EFFECT OF SOME HEAVY METALS ON THE PHYSIOLOGICAL CHANGES OF MICROALGAE

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C E R T I F I C A T E

This is to certify that this Dissertation is a bonafide record of the work done by Kum. **Elsamma Ithack** under my supervision and that no part there of has been presented before any other degree.

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PREFACE

With the large scale development of aquaculture practices around the world, there is an increasing trend to evolve noval techniques in hatchery operations and culture activities to maximise production. Mass culture of microalgae which has been experimentally carried out by plant Physiologists, Algologists and Bio-engineers as an alternate means of producing Single Cell Protein is one search innovative techniqe amenable to further development.

At present SCP is produced on a large scale by mass culturing of unicellular micro-algae such as diatoms and nannoplankters for feeding the larvae of crustaceans, molluscs and finfishes in hatcheries and has been found to be a unique factor in determining the success of the enterprise. Thus, these marine microalgae, which occupy the vital role of being primary producers in most of the food chains in natural marine ecosystem assume an equally important role outside their natural habitat also.

Living in the most dynamic of all ecosystems, these microalgae are constantly being exposed to several fluctuating environmental factors. Moreover, the aquatic ecosystem, being the most abused of all ecosystems, since it is the veritable drainage for pollutants of various grades, discharged through anthrapogenic activities, is constantly faced with the threat of containing several pollutants above a level considered safe to the system. Thus, these microalgae are more than often prone to the effects of pollutants both in the natural ecosystem and in culture systems through

contamination of the medium in which they thrive. To control the possible dangers that may be caused by transfer of pollutants along a food chain through these primary producers, it is necessary to assess the potentiality of the pollutants in bringing about appreciable changes in the physiology of these microalgae.

With this view, a study has undertaken to assess the effects of some heavy metals which are usually present in the effluents of industries, such as lead, copper and zinc on the marine microalgae Tetraselmis gracilis, Chromulina freiburgensis and the diatom Chaetoceros calcitrans which are important live feeds in hatcheries for rearing the larvae of economically important cultivable marine organisms such as crustaceans, molluscs fin-fishes and sea-cucumbers.

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INTRODUCTION

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Mariculture Importance of Micro-algae

In world aquaculture practices, the most important use of micro-algae is as live food organisms for rearing the larvae of bivalves,crustaceans and finfishes. Approximately 90% of the 12 million metric tons of aqua-culture produced animals in 1991 were reared during one or more stages using phytplankton as a feed source.

Microalgal production for feed is divided into intensive monoculture for larval stages of bivalves, shrimps and fishes and extensive culture for grow out molluscs, crustaceans, sea-cucumbers and finfishes. Favoured species of microalgae for larval feeds include species of Isochrysis, Chro-mulina, Dicrateria, Tetraselmis, Dunaliella, Chlorella, Chaetoceros, Skele-tonema and Thalassiosira. The larvae of the bivalve molluscs feed and grow best on nannoplankters especially the naked golden brown flagellates like Isochrysis, Chromulina, Pavlova and Dicrateria (Guillard 1958; Walne, 1974; Ukeles, 1975).

Algae directly or indirectly form an essential link in the food chain of fish and shell fish larvae and hence algal cultures receives importance in all hatcheries (Walne, 1970). Dicrateria, Pavlova Chaetoceros, Chromulina, Isochrysis, Tetraselmis and Chlorella are some of the phytoplankton feed species which are mass cultured in the hatcheries in pure form and they are fed individually or collectively to Rotifers, Cladocerans, Brine shrimps, Copepods, Bivalve larvae and Crustacean larvae.

Miquel (1872) and Schreiber (1927) were among the pioneers to initiate the culture of microalgae. Adequate amount of nitrate and phosphate added to the filtered seawater was used as the basic growth medium for microalgae. The components of algal culture medium (Walne, 1974) includes nutrients like nitrate, phosphate, vitamins and trace elements such as Copper, Zinc and Cobalt.

In natural aquatic ecosystem, these phytoplanktons plays a vital role as the primary producers in the basic marine food chain. The contribution of phytoflagellates to the primary productivity and the fertility of the sea has been much investigated (Raymont, 1980). Most of the investigations showed that the contribution of these phytoflagellates in terms of cell number, chlorophyll a content and carbon fixation represent sizeable proportions (50-90% of the total) irrespective of its occurance polar temporate or tropical seas.

Since the Industrial Revolution, the efforts of removing pollutants from the natural environment have not been able to keep pace with the increasing amount of waste materials and a growing population that further, aggrevates the situation. This has often resulted in the transfomation of coastal waters into sewage depots where the natural biologic balance is severly upset and in some cases totally disrupted.

The major reason for the particular sensitivity of aquatic systems to pollutants may lie in the structure of their food chains (Stumm, 1976;

1977). Compared with land systems, the relatively small biomass in aquatic environments generally occurs in a greater variety of trophic levels, whereby accumulation of Xenobiotic and poisnous substances can be enhanced. The adverse effects of waste material became acute in inland water systems, due to their traditional role as receiving bodies for effluents. In many cases harmful substances enter the food chain through primary producers and are concentrated in fish and other edible organisms particularly in nearshore areas.

Effects of Heavy Metals on Microalgae

It is well known that the ions of heavy metal are causing harmful effects to the microscopic marine life and this fact has been recognised by several workers as early as the present century. Because of their toxic properties, the role that the heavy metals play in the aquatic environment has become a subject of intense study and increasing controversy.

Heavy metals have often been termed as highly "conservative pollutants", which once added to the aquatic environment, remain there for ever and cannot be degraded into harmless substances by microbial activities as in the case of many other organic pollutants. Their occurrance in natural aquatic environment is as a result of weathering of rocks, underwater volcanic eruptions, and from a variety of human activities such as mining, processing or use of metals or substances in industries which require metals in some form.

The heavy metals which appear to be most poisonous to marine

life are Mercury, Cadmium, Silver, Nickel, Selenium, Lead, Copper,Chromium Arsenic and Zinc. They are listed here in the order of decreasing toxicity given by Ketchum et al., (1975) though this particular ranking could obviously be a matter of some debate.

According to severity of toxic action Bowen (1966) has classified heavy metals into 1) Very Toxic - effects seen at concentration below 1ppm 2) Moderately toxic - effects appear at concentration between 1ppm and 100ppm 3) Scarcely toxic - effects rarely appear except in the absence of a related essential element.

Although many metals are known to be poisonous at quite low concentrations, they are often vital as trace elements for growth and sustenance of several marine micro algae and other aquatic plants. In contrast to the non-essential trace metals such as Lead, Cadmium, Mercury, Arsenic and others, the essential metals such as Copper, Zinc, Iron and Cobalt have important biochemical functions in the aquatic organisms. They function as electron donar system or as ligends in complex enzymatic compounds. Many of these trace elements are normal constituents of marine organisms. According to Perkins (1974), the response of marine life to increasing concentration of trace elements in sea water is oligo-dynamic, ie, stimulatory at low doses and toxic at higher levels.

In the natural aquatic environment, the effect of heavy metals are first being observed in phytoplanktons as they are the primary producers in the marine food chain. Until two decades ago, interest had centred upon the lethal aspects of metal toxicity to different marine fauna and

flora. Since then, there has been an increasing awareness that the insidious build up of low-level concentrations of metals in coastal and estuarine sea areas receiving industrial effluents and sewage could be having a deliterious effect upon the growth and development of the primary producers leading to a decrease in the fertility and productivity of these regions. Further, it has been realized that the uptake of metals by the planktons provides an entry into marine food chains, the higher trophic levels of which are often used for human consumption. This has given rise to the upsurage in research into the longer term sub-lethal aspects of metal toxicity towards marine phytoplankton and the way in which metals are accumulated at the first and second trophic levels (Davies, 1979).

Investigations on heavy metal enrichment in the food chain, the concentration in organisms of various trophic levels are usually directly compared to each other, without taking the background data into consideration. The basic point of reference here is generally the water or phytoplankton. Even in unpolluted ecosystems heavy metal enrichment can takes place at each of the next highest trophic level.

The ability of natural waters to support the level of primary production expected from ambient light and nutrient conditions depends upon maintaining the available metals at a concentration between toxicity and deficiency. The relatively short life time and large surface area of phytoplankton community makes it very susceptable to changes in various concentrations and they are found to be inhibited by different heavy metals (Mandelli, 1969).

The response of a particular species of microalgae to heavy metal toxicity should by no means be regarded as a constant value, but rather as a factor subject to the influence of varying biotic and abiotic environmental conditions. Many physical and chemical factors such as temperature, oxygen content, water hardness, organic compounds, pH values, salinity and nutrient conditions may affect the toxicity of a chemical. Some biological factors like general physiologic behaviour, life cycle and life history of the organisms, species specific and individual variability can also affect the heavy metal conentrations in aquatic organisms. (Forster and Wittmann, 1979).

Since phytopankton live in a wide range of trace metal concentration it is clear that at least some species have evolved mechanisms for adaptation to grow both at high and low metal availabilities. Diatoms in metal contaminated localities tend to concentrate heavy metals from ambient waters and pass them on to higher trophic levels with deliterious effects. Adaptations of diatoms to an unfavourable metal environment include, exclusion of. heavy metals (Foster,1977), binding of the cell outside with exo-or-extra-cellular material (De Filippis end Pallaghy, 1976) altering the cell permeability to metal ions mediating a change in the metals oxidation state (Davies, 1976), detoxification by forming metal binding proteins (Stock et al., 1977) or shunting of toxic metals to metabolically intersites within the cell (Huntsman and Sunda, 1980).

Various measurements of algal response to metal toxicity include (1) Photosynthetic uptake of radio labelled carbondioxide, an indication

of the functioning of photosystem associated with chlorophyll a; (2) evolution of oxygen, a measure of rate of the Hill reaction in photosystem associated with chlorophyll b; (3) measures of relative population growth in time based on change in cell numbers, amount of chlorophyll a extracted, turbidity of a cell suspension and change in the dry weight of the culture and (4) measures of critical physiological and biochemical rates, such as synthesis of lipids, protein and nucleic acids as well as the uptake of organic and inorganic nutrients from nutrient media.

The uptake of heavy metals by aquatic plants seems to be a passive process, although one which can be influenced indirectly by metabolism. Davies (1973) showed that the kinetics of Zinc uptake by the diatom Pheodactylum tricornutum could be explained by the rapid adsorption of Zinc on to the cell membrane followed by diffusion controlling the rate of uptake and binding to proteins with the cell. Binding to protein may control the concentration in the cell because during the growth cycle, the concentration of Zinc reaches a maximum and then decreases as the amount of protein in the cell declines.

Environmental pollution by heavy metals is very often determined simply by analysing the sample by using Atomic Absorption Spectrophotometry. The metal concentration measured in organism may lie within the normal range or above it, according to the degree to pollution in the biotope. The normal range is determined by comparison with investigations conducted in areas with relatively little or no pollution.

Microalgae can be used as bio-indicators of heavy metal pollution because of their capability to accumulate and concentrate heavy metals with regard to heavy metal enrichment in the food chain, the primary producers, since they are the basic organisms represent the first stage of enrichment. When plankton algae are used as bio-indicators for metal pollution in water, it is possible that the metal concentration in the water may still be low even if the algae are highly contaminated. Algae can store heavy metals over long periods even when the metal content in the water is low, has been demonstrated for lead in Pheodactylum tricornutum (diatom) and Platymonas subcordiformis (flagellate) (Schulz - Baldes and Lewin, 1976). When compairing the metal content in the phytoplankton biomass with the metal concentration in the corresponding water, the increase in the metal concentration in the phytoplankton is not indicative of the actual metal content in the water. But depending upon their individual capacity to accumulate metals from the water, plankton algae as bio-indicators always reflect the average metal content of the water.

Micro algal culture play an important role in increasing trace metal solubilities by releasing complexing agents into the medium or on the contrary, they may enhance the incorporation of metals into particle and they foster metal sedimentation in marine environment. Each of the heavy metal either singly or in combination along with the major seasonality of environmental parameters can affect the biota as a whole and the food chain in particular.

Heavy metals such as copper and zinc which are essential for growth have toxic effects at relatively low concentrations and this can possibly inhibit productivity. Copper, Cadmium, Mercury, Lead, Zinc and possibly some other bivalent heavy metals reduce photosynthesis by causing structural damage to chloroplast (Blinn et al., 1977; Wong et al., 1979, Hollibaugh et al., 1980). Many heavy metals affect the morphology, productivity, growth and several other physiological processes of phytoplankton (Steemann Nielson and Wium Andersen 1970; Erickson, 1972; Zingmark and Miller, 1975).

One of the foremost studies to demonstrate the detrimental effect of copper to phytoplankton population was by Steemann Nielsen and Wium-Andersen, (1970) who examined the Copper tolerence of Chlorella pyrenoidesa and Nitzschia palea. Growth rate reduction with increasing copper level is one of the most prominent observations (e.g. Erickson, 1972; Jensen et al., 1976; Morel et al., 1976; Morel et al., 1978). There have also been reports of a reduction in photosynthetic rate (Steemann Nielsen et al., 1970; Overnell, 1976; Rao and Sivasubramanian, 1985) and of an increase in the rate of resipration (Rao and Sivasubramanian, 1985) as symptoms of copper toxicity.

Sunda and Guillard (1976) demonstrated that copper species that is most toxic to phytoflagellate is the cupric ion (Cu^{2+}). Sunda and Lewis (1978) suggested that growth rate in Monochrysis lutheri was negatively associated with ionic copper concentration. Canterford (1979, 1980) showed that an increase in the concentration of the chelating agent EDTA

(which complexes ionic copper) in the phytoplankton medium enhanced copper tolerance in Ditylum brightwellii.

Skeletonema costatum is a diatom that has been used extensively for copper toxicity tests. Besides centric diatoms, pennates have also been commonly used in bioassay studies. Mandelli (1969) demonstrated growth inhibition in the pennate diatom Nitzschia closterium similar to that observed for Skeletonema costatum. Toxicity of copper to Pheodactylum tricornutum was studied by Sun et al., (1990).

Lead toxicity in two marine phytoplankton are found out by Balder and Lewin (1976). Growth inhibition of chlorophycean microalgae due to lead toxicity was observed by Monahan (1976). Accumulation of Lead by Scenedesmus obliguus under non-growth condition has been observed by Fayed et al (1983). Lurden et al. (1989) studied the metal ion binding of Lead by the alga Selenastrum capricornutum. Michaels and Flegol (1990) observed the effect of Lead in marine planktonic organisms and pelagic food webs.

Passow et al. (1961) have observed that higher concentrations of Zinc affect the permeability of the plasmamembrane leading to the leakage of electrolytes. Higher concentrations of Zinc inhibit the growth of various algae (Whitton, 1970; Rana and Kumar, 1974). The kinetics of the Zinc uptake by the diatom Pheodactylum tricornutum was explained by Davies (1973) in terms of rapid absorption of metal on to cell membrane followed by diffusion controlled uptake and binding to proteins within the cell. The inhibition of carbon fixation and photosynthesis in coastal waters

as a result of Zinc uptake have also been confirmed by Davies and Sleep (1976, a,b).

Nair and Mulay (1979) has been analysed the relationship between radioactive Zinc uptake and population growth patterns of Microcystis littoralis, Chlorella vulgaris and in two species of marine chlorophycean flagellates. The tolerance level of Zinc to three algal species and Anocystis nidulans to Zinc has been detected by Jensen et al., (1974) and Shehata and Whitton (1982). The rate of environmental factors such as temperature and pH affecting the uptake of Zinc in relationship to the metabolism of Dunaliella tertiolecta has been examined by Parry and Hayword (1973).

In the Zuari estuary, Goa, Rajendran et al. (1978) had investigated the effect of metal ions such as Zinc on the photosynthesis of microplankton and nannoplankton. In Thalassiosira weissflogii, Andersen et al., (1978) found out that the Zinc ion activity in contrast to the total Zinc ions concentration was responsible for limiting the growth rate. The growth response of green alga Chlorella vulgaris, diatom, Nitzschia closterium and Navicula incerta to selected concentrations of zinc which reduced the population growth by 50% after 96 hours of exposure was estimated by Rachlin et al., (1982,1983).

Rai and Kumar (1980), Rai et al; (1981); Les and Walker (1984); Kanakavalli Susarala, (1987) and Sri Sudha (1989) studied the effect of zinc on the growth characteristics, photosynthesis and pigment composition of some phytoplankters. Michnowicz and Weaks (1984) and Harrison et al. (1984) have established the effect of pH on the toxicity of Zinc to

Selenastrum capricornutum and Chlamydomonas variabilis. Rao and Sivasubramnian (1985) had studied the effect of Zinc on the growth kinetics of marine diatoms Acnanthes haukiana; Amphora coffeaeformis; Fragilaria pinnata; Synedra tabulata, Thalassiosira fluviatilis and Triceratium dubium. Toxic effects of Copper, Zinc and lead on three species of phytoplankton Chaetoceros muelleri; Isochrysis galbana and Dicrateria inornata were studied by Ning et al; (1990).

Though some metals including heavy metals (density $>$ 5g cm^3) are needed by algae for various life processes, the physiological and metabolic requirements for heavy metals such as mercury, cadmium and lead are not properly understood (Davies, 1978; Rai et al., 1981). It could be gathered from literature that all metals with increasing concentration show inhibitory effects to microalgae leading to changes as revealed in parameters like cell number, net photosynthesis, pigment concentration and respiration rates etc.

The idea of employing algae for studying the combined effects of several metal ions is important because algae are primary producers of aquatic ecosystem and in natural waters metal ions nearly always occur in combination not in isolation leading to the phenomenon of synergism and antagonism. The complexity of phytoplankton community and the combination of many pollutants makes the toxicity studies of phytoplankton in the natural aquatic ecosystem very difficult. Because of this toxicity study of a particular heavy metal pollutant to a single species of microalgae can be possible only in the unialgal culture conditions.

The main objective of the present investigation is to find out the tolerance limit of a particular microalgae to a heavy metal or to find out the highest sublethal range of heavy metal concentrations on unialgal cultures. Secondly, to study the effect of heavy metals on the natural phytoplankton populations collected from different ecosystem such as mangroves and prawn culture fields and finally to compare the toxicity of these metals in both unialgal culture and natural phytoplankton population.

In the present investigation, the physiological response of three species of microalgae namely Tetraselmis gracilis, Chromulina freiburgensis and Chaetoceros calcitrans to three different heavy metals such as Copper, Zinc and Lead on different concentrations have been studied.

MATERIALS AND METHOD

MATERIAL AND METHODS

Microalgae selected for the study

The three species of microalgae selected for the present study are the phytoflagellates such as Tetraselmis gracilis and Chromulina freiburgensis and the diatom Chaetoceros calcitrans. Using Conway or Walne's culture medium, they are maintaining as stock cultures in the Algology Laboratory of the Institute.

According to Raymont. (1980) Tetraselmis gracilis belongs to class Prasinophyceae. The taxonomic position of Tetraselmis is as follows.

Division	-	Chlorophyta
Class	-	Prasinophyceae
Order	-	Prasinocladales
Family	-	Prasinocladaceae
Genus	-	Tetraselmis Stein
Species	-	Gracilis Kylin

Chromulina freiburgensis belongs to class Haptophyceae (Chrysophyceae)

Division	-	Chrysophyta
Class	-	Haptophyceae
Order	-	Chromulinales
Family	-	Chromulinaceae
Genus	-	Chromulina Cienkowski
Species	-	Freiburgensis Doflein

Chaetoceros calcitrans belongs to the class

Bascillariophyceae.

Division	-	Bascillariophyta
Class	-	Bascillariophyceae
Order	-	Centrales
Family	-	Chaetocereae
Genus	-	Chaetoceros Ehrenberg
Species	-	Calcitrans

Culture Media

The important culture media (Gopinathan, 1982) used for phytoplankton culture in the laboratory were

1. Schreiber's Medium (Schreiber, 1925)
2. Miquel's Medium (Miquel, 1890)
3. P.M. (Pantastico's Medium)
4. Conway or Walne's Medium (Walne, 1974)

Among these Conway or Walne's medium is used for the present experimental study. The normal constituents of Conway medium are:

Solution A - Chemicals

Potassium nitrate (KNO_3)	-	100 gm
Sodium orthophosophate (Na_2HPO_4)		20 gm
EDTA (Na)	-	45 gm
Boric acid (H_3BO_3)	-	33.4 gm
Ferric chloride ($FeCl_3$)	-	1.3 gm
manganese chloride ($MnCl_2$)	-	0.36 gm
Dist. Water	-	1 litre

Solution B - Trace Metals

Zinc chloride ($ZnCl_2$)	-	4.2 gm
Copper sulphate ($CuSo_4$)	-	4.0 gm
Cobalt chloride ($CoCl_2$)	-	4.0 gm
Ammonium molybdate	-	1.8 gm
Dist. Water	-	1 litre.

Solution C - Vitamins

Thiamine (B_1)	-	200 mg
Cyanocobalamine (B_{12})	-	10 mg

Each dissolve in 100 ml of dist. water. Added one ml of 'A' and 0.5 ml of 'B' and 0.1 ml of 'C' to 1 litre of sterilized seawater.

Culture conditions

The sea water used for the experiment is collected from the inshore waters of Cochin which is allowed to age in carbouys for few days. This aged sea water is filtered through Whatman filter paper and sterilized by boiling. This sterilized seawater is allowed to cool down. To this cooled sterilized sea water nutrients (Conway or Walne's Medium) were added.

The salinity of the seawater was maintained between 32-35 ppt; pH varied from 7.8-8.2 and dissolved oxygen content 4.5 to 5.2 ml/l. One litre capacity 'Borosil' conical flasks were used for the experiments. Duplicate samples were taken for the experimental study. The experiments were continued for 16 days.

Heavy metals

The Heavy metals, Copper, Zinc and Lead are used for the present study. Only analytical grade chemical were used.

Copper

It is taken in the form of $CuSo_4$ (Copper Sulphate). All concentrations are taken in ppm level. For the detailed experimental study copper is taken in different concentrations ranging from 5 ppm to 25 ppm. Five different concentrations of $CuSo_4$ taken, ie. 5 ppm, 10 ppm, 15 ppm and 25 ppm. For each experiment there was a control.

Zinc

The heavy metal Zinc is taken as $ZnSo_4$ (Zinc Sulphate). Five different concentrations of $ZnSo_4$ such as 30 ppm, 40 ppm, 50 ppm, 60 ppm and 70 ppm is taken for the study.

Lead

Since Lead Nitrate is ($PbNo_3$) the water soluble form of Lead, it has taken for the present study. Five different concentration of the Lead Nitrate is selected for the experiment such as, 25 ppm, 50 ppm, 75 ppm, 100 ppm and 125 ppm.

Priliminary experiments to determine the tolerance limit of microalgae to this heavy metals were carried out. The criteria for the above range finding test was mainly to study the number of cells present.

Visual changes like yellowish or pale colour of culture and clumbing of cells were also considered when observed as these changes indicated abnormal growth.

Metal iorns cause "stress" on algae. Highest effective sublethal range of metal concentrations were the choice for present investigation.

Algal cells in exponential growth phase were used for inoculation. After determining the cell concentration of the stock culture of each species, 10-20 ml of the culture was inoculated to the duplicate flasks plugged with sterlized cotton and kept in a wooden rack providing 1000 lux light. Known quantity of heavy metal stock solution were added to the medium just before inoculation. These inoculated flasks were kept under a photoperiod of 10 hrs. light and 14 hrs of darkness. The cultures were manually shaken once in twice daily to keep them in uniform suspension. The period of study for each experiment was 16 days.

Observations on cell number, primary production and chlorophyll content were carried out. Determination for growth and productivity was done in every alternate days and the analysis of Chlorophyll content once in 4 days.

Productivity Measurements

Productivity measurements were made in alternate days using Light and Dark Bottle Oxygen Technique (Gaarder and Gran, 1927). 5 ml of the culture from each flask were diluted with fresh culture medium and incubated for 3 hrs. By using Winkler's method, the oxygen content of

the bottle were determined. The oxygen values then converted into their carbon equivalents, applying a PQ of 1.25 and expressed as mgC/l/hr.

$$\text{Productivity (mgC/l/hr)} = \frac{O_2 \text{ (ml/l)} \times 0.536}{\text{PQ (1.25)} \times T}$$

Growth Estimation

For the quantitative estimation of the cells, 1 ml of the culture were taken after the thorough mixing and fixed in Lugol's iodine. The cells were counted with a calibrated heamocytometer and represented as cells x 10^4/ml.

Determination of Chlorophylls and total Carotenoids: Spectrophotometric method

Estimation of the Chlorophylls and Carotenoids were followed by the method of Parsons et al;(1984).

A known volume of the culture was filtered through a GFC Filter Paper. One to two drops of Magnesium carbonate were added to the samples while filtering to prevent acidification. The pigments were extracted by adding 10 ml of 90% acetone to each filter. The extraction was carried at low temperature for 20 hrs. The extracts were centrifuged and decanted the supernatant into a 10 cm path length Spectrophotometer cuvette and measured the extinction at the following wavelengths.

Wavelengths : 750, 664, 647, 630, 510 and 480 nm.

Calculated the amount of pigments in the sample using the revised formula of Parsons et al (1984).

For Chlorophylls.

(Ca) Chlorophyll a = 11.85 E664-1.54 E647 - 0.08 E630

(Cb) Chlorophyll b = 21.03 E647-5.43 E664 -2.66 E630

(Cc) Chlorophyll c = 24.52 E630-1.67 E664 - 7.60 E647

Where E stands for the absorbance at different wavelengths obtained above and Ca, Cb, and Cc are the amounts of Chlorophyll.

Then:

$$\mu g \text{ Chlorophyll/litre} = \frac{C \times v}{V \times 10}$$

Where v is the volume of acetone in ml, V is the volume of sample in liters and Ca, Cb, Cc are the three chlorophylls which are substituted for C.

For Carotenoids,

(Cp) Plant Carotenoids = 7.6 (E480-1.49 E510)

Where E is the absorbance at 480 and 510 nm and where Cp is substituted for C in the same equation as used above for Chlorophylls.

Two similar experiments were conducted with natural population of phytoplankton collected from two different ecosystems such as a prawn culture pond and a mangrove ecosystem. Thalassiosira, Nitzschia, Navicula Spp, Biddulphia, Fragillaria, Chlamydomonas, Scenedesmus, and Oscillotoria

were the dominated species in the ecosystems. All the above mentioned parameters were determined for comparative studies and cells were counted by using Sed-Wick Rafter chamber by the settling method.

A statistical analysis (Snedacor & Cochran 1967) also made using the computer to verify the data obtained where the three heavy metals playing any siginificant role on the growth, productivity and chlorophyll contents of the three microalgae selected for the study.

OBSERVATION AND RESULTS

OBSERVATION AND RESULTS

In the present investigation, experiments were conducted with five different concentrations of heavymetals viz; Copper, Zinc and Lead on three aquaculturly important species of microalgae such as Tetraselmis gracilis, Chromulina freiburgensis and Chaetoceros calcitrans. Observations were made on thegrowth rate, primary production and chlorophyll content for a period of 16 days. Similar experiments were conducted on natural phytoplankton population and studied all the above mentioned parameters.

EFFECT OF COPPER ON GROWTH RATE, PRIMARY PRODUCTION AND CHLOROPHYLL CONTENT OF TETRASELMIS GRACILIS

Five different concentrations of Copper (Copper Sulphate) such as 5 ppm, 10ppm, 15 ppm, 20 ppm, and 25 ppm were selected for the present study. The criteria for the selection of these concentration was based on a priliminary range finding experiment. For each experiment there was a control.

Effect on growth rate.

Growth rate of culture in all the varying concentrations of heavy metals were observed on alternate days throughout the period of study using haemocytometer.

An initial inoculum of 8×10^4 cells/ml of culture was added to control and treatments. All the concentrations showed a lag phase on

EFFECT OF COPPER ON GROWTH RATE OF TETRASELMIS GRACILIS

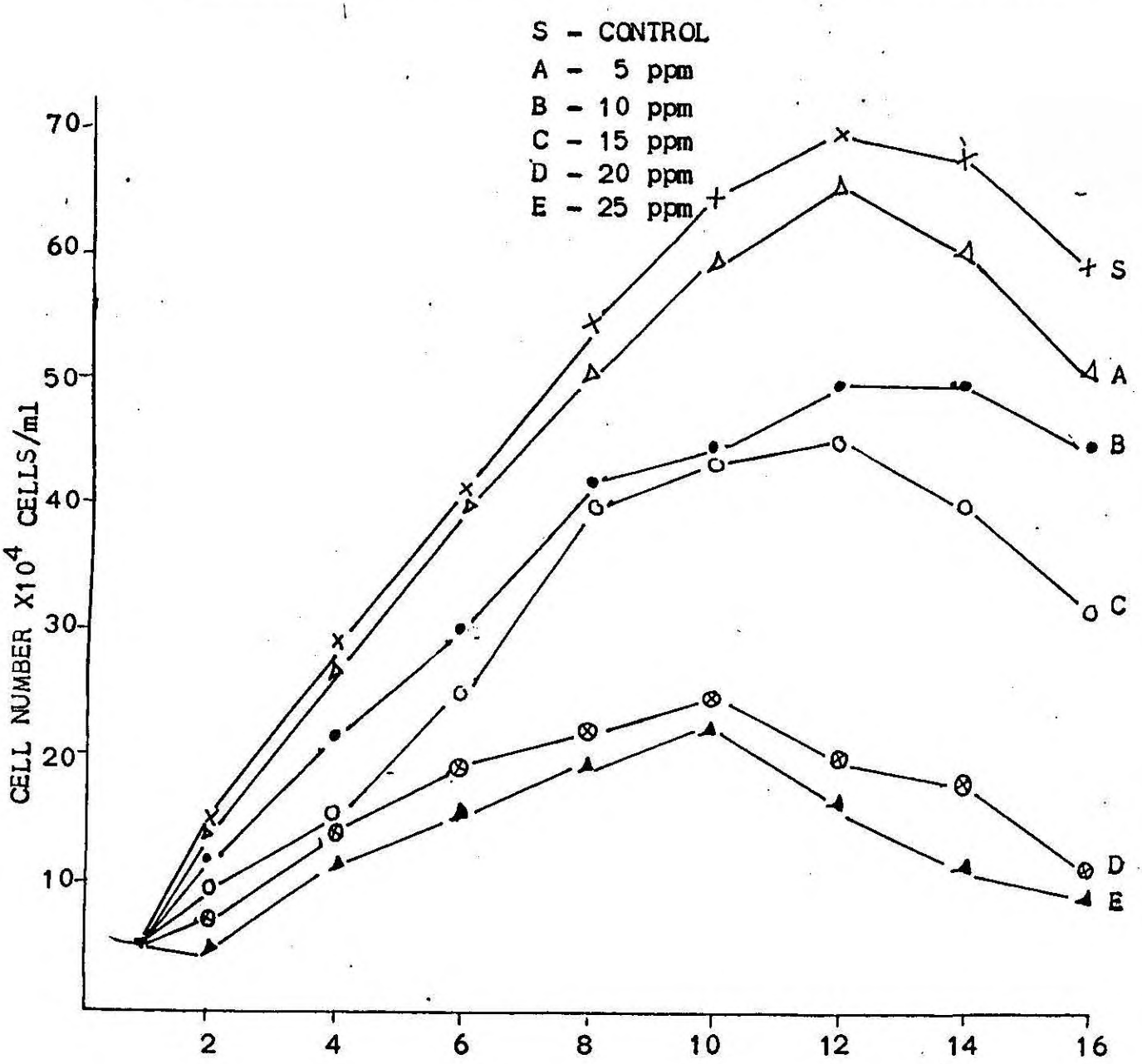

Fig. I

the 2nd day (Fig. 1). Control showed an exponential growth phase from 2nd day onwards upto 12th day and it showed a peak value of growth on the 12th day of culture (70.1 x 10^4 cells/ml). A stationary phase is observed on 14th day (68 x 10^4 cells/ml) after that growth declined.

Lowest concentration of copper (5 ppm) treated showed, a growth phase similar to that of control without much variation in cell number throughout the period of study. It revealed that 5 ppm concentration of copper have not much inhibitory effect on the cell division activity. The exponential phase showed, the maximum on the 12th day (65 x 10^4 cells/ml) after, that growth declined. On the 16th day, 5 ppm concentration showed 5% inhibition. This result explained that, the lower concentrations of copper not inhibit, the reproductive capacity of Tetraselmis gracilis.

The 10 ppm culture showed gradual growth retardation. Compared to control, cell number was less throughout the period of study. Exponential growth phase is observed from 2nd - 8th day of culture after that a stationary phase occured. Maximum numbeer of cell noted on 12th day (50.2 x 10^4 cells/ml). 27% inhibition noted on 16th day of experiment.

Growth rate in 15 ppm culture also showed similar result to that of 10ppm. Upto 8th day exponential phase and 8th-10th day stationary phases were noticed. The declining phase started fom 10th day onwards. Almost 42% of growth retardation noted on the last day of experiment.

Higher concentration of copper (20 ppm) exhibited the maximum inhibition of growth. Fourthday itself, the culture showed declining tendency and from 12th -16th day, the growth was in stationary phase. 85% inhibition of growth noted on 16th day of experiment.

Culture in the 25ppm copper revealed the toxic action of copper to arrest the dividing capacity of cell. About 90% inhibition of growth noted in this treatment on the last day of experiment. The growth declined 4th day onwards and reached the stationary phase in the last days of experiment. Maximum number of cell noted on 4th day(9×10^4 cells/ml).

Effect on primary production

Primary production in control and all the varying concentrations of heavy metals were estimated on every alternate days using Light and Dark Bottle oxygn method.

Gross production of control showed a uniform increase throughout the period of study and reached its maximum on 14th day of experiment. From 14th day onwards the total production declined (Fig.2).

Lowest concentration of copper (5 ppm) did not exhibited much effect on the photosynthetic rate of Tetraselmis. Gross and net production showed, stimulatory effect of copper on the carbon production on 10th day. On the 10th day gross and net production was 0.24 mgC/l/hr and 0.18 mgC/l/hr respectively. But after 10th day, gross and net production declined and again increased on 16th day. Respiration rate was high

EFFECT OF COPPER ON PRIMARY PRODUCTION OF TETRASELMIS GRACILIS

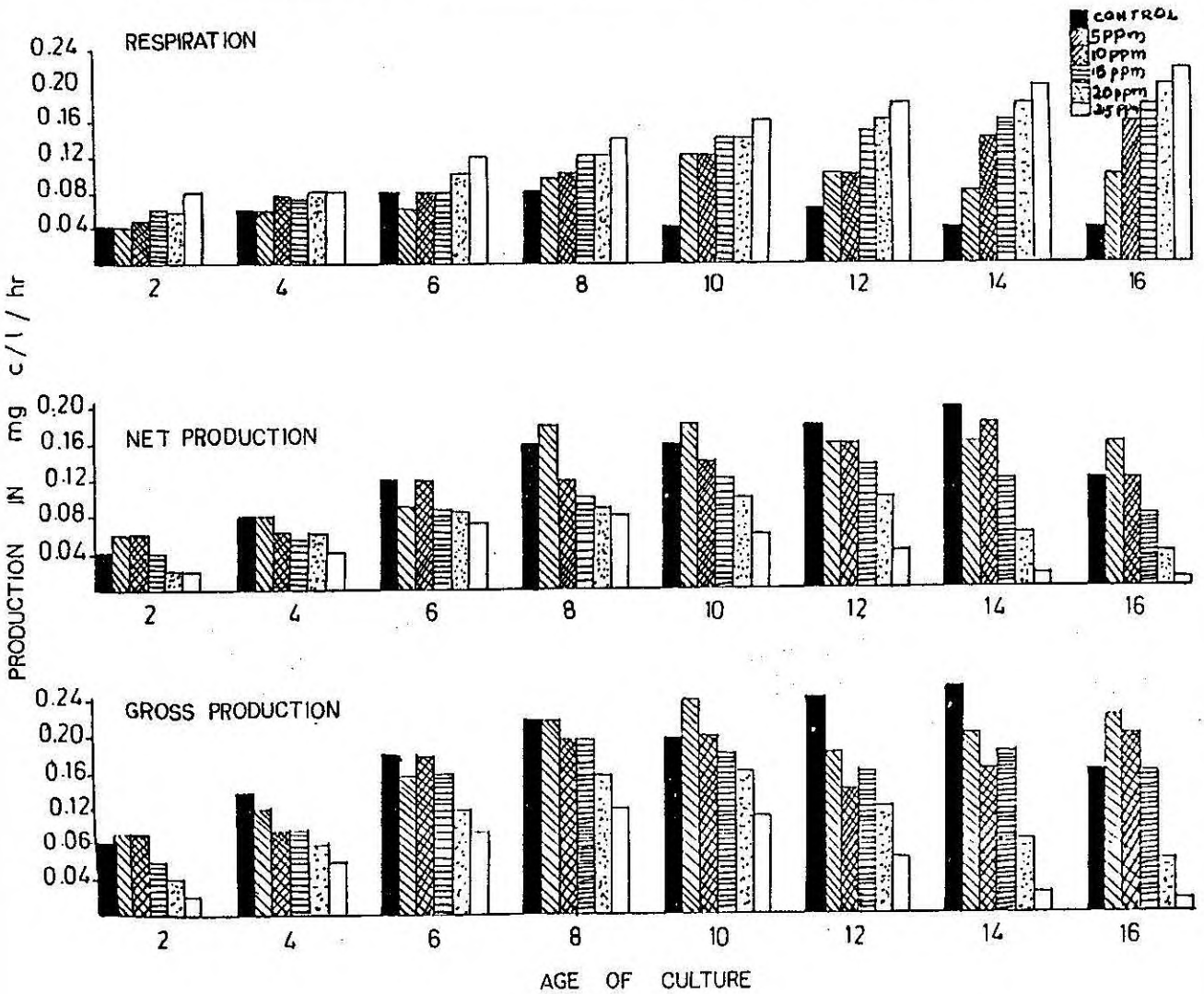

Fig. 2

on 10th and 16th day. It is cleared from this result that lower concentration of copper can stimulate the photosynthetic rate of this particular species of microalgae.

Gross and net production in the 10ppm concentration of copper was almost similar to that of control upto 10th day. But after 10th day the total production declined. Gross production was maximum on 10th day (0.20 mgC/l/hr) whereas net production showed maximum value on 14th day (0.18 mgC/l/hr) but the gross production at that day was very low, only 0.16 mgC/l/hr. The reason for this is the high respiration rate (0.14 mgC/l/hr) during that day. Throughout the period of study, the respiration rate was to be in increasing level.

Total production in the 15ppm was less compared to the above two levels of copper. Gross production was equal on 8th, 10th, 12th day of culture. But on 14th day it increased (0.18 mgC/l/hr) and again declined on 16th day. Net production was high on 12th day (0.14 mgC/l/hr) after that it declined. Reduction in the uptake of carbon was due to the high respiration rate.

Gross production was very less in the two higher concentrations of copper. In 20 ppm gross production was maximum on the 8th day and 10th day (0.16 mgC/l/hr) but the net production was maximum on the 10th day (0.10 mgC/l/hr) but after that the total production decreased considerably. Respiration rate was very high through out the period of study and reached its maximum on the 16th day (0.20 mgC/l/hr).

In the 25 ppm, almost complete inhibition of gross production noted. But the gross production showed a maximum on 8th day, after that the total production declined. On the 16th day, the gross production was only 0.01 mgC/l/hr which means 94% inhibition of gross production on the last day of experiment. The net production was also very less in the 25 ppm. It showed a peak value on 8th day after that it declined and reached a value of 0.01 mgC/l/hr. Reduction in the total primary production was due to the high respiration rate, which reached its maximum on 16th day (0.22 mgC/l/hr). It is cleared from this result that higher concentration of copper, retarded the total carbon uptake.

Effect on Chlorophyil Content

The chlorophyll a, b and c values in all the varying concentrations of copper were estimated spectrophotometrically on the 4th, 8th, 12th day, and 16th day of the growing period of the micrologal culture.

Spectrophotometric analysis of the sample for control showed gradual increase in the chlorophyll a and b content and reached its maximum on the 16th day (4420 /ug/l and 6000 /ug/l) (Fig.3).

Lowest concentration selected for the present study (5 ppm) showed an increased value of chlorophyll a (3000 /ug/l) on the 12th day, than control. But on 12th day, chlorophyll b content was equal to that of control (4800 /ug/l). Highest value for chlorophyll contents observed on 16th day. Chlorophyll a showed 5% inhibition of content, on 16th day. This result indicate that, lowest concentration of copper have not

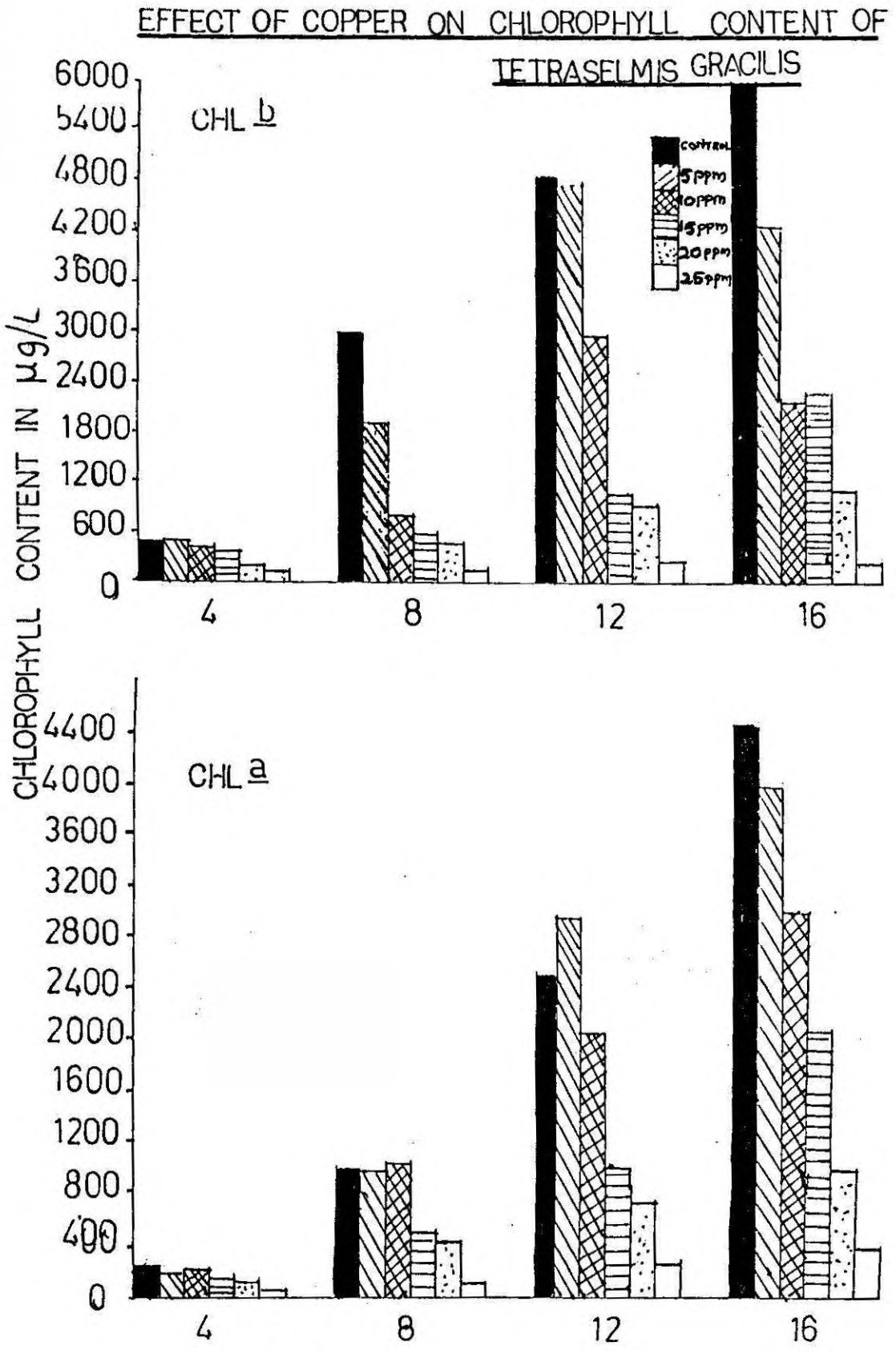
EFFECT OF COPPER ON CHLOROPHYLL CONTENT OF TETRASELMIS GRACILIS
CHL b
CHL a
CHLOROPHYLL CONTENT IN µg/L
control
5ppm
10ppm
15ppm
20ppm
25ppm
6000
5400
4800
4200
3600
3000
2400
1800
1200
600
0
4400
4000
3600
3200
2800
2400
2000
1600
1200
800
400
0
4
8
12
16
AGE OF CULTURE

Fig. 3

much inhibitory effect on the pigment content of this species of microalgae.

Chlorophyll a content of the 10 ppm copper treatment showed a maximum value on the 16th day (3000 /ug/l) but on the 12th day, the amount of chlorophyll a content was less compared to control and 5ppm concentration. The chlorophyll b content also showed an inhibitory effect on the 12th day whereas, that of 5 ppm was equal to control at that day. 12th day onwards chlorophyll b content declined (2020 /ug/l).

Analysis of these results showed that 10 ppm concentration of copper can inhibit the pigment content of Tetraselmis.

Culture in the 15 ppm copper also showed the inhibitory effect of these heavy metal on the chlorophyll content. The peak value of chlorophyll a recorded on the 16th day was 2010 /ug/l whereas that day was 4420 /ug/l, that means 55% reduction in the chlorophyll content. Chlorophyll b content was also high on 16th day (2300 /ug/l) but at that day control showed a maximum of 6000 /ug/l).

Copper showed the maximum inhibitory effect on chlorophyll content in 20 and 25 ppm. The peak value of chlorophyll a noted was on the 16th day (1000 /ug/l) but that of control was 4420 /ug/l which means 77% inhibition of pigment content occured. The chlorophyll b content of these experiment also exhibited similar result to that of 20 ppm where the peak value was observed on 16th day which showed an inhibition of 80% to that of control. Chlorophyll b in the 20 ppm showed a steady level through out the period of experiment.

In 25ppm concentration chlorophyll a and b content was very less through out the period of observation. The highest value for chlorophyll a and b recordd on 16th day (400 /ug/l and 500 /ug/l). 100% of inhibition was noted for chlorophyll a and 90% inhibition for chlorophyll b on the last day of experiment.

From these results, it is observed that, the lowest concentration (5 ppm) selected for study showed only 10% inhibition of chlorophyll content whereas that of 20 and 25 ppm showed 77 and 100% inhibition. So it is evident from the results that, higher concentration of copper can inhibit the pigment content of Tetraselmis gracilis and thus resulted the total reduction in primary production.

It is evident from the data obtained, that, at higher concentrations copper is very toxic, which can inhibit the photosynthetic rate, chlorophyll content and the growth rate. It is also evident that higher concentration can enhance the respiration, which cause the decline in total primary production. Copper can destroy the chloroplast,so that Chlorophyll contents also inhibited considerably.

PLATE I. EXPERIMENTAL SET UP

PLATE 2. EFFECT OF COPPER ON TETRASELMIS GRACILIS

EFFECT OF COPPER ON GROWTH RATE, PRIMARY PRODUCTION AND CHLOROPHYLL CONTENT OF CHROMULINA FREIBURENSIS

Varying concentrations of Copper selected for the present study was 5 ppm, 10 ppm, 15 ppm, 20 ppm and 25 pm. Selection of these concentrations was based on a priliminary range finding test. There was a control for each experiment.

Effect on growth rate

Control of the experiment indicated active exponential growth rate upto 14th day of experiment, after that cell multiplication declined. Higher number of cells noted was on 14th day (150×10^4 cells/ml).

Cell multiplication rate in 5 ppm treatment was almost equal to control upto the 8th day of culture, but after that cell number decreased (Fig. 4). From 14th day onwards a sudden retardation in cell division occured. Maximum number of cell determined was on 14th day (140×10^4 cells/ml). 14% inhibition of growth observed on final day of experiment.

Culture of Chromulina in 10 ppm treatment also expressed similar results to that of 5 ppm treatment but 14th day onwards sudden retardation of cell number obsrved. On 14th day of experiment there was only 10% inhibition of growth but on 16th day 33% reduction in growth found out.

EFFECT OF COPPER ON GROWTH RATE OF CHROMULINA FREIBURGENSIS

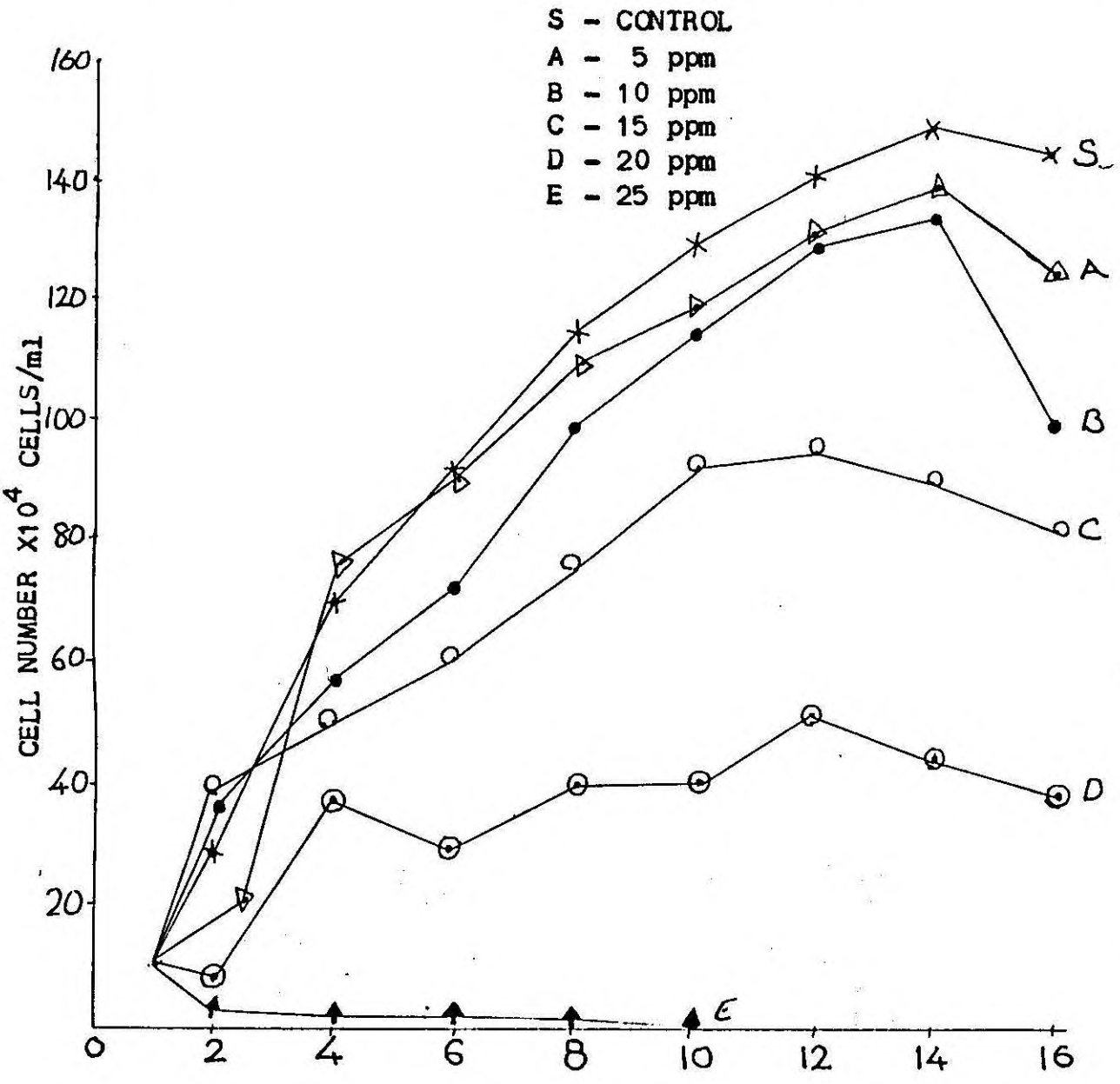

Fig. 4

Compared to control cell division rate was very less in 15 ppm treatment of Copper. From 12th day onwards death phase determined. Active growth was noted only upto 10th day of culture. Cell number of this microalga is retarded by about 45% on the last day of study period.

Growth of the algal culture in 20 ppm treatment was very much fluctuating. There was no gradual increase or decrease in the cell number but on 12th day onwards. culture in the 20 ppm treatment of copper retarded. Reason for tnis disorder of cell division may be due to the high toxic effect of copper. On 16th day, 73% of growth inhibition occured.

Higher Concentration of copper (25 ppm) much affected the cell division capacity of this microalga. On the 2nd day of experiment itself 97% of growth retardation happend. From 2nd–15th day growth was in stationery phase. Almost 100% inhibition of growth observed on 8th day of culture.

All the above mentioned factors revealed that Chromulina is the least tolerant species of microalgae to copper toxicity. On the 2nd day itself 97% of growth retardation occured in 25 ppm treatment is also indicated that lower concentrations of copper much inhibited the cell multiplication capacity of this microalga.

Effect on Primary production

Gross production of control showed gradual increase but some days it reduced due to environmental conditions. Gross production was maximum on 14th day of culture (0.30 mgC/l/hr) but on 12th day production

EFFECT OF COPPER ON THE PRODUCTIVITY OF CHROMULINA FREIBURGENSIS

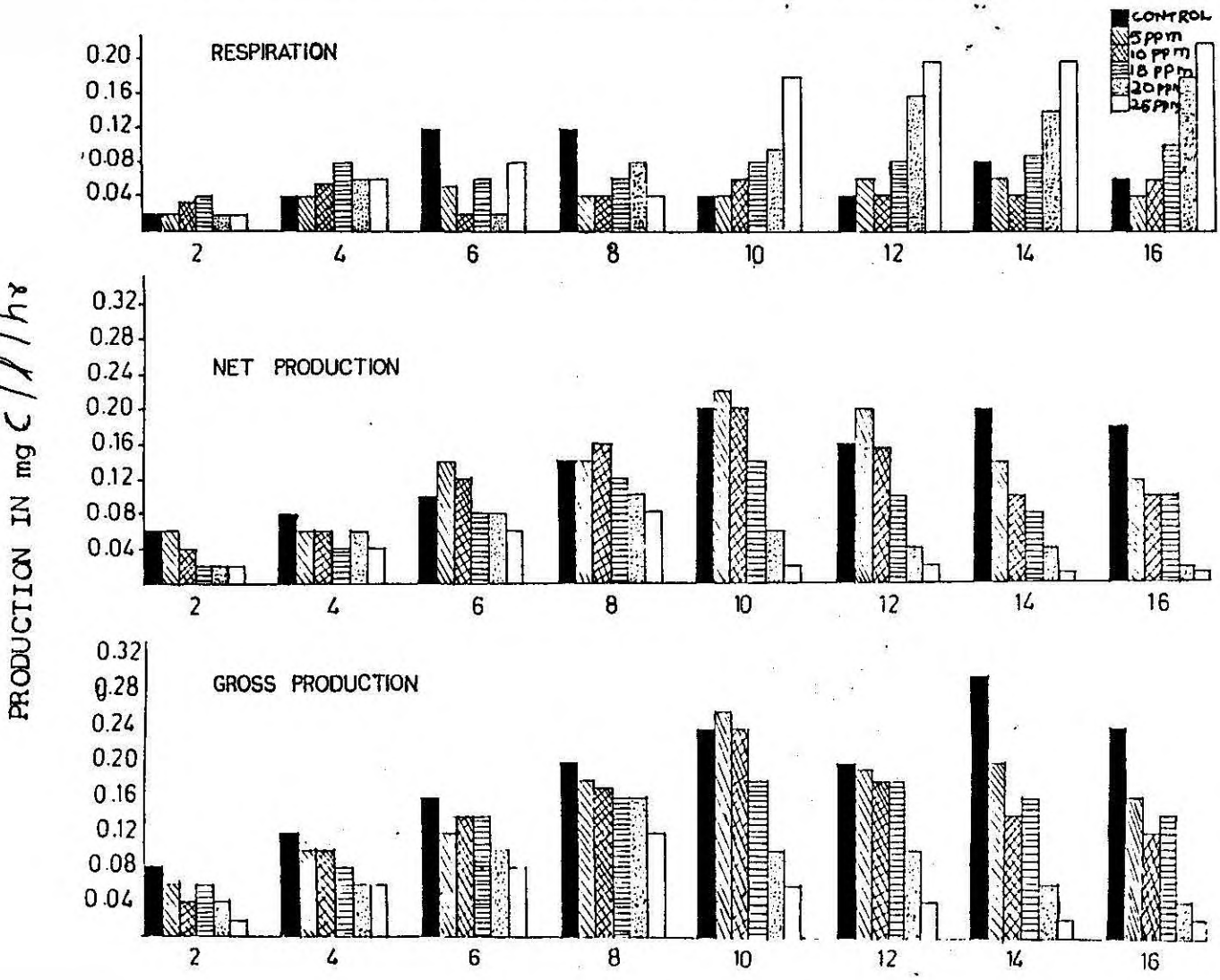

Fig. 5

was less (0.20 mgC/l/hr) . Net production was maximum on 10th and 14th day. Respiration rate showed the peak value on 6th and 8th day (0.12 mgC/l/hr) .

Primary production in 5 ppm treatment expressed less value than control, in almost all days of culture except on 10th and 12th day. (Fig. 25). There was 33% inhibition of gross production on 14th day. From 6th-12th day net production was higher than control. Respiration rate exhibited its peak value on 10th and 12th day.

Gross production inhibited by about 50% in 10 ppm treatment but net production expressed 55% of inhibition. Respiratory rate was higher than control. Reduction in the net production was because of the high respiratory action.

Culture in the 15 and 20 ppm levels revealed 41% and 83% inhibition of gross production. But net production showed 44% and 88% reduction in this two treatments which means net production very much affected by metal toxicity. When net production was less, respiratry rate expressed a very high value, so it is evident that reduction in gross and net production is due to activated respiratory mechanism.

Production of the cells in 25 ppm treatment was very much inhibited by metal toxicity. Respiratory rate was always higher than the total primary production of cells. On the last day of experiment there was 92% inhibition of gross production but net production reduced by about 94% at that day. Culture in this treatment expressed 73% enhancement

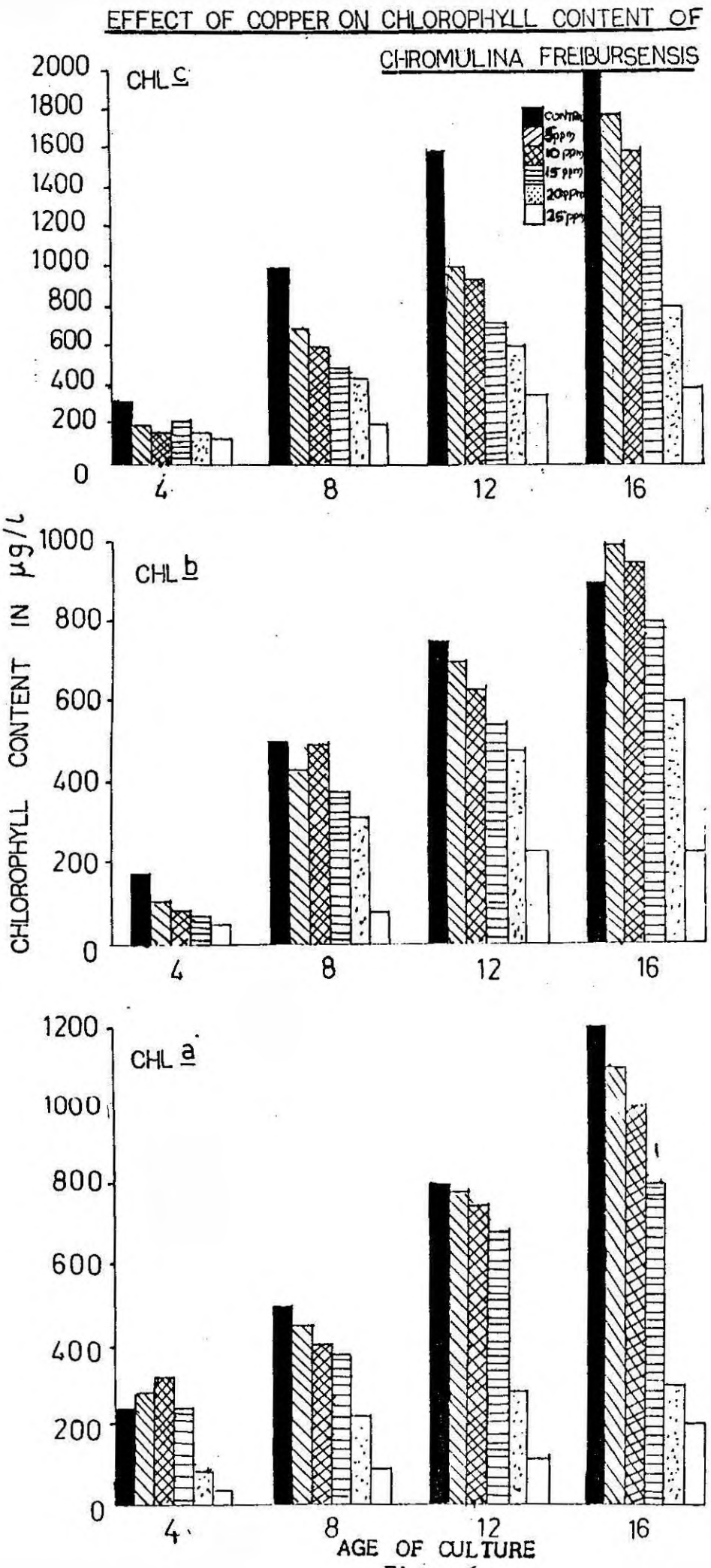
EFFECT OF COPPER ON CHLOROPHYLL CONTENT OF CHROMULINA FREIBURSENSIS
CHL c
CHL b
CHL a
CHLOROPHYLL CONTENT IN µg/l
AGE OF CULTURE
0
200
400
600
800
1000
1200
1400
1600
1800
2000
4
8
12
16

Fig. 6

of respiratory mechanism. It is determined that, respiratory value should be higher than gross and net production in all the higher doses of metal treated cultures.

The factors cleared from the above mentioned observations that as the level of heavy metal increasing total primary production will be more inhibited but the respiratory mechanism should be activated to withstand the toxic action of metal on the cells.

Effect on Chlorophyll content

Control of this experiment indicated gradual increase in Chlorophyll content, throughout the study period. Chlorophyll a. showed a peak value on 16th day (1200 µg/l). In control, amount of Chlorophyll c content was higher than the other two pigments (Fig. 6).

The 5 ppm treatment showed, less value for pigment contents than control. But compared to other treatments, it is not much affected by copper toxicity. On 4th day of culture chlorophyll a and b was greater than control but after that it expressed lower value. Chlorophyll a and c indicated 10% retardation on 16th day where as Chlorophyll c showed 10% enhancement.

Chlorophyll contents in 10 and 15 ppm concentrations was also less than control. Chlorophyll a and b revealed 20% reduction on 16th day but Chlorophyll c showed 5% increase at that day, in 10ppm treatment.

There was 75% reduction noticed for Chlorophyll a in 20 ppm treatment. Chlorophyll b and c showed 40% and 60% inhibition on 16th day of culture. It was found that Chlorophyll a is much more affected by copper, than the other two pigments.

Highest level of copper (25 ppm) selected for the study inhibited production of chlorophyll content very much. Chlorophyll a showed 80% retardation on 4th day whereas it showed 83% reduction on 16th day of culture. 75% inhibition was noticed for Chlorophyll b and 80% for Chlorophyll c on 16th day of culture.

From all the above observations it was found out that Chlorophyll a is much more affected by copper than the other two pigments. Reduction of chlorophyll value observed in all treatments may be due to the destruction of chloroplast by this heavy metal activity.

PLATE 3. EFFECT OF COPPER ON CHROMULINA FREIBURGENSIS

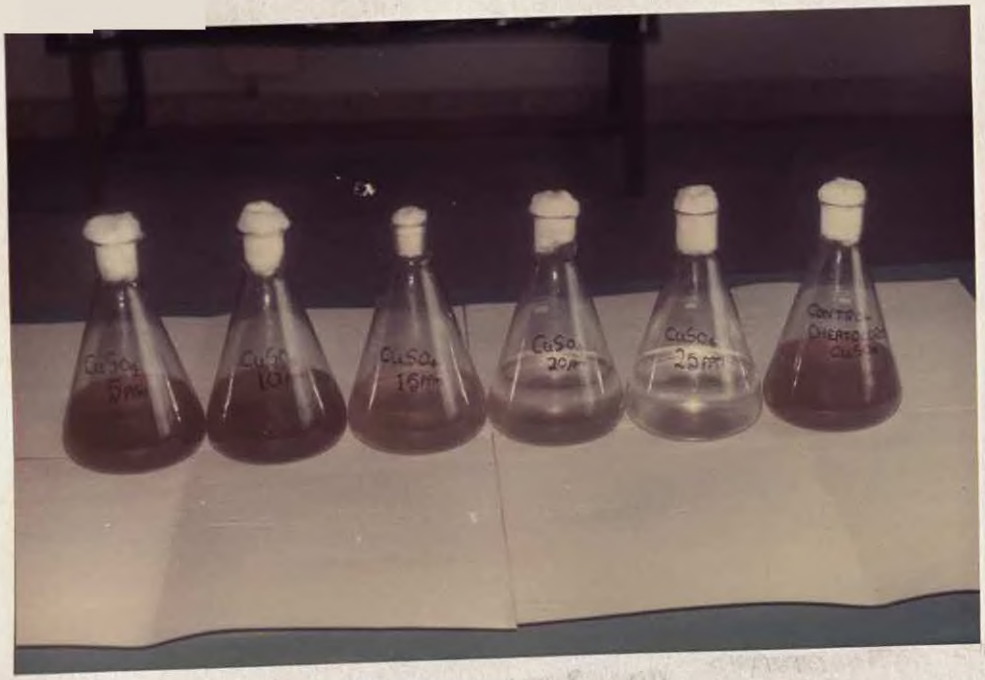

PLATE 4. EFFECT OF COPPER ON CHAETOCEROS CALCITRANS

EFFECT OF COPPER ON GROWTH RATE, PRIMARY PRODUCTION AND CHLOROPHYLL CONTENT OF CHAETOCEROS CALCITRANS.

Varying concentrations of copper in the form of Copper sulphate selected for the present study was 5 ppm, 10 ppm, 15 ppm, 20 ppm and 25 ppm. Selection of these concentrations was based on a priliminary range finding test.

Effect on growth rate

Observations on cell division made on alternate days using Haemocytometer.

Control showed an exponential growth phase from 2nd day onwards upto 16th day of culture. Maximum number of cell noted was 165.2 x 10^4 cells/ml on 16th day (Fig. 7).

The 5 ppm concentration of copper, stimulated the cell multiplication rate throughout the period of study and showed higher cell number of 180 x 10^4 cells/ml on 16th day.

Growth rate in the 10 ppm treatment was similar to control, without showing any declining tendency till the last day of study period. Cell division reached its maximum on 16th day (158.0 x 10^4 cells/ml).

The 15 ppm treatment showed exponential growth upto 8th day after that growth declined. But from 12th day onwards the growth was stationary till the last day of experiment. Maximum number of cell

EFFECT OF COPPER ON GROWTH RATE OF CHAETOCEROS CALCITRANS

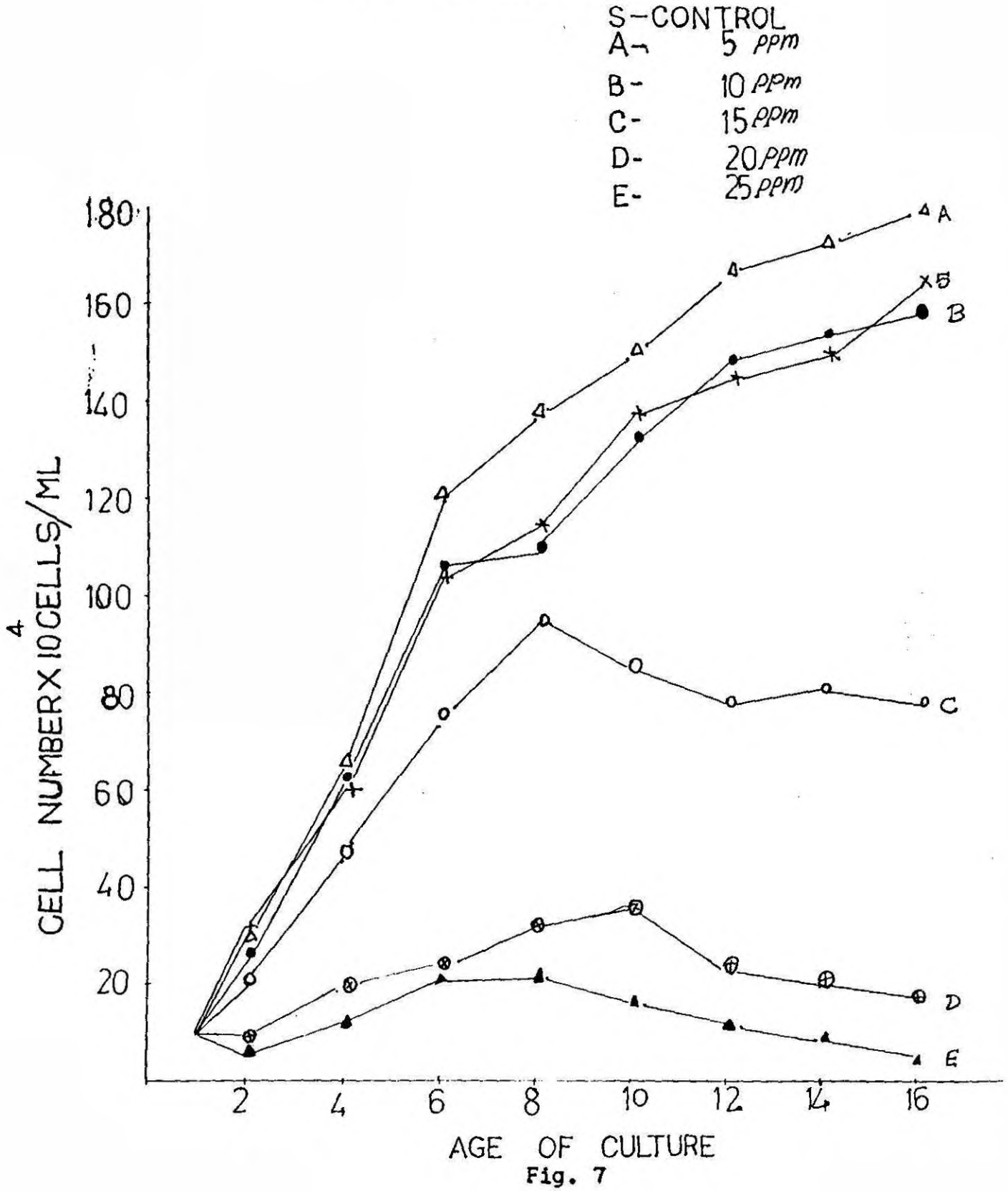

Fig. 7

was observed on 8th day (95 x 10^4 cells/ml). On 16th day, 15 ppm concentration of copper exhibited 53% inhibition of cell multiplication.

Cell division capacity of this marine diatom was much affected by 20 and 25 ppm concentrations of copper. In 20 ppm treatment active growth was there upto 10th day but in 25 ppm the culture showed declining tendency from 8th day onwards. On 16th day, 20 ppm of copper showed 88% inhibition but at the same day 25 ppm showed 96% growth retardation.

The above made observations proved that lowest concentrations of copper can stimulate the growth of this marine diatom but higher concentrations are toxic by arresting the cell multiplication process.

Effect on primary production

Primary production estimated on alternate days using Light and Dark Bottle oxygen method.

Total carbon production in control showed a gradual increase throughout the period of study. Gross and net production reached its maximum on 14th day (Fig. 8). (0.32 mgC/l/hr and 0.22 mgC/l/hr).

Primary production in the 5 ppm level of copper showed enhancement upto 10th day, after that the production decreased due to accelerated respiratory rate. Gross production showed a maximum value of 0.26mgC/l/hr on 14th day. Net production declined after 12th day. Respiration rate was going on increasing throughout the study period.

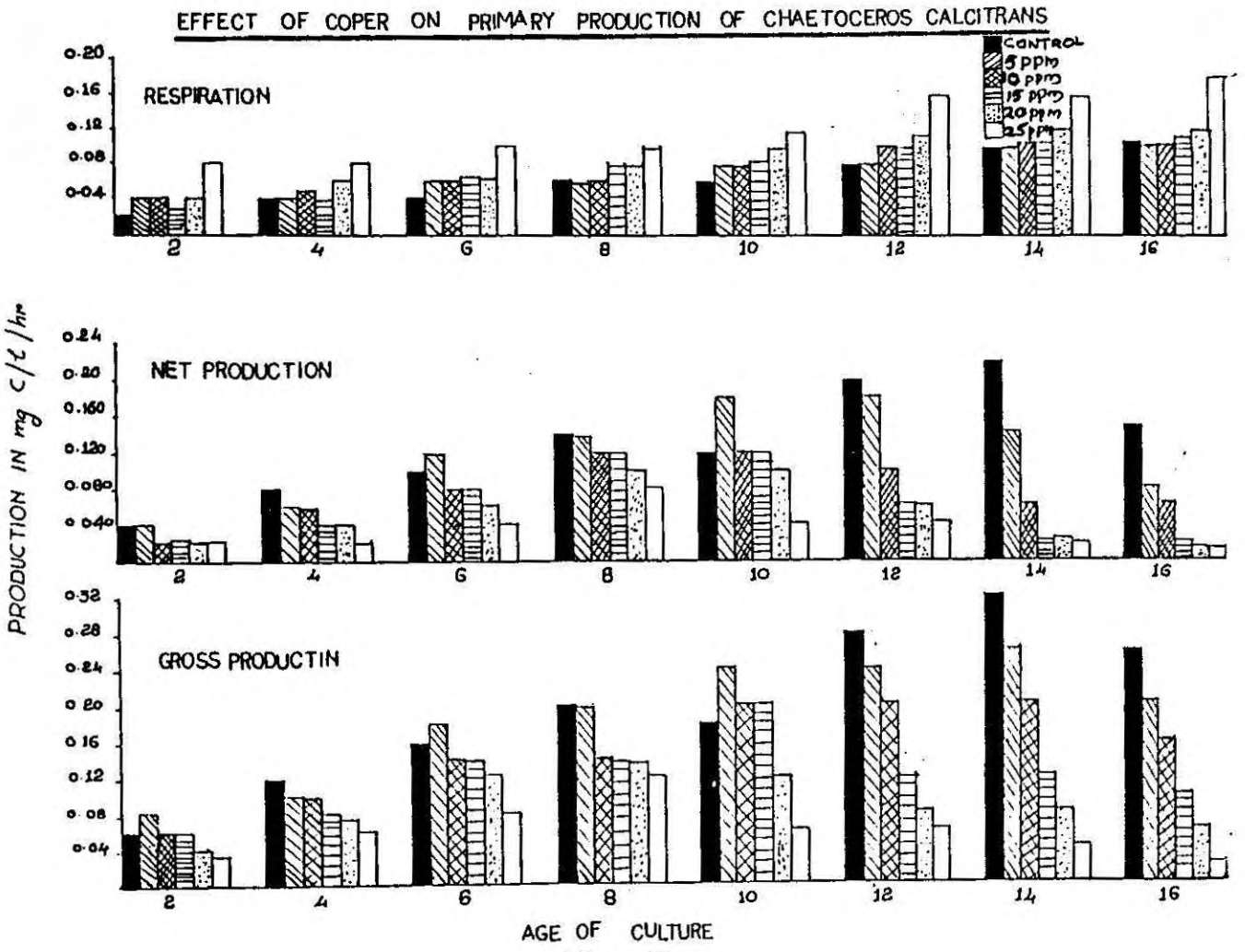
EFFECT OF COPER ON PRIMARY PRODUCTION OF CHAETOCEROS CALCITRANS
CONTROL
5 ppm
10 ppm
15 ppm
20 ppm
25 ppm
RESPIRATION
NET PRODUCTION
GROSS PRODUCTIN
PRODUCTION IN mg C/l/hr
AGE OF CULTURE

Fig. 8

Gross production in the 10 and 15 ppm level of copper was similar upto 10th day but after that gross production in 15 ppm retarded. Net production also showed similar results. 15 ppm of copper inhibited photosynthesis of this diatom by about 61%. In 15 ppm after 10th day, there was no further increase in gross production due to high respiratory rate. Both 10 and 15 ppm treatment of copper enchanced the respiratory action of this diatom.

In 20 ppm treatment, gross production retarded from 8th day onwards. Maximum production observed was on 8th day (0.130 mgC/l/hr). On 16th day, 77% inhibition of total carbon production observed. Respiratory rate was very high compared to control in all days of experiment and showed a maximum value on 14th and 16th day.

Higher concentration of copper (25 ppm) arrested the normal photosynthetic activity of this diatom throughout the period of study. 85% inhibition of total carbon production observed on 16th day of culture. Compared to control gross and net production was very low in this treatment, due to activated respiratory mechanism. Respiration rate in the treatment was enhanced throughout the period of study and reached its maximum on 16th day (0.200 mgC/l/hr).

It is found out from these observations that, lowest concentrations of copper can enhance the photosynthetic rate. But at higher levels, the production is retarded by the metal activity. It is evident from the observations that higher levels of copper is always accelerating the respiratory mechanisms of this particular marine diatom.

Effect on Chlorophyll content

Chlorophyll content is estimated on every fourth day of the experimental period using Spectrophotometric method.

Control of this experiment showed a gradual increase in Chlorophyll content and attained a peak value on 16th day. (Chlorophyll a 1050 /ug/l and b 2000 /ug/l).

Lowest concentration of copper (5 ppm) exhibited stimulatory effect on the chlorophyll content of this diatom (Fig. 9). Chlorophyll a content was higher than control in all days except on 8th day. Chlorophyll b was also stimulated throughout the period but on 16th day it declined.

In 100 ppm level of copper, the chlorophyll a content was similar to that of control upto 8th day. On 12th day the amount was greater than control but on 16th day it declined. Chlorophyll b content was higher than control upto 12th day but on 16th day Chlorophyll b content declined.

The 15 and 20 ppm of copper inhibited chlorophyll content. Throughout the period of study the amount of chlorophyll pigments was very less compared to control. Chlorophyll b content was more affected by copper than Chlorophyll a content. In 15 ppm level Chlorophyll a showed 20% inhibition whereas Chlorophyll b exhibited 60% inhibition on 16th day. Chlorophyll a content revealed 38% inhibition in 20ppm treatment but Chlorophyll b showed 70% inhibition on 16th day of experiment.

EFFECT OF COPPER ON CHLOROPHYLL CONTENT OF CHAETOCEROS CALCITRANS

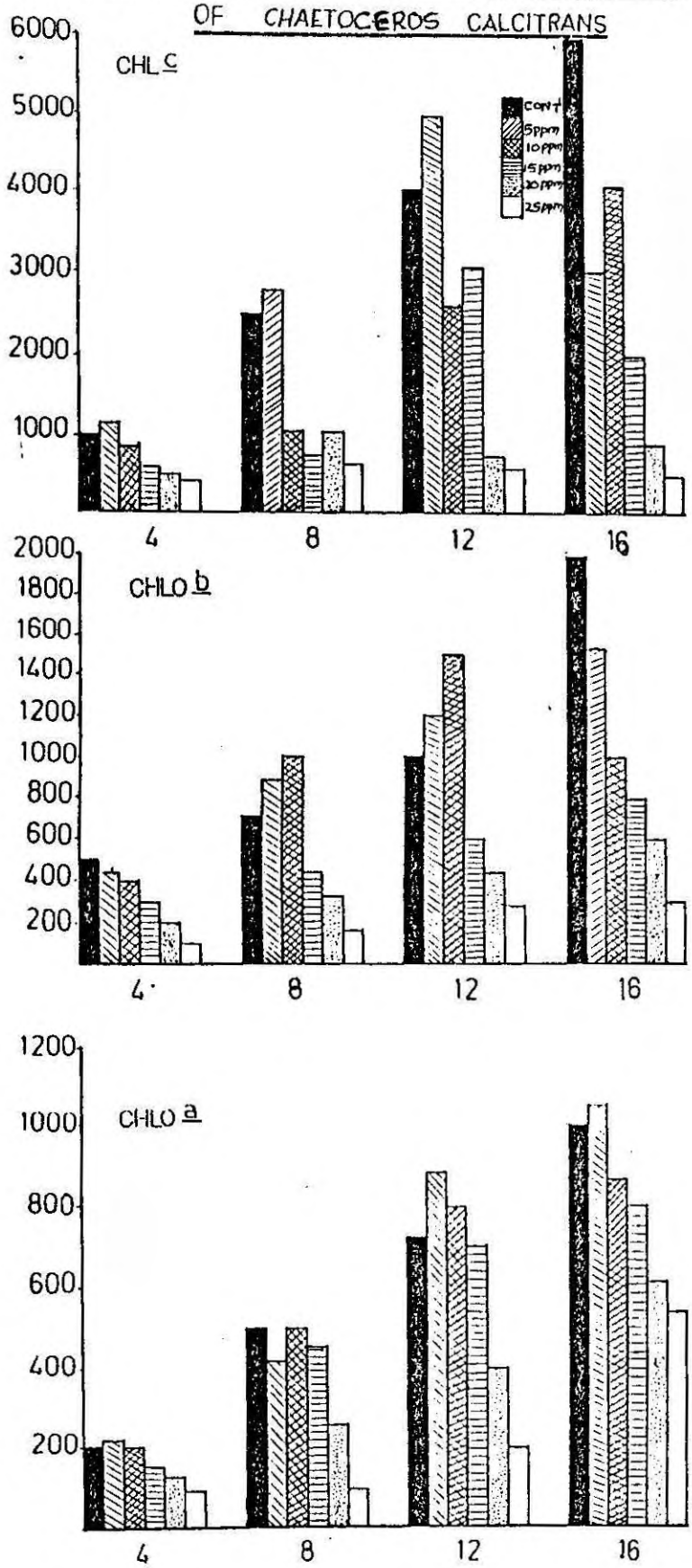

Fig. 9

Highest concentration of copper (25 ppm) inhibited the Chlorophyll content composition. Chlorophyll a content was inhibited upto 12th day but after that the same content increased (50 µg/l). But as in the above case, Chlorophyll b was very less, on 16th day Chlorophyll a content showed only 46% inhibition whereas Chlorophyll b was arrested by about 86%.

It is observed that Chlorophyll b content was much more affected by copper in Chaetoceros. But compared to other species the rate of inhibition was very less.

EFFECT OF ZINC ON GROWTH RATE, PRODUCTIVITY AND CHLOROPHYLL CONTENT OF TETRASELMIS GRACILIS

Five different concentrations of zinc sulphate such as 30 ppm, 40 ppm, 50 ppm, 60 ppm, and 70 ppm were selected for the present study. The criteria for the selection of these concentration was based on a priliminary range finding experiment. For each experiment there was a control.

Effect on Growth rate

Multiplication of cells in all the varying treatments determined on alternate days, through out the period of study.

The control showed a lag phase on 2nd day after that exponential growth phase initiated and indicated a peak value on 16th day.

In the two lower concentrations selected for study (30 and 40 ppm) cell multiplication rate was almost similar to that of control and the exponential growth phase was going on increasing till 16th day. Without showing any declining tendency (Fig.10). On 16th day, 30 and 40 ppm (65×10^4 cells/ml and 60×10^4 cells/ml) showed only 16% and 23% inhibition of cell division.

In 50 ppm treatment, the cell multiplication rate was somewhat stationary upto 8th day. On 10th day growth declined but 12th day the cell number increased and again showed declining trend. Maximum cell

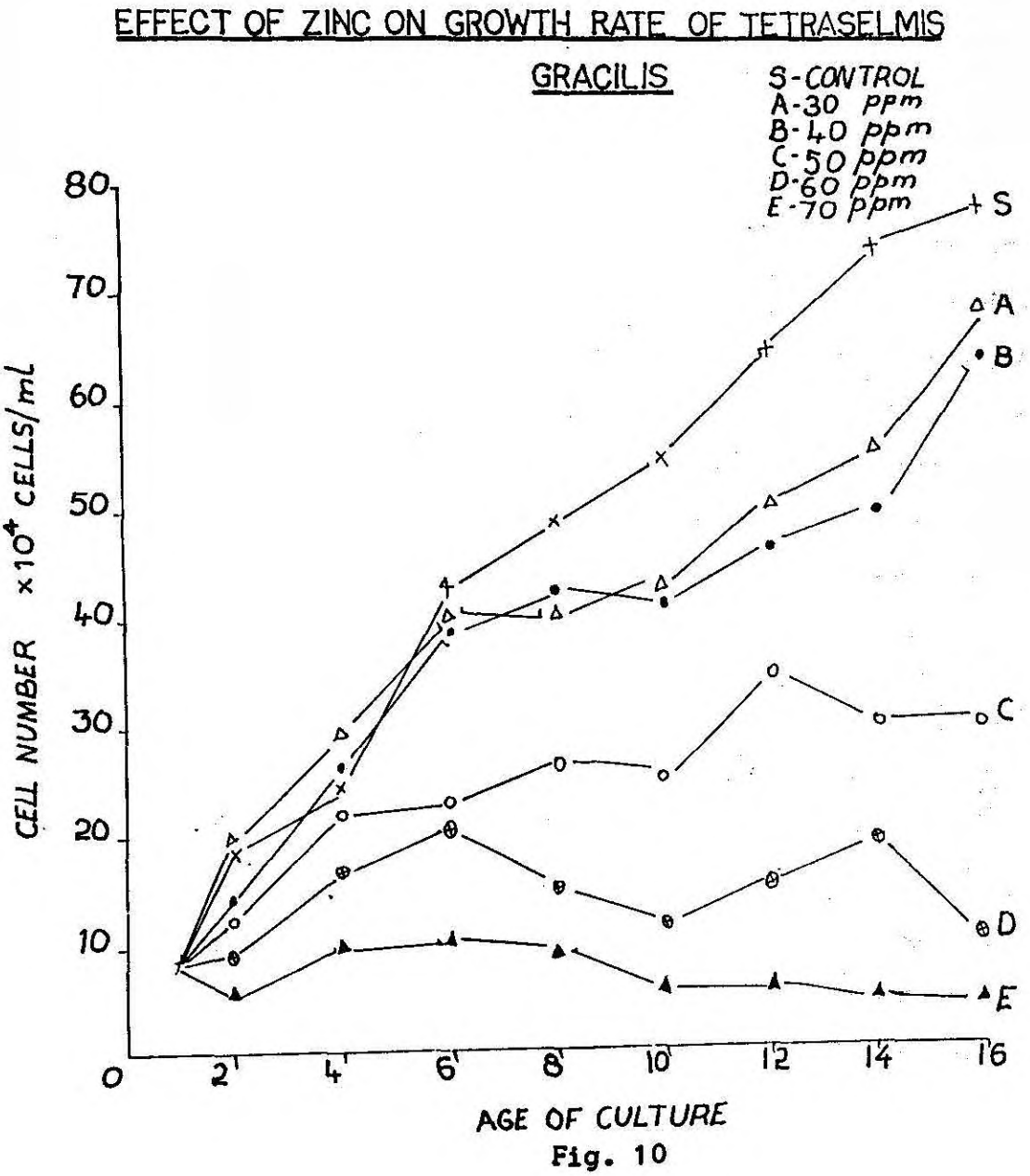
EFFECT OF ZINC ON GROWTH RATE OF TETRASELMIS GRACILIS
S-CONTROL
A-30 ppm
B-40 ppm
C-50 ppm
D-60 ppm
E-70 ppm
CELL NUMBER ×10⁴ CELLS/ml
AGE OF CULTURE
0
2
4
6
8
10
12
14
16
10
20
30
40
50
60
70
80
S
A
B
C
D
E

Fig. 10

number was observed on 12th day (35 x 10^4 cells/ml). Culture in 50 ppm treatment expressed 61% of growth inhibition on 16th day.

In the two higher concentrations (60 and 70 ppm) the rate of cell division was very less compared to control. But the highest 70 ppm also exhibited reduced growth upto the last day of experiment. In 60 ppm treatment, the cell division reduced from 6th day onwards and exhibited a stationary phase upto 12th day. But on 14th day, cell number increased and then showed a sudden retarding tendency. In 70 ppm level, division of cell retarded from 6th day onwards which exhibited a stationary phase from 10th -16th day. The maximum number of cell noted was on 6th day (10 x 10^4 cells/ml). On the last day, the growth rate was inhibited 87% and 94% in 60 and 70 ppm treatments respectively.

From the above observations, it is revealed that higher concentrations of zinc can inhibit the cell dividing capacity of this microalgae.

Effect on Primary production

Estimaton of primary production on alternate days showed, highest gross and net production for control on 14th day of experiment but the maximum value for respiration observed was on 8th day of experiment (Fig.11).

In 30 ppm treatment, gross and net production was less compared to control. Maximum value for both gross and net production observed

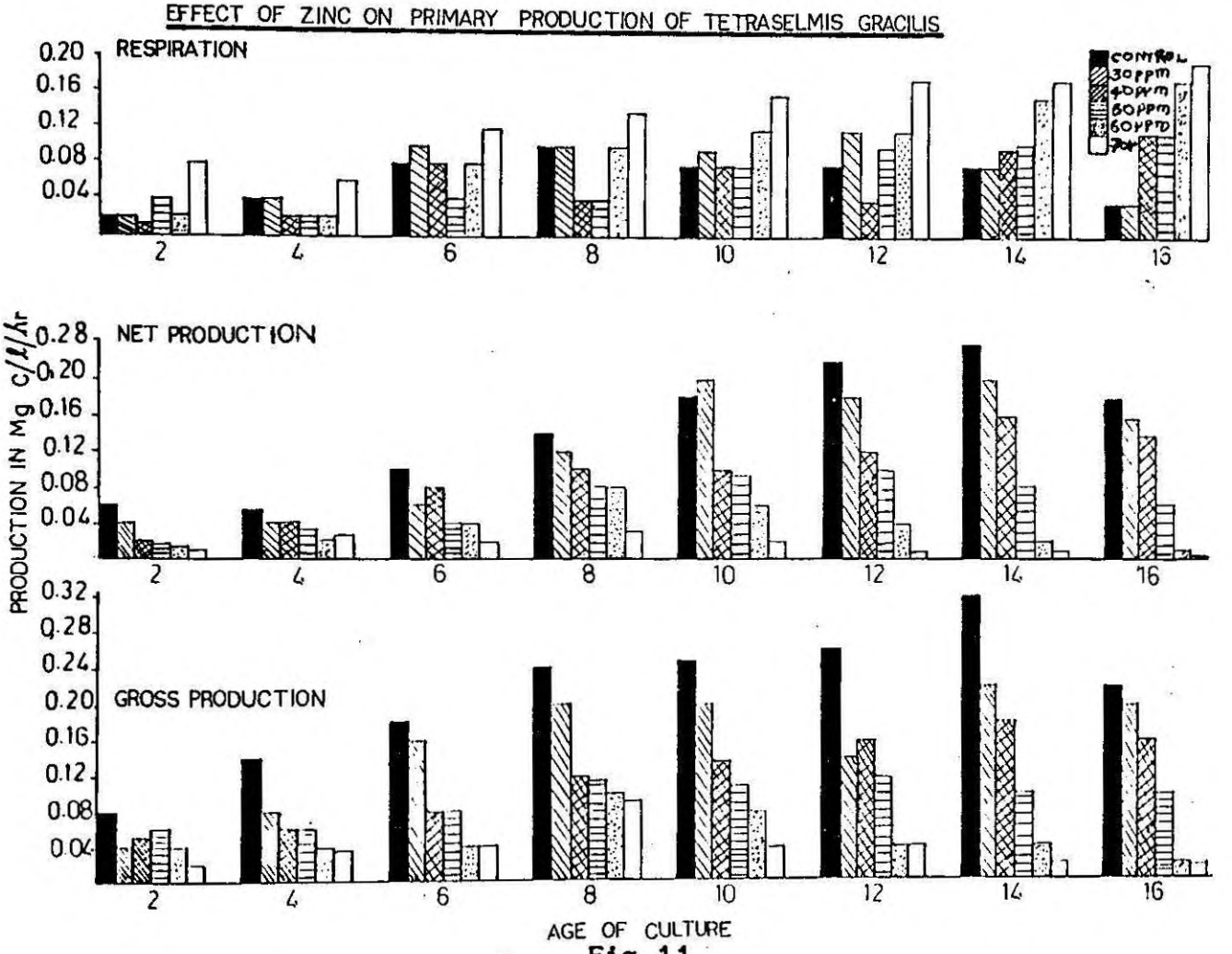
EFFECT OF ZINC ON PRIMARY PRODUCTION OF TETRASELMIS GRACILIS
RESPIRATION
NET PRODUCTION
GROSS PRODUCTION
PRODUCTION IN Mg C/L/hr
AGE OF CULTURE
CONTROL
30PPM
40PPM
50PPM
60PPM
70PPM

Fig.11

was on 10th and 14th day. (0.22 mg.Cl/hr and 0.20 mgC/l/hr). But on 12th and 14th day gross production was less compared to control, due to high respiration rate. Highest respiratory rate was observed on 12th day (0.12 mgC/l/hr).

Gross and net production in 40 ppm treatment showed a inhibitory effecct of Zinc on the primary production of this microalgal culture. Total carbon production was retarded due to the high respiratory action of algae in this treatment. Peak value for gross and net production observed was on 14th day (0.18 mgC/l/hr and 0.16 mgC/l/hr). Respiration rate showed an increasing tendency throughout the period of study.

Zinc at 50 and 60 ppm level, reduced the photosynthetic activity of Tetraselmis. In 50 ppm level gross production showed 68% inhibition of primary production on 14th day of experiment whereas the 70 ppm treatment showed 87% inhibition at that day. But in both the treatments enhancement of respiration rate was observed throughout the period of study. On 16th day 60 ppm level showed an enhanced respiration rate of 0.18 mgC/l/hr but the respiration rate of control at that day was only 0.04 mgC/l/hr.

Highest concentration of Zinc selected for the present study totally inhibited the primary production, throughout the experimental period. Compared to all other treatments, gross and net production showed very less value but at the same time the treatment exhibited an accelerated respiratory mechanism. On the last day of experiment, 91% inhibition of photosynthetic activity observed.

It is evident from all these observations that, the heavy metal Zinc can retard the total carbon production by accelerating the respiratory mechanism of the microalga Tetraselmis gracilis.

Effect on Chlorophyll content

Observations on Chlorophyll content was made on every 4th day of experimental period using spectrophotometry.

Control of the experiment showed a gradual increase in Chlorophyll content and attained a peak value on 16th day (Chlorophyll a 4600 /ug/l and Chlorophyll b 5000 /ug/l).

Lowest concentration of Zinc selected (30 ppm) showed not much inhibitory effect on the Chlorophyll content of this particular species of micro alga (Fig. 12). Chlorophyll a and b exhibited a peak value on 16th day (4600 /ug/l and 5000 /ug/l respecctively), but on 16th day Chlorophyll a content was higher than that of control. 5% increase in Chlorophyll a content observed on 16th day whereas the Chlorophyll b showed 20% inhibition at that day.

Pigment content of the 40 ppm treatment exhibited very low value compared to that of control and 30 ppm treatment. The maximum Chlorophyll a recorded on 16th day (3900 /ug/l) whereas the Chlorophyll b was maximum on 12th day (3750 /ug/l). For Chlorophyll a 11% and for Chlorophyll b 40% inhibition was observed on the last day of experiment.

EFFECT OF ZINC ON CHLOROPHYL CONTENT OF

TETRASELMIS GRACILIS

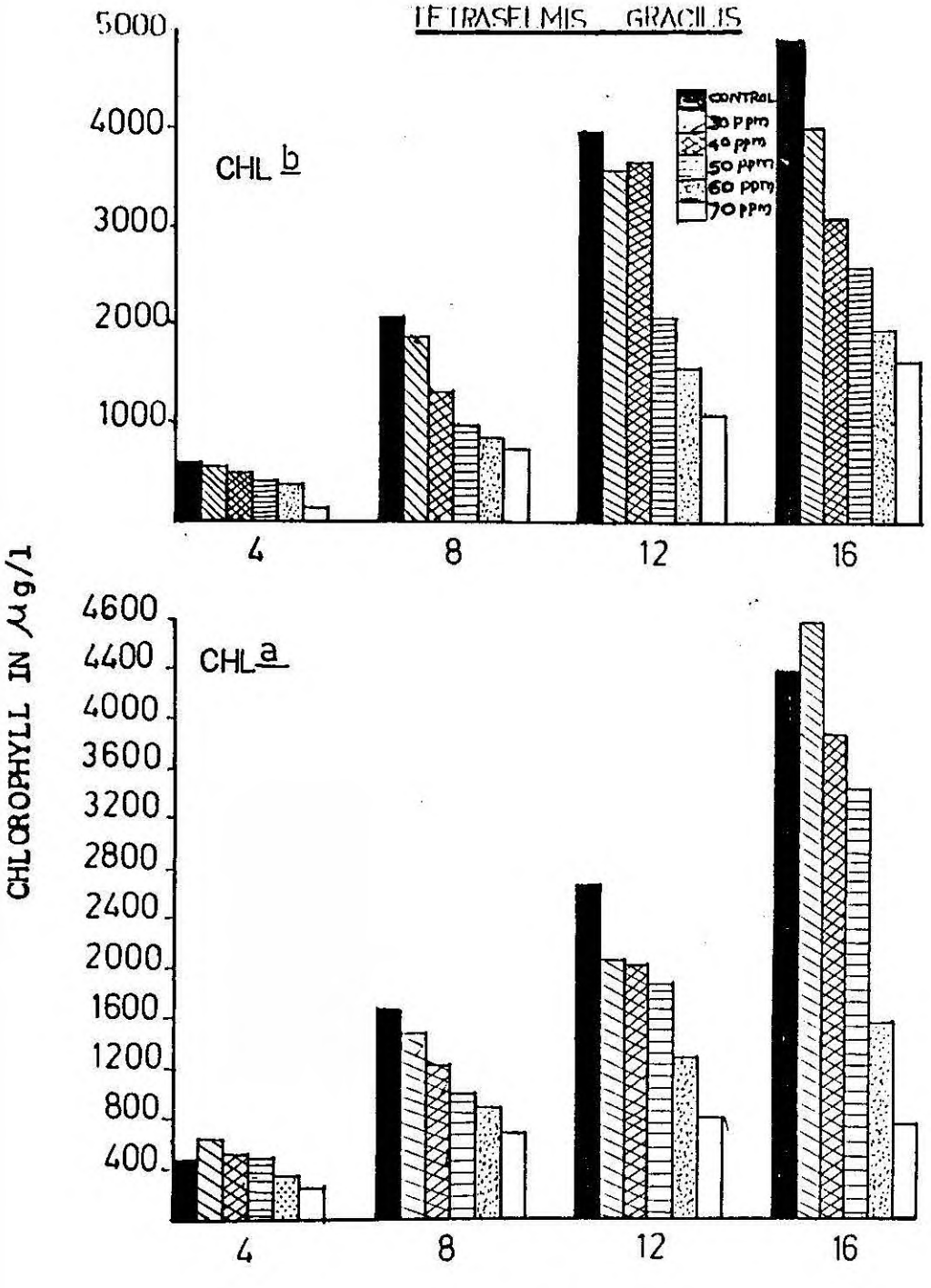

Fig. 12

Chlorophyll content in the 50 ppm treatment showed inhibitory effect due to Zinc toxicity. peak value for Chlorophyll a and b recorded on 16th day (3850 /ug/l and 2550 /ug/l). Percentage of inhibition was 21% for Chlorophyll a and 48% for Chlorophyll b.

Higher concentrations of Zinc (60 and 70 ppm) exhibited the maximum inbitory effect for the Chlorophyll pigments of this particular microalga. In 60 ppm culture, the peak value for Chlorophyll a and b was on 16th day (1600 /ug/l and 2000 /ug/l) but it did not showed much increase in value throughout the period of study. In 70 ppm treatment, Chlorophyll a content showed 83% inhibition whereas Chlorophyll b showed 69% on 16th day of experiment.

It is observed from the above data that, Chlorophyll b much inhibited by Zinc toxicity than Chlorophyll a content. It is also observed that Zinc is less toxic than Copper for Testraselmis because on 16th day, 25 ppm treatment of copper exhibited 100% inhibition of Chlorophyll content but in 70 ppm Zinc, only 83% inhibition was noted.

EFFECT OF ZINC ON GROWTH RATE, PRIMARY PRODUCTION AND CHLOROPHYLL CONTENT OF CHROMULINA FREIBURGENSIS

Five different concentrations of Zinc Sulphate selected for the present study was 30 ppm, 40 ppm, 50 ppm, 60 ppm and 70 ppm. Selection of these concentrations was based on a priliminary range finding test.

Effect on growth rate

Control of this experiment showed an exponential growth phase upto 14th day after that it entered the death phase.

Culture in the 30,40 and 50 ppm indicated active cell division upto 10th day. 10th day onwards, cell multiplication rate in all these treatments retarded which means this particular microalgae can withstand zinc toxity for 8-10 days after treatment (Fig. 13). On 16th day 37% inhibition of cell division noticed in 30 ppm whereas in 40 ppm, 40% retardation of growth determined. Culture in 50 ppm treatment exhibited 48% reduction in growth on 16th day.

When compared to control cell multiplication rate was very less in the two higher concentrations of zinc such as 60 and 70 ppm. A sudden retardation of cell number observed from 10th day onwards. About 70% inhibition of growth noticed on 60 ppm treatment but 70 ppm treatment revealed 92% of growth retardation.

PLATE 5. EFFECT OF ZINC ON TETRASELMIS GRACILIS

PLATE 6. EFFECT OF ZINC ON CHAETOCEROS CALCITRANS

EFFECT OF ZINC ON GROWTH RATE OF CHROMULINA FREIBURGENSIS

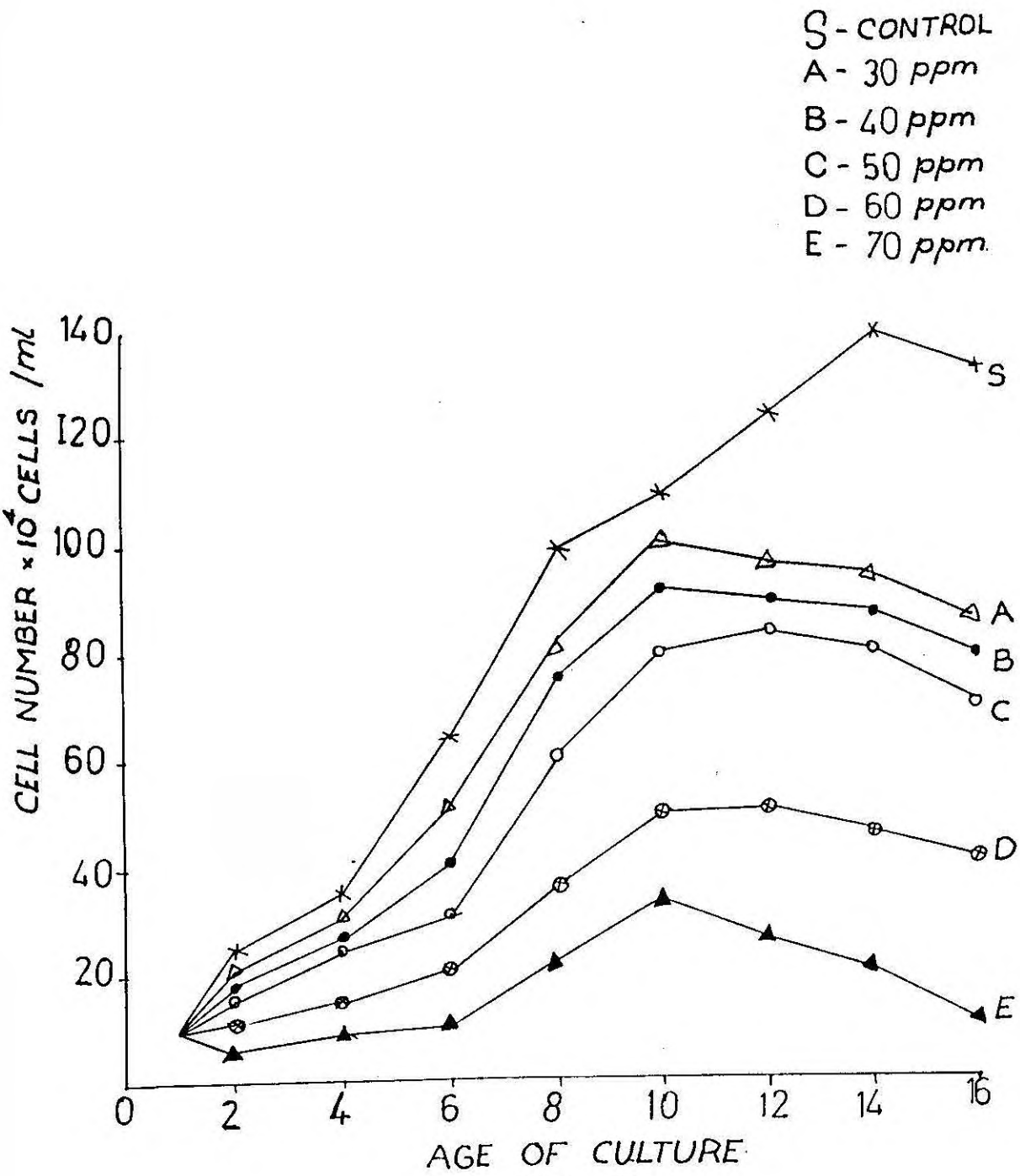

Fig. 13

All these observations revealed that both lower and higher concentrations of zinc can arrest the cell division of Chromulina. In all the 5 treatments, declining phase was started from 10th day onwards.

Effect on Primary prdoduction

Gross and net production of the control indicated a gradual increase from 2nd day onwards and reached its peak value on 12th day(0.24mgC/l/hr). Respiratory rate was going on increasing as the age of the culture increased.

In 30 ppm treatment gross production was less compared to control. 14th day onwards, total production retarded but net production showed its maximum value on 10th day after that its production declined due to the high respiratory action (Fig. 14). Respiratory mechanism was accelerated and it was always higher than the control which reached its maximum on 16th day (0.12 mg C/l/hr). This result indicated that, lowest concentration of zinc can inhibit the primary production of these species.

Primary production in 40 and 50 ppm treatment was very less compared to control and 30ppm treatment. In 40 ppm treatment gross and net production showed a peak value of 0.18 mgC/l/hr and 0.12 mg C/l/hr on 12th day. But after 12th day a sudden retardation in total production observed, due to the activated respiratory mechanism.

Gross production in 70 ppm treatment is very much retarded by zinc toxicity. Production was maximum on 8th day, after that it declined.

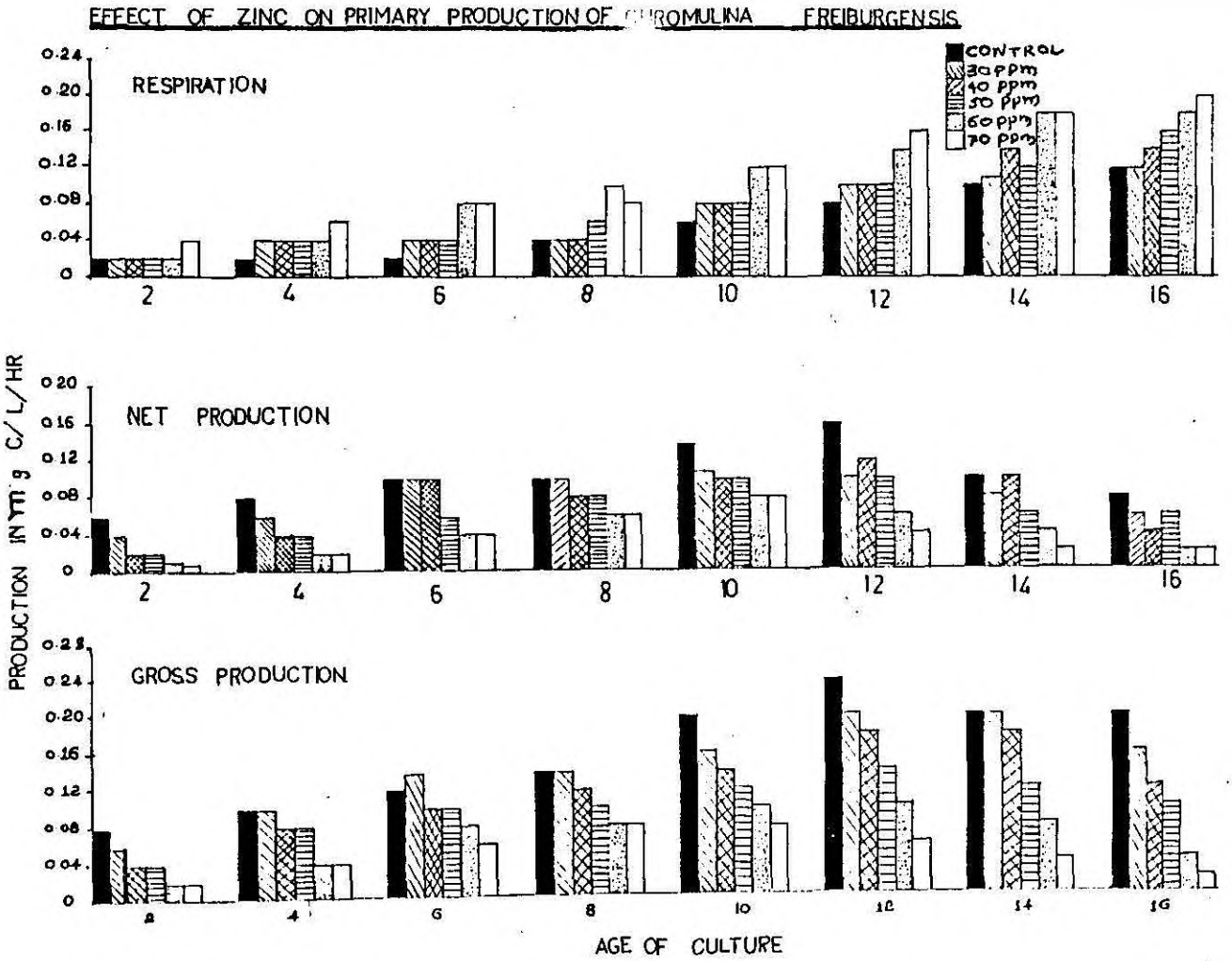
EFFECT OF ZINC ON PRIMARY PRODUCTION OF CYROMULINA FREIBURGENSIS
RESPIRATION
NET PRODUCTION
GROSS PRODUCTION
CONTROL
30 ppm
40 ppm
50 ppm
60 ppm
70 ppm
PRODUCTION IN mg C/L/HR
AGE OF CULTURE

Fig.14

Respiration rate was always higher than gross production in almost all days, in 70 ppm treatment of zinc.

Effect on Chlorophyll content

Pigment analysis was carried out in every fourth day of experiment using spectrophotometric method.

Chlorophyll content in control was gradually increasing throughout the period of study. Chlorophyll a, b and c exhibited its highest value on the last day of study (Fig. 15).

In 30 ppm treatment there was not much variation of Chlorophyll a value when compared to that of control. But Chlorophyll b expressed higher values than control upto 12th day. Chlorophyll c content was almost similar to control in this treatment. About 12% inhibition of Chlorophyll a was noted on 16th day of culture but there was only 2% retardation of Chlorophyll b on that day.

When compared to control the amount of total chlorophyll content was less in 40 and 50 ppm treatment of zinc sulphate. However, the Chlorophyll a in 40 ppm revealed 14% reduction on 16th day but at that day in 50 ppm treatment, 35% inhibition noted.

Highest concentrations of ZnSo4 (60 and 70 ppm) indicated maximum inhibition of chlorophyll pigments. 50% reduction of Chlorophyll a occured in 60 ppm treatment of zinc. Whereas, 57% was determined in 70 ppm

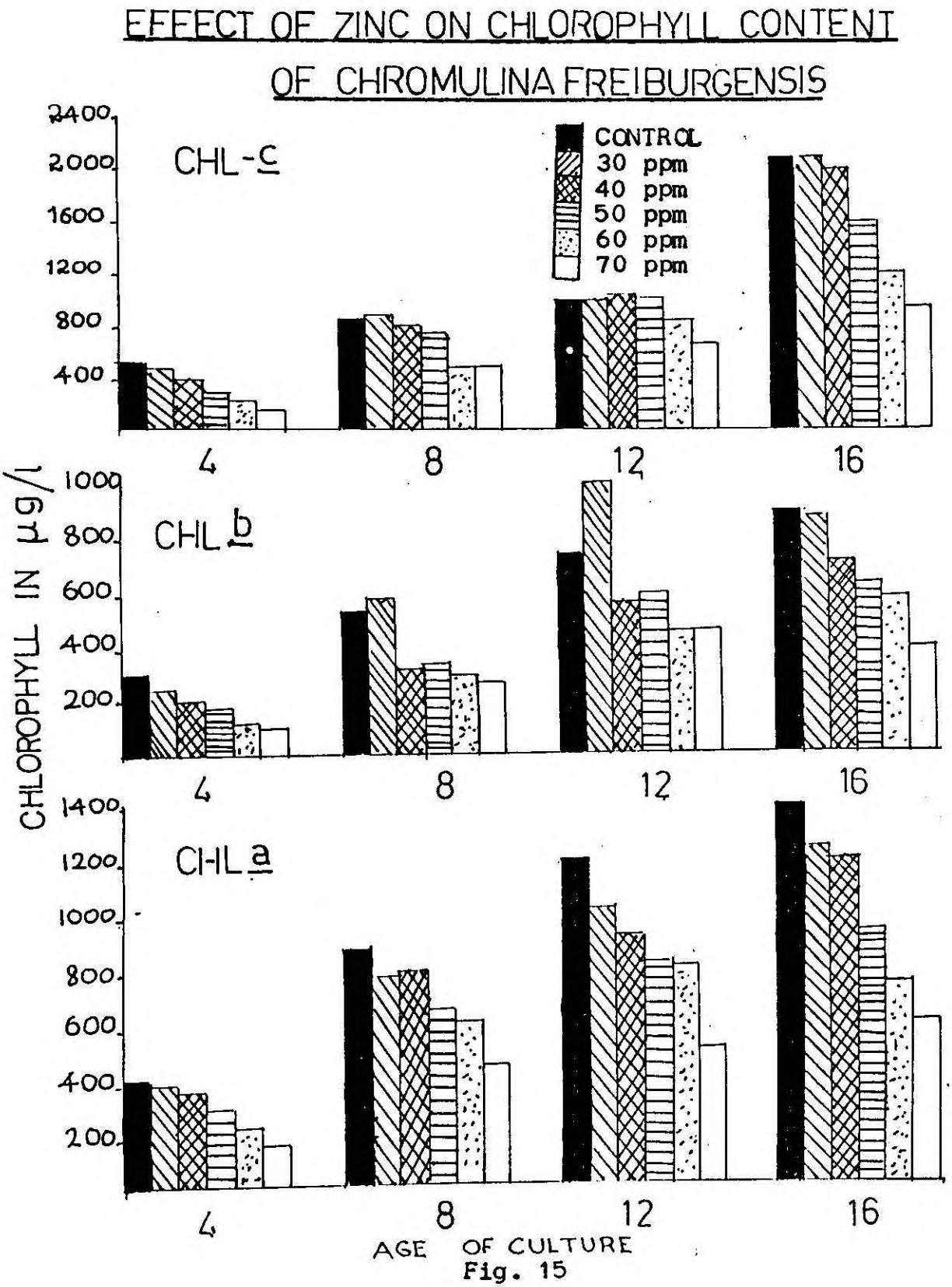
EFFECT OF ZINC ON CHLOROPHYLL CONTENT
OF CHROMULINA FREIBURGENSIS
CHL-c
CHL b
CHL a
CONTROL
30 ppm
40 ppm
50 ppm
60 ppm
70 ppm
2400
2000
1600
1200
800
400
1000
600
200
1400
4
8
12
16
CHLOROPHYLL IN µg/l
AGE OF CULTURE

Fig. 15

concentration. In 70 ppm, Chlorophyll b showed 55% inhibition but chlorophyll c expressed 56% inhibition on 16th day.

It is cleared from this observations that Chlorophyll a content is more affected by zinc toxicity than chlorophyll b and c in the particular microalga, Chromulina. It is also evident that, rate of inhibition depends on dose of heavy metal and duration of treatment.

EFFECT OF ZINC ON GROWTH RATE, PRIMARY PRODUCTION AND CHLOROPHYLL CONTENT OF CHAETOCEROS CALCITRANS

Varying concentrations of Zinc (as Zinc Sulphate) selected for the present investigation was 30 ppm, 40 ppm, 50 ppm, 60 ppm and 70 ppm. Selection of these levels were based on a priliminary range finding test.

Effect on growth rate

Growth rate was calculated on alternate days using Heamocytometer.

Control of the experiment showed an exponential growth phase from 2nd day onwards upto 16th day. Peak value of growth was noted on the last day of study period (160×10^4 cells/ml).

Lowest concentration of Zinc (30 ppm) selected for the study, did not much affected the cell multiplication rate of this marine diatom. In 30 ppm level, exponential growth phase observed upto the last day of study without showing any declining tendency. On 16th day culture in the 30 ppm level showed 21% growth inhibition.

Culture in the 40 ppm level of Zinc also exhibited exponential growth till the last day of experiment without any declining phase, but

EFFECT OF ZINC ON GROWTH RATE OF CHAETOCEROS CALCITRANS

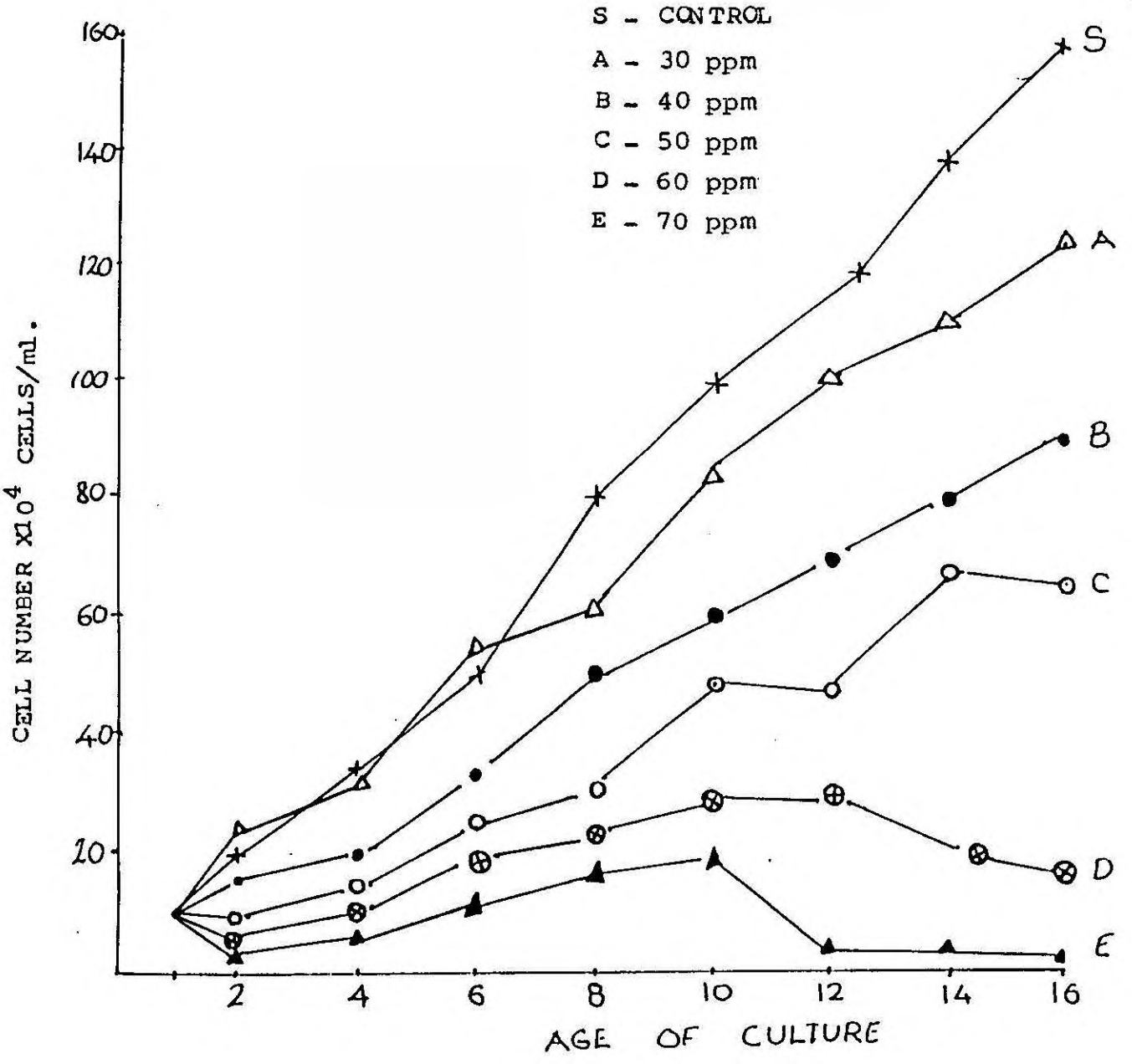

Fig. 16

the number of cells, compared to control was less (Fig. 16). On the last day of study it indicated 43% inhibition of growth. Maximum number of cell noted was on 16th day (90 x 10^4 cells/ml).

Culture in the 50 ppm level of Zinc showed active cell multiplication upto 10th day but on 10th - 12th day it expressed stationary growth. The cell division again increased on 14th day and then retarded. On 16th day, the rate of inhibition was 59% compared to control.

Cell multiplication capacity of this marine diatom retarted from 12th day onwards in 60 ppm concentration of Zinc. On the last day of experiment, growth revealed 90% of retardation.

Cell multiplication rate in the highest concentration rate of Zinc (70 ppm) inhibited from 10th day onwards. But growth was there till the final day of experiment. Growth rate showed 95% inhibition on 16th day of study.

Observations of the effect of Zinc on the cell multiplication of these marine diatom revealed that, it can grow upto 40 ppm of Zinc, without much alternation in the cell dividing capacity. The reason for this may be the rigid silicated cell wall of this diatom. In higher concentrations growth is inhibited by the metal activity, but compared to other micralgal species resistance of this diatom to Zinc toxicity was more.

Effect on Primary Production

Primary production estimated on alternate days using Light and Dark Bottle Oxygen method.

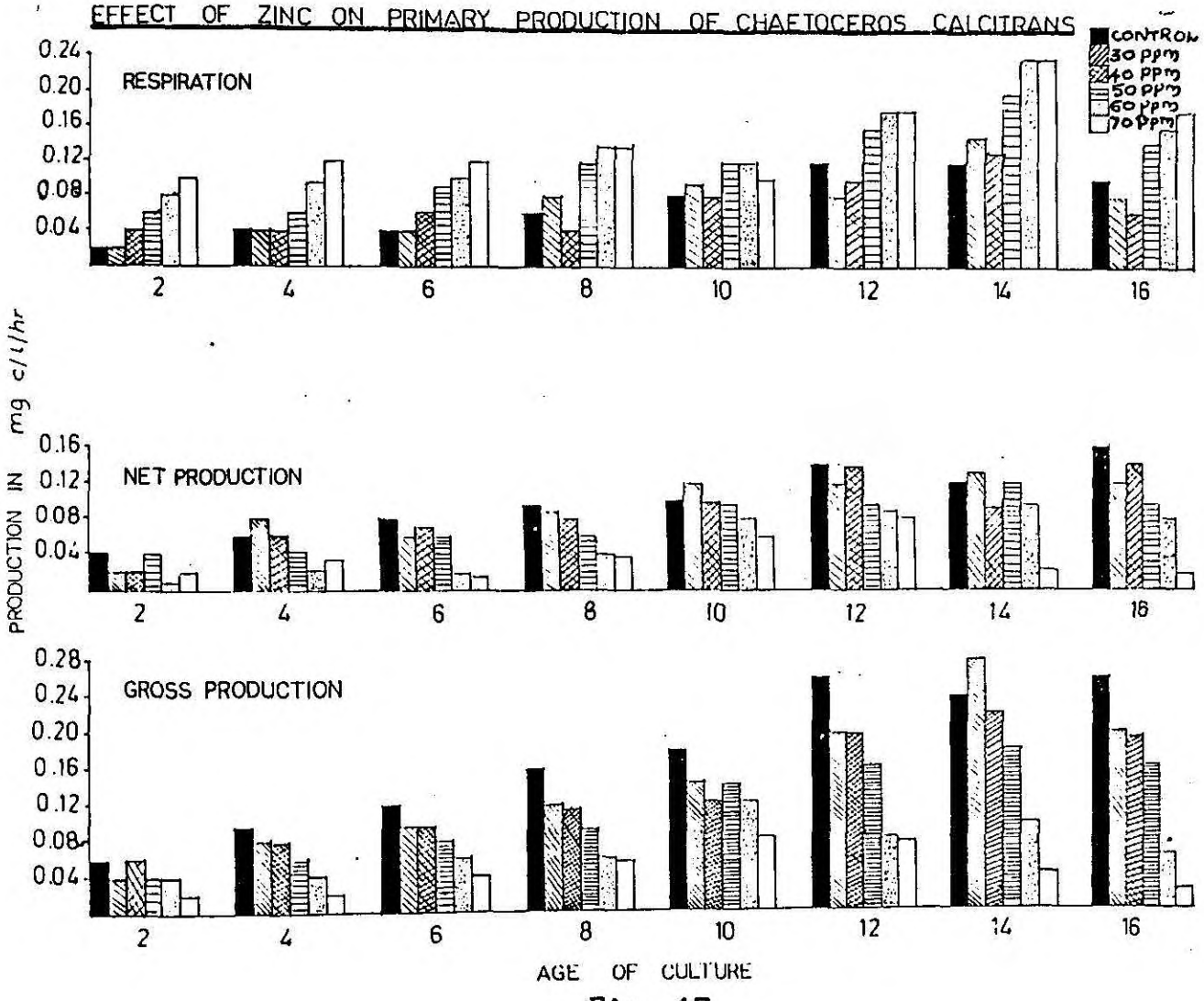
EFFECT OF ZINC ON PRIMARY PRODUCTION OF CHAETOCEROS CALCITRANS
CONTROL
30 ppm
40 ppm
50 ppm
60 ppm
70 ppm
RESPIRATION
NET PRODUCTION
GROSS PRODUCTION
PRODUCTION IN mg c/l/hr
AGE OF CULTURE

Fig. 17

For control, the peak value of gross production observed on 12th and 16th day whereas the highest net production was on 16th day of experiment. The maximum rate of respiration expressed was on 12th and 14th day (0.12 mgC/l/hr.) of culture.

Culture in the 30 ppm of Zinc revealed an increase in the highest gross and net production (0.28 mgC/l/hr and 0.144 mgC/l/hr) on 14th day which means, lower levels of the heavy metal can enhance the primary production on a certain level. But highest value of respiration also recorded on 14th day (Fig. 17), that means, reduction in the net production on these days was due to the acceleration in the respiration rate.

Compared to control, primary production in the 40 ppm level of Zinc was less. It showed a highest gross production on 14th day (0.22 mgC/l/hr) but the net production was less due to the high respiratory rate.

In the 50 ppm concentration, the culture showed a maximum gross production on 14th day after that production declined, where as the net production expressed its maximum on 14th day. Respiration rate was very high on 14th day.

Primary production of the cultures in the 60 and 70 ppm levels of Zinc revealed that higher concentration of the heavy metal can inhibit the photosynthetic activity. Gross and net production in these treatments was very less compared to the production in control. In 70 ppm treatment, from 4th to 8th day the gross production was stationery (0.06 mgC/l/hr)

EFFECT OF ZINC ON CHLOROPHYLL CONTENT OF CHAETOCEROS CALCITRANS

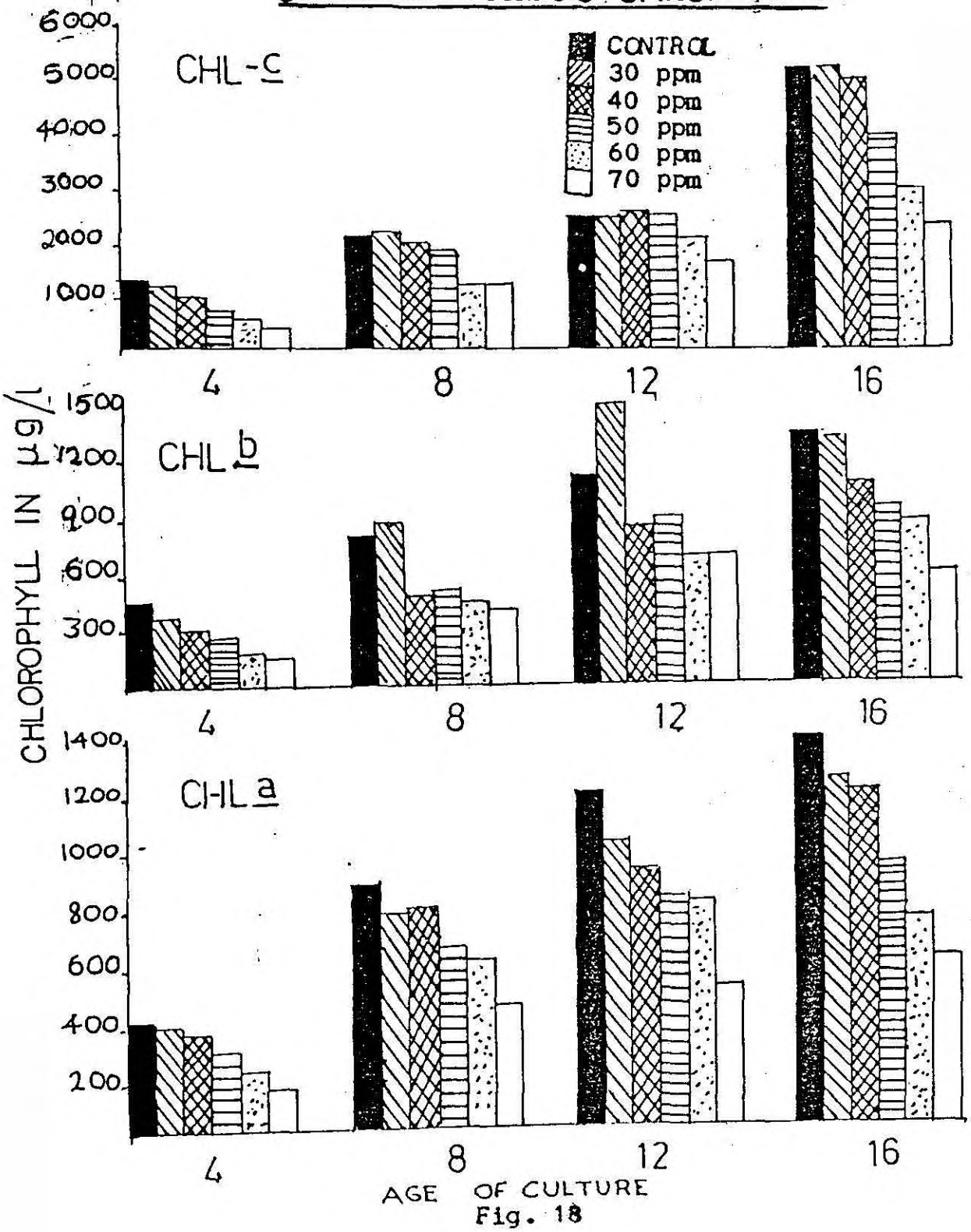

Fig. 18

and showed the maximum value on 10th day (0.12 mgC/l/hr) after that the production declined. In both of these treatments, the respiration rate was very high compared to control. Respiration rate exhibited its peak value on 12th and 14th day. In 70 ppm concentration, on 14th and 16th day net production was very less (0.02 mgC/l/hr) which was due to the increase in the respiratory process. So it is evident from the result that, higher concentration of Zinc can enhance the respiration rate of the marine diatom Chaetoceros calcitrans.

Effect on Chlorophyll content

Observation on Chlorophyll content made on every fourth day of experiment using Spectrophotometric method.

Control of this experiment showed gradual increase in Chlorophyll contents throughout the study period and reached its peak on 16th day (Fig.18). Value noted for Chlorophyll a 1400.20 µg/l, Chlorophyll b 1600.00 µg/l and Chlorophyll c 6000.28 µg/l, on 16th day.

In the lower concentration of Zinc (30 ppm) chlorophyll content was less compared to control, but upto the last day the pigment content was going on increasing without any retardation, which means, it is not much affected by metal toxicity. On the last day only 6% inhibition of Chlorophyll a was observed but Chlorophyll b showed 22% inhibition. Chlorophyll c expressed the maximum value of inhibition 32% on 16th

day, which revealed that Chlorophyll c content is more affected than other two contents of Chlorophyll.

Chlorophyll content in the 40 and 50 ppm was also less than control. In 40 ppm, Chlorophyll a exhibited 14% inhibition whereas b and c revealed 37% and 50% respectively. Here also, Chlorophyll c content is much affected by this metal toxicity. Chlorophyll a content in 50 ppm indicated 32% inhibition, but Chlorophyll b exhibited 49% retardation. Effect of 50 ppm on Chlorophyll c content was very high (83%) than Chlorophyll a and Chlorophyll b contents.

In the two highest concentration of Zinc selected for the present study (60 and 70 ppm) indicated maximum inhibitory effect on Chlorophyll content. In 70 ppm, Chlorophyll a content expressed only 50% retardation but Chlorophyll b and c exhibited 65% and 87% inhibition on the last day of experiment. Chlorophyll a and c content exhibited peak value on 12th day after that it declined but Chlorophyll c exhibited peak value on 16th day.

From these observation, it is revealed that, off the three parameters analysed, Chlorophyll content is most affected by the Zinc toxicity in this marine diatom. It is also evident that Chlorophyll c is much more inhibited by this metal than Chlorophyll a and b pigments.

EFFECT OF LEAD ON GROWTH RATE, PRIMARY PRODUCTION AND CHLOROPHYLL CONTENT OF TETRASELMIS GRACILIS

Varying levels of Lead Nitrate selected for this study was 25 ppm, 50 ppm, 75 ppm, 100 ppm and 125 ppm. These levels were chosen on the basis of a priliminary range finding test.

Effect on growth rate

Exponential growth phase observed in control was from 2nd day onwards to 12th day after that growth declined. Cell number expressed its peak value on 12th day (68 x 10^4 cells/ml).

Stimulation of growth observed in 25 ppm of treatment from 2nd - 6th day. But the growth, then declined and again increased with maximum number of cells on 14th day (69 x 10^4 cells/ml). 14th day onwards, cell multiplication rate declined. However, on 16th day, the culture indicated 16% inhibition of growth.

Culture in 50 ppm level of Lead (Fig. 19), retarded the growth by about 33%. The culture entered death phase on 12th day itself. Maximum number of cell noted was on 12th day of culture. Growth rate in the 75 ppm treatment was similar to 25 ppm upto 10th day. But from 10th day onwards, it started declining. 50% inhibition of cell number determined in 75 ppm culture. Highest number of cell noted was 45 x 10^4 cell/ml/day

EFFECT OF LEAD ON GROWTH RATE OF TETRASELMIS GRACILIS

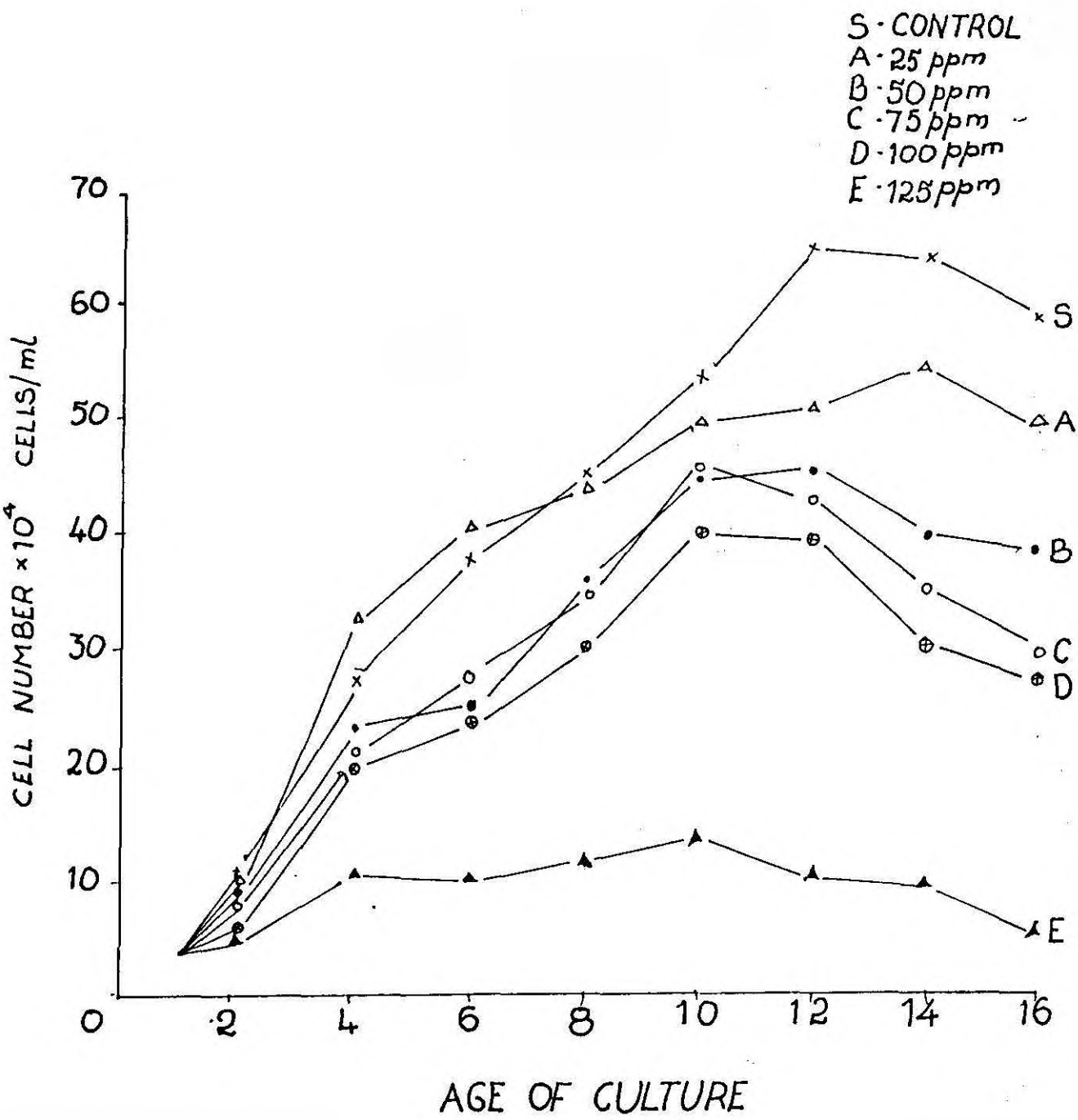

Fig. 19

Cell multiplication rate in 100 ppm level of lead was some what similar to 50 and 75 ppm. It showed stationary phase from 10th-12th day, after that entered to declining phase. Cell division was inhibited by about 53% in this treatment.

There was a vast difference observed between the cell multiplication rate of control and other treatments with that of 125 ppm level. Cell division was stationary from 6th - 10th day after that declining phase started. Maximum number of cell noted was on 10th day (14×10^4 cell/ml) It is determined that, cell division capacity of these microalgae inhibited by about 91% in 125 ppm level of Lead nitrate.

It is evident from the results that, 50% growth inhibition happend in 75 ppm level of lead but higher inhibitory effect observed was only in 125 ppm level. The gap between the growth rate of 100 ppm and 125 ppm revealed that much inhibitory action was happend in between 100 and 125 ppm levels.

Effect on Primary production

Gross and net production of control showed gradual increase from 2nd-10th day. Production was its maximum on 10th day of culture (0.36 mg C/l/hr. Respiratory action was going on increasing as the age of culture increased.

Photosynthetic rate in all the treatments was less than control (Fig. 20). On 10th day, 25 ppm treatment expressed 33% suppression

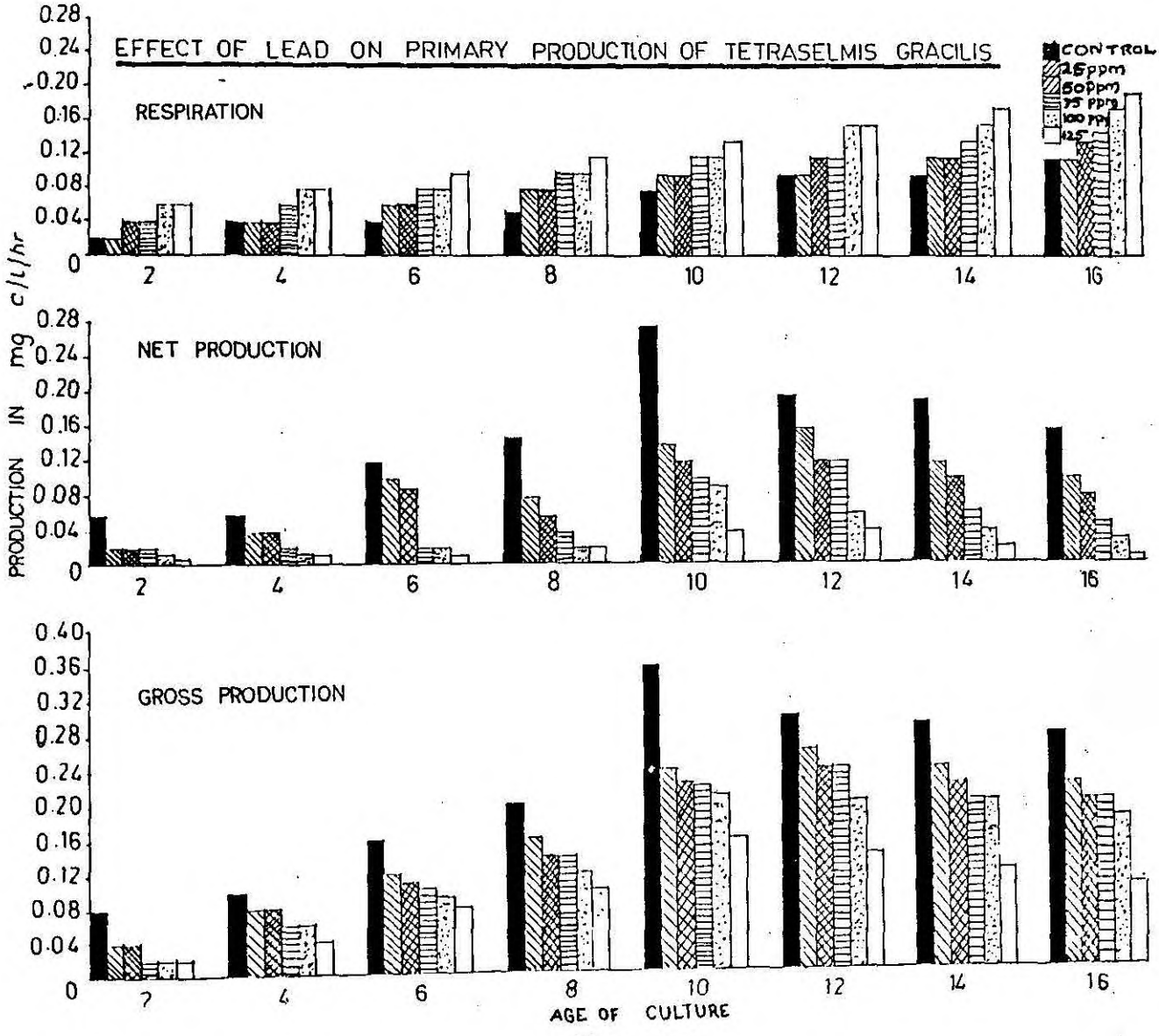
EFFECT OF LEAD ON PRIMARY PRODUCTION OF TETRASELMIS GRACILIS
CONTROL
25ppm
50ppm
75 ppm
100 ppm
125
RESPIRATION
NET PRODUCTION
GROSS PRODUCTION
PRODUCTION IN mg c/l/hr
AGE OF CULTURE
0.28
0.24
0.20
0.16
0.12
0.08
0.04
0
0.40
0.36
0.32
2
4
6
8
10
12
14
16

Fig. 20

of gross production but on 16th day it showed only 23% of inhibition. On 14th day, 17% of retardation occured in it. Reduction in the inhibition rate of gross production after 10th day may be due to acclimatization of cells towards lead toxicity. Net production also revealed similar results to that of gross production. 10th day onwards, retardation of net production determined. It was inhibited by about 50% on 10th day. 37% retardation of production noticed on 16th day. Enhancement of respiratory mechonism observed, throughout the study period. On 12th day, 33% enhancement of respiratory action occured.

Primary production in the 50 and 75 ppm, treatment much inhibited by heavy metal toxicity. Stimulation of respiratory action was found out in all the treatments. Retardation of gross production in 50 ppm level was about 28%.

In 100 ppm treatment 35% reduction in gross production determined. Whereas net production showed 81% inhibition. Respiration was enhanced by about 33% on 16th day of cultures.

Gross production in 125 ppm expressed 84% inhibition but net production reduced by about 93%. 40% stimulation of respitatory action was noted in this treatment. On 16th day, respiraton was higher than the gross production.

These factors revealed that, gross and net production in all the treatments was very less due to stimulation of respiratory action. Rate

of inhibition was depend on the dose of heavy metal and duration of exposure of cells to it.

Effect on Chlorophyll content

Control exhibited a gradual increase in Chlorophyll a and b content and expressed its peak value on 16th day of culture. (4350 /ug/l and 5500 /ug/l).

Lowest concentration of Lead nitrate (25 ppm) did not much inhibited the production of chlorophyll pigments (Fig. 21). It was almost similar to control. Highest value of Chlorophyll a and b was noted on 10th day. Chlorophyll a content was inhibited by about 3.5% on 10th day.

Chlorophyll contents in the 50 ppm treatment revealed similar results to that of 25 ppm. Highest value recorded was on 16th day for both Chlorophyll a and b (3850 /ug/l and 4875 /ug/l). Chlorophyll a showed 11.5% inhibition in this treatment.

In 75 ppm treatment observations revealed 26% retardation of Chlorophyll a content.

EFFECT OF LEAD ON CHLOROPHYLL CONTENT OF TETRASELMIS GRACILIS

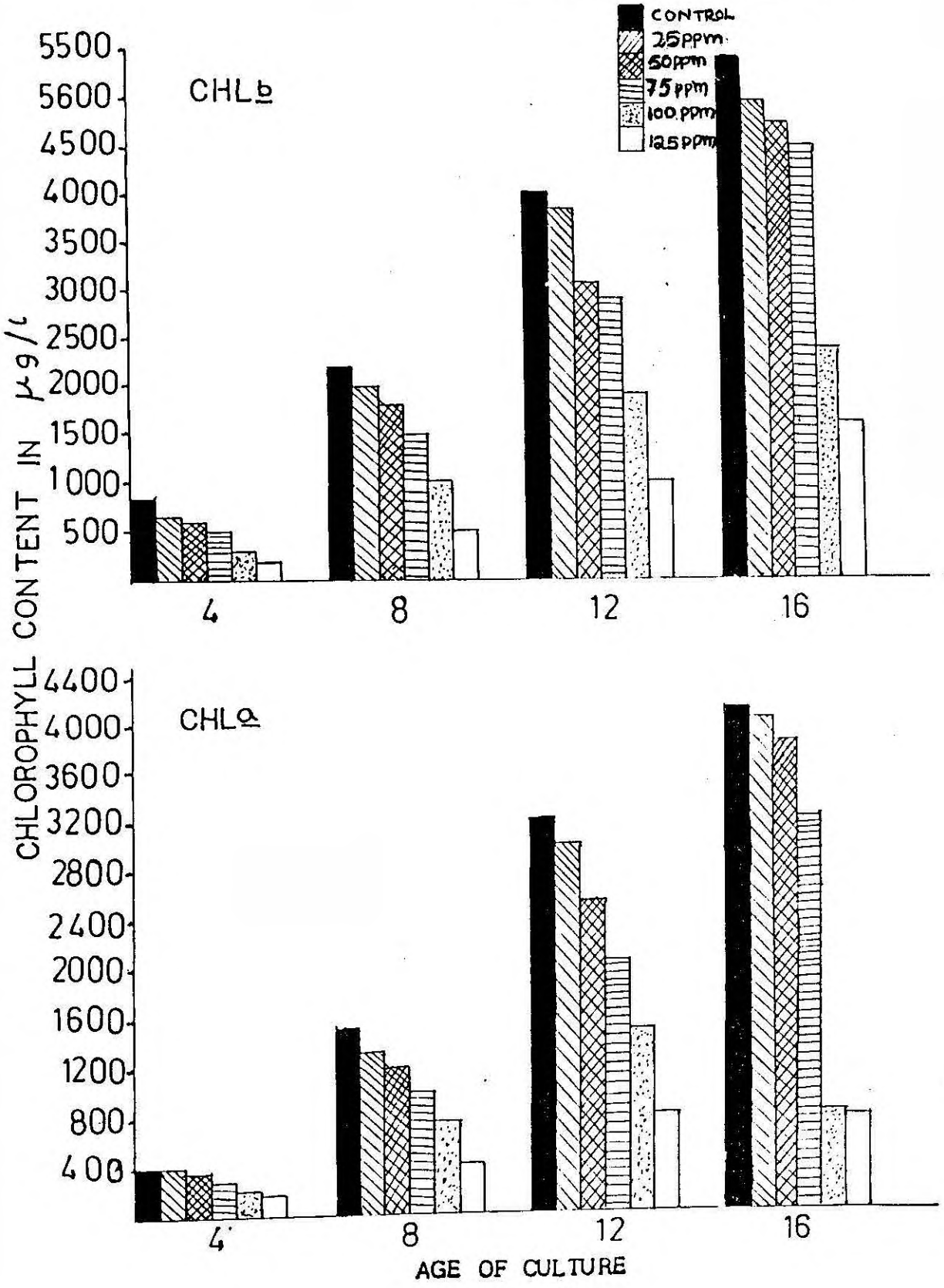

Fig. 21

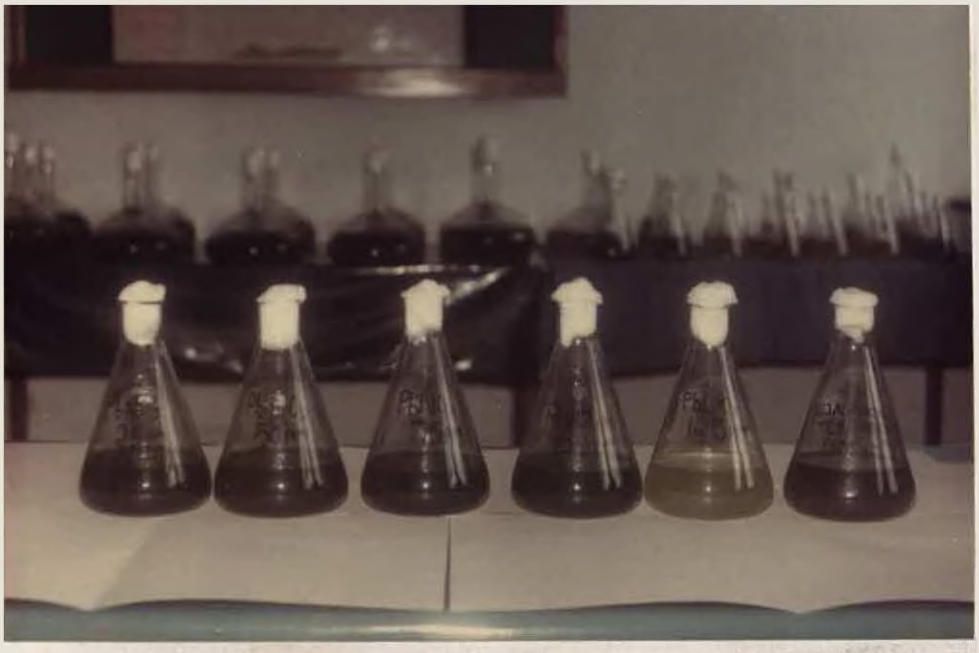

PLATE 7, EFFECT OF LEAD ON TETRASELMIS GRACILIS

PLATE 8 · EFFECT OF LEAD ON CHAETOCEROS CALCITRANS

EFFECT OF LEAD ON GROWTH RATE, PRIMARY PRODUCTION AND CHLOROPHYLL CONTENT OF CHROMULINA FREIBURGENSIS

Different levels of Lead (as Lead Nitrate) selected for the present investigation was 25 ppm, 50 ppm, 75 ppm, 100ppm and 125 ppm. Criteria for the selection of these concentrations was based on priliminary range finding experiment.

Effect on Growth Rate

Exponential growth phase noted for control was from 2nd - 14th day of culture, after that cell division declined. On 14th day control exhibited the maximum number of cell (150 x 10^4 cells/ml).

Cultures in the 25 and 50 ppm concentration of Lead indicated a slight enhancement in growth upto 8th day but a rapid retardation in cell number noted from 8th day onwards (Fig. 22). Culture in 25 ppm exhibited 43% reduction whereas in 50 ppm concentration, 53% inhibition of growth noted on 16th day.

Cell multiplication in the 75 ppm treatment was similar to that of control upto 8th day of experiment. But from 10th day onwards growth of this species is retarded leading to the death of culture. Almost 50% growth retardation happened in this treatment. Growth of this species in the 100 ppm treatment also expressed similar results to that of 75 ppm. Rate of retardation of cell number was 66% on 16th day.

Cell multiplication rate was very less in 125 ppm compared to that of control and other concentrations. From 2nd - 10th day there was slight increase in the cell number but after 10th day cell number declined. There was 93% inhibition of growth on the last day of experiment.

EFFECT OF LEAD ON GROWTH RATE OF CHROMULINA FREIBURGENSIS

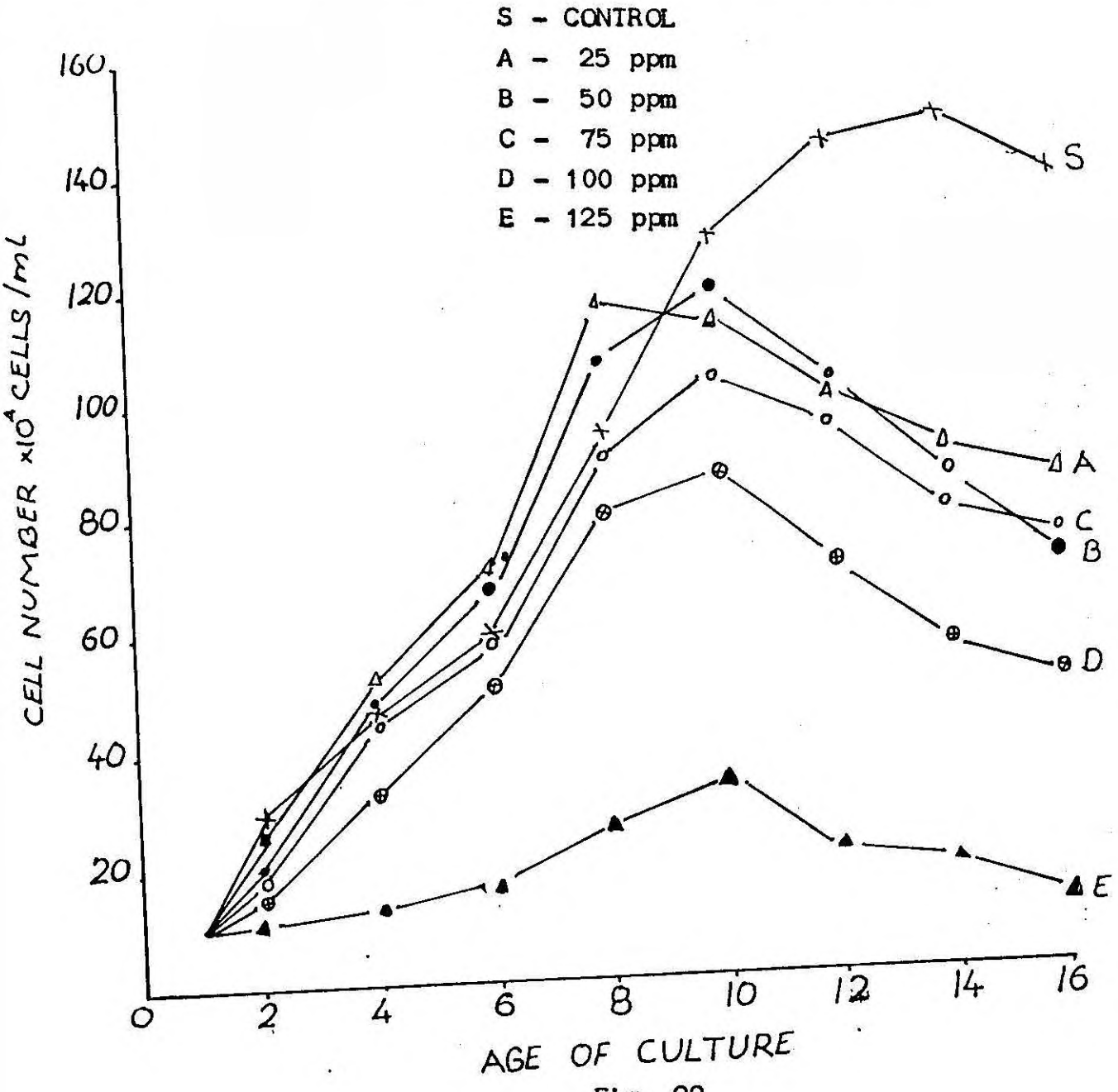

Fig. 22

It is observed from these observations that inspite of higher concentration, lowest concentration of lead, selected for the study also inhibited the cell dividing capacity of this micro-alga.

Effect on Primary Production

Primary production of the control culture showed gradual increase as the cell number increased. Highest value of gross and net production noticed was on 12th day (0.40 mg C/l/hr) and 0.30 mg C/l/hr of culture. Respiration rate gradually increased as the age of culture increased and reached the peak on 16th day (0.14 mg C/l/hr).

There was only 5% inhibition of gross and net production determined in 25 ppm treatment. Respiratory rate was some what similar to control without much enhancement or inhibition(Fig. 23).

In 50 and 75 ppm treatment, respiration rate was higher than control due to the stress condition made by metal toxicity. About 22% reduction of gross production determined in 50 ppm treatment but 75 ppm exhibited 44% of retardation. In 50 ppm treatment there was only 45% inhibition of net production but about 63% of reduction noticed in 75 ppm level of culture. Both of these treatments exhibited increased respiratory mechanism throughout the study period. Respiratory rate showed 14% enhancement in 50 ppm but 75 ppm expressed 30% increase on 16th day of culture.

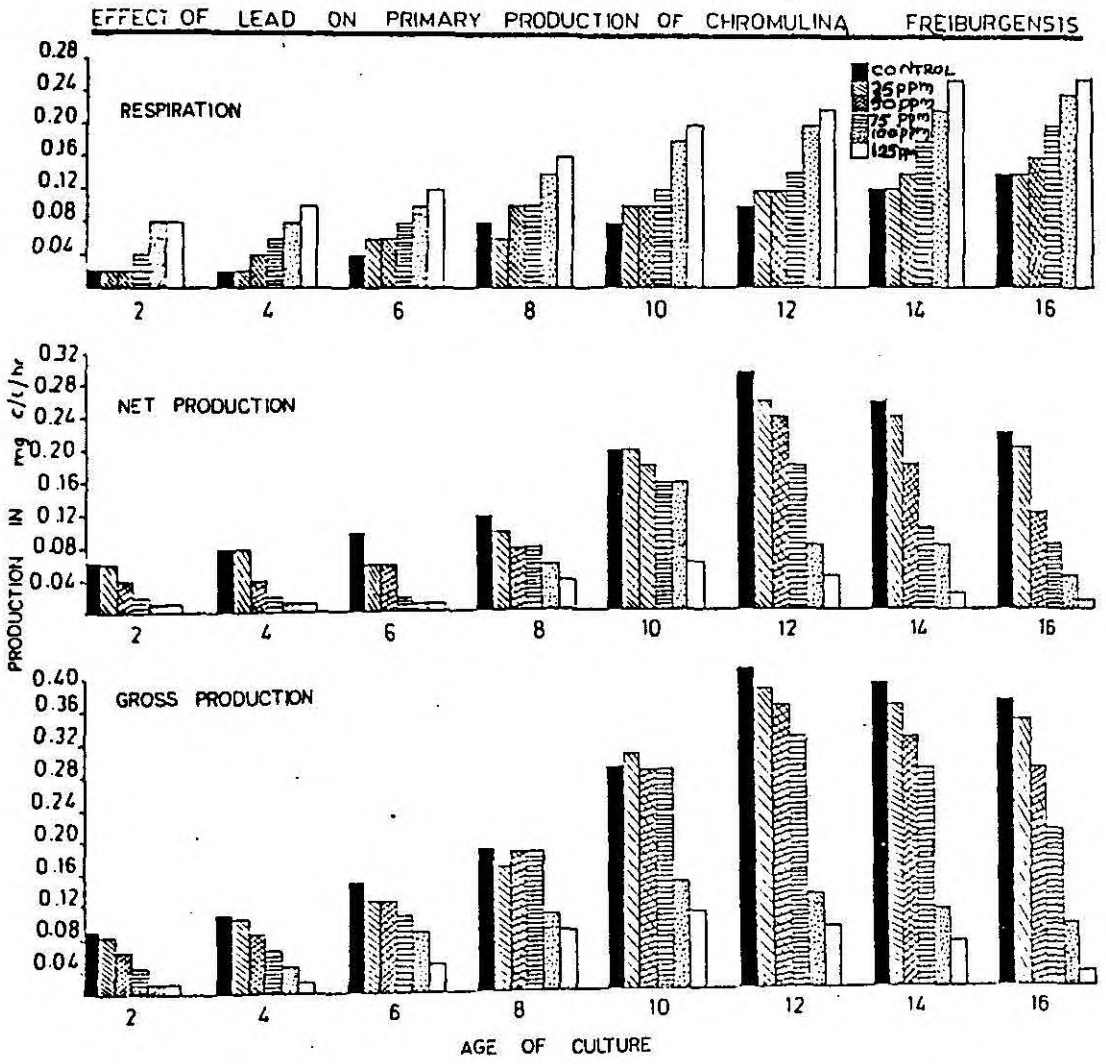
EFFECT OF LEAD ON PRIMARY PRODUCTION OF CHROMULINA FREIBURGENSIS
RESPIRATION
CONTROL
25 ppm
50 ppm
75 ppm
100 ppm
125 ppm
NET PRODUCTION
GROSS PRODUCTION
PRODUCTION IN mg c/l/hr
AGE OF CULTURE

Fig.23

Two higher concentrations of Lead (100 and 125 ppm), revealed the toxic action of metal by reducing the primary production. Total carbon production in these two treatments was very less compared to control. Gross production in 100 and 125 ppm retarded by about 77 and 93% respectively. Repiration was enhanced by about 42% and 46% in100 and 125 ppm respectively.

This observations also revealed that reduction in total carbon production is because of high respiratory mechanism in the metal treated cultures. Carbon produced was utilised for accelerated metabolic activities of cell such as respiration and other cell activities in cultures treated with higher doses of heavy metal.

Effect on Chlorophyll Content

Control showed a regular increase in Chlorophyll content throughout the study period. Chlorophyll a and b was maximum on 16th day(110 /ug/l) and 1500 /ug/l) but Chlorophyll c revealed its peak value on 12th day of culture. Amount of Chlorophyll c was higher than Chlorophyll a and b.

Upto 12th day of culture there was not much variation in Chlorophyll contents in 25 ppm treatment (Fig. 24). But on 16th day Chlorophyll a and b content expressed 23% and 15% reduction respectively. Chlorophyll c was also less and it reached its peak value on 12th day (1680 /ug/l) of culture.

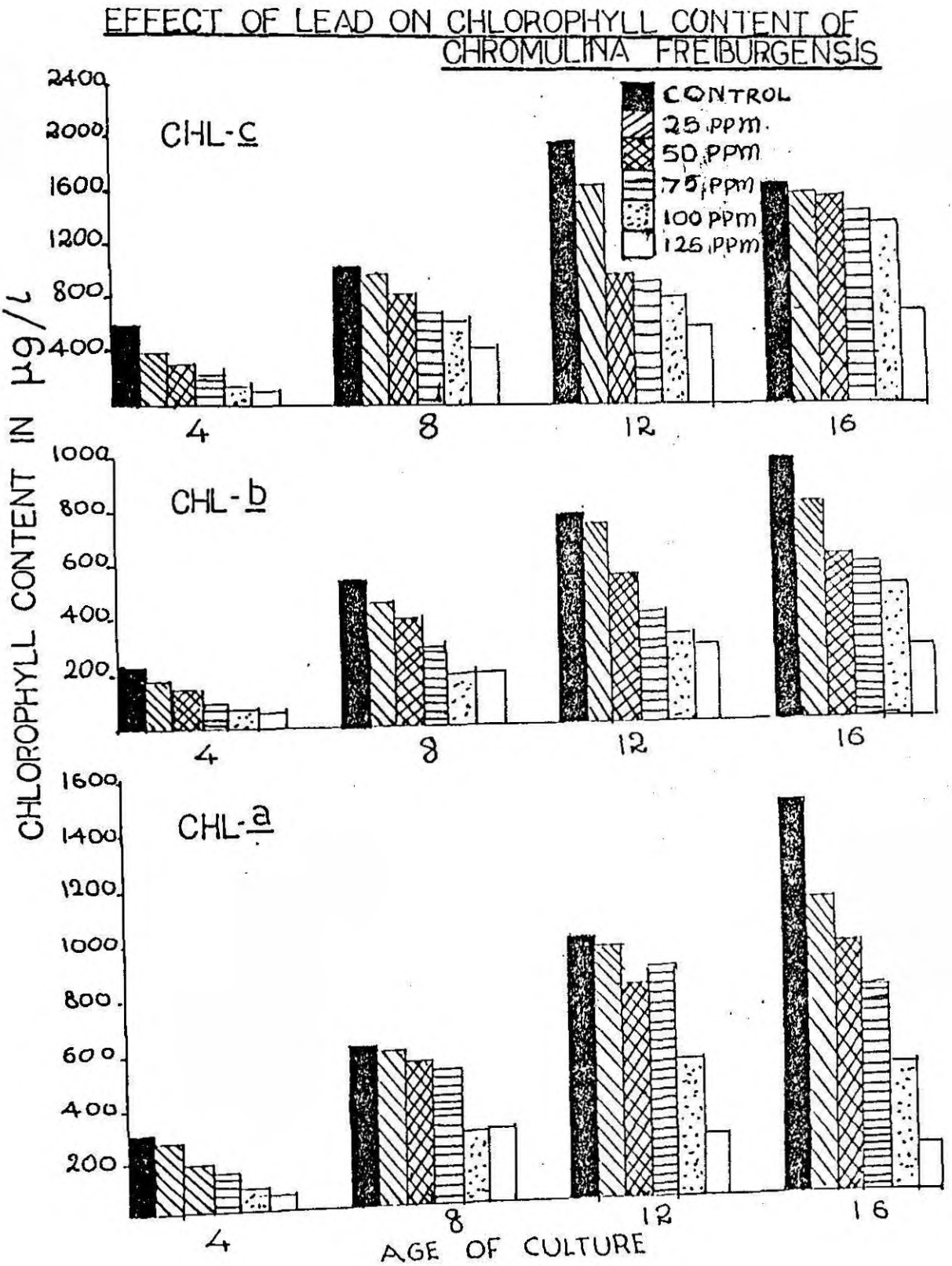
EFFECT OF LEAD ON CHLOROPHYLL CONTENT OF CHROMULINA FREIBURGENSIS
CHL-c
CHL-b
CHL-a
CONTROL
25 PPM
50 PPM
75 PPM
100 PPM
125 PPM
CHLOROPHYLL CONTENT IN µg/l
AGE OF CULTURE
2400
2000
1600
1200
800
400
1000
600
200
1400
4
8
12
16

Fig. 24

Chlorophyll content in the 50 and 75 ppm of culture was very less compared to control. Chlorophyll a in 50 and 75 ppm concentrations exhibited 43% and 46% of reduction respectively. Where as Chlorophyll b and c was inhibited by about 35% and 38% respectively.

Highest concentration of Lead selected for the study (100 and 125 ppm) revealed the maximum inhibition of Chloropnyll contents. On 16th day there was 66% retardation of Chlorophyll a in 100 ppm but 70 ppm exhibited 86% inhibition at that day.

In 100 ppm treatment there was 70% inhibition of Chlorophyll and 57% of Chlorophyll c noticed on the last day of experiment.

From the above mentioned observations it is cleared that, Chlorophyll a is much more affected by lead toxicity.

EFFECT OF LEAD ON GROWTH RATE, PRIMARY PRODUCTION AND CHLOROPHYLL CONTENT OF CHAETOCEROS CALCITRANS

Five different levels of Lead (Lead nitrate) selected for the present study was 25 ppm, 50 ppm, 75 ppm, 100 ppm and 125 ppm. Selection of these concentrations was based on a priliminary range finding test.

Effect on growth rate

An initial inoculum of 15 x 10^4 cells/ml of culture was added to the culture flasks. Counting of cells on 2nd day revealed active cell division in control and all treatments. Control showed 70 x 10^4 cells/ml on 2nd day. In control, exponential growth phase was going on increasing till 12th day, after that death phase started.

Culture in 25 ppm treatment expressed almost similar growth rate to that of control without much inhibition. On 16th day there was only 10% retardation of cell number (Fig. 25). When determining the growth of cells in all the treatments, culture in 25 ppm exhibited higher growth rate than the control.

The 50 and 75 ppm treatment expressed death phase from 10th day onwards. In 50 ppm, on 16th day there was 31% of inhibition but in 75 ppm, 37% of reduction noticed.

Cell multiplication rate in 100 ppm treatment indicated exponential growth phase upto 10th day after that death phase occured. 48% of growth retardation determined on 16th day of culture. Higher number of cell noted was on 10th day (90 x 10^4 cells/ml).

EFFECT OF LEAD ON GROWTH RATE OF CHAETOCEROS CALCITRANS

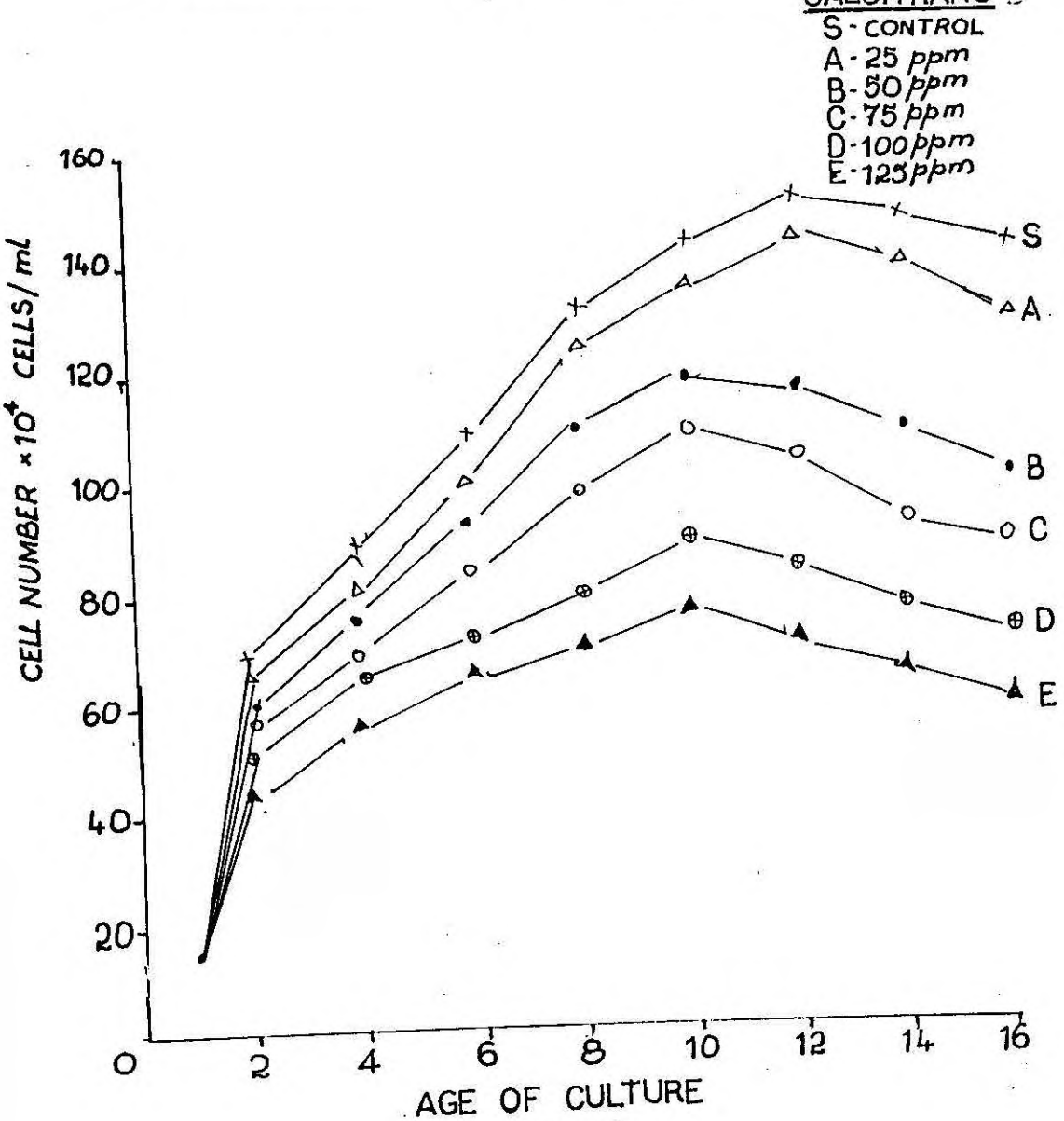

Fig. 25

Compared to all other species of microalgae treated with lead, Chaetoceros calcitrans revealed higher cell multiplication rate in 125 ppm level. Maximum cell number noted was on 10th day of culture (75×10^4 cells/ml). On 10th day, culture exhibited 48% of growth retardation but on 16th day 58% of inhibition determined.

From the above made observations, it is determined that Chaetoceros calcitrans is the most tolerant species towards lead toxicity than the other two microalgal species tested. High tolerance of this species may be due to the presence of rigid silicious cell walls, compared to the cellulose cell wall.

Effect on Primary production

As the cell number increased total production of control also revealed increasing tendency. In control peak value for gross production observed was on 12th day (0.42 mgC/l/hr) whereas net production on 10th day (0.34 mgC/l/hr).

The lowest concentration of lead nitrate (25 ppm) selected for the study, did not revealed much inhibitory effect on production. Gross and net production was almost equal to control throughout the study period. But on 8th day, there was slight increase in production than control. Only 5% inhibition of production determined on 16th day (Fig. 26).

Carbon production in the 50 ppm treatment showed similar results to that of control. Respiratory rate was going on increasing from 2nd day onwards and expressed the maximum on 14th day (0.18 mgC/l/hr).

EFFECT OF LEAD ON PRIMARY PRODUCTION OF CHAETOCEROS CALCITRANS

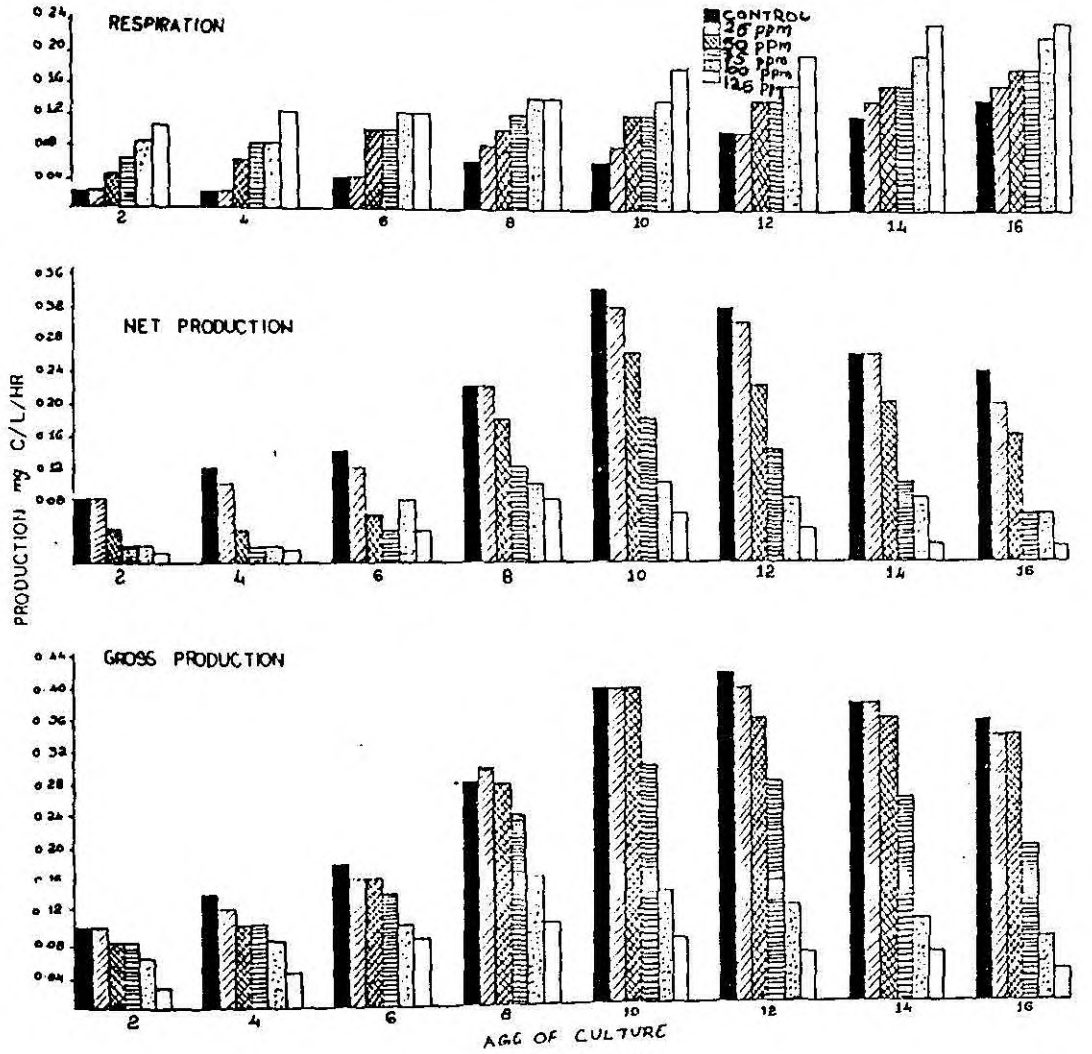

Fig.26

Here also, similar to that of 25 ppm treatment only 5% reduction of photosynthetic activity occured.

When compared to the above two levels of lead, production in 75 ppm indicated declining tendency which was due to the increased level of heavy metal. Respiratory mechanism was accelerated by metal toxicity, so that gross and net production was less.

Due to the increased level of lead nitrate, photosynthetic uptake of the cells was much inhibited in 100 and 125 ppm. Reasons for the reduction in gross production was the accelerated respiratory mechanism to withstand the abnormal toxic condition. Respiratory rate enhanced by about 33% in 100 ppm level but in 125 ppm, 41% of enhancement determined. Gross production was inhibited by 88% whereas 91% suppression of net production observed on 16th day.

It is revealed that primary production is much more inhibited by lead toxicity than growth in the species Chaetoceros calcitrans. On 16th day only 58% of growth inhibition determined but gross production expressed 91% retardation at that day. It is also revealed that lower concentrations selected have not much inhibitory effect on cell multiplication and photosynthetic rate of this marine diatom.

Effect on Chlorophyll content

Pigment analysis of control indicated gradual increase in chlorophyll content. Chlorophyll c showed higher value than other two pigments. Amount of Chlorophyll a, b and c was maximum on 16th day.

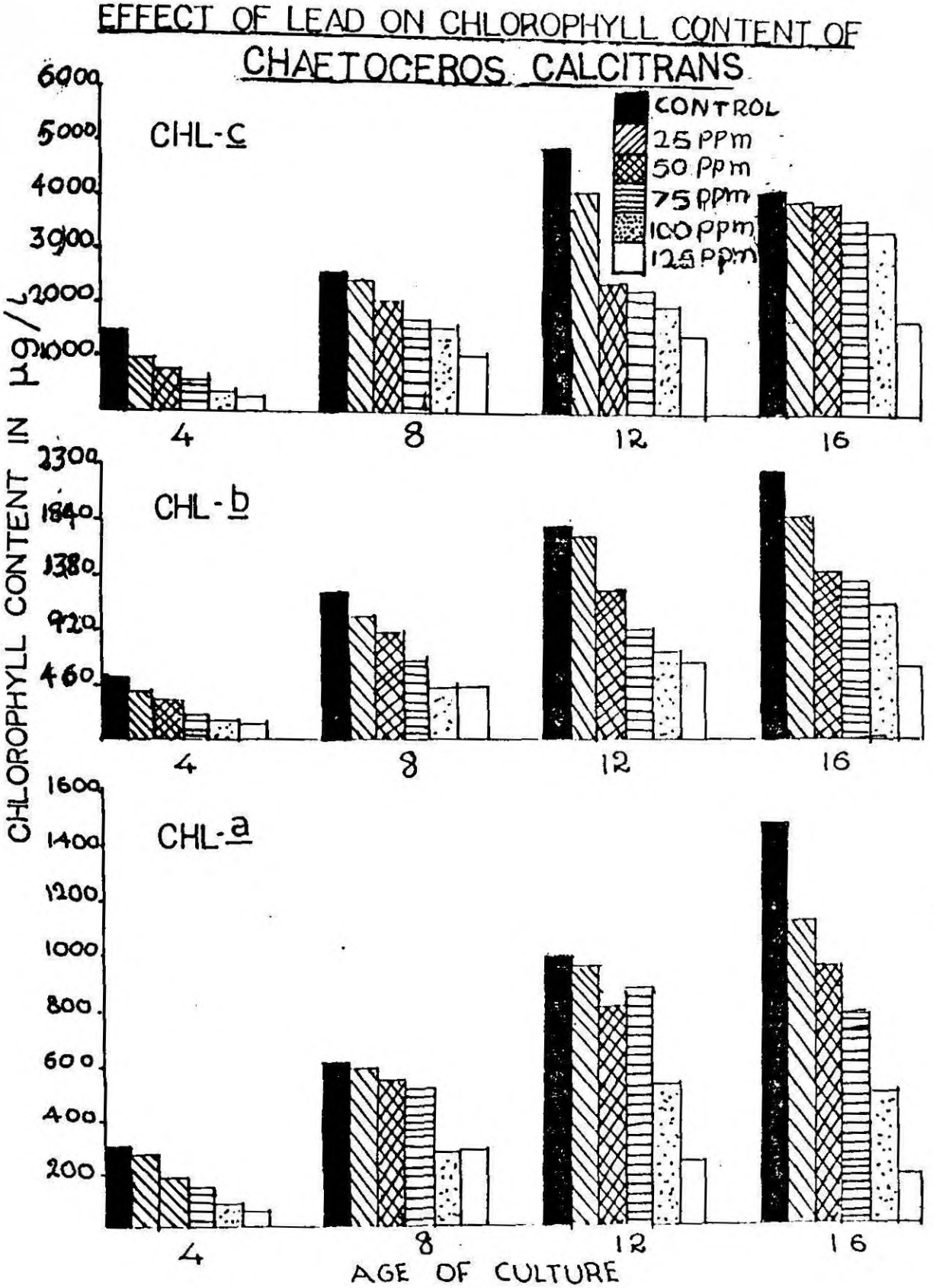
EFFECT OF LEAD ON CHLOROPHYLL CONTENT OF
CHAETOCEROS CALCITRANS
CHL-c
CHL-b
CHL-a
CONTROL
25 PPm
50 PPm
75 PPm
100 PPm
125 PPm
CHLOROPHYLL CONTENT IN µg/L
6000
5000
4000
3000
2000
1000
2300
1840
1380
920
460
1600
1400
1200
1000
800
600
400
200
4
8
12
16
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Fig. 27

Lower concentration of lead (25 ppm) did not showed, much inhibitory effect on Chlorophyll contents (Fig. 27). Chlorophyll a retarded 10% where as Chlorophyll b and c revealed 4% and 2% of inhibition respectively.

The 75 ppm treatment expressed 13% reduction in Chlorophyll a content but only 8% inhibition was there for Chlorophyll b content. Compared to Chlorophyll a and b inhibition was less in Chlorophyll c (only 3%).

There was 20% inhibition of Chlorophyll a in 75 ppm level. Chlorophyll b indicated 10% but Chlorophyll c showed only 5% inhibition on 10th day of culture. In 100 ppm treatment 33% of inhibition of Chlorophyll content revealed.

Culture in 125 ppm treatment expressed maximum inhibitory effect of lead on Chlorophyll content. Chlorophyll a is inhibited by about 55% but Chlorophyll a and c retarded by about 61% and 50% on 16th day of culture.

Estimation of Chlorophyll contents revealed that Chlorophyll a is most affected by lead than the other two pigments. For Chlorophyll c there was only 50% of inhibition observed.

EFFECT OF HEAVY METALS ON NATURAL PHYTOPLANKTON POPULATION COLLECTED FROM A PRAWN CULTURE POND

The main objective of this study was to compare the effect of heavy metals on both unialgal culture and natural phytoplankton population collected from different ecosystems. From these study it is easy to find out the effect of same levels of heavy metals on the phytoplankton of different ecosystems and on unialgal cultures of microalgae.

The important species of phytoplankton present in the water sample collected from prawn culture field at Cherai was species of Thalassiosira, Nitzschia, Navicula, Chlamydomonas, Biddulphia, Scenedesmus and Oscillotoria. Experiments were conducted in similar way to that of unialgal cultures. Five different levels of heavy metals (Copper, Zinc and Lead) selected was similar to that of concentrations taken for unialgal culture studies. Duration of the study Period was 16 days. Growth and Primary production were estimated on alternate days, whereas chlorophyll content on every fourth day.

Effect of Copper

Varying concentrations of copper selected for this study was 5 ppm, 10 ppm, 15 ppm, 20 ppm and 25 ppm. For each experiment there was a control.

Control of this experiment showed an active cell multiplication rate upto 10th day, after that growth declined. Maximum number of cell noted was on 10th day (50×10^4 cell/ml). Culture in the five ppm

PRAWN CULTURE POND - EFFECT OF COPPER ON GROWTH RATE

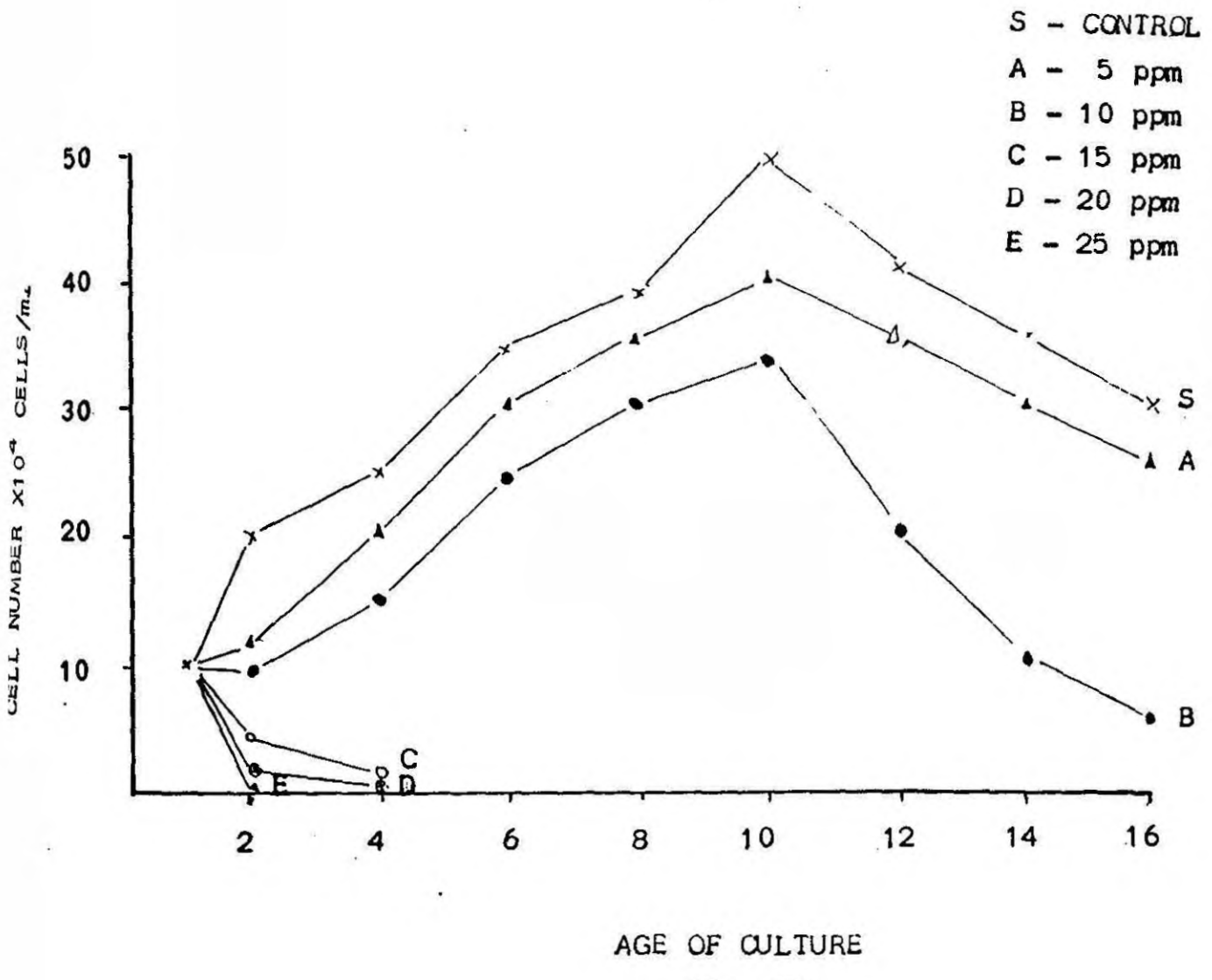
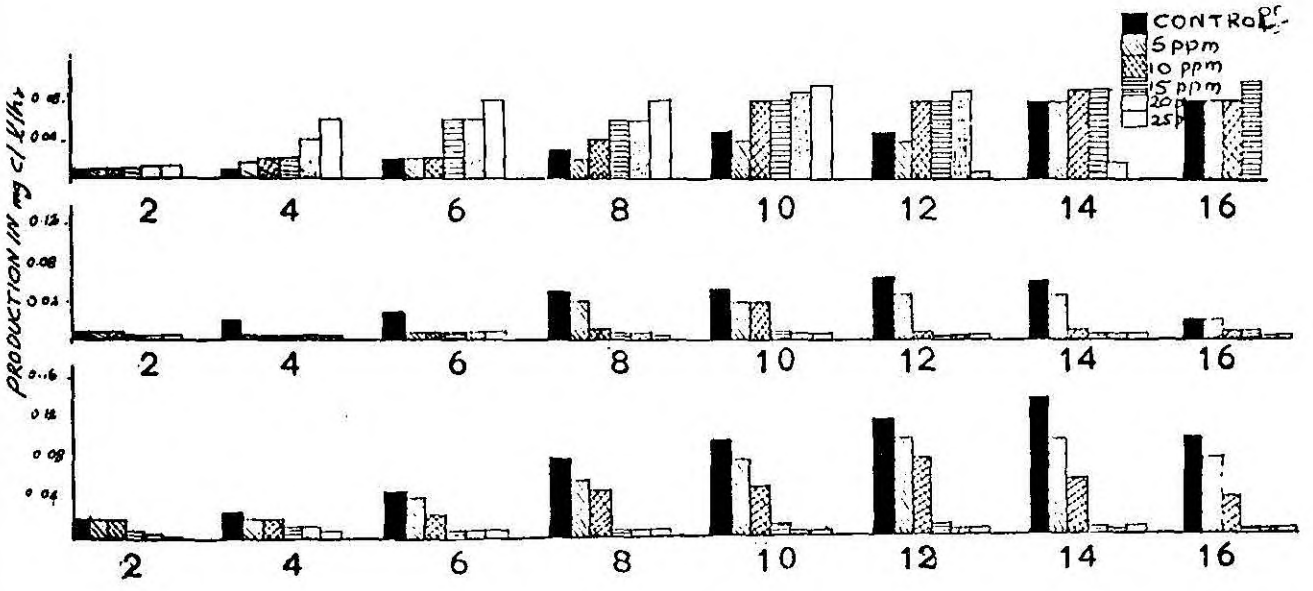


Fig. 28

PRAWN CULTURE POND - EFFECT OF COPPER ON PRIMARY PRODUCTION



AGE OF CULTURE

Fig. 29

PRAWN WLTURE POND – EFFECT OF COPPER ON CHLOROPHYLL CONTENT

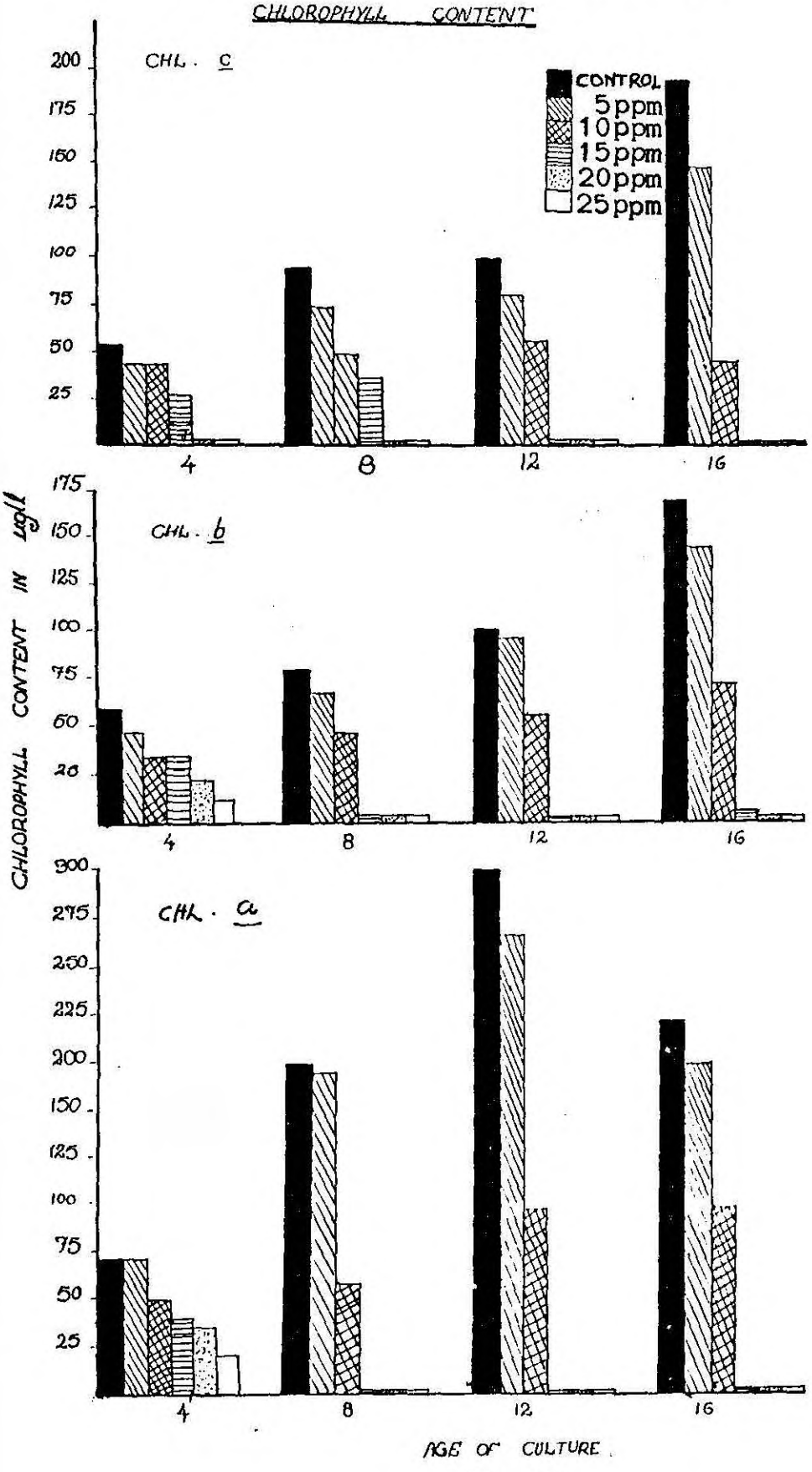


Fig.30

treatment showed not much variation in growth to that of control (Fig. 28). But in 10 ppm concentration, active cell division happened till 10th day of experiment but after that a deep retardation in growth rate observed. On 16th day 83% inhibition of growth determnined in this treatment.

In the three highest concentrations (15, 20 and 25 ppm) cell division was retarded in the first day itself, leading to the mass mortality of mixed cultures. 100% inhibition noted was on 4th day in all the three treatments.

Total carbon production in the 5 ppm treatment showed not much inhibition to that of control. But on 14th day onwards production retarded (Fig. 29). However, culture in the 10 ppm treatment revealed a significant reduction in primary production from 6th day onwards. Stimulation of respiratory action happened in these treatment. Net production was very less due to increased respiratory action. 98% retardation of primary production occured on 6th day onwards in the three higher concentrations such as 15 ppm, 20 ppm and 25 ppm. About 50% enhancement of respiratory mechanism happened in all the above treatments.

Estimation of chlorophyll content revealed that 100% inhibition of pigments in 15, 20 and 25 ppm treatments (Fig. 30). But, Chlorophyll contents in 5 ppm concentration showed about 25% inhibition. Chlorophyll c was much retarded by copper toxicity than the other two pigments. Chlorophyll c expressed 75% of retardation whereas Chlorophyll b revealed 57% in 10 ppm treatments.

These observations indicated that natural phytoplankton can tolerate very low levels of copper. Growth, primary production and chlorophyll content was almost equally inhibited by this metal toxicity.

Effect of Zinc

Five differnt concentration of zinc selected for the present study was 30 ppm, 40 ppm, 50 ppm, 60 ppm and 70 ppm.

The control of this experiment indicated an active cell division period till 10th day after that death phase occured. A similar growth pattern was observed in the two lowest concentrations and declining phase started from 10th day onwards (Fig. 31).

Culture in the 50 ppm revealed an exponential growth phase upto 6th day but after that growth was suddenly retarded leading to the complete mortality of culture. It was observed that almost 100% retardation of cell division happend in 60 and 70 ppm on 4th day. However, the total number of cell was higher than in treatments with copper.

Gross production in the culture treated with 30 ppm of Zinc was not much inhibited. Net production and respiration rate was also near to that of control. But gross production in 40 ppm also showed not much retardation but net production was inhibited much more due to accelerated respiratory process (Fig. 32). Almost 80% inhibition of total production occured in 50 ppm. From 6th day onwards, 98% inhibition of production occured in 60 and 70 ppm treatments.

PRAWN CULTURE POND - EFFECT OF ZINC ON GROWTH RATE

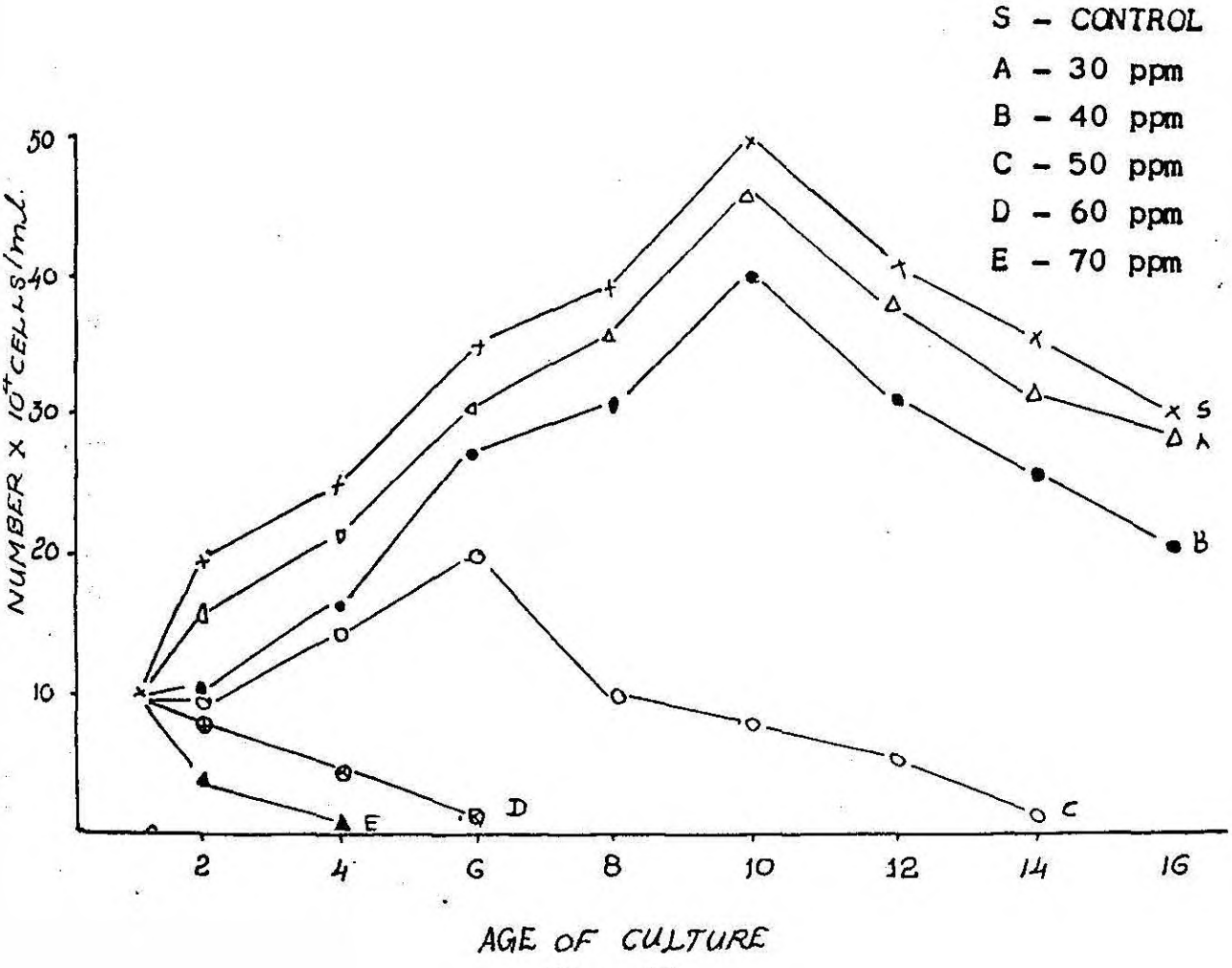
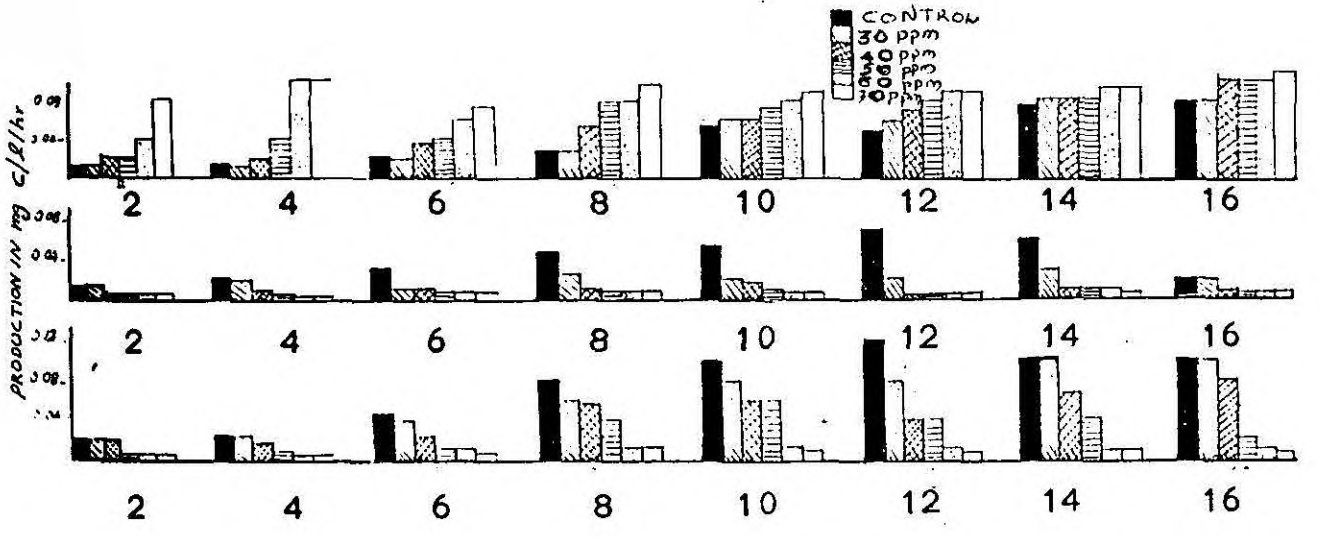


Fig. 31

PRAWN CULTURE POND - EFFECT OF ZINC ON PRIMARY PRODUCTION



AGE OF CULTURE

Fig. 32

PRAWN WLTURE POND . EFFECT OF ZINC ON CHLOROPHYLL CONTENT

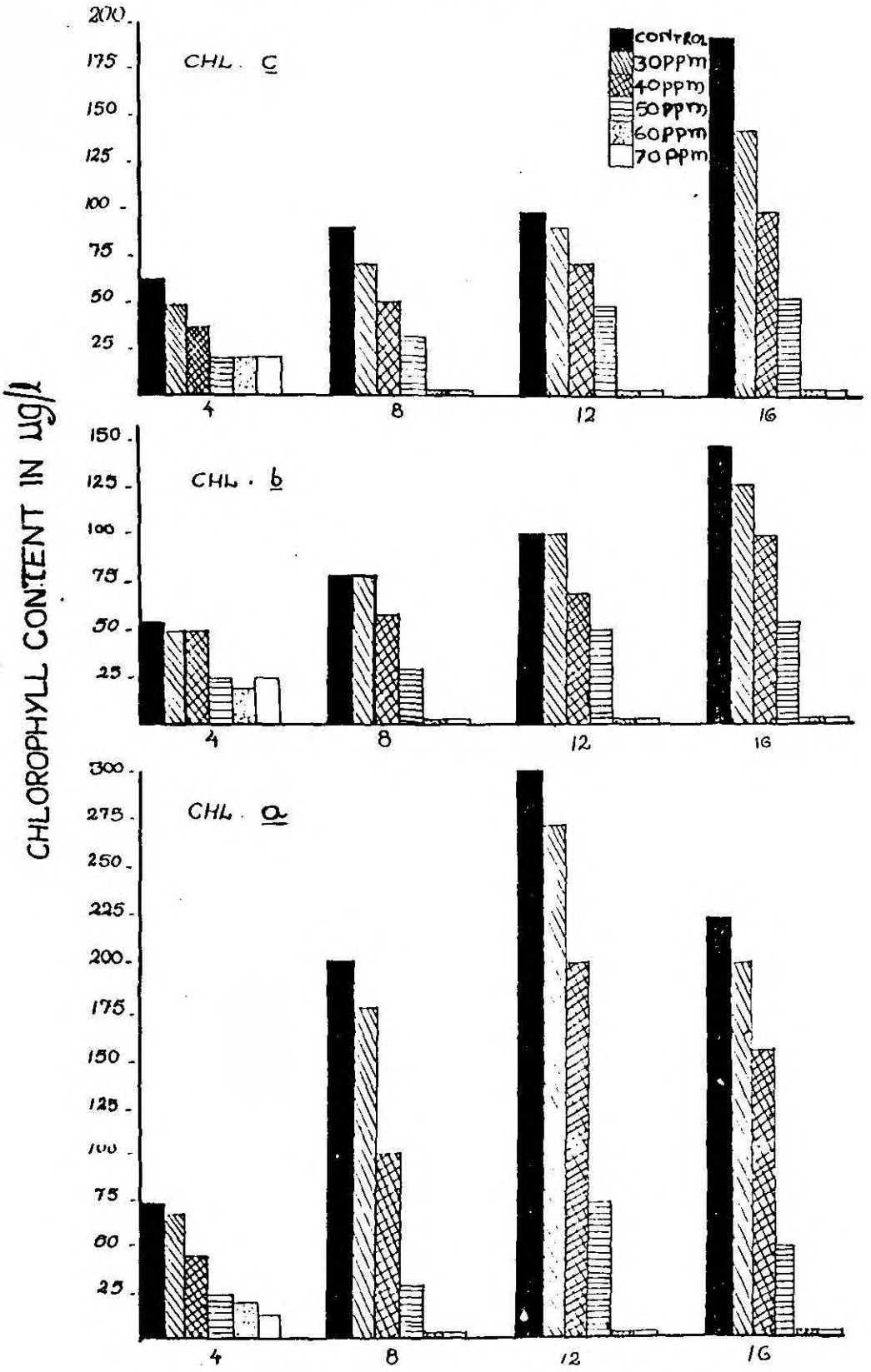


Fig. 33

Almost 100% of retardation of chlorophyll contents determined in 60 and 70 ppm treatments (Fig. 33). In 30 ppm, about 8% inhibition of Chlorophyll a content occured whereas Chlorophyll b and c revealed 16% and 27% of reduction respectively. In 50 ppm treatment 66% of retardation of Chlorophyll a indicated but Chlorophyll b and c showed 66% and 72% of retardation.

Effect of Lead

Varying concentrations of lead selected for the present investigation was 25 ppm, 50 ppm, 75 ppm 100 ppm and 125 ppm.

The 25 ppm treatment revealed somewhat similar growth rate with that of control. However, it showed declining phase from 10th day onwards (Fig. 34). Culture in the 50 ppm treatment declined from 6th day onwards and on 14th day 95% of retardation determined. Culture in all treatments (75,100 and 125 ppm) indicated almost 100% of growth inhibition on 2nd day itself.

Culture in both 25 and 50 ppm treatment did not showed much inhibitory effect of lead on primary production (Fig. 35). The production was equal to control. But in 75 ppm, 60% of inhibition observed. Stimulation of respiratory mechanism is also found out in this. On 4th day onwards 98% retardation of primary production occured in 100 and 125 ppm. Respiratory action was enhanced in all three treatments.

PRAWN CULTURE POND - EFFECT OF LEAD ON GROWTH RATE

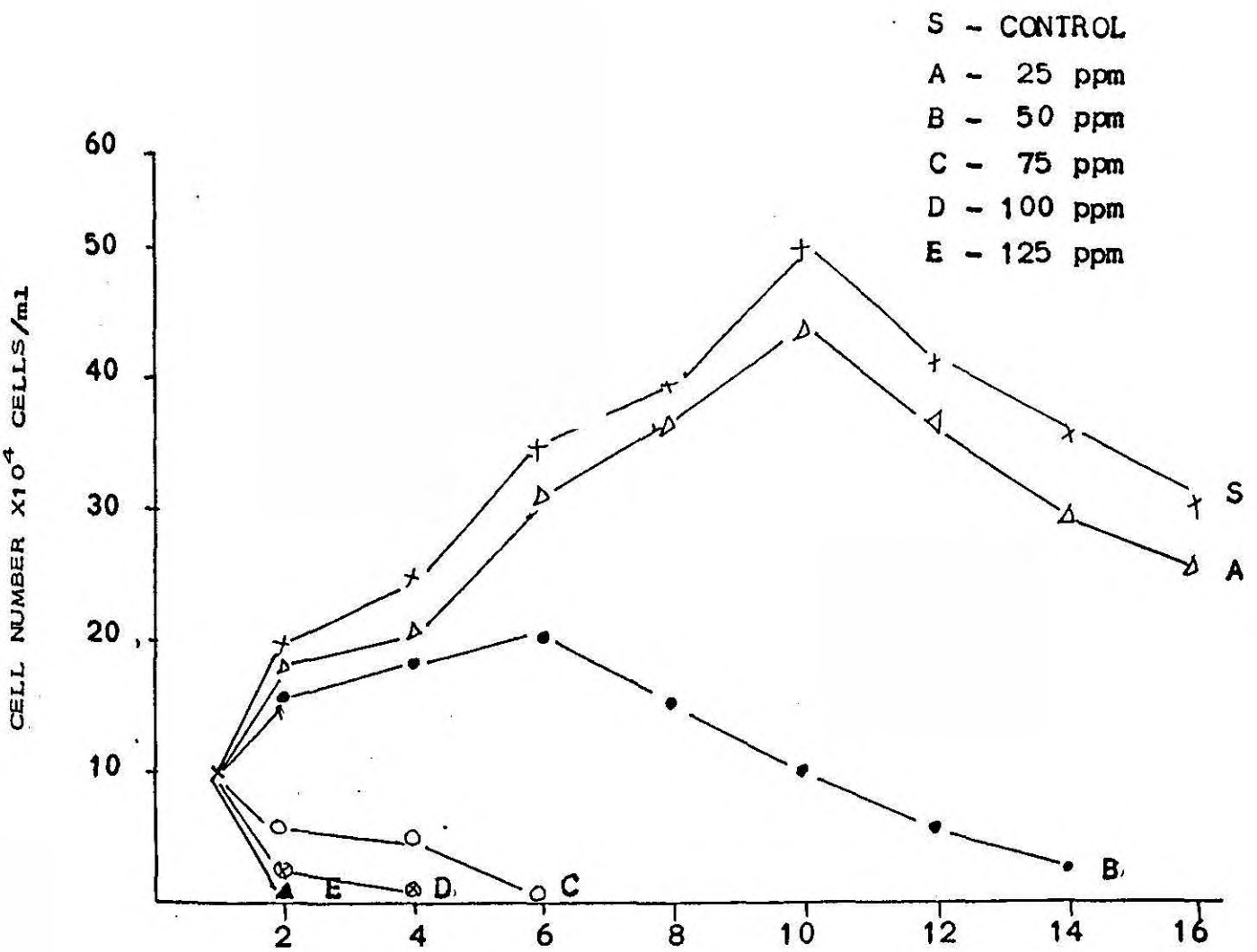
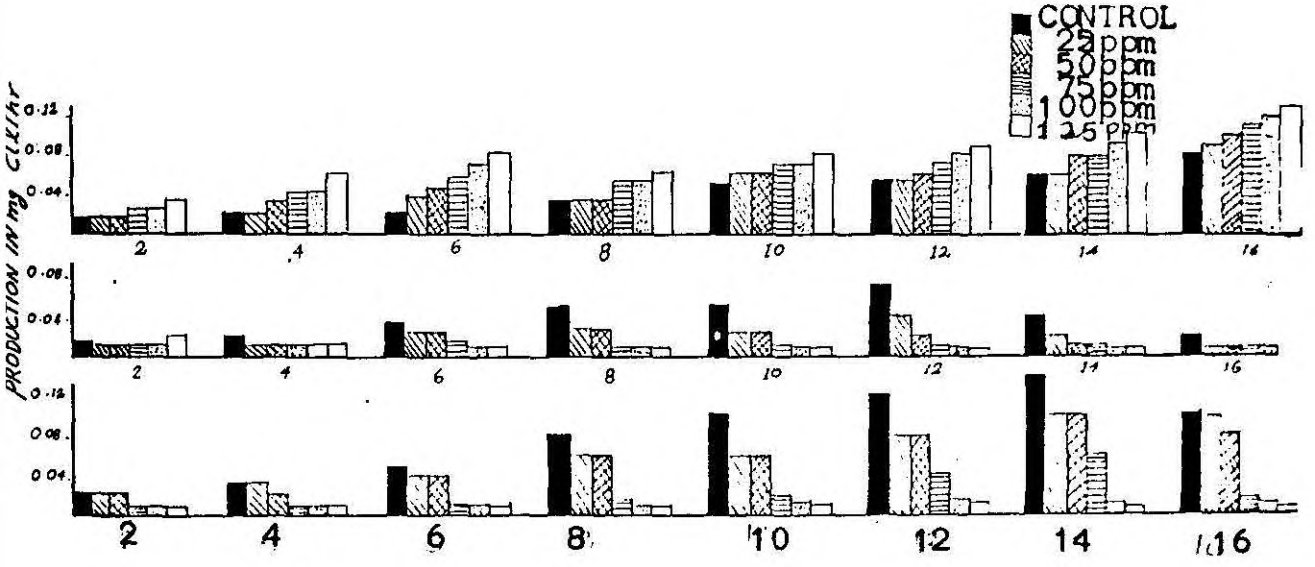


Fig. 34

PRAWN CULTURE POND - EFFECT OF LEAD ON PRIMARY PRODUCTION



AGE OF CULTURE

Fig. 35

PRAWN CULTURE POND – EFFECT OF LEAD ON CHLORPHYLL CONTENT

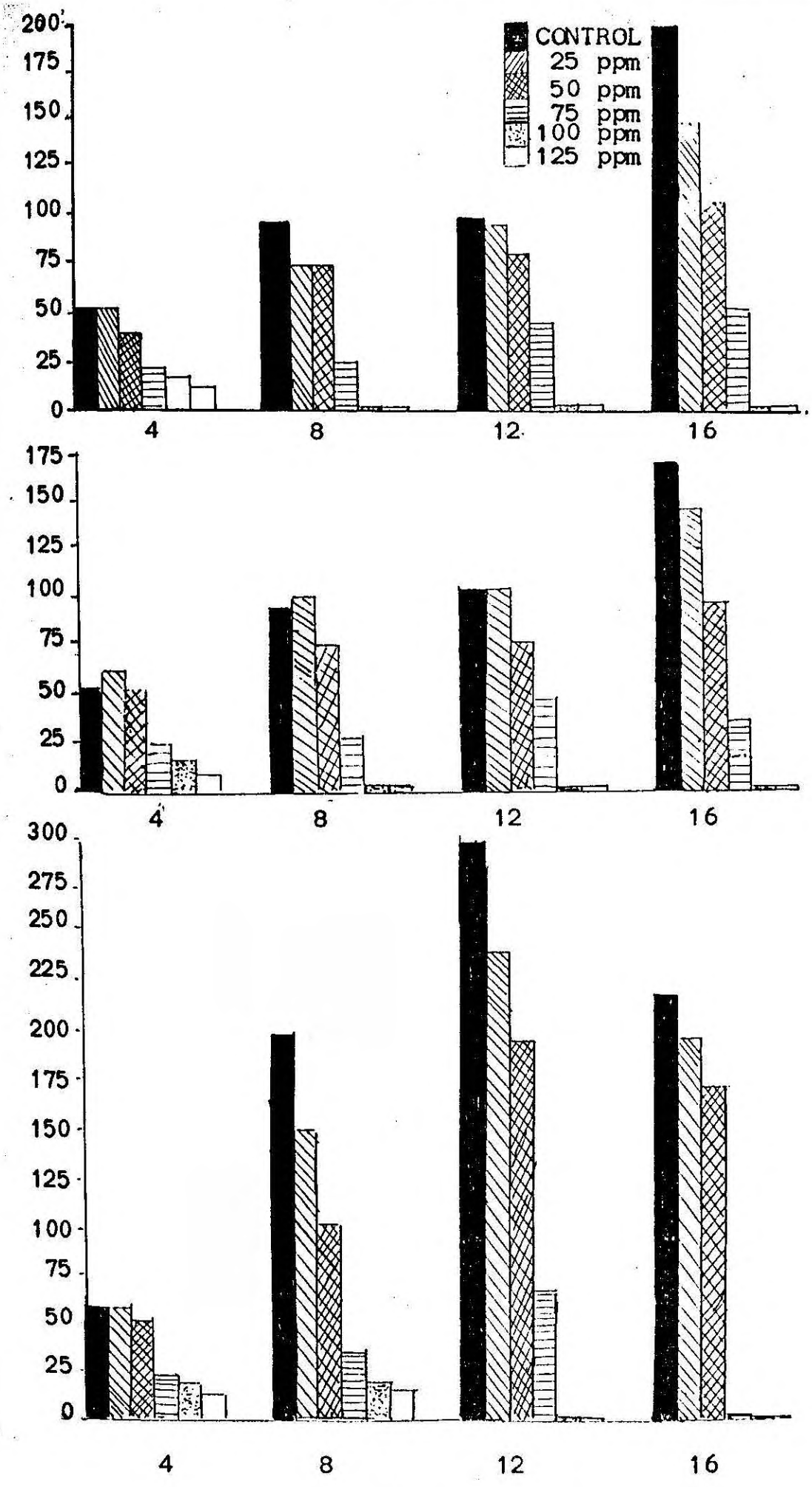


Fig. 36

Estimation of chlorophyll contents revealed that about 100% retardation in 70,100 and 125 ppm treatments (Fig. 36). 80% inhibition was noted for Chlorophyll a in 50 ppm treatments. Chlorophyll b and c was much inhibited by lead toxicity than Chlorophyll a.

EFFECT OF HEAVY METALS ON NATURAL PHYTOPLANKTON COLLECTED FROM A MANGROVE ECOSYSTEM

A similar type of experiment was conducted with natural phytoplankton collected from the mangrove ecocystem known as 'Mangalavanam'. By this study it is possible to assess the effect of some levels of different heavy metals on phytoplanktons from two different ecosystems.

Following was the main species of Phytoplankton population observed in the water sample collected from mangrove ecosystem such as species Nitzschia, Scenedesmus, Chaetoceros, Thalassisira, and Navicula.

Five different levels of heavy metals were selected. Duration of study was 16 days. Growth and primary production estimated on alternate days and chlorophyll contents on every fourth day.

Effect of Copper

Varying concentration of copper selected was 5 ppm, 10 ppm, 15 ppm, 20 ppm and 25 ppm. There was a control for each experiment.

Control exhibited an active growth rate upto 12th day of culture (55×10^4 cells/ml). About 30% inhibition of cell multiplication noted in 5 ppm treatment (Fig. 37). On 16th day about 70% and 80 % retardation

PRAWN CULTURE POND - EFFECT OF COPPER ON GROWTH RATE

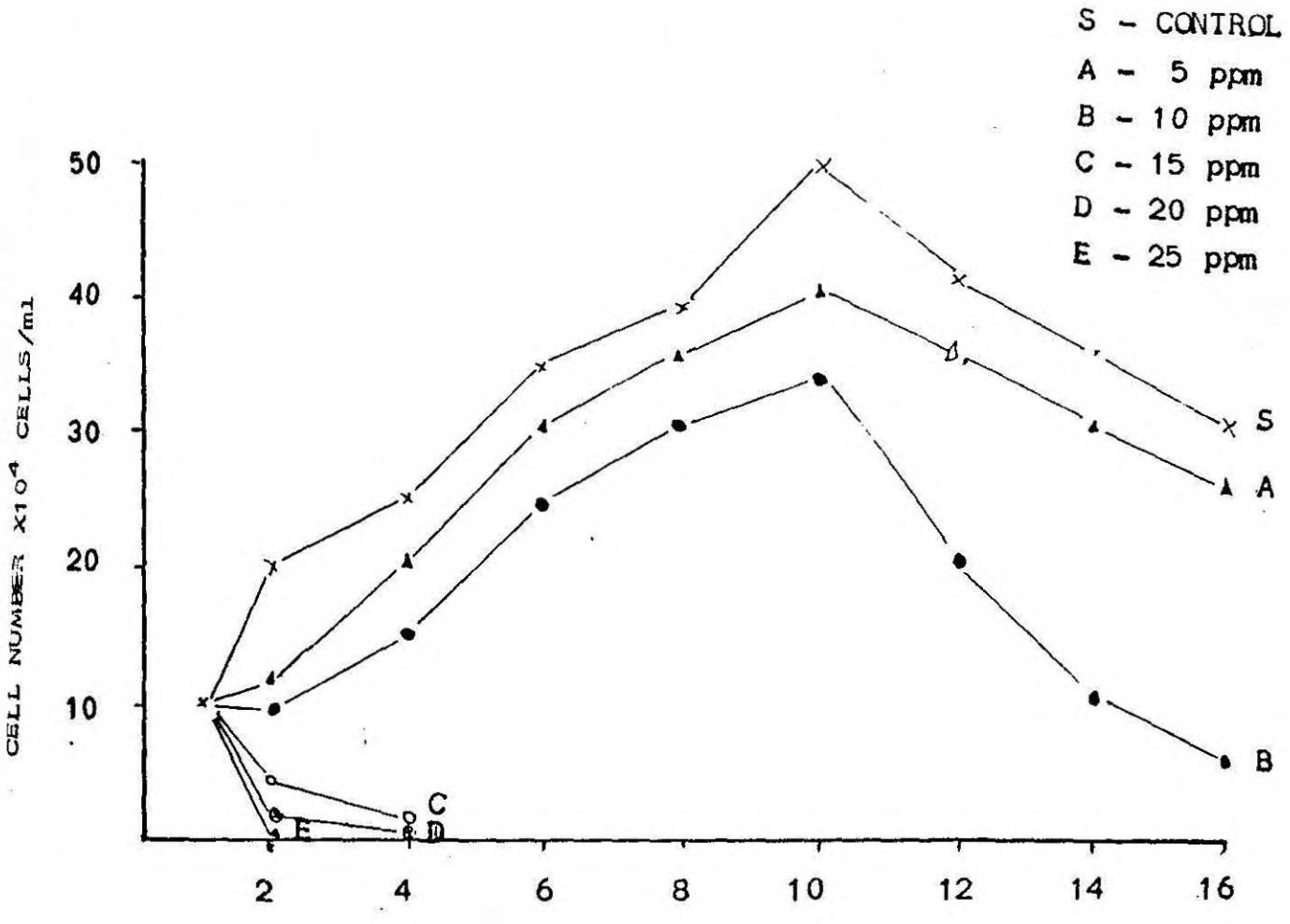


Fig. 28

MANGROVE ECOSYSTEM - EFFECT OF COPPER ON PRIMARY PRODUCTION

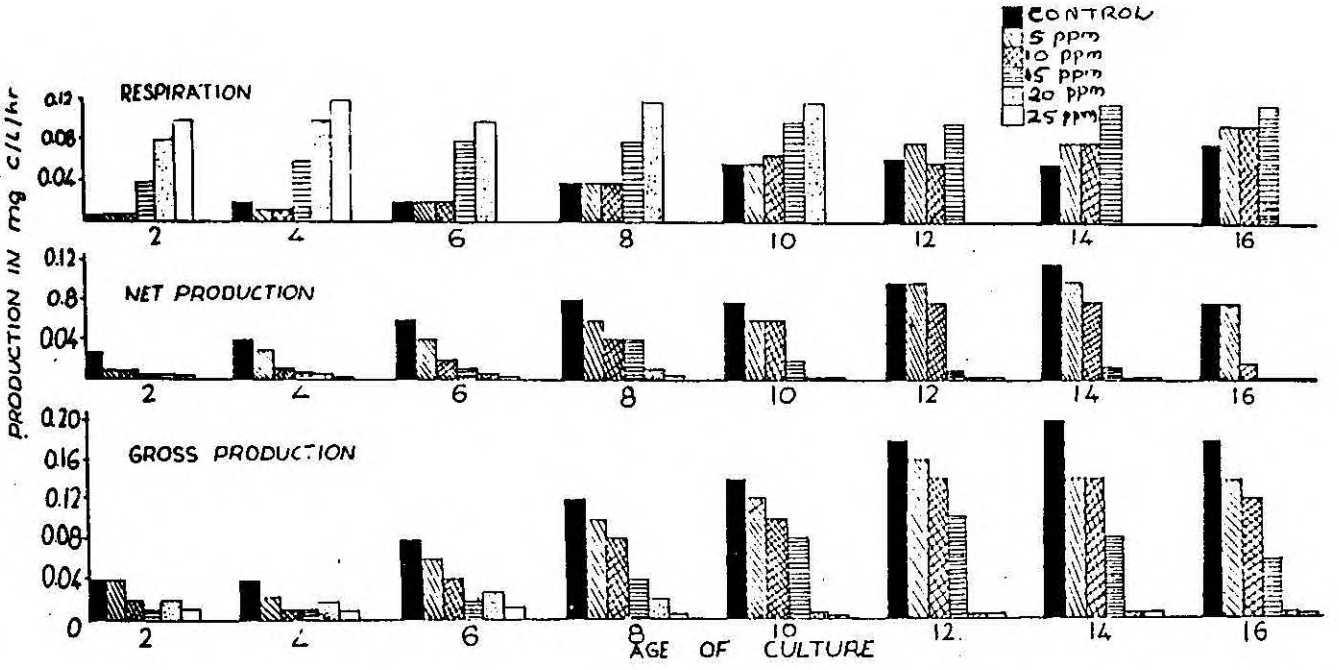


Fig. 38

MANGROVE ECOSYSTEM - EFFECT OF COPPER ON CHLOROPHYLL CONTENT

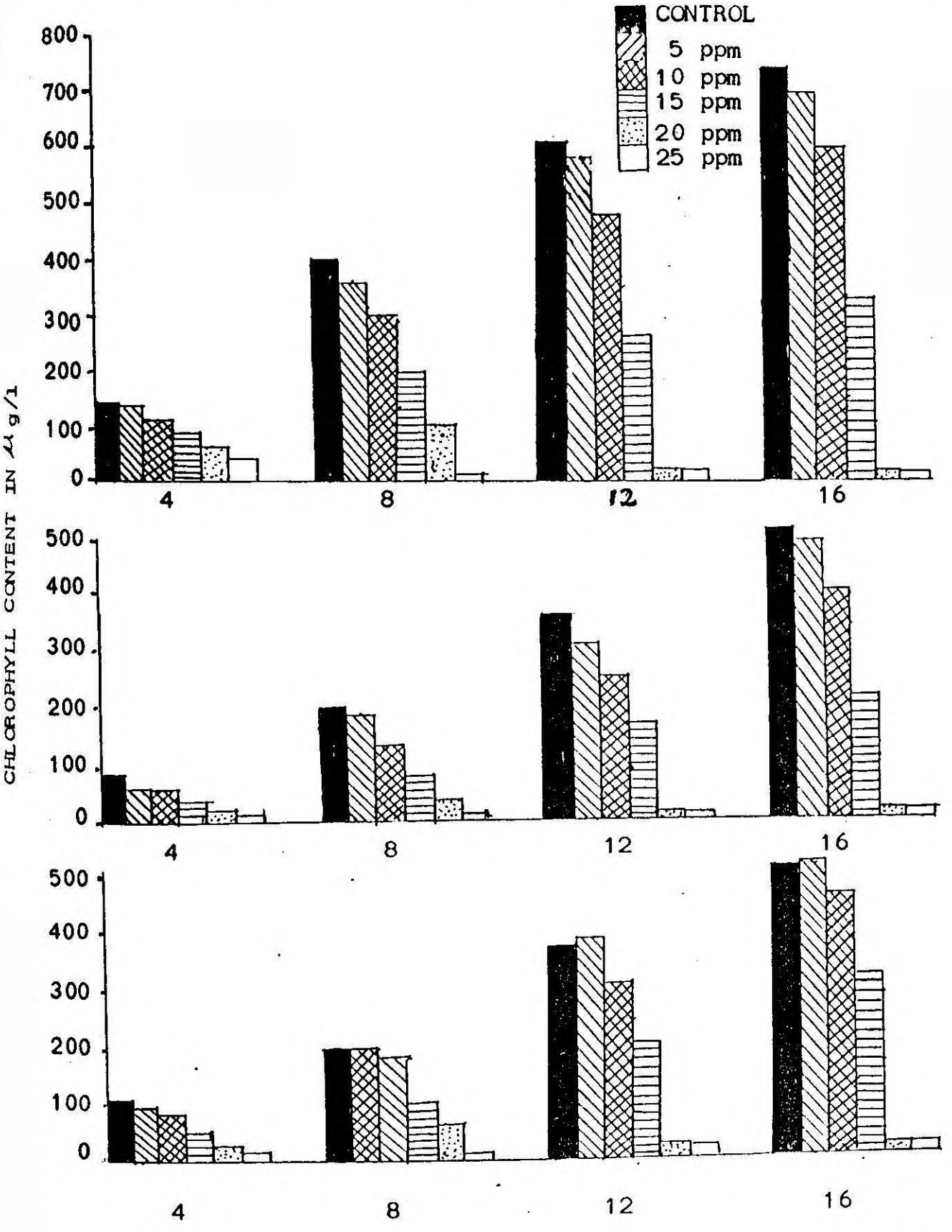


Fig. 39

of cell division noted in 10 and 15 ppm. The 20 ppm treatment expressed 98% mortality on 10th day. However in 25 ppm 100% retardation observed on 6th day.

Total carbon production in the control was almost gradually increasing throughout the study period. Gross production was very less compared to control in all the treatments. From 6th day onwards, production in 20 and 25 ppm level was nearer to zero (Fig. 38). But in 15 ppm, 66% of retardation of gross production noticed on 16th day. Net production in all the treatments was much inhibited by accelerating the respiratory mechanism. In 15, 20 and 25 ppm, net production indicated almost 98% retardation whereas in 5 ppm level, 17% of reduction in net production determined.

Reason for the total reduction in primary production was due to the accelerated respiratory mechanism. In all the treatments respiratory action revealed very high degree of enhancement. In the highest concentrations respiratory rate was above the total carbon production. 50-70% enhancement of respiration determined in higher treatments.

Chlorophyll contents are somewhat equally inhibited by copper toxicity (Fig. 39). In the higher concentrations (20 and 25 ppm) it expressed about 95% retardation from the first day of experiment itself. However, pigments in 5 and 10 ppm revealed 5-8% reduction in all the chlorophyll pigments.

These observations revealed that effect of copper on natural population of phytoplankton from the mangrove ecosystem is very high than on the unialgal cultures. All the parameters estimated revealed almost similar rate of inhibition throughout the study period.

Effect of Zinc

Varying concentrations of Zinc selected for this study was 30 ppm, 40 ppm, 50 ppm, 60 ppm and 70 ppm. Growth rate and primary production on alternate days and Chlorophyll contents on every fourth day estimated.

Control showed an active growth pattern from 2nd day onwards. Cell number was maximum on 14th day of culture (55 x 10^4 cells/ml). Culture in the 30 and 40 ppm treatments showed almost similar growth rate to that of control (Fig. 40). On 14th day it declined. There was only 5 and 10% inhibition of cell multiplication in these treatments. Culture in the 50 ppm expressed an active growth rate upto 12th day but next day onwards it suddenly declined with about 44% of inhibition. Growth in the 60 ppm was stationary upto 8th day then it declined to a very less number. About 80% of inhibition occured in this treatment. But culture in the 70 ppm declines from 6th day onwards and on 12th day 95% retardation determined. These observations revealed that 50% of inhibition of cell division happend in 50 ppm and above treatments.

Effect of this heavy metal arrested the photosynthetic rate of this mixed culture collected from mangrove area. 6th day onwards, gross production in 60 and 70 ppm was nearer to zero. Production in all other treatments also inhibited very much (Fig. 41). Net production in 50, 60 and 70 ppm revealed almost 95% of retardation. Respiratory

MANGROVE ECOSYSTEM – EFFECT OF ZINC ON GROWTH RATE

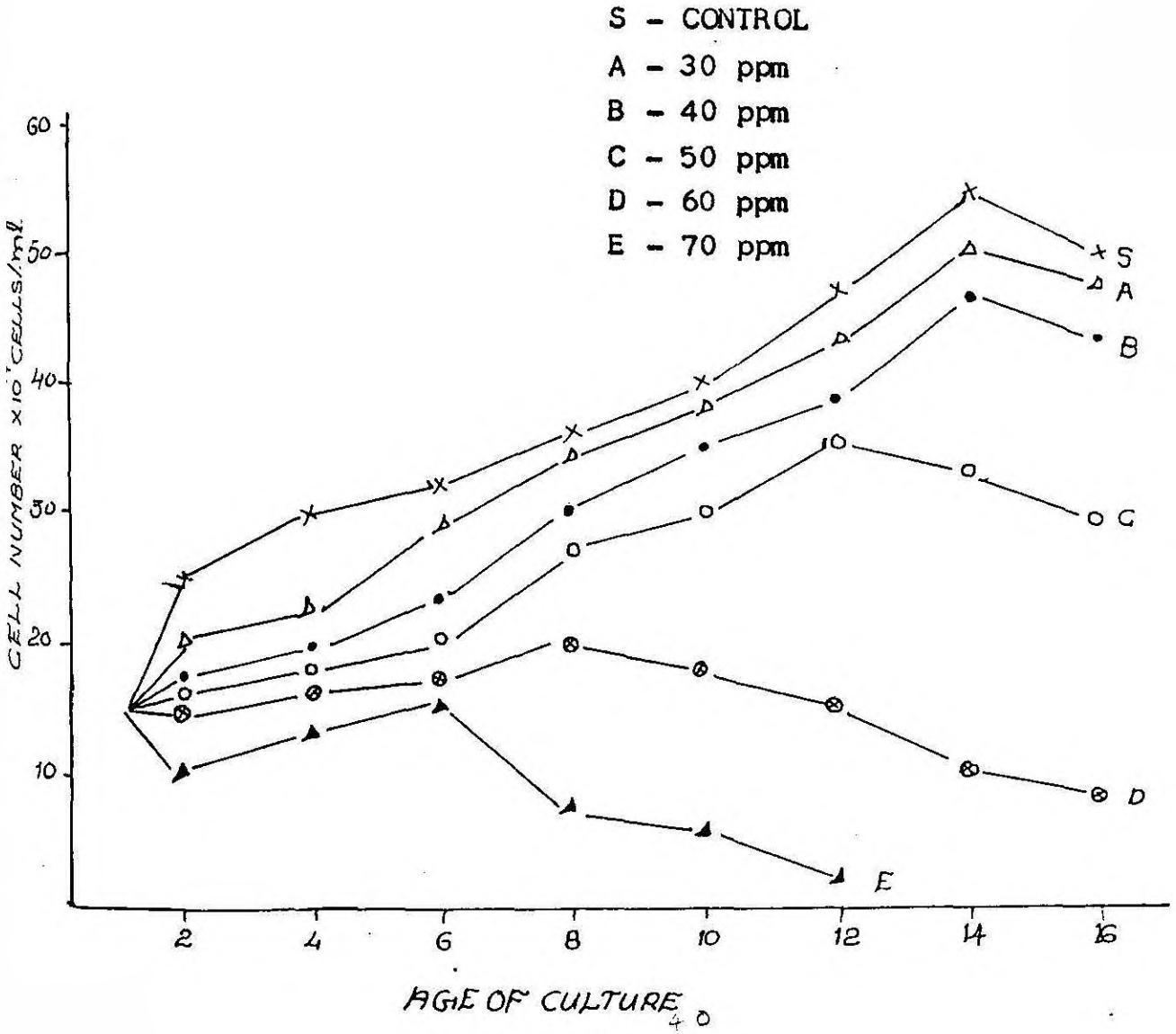
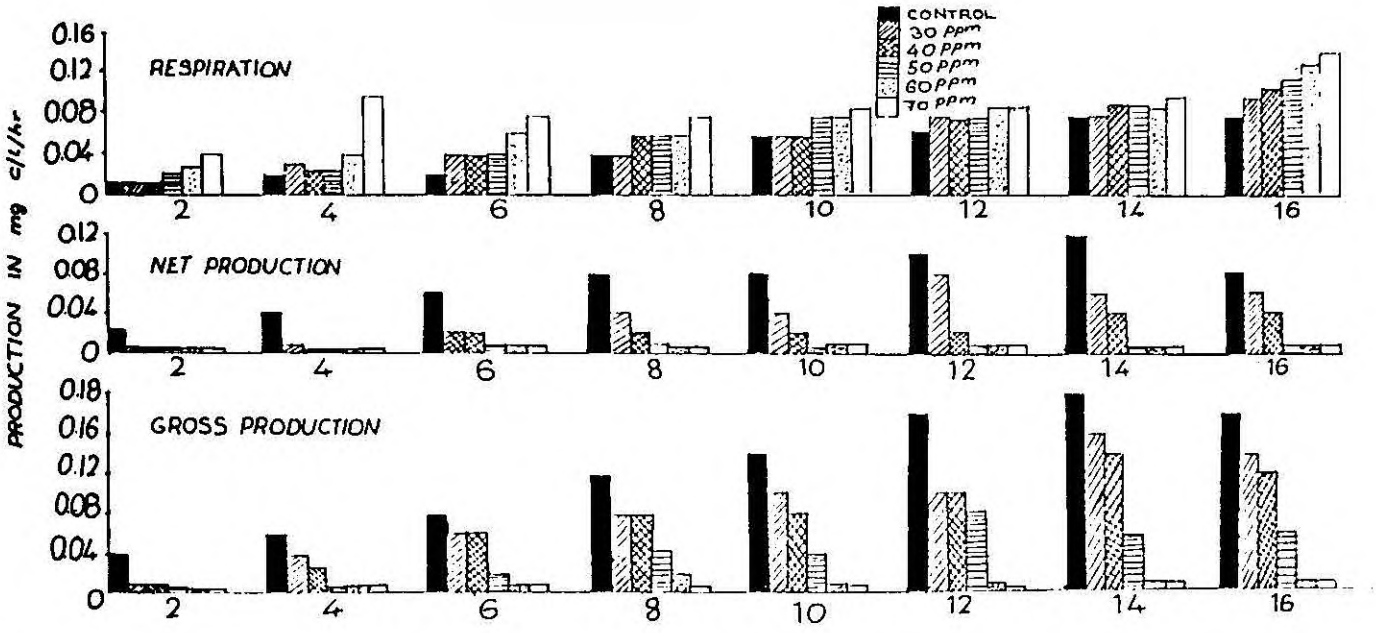


Fig. 40.

MANGROVE ECOSYSTEM - EFFECT OF ZINC ON PRIMARY PRODUCTION



AGE OF CULTURE

Fig. 41

MANGROVE ECOSYSTEM – EFFECT OF ZINC ON CHLOROPHYLL CONTENT.

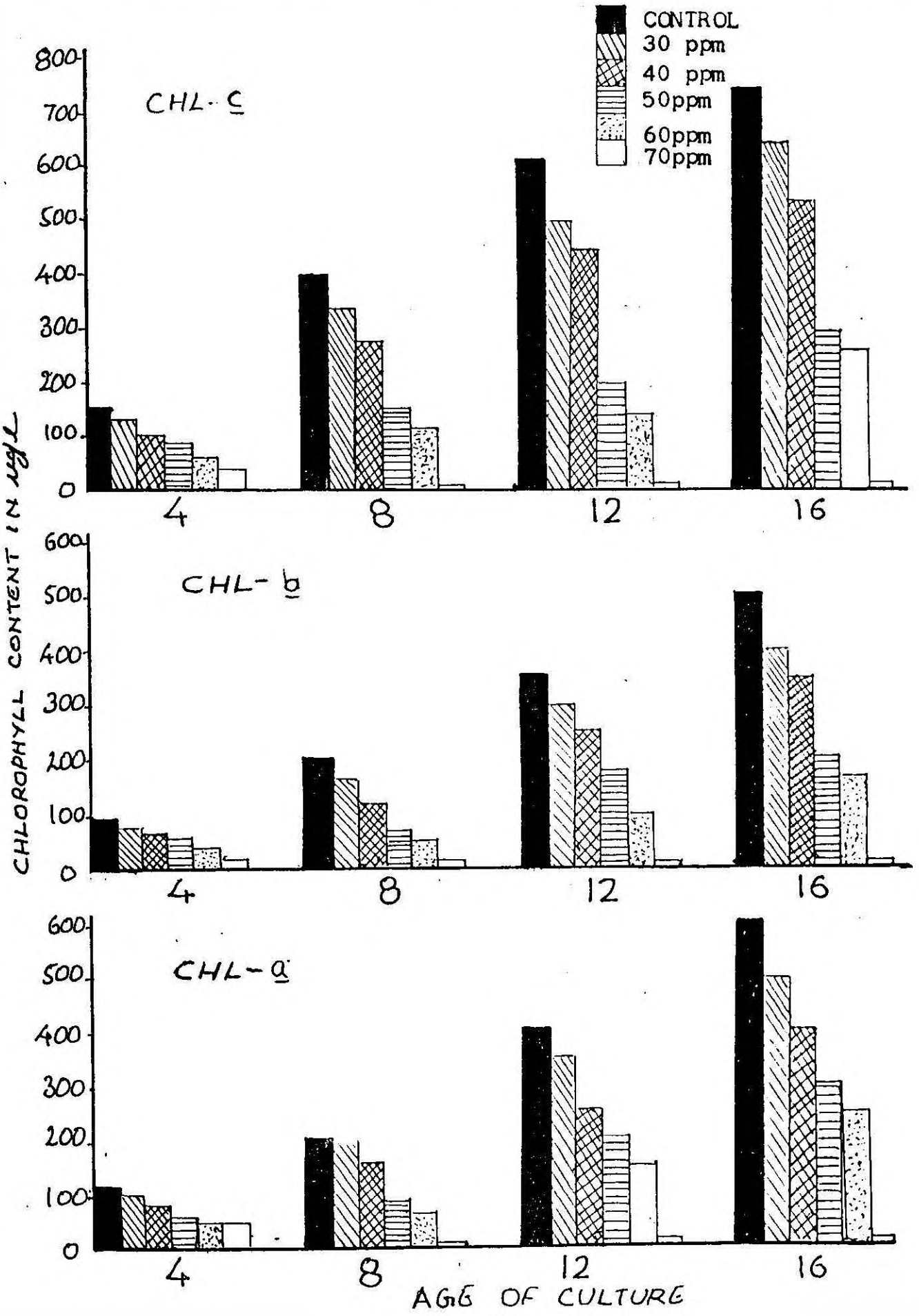


Fig. 42.

mechanism was very much accelerated in all the treatments throughout the study period. Culture in 60 and 70 ppm treatment expressed about 42 and 46% stimulation of respiratory action.

Chlorophyll pigments inhibited much more in all the treatments due to zinc toxicity (Fig. 42). In 30 ppm treatment, pigments expressed about 16% retardation of pheopigments but culture in 70 ppm treatment revealed almost 100% of retardation from 8th day onwards. In all the treatments there was not much variation in chlorophyll a content compared to control. Chlorophyll a was more affected by Zinc toxocity than the other two pigments.

However, the treatments showed very less production compared to the Chlorophyll pigments present which means that most of the pheopigments present was dead. Due to this reason production was less. Abnormality in the culture conditions may also affected the total carbon production.

Effect of Lead

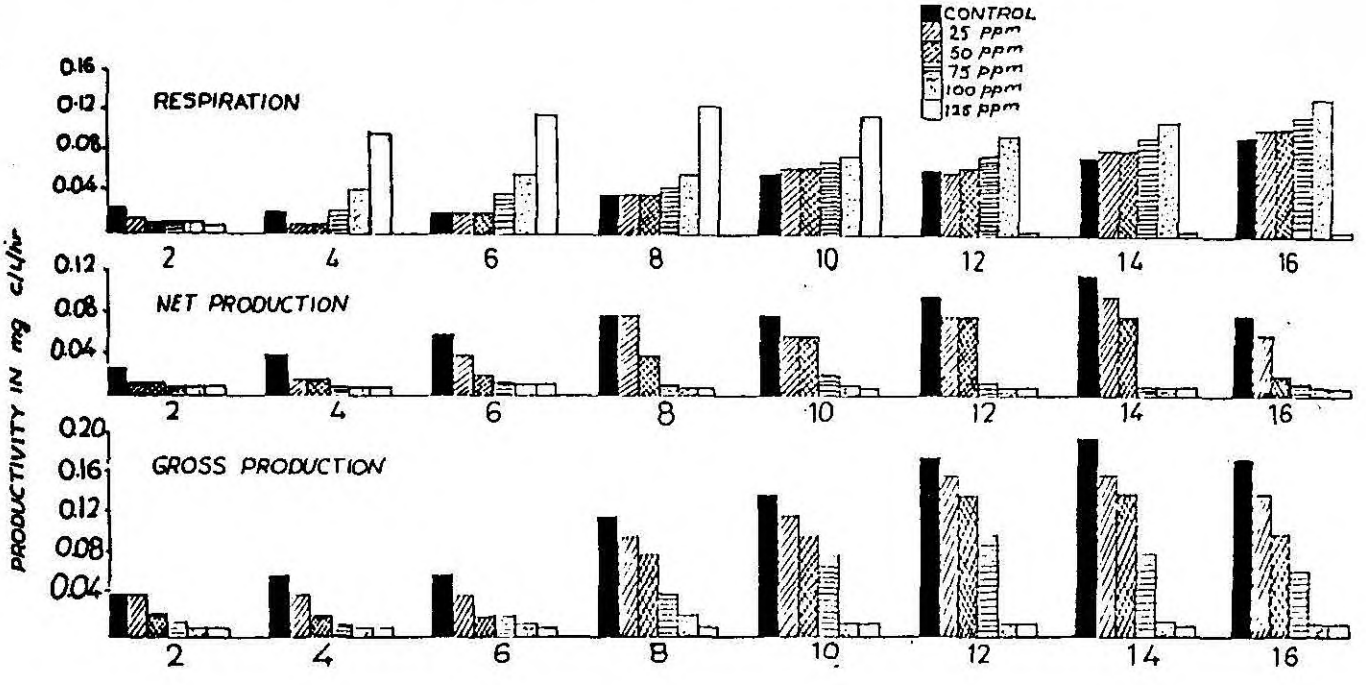
Five different concentrations of lead nitrate selected for this study was 25 ppm, 50 ppm, 75 ppm, 100 ppm and 125 ppm.

In control maximum number of cell noted was on 14th day (55 x 10^4 cells/ml). Cell division rate in 25 and 50ppm was almost parrelel to that of control, but it indicated declining tendency from 14th day onwards (Fig. 43). Rate of growth inhibition was by about 4% and 10% respectively.

MANGROVE ECOSYSTEM - EFFECT OF LEAD ON GROWTH RATE

Fig. 43

MANGROVE ECOSYSTEM - EFFECT OF LEAD ON PRIMARY PRODUCTION



AGE OF CULTURE

Fig. 44

MANGROVE ECOSYSTEM – EFFECT OF LEAD ON CHLOROPHYLL CONTENT

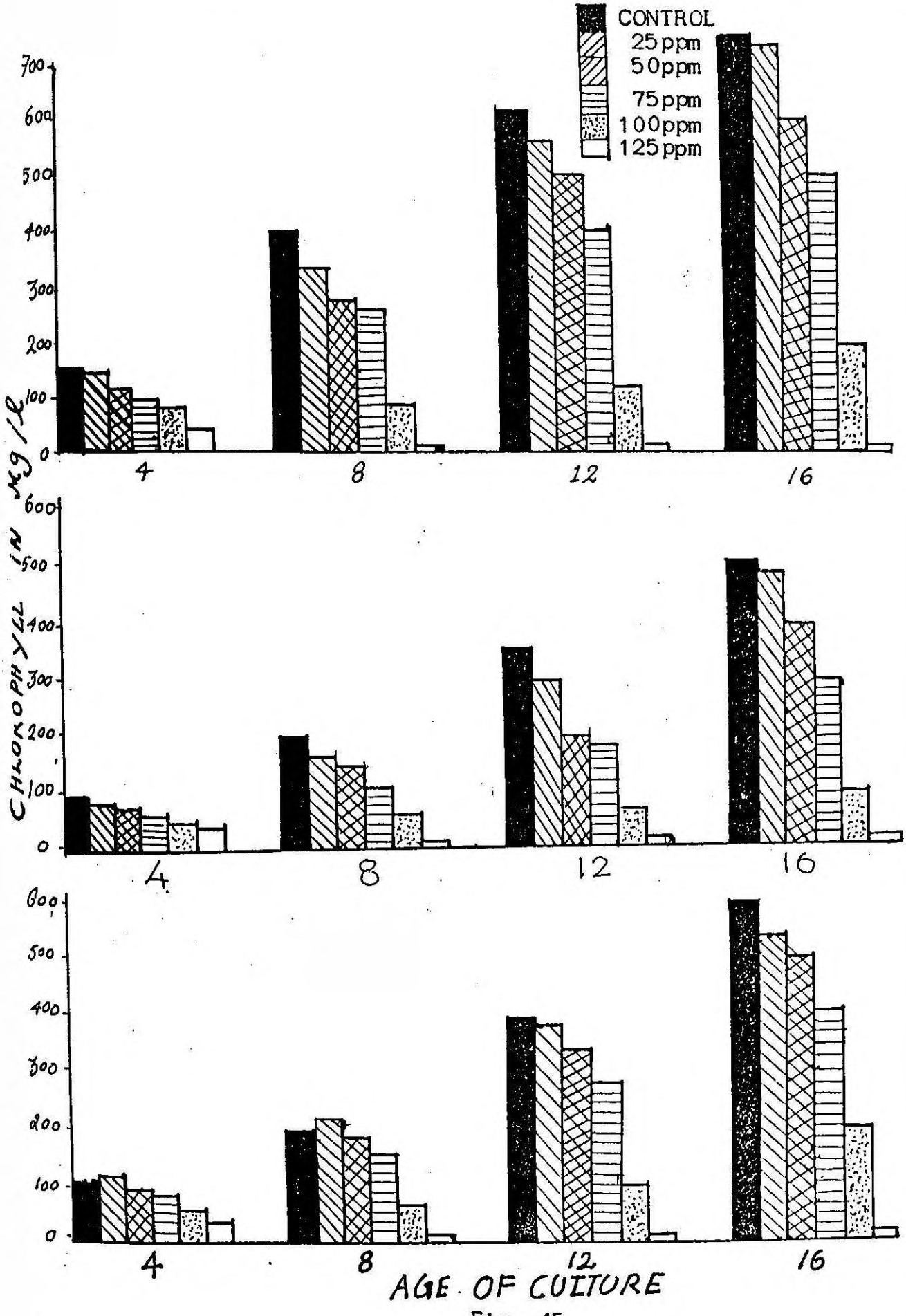


Fig. 45

However, a sudden retardation in cell division rate found out for 75 ppm, on 12th day of experiment. There was about 40% of inhibition in growth on final day but upto 12th day it expressed only 30% of retardation. Culture in 100 and 125 ppm indicated death phase from 8th and 10th day onwards. About 100% of mortality determined in100 ppm on 12th day but 75 ppm showed 84% of growth inhibition.

Primary production in 25 ppm treatment revealed 22% of inhibition. But no stimulation of respiratory mechanism occured (Fig. 44). Whereas culture in 50 ppm treatment showed 44% retardation in production. Rate of inhibition in 75 ppm was about 66%. Stimulation of respiratory action noted in this treatment, so that net production was very less. In 100 ppm treatment 95% of retardation of production determined. On 16th day 46% enhancement of respiratory action also happend in this treatment. On 6th day itself 125 ppm treatment revealed 100% inhibition of total carbon production.

Chlorophyll pigments in all the treated cultures showed retardation (Fig. 45). But when compared to the amount of Chlorophyll content, the total production was less. The reason for this was most of the Chlorophyll pigments was dead. About 66% inhibition of chlorophyll a content determined in 100 ppm where as 125 ppm showed 15% retardation. Chlorophyll a content was more affected than the other two pigments.

Of the three parameters tested, growth and primary production was most markedly affected by Zinc toxicity than Chlorophyll pigments.

STATISTICAL INTERPRETATION

The inference derived from the 2 way Analysis of variance and 't' test by the method of Snedecor and Cochran (1967) are given below:

Effect of Copper on Tetraselmis gracilis, Chromulina freiburgensis and Chaetoceros calcitrans.

(Table. 1-2)

The Analysis of variance revealed that different levels of copper has highly significant effect on the growth rate, primary production, and chlorophyll contents.

Using 't' test, when compared the means, it is found out that, 15-25 ppm level of copper has significant difference in growth rate and gross production whereas 20-25 ppm showed significant effect on respiration and chlorophyll contents of this three species of microalgae in relation to control. It is also found out that, the three species of microalgae are significantly differing from each other with respect to the copper toxicity.

Effect of Zinc on Tetraselmis gracilis, Chromulina freiburgensis and Chaetoceros calcitrans.

(Table 3 - 4).

It is revealed from the Analysis of variance that varying levels of zinc has highly significant effect on the growth rate, gross production and respiration whereas chlorophyll contents showed the significance at 1% level.

When compared the means by 't' test, it is proved that 50-70 ppm of zinc has significant effect on growth rate whereas 30-70ppm levels has significant effect on primary production. 70 ppm level of zinc was significant for chlorophyll a and 60-70 ppm for chlorophyll b and c. Species wise also it showed significant difference.

Effecct of lead on Tetraselmis gracilis, Chromulina freiburgensis and Chaetoceros calcitrans

(Table 5 - 6).

Statistical analysis of data proved that varying levels of Lead has highly significant effect on this three species of microalgae with respect to the parameters like growth rate, gross production and respiration.

Chlorophyll contents showed significance at 1% level. Species wise there was no significant difference in gross production. Hence, chlorophylla and b showed significant difference at 5% level on species wise but there was no significant difference in chlorophyll c content.

In all the species primary production showed significant difference from 50-125 ppm but growth rate showed significant difference from 75-125 ppm levels of lead in relation to control. However, chlorophyll contents revealed significant difference from 100-125 ppm.

Effect of Copper on Natural Phnytoplankton Population

(Table. 7.).

Analysis of variance revealed that ecosystem wise (Prawn culture pond and Mangrove ecosystem) there was no significant difference in

growth rate of the natural phytoplankton population with varying levels of copper. Different levels of copper revealed highly significant effect on growth rate, primary production and chlorophyll contents.

In different levels of copper all treatments employed i.e., 10-25 ppm were found to be significant in relation to control with respect to parameters like growth rate and gross production whereas respiration and chlorophyll contents indicated significant difference from 15-25 ppm levels of copper.

Effect of Zinc on Natural Phytoplankton Population
(Table. 8).

Different levels of zinc has highly significant effect on the growth rate and primary production of the natural phytoplankton population collected from two different ecosystems. In the two ecosystems, chlorophyll contents showed significance at 1% level. Ecosystem wise there was no significant difference in chlorophyll a content but chlorophyll b and c showed significant difference at 1% level.

When compared the means using 't' test it is revealed that, there is significant difference at 50-70 ppm for growth rate and primary production whereas chlorophyll contents showed significance from 60-70 ppm level of zinc.

There was significant difference between two ecosystem in relation to all the above mentioned parameters.

Effect of Lead on Natural Phytoplankton Population
(Table. 9).

Analysis of variance revealed that, lead has significant effect at 1% level on the growth rate, primary production and chlorophyll contents to natural phytoplankton population. Ecosystem wise, gross production showed significant difference at 5% level.

The natural phytoplankton population of the two ecosystems are significantly differing from each other with respect to all the parameters studied. 75-125 ppm level of lead is found to be having significant effect on growth rate, primary production and chlorophyll contents.

2 WAY ANOVA TABLE

Effect of copper on Tetraselmis, Chromulina and Chaetoceros

Table 1. Growth rate

Source	DF	SS	MS	F
Replication	7	32980.	4711.446	13.7625**
Species	2	10378.067	5189.033	15.1576**
Copper level	4	97107.700	24276.925	70.9148**
Species x levels interaction	8	23206.650	2900.856	8.4736**
Error	98	33549.250	342.339	
Total	119	197221.992		

Table 2. Gross production

Source	DF	SS	MS	F
Replication	7	0.244	0.035	21.9191**
Species	2	0.018	0.009	5.6105**
Copper Level	4	0.184	0.046	28.8276**
Species x levels interaction	8	0.001	0.000	0.0586
Error	98	0.158	0.002	
Total	119	0.602		

Effect of Zinc on Tetraselmis, Chromulina and Chaetocers

Table 3. Growth rate

Source	DF	SS	MS	F
Replication	7	37740.458	8246.637	34.8230**
Species	2	14460.950	7230.475	30.5252**
Level	4	39552.300	9888.075	41.7449**
Level x Species Interaction	8	2557.050	319.631	1.349
Error	98	23213.167	236.869	
Total	119	137523.923		

Table 4. Gross production

Source	DF	SS	MS	F
Replication	7	0.133	0.019	17.2177**
Species	2	0.77	0.039	34.9095**
Level	4	0.167	0.042	37.7324**
Level x Species Interaction	8	0.007	0.001	0.7516
Error	98	0.109	0.001	
Total	119	0.493		

Effect of Lead on *Tetraselmis*, *Chromulina* and *Chaetoceros*

Table 5 Growth rate

Source	DF	SS	MS	F
Replication	7	35171.058	5024.437	27.0186**
Level	4	27154.00	6788.500	36.5047**
Species	2	10091.850	5045.925	27.1341**
Species x levels Interaction	8	4706.900	588.362	3.1639**
Error	98	18224.317	185.962	
Total	119	95348.125		

Table 6. Gross Production

Source	DF	SS	MS	F
Replication	7	0.217	0.031	12.3936**
Level	4	0.387	0.096	38.2435**
Species	2	0.008	0.004	1.5451
Species x levels Interaction	8	0.072	0.009	3.6032**
Error	98	0.243	0.003	
Total	119	0.924		

Effect of Copper on Natural Phytoplankton Populations, From Mangrove Ecosystem and Prawn culture pood.

Table 7 Growth rate

Source	DF	SS	MS	F
Replication	7	3803.35	543.336	10.0208**
Ecosystem	4	120.050	120.050	2.2141
Level	4	7982.073	1995.519	36.8037**
Level x Ecosystem Interaction	4	804.575	201.144	
Error	63	3415.900	54.221	
Total	79	16125.950		

Effect of Zinc

Table 8 Growth rate

Source	DF	SS	MS	F
Replication	7	2548.287	364.041	5.6912**
Ecosystem	1	644.112	644.112	10.0697**
Level	4	10556.425	2634.106	41.2582**
Level x Ecosystem Interaction	4	592.325	148.681	2.3150
Error	63	4029.837	63.966	
Total	79	18370.988		

Effect of Lead

Table 9 Growth rate

Source	DF	SS	MS	F
Replication	7	2468.388	352.627	5.4560**
Ecosystem	1	577.813	577.813	8.9402**
Level	4	10196.425	2549.106	39.4411**
Ecosystem x level Interaction	4	675.123	168.781	2.6115**
Error	63	4671.737	69.631	
Total	79	77989.488		

** Significant at 1% level

* Significant at 5% level.

TOXIC EFFECT OF HEAVY METALS ON MICROALGALCULTURE

It is evident from the present investigation that copper is the most toxic and lead is the least toxic heavy metal to these three species of microalgae. The order of toxicity of the three heavy metals to Tetraselmis gracilis, Chromulina freiburgensis and Chaetoceros calcitrans was Cu > Zn > Pb.

The tolerance limit of this three species of microalgae to the heavy metals copper, zinc and lead was varying according to their physiological and morphological features. From the present investigation it is revealed that the phytoflagellate Chromulina freiburgensis is the least tolerant species to this heavy metals. The Chlorophycean, Tetraselmis gracilis is the most tolerant species to copper toxicity. However, the marine diatom Chaetoceros calcitrans was the most tolerant species towards zinc and lead toxicity. It is also revealed from the investigation that cell division and primary production were most inhibited than the chlorophyll contents in this three species of unialgal cultures due to heavy metal toxicity. Respiration rate was enhanced in all the heavy metal treated cultures.

TOXIC EFFECCT OF HEAVY METALS ON NATURAL PHYTOPLANKTON POPULATION

The present investigation proved that, of the two ecosystems (Prawn culture pond and Mangrove ecosystem) selected for the study, natural phytoplankton population collected from mangrove ecosystem were found

to be more tolerant towards copper, zinc and lead toxicity compared to the populations of prawn culture pond. Lead was found to be least toxic metal towards natural phytoplankton population of the two ecosystems. Compared to zinc and lead copper was the most toxic heavy metal to these natural populations of phytoplankton.

It is also observed from the present study that, compared to unialgal cultures of microalgae, natural phytoplankton population was more sensitive to copper, zinc and lead toxicity. As in the case of unialgal cultures, here also, cell division and primary production were more inhibited by metal toxicity than the chlorophyll pigments. Because of metal toxicity, enhancement of respiratory rate observed in all the metal treated natural phytoplankton population.

Heavy metal Toxicity to Microalgae.

Metal	Algae
Copper > Zinc > Lead	Generalization
Copper	Tetraselmis > Chaetoceros > Chromulina
Zinc	Chaetoceros > Tetraselmis > Chronulina
Lead	Chaetoceros > Tetraselmis > Chromulina
Copper > Zinc > Lead	Natural phytoplankton population
Copper, Zinc, Lead	Phytoplankton from Mangrove Ecosystem > Phytoplankton from Prawn culture pond.

DISCUSSION

DISCUSSION

The present investigation on the effect of heavy metals (Copper, Zinc and Lead) on the phytoplagellates such as Tetraselmis gracilis and Chromulina freiburgensis and the marine centric diatom Chaetoceros calcitrans has been restricted to laboratory based experiments employing a single species grown axenically in different concentrations of the heavy metals. The adoption of batch cultures allowed the simultaneous study of the effect of a wide range of metal concentrations upon cell population, all taken from the same stock culture and therefore initially in the same physiological conditions.

As the culture conditions, to which the three microalgal, species exposed were identical, the difference in their growth rates can be attributed to species specificity or upon the size of the inoculum introduced initially. The relationship between the supply of copper, Zinc and Lead and the growth of Tetraselmis, Chromulina and Chaetoceros have been considered from different aspects. The supply of these essential elements at higher concentrations limited the growth rate of this algae. The chemical or physical form in which copper, zinc and lead are present may be of significant importance in governing their toxic effect of this three species of microalgae.

In the present study of the three heavy metals, Copper is found to be most toxic whereas Zinc and Lead were found to be least toxic to both unialgal cultures of microalgae and natural phytoplankton populations.

Rao and Sivasubramanian (1985) have reported that, compared to Copper, Zinc and Lead were less toxic to five species of marine diatoms such as Acnanthes haukiana, Amphora coffeaeformis, Fragilaria pinnata, Synedra tabulata, Thalassiosira fluviatilis and Triceratium dubium. All the diatoms tolerated upto 25 ppm Zinc and Lead but copper inhibited the growth of all these marine diatoms at 10 ppm. Gachter (1976) indicated that compared to Copper, Zinc and Lead are least toxic to natural phytoplankton populations. Sorentino (1979) also got similar results with marine phytoplankton populations. In the present study, the three species studied showed growth upto 20 ppm of copper, 60 ppm of Zinc and 100 ppm of Lead.

From the present study it is revealed that there is significant difference in between species to tolerate metal toxicity. With regards to the three species considered for the present study the haptophycean flagellate Chromulina freiburgensis is found to be more sensitive to copper and zinc toxicity. While the chlorophycean flagellate Tetraselmis gracilis is found to be more tolerent to copper toxicity. But the marine centric diatom Chaetoceros calcitrans found to be least sensitive towards Zinc and Lead toxicity. This observation is supported by the fact that major trend among species in their resistence to heavy metal toxicity has been a phylogenetic one with the marine diatoms and chlorophycean members being the least sensitive whereas haptophyceans and cyanophyceans being the most sensitive (Mandelli, 1969; Erickson et al., 1970, Brand, Sunda and Guillard, 1986).

The high tolerence of the marine centric diatom Chaetoceros calcitrans to heavy metal toxicity may be due to the rigid silicious cell walls while the other two species have cellulose cell wall. The phytoflagellates Tetraselmis and Chromulina were more sensitive to metal toxicity than Chaetoceros, may be because of its losing of flagella leading to the immobility and mortality of cultures. While the sedentery form Chaetoceros did not affected in this way due to its lack of flagella and moving capacity. Loss of mobility due to copper toxicity was reported by Andersen and Morel (1978) in Gonyaulax tamarensis.

The chlorophycean member Tetraselmis gracilis is found to be more tolerant to copper toxicity than Chromulina and Chaetoceros. Greater tolerance of Tetraselmis gracilis to copper can be accounted by the organisms capacity to block the entrance of cations to the cell. This may be accomplished either through binding of the metal to the cytoplasmic membrane or to the extracellular polysaccharides or by changing the chemical form in which copper is present, so as to render it toxicologically inactive. Some biotic factors (i.e., characteristic of the organism themselves) also influence the sensitivity of microalgae to metals. Cell size may be a factor influencing microalgal sensitivity to toxicants, for example the concentration of lead that caused a 50% reduction in phytosynthesis was 15-18 ppm for Chlamydomonas reinhardtii and Navicula pelliculosa but only 5 ppm for the desmid Cosmarium botrytis which has a higher surface: volume ratio than the other algae and which may have accounted for its enhanced sensitivity to lead (Malanchuk and Gruendling, 1973).

Berland et al., (1976) reported that Tetrasalmis striata is more tolerent to copper toxicity than Chaetoceros didymus.

All the three species of microalgae such as Tetraselmis, Chromulina and Chaetoceros showed maximum inhibitory effect of growth rate, primary production and Chlorophyll content at 25 ppm of copper but there was growth upto 20 ppm concentrations. Previous studies indicated that copper at a concentration 25-30000 /ug/l was inibitory to Coccochloris elabens, Skeletonema costatum, Glendinium sp., Cylindrotheca closterium and Thalassionsira fluviatilis (Mondelli, 1969); Cryptomonas pseudobaltica, Pavlova lutheri, Pavlova pinguis, Pheoductylum tricornutum and Chlamydomonus palla (Berland et al., 1976). Asterionella glaciltis (Overnell, 1976) and Coccolithus huxleyi (Bernhard and Zatteru, 1970). From the present study it is found out that this three species can tolerate upto 25,000 /ug/l of copper.

Inhibitory levels of copper for cultures of marine phytoplankton have been ascertained by several workers (Erickson et al., 1970). Such levels were mostly 100 /ug/l but varied with species tested. Sri. Sudha (1989) reported that, copper concentrations namely 0.05 ppm and 0.10 ppm were more suitable for the growth of Isochrysis galbana but concentrations above 0.10 ppm were inhibitory to the blue green algae Synechocystis salina. Concentration of copper as low as 1 ppb was reported to be toxic to phytoplankton (Steemann Nielsen et al., 1969; Ericckson, 1972; Kanakavalli susarala 1987). Higher concentration of copper greater

than 0.15 ppm impaired the growth rate and resulted in biomass reductin. Whitton (1976) has listed several macro and micro algae to be tolerant to 1.5-2.0 mg/l copper.

Growth rate reduction with increasing copper level is one of the most prominant observations in the present study. Thomas et al. (1980) reported that inhibition of growth rate in the diatom Thalassiosira aestivalis due to copper toxicity. Concentration of 10-50 /ug/l of copper has little or no effect upon the growth rate in Coccolithus buxleyi and Pheodaehylum tricornutum. (Bernhard and Zattera, 1970). Rao and Sivasubramanian (1985) stated that inhibition of cell division of five species of marine diatoms. Skeletonema costatum and Nitzschia thermalis showed decreased growth rate due to elevated copper concentration (Metaxas and Lewis, 1990). Inhibition of growth rate observed in Chlorella pyrenoidosa and Nitzschia palea (Steemann Nielsen and Wium Andersen, 1970). From the results of Kahn and Saifullah (1986). Skeletonema costatum showed progressively decreasing growth rates due to increasing copper levels with a complete inhibition around $3.2\text{-}4.8\text{x}10^{-7}$M. Morel et al., (1978) also reported inhibition of cell division in Skeletonema costatum with increased copper concentrations. Mandelli (1969) demonstrated growth inhibition in the pennate diatom Nitzschia closterium. Fisher and Frood (1980) examined the effect of copper in the growth of Nitzschia closterium and Asterionella japonica. The growth of these two species was reduced to a third of the control for the former and a half for the latter in the presence of 8×10^{-8} M copper.

The toxic effects of copper on three species of marine phytoplankton such as Chaetoceros mulleri, Isochrysis galbana and Dicrateria inornata were studied by Ning et al., (1990). They found out that all the three species ceased growth by a plateau vaue of 7.0. Steemann Nielsen and Wium-Andersen (1970) and Erickson (1972) have reported that copper concentration as low as 5 µg/l resulted in growth inhibition of diatoms. Canterford, Buchanan and Ducker (1978) found that growth of the diatom Ditylum brightwelli inhibited by copper toxicity.

Inhibition of primary production due to copper toxicity was observed in all the three species of micro algae tested. Rao and Sivasubramian (1985) reported inhibition of primary production in five species of marine diatoms. They found out that copper inhibited photosynthesis by 95% only at 20-50 ppm level of copper. Steemann Nielsen and Wium Andersen (1971) reported that in copper treated cultures of Chlorella pyrenoidosa Pmax was reduced. Reduction in photosynthetic rate of the dinolagellate Gonyaulax tamarensis was found out by Anderson and Morel (1978). Saward et al., (1975) have noticed a depression in both standing crop of phytoplankton and rate of photosynthesis in 0.03-0.10 ppm copper. Copper enhanced microplankton and nannoplankton productivity only upto a concentration of 8 and 4 µg/l and above this level photosynthetic rate decreased enormously even upto 52% in case of nannoplankton and 73% in the case of microplankton fraction. Inhibition due to copper was much more than with other metals (Rajendran et al., 1977).

Enhancement of respiratory rate was another important observation found due to Copper toxicity in the metal treated cultures. Rao and Sivasubramaniam (1985) have reported similar results in five species of marine diatoms. Steemann Nielsen and Wium-Andersen, (1971) reported enhancement of respiratory rate at some concentrations of heavy metals on phytoplankton.

From the present investigation it is found out that chlorophyll pigments are inhibited by copper toxicity. Srisudha (1989) reported that at a higher concentration of copper, 0.15 ppm, the quantity of the pigments were found to be lower than those of control. Mandelli (1969) stata that reduction in chlorophyll pigments due to metal toxicity was caused by destruction of chloroplasts.

Copper is known to affect cell separation in Tetraselmis gracilis, resulting in multicellular aggregates. Similar effect has also been indicated by Fostner (1977) in Chlorella vulgaris. Kanasawa and Kanaswara (1969) have attributed the toxic effect of copper on cellular division due to events related to cell membrane rather than with those occurring inside the cell.

Substantial evidences indicate that the biological availability of copper can be reduced by phytoplankton exudates. Sunda and Guillard, (1976), McKnight and Morel, (1980); have demonstrated that cultures of Thalassiosira pseudonana and Skeletonema costatum excrete substances into the media that are capable of complexing copper. The proceed

discussion indicate that algae possess the capacity to produce extracellular chelators which complex and thereby detoxify the action of copper is also applicable in Tetraselmis, Chromulina and Chaetoceros.

From the present investigation it is found out that compared to copper, zinc is less toxic to Tetraselmis, Chromulina and Chaetoceros and to the natural phytplankton population. In the present study, these three species of microalgae showed growth upto 60 ppm of zinc. Bringmann and Kuhn (1959) found out that compared to copper toxicity zinc is found to be less toxic to Scendesmus sp. Zinc was less toxic than copper to Nitzschia closterium (Rosko and Rachlin, 1975). Gachter (1976) reported that zinc is less toxic compared to other heavy metals in natural phytoplankton population. Rao and Sivasubramanian (1985) also stated that zinc have less toxicity than copper to five species of marine diatoms.

The three species of microalgae such as Tetraselmis gracilis, Chromulina freiburgensis and Chaetoceros calcitrans showed growth upto 60 ppm level of zinc. Bernhard and Zattera (1970) reported that Zinc at 2.5 x 10^2 /ug/l (250 /ug/l) had no effect on the growth of Coccolithus hyxleyi. Aubert et al., (1972) reported that growth of Asterionella glacialis was not affected even at 39,000 /ug/l zinc concentration. Five species of marine diatoms such as Acnanthes haukina, Amphora Coffeaeformis, Fragilaria pinnata, Synedra tabulata, Thalassiosira fluviatilis and Triceratium dubiatum tolerated upto 30000 /ug/l of zinc (Rao and Sivasubramanian, 1985). Sri Sudha (1989) reported that 0.10 ppm of zinc inhibited the

growth of Isochrysis galbana and Synechocystis salina. But reports of previous workers have indicated that a still higher level of zinc (0.271 ppm - 7.10 ppm) caused a 50% reduction in cell division in varous species of marine diatoms and chlorophytes (Rosko and Rachlin, 1975; Rachlin et al., 1982). Break, Malnes and Jensen (1976) found that Skeletonema costatum survived higher than 400 /ug/l of zinc and it is also noted that Thalassiosira psuedonana tolerated upto 100 /ug/l.

The major symptoms of zinc toxicity to microalgal cultures were found to be the inhibition of growth rate. The three species of microalgae tested showed growth inhibition with increased level of zinc concentration.

Jensen et al. (1974) observed that though zinc is an essential element it becomes more toxic at higher levels and different species vary widely in their sensitivity to zinc toxicity. On exposure to higher concentration of 0.10 ppm zinc, Isochrysis galbana and Synechocystis salina showed reduction in growth which is a reasonable determinant of toxic effect. Andersen et al. (1978) have suggested that as a result of zinc toxicity the growth of Thalassiosira weissflogii inhibited. Rao and Sivasubramanian (1985) reported that growth inhibition in five species of marine diatoms. The marine phytoplankton species Chaetoceros mullerii, Isochrysis galbana and Dicrateria inornata expressed reduction in growth rate due to zinc ion activity (Ning et al., 1990). Canterford et al., (1980) suggested decrease in cell number of diatom Ditylum brightwelli at 200 /ug/l of zinc concentration. Anderson and Morel (1978) reported growth inhibition due to zinc ion activity in the coastal diatom Ditylum brightwelli.

Inhibition of photosynthetic rate due to zinc ion activity is observed in all the metal treated cultures of present study. Davies and Sleep (1979) have found 0.01-0.015 ppm of zinc as the minimal concentration causing detectable inhibition of carbon fixation i.e., less than 90% of control values. Zinc were reported to have very little effect on photosynthesis and respiration by marine phytoplankton even at very high concent rations (100-20,000 /ug/l). Rajendran et al., (1977) indicated that, due to zinc ion activity the photosynthesis of microplankton inhibited to a very low level. Break et al., (1976) proved inhibition of photosynthetic activity in Skeletonema and Amphidinium at 0.05-0.10 mg Zn/l. In the present investigation maximum inhibition of photosynthesis determined at 70 ppm level of zinc.

Enhancement of respiratory rate happened in all the metal treated cultures due to increased level of zinc concentration. Rao and Sivasubramanian (1985) have reported that zinc had enhanced respiratory rate by about 2.5% to 100% in five species of marine diatoms. In the present study cultures in the 50-70 ppm level of Zinc showed enhancement of respiratory action.

Dense cultures of Chaetoceros and Tetraselmis are able to resist the toxic action of zinc and the cells begin to grow eventually. This can be accounted by the fact that, the exudation of waste products from the cultures which complex the medium will thus not only compete for and thereby reduce the amount of metal takeup will also decrease the rate of incorporation into the plant cells, both processes providing a degree of protection against toxic effect of the metal (Davies, 1973).

Of the three heavy metal studied Lead was found to be least tolerant to three species of microalgae. Tetraselmis, Chromulina and Chaetoceros exhibited growth upto 125 ppm of lead whereas for copper growth rate found inhibited to the maximum in 25 ppm and for zinc in 70 ppm level. It is established that zinc and lead are least toxic to marine phytoplankton. Berland et al. (1976) reported that 2,000 /ug/l lead was lethal to Cryptomonas pseudobaltica, Pavlova pinguis, Heterothrix sp., Chaetoceros didymus, Skeletonema costatum and Chlamydomonas palla. Rao and Siva Subramanian (1985) have also stated that, five marine species of diatoms tolerated upto 2.5 - 3.0 x 10^4 /ug/l (30000 /ug/l) of lead. In the present investigation the three species studied tolerated upto 12.5 x 10^4 /ug/l lead. Canterford et al. (1980) reported that the marine diatom Ditylum brightwelli tolerated upto 750 /ug/l lead without much inhibition in growth rate. Ning et al., (1990) revealed that compared to copper and zinc, lead is least toxic to Dicrateria inornata.

Inhibition of growth rate in all the metal treated cultures due to lead toxicity is one of the most important observation in the present investigation. But all the three species tested indicated decreased growth rate upto 125 ppm. The marine diatom Chaetoceros calcitrans was found to be least sensitive to lead toxivity. The reason for this explained earlier. Growth rate and biomass of Skeletonema costatum were inhibited by dissolved Lead concentrations between 9.00005 and 0.010 mg/l making this species one of the most sensitive algal indicators of lead salt (Rivkin, 1979). Dunaliella salina was relatively unaffected at 0.3 mg pb/l but showed reduced growth and other adverse effects at 0.9 mg pb/l and higher

concentrations (Pace et al., 1977). In the range 0.25 to 2.0 mg pb/l growth was inhibited among 18 species of unicellular algae (Berland et al., 1976) and in Dunaliella tertiolecta (Stewart, 1977). At 10.0 mg pb/l, slower growth was evident among Platythamnion and Plenosporijm (Stewart, 1977). Batch cultures of Pheodactylum tricornutum, Tetraselmis and Dunaliella and Cricosphaera all grow well at less than 20.0 mg pb/l with adverse effects at higher levels (Bentley-Mowat and Reid, 1977). Rao and Sivasubramanian (1985) noted inhibited growth rate in five species of marine diatoms at 3.0×10^4 ug/l of lead. Ning et al., (1990) also observed growth inhibition in Isochrysis galbana, Chaetoceros muelleri and Dicrateria inornata due to increased level of lead ion activity.

Inhibition of photosynthesis and enhancement of respiratory actions were also observed in the present study due to higher concentrations of lead. Mills and Colwell, (1977) reported reduced oxygen uptake in 3 species of unicellular algae at 25-100 mg pb/l. Rao and Sivasubramanian (1985) stated inhibition of phytosynthetesis and enhancement of respiratory action on five species of marine diatoms at 3.0×10^4 mg/pb/l. From the present study it is revealed that compared to copper and zinc, the inhibition of photosynthetic rate was less in lead treated cultures. Lead were reported to have very little effect on photosynthesis and respiration by marine photoplankton even at very high concentrations (100 to 20,000 ug/l). Another important factor revealed from the present investigation is the inhibition of chlorophyll pigments in all the metal treated cultures. There is not much work has been done on this aspect in earlier days. Mandelli (1969) reported that reduction in chlorophyll pigments due to heavy metal toxicity is caused by the destruction of chlorophyll.

Toxicity of copper, zinc and lead to natural phytoplankton population collected from two ecosystems (Prawn culture pond and Mangrove ecosystem) were also studied. From the results it is revealed that phytoplankton population collected from Mangrove ecosystem is more tolerant towards copper, zinc and lead toxicity. It is well known that, mangrove ecosystem are more productive than other ecosystems. Compared to mangrove ecosystem, the total biomass of phytoplankton occurred in a prawn culture pond is very less. If the cell number is less, naturally the toxic effect will be more because there is not much diversification of metal ions. But in mangrove ecosystem, the total biomass of phytoplankton is more, so more surface area for the binding of metal ions. Another reason for this is phytoplanktons from mangrove ecosystem may be more tolerant towards pollutants than the prawn culture pond. The increased tolerance of microalgae to metals in most instances is physiological rather than genetic adaptation. Species diversity in the two ecosystems is another factor for difference to tolerance of metal toxicity.

As in the case of unialgal cultures, for natural phytoplankton population also copper is found to be highly toxic than zinc whereas lead is found to be least toxic to these populations. Gachter (1976) reported that compared to lead and zinc, copper is more toxic to natural phytoplankton population.

Inhibition of growth rate, primary production and chlorophyll pigments observed in all the metal treated natural phytoplankton populations as symptoms of copper, zinc and lead toxicity. Patin et al., (1974) and Ibragim and Patin (1976) measured decrease in primary production rates caused by copper, zinc and lead toxicity.

Another important fact derived from the present study is that natural phytoplankton populations are more sensitive to copper, zinc and lead toxicity than the unialgal cultures. The main reason for this is the species diversity in the natural populations. The tolerance limit of different species should be differ from each other. In batch unialgal cultures, the cell density will be very high compared to natural phytoplankton population. So naturally, the area for metal activity should be more than in natural populations. Davies (1978) reported that in dense cultures more metal is required to produce a given cellular burden. If the number of cell present in cultures is less, then the toxicity of metal should be more. There is no much work has been done in this aspect to compare the toxic effect of heavy metals on unialgal cultures and natural phytoplankton.

It is concluded from the present investigation that, if some heavy metals are required in trace amounts for growth and metabolism of micro-algae, higher concentration of them cause inhibition of growth rate and primary production which will lead to the complete dissappearance of that particular species. Moreover, the inhibition and stimulation of the growth of the micro-algae due to these heavy metals is controlled by many factors and that toxic threshold of these heavy metals is primarly a function of species composition of the microalgae.

SUMMARY

SUMMARY

This dessertation presents a comprehensive study on the effect of heavy metals namely copper, zinc and lead on three species of micro-algae such as Tetraselmis gracilis, Chromulina freiburgensis and Chaetoceros calcitrans which are of nutritive value and are used as livefood in hatchery system for rearing the larvae of crustaceans, finfishes and molluscs.

The impact of five different concentrations of heavy metals namely Copper, Zinc and Lead upon three species of microalgae and natural phyto-plankton population investigated. The physiological activity of the algae has been expressed by their growth characteristic in terms of cell concentration, quantities of chlorophyll pigments and rate of photosynthesis.

Statistical analysis of data was done by analysis of variance to study the effects of heavymetals and to know the results in significant and non-significant levels.

All the three heavy metals exhibit toxic effects on growth, productivity and pigment contents when their concentration in the medium increased but the important point is that the organism react in different ways and reveal variation in their tolerance. Of the three species, Chaetoceros calcitrans has been found to be more tolerant to zinc and lead wnereas Tetraselmis gracilis showed greater tolerance to copper. Chromulina freiburgensis is the least tolerant species to this three heavy metals.

Inhibition due to copper has been found to be higher than the other two metals even at a very low concentration. In all the metal treated cultures, growth and production was more inhibited by its toxicity than the chlorophyll pigments. Among chlorophyll pigments, chlorophyll a content was more affected than chlorophyll b and c.

Compared to unialgal cultures, natural phytoplankton population treated showed a very rapid inhibition of growth and productivity. Natural population from mangrove ecosystem is found to be more tolerant towards metal toxicity than the populations from prawn culture pond. To natural population also copper was more toxic than zinc and lead.

From the present study, it is concluded that for the successful operation of hatchery system the seawater should have very low concentration of these heavy metals, ie. below 5 ppm of copper, 30 ppm of zinc and 50 ppm of lead for rearing the larvae of economically important cultivable organisms.

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