EFFECT OF SOME TOXIC METALS ON SELECTED PHYTOPLANKTON OF KERALA WATERS

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by

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MARCH - 1987

TO

MY PARENTS

DECLARATION

The thesis submitted by me in partial fulfilment for the Degree of Doctor of Philosophy of Cochin University of Science and Technology is the authentic record of research work carried out by me under the guidance and supervision of Prof. (Dr.) K.P. Balakrishnan, Head, School of Environmental Studies, Cochin University of Science and Technology, and that the thesis has not previously formed the basis for the award of any other degree or title.

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CERTIFICATE

This is to certify that the thesis entitled "Effect of some toxic metals on selected phytoplankton of Kerala waters" is the bonafide record of the work carried out by Smt. Kanakavalli Susarla, S. under my guidance and supervision and that no part thereof has been presented for any other Degree.

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EFFECT OF SOME TOXIC METALS ON SELECTED PHYTOPLANKTON OF KERALA WATERS

CONTENTS

PREFACE

The extraction and use of metals has been the mainstay for the sustained development and progress of a nation. Metals, though fairly stable in the natural environment are found in trace quantities in water bodies. Attention has therefore been focussed to identify the metals that impair the water quality. In the last few decades the concern about the fate of these metals in the aquatic system has been gaining momentum, particularly in the industrial belts. The disasters caused by metal poisoning in recent times have prompted an indepth study of the interaction of metals with aquatic biota. Kerala, basically an agriculture oriented state has witnessed the upsurgence of various industries as a part of the nation-wide economic development programme. Cochin has been identified as the industrial capital of the state.

We are unaware of the subtle and pervasive destructive effects of the metals as pollutants on the aquatic biota when their levels in water are not high enough to cause direct toxicity.

Phytoplankton, as a distinct group of the aquatic biota, form the basis of food chain. They are regarded as a homogeneous entity, which is a useful adoption in many investigations. Neverthless, phytoplankton consists of a heterogeneous collection of organisms. The problems posed by their distribution and succession are of economic importance, since qualitative differences may have effects on the higher trophic

levels of the food chain. In natural waters, nutrient requirement varies with species resulting in their succession and is the basis of the productivity of the aquatic ecosystem. When the ecosystem is polluted the succession cycle is disrupted causing the disappearance of heterogenity leading to the imbalance in food web. The surviving resistant species may cause further ecological damage.

Attempts have been made by several scientists to investigate the effect of metals on the phytoplankton in cultures and in controlled ecosystem experiments. The results of experiments obtained under the laboratory conditions can not be directly attributed to the natural populations. But until our understanding of the natural ecosystem is complete, the predictions of the effect of any pollutant can be made only by synthesizing and co-relating the results of investigations carried out under varying laboratory conditions.

While there are umpteen number of reports regarding the effect of metals on the growth of phytoplankton, the information of their effect on the photosynthetic end products is fragmentary.

The present study is an attempt towards a better understanding of the metal-phytoplankton interactions with special reference to the physiological changes in the species.

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I N T R O D U C T I O N

The climate and topography of Kerala promote luxurient vegetation. The prevailing monsoon rains provide ideal conditions conducive to the uninterrupted agricultural operations. The short but fast flowing rivers empty their contents into the extensive back water system. Any change in the water quality at a point in the upstream therefore is likely to affect the entire lower-stream within a short time. The availability of good quality water has encouraged the establishment of multi-farious industrial complexes on or near the banks of rivers, as a part of the nationwide economic development programme in recent decades. Cochin with its fertilizer, insecticide, metallurgical, oil refining, rare earth processing, rayon and pulp manufacturing and several other industrial concerns has been identified as the industrial capital of the State. The by-products, generated by these industrial complexes together with the effluents discharged into the open streams, cause environmental degradation, particularly in water bodies, upsetting or disrupting the natural balance. The quality, quantity and nature of by-products and effluents vary with the type of industry.

Since industrial revolution, the efforts of removing pollutants from natural environment have not

been able to keep pace with the increasing amount of waste materials produced and added to the system. The increase in human population has aggravated the situation still further. Nevertheless, the growing concern for better environmental quality in recent times is to be regarded as a redeeming feature.

The water bodies are considered as a resource for developmental activities as well as acceptors for the wastes generated. The capacity of these water bodies and sediments below them, to accommodate the wastes must be continually assessed inorder to find out ways and means to maintain as well as to improve both environmental quality and industrial development.

The major reason for the particular sensitivity of aquatic systems to pollution influences lies in the structure of their food chains (Stumm 1976, 1977). Compared to the terrestrial systems, the relatively small biomass in aquatic environment generally occurs in a greater variety of trophic levels to facilitate enhanced accumulation. The quality of inland water bodies, which act as receipients of toxic wastes and effluents are thus adversely affected. Experts estimate that industrial and domestic wastes introduce about million different pollutants into natural waters (Forstner and Wittmann 1979).

Pollution is mainly caused by excessive plant nutrients as well as by sparingly degradable synthetic chemicals entering the aquatic environment through different sources. Forstner and Wittmann (1979), have identified metals as the largest group of chemical pollutants. According to Wood (1947), metals causing environmental pollution fall under three categories. Non-critical, toxic but very rare, relatively accessable and very toxic. Mercury, cadmium, lead, copper and zinc, the metals chosen for the present study come under the last category.

The water bodies in the industrial belt of Kerala receive these metals in various combinations through the by-products as well as through the effluent discharge from chloralkali plant (cadmium, mercury, zinc and lead), insecticides and inorganic chemical industries (mercury, lead, zinc and copper), Metallurgical industry (zinc, lead, cadmium and copper), Oil refinery (lead) Rock phosphate and phosphatic fertilizers (cadmium), pulp for rayon and paper industry (mercury), Textile dyeing and several small scale industries (zinc, copper, mercury, lead, cadmium, cromium and others).

Urban storm water run off has long been recognised as a major source of pollutants to surface waters (Bradford, 1977) as the concentration of lead, zinc and copper increase after storm event (Whipple et al., 1977).

All these metals have profound influence on the physiology of phytoplankton, the primary producers, in aquatic food chain and therefore affect the water quality. Hence the study of the effects of these toxicants on different phytoplanktonic organisms is imperative. As a prelude to this, an indepth study of the changes produced in the physiology and biochemical composition, by these metals individually and in combination, on the two selected algal species, S. bijugatus and N. palea was undertaken under laboratory conditions.

Preliminary experiments were carried to determine the effective range of metals producing measurable changes in the selected species grown in natural waters under laboratory conditions, since the change is dependent on background load of metal in the species selected as well as other variables in the medium.

Various parameters such as temperature, salinity, pH, nutrients, number of cells, photosynthetic pigments, carbohydrates, protein and lipid were studied to highlight the complexity of metal – phytoplankton interaction.

The results, analysed statistically indicated that different metals produced harmful effects which varied with organisms, concentration of metals and their combinations. The results also showed the antagonistic as well as synergistic action of metals in certain combinations.

Materials and Methods

The present work was carried out with pure cultures of Scenedesmus bijugatus (green alga) isolated from a small river connecting Chitrapuzha and Cochin Back water which receives effluents from sewage treatment plant at Elankulam and Nitzschia palea (Diatom) isolated from Cochin Back water.

The former species was maintained in the water collected from the river at the point where STP effluent is discharged. The water was filtered through Whatman No. 4 filter paper and sterilized. The algae was grown in the sterilized water without any addition of EDTA, Vitamines or nutrients, as the growth of this species, in the absence of above was found satisfactory. Nitzschia palea was maintained in filtered and sterilized water having a salinity of 15°/$_{oo}$ - 17°/$_{oo}$. The water from Cochin Back waters was collected when the salinity was in the above range. To this medium appropriate amount of nutrients were added as per Ketchum and Redfield (1938). Since diatoms have an absolute necessity of silicon, 70mg of Sodium metasilicate/l

was added to the medium and shaken well till dissolved.

It is well documented that addition of chelating agents like EDTA to culture medium binds the metals in solution to a large extent. Hence the experiments were conducted without adding EDTA or other chelating agents.

Axenic cultures of the selected algae were obtained using standard phycological methods. The media and the inocula were tested by standard plate count mentod for bacteria at regular intervals. The cultures were exposed to a light intensity of 17×10^{15} Quanta/cm^2/Sec. with 10 - 14 hrs light dark regime.

Stock solutions of mercuric chloride, cadmium nitrate, lead nitrate, copper sulphate, zinc sulphate and ferric chloride were prepared afresh every month to eliminate any substantial difference in storage in the metal concentration. The stock solutions were diluted prior to addition to the medium to give the desired concentrations of the metals.

Experiments were carried out in 2l boro-silicate glass culture flasks, each with 1 l medium. Known quantity of metal solutions were added to the medium just before inoculation. Nutrients were also

added wherever necessary just before inoculation. Algal cells in exponential growth phase were used for inoculation. Initial cell numbers varied between 21200 - 36000 cells/ml for Scenedesmus bijugatus and between 13200 - 64200 cells/ml for Nitzschia palea. All observations were made during the exponential growth phase, which was 12 days for Scenedesmus bijugatus and 10 days for Nitzschia palea. The mean of 25 observations contributed control values for all the parameters studied. The effect of the selected metals in different concentrations was studied in triplicate and the mean values were compared with those of control.

Preliminary experiments to determine the effective range of metals and their combinations for each organism were carried out. The criterion for the above was mainly, number of cells present. Visual changes like yellowish or pale colour of culture and clumping of cells were also considered when observed, as these changes indicated abnormal growth.

The objective of the present study was mainly the investigation of the changes in physiology and biochemical composition of the algae in presence of the metal ions as they caused "stress"

on the algae. Hence the lowest effective sublethal range of metal concentrations were the choice for present investigation.

All experiments were carried out in triplicate. Only analytical grade chemicals were used. Care was taken to minimise metal contamination.

Observations on temperature, p^H, cell numbers, production, respiration and pigments were made on alternate days, starting from second day after inoculation. Carbohydrates and proteins were estimated every other day from fourth day onwards. Salinity, phosphate and nitrate were estimated both at the beginning and end of the experiments. The lipids were estimated on the last day. All obser - vations were made after exposure of the culture to light for about three hours.

The ambient temperature was 27.5 $\pm$ 1.5°C throughout the period of experimentation.

p^H was measured by a digital p^H meter (Model 1400 of Industrial electronics corporation).

Chlorinity was estimated using Knudsen's method and salinity was read from the computed tables.

Number of cells were counted using a haemocytometer. Dense cultures were diluted before counting and actual number computed.

Gaarder and Gran's (1927) method was adopted for determining the rates of production and respiration. Modified winkler (azide) method was used to cover interference by nitrate and permanganate method, by iron. Oxygen values were converted into Carbon equivalents applying a PQ of 1.25 and expressed as mgC/l/hr. The obtained values for production were raised by 10^1 and those of respiration by 10^2 for graphic presentation.

Known volume of algal culture was filtered through GF/C (0.45um pore size). One drop of magnesium carbonate suspension was added before filtration to prevent degradation of pigments. The pigments were then extracted with 90% acetone and O.D. measured spectrophotometrically (Spectronic 21 UV-D). Equations of Lorenzen (1967) for chlorophyll a and pheophytin, jeffrey and Humphrey (1975) for Chl.b and C (c_1+c_2) and Strickland and

Parsons (1968) for carotenoids, after correcting for turbidity as required, were employed to quantify the pigments. Concentration of pigments was expressed as ug/10^6 cells. The values obtained for S. bijugatus were raised by 10^2 and those of N. palea by 10^1 for graphic representation. Carbohydrates were estimated as total free sugars using Sulphuric acid - Anthrone method (Roe, 1955). Samples were centrifuged, weighed, freezedried and extracted with 2 ml of 80% ethanol. 10 ml of the anthrone reagent was added to 1 ml of the extract and the mixture was heated in a water bath for 10 - 15 min and cooled in dark at room temperature for 30 minutes. The optical density was measured at 620 nm. The concentration of sugars was read from standard graph obtained using glucose in benzoic acid.

In the case of Nitzschia palea, the carbohydrates were fractioned and the analysis was carried out in 3 steps. The centrifuged, weighed, freezedried sample was first extracted with 0.1 N sulphuric acid and analysed as stated above to measure the acid soluble fraction. The residue at the second step was treated with 0.1 N NaOH to extract alkali soluble polysaccharide fraction which was analysed. The optical density of both these fractions was read

immediately after extraction and treatment. At the third step the insoluble residue was left in 90% sulphuric acid overnight to dissolve all the remaining carbohydrates and the optical density was determined after appropriate treatment to estimate the insoluble carbohydrate fraction.

Proteins were estimated using biuret method (Gornall et al., 1949). Samples collected by centrifugation were weighed, freezedried and were washed with 80% ethanol to deprotenize as well as to remove the pigment. Two ml of 1 N NaOH was added to the washed residue to extract proteins. To the extract 8 ml of freshly prepared biuret reagent was added and optical density measured at 540 nm. Concentration of protein was computed from standard graph obtained using Bovine Serum albumin.

When the biuret method (which measures proteins by binding to their Carbamyl group) was employed most of the treated samples of **S. bijugatus** gave higher values when compared with that of control, inspite of low production. Hence the "Heated Biuret-Folin Assay" method (Dorsey et al., 1978) was employed to measure total protein on the basis of nitrogen at the end of the growth phase. In the

case of Nitzschia palea only the latter method was employed.

Samples were concentrated by centrifugation, washed with double distilled water and stored at 4°C for one week. To each sample 5 ml of freshly prepared biuret reagent was added and heated to 100°C for 100 minutes in water bath and to this hot solution 0.5 ml of Folin reagent was added and the solution was brought to room temperature by cooling and optical density was measured at 460 nm. The concentration of proteins was computed from standard graph obtained using Bovine Serum albumin.

Lipids were measured only at the end of growth phase for control and treated samples by extracting with Chloroform methanol mixture, and determined by weighing.

Carbohydrate, protein and linid were estimated on the basis of dryweight and expressed as ug/mg. Phosphate (PO_4) and nitrate (NO_3-N) of the medium were determined before and after the experiments. For this, cultures were filtered using Whatman GF/C filters of pore size 0.45 um and the filterates were used for the estimation. Phosphate was measured by Ascorbic acid method and nitrate by

Hydrazine reduction method as specified in "Standard methods for water and waste water analysis" (APHA, AWWA, WPCF 1983).

The algae under study are known to concentrate the toxic metals used in the present work. The amount of metals accumulated were also quantified at the end of growth phase using standard methods.

For analysing mercury the samples were collected by centrifugation, washed twice with all glass double distilled water to remove traces of mercury, if any, in the samples other than those taken up by the algae and weighed. Wet digestion was carried out in Bethges apparatus, specially designed for the purpose using sulphuric acid and nitric acid. The digested samples were then oxidised using potassium permanganate and mercury concentration was determined using Mercury analyser (MA 5800 B of ECIL make). All samples except a few were analysed afresh. The remaining were kept in deep freezer and analysed within a week. In no case samples were stored after digestion. Analysis was carried out in triplicate for all samples.

In the case of cadmium, lead, copper, zinc and iron analysis was carried out only in duplicate. Samples were concentrated by centrifugation

after washing with all glass double distilled water and digested using a mixture of nitric acid and perchloric acid in the ratio of four to one. The digestion was carried out in 50 ml borosilicate conical flasks covered with funnels. The metals were concentrated by evaporating the acid to near dryness, care being taken to see that the acid did not boil. The metals were then extracted into all glass double distilled water by washing the flasks thrice, and the solution collected and stored in borosilicate containers.

Metals were quantified using AAS (model varian techtron) and expressed as ppm/100 mg of dry wt.

The data were analysed statistically using a micro computer and represented graphically. Since the toxic metals may influence the length of lag phase, growth rate in logarithmic phase and also cell density, graphical expression in the form of growth curves was selected. Gross production was also represented graphically, though was not described in the treatise to project a comprehensive picture of the effect of the selected metals on the production of the species. Variance ratios were calculated using two way analysis of variance technique and were presented in tabulated form at the end of each chapter.

3.

REVIEW OF LITERATURE

The living systems contain several elements in varying concentrations. Some of these elements in trace quantities are indispensable for normal healthy functioning of the organism. The requirement of these elements varies with species and stage of growth. The information on trace metal requirement of algae is well documented. Iron, Manganese, Molybdenum and Copper were found to be essential for all algae (Round 1973). Vanadium, Cobalt and Zinc are necessary for healthy growth and reproduction of some algal species (Noda and Horiguchi 1971).

Some of the trace metals are known to have definite functional roles. Iron is an important constituent of electron transport systems and an activator of several respiratory enzymes (Harvey, 1937). Experiments on algal culture by Goldberg (1952); Menzel and Ryther (1961); Hayward (1968); Davies (1968 and 70); Burton and Head (1970); have proved that this element is essential for phytoplankton.

Zinc acts as an activator of dehydrogenases. It was reported to help in the uptake of silicic acid

in diatoms (Hayward 1969, Rueter Jr. and Morel,1981) and for fresh water algae (Knauss and porter, 1954).

Copper is essential for algae being a constituent of plastocyanin which brings about electron transport in pigment system I (Green et al., 1939; Provasoli and Pintner 1953; Walker 1953; Johnston 1963 ; Markley et.al., 1975; Gregory., 1977).

Manganese is necessary for growth of Ditylum (Harvey, 1939) and in general important for algae (Knauss and Porter 1954; Hayward 1969).

The cationic uptake mechanisms enable these primary producers to absorb and concentrate both essential and non-essential elements to a great extent, even when the metal concentration of ambient water is very low.

It could be gathered from literature that all metals, with increase in their concentration, show inhibitory effects leading to changes as revealed in parameters like cell numbers, net photosynthesis, respiration rates, pigment concentration etc.

Toxicity studies of various metals have conclusively proved that it is not the total metal concentration but the particular physical and chemical form of the metal, that affects the organisms. The term "species" is used to designate

this effective form of metal.

Forstner and Wittmann (1979) have stated that "It is hardly possible to envisage a more abrupt change in natural systems than the one which takes place in the mixing zone between river and sea and it is expected to affect the heavy metal loads of the water. At the site of mixing two processes oxidation and complexation seem to be effective in the release of trace metals".

In an estuarine system there is continuous movement of some metals in and out of solution but little is actually lost from the system (Turekian, 1977) and that the dominant cycle of heavy metals is internal (Benninger et al., 1975; Evans et al., 1977) Natural waters are reported to possess high capacity for complexing metals.

Greater part of dissolved heavy metals transported by natural water systems under normal conditions is rapidly adsorbed on to particulate matter. But they do not remain so in the same condition and are transferred between solid and aqueous phases. Clay minerals, freshly precipitated iron hydroxides amorphous silicic acid adsorb metal ions. In aqueous phase metals may form sulphides, hydroxides, or carbonates (Forstner and Wittmann

1979). pH, temperature and salinity influence the chemical complexation of metals in sea water. At pH,7, 51% of Zn^{+} remains uncomplexed. At lower pH than sea water Cu and Zn are released into water from particulate phase (Zirino and Yamamoto, 1972).

Temperature and dissolved oxygen content are vital factors for aquatic organisms. Heavy metal toxicity increases with increase on water temperature (Lloyd, 1965). Huisman et al.,(1980) have shown that increase in toxicity of mercury,with increase in temperature,elevated respiratory activity of Scendesmus acutus. More cadmium will be released under oxidised conditions with increase in salinity. (Forstener and Whittmann (1979). They also reported that in estuaries where fresh and salt water intermix, salinity plays a dominant role in increasing the metal concentration. In case of organisms in brackish water the ion transport into organism increases with increasing salinity (Fletcher, 1970,on Neries diversicolor).

pH of the water plays an important role in the interactions between heavy metals and parameters such as hardness caused by carbonates and concentration of organic compounds. Stumm and Morgan (1970)

have stated that at higher levels of pH Lead may be precipitated.

Hühne and Kroontje (1973) have stated that pH and chloride ion concentration are important factors as the chloride and hydroxy complexes contribute to the mobilization of mercury, cadmium, zinc and lead ions in the system.

Due to osmotic regulation in aquatic organisms, the flux of ion incorporation and excretion is principally different as it is dependent on whether water is fresh, brackish or marine. (Florey, 1970). Therefore, heavy metal concentrations of fresh water, brackish water and marine organisms must be considered separately.

Besides all the factors mentioned above, metals in natural waters are also complexed by chelators. Organic substances possess a high degree of affinity towards metal ions. Many algae are known to release certain metabolites that would successfully tie up the metal ions. (Johnston 1964; Barber and Ryther, 1969; Steemann Nielsen and Wium Anderson, 1970) organic chelators seem to be necessary for the production in aquatic environment (Barber and Ryther, 1969). Grazing also may release some of the organic matter into water due to disruption of cells.

The synthetic complexons EDTA, NTA, DTPA are now widely used in industries as chelating agents for metal ions owing to their versatility (Pollard 1966; Wallace, 1971). Some are effective against metal poisoning as, NTA against copper and zinc (Sprague, 1968), EDTA against copper (Nishikawa and Tabata, 1968). In addition, both calcium and citrate have been reported to possess inhibitary effect on metal uptake by algae (Schecher and Driscoll, 1985).

The mass of algae present in water can influence the availability of metals because as primary producers they can strongly affect the dissolved metal content in open waters (Morris, 1971; Murphy et al., 1976). But mechanisms of algal metal interactions are not well understood. Various parameters like pH (Steemann Nielsen et al., 1969; Hargreaves and Whitton., 1976; Mierle and Stokes., 1976), the nature and concentration of organic solutes (Fogg and Westlake 1955; Erickson 1972; Morris and Russell., 1973; Stokes and Hutchinson., 1976) and ionic solutes (Stokes., 1974; Overnell.,1976., Mierle and Stokes;,1976), have all been observed to influence algal metal associations. Andersen and Morel (1978) have clearly stated that toxicity of

copper is due to cupric ion activity.

Two mechanisms of uptake of metal ions for algae have been postulated. One is rapid initial reaction with the surface of the cell and absorption into membranes, without intervention of metabolic processes, known as passive uptake mechanism. This has been reported for Chaetoceros spp (Glooschenko, 1969). Also for the species, Pheodactylum tricornutum and Cladophora (Burkett, 1975), Chlorella vulgaris, (Fillipis and Pallaghy, 1976). The uptake of ^{65}Zn in Dunaliella tertiolecta was influenced only by the chemistry of the medium. Passive uptake mechanisms rely on the capacity of cell membranes and intercellular substances to complex cations (Skipnes et al., 1975). It has been suggested by Maclean et al., (1972) that proteins act as cationic binding sites in membranes. The other is metabolic uptake mechanism, which is dependent on a number of external factors, such as pH, back ground metal concentrations, light intensity and chemical composition of the medium, biological variables such as the growth phase and stage of cell division (Skipnes et al., 1975). Metabolic uptake mechanisms in algae are reported by Nuzzi (1972), Burkett (1975)

Fujita & Hashizume (1975) and Kayser (1976).

A common characteristic of heavy metals is that they interact with and bind themselves to biological macromolecules, particularly enzymes. This is the determinant factor of their toxicity (Eichorn 1974) and they may inactivate the enzymes and interfere seriously with metabolic processes.

With reference to algae, metal toxicity was studied by various workers and the parameters such as cell numbers, nett photosynthesis, respiration rates, Chlorophyll, ATP, DNA, RNA, dry matter content, wet weight and Carbon balance were considered. The harmful effects produced by various metals like mercury (Kamp Nielsen, 1971; Ben Bassat et al., 1972; Nuzzi, 1972; Rice et al., 1973; Ben Bassat and Mayer, 1975; Overnell, 1975; Agarwal and Kumar, 1975; Davies, 1976), copper (Mandelli, 1969; Steemann Nielsen and Wium Anderson, 1970; Steemann Nielsen and Kamp Nielsen 1970; Martin and Olander, 1971; Erickson, 1972), Cadmium (Bruland et al., 1978), lead (Baldes and Lewin 1976; Marchetti, 1978) zinc (Parry and Hayward 1973; Jensen et al., 1974; Anderson and Morel, 1978) have been studied extensively. Growth response of phytoplankton was studied by Subba Rao (1981).

Algal cells are known to tie up the metal ions and reduce the toxicity by producing various extracellular substances. The production of extracellular acids for complexing copper in eukaryotic algae and blue greens, (Swallow et al., 1978) extracellular detoxification by organic substances released from cell (Fogg and West Lake 1955; Steeman Nielson and Kamp Nielsen 1970), and the phenomenon has been tested in Pheodactylum' tricornutum (Sunda and Guillard 1976). Once entered, metal ions are blocked by the cell wall (SteemannNielsen et al., 1969; Mirle and Stokes, 1976; Davies, 1976; Button and Hostetter, 1977) internally deposited at non metabolic sites within the cell (Silverberg et al., 1976).

Combined effects of copper and zinc (Braek et al., 1976) manganese and copper (Sunda and Huntsman, 1983) mercury and iron (Smith, 1972) have also been worked out.

The sensitivity of algal species to metals varies with species and concentrations. Tolerance for three marine phytoplankton was worked out by Jensen et al., (1974) showing copper as less toxic with zinc, zinc with magnesium. When Thalassiosira species was subjected to 200 µg/l zinc, it did not

show any effect but 200 µg/l copper was lethal and together they showed synergistic action.

Considering the ecological aspect of heavy metal pollution, species composition of phytoplankton is reportedly affected, sensitive species being replaced by tolerant ones. Amphora coffeaformis has been reported to be highly tolerant to copper (Robinson et al., 1985) and Chaetoceros to mercury (Hirota et al., 1974), Pheophyceae more resistant than chlorophyceae to heavy metals (Tsekos et al., 1972).

The relative toxicity of heavy metals for different species has been reported differently. For chlorella (Hannan and Patouillet 1972) as Hg > Ag > Cd > Pb > Cu, for Scenedesmus quadricauda (Rzewuska and Wernikowska,1974) Ag > Cu > Ni > Cd > Pb > Zn > Co > Cr and for marine phytoplankton (Berland et al., 1976) Hg > Cu > Pb, has been demonstrated.

Despite the fact that resistant species may replace sensitive ones, with increasing metal loads, productivity of the water bodies may also be impaired. Increase in the industrial waste provides an added stress. The water quality of Cochin backwater systems which is the repository of many of the industrial wastes has been investigated by several workers.

The Hydrography (Wellershaus 1971; Balakrishnan and Shynamma 1976), organic production (Qasim et al., 1972, 1974, 1979; Nair et al., 1975; Pillai et al., 1975), plant pigments (Qasim and Reddy 1967), phytoplankton (Gopinathan et al., 1974; Nair and Joseph, 1975; Joseph and Pillai, 1975) and dissolved carbohydrates (Sumitra et al., 1972) are the parameters which have attracted the attention of the scientists. Sankaranarayanan and Stephen (1978) studied the metal content of the particulate matter in Cochin backwaters. Balakrishnan and Devi (1983) discussed the disastrous effect of industrialization on Cochin backwater system.

The determination of tolerance limits of phytoplanktonic organisms which form the base of food chain, to heavy metals,is of paramount importance from biological as well as economic points of view. There is already considerable literature concerning the effect of heavy metals on the growth and development of phytoplankton in culture. The effect of metals could be assessed only by a comprehensive study of various aspects. The results of the previous investigations made,have revealed that the effect of the metals mercury, cadmium, lead, copper and zinc were

well studied but only a few parameters were taken in to consideration. It is with these in view the present work was undertaken.

4. Description of the selected phytoplanktonic species

4.1 Scenedesmus bijugatus (Turpin) Kuetzing (Chlorophyceae)
(Philipose, 1967, page 252 Fig. 164 f)

Description : Colonies flat, consisting of four to eight cells. Cells ellipsoidal with ends broadly rounded, in sub-alternating series.

Length - 18 µm

Breadth - 7 µm

Occurrence - Fresh water

The mophology of the genus was investigated by several workers, notably, Trainor (1966 and 79); Shubert (1975); Mathew and Chowdary (1977) and Siver and Trainor (1981). Reports on its use, as a source for protein were published by Venkataraman et al., (1977) and Venkataraman and Nigam (1979); as a feed for biogas plant by Nair et al., (1982) and as a means for nutrient removal from sewage effluents by Nair et al., (1981).

TABLE I

Growth parameters of S. bijugatus

Parameters	Second Day	Fourth Day	Sixth Day	Eighth Day	Tenth Day	Twelfth Day
pH	8.660	9.840	10.640	10.350	10.520	9.670
Gross production (mg C/l/hr.)	0.179	0.363	0.562	0.894	0.767	0.856
Nett production	0.143	0.330	0.539	0.853	0.706	0.763
Respiration	0.037	0.033	0.023	0.042	0.061	0.093
Chlorophyll a ($\mu g/10^6$ cells)	0.138	0.067	0.022	0.010	0.014	0.013
Chlorophyll b	0.149	0.056	0.022	0.024	0.020	0.024
Carotenoids	0.081	0.051	0.022	0.025	0.016	0.017
Pheophytin	0.025	0.010	0.004	0.025	0.014	0.016
Carbohydrate (μg/mg dry wt.)	-	48.860	13.310	16.550	12.230	9.130
Protein (Biuret)	-	20.090	6.510	15.890	8.350	5.30(
Protein Total	-	-	-	-	-	253.4
Lipid	-	-	-	-	-	221.(

====== Denotes Maximum ----- Denotes Minimum.

TABLE I

Growth parameters of S. bijugatus

Parameters	Second Day	Fourth Day	Sixth Day	Eighth Day	Tenth Day	Twelfth Day
pH	8.660	9.840	10.640	10.350	10.520	9.670
Gross production (mg C/l/hr.)	0.179	0.363	0.562	0.894	0.767	0.856
Nett production	0.143	0.330	0.539	0.853	0.706	0.763
Respiration	0.037	0.033	0.023	0.042	0.061	0.093
Chlorophyll a ($\mu g/10^6$ cells)	0.138	0.067	0.022	0.010	0.014	0.013
Chlorophyll b	0.149	0.056	0.022	0.024	0.020	0.024
Carotenoids	0.081	0.051	0.022	0.025	0.016	0.017
Pheophytin	0.025	0.010	0.004	0.025	0.014	0.016
Carbohydrate (μg/mg dry wt.)	-	48.860	13.310	16.550	12.230	9.130
Protein (Biuret)	-	20.090	6.510	15.890	8.350	5.300
Protein Total	-	-	-	-	-	253.450
Lipid	-	-	-	-	-	221.090

====== Denotes Maximum ----- Denotes Minimum.

Nitzschia palea (kutz) W. Smith (Pascher, 1930, page - 416. Fig. 801)

Description : Valves tapering from middle to sub acute ends.

Length of the cell - 55.5 µm

Breadth - 4.5 µm

Keel punctae - 18 in 10 µm

Occurrence : In brakishwater with a salinity range 12 °/₀₀ to 16 °/₀₀

The species is frequently found in polluted waters. The physiology of the species under nitrogen stress was studied by Denffer (1948) and Manny (1969).

TABLE II

Growth parameters of *N. palea*

Parameters/ Day	Second Day	Fourth Day	Sixth Day	Eighth Day	Tenth Day
pH	8.740 ------	8.960	9.240	9.270 =====	8.920
Gross production (mg.C/l/hr)	0.124 ------	0.245	0.415	0.361	0.629 =====
Nett production	0.118 ------	0.240	0.396	0.335	0.604 =====
Respiration	0.006	0.005 ------	0.019	0.026 =====	0.025
Chlorophyll a ($\mu g/10^6$ cells)	0.430	0.452 =====	0.409	0.352	0.192 ------
Chlorophyll c	0.268 =====	0.208	0.163	0.186	0.087 ------
Carotenoids	0.570	0.741 =====	0.633	0.564	0.260 ------
Pheophytin	0.180	0.191	0.180	0.193 =====	0.085 ------
Acid soluble carbohydrate (μg/mg. Dry wt.)	-	3.033 ------	3.804	7.382	14.480 ======
Alkali soluble carbohydrate	-	1.354 ------	2.048	3.970	6.809 =====
Insoluble carbohydrate	-	7.013 ------	7.179	21.500	47.220 ======
Protein	-	6.530 ------	9.930	50.650	83.380 ======
Lipid	-	-	-	-	464.84

===== Denotes Maximum; ------ Denotes Minimum

5. Metals selected and their effect on test species

The metals, mercury, cadmium, lead, copper and zinc, which enter the waters of Kerala through the effluents discharged by various industries located on the banks on rivers. The effect (Table 1) was studied in relation to the control.

TABLE 1

Sl. No.	Metal	S. bijugatus		N. palea	
		No. of treatments	Concentration in ppm	No. of treatment	Concentration in ppm
(1)	Mercury (Hg)	1	0.02	5	0.005
		2	0.03	6	0.01
		3	0.04	7	0.02
		4	0.05	8	0.03
				9	0.04
(2)	Cadmium (Cd)	10	0.01	13	0.02
		11	0.03	14	0.03
		12	0.05	15	0.04
(3)	Lead (Pb)	16	0.05	19	0.02
		17	0.1	20	0.04
		18	0.3	21	0.06
				22	0.08
(4)	Copper (Cu)	23	0.1	25	0.01
		24	0.2	26	0.02
				27	0.03
				28	0.05
(5)	Zinc (Zn)	29	0.05	33	0.02
		30	0.1	34	0.04
		31	0.3	35	0.06
		32	0.5	36	0.08

5.1.1

Effect of mercury on S. bijugatus

(Figs. I, II and III)

Sl. No. of the metal	Number of treatment	Selected concentration (in ppm)
(1)	1	0.02
	2	0.03
	3	0.04
	4	0.05

Production: (Fig. I)

The nett production of the alga was affected by mercury in all selected concentrations. In 0.02 ppm concentration the production was maximum on fourth day whereas in control it was only on eighth day. On fourth day the treated sample showed an increase of 233% over the control values of the same day. This value was 29% more than the maximum value recorded for the control. The 0.02 ppm treated sample exhibited gradual decrease in production from fourth day onwards reaching 32% lower level in relation to control, at the end of growth phase. The production of the alga was maximum on sixth day in 0.03 and 0.04 ppm treatments, the percentage increase being 38 and 7 respectively, in relation to control. It declined gradually in the former to be 53% less than control at the end of growth phase where as in the latter did not register any change upto tenth day but declined thereafter to be 35% less than that of control. When

the alga was exposed to 0.05 ppm mercury, its production was reduced to a greater extent when compared to that of control throughout growth phase. From its maximum on eighth day, which was 50% less than that of control, it declined gradually to 75% lower level on tenth day. Production maxima in 0.02, 0.03 and 0.04 ppm treatments were attained earlier than that of control.

Respiration of the treated alga eventhough varied in the early phase, was reduced in the latter growth phase without exception, whereas in control it always showed an increase. When the alga was exposed to 0.02 ppm mercury, respiration was maximum on fourth day with 239% increase over the control values. It then declined till sixth day and thereafter remained steady till tenth day. Though it increased once again towards the end of growth phase it remained 15% less than that of control. Respiration of the alga grown in 0.03 ppm mercury reached maximum on sixth day when it was 174% higher than that of control. It declined thereafter till eighth day but increased once again gradually towards the end of growth phase. It was less than that of control on the last day. However, from sixth day onwards it remained higher compared to other treatments. When the alga was cultured in 0.04 ppm mercury the respiration fluctuated with two peaks on second and sixth day. The respiration

was 16% less on second day but 113% more on sixth day in relation to control. From the maximum level on sixth day it declined to minimum level on eighth day with 76 % reduction. Respiration remained same on eighth and tenth day of growth. Further reduction, to 30% lower level was observed on tenth day. Gradual elevation in the respiration was observed, when the alga was exposed to 0.05 ppm mercury to maximum level on sixth day with 22% increase when compared to control. It then decreased till tenth day and increased thereafter towards the end of growth phase but was found reduced by 52% in relation to control on the last day.

Owing to the higher rate of nett production pH of the culture in 0.02 and 0.03 ppm treatments was found to be higher than that of control in the early phase. In general pH of the culture decreased with increase in the concentration of mercury. In no treatment it attained the maximum level of pH (10.64) reached by the control. pH of the culture was less than that of control throughout for 0.04 and 0.05 ppm treatments. Inspite of reduced rate of production in the latter growth phase, pH of the cultures in 0.02, 0.04 and 0.05 ppm treatments was observed to be higher than that of control at the end of growth phase.

Pigments: (Fig. II)

Total pigment content of the alga increased with the age of culture in all except the 0.05 ppm treatment. Increase in the concentration of mercury delayed the development of pigments and the maxima were attained subsequently later in growth phase. The increase in pheophytin followed the same pattern as that of chlorophyll a.

When the alga was grown in 0.04 and 0.05 ppm chlorophyll a and chlorophyll b were not detected on the second day of growth. Maximum amount of chlorophyll a was produced on second day by the alga in 0.03 ppm treatment and this was at a slightly higher level than the maximum level attained by control on the same day. The concentration of chlorophyll a remained higher in treated alga, at the end of growth phase. Also the concentration of chlorophyll a and chlorophyll b fluctuated with two peaks in all treatments. Chlorophyll b was less than that of control only in 0.05 ppm treatment at the end of growth phase.

Carotenoids were not suppressed in any of the treatments. The alga grown in 0.03 and 0.04 ppm mercury produced maximum amount of carotenoids on fourth day which reached a slightly higher level than that of control. When the alga was grown in 0.02 and 0.05 ppm mercury pheophytin was not detected till second day in the former

and till fourth in the latter. It was suppressed to a greater extent in 0.05 ppm treatment. Maximum amount was produced on fourth day by the alga in 0.03 ppm and 0.04 ppm treatments.

Total pigment content of the alga decreased with increase in mercury level. The alga grown in 0.03 ppm mercury produced maximum amount of all pigments.

Photosynthetic end products: (Fig.III)

When the species was exposed to mercury, the carbohydrate content increased and was higher than that of control throughout growth phase except in 0.05 ppm treatment. Total carbohydrate decreased with increasing concentration of mercury as the culture aged. The amount of carbohydrate produced was almost same in 0.02 and 0.03 ppm treatments. The percentage increase was 104 and 100 on fourth day, 142 and 167 on eighth day and 261 and 185 on twelfth day, in relation to control. Also carbohydrate level fluctuated in these two treatments. When the alga was exposed to 0.04 ppm mercury, 47% increase was noted in the carbohydrate content on fourth day. It was 3% less than that of control on eighth day. Thereafter it registered an increase towards the end of growth phase reaching 196% higher level on the last day in relation to control. The alga produced least amount of carbohydrate during the early growth phase, when

grown in 0.05 ppm mercury but gradual increase was noticed from sixth to tenth day, the maximum level being 203% higher than that of control on tenth day. It declined towards the end of growth phase and was only 31% higher than that of control on the last day.

Protein content of the alga when estimated using Biuret method, was found in greater proportion during early growth phase. Protein decreased with the ageing of culture but in all treated samples it was found to be higher than that of control, inspite of low production rate. When total protein was measured on the basis of nitrogen, most of the treated samples were found to contain less protein than that of control. The discrepency may be explained as, either the treated alga produced greater amount of carbamyl containing protein (with which biuret reagent reacted) or the cells were weak and 'leaking' rendering the protein more amenable for extraction than that of control. It is well documented that protein content of alga increases with the age of culture. But in the present investigation, when the protein was estimated using the Biuret method, it was found to decrease with the age of culture which can be explained on the basis of partial recovery of the cells from their initial 'sensitivity'. Since estimation of protein was not satisfactory as pointed out above,

total protein was separately measured for this species at the end of growth phase to give a better explanation of the metal interaction.

When measured on the basis of nitrogen total protein content of the alga decreased with increase in mercury concentration. When exposed to 0.02 ppm mercury protein was 11% more than that of control. In 0.03 ppm treatment it was equal and in the other two treatments it was reduced by 14% and 21% respectively, in relation to control.

Lipid generally increased with increasing mercury concentration and was generally higher when compared to that of control. The percentage increase was found to be 81 and 112 for the 0.02 and 0.05 ppm treatments respectively and 62 for the others.

Growth: (Fig. I)

The rate of multiplication of the alga was enhanced in the early growth phase only at 0.02 and 0.03 ppm mercury level but by sixth day, the biomass in all treatments reached higher level than that of control. From eighth day onwards no further increase was noticed. At the end of growth phase biomass in treatments was found reduced.

The nutrient uptake was studied for all the treatments. When the prepared medium was tested under

experimental conditions, without algal growth, no loss of nutrients was observed and hence it was assumed that when the test species were grown in the medium, any change in nutrient concentration could be attributed to the uptake by the algae.

The nutrient consumption of the alga was estimated at the end of the experiments. Results are given in the following table.

Selected concentration of metal	Nutrients absorbed by the alga (µg/l)		N / P
	Phosphate	Nitrate	
0.02	936	1494	1.60
0.03	932	1490	1.60
0.04	930	1484	1.60
0.05	972	679	0.70
Control	945	1290	1.37

The uptake of phosphate by alga was not affected by the metal as the difference between any two treatments and between any treatment and control was not considerable. But the nitrate uptake increased considerably with increase in metal concentration except at the highest level of mercury employed when it was reduced nearly by 50%. The N / P ratio was higher for the treated alga except when the highest level of mercury was employed.

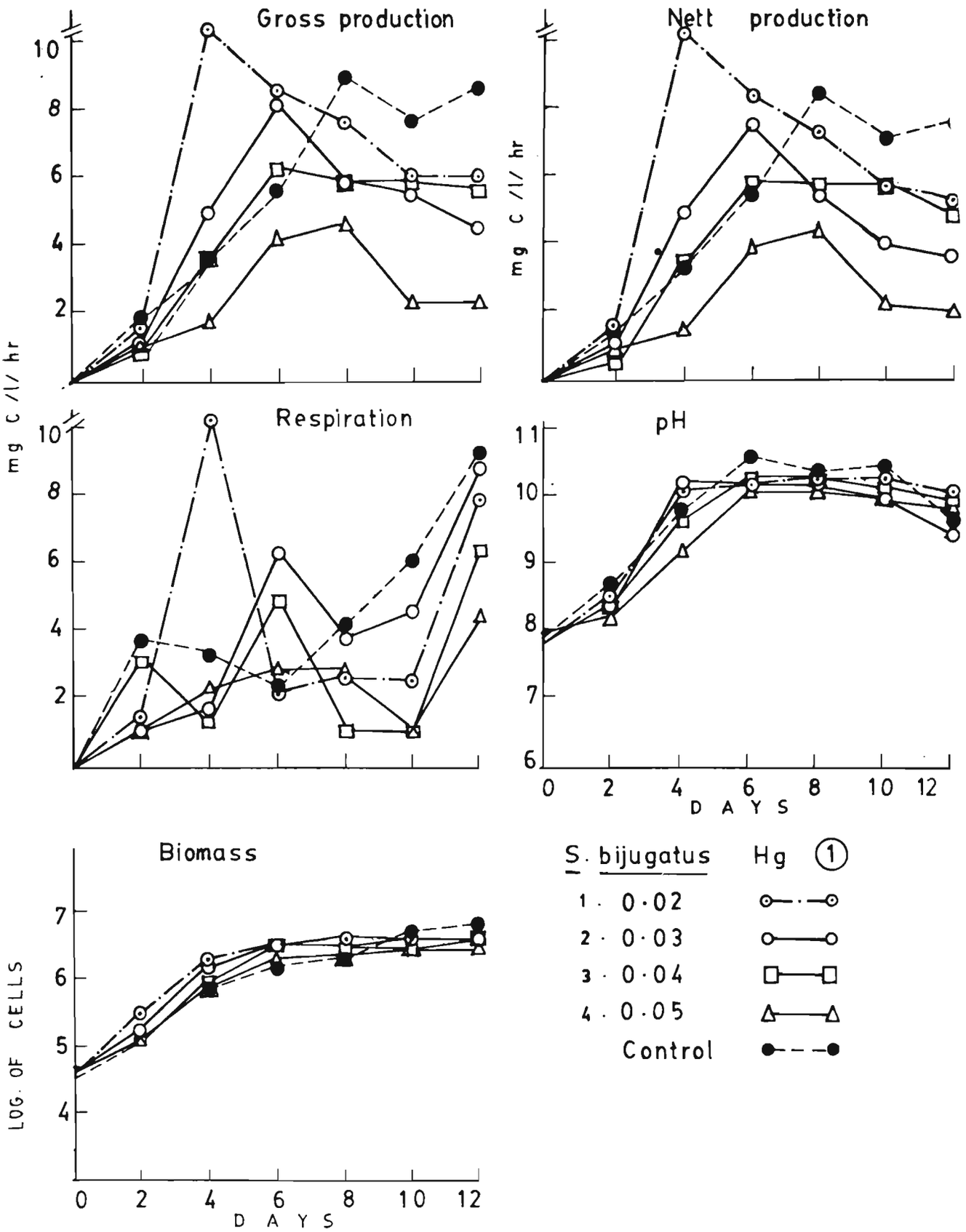

Gross production
Nett production
Respiration
pH
Biomass
mg C /l/ hr
LOG. OF CELLS
D A Y S
S. bijugatus Hg (1)
1 . 0·02
2 . 0·03
3 . 0·04
4 . 0·05
Control

Fig. I

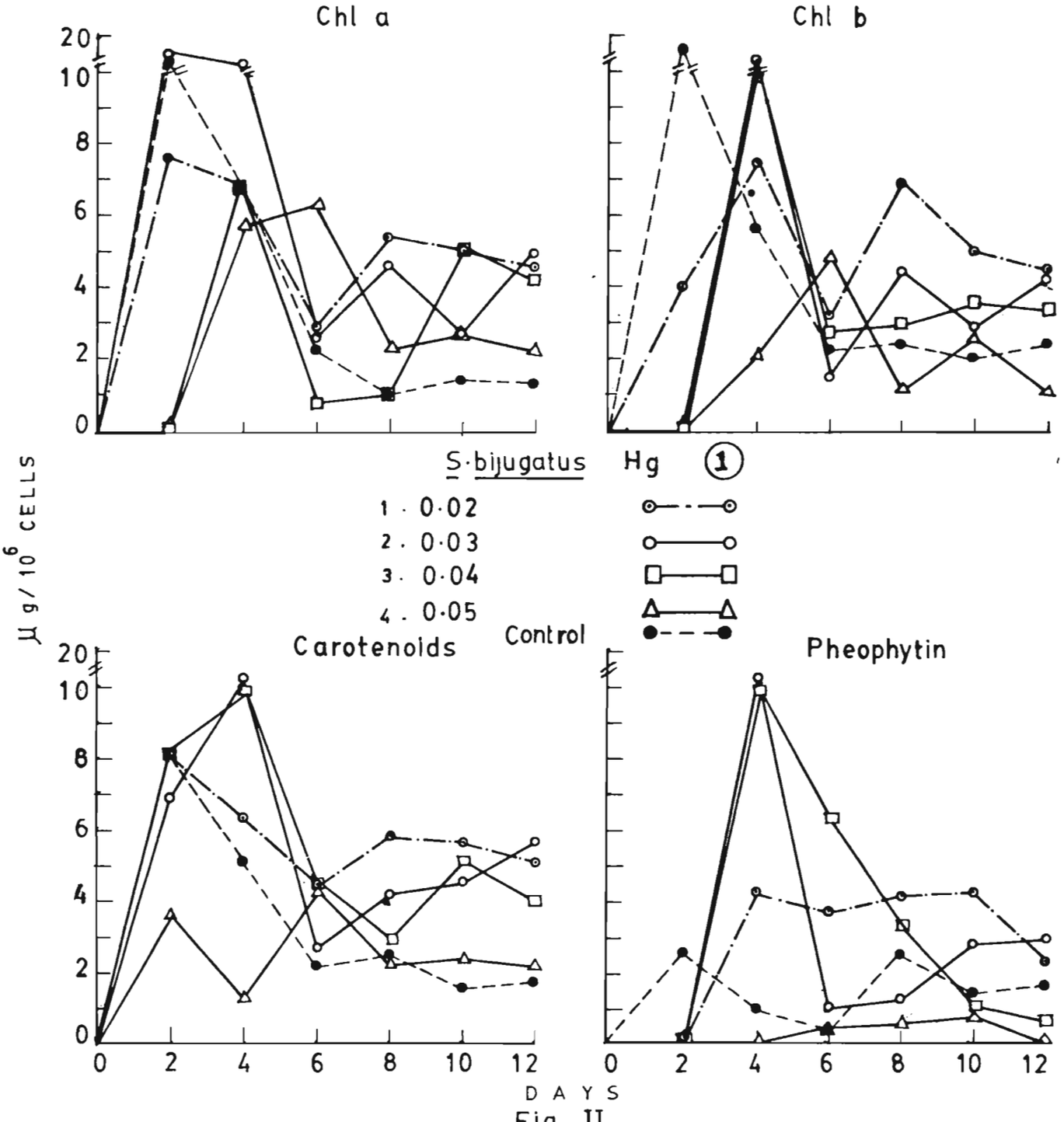
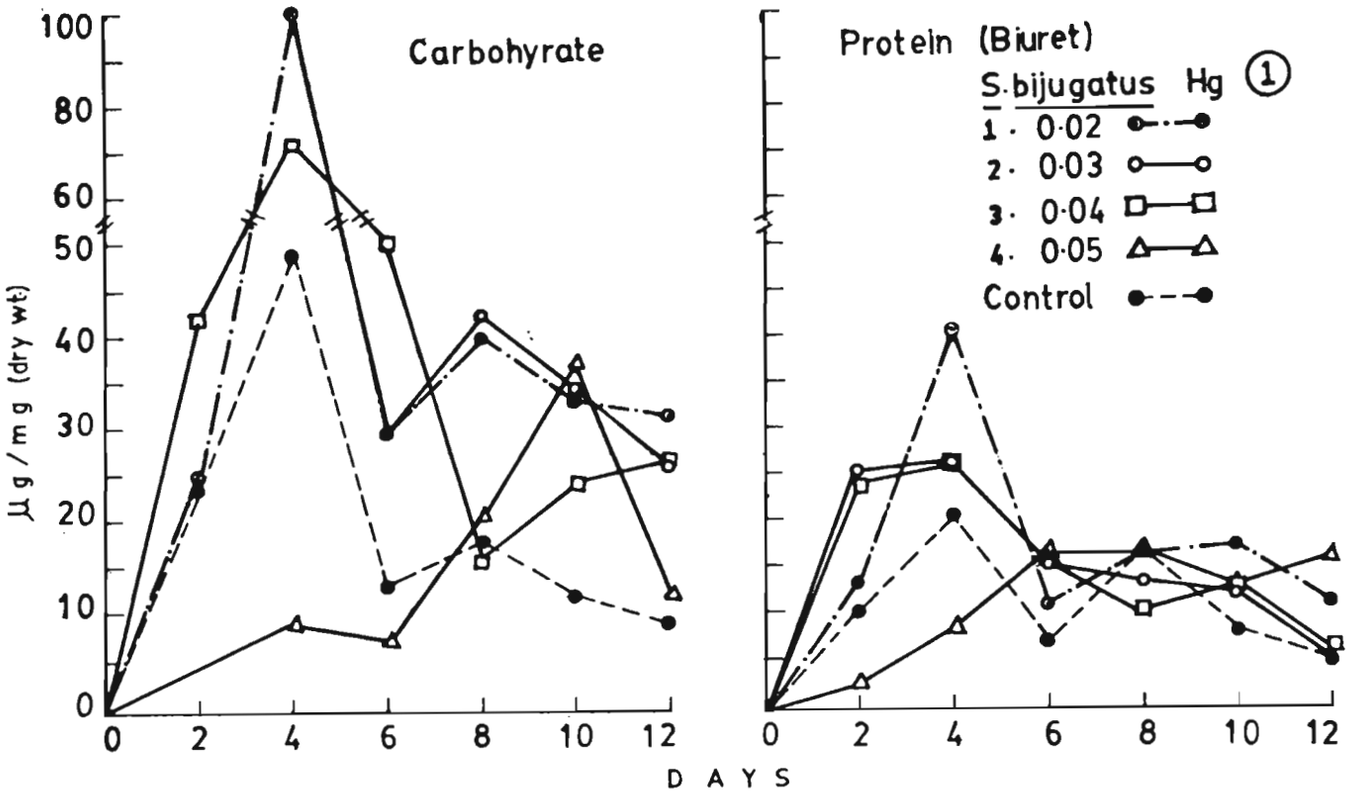

Chl a
Chl b
Carotenoids
Pheophytin
S·bijugatus Hg (1)
1 . 0·02
2 . 0·03
3 . 0·04
4 . 0·05
Control
µ g / 10^6 CELLS
DAYS
0
2
4
6
8
10
12
20

Fig. II


Carbohyrate
Protein (Biuret)
S.bijugatus Hg
1. 0·02
2. 0·03
3. 0·04
4. 0·05
Control
µg/mg (dry wt)
DAYS

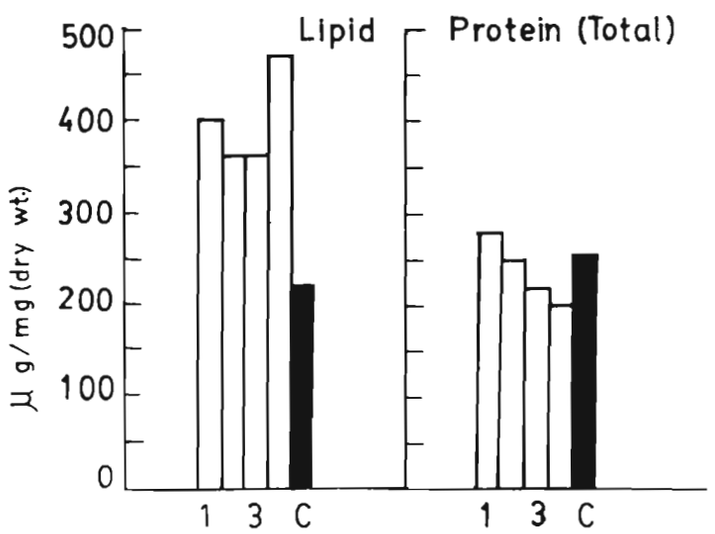

Lipid
Protein (Total)
µg/mg (dry wt)
1 3 C
1 3 C

Fig. III

Effect of mercury on N. palea

(Figs. I, II and III)

Sl. No. of the metal	Number of treatment	Selected concentration (in ppm)
(1)	5	0.005
	6	0.01
	7	0.02
	8	0.03
	9	0.05

Production: (Fig. I)

Mercury has affected the nett production of the diatom at all the concentrations studied. Production of the diatom increased gradually till the end of growth phase in 0.005 ppm and 0.01 ppm treatments. It was higher than that of control except on the second day. But on the last day it was 5% less in 0.005 ppm whereas it was 8% more in 0.01 ppm in relation to control. In the 0.02 ppm treatment, production of the alga was severely inhibited upto sixth day but recovery was observed thereafter. It gradually increased but remained 50% less than that of control at the end of growth phase. The nett production was inhibited in 0.03 and 0.05 ppm treatments.

In the 0.005 and 0.01 ppm treatments respiration of the diatom was reduced considerably in the latter phase

of growth by 20% and 44% in relation to control, respectively on the last day. Respiration fluctuated when the diatom was cultured in 0.02 ppm mercury, with 233% and 581% increase respectively on second and eighth day but at the end of growth phase it was 44% less than that of control. When the diatom was grown in 0.03 ppm mercury respiration was elevated considerably and remained higher than control except on sixth day. It was 540% and 276% more than that of control on fourth and twelfth day respectively.

The effect of mercury on production was reflected in pH. Increased production and reduced respiration have resulted in increased pH of the culture in 0.005 and 0.01 ppm treatments when compared with that of control from fourth day onwards. But in 0.02 ppm treatment pH was lowered from second to sixth day and increased subsequently with increase in production. Though the rate of production remained much reduced in this treatment, pH reached higher level at the end of growth phase when compared with control. pH of the culture in 0.03 ppm treatmentwas lower than that in 0.05 ppm due to elevated respiration and also the lack of any activity in the latter.

Pigments: (Fig. II)

The effect of mercury on the pigments varied with concentration.

The development of chlorophyll a did not exhibit any difinite pattern and also the concentration of chlorophyll a fluctuated with two peaks except in 0.01 ppm mercury. In 0.005 and 0.02 ppm treatments production of chlorophyll a was enhanced and the maxima were found on second day. This pigment was maintained at a slightly higher level than control in the diatom exposed to 0.01 ppm mercury. Chlorophyll a was less than control in the early phase in the 0.03 and 0.05 ppm treatments but towards the end of growth phase it was higher than control in the latter.

In presence of mercury, chlorophyll c (c_1 + c_2) was developed to a greater extent than control by the diatom. In 0.005 and 0.01 ppm treatments, the concentration of this pigment was maintained at a slightly higher level than control and also did not fluctuate during the growth phase. At 0.02 ppm mercury level it reached maximum on fourth day with sharp rise but declined rapidly and was not detected on sixth day. Increasing once again it reached a higher level than control on eighth and tenth days. In the diatom exposed to 0.03 ppm mercury though it reached a higher level than control in early growth phase, was not detected on sixth and eighth days but increased sharply there after and at the end of growth phase remained higher than in any other treatment.

In the 0.05 ppm mercury treated diatom it was less than control on second and fourth day but exhibited a sharp rise to maximum on sixth day. It reached slightly lower level than the one in 0.03 ppm treatment at the end of growth phase. The concentration of chlorophyll c was higher in all treatments, when compared to control on the last day.

The diatom produced more carotenoids when exposed to 0.005 and 0.01 ppm mercury, than control and the concentration was found to be higher than control through out growth phase. At 0.02 ppm level of mercury, concentration of carotenoids was less than control both at the beginning and end of growth phase. The level of carotenoids was much lower than control throughout growth phase, when the concentration of mercury employed was 0.03 ppm. In 0.05 ppm it increased from fourth day onwards reaching maximum on eighth day, declined thereafter but remained higher than control at the end of growth phase.

When the mercury level was 0.005 and 0.01 ppm phaeophytin content was more than control in early phase and was developed to a greater extent in the latter reaching maximum on second day and in the former on fourth day and decreased in both as the culture aged, but remained higher than control at the end of growth

phase. Pheophytin was detected in the early and late growth phase when the mercury level was 0.03 ppm. The diatom produced more pheophytin in 0.05 ppm than in 0.02 and 0.03 ppm but it was not detected on fourth and tenth day of the growth phase.

Photosynthetic end products: (Fig. III)

The carbohydrate content (acid soluble, alkali soluble and insoluble fractions) was less than that of control in the later growth phase.

The acid soluble fraction was slightly higher than that of control when the level of mercury was lowest (0.005 ppm), upto sixth day. At 0.01 ppm mercury level it was less than that of control throughout. This fraction was estimated only on the last two days, due to supperssed growth of the diatom, in 0.02 ppm mercury. The percentage reduction was found to be 57, 49 and 72 respectively for the 0.005, 0.01 and 0.02 treatments, at the end of growth phase. Due to the severe inhibition of growth, this fraction could not be estimated in the other two treatments.

The alkali soluble fraction did not show much variation between any two treatments and between any treatment and control during early growth phase but it was reduced by 85% in 0.005 ppm and 0.01 ppm treatments and by 67% in 0.02 ppm treatment, at the end of growth phase.

The insoluble carbohydrate fraction of the diatom was more than that of control in the early growth phase, when the mercury level was lowest but remained less than that of control in the latter phase. This fraction was less than that of control in 0.01 ppm mercury. The respective reduction was 48% and 83% in the two treatments at the end of growth phase. It was estimated only on eighth and tenth day of growth in 0.02 ppm treatment because of the inhibition of growth of the diatom. This fraction was 80% less than that of control on the last day.

Total carbohydrate content of the diatom decreased with increase in mercury level.

Total protein content of the diatom exposed to 0.005 ppm and 0.01 ppm mercury increased by 267% and 176% respectively by fourth day of growth. Though it registered an increase towards the end of growth phase, remained 40% and 69% less than control on the last day. Further reduction in protein was observed, by 83%, when the mercury level was 0.02 ppm.

Lipid content of the diatom was adversely affected in presence of the metal. The amount of lipid increased with increase in mercury upto 0.02 ppm, but did not reach the same level as that of control in any instance. The percentage reduction was 64, 58 and 48 respectively in 0.005, 0.01 and 0.02 ppm treatments.

Growth: (Fig. I)

The rate of multiplication of the diatom was not affected when the mercury level was 0.005 and 0.01 ppm. Lag phase was observed upto second day when the diatom was exposed to 0.02 ppm mercury, from which the species recovered and grew exponentially from sixth day onwards. Growth was severely inhibited in the other two treatments. The apparent lowering of cell numbers in 0.04 ppm. treatment was not due to disintegration of cells since no empty frustules were observed under the microscope. The diatom cells were seen attached to the walls of culture flask and did not enter the medium when culture flasks were shaken.

Nutrient consumption of the alga in different treatments is given in the following table.

Selected concentration of metal	Nutrients absorbed (ug/l)		N / P
	Phosphate	Nitrate	
0.005	1720	821	0.48
0.01	1700	936	0.55
0.02	1565	863	0.55
0.03	1430	380	0.27
0.04	1375	175	0.13
Control	1350	763	0.57

In general phosphate absorption of the diatom increased when exposed to mercury but gradual decrease in the uptake of this nutrient was observed with increasing metal concentration. Nitrate absorption of diatom was highest in 0.01 ppm mercury. Nitrate uptake was reduced by about half when exposed to 0.03 ppm mercury and by about 70% in 0.04 ppm treatment in relation to control.

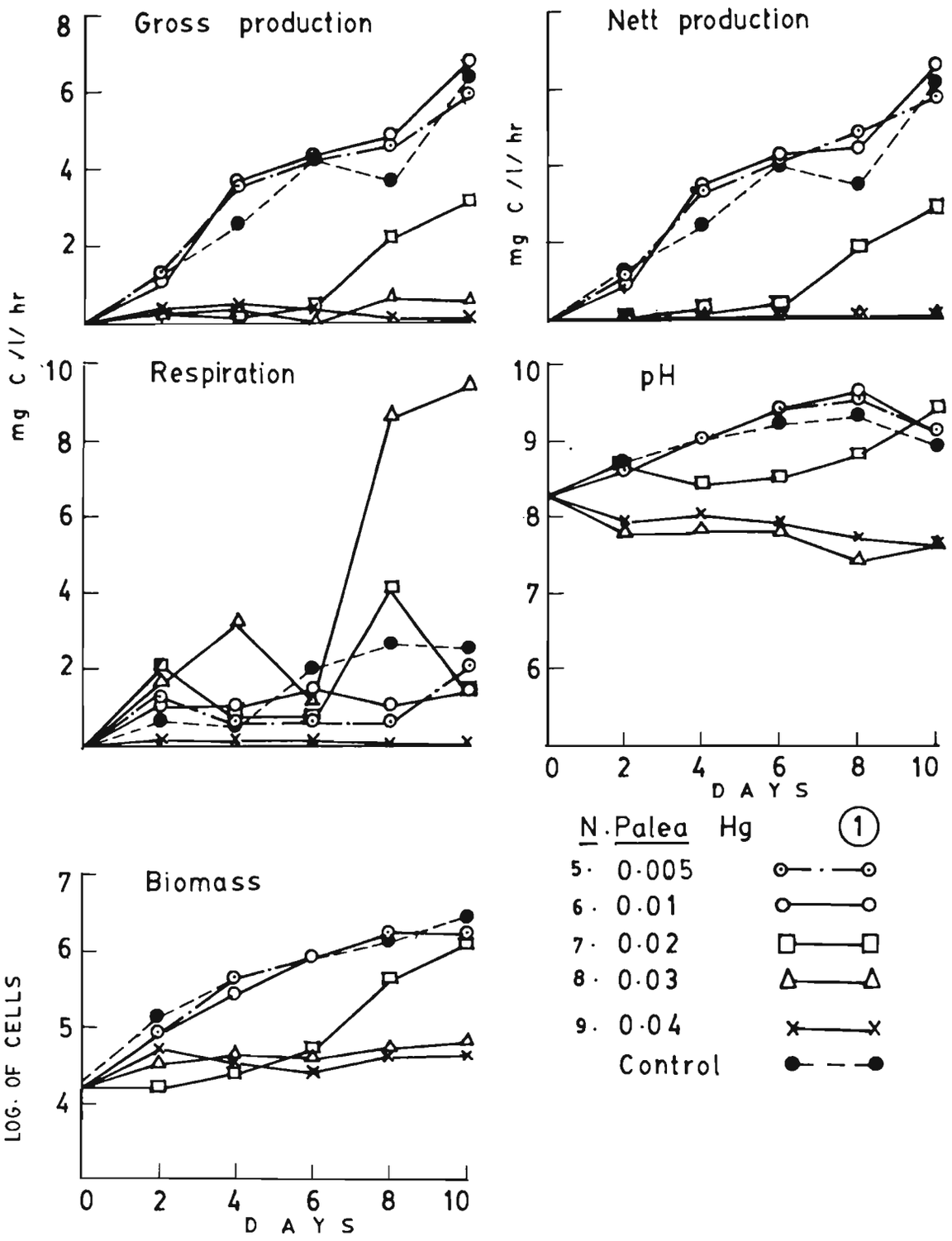

Gross production
Nett production
Respiration
pH
Biomass
mg C /l/ hr
LOG. OF CELLS
D A Y S
N. Palea Hg
5. 0·005
6. 0·01
7. 0·02
8. 0·03
9. 0·04
Control
1

Fig. 1

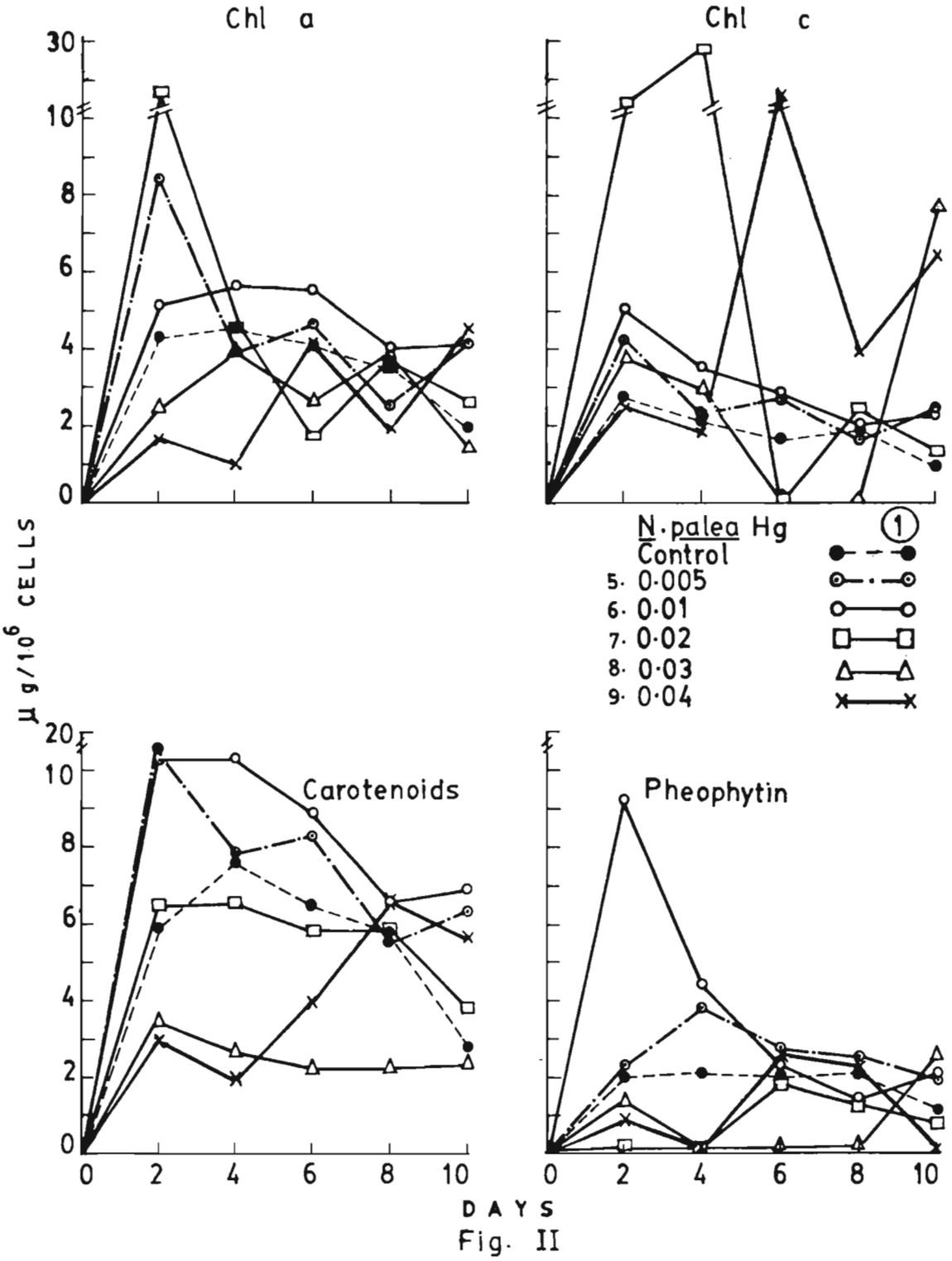

Chl a
Chl c
30
10
8
6
4
2
0
μg/10⁶ CELLS
N.palea Hg
(1)
Control
5. 0·005
6. 0·01
7. 0·02
8. 0·03
9. 0·04
Carotenoids
Pheophytin
20
10
8
6
4
2
0
0
2
4
6
8
10
DAYS

Fig. II

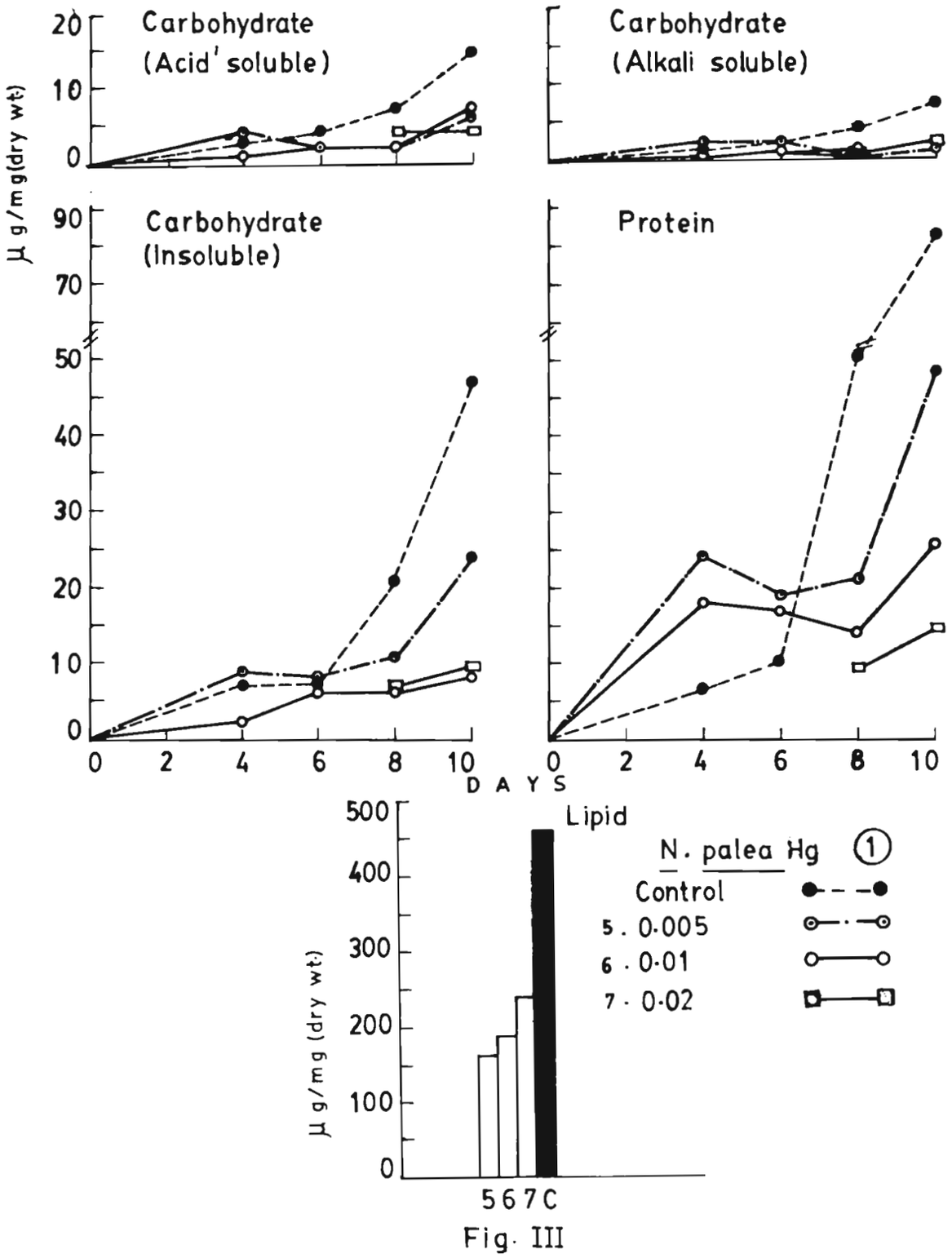

Carbohydrate
(Acid soluble)
Carbohydrate
(Alkali soluble)
Carbohydrate
(Insoluble)
Protein
µ g/mg(dry wt)
DAYS
Lipid
µ g/mg (dry wt)
N. palea Hg ①
Control
5 . 0·005
6 . 0·01
7 . 0·02
5 6 7 C

Fig. III

5.1.2

Effect of cadmium on S. bijugatus
(Figs. I, II and III)

Sl. No. of the metal	No. of treatment	Selected concentration in ppm
(2)	10	0.01
	11	0.03
	12	0.05

Production: (Fig.I)

Nett production of the alga was enhanced in the early growth phase and the production reached peak earlier than that of control in all the treatments. When the level of cadmium was 0.01 ppm and 0.03 ppm, production increased gradually till sixth day to 30% and 11% higher than that of control respectively. In both instances it declined thereafter, falling well short of control, by 45% and 40% respectively on eighth day. It increased once again towards the end of growth phase but not to the same extent as that of control and exhibited respective reduction by 12% and 8% on the last day. When the cadmium level was 0.05 ppm inspite of initial depression it increased sharply to be 58% higher than that of control by forth day and declined thereafter to 28% lower level on sixth day. Once again an increase was observed to 19% higher than that of control,at the end of growth phase.

Respiration of the alga was reduced to a large extent in 0.01 ppm cadmium treatment and was slightly higher than that of control only on sixth day. It fluctuated with two peaks, on second day and eighth day with 24% and 10% reduction respectively. It was reduced further, by 70%, at the end of growth phase. In the second treatment further reduction was observed and respiration of the alga remained less than that of control through out the growth period. From 60% lower level on second day it declined to lowest on forth day. Gradual increase in respiration was noted from sixth day onwards but on the last day it was found to be reduced by 27%. When the alga was exposed to 0.05 ppm cadmium respiration increased significantly unlike in the other two treatments except in the late growth phase. Severe fluctuation in respiration with two peaks, on second and eighth day was observed recording an increase of 165% and 528% respectively. Respiration was minimum on tenth day. It registered an increase towards the end of growth phase to 9% lower level than that of control.

Enhanced production during the early growth phase in first and second treatments was reflected in increased pH upto sixth day, which remained without much variation except on the last day when it was slightly higher in the second. In the third treatment pH of the culture increased at a low pace till forth day and

remained steady thereafter but was less than that of control. It was also lower than in other two treatments throughout growth phase, except on the last day. The maximum level of pH attained by the control (10.64) was not reached in any of the treatments.

Pigments: (Fig.II)

Pigment production was not delayed when the concentration of the metal was 0.01 ppm and 0.03 ppm. In these two treatments peaks were found on second day as in control. Total pigment concentration decreased with the aging of culture. It remained higher than that of control throughout. Between the two treatments, more chlorophyll a and chlorophyll b were produced by the alga in second treatment whereas carotenoids and pheophytin were produced to a greater extent in the first treatment. At 0.05 ppm cadmium level chlorophyll a and pheophytin were initially suppressed and very little chlorophyll b and carotenoids were produced, when compared to those of control. Neverthless pigment level in this treatment was highest during later phase of growth. Pheophytin content was minimum in this treatment during growth phase but did not differ much from that of control or other two treatments, at the end of growth phase. The level of pheophytin decreased with increase in metal concentration.

Photosynthetic end products: (Fig. III)

In all treatments, carbohydrate content of the alga was reduced in early phase. Also it fluctuated with two peaks during the growth phase. In the first treatment the two peaks were found one on other and eighth with forth day with 40% reduction and 20% increase in relation to control. In the other two treatments it reached maximum level later than that of control, on sixth and tenth days respectively with 126% and 138% increase. In the third treatment 80% increase was recorded on both sixth and tenth days of growth. But the maximum concentration of this product attained by that of control was not reached by the alga in any treatment. The variation was only marginal between any two treatments. At the end of growth phase it was higher than that of control by 53%, 75% and 86% respectively in the three treatments.

In the selected concentrations of cadmium, protein content of the alga was reduced. It did not differ between 0.01 ppm and 0.03 ppm treatments considerably but in the 0.05 ppm treatment further reduction occurred. The observed percentage reduction was 24, 18 and 40 in the three treatments respectively.

The lipid content of the alga increased in the 0.01 ppm and 0.05 ppm treatments by 30% and 75%

respectively, in relation to control whereas it was 8% less in 0.03 ppm treatment.

Growth: (Fig.I)

Growth rate of the alga was enhanced when the cadmium level was 0.01 ppm and 0.03 ppm, resulting in increased biomass in the early growth phase, when compared with that of control. From sixth to tenth day, it was same and steady in both the treatments but at the end it was slightly higher in the first treatment. The growth rate of the alga exposed to 0.05 ppm cadmium, was lowered and hence the biomass, when compared to that of control but the final yield did not differ much between the treatments. Biomass decreased with increasing cadmium concentration. Details of the nutrient uptake are given below:

Selected concentration of metal	Nutrients absorbed by the alga (µg/l)		N / P
	Phosphate	Nitrate	
0.01	1095	607	0.55
0.03	1563	1235	0.79
0.05	1461	423	0.29
Control	945	1290	1.37

Phosphate uptake of the treated alga increased where as nitrate uptake decreased. But the uptake was not directly proportional to increasing metal concentration.

Above and below 0.03 ppm level, though the uptake of both the nutrients was reduced, that of nitrate was reduced to a greater extent.

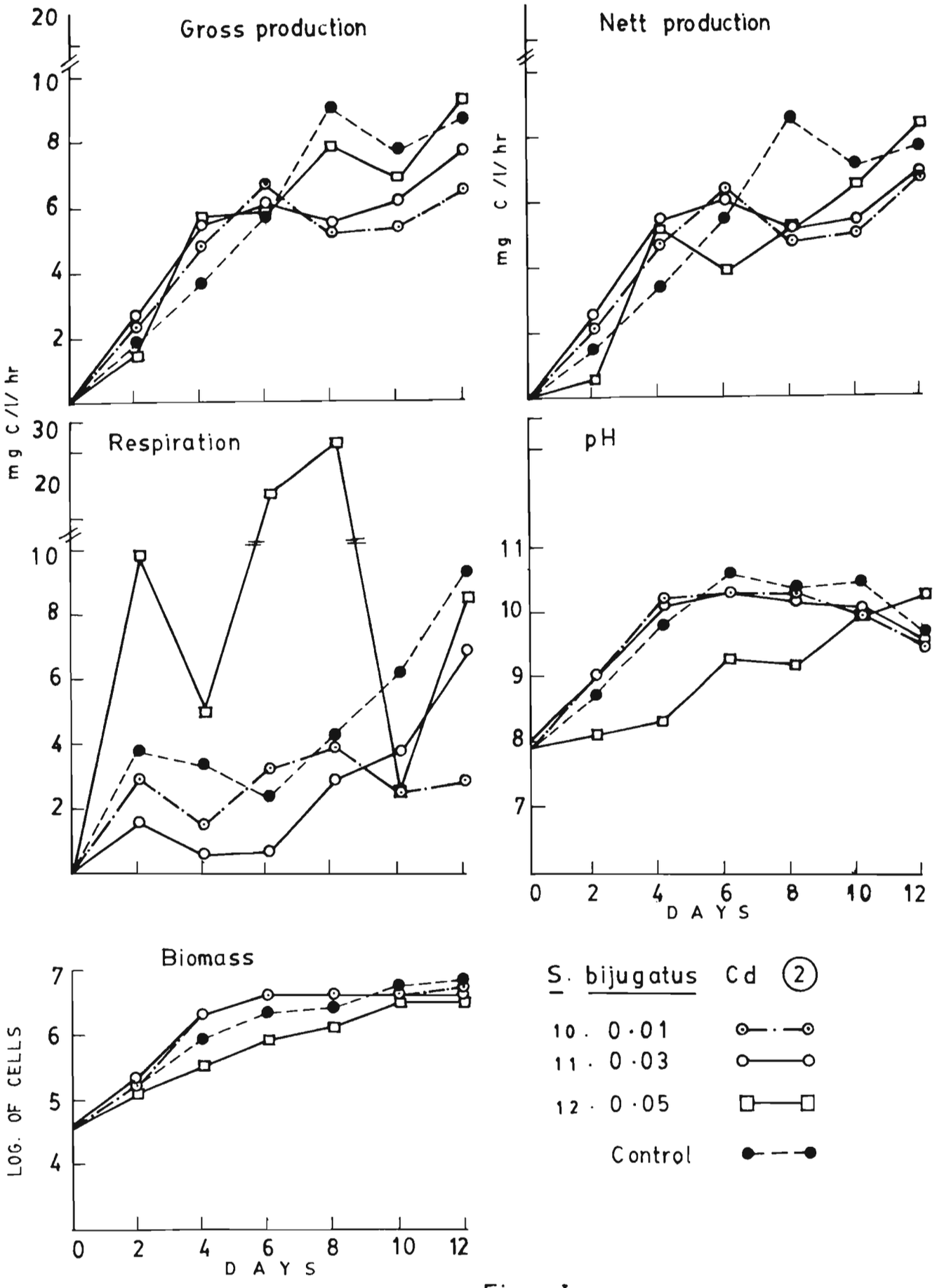

Gross production
Nett production
Respiration
pH
Biomass
mg C/l/ hr
LOG. OF CELLS
DAYS
S. bijugatus Cd (2)
10 . 0·01
11 . 0·03
12 . 0·05
Control

Fig. I

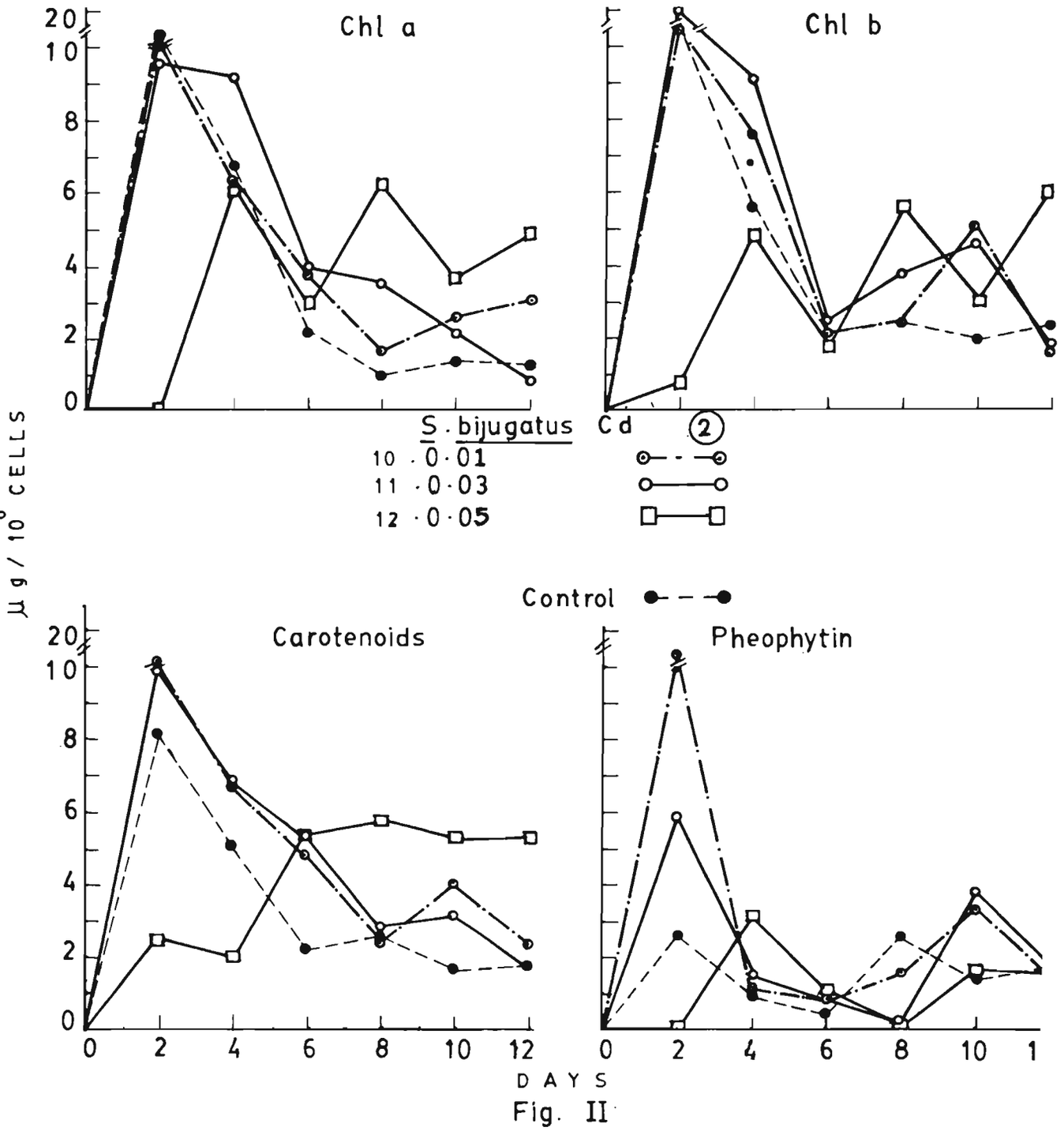
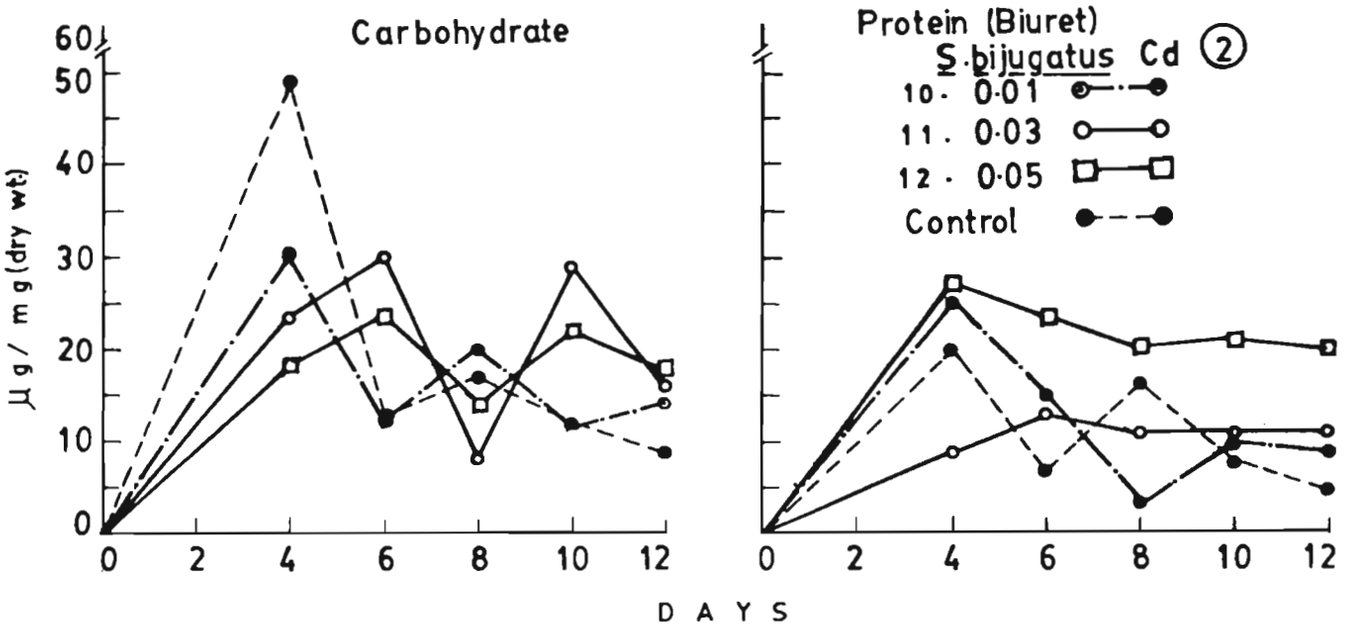

Chl a
Chl b
Carotenoids
Pheophytin
µg / 10^6 CELLS
DAYS
S. bijugatus
10 · 0·01
11 · 0·03
12 · 0·05
Cd (2)
Control

Fig. II


Carbohydrate
Protein (Biuret)
S. bijugatus Cd
10. 0·01
11. 0·03
12. 0·05
Control
µg/mg (dry wt.)
DAYS

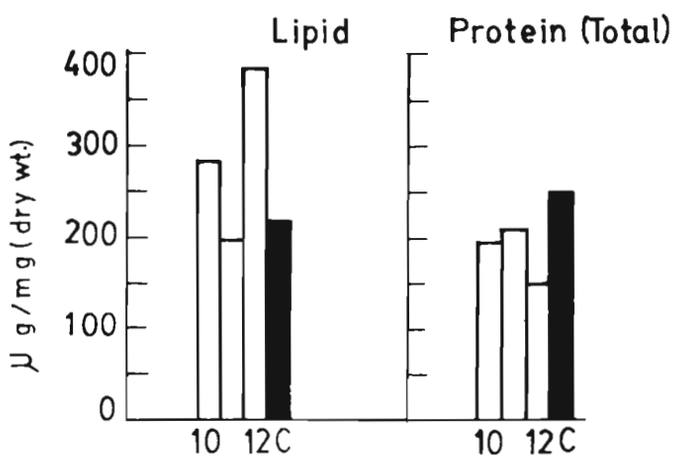

Lipid
Protein (Total)
µg/mg (dry wt.)
10
12C
10
12C

Fig. III

Effect of cadmium on *N. palea*

(Figs. I, II and III)

Sl. No. of the metal	No. of treatment	Selected Concentration (in ppm)
	13	0.02
(2)	14	0.03
	15	0.04

Production: (Fig. I)

The nett production and respiration of the diatom varied with the concentration of the metal.

When exposed to 0.02 ppm cadmium gradual increase in production was observed from fourth day onwards. In relation to control, it remained higher by 63% on fourth day, 10% on sixth day and by 1% on the last day. Production was enhanced when diatom exposed to 0.03 and 0.04 ppm levels of cadmium in the early phase as indicated by the maxima reached earlier than that of control. It increased gradually in both treatments and was 34% higher than control on fourth day, declined thereafter to be 74% and 64% less respectively on sixth day.

Once again production registered an increase, till the end of growth phase when it was 4% higher than control in 0.03 ppm treatment, but only upto eighth day in 0.04 ppm treatment remaining steady thereafter to be 31% less than control on the last day.

Respiration of the diatom was elevated to a higher level than that of control, in all treatments upto sixth day. It was increased by 300% on second day, by 94% on sixth day, declined thereafter to be 60% less than that of control at the end of growth phase, in first treatment. Respiration in 0.03 ppm and 0.04 ppm treatments was 66% more than that of control on forth day. It increased to a greater extent thereafter in 0.04 ppm treatment, upto sixth day and was 5% and 74% higher than that of control respectively. It was steady upto eighth day and once again increased by 32% at the end of growth phase in 0.03 ppm treatment. Whereas slow and steady decline was observed in 0.04 ppm treatment to 4% lower level on the last day, in relation to control.

pH of the culture remained higher than that of control in 0.02 ppm treatment owing to high rate of production. In the other two treatments it did not deviate much from that of the control though it was comparitively reduced than in early growth phase, inspite of enhanced production rate.

Pigments: (Fig.II)

At the lowest concentration of the metal chlorophyll a increased. When the diatom was exposed to 0.03 ppm and 0.04 ppm cadmium, chlorophyll a was produced to a lesser extent in early growth phase. Nevertheless, a higher concentration than that of control was attained towards the end of growth phase. Fluctuation in the level of chlorophyll a was common to all treatments. Total chlorophyll a decreased with increasing metal concentration.

Considerable increase in chlorophyll c level was noted only in first treatment on second day. From fourth day onwards in all treatments its level did not deviate much from that of control except on the last day when it was considerably higher. In all treatments an increasing tendency was recorded in the concentration of chlorophyll c towards the end of growth phase.

Enhancement in the production of carotenoids was observed in the early growth phase at 0.02 ppm. It reached maximum concentration on second day whereas in others on fourth day as in control. In all instances the level of carotenoids registered a decline from their respective maxima but increased once again towards the end of growth phase. Total carotenoids decreased with increase in cadmium concentration.

Pheophytin content of the diatom did not increase to the same extent as that of control in the early growth phase except in the last treatment. It was higher than that of control on sixth and tenth day at 0.02 ppm cadmium level, on sixth and eighth day at 0.03 ppm level and on second day at 0.04 ppm level. Total pheophytin decreased with increase in cadmium concentration.

Total pigment content of the diatom decreased with increase in metal concentration.

Photosynthetic end products: (Fig.III)

In all treated samples, acid soluble carbohydrate fraction was more than that of control in early phase. The concentration was highest in 0.03 ppm treatment followed by 0.02 ppm and 0.04 ppm treatments. At the end of growth phase the concentration of this fraction fell short of control by 50%, 16% and 27% respectively in the three treatments.

The alkali soluble fraction was severely inhibited at 0.02 ppm cadmium level and it was negligible on fourth and tenth day of growth. At 0.03 ppm and 0.04 ppm levels, the production of this fraction did not differ much from that of control except at the end of growth phase when it was 50% less.

The insoluble carbohydrate fraction of the diatom was adversely affected at 0.02 ppm and 0.03 ppm

cadmium levels in the later phase of growth. From 44% and 39% higher level on fourth day, inspite of gradual increase towards the end of growth phase it was found to be reduced to 60% and 64% lower level than that of control respectively on tenth day. In the third treatment it was 30% more than that of control on fourth day and continued to increase gradually till eighth day and quickly thereafter, surpassing that of control by ninth day. It was 77% higher at the end of growth phase in relation to control.

Protein content of the diatom was promoted in the early growth phase. 117% increase was recorded for the first and second treatments and 100% for the third treatment on sixth day. In all three, protein level declined upto eighth day to increase once again, but not to the same extent as in control and on the last day 60%, 70% and 74% reduction was registered respectively. Total protein of the diatom decreased with increase in metal concentration.

Toxic effect of cadmium is evident when the lipid, major photosynthetic product of the diatom was considered. In all treated samples it was reduced considerably. Among the treatments maximum quantity was recorded when the diatom was exposed to 0.03 ppm cadmium with only 44% reduction. In the other two treatments respective reduction of 78% and 60% was recorded.

Growth: (Fig.I)

Growth proceeded at a quicker pace in the early phase and the biomass in treatments was higher than that of control. At 0.03 ppm level cadmium delayed the multiplication of cells between second and fourth day but subsequent recovery was recorded. Neverthless, by the end of growth phase the biomass in all treatments was found reduced, when compared to that of control.

The variations in the absorption of phosphate and nitrate are given below:

Selected concentration of metal	Nutrients absorbed (µg/l)		N / P
	Phosphate	Nitrate	
0.02	1735	933	0.54
0.03	1735	922	0.53
0.04	1685	913	0.54
Control	1350	763	0.57

Both the nutrients were absorbed in greater quantity in presence of cadmium when compared to that of control. Very little variation was noticed between treatments though a tendency of reduction in uptake was found. Phosphate was taken up in greater proportion than nitrate.

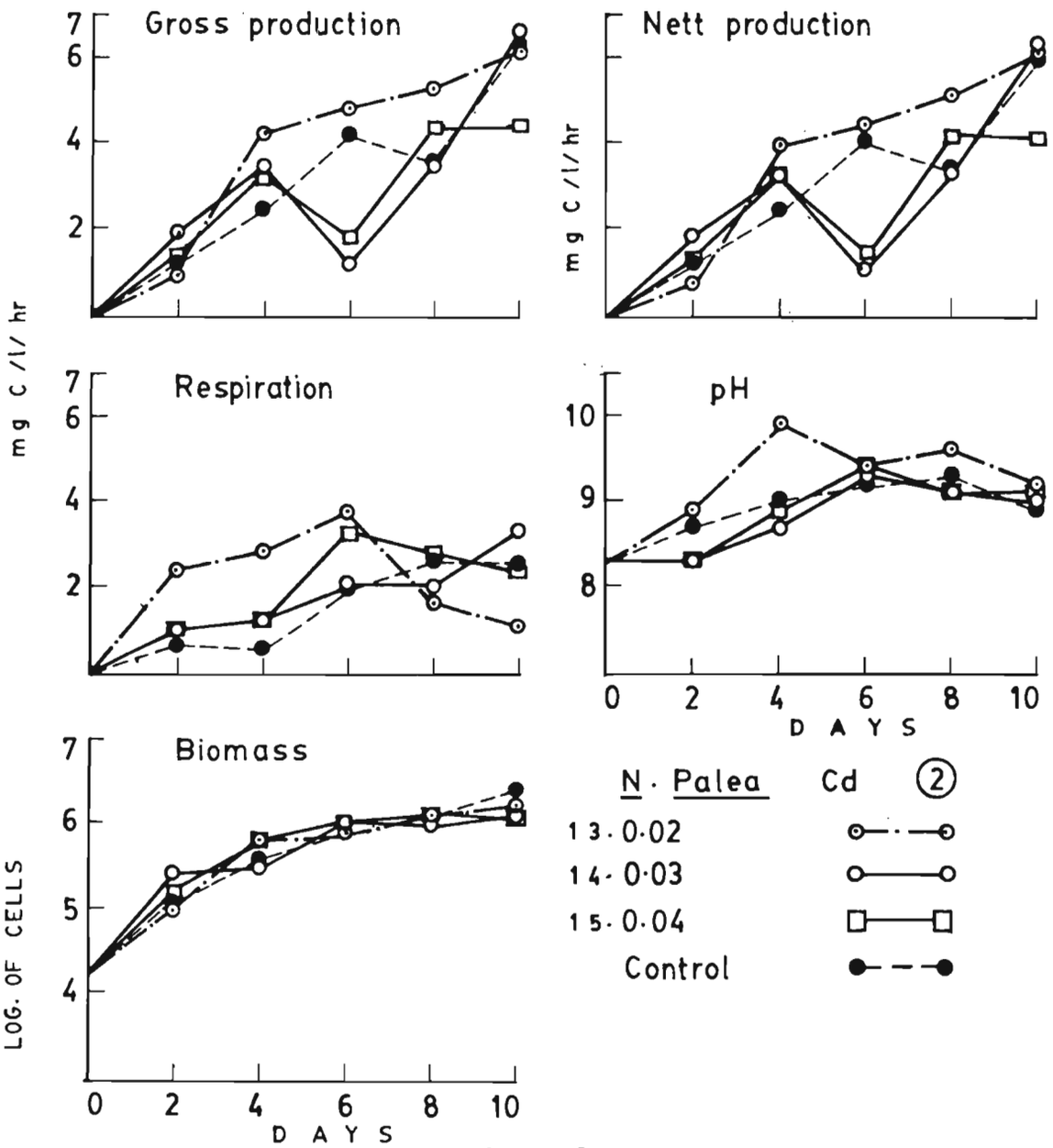

Gross production
Nett production
Respiration
pH
Biomass
mg C /l/ hr
LOG. OF CELLS
DAYS
N · Palea Cd ②
13 · 0·02
14 · 0·03
15 · 0·04
Control

Fig. I

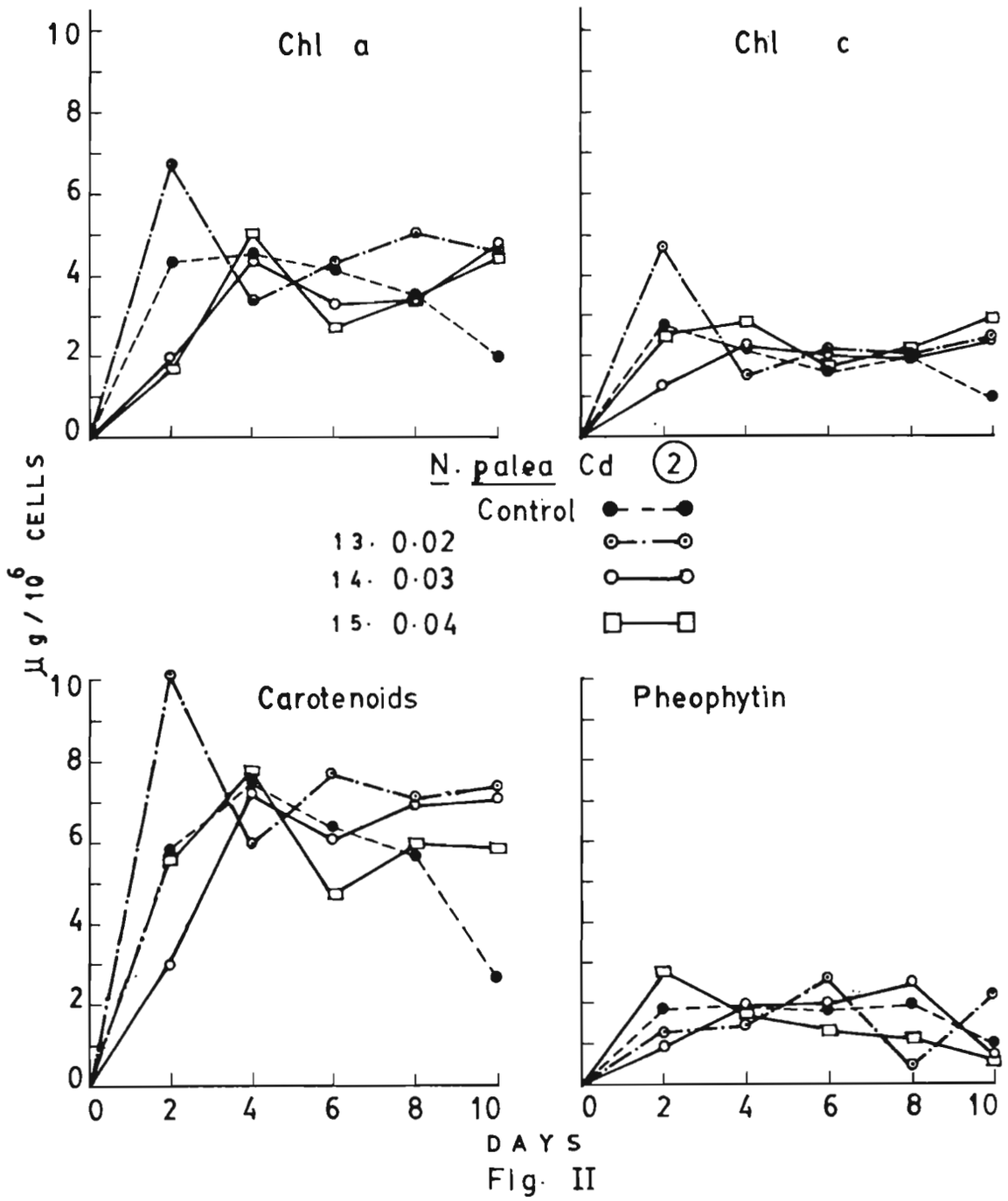

Chl a
Chl c
Carotenoids
Pheophytin
N. palea Cd (2)
Control
13. 0·02
14. 0·03
15. 0·04
µg / 10^6 CELLS
DAYS
0
2
4
6
8
10

Fig. II

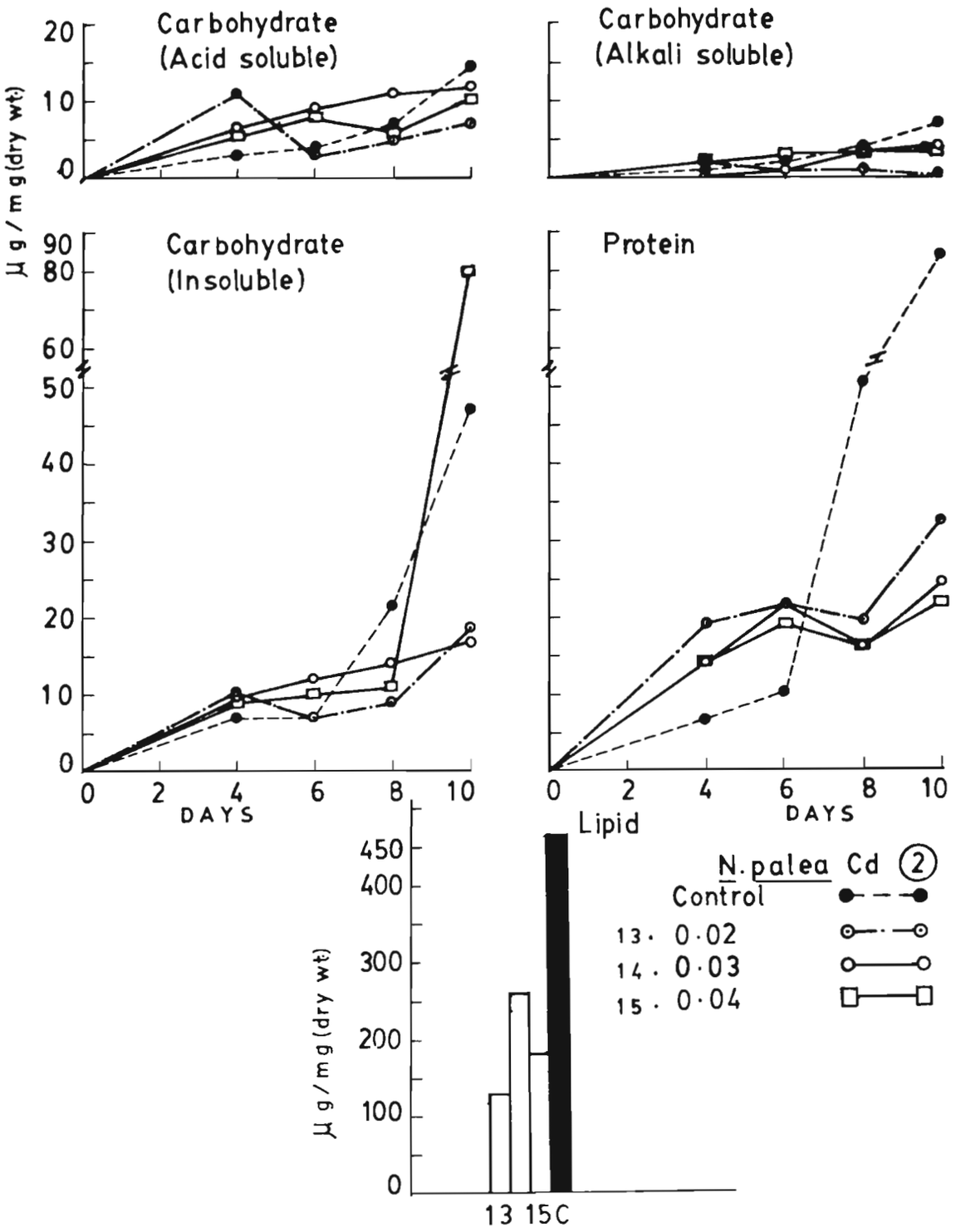

Carbohydrate
(Acid soluble)
Carbohydrate
(Alkali soluble)
Carbohydrate
(Insoluble)
Protein
Lipid
µg/mg(dry wt)
DAYS
N. palea Cd (2)
Control
13 · 0·02
14 · 0·03
15 · 0·04
13 15C

Fig. III

5.1.3

Effect of lead on S. bijugatus

(Figs. I, II and III)

Sl. No. of the metal	Number of treatment	Selected concentration (in ppm)
(3)	16	0.05
	17	0.1
	18	0.3

Production: (Fig. I)

At all test concentrations, lead enhanced the nett production in early and late growth phase.

At 0.05 ppm level lead enhanced the rate of nett production to a slightly higher level than that of control upto fourth day. Further change was not observed till sixth day. It was 6% more than that of control on fourth day. Production increased sharply from sixth day reaching 163% higher level on the last day of growth. When the concentration of lead was 0.1 ppm the nett production increased by 18% on fourth day. It declined till sixth day but increased sharply thereafter till tenth day. It was steady from tenth day onwards and was 137% more in relation to control on the last day. In 0.3 ppm treatment production increased by 72% on fourth day. It declined to minimum on sixth day with 88% reduction and increased thereafter gradually to 95% higher level by the end of growth phase.

Respiration of the alga exhibited severe fluctuation in presence of lead with two peaks on second and eighth day. Also it was elevated to a large extent.

In the first treatment respiration increased by 265% and 566% respectively on second and eighth day but declined to 66% lower level by twelfth day. In the second treatment 386% and 619% increase was observed on second and eighth day respectively. It declined thereafter sharply to minimum level on tenth day and increased once again to be 4% less than that of control by the end of growth phase. When the alga was exposed to 0.03 ppm lead, respiration was elivated by 89% on second day and by 562% on eighth day. In this instance also the respiration exhibited sharp reduction to minimum level on tenth day, increased thereafter but was 17% less than that of control on twelfth day.

pH of the culture did not vary from the initial level on second day at 0.05 and 0.3 ppm level of lead. In all treatments it increased gradually towards the end of growth phase. Among the treatments, it was least in 0.05 ppm level and highest in 0.3 ppm level during growth phase but reached same level at the end in all, which was slightly higher than the maximum reached by the control (10.64).

Pigments: (Fig. II)

Total pigment content of the alga increased in presence of the metal. All four pigments were completely suppressed upto second day in 0.1 ppm. The total pigment content was least in 0.1 ppm when compared to other treatments.

When the alga was treated with 0.05 ppm lead its chlorophyll a content was slightly higher than that of control in the later phase, where as in 0.1 ppm treatment it increased from second day to maximum level on fourth day. It remained less than that of control except on eighth and twelfth day. Concentration of chlorophyll a fluctuated with two peaks on second and sixth day when the lead concentration was 0.3 ppm and was higher at the end of growth phase, when compared to other two treatments.

Chlorophyll b was the most adversely affected pigment by this metal. All treated samples were found to contain very little chlorophyll b during growth phase. In 0.05 and 0.3 ppm, the pigment was detected on all days but it was not detected on second, sixth and eighth day in 0.1 ppm treatment. Inhibition was maximum in 0.1 ppm lead and minimum in 0.3 ppm.

Severe fluctuations were observed in carotenoid content of the treated alga. Concentration was generally

less than that of control upto fourth day. In all instances the treated alga had greater amount of cartenoids at the end of growth phase in relation to control. Inhilition of carotenoids was less in 0.3 ppm in the latter phase than in 0.05 and 0.1 ppm.

Pheophytin of alga was suppressed to a considerable extent except towards the end. It was not detected on sixth and eighth day in 0.05 ppm, upto eighth day in 0.1 ppm and from sixth to tenth day in 0.3 ppm.

The alga when grown in 0.1 ppm lead (an intermediary level) was found to produce pigments to least extent.

Photosynthetic end products: (Fig. III)

In the first and second treatments, the carbohydrate content of the alga was maximum on fourth day but with 30% and 40% reduction when compared to that of control. It decreased as the culture aged, to a 20% lower level in first treatment. In the second treatment another peak on eighth day was observed with 25% increase but it was lowered to 7% higher level by the end of growth phase. In the third treatment eventhough the carbohydrate was 60% less than that of control on fourth day it increased by 326% on tenth day. But the amount of carbohydrate dropped to 37% higher level at the end of growth phase.

Protein content of the alga was promoted by lead and the percentage increase was 18 for first and second treatments and 27 for the third treatment.

The lipid content did not show much variation. It was equal to that of control in the first treatment but was reduced by 14% in the other two treatments.

Even when the concentration of lead was high protein and lipid contents of the alga were not much affected.

Growth: (Fig. I)

The algal biomass was less than that of control throughout the growth phase in all treatments.

Selected concentration of metal	Nutrients absorbed by the alga (µg/l)		N / P
	Phosphate	Nitrate	
0.05	1132	980	0.87
0.1	1130	963	0.85
0.3	1121	960	0.86
Control	945	1290	1.37

It was found that the addition of selected concentrations of lead to the culture medium increased the absorption of phosphate whereas that of nitrate decreased when compared with control. Only marginal variation was observed between any two treatments. Corresponding to this the N / P ratio did not vary between the treatments.

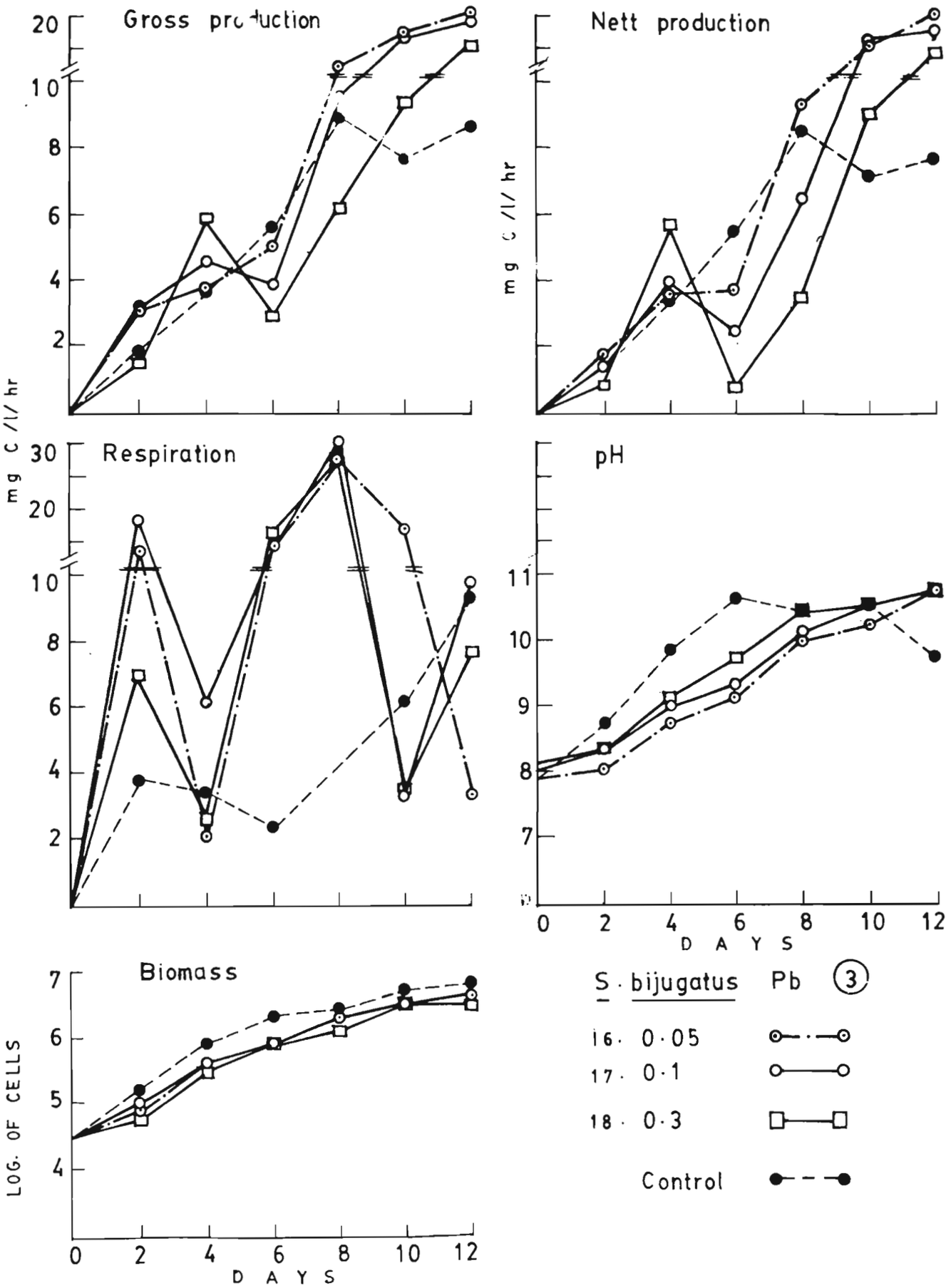

Gross production
Nett production
Respiration
pH
Biomass
mg C /l/ hr
LOG. OF CELLS
D A Y S
S. bijugatus Pb (3)
16. 0·05
17. 0·1
18. 0·3
Control

Fig. 1

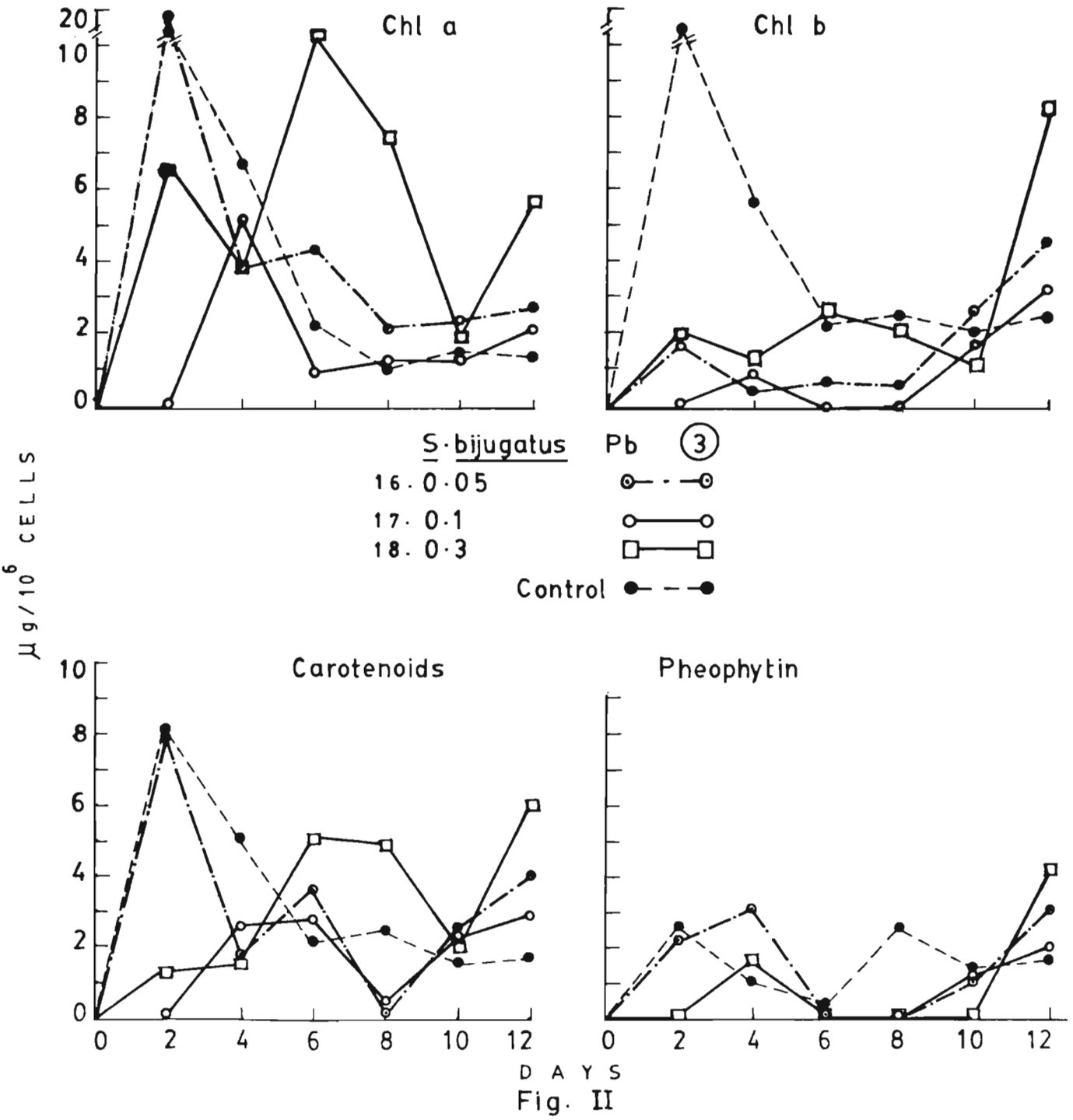
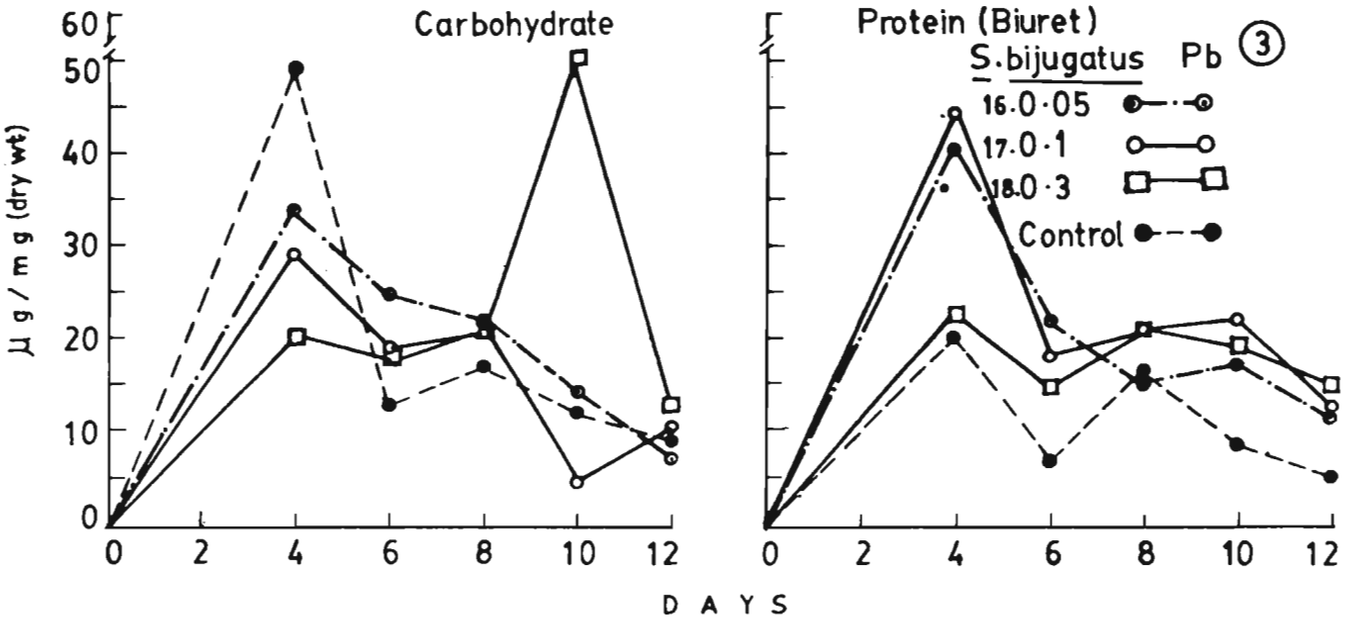

Chl a
Chl b
S·bijugatus Pb (3)
16. 0·05
17. 0·1
18. 0·3
Control
Carotenoids
Pheophytin
µg/10⁶ CELLS
DAYS

Fig. II


Carbohydrate
Protein (Biuret)
S. bijugatus Pb
16.0·05
17.0·1
18.0·3
Control
μ g / m g (dry wt)
60
50
40
30
20
10
0
0 2 4 6 8 10 12
DAYS

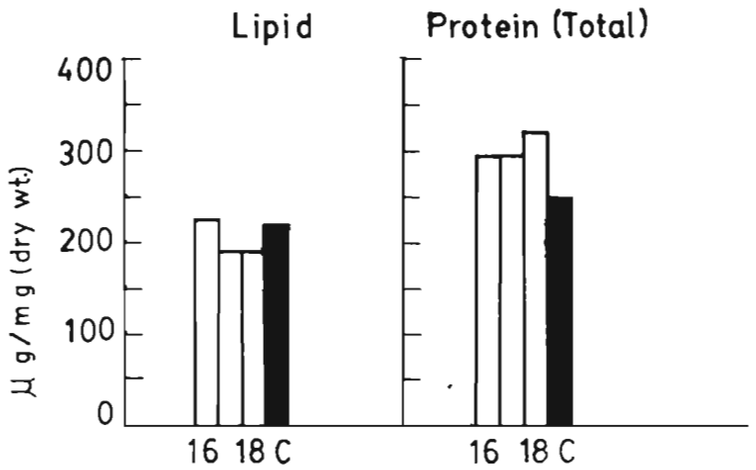

Lipid
Protein (Total)
μ g/m g (dry wt)
400
300
200
100
0
16 18 C
16 18 C

Fig. III

Effect of lead on *N. palea*

(Figs. I, II and III)

Sl. No. of the metal	Number of treatment	Selected concentration (in ppm)
③	19	0.02
	20	0.04
	21	0.06
	22	0.08

Production: (Fig. I)

The nett production of the diatom was adversely affected by lead at all dose concentrations and remained less than that of control throughout growth phase except on eighth day in the third treatment. Production reached maximum level on sixth day but remained 21% less than that of control and declined thereafter to 77% lower level by tenth day in the first treatment. When exposed to 0.04 ppm lead, production increased gradually to be equal to that of control by fourth day. It increased further to its maximum lead on sixth day but was less than that of control by 37%. The production declined thereafter and it was 70% less in relation to control on the last day. Production in 0.06 ppm and 0.08 ppm treatments was almost same upto fourth day. In both instances it reached maximum level

on eighth day with 7% increase in the former and 2% reduction in the latter, in relation to control. It declined thereafter to 73% and 75% lower level respectively at the end of growth phase.

Respiration of diatom varied with the concentration of the metal. It was higher throughout growth phase in the first treatment and exhibited an increase of 500% on second day and was minimum, at slightly higher level than control on fourth day. It increased once again gradually to 100% higher level at the end of growth phase. In the second treatment from 104% higher level on fourth day, at declined to the minimum on sixth day. It increased sharply to 176% higher level at the end of growth phase, which was highest. For the third and fourth treatments it was 100% more than that of control on second day. Very little variation was observed there after in third treatment and it exhibited 50% reduction at the end of growth phase whereas in the fourth treatment it declined to minimum level by fourth day. Though it showed gradual increase thereafter, the value was 48% less than that of control on the last day.

pH of the culture in 0.02 and 0.04 ppm treatments remained higher than that of control, throughout in the latter and from sixth day onwards in the former. In 0.06 and 0.08 ppm treatments pH remained generally less than that of control except on the last day.

Pigments: (Fig. II)

Pigment content of the diatom was considerably higher than that of control during the middle of growth.

In all the treatments the diatom developed maximum amount of chlorophyll a little later than that of control. When the maximum was reached on sixth day in the first and second treatments, it occurred on fourth day in third and fourth treatments. Considerable fluctuation in the pigment level was observed in second and third treatments. In the last treatment the concentration was equal to that of control at the end of growth phase whereas in the others, slightly higher.

The diatom grown in 0.04 ppm lead developed chlorophyll c to larger extent when compared to other treatments and was slightly less than that of control only on the last day. In the other instances the concentration of chlorophyll c was generally higher than that of control fourth day onwards. Total chlorophyll c was almost equal in first and second treatments and decreased with increase in the metal concentration. Fluctuation in the level of chlorophyll c was observed in second and third treatments.

Cartonoid content was more than that of control when the diatom was exposed to 0.02 and 0.04 ppm lead throughout growth phase, except at the end when it

was short of control in the latter. In the 0.06 and 0.08 ppm treatments it exceeded that of control by fourth day. Maximum amount of this pigment was found on fourth day in these two treatments. In the second treatment the concentration was slightly less than that of control at the end of growth phase whereas in others it was higher.

Pheophytin content of the diatom was greater than that of control throughout growth phase in the first treatment and was maximum on fourth day. In the second treatment it reached maximum level on sixth day, declined thereafter towards the end of growth phase and was not detected on the last day. In the third and fourth treatments pheophytin was less than that of control except on sixth and tenth days. In the third treatment it was not detected on second and fourth day.

Photosynthetic end products: (Fig. III)

The acid soluble carbohydrate content, was generally higher than that of control towards the end of growth phase in all treatments. The carbohydrate concentration was 86% more on sixth day when the diatom was exposed to 0.02 ppm lead. Inspite of further increase it was found to be only 66% higher on tenth day in relation to control. It was less than that of control upto sixth day in the second treatment but increased sharply

thereafter reaching 445% higher level by tenth day. At 0.06 ppm level of lead, the carbohydrate was 57% more than that of control on sixth day and decreased till eighth day, to increase once again to 72% higher level at the end of growth phase. When the diatom was exposed to 0.08 ppm lead, carbohydrate content was more than that of control. Increasing from 242% higher level on sixth day to 79% higher level by tenth day.

The concentration of alkali soluble carbohydrate of the treated diatom did not show much variation between any two treatments and also between any treatment and control. It was 44% more than that of control in the diatom exposed to 0.04 ppm lead but was reduced by 60%, 32% and 3% in the other treatments respectively, at the end of growth phase.

The insoluble carbohydrate content of the diatom increased when exposed to 0.04 and 0.08 ppm lead and decreased in 0.02 and 0.06 ppm treatments, in relation to control in early phase. It was 57% more than that of control on fourth day in the second treatment. In other treatments it was 53%, 94% and 81% more respectively on sixth day. In the above three treatments the level of carbohydrate decreased till eighth day and increased once again but was less than control by 73%, 78% and 73% respectively on the last day. In the second

treatment it increased sharply from sixth day to 30% higher level by eighth day and gradually thereafter, but not to the same extent as control and was reduced by 27% on the last day. Among the treatments insoluble fraction level was highest in this instance.

Total protein measured on the basis of nitrogen was higher than that of control in all treated samples upto sixth day. When the diatom was cultured in 0.02, 0.06 and 0.08 ppm lead, percentage increase was 101, 181 and 163 respectively on sixth day of growth. But towards the end of growth phase protein level was lowered considerably in these three treatments when compared to that of control by 75%. The diatom was exposed to 0.04 ppm lead produced maximum amount of protein when compared to other treatments. It was 252% more than that of control on fourth day and exhibited only 58% reduction at the end of growth phase.

Lipid, the major photosynthetic end product of the diatom was adversely affected at all experimental dose levels of lead. The percentage reduction was 63 and 73 for first and second treatments where as 54 for the other two.

Growth: (Fig.I)

The biomass was reduced in presence of lead inspite of initial stimulation in growth in 0.02 ppm

and 0.04 ppm treatments. Growth was delayed in the early phase at 0.06 and 0.08 ppm levels of lead, but subsequent recovery was observed when it proceeded at a better pace than that of control. Generally from sixth day onwards not much difference was observed between any two treatments and the final yield was less than that of control.

Details of nutrient uptake are given below.

Selected concentration of metal	Nutrients absorbed (µg/l)		N / P
	Phosphate	Nitrate	
0.02	1045	768	0.74
0.04	975	763	0.78
0.06	1000	765	0.77
0.08	996	760	0.76
Control	1350	763	0.57

The treated diatom absorbed phosphate to a lesser extent (by 300 µg/l) when compared to that of control.

The nitrate absorption was not affected. The N / P ratio varied little in the treatments and generally remained higher than that of control.

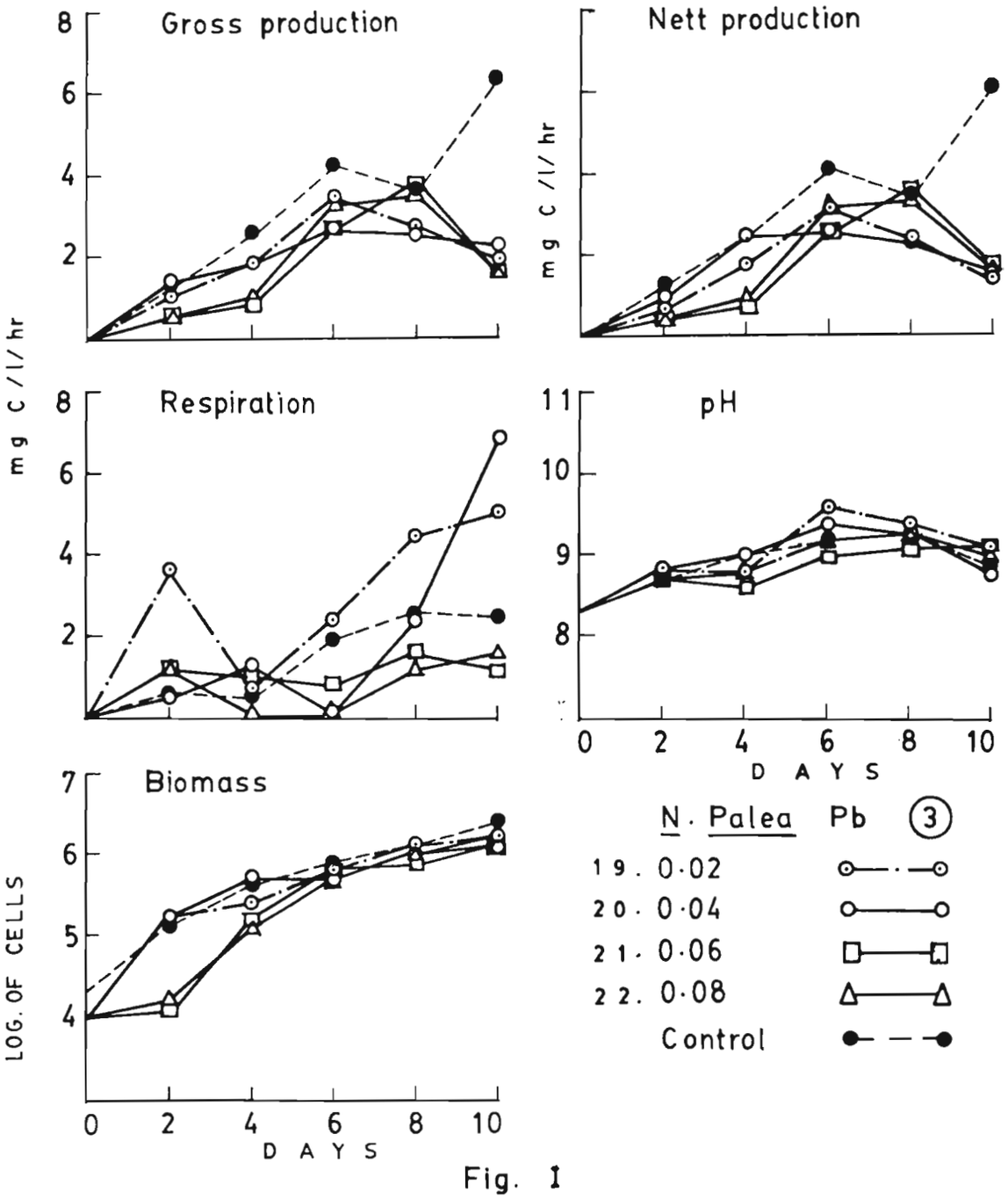

Gross production
Nett production
Respiration
pH
Biomass
mg C /l/ hr
LOG. OF CELLS
DAYS
N. Palea Pb (3)
19. 0·02
20. 0·04
21. 0·06
22. 0·08
Control

Fig. 1

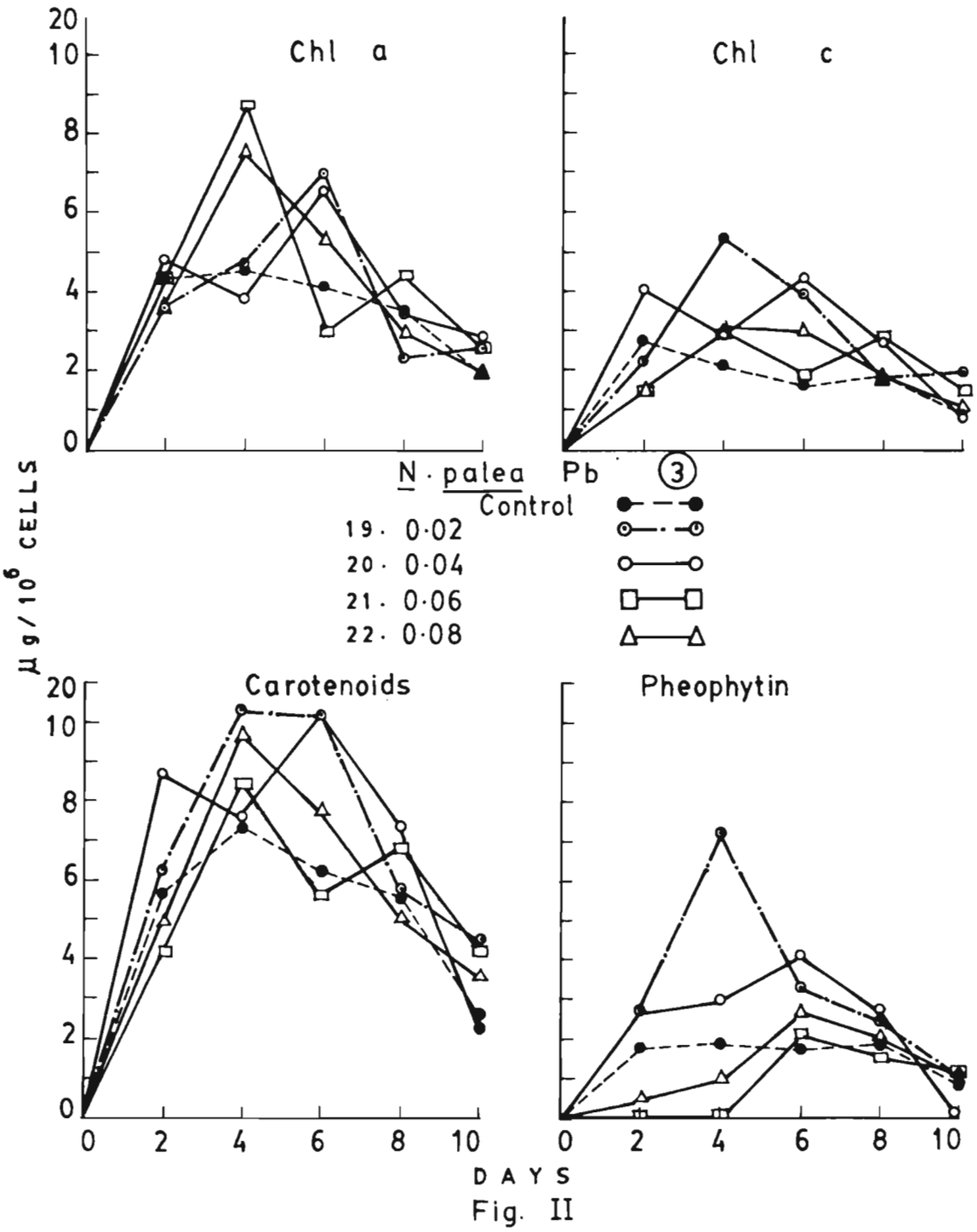

Chl a
Chl c
N. palea Pb (3)
Control
19. 0·02
20. 0·04
21. 0·06
22. 0·08
Carotenoids
Pheophytin
µg/10⁶ CELLS
DAYS
0
2
4
6
8
10
20

Fig. II

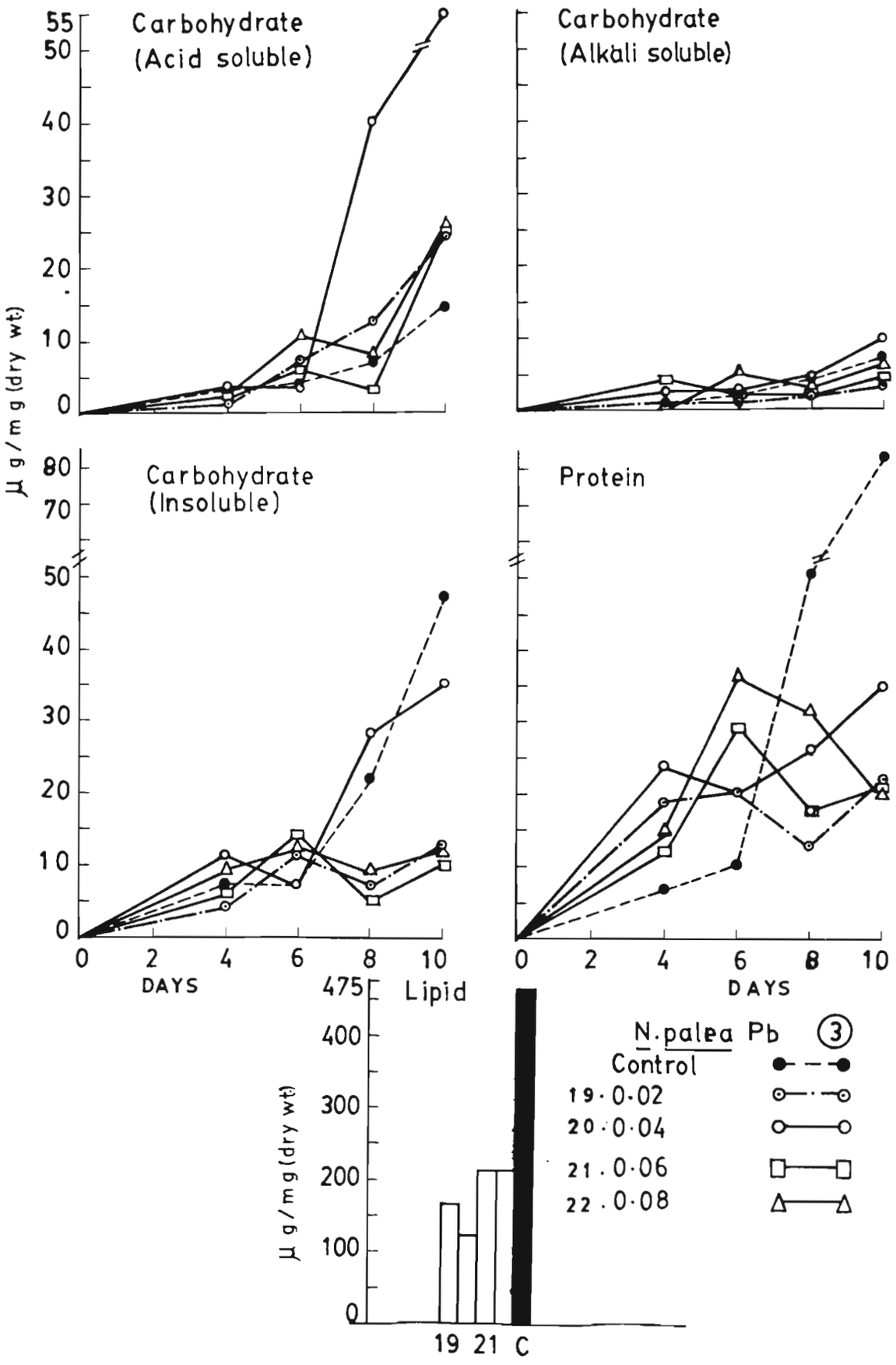

Carbohydrate
(Acid soluble)
Carbohydrate
(Alkali soluble)
Carbohydrate
(Insoluble)
Protein
Lipid
µg/mg (dry wt)
DAYS
N. palea Pb ③
Control
19 · 0·02
20 · 0·04
21 . 0·06
22 . 0·08
19 21 C

Fig. III

5.1.4

Effect of copper on S. bijugatus
(Figs.I, II and III)

Sl. No. of the metal	No. of treatment	Selected concentration (in ppm)
(4)	23	0.1
	24	0.2

Production: (Fig I)

Preliminary experiments with S. bijugatus revealed that tolerance range to copper was ten times higher than that of N. palea.

Nett production of the alga was adversely affected by copper in selected concentrations of 0.1 and 0.2 ppm. Considerable difference was observed between the two treatments in the early phase of growth. It remained less than that of control in the first treatment. 44% and 84% reduction was observed on second day in the two treatments respectively. Production was found to be 9% more in 0.1 ppm treatment whereas 52% less in 0.2 ppm treatment on fourth day. From sixth day onwards production did not vary much between the two treatments. In both, maximum production was recorded on tenth day with 10% and 23% reduction respectively in relation to control. Further reduction was observed, by 32%, in both instances on the last day.

Considerable reduction in the respiration of the alga was observed during the growth phase. It remained less than that of control throughout in the first treatment with 88% reduction at its minimum on tenth day. It increased thereafter to 38% lower level on the last day. Respiration of the alga was further reduced in the second treatment except on tenth day when it was 8% higher than that of control. It was reduced by 42% at the end of growth phase.

Consistent with low production, pH of culture in both treatments did not attain the maximum level reached by control, at any stage of growth. Inspite of high production on fourth and sixth day pH of culture in first treatment fell short of control by sixth day and did not show much variation thereafter. It was slightly higher at the end of growth phase. In the second treatment it was further lowered in the early phase. From eighth day onwards pH declined reaching the same level as that of control at the end of growth phase.

Pigments: (Fig.II)

Total pigment content of the alga increased to a large extent during the growth phase, when exposed to copper.

Chlorophyll a in both treatments remained generally higher than that of control from fourth day

onwards except when it was slightly less than that of control at the end of growth phase in the second treatment. Chlorophyll a reached maximum level on fourth day in 0.1 ppm and on second day in 0.2 ppm. It declined thereafter but from sixth day onwards it remained almost steady in the first treatment, whereas gradual reduction was noted till the end of growth phase in second treatment.

Concentration of chlorophyll b was maximum on fourth day in the first treatment. It registered gradual reduction till eighth day to a lower level than that of control, increasing thereafter to a slightly higher level by the end of growth phase. In the other treatment it fluctuated with two peaks, on second and eighth day but decreased to be just short of control by the end of growth phase.

Carotenoids reached their maximum on fourth and sixth day respectively in the two treatments, at a higher level than that of control. The level of carotenoids once again increased from eighth day onwards in the first treatment but decreased with the age of culture in second treatment.

Pheophytin content of the treated alga was higher than that of control only on fourth and sixth day of growth. It developed to a large extent at 0.1 ppm level. It was not detected on the last day of growth in either treatment.

At 0.1 ppm level of copper, chlorophyll a, chlorophyll b and carotenoids were more than those of control at the end of growth phase but when the level was 0.2 ppm only carotenoids remained higher. Also in the latter all pigments exhibited decreasing tendency towards the end of growth phase.

Photosynthetic end products: (Fig.III)

Total carbohydrate content of the alga remained same in both the treatments and was higher than that of control in later growth phase. It was 63% less than that of control on fourth day but was 268% more, at maximum on tenth day in the first treatment. In the second treatment carbohydrate content was maximum on fourth day but with 33% reduction when compared to that of control and 72% more on tenth day At the end of growth phase little variation was observed between the treatments, with 130% and 119% increase respectively in relation to control.

At 0.1 ppm level of copper, protein content of the alga was not affected. It was equal to that of control. At 0.2 ppm level, this product was lowered by 20%.

Lipid content of the alga was most affected at the selected concentration of copper. It was reduced by 64% when the species was exposed to 0.1 ppm copper where as increased by 44% at 0.2 ppm level.

Growth: (Fig.I)

Growth was stimulated by copper in the first treatment and the biomass was more than that of control upto sixth day. Thereafter it remained steady till the end of growth phase but was found to be short of control on the last day. Negligible variation in biomass was recorded between second treatment and control, upto fourth day. Further growth was not observed till sixth day but the subsequent recovery by the alga resulted in increased biomass to the same extent as in first treatment. However, considerable reduction in biomass was observed in the treatments when compared to that of control, at the end of growth phase.

Details of nutrient uptake of the alga are given below.

Selected concentration of metal	Nutrients absorbed by the alga (ug/l)		N : P
	Phosphate	Nitrate	
0.1	3536	1752	0.50
0.2	1190	648 (All NO_3 was absorbed)	0.55
Control	945	1290	1.37

Four fold increase in phosphate uptake was observed when the alga was exposed to 0.1 ppm copper.

Also nitrate uptake increased by about 50% at this level. Neverthless at a higher level of 0.2 ppm, nitrate uptake was reduced by 50%. Phosphate absorption was also considerably reduced in 0.2 ppm when compared to the other.

Nitrate level of the natural medium, at the time of experimentation with 0.2 ppm copper was found to be very low and at the end of experiment no nitrate was detected in the medium. Hence the actual uptake could not be measured.

During experiment, response of alga to copper varied with batches. In seven out of ten observations the alga reacted positively to 0.2 ppm copper. This may be due to some factor in the medium prepared from natural water, which remained active even after sterilization.

The information gathered from the present study does conclusively prove that nitrate is not the causative factor for such variations since the alga responded positively to 0.2 ppm copper when the nitrate concentration in the medium was 648 µg/l.

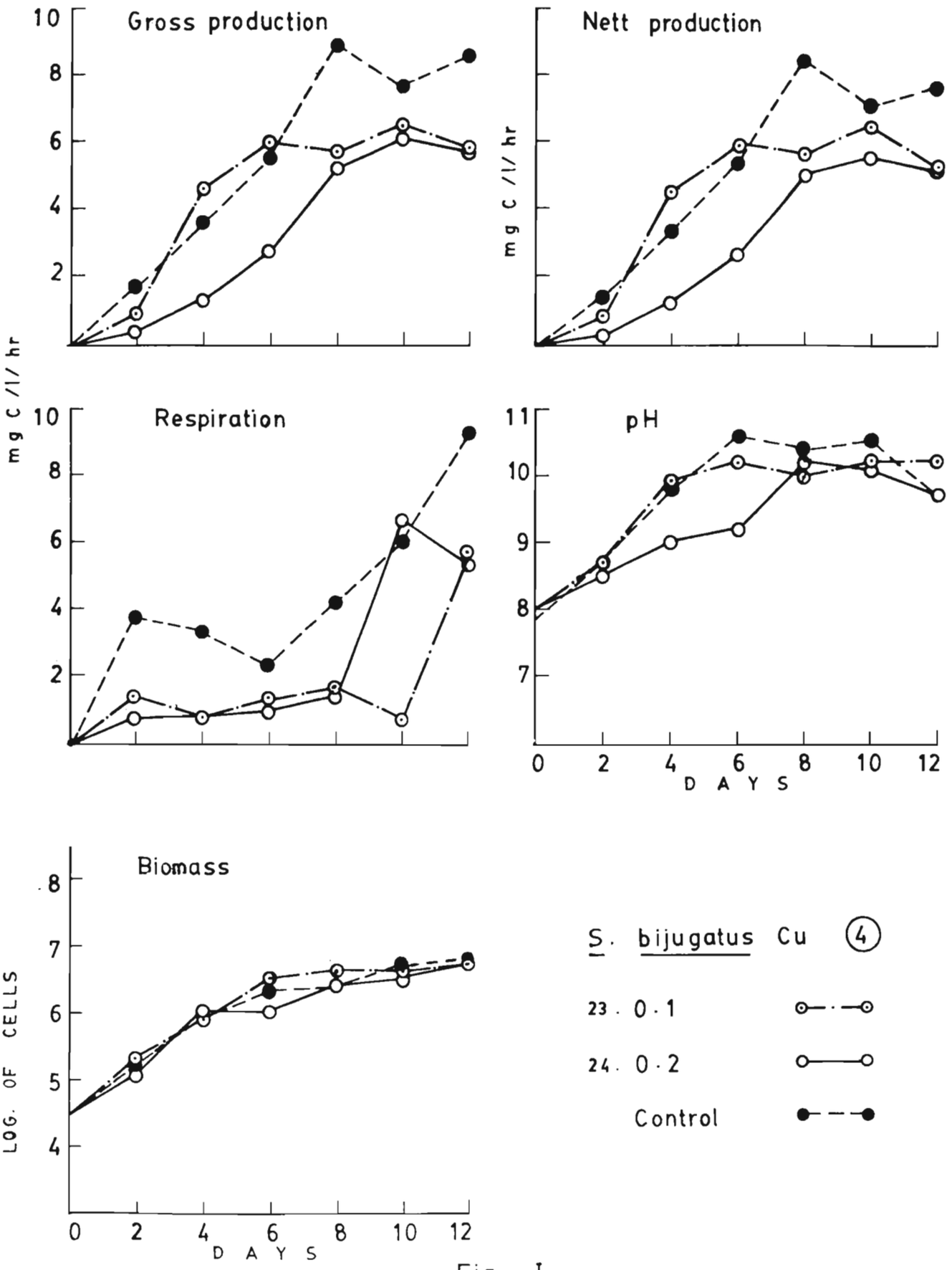

Gross production
Nett production
Respiration
pH
Biomass
mg C /l/ hr
LOG. OF CELLS
D A Y S
S. bijugatus Cu (4)
23. 0·1
24. 0·2
Control

Fig. I

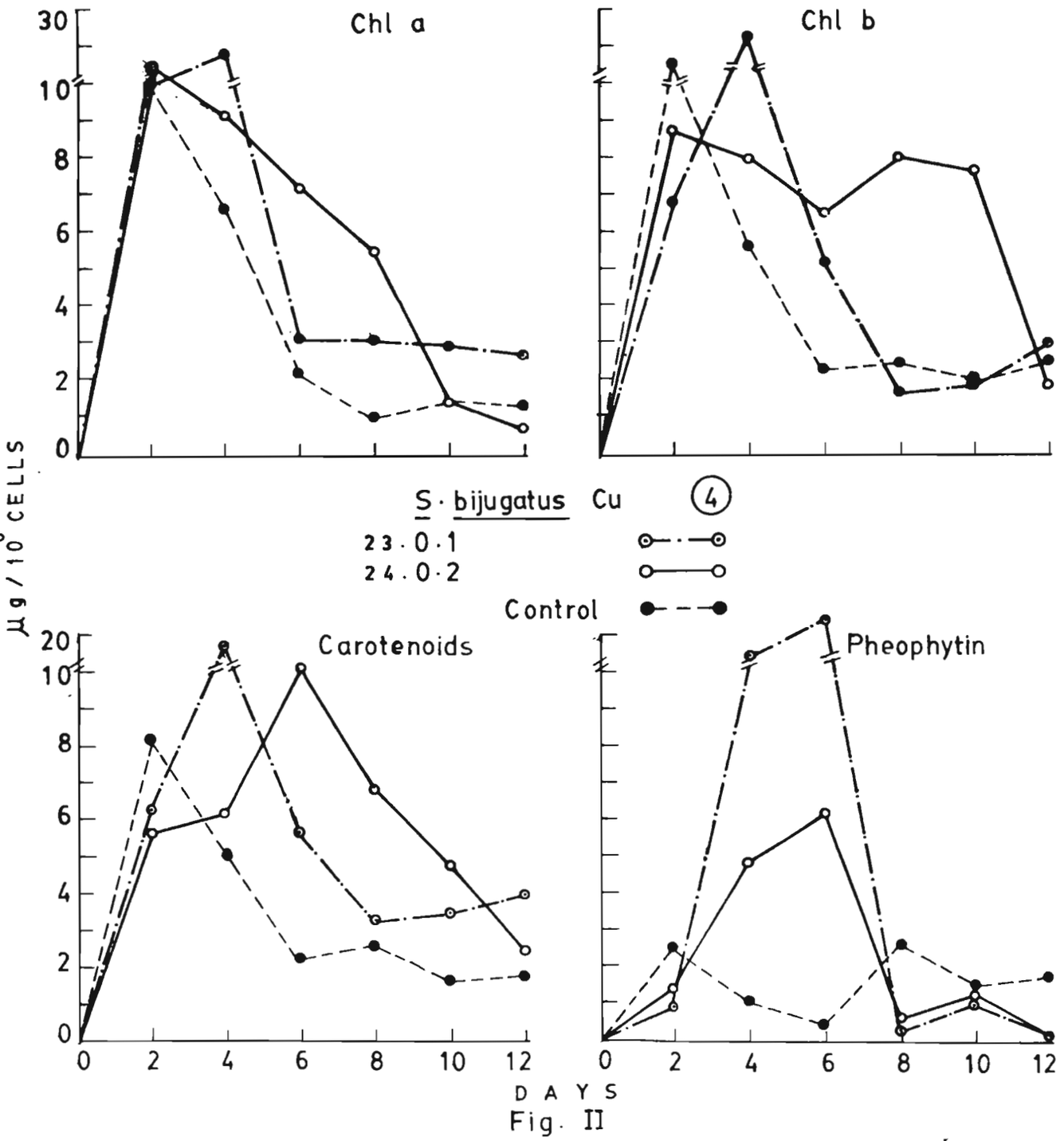
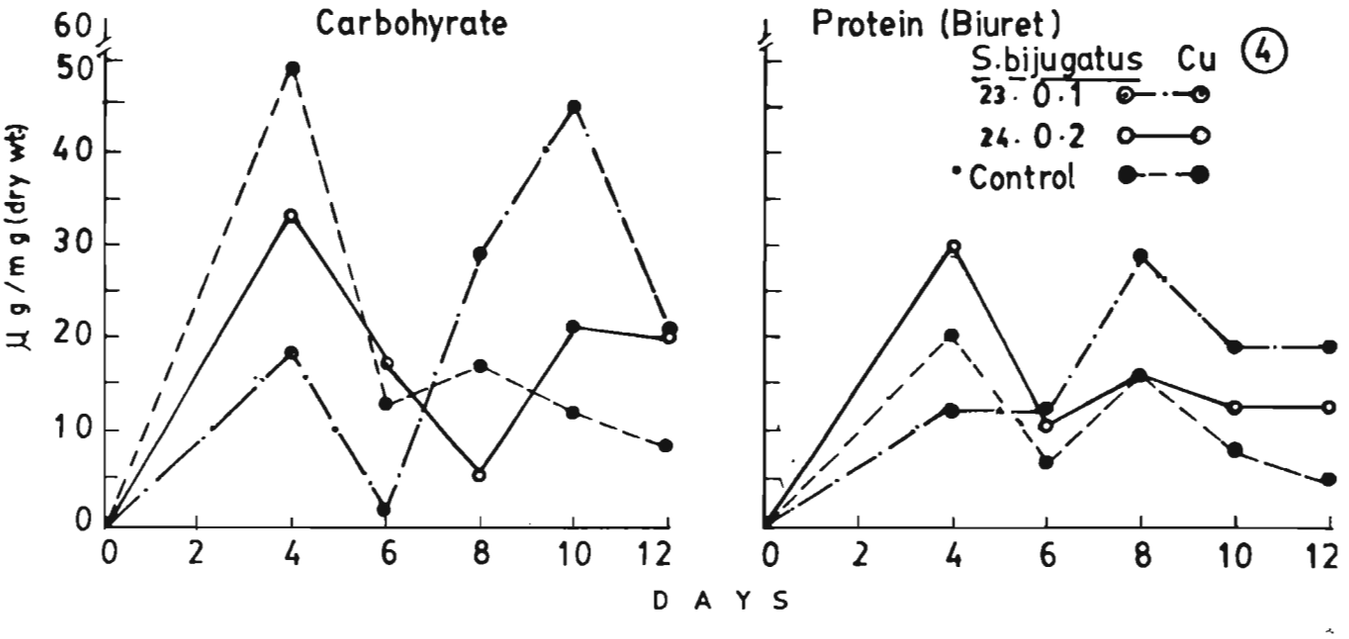

Chl a
Chl b
Carotenoids
Pheophytin
S · bijugatus Cu (4)
23 · 0 · 1
24 · 0 · 2
Control
µg / 10^6 CELLS
DAYS
0
2
4
6
8
10
12
20
30

Fig. II


Carbohyrate
Protein (Biuret)
S.bijugatus Cu
23 · 0·1
24 · 0·2
Control
4
µ g / m g (dry wt)
60
50
40
30
20
10
0
0 2 4 6 8 10 12
D A Y S

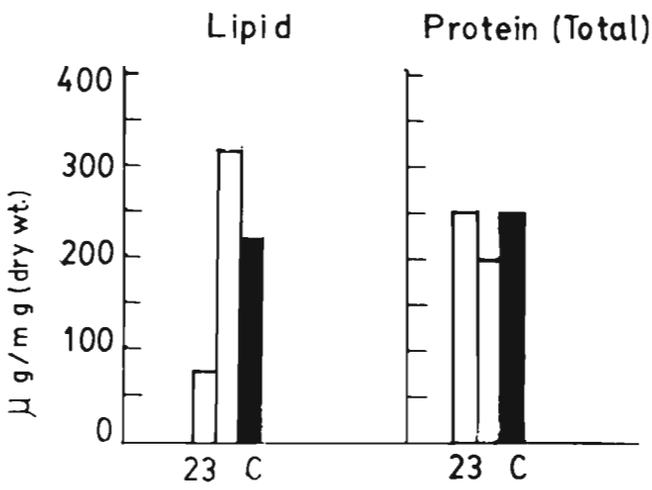

Lipid
Protein (Total)
µ g / m g (dry wt.)
400
300
200
100
0
23 C
23 C

Fig. III

Effect of copper on N. palea

(Figs. I, II and III)

Sl.No. of the metal	Number of treatment	Selected concentration (in ppm)
④	25	0.01
	26	0.02
	27	0.03
	28	0.05

Production: (Fig.I)

The nett production of the diatom was generally reduced by copper at the selected levels except at 0.02 ppm, where the production was 43% higher on fourth day. Even-though the production continued to increase, on the last day it was 2% less in relation to control. When the diatom was grown in 0.01 and 0.03 ppm copper, production increased gradually till sixth day but was less than that of control by 28%. In both instances production declined gradually thereafter and at end of growth phase it was 77% and 67% less than that of control respectively on the last day. Production was severely inhibited by copper at 0.05 ppm level upto fourth day and improved subsequently reaching maximum on eighth day with 30% reduction in relation to control. It decreased thereafter reaching lowest level by tenth day, the observed reduction being 85%.

Respiration of the diatom was elevated in the early phase of growth in presence of the metal. It was slightly higher than that of control at its maximum in the first treatment on sixth day, exhibiting 27% increase. It was reduced to 64% lower level by tenth day. At 0.03 ppm the respiration was slightly higher than that of control upto fourth day and also on last day. It was minimum on eighth day being 70% less than that of control, but was 80% more on tenth day. The respiration was elevated in 0.05 ppm treatment, and was 26% higher on fourth day, and in the 0.02 ppm treatment with an increased of 42% in relation to control. The respiration declined thereafter, in the former reaching minimum by sixth day and in the latter it was steady between sixth and eighth day. Towards the end of growth phase it increased in both instances and on tenth day it was 76% and 42% higher than that of control respectively.

pH of culture reached a higher level than that of control by sixth day in 0.01, 0.02 and 0.03 ppm treatments and continued to increase upto eighth day. Declining thereafter towards the end of growth phase, it remained higher than that of control. pH remained lowest in 0.05 ppm treatment which was higher than that of control only on the last two days. Inspite of low rate of production in 0.01, 0.03 and 0.05 ppm treatments, higher level of pH was attained.

Pigments: (Fig.II)

In general pigment content of the diatom increased in presence of this metal.

Chlorophyll a was more than that of control when 0.01 ppm and 0.02 ppm copper was employed and its concentration generally remained higher throughout growth phase except on the last day in the former treatment. In both instances maximum chlorophyll a was observed on second day. Fluctuation in pigment level was noticed only in 0.02 ppm treatment. When the copper level was 0.03 ppm and 0.05 ppm chlorophyll a reached maximum level on sixth and fourth day respectively, declined thereafter remained higher than that of control on the last day. Considerable variation in concentration of chlorophyll c occurred between any two treatments in the early phase. At 0.01 ppm and 0.05 ppm copper level concentration of chlorophyll c was lowered. It was much higher than that of control in 0.03 ppm level. In the latter growth phase the concentration of chlorophyll c did not vary much between any two treatments and remained higher than that of control at the end of growth phase.

Carotenoids were developed to a greater extent than any other pigment. The concentration of this pigment fluctuated with two peaks on second and sixth day in 0.01 ppm and 0.02 ppm and also remained higher than that

of control throughout growth phase. At 0.03 and 0.05 ppm, concentration of cartenoids was slightly lower than that of control in the early phase. Neverthless it attained a higher level than that of control reaching maximum by sixth and fourth day respectively. Towards the end of growth phase the level of carotenoids declined in all and on the last day it was lowest in first treatment and highest in the second treatment.

Pheophytin content of the diatom in all treatment but the second one did not deviate much from that of control. Only in the second treatment its level fluctuated with two peaks which are at higher levels than that of control on second and eighth day. At the end of growth phase pheophytin content of treated diatom remained slightly higher than that of control.

Photosynthetic end products: (Fig.III)

The acid soluble carbohydrate fraction of the diatom did not show much variation in the early phase between any two treatments and between any treatment and that of control. When the diatom was exposed to 0.01 ppm copper, this fraction was more than that of control from sixth day onwards and 10% higher on the last day. At 0.02 ppm level it remained less than that of control throughout. Its concentration was negligible on eighth day but increased by tenth day when it was found to be

40% less. At 0.03 ppm this fraction increased sharply from sixth day onwards and was 232% higher than that of control on tenth day. This fraction was found to be more than that of control from fourth day onwards when the diatom was exposed to 0.05 ppm copper and at the end of growth phase it reached a 100% higher level.

The alkali soluble carbohydrate fraction of the diatom was least affected, and generally did not deviate much from that of control except when it was not detected on fourth and sixth day in the first treatment and on eighth day in the second treatment.

The insoluble carbohydrate fraction of the diatom registered a gradual increase when the copper level was 0.01 ppm but was slightly higher than that of control only on sixth day. It was 53% less than that of control on the last day. This fraction was almost equal to that of control in 0.02 ppm and 0.05 ppm treatments upto fourth day. Thereafter it remained steady till eighth day in the 0.02 ppm treatment and though increased towards the end of growth phase found to be reduced by 50%. In the 0.05 ppm treatment it increased to a maximum level on sixth day, declined thereafter, was steady between eighth and tenth day of growth with 79% reduction on the last day. In 0.03 ppm treatment it was equal to that of control upto sixth day increasing thereafter to a greater extent reached maximum level on eighth day but fell 52% short of control by tenth day.

Protein content of the diatom was promoted in the early phase in relation to control. In the first and second treatments it was higher than that of control by 176% and 112% respectively on fourth day. In both instances it declined and was observed to increase once again towards the end of growth phase but remained 67% and 55% less than that of control respectively. The diatom, maintained in 0.03 ppm copper exhibited rapid increase in protein content upto fourth day and thereafter gradually increased to its maximum on eighth day with 46% reduction from where it declined to 72% lower level in relation to control. Protein content of the diatom compared to other treatments increased to a large extent when the copper level was highest (0.05 ppm) reaching its maximum on sixth day. From 176% higher level on sixth day it declined to 67% lower level on tenth day.

Adverse effect of copper was evident in the lipid content of the diatom. It was reduced by copper at all dose concentrations. Among the treatments lipid increased except in the last one. The percentage reduction was 62, 33, 24 and 64 respectively in the treatments. On the whole insoluble carbohydrate fraction, protein and lipids of the diatom were adversely affected by copper.

Growth: (Fig. I)

Growth of the diatom was stimulated in the early phase and inspite of low density inocula used, the biomass was equal to that of control by fourth day, when copper level was 0.01 and 0.02 ppm and slightly higher when copper level was 0.03 ppm. In 0.05 ppm treatment biomass remained less than that of control throughout the growth phase. It neverthless reached by sixth day the same level as recorded for other treatments. Generally from sixth day onwards little variation was observed between any two treatments and the biomass remained less than that of control till the end of growth phase.

Details of nutrient absorption of the diatom are given below.

Selected concentration of metal	Nutrients absorbed (µg/l)		N / P
	Phosphate	Nitrate	
0.01	1605	940	0.59
0.02	1895	932	0.49
0.03	1775	934	0.53
0.05	1769	936	0.53
Control	1350	763	0.57

Nutrient absorption was promoted in presence of copper. The quantity of nitrate absorbed did not vary widely with the concentration of copper but phosphate was taken up to a lesser extent at 0.01, 0.03 and 0.05 ppm levels when compared to that of 0.02 ppm treatment.

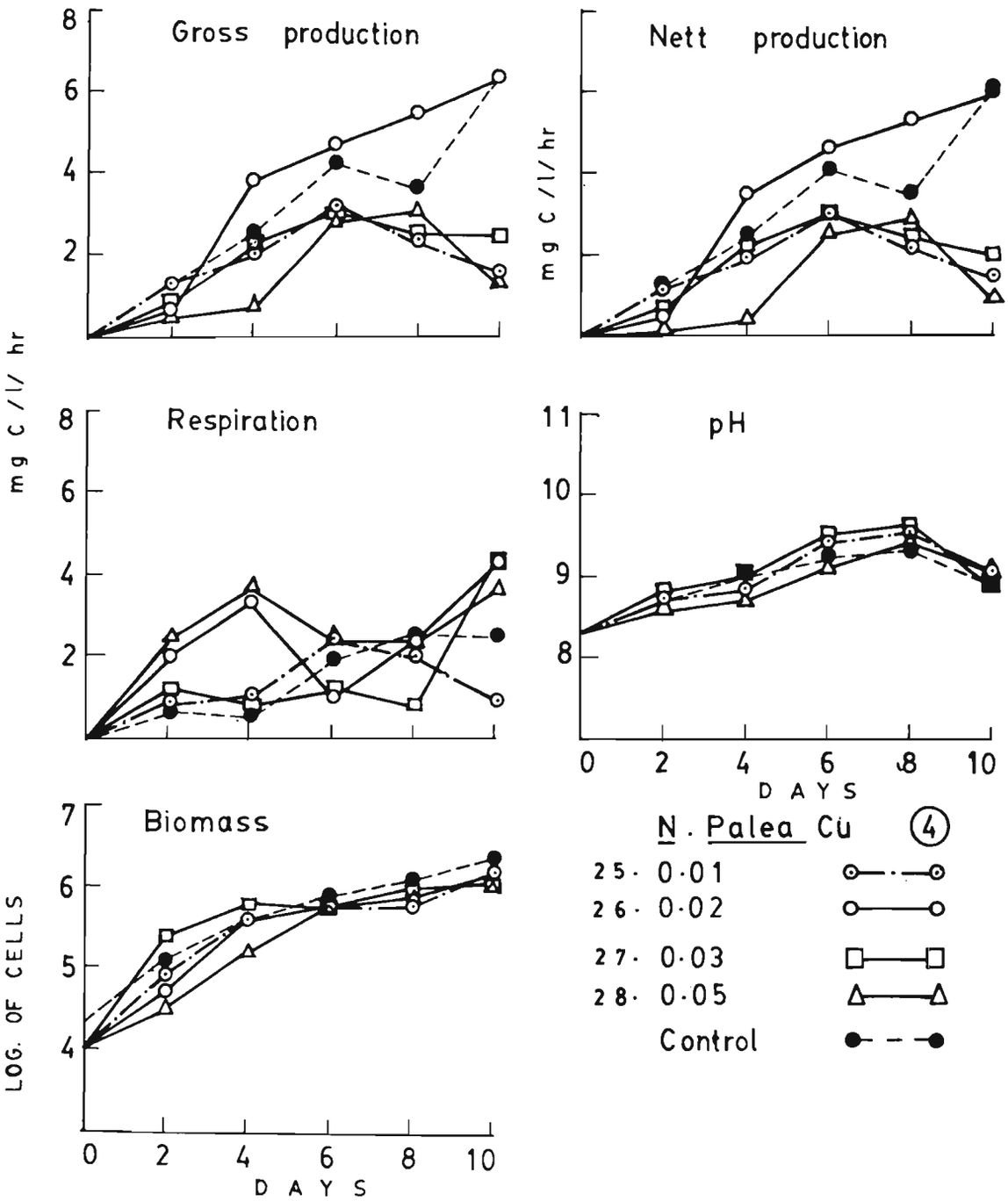

Gross production
Nett production
Respiration
pH
Biomass
mg C /l/ hr
LOG. OF CELLS
DAYS
N. Palea Cu (4)
25. 0·01
26. 0·02
27. 0·03
28. 0·05
Control

Fig. I

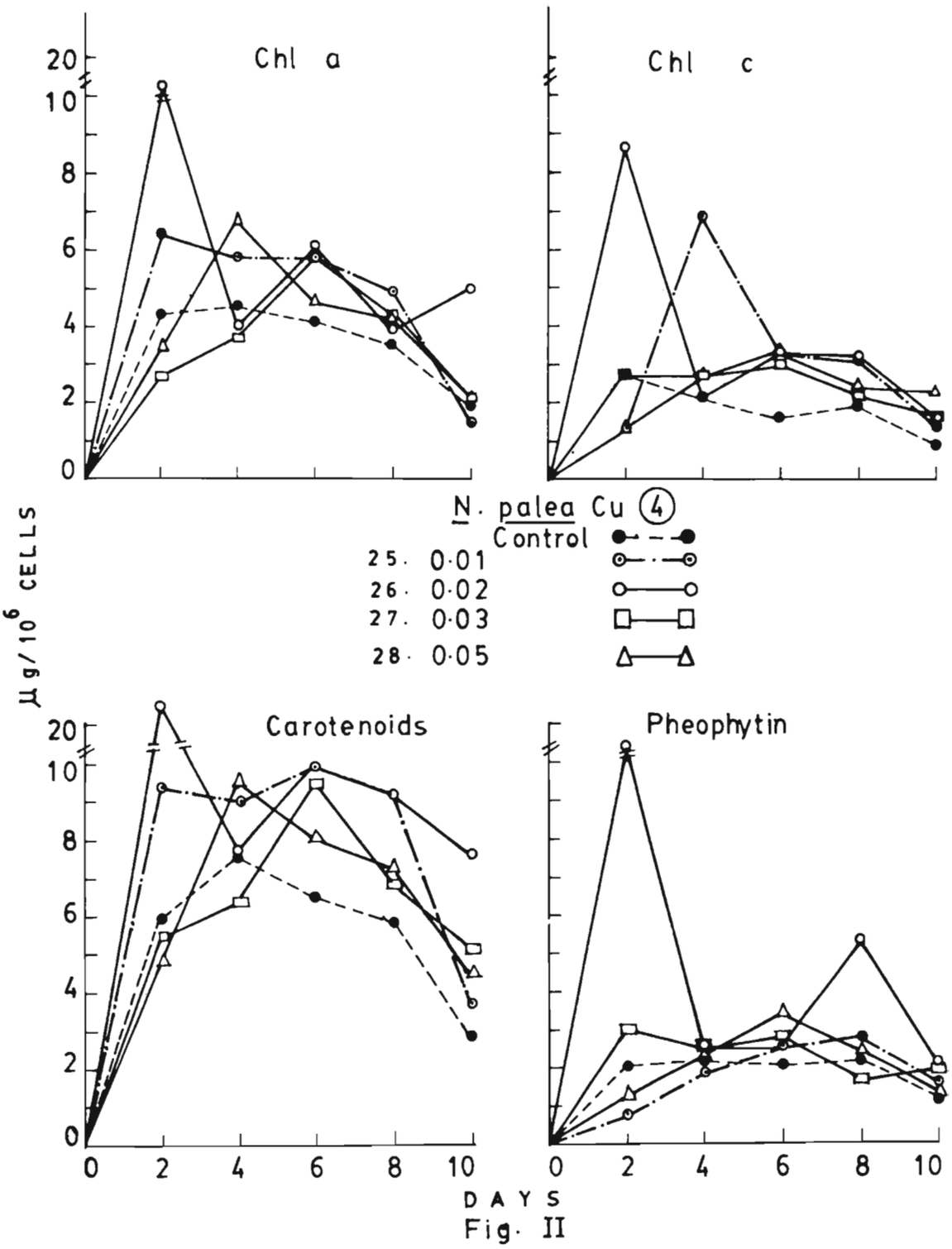
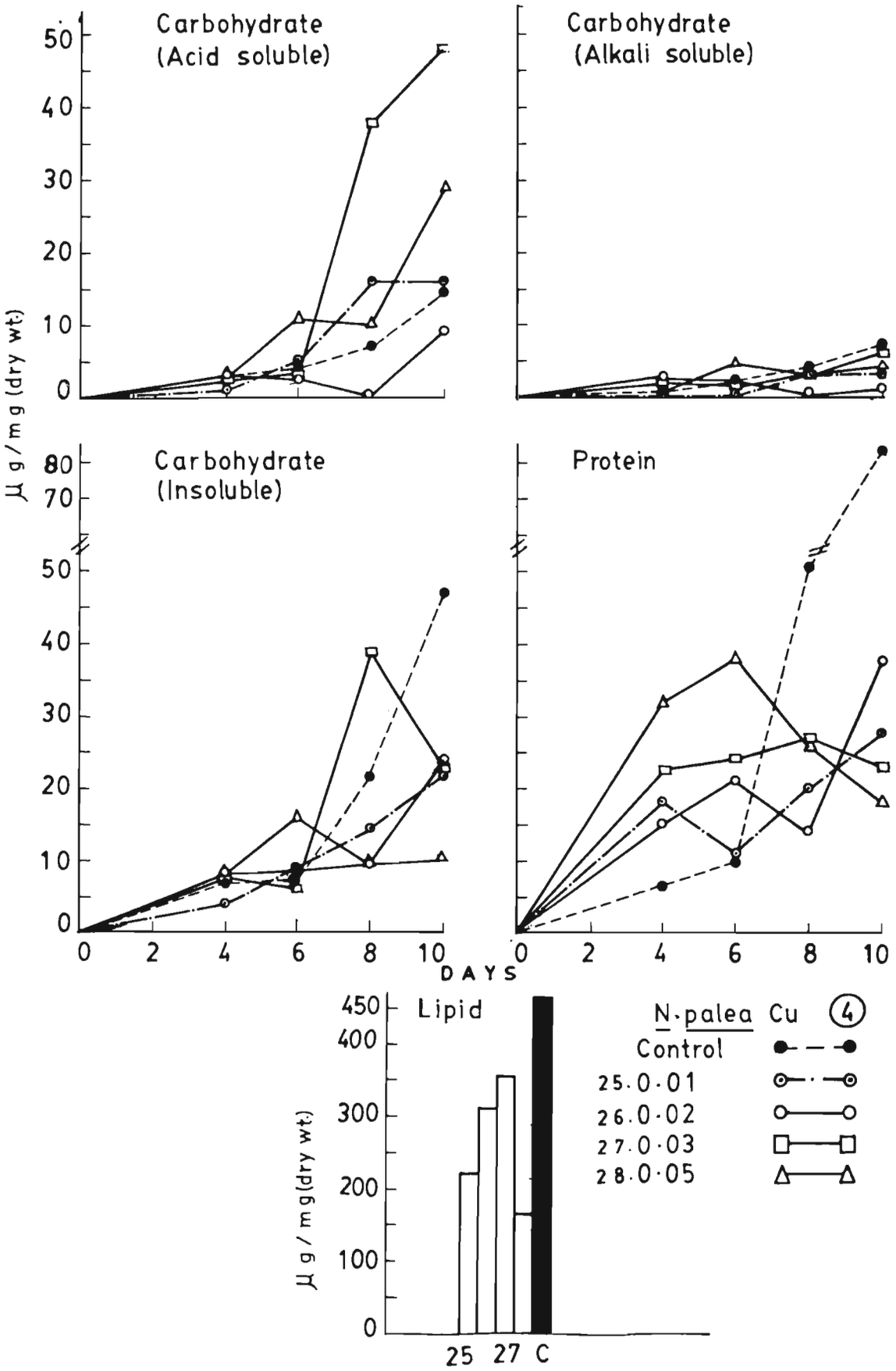

Chl a
Chl c
Carotenoids
Pheophytin
N. palea Cu (4)
Control
25. 0·01
26. 0·02
27. 0·03
28. 0·05
µg/10⁶ CELLS
DAYS
0
2
4
6
8
10
20

Fig. II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Carbohydrate
(Alkali soluble)
Carbohydrate
(Insoluble)
Protein
µg/mg (dry wt)
DAYS
Lipid
µg/mg(dry wt.)
N.palea Cu (4)
Control
25.0.01
26.0.02
27.0.03
28.0.05
25 27 C

Fig. III

5.1.5

Effect of zinc on S. bijugatus
(Figs. I, II and III)

Sl. No. of the metal	Number of treatment	Selected concentration (in ppm)
⑤	29	0.05
	30	0.1
	31	0.3
	32	0.5

Production: (Fig.1)

Nett production of the alga was enhanced in presence of zinc. In the first treatment (0.05 ppm zinc) it was slightly higher than that of control upto fourth day and though continued to increase till tenth day it was less than that of control from sixth day onwards. On tenth day it was almost equal to that of control but declined to 13% lower level at the end of growth phase. When the zinc concentration was 0.1 ppm and 0.3 ppm., production was elevated by 20% and 6% respectively on sixth day but decreased to 40% lower level in both, by eighth day. Thereafter it increased once again to 4% and 3% higher level respectively at the end of growth phase. When the alga was exposed to 0.5 ppm zinc, nett production increased to a large

extent and was much higher than that of control throughout growth phase. It increased sharply upto fourth day and thereafter gradually and was higher than that of control by 182% on sixth day, 92% on eighth day and 222% on twelfth day.

Respiration of the alga was reduced considerably except in the 0.5 ppm treatment. In 0.05, 0.1 and 0.3 ppm treatments it remained generally less than that of control throughout growth phase. At 0.05 ppm level of zinc, respiration fluctuated with two peaks. On sixth it was 35% more but on tenth day 50% less, in relation to control. It was reduced further to 76% lower level by the end of growth phase. Respiration was minimum in 0.1 ppm treatment with a reduction of 74% on sixth day and 88% on twelfth day. At 0.3 ppm level of zinc, respiration was reduced by 75% on sixth day, but increased thereafter to 44% lower level than that of control towards the end of growth phase. Zinc, at 0.5 ppm level, accelerated respiration to a very large extent. It was less than that of control on fourth day but thereafter increased sharply to maximum level by sixth day being 1743% higher. It declined thereafter till tenth day. Increasing once again it reached 1567% higher level in relation to control on the last day.

The increased rate of production and lowered rate of respiration were reflected in the higher pH

during early phase of growth upto fourth day in 0.05, 0.1 and 0.3 ppm treatments. But in 0.5 ppm, the pH of the culture was considerably lowered and was less than that of control except on twelfth day inspite of considerable increase in the rate of nett production. In the other treatments it remained less than that of control at the end of growth phase. In none of the treatments it reached the maximum level attained in the control.

Pigments: (Fig. II)

Maximum pigment concentration was noted in the early phase of growth in all treatments. Pigment content varied with the concentration of the metal and hence no definite pattern could be discerned.

Chlorophyll a was maximum on second day and was slightly more than that of control when 0.05, 0.1 and 0.3 ppm zinc was employed. But it reached to a lower level at the end of growth phase in relation to control. Fluctuation in the pigment level was noted in 0.5 ppm zinc treatment with two peaks on second and eighth day. Also delay in production of pigment was observed in this instance where the maximum amount of chlorophyll a was found on eighth day. Total chlorophyll a during the growth phase decreased with increase in metal concentration.

Chlorophyll b was suppressed in the early stage of growth and was not detected on second day in the first and second treatments. In both instances it fluctuated with two peaks, on forth and tenth day but was produced to a greater extent in the first treatment. It was less than that of control on the last day, in both.

Fluctuation in the chlorophyll b was also observed in the third treatment where it declined from maximum on second day to almost negligible level on sixth day, to increase once again to a slightly higher level than control on eighth and tenth day and decreased to lowest level at the end of growth phase. Chlorophyll b was adversely affected by 0.5 ppm zinc but as in other treatments exhibited fluctuation. It remained less than that of control throughout growth phase except on the last day, when it was equal to that of the control.

Carotenoids of the alga fluctuated during the growth phase in all treatments. The difference between that of control and treatments was more pronounced in the early phase. In all treatments maximum concentration of pigment was observed in the early phase, on fourth day in first treatment, and on second day in others. Total carotenoid content decreased with increasing concentration of the metal during the growth phase but on the last day pigment content of the alga was found to be directly related to metal concentration.

At 0.05 ppm level of zinc pheophytin content of the alga fluctuated with twopeaks on fourth and tenth day but fell short of control on twelfth day. It was not detected on second day when the level of zinc was 0.1 and 0.3 ppm but increased to a large extent reaching maximum on fourth day. It declined in both instances to a negligible level on sixth day and remained less than that of control thereafter. When the zinc level was highest (0.5 ppm) pheophytin was suppressed. It was less than that of control throughout growth phase and also was not detected on sixth and eighth day. At the end of growth phase pheophytin content in all treated samples remained less than that of control.

Total pigment content was almost equal to that of control when zinc level was 0.1 ppm. Above and below this level (0.05 and 0.3 ppm zinc) pigment content was more than that of control.

Photosynthetic end products: (Fig. III)

In general the total carbohydrate content was less than that of control in the early phase. Also the concentration of this product fluctuated with two peaks, on forth and tenth day respectively except in 0.5 ppm treatment. Carbohydrate concentration increased with increase in zinc level upto 0.3 ppm. It was maximum on forth day when the alga was exposed to 0.3 ppm zinc with

only 6% reduction when compared to control value. In the first treatment it was reduced by 60% and in second and last treatments by 53% on fourth day. The concentration of this product decreased thereafter in all treatments. But in the first three treatments it increased once again from eighth day to tenth day to 97%, 80% and 72% higher level respectively, and declined thereafter to 53%, 64% and 75% higher level respectively by the end of growth phase. In the last treatment from 53% lower level on fourth day it decreased gradually to the same level as that of control by the end of growth phase.

When the total protein was analysed on the basis of nitrogen, it was found to be less than that of control in all treatments but not much variation was observed between any two treatments. The percentage reduction was found to be 21, 26, 30 and 23 respectively in the four treatments.

The lipid content of the alga decreased with increase in zinc level. The alga grown in 0.05 ppm and 0.1 ppm zinc produced 32% more lipid. A reduction of 4% and 38% was observed when the alga was cultured 0.3 and 0.5 ppm zinc, respectively.

Zinc was found to affect all three end products adversely inspite of high rate of production.

Growth: (Fig. I)

Growth of the alga was stimulated at 0.05, 0.1 and 0.3 ppm levels and the biomass in these three treatments was more than that of control except on the last day of growth. Even when the zinc level was 0.5 ppm the growth rate and biomass were not adversely affected. Nutrient uptake of the alga is given below.

Selected concentration of metal	Nutrients absorbed by the alga (µg/l)		N / P
	Phosphate	Nitrate	
0.05	1101	612 (All the NO_3 absorbed)	0.56
0.1	1540	1188	0.77
0.3	1530	1206	0.79
0.5	1360	1200	0.88
Control	945	1290	1.37

The metal did not exert any adverse effect on the nutrient uptake of the alga. Phosphate uptake increased with increase in metal concentration except at the highest level (0.5 ppm) of zinc. The amount of nitrate absorbed did not differ much from that of control. Due to very low initial concentration of this nutrient, the actual amount of nitrate taken up by the alga could not be worked out in the case of first treatment.

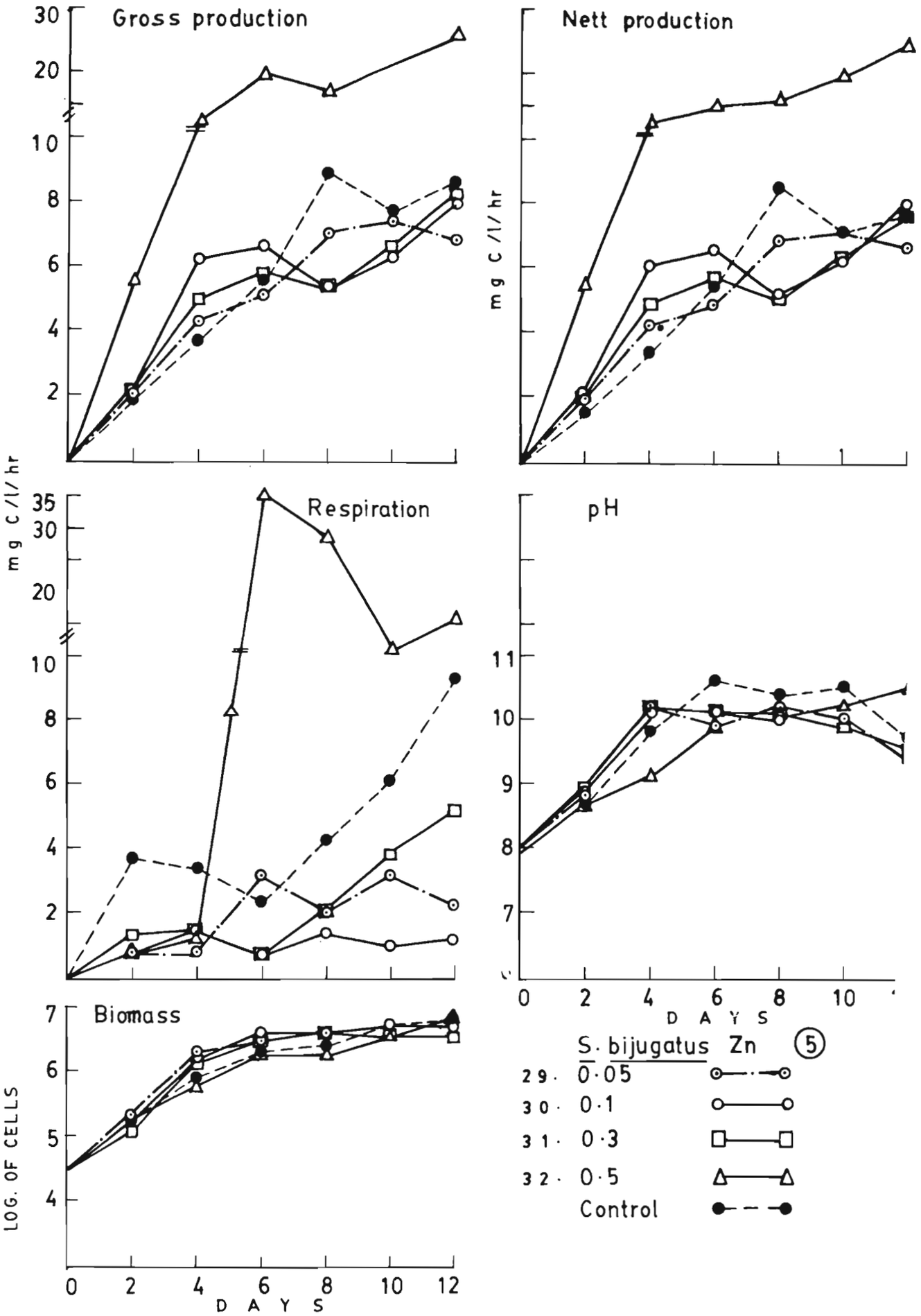

Gross production
Nett production
Respiration
pH
Biomass
mg C/l/hr
LOG. OF CELLS
DAYS
S. bijugatus Zn (5)
29. 0·05
30. 0·1
31. 0·3
32. 0·5
Control

Fig. I

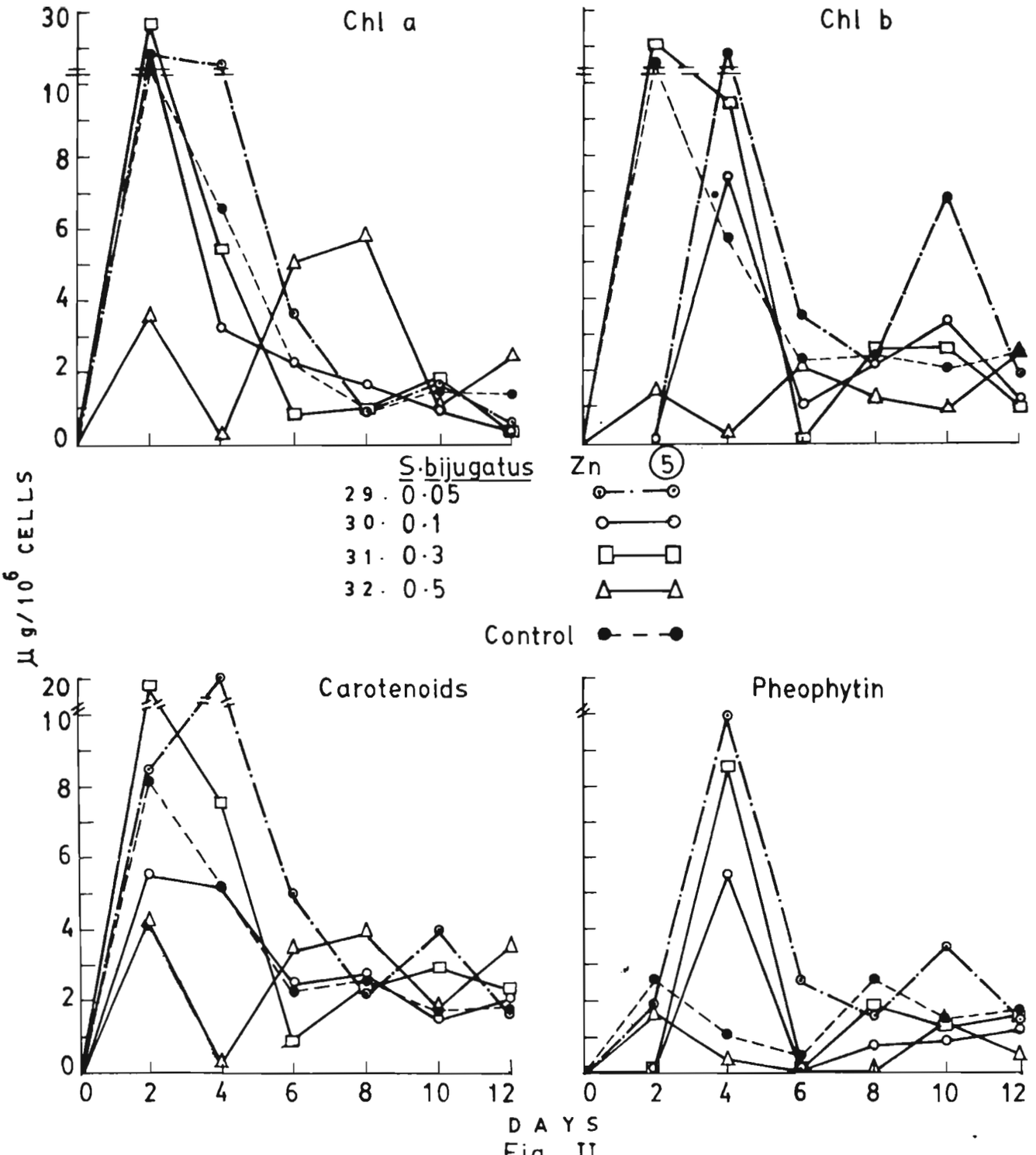
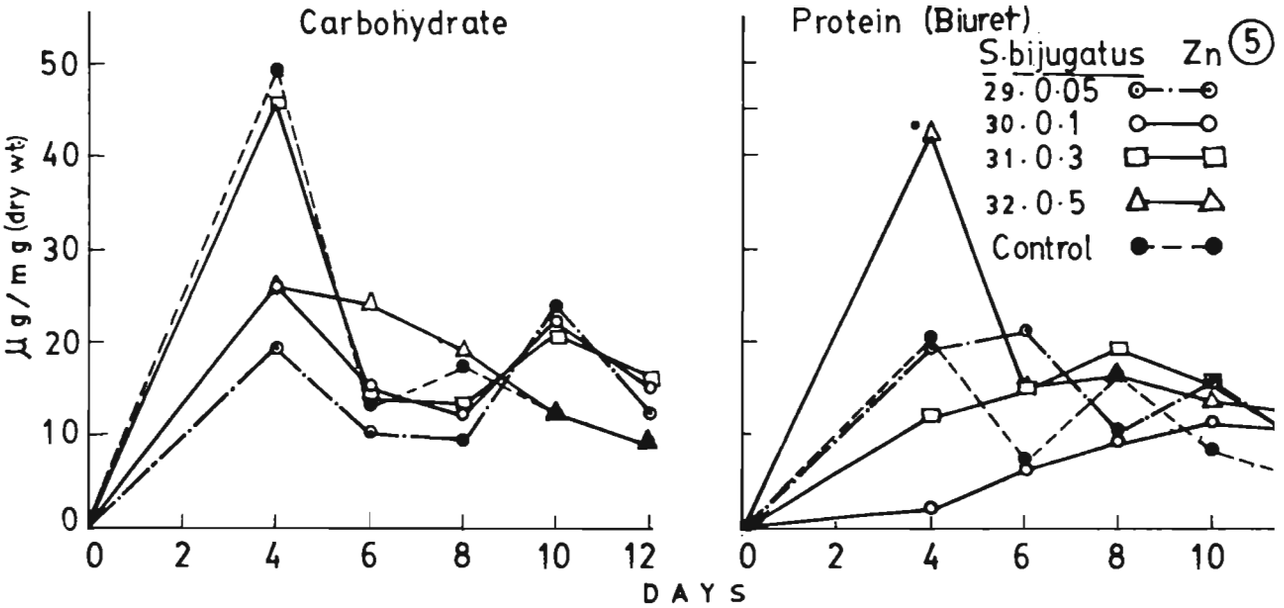

Chl a
Chl b
Carotenoids
Pheophytin
μg/10⁶ CELLS
DAYS
S.bijugatus
29. 0·05
30. 0·1
31. 0·3
32. 0·5
Zn (5)
Control

Fig. II


Carbohydrate
Protein (Biuret)
S.bijugatus Zn
29·0·05
30·0·1
31·0·3
32·0·5
Control
⑤
µg/mg (dry wt)
0
10
20
30
40
50
0 2 4 6 8 10 12
0 2 4 6 8 10
DAYS

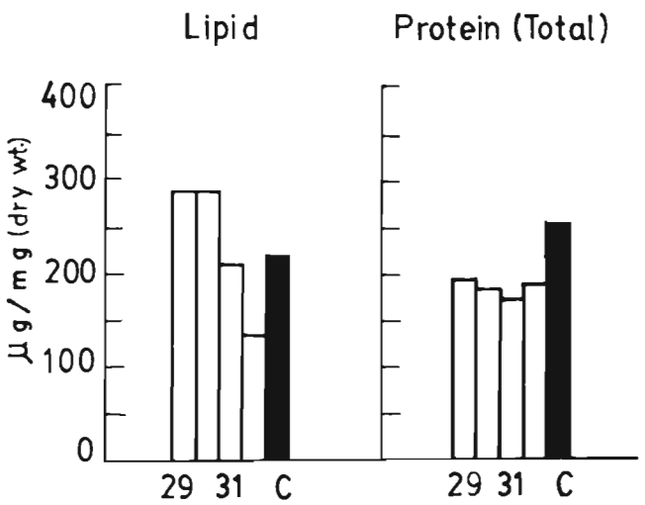

Lipid
Protein (Total)
µg/mg (dry wt)
0
100
200
300
400
29 31 C
29 31 C

Fig. III

Effect of zinc on H. palea
(Figs. I, II and III)

Sl.No.of the metal	Number of treatment	selected concentration (in ppm)
(5)	33	0.02
	34	0.04
	35	0.06
	36	0.08

Production: (Fig. I)

Zinc affected the production of the diatom adversely. The production decreased with increase in metal concentration upto 0.06 ppm level. Gradual rise in production was observed reaching maximum on sixth day with 10% reduction in the first treatment, and it declined to 77% less than that of control by the end of growth phase. Production between second and third treatments did not show much variation. It was slightly higher than that of control only on second day. From 45% lower level at its maximum on sixth day, production declined to 70% lower level at the end of growth phase in the second treatment. In the third, production reached maximum level on fourth day but remained 20% less than that of control and declined there after to 77% lower level by tenth day. Production of the diatom was suppressed to a large extent, upto fourth day when the zinc level was 0.08 ppm. From a 72% lower level on forth day production

increased, reaching maximum on eighth day at 12% higher level, and declined thereafter to 65% lower level at the end of growth phase. Production was highest in this instance at the end of growth phase when compared to other treatments.

Respiration of the diatom varied with concentration of the metal. When the level of zinc was 0.02 ppm, it fluctuated with two peaks, on fourth and eighth days with a respective increase of 300% and 150% and declined to 44% lower level by tenth day, in relation to control. When the diatom was cultured in 0.04 ppm and 0.06 ppm zinc, respiration was maximum on fourth day being 800% higher, but declined thereafter reaching 52% lower level in the former and increased to 128% higher level in the latter, by the end of growth phase. Respiration of the diatom was slightly higher than that of control when the level of zinc was 0.08 ppm, upto sixth day. Two peaks, on second and sixth day with 100% and 26% increase were recorded. It increased towards the end of growth phase to 48% higher level. Respiration of the diatom exhibited a decreasing tendency and was less than that of control at the end of growth phase in 0.02 and 0.04 ppm treatments. But in 0.06 and 0.08 ppm treatments it increased reaching higher level at the end of growth phase.

Not withstanding the lower rate of production and elevated respiration, pH of the culture in first three treatments remained generally higher than that of control upto seventh day, though declined later to a slightly lower level, by the end of growth phase. In the fourth treatment pH of the culture increased upto sixth day. It was almost steady thereafter being slightly higher than that of control at the end of growth phase.

Pigments: (Fig. II)

Pigment content of the treated diatom was more than that of control. Maximum chlorophyll a was recorded on second day in 0.02, 0.04 and 0.06 ppm treatments. In the first three, chlorophyll a was slightly higher than that of control on sixth day and declined thereafter in all treatments towards the end of growth phase. It was slightly less than that of control in first treatment and slightly more in others on the last day. Generally, total chlorophyll a increased with increase in concentration of zinc.

Chlorophyll c content of the diatom was slightly more than that of control when the zinc level was 0.02 ppm and 0.04 ppm. It was produced to a greater extent during early phase in 0.06 and 0.08 ppm treatments. Towards the end chlorophyll c level of the treated diatom

did not differ much from that of control. In the third treatment chlorophyll c remained higher than that of control throughout the growth phase.

At all experimental dose levels of this metal, the diatom produced more carotenoids than that of control. The concentration of these pigments fluctuated in all but first treatment. The carotenoid contents of the diatom was not directly correlated with the metal concentration and was slightly more in first and last treatments when compared to the other two. Generally at the end of growth phase treated diatom contained more pigment than that of control.

Total pheophytin content of the treated diatom did not show much deviation from that of control. It was initially suppressed in the first treatment. In all but first treatment it fluctuated during growth phase. It was not detected on sixth day when the zinc level was 0.08 ppm.

Photosynthetic end products: (Fig. III)

The acid soluble carbohydrate fraction increased to a large extent in treated diatom towards the end of growth phase and did not vary much between any two treatments and between any treatment and that of control in the early phase. At the lowest level of zinc, this fraction was not detected on fourth day of growth but by sixth day

it increased to be 163% more, reaching maximum on eighth day with 270% increase, but was only 38% higher at the end of growth phase. This fraction in the diatom was equal to that of control by sixth day. But in the diatom exposed to 0.04 ppm zinc it increased sharply thereafter to 369% higher level than that of control on tenth day. In 0.06 ppm zinc the diatom contained 100% more of this fraction than that of control on sixth day and increased thereafter sharply to 341% on the last day. This fraction was found to be 74% more in the diatom exposed to 0.08 ppm zinc on sixth day but declined thereafter reaching minimum level on eighth day with 57% reduction and increased once again to 52% lower level on last day in relation to control.

The alkali soluble fraction was least affected and did not differ much between any two treatments and between any treatment and that of control. The percentage reduction was 47 and 68 respectively in 0.02 ppm and 0.08 ppm treatments whereas only 3% reduction was observed in 0.04 ppm treatment and 10% increase in 0.06 ppm treatment at the end of growth phase.

The insoluble carbohydrate fraction was affected by zinc and was found to be higher than that of control in the early phase except at the lowest level of this metal. By sixth day, this fraction reached higher level than that of control in all treatments with a respective increase by 53%, 27%, 88% and 61%. In the first

treatment it increased further reaching maximum on eighth day at 33% lower level and decreased to 72% lower level at the end of growth phase. In the second and third treatments it increased sharply reaching 86% and 58% higher level respectively on eighth day. It declined in the former and increased in the latter and was 35% and 26% less than that of control respectively on the last day. In the last treatment the concentration declined to 90% lower level by the end of growth phase.

Exposure of this diatom to zinc resulted in enhanced production of protein in the early phase. Neverthless, it remained much less than that of control at the end of growth phase. When exposed to 0.02 ppm zinc, protein content increased gradually reaching a maximum level on sixth day the value being 162% more than that of control and declined thereafter to 75% lower level by the end of growth phase. Protein content was maximum on forth day in the second treatment with 329% increase. From its maximum it declined to 132% higher level by sixth day and increased thereafter gradually towards the end of growth phase exhibiting 65% reduction. Protein concentration was highest when compared to other treatments on fourth day in the third treatment with 528% increase. It was 118% higher on sixth day. It was 66%less on the last day. The diatom exposed to 0.08 ppm zinc produced least amount

of protein, compared to others. 162% increase was recorded on sixth day for this treatment. It increased further to reach maximum on eighth day but was 45% less than that of control and declined towards the end of growth phase to 78% lower level.

Among the end products, lipid was most adversely affected by zinc. It was highest in 0.04 ppm treatment when compared to others being 66% less than that of control. In the first and last treatments it was reduced by 82% and in the third by 85%.

Growth: (Fig. I)

Growth of the diatom was stimulated except at highest level of zinc. Inspite of the use of low density inoculum, biomass reached higher level than that of control in second and third treatments by fourth day. In the first three treatments biomass was equal to that of control on sixth day. It showed no further increase in second and third treatments where as in the first treatment, increased to higher level than that of control by eighth day. In the last treatment it remained less than that of control throughout but reached the same level as in second and third treatments by the end of growth phase. The final yield was reduced in all treatments when compared to that of control.

Nutrient uptake of the diatom is given below.

Selected concentration of metal	Nutrients absorbed (µg/l)		N / P
	Phosphate	Nitrate	
0.02	1655	940	0.57
0.04	1790	938	0.52
0.06	1690	9400.56	0.56
0.08	1755	940	0.54
Control	1350	763	0.57

Zinc was enhanced the uptake of phosphate and nitrate, but the former to a greater extent. Nitrate uptake did not vary with the concentration of zinc. But phosphate uptake though varied did not show any correlation with the concentration of the metal.

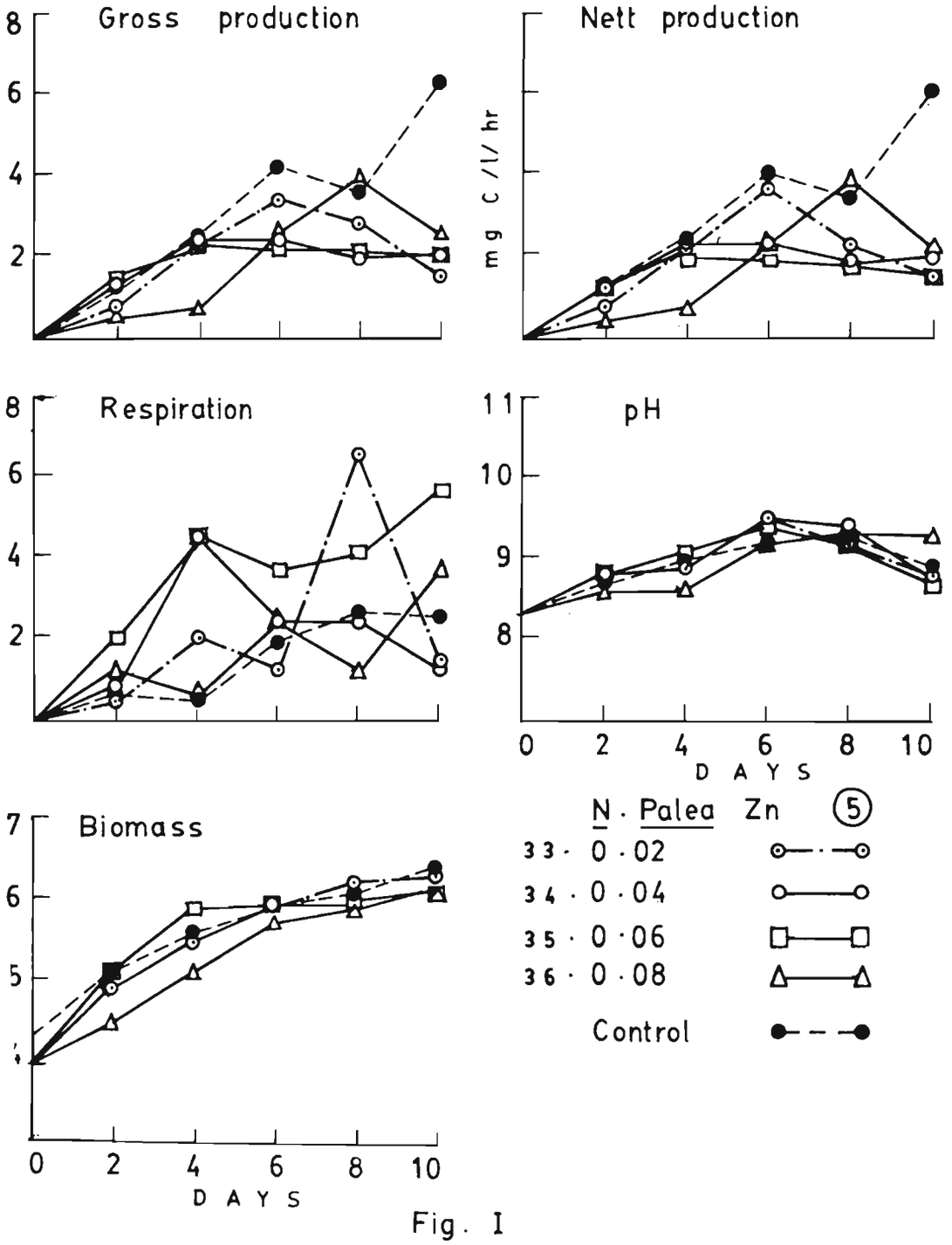

Gross production
Nett production
mg C /l/ hr
Respiration
pH
Biomass
DAYS
DAYS
N · Palea Zn ⑤
33 · 0 · 02
34 · 0 · 04
35 · 0 · 06
36 · 0 · 08
Control

Fig. I

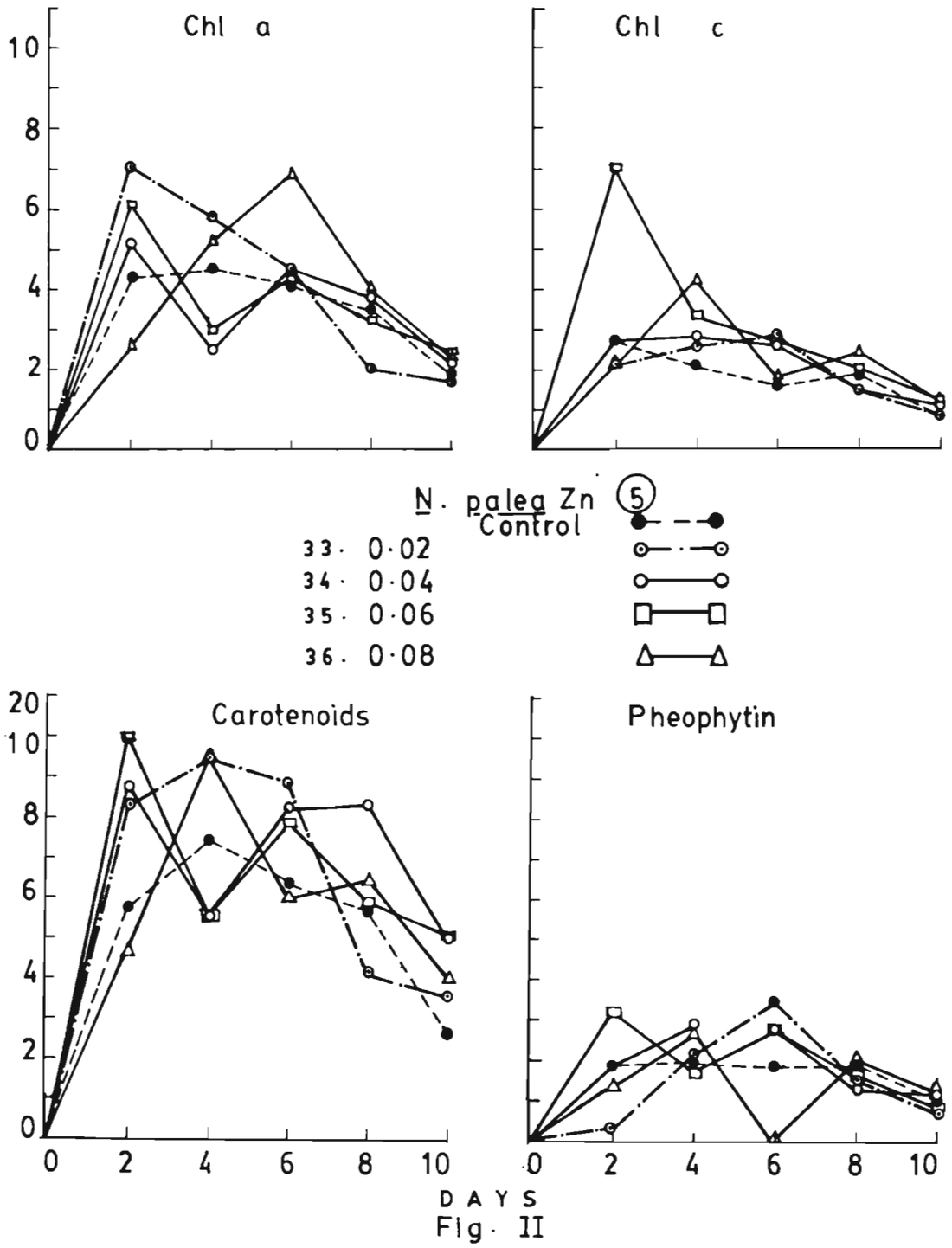

Chl a
Chl c
10
8
6
4
2
0
N. palea Zn (5)
Control
33. 0·02
34. 0·04
35. 0·06
36. 0·08
Carotenoids
Pheophytin
20
10
8
6
4
2
0
0
2
4
6
8
10
DAYS

Fig. II

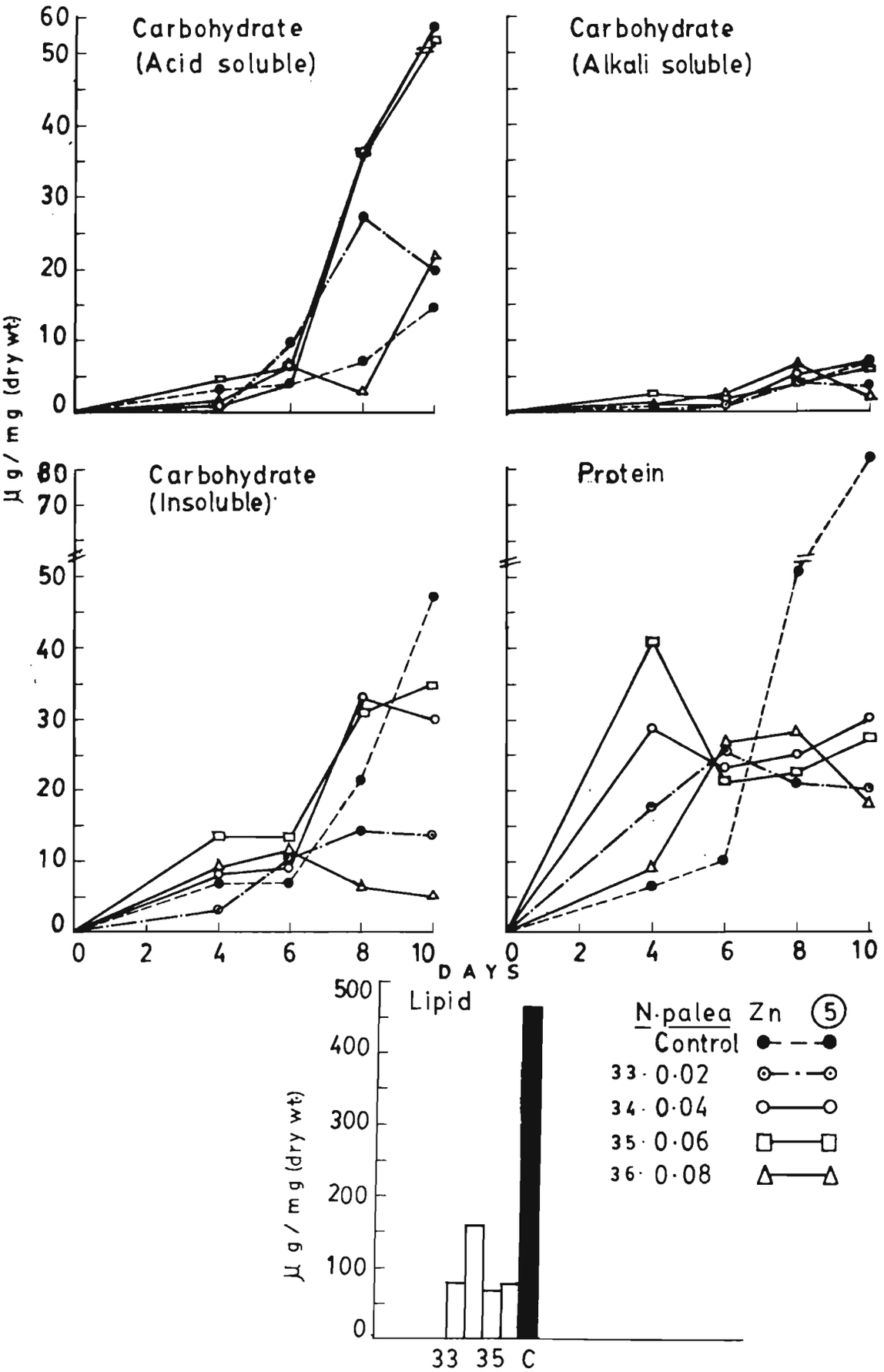

Carbohydrate
(Acid soluble)
Carbohydrate
(Alkali soluble)
Carbohydrate
(Insoluble)
Protein
µg/mg (dry wt)
DAYS
Lipid
N·palea Zn (5)
Control
33 · 0·02
34 · 0·04
35 · 0·06
36 · 0·08
33 35 C

Fig. III

DISCUSSION

Mercury:

The influence of mercury on algal growth and production was extensively studied (Kamp Nielsen, 1971; Ben Bassat et al., 1972; Nuzzi, 1972; Rice et al., 1973; Ben Bassat and Mayer, 1975). Mercury was reported to cause initial lag in growth but subsequent recovery of alga resulting in exponential growth was observed by Kamp Nielson (1971) and Aubert & Pesando (1975). Accumulation of mercury and its distribution in the cell was also investigated in short term experiments (Overnell 1975; Agarwal and Kumar 1975) and in long term experiments (Kamp Nielson 1971; Ben Bassat et al., 1972; Aubert & Pesando, 1975).

In the present investigation no lag phase was observed in the case of *S. bijugatus* but it occurred when *N. palea* was treated with 0.02, 0.03 and 0.04 ppm of mercury.

The results showed that all the pigments studied were developed to a greater extent, than in the untreated samples of both the species in most of the treatments. This is in agreement with the results obtained by Betz (1977) who reported increase in chlorophyll a, Though a definite pattern of influence could not

be discerned with individual pigments, total pigment content decreased with increasing metal concentration, but always remained higher than that of control.

Fogg (1956) found high proportion of carbon fixed photosynthetically, within 30 seconds in protein fraction in actively growing diatom Navicula pelliculosa. In the present investigation the reduction in protein in most cases except in S. bijugatus grown in 0.02 ppm mercury, in spite of high nitrate uptake, was observed. More of carbon seems to be assimilated into lipids and photosynthetic pigments with increasing concentration of mercury. The increased flow of carbon into lipids and photosynthetic pigments was reported by Harding et al. (1985), when algae were grown in decreased light intensity. Fogg (1956), Konpka and Schnur (1981) reported that nitrogen starvation promoted enhanced C^{14} flow into Polysaccharides and non-N-containing storage products. It is interesting to see that in the present investigation an increase in lipids and pigments occurred, even when nitrate and light were not limiting factors.

Kayser (1976) reported that mercuric acetate even at sublethal concentration produced impairment of growth rates, cell densities and that morphological and physiological effects of mercury are not evident immediately after addition of this toxicant. The present results agree

with the above. The toxic effect was evident only in the latter phase of growth. On the contrary it was found that mercury caused slight stimulation of growth initially.

The morphological changes such as cell enlargement, increase in cell volume and cell deformities reported by Nuzzi (1972); Davies (1973) were not observed. The cells of N. palea grown in 0.04 ppm mercury were seen attached to the culture flask and did not enter into medium when the flasks were shaken resulting in apparent decrease in cellnumber. When this culture was observed under microscope no empty frustules were noted and hence it was assumed that cell disruption did not occur.

The adverse effect of the metal at low concentration was not seen in either phosphate or nitrate absorption. But generally when production, pigments and cell numbers were low, uptake of nitrate was also low. The phosphate absorption, however, was not affected. Density of culture generally decreased with increasing mercury concentration. The difference was seen only in the latter growth phase. The biomass of S. bijugatus exhibited a gradual decrease, whereas severe suppression of growth was observed in N. palea at 0.03 and 0.04 ppm levels of mercury. At 0.02 ppm level, though an initial lag phase was present, the growth of the diatom subsequently recovered. The accumulated amount of mercury did not

differ much by either species between any two treatments when compared with other metals. Davies (1976) stated that cells detoxify mercury by precipitation and hence the uptake is restricted after first day of growth. This may be the reason for low mercury level in the test species.

Mercury appears to lower the rate of production and protein content but increase the pigments, carbohydrate and lipid except in the case of S. bijugatus when exposed to 0.02 ppm mercury. But at the same concentration it suppressed production upto sixth day. In the case of S. bijugatus when the rate of production decreased, the efficiency of the alga to build the macromolecules such as carbohydrate, protein lipid and pigments was not affected and there was only marginal reduction in biomass and protein content. S. bijugatus is more tolerant and efficient than N. palea. In the diatom, the higher rate of production at low concentration of mercury was reflected only in the increased production of carbohydrate but not protein and lipid. Increased toxicity resulted in further reduction of protein, lipid and biomass.

The toxic effect of mercury resulted in physiological disturbance as exhibited in the changes in the proportion of macromolecules and biochemical composition of cells. The effect is both qualitative and quantitative.

Cadmium:

Cadmium is not an essential micronutrient for algae (Kelley, 1968). Reports on varied effects of the metal on algae are found in literature. Measurable inhibition of growth, photosynthesis and respiration at 1 μg^{-1} level in the diatom Cylindrothica closterium was reported by Lehman and Vas Cancelos (1979). 5 uM of cadmium was reported to cause slight stimulation in growth and chlorophyll level of Chlorella ellipsoidea by Lue-Kim et al. (1980). Thomas et al. (1980) reported the interference of cadmium with cell division in Thalassiosira aestivalis. Hart and Scaife (1977) reported that cadmium at 1 ppm level inhibited growth rate appreciably in Chlorella.

In the present investigation cadmium at 0.04 and 0.05 ppm level has inhibited the growth of the test species. Increase in the level of cadmium resulted in increasing inhibition not withstanding the slight stimulation in the initial phase. At lower levels of this metal (0.01 and 0.02 ppm), nett production was enhanced but respiration was reduced. At 0.04 and 0.05 ppm cadmium both respiration and production were elevated in S. bijugatus but nett production was reduced in N. palea. Fluctuation in respiration was another effect of the metal on N. palea at 0.02 and 0.04 ppm levels. Pigment

concentration increased as the culture aged, without exception. Initial delay in production was noted in some instances. But the stimulation, in pigment development when occurred, was not reflected in the final yield of proteins and lipids. In the present toxicity study a direct correlation between photosynthetic pigments and production was not found. Increase in pigment concentration was not limited to chlorophylls. Carotenoids also exhibited the same trend where as pheophytin was not much affected.

High affinity between cadmium and algae was reported by Fayed et al., (1983) and Les and Walker (1984). Physicochemical interaction between algae and the metal was reported by Soeder et al. (1978); Hasset et al.,(1980) and Fayed et al.,(1983) leading to increased accumulation of cadmium in active growth phase than in decline phase. Diatoms Asterionella farmosa (Conway, 1978) and Phaeodactylum tricornutun (Cossa, 1976) were reported to accumulate cadmium to a very large extent, accumulation factors being 14 to 85000 and 10,000 respectively. In the present study cadmium was observed to affect the species adversely during the exponential growth phase. Les and Walker (1984) has observed the binding of all the cadmium by the blue green Chroococcus paris in 10 minutes. But the green alga Scenedesmus obliquus concentrated most of the metal in culture medium in 2 hours (Fayed et al.,1983).

Deviprasad and Deviprasad (1982) have stated that cadmium was lethal to Scenedesmus obliquus and Ankistrodesmus falcatus above 5 ppm level and for Chlorococcum sp. above 10 ppm level. The present investigation showed that cadmium was not lethal to test species at the concentration selected, but their physiological efficiency was affected adversely.

Another feature of interest was the enhanced uptake of phosphate and nitrate by test algae. Bruland et al.,(1978) found that in natural waters, high cadmium levels do not occur in plankton in the absence of significant amounts of phosphorous. Cadmium and phosphorus are correlated in microplankton. Present observation revealed increase in uptake of phosphate and nitrate by N. palea whereas uptake by S. bijugatus varied with concentration of the metal. At an intermediary level of 0.03 ppm, this species absorbed nutrients to a greater extent. Also in this treatment its behaviour was only slightly different from that of control but when grown in 0.01 ppm cadmium, it was found to be physiologically more efficient when total end products were considered.

The present study revealed that S. bijugatus was found to be more tolerant than N. palea to cadmium.

L e a d:

O'Kelley (1968, 1973) has reported that lead is not essental for the growth of algae. Stewart (1977 a) has reported that lead upto 10 mg^{1-1} produced no visual effects on the vegetative morphology or development of reproductive structures in three marine red algae but affected the cell division and cell elongation in Tiffaniella. At 10 mg^{1-1}, it was lethal to Dunaliella tertiolecta. Stewart(1977 b), Deviprasad and Deviprasad (1982) have reported that between 0.1 and 10 ppm level, lead did not show any stimulatory effect and at 10 ppm it killed Ankistrodesmus falcatus and greatly reduced the growth of Scenedesmus obliquus and Chlorococcum sp.

In the present study it was observed that lead enhanced the rate of production and respiration in S. bijugatus but in N. palea, it increased respiration alone at 0.02 and 0.04 ppm levels. Respiration of the diatom in presence of lead was high and fluctuating. Lead increased production and respiration simultaneously, when it did as in S. bijugatus or lowered both as in N. palea, as in the case of other metals.

Reports on the effect of lead on pigments of algae are scanty. Deviprasad and Deviprasad (1982) have reported increase in pigment level and have measured growth as optical density of the total pigment extract at 665 nm, on the tenth day after inoculation.

Effect of lead on pigments at the present selected levels also was not different from that produced by other metals. In general, it increased the pigment concentration in both the species. Initial suppression in all four pigments was observed when S. bijugatus was exposed to 0.1 ppm lead. Such suppression was observed only in pheophytin of N. palea at 0.06 ppm level of lead. At all test levels lead reduced the chlorophyll b to a large extent during growth phase though the situation improved towards the end of growth phase in S. bijugatus. In N. palea it was produced to a lesser extent than other pigments. The stimulation of pigments was not directly correlated with actual increase in biomass or rate of production or the total production of the species.

Reports on the effect of lead on photosynthetic end products are lacking. When treated with lead, total carbohydrate did not show any particular difference from that of control in S. bijugatus but in N. palea the acid fraction (mainly glucose units) was generally more than that of control and in particular increased to a very large extent when the level of lead was 0.04 ppm. Also in this treatment greater amount of insoluble carbohydrate was observed.

Lead was observed to produce slight stimulation on the protein production in S. bijugatus alone. But the

protein extractable by Biuret reagent remained higher than that of control. At the highest level employed (0.3 ppm) biuret protein was lowest but total protein was highest. Notwithstanding the high protein content, cells of the treated algae must have been different from the cells of control. Since Scenedesmus is known to be a sturdy species with thick cell wall, and also only 85% of protein could be extracted even after treating the healthy control cells in biuret solution for 100 minutes (Dorsey et al., 1978), the increase in protein as per Biuret method (unaccompanied by growth in terms of cell number) should be considered apparent. With regard to N. palea protein though increased in the early phase was invariably reduced by the end of growth phase.

Increase in lipid accompanied the reduction in protein in S. bijugatus. As the concentration of lead increased lipid content of the diatom increased whereas protein content was decreased in N. palea.

Working on Selenastrum capricornutum, Monahan (1976) reported that lead toxicity is dependent on pH and phosphate availability. Schulze and Brand (1978) have opined that lead killed cells mainly by depriving them of phosphate. They observed that if lead concentration was more than phosphate, population decreased.

In the present investigation it was found that even in presence of lead S. bijugatus absorbed more phosphate than that of control. In N. palea phosphate was absorbed to a lesser extent even when lead was only 0.08 ppm and phosphate was 0.9 µg/l. In either case cells were not deprived of phosphate in presence of lead but the biomass was reduced. Present results agree with those of Deviprasad and Deviprasad (1982) who reported that reduction in growth of three algal species when exposed to 1 ppm lead even when phosphate was 2.75 ppm. During the present investigation S. bijugatus was cultured in a medium where phosphate concentration was 1.1 ppm and lead concentration only 0.3 ppm.

Schecher and Driscoll (1985) working on Nostoc mucorum have reported that the uptake of lead increased at the pH range 4-7 but above 7 it decreased. Uptake was more pronounced for 0-8 hrs., but was minimal in subsequent measurements and there was rapid initial uptake which was attributed to surface associations with the algal cells. In presence of calcium, lead uptake was significantly low at higher pH of 7.2 but 26% more lead was taken up in presence of sulphate than in its absence over a pH range of 4-8.4. Citrate also decreased lead uptake.

Baldes and Lewin (1976) have observed that there was initial rapid increase in the lead associated

with Phaeodactylum tricornutum and Platymonas subcordiaformis within a few minutes. The second phase varies with the species. The lead content with slight decrease in the first phase later increases or first increases and latter decreases. This was thought to be due to reduction in lead binding sites (probably protein) within the cells as they go through their growth cycles. When the alga was exposed to metal solution for longer periods, its metal content increases. The lead ions are first adsorbed to the cell surface and then translocated to within the cell wall, to the plasma membrane and eventually to the cytoplasm.

Marchetti (1978) has reported that tetraethyl lead is more toxic than tetramethyl lead to marine organisms.

Muramoto (1980) has observed that toxicity of zinc, lead, copper and cadmium reduced when synthetic complexans were used to bind them. Increase in the ionic strength of cadmium and magnesium reduced the lead uptake (Soeder et al., 1978; Harding & Whitton, 1980). Miller (1976) reported that increase in ionic strength reduced the sensitivity of Selenastrum capricornutum to the metal

Copper:

The biotic effects of copper pollution are of particular interest because although copper is essential trace element for both plants and animals, it is strongly

toxic at quite low concentrations. For algae a micro-mutrient requirement for chelated copper has been repeatedly confirmed (Green et al., 1939, Provasoli and Pintner 1953,; Walker 1953,; Johnston 1963, 1964). Yet, in ionic form concentration as low as 1 µg/l has been reported to be toxic to the cultures of Chlorella pyrenoidosa and N. palea by Steemann Nielson et al., (1969). The toxicity of copper to different algal groups varies greatly. In general diatoms, dinoflagellates and blue green algae are reported to be more sensitive than green algae (Mandelli, 1969; Erickson et al., 1970; Saward et al., 1975).

Concentration of copper as low as 1 ppb was reported to be toxic to phytoplankton (Steemann Nielsen et al., 1969; Martin and Olander, 1971; Erickson, 1972).

At experimental dose levels copper was found to be toxic to S. bijugatus and N. palea. Their growth rates were impaired and biomass was reduced.

Steemann Nielsen and Wium Anderson (1970) have reported considerable reduction in photosynthetic rate of N. palea after 20 minutes of exposure to 1 µg Cu + 6 µg Fe, in ionic form. The reduction was seen at all light intensities and in their opinion was due to the penetration of copper into the cell. The cell membrane becomes leaky and the cells were found to loose considerable amounts of

organic matter, even upto 22%. At 2.6 µg/l photosynthesis was reduced by 50%. The "leaky" nature of the cells may be also the reason for reduced end products, inspite of better overall production indicated by increase in pH in N. palea. But both nett production and respiration were reduced in S. bijugatus.

Loss of mobility and reduction in photosynthetic rate were also reported by Anderson and Morel (1978) for Gonyaulax tamarensis. In the range of 30 and 100 µg/l, copper was observed to reduce both standing crop and rate of photosynthesis/unit chlorophyll a by Saward et al., (1975). During their investigation regarding marine food chain, no diatoms were found when copper ranged between 30-100 µg/l. During the present investigation simultaneous reduction of both nett photosynthetic and respiratory rates were observed in S. bijugatus at 0.1 and 0.2 ppm levels. But for N. palea the limit, from both biomass and quality point of view, seems to be 0.03 ppm.

Davey et al., (1973) have reported that copper was toxic to Thalassiosira pseudonana at 1 ppb level and toxicity persisted even with copper aged in artificial sea water. As stated elsewhere during the present study, the upper limit for copper at added dose concentration of 0.1 and 0.2 ppm levels varied with experiments. The factor causing such variation seems to be active in the medium

even after sterilization of the medium. Copper was the only metal which recorded such variation. Hence the upper limit as 0.2 ppm was determined on the basis of probability.

Reports on the effect of copper on pigments are lacking. All the pigments studied, chlorophyll a, b, c and carotenoids were found to be more than those of control in presence of this metal. For both test species total pigment content was maximum at the respective lower limits.

Reports on the effect of this metal on protein and lipid of algae are lacking. In the present study copper was observed to produce adverse affect on lipid content at lower level, on protein content at higher level, in S. bijugatus. Even then the total protein content was not affected. Cell numbers were found lowered indicating that the protein when produced was not utilized for sustained growth. In N. palea the anomaly observed was with regard to the end products. Both protein and lipid content of the diatom were lowered when the concentration of copper was 0.01 ppm where as increased when copper was 0.02 ppm. In all parameters estimated at 0.02 ppm level, copper produced a distinct positive effect. However this cannot be ascertained unless the interaction between copper and the growth medium is studied. On the whole S. bijugatus was found to be more tolerant than N. palea.

One mode of copper induced injury was suggested to be impairing the semi permeable osmotic barriers of cells (Harrison et al., 1977). Two peaks of copper uptake, one within the first 15 minutes and other between second and fifth day have been reported by Schenck and Hull (1985) for a dinoflagellate Gonyaulax tamarensis and efflux of copper occurs over 3 to 4 weeks. 34% of copper in both live and dead cells was found in cytoplasmic phase suggesting that copper may interfere with cell metabolism. 5 to 15 ug of copper was reported to affect photosynthesis first and presumably nitrogen fixation later by cutting off energy supplies to heterocysts in blue green algae (Horne, 1978).

EDTA was found to be effective against copper poisoning (Nishikawa and Tabata, 1968). In the pH range of 2-10, calcium reportedly has mitigating effect on the uptake of copper.

Although many recent reports discribed copper defence systems in different species, little is known about the source of the observed differences in algal sensitivity. Exclusion, tying up in cell wall, secretion of copper binding substances and production of internal sequestering sites have all been reported to be detoxifying mechanisms. Diatoms have been reported to be most sensitive organisms. (Overnell, 1976; Thomas and Seibert, 1977; Goering et al., 73, 1977).

Zinc:

Detailed work on the physiology of algae related to zinc deficiency has been carried out in Euglena by Steward (1974). Jensen et al., (1974) observed that though zinc is a necessary element it becomes rapidly toxic as its activity increases and species differ widely in their sensitivity to zinc toxicity. Anderson and Morel (1978) observed that growth rates for low zinc additions are difficult to interpret because they depend on cell's past history, on variable zinc contamination and on the depletion of zinc from the medium as cells grow. They worked out the results for cultures with higher zinc concentrations (corresponding to activities $> 10^{-12}$M.)

Parry and Hayward (1973) have reported that the uptake of zinc^{65} was temperature and pH dependent and appears to be indirectly linked with metabolism. Zinc^{65} is firmly bound to living cells. They suggested that the binding sites are "proteinacious compounds". Same authors have reported that the uptake in light at pH 9 was 50% more in 5 hours than at pH 7 and, at pH values greater than 9 uptake of Zn^{65} was markedly increased. Bachmann and Odum (1960) have reported that zinc uptake was noticed in light but not in dark and in marine seaweeds zinc is taken up in proportion to the gross oxygen production and accumulated in proportion to nett oxygen production. It was

suggested that zinc is taken up in direct proportion to the photosynthetic rate and that it accumulates in the algae as a function nett production or of their growth.

Les and Walker (1984) have reported that zinc was rapidly bound to Chroococcus paris. 90% of the metal was bound in 1 minute and nearly all within 10 minutes, but it can easily be extracted with dilute EDTA solution, suggesting the adsorption of the metal as a possibility. Also according to them zinc has lowest affinity for cells when compared to cadmium and copper. Significant reduction in the growth was also reported by 1 ppm zinc.

Allen et al., (1980) found 0.03 ppm zinc as inhibitory to the growth of Microcystis aeruginosa whereas Bartlett et al., (1974) reported 0.7 ppm zinc as algicidal to Selenastrum capricornutum.

In the present investigation zinc has affected the growth of both the species reducing the biomass though initially it exerted stimulatory influence.

Reports on the effect of zinc on pigments are lacking. In the present experimentation, zinc stimulated the production of pigments, to a greater extent in S. bijugatus than in N. palea. Whereas total amount of any one pigment increased with increasing metal concentration in S. bijugatus (except at 0.05 ppm level) it decreased in N. palea. Also at a particular concentration

of zinc the pigment was minimum but above and below 0.1 ppm in S. bijugatus and 0.04 ppm in N. palea it increased.

This metal enhanced the rate of production and reduced the rate of respiration of S. bijugatus at 0.05, 0.1 and 0.3 ppm levels and elevated both considerably at 0.5 ppm level. Interestingly the nett production rate was very high in this instance. But without exception zinc inhibited the rate of production and elevated the rate of respiration in N. palea.

At the experimental dose concentrations zinc generally reduced the protein content but carbohydrates and lipid have increased, except at 0.5 ppm level in S. bijugatus. In N. palea only the glucose fraction increased whereas protein lipid and other carbohydrate fractions decreased to a large extent at all tested levels of zinc. In both test species lipid was adversely affected. On the whole the general efficiency of both the species was reduced.

The toxic effect of the metals on the test species was not linear at the sublethal concentration employed. The species reacted differently to differe metals studied. The expression of toxicity in most instances is delayed but could be observed by the end growth phase.

The effect of mercury, cadmium, lead, copper and zinc on the diatom N. palea (Fig. IV) was found to without exception, adverse from both qualitative and quantitative aspects. S. bijugatus (Fig. IV) was foun to be more tolerant than the diatom studied, the adver effect of these metals being mostly restricted to the biomass.

Mercury, at the lowest level employed (0.02 p enhanced the production of carbohydrate, protein and lipid of S. bijugatus but reduced the biomass. At highe level (0.05 ppm) though carbohydrate and protein decrea lipid content increased to a large extent. The adverse effect caused a change in the proportion of macromolecu

At all test concentrations mercury was found t be harmful to N. palea. The effect is more qualitative than quantitative. In presence of mercury, the concentration of all three photosynthetic end products as well biomass of N. palea were reduced.

The adverse effect of cadmium is more pronounc on the protein content of both the species. Continued

reduction in biomass was observed with increase in cadmium concentration. In both species positive response was observed at 0.03 ppm cadmium level but the response was negative above and below this concentration which therefore may be regarded as optimum concentration for the production of carbohydrate, protein and lipid. However, this needs further detailed study for confirmation.

S. bijugatus responded positively to lead as far as the quality of the cells is concerned. In presence of this metal, protein content of the alga was promoted while carbohydrate and lipid were not affected. But at all test concentrations the biomass was reduced. Further reduction followed with the increase in lead concentration.

On the contrary lead exerted a total negative effect on N. palea at a much lower level.

S. bijugatus was found to have high tolerance for copper. At the highest level employed, slight reduction of protein was accompanied by increase in lipid content of the alga. At the lowest level of this metal, lipid was reduced but protein was not affected. The harmful effect was more pronounced when the photosynthetic end products were considered, though biomass was also slightly reduced.

Copper exerted a total negative influence both on biomass and photosynthetic end products of N. palea.

The effect is more pronounced at highest and lowest test concentrations. Also this diatom is less tolerant to copper than S. bijugatus.

Zinc exerted definite adverse effect on both the species at the experimental dose levels. Reduction in protein of S. bijugatus was accompanied by increase in lipid only at lower levels (0.05 and 0.1 ppm) whereas at higher levels (0.3 and 0.5 ppm) the concentration of all the three end products was reduced. Among the metals studied, zinc was found to have least effect on biomass.

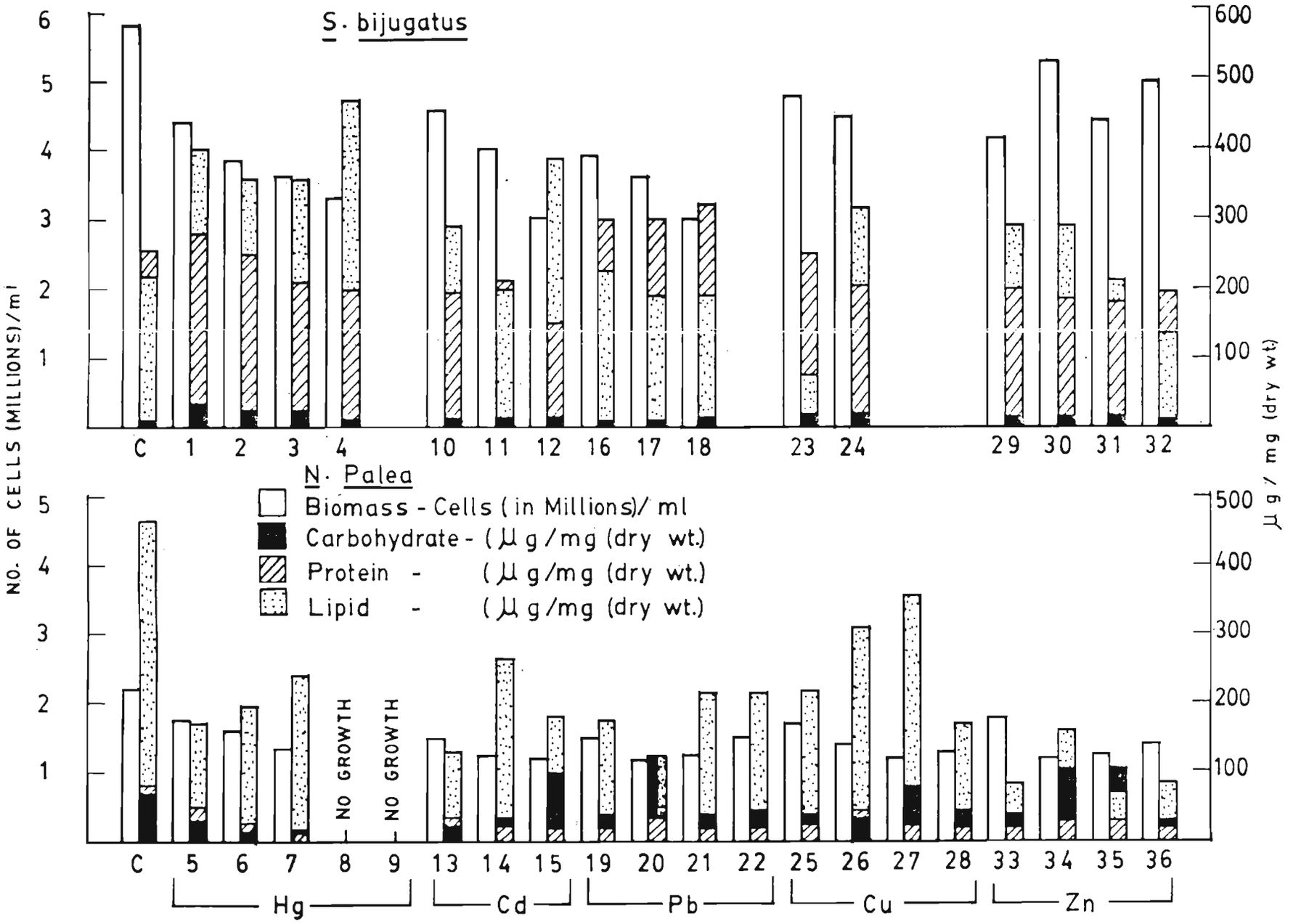
Zinc was more toxic to N. palea and the overall effect was negative except on carbohydrate concentration which increased at 0.04 and 0.06 ppm levels.

All the metals exerted harmful effect on the test species affecting the biomass and photosynthetic end products.

The relative toxicity of these metals varied with the test species. For S. bijugatus the order of toxicity was Zn > Cu > Hg > Cd > Pb when the biomass was considered and when the photosynthetic end products were taken into account, a slightly different order, Zn > Cd Cu > Hg > Pb was obtained.

The relative toxicity of the metals for N. palea was in the order Zn > Cd > Pb > Hg > Cu when either biomass or photosynthetic end products were considered.

Fig. IV


S. bijugatus
N. Palea
Biomass - Cells (in Millions)/ ml
Carbohydrate - (µg/mg (dry wt.)
Protein - (µg/mg (dry wt.)
Lipid - (µg/mg (dry wt.)
NO. OF CELLS (MILLIONS)/ml
µg/mg (dry wt)
NO GROWTH
NO GROWTH
C 1 2 3 4 10 11 12 16 17 18 23 24 29 30 31 32
C 5 6 7 8 9 13 14 15 19 20 21 22 25 26 27 28 33 34 35 36
Hg
Cd
Pb
Cu
Zn

Analysis of variance was carried out to establish the significance of the metal interaction in both species studied. (Snedecor and Cocharan, 1956).

The variance ratios obtained by the two way analysis of variance technique (Table III), for the growth parameters studied with regard to S. bijugatus were found to be significant in 96 out of 110 cases.

Mercury produced significant effect on all the parameters studied at all selected concentrations during the growth period.

At the test levels, the effect of cadmium on nett production, chlorophyll a, chlorophyll b, carotenoids and carbohydrates was not significant. But the effect was consistently significant for the period of growth.

Lead did not produce any significant effect on carotenoids. Also at the selected concentrations the effect on carbohydrate was not significant.

At the experimental dose concentrations of copper, no significant difference was noted in chlorophyll b, pheophytin and carbohydrate.

Zinc did not cause any significant change in pH, chlorophyll a and carbohydrate at the test concentrations.

The selected metals produced considerable effect on N. palea (Table IV) except in few instances. For this species 110 out of 125 values were found significant.

Mercury did not produce significant effect on chlorophyll a at any level during the growth period. The effect was considerable on chlorophyll c and pheophytin at the selected concentrations but the difference was not significant at any level for the entire growth period.

Chlorophyll c was not affected by cadmium. Chlorophyll a and carotenoids did not vary significantly during the growth phase where as the concentration of pheophytin varied considerably with the age of the culture.

Respiration of the diatom was not affected significantly by lead at different concentrations during growth period. Its effect on chlorophyll a was not significant at the selected concentrations of the metal.

Copper was the only metal to exert totally significant effect on N. palea.

At the test levels, zinc did not produce any significant difference in pH of the culture and pheophytin content of the diatom.

TABLE - III

Significance of the variance ratios in the analysis of variance table for between concentrations and between days - *S. bijugatus*

Sl. No.	Metals employed	No. of selected concentrations	pH		Gross production		Nett production		Respiration		Chlorophyll a		Chlorophyll b		Carotenoids		Pheophytin		Carbohydrate		Protein (Biuret)		Protein (Total)	Lipid
			I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II		
(1)	Hg	4	c	c	c	c	c	c	c	c	b	c	b	c	c	c	c	c	c	c	b	c	c	c
(2)	Cd	3	c	c	b	c	NS	c	c	b	NS	c	NS	c	NS	c	a	c	NS	c	c	c	c	c
(3)	Pb	3	c	c	c	c	c	c	c	c	b	c	c	c	NS	NS	a	c	NS	c	c	c	a	c
(4)	Cu	2	c	c	c	c	c	c	c	c	a	c	NS	c	b	c	NS	b	NS	c	b	c	c	c
(5)	Zn	4	NS	c	c	c	c	c	c	c	NS	c	a	c	c	c	c	c	NS	c	c	c	c	c

NS - Not significant

a - $p < 0.05$
b - $p < 0.01$
c - $p < 0.001$

I - Between concentrations
II - Between days

TABLE - IV

Significance of the variance ratios in the analysis of variance table for between concentrations and between days -

N. Palea

Sl. No.	Metals employed	No. of selected concentrations	pH		Gross production		Nett Production		Respiration		Chlorophyll a		Chlorophyll c		Carotenoids		Pheophytin		Carbohydrate (Acid soluble)		Carbohydrate (Alkali soluble)		Carbohydrate (Insoluble)		Protein (Total)		Lipid
			I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	
(1)	Hg	5	c	c	c	c	c	c	c	b	NS	NS	b	NS	c	b	c	NS	c	c	c	a	c	c	b	c	c
(2)	Cd	3	c	c	c	c	c	c	NS	c	c	NS	NS	NS	c	NS	NS	b	a	c	c	c	b	c	b	c	c
(3)	Pb	4	c	c	c	c	c	c	NS	NS	NS	c	c	c	c	c	c	c	c	c	c	c	c	c	b	c	c
(4)	Cu	4	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	b	c	a	b	c
(5)	Zn	4	NS	c	c	c	c	c	c	c	a	c	c	c	a	c	NS	c	c	c	a	c	c	c	a	a	c

NS - Not significant

a - $p < 0.05$
b - $p < 0.01$
c - $p < 0.001$

I - Between concentrations
II - Between days

The organisms growing in natural waters are adopted to the set of ecological conditions of the water. They must also be considered as adopted to the toxic metals already present in the polluted water. Hence the background load of any metal is important in metal toxicity studies.

In the present study, natural water was used as the medium. The concentration of selected metals was found to be very low in these waters. Phytoplankton are known to concentrate the metals and the concentration was generally reported to be many times higher than in the ambient waters. In the present study, the background load of metals in the species was assessed from the control. The values represent the concentration already existing in the test species. These values were subtracted from the values of metal concentration obtained by exposing the species to selected metals, to find out the actual accumulation by the species during the growth phase.

The concentration of the metals as accumulated by the species was found to be very low except in few cases because of the lower concentration of the metals employed for the present work.

Cadmium, lead, zinc and iron were detected in S. bijugatus even when it was not exposed to the

metals in the laboratory. But the concentration was very low and it was not possible to quantify. All the metals except mercury were detected in the cultures of N. palea. But quantification was not possible except in the case of zinc. The concentration of zinc in the control was found to be 0.03 ppm/100 mg dry wt. Pillai et al., (1986) reported that the concentration of copper and lead in bivalves was low. Also the level of cadmium was reported to be very low in bivalves except oysters. Zinc was reported to be the dominant metal observed in oysters of Cochin back waters. These value was subtracted for the experimental values of zinc and reported. Wherever the background level of metals could not be quantified, the observed values were considered as the actual uptake by the species, and reported.

From the present study it is seen that metals like cadmium, lead, zinc and iron are found in the water taken from the river system which receives effluents from the sewage plant and backwaters. The phytoplanktonic species, S. bijugatus and N. palea were found to be considerably affected by these metals as is revealed by change in the proportion of photosynthetic end products, even when the metals were below the detection limit. Any further addition of these metals will be injurious to the ecosystem and hence undesirable.

TABLE V

Concentration of metals in S. bijugatus at the end of growth phase

Sl. No.	Metal employed	No. of treatment	Concentration (ppm/100 mg dry wt.)
(1)	Mercury (Hg)	1	0.0089
		2	0.0038
		3	0.014
		4	0.0069
(2)	Cadmium (Cd)	10	0.034 *
		11	0.036 *
		12	0.034 *
(3)	Lead (Pb)	16	0.030 *
		17	0.030 *
		18	0.083
(4)	Copper (Cu)	23	0.106
		24	0.162
(5)	Zinc (Zn)	29	0.091
		30	0.109
		31	0.134
		32	0.160

The values represented are the average of three observations for mercury and two observations for others.

* Values are less than the indicated concentrations.

TABLE VI

The concentration of metals in N. palea at the end of growth phase

Sl. No.	Metal employed	No. of treatment	Concentration (ppm/100 mg dry wt.)
(1)	Mercury (Hg)	5	0.0035
		6	0.003
		7	0.0027
		8	Growth was highly
		9	retarded
(2)	Cadmium (Cd)	13	0.025 *
		14	0.023 *
		15	0.017 *
(3)	Lead (Pb)	19	0.024 *
		20	0.020 *
		21	0.023 *
		22	0.025 *
(4)	Copper (Cu)	25	0.010
		26	0.018
		27	0.016
		28	0.024
(5)	Zinc (Zn)	33	0.022
		34	0.046
		35	0.055
		36	0.080

The values represented are the average of three observations for mercury and two observations for others.

* Values are less than the indicated concentrations.

6. Metals selected and their effect on test species (Two metals).

The effect of the metals in combination (Table 2) studied and reported in relation to control.

TABLE 2

Sl.No. of the combination	S. bijugatus		N. palea	
	No. of treatment	Concentration in ppm	No. of treatment	Concentration in ppm
(6)	37	0.03 Hg + 0.01 Cd	39	0.005 Hg + 0.02 Cd
	38	0.03 Hg + 0.05 Cd	40	0.005 Hg + 0.04 Cd
(7)	41	0.05 Hg + 0.05 Pb	43	0.005 Hg + 0.02 Pb
	42	0.05 Hg + 0.1 Pb	44	0.005 Hg + 0.04 Pb
(8)	45	0.03 Hg + 0.01 Cu	48	0.005 Hg + 0.01 Cu
	46	0.03 Hg + 0.05 Cu	49	0.005 Hg + 0.05 Cu
	47	0.03 Hg + 0.1 Cu		
(9)	50	0.05 Hg + 0.05 Zn	52	0.005 Hg + 0.05 Zn
	51	0.05 Hg + 0.1 Zn	53	0.005 Hg + 0.08 Zn
(10)	54	0.05 Hg + 0.05 Fe	56	0.005 Hg + 0.05 Fe
	55	0.05 Hg + 0.1 Fe	57	0.005 Hg + 0.08 Fe
(11)	58	0.05 Cd + 0.05 Pb	60	0.04 Cd + 0.04 Pb
	59	0.05 Cd + 0.1 Pb	61	0.04 Cd + 0.08 Pb
(12)	62	0.05 Cd + 0.1 Zn	63	0.04 Cd + 0.02 Zn
			64	0.04 Cd + 0.04 Zn
(13)	65	0.05 Cd + 0.02 Fe	67	0.04 Cd + 0.02 Fe
	66	0.05 Cd + 0.05 Fe	68	0.04 Cd + 0.05 Fe
(14)	69	0.03 Pb + 0.05 Zn	71	0.04 Pb + 0.02 Zn
	70	0.03 Pb + 0.1 Zn	72	0.04 Pb + 0.05 Zn
(15)	73	0.3 Pb + 0.05 Fe	75	0.04 Pb + 0.02 Fe
	74	0.3 Pb + 0.1 Fe	76	0.04 Pb + 0.05 Fe

6.1.1

Combined effect of mercury and cadmium on

S. bijugatus

Sl. No. of combination	No. of the treatment	Concentration of metals (in ppm)
(6)	37	0.03 Hg + 0.01 Cd
	38	0.03 Hg + 0.05 Cd

Production: (Fig. I)

When the cadmium level was low (0.01 ppm) in the combination, rate of production was lowered upto eighth day but exceeded that of control by tenth day and remained steady thereafter. The rate of production was lowered to a large extent when the level of cadmium was raised to 0.05 ppm and remained less than that of control throughout. The percentage reduction was 13 and 81 on sixth day respectively in two treatments. On twelfth day it was found to be 7% higher than the control in the former and 70% less in the latter.

Respiration of the alga was lowered in both treatments (37 and 38) and was reduced by 68% on eighth day in relation to control, increasing thereafter in treatment 37 and decreasing in the other. At the end of growth phase it was 25% and 92% less than control respectively.

Production of the alga was reflected in the pH of the culture. Owing to enhanced production and lowered respiration, pH of the culture in treatment 37 was steady from eighth day onwards and was slightly higher than that of control at the end of growth phase. pH of the culture was comparitively low in the other due to inhibition of production but improved from eighth day onwards and remained less than that of control and treatment 37 at the end of growth phase. The maximum level of pH attained in the control (10.64) was not reached in either treatment.

Pigments: (Fig. II)

All four pigments were produced to a larger extent during the early phase of growth by the alga in treatments 37. At the maximum level chlorophyll a in the first treatment was less than that of control whereas chlorophyll b and carotenoids were more at respective maxima. At the end of growth phase concentration of chlorophyll a and chlorophyll b was less than that of control but that of carotenoids was slightly higher. Chlorophyll b and carotenoids were more than chlorophyll a in treatment 37. Pheophytin content of the alga was maximum on fourth day and was at a higher level than that of control on fourth and sixth day of growth phase, but was not detected on eighth day.

All four pigments of the alga were suppressed in the early growth phase in treatment 38 and were not detected on second day of growth. The concentration of chlorophyll a, b and carotenoids increased thereafter, but the increase of chlorophyll b was less. With the exception of pheophytin, pigments reached a higher level than those of control in the latter phase and remained higher than in treatment 37 at the end of growth phase. Severe suppression of pheophytin was noteworthy. It was not detected on second, eighth and twelfth day of growth.

Photosynthetic end products: (Fig. III)

Carbohydrate content of the treated alga was considerably less than that of control in the early growth phase, with respective reduction of 85% and 92% on sixth day. Thereafter it increased, reaching maximum on tenth day when it was 318% and 187% higher, in relation to control, respectively. In both instances it declined towards the end of growth phase but remained 152% and 163% higher than that of control respectively. At its maximum on tenth day the carbohydrate concentration of the alga in treatment 37 was slightly higher than the maximum level achieved by control on fourth day.

The protein content of the alga in treatment 37 was found to be equal to that of control whereas in the other it was reduced by 38%.

Lipid content of the alga improved by 35% when cadmium level was low and was reduced by 7% when cadmium level was high treatment 38).

Growth: (Fig. III)

The growth rate of the alga was lowered in both treatments in the early phase. In the first it improved after sixth day and from eighth day onwards all biomass was equal to control. Whereas in the other, further growth was not observed till eighth day and thereafter proceeded at a slow pace, resulting in considerable decrease in cell number at the end of growth phase.

Details of nutrient uptake of the alga are given in table.

Selected concentration of metal	Nutrients absorbed by the alga (ug/l)		N / P
	Phosphate	Nitrate	
37	2060	1490	0.72
38	2994	1788	0.60
Control	945	1290	1.37

Increase in the nutrient absorption of the alga was recorded in presence of the metals. Unlike in control more phosphate was absorbed than nitrate. Increase in

cadmium concentration has promoted nutrient uptake. Phosphate uptake was doubled when cadmium was 0.01 ppm and trebled at 0.05 ppm. The N / P ratio varied considerably between any combination and control.

Conclusion:

Inspite of slightly lowered production during most of the growth phase, when the concentration of cadmium was low, it exerted a positive effect on the lipid and carbohydrate contents of the alga without affecting the protein and growth.

But when cadmium was at higher level, treatment 38, nett production of the alga was severely inhibited, its protein content was reduced while the lipid remained unaffected. Its growth was hindered. On the whole the effect was negative.

Increase in the total pigment content seems to be a common phenomenon.

Comparison:

When the combined effect of mercury and cadmium on various parameters studied was compared with individual effects of the two metals, the adverse effect of cadmium was found to prevail at both higher and lower levels. When the cadmium level was low, treatments 37 (0.03 Hg + 0.01 Cd) the positive effect of mercury prevailed on lipid but not when the cadmium level increased, treatment 38 (0.03 Hg + 0.05 Cd).

When the cadmium level was high (0.03 Hg + 0.05 Cd) production was comparitively lowered. Respiration was not affected. All pigments were suppressed, except pheophytin, to a greater extent, whereas carbohydrates and biomass were not affected but protein and lipid were reduced.

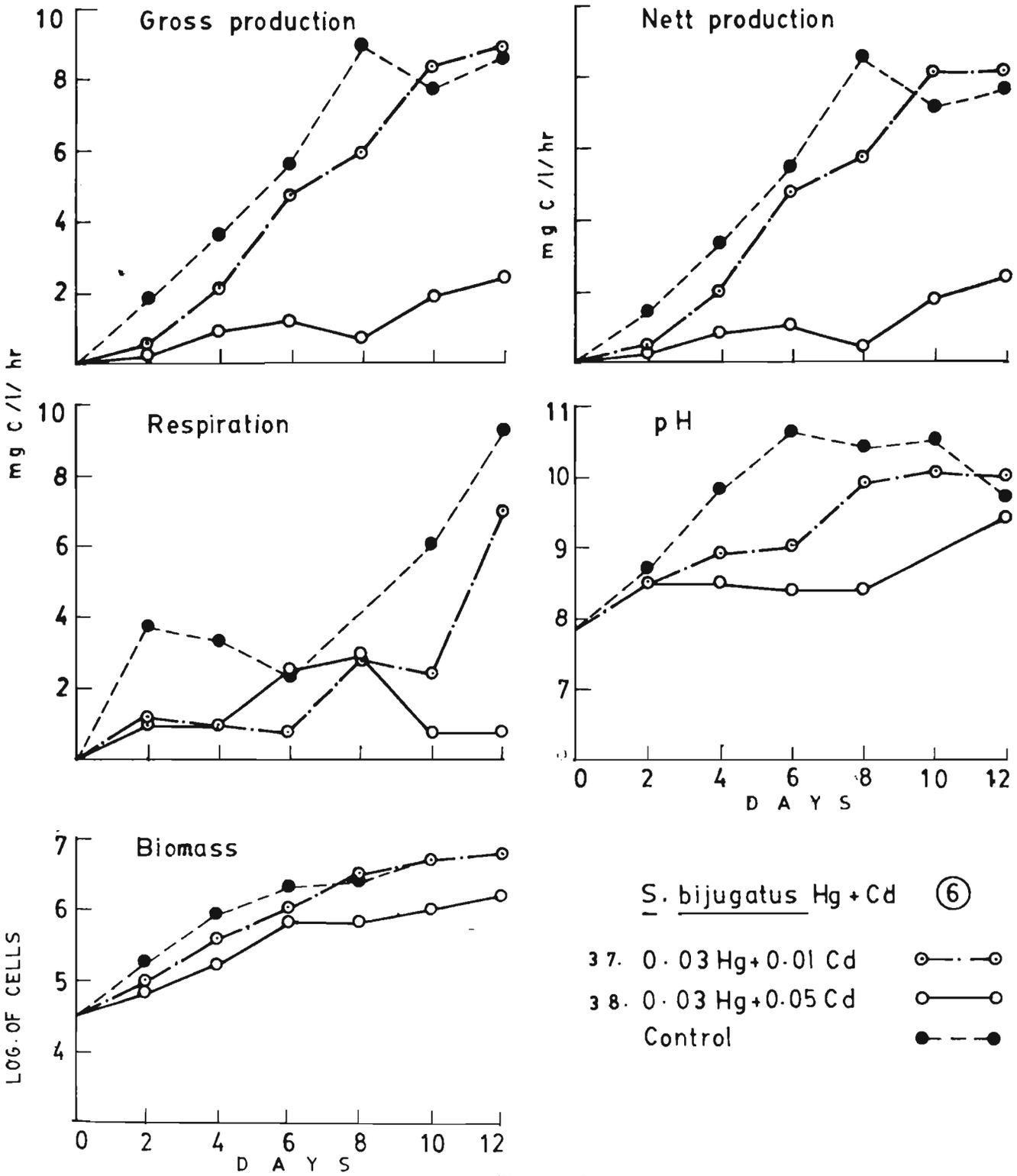

Gross production
Nett production
Respiration
pH
Biomass
mg C /l/ hr
LOG. OF CELLS
D A Y S
S. bijugatus Hg + Cd ⑥
37. 0·03 Hg + 0·01 Cd
38. 0·03 Hg + 0·05 Cd
Control

Fig. I

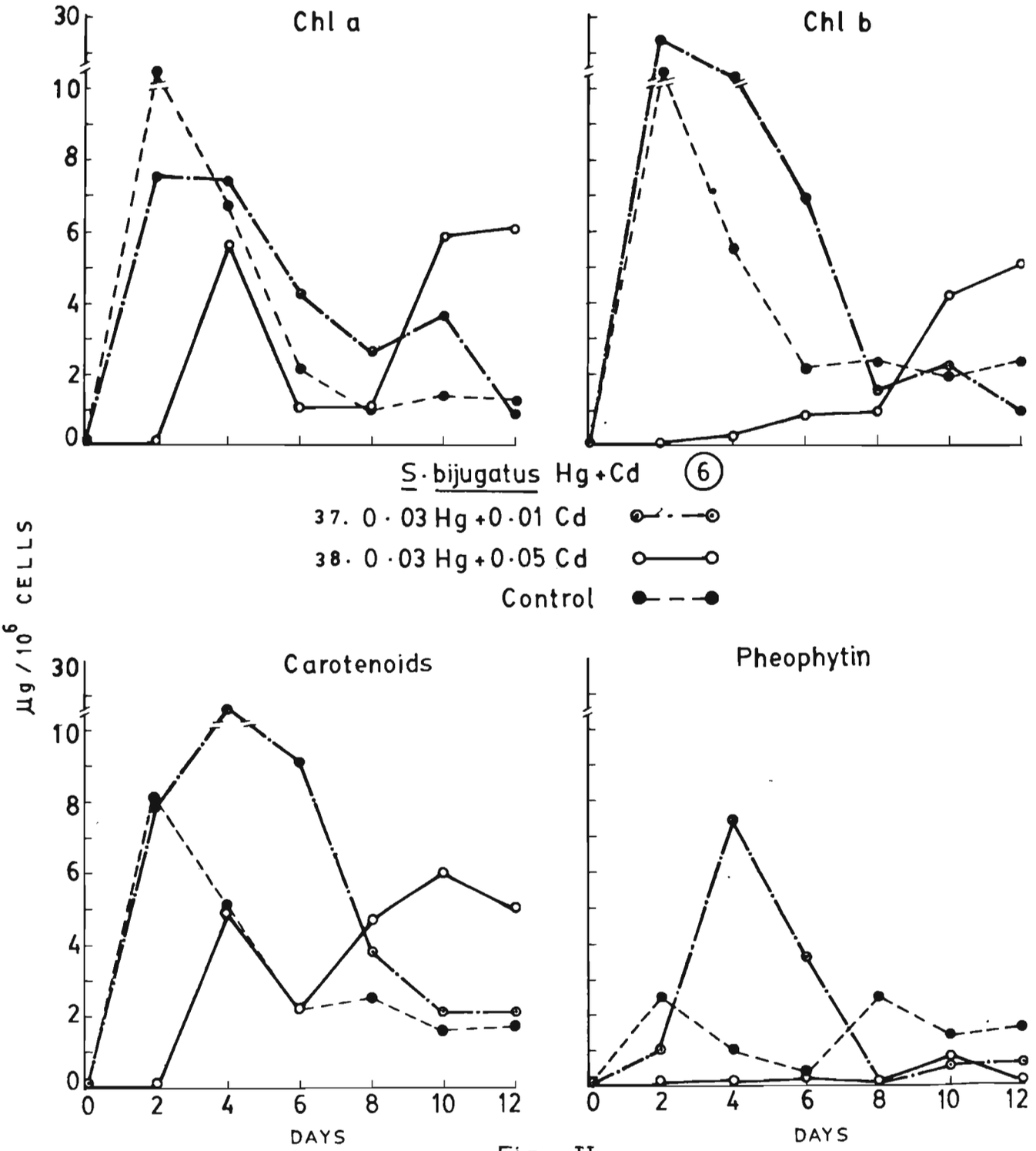
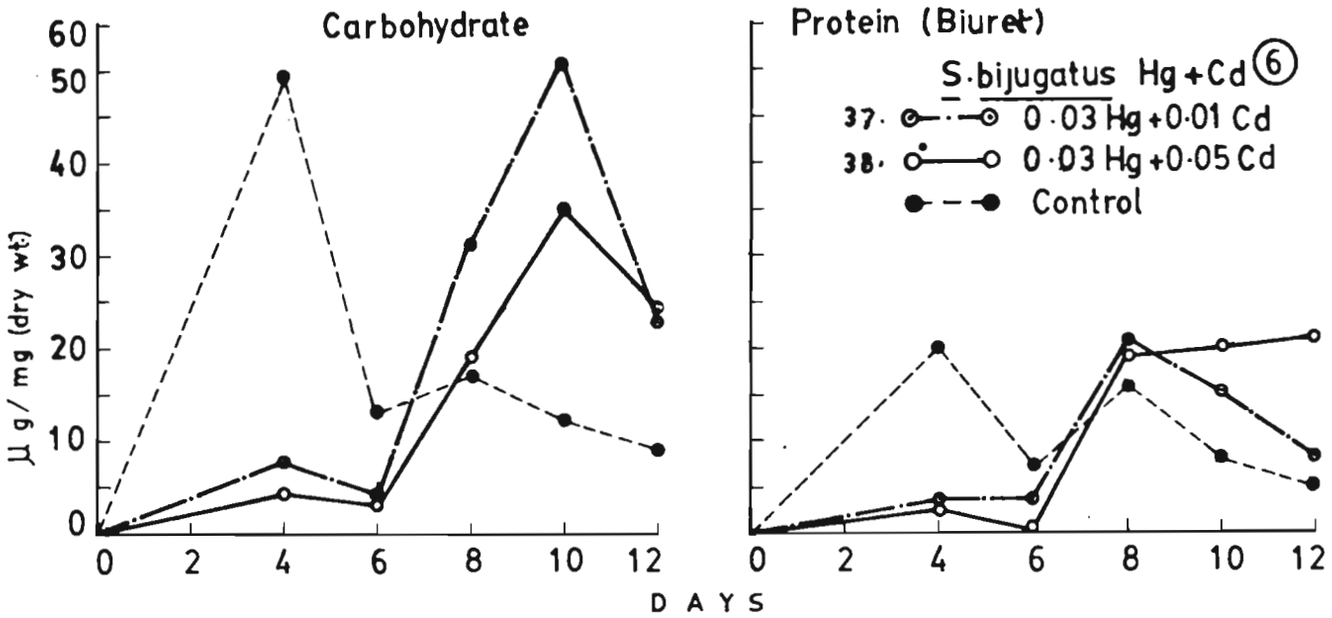

Chl a
Chl b
S. bijugatus Hg+Cd (6)
37. 0·03 Hg+0·01 Cd
38. 0·03 Hg+0·05 Cd
Control
µg / 10^6 CELLS
Carotenoids
Pheophytin
DAYS
DAYS

Fig. II


Carbohydrate
Protein (Biuret)
S. bijugatus Hg + Cd
37. 0·03 Hg + 0·01 Cd
38. 0·03 Hg + 0·05 Cd
Control
µ g / mg (dry wt)
60
50
40
30
20
10
0
0 2 4 6 8 10 12
DAYS

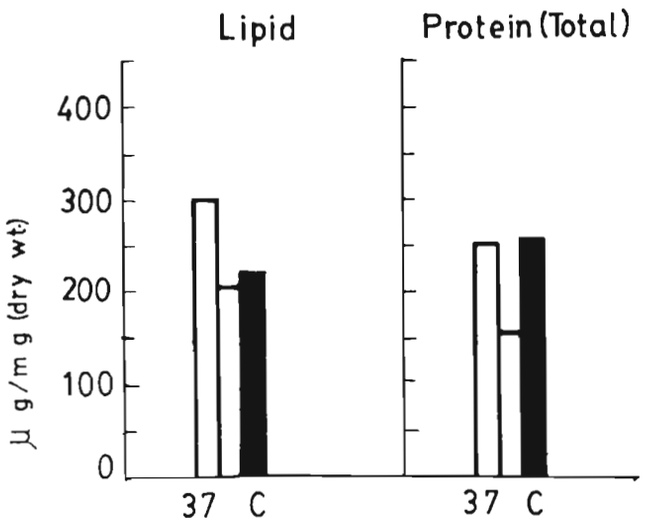

Lipid
Protein (Total)
µ g/m g (dry wt)
400
300
200
100
0
37 C
37 C

Fig. III

Combined effect of mercury and cadmium on

N. palea

Sl. No. of the combination	No. of the treatment	Concentration of metals (ppm)
(6)	39	0.005 Hg + 0.02 Cd
	40	0.005 Hg + 0.04 Cd

Production: (Fig. I)

In general, production of the diatom was adversely affected by the metals in combination. When the cadmium level was low treatment 39, production was promoted in the early phase of growth, with 375% increase at maximum on second day, whereas in the other it was reduced by 83% in relation to control. Decline in production was observed from its maximum on second day in the first, to minimum on fourth day when it was found to be slightly less than in treatment 40. From fourth day onwards production did not differ much between the two and from sixth day onwards was nearly steady till the end of growth phase, when it was found to be reduced by 56% and 60% respectively, in relation to control.

Respiration of the diatom was elevated during the early growth phase in both the treatments. The percentage increase was 460 and 640 respectively on fourth

day, in relation to control. In treatment 39 respiration continued to increase to maximum on eighth day when it was 119% higher whereas in the latter it exhibited 23% reduction. Towards the end of growth phase respiration of the diatom decreased in both treatments, but was higher, by 8% in the former and 60% less in the latter.

pH of the culture did not vary much between the two except on eighth day but remained less than that of control. It registered a gradual increase till eighth day in the former and till sixth day in the latter. At the end of growth phase it reached same level in both and was less than that of control.

Pigments: (Fig. II)

Total pigment content of the diatom increased in both the treatments when compared to that of control. Throughout the except on the last day, it remained at higher level in treatment 39 and the pigments exhibited a tendency to decrease towards the end of growth phase. But in treatment 40 only chlorophyll a and carotenoids of the diatom registered a decrease towards the end of growth phase.

Photosynthetic end products: (Fig. III)

Total carbohydrate content of the diatom was lowered in presence of these metals.

The acid soluble carbohydrate fraction of the diatom increased by 110% and 93% respectively on fourth day and declined upto sixth day when compared to that of control. In both, this fraction increased from sixth day onwards, but to a greater extent in treatment 39, reaching its maximum on eighth day with 35% increase whereas in the other it remained 55% less than that control. It dwindled to a 38% lower level in the former and increased further in the latter but 58% less than control in the last day.

The alkali soluble carbohydrate fraction of the diatom did not exhibit much variation between the treatments and between any treatment and control except on the last day of growth when it was reduced by 8% and 80% respectively.

The insoluble carbohydrate fraction of the treated diatom remained equal to that of control on fourth day. It did not differ much between the treatments though in the former it was slightly more. But at the end of growth phase was found to be 78% and 80% less than that of control, respectively.

Total protein content of the diatom registered a respective increase by 122% and 51% on sixth day. Subsequent increase in the concentration of protein was observed, though not to the same extent as in control and

on eighth day it reached almost the same level with 36% and 37% reduction respectively and declined thereafter to 67% and 64% lower level on the last day.

Lipid content of the diatom was lowered by 10% and 25% respectively.

Growth: (Fig. I)

The growth rate of the diatom was not affected in the early phase but as the culture aged it fell short of control, to a considerable extent in treatment 40. Final yield in biomass was reduced in both treatments. Details of phosphate and nitrate uptake of the diatom given below.

Selected treatments	Nutrients absorbed (ug/l)		N / P
	Phosphate	Nitrate	
39	1735	737	0.43
40	1685	664	0.39
Control	1350	763	0.57

Phosphate was absorbed to a greater extent than that of control whereas nitrate uptake was lowered. But as in control, more phosphate was taken up than nitrate. Phosphate uptake was reduced by 50 ug and nitrate uptake by 100 ug, with the increase in cadmium level. The N / P ratio showed increasing reduction with increase in cadmium level.

Conclusion:

Production of the diatom did not vary between the two treatments except for a brief period in the early growth phase but the respiration of the diatom was elevated in the first treatment. Protein content of the diatom did not vary with increase in cadmium level but lipid content was reduced and also biomass. Increase in total pigment content seems to be a common phenomenon.

Comparison:

When the combined effect of mercury and cadmium on various parameters studied was compared with individual effects of the two metals, production was found to be lowered and respiration was elevated. Concentration of chlorophyll a, chlorophyll b and carotenoids was increased. Carbohydrates were not affected, but protein content was reduced. Lipid content of the species improved to a large extent. Biomass was not affected.

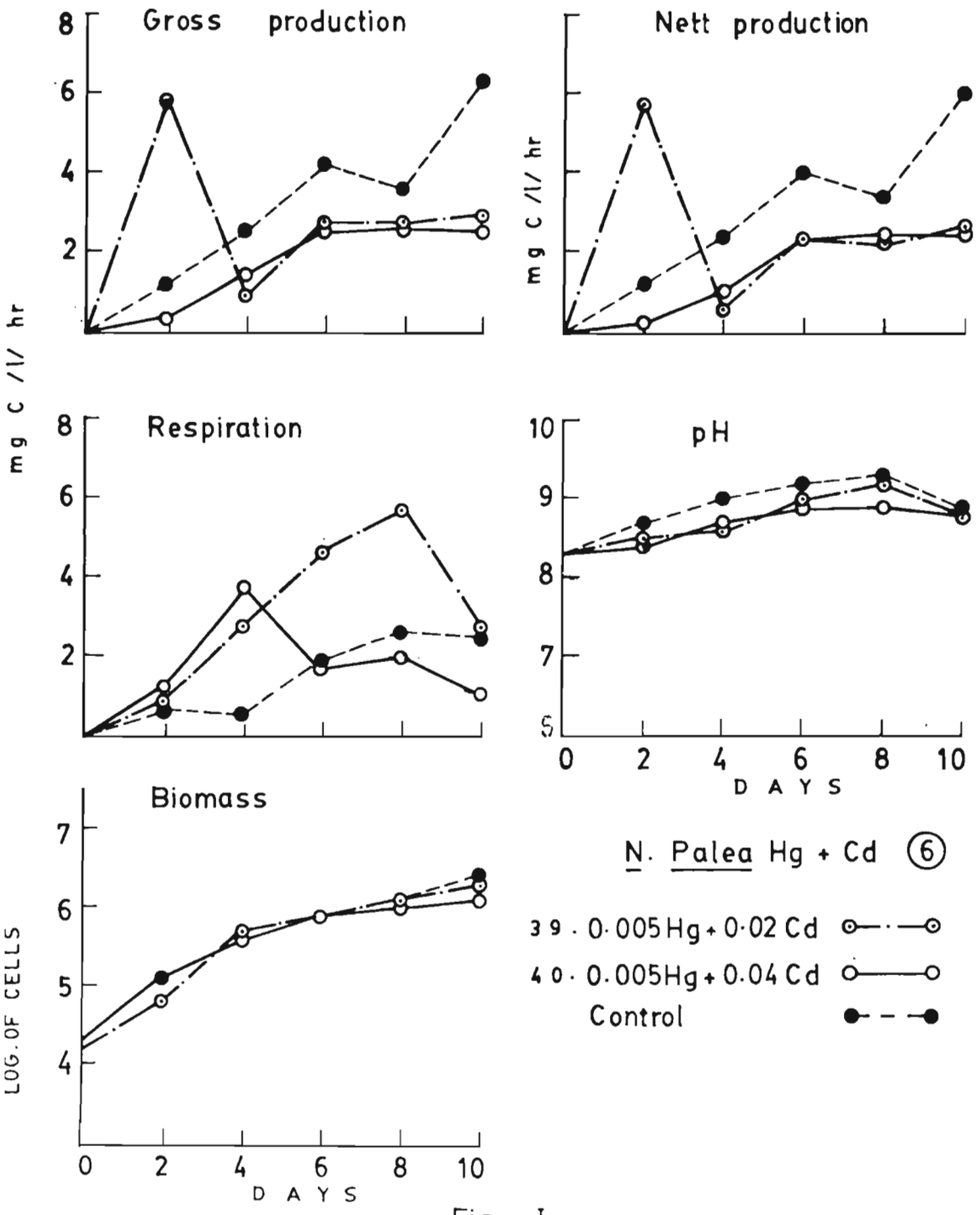

Gross production
Nett production
Respiration
pH
Biomass
mg C /l/ hr
LOG. OF CELLS
DAYS
N. Palea Hg + Cd (6)
39 . 0·005 Hg + 0·02 Cd
40 . 0·005 Hg + 0·04 Cd
Control

Fig. I

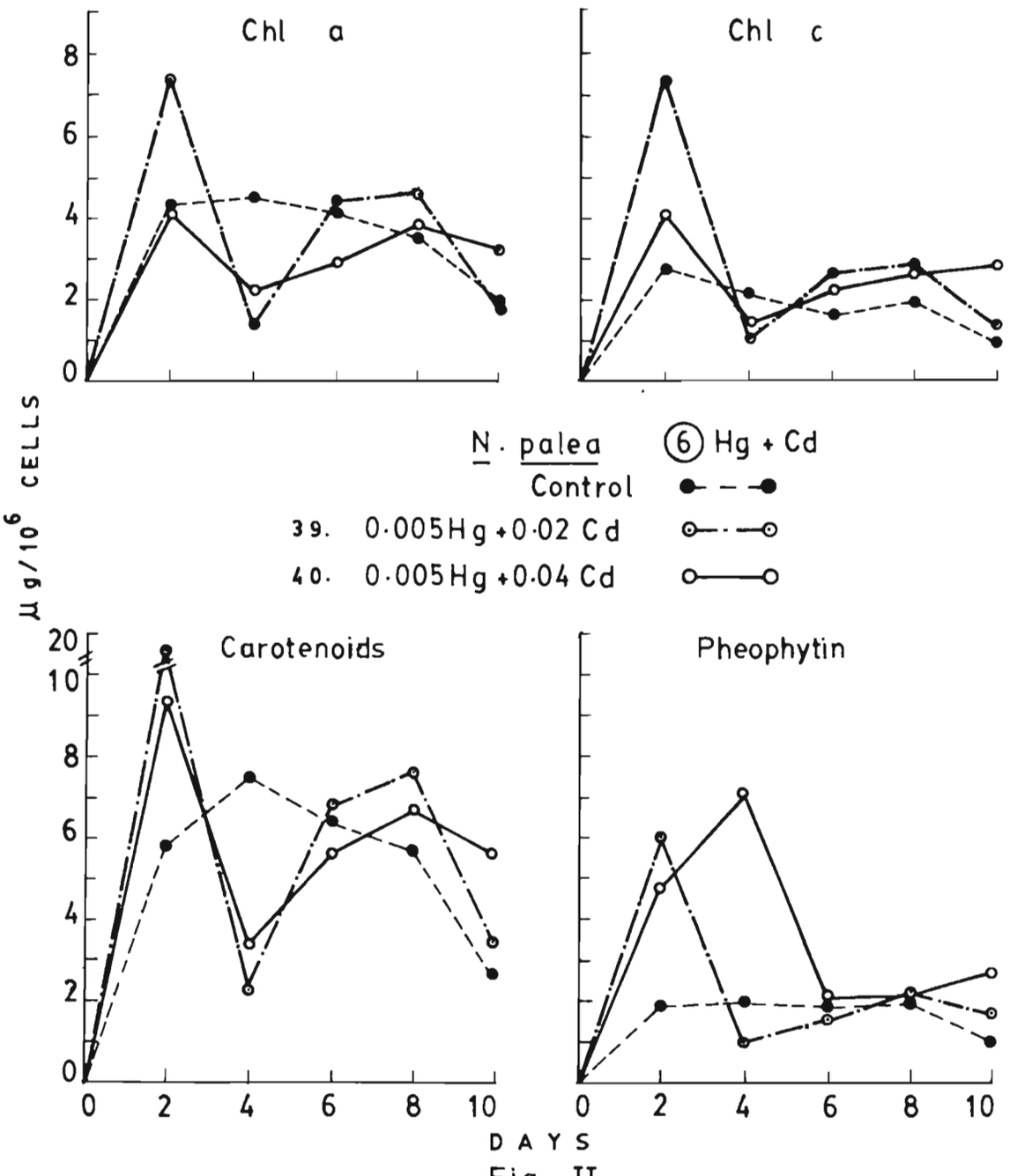

Chl a
Chl c
N. palea
Control
39. 0·005Hg +0·02 Cd
40. 0·005Hg +0·04 Cd
⑥ Hg + Cd
Carotenoids
Pheophytin
µg/10^6 CELLS
DAYS
0
2
4
6
8
10
20

Fig. II

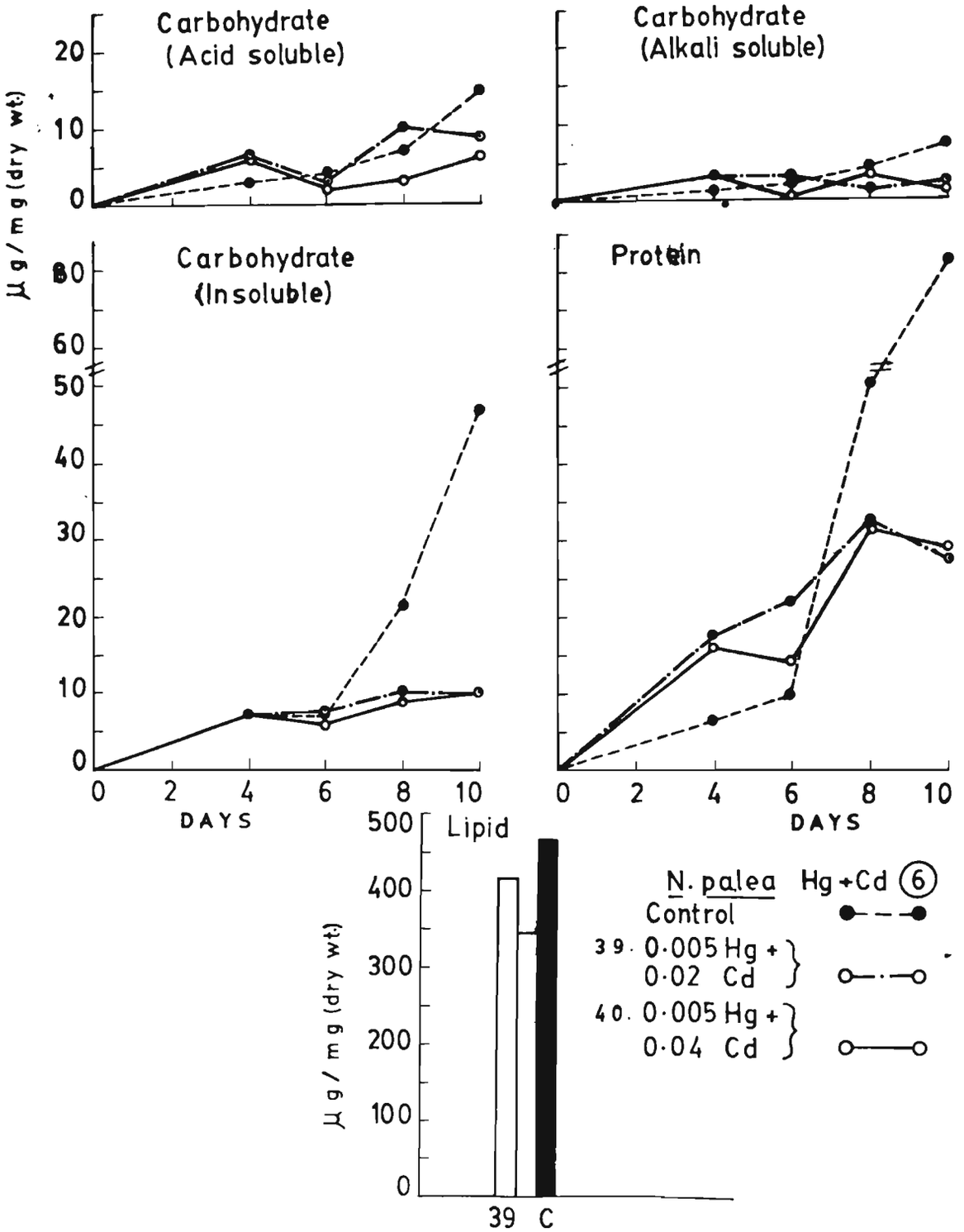

Carbohydrate
(Acid soluble)
Carbohydrate
(Alkali soluble)
Carbohydrate
(Insoluble)
Protein
µg/mg (dry wt)
DAYS
Lipid
N. palea Hg+Cd (6)
Control
39. 0·005 Hg + 0·02 Cd
40. 0·005 Hg + 0·04 Cd
39 C

Fig. III

6.1.2

Combined effect of mercury and lead on

S. bijugatus

Sl.No.of combination	No. of treatment	Concentration of metals (in ppm)
(7)	41	0.05 Hg + 0.05 Pb
	42	0.05 Hg + 0.1 Pb

Production: (Fig. I)

Production of the alga was inhibited by the metals in combination. It increased gradually to reach maximum on tenth day in treatment 41. But in the sharp rise was noted from sixth day to maximum on eighth day. It was less than control by 32% and 3% on eighth day and more by 1% and 8% on tenth day respectively. It declined in former to 10% lower level and increased in the latter to 8% higher level at the end of growth phase.

Respiration of the alga exhibited considerable fluctuation during the growth phase in both treatments which was found to be generally reduced. It exceeded that of control, only on sixth day in the first treatment and on fourth day alone in the second treatment. At the two peaks on second and sixth days it was 19% less and 43% more than control respectively in the former treatment but declined to a minimum on eighth day. Increasing once

again thereafter it reached 27% lower level than that of control at the end of growth phase. In the second treatment at the two peaks respectively on fourth and eighth days it was 36% more and 5% less than that of control. It decreased by tenth day and remained steady thereafter. It was 77% less than that of control at the end of growth phase.

pH of the culture was less than that of control in the first treatment except at the end of growth phase. In the second treatment pH was considerably lowered than that of control except on eighth day when it was equal. Inspite of increased production and reduced respiration pH decreased steadily from eighth day onwards in the second treatment.

Pigments: (Fig. II)

Pigment content of the alga was maximum in the early phase in first treatment. The total amount of any pigment in the alga did not differ much between the treatments at the end of growth phase but was slightly more in the second treatment.

In the treated alga the concentration of chlorophyll a and chlorophyll b was less than that of control at the end of growth phase whereas carotenoids and pheophytins have increased to a greater extent. Initial suppression

of chlorophyll b and pheophytin was observed in first treatment whereas pheophytin was not detected on sixth day in the second treatment. Considerable fluctuation in the pigment level was noted during growth phase in the second treatment. The metals in combination have suppressed the pigment content to some extent in the early phase.

Photosynthetic end products: (Fig. III)

Carbohydrate content of the alga was reduced in the early growth phase in both the treatments but was found to be more than that of control in the latter phase. From a respective 80% and 24% lower level than control on fourth day it increased to 285% and 130% higher level on tenth day. It was reduced in both treatments, by 185% and 64% respectively at the end of growth phase.

Total protein of the alga was less than that of control in the treatments but was found to decrease with increasing lead concentration. 21% and 47% reduction was observed in protein content of the alga in the two treatments respectively.

Lipid content of the alga was adversely affected by the metals in combination and was 46% and 48% less than control respectively in the two treatments.

Growth: (Fig. I)

Growth of the alga was stimulated between second and fourth day in both the treatments and also between sixth and eighth day in the first treatment. But the biomass was reduced slightly when compared to that of control at the end of growth phase.

Sl.No. of combination	No. of the treatment	Nutrients absorbed by the alga (µg/l)		N/P
		Phosphate	Nitrate	
(7)	41	2028	2788	1.38
	42	1980	1781	0.90
	Control	945	1290	1.37

Both the nutrients were absorbed to a far greater extent when the alga was exposed to the metals in present combination. Though 50% increase in phosphate absorption was observed. Between the treatments, uptake decreased with increase in lead concentration. The N/P ratio decreased when compared with that of control in the latter treatment.

Conclusion:

The metals in combination have reduced the production and respiration of the alga and also the chlorophyll pigments. Whereas the carbohydrate concentration has improved protein and lipid contents were adversely affected. The combination was found undesirable.

Comparison:

When the lead level was low in combination (0.05 Hg + 0.05 Pb), the positive effect of mercury was evident in reduced respiration and increased carbohydrate content. Total protein concentration was not affected by the presence of lead. The positive effect of lead was seen in increased production, increase in chlorophyll a. Pheophytin concentration was not affected by the presence of mercury.

The combination increased the carotenoid concentration of the species and decreased the lipid content.

When the lead concentration was increased in combination (0.05 Hg + 0.1 Pb), the rate of nett production increased than when either metal was employed alone. Fluctuation in respiration occurred to a lesser extent than was seen when Pb alone was employed. The influence of Pb prevailed on chlorophyll a and carbohydrate content.

The combination increased the concentration of chlorophyll b and carotenoids, and reduced the lipid and total protein content of the alga. Also biomass was lowered.

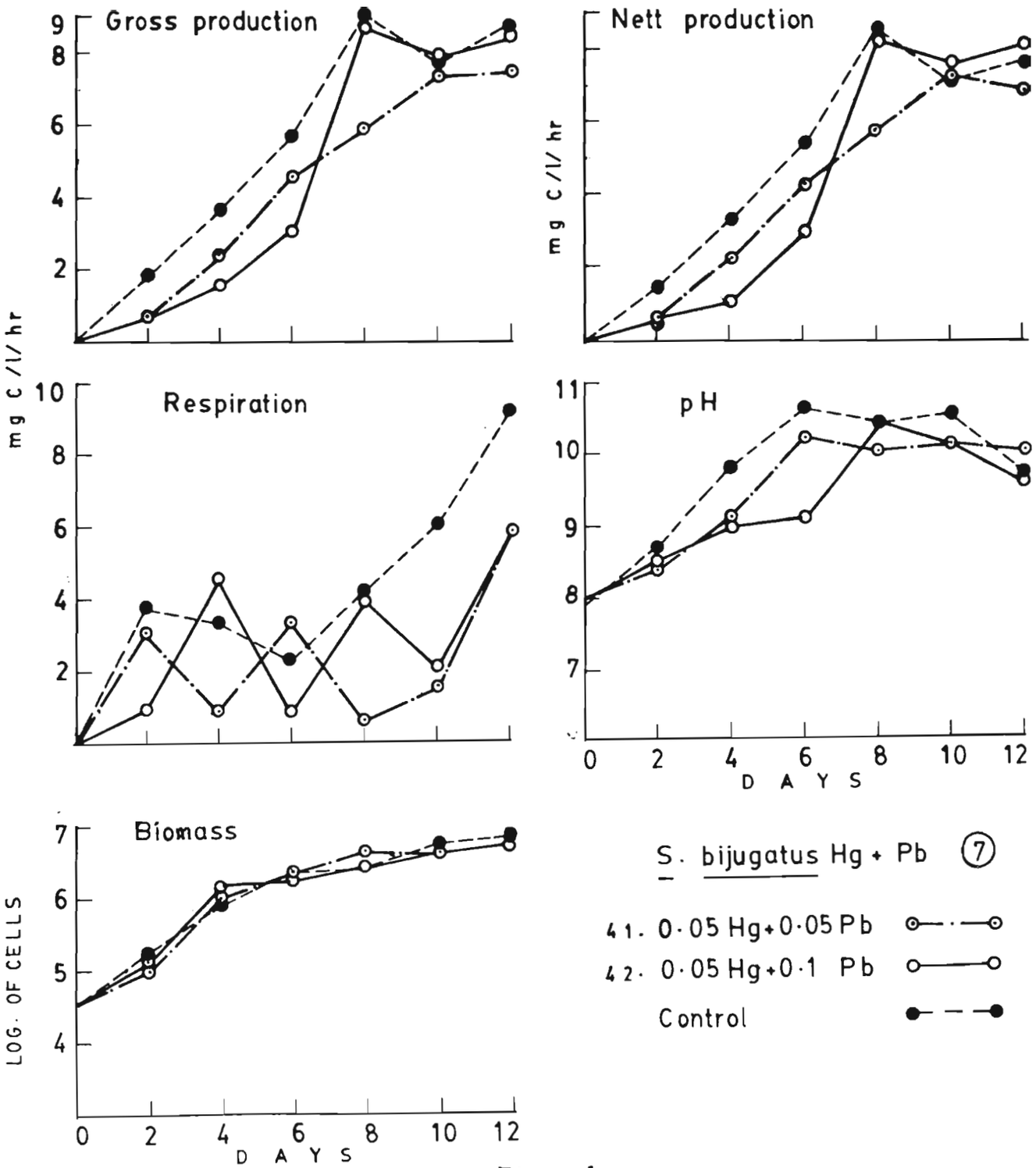

Gross production
Nett production
Respiration
pH
Biomass
mg C /l/ hr
LOG. OF CELLS
DAYS
S. bijugatus Hg + Pb (7)
41. 0·05 Hg+0·05 Pb
42. 0·05 Hg+0·1 Pb
Control

Fig. I

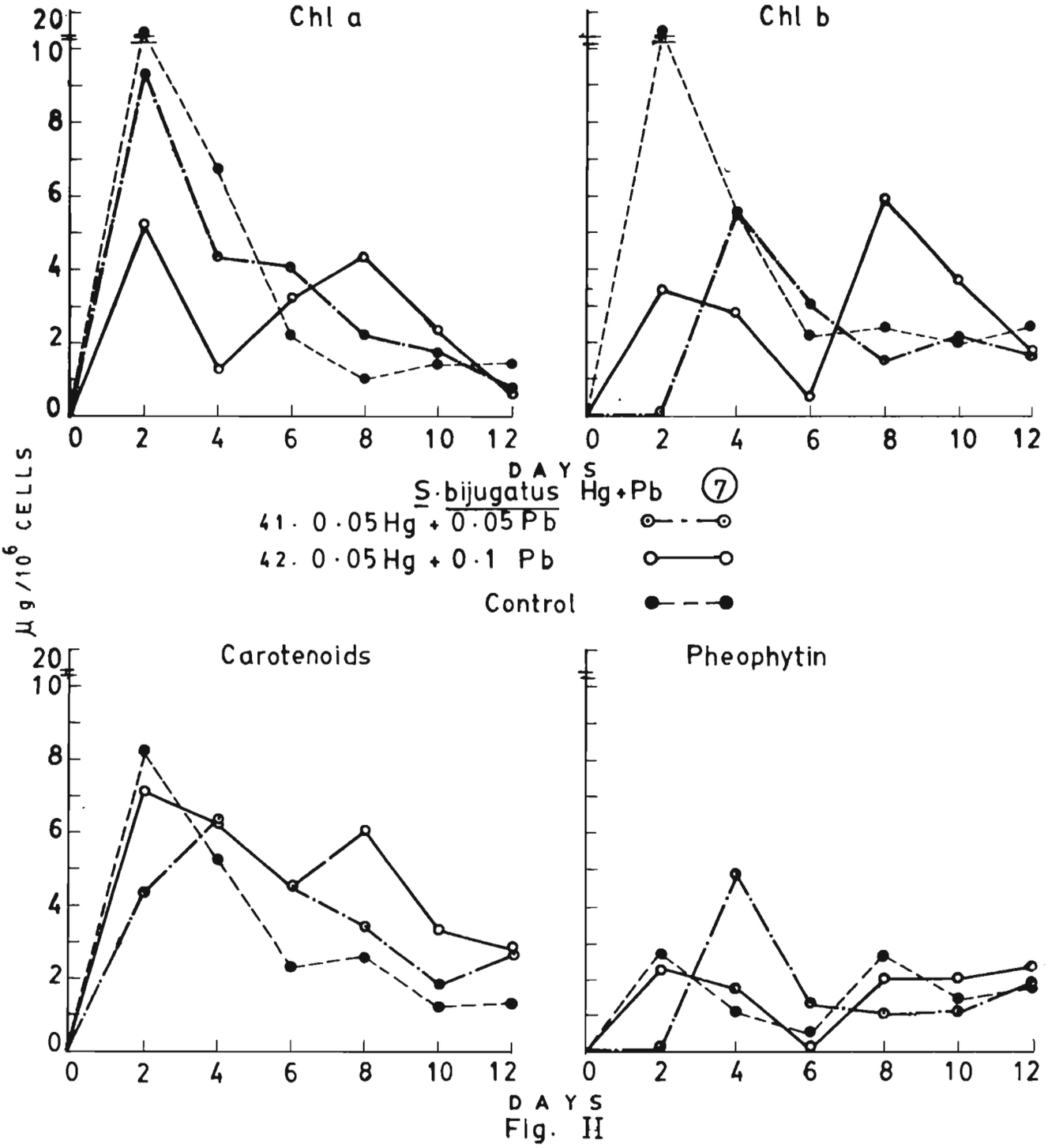
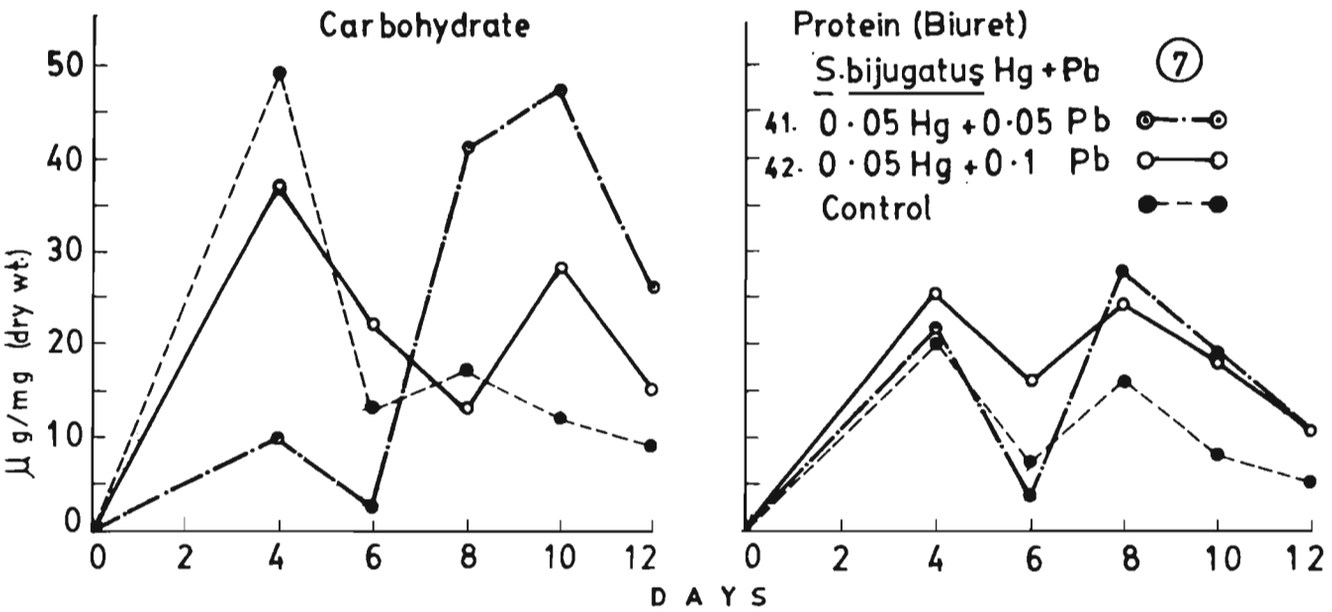

Chl a
Chl b
Carotenoids
Pheophytin
20
10
8
6
4
2
0
0 2 4 6 8 10 12
DAYS
μg/10⁶ CELLS
S. bijugatus Hg+Pb (7)
41. 0·05 Hg + 0·05 Pb
42. 0·05 Hg + 0·1 Pb
Control

Fig. II


Carbohydrate
Protein (Biuret)
S.bijugatus Hg + Pb
7
41. 0·05 Hg + 0·05 Pb
42. 0·05 Hg + 0·1 Pb
Control
µ g/mg (dry wt.)
50
40
30
20
10
0
0 2 4 6 8 10 12
DAYS

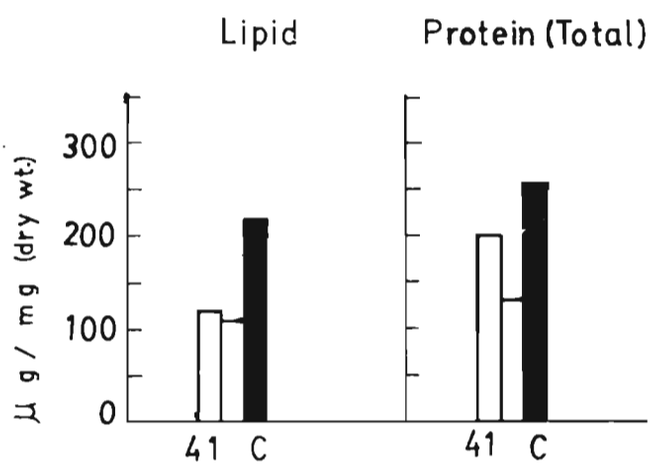

Lipid
Protein (Total)
µ g / mg (dry wt.)
300
200
100
0
41 C
41 C

Fig. III

Combined effect of mercury and lead on

N. palea

Sl. No. of combination	No. of the treatment	Concentration of metals (PPm)
(7)	43	0.005 Hg + 0.02 Pb
	44	0.005 Hg + 0.04 Pb

Production: (Fig. I)

Production of the diatom was adversely affected by the metals in combination. The overall production exhibited little variation between the treatments and was steady from fourth day onwards at 0.04 ppm lead. From 50% lower level on fourth day it increased to 5% lower level on eighth day in the first treatment, whereas in the second treatment little improvement was noted for 26% lower level to 35% lower level. Thereafter production declined in the former and very little difference was observed between the two treatments with the respective reduction of 74% and 64%, in relation to control.

Respiration of the alga in the two treatments was slightly higher than the control upto fourth day. From sixth day onwards it was equal in both the instances. On second day 100% increase was observed and it gradually declined to 60% lower level at the end of growth phase.

The pH of the culture in both the treatments was less than that of control throughout growth phase, but there was no significant difference between the treatments.

Pigments: (Fig. II)

Pigment content of the diatom was slightly more than that of control in both the treatments, at the end of growth phase. Concentration of all the pigments was minimum on fourth day in both the treatments. Chlorophyll a and carotenoids were considerably less than those of control during the middle phase of growth, and also exhibited fluctuation. Chlorophyll b and pheophytin of the treated diatom were less than that of control in early growth phase. Total pigment content of the treated diatom was slightly reduced when compared to that of control.

Photosynthetic end products: (Fig. III)

All the three carbohydrate fractions showed little variation between the two treatments but were considerably reduced than those of control.

The acid soluble carbohydrate fraction of the treated diatom was less than that of control except on fourth day in the first treatment. It was reduced by 83% and 74% respectively in the two treatments, at the end of growth phase.

The alkali soluble carbohydrate fraction of the diatom increased in the early phase when compared with that of control. It was not detected on sixth day in the second treatment. At the end of growth phase concentration of this fraction was low, reduced by 85% and 66% respectively.

Insoluble carbohydrate was the most affected product. In the first treatment from 40% higher level it declined to reach the minimum level on sixth day and increased once again to 55% lower level by eighth day. Towards the end of growth phase it was reduced further by 85% in relation to control. In the second treatment it was 20% less than control on fourth day but continued to increase to maximum on eighth day with 57% reduction and declined thereafter to 81% lower level at the end of growth phase.

The amount of each fraction at the end of growth phase in the treated diatom was nearly equal and was quite low when compared with that of control.

The protein content of the diatom was also affected adversely by the metals. It fluctuated with two peaks on fourth and eighth day in the first treatment, but found to be gradually increasing till eighth day in the other. 221% and 105% increase was found on fourth day respectively. Despite continued increase it was found

to be 35% and 39% less than control by eighth day and decreased further to 74% and 68% lower level by the end of growth phase.

The lipid content of the diatom also decreased when exposed to the metals in combination by 13%. and 19% respectively in relation to control.

All three end products were adversely affected by the metals.

Growth: (Fig. I)

The growth rate did not vary much between any treatment and control, except towards the end of growth phase. The final yield was slightly reduced. Between second and sixth day of growth, biomass was slightly higher than that of control in the second treatment.

Nutrient uptake of the diatom in the treatments is given below:

No. of the treatment	Nutrients absorbed (µg/l)		N / P
	Phosphate	Nitrate	
43	1475	743	0.50
44	1520	755	0.50
Control	1350	763	1.77

Phosphate uptake increased in the treated diatom whereas nitrate uptake was lowered, when compared to that

of control. Between the treatments, the diatom in the latter has taken up both the nutrients to greater extent. The N / P ratio was reduced in the treatments.

Conclusion:

The adverse effect of the metals was evident in the inhibition of production, pigments, biomass, carbohydrate, protein and lipid. The physiological efficiency of the diatom was generally reduced.

Comparison:

Effect of mercury prevailed on respiration and first and second fractions of carbohydrate whereas that of lead on production and third fraction of carbohydrates.

The combination has reduced the protein, lipid and biomass when compared to the effect of either metal on these parameters.

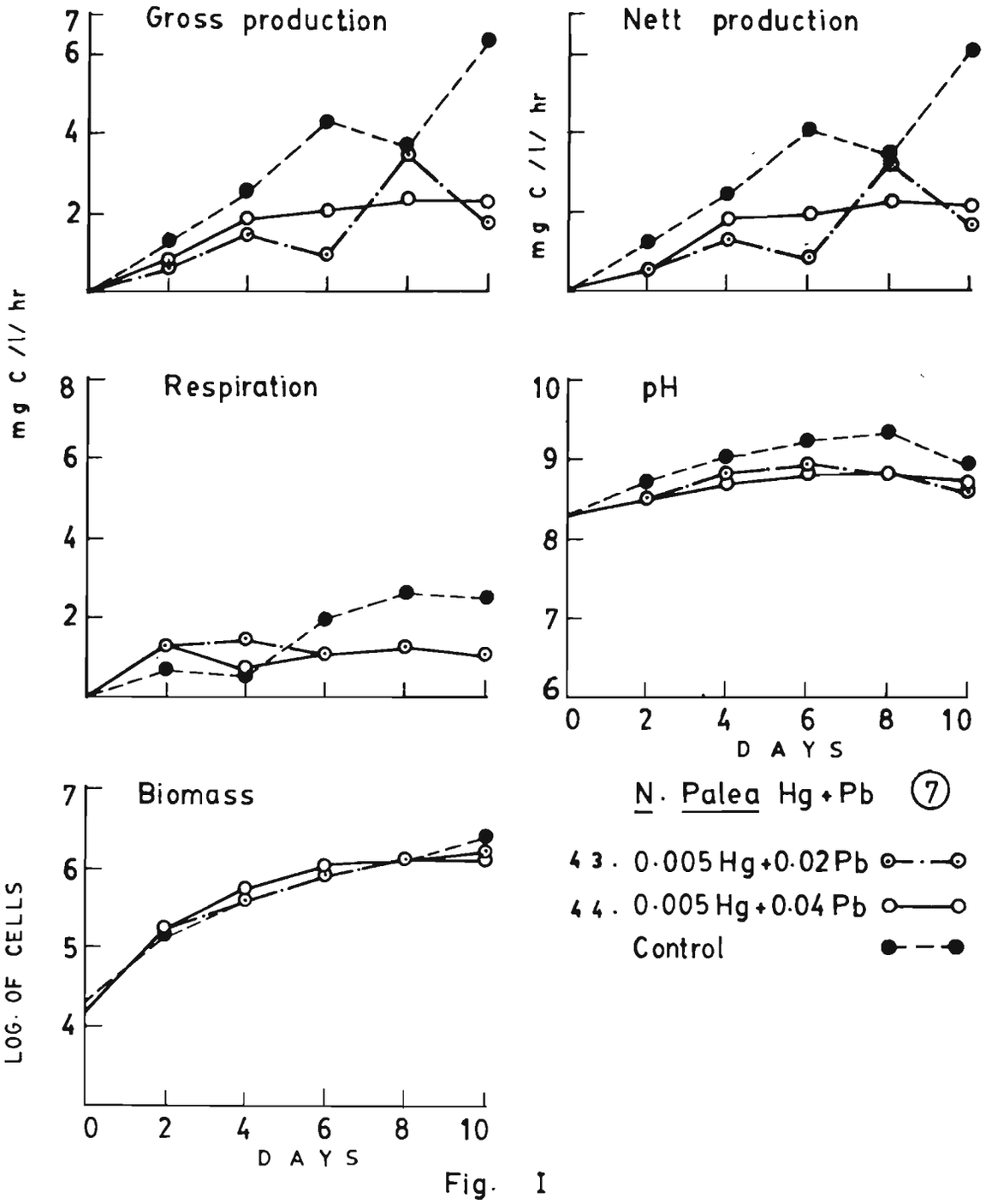

Gross production
Nett production
Respiration
pH
Biomass
mg C /l/ hr
LOG. OF CELLS
DAYS
N. Palea Hg + Pb (7)
43. 0·005 Hg + 0·02 Pb
44. 0·005 Hg + 0·04 Pb
Control

Fig. I

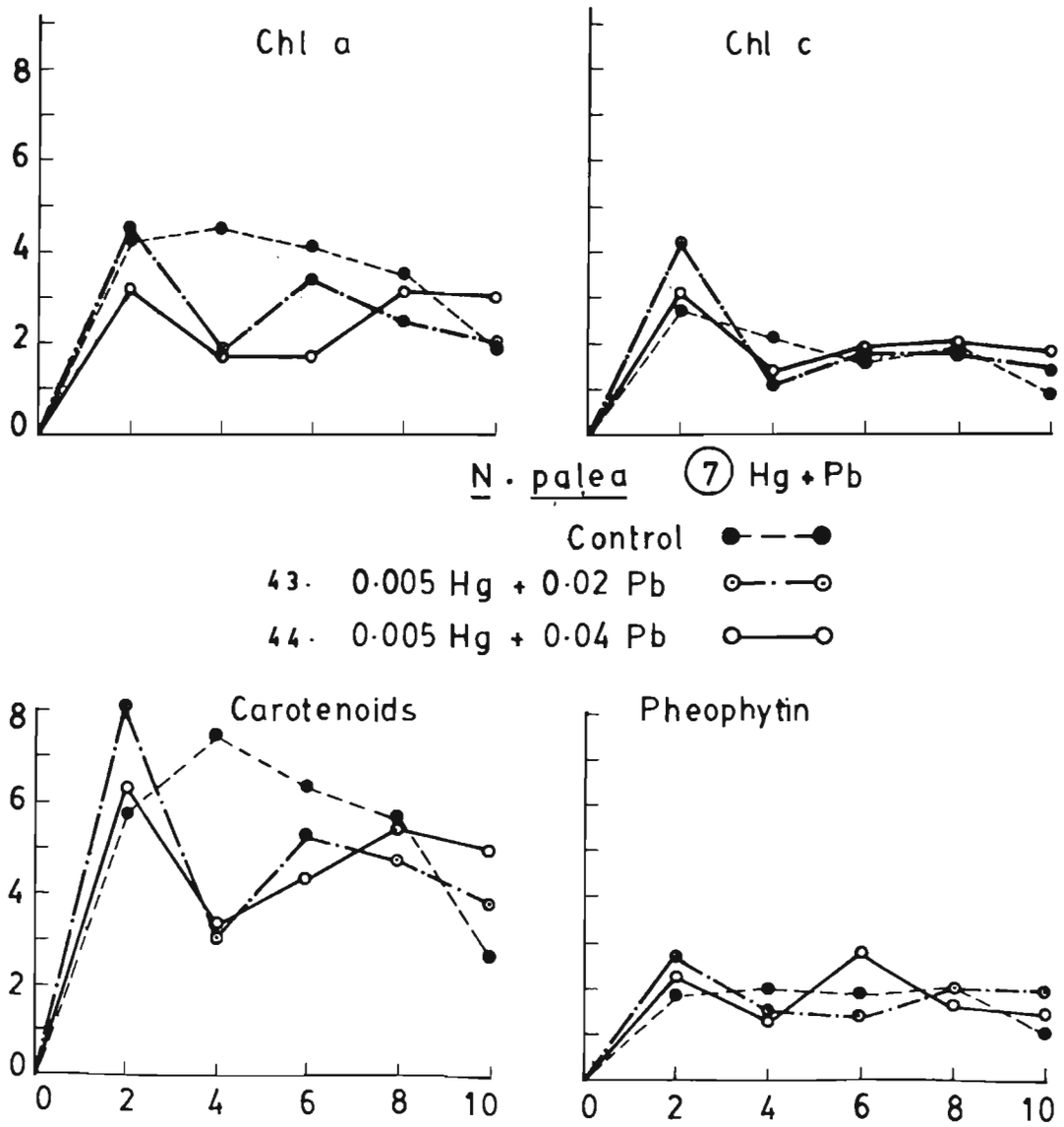

Chl a
Chl c
8
6
4
2
0
N. palea (7) Hg + Pb
Control
43. 0·005 Hg + 0·02 Pb
44. 0·005 Hg + 0·04 Pb
Carotenoids
Pheophytin
0 2 4 6 8 10
DAYS

Fig. II

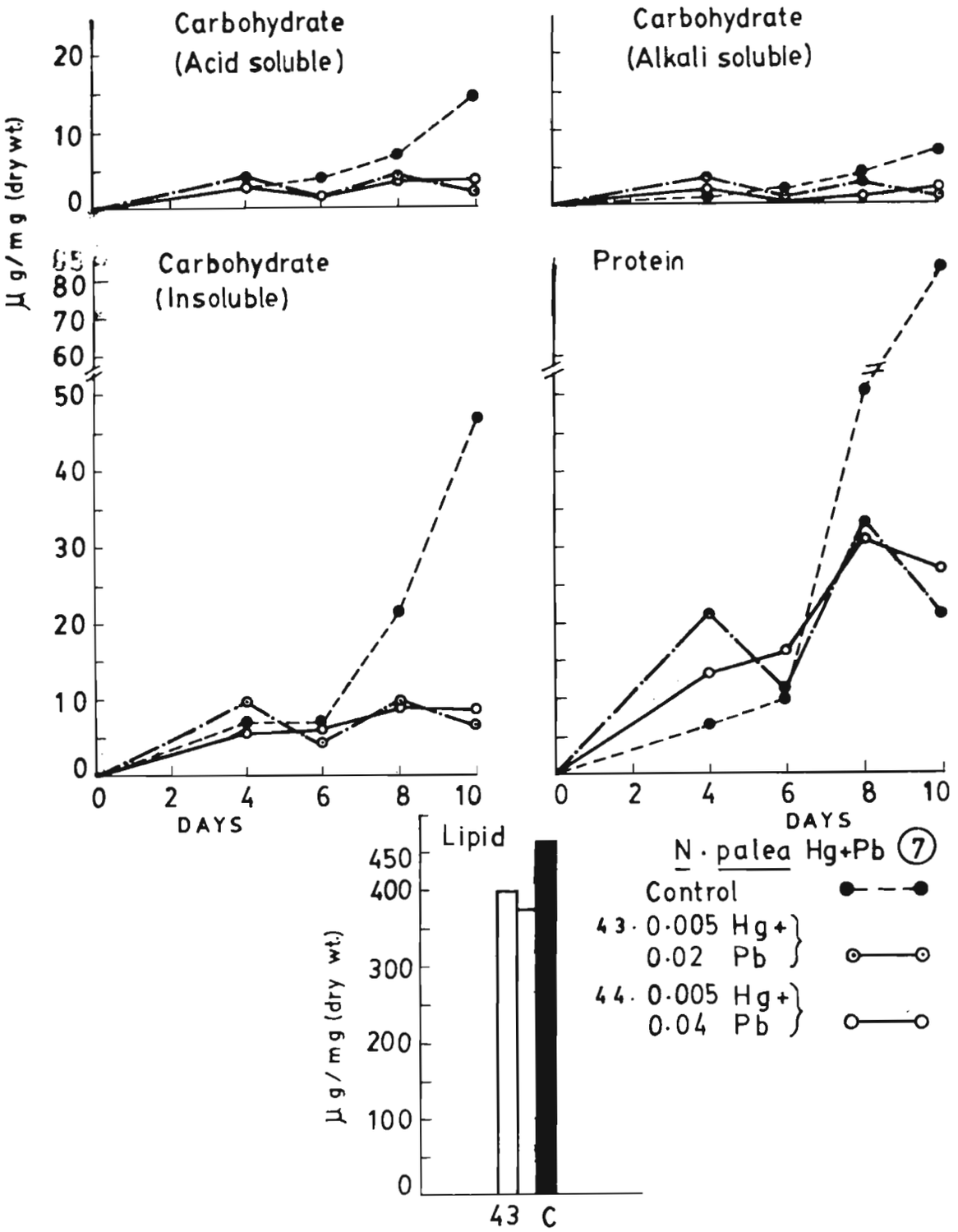

Carbohydrate
(Acid soluble)
Carbohydrate
(Alkali soluble)
Carbohydrate
(Insoluble)
Protein
µg/mg (dry wt.)
DAYS
Lipid
µg/mg (dry wt.)
43
C
N. palea Hg+Pb (7)
Control
43. 0·005 Hg+ 0·02 Pb
44. 0·005 Hg+ 0·04 Pb

Fig. III

6.1.3

Combined effect of mercury and copper ion

S. bijugatus

S. No. of combination	No. of treatment	Concentration of metals (in ppm)
(8)	45	0.03 Hg + 0.01 Cu
	46	0.03 Hg + 0.05 Cu
	47	0.03 Hg + 0.1 Cu

Production: (Fig. I)

Nett production of the alga was adversely affected in the early stages of growth and was negligible on second day in first and second treatments. It was less than that of control throughout in the second treatment. Gradual increase was noted from second to eighth day in both instances but it was 6% more in the first treatment whereas 8% less in the second treatment. Production then declined in the first treatment to 29% lower level on twelfth day. Inspite of continued increase it was 8% less in the second treatment on the last day. In the third treatment production was enhanced from second day onwards and it was 42% and 4% higher respectively on fourth and sixth day, in relation to control but fell 8% short of control by eighth day inspite of continued increase. Production remained steady thereafter and was 2% higher than that of control on the last day.

Respiration of the alga fluctuated in all treatments with two peaks. It was less than that of control throughout in the second treatment. In the first treatment it fluctuated with two peaks on fourth and eighth day of growth with 64% and 52% increase respectively in relation to control. In the second treatment it showed two peaks, on fourth and tenth day with 12% and 54% reduction respectively in relation to control. In the third treatment the respiration was considerably reduced upto fourth day but increased thereafter to 187% higher level by sixth day. It showed a second peak on tenth day with 43% reduction. Towards the end of growth phase respiration increased in the first treatment but remained 47% less than that of control. In the other two treatments it decreased to 81% and 60% lower level respectively at the end of growth phase.

Considerable variation in the pH was not observed between any two treatments except at the end of growth phase. Inspite of higher rate of production, the pH of the culture in the third treatment remained less than that of control throughout, whereas in the other two treatments it was slightly higher only at the end of growth phase. In no treatment maximum level of pH attained by the control (10.64) was reached.

Pigments: (Fig. II)

Total pigment content of the alga was not correlated with the concentration of metals. Chlorophyll a, chlorophyll b and pheophytin were not detected on second day in the first and second treatments. But these pigments were developed to greater extent in the alga in second treatment by the end of growth phase. Highest concentration of all pigments was achieved by the alga in third treatment on sixth day.

In the first treatment the concentration of chlorophyll b was minimum and was less than that of control throughout. Chlorophyll a, chlorophyll b and carotenoids exhibited an increasing tendency towards the end of growth phase in the second treatment. Concentration of carotenoids was higher than that of control in all treatments but that of pheophytin was less, in relation to control at the end of growth phase. Total pheophytin increased with increase in copper concentration.

Photosynthetic end products: (Fig. III)

The carbohydrate concentration of the alga fluctuated during the growth phase in the first and third treatments. In the first and second treatments it was low, reduced by 91% and 76% respectively on fourth day, whereas in the third treatment it was highest with 24%

increase. The carbohydrate concentration declined thereafter in all treatments. A second peak was observed on tenth day with an increase of 241% and 64% in the first and third treatments. In the second treatment the concentration remained much lower to that of control throughout growth phase with a reduction of 48% and 22% respectively on eighth day and twelfth day. In the first and third treatments, the alga showed 141% and 64% increase at the end of growth phase.

Protein content of the alga was reduced by 2%, 7% and 23% respectively in the first, second and third treatments.

Lipid content of the alga was reduced by 15%, ... and 38% respectively in the first, and third treatments, and increased by 82% in the second.

Growth: (Fig. I)

Growth of the alga was retarded by the metals in combination and the biomass was reduced towards the end the growth phase, the reduction being more in the third treatment. The growth rate was stimulated initially between fourth and sixth day in the first, between second and fourth day in second treatment and third treatments.

Details of the nutrient uptake are given in the following table.

No. of treatment	Nutrients absorbed by the alga (µg/l)		N / P
	Phosphate	Nitrate	
45	2030	1828	0.90
46	2215	5030	2.27
47	1686	1794	1.06
Control	945	1290	1.37

The treated alga absorbed both the nutrients to a greater extent than that of control. The uptake was maximum in the second treatment. Unlike in control, more phosphate was absorbed by the treated alga than nitrate. The N / P ratio varied considerably between the treatments.

Conclusion:

Nett production of the alga closely followed that of control in the second treatment and respiration was reduced. Only slight reduction in protein was observed whereas lipid increased. Combination 0.03 Hg + 0.05 Cu seems to be the limit for the alga with minimum adverse effect.

Comparison:

The influence of mercury prevailed when level of copper was lowest (0.01 ppm). As the level of copper increased, its influence was evident. At the lowest level of copper, protein was not affected but lipid was slightly reduced. At 0.05 ppm level, copper did not affect the protein but increased the lipids. At 0.1 ppm copper affected both protein and carbohydrate.

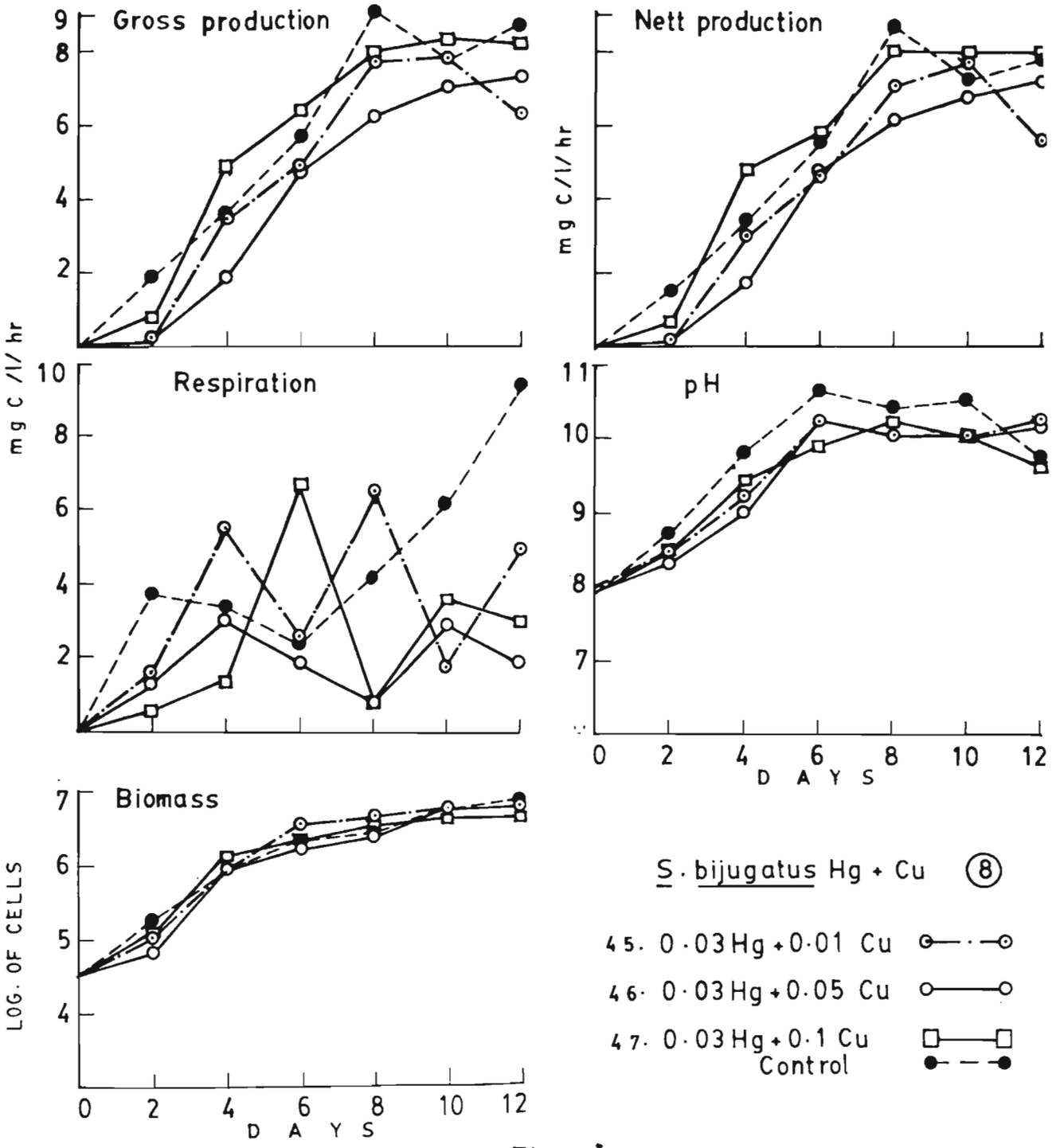

Gross production
Nett production
Respiration
pH
Biomass
mg C /l/ hr
LOG. OF CELLS
D A Y S
S. bijugatus Hg + Cu (8)
45. 0·03 Hg + 0·01 Cu
46. 0·03 Hg + 0·05 Cu
47. 0·03 Hg + 0·1 Cu
Control

Fig. I

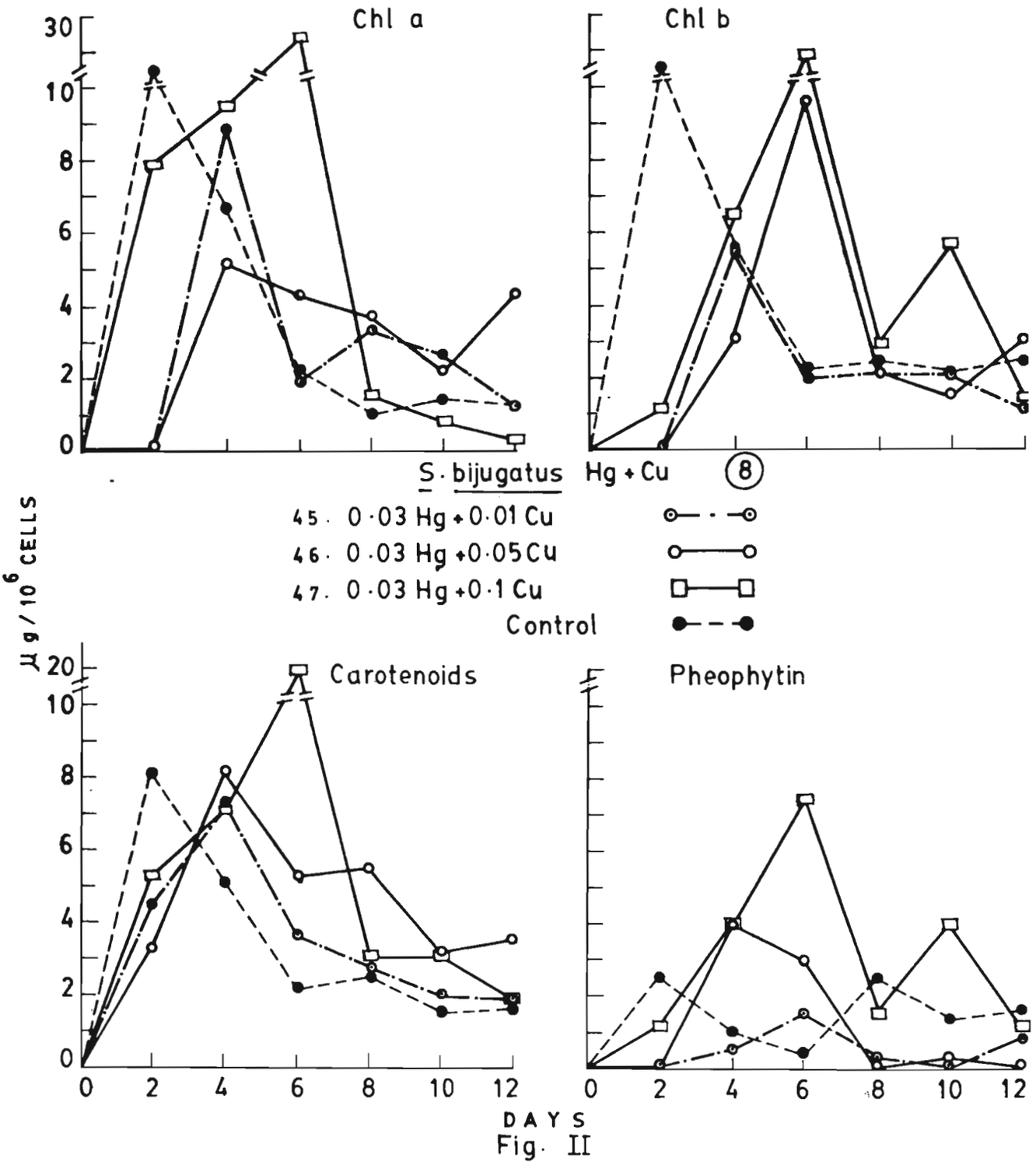
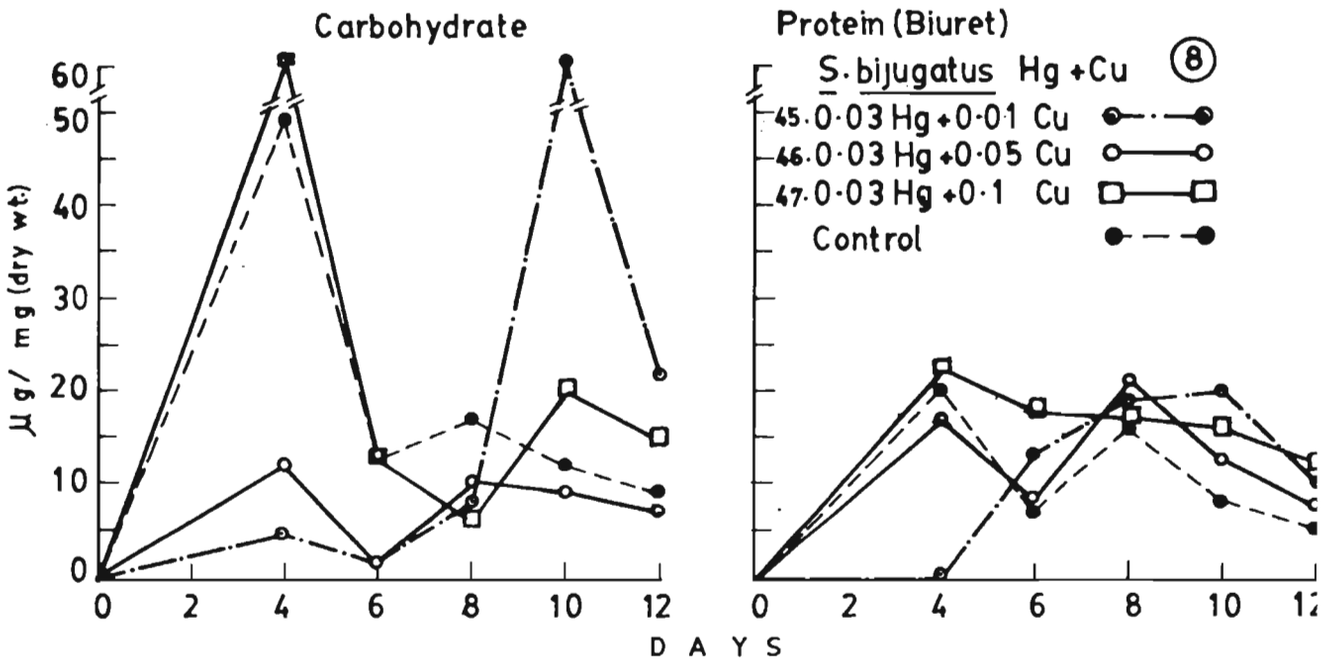

Chl a
Chl b
S. bijugatus Hg + Cu (8)
45. 0·03 Hg + 0·01 Cu
46. 0·03 Hg + 0·05 Cu
47. 0·03 Hg + 0·1 Cu
Control
Carotenoids
Pheophytin
µg / 10^6 CELLS
DAYS

Fig. II


Carbohydrate
Protein (Biuret)
S. bijugatus Hg +Cu
45. 0·03 Hg +0·01 Cu
46. 0·03 Hg +0·05 Cu
47. 0·03 Hg +0·1 Cu
Control
µ g/ mg (dry wt.)
0
10
20
30
40
50
60
0 2 4 6 8 10 12
D A Y S

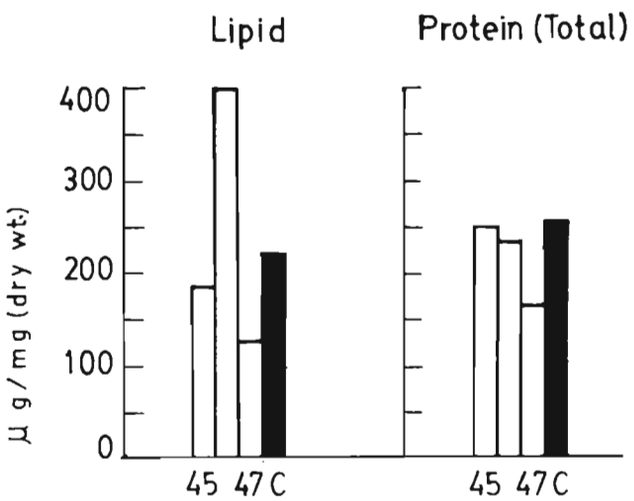

Lipid
Protein (Total)
µ g/mg (dry wt.)
0
100
200
300
400
45 47C
45 47C

Fig. III

Combined effect of mercury and copper on

N. palea

Sl. No. of combination	No. of treatment	Concentration of metals (ppm)
(8)	48	0.005 Hg + 0.01 Cu
	49	0.005 Hg + 0.05 Cu

Production: (Fig. I)

The nett production of the diatom was considerably reduced and was less than that of control throughout growth phase in both the treatments. Production was slightly higher in the first treatment compared to the other. It was 52% and 80% less respectively on sixth day in relation to control, in the two treatments. Between sixth and eighth day no considerable change occurred. From eighth day onwards production increased in both treatments but remained 38% less than that of controlon the last day.

Respiration of the diatom was higher than that of control in the early phase. It increased to a greater extent in the first treatment. Respiration was 600% higher in the first treatment on second day but in the second treatment the oxygen values in the dark bottle were higher than the initial bottle. It gradually decrease in the first treatment and increased in the second treatment to be 24% higher on sixth day. There after it

remained steady in the first treatment and was 4% less on the last day in relation to control. It declined and was negligible in the second treatment on eighth day but increased to 4% lower level by the end of growth phase.

Owing to the low production, pH of the culture in both the treatments was less than that of control and did not vary much in the two treatments except at the end. Sharp decline was noted towards the end of growth phase in the first treatment.

Pigments: (Fig. II)

Total pigment content increased when the diatom was exposed to the metals in combination. With the exception of chlorophyll a in the first treatment which remained less than that of control throughout growth phase, all pigments increased, to a greater extent in the second treatment. But at the end of growth phase the concentration of any pigment did not vary much between the two treatments and between any treatment and control.

Photosynthetic end products: (Fig. III)

The acid soluble carbohydrate fraction in the treated diatom was found to 137% and 283% more on fourth day in relation to control. It declined gradually thereafter till eighth day to 79% and 57% lower level respectively. It increased once again but was found to be 51% and 38% less than that of control on the last day.

The alkali soluble carbohydrate fraction did not vary greatly from that of control. It was slightly higher in the early phase. It was not detected from eighth day onwards in second treatment. Whereas in the first treatment from a negligible level on eighth day it increased to 81% lower level on tenth day.

The concentration of insoluble carbohydrate fraction in both the treatments was equal throughout the growth phase. It did not show much variation from that of control in the early growth phase and was reduced by 75% in relation to control on the last day.

Protein content of the diatom showed considerable increase in the early growth phase. It was 176% and 304% higher on fourth day in the two treatments respectively. It declined thereafter and was steady between sixth and eighth day. Eventhough it increased towards the end of growth phase, was found to be 49% and 68% less than that of control respectively on the last day.

The metals in combination affected the lipid content of the diatom adversely reducing it by 72% and 33% respectively, in the two treatments.

Growth: (Fig. I)

Lag in growth was noted between second and fourth day in both treatments. The biomass remained less than that of control in the two treatments.

Details of the nutrient uptake are given in the following table.

No. of treatment	Nutrients absorbed (ug/l)		N / P
	Phosphate	Nitrate	
48	1735	706	0.41
49	1725	700	0.40
Control	1350	763	0.57

Not much variation was observed between the two treatments in nutrient uptake. When compared with that of control, phosphate uptake increased by 28% but nitrate uptake was decreased by few ug. The N : P ratio varied very little between the treatments and was less than that of control.

Conclusion:

The toxic effect of the metals was evident in all the parameters studied. Both biomass and end products were lowered.

Comparison:

The common individual effect of both the metals prevailed on production on respiration. Production was lowered and respiration was elevated. Further reduction in chlorophyll a was observed than when mercury alone was

employed whereas other pigments increased. But when compared with the effect of copper, all pigments except pheophytin were found reduced. Influence of mercury on carbohydrate was evident. Protein was improved when compared with that of copper alone. Lipid was further inhibited by the metals in combination when copper level was 0.01 ppm, but increased when 0.05 ppm copper was employed. The growth rate and biomass both were lowered when compared to the effect of either metal.

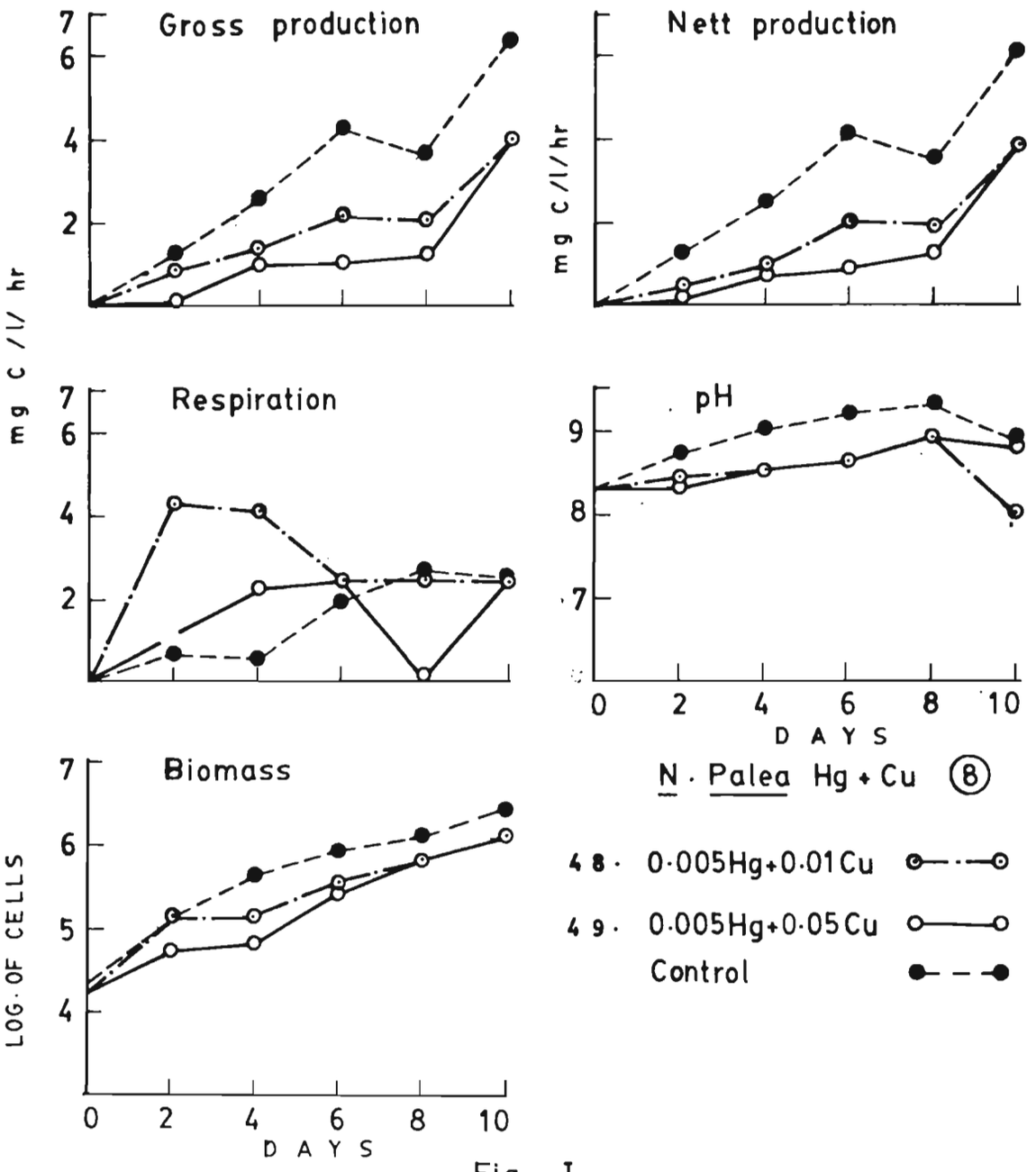

Gross production
Nett production
Respiration
pH
Biomass
mg C /l/ hr
LOG. OF CELLS
DAYS
N. Palea Hg + Cu (8)
48. 0·005Hg+0·01Cu
49. 0·005Hg+0·05Cu
Control

Fig. I

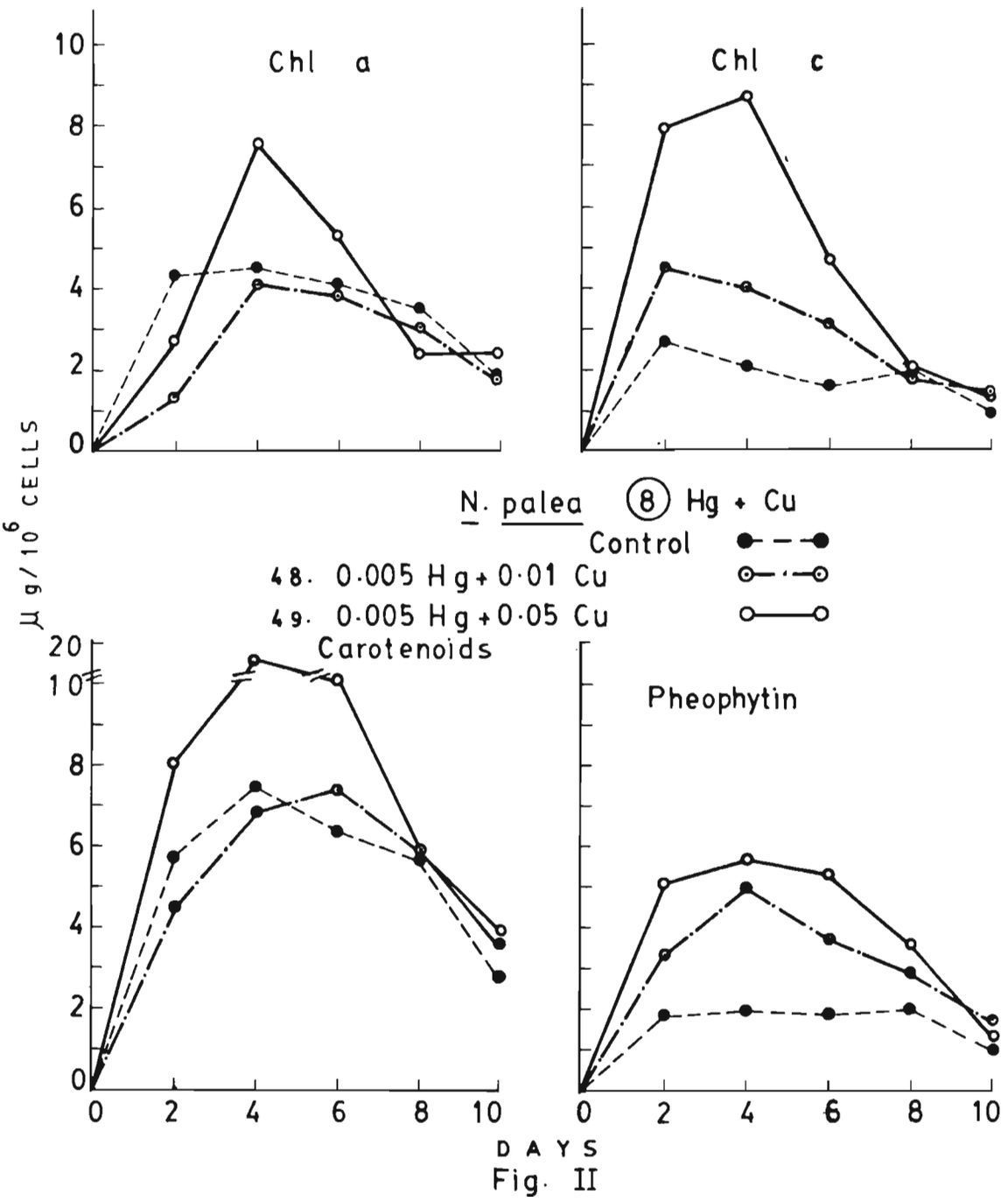

Chl a
Chl c
N. palea
(8) Hg + Cu
Control
48. 0·005 Hg + 0·01 Cu
49. 0·005 Hg + 0·05 Cu
Carotenoids
Pheophytin
μ g / 10^6 CELLS
DAYS

Fig. II

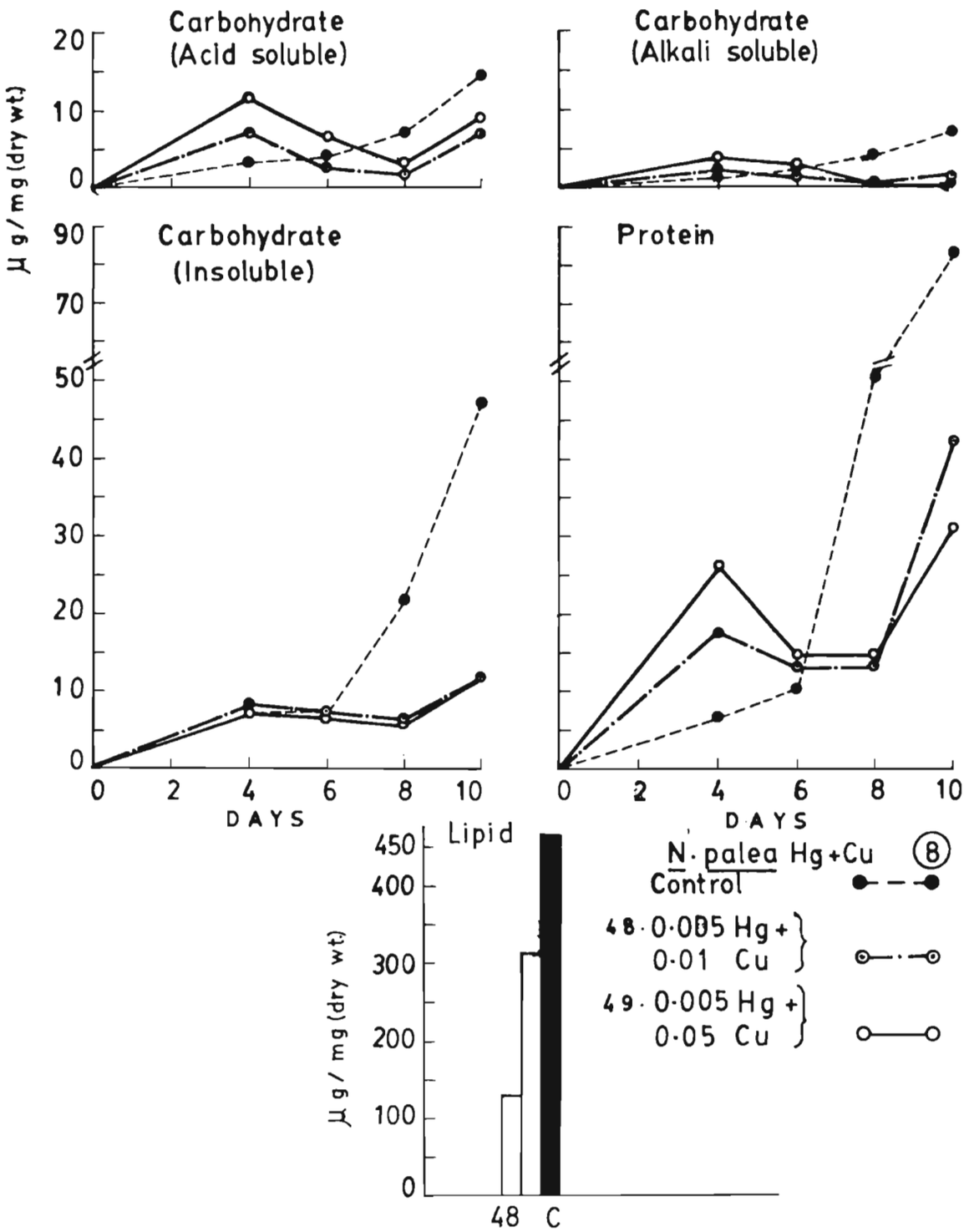

Carbohydrate
(Acid soluble)
Carbohydrate
(Alkali soluble)
Carbohydrate
(Insoluble)
Protein
µg/mg (dry wt.)
DAYS
Lipid
N. palea Hg+Cu (8)
Control
48 · 0·005 Hg + 0·01 Cu
49 · 0·005 Hg + 0·05 Cu
48
C

Fig. III

6.1.4

Combined effect of mercury and zinc on

S. bituqatus

Sl. No. of combination	No. of treatment	Concentration of metals (in ppm)
(9)	50	0.05 Hg + 0.05 Zn
	51	0.05 Hg + 0.1 Zn

Production: (Fig. I)

Production of the alga increased steadily till the end of the growth phase but remained less than that of control except on sixth day in the second treatment. In both the treatments production was reduced by 28% and 33% in relation to control on eighth day and by 3% and 2% on twelfth day respectively.

The respiration of the alga was generally reduced, to a greater extent in the first treatment, and remained less than that of control throughout growth phase. In both the treatments respiration fluctuated with two peaks on fourth and eighth day. On fourth day it was 64% and 12% less and on eighth day 50% and 5% less respectively, in relation to control. In both instances it decreased till tenth day and increased once again till twelfth day. The reduction was 60% and 31% respectively on the last day.

pH of the culture showed marginal variation between the two treatments. Except on the last day it remained less than that of control.

Pigments: (Fig. II)

Total pigment content of the alga did not vary much between the two treatments and also between any treatment and control. The maxima were attained at a latter stage, on fourth day by the treated alga whereas in control they occurred on second day. Concentration of chlorophyll a and pheophytin was negligible on fourth day in both the treatments. Also chlorophyll b was not detected on second day in the first treatment and on last day in the second treatment. Carotenoids and pheophytin were found in greater concentration in the first treatment. Chlorophyll a and carotenoids were more and chlorophyll b and pheophytin were less, in the treated alga, at the end of growth phase.

Photosynthetic end products: (Fig. III)

The carbohydrate concentration in the alga varied to a very large extent in the two treatments. In both treatments maximum concentration was reached on tenth day. On fourth day it was 24% less in the first treatment and 77% less in the second treatment. Thereafter

it dropped in both treatments and increased once again from sixth day onwards till tenth day and was 1056% higher in the first treatment and 15% higher in the second treatment. It declined thereafter in both the instances but remained 908% higher in the former and 34% less in the latter treatment.

Protein content of the alga did not vary much between the two treatments and between any treatment and control. It showed 12% increase in the first treatment and was equal in the second treatment when compared to that of control.

Lipid content of the alga improved when exposed to the metals in combination. When the zinc level was low the lipid increased by 39% and when high, by 124% in relation to control.

Growth: (Fig. I)

Growth of the alga was stimulated between second and fourth day in both the treatments. The biomass was more than that of control from fourth to eighth day. The final yield was slightly reduced.

Details of the nutrient uptake are given in the following table.

No. of treatment	Nutrients absorbed by the alga (µg/l)		N / P
	Phosphate	Nitrate	
50	2365	2070	0.88
51	2356	2106	0.89
Control	945	1290	1.37

The nutrient uptake did not vary much between the two treatments. The uptake increased when compared with that of control. Unlike in control, more phosphate was taken up by the alga than nitrate.

Conclusion:

Increase in the zinc level helped the alga to produce more lipid while the protein remained unaffected. The combination was not toxic to the alga.

Comparison:

When the present effect was compared with those obtained by employing the metals individually, the nett production was found to improve. The high respiration, as seen when zinc alone was employed, was reduced. The influence of zinc prevailed on pigments which remained at a higher level. Carbohydrate and protein improved when the metals were combined. Toxicity of mercury was reduced in presence of zinc.

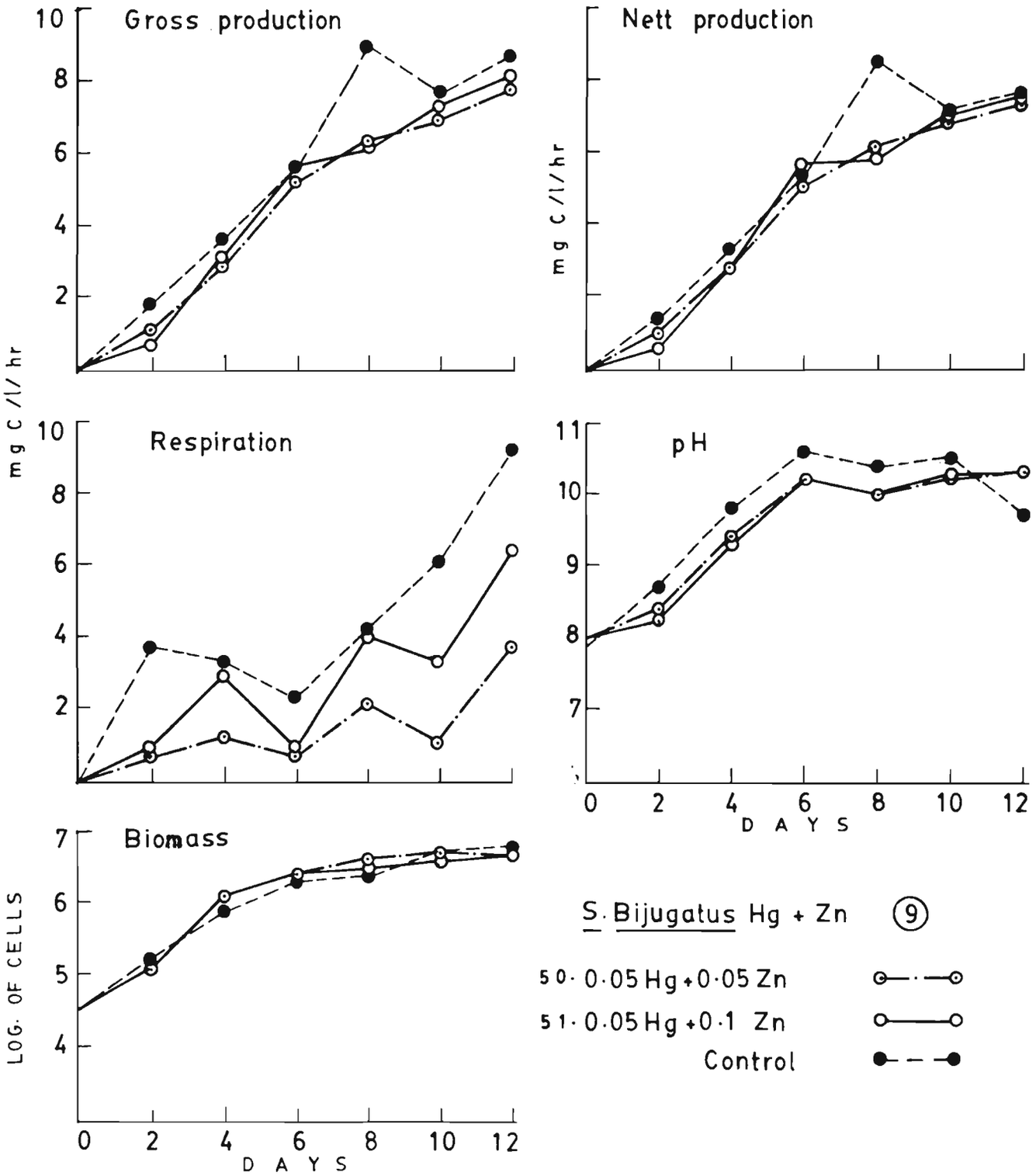

Gross production
Nett production
Respiration
pH
Biomass
mg C /l/ hr
LOG. OF CELLS
D A Y S
S. Bijugatus Hg + Zn (9)
50. 0.05 Hg + 0.05 Zn
51. 0.05 Hg + 0.1 Zn
Control

Fig. I

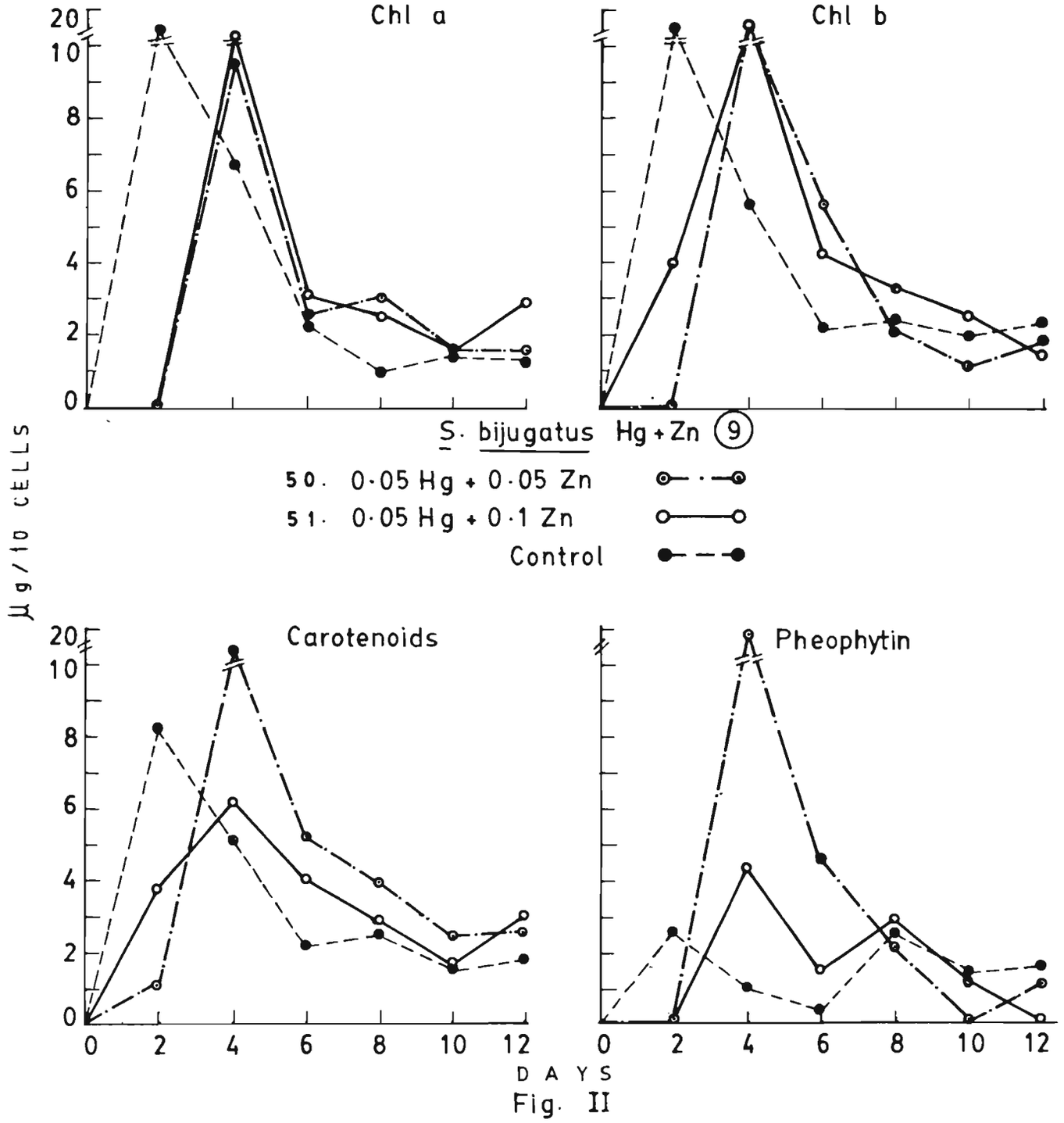

Chl a
Chl b
Carotenoids
Pheophytin
S. bijugatus Hg + Zn (9)
50. 0·05 Hg + 0·05 Zn
51. 0·05 Hg + 0·1 Zn
Control
µg / 10 CELLS
DAYS
0
2
4
6
8
10
12
20

Fig. II

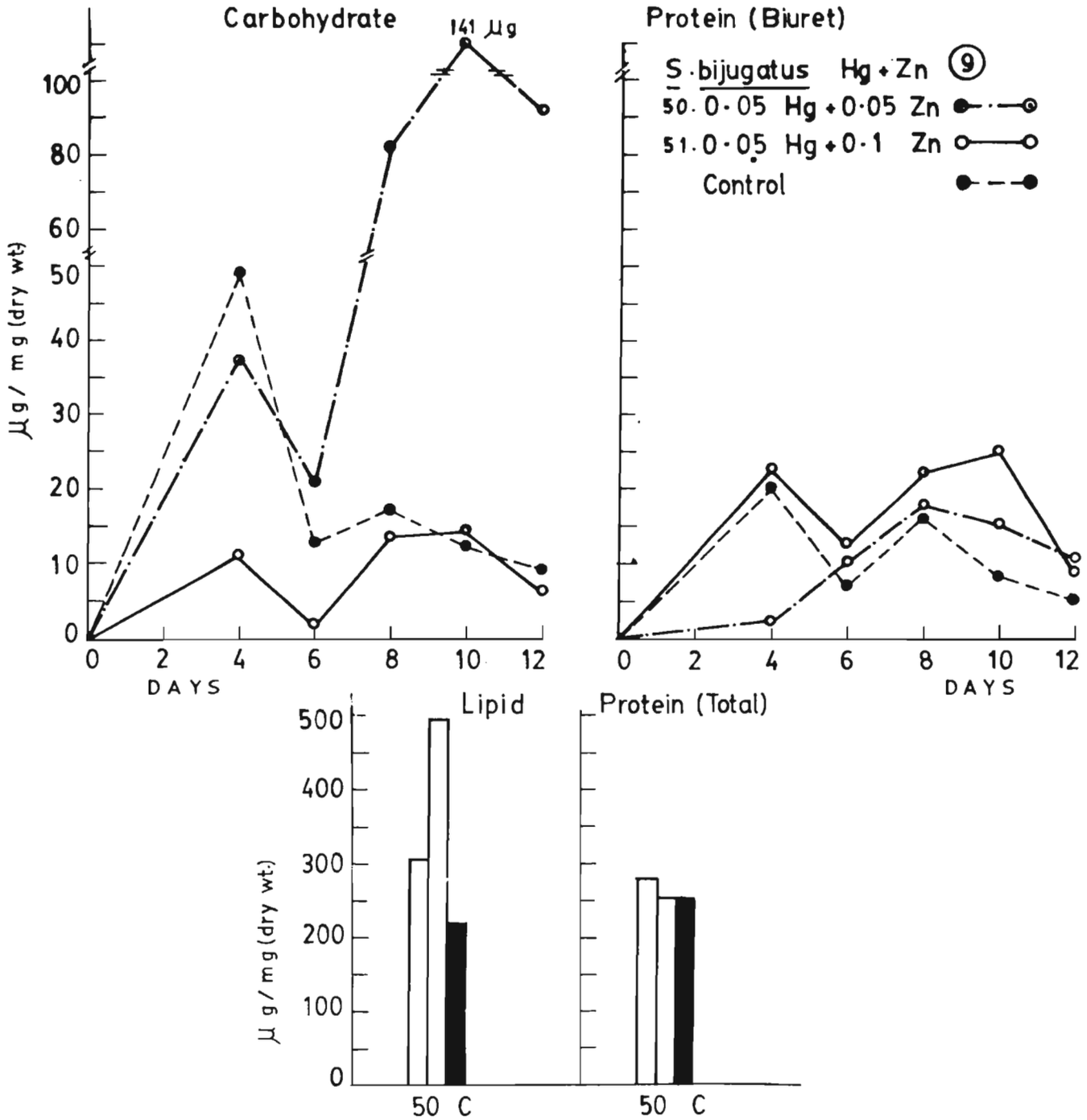

Carbohydrate
141 µg
µg / mg (dry wt)
DAYS
Protein (Biuret)
S. bijugatus Hg + Zn
50. 0·05 Hg + 0·05 Zn
51. 0·05 Hg + 0·1 Zn
Control
DAYS
Lipid
Protein (Total)
µg / mg (dry wt)
50 C
50 C

Fig. III

Combined effect of mercury and zinc on

N. palea

Sl. No. of combination	No. of treatment	Concentration of metals (ppm)
(9)	52	0.005 Hg + 0.05 Zn
	53	0.005 Hg + 0.08 Zn

Production: (Fig. I)

The production of the diatom was reduced in both the treatments. The oxygen values of the light bottle were less than the initial bottle on second day in the first treatment. Nett production of the diatom increased gradually till eighth day and quickly thereafter but was found to be 6% and 41% less in relation to control on the last day.

The respiration of the alga was higher than the control and was maximum on second day by 517% in the first treatment. It declined thereafter and was less than that of control from sixth day onwards. On eighth day the oxygen value of the dark bottle exceeded that of the initial. By tenth day the respiration was 68% less than that of control. In the second treatment the respiration was 133% more on second day. But the oxygen values in the dark bottle were higher than the initial values on fourth

and sixth day. Respiration was 8% higher by tenth day in relation to control.

Despite the variation in production, pH of the culture did not differ between the two treatments and remained less than that of control except on the last day. In neither treatment it reached the maximum level (9.27) attained by the control.

Pigments: (Fig. II)

Total pigment content of the alga increased in presence of the metals. Chlorophyll a, except on eighth day in the first treatment, remained less than that of control. Concentration of chlorophyll b was higher throughout growth phase, except on second day in first treatment. Carotenoids were generally higher in latter phase in the treatments. Pheophytin was also higher except on eighth day in first treatment. Considerable fluctuation was observed in the level of these pigments during growth phase. But the concentration of the pigments did not vary much between the two treatments and between any treatment and control at the end of growth phase.

Photosynthetic end products: (Fig. III)

The acid soluble carbohydrate fraction of the diatom was slightly more in both the treatments when compared to that of control in the early phase. But it was

found to be less than that of control from sixth day onwards. Not much variation was observed between the treatments in the level of this product. By tenth day it was 34% and 39% less than that of control respectively.

Alkali soluble carbohydrate fraction did not vary much between the two treatments and between any treatment and control. It was not detected on eighth day in the first treatment. It was less than that of control from sixth day in both the treatments. On the last day the reduction was found to be 64% and 79% respectively in relation to control.

The insoluble carbohydrate fraction was less than that of control in the early phase in the first treatment. But on sixth day it was 9% and 67% more than that of control respectively. By tenth day the concentration dropped to 74% and 76% lower level on the last day.

The protein concentration of the diatom improved in the early phase and on fourth day was 160% and 283% higher than that of control in the two treatments respectively. It declined in both the treatments thereafter and was equal on eighth day but was less than that of control. Eventhough the concentration increased further it was found to be 59% and 51% less on the last day in relation to control.

Lipid was the most affected product of the diatom. When the zinc level was low (0.05 ppm) it was reduced by 43% and when high (0.08 ppm) increased by 35%.

Growth: (Fig. I)

Growth was stimulated in both the treatments initially. In the first treatment lag was observed between second and fourth day. Inspite of gradual and continued increase, the biomass in both the treatments remained less than that of control throughout growth phase.

Details of the nutrient uptake are given below.

Selected concentration of metal	Nutrients absorbed by the alga (µg/l)		N / P
	Phosphate	Nitrate	
52	1815	865	0.48
53	1775	715	0.40
Control	1350	763	0.57

The diatom, when exposed to the metals in combination, absorbed more phosphate than that of control. But the uptake decreased with the increase in zinc concentration. Nitrate absorption increased in the first treatment but decreased in the second treatment. The N / P ratio did not vary much between the treatments and between any treatment and control.

Conclusion :

The combination is undesirable as it caused considerable change in the proportion of macromolecules like lipid, protein and carbohydrate.

Comparison :

When the effect of the metals mercury and zinc employed individually, was compared with their combined effect, production was found increased than in the former (mercury) and lowered than in the latter (zinc). Respiration was increased than with mercury and reduced than with zinc at lower level. When the zinc level is higher the influence is seen in increased respiration. Chlorophyll b and pheophytin showed an increase than when either metal employed singly. Chlorophyll a and carotenoids were less than when mercury alone was employed. The effect of mercury prevailed on carbohydrates (except on third fraction). Protein improved than when zinc alone was employed levels of zinc in combination, lipids improved to a large extent than was found when either metal was employed. Highest lipid concentration was observed in the combination 0.005 Hg + 0.08 Zn during the present work. Effect of zinc prevailed on the biomass.

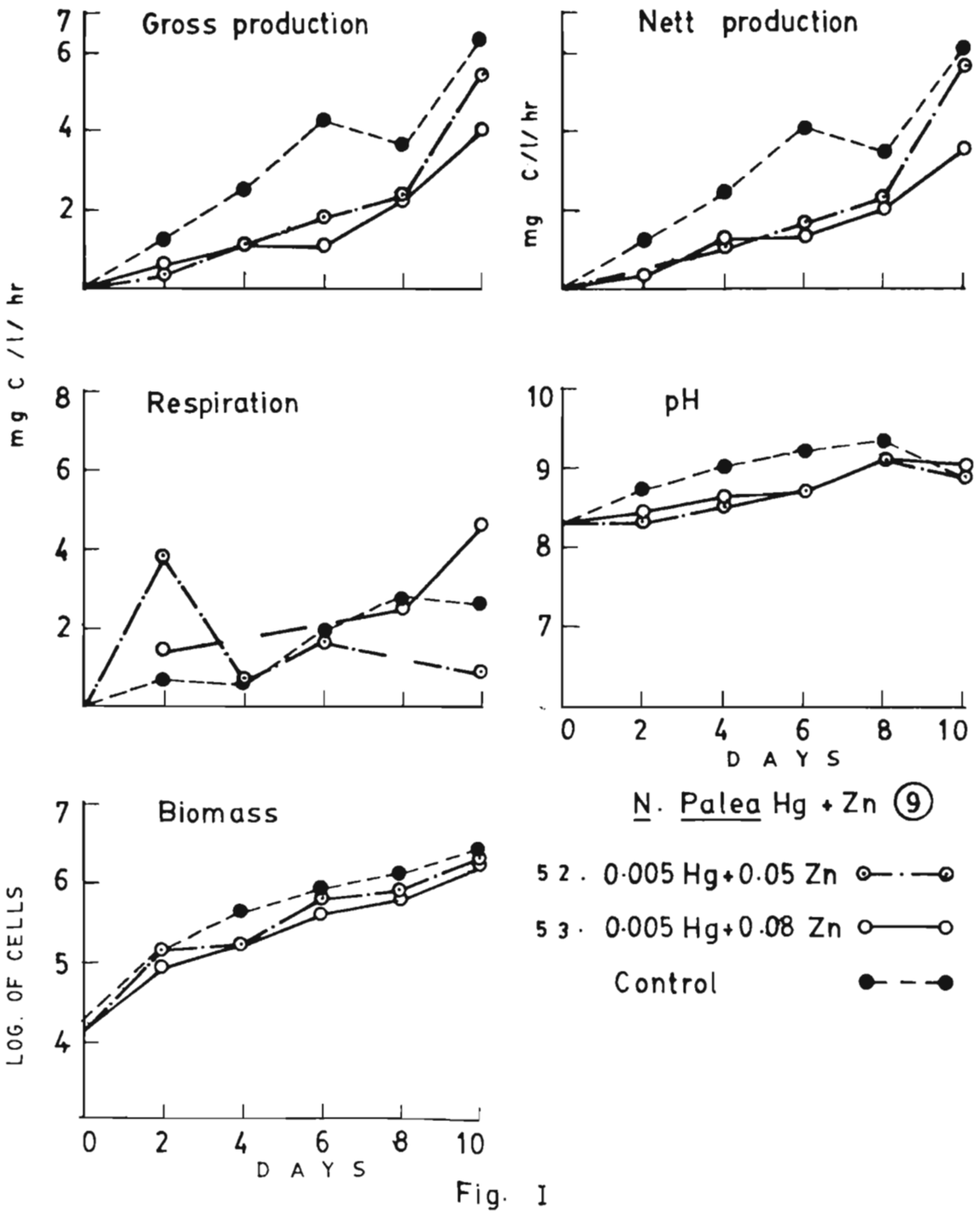

Gross production
Nett production
Respiration
pH
Biomass
mg C /l/ hr
LOG. OF CELLS
D A Y S
N. Palea Hg + Zn (9)
52. 0·005 Hg+0·05 Zn
53. 0·005 Hg+0·08 Zn
Control

Fig. I

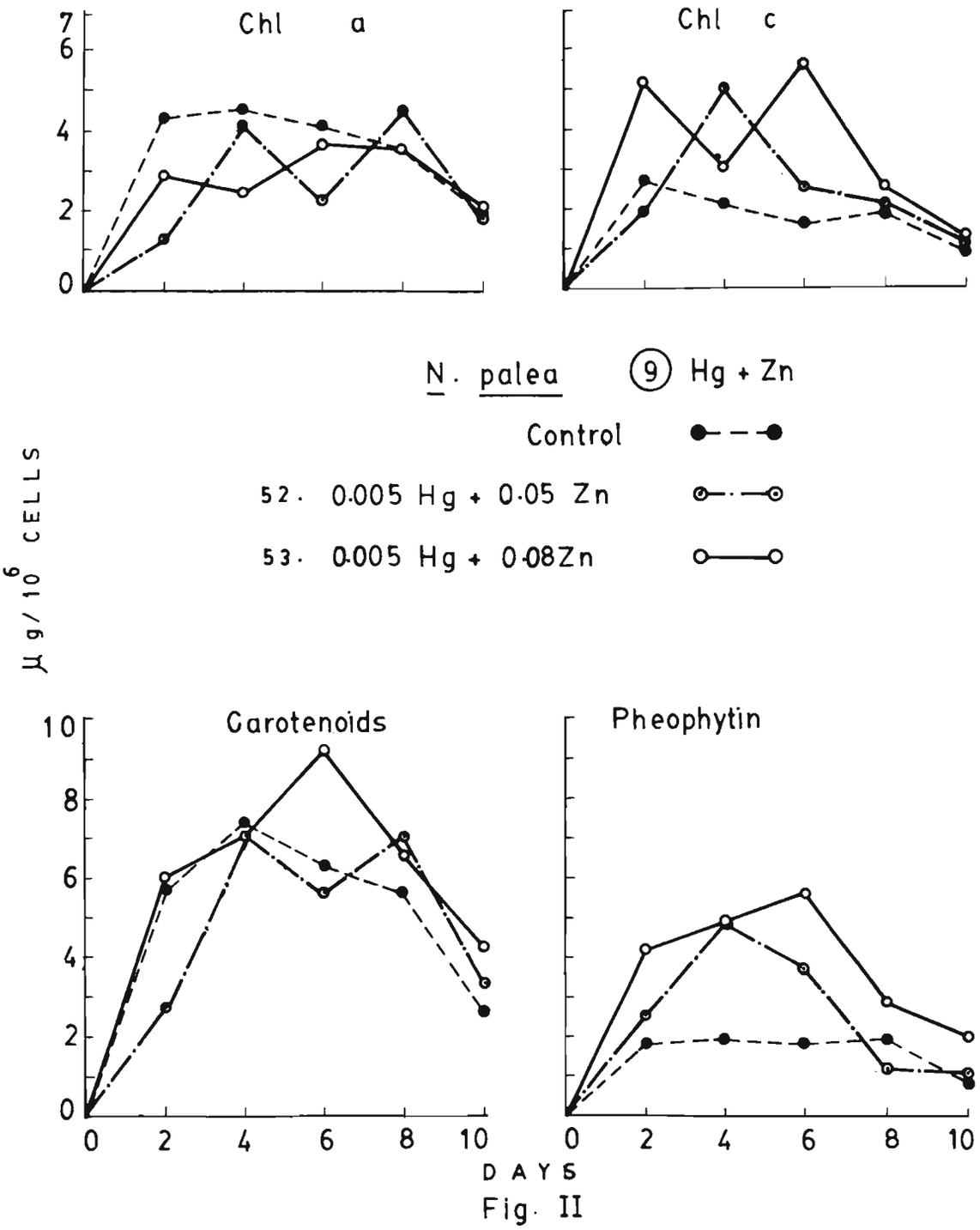

Chl a
Chl c
N. palea
⑨ Hg + Zn
Control
52. 0.005 Hg + 0.05 Zn
53. 0.005 Hg + 0.08Zn
μ g/ 10^6 CELLS
Carotenoids
Pheophytin
DAYS

Fig. II

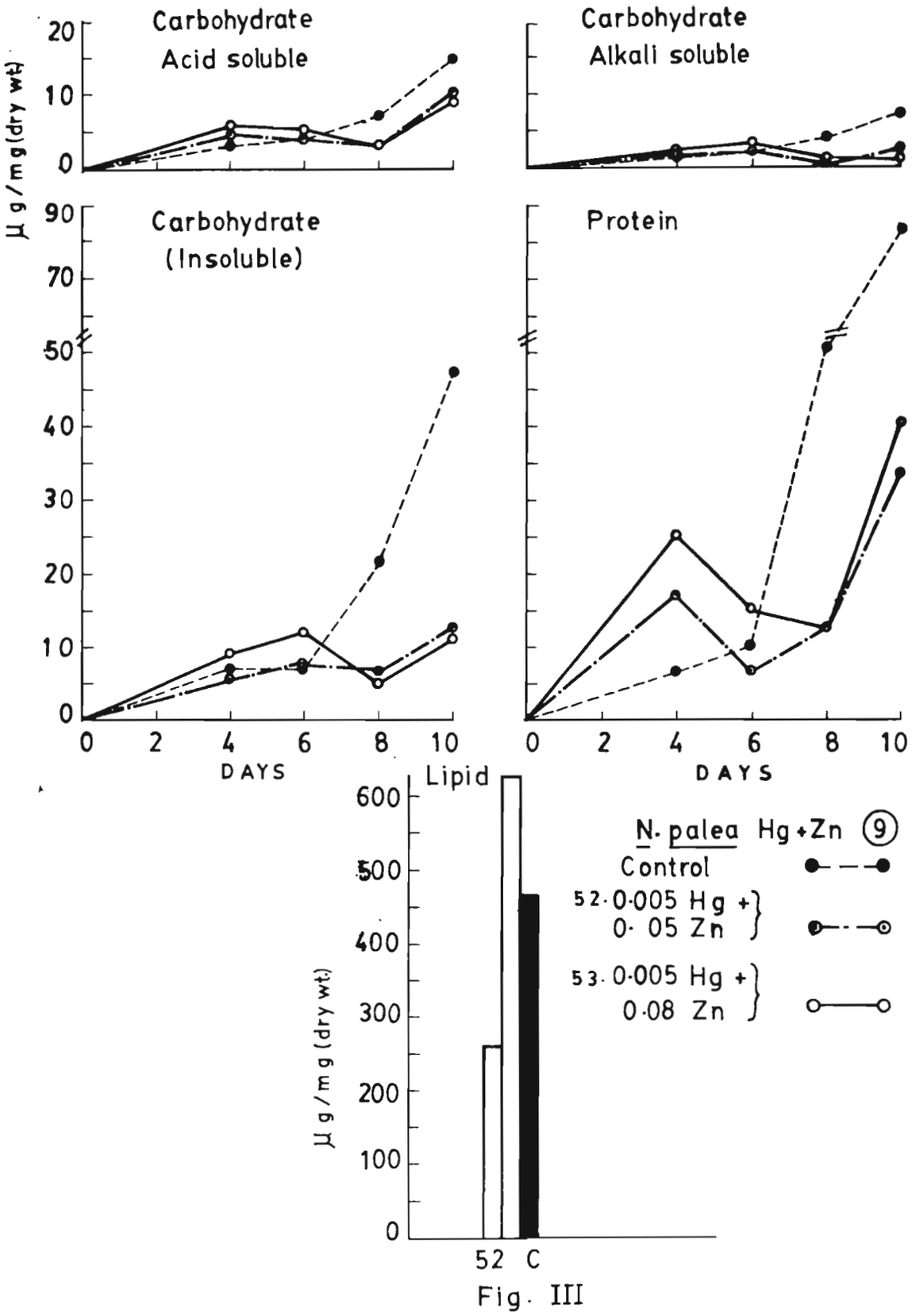

Carbohydrate
Acid soluble
Carbohydrate
Alkali soluble
Carbohydrate
(Insoluble)
Protein
µg/mg(dry wt)
DAYS
DAYS
Lipid
µg/mg (dry wt)
N. palea Hg+Zn (9)
Control
52 0·005 Hg + 0·05 Zn
53 0·005 Hg + 0·08 Zn
52
C

Fig. III

6.1.5

Combined effect of mercury and iron on S. bijugatus (Figs. I, II and III)

Sl. No. of Combination	Number of the treatments	Concentration of metals (ppm)
(10)	54	0.05 Hg + 0.05 Fe
	55	0.05 Hg + 0.1 Fe

Production: (Fig. I)

The metals in combination improved the production of the alga in the later phase of growth but was less that of control upto sixth day, when production was 27% and 58% less than that of control respectively. However from eighth day onwards it remained higher than that of control. On twelfth day 79% and 70% increase was recorded.

The respiration of the alga was lowered to a large extent in the first treatment which was 68% less than that of control on second day and was steady thereafter till twelfth day, exhibiting 87% reduction on the last day. But when the iron level was increased to 0.1 ppm the respiration also increased considerably when compared to that of control. Respiration of the alga fluctuated with two peaks on second and eighth day with an increase of 97% and 348% respectively. At the end of growth phase only 14% increase was observed.

pH of the culture in both the treatments was less than that of control in the early growth phase, reflecting the low production. But it exceeded that of control by sixth day and remained higher thereafter.

Pigments: (Fig. II)

Total pigment content of the alga in both the treatments was higher than that of control in the later phase. Chlorophyll a, chlorophyll b and carotenoids were produced to a greater extent by the alga in the first treatment. Maximum amount of chlorophyll a and chlorophyll b were produced on second day, as those of control. In the second treatment maximum amount of carotenoids was produced on fourth day whereas in the first treatment it was on second day. A second peak was registered for chlorophyll a, chlorophyll b and carotenoids on tenth day. Towards the end of growth phase pigment content of the alga exhibited a decreasing tendency. The concentration of chlorophyll b and carotenoids was less than that of control in the second treatment. Pheophytin was not detected upto fourth day as well as on twelfth day in both treatments and also on tenth day in the first treatment.

Photosynthetic end products: (Fig. III)

Carbohydrate content of the alga was less than that of control in both the treatments in early growth phase but from sixth day onwards it was produced to a greater extent than that of control. The carbohydrate content of the alga increased till the end of growth phase in the former treatment but it fluctuated with peaks on sixth and tenth day. The percentage reduction was 82 and 61 respectively on fourth day. On twelfth day 371% and 207% increase was recorded.

14% reduction in protein content was registered by the alga in first treatment whereas 11% increase by the one in second treatment.

The combination promoted the production of lipids to a large extent. 126% increase in the lipid content of the alga was recorded for the former and 104% for latter treatments.

Growth: (Fig. I)

Rate of multiplication was retarded in both the treatments when compared to that of control. But biomass was equal to that of control on eighth day. The growth thereafter was negligible in the first treatment though improved in the second. At the end of growth phase the

yield was better in second treatment than the first though in both cases biomass was less than that of control.

No. of treatment	Nutrients absorbed (in µg/l) Phosphate	Nitrate	N / P
54	1040	840	0.81
55	1036	1040	1.00
Control	945	1290	1.37

The alga absorbed more phosphate than that of control. Nitrate uptake however was lowered.

Conclusion:

Iron in combination with mercury, promoted the production of the alga but increased respiration at 0.1 ppm level. Pigment content was reduced. Protein improved at higher level. The study showed that antagonism existed between iron and mercury.

Comparison:

At both the employed levels, iron has arrested the suppression caused by mercury in production though it elevated respiration to a considerable extent at 0.1 ppm level. At lower level pigment content of the alga increased. At a higher level iron suppressed chlorophyll a to greater extent than any other pigment but increased pheophytin$. At both levels of iron, lipid and protein of the alga improved, But the biomass remained unaffected.

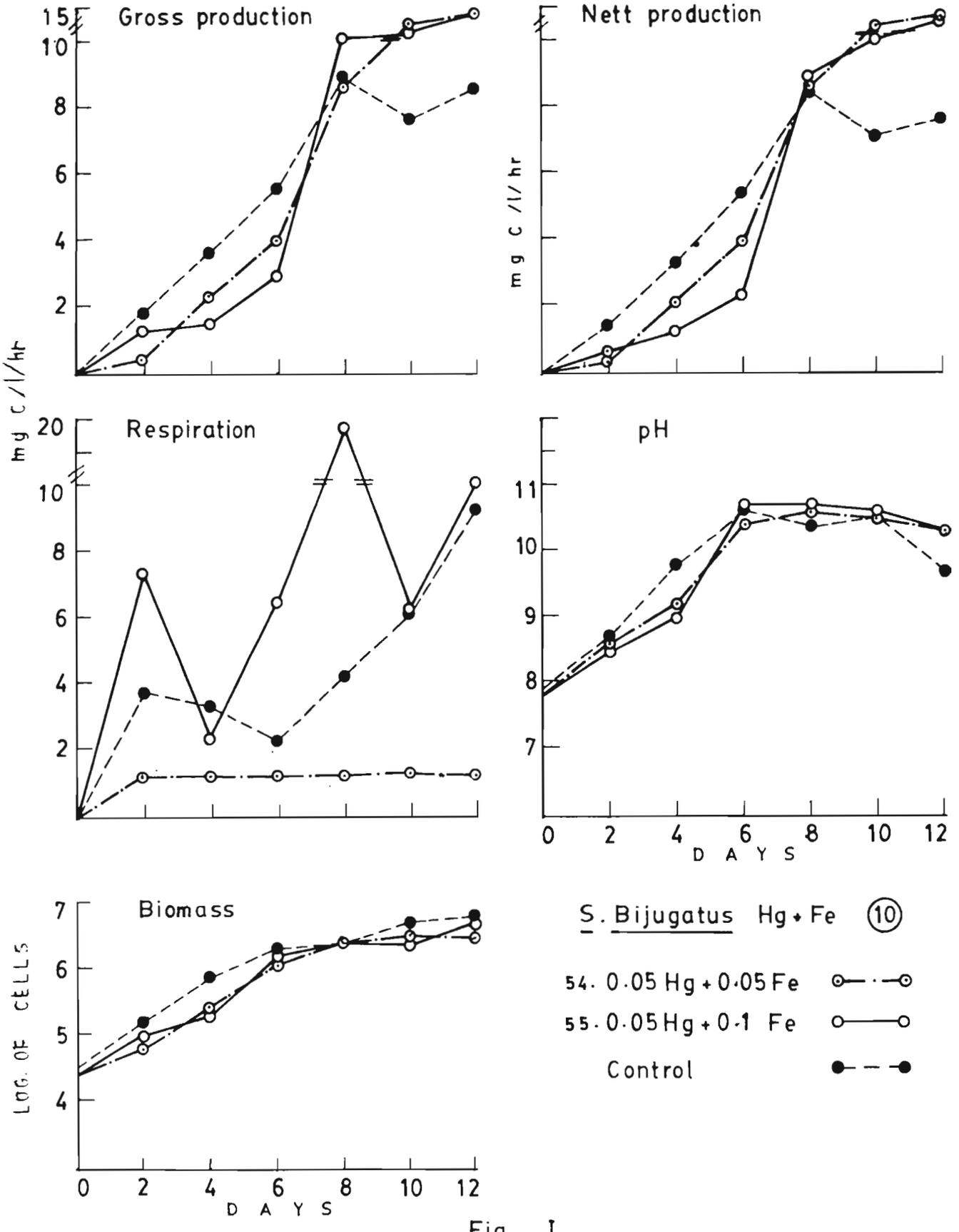

Gross production
Nett production
Respiration
pH
Biomass
mg C /l/hr
mg C /l/ hr
LOG. OF CELLS
D A Y S
S. Bijugatus Hg + Fe (10)
54. 0.05 Hg + 0.05 Fe
55. 0.05 Hg + 0.1 Fe
Control

Fig. I

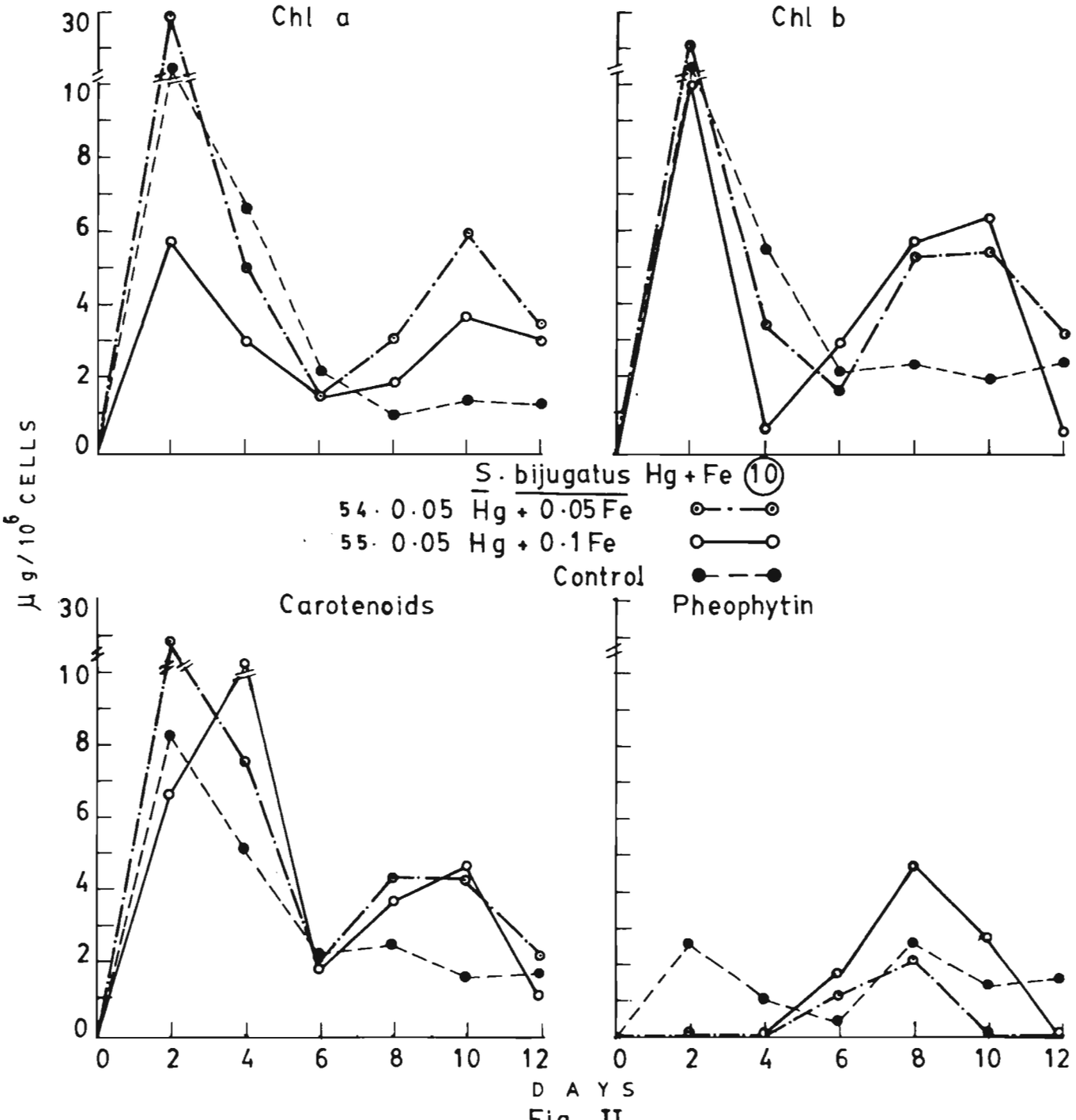
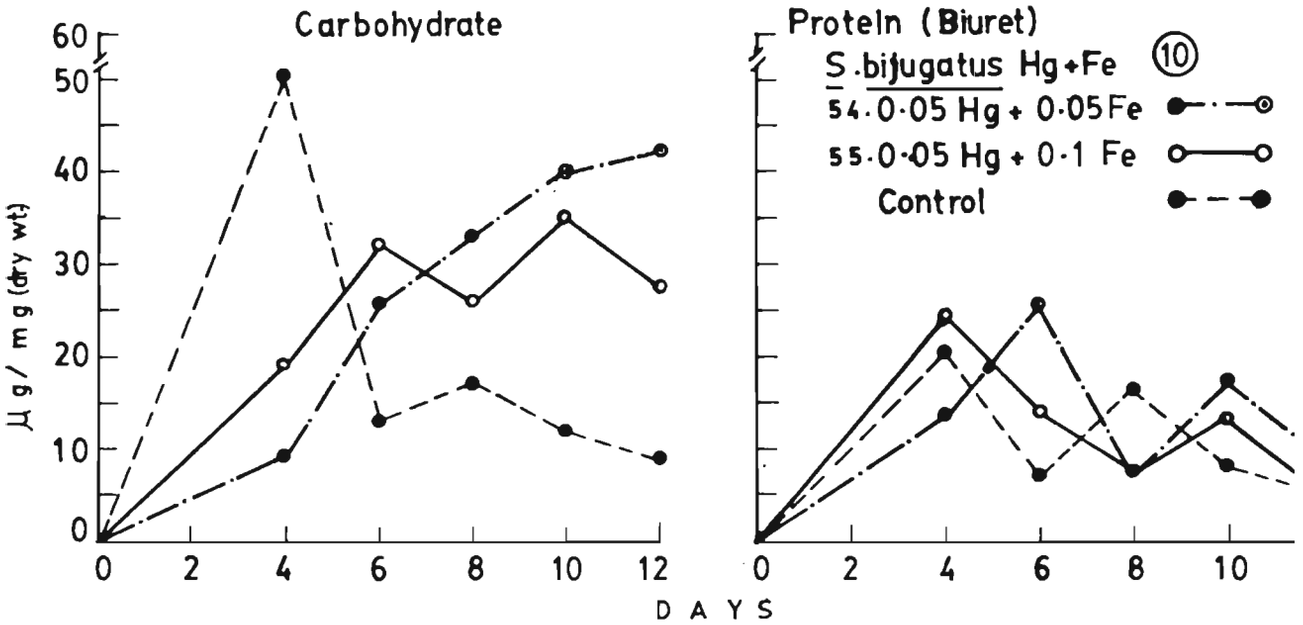

Chl a
Chl b
30
10
8
6
4
2
0
μg/10^6 CELLS
S. bijugatus Hg + Fe (10)
54 · 0·05 Hg + 0·05 Fe
55 · 0·05 Hg + 0·1 Fe
Control
Carotenoids
Pheophytin
0
2
4
6
8
10
12
DAYS

Fig. II


Carbohydrate
Protein (Biuret)
S. bijugatus Hg+Fe
54 · 0·05 Hg + 0·05 Fe
55 · 0·05 Hg + 0·1 Fe
Control
µg / mg (dry wt)
DAYS

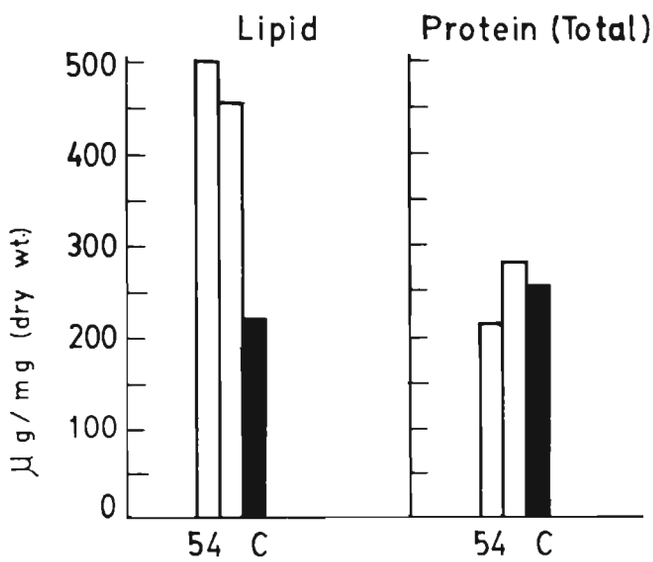

Lipid
Protein (Total)
µg / mg (dry wt)
54
C
54
C

Fig. III

Combined effect of mercury and iron on N. palea

(Figs. I, II and III)

Sl. No. of Combination	No. of treatment	Concentration of the metals (ppm)
(10)	56	0.005 Hg + 0.05 Fe
	57	0.005 Hg + 0.08 Fe

Production: (Fig. I)

The rate of nett production of the treated diatom did not vary much from that of control in the early phase, but it did not exhibit increase to the same extent as that of control after fourth day. Maximum production was recorded on eighth day for the former treatment with 18% reduction and for the latter, on sixth day with 34% reduction in relation to control. Thereafter it declined gradually in both the treatments and on tenth day it was 67% and 64% less than that of control respectively.

Respiration of the diatom in the two treatments did not differ considerably and was almost equal from fourth to eighth day. It was 54% less than that of control on eighth day, but at the end of growth phase it increased in the former treatment to 20% higher than that of control and decreased by 80% in the latter. On sixth day of growth, the oxygen values in dark bottle were higher than the initial one for both the treatments.

Owing to low production in both the treatments, pH of the culture was observed to be less than that of control throughout the growth phase. It decreased to a greater extent in the second treatment towards the end of growth phase.

Pigments: (Fig. II)

Total pigment content of the diatom in both the treatments was higher than that of control towards the end of growth phase. All four pigments were produced in larger quantity by the diatom in the latter treatment.

Maximum chlorophyll a was found on eighth day in the diatom in the first treatment whereas in the second treatment it was on the fourth day. Pigment content of the diatom decreased in both the treatments, thereafter, but remained higher than that of control.

The concentration of chlorophyll c did not vary from that of control in the first treatment upto fourth day. It increased thereafter reaching a maximum on eighth day at a higher level than that of control. In the second treatment it was slightly less than that of control till second day but increased thereafter, reaching maximum on fourth day. It then decreased gradually till the end of growth phase in both the treatments but remained higher than that of control.

The carotenoids showed a similar pattern of development as chlorophyll c in the treatments, reaching maximum level on eighth and fourth day respectively in the two treatments. The concentration of the pigment declined thereafter in both. It was much higher in the second treatment between fourth and eighth day.

Pheophytin also followed the same pattern of development as chlorophyll c, but initially the concentration was equal to that of control. Maximum concentration was attained on eighth and fourth day respectively in the two treatments. The concentration of pheophytin was almost steady from sixth day onwards in the second treatment but declined in the first one towards the end of growth phase.

Photosynthetic end products: (Fig. III)

The concentration of the acid soluble carbohydrate was slightly higher in the treated diatom when compared to that of control and gradually increased in both the treatments till eighth day, when it was 17% higher than that of control. Thereafter it was steady in both the instances but was 46% less at the end of growth phase.

In both treatments the alkali soluble carbohydrate fraction was equal to that of control on second and sixth day but was not detected on fourth day in the

first treatment. In the later growth phase it was almost equal in both treatments but on tenth day it was found to be reduced by 40% and 34% respectively.

The insoluble carbohydrate fraction of the diatom increased steadily and was higher than control on sixth day, by 46% and 78% respectively. The subsequent increase was not to the same extent as that of control and at the end of growth phase it was found reduced by 75%.

The protein content of the diatom was more in the early stage when compared with that of control. 86% and 242% increase was recorded on sixth day in the two treatments. Inspite of continued increase it was found to be 31% less on eighth day in both treatments. On the last day 52% and 42% reduction was observed respectively in the treatments in relation to control.

The lipid content of the diatom was adversely affected by the metals in combination. It was less than that of control in both the instances, by 29% and 55% respectively.

Growth: (Fig. I)

The growth rate was enhanced in the early stage. Though the diatom when exposed to these combinations produced equal number of cells to that of control by sixth day, slight retardation was recorded thereafter in both the treatments. The biomass in the former treatment was equal to that of control but in the later it was slightly lower.

No. of Treatment	Nutrients absorbed (in µg/l.) Phosphate	Nitrate	N / P
56	1700	734	0.43
57	1615	600	0.37
Control	1350	763	0.57

The diatom has absorbed more phosphate than that of control when exposed to the metals, but the nitrate absorption was reduced. The increase in iron concentration reduced the uptake of both the nutrients.

Conclusion:

The combination reduced both production and respiration. Pigment content of the diatom increased. Carbohydrate, protein, and lipid were lowered, though the biomass was not affected. Combination was found to be undesirable from the point of view of end products.

Comparison:

When the effect of the present combination was compared with that obtained by mercury alone, the production was found to be reduced but the respiration was not affected. Concentration of the pigment was lowered. Total chlorophyll a was lowered but chlorophyll c remained unaffected. When the iron level was higher carotenoids and pheophytin increased. But at the lower level they were suppressed. Mercury prevailed up on the carbohydrate concentration and biomass whereas protein and lipid showed an increase.

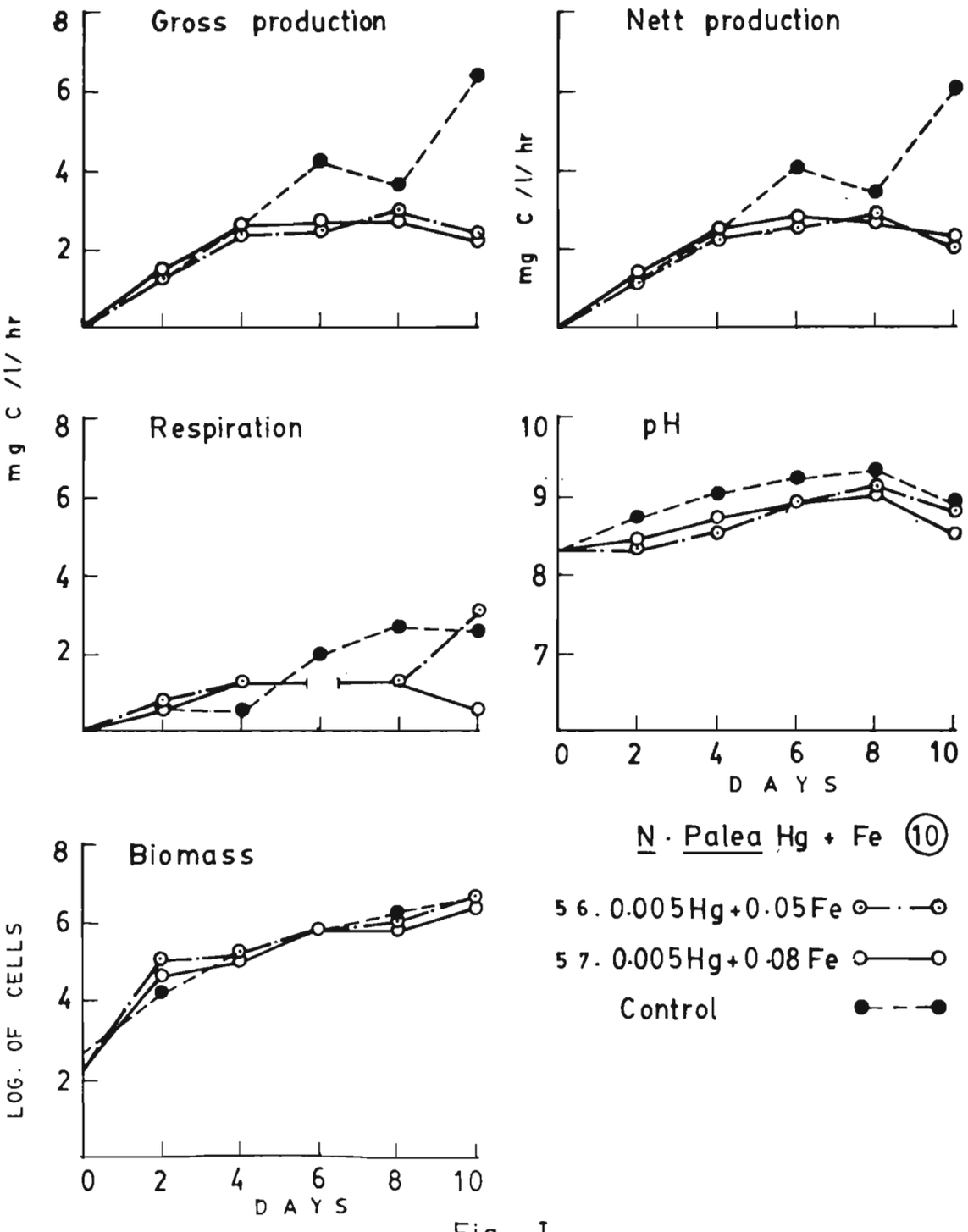

Gross production
Nett production
Respiration
pH
Biomass
mg C /l/ hr
LOG. OF CELLS
DAYS
N · Palea Hg + Fe (10)
56. 0.005 Hg + 0.05 Fe
57. 0.005 Hg + 0.08 Fe
Control

Fig. I

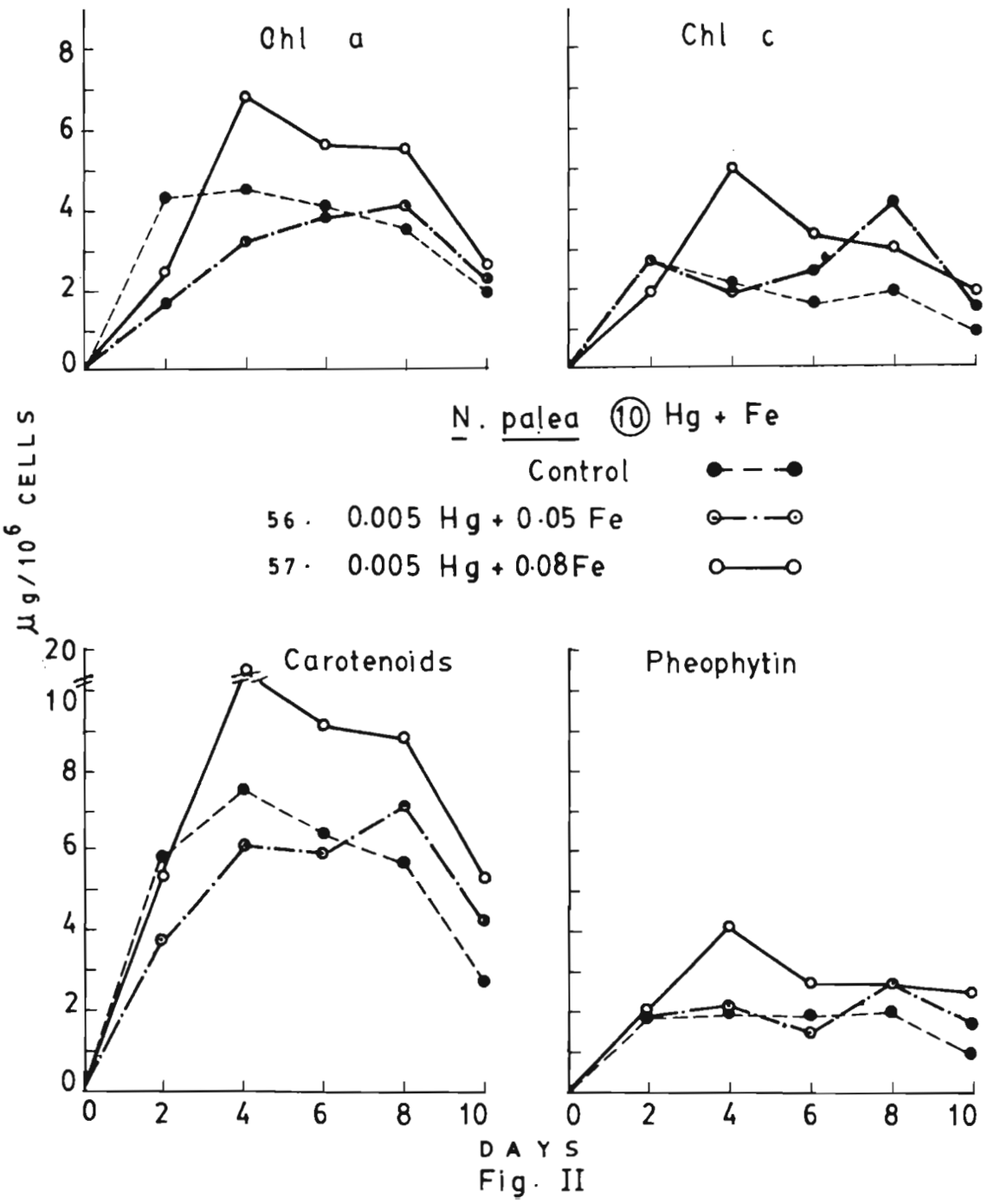

Chl a
Chl c
N. palea (10) Hg + Fe
Control
56. 0.005 Hg + 0.05 Fe
57. 0.005 Hg + 0.08 Fe
Carotenoids
Pheophytin
µg/10^6 CELLS
DAYS

Fig. II

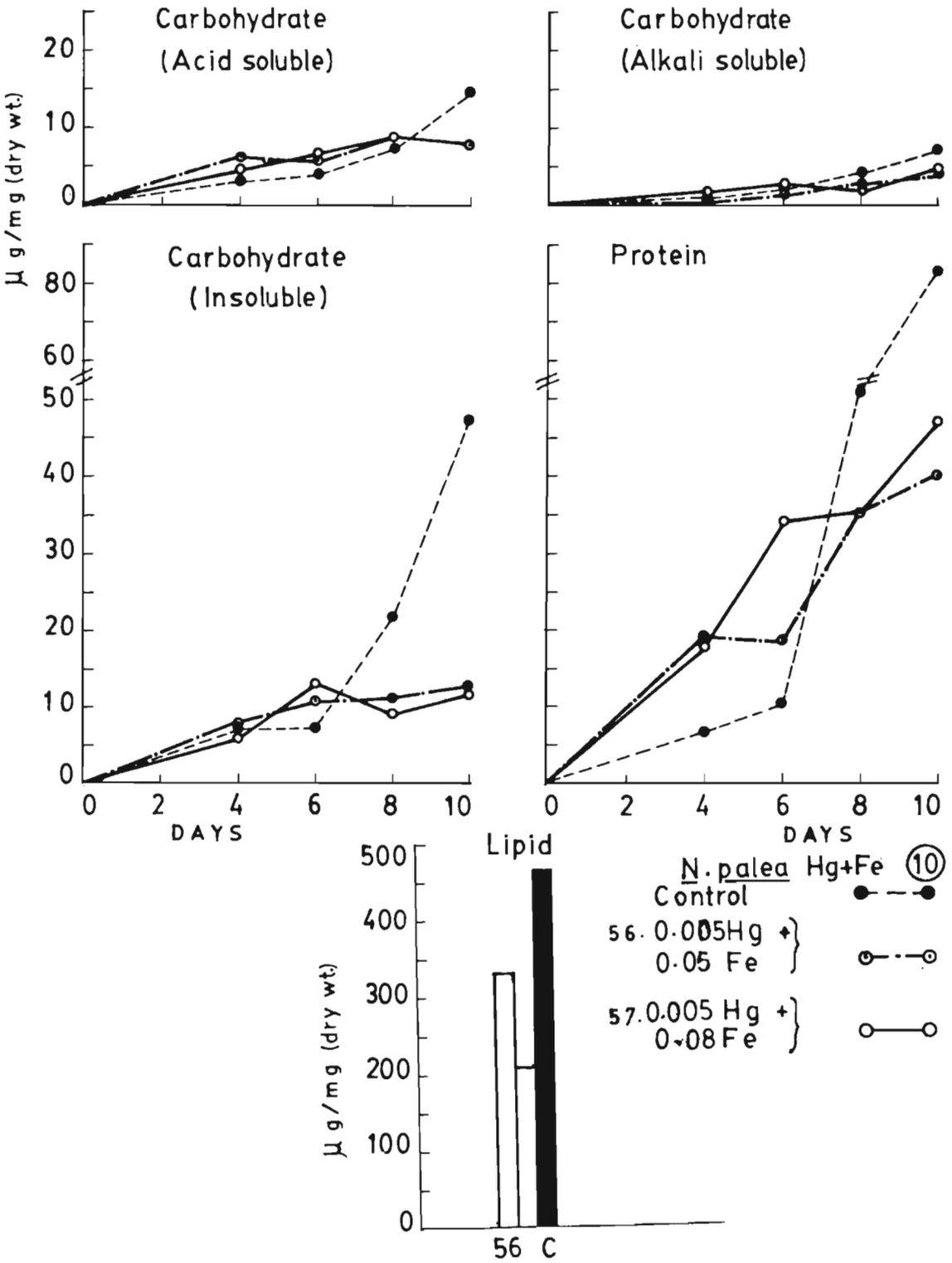

Carbohydrate
(Acid soluble)
Carbohydrate
(Alkali soluble)
Carbohydrate
(Insoluble)
Protein
µ g/mg (dry wt.)
DAYS
Lipid
N. palea Hg+Fe (10)
Control
56. 0·005Hg + 0·05 Fe
57. 0·005 Hg + 0·08 Fe
56 C

Fig. III

6.1.6

Combined effect of cadmium and lead on

S. bijugatus

(Figs. I, II and III)

Sl.No. of Combination	No. of treatment	Concentration of metals (in ppm)
(11)	58	0.05 Cd + 0.05 Pb
	59	0.05 Cd + 0.1 Pb

Production: (Fig. I)

The nett production of the alga was retarded by the metals in combination and it remained far less than that of control throughout the growth phase. The reduction was found to be 83% and 62% on second day, 95% and 82% on eighth day and 75% and 67% on the last day respectively, for the two treatments. From sixth day onwards production of the alga in the second treatment was slightly higher than that in the first treatment.

Respiration of the alga was reduced considerably and also fluctuated in the first treatment with peaks on fourth and eighth day respectively. On fourth day it was elevated by 61% and 47% respectively. It decreased to a minimum level on sixth day in the former and till eighth day in the latter. On eighth day it was 5% more in the former and 79% less in latter treatment. It was reduced by 72% and 60% at the end of growth phase.

pH of the culture in both the treatments was equal to that of control on second day. It decreased thereafter and remained much lower for the rest of the period, in relation to control.

Pigments: (Fig. II)

Pigment content of the alga increased to a large extent in both the treatments. Concentration of chlorophyll a, chlorophyll b and carotenoids was less than that of control during the early phase. The concentration of chlorophyll b and pheophytin was almost equal in the treatments at the end of growth phase. The concentration of chlorophyll a was higher in the former treatment and that of carotenoids in the latter. Considerable fluctuation was recorded in the level of pheophytin in both, but chlorophyll alone in second treatment during the growth phase. However, pheophytin was not detected on sixth day. At their respective maxima all four pigments in both the treatments attained a higher level than that of control respectively.

Photosynthetic end products: (Fig. III)

Carbohydrate content of the treated alga was less than that of control. Carbohydrate was produced to maximum extent during later phase of growth unlike that of control. No change in the concentration was noted

between sixth and eighth day in the first treatment. Considerable increase in this product was observed generally from eighth day onwards. On eighth day 28% reduction was recorded in the first treatment but 20% increase in the second. However on tenth day 170% and 252% increase was observed, in relation to control. Thereafter the level of carbohydrate declined in both instances. Neverthless it remained 130% and 250% higher than that of control respectively.

Protein content of the alga decreased to a large extent, by 68% and 83% in the treatments respectively.

The lipid content of the alga improved when compared to control by 47% and 24% respectively.

Growth: (Fig. I)

Growth of the alga was severly inhibited except in the early phase in both the treatments. The alga was found sticking to the walls of the culture flask in both treatments.

Details of the nutrient uptake are given in the following table.

No. of Treatments	Nutrients absorbed in µg/l		N / P
	Phosphate	Nitrate	
58	930	1060	1.14
59	1086	1101	1.01
Control	945	1290	1.37

The phosphate uptake decreased by few ug in the first treatment but increased by about 140 ug. in the second, when compared to that of control. Nitrate absorption decreased. With increase in concentration of lead, there was a general reduction in nitrate uptake.

Conclusion:

The metals exerted a toxic effect at the experimental concentrations and combination, reducing nett photosynthesis and protein content of the alga to a large extent though lipid content of the alga improved. Growth was retarded to a great extent.

Comparison:

When the combined effect of the metals (cadmium + lead) was compared with those obtained by employing the metals individually (cadmium and lead) production was found lowered. Respiration also was lowered but the fluctuation in the respiration exhibited when the metals were employed individually, was retained even in combination. While chlorophyll a was not affected, chlorophyll b, carotenoids and pheophytin increased considerably. Carbohydrate concentration was not affected but protein was considerably reduced when compared to that obtained with cadmium alone. The variation in the lipid concentration was marginal. Biomass was reduced.

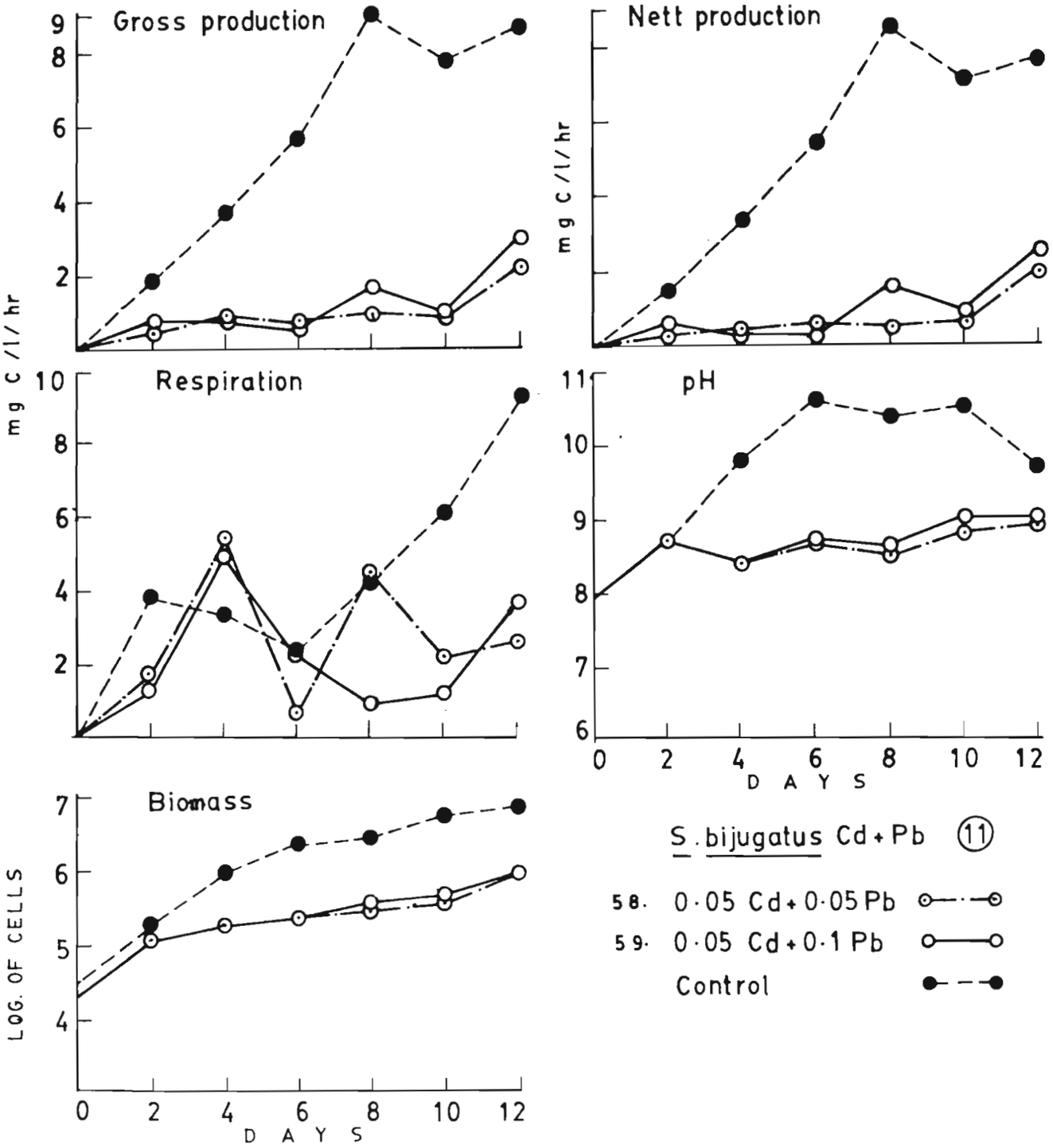

Gross production
Nett production
Respiration
pH
Biomass
mg C /l/ hr
LOG. OF CELLS
DAYS
S. bijugatus Cd + Pb (11)
58. 0·05 Cd + 0·05 Pb
59. 0·05 Cd + 0·1 Pb
Control

Fig. I

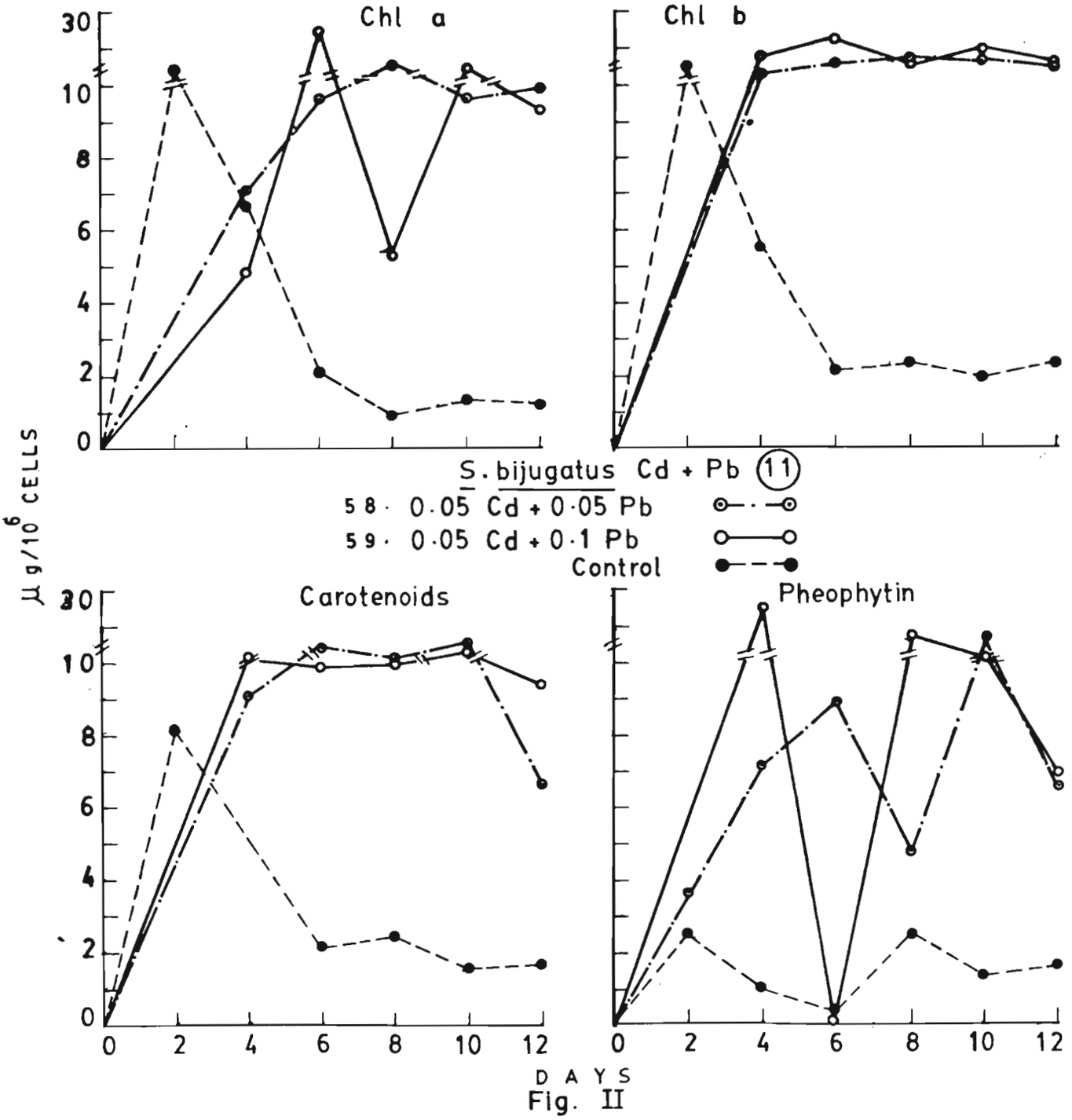
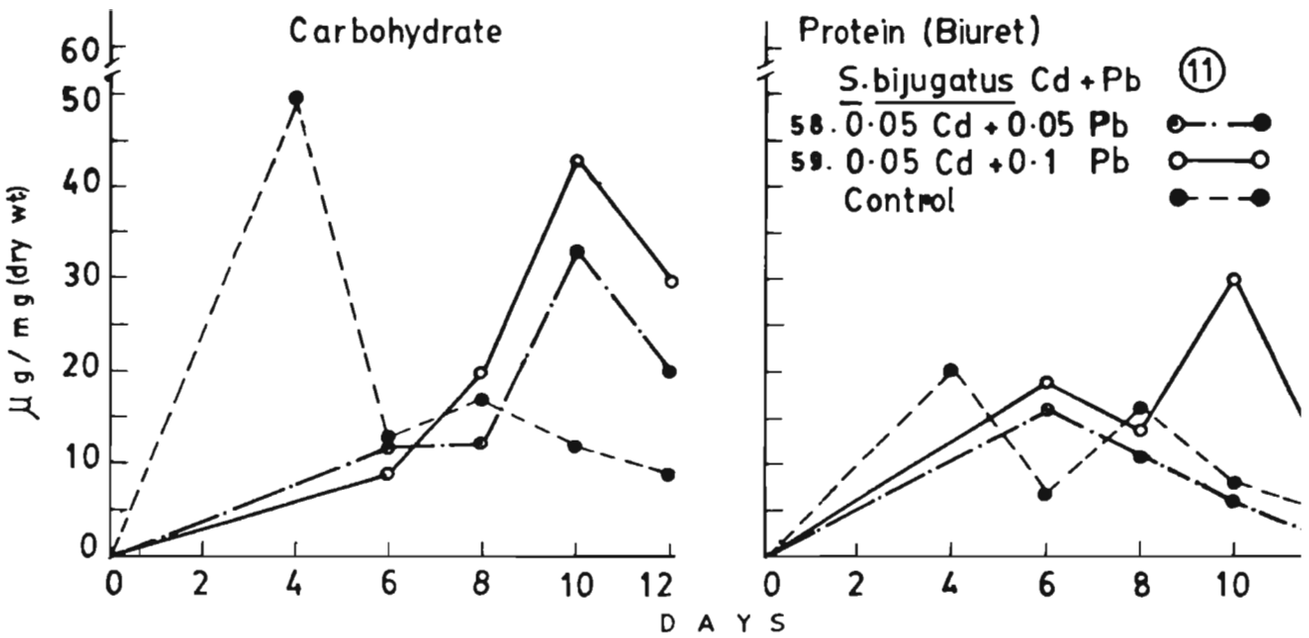

Chl a
Chl b
S. bijugatus Cd + Pb (11)
58. 0.05 Cd + 0.05 Pb
59. 0.05 Cd + 0.1 Pb
Control
Carotenoids
Pheophytin
µg/10^6 CELLS
DAYS

Fig. II


Carbohydrate
Protein (Biuret)
S. bijugatus Cd + Pb
11
58. 0·05 Cd + 0·05 Pb
59. 0·05 Cd + 0·1 Pb
Control
µg / mg (dry wt)
60
50
40
30
20
10
0
0 2 4 6 8 10 12
0 2 4 6 8 10
DAYS

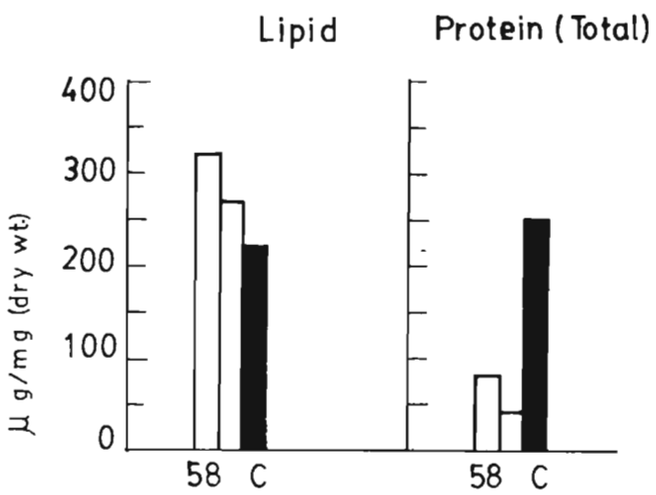

Lipid
Protein (Total)
µg/mg (dry wt)
400
300
200
100
0
58 C
58 C

Fig. III

Combined effect of cadmium and lead on *N. palea*

(Figs. I, II and III)

Sl. No. of Combination	No. of treatment	Concentration of metals (in ppm)
(11)	60	0.04 Cd + 0.04 Pb
	61	0.04 Cd + 0.08 Pb

Production: (Fig. I)

The nett production of the diatom was adversely affected by the metals in combination. It was less than that of control throughout the growth phase. Production of the diatom gradually increased in both the treatments till fourth day but was 29% and 65% less than that of control respectively. It decreased till sixth day registering 84% and 94% reduction respectively. Thereafter it improved in both the treatments but to a greater extent in the latter and was found to be reduced by 60% and 47% respectively on tenth day.

Respiration of the diatom was elevated in the early phase, with 100% and 167% increase on second day. Respiration of the diatom did not change between fourth and sixth day in both instances but was 37% less than that of control on sixth day. Thereafter in both the treatments respiration increased but to a greater extent in

the latter treatment. Once again respiration of the alga was observed to be steady between eighth and tenth day. At the end of growth phase it was reduced by 40% in the former but increased by 96% in the latter, in relation to control.

Owing to the low production rate of the diatom, pH of the culture in both the treatments was observed to be well below that of control except on eighth day when it was equal. It declined thereafter to a greater extent than that of control towards the end of growth phase.

Pigments: (Fig. II)

When the diatom was exposed to the present combination, the pigment content increased to a large extent when compared to that of control. With the exception of chlorophyll a in early phase, all pigments were produced in greater quantities by the treated diatom. Chlorophyll a was maximum in later growth phase on eighth and sixth day respectively unlike that of control.

Chlorophyll c of the diatom exhibited fluctuation during growth phase with peaks on second and sixth day respectively.

The treated diatom produced carotenoids in greater quantity than any other pigment. The pigment level of the diatom fluctuated in the first treatment.

Though chlorophyll a was reduced in treated diatom in early phase, pheophytins did not show any such reduction. At maximum level pheophytin was in greater quantity than chlorophyll in the second treatment.

The pigment level of the diatom exhibited a decreasing tendency towards the end of growth phase, as that of control.

Photosynthetic end products: (Fig. III)

The acid soluble carbohydrate fraction of the diatom in first treatment increased to a very great extent and was more than that of control throughout the growth phase. On fourth day 210% and 257% increase was recorded respectively in first and second treatments. However it decreased later in both the treatments. On sixth day it was 105% more in the first treatment but50% less in the second, than that of control. Generally from sixth day onwards it increased in both treatments and on tenth day was found to be 501% and 95% higher than that of control respectively.

The alkali soluble carbohydrate fraction of the alga did not vary considerably, from that of control in early phase but increased from sixth day onwards to a greater extent and on tenth day the percentage increase was 104 and 48 respectively.

The insoluble carbohydrate content of the diatom increased gradually in the first treatment till the end of the growth phase. It was 40% more than that of control on fourth day but was reduced by 41% on the last day. In the second treatment it was 21% higher on fourth day but declined to 71% lower level by sixth day. It increased once again towards the end of growth phase but remained 51% less than that of control on the last day.

Protein content of the diatom showed increase in the first treatment in relation to control. On sixth day 36% increase was recorded for the diatom in first treatment but 65% reduction in the other. On eighth day protein was 16% more in the first treatment but 25% less than control in the other. At the end of growth phase the percentage reduction was 24 and 16 respectively.

Lipid content of the diatom was adversely affected in both the treatments but it was reduced to a greater extent, by 50% in the former and only by 20% in the latter treatments.

Growth: (Fig. I)

Retardation in growth was noted when the diatom was exposed to the metals. The biomass was less than that of control throughout the growth phase. Growth rate of the diatom was found to be better in the former treatment in the early phase. No growth was observed between second and fourth days in the first treatment.

No. of Treatments	Nutrients absorbed (in µg/l.) Phosphate	Nitrate	N / P
60	1555	717	0.46
61	1650	777	0.47
Control	1350	763	0.57

When the concentration of lead was low the nitrate uptake was reduced. When the concentration of lead was high both the nutrients were absorbed to a greater extent than that of control and other treatment.

Conclusion:

In general the combination is not desirable since the nett production, growth rate, protein and lipid of the diatom were adversely affected.

Comparison:

When the effect of the combination was compared with the individual effects of metals, it was found that undesirable effect of both the metals prevailed in reducing production and elevating respiration. All pigments were produced to a greater extent than when either metal was employed individually. While the carbohydrate remained unaffected, protein and lipid improved to a considerable extent. It was found that the positive effect of the combination was limited to the end products, presumably due to the antagonistic action of the metals.

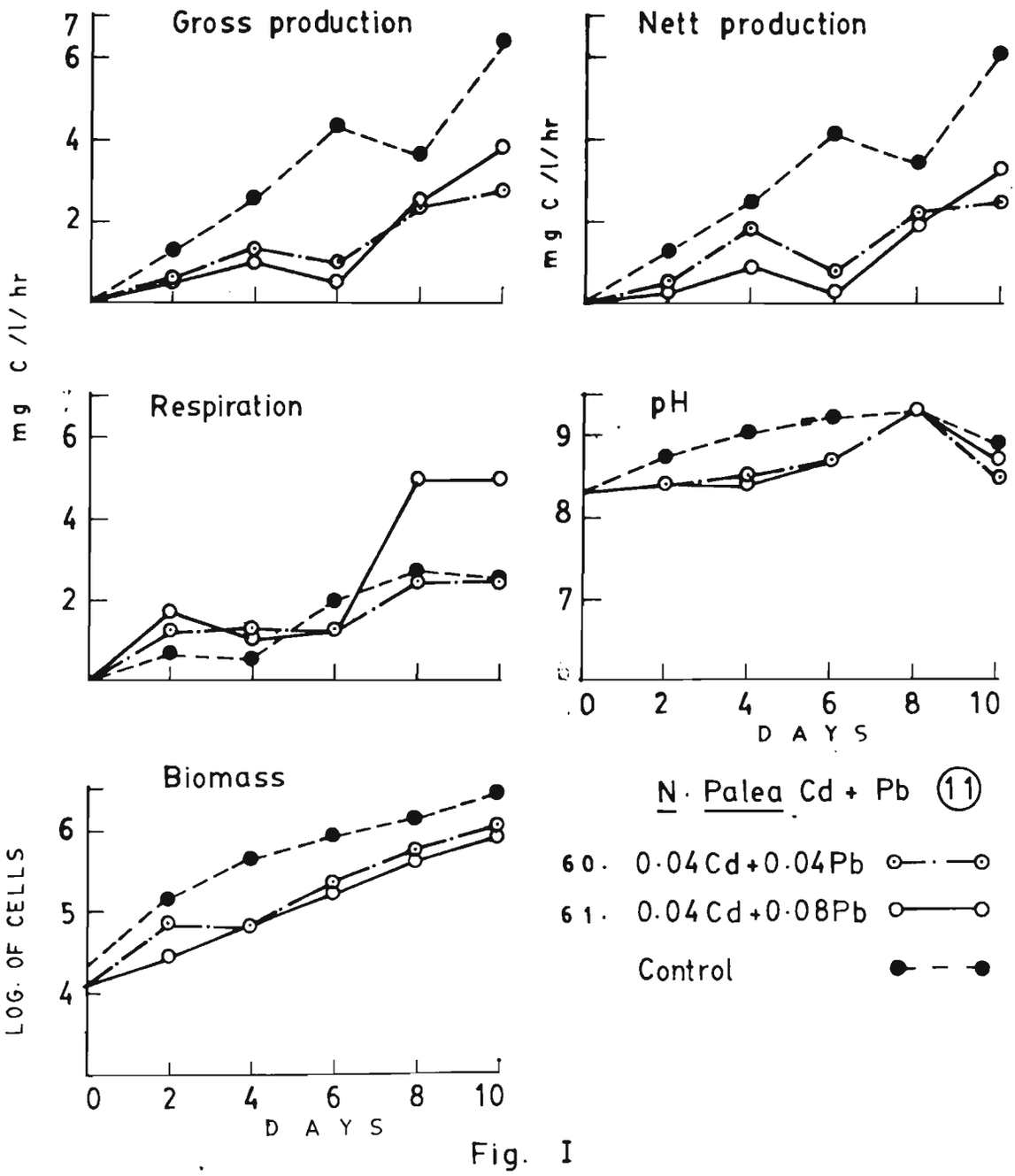

Gross production
Nett production
Respiration
pH
Biomass
mg C /l/ hr
LOG. OF CELLS
DAYS
N. Palea Cd + Pb (11)
60. 0·04Cd+0·04Pb
61. 0·04Cd+0·08Pb
Control

Fig. I

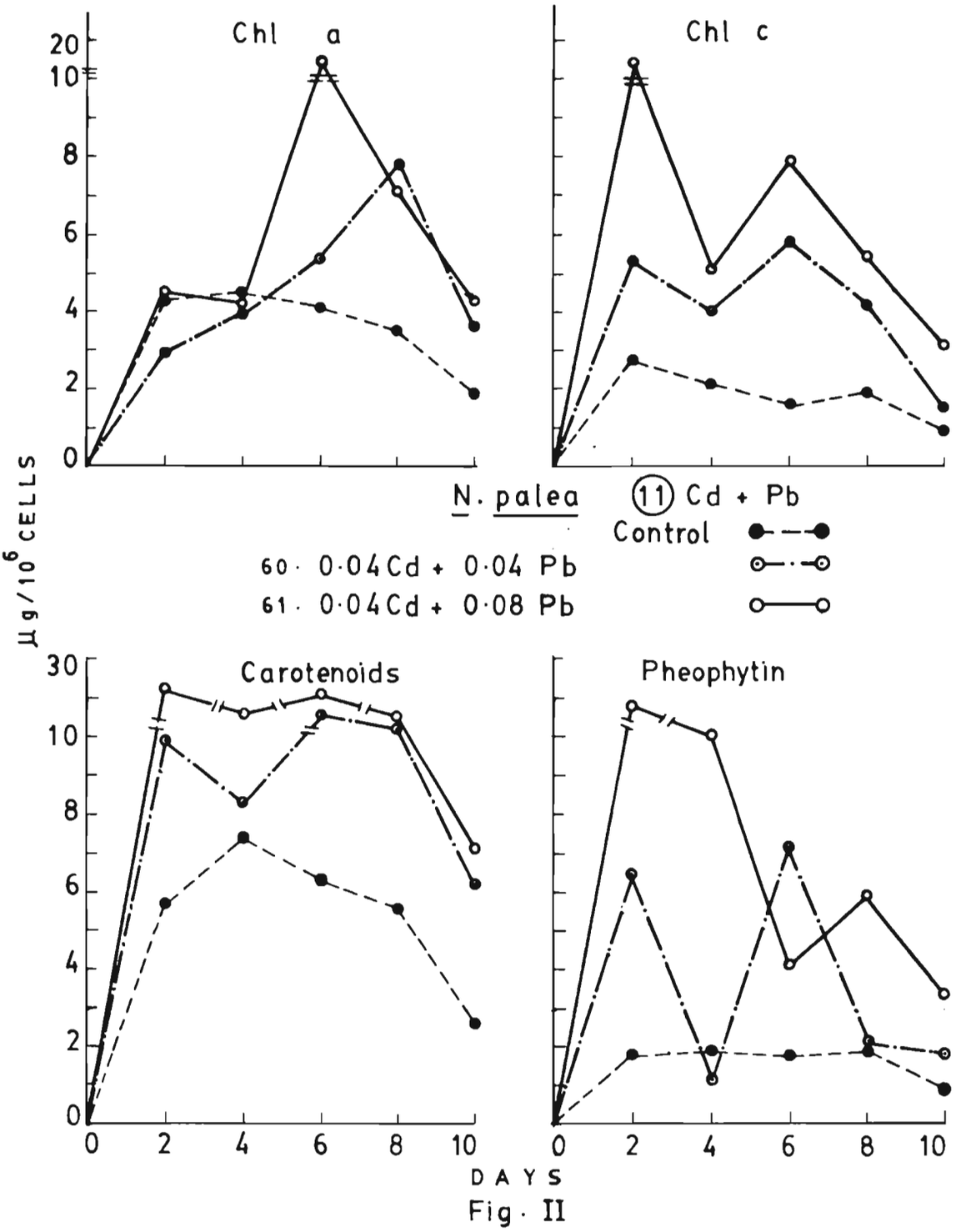

Chl a
Chl c
20
10
8
6
4
2
0
N. palea
(11) Cd + Pb
Control
60 · 0·04 Cd + 0·04 Pb
61 · 0·04 Cd + 0·08 Pb
μg/10^6 CELLS
Carotenoids
Pheophytin
30
10
8
6
4
2
0
0
2
4
6
8
10
DAYS

Fig · II

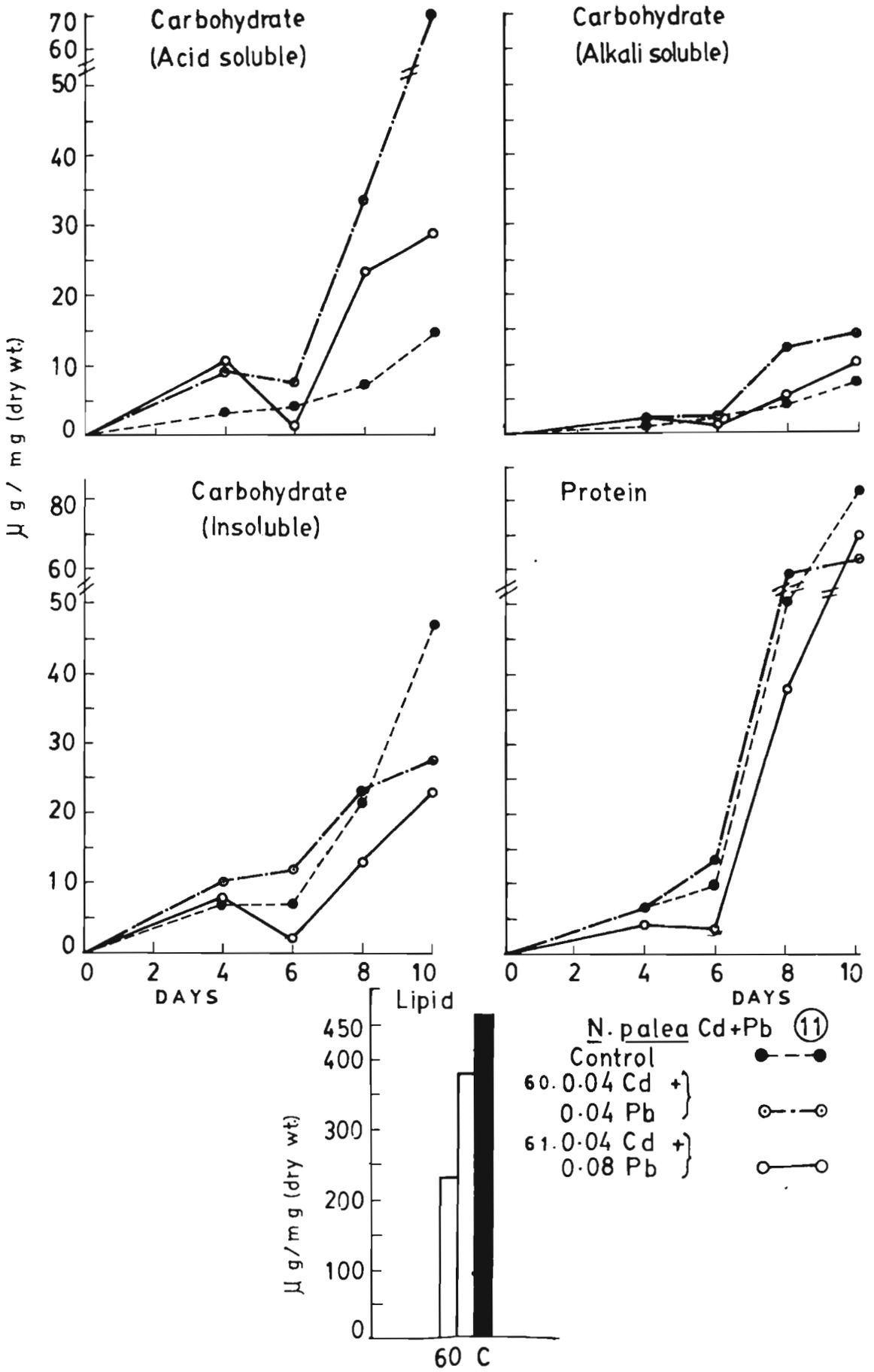

Carbohydrate
(Acid soluble)
Carbohydrate
(Alkali soluble)
Carbohydrate
(Insoluble)
Protein
Lipid
µ g / m g (dry wt.)
DAYS
N. palea Cd+Pb (11)
Control
60. 0·04 Cd + 0·04 Pb
61. 0·04 Cd + 0·08 Pb
60 C

Fig. III

6.1.7

Combined effect of cadmium and zinc on S. bijugatus

(Figs. I, II and III)

Sl. No. of combination	No. of treatment	Concentration of metals (in ppm.)
(12)	62	0.05 Cd + 0.1 Zn

Production: (Fig. I)

Production of the alga was adversely affected by cadmium and zinc in the early growth phase. However, the alga recovered from the toxic effect in the late growth phase. On second and sixth day it was less than that of control by 57% and 93% respectively. On fourth and eighth day of growth, the nett production of the alga was nil, as the oxygen values of the light bottles remained less than in the initial bottle. But by tenth day the production was 75% higher and continued to increase till the end of the growth phase. 150% increase was recorded on twelfth day in relation to control.

Respiration of the alga was elevated to a large extent and 279% increase was recorded on fourth day. Respiration continued to increase thereafter and on eighth day reached maximum with 567% increase. Though it declined thereafter till tenth day when it was 61% less, once again it increased towards the end growth phase to 51% higher level on the twelfth day.

Inspite of the very low production obtained the pH value of the culture increased gradually and continuously till the end of growth phase and was higher than that of control (10.64) on twelfth day.

Pigments (Fig.II)

Pigment content of the treated alga increased considerably in the later growth phase. Chlorophyll a, chlorophyll b and carotenoids reached their maxima on second day but only chlorophyll a and carotenoids remained higher than those of control. The amount of chlorophyll b was much less in the treated alga and was not detected on sixth day but increased thereafter to be higher than that of control on twelfth day.

Carotenoids of the alga was less than that of control only on fourth day. It fluctuated with peaks on second and sixth day.

Pheophytin of the alga was severely suppressed and when detected on twelfth day was far less than that of control.

Photosynthetic end products: (Fig.III)

Carbohydrate content of the treated alga was reduced considerably except on eighth day when it was equal to that of control. 56% reduction was registered on twelfth day. Protein content was found to be reduced by 66% but lipid content was increased by 50%.

Growth: (Fig. I)

Growth was retarded and the biomass was less than that of control throughout the growth phase. By eighth day the cells were found sticking to the glass ware and unhealthy pale green colour appeared in the culture flask.

No. of treatment	Nutrients absorbed (in µg/l)		N / P
	Phosphate	Nitrate	
62	1000	536	0.54
Control	945	1290	1.37

The alga has taken up more phosphate than that of control. But nitrate uptake was reduced by more than 50%.

Conclusion:

The nett production was reduced for most of the growth period. This was accompanied by reduction in carbohydrate, protein and biomass.

Comparison:

The combination of cadmium and zinc elevated the respiration to a large extent, which also varied considerably as in the case of cadmium alone. The combination largely suppressed pheophytin while other pigments were promoted. But carbohydrate protein and lipid remained unaltered. The addition of zinc to cadmium did not produce noticeable variation from that of cadmium alone.

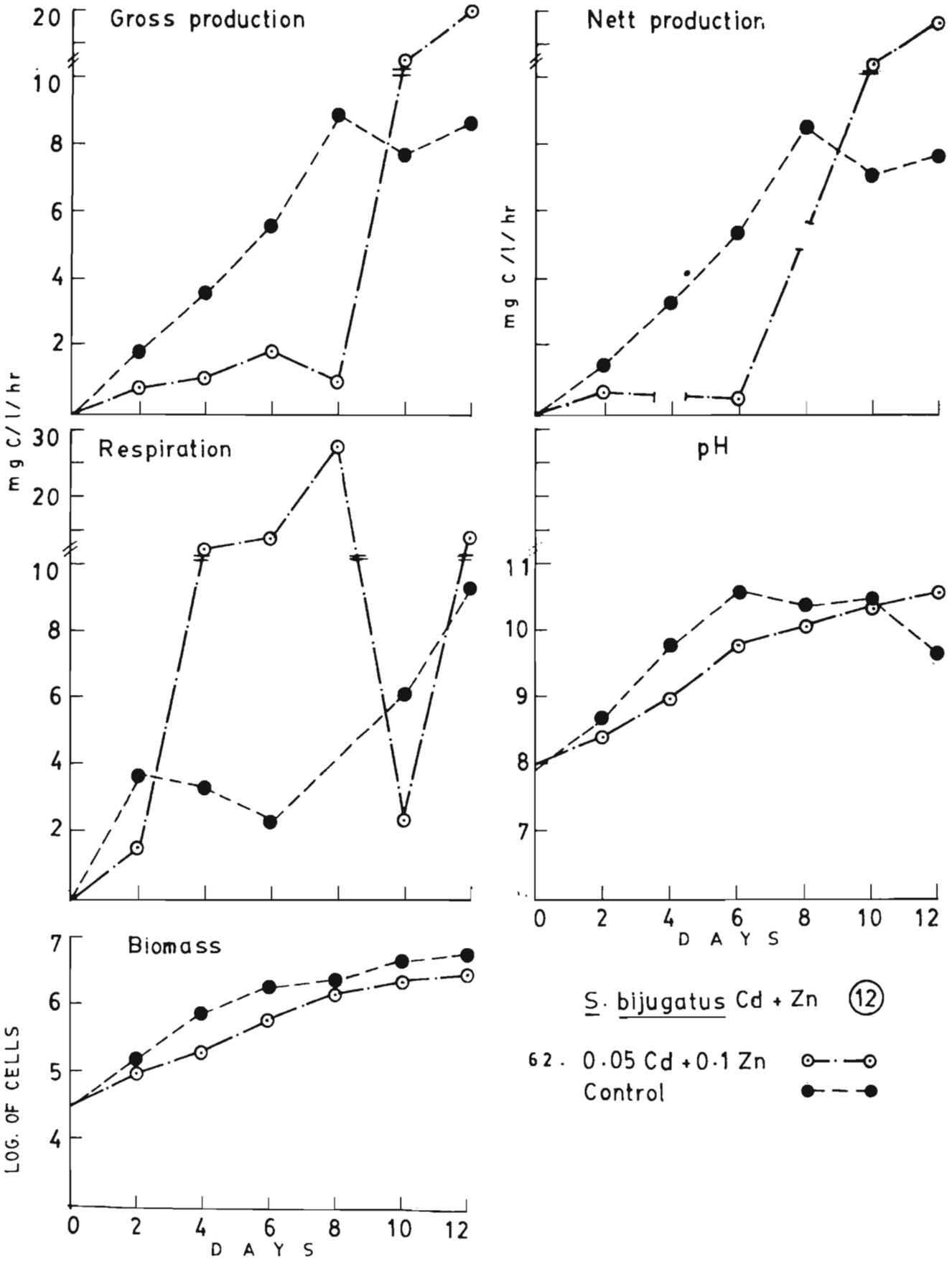

Gross production
Nett production
Respiration
pH
Biomass
mg C/l/hr
LOG. OF CELLS
DAYS
S. bijugatus Cd + Zn (12)
62. 0.05 Cd + 0.1 Zn
Control

Fig. I

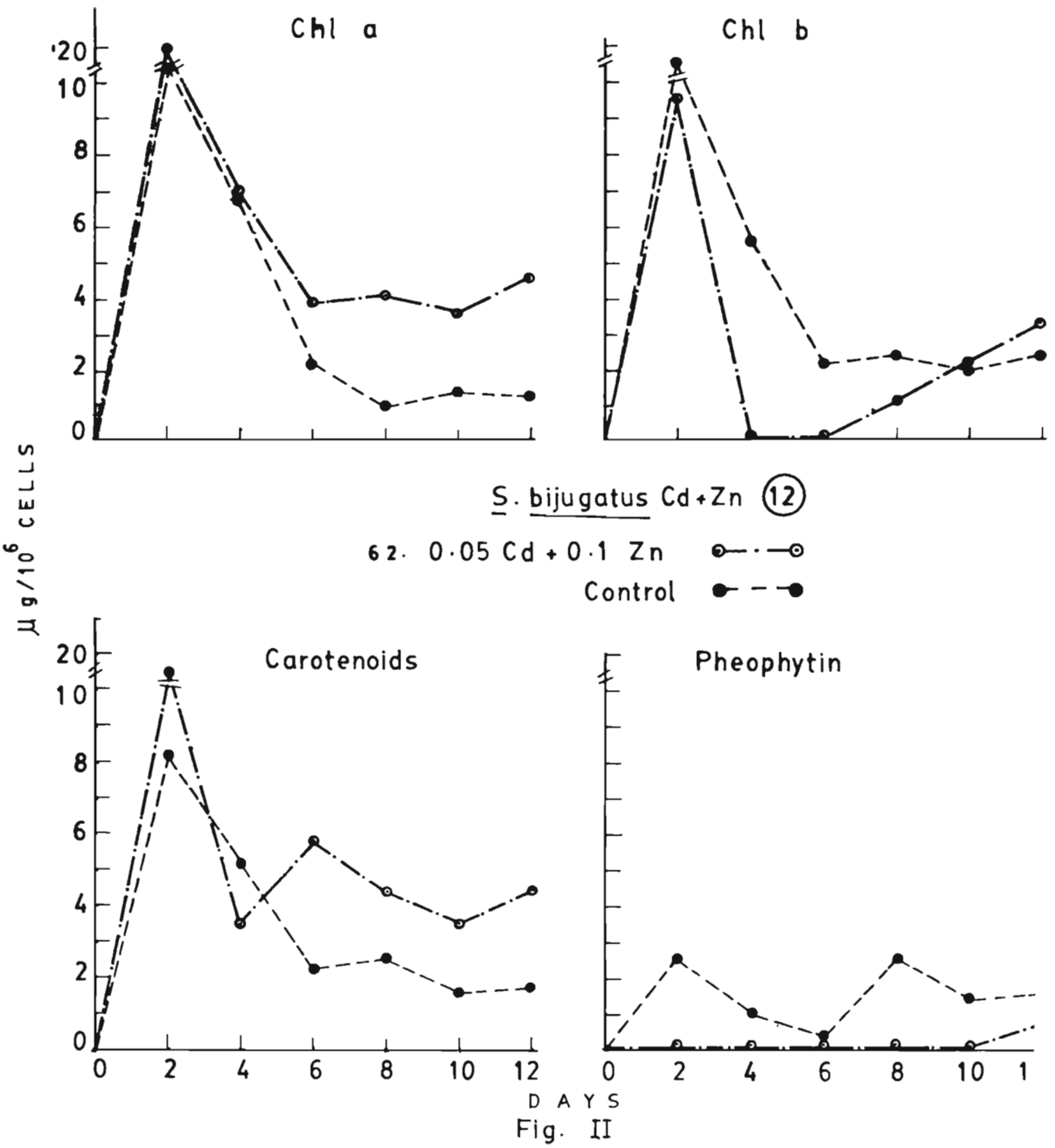
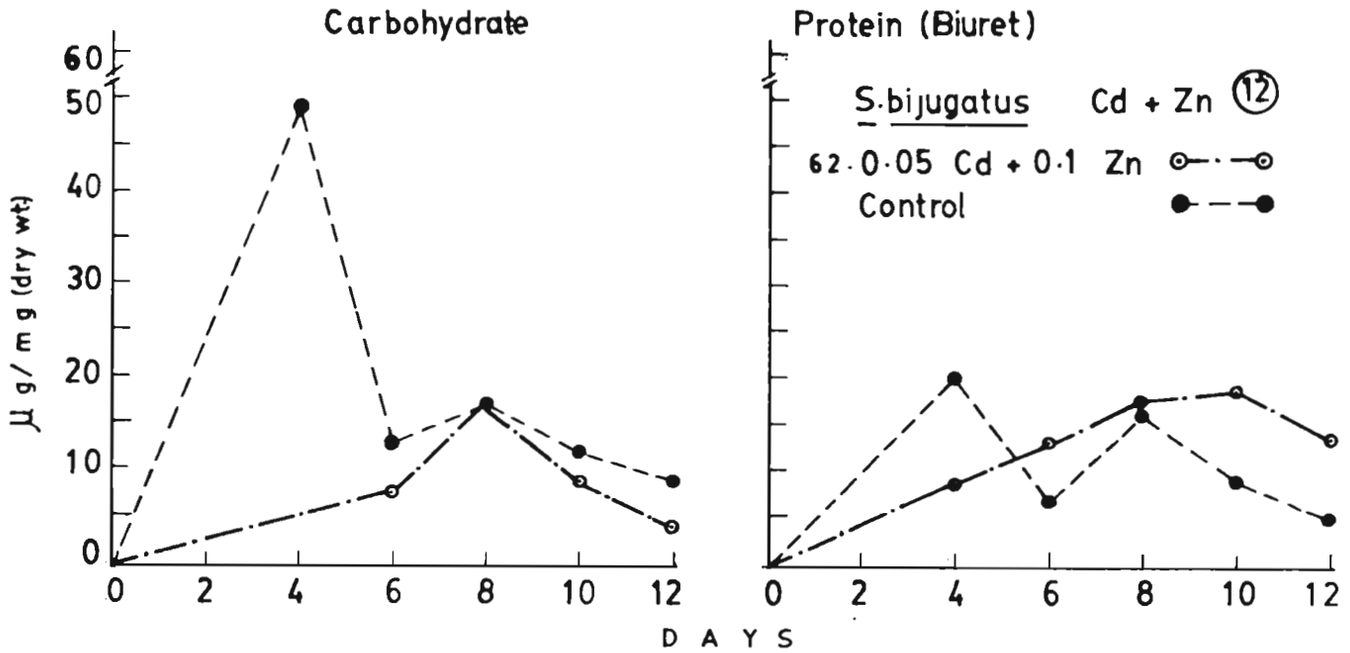

Chl a
Chl b
20
10
8
6
4
2
0
S. bijugatus Cd+Zn (12)
62. 0·05 Cd + 0·1 Zn
Control
µg/10⁶ CELLS
Carotenoids
Pheophytin
20
10
8
6
4
2
0
0 2 4 6 8 10 12
0 2 4 6 8 10 1
DAYS

Fig. II


Carbohydrate
Protein (Biuret)
S. bijugatus Cd + Zn (12)
62 · 0·05 Cd + 0·1 Zn
Control
µ g / m g (dry wt)
60
50
40
30
20
10
0
0 2 4 6 8 10 12
D A Y S

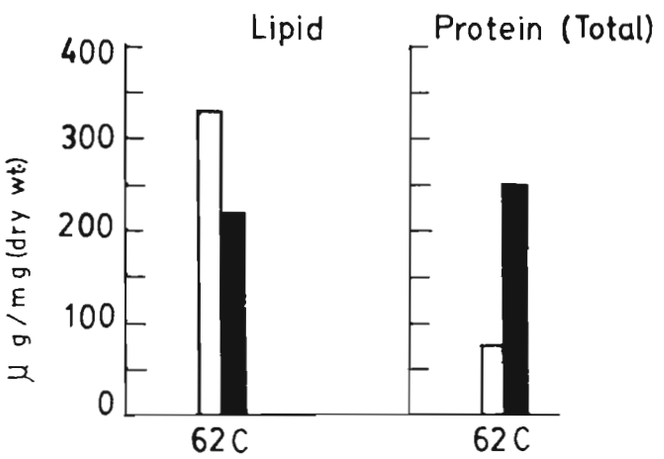

Lipid
Protein (Total)
µ g / m g (dry wt)
400
300
200
100
0
62 C
62 C

Fig. III

Combined effect of cadmium and zinc on *N. palea*

(Figs. I, II and III)

Sl. No. of combination	No. of treatment	Concentration of metals (in ppm)
(12)	63	0.04 Cd + 0.02 Zn
	64	0.04 Cd + 0.05 Zn

Production: (Fig. I)

The production of the diatom was reduced marginally in the early phase. On fourth day it was less than that of control by 5% and 32% respectively in the two treatments. Production of the diatom was same on sixth day in the two treatments, but was 48% less than control. Thereafter it increased to a greater extent in the latter and was less than that of control by 50% and 31% respectively on the last day.

Respiration of the diatom in first treatment fluctuated with peaks on fourth and eighth day with 560% and 58% increase respectively. It decreased towards the end of the growth phase but remained 32% higher than that of control. The dark bottle oxygen values were higher than the initial oxygen values in second treatment. But on sixth day it was 80% less than that of control. On eighth and tenth day of growth when oxygen value of the dark bottle remained equal to that of initial bottle.

pH of the culture in both the treatments increased gradually but was less than that of control except on tenth day.

Pigments: (Fig. II)

The pigment content of the diatom increased when it was exposed to the present combination of metals.

Concentration of chlorophyll a was marginally less than that of control in the early growth phase in both the treatments. But it increased to a greater extent in the second treatment reaching maximum on sixth day. Chlorophyll a exhibited fluctuation with peaks on fourth and eighth day in the first treatments. At the end of growth phase it did not vary between the treatments but was higher than that of control.

Chlorophyll c of the diatom in the first treatment remained more than that of control throughout the growth phase. The concentration fluctuated with peaks on second and sixth day respectively in both the treatments and exhibited a tendency to decrease towards the end of growth phase but remained higher than that of control in both instances.

Carotenoids of the diatom increased to a greater extent than any other pigment, though it did not vary much between treatments and that of control in early phase.

In both the treatments it reached a peak on sixth day and declined thereafter, to a greater extent in the former. Pheophytin content of the diatom increased considerably in the later growth phase. The concentration was less than that of control only on fourth day in the second treatment. This pigment also registered peaks on second and sixth day of growth. The level declined thereafter, till tenth day in the first but only till eighth day in the second and remained without further change till the end of growth phase.

Photosynthetic end products: (Fig. III)

The acid soluble carbohydrate fraction of the treated diatom was higher than that of control throughout the growth phase with 357% and 280% increase on fourth day and 71% and 119% increase on tenth day respectively in the two treatments.

The alkali soluble fraction also registered an increase in early phase by 214% and 293%, but the concentration decreased in the former treatment to 3% lower level at the end of growth phase whereas it was 49% higher in the latter.

The insoluble carbohydrate fraction of the diatom also increased in the early growth phase and was 100% and 96% higher respectively than that of control. It

continued to increase but not to the same extent as that of control, till tenth day, when it was 41% and 43% less respectively in the two treatments.

Protein content of the diatom was suppressed to a considerable extent in the early phase in the first treatment but improved when zinc concentration was higher on fourth day, it was 82% less than that of control in the former and 232% more in the latter. Thereafter protein increased to a considerable extent by tenth day in both instances, but it was found to be 60% and 63% less than that of control respectively.

Lipid was less than that of control by 40% and 37% respectively in the two treatments.

Growth: (Fig. I)

Growth rate was stimulated when the zinc level was high in the early phase. Thereafter it remained less than that of control. The biomass decreased in both the treatments when compared with that of control.

Details of nutrient uptake are given in the following table.

No. of treatment	Nutrients absorbed (in µg/l)		N / P
	Phosphate	Nitrate	
63	1870	818	0.44
64	1815	858	0.47
Control	1350	763	0.57

In both the treatments, nutrients were taken upto a larger extent than in the control. But between the treatments phosphate absorption decreased whereas nitrate absorption increased.

Conclusion:

The metals in combination exerted a negative effect on the physiology of the diatom by reducing nett production, protein and lipid contents and biomass.

Comparison:

When the effect of the present combination was compared with that exerted by the metals employed individually, production was found improved. Respiration was reduced when compared to the effect of zinc. Respiration was more than when cadmium alone was employed at 0.02 ppm zinc level but was much reduced at 0.05 ppm zinc level. The combination of metals increased the pigment content when compared to the effect of the metals tested individually. The acid soluble fraction was reduced when compared to the individual effect of zinc but more than when cadmium alone was employed. The alkali soluble fraction did not vary much but the insoluble fraction was found reduced. Protein and lipid were increased. Increase in zinc level reduced the biomass than when either metal was employed individually.

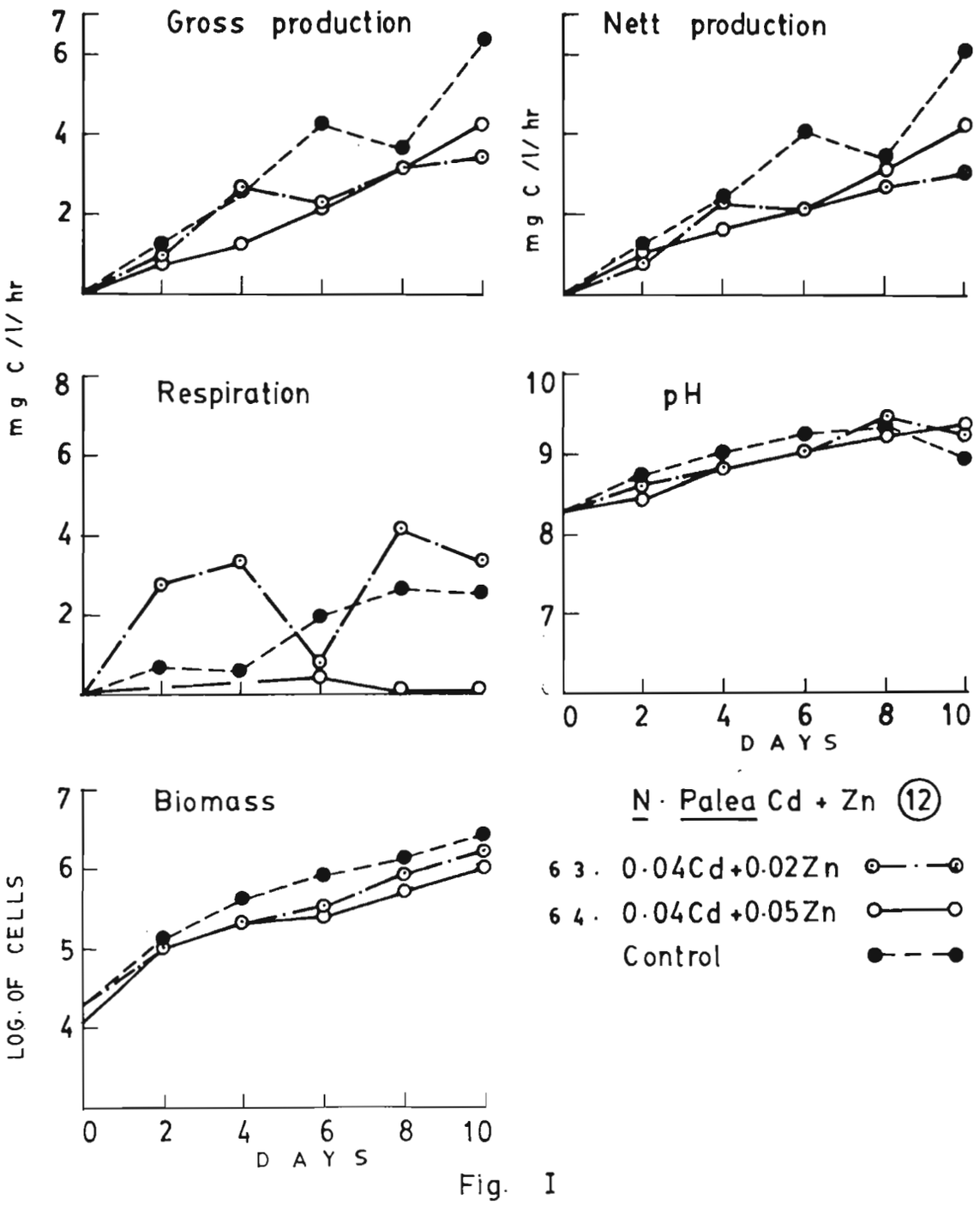

Gross production
Nett production
Respiration
pH
Biomass
mg C /l/ hr
LOG. OF CELLS
DAYS
N · Palea Cd + Zn (12)
63. 0·04Cd+0·02Zn
64. 0·04Cd+0·05Zn
Control

Fig. I

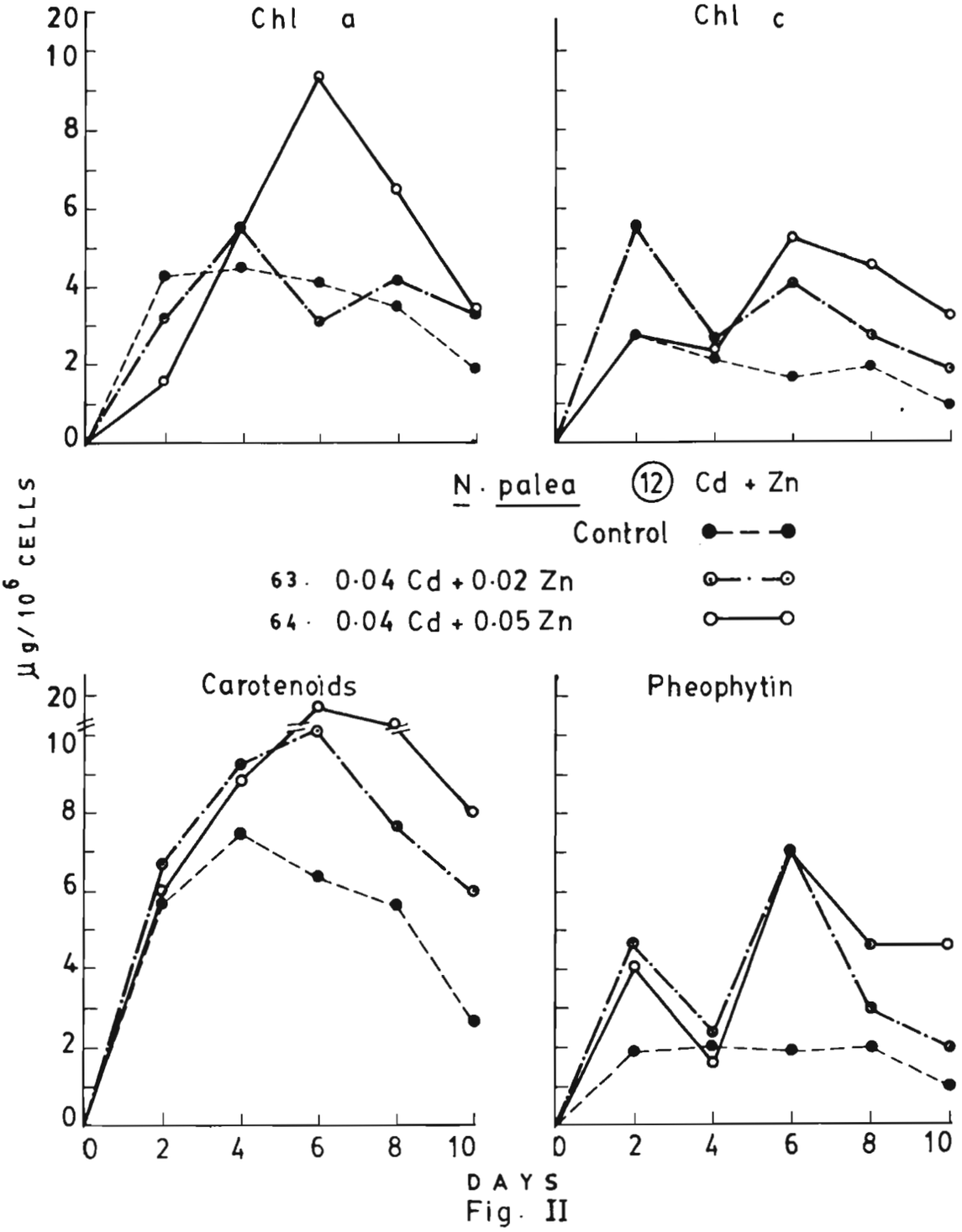

Chl a
Chl c
N. palea
(12) Cd + Zn
Control
63. 0·04 Cd + 0·02 Zn
64. 0·04 Cd + 0·05 Zn
µg/10^6 CELLS
Carotenoids
Pheophytin
DAYS

Fig. II

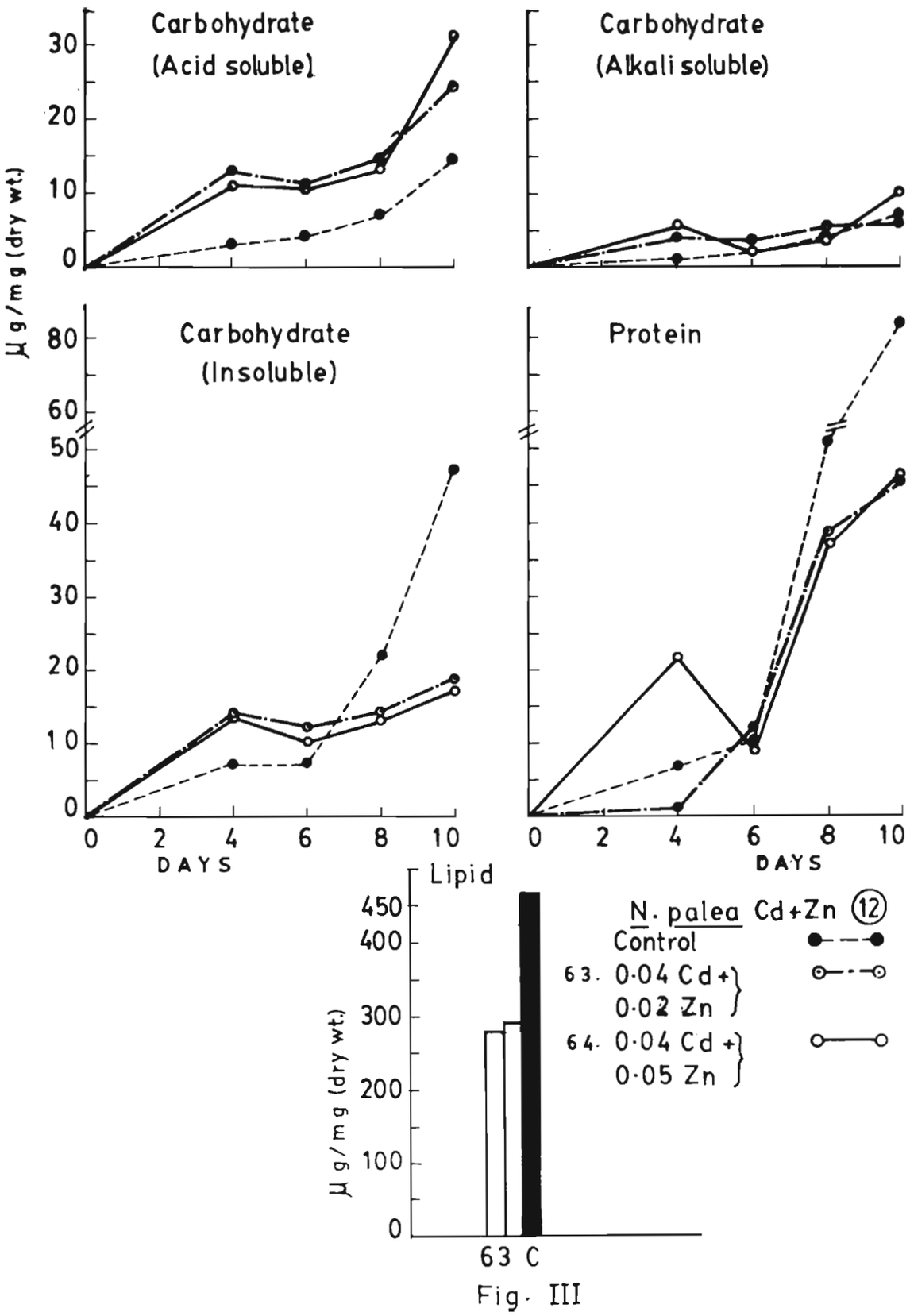

Carbohydrate
(Acid soluble)
Carbohydrate
(Alkali soluble)
Carbohydrate
(Insoluble)
Protein
µg/mg (dry wt.)
DAYS
Lipid
µg/mg (dry wt.)
N. palea Cd+Zn (12)
Control
63. 0·04 Cd + 0·02 Zn
64. 0·04 Cd + 0·05 Zn
63 C

Fig. III

.1.8

Combined effect of cadmium and iron on S. bijugatus (Figs. I, II and III)

Sl. No. of combination	No. of treatment	Concentration of metals (in ppm.)
(13)	65	0.05 Cd + 0.02 Fe
	66	0.05 Cd + 0.05 Fe

Production: (Fig. I)

Production of the alga was enhanced in the early phase. It was 143% and 79% higher than that of control on second day in the two treatments respectively. It declined thereafter, till sixth day to minimum, in the first treatment with 85% reduction and till fourth day, with 74% reduction in the second one. Once again production increased in both, till the end of the growth phase. It was 11% more in the first treatment on the last day but 10% less in the second.

Respiration of the alga increased gradually till eighth day in the first treatment. It exceeded that of control by sixth day. It was 7% higher on eighth day. It decreased thereafter upto tenth day but increased once again to 54% higher level by the end of growth phase. Respiration fluctuated in the second treatment with peaks on second and eighth day, registering 54% and 74% increas respectively. It was steady from fourth to sixth day.

In this instance also it showed reduction till tenth day and increased once again, but only to 1% higher level, at the end of growth phase.

pH of the culture was higher than that of control only on second and last day of growth. For most of the growth phase it was slightly higher in the first treatment compared to second one.

Pigments: (Fig. II)

Pigment content of the alga increased and was considerably higher than that of control except in the early growth phase.

Chlorophyll a and chlorophyll b remained much higher than that of control during most of the growth phase and carotenoids throughout. The maximum level attained by the control was exceeded in both the treatments. Also all four pigment showed fluctuation with two peaks in both the treatments. Generally, pigment concentration declined towards the end of growth phase. Pheophytin was not detected on twelfth day in both and also on tenth day in the second treatment.

In general chlorophyll a, chlorophyll b and carotenoids increased with increase in iron level towards the end of growth phase.

Photosynthetic end products: (Fig. III)

Unlike seen in the control, the carbohydrate content of the treated alga increased in the latter growth phase. On fourth day it was 90% and 70% less than that of control in the treatments respectively. It reached maximum level on sixth day with 99% and 69% increase. The concentration of this product decreased till twelfth day but remained 180% and 75% higher than that of control respectively.

The total protein was found to be higher than control by 44% and 18% respectively.

Lipid of the alga improved and was 143% and 134% more than that of control respectively.

Growth: (Fig. I)

The growth rate of the alga did not vary between the two treatments and was lower than that of control throughout the growth phase. Biomass was less than that of control throughout but reached slightly higher level in the first treatment at the end of growth phase.

Details of nutrient uptake are given in the following table.

No. of Treatment	Nutrients absorbed (in µg/l) Phosphate	Nitrate	N / P
65	860	847	0.98
66	860	388	0.45
Control	945	1290	1.37

Both the nutrients were taken up to a lesser extent than in control. Between the two treatments, phosphate absorption did not vary but nitrate absorption decreased with increase in iron level by 50%.

Conclusion:

When iron level was low respiration of the alga was low and production improved after Ten days of growth. Also the amount of carbohydrate, protein and lipid increased. Though biomass decreased in both the treatments the final yield was better when 0.02 ppm iron was employed but at 0.05 ppm it was less desirable.

Comparison:

When the effect of the present combination was compared with that obtained by employing cadmium alone, reduction in respiration was observed. Increase in pigments was found only during middle growth phase but towards the end it was not different from that exhibited when cadmium alone was employed. With addition of iron pheophytin was completely suppressed at the end of growth phase. Carbohydrate,

protein and lipid increased to a large extent but biomass was marginally lowered.

The presence of iron along with cadmium helped the species to return to near normalcy. The metals were found to be antagonistic in action.

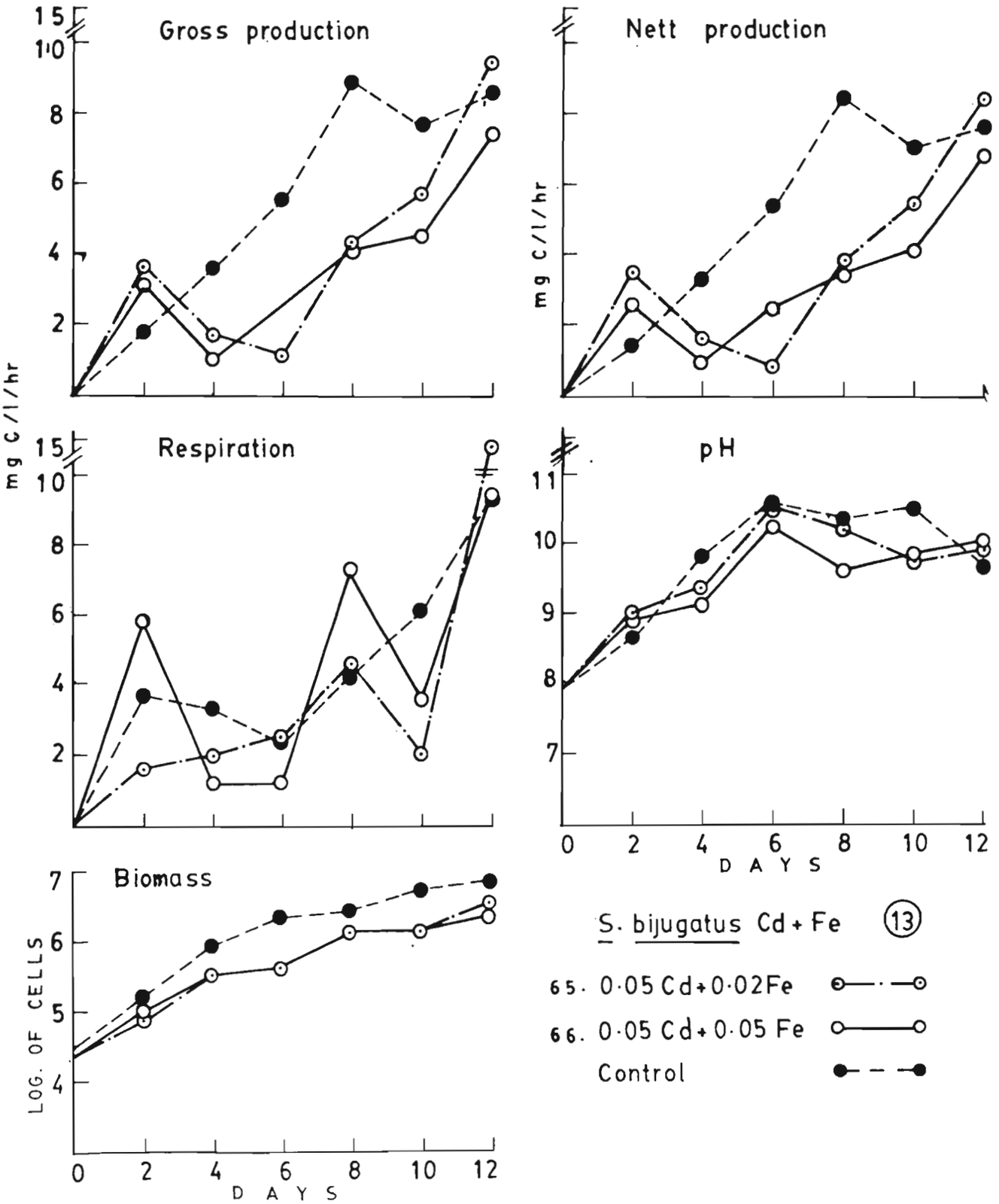

Gross production
Nett production
Respiration
pH
Biomass
mg C/l/hr
LOG. OF CELLS
DAYS
S. bijugatus Cd + Fe
13
65. 0·05 Cd+0·02Fe
66. 0·05 Cd+0·05 Fe
Control

Fig. I

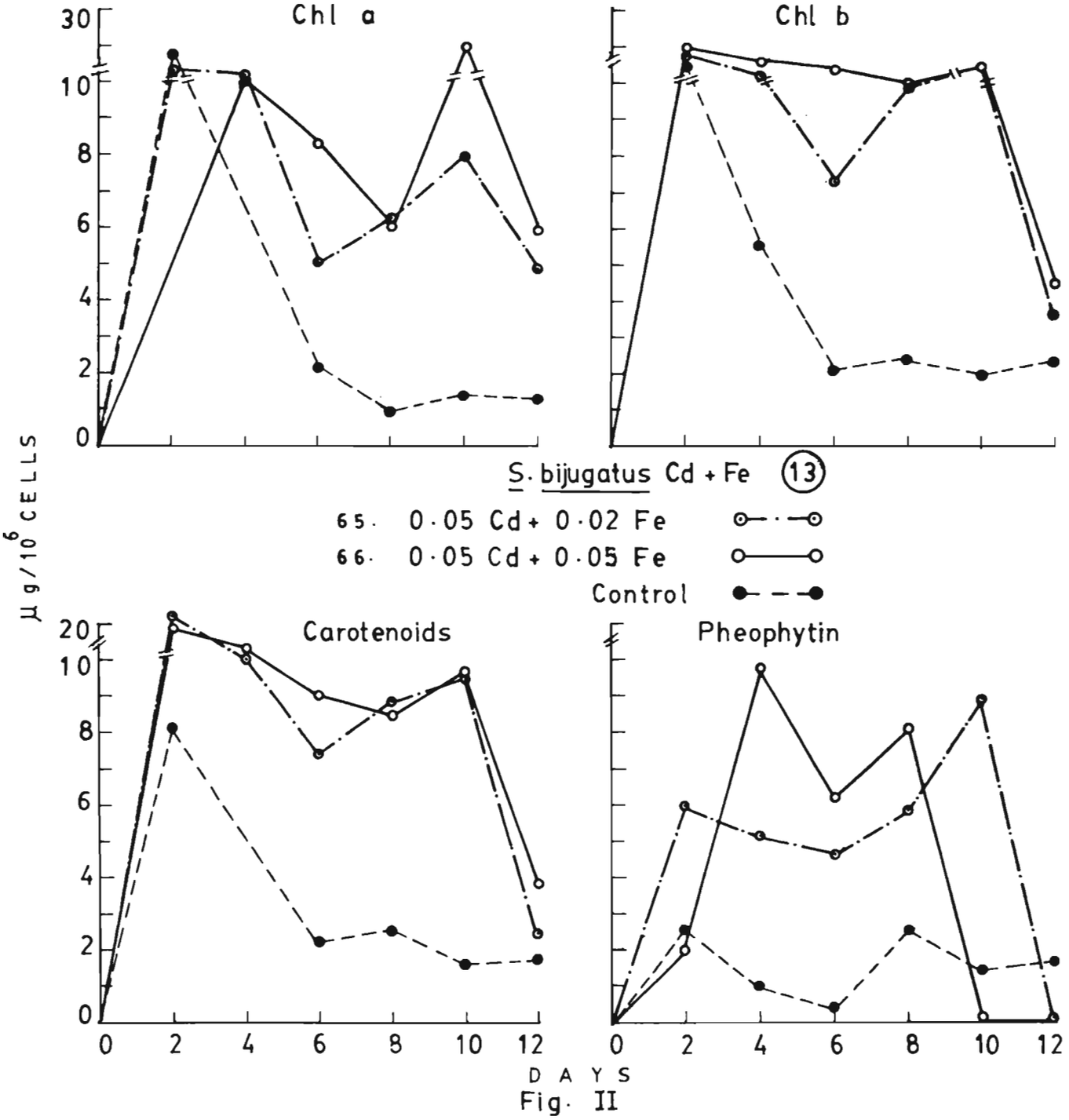
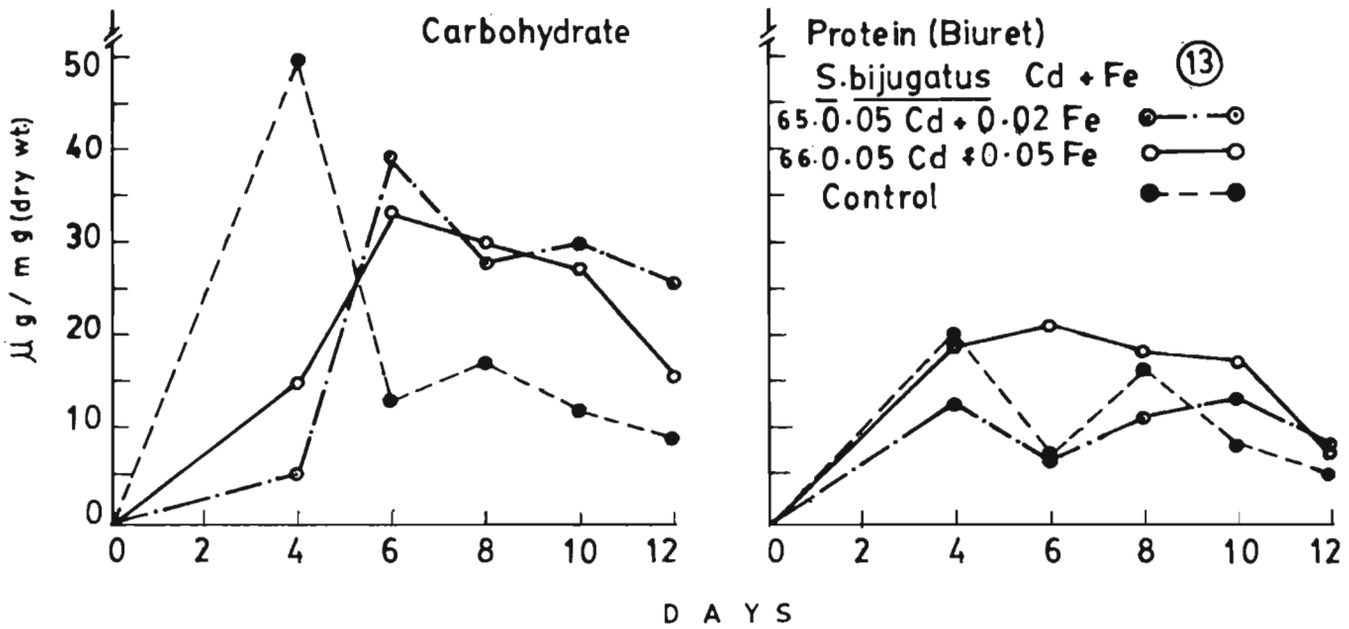

Chl a
Chl b
30
10
8
6
4
2
0
S. bijugatus Cd + Fe (13)
65. 0·05 Cd + 0·02 Fe
66. 0·05 Cd + 0·05 Fe
Control
µg/10^6 CELLS
Carotenoids
Pheophytin
20
10
8
6
4
2
0
0 2 4 6 8 10 12
DAYS

Fig. II


Carbohydrate
Protein (Biuret)
S.bijugatus Cd + Fe
65 0·05 Cd + 0·02 Fe
66 0·05 Cd + 0·05 Fe
Control
13
µ g / m g (dry wt)
0
10
20
30
40
50
0
2
4
6
8
10
12
D A Y S

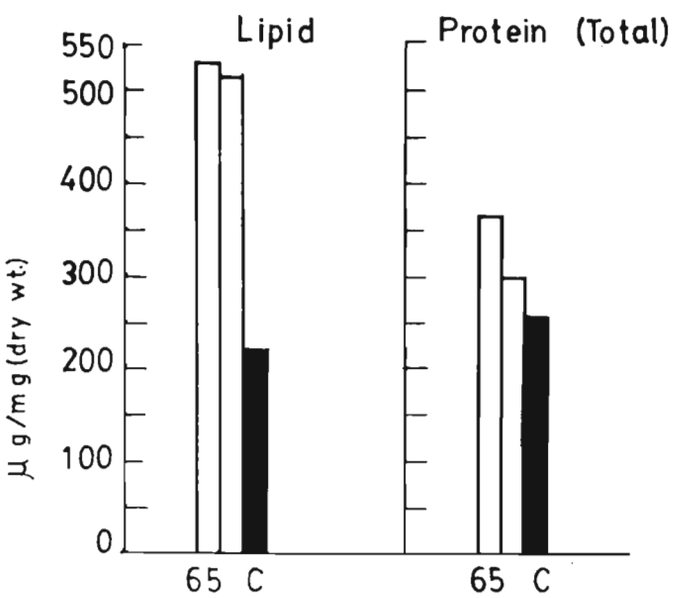

Lipid
Protein (Total)
µ g/m g (dry wt)
0
100
200
300
400
500
550
65 C
65 C

Fig. III

Combined effect of cadmium and iron on N. palea

(Figs. I, II and III)

Sl. No. of combination	No. of treatment	Concentration of metals (in ppm)
(13)	67	0.04 Cd + 0.02 Fe
	68	0.04 Cd + 0.05 Fe

Production: (Fig. I)

The rate of nett production in both the instances was reduced when compared to that of control. In the first treatment, production gradually increased till sixth day. No change was noticed between sixth and eighth day but increased once again till tenth day. The percentage reduction was 46 and 34 on eighth and tenth day respectively. Comparitively the rate of production in the second treatment was much lowered from fourth day onwards. From 69% lower level than that of control on fourth day, it decreased further, reaching minimum with 95% reduction. It increased thereafter but remained 75% less on tenth day.

Respiration of the diatom fluctuated severely in the first treatment with peaks on fourth and eighth day when it was 380% and 246% higher respectively, in relation to control. It was negligible on sixth day. It declined from eighth day but remained 48% higher than that of control.

In the other treatment it reached maximum on fourth day with 720% increase and declined thereafter to a negligible level on tenth day.

pH of the culture in the first treatment was higher than that of second treatment but was less than that of control except at the end of growth phase. It fluctuated in the second treatment and generally remained well below that of control.

Pigments: (Fig. II)

In general, pigment level of the treated diatom was much higher than that of control in the first treatment and all four pigments were maximum on sixth day. In the second treatment chlorophyll a and chlorophyll c were maximum on fourth day and pheophytin and carotenoids on second day. From the maximum level pigment content decreased in the first treatment but pheophytin fluctuated showing another peak on sixth day. In the second treatment chlorophyll c and carotenoids showed a decreasing tendency from the respective maxima, whereas chlorophyll a and pheophytin fluctuated with another peak. In both the treatments at the end of growth phase, pigment level remained higher than that of control. Carotenoids were produced to a larger extent than any other pigment in both the treatments.

Total pigment content of the diatom at the end of growth phase, with the exception of pheophytin, decreased with increase in iron level.

Photosynthetic end products: (Fig. III)

The acid soluble carbohydrate fraction was promoted in presence of the metal, to a greater extent in the first treatment. It was higher than that of control throughout growth phase with considerable increase from eighth day onwards. 76% and 144% increase was recorded on eighth and tenth day respectively. This fraction fluctuated in the second treatment with peaks on fourth and eighth day, with a respective increase of 573% and 207%. Declining from eighth day it reached the level of control on tenth day.

The alkali soluble carbohydrate was slightly higher in the treated diatom in the early phase. From sixth day it decreased in the former treatment to be 58% less and increased in the latter to be 93% more than that of control on eighth day. In both the treatments by tenth day it was found to be reduced by 7% and 47% respectively than that of control.

The insoluble carbohydrate fraction was enhanced in the early growth phase, When it was 34% and 147% more than that of control respectively, on fourth day

but was almost equal in both treatments on sixth day. The level was less than that of control after sixth day. It exhibited little variation from sixth to eighth day, increasing thereafter till the end of growth phase in the former and decreased in the latter. On both instances it was less than that of control by 56% and 82% respectively.

Protein concentration of the diatom improved to a large extent at 0.02 ppm iron level. It exhibited a gradual increase upto eighth day but was 35% less than that of control. Thereafter a sharp rise till tenth day to 7% higher level, was noted. The concentration was higher than that of control upto sixth day and on twelfth day. Protein content remained well below the level of control at 0.05 ppm iron with 55% reduction on sixth day. It reached maximum on eighth day, but with 40% reduction and declined thereafter to 84% lower level by the end of the growth phase.

Lipid content of the diatom was lowered compared to that of control in both the treatments, by 30% and 12% respectively.

Growth: (Fig. I)

Growth rates were impaired in presence of these metals, though initially, stimulation occurred in the first treatment, and retardation in the second treatment. The biomass remained much lower than that of control in both the treatments.

Details of the nutrient uptake are given in the following table.

No. of treatment	Nutrients absorbed (in µg/l) Phosphate	Nitrate	N / P
67	1090	853	0.78
68	670	655	0.98
Control	1350	763	0.57

The treated diatom absorbed less amount of phosphate than that of control. Also with the increase in iron level phosphate uptake was reduced, nearly by 50%. Nitrate uptake increased at 0.02 ppm iron but decreased at 0.05 ppm.

Conclusion:

When the effect of present combination was compared with that obtained by employing cadmium alone, it was found that when iron level was low (0.02 ppm), the respiration as well as protein content increased. But when iron level was high (0.05 ppm) nett production was lowered. The carbohydrate, protein and lipid were also reduced. Hence iron at 0.02 ppm along with cadmium appears to be less harmful than at 0.05 ppm. presumably due to the antagonistic action.

Comparison:

The rate of nett production was lowered in combinatinn than that observed when cadmium alone was employed. When the iron level was low increase in respiration was noted but when high, it was reduced. All four pigments increased. Also fluctuation was recorded when iron level was high, in chlorophyll a and pheophytin. The acid soluble carbohydrate fraction increased but insoluble fraction was reduced while the alkali soluble fraction remained unaffected. When the iron level was low, protein content increased to a large extent but when high it was not different from that obtained with cadmium alone. There was improvement in lipid with addition of iron to cadmium. But the biomass was lowered and also the growth rate in the early phase.

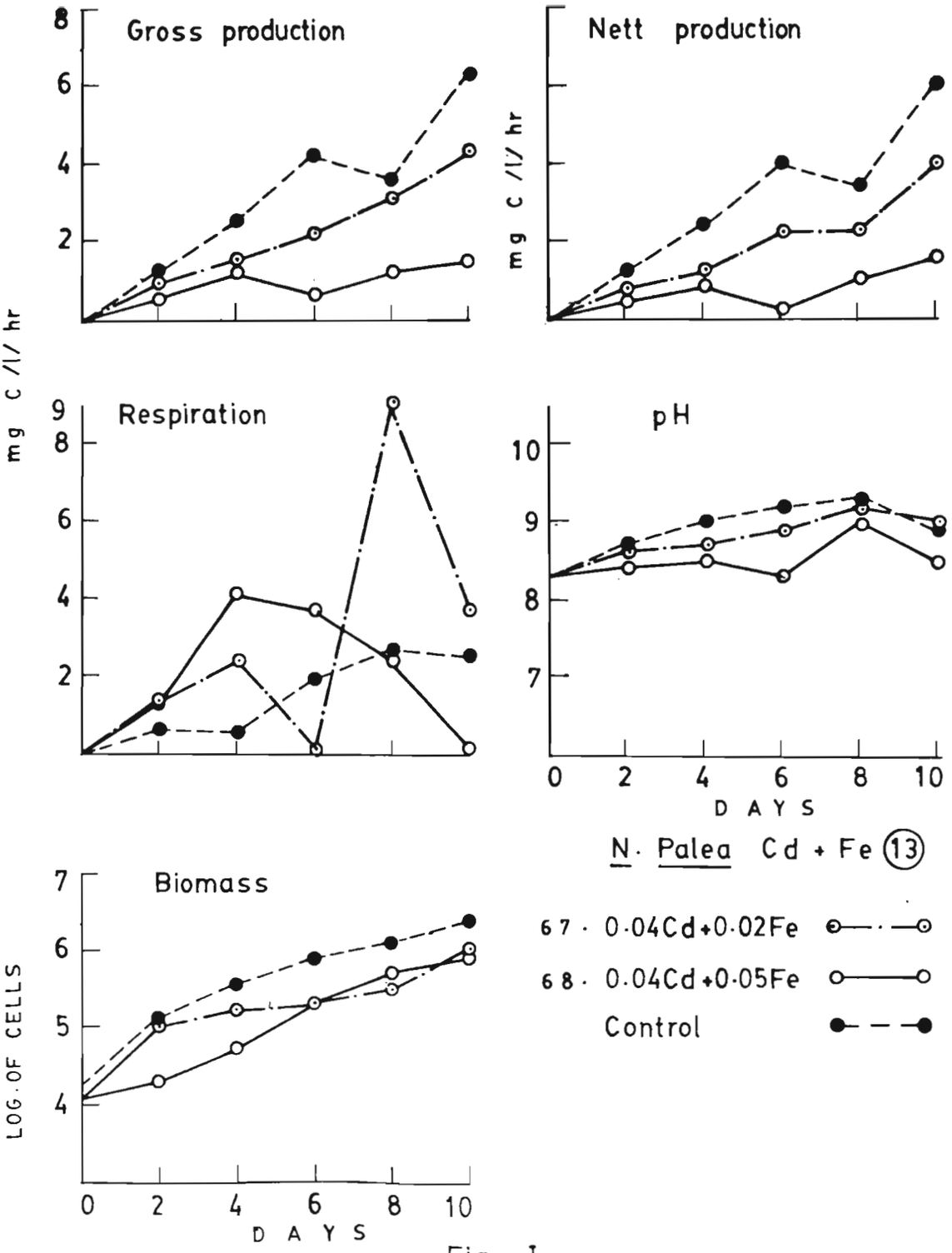

Gross production
Nett production
Respiration
pH
Biomass
mg C /l/ hr
LOG. OF CELLS
DAYS
N. Palea Cd + Fe (13)
67 · 0·04Cd+0·02Fe
68 · 0·04Cd+0·05Fe
Control

Fig. I

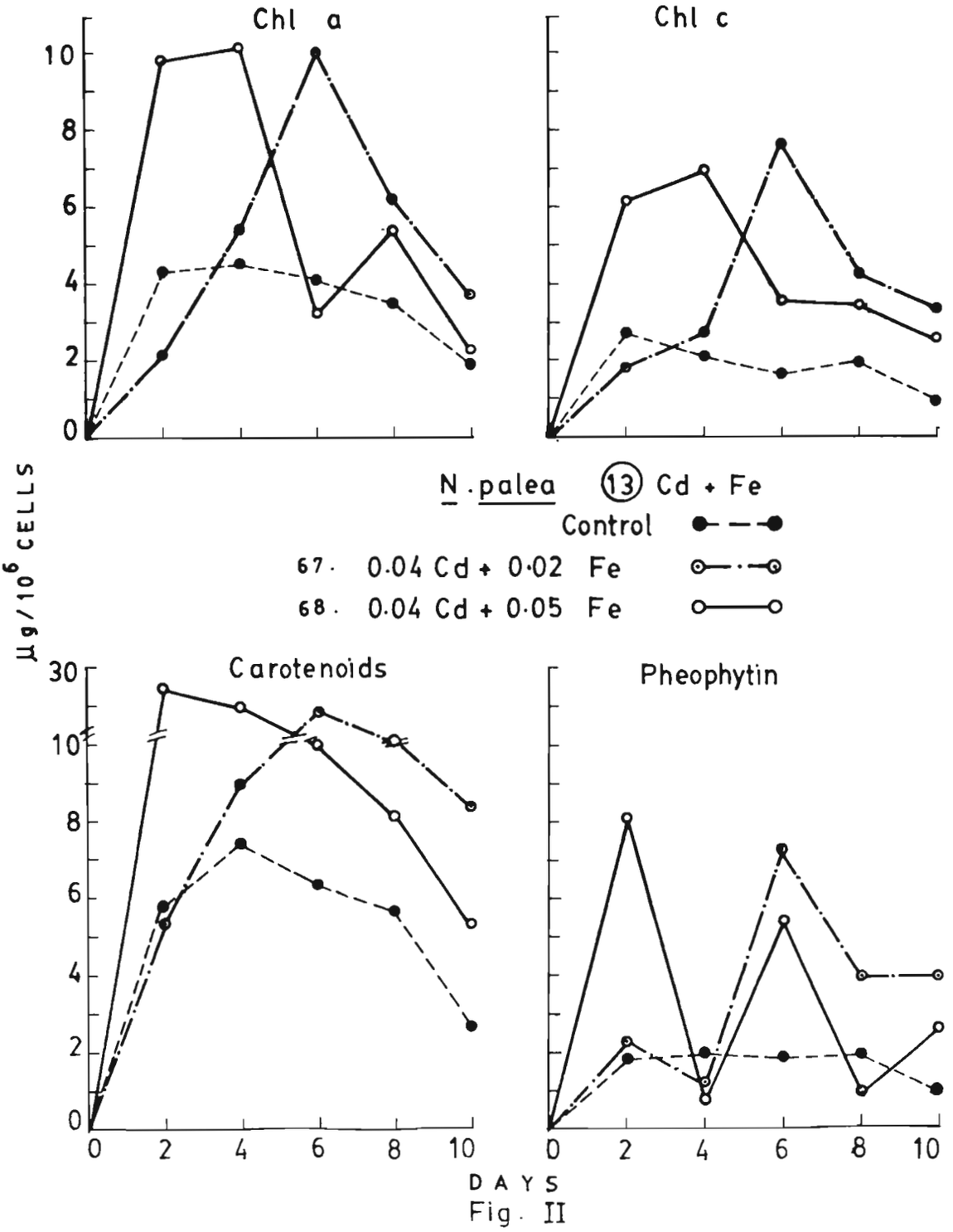

Chl a
Chl c
Carotenoids
Pheophytin
μg/10⁶ CELLS
DAYS
N. palea (13) Cd + Fe
Control
67. 0.04 Cd + 0.02 Fe
68. 0.04 Cd + 0.05 Fe

Fig. II

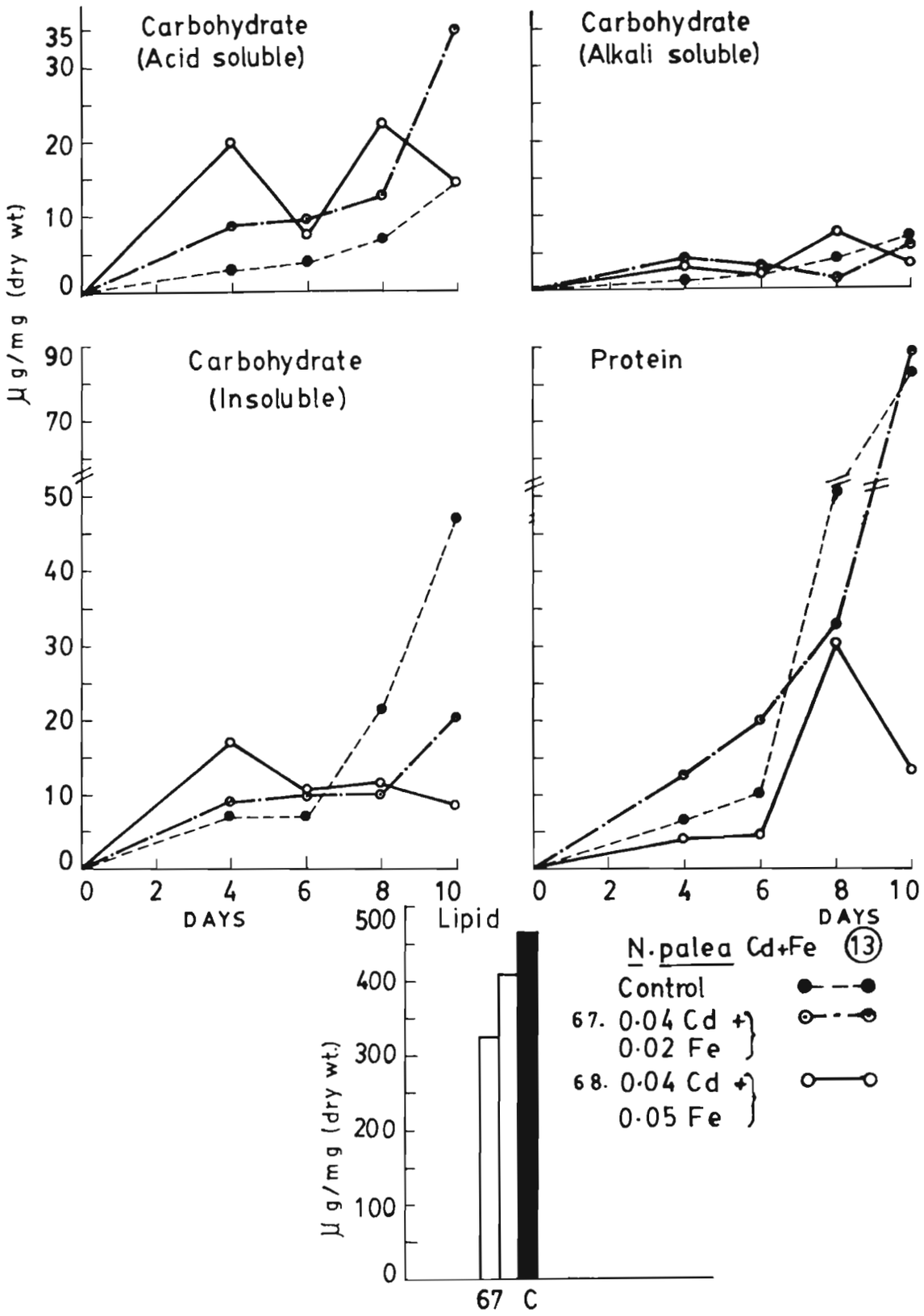

Carbohydrate
(Acid soluble)
Carbohydrate
(Alkali soluble)
Carbohydrate
(Insoluble)
Protein
µg/mg (dry wt)
DAYS
DAYS
Lipid
µg/mg (dry wt.)
67
C
N. palea Cd+Fe (13)
Control
67. 0·04 Cd + 0·02 Fe
68. 0·04 Cd + 0·05 Fe

Fig. III

6.1.9

Combined effect of lead and zinc on S. bijugatus

(Figs. I, II and III)

Sl. No. of combination	No. of treatment	Concentration of metals (in ppm)
(14)	69	0.3 Pb + 0.05 Zn
	70	0.3 Pb + 0.1 Zn

Production: (Fig. I)

The rate of nett production was lowered in both treatments when compared to that of control except on the last two days of growth. In the first treatment it was found to be 45% less than control on second day. It was nearly steady till fourth day and increased thereafter to reach maximum on twelfth day with 28% increase. In the second treatment the production was negligible till fourth day, increased thereafter till twelfth day when it was only 8% higher than that of control.

Respiration of the alga was lowered in the first treatment but elevated to a considerable extent in the second. It was 57% less than that of control on second day and remained steady till fourth day. It reached maximum on sixth day, being 44% higher than that of control and decreased thereafter, reached minimum on eighth day, but increased once again on tenth day. Rate of respiration was same on tenth and twelfth day and remained 73% less than

that of control on the last day. Respiration of the alga fluctuated in the second treatment and registered peaks on second and tenth day being 154% and 41% higher than that of control respectively. Thereafter it declined to minimum on twelfth day but remained higher than that of first treatment. It showed 60% reduction in relation to control.

pH of culture in both treatments was nearly equal to that of control on second day but further rise in pH was gradual and to a lesser extent than that of control, though reached a higher level on the last day. The maximum pH attained by control was not attained in treatment.

Pigments: (Fig. II)

Total pigment concentration was higher in the treated alga when compared to that of control. Also pigment development was delayed and it was measured from fourth day onwards. Maxima of all four pigments were attained later than those of control.

Concentration of chlorophyll a in the first treatment increased gradually till sixth day and declined thereafter reaching the same level as control on eighth day. It remained less than that of control thereafter. Chlorophyll a of alga in second treatment fluctuated with peaks on fourth and eighth day and was generally higher than that of control from fifth day onwards.

Chlorophyll b, in both treatments, was developed to nearly the same extent reaching maximum on fourth day and was higher than that of control thereafter.

Carotenoids have reached the maximum on sixth day in both treatments and thereafter remained higher than that of control. Between the treatments, it showed little variation. Pheophytins were not detected on fourth day in both the treatments and also on sixth day in the first treatment. It fluctuated with peaks on sixth and tenth day in the second treatment. In the first treatment it reached a maximum on eighth day and remained steady thereafter.

Chlorophyll a and chlorophyll b exhibited a tendency to increase towards the end of growth phase.

Photosynthetic end products: (Fig. III)

Carbohydrate content of the alga was estimated from sixth day onwards. It was found to be higher than that of control by 32% on sixth day in both. In the first treatment it increased gradually to be 305% higher than that of control at the end of growth phase, whereas in second treatment it increased by 123% to maximum on eighth day, decreasing thereafter gradually to be 240% higher on the last day of growth.

Protein was found to be 23% and 31% more than that of control in the two treatments respectively.

Lipid content of the alga was reduced by the metals in combination. It was 47% and 7% less than that of control respectively in the two treatments.

Growth: (Fig. I)

Rate of multiplication of the alga was lowered in the two treatments when compared to that of control and biomass was reduced in the treatments.

No. of treatment	Nutrients absorbed (in µg/l) Phosphate	Nitrate	N / P
69	1205	640	0.53
70	835	600	0.72
Control	945	1290	1.37

More phosphate was absorbed by the alga in the first treatment. But absorption decreased in the second treatment. About 50% reduction was observed in nitrate uptake in both the treatments.

Conclusion:

This particular combination of metals proved to be toxic from both biomass and end products points of view.

Comparison:

When the present effect was compared with the one obtained with individual metals, production and respiration were found to be less than by employing lead alone. Suppression of pheophytin was carried out to a greater extent than with lead and zinc alone. Total protein was more than that with zinc alone. Lipid was less than that when the metals were employed in isolation.

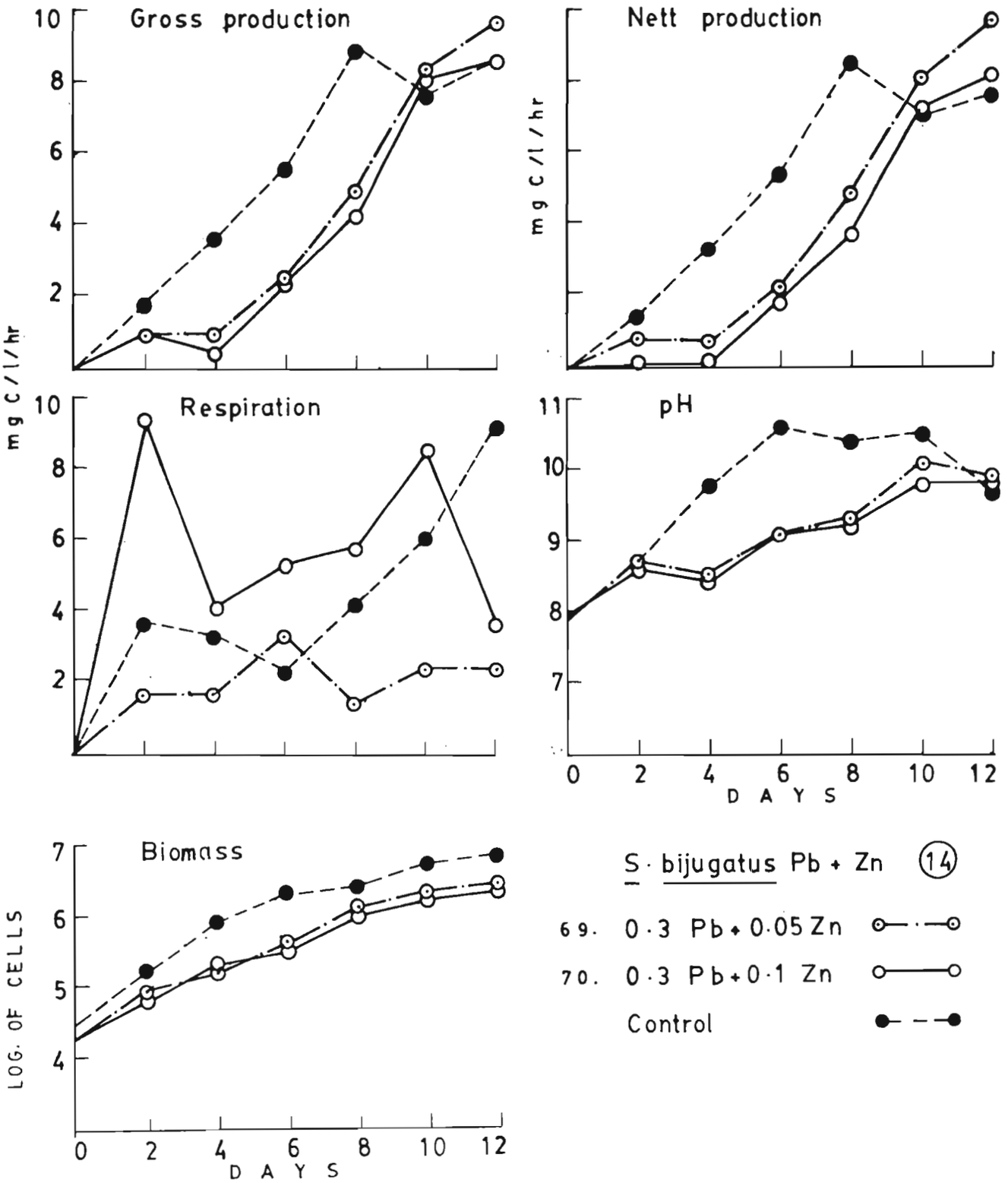

Gross production
Nett production
Respiration
pH
Biomass
mg C/l/hr
LOG. OF CELLS
DAYS
S. bijugatus Pb + Zn (14)
69. 0·3 Pb + 0·05 Zn
70. 0·3 Pb + 0·1 Zn
Control

Fig. I

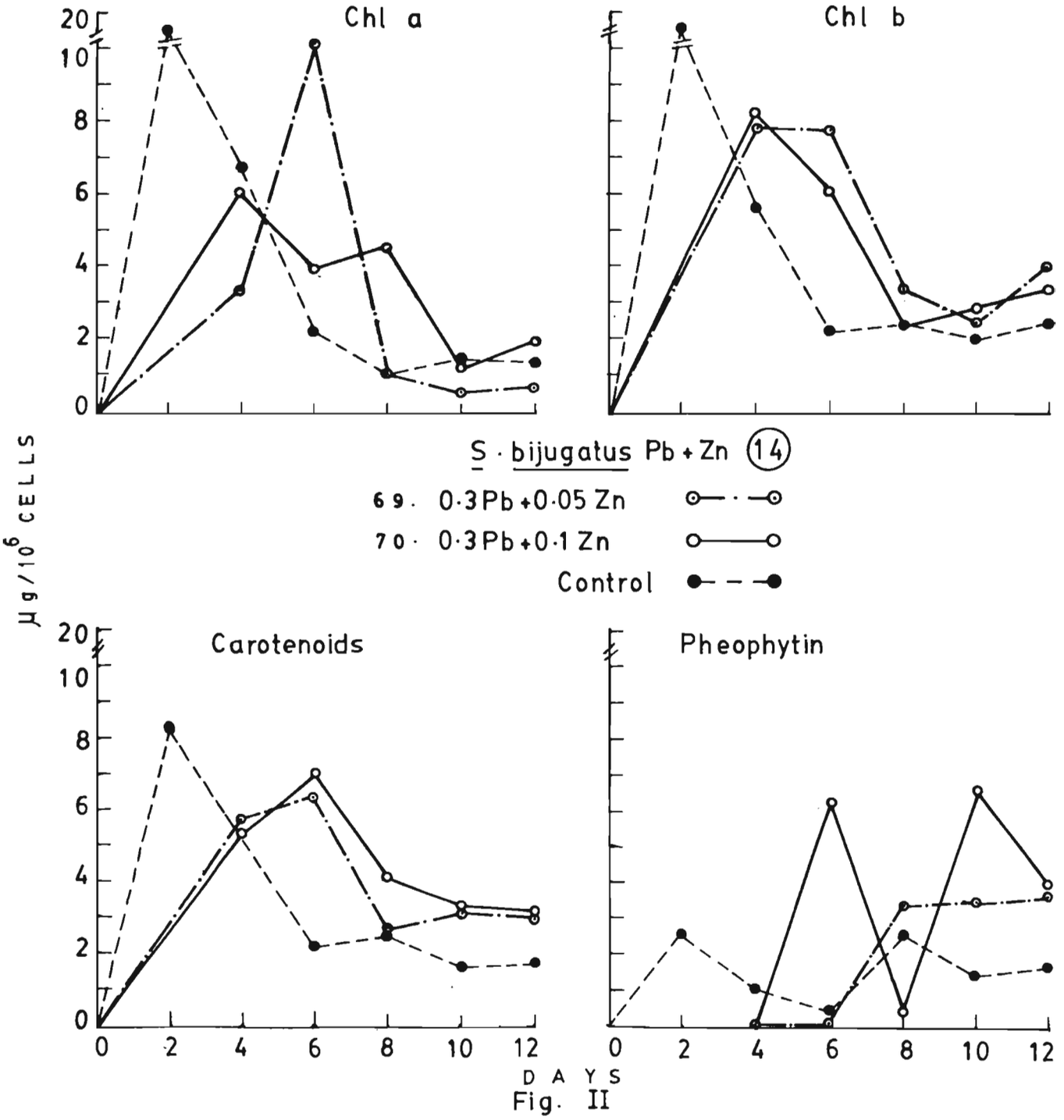
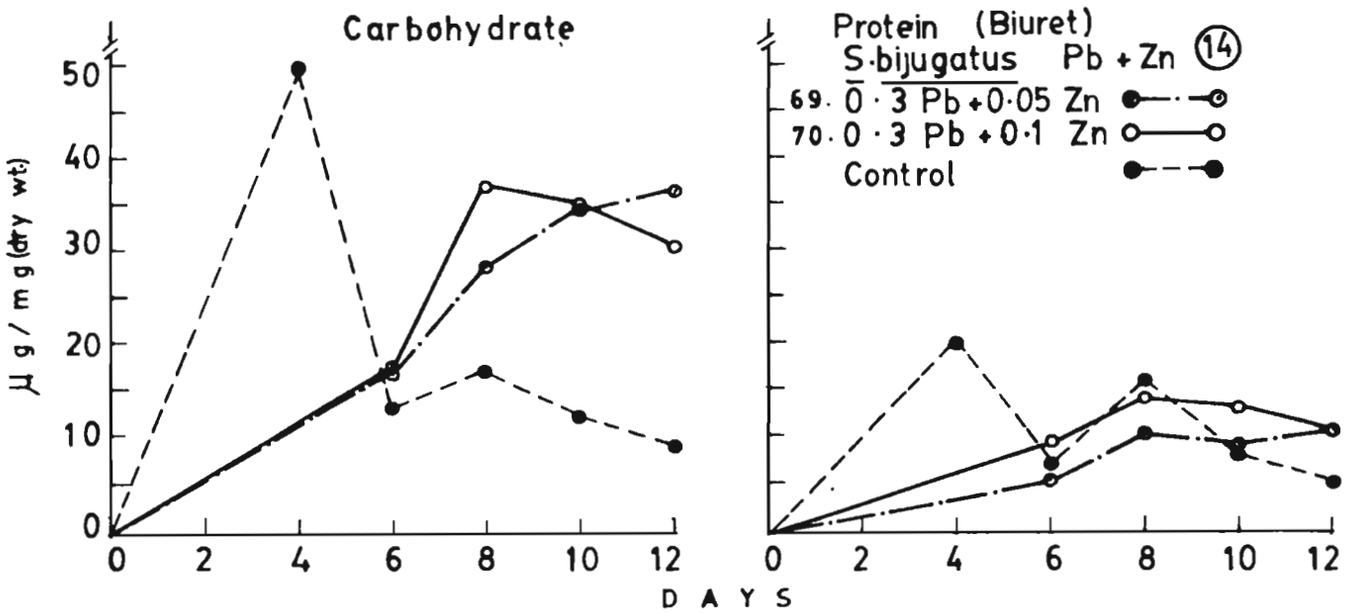

Chl a
Chl b
Carotenoids
Pheophytin
S · bijugatus Pb + Zn (14)
69. 0·3Pb + 0·05 Zn
70. 0·3Pb + 0·1 Zn
Control
µg/10⁶ CELLS
DAYS
0
2
4
6
8
10
12
20

Fig. II


Carbohydrate
Protein (Biuret)
S.bijugatus Pb + Zn
69. 0·3 Pb + 0·05 Zn
70. 0·3 Pb + 0·1 Zn
Control
14
µ g / m g (dry wt)
0
10
20
30
40
50
0
2
4
6
8
10
12
D A Y S

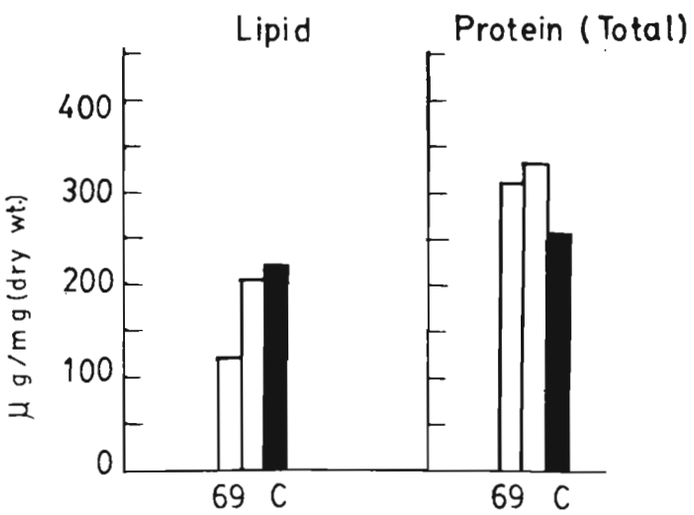

Lipid
Protein (Total)
µ g/m g (dry wt)
0
100
200
300
400
69
C
69
C

Fig. III

Combined effect of lead and zinc on N. palea

(Figs. I, II and III)

Sl. No. of combination	No. of treatment	Concentration of metals (in ppm).
(14)	71	0.04 Pb + 0.02 Zn
	72	0.04 Pb + 0.05 Zn

Production: (Fig. I)

The nett production in both the treatments remained less than that of control and was negligible on second day of growth. Thereafter it increased in both the treatments but was 45% and 39% less than that of control respectively on fourth day. Reduction was noted in both the instances from fourth to sixth day. Generally though production increased gradually from sixth day it was 56% and 43% less than control respectively on the last day of growth. From fourth day onwards production was slightly higher in the second treatment than in the first.

Respiration of the diatom was elevated in the early growth phase and was higher than the control upto fourth day. The increase was found to be 100% and 167% respectively on second day. No change in rate of respiration was noted between fourth and sixth day in the second treatment but the reduction was evident, being 47% on

sixth day. From sixth day onwards it showed a gradual increase till tenth day and was 48% higher than that of control. In the first treatment it increased from fourth to eighth day gradually but was 8% less. It declined further to 52% less than that of control on tenth day.

pH of the culture in both treatments was lowered owing to low production of the diatom. But on last day it was equal to that of control in second treatment. Maximum value of control (10.64) was not reached by the treated diatom.

Pigments: (Fig. II)

Total pigment content of the diatom increased in presence of these metals. All four pigments showed fluctuation in concentration with two peaks on second and sixth day and was higher than that of control. Concentration of chlorophyll a in the early phase did not differ much from that of control but on fourth day was found to be less. Thereafter it was generally higher throughout, except on eighth day in the second treatment. Maximum chlorophyll a was found on sixth day.

The level of chlorophyll c of the diatom remained higher than that of control throughout growth phase except on eighth day in the second treatment. In both treatments the maximum was reached on second day but was higher in the latter treatment.

Maximum amount of carotenoids was produced on sixth day in the former and on second day in the latter. In both it reached a level less than that of control on fourth day. At the end of growth phase concentration was higher in second treatment.

In both treatments maximum pheophytin was produced on second day. At the end of growth phase, the concentration was equal to that of control in the first treatment whereas it was slightly higher in the other.

All pigments exhibited a tendency to decrease in the first treatment but only chlorophyll a showed such trend in second treatment.

Photosynthetic end products: (Fig. III)

The acid soluble carbohydrate fraction was found to be more in treated diatom. On fourth day 290% and 173% increase was registered in the two treatments respectively. Concentration of this fraction declined in both the treatments till sixth day. It increased once again to maximum values of 272% and 134% on eighth day and on tenth day in the first and second treatments respectively. In the first treatment it decreased to the same level as that of control at the end of growth phase.

The alkali soluble carbohydrate fraction remained higher than that of control throughout growth phase in both

the treatments. Gradual increase till sixth day was recorded in the first treatment. It was 350% more on fourth, and by 410% on sixth, day. Marginal reduction was observed thereafter but on tenth day it was 37% higher than that of control. The concentration of this fraction in the second treatment was equal to that of first on fourth day but decreased by sixth day though remained 19% higher than that of control and increased thereafter, to be 156% on the last day of growth.

The insoluble carbohydrate fraction of the diatom also was found in greater quantity in both treatments on fourth day, with a respective increase of 166% and 139%. Fall in the concentration was noticed on sixth day in the first treatment. But subsequent increase by eighth day, to the same level of control was observed. It decreased thereafter to be 56% less than control on tenth day. In the second treatment no change was noticed in the concentration of this fraction between fourth and sixth day. It continued to increase thereafter but was found to be 3% less than that of control on eighth day and 28% on tenth day.

Protein content of the diatom increased to considerable extent when zinc was 0.05 ppm and remained throughout higher, than that of control. When the concentration of zinc was 0.02 ppm the protein content was higher except on the last day. It was equal to control on fourth

day, in first treatment and increased sharply till sixth day by 439%. The value though increased gradually thereafter it was 14% less than that of control, on the last day. In the second treatment increase recorded was 39% on fourth day, 367% on sixth day and 61% on the last day.

Lipid content of the diatom was lowered in both the treatments, by 59% and 22% respectively.

Growth: (Fig. I)

Growth rate of the diatom though was equal to that of control in the early phase no growth was noted in both treatments between fourth and sixth day and also between eighth and tenth day in second treatment. On eighth day the biomass in the second treatment was equal to that of control but the final yield was lowered at the end.

No. of treatment	Nutrients absorbed (in µg/l) Phosphate	Nitrate	N / P
71	1771	785	0.44
72	1821	600	0.33
Control	1350	763	0.57

The phosphate uptake increased in both and more phosphate taken up when zinc level was high. Nitrate uptake decreased at both levels of zinc.

Conclusion:

Reduction in photosynthesis and increase in pigment content was a common effect of both the combinations. When the level of zinc was higher lipid decreased but protein and carbohydrate increased. In general the combination was found to be undesirable.

Comparison:

When the effect of present combination was compared with those recorded when zinc and lead alone were employed, production was found to improve. When compared with that of zinc, chlorophyll a and chlorophyll b improved but carotenoids were unaffected. Pheophytin was lowered. When compared with the effect of lead alone chlorophyll a and carotenoids were found unaffected and chlorophyll b increased. But fluctuation in carotenoids and chlorophyll b were prevalent. While the acid soluble carbohydrate fraction was reduced, the alkali soluble fraction improved. When the zinc was higher, insoluble carbohydrate fraction increased. Otherwise no difference was observed. Protein and lipid increased. The biomass was not affected though initial rate of multiplication was slightly retarded.

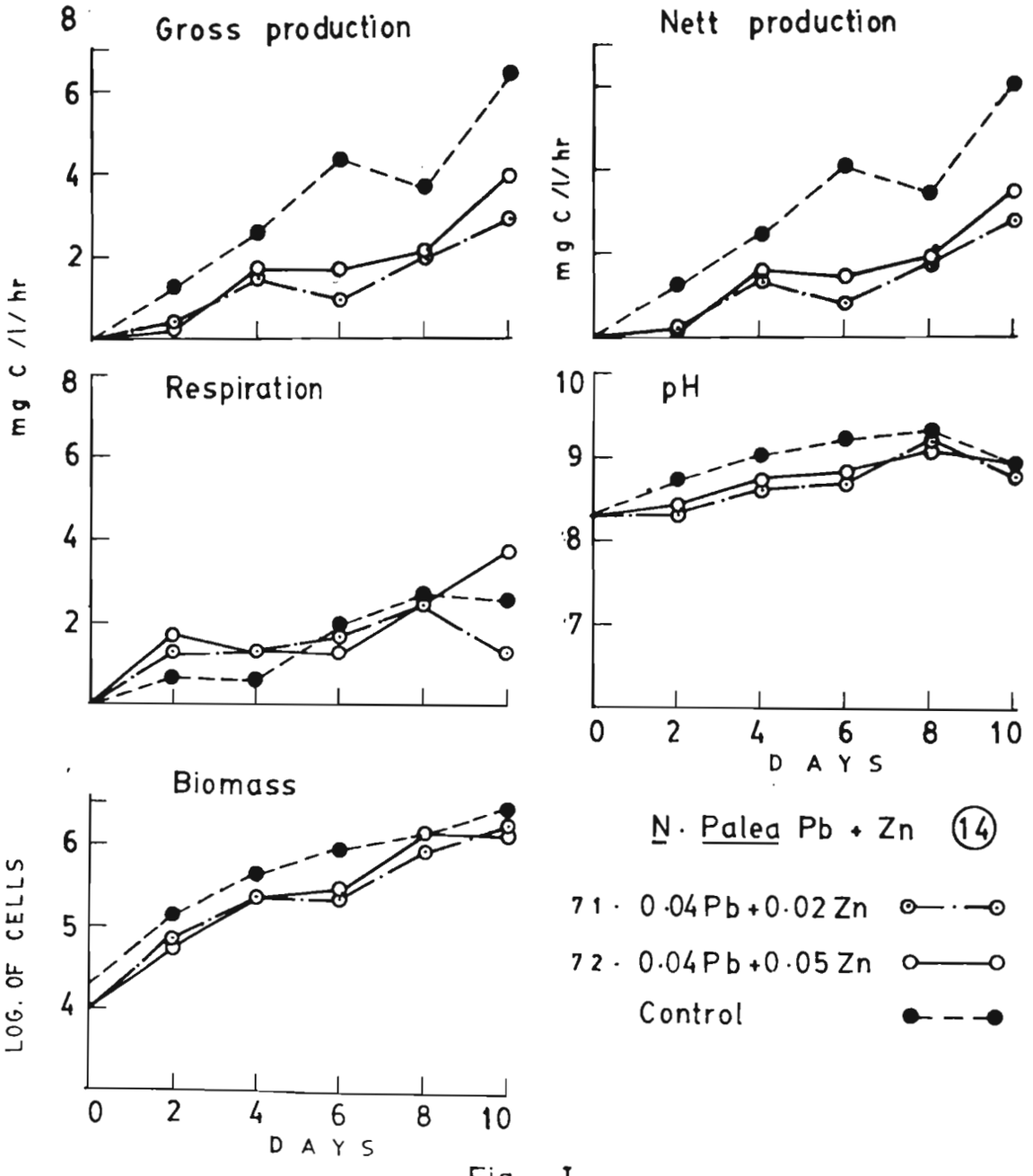

Gross production
Nett production
Respiration
pH
Biomass
mg C /l/ hr
LOG. OF CELLS
DAYS
N · Palea Pb + Zn (14)
71 · 0·04 Pb + 0·02 Zn
72 · 0·04 Pb + 0·05 Zn
Control

Fig. I

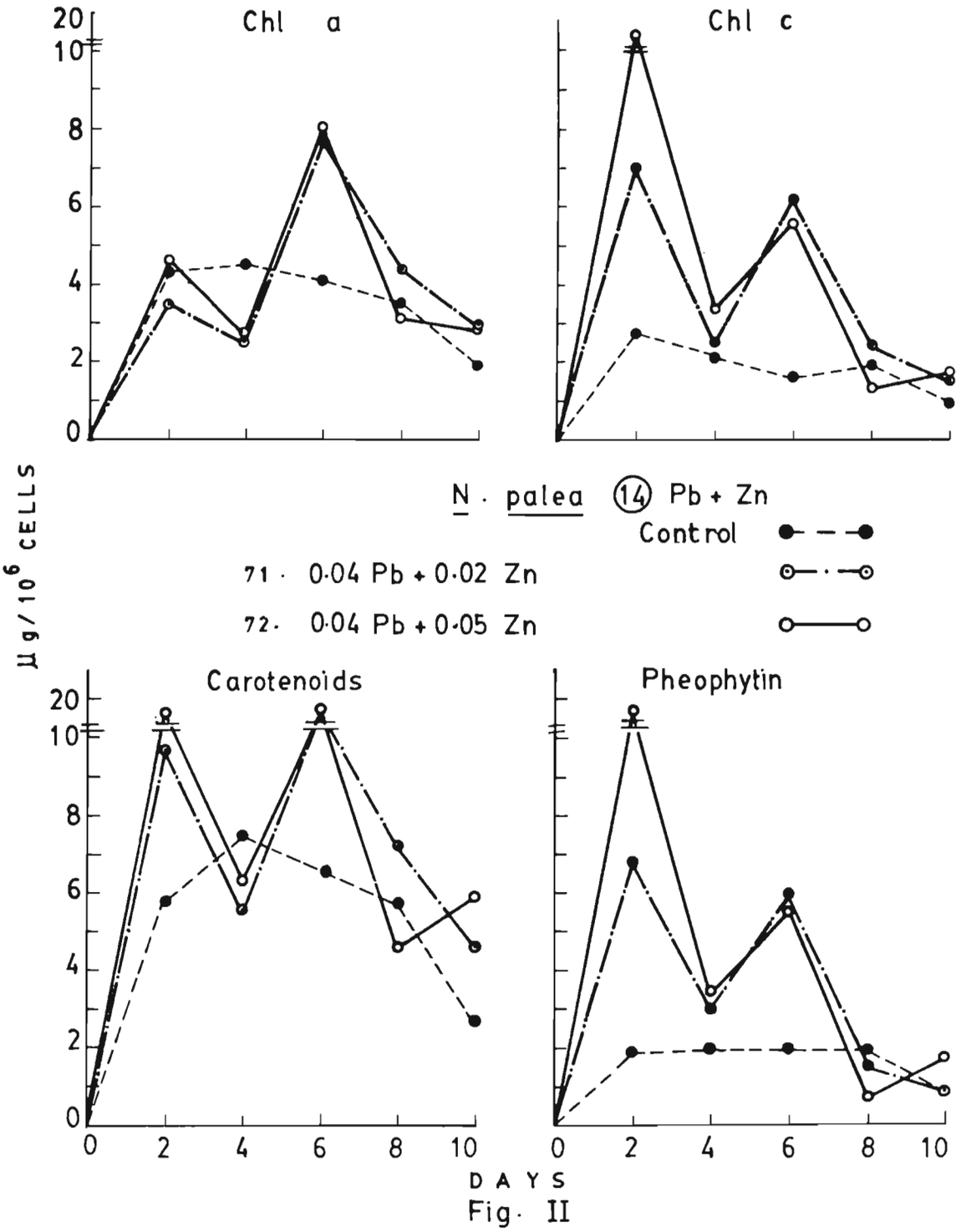

Chl a
Chl c
N. palea (14) Pb + Zn
Control
71. 0·04 Pb + 0·02 Zn
72. 0·04 Pb + 0·05 Zn
Carotenoids
Pheophytin
μg/10^6 CELLS
DAYS
0
2
4
6
8
10
20

Fig. II

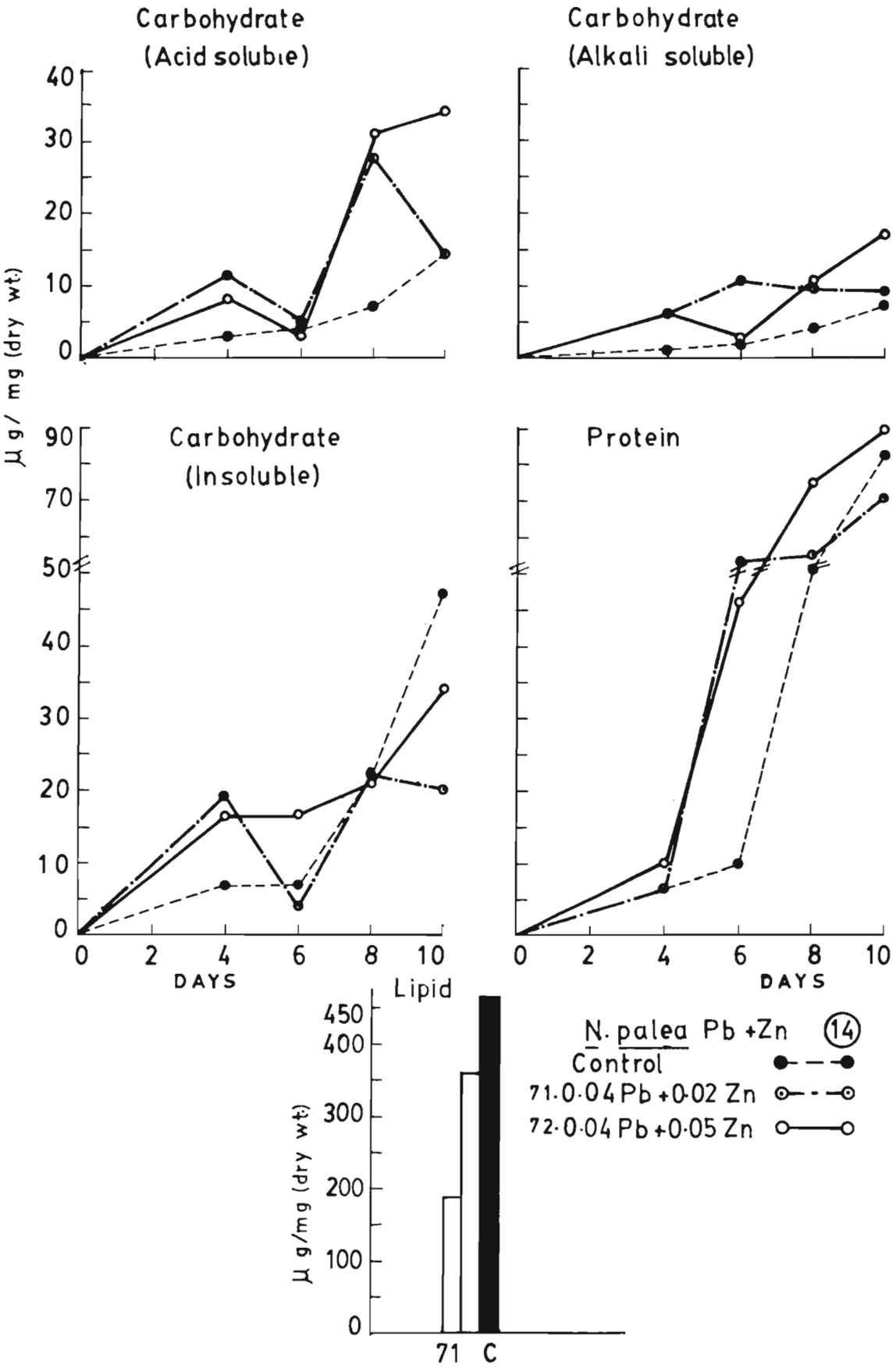

Carbohydrate
(Acid soluble)
Carbohydrate
(Alkali soluble)
Carbohydrate
(Insoluble)
Protein
µg/ mg (dry wt)
DAYS
DAYS
Lipid
µg/mg (dry wt)
71
C
N. palea Pb +Zn (14)
Control
71·0·04 Pb +0·02 Zn
72·0·04 Pb +0·05 Zn

Fig. III

6.1.10

Combined effect of lead and iron on S. bijugatus

(Figs. I, II and III

Sl. No. of combination	No. of treatment	Concentration of metals (in ppm)
(15)	73	0.3 Pb + 0.05 Fe
	74	0.3 Pb + 0.1 Fe

Production: (Fig. I)

Nett production generally was lowered during middle phase of growth. On second day, the production of the alga in first treatment was equal to that of control but increased by 36% in the other. It decreased to minimum on fourth day, increased gradually till tenth day to maximum, the value being 24% more than control in the first treatment. But it declined to 47% less than that of control on twelfth day. In the second treatment, production declining from fourth day reached minimum on sixth day and increased thereafter till the end of growth phase reaching 30% and 74% higher than control respectively on tenth and twelfth day. Very little difference was observed in the production of the alga between the treatments during the middle growth phase.

Respiration increased when exposed to the present combination of metals. In the first treatment it increased

reaching maximum on eighth day the value being 174% higher in relation to control. Sharp decline was noticed thereafter and on tenth day it was 60% less than that of control. Towards the end of growth phase it once again showed an increase but was 47% less than that of control. (For this treatment both nett production and respiration at the end were 47% less than those of control). In the second treatment respiration of the alga fluctuated, with peaks on fourth and eighth day, with an increase of 179% and 245% respectively. From its maximum on tenth day it dropped to 74% less than that of control, on the last day.

Notwithstanding the lowered rate of nett production and high rate of respiration, the pH of culture in both treatment remained slightly higher than that of control except on tenth day.

Pigments: (Fig. II)

The treated alga produced pigments to a greater extent during growth phase. But at the end only chlorophyll a was found to be more than that of control whereas chlorophyll b was reduced, caretenoids were nearly equal and pheophytin was totally suppressed.

Production of chlorophyll a was delayed and the maxima were reached on eighth day in first treatment and on fourth day in the second treatment. The level of

this pigment fluctuated to a greater extent in the second treatment. Concentration was higher than that of control from sixth day onwards in the first treatment and from fourth day onwards in the second. Towards the end of the growth phase it was almost steady in the first treatment but declined in the second treatment.

The concentration of chlorophyll b was higher than that of control from sixth day onwards in first treatment and from fourth day onwards in the second, except on the last day of growth when it was less than control in both treatments. Here too, the concentration of this pigment registered fluctuation to a greater extent in the second treatment.

Carotenoid content of the alga was slightly higher in the second treatment than in the first treatment though it followed similar pattern of development. Considerable increase was observed in the level of this pigment after second day in both the treatments. However after tenth day it declined to reach almost the same level as that of control.

Pheophytin was not detected on second, eighth and twelfth day in the first treatment and on second, fourth and twelfth day in second. This pigment exhibited severe fluctuation in the first treatment with peaks on

sixth and tenth day, whereas in the other treatment it reached maximum on sixth day and declined thereafter.

All pigments showed a decreasing trend towards the end of growth phase.

Photosynthetic end products: (Fig. III)

The treated alga produced more carbohydrate in the later phase of growth, the concentration being higher than that of control from sixth day onwards. In the first treatment it increased upto sixth day and it was 187% higher than that of control and remained steady till eighth day. It showed a gradual increase till twelfth day when it was 426% more than that of control. In the second treatment concentration of this product increased gradually reaching maximum on tenth day, when it was 269% higher than that of control, declined thereafter but remained 327% higher. But in neither treatment, the maximum concentration attained by the control was not reached.

Protein was found in greater concentration in the treated alga. It was 31% and 20% higher than that of control respectively.

The lipid content of the alga was promoted to a large extent, by 142% and 171% respectively.

Growth: (Fig. I)

The rate of multiplication was equal in early growth phase to that of control but was subsequently retarded. The biomass in both treatments did not increase from fourth to sixth day, but increased thereafter in the treatments upto tenth day. In the second treatment it increased only towards the end of growth phase.

Details of the nutrient uptake are given in the following table.

No. of treatment	Nutrients absorbed (in µg/l) Phosphate	Nitrate	N / P
73	910	500	0.55
74	928	380	0.41
Control	945	1290	1.37

The nitrate absorption decreased by 60% and 70% respectively in the two treatments but phosphate absorption was reduced only marginally.

Conclusion:

Inspite of reduced rate of production during most of growth phase the carbohydrate, protein and lipid of the alga in first treatment increased. Increase in iron level resulted in more number of cells and marginal reduction in the end products. Generally the combination helped

the alga by promoting overall production of carbohydrate, protein and lipid. But it was found to be undesirable since it caused severe disturbance in the proportion of end products.

Comparison:

When the effect of present combination was compared with that obtained by employing lead alone, the influence of lead was found to prevail on production and respiration. Addition of iron, however, brought down respiration towards the end of growth phase. The presence of iron also caused an increase in chlorophyll b, carotenoids and pheophytin. Though protein did not show any marked variation, lipid improved to a large extent with the addition of iron. Biomass however was reduced.

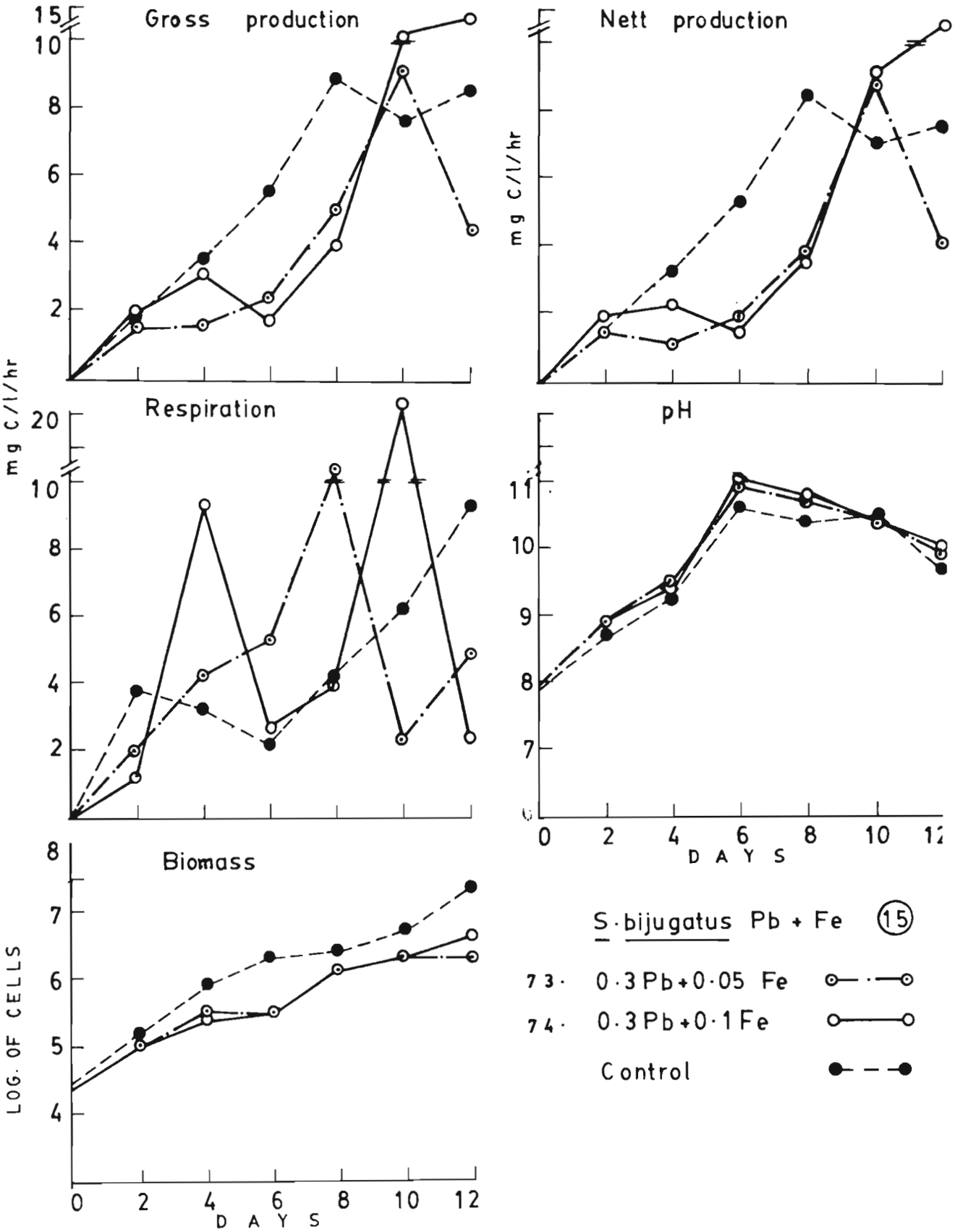

Gross production
Nett production
Respiration
pH
Biomass
mg C/l/hr
LOG. OF CELLS
DAYS
S. bijugatus Pb + Fe (15)
73. 0·3 Pb + 0·05 Fe
74. 0·3 Pb + 0·1 Fe
Control

Fig. I

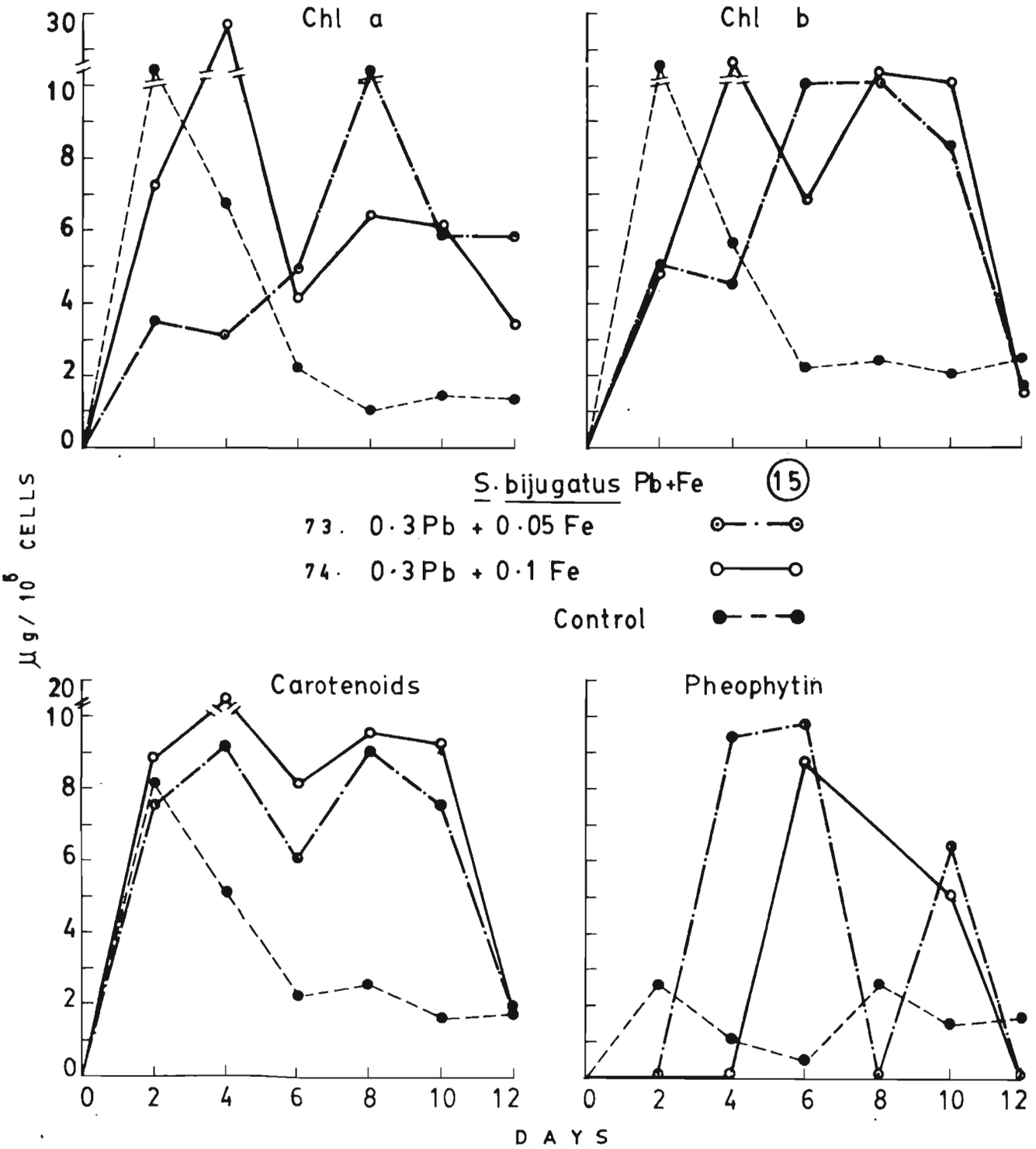
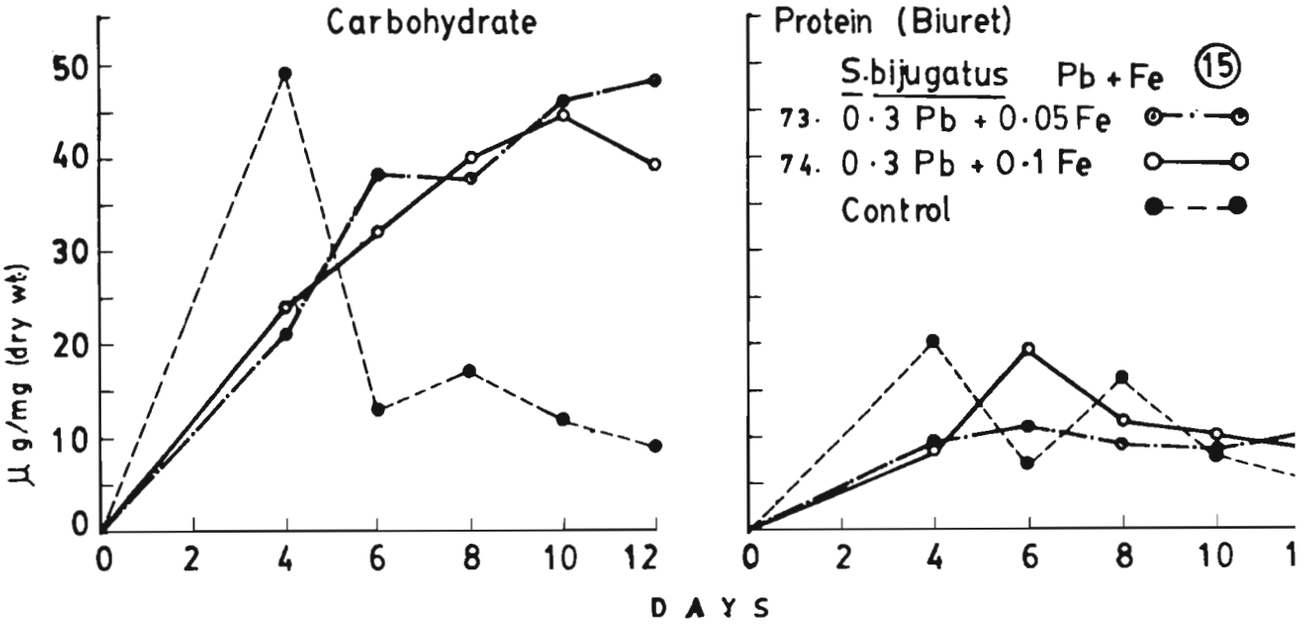
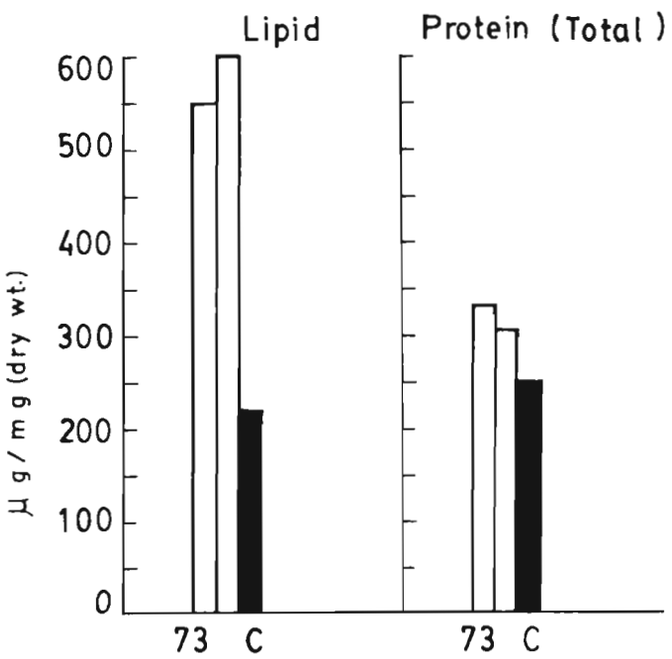

Chl a
Chl b
30
10
8
6
4
2
0
S. bijugatus Pb+Fe
15
73. 0·3Pb + 0·05 Fe
74. 0·3Pb + 0·1 Fe
Control
µg / 10^6 CELLS
Carotenoids
Pheophytin
20
10
8
6
4
2
0
0 2 4 6 8 10 12
DAYS

Fig. II


Carbohydrate
Protein (Biuret)
S.bijugatus Pb + Fe
73. 0·3 Pb + 0·05 Fe
74. 0·3 Pb + 0·1 Fe
Control
15
µ g/mg (dry wt)
0
10
20
30
40
50
0 2 4 6 8 10 12
0 2 4 6 8 10 1
DAYS


Lipid
Protein (Total)
µ g / m g (dry wt.)
0
100
200
300
400
500
600
73 C
73 C

Fig· III

Combined effect of lead and iron on N. palea

(Figs. I, II and III)

Sl. No. of combination	No. of treatment	Concentration of metals (in ppm)
(15)	75	0.04 Pb + 0.02 Fe
	76	0.04 Pb + 0.05 Fe

Production: (Fig. I)

The nett production was lowered to considerable extent in both treatments and remained less than that of control throughout the growth phase. It was negligible on the second day in second treatment but in both cases it increased till fourth day when it was 23% and 39% less than that of control respectively. It declined in the first treatment and was 85% less than that of control on sixth day. Though it increased thereafter till the end of growth phase remained 40% less than that of control. In the second treatment from fourth to eighth day there was little variation in the production but thereafter it increased to reach the same level attained by the diatom in first treatment. The percentage reduction on eighth and tenth day was 46 and 41 respectively, in relation to control.

Respiration of the diatom varied to a greater extent in the first treatment with peaks on second and

sixth day when it exhibited 100% and 95% increase respectively. It was less than that of control on fourth day and negligible on eighth day but increased considerably thereafter to reach only 4% lower level than that of control. In the second treatment respiration was negligible on second day but there after increased gradually reaching maximum on sixth day, being 74% higher than that of control. It then declined to lower level on eighth day. It increased towards the end of growth phase and was 32% higher than that of control, on the last day.

Owing to low rate of nett production, pH of the culture except on the last two days, was less than that of control. It did not vary much between the treatments.

Pigments: (Fig. II)

Total pigment content of the treated diatom was considerably higher than in control. Maximum concentration of pigment chlorophyll a, chlorophyll c and carotenoid was noted on second day as in control. Pheophytin maximum was also found on second day, whereas the control maximum was found only on fourth day. At their respective maxima all pigments were found to be much higher than that of control. The diatom in first treatment produced more pigment than in second.

Photosynthetic end products (Fig. III)

Considerable increase in the carbohydrate content of first and second fractions was noted when the diatom was exposed to the combination of metals.

The acid soluble fraction was increased by 383% and 413% respectively in the treatments on fourth day but decreased thereafter to a level higher than control by 26% and 116% on sixth day. Sharp increase was noted thereafter till the end of growth phase in the first treatment with 271% increase but only upto eighth day with 334% increase in second treatment. It declined to be only 78% higher than control on tenth day, in the second treatment. This fraction remained higher in the treated diatom throughout the growth phase.

The alkali soluble carbohydrate fraction was more than that of control, throughout the growth phase, except on sixth day in first treatment, and that on fourth day the percentage increase was 144 and 829 respectively. It declined sharply in both the treatments, On sixth day it was 29% less than the control in first and 48% more to the second. Thereafter it increased till the end of growth phase in the first treatment and was 66% higher than that of control. In the second treatment it increased only upto eighth day, being steady thereafter and was 76% higher than that of control on the last day.

The insoluble carbohydrate fraction of the diatom was higher than that of control in the early phase with 53% and 234% increase respectively, but decreased thereafter to be only 4% and 131% higher than that of control on sixth day. It increased in both the treatments to 34% and 42% less than that of control respectively.

The protein content of the diatom was slightly less than that of control except on the last day, in the first treatment and followed the same pattern as that of control. It was 37% less on sixth day but increased to 12% more than that of control at the end of growth phase. In the second treatment the protein content of the diatom remained higher than that of control except on the last day of growth. It was 15% more on fourth day and 53% on eighth day. It remained almost steady thereafter and was 8% less than that of control on the last day of growth.

Lipid content of the diatom was adversely affected with 41% and 34% reduction respectively in the two treatments, in relation to control.

Growth: (Fig. I)

The growth of the diatom was lowered in presence of the metals. The biomass was almost same in both treatments till sixth day but increased thereafter in the second treatment to reach the same level as that of control on

eighth day and remained steady till tenth day. In the first treatment gradual increase was noted till the end of growth phase. However, the biomass remained less than that of control in the second treatment. The final yield in both instances was lowered.

No. of treatment	Nutrients absorbed (in µg/l) Phosphate	Nitrate	N / P
75	1855	823	0.44
76	1815	855	0.47
Control	1350	763	0.57

Uptake of both the nutrients was enhanced in presence of the metals. Absorption of phosphate increased to a greater extent than nitrate. Between the treatments, phosphate uptake decreased and nitrate uptake increased by few µg. with increase in iron level.

Conclusion:

Inspite of reduced rate of production carbohydrate concentration of diatom in early growth phase was high. Lipids were reduced though protein was not affected. Biomass was lowered. The combination seems to be undesirable

Comparison:

When the present effect was compared with that obtained by employing lead alone, increase in production was

observed and respiration was lowered. Pigment content increased. Iron, at 0.05 ppm level in combination with lead, reduced acid soluble carbohydrate fraction but increased the alkali soluble and insoluble carbohydrate fractions. Protein and lipid content improved to a large extent. Growth however, was retarded.

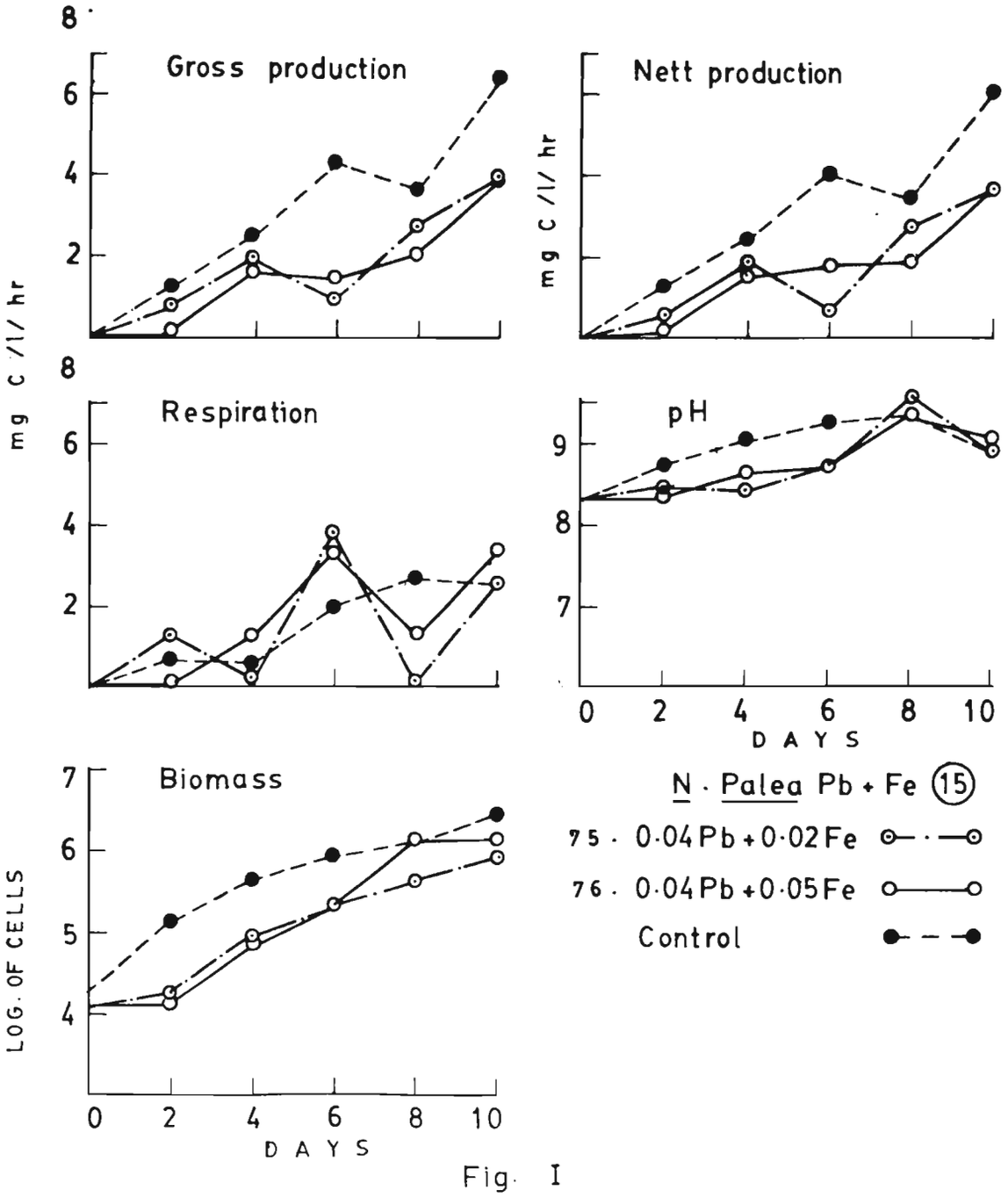

Gross production
Nett production
Respiration
pH
Biomass
mg C /l/ hr
LOG. OF CELLS
DAYS
N · Palea Pb + Fe (15)
75 · 0·04 Pb + 0·02 Fe
76 · 0·04 Pb + 0·05 Fe
Control

Fig. I

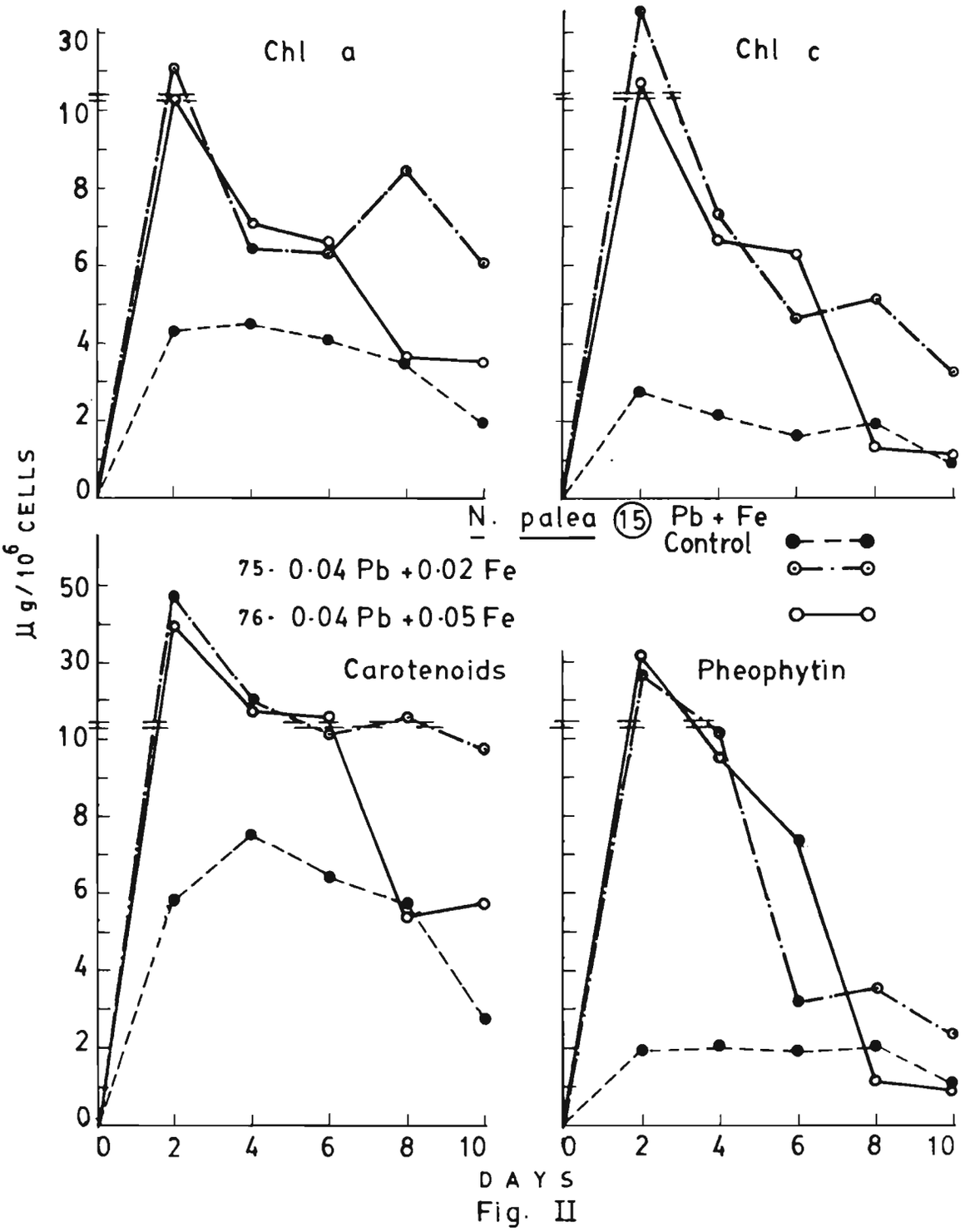

Chl a
Chl c
Carotenoids
Pheophytin
N. palea (15) Pb + Fe
Control
75- 0·04 Pb +0·02 Fe
76- 0·04 Pb +0·05 Fe
µg/10^6 CELLS
DAYS
0
2
4
6
8
10
30
50

Fig. II

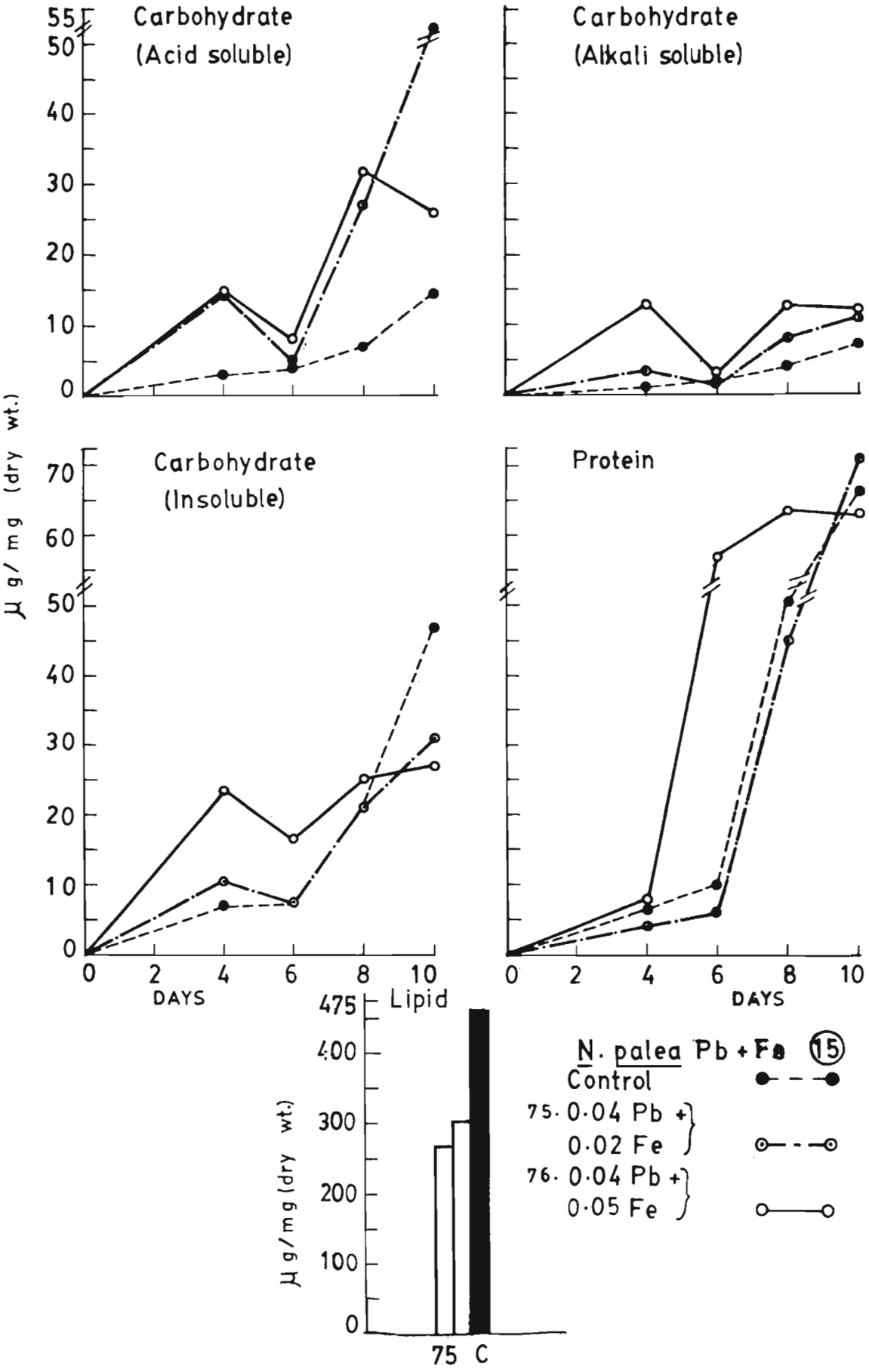

Carbohydrate
(Acid soluble)
Carbohydrate
(Alkali soluble)
Carbohydrate
(Insoluble)
Protein
µg/mg (dry wt.)
DAYS
DAYS
Lipid
µg/mg (dry wt.)
75 C
N. palea Pb + Fe (15)
Control
75. 0·04 Pb + 0·02 Fe
76. 0·04 Pb + 0·05 Fe

Fig. III

The combined effect of copper and cadmium, copper and lead, copper and zinc, copper and iron proved to be toxic to both S. bijugatus and N. palea. The culture remained totally colourless upto twelfth day and even after twenty days no colour was observed. Hence the selected parameters were not estimated. The selected concentrations for the above combinations are given below.

S. bijugatus	N. palea
0.1 Cu + 0.05 Cd	0.05 Cu + 0.02 Cd
0.1 Cu + 0.05 Pb	0.05 Cu + 0.04 Pb
0.1 Cu + 0.05 Zn	0.05 Cu + 0.05 Zn
0.1 Cu + 0.05 Fe	0.05 Cu + 0.05 Fe

Discussion

The selected combinations of two metals were found to exert toxic effect either on the end products (Carbohydrates, Proten and lipid) or on the biomass of the test species (Fig. V) when compared with control as well as the individual effect of the metals (Fig.IV). (The phenomenon see discussion Chapter IV)

The combined effect of mercury and cadmium, combination (6) (treatments 37 and 38) on S. bijugatus was adverse as the biomass was reduced to a large extent when cadmium level was high. Antagonism between the two metals was seen with N. palea (treatments 39 and 40) with increase in biomass and the major photosynthetic product, lipid.

The individual effect of lead and mercury was basically different (Fig. IV). Lead promoted protein where as mercury promoted lipid, of S.bijugatus. When these two were employed together as in combination (7) (treatments 41 and 42) the biomass of S.bijugatus increased and end products decreased. The same combination increased lipid content of N. palea (treatments 43 and 44) without affecting the biomass.

The effect of mercury and copper combination (8) on S. bijugatus (treatments 45,46

and 47) was positive as seen in increased biomass accompanied by only marginal reduction in end products. Their combined effect on N.palea did not differ from their individual effect when copper level was low (treatment 48) but when high (treatment 49) it was toxic than copper alone at the same level.

The effect of mercury and zinc combination (9) did not affect the biomass and protein of S.bijugatus (treatments 50 and 51). This is unlike their individual effect (Fig.IV). The combined effect was positive on N.palea, (treatments 52 and 53) Lipid content of the diatom increased to a large extent. Even at higher levels of zinc, the reduction in biomass was marginal.

Mercury and Iron, in combination (10) were antagonistic in action when applied to S. bijugatus (treatments 54 and 55) and N. palea (treatments 56 and 57). Improvement in biomass and end products, particularly lipid, was observed in both the species.

The roll of iron in limiting the production of natural waters is well documented (Menzel and Ryther 1961; Lewin and Ching-Hong Chen,1971). Tranter and Newwell (1963) and Shapiro (1967) have reported that iron in soluble form although chemically reactive is not necessarilly available to organisms.

During the present investigation Iron was found to mitigate the toxic effect of mercury. It promoted the production in S.bijugatus but not in N.palea. In combination with mercury it reduced pigment concentration. At higher level of 0.1 ppm, it promoted protein content and at lower level helped the alga to incorporate increased proportion of photosynthetic carbon into lipids.

Reimer et al., (1975) have reported the high affinity of iron and manganese oxides to mercury in sulphur free sediments. Iron will convert soluble mercury in water to elemental mercury. During this process hydrated feric oxide is found which is an effective co-precipitator of mercury ions. In addition,it reduces the toxic methyl mercury to elemental mercury. In the

present investigation, addition of Iron to culture has improve the alga's physiological efficiency even in presence of mercury and in combination the metals acted antagonistically.

Cadmium and lead in combination (11) were found to be synergestic in effect on S.bijugatus (treatments 58 and 59) resulting in reduction of biomass and protein. But the effect on N. palea (treatments 60 and 61) was not total. The biomass though remained reduced, improvement in the concentration of end products was observed.

The reports on the effect of cadmium and lead on algal community are scanty. Deviprasad and Deviprasad (1982) have reported an increase in growth of the green alga Ankistrodesmus falcatus when exposed 0.5 ppm each of cadmium and lead. The growth in this instance was measured by the optical density of total pigment at 665 nm. In the present investigation the combined effect of these two metals was found to be undesirable. The combination was more toxic to S.bijugatus, than to N.palea. Biomass in both the instances was found to be reduced. The combination

particularly favoured the production of pigments in both S.bijugatus and N.palea.

Cadmium and zinc together, combination (12), were found inimical to S.bijugatus (treatment 62) as seen in reduced biomass and endproducts. N.palea (treatments 63 and 64) reacted differently. The biomass remained same as when the two metals were applied singly, whereas the amount of endproducts increased.

The adverse effect of cadmium and iron, combination (13) on S.bijugatus (treatments 65 and 66) and on N.palea (treatments 67 and 68) was evident only in the lowering of biomass. The amount of protein and lipid increased in both the species.

Cadmium is known to interfere with iron nutrition of the organisms (Hewitt, 1949; Shankar and Bard, 1955). Iron significantly inhibits cadmium uptake and transfer (Hamilton and Valberg, 1974) and the kinetics of cadmium inhibition of iron transport are of a competetive type, leading to the conclusion that iron and cadmium share same transport system. Foster and Morel (1982) shown

that cadmium toxicity as reversable and the toxicity was modulated by the iron content of the growth medium. They also suggested that the cadmium is a competetive inhibitor of some iron-dependent processes or of the iron uptake system itself, though was not demonstrated. The above result was agreed upon by Harrison and Morel(1983) who worked on Thallassiosira pseudonana. At low ferric ion concentration, toxicity of cadmium due to competetive binding and interference prevail leading to iron deficiency. When iron ion concentration is high it lead to elevated iron level in the alga though the cells continued to exhibit physiological responses and biochemical activities characteristic of iron deficiency. They also stated that cadmium concentration/cell decreases simply due to dilution by the growing cell mass and there is evidently no active exclusion of cadmium from the algae.

Silicic acid uptake by the diatoms was reported to be inhibited by cadmium (Lewin, 1954). But Harrison and Morel (1983) reported the increase in the Silicic acid uptake when the cadmium level is very low. In the present investigation, the species continued to exhibit

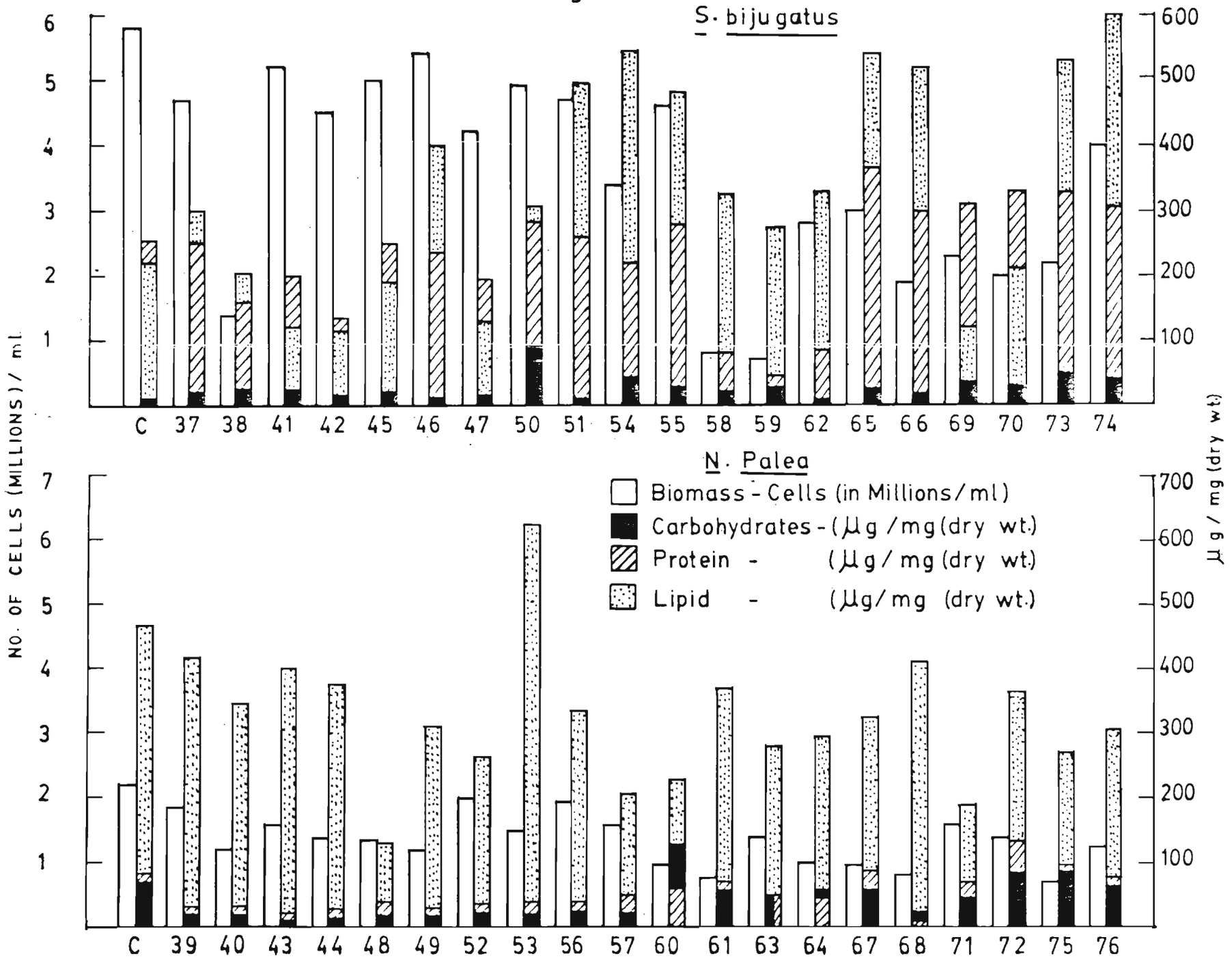
cadmium toxicity response even when the iron level in the medium was 0.05 ppm. Also the production of pigments increased when the species were exposed to these metals.

Lead and zinc when applied together, combination (14), reduced the biomass to a large extent in S.bijugatus (treatments 69 and 70) but increased the protein content of the alga. The combination did not exert any adverse effect on the biomass of N.palea (treatments 71 and 72) and increased the amount of end products.

Lead and iron together, combination (15), exerted the same effect on S.bijugatus (treatments 73 and 74) and on N.palea (treatments 75 and 76). When the iron level was low (treatments 73 and 75 respectively) the biomass was reduced. When the iron-level was high (treatments 74 and 76 respectively) the biomass was unaffected compared with their individual effect (Fig. IV) but the lipid content increased.

It can be concluded from the above study that the antagonism or synergism between the metals may be expressed either in quantitative (biomass) or qualitative (endproducts) aspects or both.

Fig. V


S. bijugatus
N. Palea
NO. OF CELLS (MILLIONS) / ml
µg / mg (dry wt)
Biomass - Cells (in Millions/ml)
Carbohydrates - (µg /mg (dry wt.)
Protein - (µg / mg (dry wt.)
Lipid - (µg/ mg (dry wt.)
C 37 38 41 42 45 46 47 50 51 54 55 58 59 62 65 66 69 70 73 74
C 39 40 43 44 48 49 52 53 56 57 60 61 63 64 67 68 71 72 75 76

Statistical analysis of the results was carried out to bring about the significance of metal species interaction.

The variance ratios calculated by the two way analysis of variance technique (Table VII) for the selected growth parameters of S.bijugatus were significant in 177 out of 220 cases.

The combination of mercury and cadmium produced significant effect on all parameters except on respiration chlorophyll a and carbohydrate at the tested levels. Pheophytin content did not vary significantly during the growth period.

The effect of mercury and lead on chlorophyll a, pheophytin and carbohydrate was not significant at the tested levels. Also this combination did not produce significant variation in pheophytin content of the alga during the growth phase.

The combination of mercury and copper produced significant effect on all the parameters studied during the growth phase.

The toxic effect of mercury and zinc, on nett production and Pigments was not significant at the test doses.

Mercury and iron did not produce any significant affect on pH, production, carbohydrate and protein at the experimental concentrations. The effect of this combination was insignificant on carbohydrate content of the alga during the growth period.

The effect of cadmium and lead on chlorophyll a, chlorophyll b and carbohydrate was found to be insignificant at the test levels.

Cadmium and zinc did not produce significant effect on pH and production of the species at the test levels. The effect on carbohydrate was negligible.

All parameters except respiration and carbohydrate were significantly affected by the combination of cadmium and iron.

At the selected levels, lead and zinc did not affect chlorophyll a, chlorophyll b and carotenoid contents of the species .

Production, respiration, chlorophyll a pheophytin and protein (biuret) were significantly affected by the combination of lead and iron at the tested dose levels. This combination also affected the carbohydrate and protein during the growth phase.

TABLE - VII

Significance of the variance ratios in the analysis of variance table for between concentrations and between days

S. bijugatus

Sl. No.	Metals employed.	No. of selected concentrations	pH		Gross production.		Nett production.		Respiration.		Chlorophyll a		Chlorophyll b		Carotenoids.		Pheophytin.		Carbohydrate		Protein (Biuret)		Protein Total	Lipid
			I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	I
(6)	Hg + Cd	2	c	c	c	c	c	c	NS	a	NS	b	b	c	b	b	b	NS	NS	b	a	c	c	c
(7)	Hg + Pb	2	c	c	c	c	c	c	c	c	NS	c	a	a	b	c	NS	NS	NS	a	c	c	c	c
(8)	Hg + Cu	3	c	c	c	c	c	c	c	a	a	b	a	a	b	c	c	b	a	b	a	a	c	c
(9)	Hg + Zn	2	b	c	a	c	NS	c	c	c	NS	c	NS	c	NS	c	NS	c	c	a	c	c	NS	c
(10)	Hg + Fe	2	NS	c	NS	c	NS	c	c	c	b	c	b	c	b	c	c	c	NS	NS	NS	c	NS	c
(11)	Cd + pb	2	c	c	c	c	c	c	c	c	c	NS	c	NS	c	a	c	a	NS	c	c	b	b	c
(12)	Cd + Zn	1	NS	c	NS	c	NS	c	c	b	c	c	a	c	c	c	c	c	NS	NS	c	c	c	c
(13)	Cd + Fe	2	c	c	c	c	c	c	NS	c	c	c	c	c	c	c	b	a	NS	NS	c	c	a	c
(14)	Pb + Zn	2	c	c	c	c	c	c	c	a	NS	c	NS	c	NS	a	a	b	b	c	b	c	b	c
(15)	Pb + Fe	2	a	c	NS	c	NS	c	NS	a	NS	a	a	a	c	c	NS	b	c	NS	NS	NS	a	c

NS - Not significant

a - $p < 0.05$
b - $p < 0.01$
c - $p < 0.001$

I Between concentrations
II Between days

The effect of selected combinations of metals was of considerable significance in the case of N.palea (Table VIII) 215 out of 250 instances were found to be significant.

At the test levels mercury and cadmium did not produce significant effect on chlorophyll a, carotenoids and pheophytin.

Mercury and lead together did not affect the respiration of the species and also the accessory pigments at the tested levels.

The effect of mercury and copper was quite significant except on respiration and alkali soluble carbohydrate fraction during,the growth phase.

Mercury and zinc together produced significant effect at the tested levels,but on respiration and acid soluble carbohydrate fraction during the growth period, their effect was not significant.

The effect of mercury and iron together was quite significant throughout the growth phase but at the tested levels it was insignificant on alkali soluble carbohydrate fraction and protein.

Cadmium and lead together produced a totally significant effect on the species.

The combination of cadmium and zinc was not significant on pH and insoluble carbohydrate fraction at the tested levels. The combination did not affect the acid soluble carbohydrate fraction and protein significantly.

At the experimental levels the effect of cadmium and iron was not significant on respiration and alkali soluble carbohydrate fraction of the species. Also the effect on chlorophyll c and carotenoids was insignificant during the growth period.

The combination of lead and zinc produced least significant effect on the species. Respiration, pheophytin content and alkali soluble carbohydrate fraction were not significantly affected. Also chlorophyll a and insoluble carbohydrate fraction were not affected at the test doses of these metals.

The effect of the combination of lead and iron on respiration, insoluble carbohydrate fraction and lipid was found to be insignificant at the tested levels.

TABLE - VIII

Significance of the variance ratios in the analysis of variance table for between concentrations and between days.

N. palea

Sl. No.	Metals employed.	No. of selected concentrations.	pH		Gross production.		Nett production		Respiration.		Chlorophyll a		Chlorophyll c		Carotenoids.		Pheophytin.		Carbohydrate (Acid soluble)		Carbohydrate (Alkali soluble)		Carbohydrate (Insoluble)		Protein		Lipid
			I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	
(6)	Hg + Cd	2	c	c	c	b	c	a	c	c	NS	c	b	c	NS	c	NS	c	b	c	b	c	c	c	a	c	c
(7)	Hg + Pb	2	c	c	c	c	c	c	NS	NS	c	c	NS	c	NS	c	NS	c	c	b	c	c	c	c	a	c	c
(8)	Hg + Cu	3	c	c	c	c	c	c	c	NS	c	c	c	c	c	c	c	c	a	c	b	NS	c	c	a	c	c
(9)	Hg + Zn	2	c	c	c	c	c	c	NS	a	b	c	c	c	b	c	c	c	NS	c	b	a	c	c	c	c	c
(10)	Hg + Fe	2	c	c	c	c	c	c	b	c	c	c	c	c	c	c	c	a	NS	c	c	c	b	c	NS	c	c
(11)	Cd + Pb	2	c	c	c	c	c	c	c	c	b	c	c	c	c	c	c	c	c	c	c	c	c	c	b	c	c
(12)	Cd + Zn	1	NS	c	c	c	c	c	c	c	a	c	c	c	c	c	c	c	NS	NS	b	c	NS	c	NS	NS	c
(13)	Cd + Fe	2	c	c	c	c	c	c	NS	b	a	a	c	NS	b	NS	a	b	c	c	NS	b	a	b	c	c	c
(14)	Pb + Zn	2	c	c	c	c	c	c	NS	NS	NS	c	c	c	c	c	NS	NS	c	c	NS	NS	NS	c	c	c	c
(15)	Pb + Fe	2	c	c	c	c	c	c	NS	c	c	c	b	c	c	c	c	c	c	c	c	c	NS	c	b	c	NS

NS - Not significant

a - $p \leq 0.05$
b - $p \leq 0.01$
c - $p \leq 0.001$

I - Between concentrations
II - Between days

TABLE IX

The concentration of metals in S. bijugatus at the end of growth phase

Sl. No. of the combination	Metals employed	No. of treatment	Concentration (ppm/100 mg dry wt.)	
(6)	Hg + Cd	37	0.0038	0.034 *
		38	0.011	0.034 *
(7)	Hg + Pb	41	0.0039	0.034 *
		42	0.0052	0.034 *
(8)	Hg + Cu	45	0.0062	Nil
		46	0.0036	0.035
		47	0.0035	0.103
(9)	Hg + Zn	50	0.0001	0.056
		51	0.0051	0.066
(10)	Hg + Fe	54	0.0004	0.043
		55	0.0053	0.164
(11)	Cd + Pb	58	0.04	0.05
		59	0.04	0.1
(12)	Cd + Zn	62	0.042	0.086
(13)	Cd + Fe	65	0.035 *	0.0157
		66	0.035 *	0.0464
(14)	Pb + Zn	69	0.052	0.052
		70	0.068	0.114
(15)	Pb + Fe	73	0.188	0.056
		74	0.212	0.10

The Values represented are the average of three observations for mercury and two observations for others.

* Values are less than the indicated concentrations.

TABLE X

The concentration of metals in *N. palea* at the end of growth phase

Sl. No. of the combination	Metals employed	No. of treatment	Concentration (ppm/100 mg dry wt.)	
(6)	Hg + Cd	39	0.006	0.027 *
		40	0.008	0.017 *
(7)	Hg + Pb	43	0.0065	0.012 *
		44	0.0055	0.015 *
(8)	Hg + Cu	48	0.001	0.011
		49	0.003	0.049
(9)	Hg + Zn	52	0.004	0.052
		53	0.004	0.062
(10)	Hg + Fe	56	0.002	0.0258
		57	0.004	0.071
(11)	Cd + Pb	60	0.033 *	0.030
		61	0.039 *	0.039
(12)	Cd + Zn	63	0.026 *	0.02
		64	0.022 *	0.063
(13)	Cd + Fe	67	0.018 *	0.013
		68	0.033 *	0.013
(14)	Pb + Zn	71	0.032 *	0.054
		72	0.023 *	0.067
(15)	Pb + Fe	75	0.030 *	0.013
		76	0.060 *	0.113

The values represented are the average of three observations for mercury and two observations for others.

* Values are less than the indicated concentrations.

6.2. Metals selected and their effect on test species (Three metals).

The effect of the metals in combination (Table 3) was studied and reported in relation to control.

TABLE 3

Sl. No. of combination	Concentration in ppm	
	S. bijugatus	*N. palea*
(16)	0.02 Hg + 0.01 Cd + 0.05 Zn	0.005 Hg + 0.02 Cd + 0.05 Zn
(17)	0.02 Hg + 0.01 Cd + 0.05 Pb	0.005 Hg + 0.02 Cd + 0.04 Pb
(18)	0.02 Hg + 0.01 Cu + 0.01 Cd	0.005 Hg + 0.05 Cu + 0.02 Cd
(19)	0.02 Hg + 0.1 Cu + 0.05 Pb	0.005 Hg + 0.05 Cu + 0.04 Pb
(20)	0.02 Hg + 0.05 Pb + 0.05 Zn	0.005 Hg + 0.04 Pb + 0.05 Zn
(21)	0.05 Pb + 0.01 Cd + 0.1 Cu	0.04 Pb + 0.02 Cd + 0.05 Cu
(22)	0.05 Pb + 0.01 Cd + 0.05 Zn	0.04 Pb + 0.02 Cd + 0.05 Zn

6.2.1

Combined effect of mercury, cadmium and zinc on

S. bijugatus

Sl.No. of the combination	Concentration of metals (in ppm)
(16)	0.02 Hg + 0.01 Cd + 0.05 Zn

Production: (Fig. I)

The nett production of the alga was enhanced in the early and late phases of growth, when compared to control. From 14% higher level on second day it decreased to its minimum, 83% lower level, on fourth day and increased gradually thereafter but was 52% less than that of control by tenth day. Considerable increase in production was observed towards the end of growth phase, exceeding that of control by 40%.

Respiration of the alga was generally reduced except on sixth and twelfth day. From 52% higher level on sixth day it declined to 43% lower level on eighth day. Thereafter it increased sharply, to 5% higher level at the end of growth phase.

Consistent with low production, pH of the culture remained well below that of control except at the end of growth phase.

Pigments: (Fig. II)

The development of pigments was delayed. As the usual green colour was lacking in the culture, pigments were measured from fourth day onwards. Neverthless, pigment content of the alga was found to increase to a considerable extent. Chlorophyll a, chlorophyll b and carotenoids were found to be much more than in control from fourth day onwards. Concentration of chlorophyll a and pheophytin fluctuated considerably during the growth phase. Pheophytin was not detected on fourth and tenth day of growth. With the exception of pheophytin, pigments exhibited a decreasing trend towards the end of growth phase. On the last day of growth, the concentration of chlorophyll a was less than that of any other pigment.

Photosynthetic end products: (Fig. III)

Total carbohydrate content of the alga was considerably less than that of control. It increased gradually from fourth day onwards reaching maximum on eighth day. From 24% higher level on eighth day it declined to 61% lower level by tenth day but once again increased to 57% higher level by the end of growth phase.

Protein content of the alga increased by 86% whereas lipid content showed reduction by 31%.

Growth: (Fig. I)

Severe suppression of growth was noted and the biomass remained far less than that of control from second to eighth day. The growth improved thereafter. But throughout the growth phase, the biomass remained less than that of control.

Sl. No. of combination	Nutrients absorbed by the alga (ug/l)		N / P
	Phosphate	Nitrate	
(16)	1510	1060	0.70
Control	945	1290	1.37

Phosphate absorption of the alga increased by 60% whereas nitrate absorption decreased by 18%.

Conclusion:

Production and respiration were generally reduced. Biomass was reduced inspite of increase in protein content. Lipid also was reduced. Not withstanding the initial delay, pigment development was positively affected. The combination is undesirable from growth point of view.

Comparison:

When the effect of the present combination was compared with that of combination (6) (0.03 Hg + 0.01 Cd)

reduction in nett production was observed for most part of the growth phase but it increased towards the end. Respiration was elevated. Considerable increase in all four pigments was observed. Carbohydrates and lipid were reduced but protein increased. Biomass was lowered.

When the effect of the present combination was compared with that of combination (9) (0.05 Hg + 0.05 Zn) the nett production was observed to be low but respiration was elevated. Increase in the level of all four pigments was observed. Carbohydrate and lipid were reduced but protein increased to a large content. Biomass was considerably reduced.

When the effect of present combination was compared with that of combination (12) (0.05 Cd + 0.1 Zn), production was found to be lowered towards the end of growth phase. Respiration was lowered considerably. All four pigments increased, particularly during latter growth phase. Carbohydrate content was not affected. Protein improved many fold whereas lipid was reduced by nearly 50%.

6.2.2

Combined effect of mercury, cadmium and lead on

S. bijugatus

Sl.No. of the combination	Concentration of metals (in ppm)
(17)	0.02 Hg + 0.01 Cd + 0.05 Pb

Production: (Fig. I)

The nett production of the alga fluctuated during the growth phase. From 28% higher level on second day, inspite of continued increase it fell short of control by 22% on fourth day. It reached lowest level on sixth day with 85% reduction, from which it increased sharply to 32% lower level by eighth day. It declined once again to 77% lower level by tenth day and registered a sharp increase thereafter, to 38% higher level at the end of the growth phase.

Respiration of the alga was less than that of control upto sixth day but was elevated to 93% higher level by eighth day. It declined to 16% lower level on tenth day. Inspite of increase it was 30% less at the end of growth phase, in relation to control.

pH of the culture remained less than that of control upto eighth day. It increased sharply to the same level as control by tenth day and remained higher thereafter, being steady upto twelfth day.

Pigments: (Fig. II)

The development of pigments was delayed and visible pale green colour appeared only on third day of growth. Neverthless, pigment content of the alga increased to a considerable extent and was more than that of control from sixth day onwards. Concentration of chlorophyll a was less than that of other pigments and that of chlorophyll b more. Both these pigments exhibited an increasing tendency towards the end of growth phase, whereas carotenoids and pheophytin decreased. Maximum concentration was observed on eighth day of growth for all pigments except pheophytin, which reached highest level on tenth day. Also pheophytin was not detected on fourth day of growth.

Photosynthetic end products: (Fig. III)

Carbohydrate concentration of the alga fluctuated during growth phase, with peaks on fourth and eighth day. From 44% lower level on fourth day it decreased to minimum, with 61% reduction on sixth day. It increased sharply to 131% higher level by eighth day and dropped once again to 13% lower level by tenth day. It increased towards the end of growth phase to be 70% more than that of control.

Protein level in treated alga increased by 68% and lipid by 16% in relation to control.

Growth: (Fig. I)

Growth of the alga was inhibited by the metals in combination. The biomass though registered a continuous increase, remained far less than that of control throughout growth phase.

Selected combination	Nutrients absorbed by the alga (ug/l)		N / P
	Phosphate	Nitrate	
(17)	1230	590	0.48
Control	945	1290	1.37

The phosphate absorption has increased nearly by 30% whereas nitrate absorption decreased by 54%.

Conclusion:

Production and respiration were lowered for greater part of growth phase but protein and lipid concentration increased. Inspite of this improvement biomass was very low. Pigment development was delayed. Neverthless, it increased considerably. On the whole the effect was undesirable from the growth point of view.

Comparison:

When the effect of the present combination was compared with that of combination (6) (0.03 Hg + 0.01 Cd) considerable reduction in nett production was observed.

Also the production exhibited fluctuation. Respiration of the alga was found elevated. Chlorophyll a was not affected but other pigments, particularly chlorophyll b and pheophytin increased to a large extent. Carbohydrate was not affected but protein increased. Lipid showed reduction. Biomass also was reduced.

When the effect was compared with that of combination (7), (0.05 Hg + 0.05 Pb) the nett production was observed to be lowered except at the end of growth phase. Respiration increased. All four pigments increased. Carbohydrate was reduced whereas protein and lipid increased. Biomass was lowered.

When compared with the effect of combination (11) (0.05 Cd + 0.05 Pb, nett production was found to improve considerably, so also respiration. Except chlorophyll b which was not affected, other pigments were lowered. Carbohydrate increased. Protein increased many fold. Lipid was reduced. Marginal increase in biomass was observed.

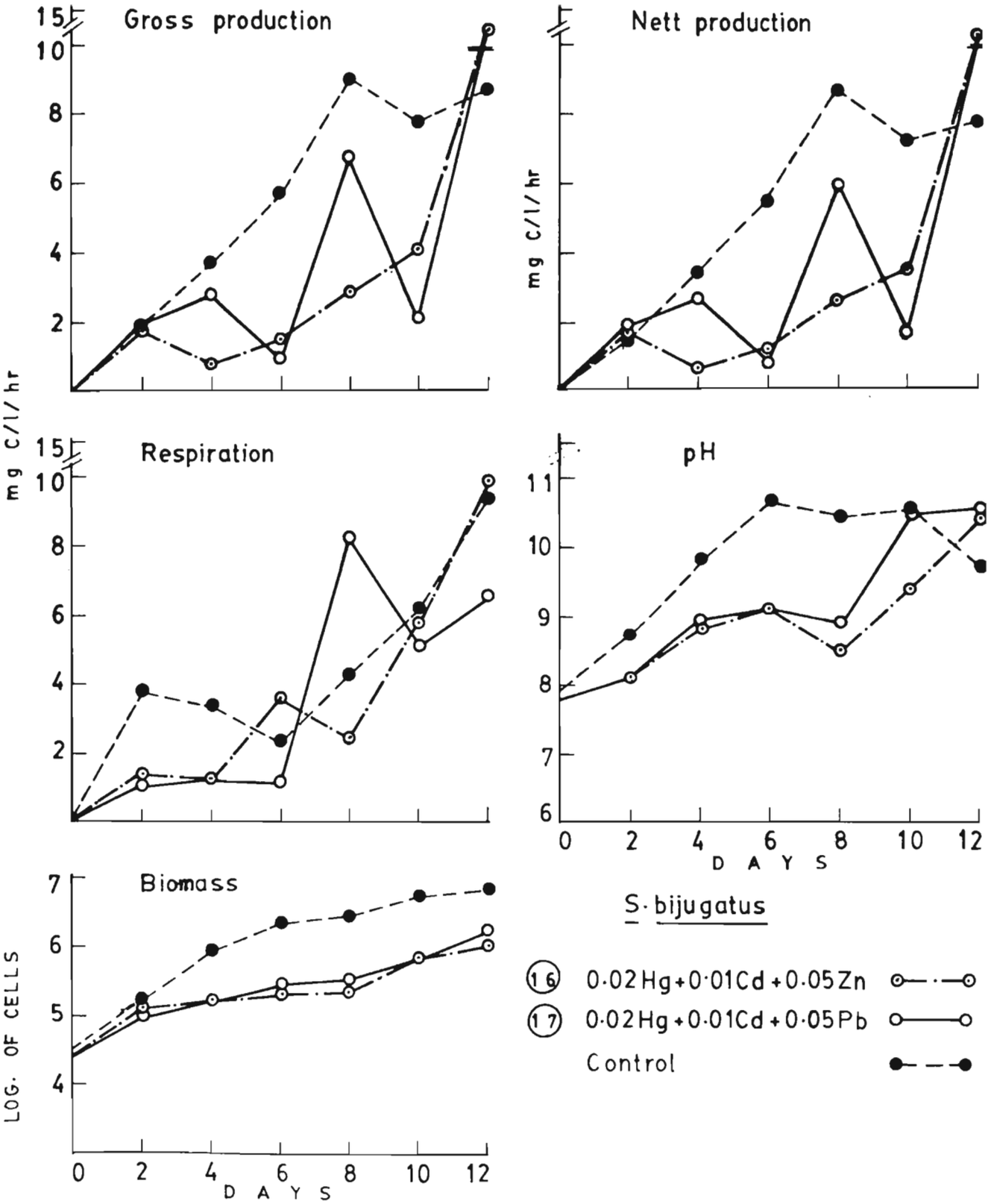

Gross production
Nett production
Respiration
pH
Biomass
mg C/l/hr
LOG. OF CELLS
DAYS
S. bijugatus
16 0·02Hg+0·01Cd+0·05Zn
17 0·02Hg+0·01Cd+0·05Pb
Control

Fig. I

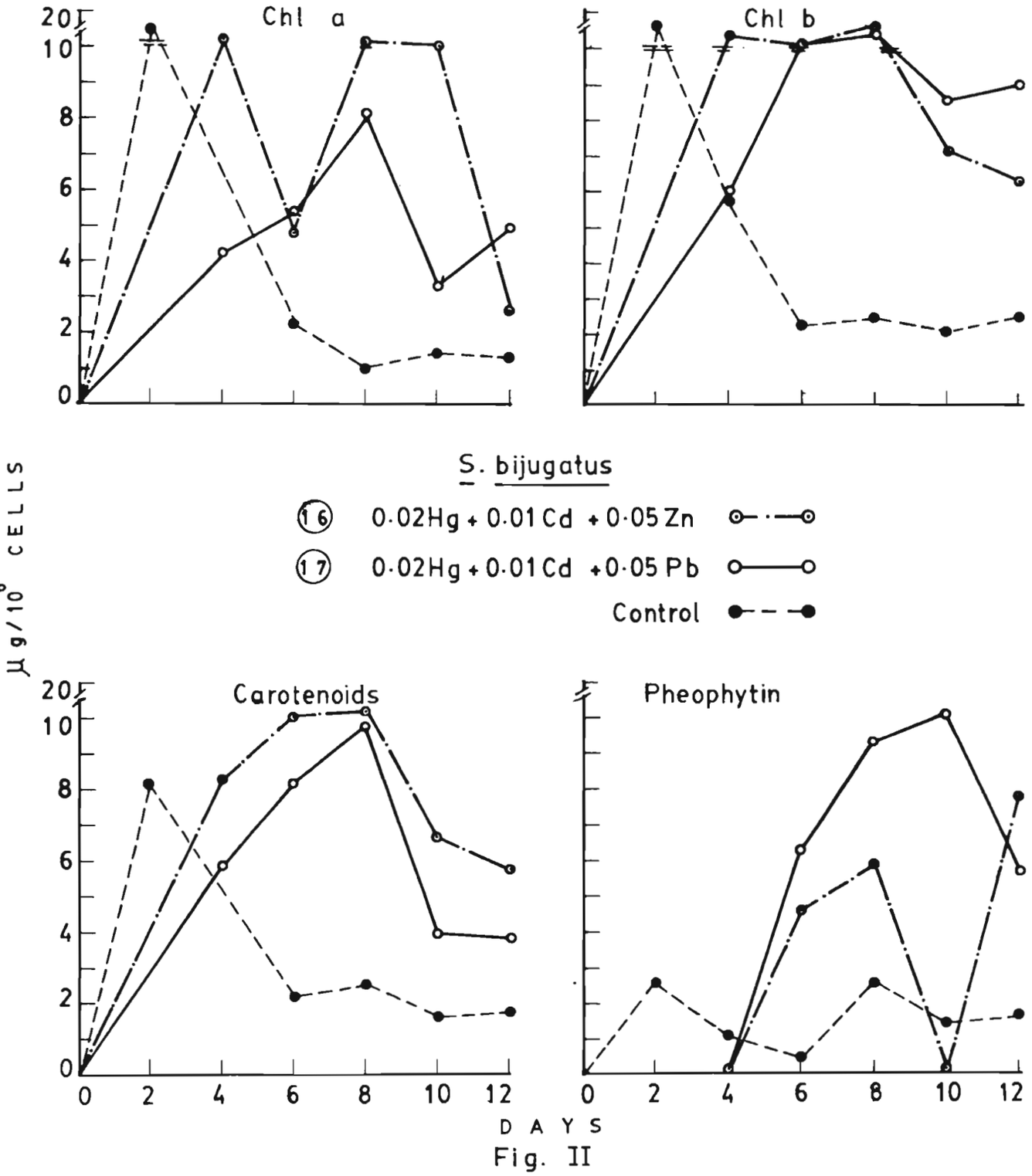
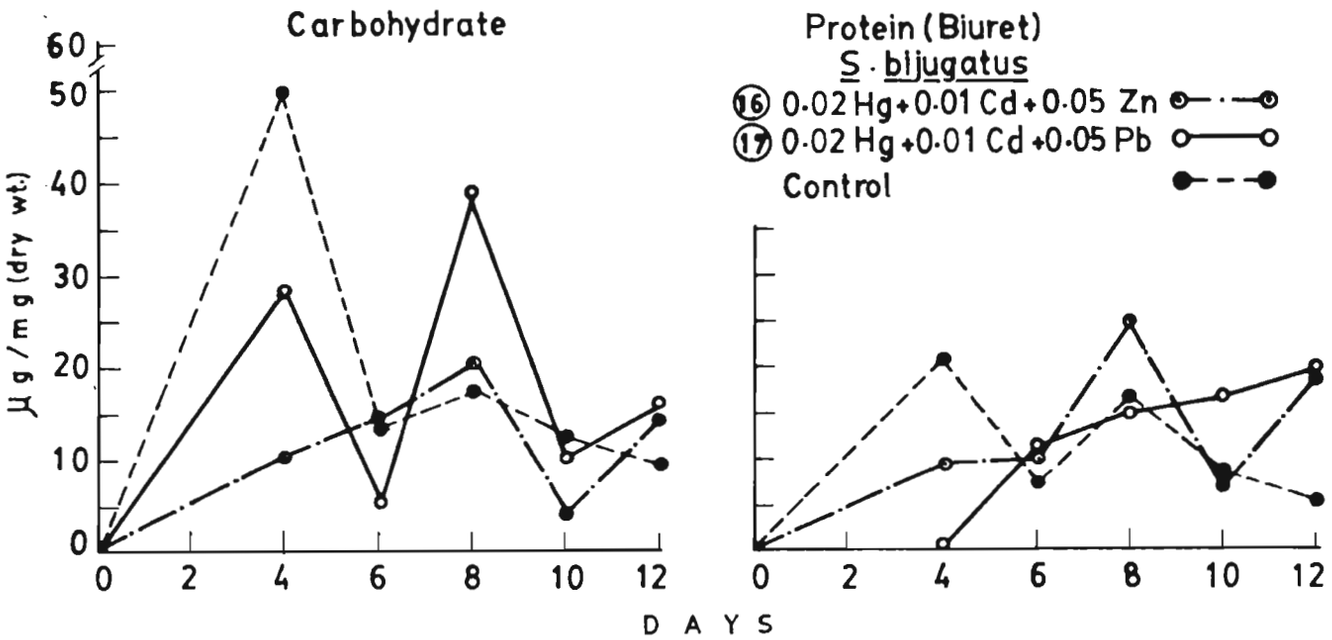

Chl a
Chl b
S. bijugatus
16 0·02Hg + 0·01Cd + 0·05Zn
17 0·02Hg + 0·01Cd + 0·05Pb
Control
µg/10⁶ CELLS
Carotenoids
Pheophytin
0 2 4 6 8 10 12
DAYS

Fig. II


Carbohydrate
Protein (Biuret)
S. bijugatus
⑯ 0·02 Hg + 0·01 Cd + 0·05 Zn
⑰ 0·02 Hg + 0·01 Cd + 0·05 Pb
Control
µg/mg (dry wt.)
60
50
40
30
20
10
0
0 2 4 6 8 10 12
DAYS

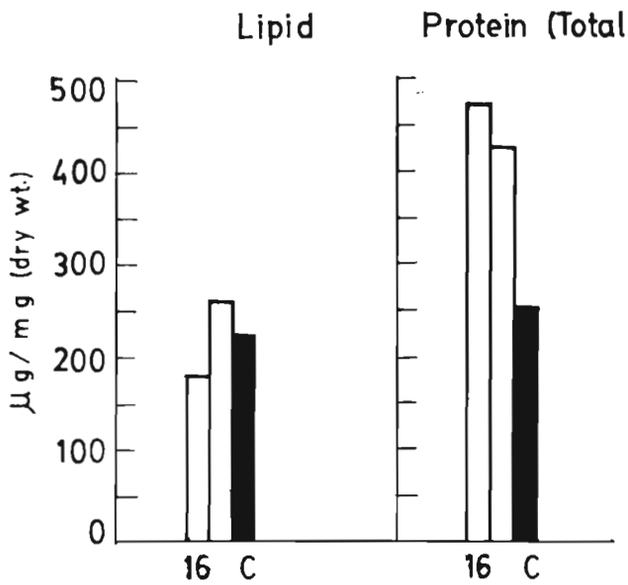

Lipid
Protein (Total)
µg/mg (dry wt.)
500
400
300
200
100
0
16 C
16 C

Fig. III

Combined effect of mercury, cadmium and zinc on *N. palea*

Sl.No. of the combination	concentration of metal (ppm)
(16)	0.005 Hg + 0.02 Cd + 0.05 Zn

Production: (Fig. I)

Nett production did not deviate much from that of control in the early phase of growth. It was 4% higher on fourth day. No considerable increase was observed thereafter. It was steady between fourth and sixth day, increasing slightly thereafter reached 14% lower level on eighth day and declined to be 68% at the end of growth phase, when compared with that of control.

Respiration of the diatom was elevated by the metals in combination. It was higher than that of control upto sixth day. From 47% higher level on sixth day it decreased to 54% lower level on eighth day and increased once again to 84% higher level by tenth day, in relation to control.

pH of the culture was lowered except when it was equal to that of control on eighth and tenth day. pH was maximum on eighth day.

Pigments: (Fig. II)

Total pigment content of the diatom was less than that of control. Throughout growth phase all pigments were found to be slightly less than those of control except on the last day. Neverthless, the difference was not considerable except in case of carotenoids. Chlorophyll a, chlorophyll c and carotenoids have reached their respective maxima on fourth, second and fourth day of growth respectively, like those observed in control.

Photosynthetic end products: (Fig. III)

The acid soluble carbohydrate fraction was more than that of control upto eiththh day. Gradual increase was observed upto eighth day when it was 17% more than that of control. Thereafter it declined to 54% lower level by the end of growth phase.

The alkali soluble carbohydrate fraction of the diatom did not differ much from that of control but remained slightly less, from sixth day onwards. 29% reduction was observed in this fraction at the end of growth phase.

The insoluble carbohydrate content of the diatom was adversely affected. The concentration was maximum on sixth day, the only instance when it was more than that of control, by 56%. Thereafter no considerable change was observed and it was 77% less than that of control at the end of growth phase.

Protein content of the diatom increased to a greater extent in the early phase of growth, when compared to that of control. The percentage increase was found to be 124 and 239 on fourth and sixth day respectively. The concentration of this product changed very little thereafter and was reduced by 56% at the end of growth phase.

Lipid content of the alga was also adversely affected and 34% reduction in this product was registered by the diatom.

Growth: (Fig. I)

The metals in combination have exerted a positive effect on the growth of the diatom and enhanced the rate of multiplication to a considerable extent in the early phase. The biomass remained more than that of control except on the last day.

Sl. No. of combination	Nutrients absorbed (µg/l)		N / P
	Phosphate	Nitrate	
(16)	1565	813	0.52
Control	1350	763	0.57

Increase in the uptake of both nutrients was observed.

Conclusion:

This particular combination of metals has reduced the rate of production and increased the rate of respiration of the diatom. The total pigment content of the diatom was reduced. The adverse effect was pronounced in the reduction of all three photosynthetic end products. No harmful effect was observed with regard to the growth of the diatom.

Comparison:

When the effect of the present combination was compared with that of combination (6) (0.005 Hg + 0.02 Cd) reduction in the early phase in nett production was noted though it was not different in the latter phase. Respiration was reduced. All four pigments were reduced. Carbohydrate was not affected but slight reduction in protein was observed. Lipid was reduced by 100 ug.

When compared with that of combination (9) (0.005 Hg + 0.05 Zn) the nett production was observed to be low. Though respiration decreased in early phase, was found elevated during latter phase. Reduction in the level of all four pigments was observed. The acid soluble and insoluble carbohydrate was reduced while the alkali soluble fraction remained unaffected. Protein content was

not affected but lipid was marginally increased. Biomass was not affected.

When compared with the effect of combination (12) (0.04 Cd + 0.05 Zn) the nett production was found to be unaffected but respiration was elevated. All the four pigments were considerably reduced. Carbohydrate and protein were unaffected but marginal increase in lipid content was observed. Biomass was improved.

Combined effect of mercury, cadmium and lead on

N. palea

Sl.No. of the combination	Concentration of metals (ppm)
(17)	0.005 Hg + 0.02 Cd + 0.04 Pb

Production: (Fig. I)

Enhancement in the rate of nett production was observed in the early growth phase and it was 10% higher than that of control on fourth day. Beyond fourth day little change in the production of the diatom was observed. It was reduced by 56% at the end of growth phase.

The respiration was also elevated in the early phase of growth. From 300% higher level on fourth day it decreased to 79% lower level by sixth day. On eighth day oxygen value in the dark bottle was higher than the initial value in two observations and nil value in one observation. At the end of growth phase respiration was found to be 44% more in relation to control.

Inspite of reduction in the rate of nett production pH of the culture closely followed that of control throughout growth phase.

Pigments: (Fig. II)

Total pigment content of the diatom was considerably lowered, though the concentration of all four pigments exceeded that of control at the end of growth phase. All pigments reached their respective maxima on second day, but only chlorophyll a was less than that of control whereas other pigments exceeded the level of control.

Photosynthetic end products: (Fig. III)

All three fractions of carbohydrate were developed to greater extent by the diatom in the early phase in this combination.

The concentration of acid soluble fraction increased gradually, remaining higher than control upto sixth day and registered 156% increase on that day. It continued to increase thereafter but was only 148% more than control on eighth day and declined to 33% lower level by tenth day.

The alkali soluble carbohydrate fraction also exhibited a similar trend and was higher than that of control by 164% and 320% on sixth and eighth day respectively. It declined to 37% lower level by the end of growth phase.

The insoluble carbohydrate fraction of the diatom also increased to a greater extent than that of

control by sixth day, registering 104% increase on that day. It was equal to that of control on eighth day and declined thereafter to 67% lower level, by the end of growth phase.

Protein was enhanced to a large extent, the concentration of which was less than that of control only on the last day of growth. It was 540% higher on fourth day and decreased to 266% higher level on sixth day. It once again registered a sharp increase but was only 78% more than that of control on eighth day. It declined towards the end of growth phase and 41% reduction was observed on the last day.

Lipid content of the diatom was adversely affected. The observed reduction in lipid was 40%.

Growth: (Fig.I)

The metals in combination have enhanced the growth rate of the diatom resulting in increased biomass. But at the end of growth phase biomass was slightly less than that of control.

Selected combination	Nutrients absorbed (µg/l)		N / P
	Phosphate	Nitrate	
(17)	1615	855	0.53
Control	1350	763	0.57

Both the nutrients were absorbed in greater amount by the treated diatom, when compared with the control.

Conclusion:

Not withstanding the reduced rate of nett production, the carbohydrate and protein of the diatom were detected in greater quantities than those of control except on the last day. The growth of the diatom was not adversely affected as only slight lowering of biomass was observed.

Comparison:

When the effect of the present combination was compared with that of combination (6) (0.005 Hg + 0.05 Cd) the nett production was found to be reduced in the early phase but not different in the latter phase. Respiration followed the same trend. Considerable reduction in the concentration of all four pigments was observed, particularly in the early phase. Acid soluble carbohydrate was unaffected but the other two fractions increased. Considerable increase in protein was observed whereas lipid was reduced by 100 ug. Biomass was unaffected.

When compared with the effect of combination (7) (0.005 Hg + 0.04 Pb) marginal increase in nett production

was observed. Respiration was reduced. Chlorophyll a was reduced to a lesser extent than other pigments. All three fractions of carbohydrate improved. Whereas protein content increased, lipid was reduced by about 50%. Biomass improved.

When compared with the effect of combination (11) (0.02 Cd + 0.04 pb), improvement in nett production and respiration was observed. Reduction in all four pigments was registered. Acid soluble carbohydrate was decreased to a larger extent than the alkali soluble fraction. The insoluble carbohydrate was not affected. Protein remained unaffected whereas lipid showed marginal increase. Biomass increased.

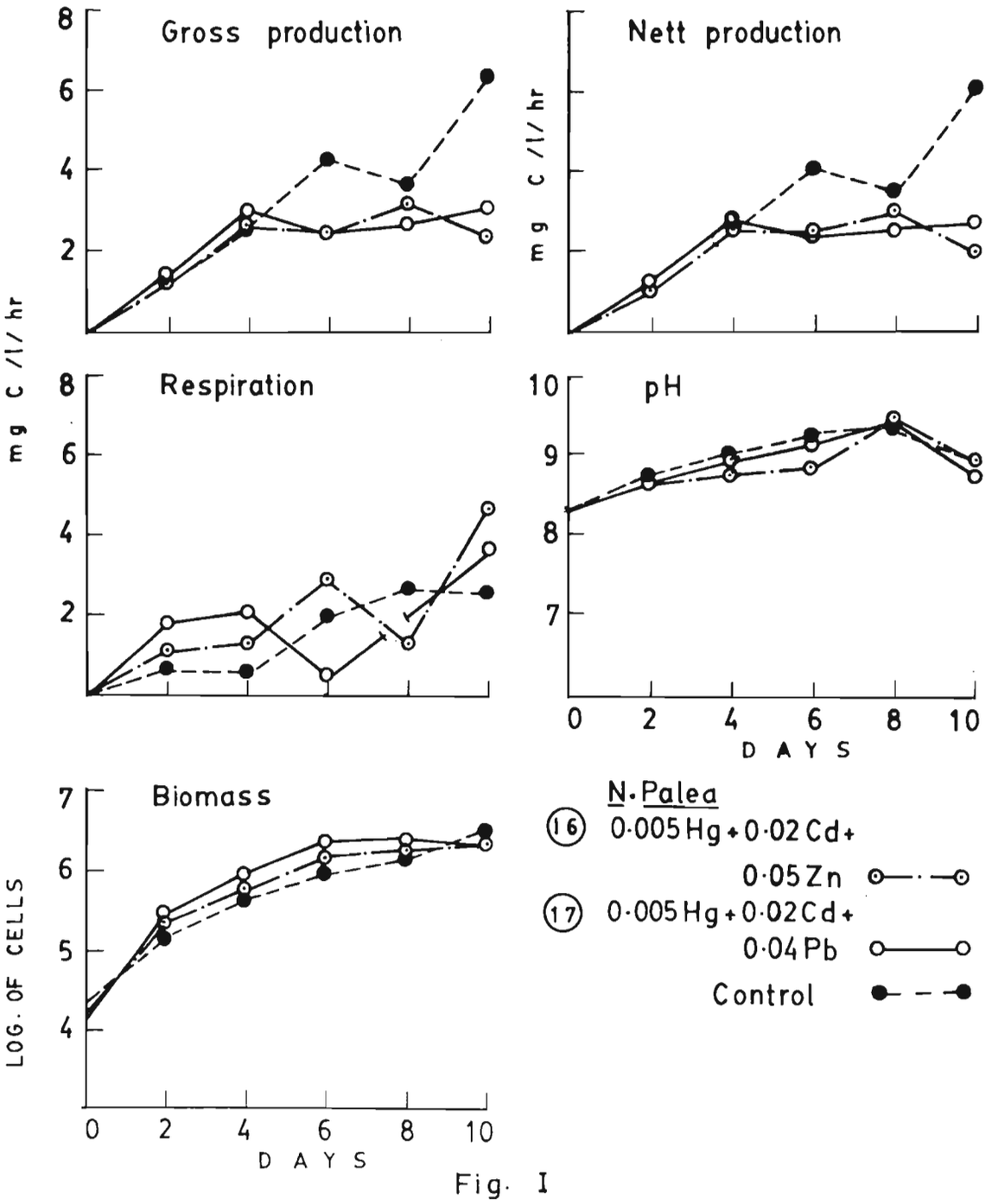

Gross production
Nett production
Respiration
pH
Biomass
mg C /l/ hr
LOG. OF CELLS
DAYS
N. Palea
16 0·005 Hg + 0·02 Cd + 0·05 Zn
17 0·005 Hg + 0·02 Cd + 0·04 Pb
Control

Fig. I

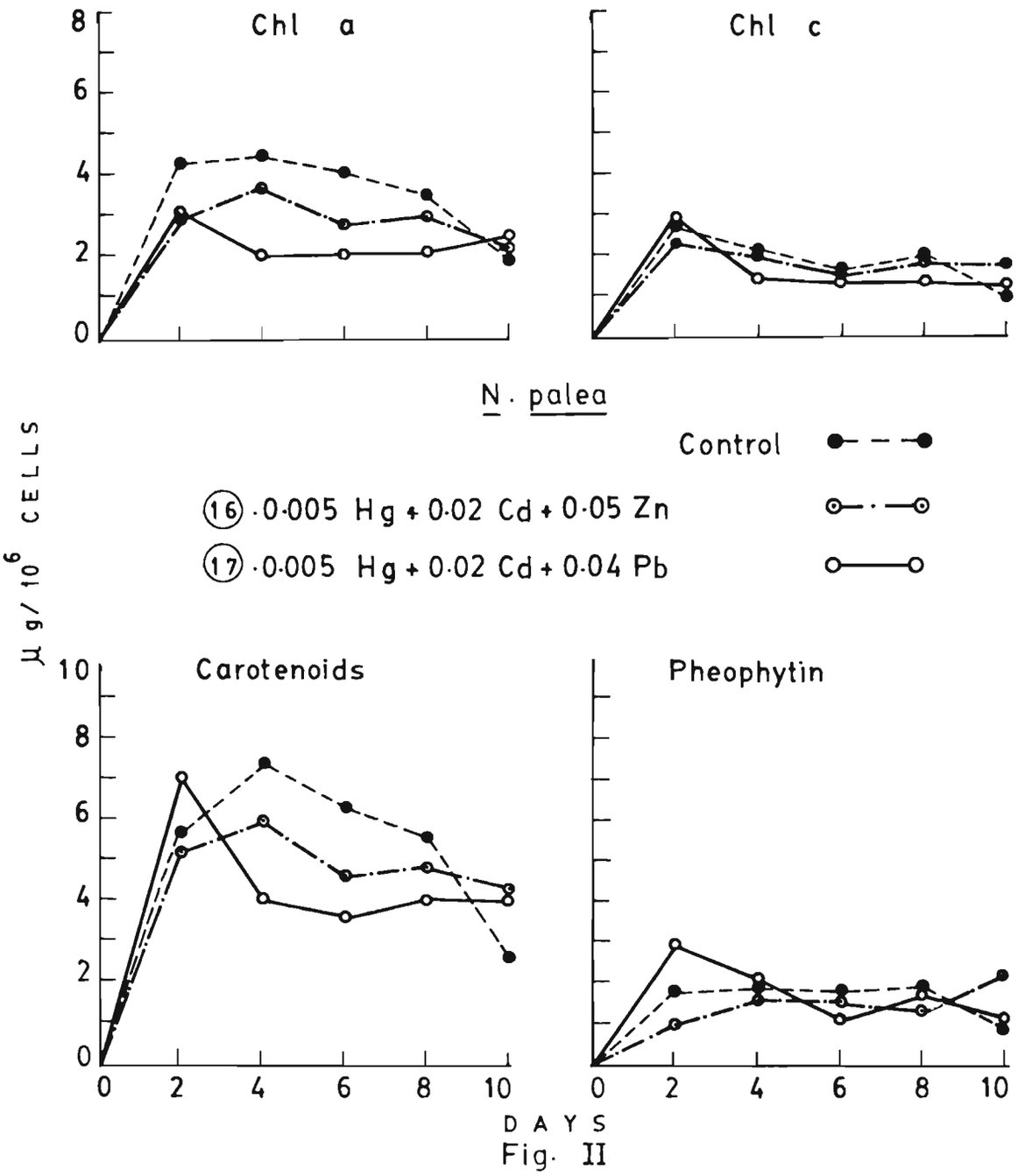

Chl a
Chl c
N. palea
Control
(16) ·0·005 Hg + 0·02 Cd + 0·05 Zn
(17) ·0·005 Hg + 0·02 Cd + 0·04 Pb
µg/10^6 CELLS
Carotenoids
Pheophytin
DAYS

Fig. II

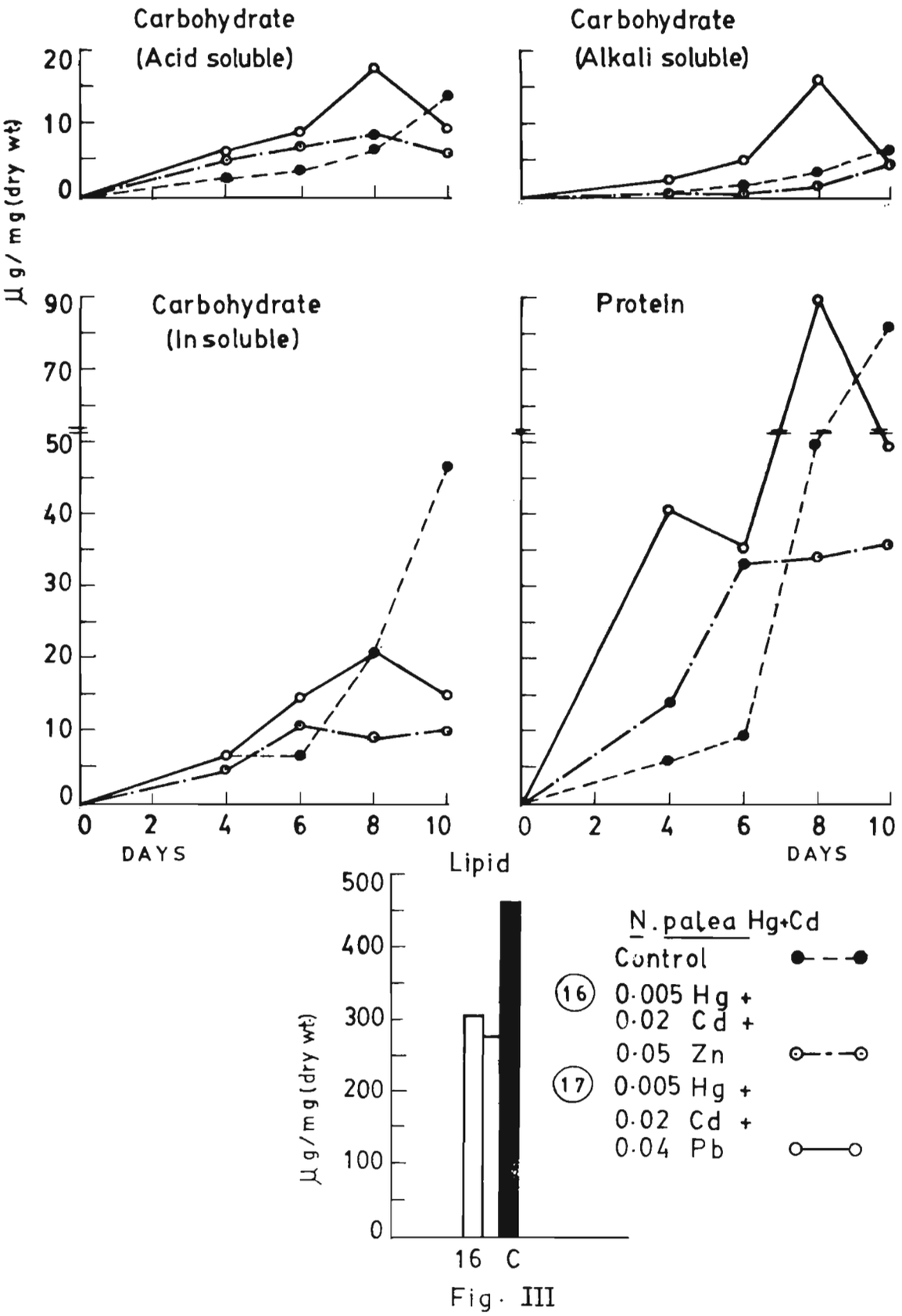

Carbohydrate
(Acid soluble)
Carbohydrate
(Alkali soluble)
Carbohydrate
(Insoluble)
Protein
µg/mg (dry wt)
DAYS
DAYS
Lipid
µg/mg (dry wt)
16
C
N. palea Hg+Cd
Control
16 0·005 Hg + 0·02 Cd + 0·05 Zn
17 0·005 Hg + 0·02 Cd + 0·04 Pb

Fig. III

6.2.3

Combined effect of mercury, copper and cadmium on S. bijugatus

Sl.No. of the combination	Concentration of metals (in ppm)
(18)	0.02 Hg + 0.1 Cu + 0.01 Cd

Production: (Fig. I)

The nett production of the alga was adversely affected by this combination and remained far less than that of control throughout. It was negligible upto fourth day and increased to be 74% less than that of control on sixth day. It decreased to 91% lower level on eighth day and remained steady upto tenth day. Increasing once again it reached 50% lower level by the end of growth phase.

Respiration of the alga was considerably lowered. It was maximum on fourth day and except for this day, it remained less than control. From 55% higher level on fourth day it dropped till sixth day and increased once again but was 24% less than that of control on eighth day. Thereafter gradual reduction in respiration was observed till the end of growth phase. 91% reduction was observed on the last day.

Consistent with low production pH of the culture remained considerably lower than that of control except on the last day of growth.

Pigments: (Fig. II)

Delay in the development of pigments was observed and all measurements were made only from fourth day onwards. Concentration of chlorophyll a, chlorophyll b and carotenoids was much higher than those of control from fourth day onwards. Carotenoid level was nearly steady during the late growth phase, whereas chlorophyll a, chlorophyll b and pheophytin exhibited considerable fluctuation. The chlorophyll a reached the maximum level on sixth, chlorophyll b on eighth, carotenoids on fourth and pheophytin on fourth day of growth respectively. Chlorophyll a and chlorophyll b exhibited a marked tendency to increase towards the end of growth phase.

Photosynthetic end products: (Fig. III)

Due to severe inhibition of growth, carbohydrate was measured only on the last day and was found to be reduced by 25% in relation to control.

Protein content of the alga was the most adversely affected product, with 93% reduction.

Lipid of the alga also reduced, by 45% in this combination.

Growth: (Fig. I)

The adverse effect of the metals in combination was clearly seen in the severe inhibition of growth. The growth was negligible upto eighth day and only slight improvement was noted towards the end of growth phase, resulting in considerable decrease in biomass.

Selected combination	Nutrients absorbed by the alga (µg/l)		N / P
	Phosphate	Nitrate	
(18)	1098	996	0.91
Control	945	1290	1.37

The uptake of phosphate increased in presence of these metals and that of nitrate decreased.

Conclusion:

The adverse effect of the metals in combination is very well pronounced in all parameters estimated except pigments. This combination was highly toxic to the alga.

Comparison:

When the effect of the present combination of metals was compared with that of combination (6) (0.03 Hg + 0.01 Cd) nett production was found to be lowered to a large extent. Respiration also was lowered. Pheophytin

was not affected but other pigments were reduced. Carbohydrate content was reduced to a large extent. Nine fold reduction in protein was observed. Lipid content was lowered by more than 50%. Biomass was reduced considerably.

When the effect of the present combination was compared with that of combination (8) (0.03 Hg + 0.1 Cu), nett production was found considerably reduced but respiration was not affected. Chlorophyll a, chlorophyll b and carotenoids increased but pheophytin was not much affected. Carbohydrate and lipid decreased though protein remained unaffected. Biomass was lowered to a considerable extent.

6.2.4

Combined effect of mercury, copper and lead on

S. bijugatus

Sl.No. of the combination	Concentration of metals (in ppm)
(19)	0.02 Hg + 0.1 Cu + 0.05 Pb

Production: (Fig. I)

The nett production of the alga was negligible upto fourth day. Increasing gradually thereafter it reached 81% lower level on eighth day. Sharp rise in production was observed from eighth day onwards and it reached 65% higher level by tenth day and further to 114% higher level by the end of growth phase.

Respiration of the alga was elevated to a large extent. It was 36% more than that of control on eighth day. Thereafter sharp rise was recorded to 474% higher level on tenth day. It declined towards the end of growth phase but remained 14% more than that of control.

pH of the culture was far below that of control upto sixth day. Consistent with higher production, it increased sharply thereafter and reached higher level than the maximum of control (10.64) on tenth day. Inspite of slight fall, pH of the culture remained higher than that of control towards the end of growth phase.

Pigments: (Fig. II)

Pigment content of the species was measured only from fourth day onwards as no colour was detected in the culture upto third day. But from fourth day onwards it was found to be higher than that of control. All four pigments registered a decline from their respective maxima, towards the end of growth phase. However, the concentration of chlorophyll b was highest when compared to other pigments in this combination. Pheophytin was not detected upto eighth day but was more than that of control on tenth and twelfth day. Total pigment content of the alga decreased with the age of the culture.

Photosynthetic end products: (Fig. III)

Growth of the species was suppressed and hence the carbohydrate was measured from eighth day onwards. From 25% higher level on eighth day it declined to 31% lower level by the end of growth phase.

Total protein content of the alga when measured at the end of growth phase was found to be 24% less than that of control.

Lipid was found to be adversely affected, with 63% reduction.

Growth: (Fig. I)

Growth of the alga was severely inhibited upto sixth day but subsequently gradual recovery was noted though not to the same extent as that of control, resulting in the reduction of biomass at the end of growth phase.

Selected combination	Nutrients absorbed by the alga (µg/l)		N / P
	Phosphate	Nitrate	
(19)	1071	512	0.48
Control	945	1290	1.37

The phosphate uptake of the alga exhibited slight enhancement whereas nitrate uptake was reduced by 40%.

Conclusion:

Though the rate of nett production was elevated and pH increased showing higher overall production than that of control, carbohydrate, protein and lipid of the alga were reduced, along with the biomass. The effect of the combination 19 on the whole was adverse on the species.

Comparison:

When the effect of the present combination of metals was compared with that of combination ⑦ (0.05 Hg + 0.05 Pb), nett production was found to be reduced in early phase, but improved in the latter phase. Chlorophyll a, chlorophyll b and carotenoids increased to a large extent but pheophytin increased only towards the end of growth phase. Carbohydrate was reduced particularly in the latter phase. Only marginal reduction was observed in protein and lipid concentration. Biomass was lowered to a large extent.

When compared with the effect of the combination ⑧ (0.03 Hg + 0.1 Cu), the nett production was observed to be far less in the early phase. Respiration increased to a large extent. Chlorophyll a, chlorophyll b and carotenoids were produced to a greater extent but pheophytin was reduced. Carbohydrate and lipid were reduced but protein was unaffected. Biomass also was reduced.

The effect of the combination of lead and copper was found to be lethal to the species. Hence, the present combination proved to be less toxic when compared with the above.

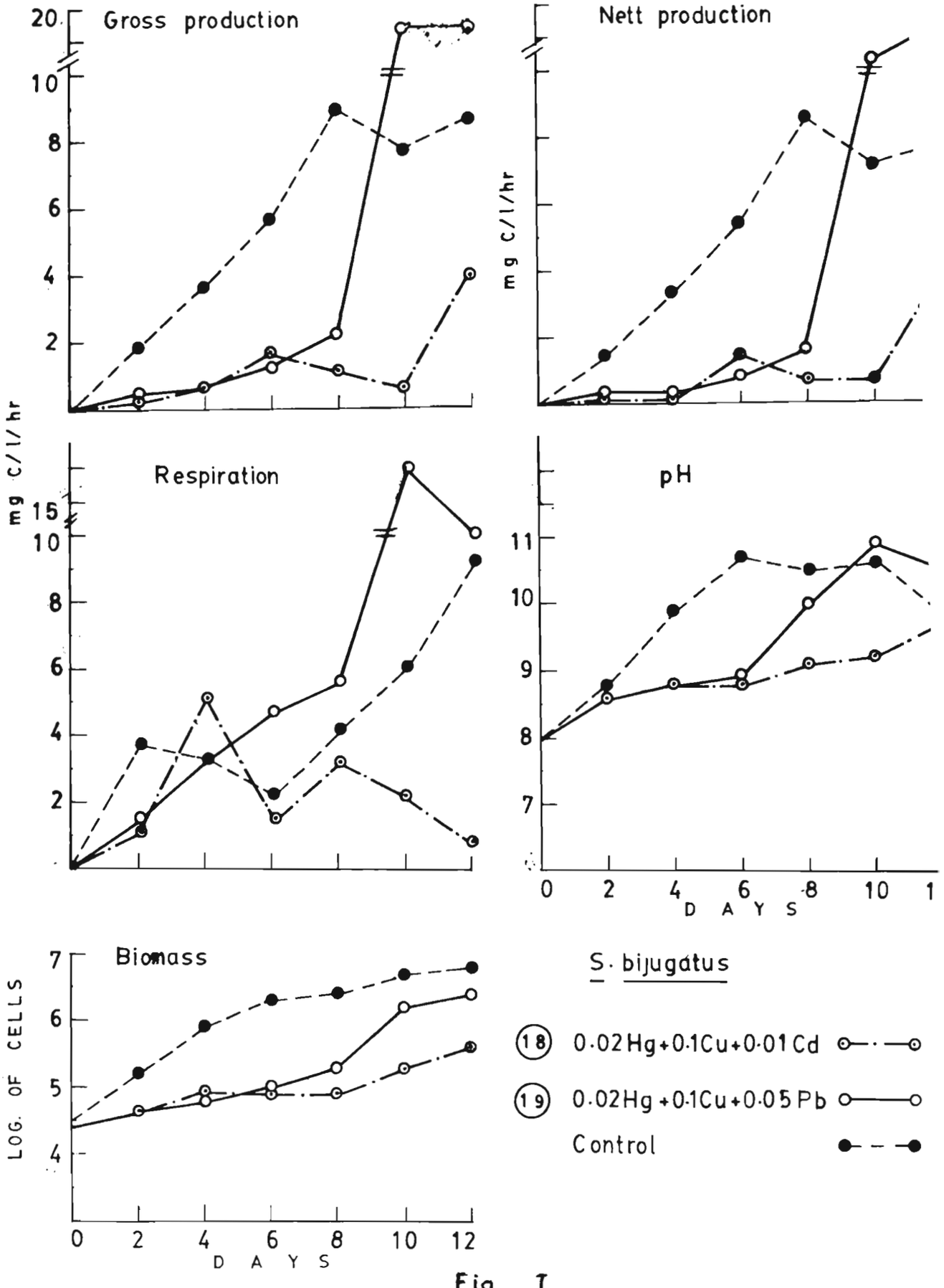

Gross production
Nett production
Respiration
pH
Biomass
mg C/l/hr
LOG. OF CELLS
DAYS
S. bijugatus
(18) 0·02Hg+0·1Cu+0·01Cd
(19) 0·02Hg+0·1Cu+0·05Pb
Control

Fig I

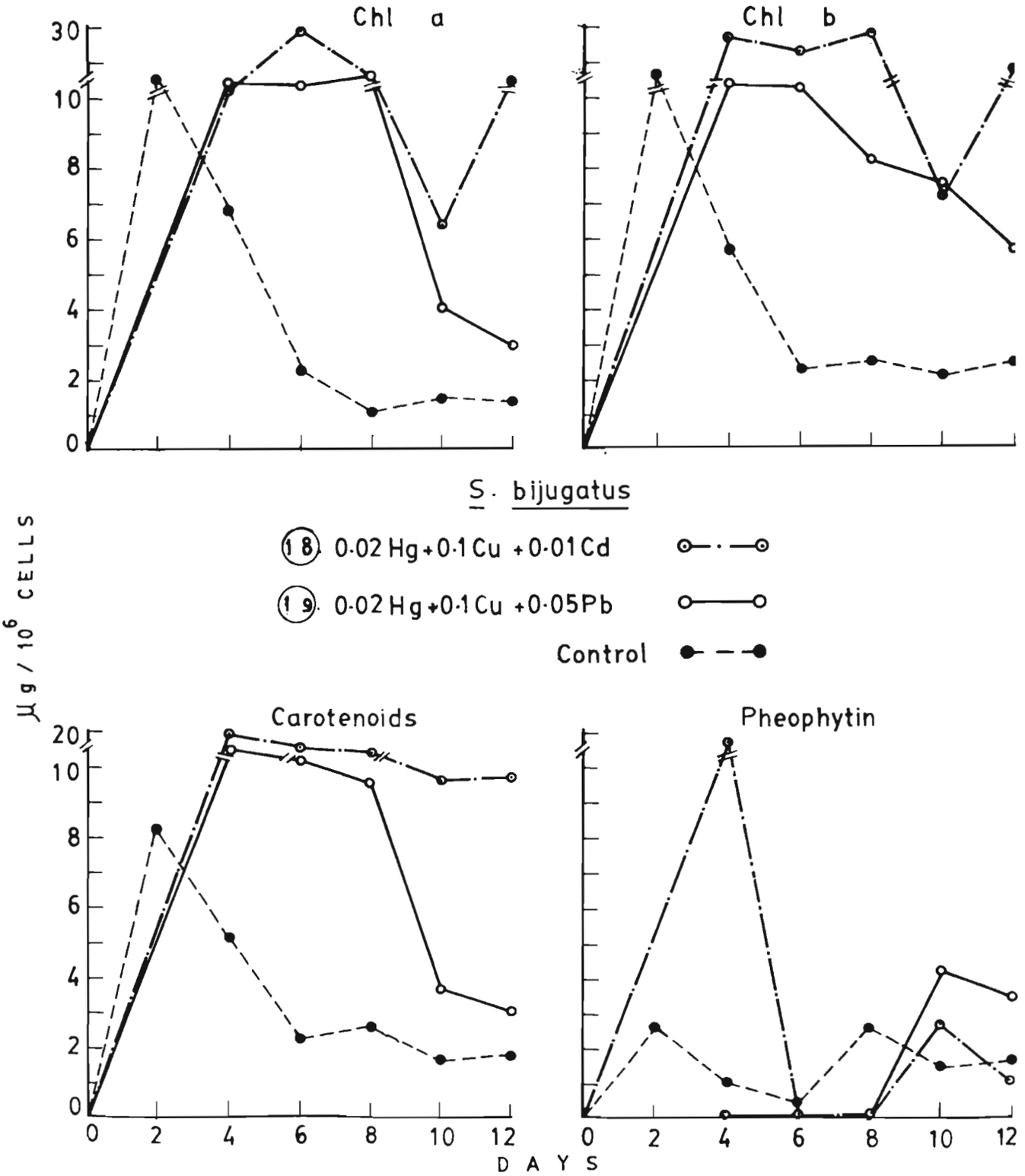
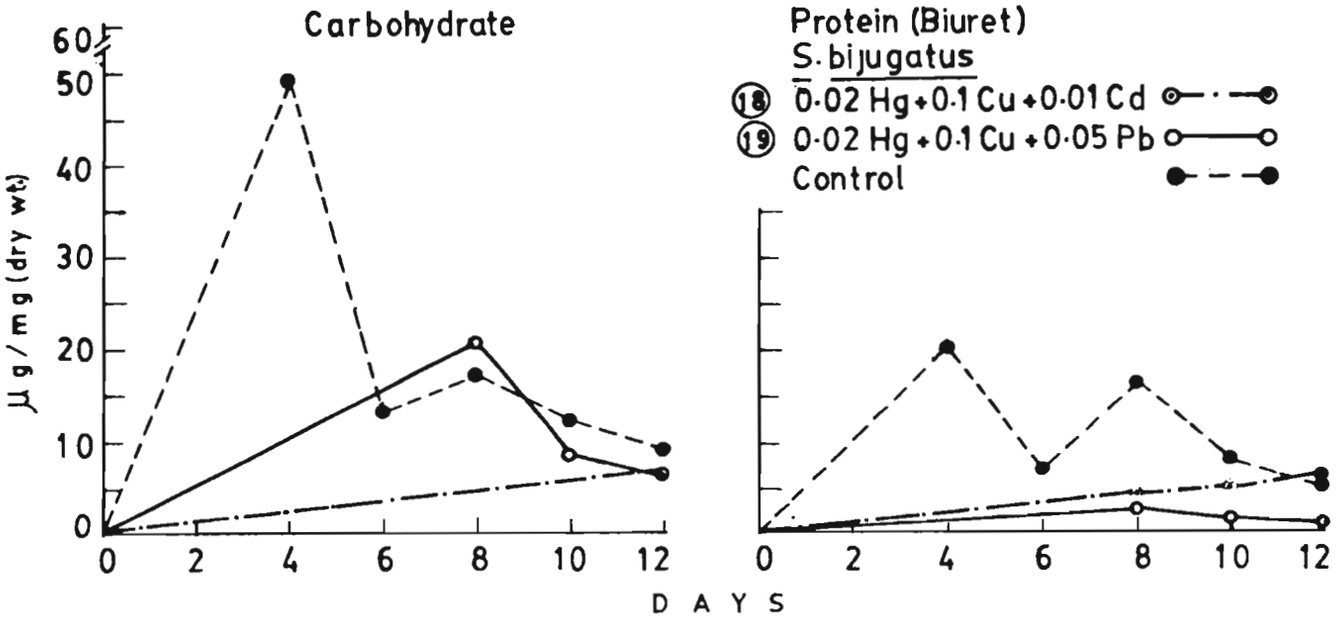

Chl a
Chl b
S. bijugatus
18 0·02 Hg + 0·1 Cu + 0·01 Cd
19 0·02 Hg + 0·1 Cu + 0·05 Pb
Control
Carotenoids
Pheophytin
µg / 10^6 CELLS
DAYS

Fig. II


Carbohydrate
Protein (Biuret)
S. bijugatus
(18) 0·02 Hg + 0·1 Cu + 0·01 Cd
(19) 0·02 Hg + 0·1 Cu + 0·05 Pb
Control
µ g / m g (dry wt.)
60
50
40
30
20
10
0
0 2 4 6 8 10 12
D A Y S

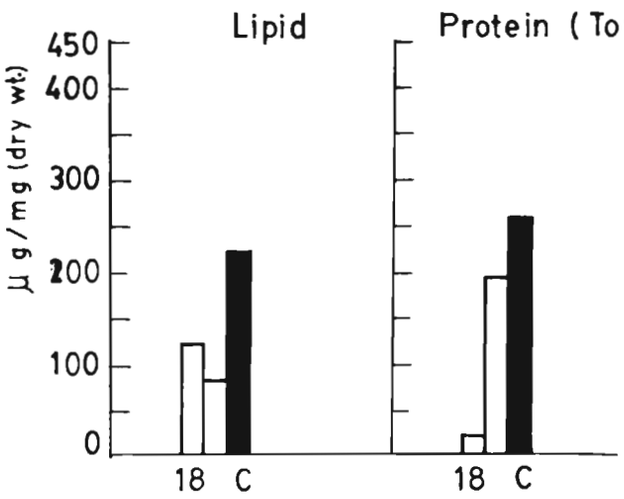

Lipid
Protein (Total)
µ g / m g (dry wt.)
450
400
300
200
100
0
18 C
18 C

Fig. III

Combined effect of mercury, cadmium and copper on *N. palea*

Sl.No. of the combination	Metals in combination (in ppm)
(18)	0.05 Hg + 0.02 Cd + 0.05 Cu

The combination was found to be highly toxic to the diatom. Pale brown colour appeared in the culture on fifth day but it disappeared by sixth day. Hence it was not possible to estimate any of the parameters selected.

Combined effect of mercury, copper and lead on *N. palea*

Sl.No. of the combination	Concentration of metals (ppm)
(19)	0.005 Hg + 0.05 Cu + 0.04 Pb

Production: (Fig. I)

Nett production of the diatom closely followed that of control upto fourth day. It remained steady between fourth and sixth day. It was 49% less on sixth day. It reached maximum level on eighth day being 19% less, and declined to 66% lower level by the end of growth phase in relation to control.

Respiration of the diatom was negligible on second day but increased sharply thereafter to reach 640% higher level by fourth day. After decreasing initially upto sixth day it increased once again 42% higher level on eighth day. It declined towards the end of growth phase and was reduced by 8% on the last day.

pH of the culture closely followed that of control upto fourth day, thereafter it gradually decreased towards the end of growth phase.

Pigments: (Fig. II)

Pigment level of the diatom increased with the age of the culture upto eighth day. All four pigments

exhibited decreasing tendency from eighth day onwards. The concentration of chlorophyll a and carotenoids exceeded those of control by sixth day whereas that of chlorophyll c by fourth day. Pheophytin content of the diatom remained higher except on fourth and sixth day of growth when it was equal to that of control. Pheophytin reached maximum concentration on second day whereas rest of the pigments on eighth day.

Photosynthetic end products: (Fig. III)

The acid soluble carbohydrate fraction of the diatom was found in greater quantity in the early phase when compared to that of control. The concentration was maximum on sixth day with 119% increase. Its level remained steady thereafter and was equal to that of control on eighth day but was found reduced by 53% on the last day.

The concentration of alkali soluble fraction did not differ much from that of control except when it was 40% less at the end of growth phase.

The insoluble carbohydrate fraction of the diatom registered a gradual increase, reaching maximum on sixth day. From 82% higher level on sixth day it decreased to 60% lower level on eighth day. Inspite of increase towards the end of growth phase it was found to be 75% less than that of control.

Production of protein was enhanced to a large extent in the early growth phase. It was 325% higher than that of control on fourth day and 191% higher on sixth day. Inspite of gradual increase it was found to be lowered by 32% and 38% respectively on eighth and tenth day of growth.

Lipid content of the diatom was found to be reduced by 44%.

Growth: (Fig. I)

Growth rate of the diatom improved in the early phase in this combination resulting in increased biomass upto sixth day when compared to that of control. But beyond this, little increase was observed in growth. Biomass at the end of growth phase, was found reduced in relation to control.

Selected combination	Nutrients absorbed (µg/l)		N / P
	Phosphate	Nitrate	
(19)	1650	782	0.47
Control	1350	763	0.57

The uptake of phosphate has increased by 19%. The treated diatom absorbed more nitrate when compared to that of control.

Conclusion:

The overall production of the diatom was lowered. All three main products of photosynthesis were reduced. Notwithstanding the initial stimulation, biomass of the species was found lowered. The effect of combination (19) was adverse on the physiology of the species.

Comparison:

When the effect of the present combination was compared with that of combination (7) (0.005 Hg + 0.04 Pb) nett production was found unaffected, but respiration was elevated. Carotenoids increased to a greater extent than the other pigments. Carbohydrate (all three fractions) were unaffected. Protein content increased but lipid was reduced by more than 100 ug. Biomass was not affected.

When compared with the effect of combination (8) (0.005 Hg + 0.05 Cu) the nett production and respiration were found unaffected. Pigments registered an increase. The acid soluble carbohydrate was lowered but other fractions remained unaffected. Martinal increase in protein was observed whereas lipid was reduced. Increase in biomass was marginal.

6.2.5 Combined effect of mercury, lead and zinc on

N. palea

Sl.No. of the combination	Concentration of metals (ppm)
(20)	0.005 Hg + 0.04 Pb + 0.05 Zn

Production: (Fig. I)

The nett production of the diatom increased gradually to maximum level on fourth day when it was 28% more than that of control. From sixth day onwards it remained less than that of control, decreasing gradually to 77% lower level by the end of the growth phase.

Respiration of the diatom was slightly higher than that of control only upto fourth day. It decreased to minimum on eighth day with 85% reduction. Though it increased towards the end of growth phase it remained 8% less than that of control.

pH of the culture remained less than that of control throughout growth phase.

Pigments: (Fig. II)

Total pigment content of the diatom decreased in this combination, when compared to that of control. Chlorophyll a remained less than that of control throughout

growth phase whereas chlorophyll c was slightly higher only on sixth and tenth day. Carotenoid concentration was higher only on the last day of growth whereas pheophytin was more on the second and last day of growth. All four pigments reached the maximum level on second day.

Photosynthetic end products: (Fig. III)

The acid soluble carbohydrate fraction registered a gradual increase from fourth day onwards. From 54% higher level on sixth day it reached 83% higher level at its maximum on eighth day. It declined towards the end of growth phase, to 69% lower level.

The alkali soluble carbohydrate fraction of the diatom did not deviate much from that of control upto sixth day. From 51% higher level on eighth day it decreased to 56% lower level by the end of growth phase.

The insoluble carbohydrate was developed to a lesser extent than that of control in the early growth phase. From 39% lower level on fourth day it reached 80% higher level on sixth day. Further increase in the concentration of this product was negligible resulting in 32% reduction by eighth day. It declined towards the end of growth phase to be 79% less, in relation to control.

Protein content of the diatom exhibited a sharp rise in presence of these metals, in early phase.

It was 135% higher than that of control on sixth day. Further increase was observed but not to the same extent as that of control and by eighth day it was 20% less. It declined thereafter and at the end of growth phase was 68% less than that of control.

Lipid content of the diatom was also adversely affected. 13% reduction was observed in this product.

Growth: (Fig. I)

Growth was stimulated in the early phase resulting in increased biomass when compared to that of control. It was steady after sixth day and at the end of growth phase it was less than that of control.

Selected combination	Nutrients absorbed (µg/l)		N / P
	Phosphate	Nitrate	
(20)	1440	797	0.55
Control	1350	763	0.57

The uptake of both the vital nutrients was enhanced by few ug in this combination.

Conclusion:

The combination of metals affected all the parameters studied adversely, except biomass.

Comparison:

When the effect of the present combination was compared with that of combination (7) (0.005 Hg + 0.04 Pb), increase in nett production was found to be limited to early phase whereas respiration increased towards the end of growth phase. All pigments exhibited slight increase particularly towards the end of growth phase. All three fractions of carbohydrate were lowered. Protein was reduced but lipid and biomass remained unaffected.

When compared with that of combination (9) (0.005 Hg + 0.05 Zn), increase in nett production was found limited to early phase. Respiration increased. All the four pigments decreased in concentration. Acid soluble and alkali soluble fractions showed marginal increase whereas the insoluble fraction remained unchanged. Protein content was reduced. Lipid also showed reduction, by 200 ug.

When compared with that of combination (14) (0.04 Pb + 0.05 Zn) the increase in nett production was found limited to early growth phase, whereas both production and respiration decreased in the latter phase. Pigments, carbohydrate and protein showed considerable reduction. Lipid was not affected. Biomass remained unaffected.

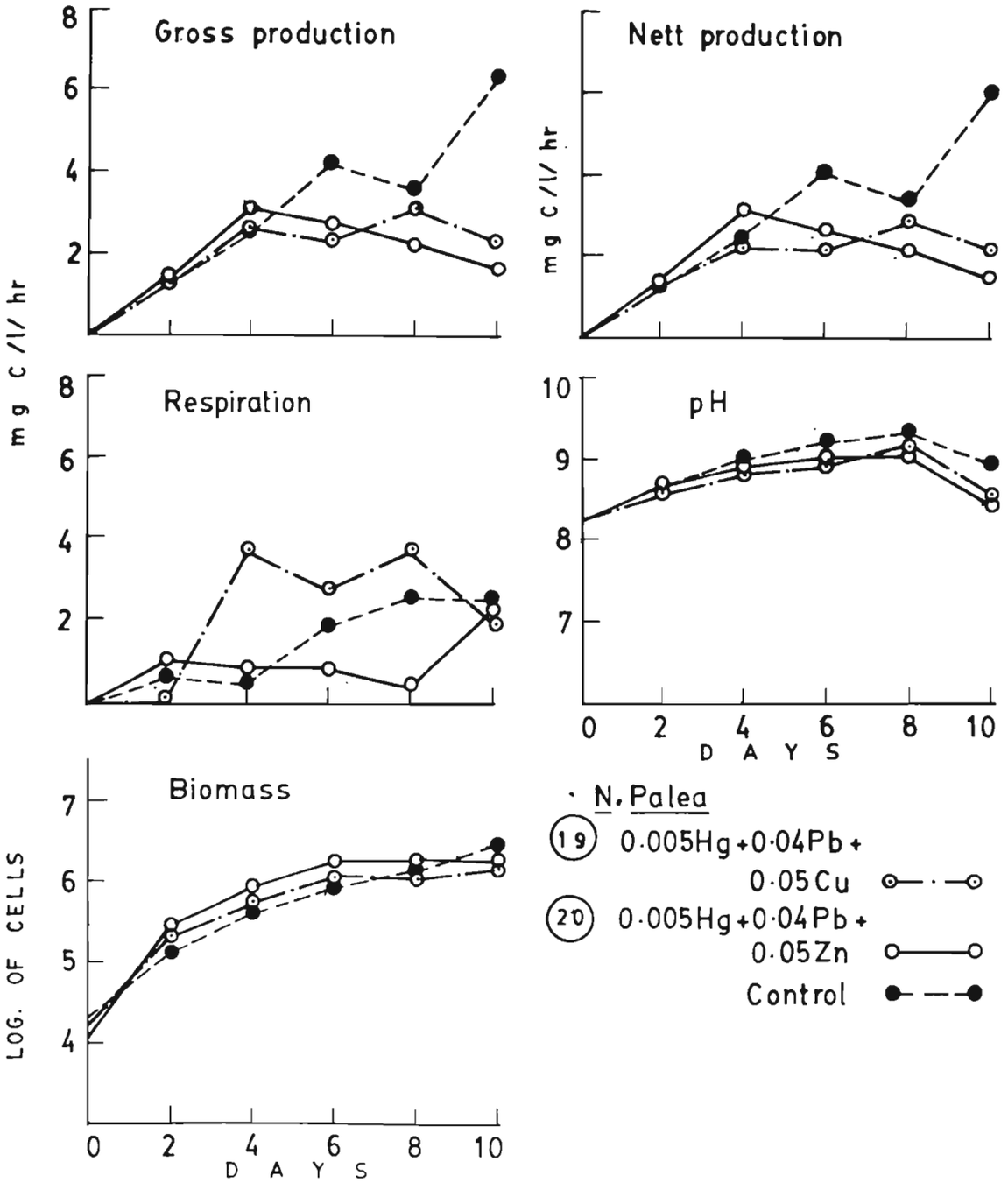

Gross production
Nett production
Respiration
pH
Biomass
mg C/l/ hr
LOG. OF CELLS
DAYS
· N. Palea
(19) 0·005Hg+0·04Pb+0·05Cu
(20) 0·005Hg+0·04Pb+0·05Zn
Control

Fig. I

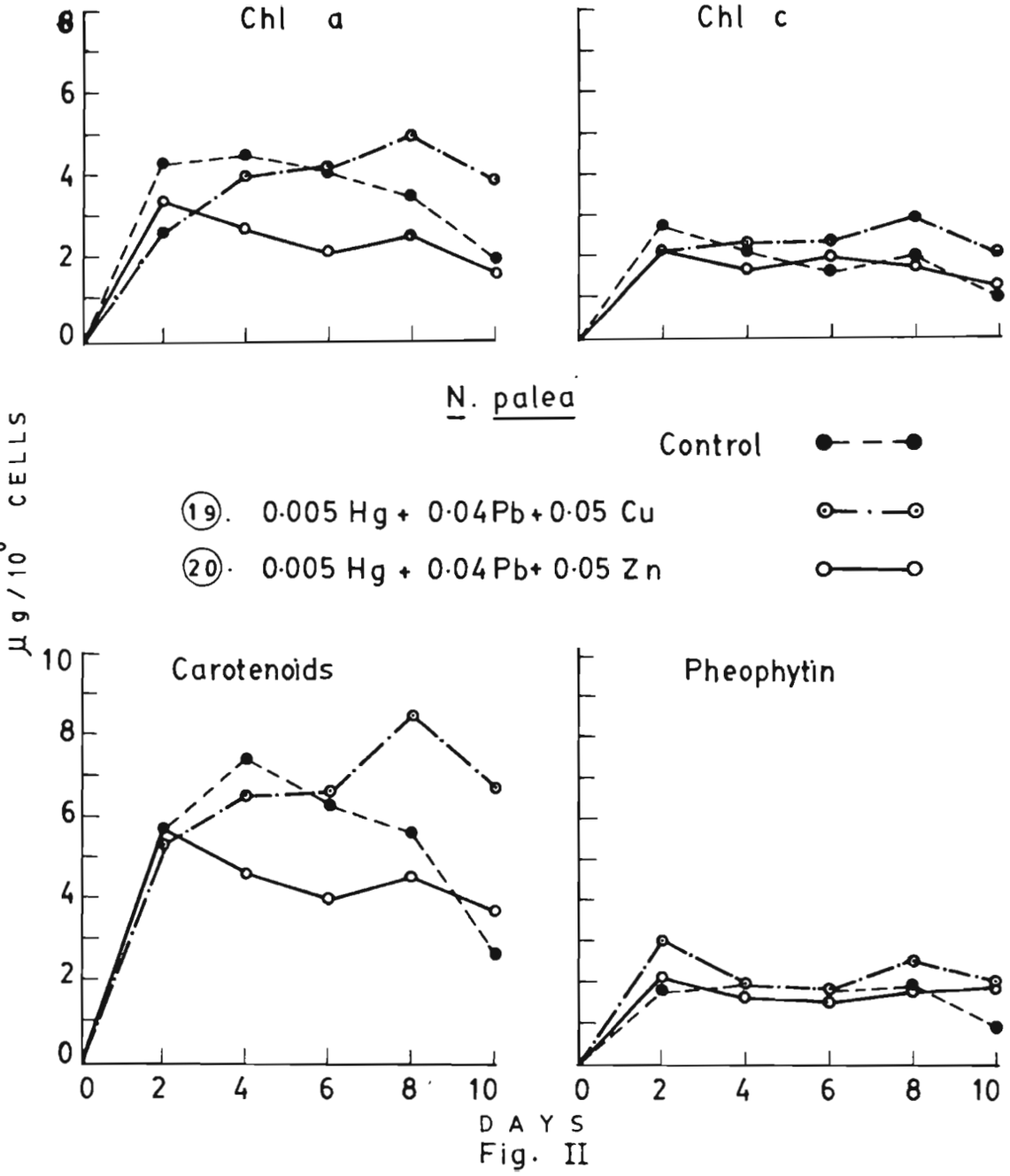

Chl a
Chl c
N. palea
Control
⑲. 0·005 Hg + 0·04 Pb + 0·05 Cu
⑳. 0·005 Hg + 0·04 Pb + 0·05 Zn
µg / 10^6 CELLS
Carotenoids
Pheophytin
0
2
4
6
8
10
DAYS

Fig. II

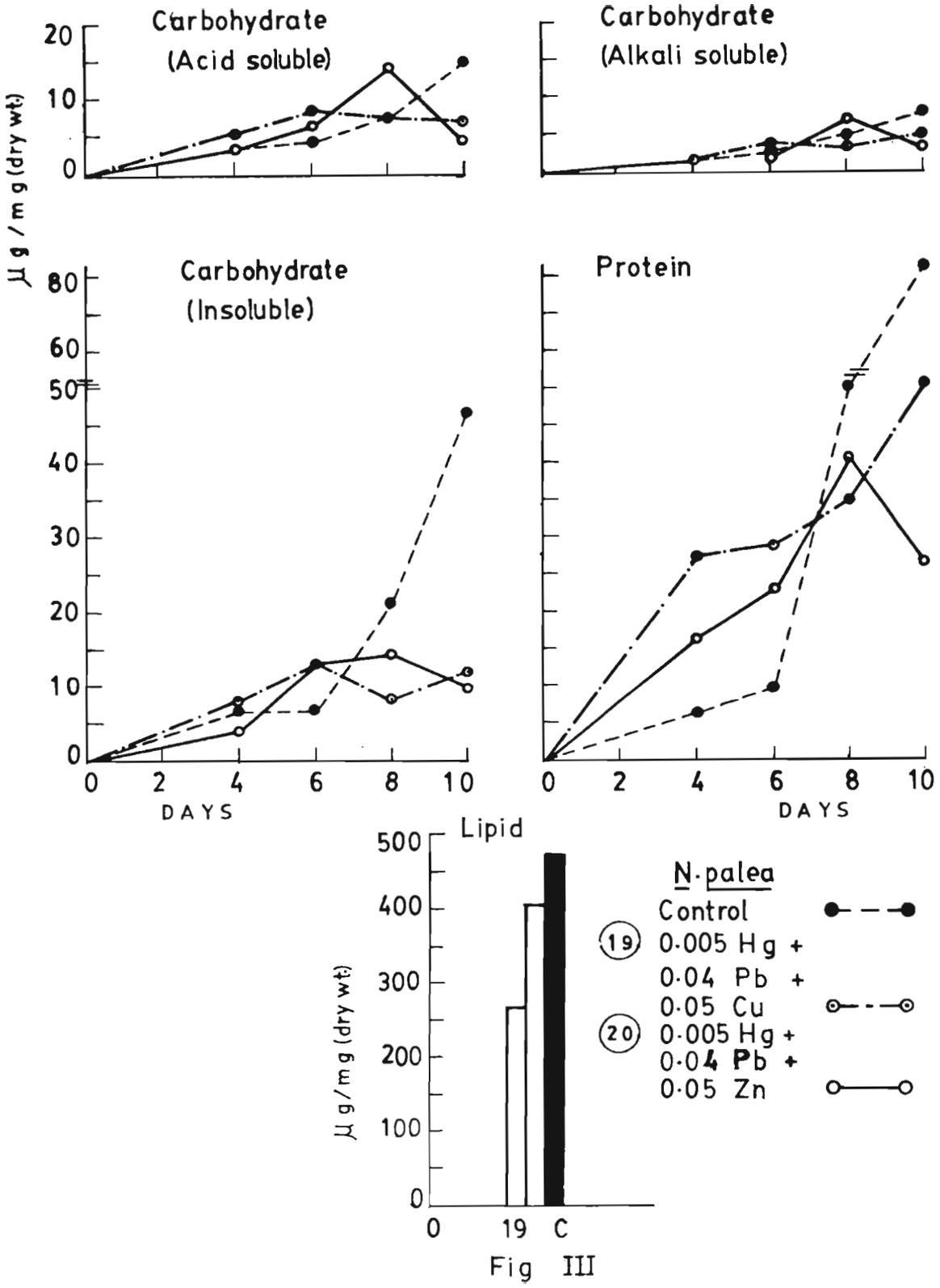

Carbohydrate
(Acid soluble)
Carbohydrate
(Alkali soluble)
Carbohydrate
(Insoluble)
Protein
µg / mg (dry wt)
DAYS
DAYS
Lipid
µg/mg (dry wt)
N. palea
Control
19 0·005 Hg + 0·04 Pb + 0·05 Cu
20 0·005 Hg + 0·04 Pb + 0·05 Zn
0
19
C

Fig III

Combined effect of mercury, lead and zinc on

S. bijugatus

Sl. No. of the combination	Concentration of metals (in ppm)
(20)	0.02 Hg + 0.05 Pb + 0.05 Zn

Production:

Nett production of the alga closely followed that of control upto sixth day but exceeded by 8% on eighth day, by 87% on tenth day and by 147% on twelfth day.

Respiration of the alga varied with peaks on second and sixth day with 5% and 4% increase respectively. It declined thereafter to minimum on eighth day with 43% reduction but increased sharply to reach the same level as control by tenth day. Inspite of continued increase it remained 17% less than that of control at the end of growth phase.

Notwithstanding the continuous increase in production, pH of the culture fluctuated during the growth phase. It was maximum on sixth day being slightly higher than that of control and declined thereafter till eighth day. Once again it exceeded that of control by tenth day and remained nearly steady thereafter at a higher level than control.

Pigments: (Fig. II)

Development of the pigments was delayed and hence the measurements were carried out from fourth day onwards. Total pigment content of the alga was severely suppressed. Chlorophyll b was not detected on fourth day and pheophytin from fourth to eighth day of growth. Pheophytin concentration was less than that of control throughout. Chlorophyll a, chlorophyll b and carotenoids were more than those of control only for a brief period during the middle of growth phase and all of them reached maximum level on sixth day of growth.

Photosynthetic end products: (Fig. III)

The carbohydrate concentration of the alga remained less than that of control throughout the growth phase except on tenth day when it was equal. The percentage reduction was found to be 49 on fourth and eighth day and 16 on the last day of growth.

Protein content of the alga was adversely affected. 36% reduction was recorded for this product.

Lipid content of the alga also was reduced, by 49%.

Growth: (Fig. I)

In general, growth of the alga closely followed that of control. No increase was noted in the cell

numbers between fourth and sixth day. The biomass however, remained slightly less than that of control at the end of growth phase.

Selected combination	Nutrients absorbed by the alga (µg/l)		N / P
	Phosphate	Nitrate	
(20)	1760	1130	0.64
Control	945	1290	1.37

The phosphate uptake of the alga increased by 86% whereas nitrate uptake was slightly lowered.

Conclusion:

Only the rate of nett production increased in presence of these metals. Lower values were obtained for all other parameters. Pigments were also suppressed to considerable extent. The combination was found to be undesirable and toxic to the species, specially when the photosynthetic end products were considered.

Comparison:

When the effect of the present combination of metals was compared with that of combination (7) (0.05 Hg + 0.05 Pb), both nett production and respiration increased to a large extent. Chlorophyll a was reduced in the early phase whereas pheophytin was suppressed to a large

extent. Chlorophyll b and carotenoids were found unchanged. Carbohydrate and protein were reduced but lipid was unaffected. Biomass was lowered.

When compared with that of combination (9) (0.05 Hg + 0.05 Zn), nett production was observed to increase to a large extent. Respiration also increased. Carotenoids were reduced to a far greater extent than the other pigments. Carbohydrate content was reduced. Nearly 50% reduction in protein was observed. Two fold reduction in lipid was observed. Biomass was lowered.

When compared with that of combination (14) (0.3 Pb + 0.05 Zn), marginal increase in production was observed. Respiration was elevated. With the exception of carotenoids, pigments were reduced. Carbohydrate was lowered. Protein was reduced by 50% while lipid was unaffected. Biomass was increased.

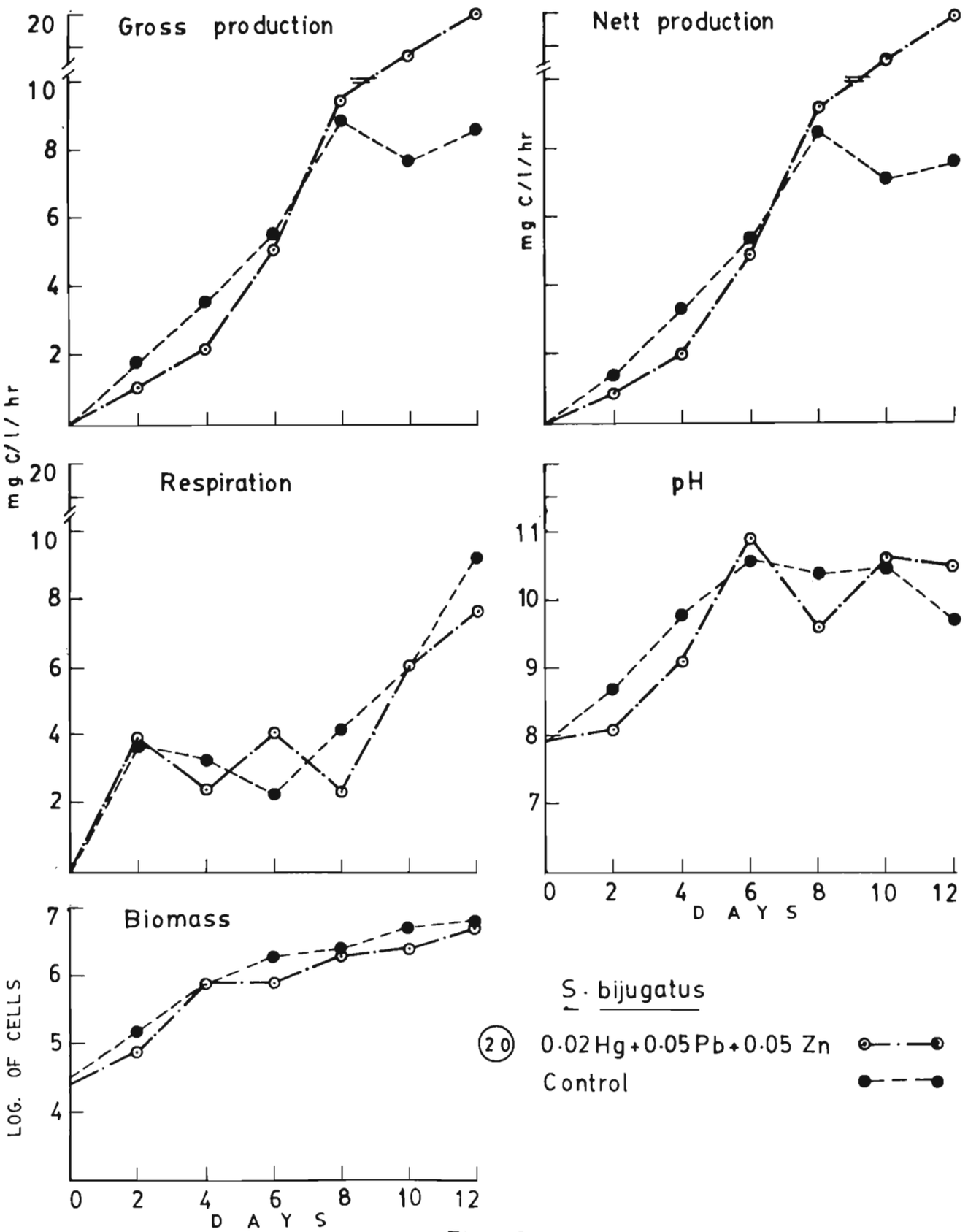

Gross production
Nett production
Respiration
pH
Biomass
mg C/l/hr
LOG. OF CELLS
DAYS
S. bijugatus
(20) 0·02 Hg + 0·05 Pb + 0·05 Zn
Control

Fig. I

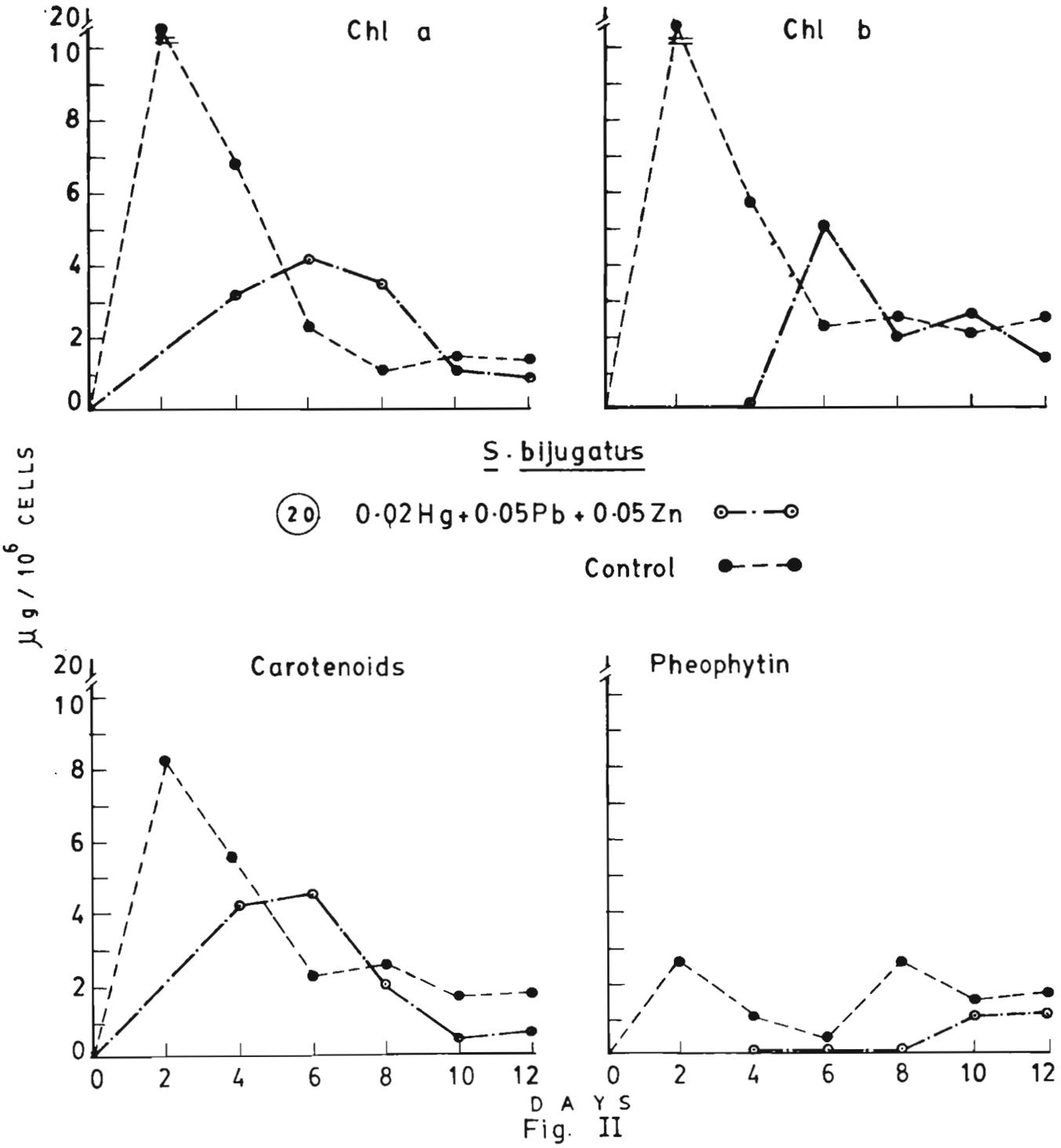
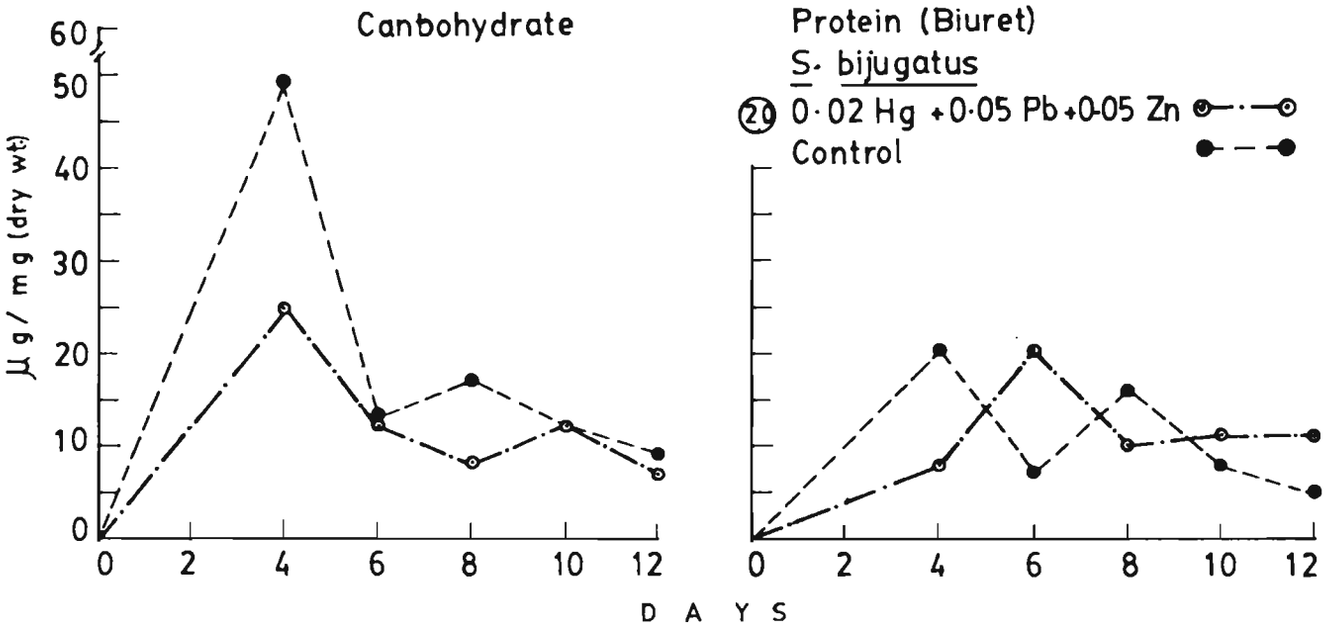

Chl a
Chl b
S. bijugatus
(20) 0·02Hg+0·05Pb+0·05Zn
Control
µg / 10^6 CELLS
Carotenoids
Pheophytin
20
10
8
6
4
2
0
0 2 4 6 8 10 12
D A Y S

Fig. II


Carbohydrate
Protein (Biuret)
S. bijugatus
20 0·02 Hg +0·05 Pb+0·05 Zn
Control
µg / mg (dry wt)
60
50
40
30
20
10
0
0 2 4 6 8 10 12
0 2 4 6 8 10 12
D A Y S

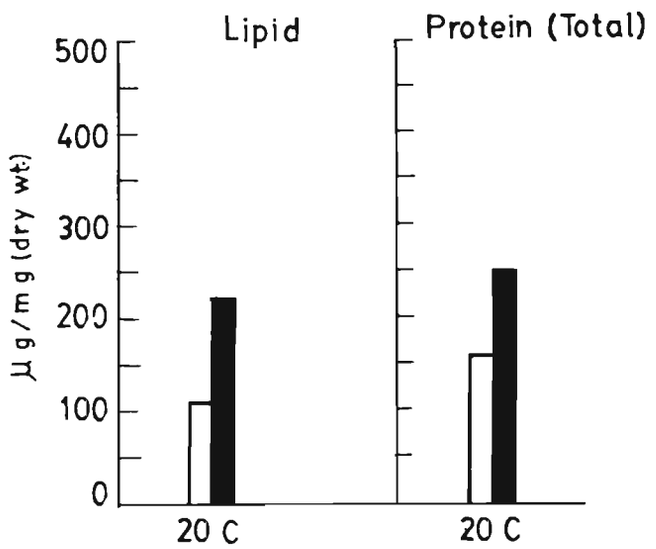

Lipid
Protein (Total)
µg/mg (dry wt)
500
400
300
200
100
0
20 C
20 C

Fig. III

6.2.6

Combined effect of lead, cadmium and copper on

S. bijugatus

Sl.No. of the combination	Concentration of metals (in ppm)
(21)	0.05 Pb + 0.01 Cd + 0.1 Cu

Production: (Fig. I)

Nett production of the alga was severely inhibited upto eighth day. 86% and 92% reduction was recorded on fourth and eighth day respectively. From eighth day onwards production increased gradually to 39% lower level in relation to control.

Respiration of the alga was elevated during the middle of growth phase. Rate of respiration was same on fourth and sixth day, but from sixth day it increased to reach maximum level on eighth day. 27% increase was recorded on fourth day. From 74% higher level on eighth day it declined to 33% lower level by tenth day and increased once again towards the end of growth phase to be 39% less than control on the last day.

pH of the culture in this treatment remained considerably less than that of control except on the last day.

Pigments: (Fig. II)

The usual green colour did not appear in the culture upto third day but by fourth day, colour appeared in all three replicates. Hence the estimation was carried out from fourth day onwards. Chlorophyll a, chlorophyll b and carotenoids exhibited considerable fluctuation during the growth period. All pigments exhibited an increasing tendency towards the end of growth phase. Pheophytin was not detected from fourth to eighth day of growth. From a lower level on tenth day it increased to a large extent by twelfth day. With the exception of carotenoids, pigments reached their respective maxima on the last day of growth. Carotenoids were maximum on fourth day. Total pigment content of the alga increased to a very large extent when compared to that of control.

Photosynthetic end products: (Fig. III)

Due to severe suppression of growth the carbohydrates were measured only on the last day and were found to be equal to that of control.

Protein content of the alga was reduced by 36% but lipid increased by 32%.

Growth: (Fig. I)

Growth of the alga was inhibited from second to fourth day and also from sixth to eighth day. During the rest of the period though the alga contined to grow the increase in biomass was not considerable.

Selected combination	Nutrients absorbed by the alga (µg/l)		N / P
	Phosphate	Nitrate	
(21)	990	942	0.95
Control	945	1290	1.36

The uptake of phosphate increased by few ug but the nitrate uptake was reduced by 26%.

Conclusion:

This combination was found to be highly toxic to the species in the initial stages. The toxic effect is more pronounced when the growth and biomass were considered. Protein content was reduced but the lipid improved. The overall photosynthetic production was not so much affected as growth.

Comparison:

When the effect of the present combination was compared with that of combination (11) (0.05 Cd + 0.05 Pd), marginal improvement in nett production was observed towards the end of growth phase. Respiration increased. Pheophytin was reduced to a greater extent than the other pigments during the middle growth phase. Carbohydrate decreased. Protein increased by 50% whereas lipid was unaffected. Marginal reduction in biomass was observed.

The present combination of metals was found to be less toxic than cadmium and copper (Cd + Cu) and cadmium and lead (Cd + Pb) to the species, since growth was completely suppressed in the above mentioned combinations.

6.2.7

Combined effect of lead, cadmium and zinc on

S. bijugatus

Sl.No. of the combination	Concentration of metals (in ppm)
(22)	0.05 Pb + 0.01 Cd + 0.05 Zn

Production: (Fig. I)

The nett production of the alga though was not inhibited, on fourth and sixth day was very low. From 30% lower level on second day it declined to 93% lower level on sixth day but increased sharply thereafter to 33% lower level by eighth day. It once again decreased slightly by tenth day and increased to 99% higher level by the end of growth phase.

Respiration of the alga was considerably reduced and remained less than that of control throughout the growth phase. Reduction was observed by 38% on second day and 27% on fourth day. It was minimum on sixth day with 52% reduction. It increased once again but was 33% less on eighth day and 21% less on the last day of growth.

The pH was less than that of control in the early phase reflecting low production. But by tenth day it increased sharply to be slightly higher than that of control and remained steady thereafter.

Pigments: (Fig. II)

Total pigment content of the alga remained much higher than that of control from fourth day onwards. The development of pigments was delayed and no visible green colour was noticed on second day. All four pigments fluctuated with peaks on fourth and tenth day of growth. The concentration of pigments showed a decreasing tendency towards the end of growth phase. Chlorophyll b was developed to a greater extent than any other pigment.

Photosynthetic end products: (Fig. III)

Carbohydrate concentration was found to be far less than that of control on fourth day but improved later and reached 23% higher level, at its maximum on sixth day. It decreased thereafter and was less than that of control on eighth day. However, it increased to 9% higher level by the end of growth phase.

Total protein content of the alga increased by 41% but lipid was reduced by 47%.

Growth: (Fig. I)

Growth of the alga was suppressed to considerable extent but no lag was observed.

Selected combination	Nutrients absorbed (ug/l)		N / P
	Phosphate	Nitrate	
(22)	1270	1084	0.85
Control	945	1290	1.37

The uptake of phosphate increased by 34% whereas that of nitrate decreased by 16%.

Conclusion:

Increase in production and protein reflect the positive influence of this combination but this in no way resulted in the increase of biomass. The combination was more toxic when the growth of the species was considered.

Comparison:

When the effect of the present combination of metals was compared with that of combination (11) (0.05 Cd + 0.05 Pb), both nett production and respiration were found increased to a large extent. The concentration of all four pigments was lowered. Carbohydrate was reduced. Four fold increase in protein was observed. Lipid content of the alga was doubled. Biomass also showed improvement.

When compared with the effect of combination (12) (0.05 Cd + 0.1 Zn), the nett production of the alga was

observed to increase and respiration was reduced to a large extent. Enhancement in the level of all four pigments was observed. Carbohydrate was unaffected. Four fold increase was found in protein. Nearly two fold reduction was observed in lipid.

When compared with the effect of combination (14) (0.3 Pb + 0.05 Zn), nett production was found unaffected in the early phase, but increased towards the end of growth phase. Respiration also was found to increase towards the end of growth phase. All four pigments exhibited reduction. Carbohydrate and lipid were reduced whereas protein showed marginal improvement. Biomass was lowered.

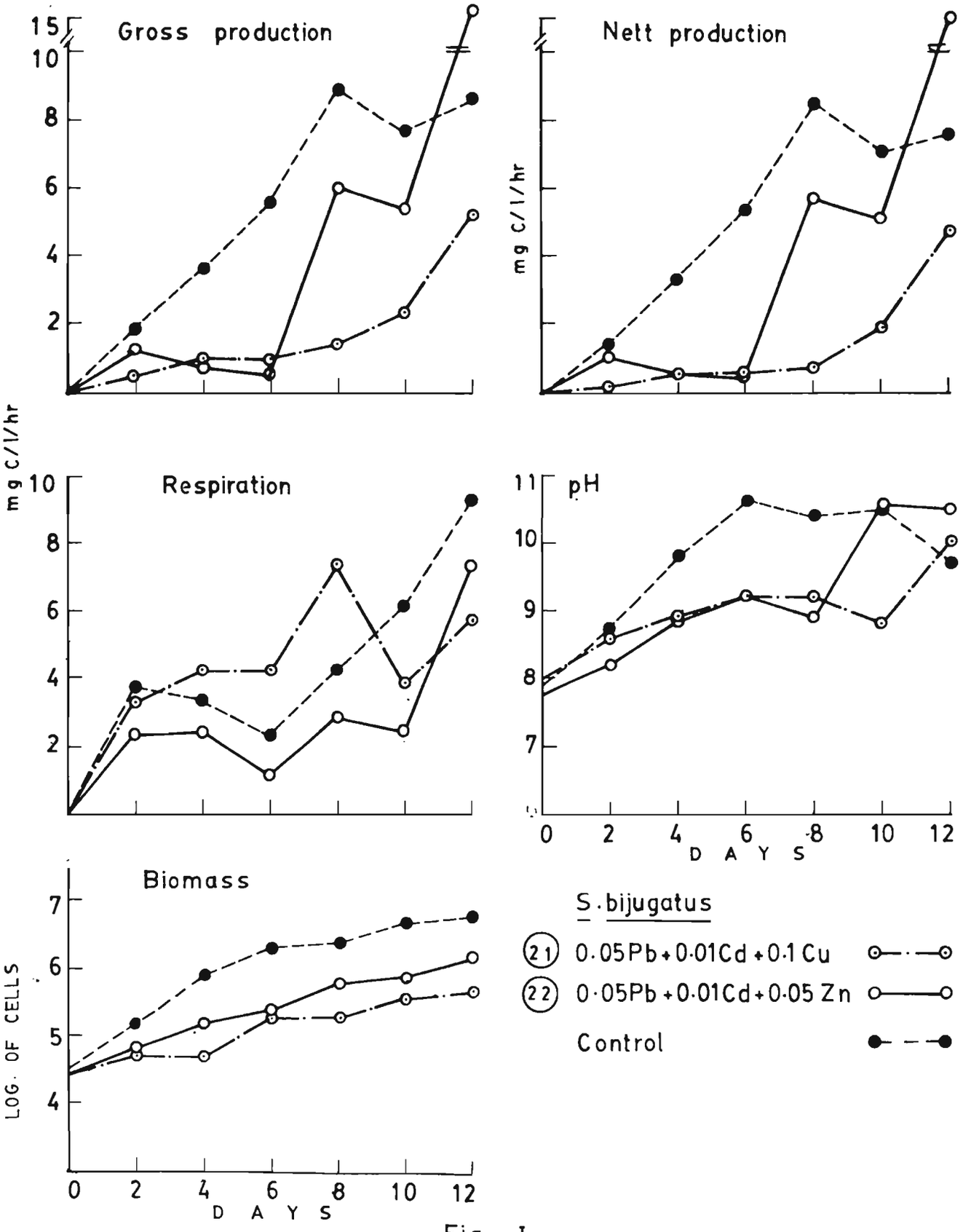

Gross production
Nett production
Respiration
pH
Biomass
mg C/l/hr
LOG. OF CELLS
DAYS
S. bijugatus
(21) 0·05Pb + 0·01Cd + 0·1 Cu
(22) 0·05Pb + 0·01Cd + 0·05 Zn
Control

Fig. I

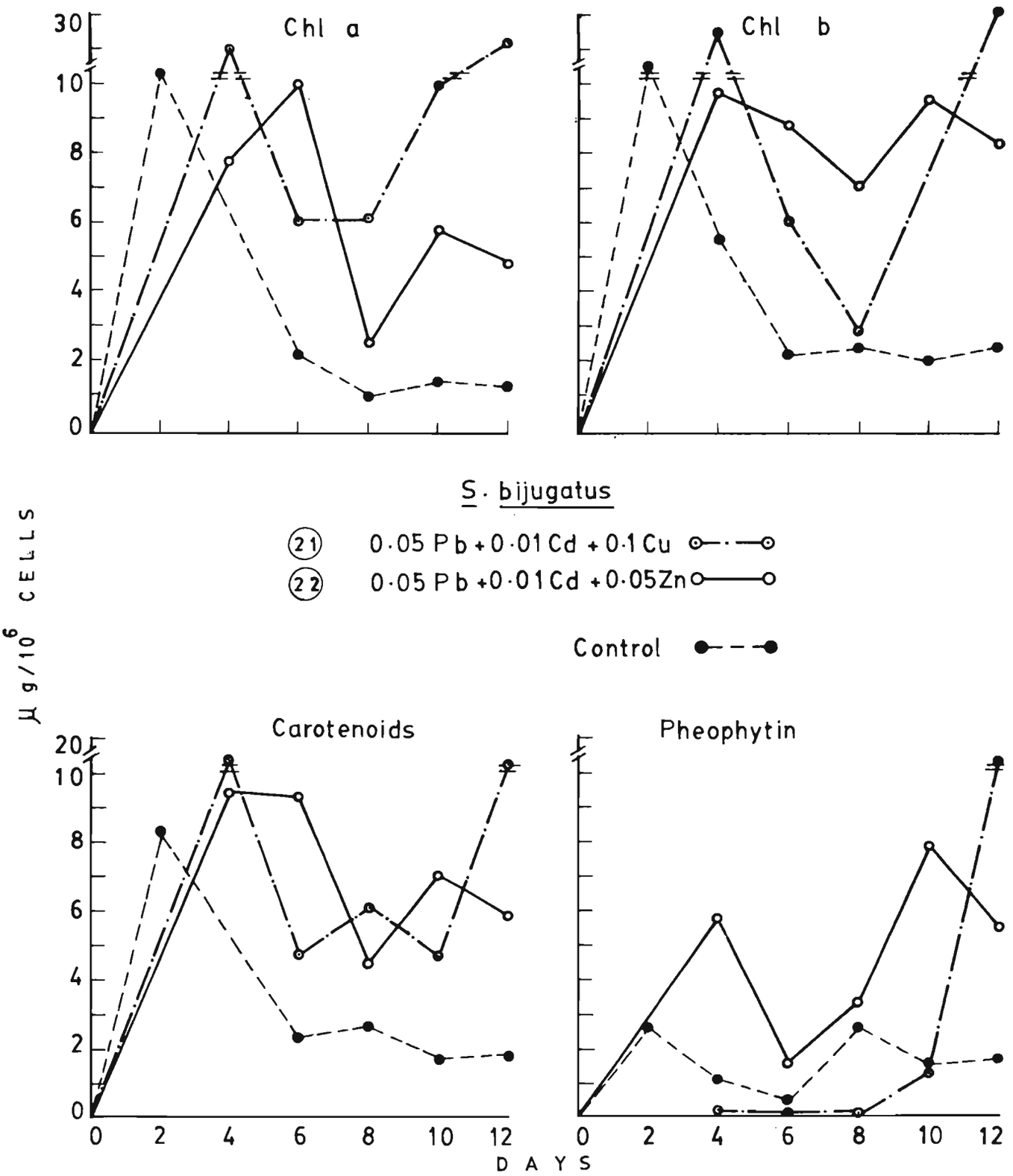
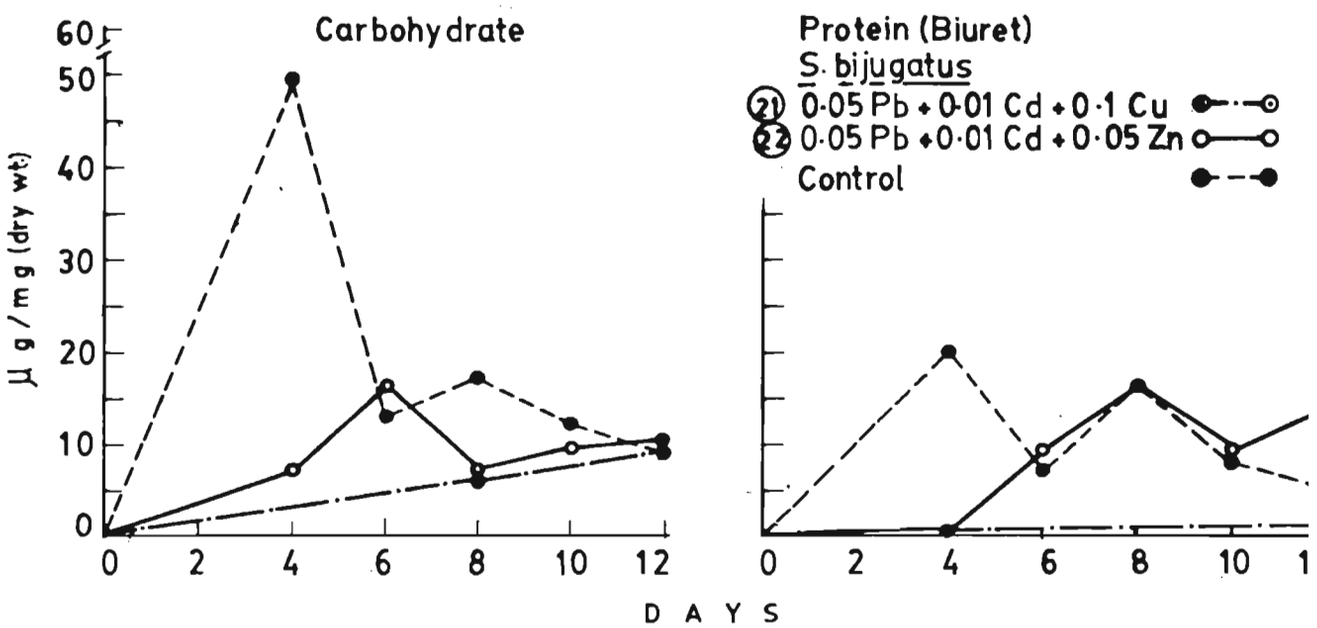

Chl a
Chl b
S. bijugatus
(21) 0·05 Pb + 0·01 Cd + 0·1 Cu
(22) 0·05 Pb + 0·01 Cd + 0·05 Zn
Control
μ g/10^6 CELLS
Carotenoids
Pheophytin
DAYS

Fig. II


Carbohydrate
Protein (Biuret)
S. bijugatus
21 0·05 Pb + 0·01 Cd + 0·1 Cu
22 0·05 Pb + 0·01 Cd + 0·05 Zn
Control
µ g / m g (dry wt.)
60
50
40
30
20
10
0
0 2 4 6 8 10 12
0 2 4 6 8 10 1
D A Y S

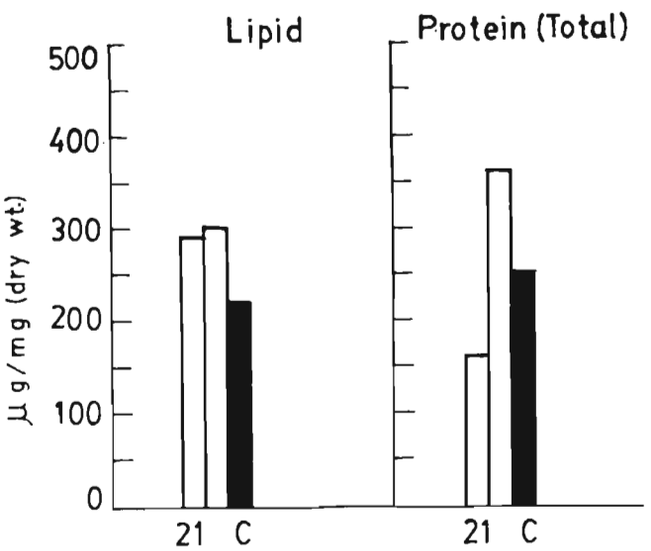

Lipid
Protein (Total)
µ g / m g (dry wt.)
500
400
300
200
100
0
21 C
21 C

Fig. III

Combined effect of cadmium, lead and copper on *N. palea*

Sl. No. of the combination	Metals in combination (in ppm)
(21)	0.02 Cd + 0.04 Pb + 0.05 Cu

This particular combination of metals was found to be highly toxic to the diatom. Growth of the diatom was completely suppressed and no perceptible change in the culture flask was observed ever after fifteen days.

Combined effect of cadmium, lead and zinc on *N. palea*

Sl. No. of the combination	Metals in combination (in ppm)
(22)	0.02 Cd + 0.04 Pb + 0.05 Zn

The metals in combination proved to be lethal to the species at the selected concentrations. Hence the selected parameters of growth were not estimated.

Discussion

The reaction of the test species varied with the combination of metals studied (Fig.VI). Few reports on the combined effect of three metals on algae were found in literature (Barlette et al., 1974). In the present study the effect of three metal combinations was found to be totally undesirable.

The effect of combinations of mercury, cadmium and zinc (Combination (16)) and mercury cadmium and lead (Combination (17)) on S.bijugatus was different from the same on N.palea. When the combination (16) was employed, lipid was reduced. But protein increased to a large extent when either combination was employed. Unlike in N.palea , the growth of S.bijugatus was severely retarded. Consequent on this, the biomass remained far less than that of control when either combination was employed. The effect of combination (16) was found to be less toxic to N.palea compared to that of combination (17). The adverse effect was evident only in the late growth phase as far as carbohydrate and protein were concerned, when combination (17) was employed. But biomas remained

nearly the same as that when combination (16) was employed. In effect, zinc or lead differed very little in combination with mercury and cadmium.

N.palea seems to be more tolerant to these two combinations ((16) and (17)) than S.bijugatus.

The combination of mercury, cadmium and copper (combination (18)) worked out to be highly toxic to S.bijugatus and lethal to N.palea. Biomas and endproducts of S.bijugatus were reduced to a large extent. Even when cadmium was replaced by lead as in combination (19) (mercury, copper and lead) the endproducts of S.bijugatus were found to be adversely affected. Lead helped the species to develop lipid when combined with mercury and cadmium (combination (17)) but reduced lipid content when combined with mercury and copper (combination (19)). Protein was affected to a large extent in S.bijugatus whereas in N.palea the toxicity was not so evident, in this respect. Biomass of both the species was lowered to a considerable extent.

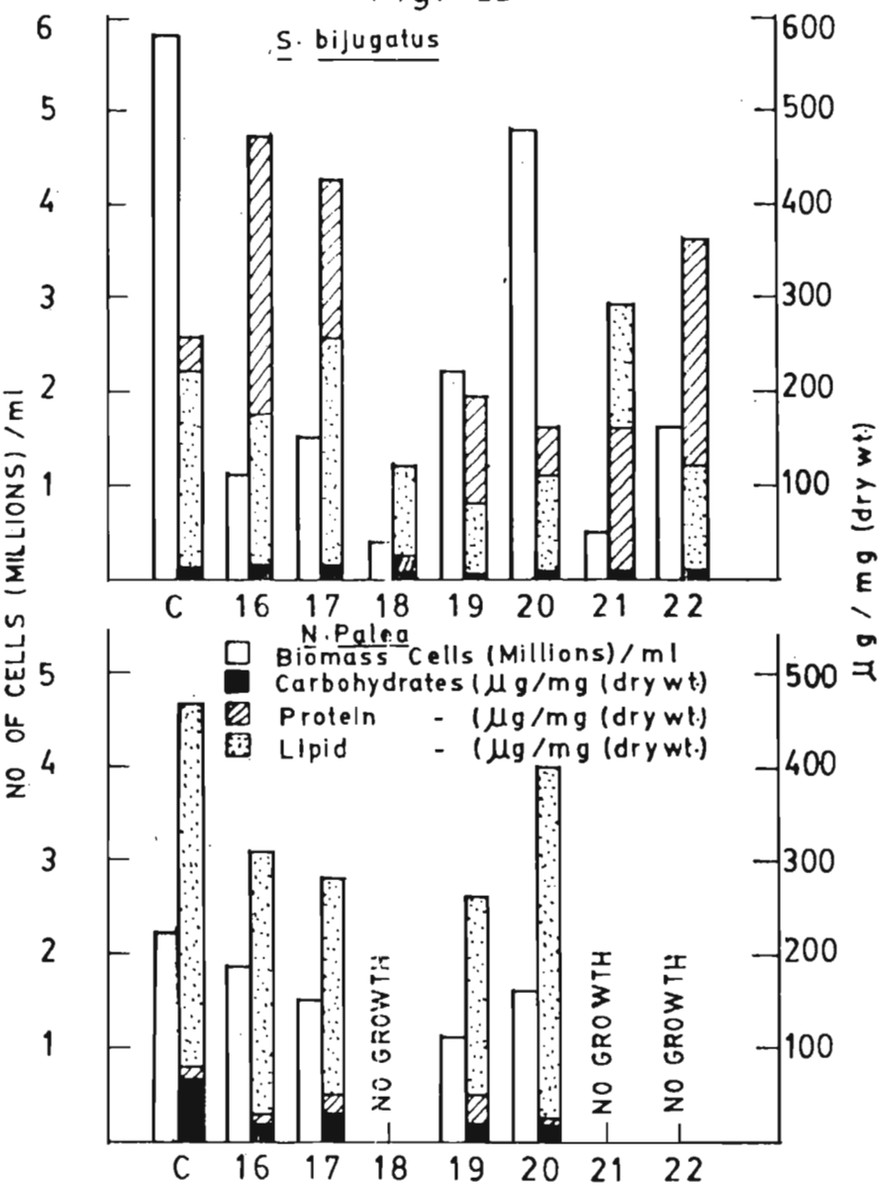
When copper was replaced by zinc as in combination (20) (mercury lead and zinc) no significant difference was noted in the concentration of endproducts but the biomass increased and pigment content was lowered in case of S.bijugatus. The effect of combinations (19) and (20) was less toxic on the biomass of N palea. Reduction in the endproducts seems to be a common effect on both the species when combination (19) was employed. When copper was replaced by zinc(combination (20)) there was no significant difference in the protein content of the diatom but increase in lipid was observed, unlike in S.bijugatus.

On the whole both the species were adversely affected by the above combination.

S.bijugatus was found to be more tolerant to lead, cadmium and copper (combination (21)) and lead, cadmium and zinc (combination (22)) than N.palea, on which the effect was found to be lethal. However considerable retardation of growth of S.bijugatus was noted, combination (22) was found to be more toxic than combination (21). The replacement of copper (in combination (21)) by zinc (in combination (22)) also brought down the pigment

content of the alga, when combination (21) was employed though the toxicity was evident in the early stage subsequent recovery was observed to a large extent. The combination (22) was one of the very few which resulted in increased production accompanied by reduced respiration.

Fig. VI


S. bijugatus
N. Palea
Biomass Cells (Millions) / ml
Carbohydrates (µg/mg (dry wt.)
Protein - (µg/mg (dry wt.)
Lipid - (µg/mg (dry wt.)
NO OF CELLS (MILLIONS) / ml
µg / mg (dry wt.)
NO GROWTH
NO GROWTH
NO GROWTH
C 16 17 18 19 20 21 22

Analysis of variance was carried out to establish the significance of the interaction of three metal combinations on the species studied.

The variance ratios obtained were significant in 73 cases out of 88 studied with regard to S.bijugatus (Table XI).

Significant difference was observed in the effect of combinations (16) and (17) except on respiration, carbohydrate and protein (biuret) at the tested levels.

The effect of combinations (18) and (19) was not significant on pheoph tin. Also during the growth period the protein was not significantly affected.

The effect of combinations (21) and (22) was most significant on the species. Only protein (biuret) content was found to be unaffected.

The effect of the three metal combination on N.palea (table XII) was significant in 42 instances out of 50 studied.

At the tested levels, the combinations (16) and (17) did not show significant effect on respiration, chlorophyll c and pheophytin. Their effect on pheophytin content of the diatom was

not significant during the period.

The effect of combinations (19) and (20) was found insignificant, in acid and alkali soluble fractions of carbohydrate and protein, at the experimental dose concentrations. The effect on alkali soluble carbohydrate fraction was not significant also during the growth period.

The combinations (18), (21) and (22) were found to be lethal to the diatom.

TABLE - XI

Significance of the variance ratios in the analysis of variance table for between concentrations and between days.

S. bijugatus

Sl. No.	Metals employed.	pH		Gross Production		Nett Production		Respiration		Chlorophyll a		Chlorophyll b		Carotenoids		Pheophytin.		Carbohydrate		Protein (Biuret)		Protein Total	Lipid
		I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	I		
(16)	Hg + Cd + Zn	c	c	c	c	b	c	NS	c	a	b	c	b	c	c	c	b	NS	c	NS	a	b	c
(17)	Hg + Cd + Pb																						
(18)	Hg + Cu + Cd	c	c	b	c	c	c	a	c	c	c	c	b	c	c	NS	NS	c	c	c	b	c	c
(19)	Hg + Cu + Pb																						
(20)	Hg + Pb + Zn	NS	c	b	c	a	c	NS	c	c	c	NS	NS	c	c	c	c	c	c	NS	NS	NS	c
(21)	Pb + Cd + Cu	c	c	c	c	c	c	b	c	c	b	b	b	c	b	b	b	b	b	NS	NS	c	c
(22)	Pb + Cd + Zn																						

NS - Not significant

a - $p < 0.05$
b - $p < 0.01$
c - $p < 0.001$

I - Between concentrations
II - Between days

TABLE - XII

Significance of the variance ratios in the analysis of variance table for between concentrations and between days.

N. palea

Sl. No.	Metals employed	pH		Gross production.		Nett production.		Respiration.		Chlorophyll a		Chlorophyll c		Carotenoids.		Pheophytin.		Carbohydrate (Acid soluble)		Carbohydrate (Alkali soluble)		Carbohydrate (Insoluble		Protein		Lipid
		I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	
(16) (17)	Hg + Cd + Zn / Hg + Cd + Pb	b	c	c	c	c	c	NS	c	c	c	NS	c	a	c	NS	NS	b	c	c	c	b	c	b	c	c
(18)	Hg + Cu + Cd	Combination Lethal																								
(19) (20)	Hg + Cu + Pb / Hg + Pb + Zn	c	c	c	c	c	c	c	c	c	b	c	c	c	b	b	c	NS	c	NS	NS	b	c	NS	c	c
(21) (22)	Pb + Cd + Cu / Pb + Cd + Zn	Combination Lethal																								

NS - Not significant

a - $p < 0.05$
b - $p < 0.01$
c - $p < 0.001$

I - Between concentrations
II - Between days

TABLE XIII

The concentration of metals in S. bijugatus at the end of growth phase

Sl. No. of the combination	Metals employed	Concentration (ppm/100 mg dry wt.)		
(16)	Hg + Cd + Zn	0.016	0.035 *	0.056
(17)	Hg + Cd + Pb	0.015	0.030 *	0.026
(18)	Hg + Cu + Cd	Growth was highly retarded		
(19)	Hg + Cu + Pb	0.021	0.095	0.025 *
(20)	Hg + Pb + Zn	0.016	0.025	0.058
(21)	Pb + Cd + Cu	0.034 *	0.034 *	0.107
(22)	Pb + Cd + Zn	0.053	0.031 *	0.065

The values represented are the average of three observations for mercury and two observations for others.

* Values are less than the indicated concentrations.

TABLE XIV

Concentration of metals in *N. palea* at the end of growth phase

Sl. No. of the combination	Metals employed	Concentration (ppm/100 mg dry wt.)		
(16)	Hg + Cd + Zn	0.002	0.052	0.063
(17)	Hg + Cd + Pb	0.003	0.039 *	0.045
(18)	Hg + Cu + Cd	Combination lethal.		
(19)	Hg + Pb + Cu	0.006	0.027 *	0.043
(20)	Hg + Pb + Zn	0.003	0.033 *	0.057
(21)	Pb + Cd + Cu	Combination lethal.		
(22)	Pb + Cd + Zn	Combination lethal.		

The values represented are the average of three observations for mercury and two observations for others.

* Values are less than the indicated concentrations.

6.3 Metals selected and their effect as test species (Four metals).

The effect of metals in combination (Table 4) was studied and reported in relation to the control.

TABLE 4

Sl.No. of combination	Concentration in ppm	
	S. bijugatus	N. palea
(23)	0.02 Hg + 0.01 Cd + 0.05 Pb + 0.05 Zn	0.005 Hg + 0.02 Cd + 0.04 Pb + 0.05 Zn
(24)	0.02 Hg + 0.01 Cd + 0.05 Pb + 0.05 Fe	0.005 Hg + 0.02 Cd + 0.04 Pb + 0.05 Fe
(25)	0.02 Hg + 0.05 Pb + 0.1 Cu + 0.05 Zn	0.005 Hg + 0.05 Cu + 0.04 Pb + 0.05 Zn
(26)	0.02 Hg + 0.05 Pb + 0.1 Cu + 0.05 Fe	0.005 Hg + 0.05 Cu + 0.04 Pb + 0.05 Zn
(27)	0.02 Hg + 0.1 Cu + 0.01 Cd + 0.05 Zn	0.005 Hg + 0.05 Cu + 0.02 Cd + 0.05 Zn
(28)	0.02 Hg + 0.1 Cu + 0.01 Cd + 0.05 Fe	0.005 Hg + 0.02 Cd + 0.05 Cu + 0.05 Fe
(29)	0.02 Hg + 0.01 Cd + 0.05 Zn + 0.05 Fe	0.005 Hg + 0.02 Cd + 0.05 Zn + 0.05 Fe
(30)	0.02 Hg + 0.05 Pb + 0.05 Zn + 0.05 Fe	0.005 Hg + 0.04 Pb + 0.05 Zn + 0.05 Fe
(31)	0.01 Cd + 0.05 Pb + 0.1 Cu + 0.05 Zn	0.02 Cd + 0.04 Pb + 0.05 Cu + 0.05 Zn
(32)	0.01 Cd + 0.05 Pb + 0.1 Cu + 0.05 Fe	0.02 Cd + 0.04 Pb + 0.05 Cu + 0.05 Fe
(33)	0.01 Cd + 0.05 Pb + 0.05 Zn + 0.05 Fe	0.02 Cd + 0.04 Pb + 0.05 Zn + 0.05 Fe

4.3.1 Combined effect of mercury, cadmium, lead and zinc on S. bijugatus

Sl.No. of the combination	Concentration of metals (in ppm)
(23)	0.02 Hg + 0.01 Cd + 0.05 Pb + 0.05 Zn

Production: (Fig. I)

The algal culture was pale and the usual green colour was lacking upto second day. Hence no estimations were carried out for various parameters. On the fourth day the nett production was negligible but it increased subsequently to 88% lower level than that of control on sixth day. Production reached maximum on eighth day but remained less than that of control by 50%. Thereafter it declined to 82% and 85% less than that of control on tenth and twelfth day respectively.

The oxygen content in the dark bottle on fourth day exceeded that of initial. But on sixth day respiration was 30% less than that of control. It was maximum on eiththh day exhibiting 114% increase. It declined gradually towards the end of growth phase and 78% reduction was observed on the last day.

pH of the culture increased gradually till the end of growth phase and was higher than that of control

on the last day, inspite of the observed inhibition in nett production and higher oxygen content in the dark bottle on fourth day.

Pigments: (Fig. II)

The concentration of all four pigments increased in this treatment. Though colour was noted in the culture flasks by second day, the chlorophyll a, chlorophyll b and carotenoids showed high concentration than that of control on fourth day. Pigment maxima were observed on eighth day. Greater amount of chlorophyll b (3.757 µg) followed by chlorophyll a (2.733 µg), carotenoids (1.34 µg) and pheophytin (1.247 µl) were recorded against the control values of 0.149 µg, 0.138 µg, 0.081 µg and 0.025 µg respectively. All four pigments exhibited decreasing tendency towards the end of growth phase but it was more pronounced in the case of pheophytin.

Photosynthetic end products: (Fig. III)

Due to low multiplication rate of the alga, the measurement of end products was possible only from eighth day onwards. The carbohydrate content showed 96% increase in relation to control on eighth day and 143% on tenth day. The concentration of this product increased further to 471% higher level by the end of growth phase.

protein content increased by 40%. Lipid content of the alga increased by 148%.

Growth: (Fig. I)

Morphological abnormality of the alga was noticed in this treatment. During early phase of growth algal cells were found attached to the bottom and walls of culture flask as a mat and did not enter into the medium when the flasks were shaken. This might be the reason for low production values obtained and also low biomass. When cells were scrapped off the glass walls and observed under the microscope, some were found to be larger in size and round, not elliptical as the healthy cell. By twelfth day clumping was noticed and flocculant mat like formation was observed. Cells were smaller, round in shape and found in single celled layers, detached from the walls of culture flask. Culture turned dark green in colour. At the end of growth phase biomass was found to be less than that of control.

Selected combination	Nutrients absorbed by the alga (ug/l)		N/P
	Phosphate	Nitrate	
(23)	1375	760	0.55
Control	945	1290	1.37

All the phosphate in medium (1375 ug) was absorbed and hence the exact amount of phosphate absorbed could not be measured. Nitrate absorption was lowered by 59%.

Conclusion:

The combination of metals produced both morphological and physiological abnormalities in the last species. The rounding off of cells along with high lipid concentration may point out the "Tying up" of the cells to unfavourable conditions.

Comparison:

Due to low multiplication rate of the alga, the measurement of end products was possible only from eighth day onwards. The carbohydrate content showed 96% increase in relation to control on eighth day and 143% on tenth day. Carbohydrate content of the alga improved considerably but protein was reduced. Lipid concentration increased to a large extent. Slight improvement in biomass was also observed.

When the present effect was compared with that of combination (17) (Hg + Cd + Pb) both nett production and respiration were found considerably lowered. Pigment content increased to a large extent. Carbohydrate and protein were lowered. Lipid content increased. Biomass was not affected.

When compared with the effect of combination (20) (Hg + Pg + Zn) nett production and respiration were found considerably lowered. Pigments increased to a very large

extent. Though the concentration of all three end products increased to a considerable extent, the biomass was found reduced to a large extent.

When compared with the effect of combination (22) (Cd + Pb + Zn) nett production was found to be considerably lowered. Respiration of the alga was elevated particularly during the middle growth phase. Further increase in the concentration of all four pigments was observed. Carbohydrate and lipid content increased. Protein did not register any change. Biomass though initially was far less, improved subsequently and at the end of growth phase was almost equal in both the combinations.

6.3.2

Combined effect of mercury, cadmium, lead and iron on S. bijugatus

Sl.No. of the combination	Concentration of metals (in ppm)
(24)	0.02 Hg + 0.01 Cd + 0.05 Pb + 0.05 Fe

Production: (Fig. I)

Nett production of the alga was adversely affected in the initial stage and was negligible on second day. But from second day onwards it improved gradually, following that of control upto eighth day. The production was further increased to 18% and 51% higher level on tenth and twelfth day respectively.

Respiration of the alga was less than that of control upto fourth day. From 40% lower level on fourth day it was elevated to 261% higher level by sixth day. From its maximum on sixth day it dropped to 95% higher level on eighth day. But on tenth and twelfth day it was 56% and 21% less than that of control.

pH of the culture was slightly higher than that of control on second day. Fall in pH was noted upto fourth day, but once again gradual increase was noted upto tenth day. On tenth and twelfth day of growth it remained higher than that of control.

Pigments: (Fig. II)

Pigment production was delayed in the initial stages. From fourth day onwards sharp increase was noted in chlorophyll a, chlorophyll b and carotenoids. Also the concentration of these three pigments fluctuated considerably with two peaks, on sixth and tenth day . Pheophytin was not detected upto eighth day. It was maximum on tenth day but the concentration was much lower than the other pigments. The concentration of all pigments decreased towards the end of growth phase but remained higher than that of control.

Photosynthetic end products: (Fig. III)

The carbohydrate content of the alga was much less in early phase. From sixth day onwards it increased and was 131% more than that of control at its peak on eighth day. It decreased gradually to 23% higher than that of control at the end of growth phase.

Increase in protein was very slight (3%) and so also lipid (6%).

Growth: (Fig. I)

Though continuous growth occurred, biomass remained less than that of control.

Selected combination	Nutrients absorbed by the alga (µg/l)		N / P
	Phosphate	Nitrate	
(24)	1085	500	0.46
Control	945	1290	1.37

Phosphate absorption increased by 140 µg but nitrate absorption decreased by 790 µg.

Conclusion:

The combination was found to be undesirable from growth point of view.

Comparison:

The effect of the present combination of metals when compared with that of combination (17) (Hg + Cd + Pb) was found to be not different with respect to nett production and respiration. But the fluctuation in the nett production was not observed whereas fluctuation in respiration was retained. Considerable fluctuation in the level of all four pigments was observed and the concentration of all four pigments was further lowered, particularly those of chlorophyll b and pheopytin, towards the end of growth phase. Carbohydrate and lipid were reduced slightly. Protein content was reduced to a large extent.

The addition of iron to the combination (17) (Hg + Cd + Pb) did not improve the situation.

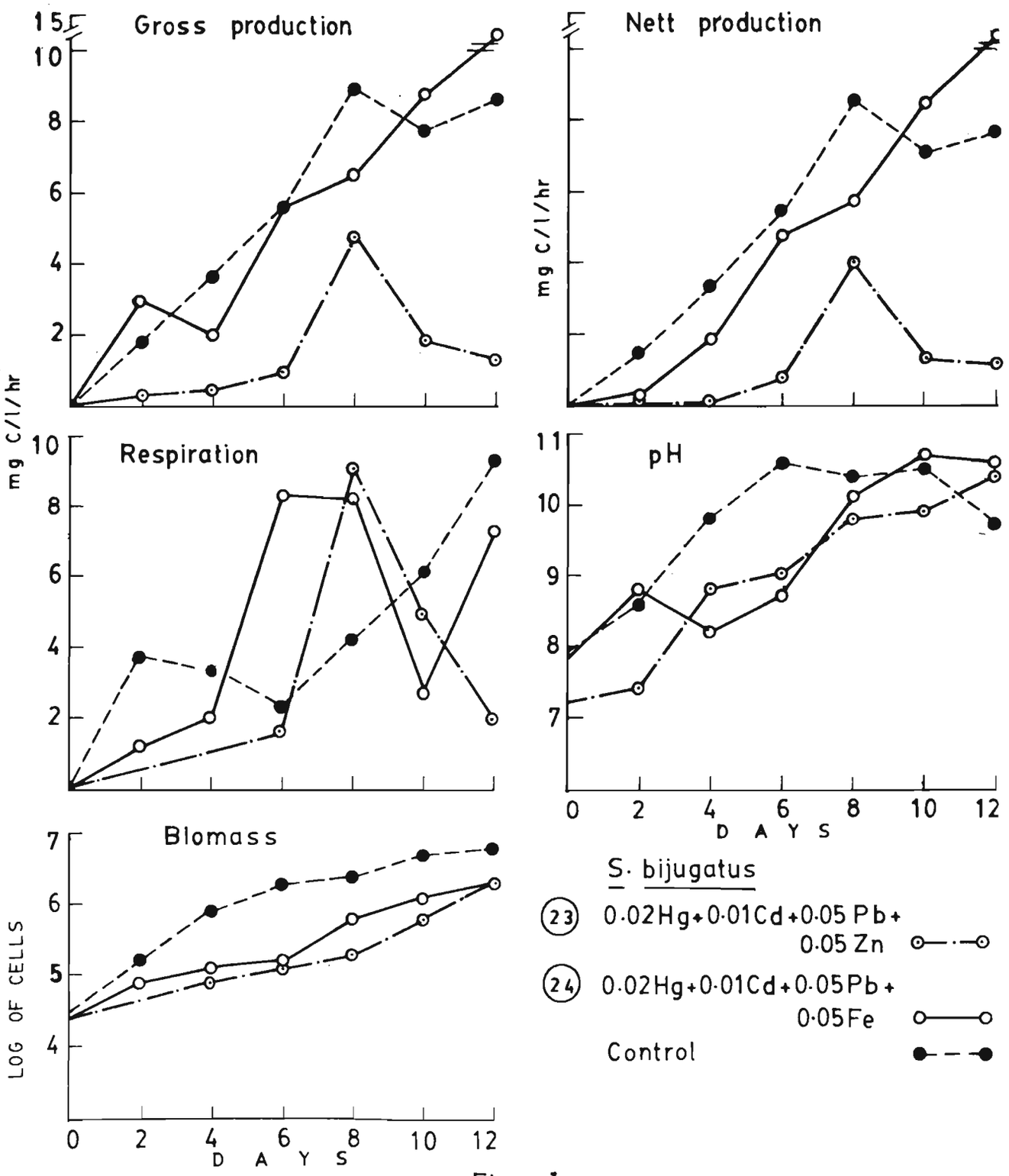

Gross production
Nett production
Respiration
pH
Blomass
mg C/l/hr
LOG OF CELLS
D A Y S
S. bijugatus
(23) 0·02Hg+0·01Cd+0·05 Pb+ 0·05 Zn
(24) 0·02Hg+0·01Cd+0·05Pb+ 0·05Fe
Control

Fig. I

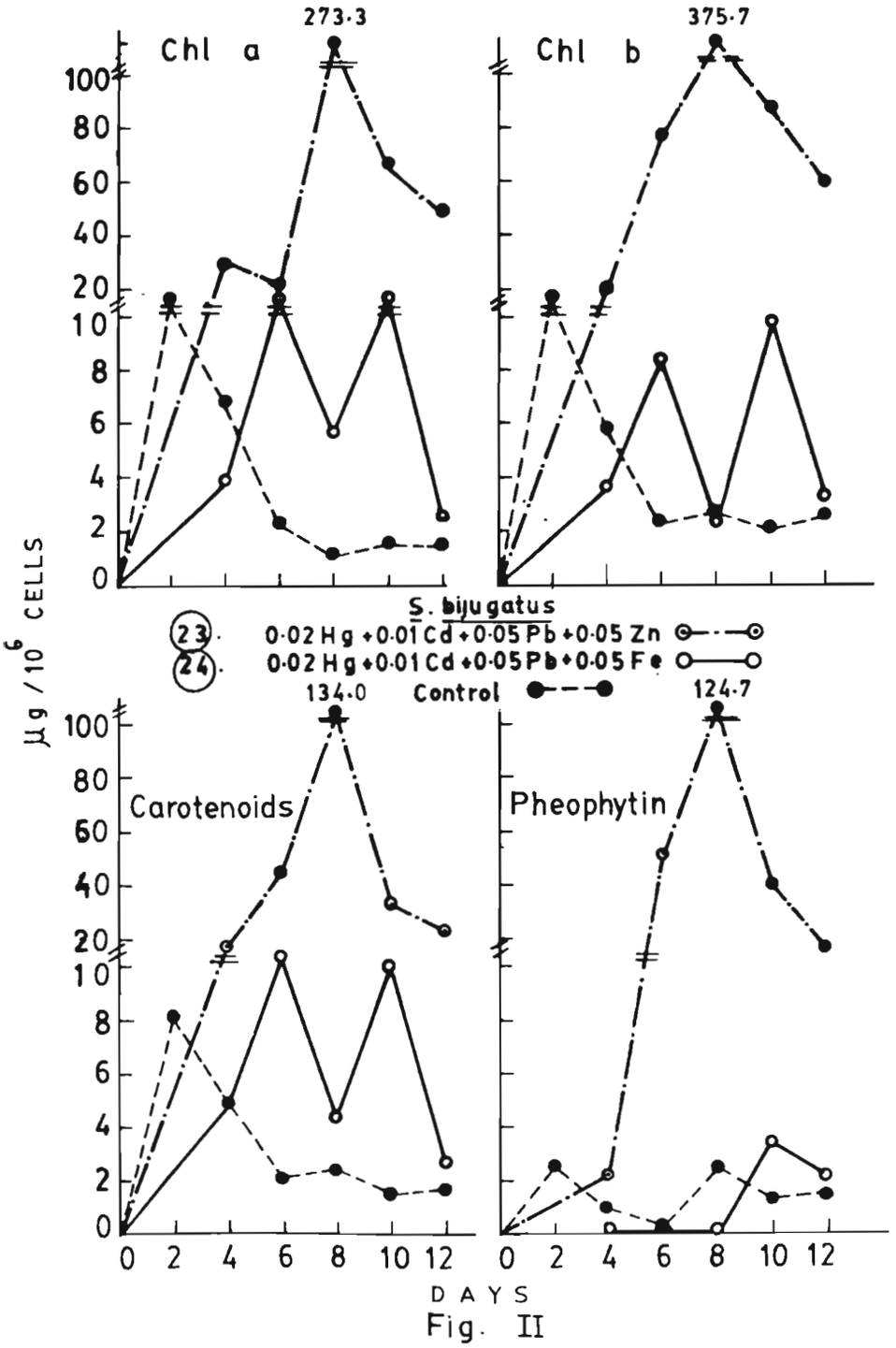
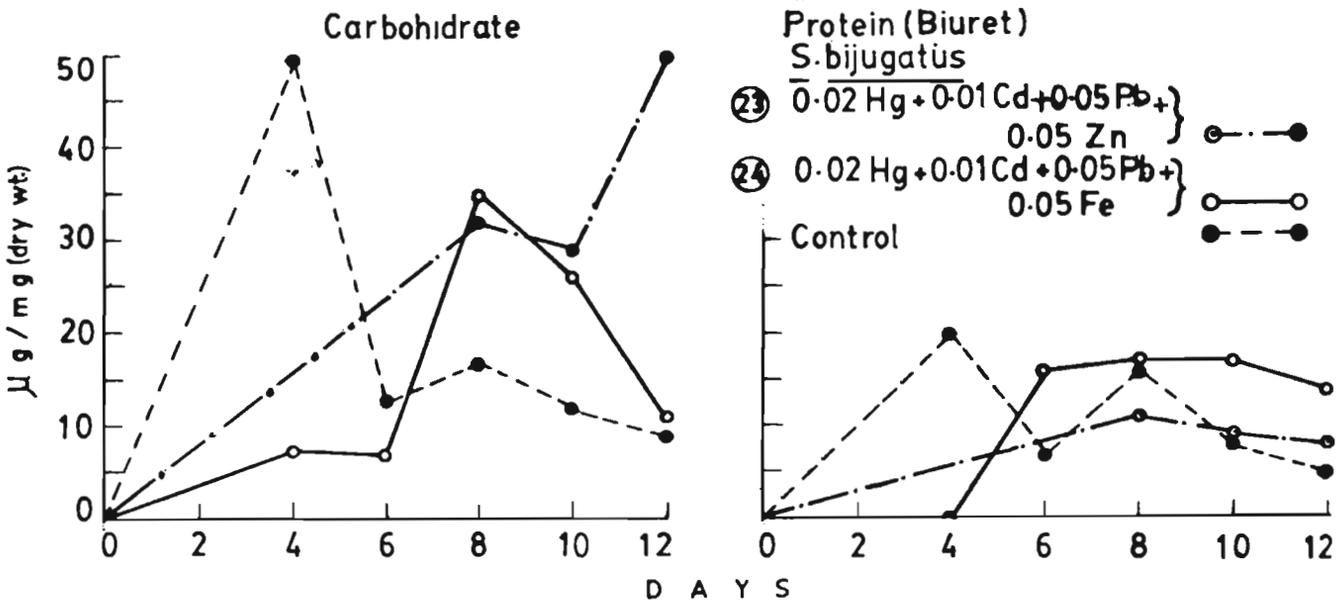

273·3
Chl a
375·7
Chl b
100
80
60
40
20
10
8
6
4
2
0
µg / 10^6 CELLS
S. bijugatus
23. 0·02 Hg +0·01 Cd +0·05 Pb +0·05 Zn
24. 0·02 Hg +0·01 Cd +0·05 Pb +0·05 Fe
Control
134·0
Carotenoids
124·7
Pheophytin
0 2 4 6 8 10 12
DAYS

Fig. II


Carbohidrate
Protein (Biuret)
S. bijugatus
23 0·02 Hg + 0·01 Cd + 0·05 Pb + 0·05 Zn
24 0·02 Hg + 0·01 Cd + 0·05 Pb + 0·05 Fe
Control
µ g / m g (dry wt.)
0
10
20
30
40
50
0
2
4
6
8
10
12
D A Y S

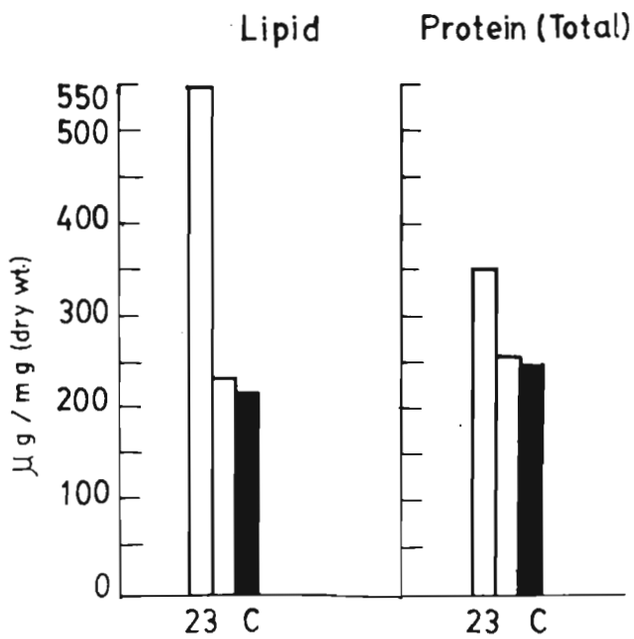

Lipid
Protein (Total)
µ g / m g (dry wt.)
0
100
200
300
400
500
550
23
C
23
C

Fig. III

Combined effect of mercury, cadmium, lead and zinc
and
mercury, cadmium, lead and iron on

N. palea

Sl. No. of the combination	Concentration of metals (in ppm)
(23)	0.005 Hg + 0.02 Cd + 0.04 Pb + 0.05 Zn
(24)	0.005 Hg + 0.02 Cd + 0.04 Pb + 0.05 Fe

In general no considerable difference in the effect was observed between combination (23) and (24). Hence they were described together.

Production: (Fig. I)

In general, nett production was considerably inhibited in both combinations and was less than that of control throughout. Between the treatments, it did not differ much. Gradual increase upto sixth day was observed to 66% lower level in both treatments and it declined to 70% by eighth day. Towards the end of growth phase it increased in both the treatments to be 46% and 56% less than that of control respectively.

Respiration of the alga differed in these two treatments. In the first treatment it remained less than

that of control throughout, with 36% reduction at the end of growth phase. The oxygen values were higher in the dark than the initial bottles on sixth and eighth day. Respiration in the second treatment increased by 233% on second day but declined thereafter to a minimum on sixth day. From 89% lower level on sixth day it increased gradually to 48% higher level on tenth day.

pH of the culture showed little variation between the two treatments and remained generally less than that of control except on the last day.

Pigments: (Fig. II)

In both the combinations pigments were developed to greater extent in the latter growth phase. The concentration of all four pigments was greater in combination 23. Among the four pigments, the carotenoids were developed to greater extent, in both cases. Pheophytin was less than that of control upto sixth day in the first whereas in the second, it was higher than that of control throughout growth phase.

Photosynthetic end products: (Fig. III)

The quantity of carbohydrates, proteins and lipids did not differ much between the two.

The concentration of acid soluble carbohydrate in combination 23 was almost equal to that of control upto eighth day whereas in the combination 24 it was slightly higher. On the last day it was 29% more in the first but 6% less in the other, in relation to control.

The alkali soluble carbohydrate fraction did not differ much between the two and also between any combination and control except when it was more than that of control at the end of growth phase, by 46% and 7% respectively.

The insoluble carbohydrate fraction was nearly equal in both. It was 17% and 40% higher respectively on sixth day but decreased to 78% and 71% lower level by eighth day, in relation to control. It increased once again towards the end of growth period but not to the same extent as observed in the control and remained reduced by 72% and 75% respectively.

Protein content of the diatom in the combination 23 showed a gradual increase upto sixth day. In combination 24 it was less than that of control upto fourth day and increased thereafter. In both instances it was higher than that of control on sixth day, by 71% and 92% respectively. It exhibited considerable increase from eighth day but remained reduced by 31% and 24% less than that of control, on the last day.

The toxic effect of these two combinations was clearly seen on the lipid which was reduced by 18% and 80% respectively.

Growth: (Fig. I)

Growth rate of the diatom was impaired in both cases. Biomass was less than that of control throughout the growth period. It was almost steady from eighth day onwards.

Selected combination	Nutrients absorbed (µg/l)		N / P
	Phosphate	Nitrate	
(23)	1700	660	0.39
(24)	1675	686	0.41
Control	1350	763	0.57

For both the treatments phosphate absorption increased and nitrate absorption decreased when compared with that of control. But no considerable difference was noted between the two.

Conclusion:

The two combinations were found to be toxic to the species. Growth and lipid formation were adversely affected.

Comparison:

When the effect of the combination (23) (Hg + Cd + Pb + Zn) was compared with that of combination (16) (Hg + Cd + Zn) the nett production was found to be reduced but respiration increased. All pigments except chlorophyll b increased considerably, particularly in the latter growth phase. All three fractions of carbohydrates and protein content increased. But two fold reduction was observed in lipid concentration. Biomass was lowered. on the whole the combination (23) was found to be less desirable than combination (16).

When compared with that of combination (17) (Hg + Cd + Pb) reduction in nett production was observed. Respiration was not affected. All four pigments increased to a large extent. All three fractions of carbohydrates were reduced. Protein content of the diatom decreased to a large extent. Two fold reduction was observed in lipids. Biomass considerably lowered. On the whole the combination (23) was found to be less desirable than combination (17).

When compared with that of combination (20) (Hg + Pb + Zn), the nett production was found to be reduced in early phase but enhanced in latter phase. Respiration was reduced. All four pigments increased, particularly in the latter phase of growth. All three

fractions of carbohydrates increased in latter growth phase. Protein content increased in the early phase in combination (20) and in latter phase in combination (23). Three fold decrease in lipid content was registered. Biomass also was considerably lowered. On the whole the combination 23 was found to be less desirable than (20).

The combination (22) (Cd + Pb + Zn) was found to be lethal and hence the combination (23) may regarded as more desirable.

When the effect of combination (24) was compared with that combination (17), nett production was found to be lowered but respiration was not affected. All four pigments were produced to a greater extent, particularly carotenoids. Though considerable increase in all three fractions of carbohydrates was observed, the concentration decreased towards the end of growth phase. During most of the growth phase, protein was found considerably reduced and at the end of growth phase when the concentration was same in both instances. Lipid content was reduced by more than 50%. Biomass was also considerably reduced.

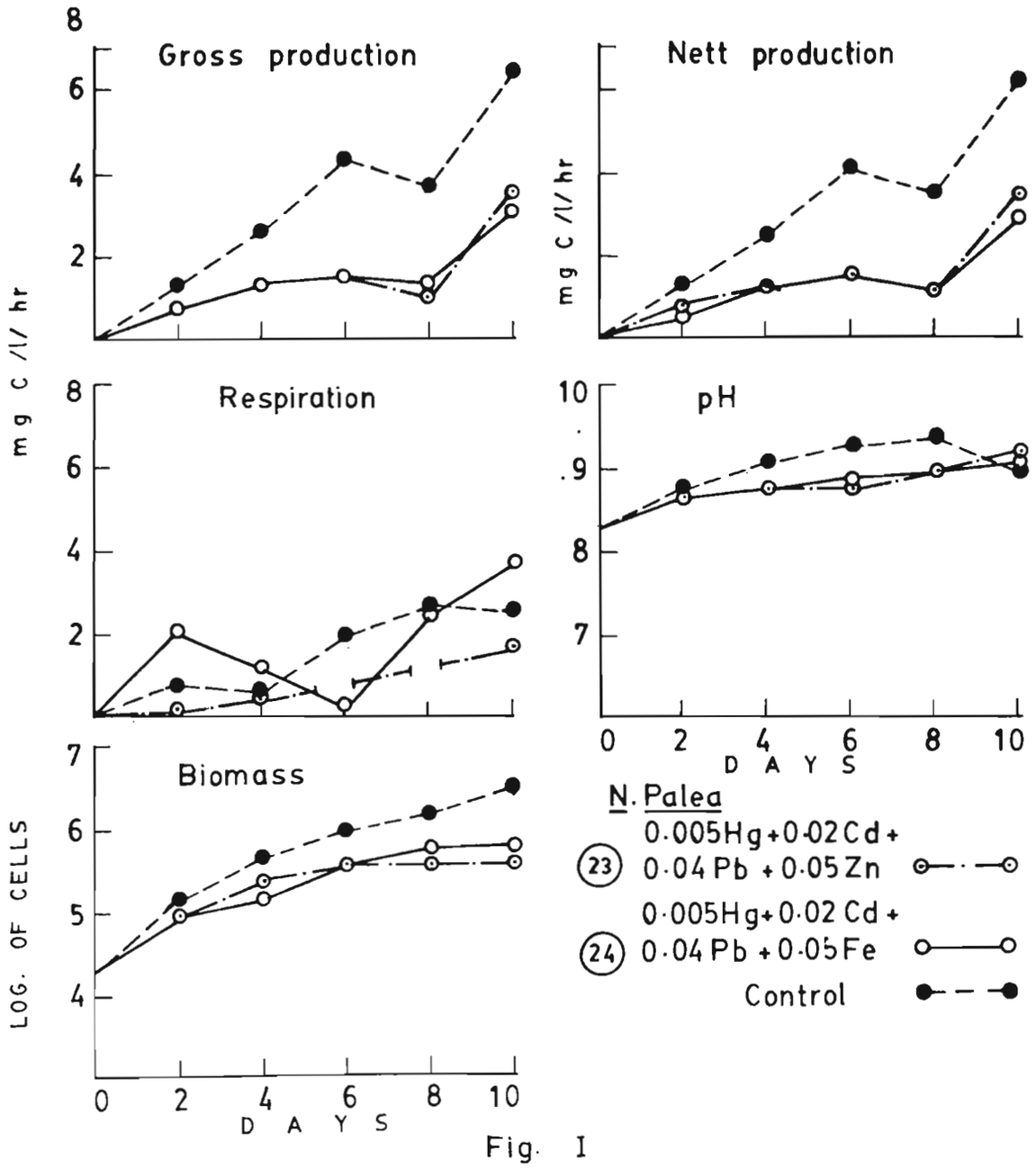

Gross production
Nett production
Respiration
pH
Biomass
mg C /l/ hr
LOG. OF CELLS
DAYS
N. Palea
0·005Hg+0·02Cd+
0·04Pb+0·05Zn
0·005Hg+0·02Cd+
0·04Pb+0·05Fe
Control

Fig. I

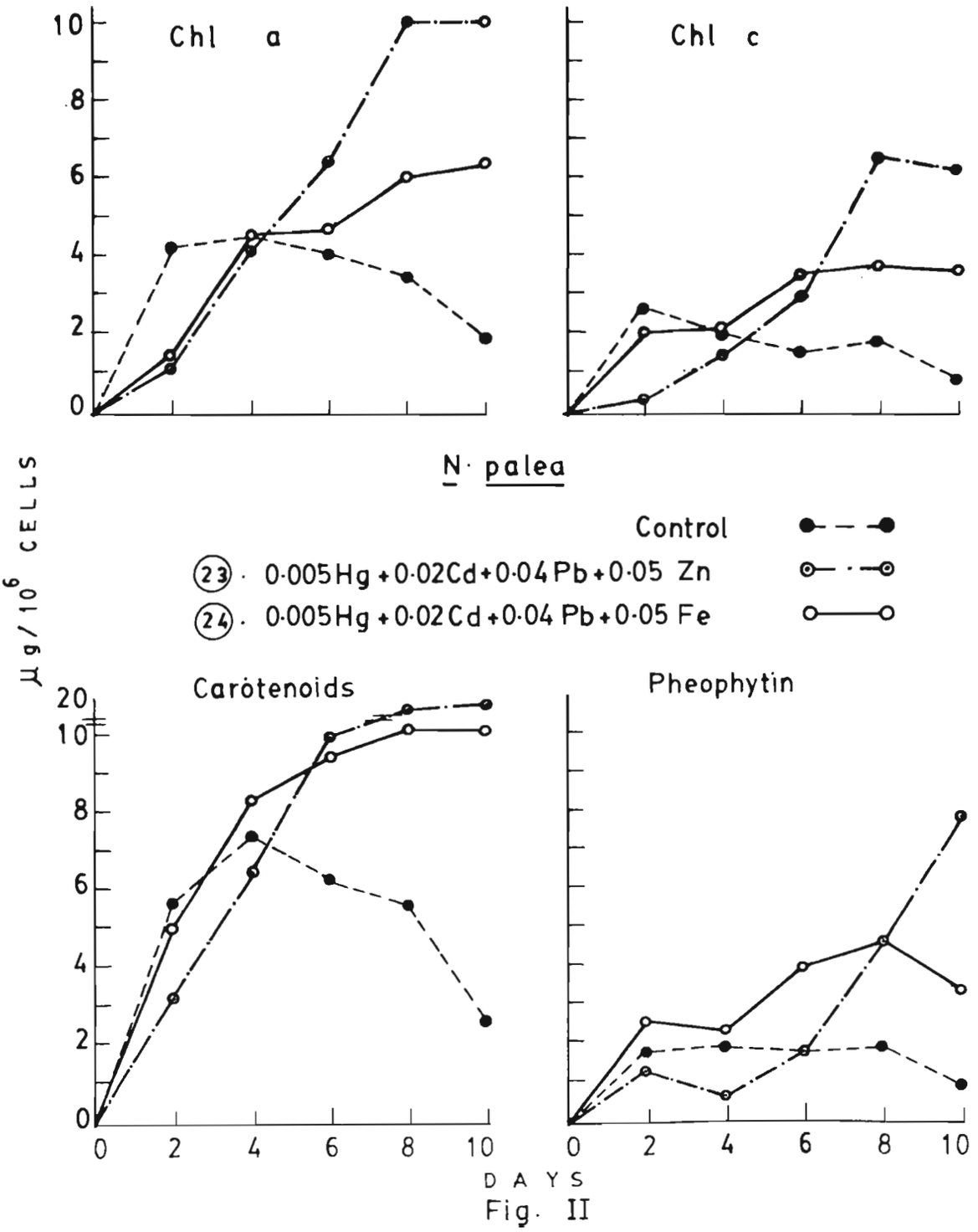

Chl a
Chl c
N. palea
Control
(23). 0·005Hg + 0·02Cd + 0·04 Pb + 0·05 Zn
(24). 0·005Hg + 0·02Cd + 0·04 Pb + 0·05 Fe
Carotenoids
Pheophytin
µg/10^6 CELLS
DAYS

Fig. II

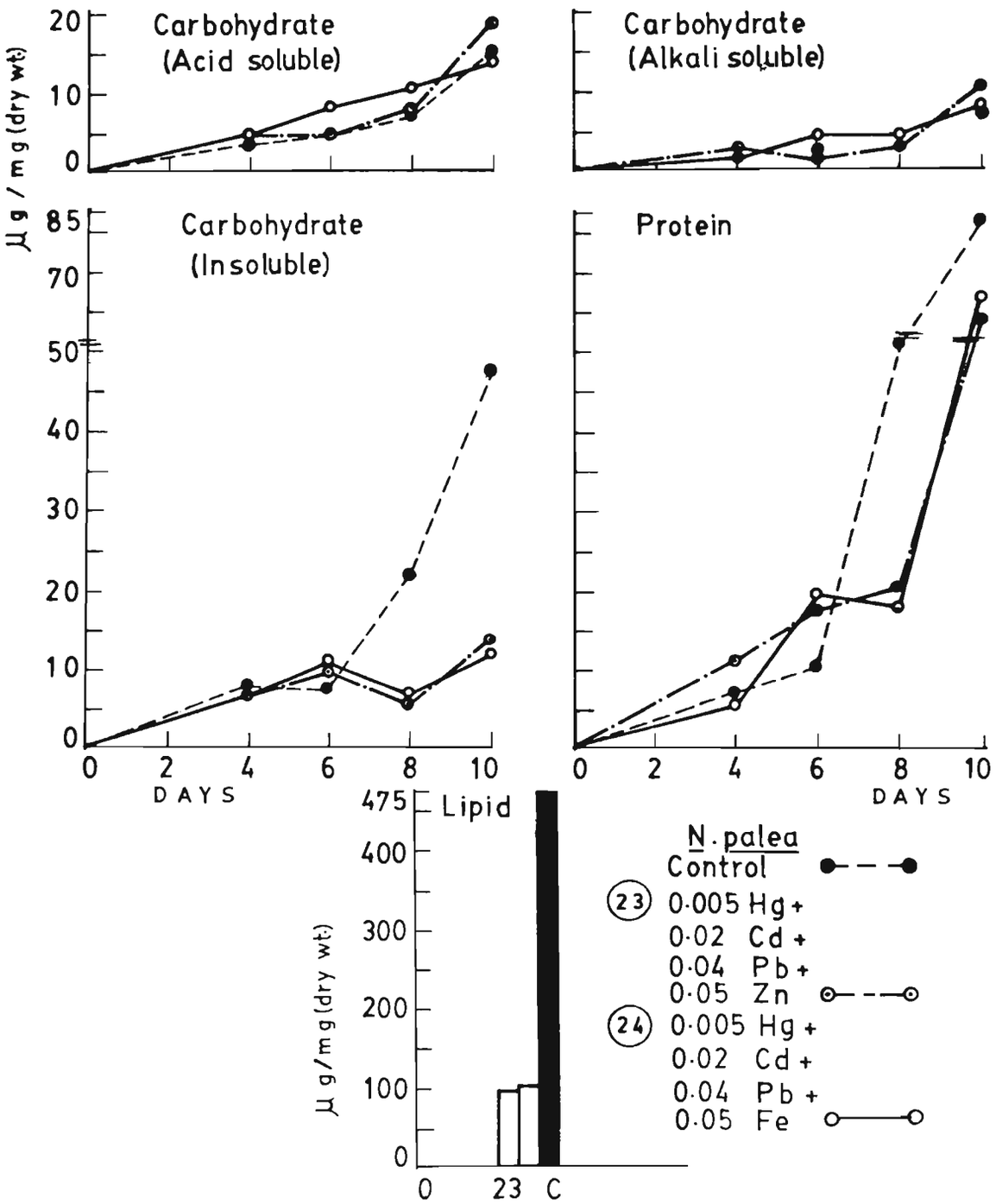

Carbohydrate
(Acid soluble)
Carbohydrate
(Alkali soluble)
Carbohydrate
(Insoluble)
Protein
µg / mg (dry wt)
DAYS
DAYS
Lipid
µg/mg (dry wt)
0
23
C
N. palea
Control
(23) 0·005 Hg +
0·02 Cd +
0·04 Pb +
0·05 Zn
(24) 0·005 Hg +
0·02 Cd +
0·04 Pb +
0·05 Fe

Fig. III

6.3.3

Combined effect of mercury, lead, copper and zinc on S. bijugatus

Sl.No. of the combination	Concentration of metals (in ppm)
(25)	0.02 Hg + 0.05 Pb + 0.1 Cu + 0.05 Zn

Production: (Fig. I)

The nett production was measured only from fourth day onwards as the alga did not grow until third day. From 78% lower level on fourth day production increased sharply to 31% higher level by sixth day. But it was only 10% higher on eighth day. From the maximum level on eighth day it declined to 16% lower level by tenth day. Inspite of slight increase observed towards the end of growth phase, production remained reduced by 18% in relation to control.

Respiration of the alga was negligible on fourth day but increased sharply by sixth day to be 78% more than that of control. It declined to lower level on eighth day and remained less than that of control for the rest of the growth period. 7% reduction was recorded on tenth day and 60% on twelfth day.

Consistent with low production, pH of the culture was very low on second day and increased thereafter gradually to maximum level on eighth day and declined towards the end of growth phase. It was slightly higher than that of control on eighth and twelfth day.

Pigments: (Fig. II)

Though the culture did not appear to have been the usual green colour on second day, all pigments measured on fourth day were found to be at their maximum level. Chlorophyll a and chlorophyll b exhibited slight reduction in concentration by sixth day and to a large extent latter, by tenth day and further to lower level than those of control by twelfth day. Concentration of carotenoids gradually decreased and on the last day fell just short of control. Pheophytin concentration was highest on fourth day and it was developed to a greater extent than any other pigment by the alga. The concentration fluctuated during the growth phase with two peaks on fourth and eighth day but declined to a lower level than that of control by the end of growth phase. Total pigment concentration of the alga was greater than that of control during middle growth phase.

Photosynthetic end products: (Fig. III)

Carbohydrates were estimated from sixth day onwards. The concentration of this product was 97% more than that of control on sixth day but dwindled to 16% lower level on eighth day and increased thereafter by 336% at the end of growth phase.

Protein content of the alga increased by 70% but lipid was reduced by 9%.

Growth: (Fig. I)

Lag in growth was observed in the initial stage but the alga recovered from the toxic effect and grew exponentially from fourth day onwards. Biomass increased quickly upto sixth day. It followed that of control closely till the end of growth phase.

Selected combination	Nutrients absorbed by the alga (µg/l)		N / P
	Phosphate	Nitrate	
(25)	1294	1120	0.87
Control	945	1290	1.37

The alga absorbed 37% more phosphate when exposed to the combination 25 than that of control. Nitrate in the medium at the time of experiment was 1120 ug/l. No nitrate was left at the end of experiment and hence the estimation of the actual amount of nitrate that could be absorbed by the alga was not possible.

Conclusion:

This combination of metals can be considered as toxic from the point of view of growth. It is interesting to note that protein content of the species increased and lipid concentration was not adversely affected.

Comparison:

When the effect of present combination was compared with that of combination (19) (Hg + Pb + Cu) rate of nett production and respiration were found to be reduced to a large extent. Considerable reduction also was recorded in the pigment content towards the end of growth phase. Carbohydrate and lipid improved. Protein content was more than doubled, Biomass also increased.

The addition of zinc to combination (19) helped to mitigate the toxicity of the same to some extent.

6.3.4

Combined effect of mercury, lead, copper and iron on S. bijugatus

Sl.No. of the combination	Concentration of metals (in ppm)
(26)	0.02 Hg + 0.05 Pb + 0.1 Cu + 0.05 Fe

Production: (Fig. I)

In this experiment the culture remained totally colourless upto fourth day and turned pale unhealthy yellowish green by sixth day. Estimation of selected parameters was carried out only from sixth day onwards.

Production of the alga was very low on sixth day, being reduced by 73% in relation to control. Inspite of slight increase it was found to be 82% less on eighth day and 54% less on tenth day. But from tenth day onwards it increased sharply, to 135% higher than control at the end of growth phase.

Respiration of the alga was less than that of control from sixth day onwards. It was 13% less on sixth day and did not register any variation upto eighth day but increased thereafter. It was maximum on tenth day, but with 33% reduction and declined to its minimum on twelfth day being 85% less than that of control.

pH of the culture remained very low on second and fourth day. It increased gradually thereafter, but

remained lower than that of control. Little improvement was noted towards the end of growth phase. It remained higher than that of control on last day.

Pigments: (Fig. II)

All four pigments of the alga increased to a very large extent during middle period of the growth phase. From sixth day onwards their concentration remained higher than that of control. Chlorophyll a reached maximum level on sixth day whereas the other pigments on eighth day. Chlorophyll b was developed to the greatest extent when compared to the others. The concentration of carotenoids and pheophytin along with chlorophylls registered a steep fall towards the end of growth phase but chlorophyll b remained at a higher level than others and total pigment content on the last day remained more, in relation to control.

Photosynthetic end products: (Fig. III)

Carbohydrates were estimated from eighth day onwards due to very low biomass. From 18% lower level on eighth day it reached maximum concentration on tenth day and was 38% more than that of control. It declined thereafter to 11% lower level by the end of growth phase.

Protein and lipid of the alga increased in this combination the former by 55% and latter by 4%.

Growth: (Fig. I)

This particular combination of metals produced a pale unhealthy yellowish green colour in the culture by eighth day. The cells were slightly distorted. Also lag in growth of the alga resulted in the low biomass upto sixth day. No further increase was observed upto eighth day but thereafter biomass increased considerably though remained less than that of control.

Selected combination	Nutrients absorbed by the alga (ug/l)		N / P
	Phosphate	Nitrate	
(26)	1296	470	0.36
Control	945	1290	1.37

Phosphate absorption of the alga increased by 37% but nitrate absorption was reduced by 64%. But this did not have much effect on the protein content. On the contrary the protein increased.

Conclusion:

The negative effect of the combination of metals was found on the growth rate of the alga rather than on protein and lipid.

Comparison:

When the effect of the present combination of metals was compared with that of combination (19) (Hg + pb + Cu), both nett production and respiration were found reduced, the latter to a large extent and it remained less than that of control throughout. Though the concentration of all four pigments increased during the middle phase of growth, towards the end, not much difference in the two combinations was noted. Pheophytin is the most affected pigment. It increased to a large extent during middle phase of growth. Suppression of this pigment upto eighth day noted when combination (19) (Hg + Pb + Cu) was employed, was not recorded in the present instance. Carbohydrate content was not affected. But both protein and lipid of the alga were found doubled. Biomass was not affected.

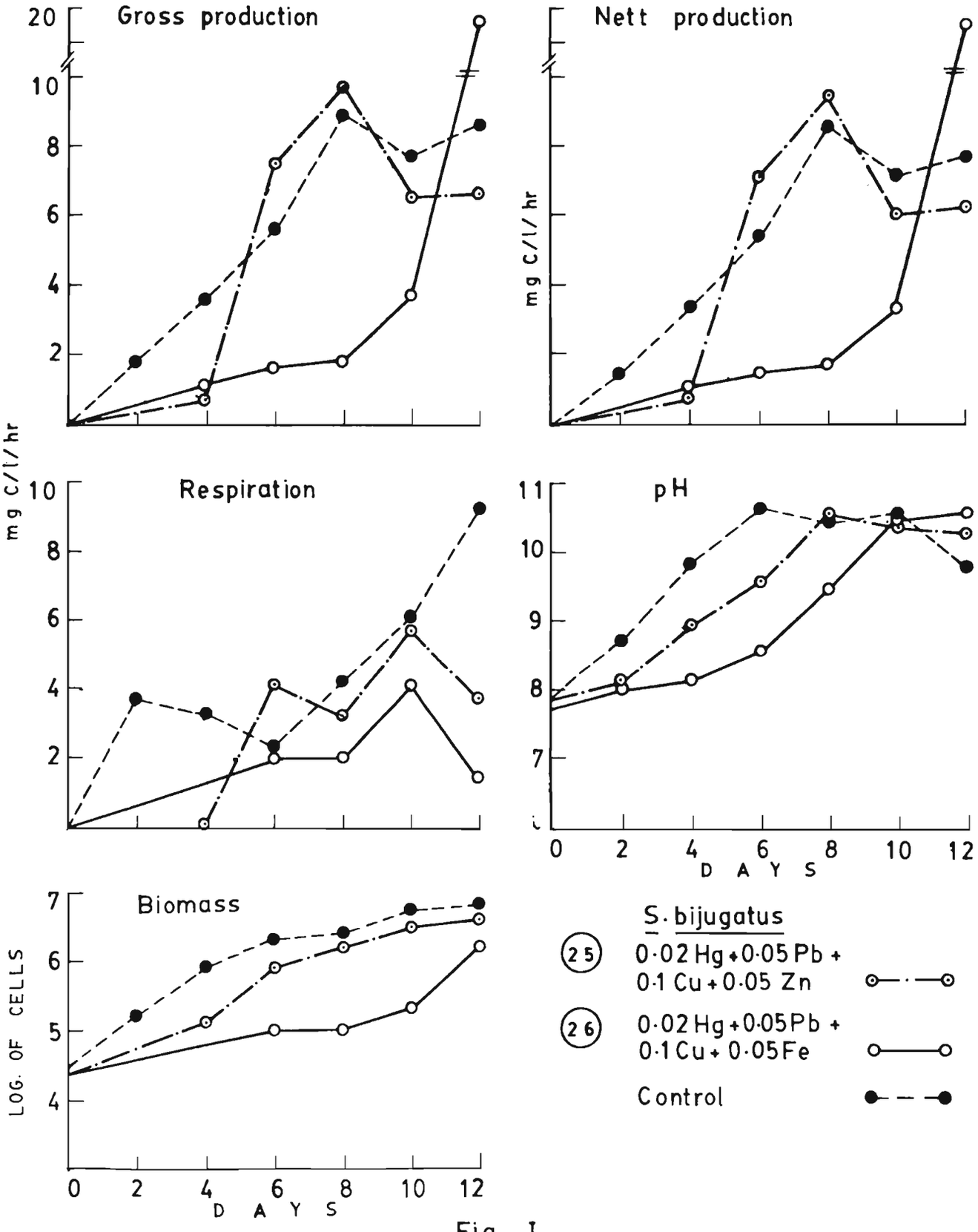

Gross production
Nett production
Respiration
pH
Biomass
mg C/l/hr
LOG. OF CELLS
DAYS
S. bijugatus
25 0·02 Hg + 0·05 Pb + 0·1 Cu + 0·05 Zn
26 0·02 Hg + 0·05 Pb + 0·1 Cu + 0·05 Fe
Control

Fig. I

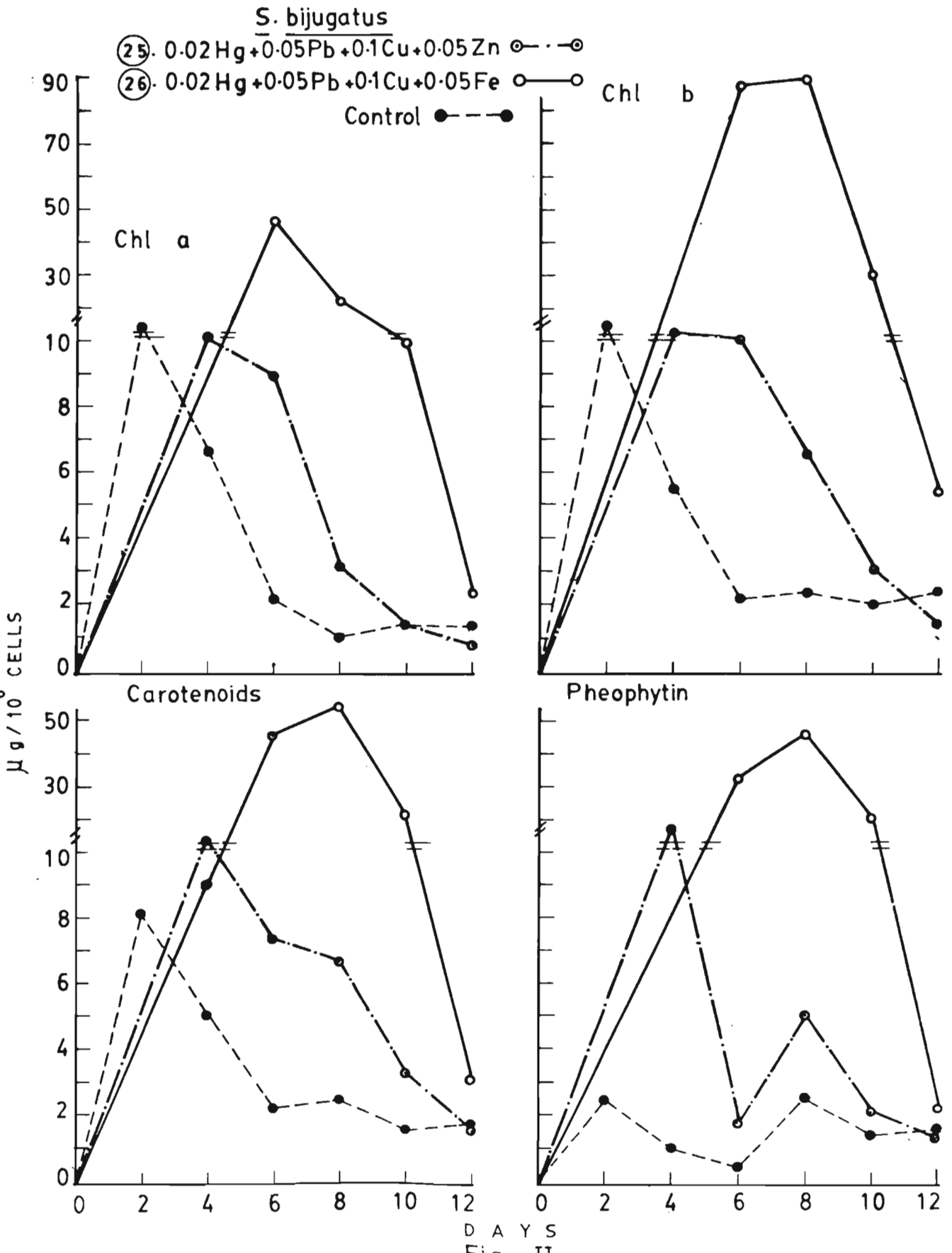
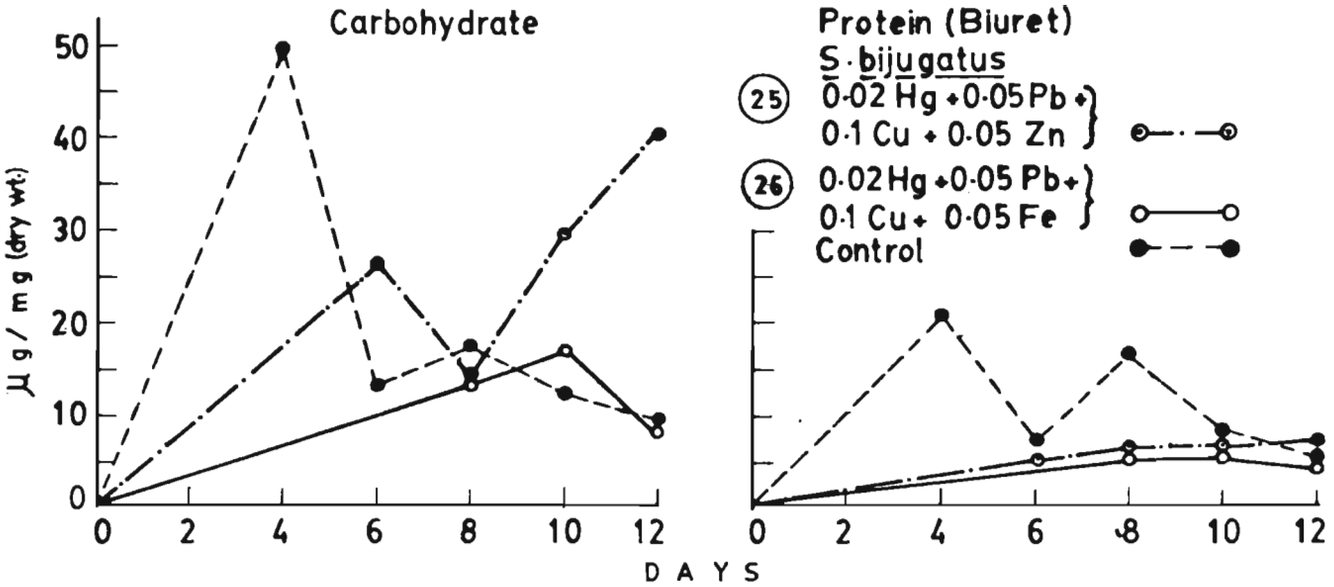

S. bijugatus
(25). 0·02Hg+0·05Pb+0·1Cu+0·05Zn
(26). 0·02Hg+0·05Pb+0·1Cu+0·05Fe
Control
Chl a
Chl b
Carotenoids
Pheophytin
µg/10^6 CELLS
DAYS

Fig. II


Carbohydrate
Protein (Biuret)
S. bijugatus
25 0·02 Hg + 0·05 Pb + 0·1 Cu + 0·05 Zn
26 0·02 Hg + 0·05 Pb + 0·1 Cu + 0·05 Fe
Control
µg / mg (dry wt.)
0
10
20
30
40
50
0 2 4 6 8 10 12
DAYS

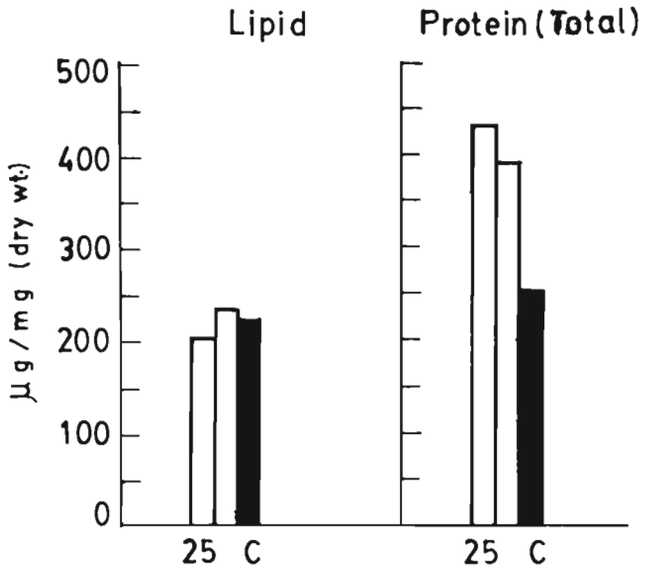

Lipid
Protein (Total)
µg / mg (dry wt.)
0
100
200
300
400
500
25 C
25 C

Fig. III

Combined effect of mercury, lead, copper and zinc

a n d

mercury, lead, copper and iron on *N. palea*

Sl.No. of the combination	Concentration of metals (ppm)
(25)	0.005 Hg + 0.04 Pb + 0.05 Cu + 0.05 Zn
(26)	0.005 Hg + 0.04 Pb + 0.05 Cu + 0.05 Fe

There was very little difference between the effects produced by the combinations (25) and (26). Hence they are described together.

Production: (Fig. I)

The nett production of the diatom was reduced to a large extent in both combination upto eighth day, in relation to control. Production in both, increased gradually upto sixth day. From 76% and 63% lower level on sixth day it was reduced to 81% and 68% respectively by eighth day. It increased thereafter, to a greater extent in the first treatment, but was found reduced by 50% and 70% respectively at the end of growth phase.

Respiration of the diatom differed considerably in the two combinations. It was negligible on fourth day and elevated to 47% higher level by sixth day

in the first. After declining to 8% lower level on eighth day, it increased once again to 112% higher level by the end of growth phase. Oxygen values obtained on second, fourth and sixth day in the second combination were higher in dark bottles than in the initial bottles. But by tenth day it exhibited 42% increase and declined to 28% by the end of growth phase.

Notwithstanding the difference in production, pH of the culture was same in both combinations upto eighth day and remained less than that of control. Thereafter it increased, to a greater extent in the first. It was higher than that of control on the last day, in both.

Pigments: (Fig. II)

In both, the pigments reached maximum concentration in the latter growth phase. Pigment content of the diatom increased to a greater extent in the first. Though a decreasing tendency was recorded in pigment concentration towards the end of growth phase in both, it was recorded to a greater extent in the second. Maximum concentration of chlorophyll a was recorded on sixth day in the first whereas chlorophyll c, carotenoids and pheophytin have attained their maxima on eighth day. In the second, all pigments except pheophytin have shown

maximum on eighth day. Pheophytin was not detected on second day in this. It reached maximum level on sixth day. The concentration of chlorophyll c and pheophytin fluctuated in the first whereas that of chlorophyll a in the second. At the end of growth phase, total pigment content was much less in the second but remained slightly more than that of control whereas it remained much higher in the first one.

Photosynthetic end products: (Fig. III)

The acid soluble carbohydrate concentration did not vary much between the two combnations but remained more than that of control throughout. The percentage increase recorded was 160 and 137 respectively on fourth day, 3 and 16 on eighth day and 21 and 7 on the last day.

The alkali soluble carbohydrate of the diatom was least affected. It did not vary much between the two combinations and between any combination and control.

Considerable reduction occurred in concentration of insoluble carbohydrate in relation to control but it did not register significant variation between the two combinations upto eighth day. It was 48% and 56% more on sixth day, but declined to 70% and 72% lower level respectively, by eighth day. This fraction increased once again towards the end of growth phase but to a far

lesser extent, compared to that of control. The reduction recorded on the last day respectively was 68% and 78%.

In general, protein content of the diatom was adversely affected. In the early stages of growth it was higher than that of control in both combinations. From 26% and 72% higher level on fourth day respectively, inspite of continued increase, the concentration of protein, fell short of control by 58% on eighth day. It increased considerably towards the end of growth phase but remained 23% and 34% less respectively, in relation to control.

Lipid was the most adversely affected product of the diatom. It was reduced by 88% in the first treatment and by 75% in the other.

Growth:

In both combinations the growth of the diatom proceeded to the same extent upto fourth day. From fourth day onwards, the biomass was greater in the second treatment and also it was only slightly less than that of control, whereas the biomass was considerably less in the first one even at the end of growth phase.

Selected combination	Nutrients absorbed by the alga (µg/l)		N / P
	Phosphate	Nitrate	
(25)	1810	732	0.40
(26)	1735	760	0.44
Control	1350	763	0.57

Phosphate absorption increased considerably, by about 34% and 25% respectively whereas nitrate absorption decreased by few µg.

Conclusion:

Both combinations proved to be toxic to the species. The addition of zinc to the combination of (19) Hg + Pb + Cu seems to increase the toxicity, than that of iron. Growth and lipid content of the diatom improved when iron was employed instead of zinc.

Comparison:

When the effect of the combination (25) (Hg + Pb + Cu + Zn) was compared with that of combination (19) (Hg + Pb + Cu) nett production of the diatom was found reduced but respiration increased towards the end of growth phase. Considerable increase in all four pigments was observed. All three fractions of carbohydrates exhibited slight increase. Protein content of the diatom though was reduced in the early phase, increased subsequently. Four fold reduction in lipid was observed. Biomass was reduced to a large extent. On the whole the combination (25) was found to be less desirable, compared to combination (16) (Hg + Pb + Cu).

When the effect of the combination (26) (Hg + Pb + Cu + Fe) as compared with that of combination (19) (Hg + Pb + Cu) the nett production was found to be reduced but respiration increased toward the end of growth phase. Though for most of the growth period all four pigments were found to be in greater quantity, towards the end of the growth phase all were reduced. The alkali soluble carbohydrate fraction was not affected, the acid soluble and insoluble carbohydrate fractions improved slightly. Protein content was not affected at the end of growth phase, though early in the period was found reduced. Lipid content was reduced by more than 50%. Biomass increased. On the whole the combination (26) was found to be more desirable than the combination (19).

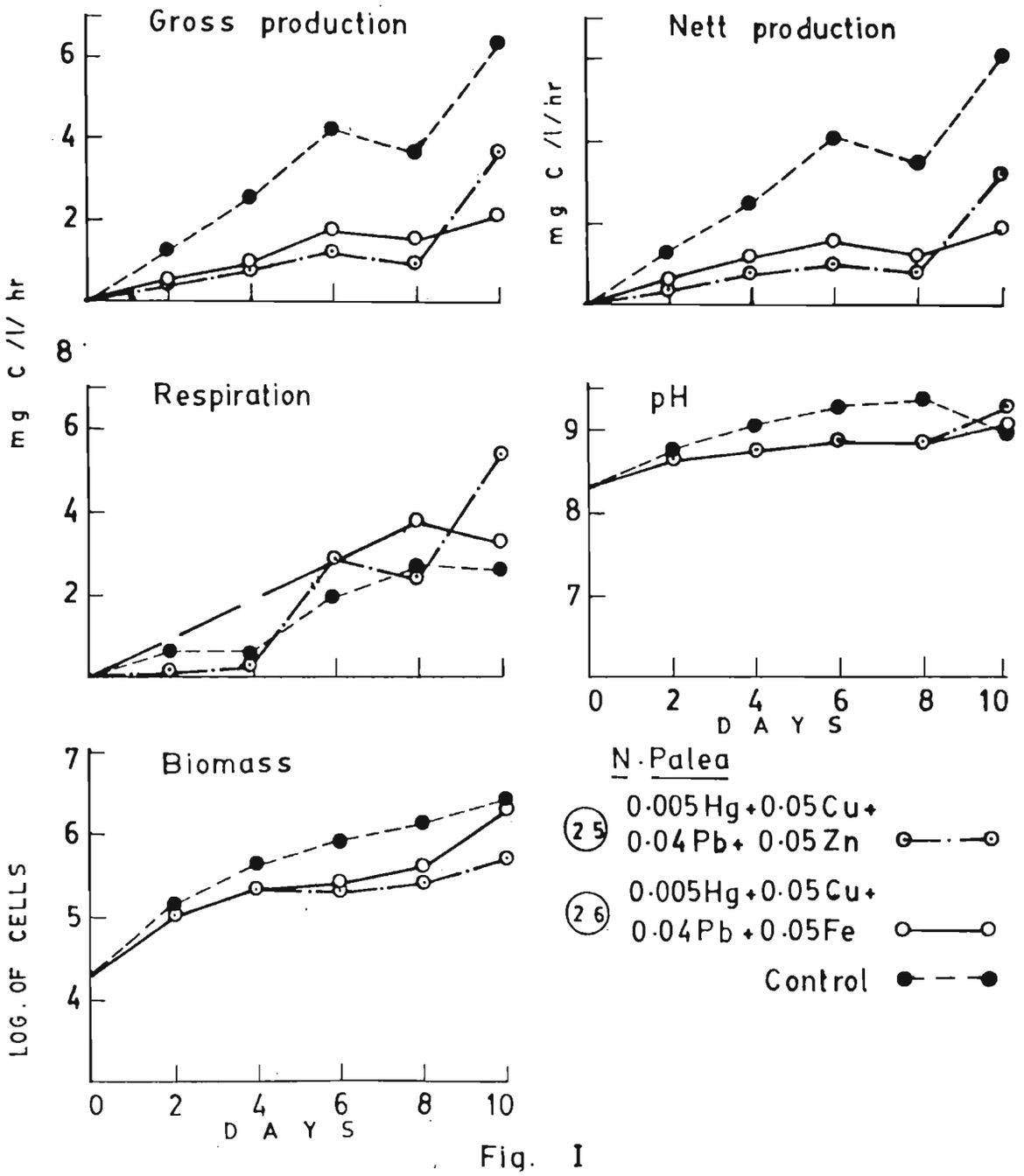

Gross production
Nett production
Respiration
pH
Biomass
mg C /l/ hr
LOG. OF CELLS
D A Y S
N. Palea
25 0·005 Hg + 0·05 Cu + 0·04 Pb + 0·05 Zn
26 0·005 Hg + 0·05 Cu + 0·04 Pb + 0·05 Fe
Control

Fig. I

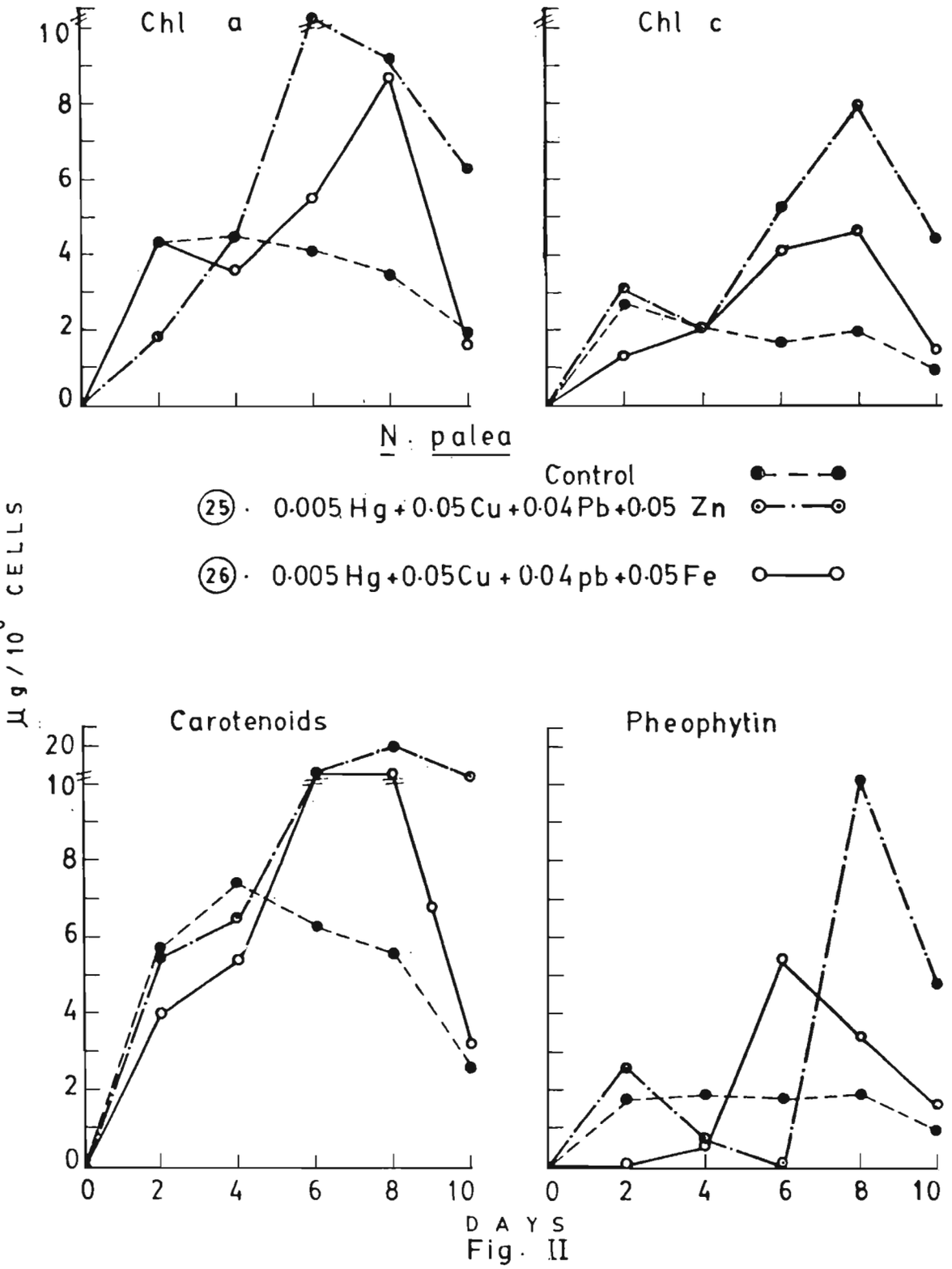

Chl a
Chl c
N. palea
Control
(25) · 0·005 Hg + 0·05 Cu + 0·04 Pb + 0·05 Zn
(26) · 0·005 Hg + 0·05 Cu + 0·04 pb + 0·05 Fe
µg / 10^6 CELLS
Carotenoids
Pheophytin
DAYS

Fig. II

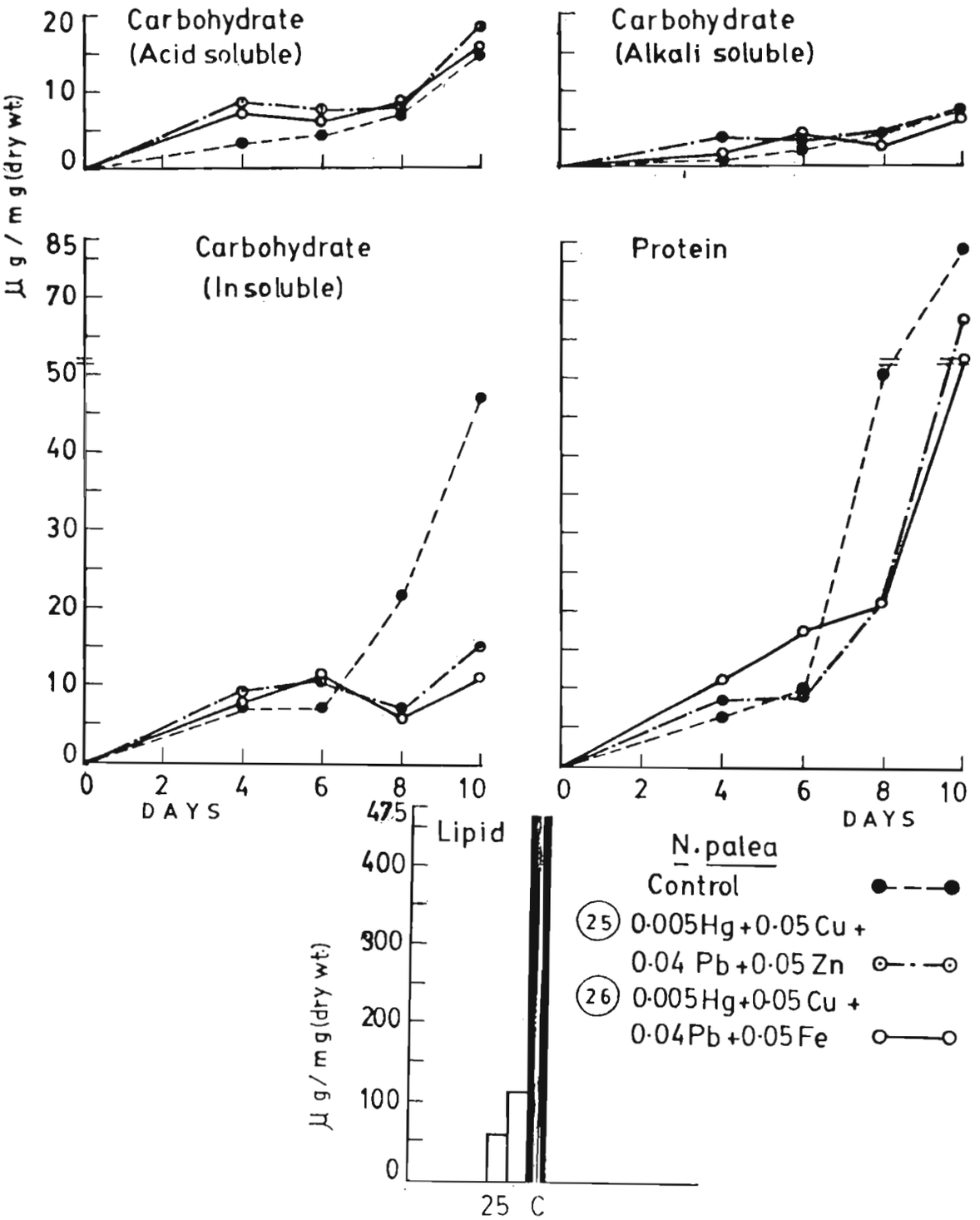

Carbohydrate
(Acid soluble)
Carbohydrate
(Alkali soluble)
Carbohydrate
(Insoluble)
Protein
Lipid
µ g / m g (dry wt)
DAYS
25 C
N. palea
Control
25 0·005Hg + 0·05 Cu +
0·04 Pb + 0·05 Zn
26 0·005Hg + 0·05 Cu +
0·04Pb + 0·05 Fe

Fig. III

6.3.5

Combined effect of mercury, cadmium, copper and zinc on S. bijugatus

Sl.No. of the combination	Concentration of metals (ppm)
(27)	0.02 Hg + 0.01 Cd + 0.1 Cu + 0.05 Zn

Production: (Fig. I)

Nett production of the alga was very low in the early phase. From 93% lower level it gradually rose to 68% lower level by eighth day but declined to 86% lower level by tenth day, further to 89% lower level by the end of growth phase.

Respiration of the alga was 15% higher than that of control on fourth day and declined to be 4% higher on sixth day. Inspite of further increase it fell short of control by 21% on twelfth day.

pH of culture increased inspite of low production to higher level than that of control by twelfth day. Except on the last two days it remained far less than that of control.

Pigments: (Fig. II)

Pigment content of the alga improved in presence of these metals and remained higher than that of control

from fourth day onwards. Chlorophyll a, b and carotenoids reached maximum concentration on sixth day whereas pheophytin on fourth day. Among the pigments chlorophyll a decreased to a greater extent than others towards the end of the growth phase. Chlorophyll b was developed to a larger extent than any other pigment during growth phase.

Photosynthetic end products: (Fig. III)

Carbohydrates were measured only on the last day due to highly retarded growth of the alga and concentration was 197% higher than that of control.

Protein content of the alga improved by 36% but lipid was reduced by 40%.

Growth: (Fig. I)

The growth was retarded considerably. The culture turned pale green in colour and not the usual dark green by eighth day. By twelfth day the culture was yellowish in colour. The cells were found slightly distorted and distended when observed under the microscope.

Selected combination	Nutrients absorbed by the alga (ug/l)		N / P
	Phosphate	Nitrate	
(27)	1302	988	0.76
Control	945	1290	1.37

The phosphate uptake of the alga increased by 38% but nitrate uptake decreased by 23%.

Conclusion:

The combination is harmful from growth point of view. Though the protein content increased it was not reflected on the growth of the alga. Also morphological abnormalities like distortion of the shape of the cell occurred. Also concentration of chlorophyll a was less than the concentration of chlorophyll b. The concentration of carotenoids and pheophytins exceeded that chlorophyll a by the end of growth phase. On the whole the combination was found to be undesirable.

Comparison:

When the effect of the present combination was compared with that of combination (16) (Hg + Cd + Zn) the rate of nett production was found to be considerably lowered. Respiration was lowered only towards the end of growth phase. The level of chlorophyll a fluctuated to a greater extent in the present instance. Also the concentration of chlorophyll a and chlorophyll b increased towards the end of growth phase. Carotenoids were not affected. Pheophytin was reduced. It showed a decreasing tendency towards the end of growth phase.

Though carbohydrate content of the alga increased towards the end of growth phase, protein was lowered by 100 µg and lipids by 50 µg. Biomass was not affected except in the early phase.

When the effect of present combination of metals was compared with that of combination (18) (Hg + Cd + Cu), the rate of nett production was found to be better towards the end of growth phase, but the rate of respiration increased to a large extent. Chlorophyll a, chlorophyll b and carotenoids were considerably lowered towards the end of growth phase, but pheophytin content increased. Carbohydrate content of the alga improved considerably. Protein content increased many fold. Lipid was not affected. Biomass increased slightly. Presence of zinc helped to mitigate the toxic effect.

6.3.6

Combined effect of mercury, cadmium, copper and iron on S. bijugatus

Sl.No. of the combination	Concentration of metals (in ppm)
(28)	0.02 Hg + 0.01 Cd + 0.1 Cu + 0.05 Fe

Production: (Fig. I)

Nett production of the alga remained less than that of control throughout growth phase. The production was 52% lower than that of control on fourth day. Even-though the production increased thereafter it was less than the control on eighth day by 32%, but decreased to 41 and 42% on tenth and twelfth day respectively.

Respiration of the alga increased sharply from 27% lower level on fourth day to its maximum, at 430% higher level by sixth day. Thereafter gradual reduction was observed which was equal to that of control on tenth day and 34% less on the last day.

pH of the culture improved gradually upto eighth day. Thereafter very little change was observed, but remained higher than that of control.

Pigments: (Fig. II)

Chlorophyll a, chlorophyll b and carotenoids were more than those of control from eighth day onwards.

All three pigments reached the maximum concentration on fourth day and declined thereafter towards the end of growth phase. Pheophytin was not detected on fourth day but by sixth day its concentration was higher than that of control. From its maximum on eighth day it declined to be slightly less than that of control on the last day.

Photosynthetic end products: (Fig. III)

Carbohydrate was measured from eighth day onwards when it was 22% higher than that of control. The concentration gradually increased and it was 90% and 248% higher on tenth and twelfth day respectively.

Protein content of the alga increased by 76% but lipid was reduced by 1%.

Growth: (Fig. I)

Growth of the alga was not suppressed at any stage and the biomass gradually increased. But remained less than that of control throughout growth phase.

Nutrient uptake of the alga is given in table.

Selected combination	Nutrients absorbed by the alga (ug/l)		N / P
	Phosphate	Nitrate	
(28)	1317	1112	0.84
Control	945	1290	1.37

Phosphate absorption of the alga increased by 39%. Actual nitrate absorption could not be estimated as all nitrate in the medium was absorbed by the alga by the end of growth phase.

Conclusion:

Inspite of increase in protein content of the alga, the growth was retarded and the combination (28) was particularly undesirable from the point of view of growth.

Comparison:

When the effect of present combination was compared with that of combination (18) (Hg + Cd + Cu), considerable improvement in both nett production and respiration was observed. The concentration of all four pigments was lowered towards the end of growth phase. Carbohydrate content increased to a large extent. Protein content increased by many fold. Lipid content of the alga was more than doubled. Biomass also improved.

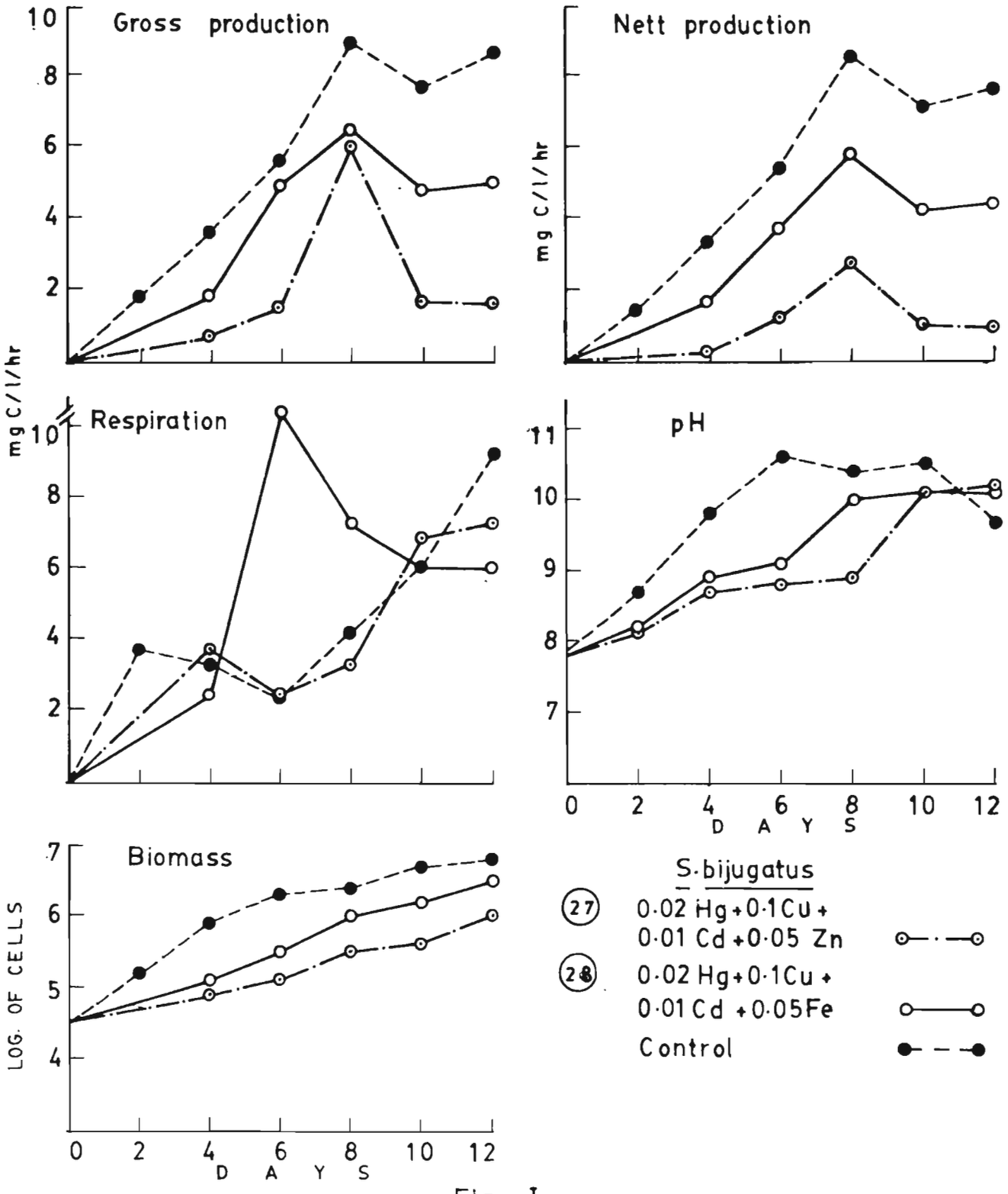

Gross production
Nett production
Respiration
pH
Biomass
mg C/l/hr
LOG. OF CELLS
DAYS
S. bijugatus
(27) 0·02 Hg + 0·1 Cu + 0·01 Cd + 0·05 Zn
(28) 0·02 Hg + 0·1 Cu + 0·01 Cd + 0·05 Fe
Control

Fig. I

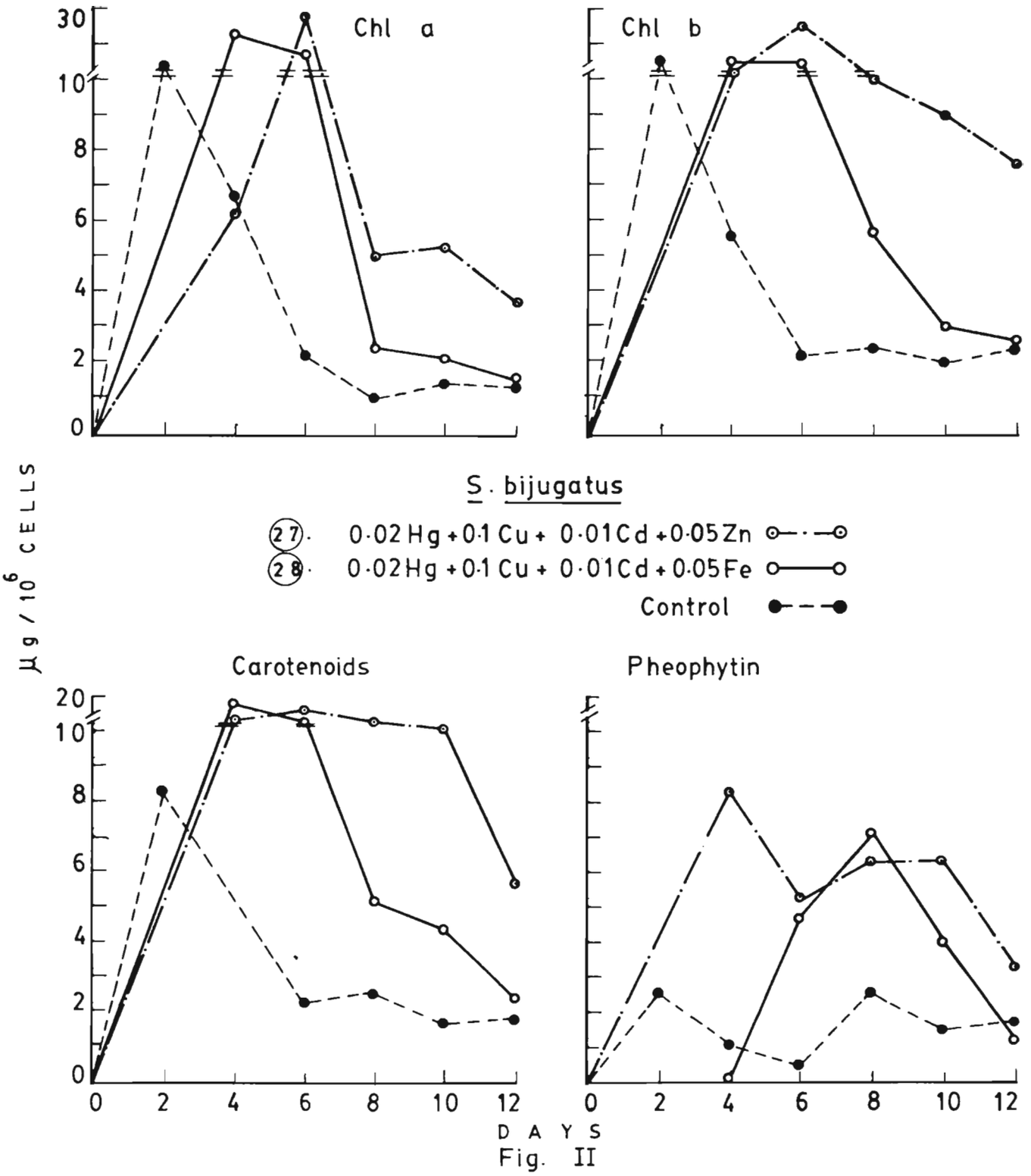
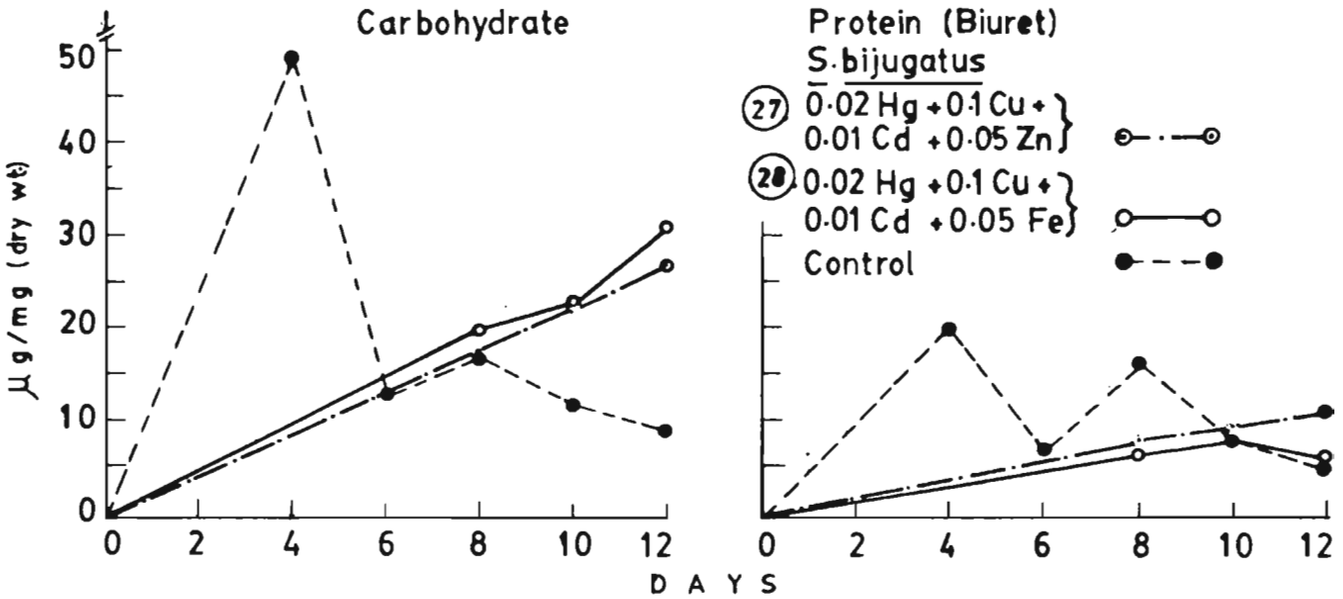

Chl a
Chl b
S. bijugatus
(27) 0·02 Hg + 0·1 Cu + 0·01 Cd + 0·05 Zn
(28) 0·02 Hg + 0·1 Cu + 0·01 Cd + 0·05 Fe
Control
Carotenoids
Pheophytin
µg / 10^6 CELLS
DAYS

Fig. II


Carbohydrate
Protein (Biuret)
S. bijugatus
(27) 0·02 Hg + 0·1 Cu + 0·01 Cd + 0·05 Zn
(28) 0·02 Hg + 0·1 Cu + 0·01 Cd + 0·05 Fe
Control
µg/mg (dry wt)
0
10
20
30
40
50
0 2 4 6 8 10 12
DAYS

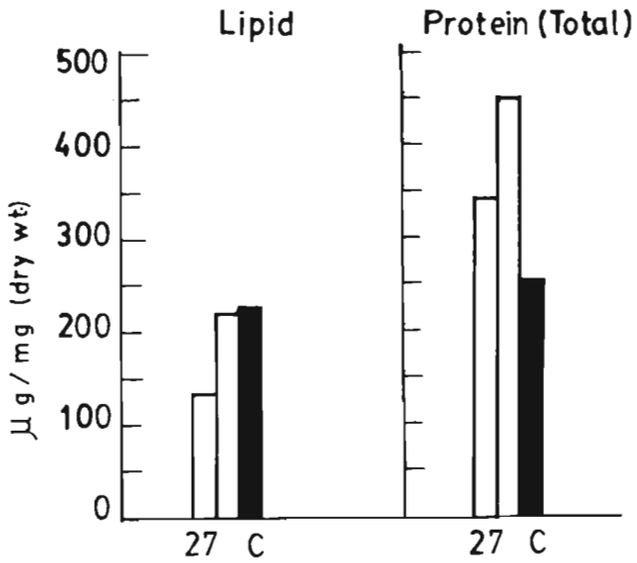

Lipid
Protein (Total)
µg/mg (dry wt)
0
100
200
300
400
500
27 C
27 C

Fig. III

Combined effect of mercury, cadmium, copper and zinc on

N. palea

Sl.No. of the combination	Metals in combination (in ppm)
(27)	0.005 Hg + 0.02 Cd + 0.05 Cu + 0.05 Zn

At the selected levels the metals in combination proved to be highly toxic to the diatom. Pale brown colour appeared in the culture by eleventh day but disappeared by twelfth day. Hence the selected parameters of growth were not estimated.

Combined effect of mercury, cadmium, copper and iron on N. palea

Sl.No. of the combination	Concentration of metals (in ppm)
(28)	0.005 Hg + 0.02 Cd + 0.05 Cu + 0.05 Fe

Production: (Fig. I)

The nett production of the diatom was adversely affected and was less than that of control throughout growth phase. The percentage reduction was 17 on fourth day, 52 on sixth day and 50 on the last day.

Respiration of the alga was 26% higher on sixth day, but declined to its minimum at 85% lower level on eighth day. It increased once again towards the end of growth phase and was 64% more in relation to control. The oxygen value in the dark bottle was more on fourth day than the initial bottle. In this treatment negative value for respiration was obtained on fourth day but one sample out of three showed the value as zero.

pH of the culture was less than that of control throughout growth phase.

Pigments: (Fig. II)

Total pigment content of the diatom increased in presence of these metals. The concentration of

chlorophyll a fluctuated with two peaks on fourth and eighth day of growth and declined towards the end of growth phase. Except on second and sixth day, it remained higher than that of control. Chlorophyll c was developed to a greater extent than chlorophyll a and also exhibited fluctuation, with peaks on second and sixth day. An increasing tendency towards the end of growth phase was exhibited. Throughout the period of growth it was more than that of control. Carotenoids of the diatom were developed to a greater extent than any other pigment and their concentration was higher throughout growth phase, than that of control. Pheophytin followed the pattern of chlorophyll c in development and fluctuated with two maxima on second and sixth day of growth. Its concentration remained higher than that of control throughout.

Photosynthetic end products: (Fig. III)

The acid soluble carbohydrate was slightly higher than that of control on fourth day. The concentration of this product increased sharply thereafter and was maximum on sixth day with 30% increase. It then decreased, but remained 67% higher on eighth day. It increased once again to 41% higher level on the last day in relation to control.

The alkali soluble fraction did not differ much between that of control and the treatment though generally it remained higher than that of control.

The insoluble carbohydrate fraction of the diatom was equal to that of control on fourth day and 78% more on sixth day. It declined to 62% lower level by eighth day, despite further increase was 68% less on the last day.

Protein content of the diatom increased gradually upto eighth day. It was 85% more on fourth day and 94% on sixth day. Inspite of further increase it fell short of control by 44% on eighth day and 36% on the last day.

The lipid content of the diatom was adversely affected by the metals in combination and was reduced by 50%.

Growth: (Fig. I)

The rate of multiplication of the diatom was enhanced in the initial stages resulting in increased biomass on second day. But further increase was slow and considerable reduction in biomass was observed towards the end of the growth period.

Selected concentration of metal	Nutrients absorbed (µg/l)		N / P
	Phosphate	Nitrate	
(28)	1675	734	0.44
Control	1350	763	0.57

Phosphate absorption of the diatom increased by 24%. Nitrate absorption was not much affected.

Conclusion:

The combination 28 is toxic to the species and the adverse effect is reflected in the reduction of biomass, protein and lipid contents.

Comparison:

The effect of combination (28) on the diatom must be concluded as more desirable than that of combination (18) (Hg + Cd + Cu) since the latter was found to be lethal. The addition of iron to combination 18 reduced the toxicity to a large extent, enabling the diatom to grow.

6.3.7

Combined effect of mercury, cadmium, zinc and iron on N. palea

Sl.No. of the combination	Concentration of metals in ppm
(29)	0.005 Hg + 0.02 Cd + 0.05 Zn + 0.05 Fe

Production: (Fig. I)

The nett production of the diatom was enhanced between second and fourth day. It was 16% more on fourth day in relation to control. But it registered 39% reduction on sixth day and increased gradually thereafter to 49% lower level on the last day.

Respiration of the diatom varied with two peaks on second and sixth day and was more than that of control throughout growth phase. The percentage increase recorded was 137 on sixth day and 164 on tenth day.

pH of the culture was higher than that of control upto fourth day owing to higher rate of production. From sixth day onwards it was less than that of control.

Pigments: (Fig. II)

Total pigment content of the diatom was reduced during the middle phase of growth. But at the end of growth phase it was more than that of control. The concentration of chlorophyll a exceeded that of control

only on last day whereas that of others was higher in early and late growth phase. A distinct tendency to increase was recorded for all four pigments. Also all four pigments were maximum on second day.

Photosynthetic end products: (Fig. III)

The acid soluble carbohydrate fraction of the diatom increased with the age of culture upto eighth day and remained steady thereafter. The percentage increase however on fourth day was 124, on sixth day 95 and on eighth day was 80. It was 26% less in relation to control on the last day.

The alkali soluble carbohydrate fraction followed the same trend as the above but did not differ much from that of control throughout the growth phase.

The concentration of insoluble carbohydrate increased from 44% lower level on fourth day to 90% higher levels by sixth day. At its maximum on eighth day it was 10% lower and declined to 74% lower level by the end of growth phase.

Protein content of the diatom increased to a large extent in the early growth phase. It was 673% higher on fourth day and declined to 257% higher level on sixth day. The concentration was maximum on fourth day. Inspite of further increase it fell short of control by 13% on eighth day and dwindled to 51% lower level by the end of growth phase. The lipid was reduced by 4%.

Growth: (Fig. I)

The growth was promoted in the early phase by the metals in combination and the biomass was higher than that of control upto eighth day. It was steady from sixth day onwards but the final yield was less than on that of control.

Selected combination	Nutrients absorbed (µg/l)		N / P
	Phosphate	Nitrate	
(29)	1695	845	0.50
control	1350	763	0.57

Both the nutrients were absorbed to a greater extent than that of control. But the increase in nitrate absorption was only by few µg whereas that of phosphate was by 26%.

Conclusion:

The combination though not as toxic as others, should be considered as undesirable as biomass and protein were reduced to some extent.

Comparison:

When the effect of the present combination of metals (Hg + Cd + Zn + Fe) when compared with that of combination (16), (Hg + Cd + Zn), the nett production was

found unaffected byt respiration of the diatom increased. The concentration of all four pigments increased. Very little improvement in the concentration of all three carbohydrate fractions was observed. In the early growth phase protein content increased to a large extent. Lipid content of the diatom increased by more than 50%. Biomass was not affected. The presence of iron in the combination, helped to mitigate the otherwise toxic effect of the combination (16) (Hg + Cd + Zn).

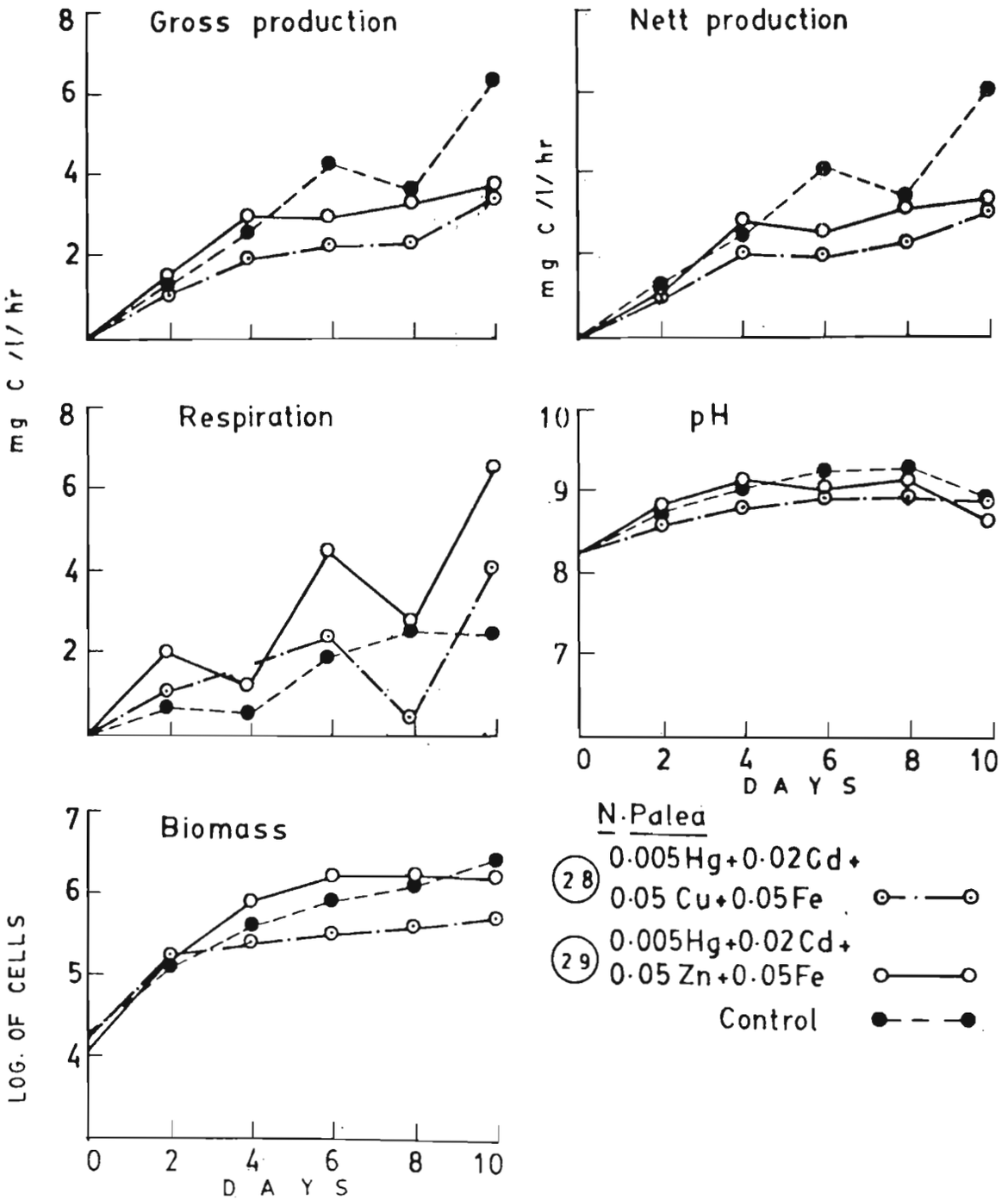

Gross production
Nett production
Respiration
pH
Biomass
mg C /l/ hr
LOG. OF CELLS
D A Y S
N. Palea
(28) 0·005Hg+0·02Cd+ 0·05 Cu+0·05Fe
(29) 0·005Hg+0·02Cd+ 0·05 Zn+0·05Fe
Control

Fig. I

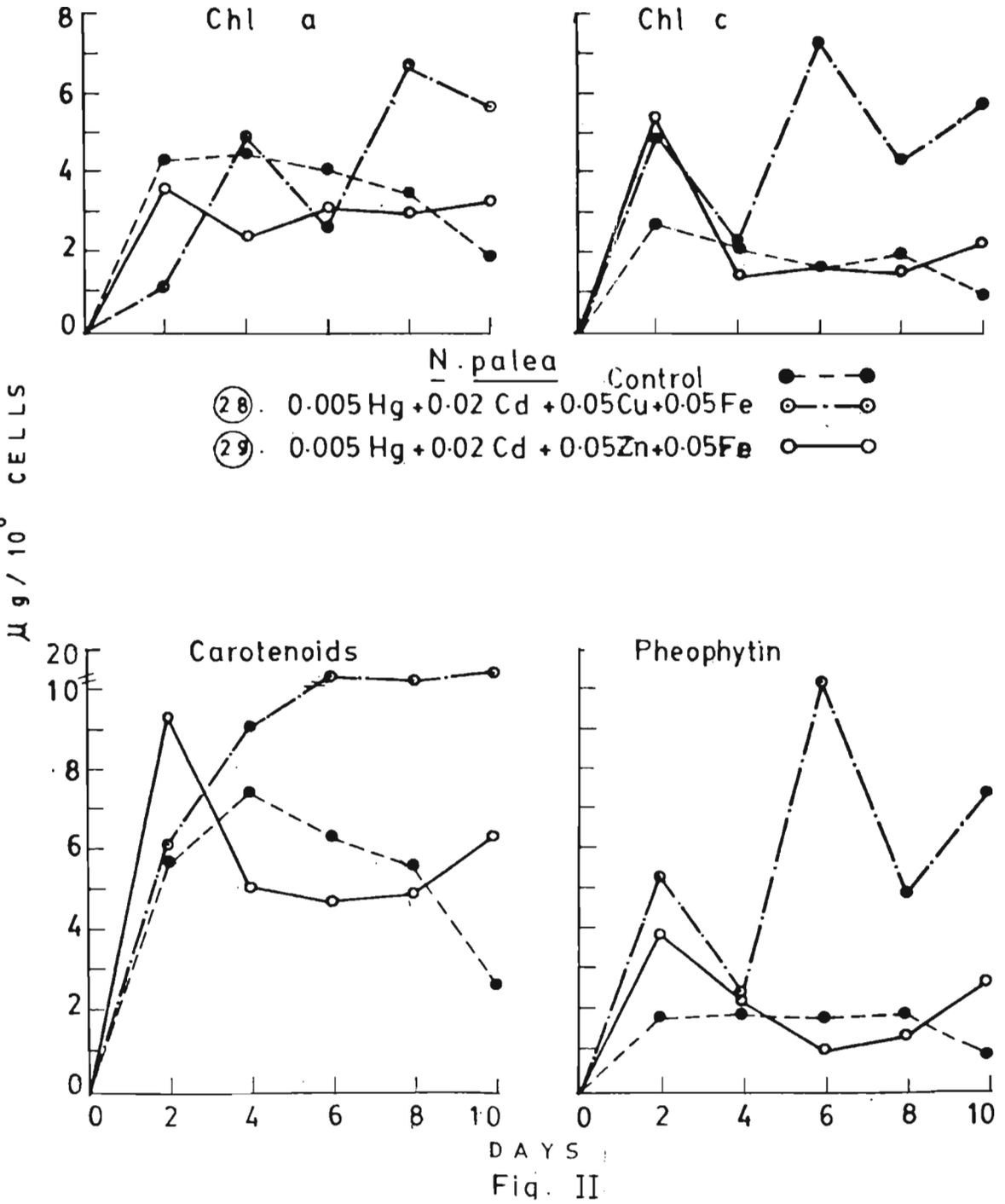

Chl a
Chl c
N. palea
Control
28. 0·005 Hg + 0·02 Cd + 0·05 Cu + 0·05 Fe
29. 0·005 Hg + 0·02 Cd + 0·05 Zn + 0·05 Fe
µg / 10^6 CELLS
Carotenoids
Pheophytin
DAYS

Fig. II

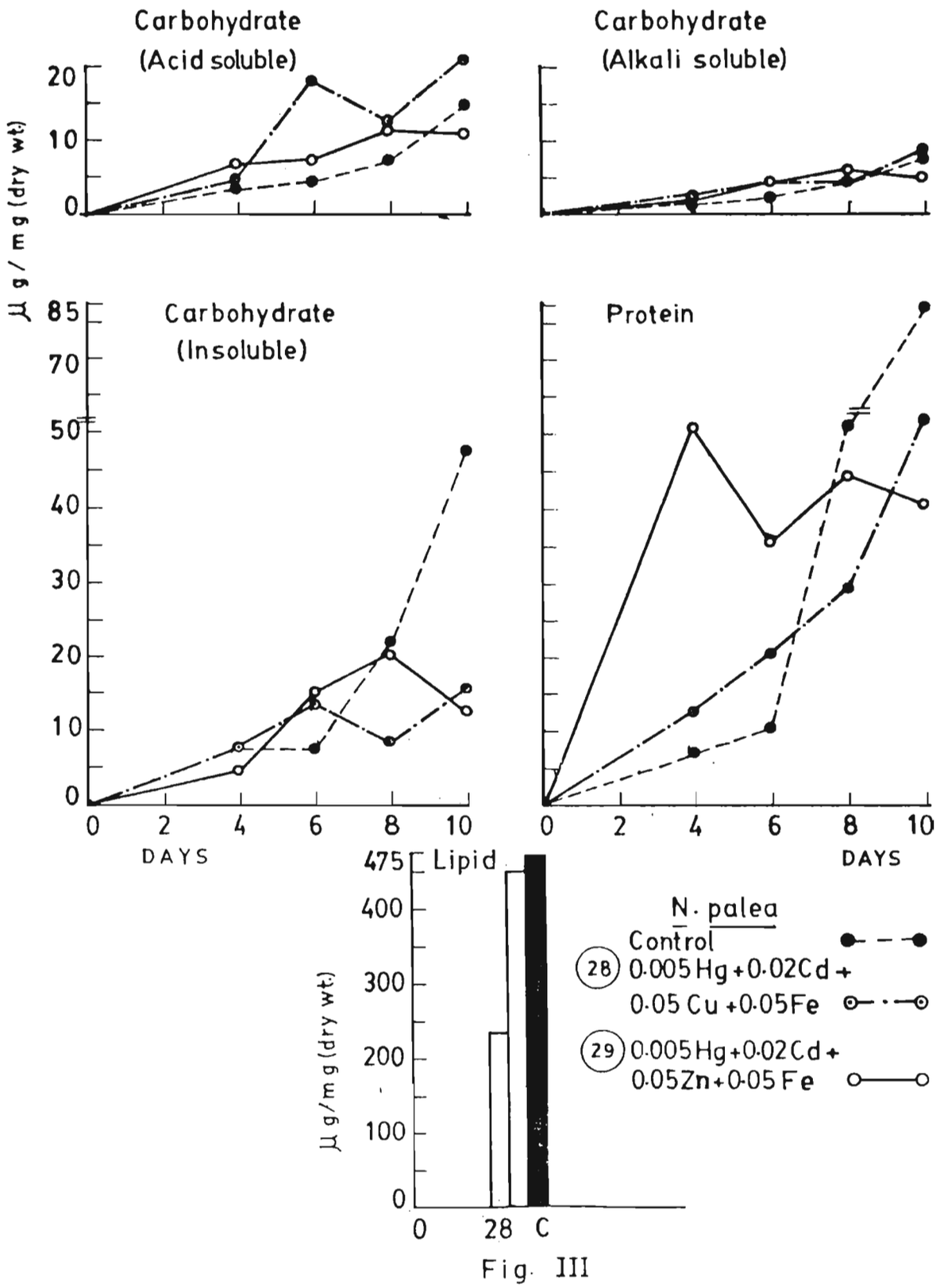

Carbohydrate
(Acid soluble)
Carbohydrate
(Alkali soluble)
Carbohydrate
(Insoluble)
Protein
µg / mg (dry wt.)
DAYS
DAYS
Lipid
µg/mg (dry wt.)
N. palea
Control
28 0·005 Hg + 0·02 Cd + 0·05 Cu + 0·05 Fe
29 0·005 Hg + 0·02 Cd + 0·05 Zn + 0·05 Fe
28
C

Fig. III

Combined effect of mercury, cadmium, zinc and iron on S. bijugatus

Sl.No. of the combination	Concentration of metals in ppm
(29)	0.02 Hg + 0.01 Cd + 0.05 Zn + 0.05 Fe

Production: (Fig. I)

The nett production of the alga was very low upto sixth day. It was reduced from fourth to sixth day and also from eighth to tenth day. It was 75% less on fourth day and 91% less on sixth day but increased to 60% lower level on eighth day. The alteration thereafter was negligible and the percentage reduction found on tenth day was 54 and on the last day 52.

Respiration of the alga was considerably reduced in this treatment. From 57% lower level on second day, it decreased to 52% lower level. It gradually increased to 64% lower level by tenth day. Thereafter it registered a sharp rise reaching 40% higher level on the last day.

pH of the culture was very low upto eighth day inspite of steady and gradual increase in production. From eighth day it rose sharply till tenth day, exceeded that of control thereafter and was slightly higher on the last day.

Pigments: (Fig. II)

Total pigment content of the alga increased to a large extent in this treatment and was much higher than that of control from fourth day onwards. Chlorophyll a reached maximum level on eighth day. Chlorophyll b and carotenoids fluctuated with two peaks on sixth and tenth day respectively. Pheophytin exhibited severe fluctuation. It was not detected on fourth and eighth day. Otherwise for the rest of the growth phase its concentration was much higher than that of control. Chlorophyll b was developed to greatest extent among the pigments. All pigments exhibited a decreasing tendency towards the end of growth phase.

Photosynthetic end products: (Fig. III)

The carbohydrate concentration was less than that of control except on the last two days. From 80% lower level on fourth day it decreased to 73% lower level. Thereafter gradual rise in the concentration of this product was noted to its maximum level on tenth day with 67% increase. At the end of growth phase it was 83% more than that of control.

Protein content of the alga increased to a very large extent, by 115% and lipid content improved by 78%.

Growth: (Fig. I)

The growth of the alga was retarded from second day onwards and the biomass was considerably reduced in relation to control, throughout growth phase.

Selected combination	Nutrients absorbed by the alga (µg/l)		N / P
	Phosphate	Nitrate	
(29)	1215	1050	0.86
Control	945	1290	1.37

The uptake of phosphate increased by 29% but that of nitrate decreased by 19%.

Conclusion:

The combination 29 was particularly harmful from growth point of view. Though the nitrate uptake decreased it did not have any adverse effect on the protein content of the alga.

Comparison:

When the effect of the present combination was compared with that of (Hg + Cd + Zn), combination (16) both nett production and respiration were found lowered. The concentration of all four pigments increased, but chlorophyll b and carotenoids developed to a greater

extent than chlorophyll a. Carbohydrate content of the alga was not affected whereas protein content improved. Lipid content was more than doubled, Biomass however, was slightly lowered.

6.3.8

Combined effect of mercury, lead, zinc and iron on S. bijugatus

Sl.No. of the combination	Concentration of the metals (in ppm)
(30)	0.02 Hg + 0.05 Pb + 0.05 Zn + 0.05 Fe

Production: (Fig. I)

The nett production of the alga was promoted in the early phase and was higher than that of control upto fourth day. From 2% higher level on fourth day it increased sharply to 88% higher level on sixth day and further to maximum, 148% higher level, by eighth day. Thereafter it declined to 89% more than that of control on tenth day. Once again it registered an increase towards the end of growth phase and was 110% higher in relation to control.

The respiration of the alga was higher on fourth, sixth and eighth day of growth in relation to control. From 48% higher level on fourth day it decreased initially till sixth day and increased sharply to maximum level, by 419% on eighth day. Thereafter it declined and was less than that of control, by 28% on tenth day. Respiration registered an increase towards the end of growth phase but remained 3% less than that of control.

Inspite of enhanced production, throughout growth phase, pH of the culture exceeded that of control only by eighth day and thereafter remained higher.

Pigments: (Fig. II)

Total pigment content of the alga was considerably reduced. Chlorophyll a, chlorophyll b and carotenoids exhibited fluctuation with two peaks, on sixth and tenth day. Their concentration was maximum on sixth day whereas that of pheophytin on fourth day. At the end of growth phase all pigments were less than that of control.

Photosynthetic end products: (Fig. III)

Carbohydrate content of the alga was reduced by 22% on fourth day, at its maximum. It declined thereafter gradually, but was 41% higher on sixth day. It registered 29% reduction on tenth day and 7% on twelfth day.

Protein content of the alga decreased by 29% whereas lipid increased by 86%.

Growth: (Fig. I)

The growth of the alga was retarded to some extent in the early phase, and the biomass was less than that of control upto sixth day. There after was nearly equal to that of control.

Selected concentration of metal	Nutrients absorbed by the alga (ug/l)		N / P
	Phosphate	Nitrate	
(30)	1225	1060	0.87
Control	945	1290	1.37

The uptake of phosphate increased by 30% whereas that of nitrate decreased by 18%.

Conclusion:

The combination was not desirable since the protein and biomass were lowered. This was also one of the very few combinations showing the reduction in the pigment content of the alga.

Comparison:

When the effect of the present combination of metals was compared with that of combination 20 (Hg + Pb + Zn), the nett production and respiration of the alga were found further increased. Further reduction in pigments was observed. Carbohydrate content of the alga improved in the early phase. Protein was not affected. Four fold increase in lipid content was observed. Biomass was not affected.

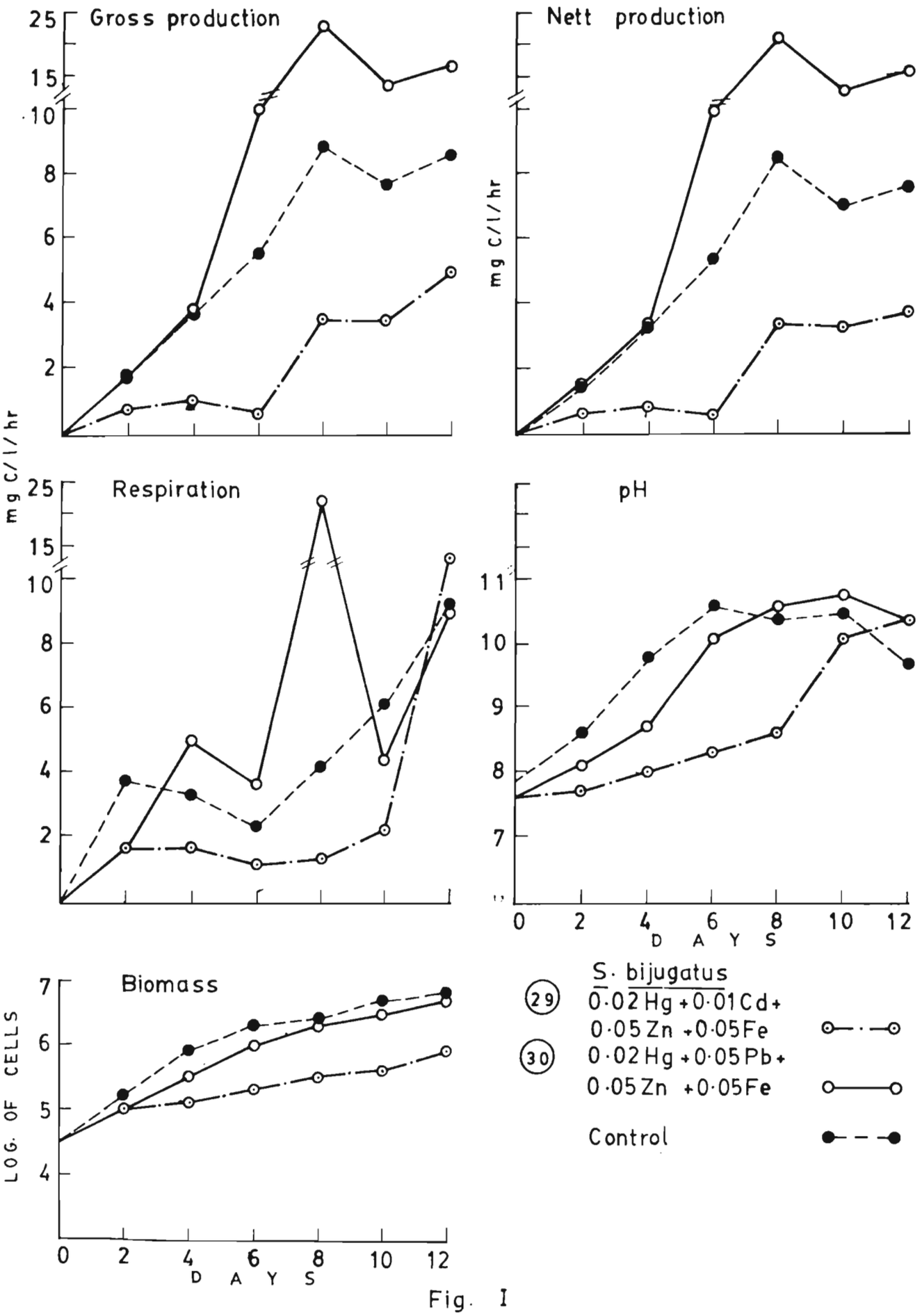

Gross production
Nett production
Respiration
pH
Biomass
mg C/l/hr
LOG. OF CELLS
DAYS
S. bijugatus
(29) 0·02 Hg + 0·01 Cd + 0·05 Zn + 0·05 Fe
(30) 0·02 Hg + 0·05 Pb + 0·05 Zn + 0·05 Fe
Control

Fig. I

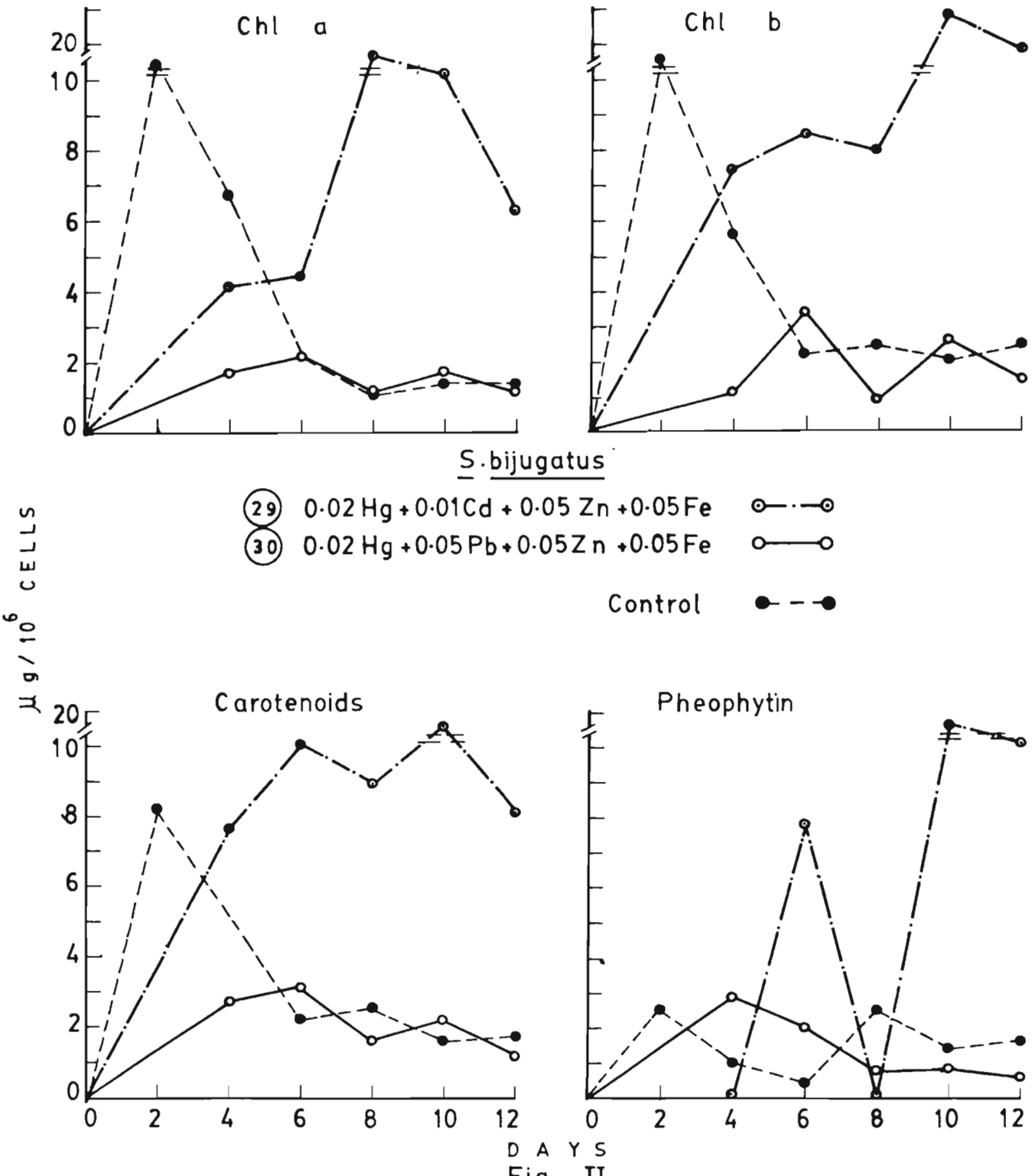
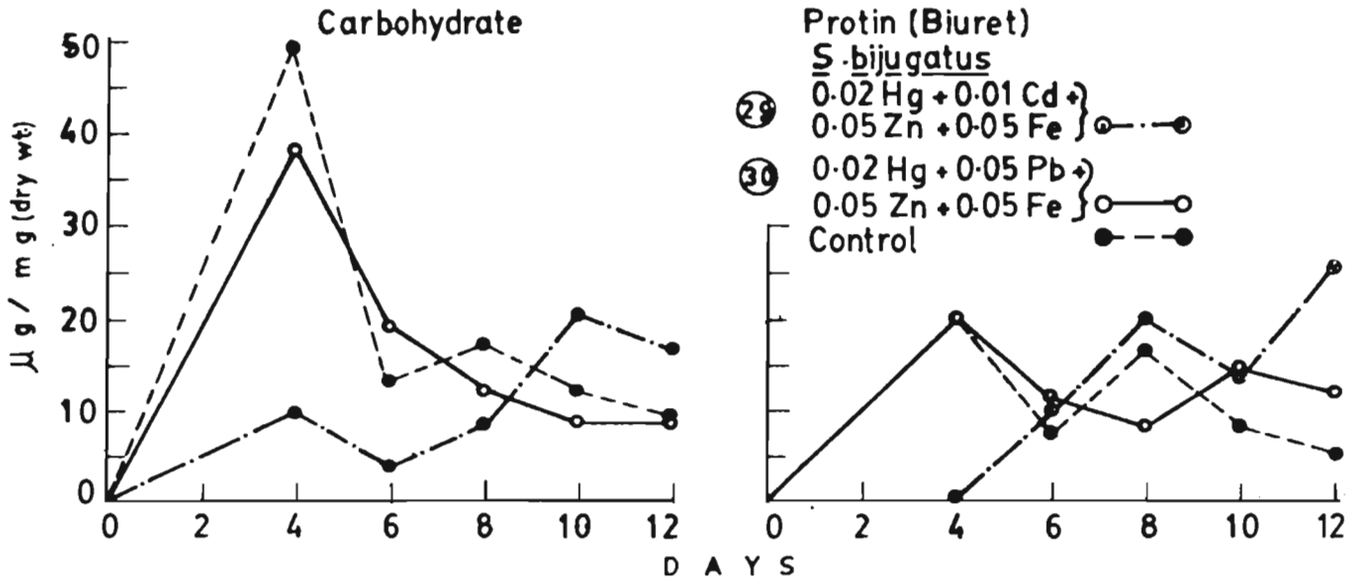

Chl a
Chl b
S. bijugatus
(29) 0·02 Hg + 0·01 Cd + 0·05 Zn + 0·05 Fe
(30) 0·02 Hg + 0·05 Pb + 0·05 Zn + 0·05 Fe
Control
μg / 10^6 CELLS
Carotenoids
Pheophytin
0 2 4 6 8 10 12
DAYS

Fig. II


Carbohydrate
Protin (Biuret)
S. bijugatus
29 0·02 Hg + 0·01 Cd + 0·05 Zn + 0·05 Fe
30 0·02 Hg + 0·05 Pb + 0·05 Zn + 0·05 Fe
Control
µ g / m g (dry wt)
0 10 20 30 40 50
0 2 4 6 8 10 12
D A Y S

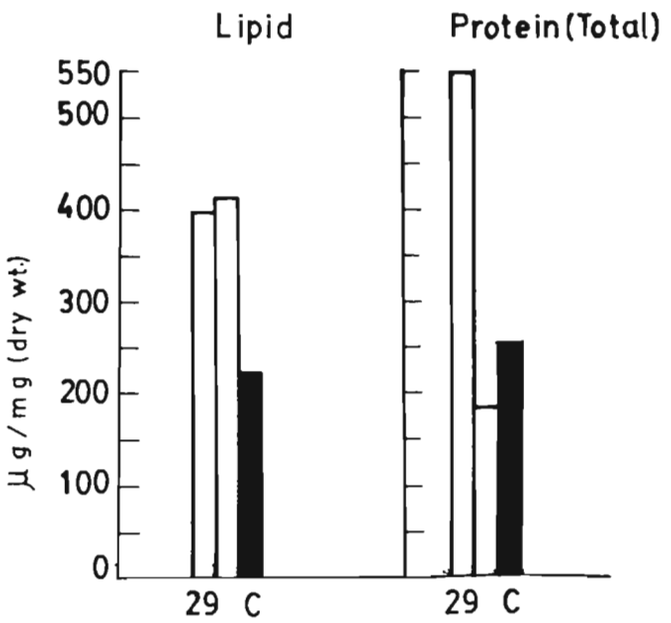

Lipid
Protein (Total)
µ g / m g (dry wt)
0 100 200 300 400 500 550
29 C
29 C

Fig. III

Combined effect of mercury, lead, zinc and iron on N. palea

Sl.No. of the combination	Concentration of metals (in ppm)
(30)	0.005 Hg + 0.04 Pb + 0.05 Zn + 0.05 Fe

Production: (Fig. I)

Nett production of the diatom was enhanced in the early growth phase. It was 41% more in relation to control on fourth day but declined to 31% lower level by sixth day and remained steady thereafter. The percentage reduction recorded was 17 on eighth day and 49 on the last day.

Respiration was elevated to a large extent. 233%, 380% and 283% increase was observed on second, fourth and sixth day respectively. Respiration was maximum on sixth day and declined quickly to 8% lower level on eighth day and further to 60% lower level on tenth day.

pH of the culture was slightly higher than that of control upto fourth day due to increased production and thereafter remained less than that of control.

Pigments: (Fig. II)

Chlorophyll a and chlorophyll c were reduced. Chlorophyll a was reduced to a greater extent than

chlorophyll c, but both pigments were slightly more than those of control at the end of growth phase. Chlorophyll a reached maximum concentration on eighth day whereas chlorophyll c on second day. Concentration of carotenoids was higher in relation to control on second and last day. It was maximum on second day. Pheophytin was produced to greater extent than any other pigment and the concentration was slightly less than that of control only on sixth and eighth day. It was maximum on fourth day. Total pigment content of the diatom exhibited an increase in relation to control by the end of growth phase.

Photosynthetic end products: (Fig. III)

The concentration of acid soluble carbohydrate fraction was higher in relation to control except on the last day. The percentage increase was 133 on fourth day, 36 on sixth day and 44 on eighth day. From its maximum level on eighth day it decreased to 42% lower level at the end of growth phase.

The alkali soluble carbohydrate fraction was the least affected and was slightly less than that of control on the last day.

The insoluble carbohydrate fraction was the most affected product. The concentration increased to maximum by 137% on fourth day and 63% on sixth day in

relation to control. Very little change was recorded thereafter but it was found to be reduced by 36% on eighth day and 74% on tenth day.

Protein content of the diatom increased to a large extent in the early phase and its concentration fluctuated with two peaks, on fourth and eighth day of growth. 457% increase was recorded on fourth day and 225% on sixth day. It increased thereafter but not to the same extent as that of control resulting in 19% reduction, at its, maximum on eighth day and 51% on tenth day.

The lipid of the diatom was least affected and only 7% reduction was observed.

Growth: (Fig. I)

Growth of the diatom was promoted in the early phase and the biomass was greater in the treatment upto eighth day, but by the end of growth phase slight reduction was observed in relation to control.

Selected concentration of metal	Nutrients absorbed by the alga (µg/l)		N / P
	Phosphate	Nitrate	
(30)	1560	940	0.60
Control	1350	763	0.57

The diatom absorbed 16% more phosphate when exposed to the metals in combination. At the end of the

experiment nitrate was not detected in the medium. Hence the actual nitrate absorption could not be estimated.

Conclusion:

The particular combination was toxic from the point of view of protein production of the diatom. The lipid and biomass also were slightly lowered. On the whole it proved to be an undesirable combination.

Comparison:

The effect of the present combination of metals was found to be not much different from that of combination (20) (Hg + Pb + Zn) in essence. The nett production increased and so did respiration. With the exception of pheophytin the concentration of all pigments remained unaffected. Pheophytin concentration was found to increase. All three carbohydrate fractions were unaffected. Protein content of the diatom increased slightly and also the lipid. Biomass was not affected. The addition of iron (Fe) to the combination Hg + Pb + Zn did not result in any considerable change.

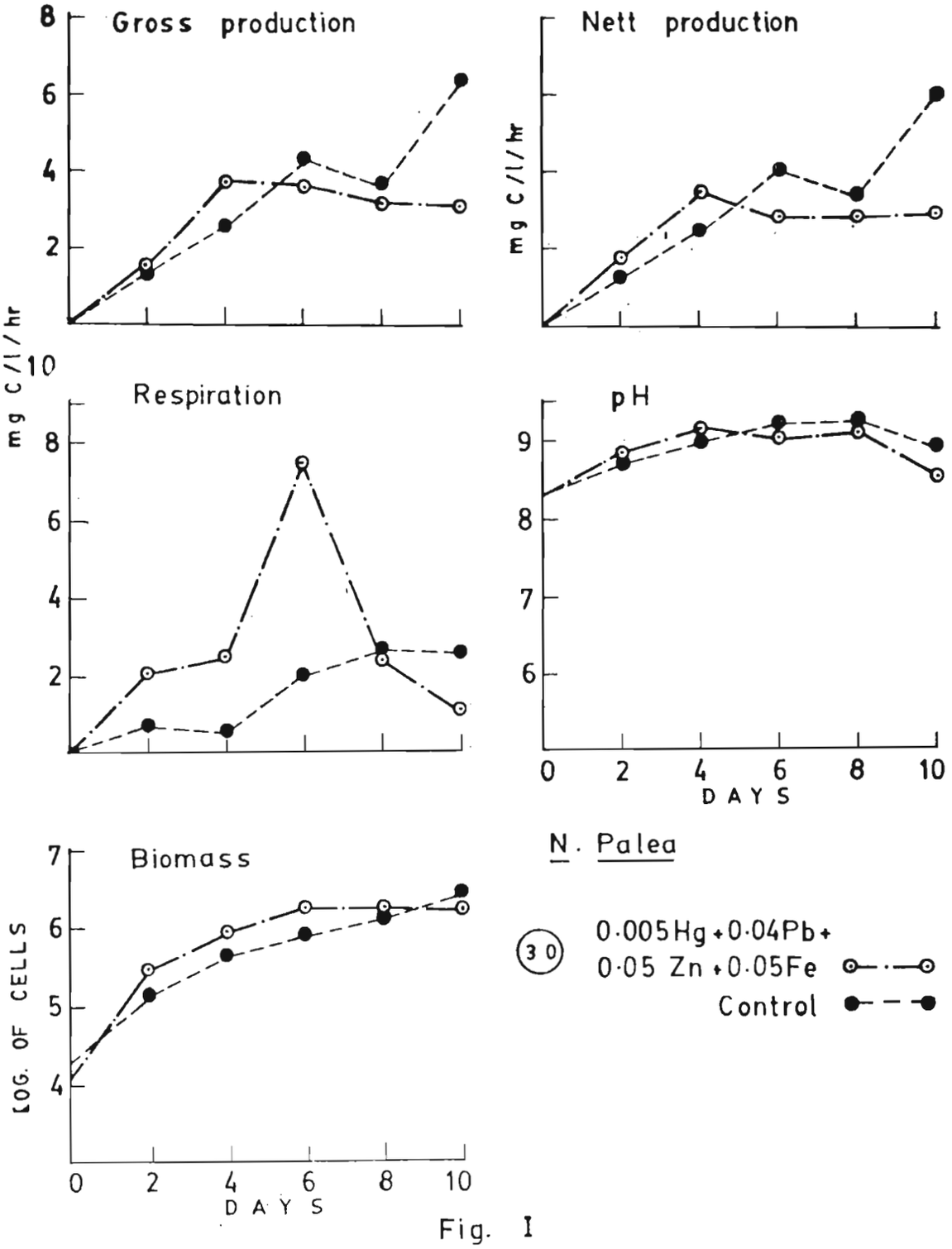

Gross production
Nett production
Respiration
pH
Biomass
mg C/l/hr
LOG. OF CELLS
DAYS
N. Palea
30
0·005Hg + 0·04Pb +
0·05 Zn + 0·05Fe
Control

Fig. I

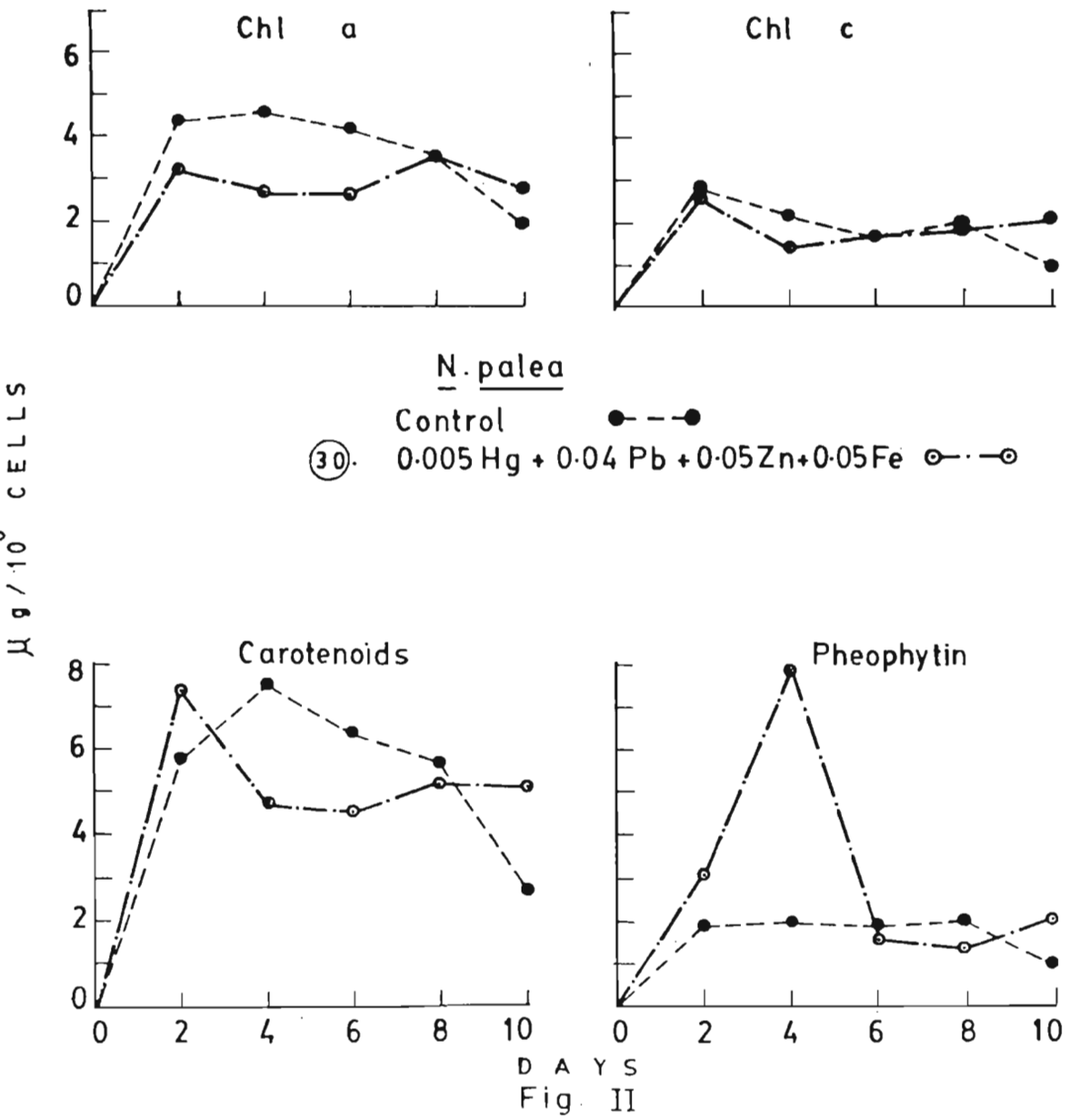

Chl a
Chl c
N. palea
Control
30. 0·005 Hg + 0·04 Pb + 0·05 Zn + 0·05 Fe
Carotenoids
Pheophytin
μg / 10^6 CELLS
DAYS
0
2
4
6
8
10

Fig. II

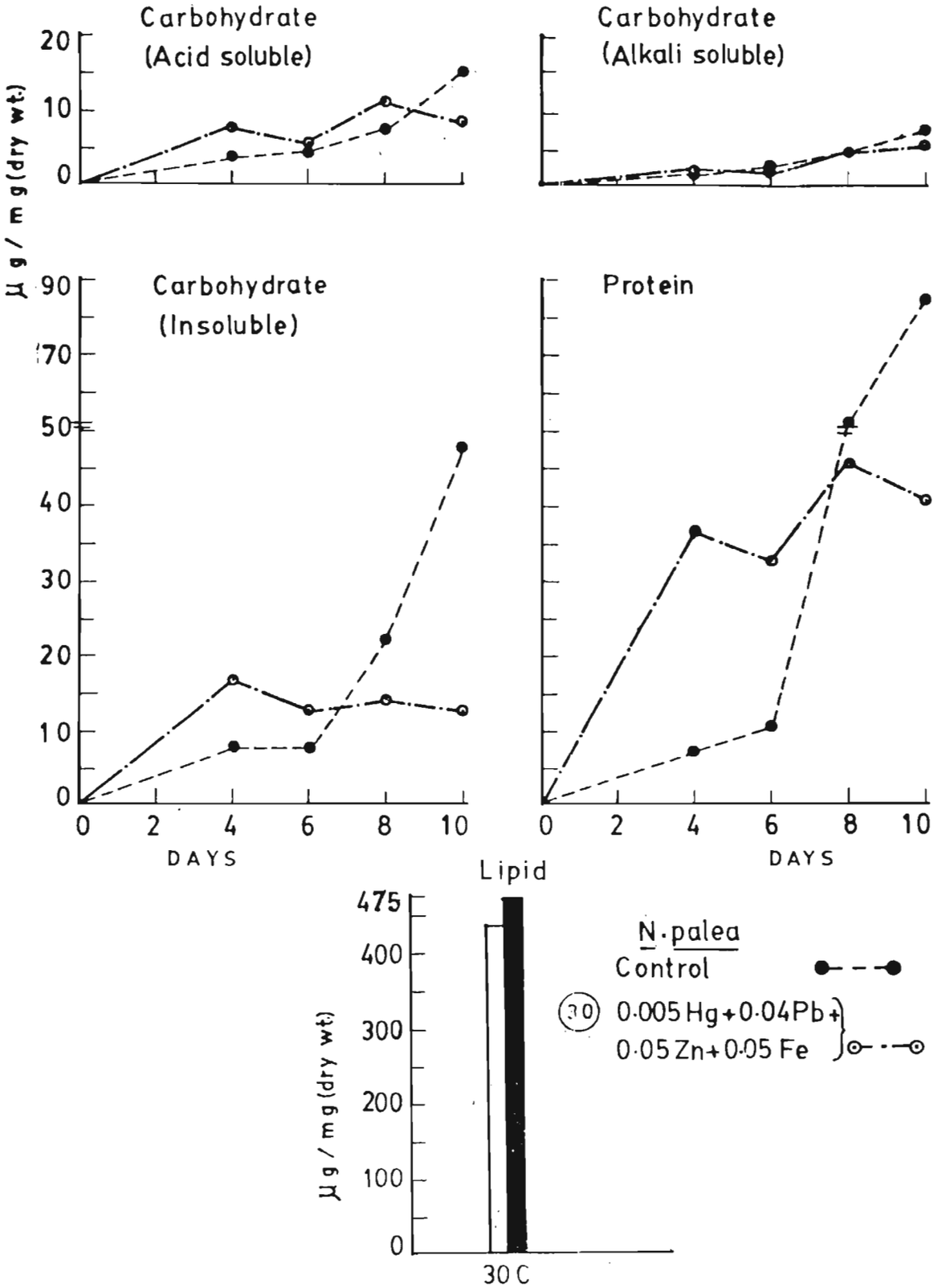

Carbohydrate
(Acid soluble)
Carbohydrate
(Alkali soluble)
Carbohydrate
(Insoluble)
Protein
µg / mg (dry wt.)
DAYS
DAYS
Lipid
µg / mg (dry wt.)
N. palea
Control
30 0·005 Hg + 0·04 Pb +
0·05 Zn + 0·05 Fe
30 C

Fig. III

6.3.9

Combined effect of cadmium, lead, copper and zinc on S. bijugatus

Sl.No. of the combination	Concentration of metals (in ppm)
(31)	0.01 Cd + 0.05 Pb + 0.1 Cu + 0.05 Zn

Production: (Fig. I)

All parameters were estimated only from sixth day onwards due to suppressed growth.

Nett production of the alga was severely suppressed and was 80% less on eighth day. It was 79% less on tenth day but increased sharply thereafter to 15% higher level by twelfth day.

Respiration of the alga was higher than that of control only on sixth day. From 78% higher level on sixth day it declined to 80% lower level by tenth day and increased once again though remained less than that of control by 15%.

No change in pH of the culture was recorded upto second day. Thereafter pH increased gradually till sixth day and declined later towards the end of growth phase.

Pigments: (Fig. II)

Total pigment content of the alga increased when compared to that of control in this combination.

The concentration of chlorophyll a fluctuated during growth phase. Concentration of chlorophyll a and carotenoids was maximum on sixth day and that of chlorophyll b and pheophytin on eighth day. Chlorophyll b was produced to a greater extent than any other pigment. Pheophytin was not detected on sixth day. Chlorophyll a and b have shown an increasing tendency whereas carotenoids and pheophytin decreased towards the end of growth phase.

Photosynthetic end products: (Fig. III)

Carbohydrates was measured from eighth day onwards when their concentration was maximum, being equal to that of control but declined to be 25% less by tenth day and increased once again to 12% higher level by the end of growth phase.

Protein content of the alga was adversely affected and was reduced by 91% in this combination.

Lipid was reduced by 47%.

Growth: (Fig. I)

Growth was measured from sixth day onwards. No growth was observed between eighth and tenth day and for the rest of the period it proceeded at slow pace. Consequently biomass was very low when compared to that of control throughout growth phase.

Selected combination	Nutrients absorbed by the alga (ug/l)		N / P
	Phosphate	Nitrate	
(31)	1020	558(more)	-
Control	945	1290	1.37

Phosphate absorption increased by few ug. The nitrate in the medium was found to increase by 558 ug at the end of growth phase. This was the only combination where the nitrate in the medium increased.

Conclusion:

The combination was highly toxic to the alga. Culture turned yellowish in colour by eighth day instead of green. Also the decrease in pH was notable inspite of sharp increase in rate of nett production towards the end of growth phase. Protein content of the alga was reduced by 91%. All these showed occurrance of severe physiological disturbances in the alga.

Comparison:

When the effect of the present combination of metals was compared with that of combination (21) (Cd+Pb+Cu) the nett production was found to be better towards the end of growth phase. Respiration was reduced. Not only the

pigment content was further reduced but also their development was delayed. But the fluctuation in chlorophyll b level was reduced. Carbohydrate content of the alga increased, but five fold reduction in protein content was observed. Also lipids were reduced by more than 50%. But the biomass increased.

When compared with the effect of combination (22) (Pb + Cd + Zn) both nett production and respiration were found lowered. Chlorophyll a was not affected but chlorophyll b, carotenoids and pheophytin were reduced. Fluctuation in pigment level during the growth phase was not observed except in the case of chlorophyll a. The concentration of carbohydrates and lipid was not affected but severe reduction in protein (13 fold) was observed. Biomass was also reduced.

6.3.10

Combined effect of copper, cadmium, lead and iron on S. bijugatus

Sl.No. of the combination	Concentration of metals in ppm
(32)	0.01 Cd + 0.05 Pb + 0.1 Cu + 0.05 Fe

Production: (Fig. I)

Nett production was found to be very low upto eighth day. 82% reduction was recorded on sixth day. Further fall to 88% lower level was observed by eighth day. But by tenth day it exceeded that of control by 9% and declined once again towards the end of growth phase. On the last day 59% reduction was observed in relation to control.

Respiration of the alga was elevated by 41% on eighth day. From its maximum level on eighth day, it decreased to 80% lower level on tenth and twelfth day.

Consistent with production, pH of the culture in this treatment increased gradually upto sixth day and again from eighth to tenth day. Fall in nett production towards the end of growth phase was accompanied by fall in pH.

Pigments: (Fig. II)

All four pigments were produced to maximum extent during later phase of growth. In this combination concentration of chlorophyll a varied with peaks on sixth and tenth day. Chlorophyll a, chlorophyll b and carotenoids reached maximum concentration on tenth day whereas pheophytin were highest on eighth day. All four pigments exhibited a tendency to decrease towards the end of growth phase. Total pigment content of the alga remained much higher than that of control from sixth day onwards.

Photosynthetic end products: (Fig. III)

The concentration of carbohydrates remained less than that of control throughout the growth phase. It was measured from eighth day onwards. From its maximum on eighth day with 17% reduction, it declined by tenth day and remained steady thereafter. On the last day 20% reduction was observed.

Protein content of the alga increased by 96% whereas that of lipid by 5% in this treatment.

Growth: (Fig.I)

The growth was gradual from sixth day onwards. Throughout growth phase the biomass was less than that of control. The culture when observed on eighth day under the

microscope, some cells were found to be slightly larger and distorted but this abnormality disappeared completely by twelfth day and all cells were normal in their shape.

Selected combination	Nutrients absorbed by the alga (ug/l)		N / P
	Phosphate	Nitrate	
(32)	1300	292	0.23
Control	945	1290	1.37

The phosphate absorption of the alga increased by 38% whereas nitrate absorption decreased to a large extent, by 77%.

Conclusion:

Inspite of increase in protein the biomass remained considerably less than that of control. The combination was particularly undesirable from growth point of view.

Comparison:

When the effect of the present combination was compared with that of combination (21) (Cd + Pb + Cu). The nett production of the alga was found improved. Reduction in respiration, particularly in the latter half of growth phase, was observed. All pigments except pheophytin were reduced. Carbohydrate content of the alga increased. Two fold increase in protein content was observed. Lipid content was lowered by few micrograms. Biomass of the alga improved to a considerable extent.

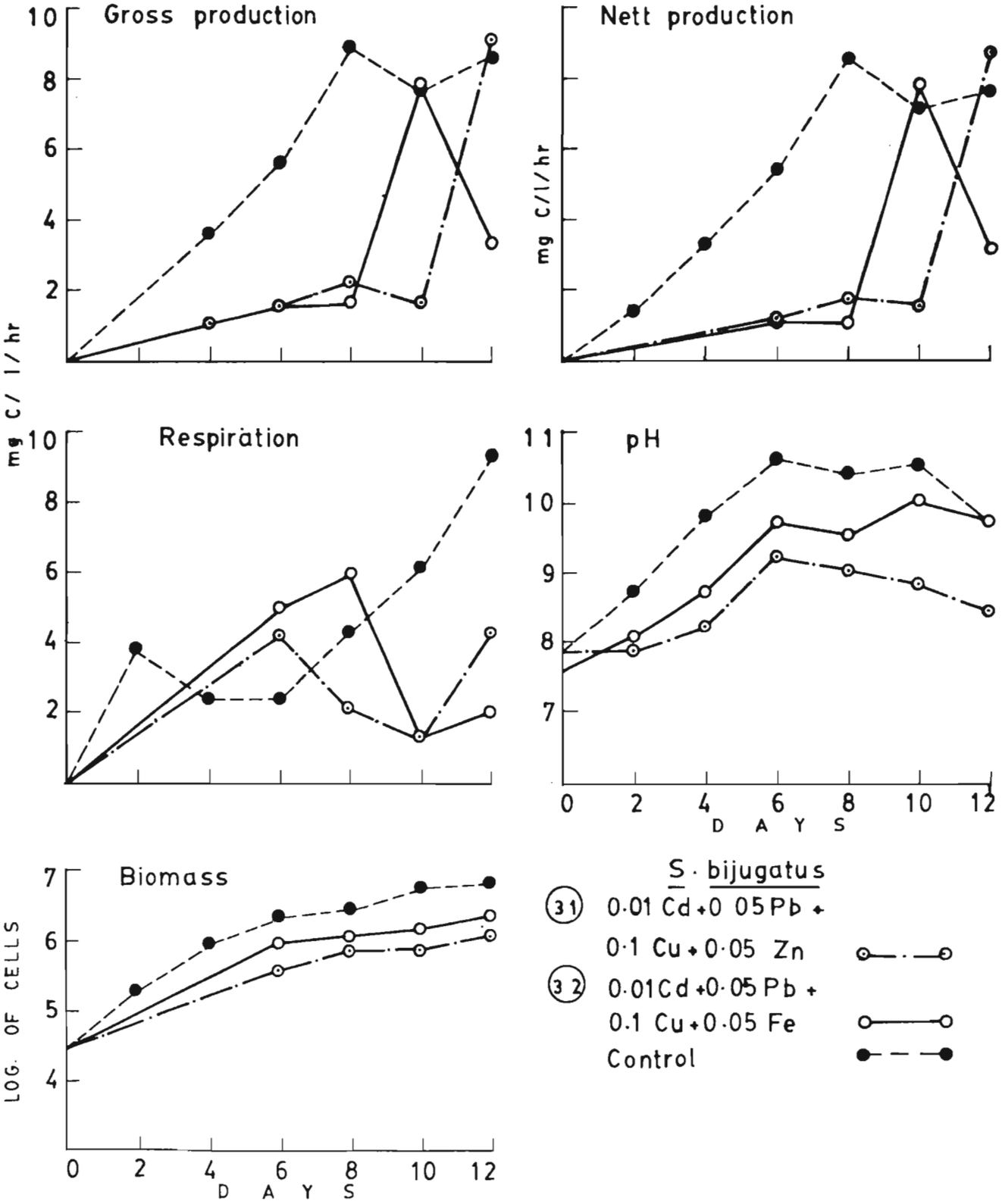

Gross production
Nett production
Respiration
pH
Biomass
mg C/ l/ hr
mg C/l/hr
LOG. OF CELLS
D A Y S
S. bijugatus
(31) 0·01 Cd +0 05Pb +
0·1 Cu + 0·05 Zn
(32) 0·01Cd +0·05 Pb +
0.1 Cu + 0·05 Fe
Control

Fig. I

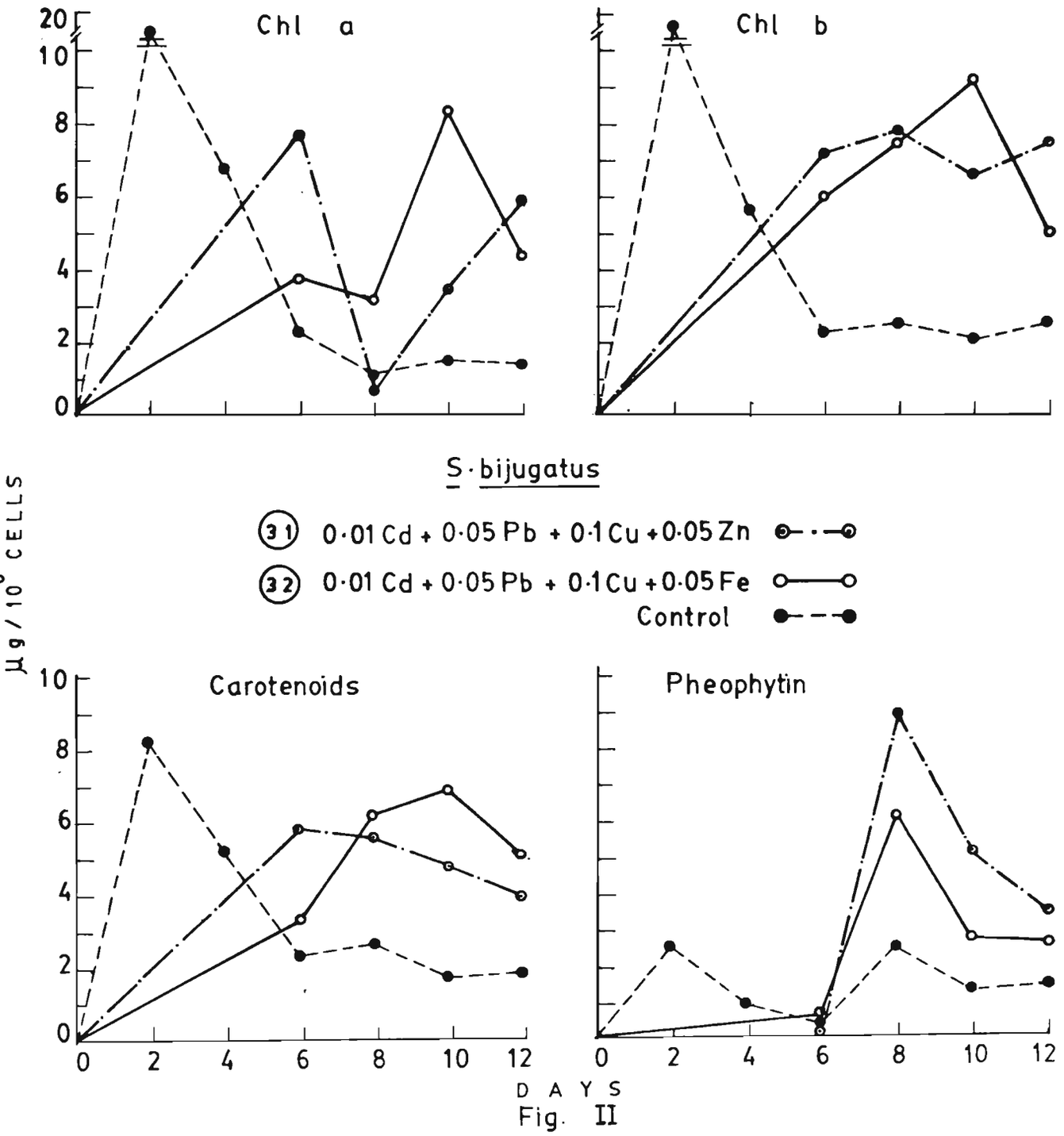
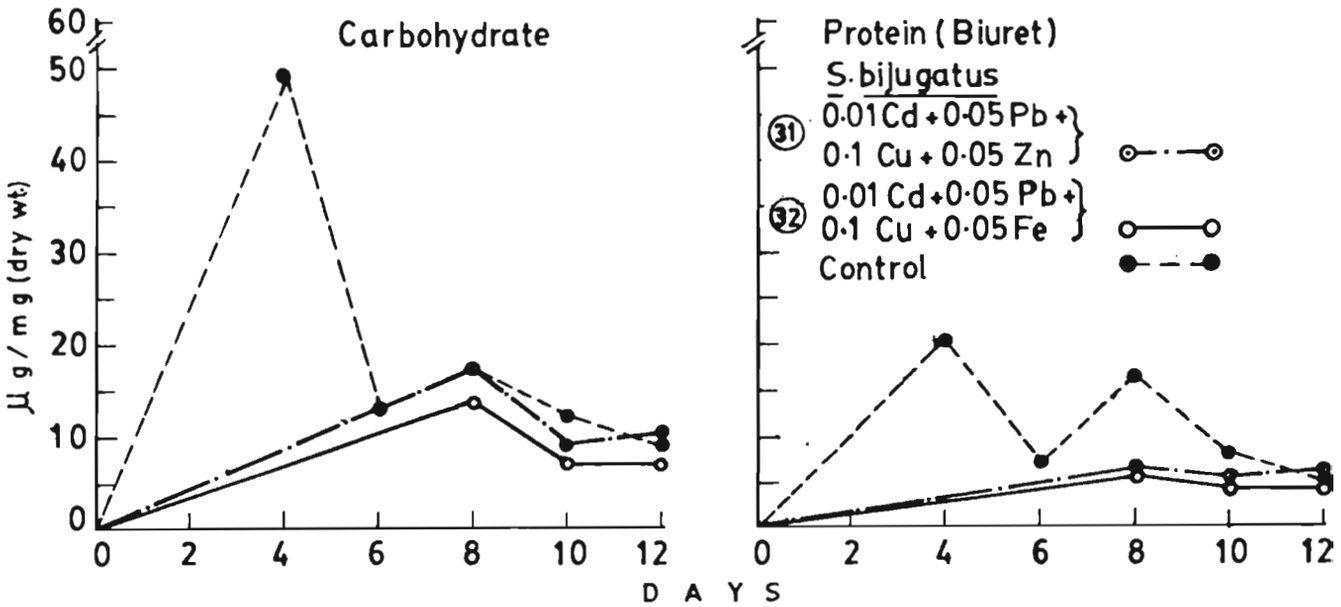

Chl a
Chl b
S·bijugatus
(31) 0·01 Cd + 0·05 Pb + 0·1 Cu + 0·05 Zn
(32) 0·01 Cd + 0·05 Pb + 0·1 Cu + 0·05 Fe
Control
Carotenoids
Pheophytin
µg / 10^6 CELLS
DAYS

Fig. II


Carbohydrate
Protein (Biuret)
S. bijugatus
31 0·01 Cd + 0·05 Pb + 0·1 Cu + 0·05 Zn
32 0·01 Cd + 0·05 Pb + 0·1 Cu + 0·05 Fe
Control
µ g / m g (dry wt.)
60
50
40
30
20
10
0
0 2 4 6 8 10 12
D A Y S

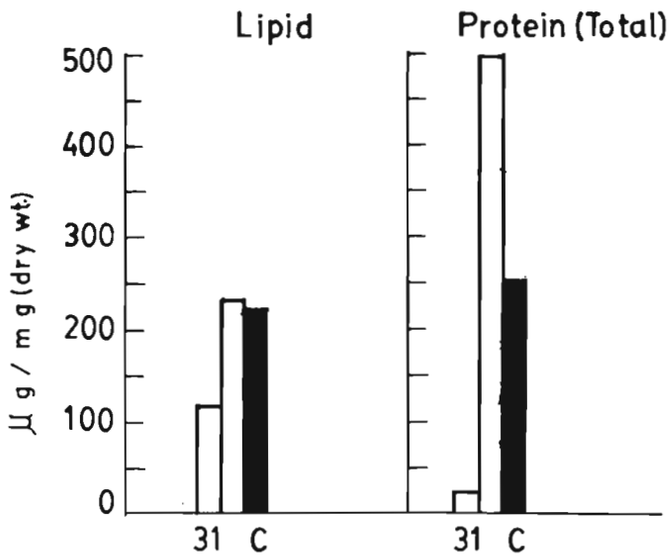

Lipid
Protein (Total)
µ g / m g (dry wt.)
500
400
300
200
100
0
31 C
31 C

Fig. III

Combined effect of cadmium, lead, copper and zinc on *N. palea*

Sl. No. of the combination	Concentration of the metals (in ppm)
(31)	0.02 Cd + 0.04 Pb + 0.05 Cu + 0.05 Zn

This combination of metals was found to be highly toxic to the diatom and hence it was not possible to estimate any of the parameters selected.

Combined effect of cadmium, lead, copper and iron on *N. palea*

Sl. No. of the combination	Concentration of the metals (in ppm)
(32)	0.02 Cd + 0.04 Pb + 0.05 Cu + 0.05 Fe

The combination of metals was found to be highly toxic to the species. Pale brown colour appeared on first day but disappeared by second day. Hence it was not possible to estimate any of the selected parameters.

6.3.11

Combined effect of cadmium, lead, zinc and iron on S. bijugatus

Sl.No. of the combination	Concentration of metals (in ppm)
(33)	0.01 Cd + 0.05 Pb + 0.05 Zn + 0.05 Fe

Production: (Fig. I)

Nett production of the alga fluctuated in the early phase of growth. The production decreased from 121% higher level than that of control on second day, to 83% lower level on fourth day and further to 92% lower level by sixth day. From sixth day onwards production increased, to 54% lower level by eighth day and further to 50% higher level by tenth day. No further change was noted and at the end of growth phase it was 41% more than that of control.

Respiration of the alga also exhibited fluctuation with peaks on second and sixth day and was elevated to 32% and 26% higher level respectively in relation to control. It declined to its minimum on eighth day with 62% reduction and increased thereafter to be equal to that of control by tenth day and further to 92% higher level on twelfth day.

pH of the culture showed a sharp increase from sixth day onwards reflecting increased production and

exceeded that of control by tenth day. The pH in this combination was higher than the maximum (10.64) attained by the control on sixth day. Slight fall in pH was recorded towards the end of growth phase.

Pigments: (Fig. II)

Pigments were estimated from fourth day onwards as the culture had no visible green colour on second day. Total pigment content of the alga remained higher than that of control in the latter phase of growth. The level of chlorophyll a varied with peaks on sixth and tenth day. It did not reach the maximum level attained by alga in control at any stage of growth. Among all pigments chlorophyll b was developed to greatest extent and its concentration remained higher than that of control from fourth day onwards. The level of carotenoids and pheophytin also registered fluctuation during growth phase. All four pigments reached maximum level on sixth day, and exhibited decreasing tendency towards the end of growth phase, except pheophytin which was not detected on eighth and tenth day of growth.

Photosynthetic end products: (Fig. III)

The carbohydrate concentration of the alga remained less than that of control except on sixth day.

From 86% lower level on fourth day it reached 73% higher level by sixth day and decreased to 57% lower level by eighth day and further to 26% lower level by twelfth day.

Protein content of the alga was reduced by 18% and lipid by 50% in this combination.

Growth: (Fig. I)

There was no initial suppression of growth but from second to sixth day it was considerably retarded. Thereafter the alga recovered and multiplied quickly. Neverthless at the end of growth phase the biomass was found to be less than that of control.

Selected combination	Nutrients absorbed by the alga (µg/l)		N / P
	Phosphate	Nitrate	
(33)	1220	1062	0.37
Control	945	1290	1.37

The nutrient uptake of the alga was affected by the metals in combination. Phosphate uptake increased by 29% whereas nitrate uptake was reduced by 18%.

Conclusion:

The combination was found to be toxic. The biomass, protein and lipids were reduced in presence of these metals, inspite of high nett production rate reflected by pH.

Comparison:

When the effect of the present combination of metals on S. bijugatus was compared with that of combination (22) (Cd + Pb + Zn), the nett production was enhanced in early phase but was reduced in the latter phase. Respiration increased to a large extent. Chlorophyll a was not affected, but chlorophyll b, carotenoids and pheophytin were considerably reduced, particularly at the end of growth phase. Carbohydrate and lipid were not affected but protein content was reduced nearly by 50%. Biomass increased.

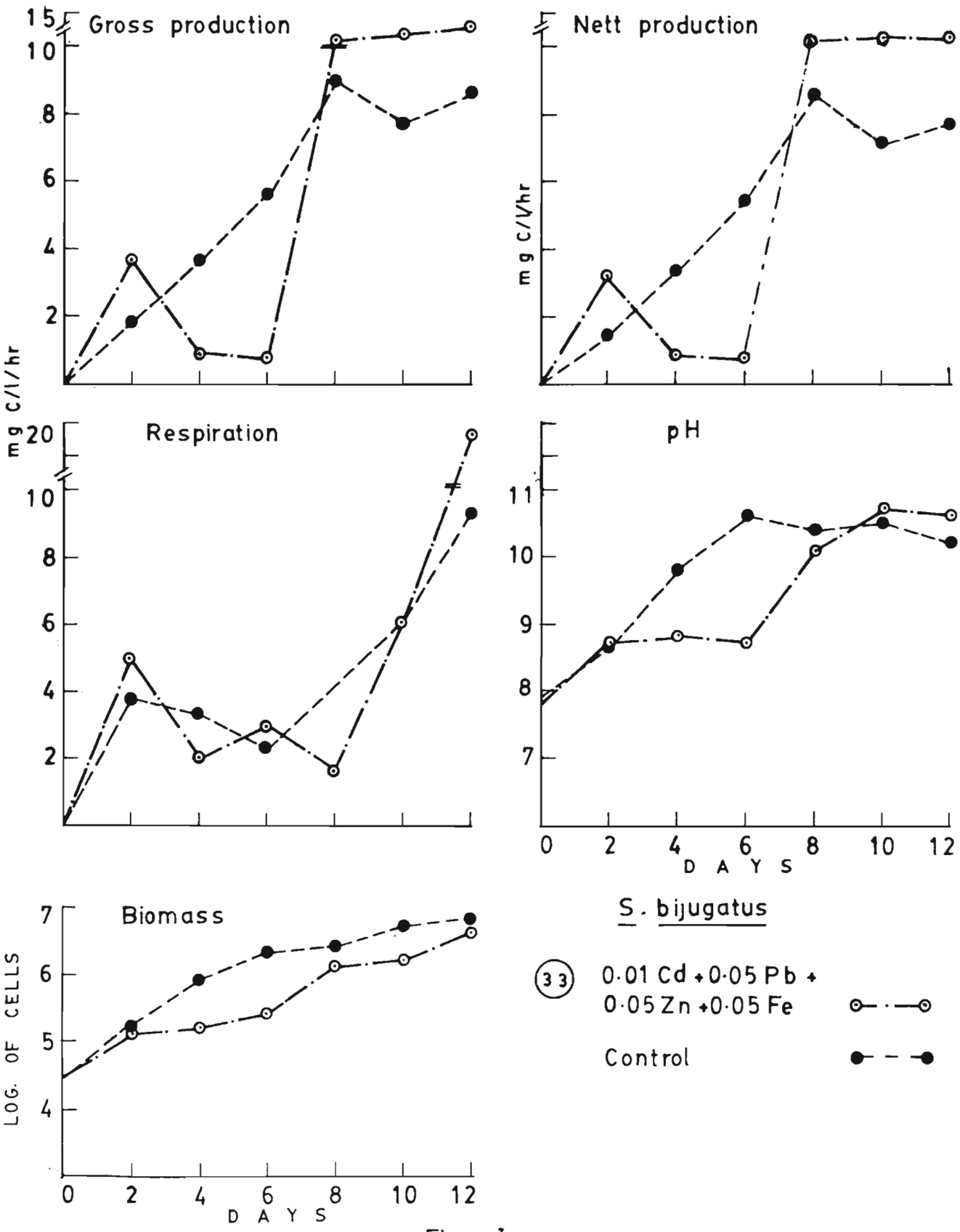

Gross production
Nett production
Respiration
pH
Biomass
mg C/l/hr
LOG. OF CELLS
DAYS
S. bijugatus
(33) 0·01 Cd + 0·05 Pb + 0·05 Zn + 0·05 Fe
Control

Fig. I

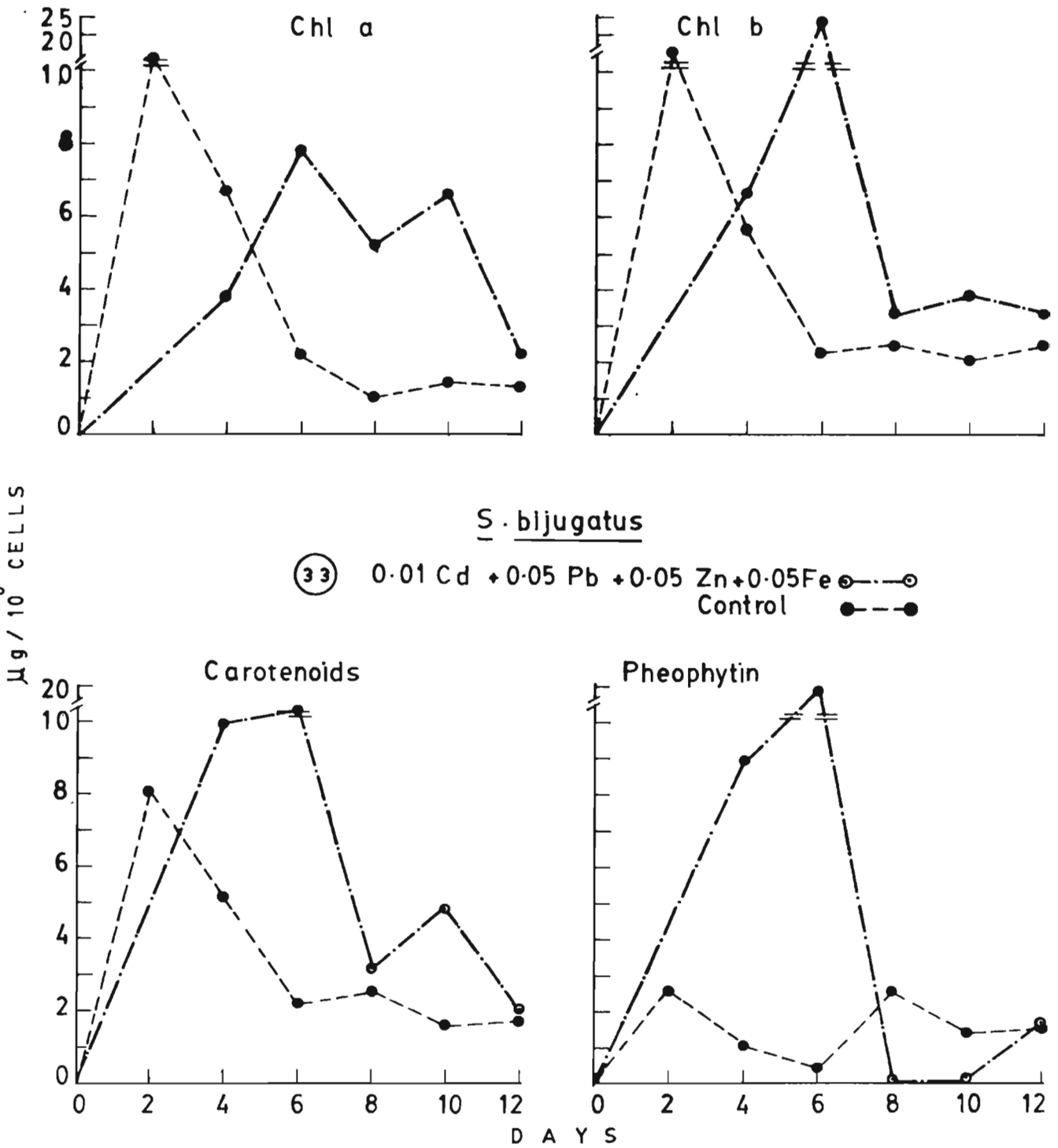
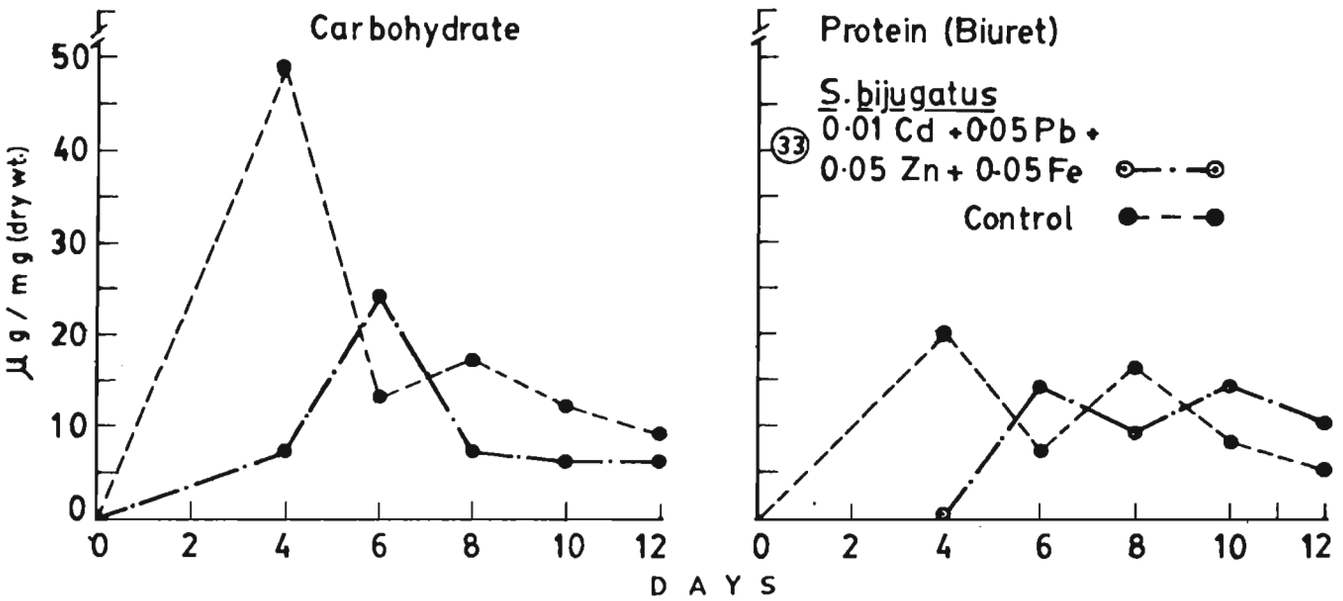

Chl a
Chl b
S. bljugatus
(33) 0·01 Cd + 0·05 Pb + 0·05 Zn + 0·05Fe
Control
Carotenoids
Pheophytin
µg / 10^6 CELLS
DAYS

Fig. II


Carbohydrate
Protein (Biuret)
S. bijugatus
0·01 Cd +0·05 Pb +
0·05 Zn + 0·05 Fe
Control
33
µ g / m g (dry wt.)
0
10
20
30
40
50
0
2
4
6
8
10
12
D A Y S

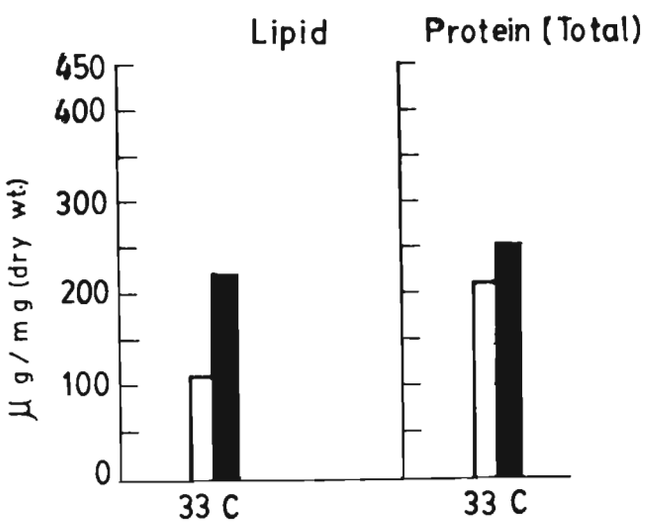

Lipid
Protein (Total)
µ g / m g (dry wt.)
0
100
200
300
400
450
33 C
33 C

Fig. III

Combined effect of cadmium, lead, zinc and iron on *N. palea*

Sl.No. of the combination	Metals in combination (ppm)
(33)	0.02 Cd + 0.04 Pb + 0.05 Zn + 0.05 Fe

The particular combination of metals proved to be highly toxic to the diatom. Hence it was not possible to estimate any of the parameters selected.

Discussion

In the natural environment metals are found along with many other compounds. The concentration of these metals depend on various other environmental factors. The interaction between the phytoplankton and metals varies with the species. Both iron and zinc have been reported to be important for the algal species and they are also known to be limiting factors for the growth of phytoplankton in the environment.

The effect of these two metals along with the selected combinations of other metals varied with the species studied (Fig. VII). S.bijugatus responded positively on more occasions than N.palea. The positive effect in all cases was however restricted to the production of carbohydrate, protein and lipid but not to the biomass.

The combination (23) (mercury, cadmium, lead and zinc) though increased the carbohydrate and protein of S.bijugatus to a large extent, when compared to that of combination (17) (mercury, cadmium and lead Fig.VI), the protein was

less and lipid was more. The addition of zinc to the combination of mercury, cadmium and lead, did not improve the growth of the alga, and the biomass in combination (23) remained far less than that of control.

Overnell (1976) stated that the effect of zinc on photosynthetic apparatus, of two strains of Sketelonema Costatum is small and that, zinc exerts its toxic effect on some part of cell metabolism, remote from photosynthesis, like cell division. Jensen et al., (1974) explained the extreme sensitivity of Skeletonema Costatum to zinc.

When zinc was replaced by iron as in combination (24) (mercury, cadmium, lead and iron) the biomass improved considerably, though not to the same extent as that of control. The cells were normal in the sense the proportion of carbohydrate, protein and lipid was same as those of control. But for the retardation in growth, iron seems to help S.bijugatus to return to near normaly. But both the combinations ((23) and (24)) proved to be highly toxic to N.palea. The addition of either iron or zinc to combination (17) (mercury, cadmium and lead) brought about

further deterioration in the situation, unlike in S.bijugatus.

Considerable recovery from the toxic effect was observed in S.bijugatus when the combination (25) mercury, copper, lead and zinc) was employed when compared to that of combination (19) (mercury, coper and lead), in biomass and all three end products. But when iron was added instead of zinc as in combination (26)(mercury copper, lead and iron), the biomass was further lowered than in combination (19) and also carbohydrate and protein. Zinc was found to be better in mitigating the toxic effect of combination (19) than iron.

On the contrary, zinc, along with mercury copper and lead, (combination (25)) was found to be more toxic to N.palea than iron, (combination (26)). Though improvement was noted in biomass lipid was reduced to a large extent.

The combination (27) (mercury, copper, cadmium and zinc) and combination (28) (mercury, copper, cadmium and iron) were found to help S.bijugatus to recover from the toxic effect of combination (18) (mercury, copper and cadmium)

but iron was found to be better than zinc in combination with mercury, copper and cadmium. When iron was employed, biomass as well as protein increased.

For N.palea, combination (27) and combination (18) were found to be lethal. The addition of zinc to combination (18) (mercury, copper and cadmium) was found to be without any positive effect whereas iron in combination with the above three metals was found to improve the situation to a large extent and made the growth of the diatom possible.

The combined effect of mercury, cadmium, zinc and iron (combination (29)) increased the protein content and lipid of S.bijugatus further than the combination (16) (mercury, cadmium and zinc) but it lowered the biomass. In N.palea also slight reduction in lipid and biomass was noted when combination (29) was employed, compared to combination (16).

Reduction in biomass seems to be common to the test species.

The effect of combination (30)(mercury, lead, zinc and iron) was less toxic on S.bijugatus when compared to that of combination

(20) (mercury, lead and zinc). Protein and lipid content of the species improved with addition of iron whereas the situation did not change in N.palea, where effect of combination (20) and (30) remained nearly the same.

When combination (31) (cadmium, lead copper and zinc) was employed, the situation deteriorated further in S.bijugatus when compared with that of combination (21) (cadmium, lead and copper). Both protein and lipid were brought down with marginal improvement in the biomass. The addition of zinc to combination (21) was undesirable, whereas the addition of iron, as in combination (32) (cadmium lead, copper and iron) improved the situation to a large extent by increasing biomass and protein.

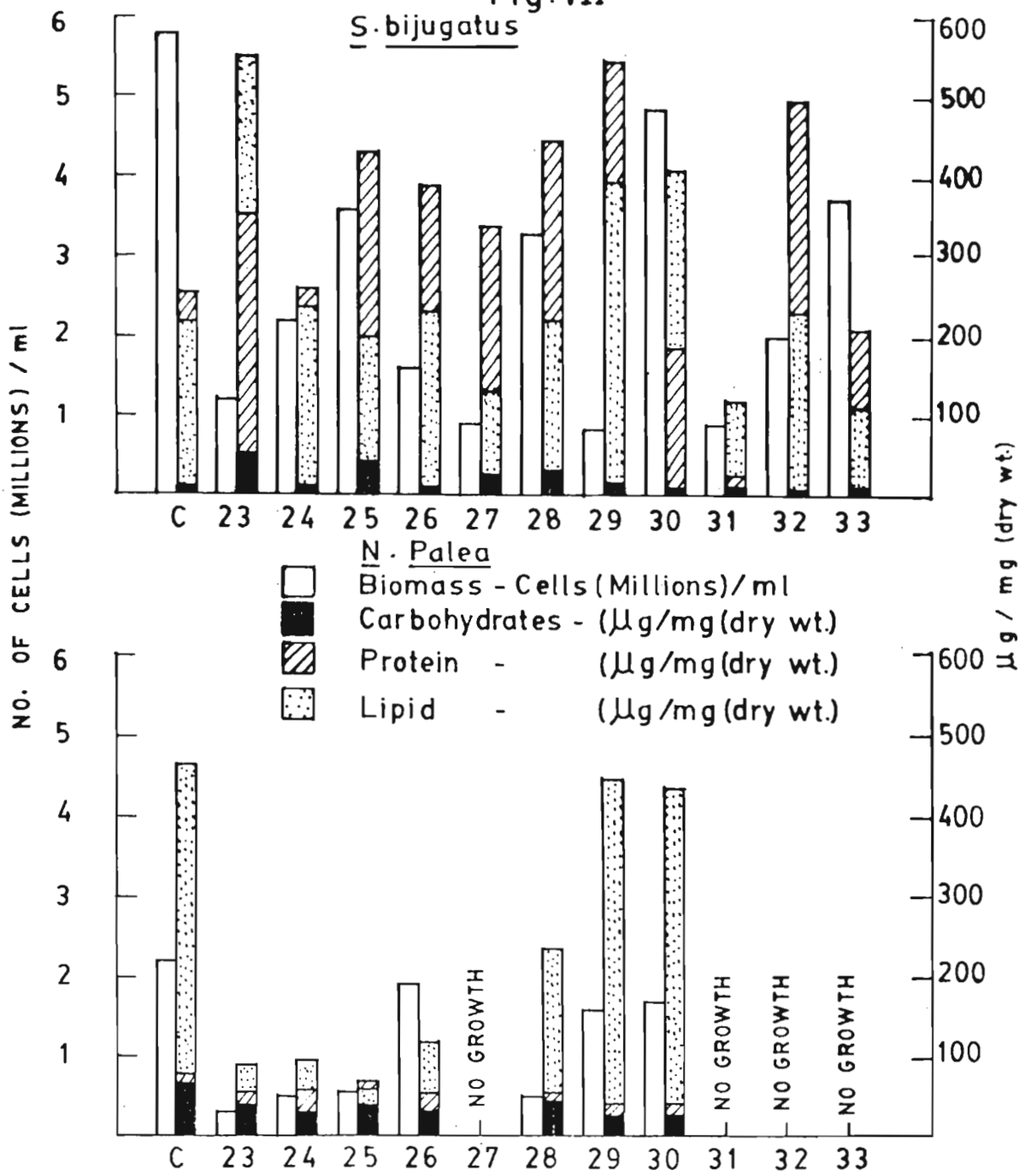
The combinations (31) and (32) were found to be lethal to N.palea along with combination (21). The addition of either zinc or iron did not help the diatom to recover from the lethal effect of combination (21).

Combination (33) (cadmium, lead, zinc and iron) improved the biomass of S.bijugatus to a large extent with slight reduction in protein.

The addition of iron to combination (22) (cadmium, lead and zinc) improved the situation considerably.

The combinations (33) and (22) were found to be lethal to N.palea. Iron was found to be ineffective in nullifying the toxicity of combination (22).

Fig. VII


S. bijugatus
N. Palea
Biomass - Cells (Millions)/ml
Carbohydrates - (µg/mg (dry wt.)
Protein - (µg/mg (dry wt.)
Lipid - (µg/mg (dry wt.)
NO. OF CELLS (MILLIONS) / ml
µg / mg (dry wt.)
C 23 24 25 26 27 28 29 30 31 32 33
NO GROWTH
NO GROWTH
NO GROWTH
NO GROWTH

Statistical analysis of the results was carried out to highlight the effect of the metals in various combinations on the test species.

The variance ratios calculated by the two way analysis of variance technique for the various parameters studied with regard to S.bijugatus (Table XV) were found to be significant in 110 out of 132 cases.

The effect of combination (23) and (24) was found to be significant except on respiration and protein (biuret) at the selected levels. The latter was not affected also during the growth period.

The effect of combination (25) and (26) was not significant on production and lipid at the dose concentrations and on protein (biuret) during the growth period.

The effect of combinations (27) and (28) was found to be significant except on carbohydrate at the tested concentration of metals and on pheophytin during the growth phase.

The effect of combination (29) and (30) was found to be highly significant except on chlorophyll b and protein (biuret) content of the alga.

The effect of combination (31) and (32) was insignificant only on respiration of the alga during the growth period and on chlorophyll a at the experimental concentrations.

The combination (33) on the whole produced least significant effect on the species. At the tested levels, production, respiration, chlorophyll a and protein (biuret) were not significantly affected. Chlorophyll a and protein (biuret) were not affected also during the growth period of the alga.

The effect of selected combinations of metals was of considerable significance in the case of N.palea (table XVI). 95 cases out of 100 studied, were found to be significant.

All the parameters studied were significantly affected by the metal combinations 23 and 24 except alkali soluble carbohydrate fraction of the diatom at the test levels.

The effect of combinations (25) and (26) was significant on respiration of the diatom at the tested concentrations.

The effect of combinations (28) and (29) was found to be totally insignificant on chlorophyll a, but only during growth period on carotenoid content of the diatom.

The effect of combination (30) on the diatom was found to be totally significant.

The combinations (27), (31), (32) and (33) were found to be lethal to the diatom.

TABLE - XV

Significance of the variance ratios in the analysis of variance table for between concentrations and between days.

S. bijugatus

Sl. No.	Metals employed	pH		Gross production		Nett Production		Respiration		Chlorophyll a		Chlorophyll b		Carotenoids		Pheophytin.		Carbohydrate		Protein (Biuret)		Protein Total	Lipid
		I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II		
(23)	Hg+Cd+Pb+Zn	b	c	c	c	c	c	NS	c	b	a	c	a	c	a	c	a	b	a	NS	NS	c	c
(24)	Hg+Cd+Pb+Fe																						
(25)	Hg+Pb+Cu+Zn	c	c	NS	c	NS	c	c	c	c	c	c	c	c	b	c	b	c	c	c	NS	c	NS
(26)	Hg+Pb+Cu+Fe																						
(27)	Hg+Cu+Cd+Zn	c	c	c	b	c	c	a	b	a	c	c	c	c	c	b	NS	NS	c	c	c	c	c
(28)	Hg+Cu+Cd+Fe																						
(29)	Hg+Cd+Zn+Fe	cc	c	c	c	b	c	c	c	c	c	NS	NS	c	a	a	c	a	c	NS	NS	c	c
(30)	Hg+Pb+Zn+Fe																						
(31)	Cd+Pb+Cu+Zn	c	c	a	a	a	a	a	NS	NS	c	a	c	a	b	a	a	c	c	c	a	c	c
(32)	Cd+Pb+Cu+Fe																						
(33)	Cd+Pb+Zn+Fe	b	c	NS	c	NS	c	NS	c	NS	NS	a	c	c	c	b	a	b	b	NS	NS	b	c

NS - Not significant

a - $p < 0.05$
b - $p < 0.01$
c - $p < 0.001$

I - Between concentrations
II - Between days

TABLE - XVI

Significance of the variance ratios in the analysis of variance table for between concentrations and between days.

N. palea

Sl. No.	Metals employed.	pH		Gross production.		Nett production.		Respiration.		Chlorophyll a		Chlorophyll c		Carotenoids.		Pheophytin.		Carbohydrate (Acid soluble)		Carbohydrate (Alkali soluble)		Carbohydrate (Insoluble		Protein		Lipid
		I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	
(23)	Hg+Cd+Pb+Zn	c	c	c	c	c	c	c	c	c	c	b	b	c	b	b	b	a	c	NS	c	c	c	a	c	c
(24)	Hg+Cd+Pb+Fe																									
(25)	Hg+Pb+Cu+Zn	c	c	c	c	c	c	NS	c	c	c	c	c	c	c	a	c	c	c	b	c	c	c	b	c	c
(26)	Hg+Pb+Cu+Fe																									
(27)	Hg+Cu+Cd+Zn	Combination Lethal																								
(28)	Hg+Cu+Cd+Fe	c	c	c	c	c	c	c	c	NS	NS	c	c	c	NS	c	a	c	c	c	c	b	c	c	c	c
(29)	Hg+Cd+Zn+Fe																									
(30)	Hg+Pb+Zn+Fe	c	c	c	b	c	b	c	c	b	a	a	a	c	c	a	c	b	c	a	a	c	c	c	c	a
(31)	Cd+Pb+Cu+Zn	Combination Lethal																								
(32)	Cd+Pb+Cu+Fe																									
(33)	Cd+Pb+Zn+Fe																									

NS - Not significant

a - $p < 0.05$
b - $p < 0.01$
c - $p < 0.001$

I - Between concentrations
II - Between days.

TABLE XVII

The concentration of metals in S. bijugatus at the end of growth phase

Sl. No. of the combination	Metals employed	Concentration (ppm/100 mg dry wt.)			
(23)	Hg + Cd + Pb + Zn	0.014	0.038*	0.036*	0.054
(24)	Hg + Cd + Pb + Fe	0.014	0.034*	0.032*	0.038
(25)	Hg + Pb + Cu + Zn	0.007	0.036*	0.148	0.053
(26)	Hg + Pb + Cu + Fe	0.007	0.034*	0.059	0.036*
(27)	Hg + Cu + Cd + Zn	0.006	0.145	0.036*	0.086
(28)	Hg + Cu + Cd + Fe	0.006	0.131	0.032*	0.036*
(29)	Hg + Cd + Zn + Fe	0.023	0.035*	0.072	0.031*
(30)	Hg + Pb + Zn + Fe	0.012	0.038*	0.020	0.033*
(31)	Cd + Pb + Cu + Zn	0.026*	0.026*	0.044	0.086
(32)	Cd + Pb + Cu + Fe	0.035*	0.036*	0.148	0.042*
(33)	Cd + Pb + Zn + Fe	0.044*	0.038*	0.041	0.043*

The values represented are the average of three observations for mercury and two observations for others.

* Values are less than the indicated concentrations.

TABLE XVIII

The concentration of metals in N. palea at the end of growth phase

Sl. No. of the combination	Metals employed	Concentration (ppm/100 mg dry wt.)			
(23)	Hg + Cd + Pb + Zn	0.003	0.029*	0.026*	0.027
(24)	Hg + Cd + Pb + Fe	0.004	0.028*	0.032*	0.08
(25)	Hg + Cu + Pb + Zn	0.002	0.035*	0.037*	0.039
(26)	Hg + Cu + Pb + Fe	0.005	0.029*	0.025*	0.12
(27)	Hg + Cu + Cd + Zn	Combination lethal.			
(28)	Hg + Cd + Cu + Fe	0.012	0.027*	0.029*	0.045
(29)	Hg + Cd + Zn + Fe	0.002	0.031*	0.039	0.044
(30)	Hg + Pb + Zn + Fe	0.002	0.027*	0.051	0.043
(31)	Cd + Pb + Cu + Zn	Combination lethal.			
(32)	Cd + Pb + Cu + Fe	Combination lethal.			
(33)	Cd + Pb + Zn + Fe	Combination lethal.			

The values represented are the average of three observations for mercury and two observations for others.

* Values are less than the indicated concentrations.

GENERAL DISCUSSION

The choice of conditions, methods and parameters engaged in toxicity assays involving metals and phytoplankton is difficult because each one of them suffers from a kind of draw back or the other. The picture of interaction of metals and phytoplankton becomes more comprehensive as the choice of parameters is increased. While growth is the most obvious parameter, the potential of the media to support growth is known to vary. The growth of S. bijugatus was found satisfactory without the addition of nutrients, chelators or vitamins to the medium, whereas the addition of nutrients to the medium was necessary to initiate and support the growth of N. palea. The addition of chelators was omitted because natural media were used to grow the test species. Also, chelators if added, may swamp the toxic effect of small quantity of metals added. Barber (1973) has reported that addition of EDTA became necessary because of the use of ultraviolate radiation to decompose organic matter in natural sea water which reduced the capacity of the water to support growth of phytoplankton. It is also known that metal toxicity is intimately connected with nutrient level of the medium (Hannan and Patouillet, 1972). The addition of chelators impairs the availability of nutrients to the plants.

Another unavoidable complication in growth experiments of phytoplankton is that the algae liberate products that are capable of forming complexes with metals. The algae are known to release considerable proportion of photosynthetic carbon into the medium in the form of organic complexes. 7% to 38% at the end of bloom (Hellebust, 1965), upto 38% (Horne et al., 1969), upto 30% (Mcknight et al., 1978; Swallow et al., 1978), upto 49% (Anderson and Zeutschel, 1970). Also these complexes are known to be liberated in greater quantities during the stationary phase. It is also not known whether phytoplankton in natural waters has a comparable stationary phase as found in cultures. Hence the effect of the metals was studied in the exponential growth phase of the test species. The results obtained with cultures of algae cannot be directly attributed to those in natural waters. But, a full understanding of the growth of the organisms in their natural habitat can be achieved only by the synthesis of the results on physiological and biochemical investigations under varying laboratory conditions.

It is well documented that assays based on growth and photosynthesis are complementary. The measurement of photosynthesis by oxygen technique seems to be preferable to C^{14} technique (Overnell 1976; Shulenberger and Reid, 1981). Since the problem of self adsorption

with tracer elements other than zinc was reported (Bachmann and Odum, 1960), the production was estimated employing both the techniques in untreated samples of S. bijugatus. The nettproduction estimated using oxygen technique was found to be 0.489 mg C/l/hr where as by C^{14} technique was found to be 0.133 mg C/l/hr (calculated as per Doty and Oguri, 1958), oxygen technique was found to be superior to C^{14} technique and hence was employed in the present study. The difference obtained in production by the above methods could be attributed to the release of extracellular products, during filteration.

The study of respiration was carried out along with photosynthesis to have a clear picture since as a catabolic process, respiration is known to be elevated in organisms in response to stress. In the present study, the selected metals were found to elevate the respiration of the test species. The increase in photosynthetic rate was not reflected in the increase in carbohydrate, protein or lipid in majority of the cases, which may be explained by elevation in respiration (which is a continuous process whereas photosynthesis is light-limited one) along with the loss of photosynthetic carbon from the cells. It is found that the rise in production unaccompanied by increase in biomass, protein and lipid cannot be considered as indicator parameter in the metal toxicity assays. The

dissolved organic compounds in the medium containing various metabolites are known to vary with time (Provasoli, 1963). This may be responsible for the higher oxygen content in dark bottle compared with the initial bottle as found in few instances during the present study.

The major photosynthetic pigment chlorophyll a, accessory pigments chlorophyll b, chlorophyll c, carotenoids and the degradation product, pheophytin were developed to a greater extent when the test species were exposed to metals except in the case of S. bijugatus in combination (20) (mercury, lead and zinc) and N. palea in combination (7) (mercury and lead) (16) (mercury, cadmium and zinc) and (17) (mercury, cadmium and lead).

Meeks (1974) has reported that S.obliquus synthesizes chlorophyll at a constant rate in the light, upto dark, in light-dark synchronized cells. But increase in the pigment content exceeding that of control may be explained as a consequence of increased carbon flow into the non-nitrogen containing products, a phenomenon known to occur when the cultures were light-limited or nitrogen-limited (Harding et. al., 1985), since metals are known to interfere with the enzymes, protein synthesis and growth. (For mercury, Benesch and Benesch 1952; Eichorn 1974; for cadmium, Gerhards and Weller 1977; for zinc, Mills . 1976; for copper, Silverberg et al., 1976). Wright (1960)

has reported that the relationship between chlorophyll and photosynthesis is known to vary inversely in natural water where nett photosynthesis is maximum when chlorophyll concentration was at an intermediate range and less when chlorophyll concentration was too high or too low. Increase in pigment content should be considered as an undesirable response which complicates the productivity assessment in polluted waters. The increase in carotenoids may be due to the above mentioned reason or due to their roll of protecting chlorophyll from photo oxidation (Bogorad, 1974). Besides the reason mentioned above, the increase in pigments may be due to the increased energy requirement of the cells to withstand the 'stress' caused by the metals. Pheophytin is best measured using fluorescence. The increase in pheophytin, as observed in the present study using Spectrophotometer, cannot be explained, as the difference in technique is known to cause considerable variation in estimation (Yentsch and Menzel 1963).

The photosynthetic end products, carbohydrate protein and lipid as estimated in the present study reflected the imbalance caused in development and physiology of the species by the selected metals and their combinations. The biomass and the end products represented as the quantitative and qualitative aspects in the present study revealed that the effect of these pollutants is total on the phytoplankton (Figs. IV, V, VI and VII).

The alteration in the proportion of carbohydrate and lipid may be the indirect effect of the metal, since metals are known to affect particularly enzymes and proteins adversely. As stated elsewhere, the increased availability of carbon, not utilised for building up protein, may be contributing to the lipid fraction, a non-nitrogen product. The carbohydrate build up may also be due to the same reason, since in healthy cells photosynthetic carbon is known to be quickly used up in building protein (Fogg, 1956).

When either iron or zinc was employed in combination with other metals, the reversal of the above mentioned situation has taken place (Biomass increased and lipid unaffected in few cases. In few others the increase in protein was not reflected on the biomass. Even when the metals iron and zinc were employed, the recovery seems to be only partial. The metal action explained as antagonistic or synergistic, projected only part of the picture since both were limited to one or two parameters studied.

There were instances, as seen in combination (23) (Hg + Cd + Pb + Zn) applied to S. bijugatus, where increase in lipid was accompanied by rounding off of the cell which may be comparable to "Tying up" of unfavourable conditions. This calls for a detailed investigation in this direction.

Except in few instances, a clearly extented lag phase was not observed in the growth of the test species. In fact many combinations caused initial stimulation in growth. In others it was stimulated at a later stage. Not withstanding the initial stimulation, the biomass of both the species was found to be lowered at the end of growth phase. The present study revealed that the lag phase was not necessarily limited to initial stages. Even if initial stimulation prevailed continued growth was not found to occur. The abnormalities such as cells sticking to the flask or forming mat-like floats, thickening of the cell walls, changing shape of cells (round and not elliptical as healthy cells) were noted.

The uptake of phosphate and nitrate though was found to be affected, did not have any direct bearing on the amount of end products. Hence the uptake may be considered as a luxurient one since phytoplankters are known to reserve both phosphates and nitrates. Also it is reported that nitrogen compounds other than amino acids are present in appreciable amounts in algae (Parsons et al., 1961).

The toxic effect was not observed immediately after the addition of the toxicant in majority of the instances. The combinations of metals were found to have complex effect on the growth and physiology of the test

species. Total imbalance in the physiology of the test species is therefore ascribable to the toxicity of the metals selected and studied. Prediction of the combined effect of metals cannot be made on the basis of information gathered from their individual effects.

Protein, carbohydrate and fat in 4:3:1 ratio is known to be suitable for zooplankton nutrition (Parsons et al., 1961). The shift in the ratio of these macromolecules may affect the productivity of the waters adversely.

8

SUMMARY

The scope and purpose of the subject were included in the introduction. The methods followed in the present investigation were described. A review of the literature concerning the metals, phytoplankton and their interaction was presented.

The selected species Scenedesmus bijugatus and Nitzschia palea were described. Their growth parameters in culture were studied with special reference to their physiology. Production and respiration of the species, pigment content, photosynthetic end products such as carbohydrate, protein and lipid and biomass in terms of cell numbers, were the parameters chosen for the present study. pH of the culture was also studied in connection with production.

Mercury, cadmium, lead, copper and zinc were selected as they are known to enter the aquatic system of Kerala through the effluents discharged by various industries located in the vicinity. The interaction of metals singly, and in combinations of either two, three or four metals on the test species was investigated.

Results of the toxicity studies of the metals on S. bijugatus and N. palea from both qualitative

and quantitative aspects were reported and discussed.

The data were statistically treated using analysis of variance to find out the significance of metal interaction on the species and results were reported.

The concentration of metals accumulated by the test species during the exponential growth phase was determined and presented in a table.

The rate of production of the test species during the exponential growth phase was found to vary considerably in presence of the metals. In a few cases the rate of production showed no direct bearing on the carbohydrate, protein and lipid content of the species. In some cases the pH of the culture also had no direct relation.

Respiration of the test species also varied with metals and their combinations.

The pigments chlorophyll a, chlorophyll b, chlorophyll c, carotenoids and pheophytin were found to increase when the species was exposed to the metals except in two cases.

Considerable variation in the concentration and proportion of carbohydrate protein and lipid of the test organisms was observed during the growth phase, when exposed to the metals.

The addition of the metals to the medium affected the growth of the species considerably and in majority of the cases biomass was reduced. It was found that the lag in growth was not restricted to the initial phase. Even when stimulation of growth occurred in early phase in a few cases, as the biomass was less than that of control, the toxic effect of metals was evident by the end of growth phase.

Nutrient uptake was found to vary but had no direct effect on the physiology of the alga. But it was found that, wher the biomass was considerably reduced, the nutrient uptake was lowered.

The results indicated that metals were either antagonistic or synergestic in action from quantitative or from qualitative aspect or in a few cases both. The positive or the negative effect of metals was evident in different parameters under different conditions and hence the effect on a single parameter cannot be considered as conclusive in the metal toxicity studies.

The effect of metals on the physiology of the species,when accumulated even in very low concentrations, was undesirable.

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