

**HEAVY METAL ABSORPTION &
UTILIZATION BY DIFFERENT
KINDS OF ALGAE**

A THESIS

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By

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To all members who collaborated with me, helped and encouraged me to carry out this thesis in this form.

I devote my thesis to colleagues and all members in Pflanzenernährung Institut der Justus-Liebig Universität, Giessen, West Germany.

" THIS THESIS HAS NOT BEEN PREVIOUSLY
SUBMITTED FOR ANY DEGREE AT THIS OR ANY
OTHER UNIVERSITY,

THE REFERENCES IN THE TEXT WILL SHOW
SPECIFICALLY THE EXTENT TO WHICH I HAVE
AVAILED MYSELF OF THE WORK OF OTHER AUTHORS."

Zeinab Kh. Ibrahim

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P R E F A C E

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Heavy metal pollution has its significant importance in areas where the effluents are not rapidly dispersed in the open sea, such as estuaries and fjords with narrow outlets. There, the heavy metal content of the waters is continuously increasing. Nowadays, the use of indicator organisms represents a rapid and relatively inexpensive tool for the qualitative detection of heavy metal contamination in fjord areas. The quality of surface waters is usually judged by the composition of its algal flora. Several investigations reported that analysis of benthic algae gives, at least, a qualitative picture of the heavy metal pollution in the area (Bryan, 1969; Butterworth et al., 1972; Fuge and James, 1974; Foster, 1976). Phillips (1976) evaluated an indicator ability of the mussel Mytilus edulis without the need for multiple analyses of water samples. The mussel apparently can act as an efficient time - integrated indicator of zinc, cadmium and lead over a wide variety of environmental conditions. With further development, this organism may be also useful for monitoring industrial effluent characteristics at the discharge site, allowing extensive control of water quality. Tunicates may also serve as marine pollution indicators for monitoring the release of industrial and/or radioactive wastes into the marine environment. Thus, Ciona intestinalis can be used as indicator for iron,

while Microcosmus sulcatus is a valuable indicator for selenium, chromium, zinc and cobalt (Papadopoulou and Kanias, 1977).

Ignatiades and Becacos-Kontos (1970) pointed out that Ciona intestinalis can resist the toxicity of oil polluted water and that the excess of suspended mud may not affect the filtering mechanism of these organisms.

When algae withdraw trace metals from the aquatic environment, the water becomes in effect " treated " or more pure, for man's reuse . Chlorella vulgaris had a higher percentage uptake of zinc but a lower percentage uptake of cobalt than did Euglena viridis and Pediastrum tetras. C. vulgaris thrived in suprisingly high concentrations of zinc. E. viridis concentrated the greatest amount of zinc, while both P. tetras and E. viridis had a higher affinity for cobalt than did Chlorella vulgaris. The three species, therefore, could accumulate trace metals to an extent that might make them toxic in food chain (Coleman et al., 1971). On the other hand, Patrick and Loutit (1976) suggested that small amounts of metals in effluents from various sources may be concentrated by bacteria and then passed to other organisms in food chain.

During recent years, cultivated soils depend entirely on sewage sludge as the main manure and plant nutrient source. Sewage sludge usually has high contents of several heavy metals and this leads to enrichment of the topsoil and thus crops may also store unfavourable levels of toxic elements such as lead,

cadmium and mercury. Andersson and Nilsson (1976) reported that the contents of zinc, copper, nickel and cadmium generally increased in the plant material as a consequence of sludge application.

The aim of this investigation is to study the response of a green (Chlorella fusca) and a blue-green (Spirulina maxima) alga to different levels of cadmium, chromium, nickel and zinc in order to compare the tolerance to, and role of, these elements, particularly in nitrogen metabolism of both organisms.

PART (I)
I N T R O D U C T I O N

(A) : HISTORICAL REVIEW

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

A. HISTORICAL REVIEW

A) CADMIUM :

Since the beginning of this century environmental pollution by the highly toxic metal cadmium has been steadily increasing. Cadmium is a serious pollutant, generated in the environment from smelting industries, attrition of automobile tires and burning of diesel and heating oil.

Other sources of cadmium are sewage sludge and related products of municipal origin which usually have high contents of heavy metals, which has limited their utilization as fertilizers in agricultures. The content of zinc, copper, nickel and cadmium generally increased in the plant materials as a consequence of sludge applications (Andersson & Nilsson, 1976). Cadmium enters the water system not only by dumping of waste water, but also through the air and drain water (Haghiri, 1973), and from phosphate fertilizers (Kropf and Geldmacher-V. Mallinckrodt, 1968; Payer et al., 1976). Like zinc or mercury, cadmium is concentrated by living organisms (Goldberg, 1957; Friberg, 1975; Huckabee and Blaylock, 1973). Cadmium toxicity in mammals is well established (Schroeder et al., 1965). A good correlation

between cardiovascular death rates and the concentration of cadmium in the air as an industrial pollutant was reported by Carroll (1966). Many experiments have shown that cadmium and its compounds can induce malignant tumors in rats (Gunn et al., 1963), causes erythrocyte destruction, testicular damage, renal degradation and hypertensive heart disease (Lagerwerff, 1972). Shibata (1977), Rugstad and Morseth (1978) confirmed the accumulation and storage of cadmium in the liver, kidney and pancreas, where it was bound to low molecular weight-protein (thionein). Like zinc, cadmium also activated normal erythrocytic ALAD (8-aminolevulinic acid dehydratase) and reversed the in vitro Pb-induced inhibition of ALAD at concentrations 40-100 fold lower than those of zinc. Cadmium is a more potent activator to normal adult human erythrocytic ALAD activity than zinc. These effects are largely attributed to binding of the metal to peripheral sulphydryl groups (Davis and Avram, 1978). Zasukhina et al. (1977) reported that cadmium chloride caused degradation of DNA in human and rat-embryo cells. According to Ivashchenko (1978), the bivalent cations increased ATPase activity of rat heart mitochondria in the following sequence : $Zn^{2+} \gg Cd^{2+} \gg Co^{2+} \gg Mg^{2+} \gg Mn^{2+} > Ca^{2+} > Ni^{2+}$. Nickel and cadmium inhibited Mg^{2+} -dependent activity of bicarbonate-stimulated ATPase. On the other hand, Matsumoto et al. (1977) reported that nickel slightly inhibited Ca-binding activity of myosin B extract from pig intestinal smooth muscle but remarkably inhibited ATPase activity.

Certain trace elements such as cadmium and lead, that may occur in various parts of the agricultural environment, have been reported to inhibit plant growth under some conditions (Haghiri, 1973 ; Miller and Koeppe, 1970 ; Page et al., 1972). Although the specific causes of the toxic effects of trace metals have not been pin-pointed, the non-beneficial effects reported for trace elements on electron energy transfer reactions in plant mitochondria may be implicated (Koeppe and Miller, 1970 ; Miller and Koeppe, 1970 ; Bittell and Koeppe, 1973). Cadmium stimulated, at low concentrations (10-50 μM), and inhibited, at higher concentrations ($> 100 \mu\text{M}$), the oxidation of NADP by isolated corn mitochondria. Additionally cadmium (250 μM) completely inhibited the phosphorylation associated with succinate oxidation. Also greater than 50% inhibition of oxidation was observed for succinate in corn mitochondria after addition of $> 0.1 \text{ mM}$ cadmium, zinc or lead for NADH; after addition of $> 0.5 \text{ mM}$ cadmium or zinc and for malate, & pyruvate after additions of $> 0.1 \text{ mM}$ cadmium (Bittell et al., 1974). Lee et al. (1976) reported that cadmium-treated soybean leaves showed higher respiration rates and activities of malate dehydrogenase, acid phosphatase, ribonuclease, deoxyribonuclease and peroxidase but decreased activity of carbonic anhydrase. Chlorosis, epinasty, abscission of leaves and decreased growth rate occurred in seedlings treated

with 1.35 μ M cadmium. However, the molecular basis of cadmium toxicity in higher plants remains largely unknown (Bazzaz et al., 1974 b ; Koeppe, 1977), while the biochemistry of the element in animal tissues is well established (Vallee and Ulmer, 1972 ; Friberg et al., 1974). The strong affinity of cadmium (and other heavy metals) for side chain ligands of protein (Vallee and Ulmer, 1972 ; Hampp et al., 1976) strongly suggests that enzyme activities are one of the first metabolic processes, the element might interfere within higher plants, too. However, little information is available concerning the effects of cadmium on plant enzymes (Mathys, 1975; Lee et al., 1976). According to Al-Wakeel (1981), cadmium severely attenuated the activity of glutamic oxaloacetic transaminase (GOT) and of glutamic pyruvic transaminase(GPT); a phenomenon that was partially alleviated(GOT)or furthered(GPT) with addition of iron. Iron attenuated peroxidase activity whereas cadmium was stimulatory. Iron partially counteracted the stimulatory effects of cadmium. Sina and Chin (1978) reported that the exposure of the acellular slime mold Physarum polycephalum to cadmium resulted in distortion of nucleolar structure and in inhibition of RNA synthesis. Nakano et al. (1978) reported that cadmium was neither involved in the normal cell metabolism of Euglena gracilis under zinc-deficient conditions nor did it replace zinc, which is essential for normal growth. According to Byrne et al. (1976), cadmium is accumulated to a

surprising extent by most fungi. Penicillium lilacinum was strongly resistant to copper, zinc, tin and cadmium, while Penicillium purpurogenum showed resistance to both cadmium and copper (Tatsuyama et al., 1975). On the other hand, yeast can naturally tolerate toxic levels of cadmium.

According to Rosko & Rachlin (1977), the effective concentrations of cadmium, copper, mercury and zinc respectively that caused 50% (EC_{50}) depression of cell division of Chlorella vulgaris : $0.06 \pm 0.001 \text{ mg l}^{-1}$, $0.18 \pm 0.003 \text{ mg l}^{-1}$, $1.03 \pm 0.03 \text{ mg l}^{-1}$ and $5.1 \pm 0.09 \text{ mg l}^{-1}$. All the metal concentrations tested, the Chlorella cells were able, in time, to overcome the stresses and resume some cell division given a certain minimum number of cells in the initial inoculum. Cadmium, copper and mercury probably exert a greater effect on cell division than on chlorophyll a content in C. vulgaris and vice versa with zinc and lead. Anikeeva et al. (1975) and Kogan et al. (1975) reported that cadmium had a negative effect on Chlorella culture : the growth rate and viability of cells was decreased, disturbing their division and resulting in increased rate of speckled mutants. Weigel & Jäger (1980a) demonstrated that cadmium accumulated mainly in the cytoplasm of roots and to a minor extent in leaf cytoplasm of bean plants. Little cadmium was bound to the cell wall

fraction or organelles. Malone et al. (1978) reported that in hydroponic experiments, 100 ug cadmium/l affected a 33% inhibition of lateral root initiation of corn. The growth of corn and soybean primary roots was not reduced at cadmium concentration of 1 mg/l, and the number of lateral roots initials in soybeans was not reduced at 2 mg cadmium/l. The toxic effects of cadmium were ameliorated by additions of zinc or iron citrate to nutrient growth solution. It was suggested by Boggess, et al. (1978) that P fertilization is not a generally effective method for reducing cadmium contamination of maize products. K and P uptake and translocation, by young barley was inhibited by chromium, nickel, copper, zinc, cadmium or lead (10^{-6} - 10^{-4} M/l) to different extents; mercury had the greatest effect, whereas zinc was almost ineffective (Oberlaender and Roth, 1978).

Wallace et al. (1977a), reported that cadmium in solution culture at 10^{-4} M decreased manganese concentrations in bush leaves at low and high concentrations of manganese (non-competitive inhibition). When manganese was decreased the concentration of iron and other ions were simultaneously increased particularly in leaves and roots. Al-Wakeel (1981), reported that cadmium largely depressed manganese absorption in wheat and radish, (Triticum aestivum L. ; Raphanus sativus L.); a phenomenon that was partially alleviated with administration of iron.

B) CHROMIUM :

The trace element chromium is known to be essential for men and animals (Lisk, 1972) through its important function in glucose metabolism. However, the essentiality of the metal to green plants is not well established, although some cultivated plants as cucumber (Bashkina, 1972). potatoes (Huffmann and Allaway, 1973b) or sugar cane (Hance, 1933), show increased productiveness when fertilized with low concentrations of chromium salts. Trivalent chromium generally has a low toxicity than hexavalent chromium compounds. Steele et al. (1977) and Steele and Rosebrough (1979) reported trivalent chromium as essential dietary trace-element for swines and turkey, respectively.

Chromium received a great attention in industrial and domestic products irrespective of the high toxicity of several of its chemical forms. Industrial production and handling of certain chromium (VI) products are associated with respiratory injury, carcinogenesis particularly in lungs, skin, ulceration and induction of allergic chromium dermatitis (Burrows, 1978;Krishna et al., 1976 ; Hill and Warren, 1979). Hutcheson et al. (1975) reported that 20 ppm chromium did not affect the vitality or metabolic function of rats. According to Kryshab (1971), exposure of animals to chromium pyrite

dust induced fibrosis accompanied by disorders in protein metabolism. 6×10^{-5} M chromium (III) did not increase the swelling of the rat liver mitochondria but formed a complex with the intrachain disulphide of insulin (Campbell and Mertz, 1963). In his opinion (Anghileri, 1969), the mechanism of chromium accumulation in tumor cells is not a simple phosphomonoesterase action but the interaction of chromium with calcium and/or nucleic acid metabolism was the main cause of such accumulation (Anghileri, 1971),

Toxic effects of chromium have also been observed in microorganisms. Lamb and Tollefson (1973) reported that cupric ions were most toxic, followed by chromate and chromic, to activate sludge microorganisms. Among lichens, Parmelia caperata and Parmelia rudecta were able to accumulate chromium particularly in the centre of the thallus (Schutte, 1977). Euglena gracilis could tolerate 1 mg/liter chromium (as chromium trioxide) for only three hours, at room temperature (Yongue et al., 1979). In the meantime, Mangi et al. (1978) considered chromium (VI) and CrO_4^{-2} as moderately toxic to several riverine algae and to a lesser extent to duck weeds. On the other hand, Petriya (1978) claimed that chromium salts, in waste waters from leather factories, at 10^{-4} M and 10^{-5} M,

stimulated growth and photosynthesis of Chlorella vulgaris whereas higher levels were inhibitory.

Bacteria seem to be more sensitive to chromium than other microorganisms. Nestmann et al. (1979) showed that chromate ion was the responsible agent causing mutagenicity in Escherichia coli and Salmonella typhimurium. According to Petrilli and De Flora (1977), all hexavalent chromium compounds caused complete inhibition of bacterial growth at doses of 400-800 ug/plate but the trivalent chromium compounds were neither toxic nor mutagenic for Salmonella typhimurium. Schott et al. (1973) showed that chromium was less effective in inhibiting growth of Streptococcus faecalis compared with thorium, iron or copper.

Fungi seem to be more tolerant to heavy metals than other microorganisms. Schmidt and Ziemer (1976) were of the opinion that the substrate, mass of inoculum and hyphal lesions are the primary influencing factors whereas adaptive or genetic phenomena do not participate in inducing high tolerance of fungi. Wood destroying fungi considerably varied in their tolerance to copper-chromium arsenate. Toxic doses of the substance drastically reduced the colonization of these fungi

(Chou & Preston, 1974) except Poria moticola that remained viable. Marsh et al. (1975) proposed that less than 25 ug per one ml medium of iron, manganese, copper, cadmium, trivalent chromium, silver or mercury partly or completely inhibited the aflatoxin production without influencing the dry weight of Aspergillus parasiticus. On the other hand, Burzynska and Maciejaska (1978) reported increased aflatoxin production by Aspergillus flavus or Aspergillus parasiticus with administration of cadmium or chromium without affecting biomass yield.

Very little had been published on the role of chromium in physiology and metabolism of plants. In some instances it is considered an indispensable micro-element (Bertand and De-Wolf, 1965, 1968). Both stimulatory and phytotoxic effects of chromium have been observed by a number of investigators. An example of the stimulatory effects, Scharrer and Schropp (1935) reported enhanced growth of maize with 0.5 ppm chromium present in the nutrient medium as chromic sulphate. Toxicity effects had been observed by Hunter and Vergnano (1953) in oats supplied with excess of 5ppm chromium as chromium sulphate. Plants suffering from chromium toxicity had small roots and narrow brownish red leaves, covered with small necrotic spots. Recently Moulinier and Mazoyer (1968), reported that chromium sulphate was very toxic to wheat and tomato, in acid soils. Rusko

(1968 a,b) noticed that application of molybdenum, chromium or zinc to grapes, before flowering, raised the total nitrogen of the grapes and chlorophyll content of leaves. Lobnik and Vidrih (1976) and Vidrih and Lobnik (1976) showed that 7 g/pot of tanning industry sludge (2.4 ppm chromium) gave the highest yield of Dactylis glomerata whereas 11.2 g/pot was inhibitory.

Usually the chromium content of plants is very low but some species can accumulate appreciable amounts of the metal (Lyon et al., 1968). Lee et al. (1977) reported that the epiphytic moss "Aerobryopsis longissima", the first chromium accumulator among bryophytes, could accumulate up to 7500 ug/g chromium. Banasova (1978), proved that Melilotus officinalis and Calamagrostis epigeios were concentrators of chromium from Dobsina locality, Czechoslovakia. Filip et al. (1979) found that Oscillatoria species was extremely resistant to chromium and could grow in chromium exposed - cultures. Chromate was reported to be absorbed freely by barley roots (Shewry and Peterson, 1975). But unless the concentration was high (0.1 mM) less than 1% of the absorbed anion was transported to the shoot. Most of the absorbed ions were found in a non-particulate form in the vacuoles. Skeffington et al. (1976) claimed that chromate uptake was active

whereas chromic uptake was passive demonstrating that the two forms did not share a common uptake mechanism.

C) NICKEL :

Because of the spread of its industry, nickel is now being continually deposited in surface soils as air-borne pollutant (Hutchinson and Whitby, 1977). Sweat and household detergents had the capacity to release nickel from stainless steel. This finding substantiates the thesis that stainless steel commodities can be sources of human skin contact allergy with nickel (Katz and Samitz, 1975).

Pathophysiological alternations of nickel metabolism have been observed in certain human diseases. D'Alonzo and Pell (1963) and Sunderman et al. (1970) reported increased concentrations of nickel in serum from patients after acute myocardial infarction. Schnegg and Kirchgessner (1976) showed that nickel deprivation profoundly impairs intestinal absorption of iron and thereby caused anemia. Moreover, Kirchgessner and Schnegg (1977) found that nickel deficiency is amended by diminished activities of malate dehydrogenase and glucose-6-phosphate dehydrogenase in rat hepatocytes.

Tseretili and Mandzhavidze (1969) reported hyperglycemia and glucosuria in patients with $\text{Ni}(\text{CO})_4$ poisoning. Sunderman (1971) proposed that : (1) the immediate toxic effects of $\text{Ni}(\text{CO})_4$ may be mediated by acute inhibition of ATPase in target tissue, and (2) the delayed toxic effects of $\text{Ni}(\text{CO})_4$ may result from inhibition of RNA polymerase activity (Sunderman and Esfahani 1968; Beach and Sunderman, 1970). Sunderman et al. showed that $\text{Ni}(\text{CO})_4$ has an inhibitory effect on induction of several enzymes in livers of rats, including phenothiazine induction aryl-hydrocarbon hydroxylase (1967a), phenobarbital induction of aminopyrine demethylase (1970) and cytochrome P-450 (1968) and cortisone induction of tryptophan pyrrolase (1967b). Nickel deficiency lead to reduced iron content in organs of rats and to greatly reduced Hb levels and erythrocyte counts (Schnegg and Kirchgessner, 1976). Gitlitz et al. (1975) reported that toxic nephropathy with aminoaciduria and proteinuria apparently develops in rats after i.p. Ni^{2+} . According to Kirchgessner, Beyer and Steinhart (1976), $8.33 \times 10^{-4}\text{M}$ or higher increased pepsin activity. Addition of iron or zinc ions had no effect.

Although nickel is regarded as a normal constituent of plant tissues (Mishra and Kar, 1974), yet it is not considered an essential element, its deficiency is not indicated by any

symptoms. 0.5 uM nickel or less slightly increased the rate of cell division in the coccolithophorid alga, Cricosphaera carterae. Higher concentrations were inhibitory with almost complete blockage at 400 uM nickel. Cell calcification was retarded at concentrations of nickel greater than 50 uM. The inhibition of cell division and calcification was reversible after the transfer of cells to control medium (Stillwell and Holland, 1977). Tanguy (1975) reported that common bacteria (Aeromonas P₃₅₈, Bacillus subtilis, Alcaligenes faecalis, Arthobacter ureafaciens), increased the solubilization of nickel and appeared to resist the resulting increased nickel concentrations.

The uptake of nickel by Umbilicaria muhlenbergii is probably physicochemical in nature. Metabolic inhibitors caused no significant decrease in nickel uptake. Dead thalli accumulated the metal to a slightly greater extent than live thalli. Nickel uptake was similar under aerobic and anaerobic conditions. Uptake was pH-dependent and increased with temperature (Neiboer et al., 1976). According to Das et al. (1978) low concentrations of nickel chloride stimulated germination rate and root-shoot length of rice (Oryza sativa L.Cv. RATNA) while higher concentrations were inhibitory. In solution culture of Phaseolus

vulgaris, a high level of iron improved growth of the plants in the presence of 2.5×10^{-5} M nickel, but not with 5×10^{-5} M nickel. The 2.5×10^{-5} M nickel decreased iron concentration in leaves, but not in other plant parts. The 5×10^{-5} M of nickel also decreased iron concentration in leaves relative to the control, although they were increased relative to those for 2.5×10^{-5} M nickel (Wallace et al., 1977 c). Giashuddin and Cornfield (1978) reported, with increasing nickel level, nitrification of sandy soils decreased, to a greater extent than did N and C mineralization. Polacco (1977) suggested that nickel is an active site component of jack bean urease and the observed stimulation of urease synthesis in soybean tobacco and rice callus by nickel strongly suggested that this metal is a component of their urease as well. Similarly, Spears et al. (1977) reported that rumen bacterial urease required nickel for maximal activity. The discovery that urease contained stoichiometric amounts of nickel lead to the suggestion that nickel is important in enzymes catalyzing reactions involved in the utilization, production or transfer of ammonia (Dixon et al., 1976).

Nickel-dependent chemolithotrophic growth of Alcaligenes (Hydrogenomonas) eutrophus is well documented (Bartha and Ordal, 1965 ; Repaske and Repaske, 1976), although no discrete

function for nickel has so far been identified. Nickel inhibition of fermentation in yeast was ascribed, in part, to inhibition of alcohol dehydrogenase (Fuhrmann and Rothstein, 1968) while excess nickel induced an unbalanced growth condition in a marine Arthrobacter sp. resulting in large, plasmolysed sphaeroplasts (Cobet et al., 1970).

D) ZINC :

Although it is well established that zinc is an indispensable microelement for plant growth (Nicholas, 1963; Taft, 1965) it is still considered among the well known marine and fresh water pollutants including cadmium and mercury (Jensen and Jernelöv, 1969; Nuzzi, 1972).

Algae (phytoplanktons) are the most important primary producers, in fresh or salt waters, and therefore are very important intermediates in the aquatic food chain. Pokrovskaya et al. (1974) reported no statistically significant difference in accumulation of manganese or zinc by Chlorella cells with increasing concentrations of the metals. According to Rachlin and Farran (1974) 2.4 ± 0.02 ppm zinc reduced the growth rate of Chlorella cells by 50% during a 96 hour exposure. In recent report, De Filippis et al. (1981a) indicated that zinc, cadmium

and mercury adversely affected cell division of Euglena, causing also chlorophyll destruction. However, the dry weight and protein content of the cells were little affected. Working with Skeletonema costatum cleve, Thalassiosaria pseudonana and Phaeodactylum tricornutum, Jensen and Rystad (1974) noticed declining relative growth rates, by administration of 50, 250 and 25.000 ug/l of zinc ions, respectively, to the three algae. At least, for one species, a significant increase in zinc uptake by the cells took place at zinc levels which did not seem to influence the growth and development of the alga. Two clones of Skeletonema c. showed significant intraspecific differences regarding the tolerance to zinc pollution. Braek et al. (1976) reported that copper and zinc acted synergistically to the algae except Phaeodactylum tricornutum, where an antagonistic effect was observed. Zinc toxicity to this alga increased at low concentrations of magnesium, indicating a common route for divalent metal ions in general. The toxicity of zinc to Hormidium rivulone (filamentous green alga), in laboratory culture media, decreased by raising magnesium, calcium or phosphate levels and increased with increased pH or cadmium. Cadmium was highly toxic, either alone or in combination with zinc (Say and Whitton, 1977).

In 1975, Singh and Garraway reported that zinc and iron greatly increased growth and sporulation of Colletotrichum

lagenarium. Larger doses of zinc, beyond 1 ppm, were toxic while iron gave beneficial effect up to 25 ppm. On the other hand, Hilgenberg and Hofmann (1977) reported that permanent light and zinc ions, have an antagonistic influence on the tryptophan synthase of Phycomyces blakesleeana. Zhironova et al. (1977) reported that the synthesis of volatile acids by Saccharomyces carlsbergensis depended on the presence of zinc and other trace elements. In presence of zinc yeast cells accumulated large amounts of Zn-protoporphyrin, that inhibited the respiratory activities as well as succinate-cytochrome c reductase and NADH-cytochrome c reductase of the mitochondria (Djavadi et al., 1977). Pryor & Marshall(1977) reported that zinc, at optimum concentration of 5×10^{-4} M, caused a 2-fold stimulation in the level of alcohol dehydrogenase induced by anaerobiosis in roots of Zea mays L. Kositsin and Khalidova (1976) proved that the carboanhydrase of tomato chloroplasts is a zinc-containing enzyme.

The results of Wandzilak & Benson (1978) established yeast nuclear RNA polymerase III as a zinc metalloenzyme while Auld and Atsuya (1976) showed that zinc is a catalytically essential component of the highly purified RNA polymerase I from yeast.

Glyoxalase I from human and porcine erythrocytes, rat liver and yeast also contained zinc in a stoichiometry of about 1 mol/mol of enzyme subunit (Aronsson et al., 1978). Failla and Weinberg (1977) published that intercellular accumulation of zinc by Candida utilis NRRL-Y-7634 was mediated by an energy and temperature-dependent highly specific process exhibiting saturation kinetics. Cells of C. utilis did not possess a zinc-efflux system; they could reduce their level of intercellular zinc only by addition of the metal into daughter cells. Zinc-deficient organisms accumulated 12 times more zinc than did cells of the same culture age grown in zinc-supplement medium.

Weismann (1977), suggested that zinc deficiency may be the cause of the Beau's lines. Pick's disease seemed to be related to enhanced concentrations of zinc in tissues (brain and red cells in humans) along with increased urinary excretion of the metal, possibly correlated with a primary or secondary distribution of zinc transport by blood proteins (Constantinidis et al., 1977). Alkaline RNase, in various tissues of the rat, was inhibited by comparatively high concentrations of zinc, in vitro, but the low concentrations were stimulatory, probably by inactivation of the inhibitor of the enzyme (Chesters and Will, 1978). Zinc also strongly stimulated γ -aminolevulinic acid

dehydrogenase (ALAD) of rabbits in vivo (Haeger-Arrønsen et al., 1976). According to Thomasino et al. (1977) , zinc is probably an important element in the ALAD system in man. Zinc may play a protective role in lead toxicity, and zinc supplementation may be a useful adjunct to chelation therapy for lead toxicity (De Ruiter et al., 1977). Cadmium might inhibit regeneration in the fore limb of the adult newt, Notophthalmus viridescens, through the inactivation of zinc metalloenzymes as result of an exchange of cadmium for zinc. Zinc chloride administered to newts prior to cadmium treatment, may prevent the replacement of zinc by cadmium (Dixon and Compher, 1977).

In higher plants, Takkar et al(1976)noticed that high P supply beyond the optimum P rate produced severe zinc-deficiency symptoms in corn plants without added zinc and a significant decrease in grain and stover yields. The main effect of P on zinc utilization by corn was to reduce the rate of zinc entry into the roots and induced zinc deficiency. Hulagur et al. (1975) reported that copper and P application reduced both content and uptake of zinc by hybrid maize grown in loamy sand . On the other hand, Gupta et al(1977) reported that the uptake and utilization of applied zinc by rice crop, increased with P

application. Mixed N and zinc fertilizers were the most effective measure of controlling apple tree rosetting coupled with the highest yield (Negreev and Tarasov, 1978).

Copper, zinc, manganese and boron increased the metabolism of protein N and organic P. There was a general increase of total and protein N in cotton plant organs, under the influence of zinc and manganese and an increase of total and organic P with zinc, copper and manganese (Tailakov, 1976). Ramirez et al. (1977) established that 1 mM zinc markedly reduced the glutamate dehydrogenase activity from Agave americana L. leaves. 50 % inhibition was reached at 10mM zinc, in undialyzed and dialyzed extracts.

PART (I)
(B) : MATERIAL AND METHODS

B. MATERIAL AND METHODS

The organisms, used in this investigation, Chlorella fusca and Spirulina maxima, were kindly supplied by Sammlung von Algenkulturen, Pflanzenphysiologisches Institut der Universität Göttingen.

CULTURE MAINTAINANCE :

A. Chlorella fusca was maintained in culture on the following nutrient solution (Kuhl, 1962):

Potassium nitrate	1011.10	mg
Sodium dihydrogen phosphate	621.00	"
Disodium hydrogen phosphate	89.00	"
Magnesium sulphate	246.50	"
Calcium chloride	14.70	"
Ferrous sulphate Fe-EDTA complex	6.95	"
Boric acid	0.061	"
Manganese sulphate	0.169	"
Zinc sulphate	0.287	"
Copper sulphate	0.00249	"
Ammonium molybdate	0.01235	"

All in one liter solution and adjusted to pH 6.

Fe-EDTA complex was prepared by dissolving 0.69g Fe SO₄.7H₂O and 0.93g disodium salt of EDTA in 80ml bidistilled water by boiling for

a short time. After the solution is cooled to room temperature it is made up to 100 ml. It contains in 1 ml the iron concentration stated above.

B. Spirulina maxima was maintained in culture on a nutrient medium of the following concentration (Aiba and Ogawa, 1977) :

Solution I :

Sodium bicarbonate	13.61	g
Sodium carbonate	4.03	g
Potassium dihydrogen phosphate	0.50	g
Distilled water	500.00	ml

Solution II :

Sodium nitrate	2.50	g
potassium sulphate	1.00	g
Sodium chloride	1.00	g
Magnesium sulphate	0.20	g
Calcium chloride	0.04	g
Ferric sulphate	0.01	g
EDTA-Na ²⁺	0.08	g
Trace element	5	ml
Distilled water	500.	ml

The two solution (I) and (II) were separately autoclaved and after cooling united together and 5×10^{-6} g vitamin B₁₂ is added.

Trace Element Stock Solution :

	<u>Concentration</u>		<u>used volume</u>	
Zinc sulphate	0.1	%	1.0	ml
Manganese sulphate	0.1	%	2.0	ml
Boric acid	0.2	%	5.0	ml
Cobalt nitrate	0.02	%	5.0	ml
Sodium molybdate	0.02	%	5.0	ml
Copper sulphate	0.0005	%	1.0	ml
Ferric sulphate			0.7	g
EDTA-Na ²⁺			0.8	g
Distilled water			981	ml

The components were prepared in two separated solutions (a + b) and autoclaved for 20 minutes. After cooling the two solutions were added to each other under aseptic conditions.

Solution a : 881 ml distilled water + 19 ml salt-stock solution
(without $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$) + 0.4g EDTA-Na²⁺

Solution b : 100 ml distilled water+0.7g $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ +0.4 g
EDTA- Na^{2+} .

Method of Experimentation :

- A. Synchronous cultures of Chlorella fusca (Pirson and Lorenzen, 1958; Kuhl and Lorenzen, 1964) were performed in 15 culture vessels of 3 cm diameter. This method is based on the light-dark changes with daily dilution of the suspension to a standard cell number with fresh culture medium.

The algae were transferred from the sterile agar slant into the culture tubes each containing 200 ml nutrient medium. After 2 light-dark changes 14: 10 hours (i.e. 2 periods of 24 hours), the culture density reached the arbitrary standard cell number of 1.5×10^6 cells per milliliter of suspension. At this time the cells have already become completely synchronous. At the beginning of the light phase, at least 98% of the cells, counted in the first light of the dark phase, have divided. After counting the cells, using the hemocytometer, the suspensions were diluted with fresh sterile culture medium to the standard cell number ($1.5 \times 10^6/\text{ml}$) which must be

attained for each new 24 hrs _cycle. Immediately after this dilution, a new growth period started under conditions of 10.000 Lux, light intensity, 28°C temperature and aeration with 1.5% v/v carbon dioxide. At the end of the dark phase the algal masses were filtered through a membrane filter (0.2 um pore-wide).

- B. Experimental cultures of Spirulina maxima were prepared by aseptically transferring 5 ml of 5-6 days old culture to 100 ml of the experimental medium. All flasks were incubated at 28-30°C under 10 hours illumination (4000 Lux) with aeration. The air was sterilized by passing over 10% copper sulphate solution followed by 4N sulphuric acid then a 30 cm length of sterile cotton wool glass cylinder followed by sterile distilled water then a sterile empty bottle to prevent back suction. At the expiry of the experimental period (10 days), the culture mass was separated from its medium by suction filtration, washed several times with bidistilled water to dissolve the solving slats. 3 replicates from each treatment (Chlorella or Spirulina) were directly put in a hot air dry oven at 100°C for 4 hours, to determine the changes in the dry weights.

Determination of the Metal Uptake :

A known weight of the dried algal mass (Chlorella or Spirulina) was digested with 10 ml ashing mixture (10 HNO₃: 1 HClO₄: 0.25 H₂SO₄) on a sand bath, till the whole mixture turned colourless. This mixture was made up to 10 ml using HNO₃ (1:2) before assaying its metals content using atomic absorption spectrophotometer (Perkin-Elmer-5000).

Determination of Total Protein Content :

The total protein content was determined as total nitrogen using Kjeldahl method and then multiply with a factor 6.25 to give the total protein content.

A known weight of dried tissue was digested with 30 ml conc. nitrogen-free sulphuric acid together with a small amount of anhydrous potassium sulphate and selenium mixture GR until the whole mixture turned colourless. The digest was quantitatively transferred to the distillation apparatus (Büch I) with 70-100 ml 40% NaOH. The distilled ammonia was received in excess N/20 HCl containing few drops of mixed indicator, then back titrated with N/20 NaOH.

Determination of Soluble protein :

Algal masses were filtered and grinded together with 25 ml fine glass perlen (0.2-0.3 mm ϕ) and 50 ml 70% ethanol

in a mill (Braun, Melsungen, West Germany) for 2 minutes. This time was enough to destroy the cell walls of Chlorella (Richter and Senger, 1964; Beiderbeck and Richter, 1969; Spiess and Richter 1966). The resultant homogenate was filtered and the residue was washed several times with 70% ethanol. The extract and washings were evaporated to dryness then digested in the same manner, mentioned for total protein content.

Determination of Insoluble Protein :

If we subtract the value of soluble protein obtained by the previous procedure from that of total protein, we can easily estimate the value for the insoluble protein.

Determination of the Amino acids :

For the amino acid assays, the fresh biomass of 2 culture vessels was kept at 100°C for 18 hours with 50 ml 6N HCl (Schieler et al., 1953). The dark resultant was filtered on active charcoal and the colourless filtrate was evaporated to dryness. The residue was dissolved in Li-citrate buffer and made up to 25 ml with the same buffer. Then the types and concentrations of the amino acids were determined using Biotronik-Amino acid analyser LC 6000 E. (Moore and Stein, 1951).

Li-Citrate Buffer :

16.9 g Li-citrate
20.0 ml Thiodiethanol
1.0 ml Phenol (10 parts phenol+ 1 part water)

This buffer must be adjusted to pH 2.0 using conc.
HCl 37%.

Pigment Estimation :

The estimation of chlorophyll a and chlorophyll b in Chlorella followed the procedures of Böger (1964). The algal masses were kept with 50 ml 96% methanol in water bath at 50°C for 30 minutes, then filtered. The residue was washed several times with 96% methanol till it became colourless. The combined methanol extracts were made up to volume, before their optical density was measured at 650 and 665 nm. The following equations were applied to calculate the pigment constituents of the experimental alga in mgs per ml.

$$\text{Chl a} = 1.65 \cdot 10^{-2} E_{665} - 0.829 \cdot 10^{-2} E_{650} \text{ mg Chl/ml.}$$

$$\text{Chl b} = 3.376 \cdot 10^{-2} E_{650} - 1.25 \cdot 10^{-2} E_{665} \text{ mg Chl/ml.}$$

$$\text{Chl a+b} = 2.55 \cdot 10^{-2} E_{650} + 0.4 \cdot 10^{-2} E_{665} \text{ mg chl/ml.}$$

For Spirulina, the procedures of Metzner et al. (1965) were followed. The pigments were extracted with methanol :

petroleum ether mixture (10:3), then filtered and evaporated. The residue was taken up with 85 % acetone and its optical density was measured at 663, 644 and 452.5 nm. The following equations were applied to calculate the pigment constituents of the experimental alga in g/L.

$$\text{Chl a} = 11.78 E_{663} - 2.29 E_{644} \quad \text{g/L.}$$

$$\text{Chl b} = 20.05 E_{644} - 4.77 E_{663} \quad \text{g/L.}$$

$$\text{Carotenes} = 4.20 E_{452.5} - 0.0264 \text{ Chla} - 0.496 \text{ Chlb} \quad \text{g/L.}$$

Carbohydrates Estimation :

The anthrone method, by Roe (1955), was applied in this investigation using glucose as a standard. 50 ml ethanol and 100 ml distilled water were poured on the algal masses and the mixture was kept in boiling water bath until the alcohol completely evaporated. 5 ml conc. HCl was added to the solution and make up to 100 ml. This mixture was refluxed for 1 hour on a sand bath then filtered.

3 ml of the filtrate + 6 ml anthrone reagent were kept in a water bath, at 15^oC, for 15 minutes, then transferred to a boiling water bath for 15 minutes, then recooled again at 15^oC for 15 minutes. The optical density of the mixture was then measured at 620 nm.

Soluble Protein Separation :

Polyacrylamide gel electrophoresis has been an extremely useful analytical tool for the separation and quantification of protein species from complex mixtures. In this investigation proteins were separated by isoelectric focusing methods (O'Farrell, 1975).

Sample Preparation :

All procedures for sample preparation must avoid conditions that result in the chemical modification of proteins. An extraction buffer, described by Schaefer (1969), was applied. It has the following composition:

1.211 g Tris
200 mg Na-EDTA
375 mg Na-tetraborate
5 g polyethylenglycol 20 000
200 mg Ascorbic acid.

All dissolved in 100 ml distilled water. The algal masses were grinded with 20 ml glass perlen (0.2-0.3nm Ø) and 10 ml of the above buffer, in a mill, for 2 minutes under cooling. The mixture was centrifugated for 2 minutes at 18,000 rpm then directly assayed or frozen for up to 2 months.

Experimental Procedure :

Equal lengths of gels were set in glass tubing (3.5 x 150 mm inside diameter) and sealed at the bottom with parafilm. The tubes were filled to approximately 80 mm. Each gel was overlayed with 25 ul gel overlay solution and after 1-2 hours, this overlayed solution was removed and replaced with 20 ul of extraction buffer or only distilled water. The gels were allowed to set for 1-2 more hours. After the polymerization of the gels, the parafilm was removed and the glass tubes were placed in the electrophoresis chamber. The water above the gels was removed and the samples were added in amounts corresponding to 50-60 ug protein. The lower reservoir (anode electrode) was filled with 10 mM $H_3 PO_4$ solution while the upper one (cathode electrode) was filled with 20 mM NaOH solution. The samples were overlayed with 10 ul of sample overlay solution. About 8 watt/8gels, 20 milliampier/8 gels and 1000 volts were applied to the isoelectric focusing system for 2.5 hours. The gels were removed from the glass tubes by pressure of water using a 5ml-syringe. The gels were washed with distilled water and kept in fixing solution (15 % TCA) over night. The fixing solution was replaced with 0.1% coomassie blue (coomassie blue R-250 in methanol : acetic acid : water 30 : 6: 30. This staining solution should be filtered before it is used) for 3/4 hour, which is replaced with a destaining solution of methanol:acetic

acid : water 30:6:30 for another 3/4 hour.

The gels could be stored in a 7% solution acetic acid in darkness for a long time.

The absorption curves of proteins in isoelectric focusing gels were measured at 500 nm using chromatogramm spectrophotometer (Zeiss, + M.Pr, splitwide 0.02 mm and split hight 1 mm with velocity 1:1).

Preparation of Enzyme Extracts from Algal Cells :

The algal fresh masses were filtered, thoroughly washed with cold distilled water, then a suitable amount of buffer (different for each enzyme) and glass perlen (0.2-0.3 mm.Ø) were added. The cells were homogenized for 2 minutes under carben dioxide cooling. The homogenate was centrifugated for 20 minutes at 18,000 rpm. The supernatant contained the enzyme extract. All operations were done under temperature between 0 -5°C. The assays were performed on a PM6 Zeiss spectrophotometer.

Estimation of Malate-Dehydrogenase (MDH):

(L-malate; NAD oxidoreductase E.C.1.1.1.37)



Malate dehydrogenase activity in the direction of the malate formation was estimated by the method of Habig and Racusen (1974) and Blackwood and Mifflin (1976) with a slight modification. The enzyme activity was estimated in the crude extract (without centrifugation) because crude extracts showed more MDH-activity than the purified ones (Berger, 1964).

Reagents :

Tris/HCl buffer	0.05 M, pH 7.5, 0.1 mM dithioerythrit.
Oxaloacetate	0.015 M
NADH	0.012 M

Enzyme Assay :

<u>Sample</u>	<u>Blank</u>
2.80 ml Tris/HCl buffer	2.9 ml Tris/HCl buffer
0.10 ml oxaloacetate	
0.05 ml NADH	0.05 ml NADH
The mixture was incubated at 25°C then add:	
<u>0.05 ml enzyme extract</u>	<u>0.05 ml enzyme extract</u>
Total volume = 3ml	3 ml

The extinction changes of coenzyme NADH were measured at 340 nm.

Glutamate Dehydrogenase Activity (GIDH):

(L-glutamate; NAD oxidoreductase deaminating E.C. 1.4.1.2)

According to Berger (1964) and Pahlich and Joy (1971), higher activity of this enzyme can be obtained by using phosphate buffer pH 7.4 for extraction instead of Tris buffer. After centrifugation the tests were performed as follows :

1.55 ml	phosphate buffer pH. 6.7
0.03 ml	NADPH 10 mg/ml
0.10 ml	ammonium acetate 6.0 M
0.05 ml	EDTA 20 mg/ml
0.20 ml	enzyme extract

This mixture with or without NADPH (blank) was incubated at 25°C for 5 minutes then the substrate (0.07 ml of 0.2 M α - Ketoglutaric acid) was added and the extinction differences (ΔE) measured at 366 nm.

PART (II)

ROLE OF HEAVY METALS IN THE GROWTH AND METABOLISM
OF CHLORELLA FUSCA AND SPIRULINA MAXIMA.

P A R T I I

ROLE OF HEAVY METALS IN THE GROWTH AND METABOLISM OF CHLORELLA FUSCA AND SPIRULINA MAXIMA

In this investigation, the author studied the effect of various concentrations ranging between 10^{-7} and 10^{-2} M of cadmium, chromium, nickel and zinc on a unicellular green alga, Chlorella fusca, and a multicellular blue green alga, Spirulina maxima. The elements were supplied in the form of their chlorides except chromium that was supplied as its trioxide.

The experiments were run for 24 hours, in case of Chlorella and continued for 10 days in case of Spirulina.

R E S U L T S

Dry Weight Gain :

Table 1 and Fig. 1 show that the fluctuations in biomass yield of Chlorella fusca, grown in media free of either of the four heavy metal ions, were minor if not insignificant. The low doses of the metals (10^{-7} or 10^{-6} M) hardly affected the dry weight gain but the larger doses seemed inhibitory depending on type of element. Chromium significantly suppressed dry weight gain at 10^{-5} M and totally arrested growth at 10^{-3} M. Cadmium, nickel and zinc behaved almost similarly though

Table (1) : Effect of various concentrations of different heavy metals on biomass of Chlorella fusca after 24 hours growth period.

(mg per algal mass)

Element concentr- ation (Molar)	Mg Biomass Yield							
	Cadmium		Chromium		Nickel		Zinc	
0	40.7		42.0		43.1		46.3	
	41.7	41.8	44.7	42.9	39.3	40.0	41.0	42.6
	43.0		42.0		37.0		40.6	
10^{-7}	38.3		36.3		43.0		42.3	
	41.7	40.3	40.3	39.6	39.0	39.3	39.6	40.4
	41.0		42.3		36.0		39.3	
10^{-6}	35.0		38.3		42.7		45.3	
	38.7	37.7	40.7	40.6	38.0	38.8	41.0	42.1
	39.3		42.7		35.7		40.0	
10^{-5}	31.0		32.3		42.0		44.3	
	35.0	33.0	36.3	35.4	37.0	38.0	41.0	42.2
	33.0		37.7		35.0		41.3	
10^{-4}	27.0		25.6		34.6		45.5	
	30.7	29.1	30.5	28.4	31.4	32.0	40.7	42.3
	29.7		29.1		29.9		40.6	
10^{-3}	26.0		-----		27.0		37.2	
	27.9	27.1	-----		22.6	23.7	34.0	37.2
	27.5		-----		21.6		40.4	
10^{-2}	18.6		-----		25.3		18.4	
	27.3	23.4	-----		20.6	20.5	20.3	19.4
	24.4		-----		15.7		19.5	
L.S.D.								
5%		4.6		4.9		6.8		4.9
1%		6.4		7.0		9.4		6.7
0.1%		8.6		10.0		13.0		9.3

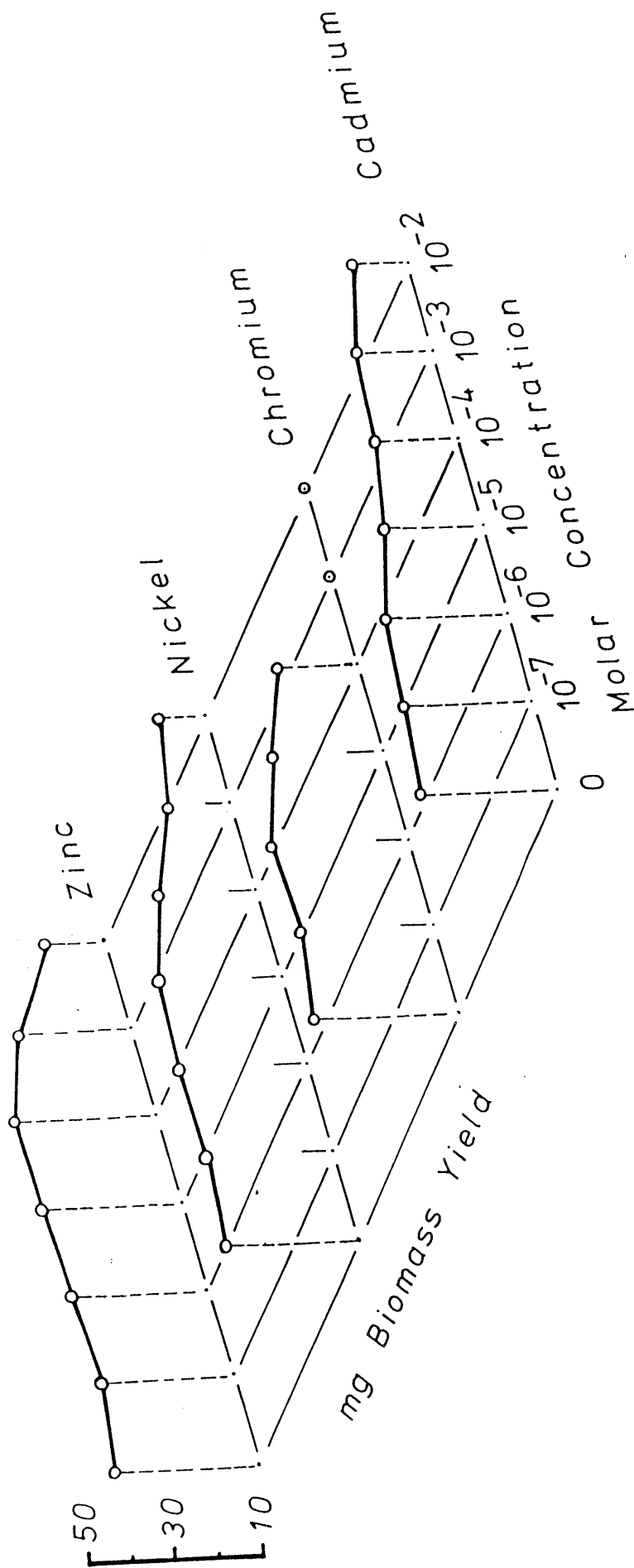


Fig.(1) : Effect of various concentrations of different heavy metals on biomass of Chlorella fusca after 24 hours growth period.

inhibition of dry weight gain started earlier (at 10^{-5} M) with administration of cadmium and latest with administration of zinc (at 10^{-2} M). The highest concentration of the three elements exerted the same suppressive effect.

On the other hand, growth of Spirulina maxima (table 1̄ and Fig. 1̄) though was arrested at 10^{-3} or above of either of the four elements yet the biomass yield significantly increased at 10^{-6} M dose; the effect being most prominent in presence of nickel and least in presence of cadmium. Larger doses did not affect the enhanced dry weight gain, in presence of zinc, but gradually attenuated biomass yield to reach the control level or even below (chromium).

Heavy Metal content :

Table 2 and Fig.2 show that significant accumulation of the added heavy metals started at 10^{-5} M concentration of chromium and at 10^{-3} M of cadmium, nickel or zinc. With further rise of concentration above these levels, Chlorella fusca accumulated larger amounts of prevailing metals, mostly of cadmium or zinc and least from chromium.

On the other hand, Spirulina maxima(table 2̄ and Fig. 2̄) started accumulation of zinc from its least concentration whereas

Table (1) : Effect of various concentrations of different heavy metals on biomass of Spirulina maxima after 10 days growth period.

(mg dry weight)

Element concentr- ation (Molar)	Mg Biomass Yield							
	Cadmium		Chromium		Nickel		Zinc	
0	113.0 105.0 97.0	105.0	113.0 105.0 97.0	105.0	113.0 105.0 97.0	105.0	113.0 105.0 97.0	105.0
10 ⁻⁷	109.7 96.3 119.7	108.6	103.7 112.0 99.3	105.0	113.7 114.0 98.0	108.8	109.3 110.3 97.3	105.6
10 ⁻⁶	130.3 125.0 114.0	123.1	116.7 122.0 110.7	116.5	120.7 131.7 138.0	130.1	115.3 127.3 119.6	120.7
10 ⁻⁵	120.7 108.7 117.0	115.5	112.7 120.7 98.0	110.5	129.3 137.0 115.3	127.2	113.0 120.0 117.3	116.8
10 ⁻⁴	116.7 114.3 101.3	110.8	79.0 92.0 84.8	85.0	112.0 110.0 99.0	107.0	121.3 111.3 124.3	119.0
10 ⁻³	----- ----- -----		----- ----- -----		----- ----- -----		----- ----- -----	
10 ⁻²	----- ----- -----		----- ----- -----		----- ----- -----		----- ----- -----	
L.S.D.								
5%		17.2		15.6		17.7		12.7
1%		24.4		22.1		25.1		18.0
0.1%		35.1		31.9		36.2		26.0

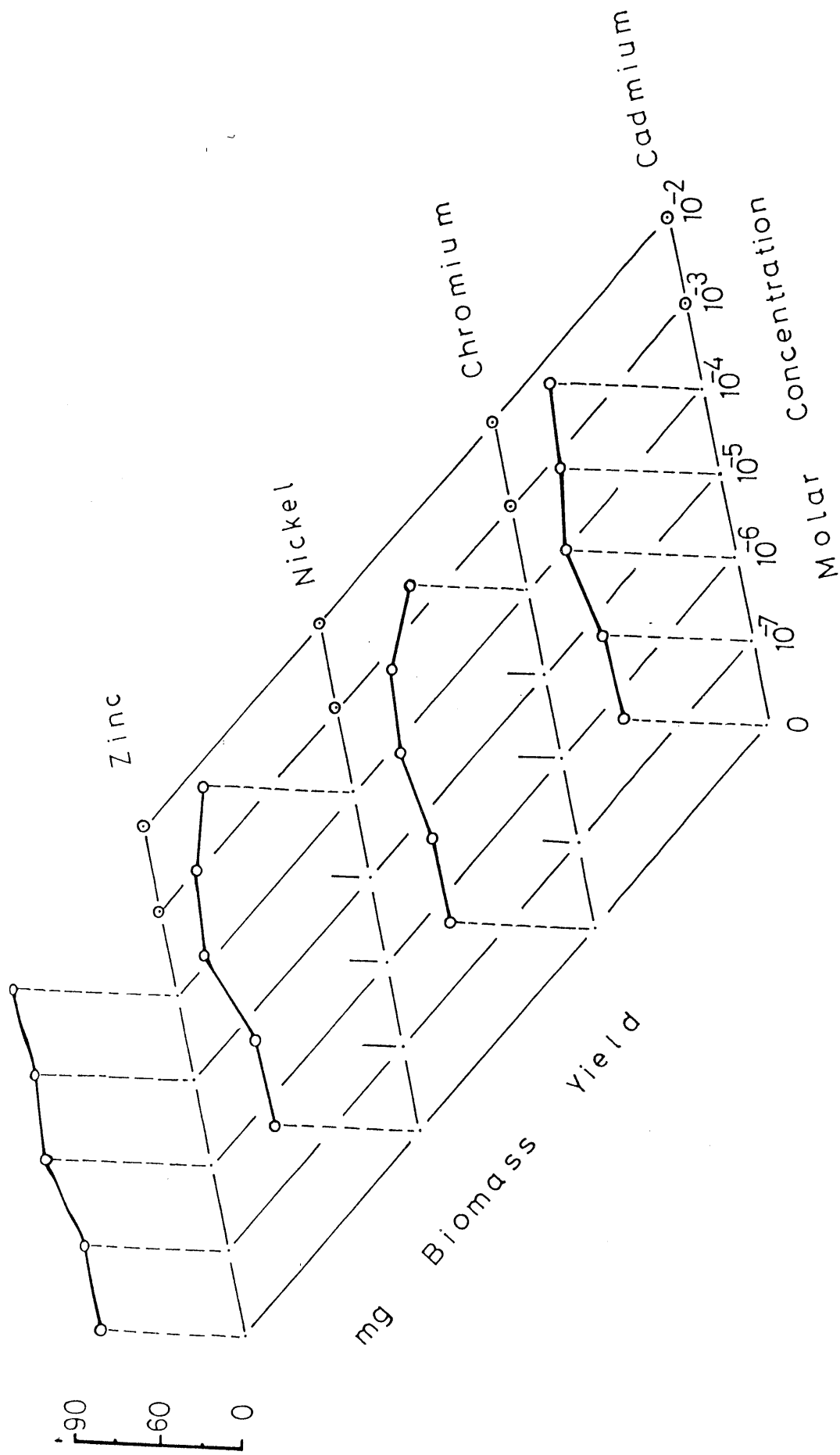


Fig. (1): Effect of various concentrations of different heavy metals on biomass of *Spirulina maxima* after 10 days growth period.

Table (2): The uptake of various concentrations of different heavy metals by Chlorella fusca after 24 hours growth period.

(ug/mg dry weight)

Element concentr- ation (Molar)	Metal uptake							
	Cadmium		Chromium		Nickel		Zinc	
0	0.13		0.25		0.06		0.18	
	0.20	0.17	0.16	0.21	0.03	0.04	0.14	0.20
	0.18		0.23		0.04		0.30	
10^{-7}	0.19		0.31		0.06		0.15	
	0.15	0.18	0.21	0.25	0.03	0.05	0.20	0.20
	0.20		0.24		0.05		0.25	
10^{-6}	0.70		0.39		0.07		0.17	
	0.60	0.77	0.35	0.37	0.04	0.06	0.24	0.20
	1.00		0.36		0.06		0.20	
10^{-5}	2.50		2.0		0.15		0.48	
	1.54	1.81	1.4	1.6	0.13	0.14	0.50	0.53
	1.39		1.4		0.15		0.61	
10^{-4}	18.53		4.1		0.71		19.1	
	12.27	14.34	4.1	4.2	0.74	0.73	10.9	14.0
	12.21		4.3		0.73		12.1	
10^{-3}	227.9		-----		2.53		258.3	
	217.0	223.5	-----		2.15	2.21	196.5	230.3
	225.5		-----		1.94		236.2	
10^{-2}	272.6		-----		9.27		461.2	
	277.8	270.3	-----		7.35	8.57	325.8	367.9
	260.5		-----		9.10		316.8	
L.S.D.								
5%		64.2		0.34		2.9		68.0
1%		88.7		0.49		4.0		94.0
0.1%		122.3		0.70		5.5		127.5

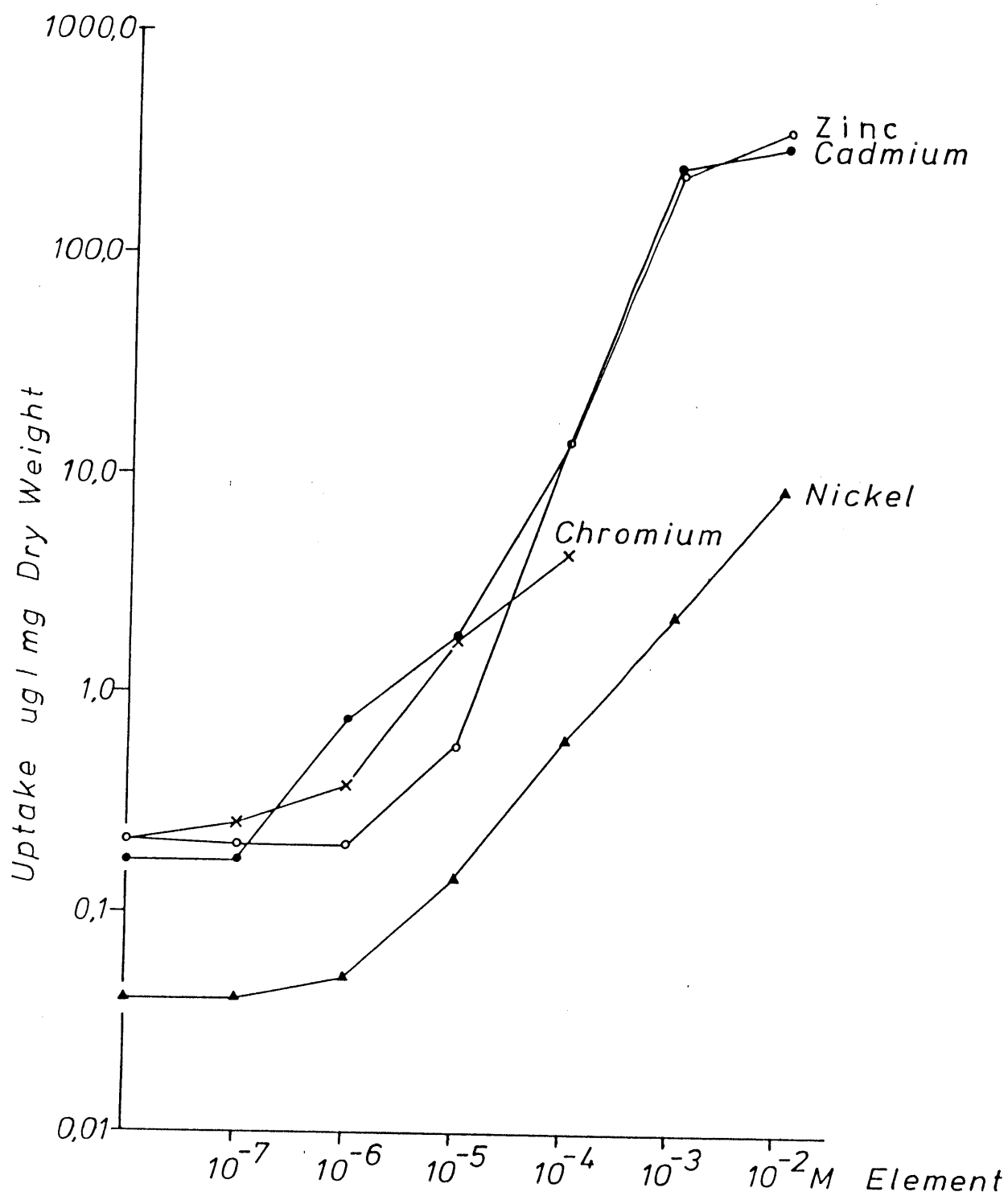


Fig.(2) : Uptake of various concentrations of different heavy metals by *Chlorella fusca* after 24 hours growth period.

Table (2): The uptake of various concentrations of different heavy metals by Spirulina maxima after 10 days growth period.

(ug/mg dry weight)

Element concentr- ation (Molar)	Metal uptake							
	Cadmium		Chromium		Nickel		Zinc	
0	0.009		0.001		0.017		0.037	
	0.008	0.009	0.002	0.002	0.018	0.016	0.036	0.035
	0.010		0.003		0.014		0.032	
10 ⁻⁷	0.009		0.002		0.010		0.109	
	0.009	0.009	0.002	0.002	0.012	0.011	0.106	0.109
	0.010		0.002		0.010		0.113	
10 ⁻⁶	0.009		0.003		0.012		0.130	
	0.009	0.009	0.004	0.003	0.010	0.012	0.131	0.127
	0.010		0.002		0.013		0.120	
10 ⁻⁵	0.040		0.030		0.013		0.120	
	0.040	0.040	0.030	0.030	0.014	0.014	0.128	0.125
	0.040		0.030		0.015		0.128	
10 ⁻⁴	0.250		2.14		0.025		0.283	
	0.240	0.257	2.10	2.15	0.022	0.022	0.234	0.258
	0.280		2.20		0.020		0.256	
L.S.D.								
5%		0.06		0.05		0.01		0.02
1%		0.08		0.06		0.02		0.03
0.1%		0.12		0.09		0.02		0.05

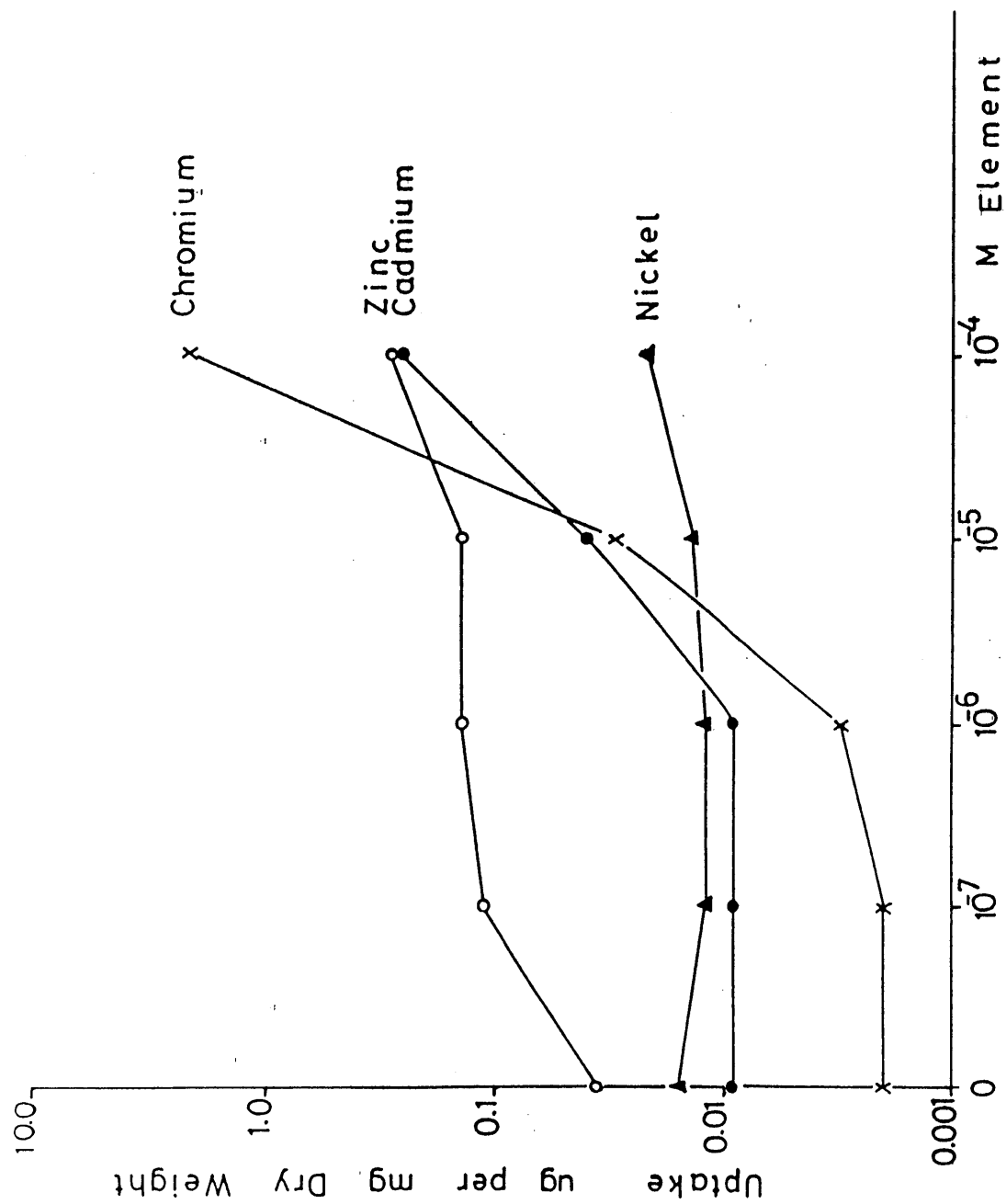


Fig. (2): Uptake of various concentrations of different heavy metals by *Spirulina maxima* after 10 days growth period.

retention of the other elements started at higher concentrations (10^{-5} M for chromium and 10^{-4} M for nickel and cadmium).

Under the same concentration (10^{-5} M or above) Chlorella accumulated larger amounts of either elements compared to Spirulina.

Protein Content :

Table 3 and Fig. 3 show that under normal controlled conditions, the insoluble components formed the major fraction of the protein content (80%) of Chlorella fusca. At lower concentrations (10^{-7} - 10^{-5} M) cadmium seemed with minor, if at all, effects on total protein content whereas the larger doses were inhibitory. Similarly nickel and zinc exerted the same effect though their inhibitory effects were apparent at 10^{-3} M. Chromium was more toxic, causing slight inhibition of protein accumulation that was furthered with increased concentration till cessation of growth at 10^{-3} M.

In spite of these fluctuations, zinc increased the ratio of insoluble protein within Chlorella cells whereas nickel seemed without effect but chromium or cadmium restored the ratio of insoluble protein to normal, with application of their largest dose though smaller doses were stimulatory.

Table (3): Effect of various concentrations of different heavy metals on protein content of *Chlorella fusca* after 24 hours growth
(ug/mg dry weight)

Element Concentration (Molar)	Protein content									
	Cadmium					Chromium				
	Soluble	Insoluble	Total	Soluble	Total	Insoluble	Soluble	Total	Insoluble	Total
0	123.4	456.2	579.6	123.4	579.6	456.2	123.4	579.6	456.2	579.6
	94.3	507.9	602.1	94.3	602.1	507.9	94.3	602.1	507.9	602.1
	107.1	466.3	573.2	107.1	573.2	466.3	107.1	573.2	466.3	573.2
10 ⁻⁷	122.3	426.4	548.7	63.4	493.2	429.8	86.0	579.0	397.1	482.2
	81.4	406.1	487.5	84.8	420.5	387.6	88.2	501.9	358.3	456.3
	75.6	428.4	504.0	56.5	500.5	444.0	82.0	608.0	420.8	483.3
10 ⁻⁶	76.9	554.7	631.6	52.1	489.4	437.3	74.5	563.0	34.6	583.2
	78.8	628.9	707.7	55.8	441.7	445.5	73.4	519.9	30.0	549.9
	87.8	520.0	607.8	55.2	497.5	442.3	74.5	570.7	36.3	607.0
10 ⁻⁵	33.9	521.2	555.1	45.9	490.3	444.4	94.7	515.5	28.2	580.6
	29.0	30.6	59.6	58.5	490.2	446.7	94.7	531.6	24.6	556.2
	28.9	529.2	558.2	41.9	475.0	433.1	94.7	589.8	28.2	618.0
10 ⁻⁴	21.7	423.7	445.4	76.6	279.4	202.8	138.8	308.9	48.8	357.7
	16.0	372.7	388.7	58.1	215.8	216.9	138.8	382.8	32.5	415.3
	18.0	335.9	353.9	46.4	274.0	227.6	146.9	558.0	28.0	586.0
10 ⁻³	25.0	228.3	253.3	---	---	---	129.7	383.5	46.4	430.0
	19.7	269.0	288.7	---	---	---	123.6	397.9	37.3	435.2
	23.0	282.9	305.9	---	---	---	123.6	387.0	55.5	442.5
10 ⁻²	57.0	198.3	255.3	---	---	---	33.7	326.2	15.8	342.0
	45.8	220.5	266.3	---	---	---	29.1	368.0	11.8	379.8
	43.0	245.0	288.0	---	---	---	45.2	330.4	5.6	336.0
L.S.D.	42.5	72.3	82.8	34.3	31.2	35.2	28.1	55.7	14.8	26.2
5%	58.2	99.4	113.4	47.9	44.3	54.1	42.7	78.3	20.4	36.2
1%	81.0	137.9	158.1	70.0	63.8	78.0	58.9	108.0	28.2	49.9

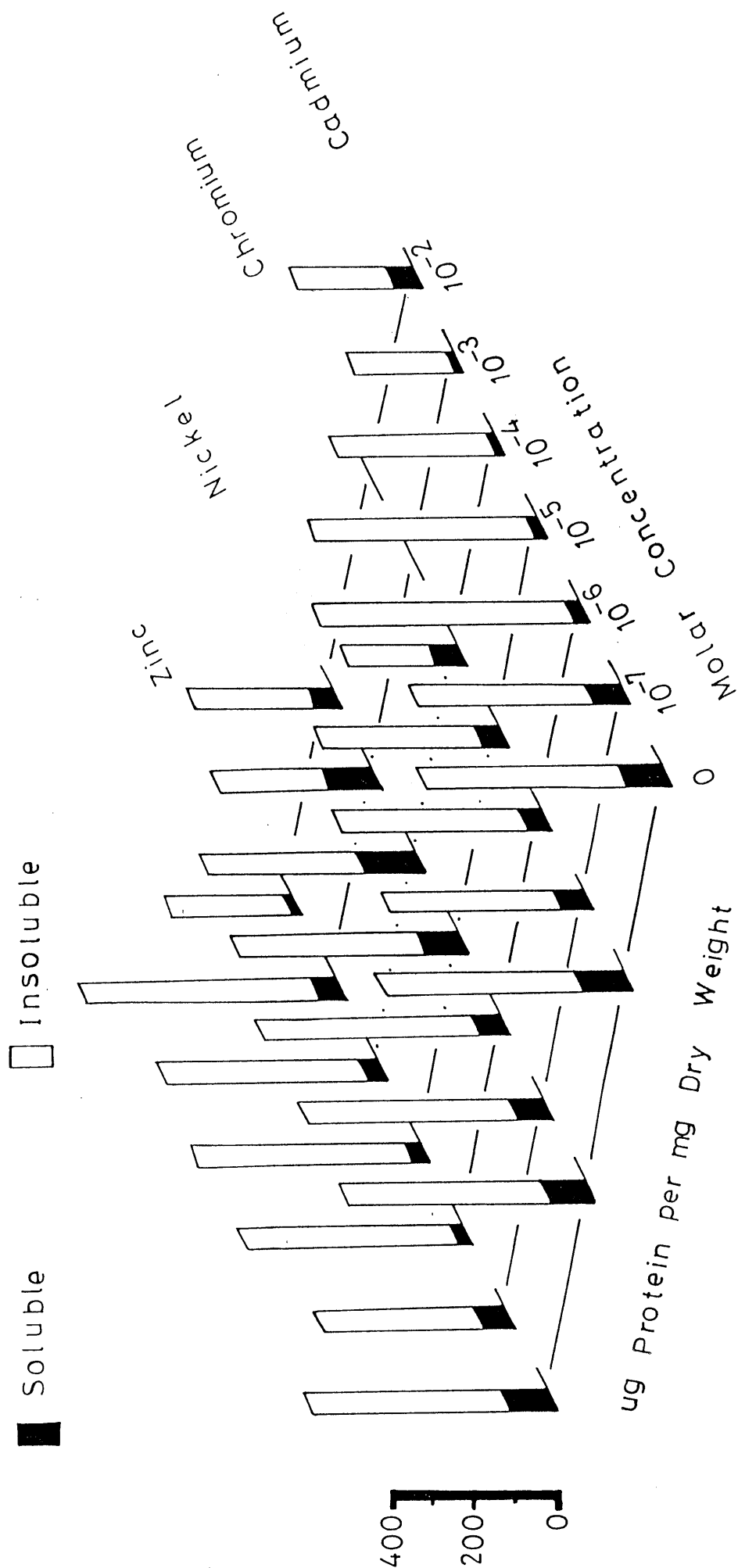


Fig.(3): Effect of various concentrations of different heavy metals on protein content of Chlorella fusca after 24 hours growth period.

Table 4 clearly shows that the four elements favoured a lower soluble protein ratio compared with controls ; a phenomenon that was furthered with increased concentration of the elements particularly zinc and least with nickel. The latter ion stimulated accumulation of this fraction at its moderate concentrations.

These observations were coupled with minor differences, at the smaller doses, in ratio of insoluble protein, relative to control whereas the larger doses were suppressive. In other words, the drop in ratio of total protein was apparently due to failure in soluble protein accumulation, with application of lower concentrations but the higher concentrations of the four elements induced lesser accumulation of both components, with soluble protein being more influenceive. In spite of this drop, there is the tendency towards enhanced insoluble protein accumulation.

Table 3, 4 and Fig. 3 clearly show that the total protein content of Spirulina maxima was indifferent to the various concentrations of either of the four metal ions.

Soluble Protein Separation :

The isoelectric focusing of the various proteins of Chlorella fusca, extracted by tris buffer (Schaefer, 1969),

Table(4): Effect of various concentrations of different heavy metals on protein content of *Chlorella fusca* after 24 hours growth period.

Element concentration (Molar)	Protein Content											
	Cadmium				Chromium				Nickel			
	Soluble	Insoluble	Total		Soluble	Insoluble	Total		Soluble	Insoluble	Total	
10 ⁻⁷	113.2	89.4	93.8	84.3	79.4	83.6	81.4	78.8	102.4	105.3	100.0	83.3
	75.3	86.2	83.3	87.8	78.3	83.0	81.3	86.2	103.1	105.3	100.0	75.2
	70.0	89.9	86.2	82.2	52.2	85.6	75.7	110.3	116.7	107.9	99.0	82.6
10 ⁻⁶	71.2	116.3	108.0	48.1	83.7	84.8	68.8	68.5	106.1	108.9	101.5	115.1
	73.0	131.9	119.1	101.0	111.0	51.0	51.0	50.2	93.4	92.6	83.7	123.8
	81.3	109.1	103.9	81.0	85.0	85.0	68.8	104.1	104.1	97.6	97.6	79.8
10 ⁻⁵	31.4	109.3	94.9	42.4	83.8	87.4	87.4	87.4	88.3	85.3	88.2	115.9
	26.8	115.2	111.8	98.8	96.4	83.8	87.4	87.4	91.6	95.3	90.9	106.6
	26.9	110.9	95.4	38.7	81.2	87.4	87.4	105.9	106.1	106.6	114.1	112.2
10 ⁻⁴	20.1	88.9	76.1	70.7	42.5	47.8	128.2	77.6	77.6	80.3	87.0	101.6
	14.8	78.2	66.4	67.7	53.7	55.7	128.2	130.7	77.0	80.3	86.5	107.0
	16.7	70.5	60.5	42.8	46.8	135.6	135.6	86.2	86.2	89.6	95.4	106.9
10 ⁻³	23.2	47.9	43.3	---	---	---	119.8	53.2	53.2	65.6	65.6	130.4
	18.2	20.9	39.1	44.9	---	---	114.1	116.0	57.5	55.3	68.0	125.2
	21.3	59.3	52.3	---	---	---	114.1	55.2	55.2	66.2	66.2	116.7
10 ⁻²	52.7	41.6	48.9	---	---	---	31.1	61.4	61.4	55.8	55.8	59.8
	40.7	44.4	49.8	50.9	---	---	26.9	33.2	71.1	64.1	62.9	63.1
	39.7	51.4	54.1	---	---	---	41.7	59.9	59.9	61.6	61.6	66.0
L.S.D.	19.8	13.4	12.0	21.1	6.7	3.7	7.8	11.6	11.6	9.4	9.4	20.0
5%	27.7	18.8	16.8	29.4	9.4	5.1	11.0	16.2	16.2	13.1	13.1	27.7
1%	36.9	26.4	23.6	41.3	13.2	7.2	15.4	22.8	22.8	18.4	18.4	38.1
0.1%										17.5	17.5	29.5

Table (3) : Effect of various concentrations of different heavy metals on protein content of Spirulina maxima after 10 days growth period.

(ug/mg dry weight)

Element concentr- ation (Molar)	Total Protein content							
	Cadmium		Chromium		Nickel		Zinc	
0	431.0		431.0		431.0		431.0	
	426.1	429.8	426.1	429.8	426.1	429.8	426.1	429.8
	432.4		432.4		432.4		432.4	
10^{-7}	451.2		378.7		412.2		468.0	
	384.3	424.1	383.5	378.7	456.0	446.8	453.2	444.7
	436.8		373.8		472.2		412.8	
10^{-6}	418.0		469.9		429.0		470.6	
	423.4	443.3	464.5	444.4	474.1	461.0	436.8	434.8
	488.4		398.9		480.0		397.1	
10^{-5}	406.0		381.0		488.5		418.2	
	345.0	394.4	345.6	370.7	442.1	466.4	404.3	416.0
	432.1		385.6		468.6		425.4	
10^{-4}	400.8		444.3		482.0		464.4	
	411.4	420.9	468.2	464.4	480.0	462.9	481.4	464.4
	450.6		480.6		426.7		447.4	
L. S. D.								
5%		68.6		49.0		49.0		11.4
1%		97.4		69.6		69.6		16.2
0.1%		140.4		100.3		100.3		23.4

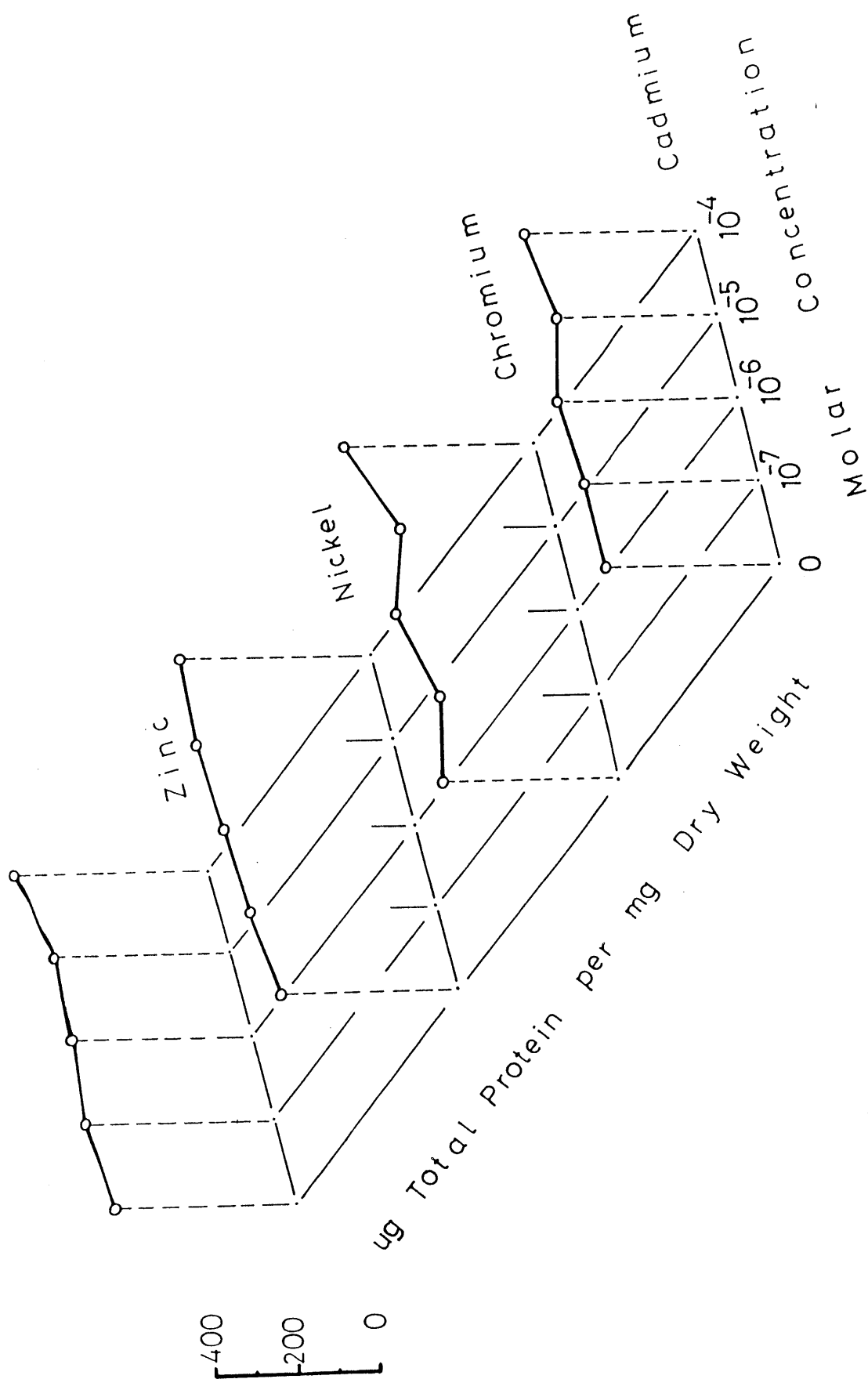


Fig.(3): Effect of various concentrations of different heavy metals on protein content of Spirulina maxima after 10 days growth period.

Table (4): Effect of various concentrations of different heavy metals on protein content of Spirulina maxima after 10 days growth period.

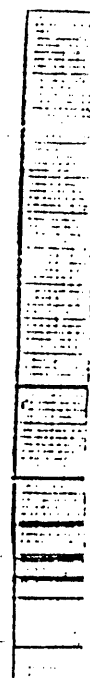
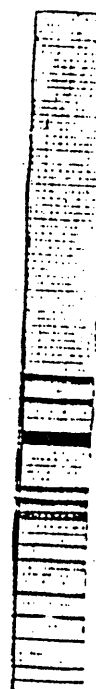
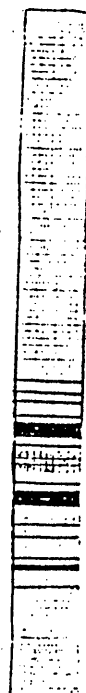
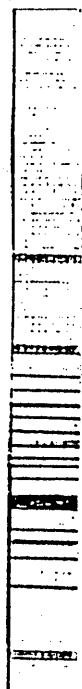
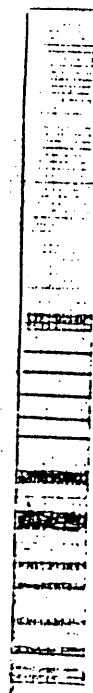
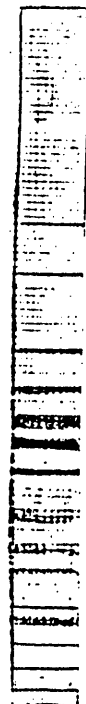
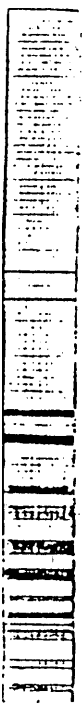
(Percent of control)

Element concentration (Molar)	Total Protein content							
	Cadmium		Chromium		Nickel		Zinc	
10^{-7}	105.0		74.8		95.9		108.9	
	89.4	98.7	89.2	80.9	106.1	104.0	105.4	103.5
	101.6		78.6		110.1		96.1	
10^{-6}	97.3		109.3		99.3		109.5	
	98.5	103.1	108.1	103.4	110.3	107.3	101.6	101.2
	113.5		92.8		111.7		92.4	
10^{-5}	94.5		88.7		113.7		97.3	
	80.3	91.8	80.4	86.3	102.9	108.5	94.1	96.8
	100.5		89.7		109.0		99.0	
10^{-4}	93.3		103.4		112.2		108.1	
	95.7	97.9	109.3	108.2	111.7	107.7	112.0	108.1
	104.8		111.8		99.3		104.1	
L.S.D.								
5%								
1%		17.2		13.2		13.2		11.8
		24.4		18.7		18.7		16.8
0.1%		35.1		27.0		27.0		24.2

using polyacrylamide diffusion gel electrophoresis reveals the fact that most of the proteins were well separated within the neutral and acidic pH range (towards the anode) whereas the alkaline proteins were few and less differentiated (towards the cathode). This is well illustrated by the sharply differentiated protein-coloured bands (using coomassie blue) located on the gels (Plate I) and better recognized by the absorption curves using the chromatogram spectrophotometer (plate II).

Numerous peaks had been observed but the most differentiated were those within the middle pH range and could be differentiated into four zones (A,B,C and D) starting from the acidic range.

Plate II shows that the control samples contain almost equally large amounts of proteins in the four sectors except " C" that contains larger numbers. Cadmium severely dropped number and/or quantity of proteins in sector A and B; more prominently in the former whereas sector C and D were hardly affected, if not increased (Sector C) particularly with application of smaller doses of the ion. In the meantime, the alkaline proteins were better differentiated and increased in quantity particularly with application of larger doses. Similar trends were observed when chromium was applied to the medium



Cadmium

Plate I_a

Control

10^{-7} M

10^{-6} M

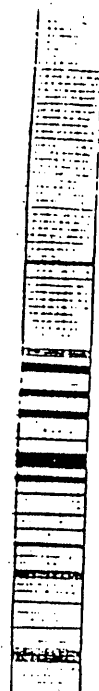
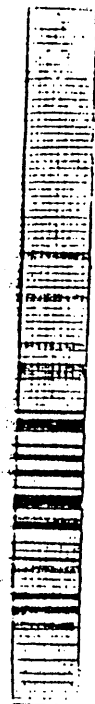
10^{-5} M

10^{-4} M

10^{-3} M

10^{-2} M

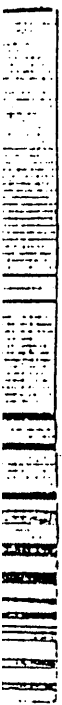
Chromium



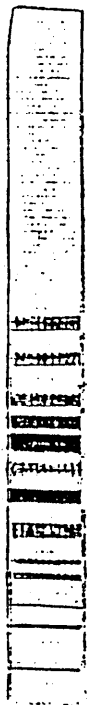
Cadmium

Chromium

Control



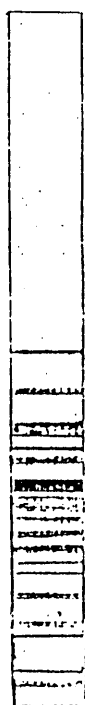
10^{-7} M



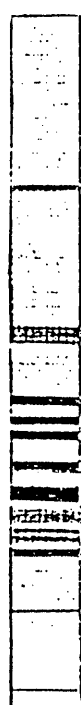
10^{-6} M



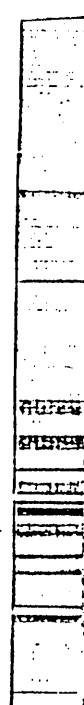
10^{-5} M



10^{-4} M



10^{-3} M



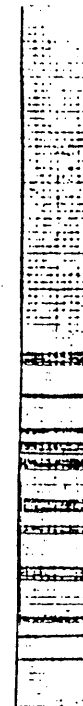
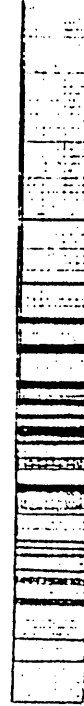
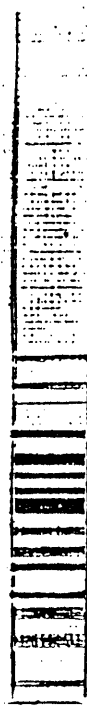
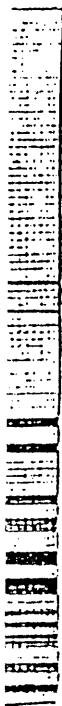
10^{-2} M

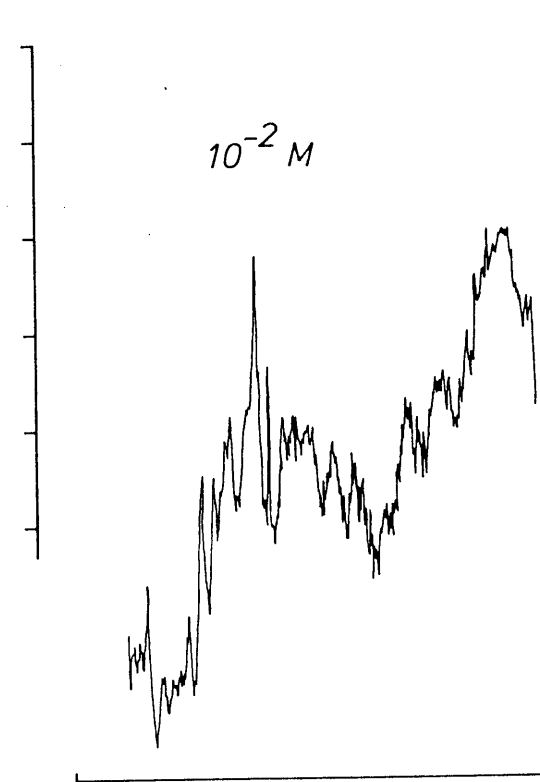
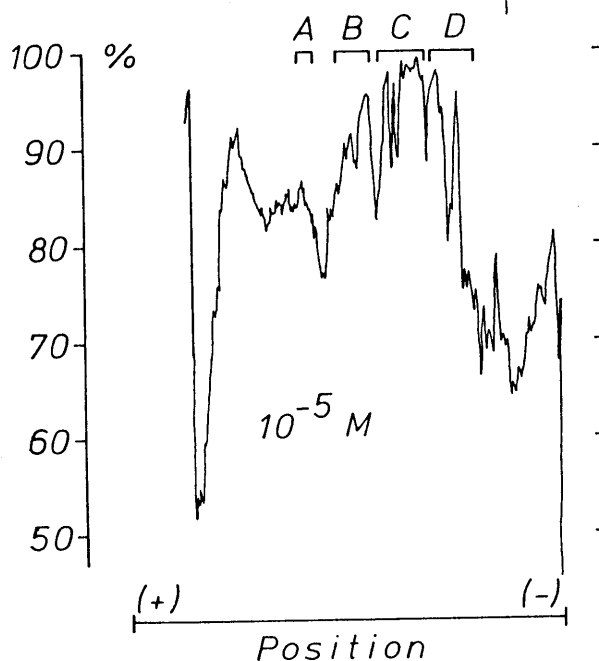
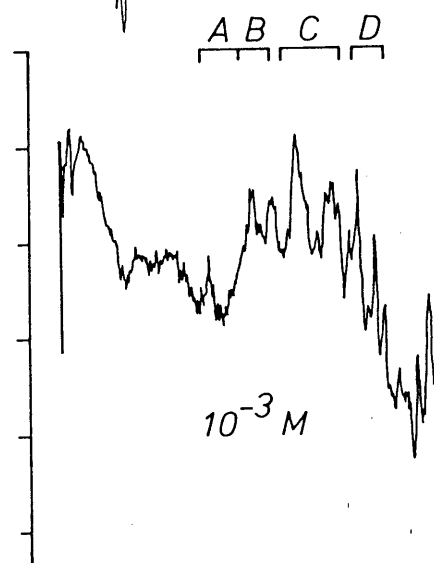
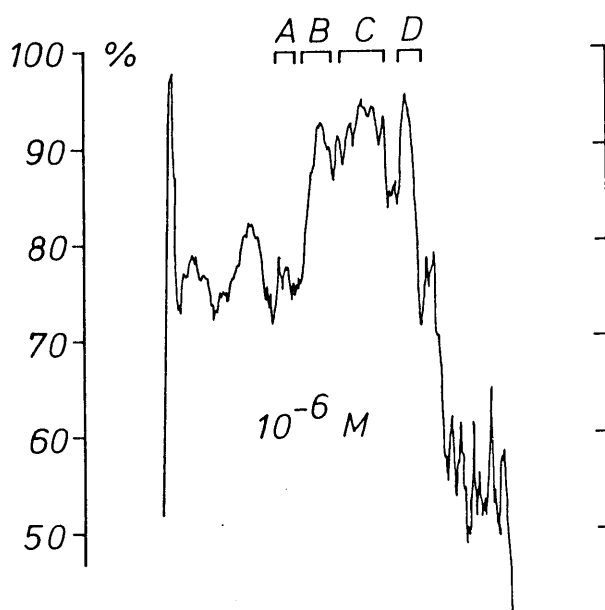
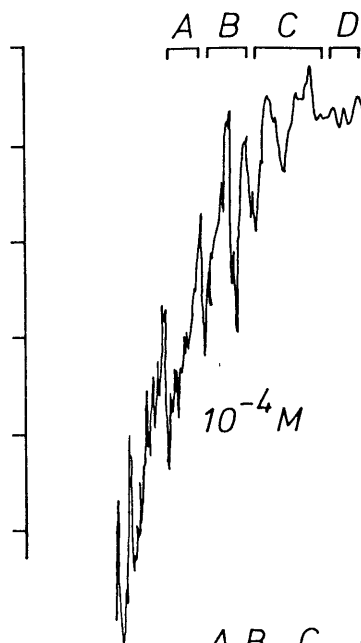
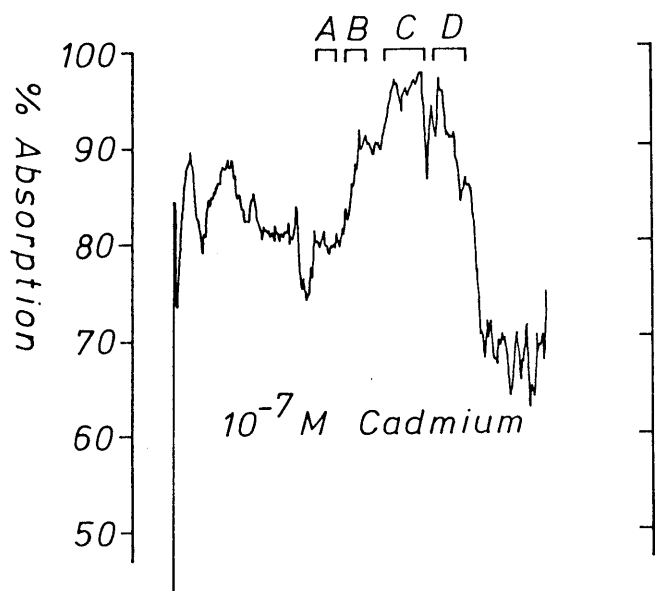


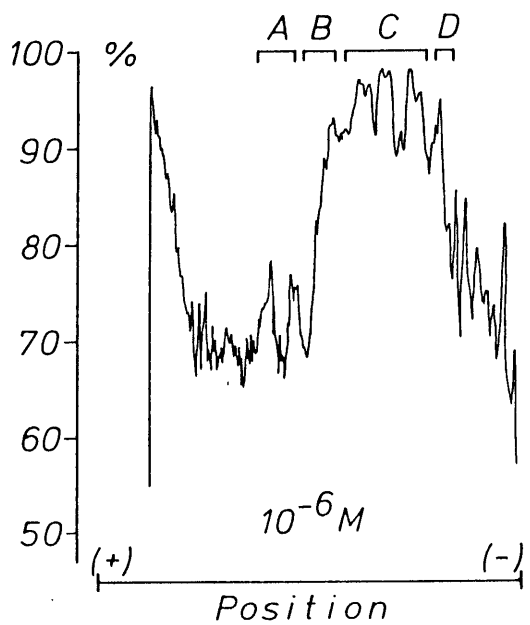
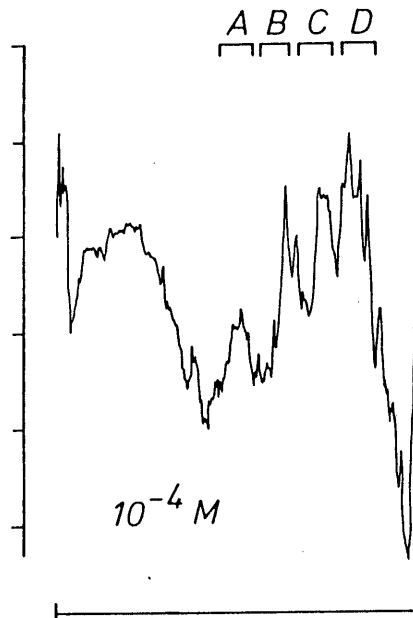
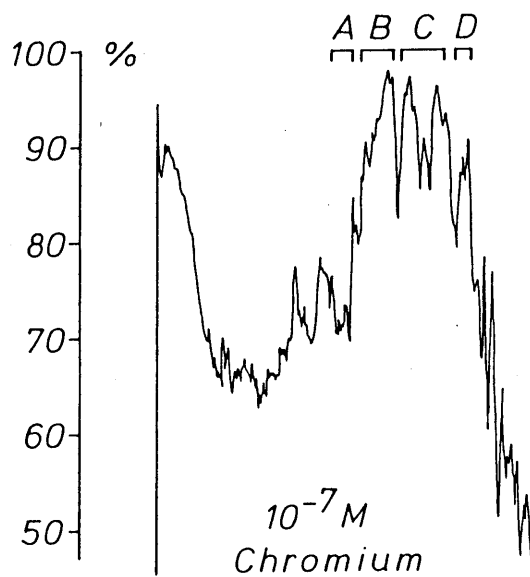
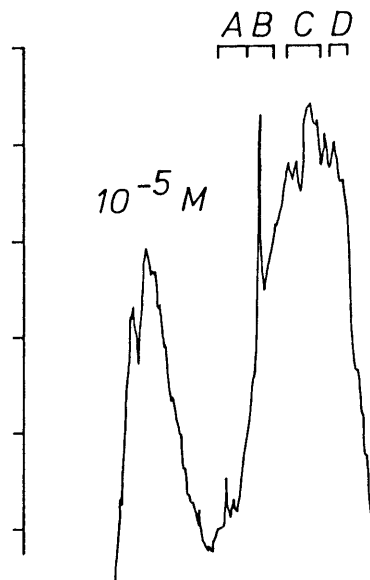
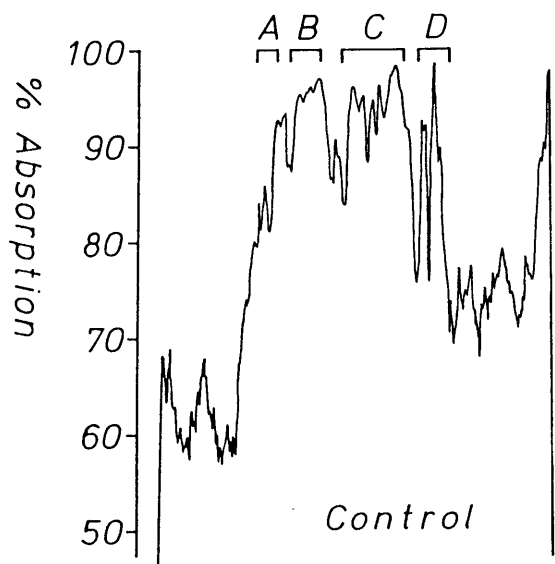
Plate I_b

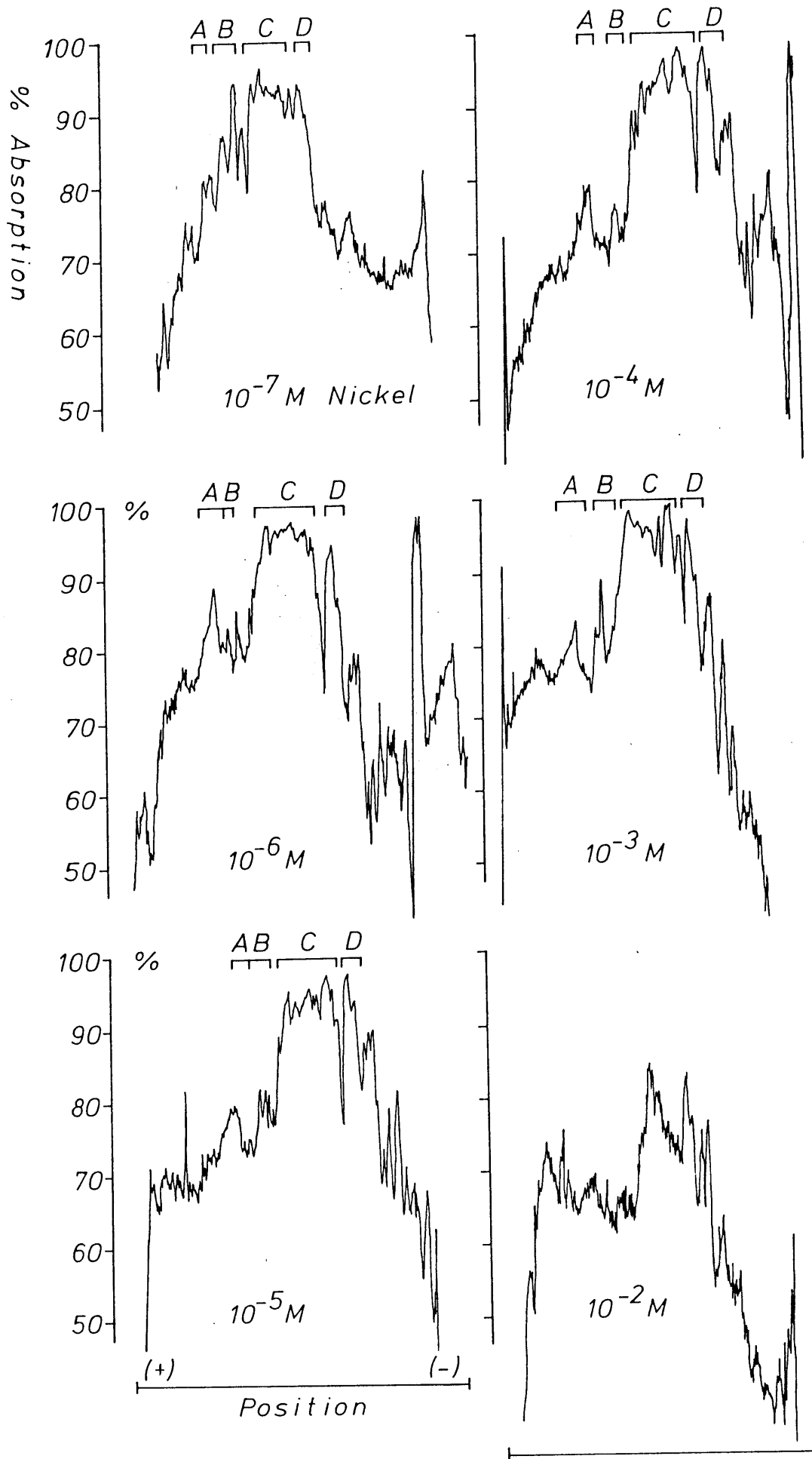
Nickel

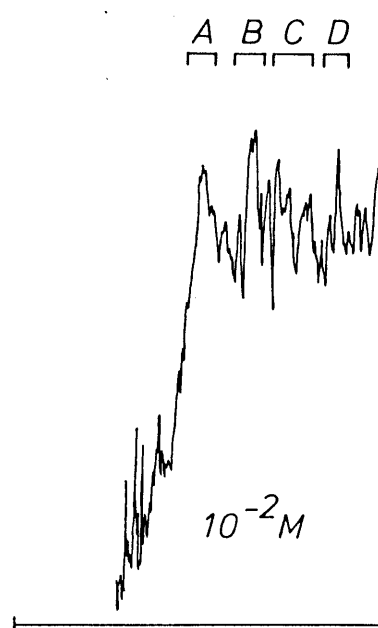
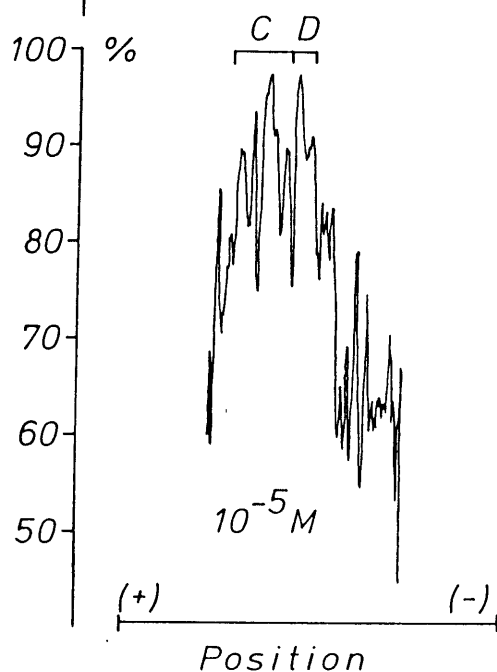
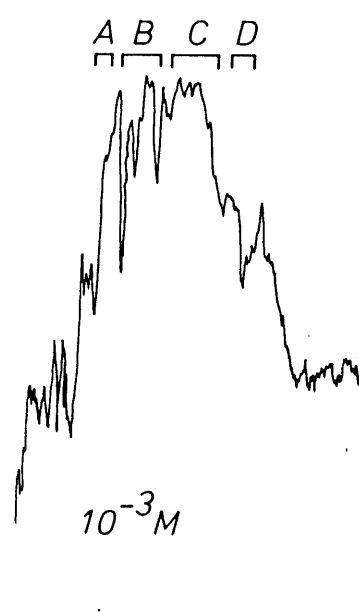
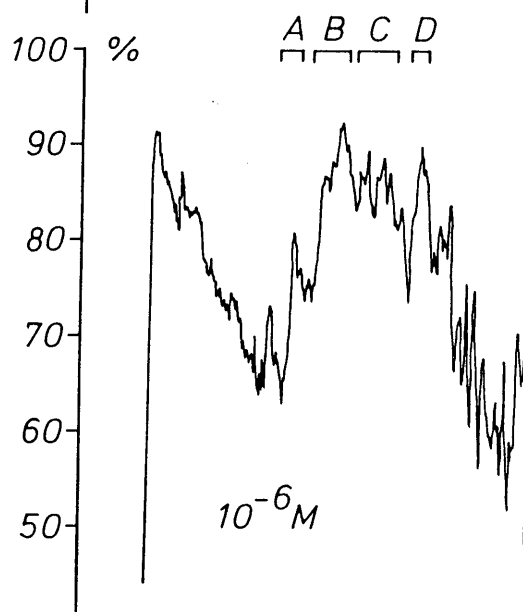
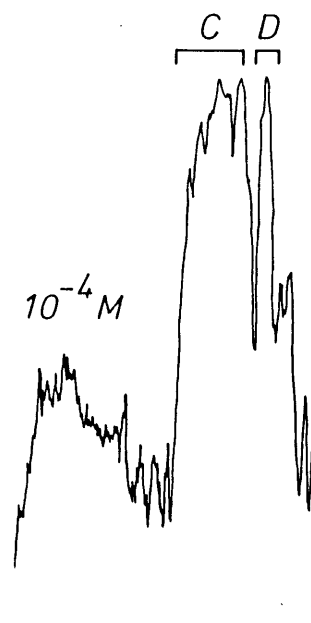
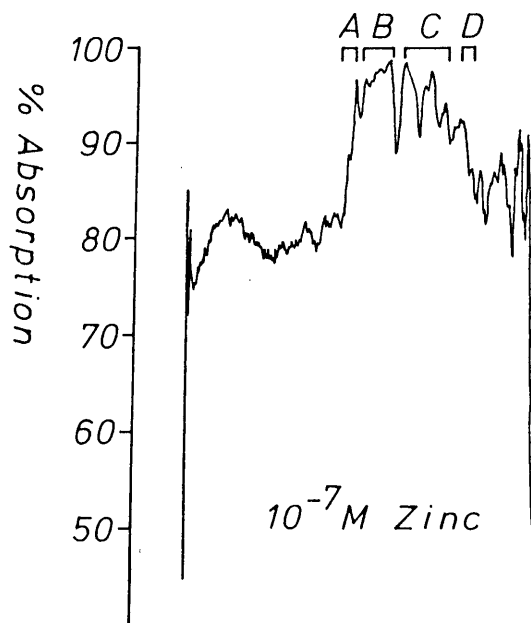
Zinc











though proteins in sector C decreased in quantity and number at the higher concentrations of the ion coupled with less differentiation of alkaline proteins, if not disappearing.

Nickel had similar effects like those of cadmium but the alkaline proteins were far better differentiated in quantity and/or quality. On the other hand, the lower concentrations of zinc (10^{-7} - 10^{-6} M) seemed to have little , if at all, effect on the soluble protein pattern of Chlorella fusca but the larger doses seemed to inhibit the formation of the more acidic proteins in sectors A and B, with little effect on those of sectors C and D. Under both conditions, alkaline proteins seemed hardly affected though increasing at 10^{-2} M concentration.

Amino Acid contents :

Table 5 and Fig. 4, show that either cadmium or chromium were suppressive to amino acid accumulation in the biomass of Chlorella fusca ; a phenomenon that was furthered with rise of

Table (5): Effect of various concentrations of different heavy metals on amino-acids concentrations of *Chloralla fusca* after 24 hours growth period
($\mu\text{g}/\text{mg}$ dry weight)

Element Concentration (Molar)	Amino - Acids										Concentrations											
	Asp.	Thr.	Ser.	Glu.	Pro.	Gly.	Ala.	Val.	Cys.	Met.	Ile.	Leu.	Phe.	Lys.	His.	Arg.	Tyr.	Orn.	Total			
0	55.1	52.4	29.5	27.8	26.9	25.6	33.4	42.5	29.7	2.2	2.3	8.0	19.6	19.7	45.1	45.7	1.5	1.6	36.3	11.1	30.1	457.8
Cd ²⁺	30.3	29.7	17.1	14.6	14.9	13.9	13.7	19.0	17.5	1.3	1.3	3.5	4.4	10.8	11.4	27.1	26.0	0.7	22.1	18.0	254.5	
	29.1	29.7	14.4	15.8	14.3	14.6	14.9	18.7	17.5	1.3	1.3	3.5	4.4	10.8	11.4	27.1	26.0	0.7	22.1	18.0	254.5	
Cr ⁶⁺	38.9	36.9	18.4	19.6	14.6	14.6	14.6	19.2	19.2	19.2	19.2	19.2	19.2	19.2	19.2	19.2	19.2	19.2	19.2	19.2	303.7	
	34.8	36.9	20.7	20.8	19.2	19.2	19.2	19.2	19.2	19.2	19.2	19.2	19.2	19.2	19.2	19.2	19.2	19.2	19.2	19.2	303.7	
10 ⁻⁷	49.1	46.2	27.1	24.8	25.5	25.5	30.3	54.5	24.0	21.3	26.0	21.3	26.0	21.3	26.0	21.3	26.0	21.3	26.0	21.3	328.1	
Ni ²⁺	43.3	46.2	27.1	24.8	25.5	25.5	30.3	54.5	24.0	21.3	26.0	21.3	26.0	21.3	26.0	21.3	26.0	21.3	26.0	21.3	328.1	
	43.3	46.2	27.1	24.8	25.5	25.5	30.3	54.5	24.0	21.3	26.0	21.3	26.0	21.3	26.0	21.3	26.0	21.3	26.0	21.3	328.1	
Zn ²⁺	49.2	49.2	27.1	24.8	25.5	25.5	30.3	54.5	24.0	21.3	26.0	21.3	26.0	21.3	26.0	21.3	26.0	21.3	26.0	21.3	402.3	
	35.7	51.5	25.8	24.5	24.5	24.5	24.5	24.5	24.5	24.5	24.5	24.5	24.5	24.5	24.5	24.5	24.5	24.5	24.5	24.5	386.0	
Cu ²⁺	50.6	46.7	27.3	25.6	23.5	23.5	30.2	56.7	23.5	23.5	30.2	56.7	23.5	23.5	30.2	56.7	23.5	23.5	30.2	56.7	449.0	
	42.7	46.7	27.3	25.6	23.5	23.5	30.2	56.7	23.5	23.5	30.2	56.7	23.5	23.5	30.2	56.7	23.5	23.5	30.2	56.7	449.0	
Co ²⁺	40.9	39.1	20.9	22.4	21.6	21.6	21.6	21.6	21.6	21.6	21.6	21.6	21.6	21.6	21.6	21.6	21.6	21.6	21.6	21.6	384.5	
	37.2	39.1	20.9	22.4	21.6	21.6	21.6	21.6	21.6	21.6	21.6	21.6	21.6	21.6	21.6	21.6	21.6	21.6	21.6	21.6	384.5	
10 ⁻⁶	47.9	47.3	23.5	24.7	24.4	24.4	24.4	24.4	24.4	24.4	24.4	24.4	24.4	24.4	24.4	24.4	24.4	24.4	24.4	24.4	330.2	
Ni ²⁺	46.7	47.3	23.5	24.7	24.4	24.4	24.4	24.4	24.4	24.4	24.4	24.4	24.4	24.4	24.4	24.4	24.4	24.4	24.4	24.4	330.2	
	46.7	47.3	23.5	24.7	24.4	24.4	24.4	24.4	24.4	24.4	24.4	24.4	24.4	24.4	24.4	24.4	24.4	24.4	24.4	24.4	330.2	
Zn ²⁺	38.5	41.4	40.0	22.6	24.6	17.3	18.8	40.1	43.1	17.4	19.0	22.9	24.0	22.9	24.0	22.9	24.0	22.9	24.0	22.9	400.3	
	38.5	41.4	40.0	22.6	24.6	17.3	18.8	40.1	43.1	17.4	19.0	22.9	24.0	22.9	24.0	22.9	24.0	22.9	24.0	22.9	400.3	
Cd ²⁺	36.0	37.0	20.2	21.7	18.5	18.5	44.0	16.7	17.4	23.7	23.6	23.6	23.6	23.6	23.6	23.6	23.6	23.6	23.6	23.6	327.7	
	36.0	37.0	20.2	21.7	18.5	18.5	44.0	16.7	17.4	23.7	23.6	23.6	23.6	23.6	23.6	23.6	23.6	23.6	23.6	23.6	327.7	
Cr ⁶⁺	39.0	37.7	21.7	21.4	20.9	19.9	47.1	18.7	17.4	24.0	23.0	23.0	23.0	23.0	23.0	23.0	23.0	23.0	23.0	23.0	315.1	
	39.0	37.7	21.7	21.4	20.9	19.9	47.1	18.7	17.4	24.0	23.0	23.0	23.0	23.0	23.0	23.0	23.0	23.0	23.0	23.0	315.1	
10 ⁻⁵	40.5	42.3	21.8	22.2	21.9	21.1	51.8	50.4	23.8	25.6	27.0	33.0	34.6	27.0	33.0	34.6	27.0	33.0	34.6	27.0	318.5	
Ni ²⁺	44.1	42.3	21.8	22.2	21.9	21.1	51.8	50.4	23.8	25.6	27.0	33.0	34.6	27.0	33.0	34.6	27.0	33.0	34.6	27.0	318.5	
	44.1	42.3	21.8	22.2	21.9	21.1	51.8	50.4	23.8	25.6	27.0	33.0	34.6	27.0	33.0	34.6	27.0	33.0	34.6	27.0	318.5	
Zn ²⁺	37.0	37.2	22.8	18.0	18.2	18.3	37.2	37.3	18.3	18.3	23.7	23.6	23.6	23.6	23.6	23.6	23.6	23.6	23.6	23.6	383.7	
	37.0	37.2	22.8	18.0	18.2	18.3	37.2	37.3	18.3	18.3	23.7	23.6	23.6	23.6	23.6	23.6	23.6	23.6	23.6	23.6	383.7	
Cd ²⁺	47.0	45.2	25.2	24.5	23.1	23.1	55.3	56.6	21.2	21.8	28.7	28.9	37.1	36.6	26.8	26.8	26.8	26.8	26.8	26.8	284.7	
	43.3	45.2	25.2	24.5	23.1	23.1	55.3	56.6	21.2	21.8	28.7	28.9	37.1	36.6	26.8	26.8	26.8	26.8	26.8	26.8	284.7	
Cr ⁶⁺	27.9	28.8	16.2	16.6	15.0	15.2	38.5	39.2	13.3	13.8	19.8	18.9	24.3	24.1	17.2	17.6	1.7	1.4	4.8	5.0	383.8	
	27.9	28.8	16.2	16.6	15.0	15.2	38.5	39.2	13.3	13.8	19.8	18.9	24.3	24.1	17.2	17.6	1.7	1.4	4.8	5.0	383.8	
10 ⁻⁴	46.2	47.8	27.7	27.4	27.5	62.8	63.2	26.3	26.4	31.3	31.6	41.9	41.6	29.5	29.1	3.7	3.7	4.7	4.0	17.6	268.8	
Ni ²⁺	49.3	47.8	27.7	27.4	27.5	62.8	63.2	26.3	26.4	31.3	31.6	41.9	41.6	29.5	29.1	3.7	3.7	4.7	4.0	17.6	268.8	
	49.3	47.8	27.7	27.4	27.5	62.8	63.2	26.3	26.4	31.3	31.6	41.9	41.6	29.5	29.1	3.7	3.7	4.7	4.0	17.6	268.8	
Zn ²⁺	40.6	40.1	23.5	25.2	19.4	17.8	18.6	45.4	47.1	20.8	20.2	23.4	23.4	23.4	23.4	23.4	23.4	23.4	23.4	23.4	455.5	
	39.5	40.1	23.5	25.2	19.4	17.8	18.6	45.4	47.1	20.8	20.2	23.4	23.4	23.4	23.4	23.4	23.4	23.4	23.4	23.4	455.5	
Cd ²⁺	20.8	24.9	15.2	13.7	10.7	12.3	28.9	30.8	10.5	10.2	13.3	13.3	13.3	13.3	13.3	13.3	13.3	13.3	13.3	13.3	344.3	
	20.8	24.9	15.2	13.7	10.7	12.3	28.9	30.8	10.5	10.2	13.3	13.3	13.3	13.3	13.3	13.3	13.3	13.3	13.3	13.3	344.3	
Cr ⁶⁺	35.7	37.1	21.1	21.4	19.7	20.3	58.3	59.3	17.4	17.2	22.7	23.4	33.7	22.0	21.2	1.9	1.7	2.3	2.6	9.3	210.3	
	35.7	37.1	21.1	21.4	19.7	20.3	58.3	59.3	17.4	17.2	22.7	23.4	33.7	22.0	21.2	1.9	1.7	2.3	2.6	9.3	210.3	
10 ⁻³	35.7	37.1	21.1	21.4	19.7	20.3	58.3	59.3	17.4	17.2	22.7	23.4	33.7	22.0	21.2	1.9	1.7	2.3	2.6	9.3	210.3	
Ni ²⁺	35.7	37.1	21.1	21.4	19.7	20.3	58.3	59.3	17.4	17.2	22.7	23.4	33.7	22.0	21.2	1.9	1.7	2.3	2.6	9.3	210.3	
	35.7	37.1	21.1	21.4	19.7	20.3	58.3	59.3	17.4	17.2	22.7	23.4	33.7	22.0	21.2	1.9	1.7	2.3	2.6	9.3	210.3	
(Zn ²⁺	28.6	30.7	16.1	17.4	10.2	11.9	31.5	32.7	18.8	15.0	20.5	19.3	23.4	24.5	16.7	17.8	2.9	2.6	2.6	2.6	356.3	
	28.6	30.7	16.1	17.4	10.2	11.9	31.5	32.7	18.8	15.0	20.5	19.3	23.4	24.5	16.7	17.8	2.9	2.6	2.6	2.6	356.3	
Cd ²⁺	19.9	16.7	12.1	10.5	10.7	29.2	27.2	8.2	8.8	13.2	12.7	16.7	17.1	10.2	9.5	0.8	0.8	0.8	0.8	0.8	163.3	
	13.4	16.7	12.1	10.5	10.7	29.2	27.2	8.2	8.8	13.2	12.7	16.7	17.1	10.2	9.5	0.8	0.8	0.8	0.8	0.8	163.3	
Cr ⁶⁺	27.1	28.7	13.7	13.5	13.7	44.2	43.9	13.8	13.4	17.6	17.3	24.2	23.8	16.7	16.3	1.9	2.2	2.5	2.5	278.2		
10 ⁻²	26.3	28.7	13.7	13.5	13.7	44.2	43.9	13.8	13.4	17.6	17.3	24.2	23.8	16.7	16.3	1.9	2.2	2.5	2.5	278.2		
Ni ²⁺	16.4	16.3	2.0	2.1	8.5	8.3	21.0	21.5	8.0	7.8	10.7	10.5	13.9	13.6	9.5	9.4	1.4	1.5	1.5	1.5	143.9	
	16.4	16.3	2.0	2.1	8.5	8.3	21.0	21.5	8.0	7.8	10.7	10.5	13.9	13.6	9.5	9.4	1.4	1.5	1.5	1.5	143.9	

L.S.D.

Cd^{2+} 5%

Cr^{6+} 0.1%

Ni^{2+} 5%

Zn^{2+} 5%

Cu^{2+} 5%

Co^{2+} 5%

Mn^{2+} 5%

Fe^{2+} 5%

Ca^{2+} 5%

Mg^{2+} 5%

K^{+} 5%

Na^{+} 5%

Cl^{-} 5%

SO_4^{2-} 5%

NO_3^{-} 5%

PO_4^{3-} 5%

$\text{H}_2\text{PO}_4^{-}$ 5%

HCO_3^{-} 5%

H_2CO_3 5%

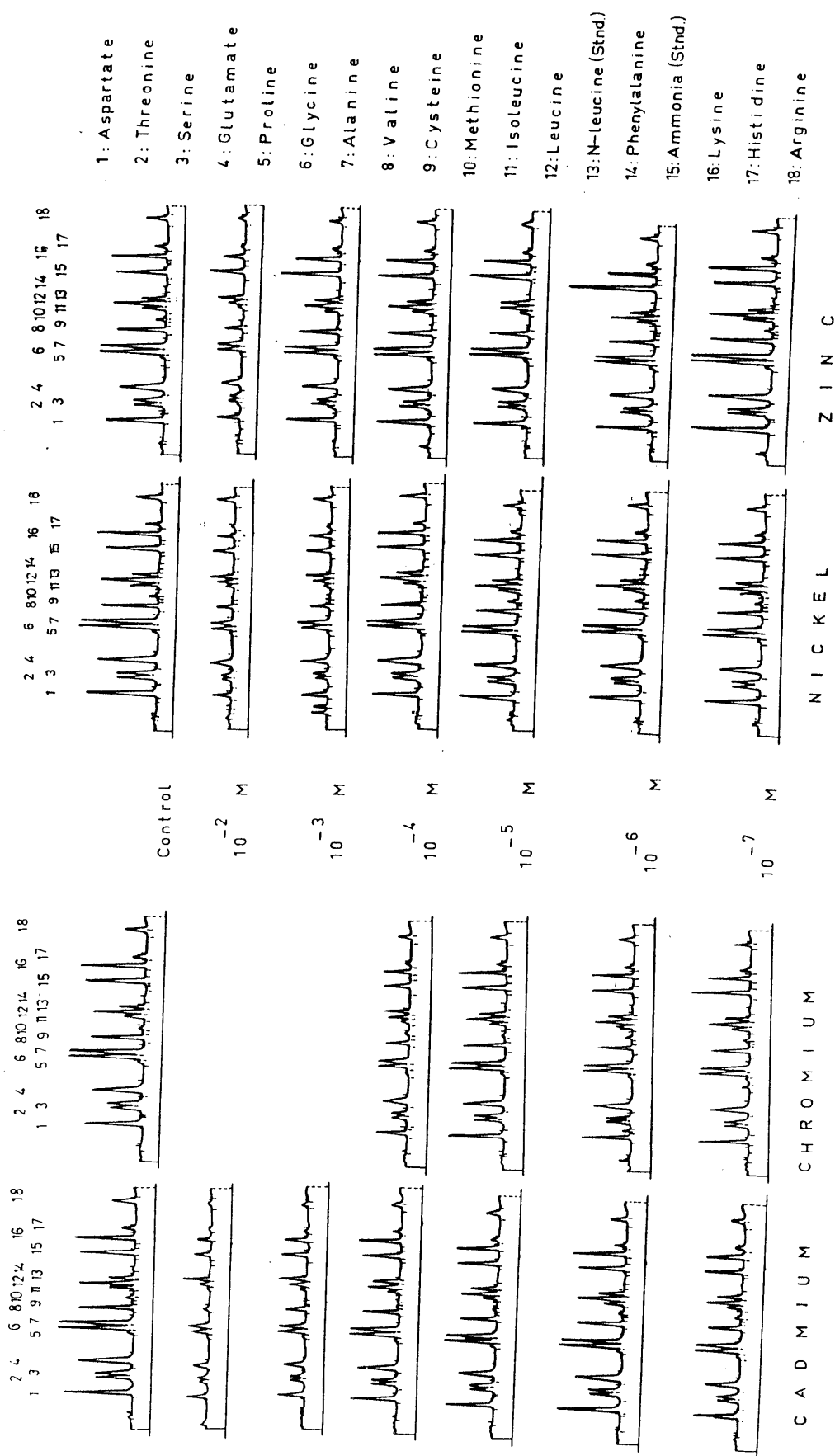


Fig.(4): Effect of various concentrations of different heavy metals on amino acids concentrations of Chlorella fusca after 24 hours growth period.

concentration particularly with the latter ion. All amino acid components participated in such event. On the other hand, nickel exerted no effect at its lower doses but the larger doses ($10^{-3}M$ or above) were suppressive to all components of the total amino acid content except arginine that accumulated in remarkably large quantities independent of concentration of nickel.

Carbohydrate Content :

Table 6 and Fig.5 show that neither cadmium nor zinc seemed to exert any effect on the total carbohydrate content of Chlorella fusca except at $10^{-2}M$ where an appreciable increase was observed, more-prominently with the latter element. Nickel behaved similarly though the increase was apparent at $10^{-4}M$ and slightly increased with rise of concentration to $10^{-2}M$. Chromium ions also initiated carbohydrate accumulation at $10^{-6}M$; a phenomenon that was furthered with increased concentration to $10^{-4}M$ above which growth stopped.

Table 6 and Fig. 5 show that within the range of $10^{-7}M$ to $10^{-4}M$, cadmium hardly affected the total carbohydrate content of Spirulina maxima whereas chromium was suppressive, more prominently at its higher concentrations. On the other hand, nickel or zinc seemed without effect till $10^{-5}M$ concentration

Table (6): Effect of various concentrations of different heavy metals on total carbohydrate content of Chlorella fusca after 24 hours growth period.

(ug/mg dry weight)

Element concentration (Molar)	Carbohydrate content							
	Cadmium		Chromium		Nickel		Zinc	
0	188.4		222.1		263.5		260.9	
	188.4	190.2	215.7	209.4	263.5	260.7	244.8	258.7
	193.7		190.4		255.1		270.3	
10^{-7}	203.0		200.0		375.6		333.5	
	230.0	213.3	206.2	204.1	321.3	351.4	357.5	346.0
	207.0		206.2		357.4		346.9	
10^{-6}	237.0		250.0		338.7		279.9	
	259.3	248.7	259.8	251.7	267.6	296.9	268.2	282.2
	250.0		245.2		284.4		298.4	
10^{-5}	180.4		287.2		293.2		203.6	
	192.8	184.5	336.4	303.6	288.3	275.1	244.5	221.6
	180.4		287.2		243.8		216.6	
10^{-4}	154.4		763.2		561.7		214.7	
	151.6	153.1	763.2	769.2	583.1	580.7	218.7	210.6
	154.4		781.3		597.2		198.5	
10^{-3}	209.4		-----		596.4		344.5	
	210.1	208.2	-----		583.2	593.6	377.5	373.5
	205.6		-----		601.2		398.4	
10^{-2}	477.5		-----		689.2		658.4	
	472.1	479.0	-----		711.3	693.7	658.4	661.3
	487.5		-----		680.7		667.0	
L. S. D.								
5%		16.2		54.4		42.5		34.0
1%		22.4		86.3		58.8		47.0
0.1%		30.9		148.6		81.0		65.0

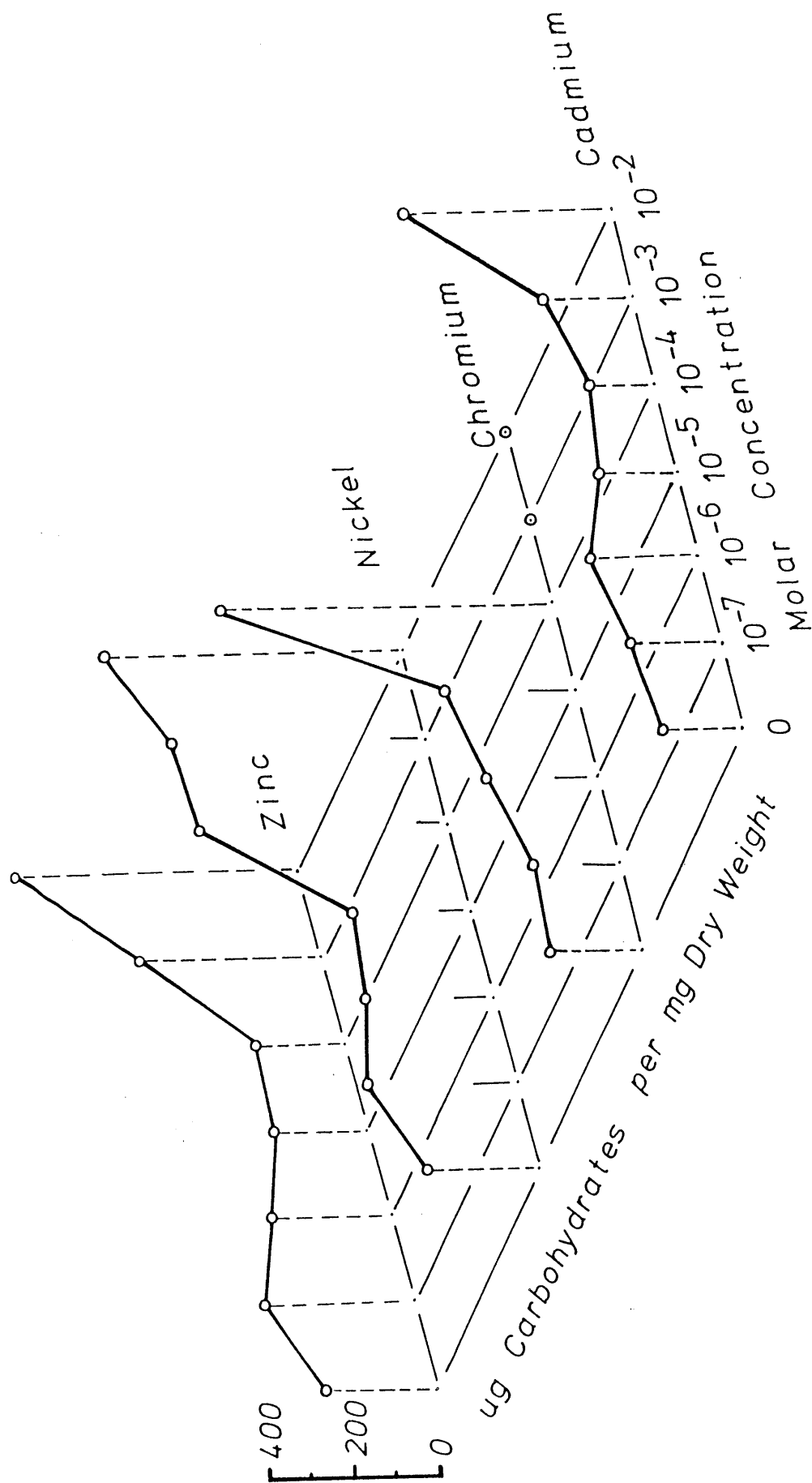


Fig.(5): Effect of various concentrations of different heavy metals on total carbohydrate content of Chlorella fusca after 24 hours growth period.

Table (6) : Effect of various concentrations of different heavy metals on total carbohydrate content of Spirulina maxima after 10 days growth period.

(ug/mg dry weight)

Element Concentration (Molar)	Carbohydrate content							
	Cadmium		Chromium		Nickel		Zinc	
0	543.4		543.4		543.4		543.4	
	536.7	541.2	536.7	541.2	536.7	541.2	536.7	541.2
	543.4		543.4		543.4		543.4	
10^{-7}	460.1		481.3		616.6		433.5	
	479.3	479.3	464.9	470.2	635.9	626.6	433.1	442.4
	498.6		464.4		627.3		460.7	
10^{-6}	528.0		520.3		552.5		527.8	
	577.2	558.4	493.1	486.2	594.5	557.2	559.4	547.6
	570.0		445.3		524.6		555.5	
10^{-5}	560.6		375.5		531.1		503.1	
	536.0	560.5	432.0	406.2	509.0	532.9	518.2	515.5
	584.9		411.0		558.6		525.3	
10^{-4}	502.3		435.4		607.3		359.6	
	588.6	547.9	420.0	410.5	650.9	623.3	354.9	351.0
	552.8		376.1		611.7		338.4	
L. S. D.								
5%	52.8		51.2		81.7		25.2	
1%	74.9		72.7		116.0		35.8	
0.1%	108.0		104.8		167.1		51.6	

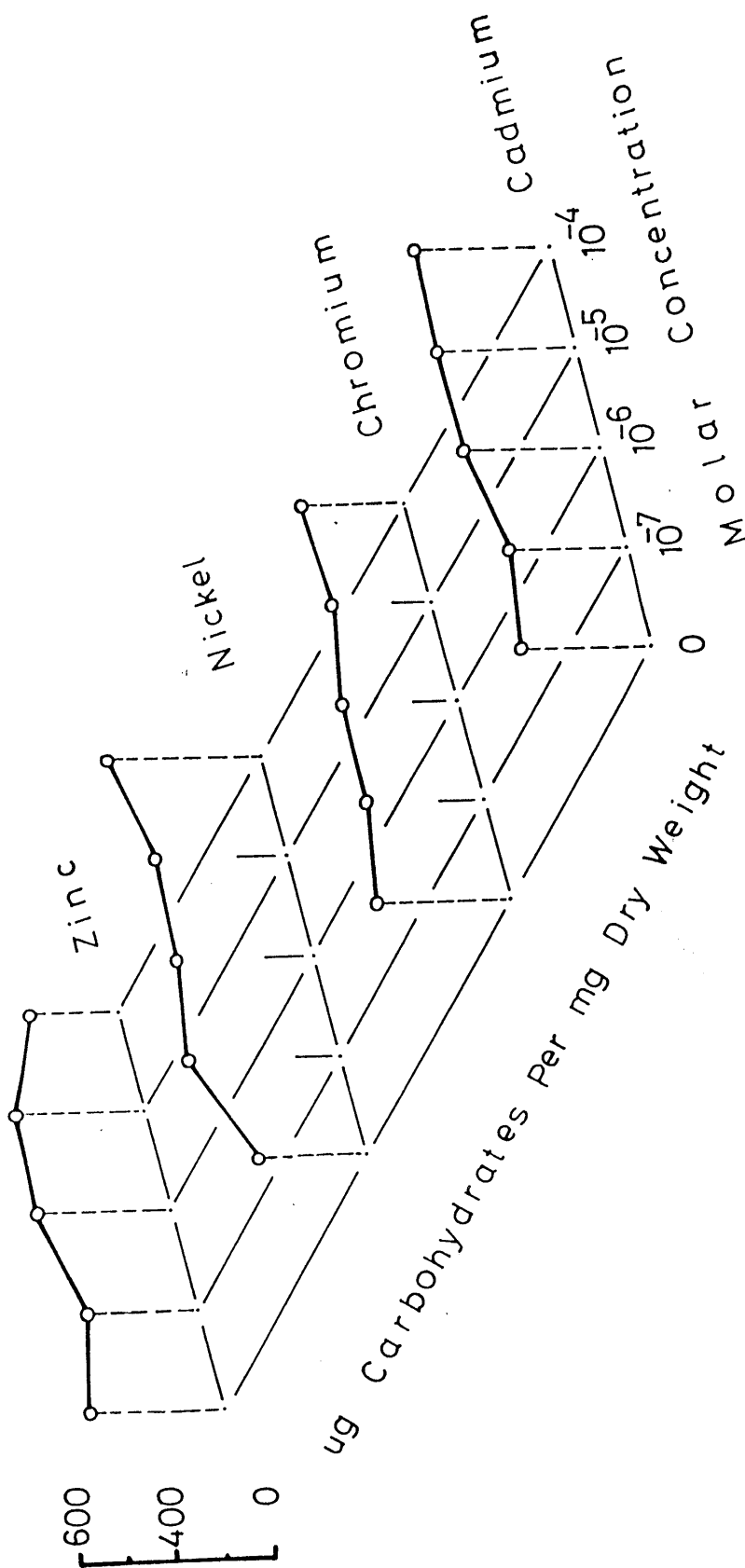


Fig. (5): Effect of various concentrations of different heavy metals on total carbohydrate content of Spirulina maxima after 10 days growth period.

above which the former enhanced whereas the latter attenuated carbohydrate accumulation.

Pigmentation :

Table 7 and Fig. 6 show that above 10^{-5} M, cadmium or chromium retarded chlorophyll a and b accumulation within the biomass of Chlorella fusca ; a phenomenon that was furthered with rise of concentration of the former ion. On the other hand, zinc showed its suppressive effect only at 10^{-2} M whereas nickel was stimulatory at either 10^{-7} or 10^{-6} M above which it was inhibitory to accumulation of both chlorophylls; more prominently at the larger doses.

These observations were more apparent in table 8 which shows that the larger doses of either elements were more suppressive to chlorophyll a than chlorophyll b accumulation except nickel where the reverse was observed.

Table 7 and Fig. 6 show that cadmium inhibited chlorophyll a, with little effects on carotenoids of Spirulina maxima whereas zinc seemed without effect, if not slightly increasing the carotenoids but nickel was stimulatory to both pigments. On the other hand, though 10^{-7} M chromium enhanced the accumulation of chlorophyll a and carotenoids, 10^{-4} M was suppressive to chlorophyll a whereas the intermediate doses exerted no effect.

Table (7): Effect of various concentrations of different heavy metals on chlorophyll content of Chlorella fusca after 24 hours growth period.
(ug/mg dry

Element Concentration (Molar)	Chlorophyll content											
	Cadmium				Chromium				Nickel			
	Chlorophyll a	Chlorophyll b	Chlorophyll a+b	Chlorophyll a+b	Chlorophyll a	Chlorophyll b	Chlorophyll a+b	Chlorophyll a+b	Chlorophyll a	Chlorophyll b	Chlorophyll a+b	Chlorophyll a+b
0	30.9 26.6 34.0	9.2 8.5 9.4	30.5 35.1 43.4	40.1 35.1 43.4	23.8 24.0 24.2	15.1 14.6 17.1	38.9 38.6 41.3	12.8 13.4 13.0	13.1 11.8 12.9	25.9 25.2 25.9	27.0 24.0 23.0	9.0 11.0 11.0
10 ⁻⁷	31.5 28.4 30.0	11.1 10.6 9.4	30.0 10.4 39.0	42.6 39.0 39.4	28.4 28.4 28.4	17.3 17.3 17.4	45.7 45.7 45.8	18.6 18.1 18.1	16.2 18.3 17.0	34.8 36.0 35.1	19.6 20.6 20.6	12.6 13.7 12.1
10 ⁻⁶	29.4 29.2 31.9	8.3 8.8 9.8	37.7 37.7 41.7	37.7 39.0 41.7	23.3 23.3 23.3	16.5 16.5 16.7	39.8 39.8 40.0	14.8 14.8 14.8	24.8 22.8 22.4	39.6 37.6 37.2	22.3 22.0 23.7	10.0 10.0 10.1
10 ⁻⁵	30.2 30.2 30.2	8.8 8.8 8.9	39.0 39.0 39.1	39.0 39.0 39.1	20.6 19.3 20.0	11.6 13.0 13.2	32.2 32.2 33.2	9.3 9.3 9.3	12.7 13.5 10.2	22.0 22.8 19.5	27.2 27.2 26.3	10.5 10.9 11.0
10 ⁻⁴	27.5 24.5 23.1	7.4 7.0 7.2	34.9 31.7 30.3	34.9 31.7 31.1	8.0 7.2 7.6	7.0 9.1 8.1	15.0 16.3 15.7	6.5 7.8 7.8	11.7 12.0 8.8	18.2 18.8 16.6	21.7 23.9 22.8	8.4 8.6 8.5
10 ⁻³	23.0 22.6 22.8	8.1 8.1 8.7	31.1 30.7 31.5	31.1 30.7 31.5	----- ----- -----	----- ----- -----	----- ----- -----	3.6 3.6 3.7	1.6 1.4 1.5	5.2 5.0 5.2	24.5 24.5 25.4	10.2 10.2 10.5
10 ⁻²	9.8 7.9 8.0	4.6 3.9 3.2	14.4 11.8 11.2	14.4 11.8 11.2	----- ----- -----	----- ----- -----	----- ----- -----	3.3 3.5 3.4	1.9 1.6 2.0	5.2 5.1 5.4	17.1 16.3 16.8	8.4 9.5 8.8
L.S.D	3.7 4.9 7.0	1.1 1.5 2.2	4.4 6.1 8.4	4.4 6.1 8.4	0.7 0.9 1.4	1.7 2.4 3.5	1.4 2.1 2.9	0.7 0.9 1.2	2.3 3.1 4.3	1.9 2.6 3.6	1.9 2.9 3.9	1.1 1.5 2.0
5% 1% 0.1%												2.0 2.8 3.8

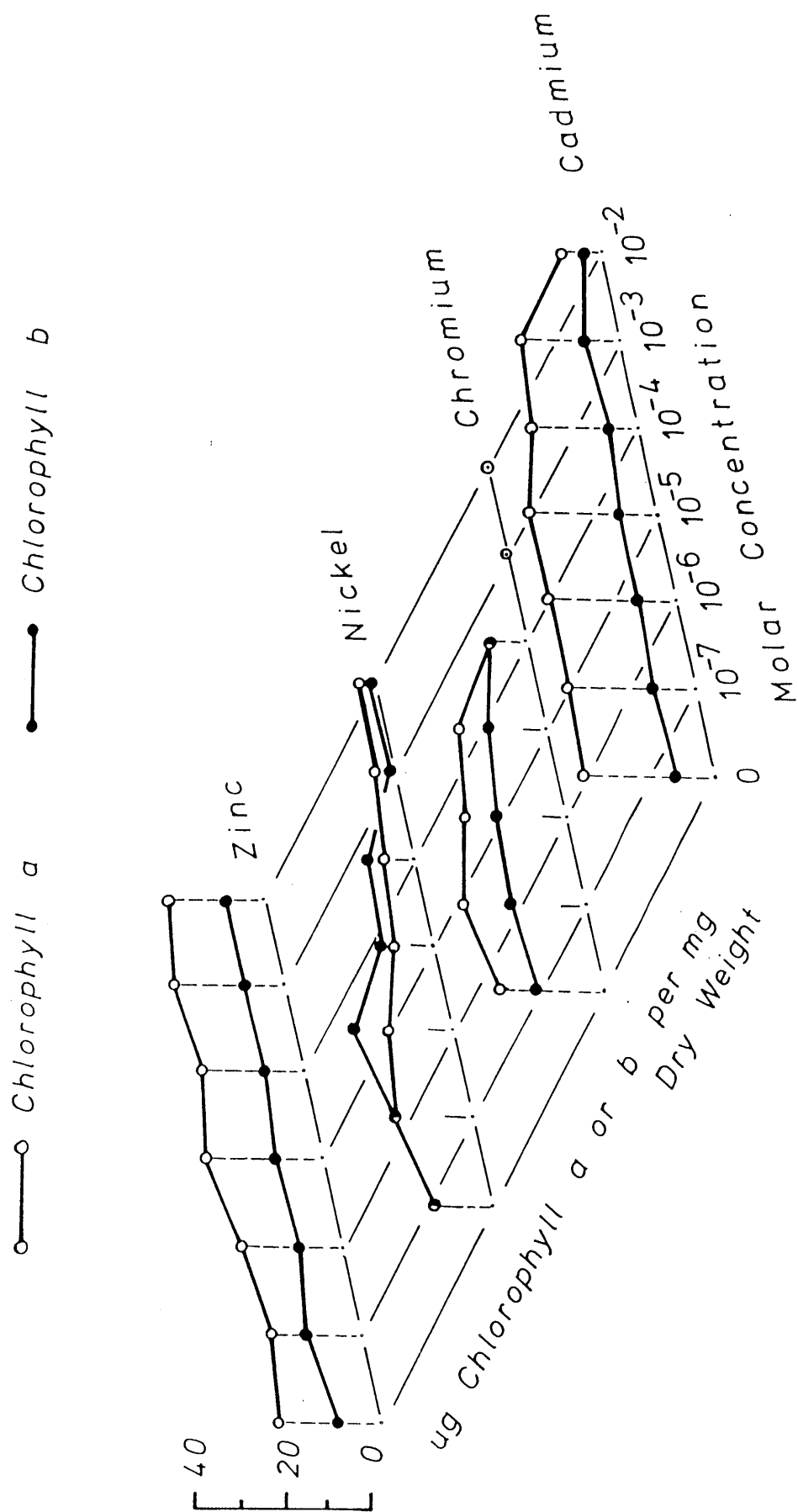


Fig.(6): Effect of various concentrations of different heavy metals on chlorophyll content of Chlorella fusca after 24 hours growth period.

Table (8): Effect of various concentrations of different heavy metals on Chlorophyll content of *Chlorella fusca* after 24 hours growth period.

(Percent of control)

Element Concentration (Molar)	Cadmium			Chromium			Chlorophyll content			Nickel			Zinc		
	Chlorophyll _a	Chlorophyll _b	Chlorophyll _{a+b}	Chlorophyll _a	Chlorophyll _b	Chlorophyll _{a+b}	Chlorophyll _a	Chlorophyll _b	Chlorophyll _{a+b}	Chlorophyll _a	Chlorophyll _b	Chlorophyll _{a+b}	Chlorophyll _a	Chlorophyll _b	Chlorophyll _{a+b}
10 ⁻⁷	98.2	116.3	107.9	118.4	118.4	118.4	115.4	115.4	115.4	137.5	136.0	136.7	135.4	135.4	135.4
	103.2	98.2	98.7	102.7	118.4	111.3	111.3	111.3	115.5	143.0	138.5	140.0	140.0	140.0	140.0
	93.2	104.5	99.8	118.4	118.4	110.5	115.7	115.7	115.5	135.0	143.7	136.6	136.6	136.6	136.6
10 ⁻⁶	97.9	97.1	95.4	97.1	97.1	97.1	100.5	100.5	100.5	111.9	113.1	113.1	113.1	113.1	113.1
	97.3	98.9	91.9	97.1	97.1	97.1	100.5	100.5	100.5	113.1	113.1	113.1	113.1	113.1	113.1
	101.4	102.2	105.6	97.1	97.1	105.9	100.5	100.5	100.5	114.1	180.5	144.8	144.8	144.8	144.8
10 ⁻⁵	99.2	97.0	98.7	98.8	86.0	74.2	81.3	81.3	81.3	71.0	100.6	85.6	85.6	85.6	85.6
	99.2	99.2	97.0	96.7	80.7	83.2	81.3	81.3	81.3	71.0	106.7	96.1	96.1	96.1	96.1
	99.3	96.1	98.9	83.0	80.7	80.7	81.3	81.3	81.3	71.0	81.1	75.9	75.9	75.9	75.9
10 ⁻⁴	81.3	77.3	88.4	34.0	43.5	43.5	37.9	37.9	37.9	49.3	92.7	70.8	70.8	70.8	70.8
	91.2	82.4	80.3	81.8	58.4	51.8	41.2	41.2	39.6	52.0	95.7	86.1	86.1	86.1	86.1
	74.5	78.7	76.7	30.8	53.5	53.5	39.7	39.7	39.7	59.7	69.9	64.6	64.6	64.6	64.6
10 ⁻³	75.5	91.7	78.7	---	---	---	---	---	---	27.6	12.8	20.2	20.2	20.2	20.2
	76.0	74.8	89.9	92.7	---	---	---	---	---	27.1	27.5	11.8	19.5	19.5	19.5
	72.8	96.6	79.8	---	---	---	---	---	---	27.9	11.8	20.2	20.2	20.2	20.2
10 ⁻²	30.2	45.3	36.5	---	---	---	---	---	---	25.1	14.9	20.2	20.2	20.2	20.2
	26.0	42.7	29.9	31.6	---	---	---	---	---	26.4	25.9	19.8	19.8	19.8	19.8
	28.2	42.5	28.4	---	---	---	---	---	---	26.1	15.5	21.0	21.0	21.0	21.0
L.S.D.	7.7	8.5	8.4	2.9	9.0	9.0	3.2	3.2	3.2	5.2	17.9	8.4	8.4	8.4	8.4
5%	10.7	11.8	11.8	4.3	12.8	12.8	4.7	4.7	4.7	7.1	24.7	11.8	11.8	11.8	11.8
0.1%	14.7	16.2	16.5	6.3	18.5	18.5	7.1	7.1	7.1	9.8	34.1	16.5	16.5	16.5	16.5

24.3
33.6
46.3

Table (7) : Effect of various concentrations of different heavy metals on pigment content of Spirulina maxima after 10 days growth period.
(ug/mg dry weight)

Element Concentration (Molar)	Pigment Content											
	Cadmium			Chromium			Nickel			Zinc		
	Chlorophyll a	Carotenes	Chlorophyll a	Carotenes	Chlorophyll a	Carotenes	Chlorophyll a	Carotenes	Chlorophyll a	Carotenes	Chlorophyll a	Carotenes
0	16.6 18.4 18.8	4.4 4.9 5.3	16.6 18.4 18.8	4.4 4.9 5.3	16.6 18.4 18.8	4.4 4.9 5.3	16.6 18.4 18.8	4.4 4.9 5.3	16.6 18.4 18.8	4.4 4.9 5.3	16.6 18.4 18.8	4.4 4.9 5.3
10 ⁻⁷	14.0 13.5 14.1	4.8 4.4 4.8	19.6 20.7 18.8	6.2 6.6 6.0	21.6 18.6 22.4	7.1 7.3 7.4	19.4 16.7 18.0	6.2 5.3 6.1	19.4 16.7 18.0	6.2 5.3 6.1	19.4 16.7 18.0	6.2 5.3 6.1
10 ⁻⁶	12.2 13.0 12.6	3.9 4.0 4.3	16.6 17.4 15.9	5.0 5.3 4.8	20.6 18.8 17.7	6.8 6.1 6.2	17.8 16.0 17.3	6.0 5.3 6.0	17.8 16.0 17.3	6.0 5.3 6.0	17.8 16.0 17.3	6.0 5.3 6.0
10 ⁻⁵	13.0 12.6 10.8	4.7 4.0 3.8	15.2 17.0 16.6	5.4 5.1 5.1	14.3 13.2 17.2	4.7 4.3 5.6	16.6 18.8 17.1	5.9 6.2 6.1	16.6 18.8 17.1	5.9 6.2 6.1	16.6 18.8 17.1	5.9 6.2 6.1
10 ⁻⁴	10.9 13.6 11.9	4.3 4.4 4.3	16.3 15.4 15.8	5.2 5.4 4.8	22.0 22.0 24.7	7.0 7.0 7.8	17.7 18.5 17.5	5.8 5.4 4.9	17.7 18.5 17.5	5.8 5.4 4.9	17.7 18.5 17.5	5.8 5.4 4.9
L.S.D.												
5%	2.0	0.7	1.7	0.6	3.4	0.9	2.1	0.8	2.1	0.8	2.1	0.8
1%	2.8	0.9	2.5	0.8	4.8	1.3	3.0	1.2	3.0	1.2	3.0	1.2
0.1%	4.0	1.4	3.6	1.1	6.9	1.8	4.4	1.7	4.4	1.7	4.4	1.7

○ Chlorophyll a
● Carotenes

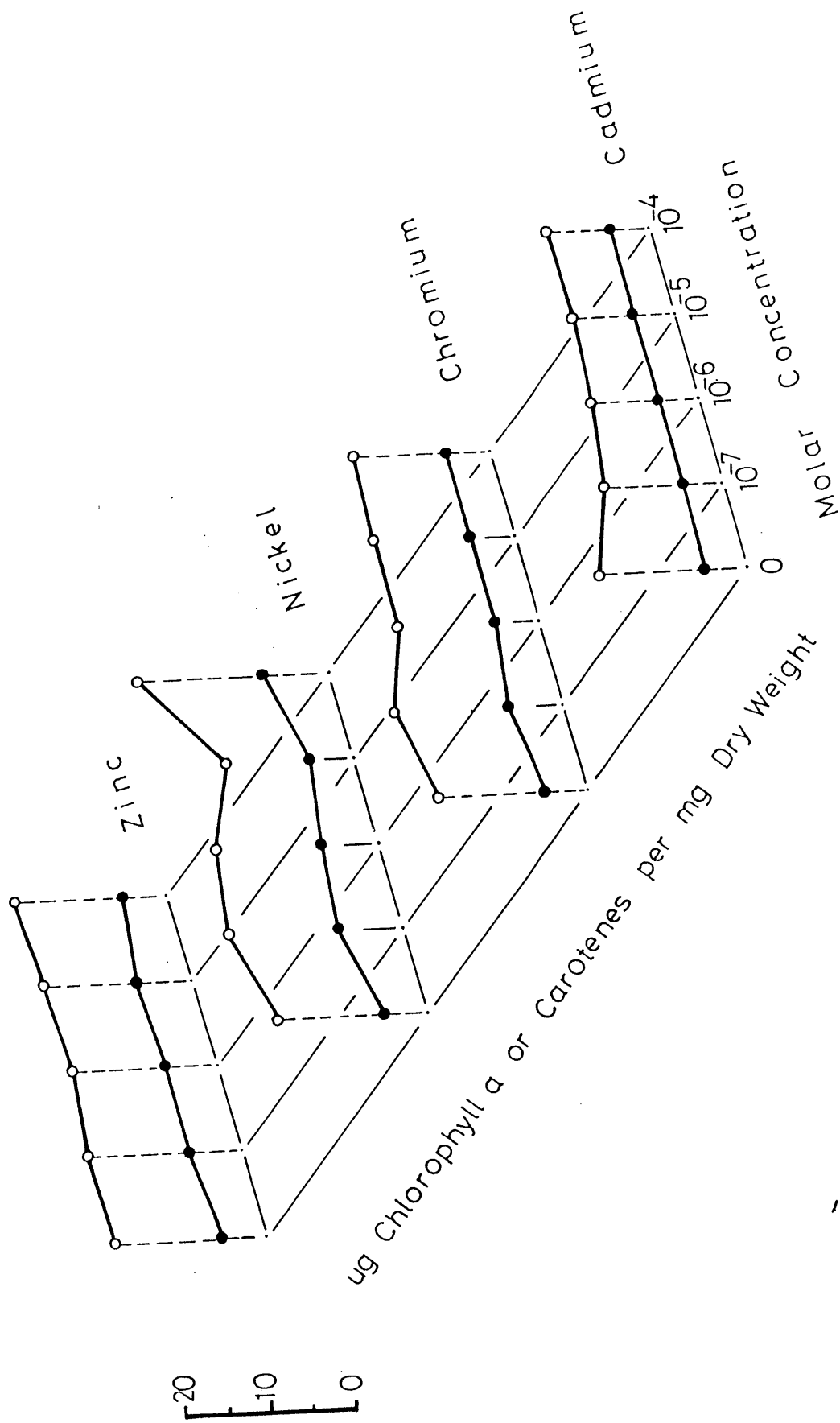


Fig. (6): Effect of various concentrations of different heavy metals on pigment content of Spirulina maxima after 10 days growth period.

These observations were further expressed in table 8 where cadmium showed the highest inhibition and nickel exerted the highest stimulatory effect on pigment accumulation.

Malate Dehydrogenase :

Table 9 and Fig.7 show that nickel largely suppressed malate dehydrogenase activity of Chlorella fusca, almost to the same extent regardless of concentration whereas cadmium or zinc were inhibitory only at 10^{-4} M or above. On the other hand, chromium stimulated malate dehydrogenase activity, more prominently at its higher concentrations.

In the meantime, the four elements were highly suppressive to malate dehydrogenase activity of Spirulina maxima, more prominently at the larger doses, with cadmium or zinc exerting the highest effect (Table 9 and Fig. 7).

Glutamate Dehydrogenase :

Table 10 and Fig. 8 show that chromium retarded glutamate dehydrogenase activity; a phenomenon that was furthered with rise of concentration of the ion. On the other hand cadmium or zinc exerted an appreciable stimulatory effect that was reversed at concentrations higher than 10^{-4} M. Similar observations were

Table (8): Effect of various concentrations of different heavy metals on pigment content of Spirulina maxima after 10 days growth period.
(percent of control)

Element Concentration (Molar)	Pigment content									
	Cadmium					Nickel				
	Chlorophyll a	Carotenes	Chlorophyll a	Carotenes	Chlorophyll a	Chlorophyll a	Carotenes	Chlorophyll a	Carotenes	Carotenes
10 ⁻⁷	78.8	98.4	115.1	127.6	121.2	146.2	108.4	127.2		
	75.4	90.5	109.5	135.9	129.1	149.3	93.3	100.8	108.0	120.3
	78.2	98.6	105.1	123.9	125.1	152.6	100.6	125.8		
10 ⁻⁶	70.4	79.4	97.2	103.5	115.1	140.6	99.4	122.7		
	72.6	82.7	88.8	108.5	103.9	126.4	88.8	108.7	118.1	
	68.2	88.0	92.6	99.6	98.9	128.3	96.7	122.9		
10 ⁻⁵	60.3	96.9	84.9	111.8	79.9	97.1	92.7	120.6		
	70.4	82.7	95.0	105.8	107.4	87.8	100.0	97.7	126.8	124.1
	72.6	77.5	92.7	104.5	96.1	115.1	95.5	125.0		
10 ⁻⁴	76.0	89.3	86.0	106.8	122.0	143.9	98.9	119.4		
	66.5	67.8	88.3	111.8	106.7	144.7	103.4	110.9	110.4	
	60.9	87.4	91.1	98.6	138.0	160.0	97.8	101.0		

L.S.D.

5%
1%
0.1%

10.4	10.7	8.6	7.2	20.2	16.3	11.4	14.9
14.8	15.2	12.2	10.2	28.7	23.2	16.2	21.2
21.3	21.9	17.6	14.6	41.3	33.4	23.4	30.5

Table (9): Effect of various concentrations of different heavy metals on malate-dehydrogenase activity of Chlorella fusca after 24 hours growth period.

(Unit/mg protein)

Element concentr- ation (Molar)	Malate - Dehydrogenase activity							
	Cadmium		Chromium		Nickel		Zinc	
0	3.72 3.29	3.51	4.71 4.31	4.51	4.38 4.04	4.21	3.24 2.84	3.04
10^{-7}	3.89 4.16	4.03	4.55 4.71	4.63	2.13 2.37	2.25	3.46 3.29	3.38
10^{-6}	3.12 3.08	3.10	6.15 5.59	5.87	2.63 2.86	2.75	3.66 3.37	3.52
10^{-5}	4.27 4.20	4.24	5.51 4.72	5.12	3.04 2.75	2.90	3.11 3.29	3.20
10^{-4}	3.29 3.09	3.19	7.19 7.22	7.21	2.53 2.47	2.50	2.17 2.13	2.15
10^{-3}	1.61 1.85	1.73	-----		2.14 2.46	2.30	4.00 4.20	4.10
10^{-2}	1.83 1.48	1.66	-----		2.74 2.69	2.55	2.50 2.60	2.55
L. S. D.								
5%		0.44		0.89		0.44		1.20
1%		0.66		1.41		0.66		1.80
0.1%		1.02		2.39		1.02		2.80

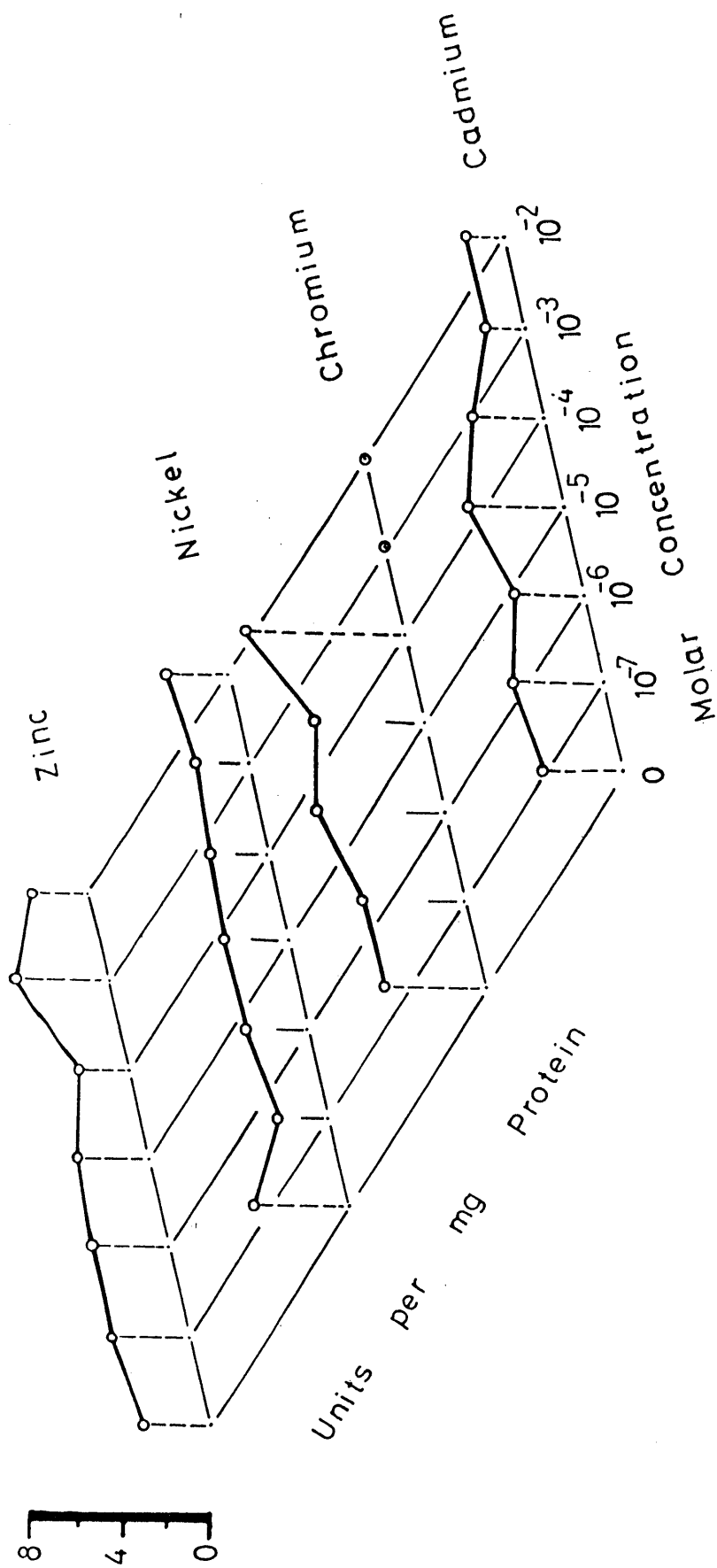


Fig.(7): Effect of various concentrations of different heavy metals on malate-dehydrogenase activity of *Chlorella fusca* after 24 hours growth period.

Table (9): Effect of various concentrations of different heavy metals on malate-dehydrogenase activity of Spirulina maxima after 10 days growth period.

(Unit /mg protein)

Element concentration (Molar)	Malate - Dehydrogenase activity							
	Cadmium		Chromium		Nickel		Zinc	
0	0.10 0.11	0.11	0.10 0.11	0.11	0.10 0.11	0.11	0.10 0.11	0.11
10 ⁻⁷	0.06 0.04	0.05	0.13 0.13	0.13	0.10 0.10	0.10	0.05 0.05	0.05
10 ⁻⁶	0.04 0.03	0.04	0.09 0.09	0.09	0.07 0.07	0.07	0.04 0.05	0.05
10 ⁻⁵	0.04 0.04	0.04	0.09 0.09	0.09	0.08 0.08	0.08	0.05 0.04	0.05
10 ⁻⁴	0.04 0.03	0.04	0.05 0.06	0.06	0.06 0.06	0.06	0.03 0.03	0.03
L. S. D.								
5%		0.02		0.02		0.01		0.01
1%		0.03		0.03		0.02		0.02
0.1%		0.04		0.04		0.03		0.03

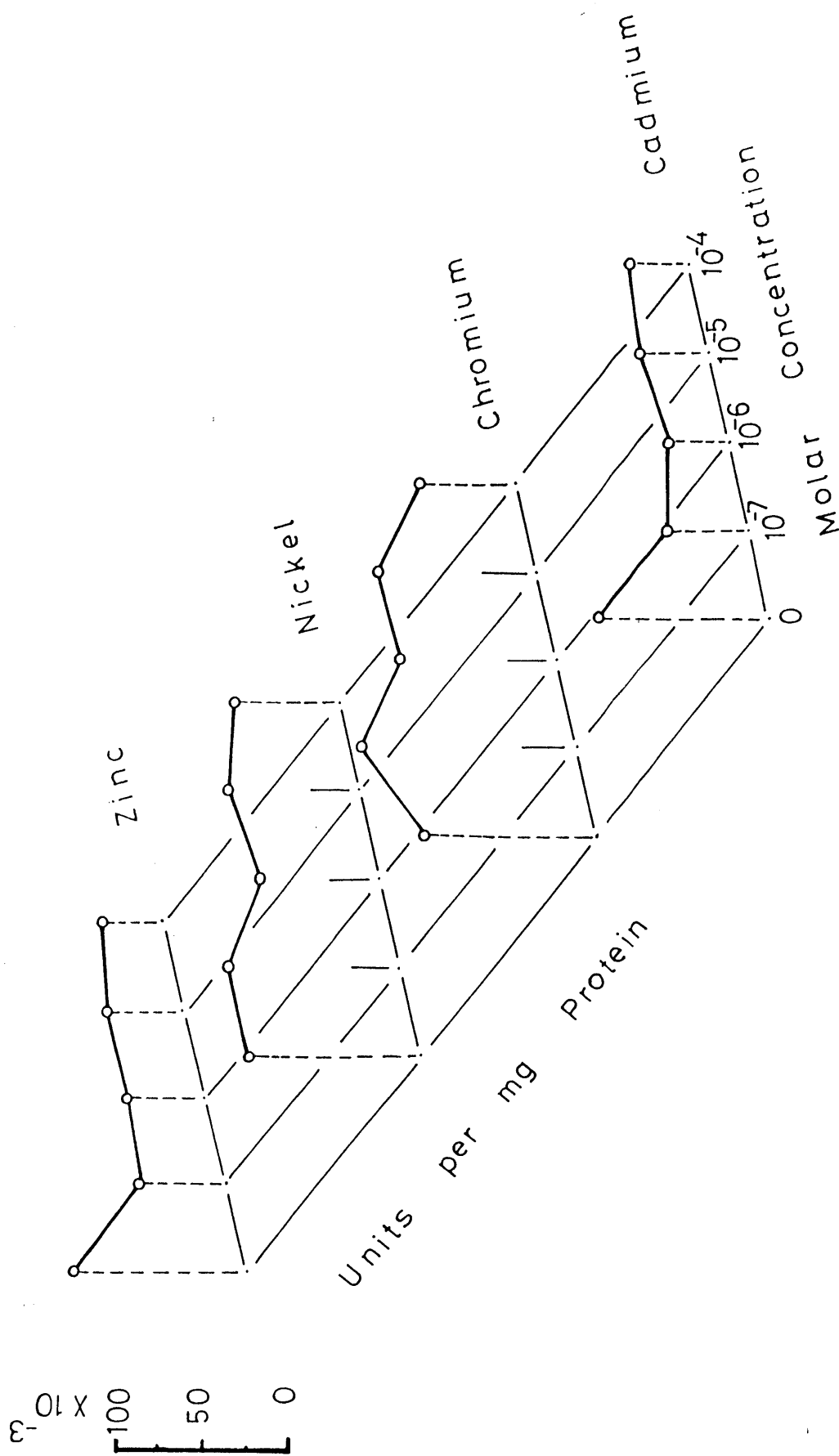


Fig.(7): Effect of various concentrations of different heavy metals on malate dehydrogenase activity of *Spirulina maxima* after 10 days growth period.

recorded for nickel though 10^{-4} M was also inhibitory.

On the other hand, the four elements seemed to be suppressive to glutamate dehydrogenase of Spirulina maxima at either of the experimental concentrations (Table 10 and Fig.8).

Table (10): Effect of various concentrations of different heavy metals on glutamate-dehydrogenase activity of Chlorella fusca after 24 hours growth period.

(Unit/mg protein)

Element concentration (Molar)	Glutamate - Dehydrogenase activity							
	Cadmium		Chromium		Nickel		Zinc	
0	0.011 0.011	0.011	0.018 0.016	0.017	0.012 0.012	0.012	0.017 0.019	0.018
10^{-7}	0.009 0.009	0.009	0.011 0.013	0.012	0.015 0.015	0.015	0.026 0.023	0.025
10^{-6}	0.007 0.007	0.007	0.016 0.014	0.015	0.013 0.012	0.013	0.021 0.019	0.020
10^{-5}	0.011 0.009	0.010	0.014 0.012	0.013	0.016 0.017	0.017	0.021 0.019	0.020
10^{-4}	0.012 0.015	0.014	0.008 0.008	0.008	0.007 0.010	0.009	0.016 0.016	0.016
10^{-3}	0.004 0.006	0.005	----- -----		0.008 0.010	0.009	0.020 0.019	0.020
10^{-2}	0.006 0.006	0.006	----- -----		0.010 0.010	0.010	0.007 0.007	0.007

L.S.D.

5%	0.003	0.003	0.003	0.003
1%	0.004	0.005	0.004	0.005
0.1%	0.007	0.008	0.006	0.008

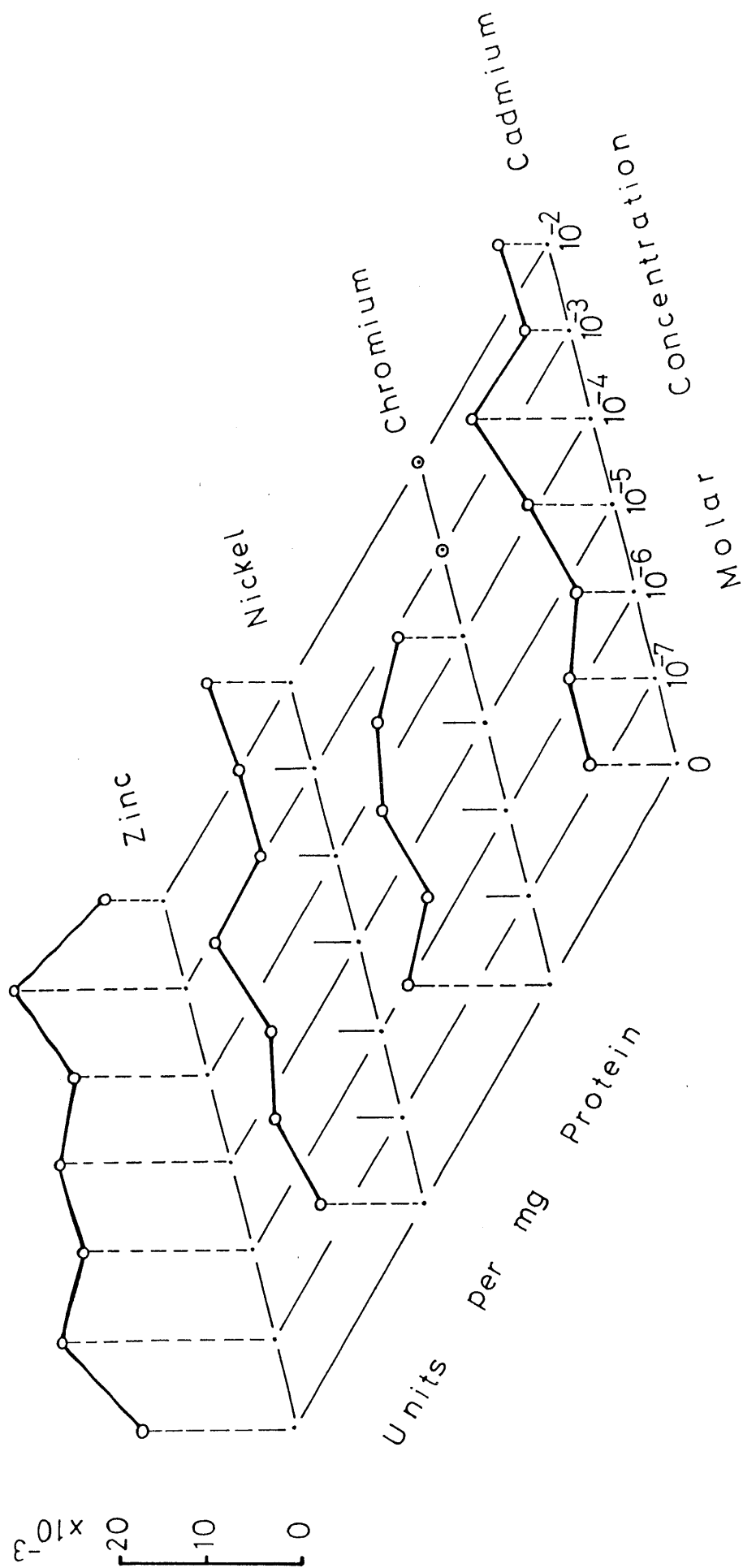


Fig.(8): Effect of various concentrations of different heavy metals on glutamate-dehydrogenase activity of *Chlorella fusca* after 24 hours growth period.

Table (10⁻⁷): Effect of various concentrations of different heavy metals on glutamate-dehydrogenase activity of Spirulina maxima after 10 days growth period.

(Unit/mg protein)

Element concentr- ation (Molar)	Glutamate - Dehydrogenase activity							
	Cadmium		Chromium		Nickel		Zinc	
0	0.003 0.003	0.003	0.003 0.003	0.003	0.003 0.003	0.003	0.003 0.003	0.003
10 ⁻⁷	0.001 0.001	0.001	0.002 0.002	0.002	0.002 0.002	0.002	0.002 0.001	0.002
10 ⁻⁶	0.002 0.001	0.002	0.002 0.002	0.002	0.001 0.001	0.001	0.002 0.001	0.002
10 ⁻⁵	0.001 0.002	0.002	0.003 0.003	0.003	0.001 0.001	0.001	0.002 0.002	0.002
10 ⁻⁴	0.001 0.002	0.002	0.002 0.002	0.002	0.001 0.002	0.002	0.001 0.001	0.001
L.S.D.								
5%		0.002		0.002		0.001		0.001
1%		0.003		0.003		0.002		0.002
0.1%		0.004		0.004		0.003		0.003

3
x 10⁴
2
0

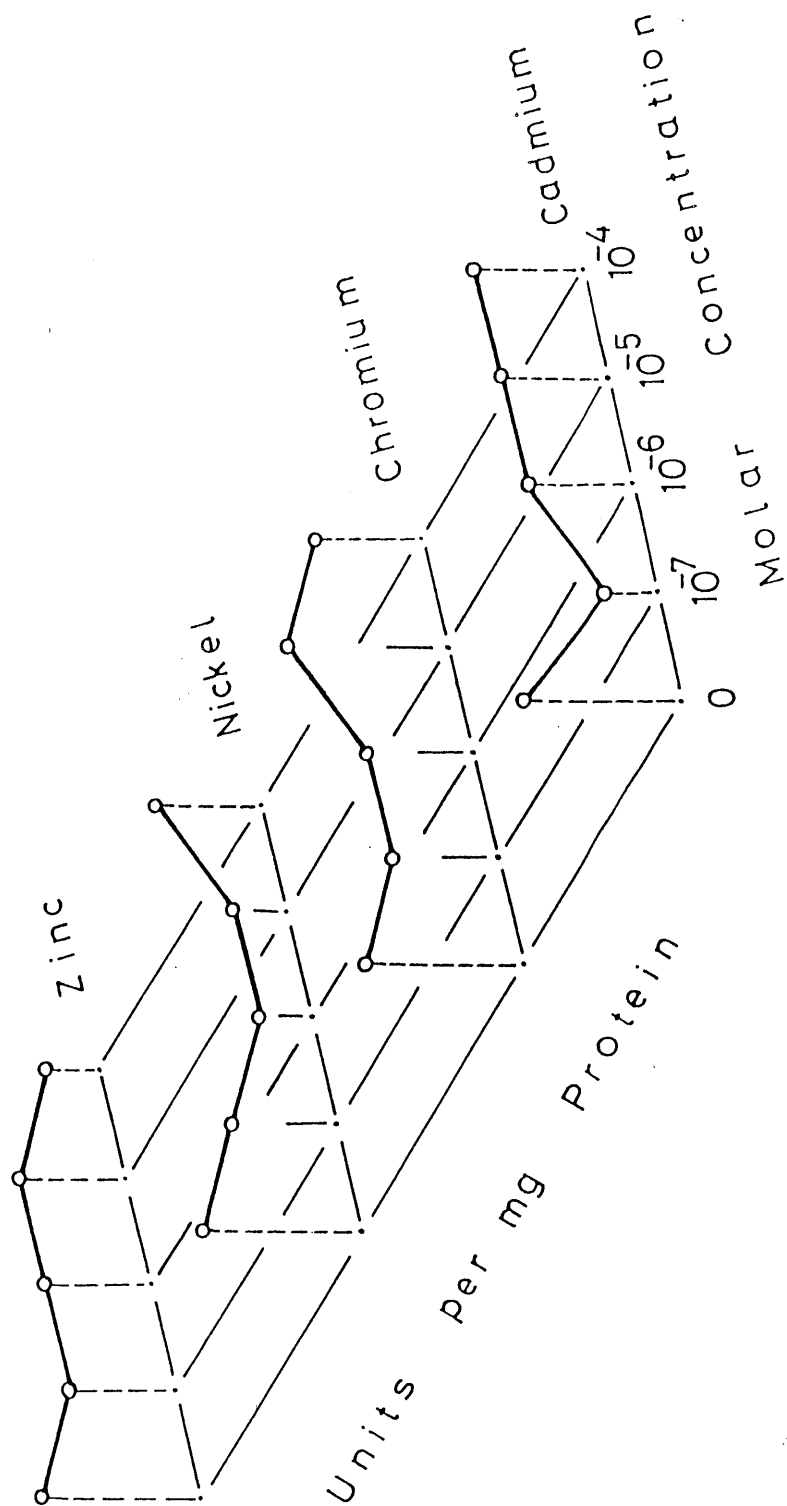


Fig.(8): Effect of various concentrations of different heavy metals on glutamate-dehydrogenase activity of Spirulina maxima after 10 days growth period.

PART(III)
DISCUSSION

PART III

DISCUSSION

The results of this investigation clearly show that, within the range of 10^{-7} - 10^{-4} M, hexavalent cationic chromium was most toxic whereas zinc seemed to have little, if at all, effect on growth criteria of Chlorella fusca. Above these limits chromium arrested growth whereas the other elements exerted almost the same inhibitory effect particularly at 10^{-2} M level. On the other hand, growth of Spirulina maxima was totally suppressed at concentrations above 10^{-4} M but was indifferent to the lower concentrations of either of the four metals except 10^{-6} M which encouraged dry weight gain. Still the difference in initial and final pH, during the growth period of both organisms has its significant role. The initial pH of Chlorella fusca media was 6 whereas that of Spirulina maxima was 9.4. Experiments with Chlorella lasted for 24 hours whereas those with Spirulina continued for 10 days.

These observations indicate that the inhibitory and/or stimulatory effects of either of these metals depend on concentration and/or type of organism. Chlorella seemed less sensitive to the ions than Spirulina.

In this connection, it may be reported that the toxicity of chromium to algae had been early recognized by Hervey (1949).

The enhanced growth rate of algae, following application of small amounts of Cr^{+6} (Hervey, 1949; Wium-Andersen, 1974) was only recorded for Spirulina at 10^{-6}M . According to Cobet et al. (1970), nickel induced an unbalanced growth condition in marine Aerobacter species resulting in large plasmolysed sphaeroblasts. On the other hand, Gehards & Weller (1977) revealed that 48 mM nickel and 72 mM cadmium did not stop the growth of Chlorella pyrenoidosa. Van Baalen and O'Donnell (1978) included nickel in the list of required trace elements for growth of blue green algae. De Filippis and Pallaghy (1976) reported that zinc and mercury stopped cell division but increased the weight of the Chlorella cells. Bonaly et al. (1978) reported that $5 \times 10^{-2}\text{M}$ cadmium completely inhibited growth of Euglena gracilis and rapidly killed all the cells but 10^{-6}M had no significant effect. Price and Vallee (1962) considered zinc as a growth limiting factor for Euglena gracilis whereas Coleman et al. (1971) described Chlorella vulgaris as a zinc tolerant organism ; 4.20 mg/l promoted but 8.70 mg/l inhibited growth of the alga. In the meantime, Rachlin and Farran (1974) showed that 2.4 ppm zinc reduced the growth of Chlorella vulgaris by 50% within 96 hours.

The results further show that the toxicity of either elements is not a function of their concentration within the organism but mainly on the alga itself as well as the metal species.

This is quite obvious from the difference in amount of accumulated particular ion in either algae and its corresponding effect on dry weight gain. Generally speaking Chlorella accumulated comparatively larger amounts of either ions compared to Spirulina before they ^{the appearance of} can exert any toxic effects.

Furthermore, although at lower concentrations (10^{-6} - 10^{-4} M) Chlorella accumulated larger amounts of cadmium compared with chromium, yet they induced almost the same inhibitory effects. In the meantime, at 10^{-4} M, inhibition by cadmium was far greater than by zinc though Chlorella accumulated equal amounts of both ions. Similarly, above 10^{-4} M, still accumulation of cadmium was larger than zinc whereas nickel was absorbed in comparatively very little amounts and yet the three ions had almost the same deleterious effects on dry weight gain.

Conversely, at 10^{-6} M both cadmium and chromium stimulated dry weight gain by Spirulina though neither of the ions appreciably, if at all, accumulated in the biomass whereas similar enhancement by nickel or zinc was accompanied by absorption of either ions particularly zinc. At 10^{-4} M though nickel was traced in the biomass yet it had almost the same negligible effects on dry weight of Spirulina as that following accumulation of the comparatively large amounts of chromium or the appreciable

amounts of either cadmium or zinc.

This indicates that these elements triggered their effects to different metabolic pathways essential for maintenance of growth activities of either organisms.

It is worth to mention that Knauss and Porter (1954) were of the opinion that the amount of an element, absorbed by Chlorella pyrenoidosa, was a function of the element and its concentration in the nutrient medium. Overnell (1975) suggested that the toxicity of cadmium to algal cells was due to inhibited oxygen evolution. Davis et al. (1978) attributed the toxicity of heavy metals, including cadmium, to their influence on the other nutrient elements, reducing their availability as well as translocation. According to Jawoski (1976) Cr VI, being a strong oxidising agent, can easily cross the biological membranes and thus is highly toxic. Das et al. (1978) showed that nickel interfered with the synthesis of enzyme proteins for catalase, peroxidase, IAA-oxidase and ascorbic acid oxidase during germination and growth of rice seedlings.

Our results also show that in spite of lower dry weight gain by the algae supplemented with high levels of the metal

ions in the nutrient media, the accumulation of such metals in the biomass was largest. This adds further support to the observations of Coleman et al. (1971), working with zinc and cobalt. They attributed their observations to the faster accumulation of the metal ion in the dead than living cells of the phytoplanktons, the number of which increased with increased concentration of the metal ions.

It should be recalled here that washing with 0.05M EDTA or hydrochloric acid did not affect the dry weight of Spirulina, cultured on the various heavy metal salts but a significant drop (reaching 50-60 %) of the original biomass weight was observed in case of cadmium or zinc supplementation to Chlorella, an indication that these elements could adhere to the outer surface of Chlorella by adsorption and/or ion exchange properties. This is in conformity with the observations of Culter and Rains (1974) that the apparent uptake of cadmium by intact barley was non-metabolic with accumulation being a function of physical adsorption and diffusion. Conversely Chandel et al. (1978) reported that uptake of zinc or copper per one gram dry weight of Phaseolus aureus and Zea mays decreased with increased concentration of the ion.

One may explain the difference in sensitivity or tolerance of the two species of algae to the four metal ions to the capacity

of either of them to eliminate the absorbed ions from the metabolic cycles through conjugation and/or precipitation. This deduction is supported by the observations of Payer & Runkel(1978) that mercury and cadmium were not only present as inorganic precipitates but also chemically bound to organic matter within the algal cells.

Table 3,3̄ and 4,4̄ revealed that up to 10^{-4} M, the four elements hardly affected the total protein content of either Chlorella or Spirulina except cadmium and chromium at 10^{-4} M that severely suppressed and 10^{-6} M cadmium that stimulated protein accumulation in Chlorella. Above these levels, the four elements but chromium- (already arrested growth)-drastically inhibited total protein accumulation in Chlorella. In spite of these fluctuations, zinc at its various tested concentrations enhanced insoluble protein accumulation a phenomenon that was also observed with cadmium till 10^{-3} M. In the meantime, chromium or nickel had a minor or insignificant effect on insoluble protein ratio.

These observations add further support to the selective action of these heavy metals dependent on algal species. They also indicate the stimulatory effects of zinc and cadmium

to complex protein formation which may be taken as a protective measure against toxicity of these two metals that were absorbed at the largest maximum rate.

The isoelectric focusing confirmed the above deductions where, within the range of 10^{-7} - 10^{-6} M the four elements had minor effects on the soluble protein pattern above which these elements disturbed the protein synthetic pattern, lowering the acidic and increasing the alkaline proteins, both in quantity and number, dependent on concentration and type of prevailing ion.

The suppression of nitrogen accumulation may be attributed to shortage of carbon skeleton resulting from low photosynthetic rate and/or nitrogen supply. Still interference of these heavy metals with absorption and utilization of other nutritive elements plays a significant role.

These observations are in agreement with those of Huang et al. (1974) who noticed decreased photosynthesis and reduction of protein content following administration of cadmium to soybeans. Working with Euglena gracilis, De Filippis et al. (1981a), showed that the total protein level increased following application of 50 uM zinc, 0.1 uM cadmium and 0.01 uM mercury. Stillwell and Holland (1979) reported that 100 uM nickel significantly decreased cell proteins in Cricosphaera carterae. Water culture experiments with soybeans (Turner and Rest, 1971) showed that 0.5 ppm anionic chromium lowered the uptake of calcium, potassium, iron, phosphorus

and manganese by roots. Oberlaender and Roth (1978) reported low potassium uptake and translocation by 7-days old barley plants following application of 10^{-6} M cadmium. According to Davis et al. (1978) uptake of bismuth, antimony or titanium, by spring barley was very low, though the yield of the plant was highly reduced. They attributed this phenomenon to reduced availability or translocation of other nutrient elements; a suggestion that may be safely applied to our results concerning the inhibitory effects of the four elements on Spirulina particularly nickel where almost no absorption was detected.

It should be noted that the four elements lowered the total amino acid content of Chlorella biomass, more prominently at their higher levels. Under all conditions, the drop in amino acid content was shared by all components except with nickel application where cysteine largely accumulated at 10^{-5} M whereas arginine highly dominated at the higher concentrations of the element, with minor effect on glutamic content. In the meantime, a failure in aspartic and methionine levels was accompanied by a decrease in lysine and isoleucine content particularly at the larger doses of the ion.

These observations may lead to the assumption that nickel encouraged arginine accumulation, the excess of which might have repressed the biosynthesis of the amino acids of the aspartate family particularly methionine. Complexing with arginine might be the counteracting mechanism against nickel toxicity.

One may record that chromium ions were able to complex with histidine, glycine or arginine (Malek et al., 1976) as well as with cysteine derivatives (De Meester and Hodgson, 1976). Similarly nickel was able to complex with cysteine, histidine or aspartic acid (Asato et al., 1975; Soestbergen and Sunderman, 1972). According to Hart and Bertram (1980), the mechanism by which Chlorella pyrenoidosa could tolerate large doses of cadmium might be related to synthesis of a metallothioneine-like protein. On the other hand, Jyung & Camp (1976) reported that the increased level of zinc in the soluble fraction in liver and duodenum of calves may be an adaptive mechanism to detoxify large quantities of zinc.

The results of tables 6, 6̄ show that the total carbohydrate content of Chlorella or Spirulina seemed indifferent to cadmium up to 10^{-4} M concentration above which a remarkable increase was observed in Chlorella. Still 10^{-6} M initiated carbohydrate accumulation by the green alga. In the meantime, chlorophyll a content of Spirulina was remarkably reduced whereas that of Chlorella was almost unaffected till cadmium reached 10^{-4} M or above where a remarkable attenuation was observed.

If these observations were coupled with those observed on nitrogen metabolism one could reach the conclusion that

cadmium though inhibited chlorophyll accumulation was stimulatory to carbohydrate assimilation in both organisms, without affecting protein synthesis, till 10^{-4} M above which cadmium adversely affected protein synthesis with the net result of increased accumulation of carbohydrates. 10^{-6} M seemed to be the optimum dose for maximum stimulation of carbohydrate and nitrogen accumulation.

It may be mentioned that Rosko & Rachlin (1977) showed that cadmium, copper, mercury reduced cell division in Chlorella vulgaris in much lower concentrations than zinc and lead. This toxicity was paralleled by a net increase in chlorophyll a per cell. On the other hand, Vorob'eva et al. (1979) reported that low concentration of cadmium and zinc stimulated photosynthesis in Chlorella pyrenoidosa, although this effect could be a secondary response due to decrease in culture density.

According to Haghiri (1973), the inhibitory effects of cadmium on growth may be attributed to lower assimilation rate as a result of reduced chlorophyll content ; a phenomenon already confirmed by Woltz and Chambliss (1979); De Filippis et al. (1981a) . Hampp et al. (1976) reported poisoning of photosynthesis of isolated chloroplasts by cadmium. It is now well established that an increase of cadmium concentration ,

significantly depressed manganese content in plant tissues of corn, bush beans, wheat and radish (Iwai et al., 1975; John, 1976; Wallace et al., 1977; Al-Wakeel, 1981), associated with low dry weights. Manganese is essential for photosystem II (Bishop, 1971) where it participates in photolysis (Anderson and Pyliotis, 1969).

Similar trends in carbohydrate fluctuations were recorded in presence of chromium ions till 10^{-4} M for Chlorella but the carbohydrate content of Spirulina severely dropped. In the meantime, chlorophyll a content of Spirulina was hardly affected whereas that of Chlorella was attenuated at the larger doses of the ion. The protein content of both organisms followed the same pattern of chlorophyll a. This indicates that the apparent effects of chromium on Chlorella are almost identical to those of cadmium though the optimum concentration ranged between 10^{-6} and 10^{-5} M whereas severe toxicity appeared at 10^{-4} M. On the other hand, chromium seemed to inhibit photosynthetic activity without affecting chlorophyll content and this reflected itself on the protein content of the alga.

It is worth to mention that Petriya (1978) showed that low concentrations of chromium (10^{-5} - 10^{-4} M) stimulated whereas higher concentrations (10^{-3} - 10^{-2} M) inhibited growth and photosynthesis of Chlorella vulgaris, whereas Stoyanov and Tungarov (1978) noticed no effect on maize. On the other

hand, Meisch & Schmitt-Beckmann (1979) reported that stimulation of growth and photosynthetic oxygen evolution by Chlorella pyrenoidosa & Chlorella WRI, in presence of small chromium doses, was accompanied by a general depression of chlorophyll synthesis.

Applying nickel or zinc to Chlorella accelerated total carbohydrate accumulation, at their lowest and highest concentrations but the moderate doses seemed without effect. The fluctuations were highly apparent and most sensitive to ion concentration in presence of nickel. Similar effects were exerted by nickel on Spirulina but the carbohydrate content of the organism seemed indifferent to zinc except at 10^{-4} M where an appreciable inhibition was observed.

If these observations were coupled with little or no effect on chlorophyll content of Spirulina together with the previous findings regarding protein accumulation one may reach the conclusion that nickel stimulated photosynthesis by Spirulina without affecting nitrogen or chlorophyll content whereas zinc hardly affected either processes except at 10^{-4} M which suppressed photosynthesis.

On the other hand, the increased total carbohydrate accumulation following nickel or zinc treatment was accompanied

by increased chlorophyll under the former or unaffected chlorophyll, under the latter ion. This was coupled with minor effects on protein content. This indicates that small doses of either ions stimulated photosynthesis whereas moderate doses exerted minor effect ; on the three components but the larger doses severely attenuated chlorophyll synthesis coupled with severe drop in protein resulting in increased carbohydrate.

These observations indicate that the large doses of the four metals had the same toxic effects on metabolism.

It should be mentioned that De Filippis (1979) reported that chlorophyll degradation following application of zinc chloride and other heavy metals to Chlorella cells was strongly correlated to their standard redox potential. De Fillipis et al. (1981a) attributed the low chlorophyll content of Euglena gracilis following application of 50 uM zinc, 0.1 uM cadmium and 0.01uM mercury to inhibition of reductive steps in the biosynthetic pathways of the pigment. In the meantime, chlorophyll b would increase; a phenomenon not highly confirmed in our results (lower concentrations of nickel only). On the contrary both chlorophyll a and chlorophyll b sharply decreased with application of the larger doses of the four ions. In a

later paper De Filippis et al. (1981b) attributed the low photosynthetic capacity of Zn-, Cd- and Hg-treated Euglena to the severe interaction of these metals with the electron transport chain; water splitting site and oxidoreductase being the most sensitive steps.

The results of this investigation further show that zinc and cadmium had similar dramatic inhibitory effects on malate dehydrogenase of Spirulina whereas chromium and nickel seemed less effective ; 10^{-7} M chromium was stimulatory whereas the same nickel concentration seemed without effect. The effect of the former ion seemed indifferent whereas that of the latter was furthered with increased concentration . This indicates non-competitive , under the former, and competitive inhibition under the latter conditions.

Similarly one may conclude that cadmium though slightly stimulated malate dehydrogenase of Chlorella, yet the larger doses competitively inhibited the enzyme whereas nickel inhibition may be regarded as non-competitive. On the other hand, zinc may be considered without effect whereas chromium seemed highly stimulatory.

On the other hand, glutamate dehydrogenase of Spirulina seemed hardly affected by either of the four elements whereas

that of Chlorella differed. Zinc exerted its inhibitory effects at 10^{-2} M; cadmium at 10^{-3} M; chromium at 10^{-4} M whereas nickel, at all concentrations, seemed without effect. 10^{-7} M zinc accelerated the enzyme activity.

According to Berger (1964), malate dehydrogenase (Krebs cycle) and glutamate dehydrogenase (amino acid metabolism) activities are independent of protein net synthesis and photosynthesis , in synchronous cultures of Chlorella under light change of 14-10 hours. Our results of Spirulina maxima totally confirm these findings but in case of Chlorella fusca the same phenomenon was quite obvious under chromium or zinc treatments where total protein decreased while malate dehydrogenase activity increased with chromium or unaffected by zinc. On the other hand, the malate dehydrogenase activity followed the trend of total protein under cadmium or nickel treatment. Similarly no correlation between malate dehydrogenase and total carbohydrates was observed except following chromium treatment where the increased activity of the enzyme was accompanied by increased total carbohydrate.

One should recall that glutamate dehydrogenase is one of the zinc metalloenzymes (Vallee and Wacker, 1970). Accordingly one could expect that cadmium would compete with zinc thus

causing a reduced activity of this enzyme (Pickett and Dean, 1976; Pickett et al., 1976; Piavaux, 1977). Weigel and Jäger, (1980b), working with bean plants reported that glutamate dehydrogenase was significantly stimulated by cadmium whereas malate dehydrogenase was inhibited. They assumed that glutamate dehydrogenase stimulation was an indirect effect since the ion did not induce de novo synthesis of enzyme proteins. Ramirez (1977) and Joy (1973) showed that high concentrations of zinc markedly reduced glutamate dehydrogenase activity in Agave americana leaves and Pisum sativum roots. Lee et al. (1976) reported that malate dehydrogenase activity increased at 0.90 and 1.35 uM cadmium.

S U M M A R Y

S U M M A R Y

The results of this investigation clearly show that, within the range of 10^{-7} - 10^{-4} M, hexavalent chromium was most toxic whereas zinc had little effect on growth of Chlorella fusca. Above these limits, chromium arrested growth whereas the other elements inhibited dry weight gain. All elements arrested growth of Spirulina maxima at 10^{-4} M whereas smaller doses seemed without effect. During the experimental period, the difference between initial and final pH was of minor significance.

Generally speaking, Chlorella accumulated larger amounts of cadmium, chromium, nickel or zinc compared with Spirulina before they can exert any toxic effect on the alga. Cadmium accumulated in larger amounts than chromium, in order to induce the same inhibitory effects on Chlorella. Nickel is hardly absorbed but exerted similar effects to zinc or cadmium when supplied at 10^{-4} M concentration.

Up to 10^{-4} M, the four elements hardly affected the total protein content of Chlorella or Spirulina. Above these levels, the four elements but chromium (already arrested growth) drastically inhibited total protein accumulation in Chlorella. Still the four elements lowered the total amino

acid content of Chlorella biomass; the drop being shared by all amino acid components except with nickel where cysteine or arginine (dependent on dose) largely accumulated with minor effects on glutamine.

At 10^{-4} M cadmium, increased the total carbohydrate and decreased the chlorophyll a content of Chlorella. Lower doses seemed without effect on carbohydrate content of either Spirulina or Chlorella but chlorophyll a of the former was reduced whereas that of the latter was not changed. Similar trends in carbohydrate fluctuations were recorded for Chlorella in presence of chromium till 10^{-4} M but the carbohydrate content of Spirulina severely dropped. Chlorophyll a of Spirulina was hardly affected whereas that of Chlorella was attenuated. The protein content followed the same pattern of chlorophyll a.

Zinc and cadmium had similar dramatic inhibitory effects on malate dehydrogenase of Spirulina whereas chromium and nickel were less effective. Cadmium though slightly stimulated malate dehydrogenase of Chlorella, yet the larger doses were competitively inhibitory. Nickel inhibition seemed non-competitive. Zinc seemed without effect whereas chromium is highly stimulatory. Glutamate dehydrogenase of Spirulina seemed hardly affected

by the four elements. Zinc exerted its inhibitory effects on glutamate dehydrogenase of Chlorella at 10^{-2} M, cadmium at 10^{-3} M, chromium at 10^{-4} M whereas nickel, at all concentrations, seemed without effect.

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R E F E R E N C E S

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ملخص رسالة دكتوراه الفلسفة

بعنوان : امتصاص بعض العناصر الثقيلة بواسطة
انواع مختلفة من الطحالب

للآنسة / زينب خليل ابراهيم

مدرس مساعد بقسم النبات - كلية العلوم - جامعة القاهرة

تظهر نتائج هذا البحث أن الكروم سداسى التكافؤ أشد العناصر
ضررا بينما الزنك أقلها ضررا لطحلب كلوريلا فوسكا وذلك فى مدى
يتراوح بين ١٠-٧-٤ جزيئى ولكن زيادة تركيز الكروم عن هذا
المدى توقف نمو الطحلب بينما العناصر الثلاث الأخرى (الزنك ، النيكل
الكادميوم) تحد من معدل زيادة الوزن الجاف للطحلب .

وباستخدام نفس العناصر مع طحلب سبيروليينا ماكسيما تبين أن
التركيزات الضعيفة تكاد لا تؤثر فى زيادة الوزن الجاف فى حين يتوقف
نمو الطحلب كلية عند تركيز ١٠-٤ جزيئى. وفى جميع الحالات فلأن
تغيرات الرقم الايدروجينى خلال فترة نمو الطحلبين تكاد تكون غير
ملحوظة .

وعلى العموم فان كلوريلا فوسكا تختزن كميات كبيرة نسبيا
من العناصر الأربعة بالمقارنة بـ سبيروليينا ماكسيما قبل ظهور أى
أعراض للتسمم كما لوحظ أن الكادميوم يتراكم بكميات اكبر من الكروم
لاظهار نفس التأثير المثبط على الكلوريلا بينما يمتص النيكل بكميات
غير ملموسة ولكن يحدث نفس التأثير الضار للزنك أو الكادميوم فى
تركيز ١٠-٤ جزيئى.

كما يتبين أن العناصر الأربعة حتى تركيز ١٠-٤ جزيئى لا تؤثر
تأثيرا محسوسا على المحتوى البروتينى للـ كلوريلا أو الـ سبيروليينا
أما التركيزات الأعلى فانها تعوق تراكم البروتين داخل الكتلة الحية
للـ كلوريلا باستثناء الكروم الذى يوقف النمو. كذلك تقلل العناصر

الأربعة من المحتوى الكلى للاحماض الامينية للكلوريل وتشارك الاحماض كلها فى هذا النقص فيما عدا حالة النيكل حيث يتراكم السيستين أو الارجنين (تبعاً لتركيز الجرعة) بكميات كبيرة دون أن يتأثر محتوى الجلوتامين.

كما تبين أن الكادميوم بتركيز 10^{-4} جزيئى يزيد من المحتوى الكربوهيدراتى ويقلل محتوى الكلوروفيل أ فى الكلوريل بينما تبدو الجرعات الأقل تركيزاً بلا تأثير على الكربوهيدرات فى حالتى الكلوريل و الاسبيرولينا ولكن تنقص كمية كلوروفيل أ من الطحلب الأول ولا تتغير فى الطحلب الثانى . ويتشابه الكروم مع الكادميوم فى تأثيره على الكربوهيدرات بالنسبة لـ الكلوريل حتى تركيز 10^{-4} جزيئى فى حين تتضاءل الكربوهيدرات بكميات ملحوظة فى حالة الاسبيرولينا، دون تغير فى محتوى الكلوروفيل أ بينما ينقص فى حالة الكلوريل، أما البروتين فإنه يتخذ نفس مسار الكلوروفيل أ .

وقد سجل للزنك والكادميوم نفس التأثير المثبط والحاد على أنزيم ماليك ديهيدروجيناز بينما الكروم والنيكل يشبطان بدرجة أقل فى حالة الاسبيرولينا أما فى حالة الكلوريل فإن الكادميوم ينشط الانزيم بدرجة قليلة بينما تعتبر الجرعات الكبيرة ذات تثبيط تنافسى أما تثبيط النيكل فيبدو لاتنافسياً بينما يظهر الزنك بلا تأثير فى حين ينشط الكروم هذا الانزيم . أما انزيم جلوتاميك ديهيدروجيناز — من الاسبيرولينا فيكاد لا يتأثر بالعناصر الأربعة فى حين تركيز 10^{-2} جزيئى من الزنك ، 10^{-3} جزيئى من الكادميوم ، 10^{-4} جزيئى من الكروم يشبط نشاط هذا الانزيم فى حالة الكلوريل بينما لا يؤثر النيكل على هذا النشاط .

امتصاص بعض العناصر الثقيلة واستخدامها بواسطة أنواع مختلفة من الطحالب

رسالة مقدمة من
زينب خليل ابراهيم
(ماجستير في العلوم ١٩٧٨ م)

للحصول على درجة
دكتوراه الفلسفة في علم النبات
(فسيولوجيا)

قسم النبات - كلية العلوم
جامعة القاهرة

١٩٨٣