STUDIES ON THE EFFECT OF SELECTED METALS ON THE PRODUCTIVITY OF TWO SPECIES OF ALGAE

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by

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CERTIFICATE

This is to certify that the thesis entitled **"STUDIES ON THE EFFECT OF SELECTED METALS ON THE PRODUCTIVITY OF TWO SPECIES OF ALGAE"** is the bonafide record of the work carried out by Smt. Manju. M.R. under my supervision and guidance in the School of Environmental Studies for the Ph.D. Degree of the Cochin University of Science and Technology and no part of this has been presented for the award of any other degree in any University.

Cochin - 682 016,
25th September, 1991

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DECLARATION

I hereby declare that this thesis entitled **"STUDIES ON THE EFFECT OF SELECTED METALS ON THE PRODUCTIVITY OF TWO SPECIES OF ALGAE"** has not previously formed the basis of the award of any degree, diploma, associateship, fellowship or other similar titles for recognition.

Cochin - 682 016,
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PREFACE

In the context of technological development in recent years, materials which are released into surface waters in various physico-chemical forms and quantities disrupt ecological relationships and cause toxicity resulting health hazards. Industries consume large quantities of water for production, but only a small fraction of it is present in the product, the rest being turned into waste water on account of different operations like cooling, washing, extraction, chemical treatments and such operations. The waste released into surface water contains enormous quantities of organic and inorganic constituents.

Several catastrophic instances caused by metal poisoning notably the massive mercury poisoning of human beings at Minamata Bay in Japan, have drawn the attention of Scientists, Governments and Public alike which pressurised them to assess the impact of toxic metals in natural waters. The discharge of trade and municipal effluents are on the increase day by day which have accelerated the biochemical cycling of many elements including heavy metals. The need to study the dynamic relationship between metals and the growth of aquatic biota, and also the effects of trace metals in different ecosystems have therefore become imminent.

The major reason for the sensitivity of aquatic systems to pollution influence is due to the structure of their food chains. In comparison with land systems, the relatively small biomass in aquatic environment generally occurs in a greater variety of trophic levels whereby accumulation of xenobiotic and poisonous substances is enhanced.

It is obvious that toxicity and the fate of water borne metal contaminants is dependent on chemical form and that the quantification of these forms could be more meaningful than measurement of total metal concentration. A potential for biomagnification of heavy metals in the food chain exists with high concentration in the primary producers. Phytoplankton lead to concentrate heavy metals to a large extent and they form the key components in the biogeochemical cycling of elements. In general the microscopic algae are considered as good monitors for chemical speciation, since they are relatively easy to maintain and are susceptible to low concentration of metal ions.

Scenedesmus abundans and Nitzschia clausii will be of interest in aquaculture. An insight into the dynamics of growth of the species and their response to heavy metal pollution will be of concern to ecologists and conservationalists as well as to law makers to set suitable criteria and standards for the effluents which are let out into inland waters, lakes and coastal environment. This can be achieved by suggesting the threshold value for the selected metals beyond which they become highly toxic, affecting the ecosystem seriously.

It is hoped that the results and conclusions drawn from the investigations described in the thesis will be of considerable help in the development of mass cultures of algae as live food in hatchery systems as well as in the pollution control of estuarine and near shore environments.

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C O N T E N T S

CHAPTER - I

INTRODUCTION

INTRODUCTION

Natural ecosystems normally have dynamic equilibrium. A small change in the abiotic and biotic components may lead to imbalances, but if these changes are within the carrying capacity of the system and of a shorter duration, the adverse effect of such a change will not be seriously reflected in the habitat. The scientific and technological advancements in recent years, in the wake of economic developments have affected the ecosystems drastically and the changes have survived for longer periods. The urge for never-ending attempts for economic development has resulted in consumption of more and more resources and generation of by-products which become wastes.

The immeasurable municipal and trade effluents reaching the aquatic system change the character of the abiotic system which affect the well being of the biotic community. In some cases, these changes may tie up with good results, but in most cases they are deliterious in nature.

The unabated and uncontrolled discharge of wastes into both fresh water and marine habitats has become a serious environmental problem all over the world. The chemical industries, mainly fertilizers and pulp factories, require large quantities of fresh water for their processing. To have access to large volume of fresh water these factories are established along the river banks. Kerala has rich potable and fishable water resources contributed by forty four monsoon-fed small rivers, with their tributeries and backwaters including lakes and adjoining water bodies.

The Cochin backwater system is one of the biggest of its kind in Kerala, which opens out to the Lakshadweep sea. This backwaters is one of the most

productive estuarine systems with an estimated annual gross production of nearly 300 gC/m^2 (Qasim et al., 1969).

The availability of fresh water in large quantities has attracted a large number of chemical industries to this area, thus making it the industrial belt of Kerala. But this also has introduced a lot of environmental problems to the State which otherwise was promoting luxurient vegetation and uninterrupted agricultural operations.

It is well documented that trace quantities of certain chemical elements exert a positive influence on the plants, animal and human life. In the case of aquatic biota, algae are the most important components of aquatic ecosystem. They account for about 60% of annual carbon dioxide fixation. They form the basis of aquatic food webs (Odum, 1959). The essential nutrients for the growth and reproduction of phytoplankton are obtained from the aquatic environment. The nutrients are classified on their quantitative requirements as macronutrients such as phosphate, nitrate and silicate and micronutrients or trace elements such as copper, zinc, molybdenum, vanadium, tin, iron, nickel and cobalt. The term "trace elements" is loosely used in the current literature to designate the elements which occur in small concentration in natural biological system (Forstner and Wittmann, 1979). The growing public concern over the deteriorating quality of the environment has led to a generalised usage when refering to trace elements. Thus for practical purposes other terms such as trace elements, trace inorganics, heavy metals and micronutrients will be treated as synonyms with the term trace elements as suggested by Forstner and Wittmann (1979).

Of these trace elements some are found to be essential for the better growth of phytoplankton and its absence will produce deficiencies in the organism. Eventhough they are essential for growth, a very small increase in amount or over supply may have toxic effect on a variety of organisms.

Certain elements such as silver, mercury, cadmium, lead and chromium are non-essential for the aquatic biota. They produce toxic effect even in very small quantities and the toxicity is a function of the chemical form. Thus metals causing environmental pollution fall under two categories - essential and non-essential elements.

The toxicity studies show that concentration needed to inhibit growth and metabolic processes such as photosynthesis varies widely and depends on factors such as chelation, pH, concentration of cells, nutrients, physiological state of cells, salinity and temperature. The direct interaction between a chemical element and biota is of interest and receives appreciable attention.

Metal contaminants are introduced mainly through direct discharge of municipal and industrial wastes into the sea or through river input. Although heavy metal levels in the open sea are usually low, high concentration may occur in coastal waters and estuaries close to effluent discharge sites.

Absorption of heavy metals is of great importance because algae constitute a primary link in food chains and can contaminate the organisms that depend directly or indirectly on them. Although the behaviour of algae in laboratory cultures can be slightly different from that of natural phytoplankton, cultures can normally serve as good models. It is also found that biological

particles can influence the distribution of heavy metals in natural water, because the functional groups on the cell surfaces are able to keep bound metal ions. The surface of algal cells have a high affinity for metal ions (Han-Bin-Xue and Wernerstumn, 1988).

In general these microscopic algae are considered as good monitors for use in testing chemical speciation since they are easy to keep and are susceptible to low concentration of metal ions.

Axenic cultures of several algae are being developed and maintained in many laboratories of the world. Many species are economically important from their application as live feed in hatcheries and culture systems. Besides, several species are used for algal assays and also test species for monitoring the effect of various pollutants. Cultures are used to study many basic processes relating to photosynthesis, growth, algal phylogeny and biomass estimation. Experiments with algal cultures are providing clues to important ecological processes such as succession pattern, mechanism of phytoplankton blooms and many other phenomenon of basic importance to plant productivity.

The present study is based on the effect of metals such as cobalt, nickel, trivalent and hexavalent chromium on two selected species of phytoplankton.

Major sources of nickel pollution in aquatic environment are the wastes from the manufacture of alloys, alkaline storage batteries, commercial fertilizers and use of sewage sludge as land-fill material. In Cochin backwaters the copper-nickel sheathing of ships to prevent the bio-fouling of wooden ship hulls wasfound to be an important source of nickel in the aquatic environment.

Cobalt, eventhough a micronutrient and an enzyme activator, is toxic at higher level. Jenkins (1980) had concluded that cobalt is one of the fourteen toxic trace elements of critical importance from the point of view of environmental pollution and health hazards. Sources of cobalt include electromagnetic wastes, paints, chemicals, sewage and industrial wastes. Atomic power plants form an important source of radio isotope of this metal to the environment.

Chromium is an important constituent of industrial and domestic wastes. Yet there exists little information regarding its effects on natural fresh water, estuarine or marine phytoplankton. Major sources of chromium are the electroplating wastes, factory wastes, industrial and textile dyes and laundry wastes which have high amounts of chromium. Textile and leather tanning industry is the major source of hexavalent chromium pollution. In the chrome alum wastes, the chromium exists in trivalent state. In the case of toxicity also, chromium varies on the valency state. The speciation studies are aimed at understanding the metal interaction in aquatic ecosystem. Dissolved chromium occurs as trivalent or hexavalent with the latter normally predominating. The hexavalent form seems to be the most toxic and toxicity varies from species to species. Thus in both species selected for study, there was marked difference in toxicity range.

All these metals, namely cobalt, nickel, trivalent and hexavalent chromium were found to have profound influence on the physiology of phytoplankton, the primary producers in the aquatic life. Works on different types of ecosystem have shown that none of the parameters like cell volume, cell number or chlorophyll individually gives a true picture of the effect of these heavy

metals on the phytoplankton. So different parameters like temperature, salinity, pH, nutrients, number of cells, photosynthetic pigments and photosynthetic end products were studied to get a clear picture of the metal phytoplankton interaction. It has been found that toxicity and fate of water borne contaminants were dependent on chemical forms and that quantifying of these forms would be meaningful than the measurement of the total metal concentration.

Metals are not usually found in the natural waters individually but in combination with many other metals. So it is necessary to find the interaction of metals in combination leading to the phenomenon of synergism and antagonism.

The thesis discusses the influence of metals such as nickel, cobalt, trivalent and hexavalent chromium on the test species primarily from the toxicological point of view, since these species will be of interest in aquaculture. Thus the specific objectives of the study are to compare the effects of selected individually added trace metals on the algal assays, to determine the possible interactive effects of a major essential element in combination with a toxic heavy metal on the algal growth potential and also to determine the accumulation of metals and its effects on food chains resulting in biomagnification.

The results analysed statistically show that there was variation in the effect of different metals on the two species. It was also found that two test species showed marked difference in its toxic effect for the same metal.

The thesis has been divided into seven chapters. The introductory chapter explains the relevance of the present investigation. Chapter two presents

the review of literature based on the work in relation to toxicity. Third chapter gives a detailed description of the material and specialised methods followed for the study. The effects of various metals selected for study - nickel, cobalt, trivalent and hexavalent chromium on the qualitative and quantitative aspects of productivity forms the subject of matter of the fourth chapter. The fifth chapter gives the impact of metals in combination on two species of algae. A general discussion and summary are included in the sixth and seventh chapters.

CHAPTER - II

HISTORIC RESUME

HISTORIC RESUME

The problem of water pollution is becoming severe day by day mainly because of the ultimate discharge of raw or partially treated industrial wastes and effluents into the aquatic ecosystem. Untreated industrial effluents have high toxic effect on the growth of various algae and it is of great importance because algae constitute the primary link in food chains and can contaminate the organisms that depend directly or indirectly on them.

Chu (1942) was a pioneer among those who set out a media having resemblance to those in which algae flourished naturally. Pringsheim (1964) also proposed various culture media such as Miquel's media, Knop's modified media and Molisch media. In these synthetic media all necessary macronutrients and some essential trace elements were found in the form of carbonates, sulphates, nitrates, chlorides and phosphates.

Nutrients play an important role in the diversity of phytoplankton in oceans (Hecky and Kilham 1988). Culture studies have established that internal cellular concentration of nutrients determine phytoplankton growth rates and the dissolved nutrient concentration in the medium was also useful in determining nutrient loading rates of aquatic ecosystem. The relative proportion of nutrient supplied to phytoplankton can be a selective force shaping phytoplankton communities and affecting the biomass yield per unit of limited nutrient.

In the different habitats such as marine and fresh water life there was marked difference in the nutrient uptake, storage capacity and growth of phytoplankton (Hecky and Kilham 1988). Phosphate plays significant role

in controlling the fertility of sea water. It occurs in the form of dissolved and particulate inorganic phosphate and as dissolved and particulate organic phosphate of biological origin (Borkar, 1976).

Studies of Piorreck et al. (1984) on two green algae such as Chlorella vulgaris and Scenedesmus obliqus and four blue green algae have shown that in all the selected algae increase in nitrate level led to an increase in the biomass, protein content and chlorophyll. But in the case of lipids there is marked decrease in dry weight at high nitrate level (Piorreck and Pohl, 1984). The influence of phosphate on the growth of phytoplankton was studied by Veldhuis and Admiraal (1987) and Mostert and Grobbelaar (1987).

Diatoms are credited as a major contribution to the primary production in the oceans. They are important basis for marine life. The difference in sinking rates between diatoms from marine and fresh water environments might contribute to the variation in silica content between marine and fresh water diatom (Titman and Kilham, 1976). Silicate plays an important part in the growth and photosynthesis of diatom (Malone et al., 1980). Blank et al. (1986) on the studies of diatom Navicula saprophilia it was observed that timing of protein synthesis and metabolic requirements, silicate in the form of silicic acid was added. Silicon deficiency also results in the depletion on lipid composition and metabolism in the diatom Cyclotella cryptica (Roessier, 1986). The importance of silica on the growth of diatom was studied by various authors like Guillard et al. (1973), Linda et al. (1984). Like the phosphates, nitrates and silicates, other macronutrients such as carbonates, sulphates and chlorides play an important role in the growth of phytoplankton.

Similar to the nutrients the environmental factors such as light, temperature and pH play an important role on the growth and toxicity of phytoplankton. Temperature and dissolved oxygen content are vital factors for aquatic organisms. It was found that when the temperature was increased there was simultaneous decrease in production due to thermal stress. (Bienfang and Johnson, 1980). Huisman et al. (1980) have shown that with the increase in temperature there was increase in toxicity of mercury resulting in the elevated respiratory activity in Scenedesmus acutus. Geider (1987) reported that chlorophyll-a increases linearly with increased light level at constant temperature. According to Kanykowski (1987) there was an inverse relationship between temperature and plant nutrients. Nutrients tend to increase with depth and temperature tend to decrease with depth.

Goldman (1977) reported that temperature has a strong influence on the chemical composition of marine phytoplankton. Cell division and nutrient uptake rates were uncoupled with respect to temperature. Rhee and Gotham (1981) had worked out with Scenedesmus species and Asteronella formosa had found that for Scenedesmus, the optimal temperature range was 20-25°C and for Asteronella formosa it was found to be 19-20°C. Temperature and light interact to modify the chemical and bio-chemical composition of a nitrogen-limited marine diatom Thalassiosira species grown at a constant dilution rate in continuous culture under a light and dark cycle (Redaye and Laws 1983). The effect of light quality on the growth, photosynthetic rate and carbon metabolism was studied by Wallen and Geen (1971). Gon claves et al. (1988) reported the effect of low temperature, light and levels of nutrients on the uptake of cadmium by the algae Selenastrum capricornutum.

Similar to other physiochemical factors variation of pH affects the toxicity and nutrient uptake (Peterson and Healey, 1985). Cadmium and copper inhibition of nutrient uptake by the green alga Scenedesmus species is highly pH dependent in an organic medium. Both metals are less toxic at low pH.

Generally the salinity in the marine environment is relatively constant and has little influence on the heavy metal concentration. In estuaries, where fresh and salt water intermix, salinity however plays a dominant role in influencing metal concentration. Moreover the high salt content alters the pH value and consequently the metal solubility (Forstner and Wittmann, 1979). Estuarine species tolerated low salinity better than the oceanic and coastal species (Brand, 1984). But most of the coastal species tolerated salinity much lower than that from which they were isolated. On the studies of estuarine species Nitzschia americana by Miller and Kanykowski (1986), it was found that both light limited and light saturated rates of photosynthesis increased as the salinity was decreased.

Besides all these physiochemical factors, the concentration of certain essential elements like iron, manganese, cobalt and zinc in small quantities enhanced the growth and the deficiency of these elements resulted in the changes in the morphology and cell structure. Zinc acted as an activator of dehydrogenase. Hayward (1968) and Rueter and Morel (1981) reported that zinc helped in the uptake of silicic acid in diatoms. It was observed that manganese enriched the estuarine phytoplankton by addition of dissolved manganese (Sanders, 1978). Payer (1972) reported the requirement of trace metals such as manganese and vanadium for the mass culturing of microalgae. Addition

of manganese was found to reduce the effect of a toxic metal. Douglas et al. (1986) studied the influence of iron on the structure of Cyanobacterium Calothrix parietina. In the medium lacking iron, the heterocyst frequency increased and colourless multicellular hairs were formed together with false branching. Kazumi et al. (1987) reported that the reduction of cell division rate produced by excess of copper on Thalassiosira pseudomona was alleviated by increasing the concentration of manganese. Cobalt is an important micronutrient for biological organisms and is a metal of wide use. It is an important constituent of vitamin B_{12} (Sharma et al., 1987). Cobalt at low concentration increased the growth and cell division.

Chelating agents were omitted from culture solutions because they have been shown to counteract inhibition of growth in toxicity studies (Hart, 1975; Hart and Scaife, 1977). Stokes et al. (1973) reported that when Scenedesmus accutiformis was grown in medium containing EDTA in addition to nickel, no significant effect of nickel on growth was observed. Hutchinson and Stokes (1975) omitted organic chelators to minimize chemical complications when investigating heavy metal toxicity to algae Tevlin (1978) reported EDTA displays a strong tendency to complex with cadmium. Effect of chelating agents on the metal toxicity to phytoplankton was reported by Fayed et al. 1983; Wunchung Wang, 1983; Oakden et al. 1984; and Bressan & Brunetti 1988. Besides affecting algae through direct involvement in metabolism, organic substances may extent important indirect effect on their growth (Kinne, 1984).

Similar to EDTA humic acid also had influence on the acute toxicity and bioavailability of trace metals (Stackhouse and Bensen, 1988). Hongve

et al. (1980) in their studies of the acute effects of copper, cadmium, lead, zinc and mercury on photosynthesis showed that there was appreciable decrease in toxicity when the metals were combined with humic acid. Similar to chelating agents the toxic effect of metals can be reduced by adding sulphur containing ascorbic acid, amino acid and reduced glutathione (Rai and Raizada, 1988). They reported that Nostoc muscorum can be protected from chromium and lead toxicity by supplementing ascorbic acid.

Algal cells are expected to liberate extra cellular products capable of forming complexes with heavy metals. Thus unicellular algae have a tendency to release considerable proportion of carbon they fix into the medium in the form of organic compounds (Hellebust, 1974). However, the extracellular polysaccharides produced by blue green algae have been shown to bind metals (Lange, 1976). Similarly in blue greens, the production of extracellular acid formed complexes with copper added to the medium (Swallow et al., 1978).

Besides the organics, certain ions such as Ca^{2+}, Mg^{2+} and K^{+} were found to protect the algae against metal poisoning in the diatom Phaeodactylum tricornutum (Overnell, 1976). Toxicity of ionic copper was ameliorated by trivalent metal ions such as manganese, cobalt, iron and chromium in the growth medium (Stauber and Florence, 1987).

Thus the studies on the heavy metal toxicity to phytoplankton showed that concentration needed to inhibit growth, metabolic processes such as photosynthesis vaired widely and depended on factors such as degree of chelation, concentration of cells, nutrients physiological state of cells, salinity temperature and concentration of organic solutes (Morris and Russel, 1973).

Mechanism of algal-metal interaction are not well understood. According to Cain and Allen (1980) for most of the algal cells, the metals first penetrate through the cell wall. Plant cell walls are normally considered as highly permeable to compounds of low molecular weight. The materials present in the algal cell wall showed a high affinity for environmental contaminants particularly heavy metals.

Gibson (1972) reported that differential response of Anabaena flosaquae and Scenedesmus quadricauda to copper was due to differential copper uptake by the cells. Organic binding also reduces availability of metals for algal uptake. (Gachter et al., 1978; and Sunda and Guillard, 1976). Studies by Anderson and Morel (1978) on the effect of copper on phytoplankton showed that it is the ionic form of the metal which is toxic to phytoplankton. Binding of heavy metals to algal surfaces were worked out by Han-Bin-Xue et al. (1988). They reported that phytoplankton cells exhibited relatively large surface area containing various functional groups such as carboxylic, amino, thio and hydroxy groups that interact with heavy metals.

The inhibition of photosynthetic activity of heavy metals are studied by Erickson, 1972; Overnell, 1976 and Fisher and Jones, 1981. The effects of heavy metals on the morphology of phytoplankton was studied by Thomas et al. 1980 and Fisher and Jones, 1981. Stauber and Florence (1987) on their studies on the copper toxicity to phytoplankton reported that metal toxicity resulted in depressed cell division, photosynthesis, growth, respiration, ATP level and in mitchondrial electron transport chain activity.

Nickel was reported to be having both stimulating and inhibiting effect on algal growth. Bertrand and DeWolf (1967) suggested that nickel may be required for plant growth. This was based on the observation that cultures of Chlorella species exposed to three microgram per liter nickel produced greater yield than the nickel free controls. But nickel was found to inhibit algal growth. Nickel inhibition of algal growth was related to the presence of free divalent nickel in the medium (Fezy et al., 1979). They suggested that the form of nickel present at equilibrium of the medium was important than the total amount added to the medium. Spencer (1981) based on the studies on the effect of nickel on seven species of fresh water algae showed that 98% of the added nickel existed as divalent nickel. Petukhov et al. (1982) reported that nickel pollution in the sea presented the greatest ecological danger for all the heavy metals based on the studies on the priority of toxicological hazards of nickel in the sea. Whitton and Shehata (1982) reported that presence of calcium or iron reduced the toxicity of nickel in blue green alga Anacystis nidulans. Deviprasad and Deviprasad (1982) reported that nickel was not lethal between 0.1 and 10 ppm in fresh water green algae Ankistrodesmis falcatus, Scenedesmus obliquus and Chlorococcum species. In Scenedesmus quadricauda and in Akistrodesmis falcatus the presence of free divalent nickel inhibited the growth (Spencer and Nicholas, 1983). According to Oliveria and Anita (1984) very minute amount of divalent nickel showed very good growth of diatom Cyclotella cryptica. But higher divalent nickel concentration produced inhibitory effect on growth. The environmental variables such as pH play a striking effect on the bioaccumulation of algae. Wang and Wood (1984)

reported that most of the algal strains accumulated nickel at an optimum pH 8.0. Watras et al. (1985) on the studies of nickel accumulation by Scenedesmus obliqus and Daphnia magna showed that growth rate declined at pH below 8 and cellular nickel quota increased.

Cobalt, a micronutrient for biological organisms, is a metal of wide use. Cobaltous ion is known to act as an alternative co-factor for magnesium and manganese in a number of enzyme systems (Mc Elroy and Nason 1954) and it may influence photosynthetic carbon fixation (Goldman, 1964; 1965). Preliminary observations of cobalt in fresh water ponds showed that cobalt and iron were higher in the hypolimnion than in the epilimnion (Benoit, 1957). Coleman et al. (1971) reported that cobalt above 0.04 milligram per liter reduced significantly the growth of three species of algae Pediastrum tetras, Chlorella vulgaris and Euglena species. Goriunova et al. (1972) reported that cobalt is necessary for the fixation of molecular nitrogen by blue greens. Chalapati Rao and Satyanarayana Rao (1974) on the studies of phytoplankton in the Bay of Bengal observed that a high value of cobalt content was observed in the surface water of the Bay of Bengal during July - December. The decrease in cobalt concentration during April resulted in the maximum phytoplankton production. But Jenkins (1980) reported that cobalt is absorbed and accumulated in very low or limited amounts by biological organisms. Under natural conditions cobalt remained in the water phase in marine environments more than five times as much as in fresh water environments (Mahara and Akirokudo, 1981) and it was also reported that cobalt values were very high in estuaries (Knauer et al., 1982). In the marine environment the cobalt was highly mobile. The

mobility was greatly influenced by environmental factors such as pH, redox potential, ionic strength, type of sediments and length of reaction time (Mahara and Akirakudo, 1981).

Morphological changes were noted at the higher levels of cobalt in blue green alga Anacystis nidulans (Whitton and Shehata, 1982). They reported that by the effect of cobalt individual cells separate soon after cell division in exponential cultures and at high levels of cobalt, cells become subspherical. Patil et al. (1986) reported that BOD removal efficiency of the alga decreased with the increase in cobalt level on Chlorella vulgaris. They reported that high concentration of cobalt (10.9 to 15 milligram cobalt per liter) lowered the activity of enzymes and oxygen production. According to Sharma et al. (1987) there was increase in toxic action with the increase in metal concentration resulting in the decrease in productivity in Spirulina platensis.

Toxicity studies of various metals had proved that it was not the total metal concentration but the particular physical and chemical form of the metal that affected the organism. The environment impact of heavy metal added to the aquatic ecosystem is controlled to a large extent by their speciation. The physiochemical form of heavy metals will determine their solubility, mobility and bioavailability. When elements exist in more than one valency state, the separation of species in each state is a commonly studied aspect of chemical speciation. It was observed that one valency state has greater toxicity than the other. Similarly in the case of chromium, hexavalent state of chromium was more toxic than trivalent chromium. (Fukai 1967; Cranstan and Murray, 1978; Nakyama et al., 1981; Anderson 1981; and Babich et al., 1982). Eventhough

chromium existed in valency two to six, the common valencies encounted are trivalent and hexavalent chromium. Smillie et al. (1981) reported that the two valency states such as trivalent and hexavalent chromium were interchangable. They reported that bacterially produced hydrogen sulphide present in the tannery wastes converts hexavalent chromium present in the marine environment to trivalent chromium. Canterford and Canterford (1980) have expressed the correlation between toxicity and metal speciation in Dictylum brightwellii. The behaviour of both the valency states of chromium in the aquatic environment was highlighted by Bartlett and Kimble 1972; Meisch and Beckmann, 1979; Campbell and Yeats 1984 and Pettine, 1990.

Harvey (1949) observed that growth of Chlorella variegatus and Chlorococcum humicola was inhibited by 1.6 to 3.2 ppm hexavalent chromium. Studies of Schroll (1978) showed that hexavalent chromium had an inhibitory effect on Chlorella pyrenoidosa whereas trivalent chromium was not so toxic. Meisch & Beckmann (1979) reported that low concentration of chromium resulted in the stimulation of growth, photosynthetic oxygen evolution and acceleration of cell division. Hexavalent chromium was toxic to several riverine algae (Mangi et al., 1978). They reported that accumulation of chromium by living and dead plant tissue has important effects on the river ecosystem. Stary et al. (1982) studied the accumulation of trivalent and hexavalent chromium in batch cultures of planktonic algae Microcystis incerta, Scenedesmus obliqus and Chlamydomonas species. It was noticed that the hexavalent chromium was not partically cumulated whereas trivalent chromium was rapidly cumulated in all types of algae. This cumulative behaviour of trivalent chromium was also reported by Stary et al. (1982) on Chlorella kessleri. Michnowicz

and Weaks (1984) reported that pH plays an important role in chromium toxicity. Toxicity of chromium, copper and nickel in Selenastrum capricornutum was reduced by increasing the pH, the acid tolerant species tolerate high concentration of metal. There exists a strong relation between the hexavalent chromium uptake and the effect of hexavalent chromium on sulphate uptake by the diatom Thalassiosira pseudomonas (Riedel, 1985). He also reported that uptake of hexavalent chromium was proportional to external chromium concentration and inversely proportional to external sulphate concentration. Studies on the effect of hexavalent chromium on the photosynthesis in Selenastrum capricornutum by Pillard et al. (1987) reported that chromium stimulated the respiration while dark carbon uptake was suppressed. The presence of ascorbic acid and sulphur containing amino acids reduce the toxic effect of hexavalent chromium in Nostoc muscorum (Rai and Raizada, 1988).

There was variation in the relative toxicities of heavy metals for different species. Studies on the toxicity of heavy metals to the marine diatom Ditylum brightwellei by Canterford and Canterford (1980) showed that the order of toxicity of metals based on the free metal concentration was $Hg^{+} > Ag^{+} > Cu^{2+} > Pb^{2+} > Cd^{2+} > Zn^{2+}$. Hongve et al. (1980) in the studies on the acute effects of Cu, Cd, Pb, Zn and Mn on photosynthesis was in the order Hg > Cu > Cd > Pb > Zn. Studies of Albinles and Walker (1983) reported the relative toxicities of Cu, Cd and Zn on Chrococcus paris was Cu > Cd > Zn. The relative toxicity varies between species. Azeez and Banerjee (1987) reported that the order of toxicity in Anacystis species was Cu > Ni > Cd > Cr whereas in Spirulina species it was Ni > Cd > Cu > Cr.

In the waterbodies metals are not present individually. They are found in combination with other metals. So it is a great need to study how combination of metal ions affect the physiological, biochemical and ecological processes of various organisms. There are several reports on the interaction of trace elements on phytoplankton (Whitton, 1972; Hutchinson, 1973; Overnell, 1975; 1976 and Henriksen and Wright, 1978).

In the case of nickel, the presence of other metals may have synergestic or antagonistic effects. Uptis et al. (1973) reported that nickel inhibition of Chlorella could be overcome by the addition of zinc. Hutchinson (1974) found that copper and nickel acted synergistically when added to cultures of fresh water green algae. Bartlett et al. (1974) studied the effects of combination of copper, zinc and cadmium on the fresh water Chlorophyte Selenastrum capricornutum. Inhibition of Scenedesmus acutiformis was greater when nickel and copper were applied in combination than predicted from the effects of either metal applied singly (Stokes, 1975). The combined effect of cadmium, lead and nickel on three species of fresh water green algae was studied by Deviprasad and Deviprasad (1982). Growth response of Fucus vesiculosus to heavy metals such as cadmium, nickel, cobalt and manganese found that manganese and cobalt reduced the inhibitory effects of cadmium and nickel (Munda and Hudnik, 1986). Stauber and Florence (1987) reported that copper toxicity was reduced by adding trivalent form of metals such as Mn^{3+}, Cr^{3+}, Co^{3+}, Fe^{3+} and Al^{3+} to the medium. Synergistic and antagonistic effects of copper, cadmium, nickel and chromium in combination was reported by Azeez and Banerjee (1987) in blue green algae Anacystis nidulans and Spirulina platensis.

The hydrographic features and water quality of Cochin backwaters which is considered as the main discharge site of the major industries in and around Cochin were studied by many workers Qasim et al. 1972; Gopinathan, 1972; Balakrishnan and Shynamma, 1976; Sankaranarayanan and Stephen, 1978. The Cochin backwaters have been studied intensively for plant pigments (Qasim and Reddy, 1967); light penetration (Qasim et al., 1968); Growth characteristics (Joseph and Nair, 1975); organic production (Qasim et al., 1969), plankton production (Pillai et al., 1975), primary productivity (Nair et al., 1975); and toxicity studies by Nair et al. 1975. The levels of copper, manganese, cobalt, nickel and zinc in the northern arm of Cochin backwaters were reported by Venugopal et al. 1982. Sankaranarayan et al. (1986) studied estuarine characteristics of the lower reaches of River Periyar.

CHAPTER - III

MATERIALS AND METHODS

MATERIALS AND METHODS

The present investigation was based on the effect of some selected metals on the productivity of two algae, the Scenedesmus abundans and Nitzschia clausii respectively.

Description, culture conditions and trends in growth of the test algae

Scenedesmus abundans, a fresh water chlorophycean algae was isolated from fresh water. Nitzschia clausii belongs to family Bacillariophyceae was isolated from Cochin backwater - the prawn culture field near Tripunithura.

The description of the test algae was given below:

Scenedesmus abundans (Kirchner) Chodat

(Philipose. 1967. Page 278-279. Fig. 184 a-d. page 277.)

Description : Colonies 2-4 celled, cells avoid to oblong-ovoid. External cells with one or more lateral spines. Cells 2-7 um broad, 6-15 um long. Spines 3-8 um long.

Occurrence : In fresh water

Nitzschia clausii (Hantzsch) Hustedt. F. 1930.

(In Paascher's Susswasserflora. Mitteleuropas Heft 10. Bacillariophyta (Diatomacea) Jene Verlag Von Gustav Fisher P.421.)

Description : Cells solitary, valves elongated sigmoid and linear with sub acute spines.

Occurrence : In brackish water with a salinity range of 12% to 16%. The species is usually found in polluted water.

Scenedesmus abundans is a two celled structure and was maintained in the laboratory in synthetic media. Synthetic fresh water media was prepared by adding the necessary macronutrients and micronutrients to the double distilled water for the growth of algae. The media was prepared using metal free double distilled water to prevent the interference of possible metal ions.

The media used for Scenedesmus abundans was a synthetic algal nutrient medium (Ward and Parrish, 1982). The media was modified by excluding the EDTA and vitamin B_{12} as they form complexes with the metal selected for study, thus reducing the actual toxicity of the metals.

Miquel's solution modified by Ketchum and Redfield (1938) was found suitable for the growth of the diatom Nitzschia clausii. The media was prepared by adding the nutrient solution such as solution (A) and solution (B) to the sea water. The probable deficiency of this media was that solution (B) was found to precipitate which will help in the growth of bacterized cultures. To overcome this, the precipitate was removed and only clear supernatant medium was used. Since silica was essential for the better growth of diatoms, silica was added to the medium in the form of sodium meta silicate.

Prior to the preparation of culture medium, the sea water collected at high tide was allowed to age in carbuoys in darkness. It was found that Nitzschia clausii grows better at a salinity of 13-17%. So throughout the experiment salinity was maintained between 14-15%. Sea water was filtered through sartorious filter paper, autoclaved, cooled and nutrient solutions added.

Through out the experiments aged sea water which was collected at the beginning was used.

The experiment was conducted is twenty numbers of two litre borosilicate flasks plugged with sterile cotton, each flask containing one litre medium. The culture was maintained at 27.5°C ± 2°C. A 10 hour light and 14 hour dark cycle was provided. A light intersity of 2400 lux was maintained by keeping the cultures under four cool white fluorescent tubes. Continuous aeration was not provided for the cultures. Instead the cultures were shaken manually every now and then to give uniform mixing of the culture. Cultures under exponential growth phase were used as innoculum. For the uniformity of the experiments, a cell density of 5.2×10^4 cells per ml and 4×10^4 cells per ml for the innoculum were maintained for Scenedesmus abundans and Nitzschia clausii respectively.

The growth phase for both species was determined. It was found that the growth phase for Scenedesmus abundans and Nitzschia clausii was 14 and 10 days respectively. Nutrient solutions were added to medium just before innoculation. The mean of twenty observations contributed control values for each parameter for each species.

The metals selected for study, such as nickel, cobalt, trivalent and hexavalent chromium were added to the medium as aqueous solutions of $NiSO_4$, $CoCl_2 \cdot 2H_2O$, $K_2Cr_2O_7$ and $CrK(So_4)_2$ for nickel, cobalt, hexavalent chromium and trivalent chromium respectively in concentrations determined by screening experiment or by preliminary trial experiments.

The intention of the study was not to measure the EC_{50} as a response of stress but the changes that are brought about in the entire physiological mechanism of the alga by observing the changes of the crucial growth parameters throughout the growth phase and subjected to stress from varying concentrations.

As a first step the toxicity ranges of the metals nickel, cobalt, trivalent chromium and hexavalent chromium were determined by screening experiments using Scenedesmus abundans in standard algal assay synthetic medium and Nitzschia clausii in natural sea water medium. These screening tests were useful to provide information about toxic ranges when the metals act alone. From the preliminary tests, a fine screening was next carried out to obtain more detailed information about the effect of metals added singly. The criteria of the screening experiment were mainly the biomass and the visual changes, such as changes in colour, clumping of cells, attaching to the bottom surfaces.

In the case of combination of metals, prior to any combination experiment, interactions could not be excluded outside the toxic range of the individual metals and it was found to occur at intermediate toxic level. The batch assays were conducted in triplicate for better precision and the mean values were compared with those of control. From the preliminary investigation, the concentration of metals at the sublethal level were selected for the present study in both species, since above lethal level the growth was found to be too low for each and every parameter.

Experiments were conducted using two, three and four metal combinations. The combination selected were detailed at the beginning of each

experiment. The concentration of metals in combination selected were always limited to those which had proved to be sublethal in experiments with individual metals. In the case of two metal combination, three types of combinations were preferred. Low concentration of one metal and the highest concentration of the other metal or medium concentration of both and lowest concentration of both (all of which were within the sublethal limit).

From the preliminary studies of three metal combination, it was observed that very low concentration of metals in combination were lethal in S. abundans. Hence three metal combination studies were carried out only for N. clausii. Three metal combination studies were carried out by addition of a third metal to the concentration of two metal combination experiments already carried out. Suitable combinations were determined by preliminary trial experiments. This also helped in comparing the three metal combination with the results of two metal combination.

Iron of fixed concentration was added to two metal combinations and effect on algae was studied in detail. A low concentration of iron was used, which was selected by preliminary trial experiments. The concentrations were 0.05 ppm and 0.2 ppm for S. abundans and N. clausii respectively.

The low concentration of all metals were treated together and its effect was determined for four metal studies.

Metal solutions were prepared fresh in double distilled water in order to prevent the interference of any other metal ions. Care was taken to ensure that the same salt and also chemicals of analytic grade were used throughout the experiments.

GROWTH PARAMETERS

Biomass determination

Preservation

The phytoplankton sample was preserved in Lugol's solution soon after taking from the flask. Lugol's solution was preferred to formalin because it preserves better the cilia and flagella of the specimen. Biomass was determined on the basis of the number of cells counted with the help of haemocytometer. Biomass was expressed as no of cells per ml. Sampling was done every alternate day from second day onwards. pH of the culture was determined using the digital pH meter. Temperature was maintained at 27.5 + 2°C through out the experiment. Chlorinity of the culture was determined by Knudsen's method and salinity was read from the computed tables. It was maintained between 14-17% through out the experiment.

Determination of the productivity

Rates of production and respiration were determined by modified Winkler method (azide modification method) from fourth day onwards on alternate days. Cultures were diluted and taken in sixty ml oxygen bottles. One set of these bottles was kept in dark room for the measurement of the rate of respiration and the other set was under light to measure the growth or production for four hours. Control solutions were maintained simultaneously. After four hours it was fixed and when the precipitate had completely settled 0.5 ml of 50% concentrated sulpuric acid was added to the bottle till the precipitate had completely dissolved and it was titrated against standard sodiumthiosulphate

using starch as indicator. Oxygen values were converted into carbon equivalents applying a photosynthetic quotient of 1.25 and it was expressed as mg/l/hour.

Determination of the quantitative variation in the pigments by spectrophotometry

Various pigments such as chlorophyll-a, chlorophyll-b, and chlorophyll-c for green and brown algae were estimated for both the species by spectrophotometric analysis using Hitachi - U - 2000 spectrophotometer.

For quantifying the pigments equations of Lorenzen (1967) for chlorophyll-a and chlorophyll-c, Jeffery and Humphrey (1975) and Strickland and Parson (1968) for carotenoids were used. Pigment concentration were expressed as mg/litre.

A known volume of the sample was filtered through millipore filter or GF/C filters after adding 1 ml of $MgCo_3$ solution while filtering to prevent degradation of pigments. The filter paper holding the sample was soaked in 90% acetone, stored in dark cool place for 20-24 hours. The sample was made upto 10 ml , centrifuged and supernatant solution was taken for measuring the pigment contents using spectrophotometer at different optical densities.

Estimation of the end products of photosynthesis

End products of photosynthesis include carbohydrate, protein and lipids.

Estimation of total carbohydrate

Total carbohydrate content of the sample was determined using the anthrone-sulphuric acid method (Roe, 1955). Samples for the estimation of total carbohydrate were taken from fourth day onwards for the diatom and

from the eighth day onwards for green algae. Samples were centrifuged, weighed and 2 ml of 80% concentrated sulphuric acid was added and kept overnight. Next day, 10 ml of anthrone reagent was added to the same and the mixture was heated in a water bath for 10-15 minutes, cooled in dark at room temperature for 30 minutes and read at an optical density of 620 nm. Concentration of the total sugar was read from the standard graph obtained using glucose in benzoic acid.

In the case of the diatom Nitschia clausii since the cell walls were silicified carbohydrates were fractioned and the analysis were carried out in three steps. The samples were first centrifuged, weighed and sulphuric acid was added and from the supernatant as above the acid fraction was measured, secondly to the residue 1.N. NaOH was added to measure the alkaline soluble fraction. Lastly to the residue 2 ml of 80% concentrated H_2SO_4 was added and kept overnight to estimate the insoluble fraction.

Estimation of protein

Protein content of the sample was determined on alternate days from fourth day onwards is the case of diatom and eighth day onwards in the case of S. abundans. For estimating the protein, heated Biuret-Folin assay method was employed (Dorsey et al., 1978).

Samples were centrifuged, washed in double distilled water, weighed and stored below 4°C for one week. After one week, to each sample was added 5 ml of Biuret reagent, shaken well and heated in a water bath for 100 minutes at 100°C. To the hot solution 0.5 ml of Folin's reagent was

added and it was suddenly cooled to room temperature and optical density was measured at 660 nm. Protein content of the sample was determined from the standard graph obtained using Bovine serum albumin.

Estimation of lipid

Lipid the end product of photosynthesis was taken on the last day of the growth phase in two algae. The samples were taken, centrifuged, freeze dried and extracted with chloroform-methanol mixture and determined by weighing after drying.

The end products of photosynthesis such as carbohydrate, protein and lipids were expressed as dry weight at µg/mg. Algal dry weight was determined on alternate days from fourth day onwards by a standard method, filtering and drying to a constant weight.

Uptake of nutrients such as phosphate and nitrate which were found to be essential for photosynthesis were determined at the beginning and end of growth phase. The sample was filtered using a GF/C filter paper and the filterate was used for the estimation of phosphate and nitrate. Phosphate content of the sample was determined by the Ascorbic acid method and the nitrate by Hydrazine reduction method (APHA, 1985).

The algae selected for the study were found to accumulate the metals which they had taken from the medium. In order to make a clear picture of the effect of toxicity, apart from the standard methods applied for chemical analysis of various parameters, it was necessary to quantify the accumulation of metals at the end of growth phase in both the species. So modern, more

sophisticated technique like Atomic Absorption Spectrophotometry was used for quantifying the trace elements in the treated algal samples.

Trace metal analysis by Atomic Absorption Spectrophotometry

All sample containers and glasswares used were washed with detergent, rinsed with water and it was soaked in concentrated nitric acid (AR grade) and kept overnight. It was then clearly washed with double distilled water in order to prevent the interference of metal ions.

For determining the accumulation of metals the samples were taken at the end of growth phase for both the algae. Cultures of microalgae were grown in triplicates at different concentration of selected metals such as nickel, cobalt, trivalent chromium and hexavalent chromium. A suitable volume was centrifuged at 7000 rpm for 15 minutes in order to separate the algae from the supernatent, it was washed thrice with double distilled water to remove excess of the metal present in the sample and it was weighed.

4 ml of concentrated nitric acid was added to these samples and then concentrated nitric acid and perchloric acid at a proportion of 4:1 was added. The digestion was carried out in small 50 ml conical flasks covered with small glass funnels kept in the sand bath. The samples were evaporated to dryness and it was washed with double distilled water and made up to 10 ml. Care was taken to prevent the solution from boiling. The samples were read using Atomic Absorption Spectrophotometer of the model Hitachi Z. 8000 Polarized Zeeman AAS and expressed as μg/100 mg of dry weight. The experiments on the accumulation of metals in algae helped in determining the uptake of

metals with reference to the increasing concentration of the metal in the medium and time duration. In the case of chromium, both trivalent and hexavalent chromium were estimated as chromium.

The data was analysed statistically using a micro computer and the different parameters were expressed in the form of growth curves (graphs) in order to give a clear picture of the effect of metals. Analysis of variance technique was employed for the statistical analysis of the experimental data collected. The purpose of the analysis is to test the significance of the variability between groups in order to identify the significant effects. Graphs will give a distorted picture if the scales are changed. In most of the recent published literature in leading journals, only significant levels are given in order to save space. The same practice is followed in this thesis also. The tables are self explanatory, hence no reference made in the text in order to avoid duplication. Levels of significance are given in the tables. The type of analysis employed helps to explain the variability in the data and identifies significant effects.

CHAPTER - IV

RESULTS AND DISCUSSION (METALS IN ISOLATION)

4. Effect of individual metals on S. abundans and N. clausii

4.1 Effect of nickel on S. abundans

Selected sublethal concentrations of nickel - 0.01, 0.02 and 0.03 ppm.

Biomass

There was an initial increase in biomass on the second day for all concentrations. But at the end of growth phase it was 5%, 24% and 14% less than the control in 0.01 ppm, 0.02, and 0.03 ppm respectively (Fig. 1). In 0.01 ppm though a general trend of increase in biomass was noticed a marginal decrease of 5% was observed towards the end of growth phase on the tenth day. In 0.02 ppm 18% increase was observed only on sixth day while it was 14%, 19%, 40% and 13% less than the control on fourth, eighth, tenth and twelfth day respectively. In 0.03 ppm except a marginal (10%) increase on the second day, it was less than the control by 8%, 29% and 3% on the sixth, tenth and twelfth day respectively. In concentrations of 0.04 ppm and above besides the decrease in the cell count, cells became enlarged, the flagellae became distinct, and in later stage the two celled structure became unicellular along with exhibition of cloning behaviour.

Production

A general trend of decrease in production was noticed in all concentrations upto sixth day. It was observed that 0.03 ppm nickel completely reduced the production (Fig. 1). In 0.01 ppm the production was more than the control from eighth day onwards. Maximum production was reported on the twelfth day unlike other concentration, in 0.01 ppm at the end of growth phase also production was 57% more than the control. But in 0.02 ppm at the end of growth phase, the production was similar to the control and in 0.03 ppm 57% decrease was observed.

In 0.02 ppm the production was 53%, 50% and 20% less than the control on the sixth, eighth and tenth day respectively. However it was almost closely following the control on twelfth and fourteenth day.

The production was seriously affected in 0.03 ppm. On the fourth, sixth, and eighth days there was considerable decrease in production and it was found to be 88%, 68% and 75%. On the tenth, twelfth and fourteenth day there was 75%, 41% and 57% decrease in production.

pH showed similar trend of increase on the twelfth day followed by a sharp decrease on the fourteenth day. Similar to production in 0.03 ppm pH was less than the control.

Respiration

In all selected concentration, the respiration was less than the control at the end of growth phase with 18%, 20% and 2% less than the control in 0.01, 0.02 and 0.03 ppm respectively (Fig.1). In 0.01 and 0.03 ppm the respiration was less than the control throughout the growth phase. In 0.02 ppm though there was 22% and 20% increase in respiration on sixth and eighth day it was less than the control from tenth day onwards. But at the end of growth phase the decrease was marginal for all concentrations. It was 18%, 20% and 2% for 0.01 ppm, 0.02 ppm and 0.03 ppm respectively.

Photosynthetic pigments

Chlorophyll-a

Unlike production and respiration, notable increase in chlorophyll-a pigment was observed in 0.01 ppm and 0.02 ppm upto twelfth day with a peak

on the tenth day. It was 160% and 260% more than the control for 0.01 and 0.02 ppm respectively. At the end of growth phase there was 17% and 58% decrease in the chlorophyll-a for 0.02 ppm and 0.03 ppm respectively while in 0.01 ppm there was 79% increase. Eventhough there was 115% and 250% increase in chlorophyll-a on fourth and twelfth day for 0.03 ppm, 15% decrease in chlorophyll pigment was noticed on the sixth and eighth day and 16% decrease on the tenth day.

Chlorophyll-b

In all concentrations chlorophyll-b was more than the control upto fourth day and also on fourteenth day where the respective values of chlorophyll-b was 177%, 224% and 21% more than the control for 0.01, 0.02 and 0.03 ppm (Fig.2). In 0.03 ppm eventhough the chlorophyll-b was 163% and 21% more than the control on the fourth and fourteenth day, it was 11%, 28%, 4% less than the control on sixth, eighth and tenth day respectively. In 0.01 and 0.02 ppm the chlorophyll-b was more than the control throughout the growth phase with a maximum of 183% more than the control on the tenth day for 0.02 ppm and 37% more on the twelfth day for 0.01 ppm respectively.

Carotenoids

At the end of growth phase it was observed that carotenoids were 62% and 122% more than the control in 0.01 ppm and 0.02 ppm respectively (Fig.2). But in 0.03 ppm it was 61% less than the control. In 0.03 ppm eventhough carotenoids were 95%, 76% and 35% more than the control on second, fourth and sixth day, it was far less than the control towards the end of growth

phase from eighth day onwards. 0.01 and 0.02 ppm was showing similar pattern of increase throughout the growth phase. Eventhough it was less than the control upto fourth day, it was more than the control from sixth day onwards with a maximum of 170% on the tenth day. Similarly in 0.02 ppm, carotenoids were closely following the control on the sixth and eighth day with a maximum at the end of growth phase on the fourteenth day.

Phaeophytin

Similar to other pigments, nickel enhanced the Phaeophytin content of the algae in 0.01 and 0.02 ppm. The peak was observed on the twelfth day, the values being 68% and 137% more than the control. In 0.01 ppm though it was less than the control on the fourth day, it was 244% and 102% more than the control on the eighth and tenth day.

In 0.03 ppm phaeophytin was less than the control though there was 20% and 38% increase on the fourth and eighth day. At the end of growth phase it was 40% less than the control.

Photosynthetic end products

Carbohydrate

Carbohydrate was estimated from eighth day onwards because the initial count was very low. The carbohydrate was more than the control on the eighth day and at the end of growth phase in all selected concentrations. At the end of growth phase it was 138%, 81% and 139% more than the control in 0.01, 0.02 and 0.03 ppm respectively (Fig.3).

Maximum carbohydrate was noticed in 0.01 ppm. The values were 244% and 138%, more than the control on eighth and fourteenth day. But

on the tenth and twelfth day, it was 17% and 52%, less than the control. In 0.02 ppm and 0.03 ppm there was 86% and 78% increase in carbohydrate on the eighth day. On the tenth day 55% and 59% decrease was noticed for both treatments in comparison with the control and the maximum increase was noticed on the last day for 0.03 ppm.

Protein

The protein content of the algae was determined from eighth day onwards. It was 120%, 101% and 171% more than the control at the end of growth phase for 0.01, 0.02 and 0.03 ppm respectively. The peak values were observed on eighth day. (Fig.3) the values being 129%, 110% and 102% more than the control. In 0.01 ppm on the tenth day there was a sharp decline by 32% less than the control followed by 120% increase on the last day. On the eighth day in 0.02 ppm and 0.03 ppm there was an increase of 110% and 102% respectively followed by 15% decrease in both on the tenth day. The protein content was maximum on the eighth day for all treated samples. In comparison with the control, protein content increased towards the end of growth phase.

Lipids were found to be less than the control in all concentrations. The maximum decrease was noticed in 0.03 ppm. It was 77%, 91% and 93% less than the control in 0.01, 0.02 and 0.03 ppm respectively.

Nutrient uptake of the algae was studied and it was found that phosphate and nitrate uptake was less than the control for all treatments. Maximum uptake was in 0.01 ppm but it was less than the control. Phosphate uptake was 50%, 80% and 84% less than the control in 0.01, 0.02 and 0.03 ppm respectively.

Fig. 1

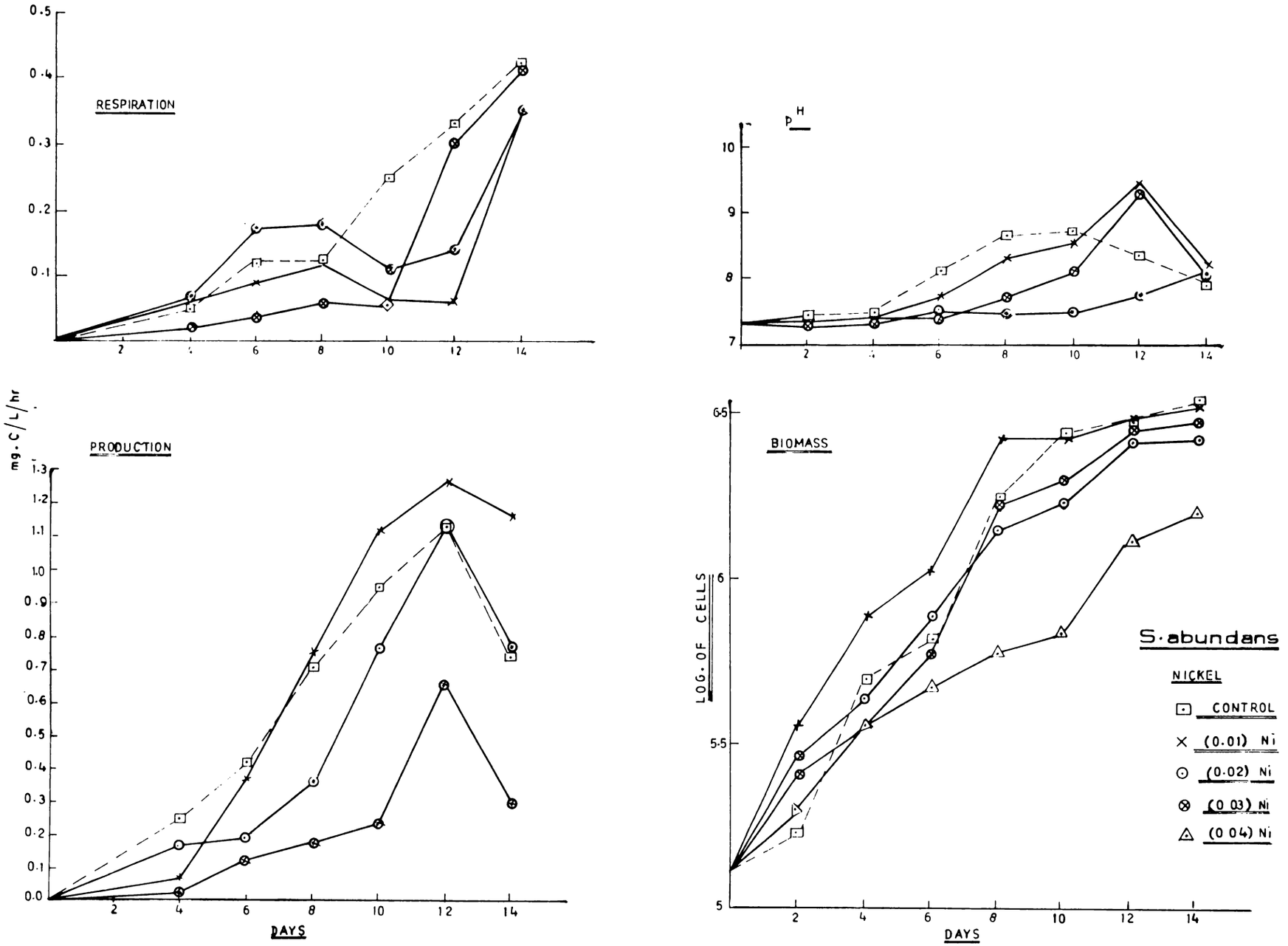
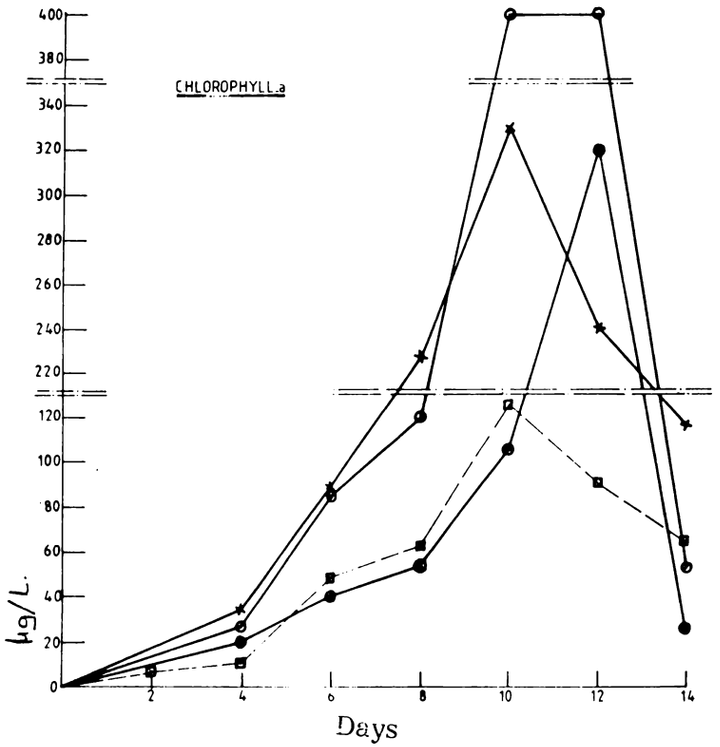
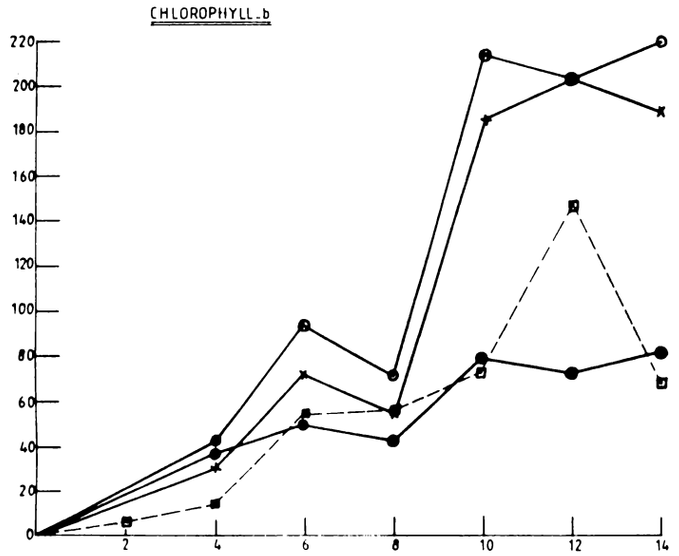
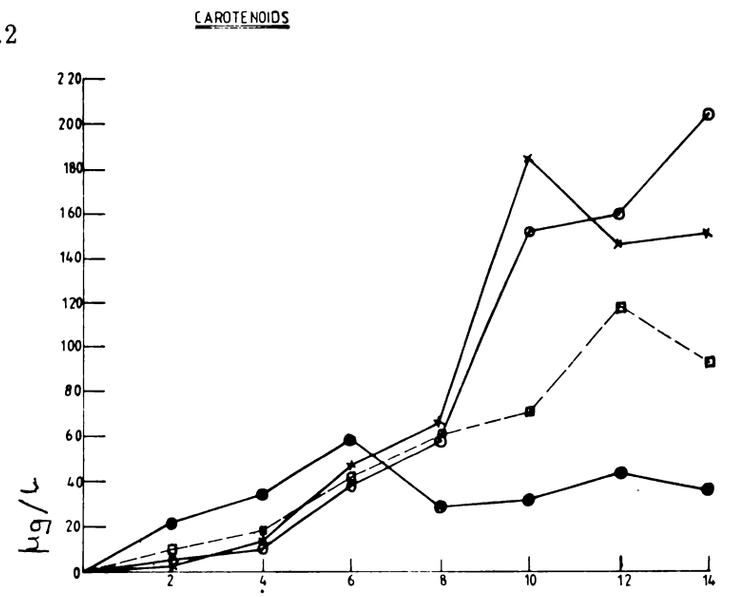
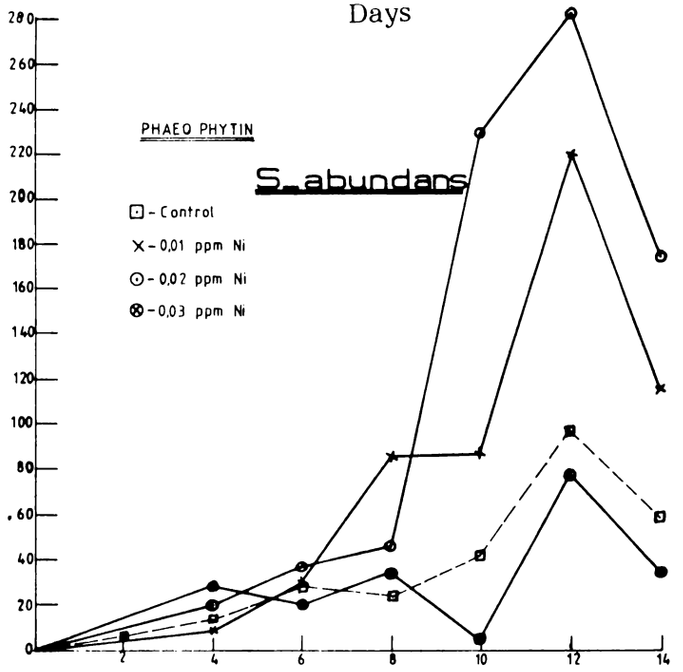

RESPIRATION
pH
PRODUCTION
mg. C/L/hr
BIOMASS
LOG. OF CELLS
DAYS
DAYS
S. abundans
NICKEL
CONTROL
(0.01) Ni
(0.02) Ni
(0.03) Ni
(0.04) Ni

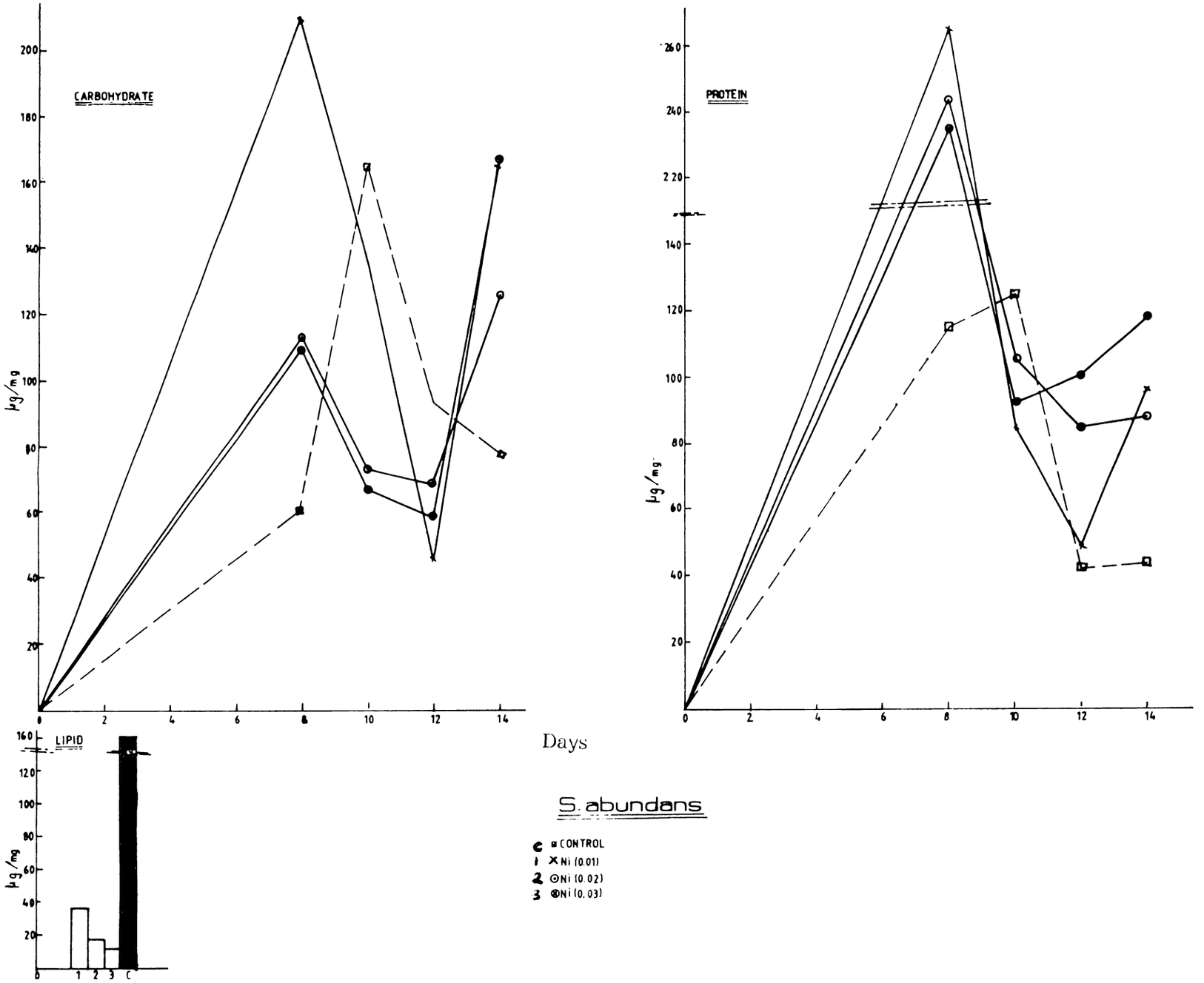
Fig.2


CHLOROPHYLL a
µg/L.
Days


CHLOROPHYLL b


CAROTENOIDS
µg/L
Days


PHAEO PHYTIN
S. abundans
- Control
- 0,01 ppm Ni
- 0,02 ppm Ni
- 0,03 ppm Ni


CARBOHYDRATE
PROTEIN
LIPID
µg/mg
Days
S. abundans
C CONTROL
1 Ni (0.01)
2 Ni (0.02)
3 Ni (0.03)

Effect of nickel on N. clausii

Selected sublethal concentrations of nickel - 0.4 and 0.6 ppm.

Biomass

The growth was more than the control through out the growth phase in 0.4 ppm but only at the beginning and at the end of growth phase in 0.6 ppm (Fig.4). In 0.4 ppm cell count was more than the control by 63% and 11% on fourth and eighth day but on the sixth day it was almost equal to the control.

In 0.6 ppm though the growth was more than the control by 54% upto fourth day, it was 28% and 14% less on sixth and eighth day respectively. However at the end of growth phase it was found to be more than the control by 5%.

Production

An increasing trend was noticed in the production upto eighth day for both concentrations. But at the end of growth phase production was 8% and 18% less than the control in 0.4 ppm and 0.6 ppm respectively. In 0.4 ppm there was 98%, 60% and 33% increase on the fourth, sixth and eighth day. Similarly in 0.6 ppm there was 59%, 40% and 48% increase on the fourth, sixth and eighth day. It was observed that eventhough there was an initial peak in production, it gradually decreased and at the end of growth phase it was lower than the control. Thus maximum decrease and increase was noticed in 0.6 ppm.

Respiration

Respiration was 34% and 45% less than the control at the end of growth phase for 0.4 ppm and 0.6 ppm respectively (Fig.4). But it was 108% and

75% more than the control on the fourth day. In 0.4 ppm 44% and 8% decrease was observed on the sixth and eighth day. Thus in 0.4 ppm respiration was less than the control from sixth day onwards though there was 108% increase on the fourth day. In 0.6 ppm 55% increase was noticed on the sixth day but towards the end of growth phase there was decrease in respiration with 7% decrease on the eighth day.

Similar to production pH also was showing an increasing trend in the first half and was found to be declining in the later half.

Photosynthetic pigments

Chlorophyll-a

A general increasing trend was noticed for both treatments upto eighth day but at the end of growth phase it was 35% and 40% less than the control for 0.4 and 0.6 ppm respectively (Fig.5). In 0.4 and 0.6 ppm there was 21% and 56% increase in chlorophyll-a on the fourth day and on the sixth day there was 12% and 34% increase for 0.4 and 0.6 ppm respectively.

Chlorophyll-c

Similar to chlorophyll-a, chlorophyll-c, was also showing a general increasing trend but at the end of growth phase it was found to be 29% and 4% less than the control for 0.4 ppm and 0.6 ppm respectively. In 0.4 ppm there was 15%, 60% and 35% increase on the second, fourth and sixth day. In 0.6 ppm the pigment content was closely following the control in the second and fourth day with 26% increase on the sixth day.

Carotenoids

Carotenoids were also showing a general increasing trend eventhough it was less than the control on the second and tenth day. At the end of growth phase 6% and 17% decrease was observed for 0.4 ppm and 0.6 ppm respectively (Fig.5). It was 41%, 47% and 75% more than the control on fourth, sixth and eighth day for 0.4 ppm and 57%, 42% and 80% more for 0.6 ppm respectively.

Phaeophytin

Phaeophytin was far less than the control at the end and in the early stage of growth for both treatments. At the end of growth it was 50% and 88% less than the control for 0.4 and 0.6 ppm (Fig.5). On the second day it was 56% and 80% less than the control and on the fourth day it was 24% and 45% less than the control for 0.4 and 0.6 ppm respectively. But for 0.6 ppm there was 53% and 22% increase on the sixth and eighth day respectively. Whereas for 0.4 ppm there was 25% decrease on the sixth day.

Photosynthetic end products

Carbohydrate

The acid soluble fraction of carbohydrate was 20% and 86% less than the control at the end of growth phase, eventhough a peak of 278% and 70% was observed on the fourth day in 0.4 ppm and 0.6 ppm respectively. In 0.4 ppm the acid fraction was more than the control upto eighth day followed by an abrupt decrease at the end of growth phase, where as in 0.6 ppm a decrease in carbohydrate was observed from eighth day onwards.

In the case of alkali soluble fraction, similar to acid fraction a peak of 251% and 167% was observed on the fourth day whereas the control was

maximum on the eighth day but towards the end of growth phase for 0.4 ppm and 0.6 ppm there was 75% and 73% decrease on the eighth day and 73% and 53% on the tenth day respectively.

Unlike the other fractions the insoluble fraction of carbohydrate was maximum at the end of growth phase. It was 453% and 287% more than the control where the control was minimum at the end of growth phase. Through out the growth phase carbohydrate was more than the control.

Protein

Protein was more than the control throughout the growth phase for both treated samples. It was 4% and 12% more than the control in 0.4 and 0.6 ppm respectively at the end of growth phase (Fig.6). Similar to the control both the treatments were maximum on the tenth day. On the fourth day it was 401% and 283% more than the control. Whereas the control was minimum on the fourth day. Thus it was observed that 0.4 ppm and 0.6 ppm nickel enhanced the protein content of N. clausii.

The lipid content of the algae was 45% and 57% less than the control in 0.4 ppm and 0.6 ppm.

The nutrient consumption of the algae was estimated at the end of growth. The uptake of phosphate for both treatments were similar to the control. Nitrate uptake was increased by 25% for 0.4 ppm whereas in 0.6 ppm it was less than the control.

Fig.4

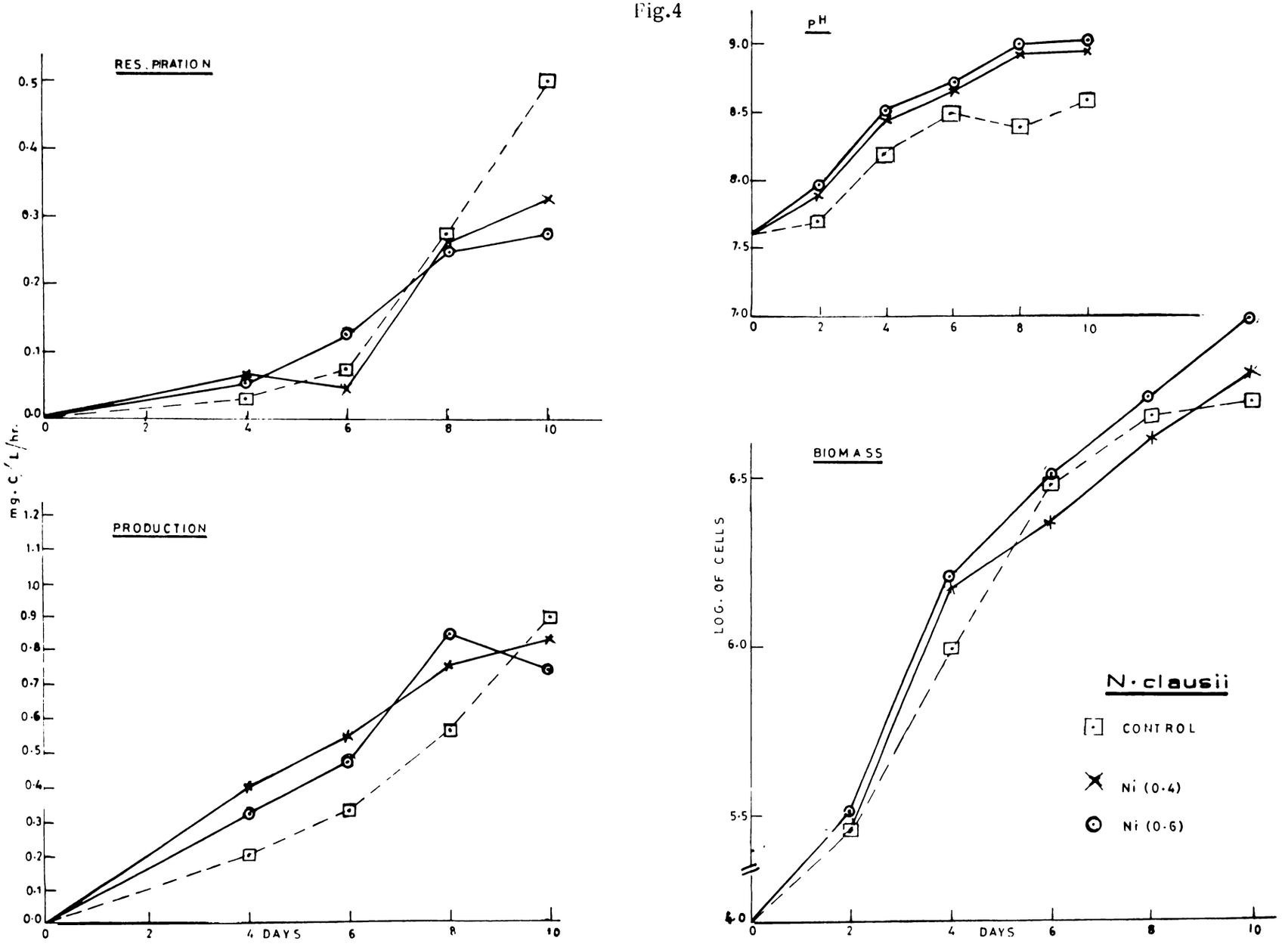
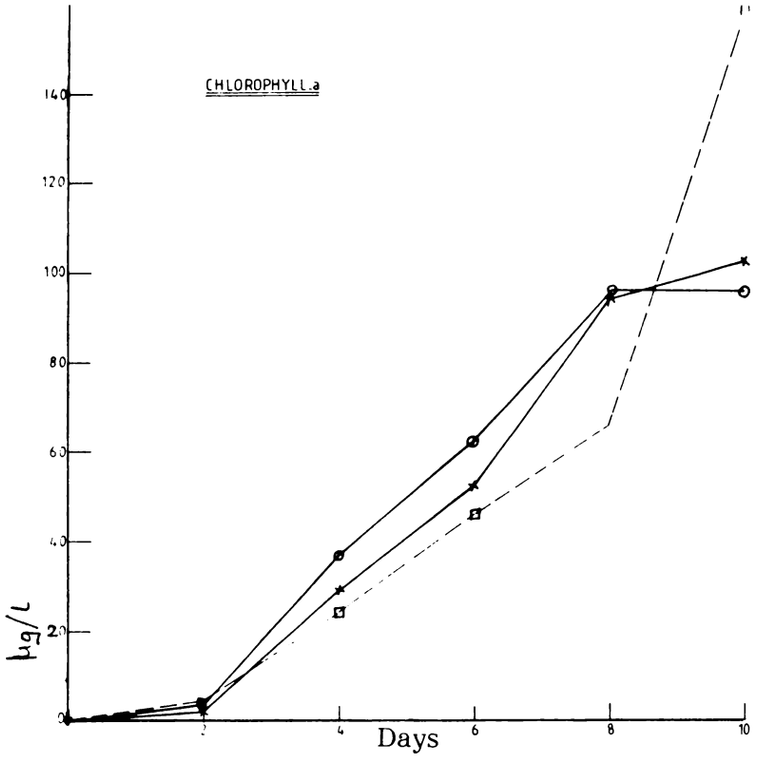
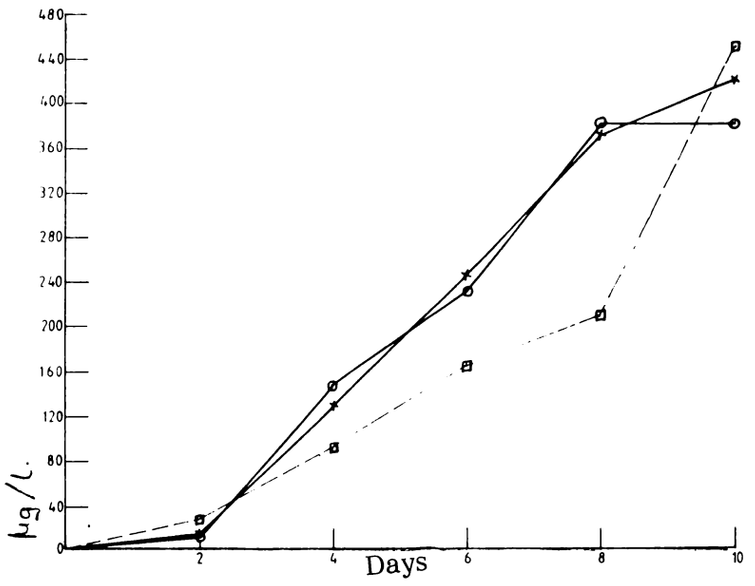
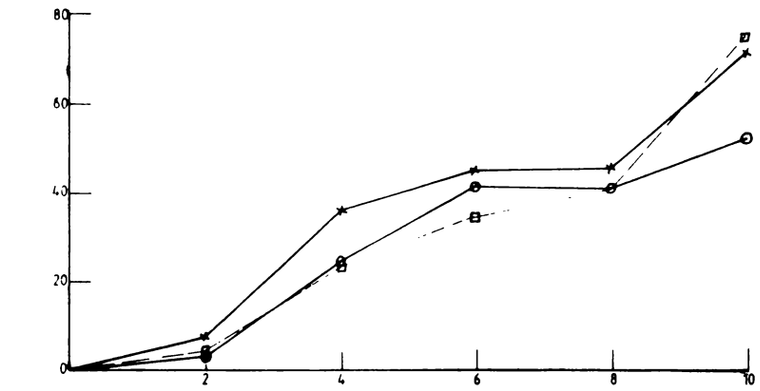

RES. PIRATION
mg. C / L/hr.
PRODUCTION
DAYS
pH
BIOMASS
LOG. OF CELLS
N. clausii
CONTROL
Ni (0·4)
Ni (0·6)

Fig.5


CHLOROPHYLL a
µg/l
Days


CAROTENOIDS
µg/l
Days


CHLOROPHYLL c

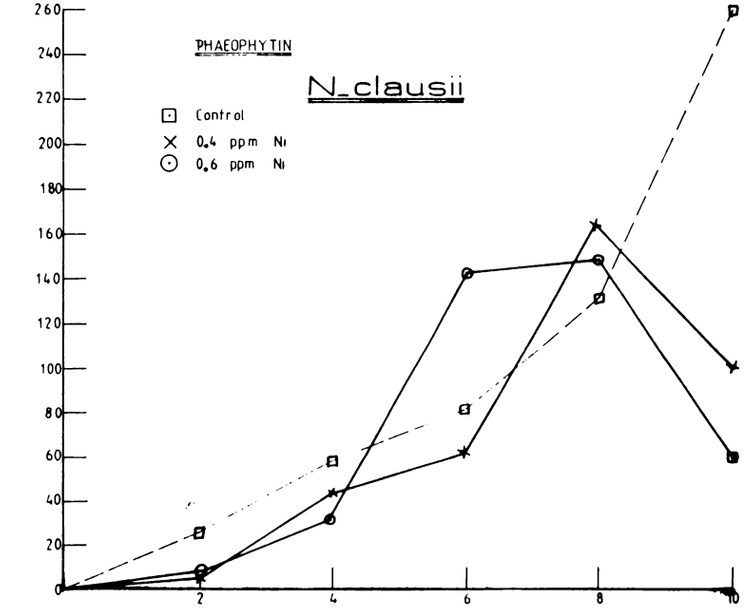
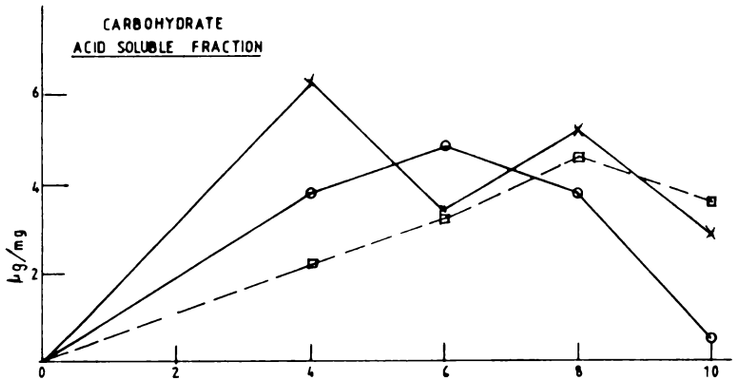
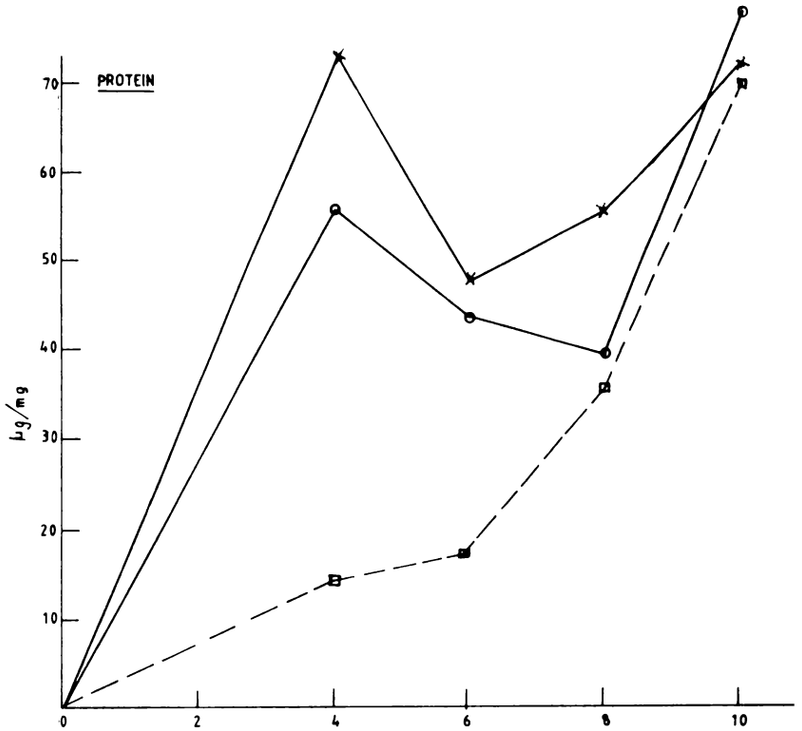
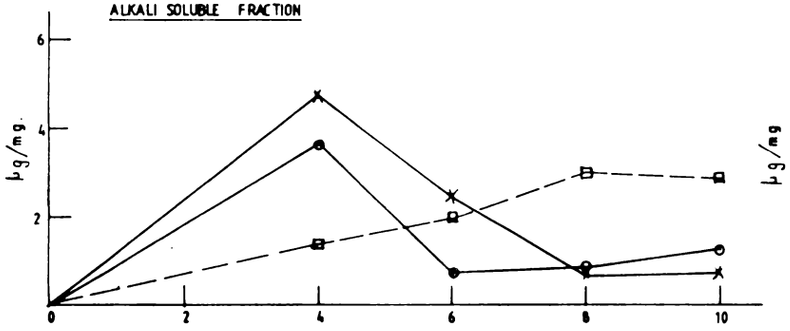

PHAEOPHYTIN
N_clausii
Control
0.4 ppm Ni
0.6 ppm Ni

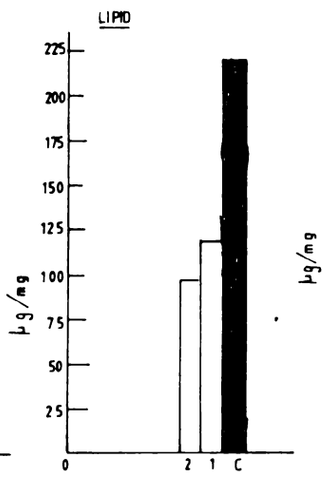
Fig.6

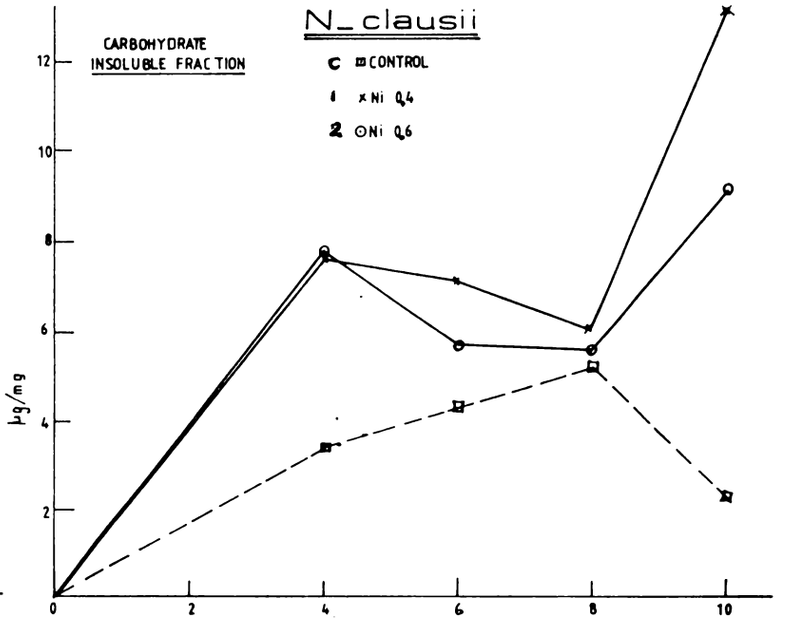

CARBOHYDRATE
ACID SOLUBLE FRACTION
µg/mg
0
2
4
6
8
10


PROTEIN
µg/mg
10
20
30
40
50
60
70
0
2
4
6
8
10

Days


CABOHYDRATE
ALKALI SOLUBLE FRACTION
µg/mg.
2
4
6
0
2
4
6
8
10


LIPID
µg/mg
25
50
75
100
125
150
175
200
225
0
2
1
C


N_clausii
CARBOHYDRATE
INSOLUBLE FRACTION
C ⊠CONTROL
1 ×Ni 0,4
2 ⊙Ni 0,6
µg/mg
2
4
6
8
10
12
0
2
4
6
8
10

4.2. Effect of cobalt on S. abundans

Selected sublethal concentrations of cobalt - 0.05, 0.1 and 0.25 ppm.

Biomass

The biomass showed a general increasing trend but it was 6%, 37% and 49% less than the control in 0.05 ppm, 0.1 ppm and 0.25 ppm respectively at the end of growth phase (Fig.7). The growth was less than the control through out the growth phase in 0.1 ppm. It was closely following the control upto eighth day with 19% and 9% decrease on the fourth and sixth day in 0.25 ppm. On the tenth day decrease was noticed with 36%, 33% and 48% less than the control on the tenth, twelfth and fourteenth day. The growth was comparatively more than the control with slight decrease at the end of growth phase. It may be noted that the lowest selected concentration of cobalt enhanced the growth whereas in 0.1 ppm and in 0.25 ppm growth was retarded.

Production

The production was less than the control through out the growth phase for all treated samples, though there was 28% increase for 0.05 ppm when compared with the control at the end of growth phase. The production was 34% and 84% less than the control at the end of growth phase for 0.1 and 0.25 ppm respectively. It was maximum on the twelfth day, but was 43% and 50% less than that of control for 0.1 and 0.25 ppm (Fig.7). pH was similar to the control in the initial stage but in the latter stage it was more than the control in 0.05 ppm. Thus changes in the production directly affected the pH.

Respiration

Respiration was 18%, 73% and 2% less than the control in 0.05, 0.1, 0.25 ppm respectively at the end of growth phase (Fig.7). Through out the growth phase respiration was less than the control at 0.25 ppm. In 0.1 ppm the respiration was more than the control upto sixth day followed by sudden decrease towards the end of growth. The peak in respiration was on the fourth day for 0.1 ppm and it was found to be 86% more than the control. It was 30% and 33% less than the control on the tenth and twelfth day respectively. In 0.05 ppm respiration was more than the control upto eighth day followed by decrease towards the end. The peak in respiration was on the sixth day and it was 60% more than the control.

Photosynthetic pigments

Chlorophyll-a

The chlorophyll-a was 148%, 93% and 30% more than the control in 0.05, 0.1 and 0.25 ppm respectively (Fig.8). It was maximum at the end of growth phase for all treated samples. In 0.05 ppm chlorophyll-a was fluctuating. It was more than the control except on the sixth and tenth day where it was less than the control. Highest value was observed on fourteenth day. In 0.1 and 0.25 ppm chlorophyll-a was less than the control upto tenth day followed by noticeable change towards the end of growth phase.

Chlorophyll-b

Similar to chlorophyll-a, chlorophyll-b was more than the control by 65%, 37% and 43% in 0.05, 0.1 and 0.25 ppm (Fig.8). Chlorophyll-b was more than the control through out the growth phase for 0.25 ppm except on the

twelfth day. In 0.1 ppm though an increasing trend was noticed it was 63% and 36% less than the control on the sixth and twelfth day. In 0.05 ppm chlorophyll-b was less than the control upto sixth day followed by an increase of 49% and 16% more than the control on sixth and tenth day respectively.

Carotenoids

A general decreasing trend was noticed in the carotenoids. At the end of growth phase in all treated samples the values were far less than the control by 35%, 37% and 65% for 0.05, 0.1 and 0.25 ppm respectively (Fig.8). In 0.05 ppm the carotenoid pigment of the algae was reduced through out the growth phase. In 0.25 ppm eventhough there was an increase upto sixth day, from eighth day onwards it was far less than the control by 22%, 39% and 70% on eighth tenth and twelfth day respectively. Whereas the control showed maximum on the twelfth day. Similarly in 0.1 ppm though the treated sample was closely following the control upto fourth day, it was less than the control through out the growth phase.

Phaeophytin

Similar to carotenoids phaeophytin was less than the control. It was 51%, 88% and 8% less than the control at the end of growth phase in 0.05, 0.1 and 0.25 ppm respectively. It was slightly more than the control on the fourth day in 0.1 ppm but was less than the control with the aging of the culture. In 0.25 ppm and 0.05 ppm the phaeophytin was less than the control from tenth day onwards. Of the three treated samples, phaeophytin was maximum by 130% on the sixth day for 0.25 ppm.

Photosynthetic end products

Carbohydrate

All the treated samples were showing similar pattern of increase and decrease. In all concentrations the carbohydrate was 69% more than the control on the eighth day, whereas the peak was on the tenth day for the control. In 0.05 ppm, 0.1 ppm and 0.25 ppm there was an abrupt decrease on the tenth day and it was found to be 66%, 61% and 79% less than the control (Fig.9). At the end of growth phase in 0.25 ppm carbohydrate was 71% less than the control. But in 0.05 ppm, the carbohydrate was similar to the control and in 0.1 ppm the carbohydrate was 42% more than the control.

Protein

It was found that unlike the control, all treated samples were 30%, 25% and 242% more than the control in 0.05 ppm, 0.1 ppm and 0.25 ppm respectively at the end of growth (Fig.9). The maximum protein content was noticed on the fourteenth day in 0.25 ppm, though a general increasing trend was noticed in 0.25 ppm. There was 82% and 64% decrease on the eighth and tenth day respectively. Similarly in 0.05 ppm, protein content was 60% and 53% less than the control on eighth and tenth day with 132% and 30% increase towards the end of growth phase on twelfth and fourteenth day. In 0.1 ppm a peak was observed on the eighth day and it was 27% more than the control, whereas on the tenth day, it was 45% less than the control. On the twelfth and fourteenth day it was 16% and 25% more than the control.

For all treatments lipids were found to be less than the control. 80% reduction in lipid content was noticed in 0.05 ppm. There was 92% and 95% decrease in lipid content in 0.1 and 0.25 ppm respectively.

The nutrient uptake of the alga treated with cobalt was less than the control for all concentrations and it was found to decrease with increase in concentration. The maximum uptake of phosphate was for the lower concentration (0.05 ppm) and it was 61% less than the control.

Fig.7

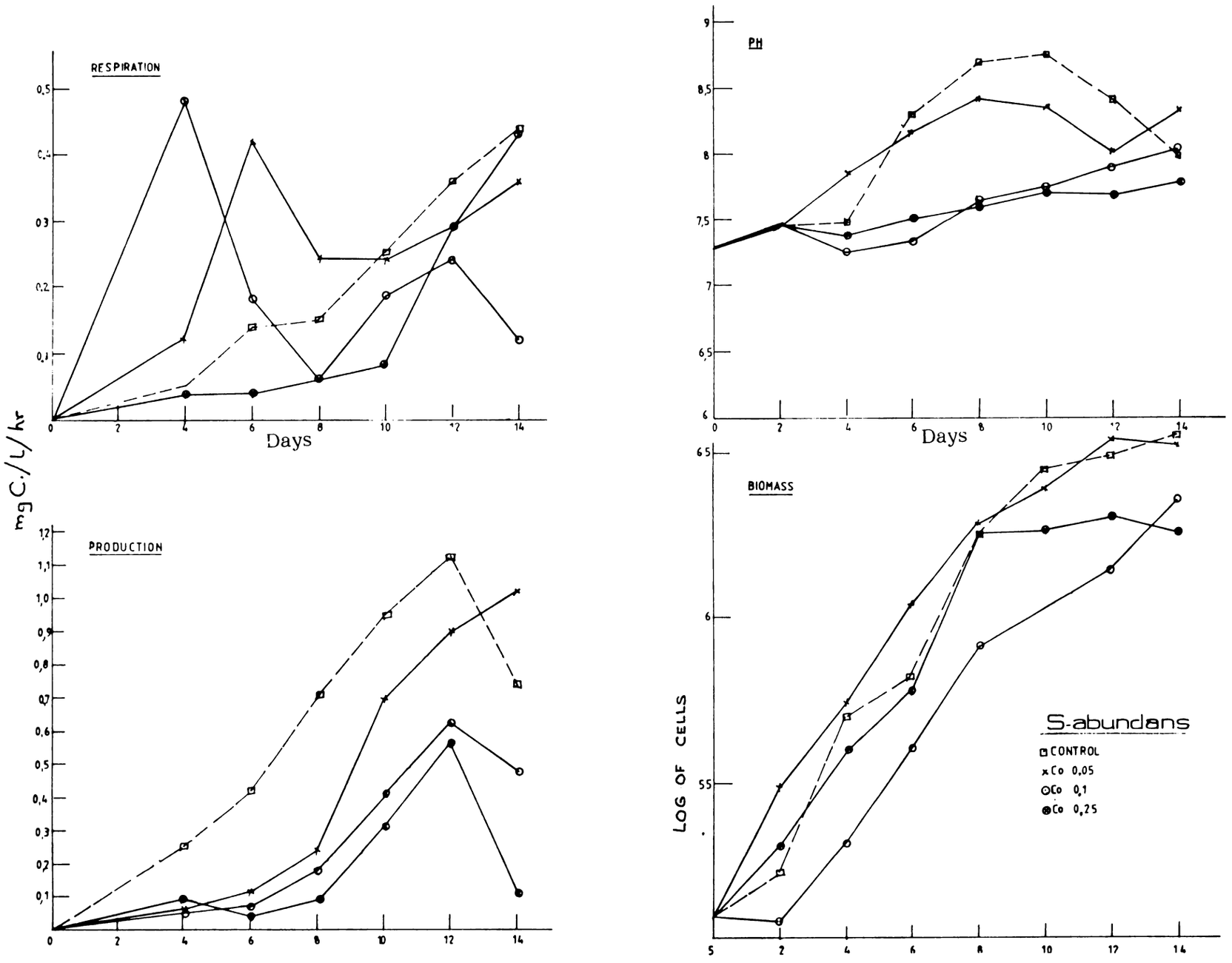

RESPIRATION
mg C./L/hr
Days
PRODUCTION
PH
Days
BIOMASS
LOG OF CELLS
S-abundans
CONTROL
Co 0,05
Co 0,1
Co 0,25

Fig.8

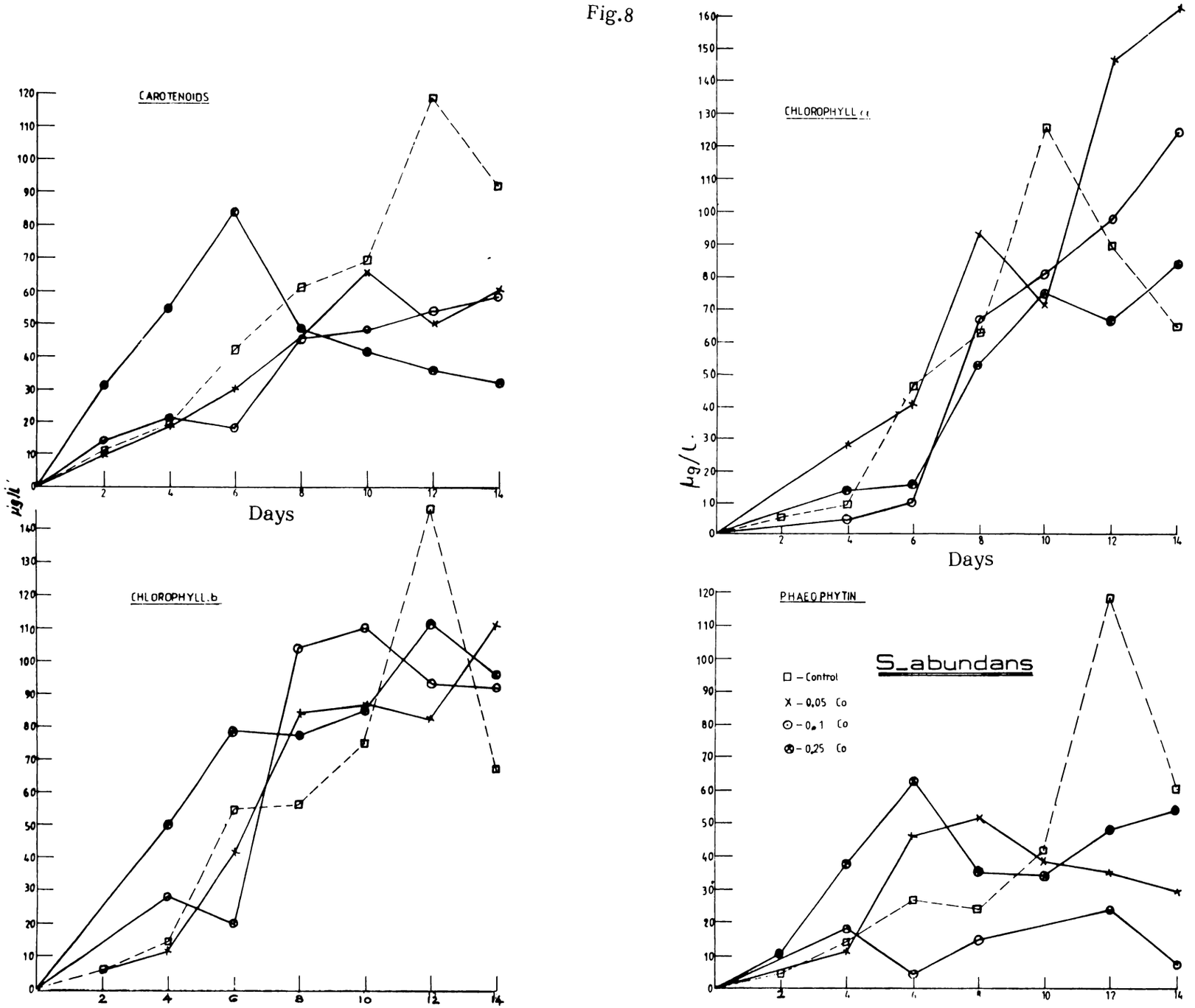
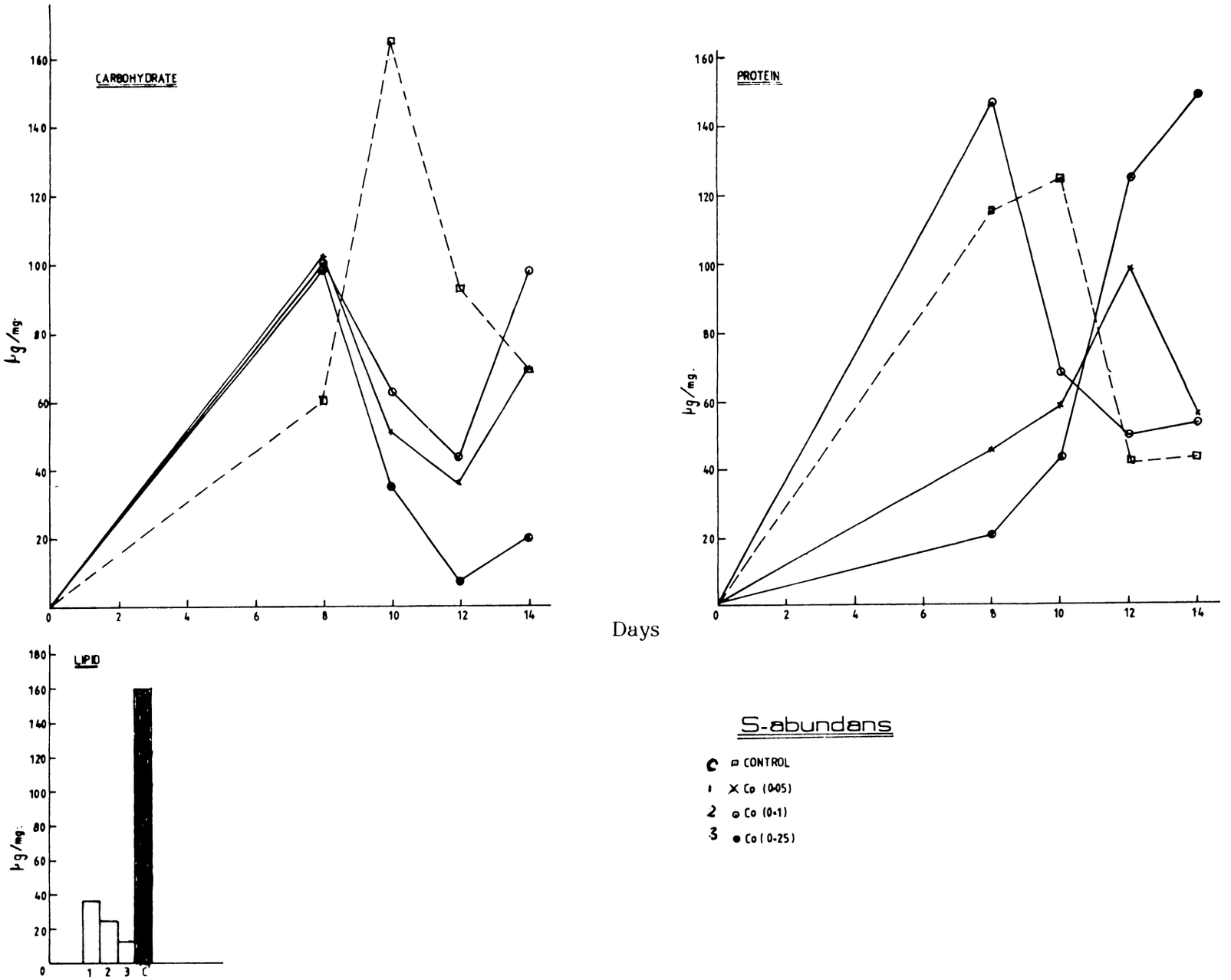

CAROTENOIDS
µg/l
Days
CHLOROPHYLL a
µg/l
Days
CHLOROPHYLL b
PHAEOPHYTIN
S_abundans
□ – Control
X – 0,05 Co
⊙ – 0,1 Co
⊗ – 0,25 Co

Fig.9


CARBOHYDRATE
PROTEIN
LIPID
µg/mg.
Days
S-abundans
C CONTROL
1 Co (0·05)
2 Co (0·1)
3 Co (0·25)

Effect of cobalt on N. clausii

Selected sublethal concentrations of cobalt - 0.3, 0.5, 0.6 ppm

Biomass

The biomass showed almost the same growth pattern in all selected concentration of the metal upto second day (Fig.10) which declined afterwards in the case of 0.6 ppm and became less than the control after four days. In 0.5 ppm the biomass increased steadily. It was more than the control through out the growth phase whereas in 0.3 ppm it was more than the control upto sixth day and later declined. At the end of growth phase there was an increase of 33% in 0.5 ppm and a decrease of 8% and 18% in 0.3 and 0.6 ppm respectively.

Production

The net production was less than the control at the end of growth phase for all concentrations, the values being 58%, 10% and 38% in 0.3, 0.5 and 0.6 ppm respectively (Fig.10). However on eighth day the production was more than the control by 11% in 0.3 ppm whereas it was less by 17% in 0.5 and 88% in 0.6 ppm. It was observed that the net production was always less than the control upto sixth day in 0.5 ppm and upto eighth day in 0.3 ppm. Similar trend was noticed in the pH also in all selected concentrations.

Respiration

Generally the respiration was less than the control at the end of growth phase. The values being 27%, 43.3% and 26% less than the control for 0.3,

0.5 and 0.6 ppm respectively (Fig.10). The respiration was always less than the control for 0.6 ppm. But in 0.3 ppm it was 45% and 24% more than the control on the sixth and eighth day, whereas in 0.5 ppm the respiration was fluctuating. It was more than the control on fourth and eighth day while on the sixth and tenth day it was less than the control.

Photosynthetic pigments

Chlorophyll-a

Though there was a general increasing trend, chlorophyll-a was less than the control for all treated samples at the end of growth phase. It was 44%, 42% and 65% less than the control in 0.3, 0.5 and 0.6 ppm respectively (Fig.11). In 0.6 ppm chlorophyll-a was less than the control through out the growth phase. In 0.3 and 0.5 ppm chlorophyll-a was 12% and 15% less than the control on the sixth day but 20% and 19% more on the eighth day.

Chlorophyll-c

All treated samples were showing an uniform pattern of increase and decrease. At the end of growth phase, it was 36%, 32% and 39% less than the control in 0.3, 0.5 and 0.6 ppm respectively (Fig.11). Chlorophyll-c was less than the control on the fourth and sixth day for all treated samples. On the eighth day there was 20%, 30% and 34% increase when compared with the control for concentrations 0.3, 0.5 and 0.6 ppm respectively. It may be noted that though there was an increase in chlorophyll-a on the eighth day in all concentration selected, chlorophyll-c was less than the control.

Carotenoids

Carotenoid content of the algae was far less than the control for all treated samples at the end of growth phase, the values showing 35%, 37%

and 65% less for 0.3 ppm, 0.5 ppm and 0.6 ppm respectively (Fig.11). In 0.6 ppm carotenoids were less than the control throughout the growth phase. In 0.3 ppm and 0.5 ppm it was fluctuating throughout the growth phase. There was an increase of 35% and 33% in 0.3 ppm and 0.5 ppm on the eighth day. It was less than the control on fourth, sixth and tenth day.

Phaeophytin

Throughout the growth phase, phaeophytin was less than the control for all treated samples. It was 83%, 75% and 89% less than the control on the tenth day for 0.3, 0.5 and 0.6 ppm respectively (Fig.11). However, in 0.3 ppm, it was 18% more than the control on the second day.

Photosynthetic end products

Carbohydrate

The acid soluble fraction of carbohydrate was 59% and 44% less than the control at the end of growth phase for 0.5 and 0.6 ppm respectively (Fig.12). Whereas it was 19% more than the control in 0.3 ppm. However it was 80%, 52% and 180% more than the control for 0.3, 0.5 and 0.6 ppm respectively on the fourth day and on the sixth day also it was more than the control but less than the fourth day values.

The alkali soluble fraction was less than the control for all treatments throughout the growth phase and by the end it was 44%, 43% and 55% less than the control in 0.3, 0.5 and 0.6 ppm respectively.

The insoluble fraction was found to be more than the control at the end of growth phase with 30%, 35% and 48% increase for 0.3, 0.5 and 0.6 ppm

respectively. The increase was only marginal upto eighth day in 0.3 ppm and attained 30% more than the control. In 0.5 ppm the insoluble fraction of carbohydrate was 40% and 30% more than the control on the fourth and sixth day followed by a sudden decrease on the eighth day. But on tenth day it was more than the control. The insoluble fraction was maximum with 65% increase on the fourth day in 0.6 ppm followed by a marginal decrease on the sixth and eighth day and at the end of growth phase, it was 48% more than the control.

Protein

Protein content of the algae was 47%, 46% and 57% less than the control on the tenth day for 0.3 ppm, 0.5 ppm and 0.6 ppm respectively (Fig.12). The control showed gradual increase upto sixth day but thereafter there was a sudden increase, reaching the maximum on tenth day. At 0.6 ppm there was marginal increase upto fourth day and it was less than the control during the rest of the growth phase. Whereas in 0.5 ppm the increase was appreciable and attained 88% over the control on the sixth day, later it was less than the control. In 0.3 ppm the protein content was less than the control upto fourth day then attaining 51% above control values on sixth day followed by decrease towards the end of growth phase. The protein content of N. clausii has been found to vary marginally with respect to control indicating higher protein values for 0.5 and 0.6 ppm till the fourth day. The protein values decreased marginally on the eighth day but drastically on the tenth day.

Lipid content of the alga was found to be less than the control for all treatments when compared with the control. It was 55%, 60% and 75% less than the control in 0.3, 0.5 and 0.6 ppm respectively.

The phosphate and nitrate uptake was more than the control for all treated samples. Maximum absorption was observed in 0.6 ppm and it was 75% and 50% more than the control for phosphate and nitrate respectively.

Fig. 10

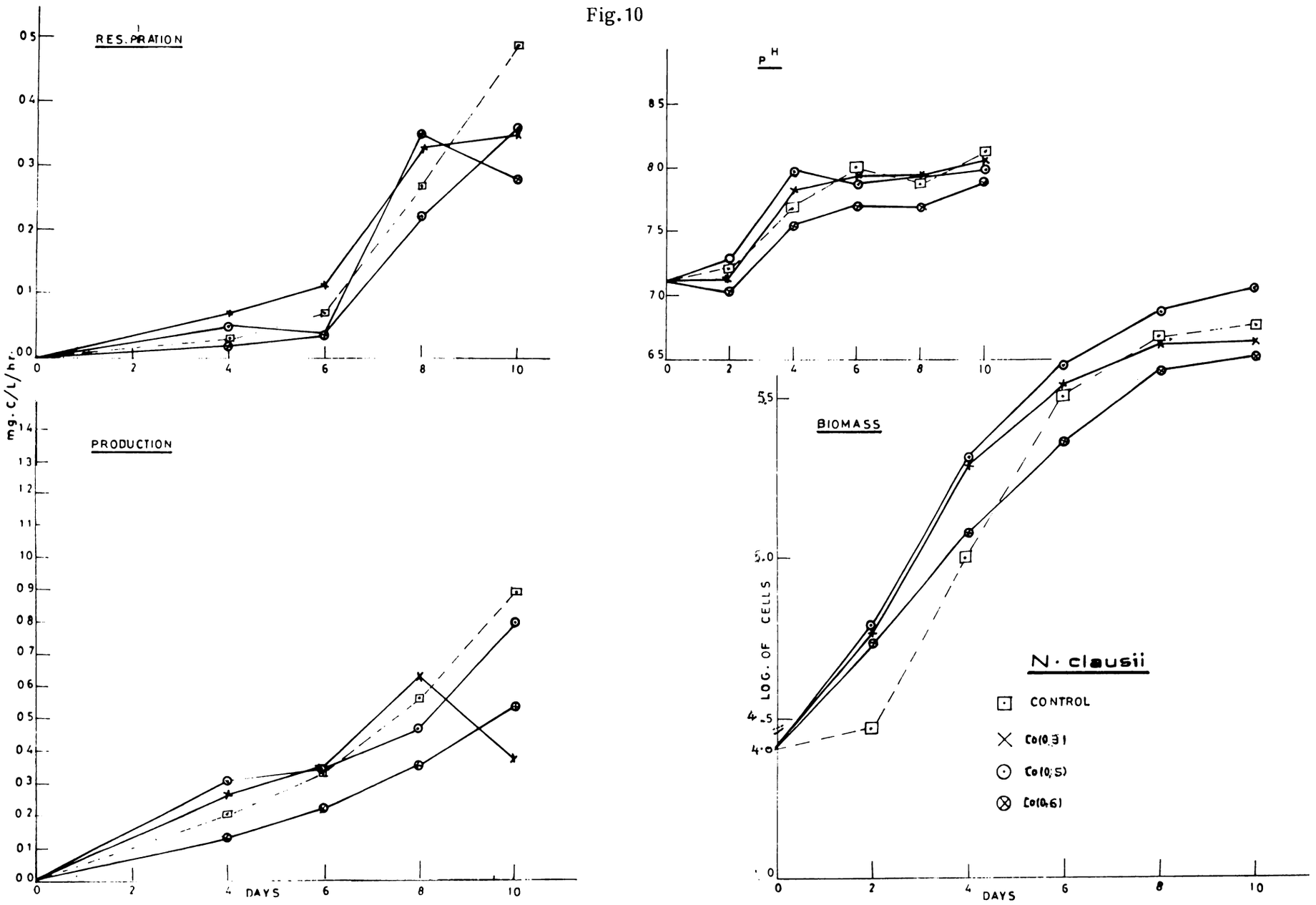
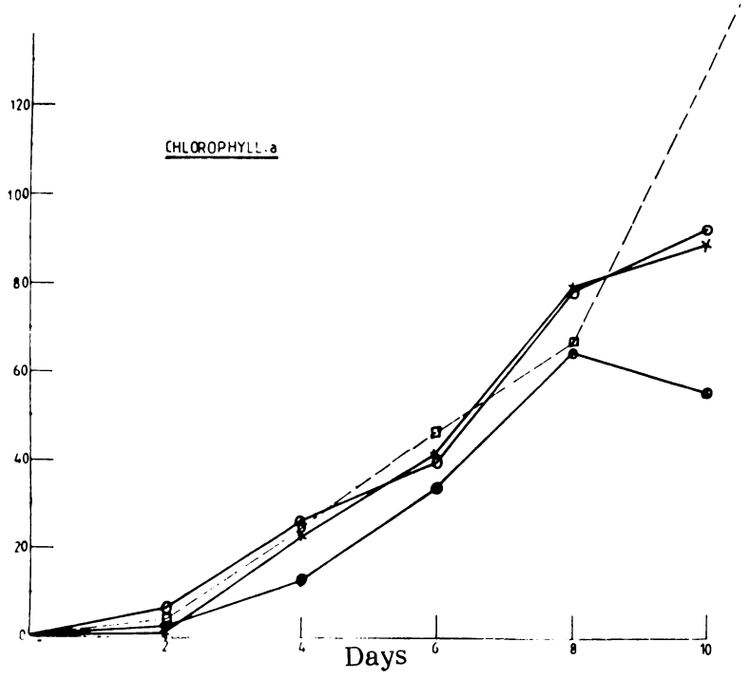
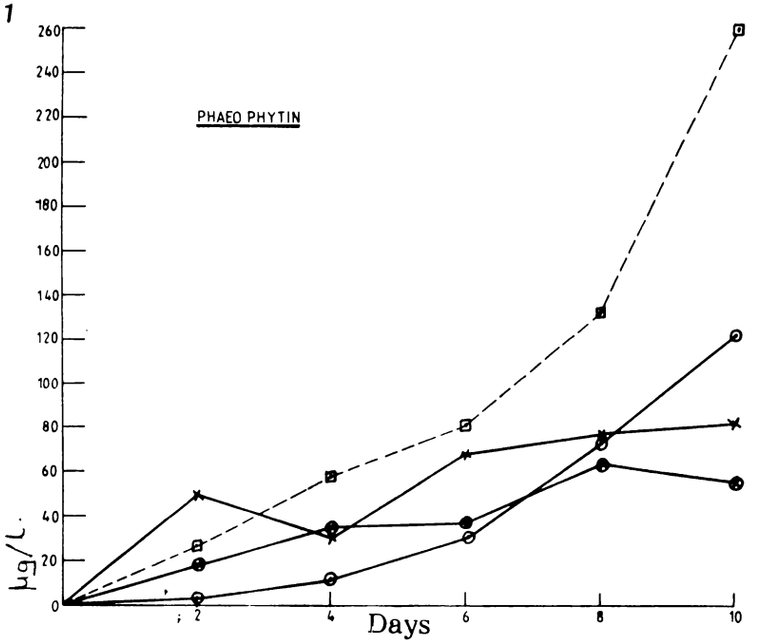
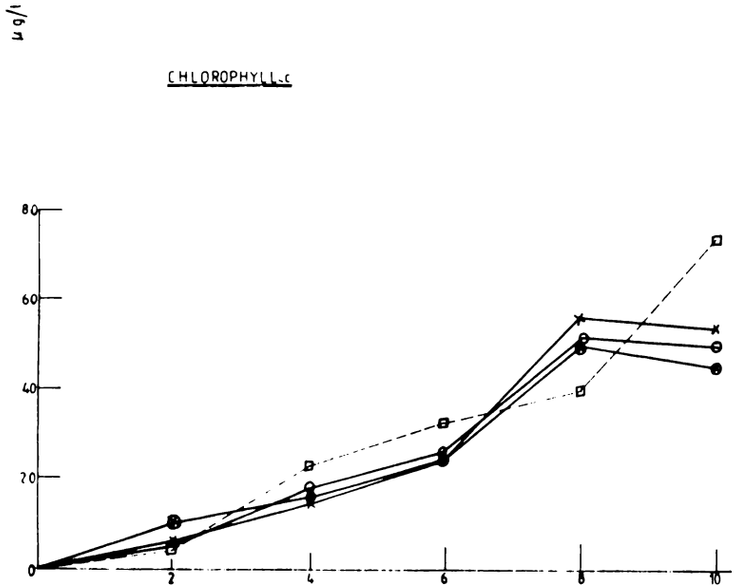

RES. PRATION
mg. C/L/hr.
PRODUCTION
DAYS
P H
BIOMASS
LOG. OF CELLS
N. clausii
CONTROL
Co(0.3)
Co(0.5)
Co(0.6)
DAYS

Fig. 11


CHLOROPHYLL.a
Days


PHAEO PHYTIN
µg/L.
Days


µg/l
CHLOROPHYLL.c

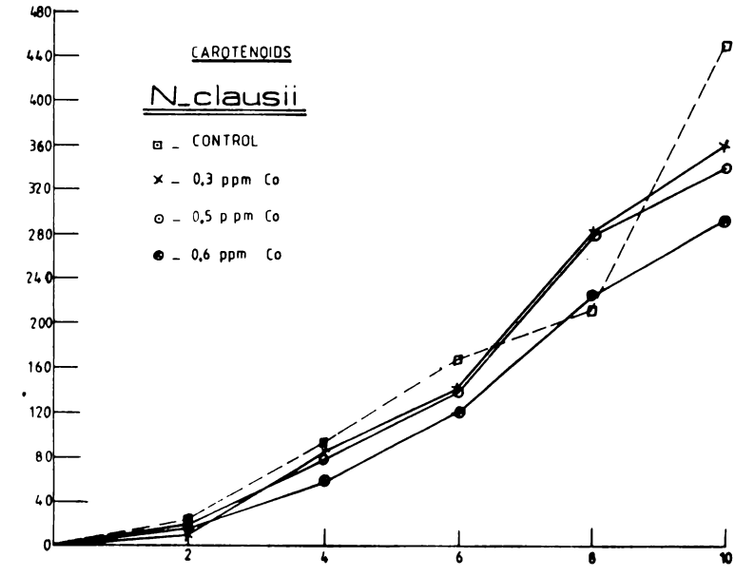
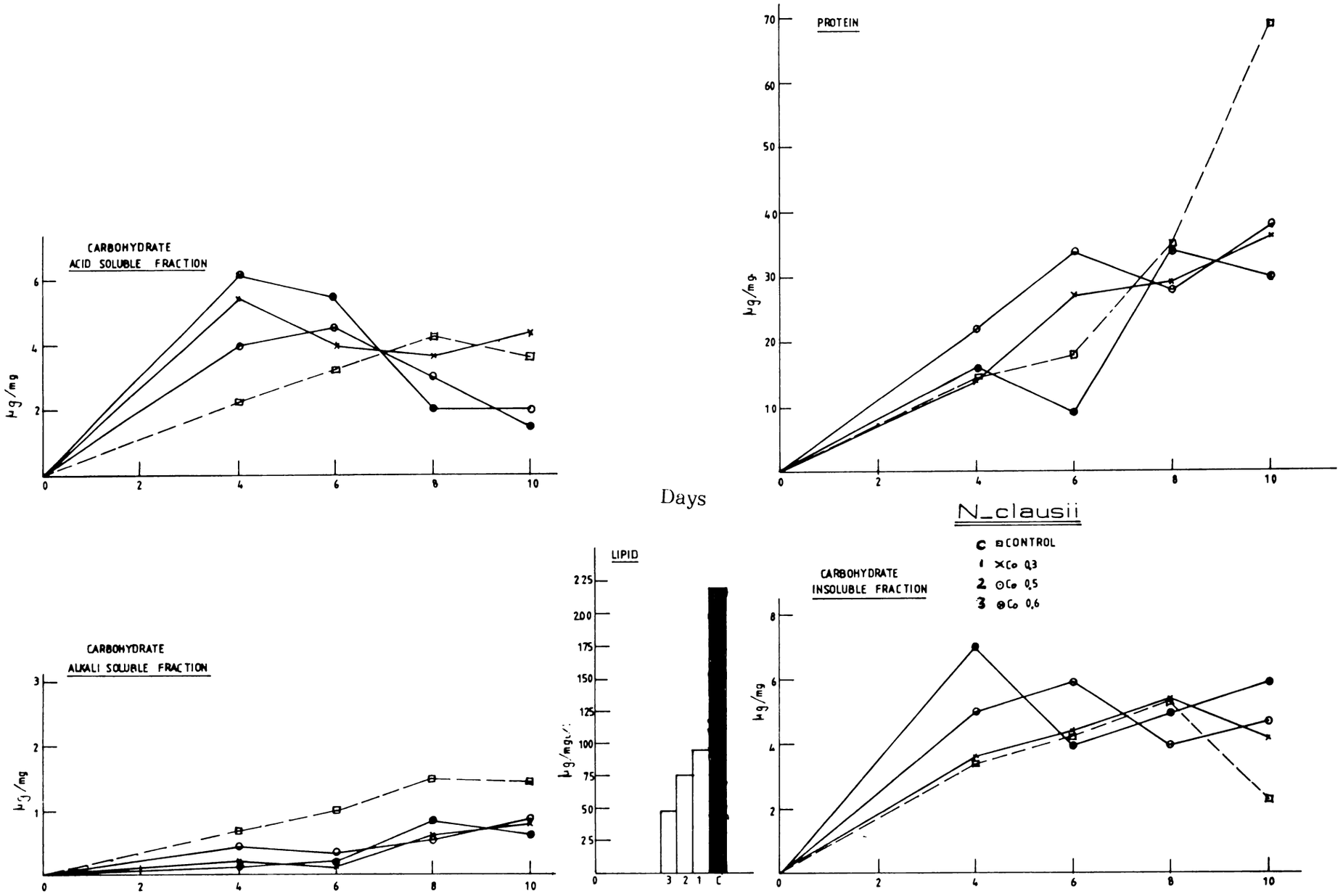

CAROTENOIDS
N_clausii
CONTROL
0,3 ppm Co
0,5 p pm Co
0,6 ppm Co

Fig. 12


PROTEIN
µg/mg
CARBOHYDRATE
ACID SOLUBLE FRACTION
µg/mg
Days
N_clausii
C CONTROL
1 Co 0,3
2 Co 0,5
3 Co 0,6
CARBOHYDRATE
ALKALI SOLUBLE FRACTION
µg/mg
LIPID
µg/mg
CARBOHYDRATE
INSOLUBLE FRACTION
µg/mg
3 2 1 C

4.3 Effect of trivalent chromium on S. abundans

Selected sublethal concentrations of trivalent chromium - 0.01, 0.02 and 0.03 ppm

Biomass

A general trend of decrease was noticed towards the end of growth phase from tenth day onwards for all treated samples (Fig.13). It was 18%, 5% and 33% less than the control at the end of growth phase. In 0.01 ppm growth was less than the control through out the growth phase. An increase of 88%, 55% and 23% was observed on the fourth, sixth and eighth for 0.02 ppm and it was 16% and 10% less than the control on tenth and twelfth day respectively. A decrease of 46%, 43% and 47% was noticed on the sixth, tenth and twelfth day. It was similar to the control on the sixth day.

Production

Production was 21% and 9% more than the control in 0.01 and 0.03 ppm at the end of growth phase but in 0.02 ppm production was less than the control throughout the growth phase (Fig.13). A decrease of 76% and 85% was observed on fourth and sixth day for 0.01 ppm but it was more than the control from eighth day onwards till the end of growth phase. In 0.03 ppm production was less than the control upto twelfth day followed by an increase of 9% at the end of growth. It was 85%, 71%, 53% and 45% less than the control on sixth, eighth, tenth and twelfth day respectively. Similar trend was followed for the pH also.

Respiration

Respiration was 60%, 40% and 47% less than the control at the end of growth phase. In 0.01 ppm respiration was less than the control throughout

the growth phase. Whereas in 0.02 ppm there was 185%, 137% and 139% increase on the sixth, eighth and tenth respectively. But it was 3% and 40% less than the control on twelfth and fourteenth day. An increase of 135% and 22% was observed on the fourth and sixth day for 0.03 ppm but it was less than the control from eighth day onwards.

Photosynthetic pigments

Chlorophyll-a

Chlorophyll-a pigment content was 37% and 10% more than the control at the end of growth phase for 0.01 and 0.03 ppm and it was similar to the control at the end for 0.02 ppm (Fig.14). In 0.01 ppm chlorophyll-a was found to be fluctuating. It was 34%, 27% and 18% more than the control on fourth, eighth and twelfth day but there was 43% and 36% decrease on sixth and tenth day respectively. An increase of 84% and 115% was noticed on second and fourth day and it was less than the control from sixth day onwards. In 0.03 ppm there was 62%, 30%, 78% decrease on sixth, eighth and tenth day. But on the twelfth day and fourteenth day there was 18% and 10% increase.

Chlorophyll-b

Chlorophyll-b was 114%, 95% and 67% more than the control for 0.01, 0.02 and 0.03 ppm at the end of growth phase (Fig.14). In 0.01 ppm chlorophyll-b was found to be fluctuating. It was 56%, 82% and 114% more than the control on fourth, tenth and fourteenth day and it was 62% and 28% less than the control on eighth and twelfth day respectively. A general trend of increase in chlorophyll-b was observed for 0.02 ppm upto tenth day. It

was 44% less than the control on the twelfth day. An increase of 219%, 6% and 67% was noticed on tenth, twelfth and fourteenth day for 0.03 ppm but it was 15% and 47% less than the control on sixth and eighth day.

Carotenoids

Compared with other photosynthetic pigments, carotenoids were lower than the control throughout the growth phase (Fig.14). At the end of growth phase it was 69%, 34% and 70% less than the control for 0.01, 0.02 and 0.03 ppm respectively. In 0.01 ppm carotenoid was 68%, 54%, 70% and 73% less than the control on sixth, eighth, tenth and twelfth day. A decrease of 36%, 50%, 52% and 61% was noticed in 0.02 ppm on sixth, eighth, tenth and twelfth day. In 0.03 ppm 78%, 80%, 9% decrease was noticed on eighth, tenth and twelfth day respectively.

Phaeophytin

Phaeophytin was 3% and 83% more than the control for 0.01 ppm and 0.03 ppm respectively but a decrease of 15% was noticed for 0.02 ppm (Fig.14). 0.01 ppm was closely following the control upto sixth day but 63% and 88% decrease was noticed on eighth and twelfth day followed by a marginal increase on the fourteenth day. Eventhough there was 115% increase on the eighth day for 0.02 ppm it was generally less than the control through out the growth phase. In 0.03 ppm phaeophytin was less than the control upto twelfth day but 84% increase was observed at the end of growth phase.

Photosynthetic end products

Carbohydrate

It was found that for all concentrations the carbohydrate content was more than the control with the peak on the eighth day (Fig.15). There was 155% and 176% increase on the eighth day for 0.01 and 0.03 ppm. In 0.03 ppm there was a sudden decrease on the tenth day and it was 45%, 69% and 38% less than the control on tenth, twelfth and fourteenth day. In 0.01 ppm there was 37% and 45% decrease on tenth and twelfth day. But 32% increase was noticed on the fourteenth day.

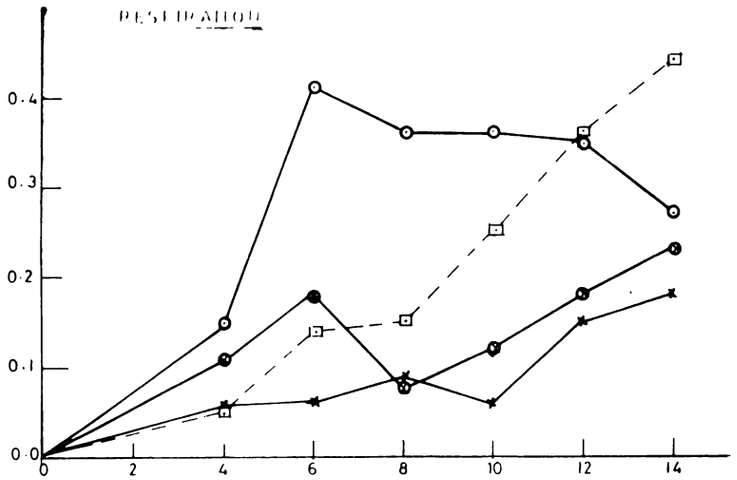
Protein

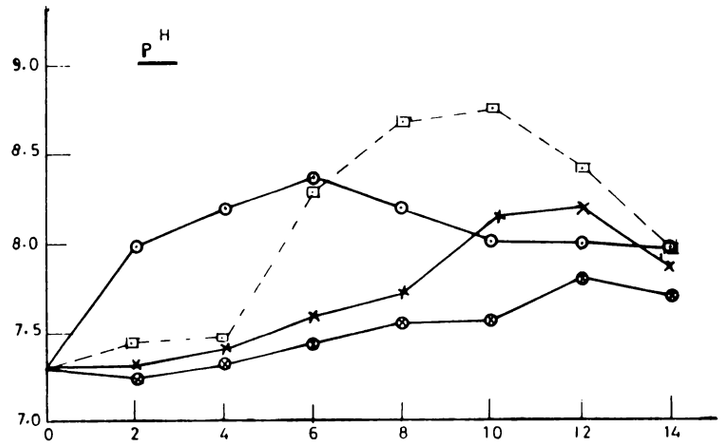
Protein content of the algae exposed to trivalent chromium was having more protein than the control through out the growth phase (Fig.15). The peak was on the eighth day and it was 92% and 164% more than the control for 0.01 and 0.03 ppm. In 0.01 ppm there was 23%, 199% and 43% increase on the tenth, twelfth and fourteenth day. In 0.03 ppm there was an increase in protein throughout the growth phase (5%, 186% and 53% increase on the tenth, twelfth and fourteenth day) inspite of the low production, low pigment content and low biomass.

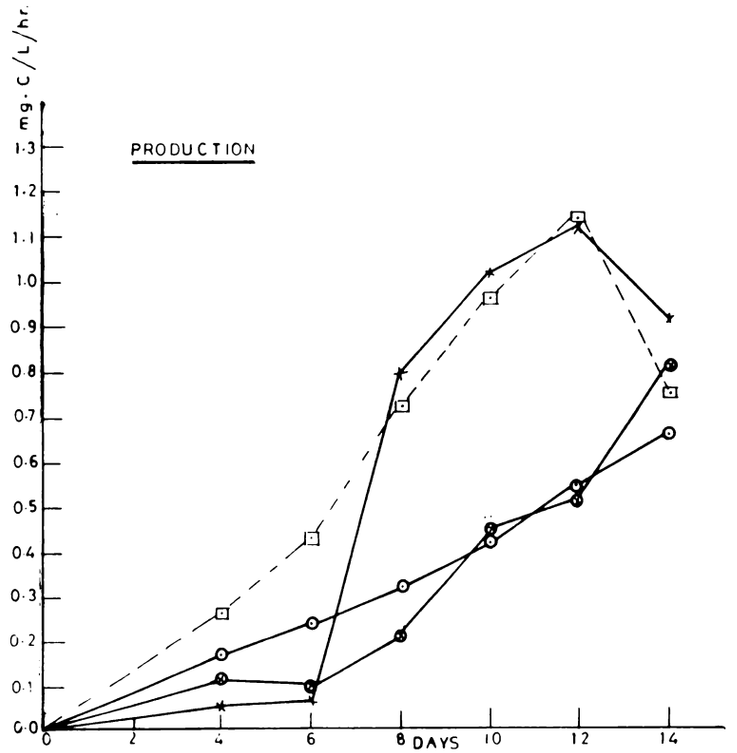
Lipid was less than the control in 0.02 and 0.03 ppm by 30% and 90% respectively and in 0.01 ppm it was similar to the control.

Of the nutrients, the phosphate uptake was 50%, 26% and 37% less than the control and the nitrate uptake was 28%, 20% and 18% less than the control for 0.01, 0.02 and 0.03 ppm respectively.

Fig. 13




P H
9.0
8.5
8.0
7.5
7.0
0
2
4
6
8
10
12
14


mg·C/L/hr.
PRODUCTION
1.3
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0
2
4
6
8 DAYS
10
12
14

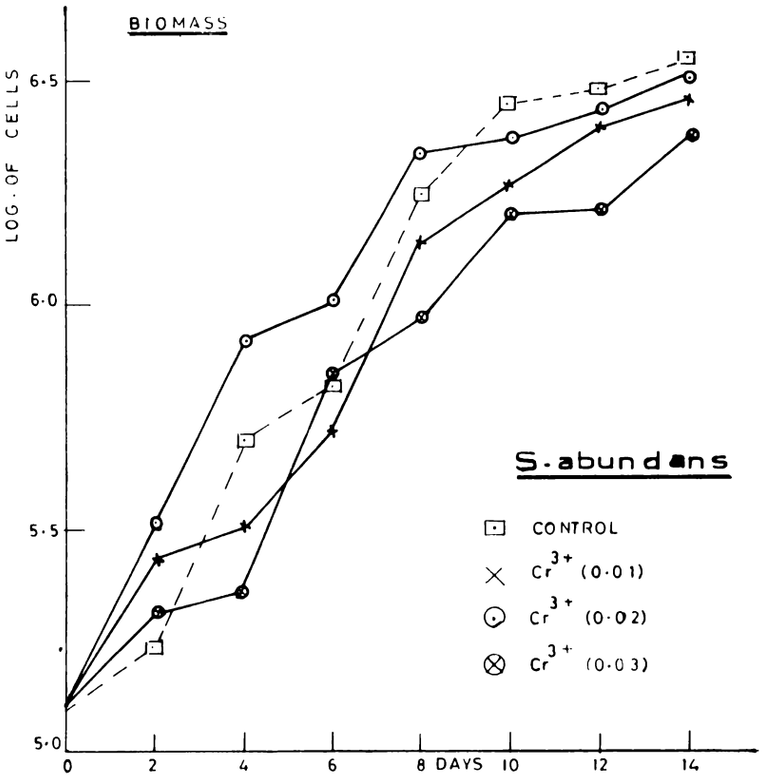
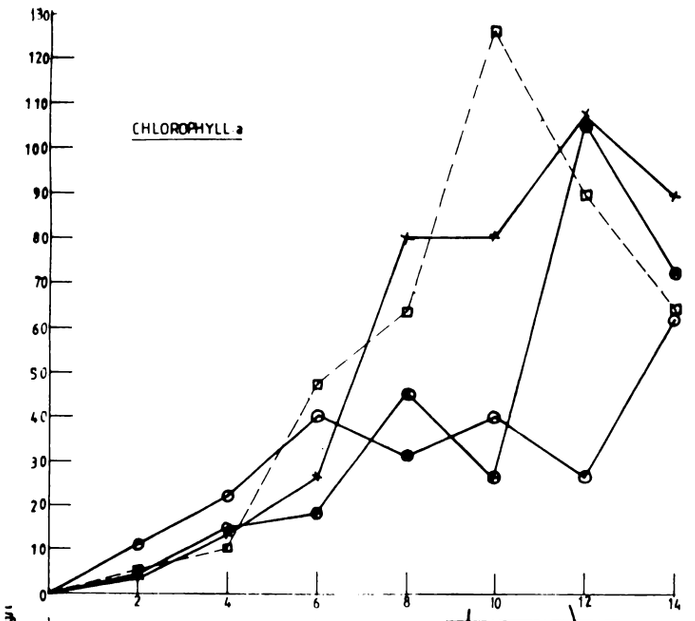
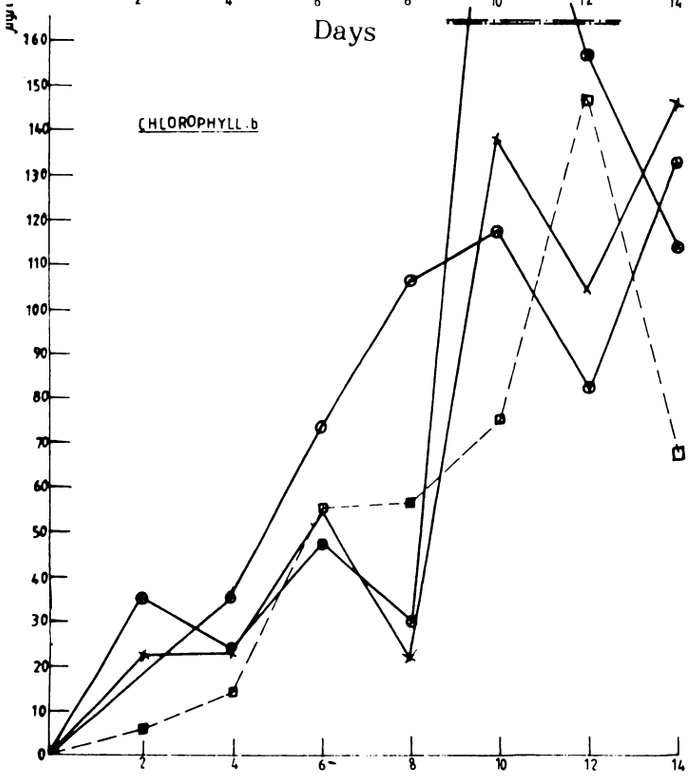
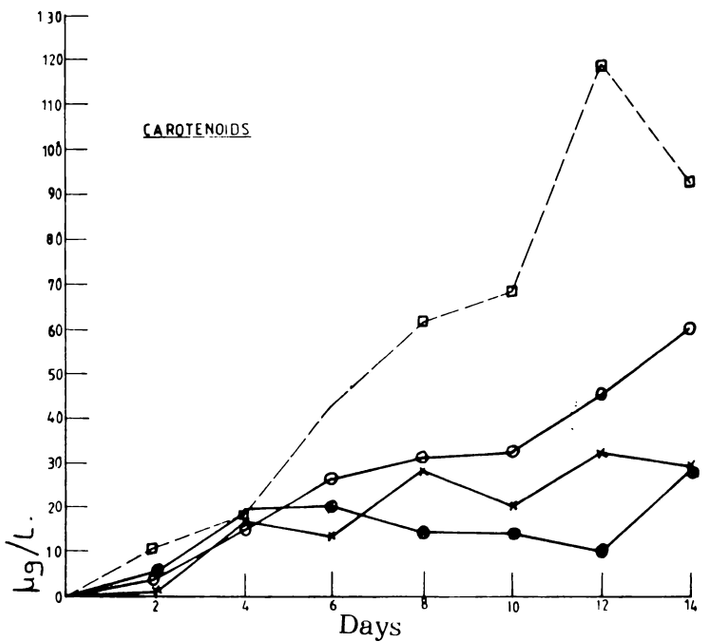

BIOMASS
LOG. OF CELLS
6.5
6.0
5.5
5.0
0
2
4
6
8 DAYS
10
12
14
S. abundans
CONTROL
Cr 3+ (0.01)
Cr 3+ (0.02)
Cr 3+ (0.03)

Fig. 14


CHLOROPHYLL a
Days


CHLOROPHYLL b


CAROTENOIDS
µg/L.
Days

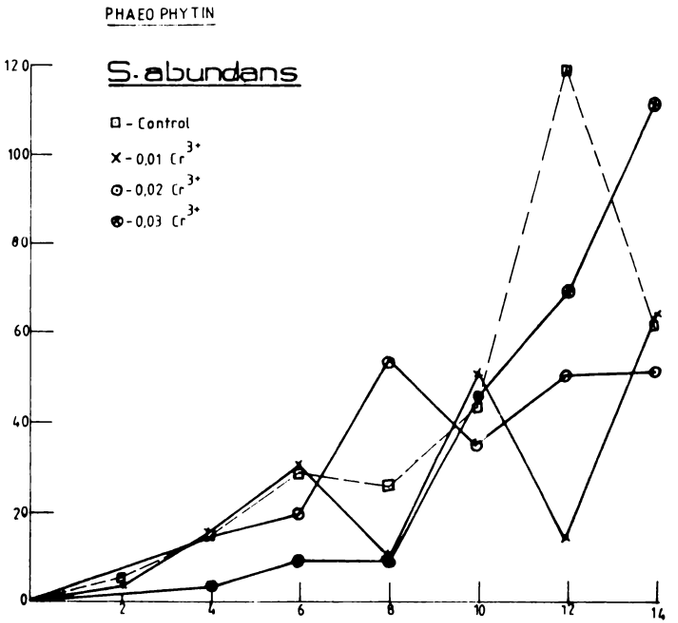
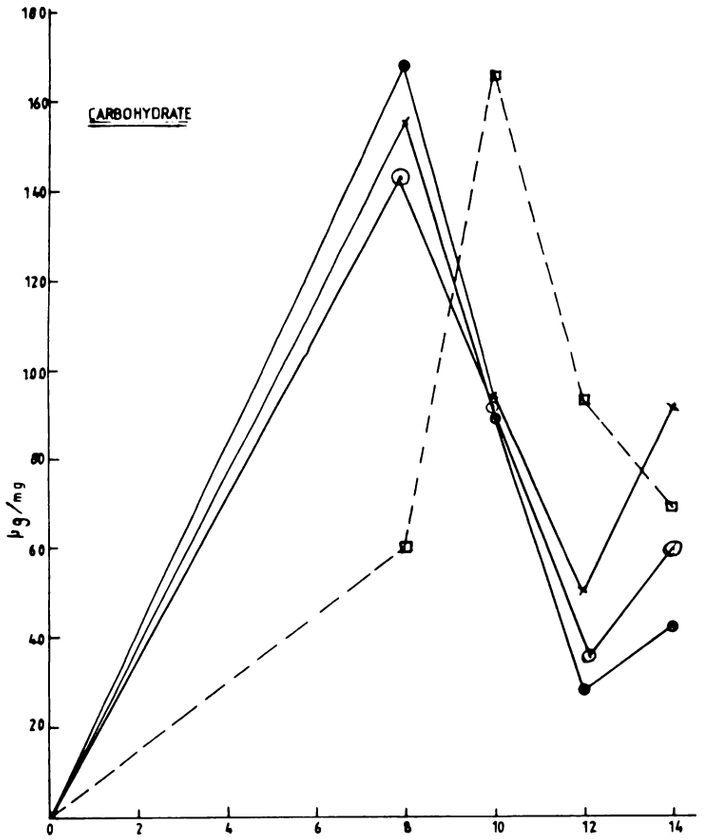
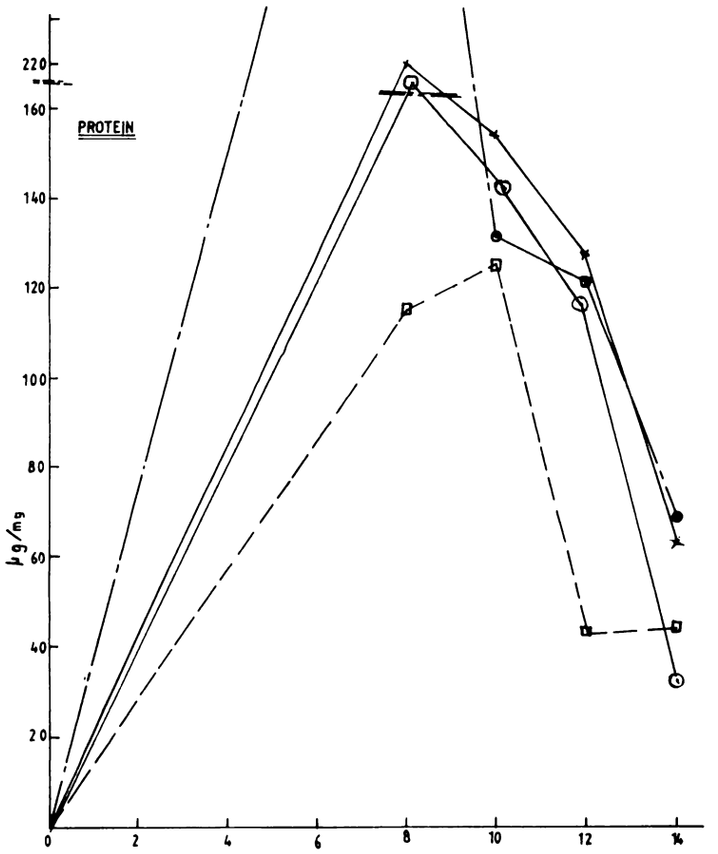
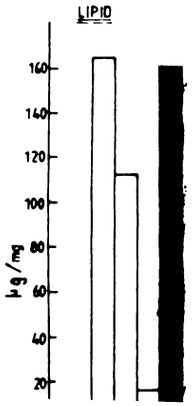

PHAEO PHYTIN
S. abundans
□ - Control
× - 0,01 Cr3+
⊙ - 0,02 Cr3+
⊛ - 0,03 Cr3+

Fig. 15





Days



S. abundans

C ⊡ CONTROL
1 × Cr^{3+}(0,01)
2 ○ Cr^{3+}(0,02)
3 ● Cr^{3+}(0,03)

Effect of trivalent chromium on N. clausii

Selected sublethal concentrations of trivalent chromium - 0.4, 0.6 and 0.8 ppm

Biomass

Biomass was more than the control at the end of growth phase for all treated samples. It was 33%, 30% and 13% more than the control for 0.4, 0.6 and 0.8 ppm (Fig.16). In 0.4 ppm through out the growth phase the biomass was more than the control with 213%, 33%, 13% and 33% more than the control on fourth, sixth, eighth and tenth respectively. In 0.6 ppm there was an initial decrease upto fourth day followed by 8% and 30% increase on sixth and tenth day. On the eighth day it was similar to the control. In 0.8 ppm the biomass was 40% less than the control on the fourth day. 47% and 14% increase was noticed on the 6th and eighth day. Thus 0.4 ppm chromium was stimulating the growth.

Production

Production was 18% and 35% more than the control in 0.4 ppm and 0.6 ppm at the end of growth phase but in 0.8 ppm there was 17% decrease in production (Fig.16). In 0.4 ppm through out the growth phase the production was more than the control. In 0.6 ppm production was 44% less than the control on the fourth day. But on the 6th and 8th day there was 23%, 73% increase. Similar to 0.6 ppm, in 0.8 ppm the production was 13% less than the control on the fourth day. Even though there was 70% and 37% increase on the sixth and eighth day, at the end of growth there was 17% decrease in production.

Respiration

Respiration was less than the control at the end of growth phase. The decrease was 51%, 52% and 31% for 0.4, 0.6 and 0.8 ppm respectively (Fig.16). In 0.4 ppm respiration was more than the control upto eighth day. The values being 99%, 92% and 25% more than the control. In 0.6 ppm it was closely following the control on the fourth and sixth day. Towards the end of growth phase, on the eighth and tenth day there was 7% and 30% decrease in respiration. In 0.8 ppm respiration was less than the control at the early stage on the fourth day and at the end of growth phase (tenth day). But 42% and 19% increase was noticed on the sixth and eighth day respectively.

Photosynthetic pigments

Chlorophyll-a

In all treated samples chlorophyll-a was less than the control at the end and early stage of growth phase. The values being 41%, 39% and 65% less than the control (Fig.17). In 0.4 ppm eventhough chlorophyll-a was less than the control on the second day it was 80%, 32% and 54% more than the control on fourth, sixth and eighth day respectively. In 0.6 ppm pigment was less than the control upto fourth day followed by 3% and 36% increase on the sixth and eighth day. Similarly in 0.8 ppm also there was 55% and 48% increase on sixth and eighth day followed by sharp decrease of 65% at the end of growth phase.

Chlorophyll-c

Similar to chlorophyll-a, chlorophyll-c was also less than the control at the end of growth phase. It was 47%, 44% and 67% less than the control

for 0.4, 0.6 and 0.8 ppm respectively (Fig.17). In 0.4 ppm the chlorophyll-c was more than the control upto eighth day. The values being 19%, 71%, 52% and 43% more than the control. But in 0.6 ppm upto fourth day chlorophyll-c was less than the control followed by 68% and 58% increase on sixth and eighth day respectively. Similarly in 0.8 ppm also chlorophyll-c was less than the control upto fourth day. 63% and 43% increase was noticed on sixth and eighth day.

Carotenoids

Carotenoids were less than the control in the early stage upto second day and at the end of growth phase. 4%, 24% and 58% decrease was noticed at the end for 0.4 ppm, 0.6 and 0.8 ppm respectively (Fig.17). In 0.4 ppm eventhough there was an initial decrease in the second day carotenoids were 96%, 43% and 72% more than the control on fourth, sixth and eighth day respectively. In 0.6 ppm carotenoids were 84% and 65% less than the control on second and fourth day but on sixth and eighth day there was 12% and 45% increase. In 0.8 ppm also similar to 0.6 ppm carotenoids were less than the control upto fourth day followed by 50% and 77% increase on sixth and eighth day. At the end of growth phase there was a sharp decrease of 58%.

Phaeophytin

Phaeophytin was marginally more than the control for 0.4 and 0.6 ppm at the end of growth phase. Whereas in 0.8 ppm there was a sharp decrease of 98% at the end of growth phase (Fig.17). In 0.4 ppm though there was an initial decrease on the second day, there was 46%, 23% and 41% increase on fourth, sixth and eighth day respectively. In 0.6 ppm there was an initial

decrease of 31% and 2% on the second and fourth day. Phaeophytin pigment content was increased from sixth day onwards till the end of growth phase. In 0.8 ppm there was 48% and 87% decrease on second and fourth day followed by 12% and 44% increase on the sixth and eighth day respectively.

Carbohydrate

The acid soluble fraction of carbohydrate was more than the control for 0.8 ppm though there was a marginal increase on the fourth and sixth day. It was 9%, 8%, 27% and 38% more than the control on fourth, sixth, eighth and tenth day respectively (Fig.18). In 0.4 and 0.6 ppm there was 46% and 20% decrease on the fourth day followed by sudden increase on the sixth and eighth day respectively. At the end of growth phase it was 8% and 64% less than the control. Thus in 0.6 ppm carbohydrate was maximum on the eighth day, it was less than the control on fourth and tenth day.

In all treated samples alkali soluble fraction was maximum on the fourth day and was less than the control with the aging of the culture. It was 182%, 273% and 399% more than the control on the fourth day for 0.4, 0.6 and 0.8 ppm respectively (Fig.18). In 0.4 ppm, carbohydrate was 14%, 87% and 85% less than the control on the sixth and eighth and tenth day respectively. In 0.6 ppm and 0.8 ppm alkali fraction of carbohydrate was 66% and 45% more than the control on the sixth day. On the eighth and tenth day it was less than the control for both treatments. On the eighth day it was 25% and 20% less than the control. On the tenth day 58% and 75% decrease was observed.

Insoluble fraction of carbohydrate was more than the control through out the growth phase for 0.4 ppm. It was maximum on the fourth day and

it was found to be 87% more than the control. On the sixth, eighth and tenth day 26%, 10% and 73% increase was observed (Fig.18). In 0.6 ppm there was only a marginal increase (3%) on the sixth day. On the fourth, eighth and tenth day there was 14%, 53% and 26% decrease. Whereas in 0.8 ppm there was 7%, 21% increase on the fourth and sixth day. On the eighth and tenth day it was 25% and 50% less than the control.

Protein

Generally protein content was more than the control for all treated samples upto sixth day (Fig.18). At the end of growth phase it was 31%, 79% and 78% less than the control for 0.4, 0.6 and 0.8 ppm. In 0.4 ppm protein was more than the control upto eighth day. It was 174% and 58% more than the control on sixth and eighth day. In 0.6 ppm it was 28% and 100% more than the control on fourth and sixth day. It was 34% and 78% less than the control at the end of growth phase on eighth, tenth day. In 0.8 ppm there was 31%, 120% and 5% increase on fourth, sixth and eighth day. There was 80% decrease in protein at the end of growth phase.

Lipid content of the alga was observed and it was less than the control for all treated samples. Minimum decrease of about 40% was noticed for 0.4 ppm.

Of the nutrients, phosphate uptake was less than the control for all treated samples. It was 33%, 50% and 62% less than the control for 0.4, 0.6 and 0.8 ppm respectively.

Fig. 16

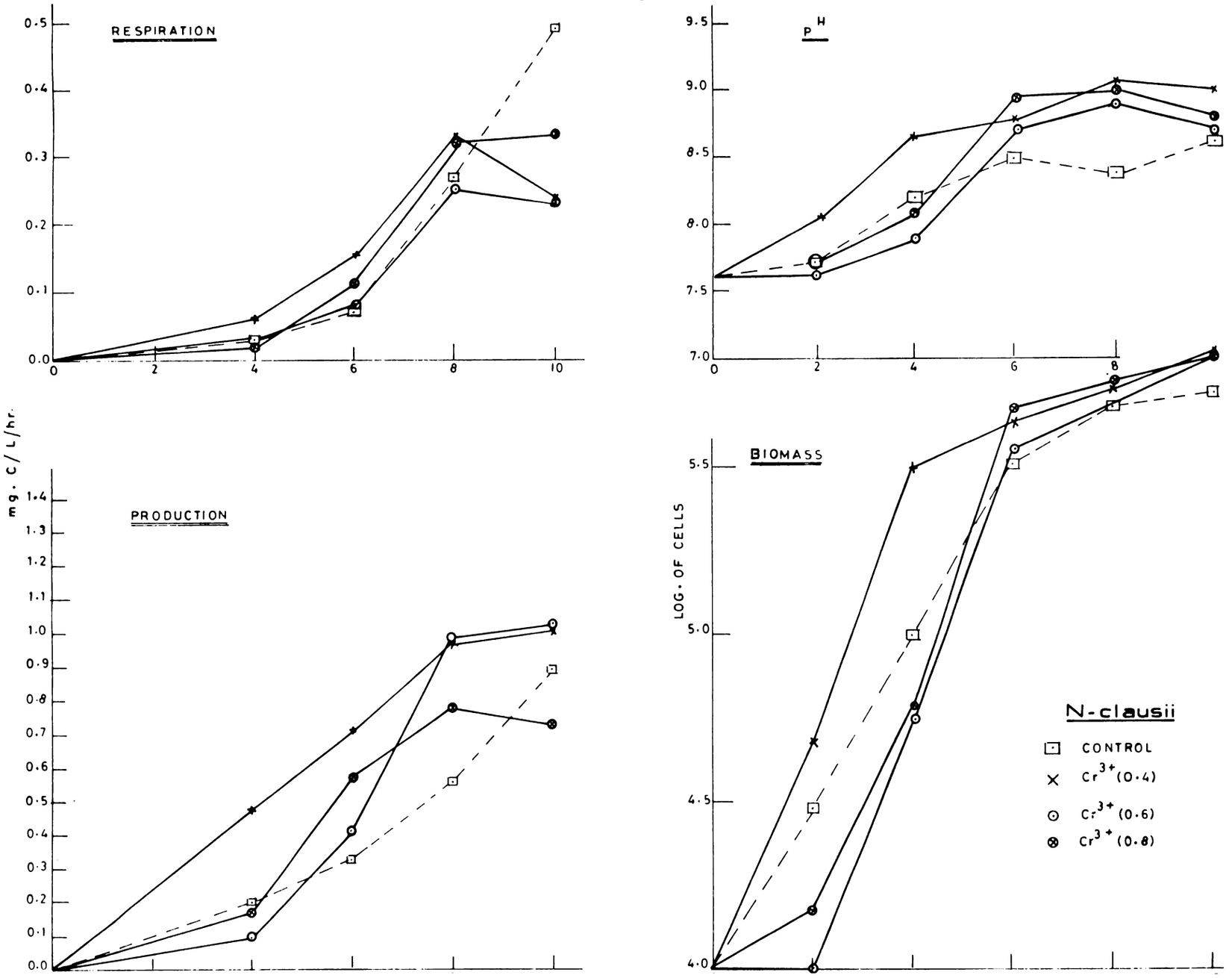
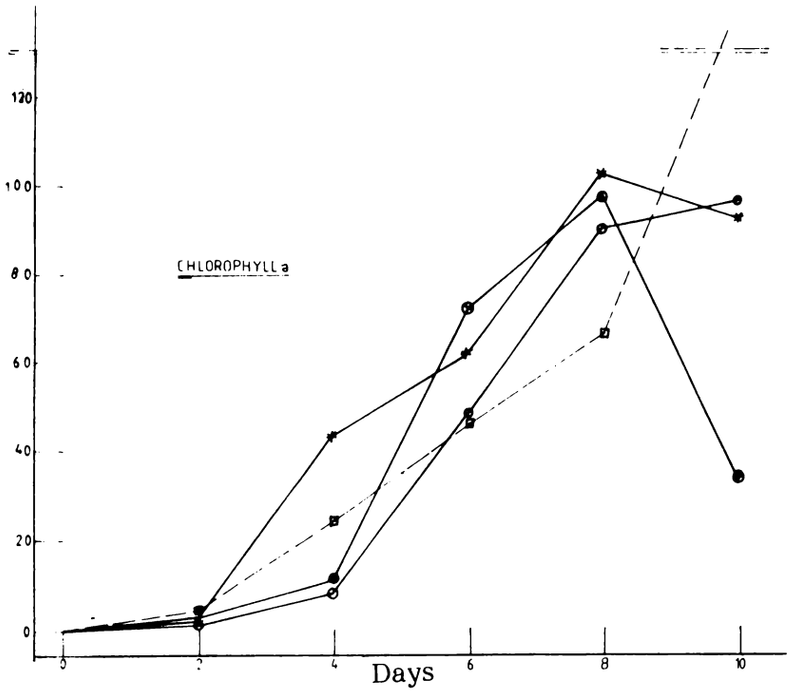
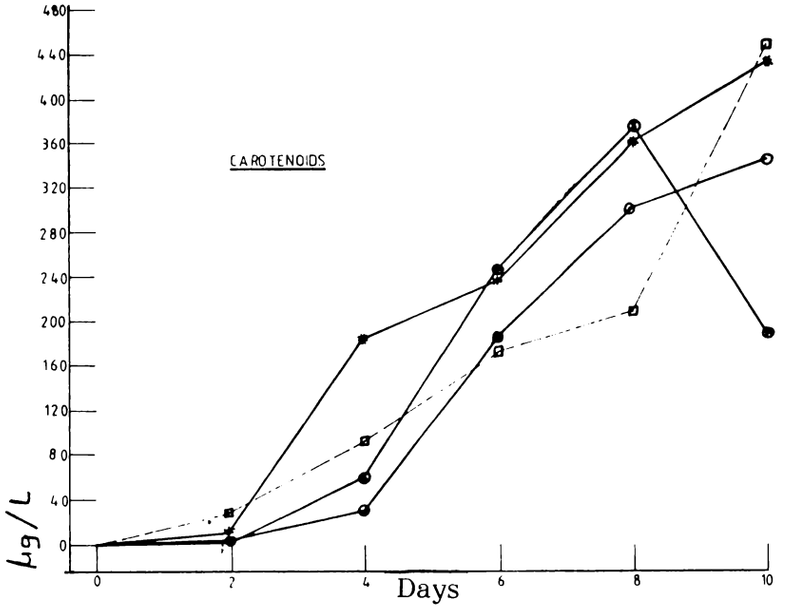
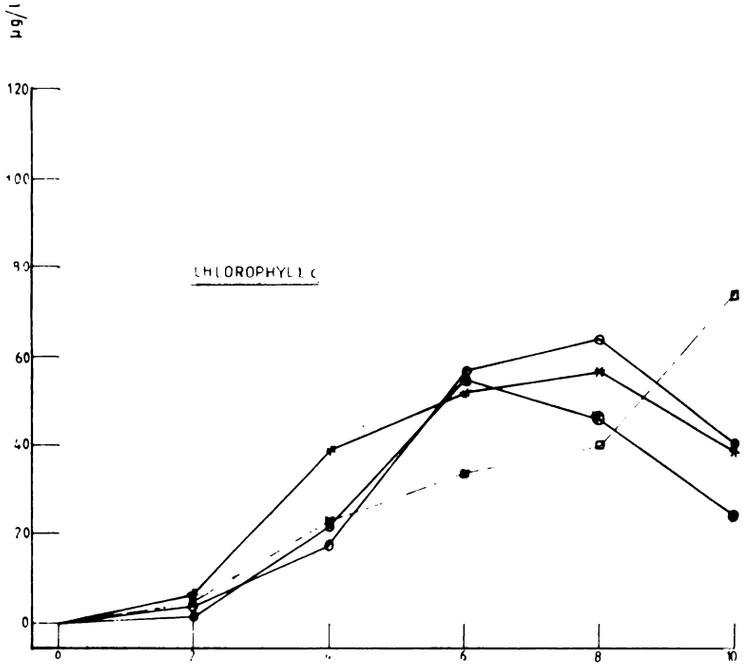

RESPIRATION
PH
PRODUCTION
mg. C / L/hr.
BIOMASS
LOG. OF CELLS
N-clausii
CONTROL
Cr3+(0.4)
Cr3+(0.6)
Cr3+(0.8)

Fig. 17


CHLOROPHYLL a
Days


CAROTENOIDS
µg/L
Days


µg/l

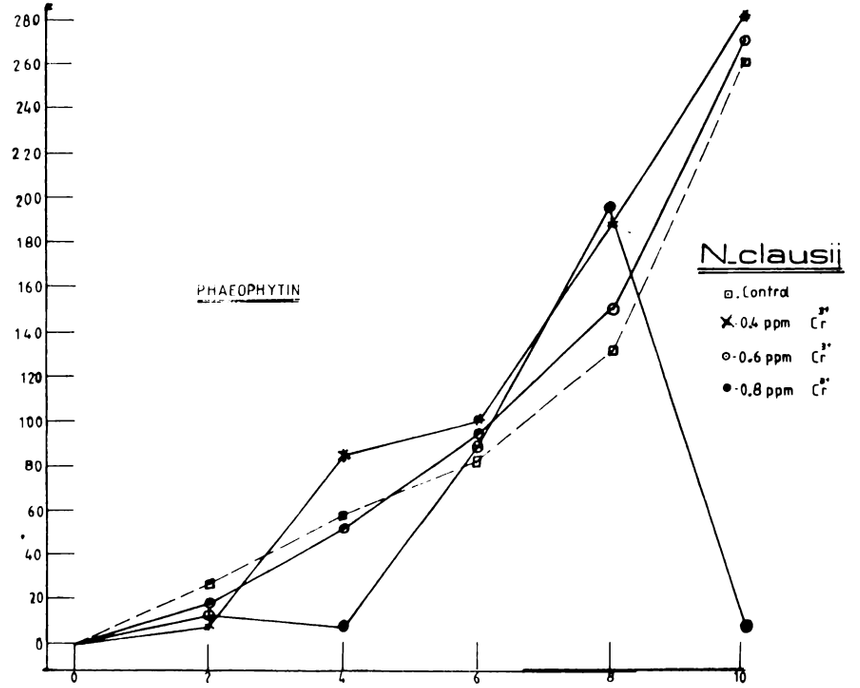
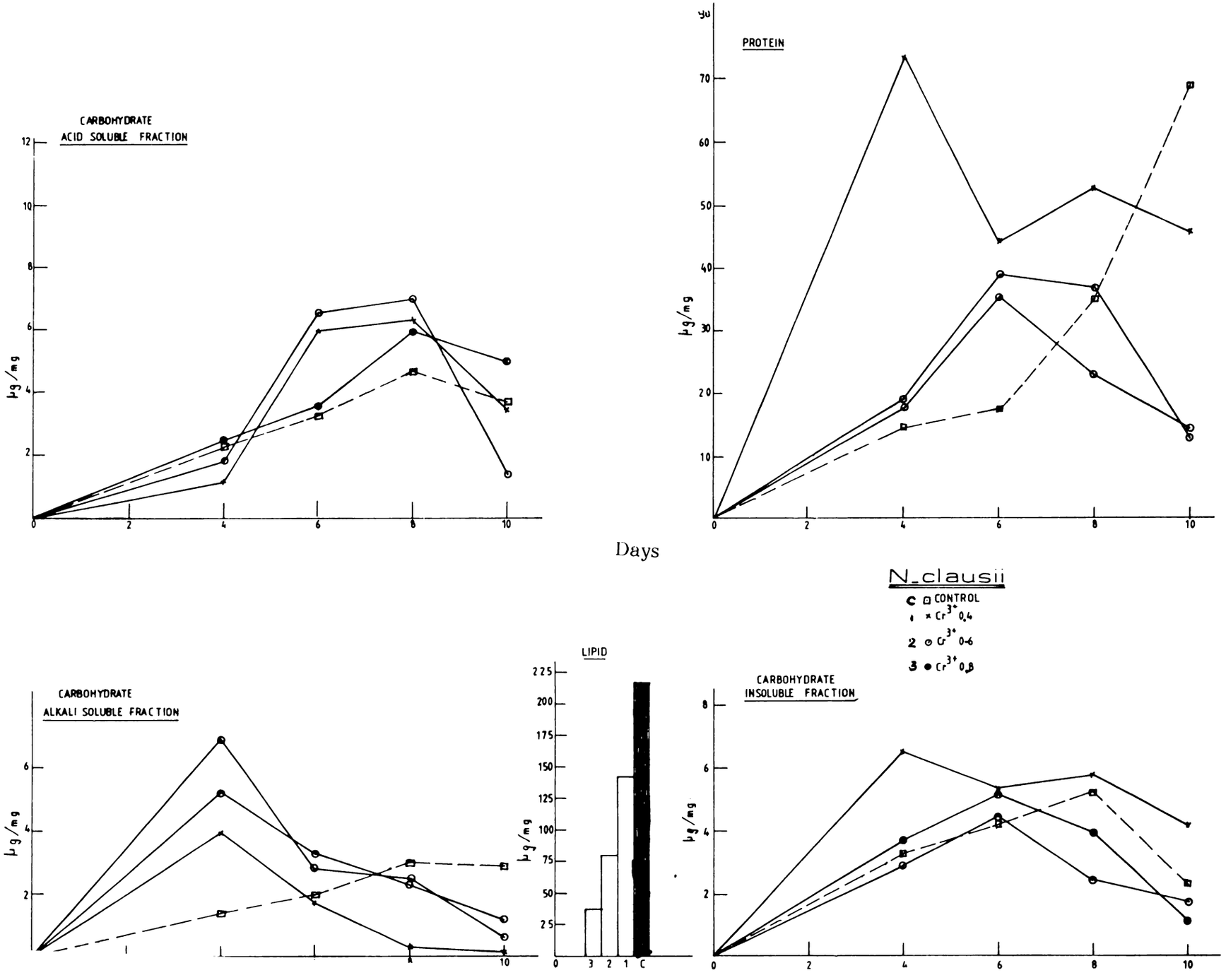

PHAEOPHYTIN
N.clausii
□. Control
✕. 0.4 ppm Cr3+
○. 0.6 ppm Cr3+
●. 0.8 ppm Cr3+

Fig. 18


CARBOHYDRATE
ACID SOLUBLE FRACTION
µg/mg
PROTEIN
µg/mg
Days
N_clausii
C □ CONTROL
1 × Cr3+ 0,4
2 ⊙ Cr3+ 0-6
3 ● Cr3+ 0,8
CARBOHYDRATE
ALKALI SOLUBLE FRACTION
µg/mg
LIPID
µg/mg
3 2 1 C
CARBOHYDRATE
INSOLUBLE FRACTION
µg/mg

4.4 Effect of hexavalent chromium on S. abundans

Selected sublethal concentrations of hexavalent chromium - 0.05, 0.1 and 0.15 ppm

Biomass

Biomass was less than the control by 13%, 3% and 37% for 0.05, 0.1 and 0.15 ppm respectively (Fig.19). In 0.05 ppm the growth was less than the control upto tenth day and was closely following the control towards the end of growth phase. The growth was found to be fluctuating in 0.1 ppm. Eventhough it was 51% less than the control on the fourth day, on the second and sixth day it was 12% and 34% more than the control and towards the end it was similar to the control. In 0.15 ppm the growth was less than the control through out the growth phase by 57%, 35% and 25% on fourth, tenth and twelfth day respectively.

Production

Production was generally more than the control with the aging of the culture for all treatments. At the end of growth it was 235%, 138% and 96% more than the control for 0.05, 0.1 and 0.15 ppm respectively (Fig.19). For 0.05 ppm the production was 52% and 57% less than the control on fourth and sixth day. 90%, 75% and 101% increase was observed on the eighth, tenth and twelfth day. Thus the peak in production was noticed in 0.05 ppm. A decrease of 76% and 66% was noticed in production on fourth, sixth and eighth day for 0.1 ppm. On the tenth and twelfth day 15% and 70% increase was observed. The production was 77%, 64% and 75% less than the control on fourth, sixth and eighth day for 0.15 ppm. On the tenth and twelfth day

17% and 37% increase in production was noticed. pH was also more than the control with the aging of the culture.

Respiration

Respiration was more than the control through out the growth phase for 0.05 and 0.1 ppm. At the end of growth phase 47% increase was observed for 0.1 and 0.15 ppm respectively and 7% increase for 0.05 ppm (Fig.19). In 0.15 ppm the respiration was more than the control upto eighth day followed by 53% decrease on the tenth day. 46% increase was noticed on the twelfth day, producing the peak at the end of growth phase .

Photosynthetic pigments

Chlorophyll-a

Generally chlorophyll-a was less than the control upto tenth day for all treated samples followed by sudden increase with the aging of the culture. A peak was observed at the end of growth phase. At the end of growth phase 168%, 272% and 44% increase was noticed for 0.05, 0.1 ppm and 0.15 ppm respectively (Fig.20). Chlorophyll-a was maximum in 0.1 ppm at the end of growth phase. In 0.15 ppm chlorophyll-a was 46%, 77% less than the control on fourth and sixth day and eighth day. 78% and 11% decrease was observed for eighth and tenth day. Thus in 0.15 ppm chlorophyll-a was less than the control through out the growth phase except on the last day when it was 44% more than the control.

Chlorophyll-b

Chlorophyll-b was 37%, 110% and 37% more than the control at the end of growth phase for 0.05, 0.1, 0.15 ppm respectively eventhough it was

less than the control upto sixth day (Fig.20). In 0.05 ppm chlorophyll-b was 16%, 25% and 36% less than the control on fourth, sixth and twelfth day. On the eighth and tenth day it was 65% and 46% more than the control. In 0.15 ppm chlorophyll-b was generally less than the control except on the tenth and fourteenth day. On the tenth day and fourteenth day 12% and 37% increase was noticed. In 0.1 ppm similar to 0.05 ppm chlorophyll-b was more than the control on the eighth, tenth, and fourteenth day. The values being 41%, 59%, 37% more than the control.

Carotenoids

In 0.1 ppm carotenoids were more than the control through out the growth phase. It was 59% more than the control at the end of growth phase whereas in 0.05 ppm and 0.15 ppm carotenoids were 12% and 24% lower than the control on fourteenth day (Fig.20). In 0.05 ppm carotenoids were less than the control, but a marginal increase of 6% and 12% was observed on the fourth and tenth day. In 0.15 ppm carotenoids were less than the control through out the growth phase. Thus it was observed that 0.1 ppm chromium stimulated, whereas 0.15 ppm completely inhibited the carotenoid production.

Phaeophytin

Phaeophytin was less than the control at the end of growth phase for all treated samples (Fig.20). In 0.05 ppm there was 24%, 88% and 98% increase on the sixth, eighth and tenth day respectively. But there was a sudden decrease of 43% on the twelfth day. In 0.1 ppm the pigment content was less than the control through out the growth phase except on the eighth and tenth day. Phaeophytin was less than the control through out the growth phase in 0.15 ppm.

Photosynthetic end products

Carbohydrate

The carbohydrate was more than the control on the eighth day and at the end of growth phase for all concentrations selected. It was 112%, 96% and 178% more than the control for 0.05, 0.1 and 0.15 ppm on the eighth day (Fig.21). At the end of growth phase 16%, 55% increase was observed for 0.05 and 0.1 ppm. For 0.15 ppm it was similar to the control. In 0.05 ppm the initial increase was followed by a peak on the tenth day which was 6% more than the control. But 12% decrease was observed on the twelfth day. In 0.01 ppm the initial increase was followed by 10% and 29% decrease on the tenth and twelfth day. In 0.15 ppm 21% and 37% decrease was observed on tenth and twelfth day.

Protein

In all treatments protein content was more than the control with the maximum peak on the eighth day. Protein content was maximum in 0.1 ppm on the eighth day. It was 129% more than the control. On the eighth day protein was 94%, 129% and 116% more than the control for 0.05, 0.1 and 0.15 ppm respectively (Fig.21). At the end of growth phase eventhough the protein content decreased, it was 71%, 92% and 52% more than the control. Thus in 0.15 ppm though the pigments were less than the control an increase in the protein content was observed.

For all treatments the lipids were less than the control. 50%, 73% and 77% decrease was noticed for 0.05, 0.1 and 0.15 ppm respectively.

In 0.05 ppm and 0.1 ppm the nitrate and phosphate uptake was more than the control whereas in 0.15 ppm nutrient uptake was reduced markedly. It was 51% less than the control.

Fig. 19

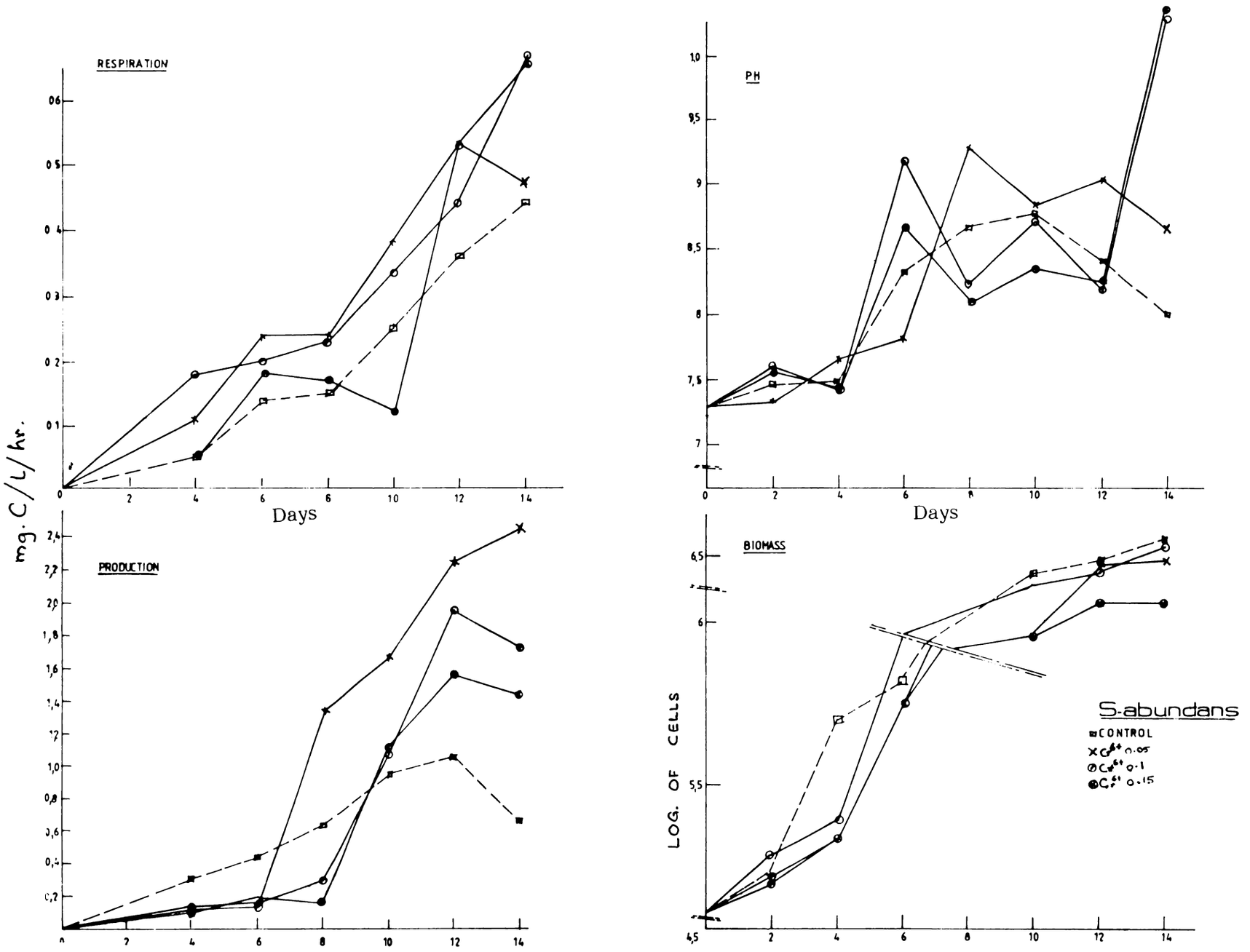

RESPIRATION
mg. C/l/hr.
Days
PH
Days
PRODUCTION
BIOMASS
LOG. OF CELLS
S-abundans
CONTROL

Fig.20

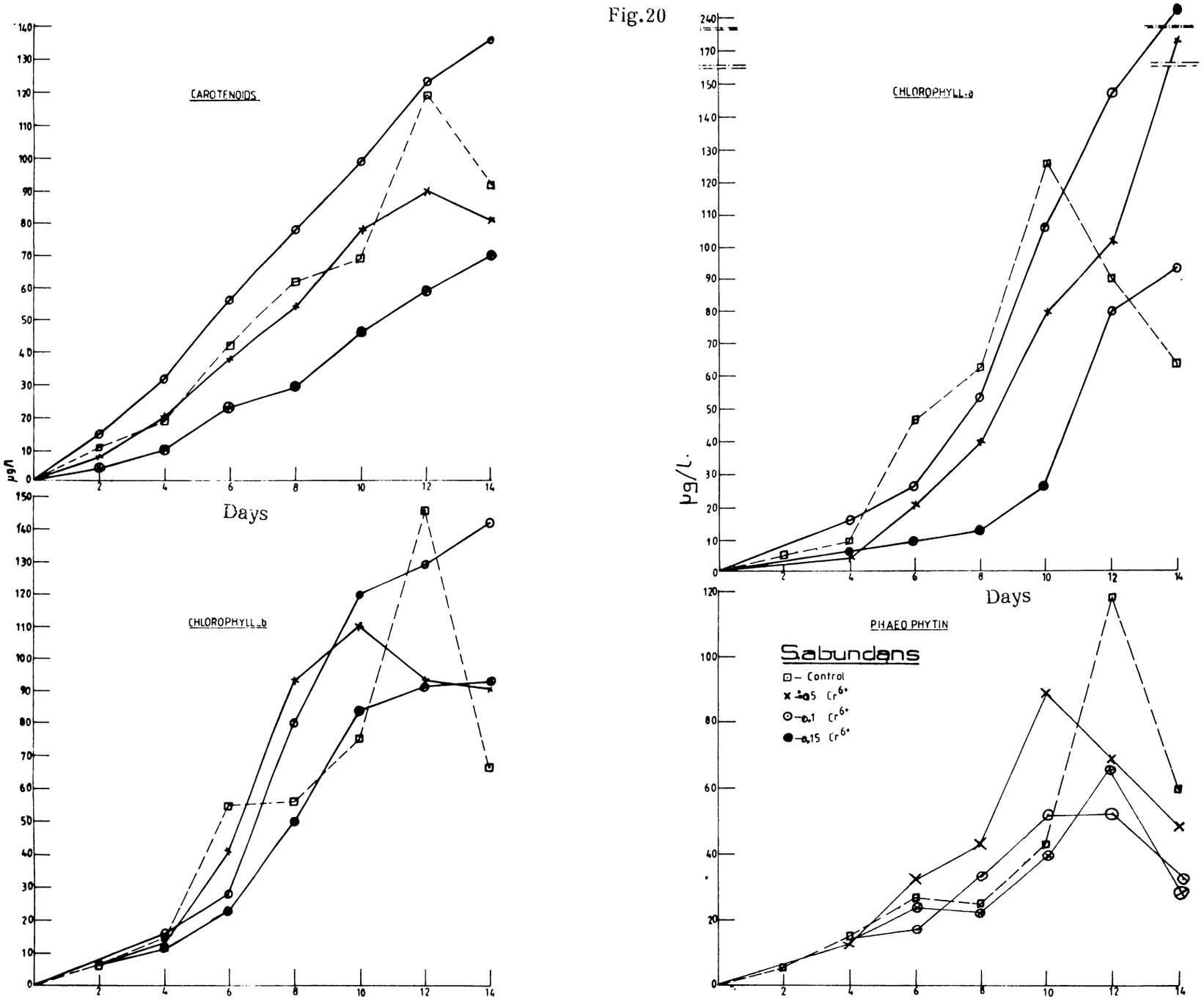
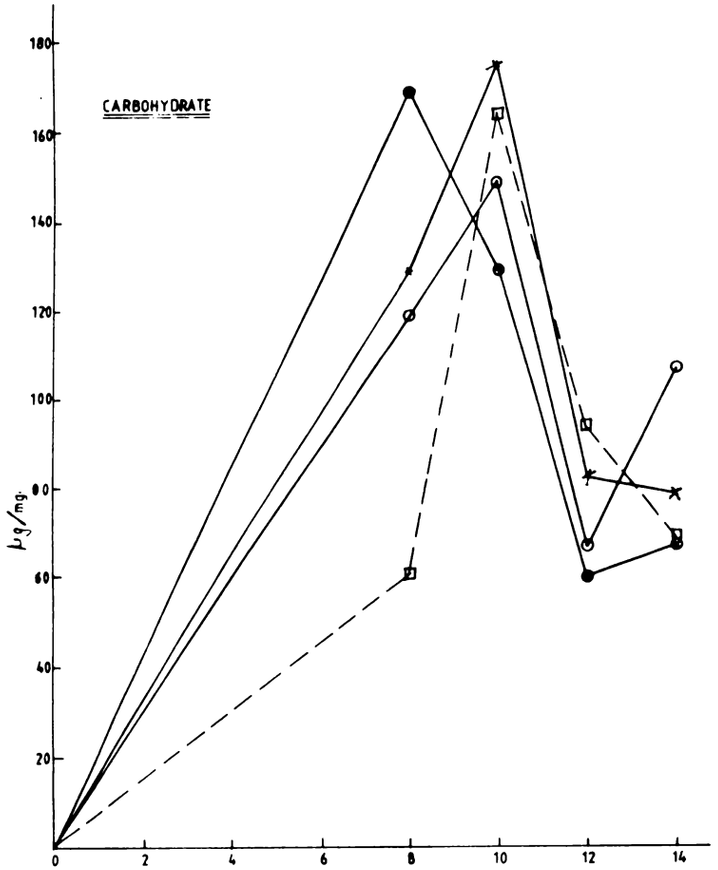
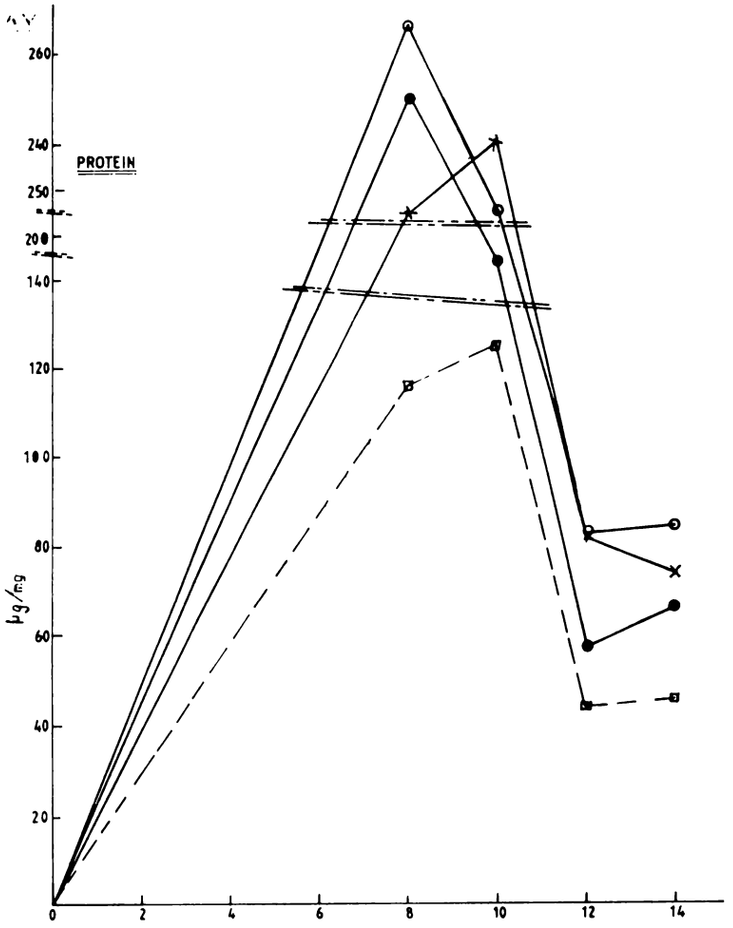
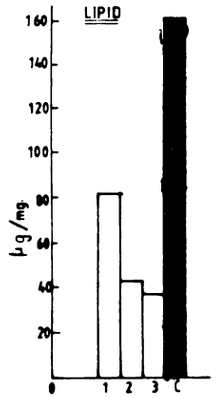

CAROTENOIDS
µg/l
Days
CHLOROPHYLL-b
CHLOROPHYLL-a
µg/l.
Days
PHAEO PHYTIN
Sabundans
□– Control
×–0.5 Cr6+
⊙–0.1 Cr6+
●–0.15 Cr6+

Fig.21





Days



S-abundans

C ▫ CONTROL

1 ✕ Cr^{6+} (0,05)

2 ○ Cr^{6+} (0,1)

3 ● Cr^{6+} (0,15)

Effect of hexavalent chromium on N. clausii

Selected sublethal concentrations of hexavalent chromium - 0.2, 0.4, 0.6 and 0.7 ppm.

Biomass

In all treated samples the growth was less than the control at the end of growth phase. In 0.2 ppm and 0.4 ppm biomass was more than the control upto sixth day. On the eighth day 15% and 17% decrease was observed in 0.2 and 0.4 ppm respectively (Fig.22). In 0.6 and 0.7 ppm the growth was less than the control throughout the growth phase. The growth was 65% and 60% lower than the control on fourth day in 0.6 and 0.7 ppm. On the eighth day it was 49% and 13% less than the control.

Production

Net production of the algae was 86% and 89% more than the control on the fourth day for 0.2 and 0.4 ppm. Whereas in 0.6 and 0.7 ppm 80% and 55% decrease was noticed (Fig.22). On the tenth day 83%, 42%, 20% decrease was observed for 0.2, 0.4 and 0.6 ppm. In 0.7 ppm 26% increase was noticed. The production was less than the control throughout the growth phase for 0.6 ppm. In 0.7 ppm eventhough the production 55% less than the control 75%, 24% and 28% increase was noticed on the sixth, eighth and tenth day respectively.

Respiration

Respiration was 30%, 82% and 32% less than the control for 0.4, 0.6 and 0.7 ppm at the end of growth phase. Whereas in 0.2 ppm respiration

was more than the control throughout the growth phase. At the end of growth phase it was 14% more than the control. In 0.4 ppm eventhough there was marked increase (81%) on the fourth day, respiration was less than the control throughout the growth phase. Similarly in 0.6 and 0.7 ppm also respiration was less than the control with the aging of the culture. In 0.6 ppm there was 54%, 35% and 81% decrease whereas in 0.7 ppm the decrease was 22%, 15% and 32% on sixth, eighth and tenth day respectively.

Photosynthetic pigments

Chlorophyll-a

In all treatments chlorophyll-a pigment was less than the control at the end of growth phase. It was 32%, 42%, 51% and 33% less than the control in 0.2, 0.4, 0.6 and 0.7 ppm respectively. In 0.6 ppm chlorophyll-a was less than the control throughout the growth phase. It was 77%, 25% and 26% less than the control on the fourth, sixth, and eighth day respectively. In 0.2 eventhough chlorophyll-a was less than the control on the second day, it was 105%, 27% and 11% more than the control on fourth, sixth and eighth day. Similarly in 0.4 ppm also 60%, 36% and 28% increase was noticed on fourth, sixth and eighth day. In 0.7 ppm chlorophyll-a was less than the control upto fourth day followed by 27% and 32% increase on the sixth and eighth day. Thus it was observed that in 0.6 ppm chlorophyll-a was less than the control throughout the growth phase.

Chlorophyll-c

Similar to chlorophyll-a, chlorophyll-c was less than the control at the end of growth phase. It was 56%, 50%, 49% and 48% less than the control

for 0.2, 0.4, 0.6 and 0.7 ppm respectively (Fig.23). In 0.2 and 0.4 ppm the chlorophyll-c was less than the control throughout the growth phase. In 0.6 ppm pigment was more than the control upto eighth day. The maximum increase in chlorophyll-c pigment... was noticed in sixth and eighth day in 0.6 ppm. It was 84% and 59% more than the control. In 0.7 ppm chlorophyll-c was closely following the control upto fourth day followed by 15% and 14% more than the control on sixth and eighth day respectively.

Carotenoids

Carotenoids were maximum at the end of growth phase for all treated samples but it was 17%, 20%, 34% and 15% less than the control for 0.2, 0.4, 0.6 and 0.7 ppm respectively (Fig.23). In 0.6 ppm carotenoids were less than the control throughout the growth phase. In 0.2 ppm though there was as initial decrease in the pigment content, it was 61%, 11% and 15% more than the control on fourth, sixth and eighth day. In 0.4 ppm similar to 0.2 ppm carotenoids were less than the control on the second day followed by 49%, 24% and 46% increase on the fourth, sixth and eighth day. In 0.7 ppm carotenoids were less than the control upto fourth day followed by 17% and 54% increase on the sixth and eighth day respectively.

Phaeophytin

A general trend of decrease was noticed in the early stage and at the end of growth phase for 0.2, 0.4 and 0.6 ppm. In 0.7 ppm at the end of growth phase pigment content was closely following the control (Fig.23). In 0.2, 0.4 and 0.6 ppm 28%, 73%, 20% decrease was observed at the end

of growth phase. In 0.2 ppm eventhough a peak of 177% was found on the sixty day, it was less than the control on second, fourth and eighth day. In 0.4 ppm phaeophytin was 44%, 30% and 73% less than the control on the fourth, eighth and tenth day. But 28% increase was noticed on the sixth day. In 0.6 ppm there was 33% increase on the fourth day followed by 80% and 40% and 20% decrease on the sixth, eighth and tenth day. But in 0.7 ppm phaeophytin was more than the control from sixth day onwards. It was 40% more than the control on sixth and eighth day. At the end it was similar to the control.

Carbohydrate

The acid soluble fraction of carbohydrate was more than the control in the early phase followed by sharp decrease towards the end of growth phase. At the end of growth phase it was 26%, 25%, 88% and 45% less than the control for 0.2, 0.4, 0.6 and 0.7 ppm respectively (Fig.24). On the fourth day there was 155%, 50%, 228%, 76% increase for 0.2, 0.4, 0.6 and 0.7 ppm. In 0.4 ppm the acid fraction was less than the control from sixth day onwards. But in all other treatments such as 0.2, 0.6 and 0.7 ppm, the decrease was from eighth day. On the eighth day there was 22%, 56% and 33% decrease for 0.2, 0.6 and 0.7 ppm respectively.

Similar to acid fraction, the alkali fraction was less than the control towards the end of growth phase on the eighth and tenth day (Fig.24). In 0.4 ppm throughout the growth phase the alkali fraction was less than the control. It was 46%, 66%, 62% and 76% less than the control on fourth, sixth, eighth and tenth day. In 0.2 ppm though there was 59% increase on

the fourth day it was less than the control throughout the growth phase. In 0.6 ppm alkali fraction was less than the control on fourth eighth and tenth day, but 69% increase was noticed on the sixth day. In 0.7 ppm there was 125% and 15% increase on the fourth and sixth day followed by a sudden decrease of 40% and 90% towards the end of growth phase on eighth and tenth day respectively.

Insoluble fraction of carbohydrate was less than the control throughout the growth phase for 0.2, 0.4 and 0.6 ppm. In 0.7 ppm there was 24% increase on the sixth day followed by 18% and 25% decrease on the eighth and tenth day (Fig.24).

Protein

Protein content was less than the control for all treated samples. It was 65%, 73%, 98% and 63% less than the control for 0.2, 0.4, 0.6 and 0.7 ppm respectively. In 0.2 and 0.4 ppm there was 119% and 79% increase on the fourth day and 111% and 66% increase on the sixth day. With the aging of the culture protein content decreased. In 0.7 ppm it was 20% lower than the control on the fourth day followed by a sudden increase of 144% and 16% on the sixth and eighth day. At the end of growth it was less than the control.

Lipid content of the algae was less than the control for all treated samples with the maximum decrease for 0.7 ppm. It was 93% less than the control. In 0.2 ppm it was 30% less than the control. In 0.4 and 0.6 ppm there was 40% and 60% decrease.

In the case of nutrient uptake, the phosphate uptake was less than the control for all treated samples. It was 76%, 72%, 60% and 52% less than the control. The nitrate uptake showed only a marginal decrease.

Fig.22

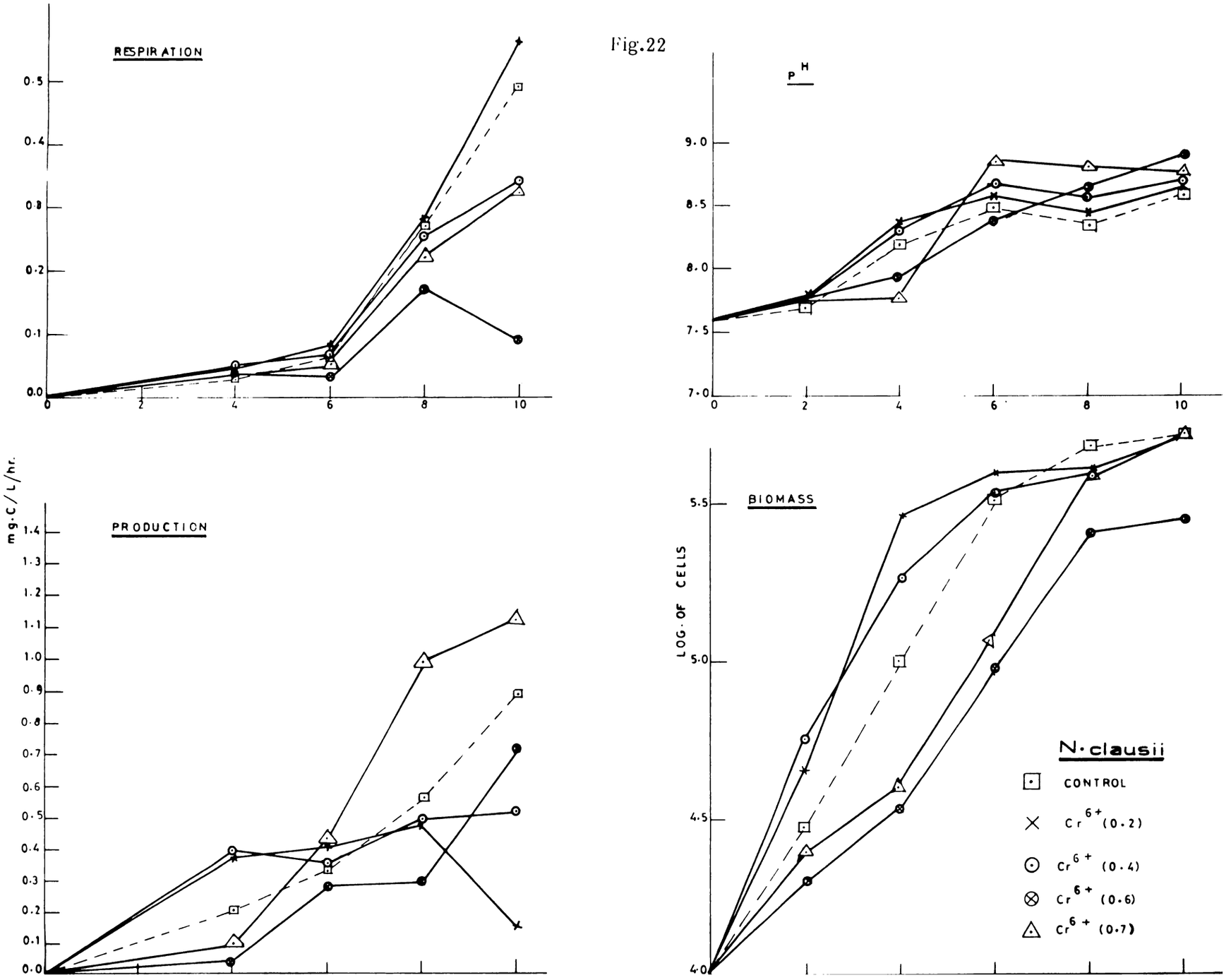
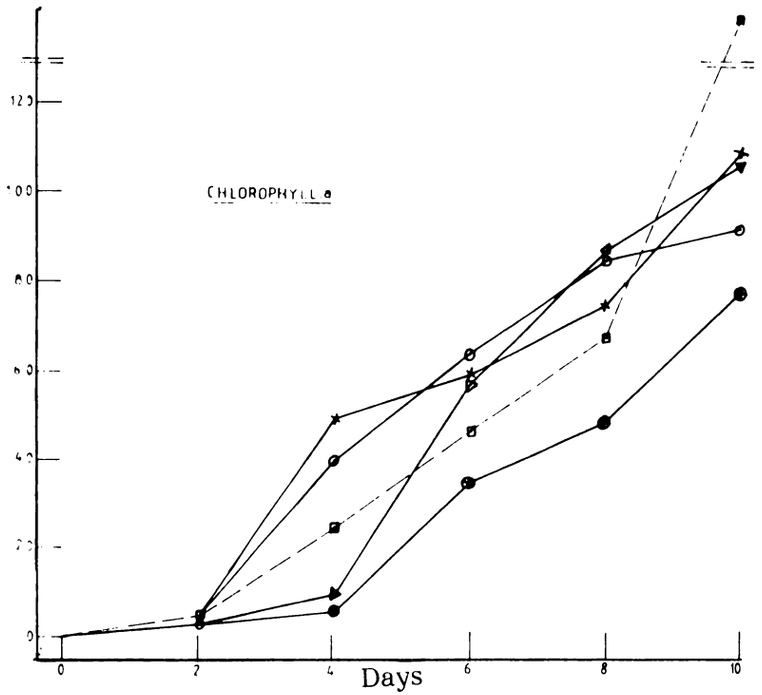
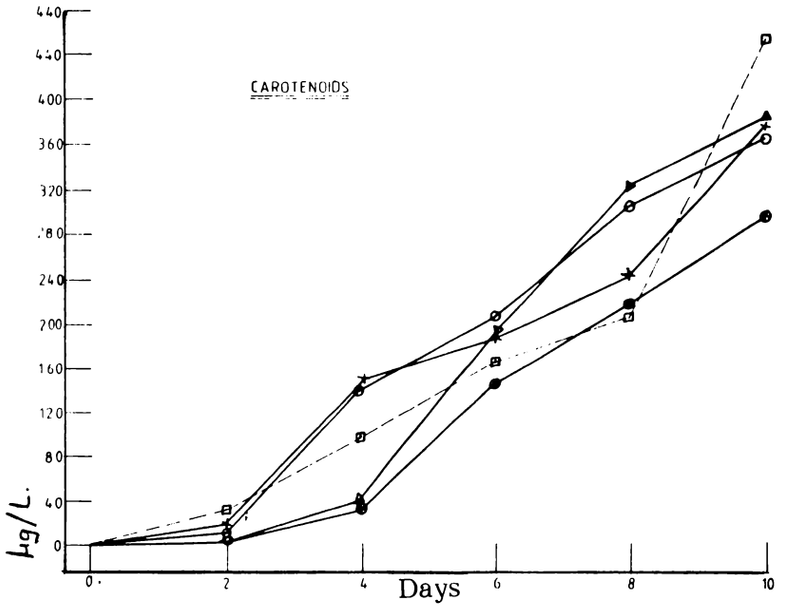
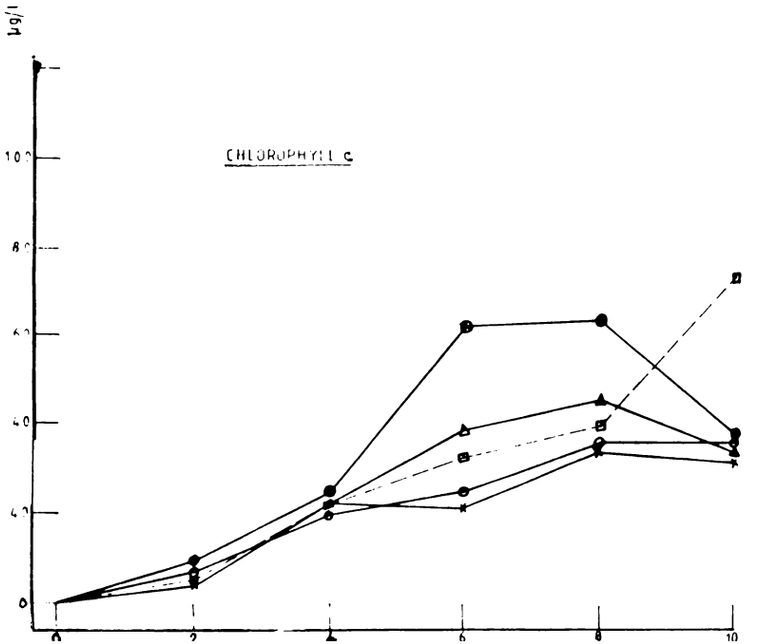

RESPIRATION
PH
PRODUCTION
mg·C/L/hr.
BIOMASS
LOG·OF CELLS
N·clausii
CONTROL
Cr6+ (0.2)
Cr6+ (0.4)
Cr6+ (0.6)
Cr6+ (0.7)

Fig.23


CHLOROPHYLL a
Days


CAROTENOIDS
µg/L.
Days


CHLOROPHYLL c

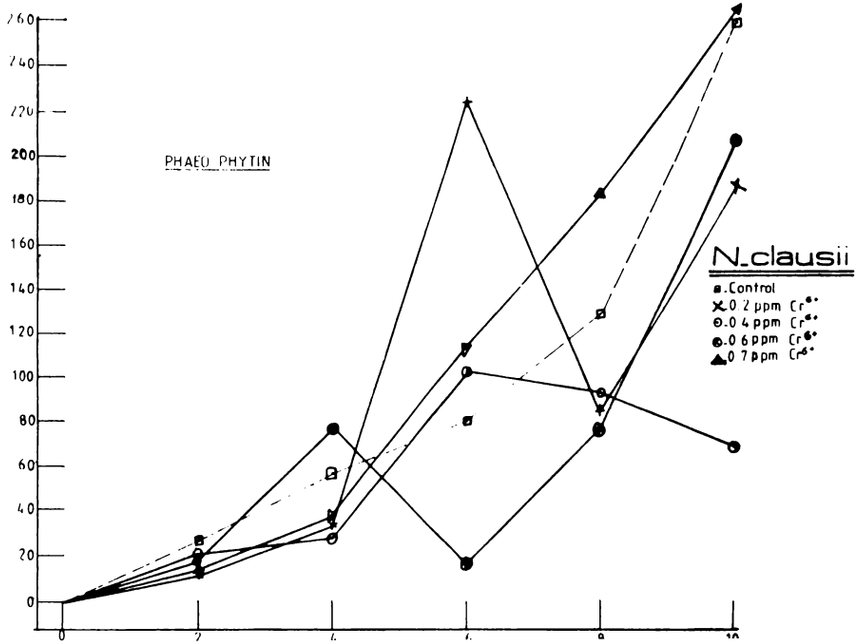
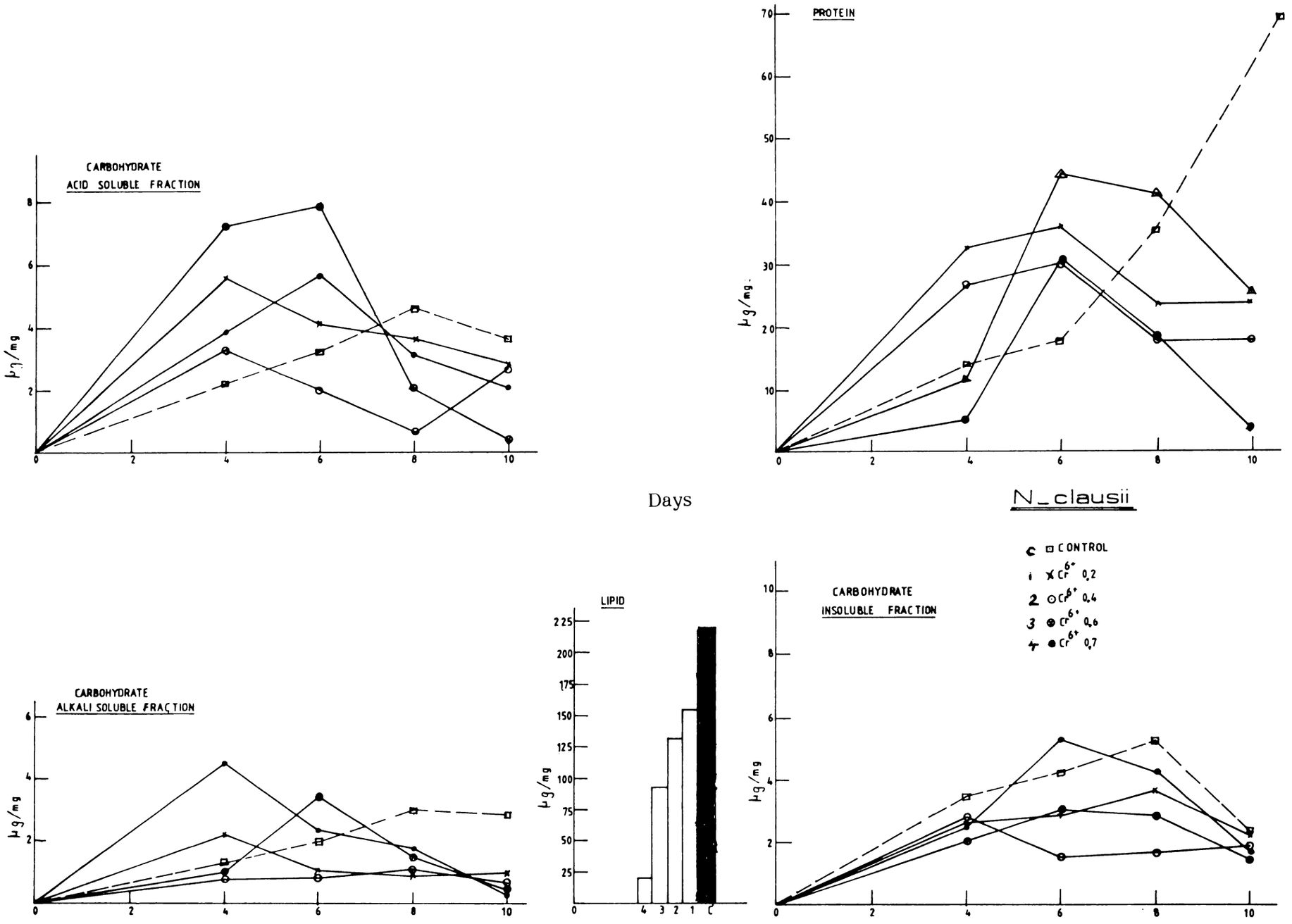

PHAEO PHYTIN
N.clausii
a.Control
0 2 ppm
0 4 ppm
0 6 ppm
0 7 ppm

Fig.24


CARBOHYDRATE
ACID SOLUBLE FRACTION
µg/mg
PROTEIN
µg/mg
Days
N_clausii
CARBOHYDRATE
ALKALI SOLUBLE FRACTION
µg/mg
LIPID
µg/mg
4 3 2 1 C
CARBOHYDRATE
INSOLUBLE FRACTION
µg/mg
C CONTROL
1 Cr6+ 0,2
2 Cr6+ 0,4
3 Cr6+ 0,6
4 Cr6+ 0,7

DISCUSSION

NICKEL

Nickel has been reported to have both stimulatory and inhibitory influence on algal growth. Bertrand and DeWolf (1967) suggested that nickel in micro-quantities may be required for plant growth, based on their observation on Chlorella species exposed to three milligram per litre nickel which produced higher yield than nickel free controls.

In the experiments on N. clausii it was observed that chlorophyll pigments and protein content were higher than the control in 0.4 ppm even-though there was decrease at the later stage.

In the present study it was found that growth of S. abundans, 0.01 ppm nickel was stimulating the growth while 0.03 ppm was proved to be toxic. The studies on the nickel toxicity indicated that nickel inhibition occurred at low concentration and was variable with species and class. Thus the sub-lethal level of nickel toxicity was not the same for green algal species S. abundans and the diatom N. clausii. The sublethal level selected for green algae was 0.01 ppm, 0.02 ppm and 0.03 ppm and for N. clausii it was 0.4 ppm and 0.6 ppm respectively.

Nickel tolerance in algae is dependent on the previous exposure of algae to nickel as well as innate tolerance. Stokes et al. (1973) compared the growth of a laboratory strain of S. abundans and a strain isolated from a nickel contaminated lake. The results showed that the lake strain tolerated higher levels of nickel.

Similar phenomenon was observed on N. clausii and S. abundans. N. clausii was collected from the polluted estuaries and S. abundans from an unpolluted fresh water pond.

In 0.03 ppm there was an abrupt decrease in the physiology of algae for S. abundans. In N. clausii such marked decrease was not noticed even in 0.6 ppm. It was found that for nickel there was no distinct toxic effect compared with other metals. It was reported that nickel was not lethal even at 10 ppm for alga (Deviprasad, 1982).

Hutchinson and Stokes (1975) presented data on the effect of nickel as nickelsulphate on some green algae such as Scenedesmus accuminatus, Haematococcus capensis, Chlorella species and Chlamydomonas species. They reported that there was decreased growth at nickel sulphate concentration between 0.5 and 0.7 milligram per liter and growth was stimulated at 0.05 milligram per liter but decreased at higher concentration of nickel.

In S. abundans well marked morphological changes were noticed at higher concentration in the preliminary experiments. The cells became enlarged, flagellae became distinct and the swimming speed reduced. Cells clumped together and the culture became yellowish in colour.

The effect of nickel on the morphology of Chlamydomonas was observed by Flavin and Slaughter (1974) and the flagellar movement was inhibited at 0.18 milligram per liter nickel acetate.

Spencer (1978) reported that growth of green alga Scenedesmus quadricauda and diatom Navicula pelliculosa was retarded at 0.1 milligram per liter divalent nickel (Ni^{++}).

In the present investigation the growth was retarded by divalent nickel (Ni^{++}) on 0.03 ppm for S. abundans and 0.6 ppm for N. clausii. The decrease in the growth was also reported by Spencer (1981). He also observed that like copper and cadmium the free nickel ion was more toxic than metal species.

Calculation of the chemical equilibrium by Fezy et al. (1979) proved that divalent nickel in the medium was associated with reduced algal growth. They suggested that the form of nickel present in the equilibrium of the medium may be as important as the total amount added to the medium.

Environmental factors play an important role on the toxicity of metals. For N. clausii the pH was more than the control through out the growth phase for both concentrations and for S. abundans, the maximum was on the twelfth day for 0.01 ppm and 0.02 ppm.

Studies on the bioaccumulation of algae by Wang and Wood (1984) showed that bioaccumulation of nickel occurred optimally at pH 8.0.

In the present investigation with N. clausii there was no well marked decrease in production and it was found to be tolerant than S. abundans. It may be due to the production of extra cellular products which detoxify the nickel (Patrick et al., 1975). The extracellular products produced may be non-specific and form complexes with other divalent cations as well (Spencer (1981)

S. abundans was sensitive to nickel. In 0.03 ppm the rate of growth, production, chlorophyll-a, chlorophyll-b, carotenoids and carbohydrate decreased. Reduced photosynthetic activity was reported for nickel at sublethal levels

(Nriagu, 1980). Notable decrease was noticed in production. Studies on the physiochemical influence on phytoplankton production by Massey (1981) showed that nickel inhibited phytoplankton production.

Hutchinson and Stokes (1975) reported that Scenedesmus accuminatus was the most sensitive fresh water species. But low concentration (0.01 ppm nickel) was found to be stimulatory for growth. Similar effect was observed for N. clausii also. Studies by Deviprasad (1982) showed that at lower concentration nickel stimulated the growth of green alga.

It was reported that nickel at a concentration of 1 ppm had no toxic effect on the marine diatom, Thalassiosira aestivalis (Deviprasad, 1982). Similar behaviour was observed for the test diatom N. clausii also.

In both selected concentrations (0.5 and 0.6 ppm) in N. clausii, the protein content was more than the control, with protein reaching the maximum even on the fourth day, eventhough there was decrease at the end of growth phase.

In the case of S. abundans also in all selected concentration (0.01, 0.02, 0.03 ppm) protein content was maximum on the eighth day. The values being 129% and 110% more than the control though there was a decrease at the end of growth phase. Stillwell and Holland (1977) had reported that five to forty micromolar nickel increased the cell protein while 400 micromolar nickel completely blocked the cell division and decreased the cell protein.

Stokes et al. (1973) reported that when Scenedesmus acutiformis was grown in a medium containing EDTA, along with nickel no significant effect

of nickel on growth was noticed. Similar behaviour was observed in natural population. Thus it was proved beyond doubt that the effect of nickel on the laboratory culture and natural population was entirely different.

Observations on S. abundans and N. clausii revealed that nickel was toxic to algae, eventhough at low concentration it was stimulatory. However toxicity varies between the two species. Nickel inhibited algal growth at 0.03 ppm and was lethal at 0.04 ppm in S. abundans. In N. clausii such well demarcation was not noticed. The toxic effect was observed at 0.6 ppm but preliminary experiments showed that it was not lethal at higher concentration upto 10 ppm though there was decrease in growth.

From the studies on the test species it was observed that algae isolated from polluted estuary may tolerate higher than four milligram nickel per liter while that from non-polluted water tolerated only upto 0.02 ppm per liter. Experiments proved that nickel toxicity was related to the presence of divalent nickel and can be influenced by other environmental factors including the presence of other metals and organic chelators. Stokes et al. (1973) observed that the addition of nickel in a medium containing EDTA was having significant effect on growth.

COBALT

Cobalt is a micronutrient for biological organisms and is essential for the growth of various algae. Wood (1974 and 1975) has classified cobalt as a group of "very toxic and relatively accessible elements". Jenkins (1980) has included cobalt in the fourteen toxic trace elements of critical importance from the point of view of environmental pollution and health hazards. At toxic level cobalt inhibits the heme biosynthesis (Tephly et al., 1978). The requirement of cobalt in some nitrogen fixing blue greens was studied by Goriunova and Maksidov (1972). The physiological role of cobalt for the algae may differ under different cultivation conditions. Cobalt was necessary for the fixation of molecular nitrogen by blue greens. Cobalt was necessary for the synthesis and accumulation of specific co-organic compounds like vitamin B_{12} (Goriunova and Maksidov, 1972).

Knauer et al (1982) has reported that cobalt values are very high in estuaries. Venugopal et al. (1982) have reported cobalt from Cochin backwaters along with copper, manganese, nickel and zinc. Mahra and Akirakudo (1981) reported that under natural conditions cobalt remainded in the water phase in marine environment more than five times as much as in fresh water environment. Studies on the metal content in Indian Ocean water by Danielsson (1979) cobalt was found in very low quantities, thus proving that cobalt is a limiting element for algae that require vitamin B_{12}.

In the present investigation it was found that 0.05 ppm cobalt enhanced the biomass in S. abundans except at the end of growth phase where there was a slight decrease. In 0.1 ppm although there was an initial increase in

biomass, it decreased towards the end. In 0.25 ppm growth was completely suppressed.

In the case of N. clausii in 0.3 ppm and 0.5 ppm biomass was more than the control throughout the growth phase. In 0.5 ppm growth was 33% more than the control. In 0.6 ppm eventhough the growth was more than the control at the early stage, with the aging of the culture, growth was decreased.

According to Cain & Allen (1980) the metals which are toxic to algae first penetrate through the cell wall and enter the protoplast. The cell walls are normally considered to be highly permeable to compounds of low molecular weight. The algal cells show a high affinity for environmental contaminants particularly heavy metals, once they enter the protoplast toxicity could be affected.

Blankenship and Wilber (1975) have reported that normal growth was evident upto 0.6 mg cobalt per liter in Circosphaera and cell division stopped above that concentration. Whitton and Shehata (1982) have shown that it was easy to increase the resistance of algae by repeated sub-culturing at inhibitary levels of the metals.

Jenkins (1980) reported that cobalt is absorbed and accumulated in very low or limited amount by biological organisms. Coleman et al. (1971) reported that cobalt above 0.04 mg/ml reduced significantly the growth of the three species of algae tested. The inhibitary effect on the early stage of growth followed by stimulatory effect on the second half of the experiment

resulting in higher biomass was reported in Spirulina platensis by Sharma et al. (1987).

Morphological changes were noted at the higher levels of metals and in some cases there was considerable diversity in the same medium. Individual cells separate soon after cell division in exponential cultures. In the case of N. clausii at high concentration cells aggregate and were found at the bottom of the flask. Whitton and Shehata (1982) have found that Anacystis species became structure less at high levels of cobalt. Studies on the effect of cobalt by Patil (1986) on the growth of Chlorella species showed that at 15 mg cobalt/litre algal cells became pale yellow and settled to the bottom and died.

In the present investigation production in S. abundans was less than the control in all concentration except an increase of 28% in 0.05 ppm at the end of growth phase. Studies on the modes of contamination of fresh water food chain by cobalt showed that concentration of Co^{60} decreased the primary productivity (Triquet, 1979).

The influence of cobalt on the growth and biochemical activity of Chlorella vulgaris was tested at four concentration (1, 5, 10 and 15 mg/l) by Patil (1986). It was observed that BOD removal efficiency of the algae decreased with the increase in cobalt level. High levels of cobalt resulted in decreasing the pH, dissolved oxygen and algal counts. Studies on the influence of Co^{57} uptake on photosynthetic activity and respiration by Parker (1969) in lake Mendota observed that the uptake was minimal. It was reported by

Olson and Christensen (1982) that at toxic level cobalt inhibited enzyme activities.

In the case of N. clausii the production was enhanced in 0.3 ppm upto eighth day and in 0.5 ppm upto sixth day but in 0.6 ppm it was less than the control throughout the growth phase. According to Goldman (1964) cobalt is an ion that may influence photosynthetic carbon fixation.

The relatively high biomass value in 0.05 ppm was not reflected in the production level in S. abundans. Though the biomass showed an increase upto eighth day in 0.05 ppm cobalt, the production increased towards the end of growth phase. The decrease in production in the first half might be attributed to the increase in respiration (Fig.7). The decrease in biomass towards the end of growth phase with simultaneous increase in production, protein and pigment content suggested that multiplication of cells was inhibited in 0.05 ppm, neverthless stimulated the production potential, protein level and chlorophyll content. Further work was proposed to be carried out to assertain the phenomenon.

In N. clausii it may be noted that in 0.3 ppm both biomass and production remained higher than the control upto sixth day indicating a stimulatory effect, but the production declined significantly on tenth day whereas the biomass was not suppressed to the same extent. The pigments chlorophyll-a, chlorophyll-c, carotenoid and phaeophytin were also less on tenth day. In 0.5 ppm eventhough the biomass was more than the control upto sixth day, photosynthetic end products such as carbohydrate and protein also showed an increasing trend in the first half of the growth phase. Protein was maximum on the first half when compared with the reduced biomass and production.

The possible reason for the decrease in the chlorophyll content by the effect of the metal at the end of growth may be due to the displacement of Magnesium from chlorophyll molecules by metal ions (Wu and Lorenzen, 1984) leading to a change in the functional characteristics. These processes result in alternation of metabolic turn over of chlorophyll in the presence of toxic metal. Cobaltous ion is known to act as alternative co-factor for Magnesium in a number of enzyme systems (Mc Elroy and Nason, 1954).

In N. clausii, eventhough the biomass was more than the control throughout the growth phase, the production was more than the control upto sixth day. Photosynthetic end products such as carbohydrate and protein also showed an increasing trend in the first half of growth phase. Protein was maximum on the first half when compared with reduced biomass and production. But in the laterhalf end products were less than the control.

Studies on the metal uptake by Anabaena variables by Jensen et al (1986) showed that metals were found in small amounts in the cytoplasm or cell wall indicating the binding by other cellular components such as protein, thus reducing the protein synthesis in the laterphase of growth. The decrease in protein synthesis may be due to high concentration of cobalt resulting in lowering the activity of enzymes. It was reported by Olson and Christensen (1982) that at toxic level cobalt inhibited the enzyme activities.

Blankenship and Wilber (1975) reported that high concentration of cobalt (6 mg/litre) reduced the cell division and protein synthesis. Stauber and Florence (1987) reported that in Nitzschia closterium copper inhibited the enzyme catalase and glutathione reductase thus reducing protein synthesis.

In S. abundans 0.1 ppm and 0.25 ppm, production and pH were less than the control throughout the growth phase. In 0.05 ppm unlike production there was as initial increase. The respiration was also higher suggesting a high metabolic rate, however it was similar to production in the later stage.

In N. clausii, the production respiration and pH in 0.6 ppm were less than the control throughout the growth phase. In 0.3 ppm and 0.5 ppm there was decrease in production in the later half which was also reflected in the pH. However the respiration was more than the control except on the tenth day in 0.3 ppm whereas in 0.5 ppm the respiration was fluctuating, being less than the control on sixth and tenth days.

Parker & Hasler (1969) reported that pH may be an important factor regulating the amount of cobalt. The reports of Peterson and Healey (1985) on the metal uptake by the green alga Scenedesmus quadricauda showed that the algae was highly pH dependent in the organic medium.

Tiller and Hodgson (1963) found that the characteristic of cobalt was strongly influenced by pH and by the type of minerals in the soil.

Cobalt, an important constituent of vitamin B_{12} which is essential for all organisms is synthesised in bacteria, fungi, blue green algae, some red and brown algae. Thus the decrease in cobalt content decreases the vitamin B_{12}.

Comparative toxicity of trivalent and hexavalent chromium

Chromium is an important trace metal occurring in industrial and domestic effluents. Yet there exists little information regarding its effects on aquatic organisms. Dissolved chromium exists as Cr^{3+} and Cr^{6+}, of which the hexavalent form seems to be more in quantity and toxicity when compared to trivalent metal.

Harvey (1949) had reported that 3.2 ppm hexavalent chromium did not inhibit the growth of algae Chlorella species whereas two species of euglenoids were inhibited by 0.32 ppm of hexavalent chromium indicating that toxicity varied from species to species.

Stokes et al. (1973) compared the growth of a laboratory strain of Scenedesmus species and a strain from a lake containing high concentration of metal. The results showed that the lake strain tolerated higher levels of metal. Studies of Mangi et al. (1978) showed that hexavalent chromium was found to be moderately toxic to several riverine algae. Nemerow (1978) has reported that the tannery wastes contain about 30 to 70 ppm chromium. Studies on the ground water pollution by Tanneries in Tamilnadu, Krishna Swamy and Haridas (1981) reported that nearly 42000 tonnes/annum of leather goods are processed on the banks of the river Palar. However, the quantitative flow of the ground water along the river has not been estimated.

Studies on the ground water pollution by Kakar and Bhatnagar (1981) reported that a high concentration of 12 mg/l of hexavalent chromium has

been found in ground water. Of the two forms of chromium, hexavalent chromium is a common component of polluted waters from industrial and metal plating areas.

Reports from the waste water treatment by Alliance leathers Private Limited, Edayar, Ernakulam District, Kerala showed that the factory discharged an effluent of about $250M^3$/day ($250M^3$ = 250,000 litres), the major pollutant was chromium which was used for tanning as chromium sulphate.

Frey et al. (1983) reported that chromium was inhibitory to growth of Thalassiosira species in fresh water. Tiwari et al. (1989) reported that the effluents from electroplating industries contain toxic metals such as chromium, nickel, cadmium, aluminium, iron, lead and alloys of zinc and that the concentration of hexavalent chromium varied in different days ranging from 2.0 and 8.9 mg/l.

In the present study, S. abundans was collected from an unpolluted fresh water pond and N. clausii was from polluted estuaries. The species exhibited different levels of toxicity to trivalent chromium and hexavalent chromium. Low concentration of trivalent chromium (0.01, 0.02, 0.03 ppm) was toxic to S. abundans. Whereas N. clausii was tolerant even at 0.8 ppm trivalent chromium and 0.7 ppm hexavalent chromium.

In S. abundans the growth was completely inhibited by 0.03 ppm trivalent chromium whereas hexavalent chromium showed better growth than the control in 0.1 ppm. The toxicity was noticed only in 0.15 ppm and the growth was completely distorted in 0.2 ppm suggesting that trivalent chromium was more

toxic to S. abundans than hexavalent chromium. It was also observed that in N. clausii hexavalent chromium was toxic at 0.6 ppm whereas trivalent chromium was not toxic even at 0.8 ppm. Ajmal et al. (1984) reported that hexavalent chromium was more toxic than trivalent chromium on the studies of the effect of trivalent chromium and hexavalent chromium on microorganisms.

The cells of N. clausii grown in 0.6 ppm chromium became attached to the culture flask and did not enter into the medium when the flasks were shaken, resulting in the apparent decrease in cell number. In some cases it was observed that there was clumping of cells and they were found at the bottom of the culture flask as a mass. When the cells were examined under microscope empty frustules were not noticed suggesting that cell disruption did not occur. In 0.03 ppm hexavalent chromium, the two celled S. abundans cells were seen singly, flagellae became distinct, cells enlarged in size, some cells lost nucleoplasm and became empty. The swelling of the cell was also exhibited by metal treated Asteronella species (Erickson, 1972 and Davies, 1976).

Morphological changes such as yellowing of cytoplasm, disruption of chloroplast, cell separation and clumping of cells were noticed by the effect of chromium on Thalassiosira aestivalis (Hollibaugh et al., 1980). They also reported that metals also interfere with cell division. In S. abundans there was clumping of cells and attachment to the bottom of the flask in 0.15 ppm hexavalent chromium. There was yellowing of cells and the culture turned yellow in colour.

In S. abundans trivalent chromium stimulated the growth in 0.02 ppm. The growth was inhibited by 0.01 and 0.03 ppm trivalent chromium. In hexavalent chromium-treated samples, the growth was less than the control for all treatments. In the case of N. clausii the growth was stimulated by hexavalent chromium in 0.2 and 0.4 ppm. In 0.6 and 0.7 ppm well marked decrease was noticed. However, in trivalent chromium samples such marked decrease was not observed, even in 0.8 ppm though there was an initial decrease in growth, an increase was observed at the end of growth phase. Thus the toxic effect of the metals noticed in the initial stage was reversed in the later stage.

This may be due to the fact that in laboratory cultures at high concentration, chromium may be adsorbed in or on cells, to reduce its concentration significantly. This may allow some surviving cells to grow, multiply and re-establish the population. This is the "Sacrificial lamb effect" reported by Mangi et al. (1978) on massed algae like tufts of Oedogonium species. Mangi et al. (1978) observed that adsorption was largely responsible for the uptake of chromium. Richards (1936) suggested that uptake occurred through the cell wall. Studies on the laboratory culture indicated that a large portion of chromium associated with algae was localised on the cell walls. Garton (1973) observed that Selenastrum capricornutum was severely inhibited by 26.6 μ molar hexavalent chromium (0.51 ppm). Wium-Andersen (1974) reported severe inhibition of growth in 5.7 μ molar/liter chromium (0.11 ppm) and slight inhibition in 0.1 μ molar/liter hexavalent chromium (0.02 ppm). The inhibition of growth of diatom by 1.9 μ molar/liter chromium (0.0365 ppm) was reported by Patrick et al. (1975). On the studies on ten heavy metals on algae Hollibaugh et al.

(1980) reported that chromium produced low growth and yield on the diatom Thalassiosira aestivalis.

In S. abundans trivalent chromium reduced the production in 0.02 and 0.03 ppm but in 0.01 ppm production was more than the control from the middle of the growth phase which was not reflected in the respiration and biomass. But in 0.02 ppm and 0.03 ppm, the production was lower than the control throughout the experiment but respiration was more than the control in 0.02 ppm upto tenth day.

The hexavalent chromium (0.05 and 0.1 ppm) treated samples showed increase in production with the aging of the culture eventhough it was less than the control in the early stage of growth phase. The increase in production was also reflected in the respiration. Respiration was more than the control throughout the growth phase.

pH of the culture also had an important role in metal uptake and thus enhancing production. It was reported by Michnowicz and Weaks (1984) that when pH level was above four, the growth and production were significantly enhanced. The effect of pH on the adsorption of chromium by Scenedesmus species in the electroplating industries was studied by Tiwari et al. (1989).

Unlike the trivalent form, the hexavalent of chromium stimulated the carbon uptake. Significant increase was noticed even in (0.05 and 0.1 ppm Cr^{6+}) the early stage of growth. With the aging of the culture, the respiration increased and the peak was noticed at the end of growth phase.

Wium-Anderson (1974) noted the inhibition of algal photosynthesis by chromium. The increase in the respiration with the aging of the culture by the effect of hexavalent chromium was also reported in Selenastrum capricornutum by Pillard et al. (1987).

According to Pillard et al. (1987) the changes by the effect of hexavalent chromium were apparent in the dark but not in the light bottles which implies that photosynthesis in the light bottles has masking effects that were present. Chromium was affecting processes that were occurring in the dark bottles.

The appearance of significant increase in carbon assimilation in the dark bottles was due to effects upon the dark carbondioxide pathway (Pillard et al. (1987) chromium has been found to stimulate carbondioxide production and growth in fungi (Babich et al., 1982).

Studies on the impact of chromium on Nostoc muscorum by Rai and Raizada (1988) showed that chromium reduced the growth, carbondioxide uptake, heterocyst production and nitrogenase activity.

In N. clausii 0.2 and 0.4 ppm hexavalent chromium, the production was increased during the first half followed by decrease in the second half whereas respiration was more than the control throughout the growth phase. But in higher concentration both respiration and production were reduced. High concentration of hexavalent chromium (0.7 ppm Cr^{6+}) reduced the production and respiration. However, respiration and production were increased in 0.8 ppm trivalent chromium.

This may be because trivalent chromium was being rapidly cumulated in all algae whereas hexavalent chromium was practically not cumulated in algae. It might be concluded that cumulation of trivalent chromium was predominantly due to chemical adsorption on the surface of algal cells. The cumulative behaviour of trivalent chromium and hexavalent chromium in batch cultures of algae was studied by Stary et al. (1982).

Studies on the adsorption of trivalent chromium and hexavalent chromium by Chlorella pyrenoidosa by Schroll (1978) found that algae had adsorbed distinct amount of trivalent chromium. There was negligible adsorption of hexavalent chromium. This suggested that unlike trivalent chromium, hexavalent chromium had an inhibitory effect on Chlorella pyrenoidosa.

Studies on the effect of chromium on alga Dunaliella bioculata by Saraiva (1976) showed that low concentration of chromium was adsorbed on the cell membrane and acted on the cell metabolism, thus affecting the respiration and photosynthesis.

Leland (1979) in his studies on toxicity of heavy metals and its bio-accumulation showed that concentration necessary to inhibit growth, metabolic processes such as photosynthesis varies widely and depends on factors such as degree of chelation, concentration of cells, pH, nutrients, physiological state of cells, salinity and temperature.

Frey et al. (1983) observed that in natural population and in cultures of Thalassiosira pseudomona, salinity exerted a strong effect on the toxicity of hexavalent chromium. The sea water can neutralise the toxicity of hexavalent chromium on phytoplankton.

According to Andersen and Morel (1978) the chemistry and toxicological mechanism of hexavalent chromium was quite different from that of other metals. In the case of cationic metals such as nickel, iron, cadmium, lead etc. the biological activity was related to the activity of the free cation rather than the total concentration. Thus they form complexes with EDTA, humic acid etc. Since hexavalent chromium was anoionic like the cationic metal ions, it has very little affinity for organic legands and were not detoxified by that (Frey et al., 1983). The mechanism of toxicity at the cell surface or with in the cell may be fundamentally distinct from that of cationic metal.

In the case of pigments in S. abundans, for trivalent chromium chlorophyll-a was more than the control at the end of growth phase while chlorophyll-b was more than the control in the early stage itself. But carotenoids and phaeophytin were less than the control throughout the growth phase.

The hexavalent chromium (0.05 and 0.1 ppm) reduced the chlorophyll-a and chlorophyll-b production in the early stage followed by increase in the later stage. The peak was observed at the end of growth of phase and it was maximum for 0.1 ppm. Whereas 0.05 ppm and 0.15 ppm reduced the carotenoids.

A general trend of increase in all pigments was observed upto eighth day eventhough there was an initial decrease on the second day. The probable reason according to Wu and Lorenzen (1984) was the displacement of Magnesium from chlorophyll molecules by metal atoms leading to a change in the functional

characteristics. These processes result in alternation of the metabolic turnover of chlorophyll in the presence of toxic metals. Saraiva (1970) reported that the initial decrease may be due to initial adsorption of chromium on the cell wall followed by acting on the cell membrane.

There was variation in different pigment content by the effect of hexavalent chromium in N. clausii. It was observed that 0.2 ppm and 0.4 ppm enhanced the chlorophyll-a and carotenoids but it was not reflected in chlorophyll-c. Chlorophyll-c was lower than the control through out the growth phase. In 0.6 ppm chlorophyll-a was lower than the control whereas carotenoids were more than the control. In 0.7 ppm it was observed that chlorophyll-a and chlorophyll-c produced an initial decrease followed by increase in pigment production.

Steeman - Nielsen and Wium-Andersen (1971) and Baker et al. (1982) had discussed the light dependent inhibition of metals in photosynthetic processes. The possible reason for the lower reduction of chlorophyll-a by heavy metals under the dark may be due to light dependent inhibition of enzymes and other factors which get activated by illumination (Baker , 1984).

Both trivalent and hexavalent chromium treated samples in S. abundans showed an initial peak in carbohydrate and proteins on eighth day. Protein was more than the control throughout the growth phase whereas carbohydrate was reduced from tenth day onwards.

Trivalent chromium stimulated protein production upto eighth day for N. clausii with a peak on the fourth day. The decrease in protein at the

end of growth phase may be due to the increased rate of adsorption of chromium with the aging of the culture. Similarly acid fraction of carbohydrate was more than the control in the early phase followed by sudden decrease towards the end. Hexavalent chromium stimulated the protein content in 0.2 and 0.4 ppm, but it was reduced with the aging of the culture. The acid soluble fraction of carbohydrate was more than the control upto middle of growth phase followed by decrease towards the end of growth for all treated samples. The maximum decrease was for 0.6 ppm whereas insoluble fraction was reduced completely.

The present study revealed that for S. abundans, trivalent chromium was more toxic than hexavalent form whereas in N. clausii the hexavalent form was more toxic than trivalent form. In S. abundans marked difference was noticed in the physiology of algae by the presence of metals whereas in N. clausii such a marked difference was not observed.

Scenedesmus abundans and Nitzschia clausii showed variation in the toxic effect, indicating that these species reacted differently to the four metals studied.

From the present investigation it was observed that the brackish water species N. clausii was more tolerant than the fresh water chlorophycean alga, S. abundans. The toxic effect showed a linear relation with different concentrations in S. abundans but not in N. clausii.

In S. abundans trivalent chromium was more toxic than nickel, cobalt and hexavalent chromium when biomass, production and end products were considered. In the case of photosynthetic pigments hexavalent chromium was

found to be more toxic than trivalent chromium, cobalt and nickel. The relative toxicity was in the order $Cr^{6+} > Cr^{3+} > Co > Ni$.

In N. clausii when the biomass and production was considered the toxicity was in the order $Cr^{3+} > Ni > Co > Cr^{6+}$. In the case of pigments, cobalt was found to be more toxic than nickel. The photosynthetic end products however, showed variation. Cobalt was highly toxic when acid fraction of carbohydrate was considered. The relative toxicity was in the order $Co > Cr^{3+} > Cr^{6+} > Ni$. In the case of protein, hexavalent chromium was highly toxic and the relative toxicity was in the order $Cr^{6+} > Co > Cr^{3+} > Ni$.

Analysis of variance was carried out to establish the significance of metal interaction in S. abundans and N. clausii. The variance ratio obtained by two way analysis technique was studied. Table 1, 2.

In S. abundans, the biomass was significant between days and between concentration in nickel treated samples. Nickel in 0.01 ppm was found to be highly significant. Production and pigments such as chlorophyll-a, chlorophyll-b, carotenoids and phaeophytin were significant between concentration and between days at 1% level. But respiration and protein content was significant with aging of culture between days at 1% level. Carbohydrate was only significant at 5% level.

In N. clausii biomass showed no significant difference between concentration but it was significant between days. In the case of production, significance was noticed between concentration and days at 1% level and the

protein content at 5% level. But respiration and pigment content was not significant between concentration but between days it was 1% significant. In the case of carbohydrate, of the three fractions of carbohydrate, the acid fraction showed significant difference between days at 5% level but the alkali fraction and the insoluble fraction showed no significant difference between concentration and days.

Cobalt produced significant effect on biomass and pH at 1% level between concentration and days in S. abundans and N. clausii. Whereas respiration and carbohydrate were not significant between concentration but only between days in S. abundans.

In N. clausii all parameters except the alkali soluble and insoluble fraction of carbohydrate were significant between days. The alkali soluble and insoluble fraction of carbohydrate were found to produce no significant difference.

In both species selected for study trivalent chromium produced significant effect between concentration and days at 1% level for pH, biomass and production. The respiration was significant between days for both species. In the case of end products, the alkali fraction was not significant in N. clausii but the acid soluble and insoluble fraction was found to be significant.

Hexavalent chromium was significant in all parameters of productivity in S. abundans. But in N. clausii the three fractions of carbohydrate were not significant.

Table 1. Results of the significance of the variance ratio between concentration and between days in S. abundans

Sl. No.	Metals employed	Selected parameters																						
		pH		Biomass		Production		Respiration		Chlorophyll-a		Chlorophyll-b		Carotenoids		Phaeophytin		Protein		Carbohydrate		Lipids		
		1	11	1	11	1	11	1	11	1	11	1	11	1	11	1	11	1	11	1	11	1		
1.	Ni	a	a	a	a	a	a	NS	a	a	a	a	a	a	a	a	a	NS	a	b	b	a		
2.	Co	a	a	a	a	a	a	NS	b	b	b	b	b	b	b	b	b	a	a	NS	b	a		
3.	Cr^{3+}	a	a	a	a	a	a	b	b	NS	a	NS	a	NS	a	NS	a	NS	a	NS	b	a		
4.	Cr^{6+}	a	a	a	a	a	a	a	a	b	b	a	a	a	a	b	b	a	a	a	a	a		

a. P < 0.01 1 Concentration

b P < 0.05 11 Days

NS Non Significant

Table 2. Results of the significance of the variance ratio between concentration and between days in N. clausii

Sl. No.	Metals employed	Selected parameters																											
		pH		Biomass		Production		Respiration		Chlorophyll-a		Chlorophyll-b		Carotenoids		Phaeophytin		Protein		Carbohydrate (1)		Carbohydrate (2)		Carbohydrate (3)		Lipids	
		1	11	1	11	1	11	1	11	1	11	1	11	1	11	1	11	1	11	1	11	1	11	1	11	1	
1.	Ni	a	a	NS	a	b	b	NS	a	NS	a	NS	a	NS	a	NS	a	a	a	b	b	NS	NS	NS	NS	a	
2.	Co	a	a	a	a	NS	a	NS	a	NS	a	NS	a	NS	a	NS	a	NS	b	NS	b	NS	NS	NS	NS	a	
3.	Cr^{3+}	b	a	b	a	b	a	NS	a	NS	a	NS	a	NS	a	NS	a	a	a	b	b	NS	NS	b	b	a	
4.	Cr^{6+}	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	NS	NS	NS	NS	NS	NS	a	

a $P < 0.01$ 1 Concentration

b $P < 0.05$ 11 Days

NS Non Significant

CHAPTER - V

RESULTS AND DISCUSSION (METALS IN COMBINATION)

5.1 Effect of combination of two metals on S. abundans and N. clausii

5.1.1 Combined effect of cobalt and trivalent chromium in S. abundans

Concentration of metals (in ppm)			Treatment number
Co 0.01	+	Cr^{3+} 0.03	1
Co 0.05	+	Cr^{3+} 0.01	2
Co 0.1	+	Cr^{3+} 0.02	3

Biomass

A general trend of decrease was noticed for all treatments of cobalt and trivalent chromium at the end of growth phase. The values being 5%, 38% and 50% less than the control (Fig.25). An increase in biomass was noticed for treatment (1) combination of 0.01 ppm cobalt and 0.03 ppm trivalent chromium upto eighth day followed by sudden decrease towards the end of growth. It was 3% and 55% more than the control on fourth and sixth day. But on the tenth day it was 32% less than the control. Eventhough there was increase in biomass on the second day for treatment (2), (a combination of 0.05 ppm cobalt and 0.01 ppm trivalent chromium) with the aging of the culture, the growth was 48%, 61%, 21% and 39% less than the control on the eighth, tenth, twelfth and fourteenth day (Fig.25). In the case of treatment (3), a combination of cobalt 0.1 ppm and trivalent chromium 0.02 ppm through out the growth phase biomass was lower than the control.

Production

It was observed that there was a decrease in net production through out the growth phase. At the end of growth phase, it was 12%, 35% and

36% less than the control for treatment (1), (2) and (3) (Fig.25). Treatment (1) was less than the control but it was closely following the control upto eighth day followed by 17%, 29% and 12% decrease on the tenth, twelfth and fourteenth day. The net production was far less than the control for treatment (2) and (3). Eventhough the production was less than the control at the end of growth phase, pH was more than the control at the end.

Respiration

The respiration was lower than the control for all treatments throughout the growth phase. At the end of growth phase it was 3%, 33% and 77% less than the control for treatment (1), (2) and (3) respectively.

Photosynthetic pigments

Chlorophyll-a

Chlorophyll-a was 338%, 70% and 104% more than the control on fourth, sixth and eighth day for treatment (1). On the tenth day it was similar to the control but at the end of growth phase it was 24% less than the control whereas treatment (2) and (3) was 65% and 24% more than the control (Fig.26). Treatment (2) and (3) was less than the control upto twelfth day. It was 46%, 33%, 15%, 57% and 1% less than the control on fourth, sixth, eighth, tenth and twelfth day respectively for treatment (2) and treatment (3) it was 46%, 65%, 58%, 57% and 21% less than the control for fourth, sixth, eighth, tenth and twelfth day respectively.

Chlorophyll-b

Chlorophyll-b was less than the control from sixth day onwards for all treatments (Fig.26). It was 14%, 74% and 76% less than the control for

treatment (1), (2) and (3) at the end of growth phase. Though there was 82% and 40% increase on second and fourth day for treatment (1) it was 46%, 31%, 40% and 81% less than the control on sixth, eighth, tenth and twelfth day respectively. Through out the growth phase chlorophyll-b was less than the control for treatment (2) and (3).

Carotenoids

Carotenoid was 16%, 27% and 12% more than the control on sixth, eighth and tenth day for treatment (1). Towards the end of growth phase it was 46% and 40% less than the control on twelfth and fourteenth day. For treatment (2) and (3) there was marginal increase of 9% and 4% at the end of growth phase eventhough it was less than the control upto twelfth day (Fig.26).

Phaeophytin

Phaeophytin was 11%, 91% and 10% less than the control at the end of growth phase for treatment (1) and (2) and (3) (Fig.26). Treatment (1) was less than the control through out the growth phase. Treatment (2) was also less than the control towards the end of growth but 97% and 20% increase was observed on second and fourth day. Similarly treatment (3) was also 97% and 56% more than the control on second and fourth day.

Photosynthetic end products

Carbohydrate

Carbohydrate was less than the control for all treated samples at the end of growth phase. It was 18%, 29% and 7% less than the control for

treatment (1), (2) and (3) (Fig.27). Carbohydrate was maximum for treatment (1). It was 52% and 66% more than the control on the eighth and tenth day but towards the end of growth it was 7% and 18% less than the control. Carbohydrate was less than the control throughout the growth phase for treatment (2) and (3). Treatment (2) was 22%, 60%, 65% and 29% less than the control on eighth, tenth, twelfth and fourteenth day respectively. Similarly treatment (3) was 21%, 77%, 68% and 7% less than the control on eighth, tenth, twelfth and fourteenth day respectively.

Protein

Unlike carbohydrate protein was less than the control for treatment (1) and (2) through out the growth phase. At the end of growth there was 81%, 25% and 27% decrease for treatment (1), (2) and (3) respectively (Fig.27). Treatment (1) was 6%, 20%, 48% and 81% less than the control on eighth, tenth, twelfth and fourteenth day. There was 31%, 57% and 25% decrease for treatment (2) on eighth, tenth and fourteenth day. On the twelfth day it was similar to control. Unlike other treatments, treatment (3) was 26% more than the control on the eighth day followed by decrease in protein content towards the end of growth.

Generally lipid was 17% more than the control for treatment (1). But treatment (2) was 30% less than the control and treatment (3) was similar to the control. There was an increase of 19%, 17% and 15% in the uptake of phosphate for treatment (1), (2) and (3) compared with the control. The nitrate uptake was similar to the control with only a marginal increase.

Fig.25

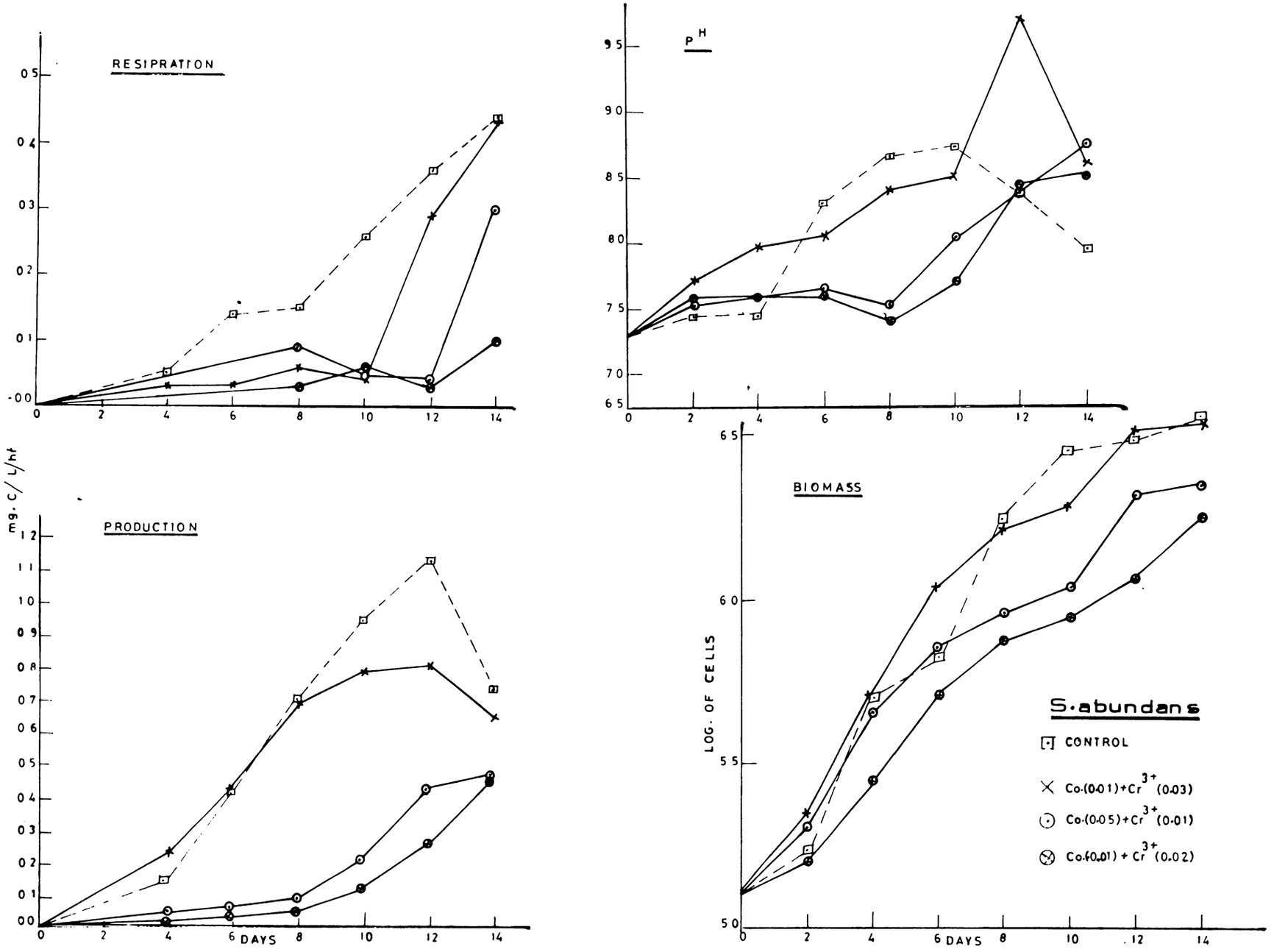

RESIPRATION
PH
PRODUCTION
mg. C/ L/hr
DAYS
BIOMASS
LOG. OF CELLS
S.abundans
CONTROL
Co.(0.01)+Cr 3+ (0.03)
Co.(0.05)+Cr 3+ (0.01)
Co.(0.01) + Cr 3+ (0.02)

Fig.26

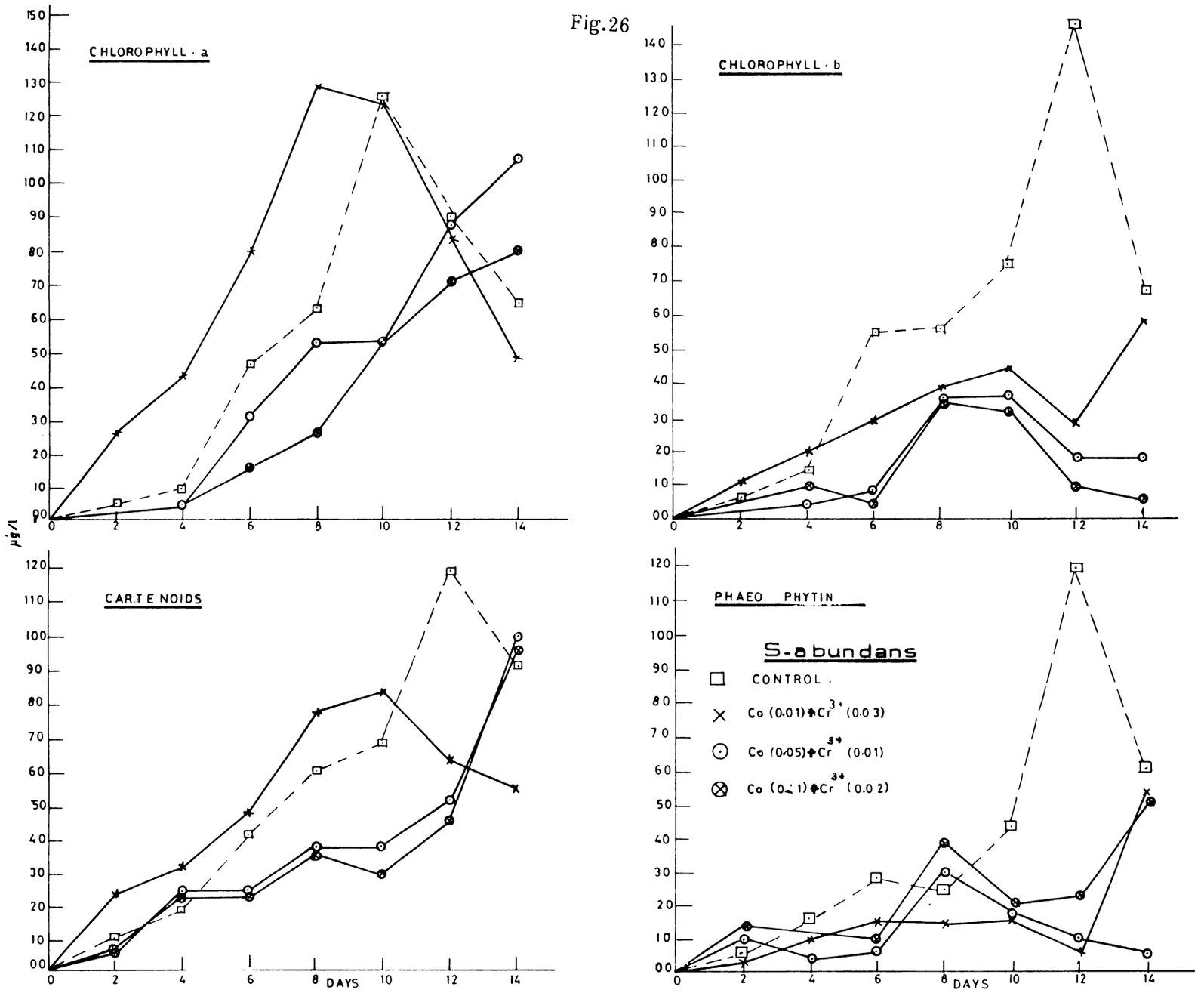
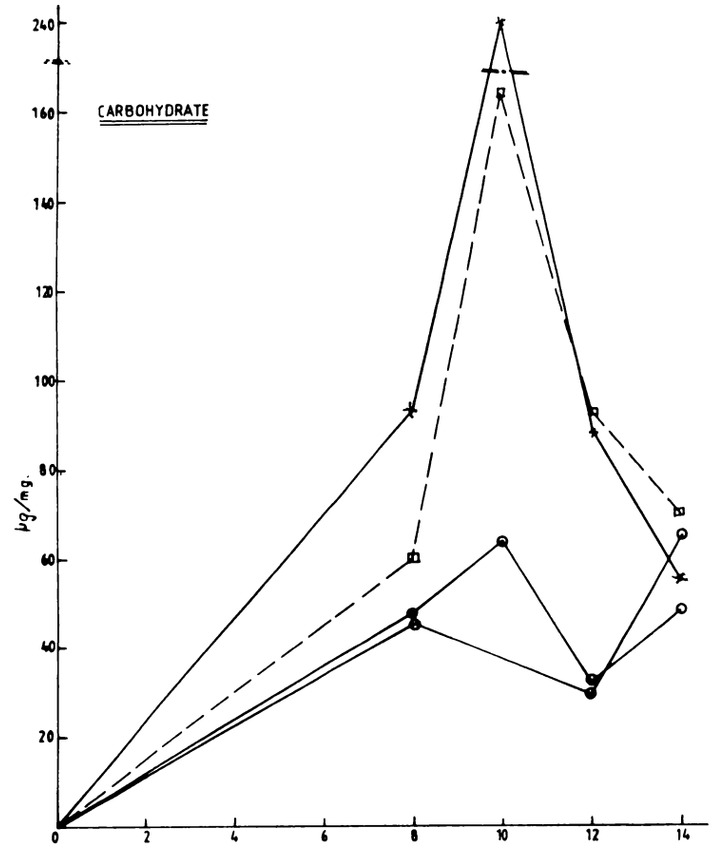
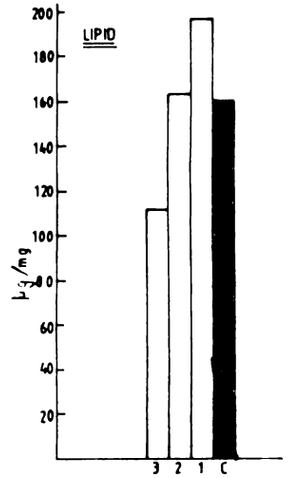
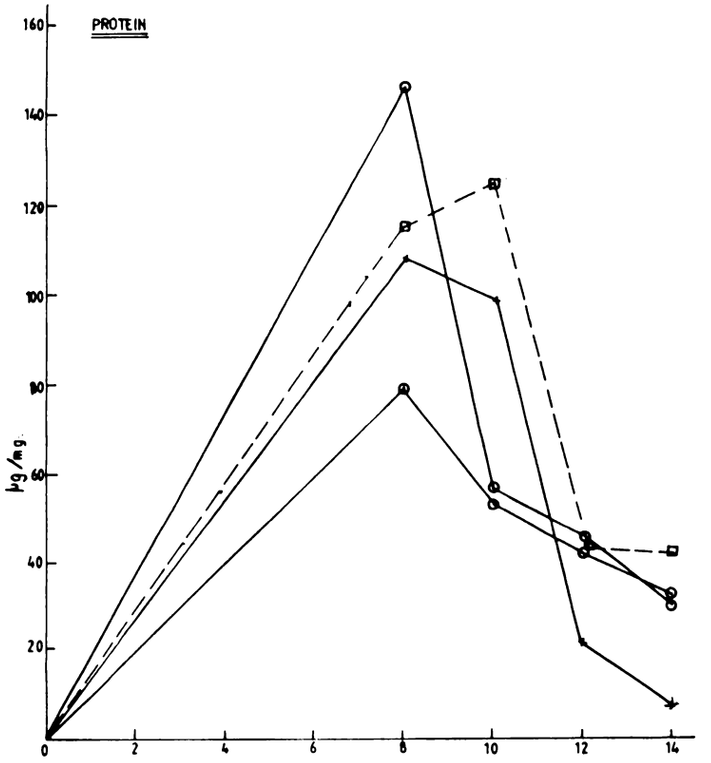

CHLOROPHYLL · a
CHLOROPHYLL · b
CAROTENOIDS
PHAEO PHYTIN
µg/l
DAYS
S-abundans
CONTROL
Co (0·01)+Cr3+ (0·03)
Co (0·05)+Cr3+ (0·01)
Co (0·1)+Cr3+ (0·02)

Fig.27







Days

S-abundans

C ⊡ CONTROL
1 × Co (0,01) + Cr^{3+} (0,03)
2 ⊙ Co (0,05) + Cr^{3+} (0,01)
3 ● Co (0,01) + Cr^{3+} (0,02)

Combined effect of cobalt and trivalent chromium in N. clausii

Concentration of metals (in ppm)	Treatment Number
Co 0.3 + Cr^{3+} 0.2	4
Co 0.5 + Cr^{3+} 0.2	5
Co 0.6 + Cr^{3+} 0.6	6
Co 0.3 + Cr^{3+} 0.8	7
Co 0.6 + Cr^{3+} 0.8	8

Biomass

A general trend of decrease in biomass was observed for treatment (5) (which was a combination of 0.5 ppm cobalt and 0.20 ppm trivalent chromium), treatment (6) (combination of 0.6 ppm cobalt and 0.8 ppm trivalent chromium). The values being 5%, 37% and 25% less than the control at the end (Fig.28). Whereas 9% and 2% increase was observed for treatment (4), a combination of 0.3 ppm cobalt and 0.2 ppm trivalent chromium and treatment (7), a combination of 0.3 ppm cobalt and 0.8 ppm trivalent chromium. Treatment (4) was more than the control on the fourth day, but 38% and 34% decrease was observed for sixth and eighth day respectively. Similarly treatment (5) was 70% more than the control on the fourth day followed by 21%, 16% and 37% decrease on sixth, eighth and tenth day respectively. Eventhough there was 20% increase on fourth day for treatment (6) treatment (8) was less than the control through out the growth phase.

Production

The production was more than the control upto eighth day for all treated samples. At the end of growth phase, the treatments (4), (5) and (7) was 25%, 15% and 68% more than the control. Whereas treatments (6) and (8) was 42% and 25% less than the control (Fig.28). Thus the maximum production was for treatment (7) and it was 68% more than the control on the tenth day. An increase of 12%, 65% and 25% was observed for treatment (4) on fourth, eighth and tenth day. But it was 22% less than the control on the sixth day. Treatment (5) was more than the control through out the growth phase. It was 143%, 44%, 126% and 15% more than the control on fourth, sixth, eighth and tenth day. Similarly treatment (7) was 86%, 13%, 94% and 68% more than the control on fourth, sixth, eighth and tenth day. But treatment (6) and (8) was less than the control at the end of growth phase. pH was also similar to production for all treated samples.

Respiration

Unlike production, respiration was less than the control on sixth, eighth and tenth day for treated samples (5), (6), (7) and (8) but treatment (4) was 15% more than the control at the end of growth phase (Fig.28). On the fourth day treatments (5) and (6) was 95% and 56% more than the control but treatments (4), (7) and (8) was less than the control. Treatment (4) was 84%, 66% and 43% less than the control on sixth, eighth and tenth day respectively. There was 10%, 67% and 68% decrease for treatment (5) on sixth, eighth and tenth day respectively. Treatment (6) was 25%, 83% and 50% less than the control on sixth, eighth and tenth day. Treatment (7) showed 14% and

71% decrease on sixth and eighth day. A decrease of 21%, 48% and 68% was observed on sixth, eighth and tenth day for treatment (8).

Photosynthetic pigments

Chlorophyll-a

Generally chlorophyll-a was lower than the control for all treatments but treatment (5) was 32%, 3% and 5% more than the control on fourth, sixth and eighth day (Fig.29). At the end of growth phase it was 23% less than the control.

Chlorophyll-c

At the end of growth phase chlorophyll-c was less than the control for all treated samples. It was 32%, 6%, 37%, 6% and 23% less than the control for treatment (4), (5), (6), (7) and (8) (Fig.29). Through out the growth phase chlorophyll-c was less than the control for all treatments but treatment (5) was 100%, 57%, 52% more than the control on fourth, sixth and eighth day. But at the end of growth phase it was 6% less than the control.

Carotenoids

Similar to other pigments carotenoids were less than the control at the end of growth phase (Fig.29). It was 16%, 3%, 30%, 9% and 21% less than the control for treatment (4), (5), (6), (7) and (8) respectively. Through out the growth phase carotenoids were less than the control for treatments (4), (6), (7) and (8) but for treatment (5), eventhough there was 46% decrease on fourth day, it was 5% and 22% more than the control on sixth and eighth day.

Phaeophytin

Through out the growth phase phaeophytin was less than the control for all treatments except on the sixth day (Fig.29). At the end of growth phase it was 25%, 68%, 55%, 75% and 71% less than the control for treatments (4), (5), (6), (7) and (8) respectively. Treatment (4) was 93%, 91% and 25% less than the control on fourth, eighth and tenth day but 74% increase was observed on the sixth day. Similarly treatment (7) was also 43% more than the control on the sixth day. It was 98%, 77% and 75% less than the control on fourth, eighth and tenth day respectively. Treatment (5), (6) and (8) was less than the control through out the growth phase.

Photosynthetic end products

Carbohydrate

Both acid soluble and alkali soluble fraction showed the peak on the fourth day followed by decrease towards the end of growth phase. Whereas the control was maximum on the eighth day.

Acid soluble fraction was 14%, 64%, 35% less than the control for treatment (4), (6) and (7) but treatment (8) was 45% more than the control at the end of growth phase (Fig.30). Carbohydrate was maximum for the treatment (7) and it was 259% more than the control. It was 42% more than the control on sixth day. On eighth and tenth day there was 25% and 35% decrease was observed for treatment (7). Acid fraction was 65%, 117%, 89% and 126% more than the control on the fourth day and 60%, 79% and 33%, increase was observed for sixth day for treatment (4), (5) and (6) respectively.

For all treatments carbohydrate was less than the control on eighth and tenth day.

Alkali soluble fraction was less than the control through out the growth phase for treatment (4). Alkali fraction was maximum on the fourth day for treatment (5), (6), (7) and (8). It was 201%, 175%, 146% and 453% more than the control for treatment (5), (6), (7) and (8) on the fourth day. But at the end of growth phase it was 35%, 17%, 51%, 48% and 42% less than the control for treatment (4), (5), (6), (7) and (8) respectively.

Unlike the other two fractions, the insoluble fraction was more than the control at the end of growth phase. It was 101%, 91%, 45%, 78% and 79% more than the control for treatment (4), (5), (6), (7), (8) respectively. Treatment (4) was 71%, 59%, 64% less than the control on fourth, sixth and eighth day. But treatment (5) was maximum (45%) on the fourth day followed by 36% and 60% decrease on the sixth, eighth day. Treatment (6), (7) and (8) was less than the control upto eighth day.

Protein

Protein was less than the control for all treated samples at the end of growth phase. But treatment (5) was 15% more than the control at the end (Fig.30). It was 23%, 25%, 26% and 17% less than the control for treatment (4), (6), (7) and (8). On the fourth day a peak was observed for all treatments. It was 60%, 293%, 96%, 189% and 202% more than the control for treatments (4), (5), (6), (7) and (8) respectively. Treatment (4) was 16% and 52% less than the control on sixth and eighth day.

Lipid content was 92%, 89%, 91%, 89% and 88% less than the control for treatment (4), (5), (6), (7) and (8) respectively. Of the nutrients, the phosphate was showing only marginal increase and decrease treatment (4), (6) and (8) was 32%, 20% and 23% more than the control while treatment (5) and (7) was 8% and 5% less than the control. The nitrate uptake was less than the control for all treatments.

Fig.28

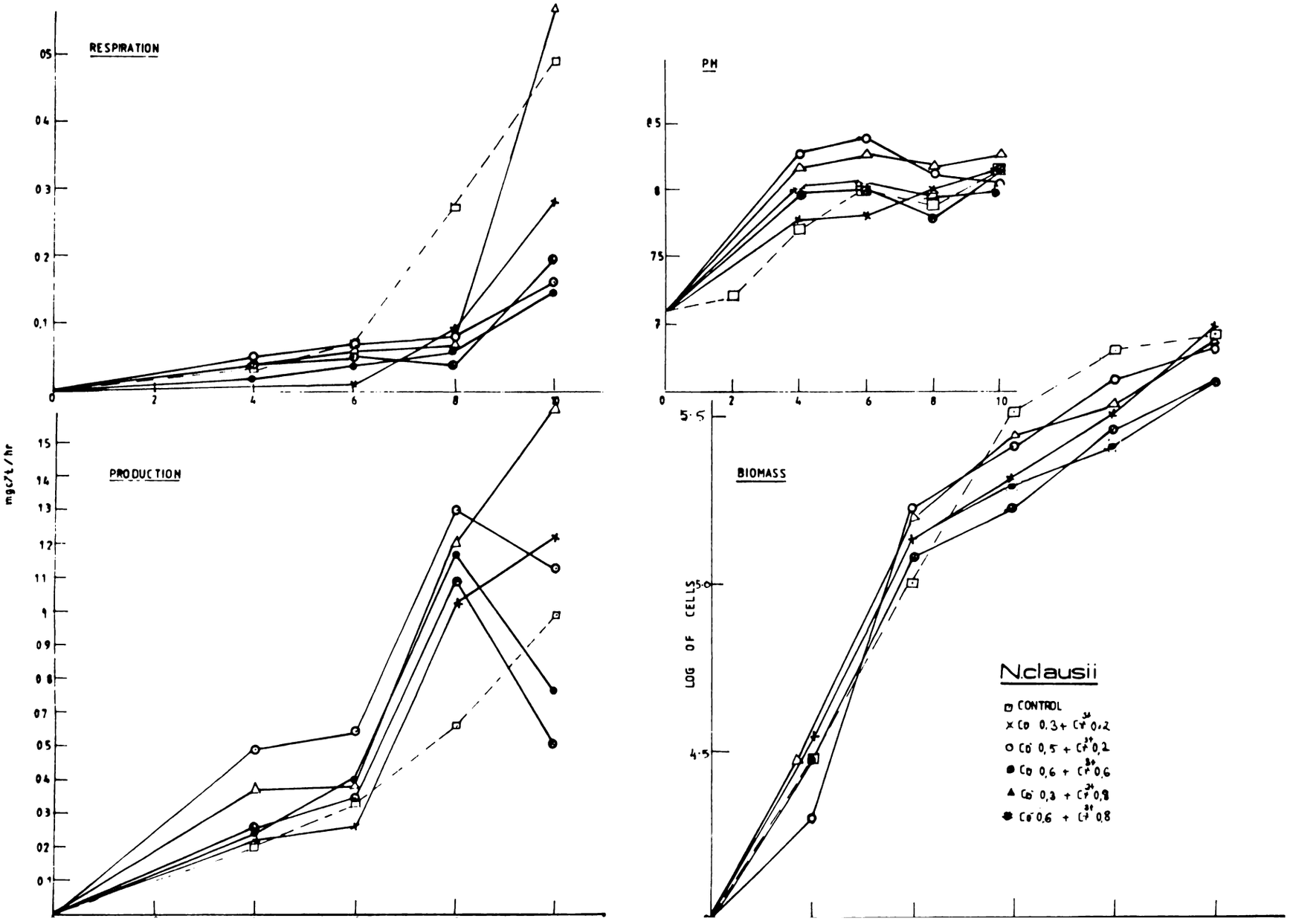
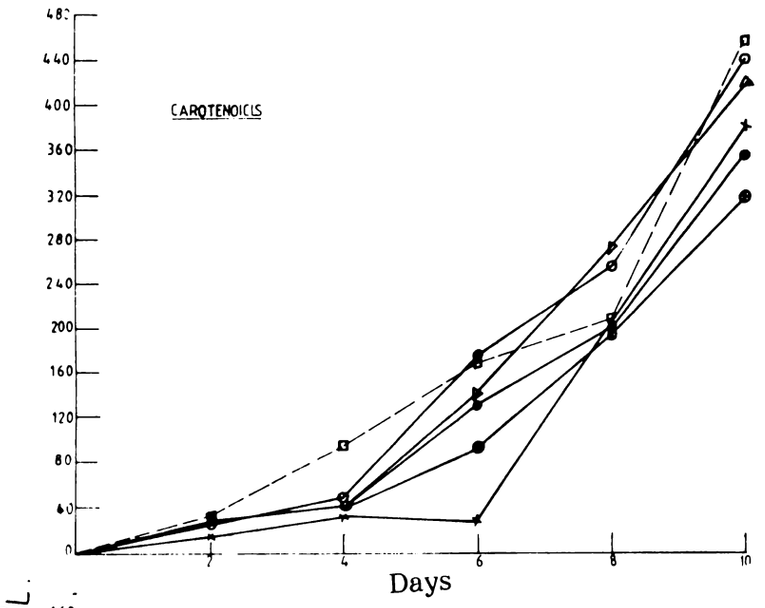
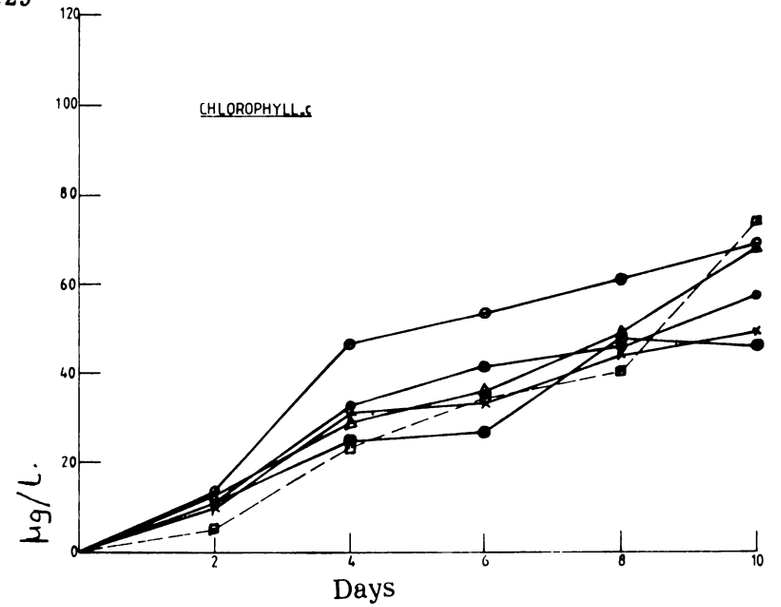
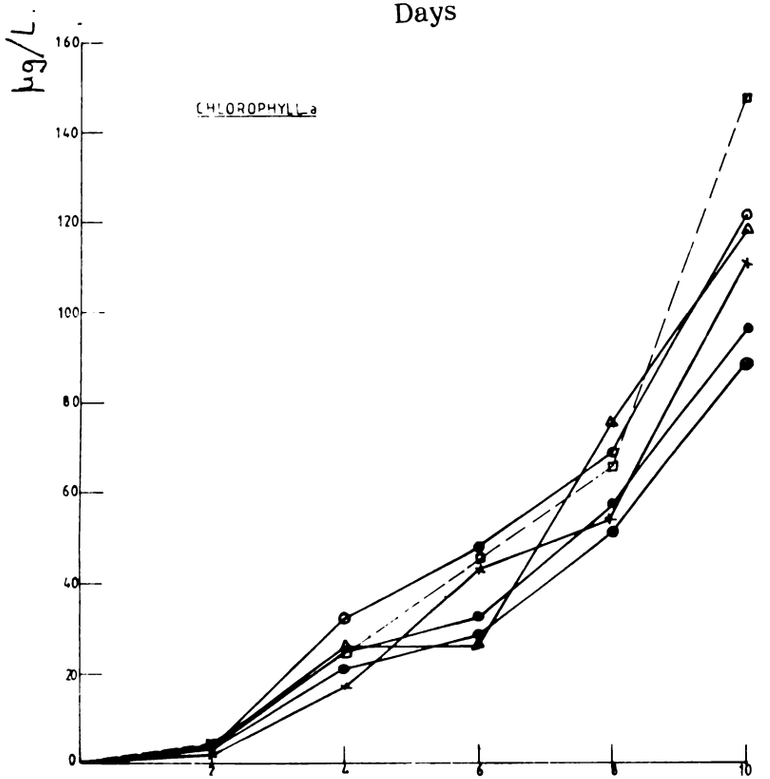

RESPIRATION
PM
PRODUCTION
BIOMASS
mgC/L/hr
LOG OF CELLS
N.clausii
CONTROL
Co 0.3 + Cr3+ 0.2
Co 0.5 + Cr3+ 0.2
Co 0.6 + Cr3+ 0.6
Co 0.3 + Cr3+ 0.8
Co 0.6 + Cr3+ 0.8

Fig.29


CAROTENOICLS
Days


CHLOROPHYLL c
µg/L.
Days


CHLOROPHYLL a
µg/L.

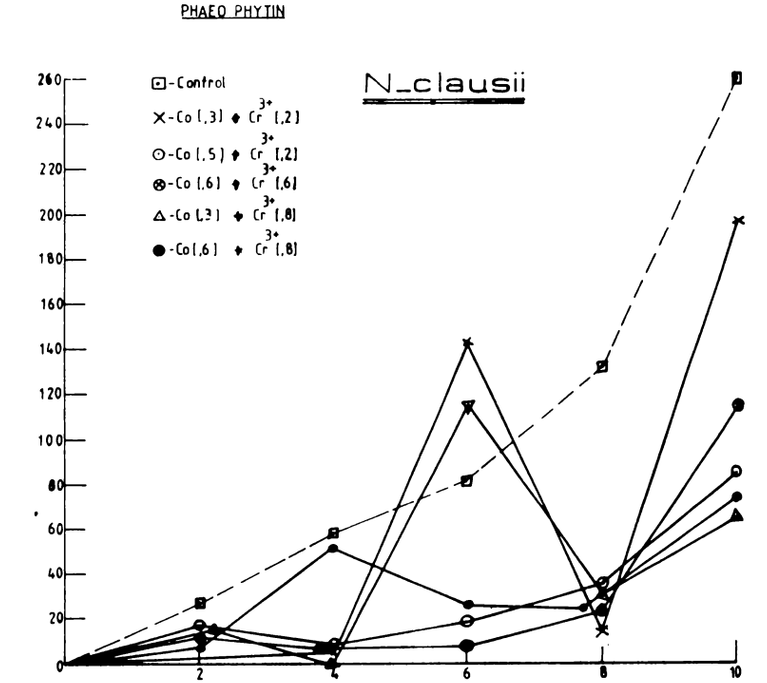
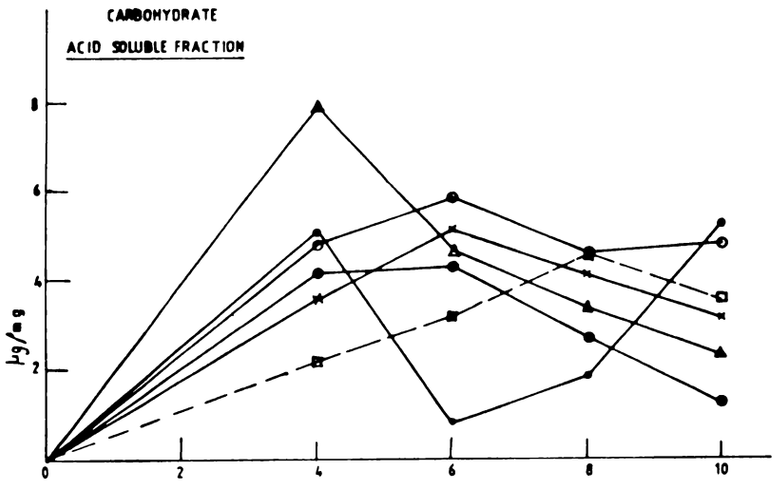
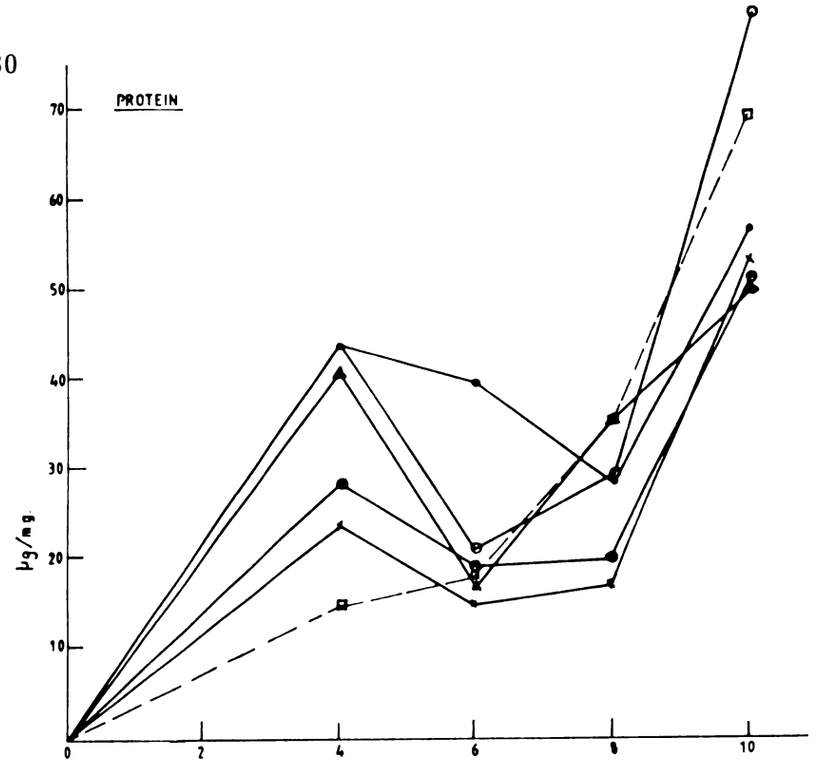

PHAEO PHYTIN
N_clausii
⊡-Control
×-Co (.3) ♦ Cr 3+ (.2)
⊙-Co (.5) ♦ Cr 3+ (.2)
⊗-Co (.6) ♦ Cr 3+ (.6)
△-Co (.3) ♦ Cr 3+ (.8)
●-Co (.6) ♦ Cr 3+ (.8)

Fig.30

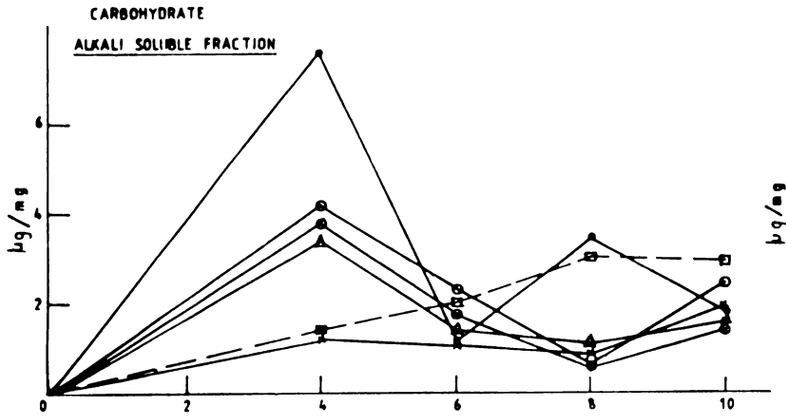


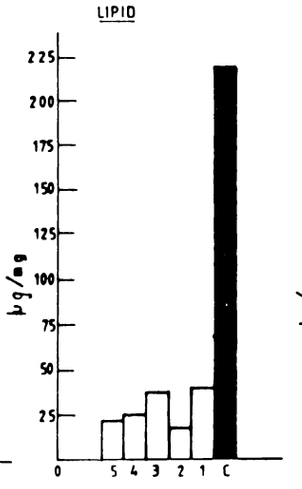


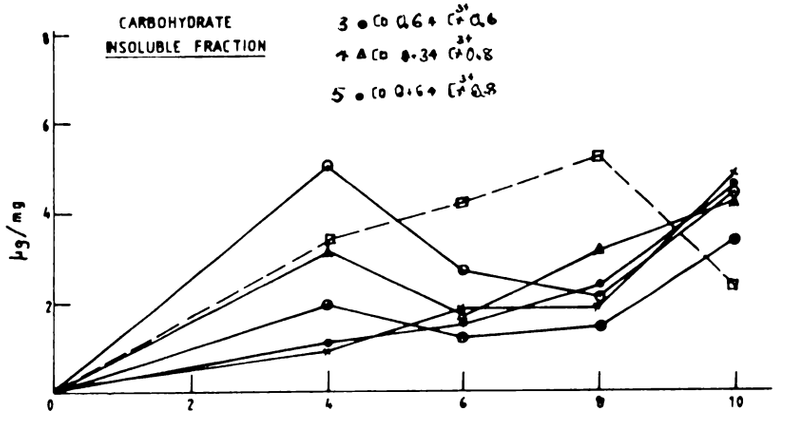
Days

N_clausii

C □ CONTROL
1 × Co 0.3 + Cr 0.2
2 ○ Co 0.5 + Cr 0.2
3 ● Co 0.6 + Cr 0.6
4 △ Co 0.34 Cr 0.8
5 ● Co 0.6 + Cr 0.8







5.1.2 Combined effect of cobalt and hexavalent chromium on S. abundans

Concentration of metals (in ppm)	Treatment Number
Co 0.01 + Cr^{6+} 0.05	9
Co 0.01 + Cr^{6+} 0.15	10
Co 0.05 + Cr^{6+} 0.05	11
Co 0.1 + Cr^{6+} 0.1	12

Biomass

There was a general decrease in the biomass at the end of growth phase for treatment (9) which in a combination of 0.01 ppm cobalt and 0.05 ppm hexavalent chromium and reatment (10) (combination of 0.01 ppm cobalt and 0.15 ppm hexavalent chromium) but there was 28% and 69% decrease for treatment (11) (combination of 0.05 ppm cobalt and 0.05 ppm hexavalent chromium and treatment (12) (combination of 0.1 ppm cobalt and 0.1 ppm hexavalent chromium). Treatment (9) was found to be fluctuating through out the growth phase. It was 46% and 8% more than the control on sixth and twelfth day whereas on fourth, eighth and tenth day there was 26%, 11% and 34% decrease. Treatment (10) was 11%, 51% more than the control on fourth and sixth day followed by 8% and 32% decrease on eighth and tenth day respectively. There was 3% and 23% increase on the fourth and sixth day for treatment (11) followed by 33%, 43% and 18% decrease on eighth, tenth and twelfth day respectively. Eventhough there was an increase on

the second day, treatment (12) was less than the control through out the growth phase.

Production

Generally the production was less than the control throughout the growth phase for all treated samples (Fig.31). The maximum inhibition was observed for treatment (11) and (12). At the end of growth phase there was 37%, 26%, 10%, 70% decrease for treatment (9), (10), (11) and (12) respectively. Treatment (9) was 22%, 10%, 15% and 25% less than the control on sixth, eighth, tenth and twelfth day. Treatment (10) was 27%, 9%, 14% and 22% less than the control on sixth, eighth, tenth and twelfth day respectively. There was a lag in production upto tenth day for treatment (11) and (12). Treatment (11) showed an increase on twelfth day but it was 39% and 10% less than the control on twelfth and fourteenth day. Substantial increase was observed in the pH at the end of growth phase. But for treatment (12) pH was less than the control throughout the growth phase.

Respiration

Respiration was less than the control for all treated samples (Fig.31). It showed an uniform decrease upto eighth day for all treatments. It was 70%, 50% and 41% less than the control on tenth, twelfth and fourteenth day for treatment (9). Treatment (10) showed 76%, 77% and 40% decrease on tenth, twelfth and fourteenth day. There was 70%, 83% and 40% decrease for treatment (11) and 52%, 58% and 86% decrease for treatment (12).

Photosynthetic pigments

Chlorophyll-a

Chlorophyll-a was more than the control throughout the growth phase for treatment (9) and (10). But (11) and (12) was less than the control through out the growth phase. At the end of growth phase treatment (9) and (10) was 10% and 24% more than the control whereas treatment (11) and (12) was 17% and 58% less than the control at the end of growth.

Chlorophyll-b

Generally chlorophyll-b was 1%, 59% and 42% less than the control for treatment (9), (10) and (11) whereas 45% increase was observed for treatment (12) (Fig.32). Treatment (9) was 71% more than the control on fourth day followed by 42%, 26%, 35% and 55% decrease on sixth, eighth, tenth, and twelfth day respectively. Similarly treatment (10) was 87% more than the control on fourth day followed by 37%, 24%, 26% and 63% decrease on sixth, eighth, tenth and twelfth day. Treatment (11) was less than the control through out the growth phase but there was 45% increase for treatment (12) eventhough it was less than the control upto twelfth day.

Carotenoids

Treatment (9), (10) and (12) was 26%, 32%, 13% less than the control at the end of growth phase, but treatment (11) was 13% more than the control (Fig.32). Treatment (9) was 38%, 7%, 13% and 31% more than the control on fourth, sixth, eighth and tenth day respectively. But towards the end of growth phase there was 37% and 26% decrease on twelfth and fourteenth

day. Similarly treatment (10) was 123%, 41%, 36% and 19% more than the control on fourth, sixth, eighth and tenth day but 30% and 32% decrease was observed on twelfth and fourteenth day. There was 43% increase on the fourth day for treatment (11) and it was less than the control upto twelfth day. Whereas treatment (12) was less than the control throughout the growth phase.

Phaeophytin

Phaeophytin showed a general trend of decrease of 86%, 59% and 57% at the end of growth phase for treatment (10), (11) and (12) but an increase of 22% was observed on the fourteenth day for treatment (9) (Fig.32). Treatment (9) was 39% more than the control on fourth day followed by decrease in phaeophytin content upto twelfth day. Treatment (10) and (12) was less than the control through out the growth phase,but treatment (11) showed 53% increase on eighth day eventhough it was less than the control throughout the growth phase.

Photosynthetic end products

Carbohydrate

There was an increase of 17%, 5% and 23% for treatment (9) (10) and (11) at the end of growth phase but treatment (12) was less than the control throughout the growth phase (Fig.33). Treatment (9) was 35%, 64% more than the control on eighth and tenth day. It was similar to the control on twelfth day. Thus treatment (9) was more than the control through out the growth phase. There was an increase of 77% and 45% on eighth and tenth day for treatment (10) but it was 17% less than the control on twelfth

day. Eventhough there was an increase of 23% at the end of growth phase it was 13%, 81% and 40% less than the control on eighth, tenth and twelfth day.

Protein

At the end of growth phase there was a decrease of 56%, 67%, 33% and 25% for treatment (9), (10), (11) and (12) respectively (Fig.33). There was an increase of 5% on the eighth day for treatment (9) followed by abrupt decrease towards the end of growth phase. Treatment (10) showed a marginal increase of 1% on eighth day followed by 17% increase on tenth day. But it was 49%, 67% less than the control on twelfth and fourteenth day. Treatment (11) was 7% and 15% more than the control on eighth and twelfth day but it was 37% less than the control on tenth day.

Lipid content was 43%, 27% less than the control for treatment (9) and (10) but 2% and 6% increase was observed for treatment (11) and (12) respectively.

Of the nutrients,50%, 37% and 43% increase in phosphate was observed for treatments (9), (10) and (11) respectively. But 78% decrease was observed for treatment (12) which was found to have less growth compared with other treatments. The nitrate uptake also showed 50% decrease for treatment (12), whereas marginal increase was observed for all treatments.

Fig.31

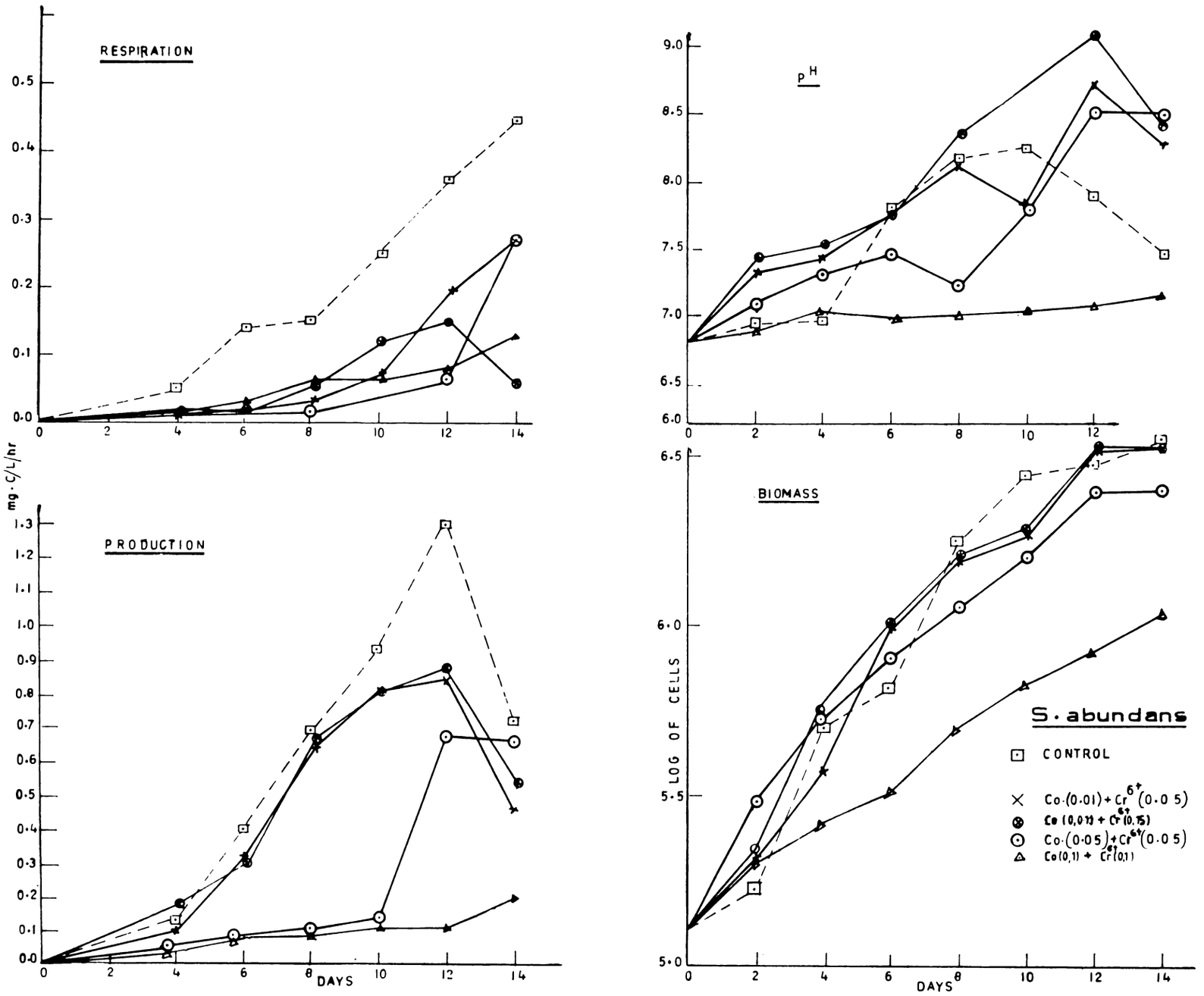

RESPIRATION
PRODUCTION
mg·C/L/hr
DAYS
pH
BIOMASS
LOG OF CELLS
DAYS
S. abundans
CONTROL
Co·(0·01) + Cr6+(0·05)
Co (0,01) + Cr6+(0,15)
Co·(0·05) + Cr6+(0·05)
Co(0,1) + Cr6+(0,1)

Fig.32

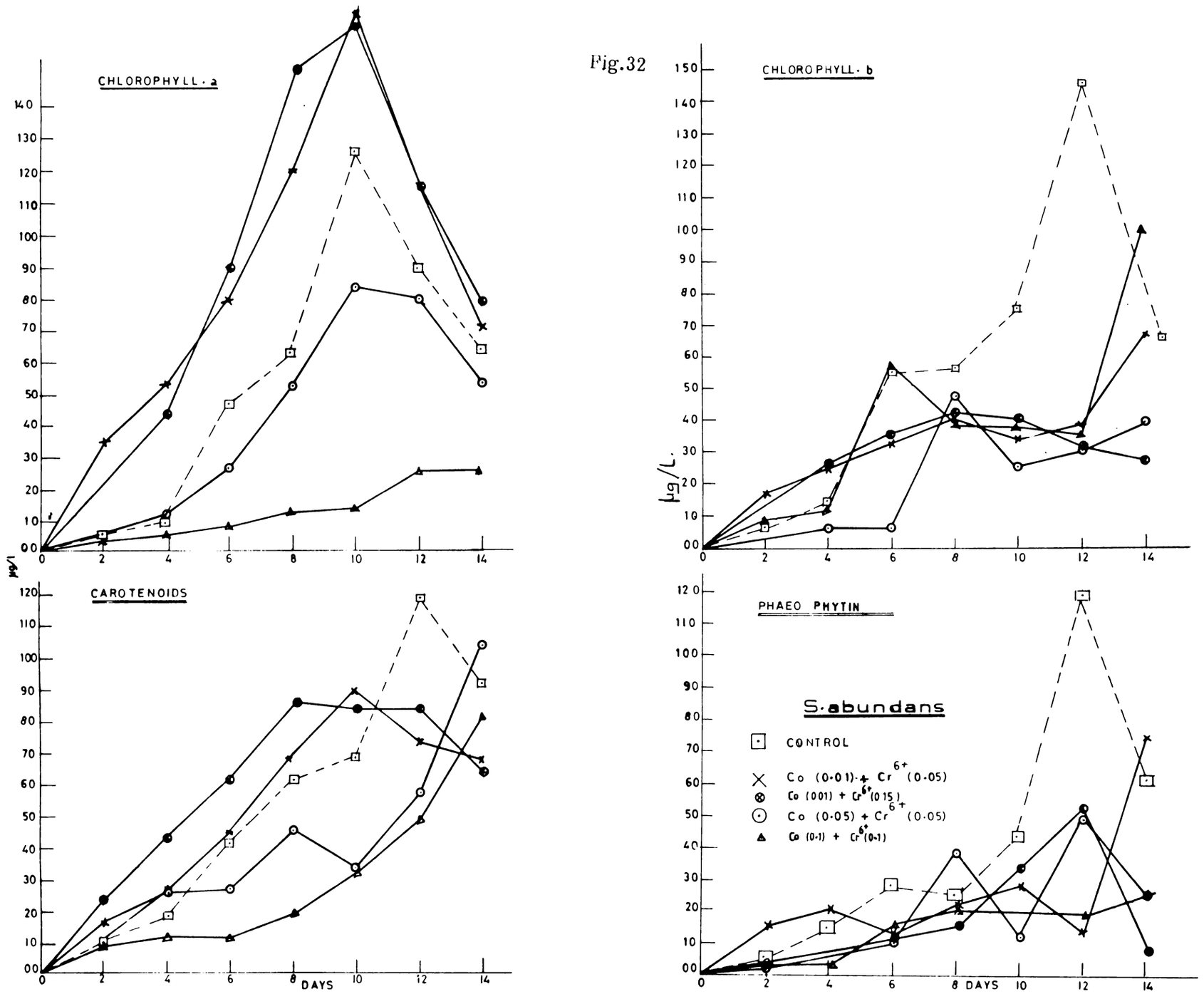
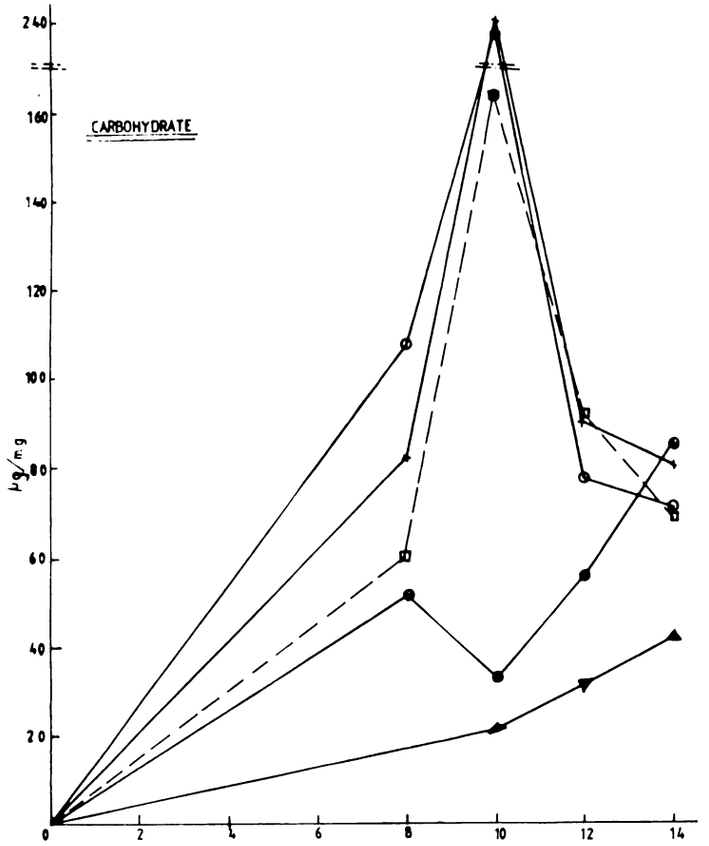
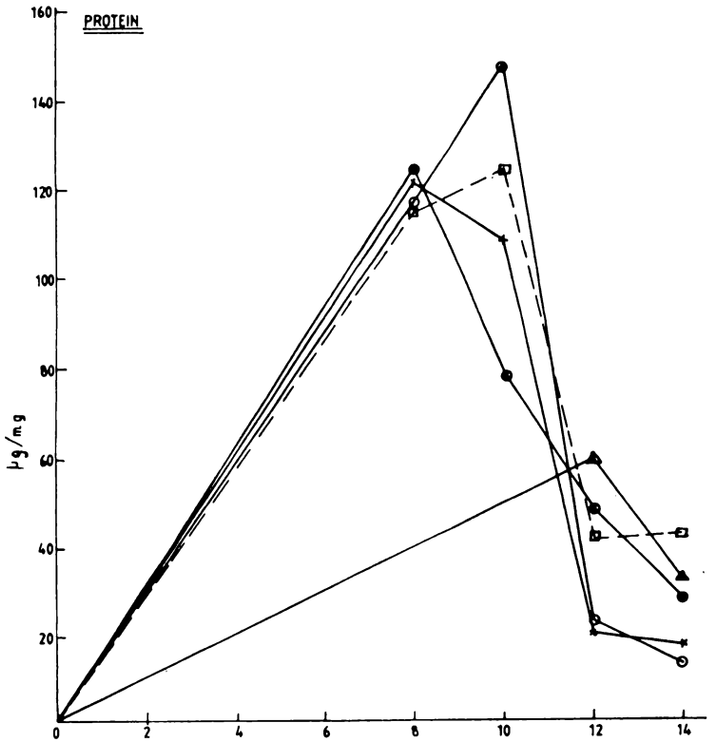
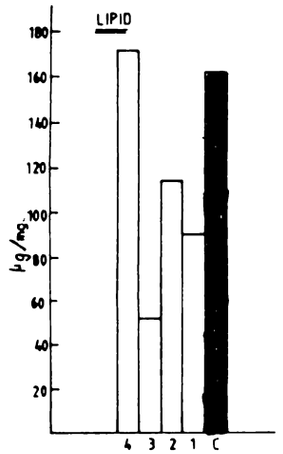

CHLOROPHYLL · a
CHLOROPHYLL · b
CAROTENOIDS
PHAEO PHYTIN
µg/l
DAYS
S. abundans
CONTROL
Co (0·01) + Cr 6+ (0·05)
Co (001) + Cr 6+ (0·15)
Co (0·05) + Cr 6+ (0·05)
Co (0·1) + Cr 6+ (0·1)

Fig.33





Days



S-abundans

C ▫ CONTROL

1 × Co (0,01) + Cr^{6+} (0,05)

2 ○ Co (0,01) + Cr^{6+} (0,15)

3 ● Co (0,05) + Cr^{6+} (0,05)

4 ▲ Co (0,1) + Cr^{6+} (0,1)

Combined effect of cobalt and hexavalent chromium on N. clausii

Concentration of metals (in ppm)			Treatment Number
Co 0.3 ppm	+	Cr^{6+} 0.2 ppm	(13)
Co 0.3	+	Cr^{6+} 0.6	(14)
Co 0.6	+	Cr^{6+} 0.2	(15)
Co 0.6	+	Cr^{6+} 0.6	(16)
Co 0.8	+	Cr^{6+} 0.6 ppm	(17)

Biomass

Generally for all treated samples the biomass was less than the control at the end of growth phase. It was 62%, 17%, 73%, 33% and 43% less than the control for treatment (13), (14), (15), (16) and (17) respectively. The treatment (13), a combination 0.3 ppm cobalt and 0.2 ppm hexavalent chromium was fluctuating. There was an initial lag in growth on second day followed by 50% and 15% increase on fourth and eighth day. But there was 8% and 62% decrease on sixth and tenth day. Treatment (14) a combination of 0.3 ppm cobalt and 0.6 ppm hexavalent chromium was less than the control throughout the growth phase. Similarly treatment (15) a combination of 0.6 ppm cobalt and 0.2 ppm hexavalent chromium, treatment (16) a combination of 0.6 ppm cobalt and 0.6 ppm hexavalent chromium and treatment (17) a combination of 0.8 ppm cobalt and 0.6 ppm hexavalent chromium were less than the control.

Production

Production was more than the control at the end of growth phase for all treated samples (Fig.34). It was 67%, 57%, 18%, 95% and 9% more than the control for treatment (13), (14), (15), (16) and (17) respectively. Treatment (13) was more than the control throughout the growth phase and it was 39%, 67%, 91%, and 67% more than the control on fourth, sixth, eighth and tenth day respectively. Treatment (14) was 41%, 25% and 2% less than the control on fourth, sixth and eighth day respectively. But it was 57% less than the control at the end. Production was fluctuating for treatment (15). It was 5% and 13% less than the control on fourth and eighth day whereas it was 44% more than the control on sixth day. Eventhough there was 26% decrease on the fourth day, treatment (16) was 21%, 26% more than the control on sixth and eighth day. Treatment (17) was less than the control upto fourth day followed by 8%, 26% and 9% increase on sixth, eighth and tenth day. Unlike production, pH showed substantial decrease at the end of growth phase for all treated samples.

Respiration

Respiration was 60% and 33% less than the control on fourth day and eighth day for treatment(14) and (15) was less than the control through out the growth phase. Treatment (16) was found to be fluctuating. It was 40% and 63% less than the control on fourth and eighth day but it 6% and 20% more than the control on sixth and tenth day respectively. Treatment (17) was less than the control upto eighth day followed by 81% increase at the end of growth phase.

Photosynthetic pigments

Chlorophyll-a

Chlorophyll-a was less than the control at the end of growth phase for all treated samples. The values being 11%, 32%, 31%, 35% and 39% less tha the control for treatment (13), (14), (15), (16) and (17) respectively. Even-though treatment (13) and (15) showed 25% and 27% increase on eighth day it was less than the control throughout the growth phase. Treatment (14), (16) and (17) was less than the control through out the growth phase.

Chlorophyll-c

Similar to chlorophyll-a, chlorophyll-c was less than the control at the end of growth phase on the tenth day for all treated samples ,but it was more than the control in the first half of growth phase. Treatment (13) and (15) and (16) was more than the control upto sixth day and on the eighth day it was similar to the control. Treatment (14) was 10% and 6% less than the control on fourth and tenth day but it was 25% and 31% more than the control on sixth and eighth day respectively. Treatment (17) was 70% more than the control on fourth day but 11%, 21% and 27% decrease was observed on sixth, eighth and tenth day respectively.

Carotenoids

Carotenoids were less than the control upto sixth day for all treated samples (Fig.35). But it was 39% and 17% more than the control on eighth day and a marginal increase of 4% and 1% was observed on the tenth day for treatment (13) and (16). Treatment (14) (15) was 29%, more than the

control on eighth day but 15% and 11% decrease was observed at the end of growth. Treatment (17) was less than the control throughout the growth phase.

Phaeophytin

Phaeophytin was less than the control at the end of growth phase for all treated samples except treatment (14) where it was 18% more than the control (Fig.35). Treatment (16), (15) and (17) was less than the control throughout the growth phase.

Photosynthetic end products

Carbohydrate

A general trend of decrease was observed at the end of growth phase, in the case of acid soluble fraction of carbohydrate (Fig.36). The acid soluble fraction was less than the control throughout the growth phase for treatment (13) but treatment (14), (15), (16) and (17) were maximum on the fourth day. Treatment (14) was 189% and 24% more than the control on fourth and sixth day but it was 26% and 25% less than the control on eighth and tenth day respectively. There was an increase of 348%, 13% and 18% observed on fourth, sixth and eighth day for treatment (15) but it was 55% less than the control at the end of growth. Treatment (16) was 297% more than the control on fourth day followed by decrease with the aging of the culture. There was an increase of 114%, 14% for treatment (17) on fourth and sixth day but it was 32% and 70% less than the control.

The alkali soluble fraction was less than the control upto eighth day for all treatments except treatment (14) (Fig.36). Treatment (14) was 32% more than the control on fourth day but it was less than the control on sixth and eighth day. At the end of growth phase treatment (13), (14) and (16) was 59%, 336% and 207% more than the control but 92% and 87% decrease was observed for treatment (15) and (17) respectively.

An initial peak was observed for the insoluble fraction of carbohydrate on the fourth day for all treatments (Fig.36) and at the end of growth phase also all treatments were more than the control. Treatment (13) was 184% and 59% more than the control on fourth and tenth day but it was less than the control on sixth and eighth day. Whereas treatment (14) was 32%, 3% and 173% more than the control on fourth, sixth and tenth day respectively. Though there was 22% decrease on sixth day, treatment (15) was 83%, 10% and 92% more than the control on fourth, eighth and tenth day respectively. Treatment (16) showed an initial peak of 61% on the fourth day followed by 34% and 27% less than the control on sixth and eighth day but it was 207% more than the control on the tenth day. Though there was a decrease of 20% on the sixth day for treatment (17), it was more than the control throughout the growth phase.

Protein

A general trend of decrease was observed in the protein content at the end of growth phase (Fig.36). It was 36%, 34%, 60%, 22% and 58% less than the control for treatment (13), (14), (15), (16) and (17). Treatment (13) showed an initial increase of 57% and 19% on fourth and sixth day, but it

was 10% and 86% less than the control. Eventhough there was 16% increase on the sixth day for treatment (14) it was less than the control throughout the growth phase. Treatment (15) was 91% and 16% more than the control but towards the end of growth it was 55% and 60% less than the control. Treatment (16) was less than the control through out the growth phase. Treatment (17) showed a peak (91%) on the sixth day but it was 6%, 25% and 58% less than the control on fourth, eighth and tenth day respectively.

Lipid content was 90%, 85%, 87%, 88% and 89% less than the control for treatment (13), (14), (15), (16) and (17) respectively.

The phosphate and nitrate uptake was more than the control for all treated samples.

Fig.34

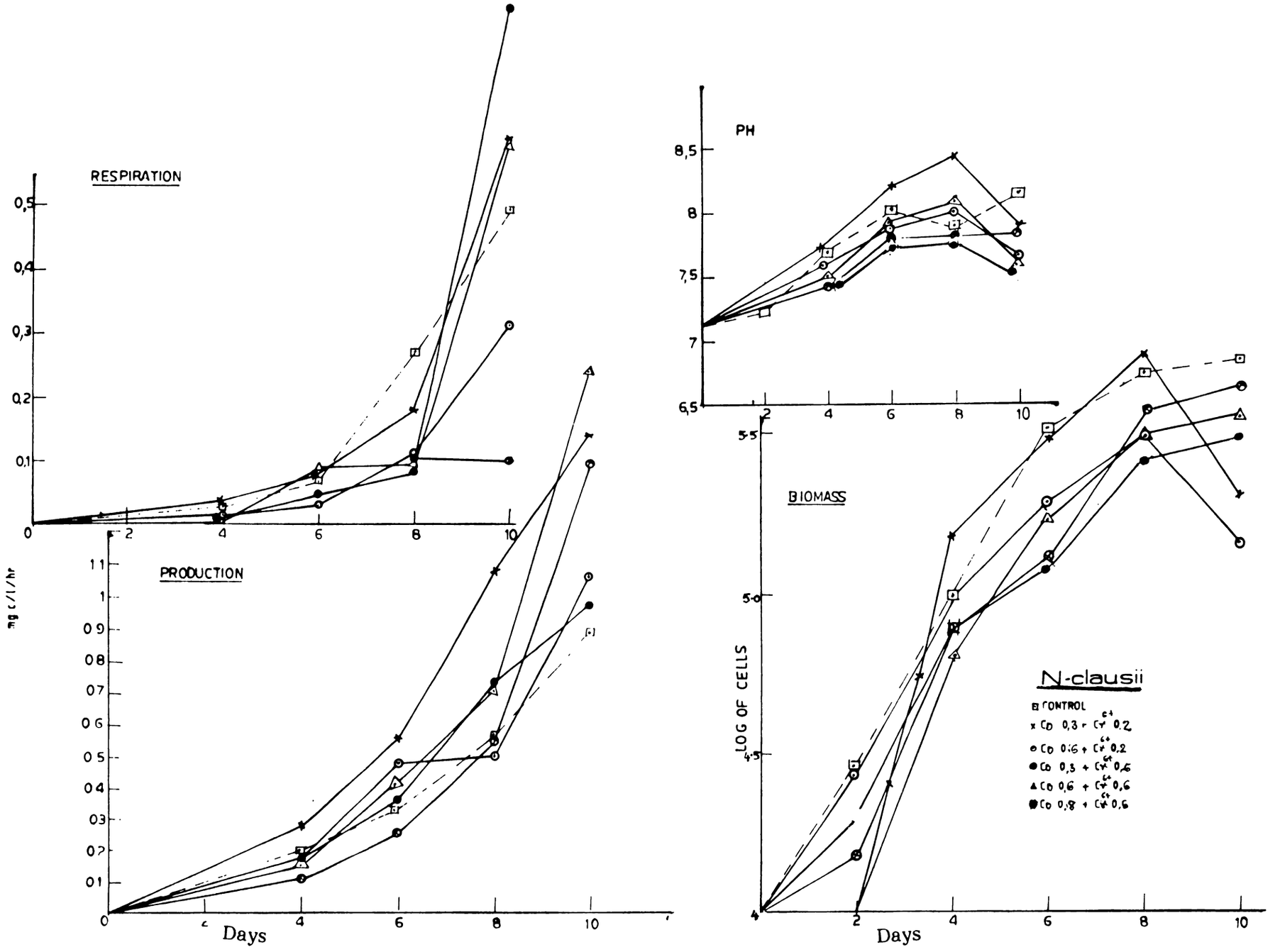
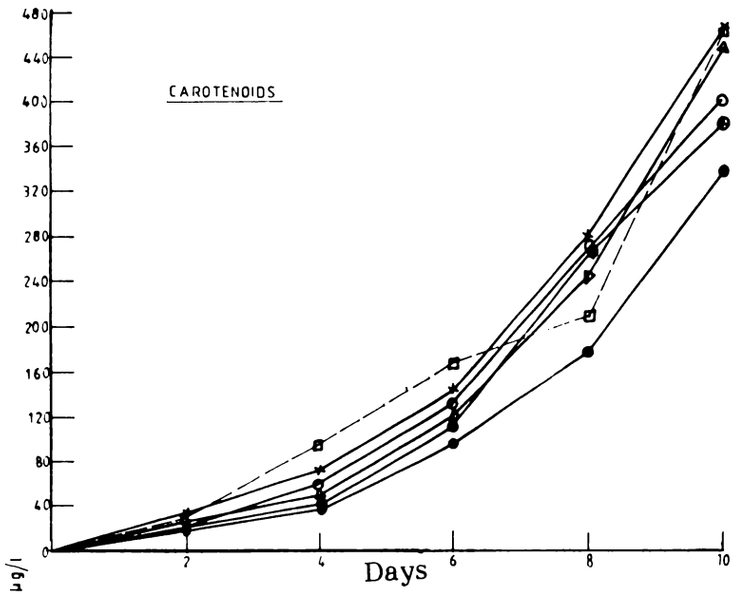
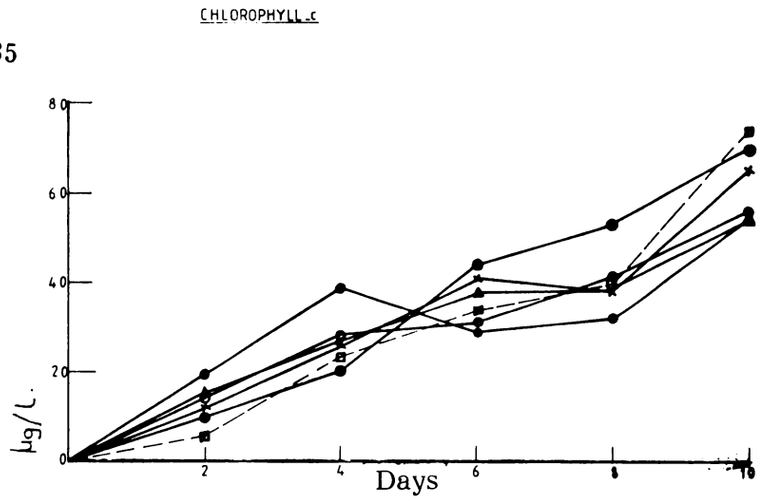
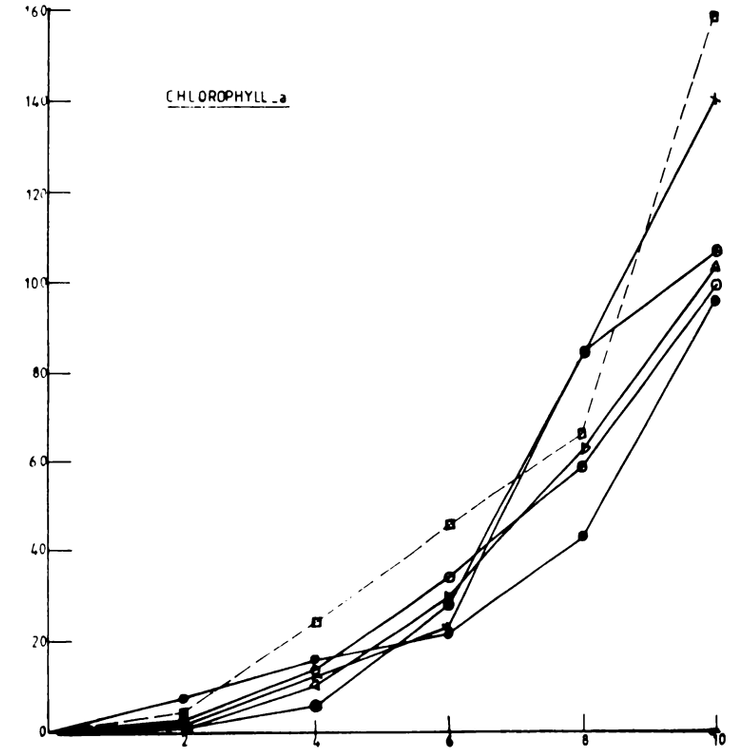

RESPIRATION
PH
PRODUCTION
BIOMASS
mg c/l/hr
LOG OF CELLS
Days
Days
N-clausii
CONTROL
Co 0,3 + Ca++ 0,2
Co 0,6 + Ca++ 0,2
Co 0,3 + Ca++ 0,6
Co 0,6 + Ca++ 0,6
Co 0,8 + Ca++ 0,6

Fig.35


CAROTENOIDS
µg/l
Days


CHLOROPHYLL_c
µg/L
Days


CHLOROPHYLL_a

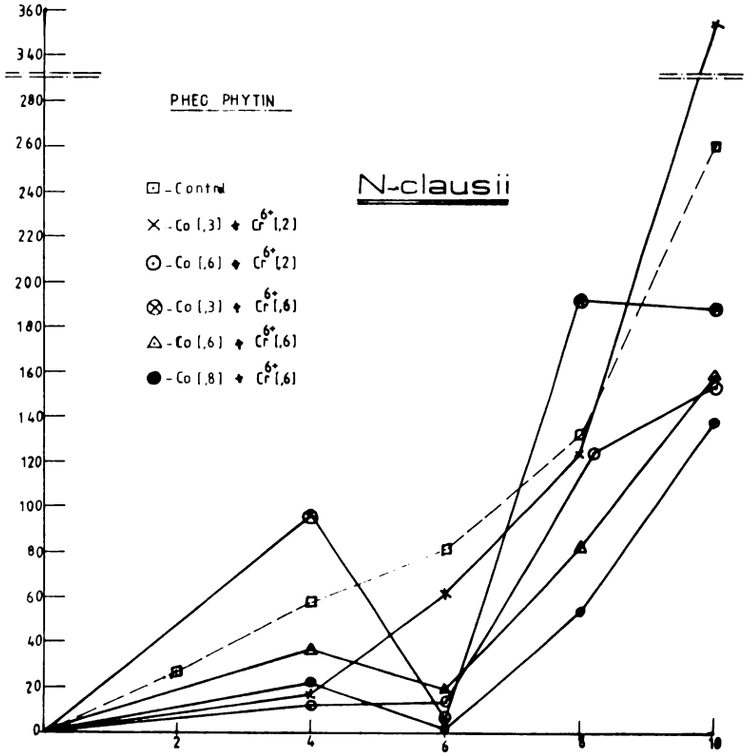
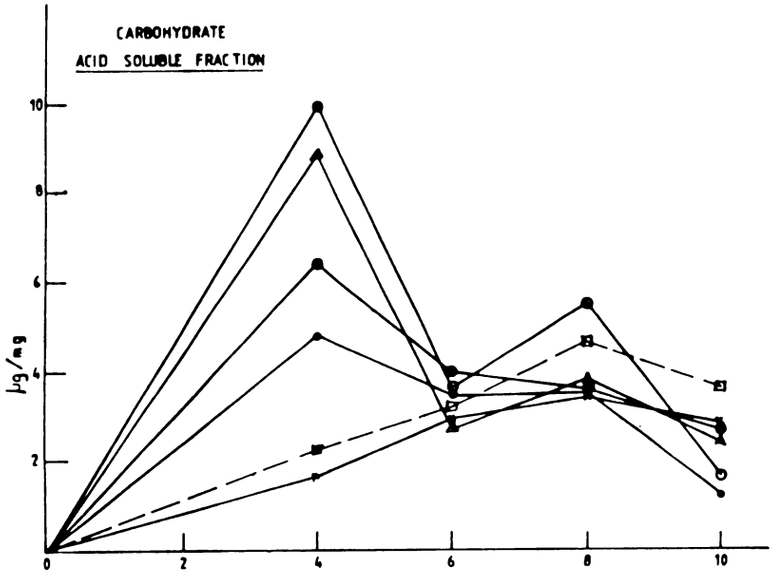
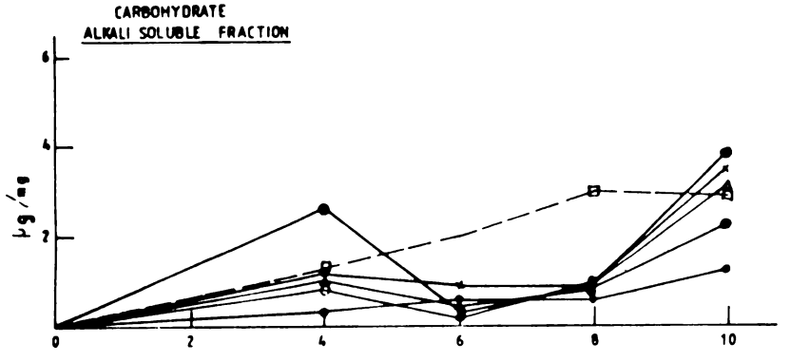
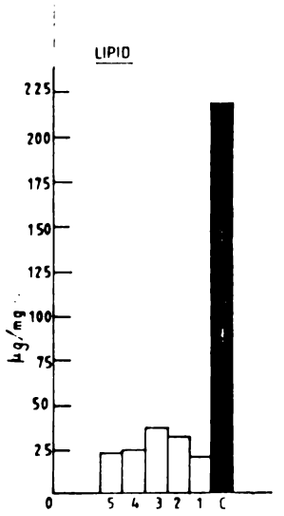

PHEO PHYTIN
N-clausii
□ - Control
× - Co (,3) + Cr6+ (,2)
⊙ - Co (,6) + Cr6+ (2)
⊗ - Co (,3) + Cr6+ (,6)
△ - Co (,6) + Cr6+ (,6)
● - Co (,8) + Cr6+ (,6)

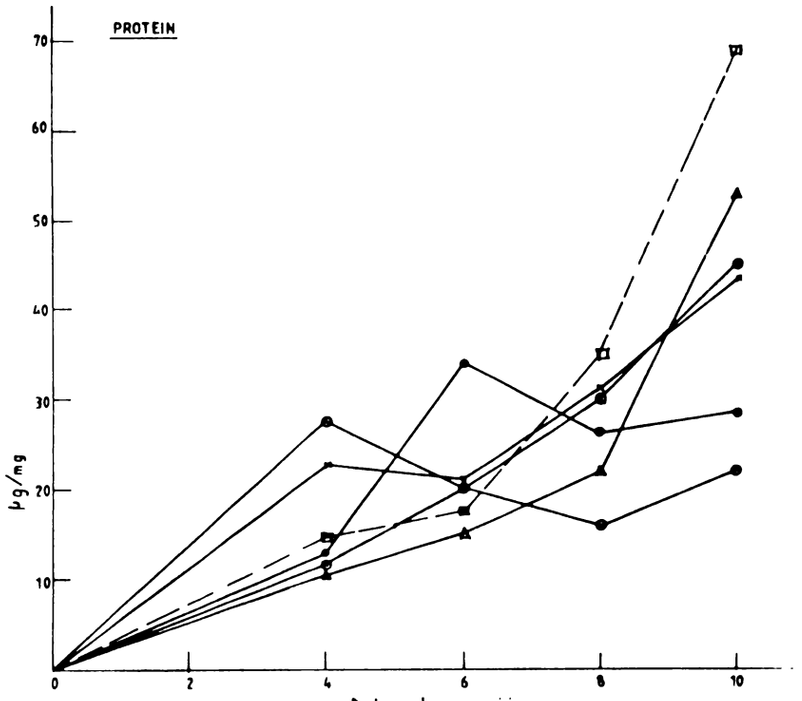
Fig.36

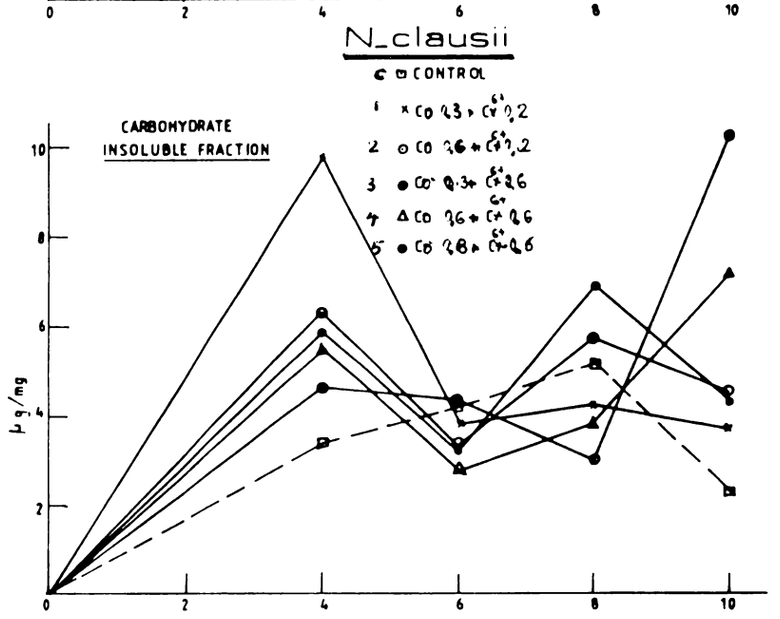

CARBOHYDRATE
ACID SOLUBLE FRACTION
µg/mg


CARBOHYDRATE
ALKALI SOLUBLE FRACTION
µg/mg


LIPID
µg/mg
5 4 3 2 1 C

Days


PROTEIN
µg/mg


N_clausii
CONTROL
CARBOHYDRATE
INSOLUBLE FRACTION
µg/mg

5.1.3 **Combined effect of nickel and trivalent chromium in S. abundans**

Concentration of metals (in ppm)	Treatment Number
Ni 0.01 + Cr^{3+} 0.01	18
Ni 0.02 + Cr^{3+} 0.02	19
Ni 0.03 + Cr^{3+} 0.03	20

Biomass

A general trend of increase was observed through out the growth phase in algae treated with a combination of metals, nickel and trivalent chromium (0.01 ppm each) referred to as treatment (18), but for a concentration of 0.02 each treatment (19) the growth was fluctuating while for a concentration of 0.03 ppm treatment (20) it was less than the control from fourth day. At the end of growth phase the biomass was more than the control for treatment (18) by 18% and less than the control by 8% and 9% for treatment (19) and (20) respectively. Treatment (18) was 20%, 65%, 52%, 14% and 33% more than the control on fourth, sixth, eighth, tenth and twelfth day respectively. Treatment (19) was 70% and 24% more than the control on second and eighth day. But it was 16% and 5% less than the control on fourth and tenth day respectively. Eventhough there was an initial increase 53% on the second day for treatment (20), it was less than the control through out the growth phase.

Production

Production was 38% and 25% less than the control at the end of growth phase for treatment (18) and (19) but treatment (20) was 6% more than

control at the end (Fig.37). There was a general decrease in production except on tenth day for treatment (18). It was 82%, 22%, 13%, 25% and 38% less than the control on fourth, sixth, eighth, twelfth and fourteenth day. The treatment (19) was less than the control through out the growth phase. However, it was almost equal to control on the sixth day. Treatment (20) was 84%, 72% and 38% less than the control on sixth, eighth and tenth day respectively. But towards the end of growth it was 9% and 6% more than the control. Similar to production, pH was also less than the control upto tenth day. But at the end of growth phase pH showed an increase for treatment (18) and (19).

Respiration

Respiration was less than the control throughout the growth phase for treatment (18) and (20) (Fig.37). At the end of growth phase treatment (18) was 20% less than the control whereas treatment (20) was 39% more than the control and treatment (19) was similar to the control. Treatment (20) was 70% and 60% and 25% less than the control on fourth, sixth and tenth day. But 7% increase was observed on the eighth day.

Chlorophyll-a

Generally chlorophyll-a was less than the control for all treated samples, eventhough there was 34% and 43% increase on the second and fourth day for all treatments. At the end of growth phase it was 31%, 58% and 17% less than the control for treatment 18, 19 and 20 respectively (Fig.38). Treatment (18) was 43%, 36% and 85% less than the control on sixth, eighth and

tenth day. Treatment (19) was similar to control upto fourth day followed by 43%, 5%, 15% and 40% less than the control on sixth, eighth, tenth and twelfth day. There was 71%, 57%, 78% and 4% decrease on sixth, eighth, tenth and twelfth day for treatment (20).

Chlorophyll-b

A general trend of increase was observed at the end of growth phase for treatment (18) and (20), though it was less than the control upto twelfth day for treatment (18) and (20). It was 40%, 98% and 81% more than the control at the end of growth phase for treatment (18), (19) and (20) respectively. Thus the treated samples were having the peak at the end of growth phase whereas the control was maximum on the twelfth day (Fig.38). Treatment (19) was fluctuating and it was 80%, 87% and 56% more than the control on fourth, eighth and tenth day. But it was 51% and 43% less than the control on sixth and twelfth day.

Carotenoids

Carotenoids were less than the control throughout the growth phase for treatment (18) and (20) and treatment (19) was found to be fluctuating. At the end of growth phase there was 47%, 17% and 8% decrease for treatment (18), (19) and (20) respectively. Treatment (19) was 50%, 36% and 57% less than the control on second, fourth and sixth day. On the eighth and tenth day it was 7% and 5% more than the control followed by sudden decrease towards the end of growth phase.

Phaeophytin

Phaeophytin was 104% and 22% more than the control for treatment (19) and (20) and it was similar to the control for treatment (18) at the end of growth phase (Fig.38). Treatment (18) was similar to the control on eighth, tenth and fourteenth day but it was 12% and 59% less than the control on fourth and twelfth day. Treatment (19) was 81%, 68% and 32% less than the control on fourth, sixth and twelfth day but it was 45%, 41% and 104% more than the control on eighth, tenth and fourteenth day respectively. Whereas treatment (20) was less than the control upto twelfth day.

Photosynthetic end products

Carbohydrate

There was a general trend of increase in carbohydrate on the eighth day. At the end of growth phase there was 20% and 27% decrease for treatment (18) and (20) and for treatment (19) there was an increase of 32% (Fig.39). Treatment (18) was 204% and 61% more than the control on eighth and twelfth day and 25% decrease was observed on tenth day. Treatment (19) was 143%, 47% and 32% more than the control on eighth, tenth and fourteenth day. Treatment (20) was 33%, 43% and 20% less than the control on tenth, twelfth and fourteenth day though there was 44% increase on eighth day. Thus the maximum carbohydrate was observed for treatment (19) and it was minimum for treatment (20).

Protein

There was an increase in protein content for treatment (18) and (20) (Fig.39) on the eighth day. Whereas treatment (19) was 38% and 33% less

than the control on eighth and tenth day. There was 65% decrease on twelfth and fourteenth day. Thus throughout the growth phase carbohydrate was less than the control. There was an increase of 42%, 44% and 22% on eighth, tenth and twelfth day respectively for treatment (18). It was 37% less than the control on the fourteenth day. Thus the maximum protein was noticed for treatment (18). Treatment (20) was 34% and 28% more than the control on eighth and twelfth day. At the end of growth phase it was similar to the control.

Lipid content of the algae was 80% and 75% less than the control for treatment (18) and (20). But treatment (19) was 30% more than the control.

In the case of phosphate and nitrate uptake, a marginal decrease was observed for all treated samples.

Fig.37

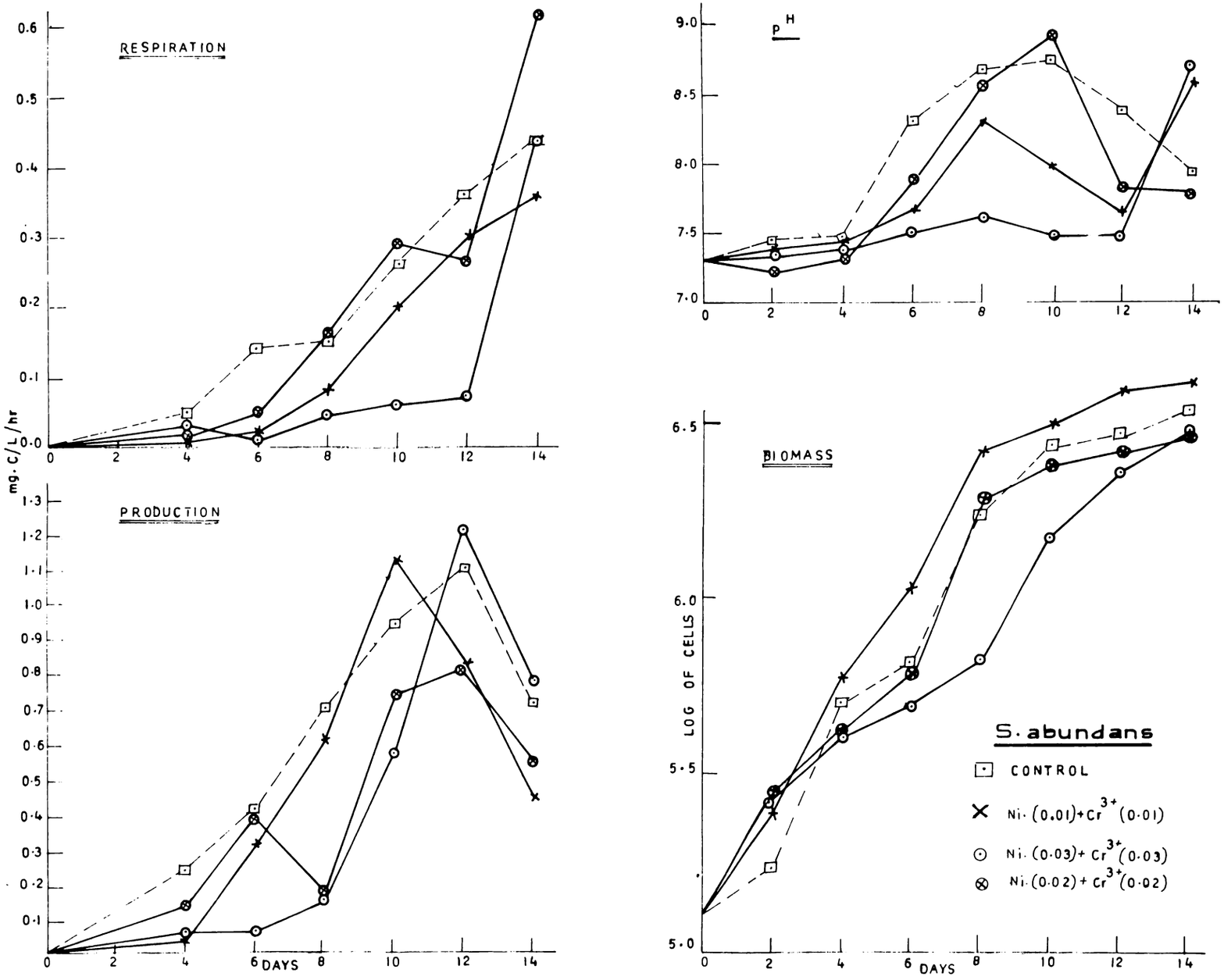

RESPIRATION
mg. C/L/hr
PRODUCTION
DAYS
pH
BIOMASS
LOG OF CELLS
S. abundans
CONTROL
Ni. (0.01)+Cr3+(0.01)
Ni. (0.03)+ Cr3+(0.03)
Ni. (0.02) + Cr3+(0.02)

Fig.38

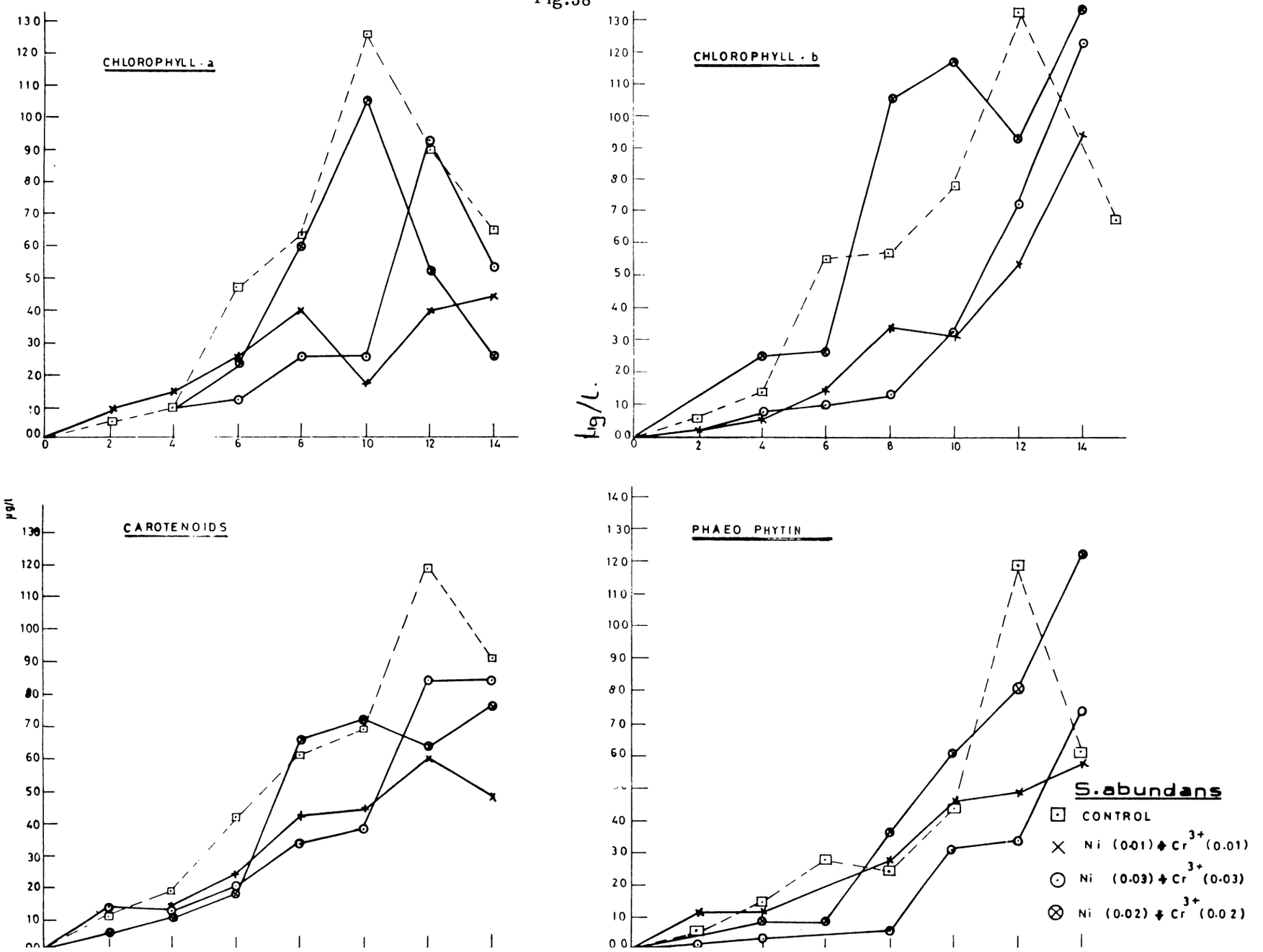
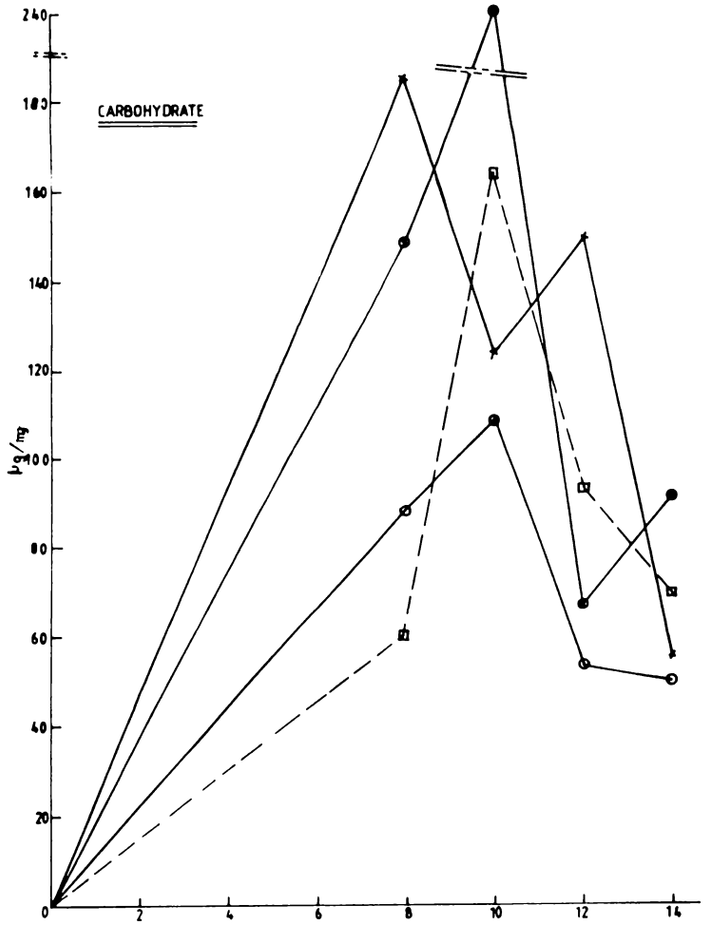
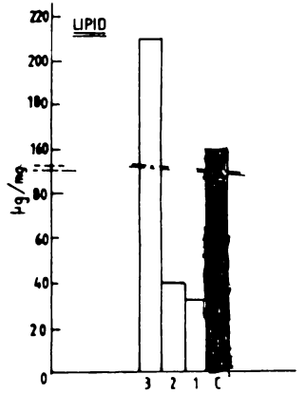
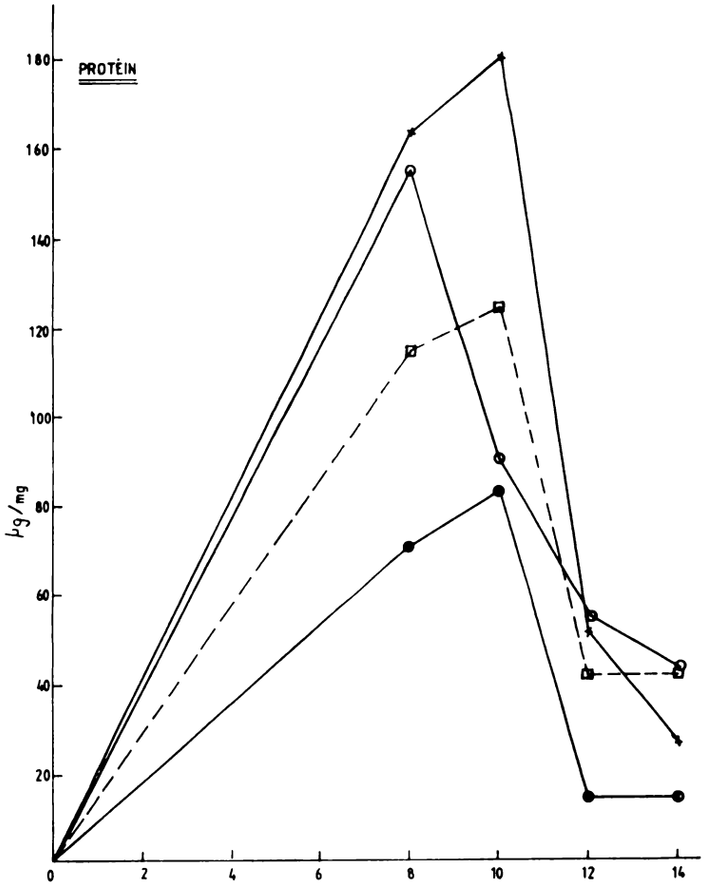

CHLOROPHYLL - a
CHLOROPHYLL - b
µg/L.
CAROTENOIDS
µg/l
PHAEO PHYTIN
S.abundans
CONTROL
Ni (0·01) + Cr 3+ (0·01)
Ni (0·03) + Cr 3+ (0·03)
Ni (0·02) + Cr 3+ (0·02)

Fig.39







Days

S-abundans

C ⊡ CONTROL

1 × Ni (0,01) + Cr^{3+} (0,01)

2 ⊙ Ni (0,03) + Cr^{3+} (0,03)

3 ● Ni (0,02) + Cr^{3+} (0,02)

5.3.2 Combined effect of nickel and trivalent chromium in *N. clausii*

Concentration of metals (in ppm)	Treatment Number
Ni 0.4 + Cr^{3+} 0.8	21
Ni 0.6 + Cr^{3+} 0.2	22
Ni 0.6 + Cr^{3+} 0.8	23

Biomass

An increase in the biomass was observed for all treatments of nickel and trivalent chromium at the end of growth phase (Fig.40). It was 99%, 66% and 68% more than the control for treatment (21) (which was a combination of 0.4 ppm nickel and 0.8 ppm trivalent chromium), treatment (22) (combination of 0.6 ppm nickel and 0.2 ppm trivalent chromium) and treatment (23) (combination of 0.6 ppm nickel and 0.8 ppm trivalent chromium). Eventhough there was an initial decrease in growth treatment (21) was 23%, 16% and 97% more than the control on fourth, sixth and eighth day respectively. Treatment (22) was 20%, 74% and 66% more than the control on fourth, eighth and tenth day respectively with 34% decrease on the sixth day. There was an increase of 85% on the fourth day followed by 20% and 33% decrease on the sixth and eighth day for treatment (23).

Production

Generally production was less than the control through out the growth phase for treatment (23). It was 4%, 31% and 51% less than the control on sixth, eighth and tenth day. At the end of growth phase there was 51%

increase for treatment (21) and 28% and 51% decrease for treatment (22) and (23) (Fig.40). Treatment (21) was showing an increasing trend with the aging of the culture but it was 29% less than the control on the fourth day. There was 27% and 13% decrease on the fourth and sixth day for treatment (22) but it was 25% less than the control on the eighth day. The pH was found to be more than the control through out the growth phase.

Respiration

A decreasing trend was observed at the end of growth phase for all treated samples. It was 64%, 25% and 48% less than the control for treatment (21), (22) and (23) respectively (Fig.40). Treatment (21) was generally less than the control but 127% increase was observed on the sixth day. Similarly treatment (22) was 8% more than the control on the sixth day. Treatment (23) was 22%, 73% and 48% less than the control on sixth, eighth and tenth day but a marginal increase of 39% was observed on the fourth day.

Photosynthetic pigments

Chlorophyll-a

Chlorophyll-a was 20%, 15% and 51% less than the control at the end of growth phase of treatment (21), (22) and (23) respectively (Fig.41). Through out the growth phase chlorophyll-a was less than the control for treatment (23). Treatment (21) was less than the control upto fourth day followed by 9% and 7% increase on sixth and eighth day. Through out the growth phase treatment (22) showed a general decrease but a marginal increase of 19% was observed on the eighth day.

Chlorophyll-c

An increase of 117% and 66% was noticed for treatment (21) and (22) at the end of growth phase. But treatment (23) was 76% less than the control at the end of growth phase. A general increase was observed through out the growth phase but there was 67% decrease on the fourth day. Similar trend was observed for treatment (22) but the peak was less than treatment (21). Treatment (23) was 89%, 33% and 55% more than the control on fourth, sixth and eighth day but an abrupt decrease of 76% was observed at the end of growth.

Carotenoids

Treatment (21) and (23) was closely following the control upto sixth day but it was less than the control (Fig.41). Treatment (21) was 11% more than the control on the eighth and tenth day. Treatment (22) was 41% and 16% more than the control on eighth and tenth day. Treatment (23) was more than the control on the second and eighth day but at the end of growth phase it was 38% less than the control.

Phaeophytin

Phaeophytin was 96%, 80% and 77% less than the control for treatment (21), (22) and (23) at the end of growth phase (Fig.41). Treatment (21) was less than the control through out the growth phase. Treatment (22) was also less than the control, but a marginal increase was observed on the fourth day.

Photosynthetic end products

Carbohydrate

Acid soluble fraction of carbohydrate was having the peak on the fourth day but it was less than the control for all treatments at the end growth phase (Fig.42). There was 449%, 157% and 112% increase on the fourth day and there was a decrease of 13%, 19% and 48% at the end of growth phase for treatments (21), (22) and (23) respectively. Treatment (23) was less than the control from sixth day onwards. Maximum decrease was observed for treatment (23).

Alkali soluble fraction showed 66%, 63% and 80% decrease at the end of growth phase (Fig.42) for treatment (21), (22), (23) respectively. Treatment (21) and (23) was less than the control through out the growth phase but treatment (22) was 50% and 44% more than the control on fourth and sixth day but towards the end of growth phase there was 80% and 63% decrease on the eighth and tenth day respectively.

Insoluble fraction was more than the control at the end of growth phase for all treated samples (Fig.42). The values being 172%, 149% and 48% more than the control for treatment (21), (22) and (23) respectively. A general trend of decrease was observed upto eighth day for treatment (23). Treatment (21) was 3% and 20% less than the control on fourth and eighth day but 24% and 172% increase was noticed on the sixth and tenth day respectively. Similar decrease of 23% and 12% was observed on the fourth and eighth day respectively, but an increase of 19% and 149% was noticed on sixth and tenth day for treatment (22).

Protein

Generally protein was less than the control for all treatments on the fourth, eighth and tenth day (Fig.42). On the fourth day there was 10%, 32% and 21% decrease and on the eighth day 12%, 37% and 63% decrease was noticed for treatment (21), (22) and (23) respectively. On the sixth day there was 43%, 17% and 116% increase for treatment (21), (22) and (23). Maximum protein was noticed for treatment (23).

Lipid content was less than the control for all treated samples. It was 91%, 82% and 87% less than the control for treatments (21), (22) and (23) respectively.

In the case of nutrients the nitrate uptake was slightly more than the control for all treated samples. Phosphate was 27% less than the control for treatment (22) and 9% and 12% increase was observed for treatment (21) and (23) respectively.

Fig.40

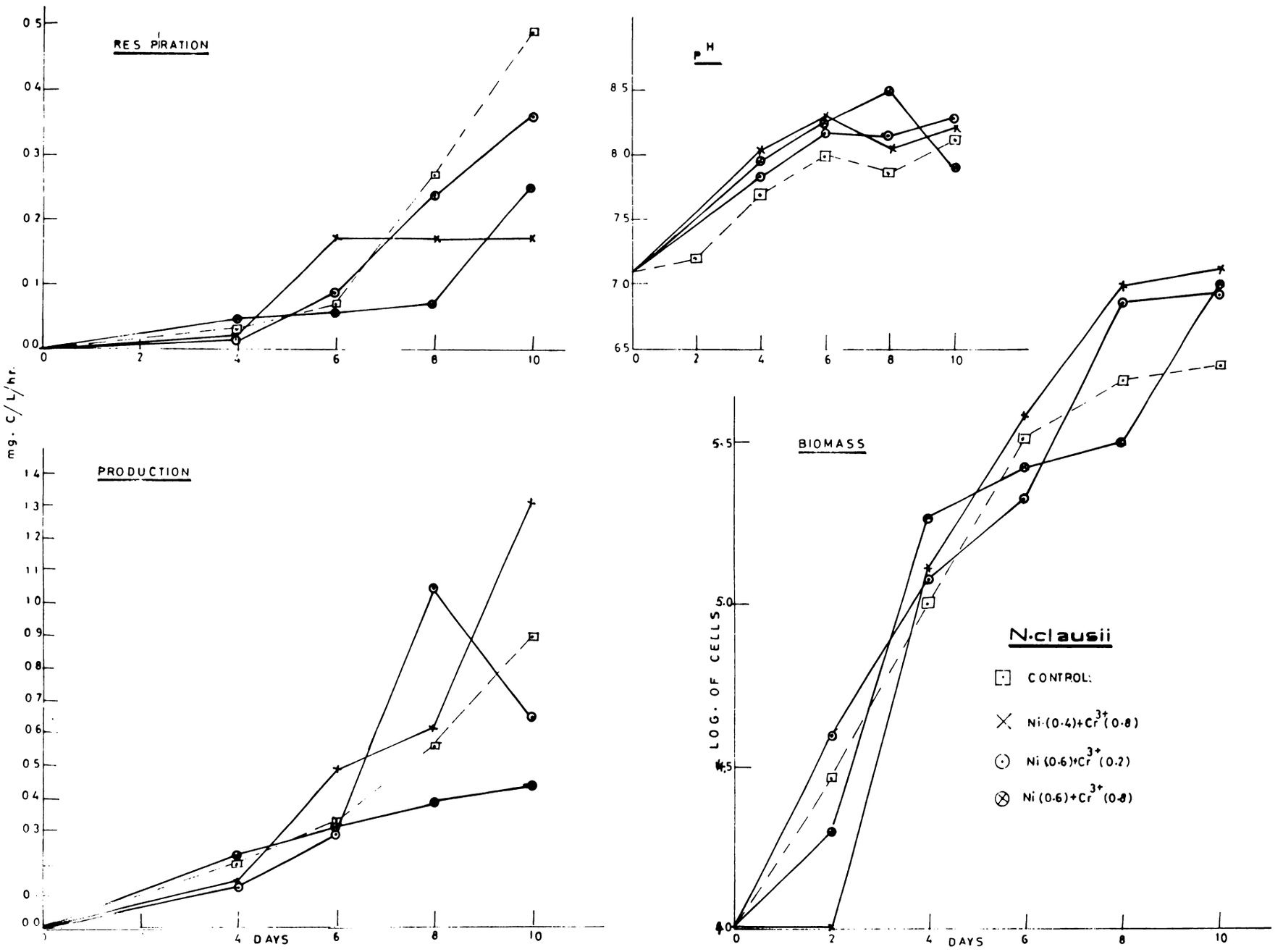
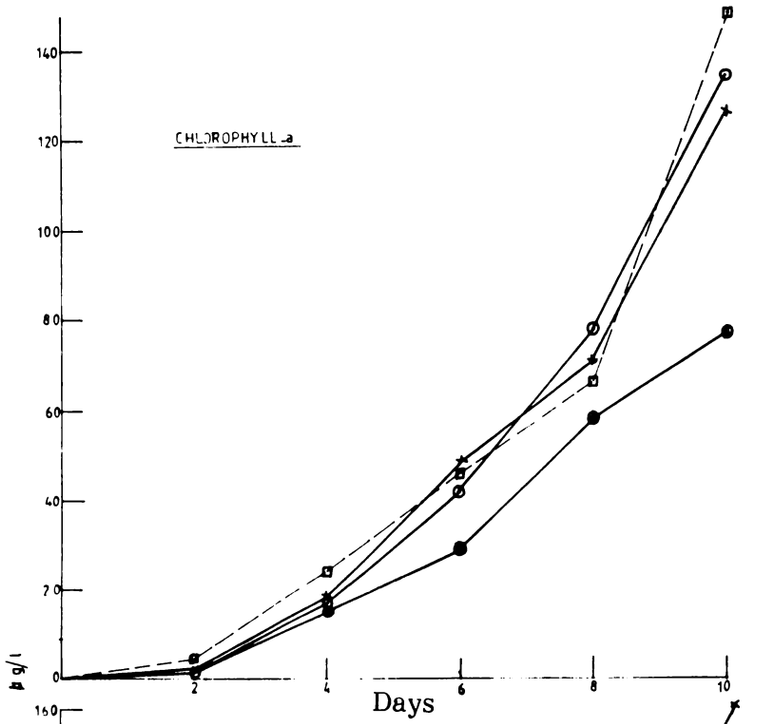
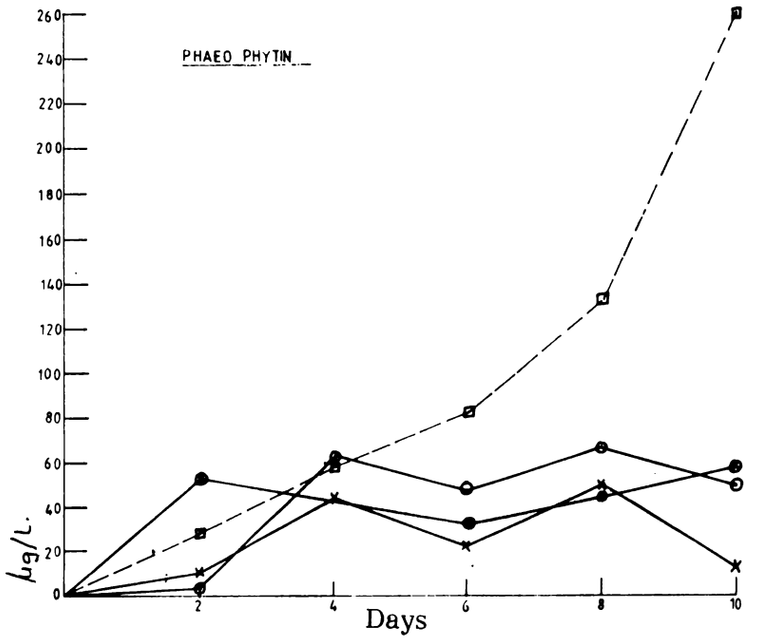
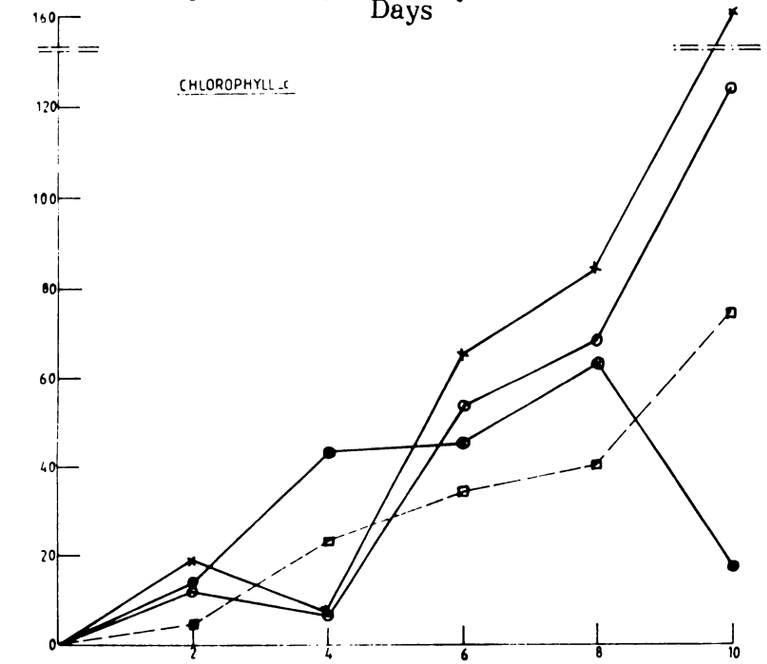

RESPIRATION
PH
PRODUCTION
BIOMASS
mg. C/L/hr.
LOG. OF CELLS
DAYS
N.clausii
CONTROL
Ni (0·4)+Cr3+ (0·8)
Ni (0·6)+Cr3+ (0·2)
Ni (0·6)+Cr3+ (0·8)

Fig.41


CHLOROPHYLL a
µg/l
Days


PHAEO PHYTIN
µg/L.
Days


CHLOROPHYLL c

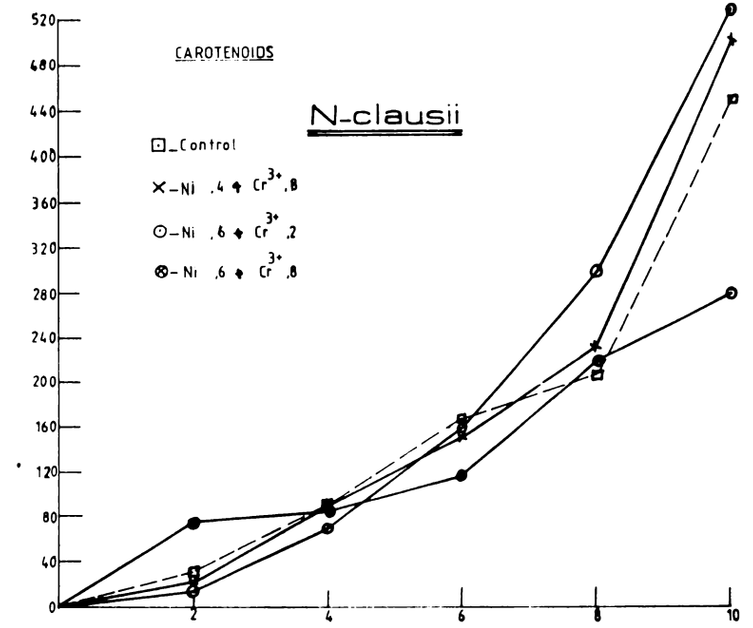
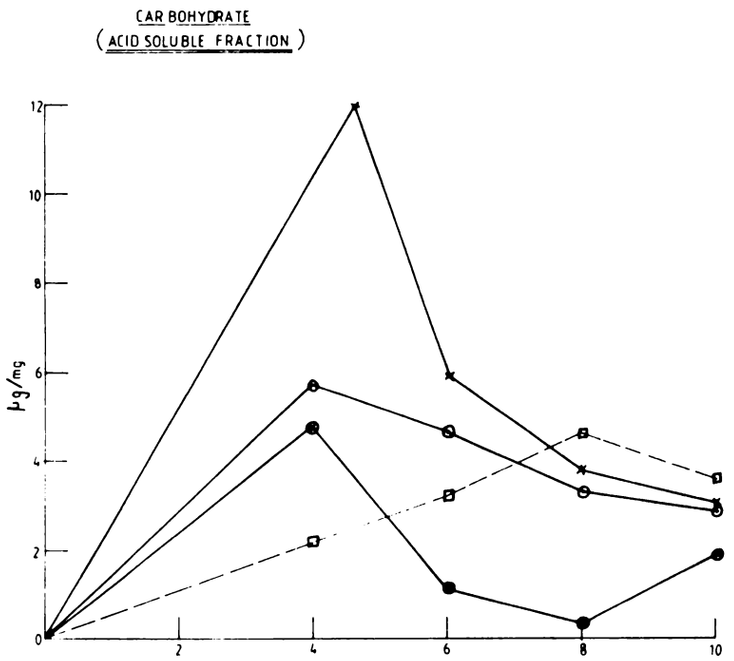
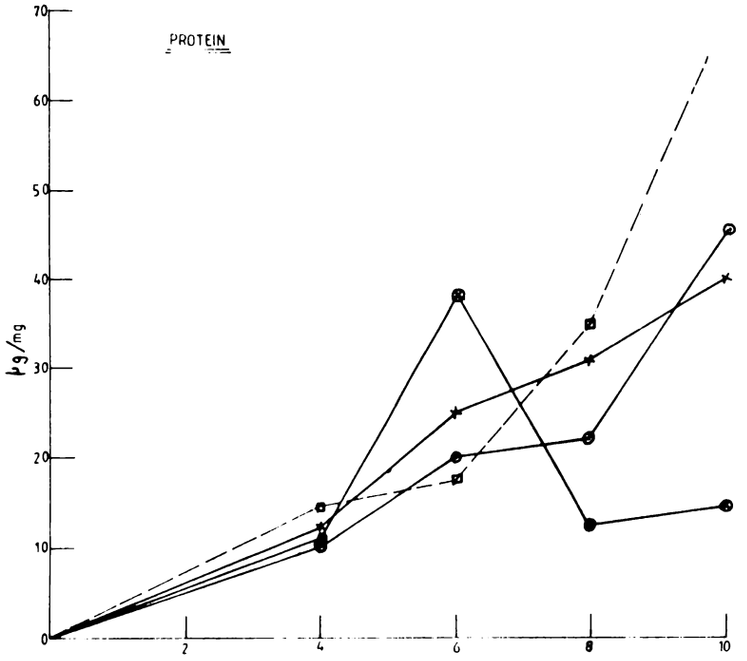

CAROTENOIDS
N-clausii
Control
Ni .4 Cr3+ .8
Ni .6 Cr3+ .2
Ni .6 Cr3+ .8

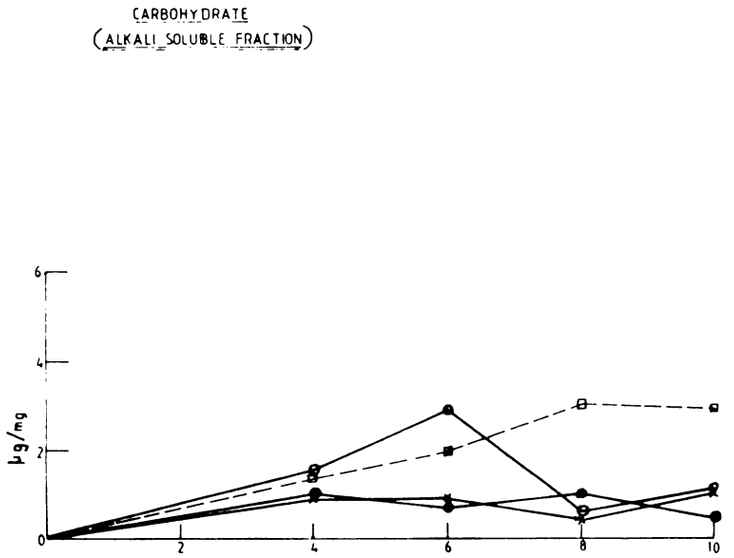
Fig.42

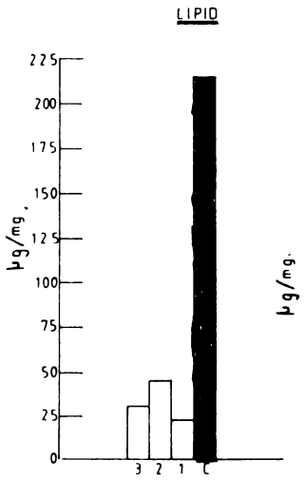


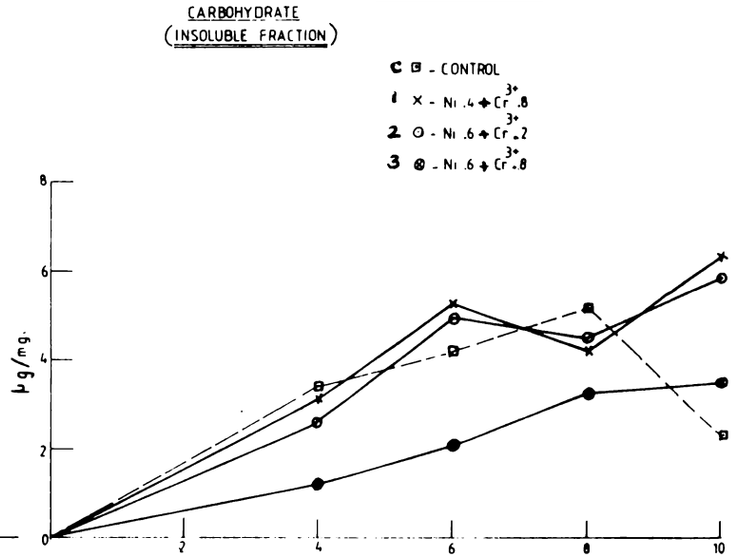


Days

N_clausii







5.1.4 Combined effect of nickel and hexavalent chromium in S. abundans

Concentration of metals (in ppm)	Treatment Number
Ni 0.03 + Cr^{6+} 0.15	24
Ni 0.01 + Cr^{6+} 0.05	25
Ni 0.02 + Cr^{6+} 0.1	26

The treatment (24) (combination of 0.03 ppm nickel and 0.15 ppm hexavalent chromium) was lethal. So the study of all parameters were made only for treatment (25) which was a combination of 0.01 ppm nickel and 0.05 ppm hexavalent chromium and (26) a combination of 0.02 ppm nickel and 0.1 ppm hexavalent chromium.

Biomass

A decrease of 7% and 19% at the end of growth phase was noticed for treatment (25) and (26) (Fig. 43). Treatment (25) was 135%, 41%, 74%, 31%, 12% and 5% less than the control on second, fourth, sixth, eighth, tenth and twelfth day respectively. Treatment (26) showed an initial increase of 18% on the second day but through out the growth phase it was less than the control.

Production

Production was less than the control for treatment (26) throughout the growth phase. At the end of growth phase both the treatments were similar to the control (Fig.43). Treatment (25) was closely following the control

on the fourth day and it was 52% more on the sixth day. But on the eighth, tenth and twelfth day there was 34%, 20% and 16% decrease was noticed for treatment (25). Similar to production pH was less than the control throughout the growth phase.

Respiration

Respiration was less than the control throughout the growth phase for treatment (25) but it was similar to the control on fourth and eighth day. There was 7%, 18% and 5% decrease on tenth, twelfth and fourteenth day (Fig.43). Respiration was found to be fluctuating for treatment (26). There was 70% decrease on the fourth day but 22%, 51% and 17% increase was observed on the sixth, eighth and fourteenth day. On the tenth and twelfth day 46% and 66% decrease was noticed.

Photosynthetic pigments

Chlorophyll-a

Treatment (25) was closely following the control upto sixth day. On the eighth day it was 27% more than the control (Fig.44). An abrupt decrease was observed from tenth day onwards. It was 16%, 40% and 58% less than the control on tenth, twelfth and fourteenth day respectively. A general trend of decrease was observed upto twelfth day for treatment (26) but it was 24% more than the control on the fourteenth day.

Chlorophyll-b

Chlorophyll-b was 98% and 81% more than the control at the end of growth phase for treatment (25) and (26) respectively (Fig.44). Treatment

(25) was 29%, 5% and 16% more than the control on fourth, eighth and tenth day. But it was 63% and 43% less than the control on sixth and twelfth day respectively. Similar trend was observed for treatment (26). There was 40% and 31% decrease on the fourth and sixth day followed by a sudden increase of 75% and 56% on the eighth day and tenth day. There was 30% decrease on the twelfth day.

Carotenoids

Generally carotenoids were less than the control for both treatments. It was 26% and 21% less than the control at the end of growth phase for treatment (25) and (26) (Fig.44). Treatment (25) was 36% and 43% less than the control on fourth and sixth day. Though there was 7% increase on the eighth day, 52% decrease was noticed on the twelfth day. Treatment (26) was 36%, 54%, 25% less than the control on fourth, sixth and eighth day. On the tenth, twelfth and fourteenth day there was 6%, 40% and 21% decrease for treatment (26). Thus the combination of metals reduced the carotenoid pigment content of the algae.

Phaeophytin

Generally phaeophytin was fluctuating for both treatments. At the end of growth phase there was 89% and 18% increase for treatment (25) and (26) respectively (Fig.44). Phaeophytin was 30%, 75% and 66% less than the control on fourth, sixth and twelfth day. On the eighth and tenth day there was 29% and 6% increase for treatment (25). Treatment (26) was 51% and 16% less than the control on sixth and twelfth day but 61% and 179% increase observed on the eighth and tenth day respectively.

Photosynthetic end products

Carbohydrate

Generally carbohydrate was more than the control in the early stage and at the end of growth phase (Fig.45). On the eighth day it was 169% and 83% more than the control and at the end of growth phase there was 56% and 10% increase for treatments (25) and (26). But there was 20% and 40% decrease for treatment (26) on tenth and twelfth day.

Protein

Protein was less than the control at the end of growth phase for both treated samples (Fig.45). It was 56% and 66% less than the control on twelfth and fourteenth day for treatment (25) and 56% and 65% less than the control for treatment (26) whereas it was showing an increase of 37% and 18% on the eighth and tenth day. Treatment (25) was 43% less than the control on the eighth day. But a peak (68%) was observed on the tenth day. Thus there was an increasing trend in protein upto tenth day followed by a sudden decrease.

Lipid content of the algae was enhanced and it was 22% more than the control for treatment (25) and it was 8% less than the control for treatment (26).

Compared with the control the phosphate uptake was less than the control for all treated sample. It was 45% less than the control for all treatment but there was only a marginal decrease in nitrate for all samples.

Fig.43

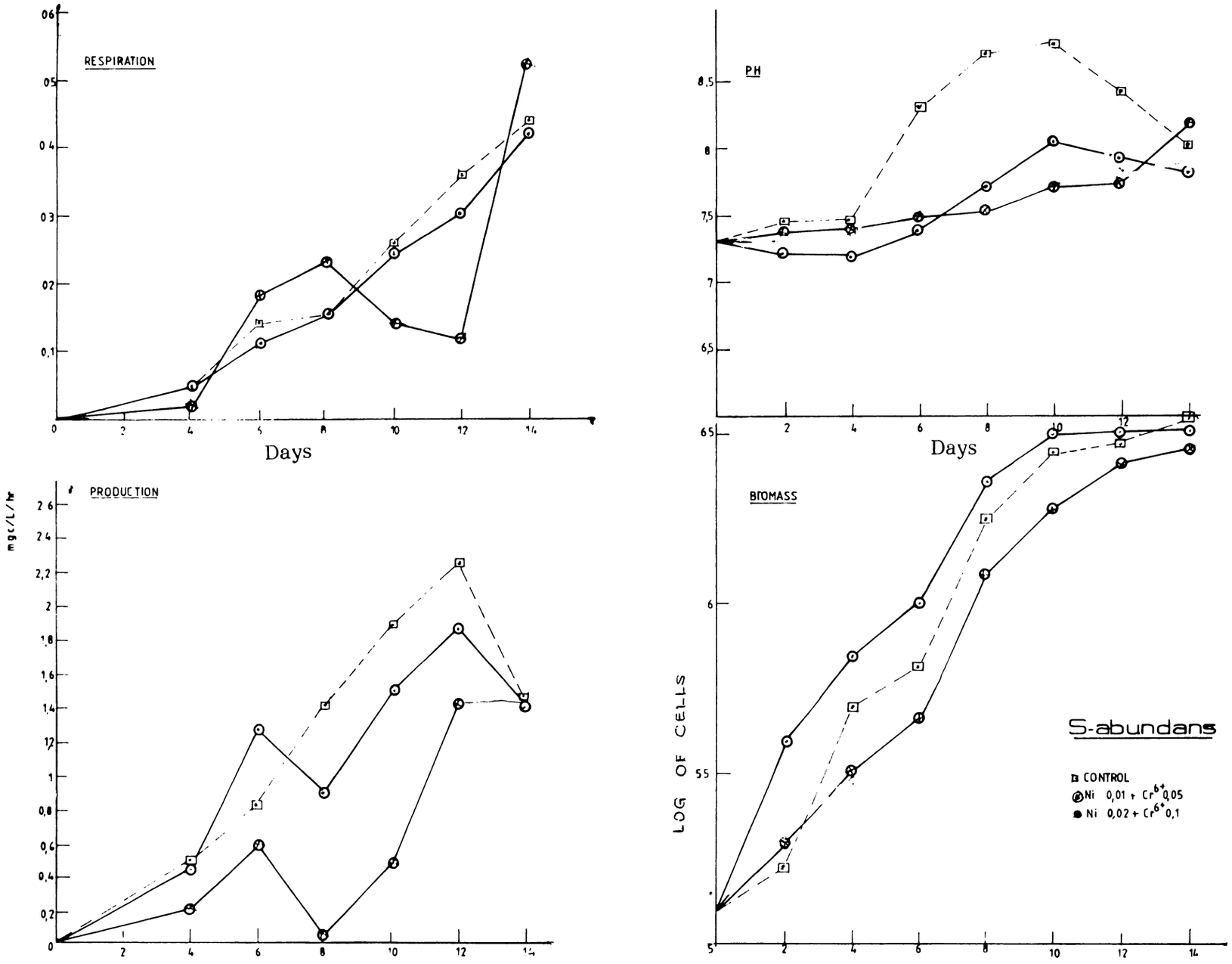

RESPIRATION
Days
PH
Days
PRODUCTION
mgC/L/hr
BIOMASS
LOG OF CELLS
S-abundans
CONTROL
Ni 0,01 + Cr6+ 0,05
Ni 0,02 + Cr6+ 0,1

Fig.44

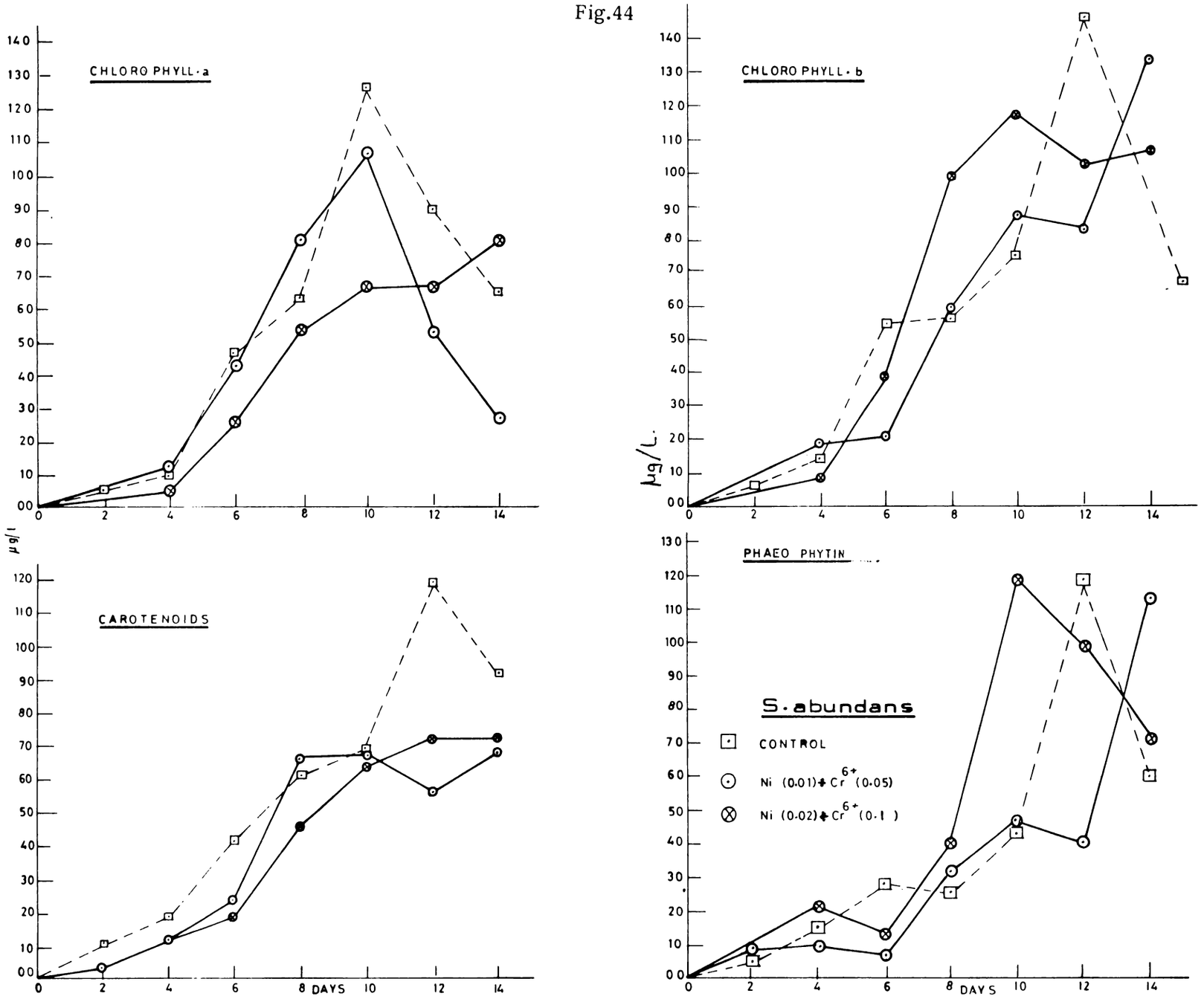
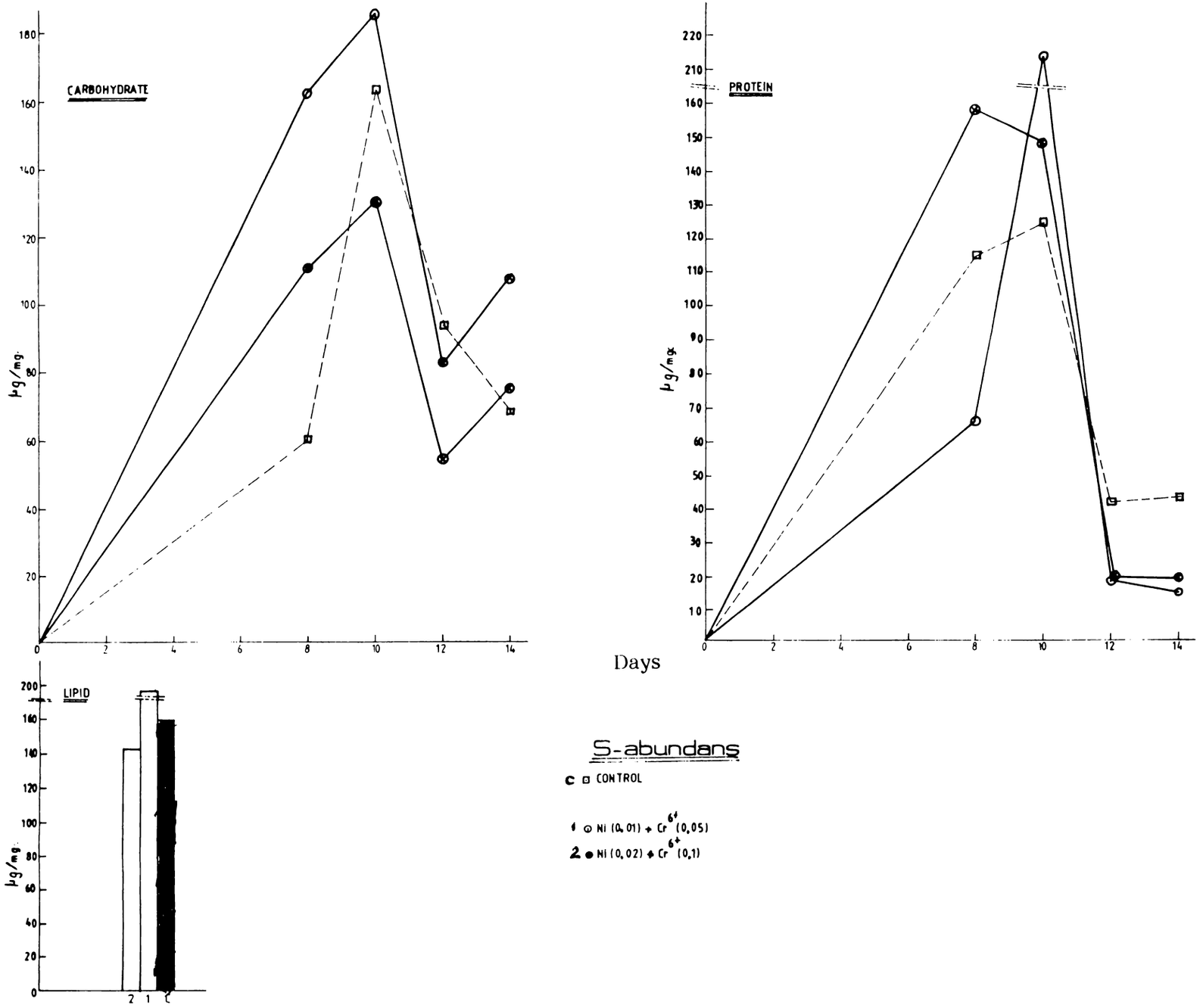

CHLORO PHYLL-a
CHLORO PHYLL-b
CAROTENOIDS
PHAEO PHYTIN
µg/l
DAYS
S.abundans
CONTROL
Ni (0.01)+Cr6+ (0.05)
Ni (0.02)+Cr6+ (0.1)

Fig.45


CARBOHYDRATE
PROTEIN
LIPID
µg/mg
Days
S-abundans
c □ CONTROL
1 ○ Ni (0,01) + Cr 6+ (0,05)
2 ● Ni (0,02) + Cr 6+ (0,1)

Combined effect of nickel and hexavalent chromium in N. clausii

Concentration of metals (in ppm)	Treatment Number
Ni 0.6 + Cr^{6+} 0.2	27
Ni 0.6 + Cr^{6+} 0.6	28
Ni 0.8 + Cr^{6+} 0.2	29

Biomass

A general trend of increase in growth was noticed for all treated samples on the fourth, eighth and tenth day, though there was an initial lag in growth on the second day (Fig.46). On the sixth day there was 12%, 10% and 17% decrease in growth for treatments (27), (28) and (29) respectively. Treatment (27), a combination of 0.6 ppm nickel and 0.2 ppm hexavalent chromium was 40%, 12% and 36% more than the control on fourth, eighth and tenth day. There was an increase of 25%, 21% and 88% for treatment (28), a combination of 0.6 ppm nickel and 0.6 ppm hexavalent chromium. 100%, 37% and 36% increase was observed for treatment (29), a combination of 0.8 ppm nickel and 0.2 ppm hexavalent chromium on fourth, eighth and tenth day respectively.

Production

Production was 82%, 95% and 48% less than the control on the fourth day for treatment (27), (28) and (29) respectively (Fig.46). At the end of growth phase there was 30% decrease for treatment (27). But treatment (28) and (29) was 38% and 28% more than the control. In treatment (27) the pro-

duction was generally less than the control but a marginal increase was observed on the sixth and eighth day respectively. There was 92%, 3% and 38% increase on the sixth, eighth and tenth day for treatment (28). Treatment (29) was 23%, 82% and 28% more than the control on sixth, eighth and tenth day. The increase in production for treatment (28) and (29) towards the end of growth was reflected in the pH also.

Respiration

Respiration was 22%, 33% and 25% less than the control on eighth day and 15%, 45% and 70% decrease was noticed on the tenth day for treatment (27), (28) and (29) respectively (Fig.46). Treatment (27) was less than the control through out the growth phase whereas treatment (28) was 180% and 22% more than the control on fourth and sixth day respectively. There was a decrease of 2% on the fourth day and 20% increase was observed on the sixth day for treatment (29).

Photosynthetic pigments

Chlorophyll-a

Chlorophyll-a was 10%, 17% and 11% less than the control for treatments (27), (28) and (29) at the end of growth phase. Treatment (27) was 38%, 30% less than the control on second and fourth day. 9% and 20% increase was noticed on the sixth and eighth day. There was a general trend of increase for treatment (28) on the fourth, sixth and tenth day. A marginal increase of 18% was observed on the eighth day. 5%, 22%, 39% increase was noticed for treatment (29) on the fourth, sixth and eighth day.

Chlorophyll-c

Chlorophyll-c was generally more than the control for treatment (27) and (28) through out the growth phase (Fig.47). Treatment (27) was 201%, 48%, 64% and 56% more than the control on second, sixth, eighth and tenth day respectively. There was 149%, 71%, 69% and 60% increase on fourth, sixth, eighth and tenth day for treatment (28). Treatment (29) was 60% less than the control on the fourth day but 49%, 126% and 57% increase was observed on the sixth, eighth and tenth day respectively.

Carotenoids

Carotenoids were 4%, 33% and 71% more than the control on the eighth day and 11%, 15% and 14% increase was noticed on the tenth day for treatment (27), (28) and (29) respectively. Treatment (27) was 44% and 17% less than the control on second and sixth day. Though there was 44% decrease on the second day, treatment (29) was more than the control through out the growth phase from fourth day onwards. Whereas treatment (28) 6% and 18% less than the control on fourth and sixth day but there was 33% and 15% increase on the eighth and tenth day respectively.

Phaeophytin

Phaeophytin was 57% and 53% less than the control at the end of growth phase for treatment (27) and (28), but treatment (29) was 4% less than the control (Fig.47). Through out the growth phase treatment (27) was less than the control. Treatment (28) was 19%, 6% more than the control on fourth and sixth day but it was 7% and 53% less than the control on eighth

and tenth day respectively. Treatment (29) was less than the control upto eighth day followed by 57% increase at the end of growth phase.

Photosynthetic end products

Carbohydrate

The acid soluble fraction of carbohydrate was showing the peak on the fourth day but it was less than the control on eighth and tenth day (Fig.48). It was noticed that there was 339%, 472% and 294% increase on fourth day and 82%, 83% and 44% on the sixth day for treatment (27), (28) and (29) but on the eighth day there was 7%, 28% and 7% decrease and 40%, 41% and 7% on the tenth day for treatment (27), (28) and (29) respectively.

The alkali soluble fraction was less than the control for treatment (28) and (29) through out the growth phase (Fig.48). Whereas treatment (27) was 45% and 53% more than the control on fourth and sixth day. But it was 80% less than the control on the eighth and tenth day.

The insoluble fraction of carbohydrate was 81%, 71% and 140% more than the control at the end of growth phase for treatment (27), (28) and (29) (Fig.48). Treatment (27) was 10% less than the control on the fourth day followed by 41%, 5% and 81% increase on sixth, eighth and tenth day respectively. There was 18% and 30% decrease on fourth and eighth day for treatment (28) but it was 71% more than the control on the tenth day. Insoluble fraction was maximum for treatment (29) and it was 48%, 74% and 140% more on the fourth, sixth and tenth day respectively.

Protein

For all treated samples the protein was more than the control on the fourth and sixth day but at the end of growth phase it was less than the

control (Fig.48). There was 110%, 10% and 127% increase on the fourth day and 83%, 55% and 69% increase on the sixth day for treatment (27), (29) and (29). But it was 46%, 60% and 27% less than the control on the tenth day.

Lipid content of the algae was 73%, 77% and 93% less than the control for treatments (27), (28) and (29) respectively.

In the case of nutrient uptake there was only a marginal increase of 7%, 6% and 8% in the phosphate uptake for treatment (27), (28) and (29) respectively, whereas nitrate uptake was less than the control through out the growth phase.

Fig.46

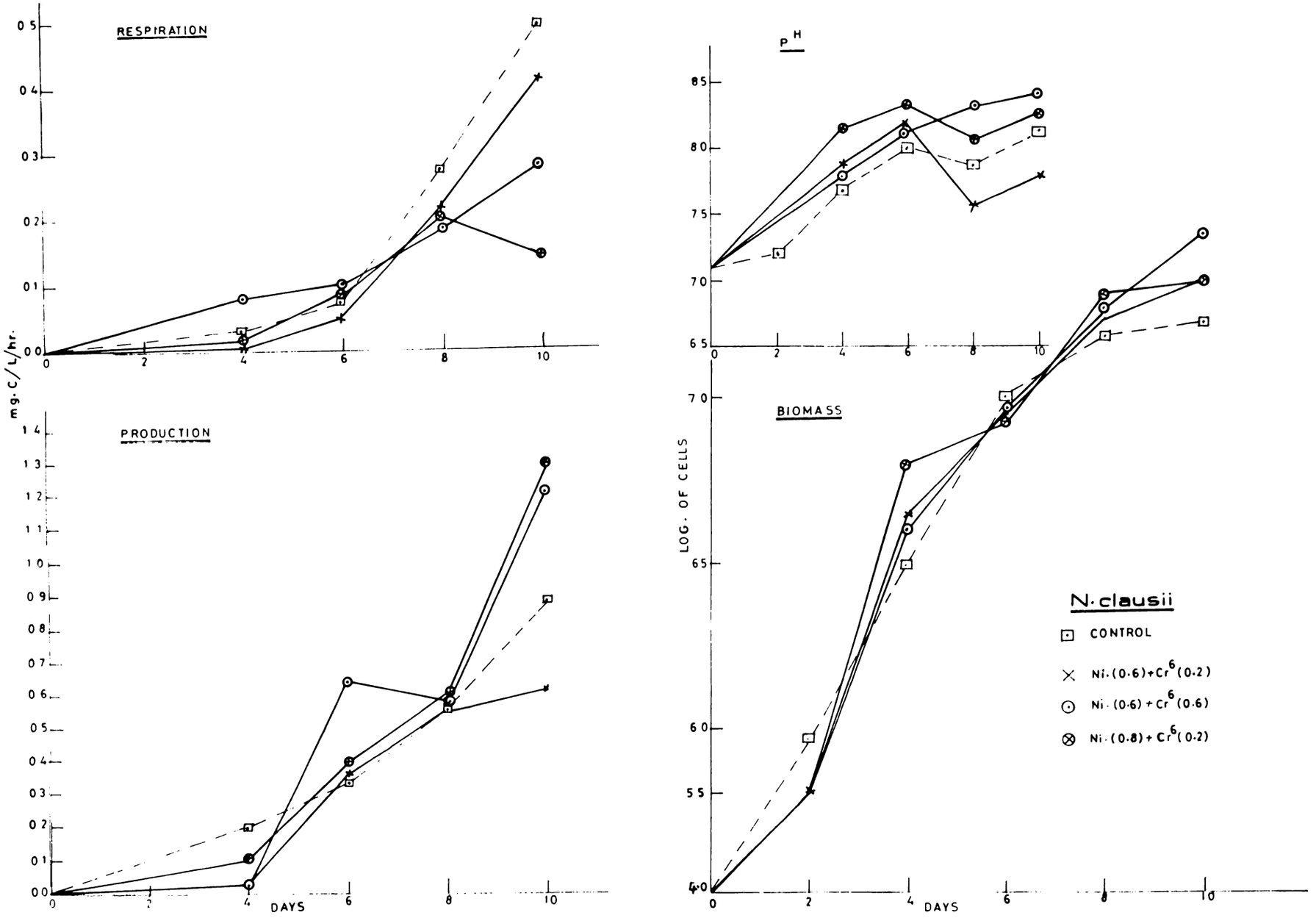
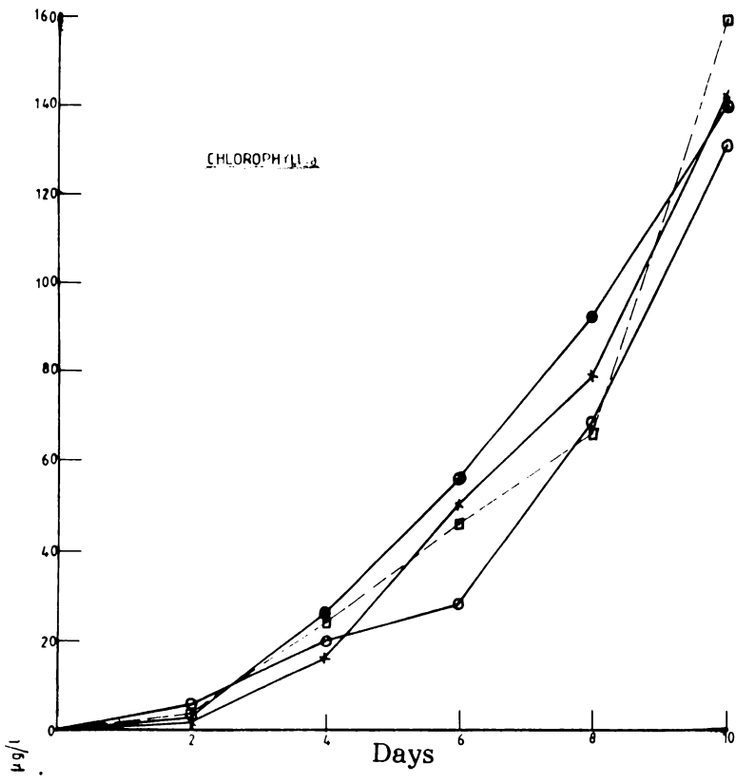
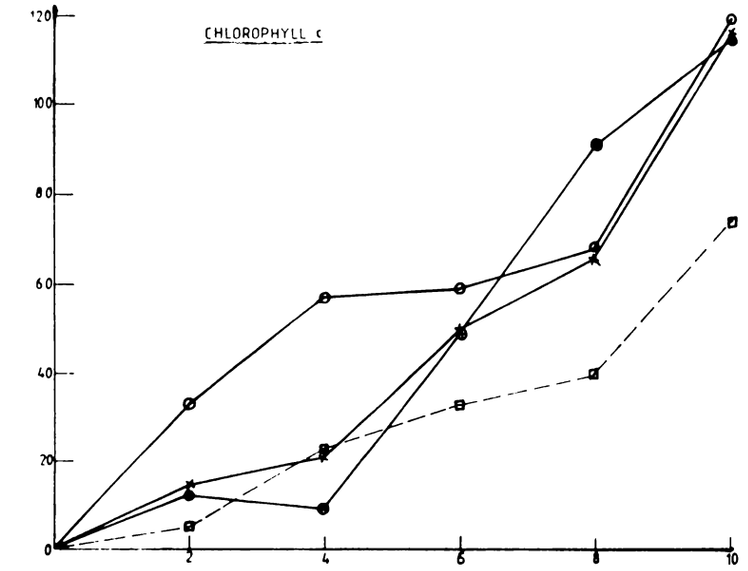
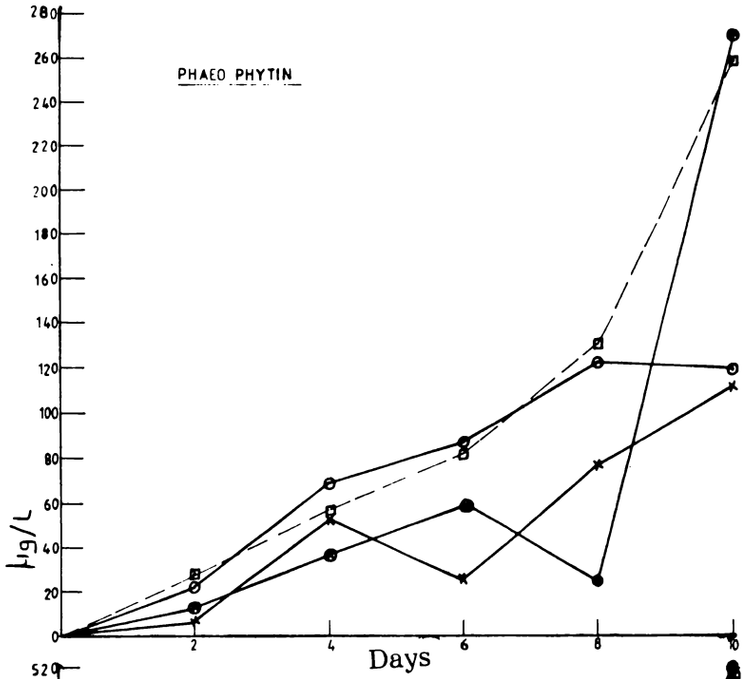

RESPIRATION
mg.C/L/hr.
PRODUCTION
DAYS
P H
BIOMASS
LOG. OF CELLS
DAYS
N.clausii
CONTROL
Ni.(0.6)+Cr6(0.2)
Ni.(0.6)+Cr6(0.6)
Ni.(0.8)+Cr6(0.2)

Fig.47


CHLOROPHYLL a
Days
µg/l


CHLOROPHYLL c


PHAEO PHYTIN
µg/L
Days

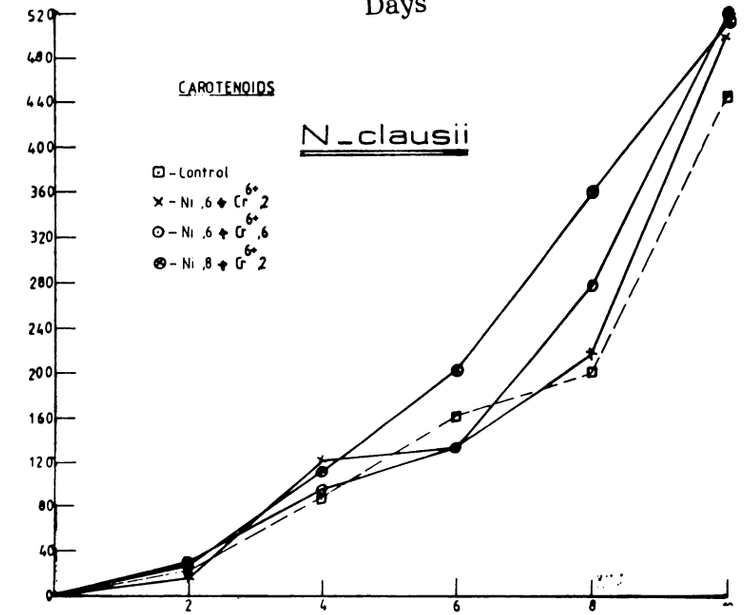
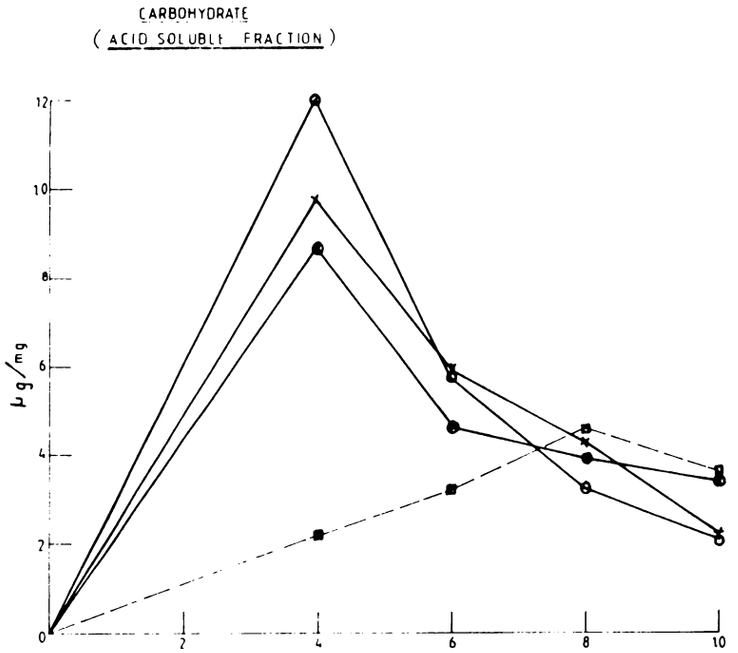
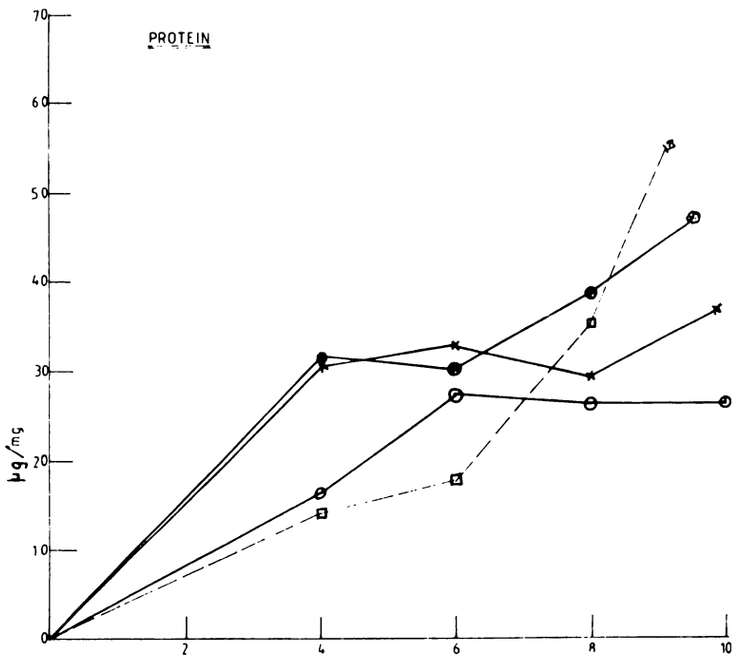
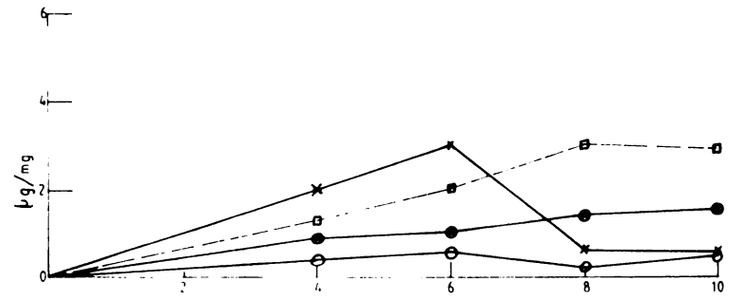

CAROTENOIDS
N_clausii
- Control
- Ni ,6 + Cr 6+ ,2
- Ni ,6 + Cr 6+ ,6
- Ni ,8 + Cr 6+ ,2

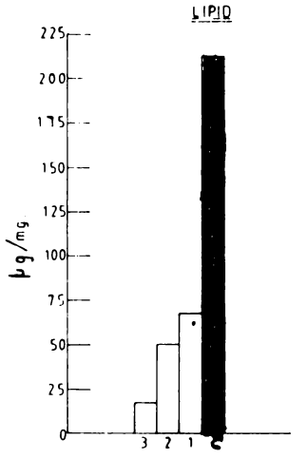
Fig.48

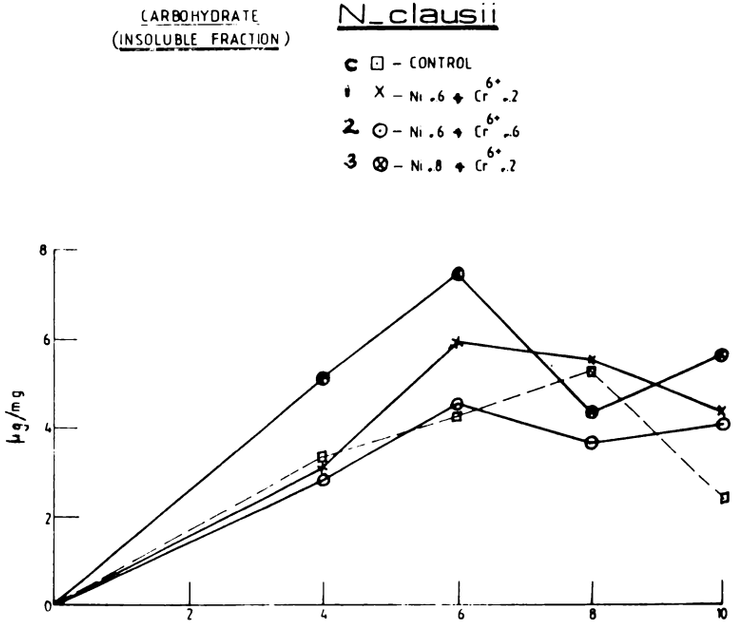

CARBOHYDRATE
(ACID SOLUBLE FRACTION)
µg/mg
0
2
4
6
8
10
12
2
4
6
8
10


PROTEIN
µg/mg
0
10
20
30
40
50
60
70
2
4
6
8
10

Days


CARBOHYDRATE
(ALKALI SOLUBLE FRACTION)
µg/mg
0
2
4
6
2
4
6
8
10


LIPID
µg/mg
0
25
50
75
100
125
150
175
200
225
3
2
1
C


CARBOHYDRATE
(INSOLUBLE FRACTION)
N_clausii
C ⊡ - CONTROL
1 X - Ni .6 + Cr 6+ .2
2 ⊙ - Ni .6 + Cr 6+ .6
3 ⊗ - Ni .8 + Cr 6+ .2
µg/mg
0
2
4
6
8
2
4
6
8
10

5.1.5 Combined effect of cobalt and nickel in S. abundans

Concentration of metals (in ppm)	Treatment Number
Co 0.01 + Ni 0.005	30
Co 0.05 + Ni 0.005	31

From the preliminary experiments it was observed that concentrations above 0.005 ppm nickel in combination with cobalt above 0.05 ppm was toxic (lethal). So very low concentration of (nickel 0.005 and cobalt 0.01) both the metals in combination was selected for study.

Biomass

The biomass was less than the control with the aging of the culture for treatment (30) (a combination of 0.01 ppm cobalt and 0.005 ppm nickel) and treatment (31) which was a combination of 0.05 ppm cobalt and 0.005 ppm nickel. Eventhough there was a marginal increase of 9% on the sixth day for treatment (30) it was 34%, 43%, 6% and 3% less than the control on the eighth, tenth, twelfth and fourteenth day. A general trend of decrease was observed for treatment (31) from fourth day onwards. It was 30% less than the control on fourth and sixth day. 64%, 71%, 53% and 49% decrease was noticed on eighth, tenth, twelfth and fourteenth day.

Production

Unlike biomass combination of metals enhanced the production by 85% and 71% at the end of growth phase for treatment (30) and (31) (Fig.49).

Treatment (30) was 18% less than the control on fourth and sixth day. On the eighth day there was 10% decrease for treatment (30). It was similar to the control on the tenth day but 10% and 85% increase was observed on the twelfth and fourteenth day. pH was less than the control on the exponential growth phase, but it was similar to the control at the end of growth.

Respiration

The respiration was less than the control for both treatments through out the growth phase (Fig.49). At the end of growth phase both the treatments were 46% less than the control. Treatment (30) was 59% and 31% less than the control on eighth and tenth day. Treatment (31) was 80% and 76% less than the control on eighth and tenth day.

Photosynthetic pigments

Chlorophyll-a

Chlorophyll-a was less than the control for treated samples with the aging of the culture. It was similar to the control upto fourth day. Treatment (30) was 29%, 30%, 58%, 10% and 17% less than the control on sixth, eighth, tenth, twelfth and fourteenth day respectively. Treatment (31) was 33%, 36%, 52%, 25% and 3% less than the control on sixth, eighth, tenth, twelfth and fourteenth day.

Chlorophyll-b

Chlorophyll-b was less than the control for treated samples with the aging of the culture (Fig.50). It was 33% and 30% less than the control on tenth day, 63% and 61% on the twelfth day and 36% and 14% decrease was

noticed on the fourteenth day for treatment (30) and (31) respectively. But there was 8% and 13% increase for treatment (30) and (31) and an increasing trend was observed upto fourth day. Thus eventhough there was an increasing trend it was less that the control with the aging of the culture.

Carotenoids

Carotenoids were generally less than the control for treated samples. But treatment (31) showed 26% increase at the end of growth and treatment (30) was 43% more than the control on the fourth day. Treatment (30) was 47%, 34%, 28%, 50% and 5% less than the control on sixth, eighth, tenth twelfth and fourteenth day. Similarly treatment (31) was 60%, 55%, 16% and 33% less than the control on sixth, eighth, tenth and twelfth day respectively.

Phaeophytin

Phaeophytin was fluctuating for the treated samples. For treatment (30) there was 34% increase and for treatment (31) 26% decrease was observed at the end of growth (Fig.50). Treatment (30) was 62% and 97% less than the control on the fourth and twelfth day but 89% and 46% increase was observed for sixth and eighth day. But treatment (31) was 73%, 29%, 91% less than the control on fourth, sixth and twelfth day. An increase was observed only on the eighth day and it was found to be 18%.

Photosynthetic end products

Carbohydrate

Carbohydrate was 43% and 8% more than the control at the end of growth phase (Fig.51) and for all the treated samples the carbohydrate was maximum at the end of growth phase. Treatment (30) was 27% more than

the control on eighth day but there was 73% and 26% decrease on the tenth and twelfth day respectively. Whereas the treatment (31) was generally less than the control upto twelfth day though there was a marginal increase of 8% at the end of growth.

Protein

There was a marginal decrease in protein at the end of growth phase and the values being 12% and 7% less than the control (Fig.51). There was marked variation between treatments in the protein content. Treatment (30) was maximum on the eighth day and it was 19% more than the control but 57% decrease was observed on the tenth day followed by marginal increase and decrease on twelfth day and fourteenth day respectively. Treatment (31) showed general trend of decrease but 18% increase was observed on the twelfth day.

Lipid content was 62% and 50% less than the control for treatment (30) and (31). In the case of nutrients, phosphate uptake was 71% and 70% less than the control for treatment (30) and (31). Whereas nitrate uptake was 50% less than the control for both treatments.

Fig.49

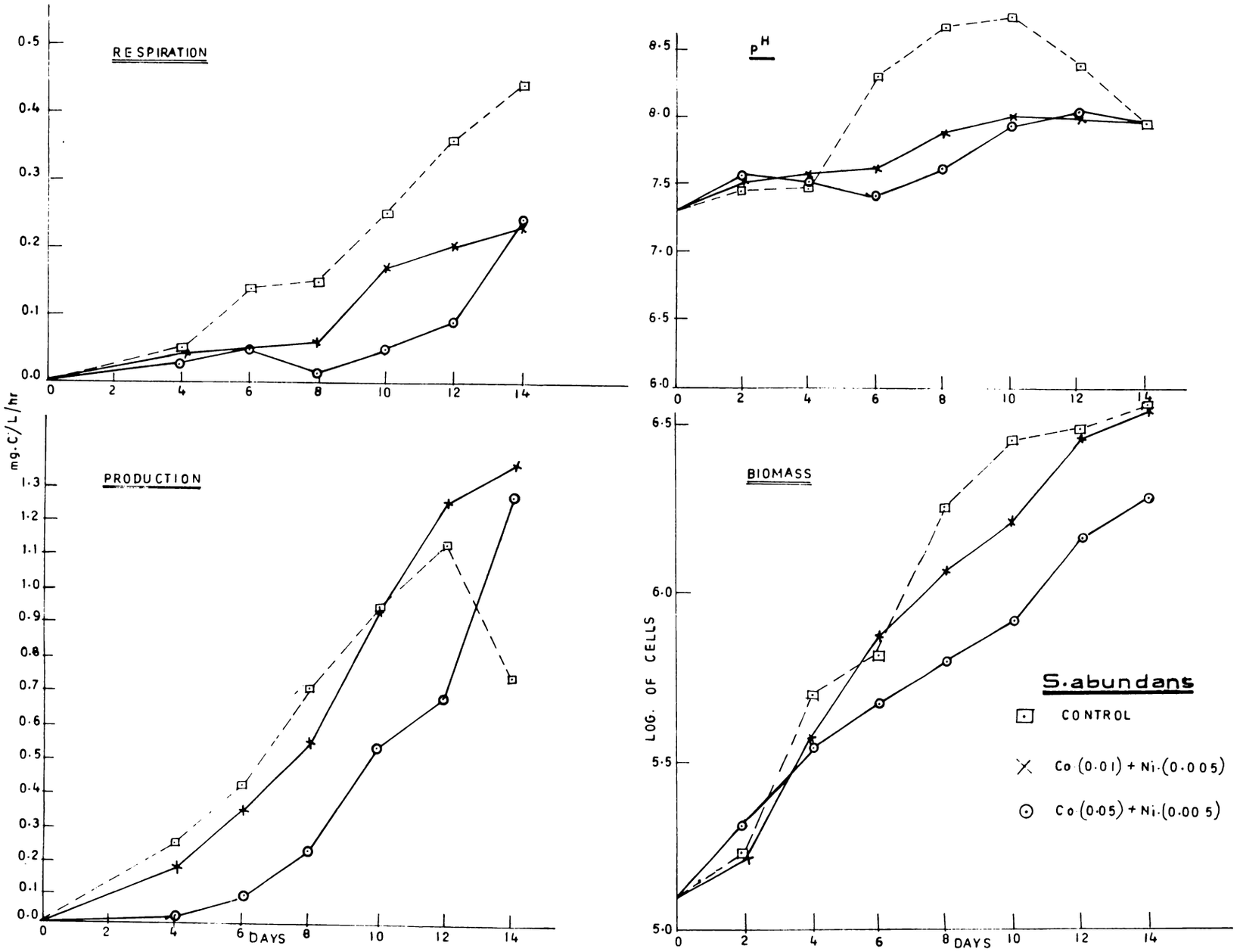

RESPIRATION
mg.C/L/hr
0.0
0.1
0.2
0.3
0.4
0.5
0 2 4 6 8 10 12 14
pH
6.0
6.5
7.0
7.5
8.0
8.5
PRODUCTION
0.0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1.0 1.1 1.2 1.3
DAYS
BIOMASS
LOG. OF CELLS
5.0
5.5
6.0
6.5
S.abundans
CONTROL
Co (0.01) + Ni (0.005)
Co (0.05) + Ni (0.005)

Fig.50

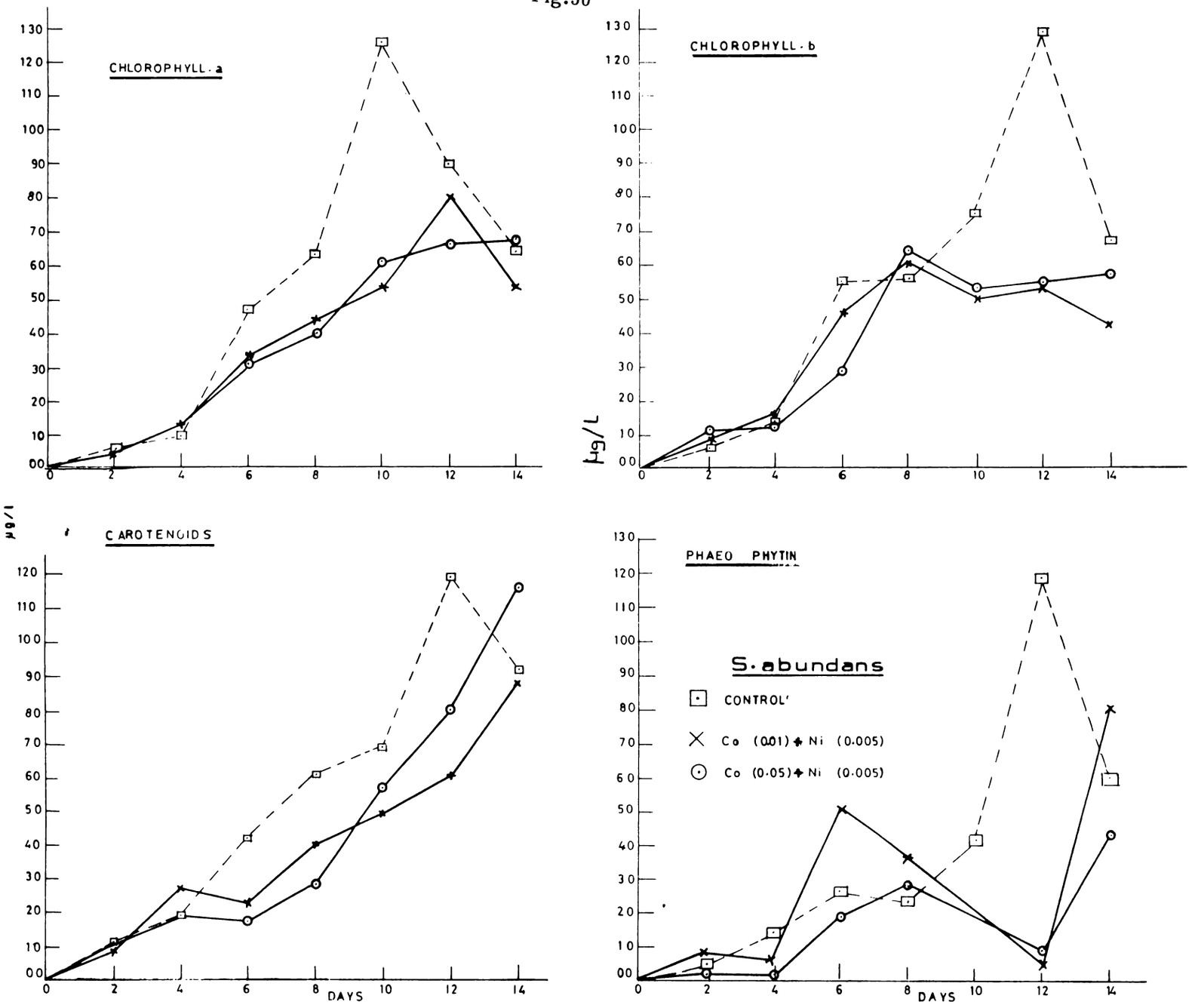
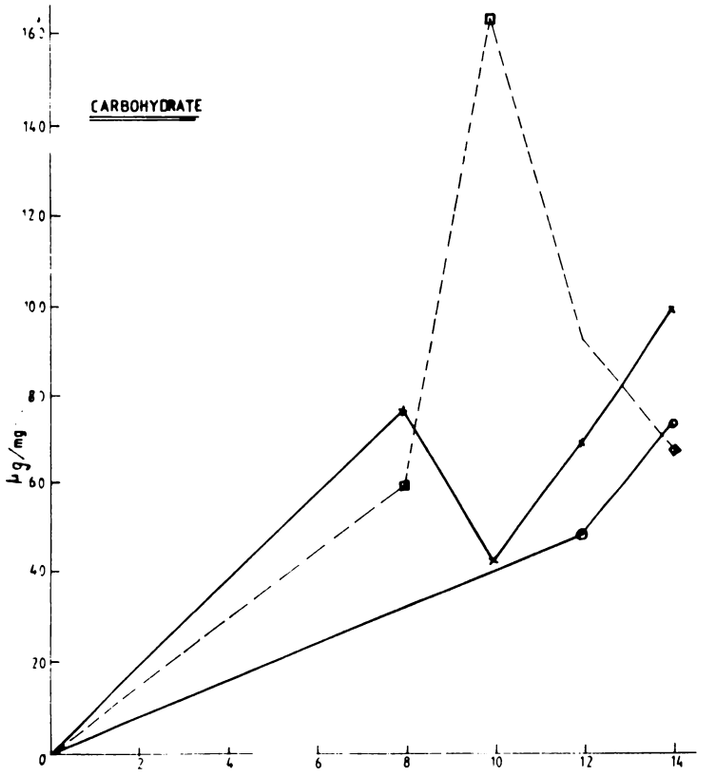
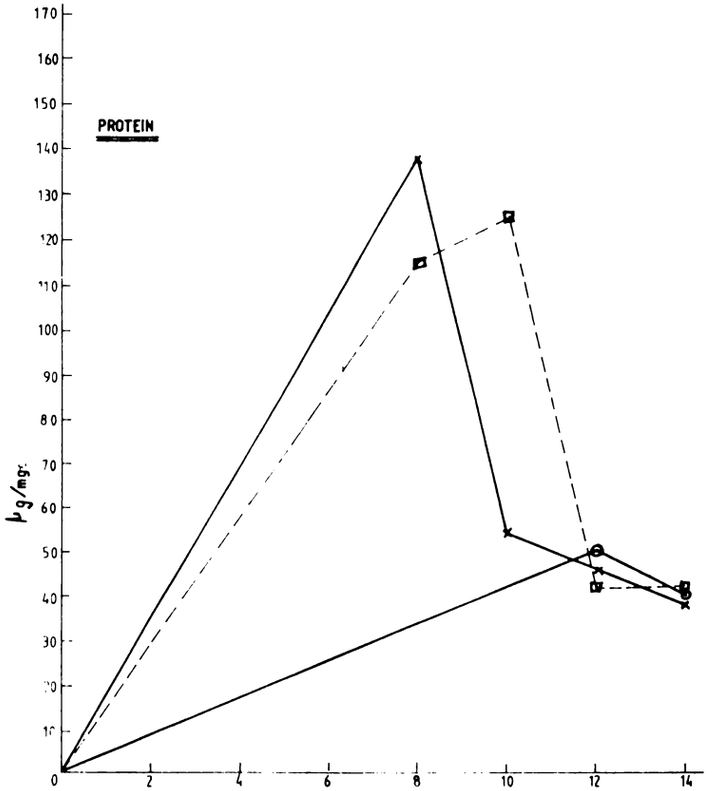
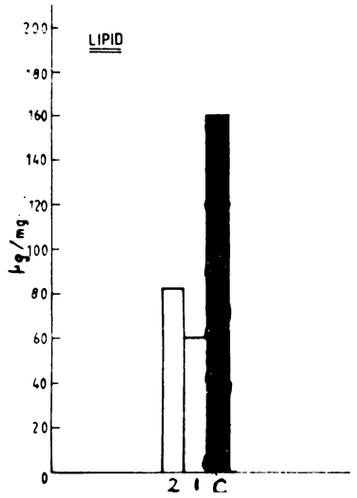

CHLOROPHYLL - a
CHLOROPHYLL - b
CAROTENOIDS
PHAEO PHYTIN
µg/l
DAYS
S. abundans
CONTROL
Co (0.01) + Ni (0.005)
Co (0.05) + Ni (0.005)

Fig.51





Days



S-abundans

C ◻ CONTROL

1 × Co (0,01) + Ni (0,005)

2 ○ Co (0,05) + Ni (0,005)

Combined effect of cobalt and nickel in N. clausii

Concentration of metals (in ppm)	Treatment Number
Co 0.3 + Ni 0.6	32
Co 0.6 + Ni 0.6	33

Biomass

Notable difference was observed in the biomass between treatments. Treatment (32) a combination of 0.6 ppm nickel and 0.3 ppm cobalt was showing marginal increase at the end of growth phase whereas there was 74% decrease at the end for treatment (33) whichwas a combination of 0.6 ppm each cobalt and nickel. Treatment (32) was found to be fluctuating. It was 25% and 4% more than the control on fourth and tenth day but there was 18% and 2% decrease on sixth and eighth day. Though there was an initial increase on the second day for treatment (33), it was 60%, 86%, 57% and 74% less than the control on fourth, sixth, eighth and tenth day respectively.

Production

A general trend of decrease in production was noticed for both treatments. At the end of growth phase treatment (32) and (33) was 25% and 94% less than the control (Fig.52). Treatment (32) was 17%, 13%, 17% less than the control on fourth, sixth and eighth day. The production was far less than the control for treatment (33). It was 53%, 84% and 88% less than the control on fourth, sixth and eighth day for treatment (33). The decrease in production for treatment (33) was noticed in the pH also through out the growth phase.

Respiration

Respiration was less than the control for both treatments on eighth and tenth day (Fig.52). On the eighth day there was 64% and 57% decrease and on the tenth day there was 61% and 67% decrease for treatment (32) and (33) respectively. Treatment (32) was similar to the control on the fourth day and 7% increase on sixth day followed by sudden decrease towards the end of growth phase. Treatment (33) was less than the control through out the growth phase.

Photosynthetic pigments

Chlorophyll-a

A general trend of decrease was observed for both treatments at the end of growth phase (Fig.53). The values being 39% and 85% less than the control for treatment (32) and (33). Treatment (32) was 55% and 42% less than the control on fourth and sixth day but 21% increase was observed on eighth day. Treatment (33) was less than the control through out the growth phase with 72%, 88% and 67% decrease on fourth, sixth and eighth day respectively.

Chlorophyll-c

Chlorophyll-c was more than the control for both treatments upto eighth day followed by 77% and 96% decrease on the tenth day for treatment (32) and (33) (Fig.53). Treatment (32) was 11%, 3% and 63% more than the control on fourth, sixth and eighth day and there was 66%, 65% and 23% increase on fourth, sixth and eighth day for treatment (33).

Carotenoids

Carotenoids were 23% and 80% less than the control for treatment (32) and (33) at the end of growth phase (Fig.53). On the second and eighth day there was 115% and 42% increase and on the fourth and sixth day there was 5% and 37% decrease for treatment (32). But for treatment (33) though there was an initial increase on the second day, carotenoids were less than the control through out the growth phase.

Phaeophytin

Generally phaeophytin was less than the control for both treatments (Fig.53). Treatment (32) was 50%, 47% and 48% and treatment (33) was 30%, 75% and 77% less than the control on sixth, eighth and tenth day respectively.

Photosynthetic end products

Carbohydrate

The acid soluble fraction of carbohydrate was less than the control through out the growth phase for treatment (32). It showed marginal decrease (2%) on fourth day followed by 56%, 78% and 25% decrease on sixth, eighth and tenth day respectively. There was 63% and 22% increase for treatment (33) on the fourth day and at the end of growth phase. But there was 53% and 47% decrease on sixth and eighth day

The alkali soluble fraction was less than the control by 78% and 91% for treatment (32) and (33) at the end of growth phase (Fig.54). Treatment (33) was less than the control through out the growth phase. Treatment (32) was 94%, 82% less than the control on the fourth and sixth day. But 45% increase was observed on the eighth day.

The insoluble fraction of carbohydrate was similar to the control for treatment (33) at the end of growth phase (Fig.54). Treatment (32) was 25%, 68% and 60% less than the control on fourth, sixth and eighth day respectively. Whereas treatment (33) showed a peak of 99% on the fourth day followed by an abrupt decrease of 67%, 56% and 66% on the sixth, eighth and tenth day respectively.

Protein

Protein was 77% and 88% less than the control for treatment (32) and (33) (Fig.54). Treatment (32) showed an initial decrease of 35% on the fourth day followed by an increase of 17% on the sixth day. There was 60% and 77% decrease on eighth and tenth day respectively. Treatment (33) was 56%, less than the control on fourth and sixth day followed by 72% decrease on the eighth day.

Lipid content was 75% and 70% less than the control for treatment (32) and (33) respectively. Phosphate uptake was 42% and 40% more than the control whereas the nitrate uptake was similar to the control.

Fig.52

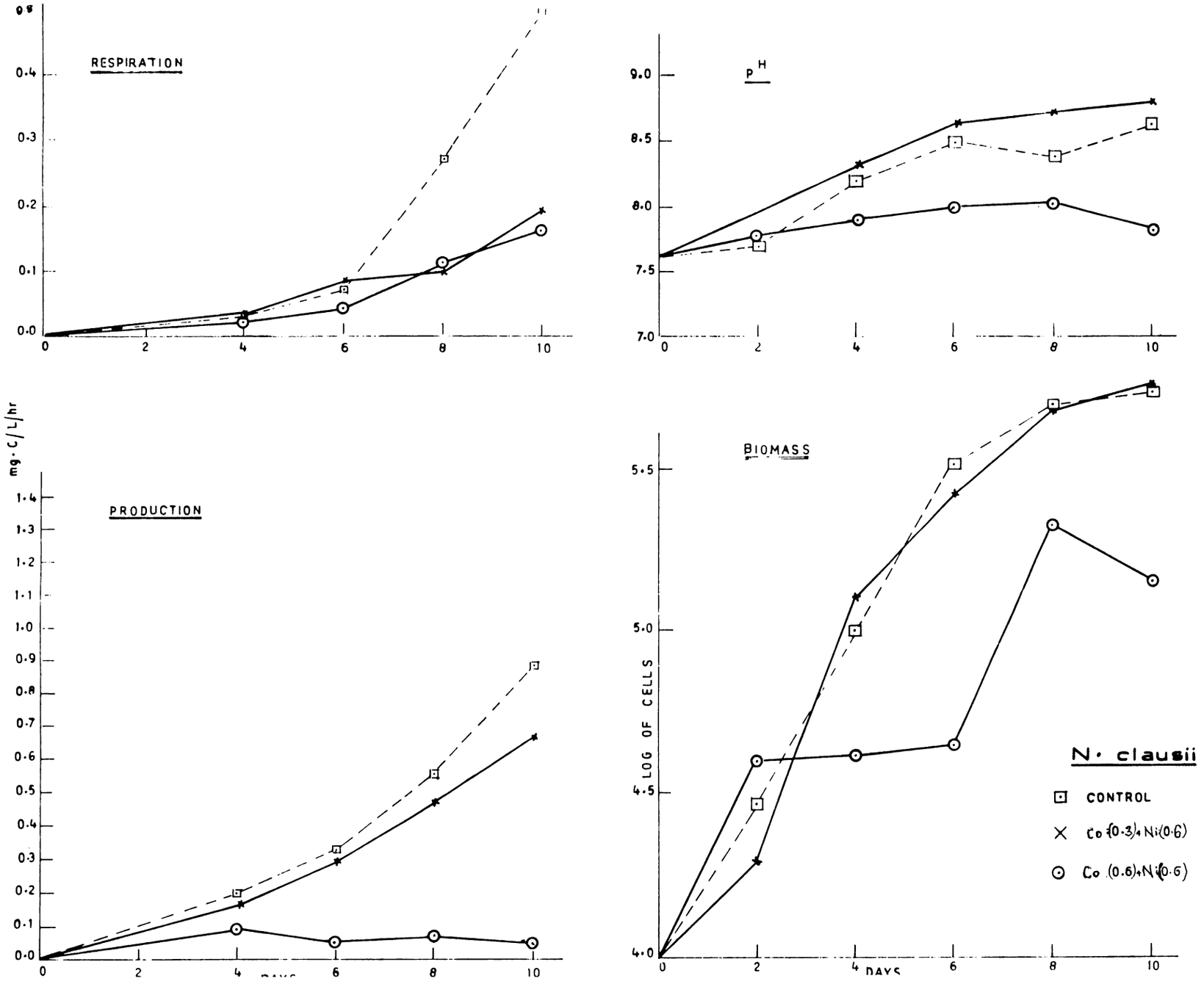
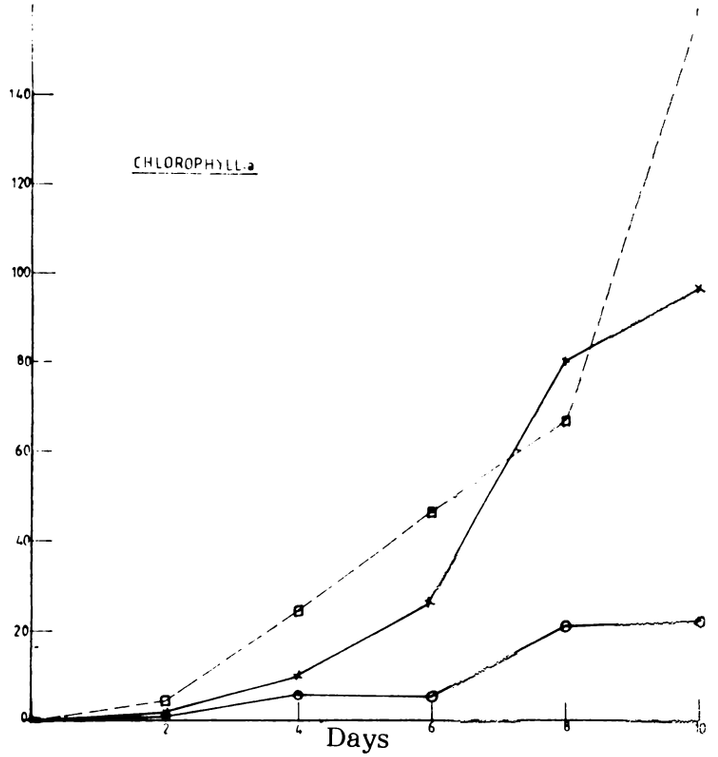
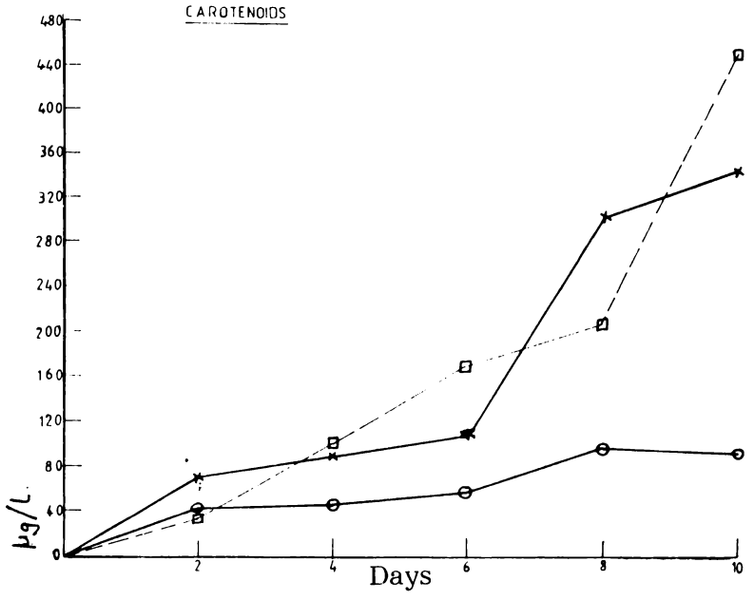
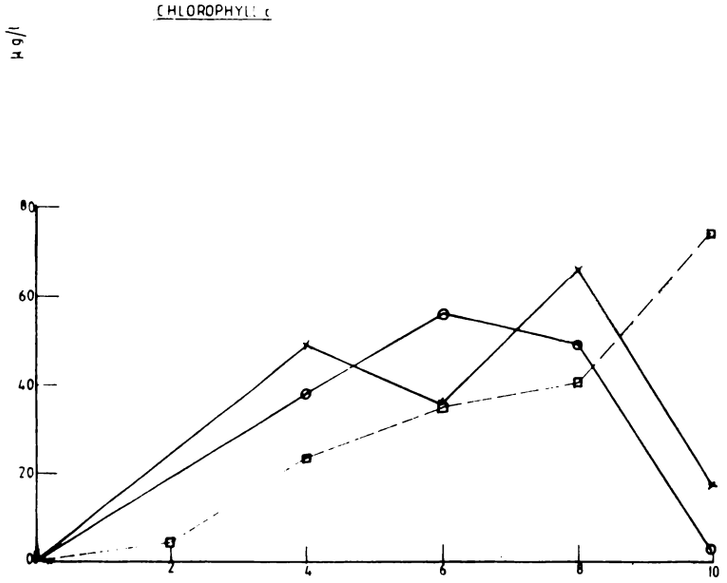

RESPIRATION
0.4
0.3
0.2
0.1
0.0
0
2
4
6
8
10
pH
9.0
8.5
8.0
7.5
7.0
mg·C/L/hr
PRODUCTION
1.4
1.3
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
BIOMASS
LOG OF CELLS
5.5
5.0
4.5
4.0
DAYS
N· clausii
CONTROL
Co (0·3)+Ni(0·6)
Co (0.6)+Ni(0·6)

Fig.53


CHLOROPHYLL-a
Days


CAROTENOIDS
µg/L
Days


CHLOROPHYLL c
µg/l

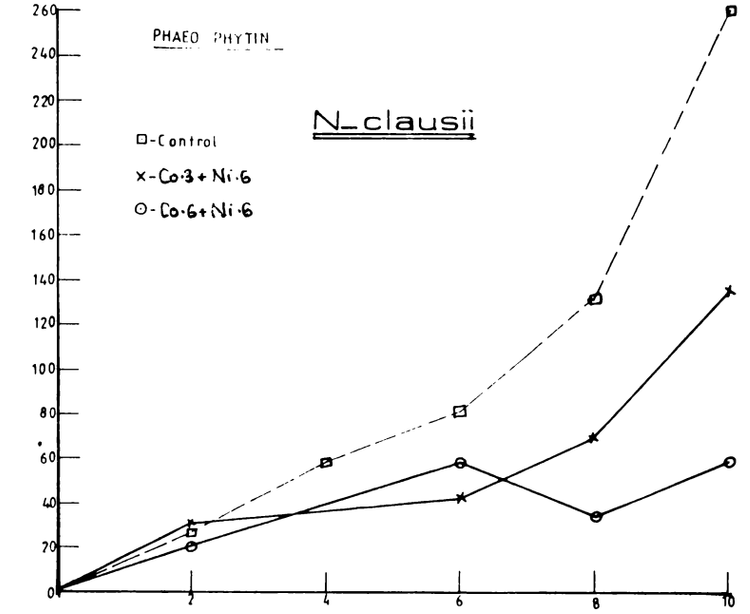
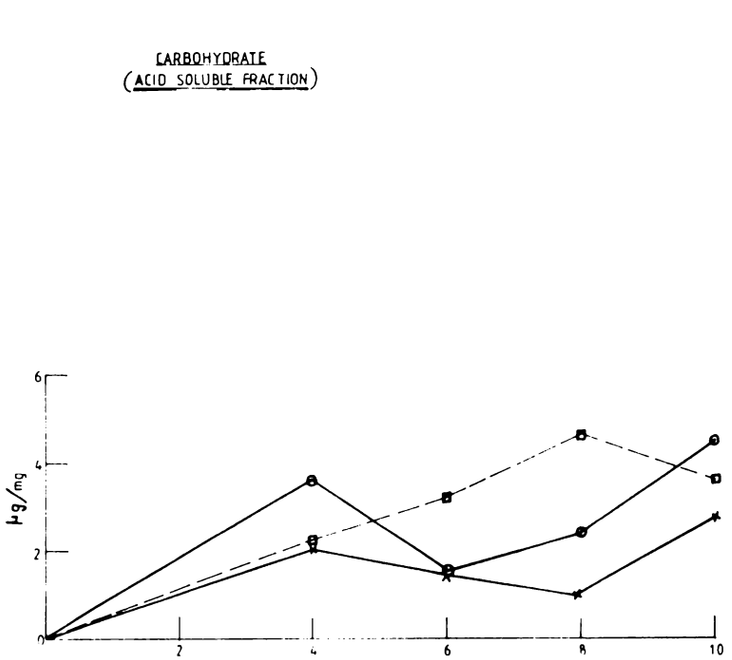
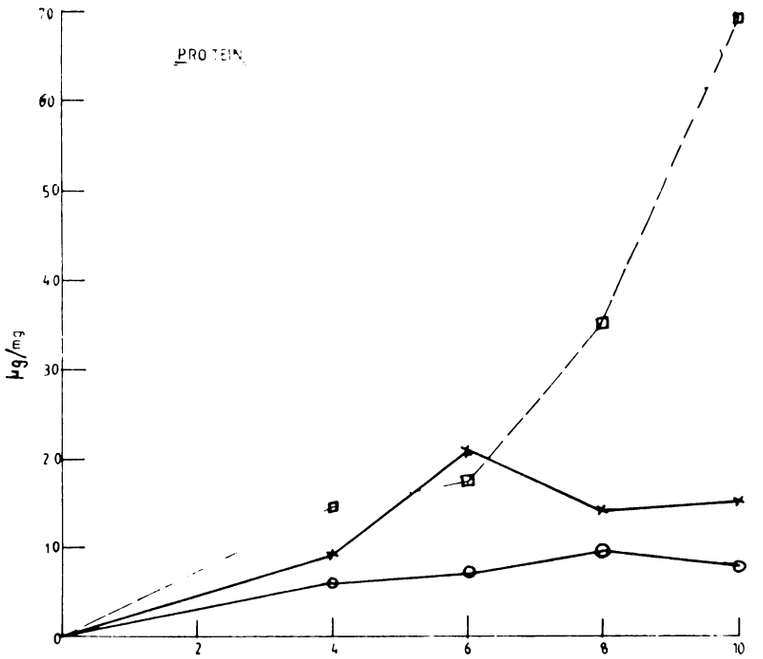
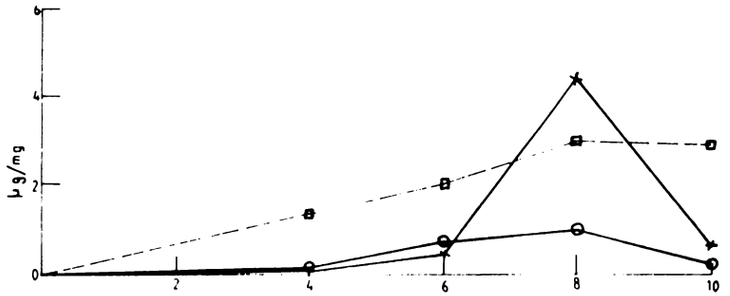

PHAEO PHYTIN
N_clausii
□-Control
×-Co·3+Ni·6
⊙-Co·6+Ni·6

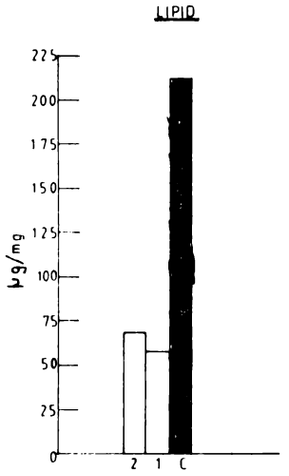
Fig.54

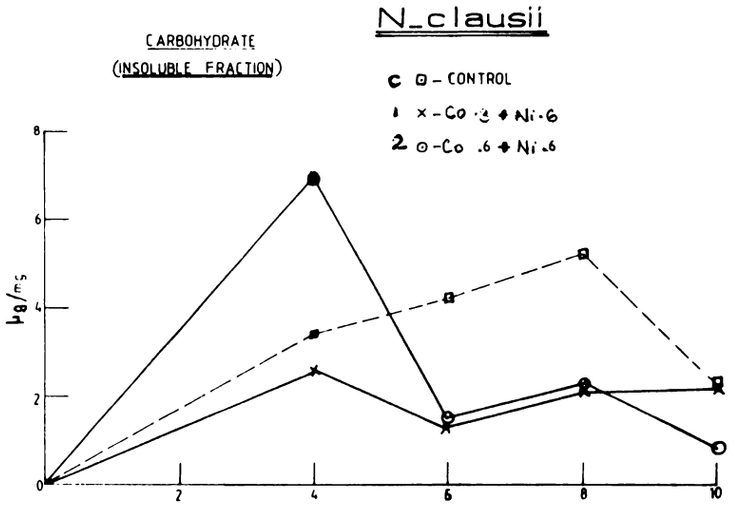

CARBOHYDRATE
(ACID SOLUBLE FRACTION)
µg/mg
0
2
4
6
2
4
6
8
10


PROTEIN
µg/mg
0
10
20
30
40
50
60
70
2
4
6
8
10

Days


CARBOHYDRATE
(ALKALI SOLUBLE FRACTION)
µg/mg
0
2
4
6
2
4
6
8
10


LIPID
µg/mg
0
25
50
75
100
125
150
175
200
225
2
1
C


N_clausii
CARBOHYDRATE
(INSOLUBLE FRACTION)
C ⊡ - CONTROL
1 × - Co .3 ◆ Ni .6
2 ⊙ - Co .6 ◆ Ni .6
µg/mg
0
2
4
6
8
2
4
6
8
10

DISCUSSION

Pollutants discharged into water bodies containing different metals might expose the microbiota to several metals either in isolation or in combination. The investigation on the toxicity of individual metals to aquatic biota has been gaining momentum. But very few studies have been made to understand the combined effects of two or more metals, despite the well known fact that heavy metals always interact in natural waters (Braek et al.,1976).

Studies of Braek et al. (1976) on the interaction of copper and zinc ions added to cultures of four species of phytoplankton observed that copper and zinc interact synergistically on three species while the interaction was antagonistic with Phaeodactylum tricornutum which suggested that relative toxicity exerted on algae varied with different species and also on the type of metals. The specific metal - metal interaction depends on the relative concentration of the toxicants and sequence of exposure to the toxicants (Nriagu, 1983). He has also reported that most phytoplankton studies have shown that the interaction is between nontoxic metal and a toxic one.

In the present investigation the combination of trivalent chromium and cobalt showed that the toxic effect was reduced and was antagonistic in S. abundans at a concentration of 0.01 ppm cobalt and 0.03 ppm trivalent chromium (Treatment 1) but 0.03 ppm trivalent chromium in isolation was toxic and during the growth phase carbohydrate, protein and lipids showed notable increase. The pigments chlorophyll-a and carotenoids also showed an increase. 0.05 ppm cobalt was found to have stimulatory effect on growth

and pigment production, whereas 0.05 ppm cobalt in combination with 0.01 ppm trivalent chromium (Treatment 2) had synergistic effect resulting in the decrease in production, pigment content and end products of photosynthesis. Thus it was proved that very low concentration of cobalt (0.01 ppm) in combination with trivalent chromium concentration at toxic level (0.03 ppm) produced antagonistic effect, whereas cobalt at a concentration of 0.05 ppm and above was synergistic in combination with 0.01 ppm and 0.02 ppm trivalent chromium (nontoxic concentration).

Studies on the manganese - copper synergism showed that copper and manganese separately had interrelated roles in photosynthesis so that excess of one metal will interfere with the photosynthetic function of the other (Christensen and Scherfig, 1979).

Combination of trivalent chromium and cobalt have not produced much variation in the biomass in N. clausii but notable increase was observed in the production whereas respiration was reduced. 0.6 ppm cobalt (toxic) in isolation reduced the production but the combination of 0.3 ppm cobalt and 0.6 ppm trivalent chromium (Treatment 6), 0.6 ppm cobalt and 0.6 ppm trivalent chromium (Treatment 7), and 0.6 ppm cobalt and 0.8 ppm trivalent chromium (Treatment 8) enhanced the production. The photosynthetic pigments such as chlorophyll-a, chlorophyll-c and protein content was stimulated in combination whereas carotenoids were reduced.

Studies of Sakaguchi et al. (1979) had reported that equimolar amounts of sodium, magnesium, manganese, cobalt, nickel and zinc added to the culture of Chlorella vulgaris reduced the toxic effect of cadmium on the culture.

The individual effect of hexavalent chromium and cobalt in S. abundans was different. Hexavalent chromium (0.05 ppm and 0.1 ppm) stimulated the production and respiration whereas the cobalt inhibited the same. But the two metals together in combination reduced the production and respiration for all treatments (9), (10), (11) and (12) while pigments, chlorophyll-a and carotenoids increased in combinations. Chlorophyll-b and phaeophytin decreased considerably. There was much variation between treatments in the case of end products of photosynthesis. Protein and carbohydrate were enhanced in treatment (9) (Cobalt 0.01 ppm and hexavalent chromium 0.05 ppm) and treatment (10) (cobalt 0.01 ppm and hexavalent chromium 0.15 ppm) whereas it was decreased considerably in treatment (11) (cobalt 0.05 ppm and hexavalent chromium 0.05 ppm) and treatment (12) (cobalt 0.1 ppm and hexavalent chromium 0.1 ppm) suggesting that concentration of cobalt was above 0.01 ppm in combination with hexavalent chromium (0.1 and 0.15 ppm), the end-products were markedly reduced whereas individual concentration of 0.05 ppm cobalt stimulated the protein and carbohydrate. The biomass showed an additive effect on algae.

In general the combination of cobalt and hexavalent chromium exhibited synergistic action on growth in N. clausii. Whereas it was antagonistic as manifested by increase in production when compared with individual metals for all treatments such as treatment (13), (14), (15), (16) and (17). In the case of respiration and pigments, the combination of metals had an additive effect on algae. The photosynthetic end products such as carbohydrate were high in combinations compared with individual concentration of cobalt.

The combination of trivalent chromium and nickel had general antagonism in the case of growth resulting in the increase in biomass in S. abundans, whereas the combination of two produced synergism resulting in the decrease in production, respiration and pigments in treatment (18) (0.01 ppm each of nickel and trivalent chromium) and treatment (20) (the 0.03 ppm each of nickel and trivalent chromium). For treatment (19) (the 0.02 ppm each of nickel and trivalent chromium) the production, respiration and pigments were less than the control. Treatment (18) and (20) enhanced the protein content of the algae whereas the carbohydrate was found to be fluctuating.

Hutchinson (1973) reported that nickel and copper acted synergistically resulting in the reduction of growth of Haematococcus capensis. Uptis et al. (1973) reported that addition of 0.05 mg nickel per liter to cultures of Chlorella species previously exposed to 50 mg aluminium per liter led to a reduction in growth.

It was reported by Stokes (1975) that inhibition of Scenedesmus acutiformis was greater when copper and nickel were applied in combination than predicted from the effects of either metal applied singly. Hutchinson and Stokes (1975) reported similar findings for Chlorella vulgaris. They stated that 0.05 mg/liter copper reduced growth of Chlorella species to 95% of the controls whereas 0.05 mg/liter nickel was stimulating the growth when these two metals were applied in combination, Chlorella species achieved the growth similar to controls.

Combination of nickel and hexavalent chromium had synergistic effect which was noticed by reduction in production and respiration in S. abundans

for treatments (25) (0.01 ppm nickel and 0.05 ppm hexavalent chromium) and treatment (26) (0.02 ppm nickel and 0.1 ppm hexavalent chromium). Similarly pigments such as carotenoids, chlorophyll-a, chlorophyll-b were decreased. But the interaction of nickel and hexavalent chromium resulted in antagonism which was indicated by increase in the rate of photosynthetic end products when compared with individual concentration of nickel for treatment (25) and (26).

Azeez and Banerjee (1987) observed that there was marked reduction in chlorophyll-a when hexavalent chromium and nickel in combination were applied to Anacystis nidulans and Spirulina platensis. Thus the presence of other metals may have synergistic effect on nickel toxicity.

There was enhanced production in treatment (28) a combination of 0.6 ppm nickel and 0.6 ppm hexavalent chromium and treatment (29) 0.8 ppm nickel and 0.2 ppm hexavalent chromium in N. clausii, whereas for treatment (27) (0.6 ppm nickel and 0.2 ppm hexavalent chromium), the production was less than the control. But the combination was found to be synergistic resulting in reduction in photosynthetic end products such as protein and lipids. In 0.6 ppm hexavalent chromium in isolation, the growth was reduced considerably but in combination with 0.6 ppm nickel stimulated the growth resulting in the increase in biomass and production.

Uptis et al. (1973) reported that nickel inhibition of Chlorella could be over come by the addition of zinc. Laboratory studies by Verma et al. (1982) on the combination of zinc, hexavalent chromium and nickel in fish

Mystis vittatus showed that combination of hexavalent chromium and nickel was highly synergistic while combination of hexavalent chromium and nickel/zinc was highly antagonistic in nature. The possible mechanism responsible for antagonistic and synergistic combination according to Verma et al. (1982) was that there was competition for critical site by less toxic metal (antagonism) or intrinisic affinity of the individual metal for the critical site (antagonism).

In S. abundans, the combination of cobalt and nickel reduced the biomass. The production was stimulated towards the end of growth phase for treatment (30) (0.01 ppm cobalt and 0.005 ppm nickel) and treatment (31) (0.05 ppm cobalt and 0.005 ppm nickel), whereas respiration was inhibited but 0.05 ppm cobalt in isolation stimulated the respiration with a peak on the sixth day. Though individual concentration of cobalt enhanced the pigment-production, combination of metals reduced chlorophyll-a, chlorophyll-c and phaeophytin.

It was reported that the combination of mercury and nickel on the growth of cyanobacterium Anabaena equalis was synergistic when both mercury and nickel were added simultaneously or when mercury was added first. But it was antagonistic when nickel was added before mercury (Stratton and Corke, 1979).

According to Deviprasad and Deviprasad (1982) combination of nickel and cadmium had interacted antagonistically in Ankistrodesmis falcatus resulting in the stimulation of growth at low concentration and the toxicity was reduced even in high concentration when compared with individual effects.

In N. clausii combination of cobalt and nickel had much variation between treatments. Low concentration of cobalt (0.3 ppm) in combination with 0.6 ppm nickel (treatment 32), growth was similar to the control, but cobalt at 0.6 ppm in combination with 0.6 ppm nickel (treatment 33) showed well marked synergism. Individual concentration of nickel stimulated the production but combination of metals interacted synergistically resulting in the reduction in production, pigment content and in the end products of photosynthesis. Thus combination of cobalt and nickel was having synergistic effect on algae.

Thus it was concluded that the combination of metals will produce synergism or antagonism qualitatively and quantitatively on parameters of productivity. Though cobalt and nickel were considered as essential elements individually for the better growth of algae, the combination of two was toxic.

The inference derived from the analysis of variance by two way analysis technique was given below. Table 3,4.

The combination of cobalt and trivalent chromium in S. abundans was significant in the case of pigments such as chlorophyll-a, chlorophyll-b and phaeophytin. Eventhough there was significant difference between days in the case of production, respiration was found to be not significant. But the combination of cobalt and trivalent chromium produced least significant effect in N. clausii.

The combination of cobalt and hexavalent chromium was significant in all parameters except respiration in S. abundans and N. clausii.

The combination of nickel and trivalent chromium and the combination of nickel and hexavalent chromium were not significant in the case of production, respiration and end products in S. abundans. Chlorophyll-b was found to be significant. But both the combinations produced significant effect in the other species N. clausii. The combination of cobalt and nickel produced least significant effect on the species. Production and respiration were not affected significantly. But end products were found to be significant. Pigments chlorophyll-a and chlorophyll-b were significant in S. abundans whereas all pigments were found to be significant between concentration and days in N. clausii.

Table 3. Results of the significance of the variance ratio between combination and between days in S. abundans

Sl. No.	Metals employed	Selected parameters																					
		pH		Biomass		Production		Respiration		Chlorophyll-a		Chlorophyll-b		Carotenoids		Phaeophytin		Protein		Carbohydrate		Lipids
		1	11	1	11	1	11	1	11	1	11	1	11	1	11	1	11	1	11	1	11	1
1.	$Co+Cr^{3+}$	a	a	a	b	a	NS	NS	NS	a	a	a	a	a	NS	a	a	a	a	a	a	a
2.	$Co+Cr^{6+}$	a	a	a	NS	a	NS	NS	NS	a	a	a	a	a	a	a	a	a	a	a	a	a
3.	$Ni+Cr^{3+}$	a	a	NS	a	NS	NS	NS	NS	a	a	a	a	a	a	a	a	NS	NS	NS	NS	a
4.	$Ni+Cr^{6+}$	a	a	NS	a	NS	NS	NS	NS	a	a	a	a	a	a	a	a	NS	NS	NS	NS	a
5.	Co+Ni	a	a	NS	a	NS	a	NS	NS	a	a	a	a	NS	NS	NS	NS	a	a	a	a	a

a P $<$ 0.01 1 Concentration

b P $<$ 0.05 11 Days

NS Non Significant

Table 4. Results of the significance of the variance ratio between concentration and between days in N. clausii

Sl. No.	Metals employed	Selected parameters																									
		pH		Biomass		Production		Respiration		Chlorophyll-a		Chlorophyll-b		Carotenoids		Phaeophytin		Protein		Carbohydrate fraction(1)		Carbohydrate fraction(2)		Carbohydrate fraction(3)		Lipids
		1	11	1	11	1	11	1	11	1	11	1	11	1	11	1	11	1	11	1	11	1	11	1	11	1
1.	$Co+Cr^{3+}$	a	a	a	a	NS	a	NS	NS	a	a	NS	NS	NS	a	NS	a	NS	a	a	a	a	a	NS	NS	a
2.	$Co+Cr^{6+}$	b	b	b	a	a	a	NS	NS	a	a	a	a	NS	a	NS	a	a	a	a	a	NS	a	NS	NS	a
3.	$Ni+Cr^{3+}$	b	b	a	a	a	a	NS	NS	a	a	a	a	NS	a	a	a	a	a	a	a	NS	NS	NS	NS	a
4.	$Ni+Cr^{6+}$	b	b	a	a	a	a	NS	NS	a	a	a	a	NS	a	a	a	a	a	a	a	NS	NS	NS	NS	a
5.	Co+Ni	a	a	NS	a	NS	a	a	NS	a	a	a	a	a	a	a	a	a	a	a	a	NS	a	NS	NS	a

a $<$ 0.01

b $<$ 0.05

NS Non Significant

1 Concentration

11 Days

5.2 EFFECT OF IRON IN COMBINATION WITH TWO METALS ON *S. ABUNDANS* AND *N. CLAUSII*

5.2.1 The effect of iron in combination with cobalt and trivalent chromium in S. abundans

Concentration of metals (in ppm)	Treatment Number
Co 0.05 + Cr^{3+} 0.01 + Fe 0.05	34

Biomass

The biomass was less than the control with the aging of the culture for the treated sample (Fig.55). Eventhough there was a marginal increase on the second day it was 4% less than the control on the fourth day. On the sixth day it was similar to control followed by sudden decrease towards the end of growth phase. It was 45% less than the control on eighth day. On the twelfth and fourteenth day 33% and 43% decrease was observed.

Production

Combinations of iron reduced the production considerably. The production was less than the control through out the growth phase (Fig.55). It was 73% less than the control at the end of growth phase. On the twelfth day eventhough there was slight increase it was 55% less than the control. On the fourth, sixth and eighth day it was 57%, 53% and 40% less than the control. The pH was also less than the control through out the growth phase.

Respiration

Respiration was less than the control through out the growth phase (Fig.55). Eventhough there was a marginal decrease at the end of growth

phase, it was 76%, 58%, 30% and 47% less than the control on fourth, sixth, eighth and tenth day.

Photosynthetic pigments

There was a general trend of decrease in pigment production.

Chlorophyll-a

Chlorophyll-a was less than the control through out the growth phase (Fig.56). At the end of growth phase there was 18% decrease when compared with the control. On the fourth and eighth day eventhough there was only a marginal decrease, it was 36%, 47%, 20% less than the control on sixth, tenth and twelfth day.

Chlorophyll-b

Chlorophyll-b was less than the control with the aging of the culture for the treated sample (Fig.56). Though it was 45% more than the control on the second day, it was closely following the control on the fourth day. There was 75% reduction at the end of growth phase. It was 57%, 27%, 63% less than the control on sixth, eighth and twelfth day. On the tenth day there was a marginal increase of 4%.

Carotenoids

Similar to other pigments there was a marginal increase on the second day followed by sudden decrease till the end of growth phase. It was 36%, 51% and 50% less than the control on sixth, eighth and tenth day. On the twelfth day though there was a peak it was 56% less than the control.

Phaeophytin

Phaeophytin was more than the control on the second and eighth day but it was 40% less than the control on the fourteenth day. Eventhough there was a marginal decrease on the fourth and tenth day, it was 23% and 59% less than the control on sixth and twelfth day.

Photosynthetic end products

Carbohydrate

A general trend of increase was noticed on the eighth day and at the end of growth phase for the treated sample (Fig.57). It was 72% and 54% more than the control on eighth and fourteenth day. Whereas on the twelfth day it was 23% less than the control.

Protein

Protein showed marked increase in the iron treated samples (Fig.57). Thus the presence of iron stimulated the protein production. Maximum protein was observed on the eighth day and it was 78% more than the control. Whereas the control was maximum on the tenth day. On the tenth day the treated sample was 10% more than the control, but on the twelfth and fourteenth day 33%, 37% decrease was noticed.

The combination of cobalt and trivalent chromium in the presence of iron enhanced the lipid production and it was 110% more than the control.

In the case of nutrients, the phosphate uptake was more than the control for the treated sample and it was 25% more than the control. Such a marked increase was not noticed for nitrate.

Fig.55

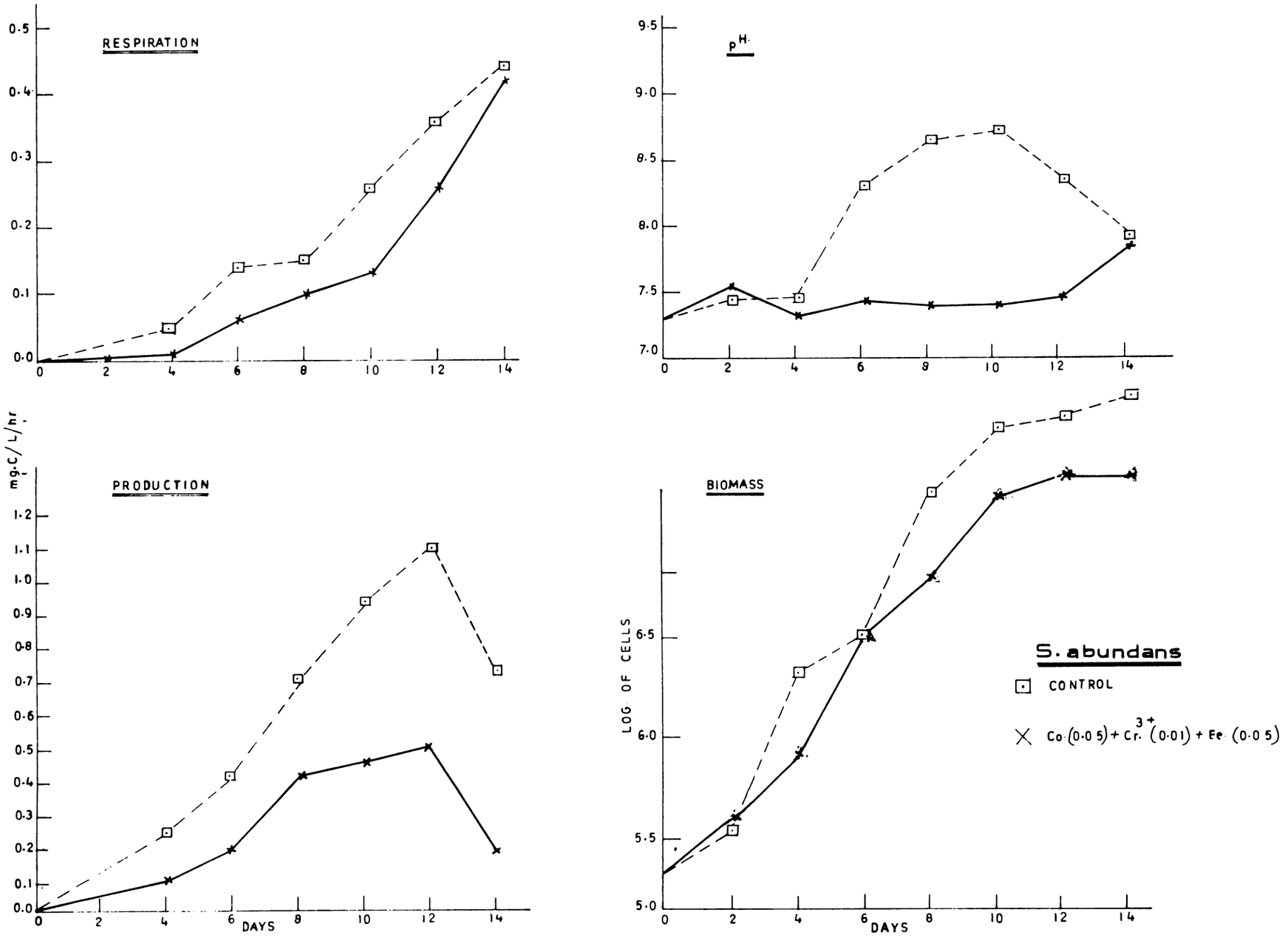

RESPIRATION
pH
PRODUCTION
mg.C/L/hr
BIOMASS
LOG OF CELLS
DAYS
DAYS
S. abundans
CONTROL
Co (0.05) + Cr 3+ (0.01) + Fe (0.05)

Fig.56

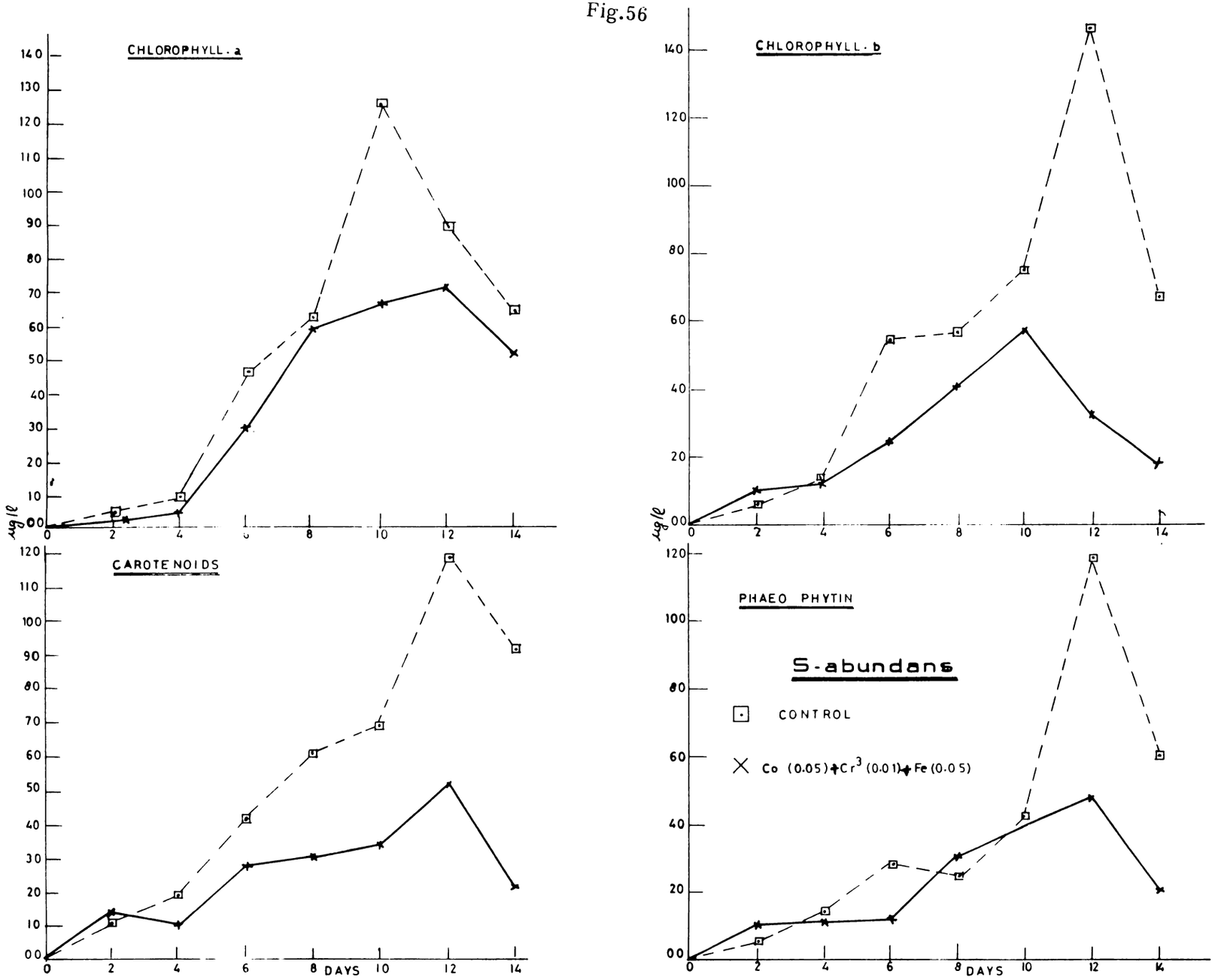
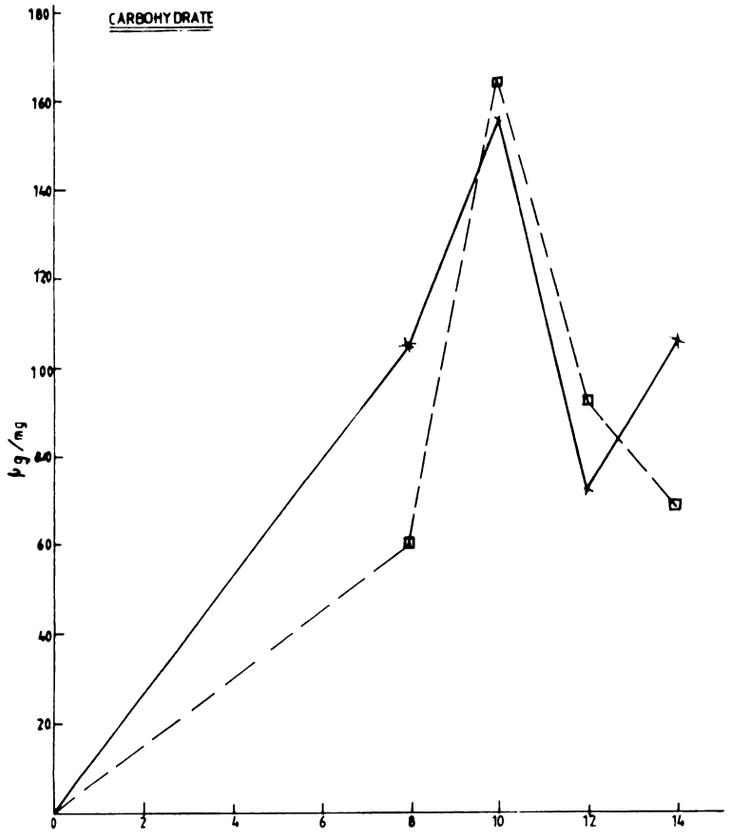
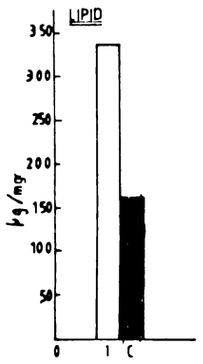
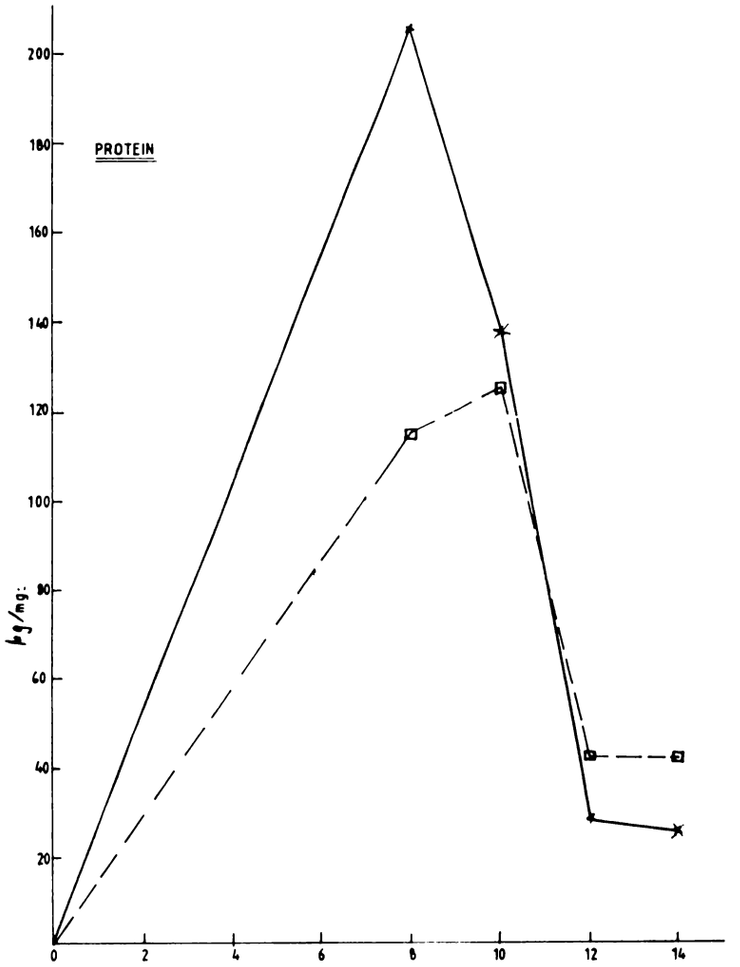

CHLOROPHYLL. a
CHLOROPHYLL. b
CAROTENOIDS
PHAEO PHYTIN
S-abundans
CONTROL
Co (0.05)+Cr3 (0.01)+Fe (0.05)
µg/l
DAYS

Fig.57







Days

S. abundans

C ⊡ CONTROL

i ×Co (0.05) + Cr^{3+}(0.01)+Fe (0.05)

Effect of iron in combination with cobalt and trivalent chromium in N. clausii

Concentration of metals (in ppm)	Treatment Number
Co 0.6 + Cr^{3+} 0.6 + Fe^{+} 0.2	35

Biomass

Growth was less than the control through out the growth phase by 10%, 37%, 22% and 26% from fourth to tenth day.

Production

There was a general trend of increase in production on sixth and eighth day and it was 42% and 38% more than the control. But on the fourth and tenth day there was 35% and 16% decrease in production. Similar trend was noticed in the case of pH also.

Respiration

Respiration was closely following the control on fourth and sixth day. But with the aging of the culture the respiration was reduced. On the eighth and tenth day 41% and 3% decrease was observed.

Photosynthetic pigments

Chlorophyll-a

Chlorophyll-a was having a marginal increase on the second and eighth day. But it was 55% and 16% and 25% less than the control on fourth, sixth and tenth day respectively.

Chlorophyll-c

Chlorophyll-c was less than the control through out the growth phase (Fig.59). It was 72%, 64%, 46% and 48% less than the control on fourth, sixth, eighth and tenth day.

Carotenoids

Carotenoids were less than the control in the first half of growth phase for the treated sample (Fig.59). It was 41% and 20% less than the control on fourth and sixth day. On the eighth day 19% increase was observed and at the end of growth phase a marginal decrease of 6% was noticed.

Phaeophytin

Increasing trend was noticed for phaeophytin with the aging of the culture. It was 14%, 12% and 11% more than the control on sixth, eighth and tenth day but 25% decrease was observed on the fourth day.

Photosynthetic end products

Carbohydrate

The three fractions of carbohydrate (acid soluble, alkali soluble and insoluble) was showing an initial peak on fourth and sixth day. Towards the end of growth phase carbohydrate was less than the control for the treated sample.

The acid soluble fraction was 559%, 271% and 7% more than the control on fourth, sixth and eighth day (Fig.60). At the end of growth phase 16% decrease was observed.

The alkali soluble fraction was 161% more than the control on the fourth day but it was 72% and 38% less than the control on the eighth and tenth day.

The insoluble fraction was 165%, 66% more than the control on fourth and sixth day. It was 16% less than the control on the eighth day followed by 53% increase at the end of growth phase.

Protein

There was marked increase in protein even in the early phase of growth upto eighth day (Fig.60). It was 353%, 117% and 5% more than the control on fourth, sixth and eighth day respectively. At the end of growth phase 66% decrease was observed.

Lipid content was 86% less than the control for the treated sample.

The nutrient uptake was found to be far less than the control. Phosphate uptake was 60% less than the control but the nitrate uptake was 28% less than the control.

Fig.58

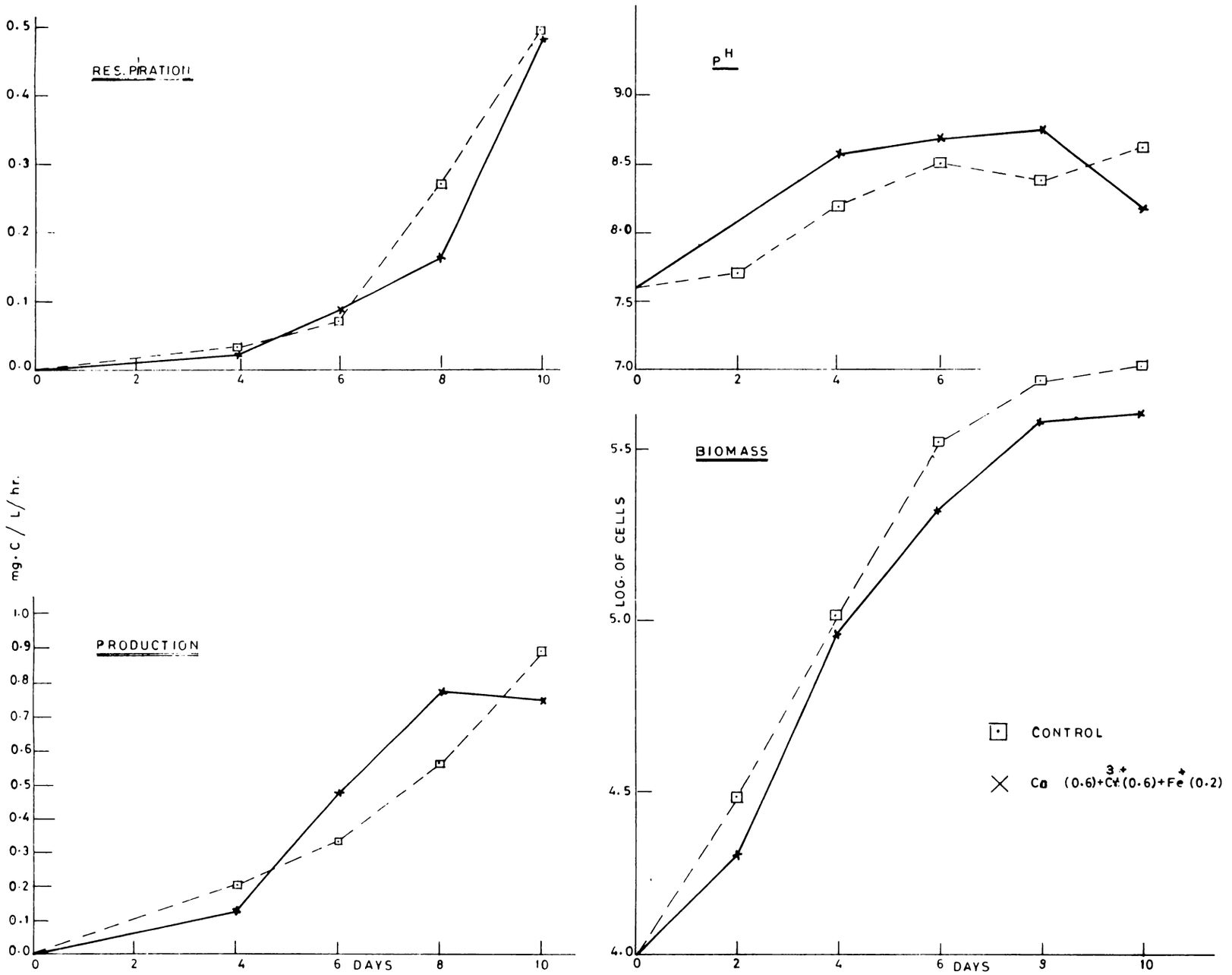
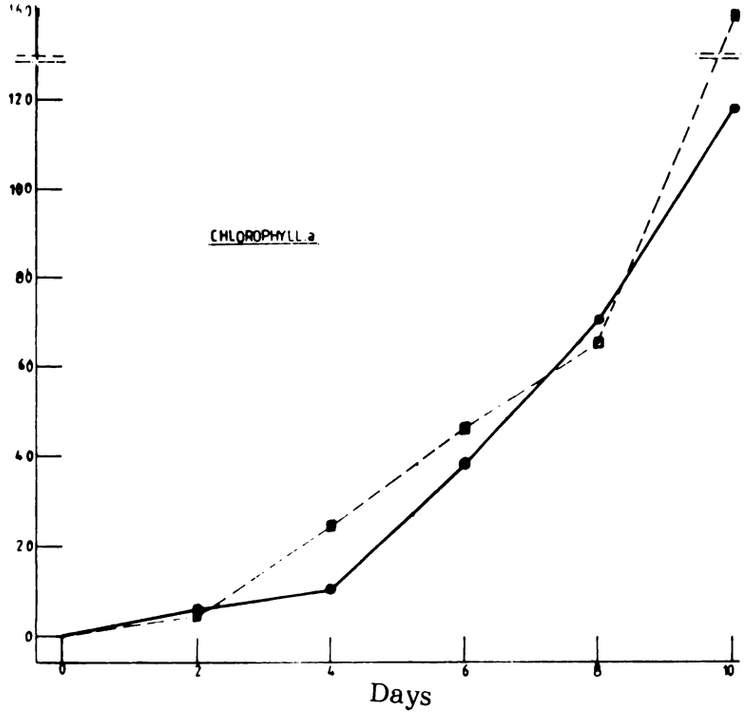
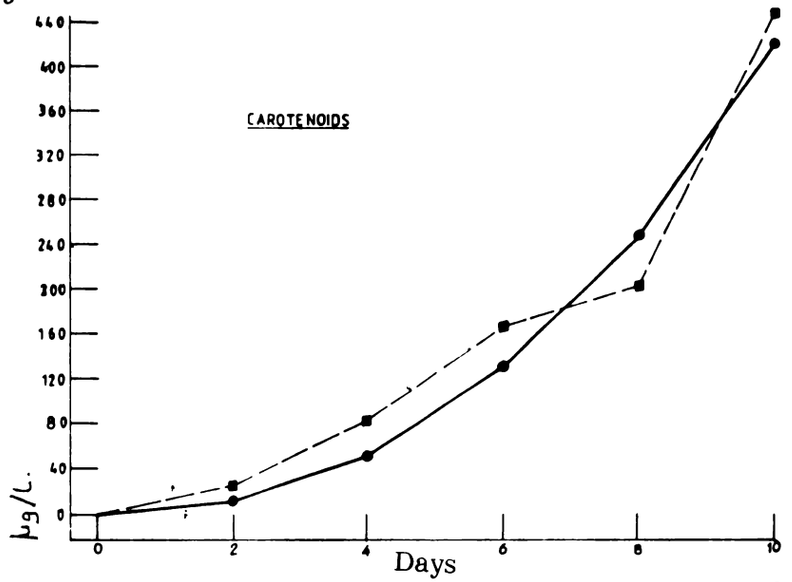
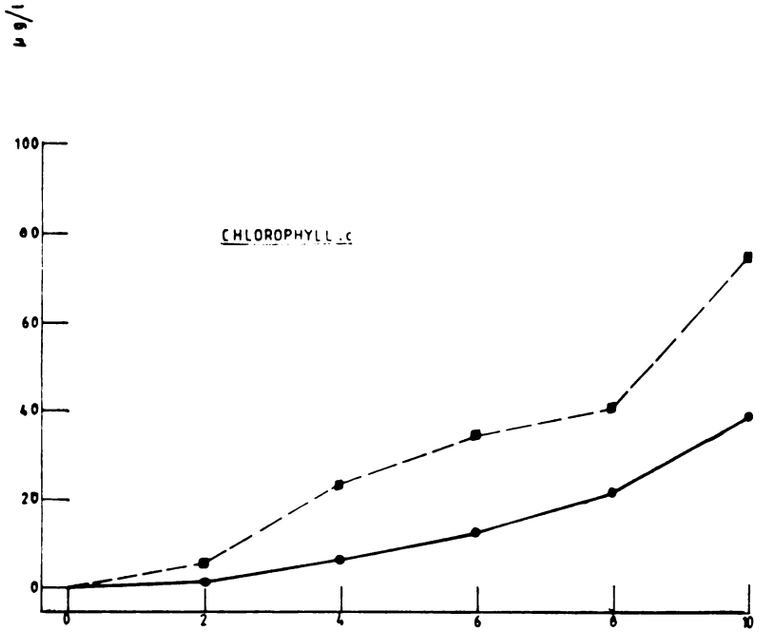

RESPIRATION
PH
PRODUCTION
mg·C / L/ hr.
BIOMASS
LOG. OF CELLS
DAYS
DAYS
CONTROL
Ca (0.6)+Cr3+(0.6)+Fe+ (0.2)

Fig.59


CHLOROPHYLL a
Days


CAROTENOIDS
µg/L.
Days


µg/l
CHLOROPHYLL c

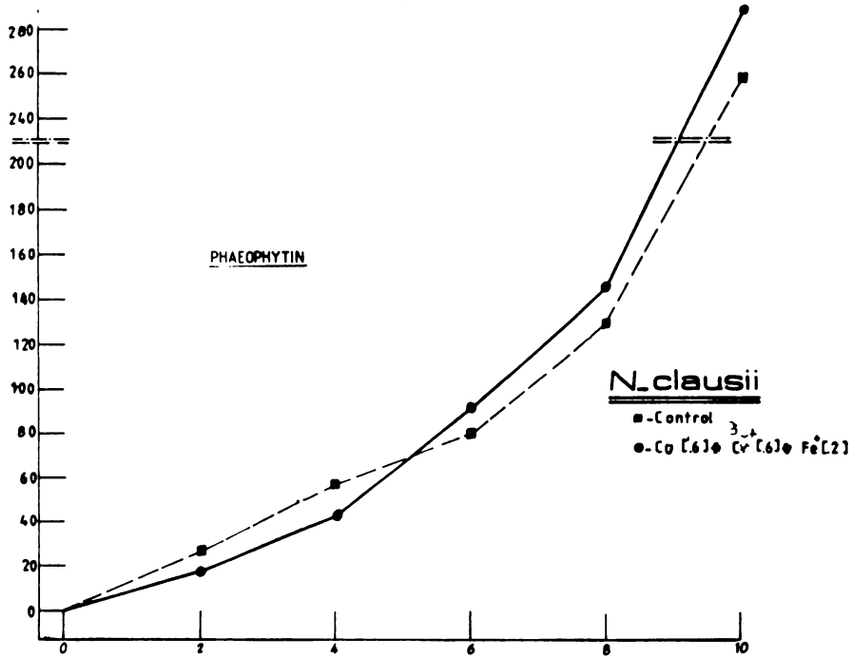
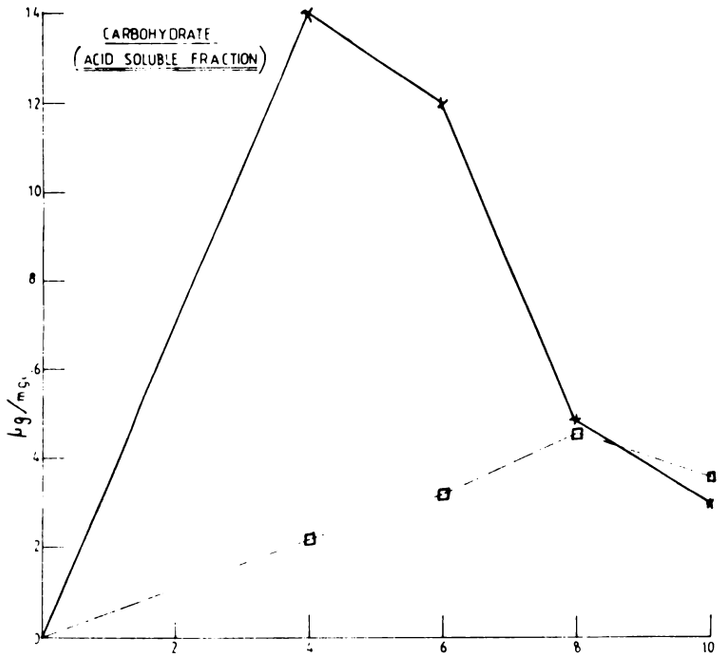
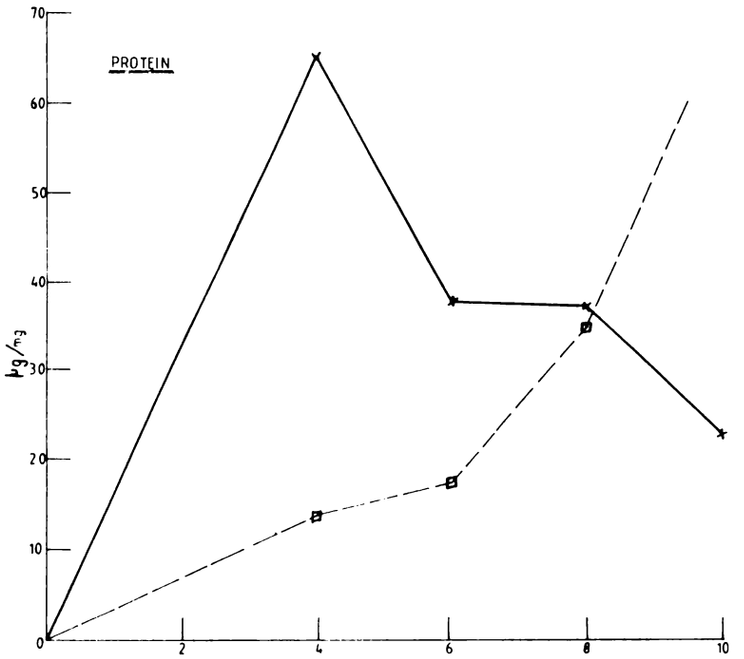
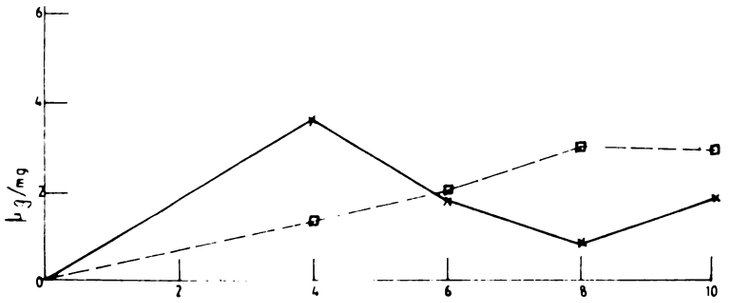

PHAEOPHYTIN
N. clausii
■-Control
●-Cu [.6]● Cr [.6]● Fe[2]

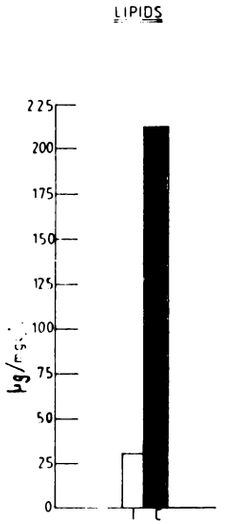
Fig.60

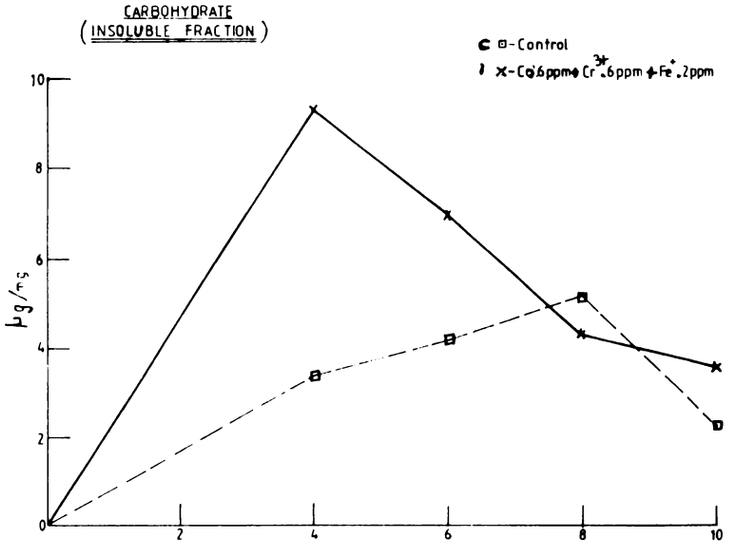




N_clausii







5.2.2 The effect of iron in combination with cobalt and hexavalent chromium in S. abundans

Concentration of metals (in ppm)	Treatment Number
Co 0.01 + Cr^{6+} 0.05 + Fe 0.05	36

Biomass

Biomass was similar to the control on second and eighth day but showed an increase of 43% on the sixth day. The growth was 24%, 25% and 33% less than the control on tenth, twelfth and fourteenth day (Fig.61).

Production

The production was showing a marginal increase upto eighth day followed by abrupt decrease towards the end of growth phase (Fig.61). It was 47%, 51% and 72% less than the control on tenth, twelfth and fourteenth day. The pH was less than the control from sixth day onwards.

Respiration

Respiration was less than the control through out the growth phase. It was 51% and 40% less than the control on twelfth and fourteenth day. A decrease of 80%, 41% and 65% was noticed on fourth, eighth and tenth day respectively.

Photosynthetic pigments

Chlorophyll-a

Chlorophyll-a was closely following the control upto eighth day for the treated sample (Fig.62). On the tenth day eventhough the pigment content was maximum, it was 33% less than the control. On the twelfth and fourteenth day it was 10% and 18% less than the control.

Chlorophyll-b

Chlorophyll-b was more than the control upto eighth day followed by an abrupt decrease from tenth day onwards (Fig.62). It was 87% and 78% more than the control on fourth and eighth day and it was similar to the control on sixth day. There was 4%, 20% and 58% decrease on tenth, twelfth and fourteenth day respectively.

Carotenoids

Carotenoids were closely following the control upto sixth day with a marginal increase on sixth day. It was 60% and 65% less than the control on eighth and fourteenth day. A decrease of 65% was observed on the tenth and twelfth day.

Phaeophytin

Phaeophytin was fluctuating through out the growth phase. It was closely following the control upto fourth day but 45% and 16% decrease was observed on sixth and twelfth day. On the eighth, and fourteenth day there was an increase of 11% and 17%.

Photosynthetic end products

Carbohydrate

Carbohydrate showed general increasing trend for the treated sample (Fig.63). It was maximum on the tenth day and the value being 325% more than the control. On the eighth and twelfth day there was 102% and 3% increase. At the end of growth phase there was a decrease of 15%.

Protein

Protein content of the sample was showing a peak on the tenth day and it was 27% more than the control, though it was 37% less than the control on the eighth day. It was 17% and 41% less than the control on the twelfth and fourteenth day respectively.

Unlike protein and carbohydrate, the lipid content of the algae was 63% less than the control.

Nutrient uptake was less than the control for treated sample. Phosphate was 13% less than the control.

Fig.61

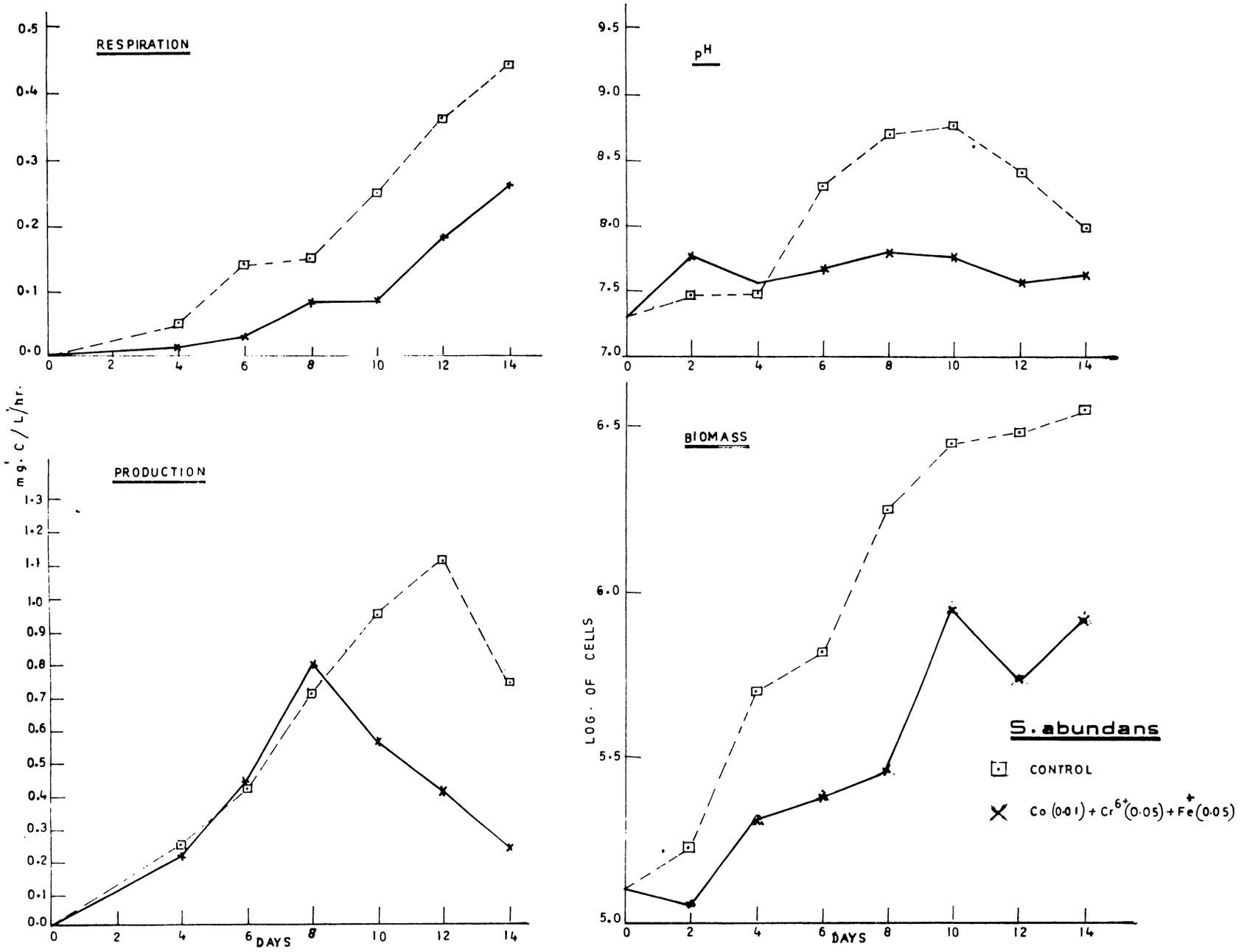

RESPIRATION
pH
PRODUCTION
BIOMASS
mg·C/L/hr.
LOG. OF CELLS
DAYS
DAYS
S. abundans
CONTROL
Co (0·01) + Cr6+ (0·05) + Fe+ (0·05)

Fig.62

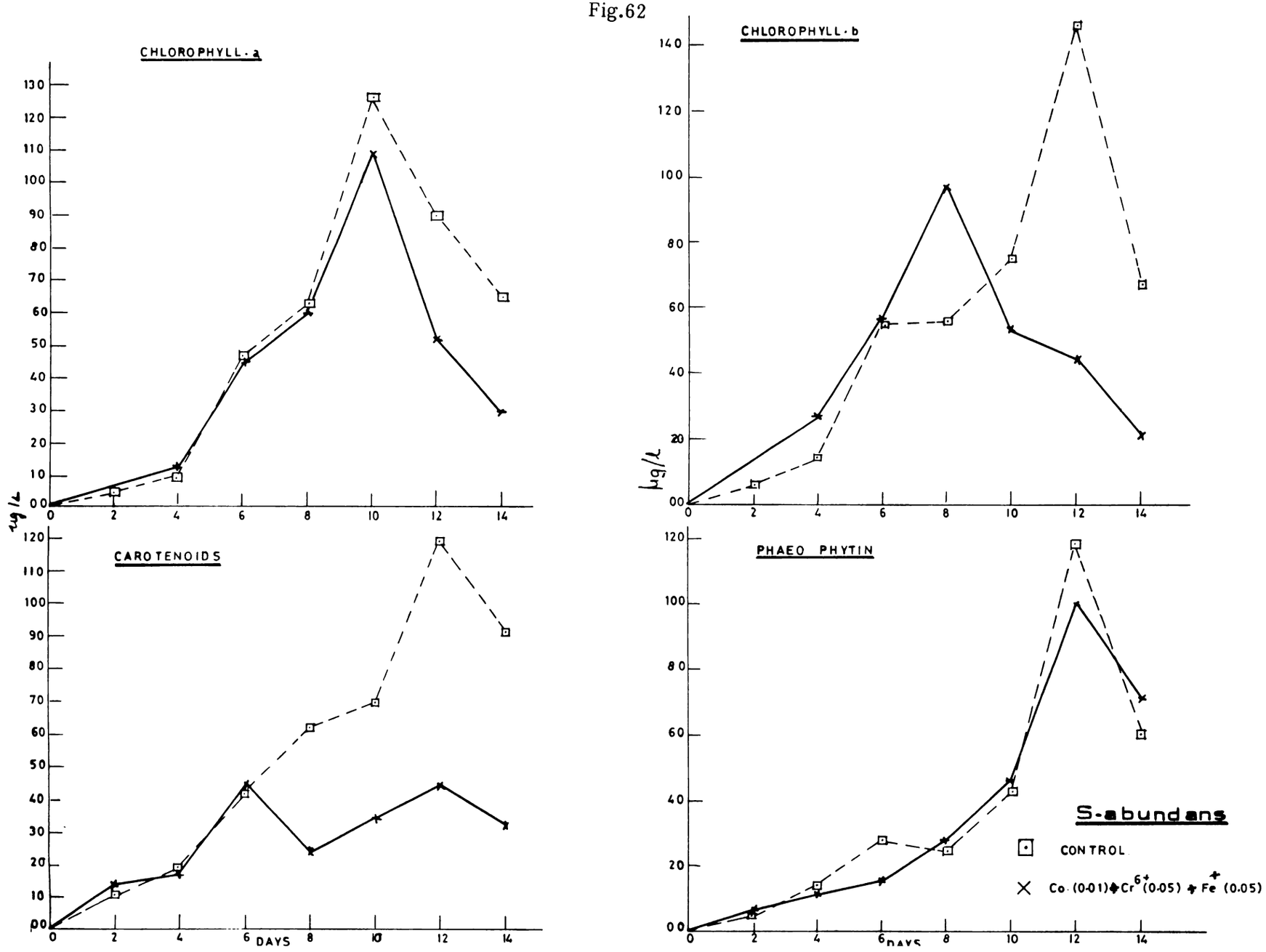
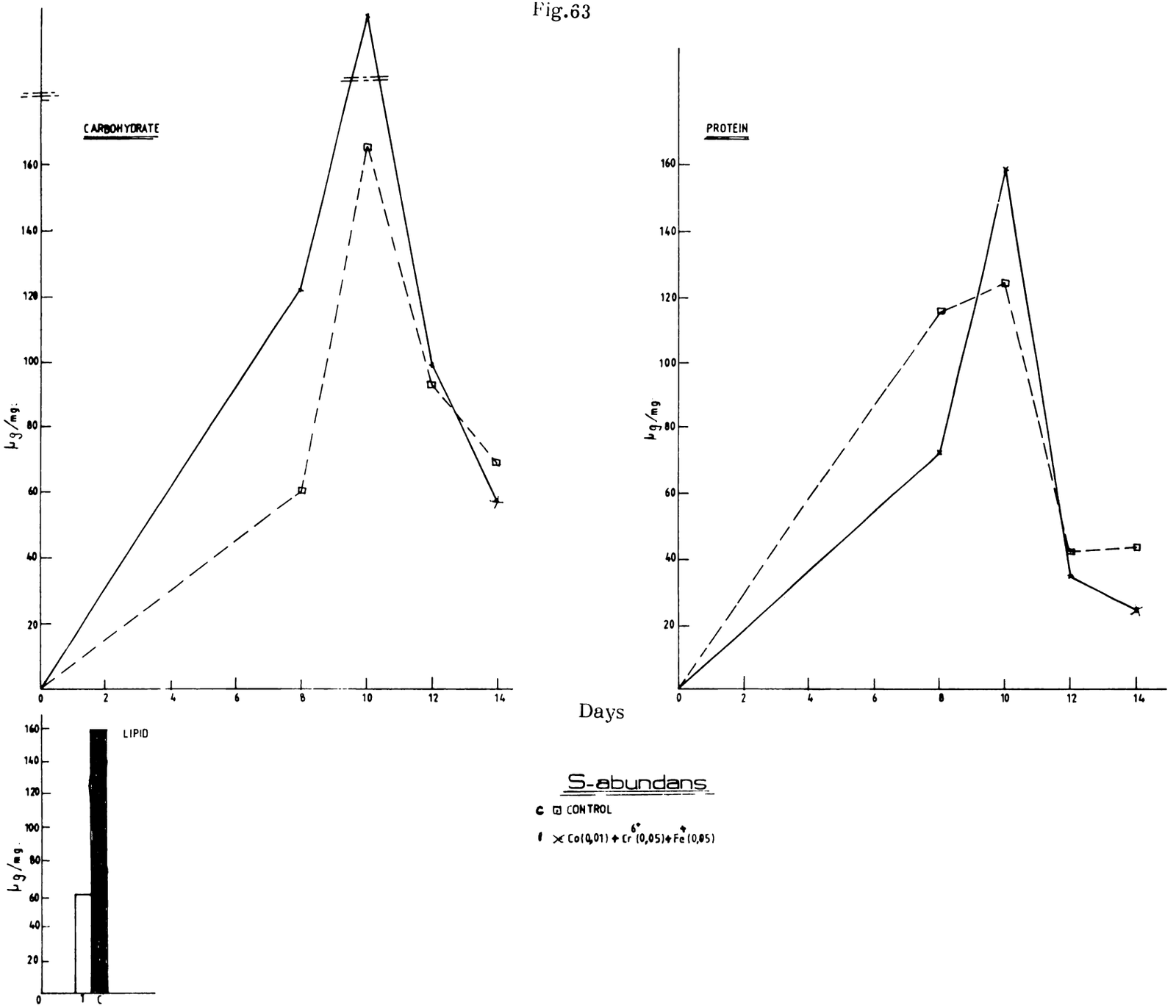

CHLOROPHYLL-a
CHLOROPHYLL-b
CAROTENOIDS
PHAEO PHYTIN
µg/l
DAYS
S-abundans
CONTROL
Co (0·01) + Cr 6+ (0·05) + Fe + (0·05)

Fig.63


CARBOHYDRATE
PROTEIN
LIPID
µg/mg
Days
S-abundans
C CONTROL
f Co(0,01) + Cr6+(0,05) + Fe+(0,05)
T
C

Effect of iron in combination with cobalt and hexavalent chromium in N. clausii

Concentration of metals (in ppm)	Treatment Number
Co 0.6 + Cr^{6+} 0.6 + Fe^{+} 0.2	37

Biomass

Biomass was showing a general decrease from fourth day onwards (Fig.64). Though it was more than the control on the second day it was 35%, 43%, 48% and 25% less than the control on fourth, sixth, eighth and tenth day.

Production

The production was showing an initial decrease of 24% on the fourth day and 7% decrease at the end of growth phase but it was showing the peak on the eighth day and it was 105% more than the control (Fig.64). Similar trend was observed in the case of pH also.

Respiration

Respiration was generally less than the control (Fig.64). It was similar to the control on the fourth day followed by 25%, 70% and 87% decrease on sixth, eighth and tenth day respectively.

Photosynthetic pigments

Chlorophyll-a

Chlorophyll-a was less than the control through out the growth though it was similar to the control on the second day (Fig.65). It was 30%, 13%,

30% and 17% less than the control on fourth, sixth, eighth and tenth day respectively.

Chlorophyll-c

Chlorophyll-c was less than the control throughout the growth phase (Fig.65). It was 40%, 32%, 33% and 37% less than the control on fourth, sixth, eighth and tenth day respectively.

Carotenoids

A general trend of decrease was observed upto eighth day for the treated sample (Fig.65). But it was 5% more the control at the end of growth phase. It was 25%, 11% and 16% less than the control on fourth, sixth and eighth day.

Phaeophytin

Phaeophytin was less than the control through out the growth phase. It was 74%, 44% and 50% less than the control on fourth, sixth and tenth day respectively.

Photosynthetic end products

Carbohydrate

A general trend of increase was noticed upto sixth day for all fractions of carbohydrate.

The acid soluble fraction was maximum on the fourth day and it was 78% and 80% less than the control on eighth and tenth day (Fig.66).

The alkali soluble fraction was 18% and 49% more than the control on fourth and sixth day. It was 64% and 55% less than the control on eighth and tenth day respectively (Fig.66).

The insoluble fraction of carbohydrate was also more than the control on fourth and sixth day. The values being 20%, 55% more than the control (Fig.66). There was 6% decrease on the eighth day followed by 86% increase at the end of growth phase.

Protein

Protein content of the diatom improved to a large extent in the presence of iron. It was more than the control upto eighth day with a peak (290%) on the sixth day (Fig.66). There was 86% and 37% increase on the fourth and eighth day respectively. But at the end of growth phase it was less than the control.

Lipid content of the algae was 90% less than the control.

The nutrient uptake was found to be more than the control. Phosphate was 44% more than the control and the nitrate uptake was similar to the control.

Fig.64

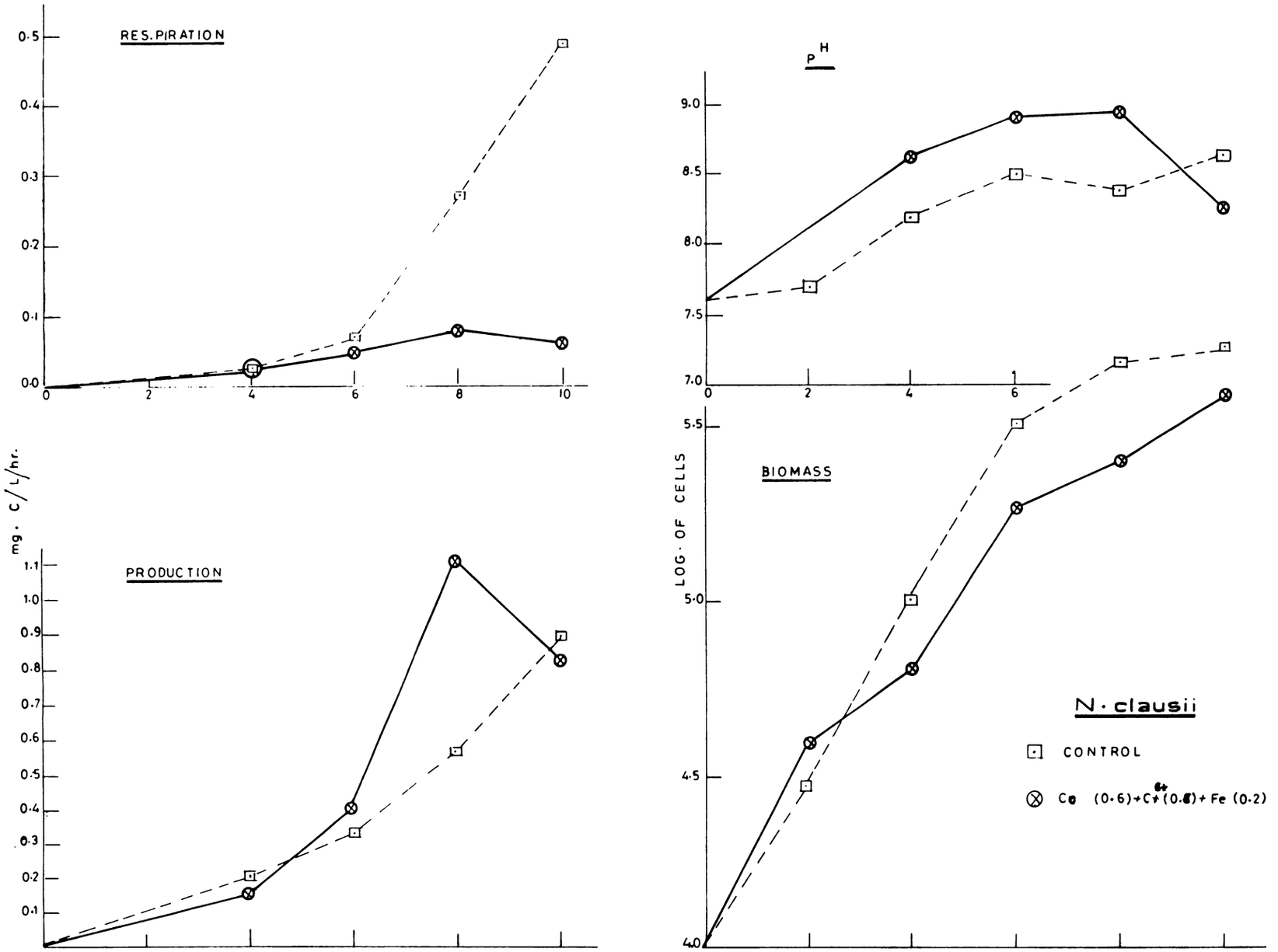
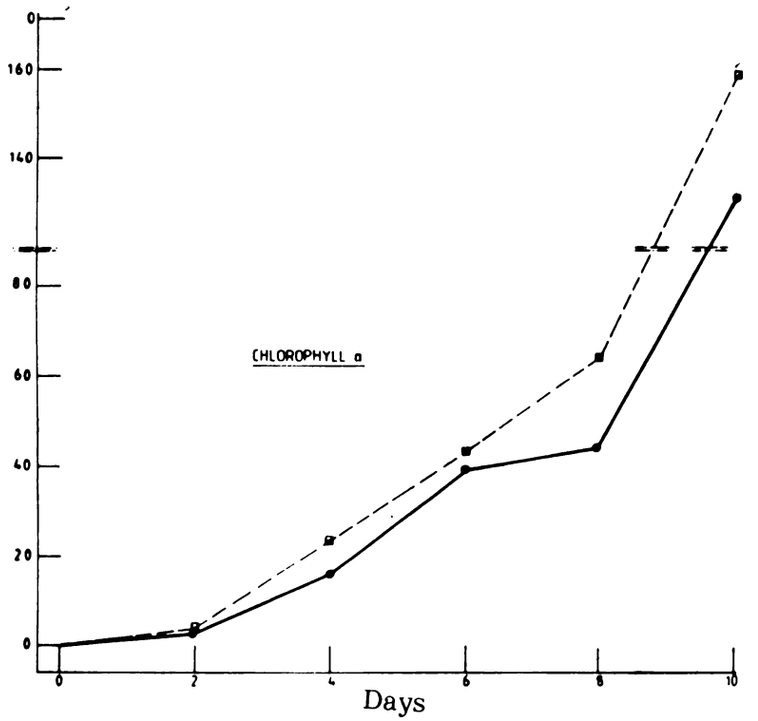
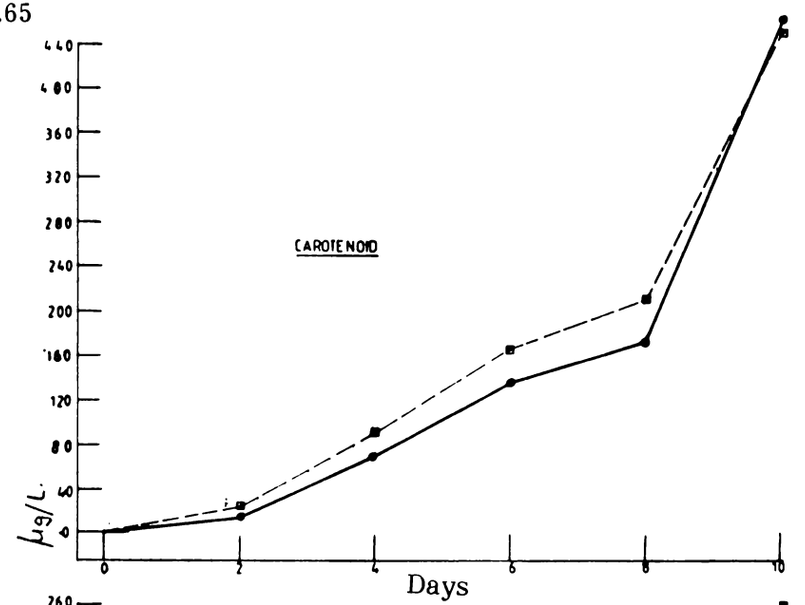
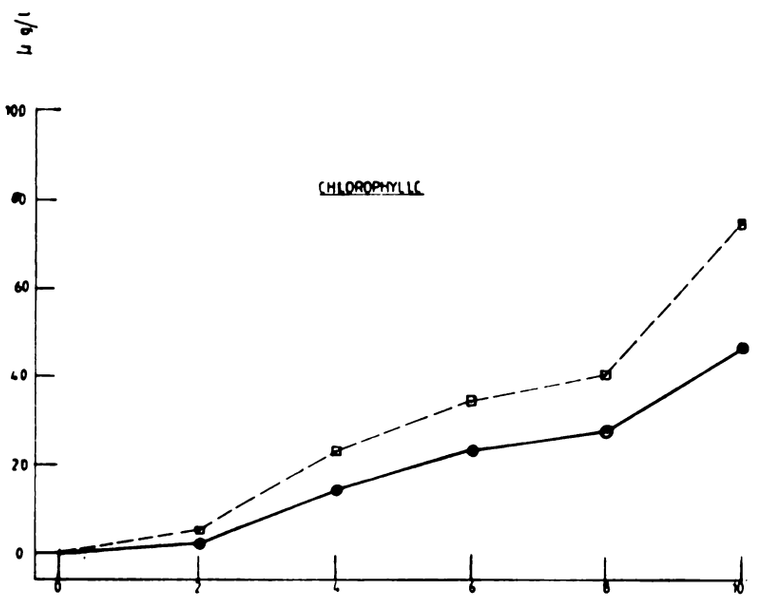

RES.PIRATION
0·5
0·4
0·3
0·2
0·1
0·0
0
2
4
6
8
10
mg · C/L/hr.
PRODUCTION
1·1
1·0
0·9
0·8
0·7
0·6
0·5
0·4
0·3
0·2
0·1
P H
9·0
8·5
8·0
7·5
7·0
0
2
4
6
LOG · OF CELLS
BIOMASS
5·5
5·0
4·5
4·0
N · clausii
CONTROL
Co (0·6)+Cr (0·6)+Fe (0·2)

Fig.65


CHLOROPHYLL a
Days


CAROTENOID
µg/L
Days


µg/l
CHLOROPHYLL c

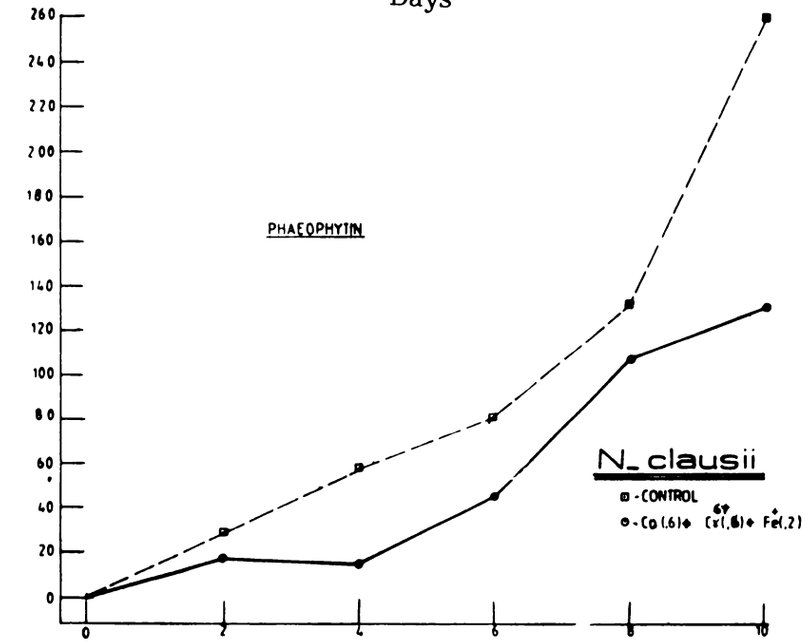
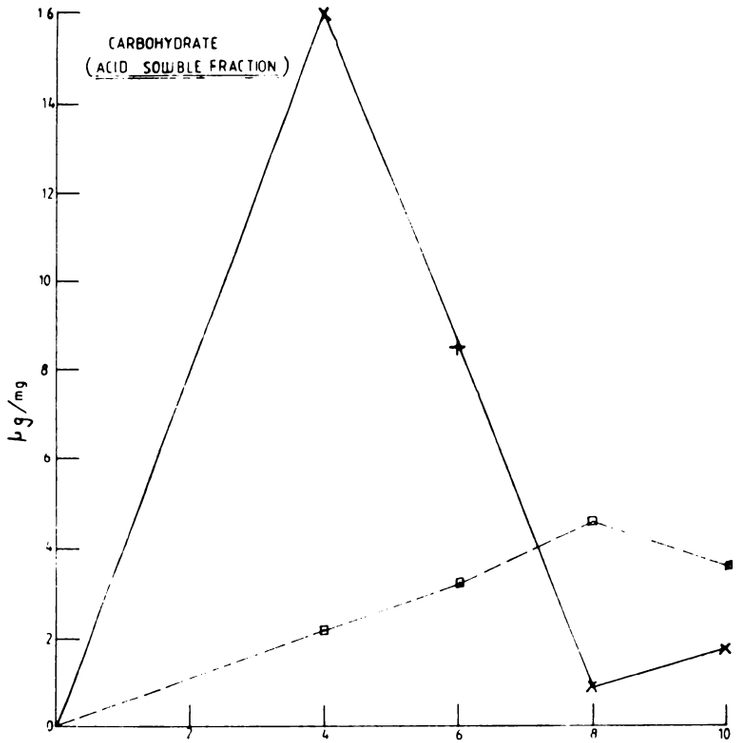
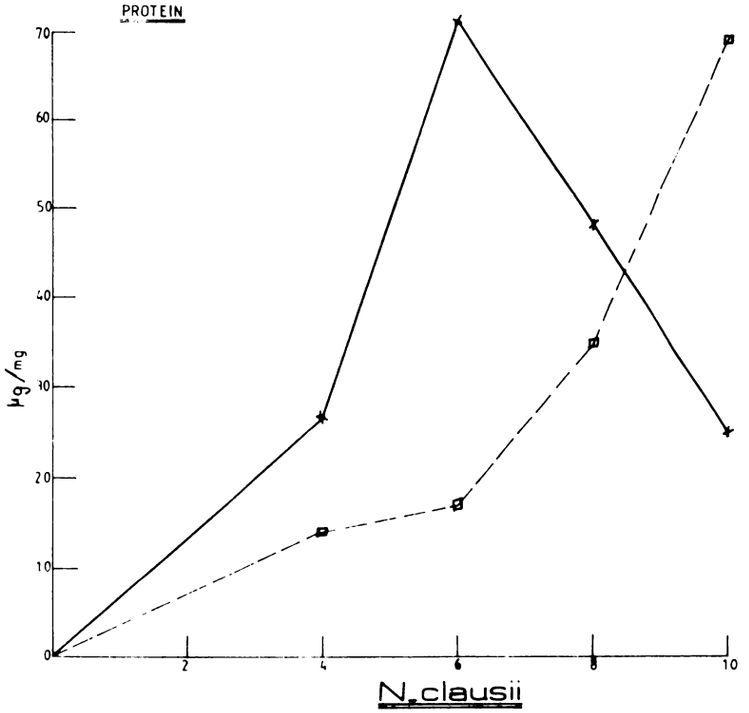
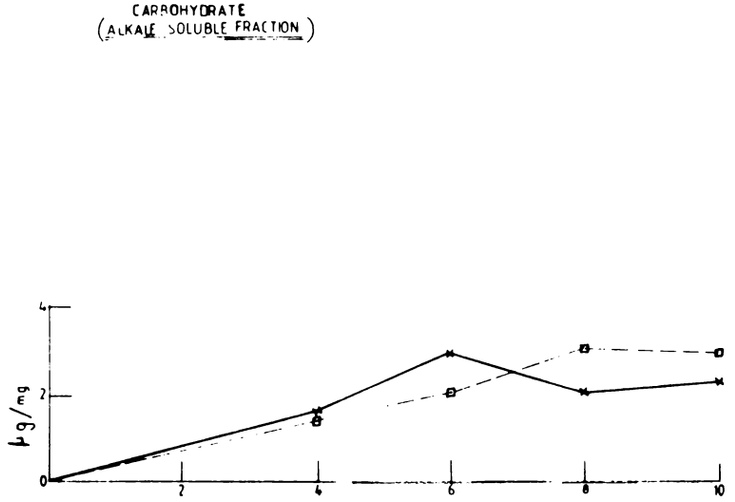

PHAEOPHYTIN
N. clausii
- CONTROL
- Co (.6) Cr (.8) Fe (.2)

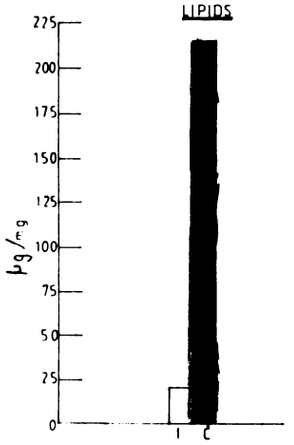
Fig.66

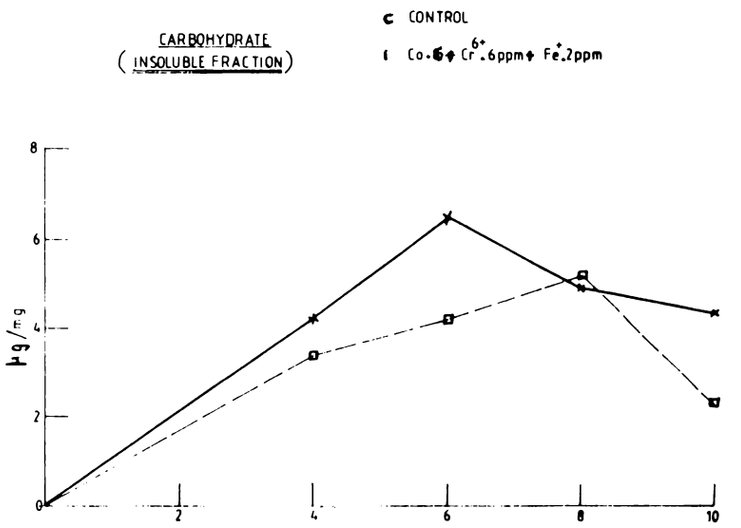

CARBOHYDRATE
(ACID SOLUBLE FRACTION)
µg/mg
0
2
4
6
8
10
12
14
16
2
4
6
8
10


PROTEIN
µg/mg
0
10
20
30
40
50
60
70
2
4
6
8
10
N.clausii

Days


CARBOHYDRATE
(ALKALE SOLUBLE FRACTION)
µg/mg
0
2
4
2
4
6
8
10


LIPIDS
µg/mg
0
25
50
75
100
125
150
175
200
225
I
C


C CONTROL
I Co.6ppm Cr6+.6ppm Fe+.2ppm
CARBOHYDRATE
(INSOLUBLE FRACTION)
µg/mg
0
2
4
6
8
2
4
6
8
10

5.2.3 The effect of iron in combination with nickel and trivalent chromium in S. abundans

Concentration of metals (in ppm)	Treatment Number
Ni 0.01 + Cr^{3+} 0.01 + Fe^{+} 0.05	38

Biomass

The growth was found to be fluctuating through out the growth phase (Fig.67). A marginal increase was observed on the second day followed by a decrease on the fourth day, and on the sixth day there was 9% increase for the treated sample. A sudden decrease was noticed from eighth day which was continued upto the end of growth. The values being 38%, 43%, 29% and 34% less than the control on eighth, tenth, twelfth and fourteenth day respectively.

Production

There was marked decrease in production through out the growth phase (Fig.67). It was 45%, 47%, 26% and 48% less than the control on the fourth, sixth, eighth and tenth day. At the end of growth phase it was 52% less than the control. pH was also less than the control through out the growth phase.

Respiration

Respiration was less than the control through out the growth phase (Fig.67). The values being 58%, 60%, 55%, 41%, and 7% less than the control on sixth, eighth, tenth, twelfth and fourteenth day respectively.

Photosynthetic pigments

Chlorophyll-a

Chlorophyll-a was similar to the control upto fourth day followed by sudden decrease towards the end of growth phase (Fig.68). At the end of growth phase 17% decrease was observed. There was 53%, 20%, 33% and 11% decrease on sixth, eighth, tenth and twelfth day respectively.

Chlorophyll-b

Chlorophyll-b was fluctuating through out the growth phase (Fig.68). 20% and 58% decrease was observed on the twelfth and fourteenth day. It was 79% and 60% more than the control on fourth and tenth day. But an increase of 42% and 2% was noticed on the sixth and eighth day.

Carotenoids

Carotenoid production decreased from early phase itself. The values being 40%, 29%, 51%, 67%, 8% and 64% less than the control on fourth to fourteenth day respectively.

Phaeophytin

Phaeophytin was closely following the control upto fourth day. But 57% decrease was noticed on the sixth day followed by 19% increase on the eighth day. Towards the end of growth phase 62%, 94% and 92% decrease was observed on tenth, twelfth and fourteenth day. Thus with the aging of the culture phaeophytin pigments decreased and maximum decrease was noticed at the end of growth phase.

Photosynthetic end products

Carbohydrate

Carbohydrate was less than the control on the eighth and tenth day, the values being 27% and 61% less than the control. But an increase by 72% and 73% was observed on the twelfth and fourteenth day.

Protein

Protein content was more than the control upto twelfth day but 14% decrease was observed on the fourteenth day. The peak in protein content was on the eighth day and it was 71% more than the control. On the tenth day 17% increase was noticed and on the twelfth day it was similar to the control.

The lipid content of the algae was 28% less than the control.

There was no marked increase in the nutrient uptake in the presence of iron. Both phosphate and nitrate uptake was similar to the control.

Fig.67

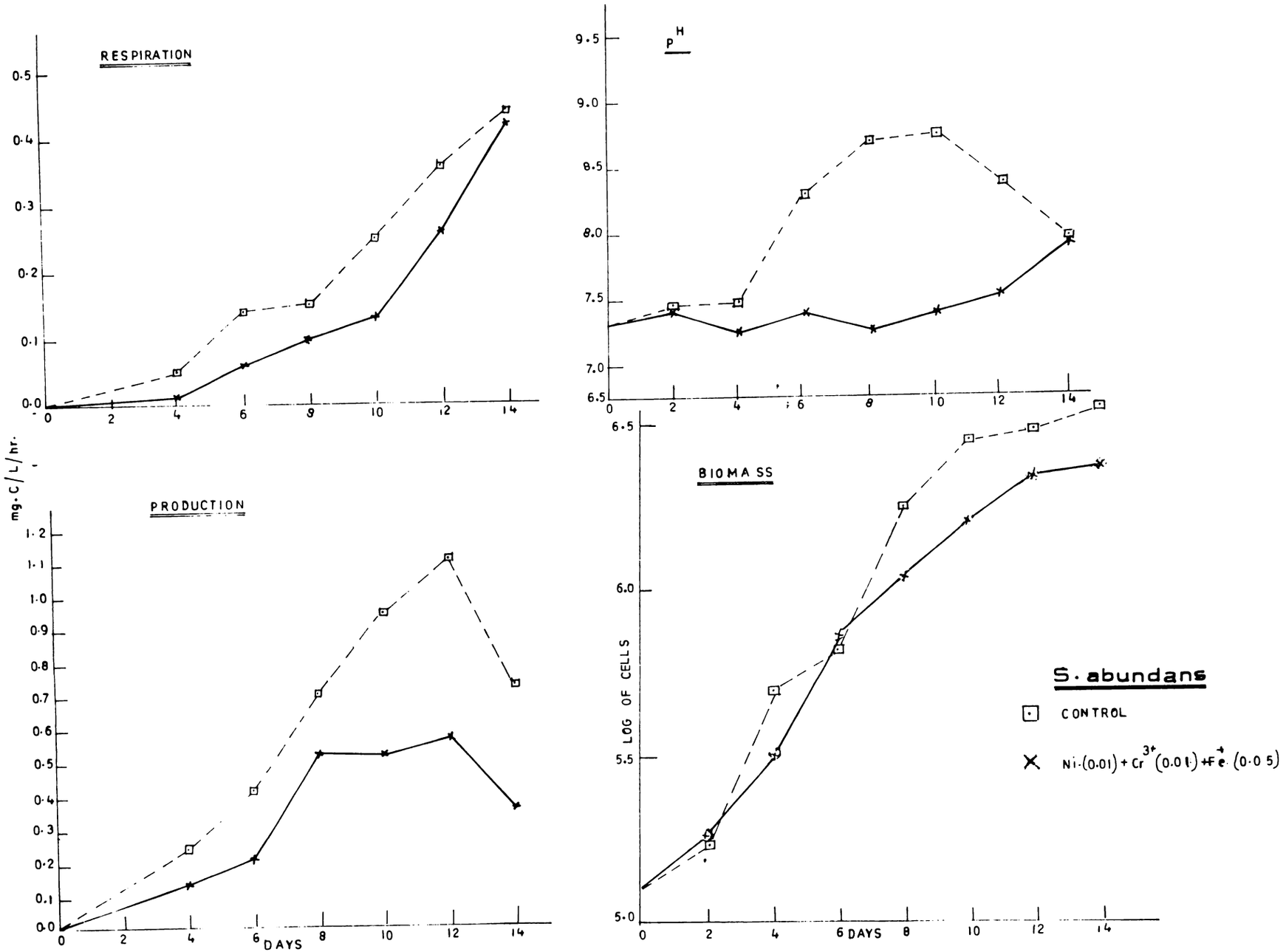

RESPIRATION
mg.C/L/hr.
PRODUCTION
DAYS
P H
BIOMASS
LOG OF CELLS
DAYS
S. abundans
CONTROL
Ni.(0.01) + Cr 3+ (0.01) + Fe+ (0.05)

Fig.68

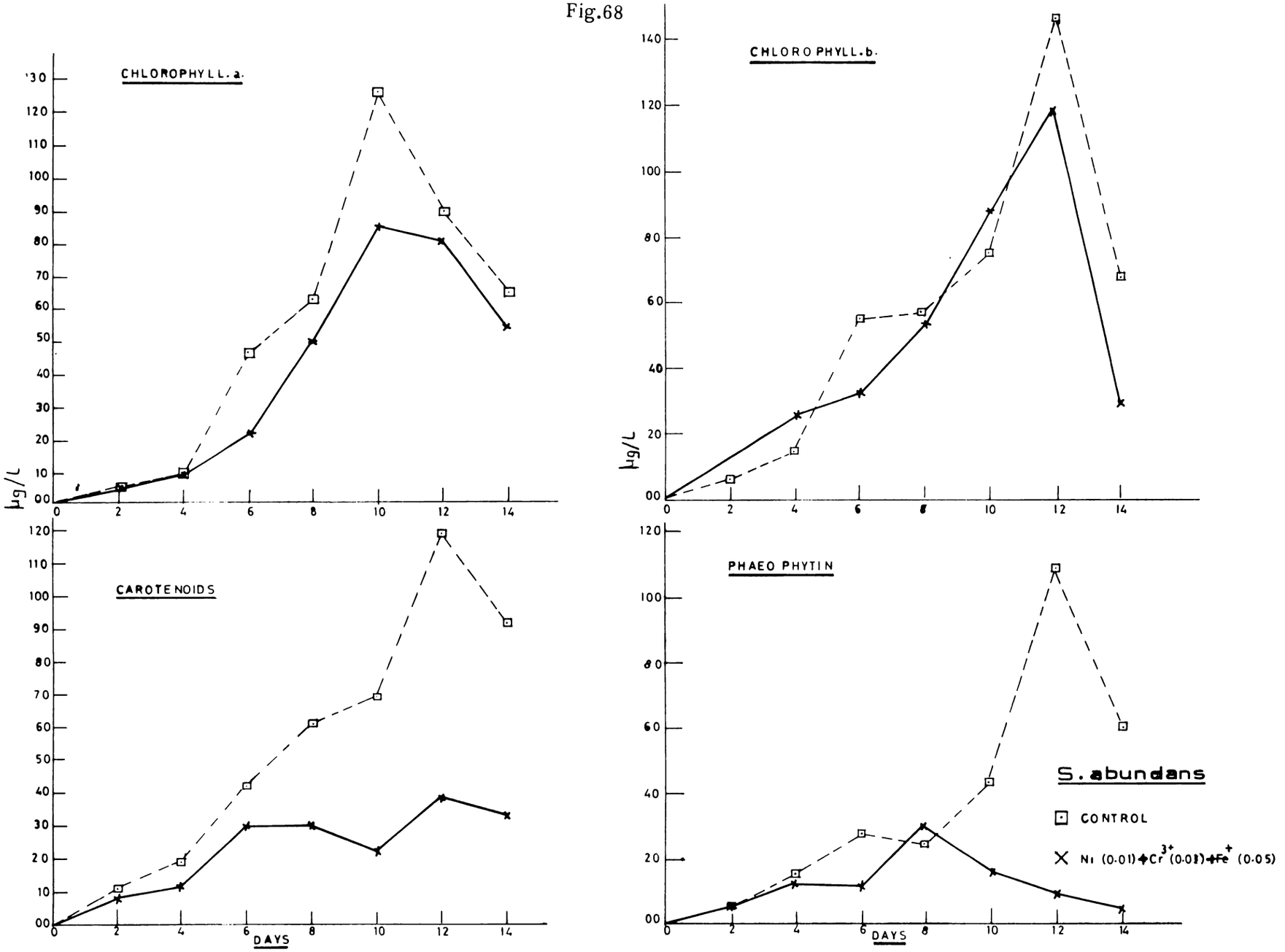
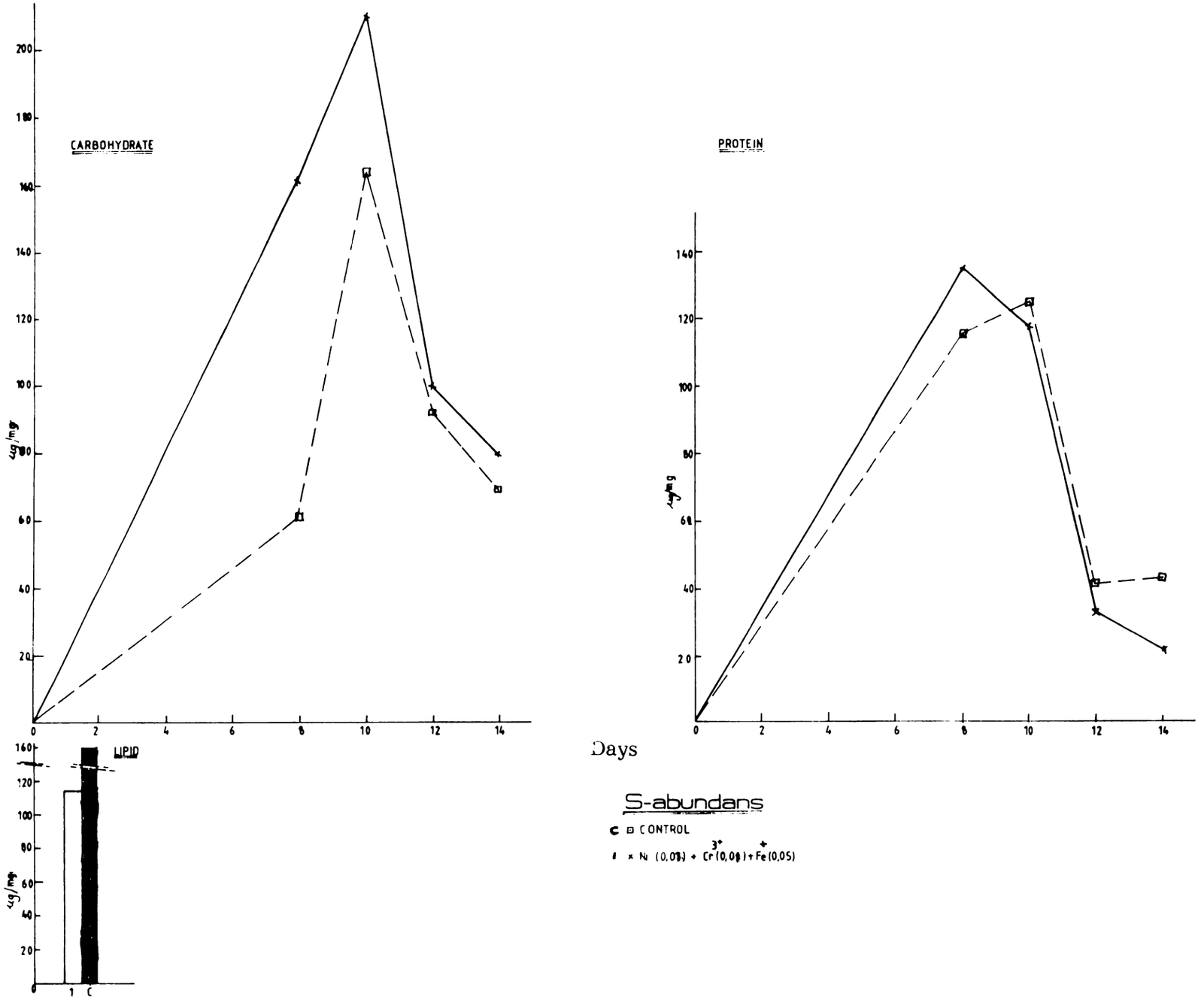

CHLOROPHYLL.a.
CHLOROPHYLL.b.
CAROTENOIDS
PHAEO PHYTIN
µg/L
DAYS
S. abundans
CONTROL
Ni (0.01)+Cr3+(0.01)+Fe+ (0.05)

Fig.69


CARBOHYDRATE
PROTEIN
LIPID
µg/mg
Days
S-abundans
C CONTROL
I × Ni (0,01%) + Cr3+ (0,01%) + Fe+ (0,05)

Effect of iron in combination with nickel and trivalent chromium in N. clausii

Concentration of metals (in ppm)	Treatment Number
Ni 0.6 + Cr^{3+} 0.8 + Fe^{+} 0.2	39

Biomass

The diatom was showing a marginal increase in biomass upto fourth day (Fig.70). Though it was similar to the control on the eighth day a decrease of 41% and 55% was noticed on the sixth day and at the end of growth phase.

Production

The net production of the diatom closely followed the control upto sixth day (Fig.70). On the eighth day a peak was observed and it was 52% more than the control. But at the end of growth phase it was 80% less than the control whereas the control was maximum at the end of growth. Similar to production pH was also more than the control upto eighth day.

Respiration

Respiration was showing a marginal increase on the fourth day but it was 40%, 70% and 28.5% less than the control on sixth, eighth and tenth day respectively.

Photosynthetic pigments

Chlorophyll-a

Chlorophyll-a was closely following the control upto fourth day for the treated sample but it was 17%, 8% and 38% less than the control on sixth, eighth and tenth day.

Chlorophyll-c

Similar to chlorophyll-a, chlorophyll-c, was less than the control with the aging of the culture (Fig.71). It was closely following the control on the second day but 61%, 62%, 15% and 68% decrease was observed on fourth, sixth, eighth and tenth day respectively.

Carotenoids

Carotenoids were less than the control through out the growth phase (Fig.71). It was 25%, 16% and 21% less than the control on second, fourth and sixth day. On the eighth day it was closely following the control but 18% decrease was observed at the end of growth phase.

Phaeophytin

Unlike other pigments, phaeophytin was more than the control upto eighth day though there was a marginal decrease initially (Fig.71). At the end of growth phase 73% decrease was observed. There was 20%, 17% and 58% increase on fourth, sixth and eighth day respectively.

Photosynthetic end products

Carbohydrate

The acid fraction and insoluble fraction of carbohydrate was showing an initial peak followed by sudden decrease with the aging of the culture.

The acid fraction was maximum on the fourth day (527%) and it was 73% more than the control on sixth day. There was 32% and 52% decrease on the eighth day and at the end of growth phase.

The alkali soluble fraction was less than the control through out the growth phase. It was 84%, 16%, 68% and 53% less than the control on fourth, sixth, eighth and tenth day respectively.

The insoluble fraction was showing a peak on the fourth day. The value being 60% more than the control. It was 29% more than the control at the end of growth phase. It was 15% and 45% less than the control on sixth and eighth day respectively.

Protein

There was an increasing trend in the protein content for the treated sample in the exponential growth phase (Fig.72). It was 143% and 165% more than the control on fourth and sixth day. Towards the end of growth it was 16% and 63% less than the control.

Unlike carbohydrate and protein,lipid content was reduced to a significant extent in the present of iron.

Phosphate uptake was found to be similar to the control whereas 20% decrease was observed in the case of nitrate uptake.

Fig.70

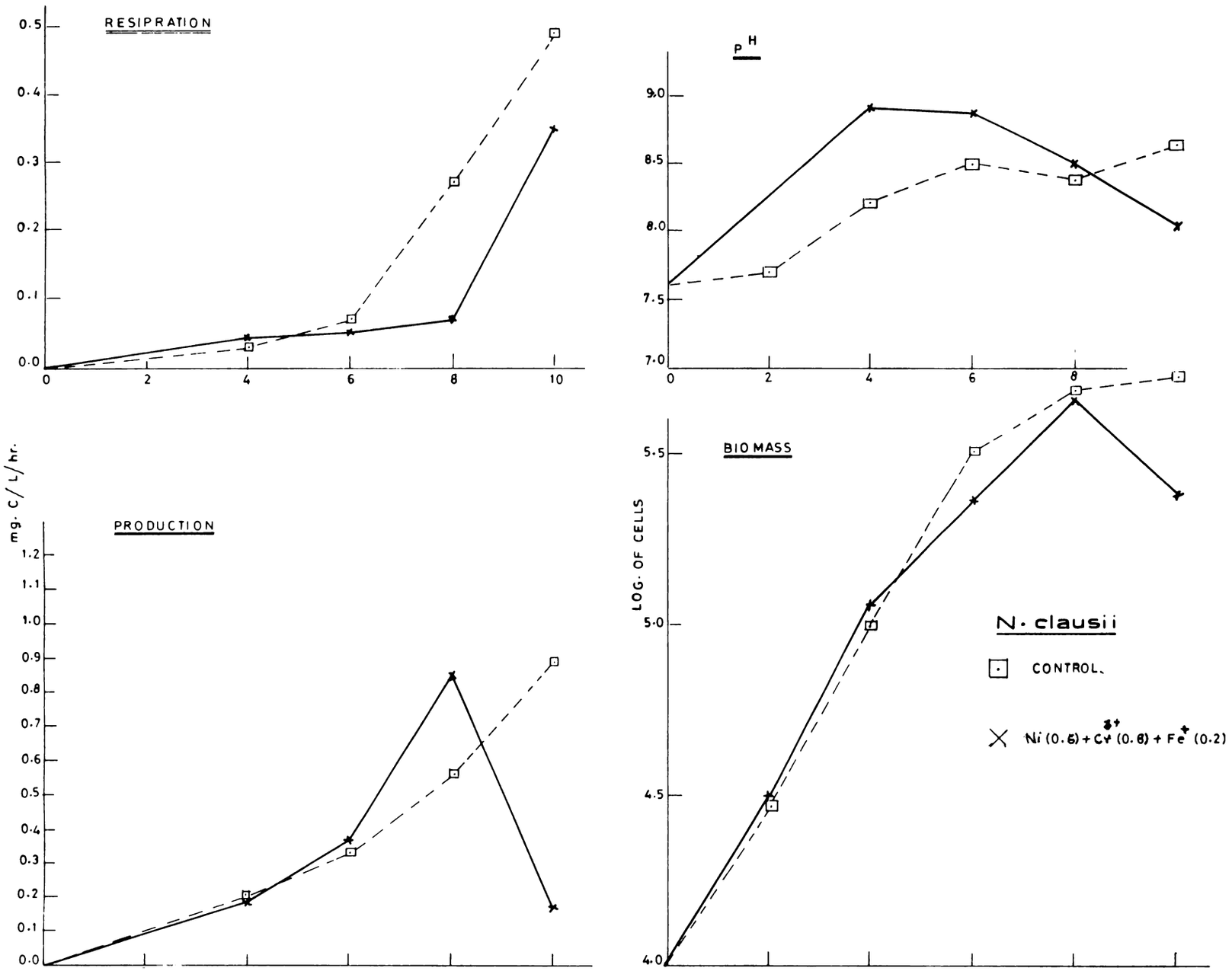
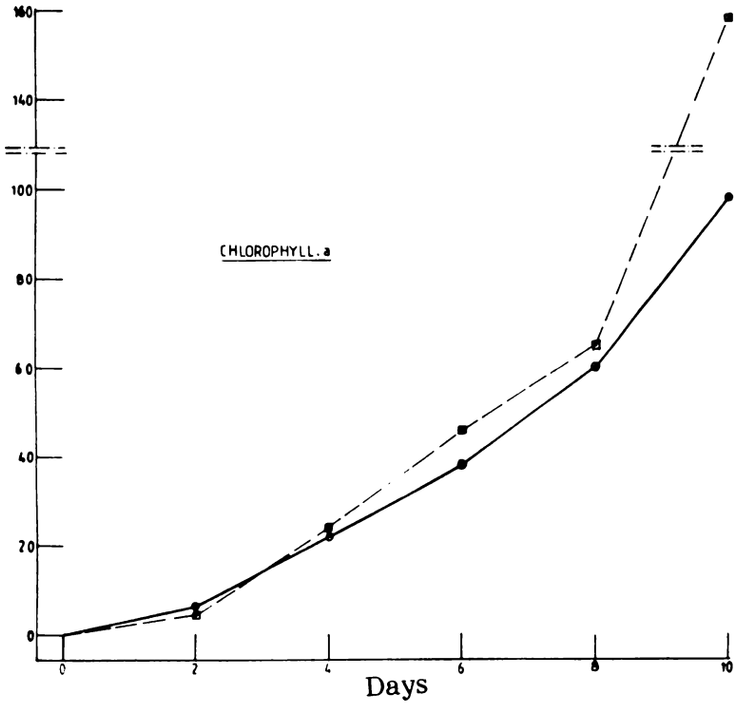
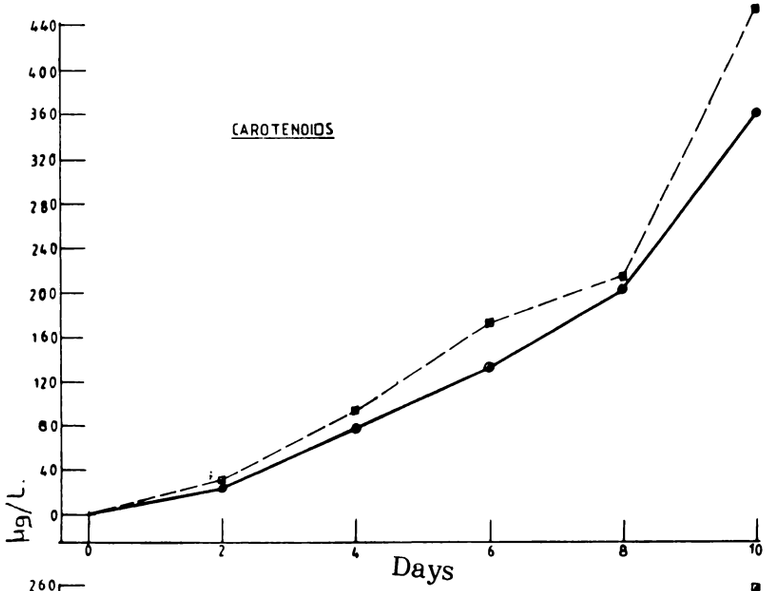
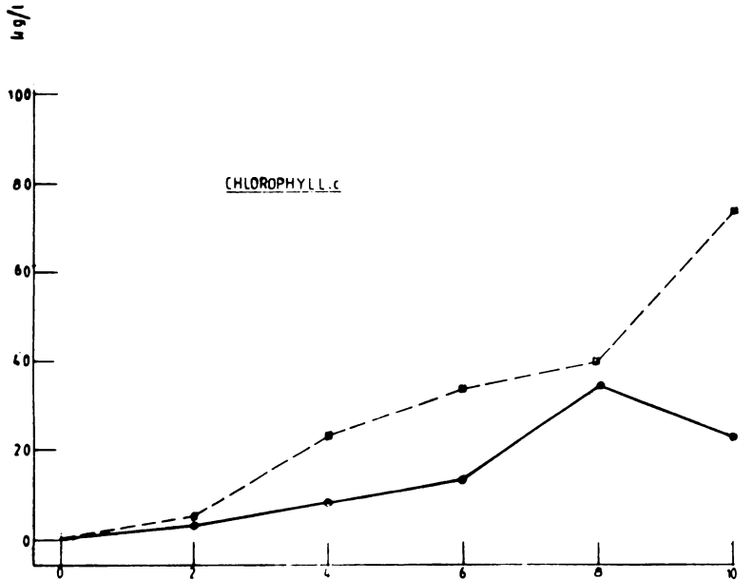
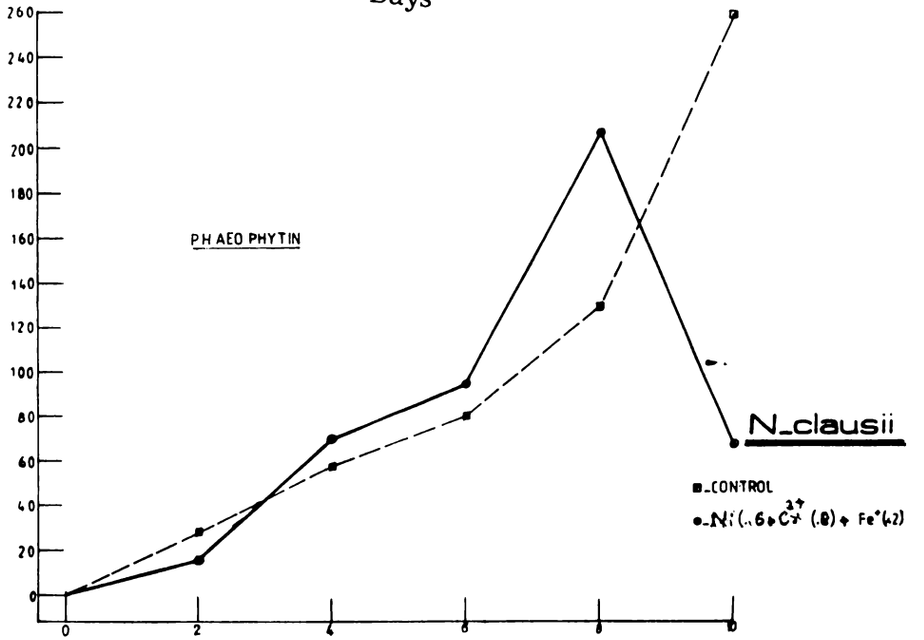

RESIPRATION
0.5
0.4
0.3
0.2
0.1
0.0
0
2
4
6
8
10
P H
9.0
8.5
8.0
7.5
7.0
0
2
4
6
8
mg. C / L / hr.
PRODUCTION
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
BIO MASS
LOG. OF CELLS
5.5
5.0
4.5
4.0
N. clausii
CONTROL.
Ni (0.6) + Cr3+ (0.6) + Fe3+ (0.2)

Fig.71


CHLOROPHYLL.a
Days


CAROTENOIDS
µg/L.
Days


µg/l
CHLOROPHYLL.c


PHAEO PHYTIN
N_clausii
■_CONTROL
●_Ni(.6+Cr3+(.8)+Fe+2)

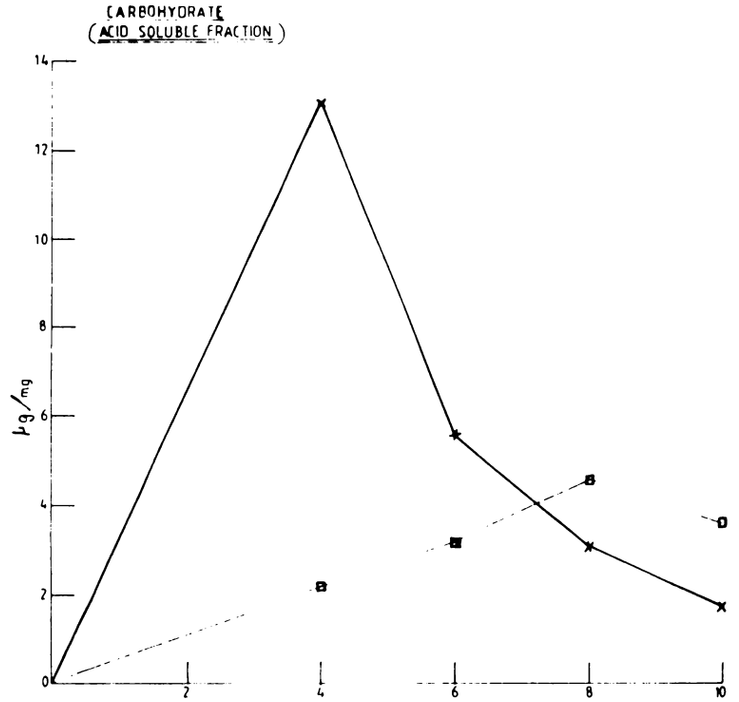
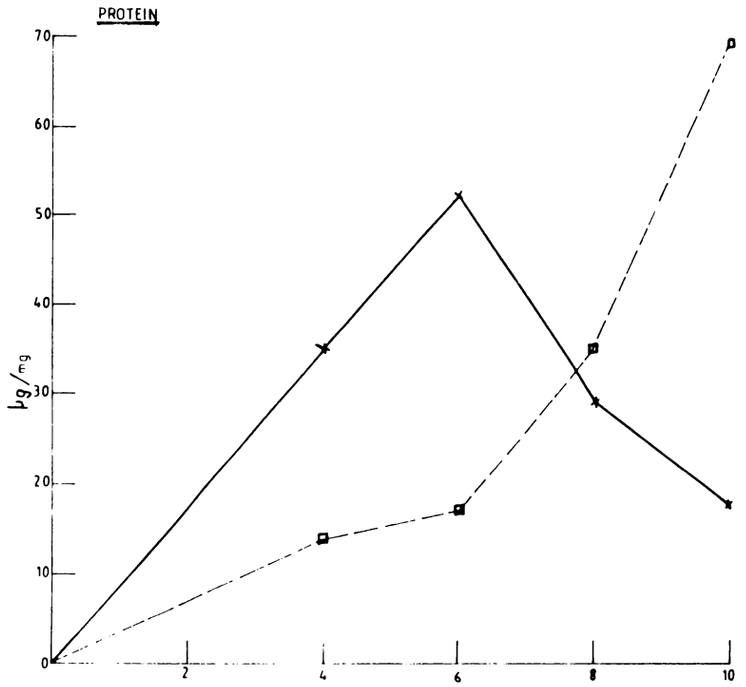
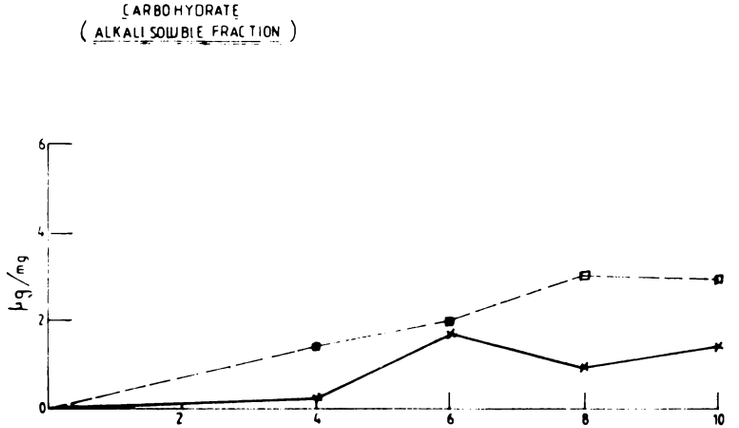


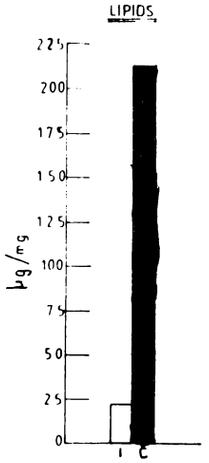
Fig.72

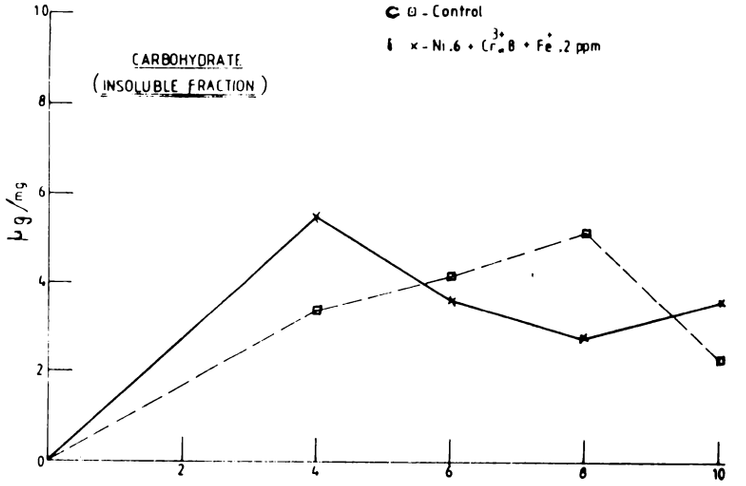


Days

N_clausii







5.2.4 The effect of iron in combination with nickel and hexavalent chromium in S. abundans

Concentration of metals (in ppm)	Treatment Number
Ni 0.01 + Cr^{6+} 0.05 + Fe 0.05	40

Biomass

The biomass was similar to the control on the second day but, 15% decrease was observed on the fourth day (Fig.73). There was 28%, 25%, 26% and 34% decrease on the eighth, tenth, twelfth and fourteenth day respectively.

Production

Production was less than the control through out the growth phase (Fig.73). It was 72% less than the control at the end of growth phase. On the fourth, eighth and twelfth day 37%, 41% and 50% decrease was observed. Similar trend was observed in the case of pH also.

Respiration

Respiration was showing a general decrease eventhough it was similar to the control on the eighth day (Fig.73). At the end of growth phase 66% decrease was observed on twelfth and fourteenth day and it was 95% and 58% less than the control on fourth and sixth day.

Photosynthetic pigments

Chlorophyll-a

Chlorophyll-a was closely following the control upto eighth day. On the tenth day though the treatment was maximum, it was 27% less than the

control. At the end of growth phase 32% decrease was noticed for the treated sample.

Chlorophyll-b

An increasing trend was observed upto tenth day for the treated sample but it was 20% less than the control on the twelfth day. It was 41% and 56% more than the control on eighth and tenth day respectively.

Carotenoids

Carotenoids were generally less than the control through out the growth phase eventhough it was similar to the control on the second day. At the end of growth phase it was 76% less than the control. On the sixth, eighth, tenth and twelfth day 38%, 48%, 33% and 61% decrease was noticed.

Phaeophytin

Phaeophytin was closely following the control upto sixth day. 34% and 16% increase was observed on the eighth and tenth day. But 63% and 82% decrease was noticed on twelfth and fourteenth day.

Photosynthetic end products

Carbohydrate

Well marked increase in carbohydrate was noticed through out the growth phase for the treated sample with a peak on the tenth day. It was 165% and 28% more than the control on the eighth and tenth day. At the end of growth phase 16% increase was noticed.

Protein

The protein was maximum on the eighth day and it was 17% more than the control (Fig.75). But it was 5%, 18% and 48% less than the control on tenth, twelfth and fourteenth day respectively. Thus eventhough there was an initial increase in protein, it was reduced with the aging of the culture.

Lipid content was 30% less than the control for treated sample.

Phosphate uptake was 55% less than the control whereas nitrate uptake was 40% less than the control.

Fig.73

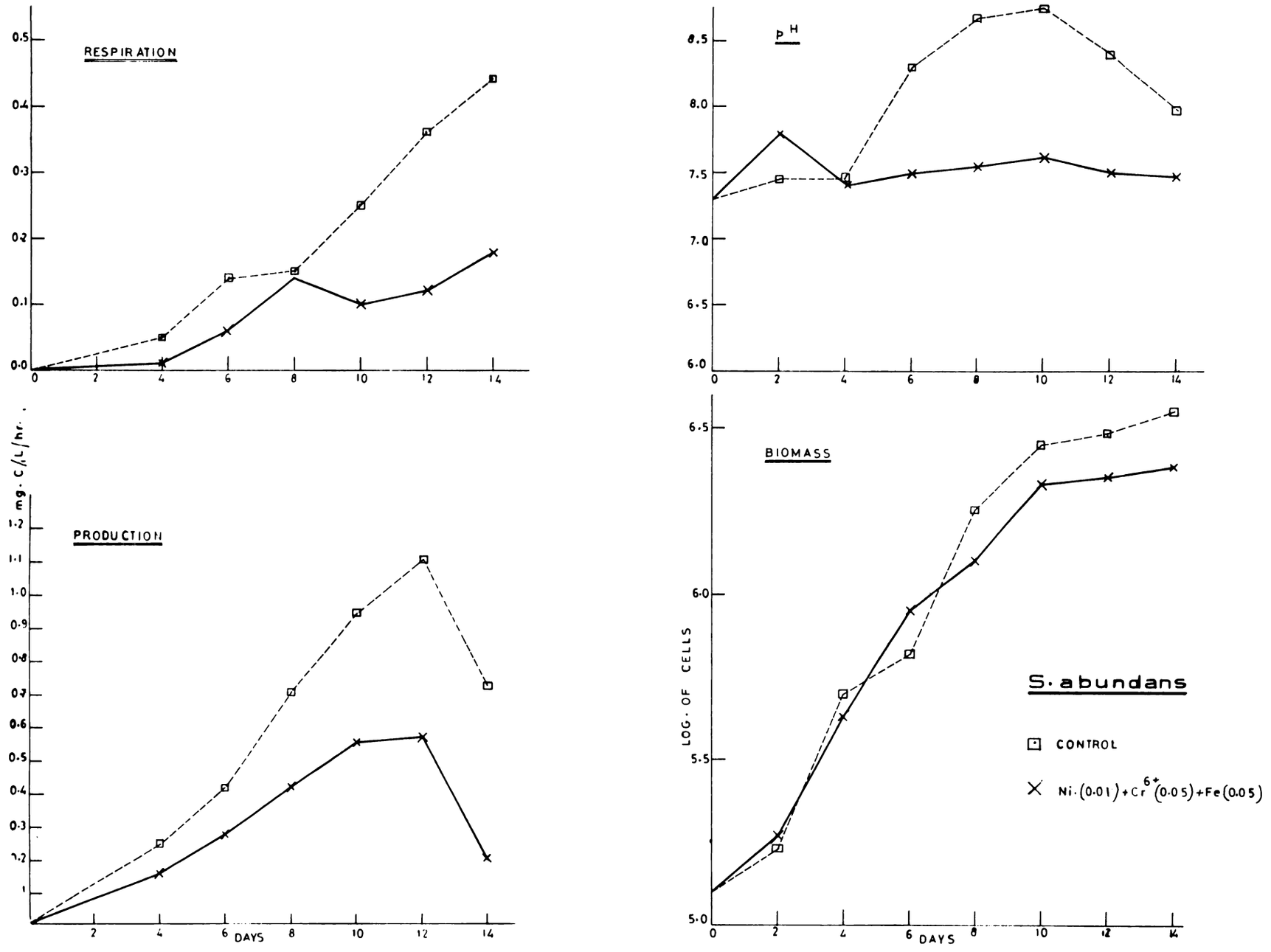

RESPIRATION
PRODUCTION
mg. C/L/hr.
DAYS
pH
BIOMASS
LOG. OF CELLS
DAYS
S. abundans
CONTROL
Ni.(0.01)+Cr 6+ (0.05)+Fe(0.05)

Fig.74

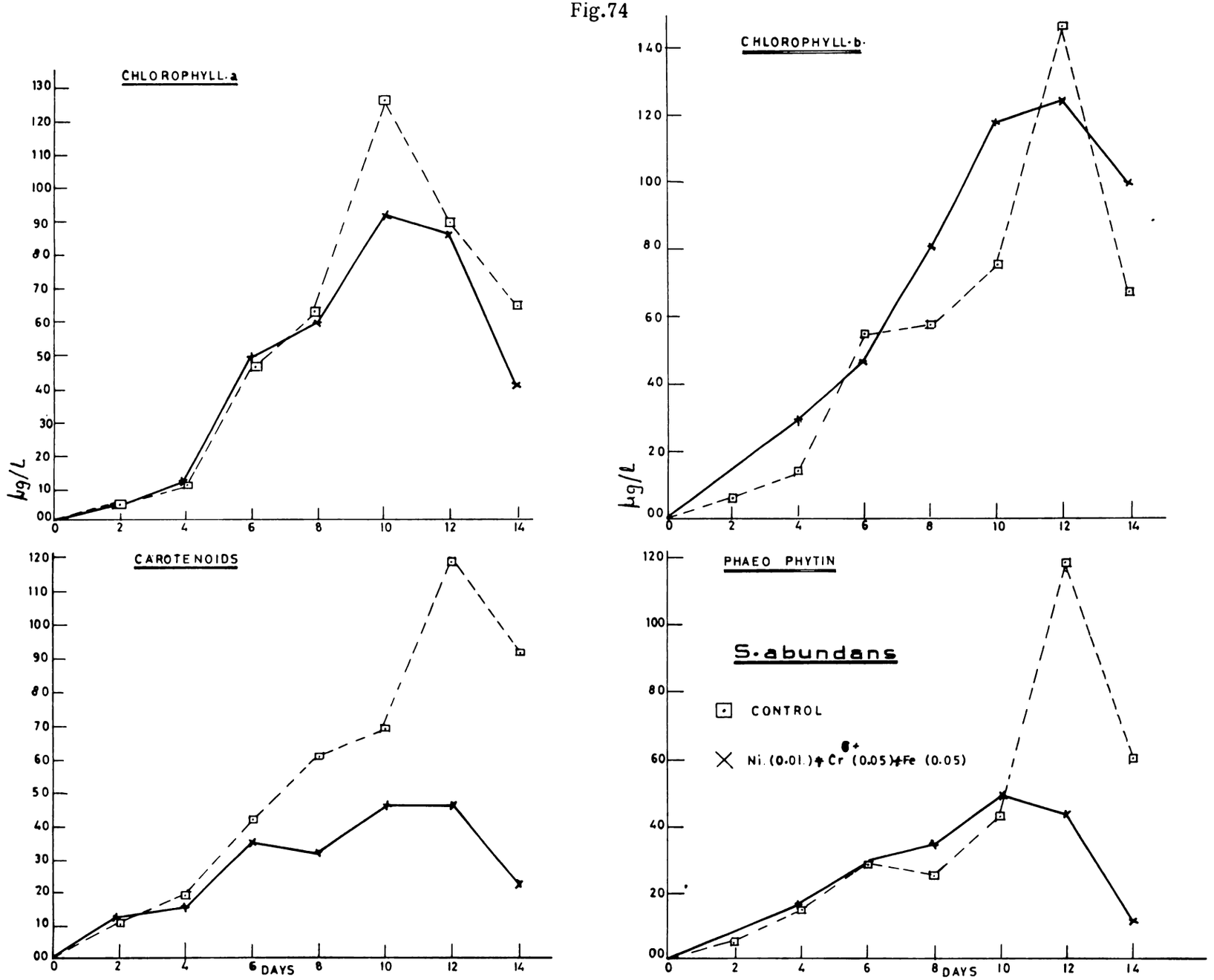
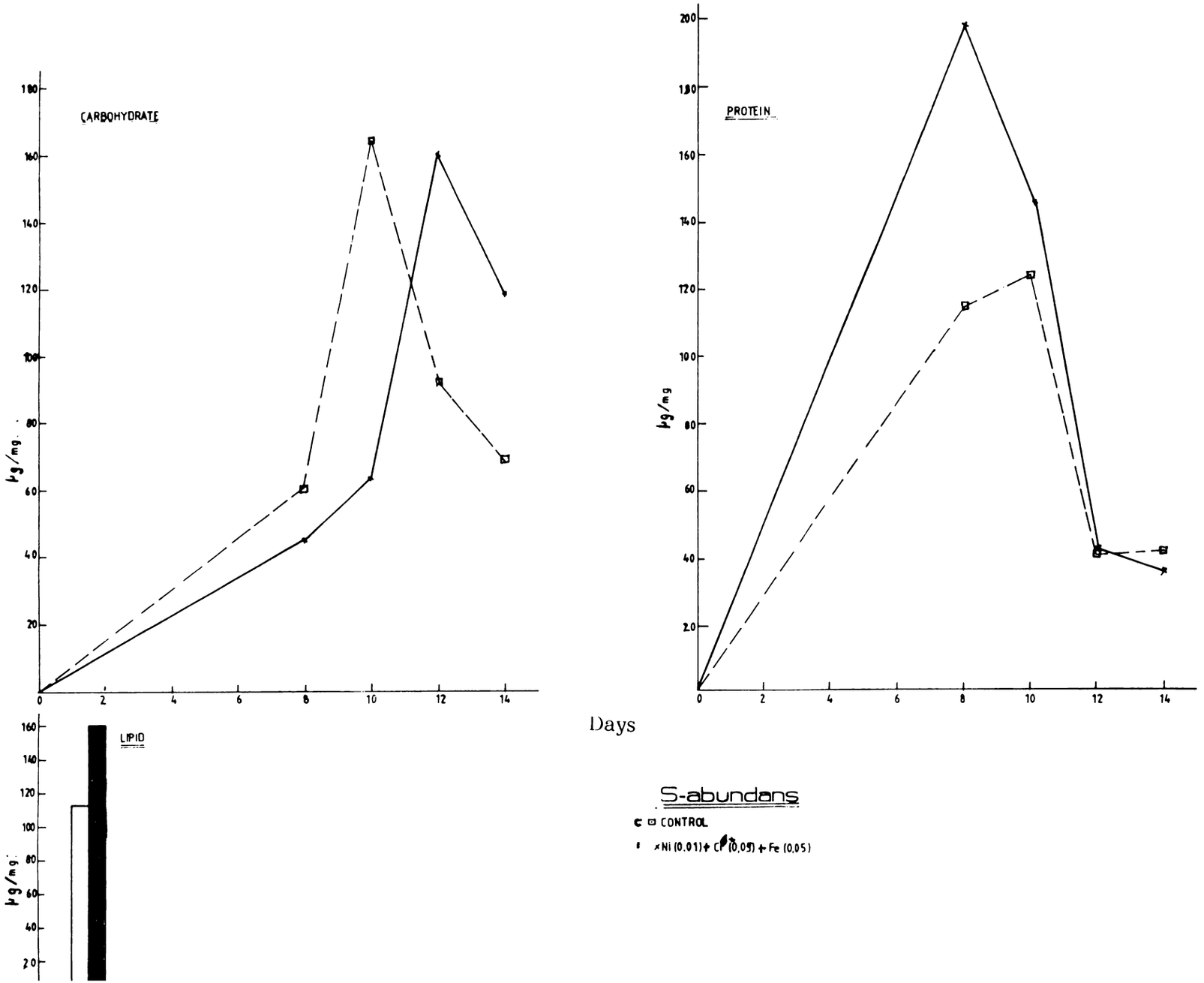

CHLOROPHYLL. a
CHLOROPHYLL-b
CAROTENOIDS
PHAEO PHYTIN
S. abundans
CONTROL
Ni. (0.01) + Cr 6+ (0.05) + Fe (0.05)
µg/L
DAYS

Fig.75


CARBOHYDRATE
PROTEIN
LIPID
µg/mg
Days
S-abundans
C CONTROL
Ni (0.01) + CP (0.05) + Fe (0.05)

Effect of iron in combination with nickel and hexavalent chromium in N. clausii

Concentration of metals (in ppm	Treatment Number
Ni 0.6 + Cr^{6+} 0.6 + Fe^{+} 0.2	41

Biomass

Biomass was less than the control through out the growth phase (Fig.76). It was 20%, 53%, 50% and 44% less than the control on fourth, sixth, eighth and tenth day respectively.

Production

The production was less than the control through out the growth phase, though there was a peak on the eighth day. It was 20% less than the control. On the fourth and sixth day, there was 59% and 50% decrease was observed. On the tenth day 60% decrease was noticed whereas the control was maximum on the tenth day. pH was also less than the control through out the growth phase.

Respiration

There was an initial increase in respiration on the fourth day and it was 38% more than the control (Fig.76). It was 54% and 71% less than the control on sixth and eighth day. At the end of growth phase it was 27% less than the control.

Photosynthetic pigments

Chlorophyll-a

Chlorophyll-a was less than the control through out the growth phase. Though it was similar to the control on the second day 56% decrease was noticed on fourth and sixth day. At the end of growth phase there was 51% decrease, whereas the control was maximum on the tenth day.

Chlorophyll-c

Similar to chlorophyll-a, chlorophyll-c, was relatively less than the control. It was 62% and 73% less than the control on sixth and eighth day. At the end of growth phase it was 70% less than the control.

Carotenoids

A general trend of decrease was noticed in the case of carotenoids (Fig.77). It was 42% and 55% less than the control on fourth and sixth day. On the tenth day 37% decrease was observed for the treated samples.

Phaeophytin

Phaeophytin was closely following the control upto fourth day. On the sixth day it was 32% less than the control. On the eighth day eventhough it was maximum, it was 30% less than the control followed by an abrupt decrease of 83% at the end of growth phase.

Photosynthetic end products

Carbohydrate

Significant increase was noticed in the acid soluble fraction of carbohydrate (Fig.78). It was maximum on the fourth day. On the sixth day it

was 256% more than the control. But towards the end of growth phase it was 25% and 50% less than the control.

The alkali soluble fraction of carbohydrate was less than the control through out the growth phase. It was 56%, 43%, 71% and 63% less than the control on fourth, sixth, eighth and tenth day respectively.

The insoluble fraction was more than the control on the fourth day and at the end of growth phase. It was showing the peak (159%) on the fourth day and 124% increase was noticed on the tenth day. But it was 25% and 27% less than the control on sixth and eighth day respectively.

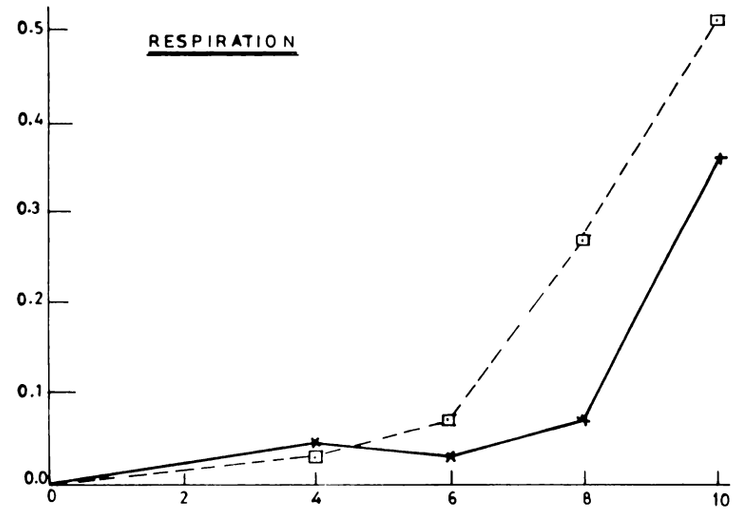
Protein

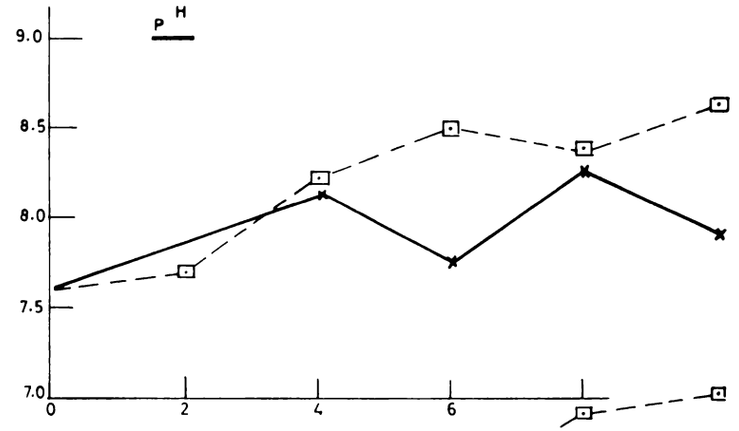
Protein content of the diatom was similar to the control on the fourth day and it was maximum on the sixth day and it was 144% more than the control. This was followed by an abrupt decrease on the eighth and tenth day. The values being 12% and 74% less than the control.

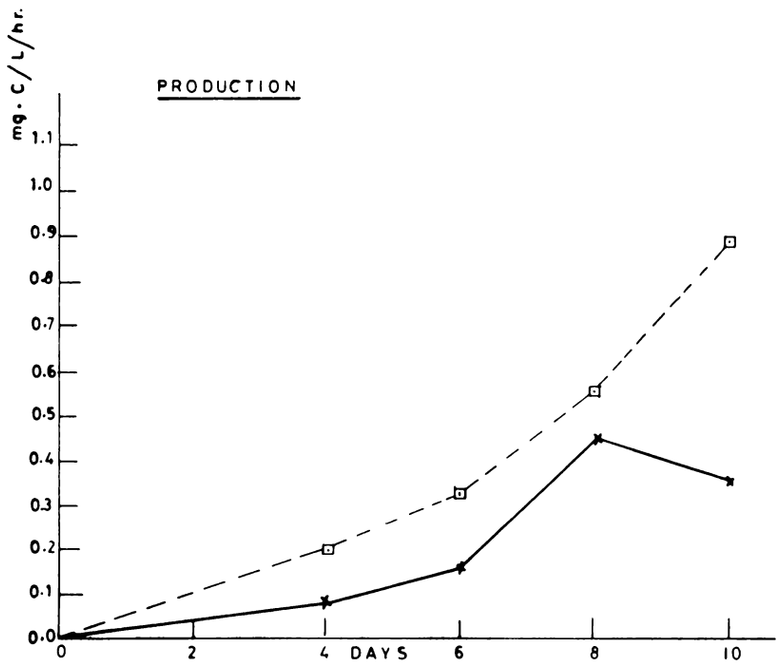
Lipid content of the algae was 88% less than the control.

Unlike production and respiration, the nutrient uptake was found to be more thant the control. Phosphate uptake was 22% more than the control.

Fig.76


RESPIRATION
0.5
0.4
0.3
0.2
0.1
0.0
0
2
4
6
8
10


PH
9.0
8.5
8.0
7.5
7.0
0
2
4
6


mg·C/L/hr.
PRODUCTION
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0
2
4
DAYS
6
8
10

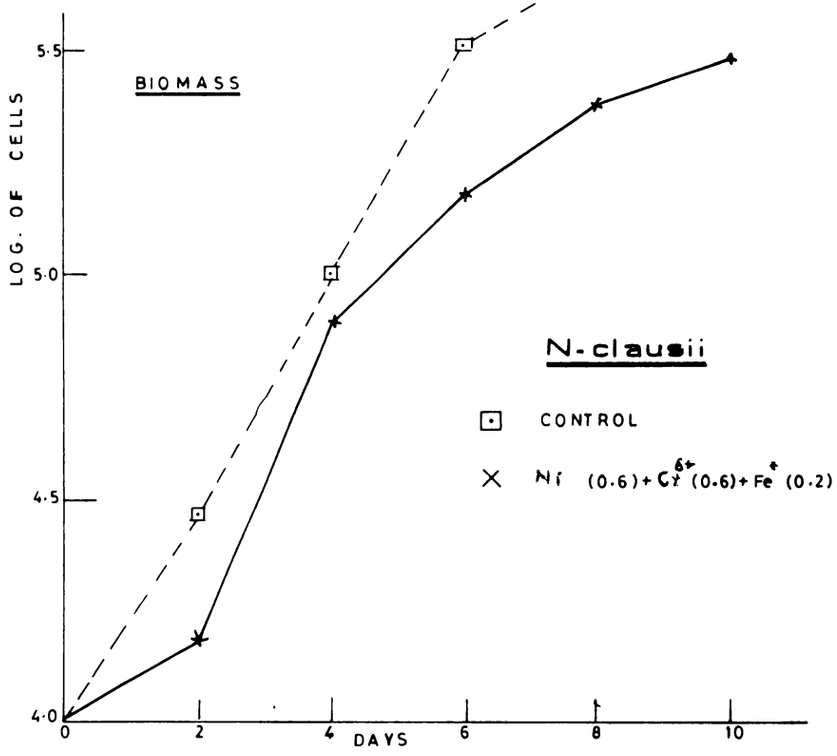
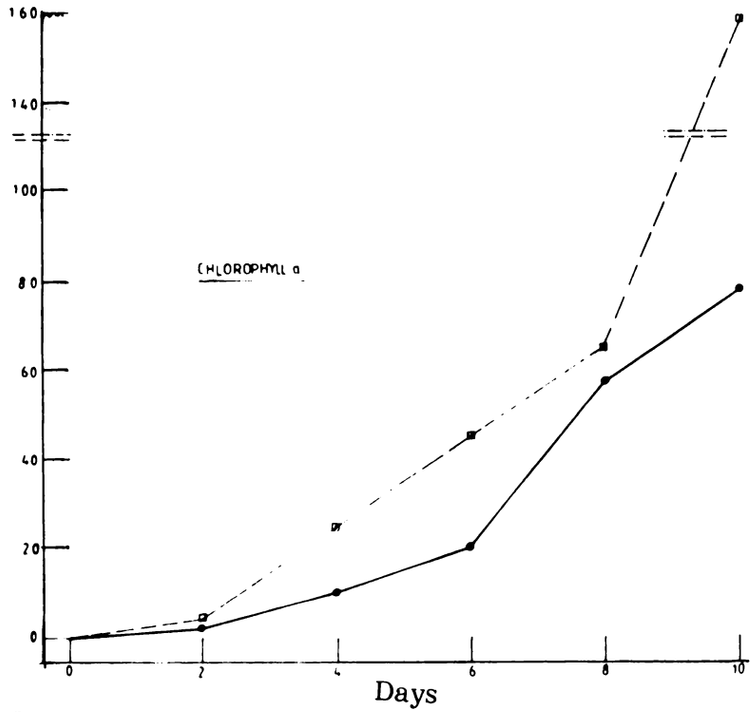
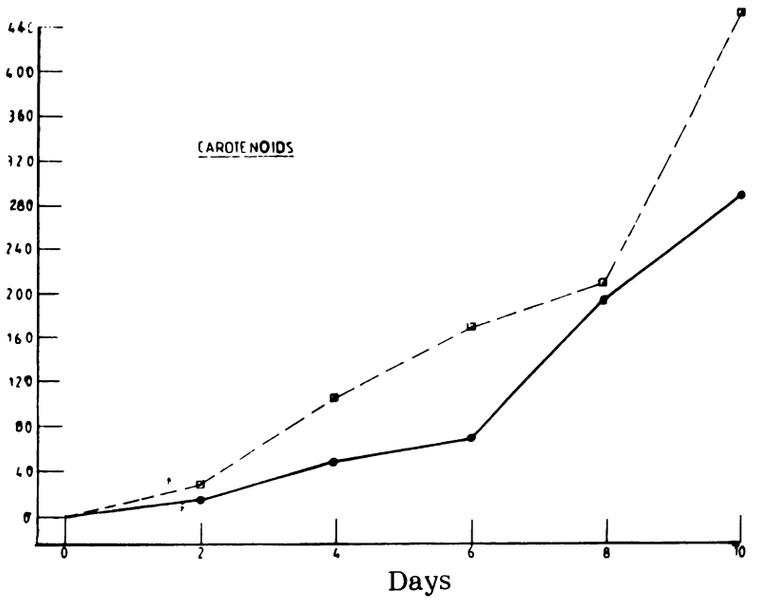
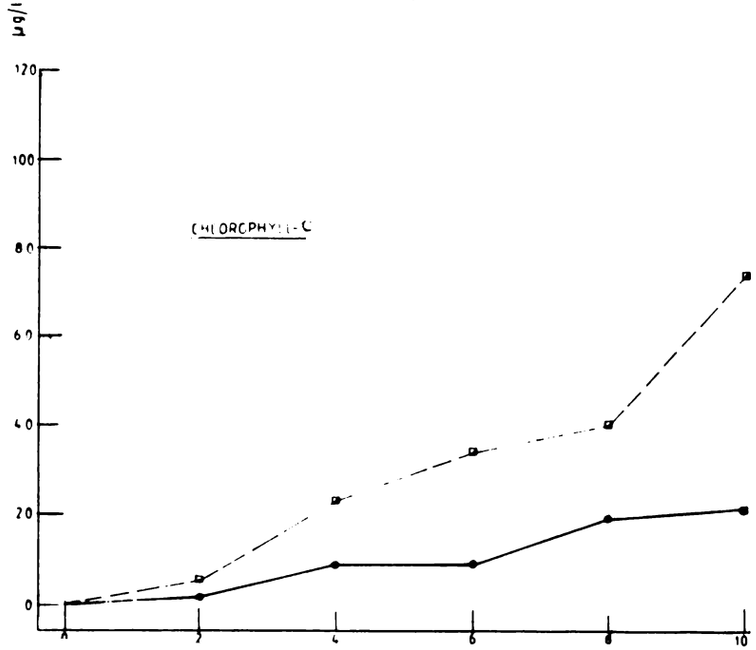

BIOMASS
LOG. OF CELLS
5·5
5·0
4·5
4·0
0
2
4
DAYS
6
8
10
N-clausii
CONTROL
Ni (0.6) + Cr6+ (0.6) + Fe+ (0.2)

Fig.77


CHLOROPHYLL a
Days


CAROTENOIDS
Days


µg/l
CHLOROPHYLL-C

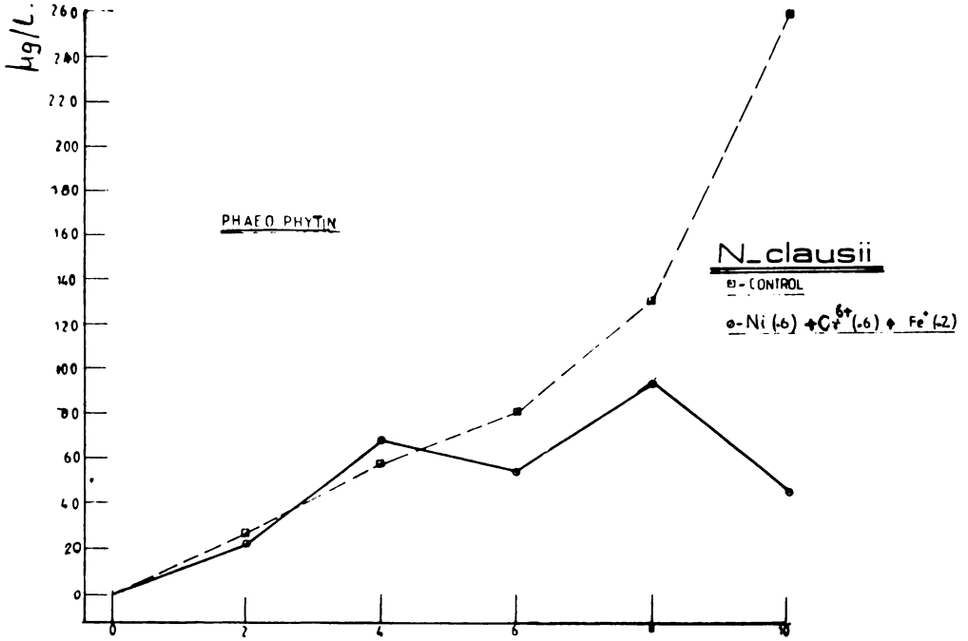
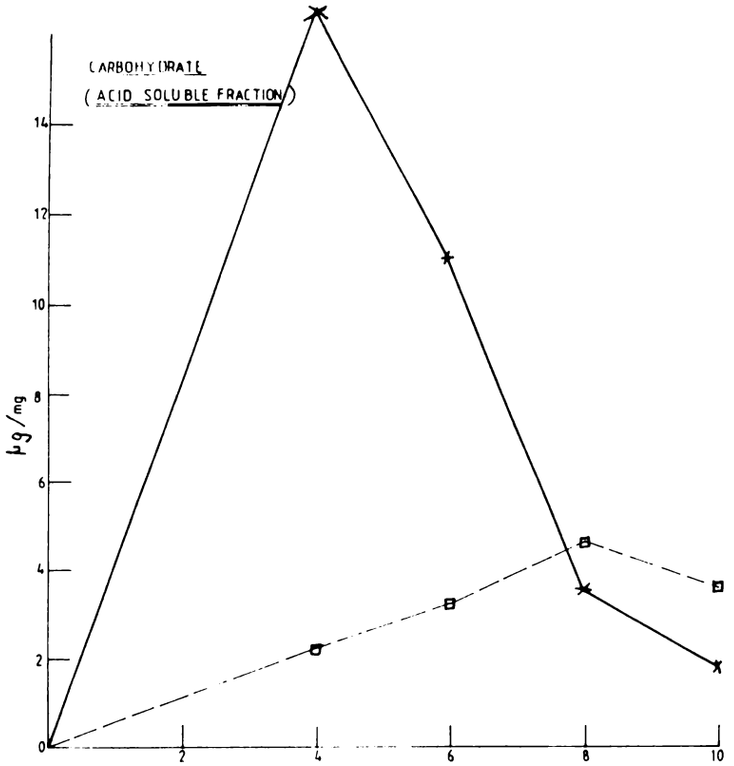
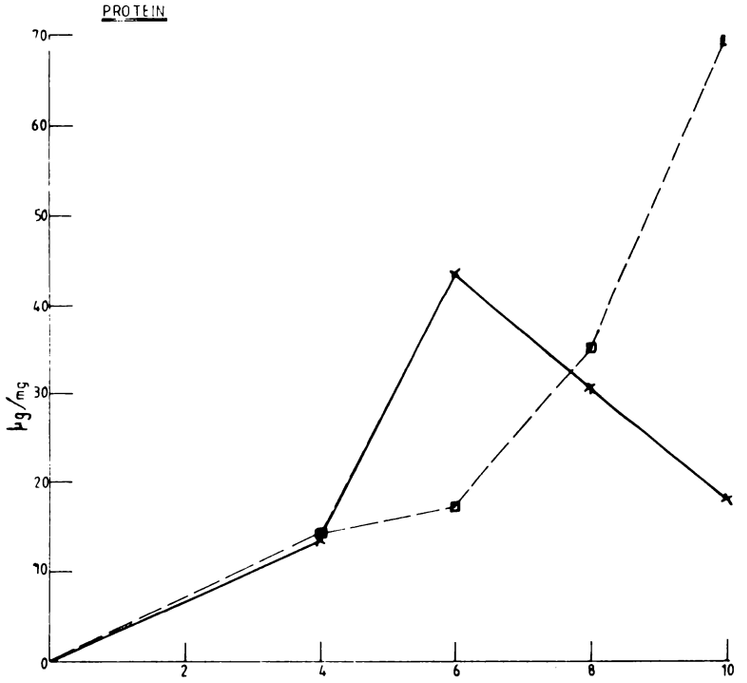

µg/L.
PHAEO PHYTIN
N_clausii
⊡ - CONTROL
o - Ni (.6) ✦ Cr6+ (.6) ♦ Fe2 (.2)

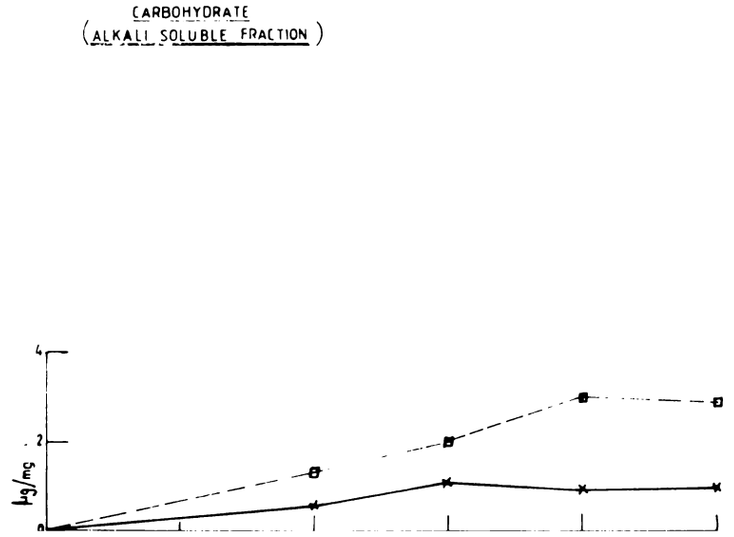
Fig.78

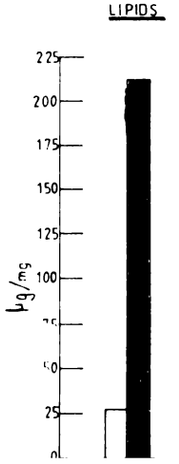


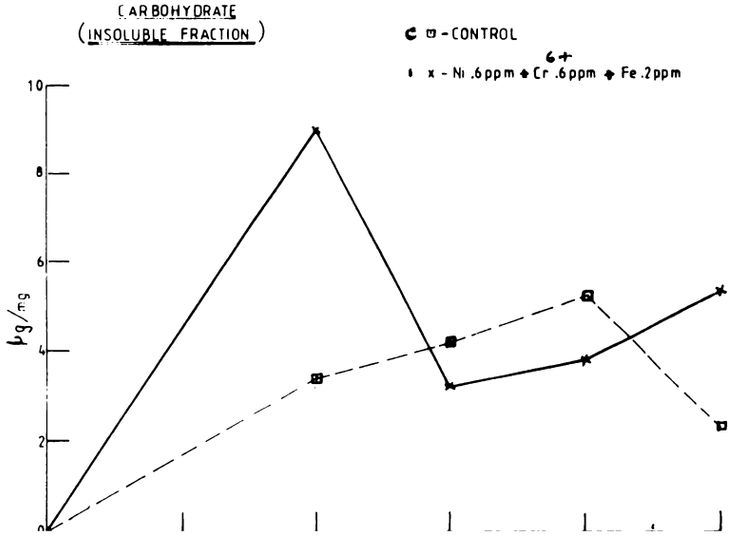
Days



N_clausii







DISCUSSION

The presence of iron was found to change the toxic effect of metals considerably. Harvey (1939) reported that growth was stopped in diatom Nitzschia closterium due to lack of iron. Goldberg (1952) in his studies on iron assimilation by marine diatoms reported that iron added must be in particulate form before it can be utilized by marine plants. Tranter and Newell (1963) and Shapiro (1967) have reported that iron in soluble form though chemically reactive was not available to organisms. The role of iron in marine ecology is receiving increasing attention in view of recent data showing that it may limit primary production in oceans. (Martin and Fitzwater, 1988).

In the present investigation iron was found to mitigate the effect of two metals in combination especially in the photosynthetic end products and production. In general iron enhanced the protein and carbohydrate contents considerably in all selected combinations. Thus the reversal of toxicity in algae in the presence of iron was observed.

From studies on the distribution of iron in North east pacific, it was reported by Martin et al., (1988) that iron was necessary for phytoplankton growth in the open sea. Hudson and Morel (1989) reported that iron is essential for nitrate utilization, chlorophyll biosynthesis and numerous other cellular functions in phytoplankton. Little is known about the mechanism of iron transport in marine photoplankton.

In S. abundans combination of cobalt and trivalent chromium in the presence of iron (treatment 34) reduced the production and respiration. However, when compared with the two metal combination of cobalt and trivalent chromium

there was improvement in the production. Combination of cobalt, trivalent chromium and iron showed wellmarked antagonism in the case of photosynthetic end products resulting in the increase in carbohydrate and protein contents. Thus the presence of iron reduced the toxicity of combination of two metals such as cobalt and trivalent chromium in fresh water forms.

In N. clausii the presence of iron in combination with cobalt and trivalent chromium (treatment 35) improved the production whereas the chlorophyll-c pigment was reduced. Antagonism was noticed in the case of photosynthetic end products whereas in the case of other pigments such as chlorophyll-a, carotenoids and phaeophytin there was not much change in the presence of iron.

Rythur and Guillard (1959) found that phytoplankton growth was enhanced by the addition of combination of trace elements and iron. In a few instances the reversal of metal toxicity to phytoplankton has been reported by Anderson and Morel (1978). Foster and Morel (1982) found that iron in combination with cadmium reduced the cadmium toxicity in Thalassiosira weissflogii. It was found that iron did not release cadmium from the cells but did prevent further uptake. Thus the presence of iron resulted in the reversal of toxicity.

In S. abundans the combination of cobalt, hexavalent chromium and iron (treatment 36) stimulated the production in the first half followed by sudden decrease with the aging of the culture. But the net production was improved in the first half when compared with the combination of cobalt and hexavalent chromium. In the case of pigments, synergism was noticed resulting in the decrease of pigments especially carotenoids. Well marked antagonism

was noticed in the case of photosynthetic end products such as carbohydrate and protein. However, lipid showed a reduction in value.

In N. clausii the combination of cobalt, hexavalent chromium and iron (treatment 37) was having synergistic effect on algae resulting in the reduction in the respiration and photosynthetic pigments. In the case of end products of photosynthesis there was significant increase in N. clausii in the presence of iron. It was reported by Rythur and Kramer (1961) that the iron requirement in coastal phytoplankton is much higher than in ocean species, a reflection of the higher iron concentration observed in coastal waters compared with the open ocean. According to Reuter and Ades (1987) iron was having a crucial role in the bioenergetics of carbon and nitrogen metabolism because it is required for the synthesis of chlorophyll and in the reduction of nitrate.

The combination of nickel, trivalent chromium and iron (treatment 38) showed significant increase in the photosynthetic end products especially carbohydrate in S. abundans. Eventhough the lipid content was less than the control, it was improved in the presence of iron when compared with the two metal combination of nickel and trivalent chromium. The stimulation of algal growth in deep sea water by added iron has been interpreted by Jackson and Morgan (1978) as the alleviation of growth inhibition resulting from ambient toxic level of copper. They found that chelators reduced cupric ion activities in sea water to nontoxic level.

Studies on the copper-manganese interaction by Sunda et al. (1981) suggested that both copper and manganese compete for the same enzymatic site. So when manganese was added to cultures treated with copper, its effect

can be reversed. Thus it was proved that growth of phytoplankton which was inhibited by the addition of copper was further enhanced or stimulated by addition of chelating agents such as EDTA, $Mncl_2$ and $Fecl_3$.

Significant changes were not observed in the case of biomass in N. clausii in the presence and in the absence of iron. Combination of nickel, trivalent chromium and iron (treatment 39) showed synergism resulting in the decrease in the pigments especially chlorophyll-c. Whereas antagonism was noticed in the case of end products resulting in the increase in the carbohydrate and protein.

Studies of Harrison and Morel (1986) on the response of marine diatom Thalassiosira species to iron stress found that growth was reduced, there was decrease in cellular iron followed by increasing rates of uptake, when iron was added to the medium. Hudson and Morel (1989) demonstrated that phytoplankton adsorb iron on cell surfaces and it was demonstrated that cell surface bound iron was directly transported into the cells. This type of transport was referred to as "internalization". According to Passow et al. (1961) the primary site of action of toxic metal is usually the cell membrane. He also suggested that the physiological antagonism between cadmium and iron was mediated by metal uptake mechanism.

Combination of nickel , hexavalent chromium and iron (treatment 40) reduced the biomass in S. abundans whereas in the absence of iron growth was improved. The presence of iron in combination with nickel and hexavalent chromium had no significant change in the pigments but the photosynthetic end products such as carbohydrate and protein was enhanced.

Murphy et al. (1984) suggested that possible reason for the reduction of the toxic effect of copper in Thalassisira weissflogii was due to the uptake of iron by the cell surface enzymes. This resulted in the antagonistic interaction between copper and iron on the growth of marine diatom. Studies of Jones et al. (1987) on the inhibition of iron uptake by certain divalent ions such as manganese cobalt, copper, nickel and zinc show that there are certain cell surface enzymes.

Combination of nickel, hexavalent chromium and iron (treatment 41) was synergistic in N. clausii resulting in the decrease in the pigment content of the algae but the combination of nickel and hexavalent chromium alone also improved the pigment content of the algae, especially chlorophyll-c. There was an initial increase in the protein content of the three fractions of carbohydrate. The acid soluble and insoluble fraction was stimulated whereas alkali fraction was inhibited.

Thus it was concluded that in the case of biomass, combinations of iron was having an additive effect on these species of phytoplankton. In most of the combinations, photosynthetic end products were stimulated whereas photosynthetic pigments especially chlorophyll-c was inhibited. Investigation is to be made for decrease in lipid in the post graduate programme.

Statistical analysis of the results was carried out to bring about the significance of metals interaction in the presence of iron. Table 5, 6.

In S. abundans, chlorophyll-a was significant for all treatments of iron. It was maximum for the combination of nickel, trivalent chromium and iron.

The combination of cobalt, trivalent chromium and iron was significant in the case of pigments and end products of photosynthesis. But respiration was found to be not significant.

In N. clausii the production was highly significant in the combination of cobalt, trivalent chromium and iron. End products of photosynthesis was also found to be significant between concentrations and days at 5% level. But, of the three fractions of carbohydrate, the alkali soluble fraction was not significant.

The combination of cobalt, hexavalent chromium and iron produced significant effect on almost all parameters of productivity except respiration in S. abundans. But protein was found to be significant only between days. In N. clausii, the combination of cobalt, hexavalent chromium and iron was highly significant in the case of production, protein, chlorophyll-a and phaeophytin. But respiration and pigments such as carotenoid and chlorophyll-c was found to be not significant. All fractions of carbohydrate except the alkali fraction was highly significant between concentrations and days. The alkali fraction was significant only between days.

The combination of nickel and trivalent chromium in the presence of iron produced significant effect in the case of pigments, carbohydrate and production in S. abundans. But protein and respiration was found to be not significant. In N. clausii the combination of nickel, trivalent chromium and iron was not significant in the case of biomass, respiration and protein. Of the pigments, chlorophyll-c and carotenoids were significant only between days.

The combination of nickel, hexavalent chromium and iron was significant in S. abundans when the pigments and end products of photosynthesis was concerned. But the protein was significant only between days. The respiration was found to be not significant. In N. clausii the combination of nickel, hexavalent chromium and iron was not significant in the case of production. Respiration showed significant effect only between days. Biomass and pigments such as chlorophyll-a and phaeophytin were highly significant.

Thus the combination of metals in the presence of iron produced significant effect on pigments and carbohydrate.

Table 5. Results of the significance of the variance ratio between concentration and between days in S. abundans

Sl. No.	Metals employed	Selected parameters																						
		pH		Biomass		Production		Respiration		Chlorophyll-a		Chlorophyll-b		Carotenoids		Phaeophytin		Carbohydrate		Protein		Lipids		
		1	11	1	11	1	11	1	11	1	11	1	11	1	11	1	11	1	11	1	11	1		
1	Co^{+} Cr^{3+} + Fe	a	a	a	a	b	b	NS	a	b	a	a	a	b	a	b	b	b	b	NS	b	a		
2.	Ni^{+} Cr^{3+} + Fe	a	a	a	a	a	a	NS	a	b	a	a	b	a	b	a	b	a	b	NS	b	a		
3.	Co^{+} Cr^{6+} + Fe	a	a	a	a	a	b	NS	a	a	b	b	b	b	b	b	a	b	b	NS	b	a		
4.	Ni^{+} Cr^{6+} + Fe	a	a	a	a	a	a	NS	a	a	b	a	a	a	a	a	a	a	a	NS	b	a		

a $P < 0.01$
b $P < 0.05$
NS Non Significant

1 Concentration
11 Days

Table 6. Results of the significance of the variance ratio between concentration and between days in N. clausii

Sl. No.	Metals employed	Selected parameters																									
		pH		Biomass		Production		Respiration		Chlorophyll-a		Chlorophyll-c		Carotenoids		Phaeophytin		Carbohydrate fraction(1)		Carbohydrate fraction(2)		Carbohydrate fraction(3)		Protein		Lipids
		1	11	1	11	1	11	1	11	1	11	1	11	1	11	1	11	1	11	1	11	1	11	1	11	1
1.	$Co+Cr^{3+}+Fe$	NS	a	NS	NS	a	a	NS	a	a	a	NS	a	NS	a	NS	a	a	a	NS	a	a	a	a	a	a
2.	$Ni+Cr^{3+}+Fe$	NS	a	NS	NS	a	a	NS	a	a	a	NS	a	NS	a	NS	a	a	a	NS	a	a	a	NS	NS	a
3.	$Co+Cr^{6+}+Fe$	NS	a	NS	NS	a	a	NS	a	a	a	NS	a	NS	b	NS	b	a	a	NS	a	a	a	NS	NS	a
4.	$Ni+Cr^{6+}+Fe$	NS	a	a	a	a	a	NS	a	a	a	NS	b	NS	b	NS	a	a	a	NS	a	a	a	a	a	a

a $P < 0.01$

b $P < 0.05$

NS Non Significant

1 Concentration

11 Days

5.3 EFFECT OF COMBINATION OF THREE METALS ON *N. CLAUSII*

5.3.1 Combined effect of cobalt, nickel and trivalent chromium in *N. clausii*

Concentration of metals (in ppm)	Treatment Number
Co 0.3 + Ni 0.4 + Cr^{3+} 0.8	42
Co 0.3 + Ni 0.6 + Cr^{3+} 0.2	43

Biomass

Biomass was more than the control upto fourth day for both treatments but it was less than the control with the aging of the culture (Fig.79). Treatment (42) (a combination of 0.3 ppm cobalt, 0.4 ppm nickel and 0.8 ppm trivalent chromium) was 66%, 20% and 32% less than the control on the sixth, eighth and tenth day. Treatment (43), a combination of 0.3 ppm cobalt, 0.6 ppm nickel and 0.2 ppm trivalent chromium was 47%, 35%, 23% less than the control on sixth, eighth and tenth day respectively.

Production

Production was less than the control through out the growth phase for treatment (42). It was 10%, 25%, 52% and 45% less than the control on fourth, sixth, eighth and tenth day respectively. Treatment (43) was 53% more than the control on the fourth day but it was 26% and 45% less than the control on sixth and eighth day. At the end of growth phase it was similar to control. pH was more than control through out the growth phase for treatment (42). But it was less than the control for treatment (43).

Respiration

Both the treated samples were more than the control upto sixth day, but it was less than the control with the aging of the culture (Fig.79). Treatment (42) was similar to the control on the fourth day and it was 7% more than the control on sixth day. On eighth and tenth day it was 25% and 32% less than the control. Treatment (43) was 56% and 63% less than the control on eighth and tenth day respectively.

Photosynthetic pigments

Chlorophyll-a

Chlorophyll-a was less than the control through out the growth phase for treatment (42). It was 50%, 60%, 13% and 49% less than the control on fourth, sixth, eighth and tenth day respectively. Treatment (43) was 28%, 42%, 35% less than the control on fourth, sixth and tenth day but 37% increase was observed on the eighth day.

Chlorophyll-c

Similar to chlorophyll-b, chlorophyll-c was more than the control upto sixth day for both treatments but with the aging of the culture it was less than the control (Fig.80). Treatment (42) was 136% and 116% more than the control on fourth and sixth day, but 61% and 76% decrease was observed on eighth and tenth day respectively. Treatment (43) was 127% and 36% more than the control on fourth and sixth day. On eighth and tenth day 45% and 70% decrease was noticed.

Carotenoids

Carotenoids showed an initial increase of 91% on the second day for treatment (42) but it was less than the control through out the growth phase. It was 8%, 40%, 7% and 40% less than the control on fourth, sixth, eighth and tenth day. Treatment (43) was found to be fluctuating. It was 157%, 12% and 50% more than the control on second, fourth and eighth day but 28% and 21% decrease was noticed on the sixth and tenth day respectively.

Phaeophytin

Combination of metals reduced the phaeophytin. It was less than the control for both treatments through out the growth phase except on the eighth day (Fig.80). At the end of growth phase 30% and 26% decrease was observed for treatment (42) and (43) respectively.

Photosynthetic end products

Carbohydrate

The acid soluble fraction of carbohydrate was 30%, 16% and 30% less than the control on fourth, eighth and tenth day for treatment (42). Treatment (43) showed an initial increase of 51% on the fourth day but it was 21%, 64% and 80% less than the control on sixth, eighth and tenth day respectively.

The alkali soluble fraction was less than the control through out the growth phase for both treatments (Fig.81). It was 95%, 69%, 20% and 80% less than the control on fourth, sixth, eighth and tenth day for treatment (42). Treatment (43) was 82%, 49%, 37% and 81% less than the control. The insoluble fraction was also less than the control through out the growth phase.

Protein

Protein was less than the control through out the growth phase for treatment (42), though it was similar to control on sixth day. It was 61%, 73% and 66% less than the control on fourth, eighth and tenth day respectively. Treatment (43) was closely following the control on fourth day and it was 62% more than the control on sixth day. But 37% and 75% decrease was noticed on eighth and tenth day respectively.

Lipid content was 72% and 88% less than the control for treatment (42) and (43) respectively.

Nutrient uptake was more than the control for both the treated samples. The phosphate uptake was 37% and 30% more than the control.

Fig.79

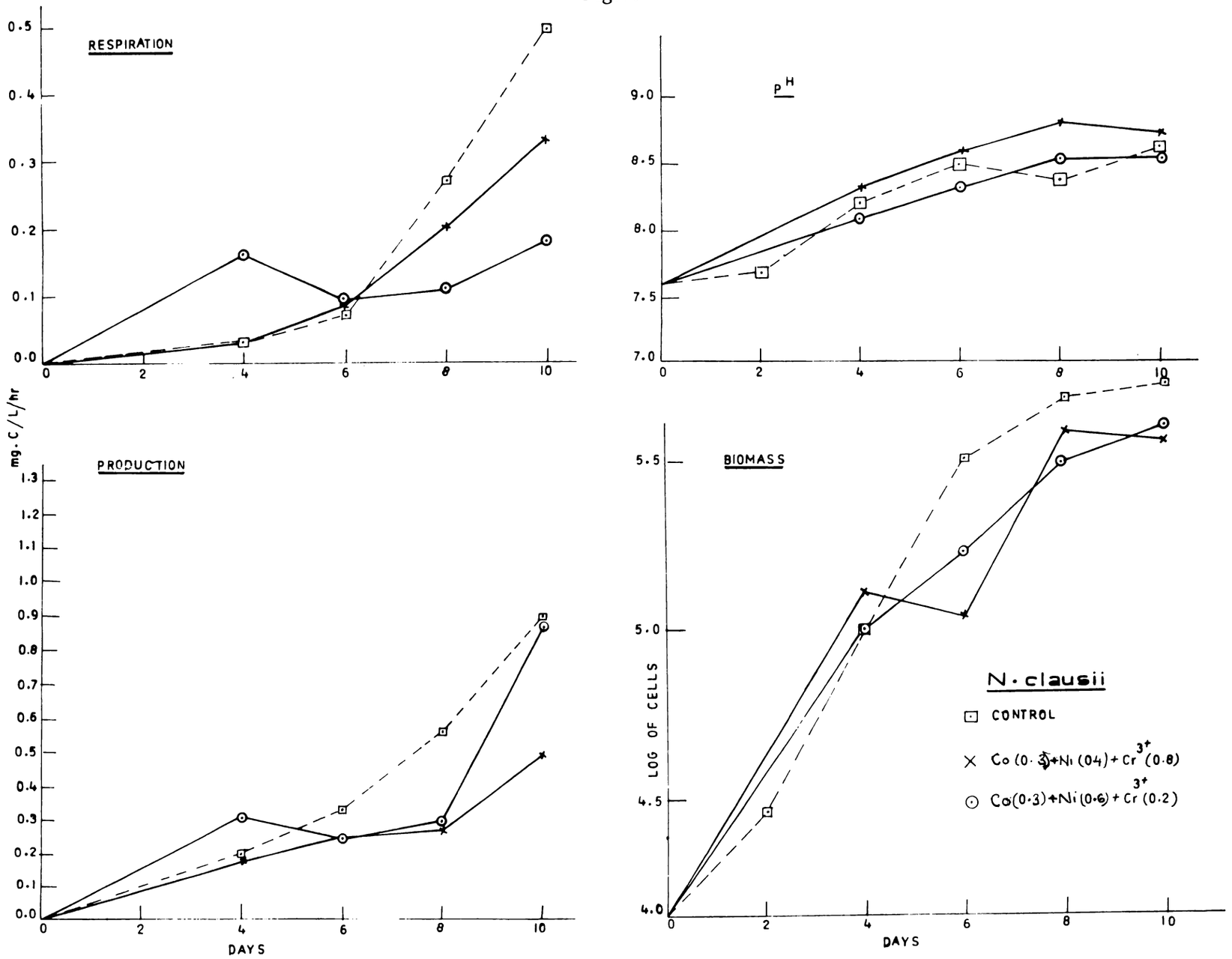
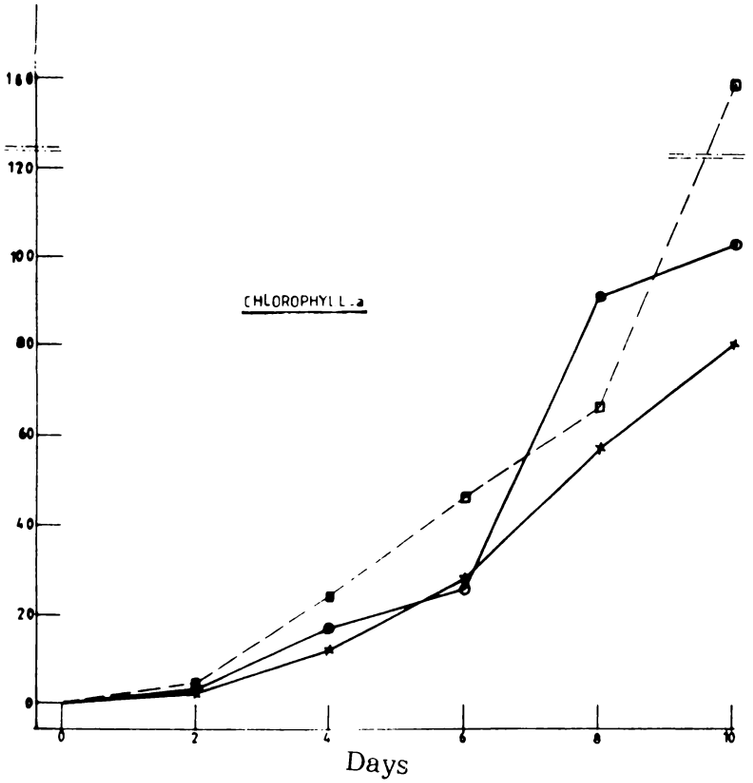
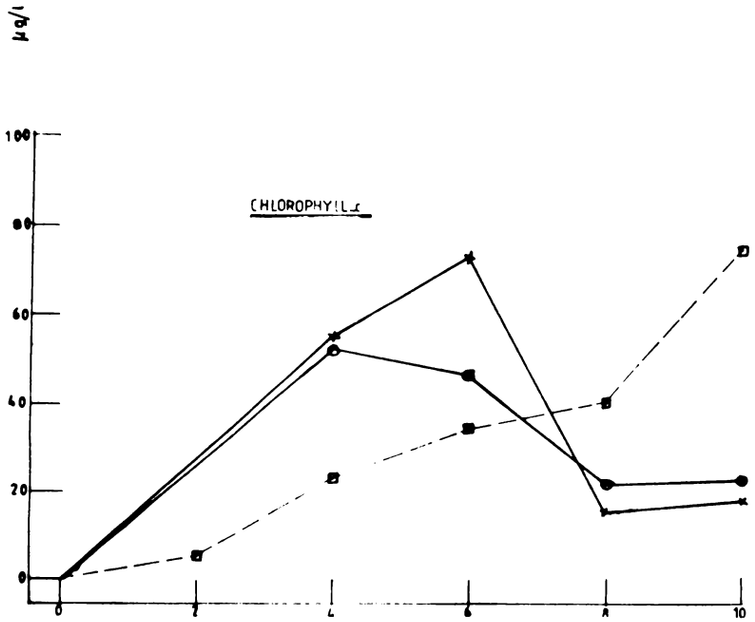
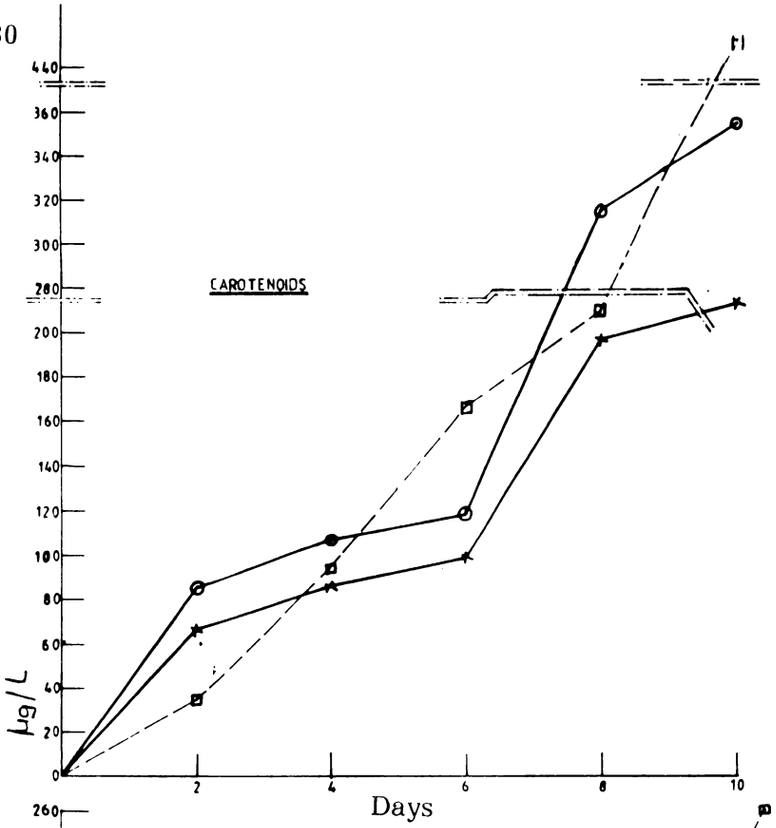

RESPIRATION
PH
PRODUCTION
BIOMASS
mg. C/L/hr
LOG OF CELLS
DAYS
DAYS
N. clausii
CONTROL
Co (0·3)+Ni (0·4)+Cr 3+ (0·8)
Co(0·3)+Ni(0·6)+Cr 3+ (0·2)

Fig.80


CHLOROPHYLL a
Days


µg/l
CHLOROPHYLL c


CAROTENOIDS
µg/L
Days

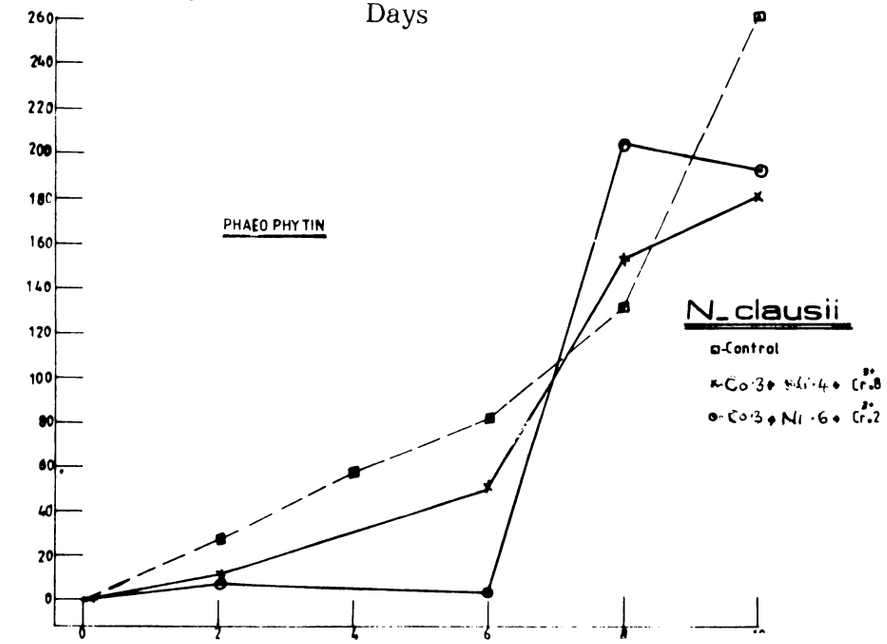
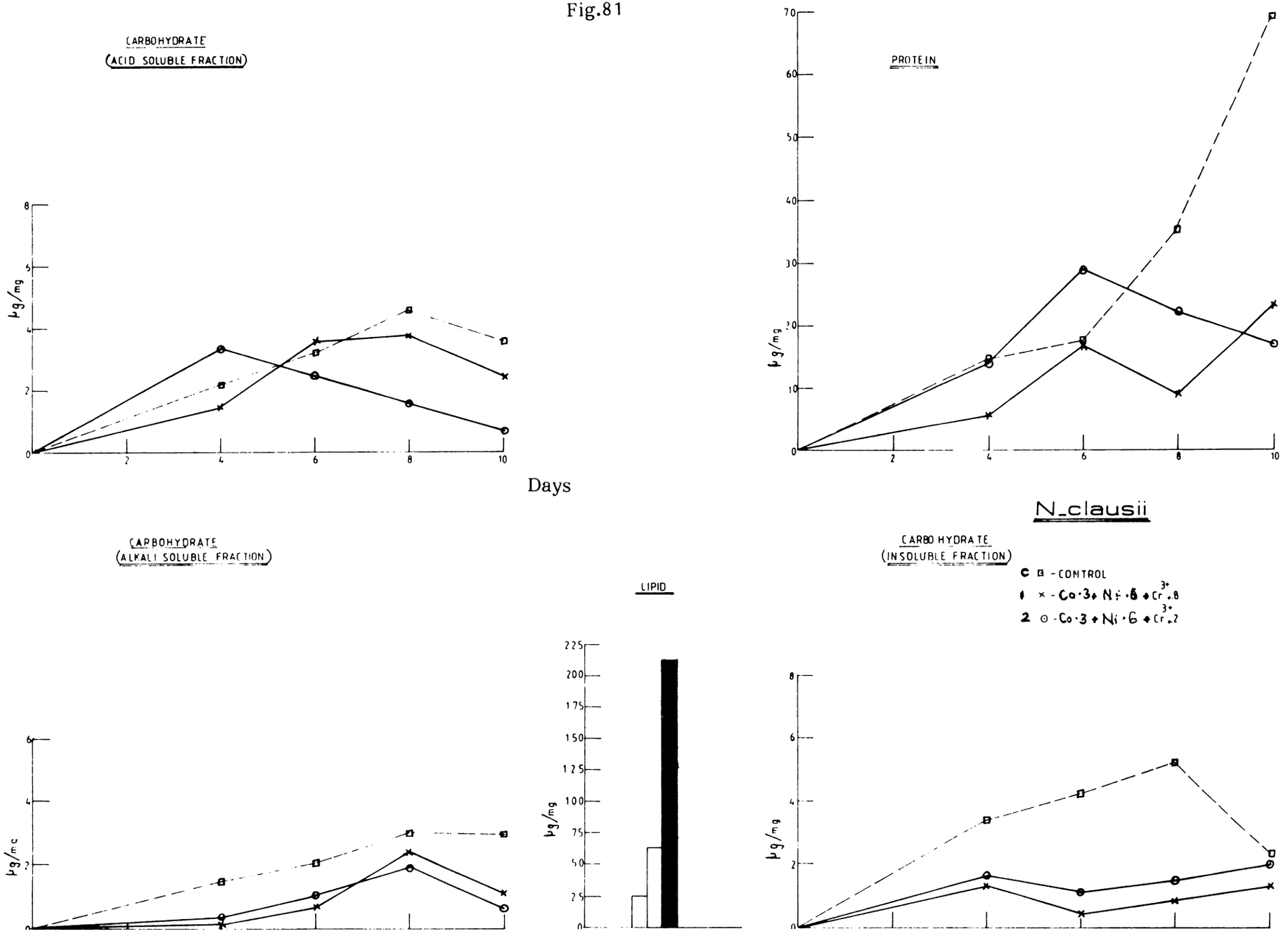

PHAEO PHYTIN
N_clausii
Control

Fig.81


CARBOHYDRATE
(ACID SOLUBLE FRACTION)
µg/mg
PROTEIN
µg/mg
Days
N_clausii
CARBOHYDRATE
(ALKALI SOLUBLE FRACTION)
µg/mg
LIPID
µg/mg
CARBO HYDRATE
(INSOLUBLE FRACTION)
µg/mg
C ◻ - CONTROL
1 × - Co·3 + Ni·6 + Cr3+·8
2 ⊙ - Co·3 + Ni·6 + Cr3+·2

5.3.2 Combined effect of cobalt, nickel and hexavalent chromium in *N. clausii*

Concentration of metals (in ppm)	Treatment Number
Co 0.3 + Ni 0.4 + Cr^{6+} 0.6	44
Co 0.3 + Ni 0.6 + Cr^{6+} 0.2	45

Biomass

There was an initial increase in the biomass on the second day for both treatments. Treatments (44) a combination of 0.3 ppm cobalt, 0.4 ppm nickel and 0.6 ppm hexavalent chromium was 20%, 60%, 61% and 51% less than the control on fourth, sixth, eighth and tenth day respectively, whereas treatment (45) (a combination of 0.3 ppm cobalt, 0.6 ppm nickel and 0.2 ppm hexavalent chromium) was similar to the control on fourth day but it was 44% and 8% less than the control on sixth and eighth day. At the end of growth phase 12% increase was observed.

Production

Production was less than the control through out the growth phase for treatment (44). It was 80%, 28%, 42% and 53% less than the control on fourth, sixth, eighth and tenth day respectively. But treatment (45) was 46% and 42% less than the control on fourth and tenth day. On sixth day it was similar to the control and 19% increase was observed on the eighth day. Similar to production pH was less than the control at the end of growth phase for both treatments eventhough there was a peak on the tenth day.

Respiration

Respiration was less than the control through out the growth phase for treatment (44) (Fig.82). It was 40%, 46%, 50% and 70% less than the control on fourth, sixth, eighth and tenth day respectively. Though there was 15% increase on sixth day, 40%, 45% and 42% decrease was observed on fourth, eighth and tenth day respectively for treatment (45).

Photosynthetic pigments

Chlorophyll-a

Through out the growth phase treatment (44) was less than the control. It was 47%, 23%, 18% and 38% less than the control on fourth, sixth, eighth and tenth day respectively. Treatment (45) was 27%, 17% and 50% less than the control on fourth, sixth and tenth day and on the eighth day it was slightly more than the control.

Chlorophyll-c

Chlorophyll-c was less than the control through out the growth phase for both treatments. It was 70%, 67%, 20% and 34% less than the control on fourth, sixth, eighth and tenth day for treatment (44) and 61%, 62%, 21% and 53% decrease was observed for treatment (45) on fourth, sixth, eighth and tenth day respectively.

Carotenoids

Carotenoids were generally less than the control for both treatments except on the eighth day (Fig.83). On the eighth day 6% and 47% increase was noticed for treatment (44) and (45) respectively. Treatment (44) was

45%, 27% and 16% less than the control on fourth, sixth and tenth day. Treatment (45) was 36%, 16% and 32% less than the control on fourth, sixth and tenth day respectively.

Phaeophytin

There was variation in its effects between two treatments but at the end of growth phase phaeophytin was less than the control for both treatments. Treatment (44) was 11%, 35% and 36% less than the control on fourth, eighth and tenth day. 19% decrease was noticed on the sixth day for the treated sample, whereas treatment (45) was 44%, 17% and 46% more than the control on fourth, sixth and eighth day but 63% decrease was observed at the end of growth phase.

Photosynthetic end products

Carbohydrate

The acid soluble fraction of carbohydrate showed a general trend of increase through out the growth phase for treatment (44). It was 32%, 22%, 1% and 17% more than the control on fourth, sixth, eighth and tenth day. Whereas treatment (45) was less than the control through out the growth phase and the values being 10%, 16% and 37% less than the control on fourth, eighth and tenth day. It was similar to control on sixth day.

The alkali soluble fraction showed a marginal increase on fourth day for treatment (44) but both the treatments were less than the control through out the growth phase.

Similarly the insoluble fraction was also less than the control for both treatments through out the growth phase.

Protein

There was an initial increase in protein content but it was 30% and 51% less than the control at the end of growth phase for treatment (44) and (45) respectively. Treatment (44) was 220%, 82% and 64% more than the control on fourth, sixth and eighth day. Similarly, treatment (45) was 267%, 145% and 124% more than the control on fourth, sixth and eighth day respectively.

Lipid content was less than the control for both treated samples. In the case of nutrients, the phosphate uptake was 13% and 50% more than the control for treatment (44) and (45) respectively, whereas the nitrate uptake was showing only a marginal increase.

Fig.82

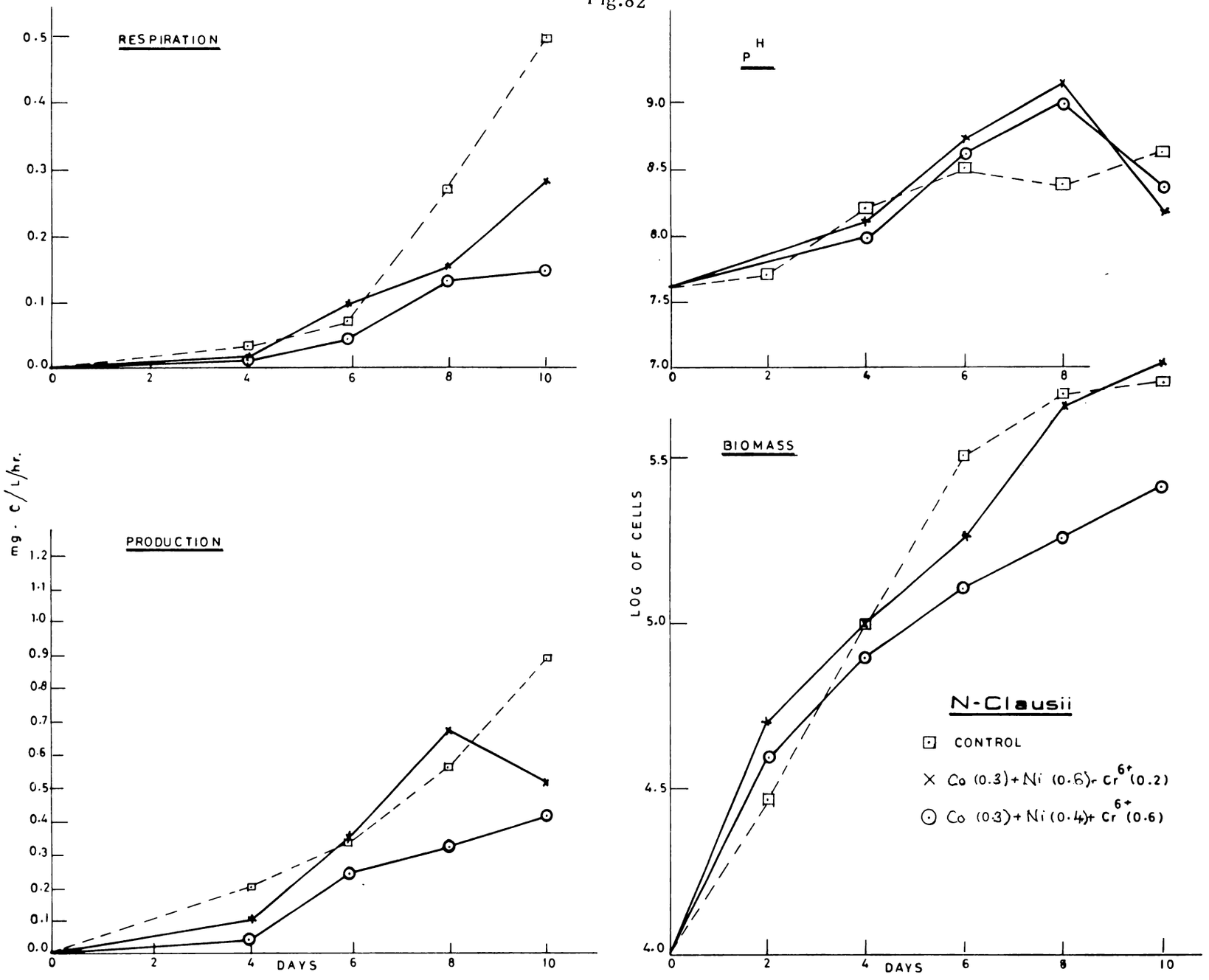
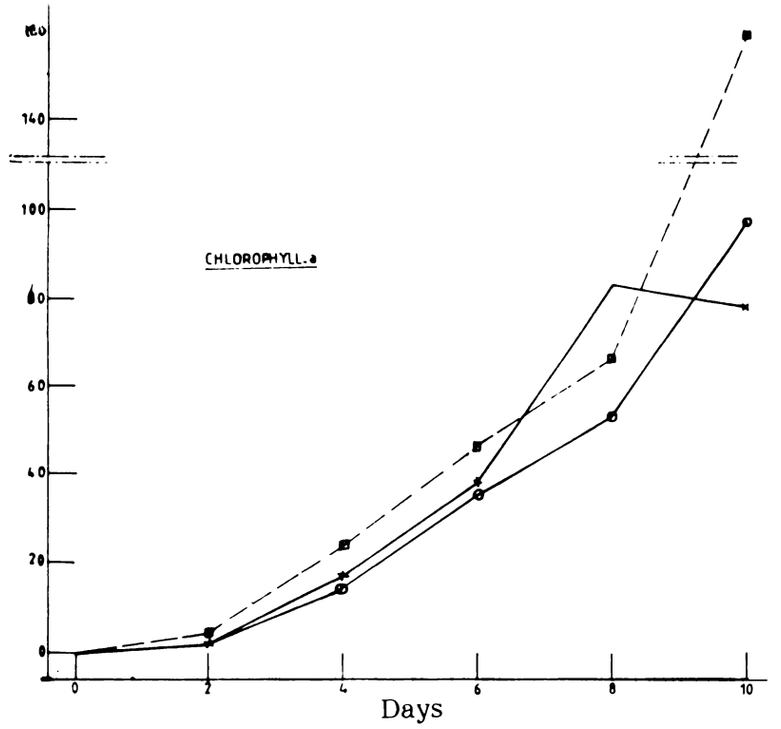
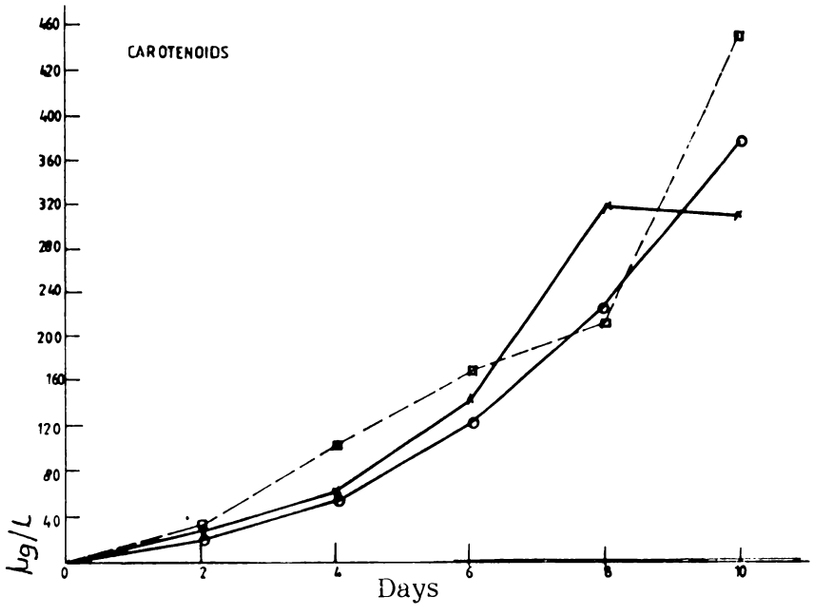
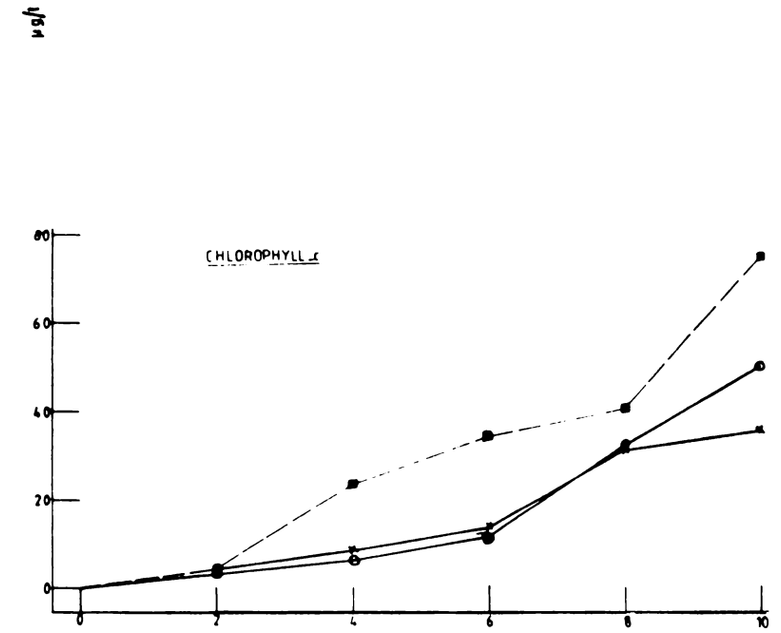

RESPIRATION
PH
PRODUCTION
BIOMASS
mg · C/L/hr.
LOG OF CELLS
DAYS
DAYS
N-Clausii
CONTROL
Co (0.3) + Ni (0.6) - Cr 6+ (0.2)
Co (0.3) + Ni (0.4) + Cr 6+ (0.6)

Fig.83


CHLOROPHYLL-a
Days


CAROTENOIDS
µg/L
Days


µg/l
CHLOROPHYLL-c

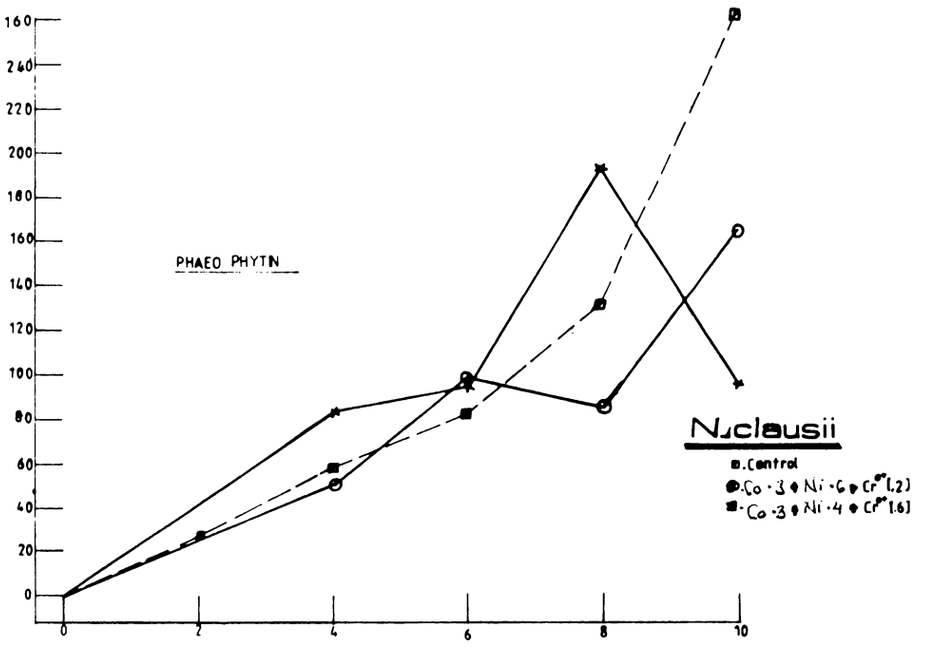
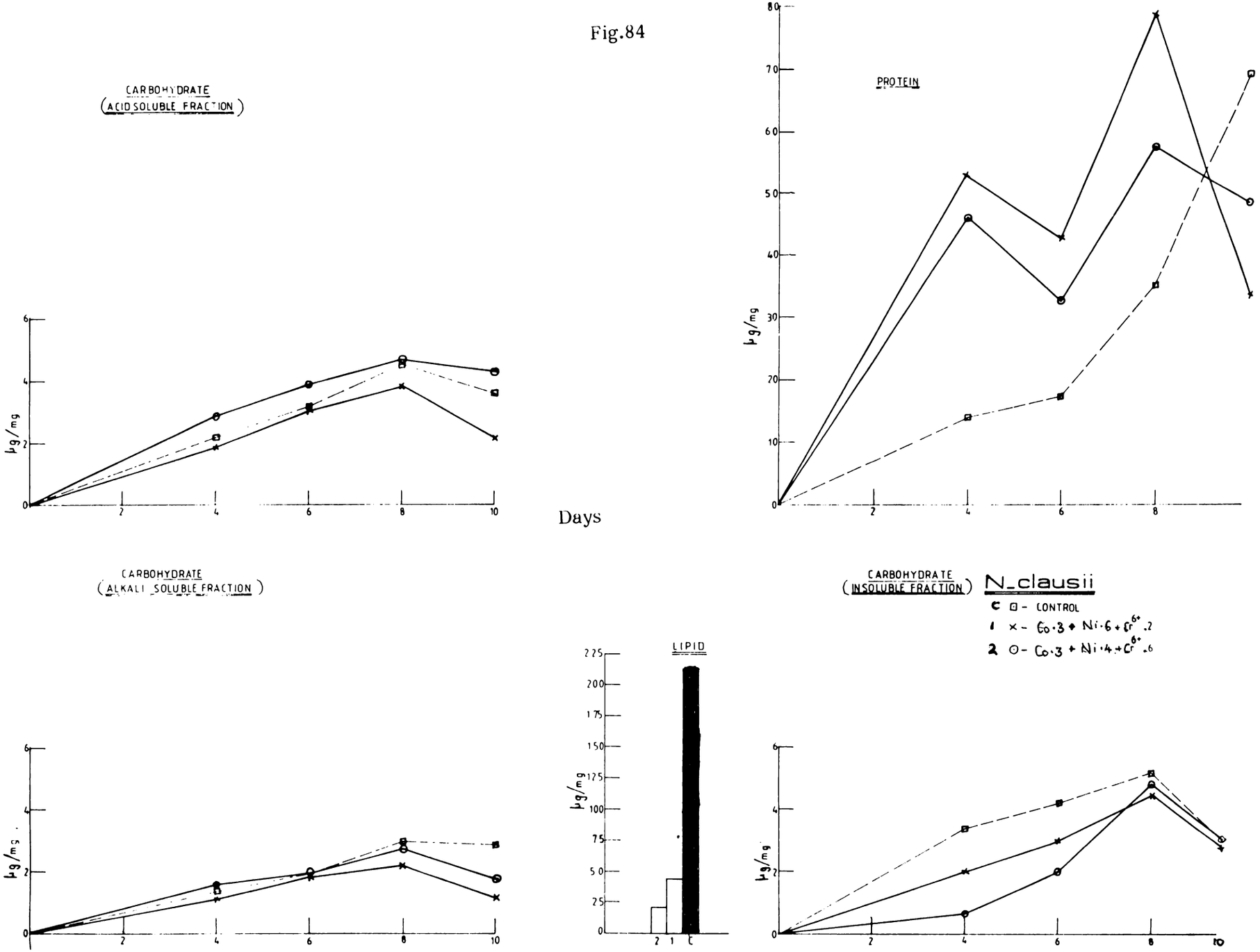

PHAEO PHYTIN
N.clausii
control
Co·3 Ni·6 Cr(1.2)
Co·3 Ni·4 Cr(1.6)

Fig.84


CARBOHYDRATE
(ACID SOLUBLE FRACTION)
µg/mg
Days
PROTEIN
CARBOHYDRATE
(ALKALI SOLUBLE FRACTION)
LIPID
CARBOHYDRATE
(INSOLUBLE FRACTION)
N_clausii
C ⊡ - CONTROL
1 × - Co·3 + Ni·6 + Cr6+ ·2
2 ⊙ - Co·3 + Ni·4 + Cr6+ ·6

5.3.3 **Combined effect of cobalt, trivalent and hexavalent chromium in N. clausii**

Concentration of metals (in ppm)	Treatment Number
Co 0.3 + Cr^{3+} 0.2 + Cr^{6+} 0.2	46
Co 0.3 + Cr^{3+} 0.2 + Cr^{6+} 0.6	47

Biomass

Both the treatments showed an initial increase but it was less than the control with the aging of the culture (Fig.85). Treatment (46) was closely following the control with 5%, 25%, 7% decrease on fourth, sixth and eighth day. At the end of growth phase it was similar to control, whereas treatment (47) was 25%, 61%, 70% and 47% less than the control on fourth, sixth, eighth and tenth day.

Production

A general trend of decrease was noticed for both treatments. But treatment (46) was 35%, 12% and 27% less than the control on fourth, sixth and tenth day respectively. But 18% increase was noticed on the eighth day. Treatment (47) was 67%, 33%, 44% and 61% less than the control on fourth, sixth, eighth and tenth day. Similar trend was observed in the case of pH also.

Respiration

A general trend of decrease was noticed in the respiration. Treatment (46) was having an initial increase, but it was 77%, 78% and 58% less than

the control on sixth, eighth and tenth day respectively. Treatment (47) was 60%, 39%, 89% and 88% less than the control on fourth, sixth, eighth and tenth day respectively.

Photosynthetic pigments

Chlorophyll-a

Chlorophyll-a was less than the control on fourth and tenth day for treatment (46). The values being 40% and 19% less than the control, 18% increase was observed on sixth and eighth day respectively. Treatment (47) was 55%, 45%, 33% and 30% less than the control on fourth, sixth, eighth and tenth day respectively.

Chlorophyll-c

Chlorophyll-c was less than the control for both treatments through out the growth phase. Treatment (46) was 53%, 60%, 40% and 26% less than the control and treatment (47) was 58%, 65%, 45% and 31% less than the control on fourth, sixth, eighth and tenth day respectively.

Carotenoids

A general trend of increase was noticed at the end of growth phase for both treatments (Fig.86). Treatment (46) was 40% and 8% less than the control on fourth and sixth day but 43% and 6% increase was noticed on eighth and tenth day respectively. Treatment (47) was 53%, 42% and 24% less than the control on fourth, sixth and eighth day. But at the end of growth phase 3% increase was observed.

Phaeophytin

There was variation between treatments in the phaeophytin content. Treatment (46) was 7% and 13% less than the control on fourth and tenth

day. But 96% and 123% increase was found on sixth and eighth day. Treatment (47) was less than the control through out the growth phase except on the eighth day. The values being 52%, 7% and 8% less than the control on fourth, sixth and tenth day, on the eighth day 14% increase was observed.

Carbohydrate

The acid soluble fraction was showing an initial peak for both treatments. It was 71% and 16% more than the control on fourth and eighth day for treatment (46). On the sixth day though there was only a marginal decrease, it was 35% less than the control at the end of growth phase. Treatment (47) was 283% more than the control on fourth day but it was 40%, 61% and 35% less than the control on sixth, eighth and tenth day respectively.

The alkali soluble fraction was less than the control for both treatments but 11% increase was noticed on the eighth day for treatment (46).

The insoluble fraction was 41% and 26% less than the control for treatment (46) on fourth and sixth day. But 34% and 55% increase was noticed on the eighth and tenth day. Treatment (47) was showing an initial increase of 32% on the fourth day but 51%, 28% and 18% decrease was noticed on sixth, eighth and tenth day respectively.

Protein

Combination of three metals increased the protein production considerably (Fig.87). Treatment (48) was 254%, 168% and 128% more than the control on fourth, sixth and eighth day. Treatment (47) was 285%, 41% and 51% more than the control on fourth, sixth and eighth day. At the end of growth phase 11% and 53% decrease was observed for treatment (46) and (47) respectively.

Lipid content was 82% and 70% less than the control for treatment (46) and (47) respectively. There was 64% and 58% increase in phosphate uptake and 20% and 32% increase in nitrate uptake when compared with the control.

Fig.85

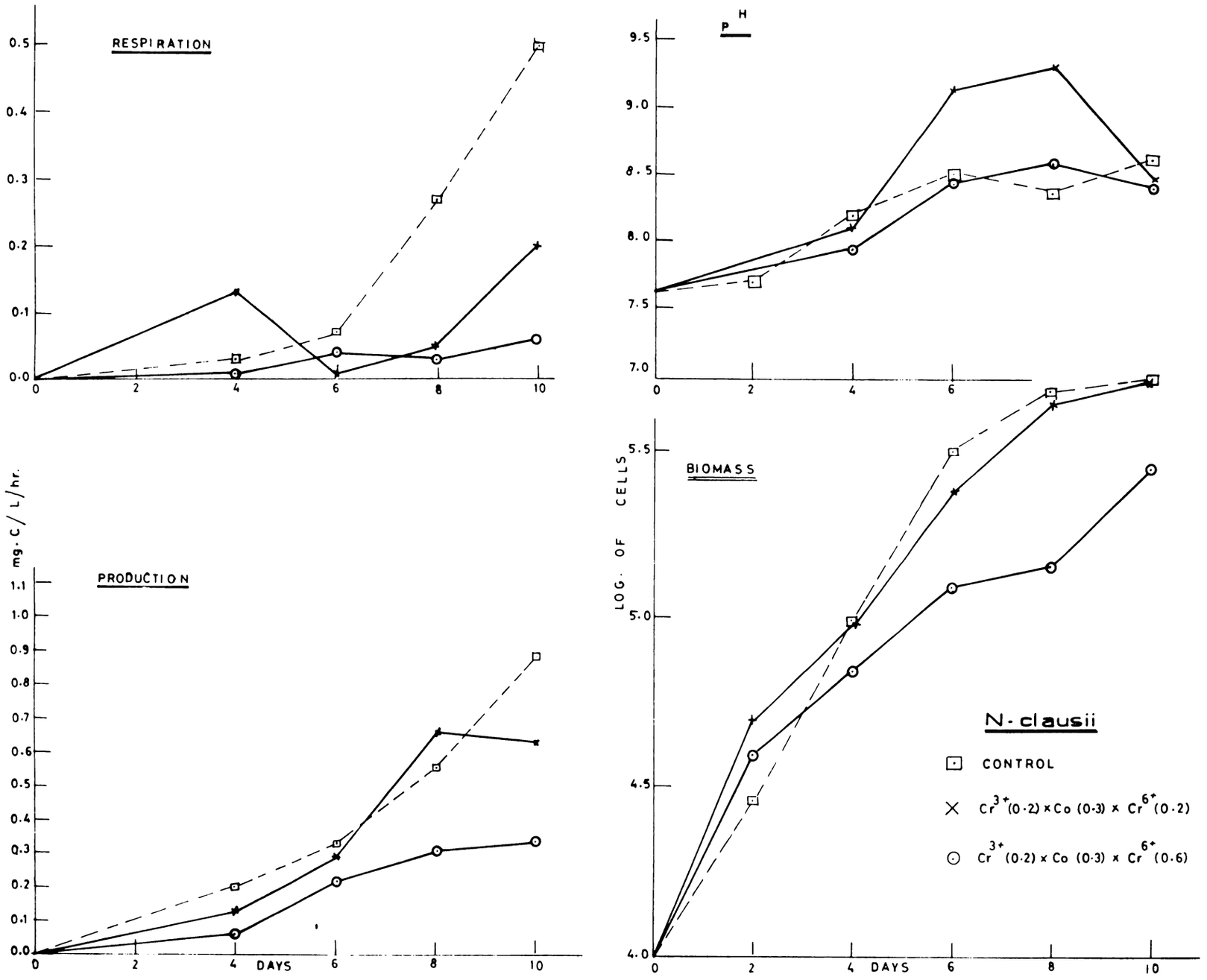

RESPIRATION
PH
PRODUCTION
mg. C / L / hr.
BIOMASS
LOG. OF CELLS
DAYS
N. clausii
CONTROL
Cr3+ (0·2) × Co (0·3) × Cr6+ (0·2)
Cr3+ (0·2) × Co (0·3) × Cr6+ (0·6)

Fig.86

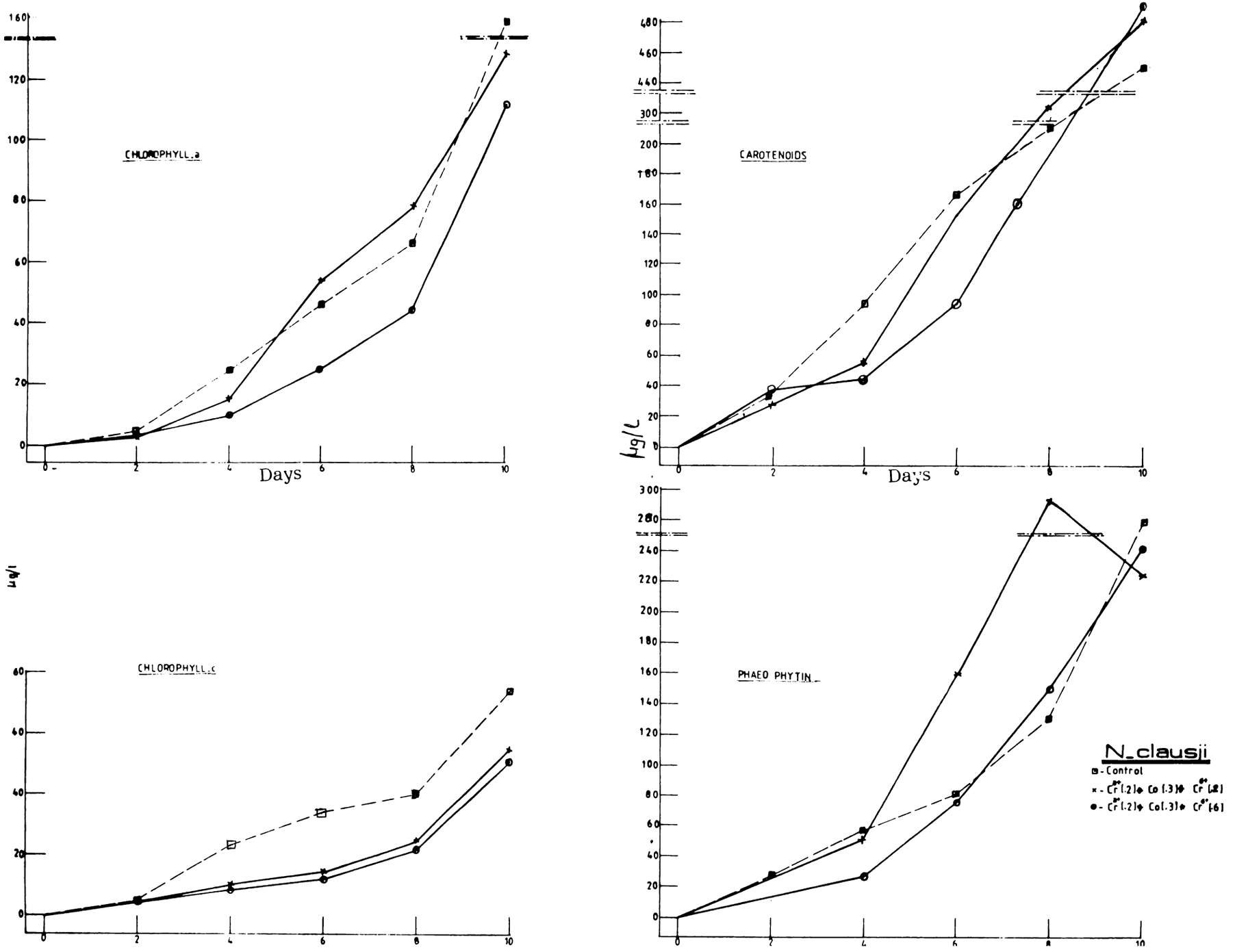
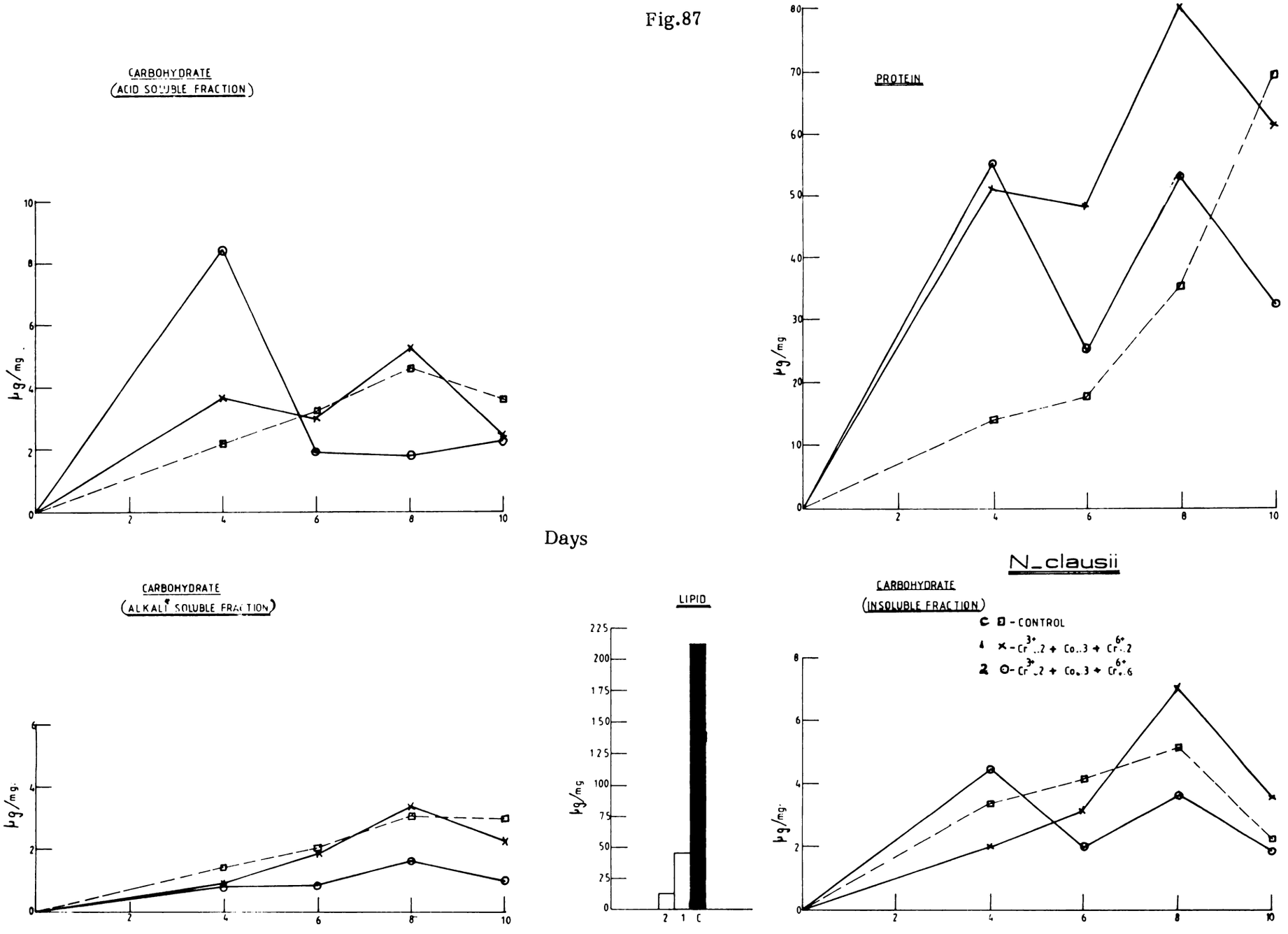

CHLOROPHYLL a
CAROTENOIDS
PHAEO PHYTIN
CHLOROPHYLL c
Days
µg/l
N. clausii
- Control

Fig.87


CARBOHYDRATE
(ACID SOLUBLE FRACTION)
µg/mg
PROTEIN
µg/mg
Days
N_clausii
CARBOHYDRATE
(ALKALI SOLUBLE FRACTION)
µg/mg
LIPID
µg/mg
CARBOHYDRATE
(INSOLUBLE FRACTION)
C - CONTROL
4 - Cr3+..2 + Co..3 + Cr6+..2
2 - Cr3+..2 + Co..3 + Cr6+..6
µg/mg

5.3.4 Combined effect of nickel, trivalent and hexavalent chromium in N. clausii

Concentration of metals (in ppm)	Treatment Number
Ni 0.6 + Cr^{3+} 0.2 + Cr^{6+} 0.6	48

Biomass

Growth was more than the control on the second day but with the aging of the culture it was reduced (Fig.88) for treatment (48), a combination of 0.2 ppm trivalent chromium, 0.6 ppm nickel, 0.6 ppm hexavalent chromium. It was similar to the control on fourth day followed by 60%, 28% and 12% decrease on sixth, eighth and tenth day respectively.

Production

The production was less than the control through out the growth phase (Fig.88). It was 55%, 24% and 50% less than the control on sixth, eighth and tenth day respectively. pH was found to be less than the control on fourth and tenth day.

Respiration

A general trend of decrease was noticed in the case of respiration (Fig.88). It was 18% less than the control on the fourth day. It was 53%, 88%, 92% less than the control on fourth, eighth and tenth day. Thus with the aging of the culture, the respiration was decreased.

Photosynthetic pigments

Chlorophyll-a

Chlorophyll-a was less than the control through out the growth phase eventhough it was similar to the control on eighth day (Fig.89). It was 27%, 20% and 92% less than the control on fourth, sixth and tenth day respectively.

Chlorophyll-c

Chlorophyll-c was completely reduced through out the growth phase (Fig.89). It was 76%, 51% and 11% less than the control on sixth, eighth and tenth day respectively.

Carotenoids

An increasing trend was noticed with the aging of the culture for the treated sample. But it was 31% and 28% less than the control on fourth and sixth day. On the eighth and tenth day, 15% and 3% increase was observed.

Phaeophytin

Similar to carotenoids an increasing trend was noticed in the exponential growth phase. It was closely following the control on the fourth day. On the sixth and eighth day 70% and 83% increase was noticed but at the end of growth phase 62% decrease was observed for the treated sample.

Photosynthetic end products

Carbohydrate

The acid soluble fraction of carbohydrate was maximum on the fourth day and it was 401% more than the control followed by 10%, 27% and 26% less than the control on sixth, eighth and tenth day respectively (Fig.90).

The alkali soluble fraction was less than the control through out the growth phase. The values being 80%, 10%, 38% and 40% less than the control on fourth, sixth, eighth and tenth day respectively.

Similar to the alkali fraction, the insoluble fraction was less than the control, but 33% increase was observed at the end of growth phase. It was 51%, 45% and 35% less than the control on fourth, sixth and eighth day respectively.

Protein

Combination of metals stimulated the protein content of the algae (Fig.90). It was maximum on the fourth day. The value being 265% more than the control, 140% and 37% increase was noticed on the sixth and eighth day. But at the end of growth phase 20% decrease was observed for the treated sample.

Lipid content was 82% less than the control for the treated sample. Eventhough there was decrease in the end products, uptake of nutrients was found to be more than the control.

Fig.88

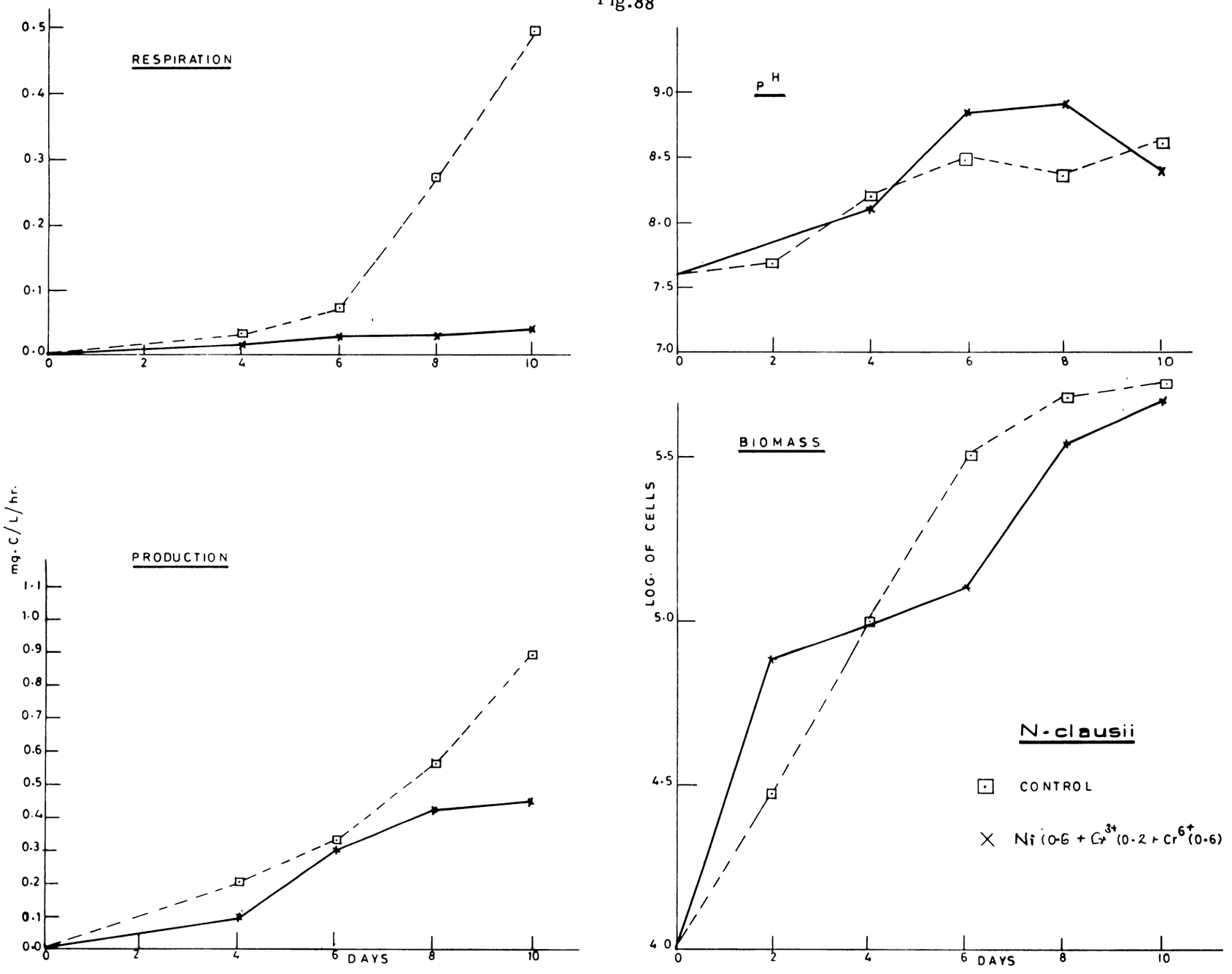
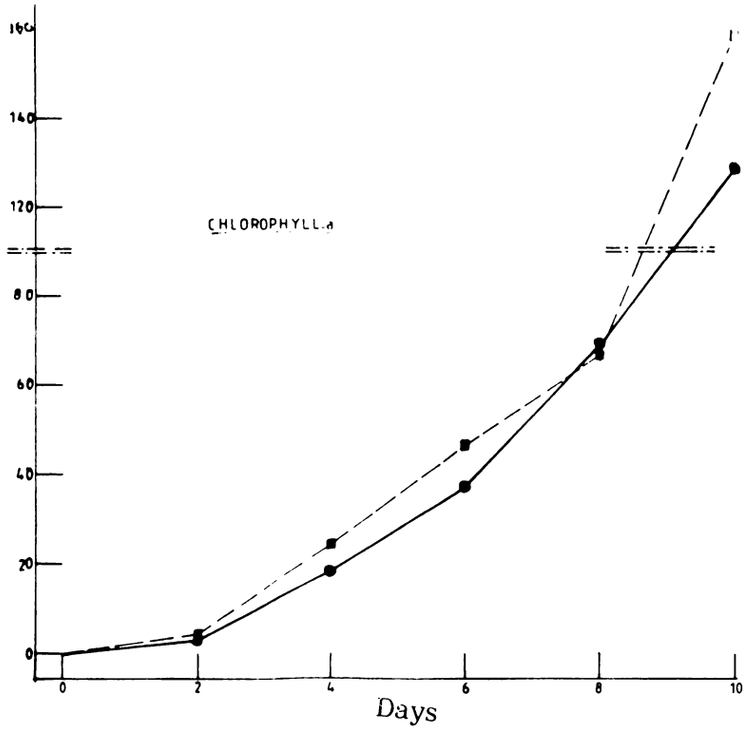
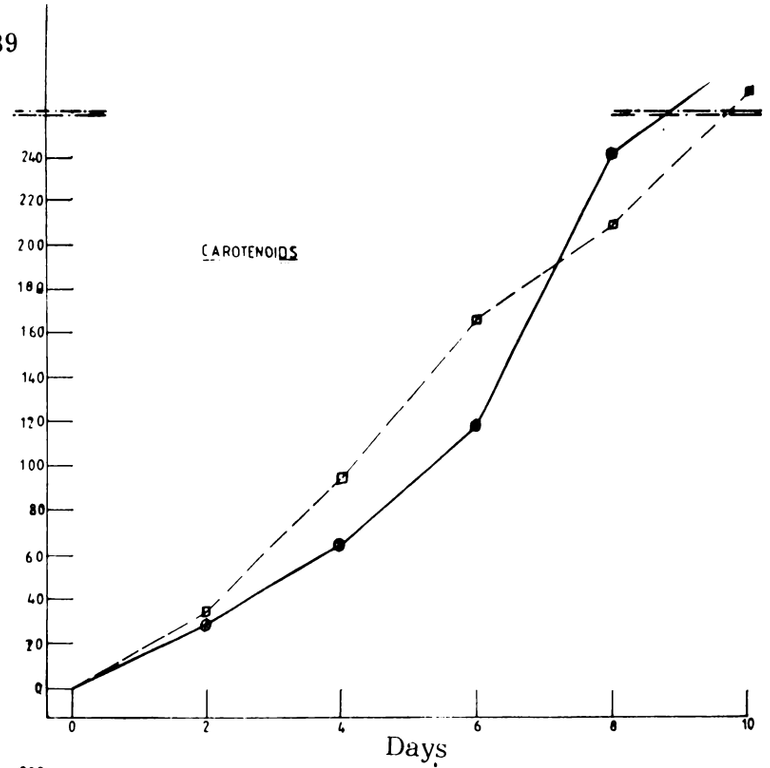
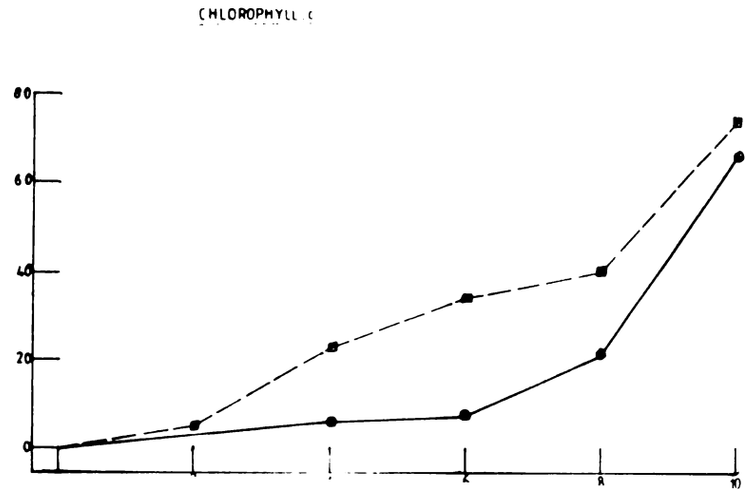

RESPIRATION
0.5
0.4
0.3
0.2
0.1
0.0
0
2
4
6
8
10
PH
9.0
8.5
8.0
7.5
7.0
PRODUCTION
mg. C/L/hr.
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
DAYS
BIOMASS
LOG. OF CELLS
5.5
5.0
4.5
4 0
N-clausii
CONTROL
Ni (0·6 + Cr3+ (0·2 + Cr6+ (0·6)

Fig.89


CHLOROPHYLL a
Days


CAROTENOIDS
Days


CHLOROPHYLL c

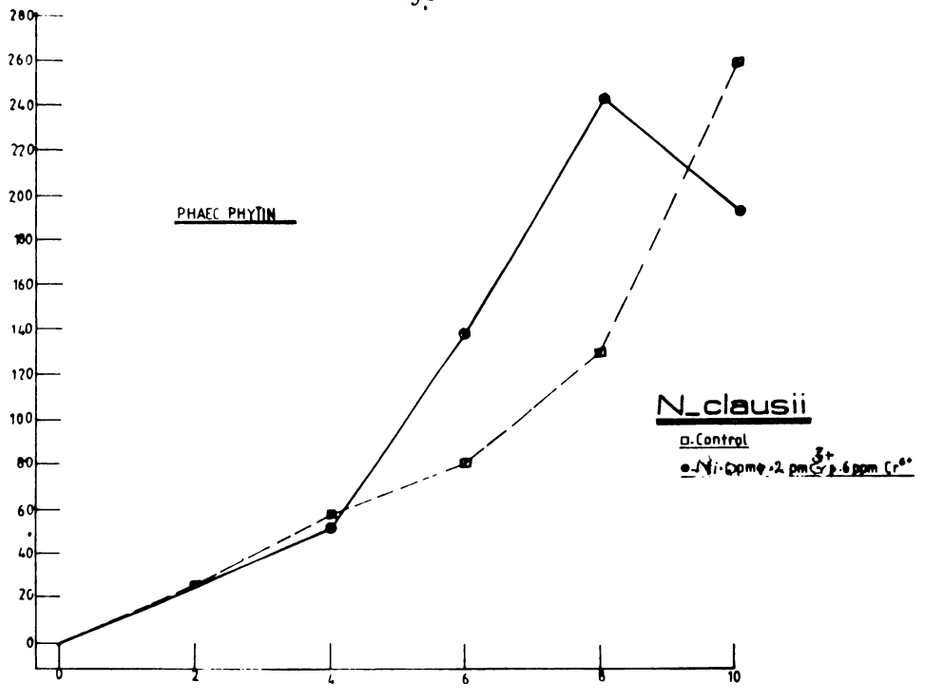
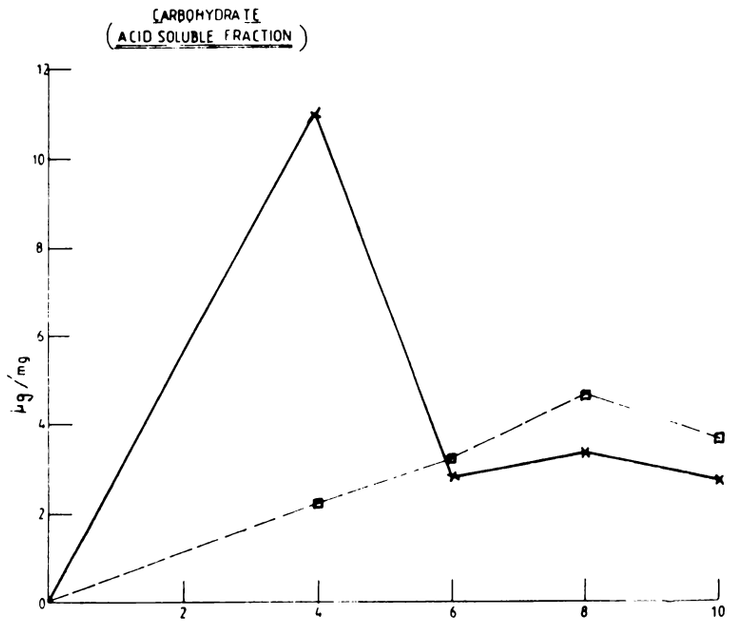
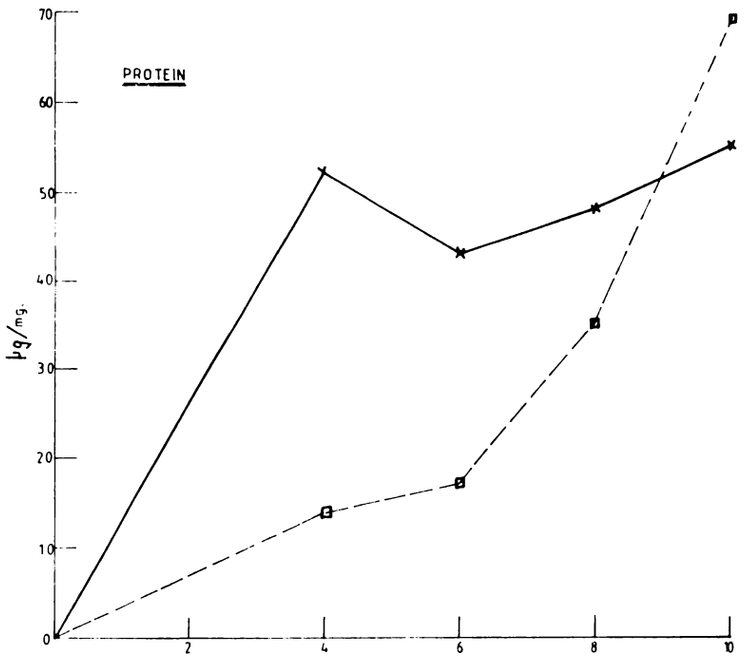

PHAEOPHYTIN
N_clausii
□ Control
● Ni 6ppm + 2 ppm Cr3+ + 6 ppm Cr6+

Fig.90



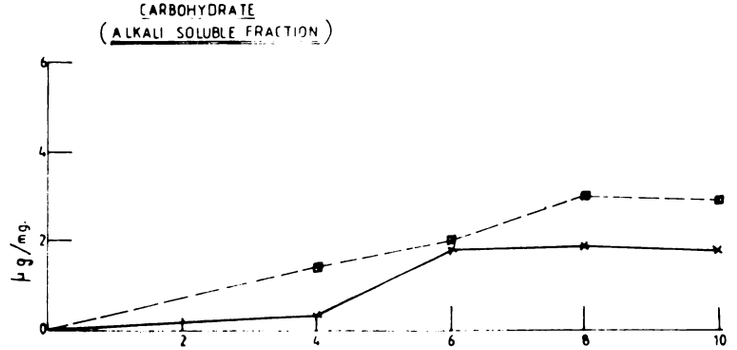


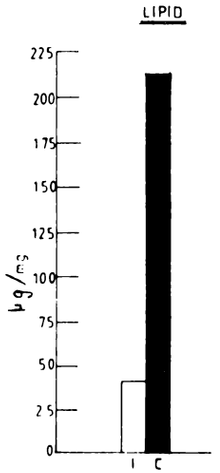
Days

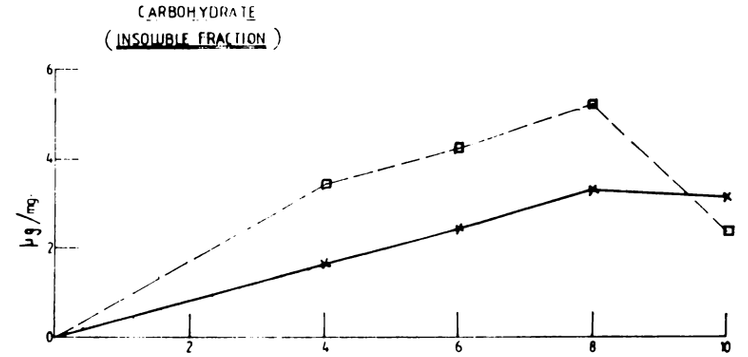
N_clausii

C □ - Control

I × - Ni +6 ppm + Cr^{3+} +2ppm + Cr^{6+} +6ppm







DISCUSSION

Increasing usage of heavy metals and their widespread dissemination in the aquatic environment has stimulated many studies on the effects of heavy metals in combination on phytoplankton. Trace metal interactions have not been studied extensively in phytoplankton. Only few reports have been found in comparing the effects of toxic metals added individually with their effects when combined (Krock and Mason, 1971 and Braek, 1976). So in the present study the effect of three metal combination was found to be totally most essential.

There was considerable variation in the effect of metals employed either individually or in combination. In the case of N. clausii combination of cobalt, nickel and trivalent chromium (treatment 42 and 43), produced significant increase in chlorophyll-c, whereas for all other three metal combinations chlorophyll-c was reduced. It was observed that nickel and cobalt together reduced the protein and carbohydrate content of the algae. But in the presence of trivalent chromium there was improvement in the end products. This may be due to competition between nickel and cobalt with trivalent chromium for same active site on the enzymes. Such a competition would reduce the probability of cobalt and nickel becoming toxic in the presence of trivalent chromium. Similar effect was noticed for lead-manganese antagonism in Chlorella stigmatophora by Christensen and Scherfig (1979).

Li (1979) showed an increase in cellular nitrogen as a result of increased cell protein in cadmium-stressed Thalassiosira weissflogii in continuous culture.

Rice et al. (1973) have suggested that changes in cell metabolism as a result of metal toxicity may cause changes in gross cell composition.

The combination of cobalt, nickel and trivalent chromium reduced the production and respiration considerably. Studies on the heavy metal tolerance of marine phytoplankton by Braek et al. (1976) showed that joint effect of copper and zinc on marine phytoplankton could not be predicted on the basis of the toxicity of individual metals. Bartlett et al. (1974). studied the effects of combination of copper, zinc and cadmium on the fresh water chlorophyta Salenastrum capricornutum.

The combination of nickel, cobalt and hexavalent chromium (treatment 44 and 45) reduced the pigment content. Similarly production and respiration were reduced but the production was improved in the two metal combinations of cobalt and hexavalent chromium and combination of nickel and hexavalent chromium. But in the presence of nickel together with cobalt and hexavalent chromium there was considerable decrease in production.

Studies of the cadmium toxicity on Thalassiosira weissflogii by Foster and Morel (1982) reported that cadmium toxicity was reversed in the presence of iron. The combination of nickel, cobalt and hexavalent chromium showed significant increase in protein content but the absence of hexavalent chromium (combination of cobalt and nickel) reduced the protein.

Braek et al. (1976) on their studies on Phaeodactylum tricornutum found that the effect of combination of metals was greater than the sum of effects caused by the components applied separately. Deviprasad and Deviprasad

(1982) observed interaction between (copper and nickel) and (cadmium and lead) in Ankistrodesmis falcatus. It was found that copper and nickel together decreased toxicity below that was observed with individual metals.

Combination of cobalt, trivalent and hexavalent chromium (treatment 46 and 47) reduced the production and respiration. Chlorophyll-c was also adversely affected. But in the absence of hexavalent chromium (combination of cobalt and trivalent chromium) there was significant increase in chlorophyll-c and phaeophytin content. Well marked antagonism was noticed, resulting in the increase in the end products of photosynthesis such as a protein and carbohydrate.

Braek et al. (1976) suggested that competition of two metals for a common uptake site probably resulted in a complex antagonistic effects on the growth of phytoplankton species.

The interaction of cobalt, trivalent and hexavalent chromium was synergistic resulting in the reduction in production and respiration. This may probably be due to enhanced toxicity of one metal in the presence of another metal resulting in the increased permeability of the plasma membrane when stressed by several toxicants (Nriagu, 1983).

Combination of nickel, trivalent and hexavalent chromium (treatment 48) completely suppressed the respiration. Though there was an increasing trend, production was less than the control through out the growth phase. Similarly chlorophyll-c pigment content was reduced but significant increase

was noticed in the combination of nickel and trivalent chromium. Treatment (48) produced antagonism resulting in the increase in the protein content.

Hutchinson (1973) reported that tolerance of nickel may be complicated by the presence of other heavy metals and it was suggested that for many algae nickel and copper mutually enhance the toxicity of each other and it is an example of heavy metal synergism.

Analysis of variance was carried out to establish the significance of the interaction of three metal combination on N. clausii. Table 7.

The combination of cobalt, nickel and trivalent chromium was not significant in the case of biomass, pigments and production. Respiration was found to be significant only between days. Carbohydrate was found to be highly significant.

The combination of cobalt, nickel and hexavalent chromium was highly significant in the case of production, pigments, biomass and carbohydrate. But carotenoids were found to be not significant.

The combination of cobalt, trivalent and hexavalent chromium was significant in the case of production, pigments and carbohydrate. But it was not significant for respiration, protein and biomass.

The combination of nickel, trivalent and hexavalent chromium was highly significant in the case of biomass, production and end products of photosynthesis were considered. But respiration was significant only between days.

Table 7. Results of the significance of the variance ratio between concentration and between days in N. clausii

Sl. No.	Metals employed	Selected parameters																									
		pH		Biomass		Production		Respiration		Chlorophyll-a		Chlorophyll-b		Carotenoids		Phaeophytin		Carbohydrate fraction(1)		Carbohydrate fraction(2)		Carbohydrate fraction (3)		Protein		Lipids
		1	11	1	11	1	11	1	11	1	11	1	11	1	11	1	11	1	11	1	11	1	11	1	11	1
1.	Co+Ni+Cr^{3+}	NS	a	NS	NS	NS	NS	NS	b	a	b	NS	NS	NS	NS	NS	NS	a	b	NS	b	a	b	NS	b	a
2.	Co+Ni+Cr^{6+}	NS	a	b	b	a	a	NS	b	a	b	NS	NS	NS	b	NS	b	a	b	NS	b	a	b	NS	b	a
3.	Co+Cr^{3+}+ Cr^{6+}	NS	a	NS	NS	a	a	NS	b	a	b	NS	b	NS	b	NS	b	a	b	NS	b	a	b	NS	b	a
4.	Ni+Cr^{3+}+ Cr^{6+}	NS	a	NS	b	NS	NS	NS	b	b	b	NS	b	NS	b	NS	b	a	b	NS	b	a	b	NS	b	a

a P < 0.01
b P < 0.05
NS Non Significant

1 Concentration
11 Days

5.4 EFFECT OF COMBINATION OF FOUR METALS ON *S. ABUNDANS* AND *N. CLAUSII*

5.4.3 Combined effect of cobalt, nickel, trivalent and hexavalent chromium in S. abundans

Concentration of metals (in ppm)	Treatment Number
Co 0.01 + Ni 0.005 + Cr^{3+} 0.01 + Cr^{6+} 0.05	49

Biomass

These was well marked decrease in the biomass through out the growth phase (Fig.91). It was 60%, 63%, 83%, 86% and 82% less than the control on fourth, sixth, eighth, tenth and twelfth day. At the end of growth phase 76% decrease was observed.

Production

There was complete suppression in the production (Fig.91) 92%, 95%, 84%, 94% and 91% decrease was observed on sixth, eighth, tenth, twelfth and fourteenth day. pH was also less than the control through out the growth phase.

Respiration

Similar to production, respiration was reduced completely (Fig.91). At the end of growth phase 94% decrease was observed. It was 92%, 88%, 89% and 94% less than the control on sixth, eighth, tenth and twelfth day.

Photosynthetic pigments

Chlorophyll-a

There was an initial increase of 55% on the fourth day followed by sudden decrease with the aging of the culture (Fig.92). Though it was closely

following the control at the end of growth (with a marginal decrease of 6% on the fourteenth day), it was 62%, 55%, 77% and 33% less than the control on sixth, eighth, tenth and twelfth day respectively.

Chlorophyll-b

Through out the growth phase chlorophyll-b was less than the control. It was 83%, 76%, 63%, 52% less than the control on sixth, eighth, tenth and twelfth day. At the end of growth phase 10% decrease was observed.

Carotenoids

Similar to chlorophyll-b, carotenoids were less than the control through out the growth phase. It was 46%, 53%, 67%, 64%, 70% less than the control on fourth, sixth, eighth, tenth and twelfth day respectively. At the end of growth phase 67% decrease was observed for the treated sample.

Phaeophytin

Phaeophytin was 73% and 60% less than the control on fourth and sixth day. Eventhough there was 53% and 12% increase on eighth and tenth day, 50% and 33% decrease was observed on the twelfth and fourteenth day.

Photosynthetic end products

Carbohydrate

There was total suppression in carbohydrate (Fig.93). It was 75%, 96%, 89% and 72% less than the control on eighth, tenth, twelfth and fourteenth day.

Protein

Protein was less than the control through out the growth phase. Maximum protein was on the eighth day but it was 49% less than the control. It was 58%, 68% and 11% less than the control on tenth, twelfth and fourteenth day respectively.

Lipid was found to be comparatively high for four metal combinations. It was 83% more than the control. Of the nutrients, the phosphate and nitrate uptake were 50% and 40% less than the control.

Fig.91

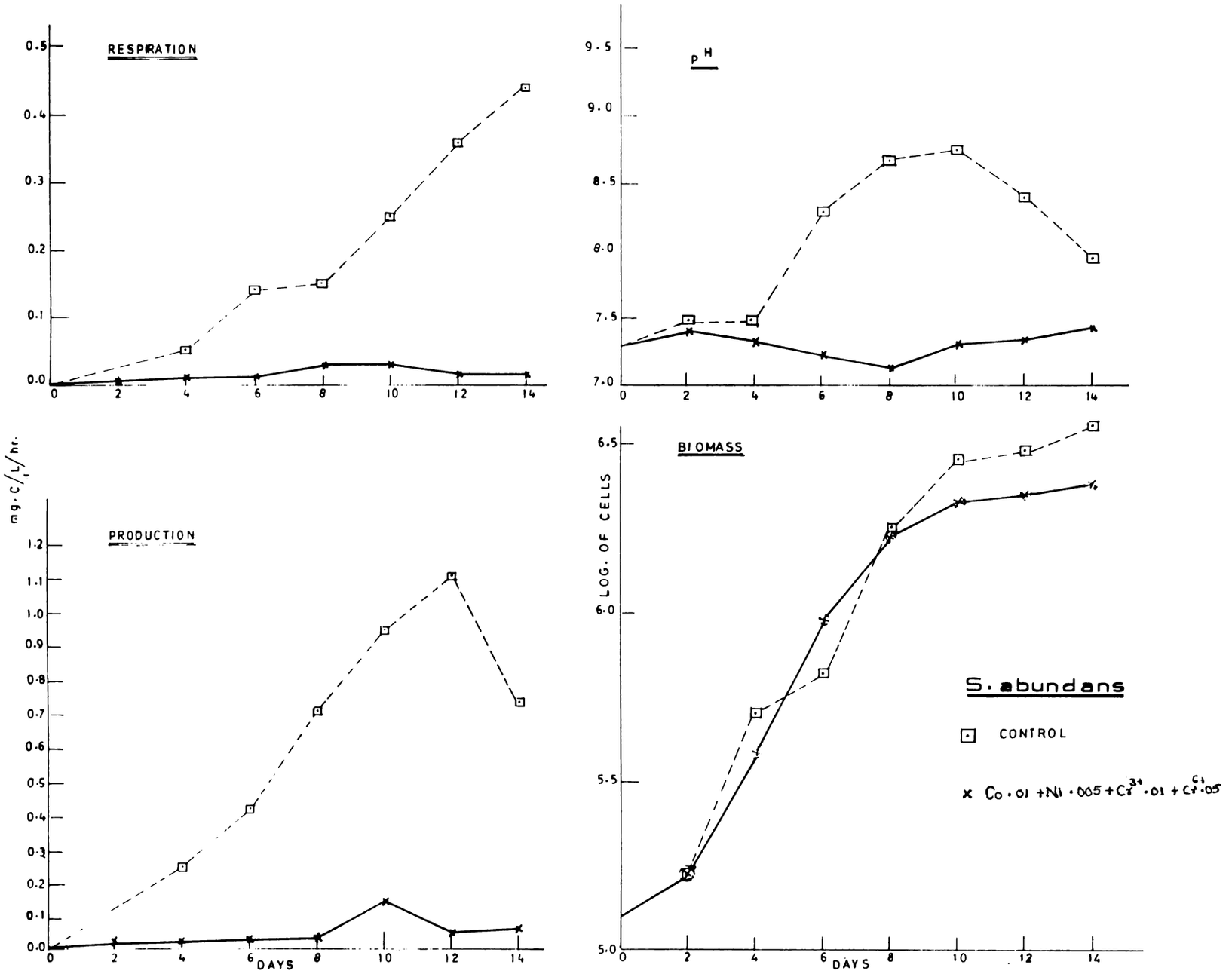
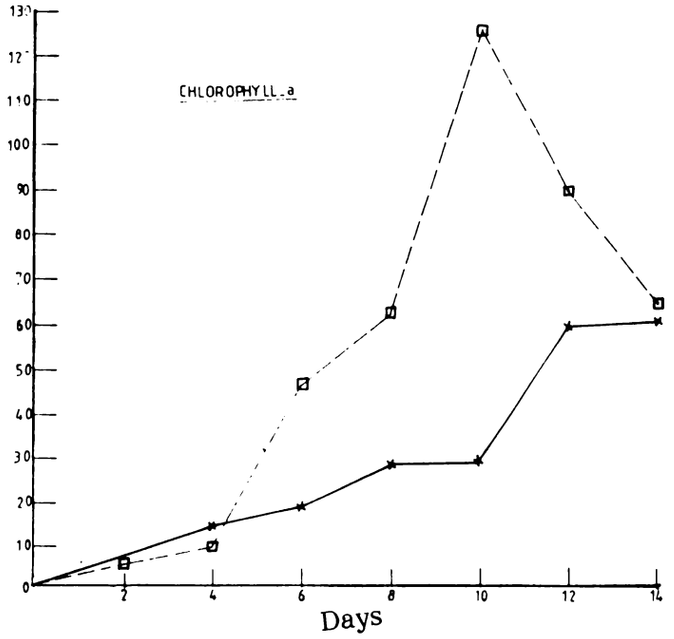
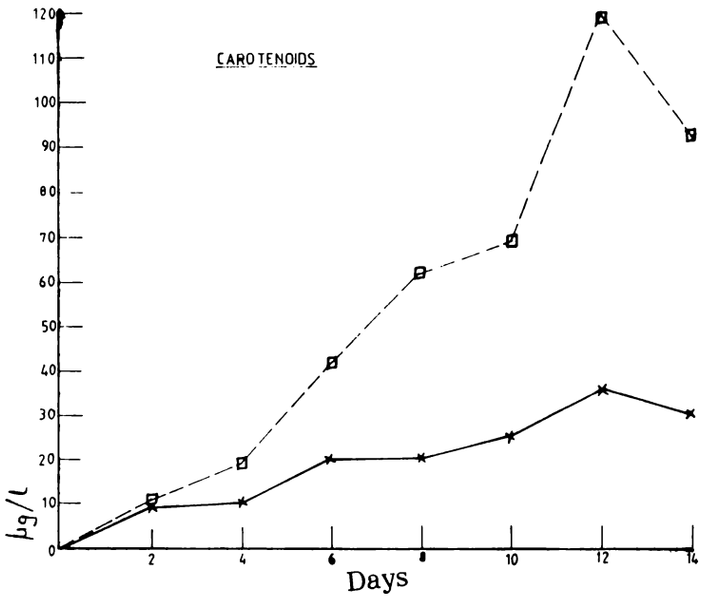
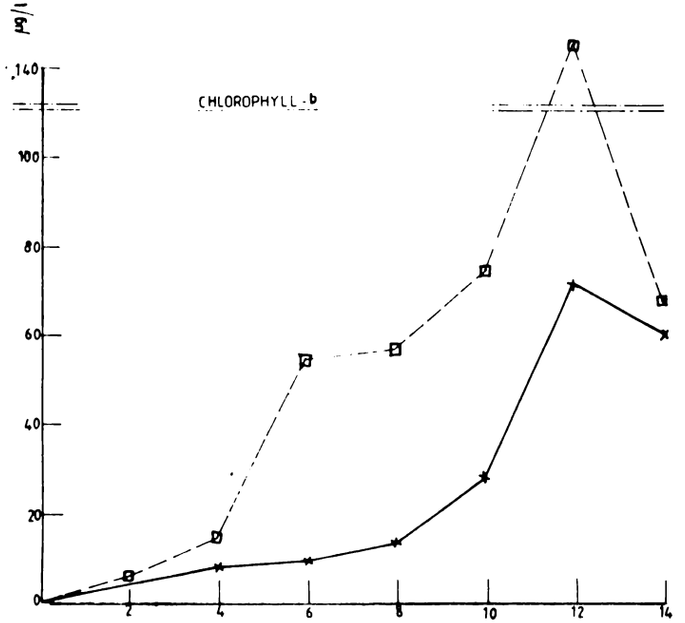

RESPIRATION
PH
PRODUCTION
BIOMASS
mg·C/L/hr.
LOG. OF CELLS
DAYS
DAYS
S. abundans
CONTROL
× Co·01 +Ni·005 +Cr3+·01 + Cr6+·05

Fig.92


CHLOROPHYLL a
Days


CAROTENOIDS
µg/l
Days


µg/l
CHLOROPHYLL b

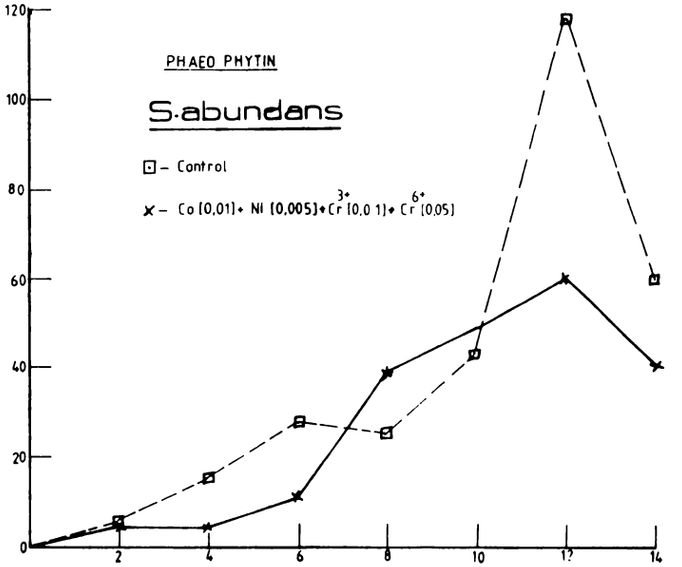
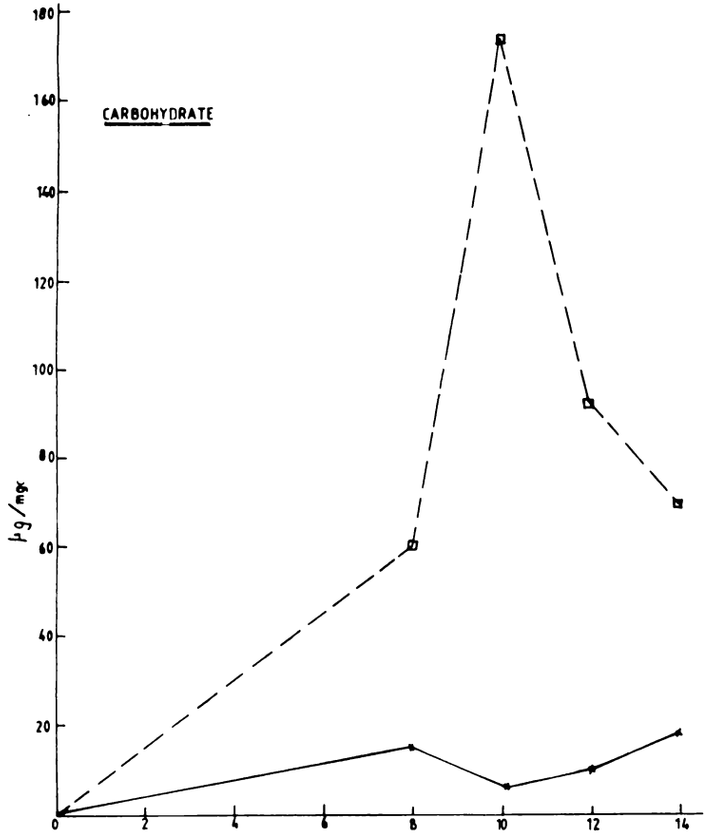
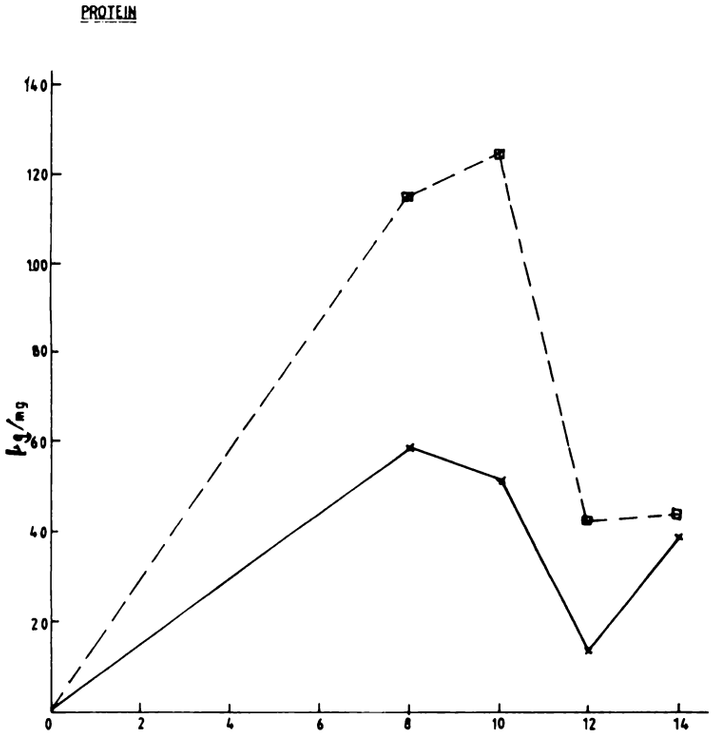
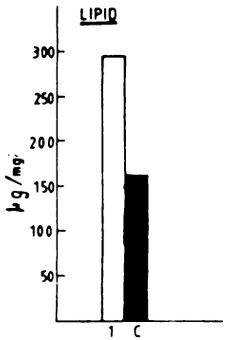

PHAEO PHYTIN
S.abundans
□ – Control
× – Co [0,01] + Ni [0,005] + Cr 3+ [0,01] + Cr 6+ [0,05]

Fig.93





Days



S-abundans

C ◻ CONTROL

I ✕ Co·01 + Ni·005 + Cr^{3+} (0.01) + Cr^{6+} (0,05)

Combined effect of cobalt, nickel trivalent and hexavalent chromium in N. clausii

Concentration of metals (in ppm)	Treatment Number
Co 0.3 + Ni 0.4 + Cr^{3+} 0.2 + Cr^{6+} 0.2	50

Biomass

A general trend of decrease was noticed for the treated sample when compared with the control (Fig.94). It was showing a lag upto second day. There was 45%, 50%, 41% and 50% decrease on fourth, sixth, eighth and tenth day respectively.

Production

Production was 58% and 49% less than the control on fourth and tenth day. It was similar to the control on the sixth day and 44% increase was observed on the eighth day. Eventhough pH was showing an increasing trend, it was less than the control at the end of growth phase.

Respiration

Respiration showed an initial increase of 62% on the fourth day (Fig.94) but it was 85%, 71% and 75% less than the control on sixth, eighth and tenth day respectively.

Photosynthetic pigments

All pigments were less than the control through out the growth phase for four metal combination.

Chlorophyll-a

Chlorophyll-a was similar to the control on the second day but it was 45%, 32% and 33% less than the control on fourth, sixth and eighth day. On the tenth day though there was an increasing trend, it was 6% less than the control.

Chlorophyll-c

Chlorophyll-c was less than the control through out the growth phase (Fig.95). It was 74%, 78%, 60% and 29% less than the control on fourth, sixth, eighth and tenth day.

Carotenoids

Similar to chlorophyll-c, carotenoids were also less than the control through out the growth phase (Fig.95). It was 49%, 36%, 26% and 18% less than the control on fourth, sixth, eighth and tenth day respectively.

Phaeophytin

Phaeophytin was less than the control upto sixth day but with the aging of the culture it was more than the control (Fig.95). It was similar to the control on fourth day but 18% decrease was noticed on the sixth day,on the eighth and tenth day 14% and 13% increase was observed for the treated sample.

Photosynthetic end products

Carbohydrate

The acid soluble and insoluble fraction of carbohydrate showed an increase in the first half of the growth but in the second half it was less than the

control. The alkali soluble fraction was less than the control through out the growth phase.

The acid soluble fraction was 1% and 31% more than the control on fourth and sixth day. But on eighth and tenth day 86% and 67% decrease was observed.

The alkali soluble fraction was 64%, 18%, 80% and 50% less than the control on fourth, sixth, eighth and tenth day respectively.

The insoluble fraction was closely following the control on fourth day, 17% decrease was observed on the sixth day. But 35% and 43% increase was noticed on eighth and tenth day respectively.

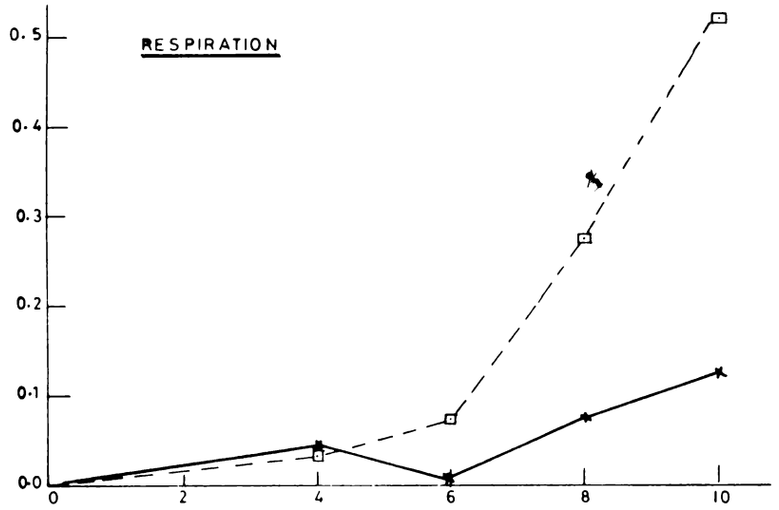
Protein

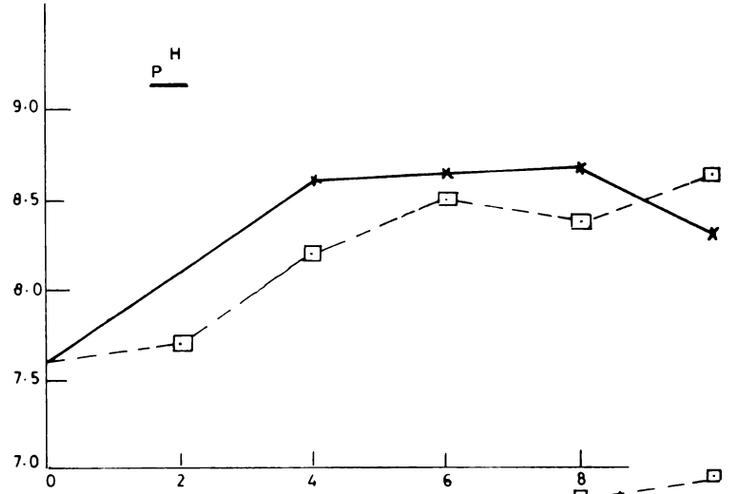
Protein content was increased and it was more than the control in the first half of growth phase. Protein content was maximum on the sixth day and it was 152% more than the control. On the fourth day it was 97% more than the control. 48% and 72% decrease was observed on the eighth and tenth day for the treated sample.

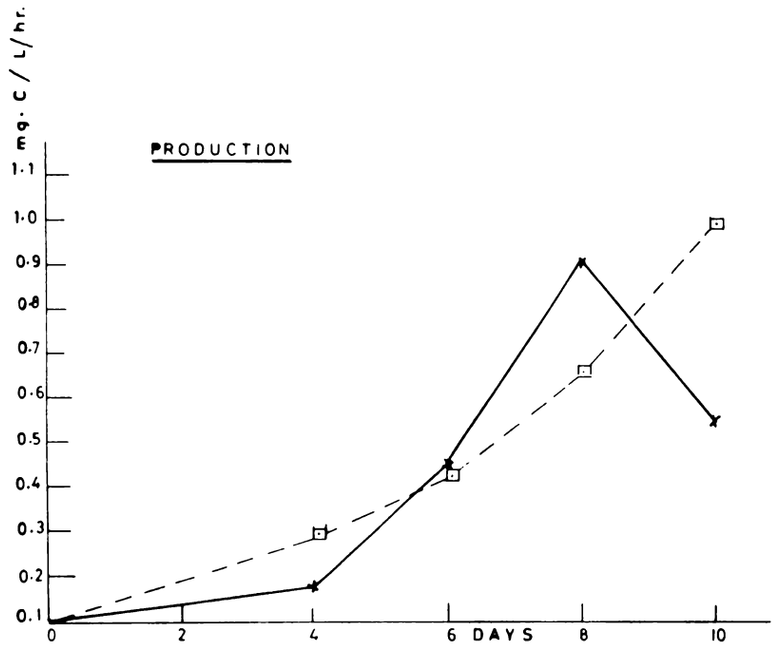
There was considerable decrease in the lipid content of the algae. In N. clausii maximum decrease in lipid content was also for the four metal combination of cobalt, nickel, trivalent and hexavalent chromium.

Eventhough there was decrease in all parameters of productivity by the effect of four metals in combination, the nutrient uptake was found to the maximum. There was 66% and 70% increase in the uptake of phosphate and nitrate by N. clausii.

Fig.94


RESPIRATION
0·5
0·4
0·3
0·2
0·1
0·0
0
2
4
6
8
10


P H
9·0
8·5
8·0
7·5
7·0
0
2
4
6
8


PRODUCTION
mg·C / L/hr.
1·1
1·0
0·9
0·8
0·7
0·6
0·5
0·4
0·3
0·2
0·1
0
2
4
6 DAYS
8
10

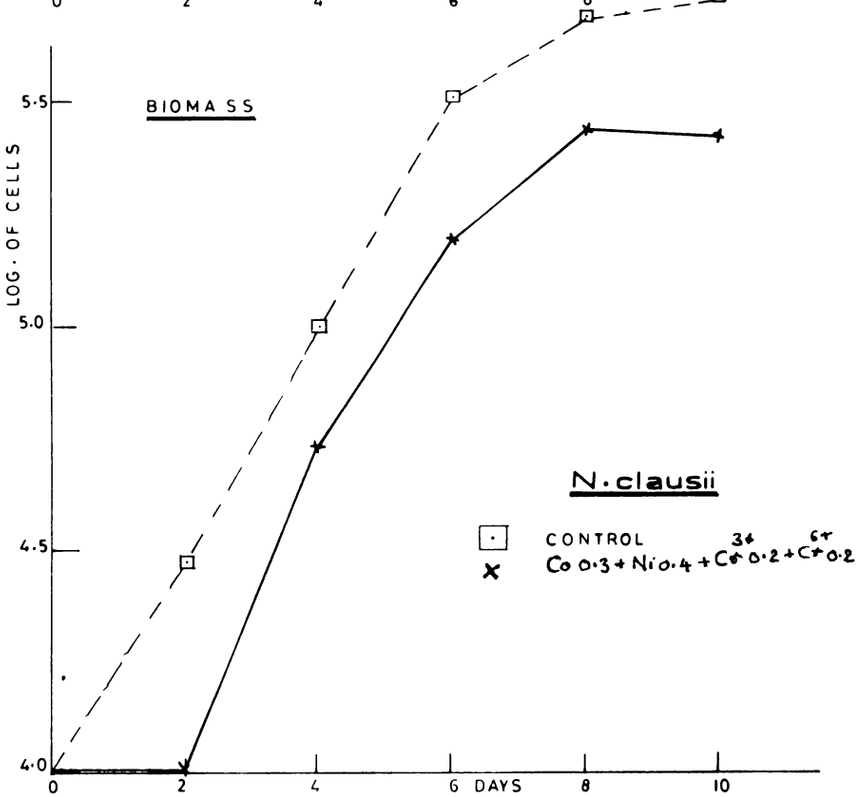
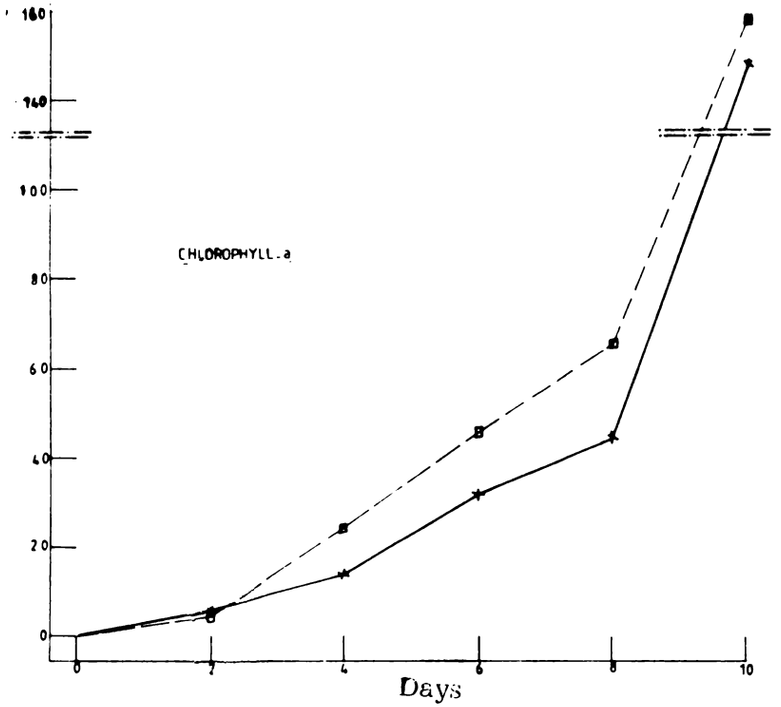
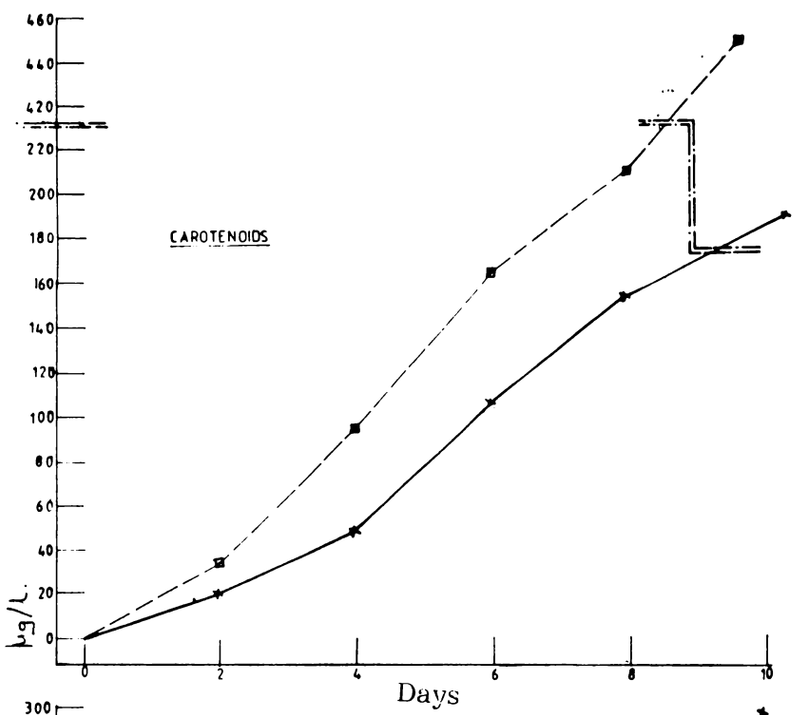
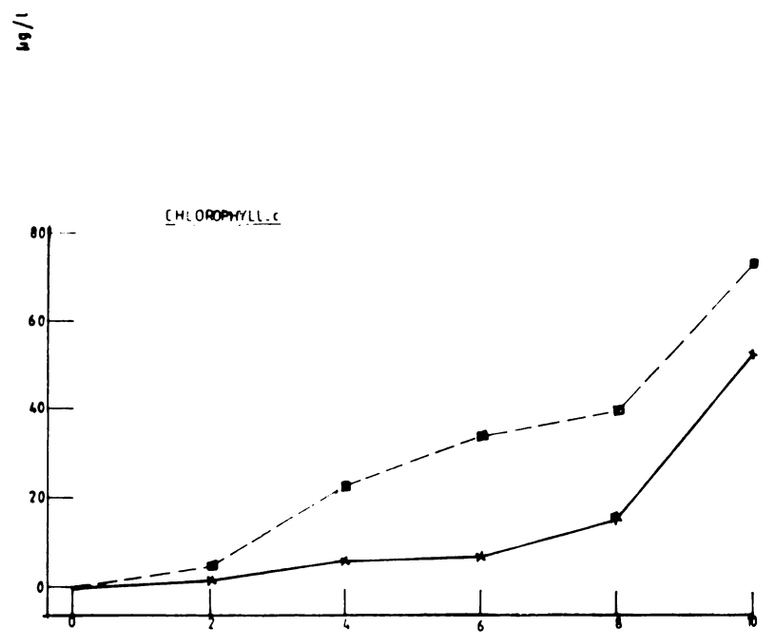

BIOMASS
LOG. OF CELLS
5·5
5·0
4·5
4·0
0
2
4
6 DAYS
8
10
N. clausii
CONTROL
Co 0·3 + Ni 0·4 + Cr 0·2 + Cr 0·2

Fig.95


CHLOROPHYLL.a
Days


CAROTENOIDS
µg/L
Days


CHLOROPHYLL.c
µg/l

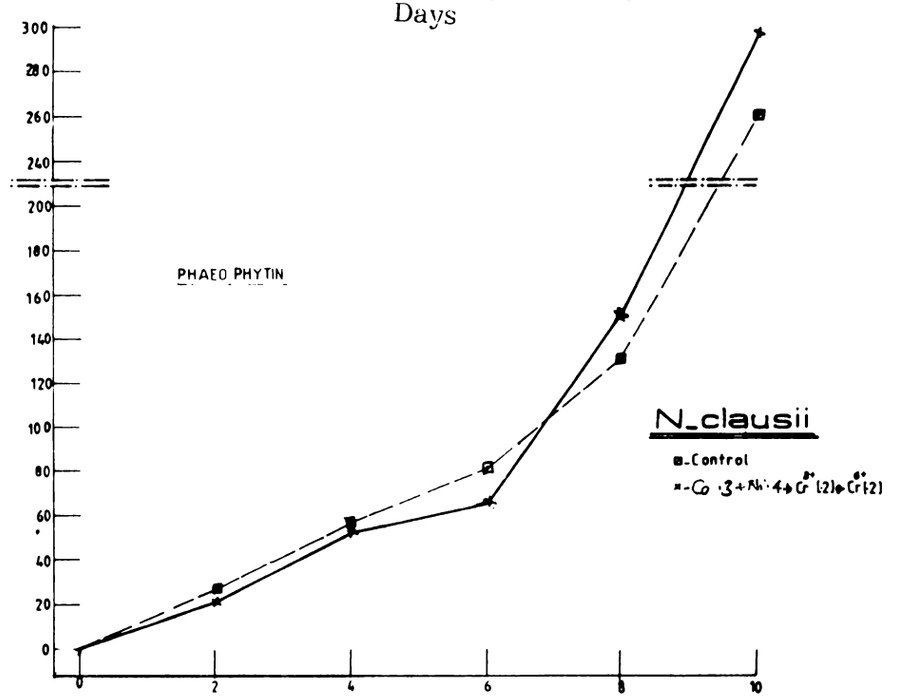
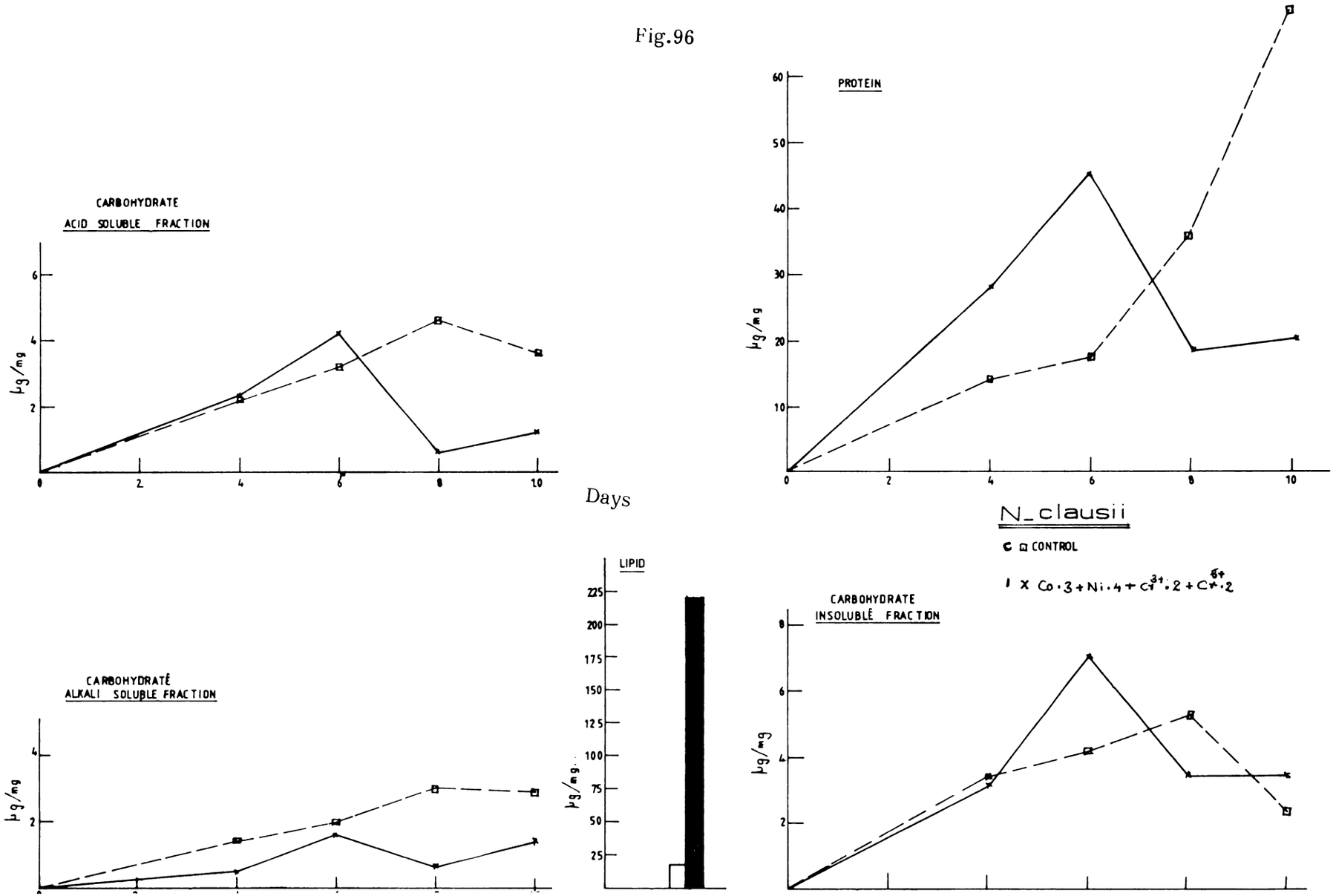

PHAEO PHYTIN
N_clausii
■-Control

Fig.96


CARBOHYDRATE
ACID SOLUBLE FRACTION
µg/mg
Days
PROTEIN
N_clausii
C □ CONTROL
1 X Co·3+Ni·4+Cr3+·2+Cr6+·2
LIPID
µg/mg
CARBOHYDRATE
ALKALI SOLUBLE FRACTION
CARBOHYDRATE
INSOLUBLE FRACTION

DISCUSSION

In natural waters, the metals are present together in solution, when mixed together they have supplemental synergistic or antagonistic effect on biota (Eaton, 1973; Sprague and Ramsay, 1965). Studies of Braek et al. (1976) indicated that complex effects on growth and development of phytoplankton may be expected when mixtures of heavy metals were introduced into the marine habitat. The effect of combination of metals cannot be based on result obtained with metals applied separately.

In the present study, it was observed that growth was less than the control throughout the growth phase for both species.

Several authors have found that heavy metals cause prolongation of the lagphase more or less in proportion to the dosage followed by normal growth (Steeman-Nielson and Wium-Andersen 1970 and Bartlett et al. (1974). The common explanation for this phenomenon seems to be that the medium is modified during the first part of the experiment either by exudation from living cells or by leaching from dead cells to render the heavy metal less toxic by some sort of chelation and that the residual cells then can grow normally in the later half of experiment. According to Braek et al. (1976) reduced growth rate and lowered final cell yields were the main results of heavy metal toxicity.

In S. abundans, the production and respiration was completely suppressed in treatment (49). In N. clausii treatment (50) also similar trend was observed but production was slightly more than the control on the sixth and eighth day respectively.

In the studies on the effect of mixture of metals on fresh water algae by Wong et al. (1978), it was reported that the presence of a mixture of metals in the growth medium inhibited about 70% of the primary productivity in Scenedesmus quadricauda. Obviously the mechanism involved in the multiple metal toxicity are very complex. In the studies on manganese - copper synergism by Christensen and Scherfig (1979) it was found that excess of one metal will interfere with the photosynthetic function of the other.

In both S. abundans and N. clausii all pigments were less than the control through out the growth phase. This may be due to the joint effect of a group of four metals. Studies on the effect of a group of metals on Thalassiosira aestivalis by Hollibaugh et al. (1980) found that synergistic effect was produced with respect to toxicity on phytoplankton. It was observed that the toxic effect of four metal combination was more when compared with individual effects of cobalt, nickel, trivalent and hexavalent chromium and also two metal combination of these metals.

On the studies of Skeletonena costatum it was concluded that the effect of combination of metals was greater than the sum of metals caused by the components applied separately (Braek and Jensen, 1976). The complex effects on growth and development of the phytoplankton may be expected when mixture of heavy metals were introduced into the marine habitat. Predictions of joint effects cannot be based on results obtained with the metals applied separately.

In the case of photosynthetic end products such as carbohydrate and protein, notable decrease was observed in S. abundans. Whereas in N. clausii protein and carbohydrate were more than the control in the first half of growth

phase but alkali soluble fraction of carbohydrate was completely suppressed. Notable decrease in lipid content was also observed. Whereas in S. abundans the lipid was found to be more than the control. This may be due to tying up of cells against the toxic effect of metals.

Analysis of variance was carried out to establish the significance of variance for four metal combinations in both the species studied. Table 9.

The combination of cobalt, nickel, trivalent and hexavalent chromium was highly significant in the case of biomass, and pigments in S. abundans. But respiration and protein was significant only between days. The end products such as carbohydrate and lipids were also significant.

In N. clausii, the combination of cobalt, nickel, trivalent and hexavalent chromium was significant in the case of biomass and production. Respiration, pH, chlorophyll-c and carotenoids were significant only between days. The end products of photosynthesis such as protein and carbohydrate were highly significant.

Table 8. Results of the significance of the variance ratio between concentration between days in S. abundans

Sl. No.	Metals employed	Selected parameters																									
		pH		Biomass		Production		Respiration		Chlorophyll-a		Chlorophyll-b		Carotenoids		Phaeophytin		Carbohydrate		Protein			Lipids
		1	11	1	11	1	11	1	11	1	11	1	11	1	11	1	11	1	11	1	11	1					
1.	Co+Ni+ Cr^{3+}+ Cr^{6+}	a	a	a	a	NS	a	NS	b	a	b	a	a	NS	a	a	a	a	a	NS		b	b				

Results of the significance of the variance ratio between concentration and between days in N. clausii

Sl. No.	Metals employed	Selected parameters																											
		pH		Biomass		Production		Respiration		Chlorophyll-a		Chlorophyll-c		Carotenoids		Phaeophytin		Carbohydrate fraction(1)		Carbohydrate fraction(2)		Carbohydrate fraction(3)		Protein		Lipids	
		1	11	1	11	1	11	1	11	1	11	1	11	1	11	1	11	1	11	1	11	1	11	1	11	1	
1.	Co+Ni+ Cr^{3+}+ Cr^{6+}	a	a	a	a	a	a	NS	a	a	a	NS	a	NS	a	a	a	a	a	NS	NS	a	a	a	a	a	

a P < 0.01
b. P < 0.05
NS Non-Significant

1 Concentration
11 Days

5.5 Quantitative study of the metal uptake as determined by AAS

The metal concentration of algae may be a better reflection of the presence of these metals in the environment than single or occasional measurement of the metal concentration determined directly from the waters. Phytoplankton provide great surface area by which pollutants may be concentrated from water.

According to Sakagunchi et al. (1979) and Hasett et al. (1980) metal absorption is a physio chemical process not mediated by metabolic processes. The absorption and accumulation of metals by phytoplankton have been reviewed in depth by Davies (1978). From the studies on the uptake of zinc in Phaeodactylum tricornutum and mercury in Isochrysis galbana Davies (1978) reported that uptake is a passive process involving rapid adsorption on to the cell membrane followed by diffusion controlled transport into cytoplasm at rates proportional to the concentration of surface bound metal.

In the present investigation it was observed that accumulation of all metal ions increases as the level in the medium was increased. The weight of the metal/milligram dry weight of algae increased with increase of metal in the medium and generally decreased with the age of the culture.

The organisms growing in natural waters are adapted to the environmental condition and the metal concentration of that water. So the algae growing in polluted water was adapted to the metal concentration in that medium. So the initial concentration is to be considered while studying its toxic effect. In the present study, concentration of metals accumulated was found to be too low because of very low concentration added to the test medium. Skaar et al. (1974) and Eide et al. (1979) reported that concentration of nickel in

the cells of Phaeodactylum tricornutum was proportional to that in the medium upto a level of 750 ppb.

Eventhough it was reported by Fayed et al. (1983) that most of the metal accumulation took place in the first two hours, the samples for the present study were taken only at the end of growth phase. The uptake was not taken from aged cultures, since long term experiments were complicated by factors like aging of cells and in the case of batch cultures accumulation of organic extra cellular material had taken place. According to Hasett et al. (1980) there was significant increase in metal accumulation with the increase in metal : algal exposure ratio.

In the present investigation, the trivalent and hexavalent chromium were estimated as total chromium in three metal and four metal combination studies.

Binding to protein controlled the level of nickel in the cell because during population growth cycle, the concentration reached the maximum and decreased as the amount of protein in the cell declined (Skaar et al., 1974). It was also reported by Skaar et al. (1974) that in phosphate starved cells, the capacity for nickel accumulation was low and it was enhanced by pre-treatment with phosphate due to the synthesis of new binding sites.

Thus it was concluded that toxicity of metals was strongly dependent on the composition of the culture medium, density of the culture and metal losses through adsorption, precipitation or volatilization. Some values were found higher than initial concentration. Further detailed study is proposed. If the higher values is due to metal contamination in the culture media, it would have reflected in all readings.

Table 9. Uptake of metals in S. abundans and N. clausii as determined by A.A.S.(µg/100 mg dry wt algae)

S. abundans		N. clausii	
Metal concentration (ppm)	Accumulation	Metal concentration (ppm)	Accumulation
Nickel		Nickel	
0.01	0.0054	0.4	0.0975
0.02	0.031	0.6	0.1723
0.03	0.025		
Cobalt		Cobalt	
0.05	0.0678	0.3	0.0843
0.1	N.E.*	0.5	0.1129
0.25	N.E.*	0.6	0.1612
Cr^{3+}		Cr^{3+}	
0.01	0.004	0.4	0.021
0.02	0.003	0.6	0.031
0.03	0.0054	0.8	0.047
Cr^{6+}		Cr^{6+}	
0.05	0.002	0.3	0.003
0.1	0.0082	0.6	0.008
0.15	0.017	0.7	0.011

*N.E. - Not Estimated

Table 10. Uptake of metals in S. abundans as determined by AAS (µg/100 mg dry wt. algae)

Metal concentration (in ppm)	Accumulation	
Co and Cr^{3+}	Co	Cr^{3+}
Co 0.01 + Cr^{3+} 0.03	0.0105	0.01552
Co 0.05 + Cr^{3+} 0.01	0.0336	0.022
Co 0.1 + Cr^{3+} 0.02	0.09221	0.0118
Co and Cr^{6+}	Co	Cr^{6+}
Co 0.01 + Cr^{6+} 0.05	0.0112	0.004
Co 0.01 + Cr^{6+} 0.15	0.022	0.098
Co 0.05 + Cr^{6+} 0.05	0.0413	0.0186
Co 0.1 + Cr^{6+} 0.1	0.08	0.0189
Ni and Cr^{3+}	Ni	Cr^{3+}
Ni 0.01 + Cr^{3+} 0.01	0.001	0.002
Ni 0.02 + Cr^{3+} 0.02	0.0272	0.0076
Ni 0.03 + Cr^{3+} 0.03	0.079	0.0095
Ni and Cr^{6+}		
Ni 0.01 + Cr^{6+} 0.05	0.0027	0.0027
Ni 0.02 + Cr^{6+} 0.1	0.0146	0.029
Ni 0.03 + Cr^{6+} 0.15	0.046	0.0089
Co and Ni	Co	Ni
Co 0.01 + Ni 0.005	0.0047	0.006
Co 0.05 + Ni 0.005	0.008	0.0262

Table 11. Uptake of metals in N. clausii as determined by AAS

(µg/100 mg dry wt : algae)

Metal concentration (in ppm)	Accumulation	
Co and Cr^{3+}	Co	Cr^{3+}
Co 0.3 + Cr^{3+} 0.2	0.1179	0.0304
Co 0.5 + Cr^{3+} 0.2	0.1346	0.029
Co 0.6 + Cr^{3+} 0.6	0.1669	0.032
Co 0.3 + Cr^{3+} 0.8	0.1137	0.033
Co 0.6 + Cr^{3+} 0.8	0.1841	0.03
Co and Cr^{6+}	Co	Cr^{6+}
Co 0.3 + Cr^{6+} 0.2	0.0908	0.0072
Co 0.3 + Cr^{6+} 0.6	0.0145	0.0029
Co 0.6 + Cr^{6+} 0.2	0.1726	0.0035
Co 0.6 + Cr^{6+} 0.6	0.1632	0.005
Co 0.6 + Cr^{6+} 0.6	0.1852	0.005
Ni and Cr^{3+}	Ni	Cr^{3+}
Ni 0.4 + Cr^{3+} 0.8	0.1149	0.008
Ni 0.6 + Cr^{3+} 0.2	0.1390	0.021
Ni 0.6 + Cr^{3+} 0.8	0.1370	0.024
Ni and Cr^{6+}	Ni	Cr^{6+}
Ni 0.6 + Cr^{6+} 0.2	0.1313	0.0028
Ni 0.6 + Cr^{6+} 0.6	0.1361	0.0056
Ni 0.8 + Cr^{6+} 0.2	0.1657	0.003
Co and Ni	Co	Ni
Co 0.3 + Ni 0.6	0.1084	0.1239
Co 0.6 + Ni 0.6	0.1476	0.1344

Table 12. Uptake of metals in S. abundans as determined by AAS

(µg/100 mg dry wt: algae)

Metal concentration (in ppm)	Accumulation	
	Co	Cr^{3+}
Co 0.05 + Cr^{3+} 0.01 + Fe 0.05	0.009	0.008
Co 0.01 + Cr^{6+} 0.05 + Fe 0.05	0.004	0.006
Ni 0.01 + Cr^{6+} 0.05 + Fe 0.05	0.005	0.0057
Ni 0.01 + Cr^{6+} 0.05 + Fe 0.05	0.0082	0.013

Uptake of metals in N. clausii as determined by AAS

(µg/100 mg dry wt: algae)

Metal concentration (in ppm)	Accumulation	
	Co	Cr^{3+}
Co 0.6 + Cr^{3+} 0.6 + Fe 0.2	0.1035	0.0281
Co 0.6 + Cr^{6+} 0.6 + Fe 0.2	0.084	0.012
Ni 0.6 + Cr^{3+} 0.8 + Fe 0.2	0.1334	0.0289
Ni 0.6 + Cr^{6+} 0.6 + Fe 0.2	0.1390	0.0113

Table 13. Uptake of metals in N. clausii as determined by AAS (µg/100 mg dry wt: algae)

Metal concentration (in ppm)	Accumulation		
	Co	Ni	Cr^{3+}
Co 0.3 + Ni 0.4 + Cr^{3+} 0.8	0.0895	0.0913	0.0064
Co 0.3 + Ni 0.6 + Cr^{3+} 0.2	0.0886	0.1181	0.007
	Co	Ni	Cr^{6+}
Co 0.3 + Ni 0.4 + Cr^{6+} 0.6	0.1066	0.0944	0.0032
Co 0.3 + Ni 0.6 + Cr^{6+} 0.2	0.0974	0.1264	0.0083
	Co	Cr^{3+}	Cr^{6+}
Co 0.3 + Cr^{3+} 0.2 + Cr^{6+} 0.2	0.0933	0.006	0.0069
Co 0.3 + Cr^{3+} 0.2 + Cr^{6+} 0.6	0.1231	0.0057	0.012
	Ni	Cr^{3+}	Cr^{6+}
Ni 0.6 + Cr^{3+} 0.2 + Cr^{6+} 0.6	0.0915	0.0036	0.0068

Table 14. Uptake of metals in S. abundans and N. clausii as determined by AAS (µg/100 mg dry wt: algae)

Metal concentration (in ppm)	Accumulation		
S. abundans	Co	Ni	Cr^{3+} + Cr^{6+} *
Co 0.01 + Ni 0.005 + Cr^{3+} 0.01 + Cr^{6+} 0.05	0.001	0.0025	0.014
N. clausii			
Co 0.3 + Ni 0.4 + Cr^{3+} 0.2 + Cr^{6+} 0.2	0.0096	0.0667	0.0185

* The two forms of chromium was estimated as total chromium.

CHAPTER - VI

GENERAL DISCUSSION

GENERAL DISCUSSION

The effect of heavy metals on the growth, biochemical and physiological properties as well as its accumulation of metals in algal biomass has been studied by a number of authors. The use of algal toxicity tests to monitor the biological impact of pollutants is important because algae are primary producers in aquatic systems. Such tests provide an approach in determining compatible levels of trace elements.

The present investigation on the effect of heavy metals cobalt, nickel, trivalent and hexavalent chromium on S. abundans and N. clausii was restricted to laboratory based bioassays employing a single species grown axenically.

The use of batch cultures allowed the simultaneous study of the effect of a wide range of metal concentration upon the fixed cell concentration of the innoculum. The use of an innoculum of uniformly low density in these investigations reduced the possibility of an algal overload and helped to have an exact picture of the effect of metals on the physiology of algae. Harris et al. (1970) reported that toxicity of mercuric compounds decreased with increasing cell concentration. Ben-Bassat et al. (1972) indicated that cell concentration affected the toxicity of mercury - II to Chlorella pyrenoidosa.

In the present study, the growth of S. abundans and N. clausii were satisfactory in the presence of nutrients. But chelating agents were omitted from culture because they have been shown to counteract inhibition of growth in toxicity studies. (Hart, 1975; Hart and Scaife, 1977). Hutchinson and Stokes (1975) omitted organic chelators to minimise chemical complications when

investigating heavy metal toxicity to algae. Gardiner (1974 a,b) and Tevlin (1978) reported that EDTA displayed a strong tendency to complex with cadmium.

In the toxicity studies, N. clausii was found to be more tolerant to metals than S. abundans. This may be due to the fact that S. abundans was collected from an unpolluted fresh water pond whereas N. clausii was from a polluted estuary.

Goldberg (1976) has reported that estuaries are often the most heavily contaminated marine regions with regard to heavy metals. It has also been found that phytoplankton inhabiting estuarine water are significantly more tolerant to certain heavy metals than their oceanic counterparts. According of Guillard and Kilham (1977) the cells inhabiting coastal or estuarine waters are more opportunistic and more tolerant to abiotic stresses than their counterparts from oceanic waters. Antonovics (1971) studied higher plants and concluded that tolerance from one metal does not generally confer tolerance to another metal.

Nickel and cobalt, though considered as essential elements are toxic at higher concentrations. Nickel was stimulatory in 0.01 ppm but was toxic in 0.03 ppm for S. abundans whereas in N. clausii such a marked variation was not observed.

In 0.01 and 0.02 ppm nickel, all pigments were showing a substantial increase with the aging of the culture. The end products of photosynthesis such as carbohydrate and protein were also showing an increase in the early phase in S. abundans. The increase in protein in the early stage of growth might be due to the fact that production of protein was governed by the amount

of nitrate in the medium. This is in accordance with the observation of Myklestad (1974), as the concentration of protein was almost constant after the medium had become depleted of nitrate.

An increase in the pigment content in S. abundans exceeding that of control may be explained as a consequence of increased carbon flow (Sudha, 1989). According to Lustigman (1986) the possible reason for the increased amount of both carotenoids and chlorophyll may be due to accumulation of both pigments as a result of delayed cell division. This he observed in the toxicity studies of copper on Dunaliella tertiolecta.

Photosynthetic end products such as protein and carbohydrate showed marked increase for S. abundans and N. clausii by the effect of nickel. It was reported that 5 to 50 micromolar nickel was found to increase the cell protein in Circosphaera carterae (Stillwell and Holland, 1977). The variation found in the quantity of pigment and photosynthetic end products during growth phase may be due to the entry of metal into the cell resulting in the channelization of the photosynthetic energy into different components at different times (Satya and Balakrishnan, 1988)

Unlike nickel, cobalt reduced the pigment production in both species. The possible reason for the decrease in the chlorophyll content by the effect of metal at the end of growth may be due to displacement of magnesium from chlorophyll molecules by metal ions (Wu and Lorenzen, 1984 and Gross et al., 1970). In the case of end products of photosynthesis, the decrease in carbohydrate may be due to respiration of water extractables carbohydrates by phytoplankton (Handa and Tominagan, 1969 and Hitch cock, 1977).

There was variation in the toxic effect by both forms of chromium. Eventhough there were reports that hexavalent chromium was more toxic than trivalent form, in S. abundans the reverse was observed. The trivalent form was more toxic in S. abundans whereas in N. clausii the hexavalent form was toxic.

Significant increase in production and respiration was noticed in both species by the effect of hexavalent chromium. Thus inhibition of cell division without any decrease in photosynthesis was observed. Fischer and Jones (1981) showed that in Asteronella japonica copper treated cells photosynthesised at normal rates, with cells continuing to enlarge when fixed carbon could not be excreted or utilised in cell division.

Respiration was more than the control through out the growth phase for hexavalent chromium treated samples. With the aging of the culture, the respiration increased with the peak at the end of growth phase. The present explanation for the appearance of significant increase in carbon assimilation in the dark bottles was due to effects upon the dark carbon dioxide pathway (Pillard, 1987). Babich et al. (1982) also reported that hexavalent chromium stimulated the carbon dioxide production.

There was decrease in production and respiration at higher concentration (0.02 and 0.03 ppm) of trivalent chromium. This may be due to the cumulative behaviour of trivalent chromium (Stary et al., 1982). But a substantial increase was observed in the end products of photosynthesis such as carbohydrate and protein by the effect of trivalent chromium. The increase in the carbohydrate

in N. clausii may be due to the synthesis of acid soluble glucan, which is probably the common reserve polysaccharide in diatom (Myklestad, 1974).

Toxic effect of trivalent chromium (0.03 ppm) was reversed by addition of cobalt in S. abundans. 0.03 ppm trivalent chromium was toxic in isolation. But toxicity was reduced by 0.01 ppm cobalt. In all combinations selected for study, the cobalt above 0.01 ppm reduced the growth and end products in S. abundans. Thus eventhough 0.05 ppm hexavalent chromium stimulated the growth and pigments, it was reduced by the effect of 0.05 ppm cobalt. But in N. clausii the concentration selected for individual study was used for combination. Nickel and cobalt are essential elements for growth. But both in combination was toxic. An improvement was observed in very low concentration of both metals in S. abundans.

Hutchinson (1973) suggested that for many algae nickel and copper mutually enhance the toxicity of each other and it is an example of heavy metal synergism.

Combinations of nickel and chromium reduced the pigments and the production. Verma et al. (1982) reported that in Mystis vittalis the combination of hexavalent chromium and nickel was highly synergistic. Azeez and Banerjee (1987) on their studies on Anacystis nidulans and Spirulina plantensis reported that the presence of other metals had synergistic effect on nickel toxicity.

The three metal combination experiments showed that production and respiration was reduced considerably but well marked increase in protein and carbohydrate was observed for all selected combinations except the combination

of nickel, cobalt and trivalent chromium in N. clausii. The possible reason for the increase in protein in the three metal combinations may be attributed to the sensitivity of the cells to the metals due to which the cells were rendered amenable for easy extraction of protein (Dorsey et al., 1977).

The combination of four metals reduced all parameters of growth. There was lag in growth in S. abundans and N. clausii. Photosynthetic pigments and end products such as protein and carbohydrate were reduced. But there was a notable increase in the major end product of photosynthesis such as lipids in S. abundans. This may be due to accumulation of the final end product of photosynthesis due to tying up of cells against stress caused by the combined effect of metals. Presence of iron also reduces the toxic effect as far as the formation of end products are considered.

Steemann - Nielson and Kamp Nielsen (1970) found evidence that natural communities counteract the influence of heavy metal toxicity either in a chemical or physical way.

In addition to species tolerance there are other factors that offset metal toxicity including metal interactions, algal competitions, the ratio of an excess metal to other metal and nutrient level. Fortunately living organisms have the capacity to counter act these factors in the natural environment upto a certain degree and it is for the monitoring and enforcing agencies to see that threshold levels are not crossed.

SUMMARY

The thesis embodies the results of a study on the variations in the parameters of productivity of two test species, a chlorophycean alga and a diatom. The chlorophycean alga Scenedesmus abundans was isolated from a fresh water pond whereas the diatom Nitzschia clausii was from the Cochin backwaters. Their growth parameters and their variations due to the effect of addition of some heavy metals have been studied. The growth parameters include biomass, production, respiration, photosynthetic pigments and end products of photosynthesis. The cell numbers were estimated by using a haemocytometer and production and respiration by oxygen light and dark technique. Spectrophotometric analysis for pigments, anthrone method for carbohydrate and heated biuret method for protein were the different methods employed in the present investigation.

The present study is confined to nickel, cobalt, trivalent and hexavalent chromium. Different metals are discharged from various industries in and around Cochin. The effects of these metals individually and in combination were studied. Experiments to determine the effects of interaction of metals in combination enabled the assessment of the antagonistic and synergistic effect of metals on test species. The concentration or accumulation of metals on algae was determined by Atomic Absorption Spectrophotometry.

The effect of metals showed marked difference between concentrations in S. abundans whereas in N. clausii such a clear cut variation was not observed. All metals were found to enhance the growth at low concentrations but were toxic at high concentrations.

Nickel stimulated the growth, pigment content and production in low concentration (0.01 ppm) but was toxic at high concentration (0.03 ppm) to S. abundans resulting in the decrease in growth, production, pigment content and end products. But in N. clausii, eventhough 0.6 ppm was toxic, such a marked variation similar to S. abundans was not observed.

The relatively high biomass observed in the early phase of growth was not reflected in the production and pigments in S. abundans even in low concentration of cobalt. In N. clausii though there was an initial increase, at the end of growth phase, production and pigments were reduced.

Of the two forms of chromium, the trivalent one was found to be more toxic in S. abundans than hexavalent chromium. All productivity parameters showed reduction. Morphological changes occur like two celled structure becoming unicellular . However, in N. clausii growth was reduced only at a higher concentration of 0.8 ppm. On the other hand hexavalent chromium was more toxic to N. clausii than trivalent form. The increase in respiration was observed in the early phase in both species with peak at the end of growth phase.

In S. abundans the combination of low concentration of cobalt and high concentration of trivalent chromium was antagonistic while the same concentrations in isolation were toxic. But 0.05 ppm cobalt in combination with 0.01 ppm trivalent chromium had a synergistic effect resulting in the decrease in production, pigment content and end products of photosynthesis.

Combination of trivalent chromium and cobalt in N. clausii enhanced the production and photosynthetic pigments such as chlorophyll-a, chlorophyll-c and protein content, whereas carotenoids were reduced. Hexavalent chromium

stimulated the production and respiration, whereas cobalt inhibited the same. But the two metals together in S. abundans reduced the production, respiration, chlorophyll and phaeophytin. There was much variation between treatments in the case of end products of photosynthesis.

In concentrations above 0.01 ppm cobalt, in combination with hexavalent chromium, the end products were markedly reduced whereas individual concentration of the same stimulated the protein and carbohydrate. The combination of cobalt and hexavalent chromium was synergistic for growth in N. clausii but was antagonistic for photosynthetic end products such as carbohydrate.

The combination of nickel and trivalent chromium had general antagonism for growth resulting in the increase in biomass in S. abundans. But combination produced synergism resulting in the decrease in production, respiration and pigments. Combination of nickel and hexavalent chromium had synergistic effect resulting in the reduction of production, respiration and pigments in S. abundans and antagonism was noticed in the case of photosynthetic end products.

Combination of cobalt and nickel produced general synergism resulting in the decrease in biomass, production, respiration and pigments in S. abundans. In N. clausii combination of cobalt and nickel had much variation between treatments. Though cobalt and nickel were considered as essential elements individually for the better growth of algae, the combination of the two was toxic.

When iron was employed in combination with the two metal combination, it resulted in a substantial increase in the end products of photosynthesis such

as carbohydrate and protein in both species. Combination of iron together with cobalt and hexavalent chromium stimulated the production in the first half of growth phase in S. abundans, but was reduced in the latter half.

The three metal combination experiments with N. clausii showed that production and respiration reduced considerably but well marked increase in protein and carbohydrate was observed. The combination of nickel, cobalt and trivalent chromium reduced the production and respiration considerably whereas combination of two metals such as cobalt and trivalent chromium stimulated the production. The combination of nickel, cobalt and hexavalent chromium reduced the production, respiration and pigment content. But the two metal combination of cobalt and hexavalent chromium improved the production. The protein content improved in the combination of nickel, cobalt and hexavalent chromium but it was reduced in the combination of cobalt and nickel.

The interaction of cobalt, trivalent and hexavalent chromium and combination of nickel, trivalent and hexavalent chromium showed marked synergism resulting in the reduction in production, respiration and pigments. But significant increase in pigment content was noticed in the combination of nickel and trivalent chromium.

The combination of four metals selected for study, reduced all the growth parameters of productivity resulting in the total suppression of growth in S. abundans and in N. clausii. But a notable increase in lipid content was noticed in S. abundans which was not observed in N. clausii.

It was thus concluded that species tolerance, metal interactions, ratio of excess metal to another, algal competition and nutrient level along with the growth phase determine the tolerance levels and toxicity.

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*Not referred to the original.

APPENDIX

Table - 15 Effect of nickel on different parameters of productivity in S. abundans

Days	Control					Ni - 0.01ppm					Ni - 0.02ppm					Ni - 0.03ppm				
	a	b	c	d	e	a	b	c	d	e	a	b	c	d	e	a	b	c	d	e
2nd	16.95	—		5.79	6.141	35.4	—		33.55		29					25.6				
4th	50.2	.253	.0503	9.92	14.36	77.26	.0704	0.058	33.85	30.16	43	.177	.059	26.73	42.38	36	.030	.029	21.38	37.86
6th	66	.422	.145	47.12	56.58	105	.379	0.089	86.87	72.66	78	.1966	.177	84.64	95.75	61	.135	.044	40.10	49.26
8th	176	.714	.150	63.01	55.18	269	.754	.1206	227.20	57.4	143	.362	.1809	120.29	71.84	177	.181	.058	53.46	41.01
10th	283	.955	.256	126.57	54.41	273	1.129	.058	329.67	184.4	169	.766	.118	400.95	213.01	202	.239	.060	106.92	78.16
12th	304.6	1.132	.361	89.86	86.71	311	1.277	.060	240.57	202.54	264	1.120	.147	400.95	202.54	297	.663	.302	320.76	71.55
14th	355	.738	.444	64.63	67.53	336	1.162	.364	115.83	187.52	271	.748	.358	53.46	219.04	303	.318	.439	26.73	81.73

a - Biomass (Cell concentration/ml x 10^3) b - Production mgc/l/hr, c - Respiration mgc/l/hr.

d - Chlorophyll - a - µg/l e - Chlorophyll - b µg/l

	f	g	h	i	f	g	h	i	f	g	h	i	f	g	h	i
2nd	11.31	4.87			2.8	-			4.2	-	-		22.13			
4th	18.86	14.34			15.2	8.28	-		14	19.78		-	33.2	27.53		-
6th	42.16	27.25	—		46	28.06	—		38	38.76			57.3	20.71		
8th	61.45	24.85	60.93	115.49	66	85.54	209.59	264.94	59.3	46.77	113.85	243.01	28	33.85	108.74	234.19
10th	68.5	42.76	164.11	125	184	86.42	135.21	84.74	152	229.88	73.39	105.95	32	4.01	66.66	91.55
12th	118.66	118.67	93.93	42.61	145	200.47	44.7	48.83	160	282.44	69.78	84.84	44	78.18	57.66	99.37
14th	91.8	60.05	69	43.57	149.3	116.72	164.73	96.12	204	175.08	124.96	87.92	36	36.09	165.35	118.12

g - Carotenoid µg/l, g - Phaeophytin µg/l, h - Carbohydrate µg/mg, i - Protein µg/mg

Table - 16 Effect of nickel on different parameters of productivity in N. clausii

Days	Control						0.4						0.6					
	a	b	c	d	e	f	a	b	c	d	e	f	a	b	c	d	e	f
2nd	3	–	-	43.53	46.53	33.37	3	-	--	24.94	39.24	17.06	3		–	30.29	74.82	17.3
4th	10	.2037	.030	241.05	230.3	93.94	5	.4050	.063	294.94	243.24	132.6	16	.3251	.053	378.67	368.8	148
6th	32.5	.3389	.079	463.3	338.9	165.2	24	.5455	.044	521.23	429.96	243	32	.4746	.122	621.47	458.9	235
8th	48.5	.5667	.272	658.8	403.2	209.9	42	.7570	.251	935.55	405.62	369	34	.843	.254	962.28	456.8	378
10th	53.1	.8943	.495	1583.8	740.1	450.6	65	.8259	.324	1029.1	525.3	422.6	57	.735	.272	955.60	713.9	374

a - Biomass (Cell concentration/ml x 10^3) b - Production mgc/l/hr, c - Respiration mgc/l/hr,
d - Chlorophyll-a µg/l e - Chlorophyll-c µg/l f - Carotenoids µg/l

	g	h	i	j	k	g	h	i	j	k	g	h	i	j	k
2nd	26.96		-		-	4.8					5.8		–	–	–
4th	58.3	2.21	1.38	3.47	14.49	37.4	8.379	4.85	7.64	72.64	24	3.78	3.69	7.7	55.63
6th	81.26	3.23	2.01	4.26	17.88	74.1	3.41	2.54	7.13	47.91	143	4.85	.794	5.74	43.07
8th	131.77	4.63	3.04	5.22	35.25	152.4	5.29	.743	6.01	55.41	150.6	3.85	.826	5.69	39.28
10th	260	3.68	2.9	2.369	69.02	100.5	2.95	.778	13.11	72.08	62.1	.522	1.37	9.18	77.92

g - Phaeophytin µg/l h - Carbohydrate acid - fraction - µg/mg
i - Carbohydrate - alkali fraction µg/mg, j - Carbohydrate - insoluble fraction µg/mg, k - Protein µg/mg

Table - 17

Effect of Cobalt on different parameters of Productivity in S. abundans

Days	Control					Co - 0.05					Co - 0.1					0.25			
	a	b	c	d	e	a	b	c	d	e	a	b	c	d	e	a	b	c	d	e
2nd	16.95	-	–	5.79	6.141	31	–	–	-	-	12	-	-	–	-	21	-	-	–	–
4th	50.2	.2534	.050	9.92	14.36	54.7	.060	.119	28.51	12.01	21	.059	.482	5.35	28.95	40.3	0.088	.044	13.37	50.52
6th	66.	.422	.144	47.12	56.58	109.2	.118	.419	40.09	42.94	40	.060	.178	10.69	20.48	60	0.069	.044	16.04	79.8
8th	176	.7136	.150	63.01	55.18	193	.241	.241	93.55	84.77	81	.181	.060	66.82	104.02	177.6	0.088	.060	53.46	77.05
10th	283	.955	.256	126.57	54.41	243	.708	.241	71.28	87.52	107	.413	.177	80.19	110.21	182	.312	0.089	75.73	86.4
12th	304.6	1.132	.362	89.86	86.71	347	.905	.294	147.02	83.66	138	.639	.241	97.92	93.04	201.6	.574	.298	66.83	111.45
14th	355	.738	.444	64.63	67.53	333	1.025	.362	160.37	111.74	223	.483	.121	124.74	93.02	182.3	.119	.436	84.64	96.59

a - Biomass (Cell concentration /mlx10^3) , b - Production mgc/l/hr, c - Respiration mgc/l/hr

d - Chlotophyll-a /ug/l e - Chlorophyll-b /ug/l

	f	g	h	i	f	g	h	i	f	g	h	i	f	g	h	i
2nd	11.31	4.86	–	–	10	–	–	–	14	–	–	–	31.2	–	–	–
4th	18.86	14.34	–	–	19.2	13.36	–	–	20.8	17.1	–	–	54.4	37.95	–	–
6th	42.16	27.25	–	-	30	46.77	–	–	17.6	4.81	–	–	84	62.81	–	–
8th	61.45	24.85	60.93	115.49	46.6	52.12	103.2	45.84	46	15.06	102.6	147.56	48	35.19	103.54	20.78
10th	68.5	42.76	164.11	125	64.6	40.48	51.72	59.07	48	24.06	63.4	69.16	42	34.08	34.49	44.04
12th	118.66	118.67	93.93	42.61	50	35.42	36.37	99.2	54	24.06	44.89	49.78	36	47.44	7.27	125.74
14th	91.8	60.05	69	43.57	60	29.4	75.01	56.65	58	7.35	97.82	54.42	32	54.79	20	149.08

g - Carotenoid /ug/l, g - Phaeophytin /ug/l, h - Carbohydrate /ug/mg, i - Protein /ug/mg

Table - 18 Effect of cobalt on different parameters of productivity in N. clausii

Days	Control						Co - 0.3						Co - 0.4						Co - 0.5					
	a	b	c	d	e	f	a	b	c	d	e	f	a	b	c	d	e	f	a	b	c	d	e	f
2nd	3	–	–	45.5	46.53	33.37	9	–	–	10.6	61.8	15	6	–	–	64.1	54.8	24	5.5	–	–	32.1	96.4	19
4th	10	.204	.030	241	230.3	93.9	19.5	.276	.072	222.7	173	89.3	21	.313	.059	247.2	194	83	12	.130	.024	133.6	175	59
6th	33	.339	.079	463.3	338.9	165.2	35	.352	.114	405	223.6	145	42	.332	.043	394.2	234	146	24	.226	.048	334.1	243	119
8th	48	.567	.272	658.8	403.2	210	48	.633	.339	793	484.1	285	61	.472	.354	788.5	524	280	40	.352	.223	641.5	499	225
10th	53	.894	.495	1583	740.1	451	49	.373	.358	882	474.2	35 7	71	.796	.280	922.1	505	349	44	.548	.368	558.3	451	291

a - Biomass (cell concentration/ml x 10^3, b - Production mgc/l/hr, c - Respiration mgc/l/hr, d - Chlorophyll - a ʌug/l,

e - Chlorophyll - c - ʌug/l, f - Carotenoids ʌug/l.

	g	h	i	j	k	g	h	i	j	k	g	h	i	j	k	g	h	i	j	k
2nd	26.96	–	–	–	–	49.18	–	–	–	–	3.2	–	–	–	–	20.31	–	–	–	–
4th	58.26	2.21	1.38	3.47	14.49	12.63	5·5	.451	3.65	14.28	12.03	4·0	.885	5.04	22.12	53.24	6·2	.167	7.48	16.14
6th	81.25	3.24	2.00	4.25	17.88	54.79	4·1	.267	4.46	26.96	30.73	5·0	.753	6.99	33.79	55	5·5	.381	4.17	9.05
8th	131.77	4.63	3.04	5.22	35.25	61.47	4·2	1.34	5.43	29.06	56.13	3·2	1.16	4.30	28.06	55·8	2·3	1.743	5.92	34.02
10th	260	3.68	2.92	2.37	69.02	109.59	4·4	1.61	4.2	36.37	122.9	2·9	1.66	4.98	37.38	56.13	2·0	1.31	6.76	29.7

g - Phaeophytin ʌug/l, h - Carbohydrate acid fraction ʌug/mg, i - Carbohydrate alkali fraction ʌug/mg,

j - Carbohydrate insoluble fraction ʌug/mg, k - Protein ʌug/mg.

Table - 19 Effect of trivalent chromium on different parameters of productivity in S. abundans

Days	Control					cr^{3+} - 0.01 ppm					cr^{3+} - 0.02 ppm					cr^{3+} - 0.03 ppm				
	a	b	c	d	e	a	b	c	d	e	a	b	c	d	e	a	b	c	d	e
2nd	16.9	–	–	5.79	6.14	27.6	–	–	–	–	32	–	–	–	–	21	–	–	–	–
4th	50.2	.254	.050	9.92	14.36	32	.059	.059	13.36	22.45	85	.177	.148	21.38	34.56	23	.118	.118	14.25	23.0
6th	66	.422	.144	47.12	56.58	53	0.060	.060	26.73	54.49	103	.236	.413	40.38	72.37	67	.059	.177	17.8	46.96
8th	176	.714	.150	63.01	55.18	137	.774	.089	80.19	21.47	219	.317	.358	31.47	106.77	94	.206	.089	44.55	29.72
10th	283	.956	.256	126.57	54.41	186	1.013	.060	80.19	137.4	236	.413	.357	40.09	117.2	159	.446	.1206	26.73	239.75
12th	304.6	1.133	.362	89.86	86.71	251	1.117	.151	106.92	104.76	272	.537	.353	26.73	81.73	161	.505	.178	106.92	156.85
14th	355	.738	.444	64.63	67.53	290	.895	.181	89.10	145.03	336	.654	.265	62.37	132.1	239	.807	.236	71.28	113.38

a - Biomass (Cell concentration/ml x 10^3) b - Production mgc/l/hr, c - Respiration mgc/l/hr
d - Chlorophyll-a - µg/l e - Chlorophyll - b µg/l

	f	g	h	i	f	g	h	i	f	g	h	i	f	g	h	i
2nd	11.31	4.869	–	–	1.8	5.88	–	–	3	–	–	–	4	–	–	–
4th	18.8	14.34	–	–	18.4	14.96	–	–	15.7	14.16	–	–	17.86	2.67	–	–
6th	42.1	27.25	–	–	13.3	30.7	–	–	26.6	18.71	–	–	20	8.93	–	–
8th	61.45	24.85	60.93	115.49	28	9.166	155.6	221.55	30.6	53.43	140	210	13.3	8.01	168.6	305.66
10th	68.5	42.76	164.11	125	20	49.89	96.96	154.06	32.6	33.41	92	140	13.3	43.67	89.18	131.61
12th	118.6	118.67	93.93	42.61	32	13.36	51.83	127.55	45.3	49.89	33	180	10.6	68.15	28.74	122.27
14th	91.8	60.06	69	43.57	29	62.36	91.6	62.48	60	50.78	68	32	29.3	110.47	42.72	66.81

f - Carotenoid µg/l, g - Phaeophytin µg/l, h - Carbohydrate µg/mg, i - Protein µg/mg

Table - 20 Effect of trivalent chromium on different parameters of productivity in N. clausii

Days	Control						cr^{3+} 0.3						cr^{3+} 0.6						cr^{3+} 0.8					
	a	b	c	d	e	f	a	b	c	d	e	f	a	b	c	d	e	f	a	b	c	d	e	f
2nd	3	–	–	43.5	46.5	33.4	5	–	–	26.7	55.7	18.4	1	–	–	5.34	43.1	5.2	2	–	–	21.3	36.93	4
4th	10	.203	.031	241	230.3	93.9	31	.479	.060	434.3	396	183	6	.113	.035	73.5	169.7	32	6	.176	.024	113.6	225.9	59
6th	33	.339	.078	463.5	339	165.2	43	.717	.151	615	517	236	35	.417	.065	481	570.1	186	48	.577	.112	721.7	554	249
8th	49	.567	.272	659	403	210	55	.978	.339	1016	580	361	49	.983	.254	895	638.2	304	56	.782	.324	975.6	463	371
10th	53	.894	.494	1583	740	451	71	1.061	.241	922	392	432	70	1.209	.237	962	410.11	346	60	.737	.339	340.8	241	189

a - Biomass (cell concentration/ml x 10^3), b - Production mgc/l/hr, c - Respiration mgc/l/hr, d - Chlorophyll - a µg/l,
e -Chlorophyll - c µg/l, f - Carotenoids µg/l.

	g	h	i	j	k	g	h	i	j	k	g	h	i	j	k	g	h	i	j	k
2nd	26.9	–		–	–	8.4	–	–	–	–	18.5	–	–	–	–	13.9	–	–	–	–
4th	58.3	2.21	1.38	3.48	14.5	85.5	1.175	3.9	6.51	76.2	57.5	1.78	5.16	2.99	18.65	7.3	2.43	6.89	3.74	19
6th	81.25	3.24	2.01	4.25	17.8	100	6.07	1.72	5.39	48.9	96.1	6.55	3.33	4.4	35.8	91.6	3.5	2.94	5.16	40
8th	131.7	4.63	3.04	5.22	35.3	187.5	6.26	.376	5.77	56.04	149	6.99	2.26	2.45	23.1	190	5.9	2.43	3.94	37.2
10th	260	3.68	2.92	2.37	69.0	267	3.39	.370	4.11	47.2	266	1.33	1.21	1.747	14.8	6.7	5.08	.720	1.19	13.8

g - Phaeophytin µg/l, h - Carbohydrate acid fraction µg/mg, i - Carbohydrate alkali fraction µg/mg,
j - Carbohydrate insoluble fraction µg/mg, k - Protein µg/mg.

Table - 21 Effect of hexavalent chromium on different parameters of Productivity in S. abundans

Days	Control					Cr^{6+} - 0.05 ppm					Cr^{6+} - 0.1 ppm					cr^{6+} - 0.15 ppm				
	a	b	c	d	e	a	b	c	d	e	a	b	c	d	e	a	b	c	d	e
2nd	16.95	–	–	5.79	6.141	16.4	–	–	–	–	19	–	–	–	–	16	–	–	–	–
4th	50	.2534	.050	9.92	14.359	21	.121	.118	5.346	12.04	24.6	0.06	.181	16.04	14.4	22	.059	.058	5.346	12.29
6th	66	.42207	.145	47.12	56.58	56	.178	.239	21.38	41.34	88.5	0.120	.209	26.73	28.95	59	.151	.181	10.69	23.04
8th	176	.71364	.150	63.01	55.18	141	1.35	.241	40.10	93.84	172	.236	.236	53.46	80.11	111	.181	.177	13.37	50.52
10th	283	.95455	.256	126.57	54.41	180	1.67	.385	80.19	110.20	246	1.097	.327	106.92	120.10	185	1.121	.120	26.73	84.77
12th	305	1.1328	.362	89.86	86.71	300	2.28	.537	102.29	93.84	291	1.94	.439	147.02	129.67	229	1.56	.531	80.19	93.84
14th	355	.73818	.444	64.63	67.53	309	2.48	.478	173.75	92.72	346	1.76	.657	240.56	142	223	1.45	.663	93.56	83.02

a - Biomass (Cell concentration /mlx10^3), b - Production mgc/l/hr, c - Respiration mgc/l/hr
d - Chlorophyll-a /ug / l, e - Chlorophyll-b /ug/l

	f	g	h	i	f	g	h	i	f	g	h	i	f	g	h	i
2nd	11.31	4·869	–	–	8.1	5·88	–	–	15.1	5·78	–	–	5.3	5·88	–	–
4th	18.86	14·34	–	–	20	15·36	–	–	32	14·16	–	–	10.1	14·34	–	–
6th	42.16	27·25	–	–	37.3	38·85	–	–	56	18·71	–	–	23.2	27·25	–	–
8th	61.45	24·85	60.93	115.49	54	46·77	129.74	224.49	78	33·41	119.4	264.94	29	24·85	169.68	249.9
10th	68.5	42·76	164.11	125	78	86·42	174.36	240.8	99	49·89	148.49	223.53	46.2	42·76	129.4	167.06
12th	118.66	118·76	93.93	42.61	90	68·15	82.53	81.52	123.1	50·1	66.92	81.40	59	68·9	58.76	57.05
14th	91.8	60·06	69	43.57	81	50·78	79.84	74.92	146	29·4	107.34	84.02	70	48·1	67.73	66.33

f - Carotenoid /ug/l, g - Phaeophytin /ug/l, h - Carbohydrate /ug/mg, i - Protein /ug/mg

Table - 22 Effect of hexavalent chromium on different parameters of productivity in N. clausii

Days	Control						Cr^{6+} 0.4						Cr^{6+} 0.6						Cr^{6+} 0.7					
	a	b	c	d	e	f	a	b	c	d	e	f	a	b	c	d	e	f	a	b	c	d	e	f
2nd	3	-	-	43.5	46.5	33.3	6	-	-	48.1	76.5	16	2	-	-	32	96.2	6.8	3	-	-	42	48.9	6
4th	10	.204	.031	241	230.3	93.9	19	.386	.055	388	202	140	4	.045	.037	53	254	31	4	.091	.031	94	225	40
6th	33	.339	.078	463	339	165.2	34	.358	.065	628	253	205	10	.281	.037	347	626	145	12	.423	.061	588	392	193
8th	49	.567	.272	659	403	209.9	40	.497	.254	842	369	308	26	.295	.176	481	641	216	42	.995	.229	868	462	322
10th	53	.894	.495	1583	740	451	-	.517	.344	909	369	363	29	.716	.091	768	380	295	52	1.13	.337	1055	347	383

a - Biomass (cell concentration/ml x 10^3) b - Production mgc/l/hr, c - Respiration mgc/l/hr, d - Chlorophyll - a µg/l,

e - Chlorophyll-c µg/l, f - Carotenoids µg/l.

	g	h	i	j	k	g	h	i	j	k	g	h	i	j	k	g	h	i	j	k
2nd	26.96	-	-	-	-	22.98	-	-	-	-	19.3	-	-	-	-	17.1	-	-	-	-
4th	58.3	2.21	1.38	3.48	14.49	32.07	3.33	.736	2.71	26.7	77.5	7.27	.993	2.02	5.46	33.4	3.89	4.49	2.64	12.5
6th	81.26	3.24	2.01	4.25	17.8	104.2	2.11	.680	1.56	30.2	16.1	7.8	3.405	3.03	30.1	114.9	5.6	2.32	5.28	44.5
8th	131.7	4.63	3.04	5.22	35.3	93.5	.601	1.13	1.67	18.2	78.8	2.00	1.55	2.83	23.1	184	3.06	1.79	4.29	41.2
10th	260	3.68	2.92	2.37	69.02	70.8	2.73	.705	1.89	18.4	209.8	.460	.483	1.45	3.70	260	2.02	.315	1.78	25.04

g - Phaeophytin µg/l, h - Carbohydrate acid fraction µg/mg, i - Carbohydrate alkali fraction µg/mg, j - Carbohydrate insoluble fraction µg/mg

k - Protein µg/mg.

Table - 23 Effect of Cobalt and trivalent chromium on different parameters of productivity in S. abundans

Days	Control					Co 0.01 + cr^{3+} 0.03					Co 0.05 + cr^{3+} 0.01					Co .1 + cr^{3+} 0.02				
	a	b	c	d	e	a	b	c	d	e	a	b	c	d	e	a	b	c	d	e
2nd	16.95	-	-	5.79	6.141	22	-	-	26.73	-	20.6	-	-	-	-	16.4	-	-	-	-
4th	50.2	.2534	.0502	9.92	14.35	52	.249	.0294	43.44	20.12	46.2	.059	-	5.346	4.84	2.8	.029	-	5.346	10.78
6th	66	.4220	.1446	47.12	56.58	110	.427	.030	80.19	29.46	73.5	.060	-	31.86	8.95	52.3	.044	-	16.71	5.64
8th	176	.7136	.1503	63.01	55.18	164	.737	.059	129.2	38.55	92	.103	.0904	53.46	35.77	76.6	.0607	.0306	26.73	35.76
10th	283	.954	.2564	126.57	54.41	193	.786	.0442	124.74	44.58	110	.2238	.059	53.46	35.51	89.3	.135	.0612	53.46	31.65
12th	304.6	1.132	.3615	89.86	86.71	321	.806	.2946	84.65	27.26	203.5	.447	.030	89.10	17.08	119	.273	.0306	71.28	9.65
14th	355	.738	.444	64.63	67.53	337	.648	.4325	49.01	57.8	218	.476	.2979	106.92	17.08	117.6	.466	.1014	80.19	16.08

a - Biomass (Cell concentration /ml x 10^3) b - Production mgc/l/hr, c - Respiration mgc/l/hr
d - Chlorophyll. a /ug/l e - Chlorophyll. b /ug/l

	f	g	h	i	f	g	h	i	f	g	h	i	f	g	h	i
2nd	11.31	4.869	-	-	24	4.80	-	-	7.2	9.62	-	-	6.4	-	-	-
4th	18.86	14.34	-	-	32	10.08	-	-	23	4.05	-	-	24	9.62	-	-
6th	42.16	27.25	-	-	49.3	14.6	-	-	23	6.34	-	-	24	6.9	-	-
8th	61.45	24.85	60.95	115.49	78	14.3	93.16	108.35	36	30.06	47.52	79.67	38	38.75	47.86	146.52
10th	68.5	42.76	164.16	125	84	14.5	272.64	99.34	30	16.03	64.81	53.83	38	16.03	37.14	57.15
12th	118.66	118.67	93.93	42.61	64	5.02	87.61	21.96	46	8.011	32.99	42.66	52	20.04	30.28	46.07
14th	91.8	60.06	69	43.57	56	53.39	56.59	8.118	100	5.34	49.26	32.86	96	53.46	64.76	31.97

f - Carotenoid /ug/l, g - Phaeophytin /ug/l, h - Carbohydrate /ug/mg, i - Protein /ug/mg

Table - 24 a. Combined effect of cobalt and trivalent chromium on different parameters of productivity in N. clausii

Days	Control						Co 0.3 + cr^{3+} 0.2						Co 0.5 + cr^{3+} 0.2						Co 0.6 + cr^{3+} 0.2					
	a	b	c	d	e	f	a	b	c	d	e	f	a	b	c	d	e	f	a	b	c	d	e	f
2nd	3	–	–	43.5	46.5	33.4	2	–	–	42.8	133.8	24.8	3	–	–	37.4	115.8	22.4	1	–	–	16.1	120	28
4th	10	.203	.031	241.1	230.3	93.9	17	.496	.058	320.8	464.3	50	16	.379	.036	267.3	291.7	40	15	.284	.012	120.3	255	72
6th	33	.339	.079	463.3	336.9	165	26	.543	.071	481.1	532	174	28	.386	.067	267.3	359	140	30	.567	.072	227.2	405	144
8th	49	.567	.272	658.9	403.2	210	41	1.28	.088	694.9	613.8	254	34	1.102	.077	761.8	498	270	56	1.09	.181	828.6	380	292
10th	53	.894	.495	1584	740	451	50	1.036	.161	1216.2	693.4	438	54	1.51	.572	1189.5	696	412	20	1.5	.603	1403	646	468

a - Biomass (cell concentration/ml x 10^3) b - Production mgc/l/hr, c - Respiration mgc/l/hr, d- Chlorophyll - a µg/l,

e - Chlorophyll - c µg/l, f - Carotenoids - µg/l

	g	h	i	j	k	g	h	i	j	k	g	h	i	j	k	g	h	i	j	k
2nd	26.9	–	–	–	–	17.11	–	–	–	–	14.9	–	–	–	–	–	–	–	–	–
4th	58.3	2.21	1.38	3.48	14.5	6.68	4.81	4.16	5.05	43.9	1.34	7.95	3.39	3.29	41.9	17.4	1.62	1.21	9.88	22.8
6th	81.27	3.24	2.01	4.25	17.9	18.7	5.81	2.31	2.71	20.8	116.3	4.61	1.13	1.70	17.6	61.5	2.87	.892	4.02	21.5
8th	131.7	4.63	3.04	5.23	35.3	34.7	4.63	.794	2.11	26.59	30.7	3.43	1.04	3.11	35.1	125.6	3.47	.936	4.21	31.8
10th	260	3.68	2.92	2.37	69.1	84.19	4.84	2.43	4.54	80.4	66.8	2.41	1.51	4.22	50.8	354.2	2.79	3.53	3.78	43.8

g - Phaeophytin µg/l, h - Carbohydrate acid fraction µg/mg, i - Carbohydrate - alkali fraction µg/mg,

j - Carbohydrate insoluble fraction µg/mg, k - Protein µg/mg.

Table - 24 b. Contd... Combined effect of cobalt and trivalent chromium on different parameters of productivity in N. clausii

Days	Control						Co 0.3 + cr^{3+} 0.8						Co 0.6 + cr^{3+} 0.8					
	a	b	c	d	e	f	a	b	c	d	e	f	a	b	c	d	e	f
2nd	3	-	-	43.5	46.5	33.4	1	-	-	32.1	139.6	30	3	-	-	37.4	140.5	28
4th	10	.2037	.031	241.1	230.3	93.9	7	.151	.018	106.9	275.1	44	9	.195	.012	133.7	286.9	60
6th	33	.339	.078	463.3	338.9	165.2	17	.410	.083	294	379.1	122	20	.489	.035	347.5	317.3	134
8th	49	.567	.272	658.9	403.2	210	31	.716	.099	641.5	394.5	246	31	.492	.118	594.7	404.7	272
10th	53	.894	.495	1584	740.1	451	36	1.75	.596	1035.8	539.4	458	14	1.06	.314	1089.3	557.3	400

a - Biomass (Cell concentration/ml x 10^3) b - Production mgc/l/hr, c - Respiration mgc/l/hr
d - Chlorophyll - a /ug/l, e - Chlorophyll-b /ug/l, f - Carotenoids - /ug/l

	g	h	i	j	k	g	h	i	j	k	g	h	i	j	k
2nd	26.9	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4th	58.3	2.2113	1.38	3.48	14.5	37.4	8.81	1.04	5.59	11.6	17.4	9.91	.836	6.36	27.7
6th	81.26	3.24	2.00	4.25	17.8	12.1	2.88	.351	2.81	15.1	14.7	3.67	.234	3.30	20.9
8th	131.8	4.63	3.038	5.23	35.3	82.8	3.64	.927	3.86	22.4	128.3	5.51	.910	5.78	16.1
10th	260	3.68	2.92	2.37	69.1	156.4	2.25	3.17	7.29	53.5	153.6	1.67	2.319	4.55	27.2

g - Phaeophytin /ug/l, h - Carbohydrate - acid fraction /ug/mg, i - Carbohydrate - alkali fraction /ug/mg,
j - Carbohydrate - insoluble fraction /ug/mg, k - Protein /ug/mg

Table - 25 Effect of conbination of cobalt and hexavalent chromium on different parameters of productivity in S. abundans

Days	Control					Co 0.01 + cr^{6+} 0.05					Co 0.01 + cr^{6+} 0.15				
	a	b	c	d	e	a	b	c	d	e	a	b	c	d	e
2nd	16.9	-	-	5.79	6.14	21	-	-	35.64	17.18	22	-	-	-	-
4th	50.2	.2534	.0502	9.92	14.36	37	.1167	.0147	53.46	24.64	56	.1915	.011	44.55	26.89
6th	66.0	.422	.144	47.12	56.58	97	.3281	.0153	80.19	32.85	100	.306	.030	89.10	35.42
8th	176	.7136	.150	63.01	55.18	155	.644	.030	120.29	40.95	161	.649	.0612	151.47	42.23
10th	283	.9545	.256	126.57	54.41	185	.808	.0747	169.29	34.98	192	.823	.0607	164.84	40.48
12th	304	1.132	.362	89.86	86.71	331	.845	.1809	115.83	38.55	320	.878	.0808	115.84	31.94
14th	355	.7381	.444	64.63	67.53	330	.458	.2596	71.28	67.16	335	.549	.264	80.19	27.26

a - Biomass (Cell concentration/ml x 10^3) p - Production mgc/l/hr, c - Respiration mgc/l/hr.
d - Chlorophyll . a ⁄ug/l e - Chlorophyll . b ⁄ug/l

	f	g	h	i	f	g	h	i	f	g	h	i
2nd	11.31	4.8€	-	-	16.8	15.04	-	-	22.3	-	-	-
4th	18.86	14.34	-	-	26	20.01	-	-	42	-	-	-
6th	42.16	27.25	-	-	45.4	12.8	-	-	59.6	-	-	-
8th	61.45	24.85	60.93	115.49	70	23.3	82.22	122.28	84	16.03	107.9	116.8
10th	68.5	42.76	164.16	125	90	26.7	269.6	109.3	82	33.3	238.12	147.06
12th	118.66	118.67	93.93	42.6	74	12.02	93.18	20.12	82	54.03	77.93	22.14
14th	91.8	60.05	69	43.57	68	73.5	81.17	18.8	62	8.009	72.8	14.27

f - Carotenoid ⁄ug/l, g - Phaeophytin ⁄ug/l, h - Carbohydrate ⁄ug/mg, i - Protein ⁄ug/mg

Table - 26 a. Combined effect of cobalt and hexavalent chromium on different parameters of productivity in N. clausii

Days	Control						Co 0.3 + cr^{6+} 0.2						Co 0.3 + cr^{6+} 0.6						Co 0.6 + cr^{6+} 0.2					
	a	b	c	d	e	f	a	b	c	d	e	f	a	b	c	d	e	f	a	b	c	d	e	f
2nd	3	-	-	43.5	46.5	33.4	2	-	-	37.4	139.8	19.2	4	-	-	21.38	123.3	176	1	-	-	26.7	192.7	19.2
4th	10	.204	.030	241.1	230.3	93.9	20	.106	.029	254	92.2	116	12	.147	.024	173.7	72.4	72	13	.145	.024	180.4	75.3	93
6th	33	.339	.078	463.3	338.9	165.2	27	.419	.094	569	503.9	206	22	.294	.086	427.7	533.7	160	38	.484	.178	481.4	650.5	156
8th	49	.567	.272	658.9	403.2	210	67	1.03	.206	916	913.1	360	85	1.04	.245	788.5	683.3	296	96	.611	.178	708.4	836.5	234
10th	53	.894	.495	1584	704.1	451	73	1.15	.148	1403.3	1161.9	514	89	.643	.368	1349.9	1232.2	526	106	1.36	.177	1269.7	1607.9	504

a - Biomass (cell concentration/ml x 10^3), b - Production mgc/l/hr, c - Respiration mgc/l/hr, d - Chlorophyll-a µg/l,

e - Chlorophyll - b µg/l, f - Carotenoids µg/l.

	g	j	i	j	k	g	h	i	j	k	g	h	i	j	k	g	h	i	j	k
2nd	26.9	-	-	-	-	14.16	-	-	-	-	4.8	-	-	-	-	10.7	-	-	-	-
4th	58.3	2.21	1.38	3.46	14.5	38.7	8.72	.922	5.17	32.9	61.5	5.70	2.07	2.67	9.78	44.8	12.15	.920	3.39	13.05
6th	81.27	3.24	2.01	4.25	17.9	60.2	4.66	.991	7.4	30.2	48.8	4.64	2.91	5.08	20.98	22.9	5.93	.859	5.31	25.7
8th	131.8	4.63	3.04	5.23	35.3	26.7	3.95	1.41	4.39	38.7	67.5	3.40	.628	4.56	22.05	51.5	3.85	.436	4.20	30.9
10th	260	3.68	2.93	2.37	69.02	271	3.44	1.48	5.69	49.9	49.5	2.96	1.081	5.92	45.8	12.5	3.18	.989	6.45	40.5

g - Phaeophytin µg/l, h - Carbohydrate - acid fraction µg/mg, i - Carbohydrate - alkali fraction µg/mg,

j - Carbohydrate - insoluble fraction µg/mg, k - Protein µg/mg.

Table-26 b. Contd,,, Combined effect of cobalt and hexavalent chromium on different parameters of productivity in N. clausii

Days	Control						Co 0.6 + cr^{6+} 0.6						Co 0.8 + cr^{6+} 0.6					
	a	b	c	d	e	f	a	b	c	d	e	f	a	b	c	d	e	f
2nd	3	-	-	43.5	46.5	33.4	2	-	-	-	-	77	2	-	-	-	-	72
4th	10	.204	.031	241.1	230.3	93.9	19	.229	.042	160.4	437.3	92	13	.169	.031	106.9	487.9	89
6th	33	.339	.079	463.3	338.9	165.2	26	.326	.061	294	452.9	116	27	.293	.084	267.3	351.3	104
8th	49	.567	.272	658.9	403.2	210	33	.392	.073	581	627.7	220	48	.472	.097	801.9	658.9	300
10th	53	.894	.495	1584	740	451	89.5	.437	.256	768.5	173.8	278	56	.673	.192	968.9	167.7	346

a - Biomass (cell concentration/mg x 10^3) b - Production mgc/l/hr, c - Respiration mgc/l/hr,

d - Chlorophyll- a ug/l, e - Chlorophyll- b ug/l, f - Carotenoids ug/l

	g	h	i	j	k	g	h	i	j	k	g	h	i	j	k
2nd	26.9	-	-	-	-	53.4	-	-	-	-	28.8	-	-	-	-
4th	58.3	2.211	1.38	3.48	14.5	42.6	4.71	.970	1.2	11.4	30.5	2.17	.092	2.63	9.3
6th	81.26	3.24	2.01	4.25	17.9	32.07	1.14	.774	2.11	38.7	40.8	1.43	.358	1.35	21.1
8th	131.7	4.63	3.04	5.23	35.3	49.4	.325	1.004	3.38	12.9	69.9	1.02	4.42	2.11	14.1
10th	260	3.68	2.922	2.37	69.1	58.8	1.89	.579	3.51	14.2	136.3	2.78	.625	2.35	15.65

g - Phaeophytin ug/l, h - Carbohydrate - acid fraction ug/mg, i - Carbohydrate - alkali fraction ug/mg,

j - Carbohydrate - insoluble fraction ug/mg, k - Protein ug/mg

Table - 27 Effect of combination of nickel and trivalent chromium on different Parameters of Productivity in S. abundans

Days	Control					Ni 0.01 + cr^{3+} 0.01 ppm					Ni 0.02 + cr^{3} 0.02 ppm					Ni 0.3 + cr^{3} ppm				
	a	b	c	d	e	a	b	c	d	e	a	b	c	d	e	a	b	c	d	e
2nd	16.95	-	-	5.791	6.14	25	-	-	10.69	2.972	29	-	-	-		26	-	-	-	-
4th	50.2	.2534	.050	9.92	14.37	605	.0447	.014	14.25	5.616	42	.155	.014	10.69	25.97	41.1	.049	.030	10.69	7.81
6th	66	.4220	.144	47.12	56.58	110	.032	.015	26.73	15.4	74.4	.428	.059	26.73	26.97	50	.0663	.014	13.37	9.78
8th	176	.7137	.150	63.01	55.18	268	.6173	.075	40.10	34.4	219	.192	.162	60.15	105.95	68	.162	.044	26.73	12.11
10th	283	.9545	.256	126.57	54.41	324	1.149	.197	17.82	31.65	271.3	.757	.192	106.92	117.24	154	.589	.058	26.73	31.65
12th	305	1.132	.361	89.86	86.71	405	.853	.294	40.09	53.94	306	.826	.265	53.46	93.02	235	1.238	.078	93.55	72.66
14th	355	.73818	.444	64.63	67.53	422	.455	.358	44.55	94.65	326	.560	.619	26.73	133.74	322	.786	.442	53.46	122.74

a - Biomass (Cell concentration/ml x 10^{3}), b - Production mgc/l/hr, c - Respiration mgc/l/hr
d - Chlorophyll-a μg/l, e - Chlorophyll . b μg/l

	f	g	h	i	f	g	h	i	f	g	h	i	f	g	h	i
2nd	11.31	4.86	-	-	12.3	11.48	-	-	5.6	-	-	-	13.6	-	-	-
4th	18.86	14.34	-	-	13.6	12.56	-	-	12	9.88	-	-	11.2	3.20	-	-
6th	42.16	27.25	-	-	24	24.94	-	-	18	8.68	-	-	20.4	5.34	-	-
8th	61.45	24.85	60.93	115.49	42	24.94	185.48	164.48	66	36.08	148.35	71.05	34	5.34	88.2	155.01
10th	68.5	42.76	164.11	125	44	42.76	123.92	180.75	72	60.58	242.16	83.64	38	31.4	109.5	90.72
12th	118.66	118.67	93.93	42.61	60	48.14	151.85	52.34	64	80.18	66.08	14.86	84	33.4	53.38	54.55
14th	91.8	60.06	69	43.57	48	57.92	55.18	27.33	76	122.95	91.5	15.08	84	73.5	50.26	43.98

f - Carotenoid μg/l, g - Phaeophytin μg/l, h - Carbohydrate μg/mg, i - Protein μg/mg

Table - 28 Combined effect of nickel and trivalent chromium in N. clausii

Days	Control						Ni .4 + cr^{3+} .8						Ni .6 + cr^{3+} .2						Ni .6 + cr^{3+} .8					
	a	b	c	d	e	f	a	b	c	d	e	f	a	b	c	d	e	f	a	b	c	d	e	f
2nd	3	–	-	43.5	46.5	33.4	1	-	-	26.7	192	19	4	-	-	21.3	123	18	2	-	-	-	132	77
4th	10	.2037	.030	241	230	93.9	13	.145	.024	180	75	93	12	.147	.024	174	72	72	19	.229	.042	160	437	92
6th	33	.339	.079	463	339	165	38	.484	.178	481	650	156	21.5	.294	.085	428	533	160	26	.326	.061	294	453	116
8th	49	.567	.271	658	403	210	96	.611	.176	708	836	234	85	1.04	.244	789	683	296	33	.392	.073	581	628	220
10th	53	.894	.494	1584	740	451	106	1.36	.177	1270	1608	504	89	.643	.367	1350	1232	526	90	.437	.256	768	174	278

a - Biomass (cell concentration/ml x 10^3), b - Production mgc/l/hr, c - Respiration mgc/l/hr, d - Chlorophyll - a µg/l,
e - Chlorophyll-c µg/l, f - Carotenoids µg/l.

	g	h	i	j	k	g	h	i	j	k	g	h	i	j	k	g	h	i	j	k
2nd	26.96	–	-	–	–	10.69	-	-	–	–	4.8	-	–	-	-	53.4	–	–	-	–
4th	58.26	2.21	1.38	3.48	14.5	44.7	12.15	.920	3.39	13.1	61.5	5.69	2.07	2.67	9.78	–	4.71	.97	1.2	11.4
6th	81.26	3.24	2.00	4.26	17.8	22.68	5.93	.86	5.3	25.7	48.8	4.64	2.9	5.07	20.9	32.07	1.14	.77	2.11	38.7
8th	131.77	4.63	3.04	5.23	35.3	51.5	3.85	.44	4.2	30.9	67.5	3.40	.628	4.56	22.1	49.4	.325	1.01	3.38	12.9
10th	260	3.68	2.9	2.37	69.1	12.5	3.18	.99	6.44	40.5	49.4	2.96	1.08	5.91	45.8	55.8	1.89	.579	3.51	14.2

g - Phaeophytin µg/l, h - Carbohydrate acid fraction µg/mg, i - Carbohydrate alkali fraction µg/mg,
j - Carbohydrate insoluble fraction µg/mg, k - Protein µg/mg.

Table - 29 Effect of combination of nickel and hexavalent chromium on different parameters of productivity in S. abundans

Days	Control					Ni 0.01 + cr^{6+} 0.05 ppm					Ni 0.02 + cr^{6+} 0.1 ppm				
	a	b	c	d	e	a	b	c	d	e	a	b	c	d	e
2nd	16.9	–	–	5.791	6.141	40	–	–	–	–	20	–	–	–	–
4th	50.2	.2534	.053	9.92	14.35	71	.249	.012	12.47	18.61	32	.117	.0153	53 46	8.58
6th	66	.422	.1446	47.12	56.58	115.2	.642	.1179	43.44	20.1	46.7	.306	.177	26.73	38.12
8th	176	.7136	.1502	63.01	55.18	232	.468	.149	80.19	59.73	125	.030	.2282	53.46	99.34
10th	283	.954	.256	126.57	54.41	319	.758	.238	106.92	87.52	195	.259	.1366	66.83	117.24
12th	304.6	1.132	.361	89.86	86.71	322	.944	.294	53.46	83.66	263	.729	.1215	66.83	102.38
14th	355	.738	.444	64.63	167.53	330	.724	.422	26.73	133.74	287	.731	.520	80.19	126.6

a - Biomass (Cell concentration/m^1 x 10^3) b - Production mgc/l/hr, c - Respiration mgc/l/hr
d - Chlorophyll - a ug/l e - Chlorophyll - b ug/l

	f	g	h	i	f	g	h	i	f	g	h	i
2nd	11.31	4.86	–	–	4.1	0.10	–	–	4.2	–	–	–
4th	18.86	14.34	–	–	12	9.97	–	–	12	21.09	–	–
6th	42.16	27.25	–	–	24	6.68	–	–	19	13.23	–	–
8th	61.45	24.85	60.93	115.49	66	32.07	164.33	66.34	46	40.09	111.56	158.76
10th	68.5	42.76	164.16	125	68	45.43	187.5	211.15	64	119.39	131.81	148.09
12th	118.66	118.67	93.93	42.6	56	40.09	83.7	18.56	72	99.7	55.63	19.34
14th	91.8	60.06	69	43.57	68	113.60	108.29	15.11	72	71.27	76.4	19.59

g - Carotenoid ug/l, g - Phaeophytin ug/l, h - Carbohydrate ug/mg, i - Protein ug/mg

Table - 30 Combined effect of nickel and hexavalent chromium in N. clausii

Days	Control						Ni .6 + cr^{6+} .2						Ni .6 + cr^{6+} .6						Ni .8 + cr^{6+} .2					
	a	b	c	d	e	f	a	b	c	d	e	f	a	b	c	d	e	f	a	b	c	d	e	f
2nd	3	-	-	43.5	46.5	33.3	2	-	-	26.7	140.5	18.4	2	-	-	53.4	33.5	30	2	-	-	37.4	139	19.2
4th	10	.204	.030	241	230.3	93.9	14	.035	.014	167	210.5	124	13	.012	.084	200	574	88	20	.106	.029	254	92	116
6th	33	.339	.078	463	339	165	29	.369	.059	508	503	136	30	.654	.096	281	579	135	27	.419	.094	568	504	206
8th	49	.567	.271	659	403	210	55	.566	.210	795	664	220	59	.588	.181	782	685	280	67	1.03	.206	916	913	360
10th	53	.894	.494	1584	740	451	73	.627	.417	1417	1160	500	100	1.24	.272	1310	1191	520	73	1.15	.148	1403	1161.9	514

a - Biomass (cell concentration/ml x 10^3) b - Production mgc/l/hr, c - Respiration mgc/l/hr, d - Chlorophyll-a µg/l,

e - Chlorophyll - c µg/l, g - Carotenoids µg/l.

	g	h	i	j	k	g	h	i	j	k	g	h	i	j	k	g	h	i	j	k
2nd	26.96	-	-	-	-	6.95	-	-	-	-	23.2	-	-	-	-	14.1	-	-	-	-
4th	58.2	2.211	1.38	3.47	14.5	56.1	9.72	2.01	3.13	30.5	69.5	12.65	.418	2.88	16.0	38.7	8.7	.922	5.17	32.9
6th	81.26	3.24	2.01	4.25	17.8	25.4	5.92	3.07	5.99	32.77	86.2	5.95	.627	4.54	27.9	60.14	4.67	.991	7.4	30.2
8th	131.7	4.63	3.04	5.22	35.3	77.5	4.33	.627	5.52	29.7	122.3	3.3	.179	3.63	26.9	26.7	3.95	1.41	4.3	38.8
10th	260	3.68	2.92	2.37	69.1	112.3	2.3	.620	4.31	37.3	120,2	2.18	.511	4.05	26.9	271	3.44	1.48	5.69	49.95

g - Phaeophytin µg/l, h - Carbohydrate acid fraction µg/mg, i - Carbohydrate alkali fraction µg/mg, j - Carbohydrate insoluble fraction µg/mg

k - Protein µg/mg.

Table - 31 Effect of combination of cobalt and nickel on different parameters of Productivity in S. abundans

Days	Control					Co 0.01 + Ni 0.005ppm					Co 0.05 + Ni 0.005ppm				
	a	b	c	d	e	a	b	c	d	e	a	b	c	d	e
2nd	16.9	-	-	5.79	6.14	16.3	-	-	5.346	7.81	20	-	-	5.346	11.88
4th	50.2	.2534	.0502	9.92	14.359	37	.1659	.0306	13.37	15.83	35	.015	.044	13.37	13.49
6th	66	.422	.1446	47.12	56.58	72	.344	.059	33.42	46.51	46.7	.073	.059	31.19	29.31
8th	176	.7136	.1502	63.01	55.18	115.6	.641	.060	44.55	61.37	63	.221	.029	40.10	64.12
10th	283	.954	.2564	126.57	54.4	159.8	.935	.177	53.46	50.15	79.9	.531	.059	60.15	52.14
12th	304.6	1.132	.361	89.86	86.7	283.7	1.252	.209	80.19	53.12	143	.678	.088	66.83	55.87
14th	355	.738	.444	64.63	67.5	347	1.37	.239	53.46	42.94	184	1.268	.235	66.83	57.8

a - Biomass (Cell concentration / ml x 10^3) p - Production mgc/l/hr, c - Respiration mgc/l/hr
b - Chlorophyll. a µg/l, e - Chlorophyll -b µg/l

	f	g	h	i	f	g	h	i	f	g	h	i
2nd	11.31	4.86	-	-	8	-	-	-	10.4	-	-	-
4th	18.86	14.34	-	-	27	5.88	-	-	19	5.88	-	-
6th	42.16	27.25	-	-	22	5.34	-	-	17	3.78	-	-
8th	61.45	24.85	60.93	115.49	40	51.78	77.44	137.82	28	19.35	-	-
10th	68.5	42.76	164.11	125	49	36.5	42.75	54.31	57	29.5	-	-
12th	118.66	118.67	93.93	42.6	60	4.009	69.15	45.49	80	9.34	49.66	50.26
14th	91.8	60.05	69	43.57	88	81.0	99.15	38.17	116	44	74.57	40.54

f - Carotenoid µg/l, g - Phaeophytin µg/l, h - Carbohydrate µg/mg, i - Protein µg/mg.

Table - 32 Combined effect of cobalt and nickel on different parameters of productivity in N. clausii

Days	Control						Co 0.3 + Ni 0.6						Co 0.6 + Ni 0.6					
	a	b	c	d	e	f	a	b	c	d	e	f	a	b	c	d	e	f
2nd	3	–	–	43.5	46.5	33.4	2	–	–	–	–	72	4	–	–	–	–	40
4th	10	.203	.031	241	230	93.9	13	.169	.031	106.9	488	89	5	.094	.024	66.8	383.2	4.5
6th	33	.339	.078	463	339	165.2	27	.293	.084	267.3	351	104	7	.053	.047	53.4	560.4	56
8th	49	.567	.271	659	403	210	48	.472	.096	801.9	659	300	21	.071	.117	213.8	495.5	95
10th	53	.894	.495	1584	740	451	56	.673	.192	968.9	167.7	346	14	.059	.162	227.2	28.45	90

a - Biomass (Cell concentration/ml x 10^3) b - Production mgc/l/hr, c - Respiration mgc/l/hr
d - Chlorophyll-a µg/l e - Chlorophyll-c µg/l, f - Carotenoids µg/l

	g	h	i	j	k	g	h	i	j	k	g	h	i	j	k
2nd	26.9	–	–	–	–	28.7	–	–	–	–	29.4	–	–	–	–
4th	58.8	2.21	1.38	3.48	14.5	30.2	2.17	.092	2.63	9.29	35.6	3.61	.083	6.92	6.38
6th	81.26	3.24	2.01	4.25	17.9	40.1	1.42	.358	1.35	21.02	57.5	1.52	.725	1.41	7.9
8th	131.78	4.63	3.04	5.23	35.3	69.9	1.03	4.41	2.11	14.08	33.4	2.44	–	2.29	9.6
10th	260	3.68	2.92	2.369	69.02	136.3	2.78	.625	2.35	15.65	58.8	4.51	.255	.798	8.2

g - Phaeophytin µg/l, h - Carbohydrate - acid fraction µg/mg, i - Carbohydrate alkali fraction µg/mg
j - Carbohydrate Insoluble fraction µg/mg, k - Protein µg/mg

Table-33 Effect of iron in combination with the metals on different parameters of Productivity in S. abundans

	Control					Co + Cr^{3+} + Fe					Ni + Cr^{3} + Fe					Ni + Cr^{6+} + Fe					Ni + Cr^{6+} + Fe				
	a	b	c	d	e	a	b	c	d	e	a	b	c	d	e	a	b	c	d	e	a	b	c	d	e
2nd day	169	-		5.791	6.141	182	-	-	-	-	176	-	-	-	-	194	-	-	-		186	-	-		-
4th day	502	.254	.050	9.92	14.359	281	.1086	.0121	5.346	12.2	315	.142	.012	10.69	25.75	386	.224	.024	12.69	26.89	425	.161	.011	10.6	29.8
6th day	660	.422	.145	47.12	55.18	646	.1961	.0603	29.8	24.2	720	.225	.060	21.7	32.4	950	.442	.029	46.4	56.45	89	.282	.060	49.6	48.8
8th day	1760	.714	.150	63.01	56.58	985	.422	.106	59.6	40.5	1090	.531	.059	50.6	54.8	1710	.796	.089	60.19	98.39	1265	.422	.060	62.011	79.8
10th day	2830	.955	.256	126.57	75.14	1720	.468	.136	66.83	56.8	1590	.521	.118	84.65	87.52	2120	.560	.089	108	52.6	2140	.566	.149	92.8	117.24
12th day	3046	1.132	.362	89.86	146.96	2040	.513	.256	72.1	31.65	2165	.580	.212	80.19	117.24	2290	.413	.177	50.6	44.4	2250	.567	.120	80.6	124
14th day	3550	.738	.444	64.63	67.53	1990	.196	.422	52.8	17.08	2335	.361	.413	53.46	217.8	2335	.236	.265	29.8	20.8	2375	.206	.184	40.09	98.6

a - Biomass (Cell concentration /ml x 10^{3}) b - Production mgc/l/hr c - Respirationmg c / l / hr, d - Chlorophyll-a /ug / l

e - Chloro phyll-b /ug / l.

	f	g	h	i	f	g	h	i	f	g	h	i	f	g	h	i	f	g	h	i
2nd day	11.31	4.87	-	-	13.6	9.62	-	-	8.3		-	-	16.8	5.8	-	-	15.2		-	-
4th day	18.86	14.34			9.6	11.13		-	11.2	12.56	-		13.6	10.6	-		11.6	14.9	-	-
6th day	42.16	27.25	-	-	27	11.2	-	-	30	11.48	-	-	44	14.96	-		35	29.8	-	-
8th day	61.45	24.85	60.95	115.49	39	30.08	104.9	206.7	30	29.6	44.4	198.4	24	27.6	123.11	72.37	32	33.4	161.51	135.39
10th day	68.5	42.76	164.16	125	34	40.6	155.01	138.02	22	16.03	63.86	146.81	34	40.7	271.3	159.13	46	49.8	211.61	118.19
12th day	118.66	118.67	93.93	42.61	52	48.11	72.3	28.29	38	8.01	161.66	42.25	44	99.8	97	35.3	46	43.8	100.6	34.56
14th day	91.8	60.06	69	43.57	22	20.66	106.23	26.96	33	5.34	119.56	37.48	32	70.6	58.33	25.8	22	10.47	80.48	22.77

f - Carotenoid /ug / l, g - phaeophytin /ug / l, h - Carbohydrate /ug /mg i - Protein /ug /mg

Table - 34 a. Effect of iron in combination with the metals on different parameters of productivity in N. clausii

Days	Control						Co .6 + cr^{6+} .6 + Fe^{+} .2						Ni .6 + cr^{6+} .6 + Fe^{+} .2					
	a	b	c	d	e	f	a	b	c	d	e	f	a	b	c	d	e	f
2nd	3	-	-	43.53	46.53	34	4	-	-	42.7	22.9	18	2	-	-	32.1	15.6	17.2
4th	10	.2037	.030	241	230.3	93.9	7	.1549	.036	167.1	138.9	71	8	.083	.041	106.9	86.3	54
6th	33	.338	.078	463.3	338.9	165.2	19	.4069	.058	400.9	228.8	147	15	.168	.036	200.5	89.3	74
8th	49	.566	.272	658.9	403.2	209.9	25	1.166	.081	434	269.5	176	24	.450	.078	574.7	205	198
10th	53	.894	.495	1583.7	740.1	450.6	40	.8378	.060	1309.7	461.06	476	30	.362	.362	775.17	225.1	284

a - Biomass (Cell concentration/mg x 10^3) b - Production mgc/l/hr, c - Respiration mgc/l/hr,
d - Chlorophyll-a µg/l, e - Chlorophyll - c - µg/l f - Carotenoids µg/l

	g	h	i	j	k	g	h	i	j	k	g	h	i	j	k
2nd	26.96	-	-	-	-	16.57	-	-	-	-	22.72	-	-	-	-
4th	58.26	2.21	1.38	3.47	14.5	-	16.16	1.63	4.2	26.96	68.15	17.69	.597	9.33	14.43
6th	81.25	3.24	2.01	4.25	17.8	45.44	8.53	2.99	6.59	71.33	55.46	11.55	1.143	7.09	43.79
8th	131.77	4.63	3.04	5.22	35.3	108.25	.989	1.077	4.91	48.32	93.55	3.51	.867	4.36	30.89
10th	260	3.68	2.92	2.36	69.02	130.97	.712	1.317	4.42	25.82	45.44	1.84	1.053	3.63	18.91

g - Phaeophytin µg/l, h - Carbohydrate acid fraction µg/mg, i - Carbohydrate - alkali fraction µg/mg,
j - Carbohydrate insoluble fraction µg/mg, k - Protein µg/mg.

Table - 34 b. Contd,,, Effect of iron in combination with the metals on different parameters of productivity in N. clausii

Days	Control						Co .6 + cr^{3+} 0.6 + Fe^{+} .2						Ni .6 + Cr^{3+} .8 + Fe^{+} .2					
	a	b	c	d	e	f	a	b	c	d	e	f	a	b	c	d	e	f
2nd	3	-	-	43.5	46.53	33.4	2	-	-	50.76	10.19	19.2	3	-	-	58.8	27.06	25.2
4th	10	.204	.030	241	230.3	93.9	9	.133	.036	106.9	64.8	55	12	.201	.0413	227.2	88.8	79
6th	33	.339	.079	463.3	339	165.2	21	.483	.085	387.5	120.4	132	23	.381	.047	380.9	127.5	129
8th	49	.567	.272	658.8	403	209.9	38	.784	.161	708.3	217.4	250	46	.865	.078	601.4	345.5	206
10th	53	.894	.494	1583.7	740	450.6	39	.754	.483	1176.1	380.2	424	24	.177	.353	975.6	234.78	368

a - Biomass (Cell concentration/ml x 10^3) b - Production mgc/l/hr, c - Respiration mgc/l/hr, d - Chlorophyll-a - µg/l,
e - Chlorophyll - c µg/l, f - Carotenoids µg/l.

	g	h	i	j	k	g	h	i	j	k	g	h	i	j	k
2nd	26.96	-	-	-	-	18.9	-	-	-	-	15.76	-	-	-	-
4th	58.3	2.21	1.381	3.47	14.5	43.4	14.5	3.61	9.33	65.7	70.3	13.8	.209	5.58	35.29
6th	81.26	3.24	2.01	4.25	17.9	92.63	12.08	1.79	7.09	38.8	95.5	5.6	1.67	3.61	52.8
8th	131.7	4.63	3.04	5.23	35.3	147	4.95	.838	4.36	37.2	208.5	3.14	.947	2.87	29.4
10th	260	3.68	2.92	2.369	69	290.0	3.08	1.79	3.63	23.4	68.16	1.73	1.367	3.06	18.14

g - Phaeophytin µg/l, h - Carbohydrate acid fraction µg/mg, i - Carbohydrate - alkali fraction µg/mg,
j - Carbohydrate insoluble fraction µg/mg, k - Protein µg/mg.

Table - 35 Combined effect of nickel, cobalt and trivalent chromium on different parameters of productivity in N. clausii

Days	Control						Ni (0.4) + Co (0.3) + cr^{3+}(0.8)						Ni (0.6) + Co (0.3) + cr^{3+} (0.2)					
	a	b	c	d	e	f	a	b	c	d	e	f	a	b	c	d	e	f
2nd	3	-	-	43.53	46.5	33.4	-	-	-	-	-	64	-	-	-	-	-	86
4th	10	.204	.030	241.1	230.3	93.9	13	.184	.036	120.3	545.4	86	10	.312	.167	173.7	525	106
6th	33	.339	.078	463.3	338.9	165.2	11	.256	.084	187.1	734	98	17	.251	.086	267.3	463	118
8th	49	.567	.272	658.9	403.2	210	39	.271	.206	574.7	154	196	32	.306	.118	908.8	218.6	316
10th	53	.894	.495	1583	740.1	451	36	.491	.332	801.9	178	272	41	.873	.184	1029.1	224	354

a- Biomass (Cell concentration/ml x 10^3) b - Production mgc/l/hr c - Respiration mgc/l/hr
d- Chlorophyll - a µg/l e - Chlorophyll - c µg/l, f - Carotenoids µg/l

	g	h	i	j	k	g	h	i	j	k	g	h	i	j	k
2nd	26.9	-	-	-	-	12.7	-	-	-	-	10.7	-	-	-	-
4th	58.3	22 1	1.38	3.47	14.5	-	1.53	.070	1.36	5.62	-	3.35	.251	1.62	14.3
6th	81.3	3.24	2.01	4.25	17 9	49.4	3.67	.638	.409	17.7	3.4	2.55	1.03	1.14	29.1
8th	131.7	4.63	3.03	5.22	35.3	153.6	3.87	2.45	.876	9.27	204.5	1.63	1.89	1.41	22.2
10th	260	3.68	2.92	2.37	69.1	179.8	2.58	.598	1.38	23.12	191.7	.763	.562	2.01	17.5

g - Phaeophytin µg/l, h - Carbohydrate - acid fraction µg/mg, i - Carbohydrate alkali fraction µg/mg,
j - Carbohydrate insoluble fraction µg/mg, k - Protein µg/mg

Table - 36 Combined effect of nickel, cobalt and hexavalent chromium on different parameters of productivity in N. clausii

Days	Control						Ni .6 + Co .3 + cr^{6+} .2						Ni .4 + Co .6 + cr^{6} .6					
	a	b	c	d	e	f	a	b	c	d	e	f	a	b	c	d	e	f
2nd	3	-	-	43.5	46.5	33.4	5	-	-	-	-	32	4	-	-	-	-	28
4th	10	.204	.030	241	230.3	93.9	10	.1086	.018	173.7	88.9	60	8	.0416	.017	140	67.1	51
6th	33	.339	.079	463	338.9	165	18	.349	.091	380.9	127.5	138	13	.241	.042	354	108.2	120
8th	49	.567	.272	658	403.2	210	45	.678	.151	828.6	317.8	310	19	.325	.134	534	323	224
10th	53	.894	.495	1583	740	451	60	.5128	.287	788.5	345	304	26	.419	.148	975.6	486.5	374

a - Biomass (Cell concentration/ml/10^3) b - Production mgc/l/hr, c - Respiration mgc/l/hr, d - Chlorophyll-a µg/l,
e - Chlorophyll-c µg/l, f - Carotenoids µg/l

	g	h	i	j	k	g	h	i	j	k	g	h	i	j	k
2nd	26.96	-	-	-	-	84.19	-	-	-	-	-	-	-	-	-
4th	58.26	2.21	1.38	3.47	14.5	84.19	1.98	1.13	2.05	53.3	54.36	2.94	1.51	.615	46.4
6th	81.26	3.24	2.01	4.25	17.8	95.5	3.26	1.87	3.15	43.9	96.89	3.98	2.03	2.01	32.6
8th	131.77	4.63	3.04	5.23	35.3	192.4	3.86	2.25	4.54	78.9	85.5	4.68	2.85	4.84	57.9
10th	260	3.68	2.92	2.37	69.1	94.89	2.31	1.21	2.35	33.8	164.7	4.31	1.83	2.55	48.6

g - Phaeophytin - µg/l, h - Carbohydrate - acid fraction µg/mg, i - Carbohydrate - alkali fraction µg/mg,
j Carbohydrate insoluble fraction µg/mg, k - Protein µg/mg.

Table - 37 Combined effect of cobalt, trivalent and hexavalent chromium on different parameters of productivity in N. clausii

Days	Control						Co .3 + cr^{3+} .2 + cr^{6} .2						Co .3 + cr^{3+} .2 + cr^{6+} .6					
	a	b	c	d	e	f	a	b	c	d	e	f	a	b	c	d	e	f
2nd	3	-	-	43.5	-	34	5	-	-	-	-	28	4	-	-	-	-	36
4th	10	.204	.031	241.1	230.3	94	10	.132	.133	147	106.9	56	7	.066	.012	106.9	96.2	44
6th	33	.339	.079	463.3	339	165	25	.295	.018	547.9	138.4	152	13	.227	.048	253.9	117.9	95
8th	49	.567	.272	658.9	403	209.9	45	.669	.059	779.8	240.7	302	15	.314	.030	441	221.6	160
10th	53	.894	.495	1583.7	740	451	53	.649	.206	1283	548.1	480	28	.344	.060	1122.6	508	466

a - Biomass (cell concentration/ml x 10^{3}) b - Production mgc/l/hr, c - Respiration mgc/l/hr.
d - Chlorophyll-a µg/l , e - Chlorophyll - c µg/l, f - Carotenoids µg/l

	g	h	i	j	k	g	h	i	j	k	g	h	i	j	k
2nd	26.96	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4th	58.26	2.21	1.38	3.47	14.5	53.9	3.78	.910	2.02	51.3	27.4	8.47	.882	4.59	55.85
6th	81.25	3.23	2.01	4.25	17.8	159.7	3.05	1.837	3.17	48.1	76.18	1.97	.760	2.05	25.3
8th	131.7	4 62	3.04	5.22	35.3	294	15.37	3.37	7.04	80.5	151	1.81	1.41	3.73	53.3
10th	260	3.68	2.92	2.369	69.02	225	2.41	2.22	3.68	61.14	243	2.42	.89	1.93	32.21

g - Phaeophytin - µg/l, h - Carbohydrate acid fraction µg/mg, i - Carbohydrate - alkali fraction µg/mg,
j - Carbohydrate insoluble fraction µg/mg, k - Protein µg/mg

Table - 38 Combined effect of nickel, trivalent and hexavalent chromium in N. clausii

Days	Control											Ni 0.6 + cr^{3+} 0.2 + cr^{6+} 0.6									
	a	b	c	d	e	f	g	h	i	j	k	a	b	c	d	e	f	g	h	i	j	k
2nd	3	-	-	43.5	46.5	33.4	26.96	-	-	-	-	8	-	-	-	-	29	-	-	-	-	-
4th	10	.203	.031	241	230.3	93.9	58.26	2.211	1.38	3.48	14.5	9	.0918	.025	173.7	61.1	64	52.1	11.09	.274	1.69	52.9
6th	83	.339	.079	463	339	165.2	81.26	3.24	2.01	4.25	17.9	13	.306	.037	368	79.6	118	138.3	2.89	1.79	2.37	43.1
8th	49	.567	.272	659	403	210	131.8	4.63	3.04	5.22	35.3	35	.429	.031	682	209	242	241.9	3.36	1.86	3.36	48.4
10th	53	.894	.495	1584	740	451	260	3.68	2.92	2.37	69	47	.459	.043	1270	656	468	193.8	2.72	1.74	3.15	55.3

a - Biomass (cell concentration/ml x 10^3) b - Production mg/l/hr, c - Respiration mgc/l/hr, d - Chlorophyll-a µg/l, e - Chlorophyll-c µg/l,

f - Carotenoids µg/l, g - Phaeophytin µg/l, h - Carbohydrate - acid fraction µg/mg,

i - Carbohydrate - alkali fraction µg/mg, j - Carbohydrate - insoluble fraction µg/mg,

k - Protein µg/mg

Table - 39 Combined effect of cobalt, nickel, trivalent and hexavalent chromium in S. abundans

Days	Control									Co 0.04 Ni 0.005 + cr^{3+} 0.01 + cr^{6+} 0.05								
	a	b	c	d	e	f	g	h	i	a	b	c	d	e	f	g	h	i
2nd	16.9	-	-	5.79	6.14	11.3	4.86	-	-	11.1	-	-	-	-	9.6	5.8	-	-
4th	50.2	.253	.050	9.9	14.36	18.8	14.3	-	-	20.5	.024	.011	15.4	7.81	10	3.8	-	-
6th	66	.422	.144	47.2	56.58	42.2	27.3	-	-	24	.0306	.012	17.9	8.9	20	11.1	-	-
8th	176	.713	.1502	63.1	55.2	61.5	24.8	60.9	115.5	29	.0306	.031	28.7	13.1	20	38.1	15.7	58.9
10th	283	.955	.256	126.6	54.4	68.5	42.7	164.1	125	39	.1502	.031	28.7	27.8	25	48.1	6.9	51.6
12th	305	1.132	.362	89.8	86.7	118.6	118.6	93.9	42.6	54	.061	.024	59.8	70.6	36	59.1	9.8	13.6
14th	355	.738	.444	64.6	67.5	91.8	60.05	69	43.6	84	.061	.024	60.6	60.2	30	40.1	19.1	38.7

a - Biomass (cell concentration/ml 10^3) b - Production mgc/l/hr, c - Respiration mgc/l/hr, d - Chlorophyll-a ug/l, e - Chlorophyll-b /ug/l,
f - Carotenoids /ug/l, g - Phaeophytin /ug/l, h - Carbohydrate /ug/mg, i - Protein /ug/mg

Combined effect of cobalt, nickel, trivalent and hexavalent chromium in N. clausii

Days	Control											Co 0.3 + Ni 0.4 + cr^{3+} 0.2 + cr^{3+} 0.2										
	a	b	c	d	e	f	g	h	i	j	k	a	b	c	d	e	f	g	h	i	j	k
2nd	3	-	-	43.5	46.5	33.4	26.96	-	-	-	-	1	-	-	58.8	23.3	20	21.4	-	-	-	-
4th	10	.203	.030	241	230.3	93.9	58.3	2.21	1.38	3.47	14.4	6	.085	.048	134	62.01	48	58.8	2.25	.492	3.41	28.6
6th	33	.339	.079	463.3	339	165	81.27	3.24	2.00	4.25	17.9	16	.350	.012	314	72	106	66.2	4.25	1.65	7.14	45.2
8th	49	.567	.272	659	403	210	131.7	4.63	3.03	5.22	35.3	29	.816	.078	441	163	154	151.1	.642	.602	3.4	18.2
10th	53	.894	.495	1584	740	451	260	3.68	2.92	2.37	69	27	.459	.122	1484	526	368	295.4	1.209	1.44	3.4	19.1

a - Biomass (cell concentration/ml x 10^3), b - Production mgc/l/hr, c - Respiration mgc/l/hr, d - Chlorophyll-a /ug/l, e - Chlorophyll-c /ug/l,
f - Carotenoids /ug/l, g - Phaeophytin /ug/l, h - Carbohydrate - acid fraction /ug/mg, i - Carbohydrate alkali fraction /ug/mg,
j - Carbohydrate - insoluble fraction /ug/mg, k - Protein /ug/mg.