	06.90	07.10	04.55	06.21	04.75	05.32
0.050	11.55	14.40	09.03	10.10	05.90	07.40
0.100	14.90	16.30	10.60	12.80	06.80	08.12
0.150	15.00	23.80	11.92	17.10	07.32	09.26

in the uptake and accumulation of copper and nickel by B-4 *Scenedesmus*. Moreover Hall *et al.*, (1989a,b) have also shown that sublethal levels of Cu^{2+} activity resulted in an increase in cellular copper concentration in nutrient limited cultures of *Chlorella vulgaris* and *Chlamydomonas geitleri*. Larger cell number may account for the rapid absorption and elevated cellular metal concentration in the blue green alga.

Most work on metal uptake has been concerned with rather rapid uptake - usually over a number of hours. The present investigation has dealt with the uptake during prolonged growth of the cultures (eight days) and our absolute values can thus not be compared directly with those of Hassall(1963), Mandelli (1969) and Button and Hostetter (1977). Long term experiments are complicated by such factors as aging of cells, and in batch cultures accumulation of organic extracellular material takes place. The latter may account for the decreased metal uptake in older cultures of eight days.

4.7. Statistical interpretation of data:

The inferences derived from the analysis of variance by split-plot design method (Steel and Torrie, 1960) are discussed below:

Copper:

Isochrysis galbana.

In different salinities, all treatments employed i.e. 0.050 to 0.150 ppm were found to be significant in relation to control with respect to

parameters like cell concentration, chlorophyll c and production. 0.050 ppm and 0.100 ppm concentration was significant for chlorophyll a and carotenoids.

At different pH, the selected treatments were found to be significant for biomass and carotenoids but 0.050 ppm concentration was significant for chlorophyll a, c and production.

Synechocystis salina.

Copper concentrations ranging from 0.020 to 0.150 ppm were found to be significant in relation to control for all the parameters in different salinities. But between the higher copper treatments namely 0.100 ppm and 0.150 ppm there was no significance for non-chlorophyllous pigments alone.

At different pH, 0.020 to 0.070 ppm copper concentrations were significant for non-chlorophyllous pigments and carbon production whereas 0.020 ppm and 0.050 ppm was found to be significant for biomass and chlorophyll a.

Manganese:

Isochrysis galbana.

All the three treatments 0.050 ppm, 0.100 ppm and 0.150 ppm were significant for carotenoids in different salinities but 0.050 ppm concentration was significant in relation to control for biomass, chlorophyll a and c.

At different pH, the three selected concentrations were significant in relation to control for all the parameters except for chlorophyll a, wherein 0.100 ppm and 0.150 ppm was significant.

Synechocystis salina.

In different salinities, between the lower concentrations of manganese namely 0.020 ppm to 0.070 ppm there was least significance for biomass, non-chlorophyllous pigments and production but the three treatments were found to be significant in relation to control.

At different pH, each individual treatment ranging from 0.050 to 0.100 ppm was found to be significant in enhancing the growth rate and physiological activity of the blue green alga.

Zinc:

Isochrysis galbana.

Each of the selected treatment i.e. 0.010 to 0.150 ppm was found to be significant in accelerating the pigment content and metabolic activity of the flagellate but for biomass alone, 0.150 ppm did not have much impact in relation to control.

At different pH, 0.050 ppm to 0.150 ppm concentrations showed significance for cell concentration and chlorophyll a whereas 0.050 ppm and 0.100 ppm was significant for carotenoids and production.

Synechocystis salina.

In different salinities and at different pH, each of the selected treatment namely 0.020 ppm, 0.050 ppm and 0.070 ppm was found to be significant in relation to control for all the studied parameters.

CHAPTER V

5. GENERAL DISCUSSION

The present investigation on the effect of trace metals (Cu, Mn and Zn) on *I. galbana* and *S. salina* has been restricted to laboratory based bio-assays employing a single species grown axenically. The adoption of batch cultures allowed the simultaneous study of the effect of a wide range of metal concentrations upon cell populations all taken from the same stock culture and therefore initially in the same physiological condition.

The relationship between the supply of Cu, Mn and Zn and the growth of *I. galbana* and *S.salina* have been considered from different aspects. The supply of these essential elements at higher concentrations limited the growth rate of the algae. The physical or chemical form in which Cu, Mn and Zn are present may be of importance in governing their availability to the two species.

As the culture conditions to which the two species were exposed were identical, the difference in their growth rates can be attributed to species specificity or upon the size of the inoculum introduced initially. On addition of the three metals, cells of the blue green alga attained very high densities than the flagellate. Higher growth

rate in the blue green alga is in accordance with the fact that smaller species have the capacity to grow faster (Raymont, 1980).

The blue green alga was found to be more sensitive to copper and zinc than the flagellate. Variations in the metal sensitivity of the algal species were found to be due to differential metal reactivity of cell surfaces and sometimes to diverse abilities of the cells of accommodate higher cellular metal concentration on the basis of cell size and density (Fisher *et al.*, 1984).

Under the optimum concentration of all the three metals, an increasing trend in cell concentration, amount of chlorophyll a, c, carotenoids and the rate of carbon fixation were observed in the exponential phase. Cossa (1976) and Morel *et al.*, (1978) have demonstrated that the exponentially growing cells generally take up metals at a higher rate than do cells in the stationary growth phase.

An increase in the pigment content exceeding that of control may be explained as a consequence of increased carbon flow into non-nitrogen containing products, a phenomenon known to occur in light or nitrogen limited cultures. The other possible reason for the hike in pigment content may be due to the increased energy requirement of the cells to withstand the 'stress' caused by the metals. Such an enhancement of pigment concentration has been also observed by Lustigman, (1986) in

Dunaliella tertiolecta as a result of copper toxicity. An increase in the proportion of carotenoids in older cultures may be due to the depletion of major nutrients such as nitrate, phosphate (Droop, 1973) or it may be due to their role in protecting the photo-oxidation of chlorophyll (Bogorad, 1974).

Characterisation of various pigment fractions by chromatographic separation has indicated a disparity in the R_F values in the flagellate as well as in the blue green alga with similar concentrations of Cu, Mn and Zn. Non-uniformity in the absorption peak of diadinoxanthin may be due to the activity of metal ions. Such a shift in the absorption peak to shorter wave lengths has been noted in chlorophyll suggesting that the magnesium of the chlorophyll molecule may have been displaced by the toxic metal (Gross *et al.*, 1970).

Prolongation of the lag period in *I. galbana* and *S. salina* was observed only at a higher concentration of copper and zinc. It has been noticed that many selected concentrations stimulated growth at initial stages and in some the cell activity was resumed towards the end of the growth phase. This block in the initial cell division may be only a temporary phenomena, but may not have any effect on the division during the exponential phase (Morel *et al.*, 1978). The effective concentration of metals in culture may also be decreased by precipitation, absorption onto the culture vessels and chelation by extracellular products.

The documentation of the cellular changes brought about by short term exposure of *I. glabana* and *S.salina* to copper is of significance in assessing the effects at cellular level induced by metal pollutants. The structural disorganisation can be explained by the fact that the eukaryotic algae like the flagellate with all the organelles and structural complexity may be better able to separate toxic metals spatially from sensitive metabolic sites than the prokaryotic cyanophycean member which has a simple relatively undivided structure which does not allow them to confine toxic metal in parts of the cell where they will be harmless (Brand *et al.*, 1983). The above evidence may account for the ultrastructural disorganisation in *S. salina* whereas no apparent distortion was noticed in *I. galbana*.

Environmental factors such as salinity, pH and temperature modified the toxicity of metals to algae. Though the optimum salinity level for the two species differs, 25 ppt for *I. galbana* and 15 ppt for *S. salina*, both the species showed adaptability to a range of salinity tested and this is a clear indication how algae are able to regulate its metabolic activity to the requirement of the environment. The blue green alga , *S. salina* is mostly represented in fresh waters (Desikachary, 1959). This basic affinity to fresh water environment may be responsible for the better growth of the species at 15 ppt. The suggestion of Frostner and Prosi (1979) that an increase in salinity leads to competition between

absorbed heavy and dissolved cations, latter partially replacing the heavy metals thereby decreasing their bioavailability may be applicable only to *I. galbana* and not to *S. salina.*

Marine species in culture are reported to tolerate 6.8 to 9.6 pH levels with optimum at pH 8.0 (Kain and Fogg,1958). pH was found to be an important physico-chemical factor influencing the transformation of Cu, Mn and Zn and also its toxicity to *I. galbana* and *S. salina.*

The optimum temperature required for the activity of the metal ion to maximise the rate of carbon production varied from 25°C to 30°C for *S. salina* and *I. galbana.* This variation in the temperature optima may be due to the fact that the enzymatic processes controlling the cell division and photosynthesis are different (Innis and Ingraham, 1978).

Although we still lack the necessary field methods to directly measure the effects of trace metals in natural phytoplankton assemblages, it is clear that interactions between trace metal toxicity and nutritional metals could play a significant role in regulating phytoplankton growth in natural systems. Hence field studies with natural phytoplankton communities will enable realistic interpretations to problems of algal blooms and their dynamics in natural ecosystems.

SUMMARY

6. SUMMARY

The thesis presents a comprehensive study on the importance of trace elements namely copper, manganese and zinc on *I. galbana* and *S. salina* which are of nutritive value and are used as live food for rearing the shellfish larvae and for developing mass cultures in the hatchery systems.

The flagellate and the blue green alga isolated from the coastal region off Cochin, were grown in enriched seawater and maintained in the logarithmic growth phase of the life cycle.

The impact of varying conditions of environmental parameters (salinity, pH and temperature) and their role on the rate of metal uptake have been investigated. The physiological activity of the algae has been expressed by their growth kinetics in terms of cell concentration, quantities of chlorophyll and carotenoid pigments and rate of photosynthesis has been measured by the radioisotope technique.

The concentration of the three trace metals Cu, Mn and Zn in extracted seawater samples and algae has been determined by Atomic Absorption Spectrophotometry. Differential Pulse Anodic Stripping Voltammetry was employed to study the existence of copper complexes in the two species.

Experiments to assess the effect of interaction of metals in different combinations on *I. galbana* and *S. salina* have been conducted in order to understand the phenomenon of synergism and antagonism.

Transmission Electron Microscope studies have been undertaken using Carl Zeiss TEM to investigate the response of these microalgae to metals at cellular level.

Results of the trace metal studies on *I. galbana* and *S. salina* from both qualitative and quantitative aspects are reported and discussed.

Statistical analysis of data was done by analysis of variance using split-plot design method.

All the three metals exhibit toxic effects on growth when their concentrations in the medium increase, but the important point is that the organisms react in different ways and reveal variation in their tolerence. Of the two species, *I. galbana* has been found to be more tolerant to copper and zinc and *S. salina* shows a greater tolerance to manganese. In all the experiments toxicity has been indicated by a general delay in growth. The optimum concentration of Cu, Mn and Zn which enhances the growth rate of *I. galbana* and *S. salina* has been shown to differ under the influence of salinity, pH and temperature.

Manganese has been found to play a more important role than copper and zinc in accelerating the growth of the flagellate and the blue green alga. Inhibition due to copper has been found to be higher than the other two metals even at a lower concentration. Moderately higher concentration of Mn and Zn does not affect the growth of the two species.

The influence of trace metals on growth measurements of the two microalgae indicates that they exhibit peak growth and activity from four to twelve days of inoculation and thereafter the growth rate declines gradually within fourteen to twenty days when the two species attain senescence.

Quantitative evidence has been found for the complexation of Cu, Mn and Zn in the culture media by extracellular ligands produced by algae. An increase in metal complexation correlates well with a decrease in metal toxicity and a decrease in metal uptake in the culture medium. The detoxification of trace metals may represent an important physiological function of extracellular products.

The pigments, chlorophyll a, chlorophyll c and carotenoids are found to increase and they even exceed that of control in lower concentrations of copper and zinc and in higher concentrations of manganese.

The rate of carbon fixation by the flagellate and the blue green alga is affected by the action of different concentrations of Cu, Mn and Zn. The rate of ^{14}C uptake is comparatively higher in optimum concentrations of manganese than in copper or zinc for both *I. galbana* and *S. salina*.

The rate of copper, manganese and zinc uptake by the algal cells determined by AAS shows that the weight of the metal per mg dry weight of the alga increases with an increase of metal in the medium and generally decreases with the age of the culture.

Anodic stripping voltammetry has confirmed the presence of copper complexes in both the species exposed to different photoperiods with interspecies difference.

Electron microscope studies have illustrated the extent of intracellular disorganisation induced by sublethal dose of copper on *I. galbana* and *S. salina*. Drastic changes have not been observed. Discontinuity of the cell membrane and photosynthetic lamellae have been noticed at certain regions of the cell in *S. salina* whereas appreciable disruption in the cellular structure is not visible in *I. galbana*. The mechanism of cellular detoxification by an increase in the number of lipid inclusions in *I. galbana* and an increase in the size of polyhedral bodies in *S. salina* may be of importance in reducing the toxic burden of the metal to algae.

Investigations of metal interactions indicate that with changes in metal ion combination there is an alteration in the algal response. Synergistic and antagonistic behaviour of metal ions has been detected in the two species.

The modification of metal toxicity is controlled by environmental factors like salinity, pH and temperature. The optimum salinity level for the two species differs with the flagellate growing best at 25 ppt and the blue green alga shows better growth at 15 ppt. A linear relationship exists between pH and toxicity of copper in *I. galbana* but in *S. salina*, they exhibit an inverse relationship. The inverse relationship between pH and toxicity is also applicable to manganese and zinc for both the species.

The optimum temperature required for the activity of metal ions to maximise the rate of carbon production varies from 25°C - 30°C for *S. salina* and *I. galbana*.

The above findings indicate that interactions between trace metal toxicity and nutritional metals could play a significant role in regulating phytoplankton growth in natural systems.

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APPENDIX

Table 13: EFFECT OF COPPER ON THE PHYSIOLOGICAL ACTIVITY OF *I. GALBANA* IN DIFFERENT SALINITIES

Days of Growth	Cu Concn (ppm)	15 ppt					25 ppt					35 ppt				
		a	b	c	d	e	a	b	c	d	e	a	b	c	d	e
2	0.05	120	0.65	1.40	1.52	1.23	130	0.76	1.70	1.74	1.44	180	0.84	1.93	1.98	1.94
	0.10	160	0.72	1.80	1.62	1.26	250	0.91	1.90	2.10	1.86	280	0.95	1.94	2.18	1.92
	0.15	80	0.48	1.02	1.30	1.56	100	0.49	1.22	1.40	1.30	120	0.52	1.43	1.62	1.32
	CON	40	0.35	0.94	0.91	1.26	90	0.36	0.95	0.96	1.32	120	0.37	0.98	0.96	1.28
4	0.05	300	0.93	2.06	2.10	1.98	340	0.97	2.42	2.20	2.58	550	1.02	2.62	2.70	2.32
	0.10	500	1.20	2.80	2.90	2.06	480	1.18	2.60	2.80	2.66	500	1.20	2.80	2.81	2.70
	0.15	200	0.88	1.42	1.90	1.98	250	0.90	1.50	2.00	2.04	300	1.06	1.83	2.20	2.28
	CON	180	0.83	1.03	1.25	2.04	190	0.84	1.28	1.34	2.16	200	0.82	1.21	1.28	2.58
6	0.05	1300	2.60	4.49	4.30	5.16	1400	2.85	4.52	4.30	5.66	1600	2.94	4.87	4.22	5.28
	0.10	650	2.00	3.60	3.80	3.30	950	2.60	3.80	4.00	3.68	1050	2.90	4.00	4.11	3.81
	0.15	560	1.60	3.66	3.20	3.48	640	2.00	3.80	3.82	4.02	600	2.50	3.80	3.75	4.26
	CON	680	1.76	1.72	2.00	4.33	700	1.93	1.90	1.86	4.32	750	1.85	1.60	1.50	4.32
8	0.05	600	3.60	4.46	4.08	5.26	620	3.50	4.61	4.45	6.00	720	3.53	4.63	4.86	6.20
	0.10	1100	3.86	4.86	4.22	4.60	1120	3.90	4.58	4.58	5.08	1700	3.20	4.98	4.79	5.48
	0.15	550	2.55	3.78	3.28	4.18	700	2.75	4.10	4.10	5.24	610	2.60	3.69	3.92	4.60
	CON	400	2.55	2.90	3.20	4.58	420	2.30	3.79	3.79	5.10	440	2.81	2.08	3.86	5.80
10	0.05	520	3.88	5.53	5.58	5.48	600	3.66	5.08	5.18	6.43	700	3.92	5.67	5.57	6.50
	0.10	680	4.74	5.30	5.08	4.89	650	4.92	5.20	5.08	5.26	660	3.83	5.30	5.03	5.42
	0.15	510	3.63	4.61	4.70	4.46	520	3.66	4.87	4.27	5.48	580	3.88	4.80	4.77	5.76
	CON	430	3.77	4.41	5.35	4.80	460	3.52	4.65	5.13	5.43	485	3.27	4.69	5.29	6.08

Table 13 (contd...)

Days of Growth	Cu Concn (ppm)	15 ppt					25 ppt					35 ppt				
		a	b	c	d	e	a	b	c	d	e	a	b	c	d	e
	0.05	550	4.71	5.78	6.44	6.00	580	4.33	5.68	5.88	6.64	600	4.85	6.29	6.69	7.02
	0.10	650	4.60	5.80	5.24	5.46	660	4.78	5.92	5.69	5.60	665	4.94	5.98	5.92	5.72
12	0.15	500	3.92	4.94	4.86	5.12	510	4.04	4.99	4.81	5.68	540	4.17	5.05	4.96	5.92
	CON	420	4.50	5.12	5.42	5.44	440	4.69	5.14	5.66	5.56	480	4.62	5.26	5.88	6.48
	0.05	500	4.75	6.13	5.99	7.52	560	4.28	6.80	5.57	7.86	580	4.43	6.65	4.72	7.78
	0.10	660	4.82	6.36	5.86	6.78	650	4.90	6.43	5.70	6.62	650	4.98	6.82	5.90	6.86
14	0.15	450	4.61	5.87	5.30	6.62	470	4.74	5.06	5.55	6.82	520	4.71	5.12	5.63	6.92
	CON	410	4.46	6.06	5.52	6.53	430	4.84	6.26	5.83	6.80	460	4.91	6.18	5.98	7.62
	0.05	480	4.78	6.92	5.54	6.50	510	3.68	7.27	6.95	6.72	550	3.96	7.48	4.51	6.80
	0.10	620	3.91	5.52	4.86	6.20	640	4.00	6.10	4.88	6.34	640	4.13	6.16	5.00	6.43
16	0.15	460	4.45	6.10	4.82	5.50	465	4.61	6.20	4.91	6.52	500	4.60	6.11	4.88	6.61
	CON	380	4.32	5.42	4.19	5.43	420	4.12	5.58	4.46	5.72	440	4.45	5.72	4.66	6.62
	0.05	450	3.56	6.20	4.85	6.01	470	3.49	6.38	5.81	6.21	480	3.50	6.30	4.42	6.18
	0.10	600	3.65	5.50	4.42	5.60	630	3.86	5.80	4.72	5.83	630	3.96	5.93	4.84	5.88
18	0.15	410	3.44	5.82	4.42	4.50	460	4.48	5.70	4.56	5.64	480	4.56	5.81	4.46	5.68
	CON	375	3.42	5.20	3.86	4.81	410	3.40	5.36	3.90	4.91	430	3.73	5.21	3.82	5.30
	0.05	420	3.43	5.02	4.42	5.20	450	3.44	5.12	5.22	5.36	470	3.40	5.28	4.32	5.42
	0.10	580	3.58	4.64	4.26	4.52	600	3.72	4.86	4.64	4.78	620	3.80	4.98	4.78	4.96
20	0.15	380	3.02	4.43	4.01	4.00	440	3.82	4.80	4.20	4.96	460	4.10	4.90	4.13	5.02
	CON	360	2.91	4.34	3.10	4.02	380	3.00	4.63	3.15	4.13	420	3.68	4.86	3.38	4.52

Con. - Control a - Cell concentration/ml x 10^3 b - Chlorophyll 'a' $\mu g/10^6$ cells

C - Chlorophyll 'c' $\mu g/10^6$ cells d - Carotenoids $\mu g/10^6$ cells e - Production mgC/l/hr.

Table 14. EFFECT OF COPPER ON THE PHYSIOLOGICAL ACTIVITY OF I. GALBANA AT DIFFERENT pH

Days of Growth	Cu Concn (ppm)	pH 6.0					pH 7.0					pH 8.0				
		a	b	c	d	e	a	b	c	d	e	a	b	c	d	e
	0.05	750	4.15	2.95	4.91	4.58	640	2.46	2.30	3.02	4.32	450	2.12	1.88	2.96	4.10
	0.10	210	4.46	3.12	3.80	2.58	270	2.62	3.42	3.78	2.62	300	2.90	2.50	2.40	2.60
4	0.15	200	4.31	3.02	3.56	2.16	250	2.53	3.14	3.00	2.28	240	2.40	3.08	2.94	2.12
	CON	350	3.83	2.40	3.00	4.02	360	4.52	3.86	4.42	4.16	380	4.30	3.84	4.40	4.20
	0.05	650	7.29	3.68	6.92	4.91	540	3.82	2.80	4.71	4.81	380	3.11	2.23	3.85	4.62
	0.10	180	8.78	4.43	5.60	2.98	230	4.81	3.97	4.85	2.58	380	4.28	3.85	3.50	2.13
8	0.15	160	7.10	3.54	6.18	2.40	175	3.42	2.83	4.35	2.32	170	3.20	2.64	3.00	2.04
	CON	280	5.60	4.40	6.56	4.33	300	5.56	4.50	4.83	4.41	290	5.62	4.80	4.88	4.50
	0.05	300	6.32	8.85	10.67	3.20	400	5.20	6.47	6.50	3.12	320	4.72	5.77	5.88	3.02
	0.10	180	7.69	4.33	9.17	2.76	290	4.94	3.94	4.66	2.41	250	4.80	3.46	3.60	2.02
12	0.15	150	7.12	3.48	8.82	2.38	225	3.36	2.74	4.30	2.26	200	2.98	2.42	3.47	1.94
	CON	250	6.14	3.42	8.42	3.56	310	4.56	2.43	7.10	3.60	300	5.97	3.20	7.67	3.64
	0.05	600	2.80	3.13	3.67	2.58	480	4.18	3.93	3.33	2.44	360	3.96	3.42	3.00	2.38
	0.10	400	2.97	1.88	5.00	2.70	320	4.37	3.39	3.09	2.62	380	3.87	3.23	2.61	2.54
16	0.15	300	2.47	1.72	4.24	2.29	275	3.18	2.56	4.04	2.16	250	2.84	2.32	3.36	2.10
	CON	240	3.02	2.48	5.50	2.84	260	3.83	2.61	5.75	2.91	270	3.77	2.57	5.39	2.70
	0.05	500	1.01	3.39	2.30	2.30	460	1.12	3.02	2.12	2.11	310	1.09	2.43	1.98	2.10
	0.10	350	2.50	1.94	2.50	2.42	300	3.83	3.38	3.65	2.20	330	3.06	3.04	2.50	2.24
20	0.15	280	2.10	1.31	2.43	2.15	265	2.16	1.18	2.32	1.88	240	2.00	1.12	2.26	1.90
	CON	200	1.86	2.60	2.22	2.48	230	2.31	2.52	2.57	2.50	250	2.53	2.39	2.86	2.52

Con - Control a - Cell concentration/ml x 10^3 b - Chlorophyll 'a' $\mu g/10^6$ Cells.

c - Chlorophyll 'c' $\mu g/10^6$ Cells d - Carotenoids $\mu g/10^6$ Cells e - Production mgC/l/hr.

Table 15. EFFECT OF COPPER ON THE PHYSIOLOGICAL ACTIVITY OF S. SALINA AT DIFFERENT pH

Days of Growth	Cu Concn (ppm)	pH 6.0				pH 7.0				pH 8.0			
		a	b	c	d	a	b	c	d	a	b	c	d
	0.02	275	1.88	2.24	1.63	325	2.28	2.64	2.10	345	2.32	2.60	2.00
	0.05	225	1.56	1.98	1.50	280	1.76	2.06	1.86	300	1.80	2.18	1.92
4	0.07	185	1.08	1.12	1.22	245	1.40	1.54	1.54	255	1.45	1.60	1.66
	CON	200	1.24	1.55	1.48	250	1.62	1.80	1.63	275	1.76	1.92	1.75
	0.02	550	2.76	3.57	3.20	650	3.16	3.90	3.58	665	3.28	3.94	3.50
	0.05	500	2.32	3.48	2.96	625	2.84	3.76	3.22	650	2.91	3.80	3.38
8	0.07	400	1.87	2.14	2.40	550	2.66	2.95	2.77	575	2.74	3.02	2.81
	CON	450	2.40	3.10	2.72	575	3.00	3.54	2.94	600	3.11	3.42	3.02
	0.02	3000	5.30	6.56	7.12	3500	5.88	6.72	7.04	3750	5.80	6.75	7.10
	0.05	2750	4.72	6.02	5.80	3200	4.90	6.34	5.95	3500	4.96	6.52	5.90
12	0.07	2450	3.54	4.30	4.94	2800	3.74	4.98	5.36	3000	3.82	5.10	5.42
	CON	2500	4.67	6.18	5.44	3000	4.82	6.24	5.70	3500	4.90	6.16	5.84
	0.02	6000	3.16	4.42	5.30	6500	3.54	4.61	5.74	6600	3.66	4.72	5.86
	0.05	5500	2.83	3.96	4.95	6000	2.92	4.10	5.26	6500	2.90	4.24	5.40
16	0.07	5000	2.06	3.24	3.88	5200	2.28	3.50	4.51	5500	2.34	3.60	4.62
	CON	5200	2.76	4.02	4.40	5500	2.85	4.40	4.86	6000	2.88	4.38	4.88
	0.02	5550	2.13	2.91	3.36	6000	2.38	3.12	3.85	6500	2.40	3.41	3.80
	0.05	5000	1.74	2.46	3.12	5400	2.06	2.53	3.20	5500	2.15	2.62	3.34
20	0.07	4600	0.98	1.88	2.64	5000	1.14	2.30	2.55	5200	1.20	2.44	2.65
	CON	4800	1.64	2.62	3.10	5000	1.76	2.89	3.16	5500	1.81	2.75	3.10

Con - Control, a - Cell concentration/ml x 10^3 b - Chlorophyll 'a' $\mu g/10^6$ Cells

C - Non-chlorophyllous pigments $\mu g/10^6$ Cells d - Production mgC/l/hr

Table 16: EFFECT OF MANGANESE ON THE PHYSIOLOGICAL ACTIVITY OF I. GALBANA IN DIFFERENT SALINITIES

Days of Growth	Mn Concn (ppm)	15 ppt					25 ppt					35 ppt				
		a	b	c	d	e	a	b	c	d	e	a	b	c	d	e
	0.05	220	0.89	1.31	1.38	1.72	260	0.94	1.52	1.62	1.86	250	0.91	1.39	1.42	1.80
	0.10	160	0.71	1.06	1.10	1.26	185	0.75	1.23	1.40	1.32	180	0.73	0.99	1.08	1.30
2	0.15	120	0.65	0.95	0.96	1.23	150	0.69	0.97	0.96	1.30	175	0.67	0.96	0.97	1.26
	CON	120	0.66	0.94	0.96	1.20	160	0.68	0.95	0.98	1.28	150	0.68	0.94	0.98	1.24
	0.05	280	0.96	2.01	2.14	1.98	320	1.12	2.34	2.47	2.28	310	1.08	2.16	2.32	2.06
	0.10	240	0.91	1.80	1.95	1.82	265	0.98	1.96	2.12	1.90	240	0.97	1.72	2.01	1.84
4	0.15	200	0.87	1.76	1.90	1.80	230	0.95	1.84	1.99	1.82	225	0.95	1.64	1.79	1.80
	CON	250	0.88	1.91	1.98	1.86	290	0.97	1.92	2.10	2.04	280	0.96	1.81	2.04	2.00
	0.05	500	1.80	4.08	4.20	3.30	540	2.42	4.30	4.49	3.68	520	2.20	4.26	4.40	3.26
	0.10	320	1.40	3.56	3.71	2.91	345	1.98	3.69	3.82	3.12	340	1.87	3.52	3.71	2.88
6	0.15	330	1.45	3.66	3.60	3.02	360	2.06	3.75	3.88	3.20	355	1.94	3.61	3.75	3.14
	CON	370	1.60	3.70	3.82	3.12	410	2.18	3.80	3.90	3.26	400	2.01	3.68	3.81	3.24
	0.05	610	3.50	5.06	5.12	4.26	650	4.22	5.24	5.48	4.64	630	4.02	5.06	5.18	4.42
	0.10	440	2.72	4.46	4.77	3.84	465	3.16	4.64	4.87	4.02	460	3.10	4.53	4.72	3.96
8	0.15	420	2.58	4.71	4.58	3.62	450	2.85	4.52	4.71	3.86	440	2.70	4.41	4.64	3.80
	CON	450	2.85	4.71	4.80	3.90	490	2.98	4.88	4.86	4.12	480	2.84	4.62	4.75	4.06
	0.05	770	4.66	6.11	6.20	5.12	850	4.92	6.24	6.40	5.76	830	4.80	6.10	6.02	5.62
	0.10	590	3.88	5.55	5.68	4.32	610	4.30	5.62	5.81	4.40	580	4.22	5.42	5.70	4.38
10	0.15	580	3.63	5.48	5.63	4.22	600	4.28	5.52	5.74	4.30	560	4.16	5.30	5.61	4.20
	CON	550	3.57	5.51	5.60	4.18	590	4.10	5.63	5.90	4.32	580	4.20	5.50	5.83	4.28

Table 16 (contd...)

Days of Growth	Mn Concn (ppm)	15 ppt					25 ppt					35 ppt				
		a	b	c	d	e	a	b	c	d	e	a	b	c	d	e
	0.05	840	4.98	6.62	6.83	6.20	1010	5.12	6.81	7.08	7.02	950	4.93	6.70	6.92	6.72
	0.10	670	4.42	5.90	6.01	5.82	700	4.70	6.12	6.43	6.12	675	4.52	5.94	6.02	5.94
12	0.15	640	4.13	5.36	5.88	5.74	680	4.40	5.71	5.94	6.02	685	4.28	5.61	5.82	5.86
	CON	680	4.56	5.99	6.22	6.14	710	4.68	6.20	6.59	6.28	750	4.61	6.06	6.28	6.32
	0.05	800	4.76	6.73	6.91	6.00	895	5.01	6.92	7.24	6.80	850	4.82	6.80	6.94	6.50
	0.10	610	4.30	6.10	6.22	5.78	640	4.61	6.20	6.50	5.92	620	4.40	5.98	6.16	5.82
14	0.15	585	4.06	5.40	5.80	5.68	600	4.32	5.88	5.90	5.81	560	4.21	5.72	5.90	5.70
	CON	600	4.42	6.16	6.20	5.70	620	4.50	6.38	6.71	5.90	610	4.46	6.15	6.39	5.88
	0.05	680	4.10	6.52	6.83	5.52	700	4.82	6.62	6.97	5.76	685	4.41	6.43	6.79	5.60
	0.10	520	3.81	5.94	6.02	5.28	550	4.15	6.08	6.20	5.42	540	4.02	5.72	5.91	5.36
16	0.15	540	3.62	5.38	5.76	5.34	570	3.91	5.65	5.88	5.54	560	3.76	5.40	5.62	5.50
	CON	510	3.90	5.90	6.02	5.18	545	4.18	6.13	6.18	5.22	520	4.10	5.95	6.04	5.20
	0.05	640	3.96	6.30	6.54	5.48	660	4.16	6.42	6.73	5.88	640	4.12	6.28	6.50	5.42
	0.10	480	3.62	5.71	5.83	5.20	510	4.81	5.85	5.98	5.32	500	3.74	5.52	5.77	5.30
18	0.15	500	3.43	5.22	5.70	5.32	520	3.72	5.41	5.80	5.46	510	3.51	5.24	5.54	5.46
	CON	475	3.68	5.75	5.88	5.10	500	3.80	5.92	5.84	5.20	480	3.86	5.61	5.87	5.12
	0.05	550	3.50	5.67	6.08	5.24	605	3.92	5.81	6.22	5.48	580	3.70	5.78	6.01	5.34
	0.10	460	3.36	5.28	5.27	5.12	470	3.51	5.43	5.48	5.26	465	3.42	5.30	5.21	5.20
20	0.15	450	3.20	4.85	5.14	5.16	460	3.26	4.98	5.06	5.20	470	3.28	4.80	5.10	5.28
	CON	430	3.21	5.22	5.18	4.96	465	3.42	5.39	5.23	5.02	440	3.34	5.10	5.20	4.89

Con. - Control a - Cell concentration/ml x 10^3 b - Chlorophyll 'a' $\mu g/10^6$ cells.

c - Chlorophyll 'c' $\mu g/10^6$ cells d - Carotenoids $\mu g/10^6$ cells e - Production mgC/l/hr.

Table 17. EFFECT OF MANGANESE ON THE PHYSIOLOGICAL ACTIVITY OF I. GALBANA AT DIFFERENT pH

Days of Growth	Mn Concn (ppm)	pH 6.0					pH 7.0					pH 8.0				
		a	b	c	d	e	a	b	c	d	e	a	b	c	d	e
	0.05	300	2.42	2.85	3.14	3.02	340	2.94	3.12	3.62	3.24	350	2.96	3.10	3.66	3.52
	0.10	350	2.88	3.40	3.95	3.30	400	3.26	3.94	4.21	3.62	425	3.20	4.02	4.33	3.76
4	0.15	325	2.60	3.28	3.90	2.91	385	3.08	3.62	4.18	3.16	390	3.14	3.70	4.12	3.23
	CON	250	2.32	2.91	2.98	1.80	280	2.90	3.44	3.80	2.80	300	2.92	3.50	3.78	2.91
	0.05	600	4.80	5.12	6.28	6.06	650	5.88	6.20	7.30	6.50	675	5.80	6.32	7.28	6.64
	0.10	750	5.16	6.80	7.60	6.94	780	6.52	7.90	8.51	7.32	800	6.61	8.24	8.50	7.48
8	0.15	700	4.98	6.40	6.72	5.88	750	6.10	7.24	8.20	6.40	765	6.15	7.30	8.22	6.51
	CON	510	4.60	5.80	6.12	3.64	550	5.76	6.60	7.60	5.54	580	5.75	6.68	7.72	5.70
	0.05	810	4.92	5.40	6.70	6.88	825	5.90	6.28	7.45	7.21	820	5.91	6.36	7.40	7.42
	0.10	885	5.56	6.92	8.24	8.02	1020	6.72	8.04	8.70	8.95	1050	6.74	8.21	8.92	9.10
12	0.15	840	5.18	6.56	6.88	6.40	1000	6.24	7.42	8.32	7.04	1010	6.20	7.50	8.40	7.16
	CON	650	4.73	5.98	6.01	5.77	700	5.80	6.73	7.65	6.12	715	5.88	6.70	7.60	6.40
	0.05	775	3.81	4.70	5.71	6.62	785	4.45	5.36	6.04	6.80	780	4.52	5.40	6.12	6.96
	0.10	810	4.76	5.80	6.83	7.83	1000	5.60	6.52	6.80	8.14	1025	5.65	6.58	6.83	8.24
16	0.15	800	4.13	5.62	5.20	6.20	950	5.48	6.10	6.62	6.72	975	5.50	6.12	6.58	6.88
	CON	575	4.10	5.28	5.16	5.41	650	4.57	5.86	6.14	5.60	660	4.64	5.80	6.10	5.76
	0.05	650	3.62	4.02	5.24	6.01	665	3.72	4.64	5.30	6.48	675	3.70	4.62	5.34	6.61
	0.10	700	4.10	4.83	5.98	6.84	725	4.36	5.20	6.18	7.70	700	4.42	5.24	6.10	7.83
20	0.15	675	3.68	4.42	4.72	5.71	680	3.94	4.80	5.90	6.51	685	3.90	4.85	5.92	6.75
	CON	500	3.50	4.32	4.80	5.08	610	3.82	4.51	5.72	5.12	620	3.88	4.60	5.70	5.20

Con. - Control a - Cell concentration/ml x 10^3 b - Chlorophyll 'a' $\mu g/10^6$ cells.

c - Chlorophyll 'c' $\mu g/10^6$ cells d - Carotenoids $\mu g/10^6$ cells e - Production mgC/l/hr.

Table 18. EFFECT OF MANGANESE ON THE PHYSIOLOGICAL ACTIVITY OF S.SALINA AT DIFFERENT pH

Days of Growth	Mn Concn (ppm)	pH 6.0				pH 7.0				pH 8.0			
		a	b	c	d	a	b	c	d	a	b	c	d
4	0.05	225	1.30	1.86	1.48	250	1.52	2.21	1.54	300	1.60	2.36	1.61
	0.10	300	1.58	2.26	1.64	330	1.83	2.60	1.70	400	1.94	2.65	1.78
	0.15	350	2.16	2.64	1.78	375	2.65	2.94	1.82	450	2.70	3.04	1.95
	CON	250	1.44	1.84	1.52	275	1.56	2.10	1.65	300	1.66	2.14	1.70
8	0.05	545	2.84	3.58	3.26	600	3.08	4.12	3.20	650	3.10	4.20	3.28
	0.10	600	3.40	3.91	3.74	675	3.74	4.94	3.81	725	3.82	5.16	3.85
	0.15	725	3.65	4.16	4.08	800	3.95	5.30	4.10	850	4.06	5.54	4.20
	CON	550	2.90	3.62	3.50	585	3.16	4.34	3.60	810	3.50	4.40	3.71
12	0.05	2800	4.23	5.36	5.44	3000	4.36	5.42	5.48	3200	4.42	5.50	5.50
	0.10	3000	4.41	5.94	5.98	4000	4.90	6.04	6.01	4500	5.06	6.14	6.12
	0.15	3700	5.16	6.14	6.26	4500	5.61	6.28	6.30	4600	5.74	6.32	6.36
	CON	3000	4.52	5.77	5.60	3250	4.70	5.83	5.54	3500	4.82	5.90	5.60
16	0.05	5000	3.14	4.20	4.32	5400	3.20	4.18	4.41	5500	3.36	4.20	4.45
	0.10	6000	3.50	4.81	4.80	6200	3.62	4.88	4.88	6500	3.70	4.80	4.94
	0.15	6500	4.27	5.08	5.14	6700	4.25	5.10	5.16	6800	4.20	5.30	5.28
	CON	5500	3.46	4.60	4.65	5800	3.50	4.66	4.70	6000	3.45	4.68	4.77
20	0.05	3800	1.96	2.14	3.10	4000	2.44	3.12	3.12	4200	2.50	3.16	3.16
	0.10	5000	2.30	3.65	3.94	5400	2.71	3.24	3.90	5600	2.74	3.28	3.92
	0.15	5500	2.52	3.94	4.20	5700	2.94	4.30	4.26	5700	3.01	4.26	4.30
	CON	4000	2.01	2.82	3.20	4400	2.53	3.40	3.30	4500	2.62	3.44	3.36

Con - Control, a - Cell concentration/ml x 10^3 b - Chlorophyll 'a' $\mu g/10^6$ Cells

C - Non-chlorophyllous pigments $\mu g/10^6$ Cells d - Production mgC/l/hr

Table 19. EFFECT OF ZINC ON THE PHYSIOLOGICAL ACTIVITY OF I. GALBANA IN DIFFERENT SALINITIES

Days of Growth	Zn Concn (ppm)	15 ppt					25 ppt					35 ppt				
		a	b	c	d	e	a	b	c	d	e	a	b	c	d	e
	0.01	200	0.87	1.28	1.40	1.68	290	0.95	1.76	1.84	1.90	265	0.94	1.52	1.74	1.82
	0.05	150	0.70	1.02	1.20	1.36	250	0.89	1.40	1.54	1.70	260	0.88	1.32	1.50	1.66
2	0.10	120	0.69	0.98	1.04	1.28	180	0.75	1.28	1.42	1.66	160	0.74	1.10	1.38	1.50
	0.15	250	0.91	1.42	1.61	1.72	170	0.71	1.04	1.39	1.54	140	0.72	0.99	1.40	1.38
	CON	200	0.88	1.32	1.41	1.62	240	0.91	1.52	1.72	1.81	230	0.90	1.30	1.61	1.68
	0.01	280	0.97	1.96	2.20	1.92	400	1.46	2.42	2.54	2.40	390	1.32	2.21	2.50	2.38
	0.05	190	0.81	1.32	1.52	1.50	320	0.99	2.04	2.24	2.12	310	0.98	1.98	2.22	2.08
4	0.10	180	0.74	1.04	1.26	1.34	200	0.86	1.68	1.76	1.94	205	0.84	1.55	1.62	1.82
	0.15	370	1.22	2.18	2.42	2.14	210	0.78	1.56	1.68	1.83	200	0.75	1.60	1.44	1.76
	CON	260	0.96	1.88	1.99	1.81	290	0.98	1.98	2.14	1.96	280	0.97	1.91	2.08	2.00
	0.01	360	1.52	2.42	2.76	2.88	550	2.32	3.40	3.49	3.61	520	2.36	3.26	3.24	3.54
	0.05	250	1.31	2.10	2.30	2.56	440	1.84	2.66	2.80	3.02	430	1.51	2.75	2.69	2.99
6	0.10	280	1.20	2.01	2.24	2.34	310	1.45	2.20	2.28	2.84	300	1.44	2.20	2.10	2.80
	0.15	490	2.66	3.12	3.32	3.40	340	1.34	2.16	2.30	2.72	310	1.10	2.21	2.18	2.68
	CON	370	1.60	2.50	2.48	2.50	430	1.81	2.71	2.58	2.79	410	1.64	2.50	2.40	2.70
	0.01	660	3.42	4.80	5.02	5.60	750	4.38	5.90	6.24	6.04	740	4.21	5.82	5.84	6.00
	0.05	480	2.60	4.41	4.64	4.48	700	3.61	5.01	5.31	5.92	690	3.50	4.98	5.29	5.20
8	0.10	450	2.45	4.02	4.50	4.29	520	2.84	4.74	4.89	4.46	500	2.78	4.70	4.71	4.40
	0.15	680	4.12	5.72	5.98	5.80	490	2.62	4.31	4.76	4.42	495	2.54	4.24	4.30	4.38
	CON	450	3.20	4.91	4.71	4.31	500	3.40	5.16	4.98	4.60	490	3.26	5.10	5.02	4.66
	0.01	990	4.91	6.32	6.65	6.80	1060	5.30	6.61	6.76	6.92	1020	5.22	6.24	6.52	6.70
	0.05	770	4.36	5.41	5.62	6.01	840	4.62	5.82	6.00	6.32	810	4.54	5.76	5.88	6.20
10	0.10	650	3.68	4.82	5.04	5.78	720	3.94	4.98	5.52	5.90	705	3.81	4.84	5.40	5.72
	0.15	600	3.42	4.40	4.81	5.42	680	3.82	4.62	5.20	5.84	660	3.73	4.52	5.08	5.54
	CON	660	4.10	5.50	5.76	6.20	630	4.50	5.80	5.84	6.40	620	4.40	5.61	5.68	6.18

Table 19 (contd..)

Days of Growth	Zn Concn (ppm)	15 ppt					25 ppt					35 ppt				
		a	b	c	d	e	a	b	c	d	e	a	b	c	d	e
12	0.01	1100	5.42	6.96	7.26	7.54	1300	5.60	7.28	7.42	7.72	1260	5.54	7.20	7.26	7.20
	0.05	850	4.60	5.80	6.12	6.40	880	4.72	5.96	6.30	6.62	860	4.68	5.84	6.24	6.50
	0.10	700	3.91	4.98	5.44	6.02	740	4.06	5.14	5.62	6.24	720	4.00	5.08	5.58	6.16
	0.15	680	3.72	4.61	5.10	5.96	710	3.84	4.82	5.48	6.10	690	3.72	4.72	5.32	5.91
	CON	690	4.08	5.76	5.92	6.64	700	4.22	5.90	6.01	6.60	680	4.16	5.86	5.96	6.32
14	0.01	1050	5.16	6.74	6.94	7.12	1240	5.40	6.90	7.16	7.50	1200	5.32	6.74	7.02	7.34
	0.05	810	4.48	5.64	6.02	6.18	840	4.56	5.78	6.18	6.40	820	4.50	5.60	6.10	6.22
	0.10	670	3.72	4.76	5.10	5.81	690	3.84	4.82	5.34	6.00	680	3.78	4.71	5.28	5.92
	0.15	660	3.61	4.54	4.82	5.76	680	3.72	4.74	5.06	5.92	650	3.65	4.50	4.96	5.80
	CON	630	3.82	5.56	5.82	6.28	660	4.10	5.76	5.98	6.42	640	4.02	5.62	5.90	6.30
16	0.01	860	4.98	6.50	6.78	6.82	880	5.20	6.62	6.92	7.20	870	5.10	6.51	6.90	7.00
	0.05	740	4.30	5.28	5.84	6.00	760	4.42	5.60	5.90	6.20	750	4.38	5.52	5.88	6.08
	0.10	620	3.61	4.65	5.00	5.64	640	3.76	4.74	5.10	5.93	630	3.70	4.70	5.02	5.72
	0.15	610	3.54	4.42	4.72	5.48	630	3.68	4.56	4.88	5.80	620	3.62	4.50	4.80	5.68
	CON	610	3.76	5.41	5.62	5.90	640	3.98	5.51	5.70	6.28	625	3.80	5.42	5.68	6.10
18	0.01	780	4.64	6.41	6.22	6.24	800	4.82	6.50	6.80	6.90	800	4.70	6.40	6.70	6.60
	0.05	680	4.08	5.04	5.66	5.92	700	4.24	5.46	5.81	6.16	690	4.16	5.40	5.72	6.02
	0.10	590	3.52	4.46	4.89	5.60	620	3.68	4.68	5.00	5.80	610	3.60	4.62	4.96	5.71
	0.15	580	3.40	4.32	4.54	5.32	600	3.52	4.42	4.70	5.71	590	3.48	4.32	4.68	5.56
	CON	580	3.58	5.32	5.41	5.61	610	3.76	5.48	5.68	5.72	600	3.62	5.36	5.60	5.78
20	0.01	700	4.20	6.01	5.96	6.02	740	4.40	5.88	6.20	6.60	720	4.36	5.96	6.08	5.90
	0.05	600	3.88	4.86	5.20	5.81	640	3.96	4.92	5.32	6.00	620	4.01	4.85	5.21	5.80
	0.10	540	3.42	4.12	4.62	5.54	560	3.56	4.20	4.78	5.74	540	3.40	4.16	4.60	5.46
	0.15	520	3.36	4.08	4.40	5.10	580	3.40	3.96	4.52	5.38	560	3.32	4.00	4.48	4.98
	CON	510	3.30	5.16	5.08	5.24	550	3.52	4.90	5.40	5.60	520	3.50	5.02	5.26	5.22

Con. - Control a - Cell concentration/ml x 10^3 b - Chlorophyll 'a' $\mu g/10^6$ cells.

c - Chlorophyll 'c' $\mu g/10^6$ cells d - Carotenoids $\mu g/10^6$ cells e - Production mgC/l/hr.

Table 20. EFFECT OF ZINC ON THE PHYSIOLOGICAL ACTIVITY OF I. GALBANA AT DIFFERENT pH

Days of Growth	Zn Concn (ppm)	pH 6.0					pH 7.0					pH 8.0				
		a	b	c	d	e	a	b	c	d	e	a	b	c	d	e
	0.05	310	1.45	2.23	2.46	3.14	400	1.56	2.40	2.62	3.48	450	1.61	2.58	2.52	3.56
	0.10	300	1.32	2.02	2.31	2.90	350	1.40	2.18	2.41	3.02	375	1.42	2.30	2.12	3.10
4	0.15	285	1.30	1.99	2.24	2.88	310	1.38	2.16	2.32	2.90	2.90	350	1.40	2.26	2.18
	CON	260	1.02	1.61	1.80	1.92	300	1.26	1.74	1.88	2.48	320	1.32	1.88	2.66	2.71
	0.05	620	3.85	5.81	5.60	6.26	810	3.34	5.82	5.81	6.65	1005	3.40	5.90	5.92	6.60
	0.10	600	3.72	5.40	5.30	5.81	720	3.08	5.36	5.62	5.92	800	3.22	5.42	5.75	5.98
8	0.15	575	2.60	4.76	4.84	5.60	700	2.80	4.10	4.93	5.70	710	2.84	4.23	5.02	5.77
	CON	540	3.40	5.16	4.98	4.60	610	3.52	4.48	4.70	4.78	650	3.61	4.51	4.86	5.10
	0.05	710	4.94	6.81	6.98	7.12	895	5.02	6.95	7.40	7.48	1020	5.20	6.88	7.53	7.50
	0.10	685	4.82	5.82	5.56	6.58	760	4.91	5.99	6.76	6.83	850	4.93	5.90	6.91	6.80
12	0.15	620	3.42	5.08	4.90	5.84	740	3.66	5.41	5.93	5.92	760	3.60	5.45	6.03	5.98
	CON	650	3.72	5.43	5.22	4.81	820	3.82	5.82	5.16	4.96	840	3.81	5.83	5.12	5.23
	0.05	665	3.42	4.80	5.02	5.60	800	4.60	5.10	6.26	6.40	895	4.74	5.22	6.35	6.45
	0.10	650	3.16	4.12	4.62	5.54	705	4.18	4.83	5.63	5.74	775	4.26	4.96	5.60	5.78
16	0.15	600	2.66	3.10	3.32	3.40	700	2.76	4.56	4.92	4.16	720	2.84	4.65	4.94	4.24
	CON	600	2.60	4.41	4.64	4.48	750	3.12	4.72	4.98	4.78	775	3.26	4.70	5.02	4.82
	0.05	620	3.38	4.72	5.0	5.58	775	3.92	4.80	5.34	5.62	810	3.90	4.83	5.30	5.66
	0.10	600	3.10	4.16	4.61	5.41	700	3.63	4.22	4.46	5.50	715	3.66	4.25	4.42	5.45
20	0.15	565	2.58	3.08	3.34	3.20	650	2.62	3.36	3.57	3.78	675	2.60	3.30	3.51	3.83
	CON	550	2.54	4.30	4.64	4.24	610	2.66	4.48	4.78	4.30	645	2.72	4.58	4.70	4.32

Con. - Control a - Cell concentration/ml x 10^3 b - Chlorophyll 'a' $\mu g/10^6$ cells.

c - Chlorophyll 'c' $\mu g/10^6$ cells d - Carotenoids $\mu g/10^6$ cells e - Production mgC/l/hr.

Table 21. EFFECT OF ZINC ON THE PHYSIOLOGICAL ACTIVITY OF S.SALINA IN DIFFERENT SALINITIES

Days of Growth	Zn Concn (ppm)	15 ppt				25 ppt				35 ppt			
		a	b	c	d	a	b	c	d	a	b	c	d
	0.02	55	0.38	0.41	0.52	120	0.41	0.46	0.65	100	0.41	0.43	0.61
	0.05	95	0.62	0.59	0.68	150	0.68	0.61	0.82	140	0.61	0.61	0.75
2	0.07	80	0.51	0.50	0.62	100	0.58	0.56	0.80	110	0.56	0.52	0.72
	CON	50	0.41	0.42	0.58	90	0.45	0.46	0.78	85	0.41	0.44	0.74
	0.02	250	1.20	1.61	1.24	310	1.60	2.00	1.48	300	1.55	1.91	1.32
	0.05	285	1.86	1.52	1.86	350	1.64	2.24	2.62	345	1.70	2.06	1.96
4	0.07	200	1.08	1.12	1.13	280	1.32	1.88	1.36	270	1.22	1.76	1.20
	CON	250	1.16	1.27	1.32	300	1.26	1.48	1.51	275	1.20	1.35	1.50
	0.02	500	2.40	2.32	2.50	810	3.32	4.02	2.80	785	3.04	3.86	2.60
	0.05	560	3.20	3.00	2.72	1000	4.30	5.51	4.42	950	4.16	4.62	4.10
6	0.07	440	2.16	2.00	2.26	750	2.62	3.70	2.66	700	2.42	3.49	2.40
	CON	500	2.24	2.06	2.60	800	2.60	3.56	3.21	750	2.36	3.24	3.02
	0.02	2000	4.80	4.66	5.10	3050	5.51	7.76	5.60	2750	5.32	7.61	5.20
	0.05	2300	6.42	6.20	5.61	4000	6.60	8.10	6.81	4050	6.55	8.02	6.14
8	0.07	1750	4.36	4.18	4.52	2850	5.28	6.40	5.24	2800	5.20	6.36	4.98
	CON	2010	4.40	4.53	5.20	2800	5.31	6.88	5.40	2700	5.18	6.72	5.16
	0.02	3000	3.81	3.62	4.96	3200	5.40	6.38	5.31	3050	5.10	6.01	4.80
	0.05	3200	5.38	5.10	5.32	3500	5.78	6.30	6.02	3400	5.40	7.52	5.94
10	0.07	2800	3.20	3.10	4.20	3000	3.84	5.40	4.82	2950	4.51	5.18	4.62
	CON	3050	3.46	3.60	5.02	3200	4.20	5.56	5.10	2800	4.62	5.43	5.76

Table 21 (contd...)

Days of Growth	Zn Concn (ppm)	15 ppt				25 ppt				35 ppt			
		a	b	c	d	a	b	c	d	a	b	c	d
12	0.02	4000	2.64	2.70	4.10	4300	4.31	5.22	5.01	4200	4.18	5.20	4.52
	0.05	4500	4.13	4.28	5.12	4800	4.66	5.46	5.84	4650	4.32	5.38	5.62
	0.07	3500	2.10	2.26	4.00	3800	2.90	4.34	4.32	3700	2.64	4.12	4.30
	CON	3600	2.30	2.52	4.96	4000	3.15	4.41	4.64	3800	3.08	4.40	4.70
14	0.02	4500	2.18	2.30	3.82	5000	3.96	4.99	4.76	4800	3.74	4.50	4.10
	0.05	5000	3.02	3.21	4.63	5500	4.18	5.02	5.12	5200	4.06	4.88	5.06
	0.07	4000	2.00	2.14	3.75	4500	2.62	3.84	3.98	4300	2.50	3.63	3.86
	CON	4200	2.11	2.36	4.32	4600	2.81	3.92	4.40	4400	2.76	3.75	3.90
16	0.02	4800	1.80	1.96	3.91	5500	2.43	3.61	4.81	5400	2.69	3.28	4.08
	0.05	5500	2.12	2.48	4.72	6200	3.02	4.52	5.16	6100	3.14	3.62	5.16
	0.07	4200	1.65	1.81	3.84	4800	1.44	2.65	4.02	4600	1.31	2.54	3.92
	CON	4400	1.76	1.98	4.40	4600	1.83	2.62	4.46	4500	1.58	2.60	4.08
18	0.02	4500	0.98	1.16	2.14	4800	1.32	2.42	2.60	5000	1.28	2.14	2.85
	0.05	5000	1.28	1.30	3.32	6000	2.08	3.16	3.52	5400	2.10	2.98	3.16
	0.07	4000	0.84	0.91	2.08	4500	0.85	1.53	2.16	4300	0.84	1.43	1.94
	CON	4200	0.97	0.98	2.86	4600	0.99	1.45	3.10	4400	0.96	1.38	2.18
20	0.02	4000	0.64	0.96	1.63	4400	0.97	1.18	1.98	4200	0.94	1.12	1.82
	0.05	4600	0.91	0.98	2.12	5000	1.30	2.04	2.50	4600	1.26	1.86	2.04
	0.07	3800	0.56	0.81	1.40	4100	0.61	0.89	1.56	4000	0.61	0.85	1.48
	CON	4000	0.63	0.86	1.76	4200	0.67	0.95	1.84	4400	0.82	0.96	1.65

Con - Control, a - Cell concentration/ml x 10^3 b - Chlorophyll 'a' $\mu g/10^6$ Cells

C - Non-chlorophyllous pigments $\mu g/10^6$ Cells d - Production mgC/l/hr

Table 22. EFFECT OF ZINC ON THE PHYSIOLOGICAL ACTIVITY OF S. SALINA AT DIFFERENT pH

Days of Growth	Zn Concn (ppm)	pH 6.0				pH 7.0				pH 8.0			
		a	b	c	d	a	b	c	d	a	b	c	d
	0.02	285	1.61	2.04	1.66	300	1.72	2.16	1.71	325	1.78	2.34	1.96
	0.05	300	1.75	2.26	1.98	315	1.80	2.30	2.16	345	1.84	2.36	2.20
4	0.07	265	1.38	1.85	1.34	285	1.46	1.90	1.42	300	1.50	1.98	1.46
	CON	255	1.40	1.81	1.64	280	1.54	2.15	1.70	300	1.68	2.24	1.90
	0.02	566	2.65	3.72	3.72	595	2.70	4.56	3.81	610	2.74	4.61	3.76
	0.05	600	2.90	3.88	3.86	625	2.96	4.90	3.90	630	3.02	4.94	3.88
8	0.07	545	2.10	3.08	3.00	550	2.14	3.21	3.14	555	2.20	3.30	3.12
	CON	565	2.96	3.74	3.62	580	3.10	4.32	3.75	600	3.45	4.46	3.76
	0.02	2800	5.20	7.04	6.20	3400	5.28	7.12	6.34	3500	5.36	7.18	6.40
	0.05	3500	5.84	7.61	7.44	3700	5.90	7.70	7.40	3800	5.96	7.65	7.38
12	0.07	3000	4.31	6.10	6.10	3500	4.48	6.28	6.05	3600	4.50	6.32	6.10
	CON	3000	4.61	5.89	5.74	3200	4.65	5.80	5.58	3400	4.85	5.94	5.63
	0.02	3500	4.96	6.83	6.45	5600	4.98	6.88	6.50	6000	5.04	6.91	6.62
	0.05	7000	5.56	7.21	7.92	7200	5.60	7.30	8.10	7400	5.62	7.41	8.16
16	0.07	6000	4.10	5.82	6.50	6400	4.12	5.98	6.64	6500	4.20	5.84	6.60
	CON	5400	3.98	4.75	4.90	5600	3.56	4.65	4.80	5800	3.50	4.62	4.81
	0.02	5000	5.02	6.80	6.30	5200	5.14	6.84	6.42	5300	5.04	6.72	6.40
	0.05	6500	5.63	7.02	6.88	6700	5.66	7.14	6.90	6800	5.70	7.00	6.94
20	0.07	5500	4.45	5.75	6.20	5800	4.50	5.80	6.31	6000	4.54	5.86	6.40
	CON	4050	2.24	2.91	2.85	4200	2.50	3.60	3.45	4500	2.65	3.51	3.46

Con - Control, a - Cell concentration/ml x 10^3 b - Chlorophyll 'a' $\mu g/10^6$ Cells

C - Non-chlorophyllous pigments $\mu g/10^6$ Cells d - Production mgC/l/hr