# IMPACT ASSESSMENT OF BIOCIDES ON MICROALGAE - A STUDY IN VITRO

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**DECEMBER 1993**

DEDICATED TO MY PARENTS

# C E R T I F I C A T E

This is to certify that the thesis entitled **"IMPACT ASSESSMENT OF BIOCIDES ON MICROALGAE - A STUDY IN VITRO" is the bonafide record of the work carried out by Mrs. V.M. ASMA** 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 **'IMPACT ASSESSMENT OF BIOCIDES ON MICROALGAE - A STUDY IN VITRO"** 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

Man is becoming more and more concerned about his surroundings. One main reason for this is the increasing threat to the health and cleanliness of the environment on account of pollution from manifold sources. Environmental pollution is a global phenomenon causing, in many instances, rather permanent damages to the aerial, terrestrial and aquatic environments. The pollution has acquired such dimensions that the very existence of life on earth is threatened. The release into the nature, of various kinds of chemicals in the form of solids, liquids and gases is an ever increasing problem and efforts are being made all over the world, especially by the developed nations to combat it on a war footing. However due to various reasons the developing or underdeveloped countries are not able to properly conceive the seriousness of the problem and fight against it.

In India also the problems of pollution are acquiring serious dimensions. One major area which is highly threatened is the aquatic environment, both fresh water and marine which houses myriads of living organisms especially the green matter or the phytoplankton which form the basis of all aquatic life. Large quantities of several biocides in the form of insecticides, fungicides and herbicides are being extensively used for crop protection and their residues ultimately reach the aquatic environment and affect those life forms at all levels of food web.

Until now there is no proper understanding of the magnitude of pollution or the damages caused by the pollutants to the living organisms of the aquatic ecosystem.

Therefore specialised knowledge is required to understand the problems of preservation and improvement of environment. Since the algae are extremely important components of aquatic ecosystem, it is important to examine the effects of pollutants on algae.

Algal assays contribute to the efficient analysis of water quality and are necessary to obtain appropriate quantitative data expressing the relationship between the pollution load and the biological response of the receiving water. Algal assays are the source of relevant and quantative information about the availability of chemical substances to algae and their different stimulative or inhibitory effects.

The origin of algal bioassays can be traced to the work of Prof. Martinus Beijerinck (1899) who was the first to obtain a pure (axenic) culture of algae. Axenic cultures are an important component to algal bioassay culture methods.

The use of different pollutants such as biocides are increasing day by day and it has been reported that in India within the last 30 years the use of biocides has become 40 times higher i.e, from 2,000 tonnes to 80,000 tonnes. Eventhough these biocides afford remarkable benefits to mankind by increasing crop yields, protecting forests and also by con-

trolling arthropod vectors of serious human disease, they may produce adverse effects on the ecosystem. Most of the biocides are usually applied to the terrestrial habitats, but by accidental fall out of spray from agricultural treatments, fall out from atmosphere and also by surface run off from agricultural land, they may ultimately reach non-target organisms in the aquatic ecosystem. The results of pesticide residue analysis indicate that the pesticides can reach non target organisms in the aquatic environment and give indications of biological reservoirs of pesticides in the environment.

The present study was undertaken to make a detailed investigation for the assessment of specific impact of commonly used biocides at the lower trophic level of food chain i.e., microalgae by using batch culture techniques in the laboratory. Microalgal representatives from three habitats i.e., fresh water, estuarine and marine were investigated. The different biocides selected are of common use in the agricultural practices.

Because of the importance of microalgae as live feed for larval and postlarval stages of different aquatic organisms, the fluctuations in algal populations as a result of biocide treatment will surely affect the food chain. These studies are also of significance in setting the criteria and standards for water quality management by suggesting threshold values of different biocides tested, beyond which they affect the ecosystem adversely.

The research work for the thesis was started after the completion

of six months course programme in mariculture. During the course work the candidate got familiarized with different research techniques which were useful to carry out this work. Most of the facilities for doing the work were available at the Algology Laborartory of the Central Marine Fisheries Research Institute. However, for analysing the pesticide residues using Gas Chromatography, the candidate made use of the facilities available at the Indo Cargo Surveyors, Cochin.

The thesis has been divided into six chapters. The Introductory chapter explains the relevance of research work undertaken. A review of work already carried out on the effects of biocides on microalgae is presented in the second chapter. Chapter three gives a detailed description of the material and methods followed for the study.

The fourth chapter gives the results and discussion of all the experiments carried out. This chapter mainly focuses on five important aspects. The first part of the chapter gives the results of bioassay studies which are essential to find out the effective concentration of biocides which inhibit fifty percent growth of microalgae. The second part gives an idea of the effect of five biocides and their combinations on the physiological aspects of three microalgal cultures. The third part explains the effect of biocides on the protein and carbohydrate contents of microalgae. As bioaccumulation studies are very important particularly in the case of organochlorines, an experiment was conducted with one microalgae and the results of this study are also given in chapter four. Last part of this chapter gives important morphological changes observed as a result

of biocide application.

A detailed general discussion about all the experiments carried out is included in chapter five. The last chapter embodies the salient conclusions generated out of the study.

It is hoped that the results and conclusions drawn from these investigations will be useful in the pollution control of estuarine and nearshore environments, as well as for improving the culture aspects of marine algae as live food in hatchery systems

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# CHAPTER 1

# INTRODUCTION

Microalgae are the microscopic photosynthetic plant components in the aquatic ecosystems. They incorporate solar energy into biomass, produce oxygen that gets dissolved into water, function in cycling and mineralization of chemical elements and serve as food for herbivorous and omnivorous animals. When they die, they sink to the bottom where their chemical constituents are transformed, solubilized and recycled into the water. These functions depend on phytoplankton population dynamics which in turn depends upon seasonal variability in temperature, intensity of solar radiation, nutrient concentration in the water and grazing by animals.

Algae comprise a large and heterogenous group of benthic and planktonic species that occur together in often complex communities. Members of the following classes are often prominent in natural assemblages Chlorophyceae (green algae), Bacillaro phyceae (Diatoms) Cyanophyceae (blue-green algae), Rhodophyceae (red algae) Chrysophyceae (golden brown algae) Haptophyceae, Crespedophyceae, Xanthophyceae, Prasinophyceae, Pyrrophyceae (dinoflagellates), Cryptophyceae, and Euglenophyceae (Raymond, 1980) Green algae, blue green algae and diatoms are commonly dominant in algal communities.

Popularisation and commercial application of photosynthetic biomass production systems, like cultivation of algae are more relevant now than

ever before in the International context of energy shortage, water disposal problems, environmental protection, alternative food additives and cheaper sources of feed proteins (Venkataraman, 1990). Production of microalgae for more varied and newer applications like aquacultural practices has come of age.

Natural and anthropogenic alterations of water quality can bring about changes in species composition of the algal community, rates of production, biomass and water chemistry. If water quality is altered by toxicants or growth stimulates from industrial, agricultural or municipal sources, normal algal function may be upset, causing gross changes in structure and function of the receiving aquatic ecosystem.

As inhabitants of environments which are directly or indirectly entered by biocides there are opportunities for algae to encounter these biocides. An awareness of the potential for biological activity of these toxicants should coincide the important micro-algae in the aquatic ecosystem.

Chemical nature of our environment has been altered for the past several years by the addition of several hundred thousand synthetic chemical compounds. Some of these were used as pesticides although their general lack of specificity suggests that the term 'biocide' may be more appropriate (Carson 1962). The environmental persistance, accumulation and effects (singular, additive or synergistic) of these chemicals in the biosphere are poorly understood.

Only after the book "Silent Spring" (Carson, 1962), was published did the general public become aware of the seemingly pervasive nature of some of the pesticides and their undesirable effects on non-target species or ecosystem. A year later, Butler and Springer (1963) described the potential hazards of pesticides to coastal areas in a paper entitled "Pesticides a new factor in coastal environments".

The biocides that have caused most concern as pollutants are synthetic organic compounds which have only come into use since the 1939-45 war, (Colin Walker 1975). The earlier history of pesticide use has been recorded by several authors (Metcalf 1955, and Martin, 1964). Naturally occurring substances such as pyrethrum, nicotene and rotenone were the principal insecticides in the mid 18th century. Since 1940 two important groups of synthetic innsecticides have emerged, the organochlorine compound and the organophosphorus ester or organophosphates.

In India the pesticide industry was started with the import of 'BHC' in 1952, (Agarwal, 1986). By 1958, India was manufacturing five basic pesticides for a total production of 5,460 metric tonnes. By 1977, there were 42 different pesticides manufactured in the country that totalled 40,658 metric tonnes. The production of pesticides during 1978 was 44,000 metric tonnes of which HCH (BHC) and DDT accounted for 76.4 per cent.

It has been reported that Rs.850 crores worth of pesticides are sold in India every year. However, India uses only 400 gms per hectare

while in Japan it is 11,000 grams per hectare. India now produces 67,000 tonnes of pesticides. In India there are about 350 units engaged in the manufacture of these harmful toxicants.

According to the reports of World Health Organisation, every year 10,000 death and 4,00,000 cases of intoxication are caused from these pesticides. In India within the last 30 years the use of pesticides has beome 40 times higher i.e., from 2,000 tonnes to 80,000 tonnes.

It has been reported that the total quantity of pesticides used annually in Kerala is about 1000 tonnes, of which about 250 tonnes are used in the rice fields of Kuttanad alone.

Pesticides are commercially valuable because of their toxic properties. Although they are used primarily for specific purposes they have the ability to exert biological effects on all living organisms. In addition to their toxic characteristics, some compounds display carcinogenic properties, and have become of increasing concern to public health because of their uncontrolled ubiquitous distribution which now extended far beyond their intended use. The major factor which accounts for public and scientific concern, about the pesticides, is their high biological activity.

Patterns of pesticide usage are changing in this country and these changes are reflected in amounts of various pesticides produced annually. Pesticides are likely to affect freshwater ecosystems more severely than terrestrial ones. The main sources in water include accidental fall out of spray from agricultural treatments, fall out from the atmosphere,

and surface run-off from agricultural land. Apart from their wide spread and important use in agriculture, the herbicides are also used for aquatic weed control. The results of pesticides residue analysis indicate that pesticides can reach non-target organisms in the aquatic environment and give indications of biological reservoirs of pesticides in their environment.

The biocides can have toxic effects on many different types of organisms and effect biological processes at the cellular, population community and ecosystem levels of organisation (Boyle 1984). There is a definite and continuing need for assessment and mitigation of the impact of toxicants, and so all legal, social, economic and biological aspects should be considered.

The direct, short term effects of biocides on aquatic algae have been the subject of a myriad of different types of laboratory studies. The role of algae in the disappearance and degradation of environmental contaminants has only recently been addressed.

The effect of various pollutants on algae based upon laboratory and field investigations are reported already. But the work within the country is very little. Eventhough there are published reports, most of them are short term tests and also the effect on the biochemical compounds of algae is very limited.

As the use of biocides is increasing day by day, the effects of these toxicants on aquatic organisms particularly at the lower trophic

level need in depth study. One lacuna in such studies is the lack of adequate knowledge on the impacts of these biocides on individual species.

The present work was undertaken to make a detailed investigation for the assessment of specific impact of organochlorine, organophosphorous and carbamate insecticides, one herbicide and fungicide on micro algae. All the five biocides were selected after making an inventory survey among agricultural officers and farmers and it was found that the five biocides selected for the study are of common use in paddy fields and other crops. As the aquatic system often contains mixture of biocides, two types of biocide mixtures were also selected for the study. Marine, estuarine and freshwater species of microalgae were tested in the present study

Since the algal bioassays are very essential to determine the concentration of toxicant which inhibit the growth of algae by 50%, algal bioassay tests were conducted with two species of microalgae. Algalbioassays are generally more rapid and less demanding of facilities, space and time of personnel. Important factors governing the usefulness of algal bioassays include the sensitivity and stability of strains and the ease and rapidity with which the organisms are cultured by which growth or other indices of physiological state can be measured.

The uptake and concentration of environmental contaminants by biotic and abiotic compartments are important in understanding the toxicity of various compounds and their distribution in the aquatic ecosystem.

The phenomenon of uptake and concentration of a chemical by organisms is commonly termed bioaccumulation.

The problem of environmental contamination by persistent chlorinated pesticides is of major concern due to the presence of their residues in the environment and in the human tissues. In developing countries like India, organochlorines are extensively being used in agriculture and vector control programmes. Of the two important organochlorines used in India, DDT and BHC, the use of DDT shows a downward trend, but the trend for BHC remains constant. The cumulative effects of these chlorinated pesticides on our coastal environment can be expected to be considerable. Organochlorine pesticides are in general fat soluble and hence can be concentrated by various organisms.

Widely located at the base of the food chain, and in general having a high surface area: volume cell characteristics, algal populations have a significant potential for absorption and consequent reaction with the pesticides. This has been especially recognised with respect to the persistent lipid partitioning organochlorine insecticides with the phytoplankton which are so important in pelagic food chains, reflecting the general concern for biological magnification of organochlorine insecticide. Eventhough there are reports about the bioaccumulation of different organochlorines and other pesticides, most of them are based on short term experiments. So in order to find out the long term effects, particularly the bioaccumulation of organochlorines experiment was conducted using as chromatographic method and the results are explained elsewhere.

By using photomicrography, morphological changes observed as a result of biocide effect are also explained.

The main objective of the present study was primarily to determine the safe level of application factor of each biocide after considering the various physiological effects, alterations in proteins, carbohydrates and morphological effects of particular biocide. The application factor of each biocide tested should be having the least impact on the ecosystem. Another aim of the investigation was to find out which is most toxic among the five biocides tested; and also to find out that, among freshwater estuarine and marine species of microalgae, which one is most resistant to the pollutants. This study will also focus on the importance of micro-algae in pollution control.

# CHAPTER II

# REVIEW OF LITERATURE

In this chapter on literature review, greater stress has been given to the earlier work done on important organic biocides and their effects on microalgae. Eventhough very few literature are available on this aspect during 1960s, Most of the earlier works done on this aspect are after 1970s, and hence literature was collected from that period onwards. For some organic biocides consistent data have been obtained by different workers with regard to their effects on microalgae. For a few biocides, the studies have included both pure culture and natural population. In view of the difference in chemical and toxicological properties of different biocides and the variation in experimental procedures of different workers (eg: axenic and non-axenic culture), caution is advised in comparing some of the data.

The biocides used for the present study come under three groups; insecticides, herbicides and fungicides.

## 2.1. INSECTICIDES

Based on their chemical nature, the insecticides are classified into three groups; (a) Organochlorines (b) Organophosphates and (c) Carbamates.

a) Organochlorines:

As the organochlorines are widely used for the past four decades, the toxicological effects of these insecticides have been studied by various investigators.

Raghu and Macrae (1967) reported that Y-BHC used for insect control in rice fields, promoted algal growth than in untreated fields, and this result was established in pot experiments. The insecticides selectively stimulated the indigenous blue green soil algae.

Wurstur (1968), who emphasized the important contribution of phytoplankton to global photosynthesis and the consequences of interference with this process, found that concentration of DDT below 10 ppb inhibited photosynthesis in marine planktonic algae. The toxicity of DDT to the diatom Skeletonema costatum, increased with decreasing cell concentration and it was concluded that low levels of DDT in natural waters might have deleterious effects on the phytoplankton. A similar conclusion was reached by Sodergren (1968) who reported that growth of Chlorella sp. was affected by less than 0.3 ppb DDT.

A study was carried out to determine what affect the entry of three broad spectrum insecticides may have on algal population (Christie, 1969). The organochlorine tested was DDT. It was found that the three pesticides, varied in their degree of toxicity to algae and also in the extent to which they were degraded in the presence of algae.

DDT uptake and metabolism by marine diatom were reported (Keil and Priester, 1969), and it was concluded that the diatom was capable of absorbing and concentrating DDT above the level in sea water. DDT was metabolised by this organism to DDE.

Algal species isolated from various marine environments responded differentially to DDT (Menzel et al., 1970) whereas the estuarine naked green flagellate Dunaliella tertiolecta was insensitive to 1000 ppb DDT. Photosynthetic $^{14}C$ uptake by the marine diatom Skeletonema costatum and the coccolithophorid Coccolithus huxleyi was significantly reduced to above 10 ppb DDT. Menzel et al., (1970) further showed that cell division in the diatom, but not in Coccolithus, was prevented by 100 ppb DDT. The most DDT sensitive organism tested by Menzel et al., (1970) was also a marine diatom, Cyclotella nana, in which $^{14}C$ uptake and growth were affected at DDT levels of 1 ppb and 100 ppb respectively.

Growth and $^{14}C$ assimilation in low-density population of Scenedesnus quadricauda were inhibitd by 0.1 ppm DDT. (Stadnyk et al., 1971). But, contrary to this report Morgan (1972) reported that growth of green algae Chylamydomonas reinhardii was not appreciably affected by DDT at levels upto 20 ppm and there was no significant effect on $^{14}C$ uptake.

Mosser et al., (1972 a) confirmed that Dunaliella tertiolecta was unaffected by 1000 ppb DDT and that other organisms, including Euglena gracile and Chlamydomonas reinhardii were also relatively resistant.

Mosser et al., (1972 b) investigated the effects of DDT on mixed cultures of marine algae, containing a 'sensitive', diatom (Thalassiosira pseudonana) and a resistant green alga (Dunaliella tertiolecta) in equal proportions. It was found that the growth of D. tertiolecta was not inhibited at any DDT level tested. However, T. pseudonana grew faster and soon out numbered D. tertiolecta. D. tertiolecta in control cultures, was affected by 100 ppb of DDT to the extent that its competitive success was significantly diminished, eventhough T. pseudonana was unaffected by 10 ppb DDT in the pure culture. Mosser et al., (1972 b) pointed out that DDT can occur in natural waters at levels equivalent to those which caused a marked change in species ratio in their experiments and considered the ecolgical implication of such a pesticide induced alternations in phytoplankton populations, via effects on the selectively grazing zooplankton.

Photsynthetic $^{14}C$ fixation by Scenedesmus quadricauda was not significantly inhibited by DDT at concentrations upto 1 ppm, although the metabolite DDE was inhibitory (Luard 1973).

Recognising that in nature, organisms may be exposed to several toxicants simultaneously, Mosser et al., (1974) investigated the effects of mixture of organochlorines on the growth of marine diatom Thalassiosira pseudonana. Because of their ubiquity, chlorinated hydrocarbons were considered to be likely environmental contaminants. Results indicated that interaction between the organochlorines may occur, causing their toxicity to phytoplankton. DDE, the universal pollutant derived from

DDT, and polychlorinated biphenyls (PCB's are industrial organochlorines) were far more inhibitory to T. pseudonana in combination than they were individually. In complete contrast, the addition of DDT (500 ppb) to cultures, whose growth had been prevented by PCB's (50 ppb) substantially restored growth (Mosser et al., 1974).

Butler et al., (1975a) reported the effects of various concentrations of insecticides on the growth of 36 isolates of planktonic algae. Among the different inseticides tested, organochlorine insecticide was found to be more toxic. Powers et al., (1975) reported the toxicity of DDE to a marine dinoflagellate and it was found to be highly toxic to Exuviella baltica. Concentrations as low as 0.1 parts per thousand million ($10^{9}$ pt m) significantly inhibitd growth for at least 24 hur as measured by cell number and growth rate.

DDT and PCB's have been observed to reduce the rate of cell division, in marine phytoplankton, thereby indirectly reducing the total photosynthetic carbon fixation in treated cultures (Fisher 1975). Total marine photosynthesis will likely remain undiminished by these compounds, although alteration in phytoplankton communities through selective toxicity could affect herbivore population.

The effect of endrin on primary production in a pond ecosystem was reported (Nassar, 1976). The results clearly indicated that endrin did affect the primary production of phytoplankton, while its higher concentration affected the rate of oxygen consumption of phytoplankton. Dieldrin

induced destruction of marine algal cells was reported by Powers et al., (1977). Treatment of axenic cultures of the marine dinoflagellate Exuniella baltica with 10 parts per thousand million ($10^9$ ptm) of dieldrin inhibited growth rate and caused large number of cells to disintegrate within 12 hours of exposure.

Subramanian et al., (1979) reported the effect of low concentration of DDT on the growth and production of marine diatom Skeletonema costatum. At 1 ppb concentration there is no marked change. Above 4 ppb a marked reduction in cell number was noticed. Concentrations above 10 ppb DDT impared growth rate.

The effect of low concentration of organochlorine insecticide DDT was compared with organophosphate and carbamate insecticides (Ramachandran et al., 1980). Results clearly indicated that organochlorine was toxic than other insecticides as reported by other workers.

An organochlorine insecticide permethrine and its carrier solvent was found to affect microalgae additively, synergistically and antagonistically when tested for photosynthesis in green algae, as reported by Stratton and Corke (1981). Interaction of DDT with two species of fresh water algae was reported (Goulding and Ellis, 1981). The amount of inhibition varied with time and with the method of growth assessment. Bioaccumulation studies showed that both the algae accumulated $^{14}C$ DDT.

Field studies were also conducted by several workers. Rajendran and Venugopalan (1983) conducted in situ studies with natural phytoplankton

to find the effect of pesticides on them. In additions to organochlorine insecticides, organophosphates and carbamates were also tested. As observed by various other workers Rajendran and Venugopalan (1983) also found that the primary production was inhibited considerably by these pesticides.

By using a mortal stain - Evan's blue, Walsh (1983) reported the cell death of marine diatom exposed to the pesticides. All the pesticides tested, particularly organochlorine insecticides caused death of cells, but significant mortality occurred only at concentrations higher than the $EC_{50}$ values calculated from population growth studies.

Regarding the effect of biocides on cellular composition or micro-algae, only very little reports are available. Goulding et al., (1984) reported the effect of DDT on the cellular composition of Chlorella fusca. Results showed that DDT caused a decrease in cell size by 46%, When measured by biovolume, and 43% when based on dry weight. Patil et al., (1985) studied and discussed the effect of DDT on parimary productivity of phytoplankton of a pond ecosystem. It has been observed that at higher concentrations of DDT, the rate of oxygen consumption increased and the reserve food material was metabolised.

A number of organochlorine insecticides were found to inhibit the growth of Ankistrodesmus fusiformis, in vitro (de Fatia celeste, and Caceres, 1986). It was found that at 2 ppm concentration of gamma BHC the growth of the algae was inhibited. The results clearly suggested that gamma BHC an interferred with the physiology of this organism on concent[illegible]er than 1 ppm.

Lal et al., (1987) reported the effect of DDT, fenitrothion and chlorpyrifos on growth, photosynthesis and nitrogen fixation in Anabaena (Arm 310) and Aulosira fertilissima. DDT inhibited the growth of Anabaena where as it was stimulatory to Aulosira. Fenitrothion and chlorpyrifos wre quite toxic even at concentrations of 100 times less than DDT

The effect of pesticides on the filamentous forms of algae is very rare. One such report was published by Awasti and Singh (1987). They reported on the effect of DDT and BHC on filamentous green algae Spirogyra cylindrica. As in the case of unicellular microalgae, this alga was sensitive to both the insecticides even at low concentrations. The effect of DDT was more severe than BHC.

Field studies were conducted to find out the combined effects of chloro organic pesticides on the primary production of phytoplankton communities (Savinova and Savinov, 1987). Only short term experiments were possible in the field and the results showed that the concentration of each toxicant brought the level of primary production down to 53-77% of the control.

Regarding the study of the effect of toxicant on biochemical compounds, Yu Huzhao (1987) reported the effect of DDT and BHC on amino-acid contents of Chlorella vulgaris. Experimental results show that the treatment with 0.1 ppm of DDT and BHC had very little effect on amino acid content. However, after being treated with 1-10 ppm of DDT and BHC, the aminoacid content increased.

Yasuno et al., (1987) were not able to find out any toxic effect of the organochlorine insecticide permethrin. They conducted a field study in an enclosure system containing phytoplankton and zooplankton. The insecticide addition did not significantly change the primary production, respiration, chlorophyll a concentration and sedimentation.

Piska and Waghray (1990) compared the toxic effect of an organochlorine insecticide endosulfan with two other insecticides and found that endosulfan was most toxic than the other insecticide tested.

Fate and biological affects of lindane and deltamethrin in fresh water mesocosms were reported (Caquet et al., 1992). The phytoplanktonic and periphytic communities were positively affected by the treatment.

Polychlorinated biphenyls:

Polychlorinated biphenyls which are similar in structure, persistence and biological effects to some organochlorine pesticides have been widely detected as environmental pollutants. Although they are not considered as pesticides, the PCB's find numerous industrial uses and they may be analytically confused with DDT (Keil et al., 1971). It is important to investigate their reaction with the biota. Several workers have reported that PCB's are toxic to algae and some evidence shows that they are being more toxic than DDT.

The marine diatom Cylindrotheca Closterium took and concentrated the PCB mixture (Aroclor, 1242) which at 10 ppb reduced growth, RNA

and chlorophyll levels in this organism (Keil et al., 1971). Growth rates of marine diatoms Thalassiosira pseudonana and Skeletonema costatum were reduced in the presence of 25 ppb PCB's and severely inhibited by 100 ppb, at which level DDT was only slightly inhibitory (Mosser et al., 1972a). By comparison with the diatoms, other algae were less sensitive to PCB's and DDT, suggesting that selective inhibition of sensitive phytoplankton species by organochlorines might alter the species composition in natural algal communities. This view was endorsed by Mosser et al., (1972 b), who found that at 1 ppb PCB's affected the species ratio in a mixture of T. pseudonana (sensitive) and D. tertiolecta (relatively resistant)

Moore and Harriss (1972) reported on the first 'in situ' bioassay of short term effects of polychlorinated biphenyl compounds on natural phytoplankton communities. The effect of PCB's reported in these studies suggested that the toxic effects of organochlorines were more acute 'in situ' at a community level than for single species laboratory cultures. This view was further recommended by Moore and Harriss (1974), that natural, mixed phytoplankton communities were more sensitive to PCB's than pure or mixed laboratory cultures. It was found that the greatest sensitivity to PCB was in net plankton and not in nannoplankton

Uptake of PCB's by marine phytoplankton was reported by Harding and Philips (1978 b) who found that there was a definite relationship between cell density and accumulation of PCB's. Effects of PCB on marine phytoplankton photosynthesis and cell division was also reported

by Harding and Phillips (1978b). PCB concentration as low as 1.0 $\mu gl^{-1}$ reduced cell division of Thalassiosira pseudonana and Isochrysis galbana. A similar observation was reported by Michaels et al., (1982).

Responses of marine diatom Thalassiosira rotula to PCB's was reported by Reiriz et al., (1983). The results of this experiments concluded that, the growth phase from which the inoculum came, had a determining influence on the sensibility of microorganisms to the toxic substance. This sensitivity was highest in exponential phase.

Regarding the biochemical aspects of microalgae, Bazulic et al., (1988) reported the PCB effects on production of carbohydrates, lipids and proteins in marine diatom Phaeodactylum tricornutum. 'Arclor 1254' had an influence on biochemical composition of phytoplankton.

b) Organphosphates:

Of the wide range of pesticides screened, organophosphorus compounds were found to be relatively non-toxic to marine phytoplankton (Ukeles, 1962), Christie (1969) observed that 100 ppm malathion had little significant effect on the green alga Chlorella pyrenoidosa. Moore (1970) found that 'malathion' and 'parathion' were relatively non-inhibitory to the flagellate Euglena gracilis.

It is seen that organophosphorus insecticides are relatively non-toxic to microalgae. However, the finding that some of the "newer" organophosphorus insecticides, as reported by Derby and Ruber (1971),

were found to depress the oxygen evolution in algal cultures of four species of marine phytoplankton and signified the need to obtain further data on the antialgal activity of this group of insecticides. Indeed, at 2.5 ppb, Dursban had been found to stimulate growth of blue green algae in artificial ponds (Hulbert et al., 1972).

Butler et al., (1975b reported on the effects of various concentrations of diazinone, an organophosphate and the results showed that diazinon was toxic at concentrations of 10 ppm. They also reported the capability of diazinon being absorbed by the several isolates of microalgae. Organophosphate was found to be less toxic than other insecticides tested (Butler et al., 1975b. But contradictory to this report, Ramachandra et al., (1980) reported that organophosphates were more toxic than carbamate insecticides but less toxic than organochlorines. Murray and Guthrie (1980) reported that, eventhough, the organophosphates showed an initial inhibition of growth, at a later stage the algal population approached or exceeded those of control by some measures.

There was an interesting report that algae could utilize the elements of insecticides or its biodegraded metabolites (Rath and Misra, 1981). 'Dimecron - 100', an organophosphorus insecticide was tested for its nutritional value in relation to growth of Oscillatoria obscura at 400 ppm. A significant increase in growth in relation to optical density, dry weight and total chlorophyll content was noted in the Dimecron treated algae (Rath and Misra, 1981).

The effects of methyl parathion on the growth, cell size, pigment and protein content of Chlorella protothecoides was reported (Saroja and Bose, 1982). Slight or moderate inhibition with respect to cell number, packed cell volume, pigment content and protein content was noticed at 10 ppm and 20 ppm. 30 ppm and higher concentrations inhibited the growth severely. Chlorophyll was found to be the most sensitive parameter. There was another report about the effect of methyl parathion (Rajendran and Venugopalan, 1983). Field study was conducted to find out the effect of methyl parathion, malathion - two organophosphates and other insecticides. Primary production was found inhibited considerably by these pesticides.

It is important to know that the insecticide 'malathion' can have a toxic effect on unicellular chlorophyllous marine phytoplanktonic organisms and that it is concentrated by the first link of the trophic chain (Prevot and Soyer, 1985). Malathion was found toxic to the dinoflagellate Prorocentrum micans even at 10 ppm, causing a clear decrease of chlorophyll and an increase of the oxygen consumption of phytoplankton population at 1.27 to 5.07 ppm of malathion. At this concentration of toxicants they reported a significant reduction in dissolved oxygen and free $Co_2$ of water.

Influence of the organophosphorus insecticide DDVP on aquatic ecosystem was reported by Pal and Konar (1985). DDVP at 0.014 to 1.424 ppm did not alter dissolved oxygen, pH, temperature, colour and odour of water primary productivity of water and populations of phytoplankton and zooplankton were significantly reduced at these concentrations. Results

of these experiments concluded that frequent spillage of DDVP into fish pond or in natural water would be detrimental to basic ecosystem parameters which are responsible for high fish yield.

In a field study conducted by Pal and Konar (1989) it was found that continuous drainage of phosphamidon into water hampered the growth and production of food chain organisms even at low concentration. They found that phytoplankton population decreased at 0.4257 ppm.

In a laboratory study, Raine et al., (1990) reported that Nuvan(R) - was toxic to phytoplankton at a concentration of 1 ppm. They compared the results of 'in vitro' study with that of natural populations. The effect of monocrotophos was reported by Piska and Waghray (1990) and found that gross and net production decreased gradually in treated samples.

Physiological alterations induced by an organophosphorus insecticide 'trichlorfon' on Anabaena have been reported by Marco et al., (1990). The addition of trichlorfon to nitrate containing cultures of Anabaena resulted in a decrease in the content of all the main nitrogen compounds and an increase on the carbohydrate fraction per unit dry weight, cell division, and morphology were altered. All these trichlorfon induced alterations were noticeable from the first 24 hours of treatment, but inhibition of growth did not occur until the fourth day.

Pollution of aquatic ecosystem by the pesticide 'methyl parathion' was studied by Pal and Konar (1990). A 90 day observation was carried

out and found that primary productivity and phytoplankton population significantly decreased at all exposures. Toxicity studies were performed with three different species of fresh water microalgae, to study the direct effects of the insecticide. Dursban(R) 4 E had no appreciable effect on the growth of non-limited algae at concentrations relevant for field situations. For phosphorus limited algae, however, significant and dissimilar effects were found (Van Donk et al., 1992).

c) Carbamates:

Microalgae were also susceptible to carbaryl insecticides, which was lethal to two species at 1 ppm and to all five species tested at 10 ppm (Ukeles 1962). Christie (1969) compared the toxicity to carbaryl sevin with two other insecticides and found that three pesticides varied in their degree of toxicity to algae and also in the extent to which they are degraded in the presence of algae. At a lower level i.e., 0.1 ppm, carbaryl was found to stimulate growth and $^{14}C$ assimilation in low density populations of the fresh water algae Scenedesmus quadricauda (Stadynk et al., 1971). Bulter et al., (1975b) while testing the toxicity of various insecticides to 36 isolates of planktonic algae, found that carbaryl was toxic at a concentration of 25 ppm.

Ramachandran et al., (1980) compared the toxic effect of sevin with other insecticides and it was found that sevin was least toxic than the other two insecticides. In a field study conducted by Rajendran and Venugopalan (1983) it was reported that primary production was inhibited

considerably by the insecticides including carbaryl sevin. The effect of Carbaryl on single species and on communities made up of three and five species has been reported by Maly and Ruber (1983).

Regarding the effect of insecticides on the reproductive• stage of microalgae, Cain and Cain (1984) reported the effect of carbaryl and propoxur (both are carbamates) on zygospore germination and growth of green alga . Chlamydomonas moewusii. Carbaryl produced signifiant growth inhibition over the concentration range tested. But one important conclusion they reported is that concentration which adversely affected growth had no significant effect on zygospore germination.

Kentzer et al., (1984) reported on the toxic effects of carbaryl against laboratory strain of phytoplanktonic algae, Chlorella and Anacystis. Carbaryl in concentration of 10 ppm was found to inhibit the cell multiplication and total production of chlorophyll a. But contrary to this report, Khalil and Mostafa (1986) reported that, methomyl which is also a carbamate insecticide showed no significant effect on the growth of fresh water alga Phormidium fragile upto a concentration of 112.5 ppm. But there was a gradual decrease in all nitrogen fraction, total carbohydrate content, chlorophyll a and carotene content. The same carbaryl insecticide methomyl - $^{14}C$ labelled, was found to inhibit the growth and total carbohydrate content of Nostoc muscorum and Tolypothrix tenuis at a concentration of 100 ppm (Kobbia et al., 1991).

## 2.2. HERBICIDES:

There are many reports of the direct effect which herbicides can exert upon microalgae. Recognizing the importance of evaluating the tolerance of marine phytoplankton to pesticides, used on commercial shell fish beds Ukeles (1962) tested a wide range of toxicants, including phenyl urea herbicides, for effects on the growth of marine algae in an enriched sea water medium. Diuron, lethal to all but one species at 0.004 ppm was the most toxic of the phenyl ureas and the relative order toxicity was diuron > monuron > neburon > fenuron (Ukeles, 1962).

Available data on 2, 4 D and 2, 4, 5 T suggest that only at high concentration does 2, 4 D have adverse effects on algal population natural or cultured. Stimulation of algal growth and photosynthesis was observed with 10 ppm 2, 4 D (Walsh et al., 1970).

Evidence for atrazine inhibitory action on algal photosynthesis has come from Walsh (1972), and Hollister and Walsh (1973). Inhibition of algal photosynthesis has been established with monuron (Walsh, 1972). According to Hollister and Walsh (1973) the effects of $O_2$ - evolution of several different types of marine phytoplankton, the diatom, are generally less sensitive than other algal types to phenyl urea and triazine herbicides.

The effect of various concentrations of Atrazine and 2, 4, D was tested on the growth of 36 isolates of planktonic algae by Butler et al., (1975 b) and found that atrazine was more toxic than 2, 4, D.

Effect of pesticides on filamentous forms of algae is very rare. Singh et al., (1978) reported that two nitrogen fixing, filamentous species of cyanobacteria were sensitive than a unicellular non-fixing species to the herbicide, Alachlor.

By comparing the salt marsh edaphic algae in culture, microecosystems and in the field Plumley and Davis (1980) reported that at 2.2 ppm concentration of the herbicide atrazine, the rate of photosynthesis, chlorophyll content and cell numbers in unialgal cultures were redued. But, the result with lower concentration indicated an ability to maintain chlorophyll production and cell division with reduced photosynthesis.

Bednarz (1981 a) reported the sensibility of a green algae and 3 blue green algae to six herbicides and 3 other insecticides. The effect of 2, 4, D acid on green and blue green algae in unialgal and mixed cultures was reported by Bednarz (1981 b). Low concentration of 2, 4, D usually stimulated the growth of algae, but higher concentrations inhibited or stopped the growth. It was concluded that tolerant species decreased the toxicity of the herbicide in the medium thus helping the more sensitive algae to survive. The evaluation of the adaptation ability of some green algae to 2, 4, D monuron, and diuron admixtures, under laboratory conditions was also reported by Bednarz (1981 c). The results of these experiments showed that, the toxicity action of monuron, diuron, simazine, atrazine and 2, 4, D upon Ankistrodesmus Chlorella, Dictyosphaerum Scenedesmus and Hormidium was irreversible, even if the algae were transferred to media free of these substances.

In a field study, conducted by De Noyelles et al., (1982) comparison was made between atrazine treated ponds and control ponds. Atrazine at concentrations of 20 to 500 ppb inhibited photosynthesis and both concentrations depressed phytoplankton growth in the ponds within a few days. Laboratory tests verified the effects on other species at concentrations of atrazine as low as 1 to 5 ppb.

Impact of the herbicide Magnacide - H (2 propenal) on algae was studied by Fritz Sheridan (1982). The concentration of magnacide - H to effect a 50% reduction in photosynthesis was different for 3 algal species tested for different temperatures. Study on physiological response of the blue green algae to 5 herbicides was carried out (Mehta and Hawxby 1983).

The toxicity of the herbicide stam - f - 34 (propanil) on Nostoc calcicola was reported by Pandey et al., (1984). The herbicide caused an inhibition of the nitrogen fixing capability of alga which is concentration dependent and lethal at 30 ppm. Effects of Atrazine and its degradation products, alone and in combination on phototrophic microorganisms were reported by Stratton (1984). Atrazine was significantly more toxic than its degradation products.

Meyerhoff et al., (1985) compared the chronic toxicity of tebuthiuron to an alga, Selenastrum capricornutum, a cladoceran and the fat head minnow and found that the alga was the most sensitive among the three groups.

Regarding the effect of toxicant on biochemical compounds of microalgae, Salama et al., (1985) investigated the effect of herbicide 'Amitrole' on growth, carbohydrate and nitrogeneous compounds in two blue green algae and found that lower and moderate levels i.e., upto 3.6 ppm induced more accumulation of carbohydrate.

In addition to laboratory studies, field studies were also conducted to find out the effect of atrazine, Moorhead and Kosinski (1986) reported on the effect of atrazine on the productivity of algal communities in artificial stream. Net community productivity was fairly low, indicating high respiration relative to oxygen production. Krieger and Baker (1986) also reported on the effects of herbicides on stream algal productivity and nutrient uptake. Detoxification of herbicides by blue green algae was reported by Chinnaswamy and Patel (1986).

Hersh and Crumpton (1987) conducted a study of naturally occurring atrazine tolerance to algae from different Iowa springs. The main purpose of this experiment were to develop a quick method of assessing the effects of an algal growth and also to investigate an ecologically meaningful end point for toxin growth experiments. Atrazine induced photosynthetic inhibition of Cyclotella meneghiniana (Bacillarophyta) has been reported by Millie and Hersh (1987). Mayasich (1987) also reported on the growth responses of Nannochloris oculata Droop and Phaeodactylum tricornutum Bohlin to the herbicide atrazine as influenced by light intensity and temperature in unialgal and bialgal assemblage.

algal assays were highly efficient in detection of biological potential.

Simple marine algal bioassay method has been described for short and long term studies on pesticides and industrial wastes (Walsh and Alexander, 1980). It can be used for rapid screening of a variety of substances with single species and multiple species tests which would give relative toxicities of the pollutants tested. Following this method, 96 hour $EC_{50}$ values for some pesticides and diatom Skeletonema have been reported by the above authors.

Acute static toxicity tests were conducted with six insecticides (Ambush$^R$, Bux$^R$, Dursban$^R$, Fentrifanil$^R$, Larvin$^R$ and Pydrin$^R$) and one herbicide (Borthwick et al., 1981), algal bioassays were conducted with marine algae to determine the concentration of pesticide that would inhibit population growth by 50% in 96 hour. It was found that the synthetic pyrethroids - Ambush and Pydrin were the most toxic of the seven pesticides tested.

Based on photosynthesis (oxygen evolution), $EC_{50}$ was determined from short assays of 5 minutes duration. Green algae isolated at random from an atrazine contaminated stream exhibited similar tolerance to atrazine (range of $EC_{50}$ 42-125 $\mu g/l$) compared to algae isolated at random from a non-contaminated stream (range of $EC_{50}$s : 35 - 152 $\mu g/l$) (Hersh and Crumpton, 1989).

Effects of herbicides on photosynthetic electron transport in algal systems were reported by Samuel and Bose (1987). Pyridazinone herbicides Sandoz 9785, Sandoz 9789 and Sandoz 6706, inhibited photosystem II electron transport in Chlorella protothecoides, when the herbicides were added to assay medium. The inhibitory efficiency varies with the algal species and the nature of substitution.

Characterisation of the adaptation responses of Anacystis nidulans to growth in the presence of sublethal doses of herbicide was reported by Hatfield et al., (1989) who found that the contents of accessory pigments phycocyanin increases in relation to chlorophyll.

Mishra and Pandey (1989) reported on the toxicity of three herbicides to some nitrogen fixing cyanobacteria like Nostoc Linckia, N. calcicola and Anabeana doliolum. These cyanobacteria were found to be tolerant to 2, 4, D than to Machete and Saturn. Price et al., (1989) reported that the run off from agricultural field treated with tebuthiuron above 2.24 kg/hectare concentration might adversely affect the green algal community of Playa lakes.

The primary and secondary effects of simazine and terbutryne on fresh water marsh periphyton have been studied and reported by Gurney and Robinson (1989).

The effects of the pyridinone herbicides fluirdone on the growth, pigment content and composition and photosynthetic capability of Oscillatoria agardhii Gomount were investigated by Millie et al., (1990). Fluridone

concentration ranging from 0 t o 100 ppb decreased biomass, chlorophyll a and total carotenoid contents with increasing fluridone concentration. Francois and Robinson (1990) examined the toxicity of three triazine herbicides (atrazine, Simazine and terbutryn) in unialgal batch cultures of Chlamydomonas. Among the three herbicides, Terbutryn was the greatest inhibitor of growth and $Co_2$- fixing.

Impact of an organophosphate herbicide (Glyphosate$^{(R)}$) on periphyton communities developed in experimental streams was reported by Austin et al., (1991). The addition of Glyphosate to a periphyton community appeared to have little effect on subsequent successional patterns. Abou-Waly (1991) investigated on the response of Scenedesmus sp., to three phenyl ureas, namely maloran, dicuran and patoran. Among these herbicides maloran was found to be highly toxic compared to other two. Abou Waly et al., (1991 a) conducted experiments with Atrazine and hexazinone to unialgal cultures of Anabaena flos-aquae and Selenastrun capricornutum. Reduction in growth was observed with an increase of atrazine concentration Hexazinone treated cultures of S. capricornutum had substantially recovered by 7 days after treatment.

## 2.3. FUNGICIDES:

Compared to insecticides and herbicides the literature on the effect of fungicides on microalgae is very little

Ethyl mercury phosphate was lethal to all marine phytoplankton species tested when incorporated at a level of 60 ppb in the culture media (Ukeles, 1962), Harriss et al. (1970) found that three organomercury fungicides, at less than 1 ppb, reduced growth and photosynthesis in the marine diatom Nitzchia delicatissima and also in a natural population of fresh water phytoplankton. These results indicated that at least some marine and fresh water phytoplankton speies were sensitive to organo mercury compounds at levels below those proposed for water quality standards and hence suggested that entry of such compounds into natural waters should be prevented.

Somasekhar and Sreenath (1984) reported on the effect of two fungicides on primary production in a pond ecosystem. In comparison with the control the gross production and net production was found to decrease gradually with the increase in concentration of the toxicant, reaching a zero value at 1000 ppm. The effect of organic fungicide Dithane $M_{45}$ and Cynkotox on some unicellular algae was reported by Kosawska and Falkowski (1984) Dithane $M_{45}$ depressed 50% cell multiplication and total production of chlorophyll at 0.2 to 0.6 ppm in Chlorella and Scenedesmes and at 2 ppm in Anacystis nidulans.

The effect of mercuric chloride and 'Emisan 6' on the photosynthetic efficiency of Westiellopsis prolifica was studied by Rath et al., (1985) and found that the mercury based pesticide toxicity was totally concentration dependent. At lower concentration, mercury acted as a growth regulator and at higher concentration it acted as a growth inhibitor.

Rath et al., (1986) reported the effect of the insecticide Emisan-6 on the nitrogen metabolism of the alga - W. prolifica. They concluded that the effect was totally dependent upon the dose and duration of the experiment.

## 2.4. BIOASSAY STUDIES TO DETERMINE $EC_{50}$ VALUES.

Biological assays are essential for evaluating the toxicity of chemicals and are an important tool in detecting and quantifying environmental pollutants such as persistent pesticide residues. A wide range of toxicity tests have been developed in the recent decades utilizing different organisms such as algae, crustaceans, molluscs and fishes to predict the probable effect of new chemicals and effluents on aquatic ecosystem (Sprague, 1973; Walsh et al., 1980; Reish and Oshida 1986).

It was reported that (Walsh, 1972, Hollister and Walsh 1973) green algae are very susceptible to one herbicide - atrazine. Its $EC_{50}$ value ranges from 0.06 to 0.16 mg/l (ppm). Hollister and Walsh, (1973) stated that when bioassay analyses are conducted for the effects of herbicides on marine unicellular algae, two factors are particularly important

1. The response in relation to taxonomic position and
2. The wide range of responses by individual species within a given family.

The marine unicellular algae Skeletonema costatum, Amphiprora paludosa and Phaeodactylum tricornutum were exposed to dimethoate and bayluside in laboratory bioassays (Ibrahim, 1983). There was variation

in the growth response of the species and metabolism of metabolic products Walsh (1983) reported the cell death and inhibition of population growth of marine unicellular algae by pesticides. All the pesticides caused death of cells, but significant mortality occurred only at concentrations higher than $EC_{50}$ calculated from population growth studies.

Walsh and Merrill (1984) conducted short term static tests which estimated the growth rate responses by measurements of change of biomass, chlorophyll content, cell number or fluorescence. Rates of primary production have also been estimated by measurement of oxygen evolution or uptake of $^{14}C$.

The 96 hour $EC_{50}$ limits of five species of diatoms to the commonly used organochlorine pesticides, DDT and Heptachlor was studied in laboratory cultures by Rajaretnam et al., (1987). The impact of pesticides on the diatom was measured in terms of population growth, chlorophyll-a content and carbon content. Statistical characterization of atrazine (herbicide) induced phtosynthetic inhibition of Cyclotella meneghiniana was reported by Millie and Hersh, 1987).

In the static algal toxicity tests, toxicants are usually added to cultures with low numbers of algae and population growth is measured over a period of time; often 96 hour. Wright (1978) compared the toxicity of Atrazine herbicide on fresh water algae to other triazine herbicides. Cain et al. (1979) compared results of fresh water algal tests to chemical analyses of effluent from a sewage treatment plant and concluded that

Toxicity testing with fresh water algae in river Periyar in Kerala was reported by Joy (1990). The effect of effluent from a fertilizer factory was studied on two species of unicellular algae to predict the probable effect of continued discharge of this complex waste on the microphytic flora. $EC_{50}$ values of the effluent on growth of Nitzchia palea and Oocystis pusilla were reported.

The acute toxicity of commercial herbicide, Paraquat was determined by 96 hour static bioassay on the fresh water chlorophytes (Ibrahim, 1990). The 96 hour $EC_{50}$ values of Paraquat for reducing growth and metabolic products of three algae were determined. The three algae and their test parameters responded differentially to Paraquat and it was observered that Paraquat has inhibitory effect on the primary producers.

Abou Waly et al., (1991 a) reported the growth responses of fresh water algae Anabeana flos-aquae and Salenastrum capricornutum to Atrazine and Hexazinone. The $EC_{50}$ values were calculated for each herbicide at 3, 5 and 7 days after treatment. The $EC_{50}$ values generally increased with time.

## 2.5. BIOACCUMULATION STUDIES:

The introduction of gas chromatography in 1952 for residue analysis made it possible to detect and determine the concentration of organochlorine compounds in very small quantities. The presence of many pesticides can be detected at the parts per trillion level. Residues of pesticides occur in biological and physical components of coastal and oceanic environments and some of the residues have been implicated in degradation of

portions of these environments. Analysis of pesticide residues indicates that pesticides can reach non-target organisms in the environment and give indication of biological reservoirs of pesticides in the environments.

Activated by Rachel Carson's 'Silent Spring', and growing public anxiety about pollution residue analysts have carried out a large number of surveys since 1960.

Some algal species show a marked capacity to concentrate DDT from the surrounding medium, although the degree of accumulation varies with DDT concentration and the algal species (Sodergren, 1968; Vance Drummond, 1969; Keil and Priester, 1969; Cox, 1970; Rice and Sikka, 1973 b).

Sodergren (1968) studied the mechanism of DDT uptake by Chlorella sp., and found that $^{14}C$ DDT at a concentration of 0.6 ppb was rapidly taken up i.e., within 15 seconds by Chlorella cells. The author concluded that the rate of penetration of DDT into algal cells was probably equal to its rate of diffusion in water and noted that DDT accumulation in the algae in continuous culture induced morphological changes and cell clumping.

Vance and Drummond (1969) incorporated higher pp-DDT concentration upto 20 ppm in cultures of green and blue green algae. The algae were generally quite resistant to the toxic effects, eventhough DDT was concentrated at least 100 fold from the medium. They also degraded the pesticide to a slight extent. These authors considered that whereas

algae are generally more resistant than higher members of the food chain to the effects of chlorinated pesticides, they are very efficient potentiators of pesticide residues within the food web. Christie (1969) reported the uptake of DDT, Sevin and malathion on algal populations. Results showed that degradation of $^{14}C$ DDT occurred only in the presence of live algae. Uptake of $^{14}C$ malathion (100 ppm) was reported by axenic cultures of Chlorella pyrenoidosa. This species also took up $^{14}C$ Sevin Carbaryl from the medium, but the compound was not altered appreciably by the alga in acidic medium and above 0.1 ppm inhibited the growth (Christie, 1969).

Keil and Priester (1969) reported that the marine diatom Cylindrotheca closterium concentrated DDT 190 fold from a medium containing 0.1 ppm of insecticide. In view of their tendency for intracellular storage of oil, it was considered possible that diatoms might serve as "pick-up" organisms for oil soluble pesticides, which might then be detoxified (Keil and Priester, 1969). Like DDT, its metabolite pp-DDE was accumulated to a high degree by Chlorella cells, only a small part of added DDE remaining in the aqueous medium (Sodergren, 1971).

Regarding the uptake of pesticides from the culture medium, Krikwood and Fletcher (1970) reported the absorption of MCPB by a unicellular alga to a greater extent than MCPA. Greatest uptake occurred in Chlamydomonas globosa an alga of relatively large size and thin cell wall. They concluded that the uptake of herbicide was optimal at pH values favouring their movement as undissociated molecules.

It was reported that DDT residues in marine phytoplankton increased from the year 1955 to 1969 (Cox, 1970). DDT and its metabolites were noticed in all the samples analysed.

Six species of axenic marine algae, representing four taxonomic division, rapidly took up $^{14}C$ dieldrin but formed no detectable metabolites of the insecticide (Rice and Sikka, 1973 b). This indicated, as had been suggested by Menzel et al., (1970), that the algae might either incorporate dieldrin as small particles or that saturation was maintained while the algae concentrated the pesticide from solution. Total dieldrin uptake increased with increasing cell concentration (Rice and Sikka, 1973 b), but uptake was not correlated with number of cells per unit mass. However Rice and Sikka (1973 b) did not detect any algal metabolites of dieldrin. Photodieldrin has been identified as product of $^{14}C$ dieldrin in some microbial cultures isolated from dieldrin contaminated lake water (Matsumura et al., 1970).

Uptake of methoxychlor (methoxy-DDT) by actively growing green algae and diatoms was reported by Butler et al., (1975b). They also reported the uptake of diazinon, another phosphorothionate insecticide and acaricide by several algae. The cultures of algae removed significant amount of 2, 4, D from a medium containing 0.01 ppm 2, 4, D. But algal degradation of atrazine was not detected by Butler et al. (1975b). However, under optimal light conditions of Scenedesmus absorbed amitrole and metabolised.

Schauberger and Wildman (1977) investigated the bioconcentration of dieldrin and aldrin, both organochlorine insecticides, in fresh water blue green algae and found that there was a great difference in uptake

blue green algae and found that there was a great difference in uptake among three species. Moreover, they found that the pattern of bioconcentration ratios of dieldrin and aldrin was different among these three species However, these differences in uptake could not be related to difference in toxic effects on the alga.

Polychlorinated biphenyls are considered to be industrial organochlorines. PCB uptake by marine phytoplankton has also been reported (Harding and Phillips, 1978b) The relationship between cell density and accumulation of PCB was also investigated.

Glooschenko et al., (1979) showed that Scenedesmus quadricauda accumulated chlordane at the same rate alive or when heat killed and that bioaccumulation was essentially complete in 24 hours. These studies suggest that bioaccumulation in algae is not affected by rate of cell growth or metabolism at least for organic compounds.

Using a model laboratory ecosystem Miyamoto et al., (1979) showed that, the bioaccumulation ratio of algae was much greater than for other representative aquatic organisms for DDT and total DDT residue, which included the degradation products DDD and DDE. These investigators concluded that the bioaccumulation ratio may increase with time and algae may represent a chronic reservoir of contamination by the prolonged addition of a chemical through food chain to fish.

Adsorption of an organophosphorus insecticide 'Fenitrothion' by planktonic and benthic algae was reported by Lakshminarayana and Bourque

(1980). These studies showed that fenitrothion will be absorbed or actively taken up by plankton, particularly the phytoplankton.

In a comparative study of the uptake of the organochlorine, DDT and the organophosphate Fenvalerate by Daphnia, snails. fish and algae in a model ecosystem, Ohkawa et al. (1980) showed that algae had the highest bioaccumulation ratio for DDT and a subsequentially high ratio for Fenvalerate. The interaction of DDT with two species of fresh water algae was studied by Goulding and Ellis (1981). Both the algae, Anabaena variabilis and Chlorella fusca accumulated $^{14}C$ DDT. Neither alga significantly metabolized DDT although cells of C. fusca contained a small amount of DDE after 480 hour incubation with the insecticide.

The bioconcentration of two polychlorinated biphenyls such as Aroclor 1232 and Aroclor-1248 by live and dead cells of diatom Thalassiosira rotula has been reported (Murado et al., 1984). PCBS were concentrated in T. rotula and metabolic transformation of PCBs were also noticed.

Accumulation, degradation and biological effects of lindane on Scenedesmus obliques was reported by Lin-Yi-Xing and Sun-Bo-Zen (1987). This particular species possess certain accumulating Capacty for $\gamma$-BHC, which is higher at 1 $mgl^{-1}$ than at 10 $mgl^{-1}$.

Compared to organochlorine insecticides, organophosphates are considered to be easily degradable. The capabilities of five algal species to degrade two organophosphate insecticides, such as monocrotophos and

quinalphos have been determined (Megharaj et al., 1987). It is evident that green and blue green algae are equally potential in detoxifying these insecticides.

Dhanraj et al., (1989) reported on the bioconcentration and metabolism of aldrin and phorate two blue green alga, Anabaena sp. and Aulosira fertilissima. They showed marked ability to bioconcentrate aldrin and phorate from culture medium. Aldrin was metabolized to dieldrin by both blue green algae, but no metabolism was noticed in the case of phorate.

There are reports about the concentration of different organochlorine pesticides in the sediments of the Arabian sea and in the sediments along the east coast of India (Sarkar and Sen Gupta, 1987, 1988). Chlorinated pesticide residues in sediments from the Arabian Sea along the Central West Coast of India indicated that the residue levels of all the organo chlorine pesticides detected were in the order : Dieldrin < pp - DDD< op - DDE < pp - DDT < pp - DDE < aldrin < BHC (Sarkar and Sen Gupta, 1987). The same investigators reported on the pesticide residues in the sediments of east coast of India also (Sarkar and Sen Gupta, 1988) and they reported that apart from DDT and its isomers, residues of gamma-BHC, aldrin and dieldrin were recorded from a number of places. Their concentration values are high mainly in river mouths which indicate that these pesticides are of land origin.

# CHAPTER III

# MATERIALS AND METHODS

## 3.1 ORGANISMS AND THEIR MAINTENANCE

The microalgae selected for the present study were two unicellular algae and a mixture of microalgae. The two unicellular algae were:

1. Tetraselmis gracilis
2. Dicrateria inornata

Both the strains are from CMFRI Algology Laboratory, and are used as live food in aquaculture practices. Taxonomic position of the selected species are as follows:

Tetraselmis gracilis

| | | |
|---|---|---|
| Class | - | Prasinophyceae |
| Order | - | Prasinocladales |
| Family | - | Prasinocladaceae |

Dicrateria inornata

| | | |
|---|---|---|
| Class | - | Haptophyceae |
| Order | - | Isochrysidales |
| Family | - | Gephyrocapsaceae |

3. Mixture of Microalgae

Microalgal samples were collected from different paddy fields, and they were enriched with nutrients and allowed to grow in the laboratory, under culture conditions. This was maintained as mixed culture of freshwater

microalgae in the laboratory and it was dominated by Chlorophycean members. Important strains in this culture were:

1. Chlorella ovalis - Chlorophyceae
2. Scenedesmus indicus - Chlorophyceae
3. Selenastrum gracile - Chlorophyceae
4. Nitzchia longissima - Bacillarophyceae

3.1.1. Culture condition:

Sea water medium: Prior to preparation of the culture medium the sea water collected from offshore was allowed to age in carbuoys. Further sea water was filtered through Sartorius filter paper, and then boiled. After boiling upto 100° C, it was allowed to cool down one overnight. The cool sterilized sea water was then enriched with the proper nutrient medium, and this medium was transferred to sterilized culture flasks. However, the cultures were not bacteria free.

Fresh water: For culturing mixed culture of fresh water microalgae, the freshwater was collected from non-contaminated ponds, filtered and sterilized As in the case of sea water, the cool sterilized fresh water was then enriched with the nutrients and transferred to culture flasks.

3.1.2. Culture media:

The important culture media (Gopinathan, 1982) used for nanoplankton flagellate culture in the laboratory were:

1. Schreibers medium
2. Miquels medium
3. Pantastico's medium
4. Walnes or Conways medium

Among the above media, Walnes medium (Walne, 1974) was found to be the best for the growth of microalgae. In the present study, Walne's medium was selected for further experiments.

The composition of Walne's medium is as follows:

Solution A:

| | | |
|---|---|---|
| $KNO_3$ | : | 100gm |
| $Na_2 HPO_4$ | : | 20gm |
| EDTA | : | 45 gm |
| $H_3BO_3$ | : | 33.4gm |
| $Fecl_3$ | : | 1.3gm |
| $Mncl_2$ | : | 0.36gm |
| Distilled water | : | 1 litre |

Solution B:

| | | |
|---|---|---|
| $Zncl_2$ | : | 4.2gm |
| $Cocl_2$ | : | 4.0gm |
| $CuSo_4$ | : | 4.0 gm |
| Ammonium molybdate | : | 1.8gm |
| Distilled water | : | 1 litre |

Solution C:

Vitamin B1 (Thiamine ) : 200mg

Vitamin B12 (Cyanocobalamine) 100mg

Each of the chemicals was dissolved separately in 100 ml distilled water.

Solutions A, B & C were prepared in different reagent bottles. Added 1 ml of A, 0.5 ml of B and 0.1 ml of C to 1 litre of filtered and sterilized sea water.

The salinity of the sea water used to prepare the medium was 30-35 ppt, for culture of Dicrateria inornata, whereas Tetraselmis gracilis was grown at 20-25 ppt.

Freshwater culture media:

Modified medium of Ward and Parrish (1982) was used for culturing the fresh water microalgae.

1. Macronutrients:

| | | |
|---|---|---|
| $NaNo_3$ | : | 6.37g |
| $Mgcl_2.6H2O$ | : | 3.05g |
| $MgSO_4.7H_2O$ | : | 3.675g |
| $Cacl_2.7H_2O$ | : | 1.1g |
| $K_2HPO_4$ | : | 0.261g |
| Distilled water | : | 1 litre |

2. Micronutrients

| | | | |
|---|---|---|---|
| 1. | $CoCl_2$ | : | 0.078g/100 ml |
| | $Cucl_2$ | : | 0.9g/100ml |

Dilute 1 ml of each to 1 litre for working stock solution.

| | | | |
|---|---|---|---|
| 2. | $H_3BO_3$ | : | 0.185g |
| | $MnCl_2$ | : | 0.264g |
| | $FeCl_3$ | : | 0.096g |
| | $Na_3$ EDTA | : | 0.3g |
| | Distilled water | : | 1 litre |

To this add 1 ml of each micronutrients - stock solution

| | | | |
|---|---|---|---|
| 3. | $Na_2Sio_3$ | : | 1.2g |
| | Distilled water | : | 100ml |

The maintenance and test medium was prepared by adding 4 ml of macronutrient solution and 1 ml of micronutrient stock solution and 1 ml of silicate solution to 1 litre of sterilized fresh water.

The stock culture and experiment cultures were maintained in 'corning' conical flasks plugged with sterilized cotton. They were maintained in the exponential phase. The stock cultures were used to reculture every 14 days in the case of marine species, and every 7 days in the case of fresh water mixed culture. All stock and test cultures were illuminated with day light flourescent tubes. The light : dark period within the chamber was 10 : 14 hours with a mean light intensity of $34.61 \times 10^{15}$ quanta x $cm^{-2} \times sec^{-1}$. The ambient temperature ranged from $27^{\circ}$ C to $32^{\circ}$ C.

Aeration was not provided to the cultures. Instead the cultures 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 in the case of marine species. But freshwater species showed a tendency to settle down after one week. Experimental set up was as shown in the photograph (Plate 1).

## 3.2. BIOCIDES

Biocides used for the present study come under three categories:

1. Insecticides : 3 Nos
2. Herbicides : 1 No
3. Fungicides : 1 No.

In the addition to these three groups two mixtures of biocides were also used. They are:

1. Mixture of all the five biocides
2. Mixture of one insecticide, one herbicide and one fungicide.

### 1. Insecticides:

There are three important group of insecticides. They are:

1. Organochlorines
2. Organophosphates
3. Carbamates

## PLATE 1

a) Experimental set up.

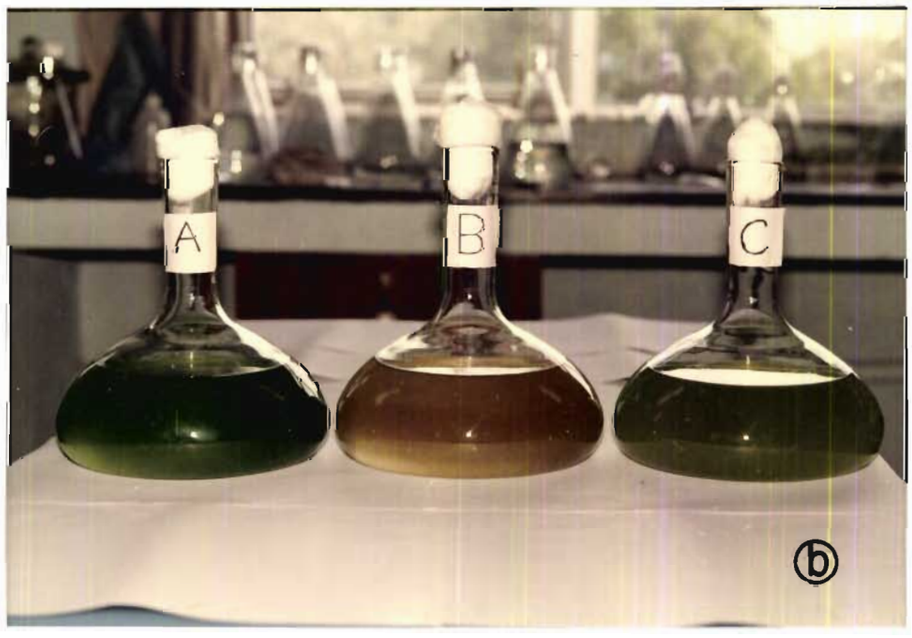
b) Stock culture

A. Tetraselmis gracilis

B. Dicrateria inornata

C. Mixed culture of freshwater algae.


a


A
B
C
b

One insecticide from each group was used for the present study. 'BHC' as organochlorine, 'Nuvacron' as organophosphate and 'Carbaryl sevin' as carbamate insectcide. The Herbicide used was 'Gramaxone', and the fungicide was 'Cuman L'(R). These five biocides were selected for the present study were purchased from the agrochemical shops. All of them were commercial grade. The detailed description about the biocides are as follows:

1. 'BHC': Benzene hexa chloride:- Gamma isomer - (Lindane) - commercial grade BHC 50% chemical name - Hexachlorocyclohexane.

2. 'Nuvacron' (Organophosphate) - Monocrotophos 36% SL - Commercial grade.

Insecticide with systemic and contact action based on Monocrotophos. Chemical name - Dimethyl Phosphate of 3-hydroxy-N-methyl-cis-crotonamide.

A water soluble organophosphorous concentrate containing 360 gms monocrotophos as active ingredient in a kg. of product.

3. 'Carbaryl sevin': Carbamate insecticide - 50% W.D.P. Commercial grade.

Composition: : W/W

Carbaryl (A.I.) : 50%

Adjcevants & carriers : 50%

Chemical Name - 1-Naphthyl N-methyl carbamate

2. Herbicide:

4. 'Gramaxone': Paraquat dichloride 24% WSC commercial grade.

| Composition: | | W/W |
|---|---|---|
| Paraquat dichloride | : | 24 |
| Other ingredients | : | 76 |

Chemical name - 1-1-Dimethyl-4-4-bipyridilium dichloride

5. Fungicide: 'Cuman L'(R). Ziram 27% SC Commercial grade

An organic collodial liquid fungicide containing 270gms zinram (zinc dimethyl dithiocarbonate) as active ingredient in a kg of product. Chemical name - Zinc dimethyl dithiocarbonate

## 3.3. GROWTH MEASUREMENTS

### 3.3.1. Measurements of cell concentration:

The cells were fixed in Lugol's iodine solution and counted with a carlibrated Haemocytometer (improved, Neabaur, 0.1 mm deep, fein optic, made in GDR). Four counts were made from each sample to ensure counting accuracy and then mean value was taken.

### 3.3.2. Productivity measurements:

Productivity measurements were made using light and dark bottle oxygen method (Gaarder and Gran, 1927). From each experimental flask 3 samples - initial, light and dark - were analysed for dissolved $O_2$ content

using Winklers method. The oxygen values were then converted into their carbon equivalents.

Productivity as determined by using the formula

$$mgc/l/hr = \frac{O_2 ml \times 0.536}{PQ \times T}$$

The PQ (Photo synthetic quotient) is 1.25

Gross production, net production and Respiration were calculated using this method (Strickland and Parsons, 1968).

3.3.3. Determination of quantitative variation of algal pigments by Spectrophotometry

The quantity of pigments was also used as an index of physiological activity. The concentrations of chlorophylls and carotenoids were estimated by Spectrophotometric (Spectronic 1001) analysis of pigment extract. For marine species the pigment values were estimated as described by Parsons et al., (1984). For the estimation of fresh water pigments the method followed by Sartory and Grobbelaar (1984) was used.

A known volume of the culture was filtered through Millipore HA filters of pore size 0.4 /u. One or two drops of $MgCo_3$ were added to the sample, while filtering, to prevent acidification. The pigments were extracted by adding 10 ml of 90% acetone to each filter paper. The extraction was carried out at low temperature for 20 hours. The extracts were centrifuged extinction of the clear solution was measured by Spectronic otometer.

For the estimation of fresh water phytoplankton pigments hot methanol was used instead of acetone (Sartory and Grobbelaar 1984).

The absorbance of the clear pigment extract was measured against the blank at different wavelengths such as 750, 664, 647, 630, 510 and 480 n.m. Concentration of various pigments (chlorophylls and carotenoids) were then calculated using the equation given by Parson's et al., (1984).

3.3.4. Biochemical compounds:

1. Protein (Dorsey et al., 1978).

Algal cells were collected after centrifugation of the culture and the algal pellet was washed with an isotonic solution of Ammonium molybdate The protein was extracted with appropriate reagents at 100° C for 100 minutes. The concentrations and final volumes of the reagents were as published by Winkfors et al., (1984). After the heated biuret-folin colour development was complete, this colour was measured in Spectronic 1001 Spectrophotometer at 660 n.m. Protein nitrogen values were determined by interpolation from a standard curve obtained with prepared solutions of bovine serum albumin. Total protein was then calculated using a conversion factor of 6.25 generally accepted for most algal species (Dorsey et al., 1978).

ii) Carbohydrate (Kochert 1978)

Carbohydrate determinations were made using phenolsulphuric acid method for analysis of algae as reported by Kochert (1978), based on procedures developed by Dubois et al., (1956).

Algal culture samples were collected and washed with isotonic solution by repeated centrifugation. Cells to be analysed were then homogenized in 1 ml 80% sulphuric acid as in Myklestad and Haug (1972), and the total amount of carbohydrate in the solution was measured by the phenol sulphuric acid method using glucose as standard.

## 3.4. ALGAL BIOASSAY PROCEDURE

Each species of microalgae was exposed to the pesticides in various concentration to study the effect of pesticides on each microalgal species. Short term bioassay test was almost always of the static type (Reish and Oshida, 1986).

Initially a wide range of pesticide concentrations were used to determine the effect on the microalgae by using the method - range finding test, as outlined by Ward and Parrish (1982) and Reish and Oshida (1986). Using the results of range finding test, pesticide concentrations of the narrow range were prepared and its effect on each microalgal species was determined using - Definitive test (APHA, 1980).

All the tests were conducted as follows: clean, sterilized 500 ml conical flasks were filled with 200 ml of culture medium. Then the required strength of pesticide was added to the medium by using micropipett. While transferring the pesticide solution, the pipetts were rinsed with the pesticide solution of particular conentration prior to delivery in order to obtain good reproducible aliquots. After the pesticide concentration was added, known number of microalgal cells were inoculated from

a stock culture of exponential growth phase. The contents were hand shaken and incubated. Each concentration was established in triplicate. Control was also maintained of the same volume used for the test. All the cultures were periodically hand shaken to keep the cells in suspension.

## 3.5. BIOACCUMULATION STUDIES:

Only one insecticide - organochlorine, 'BHC' was selected for the study of bioaccumulation by the microalgae, - Tetraselmis gracilis. The method followed by Schauberger and Wildman (1977) was adopted to find out the bioaccumulation of the organochlorine insecticide.

The alga was harvested by centrifugation, lyophilized to ensure uniformity among samples and extracted 3 times with 2.5ml acetonitrile. An equal volume of 2% aqueous $Na_2So_4$ was added to the collected supernatant. The mixture was extracted 3 times with 2 ml hexane. The extracts were evaporated to 1 ml under nitrogen.

Clean up procedure:

A glass column of size 200 mm x 14 mm was packed with florisil and anhydrous $Na_2SO_4$ (1 : 1). After elution with 200 ml 6% ethyl ether/ petroleum ether (v/v), and then with 200 ml 15% ethyl ether/petroleum ether (v/v), the elute was evaporated to 1 ml on a steam bath.

Chromotographic analysis was performed on a Chemito-8510 Gas chromotograph equipped with 1 bar stainless steel column packed with 5% SE 30, and Ni 63 as electron capture detector and nitrogen as carrier gas

The operating temperatures for the various components of the gas chromatogram were as follows:

| | |
|---|---|
| Oven temperature | $200^{o}$ C |
| Injector temperature | $230^{o}$ C |
| Detector temperature | $250^{o}$ C |

Thre bioaccumulation of the organochlorine pesticide was find out using the formula given by AOAC (1984).

$$\text{Each residue ppm} = \text{Conc.of Std.} (\mu g/ml) \times \frac{\text{Peak size of sample}}{\text{Peak size of standard}} \times \frac{\mu L \text{ Std}}{\mu L \text{ sample}} \times \frac{\text{Dilution volume}}{\text{Weight of sample}}$$

## 3.6. PHOTOMICROGRAPHY

In order to understand the morphological deformities of microalgal cells as a result of biocide treatment, photomicrographs were taken. The photomicrographs of the algal cells which were fixed in 50% glycerine, were taken on Olympus Universal Research Microscope (Vonvox model P.M. 10 A.D.) equipped with an automatic exposure system. Kodak colour film was used for taking photographs (Kodak colour 35 mm 100 ASA).

## 3.7. STATISTICAL ANALYSIS

The effective concentration of biocide that would inhibit the growth of test algae by 50% as compared to control growth as $EC_{50}$ and was calculated by probit procedure using computer (SAS Institute, 1985). The advantage of probit method is that it provides a more reliable statistical

treatment of data (Reish and Oshida. 1986).

In order to understand the effect of various concentrations of different biocides and their mixture on three microalgal culture, two way analysis of variance (ANOVA) technique was employed (Snedecor and Cochran, 1967) and the F value was taken at 5% and 1% significant level. In Anova Tables, different concentrations of toxicant were considered as treatments, and between days as replicates.

# CHAPTER IV

# RESULTS AND DISCUSSION

## 4.1. RESULTS OF 96 HOUR BIOASSAY TESTS - $EC_{50}$ VALUES OF 3 BIOCIDES FOR 2 SPECIES OF MICROALGAE IN CULTURE

### I. Organophosphate insecticide - 'Nuvacron'

a) Population growth

Table 1 shows that the population growth of Tetraselmis gracilis and Dicrateria inornata decreased with increasing concentration of 'Nuvacron', while low concentration enhanced their growth.

In the case of T. gracilis after 24 hours of growth in 25 and 50 ppm concentration of 'Nuvacron' showed an increase of 5.9% and 2.2% over control, and when it reached 125 ppm, a reduction of 78.26% growth was observed. The $EC_{50}$ value was 94.4 ppm. After 48 hours of growth the $EC_{50}$ value was 75.5 ppm. After 72 hours of growth, the $EC_{50}$ value further decreased and reached 74.9 ppm. After 96 hours of growth the $EC_{50}$ value remained static at 74.99 ppm.

Dicrateria inornata also showed enhanced growth over control at 25 and 50 ppm, throughout the 96 hour period. On a population growth basis, the $EC_{50}$ values calculated were, 79.43, 81.28, 74.99 and 74.13 ppm for 24, 48, 72 and 96 hour respectively.

b) Net carbon production

Table 2 shows the net carbon production at different concentrations of 'Nuvacron' for T. gracilis and D inornata. As in the case of population growth, the carbon production was also found stimulated at the lower concentration of 25 ppm 'Nuvacron' in T. gracilis and D. inornata.

T. gracilis showed an increase by about 6.34% carbon production over control at 25 ppm after 24 hour growth. At 125 ppm concentration of 'Nuvacron', this species showed no carbon production, after 24 hour. But afterwards the same concentration showed low values of carbon production. The $EC_{50}$ values calculated were 74.98, 75.85, 68.39 and 69.43 ppm for 24, 48, 72 and 96 hour respectively.

In the case of D. inornata, 25 ppm concentration showed an increase in carbon production in all the 4 days. But the percentage increase was varying. At 125 ppm concentration this species did not show any carbon production upto 48 hour. On net carbon production basis, $EC_{50}$ values of 'Nuvacron' were 54.33, 61.66, 66.07 and 68.39 ppm for 24, 48, 72 and 96 hour respectively.

c) Pigment production

In 25 ppm concentration of Nuvacron T. gracilis showed a little stimulation of only 3.6%, increase in chlorophll a production. In the case of chlorophyll b 9.4% increase was observed at 25 ppm concentration. But in the case of D. inornata under all concentrations tested, the 'Nuvacron' lowered the chlorophyll content (Table 3 and 4a).

$EC_{50}$ values for inhibition of chlorophyll a content by 'Nuvacron' were 91.2 and 62.66 ppm for T. gracilis and D. inornata respectively. This result shows that D. inornata is more sensitive compared to T. gracilis. For chlorophyll b, which is specific for T. gracilis, the $EC_{50}$ value was 89.13 ppm, showing that chlorophyll b was more sensitive to 'Nuvacron' than chlorophyll a in T. gracilis. For chlorophyll c, which is an accessory pigment of D. inornata, the $EC_{50}$ calculated was 45.71 ppm.

II. **Herbicide - 'Gramaxone'**

a) Population growth

Table 5 shows that the population growth of T. gracilis and D. inornata decreased with increasing concentration of 'Gramaxone', while low concentration of about 0.1 ppm enhanced their growth.

A concentration of 0.1 ppm of 'Gramaxone' showed an increase by 13.68% in the growth of T. gracilis during a period 24 hours. After 48 hour, the percentage stimulation decreased reaching 4.21%, and after 72 and 96 hour no stimulation in growth was observed. $EC_{50}$ values calculated were 0.987, 0.904, 0.707 and 0.841 ppm for 24, 48, 72 and 96 hour respectively.

D. inornata showed an increase of about 5.05% in its growth at 0.1 ppm after 24 hour. But after 48 hours the percentage stimulation decreased as in the case of T. gracilis. But unlike the case of T. gracilis, the particular species showed that EC50 values decreased with the number of days of growth. The $EC_{50}$ values of 'Gramaxone' were 0.799, 0.72, 0.564 and

and 0.443 ppm for 24, 48, 72 and 96 hours respectively.

b) Net carbon production

Table 6 shows the effect of different concentrations of 'Gramaxone' on net carbon production by the two species of microalgae.

T. gracilis showed an increase in carbon production by about 18.22% over control at 0.1 ppm concentration of 'Gramaxone' after 24 hours of growth. After this period no increase in production was observed. The same species did not show any carbon production at 1.6 ppm concentration after 24 hour period, but afterwards very little production was observed in this particular concentration. The $EC_{50}$ values calculated for net carbon production were 0.756, 0.432, 0.383, 0.441 ppm for 24, 48, 72 and 96 hour respectively.

In the case of D. inornata about 30.77% increase in carbon production was observed at 0.1 ppm concentration after 24 hours of growth. But at 1.6 ppm concentration no carbon production was observed. The $EC_{50}$ values calculated for net carbon production were 0.423, 0.305, 0.335 and 0.255 ppm for 24, 48, 72 and 96 hours respectively.

c) Pigment production

The 'Gramaxone' at 0.1 ppm concentration stimulated the pigment production of T. gracilis upto 4.4% over control. But other concentrations tested showed, no stimulation in pigment production (Table 3 and 4b).

The 96 hour $EC_{50}$ values calculated for chlorophyll a were 0.510 ppm for T. gracilis and 0.518 ppm for D. inornata. It showed that almost the same concentration of 'Gramaxone' caused 50% inhibition of chlorophyll a production in the two species of microalgae (Table 3a). In the case of D. inornata unlike in the case of T. gracilis no stimulation of pigment production was observed. It was found that the pigment production was observed. It was found that the pigment production decreased as the toxicant concentration was increased.

For T. gracilis, the 96 hour $EC_{50}$ value calculated was 0.494 ppm for chlorophyll b. In the case of D. inornata the $EC_{50}$ value for chlorophyll c production was 0.395 ppm

III. **Fungicide - 'Cuman' L(R).**

a) Population growth:

Table 7 shows the effect of various concentrations of fungicide on population growth of two species of microalgae.

In the case of T. gracilis, 0.005 ppm concentration caused a stimulation in growth by 13.27% over control after 24 hour period. After that no stimulation of growth was observed for any concentration of fungicide tested. There was a gradual decrease in the $EC_{50}$ values with time. i.e., 0.045, 0.024, 0.02, 0.02 ppm for 24, 48, 72 and 96 hour respectively.

D. inornata showed no stimulation of growth even at very low concentrations of fungicide. The rate of reduction of growth increased with increasing concentration of fungicide. The $EC_{50}$ values calculated were 0.026, 0.022, 0.018, and 0.016 ppm for 24, 48, 72 and 96 hour respectively.

b) Net Carbon production

Table 8 shows the effect of fungicide on carbon production of two species of microalgae.

T. gracilis showed an increase in carbon production at 0.005 ppm concentration of fungicide. After 48 hours, the increase was about 27.3% But at 0.08 ppm concentration no carbon production was observed. The $EC_{50}$ values calculated were 0.018, 0.018, 0.015 and 0.016 ppm for 24, 48, 72 and 96 hours respectively.

D. inornata showed that under all concentrations tested, 'Cuman L.(R)' lowered the carbon production. At 0.04 ppm no carbon production was observed after 24 hours of the experiment. Afterwards low values for carbon production was observed at 0.04 ppm concentration. But for 0.08 ppm concentration no carbon production was observed throughout the 96 hour period. The $EC_{50}$ values calculated were 0.005, 0.007, 0.007 and 0.009 ppm for 24, 48, 72 and 96 hour respectively.

c) Pigment production:

Table 3, 4a and b shows the effect of fungicide 'Cuman L(R) on the pigment production in the two species of microalgae.

T. gracilis at 0.005 ppm concentration showed an increase of 11.39% pigment production over control after 96 hours of growth. For all other concentrations tested, a gradual devcrease in production was observed. The 96 hour $EC_{50}$ values calculated for chlorophyll a were 0.021 ppm for T. gracilis and 0.006 ppm for D. inornata (Table 3).

The 96 hour $EC_{50}$ value of Cuman L(R) for chlorophyll b production in T. gracilis was 0.025 ppm (Table 4a). The 96 hour $EC_{50}$ values of Cuman L(R) for Chlorophyll c production in D. inornata was 0.007 ppm (Table 4b).

Comparison of $EC_{50}$ values calculated from population growth and net carbon production

Table 9 gives a comparison of $EC_{50}$ values calculated from population growth and net carbon production between the two species of microalgae.

In the case of 'Nuvacron' the $EC_{50}$ value calculated for growth was higher than that calculated for production studies. It shows that production was the sensitive parameter compared to growth.

With the herbicide 'Gramaxone', the $EC_{50}$ values calculated for growth, and net carbon production was found varying. The concentration of 'Gramaxone' that inhibited 50% growth decreased upto 72 hour period, but subsequently it showed an increasing trend. The same was the case with production also. The 96 hour $EC_{50}$ value calculated for population growth was 0.841 ppm and that for net carbon production was 0.441 ppm, showing that the production was affected much more than population growth.

But coming to Fungicide 'Cuman L$^{(R)}$', the population growth studies showed that there was a gradual decrease in the $EC_{50}$ values from 0.045 ppm to 0.02 ppm (24 hour to 96 hour). The $EC_{50}$ values calculated for production was found to be lower than that calculated for population growth.

The microalgae D. inornata showed that $EC_{50}$ value calculated for growth was higher than that calculated for production studies with the insecticide 'Nuvacron'. The $EC_{50}$ value calculated for net carbon production was found to be increase with age of culture i.e. from 54.33 ppm (24 hour) to 68.39 ppm (96 hour).

The $EC_{50}$ values calculated for 'Gramaxone' was found to decrease with the age of culture, i.e. from 0.799 ppm for 24 hour to 0.443 ppm for 96 hour. The $EC_{50}$ values obtained for production studies was less than that was obtained for population growth, the respective values being 0.255 ppm and 0.443 ppm.

With the fungicide 'Cuman L$^{(R)}$', the particular concentration which inhibited the growth of the microalgae D. inornata to about 50% was found to decrease with the age of culture, i.e., from 0.026 for 24 hour to 0.016 for 96 hour. But during production studies it was found to increase with the age of culture i.e. from 0.005 to 0.009 ppm. In this case also $EC_{50}$ value calculated for production studies was less than that calculated for population growth.

These data revealed that in almost all cases production was found to be the sensitive parameter compared to population growth

96 hour $EC_{50}$ values of three biocides - for growth, production and chlorophyll 'a' of two species of microalgae:

Table 10 shows, the 96 hour $EC_{50}$ values of three biocides for three parameters in the two species of microalgae. It was observed that for each biocide tested the 96 hour $EC_{50}$ values were higher with respect to T. gracilis showing that T. gracilis was the resistant species than D. inornata. 'Nuvacron' at 74.99 ppm reduced 50% growth in T. gracilis, but in D. inornata 50% growth inhibition took place in 74. 13 ppm. Same was the case with production and chlorophyll. In T. gracilis 50% reduction in carbon production was observed at 69.43 ppm but $EC_{50}$ value for the depression of Chlorophyll a was 91.2 ppm. In the case of D. inornata $EC_{50}$ value for production was 68.39 ppm and that for chlorophyll a was 62.66 ppm.

For the herbicide 'Gramaxone', 96 hour $EC_{50}$ values alculated for three parameters of growth, production and chlorophyll a content respectively for T. gracilis were 0.841, 0.441 and 0.51 ppm. But for D. inornata it was 0.443, 0.255, and 0.518 ppm for growth, production and chlorophyll a content respectively.

For fungicide 'Cuman L.$^{(R)}$', the 96 hour $EC_{50}$ values calculated for three parameters of growth, production and chlorophyll a, T. gracilis showing that production was the sensitive parameter, and chlorophyll a

was found to be resistant among the three. But, for D. inornata it were 0.016, 0.009 and 0.006 ppm for growth, production and chlorophyll a content showing chlorophyll a as the most sensitive, and growth the most resistant parameter among the three tested.

## DISCUSSION

Short term static type of bioassay experiments on microalgae are of much value in assessing their response to various concentration of biocides. Such experiments will provide first hand information concerning health status of an aquatic environment under stress from pollution. To understand the pesticide effect precisely and for the assessment of toxicity, Walsh and Alexander (1980), Walsh and Merril (1984), Ibrahim (1983, 1990). Rajaretnam et al. (1987) have suggested the use of more than one parameter in algal bioassay tests.

The three biocides tested here, i.e, 'Nuvacron', 'Gramaxone' and 'Cuman L'$^{(R)}$, affected growth, carbon production and pigment content of two species of microalgae. The results showed that the growth of T. gracilis and D. inornata was stimulated in low concentration of biocides except for fungicide with D. inornata. Literature on algal stimulation by the toxicants are scanty. Ibrahim (1983), showed that low concentrations of pesticides stimulated growth and metabolic products of Phaeodactylum tricornutum, a phenomenon that was reversed at high concentration. Rajaretnam et al., (1987) observed that the growth of Chaetoceros didynus and Thalassiosira nitzchoides was stimulated in low concentration of DDT and Heptachlor. The capacity of certain toxicant to stimulate the algal

growth leads to the assumption that the microalgal species investigated here, particularly T. gracilis had the ability to metabolize the toxicants and the resulting products or metabolites may act as some kind of nutrient for the growth of algae. This confirms the findings of Butler et al.. (1975a) who speculated that certain algal strains might metabolize the pesticides. Rath and Misra (1981) also report that algae could utilize the elements of insecticides or its biodegraded metabolites.

Population growth inhibition is a reasonable determinant of toxic affects (Fisher and Frood, 1980). In the present study, the growth of the two microalgal species tested was inhibited with the increase of toxicant concentration. Eventhough some growth stimulation was apparent in low concentration of biocides, higher concentrations invariably affected the growth of the microalgae.

The data obtained reveal that the herbicide - 'Gramaxone' and fungicide 'Cuman L.$^{(R)}$ were more toxic than, the organophosphate insecticide - 'Nuvacron'. This is in confirmatory with the findings of Ukeles (1962) in that, among the wide range of pesticides screened, organophosphorus compounds were found to be relatively non-toxic to marine phytoplankton. Christie (1969) also observed that 100 ppm 'Malathion' - an organophosphate insecticide had little significant effect on the green alga.

Carbon production which is another parameter tested here, varied with increasing concentration of biocides tested. With some exceptions the production was severely affected than the population growth of the two species of microalgae (Table 8). As in the case of population growth

studies, the insecticide 'Nuvacron' was found to be less toxic compared to 'Gramaxone' and 'Cuman L.(R)'. 'Cuman L.(R), the fungicide, was found to be the most toxic biocide tested. 'Cuman L.(R) at 0.016 ppm for T. gracilis and 0.009 ppm for D. inornata caused 50% reduction in carbon production after 96 hour period. Eventhough there are reports about algal bioassays, almost all investigators have reported on the parameters like growth, pigment content, protein content etc. (Rajaretnam et al., 1987, Ibrahim, 1983, 1990). Reports about the effect of toxicants on carbon production are very rare. Walsh and Merril (1984) made observations of rate of primary production in bioassay studies. Hersh and Crumpton (1989) reported $EC_{50}$ values based on photosynthesis - oxygen evolution.

In bioassay studies the amount of pigment content of test organism has been an important parameter. Chlorophyll a, b and c content in biocide treated cultures was lower than in controls except for T. gracilis. In T. gracilis the lower concentration of the three biocides tested showed an increase in pigment production. Similar results have been reported by Francois and Robinson (1990) who observed slight stimulation of chlorophyll at the lowest concentration of the herbicide tested. They concluded that strong stimulation in chlorophyll synthesis which was observed with herbicide exposure was a tolerance mechanism.

But for D. inornata all the concentrations of toxicants tested showed decrease in chlorophyll values. The percentage inhibition exhibited by chlorophyll was slightly higher than that exhibited by population growth in the

particular biocide concentration. The low concentration of 'Nuvacron' and 'Gramaxone' stimulated the growth rate of D. inornata, but the pigment estimation showed inhibited response. This was mainly due to the reduced concentration of chlorophyll content per cell in treated cells. This confirms the finding of Rajaretnam et al., (1987).

Bioassay tests with herbicides using microalgae have been already reported (Walsh, 1972, Hollister and Walsh 1973). Green algae are very suspectible to the herbicide-atrazine. Its $EC_{50}$ values ranged from 0.06 to 0.16 mg/l (ppm)

The herbicide 'Gramaxone' (Paraquat) which has been used in the present investigations was already tested with chlorophytes and the results reported by Ibrahim (1990). The 96 hour $EC_{50}$ values of Paraquat were determined for growth and metabolic products in the three algae. The 96 hour $EC_{50}$ values calculated for each species were low compared to the values reported here. That may be because of two reasons. One reason is that the species is different. Another reason is that 'Gramaxone' used was a local product. Ibrahim (1990) used the Gamaxone that contained 40% paraquat and 10% of a mixture of two detergents Lissapol NX and DS 4392 or Ethomene 525. The 'Gramaxone' used in the present studies was a commercial grade purchased from a local agrochemical shop and it contained 24% water soluble concentrate and 76% other ingredients.

Among the three biocides tested here, the fungicide 'Cuman $L^{(R)}$' was found to be the most toxic one. Bioassay tests with fungicides using microalgae are very rarely conducted. However, there are a few reports

about the effect of fungicide on microalgae (Ukeles, 1962; Harris et al.. 1970; Somasekar and Sreenath (1984). Kosawska and Falkowski (1984) reported that the effect of organic fungicide Dithane M45 depressed 50% cell multiplication and total inhibition of production of chlorophyll a at 0.2 ppm to 0.6 ppm in Chlorella and Scenedesmus and at 2 ppm in Anacystis nidulans.

The present study showed that the $EC_{50}$ values with respect to each biocide and algal species may vary (Table 8). Nuvacron with Tetraselmis gracilis showed that $EC_{50}$ values for growth decreased with time. But, for production estimation, the $EC_{50}$ value was not showing any definite trend. The same insecticide with D. inornata showed that with respect to growth, the $EC_{50}$ values increased with time upto 48 hour, afterwards it was decreased. But, for production, $EC_{50}$ value increased upto 96 hour.

'Gramaxone', the herbicide tested here with T. gracilis showed that $EC_{50}$ value decreased with time upto 72 hr and then increased after 696 hour. The same was the case with production also. But D. inornata with the same herbicide showed that $EC_{50}$ values decreased gradually with the age of culture upto 96 hour of growth. The same species for production estimation did not show any gradual decrease.

With the fungicide Cuman $L^{(R)}$, the growth in T. gracilis showed gradual decrease in the time. But, for production, no such phenomenon was observed. The same fungicide with D. inornata showed an interesting result in that the $EC_{50}$ value decreased with time for growth but increased

with time for production. Thus the present investigation shows that there is no rule in respect of $EC_{50}$ values with time. However, Walsh (1983) reported increased $EC_{50}$ values on each day after exposure, except for Chlorella sp., in which the values for third and fourth days were not statistically different, which suggested that physical, chemical or biological factors either singly or combination may affect toxicity in static algal tests with pesticides. He concluded that since the purpose of toxicity tests is to detect toxicity without consideration of fate of toxicant, $EC_{50}$ values derived on the second day of exposure in acute tests was the best approximate toxicity. After 2nd day, the fate of toxicant become important in assessment of toxicity. Toxicants may decompose or adsorb to the glass walls of exposure vessels. Certain pesticides may be degraded or metabolized as reported by Butler et al., (1975a and Rath and Misra (1981). These metabolites may act as nutrients or as more toxic substances than the parent compound.

Abou Waly et al. (1991 a) concluded that the $EC_{50}$ values generally increased with time except for one species Salenastrum capricornutum with the herbicide Atrazine, where there was a gradual decrease of $EC_{50}$ value with time.

The present study revealed that there was variation in growth responses of two species with three biocides (Table 10). These observations were in conformative with those of Ibrahim (1983, 1990) and Rajaretnam et al., (1987). The 50% effective dose values reported by several authors

varied greatly, such varied responses may be due to the interspecific differences of the test organism (Menzel et al., 1970, Mosser et al., (1972a,b), Fisher et al., 1974). The amount of inoculum and the duration of exposure period determine the $EC_{50}$ values, because a very high initial inoculum can markedly reduce the degree of inhibition (Goulding and Ellis, 1981).

The data also revealed the wide range of responses of the test algae to the biocides within the same species. This agrees with the findings of Hollister and Walsh (1973) who observed the same variations in the case of herbicides on algae. They suggested the use of several species from different families in algal bioassay studies to obtain a realistic data.

The wide variation in $EC_{50}$ values for population growth, carbon production and pigment content of the two microalgae tested with each biocide lead to the conclusion that the chemical constituents of algae should be taken into consideration in algal bioassay testswith the toxicants to obtain a complete picture of their toxic effects. These type of bioassay studies can be extended to other marine and fresh water algae

TABLE- 1. $EC_{50}$ values of an organophosphate insecticide 'Nuvacron' on population growth of microalgae.

| Duration of Experiment | Observations | Test organisms | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Tetraselmis gracilis | | | | | | Dicrateria inornata | | | | | |
| | | Nuvacron concentration ppm | | | | | | | | | | | |
| | | 0 | 25 | 50 | 75 | 100 | 125 | 0 | 25 | 50 | 75 | 100 | 125 |
| 24 Hour | Number of cells x $10^4$/ml | 9.2 | 9.75 | 9.4 | 6.65 | 4.00 | 2.00 | 19.2 | 20.8 | 19.4 | 10.0 | 7.4 | 0.8 |
| | % response | 100 | 105.9 | 102.2 | 72.3 | 43.8 | 21.74 | 100 | 108.3 | 101.0 | 52.1 | 38.5 | 4.2 |
| | % increase(+)/ decrease (-) | 0 | + 5.9 | + 2.2 | - 27.7 | - 56.52 | - 78.26 | 0 | + 8.3 | + 1.0 | - 47.9 | - 61.5 | - 95.8 |
| | $EC_{50}$ ppm | 94.4 | | | | | | 79.43 | | | | | |
| 48 Hour | Number of cells x $10^4$/ml | 21.6 | 21.8 | 21.7 | 11.9 | 6.2 | 2.9 | 34.0 | 36.8 | 34.2 | 19.8 | 12.8 | 1.0 |
| | % response | 100 | 100.9 | 100.5 | 55.9 | 28.7 | 13.43 | 100 | 108.2 | 100.5 | 58.2 | 37.7 | 2.9 |
| | % increase(+)/ decrease(-) | 0 | + 0.9 | + 0.5 | - 44.1 | - 71.3 | - 86.57 | 0 | + 8.2 | + 0.5 | - 41.8 | - 62.3 | - 97.1 |
| | $EC_{50}$ ppm | 75.5 | | | | | | 81.28 | | | | | |
| 72 hour | Number of cells x $10^4$/ml | 36.5 | 37.0 | 36.0 | 20.1 | 10.2 | 3.2 | 58.0 | 59.8 | 58.2 | 30.0 | 19.9 | 1.2 |
| | % response | 100 | 101.4 | 98.6 | 55.07 | 27.95 | 8.76 | 100 | 103.1 | 100.3 | 51.7 | 34.3 | 2.1 |
| | % increase (+)/ decrease (-) | 0 | + 1.4 | - 1.4 | - 44.9 | - 72.05 | - 91.24 | 0 | + 3.1 | + 0.3 | - 48.3 | - 65.7 | - 97.9 |
| | $EC_{50}$ ppm | 74.9 | | | | | | 74.99 | | | | | |
| 96 Hour | Number of cells x$10^4$/ml | 47.0 | 47.8 | 40.0 | 28.5 | 18.0 | 5.8 | 75.0 | 76.1 | 75.1 | 39.8 | 28.2 | 1.4 |
| | % response | 100 | 101.7 | 85.11 | 60.63 | 38.29 | 12.34 | 100 | 101.3 | 100.1 | 53.1 | 37.6 | 1.86 |
| | % increase (+)/ decrease (-) | 0 | + 1.7 | - 14.89 | - 39.31 | - 61.71 | - 87.66 | 0 | + 1.3 | + 0.1 | 46.9 | 62.4 | 98.14 |
| | $EC_{50}$ ppm | 74.99 | | | | | | 74.13 | | | | | |

TABLE-2. $EC_{50}$ values of an organophosphate insecticide 'Nuvacron' on net carbon production of microalgae.

| Duration of Experiment | Observations | Test organisms | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Tetraselmis gracilis | | | | | | Dicrateria inornata | | | | | |
| | | Nuvacron concentration ppm | | | | | | | | | | | |
| | | 0 | 25 | 50 | 75 | 100 | 125 | 0 | 25 | 50 | 75 | 100 | 125 |
| 24 Hour | Net carbon production mgC/l/hr. | 0.043 | 0.045 | 0.033 | 0.028 | 0.008 | 0 | 0.049 | 0.052 | 0.038 | 0.025 | 0.011 | 0 |
| | % response | 100 | 106,34 | 76.53 | 65.96 | 18.77 | 0 | 100 | 106.7 | 77.71 | 51.94 | 22.69 | 0 |
| | % increase(+)/ decrease (-) | 0 | + 6.34 | - 23.47 | - 34.1 | - 81.23 | 0 | 0 | + 6.7 | - 22.3 | - 48.06 | - 77.31 | 0 |
| | $EC_{50}$ ppm | | | | 74.98 | | | | | | 54.33 | | |
| 48 Hour | Net carbon production mgC/l/hr | 0.0653 | 0.071 | 0.057 | 0.039 | 0.010 | 0.006 | 0.088 | 0.089 | 0.060 | 0.038 | 0.016 | 0 |
| | % response | 100 | 108.7 | 87.44 | 59.7 | 15.31 | 9.18 | 100 | 102.3 | 68.26 | 43.23 | 18.54 | 0 |
| | % increase(+)/ decrease(-) | 0 | + 8.7 | - 12.56 | - 40.3 | - 84.6 | - 90.8 | 0 | + 2.3 | - 31.74 | - 56.77 | - 81.46 | 0 |
| | $EC_{50}$ ppm | | | | 75.85 | | | | | | 61.66 | | |
| 72 hour | Net carbon production mgC/l/hr | 0.1341 | 0.164 | 0.098 | 0.064 | 0.018 | 0.008 | 0.135 | 0.138 | 0.098 | 0.060 | 0.034 | 0.006 |
| | % response | 100 | 122.4 | 73.08 | 47.73 | 13.42 | 5.97 | 100 | 109.4 | 72.16 | 44.18 | 25.04 | 4.49 |
| | % increase (+)/ decrease (-) | 0 | + 22.4 | - 26.92 | - 52.3 | - 86.58 | - 94.0 | 0 | + 9.4 | - 27.8 | - 55.8 | - 74.96 | - 95.51 |
| | $EC_{50}$ ppm | | | | 68.39 | | | | | | 66.07 | | |
| 96 Hour | Net carbon production mgC/l/hr | 0.196 | 0.208 | 0.151 | 0.088 | 0.062 | 0.02 | 0.216 | 0.204 | 0.163 | 0.108 | 0.048 | 0.008 |
| | % response | 100 | 106.12 | 77.04 | 44.89 | 31.63 | 10.2 | 100 | 103.4 | 75.5 | 49.9 | 22.2 | 3.69 |
| | % increase (+)/ decrease (-) | 0 | + 6.12 | - 22.96 | - 55.11 | - 68.37 | - 89.8 | 0 | + 3.4 | - 24.5 | - 50.1 | - 77.8 | - 96.3 |
| | $EC_{50}$ ppm | | | | 69.43 | | | | | | 68.39 | | |

TABLE-3. $EC_{50}$ values of three biocides on Chlorophyll 'a' content of microalgae.

(1) Organophosphate Insecticide - 'Nuvacron'.

| Observation | Test Organisms | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Tetraselmis gracilis | | | | | | Dicrateria inornata | | | | | |
| | 'Nuvacron' concentration (ppm) | | | | | | | | | | | |
| | 0 | 25 | 50 | 75 | 100 | 125 | 0 | 25 | 50 | 75 | 100 | 125 |
| Chlorophyll 'a' /ug/l | 1232 | 1276 | 1120 | 846 | 514 | 228 | 439.5 | 371.6 | 343.8 | 208.8 | 118.8 | 20.8 |
| % response | 100 | 103.6 | 90.9 | 68.67 | 41.72 | 18.50 | 100 | 84.55 | 78.23 | 47.50 | 27.04 | 4.7 |
| % increase (+)/ decrease (-) | 0 | + 3.6 | - 9.1 | - 31.33 | - 58.28 | - 81.5 | 0 | - 15.45 | - 21.77 | - 52.50 | - 72.96 | - 95.3 |
| $EC_{50}$ (ppm) | 91.20 | | | | | | 62.66 | | | | | |

(2) Herbicide - 'Gramaxone'

| Observation | Test Organisms | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Tetraselmis gracilis | | | | | | Dicrateria inornata | | | | | |
| | Gramaxone Concentration (ppm) | | | | | | | | | | | |
| | 0 | 0.1 | 0.2 | 0.4 | 0.8 | 1.6 | 0 | 0.1 | 0.2 | 0.4 | 0.8 | 1.6 |
| Chlorophyll 'a' /ug/l | 1154 | 1169 | 905.7 | 721.9 | 318.9 | 226.7 | 422.3 | 404.0 | 317.3 | 290.8 | 156 | 37.5 |
| % response | 100 | 104.4 | 78.5 | 62.6 | 27.6 | 19.6 | 100 | 95.7 | 75.2 | 68.9 | 36.9 | 8.89 |
| % increase (+)/ decrease (-) | 0 | + 4.4 | - 21.5 | - 37.4 | - 72.4 | - 80.4 | 0 | - 4.3 | - 24.8 | - 31.1 | - 63.1 | - 91.1 |
| $EC_{50}$ (ppm) | 0.51 | | | | | | 0.518 | | | | | |

(3) Fungicide - 'Cuman $L^{(R)}$,

| Observation | Test Organisms | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Tetraselmis gracilis | | | | | | Dicrateria inornata | | | | | |
| | Cuman $L^{(R)}$ Concentration (ppm) | | | | | | | | | | | |
| | 0 | 0.005 | 0.01 | 0.02 | 0.04 | 0.08 | 0 | 0.005 | 0.01 | 0.02 | 0.04 | 0.08 |
| Chlorophyll /ug/l | 1233 | 1373.7 | 1190.8 | 528.8 | 93.4 | 56.3 | 428.8 | 248.9 | 104.3 | 38.61 | 20.8 | 0 |
| % response | 100 | 111.4 | 96.6 | 42.9 | 7.6 | 4.6 | 100 | 58.05 | 24.3 | 9.0 | 4.85 | 0 |
| % increase (+)/ decrease | 0 | + 11.4 | - 3.4 | - 57.12 | - 92.4 | - 95.4 | 0 | - 41.95 | - 75.7 | - 91.0 | - 95.15 | 0 |
| $EC_{50}$ (ppm) | 0.021 | | | | | | 0.006 | | | | | |

TABLE-4a. $EC_{50}$ Values of three biocides on Chlorophyll 'b'

(1) 'Nuvacron'

| Observations | Tetraselmis gracilis | | | | | |
|---|---|---|---|---|---|---|
| | Nuvacron concentration (ppm) | | | | | |
| | 0 | 25 | 50 | 75 | 100 | 125 |
| Chlorophyll 'b' /ug/l | 786 | 938 | 716 | 527 | 318 | 158 |
| % response | 100 | 119.4 | 91.1 | 67.05 | 40.46 | 20.10 |
| % increase(+)/ decrease (-) | 0 | + 9.4 | - 8.9 | - 32.9 | - 59.5 | - 79.9 |
| $EC_{50}$(ppm) | 89.13 | | | | | |

(2) 'Gramaxone'

| Observations | Tetraselmis gracilis | | | | | |
|---|---|---|---|---|---|---|
| | Gramaxone concentration(ppm) | | | | | |
| | 0 | 0.1 | 0.2 | 0.4 | 0.8 | 1.6 |
| Chlorophyll 'b' /ug/l | 986.9 | 985.6 | 880.8 | 477.6 | 277.6 | 110.6 |
| % response | 100 | 99.9 | 89.2 | 48.4 | 28.1 | 11.2 |
| % increase(+) /decrease(-) | 0 | - 0.14 | - 10.8 | - 51.6 | - 71.9 | - 88.8 |
| $EC_{50}$(ppm) | 0.494 | | | | | |

(3) 'Cuman $L^{(R)}$

| Observations | Tetraselmis gracilis | | | | | |
|---|---|---|---|---|---|---|
| | Cuman L concentration (ppm) | | | | | |
| | 0 | 0.005 | 0.01 | 0.02 | 0.04 | 0.08 |
| Chlorophyll 'b' /ug/l | 1012 | 1134 | 1008 | 616 | 117.1 | 75.8 |
| % response | 100 | 112.1 | 99.6 | 60.9 | 11.6 | 7.5 |
| % increase(+)/ decrease(-) | 0 | + 12.1 | - 0.42 | - 39.13 | - 88.4 | - 92.5 |
| $EC_{50}$(ppm) | 0.025 | | | | | |

TABLE-4b. $EC_{50}$ values of three biocides on chlorophyll 'c'

(1) 'Nuvacron'

| Observations | Dicrateria inornata | | | | | |
|---|---|---|---|---|---|---|
| | Nuvacron concentration (ppm) | | | | | |
| | 0 | 25 | 50 | 75 | 100 | 125 |
| Chlorophyll 'c' /ug/l | 134.7 | 92.2 | 82.3 | 58.8 | 18.4 | 0 |
| % response | 100 | 68.5 | 61.1 | 43.7 | 13.66 | 0 |
| % increase(+) /decrease(-) | 0 | - 31.5 | - 38.9 | - 56.3 | - 86.34 | 0 |
| $EC_{50}$ (ppm) | 45.71 | | | | | |

(2) 'Gramaxone'

| Observations | Dicrateria inornata | | | | | |
|---|---|---|---|---|---|---|
| | Gramaxone concentration (ppm) | | | | | |
| | 0 | 0.1 | 0.2 | 0.4 | 0.8 | 1.6 |
| Chlorophyll 'c' /ug/l | 129 | 110.3 | 82.1 | 70.7 | 42.3 | 16.2 |
| % response | 100 | 85.4 | 63.5 | 54.7 | 32.7 | 12.6 |
| % increase(+) /decrease (-) | 0 | - 14.6 | - 36.5 | - 45.3 | - 67.3 | - 87.4 |
| $EC_{50}$(ppm) | 0.395 | | | | | |

(3) 'Cuman $L^{(R)}$

| Observation | Dicrateria inornata | | | | | |
|---|---|---|---|---|---|---|
| | Cuman L Concentration (ppm) | | | | | |
| | 0 | 0.005 | 0.01 | 0.02 | 0.04 | 0.08 |
| Chlorophyll 'c' /ug/l | 131.4 | 88.8 | 42.8 | 16.2 | -ve | -ve |
| % response | 100 | 67.6 | 32.6 | 12.3 | - | - |
| % increase(+) /decrease(-) | 0 | - 32.4 | - 67.4 | - 87.7 | - | |
| $EC_{50}$(ppm) | 0.007 | | | | | |

TABLE-5. $EC_{50}$ values of herbicide 'Gramaxone' on population growth of microalgae.

| Duration of Experiment | Observations | Test organisms | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Tetraselmis gracilis | | | | | | Dicrateria inornata | | | | | |
| | | Gramaxone concentration (ppm) | | | | | | | | | | | |
| | | 0 | 0.1 | 0.2 | 0.4 | 0.8 | 1.6 | 0 | 0.1 | 0.2 | 0.4 | 0.8 | 1.6 |
| 24 Hour | Number of cells x $10^4$/ml | 9.5 | 10.8 | 9.0 | 7.3 | 5.8 | 3.0 | 19.8 | 20.8 | 19.0 | 16.0 | 9.6 | 4.6 |
| | % response | 100 | 113.7 | 94.7 | 76.8 | 61.1 | 31.6 | 100 | 105.0 | 95.9 | 80.8 | 48.5 | 20.2 |
| | % increase(+)/ decrease (-) | 0 | + 13.7 | - 5.3 | - 23.2 | - 38.9 | - 68.4 | 0 | + 5.0 | - 4.1 | - 19.2 | - 51.5 | - 79.8 |
| | $EC_{50}$ ppm | 0.987 | | | | | | 0.799 | | | | | |
| 48 Hour | Number of cells x $10^4$/ml | 21.4 | 22.3 | 20.8 | 16.8 | 10.7 | 6.6 | 38.2 | 39.4 | 37.6 | 22.0 | 18.6 | 9.0 |
| | % response | 100 | 104.2 | 97.2 | 78.5 | 50.5 | 30.8 | 100 | 103.1 | 98.4 | 57.6 | 48.7 | 23.6 |
| | % increase(+)/ decrease(-) | 0 | + 4.2 | - 2.8 | - 21.5 | - 49.5 | - 69.2 | 0 | + 3.1 | - 1.6 | - 42.4 | - 51.3 | - 76.4 |
| | $EC_{50}$ ppm | 0.904 | | | | | | 0.72 | | | | | |
| 72 hour | Number of cells x $10^4$/ml | 38.6 | 36.5 | 32.0 | 28.9 | 16.8 | 9.2 | 68.2 | 67.0 | 54.6 | 34.1 | 29.2 | 14.0 |
| | % response | 100 | 94.5 | 82.9 | 74.9 | 43.6 | 23.8 | 100 | 98.2 | 80.1 | 50.0 | 42.8 | 20.5 |
| | % increase (+)/ decrease (-) | 0 | - 5.44 | - 17.1 | - 25.1 | - 56.4 | - 76.2 | 0 | - 1.86 | - 19.9 | - 50.0 | - 57.2 | - 79.5 |
| | $EC_{50}$ ppm | 0.707 | | | | | | 0.564 | | | | | |
| 96 Hour | Number of cells x $10^4$/ml | 47.2 | 46.0 | 42.5 | 39.0 | 20.8 | 14.0 | 84.6 | 83.0 | 61.2 | 41.0 | 24.0 | 12.0 |
| | % response | 100 | 97.5 | 90.1 | 82.6 | 44.1 | 29.7 | 100 | 98.1 | 72.3 | 48.5 | 28.4 | 14.2 |
| | % increase (+)/ decrease (-) | 0 | - 2.5 | - 9.9 | - 77.4 | - 55.9 | - 70.3 | 0 | - 1.9 | - 27.7 | - 51.5 | - 71.6 | - 85.8 |
| | $EC_{50}$ ppm | 0.841 | | | | | | 0.443 | | | | | |

TABLE-6. $EC_{50}$ values of herbicide 'Gramaxone' on net carbon production of microalgae.

| Duration of Experiment | Observations | Test organisms | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Tetraselmis gracilis | | | | | | Dicrateria inornata | | | | | |
| | | Gramaxone concentration (ppm) | | | | | | | | | | | |
| | | 0 | 0.1 | 0.2 | 0.4 | 0.8 | 1.6 | 0 | 0.1 | 0.2 | 0.4 | 0.8 | 1.6 |
| 24 Hour | Net carbon production mgC/l/hr. | 0.048 | 0.057 | 0.041 | 0.032 | 0.024 | -ve | 0.047 | 0.061 | 0.039 | 0.016 | 0.016 | -ve |
| | % response | 100 | 118.2 | 84.5 | 65.2 | 49.7 | - | 100 | 130.8 | 85.0 | 34.8 | 34.8 | - |
| | % increase(+)/ decrease (-) | 0 | + 18.2 | - 15.5 | - 34.8 | - 50.3 | - | 0 | + 30.8 | - 14.9 | - 65.2 | - 65.2 | - |
| | $EC_{50}$ ppm | 0.756 | | | | | | 0.423 | | | | | |
| 48 Hour | Net carbon production mgC/l/hr. | 0.069 | 0.064 | 0.046 | 0.038 | 0.021 | 0.008 | 0.091 | 0.085 | 0.065 | 0.024 | 0.016 | -ve |
| | % response | 100 | 91.8 | 67.8 | 55.4 | 30.1 | 11.9 | 100 | 93.9 | 71.9 | 26.9 | 17.9 | -ve |
| | % increase(+)/ decrease(-) | 0 | - 8.2 | - 32.2 | - 44.0 | - 69.9 | - 88.2 | 0 | - 6.1 | - 28.1 | - 73.1 | - 82.1 | -ve |
| | $EC_{50}$ ppm | 0.432 | | | | | | 0.305 | | | | | |
| 72 hour | Net carbon production mgC/l/hr | 0.144 | 0.133 | 0.090 | 0.073 | 0.031 | 0.019 | 0.132 | 0.114 | 0.100 | 0.050 | 0.024 | 0.008 |
| | % response | 100 | 92.5 | 66.5 | 50.6 | 21.2 | 12.9 | 100 | 84.14 | 76.13 | 42.5 | 18.43 | 6.19 |
| | % increase (+)/ decrease (-) | 0 | - 7.,5 | - 33.5 | - 49.4 | - 78.8 | - 87.1 | 0 | - 15.86 | - 23.9 | - 57.5 | - 81.57 | - 93.81 |
| | $EC_{50}$ ppm | 0.383 | | | | | | 0.335 | | | | | |
| 96 Hour | Net carbon pr-oduction mgC/l/hr. | 0.297 | 0.281 | 0.253 | 0.148 | 0.082 | 0.019 | 0.198 | 0.163 | 0.122 | 0.074 | 0.016 | 0.008 |
| | % response | 100 | 94.7 | 85.2 | 49.9 | 27.5 | 6.13 | 100 | 82.5 | 61.8 | 37.4 | 8.19 | 4.0 |
| | % increase (+)/ decrease (-) | 0 | - 5.3 | - 14.8 | - 50.1 | - 72.5 | - 93.7 | 0 | - 17.5 | - 38.2 | - 62.6 | - 91.8 | - 96.0 |
| | $EC_{50}$ ppm | 0.441 | | | | | | 0.295 | | | | | |

TABLE-7. $EC_{50}$ values of fungicide 'Cuman $L^{(R)}$' on population growth of microalgae

| Duration of Experiment | Observations | Test organisms | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Tetraselmis gracilis | | | | | | Dicrateria inornata | | | | | |
| | | 'Cuman L.$^{(R)}$' concentration (ppm) | | | | | | | | | | | |
| | | 0 | 0.005 | 0.01 | 0.02 | 0.04 | 0.08 | 0 | 0.005 | 0.01 | 0.02 | 0.04 | 0.08 |
| 24 Hour | Number of cells x $10^4$/ml | 9.8 | 11.1 | 9.7 | 6.65 | 4.8 | 2.6 | 19.2 | 15.5 | 13.2 | 10.6 | 7.1 | 4.0 |
| | % response | 100 | 113.3 | 98.9 | 67.8 | 48.9 | 26.5 | 100 | 80.7 | 68.8 | 55.2 | 36.9 | 20.3 |
| | % increase(+)/ decrease (-) | 0 | + 13.3 | − 1.1 | − 32.1 | − 51.1 | − 73.5 | 0 | − 19.3 | − 31.2 | − 44.7 | − 63.1 | − 79.2 |
| | $EC_{50}$ ppm | 0.045 | | | | | | 0.026 | | | | | |
| 48 Hour | Number of cells x $10^4$/ml | 21.8 | 20.2 | 19.3 | 12.7 | 5.0 | 3.0 | 36.8 | 33.4 | 27.8 | 20.4 | 9.8 | 5.2 |
| | % response | 100 | 92.7 | 88.5 | 58.3 | 22.9 | 13.8 | 100 | 90.8 | 75.5 | 55.4 | 26.6 | 14.1 |
| | % increase(+)/ decrease(-) | 0 | − 7.3 | − 11.5 | − 41.7 | − 77.1 | − 86.2 | 0 | − 9.2 | − 24.5 | − 44.6 | − 73.4 | − 85.9 |
| | $EC_{50}$ ppm | 0.024 | | | | | | 0.022 | | | | | |
| 72 hour | Number of cells x $10^4$/ml | 39.0 | 36.6 | 32.8 | 19.8 | 4.9 | 2.6 | 66.8 | 61.9 | 54.6 | 30.4 | 6.8 | 4.4 |
| | % response | 100 | 93.9 | 84.1 | 50.8 | 12.6 | 6.7 | 100 | 92.5 | 81.7 | 45.5 | 10.2 | 6.6 |
| | % increase (+)/ decrease (-) | 0 | − 6.1 | − 15.9 | − 49.2 | − 87.4 | − 93.3 | 0 | − 7.5 | − 18.3 | − 54.5 | − 89.8 | − 93.4 |
| | $EC_{50}$ ppm | 0.02 | | | | | | 0.018 | | | | | |
| 96 Hour | Number of cells x $10^4$/ml | 47.1 | 45.2 | 42.8 | 24.2 | 3.9 | 2.0 | 84.8 | 72.8 | 66.6 | 40.4 | 6.0 | 4.0 |
| | % response | 100 | 95.9 | 90.9 | 51.4 | 8.3 | 4.2 | 100 | 82.8 | 78.5 | 47.6 | 7.08 | 4.72 |
| | % increase (+)/ decrease (-) | 0 | − 4.1 | − 9.1 | − 48.6 | − 91.7 | − 95.8 | 0 | − 17.2 | − 21.5 | − 52.4 | − 92.92 | − 95.28 |
| | $EC_{50}$ ppm | 0.02 | | | | | | 0.016 | | | | | |

TABLE-8. $EC_{50}$ values of fungicide 'Cuman $L^{(R)}$' on net carbon production of microalgae.

| Duration of Experiment | Observations | Test organisms | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Tetraselmis gracilis | | | | | | Dicrateria inornata | | | | | |
| | | 'Cuman L.$^{(R)}$' concentration (ppm) | | | | | | | | | | | |
| | | 0 | 0.005 | 0.01 | 0.02 | 0.04 | 0.08 | 0 | 0.005 | 0.01 | 0.02 | 0.04 | 0. |
| 24 Hour | Net carbon production mgC/l/hr | 0.041 | 0.043 | 0.033 | 0.016 | 0.008 | -ve | 0.056 | 0.028 | 0.016 | 0.008 | 0 | -v |
| | % response | 100 | 103.4 | 79.1 | 39.6 | 19.9 | -ve | 100 | 50.3 | 28.9 | 14.6 | 0 | -v |
| | % increase(+)/ decrease (-) | 0 | + 3.4 | - 20.9 | - 60.4 | - 80.1 | -ve | 0 | - 49.7 | - 71.1 | - 85.4 | 0 | -v |
| | $EC_{50}$ ppm | | | | 0.018 | | | | | | 0.005 | | |
| 48 Hour | Net carbon production mgC/l/hr | 0.064 | 0.082 | 0.049 | 0.022 | 0.016 | 0.008 | 0.099 | 0.068 | 0.029 | 0.016 | 0.008 | -v |
| | % response | 100 | 127.3 | 76.3 | 34.9 | 25.4 | 12.8 | 100 | 68.96 | 30.13 | 16.58 | 8.29 | -v |
| | % increase(+)/ decrease(-) | 0 | + 27.3 | - 23.7 | - 65.1 | - 74.6 | - 87.2 | 0 | - 31.04 | - 69.87 | - 83.5 | - 91.71 | -v |
| | $EC_{50}$ ppm | | | | 0.018 | | | | | 0.007 | | | |
| 72 hour | Net carbon production mgC/l/hr | 0.142 | 0.141 | 0.048 | 0.02 | 0.014 | 0.008 | 0.149 | 0.095 | 0.043 | 0.025 | 0.008 | -v |
| | % response | 100 | 99.3 | 33.80 | 14.08 | 9.86 | 5.63 | 100 | 64.24 | 28.82 | 17.71 | 5.52 | -v |
| | % increase (+)/ decrease (-) | 0 | - 0.7 | - 66.2 | - 85.92 | - 90.14 | - 94.37 | 0 | - 35.76 | - 71.2 | - 82.29 | - 94.48 | -v |
| | $EC_{50}$ ppm | | | | 0.015 | | | | | 0.007 | | | |
| 96 Hour | Net carbon production mgC/l/hr. | 0.287 | 0.269 | 0.237 | 0.099 | 0.024 | -ve | 0.214 | 0.165 | 0.989 | 0.046 | 0.027 | -v |
| | % response | 100 | 93.79 | 82.53 | 34.5 | 8.5 | -ve | 100 | 77.34 | 46.3 | 21.72 | 12.6 | -v |
| | % increase (+)/ decrease (-) | 0 | - 6.21 | - 17.47 | - 65.5 | - 91.5 | -ve | 0 | - 22.6 | - 53.7 | - 78.3 | - 87.4 | -v |
| | $EC_{50}$ ppm | | | | 0.016 | | | | | 0.009 | | | |

TABLE-9. $EC_{50}$ Values of three biocides on two species of microalgae for growth and net carbon production.

| Duration of experiment | Observation | Biocides | | | | | |
|---|---|---|---|---|---|---|---|
| | | 'Nuvacron' | | 'Gramaxone' | | 'Cuman L'(R) | |
| | | T. gracilis | D. inornata | T. gracilis | D. inornata | T. gracilis | D. inorna |
| 24 hour | Growth | 94.4 | 79.43 | 0.987 | 0.799 | 0.045 | 0.026 |
| | Production | 74.98 | 54.33 | 0.756 | 0.423 | 0.018 | 0.005 |
| 48 hour | Growth | 75.5 | 81.28 | 0.904 | 0.72 | 0.024 | 0.022 |
| | Production | 75.85 | 61.66 | 0.432 | 0.305 | 0.018 | 0.007 |
| 72 hour | Growth | 74.9 | 74.99 | 0.707 | 0.564 | 0.020 | 0.180 |
| | Production | 68.39 | 66.07 | 0.383 | 0.335 | 0.015 | 0.007 |
| 96 hour | Growth | 74.99 | 74.13 | 0.841 | 0.443 | 0.020 | 0.016 |
| | Production | 69.43 | 68.39 | 0.441 | 0.255 | 0.016 | 0.009 |

TABLE-10. 96 hour $EC_{50}$ values of three biocides for growth, production and chlorophyll *a* of two microalgae.

| Biocide | Observation | Test organism | |
|---|---|---|---|
| | | Tetraselmis gracilis | Dicrateria inornata |
| 'Nuvacron' $EC_{50}$ (ppm) | Growth | 74.99 | 74.13 |
| | Production | 69.43 | 68.39 |
| | Chlorophyll *a* | 91.2 | 62.66 |
| 'Gramaxone' $EC_{50}$ (ppm) | Growth | 0.841 | 0.443 |
| | Production | 0.441 | 0.255 |
| | Chlorophyll *a* | 0.51 | 0.518 |
| 'Cuman L(R)' $EC_{50}$ (ppm) | Growth | 0.020 | 0.016 |
| | Production | 0.016 | 0.009 |
| | Chlorophyll *a* | 0.021 | 0.006 |

## 4.2. IMPACT OF FIVE BIOCIDES AND THEIR COMBINATIONS ON THE PHYSIOLOGY OF MICROALGAE IN CULTURE.

### 1. GROWTH

The effect of pollutants on algae can be measured by various methods. Measuring of relative population growth in time based on changes in cell number is one important method followed in the present investigation. The changes in the cell number of microalgae in culture would show the effect of pollutant on microalgal culture. Three insecticides, one herbicide, one fungicide, a combination of these five biocides, and another combination of one insecticide, one herbicide and one fungicide were used for the study. Monoculture of two microalgae of which one was marine and the other estuarine and a mixed culture of freshwater microalgae were used for the investigation.

**Effect of inseticides on growth of microalgae**

1. Organochlorine insecticide – Benzene Hexa Chloride - 'B. H. C'.

The results of range finding test of 'B.H.C.' with respect of three microalgal culture under investigation showed that concentration of BHC above 5 ppm totally inhibited the growth of microalgae. Therefore the sublethal concentrations of 'BHC' selected for the present investigation were, 0.5, 1, 2 and 4 ppm.

Because of its low water solubility, the first stock solution of 'B.H.C' was prepared in acetone. So two controls were kept; one without acetone in the medium and the other one with acetone. The three cultures treated with different concentration of 'B.H.C' and the effect of toxicant on each algal species are as follows:-

A. Tetraselmis gracilis

Under four different concentrations of insecticide tested, the flagellate was found to grow rapidly at lower concentrations of insecticide. Both the control cultures i.e, one with acetone and the other without acetone in the medium showed a decline in growth after 14 days of experimentation (Fig. 1a). Acetone has been found to have no inhibitory or stimulatory effect on the growth of microalgae.

The four different concentration of 'BHC' tested were 0.5, 1, 2 ad 4 ppm. The lower concentration of 0.5 ppm showed an enhancement of growth over control, reaching the highest value of 78 x $10^4$ cells/ml on the 14th day. Afterwards the growth declined. At 1 ppm concentration growth was almost similar to control upto the 4th day. Subsequently decrease in cell number compared to control was observed. Two higher concentrations tested showed a declined growth at the beginning. Afterwards they showed an enhancement of growth, and when the culture reached 20 days of growth, the cell number of the culture at 4 ppm concentration, exceeded the control and all the lower concentration tested.

statistical interpretation using analysis of variance revealed that, there was significant variation in growth of T. gracilis with respect to different concentrations and also on different days. The significance was at 1% level (Table 11).

B. Dicrateria inornata

As in the case of estuarine form of microalga T. gracilis, this marine form also showed almost same effect of 'BHC' at lower concentrations. The lower concentration of 0.5 ppm showed an enhancement of growth (Fig. 1b). But all other concentration tested showed an inhibition of growth rate compared to control. Control cultures showed its peak growth on the 14th day. But the 2 ppm concentration showed its peak growth on the 16th day of growth. At 4 ppm concentration of 'BHC' this species showed an irregular tendency of growth during the 20 days observation and the peak growth was on the 18th day. Unlike T. gracilis this species at 4 ppm concentration showed a declined growth after the 18th day of culture.

Analysis of variance revealed that the four different concentration of 'BHC' had significant variation in the growth of D. inornata (Table 11).

C. Mixed culture of freshwater microalgae

The mixed culture was composed of four species, Chlorella ovalis, Selenastrum gracile, Scenedesmus indicus, and Nitzschia longissima. The mixed culture was dominated by Chlorella ovalis by about 65%. The diatom Nitzchia longissima was found to be represented by about 5% of the total number of cells. After reaching the 10th day, the culture showed a decline in growth. Control and acetone showed same trend in growth.

All the concentrations of 'BHC' tested showed an inhibitory effect on the growth of four species, compared to control (Fig. 2). At 4 ppm concentration the diatom could not survive even for one day. Concentration of 4 ppm was found inhibitory to Selenastrum gracile and Scenedesmus indicus and the culture was represented by C. ovalis only, after the 16th day of growth.

Analysis of variance indicated that the effect of 'BHC' on four different species of microalgae in the mixed culture was significant at 1% level between different concentrations and between days (Table 11).

2. Organophosphate insecticide - 'Nuvacron'

Based on the results of bioassays and also after considering the result of range finding test for 'Nuvacron' of mixed culture of freshwater microalgae the four different sublethal concentrations of 'Nuvacron' selected were 25, 50, 75 and 100 ppm. Compared to organochlorine, the organophosphate was found to be less toxic. Nuvacron is water soluble. The effects of 'Nuvacron' on the cultures of three microalgae were as follows.

A. Tetraselmis gracilis

The lower concentrations tested showed an enhanced growth over control throughout the experimental period (Fig. 3a). The 50 ppm concentration showed an increased growth rate upto the 4th day, but afterwards, decreased growth rate compared to control was observed. The two higher concentrations of 75 and 100 ppm showed a declined growth at the beginn-

ing of the experiment, but later the cell number gradually increased. In the 75 ppm concentration, the exponential phase reached its peak on the 16th day of experiment. However, in the 100 ppm concentration, the cell number went on increasing. It was observed that after 20 days growth, the control flask showed the lowest number of cells than the four different concentrations of 'Nuvacron'.

Analysis of variance indicated that the effect of 'Nuvacron' was significant at 1% level (Table 11).

B. Dicrateria inornata

As shown in Fig. 3 b this species was found to be sensitive to 'Nuvacron' than T. gracilis. The lower concentration of 25 ppm showed an enhancement of growth over control upto the 14th day and then declined. The 50 ppm showed little enhancement only upto the 4th day. Afterwards the growth rate was less compared to control. Eventhough it reached the peak growth on the 14th day, the cell number observed was only 185 x $10^4$ cells/ml. But the same for control was 240 x $10^4$ cells/ml. The microalgae treated with 75 and 100 ppm concentration of 'Nuvacron' showed very diminished growth. The peak growth observed was on the 12th day and the number of cells observed on this day was very low i.e., about 137.5 x $10^4$ cells/ml for 75 ppm and 100 x $10^4$ cells/ml for 100 ppm. The cell number gradually decreased after the 12th day.

The analysis of variance revealed that, there was significant relationship between different concentrations of Nuvacron, on growth of D. inornata.

C. Mixed culture of Freshwater microalgae

Compared to the other two monocultures, mixed culture of fresh water algae was found to be more sensitive to 'Nuvacron'.

All the four concentrations tested were found inhibitory to the growth of fresh water algae. But 25 ppm concentration showed an almost similar growth compared to control on the 12th and 14th days. The diatom N. longissima survived upto 10th day in 75 and 100 ppm concentration. The control, 25 and 50 ppm concentration of 'Nuvacron' showed the exponential phase on the 10th day of experiment, but for 75 and 100 ppm the peak growth was observed on the 12th day. The dominant species Chlorella ovalis showed an increased growth rate compared to all other species.

The effect of four different concentrations of 'Nuvacron' was found to be significant at 1% level for the 4 species. Except for N. longissima the day wise effect was found to be significant at 1% level. However for N. longissima a 5% significance was indicated (Table 11).

3. Carbamate insecticide - 'Carbaryl Sevin'.

The results of range finding test for 'carbaryl sevin' with respect to the three microalgal culture revealed that above 8 ppm concentration the growth of microalgae was totally inhibited, especially in D. inornata. While considering this aspect the selected concentration of 'carbaryl sevin' were 2, 4, 6 and 8 ppm.

As this insecticide was water insoluble two controls were set up. But the solvent acetone showed no inhibitory or stimulatory effect to the

microalgae.

A. Tetraselmis gracilis

2 ppm concentration of 'Carbaryl sevin' showed stimulation of growth over control reaching 80 x $10^4$ cells/ml on the 14th day. Afterwards the cell number declined. The 4 ppm concentration showed little enhancement of growth upto the 4th day and then the growth rate was low compared to the control (Fig. 5a). The higher concentration tested i.e, 6 ppm and 8 ppm showed decreased growth rate at the beginning of the experiment. For 6 ppm the peak growth was observed on the 16th day. But the 8 ppm concentration of 'Carbaryl' sevin showed the peak growth on 20th days of experiment.

Statistical analysis using ANOVA revealed that the effect of carbaryl was significant between concentrations and between days at 1% level (Table 11).

B. Dicrateria inornata

Fig. 5 b shows the effect of various concentrations of 'carbaryl sevin' on growth of D. inornata for 20 days period. Unlike T. gracilis, the insecticide did not show any stimulation of growth even for the lowest concentration tested. Concentration of 2 ppm shows the cell number 185 x $10^4$ cells/ml on the 14th day compared to 225 x $10^4$ cells/ml for control on the same day. In 4 ppm concentration, the peak growth was observed on the 16th day, after which a sudden decrease in cell number was observed. For 6 ppm and 8 ppm concentration eventhough there was inhibition of

growth at the beginning of the experiment, after 14th day the cell number gradually increased.

Analysis of variance revealed that there was significant variation in growth of D. inornata between treatments and between different days. The significance was at 1% level (Table 11).

C. Mixed Culture of Fresh water microalgae.

On the 2nd day of experiment, the 2 ppm concentration sowed almost similar growth as that of control. Unlike the control cultures, the insecticide treated cultures showed the peak growth on the 14th day. (Fig. 6). The diatom N. longissima did not survive in the 8 ppm concentration of insecticide. At 6 ppm concentration one or two cells of diatom was observed upto the 8th day. The figure shows that the Chlorella ovalis was not much affected by this toxicant.

Statistical analysis of data revealed for Chlorella ovalis was that both treatment wise and day wise the effect was significant at 1% level. But for Selenastrum gracile the different concentration tested was significant at 5% level. But no significant variation in growth was observed between days. For S. indicus and N. longissima both treatment-wise and day-wise the effect of 'Carbaryl Sevin' was significant at 1% level (Table 11)

II. **Effect of herbicide on growth of microalgae**

4. Herbicide - 'Gramaxone':

As in the case of insecticide 'Nuvacron', the results of bioassay

FIG.1

EFFECT OF B.H.C. ON GROWTH OF MICROALGAE

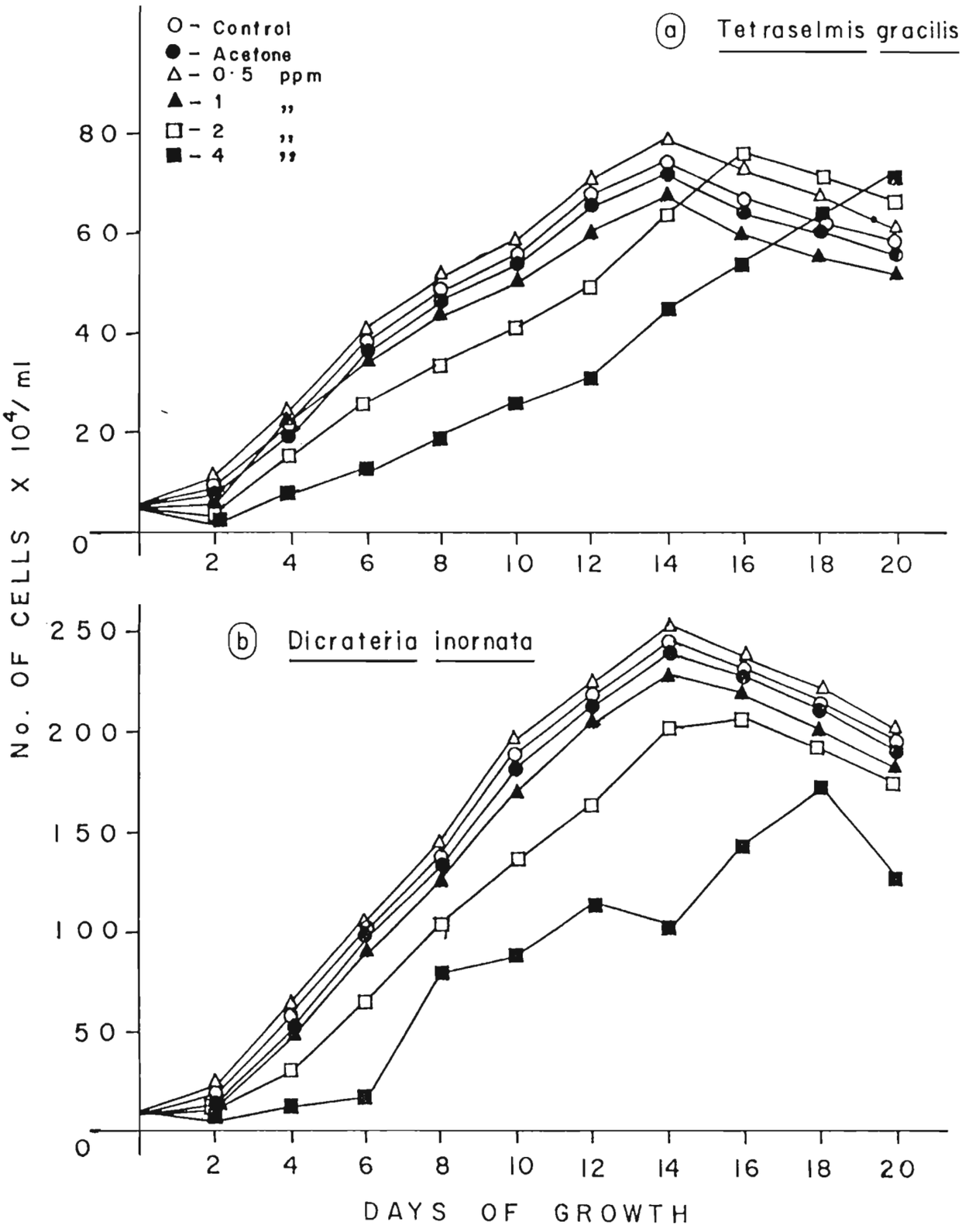


FIG.2

EFFECT OF 'B.H.C' ON GROWTH OF MIXED CULTURE OF FRESH WATER MICROALGAE

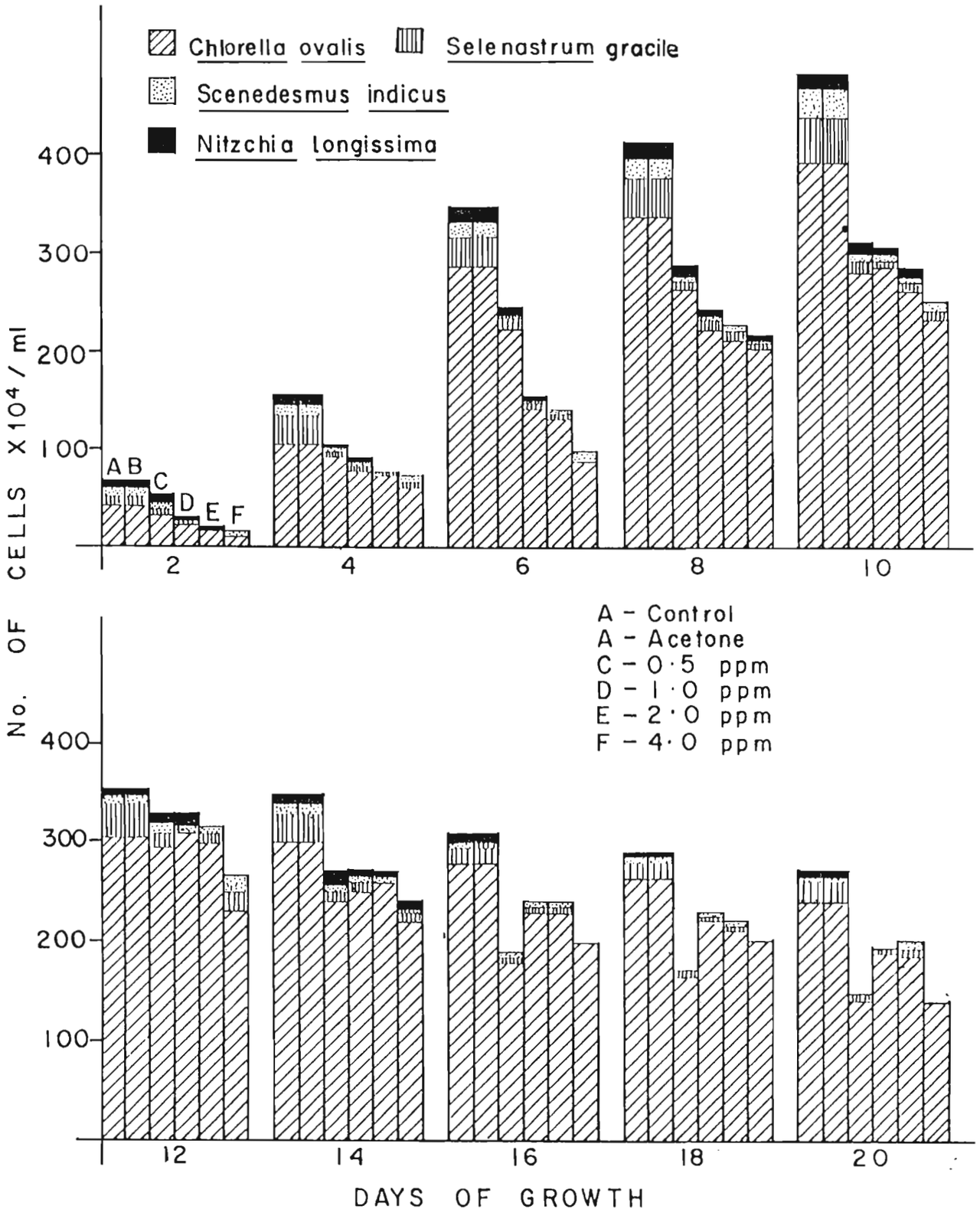


FIG.3

EFFECT OF NUVACRON ON GROWTH

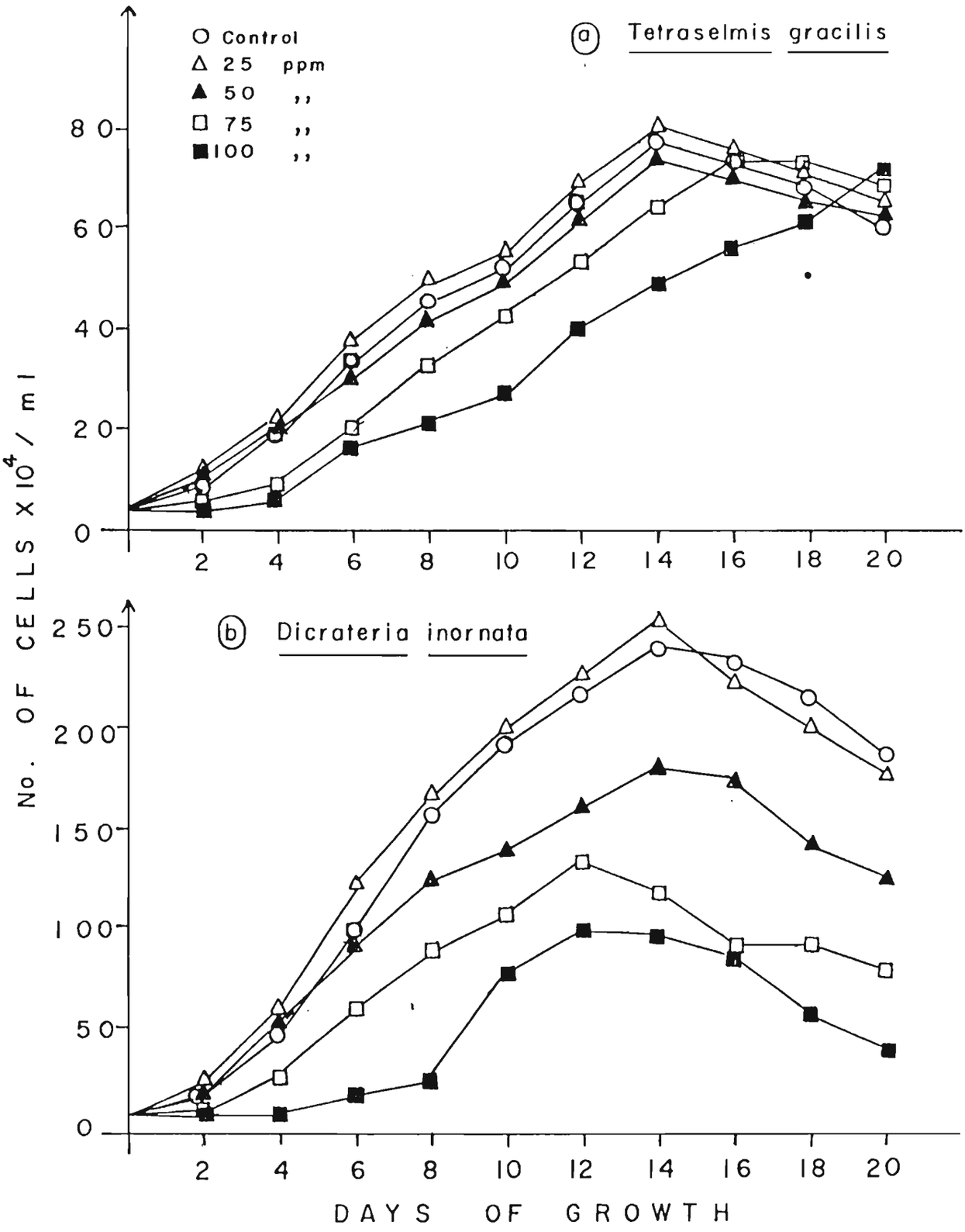


FIG.4

EFFECT OF 'NUVACRON' ON GROWTH OF FRESH WATER MIXED CULTURE OF MICROALGAE

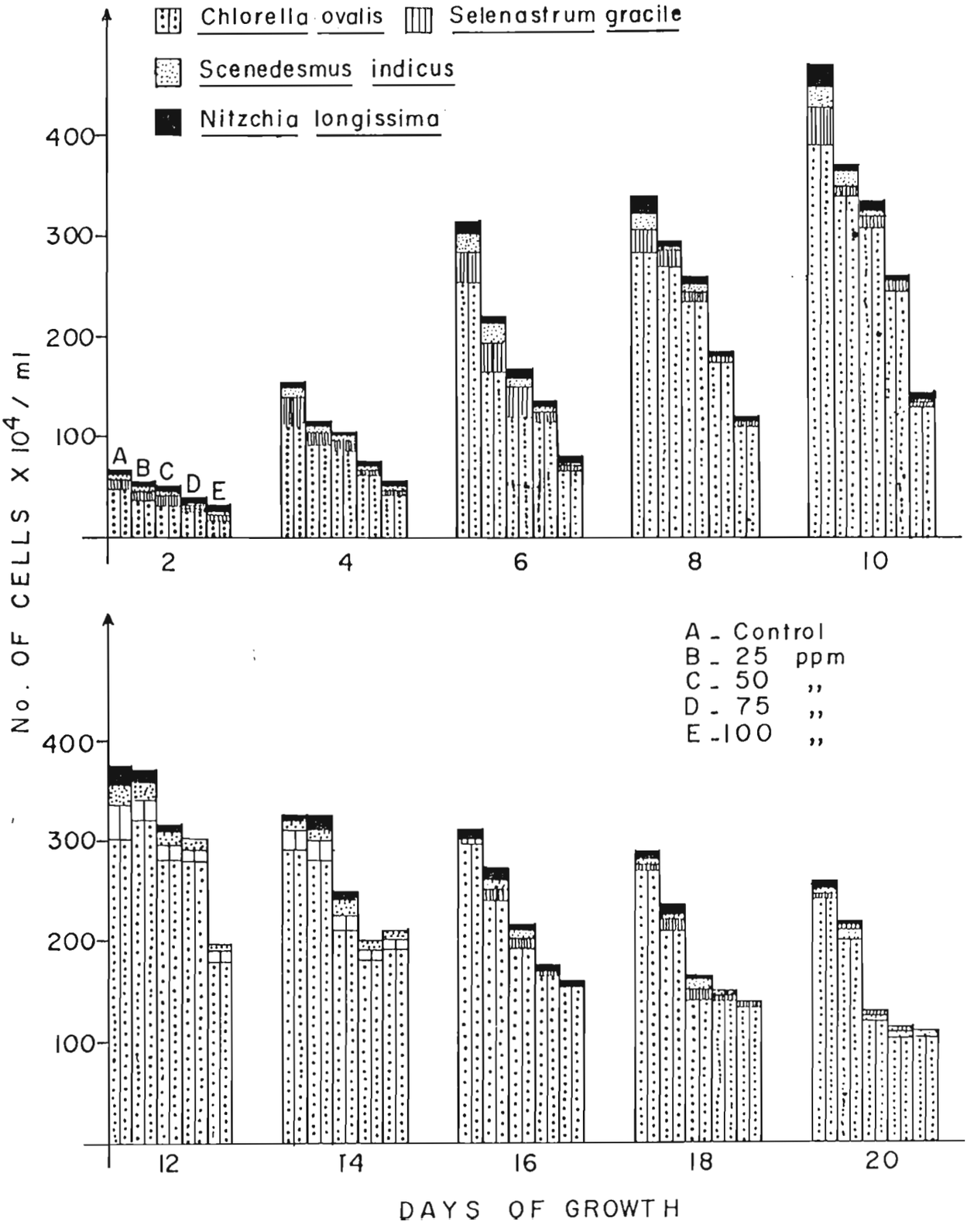


FIG.5

EFFECT OF CARBARYL SEVIN ON GROWTH

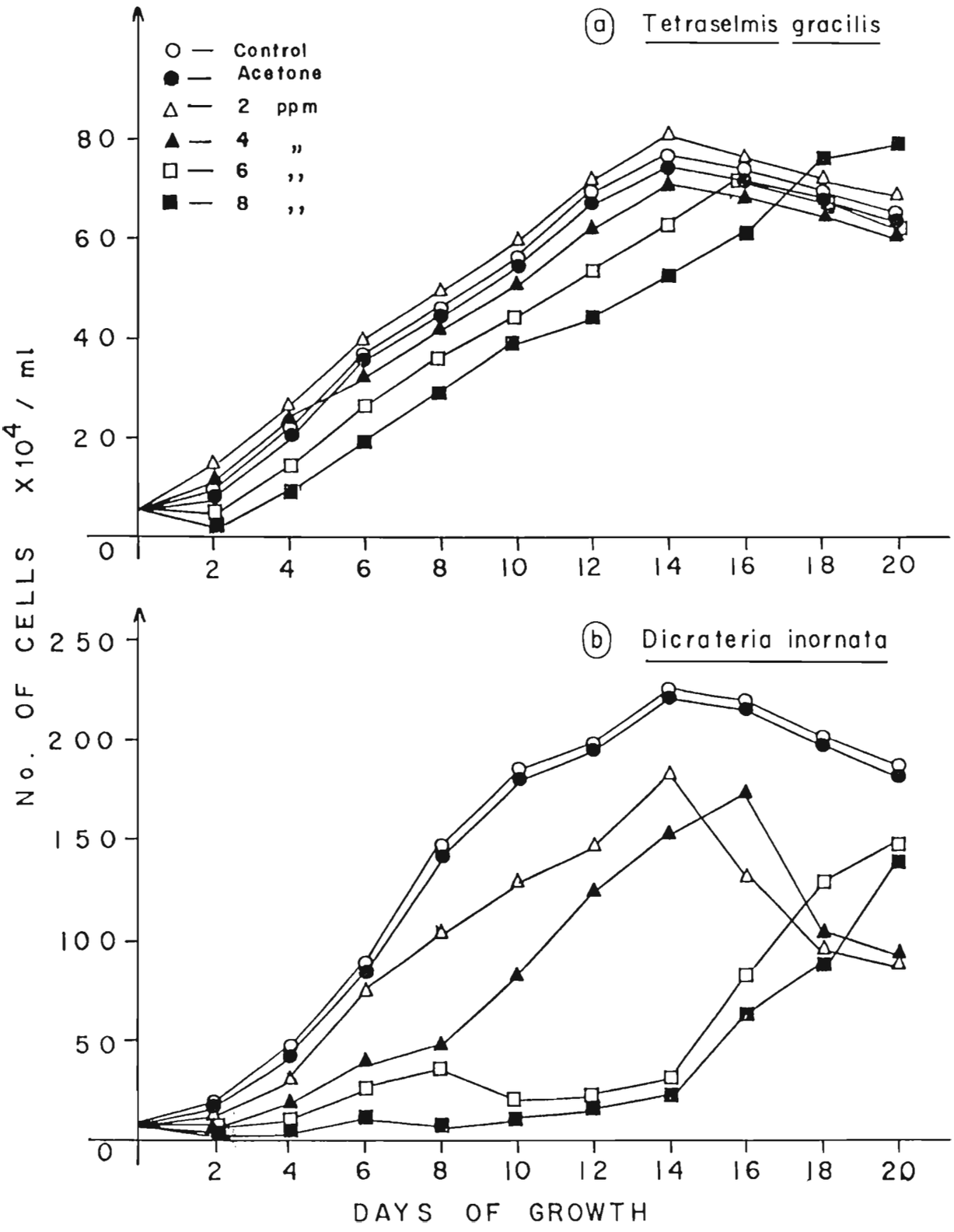
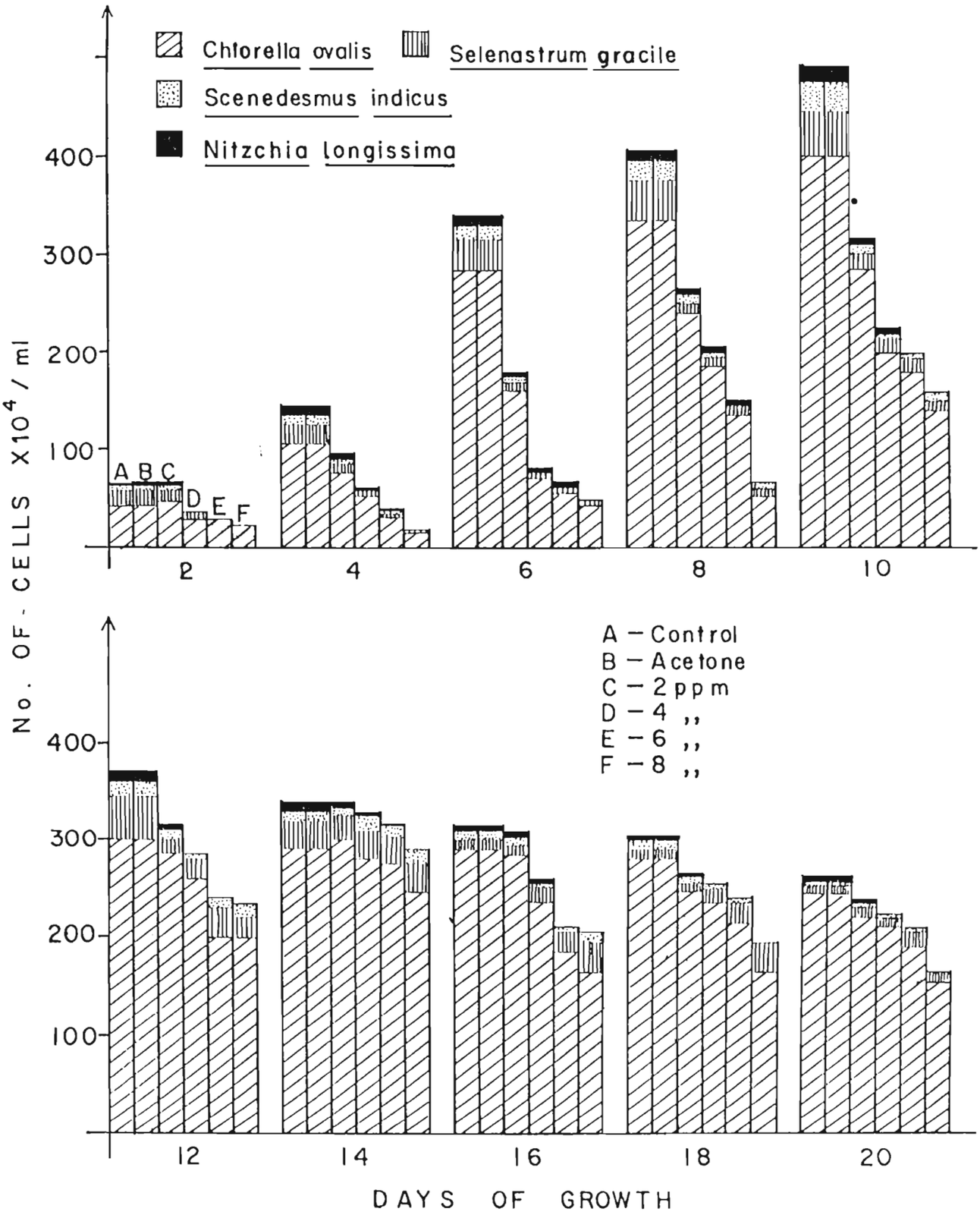


FIG.6

EFFECT OF 'CARBARYL SEVIN' ON GROWTH OF MIXED CULTURE OF FRESH WATER MICROALGAE



for 'Gramaxone' revealed that the $EC_{50}$ values might vary with respect to each parameter. But when the freshwater culture was considered, it was seen that it can tolerate around 1.6 ppm. After considering the effect of 'Gramaxone' on three cultures under investigation the selected concentrations of 'Gramaxone' for experiments were 0.2, 0.4, 0.8 and 1.6 ppm. The effects of these concentrations of 'Gramaxone' on each microalgae are as follows:

A. Tetraselmis gracilis

Fig. 7 a shows the effect of four different concentrations of 'Gramaxone' on the growth of T. gracilis. At the beginning, the two lower concentrations showed an enhancement of growth over control. But when the cultures attained 6 days of growth they showed decreased growth rate compared to control. Concentrations of 0.8 and 1.6 ppm 'Gramaxone' showed an irregularity in the growth of microalgae. At the beginning, eventhough the growth was very low compared to control, the cell number gradually increased until 8th day of observation after which the growth was inhibited. But when it attained the 12th day of growth, the cells showed an enhancement in growth and it continued throughout the experimental period.

Statistical interpretation of data, using analysis of variance showed that both treatment wise and day wise the effect of 'Gramaxone' on algae was significant at 1% level (Table 12).

B. Dicrateria inornata

This particular species showed variation in its growth with the effect of 'Gramaxone'. Concentrations of 0.2 ppm and 0.4 ppm showed peak growth on the 12th day of experiment, but the control culture attained its peak on the 14th day (Fig. 7 b). The two higher concentrations tested showed tan irregularity in their growth. The cell number increased upto the 4th day. When the culture reached the 16th day of growth the cell number showed an enhancement and the trend continued upto the 20th day. Concentration of 1.6 ppm showed a declined growth upto the 14th day, and then the cell number gradually increased.

Analysis of variance indicated that both treatmentwise and daywise the effect of 'Gramaxone' was significant at 1% level (Table 12).

C. Mixed culture of Freshwater microalgae

Figure 8 shows the effect of four different concentrations of 'Gramaxone' on the growth of mixed culture of freshwater microalgae. On the 2nd day of experiment the 0.2 ppm concentration showed an enhancement of growth. Selenastrum gracile showed an increased growth rate over control. From 4th day onwards no enhancement was noticed for this particular concentration. Concentrations of 0.8 ppm and 1.6 ppm showed low growth rate compared to control. The diatom species survived with very low cell number upto the 10th day of growth. Concentration of 1.6 ppm showed its peak growth on the 12th day of experiment. N. longissima survived upto the 12th day in 0.4 ppm and upto 14th day in 0.2 ppm concentration of 'Gramaxone'.

FIG.7

EFFECT OF 'GRAMAXONE' ON GROWTH

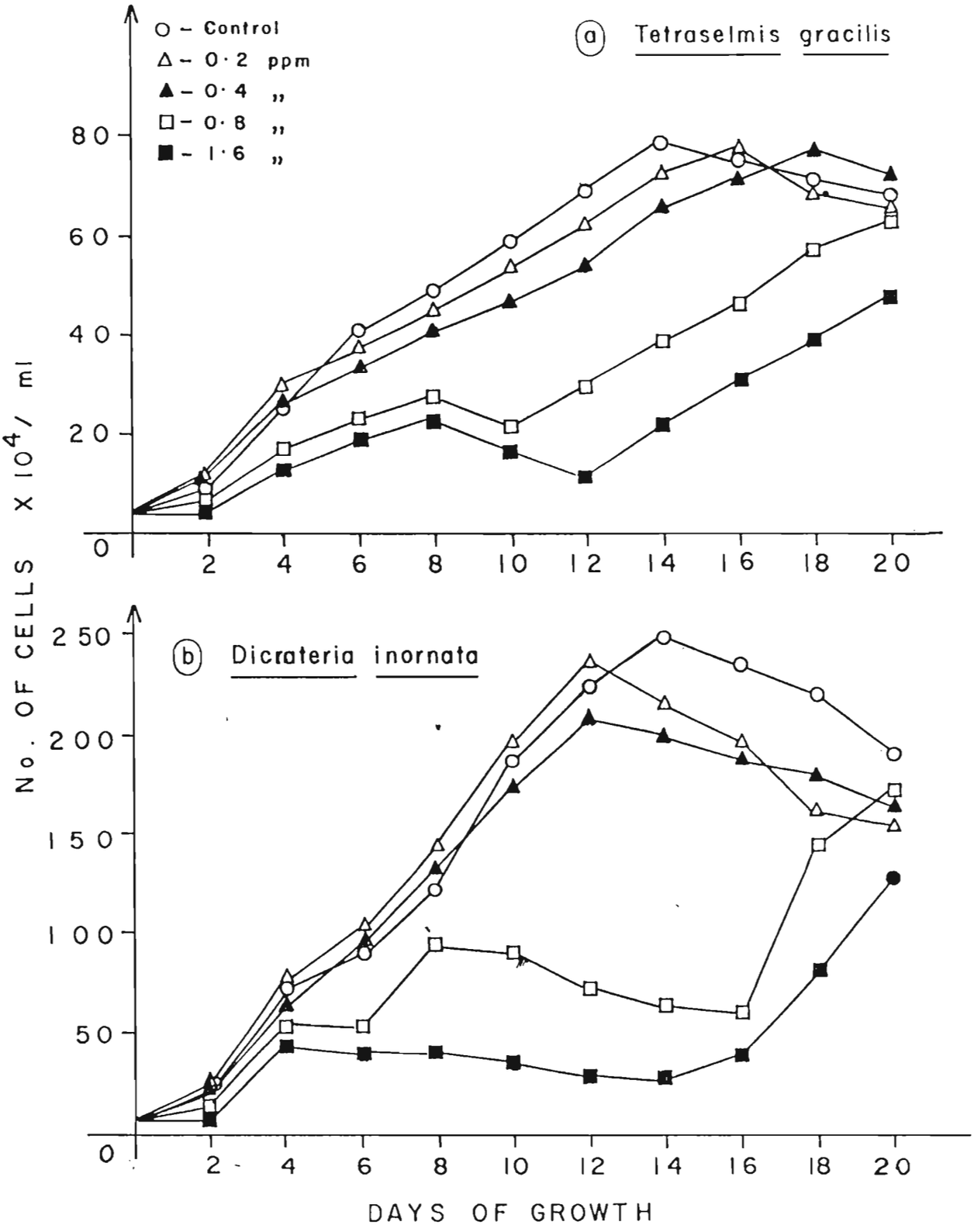
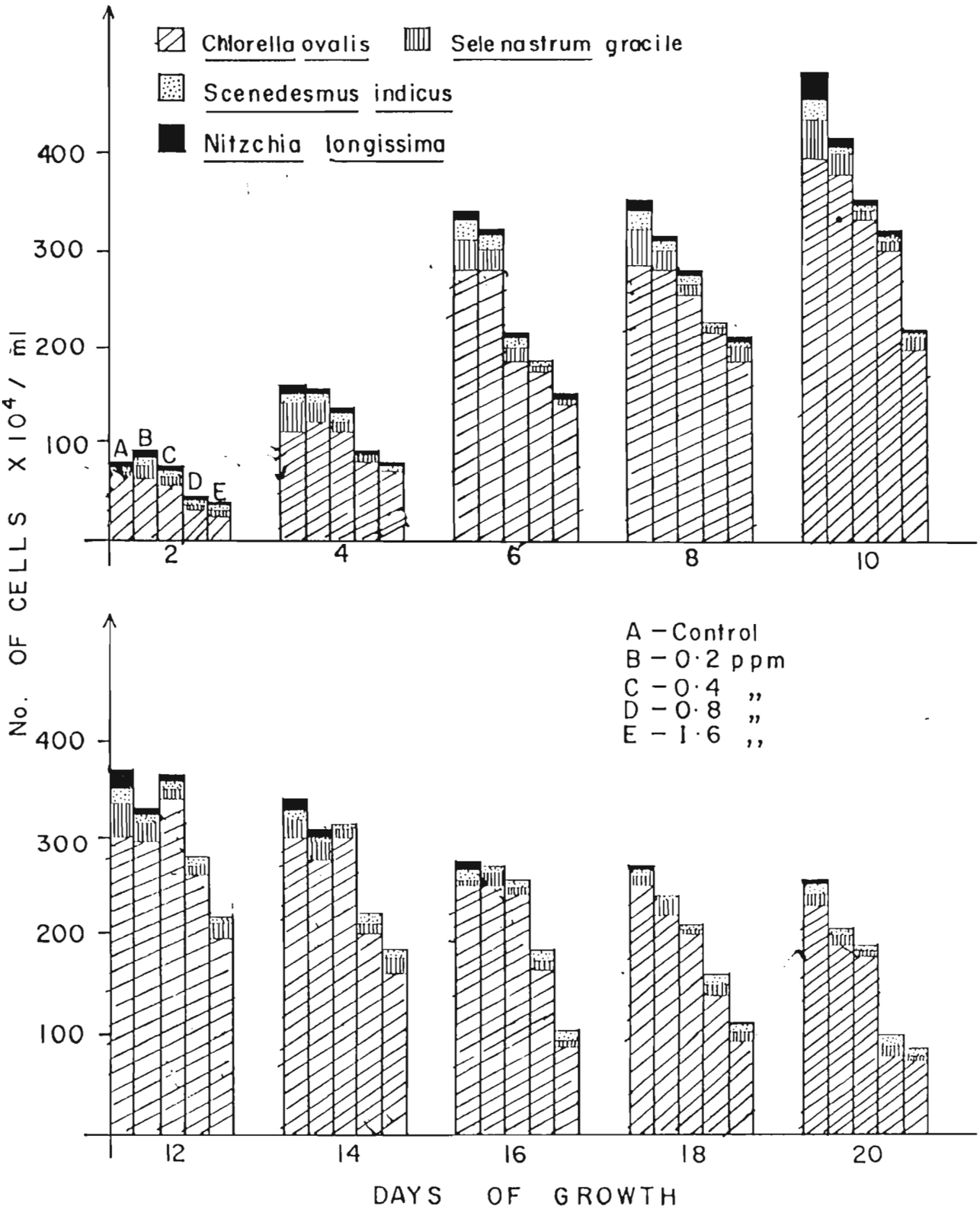


FIG.8

EFFECT OF 'GRAMAXONE' GROWTH OF MIXED CULTURE OF FRESH WATER MICROALGAE



Analysis of variance indicated that there was highly significant variation in growth between treatments and betwee days for C. ovalis. Selenastrum gracile showed highly significant variation in growth between treatment. But this species did not show any significant difference in growth between days. The same result was obtained with S. indicus and N. longissima also (Table 12).

## III. Effect of fungicide on growth of microalgae

### 5. Fungicide - 'Cuman $L^{(R)}$

The results of bioassay tests using two microalgae revealed that the $EC_{50}$ values may vary with respect to each parameter for each species. After considering the results of bioassays and range finding tests with mixed culture of freshwater microalgae, the sublethal concentrations of 'Cuman $L^{(R)}$ selected for the investigation were 0.005, 0.01, 0.02 and 0.04 ppm. 'Cuman $L^{(R)}$, is a water soluble chemical. The effects of four different concentration of this fungicide on microalgae are as follows:

#### A. Tetraselmis gracilis:

Under four different concentrations tested, the flagellate was found to grow rapidly at the lower concentration of 0.005 ppm of 'Cuman $L^{(R)}$, (Fig. 9 a). The peak growth was observed on the 14th day, and then declined. Concentration of 0.01 ppm showed diminished growth compared to control and the exponential stage was reached on the 16th day only. The two higher concentrations tested showed very low growth rate at the beginning

of the experiment upto the 8th day i.e only 16% of control for 0.02 ppm and only about 10% growth for 0.04 ppm. Afterwards the cell number gradually increased throughout the experimetal period, and when it attains 20 days growth all the experimental flasks showed almost the same rate of growth.

Analysis of variance indicated that there was highly significant variation in growth between the four treatments. It also revealed that between days, the effect of fungicide on growth was highly significant (Table 12).

B. Dicrateria inornata

Fig. 9b shows the variation in growth of microalgae as a result of fungicide treatment. Even at the lowest concentration of 0.005 ppm tested, the species did not show any enhanced growth over control. Concentrations of 0.01 ppm showed very low cell number compared to control and the peak growth was observed on the 16th days of growth. After that period the growth declined. At 0.02 ppm concentration of fungicide the growth of microalgae at the beginning showed diminished growth. But as it grew the cell number gradually increased and this tendency was continued throughout the experimental period. Coming to 0.04 ppm concentration the growth of algae was very severely affected.

Statistical interpretation using analysis of variance showed that there was highly significant variation in growth rate between treatments and between days. (Table 12)

FIG.9

EFFECT OF CUMAN L® ON GROWTH

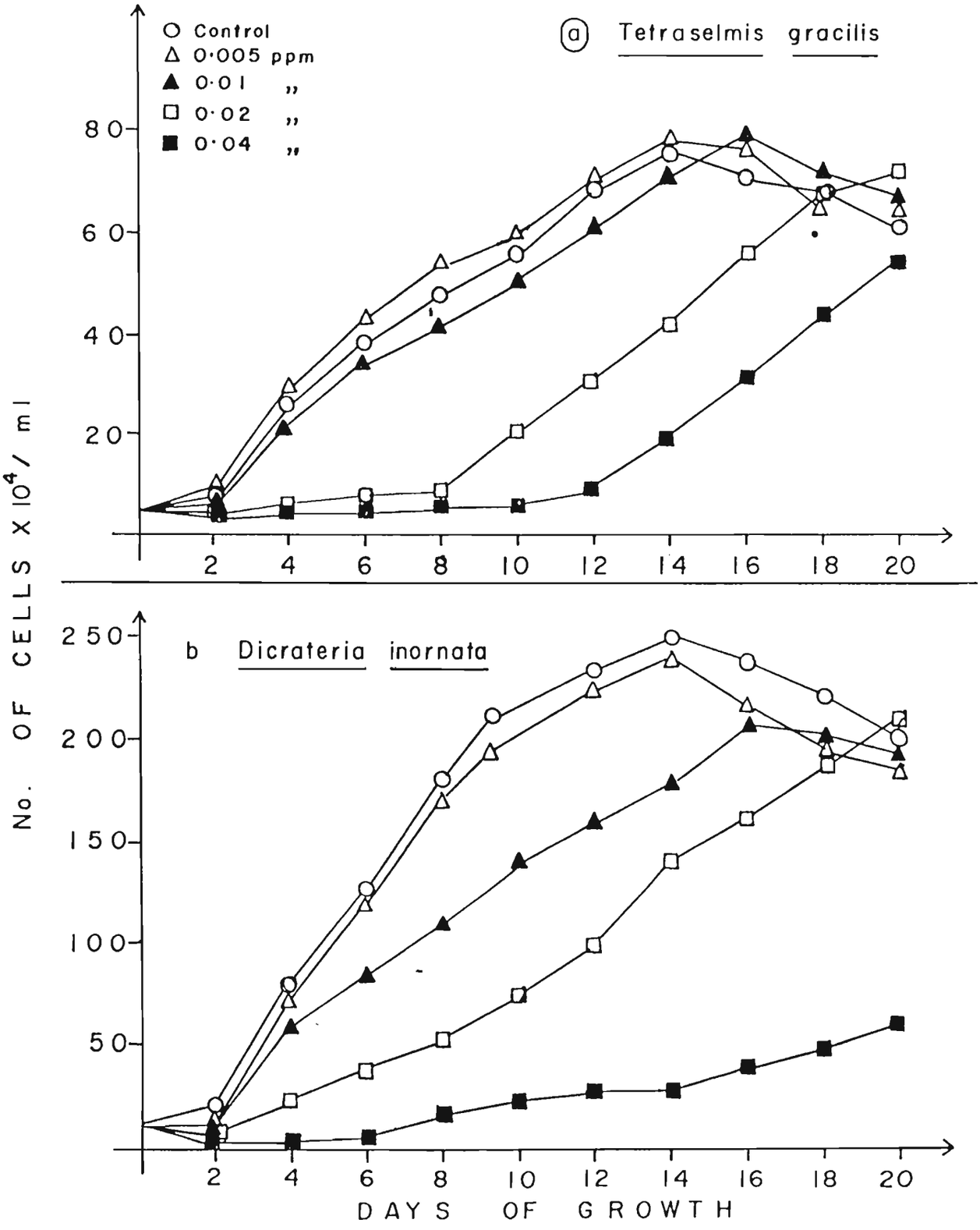
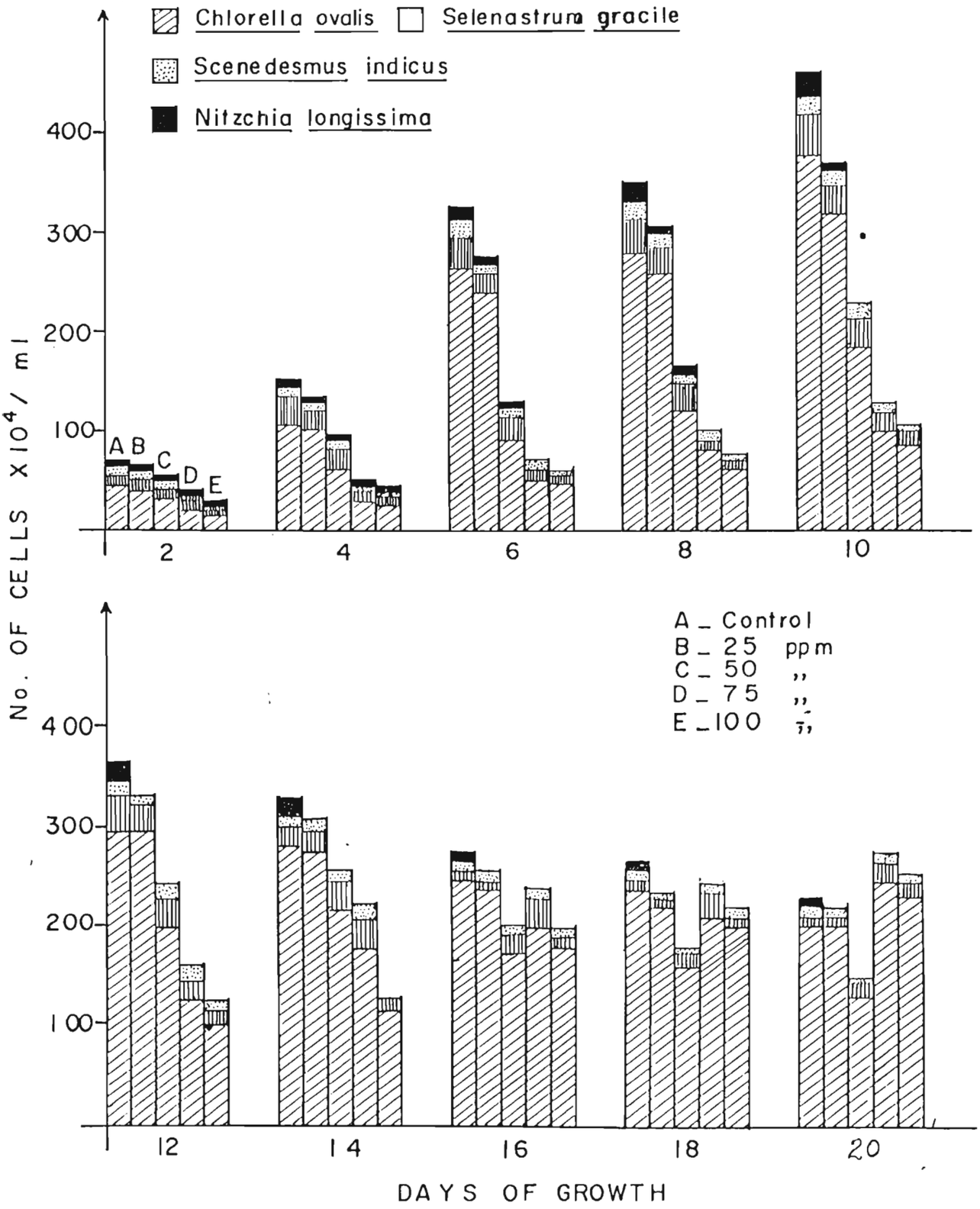


FIG.10.

EFFECT OF CUMAN L® ON GROWTH OF FRESH WATER MIXED CULTUREOF MICROALGAE



C. Mixed culture of freshwater microalgae

Figure 10 shows the effect of various concentrations of fungicide on the growth of mixed culture of freshwater microalgae. This fungicide did not show any stimulation even for the lowest concentration of 0.005 ppm The highest concentration tested showed reduced growth at the beginning. But as the culture grew this inhibition of growth was changed and the cells grew gradually and when it reached the 20th day of growth the highest concentration tested showed enhanced growth compared to control and the lower concentration tested. The N. longissima with very low cell number survived for 4 days only, with the higher concentrations. When the culture attained 10 days growth N. longissima was present in control ad 0.005 ppm concentration only. After the 12th day of growth the diatom did not survive in any of the fungicide treated flasks.

Analysis of variance revealed that the growth of Chlorella ovalis had highly significant response with the four treatments and also between days. But in the case of Selenastrum gracile and Scenedesmus indicus the effect of fungicide was not significant over growth. The diatom species tested showed significant difference in their growth with the four different concentration of fungicide. But over days there was no significant variation in growth (Table 12).

IV. **Effect of mixture of biocides on microalgae**

6. Mixture of 5 biocides

Mixture of biocides was prepared using three insecticides, one herbicide and one fungicide. Range finding test was conducted using this mixture of biocides for three microalgal culture under investigation and

the test showed that the growth of algae was totally inhibited above 0.5 ppm concentration. The sub lethal concentrations of this mixture of biocide used for the present investigation were 0.05,0.1,0.2 and 0.4 ppm.The effects of these concentrations on the microalgae are as follows:

A. Tetraselmis gracilis

Figure 11 a shows the effect of various concentrations of biocide mixture on the growth of microalgae. The lowest concentration of 0.05 ppm showed an enhancement of growth over control. In the beginning 0.1 ppm concentration of mixture showed a little enhancement over control and the same concentration showed diminished growth for the rest of the days. The peak growth was observed on the 16th day. The two higher concentrations tested showed very low cell number at the beginning, but as the culture grew the cell number gradually increased. After 20 days of growth, the highest concentration showed the maximum cell number compared to all other treatments.

Analysis of variance revealed that there was highly significant variation in growth of microalgae with different concentrations of biocide mixture (Table 12).

B. Dicrateria inornata:

With the mixture of five biocides D. inornata showed variation in growth rate which is in comparison with that of T. gracilis. The lower concentration of 0.05 ppm showed enhanced growth rate throughout the experimental period (Fig. 11 b). The higher concentration of 0.2 ppm and

and 0.4 ppm showed diminished growth at the beginning but as the culture grew, the cell number gradually increased and it showed the highest cell number over other treatments after 20 days of growth.

Analysis of variance revealed that there was highly significant variation in growth of microalgae with four treatments for the 20 days growth (Table 12).

C. Mixed culture of freshwater microalgae.

Figure 12 shows the effect of mixture of biocides on mixed fresh water algae. The lowest concentration of 0.05 ppm enhanced the growth in the mixed culture, particularly that of Selenastrum gracile, on the 2nd day only. On the 4th day the lowest concentration showed the same number of cells as that of two controls. Afterwards the growth diminished. Concentration of 0.1 ppm showed low growth rate compared to control and the peak growth was observed on the 14th day. But the diatom sp. did not survive in this concentration after 10th day. Concentration of 0.2 ppm and 0.4 ppm mixture of biocide showed very low growth rate. N. longissina didn't survive after the 2nd day of experiment. At 0.4 ppm concentration, when the culture reached 16th day of growth,it was represented by C. ovalis only.

Analysis of variance revealed that in the case of C. ovalis there was highly significant variation in growth rate, between treatment and between days. The same was the case with other three species. But between days the effect of biocide mixture on growth of N. longissima was not significant (Table 12).

7. Mixture of 3 biocides

One insecticide - 'Nuvacron', one herbicide - 'Gramaxone', and one fungicide. 'Cuman L$^{(R)}$' were mixed together and the effect of this mixture on the three microalgal culture was investigated. The range finding test conducted using this mixture showed that the growth of microalgae was totally inhibited above 0.5 ppm. The effect of sublethal concentration of mixture of the three biocides on microalgae are as follows:

A. Tetraselmis gracilis

Figure 13 a shows the effect of four different concentrations of mixture of three biocides on growth of T. gracilis. The lowest concentration of 0.05 ppm showed an enhancement of growth upto the 6th day. Afterwards upto the 12th day growth rate was less compared to control. The culture showed its peak growth on the 14th day. Concentration of 0.1 ppm showed diminished growth over control, the peak growth was attained on the 18th day only, i.e., two days after normal period. The two higher concentrations tested showed very less cell number compared to control at the beginning. Afterwards the culture grew gradually and when it reached 20 days of growth the culture showed the highest cell number compared to control.

Analysis of variance revealed that there was highly significant variation in growth of microalgae with different concentrations of toxicant and also between different days (Table 12).

B. Dicrateria inornata

This species showed variation in growth rate as a result of treatment with three biocide mixture. The lowest concentration of 0.05 ppm showed an enhanced growth upto the 6th day. All other concentration tested showed diminished growth. For the higher concentration tested, eventhough there was a diminished growth at the beginning, as the culture grew, the initial inhibition was got over and when the culture attained 20 days of growth all the flasks showed almost the same cell number (Fig. 13 b).

Analysis of variance revealed that there was highly significant variation in growth of microalgae with different concentration of toxicant mixture and also between different days (Table 12).

C. Mixed culture of freshwater micro algae

Figure 14 shows the variation in growth of mixed culture of micro algae as a result of treatment of three biocide mixture. The lower concentration of 0.05 ppm showed an enhanced growth upto the 8th day. Afterwards no increased growth rate compared to control was observed. The culture attained its peak growth on the 10th day. The two higher concentrations tested showed very diminished growth rate at the beginning. But afterwards the cells grew rapidly and attained peak growth on 14th and 16th day for 0.2 ppm and 0.4 ppm respectively. At 0.4 ppm concentration N. longissima survived upto the 4th day only when the culture attained the 14th day, the diatom sp. was represented in control and 0.05 ppm concentration only.

FIG. II

EFFECT OF MIXTURE OF 5 BIOCIDES ON GROWTH OF MICROALGAE

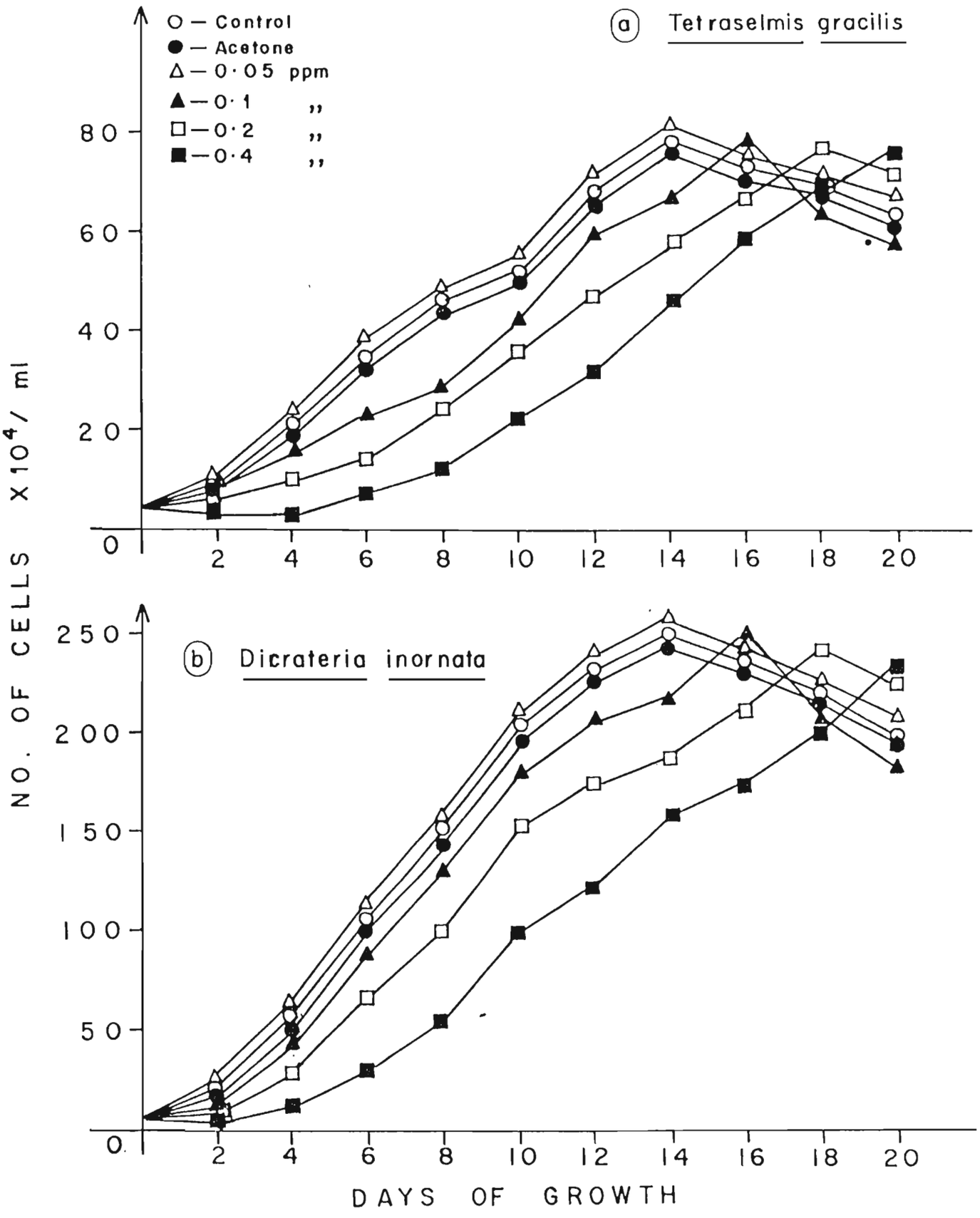


FIG. 12

EFFECT OF MIXTURE OF 5 BIOCIDES ON GROWTH OF MIXED CULTURE OF FRESH WATER ALGAE

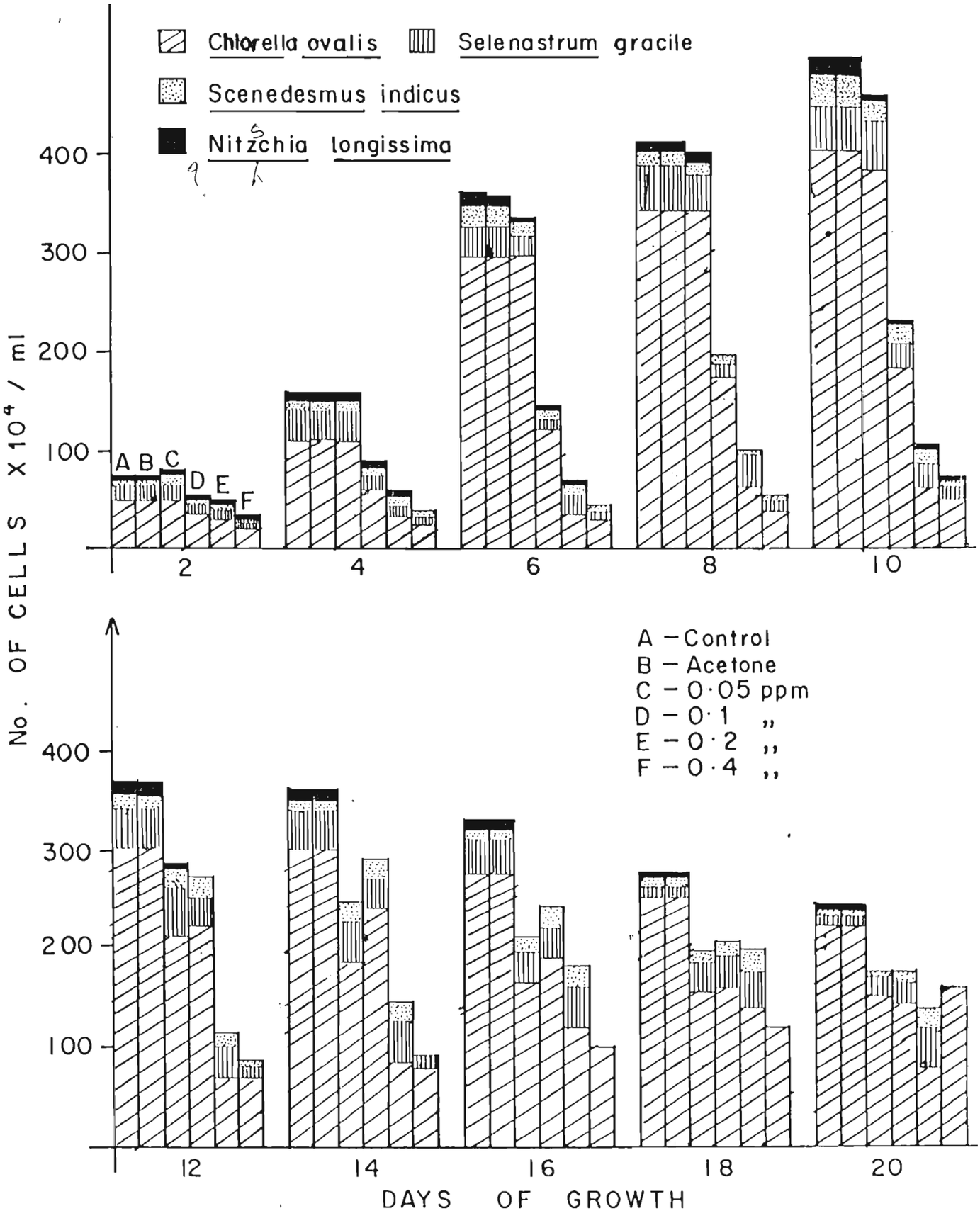
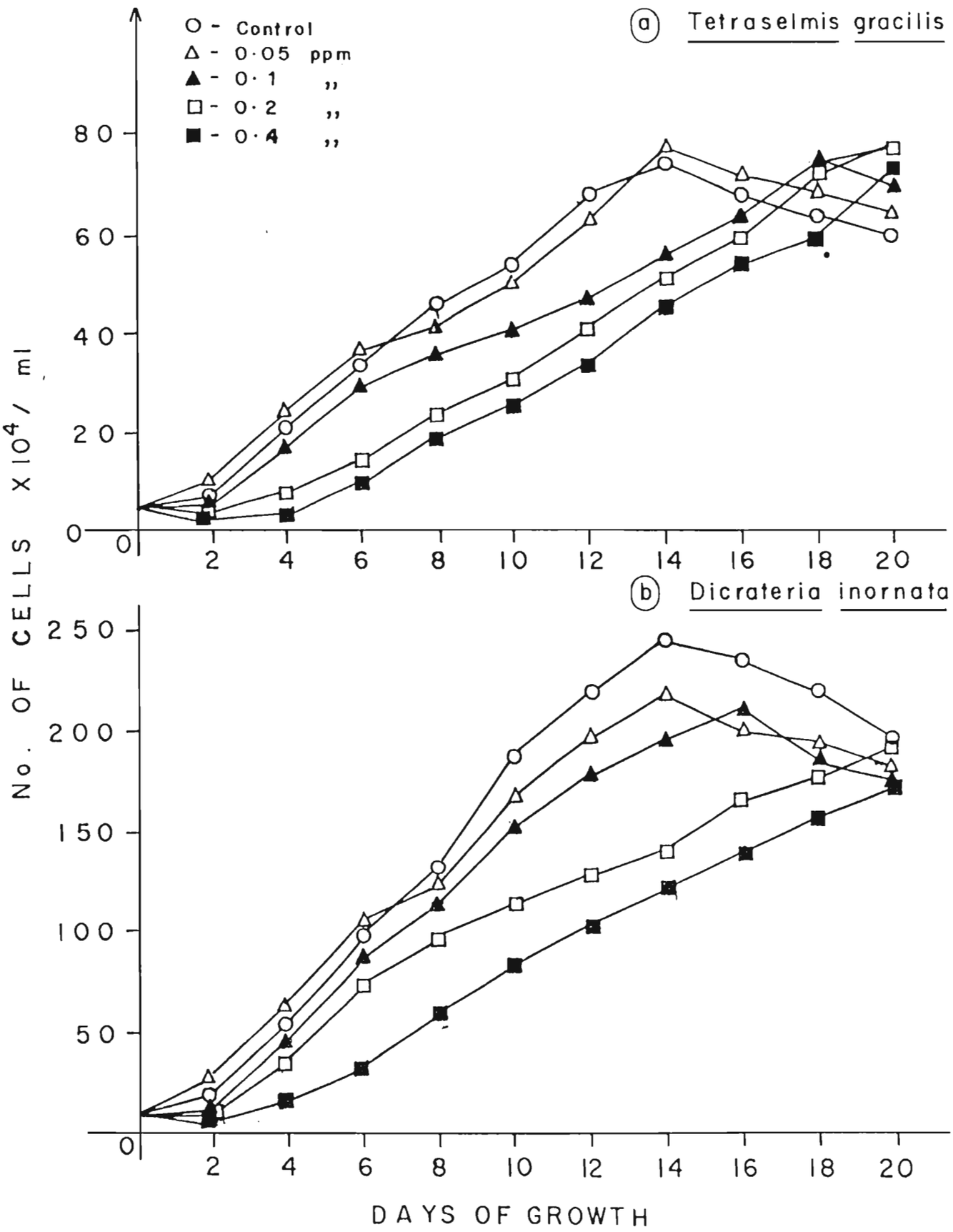


FIG.13

EFFECT OF MIXTURE OF 3 BIOCIDES ON GROWTH



EFFECT OF MIXTURE OF 3 BIOCIDES ON GROWTH OF MIXED CULTURE OF FRESH WATER MICROALGAE

FIG.14

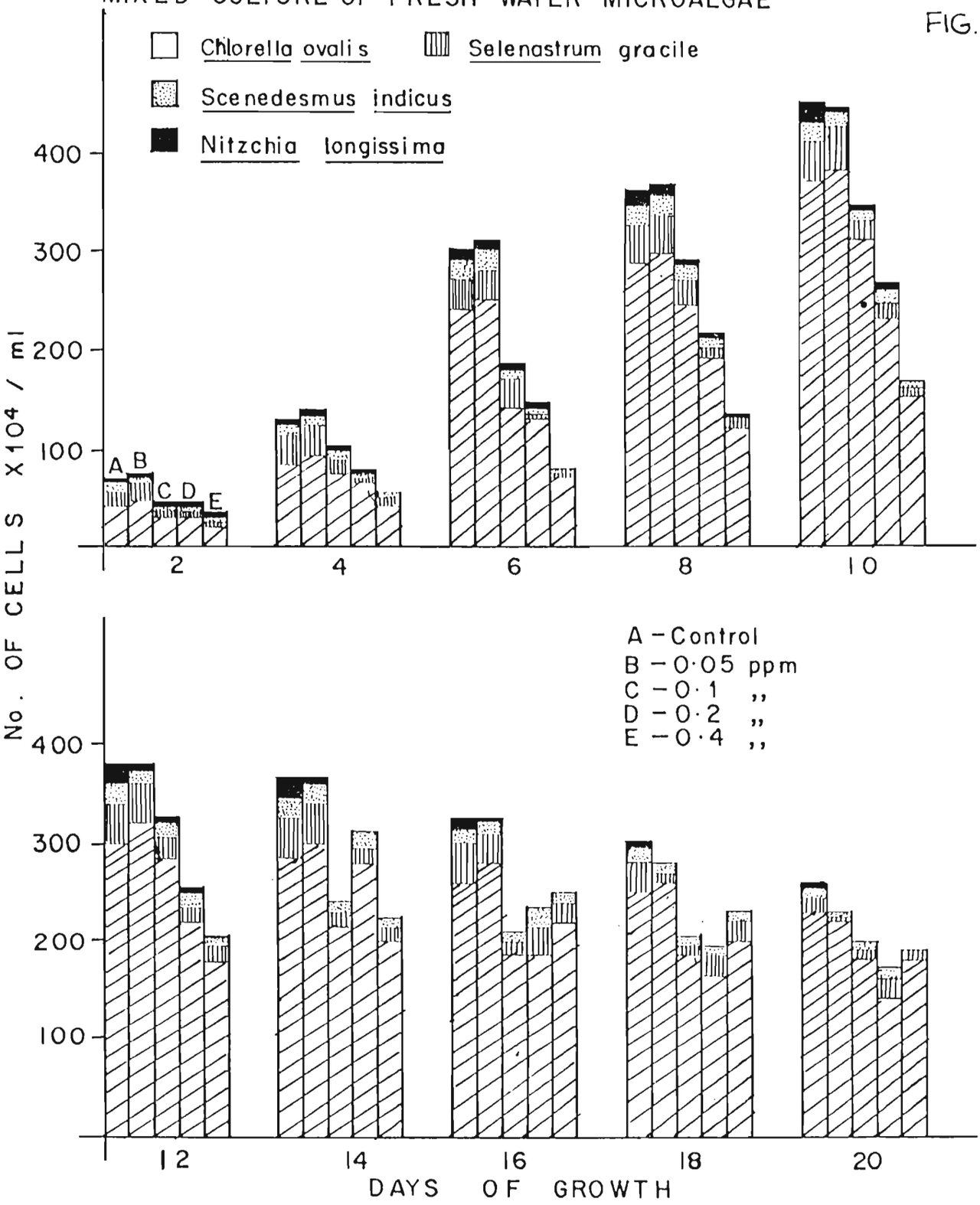


TABLE-11. ANOVA TABLE FOR GROWTH OF MICROALGAE WITH THREE INSECTICIDES.

| Insecticide | Source | D.F | Tetraselmis gracilis | | | Dicreteria inornata | | | Fresh water mixed culture | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | Chlorella ovalis | | | Selenastrum gracile | | | Scenedesmus indicus | | | Nitzenia longissima | | |
| | | | S.S | M.S | F | S.S | M.S | F | S.S. | M.S | F | S.S | M.S | F | S.S | M.S | F | S S | M S | F |
| 'B H C ' | Treatment | 5 | 0.2399 | 0.0479 | 11.91** | 4.3676 | 0.8735 | 32.85** | 8.4828 | 1.6966 | 19.59** | 0.0824 | 0.0165 | 9.65** | 0.4967 | 0.0994 | 18.54** | 0.0438 | 0.0088 | 13.18** |
| | Replicate | 9 | 2.5241 | 0.2805 | 69.61** | 29.5813 | 3.2868 | 123.59** | 41.3862 | 4.7096 | 54.39** | 0.1107 | 0.0123 | 7.20** | 0.2538 | 0.0282 | 5.26** | 0.0329 | 0.0037 | 5.50** |
| | Error | 45 | 0.1813 | 0.0040 | | 1.1967 | 0.0266 | | 3.8959 | 0.0866 | | 0.0768 | 0.0017 | | 0.2411 | 0.0054 | | 0.0298 | 0.0007 | |
| 'Nuvacron ' | Treatment | 4 | 0.1679 | 0.0419 | 14.70** | 9.1327 | 2.2874 | 36.18** | 11.7288 | 2.9322 | 25.23** | 0.0494 | 0.0124 | 14.39** | 0.2734 | 0.0684 | 14.59** | 0.0473 | 0.0118 | 4.89** |
| | Replicate | 9 | 2.4677 | 0.2742 | 96.02** | 13.8467 | 1.5385 | 24.38** | 29.2565 | 3.2507 | 27.97** | 0.0763 | 0.0085 | 9.88** | 0.1352 | 0.0150 | 3.21** | 0.0479 | 0.0053 | 2.21* |
| | Eroor | 36 | 0.1028 | 0.0029 | | 2.2714 | 0.0631 | | 4.1840 | 0.1162 | | 0.0309 | 0.0009 | | 0.1686 | 0.0047 | | 0.0870 | 0.0024 | |
| 'Carbaryl Sevin ' | Treatment | 5 | 0.0357 | 0.0089 | 20.41** | 11.902 | 2.3805 | 17.79** | 15.6852 | 3.1370 | 17.72** | 0.0917 | 0.0183 | 2.55** | 0.2043 | 0.0409 | 4.92** | 0.0518 | 0.0104 | 15.93** |
| | Replicate | 9 | 0.2997 | 0.2555 | 583.96** | 13.434 | 1.4926 | 11.10** | 41.7742 | 4.6416 | 26.22** | 0.1209 | 0.0134 | 1.87** | 0.2723 | 0.0303 | 3.64** | 0.0181 | 0.0020 | 3.09** |
| | Error | 45 | 0.0158 | 0.0004 | | 6.0215 | 0.1338 | | 7.9656 | 0.1770 | | 0.3239 | 0.0072 | | 0.3738 | 0.0083 | | 0.0293 | 0.0007 | |

** Significant at 1% level, * - Significant at 5% level, N.S. - Not Significant

TABLE-12. ANOVA TABLE FOR GROWTH OF MICROALGAE WITH FOUR TOXICANTS.

| Toxicant | Source | D.F | Tetraselmis gracilis | | | Dicrateria inornata | | | Fresh water mixed culture | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | Chlorella ovalis | | | Selenastrum gracile | | | Scenedesms indicus | | | Nitzchia longissima | | |
| | | | S.S | M.S | F | S.S | M.S | F | S.S. | M.S | F | S.S | M.S | F | S.S | M.S | F | S S | M S | F |
| Herbicide Gramaxone | Treatment | 4 | 0.7518 | 0.1879 | 25.79** | 8.5168 | 2.1292 | 12.54** | 10.3025 | 2.5756 | 34.47** | 0.0347 | 0.0087 | 5.17** | 0.2014 | 0.0504 | 10.07** | 0.0628 | 0.0157 | 8.77** |
| | Replicate | 9 | 1.5411 | 0.1712 | 23.49** | 9.9486 | 1.1054 | 6.51** | 31.9725 | 3.5525 | 47.55** | 0.0240 | 0.0027 | 1.59 N.S | 0.0751 | 0.0083 | 1.67 N.S | 0.0186 | 0.0021 | 1.16 NS |
| | Error | 36 | 0.2624 | 0.0073 | | 6.1104 | 0.1697 | | 2.6897 | 0.0747 | | 0.0603 | 0.0017 | | 0.1799 | 0.0050 | | 0.0643 | 0.0018 | |
| Fungicide (R) Ciman L | Treatment | 4 | 0.9537 | 0.2384 | 20.36** | 15.3953 | 3.8488 | 34.51** | 13.7868 | 3.4467 | 12.88** | 0.0193 | 0.0048 | 2.4 N.S | 15.4124 | 3.8531 | 0.88 N.S | 0.0646 | 0.0161 | 8.49** |
| | Replicate | 9 | 1.8905 | 0.2101 | 17.94** | 14.7319 | 1.6369 | 14.67** | 20.1924 | 2.2436 | 8.38* | 0.0346 | 0.0039 | 1.99 N.S | 41.9966 | 4.6663 | 1.06 N.S | 0.0154 | 0.0017 | 0.89 N.S |
| | Eroor | 36 | 0.4215 | 0.0117 | | 4.0149 | 0.1115 | | 9.6373 | 0.2677 | | 0.0695 | 0.0019 | | 158.110 | 4.3919 | | 0.0684 | 0.0019 | |
| Mixture of 5 Biocides | Treatment | 5 | 0.2470 | 0.0494 | 11.62** | 0.8813 | 0.1763 | 5.29** | 25.232 | 7.0465 | 20.52** | 0.1354 | 0.0271 | 7.84** | 0.5566 | 0.1113 | 15.75** | 0.1289 | 0.0258 | 4.56** |
| | Replicate | 9 | 3.1283 | 0.3476 | 81.75** | 30.8203 | 3.4245 | 102.94** | 20.848 | 2.3165 | 6.75** | 0.1035 | 0.0115 | 3.33** | 0.2996 | 0.0333 | 4.71** | 0.0528 | 0.0059 | 1.04 N.S |
| | Error | 45 | 0.1913 | 0.0043 | | 1.4970 | 0.0333 | | 15.450 | 0.3433 | | 0.1554 | 0.0035 | | 0.3179 | 0.0071 | | 0.2545 | 0.0057 | |
| Mixture of 3 Biocides | Treatment | 4 | 0.1732 | 0.0433 | 8.38** | 3,3039 | 0.8260 | 20.14** | 7.0271 | 1.7568 | 16.24** | 64.142 | 16.036 | 1.04 N.S | 0.3656 | 0.0914 | 15.97** | 0.0463 | 0.0116 | 6.77** |
| | Replicate | 9 | 2.4794 | 0.2755 | 53.32** | 19.175 | 2.1306 | 51.95** | 30.437 | 3.3819 | 31.26** | 141.395 | 15.711 | 1.02 N.S | 0.1995 | 0.0222 | 3.87** | 0.0147 | 0.0016 | 0.96 N.S |
| | Error | 36 | 0.1859 | 0.0052 | | 1.477 | 0.0410 | | 3.8947 | 0.1082 | | 556.91 | 15.469 | | 0.2061 | 0.0057 | | 0.0616 | 0.0017 | |

** - Significant at 1% level, * - Significant at 5% level, N.S. - Not significant

Analysis of variance revealed that C. ovalis and S. indicus had highly significant variation in growth rate with the four different concentration and also between days. But for Selenastrum gracile the effect of toxicant on growth was not significant. For N. longissima the different concentration tested was found to be highly significant. But between days there was no significant variation (Table 12).

## DISCUSSION

### 1. Organochlorine insecticide - 'BHC'

Because of the widespread use of organochlorines during the past several decades, the toxic effects of these insecticides have been studied by various investigators ( c.f. Rajaretnam et al., 1987.)

In the present study due to the low water solubility of the insecticide acetone treated control was also tested to find out whether acetone had ay toxic effect on microalgae. From the result it was observed that acetone has no inhibitory or stimulatory effect on algae. This was in conformity with the findings of Ramachandran et al., (1980), Stratton and Croke (1981) and Rajendran and Venugopalan (1983). They reported that the acetone treated cultures had the same growth rate as in control.

Except for mixed culture of freshwater microalgae the lowest concentration of 'BHC' tested showed an enhancement of growth over control. As already indicated in the literature review, the different species of phyto-

plankton responded differently to the organochlorines(c.f.Rajaretnam et al., 1987). This has been found in this investigation also. Algal stimulation by organochlorines was already reported by different investigators (Raghu and Mac Rae, 1967; Menzel et al., 1970; Mosser et al., 1972 a; Rajaretnam et al., 1987).

The present investigation shows that in T. gracilis the 0.5 ppm concentration of BHC showed an enhanced growth than control throughout the experimental period. The same was the case with the marine species D. inornata. Concentration of 1 ppm of 'BHC' showed an enhanced growth upto 4th day in T. gracilis. However for this concentration, D. inornata showed no enhancement in growth. But the growth rate was almost similar to control . Algal stimulation by organochlorines has been already dealt with (Ukeles 1962) reported that at 5 ppm 'Y - BHC' as lindane did not prevent the growth of marine phytoplankton cultures and at 1 ppm even stimulated the growth of Protococcus sp. Raghu and Mac Rae (1967) reported that Y - BHC promoted algal growth in rice fields. DDT exhibited no toxic properties upto a concentration of 100 mg/l as reported by Christie (1969). Mosser et al., (1972 b) reported that upto 100 ppb concentration of DDT, Dunaliella tertiolecta showed increased growth rate over control. Patin (1982) observed a fairly varigated pattern of reaction of phytoplankton cells in the presence of chloroorganic toxicants in the medium. In several cases they observed enhanced (more than 100 per cent) rates of cell division.

Rajaretnam et al., (1987) showed that the growth of diatoms was stimulated in lower concentration of DDT and Heptachlor-two organochlorine

pesticides. Lin Yi Xing and Sun Bozen (1987) showed that the growth did not differ much from control values at 0.1-10 $mgl^{-1}$ concentration of lindane.

In the present study the growth increase on account of low concentration of 0.5 ppm of BHC may be because the algal strains, particularly T. gracilis and D. inornata utilized this as nutrient which favoured and growth of algae. But in the case of freshwater species no stimulation of growth was observed.

Population growth inhibition is a reasonable determinant of toxic effects (Fisher and Frood, 1980). In the present study the growth of all the species was inhibited with the increase of 'BHC' concentration. Even though some growth stimulation was apparent in low concentrations of pesticides for T. gracilis and D. inornata, the higher concentration invariably affected the growth of all species, particularly freshwater species. There are plenty of reports regarding the inhibitory action of organochlorines. Ukeles (1962) demonstrated that DDT could depress the reproduction of Proto coccus of 50% at 600 ppb. The effect on growth of diatom Skeletonema costatum by DDT was repeatedly reported by many authors (Menzel et al., 1970; subramanian et al., 1979; Rajaretnam et al., 1987).

The pesticides primarily affect the cell division as reported by Fisher (1975). The results of present investigation shows that the cell division is delayed and the exponential growth of the culture attained one or two days after the normal period indicating the extended lag phase of growth of treated cultures.

The mixed culture of freshwater microalgae was dominated by Chlorella ovalis. The other three species was represented by only very limited number of cells. It was observed that the species with lower cell number was severelly affected by the effect of 'BHC'. This was in conformity with the result of Wurstur (1968) who reported that the toxicity to diatoms increased as cell concentration decreased. This inhibition may be of ecological importance

The results of the effect of 'BHC' on microalgae, particularly the estuarine form T. gracilis showed that the concentration of BHC at 2 ppm and 4 ppm had inhibited growth at the beginning but later they recovered and enhanced growth when the culture attained 20 days growth. This result was in line with that of Powers et al., (1975), that organochlorine toxicant is absorbed or adsorbed by the cells and the degree of growth inhibition is presumably determined by the concentration of toxicant per cell. As the cells divide, the concentration of toxicant per cell is decreased. This process would reduce growth inhibition, allowing cell growth rates ultimately return to normal.

Ramachandran et al., (1980) reported the growth inhibition of diatom with 1 to 50 ppb concentration of DDT. Rajendran and Venugopalan (1983) found that lindane and endosulfan decreased the cell number even at 2 ppb concentration. DDT at 0.1 ppm concentration inhibited the growth of Chlorella fusca as reported by Goulding and Ellis (1981). They concluded that the amount of inhibition varied with time and also the method of growth assessment. Lin Yi Xing and Sun-Bozen (1987) reported that lindane at a concentration of 50-100 $mgl^{-1}$ growth of microalgae was inhibited.

The above reports show that eventhough all these pesticides come under organochlorines, they differ in their toxicity. The difference in the concentration of organochlorines as reported by various investigators may be due to difference in species tested and also due to difference in growth assessment.

The three microalgal cultures presently experimented showed various effects as a result of 'BHC' treatment. The reduction in growth rate ultimately would lead to decreased production and extinction of organism, and also would affect the whole production sequence of that particular ecosystem. The 'BHC' contamination above 5 ppm concentration may lead to total imbalance in the natural ecosystem.

2. **Organophophate insecticide - 'Nuvacron'**

Most of the earlier reports suggest that organophosphates are less toxic than organochlorines (Ukeles, 1962; Ramachandran et al.,1980; Rajendran and Venugopalan, 1983). This aspect was also studied in the present investigation.

The effect of four different concentrations of 'Nuvacron'on growth of three microalgal culture showed varying results. Concentration of 25 and 50 ppm of 'Nuvacron' exhibited stimulation of algal growth in T. gracilis and D. inornata. But the stimulation by 25 ppm concentration was evidenced throughout the experiment. For 50 ppm concentration after initial stimulation, the cell number declined over control. The stimulation of algal growth

by organophosphates was already reported (Rath and Misra, 1981; Ibrahim 1983; Von Donk et al., 1992). Rath and Misra (1981) tested an organophosphate insecticide Dimecron 100 for its nutritional value in relation to growth of Oscillatoria at 400 ppm, and observed increased growth rate in 'Dimecron' treated algae. Ibrahim (1983) also reported that two organophosphates, Bayluscide at concentrations of 10 to 20 ppb, and Dimethoate at concentrations of 2.5 and 5 ppm enhanced the growth of Phaeodactylum tricornutum. Butcher et al., (1977) and Von Donk et al., (1992) also have the opinion that organophosphate insecticide Dursban 4E stimulated the phosphorus limited green algae at a lower concentration of 0.03 $mgl^{-1}$.

Stimulation of algal growth by organophosphates lead to the assumption that the algal strains might have the ability to metabolize the lower concentrations of insecticide. This confirms the findings of Butler et al., (1975 a) who speculated that certain algal strains might metabolize the pesticides. Butcher et al., (1977) have the opinion that the algal stimulation as a result of organophosphate application may be due to an increase in nutrients especially. phosphorus resulting from degradation of compound. It is an established fact that unlike organochlorines, organophosphates can be easily degraded. The degradation and metabolism of organophosphate insecticide by microalgae has been reported by Megharaj et al., (1986) and Lakshminarayana and Bourque (1980).

Mixed culture of freshwater microalgae under investigation showed no stimulatory effect as a result of 'Nuvacron' application. It may be related to the fact that these algal strains may not have the capacity to degrade the particular insecticide.

For 75 and 100 ppm concentration of 'Nuvacron' the growth of algae was inhibited. But in T. gracilis the initial inhibition of growth was changed and as the culture grew the number of cells also increased and when it reached the 20th day, the cell number exceeds that of control values. It showed that after the initial inhibition of toxicants the cells acclimatized to the toxicants and also the toxicants would have undergone degradation and the degraded metabolites might have acted as bioactive substance for the test organism. The results showed that the estuarine species T. gracilis was resistant and it could tolerate the 'Nuvacron' concentration even above 100 ppm. This was in conformity with the findings of christie (1969) who found that 100 ppm 'malathion' had little significant effect on the green alga Chlorella pyrenoidosa.

For the marine species D. inornata 75 and 100 ppm concentration may be considered as algistatic in its effect. Initial growth for 8 days showed a decline in growth but afterwards the growth rate increased upto the 14th day. Later it declines again, this explains the fact that 75 and 100 ppm concentrations have algistatic effect upto the 8th day. After 14th day of experiment the growth again declined showing that this particular species, unlike T. gracilis could not utilize the large amount of metabolites after a certain level.

In the mixed culture the dominant species C. ovalis was not much affected by 'Nuvacron'. But the growth of other species especially N. longissima was affected. This species did not survive in the culture after

10th day of experiment. One the reasons may be that the diatom was represented by only very little cell number.

Growth inhibition by organophosphates has been reported by several investigators. Ramachandran et al., (1980) reported that, Dimethoate inhibited the growth of diatom even at very low concentration of 1 ppb. The 10 ppm concentration of 'Malathion' was found to inhibit the growth of Chlorella as reported by Saroja and Bose (1982). Ibrahim (1983) also reported that two organophosphates 'Dimethoate' and Bayluside inhibited the growth of unicellular algae. Prevot and Soyer (1985) have confirmed the findings of Saroja and Bose (1992) who found that even at 10 ppm of 'Malathion' the growth of dinoflagellate was inhibited.

The inhibition of growth of microalgal culture may be because of the decreased growth rate as a result of the delayed cell division due to the 'Nuvacron' application.

The present investigation using 'Nuvacron' with three microalgal culture leads to the conclusion that eventhough T. gracilis showed some tolerance to the 'Nuvacron' above 100 ppm, the other species, particularly fresh water populations were seriously affected by concentrations above this level.

3. Carbamate insecticide 'carbaryl sevin'

Due to low water solubility, acetone was used as the first solvent. As already stated in the case of 'BHC', the acetone treated cultures showed similar growth as in control.

In the case of T. gracilis the lower concentration of 2 ppm showed an enhancement of growth over control throughout the experimental period. But the other two cultures tested i.e., D. inornata and the mixed fresh water culture did not show any stimulation of growth even with the lowest concentration tested.

Reports about stimulation of algal growth by carbaryl insecticide are scarce. Stadnyk et al., (1971) have noticed stimulation in cell number when cultures of Scenedesmus quadricauda were treated with 0.1 and 1 ppm carbaryl. Ramachandran et al. (1980), Rajendran and Venugopalan (1983) reported that the carbamate pesticide sevin exerted no significant effect upto 20 ppb concentration.

The present studies revealed that, in the case of T. gracilis 6 ppm and 8 ppm concentration of sevin has diminished growth at the beginning. But with the age of the culture, the growth rate also increased. Under the four different concentrations tested D. inornata and mixed fresh water culture showed growth inhibition at the beginning.

Growth inhibition by carbaryl insecticide has already been reported. But different concentrations of sevin which inhibit the growth of different microalgae may vary. Ukeles (1962) found that carbaryl toxicity was dependent upon the concentration and the alga being tested. Growth of five unicellular algae was stopped (algicidal) or cell division prevented (algistatic) by 10 ppm carbaryl. Christie (1969) found that carbaryl was more

toxic in an acid environment and at pH 6 any concentration above 0.1 ppm would inhibit the growth of Chlorella pyrenoidosa. Butler et al., (1975b) reported an inhibited growth at 10 ppm and at 25 ppm growth was suspended They concluded that because carbaryl is unstable and is subject to high rates of photolysis and hydrolysis, it is likely that the breakdown products are influencing the algal growth.

Kentzer et al., (1984) reported that at a concentration of 10 ppm, the carbaryl sevin inhibited the cell multiplication of Chlorella and Anacystis in the laboratory culture. The present investigation also showed that concentration above 8 ppm stopped growth for the three microalgal culture. But this was in contradiction to the existing reports of Khalil and Mostafa (1986) and Kobbia et al., (1991). They reported that a carbamate insecticide "methomyl" showed no significant effect on the growth of fresh water alga - Phormidium fragile upto a concentration of 112.5 ppm. But according to them the same insecticide inhibited growth of Nostoc muscorum and Tolypothrix tenuis at a concentration of 100 ppm.

Thus it can be concluded that the effect of carbaryl sevin on microalgae will depend on many factors. The results of present investigation revealed that in concentrations above 8 ppm the growth of algal population may be eliminated.

4. **Herbicide - 'Gramaxone'**

Apart from their widespread use in agriculture, the herbicides are also used for aquatic weed control. It is therefore important that these

herbicides be assessed for any deleterious effects on the ecology and quality of treated waters.

The four different concentration of 'Gramaxone' employed in three cultures showed varying results. With T. gracilis the two lower concentration of 0.2 and 0.4 ppm showed an enhancement of growth over control upto 4th day, and then growth diminished compared to control. But in the case of D. inornata concentration of 0.2 ppm showed enhanced growth through out the 20 days period. For mixed culture little enhancement was observed at the beginning for 0.2 ppm concentration. So the result revealed that 0.2 ppm of 'Gramaxone'had some stimulatory effect on growth of algae.

Algal stimulation by herbicides has already been reported. Walsh et al., (1970) observed stimulation of algal growth and photosynthesis with 10 ppm 2, 4, D. This was also supported by Bednarz (1981 a), who found that low concentration of 2, 4 D, usually stimulated the growth of algae. The stimulation of algal growth with simazine has been reported already (Francois and Robinson, 1990). Austin et al., (1991) reported that, an organophosphate herbicide 'Glyphosate (R), enhanced growth above control values which suggested that the phosphate constituent of this herbicide might be acting as a nutrient.

The inhibitory effect of 'Gramaxone' follows a peculiar character as revealed by the result. The two species tested i.e, T. gracilis and D. inornata showed that concentration of 0.8 and 1.6 ppm effected almost the same growth rate compared to control upto 8th day in T. gracilis and

only upto 4th day in D. inornata. Afterwards the number of cells decreased showing declined growth. But when it attained a growth of 12 days again the cell number increased. this was in agreement with the finding of Abouwaly et al., (1991 a) who found that the herbicide Hexazinone treated cultures of Selenastrum capricornutum recovered substantially in 7 days time after treatment.

There are good amount of literature on the inhibition of growth by herbicides. Growth of unicellular algae was inhibited by two herbicides MCPB and MCPA as reported by Krikwood and Fletcher (1970). Atrazine and 2, 4, D were also found to inhibit the growth of algae at different concentrations (Butler et al., 1975 a). Plumley and Davis (1980) reported that at 2.2 ppm concentration of herbicide 'atrazine', the cell number in unialgal cultures was reduced. Inhibition of growth rate by low concentration of atrazine was reported by stratton (1984) and Mayasich et al., (1986).

Bednarz (1981 b) reported that the various effects of herbicide on microalgae are rather caused by the action of extra cellular secretion of algae. It is also possible that the joint action of pesticides and extracellular secretions can increase the sensibility of algae to the pesticides.

Millie et al., (1990) reported that herbicide - fluridone inhibited biomass in Oscillatoria agardhii upto a concentration of 100 ppb. The decrease in cell biomass with increasing fluridone concentration coincided with the reduction in growth. Francois and Robinson (1990) also reported the inhibitory effect of herbicide 'Terbutryn' on Chlamydomonas geitleri.

The use of herbicide for the present investigation 'Gramaxone' (Paraquat) and already tested and results were reported by Ibrahim (1990). Contrary to his reports, in the present investigations, the inhibitory concentration of 'Gramaxone' was found varying and this has been reported in the section dealing with the results of bioassay studies.

Abouwaly et al., (1991 b) reported that reduction in growth was observed with an increase of atrazine concentration.

As reported by other workers, the present investigation clearly showed that at higher concentrations upto 1.6 ppm the decrease in cell biomass retarded as a result of reduction in growth rate. The difference of inhibition observed for different algae is not likely a function of variation in tolerance among genotypes. It can be concluded that above 1.6 ppm concentration of 'Gramaxone' the growth of microalgae was totally eliminated.

5. **Fungicide - 'Cuman L$^{(R)}$'.**

Information on the effects of fungicides on microalgae are very much limited. The present investigation showed that T. gracilis at a concentration of 0.005 ppm made little enhancement of growth over control through out the experimental period. This was in confirmaty with the results of Rath et al., (1985, 1986). They reported that at lower concentrations, mercury based fungicides may act as growth regulator and at higher concentration it acted as a growth inhibitr. They concluded that the effect was totallay independent upon the dose of the toxicant and the duration of application.

Concentration 0.02 and 0.04 ppm of 'Cuman L(R)' was found to inhibt the growth of microalgae especially in D. inornata. Depression of growth rate by fungicide is already reported. Ethyl mercury phosphate was lethal to all marine phytoplankton species tested when incorporated at a level of 60 ppb in the culture media (Ukeles, 1962). Harriss et al., (1970) found that the organomercury fungicides, at less than 1 ppb reduced growth and photosynthesis of marine diatom Nitzchia delicatissima and also in a natural population of fresh water phytoplankton. Kosawska and Falkoswki (1984) reported that the fungicide Dithane M45 depressed 50% cell multiplication at 0.2 to 0.6 ppm in Chlorella and Scenedesmus and at 2 ppm in Anacystis nidulans.

The present investigation and results of previous authors revealed that different fungicides had varying effects to different species of micro-algae. The growth of microalgae especially D. inornata was seriously affected above 0.04 ppm concentration.

**Mixture of Biocides:**

Recognizing that in nature, organisms may be exposed to several toxicants simultaneously, experiments were conducted with two mixtures of biocides. The synergistic effect of biocide was found to vary with the effect of each biocide independently

**Mixture of 5 biocides:**

Mixture of all the five biocides tested, showed varying effects on the three species of microalgae. The lowest concentration of 0.5 ppm

stimulated the growth of algae especially in T. gracilis and D, inornata The higher concentration of 0.2 and 0.4 ppm were found to be inhibitory at the beginning but later the algae recovered from the inhibitory action of toxicant mixture. With the mixed fresh water culture the species diversity was very much affected by the toxicant mixture. Above 0.4 ppm of toxicant mixture the growth of microalgal culture was totally inhibited.

**Mixture of 3 biocides:**

Mixture of one insecticide, one herbicide and one fungicide showed almost same pattern of growth with the microalgal culture at the same concentrations tested for mixture of 5 biocides. But the percentage of inhibition showed some variations. With the freshwater culture the two mixtures showed varying results.

When the independent effect of each biocide was considered the synergistic effect of the mixture of biocide showed varying results. In this case the synergistic effect was found to be higher compared to the indedendent effect of each biocide, except for fungicide 'Cuman $L^{(R)}$. This fungicide was found to be highly toxic above 0.04 ppm. But in the mixture, the algae showed to withstand upto 0.4 ppm, for the two mixtures tested. The most obvious result was with the organophosphate insecticide. 'Nuvacron' which was found to be tolerable even at 100 ppm. But in the mixture, after 0.4 ppm it was very much affected.

Eventhough considerable amount of literature is available about the effect of different biocides on microalgae, the reports about the synergistic

effect of biocide is very little. The only one report is that of Mosser et al (1974). They investigated the effects of mixture of organochlorines on the growth of marine diatom Thalassiosira pseudonana, and it was found that the interaction of mixture of organochlorines was far more inhibitory to the algae than they were individually. This was in agreement with the results of present investigation.

From the results of the present investigation it is very clear that the effect of each biocide on the growth of three microalgal culture varied greatly. The effect of biocide and their mixtures was dependent upon a number of interrelated factors.

Three microalgal cultures investigated here are from three different habitats. T. gracilis is an estuarine form, D. inornata a marine species, and the mixed culture raised through the collection from the paddy field and they are almost freshwater species. The results of the present investigation showed that among these three cultures, the estuarine form is considered as the most resistant species. Same is the finding of other investigators like Menzel et al., (1970) and Patin (1982). They are of the opinion that euryhaline forms are much more resistant than oceanic species. In general, euryhaline forms of organisms are considered to be sturdy species, because, every now and then, they are subjected to changes in the environmental conditions. In this situation they might have acquired some resistance to the external stress. The present results clearly indicate that eventhough the biocides are causing some initial inhibition in the growth of T. gracilis, later this species got acclimatized and it showed the same growth rate as control during the end of the experiment.

Patin (1984) is of the opinion that certain freshwater forms of phytoplankton are more resistant to the effect of chloro organic compounds, even though certain species may well be selectively sensitive to individual toxicants The present result agrees with this statement. In nature, the phytoplankton community is represented by an array of different species. In this population some species may be dominant and yet some other may be represented by only very low numbers. Results of this investigation showed that the dominant species, was Chlorella ovalis which can withstand the effect of pollutants, but other species, which were only nominally represented in the culture, especially the diatom N. longissima are very much affected by the action of biocides. This type of result has been reported by some investigators like Wurstur (1968), Mosser et al., (1972b) and Moore and Harriss (1974). They are of the opinion that for a mixed culture, selective toxicity will result in changes in community structure and in the production of certain size classes of species, which may be critical food for higher trophic levels.

Among the five biocides tested, the fungicide Cuman $L^{(R)}$ was found to be most toxic i.e., above 0.04 ppm, the growth of microalgae was severely affected. The least toxic one among the five was the organophosphate insecticide as reported by various other investigators (Ukeles, 1962; Christie, 1969; Hulbert et al., 1972; Butler et al., 1975 a). In general organo phosphates are considered to be less toxic to micro algae. Rath and Misra (1981) even tested the nutritive value of an organophosphate insecticide at 400 ppm concentration and found that the algae could utilize the elements

of organophosphate insecticide. The reason for the less toxic nature of this insecticide is because that it can easily undergo degradation. Organophosphates are not persistent pollutants like organochlorines.

The three insecticides investigated here come under three categories; organochlorine, organophosphate and carbamate. The toxicity of these insecticides are in the order organochlorine > Carbamate > organophosphates But the findings of Christie (1969) showed that carbamate insecticide was more toxic than organochlornes and organophosphates. Ramachandran et al., (1980) and Rajendran and Venugopalan (1983) are of the opinion that carbamates are less toxic than organochlorines and organophosphates, the order of toxicity being organochlorine > organophosphate > Carbamates. The difference in the toxicity order of insecticides may be because of difference in the quality of insecticide used and also be difference in species tested. All insecticides under organochlorine, or organophosphate may not respond similarly as already stated. the comparison is very difficult in view of the difference in chemical and toxicological properties of different biocides and the variation in experimental procedures of different workers.

Murray and Guthrie (1980) reported that eventhough the pesticides showed an initial inhibition of growth, at a later stage the algal population approached or exceeded those of control. The present results agree with this statement.

The results clearly show that the different biocides tested react differentially with three cultures. Lower concentration may cause stimulation

But the stimulation of algal growth may also cause problems in the natural environment. As reported by Hurlbert et al., (1972) excessive algal production may cause problem of eutrophication. Excessive algal production may be because of increased nutrients which may be the results of insecticide application. Since the water draining from agricultural land bears both nutrients and insecticides, they may pose double threat to the aquatic environment.

## 2. CARBON PRODUCTION

In order to have a proper understanding on the potential direct effects of environmental contaminants like biocides on aquatic algae, it is important to first consider examples of these effects on processes at the cellular levels of organization.

As the carbon production of microalgal culture is an important measure of the physiological state of an organism, the difference in carbon production rate would clearly show the effect of various concentrations of pollutants. Both gross and net carbon production were estimated for each algal culture as an effect of biocide treatment.

The values of gross and net carbon production for three microalgal cultures investigated showed makred variations. In the case of T. gracilis which is an estuarine form of phytplankton, the maximum value of production was observed on 14th day of experiment. The values were 0.48 mgc/l/hr for gross production and 0.44 mgC/1/hr for net production (Fig. 15 and 16). But D. inornata showed maximum value of 0.56 mgC/l/hr for gross and 0.55 mgC/l/hr for net production. (Fig. 17 and 18). In the case of mixed culture of freshwater algae as shown in the figure 19 and 20 highest rates were on the 10th day of experiment. i.e. 0.4 mgC/l/hr for gross production and 0.38 mgC/l/hr for net production.

### 1. Organochlorine insecticide - 'B.H.C.'

With the organochlorine insecticide 'B.H.C.', control and acetone treated cultures showed the same trend in carbon production in the three microalgal cultures.

In T. gracilis 0.5 ppm concentration was found to stimulate the carbon production. Hence the highest value of gross carbon production which was 0.58 mgC/l/hr observed on 14th day of experiment. This was about 20% more than for control on the same day. The increased gross production at 0.5 ppm was observed upto the 18th day of experiment (Fig. 15a). Eventhough there was a well marked increase in gross carbon production with the 0.5 ppm concentration, the net carbon production did not show an increase over control (Fig. 16a). Upto the 10th day of experiment, net carbon production at 0.5 ppm concentration showed slightly increased values than control i.e. about 12.5% increase in production on 10th day. But when the culture attained 14th day, the net production values in control and in 0.5 ppm treated cultures were the same. The difference in gross and net production values of treated cultures showed that the respiratory rate was high in pesticide treated culture than control.

Concentrations of 1,2 and 4 ppm of B.H.C. invariably affected the carbon production of T. gracilis. In 4 ppm concentration, the culture shows low value of gross carbon production, and net carbon production observed at the beginning of the experiment was zero, indicating that the respiratory value was very high, compared to all other treatments. Another peculiarity observed in this case was that, at 2 and 4 ppm concentration very low production in both gross and net carbon was effected at the beginning of the experiment. But with the age of culture, the production increased.

Analysis of variance revealed that, there was significant variation

in gross and net carbon production under four different concentrations and also over days. Significance was at 1% level. (Table 13 & 14). But for respiration, statistics showed no significant variation (Table 15).

The marine form D. inornata also showed variation in carbon production as a result of treatment with different concentrations of 'B.H.C.' The 0.5 ppm concentration shows increased carbon production than control throughout the experimental period. The highest value of gross production was observed in the 14th day of experiment i.e, 0.62 mgC/l/hr the corresponding net production of 0.58 mgC/l/hr was about 7.4% more over control. The highest concentration of 2 ppm and 4 ppm always showed very low values of production. Unlike in the case of T. gracilis, the carbon production was not at all increased with the age of culture in pesticide treated cultures especially for 2 and 4 ppm (Fig. 17a, and 18a). The pesticide treated cultures showed increased rate of respiration throughout the experimental period.

Analysis of variance showed that there was significant variation in gross and net carbon production of D. inornata, under four different concentrations, and also over days. The significance was at 1% level. But there was no significant variation in respiration (Table 13, 14, and 15)

Mixed culture of fresh water microalgae with the four different concentrations of 'B H C' showed low values of carbon production compared to control. No increased carbon production over control was observed

even at the lowest concentration tested. The percentage inhibition varied with the age of culture. The highest carbon values for 0.5 ppm observed on the 10th day of experiment was 0.34 mgC/l/hr (Gross production) and 0.32 mgC/1/hr (net production) which was about 16% lower than control observed on the same day. Concentration of 2 and 4 ppm of 'B.H.C.' was found to inhibit the carbon production especially at the beginning of the culture (Fig. 19a and 20a). While the gross production was represented by low values, the net production was zero indicating high respiratory rate. As in the case of T. gracilis this culture also showed the peculiarity that, after an initial inhibition in 2 and 4 ppm 'BHC' concentration, the cultures regained their normal activity and the carbon production increased when the culture attained 18 days growth. The highest production at 4 ppm was observed on the 18th dy of experiment i.e., 0.15 mgC/l /hr gross production and 0.13 mgC/l/hr net production.

Analysis of variance revealed that there was significant variations in gross carbon production as a result of 'B.H.C.' treatment. (Table 13). But the net production showed a mixed trend, significant at 5% level (Table 14). There was no significant variation in respiration as a result of 'B.H.C. Treatment (Table 15).

2. **Organophosphate insecticide–'Nuvacron'**

Four different concentrations of 'Nuvacron' was seen to affect the carbon production in T. gracilis (Fig. 15b and 16b).

'Nuvacron' at 25 ppm showed an increased carbon production than

control. But the percentage of increase was varied on each day. The highest gross production was observed on the 14th day of experiment. At 25 ppm concentration the gross carbon production was 0.5 mgC/l/hr, and net production value 0.48 mgC/l/hr. i.e., net production was about 11.63% higher than that observed for control cultures. 'Nuvacron' of 100 ppm showed very low values of carbon production at the beginning i.e., on the 2nd day only about 12% of control value was observed. The same day the net production observed was only 0.008 mgC/l/hr indicating high respiratory rate at 100 ppm concentration. But with the age of culture, the earlier inhibition of high concentration had changed and the highest value was observed on the 20th dy of experiment i.e., 0.32 mgC/l/hr of gross production and 0.26 mgC/l/hr of net production.

Statistical interpretation using analysis of variance revealed that there was highly significant variation in gross and net carbon production as a result of 'Nuvacron' treatment (Table 13 and 14). But there was no such variation in the respiratory rate as a result of insecticide treatment (Table 15).

The rate of carbon production of D. inornata showed variation with four different concentrations of 'Nuvacron' (Fig. 17b and 18b). The lower concentration of 25 ppm showed an increased carbon production than control only at the beginning of the experiment. But later the production decreased compared to control. The highest production was observed on the 14th day, the values being 0.43 mgC/l/hr for gross production

and 0.32 mgC/l/hr for net production, which was only 62.75% of control value on the same day. Concentrations of 50, 75 and 100 ppm showed low values for gross carbon production, and the net carbon production was very much affected. For 100 ppm, the maximum amount of net carbon production was only 0.12 mgC/l/hr observed on the 14th day, but on the same day the control shows 0.57 mgC/l/hour. Unlike in the case of T. gracilis, the earlier inhibition was not at all recovered in this species, and when the culture attained 20 days of growth the net production observed was zero.

Analysis of variance revealed that there was significant variation in gross and net carbon production as a result of 'Nuvacron' treatment. (Table 13 and 14). But the respiration showed no significant variation (Table 15).

Figure 19b and 20b explain the variation in gross and net production values of freshwater culture as a result of 'Nuvacron' treatment. The four different concentrations of 'Nuvacron' showed variation in carbon production. No increased production was observed even at low concentration of 25 ppm. At this concentration the highest net production was observed on this 10th day of experiment i.e., only 0.28 mgC/l/hr compared to0.4 mgC/l/hr observed for control cultures on the same day. For 50 ppm the highest production was observed on the 12th day. Concentration of 75 ppm showed maximum carbon production on the 16th day of experiment. But with the higher concentration, the net carbon production showed very low values, the maximum amount it could produce was only 0.05 mgC/l/hr, which remained unchanged from 14th to 20th day of experiment.

Analysis of variance revealed that there was significant variation in gross and net carbon production as a result of four different concentration of 'Nuvacron' (Table 13 and 14). But there was no significant variation in the respiratory rate (Table 15).

3. **Carbamate insecticide-'Carbaryl sevin'**

The effect of four different concentrations of 'carbaryl sevin' on gross and net production of three microalgal cultures showed variation in their values. As observed in the case of 'BHC', in the control and acetone treated control, the carbon production did not vary.

With T. gracilis, the 2 ppm concentration of Carbaryl sevin stimulated the gross carbon production on the 2nd and 4th day. But corresponding net production values were very low compared to control showing that the insecticide treated cultures had high respiratory rate compared to control. The highest value for net carbon production of 0.47 mgC/l/hr at 2 ppm concentration was observed on the 14th day of experiment (Fig. 15c and 16c). For 4 ppm concentration the maximum production observed was on the 16th day of experiment. In concentrations of 6 and 8 ppm net production was zero, especially at the beginning of the experiment. This indicated an increased respiratory rate of insecticide treated culture. Concentration of 8 ppm always shows very low value of net carbon production and the maximum value observed was 0.22mgC/l/hr which remained unchanged from 16th to 20th day of experiment.

Analysis of variance revealed that there was significant variation in gross and net carbon production of T. gracilis with four different con-

centration, over days. (Table 13 and 14). Between treatments, there was no significant variation in respiratory rate. But, over the days, the 'Carbaryl sevin 'showed significant variation in respiratory rate (Table 15).

Fig. 17c and 18c explain the variation in carbon production of D. inornata as a result of 'carbaryl sevin' treatment. The four different concentrations tested shows low values of carbon production compared to control. But the percentage reduction varied with the age of culture. At 2 ppm concentration the maximum gross production of 0.3 mgC/l/hr was observed on the 14th day of experiment and the net production observed was also 0.3 mgC/l/hr showing that the respiratory rate was zero. Compared to control the percentage inhibition of net production was about 37.5%. In 4 ppm concentration, the maximum net production was observed on 16th day which was 0.2 mgC/l/hr but afterwards the production decreased. In 6 ppm and 8 ppm the values of carbon production was very low and the maximum production was observed on 20th day of experiment. The amount of carbon produced was only 0.1 mgC/l/hr.

Analysis of variance indicated that there was significant variation (significant at 1% level) in gross and net carbon production as a result of insecticide treatment (Table 13 and 14). But the respiratory rate showed 5% significance between four different concentrations, and at 1% significance over days (Table 15).

The variation in carbon production of freshwater culture as a result of 'carbaryl' treatment is shown in the figure 19c and 20c. No increased

production was observed even with the low concentration tested. The 2 ppm concentration showed its maximum gross production on the 10th day, the value being 0.33 mgC/l/hr for gross and the corresponding net carbon production was 0.32 mgC/l/hr, which is 88.89% of control production. The 4 ppm concentration showed maximum production on 12th day and the net carbon production value observed was only 0.14 mgC/l/hr. In concentration of 6 ppm and 8 ppm showed very low values of carbon production. For 8 ppm the production was beyond the detectable limit upto 4th day. But after wards the same culture showed very low values of carbon production. The maximum net carbon production at 8 ppm concentration was observed on 16th day the value being 0.08 mgC/l/hr. It was observed that the insecticide treated cultures shows increased respiratory rate than control.

Analysis of variance revealed that there was significant variation in gross and net carbon production of mixed culture, as a result of insecticide treatment (Table 13 and m14). But the variation in respiration was not significant (Table 15).

4. **Herbicide - 'Gramaxone'**

With the herbicide 'Gramaxone' the carbon production of three microalgal cultures varied very much.

With four different concentrations of 'Gramaxone' T. gracilis showed difference in carbon production. Fig. 15 d and 16 d. The lowest concentration of 0.2 ppm showed an increased carbon production than control only on the 2nd day of observation. But corresponding net production

value showed no increase than control. At 0.2 ppm concentration, the maximum production was observed on the 14th day, the values being 0.5 mgC/l/hr for gross production and 0.44 mgC/l/hour for net production. It was about 95.65% of that observed for control culture. At 0.4 ppm concentration the maximum production was observed on the 16th day. Concentration of 0.8 and 1.6 ppm showed the peculiarity that, eventhough they are showing low values for gross carbon production, net production was zero. This may be because of increased respiratory rate of treated cultures. At 1.6 ppm concentration the maximum net carbon production was observed on the 20th day of experiment, the value being 0.08 mgC/l/hr.

Analysis of variance revealed that there was significant variation in gross and net carbon production of T. gracilis. as a result of 'Gramaxone' treatment (Table 13 and 14). The respiratory rate was found to be significant at 5% level (Table 15).

The herbicide showed difference in the production values with D. inornata, (Fig. 17d and 18d). The lower concentration of 0.2 ppm showed an increased production than control at the beginning of the experiment. But the corresponding net production did not show any increase. The difference in gross and net production was due to the difference in respiratory rate. In 0.2 ppm concentration the maximum production was observed on the 14th day, the values being 0.48 mgC/l/hr for gross production, only 0.4 mgC/l/hr for net production which is 88.89% of the control production. The 0.4 ppm concentration showed the maximum production on the 14th day and the value was 0.3 mgC/l/hr. Concentrations

of 0.8 ppm and 1.6 ppm showed low values for net carbon production. For 1.6 ppm the maximum net carbon production was observed on the 20th day of experiment, the value being 0.14 mgC/l/hr.

Analysis of variance indicated that there was significant variation in gross and net carbon production, between treatments and also between days. (Table 13 and 14). There was no significant variation in respiration between treatments. But between days the variation in respiration was found to be significant at 5.%level. (Table 15).

With the mixed culture of fresh water microalgae, the different concentrations of 'Gramaxone' showed variation in carbon production. Even with the lowest concentration tested no increased production was observed. But when the culture attained 12th day of growth, the maximum gross production was observed with 0.8 ppm concentration (Fig. 19d and 20d). But the net carbon production was varied with different concentration In 0.2 ppm concentration the maximum net carbon production was observed on 12th day of experiment. For 0.4 ppm the maximum production was observed on 12th day. The 0.8 ppm concentration showed maximum production on the 12th day of experiment. At 1.6 ppm concentration the net carbon production was zero upto 4th day showing increased respiratory rate. The maximum production was observed was 0.075 mgC/l/hr.

Analysis of variance revealed that there was significant variation in gross and net carbon production between treatments and also between days (Table 13 and 14). But there was no significant variation in respiration

as a result of herbicide treatment (Table 15).

5. **Fungicide - 'Cuman L.**(R)

The four different concentrations of fungicide affected the carbon production of three microalgal culture under investigation.

In the case of T. gracilis the concentration of 0.005 ppm showed little increase over control at the beginning of the experiment (Fig. 15e and 16e). At this concentration the highest value obtained was 0.5mgC/l/hr for gross production and 0.44 mgC/l/hr for net production which was 97.78% of control. With 0.01 ppm concentration the highest value obtained for net production was 0.40 mgC/l/hr. The concentrations of 0.02 and 0.04 ppm was found to reduce the carbon production very much. At the beginning of the experiment these cultures showed very low values of gross carbon production and the net production was also less. This showed that at higher concentration, the respiratory rate increased. At 0.02, and 0.04 ppm. the highest net production was observed on the 20th day of experiment, the values being 0.26 mgC/l/hr and 0.12 mgC/l/hr respectively.

Analysis of variance revealed that there was highly significant variation in gross and net carbon production, between treatment and between days (Table 13 and 14). The respiratory rate showed significant variation between treatments, but between days there was no significant variation in respiratory rate (Table 15).

The fungicide 'Cuman L$^{(R)}$' was found to inhibit the carbon production of D. inormata to a more extent than that observed in T. gracilis (Fig. 17e and 18e). At 0.005 ppm concentration the highest value of carbon production was observed on 16th day and the gross production was 0.4 mgC/l/hr while net production was 0.32 mgC/l/hr. At 0.01 ppm the highest value obtained for net production was 0.2 mgC/l/hr and it was on the 16th day of experiment, concentration of 0.02 and 0.04 ppm showed very low values for carbon production. In the early period of the culture they showed low values for gross carbon production and the net production was negligible or zero. This indicated high respiratory rate at higher concentrations of fungicide. But with the age of culture, these concentration showed an increased production than at the beginning and when the culture attained 20 days growth, these cultures showed the highest production.

Analysis of variance indicated that there was significant variation in gross and net carbon production of D. inornata, between treatments and over days (Table 13 and 14). The variation in respiratory rate was found to be significant at 5% level, between treatments. But over days there was no significant variation in respiration (Table 15).

The freshwater culture showed various patterns of carbon production as a result of fungicide treatment (Fig. 19e and 20 e). Upto the 12th day of experiment, the four different concentrations tested showed low values of carbon production compared to control. But afterwards the

treated cultures showed little enhancement over control. At 0.005 ppm the highest value was observed on the 10th day, the values being 0.34 mgC/l/hr for gross production and 0.3 mgC/l/h for net production which was 88.24% of control values observed on the same day. For 0.01 ppm the peak value obtained for net production was 0.22 mgC/l/hr on the 14th day. At the beginning of the experiment, the highest concentration tested showed very low values for net carbon production and this may be because of increased respiratory rate. But with the age of culture this condition had changed and the peak value of net production at 0.02 ppm observed on 16th day was 0.15 mgC/l/hr. The 0.04 ppm concentration gave the highest value of 0.08 mgC/l/hr on the 20th day of experiment.

Analysis of variance revealed that there was significant variation in gross carbon production between treatments and between days (Table 13). For net carbon production, between treatments the significance was at 1% level and between days the significance observed was at 5% level (Table 14). Between treatments the variation in respiratory rate was not significant. But, between days there was 5% significance in variation (Table 15).

6. **Mixture of 5 biocides**

The effect of four different concentrations of five biocide mixture were found to inhibit the rate of carbon production in three microalgal cultures. Control and acetone treated control showed same trend in gross and net carbon production.

T. gracilis showed an increase in carbon production compared to control at the low concentration of 0.05 ppm only on the 2nd day observation (Fig. 15 f and 16f). But the corresponding net carbon production observed was below control values. This difference showed increased respiratory rate of toxicant treated algae. The highest value of net carbon production at 0.05 ppm observed was on 14th day and it was 0.42 mgC/l/hr which was 87.5% of control on the same day. At 0.01 ppm concentration the maximum production of 0.42 mgC/l/hr was on the 16th day of experiment. As observed in the case of each biocide, the mixture of biocide also showed very low values of net carbon production with the highest concentration tested because of increased respiratory rate. At 0.2 ppm the highest net production was observed on 18th day the value being 0.36 mgC/l/hr. The highest concentration of 0.4 ppm showed the maximum production of 0.22 mgC/l/hr on the 18th day and this remained unchanged upto the 20th day of experiment.

Analysis of variance indicated that there was significant variation in gross and net carbon production between treatments and between days (Table 13 and 14). But there was no significant variation in respiratory rate (Table 15).

The four different concentrations of biocide mixture was found to inhibit the carbon production of D. inornata (Fig. 17f and 18f). The concentration of 0.05 ppm showed an increase in gross carbon production upto the 6th day of experiment corresponding net carbon production was low compared to control. This showed increased respiratory rate of toxicant treated cultures. The highest value of carbon production at 0.05 ppm

was observed on the 16th day, the values being 0.49 mgC/l/hr for gross production and 0.45 mgC/l/hr for net production. At 0.01 ppm the highest net production observed was 0.42 mgC/l/hr on 16th day of experiment concentrations of 0.2 and 0.4 ppm show very low values of net carbon production in the beginning, indicating a high respiratory rate. At 0.2 ppm concentration a maximum production of 0.22 mgC/l/hr was obtained on the 20th day of experiment. At 0.4 ppm production of 0.12 mgC/l/hour was observed on the 20th day of experiment.

Analysis of variance revealed that there was significant variation in gross and net carbon production of D. inornata, between treatments and also between days (Table 13 and 14). But the variation in respiratory rate was not significant. (Table 15).

The mixed culture showed various values for carbon production at four different concentrations tested (Fig. 19f and 20f). The low concentration of 0.05 ppm showed a little increase in carbon production compared to control on the 2nd day of observation. But the corresponding net production was lower than control. The highest value of net carbon production at 0.05 ppm concentration was observed on 10th day of experiment and it was 0.30 mgC/l/hr, which is 88.89% of control values observed on the same day. In 0.1 ppm concentration a maximum net production of 0.22 mgC/l/hour was noticed on 12th day of experiment but in 0.2 and 0.4 ppm only very low values of carbon production was seen compared to control. The highest production at 0.2 ppm was observed on the 14th day and it was 0.1 mgC/l/hr. At 0.4 ppm highest production observed was 0.075 mgC/l/hr on the 20th day of experiment.

Analysis of variance revealed that the four different concentrations of biocide mixture did not show any significant variation in gross and net carbon production. But between days, gross production showed highly significant variation (Table 13 and 14). The respiratory rate also showed significant variation between treatments. But between days there was no significant variation in respiration (Table 15).

7. **Mixture of 3 biocides**

Mixture of one insecticide, one herbicide and one fungicide at four different concentration showed low values for production compared to control.

With T. gracilis this biocide mixture showed varying values for carbon production (Fig. 15g and 16g). At 0.05 ppm, the highest production was observed on the 14th day and it was 0.39 mgC/l/hr, for gross production and 0.32 mgC/l/hr for net production which is 65.31% of that observed for control on the same day. At 0.1 ppm the maximum value of net production obtained was 0.3 mgC/l/hr on 16th day. Concentrations of 0.2 and 0.4 ppm yielded zero value for net carbon production on the 2nd and 4th day showing increased respiratory rate. Afterwards production values showed little increase with the age of the culture. The highest value of net production obtained at 0.2 ppm was 0.3 mgC/l/hr on 16th day of experiment. At 0.4 ppm. the maximum production observed was 0.3 mgC/l/hr which was on the 20th day of experiment.

Analysis of variance revealed that there was significant variation in gross and net carbon production of T. gracilis, under four different concentrations and also between days (Table 13 and 14). The respiration showed 5% sighificance between treatments. But, between days the effect of biocide mixture on respiratory rate was not significant (Table .15).

With the species D. inornata, the various concentration of biocide mixture showed varying value of carbon production, as shown in the figures (Fig. 17g and 18g). The lowest concentration of 0.05 ppm showed little increase in production than control upto the 6th day of experiment. At this concentration the highest value was observed on the 14th day of experiment, the values being 0.45 mgC/l/hr for gross and 0.39mgC/l/hr for net production, which was 86.67% of control observed on the same day. At 0.1 ppm, the highest value of net production observed as 0.36 mgC/l/hr on 16th day. Concentrations of 0.2 and 0.4 ppm showed very low values of net carbon production indicating a high respiratory rate at this concentration. The maximum net carbon production at 0.3 ppm was 0.24 mgC/l/hr on the 20th day of experiment. The maximum value obtained for net carbon production at 0.4 ppm was 0.19 mgC/l/hr on the 20th day.

Analysis of variance revealed that there was significant variatrion in gross and net carbon production of D. inornata, between treatments and also between days (Table 13 and 14). But there was no significant variation in the respiratory rate of D. inornata.

The freshwater culture showed different rates of carbon production as a result of toxicant treatment (Fig. 19g and 20g). In 0.05 ppm concentration a maximum gross production of 0.38 mgC/l/hr was seen on the 10th day, and the corresponding net production was 0.38 mgC/l/hr, the respiratory value being zero. At 0.1 ppm, the maximum value of net roduction obtained was 0.18 mgC/l/hr on the 12th day of experiment. The highest concentration of 0.2 and 0.4 ppm showed very low values of net carbon production at the beginning of the culture. But afterwards the net carbon production showed an increasing trend with the age of culture. At 0.2 ppm the maximum production observed was 0.15 mgC/l/hr on the 16th day of experiment. At 0.4 ppm the maximum production was only 0.10 mgC/l/hr on the 16th day of experiment.

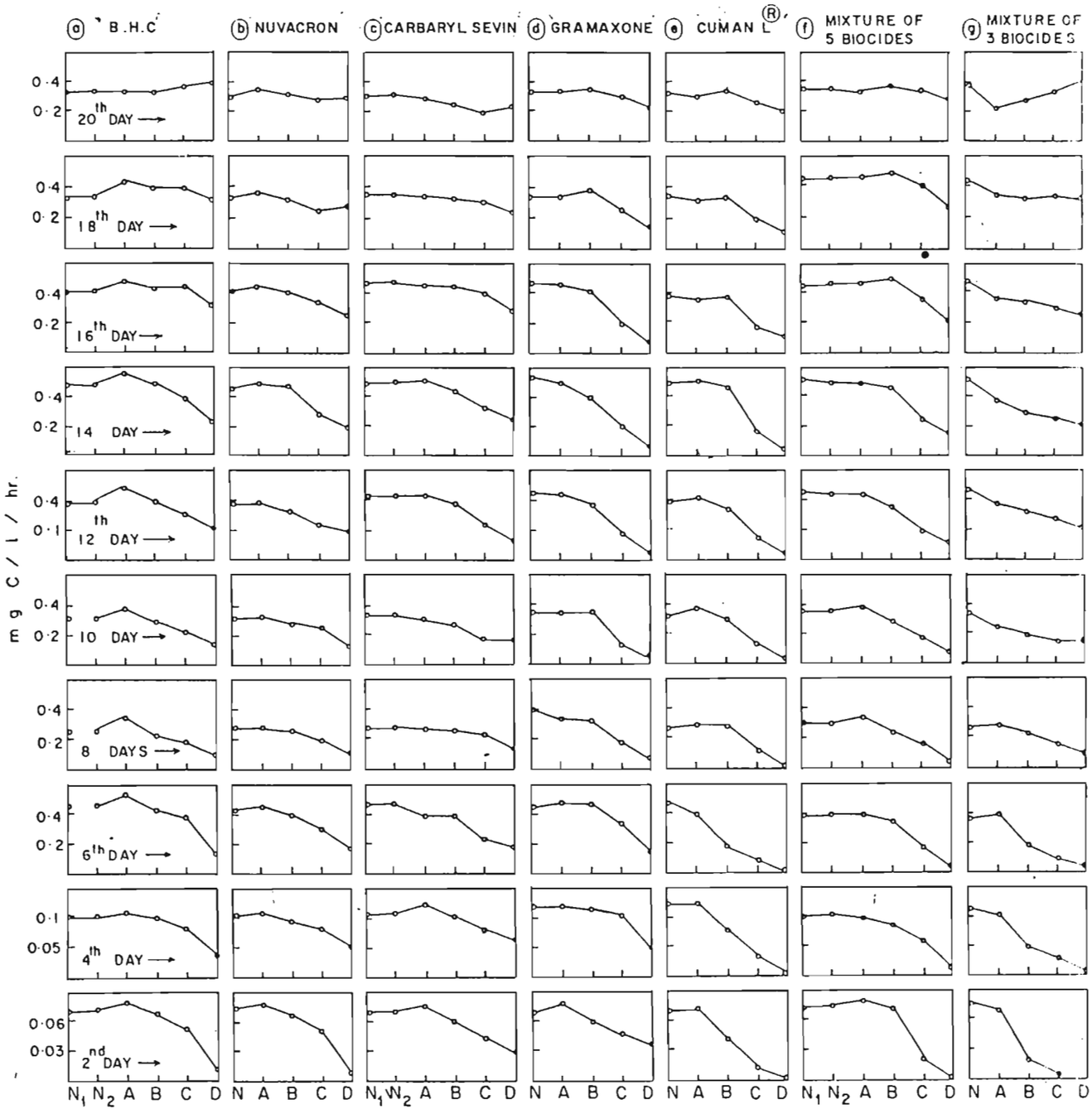
Analysis of variance showed that there was significant variation in gross and net carbon production of freshwater culture, between treatments in different days (Table 13 and 14). But there was no significant variation in the respiratory rate of culture as a result of toxicants in mixture (Table 15).

## DISCUSSION

Evethough, volumious literature is available on growth rate of microalgae as a result of biocide treatmet, very little is kown about

FIG.15

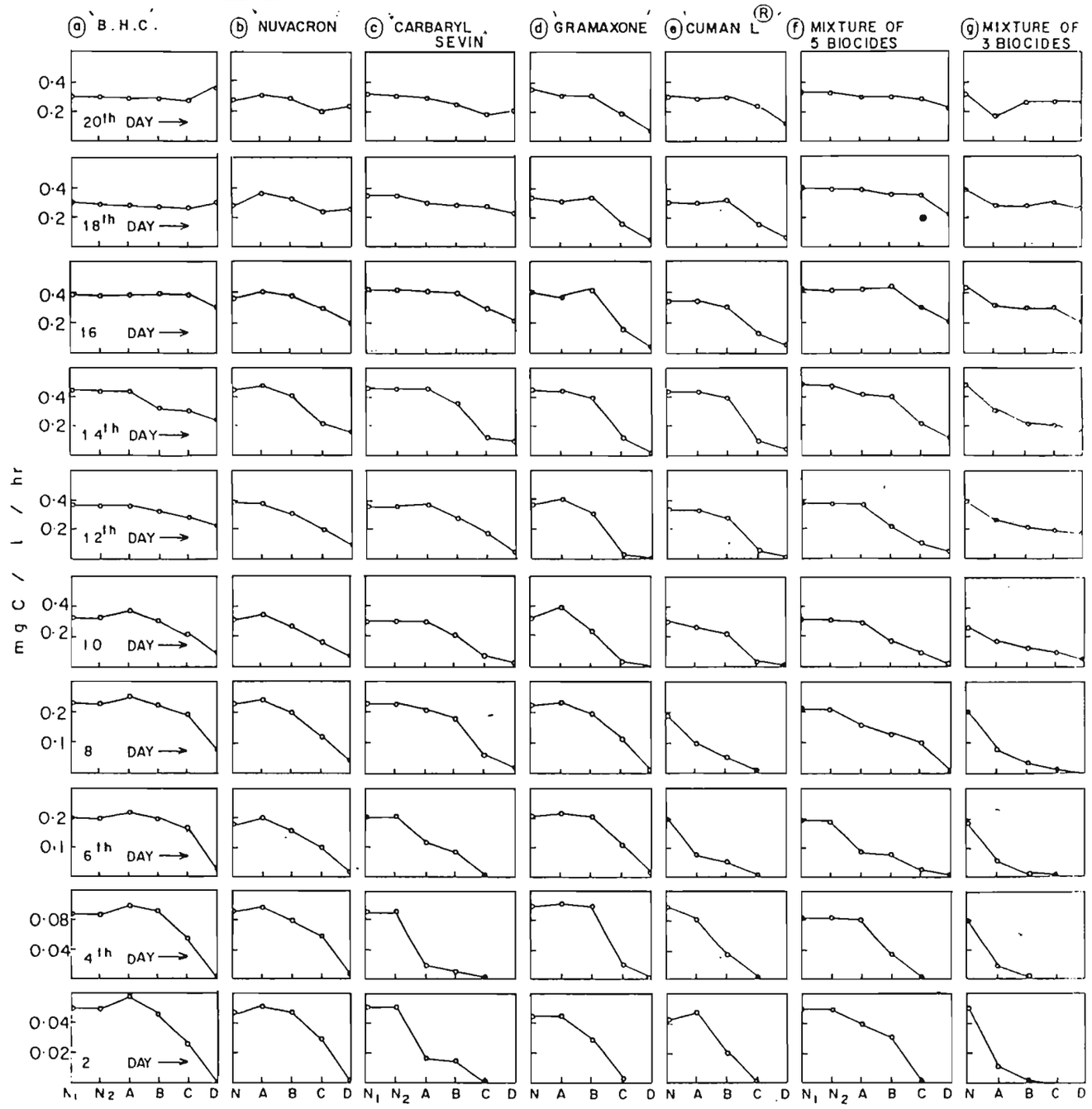
EFFECT OF SEVEN TOXICANTS ON GROSS CARBON PRODUCTION OF TETRASELMIS GRACILIS




| | | | | | | |
|---|---|---|---|---|---|---|
| $N_1$ — CONTROL | N–CONTROL | $N_1$ – CONTROL | N–CONTROL | N–CONTROL | $N_1$ – CONTROL | N–CONTROL |
| $N_2$ — ACETONE | | $N_2$ – ACETONE | | | $N_2$ – ACETONE | |
| A — 0·5 ppm | A — 25 ppm | A — 2 ppm | A — 0·2 ppm | A — 0·005ppm | A — 0·05 ppm | A — 0·05ppm |
| B — 1 ppm | B — 50ppm | B — 4 ppm | B — 0·4ppm | B — 0·01 ppm | B — 0·01 ppm | B — 0·1 ppm |
| C — 2 ppm | C — 75ppm | C — 6 ppm | C — 0·8ppm | C — 0·02 ppm | C — 0·2 ppm | C — 0·2 ppm |
| D — 4 ppm | D — 100 ppm | D — 8 ppm | D — 1·6ppm | D — 0·04 ppm | D — 0·4 ppm | D — 0·4 ppm |

FIG.16

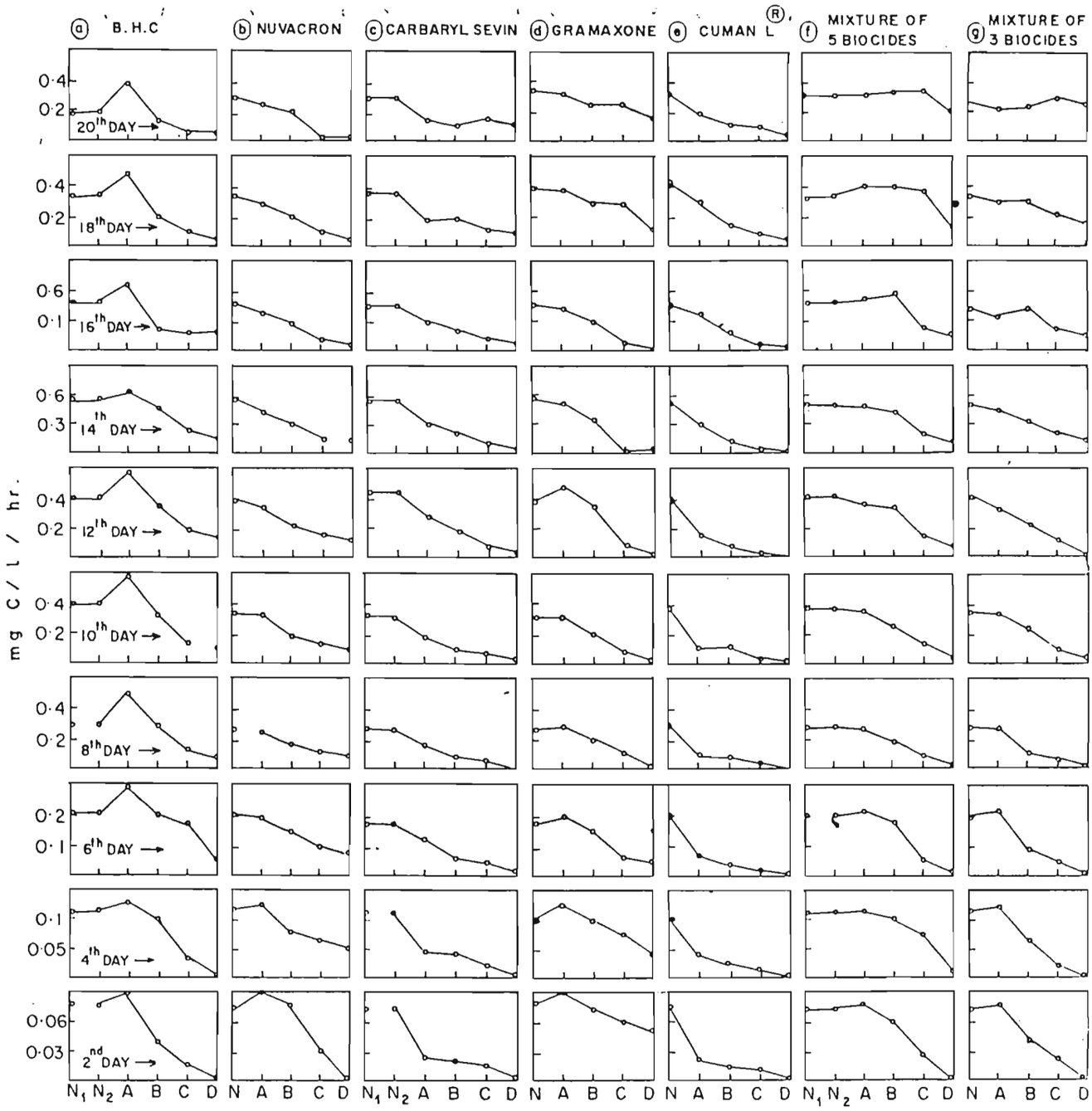
EFFECT OF SEVEN TOXICANTS ON NET CARBON PRODUCTION OF TETRASELMIS GRACILIS




| | | | | | | |
|---|---|---|---|---|---|---|
| N - CONTROL | N - CONTROL | $N_1$ - CONTROL | N - CONTROL | N - CONTROL | $N_1$ - CONTROL | N - CONTROL |
| $N_2$ - ACETONE | | $N_2$ - ACETONE | | | $N_2$ - ACETONE | |
| A - 0·5ppm | A - 25 ppm | A - 2 ppm | A - 0·2 ppm | A - 0·005ppm | A - 0·05 ppm | A - 0·05ppm |
| B - 1 ppm | B - 50 ppm | B - 4 ppm | B - 0·4 ppm | B - 0·01 ppm | B - 0·1 ppm | B - 0·1 ppm |
| C - 2 ppm | C - 75 ppm | C - 6 ppm | C - 0·8 ppm | C - 0·02 ppm | C - 0·2 ppm | C - 0·2 ppm |
| D - 4 ppm | D - 100 ppm | D - 8 ppm | D - 1·6 ppm | D - 0·04 ppm | D - 0·4 ppm | D - 0·4 ppm |

FIG.17.

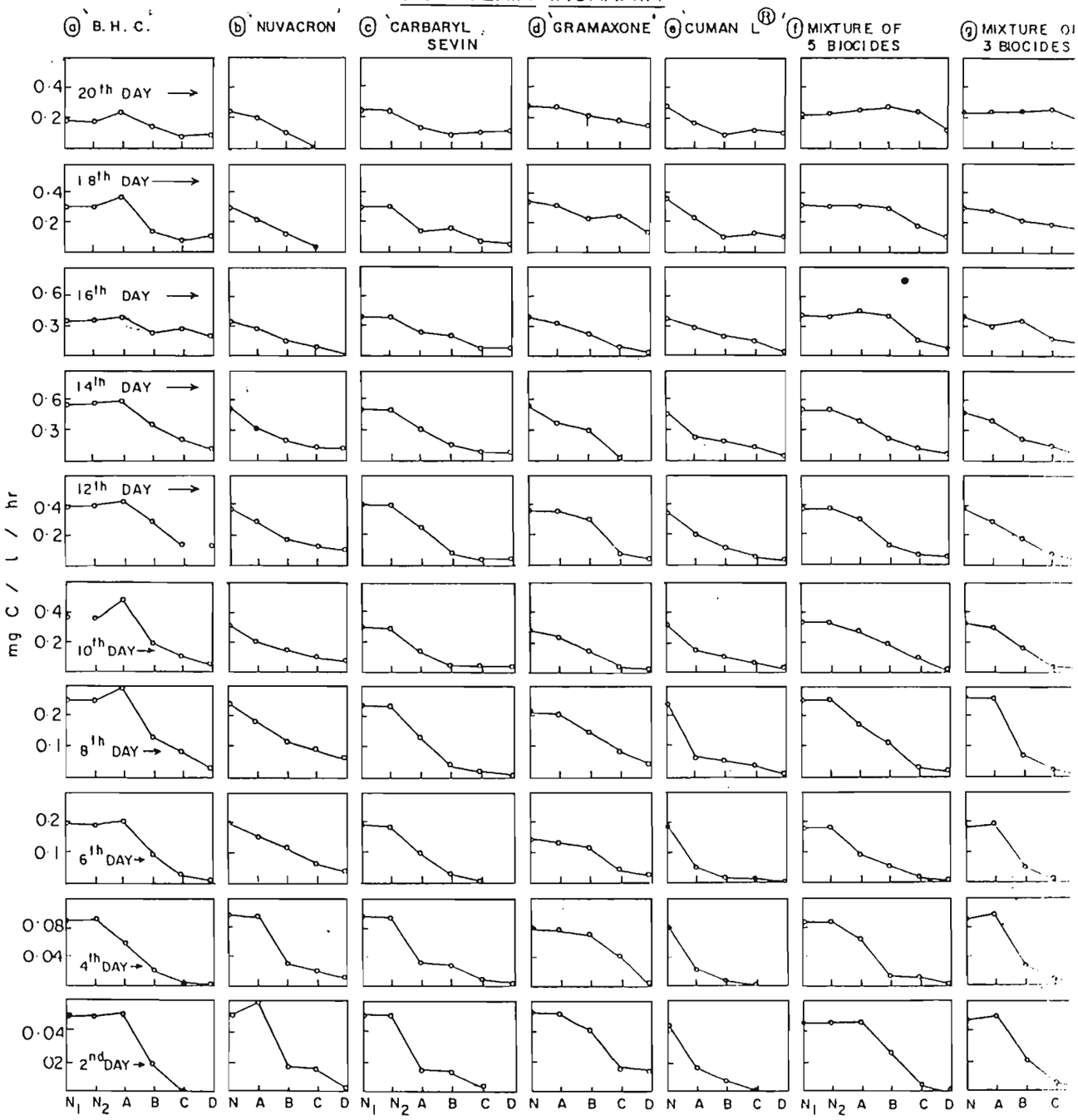
EFFECT OF SEVEN TOXICANTS ON GROSS CARBON PRODUCTION OF DICRATERIA INORNATA




| | | | | | | |
|---|---|---|---|---|---|---|
| $N_1$ — CONTROL | N–CONTROL | $N_1$ – CONTROL | N–CONTROL | N–CONTROL | $N_1$ –CONTROL | N–CONTROL |
| $N_2$ — ACETONE | | $N_2$ –ACETONE | | | $N_2$ –ACETONE | |
| A— 0·5 ppm | A— 25 ppm | A— 2 ppm | A— 0·2 ppm | A —0·005ppm | A—0·05 ppm | A—0·05ppm |
| B— 1 ppm | B— 50ppm | B— 4 ppm | B— 0·4ppm | B—0·01 ppm | B—0·01ppm | B—0·1 ppm |
| C— 2 ppm | C— 75ppm | C— 6 ppm | C— 0·8ppm | C—0·02 ppm | C—0·2 ppm | C—0·2 ppm |
| D— 4 ppm | D — 100ppm | D— 8 ppm | D — 1·6 ppm | D—0·04 ppm | D—0·4 ppm | D—0·4 ppm |

FIG. 1 8

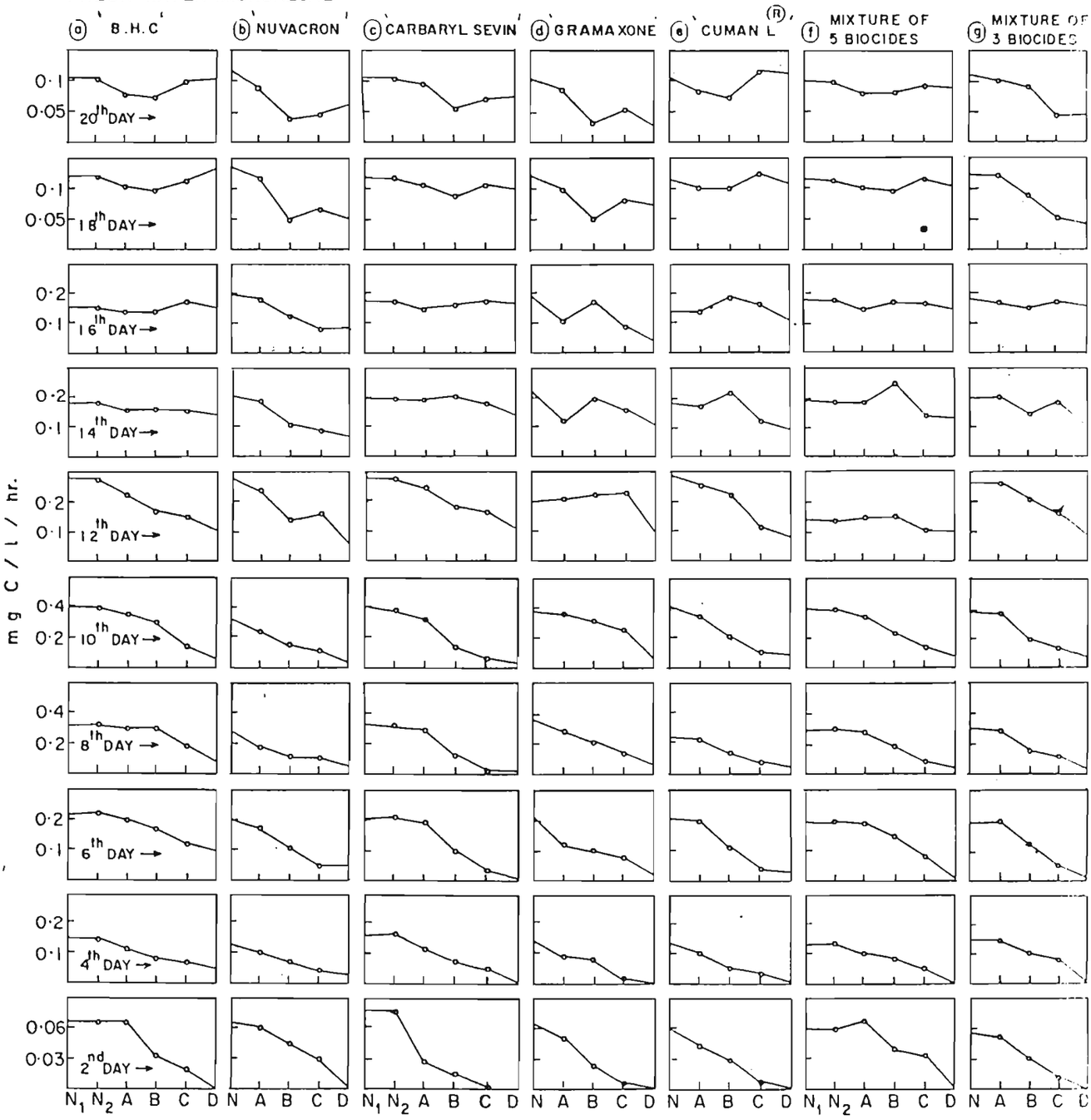
EFFECT OF SEVEN TOXICANTS ON NET CARBON PRODUCTION OF DICRATERIA INORNATA




| | | | | | | |
|---|---|---|---|---|---|---|
| N - CONTROL | N - CONTROL | $N_1$ - CONTROL | N - CONTROL | N - CONTROL | $N_1$ - CONTROL | N - CONTR |
| $N_2$ - ACETONE | | $N_2$ - ACETONE | | | $N_2$ - ACETONE | |
| A - 0·5 ppm | A - 25 ppm | A - 2 ppm | A - 0·2 ppm | A - 0·005 ppm | A - 0·05 ppm | A - 0·05 pp |
| B - 1 ppm | B - 50 ppm | B - 4 ppm | B - 0·4 ppm | B - 0·01 ppm | B - 0·1 ppm | B - 0·1 pp |
| C - 2 ppm | C - 75 ppm | C - 6 ppm | C - 0·8 ppm | C - 0·02 ppm | C - 0·2 ppm | C - 0·2 pp |
| D - 4 ppm | D - 100 ppm | D - 8 ppm | D - 1·6 ppm | D - 0·04 ppm | D - 0·4 ppm | D - 0·4 pp |

FIG.19

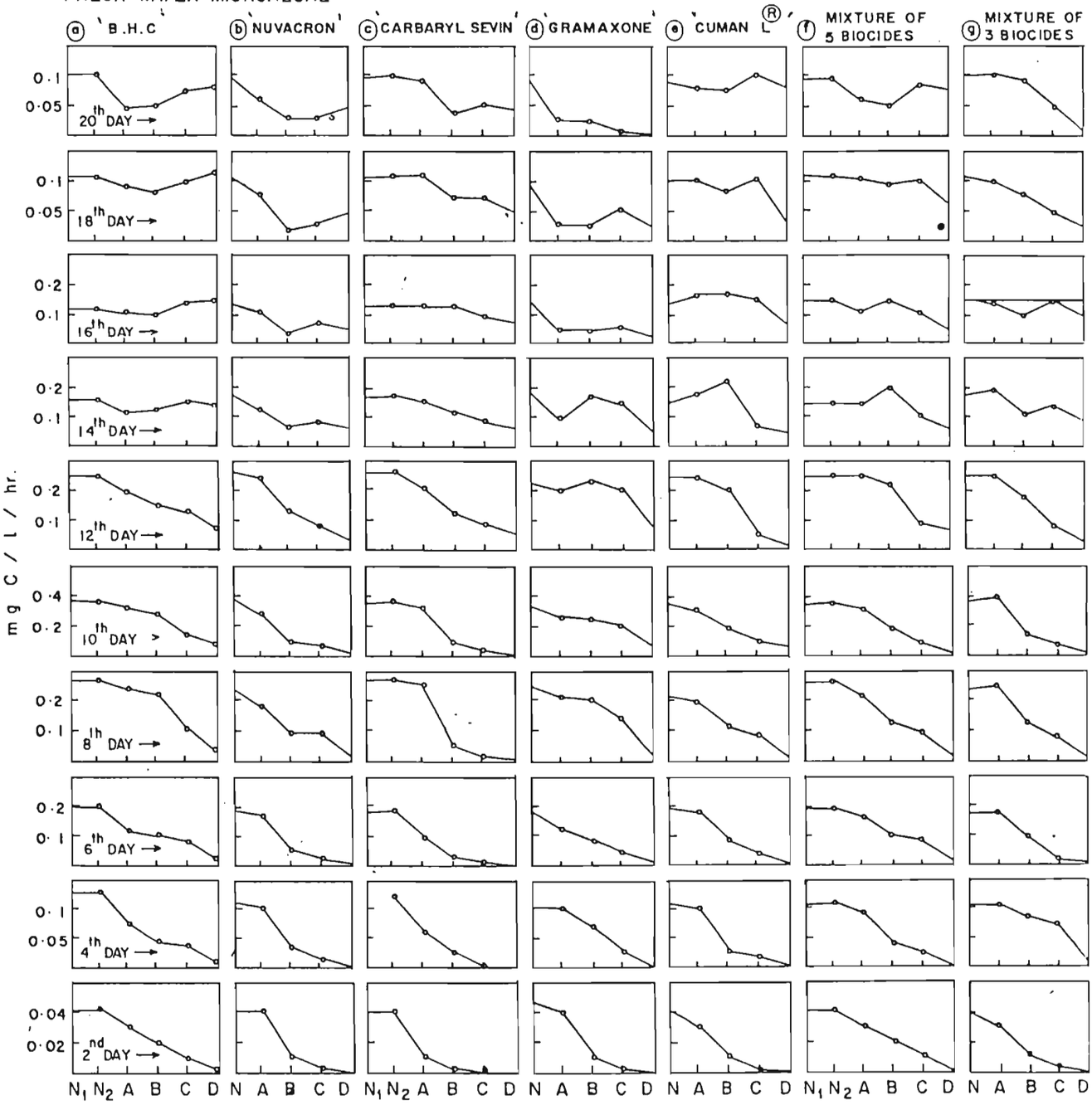
EFFECT OF SEVEN TOXICANTS ON GROSS CARBON PRODUCTION OF MIXED CULTURE OF FRESH WATER MICROALGAE




| | | | | | | |
|---|---|---|---|---|---|---|
| $N_1$ — CONTROL | N-CONTROL | $N_1$ - CONTROL | N-CONTROL | N-CONTROL | $N_1$ - CONTROL | N-CONTROL |
| $N_2$ — ACETONE | | $N_2$ - ACETONE | | | $N_2$ - ACETONE | |
| A— 0·5 ppm | A— 25 ppm | A— 2 ppm | A— 0·2 ppm | A—0·005 ppm | A—0·05 ppm | A—0·05 ppm |
| B— 1 ppm | B— 50 ppm | B— 4 ppm | B— 0·4 ppm | B—0·01 ppm | B—0·01 ppm | B—0·1 ppm |
| C— 2 ppm | C— 75 ppm | C— 6 ppm | C— 0·8 ppm | C—0·02 ppm | C—0·2 ppm | C—0·2 ppm |
| D— 4 ppm | D— 100 ppm | D— 8 ppm | D— 1·6 ppm | D—0·04 ppm | D—0·4 ppm | D—0·4 ppm |

FIG.20

EFFECT OF SEVEN TOXICANTS ON NET CARBON PRODUCTION OF MIXED CULTURE OF FRESH WATER MICROALGAE




| (a) B.H.C | (b) NUVACRON | (c) CARBARYL SEVIN | (d) GRAMAXONE | (e) CUMAN L® | (f) MIXTURE OF 5 BIOCIDES | (g) MIXTURE OF 3 BIOCIDES |
|---|---|---|---|---|---|---|
| $N_1$ – CONTROL | N – CONTROL | $N_1$ – CONTROL | N – CONTROL | N – CONTROL | $N_1$ – CONTROL | N – CONTROL |
| $N_2$ – ACETONE | | $N_2$ – ACETONE | | | $N_2$ – ACETONE | |
| A – 0·5 ppm | A – 25 ppm | A – 2 ppm | A – 0·2 ppm | A – 0·005 ppm | A – 0·05 ppm | A – 0·05 ppm |
| B – 1 ppm | B – 50 ppm | B – 4 ppm | B – 0·4 ppm | B – 0·01 ppm | B – 0·01 ppm | B – 0·1 ppm |
| C – 2 ppm | C – 75 ppm | C – 6 ppm | C – 0·8 ppm | C – 0·02 ppm | C – 0·2 ppm | C – 0·2 ppm |
| D – 4 ppm | D – 100 ppm | D – 8 ppm | D – 1·6 ppm | D – 0·04 ppm | D – 0·4 ppm | D – 0·4 ppm |

TABLE-13.

ANOVA TABLE FOR GROSS CARBON PRODUCTION

| Toxicant | Source | D.F | Tetrasclmis gracilis | | | Dicrateria inornata | | | Mixed culture of fresh water algae | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | S.S | M.S | F | S.S | M.S | F | S.S | M.S | |
| 'B H C' | Treatment | 5 | 0.1256 | 0.0251 | 14.37** | 0.9331 | 0.1866 | 33.96** | 0.0819 | 0.0164 | 5.[illegible] |
| | Replicate | 9 | 1.0566 | 0.1174 | 67.13** | 0.8019 | 0.0891 | 16.21** | 0.2273 | 0.0253 | 7.[illegible] |
| | Error | 45 | 0.0787 | 0.0018 | | 0.2473 | 0.0055 | | 0.1469 | 0.0033 | |
| 'Nuvacron' | Treatment | 4 | 0.0795 | 0.0199 | 11.18** | 0.5252 | 0.1313 | 32.25** | 0.1622 | 0.0406 | 37.[illegible] |
| | Replicate | 9 | 0.5934 | 0.0659 | 37.09** | 0.1468 | 0.0163 | 4.01** | 0.1057 | 0.0118 | 10.[illegible] |
| | Error | 36 | 0.0639 | 0.0018 | | 0.1466 | 0.0041 | | 0.0385 | 0.0011 | |
| 'Carbaryl Sevin' | Treatment | 5 | 0.1267 | 0.0317 | 23.77** | 0.4676 | 0.0935 | 24.75** | 0.2628 | 0.0526 | 14.6 |
| | Replicate | 9 | 1.1521 | 0.1280 | 96.03** | 0.3637 | 0.0404 | 10.69** | 0.1693 | 0.0188 | 5.2 |
| | Error | 45 | 0.0479 | 0.0013 | | 0.1700 | 0.0038 | | 0.1609 | 0.0036 | |
| 'Gramaxone' | Treatment | 4 | 0.7701 | 0.1925 | 26.77** | 0.5507 | 0.1377 | 18.62** | 0.1095 | 0.0274 | 4.0* |
| | Replicate | 9 | 0.5477 | 0.0609 | 8.46** | 0.3224 | 0.0358 | 4.84** | 0.3420 | 0.0380 | 5.6* |
| | Error | 36 | 0.2589 | 0.0072 | | 0.2662 | 0.0074 | | 0.2424 | 0.0067 | |
| 'Cuman L(R)' | Treatment | 4 | 0.6845 | 0.1711 | 27.84** | 0.6657 | 0.1664 | 26.38** | 0.1458 | 0.0365 | 8.0* |
| | Replicate | 9 | 0.5018 | 0.0558 | 9.07** | 0.2852 | 0.0317 | 5.02** | 0.1628 | 0.0181 | 3.9* |
| | Error | 36 | 0.2213 | 0.0062 | | 0.2271 | 0.0063 | | 0.1632 | 0.0045 | |
| Mixture of 5 Biocides | Treatment | 5 | 0.4111 | 0.0822 | 34.73** | 0.5313 | 0.1063 | 20.40** | 0.1109 | 0.0222 | 0.2* |
| | Replicate | 9 | 1.3337 | 0.1482 | 62.60** | 0.7393 | 0.0821 | 15.77** | 0.9139 | 0.1016 | 1.3* |
| | Error | 45 | 0.1065 | 0.0024 | | 0.2344 | 0.0051 | | 3.3914 | 0.0754 | |
| Mixture of 3 Biocides | Treatment | 4 | 0.1732 | 0.0433 | 8.38** | 0.5221 | 0.1305 | 24.84** | 0.0863 | 0.0216 | 11.4** |
| | Replicate | 9 | 2.4794 | 0.2755 | 53.32** | 0.2918 | 0.0324 | 6.17** | 0.1619 | 0.0179 | 9.5** |
| | Error | 36 | 0.1859 | 0.0052 | | 0.1891 | 0.0053 | | 0.0676 | 0.0019 | |

* Significant at 5% level, ** Significant at 1% level, N.S. Not significant

TABLE-14.
ANOVA TABLE FOR NET CARBON PRODUCTION

| Toxicant | Source | D.F | Tetraselmis gracilis | | | Dicrateria inornata | | | Mixed culture of fresh water algae | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | S.S | M.S | F | S.S | M.S | F | S.S | M.S | F |
| BHC | Treatment | 5 | 0.0741 | 0.0148 | 8.99** | 0.7116 | 0.1423 | 22.25** | 0.1174 | 0.0235 | 3.19* |
| | Replicate | 9 | 0.8674 | 0.0964 | 58.51** | 0.4924 | 0.0547 | 8.55** | 0.1460 | 0.0162 | 2.18* |
| | Error | 45 | 0.0741 | 0.0017 | | 0.2879 | 0.0064 | | 0.3350 | 0.0074 | |
| Nuvacron | Treatment | 4 | 0.1973 | 0.0493 | 16.67** | 0.3769 | 0.0942 | 32.70** | 0.2542 | 0.0643 | 12.36* |
| | Replicate | 9 | 0.6658 | 0.0739 | 25.00** | 0.0823 | 0.0091 | 3.16** | 0.1765 | 0.0196 | 3.77* |
| | Error | 36 | 0.1065 | 0.0029 | | 0.1037 | 0.0029 | | 0.1873 | 0.0052 | |
| Carbaryl Sevin | Treatment | 5 | 0.1720 | 0.0430 | 19.76** | 0.4467 | 0.0893 | 28.52** | 0.2334 | 0.0467 | 14.49* |
| | Replicate | 9 | 0.8286 | 0.0921 | 42.29** | 0.2361 | 0.0262 | 8.37** | 0.1041 | 0.0116 | 3.59* |
| | Error | 45 | 0.0784 | 0.0022 | | 0.1409 | 0.0031 | | 0.1450 | 0.0032 | |
| Gramaxone | Treatment | 4 | 0.8208 | 0.2052 | 38.37** | 0.5044 | 0.2161 | 14.92** | 0.1292 | 0.0323 | 15.27* |
| | Replicate | 9 | 0.3759 | 0.0418 | 7.81** | 0.2515 | 0.0279 | 3.31** | 0.2359 | 0.0262 | 12.39* |
| | Error | 36 | 0.1925 | 0.0054 | | 0.3043 | 0.0085 | | 0.0761 | 0.0021 | |
| Cuman L(R) | Treatment | 4 | 0.4532 | 0.1133 | 21.36** | 0.5883 | 0.1471 | 41.29** | 0.1235 | 0.0309 | 7.54* |
| | Replicate | 9 | 0.4073 | 0.0453 | 8.53** | 0.1853 | 0.0206 | 5.78** | 0.0927 | 0.0103 | 2.51* |
| | Error | 36 | 0.1909 | 0.0053 | | 0.1282 | 0.0036 | | 0.1474 | 0.0041 | |
| Mixture of 5 Biocides | Treatment | 5 | 0.3018 | 0.0604 | 20.88** | 0.4736 | 0.0947 | 30.56** | 0.6021 | 0.1204 | 1.77 |
| | Replicate | 9 | 1.2424 | 0.1380 | 47.75** | 0.4548 | 0.0505 | 16.30** | 0.7618 | 0.0846 | 1.25 |
| | Error | 45 | 0.1301 | 0.0029 | | 0.1395 | 0.0034 | | 3.0551 | 0.0679 | |
| Mixture of 3 Biocides | Treatment | 4 | 0.0748 | 0.0187 | 7.72** | 0.3865 | 0.0966 | 27.78** | 0.1188 | 0.0297 | 14.90* |
| | Replicate | 9 | 1.2131 | 0.1348 | 55.65** | 0.2155 | 0.0239 | 6.88** | 0.0118 | 0.0132 | 6.63* |
| | Error | 36 | 0.0872 | 0.0024 | | 0.2152 | 0.0035 | | 0.0715 | 0.0019 | |

* Significant at 5% level, ** Significant at 1% level, N.S. Not significant

TABLE-15.
ANOVA TABLE FOR RESPIRATION

| Toxicant | Source | D.F | Tetraselmis gracilis | | | Dicrateria inornata | | | Mixed culture of fresh water algae | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | S.S | M.S | F | S.S | M.S | F | S.S | M.S | F |
| B H C | Treatment | 5 | 0.0059 | 0.0012 | N.S. 1.29 | 0.2997 | 0.0599 | N.S 0.81 | 0.0408 | 0.0082 | N.S 0.54 |
| | Replicate | 9 | 0.0144 | 0.0016 | N.S 1.73 | 0.7228 | 0.0803 | N.S 1.08 | 0.1866 | 0.0207 | N.S 1.36 |
| | Error | 45 | 0.0415 | 0.0009 | | 3.334 | 0.0741 | | 0.6785 | 0.0151 | |
| Nuvacron | Treatment | 4 | 5.659 | 1.415 | N.S 1.13 | 0.03050 | 0.0076 | N.S 0.59 | 0.0041 | 0.0011 | N.S 2.28 |
| | Replicate | 9 | 10.865 | 1.207 | N.S 0.96 | 0.1351 | 0.0150 | N.S 1.17 | 0.0050 | 0.0006 | N.S 1.22 |
| | Error | 36 | 45.22 | 1.256 | | 0.4629 | 0.0129 | | 0.0166 | 0.0005 | |
| Carbaryl Sevin | Treatment | 5 | 0.0093 | 0.0023 | N.S 1.62 | 0.0119 | 0.0024 | * 3.22 | 0.0304 | 0.0061 | N.S 1.65 |
| | Replicate | 9 | 0.0517 | 0.0058 | ** 3.99 | 0.0210 | 0.0023 | ** 3.16 | 0.0980 | 0.0109 | N.S 1.38 |
| | Error | 45 | 0.0517 | 0.0014 | | 0.0333 | 0.0007 | | 0.2605 | 0.0058 | |
| Gramaxone | Treatment | 4 | 0.0144 | 0.0036 | * 3.03 | 0.0046 | 0.0011 | N.S 1.19 | 0.0183 | 0.0046 | N.S 1.74 |
| | Replicate | 9 | 0.0272 | 0.0030 | * 2.54 | 0.0187 | 0.0021 | * 2.19 | 0.0209 | 0.0023 | N.S 0.89 |
| | Error | 36 | 0.0428 | 0.0012 | | 0.0343 | 0.0009 | | 0.0950 | 0.0026 | |
| Cuman L(R) | Treatment | 4 | 0.0422 | 0.0106 | ** 4.59 | 0.0083 | 0.0021 | * 3.43 | 0.0024 | 0.0006 | N.S 2.32 |
| | Replicate | 9 | 0.0343 | 0.0038 | N.S 1.66 | 0.0044 | 0.0005 | N.S 0.80 | 0.0052 | 0.0006 | * 2.22 |
| | Error | 36 | 0.0828 | 0.0023 | | 0.0218 | 0.0006 | | 0.0093 | 0.0003 | |
| Mixture of 5 Biocides | Treatment | 5 | 0.0104 | 0.0021 | N.S 1.91 | 0.0883 | 0.0177 | N.S 1.35 | 0.0064 | 0.0013 | * 2.71 |
| | Replicate | 9 | 0.0196 | 0.0022 | N.S 1.99 | 0.1734 | 0.0193 | N.S 1.48 | 0.0075 | 0.0008 | N.S 1.76 |
| | Error | 45 | 0.0492 | 0.0011 | | 0.5872 | 0.0131 | | 0.0212 | 0.0005 | |
| Mixture of 3 Biocides | Treatment | 4 | 0.0121 | 0.0030 | * 3.25 | 0.0179 | 0.0045 | N.S 1.76 | 0.0029 | 0.0007 | N.S 1.82 |
| | Replicate | 9 | 0.0179 | 0.0019 | N.S 2.15 | 0.0229 | 0.0025 | N.S 0.99 | 0.0059 | 0.0007 | N.S 1.67 |
| | Error | 36 | 0.0334 | 0.0009 | | 0.0917 | 0.0026 | | 0.0141 | 0.0004 | |

* Significant at 5% level, ** Significant at 1% level, N.S. Not significant

its effects on photosynthetic process. Since a substantial part of world's photosynthesis is performed by phytoplankton (Yentsch, 1963), interference with this process could be important to the biosphere.

Results of present investigation made it clear that, the four different concentrations of each biocide tested had significant effect on both gross and net carbon production. Respiratory rate was also found to be affected by the biocide treatment.

1. **Organochlorine insecticide-'BHC'**

Because of the low water solubility of 'BHC', acetone treated cultures were also tested. The acetone was found to have no stimulatory or inhibitory action on carbon production of microalgal culture. This was earlier verified and established by Ramachandran et al., 1981, and Rajendran and Venugopalan (1983). They observed that there was no effect of solvent (acetone) on primary production.

The lowest concentration of 'BHC' tested showed increased carbon production than control in T. gracilis and D. inornata. Increased carbon production at low concentration of organochlorines has not been reported earlier. The increased carbon production in low concentration of the biocide may be due to the increase of photosynthesizing cells, than that was present in the control. As already stated, the 0.5 ppm concentration of 'BHC' showed an increased growth rate than control.

It was found that, at 1 to 4 ppm concentration of 'BHC', the gross and net carbon production was inhibited considerably. The percentage inhibition increased with the increase in the concentration of 'BHC'. Reduction in the rate of photosynthesis in a number of microalgal species has been reported for organochlorine pesticides (Wurstur •1968; Menzel et al., 1970; Subramanian et al., 1979, Ramachandran et al., 1981; Rajendran and Venugopalan 1983. Wurstur (1968) reported that concentrations of DDT as low as few parts per billion in water reduced photosynthesis in laboratory cultures. Menzel et al., 1970 reported that concentration above 10 ppb reudced the $^{14}C$ uptake significantly.

The present finding that organochlorine insecticide inhibited photosynthesis per cell in phytoplankton is consistent with the similar conclusions of Mac Farlane et al., (1972) Fisher 1975; Harding and Phillips (1978b). Their findings are also compatible with the fact that several organochlorine compounds inhibit the photosynthetic process possibly by interfering with electron transport (Lawler and Roger, 1967; Biggs et al., 1978). Fisher (1975) reported that Poly chlorinated biphenyls did not inhibit photosynthesis per cell and that, decreased carbon fixation resulted from reduced number of photosynthesizing cells. But contrary to this, Harding and Phillips (1978b) reported that Polychlorinated biphenyls caused an immediate inhibition of photosynthesis which resulted in reduced growth. Michaels et al., (1982) concluded that inhibited photosynthesis by phytoplankton cultures, apparently resulted both from reduced photosynthesis per cell, and a smaller number of photosynthesizing viable cells in treated cultures than in control. The results of present investigation agree with this statement.

By adopting in situ' method of investigation, there are reports about the decrease in primary production by pesticides (Nassar, 1979, Rajendran and Venugopalan, 1983, Patil et al., 1985). They are of the opinion that at higher concentration the primary production was very much affected. The decrease in gross production is because at higher concentration large population of the autotrophs are practically killed due to the lethal effect of pesticide. (Thosar and Das, 1984).

The present investigation showed that 2 ppm and 4 ppm concentrations of 'BHC' increased the respiratory rate, as a result of which the net production observed was zero, especially at the beginning of the experiment. This was in conformative with the findings of Patil et al., (1985) that, at higher concentrations of DDT, the reserve food material is metabolized and hence the net production became nil. The findings of Piska and Waghray (1990) also support the conclusion, that gross and net production were found to decrease gradually in treated samples when compared to control, and the respiratory rate increased gradually.

Another aspect observed during the present investigation is that, except in D. inormata, with the age of culture, the carbon production was found to increase in treated culture especially at 2 and 4 ppm. This may be due to the reason that after initial inhibition, with the age of culture, the cell number increased. This increased number of photosynthesizing cells could produce more carbon.

2. **Organophosphate - Nuvacron'**

Except mixed culture, the 25 ppm concentration of 'Nuvacron' showed an increased carbon production than that observed for control cultures. It was found that at this concentration the cell number also increased than in control. The increased number of cells may be responsible for the increased carbon production at 25 ppm concentration.

Concentration of 50, 75 and 100 ppm of 'Nuvacron' was found to inhibit the carbon production of three algal cultures under investigation. Several organophosphorus insecticides such as Dursban, Baytex and Abate were found to reduce photosynthetic activity in aquatic algae (Derby and Ruber 1970; Brown et al., 1976), by inhibiting the Hill reaction. Derby and Ruber reported that photosynthesis in four estuarine phytoplankters was inhibited by Baytex and Abate at concentrations of 0.01 to 1 ppm. But they are of the opinion that the response varied with the algal species tested. The results of present investigation also show that the response varied with each microalgae tested.

Methyl parathion and malathion, two organophosphates, were found to inhibit the primary production as reported by Rajendran and Venugopalan (1983). They concluded that the primary effect of pesticide might be on cell division and growth and the decrease in the photosynthetic rate was merely a reflection of the effect as reported by various authors.

Raine et al., (1990) reported that the organophosphate insecticide, Nuvan$^{(R)}$, inhibited photosynthetic carbon dioxide fixation by natural

assemblages of phytoplankton. The inhibition was dose related.

At the Nuvacron concentrations at 75 and 100 ppm, very low values of net production were obtained. This may be because of the reason that as result of increased respiratory rate the complete metabolism of food materials takes place. This was in conformity with the findings of Saha and Singh (1981) that, Malathion, at higher concentration metabolized the reserve food materials and because of the consumption of more oxygen increased respiratory rate was seen.

Monocrotophos, an organophosphate insecticide, was found to inhibit the carbon production (Piska and Waghray, 1990). At and above 100 ppm no net production was observed. This was confirmative with the findings of present investigation. Gross production in the case of treated series descreased with increased concentrations, whereas net production decreased gradually reaching to zero value at high concentration. Thus respiration increased considerably.

3. **Carbamate insecticide -'carbaryl sevin'**

Above 2 ppm concentration of 'carbaryl sevin', the carbon production was very much affected in the three microalgal culture experimented. At 2 ppm concentration, the gross production of T. gracilis showed very little increase than control. Stadynk et al. (1971) reported that carbaryl stimulated $^{14}C$ assimilation in low density population of fresh water alga. But the net production values were low compared to control. This shows an increased respiratory rate of insecticide treated culture.

Concentrations of 4, 6 and 8 ppm of carbaryl sevin invariably affected the gross and net carbon production of microalgae. Inhibition of carbon production by carbamate insecticide has been reported by various investigators. (Derby and Ruber, 1970, Rajendran and Venugopalan 1983). 'Baygon, a carbamate insecticide at concentration of 0.01 to 10 ppm inhibited photosynthesis (Derby and Ruber, 1970).

Rajendran and Venugopalan (1983) tested the effect of carbaryl sevin on primary production by adopting in situ method and it was found that the carbon production was very much affected by 'Carbaryl sevin' even at 20 ppb level. They are of the opinion that a decrease in cell number would affect the photosynthetic rate.

The present data agree with this opinion. But the concentration of Carbaryl sevin used in both the tests show wide difference. Rajendran and Venugopalan (1983) tested upto 20 ppb only and at this concentration only 45.64% production was observed compared to control. During the present investigation the production showed very little inhibition upto 2 ppm but after that serious inhibition was seen. The difference in the required concentration may be because of the reason that, the above mentioned authors used the technical grade Sevin which is 100% pure.. But in the present case commercial grade sevin was used which contain 50% active ingredient. Another reason may be that, as reported by various investigators, the inhibition of primary production varied with the algal species.

As reported in the case of organoclorine and organophosphate insecticides the carbaryl insecticide also showed increased repiration especially at the beginning of the culture. At the beginning, the cell number was low in the treated cultures especially at 6 and 8 ppm. As a result of increased respiratory rate, the carbon produced was completely metabolized which ultimately lead to negligible or nil values for net production.

4. **Herbicide - Gramaxone**

The four different concentration of 'Gramaxone' showed inhibition of carbon production with three microalgal culture under investigation. The lower concentraion tested was 0.2 ppm under which eventhough an increase in gross production was observed, the net production was less compared to control. Reports about photosynthetic inhibition by herbicides are plenty. Walsh (1972) listed a variety of commercial herbicides that inhibit photosynthesis. Two triazine herbicides, atrazine and simazine, inhibited photosynthesis in aquatic algae by blocking electron transport in the Hill reaction in photosystem II (Hawxby et al., 1977).

Plumly and Davis (1980) reported that 2.2 ppm concentration of atrazine reduced the rate of photosynthesis in unialgal cultures of Nitzchia sigma. Results with low concentration indicate an ability to maintain chlorophyll production and cell division but with reduced photosynthesis. The same herbicide atrazine showed an almost complete inhibition of photosynthesis at 500 ppb concentration as reported by De Noyelles et al., (1982). Another herbicide 'Magnacide-H was' found to inhibit the photo-

synthesis in microalgal cultures (Fritz Sheridan (1982). As reported by Hawxby et al., (1977), Hersh and Crumpton (1989) concluded that herbicides inhibited photosynthesis by blocking electron transport between photosystem II and I.

The present investigation showed one peculiarity that with the age of culture, the inhibitory effect changed and at 1.6 ppm the highest production was observed which was on 20th day of experiment. This was in agreement with the findings of Moorhead and Kosinki (1986) that after one week the initial inhibition of primary production changed as a result of herbicide treatment.

Concentration of 0.8 and 1.6 ppm of 'Gramaxone' showed increased respiratory rate. Moorhead and Kosinki (1986) also report high respiration relative to oxygen production and net community productivity values were fairly low.

Recently, Abou waly et al., (1991b) also report that with two herbicides, atrazine and hexazinone, the $^{14}$ uptake was inhibited, and this inhibition was generally related to the herbicide dose.

5. **Fungicide - 'Cuman L.$^{(R)}$'**

The four different concentrations of fungicide were found to inhibit the carbon production of three microalgal cultures. But in T. gracilis, 0.005 ppm was found to produce more carbon than control, only at the beginning of the culture. This increased carbon production may be because

at this concentration the cell number increased than in control.

Concentration of 0.01, 0.02 and 0.04 ppm of fungicide inhibited the gross and net carbon production of microalgae. However, the percentage inhibition was found to vary with respect to dose of the fungicide. The three microalgae under investigation responded differently to the fungicide.

Compared to other biocides like insecticides and herbicides, literature about the effect of fungicide on microalgae is very rare. Harriss et al. (1970) reported that organomercurial fungicide of concentration as low as 0.1 part per billion in water reduced photosynthesis. This shows the high toxic nature of the fungicide tested. But the fungicide tested here showed that above 0.005 ppm (5 ppb) the photosynthesis was very much affected. The difference in concentration required may be due to the difference in quality of the fungicides tested.

Another report about effect of fungicide on primary productivity is that of Somasekhar and Sreenath (1984). The fungicide tested were Brassicol and Benlate from 100 to 1000 ppm. They reported that in comparison with the control, the gross production in the case of treated series decreased with increase in concentration. Whereas the net production decreased gradually reaching a zero value at 1000 ppm.

The present investigation showed that a higher concentration of 0.02 and 0.04, an increase in respiratory rate occurred. At higher concentration of fungicide, probably the reserve food material is metabolized

among phytoplankton and hence the net production become low. This was in line with the findings of Somasekhar and Sreenath (1984).

It was observed that with the age of culture, the production increased especially at 0.2 and 0.4 ppm concentration. As already reported in the results of growth studies, the cell number was found to increase in 0.2 and 0.4 ppm concentration. This increased cell number might have resulted in an increased production of carbon.

6. **Mixture of 5 biocides**

The three microalgal cultures under investigation showed that as a result of the synergistic effect of biocide the carbon production was decreased. The synergistic effect of biocide mixture on phytoplankton production has not been reported earlier.

In this case the inhibition of carbon production was dose related, greater inhibition occurred with high concentration. As observed in the case of each biocide, the mixture of biocides also showed very little net production values at higher concentrations and it was due to increased repiratory rate.

Reduction in carbon production may be due to reduced growth rate. As reported earlier the higher concentration of biocide mixture reduced the growth rate of culture. Treated cultures showed reduced number of photosynthesizing viable cells in the culture. Due to less

number of photosynthesizing cell the production was also at a low rate.

7. **Mixture of 3 biocides**

Compared to the mixture of 5 biocides, the three biocide mixture shows more toxic effect at the same concentrations. The percentage inhibition of carbon production was more with mixture of 3 biocides.

As a result of reduced growth rate of toxicant treated cultures, the cell number was reduced. This reduced cell number was responsible for the decreased carbon production in treated culture than in control cultures.

From the data on carbon production of three microalgal cultures, it was clear that the seven toxicant tested varied in their effect on microalgal culture. Among the 5 biocides tested the fungicide was more toxic than other biocides. The least toxic one was organophosphate insecticide 'Nuvacron'.

As already stated the culture of the estuarine form T. gracilis was more resistant than the other two cultures. In most cases freshwater culture showed maximum inhibition for carbon production. But recovery from the earlier inhibition of carbon production in D. inornata did not take place with the age of culture as observed in T. gracilis and freshwater mixed culture of microalgae.

It was observed that the different concentrations of biocide which retarded photosynthesis were found to inhibit the growth of algae. But

the percentage of inhibition was more with respect to production. As already mentioned with the results of 96 hours bioassay tested production is considered as a sensitive parameter than growth. This is in conformity with the findings of Plumly and Davis (1980) who stated that low concentration of herbicide exhibited an ability to maintain cell division with reduced photosynthesis. As already discussed most biocides affect the photosynthetic electron transport system in chloroplasts of microalgae because photosynthesis involves a series of reactions and external stress of any kind can reduce their photosynthetic activity.

## 3. PIGMENT CONTENT

The effect of various concentrations of biocides on pigments such as chlorophylls and carotenoids were investigated.

Chlorophyll a

### 1. Organochlorine insecticide - B.H.C.

The effect of various concentration of 'BHC' on the chlorophyll a content of T. gracilis is shown in Fig. 21a. The acetone treated cultures showed the same trend in pigment content as observed for control cultures. Concentration of 0.5 ppm of 'BHC' stimulated the pigment content of algae upto the 8th day of experiment. The 2 ppm and 4 ppm concentrations of 'BHC' yielded very low values of chlorophyll a at the beginning of the experiment, but later the values were found to increase from 0.9 $\mu g/10^6$ cells on the 4th day to 1.9 $\mu g/10^6$ cells on the 20th day of experiment.

Statistical interpretation of data, using analysis of variance showed that, between treatments, the variation in chlorophyll a content was significant at 5% level. But between day the variation showed high significance (Table 16).

As in the case of T. gracilis, D. inornata also shows stimulated production of chlorophyll a for 0.5 ppm concentration of 'B.H.C.' (Fig. 21b). But in higher concentrations of 'B.H.C.', the trend of chlorophyll a production was different from T. gracilis i.e. at this concentration the chloro-

phyll values increased upto the 12th day of experiments, and then the pigment content decreased with the age of culture.

Analysis of variance showed that both treatment wise and daywise the variation in chlorophyll a content was highly significant (Table 16).

Unlike in the case of the two other pure cultures, this mixed freshwater culture showed that there was no stimulation of chlorophyll a content at the beginning of the experiment. But after the 12th day the treated cultures showed more pigment content than control. Concentrations of 2 ppm and 4 ppm of 'BHC' showed the highest production of chlorophyll a on the 12th day of experiment but later the pigment content decreased.

Analysis of variance showed that there was no significant variation in cchlorophyll a content of freshwater culture between treatments. But between days the variation in chlorophyll a content was highly significant (Table 16).

2. **Organophosphate insecticide - 'Nuvacron'**

The three culture investigated here responded differently to this insecticide (Fig. 22).

'Nuvacron' of 25 ppm caused an increase in chlorophyll 'a' content than control in T. gracilis and mixed freshwater culture, only at the beginning of the experiment. But in the case of D. inornata 25 ppm showed increased chlorophyll 'a' content than control on the 12th day of observation

At 100 ppm concentration of 'Nuvacron', the chlorophyll a content was not very much affected in T. gracilis and mixed freshwater culture. But in the case of marine species D. inornata, 75 and 100 ppm concentrations showed very low values of chlorophyll a.

Statistical analysis showed that in T. gracilis there was no significant variation in chlorophyll a content between treatments. But between days there was significant variations. In D. inornata, between treatments, the variation was highly significant, but between days it was not significant. In the case of mixed freshwater culture between treatments there was no significant variation in chlorophyll a content. But between days the chlorophyll a content was found to be highly significant (Table 16).

### 3. Carbamate insecticide - 'Carbaryl Sevin'

Fig. 23 shows the variation in clorophyll a content of three cultures under four different concentration of 'Carbaryl Sevin'. As shown in the figure, the chlorophyll a content of T. gracilis was not much affected. Increased chlorophyll a content than control was observed for the 2 and 4 ppm concetrations on the 4th and 8th day. Observation made onthe 20th day showed the highest value of chlorophyll a content in 8 ppm concentration. But in the case of D. inornata, the chlorophyll a content was not stimulated by the lower concentrations of carbaryl sevin. The four different concentrations tested shows low values for chlorophyll a than control. For 8 ppm the maximum chlorophyll a content was observed on the 20th day of experiment. In the case of mixed culture of freshwater algae eventhough no stimulation of chlorophyll a was observed at the

beginning the four different concentrations gave chlorophyll value which was above 50% of that observed for control cultures. When the culture attained 16th day growth the 6 ppm and 8 ppm of carbaryl sevin showed the highest values of chlorophyll a.

Statistical interpretation of data using analysis of variance indicated that, in the case of T. gracilis, the variation of chlorophyll a content was not significant between treatments. But between days the variation was highly significant. In D. inornata, between treatments the variation was highly significant but between days the variation was significant at 5% level. Coming to freshwater culture, there was no significant variation in chlorophyll a content between treatments. But between days there was highly significant variation in chlorophyll a content (Table 16).

4. **Herbicide - 'Gramaxone'**

Fig. 24 shows the effect of four different concentration of 'Gramaxone' on chlorophyll a content of three microalgal culture.

At the beginning of the experiment, eventhough higher concentration of 0.8 and 1.6 ppm showed some decreased production of chlorophyll a compared to control, the difference was not well marked. In the case of T. gracilis 0.2 and 0.4 ppm concentration showed an increased chlorophyll a content than control from the 8th day onwards. At concentration of 1.6 ppm, the highest chlorophyll a content was observed on 4th day of experiment, after that the chlorophyll a content decreased upto the 16th day, and the 20th day observation showed little increase than on the 16th day. D. inornata also showed reduced chlorophyll a content than

control. In the case of mixed culture of freshwater algae, 0.2 ppm showed increase in chlorophyll a content than control on the 4th day. After the 8th day of experiment. the chlorophyll a content was found to decrease in both control and treated cultures.

Statistical treatment of data using analysis of variance showed that, the three microalgal cultures under investigation shows, significant variation in chlorophyll a content, between treatments. But between days T. gracilis showed significance at 5% level. In D. inornata, the variation was not significant. But in the case of freshwater algae there was variation between days in chlorophyll a content and was highly significant (Table 16).

## 5. Fungicide - Cuman L(R)

The effect of different concentrations of fungicide Cuman L(R) on chlorophyll a content of three microalgal cultures is shown in figures 25.

In the case of T. gracilis the lowest concentration of 0.005 ppm showed an increase in chlorophyll a content than control. On the 4th day of observation 0.005 ppm and 0.01 ppm showed the peak value of 3.7 $\mu g/10^6$ cells on 12th day observation. The highest concentration of 0.04 ppm concentration shows the peak value of 2.5 $\mu g/10^6$ cells on the 20th day. Unlike T. gracilis chlorophyll a content of D. inornata was very much affected by fungicide treatment. The lowest concentration of 0.005 ppm showed the maximum value of 1.8 $\mu g/10^6$ cells on 12th

day which was only 69.23% of control observed on the same day. The highest concentration gave very low values of chlorophyll a upto the 12th day of observation. Afterwards little enhancement was noticed and the highest value of 0.5 $\mu g/10^6$ cells was observed on the 20th day. But in the case of mixed culture of freshwater algae 4th, and 8th day observations showed low values of chlorophyll a content compared to control. But with the age of culture, the variation was not well marked, as shown in fig. 25c.

Analysis of variance revealed that in the case of T. gracilis, both treatment wise and daywise the variation in the chlorophyll a content was highly significant. In the case of D. inornata the variation was highly significant between treatment. But between days the variation was not significant. The freshwater culture showed significance at 5% level between treatment and between days the variation in chlorophyll a was highly significant(Table 16)

6. **Mixture of 5 biocides**

Fig. 26 shows the effect of mixture of 5 biocides on the chlorophyll a content of three microalgal cultures. No increase on chlorophyll a content was observed in any of the cultures tested. Eventhough the treated cultures showed low values of chlorophyll a compared to control, the percentage of inhibition was very less. With the highest concentration tested the three cultures especially T. gracilis showed increased chlorophyll a content with the age of the culture.

Analysis of variance revealed that in the case of T. gracilis, between treatments, the variation in chlorophyll a content was not significant, but between days, highly significant variation in chlorophyll a was observed D. inornata showed highly significant variation in chlorophyll a content between treatments and between days. In the case of mixed culture, the variation in chlorophyll a content was not significant between treatments but between days the variation was significant at 5% level (Table 16).

## 7. Mixture of 3 biocides

The effects of four different concentrations of mixture of three biocides on chlorophyll a content of three microalgal culture are presented in Fig. 27. In T. gracilis and fresh water algal culture, the lower concentrtion of 0.05 ppm, showed an increased chlorophyll a content than control, at the beginning of the experiment. The higher concentration tested showed low values of chlorophll a at the beginning, but afterwards with the age of culture it increased.

Analysis of variance revealed that in the case of T. gracilis, there was no significant variation in chlorophyll a content between treatment, but between days there was highly significant variation. D. inornata showed highly significant variation in chlorophyll a content between treatments and also between days. The mixed culture of microalage showed that between treatments the variation was significant at 5% level, and between days the significance was very high (Table 16).

FIG.21

EFFECT OF 'BHC' ON CHLOROPHYLL a CONTENT OF MICROALGAE

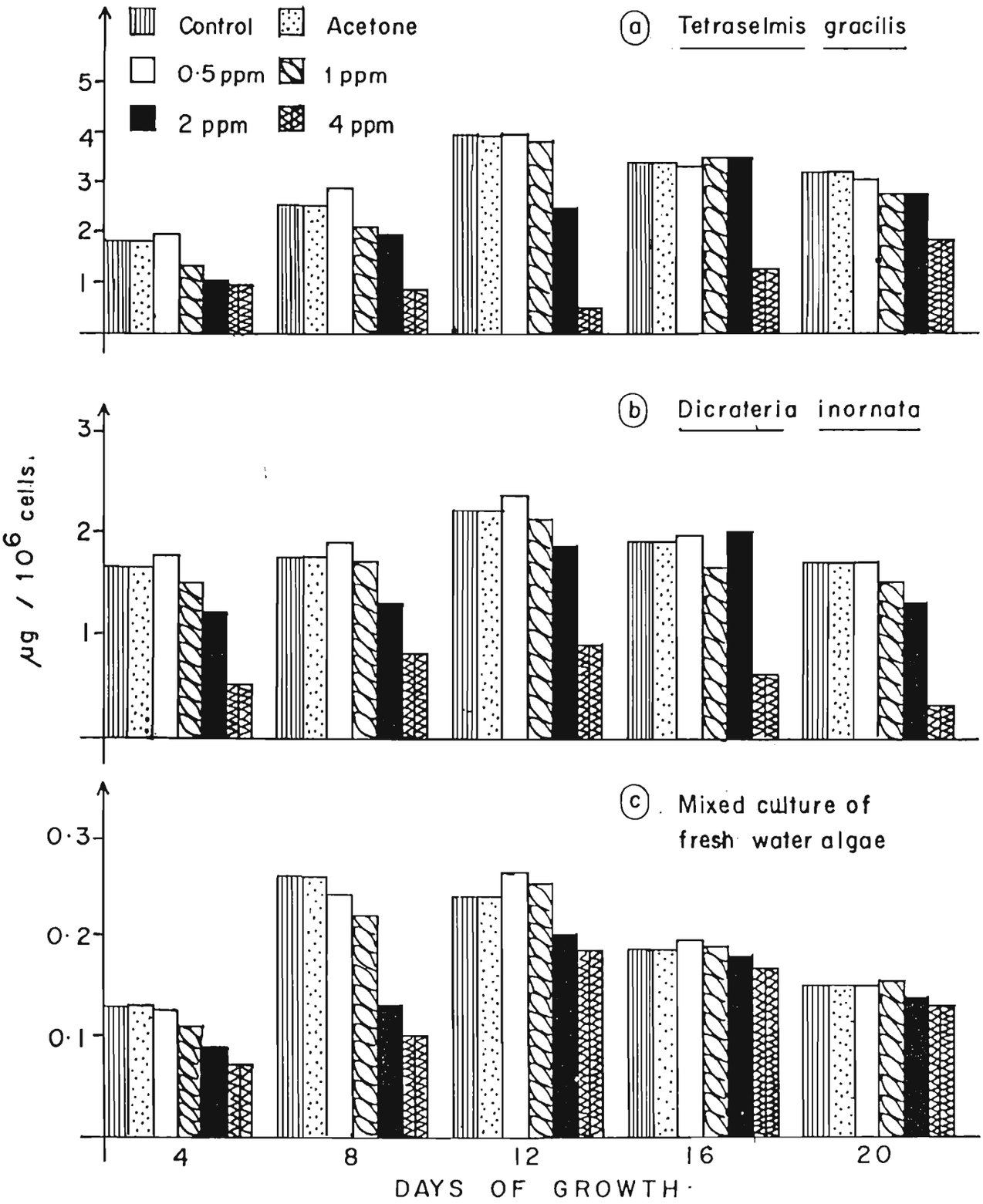


FIG.22

EFFECT OF 'NUVACRON' ON CHLOROPHYLL a CONTENT OF MICROALGAE

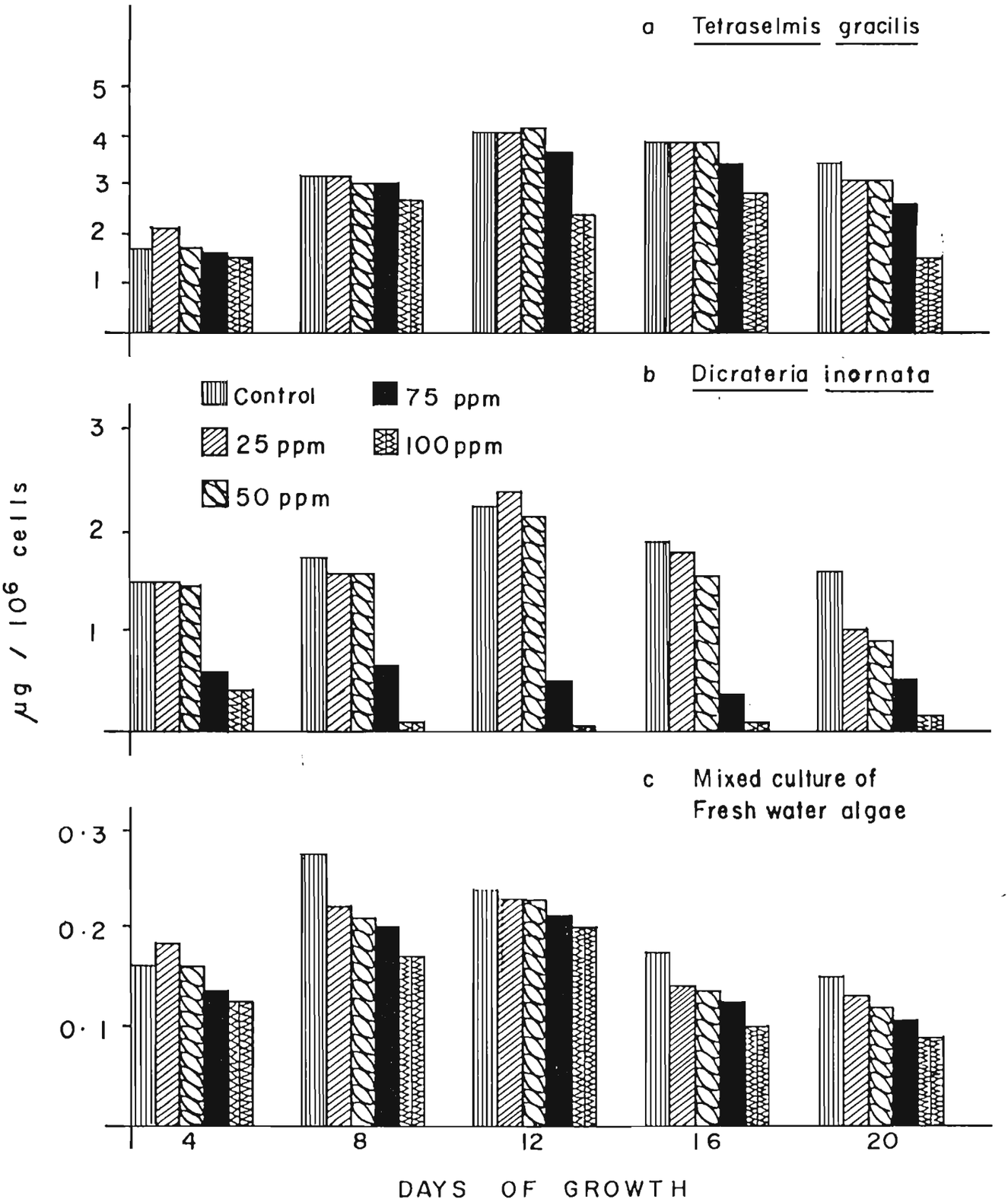


FIG.23.

EFFECT OF 'CARBARYL SEVIN' ON CHLOROPHYLL a CONTENT OF MICROALGAE

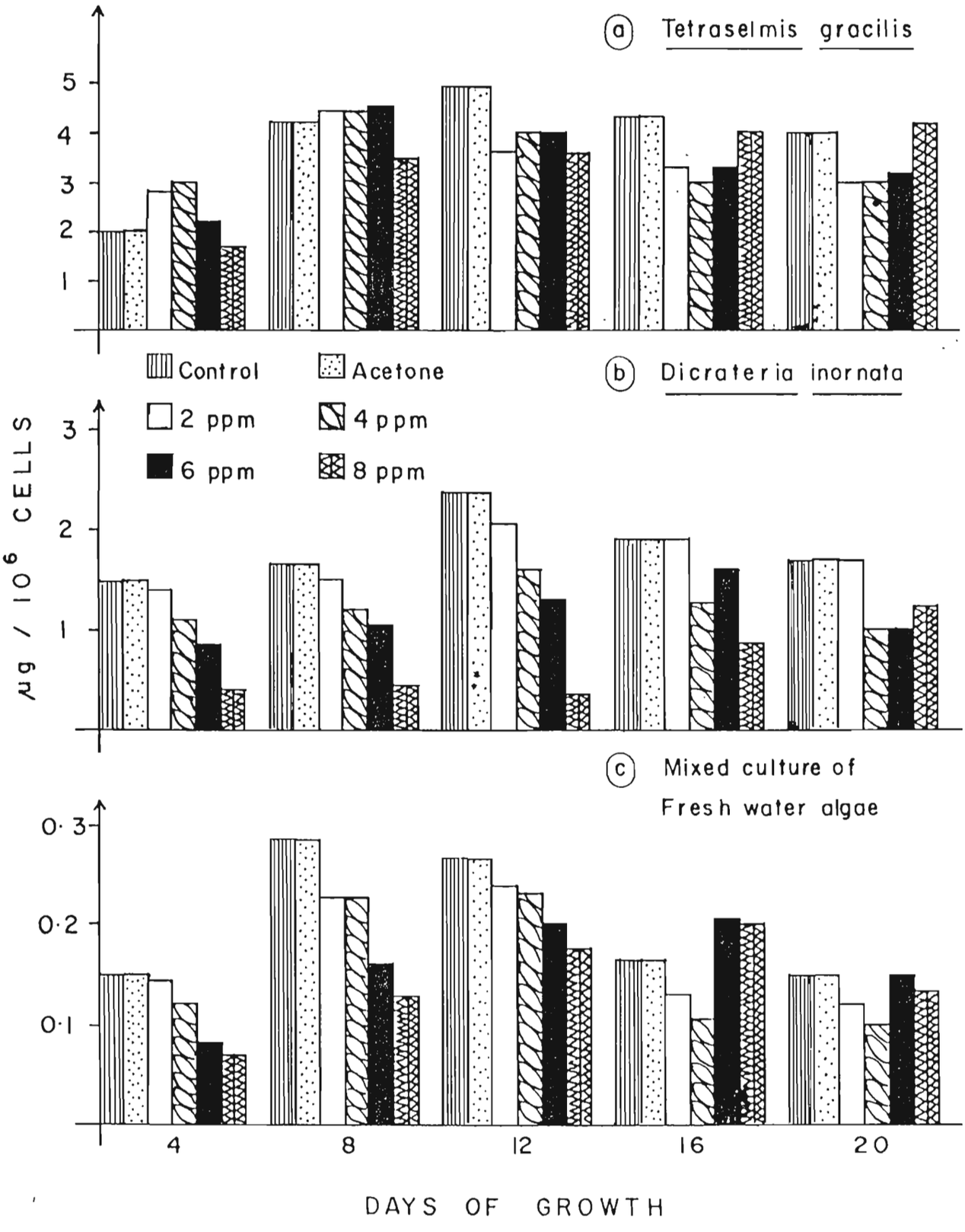


FIG.24

EFFECT OF 'GRAMAXONE' ON CHLOROPHYLL a CONTENT OF MICROALGAE

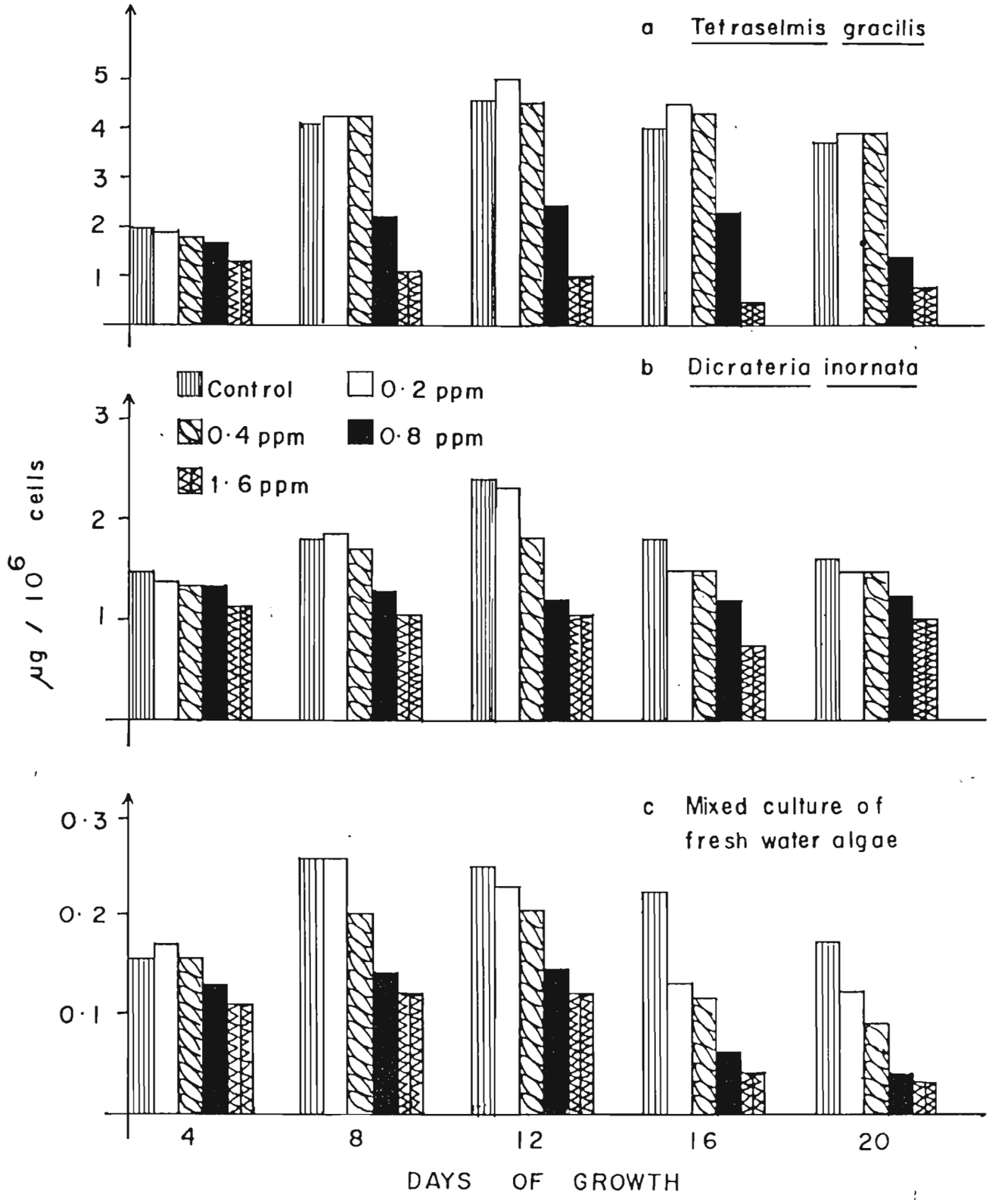


FIG.25

EFFECT OF CUMAN L® ON CHLOROPHYLL a CONTENT OF MICROALGAE

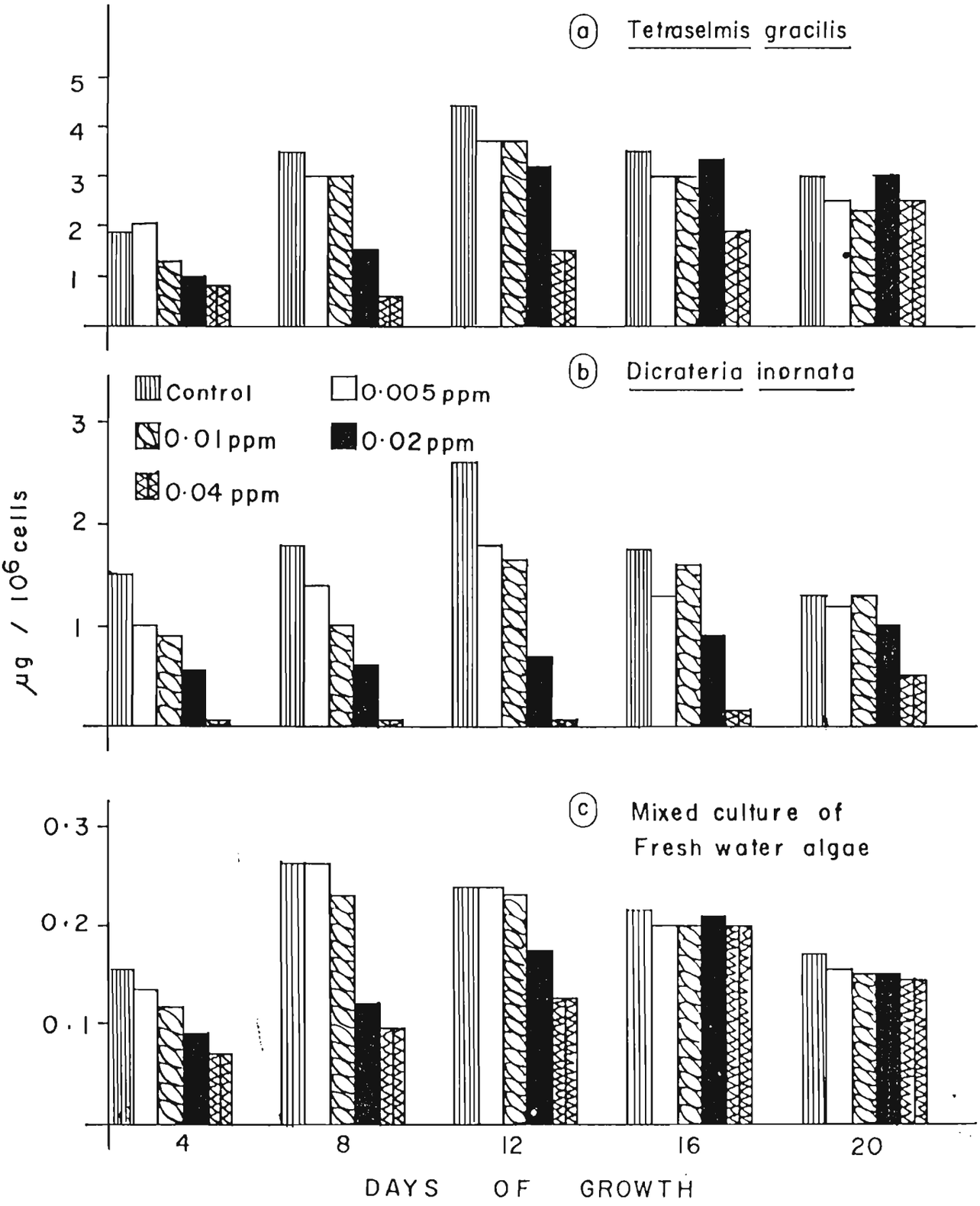


FIG.26.

EFFECT OF MIXTURE OF 5 BIOCIDES ON CHLOROPHYLL a CONTENT OF MICROALGAE

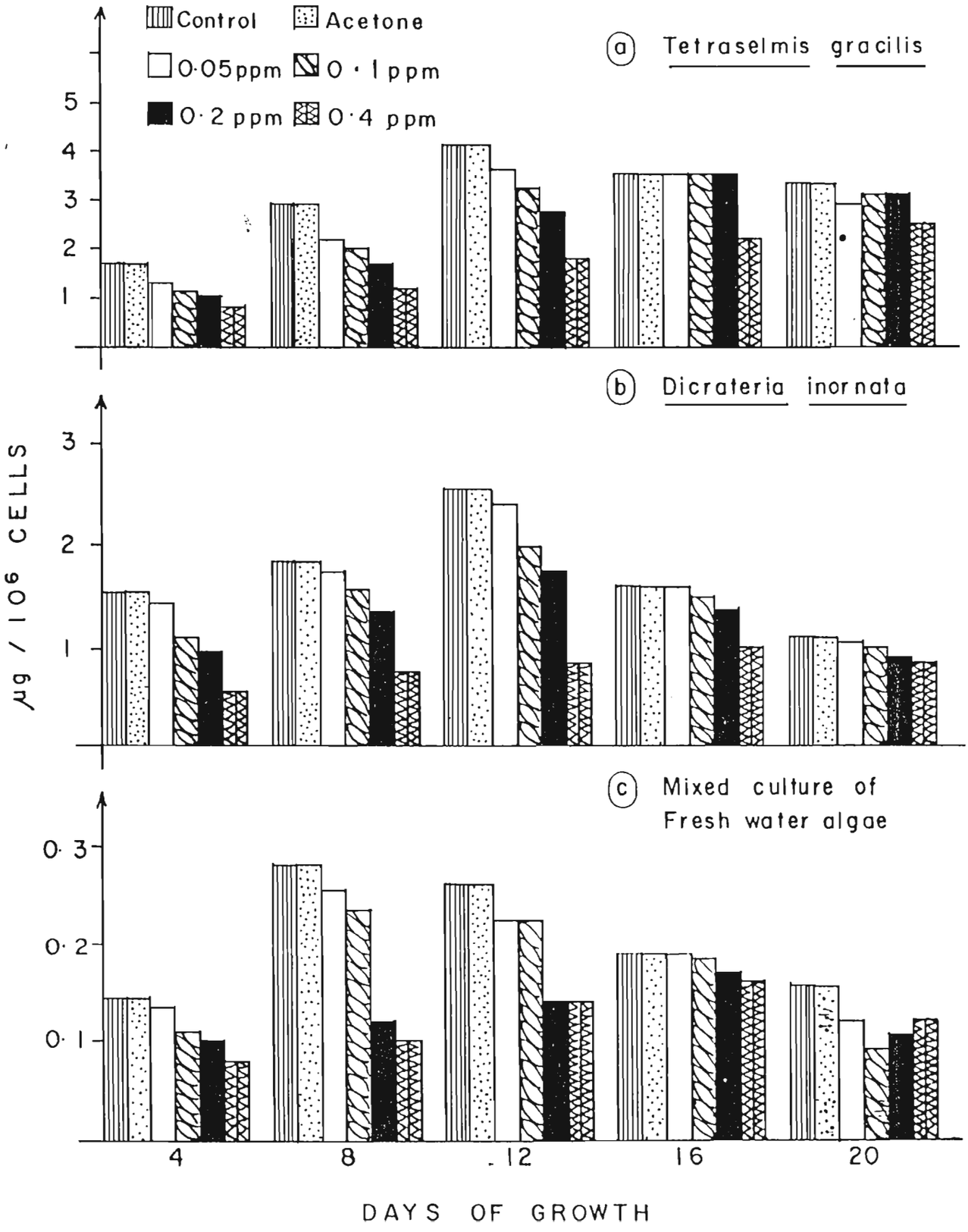


FIG.27

EFFECT OF MIXTURE OF 3 BIOCIDES ON CHLOROPHYLL a, CONTENT OF MICROALGAE

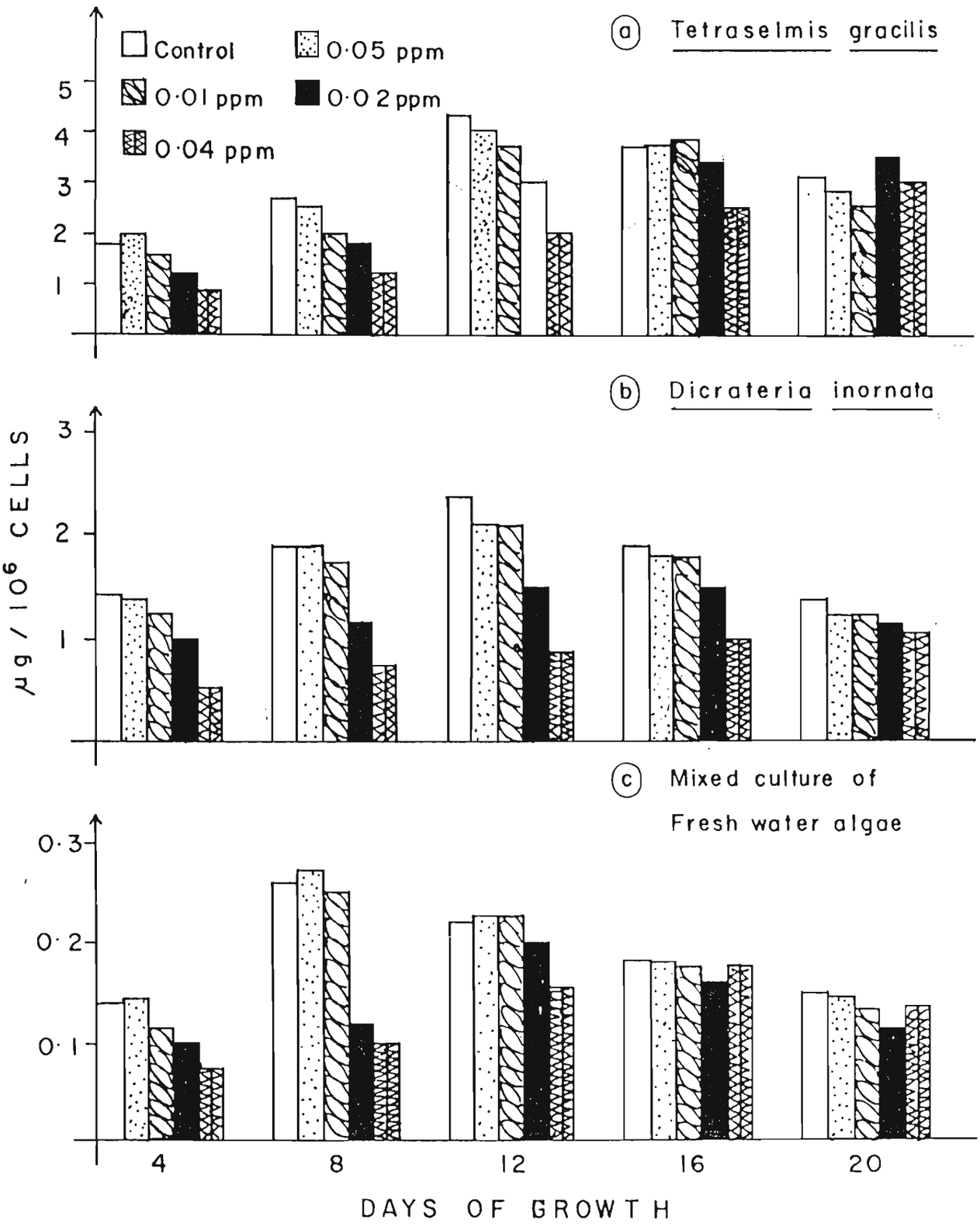


TABLE-16.
ANOVA TABLE FOR CHLOROPHYLL a'

| Toxicant | Source | D.F | Tetraselmis gracilis | | | Dicrateria inornata | | | Mixed culture of fresh water algae | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | S.S | M.S | F | S.S | M.S | F | S.S | M.S | F |
| BHC | Treatment | 5 | 9.035 | 1.8069 | 4.049* | 8.4723 | 1.6945 | 127.71** | 0.0099 | 0.0019 | N.S 0.74 |
| | Replicate | 4 | 19.1147 | 4.7787 | 10.71** | 1.4109 | 0.3527 | 26.58** | 0.0692 | 0.0173 | 6.41** |
| | Error | 20 | 8.925 | 0.4463 | | 0.2654 | 0.0133 | | 0.0539 | 0.0027 | |
| Nuvacron | Treatment | 4 | 0.5385 | 0.1346 | N.S 0.58 | 14.1342 | 3.5335 | 36.22** | 0.0045 | 0.0011 | N.S 2.09 |
| | Replicate | 4 | 3.3434 | 0.8356 | 3.62* | 1.1288 | 0.2822 | N.S 2.89 | 0.0452 | 0.0113 | 21.18** |
| | Error | 16 | 3.6962 | 0.2310 | | 1.5609 | 0.0976 | | 0.0085 | 0.0005 | |
| Carbaryl Sevin | Treatment | 5 | 1.0299 | 0.2059 | N.S 0.48 | 5.9849 | 1.1969 | 16.19** | 0.0098 | 0.0019 | N.S 1.29 |
| | Replicate | 4 | 12.5133 | 3.1283 | 7.21** | 1.1289 | 0.2822 | 3.82* | 0.0557 | 0.0139 | 9.15** |
| | Error | 20 | 8.6745 | 0.4337 | | 1.4785 | 0.0739 | | 0.0304 | 0.0015 | |
| Gramaxone | Treatment | 4 | 44.681 | 11.1702 | 20.13** | 1.5905 | 0.3976 | 6.79** | 0.0586 | 0.0146 | 18.77** |
| | Replicate | 4 | 8.245 | 2.0613 | 3.71* | 0.4069 | 0.1017 | N.S 1.74 | 0.0474 | 0.0119 | 15.19** |
| | Error | 16 | 8.880 | 0.5550 | | 0.9360 | 0.0585 | | 0.0125 | 0.0008 | |
| Cuman L(R) | Treatment | 4 | 11.607 | 2.9018 | 8.99** | 8.5957 | 2.1489 | 10.32** | 0.0205 | 0.0051 | 4.33* |
| | Replicate | 4 | 19.3228 | 4.8307 | 14.97** | 0.8473 | 0.2118 | N.S 1.02 | 0.0329 | 0.0082 | 6.95** |
| | Error | 16 | 5.1633 | 0.3226 | | 3.3327 | 0.2083 | | 0.9189 | 0.0012 | |
| Mixture of 5 Biocides | Treatment | 5 | 2.3651 | 0.4730 | N.S 1.59 | 3.4276 | 0.6855 | 5.14** | 0.0218 | 0.0044 | N.S 1.66 |
| | Replicate | 4 | 15.4314 | 3.8579 | 12.99** | 2.8743 | 0.7186 | 5.39** | 0.0439 | 0.0109 | 4.20* |
| | Error | 20 | 5.9422 | 0.2971 | | 2.6679 | 0.1334 | | 0.0524 | 0.0026 | |
| Mixture of 3 Biocides | Treatment | 4 | 0.9934 | 0.2483 | N.S 0.94 | 3.9349 | 0.9838 | 28.51** | 0.0168 | 0.0042 | 3.14* |
| | Replicate | 4 | 23.1072 | 5.7768 | 21.86** | 0.7203 | 0.1801 | 5.22** | 0.0295 | 0.0074 | 5.51** |
| | Error | 16 | 4.2279 | 0.2642 | | 0.5522 | 0.0345 | | 0.0214 | 0.0013 | |

* Significant at 5% level, ** Significant at 1% level, N.S. Not significant

Chlorophyll 'b'

Chlorophyll b is an accessory pigment in chlorophytes. Out of the three microalgal cultures investigated, T. gracilis and mixed culture of freshwater algae have the chlorophyll b content in their chloroplast. Like chlorophyll a, chlorophyll b was also affected with the biocide treatment.

1. **Organochlorine insecticide - B.H.C.**

Fig. 28 a shows the effect of four different concentrations of 'BHC on chlorophyll b content of T. gracilis and mixed culture of freshwater algae. Both control and acetone treated control did not show any variation in their chlorophyll b content. In T. gracilis 0.5 ppm of 'BHC' showed increased chlorophyll b content than control. The observation showed 1.5 $\mu g/10^6$ cells for 0.5 ppm but on the same day the control showed only 1.1 $\mu g/10^6$ cells. In the case of mixed culture no increased chlorophyll b content was observed even with the lowest concentration tested. The higher concentration tested showed low values of chlorophyll b content at the beginning. But afterwards with the age of culture, this inhibition reduced.

Analysis of variance revealed that in the case of T. gracilis, there was no significant variation in chlorophyll b content between treatments. But between days the significance was at 1% level. In the case of mixed culture freshwater algae, between treatment and between days the variation was significant at 1% level. (Table 17a).

## 2. Organophosphate insecticide - 'Nuvacron'

The effect of four different concentrations of 'Nuvacron' on chlorophyll b content of two microalgal culture is explained in Fig. 28b. The chlorophyll b content in T. gracilis was not much affected with the 'Nuvacron' treatment, but little variation in chlorophyll b content was noticed especially on the 4th and 20th day of observation. With organophosphate insecticide the chlorophyll b content of mixed freshwater culture was very much affected. Observation made on the 4th and 8th day showed that all the cultures treated with four different concentrations of pesticide had chlorophyll b content below 50% of that control.

Analysis of variance revealed that in the case of T. gracilis, between treatment the variation in chlorophyll b content was significant at 5% level, and between days the significance was at 1% level. In the case of mixed fresh water culture, both treatment wise and daywise, the variation in chlorophyll b content was significant at 1% level (Table 17a).

## 3. Carbamate insecticide - 'Carbaryl sevin'

Fig 29 a presents the difference in chlorophyll b values as a result of 'Carbaryl sevin' treatment. As shown in the Fig. it was observed that the chlorophyll b content of T. gracilis was not much altered by this insecticide. Stimulation in chlorophyll b was observed with the lower concentration especially at the beginning of the experiment. But coming to mixed culture, chlorophyll b values showed significant variation with the 'Carbaryl ' treated cultures. The insecticide treated cultures showed low values of chlorophyll b content compared to control.

Analysis of variance revealed that in the case of T. gracilis , between treatments there was no variation in chlorophyll b content, but between days the variation was highly significant. In the case of mixed culture of freshwater algae, the variation was highly significant between treatments, and between days the variation in chlorophyll b content was not significant (Table 17a).

4. **Herbicide 'Gramaxone'**

The variation in chlorophyll b content as a result of herbicide treatment is shown in Fig. 29b. The lower concentration of 0.2 and 0.4 ppm effected increased chlorophyll b content than control. With a concentration of 1.6 ppm the chlorophyll b remained the same upto the 12th day, then after decrease it again increased on 20th day of observation. The herbicide was found to affect the chlorophyll b production in the mixed culture. The herbicide treated cultures showed low values of chlorophyll b content compared to control. The inhibition was very marked with the age of culture.

Analysis of variance revealed that in the case of T. gracilis both treatment and day wise there was highly significant variation in chlorophyll b content. But in the case of mixed culture the variation was not significant (Table 17a).

5. **Fungicide - Cuman L(R)**

Fig. 30 shows the variation in chlorophyll b content of two micro-algal culture as a result of fungicide treatment. In T. gracilis the lower

concentration of fungicide showed increased amount of chlorophyll b values than that of control cultures. With the other biocides tested, in this case also the highest concentration showed an initial inhibition. With the same fungicide, the mixed culture showed no increased chlorophyll b content even at the lower concentrations tested. The four different concentration tested invariably affected the chlorophyll b content of the microalgae.

Analysis of variance revealed that, two microalgal culture under investigation show highly significant variation in chlorophyll b content, both treatment wise and daywise (Table 17a).

6. **Mixture of 5 biocides:**

Fig. 31a shows the effect of mixture 5 biocides on chlorophyll b content of two microalgal culture. In the case of T. gracilis the chlorophyll b was not much affected by toxicant treatment. But with freshwater culture, the higher concentration of 0.4 ppm gave very low values of chlorophyll b. The peak value observed in this concentration was 0.04 $\mu g/10^6$ cells on the 12th day and afterwards there was a decreasing tendency.

Analysis of variance revealed that, in the case of T. gracilis., between treatment the variation in chlorophyll b content was not significant. But between days the variation was highly significant. Mixed culture showed highly significant variation, between treatments and between days (Table 17a).

7. **Mixture of 3 biocides:**

Fig. 31b. shows the chlorophyll b content was effected with the toxicant treatment. At the beginning of the culture, the lower concentration caused a little increase in chlorophyll b content compared to control. It was observed that when the culture reached the 16th and 20th day of growth, the toxicant treated cultures showed high values of chlorophyll b than control. In the case of mixed culture, as observed with T. gracilis, the earlier inhibition was removed with age of culture.

Analysis of variance indicated that, in T. gracilis, between treatment the variation was significant at 5% level, and between days 1% significance was observed. But in the case of mixed culture the variation was highly significant between treatment and between days the variation was significant at 5% level (Table 17a).

Chlorophyll c

In addition to chlorophyll a, D. inornata contain chlorophyll c, also as a necessary pigment in their chloroplast. As observed in the case of chlorophyll a content of D. inornata chlorophyll c also was found to be affected with the biocide treatment (Fig. 32 and 33).

With the organochlorine insecticide, 'BHC', 0.5 ppm concentration showed a little increase in chlorophyll c content than control on the 8th and 12th day of experiment. When the highest concentration of 4 ppm was tested the chlorophyll c content decreased very much compared to

control. Analysis of variance revealed that both treatment wise and daywise the variation in chlorophyll c content was significant at 1% level (Table 17b).

The organophosphate insecticide 'Nuvacron' showed that, eventhough at concentrations of 25 and 50 ppm, the chlorophyll c did not show much difference, in the higher concentration of 75 and 100 ppm the chlorophyll c content was very much reduced. Less than 0.1 $\mu g/10^6$ cells was observed at 100 ppm. But the statistical results showed that the variation in chlorophyll c content was not significant (Table 17b).

'Carbaryl sevin' treatment showed that eventhough there was marked variation in chlorophyll c content of insecticide treated cultures, at the beginnning of the experiment, with the age of culture the variation was not well marked. Analysis of variance showed that between treatment the variation was not significant, but between days the significance was at 5% level (Table 17b).

Culture treated with 0.2 ppm of Herbicide, 'Gramaxone' showed very little increase of chlorophyll b on the 8th and 12th day of observation. On the 4th day the chlorophyll c content of herbicide treated cultures was not much affected. But afterwards the chlorophyll c content declined at 1.6 ppm concentration of 'Gramaxone'. Analysis of variance showed that between treatments the variation in chlorophyll c content was significant at 1% level, but between days the variation was not significant. (Table 17b).

FIG.28

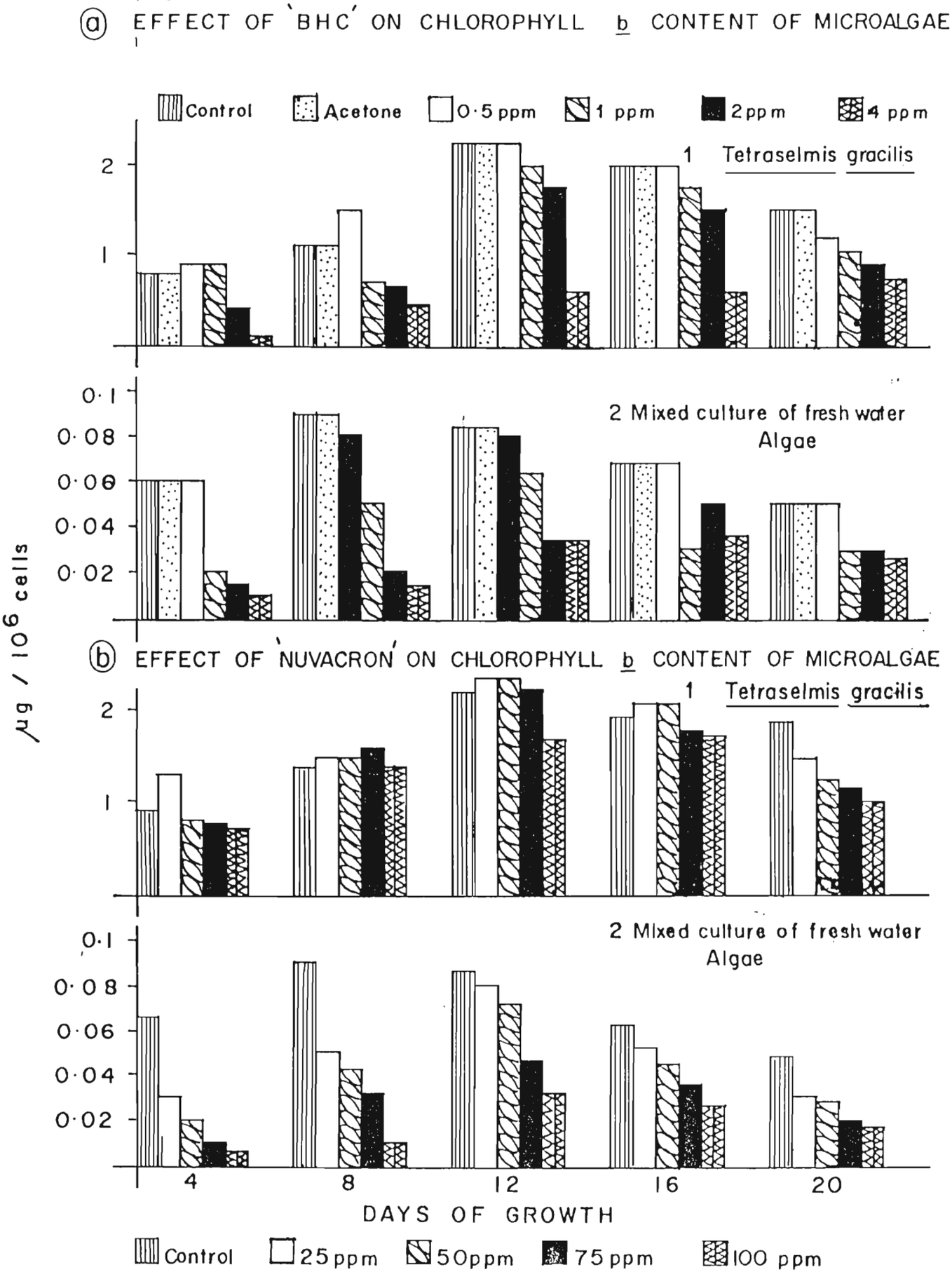

(a) EFFECT OF 'BHC' ON CHLOROPHYLL b CONTENT OF MICROALGAE
Control
Acetone
0·5 ppm
1 ppm
2 ppm
4 ppm
1 Tetraselmis gracilis
2 Mixed culture of fresh water Algae
µg / 10⁶ cells
(b) EFFECT OF 'NUVACRON' ON CHLOROPHYLL b CONTENT OF MICROALGAE
1 Tetraselmis gracilis
2 Mixed culture of fresh water Algae
DAYS OF GROWTH
Control
25 ppm
50 ppm
75 ppm
100 ppm

FIG.29

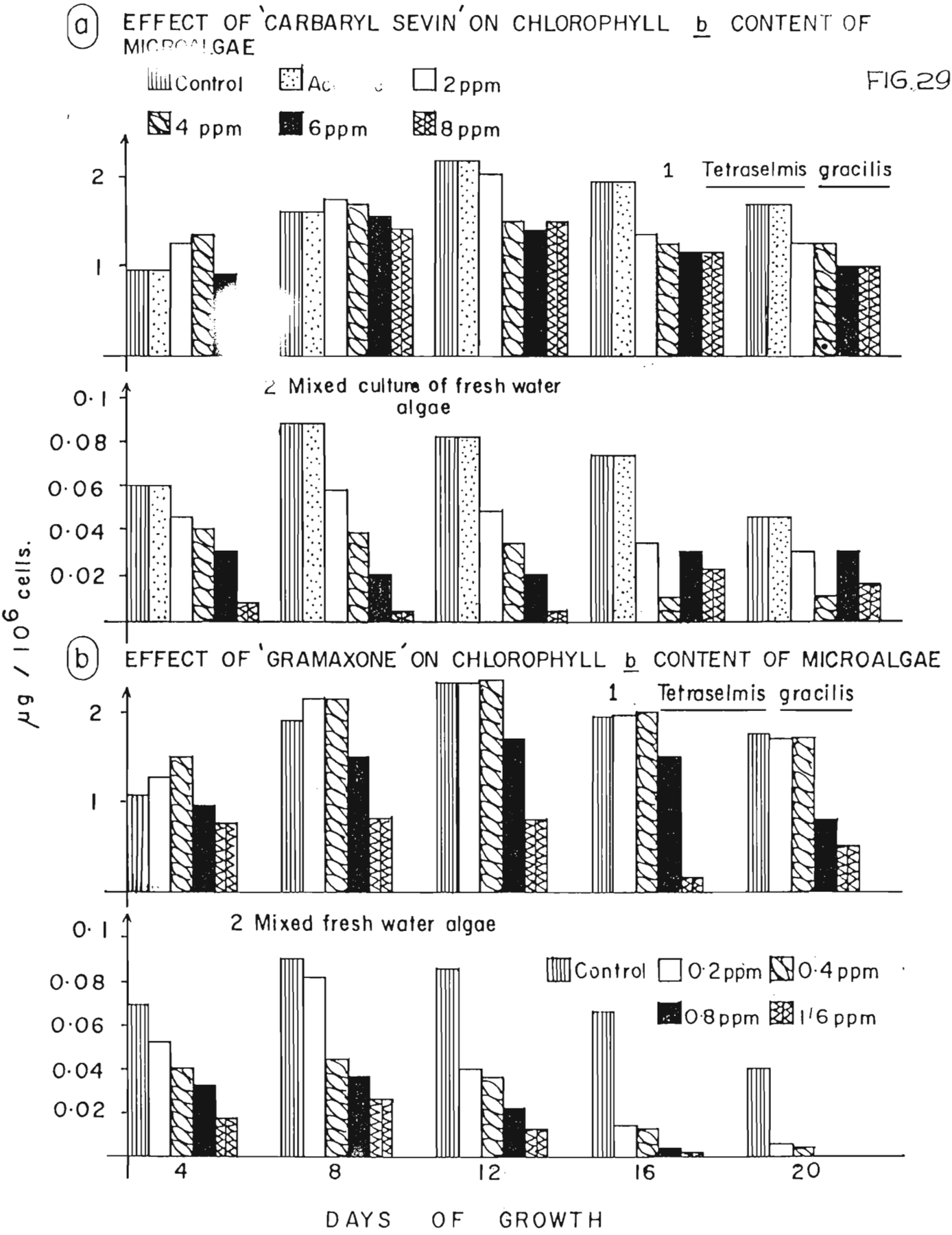

(a) EFFECT OF 'CARBARYL SEVIN' ON CHLOROPHYLL b CONTENT OF MICROALGAE
Control
Ac
2 ppm
4 ppm
6 ppm
8 ppm
1 Tetraselmis gracilis
2 Mixed culture of fresh water algae
(b) EFFECT OF 'GRAMAXONE' ON CHLOROPHYLL b CONTENT OF MICROALGAE
1 Tetraselmis gracilis
2 Mixed fresh water algae
Control
0·2 ppm
0·4 ppm
0·8 ppm
1·6 ppm
µg / 10⁶ cells.
DAYS OF GROWTH

FIG.30

EFFECT OF CUMAN L® ON CHLOROPHYLL b CONTENT OF MICROALGAE

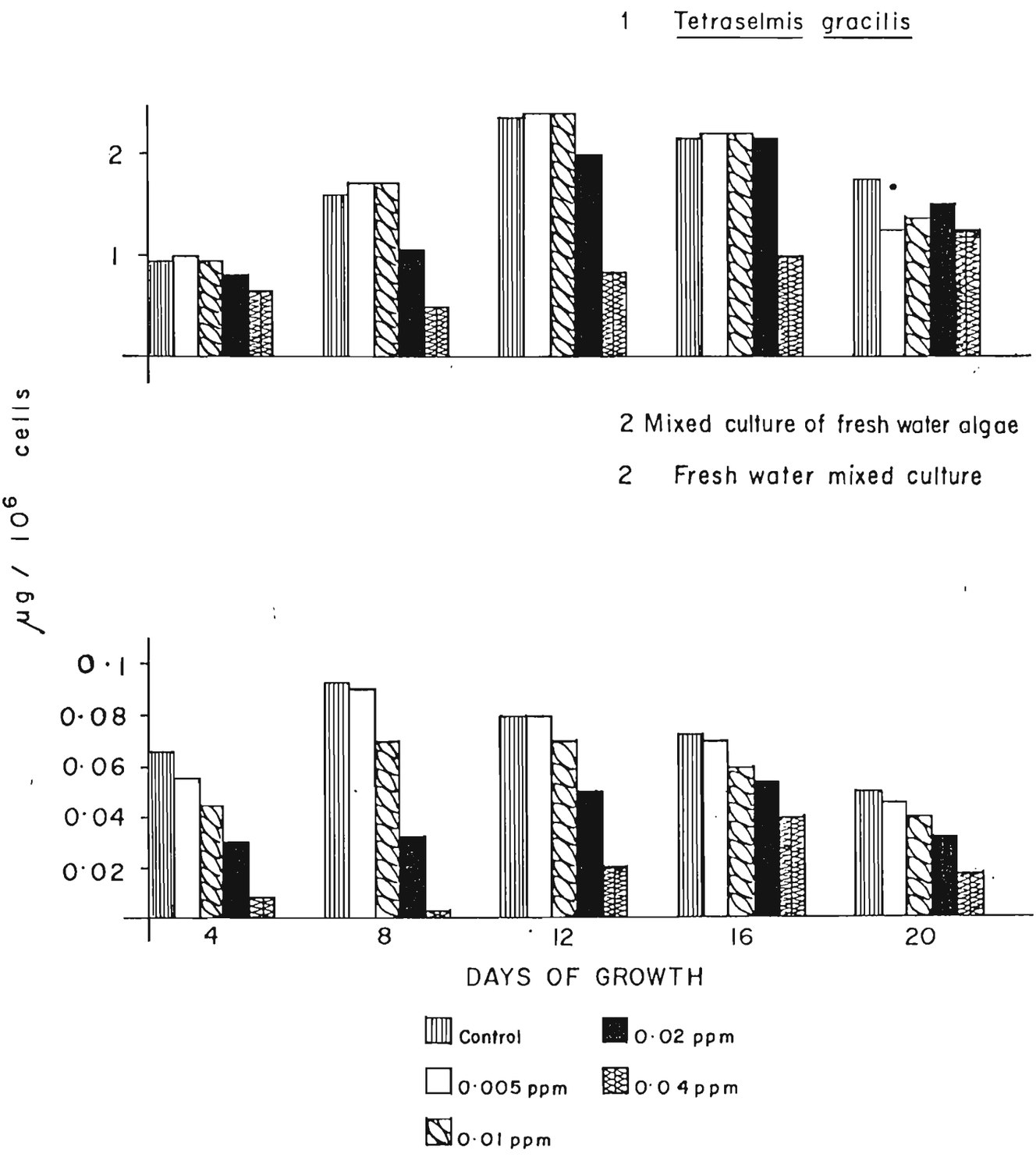
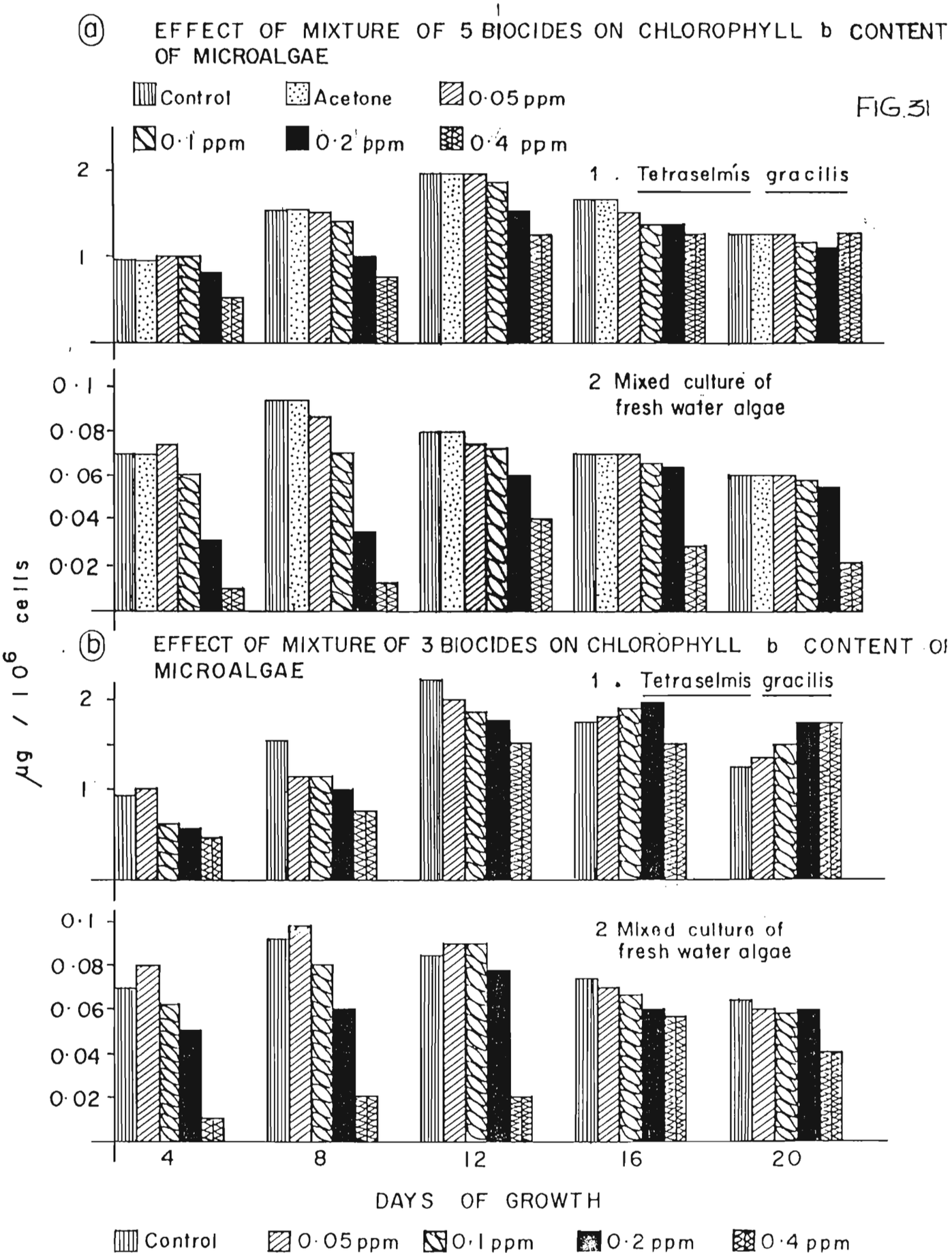
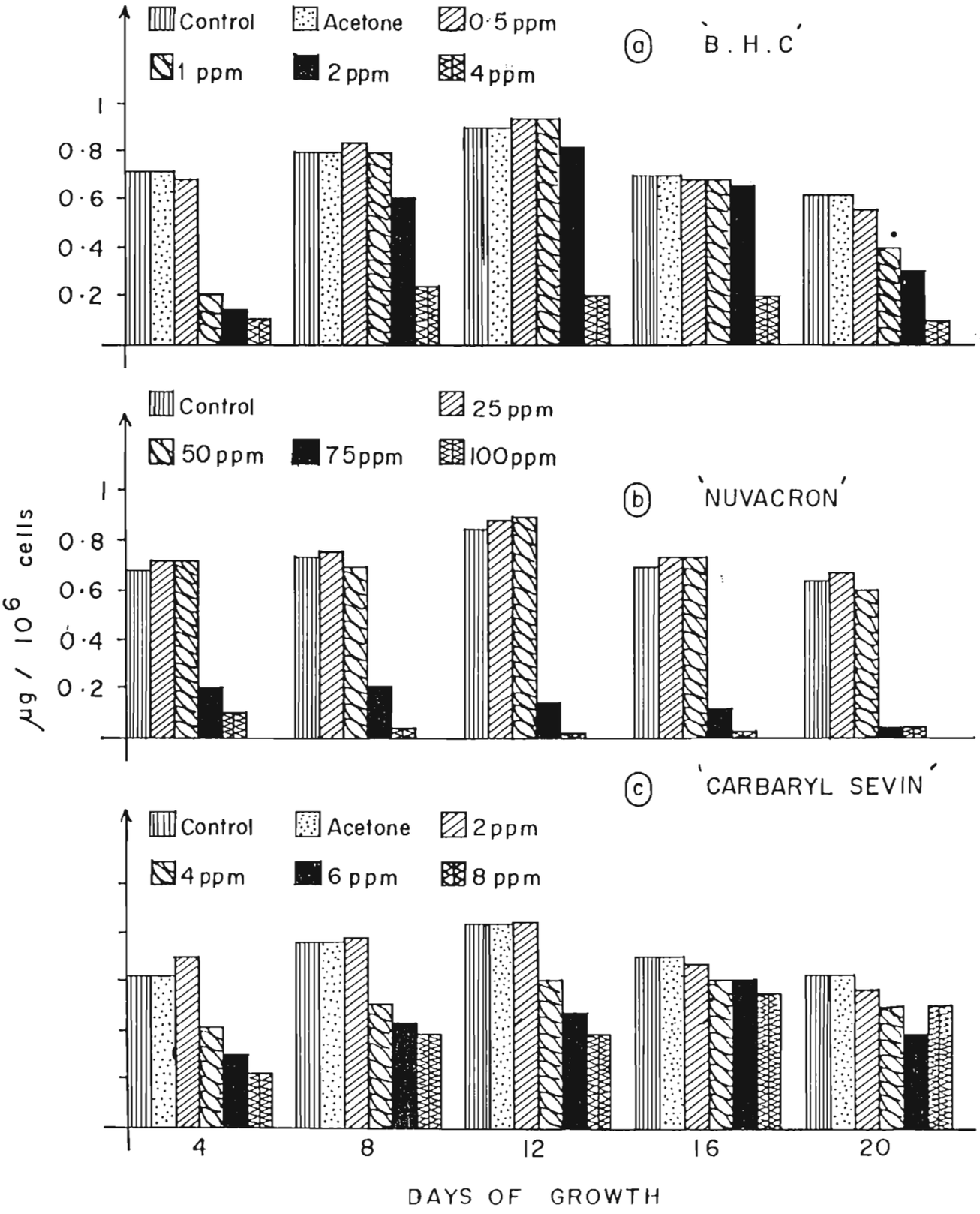


FIG.31


(a) EFFECT OF MIXTURE OF 5 BIOCIDES ON CHLOROPHYLL b CONTENT OF MICROALGAE
Control
Acetone
0·05 ppm
0·1 ppm
0·2 ppm
0·4 ppm
1 . Tetraselmis gracilis
2 Mixed culture of fresh water algae
(b) EFFECT OF MIXTURE OF 3 BIOCIDES ON CHLOROPHYLL b CONTENT OF MICROALGAE
1 . Tetraselmis gracilis
2 Mixed culture of fresh water algae
µg / 10⁶ cells
DAYS OF GROWTH
4
8
12
16
20
Control
0·05 ppm
0·1 ppm
0·2 ppm
0·4 ppm

EFFECT OF THREE BIOCIDES ON CHLOROPHYLL c CONTENT OF DICRACTERIA INORNATA

FIG.3



EEFECT OF FOUR TOXICANTS ON CHLOROPHYLL c CONTENT OF DICRATERIA INORNATA

FIG.33

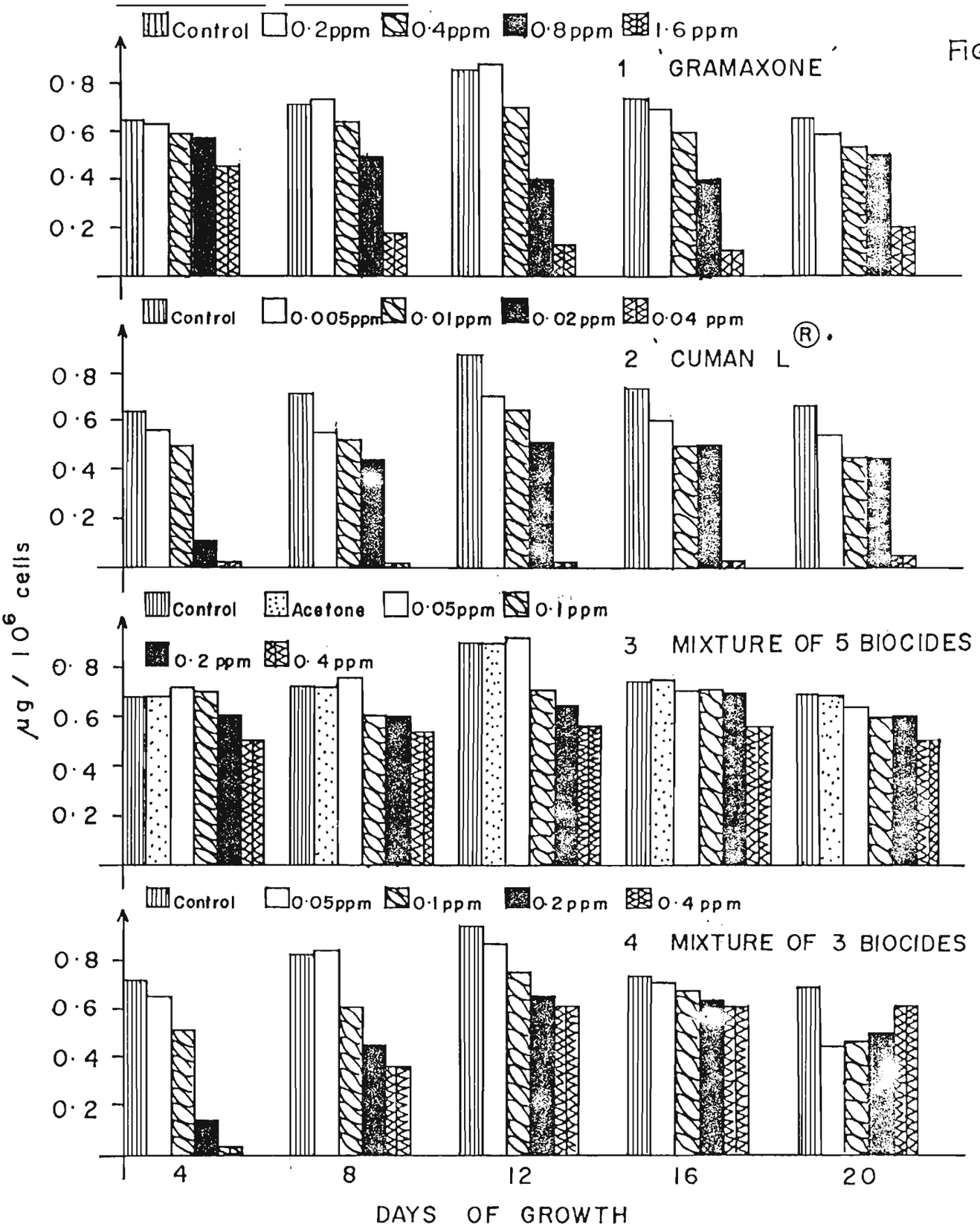


TABLE-17.
ANOVA TABLE FOR (a) CHLOROPHYLL 'b' (b) CHLOROPHYLL 'c'

| Toxicant | Source | D.F | Tetraselmis gracilis | | | Mixed culture of fresh water algae | | | Dicrateria inornata | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | S.S | M.S | F | S.S | M.S | F | S.S | M.S | F |
| B H C | Treatment | 5 | 2.8118 | 0.5624 | 1.96 N.S | 0.0125 | 0.0025 | 13.94 ** | 1.2325 | 0.2465 | 7.30 ** |
| | Replicate | 4 | 9.3705 | 2.3426 | 8.17 ** | 0.0039 | 0.0009 | 5.49 * | 0.6178 | 0.1544 | 4.58 ** |
| | Error | 20 | 5.7352 | 0.2868 | | 0.0036 | 0.0002 | | 0.6750 | 0.0338 | |
| Nuvacron | Treatment | 4 | 2.8214 | 0.7054 | 4.12 * | 0.0065 | 0.0016 | 12.19 ** | 94872.1 | 23718 | 1.01 N.S |
| | Replicate | 4 | 8.7839 | 2.1959 | 12.83 ** | 0.0056 | 0.0014 | 10.48 ** | 94455.1 | 23613 | 1.00 N.S |
| | Error | 16 | 2.7379 | 0.1711 | | 0.0021 | 0.0001 | | 37768 | 23605 | |
| Carbaryl Sevin | Treatment | 5 | 1.7677 | 0.3535 | 1.60 N.S | 0.0188 | 0.0038 | 9.17 ** | 0.2038 | 0.0408 | 1.97 N.S |
| | Replicate | 4 | 4.8154 | 1.2039 | 5.46 ** | 0.0016 | 0.0004 | 0.99 N.S | 0.2766 | 0.0692 | 3.35 * |
| | Error | 20 | 4.4073 | 0.2204 | | 0.0082 | 0.0004 | | 0.4134 | 0.0207 | |
| Gramaxone | Treatment | 4 | 23.665 | 5.9161 | 19.41 ** | 0.0979 | 0.0245 | 0.98 N.S | 0.3206 | 0.0802 | 7.66 ** |
| | Replicate | 4 | 6.7392 | 1.6848 | 5.53 ** | 0.1288 | 0.0322 | 1.29 N.S | 0.0492 | 0.0123 | 1.17 N.S |
| | Error | 16 | 4.8773 | 0.3048 | | 0.3981 | 0.0249 | | 0.1675 | 0.0105 | |
| Cuman L(R) | Treatment | 4 | 6.6219 | 1.6555 | 5.33 ** | 0.0241 | 0.0060 | 13.82 ** | 1.9484 | 0.4871 | 31.49 ** |
| | Replicate | 4 | 8.8678 | 2.2169 | 7.14 ** | 0.0098 | 0.0025 | 5.63 ** | 0.1993 | 0.0498 | 3.22 * |
| | Error | 16 | 4.9688 | 0.3106 | | 0.0069 | 0.0004 | | 0.2475 | 0.0155 | |
| Mixture of 5 Biocides | Treatment | 5 | 4.0440 | 0.8088 | 2.52 N.S | 0.02 9 | 0.0046 | 8.50 ** | 0.0178 | 0.0036 | 0.46 N.S |
| | Replicate | 4 | 5.6807 | 1.4202 | 4.43 ** | 0.0159 | 0.0039 | 7.39 ** | 0.1502 | 0.0376 | 4.89 ** |
| | Error | 20 | 6.4137 | 0.3207 | | 0.0108 | 0.0005 | | 0.1533 | 0.0077 | |
| Mixture of 3 Biocides | Treatment | 4 | 5.1551 | 1.2888 | 3.45 * | 0.0144 | 0.0036 | 7.74 ** | 0.1318 | 0.0329 | 1.34 N.S |
| | Replicate | 4 | 13.613 | 3.4034 | 9.11 ** | 0.0075 | 0.0019 | 4.00 ** | 0.1333 | 0.0333 | 1.25 N.S |
| | Error | 16 | 5.978 | 0.3736 | | 0.0075 | 0.0005 | | 0.4281 | 0.0268 | |

* Significant at 5% level, ** Significant at 1% level, N.S. - Not significant.

The fungicide Cuman $L^{(R)}$ was found to decrease the amount of chlorophyll c content in microalgae on the 4th day of observation. Concentrations of 0.02 and 0.04 ppm showed marked decrease but afterwards the 0.02 ppm concentration enhanced chlorophyll c production (Fig. 33.2). But the concentration of 0.04 ppm inhibited the chlorophyll c content. Analysis of variance revealed that between treatments the variation in chlorophyll c content was significant at 1% level and between days the significance at 5% level (Table 17b).

With the mixture of 5 biocides, the chlorophyll c content was not much affected, as shown in the fig. 33.3. Analysis of variance also showed there was no significant variation between treatment. But between days the significance was at 1% level. The mixture of 3 biocides showed very marked difference in the chlorophyll c content at the beginning. But with the age of culture, the chlorophyll c content of treated cultures showed enhancement. Statistical analysis showed that the variation in chlorophyll 'c' content was not significant (Table 17b).

Carotenoids:

These are accessory pigments in the phytoplankton which help the process of photosynthesis. An attempt was made to assess the effect of biocides on the carotenoid content of the microalgae tested.

It was observed that in T. gracilis and D. inornata, the carotenoid content increased with the age of culture. But control cultures of

freshwater algae showed an increase in carotenoid production upto the 8th day but it decreased with the age of culture.

Different biocides tested was specific in their action on carotenoid content of algae. Carotenoid content of microalgal cultures were found to be affected with the biocide treatment.

1. **Organochlorine insecticide - 'B.H.C.'**

Fig. 34 shows the effect of 4 different concentrations of B.H.C. on carotenoid content of three microalgal culture. In T. gracilis the lower concentration of 0.5 and 1 ppm showed very little stimulation in carotenoid content. At 1 ppm the peak value was obtained on 12th day and it was 2.0 $\mu g/10^6$ cells, and afterwards it decreased. At higher concentration of 4 ppm eventhough earlier inhibition was noticed, the carotenoid content increased with the age of culture. Statistical analysis showed that there was highly significant variation in carotenoid content of T. gracilis both treatment wise and daywise (Table 18).

'B.H.C.' treated cultures of D. inornata showed increased carotenoid content especially on 8th and 12th day of experiment. The lower concentration of 0.5 ppm always showed increased amount of carotenoid content compared to all other concentration tested. Observation on the 20th day showed that the carotenoid content was very low at 1,2 and 4 ppm concentration. Analysis of variance revealed that between treatments the variation in carotenoid content was not significant, but between days the variation was highly significant (Table 18).

In the case of mixed freshwater culture, the 'B.H.C.' treated cells showed increased carotenoid content than control especially at the lowest concentration. With higher concentrations tested eventhough some inhibition was observed at the beginning the pesticide treated cultures showed increased carotenoid content with the age of culture. Analysis of variance showed that between treatments and between days the variation in carotenoid content was not significant (Table 18).

2. **Organophosphate insecticide - 'Nuvacron'**

The different concentrations of 'Nuvacron', tested affected the carotenoid content of three microalgal cultures (Fig. 35). In the case of T. gracilis except 100 ppm all other concentrations tested showed an increased carotenoid content than control upto the 16th day of experiment. But when the culture attained 20 days of growth, the control cultures showed maximum amount of 1.92 $\mu g/10^6$ cells while all the treated cultures showed less values. Analysis of variance indicated that the variation in carotenoid content was not significant (Table 19).

For D. inornata, the 25 ppm concentration showed increased carotenoid content than control upto the 16th day. At 50 ppm concentration the carotene content was not so much affected. But 75 and 100 ppm concentrations affected the carotenoid content of microalgae very much. Less than 0.05 $\mu g/10^6$ cells was observed at 100 ppm, throughout the experimental period. Analysis of variance revealed that the variation in the carotenoid content as a result of 'Nuvacron' treatment was highly significant (Table 18).

Concentration of 25 ppm 'Nuvacron' showed a marked increase in the carotenoid content of freshwater microalgae during the 4th day. After wards the treated cultures yielded low values of carotenoid content than control. Analysis of variance revealed that, the variation in the carotenoid content as a result of 'Nuvacron' treatment was not significant (Table 18).

3. **Carbamate insecticide - 'Carbaryl Sevin'**

Fig. 36 shows the effect of 'Carbaryl Sevin' on the carotenoid content of microalgae. As already reported in the case of 'B.H.C.'. control and acetone treated cultures showed the same values of carotenoid content. In the case of T. gracilis, the carotenoid content was not much affected. The lower concentration of 2 and 4 ppm showed increased carotenoid content than control especially at the beginning of the culture. With 8 ppm concentration, the culture showed maximum amount of carotenoid content on the last day of experiment. Analysis of variance revealed that between treatments, the variation in carotenoid content was not significant, but between days the variation was highly significant (Table 18).

Unlike T. gracilis the carotenoid content of treated cultures of D. inornata showed very low values than control and acetone treated control. As in the case of control, the treated cultures also showed an increase in carotenoid content with the age of culture. Concentrations of 4 and 6 ppm showed the low value of 0.2 $\mu g/10^6$ cells upto 16th day. Concentration of 8 ppm carbaryl sevin was quite inhibitory to the carotenoid content of D. inornata. The carotenoid content of these cultures

was beyond detectable limits. Analysis of variance revealed that, between treatments, the variation in carotenoid content was highly significant, but between days the variation was found to be significant, at 5% level (Table 18).

In the case of mixed culture of freshwater algae, eventhough the treated cultures showed decrease in the carotenoid content than the control, the effect was not very marked as in the case of D. inornata. The highest concentration of 8 ppm showed more than 50% of the control values throughout the experimental period. Analysis of variance indicated that between treatments the variation was not significant, but between days the variation was significant at 5% level (Table 18).

4. **Herbicide 'Gramaxone'**

Effect of four different concentrations of 'Gramaxone' on the Carotenoid content of three microalgal cultures is shown in fig. 37. T. gracilis shows increased carotenoid content in 0.2 and 0.4 ppm concentrations upto the 11th day and the maximum carotenoid content observed was 1.2 $\mu g/10^6$ cells during 12th day of experiment. Afterwards the carotenoid content decreased. Concentration of 1.6 ppm of herbicide was quite inhibitory to carotenoid pigment of T. gracilis. Analysis of variance revealed that the variation in carotenoid content was highly significant between treatments but between days the variation was significant at 5% level (Table 18).

There was marked variation in the amount of carotenoid content of D. inornata as a result of herbicide treatment. The lowest concentration of 0.2 ppm gave some enhanced values than control upto the 8th day. At 0.8 and 1.6 ppm of 'Gramaxone', the carotenoid content was below 0.1 $\mu g/10^6$ cells throughout the experimental period. As in the case T. gracilis 1.6 ppm was found to be totally inhibitory to the carotenoid content of D. inornata. Analysis of variance revealed that the variation in carotenoid content was highly significant between treatments, but between days the variation was significant at 5% level (Table 18).

But compared to other two species, the carotenoid content of freshwater culture was not much affected (Fig. 37c). But in this case no increased carotenoid content was observed even with the lowest concentration tested. Eventhough the treated cultures showed decreased carotenoid content than control values, the variation was not well defined as in the case of D. inornata and T. gracilis. Analysis of variance revealed that between treatments, the variation was significant at 5% level and between days the variation was significant at 1% level (Table 18).

## 5. Fungicide - Cuman L.(R)

Fig. 38 shows the effect of fungicide Cuman L(R), on the carotenoid content of three microalgal cultures. In T. gracilis 0.005 ppm showed very little increase in carotenoid than control upto 12th day, afterwards it showed less values than control. Concentration of 0.01 ppm showed slight enhancement in carotenoid content during 8th and 12th day of observation. The carotenoid content was below detectable limited at

0.02 and 0.04 ppm especially at the beginning of the culture. At 0.04 ppm concentration the maximum carotenoid content of 0.2 $\mu g/10^6$ cells was observed on the 20th day of experiment. Analysis of variance showed that the variation was significant at 5% level between treatments and between days the variation was significant at 1% level (Table 18).

In the case of D. inornata, the four different concentration of 'Cuman $L^{(R)}$ showed decreased values of carotenoid content. As in the case of control, the fungicide treated cultures also showed increased carotenoid contnt with the age of culture. No carotenoid content was observed in the 0.04 ppm concentration. At 0.02 ppm upto 12th day the carotenoid content was beyond detectable limits but afterwards very low values of carotenoid content were observed compared to control. Analysis of variance revealed that there was high significant variation in carotenoid content, as a result of fungicide treatment (Table 18).

The four different concentration of fungicide tested showed variations in the carotenoid content of mixed culture of freshwater algae. But the effect was not well marked as in the case of D. inornata. The lower concentration of 0.005 ppm showed little increase in carotenoid content on the 8th and 12th day of experiment. Analysis of variance revealed that, both treatment wise and day wise the variation in the carotenoid content was highly significant.

### 6. Mixture of 5 biocides

Fig. 39 represents the difference in the carotenoid content of three microalgal cultures as a result of treatment with a mixture of 5 biocides.

In T. gracilis with the exception of 12th day observation, the four different concentrations tested, showed decreased carotenoid content than in control. This 4th day observation showed that with concentrations of 0.2 and 0.4 ppm of biocide mixtures the carotenoid content was below detectable limits. But with the age of culture the carotenoid content of these cultures increased and reached the peak values of 0.4 $\mu g/10^6$ cells on 20th day of experiment. Analysis of variance revealed that both treatment wise and day wise the effect of biocide mixture on carotenoid content was highly significant (Table 18).

On the 4th day of experiment with D. inornata treated with 0.2 and 0.4 ppm concentrations of 5 biocide mixture shows that the carotenoid content was below detectable limits. But as observed in the case of T. gracilis, the carotenoid content increased for this two concentration with the age of culture. On the 12th day of observation the cultures treated with 0.05 and 0.1 ppm shows increased carotenoid content than in control. Analysis of variance revealed that both treatmentwise and daywise the variation in the carotenoid content was highly significant (Table 18).

As shown in Fig. 39c it was observed that the carotenoid content of mixed culture of freshwater algae was not affected severely with the treatment of biocides as in the case of T. gracilis and D. inornata. One peculiarity observed in this case was that the treated cultures showed maximum carotenoid content on the 4th day. With the age of culture, The carotenoid content of treated cultures decreased in 0.4 ppm concentration and the values were 0.085 $\mu g/10^6$ cells on 4th day and 0.015 $\mu g/10^6$

cells on 20th day. Analysis of variance revealed that the variation in carotenoid content as a result of toxicant treatment was highly significant (Table 18).

7. **Mixture of 3 biocides**

Unlike the mixture of 5 biocides, the three biocide mixture was more toxic to the carotenoid pigment of three microalgal culture. All the toxicant treated cultures showed decreased carotenoid content than control values (Fig. 40).

In the case of T. gracilis the 4th and 8th day observation no carotenoid content was observed in the culture at 0.2 and 0.4 ppm concentration But after 12th day the carotenoid content of the treated cultures showed increased values, but it was less than that observed for control cultures. Analysis of variance revealed that between treatments the variation in carotenoid content was not significant, but between days the variation was highly significant (Table 18).

In D. inornata the concentration of 0.4 ppm biocide mixture was found to be quite inhibitory to the carotenoid content. All other concentrations tested showed an inhibitory response in the beginning but afterwards the cultures showed the presence of carotenoids in their cells. Analysis of variance showed that, the effect of 3 biocide mixture on the carotenoid content of D. inornata was highly significant (Table 18).

FIG.34

EFFECT OF 'B.H.C' ON CAROTENOID CONTENT OF MICROALGAE

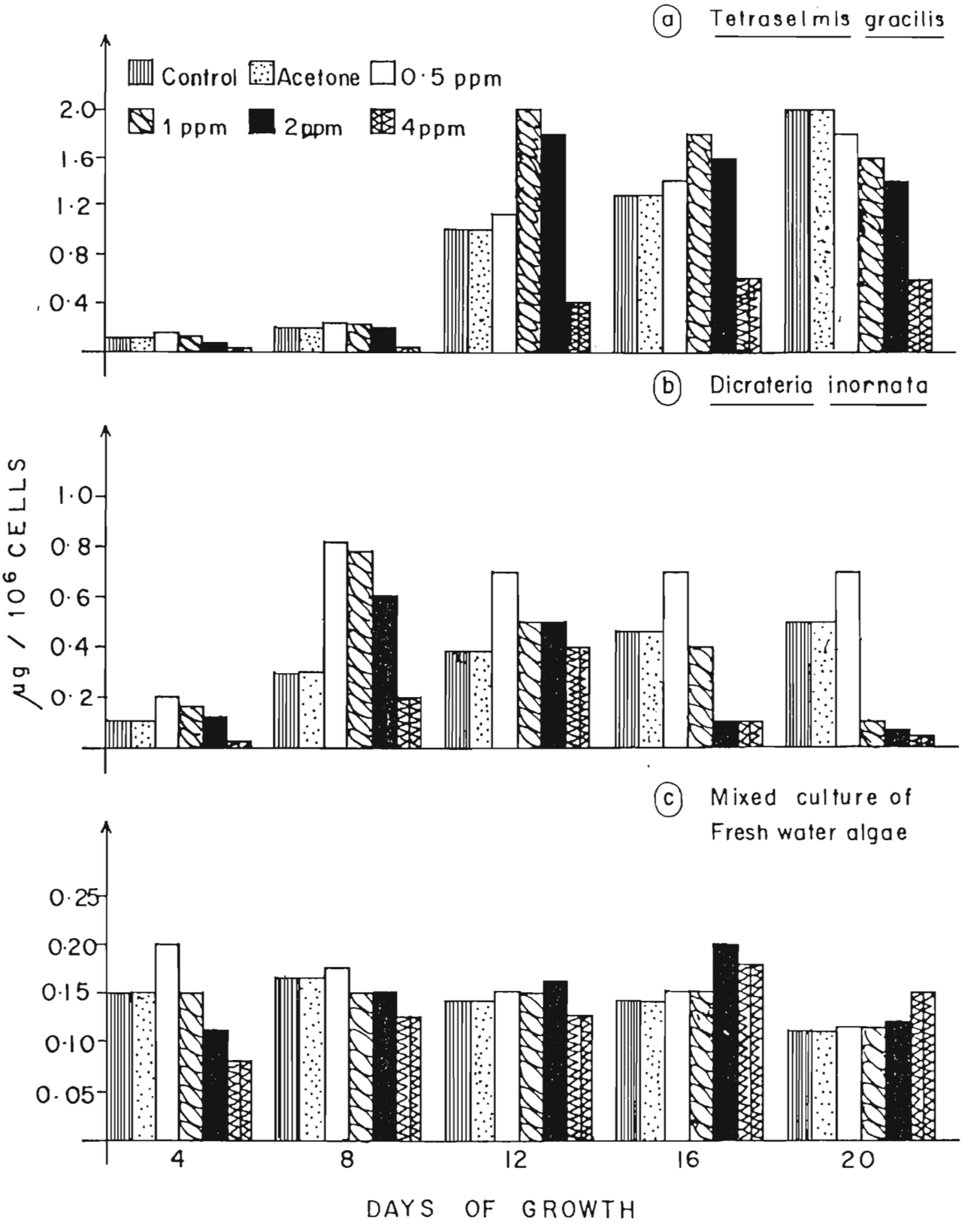
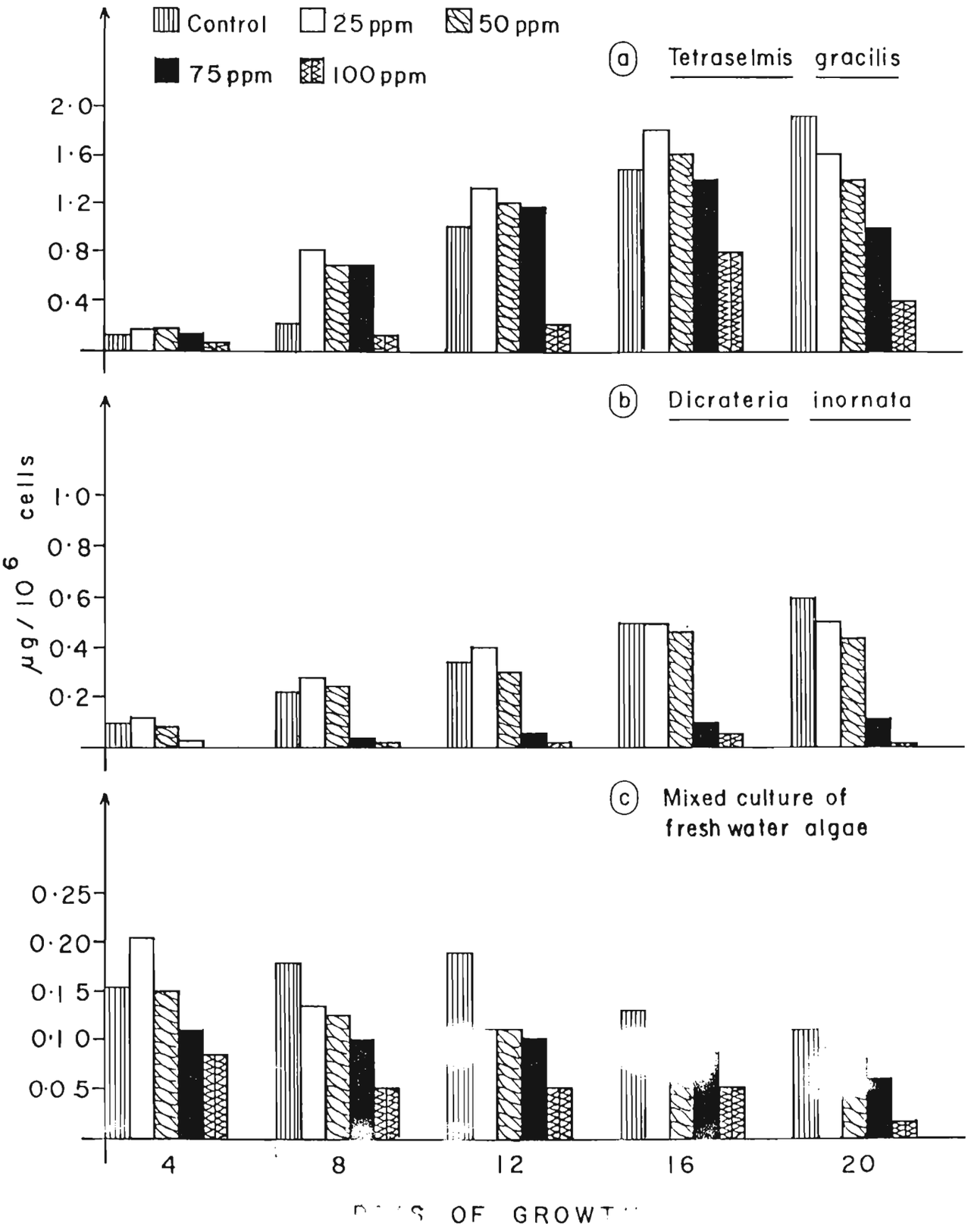


FIG.35

EFFECT OF 'NUVACRON' ON CAROTENOID CONTENT OF MICROALGAE



EFFECT OF 'CARBARYL SEVIN' ON CAROTENOID CONTENT OF MICROALGAE

FIG. 36

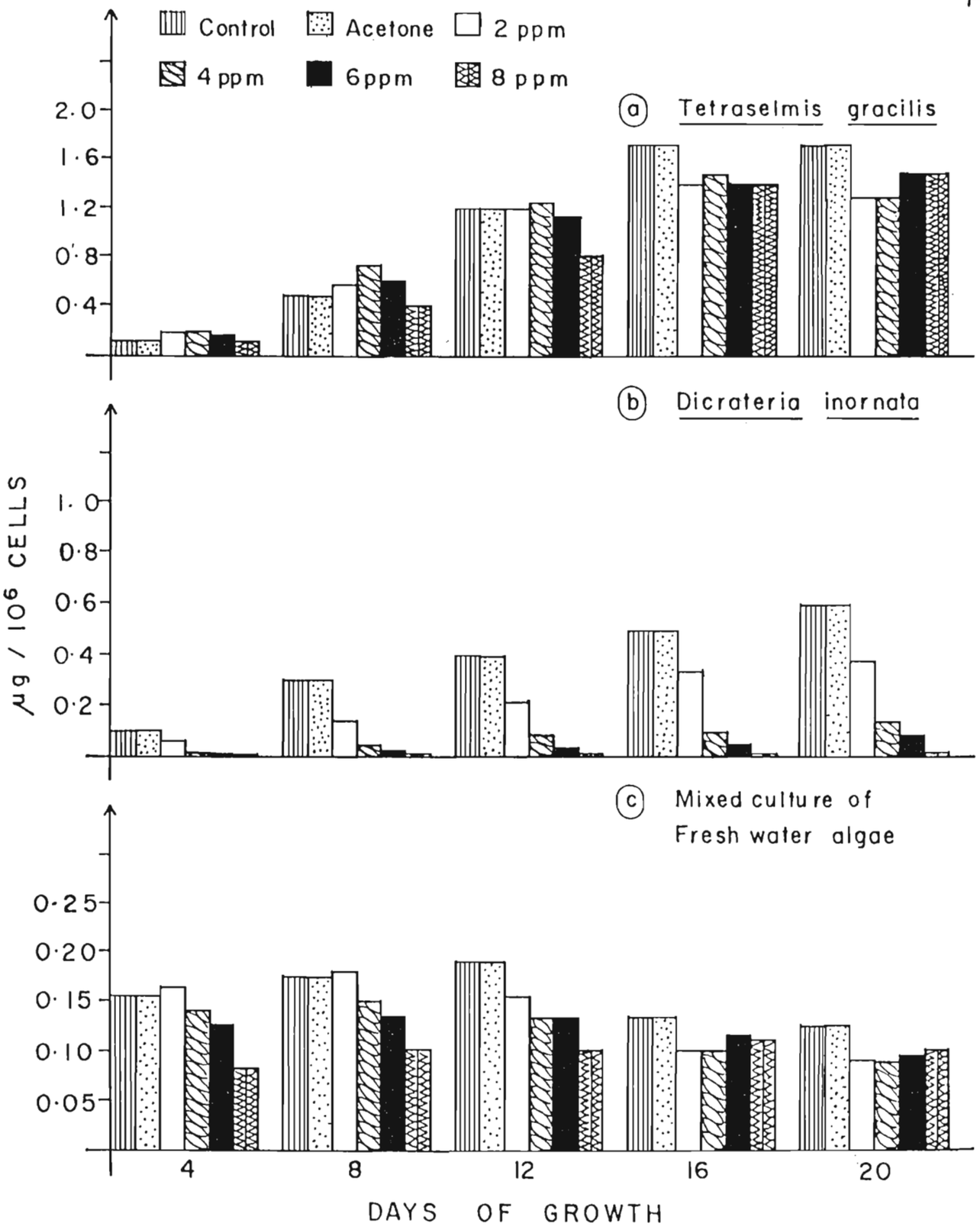


FIG.37

EFFECT OF 'GRAMAXONE ON CAROTENOID CONTENT OF MICROALGAE

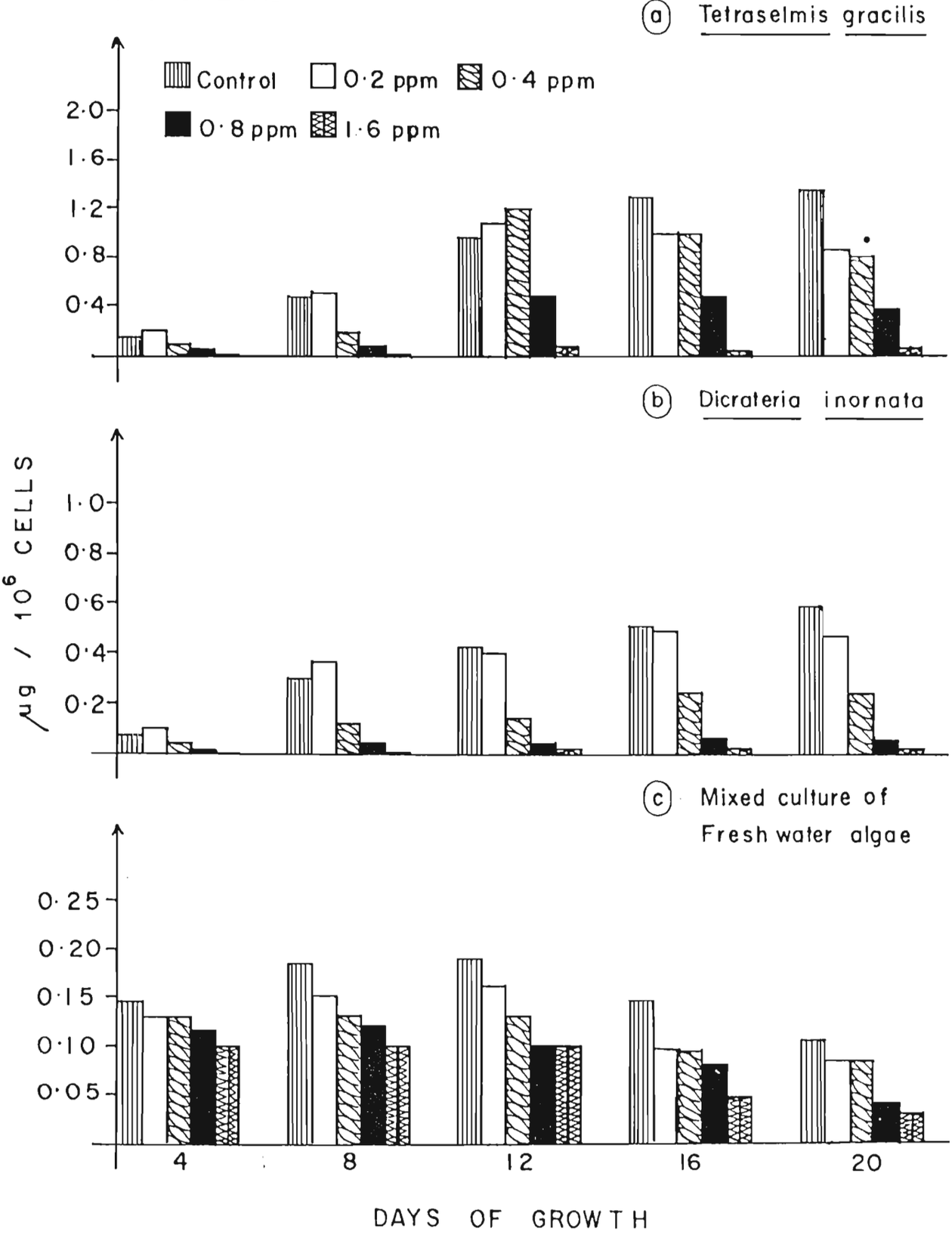


FIG.38

EFFECT OF CUMAN L® ON CAROTENOID CONTENT OF MICROALGAE

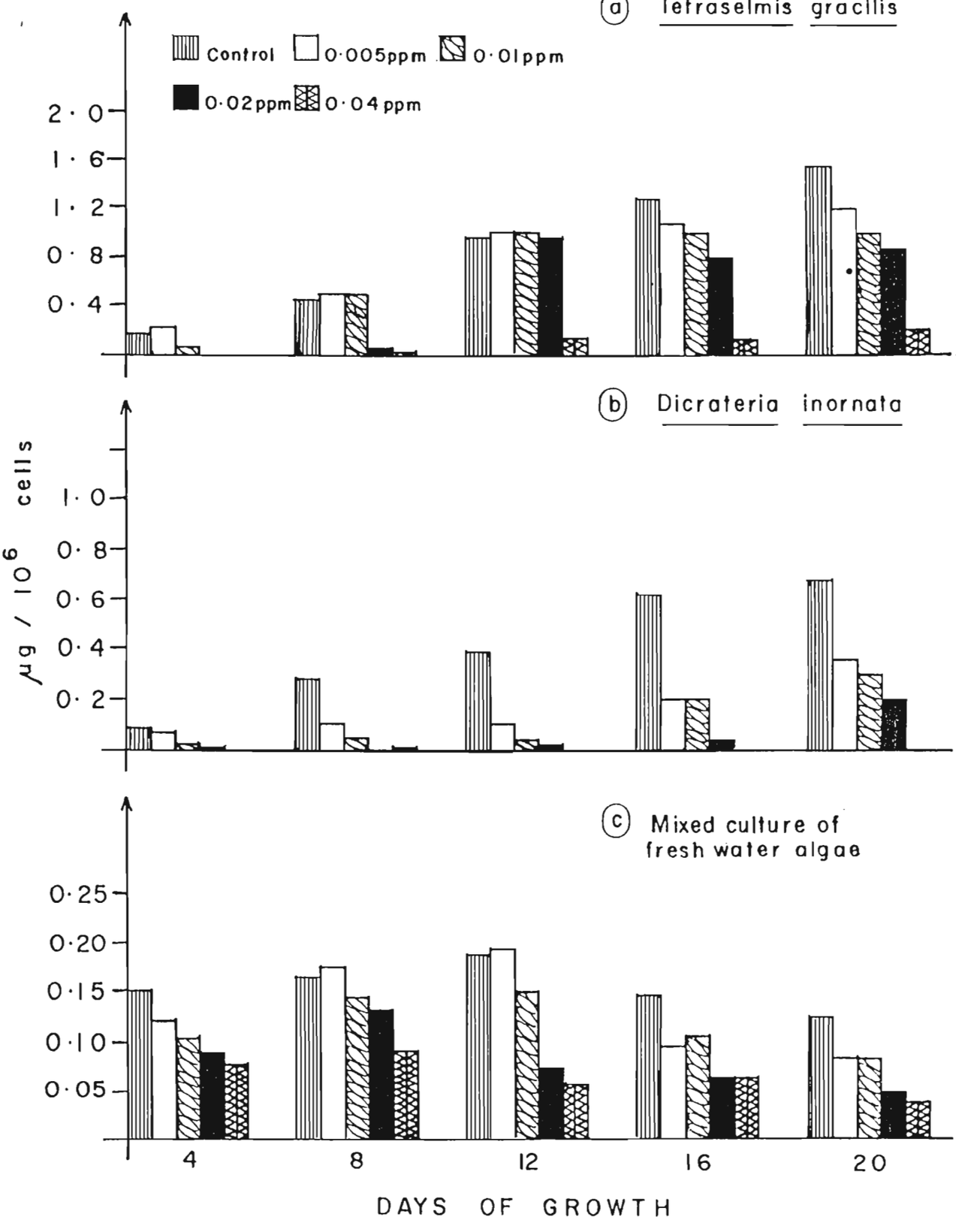
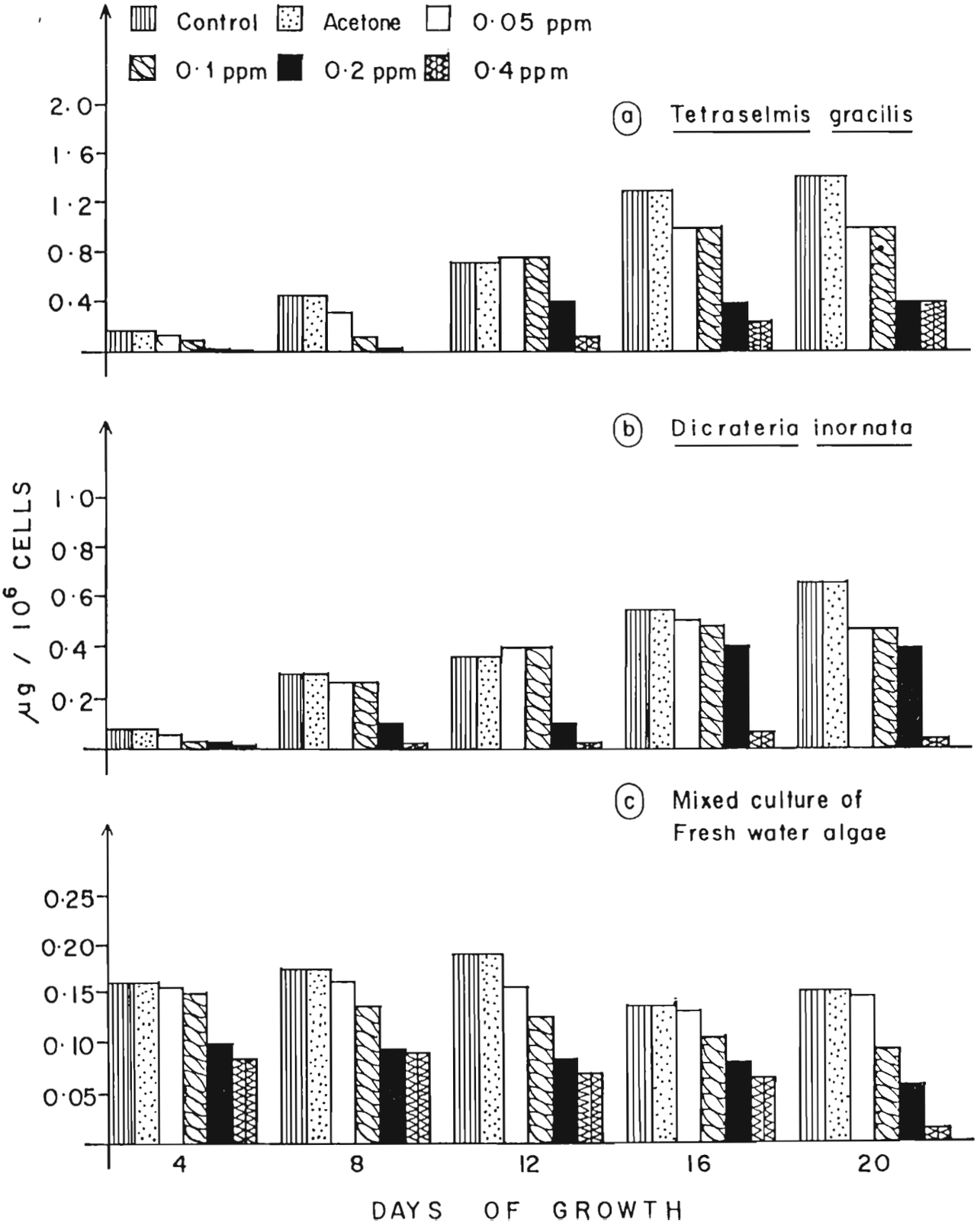


FIG.39

EFFECT OF MIXTURE OF 5 BIOCIDES ON CAROTENOID CONTENT OF MICROALGAE



EFFECT OF MIXTURE OF 3 BIOCIDES ON CAROTENOID CONTENT OF MICROALGAE

FIG.40

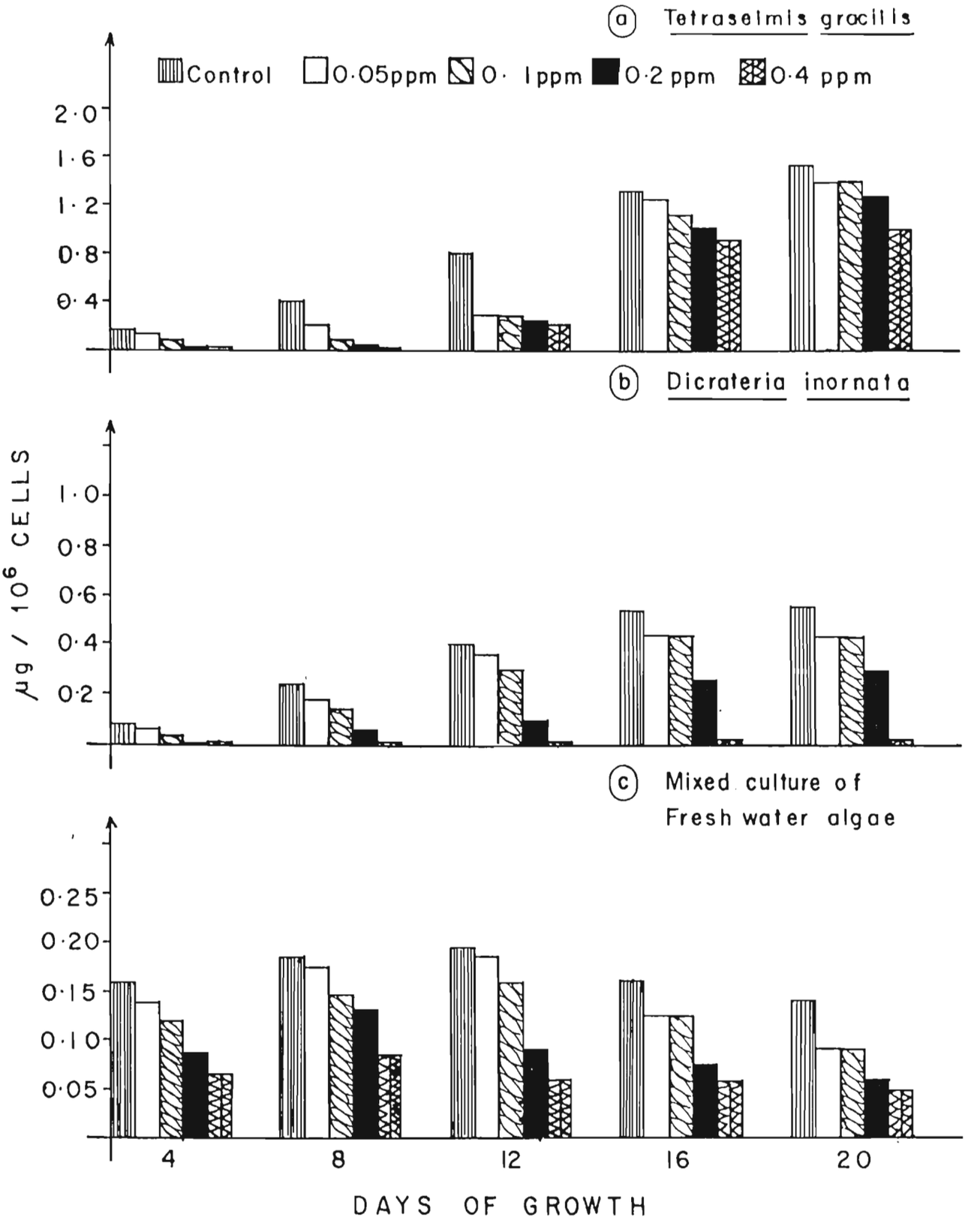


TABLE-18.
ANOVA TABLE FOR CAROTENOIDS

| Toxicant | Source | D.F | Tetraselmis gracilis | | | Dicrateria inornata | | | Mixed culture of fresh water algae | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | S.S | M.S | F | S.S | M.S | F | S.S | M.S | F |
| B H C | Treatment | 5 | 5.2641 | 1.0528 | 6.31** | 0.4781 | 0.0956 | 2,42 N.S | 0.0030 | 0.0006 | 0.69 N.S |
| | Replicate | 4 | 13.1072 | 3.2768 | 19.62** | 0.9985 | 0.2496 | 6.31** | 0.0063 | 0.0016 | 1.80 N.S |
| | Error | 20 | 3.3398 | 0.1669 | | 0.7916 | 0.0396 | | 0.0176 | 0.0009 | |
| Nuvacron | Treatment | 4 | 1.4348 | 0.3587 | 0.35 N.S | 0.5619 | 0.1405 | 5.76 N.S | 0.0098 | 0.0025 | 1.77 N.S |
| | Replicate | 4 | 8.7399 | 2.1849 | 2,14 N.S | 0.2582 | 0.0646 | 4,40* | 0.0146 | 0.0037 | 3.58* |
| | Error | 16 | 16.3700 | 1.0231 | | 0.3904 | 0.0244 | | 0.0223 | 0.0014 | |
| Carbaryl Sevin | Treatment | 5 | 0.5079 | 0.1016 | 1,68 N.S | 0.7968 | 0.1594 | 10.87** | 0.0038 | 0.0008 | 0.74 N.S |
| | Replicate | 4 | 15.677 | 3.9194 | 65.06** | 0.2582 | 0.0646 | 4.40* | 0.0146 | 0.0037 | 3.58* |
| | Error | 20 | 1.2048 | 0.0602 | | 0.2934 | 0.0147 | | 0.0204 | 0.0010 | |
| Gramaxone | Treatment | 4 | 4.1488 | 1.0372 | 5.16** | 0.4328 | 0.1082 | 5.51** | 0.0057 | 0.0014 | 3.57* |
| | Replicate | 4 | 3.4758 | 0.8689 | 4.33* | 0.3417 | 0.0854 | 4.35* | 0.0323 | 0.0081 | 20.36** |
| | Error | 16 | 3.1231 | 0.2008 | | 0.3143 | 0.0196 | | 0.0063 | 0.0004 | |
| Cuman L(R) | Treatment | 4 | 2.9367 | 0.7342 | 3.58* | 0.4368 | 0.1092 | 4.41** | 0.0216 | 0.0054 | 13.89** |
| | Replicate | 4 | 11.5765 | 2.8941 | 14.12** | 0.7169 | 0.1792 | 7.24** | 0.0148 | 0.0037 | 9.53** |
| | Error | 16 | 3.2799 | 0.2050 | | 0.3959 | 0.0248 | | 0.0052 | 0.0004 | |
| Mixture of 5 Biocides | Treatment | 5 | 2.3189 | 0.4638 | 4.52** | 0.9110 | 0.1822 | 5.27** | 0.0497 | 0.0099 | 31.37** |
| | Replicate | 4 | 16.3217 | 4.0804 | 39.75** | 0.9776 | 0.2444 | 7.06** | 0.0195 | 0.0049 | 15.34** |
| | Error | 20 | 2.0531 | 0.1027 | | 0.6918 | 0.0346 | | 0.0063 | 0.0003 | |
| Mixture of 3 Biocides | Treatment | 4 | 0.2005 | 0.0501 | 2.56 N.S | 0.7345 | 0.0184 | 6.76** | 0.0331 | 0.0083 | 26.18** |
| | Replicate | 4 | 17.7618 | 4.4405 | 227.09** | 0.4598 | 0.1149 | 4.24** | 0.0090 | 0.0023 | 7.12** |
| | Error | 16 | 0.3128 | 0.0196 | | 0.4344 | 0.0272 | | 0.0051 | 0.0003 | |

* Significant at 5% level, ** Significant at 1% level, N.S. Not significant

In the case of mixed culture of freshwater algae, the percentage inhibition of carotenoid content was less compared to D. inornata. The highest concentration of 0.4 ppm showed the maximum carotenoid content of 0.885 $\mu g/10^6$ cells on the 8th day of experiment but afterwards it decreased. Analysis of variance revealed that both treatment-wise and daywise the variation in the carotenoid content was highly significant (Table 18).

## DISCUSSION

The most important component of the chloroplast lamellae are the coloured organic compounds of photosynthetic pigments. The photosynthesizing cells of microalgae contain very large amounts of pigments. The pigment content of microalgae has great importance in productivity studies because of the gross photosynthetic potential is estimated using these pigment compounds.

The main pigments associated with photosynthesis are the chlorophylls and carotenoids. Chlorophyll a is the main photosynthetic pigment which is present in the three microalgal culture under investigation. But chlorophyll b is the accessory pigment of T. gracilis and freshwater algae, and chlorophyll c is the accessory pigment of D. inornata. The accessory pigment carotenoids, occur in the three microalgal cultures investigated here.

Wide variations in the concentrations of chlorophyllous and non-chlorophyllous pigments were recognized throughout the experimental

period in relation to the various concentrations of biocide treatment.

In most cases, the lowest concentration tested, showed an increased pigment content than control especially at the beginning of the culture. But the percentage increase observed was dependent upon the biocide and the species tested. It was observed that, the two green algal cultures tested i.e. T. gracilis and mixed culture of freshwater algae shows increased chlorophyll a content as a result of biocide treatment. Reports about the increased pigment content as a result of toxicant treatment are very rare. Ibrahim (1983) while testing the toxicity of two insecticides 'Dimethoate' and Bayluscide, reported that low concentration of Dimethoate increased the chlrophyll content of Amphiprora paludosa and Phaeodactylum tricornutum. Another report by Rajarethnam et al., (1987) is that in Thalassiosira fluviatilis and T. nitzchoides, the low concentration of an organochlorine insecticide 'Heptachlor' increased the pigment production. But the reason for increased pigment production was not reported in these cases. Francois and Robinson (1990) reported on the slight stimulation in chlorophyll occurring even at the lowest concentration of herbicides such as 'Atrazine, terbutryn and simazine. They concluded that the stimulation of chlorophyll synthesis could be a tolerance mechanism. The results of the present investigations agree with this conclusion.

Lin Yi-Xing and Sun Bo-zen (1987) reported that, upto 10 ppm concentration of lindane ( $\gamma$ -BHC), the chlorophyll and carotene contents in Scenedesums obliquus do not differ much from control. But the same

insecticide $\gamma$ BHC tested in the present series of experiments showed that the pigment content was affected above 1 ppm concentration. This may be because of the difference in the composition of insecticide and also due to the difference in species. The insecticide BHC innvestigated here is a local product.

Inhibition of pigment production as a result of biocide treatment has been already reported by variuous authors. The results of the present investigation revealed that, the higher concentration of all the biocides tested invariably affected the pigment content of three microalgal culture, especially at the beginning of the culture. But the percentage inhibition was dependent upon nature of the biocide and the species tested. This was in confirmity with the findings of Wurstur (1968) who found that, the effect of DDT on chlorophyll is dependent upon the algal species involved and nature of pesticide used.

Schauberger and Wildman (1977) reported that the chlorinated insecticide aldrin and dieldrin at 100 µg/l decreased the pigment absorption. As observed in the present investigation the initial inhibition of pigment content has been reported by Goulding and Ellis (1981) also. Saroja and Bose (1982) while testing the toxicity of an organophosphate insecticide methyl parathion, found that 10 to 30 ppm concentration of insecticide inhibited Chlorophyll content than any other parameters measured. They concluded that inhibition of photosynthetic pigment was, as a result of the inhibition of photosynthetic activities.

The results of the present investigation also lead to the conclusion that the inhibited response to pigments at higher concentrations of biocides

is mainly due to reduced concentration of chlorophyll content per cell in biocide treated cells, as reported by Rajarethnam et al., (1987).

It was observed that in most cases, the earlier inhibition of pigment production at higher concentrations of biocides, altered with the age of culture. With higher concentrations of almost all biocides tested, the percentage inhibition was high at the beginning of the culture. Afterwards recovery was observed. This was in conformity with the findings of Goulding and Ellis (1981) who found that the initial inhibition was more marked and recovery occured after 20 days of the experiment. This type of observation has been reported by Abou Waly et al., (1991 a,b) also. They are of the opinion that after an initial inhibition, a strong recovery in clorophyll content occurred after day one, and on day '7' when the chlorophyll activity exceeded controls.

The chlorophyll a/b ratio in T. gracilis and mixed culture of fresh-water algae, and chlorophyll a/c ratio in D. inornata were also found to be affected by biocide treatment. But each biocide tested was specific in its action on microalgae. The inhibitory or stimulatory action of each biocide on pigment production was dependent upon dose and duration of experiment. Changes in the chlorophyll ratios of microalgae could lead to changes in the photosynthetic unit size and in the photochemical reaction of two photosystems as reported by Saroja and Bose (1982).

Non Chlorophyllous pigments like carotenoids may also serve as potential light harvesting pigments and these accessory pigments in algae

and higher plants increase the absorption spectra over wider wavelength than would exist if chlorophyll was the sole-absorbing pigment (Harris 1978). So change in the carotenoid content of microalgae will affect the photosynthetic process.

The different concentrations of biocides were found to affect the carotenoid content of microalgae. But compared to chlorophyll pigments, this non-chlorophyllous pigment was not affected severely. This was in conformity with the findings of Saroja and Bose (1982).

Millie et al., (1990) tested the toxic effect of carotenoid inhibiting herbicide, fluridone on Oscillatoria agardhii and found that the reduced carotenoid content led to the inhibition of photosynthetic efficiency.

The three microalgal culture under the present investigation, showed that upto a certain period the chlorophyllous pigments increased and then declined with the age of culture. But the non-chlorophyllous pigments like carotenoids increased with the age of culture. The peculiarity observed with the biocide treated cells is that, in most cases, the treated cells especially at higher concentrations show less chlorophyllous pigments and more carotenoid pigments especially at the beginning of the culture. This leads to the assumption that, in the absence of major chlorophyllous pigments, these accessory pigment would help in the photosynthetic process of treated cultures.

The treated cultures shows inhibition of pigment content and photo-synthetic rate. But the peculiarity observed was that, the percentage

inhibition was high with respect to photosynthetic rate. It shows that eventhough pigment production was there, the activity of pigments was inhibited. Abouwaly et al., (1991b) also report that chlorophyll activity was inhibited more than chlorophyll content. In the present investigation the quantity of pigments was estimated in all cases. In this estimated pigments dead chlorophyll may also come.

Photosynthetic rate per unit chlorophyll is often used as an indicator of the physiological state of an algal assemblage (Perry et al., 1981) If chlorophyllous and non-chlorophyllous pigments are altered by the toxicant treatment, it will adversely influence rate of photosynthesis, and will decrease the oxygen production of the microalgal cultures. So the alterations in pigment content could ultimately lead to an unbalanced physiological state.

The present investigation leads to the conclusion that each biocide tested has varying effects on the pigment content of microalgal culture. But the mode of action of each biocide on the pigment content of microalgae needs indepth study. This type of study will help to find out the actual reason for the inhibition of pigments in treated cultures.

## 4.3. IMPACT OF 5 BIOCIDES AD THEIR COMBINATIONS ON PROTEIN AND CARBOHYDRATE CONTENT OF MICROALGAE.

As a result of biocide treatment, the biochemical compounds like protein and carbohydrate content of three microalgal culture showed varied responses. But the percentage of inhibition or stimulation was found variable with different biocides tested.

### 1. Organochlorine insecticide - 'B.H.C.'

Fig. 41 shows the variation in the biochemical compounds such as protein and carbohydrate content of three microalgal culture as a result of 'B.H.C.' treatment. Control and acetone treated control yielded same values for protein and carbohydrate content. It was found that protein content of the three microalgal culture decreased with the age of culture, whereas the carbohydrate content showed increased values.

In the case of T. gracilis the lowest concentration of 0.5 ppm B.H.C. resulted in a stimulation in protein content throughout the experimental period. D. inornata showed stimulation only on the 5th day of the experiment. But in the case of mixed culture of freshwater algae no increased protein content than control was observed even with the lowest concentration of 0.5 ppm. Concentrations of 1,2 and 4 ppm of 'B.H.C'. shows inhibitory effect. But the percentage inhibition was found to be variable in the three microalgal cultures. In the case of T. gracilis the 4 ppm concentration of 'B.H.C.' showed that after the initial inhibition the protein content increased with the age of the culture. But the protein content was very less compared to control and other treated cultures.

In the case of D. inornata a marine alga, the highest concentration of 2 and 4 ppm showed peak value on the 10th day and afterwards it decreased Compared to freshwater culture, the percentage inhibition was very high in D. inornata.

Statistical interpretation of data, using analysis of variance showed highly significant variations in protein content as a result of B.H.C. treatment (Table 19).

As shown in the Fig. 41, the carbohydrate content of the three microalgal cultures showed variations as a result of 'B.H.C.' treatment. As observed in the case of protein content, the carbohydrate content also showed no variation in the control and acetone treated cultures. In the case of T. gracilis, the lowest concentration of 0.5 ppm enhanced carbohydrate content than control upto the 15th day. But with the other two cultures enhancement was observed on the 15th and 20th day of experiment. Concentrations of 1,2 and 4 ppm of B.H.C. showed inhibitory effect. The percentage of inhibition was well marked in the beginning of the culture but with the age of culture, the percentage of inhibition was reduced, except in D. inornata.

Analysis of variance revealed that in the case of T. gracilis and D. inornata, there was highly significant variation in carbohydrate content as a result of 'B.H.C.' treatment. But in the case of freshwater culture, the variation was not significant between treatment, but between days there was highly significant variation in the carbohydrate content of treated algae (Table 20).

## 2. Organophosphate insecticide - 'Nuvacron'

The effect of four different concentrations of 'Nuvacron' on protein and carbohydrate content of three microalgal culture is given in Fig. 42. Compared to 'B.H.C.', the toxic effect of 'Nuvacron' was very low.

The protein content of three microalgal culture under investigation showed varied responses. In the case of T. gracilis, the 'Nuvacron' treated cultures showed increased protein content. The peculiarity observed in this case is that at 25 and 50 ppm protein content increased than in control upto the 15th day of experiment. But on the 5th day, at 75 and 100 ppm concentration of the protein values were low but afterwards, the protein content increased and this trend continued upto the 15th day. In the case of D. inornata, 25 ppm concentration showed increased protein content than control throughout the experimental period. Concentrations of 75 and 100 ppm of 'Nuvacron' were found inhibitory. In the case of freshwater culture, 50, 75 and 100 ppm concentration of 'Nuvacron' showed low values of protein content compared to control. But the percentage of decrease was less compared to D. inornata.

Statistical interpretation of data using analysis of variance showed that, in the case of T. gracilis, between treatments there was no significant variation in protein content, but between days, the variation was highly significant. But the other two cultures showed variation in protein content to a highly significant level between treatments and between days (Table 19).

The carbohydrate content of three microalgal cultures was found to be affected with 'Nuvacron' treatment. The lower concentration of 25 ppm showed stimulatory effect especially in T. gracilis and D. inornata. In the case of mixed culture of freshwater algae, stimulation was observed only on the 5th day of experiment. Concentrations of 50, 75 and 100 ppm showed inhibitory response with respect to the carbohydrate content of three microalgal cultures. Compared to other two cultures, the percentage inhibition at 100 ppm was wellmarked in the case of D. inornata.

Analysis of variance revealed that, in the case of T. gracilis, there was highly significant variation in carbohydrate content as a result of 'Nuvacron' treatment. Between treatment, D. inornata showed highly significant variation, but between days, the variation was not significant. In the case of freshwater culture, between treatments, the significance in variation was at 5% level and between days there was highly significant variation in carbohydrate content (Table 20).

### 3. Carbamate insecticide 'Carbaryl Sevin'

Fig 43 shows the effect of carbamate insecticide - 'Carbaryl Sevin' on biochemical compounds of three microalgal culture. This insecticide showed stimulatory effect at lower concentrations and inhibitory effect at higher concentrations tested. In the case of T. gracilis the lower concentration of 2 ppm showed an increased protein content than control. At 6 ppm concentration, after initial inhibition, it showed an enhanced protein content on the 10th day of observation. At 8 ppm concentration

about 50% inhibition was observed at the beginning and afterwards, the percentage inhibition was decreased with the age of culture. In the case of D. inornata and freshwater mixed culture, the four different concentrations of carbaryl sevin showed inhibitory response. But the percentage of inhibition was found varied, and it was well marked in the case of D. inornata.

Analysis of variance revealed that the three microalgal culture under investigation had highly significant variation in protein content, between four different concentrations of 'carbaryl sevin'. Between days, T. gracilis and D. inornata showed highly significant variation, but in mixed culture of freshwater algae the significance was at 5% level (Table 19).

Concentration of 2 ppm carbaryl sevin was found to stimulate the carbohydrate content in T. gracilis and D. inornata. But in the case of freshwater culture no such stimulation was observed even at the lowest concentration tested. Concentrations of 4, 6 and 8 ppm invariably affected the carbohydrate content of microalgae. But the percentage inhibition showed variation. Another peculiarity observed was that, in the case of D. inornata, the cultures treated with 6 and 8 ppm concentration of 'carbaryl sevin', the carbohydrate content decreased with the age of culture.

Analysis of variance revealed that in the case of T. gracilis, the variation in carbohydrate content was significant at 5% level, between treatments, but between days the variation was highly significant. In

the case of D. inornata, between treatments the variation was highly significant and between days it was not significant. Mixed culture of freshwater algae showed no significant relation between treatments, but between days highly significant variation was observed (Table 20).

4. **Herbicide - 'Gramaxone'**

The effects of four different concentrations of 'Gramaxone' on biochemical content of three microalgal culture are given in Fig. 44.

In this case no stimulation of protein content was observed even with the lowest concentration of 0.2 ppm. As in the case of control, the treated cultures also showed reduction in the protein content with the age of culture. But in the case of D. inornata and freshwater culture the peak value was observed on the 10th day of experiment. The percentage of inhibition of protein content, especially at 0.8 and 1.6 ppm concentration was high in D. inornata than in two other cultures.

Analysis of variance revealed that, the three microalgal culture under investigation had highly significant variation in their protein content as a result of 'Gramaxone' treatment (Table 19).

Unlike in the case of protein content, the carbohydrate content of microalgal cultures showed stimulation with the lowest concentration of 0.2 ppm of 'Gramaxone'. In the case of mixed culture of freshwater algae, the stimulated production was observed only on the 15th day onwards Concentrations of 0.4, 0.8 and 1.6 ppm of 'Gramaxone' was found inhibitory to the carbohydrate content of microalgae. A very marked inhibition

was observed in the case of D. inornata at the higher concentrations of 0.8 and 1.6 ppm.

Analysis of variance revealed that, except D. inornata, the other two cultures had highly significant variation in carbohydrate content, between treatments. But between days the variation in carbohydrate content was not significant, except in T. gracilis (Table 20).

5. **Fungicide - Cuman L(R)**

Fig. 45 shows the variations in biochemical compounds of three microalgal cultures as a result of fungicide treatment.

As observed in the case of herbicide, the fungicide also showed no stimulated protein content than control even with the lowest concentration of 0.005 ppm. The four different concentrations of 'Cuman L(R) invariably affected the protein content of microalgal cultures. The percentage inhibition was very marked in the case of D. inornata. Another pecularity observed in this case was that, at concentration of 0.02 and 0.04 ppm the microalgal cultures particularly T. gracilis and D. inornata showed peak values of protein content on the 20th day of experiment.

Analysis of variance revealed that between treatments the variation in protein content was highly significant in three microalgal cultures. But between days the variation was not significant in T. gracilis and D. inornata. In mixed culture of freshwater algae the variation was found significant at 5% level (Table 19).

In the case of T. gracilis 0.005 ppm concentration of Cuman L(R) showed stimulated carbohydrate production than in control. The freshwater culture showed stimulated production only on the 5th day of observation. Concentrations of 0.01, 0.02 and 0.04 ppm of Cuman L(R) were found inhibitory to the carbohydrate synthesis of microalgal cultures.. More than 50% inhibition was observed at 0.04 ppm concentration of Cuman L(R)

Analysis of variance revealed that between treatments, the variation in Carbohydrate content was highly significant except in freshwater mixed culture. But the variation was not significant between days except in T. gracilis (Table 20).

6. **Mixture of 5 biocides:**

The synergistic effect of 5 different biocides on biochemical compounds of three microalgal culture is depicted in fig. 46.

The mixture of 5 biocides at 0.05 ppm showed an enhanced protein content on the 5th day of experiment, but afterwards the protein content decreased compared to control. Concentrations of 0.1, 0.2 and 0.4 ppm of biocide was found inhibitory to the protein content of three microalgal cultures. More than 50% inhibition was observed with 0.2 and 0.4 ppm especially at the beginning of the experiment. Another peculiarity observed was that, in the case of mixed culture of freshwater algae and T. gracilis, 0.2 and 0.4 ppm concentration of biocide mixture treated cultures showed the peak value on the 20th day of experiment, i.e., with the age of culture, the protein increased.

Analysis of variance revealed that, between treatments the variation in protein content was highly significant except in T. gracilis, but between days, there was no significnt variation except in D. inornata which showed significance at 5% level (Table 19).

Carbohydrate content of biocide treated cultures showed variations. At 0.05 ppm concentration, the biocide mixture showed increased carbohydrate content compared to control in the three micrialgal cultures. But the percentage of increase was high in T. gracilis. All other concentrations tested showed inhibitory response in the three microalgal culture. At 0.4 ppm the carbohydrate content was very much inhibited.

Analysis of variance showed that, the three microalgal culture had highly significant variation in carbohydrate as a result of treatment with 3 biocide mixture (Table 20).

7. **Mixture of 3 biocides:**

Fig. 47 shows the variation in the biochemical compounds of three microalgal cultures as a result of 3 biocide mixture treatment.

Compared to the 5 biocide mixture the three biocide mixture was more toxic. No increased protein content was observed even with the lowest concentration of 0.05 ppm. The protein content of treated cultures was inhibited. Percentage of inhibition was high in the case of 0.2 and 0.4 ppm concentration. Another pecularity observed was that at these

concentrations, after the initial inhibition, the protein increased with the age of culture.

Analysis of variance revealed that, the three microalgal cultures had highly significant variation in protein content between treatments. But between days, the variation in protein content was not significant (Table 19).

Unlike in the case of protein, the carbohydrate content was not so much affected with toxicant treatment. At 0.05 ppm this biocide mixture showed an increased carbohydrate content than control especially in T. gracilis. The other two cultures shows increased carbohydrate content at 0.05 ppm on 5th day only. Concentrations of 0.1, 0.2 and 0.4 ppm showed inhibitory effect. But the percentage of inhibition was high with the highest concentration. As observed in the case of control cultures, the treated cultures also showed increased carbohydrate content with the age of culture. But in D. inornata the 0.04 ppm concentration the carbohydrate remain unchanged.

Analysis of variance revealed that, in the case of T. gracilis and mixed culture of freshwater algae, the variation in carbohydrate content as a result of toxicant treatment was significant at 1% level. In the case of D. inornata between treatments, there was highly significant variation, but between days the variation was significant at 5% level (Table 20).

## DISCUSSION

The biochemical compounds of the three microalgal cultures showed variation as a result of biocide treatment. This variation in content of biochemical compounds such as protein and carbohydrate was related to the dose of the toxicant and duration of the experiment.

The protein content of the algae is a direct function of the amount of available nitrogen in the growth medium. The protein content is quite high in cells during vigorous exponential growth. But in the case of carbohydrate, the microalgae produce more carbohydrate as the cultures age. The untreated cultures of three microalgae showed this type of result i.e., the protein content decreased with the age of culture and carbohydrate content increased with age of culture.

Eventhough there is sufficient literature about the effect of different biocides on physiological changes of microalgae, it is very rare for the effects on biochemical contents of microalgae.

The present results revealed that some of the biocide tested have stimulation of protein content with the lowest concentration tested. This may be because of the reason that, the increased cell number at this concentration may tend to produce an increased protein content than control. The treated cells at lower concentrations of different biocides had a stimulatory effect. The cells showed a tendency to absorb more nutrients from the medium. As observed in the case of 'Nuvacron'treatment

on T. gracilis, the results showed increased protein content in treated cultures. As already given, 'Nuvacron', the organophosphate insecticide tested here, is a degradable insecticide. This insecticide may undergo biodegradation and the resulting compounds may act as nutrients in the medium. Naturally the treated algal cells may tend to absorb more nutrients so that eventually resulted in the high protein content of treated cultures.

Cochrane (1958) reported that the increase in total protein-N is the real sign of increase in cytoplasm. DCMU treated cultures of Euglena showed increased protein content per cell. (Calvaryrac et al., (1979). But no other reports are available for the actual mechanism of the stimulation in protein content as a result of biocide treatment.

Goulding et al. (1984) reported that the amount of protein per cell was unaffected by DDT treatment. At the higher concentrations almost all biocide tested here showed variations in biochemical content. The percentage of inhibition of proteins and carbohydrate was found to increase with increase in the toxicant concentration. A marked inhibition was observed in the beginning of the experiment.

The peak production of biochemical compound, especially in the case of protein contents, was postponed to later days of growth. This was in conformity with the findings of Saroja and Bose (1982) that, the 'methyl parathion' at 10 and 20 ppm had the peak production of protein content on 19th day of experiment.

Collyer and Fogg (1955), Lewin and Guillard, (1963) are of the opinion that the decrease in the protein levels was caused by an insufficient nitrogen level in nutritive medium. But during the present investigations, all the control and treated cultures were supplied with same quantity of nutrients. The toxicant concentration may vary in each culture. At the higher concentrations, perhaps due to the external stress these algal cells might have proper conditions to absorb the nutrients from the medium. This may result in the decreased protein and carbohydrate content at higher concentrations.

Ibrahim (1983) reported that the protein and carbohydrate contents of microalgae were affected with insecticide treatment. Bazulic et al., (1988) also reported on the toxic effect of polychlorinated biphenyls on the biochemical composition of phytoplankton Phaeodactylum tricornutum.

The present results reveal that it was the protein content of microalgae which was affected with biocide treatment than carbohydrate. This was in conformity with the findings of Ibrahim, (1983) and Bazulic et al., (1988). Bazulic et al. (1988) reported that total carbohydrate content was slightly higher in contaminated cultures. The present results also obtained similar result at the lower concentrations of biocides. With the age of culture the carbohydrate increased in both control and treated cultures.

Carbohydrate and protein content of three chlorophytes tested showed similarity in their response to paraquat (Ibrahim 1990). This herbicide has an inhibitory effect on microalgae.

The results of effect of biocides on biochemical compounds of microalgae, leads to the conclusion that the protein content was affected more than carbohydrate with biocide treatment. Each biocide tested, and mixture of biocides tested was specific in their action, and the percentage of stimulation or inhibition was related to the dose of the toxicant and duration of the experiment.

FIG. 41

EFFECT OF B.H.C. ON PROTEIN AND CARBOHYDRATE CONTENTS OF MICROALGAE

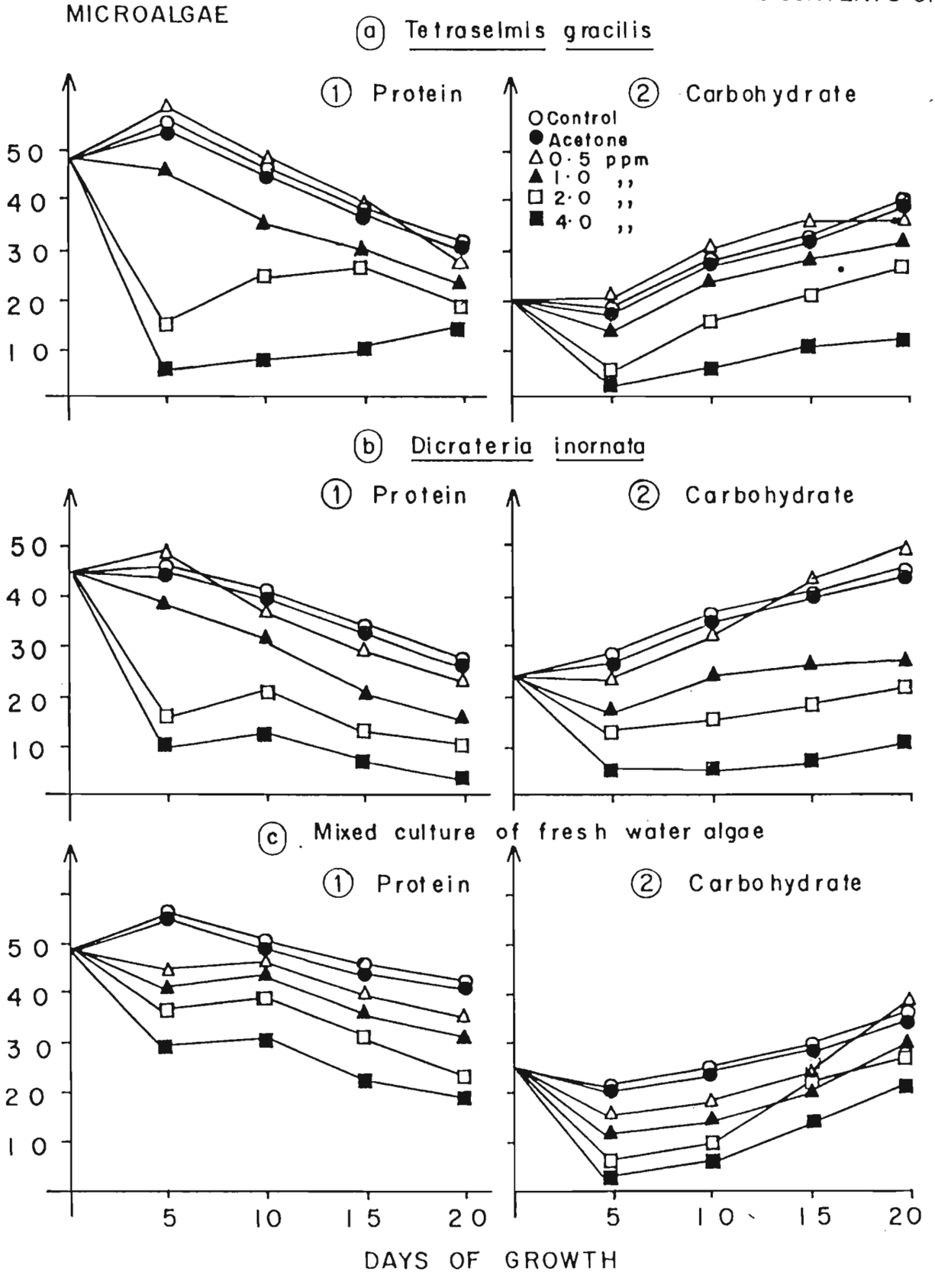


FIG. 42

EFFECT OF 'NUVACRON' ON PROTEIN AND CARBOHYDRATE CONTENTS OF MICROALGAE

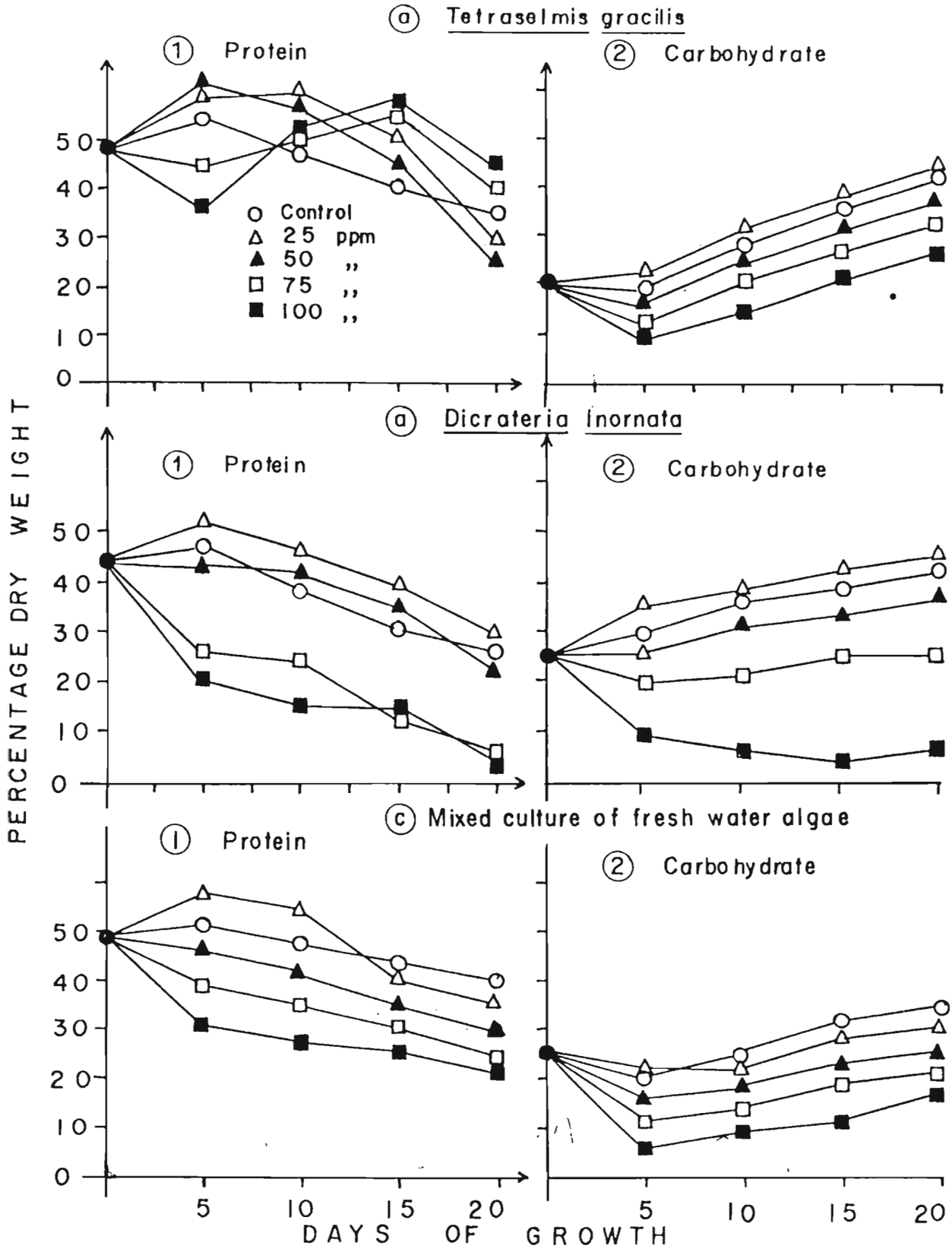


FIG.43

EFFECT OF 'CARBARYL SEVIN' ON PROTEIN AND CARBOHYDRATE CONTENTS OF MICROALGAE

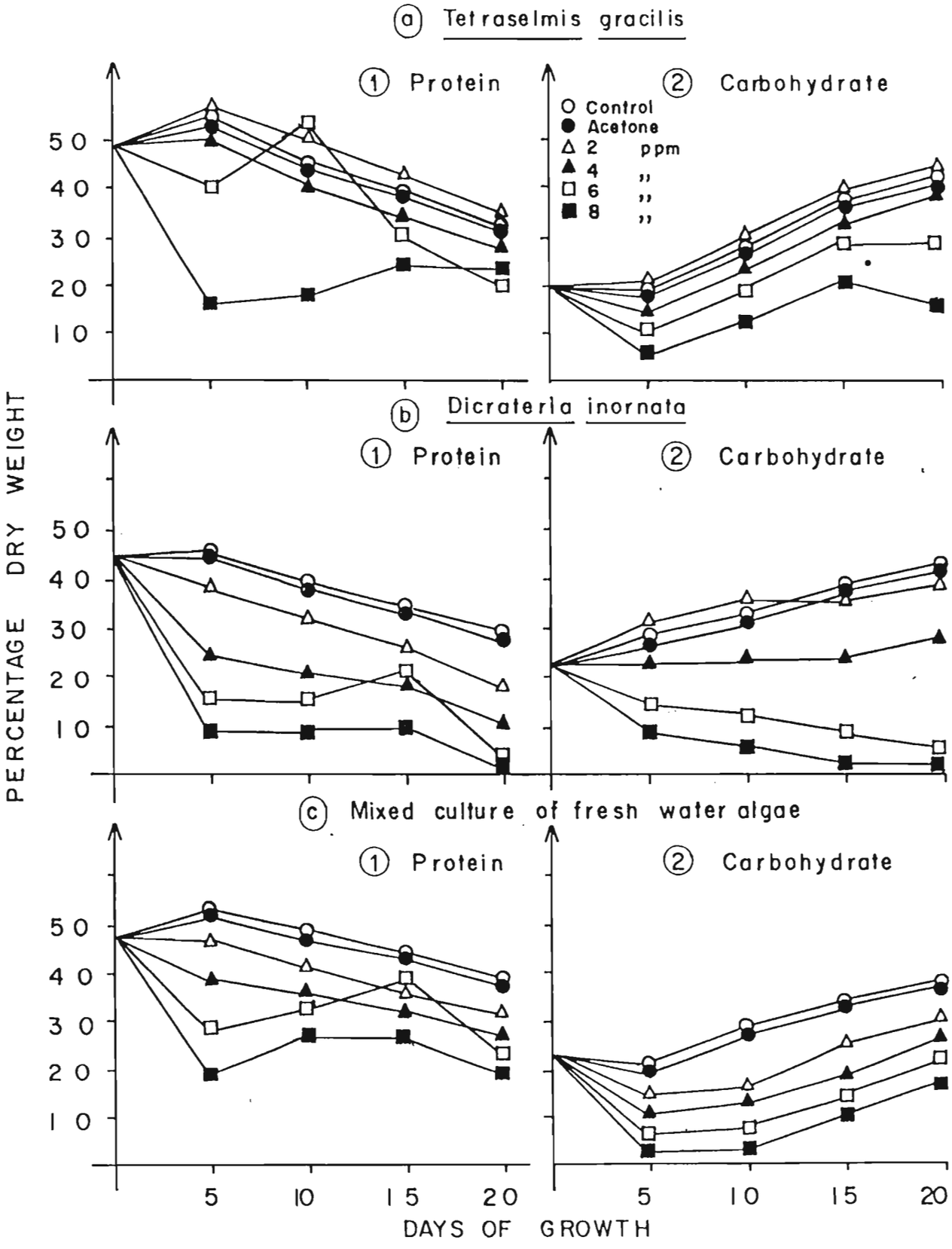
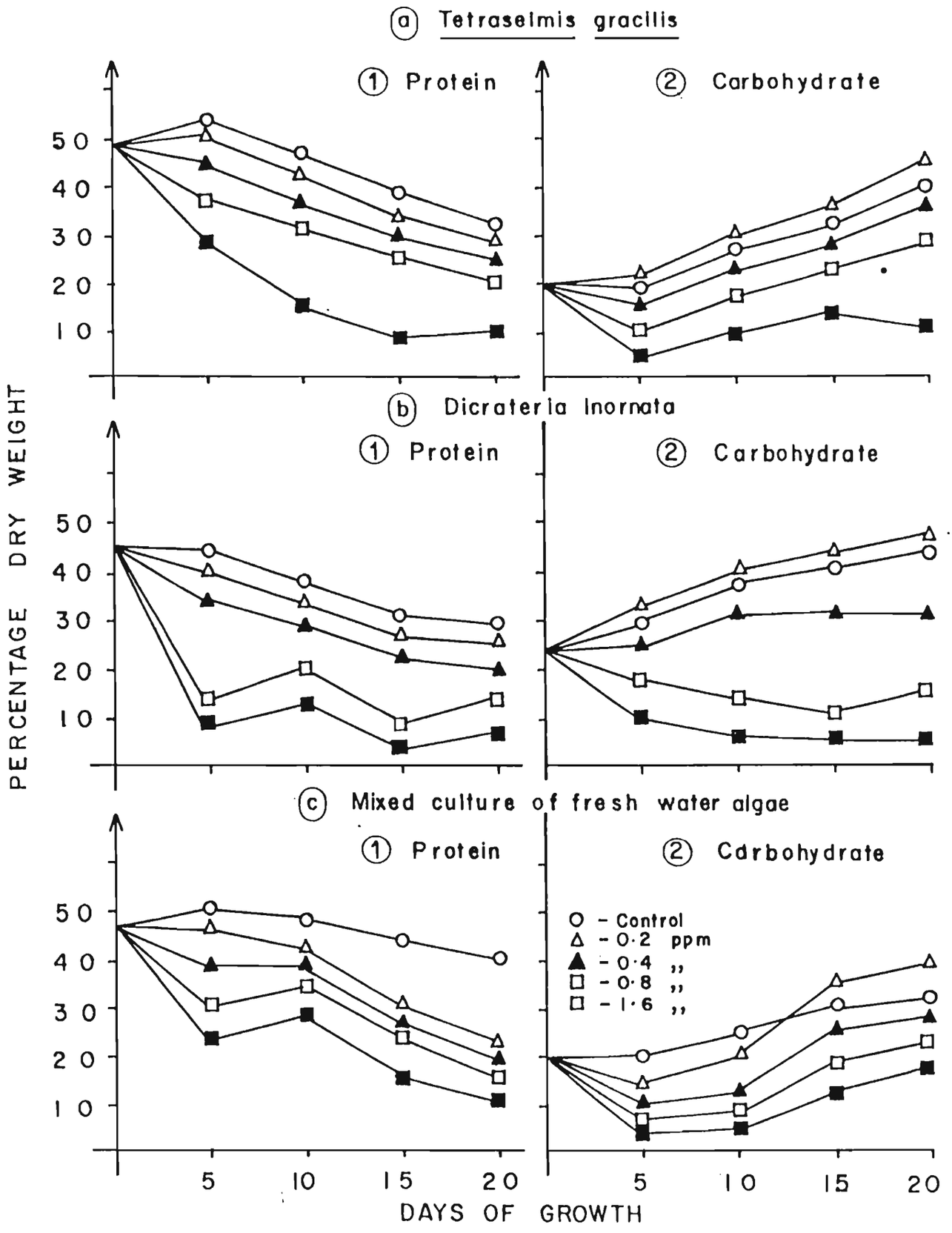


FIG. 44

EFFECT OF 'GRAMAXONE' ON PROTEIN AND CARBOHYDRATE CONTENTS OF MICROALGAE




CONTENTS OF MICROALGAE

(a) Tetraselmis gracilis

FIG. 45

EFFECT OF CUMAN L® ON PROTEIN AND CARBOHYDRATE CONTENTS OF MICROALGAE

FIG.45

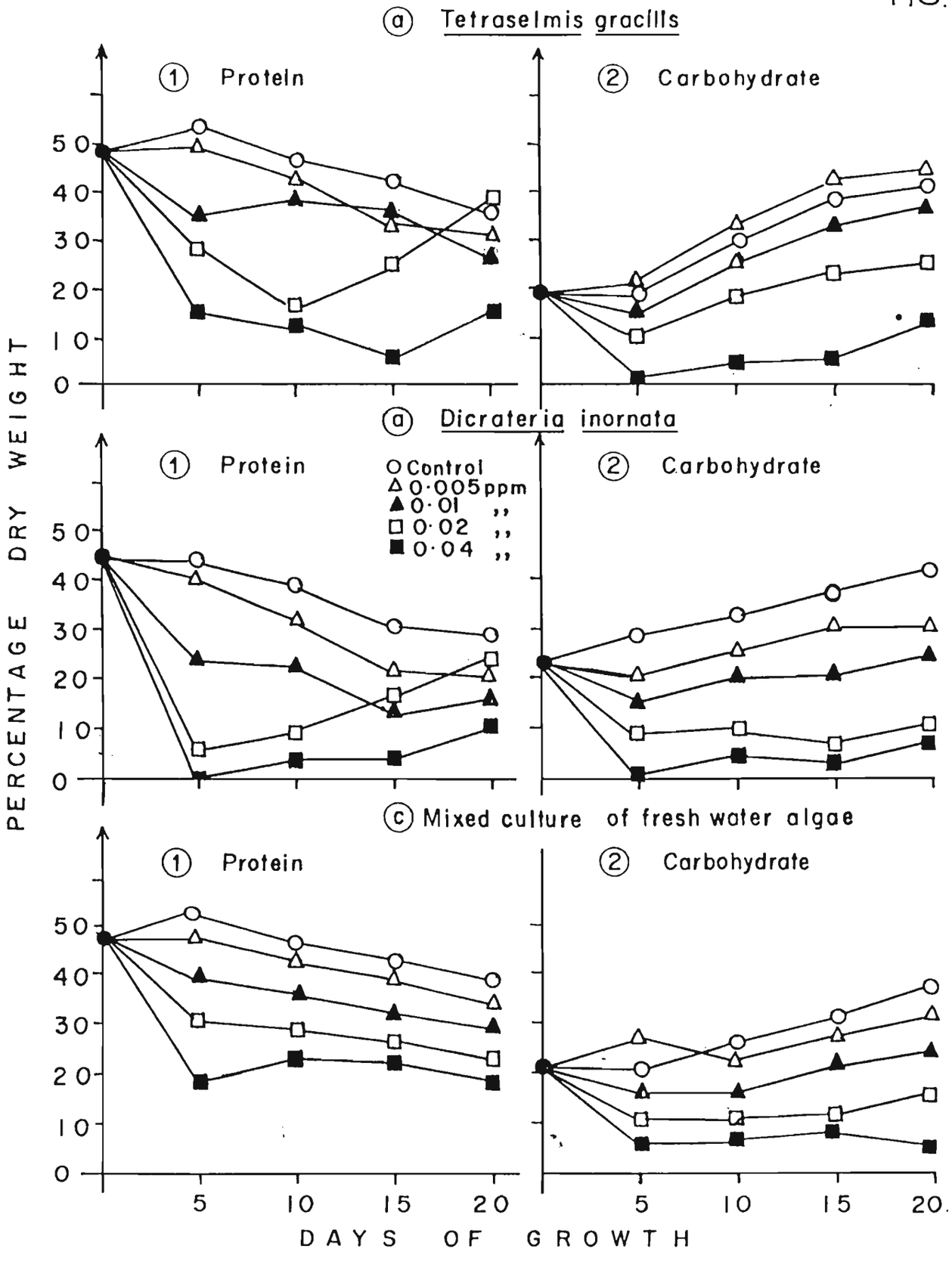


FIG.46

EFFECT OF MIXTURE OF 5 BIOCIDES ON PROTEIN & CARBOHYDRATE CONTENTS OF MICROALGAE

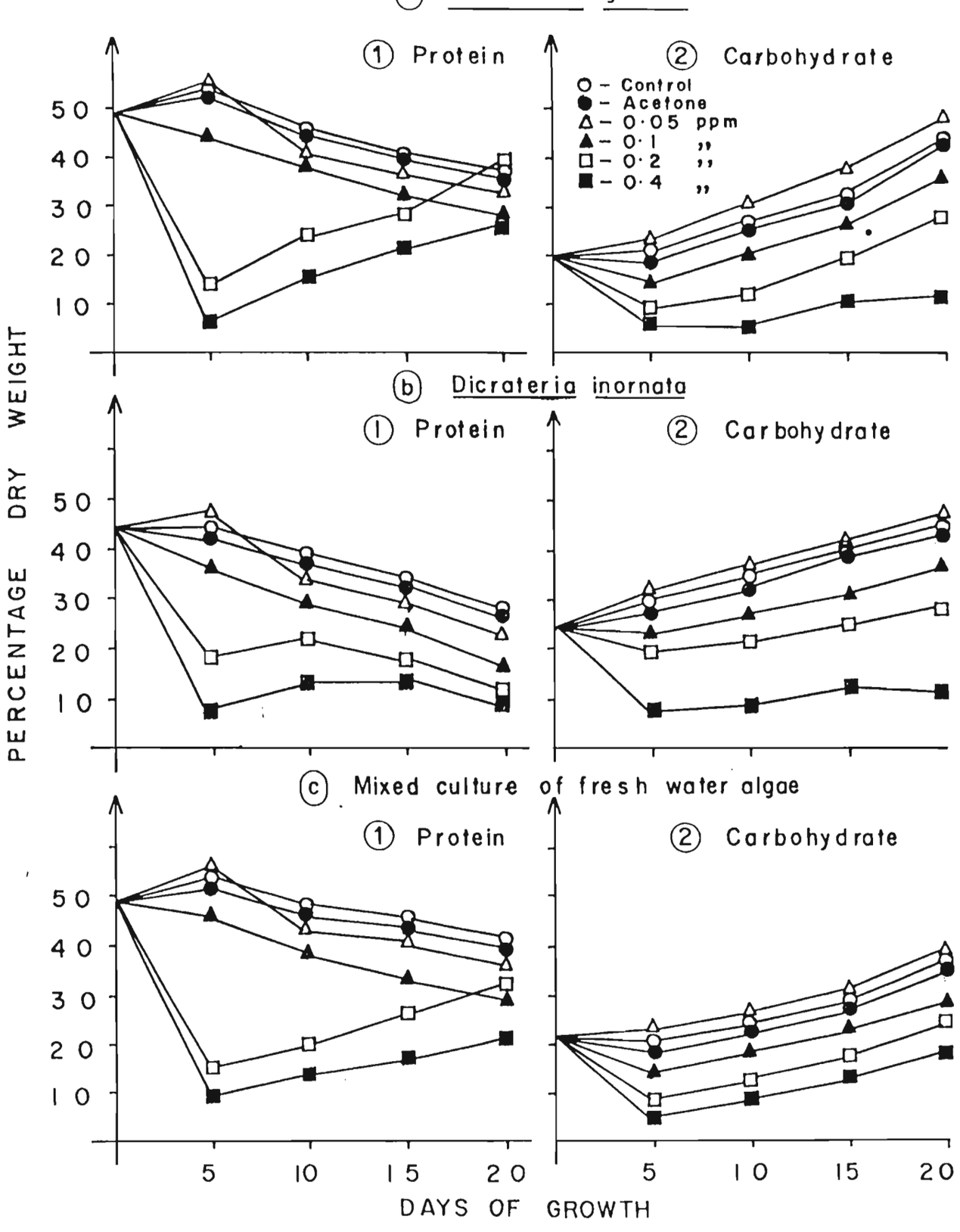


FIG.47

EFFECT OF MIXTURE OF 3 BIOCIDES ON PROTEIN AND CARBOHYDR CONTENTS OF MICROALGAE

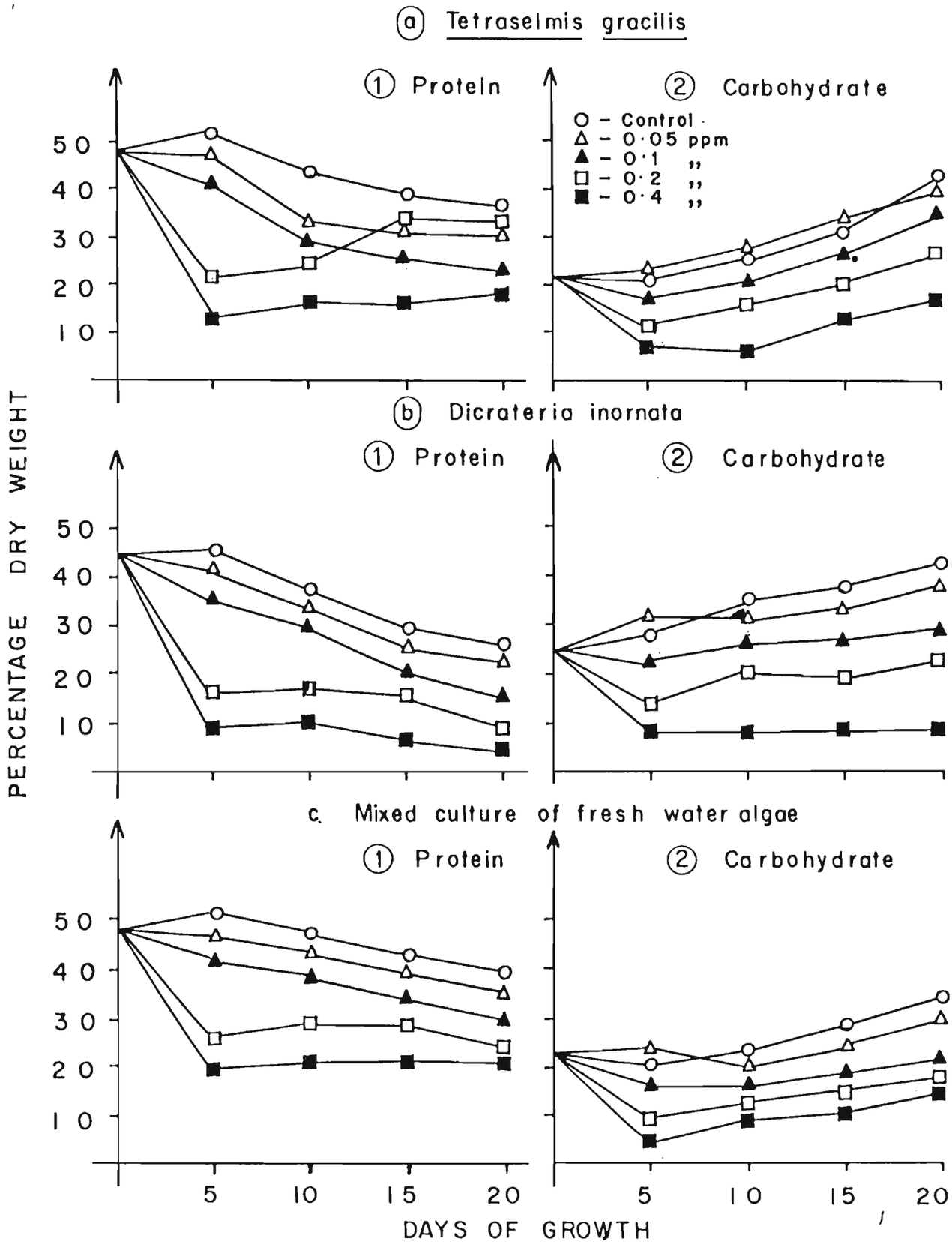


TABLE-19.
ANOVA TABLE FOR PROTEIN

| Toxicant | Source | D.F | Tetraselmis gracilis | | | Dicrateria inornata | | | Mixed culture of fresh water algae | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | S.S | M.S | F | S.S | M.S | F | S.S | M.S | F |
| B H C | Treatment | 5 | 5746.9 | 1149.4 | 18.29** | 2102.4 | 420.48 | 56.31** | 1479.2 | 295.8 | 49.71** |
| | Replicate | 3 | 481.95 | 160.65 | 2.56** | 938.6 | 312.88 | 41.89** | 616.10 | 205.36 | 34.53** |
| | Error | 15 | 942.5 | 62.83 | | 112.01 | 7.467 | | 89.22 | 5.947 | |
| Nuvacron | Treatment | 4 | 396.10 | 99.03 | 1.26N.S | 2749.6 | 687.41 | 61.15** | 1419.4 | 354.8 | 16.77** |
| | Replicate | 3 | 1459.5 | 486.49 | 6.17** | 1060.12 | 353.37 | 31.44** | 692.6 | 230.88 | 10.91** |
| | Error | 12 | 945.5 | 78.79 | | 134.89 | 11.241 | | 253.8 | 21.15 | |
| Carbaryl Sevin | Treatment | 5 | 1562.5 | 312.5 | 5.08** | 2628.0 | 525.6 | 34.54** | 1109.6 | 221.92 | 8.87** |
| | Replicate | 3 | 1144.6 | 381.5 | 6.21** | 645.5 | 215.2 | 14.14** | 255.5 | 85.16 | 3.40** |
| | Error | 15 | 922.4 | 61.49 | | 228.2 | 15.22 | | 375.3 | 25.02 | |
| Gramaxone | Treatment | 4 | 2435.5 | 608.86 | 17.46** | 3099.4 | 744.9 | 15.78** | 1060.9 | 265.24 | 15.47** |
| | Replicate | 3 | 1319.9 | 439.98 | 12.61** | 742.3 | 247.5 | 5.04** | 541.46 | 180.48 | 10.53** |
| | Error | 12 | 418.7 | 34.89 | | 589.3 | 49.11 | | 205.68 | 17.14 | |
| Cuman L(R) | Treatment | 4 | 2986.6 | 746.6 | 9.35** | 2493.9 | 623.5 | 9.77** | 1403.4 | 350.84 | 29.87** |
| | Replicate | 3 | 278.05 | 92.68 | 1.16N.S | 95.09 | 31.69 | 0.49N.S | 173.3 | 57.78 | 4.92** |
| | Error | 12 | 958.7 | 79.89 | | 765.5 | 63.79 | | 140.96 | 1.75 | |
| Mixture of 5 Biocides | Treatment | 5 | 978.3 | 195.67 | 1.45N.S | 1853.7 | 370.7 | 10.80** | 3562.0 | 713.62 | 13.87** |
| | Replicate | 3 | 23.14 | 7.714 | 0.06N.S | 415.69 | 138.56 | 4.04** | 47.07 | 15.691 | 0.31N.S |
| | Error | 15 | 2030.6 | 135.37 | 0.06 | 514.74 | 34.32 | | 771.64 | 51.443 | |
| Mixture of 3 Biocides | Treatment | 4 | 1015.34 | 253.84 | 5.87** | 1566.0 | 391.5 | 8.32** | 2061.4 | 515.36 | 26.87** |
| | Replicate | 3 | 96.96 | 32.32 | 0.75N.S | 249.9 | 83.31 | 1.77N.S | 104.74 | 34.91 | 1.82N.S |
| | Error | 12 | 518.67 | 43.22 | | 564.9 | 47.07 | | 230.36 | 19.19 | |

* Significant at 5% level, ** Significant at 1% level, N.S. Not significant

TABLE-20
ANOVA TABLE FOR CARBOHYDRATE

| Toxicant | Source | D.F | Tetraselmis gracilis | | | Dicrateria inornata | | | Mixed culture of water algae | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | S.S | M.S | F | S.S | M.S | F | S.S | M.S |
| B H C | Treatment | 5 | 1686.03 | 337.21 | 64.33** | 3171.2 | 634.2 | 62.95** | 46.58 | 9.316 |
| | Replicate | 3 | 1228.1 | 409.38 | 78.09** | 625.70 | 208.6 | 20.69** | 1740.28 | 508.09 |
| | Error | 15 | 78.63 | 5.24 | | 151.14 | 10.08 | | 148.53 | 9.902 |
| Nuvacron | Treatment | 4 | 156.51 | 39.126 | 11.46** | 2742.0 | 685.51 | 44.47** | 167.32 | 41.83 |
| | Replicate | 3 | 940.24 | 313.415 | 91.79** | 67.08 | 22.36 | 1.45 N.S | 1110.86 | 370.29 |
| | Error | 12 | 40.97 | 3.414 | | 184.99 | 15.42 | | 131.14 | 10.93 |
| Carbaryl Sevin | Treatment | 5 | 440.88 | 88.175 | 3.64* | 4683.01 | 936.60 | 19.81** | 72.03 | 14.41 |
| | Replicate | 3 | 1737.8 | 579.26 | 23.93** | 103.47 | 34.49 | 0.729 N.S | 1139.09 | 379.69 |
| | Error | 15 | 363.11 | 24.207 | | 709.24 | 47.28 | | 77.55 | 5.17 |
| Gramaxone | Treatment | 4 | 620.91 | 155.23 | 18.06** | 3313.06 | 828.26 | 31.01** | 243.39 | 60.85 |
| | Replicate | 3 | 1183.61 | 394.54 | 45.89** | 43.809 | 14.603 | 0.55 N.S | 1396.01 | 465.34 |
| | Error | 12 | 103.16 | 8.596 | | 320.57 | 26.713 | | 264.89 | 22.07 |
| Cuman L(R) | Treatment | 4 | 3952.3 | 988.08 | 20.41** | 1208.53 | 302.13 | 11.24** | 95.913 | 23.98 |
| | Replicate | 3 | 1051.8 | 350.60 | 7.24** | 219.02 | 73.006 | 2.72** | 432.68 | 144.23 |
| | Error | 12 | 580.9 | 48.41 | | 322.59 | 26.88 | | 660.39 | 55.03 |
| Mixture of 5 Biocides | Treatment | 5 | 507.82 | 101.56 | 15.18** | 1410.3 | 282.07 | 66.68* | 267.03 | 53.41 |
| | Replicate | 3 | 1546.47 | 515.49 | 77.07** | 555.9 | 185.32 | 43.81** | 1037.62 | 345.87 |
| | Error | 15 | 100.33 | 6.689 | | 63.45 | 4.230 | | 40.93 | 2.73 |
| Mixture of 3 Biocides | Treatment | 4 | 623.27 | 155.82 | 9.73** | 1594.2 | 398.54 | 38.61** | 286.13 | 71.53 |
| | Replicate | 3 | 714.34 | 238.11 | 14.88** | 118.33 | 39.44 | 3.82* | 412.64 | 137.55 |
| | Error | 12 | 192.08 | 16.007 | | 123.88 | 10.32 | | 27.84 | 2.32 |

* Significant at 5% level, ** Significant at 1% level, N.S. Not significant

## 4.4. MORPHOLOGICAL CHANGES EFFECTED BY BIOCIDES

A study was made on the morphological changes brought about on the cell characteristic by the 5 biocides used for the experiments. For the purpose of the study photomicrography of the treated algal cells were taken using an 'Olympus' Universal researchnmicroscope (Vonvox model P M 10 A.D) equipped with an automatic exposure systems.

### 1. Tetraselmis gracilis

Normal cells of Tetraselmis gracilis are green and oval shaped with an anterior furrow from which arises four flagellae (Plate 2a). However the flagellae are not visible in the photograph.

Morphological deformities were observed as a resuilt of biocide treatment and the same are as follows:

The organochlorine insecticide - 'BHC' at 4 ppm concentration caused the breakdown of the cell wall, and as a result the cell lost its oval shape. Cells with different sizes were also observed (Plate 2b).

No remarkable change was observed in cells treated with 25 ppm concentration of 'Nuvacron' (Plate 2c) one cell under the process of division is seen in the photograph.

The carbamate insecticide 'carbaryl sevin' did not have any marked effect in the shape of the cells. But some cells become larger than their normal size (Plate 3a).

Plate 3b shows the irregularity caused in the size of the T. gracilis cells treated with herbicide. Some of the cells become swollen. Shape of the cells was also altered compared to normal cells.

As a result of fungicide treatment i.e., Cuman $L^{(R)}$, the microalgal cells underwent morphological changes. At 0.02 ppm concentration of 'Cuman $L^{(R)}$', the cell wall of some of the cells brokendown and they showed a tendency to clump together (Plate 4a). Plate 4 b clearly shows that 'Cuman $L^{(R)}$' at 0.04 ppm concentration caused the clumping of algal cells into separate groups.

2. Dicrateria inornata

Normal cells of Dicrateria inornata are brownish yellow in colour and rounded in shape having two equal flagellae. But the flagellae are not seen in the photograph (Plate 5 a).

As in the case of T. gracilis, this species also showed morphological deformities as a result of biocide treatment. Plate 5b shows cells with broken and irregular walls as a result of 'BHC' treatment at 4 ppm concentration. Cells treated with 'Nuvacron' of 25 ppm become more brownish. No broken cell walls were observed but cells with irregular size are seen in the photograph (Plate 5c).

Unlike in the case of T. gracilis the 'carbaryl sevin' treated cells of D. inornata showed broken cell walls. Carbaryl sevin at 8 ppm concentration caused the disruption of cell wall (Plate 6a).

Plate 6 b reveals the effect of 'Gramaxone', the herbicide on cell size of D. inornata. One of the cells become very much gigantic compared to other cells.

Most of the algal cells treated with 0.04 ppm concentration of the fungicide became clumped together and formed separate groups as shown in the photomicrograph (Plate 6 c).

### 3. Mixed culture of fresh water algae.

The mixed culture of freshwater microalgae was dominated by Chlorella ovalis cells. Scenedesmus cells are also seen in the photograph (Plate 7 a) but other two species are not seen in the photograph.

As a result of 'Nuvacron' treatment, the cells showed a tendency to group together. Only Chlorella cells are seen in the Plate 7b.

'Gramaxone' treated cells also showed clump formation. Some of the cells became more green coloured compared to other normal cells (Plate 8 a).

Separate groups of cells were formed as a result of fungicide treatment(Plate 8b)along with normal cells.

## DISCUSSION

Morphological deformities like disintegration of cell wall and disarrangement of chloroplast are reported by various authors as a result of

biocide treatment (Sodergren, 1968; Mac Farlane et al., 1972; Ramachandran et al., 1980).

The results obtained in the present study showed that the cell wall of microalgae namely T. gracilis and D. inornata, treated with 'B.H.C' were in a broken state. As a result of 'BHC' treatment at 4 ppm concentration, the cell walls became wrinkled and broken, so that the shape of the cells become irregular. This result was in conformatory with the findings of other investigators. Sodergren (1968) reported that Chlorella species agglomerated and became wrinkled and hexagonal in the presence of DDT, shape loss occured in Skeletonema costatum (subramanian et al., 1979), and deformity and changes in the chloroplast structure were recorded in the diatom Nitzchia delicatissima (Mac farlane et al., 1972).. But Goulding and Ellis (1981) did not obsrve any broken cell, but they reported that DDT treated cells were considerably smaller and more ovoid than control cells. Goulding et al., (1984) concluded that, the effect of DDT on cell size and shape in Chlorella fusca was more related to the markedly decreased levels of fatty acids, chlorophyll 'a' and other components in the treated cells.

The disruption of cell wall may be because of the interaction of 'BHC' with lipid materials of the cell membrane as reported by Mac Farlane et al., 972). Powers et al., (1977) reported on the dieldrin,an organochlorine induced destruction of marine algal cells which resulted in concomitant decrease in size of survivors and their progeny

There are only very few reports of changes in cell size and shape as a result of treatments with biocides other than organochlorine insecticides The T. gracilis cells did not show any particular change at 25 ppm concentration of 'Nuvacron'.

The results of the present investigation showed gigantism in some of the cells as a result of 'Gramaxone' treatment. This was in consonance with the findings of Walsh and Alexander (1980) who found that, as a result of 'ethoprop' treatment, giant cells of 2 to 5 times larger than normal cells occurred in Skeletonema costatum. The gigantism of the cells observed in the present investigation may be because of two reasons. One is the increased production of pigments and other cellular components. Another reason may be the absorption of pesticide from the medium into the cells.

The fungicide treated cells of the three cultures investigated showed a tendency for clump formation. At 0.04 ppm concentration of Cuman L(R), the cells of the microalgae became sedentary and some of the cells lost cell wall also. In adverse condition, the individual algal cells may form groups to withstand the effect of fungicide. Hence clump formation occurs.

Canterford (1980) reported on the mercury induced development of abnormal cells and ultra structural changes in the diatom Ditylum brightwelli He was of the opinion that, a true ' ' phase occurred when the cells in the inoculum were viable but not in a condition to ide. The lag phase represents a period of detoxification and reconstitution of the cell. Apparently the higher the toxicant concentration and exposure time, longer

the period of reconstitution prior to cell division. No cell division occurred until the cell was completely normal. This statement was in line with the present observation.

Most of the morphological deformities reported here were found to occur at the beginning of the culture usually after 4 days of growth. Later as the age of the culture advanced the cells showed a tendency to regain the normal size. As already reported in the results of growth studies most of toxicants are toxic at the beginning. The algae became acclimatized to the toxicant medium and after 20 days of growth, most of the toxicants were not found to be much toxic especially in the case of miroalgae, T. grailis. The reduced cell number at the beginning indicated the delayed cell division, and with that the lag phase was very much extended. During this lag phase detoxification of the toxicant took place and the deformities to the cells disappeared and normal cells were formed.

The five biocides investigated here affected the morphology of phytoplankton cells in different ways; some cause disruption of cell wall, some others caused chlorophyll destruction, yet other caused gigantism of cells and also clumping of cells. All these effects of biocides are considered abnormal compared to normal cells of phytoplankton. Whether these deformities of the morphology will change the internal structure of the cell, especially that of the chlorophyll structure need indepth study.

In a natural ecosystem, where a number of species of phytoplankton are present such type of morphological changes of the component

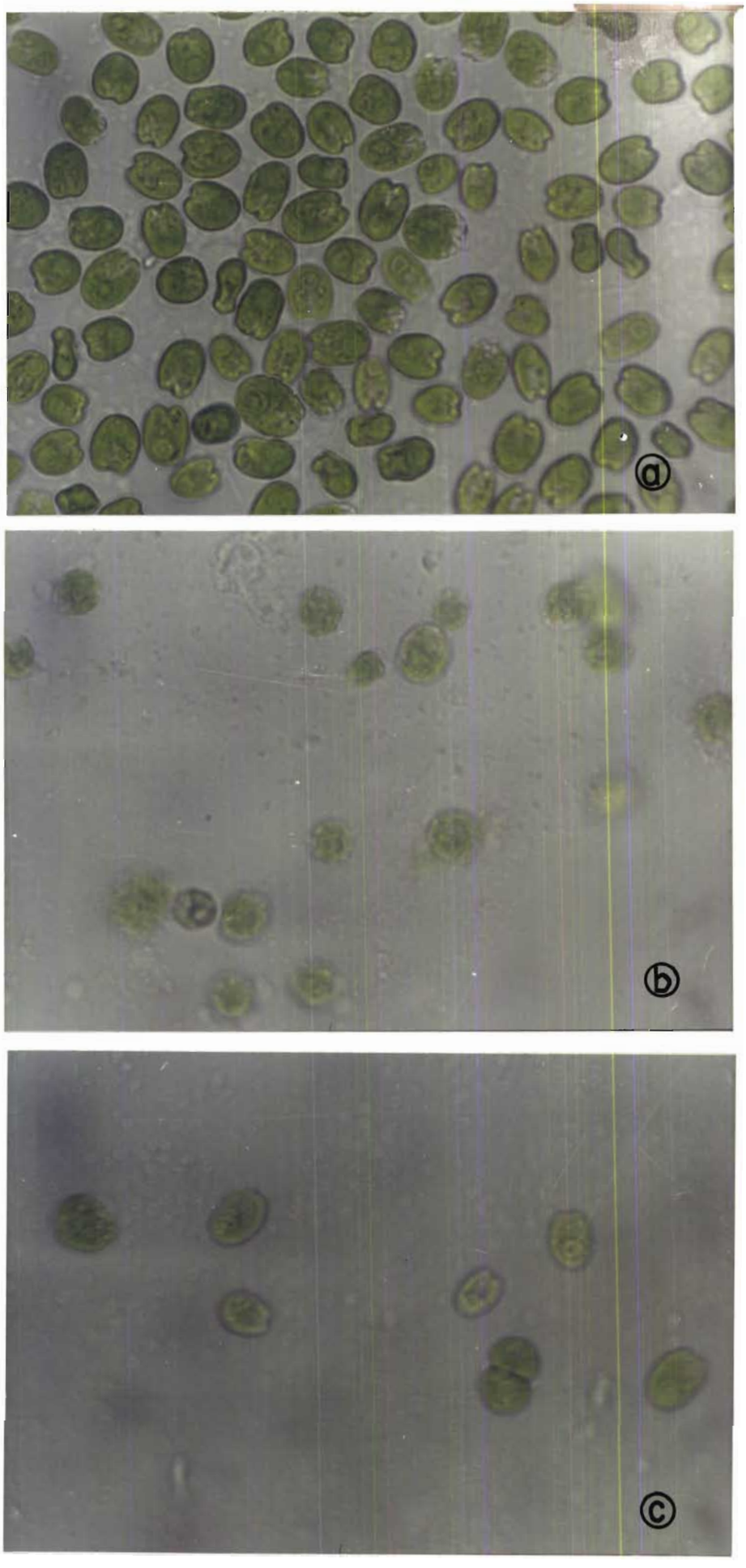
phytoplankton would affect the species diversity of the particular ecosystem. As reported by Powers et al., (1977) pollutant induced cell distintegration, size reduction and growth inhibition among sensitive phytoplankters in natural waters could affect those consumers that feed selectively on algal species.

PLATE 2

a) Photomicrograph showing Tetraselmis gracilis cells X 400

b) Photomicrograph showing 'B.H.C.' treated cells of T. gracilis X 400.

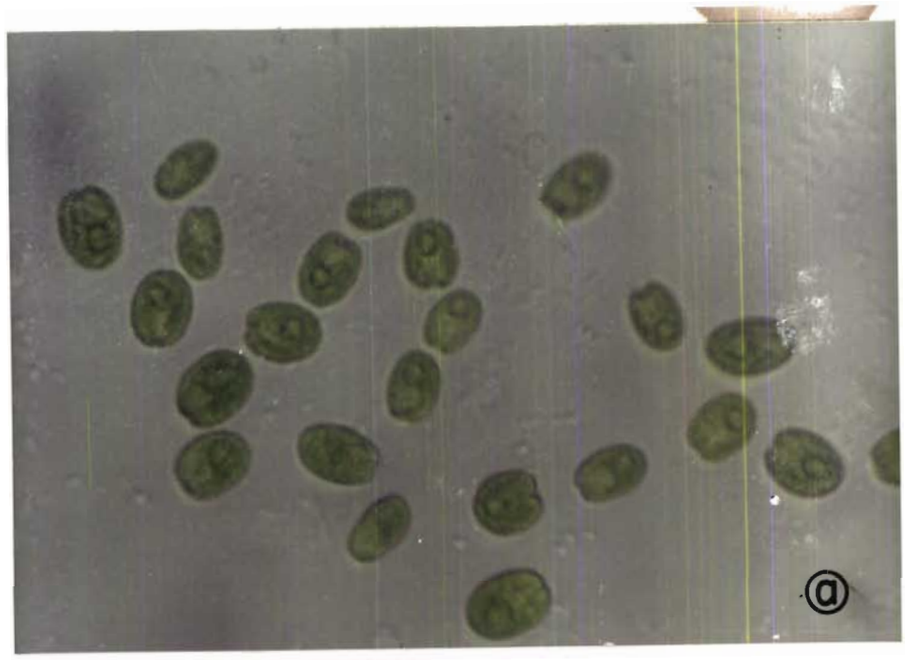
c) Photomicrograph showing 'Nuvacron' treated cells of T. gracilis X 400.

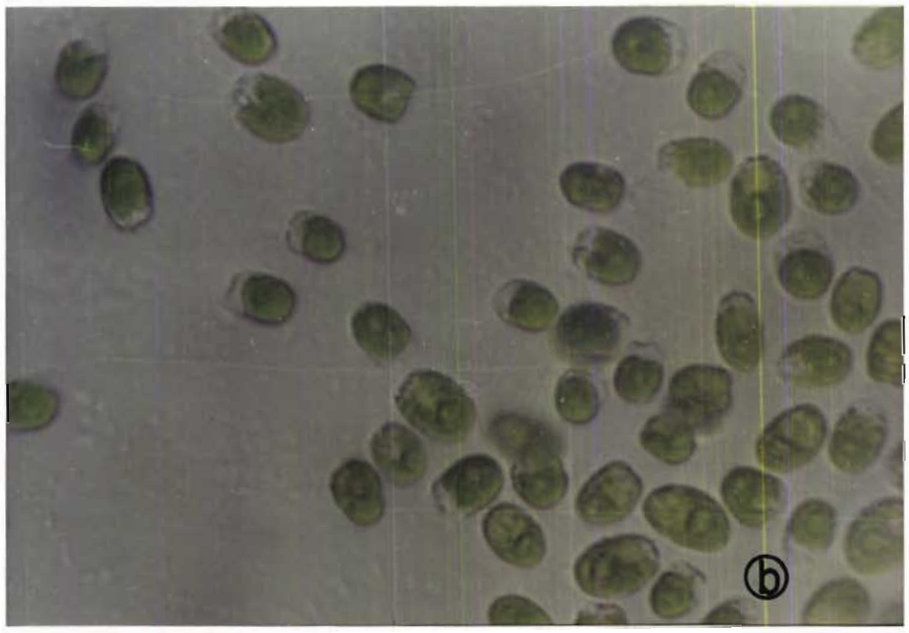

a
b
c

## PLATE 3

a) Photomicrograph showing 'Carbaryl Sevin' treated cells of T. gracilis X 400.

b) Photomicrograph showing 'Gramaxone' treated cells of T. gracilis X 400.

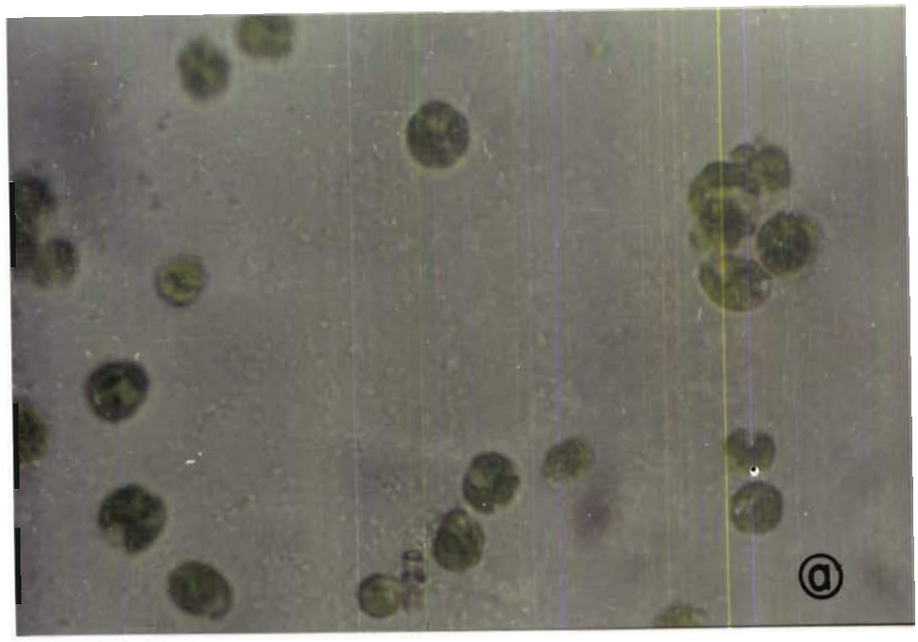


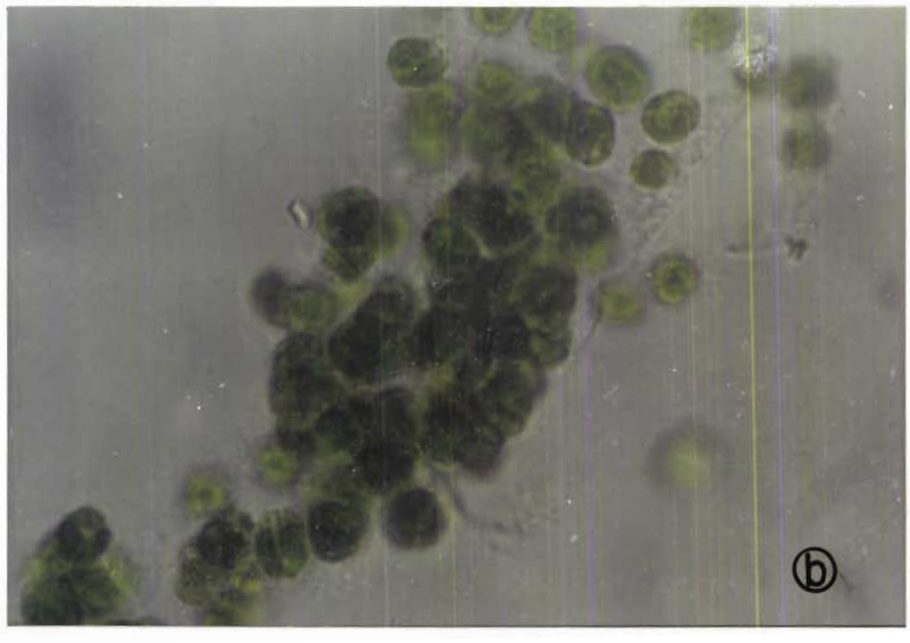



## PLATE 4

a) Photomicrograph showing 'Cuman L(R) treated cells of T. gracilis (at 0.02 ppm) X 400

b) Photomicrograph showing 'Cuman L(R) treated cells of T. gracilis (at 0.04 ppm) X 400



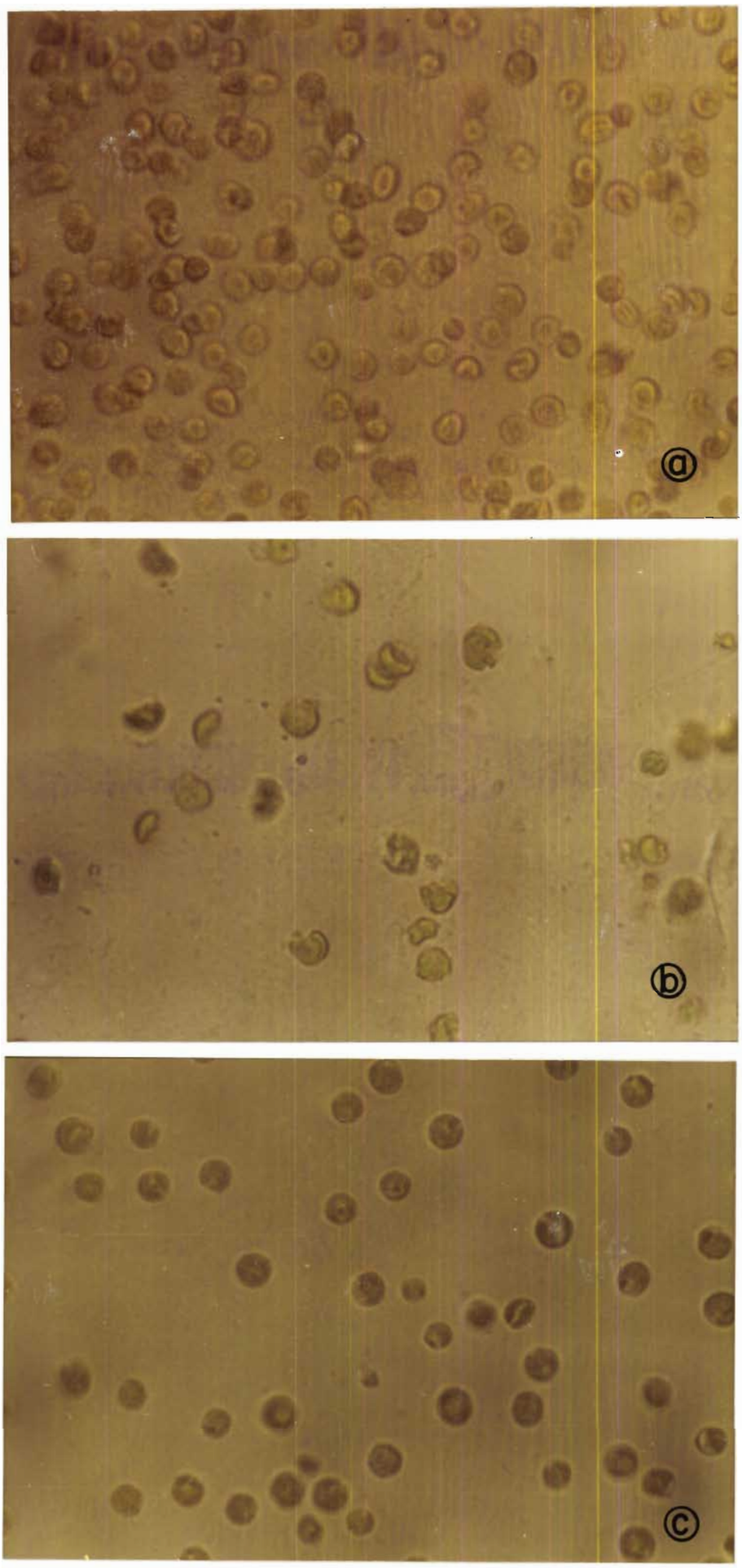



## PLATE 5

a) Photomicrograph showing Dicrateria inornata cells X 400

b) Photomicrograph showing 'B.H.C' treated cells X 400

c) Photomicrograph showing 'Nuvacron' treated cells X 400

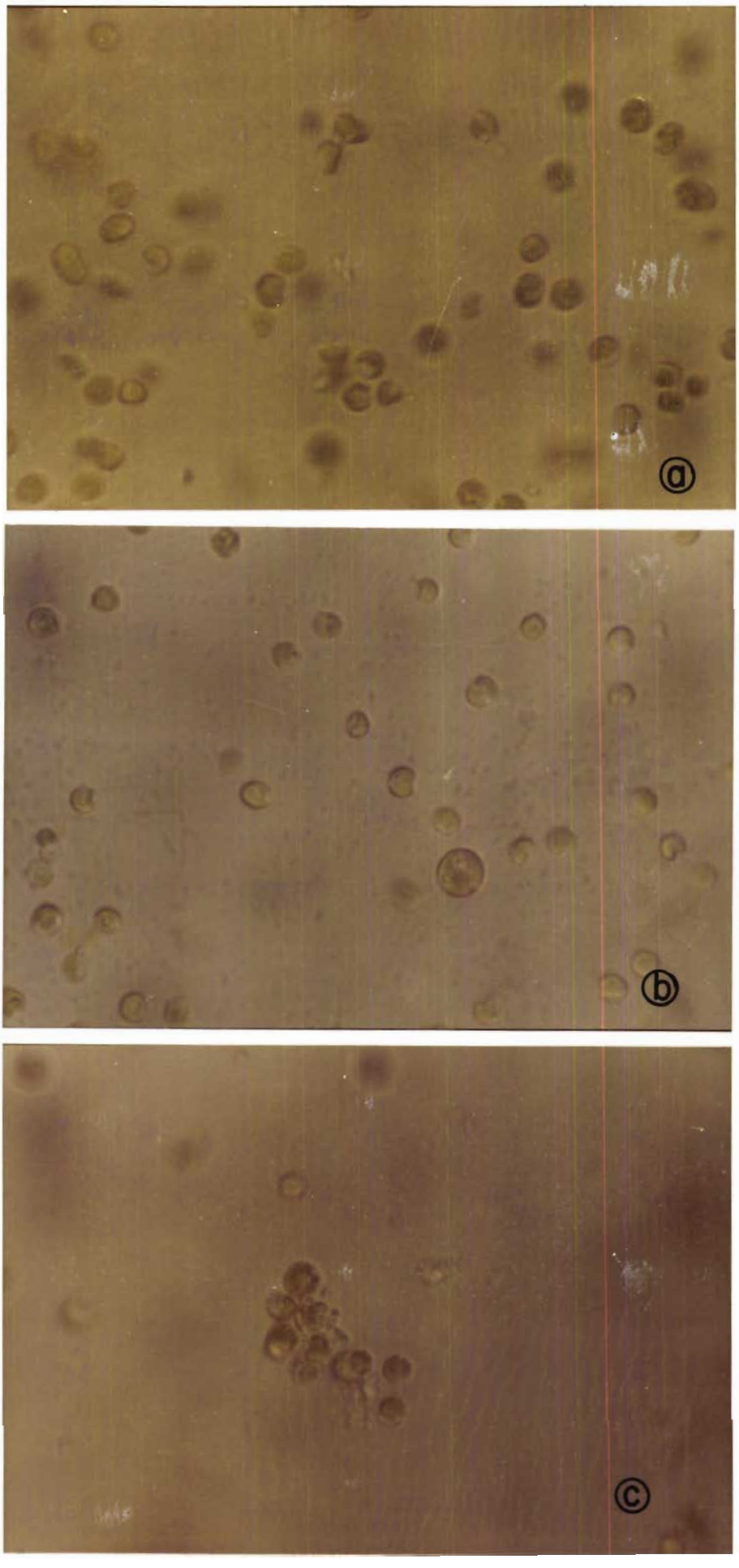

a
b
c

## PLATE 6

a) Photomicrograph showing 'Carbaryl Sevin' treated cells of D. inornata X 400

b) Photo micrograph showing 'Gramaxone' treated cells of D. inornata X 400

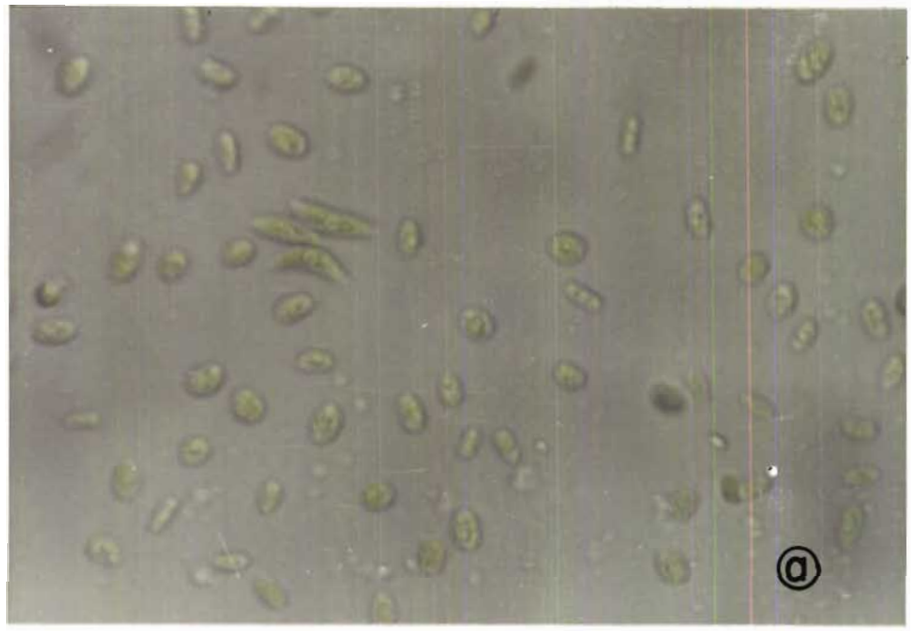
c) Photomicrograph showing 'Cuman L(R)' treated cells of D. inornata X 400 .

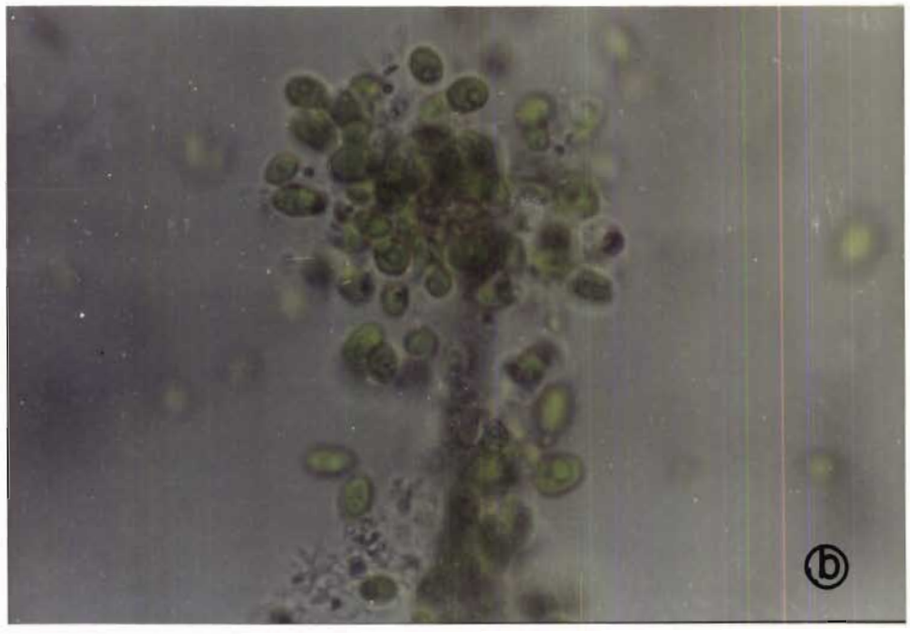

a
b
c

## PLATE 7

a) Photomicrograph showing untreated cells of Chlorella ovalis and Scenedesmus indicus X 400

b) Photomicrograph showing 'Nuvacron' treated cells of Chlorella ovalis X 400

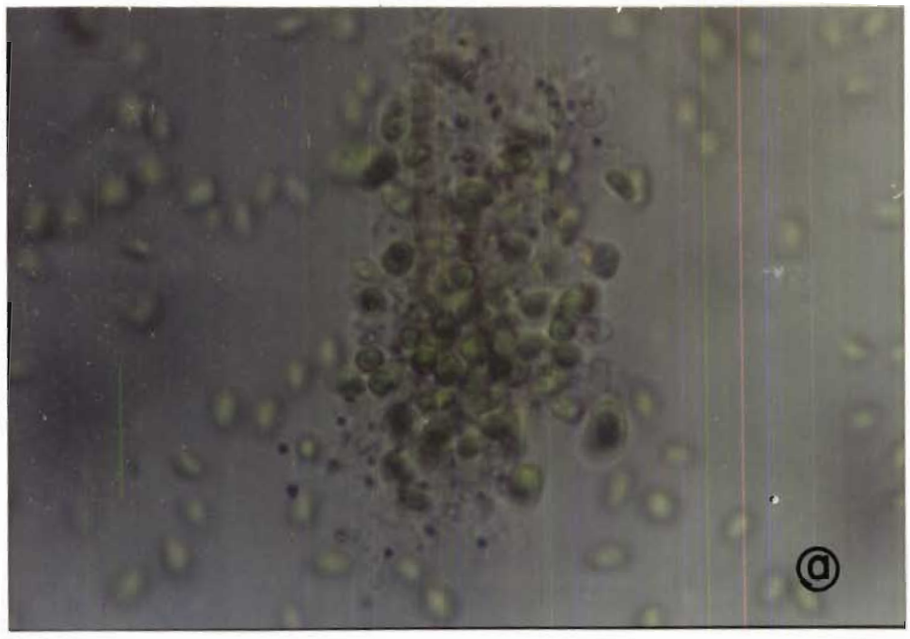


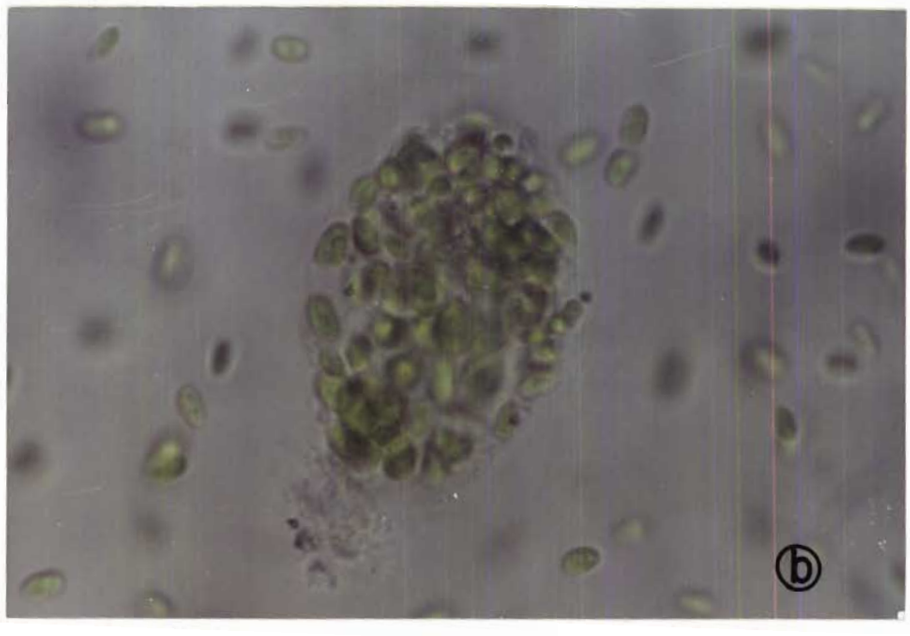



## PLATE 8

a) Photomicrograph showing 'Gramaxone' treated cells of Chlorella ovalis X 400

b) Photomicrograph showing 'Cuman L(R)' treated cells of Chlorella ovalis. X 400.



## 4.5. ACCUMULATION OF AN ORGANOCHLORINE INSECTICIDE 'BHC' IN THE MICROALGA TETRASELMIS GRACILIS

The ability of microalgae Tetraselmis gracilis to accumulate 'BHC' from aqueous solution has been demonstrated.

As shown, in the table (Table 21) the particular algal species concentrated BHC from aqueous solution. Control and acetone samples with no pesticides in the medium did not accumulate BHC. It was observed that the percentage of 'BHC' accumulated, to what actually present in the medium was found to increase with increase in the concentration of BHC in the medium. Algae grown in 0.5 ppm of BHC in the medium accumulated 0.106 ppm, i.e., about 21.2% of that was present in the medium. But when it reached 4 ppm concentration, it was observed that about 29.43% was accumulated after 10 days of exposure.

After 20 days of exposure of the microalgae to the insecticide 'BHC' it was observed that nearly 86% of the toxicant present in the medium was concentrated by the algae at 4 ppm concentration. For 2 ppm concentration 50% was accumulated, for 1.0 ppm concentration 36.5% was accumulated, and in the case of 0.5 ppm concentration only 35.4% of the toxicant present in the medium was concentrated after 20 days exposure.

From the results, it was observed that as the concentration of 'BHC' in the culture medium increased, the amount of BHC accumulated also increased. Further, long term exposure of the algal cells to the organochlorine insecticide, 'BHC' increased the chance for the accumulation of

TABLE- 21. Concentration of organochlorine insecticide - 'BHC' extracted from microalga - Tetraselmis gracilis

| Initial concentration of 'BHC' in the medium in ppm | BHC extracted from alga - ppm | | | |
|---|---|---|---|---|
| | After 10 days exposure | | After 20 days exposure | |
| | Conc. of BHC accumulated | Percentage accumulated | Conc. of BHC accumulated | Percentage accumulated |
| 0.5 | 0.106 | 21.2 | 0.177 | 35.4 |
| 1.0 | 0.253 | 25.3 | 0.365 | 36.5 |
| 2.0 | 0.586 | 29.3 | 1.0 | 50.0 |
| 4.0 | 1.176 | 29.43 | 3.423 | 85.58 |

the 'BHC' i.e, accumulation is enhanced with lengthened duration of contact.

## DISCUSSION

As indicated in the literature review there are several reports concerning the bioaccumulation of organochlorine insecticide by the microalgae in laboratory cultures. It was not surprising that different species responded differently to the insecticide.

It was observed that, the accumulation is enhanced with longetr resident time to the insecticide. A similar observation has been reported by Lin Yi-Xing and Sun Bo-zen (1987). They tested the bioaccumulation of $\gamma$-BHC after 1, 3, and 5 days of exposure and found high accumulation after 5 days of exposure.

Rice and sikka (1973 b) reported the uptake of dieldrin another organochlorine insecticide by Tetraselmis chiu. As observed in the present study, they also reported that uptake of dieldrin increased linearly with an increase in dieldrin concentration. But the accumulation of toxicant reported was many times higher than the original concentration in the culture medium. This is in contradiction to the present report. However, it may be due to the nature of pesticide used.

The same insecticide $\gamma$-BHC was tested in Chlorella pyrenoidosa also (Sodergren, 1971). The insecticide, which contained atleast 99% gamma BHC was less readily accumulated by Chlorella pyrenoidosa from aqueous medium containing 0.18 ppb lindane than DDT, which again has much lower affinity for water (Sodergren, 1968).

Another organochlorine insecticide, DDT was found to accumulate in marine diatom to about 265 times to that present in the culture medium, as reported by Keil and Priester (1969). Contrary to the present investigation, diatoms accumulate DDT many times more than what actually is present in the medium. This may be because the diatoms store food as oil and leucosin. But the species which was investigated during the present study stores food as starch. The oil and leucosin may serve as storage or 'pick-up' substances for oil soluble insecticides (Keil and Priester, 1969).

The present investigation shows that algae has the capacity to concentrate certain amount of organochlorine insecticide from the medium in which they are grown. The uptake of organochlorine by Tetraselmis gracilis could be considered as adsorption into the cell followed by absorption into cell as reported by various authors (Sodergren, 1968; Rice and Sikka, 1973b).

Because of its prolific growth this particular species would have a larger exposed surface to absorb the insecticide. If this species is kept in the same medium for a long time there is a tendency to absorb more and more toxicants into the cell. That is why the rate of accumulation is high after 20 days of exposure when compared to 10 days exposure. The rate of magnitude of bioconcentration of chemicals is governed by different chemial and physical process that vary among different taxa of algae (Sodergren, 1968, 1971, Rice and Sikka, 1973b).

The uptake of BHC from the growing medium by algal cells may be the result of several processes. The insecticide may be metabolically active and may act as an essential nutrient or mineral, get transported across the cell membrane and thus enter into biochemical processes (Boyle, 1984). As pointed out earlier, the lower concentration of BHC, was found to enhance growth of algae which may be due to its functioning as an additional nutrient for the growth.

Regarding the behaviour of 'BHC', an organochlorine insecticide it is considered that all organochlorines are 'heavy chemicals' (Addison, 1976). One of the reasons for its persistence and accumulation is the inability of most of the biological degradative forces to act on this heavy chemical. But Lin Yi-Xing and sun-Bo-zen (1987) reported that there is simultaneous lindane ( $\gamma$ -BHC) accumulation and degradation in the alga.

Glooschenko et al., (1979) suggest that bioaccumulation in algae is not affected by rate of cell growth or metabolism. But contrary to this report Lakshminarayana and Bourque (1980) suggest that the degree of absorption of organochlorines by plankton depends on various environmental conditions, particularly their population number, seasonal variation, bloom formation, growth rate etc.

Among the organochlorine pesticides detected in the coastal sediment from the Arabian sea (Sarkar and Sengupta, 1987) the concentration of BHC

was more, compared to other organochlorines. So there is chance for accumulation of this chemical in natural phytoplankton and thus get incorporated in the other members of food chain. Although Tetraselmis gracilis, which was used in the experiments during the present studies is a laboratory culture, it is one of the representative genus found in natural waters. Joseph and Nair (1975) considered this as an estuarine form of phytoplankton. So chances of accumulation of organochlorine from natural waters by this alga are high. The accumulation of 'BHC' by Tetraselmis gracilis may be an important link in providing or transferring this chlorinated hydrocarbon from agricultural run off to the aquatic environment. Significant amount of this insecticide may be removed from the water column via absorption or uptake by the alga. The role played by this alga in the exchange of pesticide must be considered in aquatic environments.

Other than bioaccumulation, another process that is biodegradation is also important in understanding the interaction of toxic chemicals on aquatic ecosystem. Algae appear to act directly in the degradation of organic chemicals. Thus algae may be an important agent in the mitigation of contaminating effects on other organisms by sorption or rendering the toxic chemical to a harmless form by degradation.

Eventhough, the present investigation shows the capabilities of algal species to accumulate the insecticide, tests on single species of algae are of limited applicability in assessing the effects of pollutants on algal communities. The reason is that the algal communities are composed of an array of species with different sensitivities.

Further research is needed to understand the magnitude to which algae are able to bioconcentrate or biodegrade pesticides. It is important in understanding how the pesticides move through food chain, and also how algae provide some sort of protection to other organisms in the aquatic ecosystem.

With the liberalization of global trade and tariff it is possible that several toxic pesticides might reach the country in the name of plant protection and increasing agricultural production. Some of this algae can remain as very useful sentinels for protecting the ecosystem from toxicity and bioaccumulation.

# CHAPTER V

# GENERAL DISCUSSION

The present investigation on the impact of five different biocides and their combinations on three microalgal cultures was restricted to laboratory based bioassays. The adoption of batch cultures allowed the simultaneous study of the effect of different concentrations of toxicants upon the physiological and biochemical parameters like proteins and carbohydrates of three microalgal cultures. The inoculum for the present investigation was taken from the same stock culture and therefore initially they were in the same physiological condition.

There is no doubt that the observed alterations in physiological and biochemical parameters like proteins and carbohydrate contents of microalgae were caused by the biocide treatment. Since control and treated cultures were derived from the same stock culture and as the experiments were performed under the same strictly controlled conditions (nutrients, light and temperature), the differences observed with the microalgae can only be attributed to the action of biocides.

Among the three microalgal cultures tested, it was observed that the microalgal cells with smaller size had the capacity to grow faster. D. inornata grew faster than T. gracilis. Mixed culture of fresh water algae though it was composed of four species, the species smaller in size, namely Chlorella ovalis showed high growth rate. This is in agreement with the fact that smaller species have the capacity to grow faster (Raymont, 1980).

The results of the 96 hour bioassay tests revealed the wide range of responses of the test algae to the biocides within the same species. The 50% effective dose values reported by several authors varied greatly and such varied responses may be due to the interspecific difference of the test organism (Menzel et al., 1970; Mosser et al., 1972 a, b, Fisher et al., 1974). Goulding and Ellis (1981) reported that the amount of inoculum and the duration of exposure period determined the $EC_{50}$ values, because a very high initial inoculum can markedly reduce the degree of inhibition. But in the present investigation the amount of inoculum for each treatment was taken from the same stock culture and also the amount of inoculum added to each treatment flask was the same.

The four different concentrations of the seven toxicants, affected the physiological and biochemical parameters, like proteins and carbohydrate content of three microalgal cultures in different ways. Both stimulatory and inhibitory actions were observed in almost all cases, depending on the concentration of the toxicant

Out of the four different concentrations selected for each toxicant, the lower concentration of most of biocides caused a stimulatory response especially in the case of T. gracilis. The other two cultures tested i.e., D. inornata and mixed culture of fresh water algae also showed a stimulatory response but the percentage stimulation was less compared to T. gracilis.

There is a controversy among different investigastors about the actual reason for the stimulation of algal growth as a result of biocide treatment. Some are of the opinion that the stimulation could be attributed to the fact

that, certain algal strains might metablize the pesticide for their growth (Butler et al., 1975; Rath and Mishra, 1981). This conclusion may be correct especially in the case of easily degradable biocides like organophosphates. But during the present investigation the stimulation of growth at 0.5 ppm of 'B.H.C.', was also noticed. B.H.C. is persistent organochlorine insecticide. Reports about algal growth stimulation by lower concentrations of organochlorines are rare (Patin et al., 1982, and Rajaretnam et al., 1987). But they failed to explain the actual reason for the transient stimulation of photosynthesis and cell division of algae. The algal growth stimulation at the lower concentration of the most of the biocides shows that at particular concentration they may act as an additional nutrient in the medium.

Francois and Robinson (1990) are of the opinion that the obvious stimulation in growth and $Co_2$ fixation observed with one herbicide - simazine treatment was perhaps due to the stimulatory effect of herbicide upon protein synthesis. This conclusion may be true in the case with some of the biocides tested in the present investigation. But in some cases though the treated cultures showed increased growth rate than control, the protein synthesis may have been inhibited.

Recently Van Donk et al., (1992), have reported that the stimulatory effect of Dursban(R), 4E, an organophosphate, insecticide, may be due to the direct effect of the carrier substances in Dursban(R) 4E. This supports the conclusion of Lewis (1986) that the carrier contains surfactants and it is conceivable that these substances may exert their influence on the physiology of algal cells.

Almost all the biocides tested here were of commercial grade, and they contained adjuvants and carrier substances other than active ingredients. These carriers may be responsible for the sudden stimulation of growth in algae in lower concentrations. But the chemical action of these adjuvants and carriers needs further explanation. Anyway, the carriers of different biocides tested may have their role in affecting the growth of algae which needs further study

In order to find out the actual reason for the stimulation of algal growth as a result of toxicant treatment, through understanding of action of each and every biocide on the cellular components of microalgae needs to be explained.

As a result of biocide treatment the rate of carbon production was very much affected. Lower concentrations of some of the biocides shows stimulatory response. In almost all cases net carbon production was round to be affected seriously, due to increased respiratory rate which leads to complete metabolism of carbohydrate.

The increase in the pigment content was also observed at the lower concentrations of biocide treatment. According to Francois and Robinson (1992) the increase in the pigment content is a tolerance mechanism against the action of herbicide. One peculiarity observed was that, the green algal members showed higher stimulation in pigment content than in D. inornata. High chlorophyll concentration indicated severe eutrophication of waters in the polluted area (Raman and Phani Prakash 1989). Exponentially growing

cells showed high chlorophyll pigments, but with the age of culture the proportion of carotenoids was found increase which may be due to the depletion of major nutrients such as nitrate and phosphate (Droop, 1973) or it may be due to their role in protecting the photoxidation of chlorophyll (Bogorad, 1974).

When we compare the percentage inhibition of pigments with that of carbon production, it was observed that percentage inhibition was high with respect to the carbon production. It shows that even though the treated cultures show capacity to production of pigments, the activity of pigments especially chlorophyll activity was inhibited that ultimately leads to decrease in carbon production. Among the estimated pigments, dead chlorophyll may also come. This statement was in line with those of Abou waly et al., (1991 b).

With the increase in the concentration of biocides, the growth and metabolism of algae were inhibited. But the percentage inhibition varied with different concentrations and also with the age of culture. One peculiarity observed was that almost all biocides tested had severe effect at the start of the culture. But in the case of herbicide 'Gramaxone', the effect was not well marked, at the beginning, but when the culture attained 10 days growth, the percentage of inhibition became more and again after two or three days an increased growth rate was observed.

The inhibition of growth and metabolism of microalgae have been already reported. But there is controversy between different investigators about the reason for inhibition. Fisher (1975) is of opinion that the

pollutants do not inhibit photosynthesis per cell, but decreased carbon fixation resulted from reduced number of photosynthesizing cells. But contrary to this, Harding and Phillips (1978b) concluded that the immediate inhibition was on photosynthesis which resulted in reduced cell growth. Plumley and Davis (1980) also support this view and they reported that the lower concentration of herbicide atrazine indicated an ability to maintain chlorophyll production and cell division with reduced photosynthesis. The present data also agree with this statement. The carbon production was found to be more affected than any other parameter tested. Since a substantial part of world's photosynthesis is performed by phytoplankton (Yentsch 1963), interference with this process could be important to the biosphere.

Experiments with mixed culture of microalgae, which represent the natural population lead to the conclusion that selective toxicity will result in changes in community structure and in the production of certain size classes of phytoplankton which may be critical food for higher trophic levels. This type of result was reported by Moore and Harris (1974). Another conclusion derived from studies with mixed culture is that toxicity increased with decrease in the cell concentration. In the present study Nitzchia longissima, representing by 5% of total population, was affected severely. This signifies that a natural population with vast diversity will be affected by biocide treatment. Species which are dominant in the community were not very much affected. As reported by Mosser et al., (1972) on the possible effects of toxicants on algal community structure and seasonal succession, a depression of growth rate at any concentration should be considered environmentally meaningful.

As reported in various other investigations the toxicity of a pollutant depends on the duration of the experiment. In the present case after an initial inhibition of algal growth and metabolism the treated algae returned to the normal stage. These types of results were already reported by various investigators (Power et al., 1975; Goulding and Ellis, 1981, Patin et al., 1982; Abou Waly et al., 1991 a, b).

Detoxification of biocides after initial toxification may be because of the function of extra cellular products. Bednarz (1981 b) was of the opinion that joint ation of pesticides and extra cellular secretions can increase the sensibility of algae to pesticides. Srisudha (1989) also reported that detoxification of trace metals may represent an important physiological function of extracellular products.

In the case of long term studies with different pollutants, the conclusions of Stockner and Antia (1976) become very important. They are of the opinion that, the short term 'shock' response should be clearly distinguished from the long term 'habituation' response of phytoplankters to the test chemical in the bioassays. The candidate's data agree with this statement. It was observed that the short term response would indicate the relatively immediate tolerance potential. But only the long term response, that is expected to be ecologically realistic for adaptation to environmental protection against pollution and eutrophication. Short term studies may be technically convenient for laboratory and field manipulations. But the phytoplanktons in seas and lakes have infinite time to adapt to all forms of environmental stress.

According to Walsh (1983) the long term studies may be ineffective for detection and quantification of toxicity because the fate and behaiour of toxicant in exposure vessels can affect toxicity. This is in conformity to the findings of Hansen (1980) that within the concentration range of 10-100 /ug/litre a portion equal to 2.3% of the lindane was adsorbed onto the glass wall. Whether the biocides used in the present investigation were adsorbed on to glass walls needs further study.

Patin et al., (1982) are of the opinion that short term experiments do not yield a complete picture of the possible consequences of the toxicant in the medium inhabited by unicellular algae. But unlike the results of present studies they observed that toxicity of DDT became considerably higher in long term experiments as compared with short term experiments.

But Abou Waly et al., (1991a, b) reported that biomass production and $^{14}C$ uptake were inhibited by both herbicides on the Ist day and began recovering on day 3 over different concentration.

All these observations and also the results of the present study lead to the conclusion that long term studies will be more appropriate to understand the specific effect and also the fate of the toxicant in the medium.

In order to understand the specific action of each biocide, the decision to use more than one parameter is justified (Goulding and Ellis 1981). It was also noted that the time course of inhibition for one parameter is different from other parameters. As reported by Saroja and Bose (1982)

the present investigation also suggest that each biocide tested has multiple sites of action on cell metabolism.

The inhibition of algal growth by different concentrations of biocides will lead to the alterations in the community structure in a natural ecosystem The stimulation of algal growth can also make alterations in the ecosystem. The present investigation shows that, the lower concentrations of most of the biocides stimulate the algal production and also another stimulation of algal growth was observed at the end of experiment. As already reported some of the biocides can easily undergo degradation, and the resultant product may act as nutrients to the culture medium. Butcher et al., (1977) reported an increase in the nutrients especially phosphorus resulting from degradation of organophjosphorus insecticide.

A conclusion similar to that drawn by Hurlbert et al., (1972) can be made that the insecticides are capable of aggragating eutrophication problems. Water draining from agricultural land pose a double threat to the aquatic environment in that, they bear both insecticide and excess nutrients. Excess nutrients will always cause eutrophication which may lead to oxygen depletion in the water.

The results of bioaccumulation studies are of special interest. It was observed that the estuarine form T. gracilis is able to accumulate the organochlorine insecticide 'BHC' from the growing medium. A percentage of 35.57 was accumulated at 4 ppm concentration. Hansen (1980) reported more than 90% lindane ( $\gamma$ BHC) uptake by the primary producer Chlorella

But the interesting result was that eventhough this algal species accumulate this toxicant in the cell they can grow in the same medium. Results of the 20th day observation show that at 4 ppm concentration of 'BHC', the cell number exceeded that of control. Perhaps it may due to the reason that, eventhough at the beginning this concentration showed an inhibited growth, with the age of culture, this species became acclimatized and the toxicant was concentrated in the cell. This concentration may be insufficient to cause a severe inhibition. This type of observation leads to the conclusion that the resistant form of microalga like T. gracilis can be used as very useful material for waste water treatment.

Among the three microalgal cultures investigated here, T. gracilis the estuarine form was found to be the most resistant strain. The other two forms, i.e., marine and fresh water strains showed variation in their response with respect to different parameters.

Among the different biocides tested the fungicide 'Cuman L$^{(R)}$' was found to be the most toxic. Above 0.1 ppm this biocide caused total lethality in the three microalgae tested. Organophosphate insecticide 'Nuvacron' was the least toxic biocide tested.

The combination of different biocides gave varying results. The combination of 3 biocides, i.e, one organophosphate insecticide 'Nuvacron', herbicide - 'Gramaxone' and fungicide - 'Cuman L$^{(R)}$' was more toxic than the combination of all the 5 biocides tested at the same four concentrations. When the synergistic effect of biocide mixture was taken into consideration

it was observed that except fungicide, the toxicity of all other biocides tested caused more toxic effect in their combination. The orgaophosphate insecticide 'Nuvacron' even at 100 ppm was not lethal to the microalgae, but when it was mixed with other biocides, the toxicity increased and lethality occurred above 0.5 ppm of this mixture.

As the nature often contains mixtures of biocides in different combinations this type study needs further clarification. The interactions of different biocides needs indepth study especially regarding their synergistic effects.

When we consider the different physiological, biochemical and morphological alterations induced by different biocides, the prediction of an application factor is very much difficult. Because of the reason that each and every alga, and its test parameters respond differently to the action of biocides. But when we take into consideration, the lethal limit of each biocide tested herewith respect to three microalgae, the possible application factor may be as follows:

| | | |
|---|---|---|
| 1. | B.H.C. | - Below 5 ppm |
| 2. | 'Nuvacron' | - Below 130 ppm |
| 3. | Carbaryl sevin | - Below 10 ppm |
| 4. | Gramaxone | - Below 2 ppm |
| 5. | Cuman $L^{(R)}$ | - Below 0.1 ppm |

In the case of mixture of these biocides, below 0.5 ppm is recommended.

The farmers apply these different biocides in the following concentrations.

| | | |
|---|---|---|
| 1. B.H.C. | - | 5000 ppm/acre |
| 2. Nuvacron | - | 500 ppm/acre |
| 3. Carbaryl Sevin | - | 2500 ppm/acre |
| 4. Gramaxone | - | 2500 ppm interrestrial habitat and 1.5 ppm in water |
| 5. Cuman $L^{(R)}$ | - | 1000 ppm /acre |

The microalgae tested here, especially T. gracilis and D. inornata are considered as pollution indicators. Still, this species may be adversely affected by biocides after a threshold limit.

Thus the rate applied in the field is always on high side. On the basis of information already available and the results obtained during the present study it can be concluded that the variable response of algae to different biocides and their combination depend upon many factors. The significant role played by the chemical structure of compounds, the pathways of action and the variability of species.

Programmes directed towards detecting large scale spatial differences and relatively long term temporal changes of the order of a year or more are required for the evaluation of the stress carried by the organochlorine pesticides.

It is hoped that field studies on phytoplankton communities will enable a realistic interpretation to the problems of biocide treatments and their interactions in the ecosystem.

# CHAPTER VI

# SUMMARY AND CONCLUSIONS

The impact of some commonly used biocides on three microalgal cultures was investigated. The two unicellular algae selected are of importance for mariculture as larval and post larval food in hatchery systems. The fresh water algae collected from paddy fields, formed a mixtur representing a natural population.

In order to find out the effective concentration of biocides that would inhibit the growth of algae by 50%, 96 hour bioassay tests were conducted. The number of cells, and rates of carbon production were determined after 24, 48, 72 and 96 hour and they were compared with that of control. The variations in pigment contents were also estimated. The significance of using three parameters is that one parameter does not give accurate results.

The results of 96 hour bioassay tests revealed that there was variation in growth responses of microalgal species with three biocides. In almost all cases the rate of carbon production was found to be more affected than other parameters. It was also revealed that $EC_{50}$ values varied with the duration of experiment.

The 96 hour bioassay tests did not give a real picture of the impact of various biocides. Therefore, long term experiments were conducted to

understand the effect of different biocides on physiological and biochemical characters of three microalgal cultures. Each experiment was of 20 days duration. A total 21 sets of experiments were conducted

Among the physiological parameters, growth, rate of carbon production, pigments such as chlorophyll a and b in green algae and chlorophyll a and c in brown algae as well as carotenoids were estimated during the period of experiments lasting 20 days. Biochemical parameters such as protein and carbohydrate contents were also estimated.

The five different biocides selected were of common use in agricultural fields. Hence their residues may be ultimately reaching the aquatic ecosystem. In nature mixtures of biocides occur and so combinations of five different biocides were also tested.

Due to the low water solubility in the case of 'B.H.C' and 'Carbaryl sevin', acetone was used as the solvent. Results showed that acetone has no stimulatory or inhibitory effect on microalgal culture.

The growth of algae was found to be affected by the presence of biocide. The effect of different concentrations was specific for each biocide The lower concentration of most of the biocides caused a stimulatory effect especially in T. gracilis.

The percentage inhibition of algal growth was found to increase with increased concentration of biocide. It was also observed that almost all

biocides showed maximum toxic effect in the culture at the commencement of application. With the ageing of culture the toxic effect decreases. So it can be concluded that most of the biocides show an immediate shock response followed by a habituation response.

The results of experiments with mixed culture of fresh water algae showed that species composition was affected. In the natural population of phytoplankton, the chlorophycean members were found dominating, Chlorella ovalis was the dominant species. The other species especially Nitzchia longissima was only nominally represented and this species was found seriously affected by biocide treatment.

The rate of carbon production was found to be affected by biocide treatment. In this case also lower concentration of some of the biocides showed stimulation in carbon production. But the percentage stimulation was less compared to that observed for growth of microalgae.

With the increase in the concentration of biocide, the carbon production was inhibited. The maximum inhibition was observed at the beginning of the experiment. Compared to gross carbon production, net carbon production was less. The difference in gross and net carbon production may be due to the fact that, at higher concentrations of biocide treatment, due to increased respiratory rate, the reserve food material is completely metabolized and that leads to decrease in net production. The percentage inhibition in carbon production was found decreased with the age of culture.

The pigment content was also affected by biocide treatment. At lower concentrations the chlorophyll pigment was stimulated especially in T. gracilis and mixed culture of fresh water algae. The higher concentrations showed very low values for pigments especially at the beginning of the culture.

In the case of control cultures, after an expoential phase, the chlorophyll pigments decreased but carotenoid content increased. But a reverse condition was observed in treated cultures especially at higher concentrations. In the case of D. inornata the pigment content was very much affected.

Another peculiarity observed was that even though the treated cultures show inhibitory response with respect to pigment, the percentage inhibition was less compared to photosynthetic rate. It shows that chlorophyll activity was inhibited. Among the estimated pigments, dead chlorophyll may also come.

The biochemical products like proteins and carbohydrate of algae were found affected by biocide treatment. In the case of control cultures, the protein content was found to decrease with age of culture and carbohydrate content was found to increase. The lower concentrations of some of the biocides tested showed stimulation of proteins and carbohydrates. But the percentage stimulation was high with respect to carbohydrate content. This showed that carbohydrate was resistant to biocides than protein. With the increase in the concentration of biocides, the protein

and carbohydrate content was found inhibited. One peculiarity observed with 'Nuvacron' treatment is that in the case of T. gracilis the protein synthesis was not much affected even at 100 ppm concentration of 'Nuvacron This may be because of the fact that, the 'Nuvacron' undergo degradation and these metabolites may act as nutrients in the medium. These increased nutrient uptake may ultimately leads to increased protein content. Thus it can be concluded that there was a relationship between the available nutrients and protein synthesis in microalgae.

Morphological deformities like cell well breakage, chloroplast disarrangement, gigantism of some of the cells, and even clumping of cells were also observed as a result of biocide treatment.

The results of bioaccumulation studies revealed that the estuarine form of an alga, T. gracilis, had an ability to absorb the organochlorine insecticide from the medium and got accumulated in it. The degree of accumulation was found to increse with the concentration of toxicant in the medium and lengthened duration of contact. It was observed that nearly 86% of the toxicant present in the medium was concentrated by the alga at 4 ppm concentration.

Statistical analysis of data was done using analysis of variance. In most cases the variations in growth responses of algae as a result of biocide treatment were highly significant.

Out of the three microalgal cultures investigated the estuarine form T. gracilis was found to be a resistant species. Because of this character

and also due to the capacity to accumulate toxicant this alga can be recommended for waste water treatment as scavengers.

Among the different biocides, the fungicide - 'Cuman L.(R) was found most toxic. The organophosphate insecticide 'Nuvacron' was less toxic and because of its highly degradable nature it can be recommended for field use.

The combination of biocides showed more toxicity than individual biocides, except the fungicide 'Cuman L(R).

It was observed that the lethal limit of five different biocides tested here was low as compared to the concentrations of biocides that farmers are applying in the field. So caution is advised in applying different biocides in the field.

The above findings revealed that the interaction between different biocides and the microalgae could play a significant role in the alterations in aquatic ecosystem.

Further research is needed especially in the case of biodegradation and bioaccumulation characters of microalgae. This area should focus on the identification of kind of algae that take up different pollutants into their cells and how the pollutants are degraded or are passed on to consumer organisms.

With the liberalization of global trade and tariff it is possible that several toxic pesticides might reafch the country in the name of ,plant protection and increasing agricultural production. Some of this algae can remain as very useful sentinels for protecting the ecosystem from superfluous toxicity and bioaccumulation.

The protection of aquatic resources requires the quantities of biocides used to be held to the minimum possible. It is hoped that the use of natural biocides and also by adopting the biological control of pests would provide an answer for a safer environment in future years.

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