MULTIDISCIPLINARY ENVIRONMENTAL STUDIES

ROLE OF FRESH WATER ALGAE & WEEDS IN THE CONCENTRATION OF HEAVY METALS

FINAL REPORT

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PREFACE

Understanding of the interactions between pollutants and the components of contact systems has been identified as prerequisite in pollution control and abatement programing, establishing environment quality criteria and setting up predictive models on the movement of pollutants in the biosphere.

With the increase in the amount and diversity of industrial pollution the interaction between heavy metals and the components of aquatic ecosystem is of great importance since these systems produce food utilized by man.

This report deals with the role of the phytic component of an aquatic ecosystem in concentration of heavy metals, discussing mechanisms and factors involved in metal accumulation process.

Principal Investigator

SAMI Fayed

ABSTRACT

Pollutants such as heavy metals are finding their way in water supplies involving numerous and descrete public health hazards the most dangerous of which are the mutagenic changes leading to transformations to future generations. The conventional methods adopted in waste treatment can cut down metal content to a quite low level; however, of environmental importance is their level in water that is withstandable by aquatic life. Being of refractory nature these pollutnats accumulate and build up in the food chain and are thus transmitted in high concentrations in human food.

This work has been devoted to describe the pattern of interaction between cadmium, lead, copper and zinc and the phytic component of the aquatic ecosystem namely algae and higher aquatic plants; and to evaluate the role of these plants in removing heavy metals from waters considering the plant capacity for metals accumulation and their removal rate.

It has been planned to obtain knowledge that could be useful in making decisions concerning: 1) aquatic weeds management and utilization of collected plants
2) Design criteria of wastewater treatment ponds
3) Permissible limits of heavy metals in the aquatic environment.

Investigations were made in laboratory scale static and continuous flow systems composed of water-metal-plant. Metal accumulation from well defined synthetic waters of varied compositions and from a natural water, namely Nile water, was assessed. Scenedesmus obliquus from the algae and Eichhornia crassipes from the higher aquatic plants were employed throughout this study.

The results obtained emphasized the high capacity of algae and free roots of aquatic macrophytes in metal accumulation and revealed that accumulation takes place mostly by physical processes and is a function of metal: plant material exposure ratio. However, metal accumulation can be retarded by the presence of certain water constituents that possess chelating properties.

Therefore, the following recommendations were made:

1. Plant growth can be employed to rid water from heavy metals taking into account mainly detention time and metal: plant exposure ratio.

   Collected plants exposed to metals should be disposed of in a confined place where heavy metals can be further concentrated to prevent their recycling in the environment. Also, utilization of fish grown in such habitat should be restricted. This biotechnology should not be expected to result into high metal removal rates with waters of high organic content.

2. Water ways authorities should not consider aquatic plants infinitly as a foe. Water weeds are in some cases beneficial in the sense of water purification and what is needed might be mechanical removal of the excessive growths of these plants from the infested water ways.

3. Permissible levels of heavy metals in water supplies should be revised taking into account their high concentration factor in the primary producers. This may lead to suggest forbidding disposal of the wastes that carry hazardous heavy metal at any levels into water supplies.

I. INTRODUCTION

The existance of heavy metals in water is associated with either natural processes or man's activities. Two important natural processes contributing heavy metals to natural waters are chemical weathering and soil leaching. The discharge of wastewaters generated by industrial activities may be the main source of water contamination by heavy metals over the background level. The concentration of heavy metals in water has been correlated with urbanization and run off (Durms, 1974). Arsenic, cadmium, chromium, copper , lead, mercury, nickel and zinc are used'in industry and thus may reach to water supplies (Table 1.). In addition, contribution from non point sources such as rainfall may lead to elevation of metal content of rivers.

As these pollutants reach an aquatic ecosystem they undergo physicochemical changes that determine their access to and effect on aquatic life. Forms of occurrence of metal species in natural waters differ from free metal ions, inorganic ion paris , inorganic complexes, organic complexes chelates,metal complex bound to high molecular organic matter, metal species in the form of highly dispersed colloids to metal species sorbed on colloides. These range from less than 10 A^{o} to 1000 A^{o} .

While heavy metals are usually found in natural waters in low concentrations an amount sinks in the sediment and another amount is trapped by substrates in the water body such as non living particulates and living

Table (1) Uses and Sources of Selected Heavy Metals.

Metal	Use or Source
Cadmium	Batteries, tires, motor oils, heating oils, paints plastics, coal, water mains and pipes, photography, various chemical production industry.
Copper	Ink manufacturing, dyes and pigments, electroplating industry, pharmaceutical industry, taxtile industry, tanning industry, various chemical production industry.
Lead	Batteries, dyes and paints, auto exhaust, photography, textile plants.
Zinc	Embalming, wood preservation, water mains and pipes, paints, electroplating industry, pulp and paper mills, dye and tanning industry, various chemical production industry.
Chromium	Alloys, refractories, catalysts, photography, paints, bacteriocides, electroplating industry pulp and paper mills, dye and tanning industry,
Mercury	Coal, fungicides, electrical batteries, embalming, ink manufacturing, electroplating industry, pharmaceutical industry, pulp and paper mills, dye and tanning industry.
Mercury	Coal, fungicides, electrical batteries,ambalming: ink manufacturing, electroplating industry, pharmaceutical industry, pulp and paper mills, dye and tanning, textile plants.

plants and animals, However, high levels of heavy metals have been detected in fresh waters (Florence, 1977).

Because of their refractory nature that makes them pass to the ultimate of the food chain heavy metals have been gaining great concern. Among the living matter in surface water algae and higher plants constitute the primary production of water. In the last ten years attention of people interrested in water environment has been payed to the interactions between aquatic plants and water pollutants.

The phenomenon of concentrating pollutants by aquatic plants has been noticed in ponds created to dump wastewater effluents where aquatic plants flourish on behalf of nutrient content to water (8,52,53,54). It is usually planned to collect these plants and their fate is determined according to needs and feasibility of their utilization. There has been an emphasis on converting aquatic weeds for animal feed, as fertilizers or for energy production(45,55). However, contamination of these plants by heavy metals may limit their beneficial spectrum especially in the area of food supply.

On the other hand, infestation of surface waters by aquatic weeds has been regarded sometimes hazardious from the public health point of view being hosts of snails and creates problems in irrigation systems (21,23,29).

The weeds found in the Egyptian waterways are: Eichhornia crassipes and Pistia stratiotes (Floating plants), Ceratophyllum demersum,Najas spp.,Ottelia alismoides,Potomogeton spp. and Sanichellia palustris (submersed plants), and Cyperus alopecuroides, Echinochloa stagnina, Nymphaea Spp. Panicum repens, Polygonum serrulatum and Typha spp. (ditchbank and emergent plants) (30). Length in km of water ways infested by weeds in the different parts of Egypt according to a survey conducted in April 1975 is shown in table No. 2 (30). The Ministry of Irrigation applies chemical, mechanical and manual means of control in order to get waterways free. According to the reports of the Ministry of Irrigation the total financial resources needed for the control of aquatic weeds in Egypt in the fiscal year 1975 reached 8.75 million dollars. However, chemicals used in weed control have certain impact on water quality (46).

Though phytoplankton sometime constitutes only a small part of the total plant production in an aquatic ecosystem, in terms of biomass, compared to the higher plants,yet these microorganisms provide greater surface areas. Nile water phytoplankton amounts to 10^6 organism per litre in the average. These organisms may transfer hazardious contaminants mentioned before to man, animal and birds. People especially in rural areas utilize fish caught from drains where they may feed on contaminated phytoplankton and aquatic weeds.

In Japan, analysis of metal content per unit weight of each food by food group revealed (18) that the highest As, Cu and Pb contents were found in seaweeds, fish and shellfish. Although fish and shellfish constituted 6.6% of the "menu" yet the percentages of As, Cu, Pb and Zn contributions by this food group were 45, 13, 17 and 12 respectively. Based on the information of the FAO in 1971 on the supply of foods by food groups in various countries it was estimated (17) that the average daily intake of metals from food for a person in Egypt is 61 ug As, 2.4 mg Cu, 118 ug Pb, 10.3 mg Mn and 10.5 mg Zn.

Table (2) Length (in Km) of Water-ways Infested by Weeds in the Different Parts of Egypt. (Survey conducted in April 1975)

Geographical region	Total length	Type of waterway		Type of prevailing weeds		
		Drain	Canals	Floating	Submersed	Ditchbank
1. West Delta	7254	3929	3325	1735	3975	1544
2. Middle Delta	8505	4332	4173	1061	5599	1845
3. East Delta	10147	4723	5424	1118	6642	2387
4. Middle Egypt	9269	4516	4753	1055	5515	2699
5. Upper Egypt	4941	756	4185	0	4484	457
Total	40116	18256	21860	4969	26215	8932

The data in this table was collected from the reports of the Ministry of Irrigation, then tabulated, classified, summarized and interpreted according to geographical regions.
(From Reference No. 30)

Analysis of metal content of red mullet (Mullus barbatus), a marine fish and Nile bolti (Tilapia Spp.), a fresh water fish, caught from Egyptian waters showed that the former contained 9.62 mg Cu, 15.64 mg Zinc, 3.25 mg Lead and 4.17 mg Hg per kg dry fish while the latter contained 9.64 mg Cu, 24.95 mg Zn, 4.11 mg Lead 4.5 mg Hg per kg dry fish (2).

Among the heavy metals which present the greatest threat to human health are cadmium, mercury, nickel and lead (5, 46). Their relative effect on man depends mainly on the mechanism of their metabolism. The chemical environment with its overwhelming number and types of substances provides the possibility for synergestic or sometimes antagonistic effects. For example both zinc and calcium may be protective against cadmium toxicity. The common chronic health effect of heavy metals on general population is likely to be on the Kidneys (46).

II. GOAL AND OBJECTIVES

Goal: Evaluation of aquatic plants in removing heavy metals from water.

Objectives:
1. Determination of the uptake rate of heavy metals by aquatic plants.
2. Determination of the accumulation capacity of aquatic plants for heavy metals.

The thus gained information may be useful in taking decisions concerned with:

1. Aquatic weeds management in the River Nile and its branches.

2. Permissible limits of heavy metals in aquatic environment.
3. Design criteria of wastewater treatment ponds.
4. Utility of harvested plants from waste treatment ponds.
5. Permissible numbers of micro algae in drinking waters.

III. WORKING HYPOTHESES AND CONSIDERATIONS

- Dealing with a living organism, it should be kept in a nonnutritive medium in order to maintain constant exposure ratios during the experiments of studying accumulation rates.
- For the same reason the plant should be kept under illumination in order to minimize catabolic activities which adds metabolites to the surrounding medium. These metabolites might alter the forms of the metal of concern and thus its accumulation rate.
- Naturally, heavy metals exist in surface waters at a very low level and the chemical composition of a water determines the forms in which the metal exists; and in turn the metal form determines the mechanism by which the plant accumulates the metal.
- Cobalt, Copper, Zinc, Manganese, and Iron are required for plant growth in minute concentrations and must be taken up by plant by an active transport mechanism.
- In natural water, non living matter associated with plant surfaces contributes to the process of metal accumulation.

- Heavy metals do not get access to water separately; they do not come out in a wastewater in a simple form and they are usually associated with dissolved and particulate organic matter. That is to say, chemical analysis of a wastewater sample for heavy metals does not provide expectations with regard to their accumulation by plants in the receiving water.

- Heavy metals carried by phytoplankton change their specific densities and may make them loose their boayancy properties. This may accelerate phytoplankton sinking and affect normal phytoplankton succession especially in the sytems where difference in specific densities is utilized in separating algae from water.

- Of environmental importance is the level of hazardeous heavy metals in a water that is withstandable by aquatic plants and animals which may be ultimately utilized by man.

- The physiological state of aquatic plants determines their surface structure and the composition of their surface water interface which is the substrate of heavy metal accumulation by plant. Propagation of some aquatic plants is seasonal, Some others are affected physiologically by inadequacy of some nutrients and other plants by their surplus.

IV. PLAN OF WORK

The plan of this work was designed to deal with micro-algae and macrophytes and to proceed within the following frames:

A. Microalgae

1. Studying the mechanism (s) of heavy metals removal by algae.
2. Studying the accumulation of heavy metals by the test alga _Scenedesmus_ sp. under varied chemical conditions in static system in a non-nutritive medium.
3. Studying the accumulation of heavy metals in the test alga growing in a nutritive medium in a semi-continuous flow system.

B. Macrophytes

1. Field Investigation: Analysis of aquatic weeds samples picked up from selected locations in the River Nile and its branches for the determination of Pb, Cd, Cu and Zn concentrations.
2. Studying the accumulation of heavy metals in the aquatic macrophyte _Eichhornia crassipes_ in a static system in a non-nutritive medium under varied chemical conditions
3. Studying the accumulation of heavy metals in _Eichhornia_ crassipes under nutritive conditions in a continuous plug flow system.

V. MATERIAL AND METHODS

The working system was composed of plants (algae or macrophytes), water (prepared with varied composition or natural Nile water) and the metals Copper, Zinc, Cadmium and Lead separately or in combination added as their salts as mentioned in VI.

Algae

Scenedesmus sp. was used as test alga. This organism was isolated from Nile water and continuously cultured in a batch system under optimum conditions in the laboratory. The culturing medium was the AAM (44) with the concentration of micrcnutrients (trace elements) at their $\frac{1}{3}$ strength. All the chemicals used in preparation of the medium were of AR grade in order to assure minimum contamination by heavy metals. For the same reason air was delivered to the culture flask after beenfiltered through glass wool and activated carbon and washed in 0.1 N sulfuric acid solution and finally in distilled water. Precautions were made to minimize bacterial growth.

After seven days incubation the algae were examined microscopically to be sure that they were in a good physiological state and that the culture was not contaminated by other algae. Algae in the culture were then separated by leaving the culture settle under faint illumination in order to maintain anabolic activity. The supernatant water was replaced by distilled water to which 15 mg KCl and 15 mg $NaHCO_3$ mg per liter were added. This wash was repeated before algae were used in metal accumulation studies. By this wash algae was supposed to get free from surrounding metabolites and excess nutrients. It was found that algae excrete organic ligands of different metal complexing capacities (27, 48). No further treatment e.g. by EDTA was made since it is believed (34) that this chelating coumpound may cause a loss of integrity of the cell wall-plasma membrane structure.

In some experiments, seston including phytoplankton isolated from Nile water was used instead of the test alga.

Sedgwick Rafter technique (42) was used to separate seston from water,

Macrophytes

Eichhornia crassipes was selected as the test plant used all over the study of heavy metal accumulation by aquatic macrophytes. It was estimated that this plant infested 40116 km of the water ways in Egypt in April 1975 (30).

To obtain test plants of similar age and of similar physiological state to be employed in this study was one of the objectives. However, trials to cultivate this plant under laboratory conditions did not succeed. The plant flourished in the beginning but after sometime (about six weeks) was subject to infection by some types of aquatic funji and thus deteriorated. At last, the test plant was obtained from a man-made pond about 100 m^2 surface area and about 2 metres depth. This pond is supplied with Nile water and receives seepage water from the surrounding garden (The Botanical Garden). The *Eichhornia* plants are maintained there as one of the aquatic ornamental plants. No point source of pollution by heavy metals exists in the area except precipitation from the atmosphere if any. Analysis of water samples from the pond is given in table 3.

Water

The basic synthetic water used in the study was intended to be a simple one. Because lysis of plant cells could take place when introduced in distilled water proper 15 mg Na HCO_3 per liter of redistilled water in allglass

Table (3) Chemical Characteristics of the Pond Water from which *Eichhornia* crassipes Was Isolated.

Electric conductance, umho/cm	410
pH	6.8
Total hardness, as Ca CO_3 mg/l	134
Calcium " " mg/l	84
Total alkalinity as Ca Co_3	188
Chlorides, as Cl, mg/l	28
Sulfates, as SO_4, mg/l	20.8
Total phosphorus, as P, mg/l	0.220
Ortho phosphate, as P, mg/l	0.038
Nitrate, as N, mg/l	0.390
Copper, ug/l	2.30
Zinc, ug/l	5.20
Cadmium, ug/l	0.30
Lead, ug/l	0.40

apparatus was used as the basic synthetic water. This simplicity in the test water composition must have minimized the number of inorganic ligands under the experimental conditions.

Some of the experiments were conducted to reveal the effect of the chemical composition of water on metal accumulation by plants. In this concept, calcium and magnesium salts and chelating agents were identified the most important chemical factors in natural waters. These were added separately or in combination to the basic synthetic water, Calcium was added as calcium chloride and magnesium as magnesium sulfate. The sodium salt of ethylene diamine tetraacetic acid (EDTA) was used as a model compound for chelating agents.

Filtered Nile water was used in some experiments. This water was analyzed according to the american standard methods for water analysis (42).

Heavy metals

Copper as copper sulfate, zinc as zinc chloride, cadmium as cadmium chloride and lead as lead nitrate were the metals salts used in this study. Dilute solutions of these salts were added separately or in combination to the test waters before introducing the test plants.

To study accumulation of a heavy metal by algae the best way was to analyze the exposed algae for the metals content. Isolation of algae from contact medium can be achieved by centrifugation, filtration through membrane filters or settling. All of these means are liable to remove heavy metals as well. A comparison between the effect of centrifugation and filtration through membrane filters on the contrations of selected heavy metals was made in absence of algae. The results obtained (not presented here) indicated that both techniques removed part of the heavy metals but removal by centrifugation at 1000 rpm was considered nonsignificant. Isolation of algae by leaving the suspension to settle down needs a long time that was not allowed under the experimental conditions. Therefore, it was decided to apply centrifugation technique to isolate algae from the test waters.

After separation of test algae by centrifugation they were transfered from the centrifuge tube to a pre-weighed beaker by a minimum amount of redistilled water, (after discarding the supernatant water). The algae transfered to the beaker were dried on a water bath followed by drying in an oven (105°C) for ½ hour, cooled and weighed. Digestion of algae was carried out under flux using HNO_3 - H_2O_2. After a clear solution was obtained and excess acid was expelled the solution of digested algae was filtered through acid washed filter paper and the filtrate completed to 25 ml by redistilled water and then transfered to a 50 ml-plastic bottle for metal content determination.

Experimental macrophytes were dried in an oven at 105°C. The shoot was separated from the root of each and weighed separately. Each of them was ground up separately in a stainless steel grinder. Weighed portions (0.5-1.0 gm) of the dried and ground plants were ignited in a porcelain crucible with a cover in a muffle furnace at 550°C for 4 hours. The residue was dissolved by 2:1 HNO_3 untill a clear solution was obtained. After filtration through acid washed filter paper the filtrate was completed to 50 ml by redistilled water and transfered to a plastic bottle for metal content determination.

Metals concentrations were determined using a Perkin-Elmer Model 370 atomic absorption spectrophotometer equiped with a Heated Graphite Atomizer Model 2100 and deuterium arc background corrector.

Element	Wave length nm	Slit width mm	Flame gas
Cadmium	228.8	0.7	Air-acetylene
Copper	324.8	0.7	"
Lead	283.3	0.7	"
Zinc	213.9	0.7	"

The conditions were adjusted so that a standard contained 2 mg Cd/l gave an absorbance reading of about 0.35 absorbance units; a standard contained 5 mg Cu/l gave an absorbance reading of about 0.25 absorbance units; a standard contained 20 mg Pb/l gave an absorbance reading of about 0.18 absorbance units; and a standard contained 0.5 mg Zn/l gave an absorbance reading of about 0.12 absorbance units. For the standard conditions of this apparatus described above the working range was linear up to concentrations of approximately 2, 5, 20 and one mg/l of Cd, Cu, Pb and Zn respectively.

VI EXPERIMENTAL

VI.1 Accumulation of Heavy Metals in Algae

VI.1.1. Algal Assays with Heavy Metals

The growth response of a test alga, Scenedesmus sp., in absence and in presence of Cd, Pb, Zn and Cu seperately in the standard Algal Assay Medium in batch cultivation was investigated by employing the procedure adopted by the USEPA (1974). Algal growth was followed up by determining chlorophyll a concentration in 50-ml samples. Pigments extraction was made by hot methanol.

The aim behind these experiments was to obtain information on the maximum level of metals that do not considerably affect growth of algae under fresh water conditions. Besides those available in the literature these information were useful in selecting metal doses applied in metal accumulation studies.

VI.1.2. Mechanism of Metal Accumulation by Algae

VI.1.2.1 Boundage of Metals Accumulated in Algae

An experiment was carried out to show to what extent could the metal be bound to algal surface. In this experiment, to algal suspensions in distilled water (+ 86 mg/l $NaHCO_3$) metal solutions were added in amounts as mentioned in table 9. Three replicates were made for each metal forming three sets. After 24 hours shaking, algae were separated from each flask by centrifugation at 1000 rpm for five minutes. Algal contents of one set were digested for determination of metal content.

Algae separated from the second set were resuspended in distilled water (+ 86 mg/l $NaHCO_3$) and shaked for 24 hours. Algae separated from the third set were resuspended in distilled water (+ 86 mg/l $NaHCO_3$) to which 3.6 mg/l of EDTA were added to each flask. After 24 hours shaking the algae separated from each of the flasks in the second and third sets were digested and analyzed for metal content.

VI.1.2.2. Effect of Cell Death on Metal Accumulation in Algae

In this experiment accumulations of metals in live and denatured UV-treated algae were compared. The aim of this comparison has been to reveal if the vitality of algae plays any role in the removal processes, i.e. to reveal if there is any physiological forces that draw these metals to the algal surface. A suspension of the algae in distilled water (+ 86 mg/l $NaHCO_3$) was divided into two equal amounts. One of them was introduced into a wide dish to make a thin film. This was subjected to UV irradiation at a distance of 20 cm for one hour with stirring the contents frequently. The subjected algae were then isolated by centrifugation and resuspended in distilled water (+ 86 mg/l $NaHCO_3$) and made up to the initial volume (250 ml). Metal solutions ($Cu\ SO_4$, $Zn\ Cl_2$, $Cd\ Cl_2$ or $Pb\ (NO_3)2$) was added to both suspensions in the amounts mentioned in table 10. After 24 hours shaking, algae in the test flasks were isolated by centrifugation and digested for metals content determinations.

VI.1.2.3. Effect of Cell Wall Structure on Metal Accumulation

Comparison was made between the accumulations of cadmium by two types of algae of different cell wall sctructures namely the green alga <u>Scenedesmus</u> sp. of cellulosic cell wall and the diatom <u>Melosira</u> sp. of silicious cell wall. The first alga was available from laboratory culturing. The diatoms were obtained as a natural unialgal community. Algae were separated by centrifugation and washed in distilled water containing 15 mg/l $NaHCO_3$.

To the algal suspensions equal amounts of cadmium chloride solution were added. After shaking for two hours the algae in the flasks were separated, dried, weighed, digested and analyzed for cadmium content.

VI.1.2.4. Effect of the Physiological State of Cells on Metal Accumulation in Algae.

Algae in the logarithmic growth phase are known to possess electric charges of higher maguitude than those in the decline phase. The capabilities of algae in the two physiological states to remove heavy metals were compared. In this experiment the test alga was cultured in the standard Algal Assay Medium (EPA, 1971) in a batch system and on the tenth day another batch was started. After 4 days algae in both cultures were isolated by centrifugation. Algae in the logarithmic and decline growth phases were thus obtained. These were then resuspended in distilled water (+ 86 mg/l $NaHCO_3$) and distributed in test flasks (at 250-ml portions) to receive metal solutions in the amounts shown in table 12. After 2 hours shaking the algae were separated by centrifugation and digested to determine metal contents.

VI.1.3. Accumulation of Metals by Algae in Non-nutritive Medium in Static Systems.

In order to keep constant metal: algae ratios during experimentation a non-nutitive medium was used, i.e. a medium free from phosphate and nitrate radicals. Moreover, algae used in these experiments were quite starved.

VI.1.3.1. Effect of Exposure Ratio on Metal Accumulation by Algae .

The capacity of the test algae for heavy metals accumulation was investigated in distilled water to which $NaHCO_3$ (86 mg/l) was added to raise the pH to 7. Four dilutions for the initial algal suspension were made to obtain five concentrations of algae. Equal amounts of the metal solution concerned were added to the test flasks (500 ml) which contained 250 ml portions of the algae suspensions. Thus, five different metal: algae exposure ratios were obtained: After shaking for 24 hours algae were separated by centrifugation and digested to be analyzed for the metal content.

VI.1.3.2. Effect of Different Constituents of Water on Metal Accumulation in Algae.

The accumulation of Cd, Pb, Zn and Cu (separately) by Scenedesmus sp. in batch system after one, two and 24 hours exposure in synthetic waters of different chemical compositions spiked with low concentrations of metals was investigated. In these experiments the algal suspension in distilled water (+ 15 mg $MaHCO_3$ per liter) was distributed at 250-ml portions in four sets of 500-ml conical flasks. Three of these sets of flasks received more additions. To the second set 10 mg/l Mg as Mg SO_4 and 5 mg/l Ca as Ca Cl_2 were added. To the third set one mg/l Na_2-EDTA was added. To the fourth set both Mg and Ca salts solutions and Na_2-EDTA solution were added at the same concentrations as in the second and third sets. The metal solution was then added to the test flasks. Concentrations of algae in suspension and concentrations of metals added are mentioned in the tables 17-20. The concentration of algae ranged between 30 and 70 mg/250 ml, on dry weight basis. Cu as Cu SO_4 and Zn as Zn Cl_2 were added at 50 ug/250 ml suspension. Cd as Cd Cl_2 and Pb as Pb $(NO_3)_2$ were added at 100 ug/250 ml algae suspension. The flasks were then shaked at 60 oscillations per minute under mild illumination and a constant temperature of 24°C. One of the flasks from each set was taken after the elapse of 1,2 and 24 hours. The contents of the flasks were subjected to centrifugation in glass cups at 1000 rpm for five minutes. The thus separated algae were dried, weighed and digested for metal content determination.

V1.3.3. Metal Accumulation From Different Media

Accumulations of heavy metals by the test algae suspended in distilled water (+ 15 mg/l $NaHCO_3$) in filtered(through 45 mu MF) Nile water and filtered biologically treated synthetic sewage effluent were compared. Chemical compositions of the latter two are presented in table (21). Pb, Cd, Cu and Zn were below the detection limite by the flameless technique. Metal solutions were added on algal suspensions (250 ml) in conical flasks (500 ml) and shaked for two hours after which algae were separated by centrifugation, dried, weighed and digested for metal analysis.

V1.1.3.4. Effect of pH on Metal Accumulation

The Effect of pH on accumulation of metals by algae in the range 6-8 was investigated. The test algae were suspended in distilled water to which NaCl (15 mg/l) was added. This suspension (108.4 mg/l dry algae) was divided into two flasks.

To one of them $NaHCO_3$ solution was added to raise the pH to near 8. The contents of each flask was then distributed into 4 500-ml flasks which then received the amounts of metals mentioned in table 23. The flasks were then shaked for 2 hours after which the final pH was determined and algae were separated for metal analysis.

Vl.1.3.5. Accumulation of The Four Metals in Combination

In polluted water systems metals are usually found in combinations. Experiments were conducted to assess the accumulation of the four metals by the test alga.

Equimolar (10^{-6} and $2x10^{-6}$M) concentrations of Cu(as Cu SO_4), Cd (as Cd Cl_2), Zn (as Zn Cl_2) and Pb (as $Pb(NO_3)_2$) were added to an algal (Scenedesmus sp.)suspension in $NaHCO_3$ (100 mg/l) solution. Beside determining algal concentration the suspension was distributed at 250-ml portions and shaked for two hours under moderate illumination. Algae were then separated, digested and analyzed for metals content.

VI.1.3.6. Effect of Anionic Radicals on Metals Accumulation

Natural waters contain salts of the anionic radicals HCO_3^-, Cl^-, SO_4^{--}, and PO_4^{---}. The effect of the presence of these radicals on accumulation of cadmium and lead, separately, by the test algae was investigated.

To algal suspensions in distilled water (500-ml protions in one liter-conical flasks) one ml of equimolar solutions of the sodium salts of Cl^-, HCO_3^-, $H_2PO_4^-$ or SO_4^{--} was added to the algal suspensions. Then, one ml of 10^{-3}M Cd, Cu or Pb solution was added to each of the test flasks. The contents of each of the flasks were divided into two 500-ml flasks as duplicates. After shaking for two hours algae were separated by centrifugation, digested and analyzed for metal content.

VI.1.3.7. Accumulation of Metals by Suspended Matter in Water

In natural waters there are algae in different physiological states, zooplankton, debris and inorganic formulations collectively called seston. All these suspended matter get contact with metals in water. The tendency of seston to remove heavy metals was investigated. Seston in a Nile water sample was isolated and concentrated 10 times by settling technique. The concentrate was distributed at 250-ml portions in conical flasks which then received the amounts of heavy metals solutions as mentioned in table 27.

After shaking for 2 hours suspended matter was isolated, dried, weighed, digested and then analyzed for metal contents, The concentration of seston was determined by filtering a known volume of the test seston suspension on a preweighed 0.45 mu membrane filter, dried at 105 °C and weighed. Ash content was determined by igniting the particulates retained on the membrane filter at 550 °C. Algal content in the seston was determined in terms of chlorophyll (a) content. Extraction of chlorophyll was made by 90% acetone.

Analysis of Nile water for pH, total alkalinity and total hardness was carried out according to the american standard methods (42).

VI.1.4. Accumulation of Metals by Algae Grown in Semi-Continuous Flow System

Metabolites released by algae during their growth in nutritive media may interfere in metal accumulation; a factor that is not liable to exist in case of metal accumulation by alge introduced in non-nutritive media. Therefore, it was planned to investigate the accumulation of heavy metals in algae grown in a semi-continuous flow system.

VI.1.4.1. Comparison between Metals Accumulations by *Scenedesmus* grown in Semi-Continuous Flow System.

A semicontinuous flow system was conducted at 0.25, 0.50 and 0.75 l/day. One-litre portions of algae suspended in the Algal Assay Medium were introduced in 2-L flasks. Treated air was supplied to the test flasks which were subjected to continuous illumination. Each day a certain volume of the suspension was withdrawn and the same volume of the growth medium was introduced instead. Each day the concentration of algae in the withdrawn portion was determined and presented as dry algae per liter. pH was also determined each time. When a steady state was reached heavy metals were added to the media supplied to the test flasks. The system was run for 15 days. The concentration of metals increased in the test flasks day after day. Zn, Cd and Pb was added at 400 or 200 µg/l and Cu at 200 or 100 µg/l in the substituting feed medium. Part of the 250 ml portions withdrwan every day was centrifuged to separate algae. These were dried, weighed and digested to determine metal content.

VI.1.4.2. Effect of Dilution Rate on Metal Accumulation

A set of experiments were conducted in a semi-continuous flow system to study the effect of algal growth rate on the accumulation of cadmium by the test alga.

These experiments were run as mentioned in VI.1.4.1. except that the variable here was the dilution rate. The experiment was run first by metal-free growth medium until steady states were reached. Then cadmium at a concentration of 200 ug/l was added to the feed medium.

VI.2. Accumulation of Heavy Metals by Aquatic Macrophytes

VI.2.1. Field Investigation for Metals Content of Aquatic Macrophytes

Water, macrophytes and sediment samples were collected from the River Nile at the site where wastewaters from iron and steel and nitrogenous fertilizers are discharged. Samples were also collected from the Rosetta branch at the location where wastewaters from soda and soap, pesticides and phosphate fertilizers discharge their wastes. From a canal (Rayah Eltawfiki) separates from the river, namely, at the site where an electronic and metal factory is located samples were also taken. At each of the above mentioned locations two samples were collected, one upstream and the other downstream the wastewater discharge point. Both floating and rooted hydrophytes were represented if available.

In addition, 12 aquatic plants samples were collected from Alexandria district, a heavily industrialized area. Some wastewaters are discharged into Mahmaudia canal, others find their way to Smouha drain, while many others are directed to the sea through the sewerage system to outfall at Qayetbay.

As a background,a sample was collected from the Dam Lake, an area upstream any industrial activities, to be compared with the downstream samples with regard to metals content.

VI.2.2. Heavy Metal Accumulation by Eichhornia Crassipes in a Static System.

VI.2.2.1. Effect of Exposure Ratio on Metal Accumulation in Synthetic Water and Natural Nile Water.

1.5 litres-portions of filtered Nile Water were introduced into 2 litres-plastic jars. Nile water was filtered through filter paper Whatmann No.1 using a basket centrifuge to remove suspended matter. Analysis of Nile water (averaged over the whole period of study) is given in table 36. In another set of jars 1.5 litres-portions of $NaHCO_3$ solution (0.005 %) were introduced. Aliquots of the metal solution under investigation,(Cu, Cd, Zn or Pb) were added to the jars to give the initial concentrations of the metals as mentioned in the tables 37-40. After well mixing, Eichhornia crassipes plants were introduced into the jars and these were kept under illumination from cool white fluourescent tubes. After 4 days exposure, the plants were removed and dried for metal content determination following the steps for metal content determination in V.

VI.2.2.2. Effect of Chemical Composition of Water on Metal Accumulation.

Cadmium as cadmium chloride or lead as lead nitrate was added at the rate of one mg metal per liter to 2 liters-jars, each contained 1.5L of the following solutions in triplicates:

1. Distilled water + 60 mg $NaHCO_3/l$
2. Distilled water + 60 mg $NaHCO_3$ + 5 mg Ca as $CaCl_2$ per liter + 10 mg Mg as Mg SO_4 per liter.
3. Distilled water + 60 mg $NaHCO_3$ per liter + 6 mg EDTA (disodium salt) per liter.
4. Distilled water + $NaHCO_3$ + EDTA + Ca and Mg salts as mentioned in 1,2 and 3.

Into these jars *Eichhornia* crassipes plants were introduced. After one day exposure the plants were removed and dried for metal content determination following the steps mentioned in ..... V.

VI.2.2.3. Effect of Disodium EDTA on Metal Accumulation by *Eichhornia* crassipes.

Cadmium as cadmium chloride or lead as lead nitrate was added at a rate of one mg metal per litre to 2 liters-jars each contained 1.5 liters of $NaHCO_3$ solution (60 mg $NaHCO_3/l$), in triplicates, which received EDTA solution at the concentrations 1,6,12 or 24 mg/l giving thus 4 sets of jars.

Into these jars Eichhornia crassipes plants were introduced. For each set of jars the test plant was removed after one, two or 24 hours exposure time. These plants were dried for metal content determination.

VI.2.2.4. Accumulation of Cu, Cd, Zn and Pb by Eichhornia Crassipes .

Into jars contained 1.5 L of water (60 mg $MaHCO_3$/l solution) the equimolar concentrations 2,4,8 or 16 x 10^{-6}M of Cu (as Cu SO_4), Zn (as Zn Cl_2), Cd (as Cd Cl_2) and Pb (as $Pb(NO_3)_2$) were added. Eichhornia crassipes plants were then introduced. After one day exposure the plants were removed and dried for metals content determination.

VI.2.3. Accumulation of Metals by Eichhornia Crassipes in a Continuous Plug Flow System.

A Laboratory scale gutter (No.1) 5 m length, 25 cm width and 30 cm depth was filled with settled Mile water. Fifty Eichhornia crassipes plants were then inserted into the water. Continuous flow of settled Nile water into the gutter at one of its ends was performed using a peristaltic dosing pump at a rate ranged between 12 and 13 liters per day . Another similar gutter (No.2) without plant was supplied with Nile water from the same container and at the same rate serving as a control experiment. Both of the two gutters were subjected to illumination for 14 hours daily. Cool white fluorescent tubes plus tungston lamps were the sources of light giving an intensity of about 4000 lux at the plants. After the elapse of 15 days the feed water was injected by equimolar solutions of Cd (as Cd Cl_2), Cu (as Cu SO_4) and Pb (as $Pb(NO_3)_2$) to give a final concentration of 0.5×10^{-6}M for each metal. After 9 days the concentration of the metals were increased to the double futher on. The gutters were externally marked into four successive zones in order to identify sampling sites. Water samples (50 ml) were withdrawn and plant samples were picked up for analysis from the different zones in the time shown in the corresponding tables. Water samples were acidified and plant samples were dried weighed and digested for metals content determination.

Physicochemical characteristics of Nile water before and after metals addition compared with characteristics of effluents of the gutters after 5 weeks flow were determined.

VI.2.4. Accumulation of Metals by Eichhornia crassipes in a Flow Completely Mixed System.

This experiment was carried out in a plastic dish 40 litres-capacity with 30 cm depth. The dish was filled with settled Nile water and 10 Eichhornia crassipes plants were introduced in it. The dish was continuously feeded by the test water at a rate of 6-6.2 liters/day using a peristaltic dosing pump. A stainless steel stirrer kept the water of the dish in turbulance. The outflow came out from a nozzle at the rim of the dish. The dish was subjected to illumination for 14 hours daily from cool white fluorescent tubes plus tungston lamps to give a light intensity of about 4000 lux. After the elapse of two weeks the feed water was injected with equimolar solutions of Cu (as Cu SO_4), Cd (as Cd Cl_2) and Pb (as Pb $(NO_3)_2$) to give a final concentration of 0.5 x 10^{-6}M for each metal. After 6 days the concentrations of the metals added to the feed water were raised to 10^{-6}M. Water samples (50 ml) were withdrawn from the feed tank (7 litres) and from the experimental dish; acidified and kept in plastic bottles for metal analysis. After 11 days from the begining of metals addition to feed water, plant samples were picked up for metal content determination in the time mentioned in the corresponding table.

VII. RESULTS AND DISCUSSION

VII.1. Accumulation of Heavy Metals in Algae

VII.1.1. Algal Assays with Selected Heavy Metals

Growth curves obtained from the assays concucted in absence and pressence of heavy metals are shown in Fig. 1-4 The aim behind these assays was to determine working ranges of metals concentrations that would not be lethal for the test alga growing under its optimum conditions and thus could be applied in the subsequent metals accumulation studies. Chlorophyll (a) content was the parameter used to assess physiological stresses.

In case of addition of high doses of Cu growth curves were characterized by a lag phase. The presence of Cu had effects on both the growth rate and the maximum growth . Among the growth inhibitory effects the metal might interfere with availability of an essential nutrient perhaps phosphate. The presence of Cu was inhibitory effective at as low as 40 ug/l and the dose 100 ug/l showed a similar result.

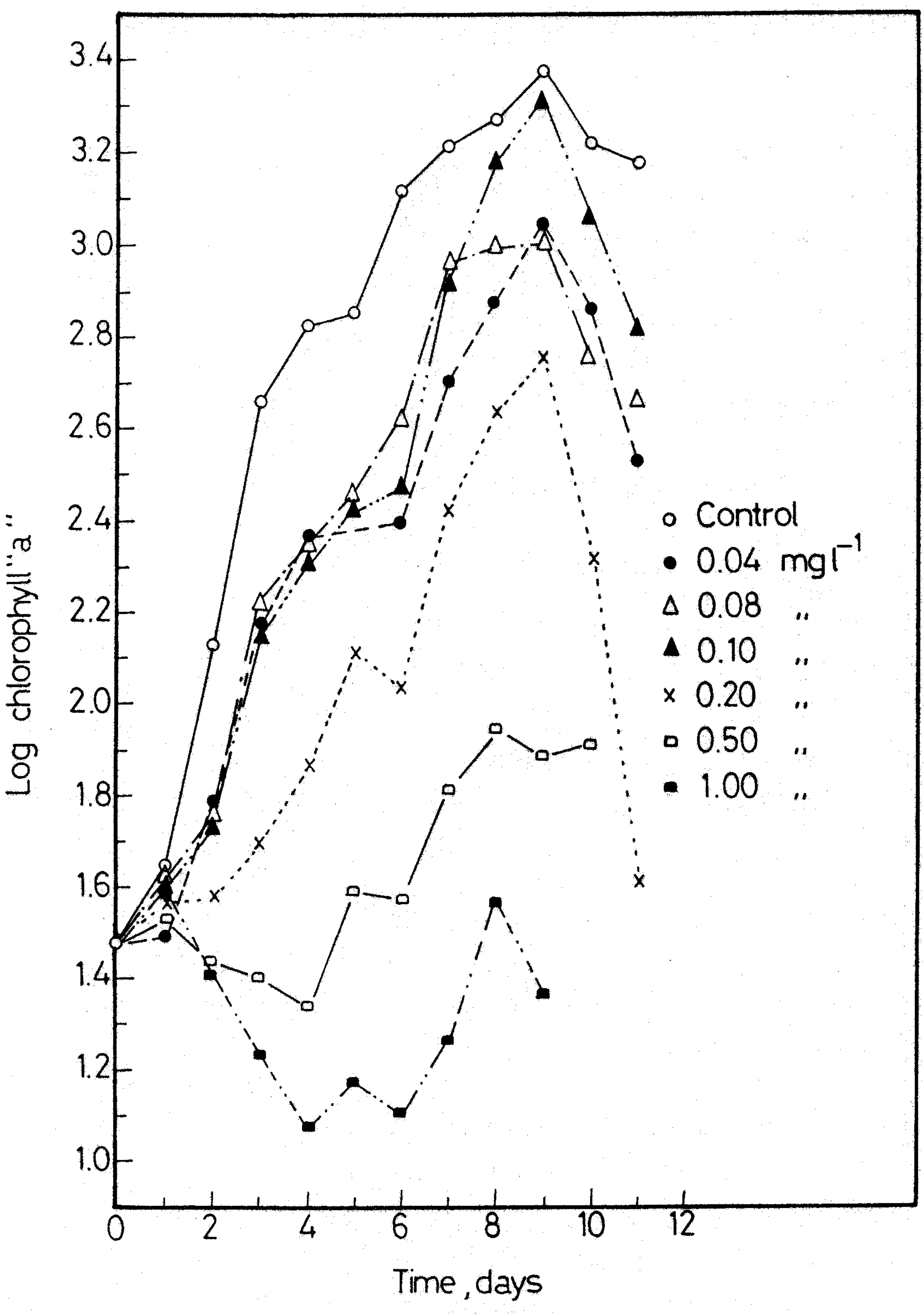


Fig.(1) Growth of Scenedesmus in Nutrient Solution Containing Copper.

Table(4) Effect of Copper on Scenedesmus Growth

Cu dose	Incubation Days									
	1 st		2 nd		3 rd		4 th		5 th	
	Ca	pH	Ca	pH	Ca	pH	Ca	pH	Ca	pH
Control	46.6	7.8	116.54	8.75	370.3	9.95	679.6	10.2	718	10.5
Control (-T.E.)	45.0	7.9	133.01	9	453.72	10	668.88	10.3	732.56	10.4
0.04 mgl^{-1}	31.16	7.8	61.08	8.45	151.88	9.4	234.24	9.4	253.88	9.4
0.08 "	41.32	7.8	55.80	8.6	166.79	9.2	230.32	9.3	289.12	9.65
0.10 "	40.60	7.8	54.84	8.55	178.56	9.2	203.12	9.15	271.88	9.4
0.20 "	37.24	7.7	38.04	7.75	50.48	9.1	73.56	9.	133.32	9.3
0.50 "	34.52	7.5	27.72	8.0	25.56	8.4	22.04	8.35	39.36	8.4
1.0 "	37.64	7.4	25.56	7.75	17.36	7.9	12.08	7.7	15.20	7.75

Ca: Chlorophyll "a"

Cu as Cu SO_4

Initial inoculum: 30.3 ug/1 chlorophyll "a"

T.E: Trace Elements

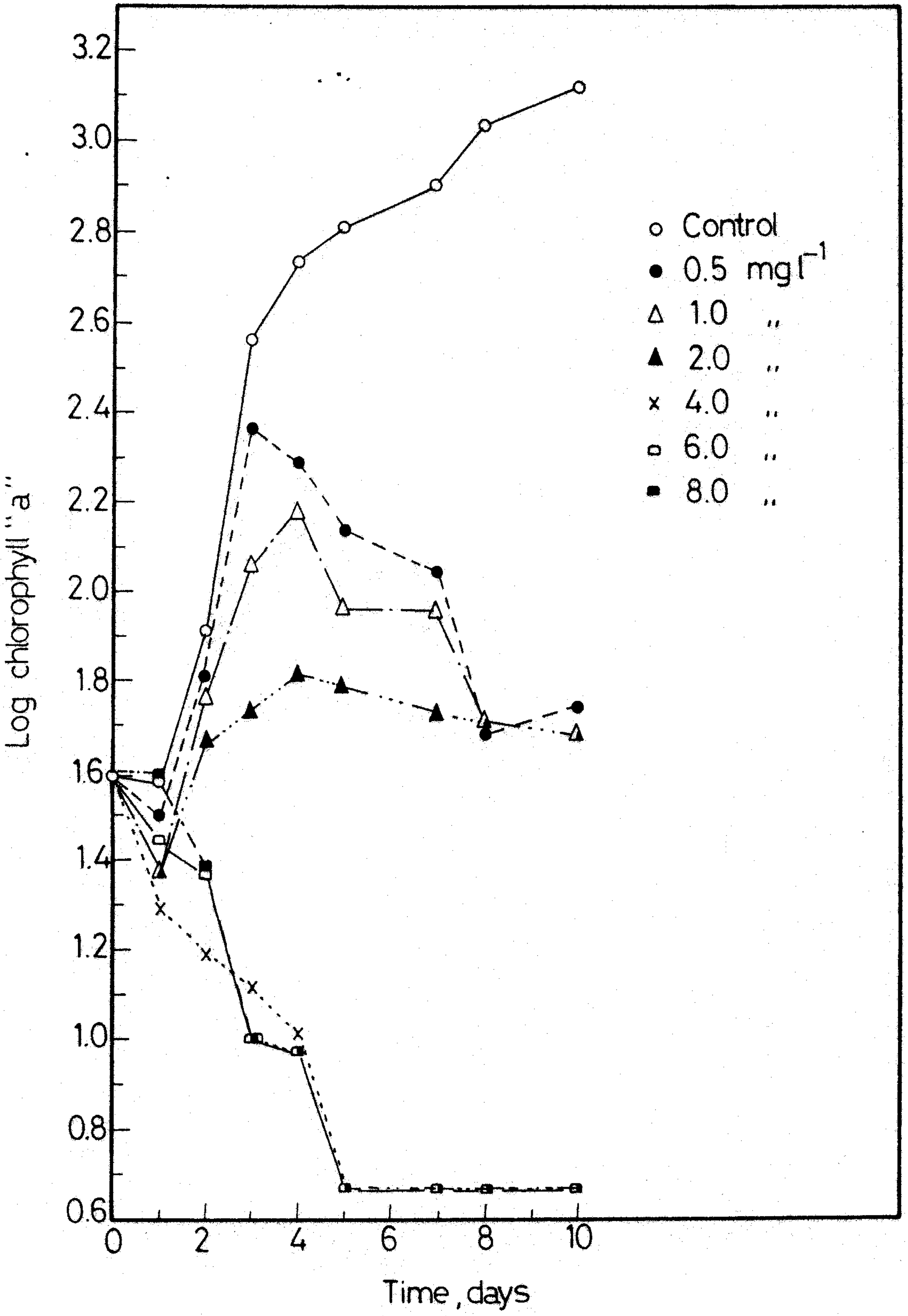


Fig. (2) Growth of Scenedesmus in Nutrient Solution Containing Zinc.

Table (5) Effect of Zinc on *Scenedesmus* Growth

Zn dose	Incubation Days									
	1 st		2 nd		3 rd		4 th		5 th	
	Ca	pH	Ca	pH	Ca	pH	Ca	pH	Ca	pH
Control	46.16	7.8	92.04	8.5	309.06	9.65	794	10.3	-	10.2
Control (-T.E.)	37.28	7.92	81.92	8.8	364.61	10.0	540.24	10.4	638.96	10.3
.5 mgl^{-1}	31.96	7.5	64.76	8.2	232.88	9.6	195.28	10.25	139.16	10.2
1.0 "	23.24	7.6	57.2	8.15	113.56	9.0	150.72	9.7	93.6	9.9
2.0 "	23.24	7.8	45.68	8.0	54.04	8.5	64.32	8.7	61.04	8.6
4.0 "	19.68	7.6	15.44	7.7	13.2	7.6	10.32	7.4	4.68	7.75
6.8 "	27.28	7.5	23	7.5	10.08	7.3	6.24	7.4	4.68	7.4
8.0 "	39.76	7.45	24	7.35	10.08	7.15	6.24	7.2	4.68	7.3

Ca = Chlorophyll "a"

Zn : as $Zn\ Cl_2$

Initial inoculum : 38.65 ug/1 chlorophyll "a"

T.E. = Trace Elements

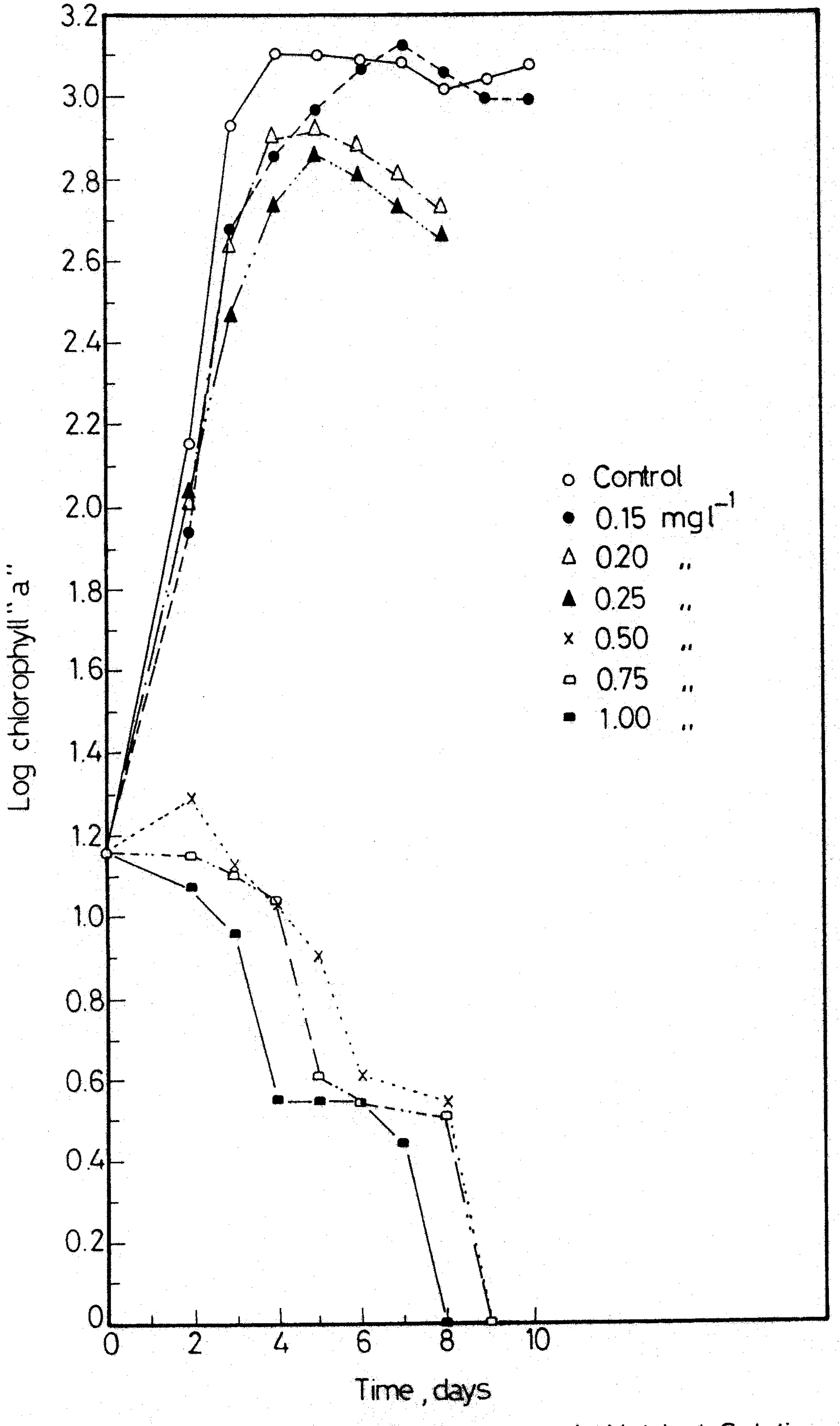


Fig. (3) Growth of Scenedesmus in Nutrient Solution Containing Cadmium.

Table (6) Effect of Cadmium on Scenedesmus Growth

Cd dose	Incubation Days							
	2 nd day		3 rd day		4 th day		5 th day	
	Ca	pH	Ca	pH	Ca	pH	Ca	pH
Control	143.36	8.8	848.28	9.1	1263.66	10.6	1267.48	10.6
0.15 mgl^{-1}	86.24	9.2	479.84	10.2	719.12	10.6	944.88	10.7
0.20 "	102.76	9.1	437.23	9.95	804.83	10.6	834.16	10.4
0.25 "	107.24	8.6	287.68	9.1	540.68	10.3	724.88	10.65
0.5 "	19.68	8.7	13.41	8.45	10.78	8.45	8.06	8.5
0.75 "	14.06	8.4	12.71	8.25	10.896	8.5	4.096	8.7
1.0 "	11.87	8.5	9.22	8.20	3.58	8.7	3.58	8.45

Ca : Chlorophyll "a"

Cd as $CdCl_2$

Initial inoculum : 14.59 ug/1 chlorophyll "a"

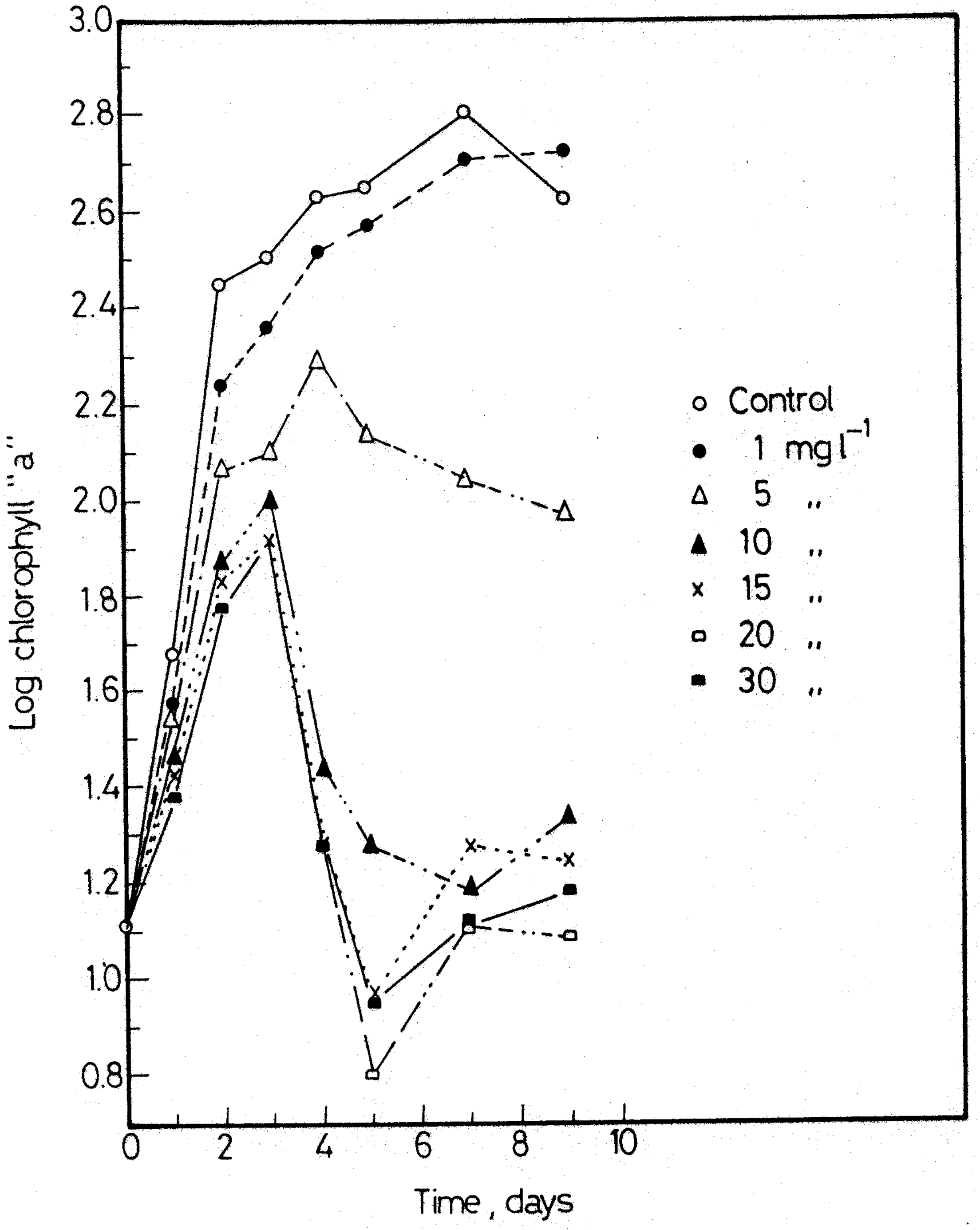


Fig. (4) Growth of Scenedesmus in Nutrient Solution Containing Lead.

Table (7) Effect of Lead on *Scenedesmus* Growth

Pb dose	Incubation Days									
	1 st day		2 nd day		3 rd		4 th day		5 th day	
	Ca	pH	Ca	pH	Ca	pH	Ca	pH	Ca	pH
Control	38.64	8.9	248.32	10.3	469.06	10.9	462.64	11	517.92	11
1 mgl^{-1}	37.04	8.1	172.54	10.5	229.20	11.05	329.02	11.1	369.04	11.1
5 "	34.52	9.0	117.56	10.34	125.61	11.0	224.31	11.1	136.75	10.95
10 "	28.84	8.5	74.28	10.0	100.8	10.7	27.44	10.6	19.16	10.6
15 "	26.36	8.3	69.6	10.1	83.64	10.8	19.24	10.7	9.28	10.6
20 "	26.36	8.8	68.44	10.1	85.44	10.75	19.24	10.7	5.64	10.6
30 "	23.92	8.4	59.48	9.9	86.04	10.7	19.16	10.6	9.2	10.6

Ca : Chlorophyll "a"

Pb as Pb $(NO_3)2$

Initial inoculum : 12.88 ug/1 chlorophyll "a"

Table (8) Effect of Metals on the Maximum Growth Rate and Maximum Growth of Scenedesmus Sp.

1- Copper

Cu Conc. mgl^{-1}	0	0.04	0.08	0.1	0.2	0.5	1.0
Growth rate, day^{-1}	0.9	0.54	0.57	0.59	0.17	0	0
Max. Growth, ugl^{-1}	1899.4	1120.3	1268.6	2024.5	573.8	89.40	37

2- Zinc

Zn Conc., mgl^{-1}	0	0.5	1.0	2.0	4.0	6.0	8.0
Growth rate, day^{-1}	0.75	0.6	0.36	0.11	0	0	0
Max. Growth, ugl^{-1}	1307.9	195	150	64	-	-	-

3- Cadmium

Cd Conc., mgl^{-1}	0	0.15	0.2	0.25	0.5	0.75	1.5
Growth rate, day^{-1}	1.35	1.16	1.13	0.99	0	0	0
Max. Growth, ugl^{-1}	1267.5	1355	834	724	-	-	-

4- Lead

Pb Conc., mgl^{-1}	0	1	5	10	15	20	30
Growth rate, day^{-1}	1.07	0.96	0.76	0.68	0.62	0.63	0.63
Max. Growth, mgl^{-1}	644	512	224.3	100.8	83.6	85.4	86.04

Maximum Growth : as ug chlorophyll "a"

Drastic effect resulted from the presence of 200 ug Cu/l. Therefore, the dose of Cu used in the accumulation studies was likely kept at 100 ug/l, in most cases. Nevertheless, different types of algae exhibited different responses to Cu (10,25,37).

In case of Cd addition to the growth medium a clear difference was noticed in the growth response at the doses 250 and 500 ug/l (Fig.3). The growth curve in presence of the dose 250 ug/l coincided with the growth curve obtained from the control flask. In presence of 500 ug/l and higher complete inhibition of growth occurred. Therefore, concentrations at or below 200 ug/l Cd were considered appropriate for metal accumulation studies. Reported toxic concentrations of cadmium to different algal species vary widely (10,41,56, 57).

The lowest dose of Pb (Fig.4) tested (1 mgl^{-1}) affected slightly the maximum growth through did not affect the maximum growth rate. Therefore an intermediate dose, that is 400 ug/l, was chosen for Pb accumulation studies. No inhibitory action by lead at 1.0 mg/l was detected on the growth of Coelastrum in a turbidostat system (Soeder et al, 1978) and 1.5 mg/l had no influence on the growth of Pediastrum tetras(Wettern et al, 1976).

The addition of 500 ug Zinc per liter to the growth medium resulted (Fig.2) in a sharp drop in algal growth after three days incubation. Clear inhibition of algalgrowth starting from the begining of incubation was displayed by 2 mg Zn/l. Thus, doses below 500 ug/l were considered safe in the metal accumulation studies as far as contact time was less than 3 days.

However, the information gained from these assays could not be considered valid under other chemical conditions since it is known that the chemical environment affects the form of metal and thus its toxicity. For example, it has been indicated that the sensitivity of algae to metals is inversely proportional to the ionic strength of the medium (Miller et al, 1976). Also, there are evidences that metal toxicity is controlled by the presence of chelating compounds (Van Den Berg et al, 1979).

VII.1.2. Mechanism of Metal Accumulation in Algae

The measured pollutant accumulation is a meaningful criterion for the food chain aspects of polution and for its fate in the environment and the following experiments were a contribution for understanding the accumulation mechanisms.

VII.1.2.1. Boundage Extent of Metals Accumulated in Algae

Table 9 shows that only a low percentage of the metals accumulated by the algae were released on resuspending the algae in distilled water and shaking for 24 hours; while on washing with EDTA solution most of the accumulated metals were mobilized. This is in agreement with the results obtained by Schreffler et al (1977); Weber et al (1978), Sandmann and Boger (1980), Nakajima el al (1979); Sakaguchi et al (1979). Hassett et al (1980) showed that lead was more bound to different types of algae than cadmium. Horikoshi et al (1981) also reported that EDTA was capable of mobilizing metals from bacteria, fungi, yeast and actinomycetes.

Table(9) Release of Heavy Metals From Algae

Metal added M	ug M. added/ 250 ml	Loaded algae by H.M.			Loaded algae washed with D.W(1)			Loaded algae washed with EDTA solution (2)			Metal retained in (1), %	Metal retained in (2) %
		Dry Wt. digested algae, gm	ug M. in digested algae	ug M./ gm dry algae	Dry Wt. degested algae	ug M. in digested algae	ug M./ gm dry algae	Dry Wt. digested algae	ug M. in digested algae	ug M./ gm dry algae		
Cu	44.1	0.0391	23.96	612.79	0.0401	20.83	519.45	0.0347	5.00	144.09	88.77	23.51
Zn	44.2	0.0447	28.90	646.53	0.0455	22.60	531.76	0.0347	9.80	255.21	82.48	39.47
Cd	100.8	0.0410	65.07	1587.07	0.0381	63.01	1653.81	0.0320	21.07	658.44	104.20	41.49
Pb	88.4	0.0473	78.57	1661.10	0.0438	66.67	1522.15	0.0395	38.77	981.66	91.64	59.10

Concentration of algae in suspension : 50 mg/250 ml

(1) Distilled water + Na HCO_3 (86 mg/l).

(2) Distilled water + Na HCO_3 + EDTA (3.6 mg/l).

VII.1.2.2. Effect of Cell Death on Metal Accumulation

Metal accumulation by living algae was compared with that displayed by UV-treated algae to reveal if the vitality of the cells plays any role in the accumulation process, i.e. to reveal if there is any physiological forces that draw these metals to the algal surfaces. The results in table 10 showed that nontreated and treated algae by UV irradiation accumulated more or less equal amountsof metals indicating that no internal forces are involved in the accumulation process. This suggestion could be supported by the findings of Sakaguchi et al (1979) who reported that heat killed Chlorella took up cadmium to a greater degree than living ones. Also, it has been reported by Sakaguchi et al (1981), that the capacity of Chlorella cells to accumulate molybdenum was increased by heat treatment. This can be explained that the cells exposed to such treatments loose some of their contents,thus, their surface/weight ratio increases.

VII.1.2.3. Effect of Cell Wall Structure on Metal Accumulation.

Cadmium accumulation by the green alga Scenedesmus obliquus was compared with the accumulation by Melosira granulata on dry weight basis, bearing in mind that accumulation is likely to be a matter of surface area than weight.

The calculated volumes of Melosira granulata and Scenedesmus quadricauda were found to be 60.000 u^3 and 1000 u^3 respectively (cited in Wollenweider, 1969). This means that one gram of the former provides less

Table(10) Removal of Heavy Metals by Live and UV Treated Algae

Metal M	Amount of metal added ug/250 ml	Live algae (.09652 gm)				UV treated algae (.07377 gm)			
		dig. algae gm	ug.M. in dig. alg.	ug M. acc. by dig. alg.	ug M. acc. by all alg.	dig.alg. gm	ug M. in dig. alg.	ug M. acc. by dig. alg	ug M. acc. by all alg.
Zn	44.2	0.0884	56.32 (-22.69)	33.63	36.72	0.0685	57.14 (-17.58)	39.56	42.60
Cd	50.4	0.0885	48.37	48.37	52.75	0.0690	47.10	47.10	50.36
Cu	44.1	0.0895	35.31	35.31	38.08	0.0679	40.0	40.00	43.45
Pb	44.2	0.0886	43.89	43.89	47.81	0.0682	45.06	45.06	48.74

Heavy metal content of algae

Zn : 256.69 ug/gm

Cd : 0.00 ug/gm

Cu : 0.00 ug/gm

Pb : 0.00 ug/gm

Between brackets are the initial Zn content in algae.

Algae suspended in distilled water + 86 mg/l Na HCO_3 (pH 7.05).

Table (11) Accumulation of Cadmium by Two Different Types of Algae

Type of algae	Concentration of algae gm/250 ml	Cadmium added mg/250 ml	Exposure ratio, mg Cd/gm algae	Cadmium accumulated mg	Average Cd accum. mg	Cadmium accumulated mg/gm
Melosira	0.03275	0.378	11.54	0.2345	0.230	7.023
	0.03275	0.378	11.54	0.2252		
Scenedesmus	0.01325	0.378	28.53	0.0969	0.1013	7.645
	0.01325	0.378	28.53	0.1058		

surface area than an equivalent weight of the latter, assuming equal values for their specific gravity. The results given in table 11 showed that cadmium accumulated by the diatom was less than that accumulated by the green alga but not in a ratio matching the reverse of the ratio between their cell volumes. It must be mentioned that the exposure ratio as will be discussed later must have had an effect on the accumulated cadmium. Assuming a linear relationship between exposure ratio and metal accumulated per unit weight of algae, then at an exposure ratio equaled 11.54, one gm Scenedesmus must have accumulated much less amount of cadmium than did Melosira. However this comparison is not fare since the two types of algae did not grow under the same growth conditions. It is believed that metals are primarily bound to adsorption sites of the extracellular compounds (Mangi et al, 1978; Laube et al 1980; and Hassett et al, 1980). Composition of these compounds depends on growth medium and specific metabolic activities. Variation in the capacity to sorb metals by different alga species has been doccumented (14, 33, 36).

VII.1.2.4. Effect of the Physiological State of Cells on Metal Accumulation in Algae

Algae in the logarithmic growth phase are known to possess electric charges of higher magnitude than do those in the decline phase. Capabilities of metal accumulation by the test alga in the two physiological states were compared. Algae in the active growth phase proved to be a better accumulator for metals than are those in the decline phase (table 12). This difference in accumulation capacity was noticed also by Hassett et al (1980). However, when Jennett et al (personal communication) compared exponentional growth phase cells versus stationary phase cells they found several anomalous behaviour. Lead accumulation by young cells of *Nostoc* and *Oscilatoria* were higher than old cells while the opposite happened with *Chlamydomonas*. Since the composition of the surrounding differs according to cell activity interaction between metal and cell surface can be different according to physiological state.

The available results and information must, thus, lead to suggest that metal accumulation by algae is a process that takes place on the cell surface and is controlled by the composition of this surface which is determined by the types of alga and its physiological state. The results should support the reports (16, 36, 41) which stated that sorption or uptake of lead and cadmium by algae is essentially a passive process not mediated by metabolic processes. Studying the nature of bonding between metalic ions and algal cell walls Crist et al (1981) suggested covalent and ionic charge bonding. Ionic charge bonding has been thought to arise from a surface charge generated on the α and B carboxyl groups and covalent bonding on the amino, imide and imidazol groups. These groups are constituents in the matrix of polysaccharides and protein in the outer layers of the cell.

Some reports (Laube et al, 1980 and Wong et al, 1980) however, tend to say that metals may go further to the inside of the cell. Examination by electronic microscope revealed that when lead was added in the inorganic ligand it was found only in the vicinity of the cell wall (Lorch, 1977) while when added as tetramethyl lead it penetrated the cell wall and was deposited within concreton bodies of the algae (Silverberg et al, 1977).

Table (12) Metals Accumulation By Algae In The Logrithmic and The Decline Growth Phases After 2 Hours Exposure Time.

Algal phase	Metal	Added metal Ug/l	Concentration of algae mg/l	Ug M. accum by algae in the flask	Ug M. removed by algae, Ug/gm
Logarithmic	-	-	0.0686	-	-
	Pb	315.93	0.0686	153.12	2232.07
	Cu	357.14	0.0686	343.00	5000.00
	Zn	360.00	0.0686	351.73 (-17.75)	4868.51
	Cd	360.00	0.0686	238.26	3473.18
Decline	-	-	0.0957	-	-
	Pb	315.93	0.0957	185.20	1935.21
	Cu	178.57	0.0957	159.66	1668.34
	Zn	315.00	0.957	120.48 (-27.27)	9973.98
	Cd	360.00	0.0957	181.07	1892.06

Algae in logarithmic phase contained 258.72 ug Zn/gm

Algae in Decline phase contained 285.00 ug Zn/gm

Pb, Cu and Cd content of both were below detection limits.

Test medium: distilled water + 86 mg/l $NaHCO_3$ (pH 7.0)

Since some of the heavy metals like zinc and copper are required for algal growth there must be a mechanism for their transfer from the surface to the inside of the cell.

VII.1.3. Accumulation of Metals by Algae in Non-nutritive Medium in Static System.

VII.1.3.1. Effect of Exposure Ratio on Metal Accumulation

Metals accumulations by the same test algae from the same stock culture were compared at five metal: algae ratios. The results (Fig. 5) indicated a linear relationship between metal: algae exposure ratio and metal accumulated per unit weight of algae. Under the experimental conditions 7.7 mg Cu, 5.2 mg Zn, 16.2 mg Pb or 12.2 mg Cd could be accumulated by one gram of algae (Table 13 to 16). The positive correlation between cell content and concentration in the medium was mentioned in the reports of most of investigators (1,12,13,15,19,35,40,41,57). This phenomenon supports the suggestion that metal accumulation by algae is rather a physicochemical process that takes place on the surface than a biological procesess. Frey et al (1978) described the metal - algae interaction as amenable to Langmiur adsorption type of analysis. Also, Horikoshi et al (1981) reported that uptake of uranium by actinomycetes followed Freundlich isotherm.

The fate of accumulated metals in algae through the trophic levels of the aquatic food web can be illustrated diagramatically for cadmium as an example.

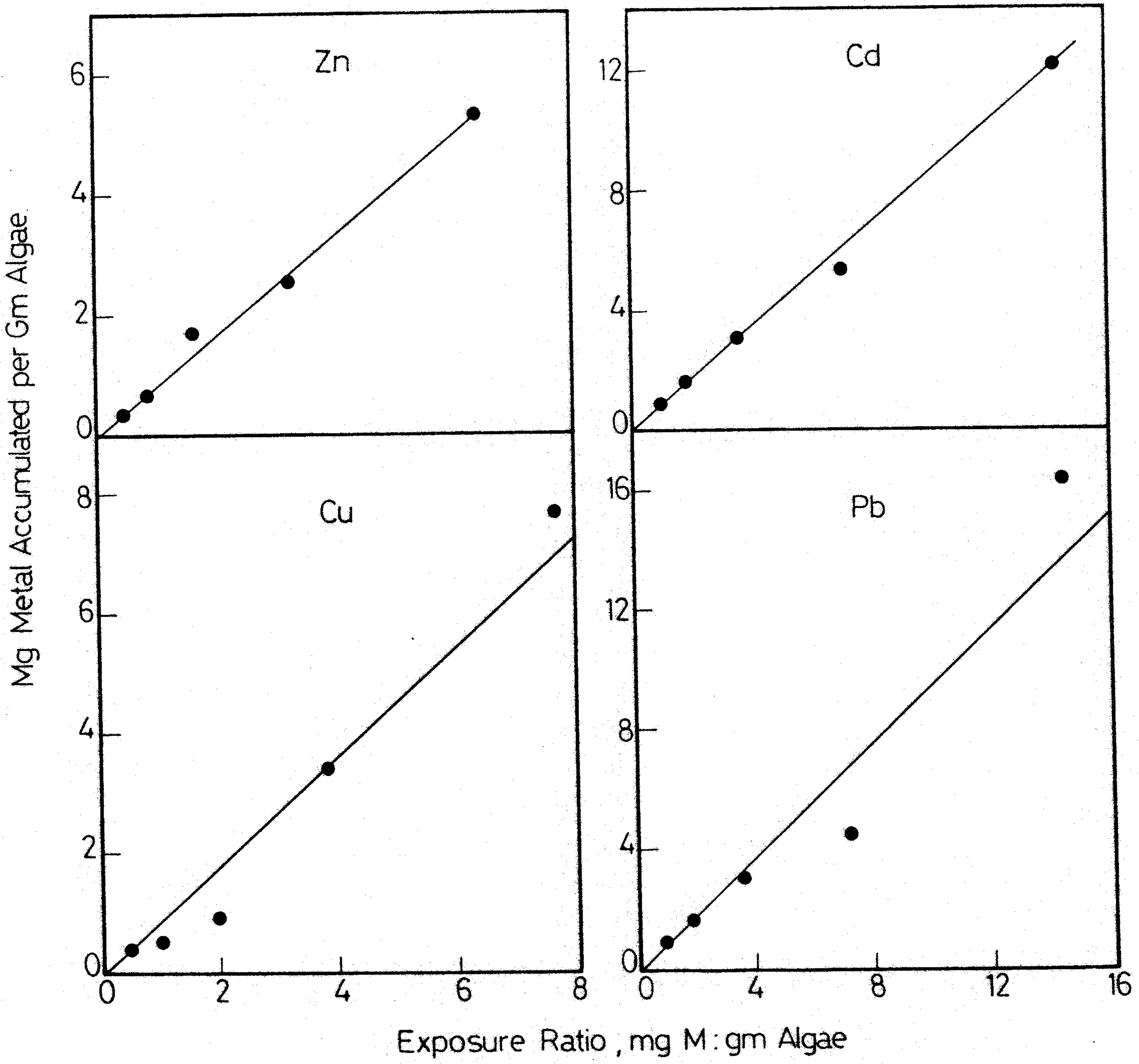


Fig. (5) Effect of Exposure Ratio on Metal Accumulation by Non-growing Algae.

Table (13): Copper Accumulation By Algae of Different Concentrations In 24 Hours

No. of flask	Wt. of algae in flask (250 ml susp.) gm	Cu: algae exposure ratio mg/gm	Wt. digested algae, gm	ug Cu in dig. algae	ug Cu removed by algae in flask	% Removal	mg Cu accum. in gm algae
1	0.0922	0.447	0.0872	27.91	29.51	59.0	6.320
2	0.0461	0.954	0.0392	19.42	22.84	45.6	0.495
3	0.0231	1.909	0.0177	18.23	23.79	47.6	0.959
4	0.01150	3.818	0.0067	22.57	38.74	77.4	3.368
5	0.0057	7.636	0.0031	24.06	44.24	88.4	7.7613

Algae suspended in distilled water adjusted to pH 7.0 by Na HCO_3 (86 mg/1)

Cu content of initial algae: 0.0 ug Cu/gm dry algae

Amount of Cu added (as Cu SO_4) to each flask: 44 ug/250 ml

Table(14) Zinc Accumulation By Algae of Different Concentrations In 24 Hours

No. of flask	Wt. of algae in flask (250 ml susp.) gm	Zn: algae exposure ratio mg/gm	Wt. digested algae, gm	ug Zn* in dig. algae	ug Zn removed by algae in flask	% Removal	mg Zn accum. in gm algae
1	0.1102	0.400	0.1000	59.23	35.88	71.76	0.32559
2	0.0551	0.800	0.0507	47.76	37.76	75.52	0.68530
3	0.0275	1.600	0.0252	49.25	46.13	92.26	1.67745
4	0.0137	3.201	0.0124	34.79	34.64	69.28	2.52847
5	0.0068	6.403	0.0049	26.74	35.83	71.66	5.26912

* Including Zn content of initial algae: 256.69 ug Zn/gm algae.

pH of suspension 7.1

Amount of Zn added (as Zn Cl_2) to each flask: 44.1 ug/250 ml

Table (15) Cadmium Accumulation By Algae of Different Concentrations In 24 Hours

No. of Flask	Wt. of algae in flask (250 ml susp.) gm	Cd algae exposure ratio mg/gm	Wt. digested algae, gm	ug Cd in dig. algae	ug Cd removed by algae	% Removal	mg Cd accum.in gm algae
1	0.1126	0.888	0.0977	83.55	96.29	96.3	0.85517
2	0.0563	1.776	0.0498	85.90	97.11	97.1	1.72490
3	0.0281	3.559	0.0263	81.20	86.76	86.8	3.08745
4	0.0140	7.143	0.0139	75.21	75.75	75.8	5.41079
5	0.0070	14.286	0.0039	47.75	85.71	85.7	12.24359

Cd content of initial algae : 0.0 ug Cd/gm dry algae

pH of suspenion 7.05

Amount of Cd added (as Cd Cl_2) to each flask : 100.4 ug/250 ml.

Table (16) Lead Accumulation By Algae of Different Concentrations in 24 Hours

No. of flask	Wt. of algae in flask (250 ml susp.) gm	Pb:algae exposure ratio mg/gm	Wt. digested algae, gm	ug Pb in dig. algae	ug Pb removed by algae in flask	% Removal	mg Pb accum. in gm algae
1	0.0984	0.898	0.0885	97.88	88.81	88.8	0.90260
2	0.0492	1.797	0.435	73.20	82.79	82.8	1.68276
3	0.0245	3.593	0.0204	61.64	74.33	74.3	3.02157
4	0.0123	7.187	0.0090	40.00	54.66	54.7	4.44444
5	0.0061	14.374	0.0026	42.12	98.82	98.8	16.20000

Pb content of initial algae : 0.0 ug Pb/gm algae

pH of Suspension : 7.1

Amount of Pb added (as Pb nitrate) to each flask : 88.4 ug/250 ml.

Water	Algae	Herbivore	Primary Predator
	CF ; 30500	CF : 3	CF : 3
0.4 µg/ml	12.2 mg/gm	36.6 mg/gm	109.8 mg/gm

VII.1.3.2. Effect of Different Constituent of Water on Metal Accumulation in Algae.

The results proved that metal accumulation by algae was greatly retarded by the presence of EDTA (Table 17-20). This compound is a synthetic model ligand of aminocarboxylic acid structures the could be functioning groups in natural organic ligands. When a metal solution is added to an algal suspension contatining compounds of chelating power like EDTA the metal ion can be attracted by: the organic ligands, the inorganic ligands (e.g. OH and CO_3) and adsorption sites on algal surface due to presence of the matrix of polysaccharides and proteins in the outer layer of the cell and extracellular metabolic products that may have chelating properties too (27,48) .

It seems from the results that EDTA had the most attracting power under the experimental conditions. Also, it apperated that the metal - EDTA complexes had poor affinity to algal surfaces. This was also observed by Ahlf et al (1980) for algae and Rainbow et al (1980) for barnacles.

From the tables it appeared that the presence of the divalent cations calcium and magnesium did not reduce the power and capacity of EDTA to chelate heavy metals and thus metal accumulation by algae was retarded by the same manner

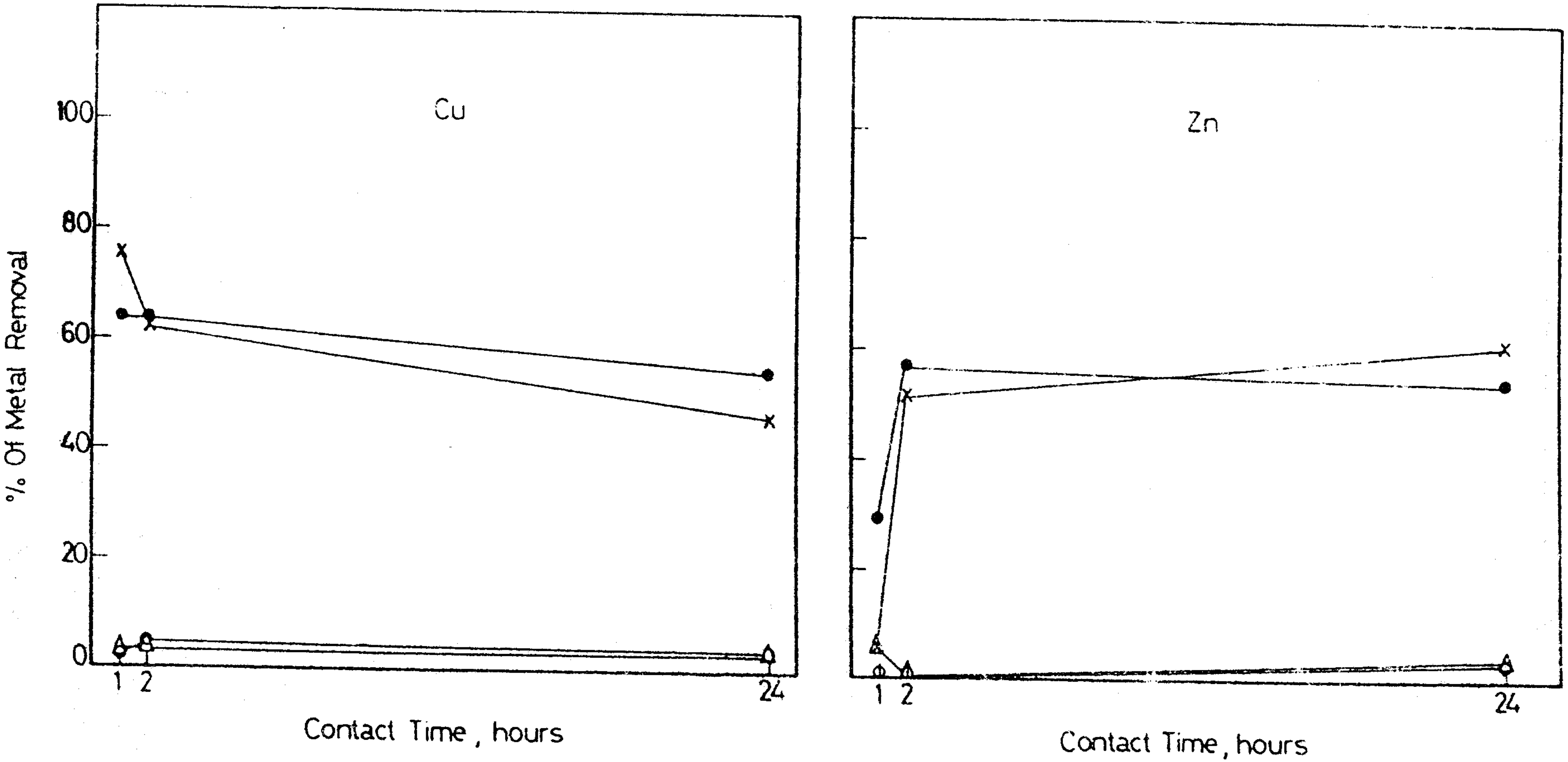


Fig.(6a) Rate of Metal Removal by Algea Under Different Chemical Environment.

●——● In distilled water (+ $NaHCO_3$)

x——x In distilled water + Ca and Mg salts

o——o In „ + Na_2 - EDTA

Δ——Δ In „ + Ca and Mg salts + Na_2 - EDTA

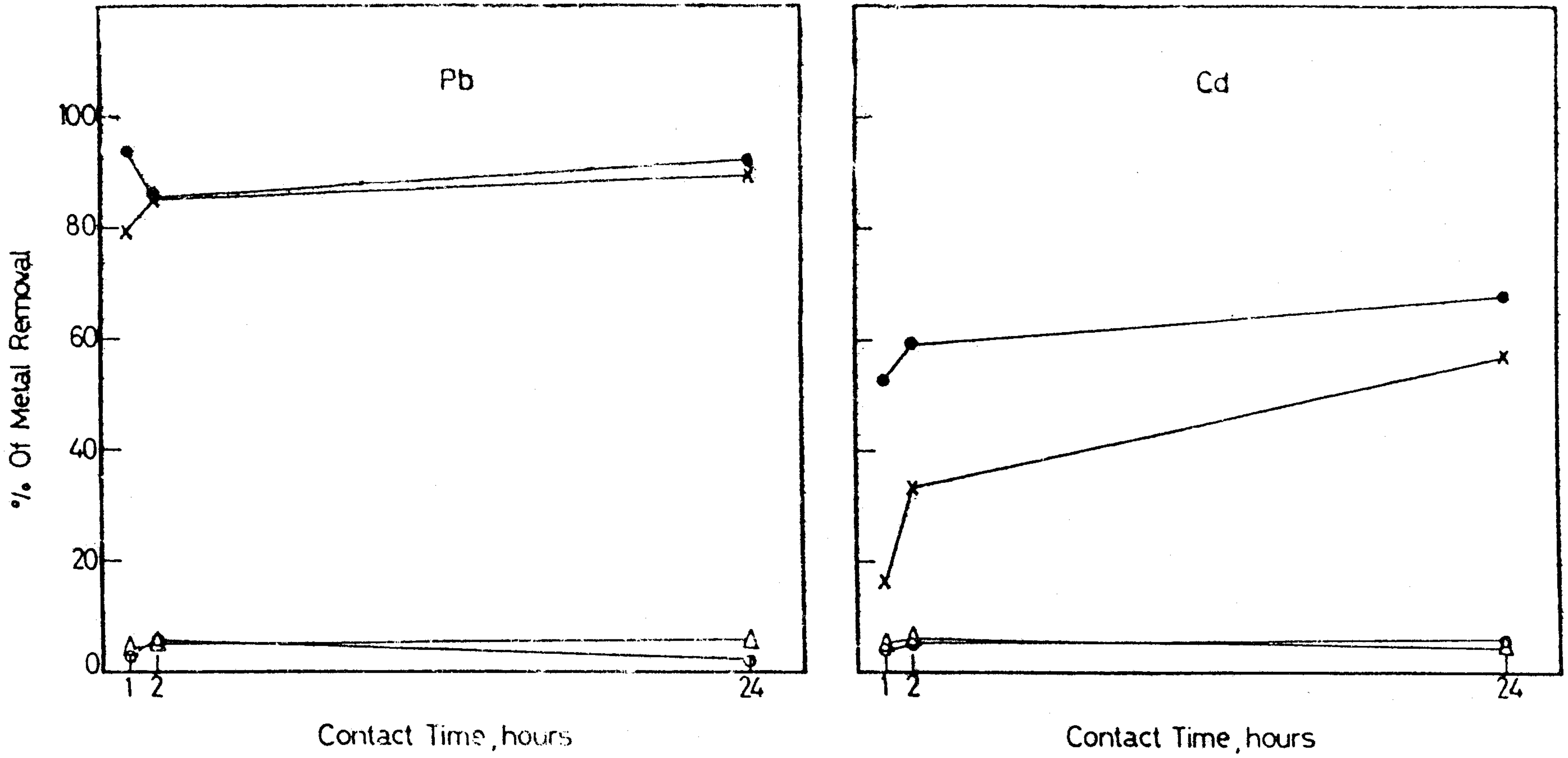


Fig. (6 b) Rate of Metal Removal by Algea Under Different Chemical Enviroment.

●——● In distilled water (+ $NaHCO_3$)

×——× In distilled water + Ca and Mg salts

○——○ In " + Na_2 - EDTA

△——△ In " + Ca and Mg salts + Na_2 - EDTA

Table (17) Rate of Copper Accumulation By Algae In Waters of Different Compositions.

No. of flask	Contact time. hour	Wt. algae digested, gm	Cu content in digested algae, ug	ug Cu/ gm dry algae	ug Cu accumulated by 55 mg dry algae	% Removal
1	1	0.0425	25.00	588.23	32.35	64.70
4		0.0456	31.68	694.74	38.21	76,42
7		0.0450	1.32	29.33	1.47	2.94
10		0.0459	1.51	32.90	1.81	3.62
2	2	0.0410	23.68	577.56	31.76	63.52
5		0.0438	25.00	570.78	31.39	62.78
8		0.0452	1.75	38.72	2.13	4.26
11		0.0482	1.53	31.74	1.74	3.48
3	24	0.0430	21.05	489.53	26.92	53.84
6		0.0497	21.05	423.54	23.29	46.58
9		0.0523	1.43	27.34	1.50	3.00
12		0.0499	1.51	30.26	1.66	3.32

Cu added to each flask (250 ml algae suspenion) = 50 ug Cu as Cu SO_4.

Flask No. 1,2,3 algae suspended in distilled water (+ 15 mg/l $NaHCO_3$)

4,5,6 algae suspended in distilled water + Ca and Mg salts

7,8,9 algae suspended in distilled water + EDTA

10,11,12 algae suspended in distilled water + Ca and Mg salts+EDTA

Cu content of test algae : 0.0 ug Cu/gm dry algae.

Concentration of Algae in suspension : 220 mg/l

Table (18) Rate of Zinc Accumulation by Algae In Waters of Different Chemical Compositions.

No. of flask	Contact time, hour	Wt. algae digested, gm	Zn content in digested algae ug	ug Zn/gm dry algae	ug Zn zccumulated by 30 mg	% Removal
1	1	0.0241	17.50	726.14	14.08	29.1
4		0.0256	9.00	351.56	2.85	5.7
7		0.0265	4.25	160.38	0.00	0.0
10		0.0243	8.50	349.79	2.79	5.5
2	2	0.0241	29.25	1213.69	28.71	57.4
5		0.0214	24.00	995.85	25.94	51.9
8		0.0272	6.75	248.16	0.00	0.0
11		0.0254	4.25	167.32	0.00	0.0
3	24	0.0312	35.00	1121.79	25.95	54.6
6		0.0283	36.25	1280.92	30.73	61.5
9		0.0300	9.50	316.67	1.80	3.6
12		0.0290	9.25	318.97	1.87	3.7

Zn added to each flask : 50 ug Zn as $ZnCl_2$

No. 1,2,3 Algae suspended in distilled water (+ 15 mg/l $NaHCO_3$)

No. 4,5,6 Algae suspended in distilled water + Ca and Mg salts

No. 7,8,9 Algae suspended in distilled water + EDTA

No. 10,11,12 Algae suspended in distilled water + Ca and Mg + EDTA

Zn content of 30 mg algae = 7.7 ug Zn

Concentration of Algae in suspension : 120 mg/l -

Table (19) Rate of Cadmium Accumulation By Algae In Waters of Different Chemical Compositions.

No. of flask	Contact time, hours	Wt. algae digested gm	Cd content in digested algae	ug Cd/gm dry algae	Cd accumulated by 40 mg dry algae	% Removal
1	1	0.0333	44.0	1321.32	52.84	52.84
4		0.0307	12.6	410.42	16.40	16.40
7		0.0359	4.0	11.42	4.44	4.44
10		0.0364	4.8	131.87	5.28	5.28
2	2	0.0355	52.4	1476.06	59.04	59.04
5		0.0344	28.8	837.21	33.48	33.48
8		0.0396	5.45	137.63	5.48	5.48
11		0.0296	5.45	184.12	7.36	7.36
3	24	0.0356	60.95	1712.08	68.48	68.48
6		0.0408	58.2	1426.47	57.04	57.04
9		0.0376	6.15	163.56	6.52	6.52
12		0.0427	5.08	118.97	4.76	4.76

Cd added to each flask : 100 ug Cd as Ca Cl_2

No. 1,2,3 Algae suspended in Distilled water

No. 4,5,6 Algae suspended in Distilled water + Ca and Mg salts

No. 7,8,9 Algae suspended in Distilled water + EDTA

No. 10,11,12 Algae suspended in Distilled water + Ca and Mg + EDTA

- Cd content of test algae :0.0ug Cd/gm dry wt.

Concentration of Algae in suspension : 160 mg/l

Table (20) Rate of Lead Accumulation By Algae In Waters of Different Chemical Compositions

No. of flask	Contact time, hours	Wt. algae digested, gm	Pb content in digested algae, ug	ug Pb/ gm dry algae	ug Pb accumulated by 70 mg dry algae	% Removal
1	1	0.0546	73.26	1341.76	93.93	93.92
4		0.0655	74.42	1136.18	79.53	79.53
7		0.0655	2.50	38.17	2.67	2.67
10		0.0585	3.30	56.41	3.95	3.95
2	2	0.0569	69.77	1226.19	85.83	85.83
5		0,0522	63.95	1225.10	85.76	85.76
8		0.0509	4.20	82.51	5.77	5.77
11		0.0582	4.20	72.16	5.05	5.05
3	24	0.0465	61.63	1325.38	92.77	92.77
6		0.0483	61.62	1275.78	89.30	89.30
9		0.0522	1.70	32.57	2.28	2.28
12		0.0672	6.60	98.21	6.87	6.87

Pb added to each flask = 100 ug Pb as Pb nitrate

Flask No. 1,2,3 Algae suspended in distilled water (+ 15 mg/l $NaHCO_3$)

4,5,6 Algae suspended in distilled water + Ca and Mg salts

7,8,9 Algae suspended in distilled water + EDTA

10,11,12 Algae suspended in distilled water + EDTA + Ca + Mg salts

Pb content of test algae : 0.0 ug Pb/gm dry algae.

Concentration of Algae in suspension : 280 mg/l

as occurred in absence of calcium and magnesium. The results meant that the heavy metals cadmium, copper, zinc and lead could, under the experimental conditions, replace calcium and magnesium in their complexes with EDTA.

The same tables show that metal accumulation after 24 hours contact time in absence and presence of calcium and magnesium (tests no. 1,2,3 and 4,5,6) were almost the same except lower accumulations of cadmium in presence of Ca^{+2} and Mg^{+2}.

Those results indicate that calcium and magnisium in soft waters will not hinder metal accumulation by algae. However, Soeder et al (1978) reported that reduction of lead sorption was achieved by increasing the concentration of calcium in the medium from 15 to 250 mg/l and the concentration of magnesium from 5 to 50 mg/l. Ion discrimination effect was especially evident if Ca^{+2} and Mg^{+2} were added simultaneously with the lead. Harding and Whitton (1981) also found that high levels of calcium and magnesium resulted in decreased uptake of zinc. The increase in ionic strength in general was found by Miller et al (1976) to reduce the senstivity of Selenastrum capricornatum to zinc, copper and cadmium. Nevertheless, Sailer et al (1980) found that the metal ion bonding follwed the order $Cu > Zn > Mg > Ca$.

The tables revealed also that most of the metals accumulations took place in the first two hours contact (fig. 6). This rapid interaction between the metals and algae reflects high affinity to each other and denotes to a physicochemical nature of interaction rather than a biological one. These results came coinciding with the findings of Soeder et al (1978) and Nakajima et al (1979 .

VII.1.3.3. Metals Accumulation from Different Waters

Comparison was made between metal accumulations by algae suspended in distilled water, filtered Nile water and filtered biologically degraded synthetic organic wastewater (table 21). It was presumed that the last water contained biological metabolites of chelating properties different from those of Nile water and of higher concentrations. On the other hand, Nile water contained higher concentration of calcium and magnesium.

The comparison presented in table 22 showed that metal accumulations from distilled water and filtered Nile water were almost of the same magnitude; while in the degraded synthetic wastewater the accumulation was much less except for copper which could be accumulated by the algae in close amounts. In distilled water the magnitude of accumulation was in the order Cd= Cu> Zn> Pb; in filtered Nile water the order was Cd> Cu> Zn = Pb and in the degraded wastewater the order changed to Cu> Cd> Zn> Pb.

VII.1.3.4. Effect of pH on Metal Accumulation

The pH of natural waters lies in the range 6.5 - 8.5. The effect of variation in pH within this range on metal accumulation by algae was investigated, The results (table 23) showed that there was no significant difference in lead or zinc accumulations with the change in pH; while differences in metal accumulation were detected in cases of cadmium and copper. Similarly, Hassett et al (1980) found that the concentration factors for cadmium by 11 day old Scenedesmus obliquus were slightly different in the pH range 6 - 8. They found that different algae

Table (21) Chemical Analysis of Nile Water (NW) and Biologically Treated Synthetic Sewage Effluent (S.E).

	NW	S.E	
pH	7.2	5.3	
E.C	745	215	u mho Cm^{-1}
T. alkalinity	140	3	mg/l ac CaC_3
T. Hardness	132	18	mg/l as $CaCO_3$
Ca Hardness	48	8	mg/l as $CaCO_3$
Mg Hardness	84	10	mg/l as $CaCO_3$
Cl^-	22	80	mg/l as Cl^-
SO_4^{--}	17.5	10	mg/l as SO_4^-
PO_4^{---}	0.060	0.4875	mg/l as P
NO_3^-	0.038	2.5	mg/l as N

Table (22) Heavy Metals Accumulation By Algae From Different Types of Waters.

Metal M.	Amount added	Distilled Water			Biology. Treat. Syn. Sew.			Filtered Nile Water		
		dig. alg., gm	ug M. in dig. alg.	ug M. acc. in all. alg.	dig. alg., gm	ug M. in dig. alg.	ug M. acc. in all alg.	dig. alg. gm	ug M. in dig. alg.	ug M. acc. in all alg.
Pb	44.23	0.0158	22.22	23.91	0.0121	9.00	9.37	0.0149	23.33	25.54
Cd	50.40	0.0111	37.68	57.71	0.0034	5.94	22.01	0.0160	39.31	40.54
Zn	44.10	0.0111	22.46 (2.85)	30.03	0.0094	13.27 (2.41)	14.56	0.0139	27.63 (3.57)	25.56
Cu	41.20	0.0120	27.50	38.96	0.0053	12.500	29.72	0.0140	27.50	32.41
contact algae in 250 ml, gm			0.0170			0.0126			0.0165	

Algae content of Zn = 256.69 ug/gm dry algae

In brackets are the initial content of Zn in digested algae.

Table (23) Effect of pH on Heavy Metals Accumulation By Algae

Metal M.	Amount added ug/l	Final pH	ug M. accum. in test algae	Accumlation, ug/gm dry algae
Cd	201.0	6.45	89.25	823.36
		7.90	117.14	1080.62
Cu	178.0	6.30	169.21	1561.03
		7.80	147.54	1361.07
Pb	176.9	6.4	131.46	1212.70
		8.0	136.33	1257.65
Zn	176.4	6.9	142.00	1309.98
		7.8	142.55	1315.07

Concentration of algae: 108.4 mg/l

Initial content of Zn : 256.69 ug/gm dry algae.

respond differently to the effects of pH and they suggested that their data reflect differences in the composition of the cell coverings of the various algae employed in the study.

VII.1.3.5. Accumulation of Cadmium, Copper, Zinc and Lead in Combination in the Test Algae

The test algae suspended in distilled water (+ 100 mg/l $NaHCO_3$) could accumulate the four metals in the same time. However, the highest percentage of accumulation was that of zinc and the lowest was that of lead (table 24). This preferential adsorption of heavy metals by the test alga might differ with respect to other algae as has been shown by Hassett et al (1980). Table 24 shows also that with the increase in metals: algae exposure ratio the total metals accumulated increased but the amount of one metal accumulated per cent added of that metal decreased. This result is different from that obtained on unimetal/ algae experiments indicating an antagonistic effect when in combination. Table 25 revealed that, on a molar basis, the amount of one metal accumulated per cent total metal accumulated was substantially the same at the different initial concentrations added. The affinity of metals to the algal surface followed the order $Zn > Cu = Cd > Pb$ which did not change with change in initial metal added.

VII.1.3.6. Effect of Anionic Radicals on Metals Accumulation

To algal suspensions in distilled water sodium salts of different anionic radicals were added in such a high molar concentration that the added radical must

Table (24) Accumulation of Cu, Cd, Zn and Pb by <u>Scenedesmus</u>

Amount of metal added	Amount of metal accumulated, ug				Metal accumulated per cent added metal.			
	Cu	Cd	Zn	Pb	Cu	Cd	Zn	Pb
$1x10^{-6}M$	17.1	39.9	32.6	42.6	26.9	36.5	49.9	20.6
	23.5	39.4	38.8	74.9	36.9	35.0	59.4	23.1
	23.6	39.3	39.9	46.6	37.1	36.0	61.1	22.5
Average	21.4	39.5	37.1	45.7	33.6	35.1	56.8	22.1
$2x10^{-6}M$	37.8	62.6	54.4	67.3	29.7	27.8	41.6	16.2
	35.7	68.8	55.0	64.8	28.1	30.6	42.1	15.6
	34.4	65.5	53.1	63.2	27.0	29.1	40.7	15.3
Average	36.0	65.6	54.2	65.1	28.3	29.2	41.5	15.7

PH of algal suspension = 6.9

Concentration of algae = 0.636 gm/l

Contact time = 2 hours

* Zn content of test alga (250 ug/gm) was substracted.

$1x10^{-6}M$ metal : 63.6 Cu, 65.3 Zn, 112.4 Cd and 207.2 Pb ug/l.

$2x10^{-6}M$ metal : 127.2 Cu, 130.6 Zn, 224.8 Cd and 414.4 Pb ug/l

Table (25) Metal Accumulated Percent Total Accumulated Metals

Metal M	Metal added, ug	Average metal accum., ug	Metal accumulated percent metal added (average)	Metal accumulated* percent total metal accumulated
Cu	63.6	21.4	33.6	23.0
	127.2	36.0	28.3	24.9
Cd	112.4	39.5	35.1	23.6
	224.8	65.6	29.2	25.3
Zn**	65.3	37.1	56.8	38.5
	130.6	54.2	41.5	36.2
Pb	207.2	45.7	22.1	14.9
	414.4	65.1	15.7	13.5

* On molar basis

pH of algal suspension = 6.9

Concentration of algae : .0636 gm/l

Contact time = 2 hours

** Zn content of the test algae (250 ug/gm) was substracted have dominated. According to the acidity of the added radical the OH ion concentration was given in solution. Further, the addition of metal solutions to the suspension must have altered the OH ion concentration giving rise to different pH values. This was indicated by the final pH values mentioned in table 26. Therefore the metal species formed in these solutions must have been determined by the combined effect of the anionic radical added and the OH created in solution. Table 26 shows different anion effects on metal accumulation by algae according to the metal added. The greatest differences were exhibited in the case of lead and the least were detected with copper. In natural waters, however, where the system is usually a complex one containing several anions and cations it would not be easy to predict which metal species could be formed. However, non of the anions seemed to have drastic inhibitory effect on metal accumulation. This is against what has been reported by Wong et al (1980) who noticed that the presence of NaCl suppressed cadmium uptake by Chlorella salina. Slowik et al (1977) found that copper accumulation rate in presence of $Cu\ Cl_2$ was higher than that with $Cu\ SO_4$ while there was not much difference in lead accumulation in the presence of the chloride and nitrate forms of lead. It has been reported (cited by Wong et al, 1980) that when cadmium was tested in the form of nitrate, carbonate, acetate and chloride the element was slightly more toxic as nitrate and acetate than as chloride or carbonate.

VII.1.3.7. Metals Accumulation by Suspended Matter in Nile Water.

Seston in natural water is composed of particulates of inorganic and organic nature. Organic particulates may be living or non living, of plant or

Table (26) Metal Accumulation by Algae under Different Anionic Conditions

Metal added, 4.0 uM	Exposed algae concentration gm/l	Anion radical present, 4.0. mM	pH final	Metal accumulated mg/gm
Cadmium,	0.067	$NaHCO_3$	8.9	2.149
		Na Cl	6.7	1.480
		Na_2SO_4	7.1	1.552
		NaH_2PO_4	8.6	1.433
Copper	0.042	$NaHCO_3$	8.9	2.000
		Na Cl	6.3	2.171
		Na_2SO_4	7.1	2.362
		NaH_2PO_4	8.6	2.171
Lead	0.051	$NaHCO_3$	9.0	9.6268
		Na Cl	6.8	13.2656
		Na_2SO_4	7.0	15.0156
		NaH_2PO_4	8.6	11.6016

animal origin, the so called debris. Algae in some cases constitute one fraction of the total suspended matter. Due to their low specific density compared to that of inorganic particulates, algae usually constitute a small fraction of the total seston on dry weight basis though they may provide large surface areas for metal accumulation. The aim behind this experiment was to assess the contribution of algae in metal accumulation by Nile water seston. The concentration of chlorophyll (a) in the seston investigated was 146.2 ug/l corresponding arbitrarily to 7.31 mg/l dry (105 ^{o}C) algae. Assuming that all types of algae in the seston had similar accumulation capacity as the test alga, Scenedesmus sp, and assuming that all algae were in the logarithmic growth phase, then according to table 12 the amount of algae in the seston - 7.31 mg/l - must have accumulated 16.3 ug Pb, 36.5 ug Cu, 35.6 ug Zn or 25.4 ug Cd. Comparing these amounts with the amounts accumulated by the whole seston, being 297.99 ug Cu, 349.92 ug Cd, 367.35 ug Pb or 297.33 ug Zn (table 27) indicated that living algae in Nile water had a small contribution in metal accumulation compared to other particulates suspended in the water. In this respect Babich and Stotzky (1977) reported that the presence of clay minerals decreased the toxic effects of cadmium on bacteria and fungi.

VII.1.4. Metal Accumulation by Algae grown in a Semi-continuous Flow System

In the foregoing experiments it was necessary to maintain a constant concentration of algae during the contant time (2-24 hours). This has been thought to be fulfilled by keeping algal activities at a minimum. The results obtained under these conditions can be useful in

Table (27) Metal Accumulation By Nile Water Seston After 2 Hours Exposure

Metal M.	M. Content of of seston ug/gm	Exposure concentration ug/l	M. content of seston/l after exposure, ug	M. accum. by seston/l,ug	ug M. accum. per gm seston
Cu	49.54	357.14	347.17	297.99	300.15
Cd	0.00	360.00	349.92	349.92	352.46
Pb	51.07	315.93	418.05	367.35	370.01
Zn	193.31	315.00	489.25	297.33	299.49

M.= Metal

Concentration of seston: .9928 gm/l, on **dry** Wt. (105°C) basis

Chlorophyll 'a' concentration: 146.2 ug/l

% ash (550°C): 94%

pH: 7.8

Total alkalinity of Nile water (as $CaCO_3$) : 142 mg/l

Total Hardness of Nile water (as $CaCO_3$) : 136 mg/l comparative studies but these conditions do not simulate the natural water systems where due to living organisms activities different metabolites are released which possess different chelating powers (Baccini and Suter, 1977; Van Den Berg and Kramer, 1979 and Van Den Berg et al, 1979). There is adequate evidence that a variety of different organic substances including carbohydrates, fatty acids, amino acids, polypeptides, growth substances, vitamines and enzymes, is liberated into the media from healthy algal cells in laboratory cultures. The presence in natural waters of appreciable concentrations of many of these substances has been established. Interactions between metals that reach a water and these substances could be a determinental factor in metal accumulation by algae. The forthcoming discussion deals with metal accumulation by growing algae under laboratory conditions which must be also different from field conditions in many aspects. The results will be compared with the results obtained on metal accumulation by nongrowing algae.

VII.1.4.1. Comparison Between Metals Accumulations by Scenedesmus Grown in Semi-Continuous Flow System.

Under the same growth conditions namely, nutrients content in the medium, illumination and temperature, the results indicated that the growth of the test alga Scenedesmus sp. was retarded most by cadmium (tables 28 - 30).

Comparing the steady state algae biomass under the same conditions of dilution rate and metal concentration (table 31) resulted in the following order of growth retarding effects of metals on the test alga.

Table (28) Metal Accumulation By Algae Grown In A Semicontinuous Flow System OF 0.25 Liter Per Day.

Day	Cd				Pb			
	Conc. M. ug/l	Conc. algae mg/l	M. accum. in algae/l ug/l	pH	Conc. M ug/l	Conc. algae mg/l	M. accum. in algae/l ug/l	pH
		0.093		7.5		0.095		7.5
		0.095		7.7		0.103		7.8
		0.109		7.2		0.095		7.2
1	100.0	0.095	31.3	7.6	100	0.103	21.9	7.6
2	175.0	0.078	98.3	7.7	175	0.100	186.7	7.7
3	231.3	0.075	-	7.7	231.3	0.105	156.1	7.7
4	273.4	0.089	185.4	7.4	273.4	0.104	206.7	7.5
5	305.0	0.075	127.1	7.4	305.0	0.082	209.6	7.4
6	328.7	0.066	-	7.4	328.7	0.080	-	7.5
7	346.5	0.098	196.6	7.5	346.5	0.122	195.6	7.7
8	359.8	0.040	212.6	7.5	359.8	0.086	254.2	7.6
9	370.0	0.029	154.7	7.6	370.0	0.074	244.3	7.9
10	377.5	0.017	162.4	7.5	377.5	0.073	249.8	7.9
11	383.0	0.023	-	7.5	383.0	0.090	197.7	7.8
12	387.0	0.024	182.6	7.5	387.0	0.085	219.1	7.9
13	390.0	0.028	159.9	7.4	390.0	0.091	189.4	7.7
14	392.5	0.011	172.4	7.3	392.0	0.075	178.0	7.9
15	394.4	0.011	168.2	7.2	394.4	0.077	205.2	7.7

M = metal

Table (28) Continued

Day	Cu				Zn			
	Conc. M ug/l	Conc. algae mg/l	M. accum. in algae/l ug/l	pH	Conc. M ug/l	Conc. algae mg/l	M. accum. in algae/l ug/l	pH
		0.117		7.4		0.087	22.3	7.4
		0.107		7.5		0.096	24.6	7.7
		0.092		7.6		0.088	22.6	7.7
1	50.0	0.121	47.7	7.5	100.0	0.111	75.1	7.4
2	87.5	0.109	68.9	7.7	175.0	0.110	148.7	7.9
3	115.6	0.102	55.1	7.7	231.3	0.093	139.2	7.9
4	136.7	0.091	79.5	7.4	273.4	0.098	200.8	7.5
5	152.5	0.072	93.7	7.3	305.0	0.085	199.7	7.4
6	164.4	-	-	-	328.7	0.064	168.8	7.7
7	173.3	0.095	114,6	7.6	346.5	0.064	168.8	7.7
8	180.0	0.064	114.3	7.6	359.8	-	-	-
9	185.0	0.055	88.8	7.7	370.0	0.059	201.1	7.8
10	188.7	0.056	72.1	7.8	377.5	0.046	182.5	7.9
11	191.5	0.083	71.9	7.7	383.0	0.062	168.3	7.7
12	193.6	0.100	81.1	7.8	387.0	0.063	180.2	8.0
13	195.2	0.104	84.1	7.6	390.0	-	-	-
14	196.4	0.098	90.4	7.8	392.0	0.038	163.5	8.4
15	197.3	0.111	95.2	7.4	394.4	0.038	172.4	8.5

M = metal

Table (29) Metals Accumulation By Algae Grown In A Semicontinuous Flow System of 0.50 Liter Per Day.

Day	Cd				Pb			
	Conc. M, ug/l	Conc. algae gm/l	M. accum. in algae/l ug/l	pH	Conc. M, ug/l	Conc. algae gm/l	M. accum. in algae/l ug/l	pH
		0.071		7.5		0.082		9.8
		0.077		8.0		0.087		9.2
		0.067		7.9		0.076		8.5
1	100	0.051	55.1	7.5	100	0.081	53.6	8.2
2	150	0.059	51.2	7.2	150	0.096	62.2	9.0
3	175	0.029	111.5	7.3	175	0.067		9.0
4	187.5				187.5			
5	193.7	0.037	121.5	7.1	193.7	0.084	75.5	8.9
6	196.9	0.0415	79.3	7.7	196.9	0.098	90.4	8.3
7	198.4	0.032	87.7	7.3	198.4	0.045	117.4	7.8
8	199.2	0.030	65.7	7.2	199.2	0.055	67.6	9.0
9	199.6	0.036	63.8	7.5	199.6	0.074		8.5
10	200	0.023	68.3	7.1	200.	0.063	88.2	7.9
11	200				200.			
12	200				200			
13	200	0.021	52.6	7.1	200	0.069	95.6	8.6
14	200	0.025	-	7.1	200	0.066	81.3	9.1
15	200	0.025	51.1	7.1	200	0.065	95.4	9.0

M = Metal

Table (29) Continued

Day	Cu				Zn			
	Conc. M ug/l	Conc. algae gm/l	M accum. in algae/l ug/l	pH	Conc. M ug/l	Conc. algae gm/l	M accum. in algae/l ug/l	pH
		0.075		8.0		0.0835		7.6
		0.082		8.8		0.0840		8.0
		0.078		9.0		0.0820		7.8
1	50	0.064	64.3	8.3	100	0.0710	98.0	7.8
2	75	0.099	66.0	8.0	150	0.0920	115.0	8.0
3	87.5	0.066	88.3	8.4	175	0.0725	80.2	8.4
4	93.7				187.5			
5	96.9	0.090	98.5	7.8	193.7	0.0855	110.4	7.5
6	98.4				196.9	0.0595	109.2	7.5
7	99.2	0.060	89.6	7.5	198.4	0.0550	114.3	7.3
8	99.6	0.067	82.9	7.7	199.2	0.0540	112.3	7.7
9	99.8	0.074	86.2	7.2	199.6	0.0795	118.3	7.3
10	99.9	0.054	92.9	7.1	200	0.0660	141.0	7.4
11	100							
12	100							
13	100	0.058	79.9	7.2	200	0.0705	153.6	7.5
14	100	0.0650	93.0	7.2	200	0.0805	137.6	7.7
15	100	0.064	71.2	7.8	200	0.0780	132.6	8.4

Table (30) Metals Accumulation By Algae Grown In a Semicontinuous Flow System of 0.75 liter Per Day.

Day	Cd				Pb			
	Conc. M, ug/l	Conc. algae gm/l	M. accum. in algae/l ug/l	pH	Conc. M, ug/l	Conc. algae gm/l	M. accum. in algae/l ug/l	pH
		0.065		7.5		0.060		7.8
		0.065		7.1		0.061		7.3
		0.062		7.5		0.058		8.2
1	150.0	0.046	51,7	7.3	150.0	0.074	110.1	7.2
2	187.5	0.010	22.7	7.0	187.5	0.034	124.2	7.1
3	196.9	0.006		7.0	196.9	0.029		7.4
4	199.2	0.008	12.4	7.0	199.2	0.037	80.6	7.3
5	199.8	0.009	11.2	7.0	199.8	0.042	85.6	7.3
6	199.9	0.007	10.7	7.0	199.9	0.044	110.8	7.1
7	200	0.010	14.2	7.0	200	0.037	110.0	7.2
8	200	0.009	11.4	7.0	200	0.036	66.3	7.3
9	200	0.011	17.4	7.0	200	0.044	39.0	7.3

M = Metal

Table (30) Continued

Day	Cu				Zn*		
	Conc. M, ug/l	Conc. algae gm/l	M. accum. in algae, ug/l	pH	Conc. of M., ug/l	Conc. of algae, gm/l	pH
		0.060		8.0		0.0680	7.8
		0.063		7.6		0.0565	7.3
		0.056		8.3		0.0800	8.0
1	150	0.049	37.8	7.2	150	0.0910	7.1
2	187.5	0.022	40.2	7.1	187.5	0.0510	7.2
3	196.9	0.010		7.0	196.9	0.0450	7.4
4	199.2	0.012	43.2	7.2	199.2	0.0565	7.2
5	199.8	0.009	33.2	7.2	199.8	0.0565	7.4
6	199.9	0.010	37.5	7.1	199.9	0.0590	7.3
7	200	0.009	41.2	7.0	200	0.0520	7.5
8	200	0.009	40.8	7.0	200	0.0525	7.9
9	200	0.012	36.8	7.0	200	0.0525	8.7

* Zn accumulated in algae not derermined.

Dilution Rate		Metal Concentration		Order of Effect
0.25	day^{-1}	400	ug/l	Cd>Zn>Pb
0.50	day^{-1}	200	ug/l	Cd>Pb>Zn
0.75	day^{-1}	200	ug/l	Cd>Cu>Zn>Pb

The same table (31) revealed that at the same metal: algae exposure ratio the unit weight of the washed non-growing algae accumulated higher amounts of metals than did the growing algae in the steady state growth conditions, indicating that the cultures in the latter cases contained substances that retarded metal accumulation.

Fig. 7 for Copper and Fig 12 for Zinc showed that the effects of these two metals seemed to be overcomed and algae could reattain their growth activities after a lag of time. This phenomenon was also reported by Miller et al, 1976.

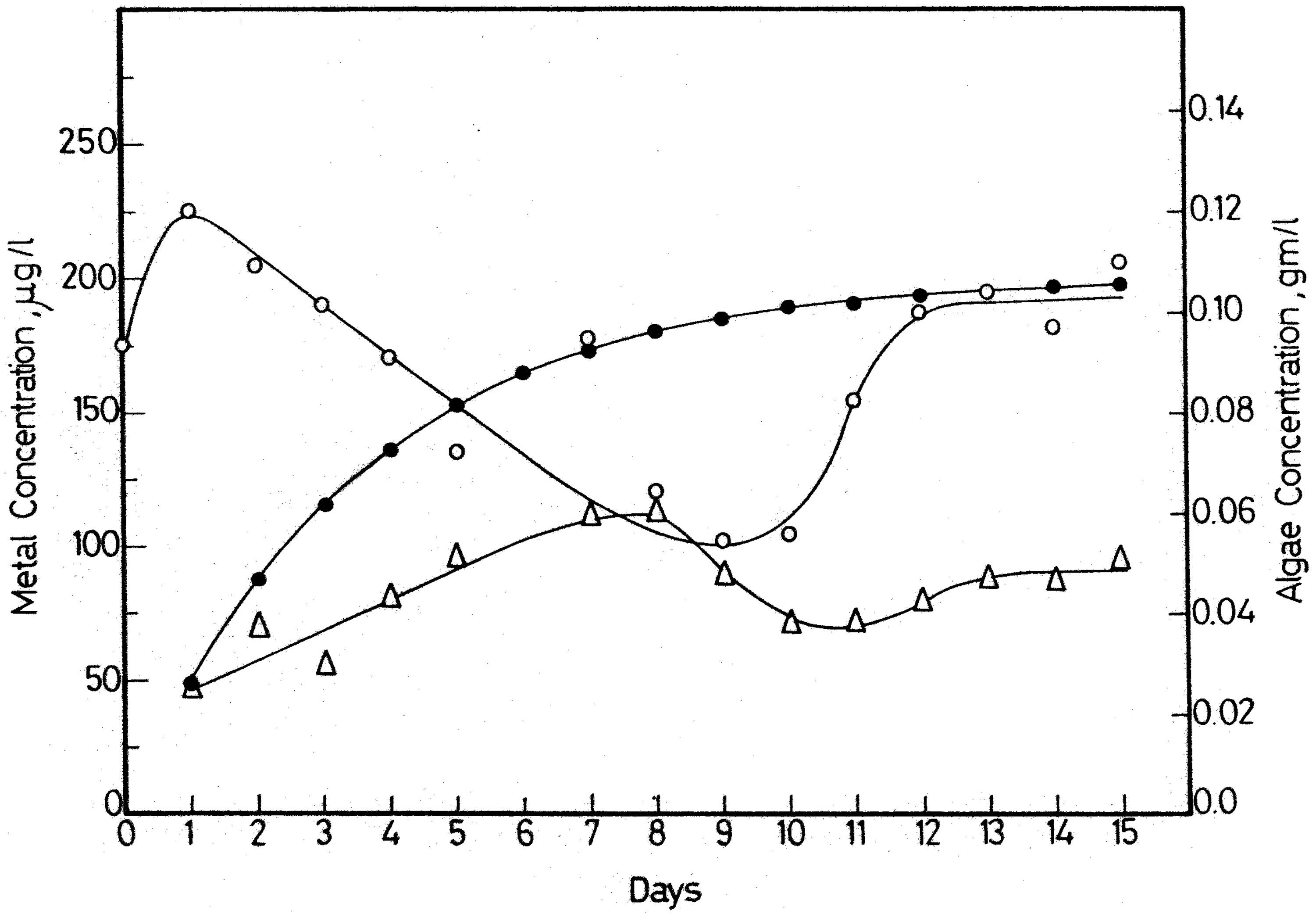
It might be deduced from Fig. 15-18 that inhibition of Scenedesmus growth under the experimental conditions took place at the metal: algae ratios (mg metal / gm algae): 2.67, 1.60, 1.10 and 1.07 for lead, zinc, copper and cadmium respectively.

It should be pointed out to the morphological changes in Scenedesmus cells that occurred as a result of adding metals to the growth medium. While no changes were detected with Pb and Zn changes occurred with cadmium and copper (Fig. 19-22). With cadmium the four cells of the coenobium got rearranged in a centric manner with an intrusion in each cell like a collapse. With copper the cells took a round shape and tended to

Table (31) Steady State **Biomass** and Metal Accumulation in the Algae at Different Metal Concentrations and Different Dilution Rates.

Metal	Metal Concentration in Reactor mg/l	Dilution Rate day^{-1}	Biomass gm/l	Exposure Ratio mg/gm	Metal in Algae/l mg/l	Metal Accumulation mg/gm in growing algae	Metal Accumulation mg/gm in nongrowing algae
Pb	0.400	0.25	0.0813	4.92	0.215	2.64	4.70
Zn	0.400	0.25	0.0500	8.00	0.180	3.60	7.20
Cu	0.200	0.25	0.1020	1.96	0.080	0.78	1.70
Cd	0.400	0.25	0.0187	21.4	0.165	8.82	18.5
Cu	0.100	0.50	0.0653	1.53	0.086	1.32	1.30
Zn	0.200	0.50	0.0771	2.59	0.140	1.80	2.10
Pb	0.200	0.50	0.0667	3.00	0.087	1.30	2.70
Cd	0.200	0.50	0.0240	8.30	0.050	2.10	7.20
Cu	0.200	0.75	0.0106	18.87	0.037	3.5	17.15
Zn	0.200	0.75	0.0520	3.85	-	-	3.15
Cd	0.200	0.75	0.0090	22.20	0.012	1.39	19.20
Pb	0.200	0.75	0.0453	4.40	0.087	1.92	4.10

* From Figure No. 5



Steady State
Exposure Ratio : 1.96 mg/gm

Metal Accumulation per gram
Algae : 0.78 mg/gm

Fig. (7) Changes in Algae Concentration o——o, Copper Concentration in the Reactor ●——● and Accumulated Copper in Algae Δ—Δ at the Flow Rate 0.25 l/Day.

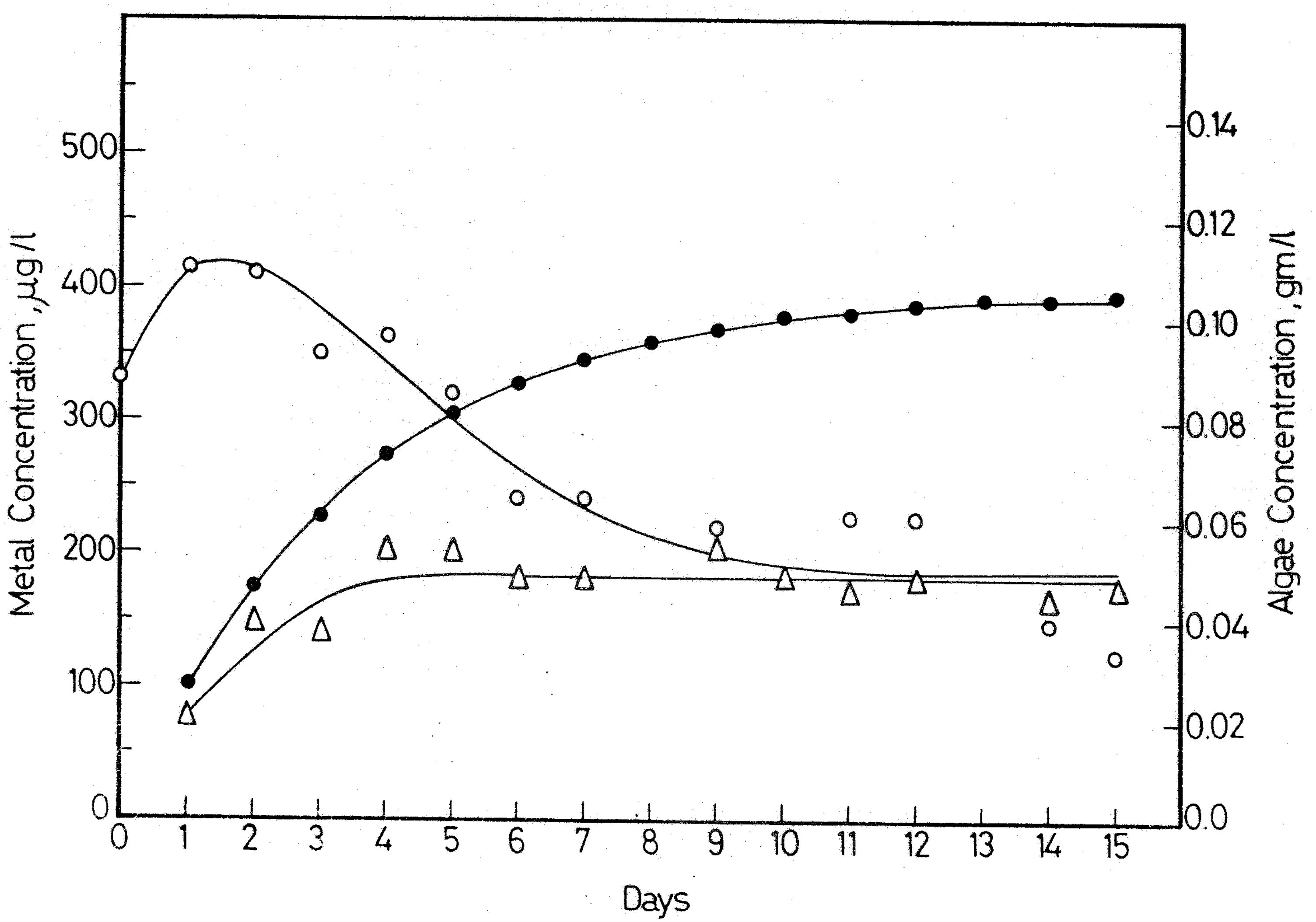


Fig.(8) Changes in Algae Concentration o——o; Zinc Concentration in the Reactor ●——● and Accumulated Zinc in Algae Δ——Δ at the Flow Rate 0.25 l/Day.

Steady State
Exposure Ratio : 8.0 mg/gm

Metal Accumulation per gram
Algae : 3.6 mg/gm

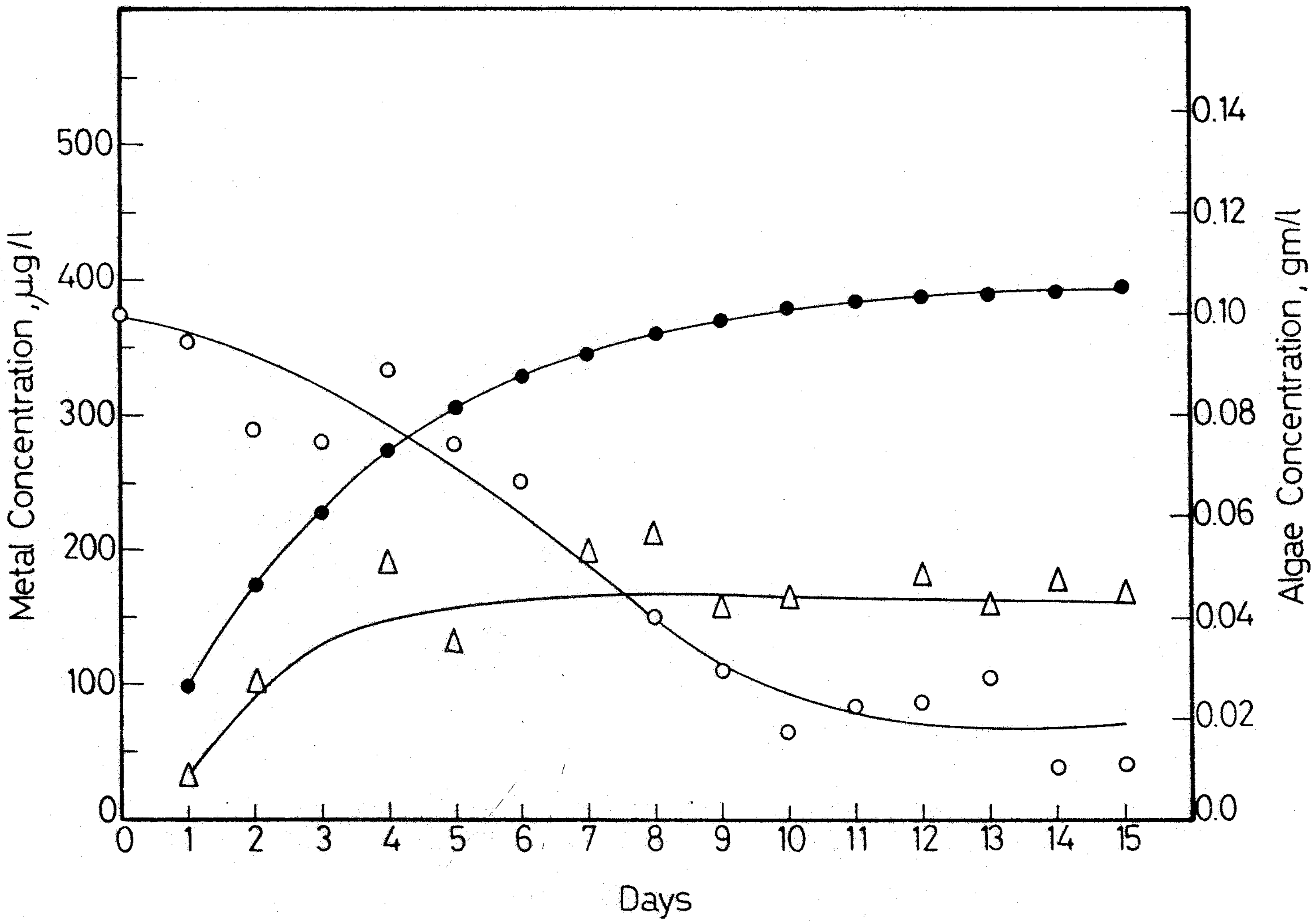


Fig. (9) Changes in Algae Concentration ○——○ ; Cadmium Concentration in the Reactor ●——● and Accumulated Cadmium in Algae △—△ at the Flow Rate 0.25 l/Day.

Steady State
Exposure Ratio : 21.4 mg/gm

Metal Accumulaion per gram
Algae : 8.82 mg/gm

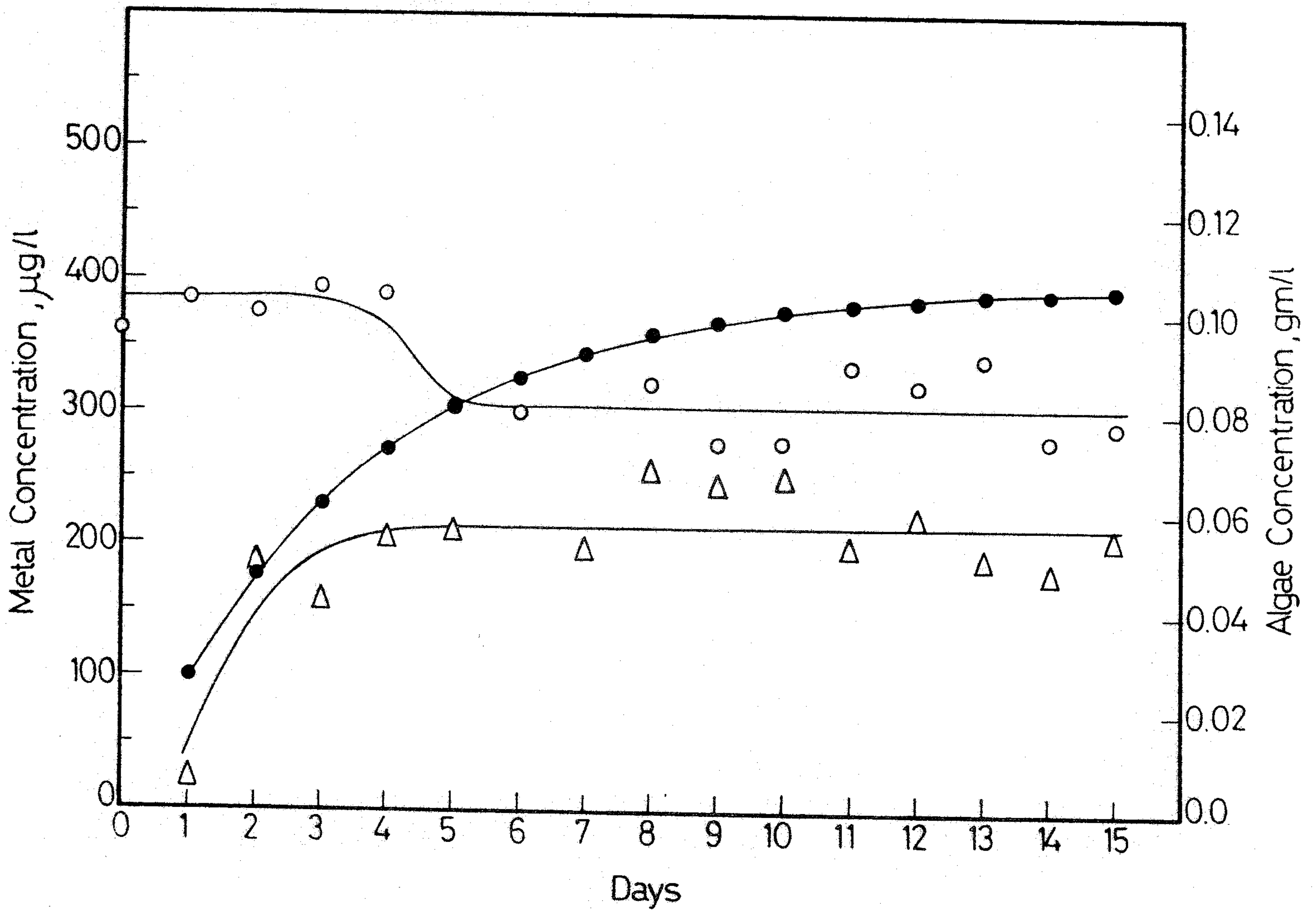
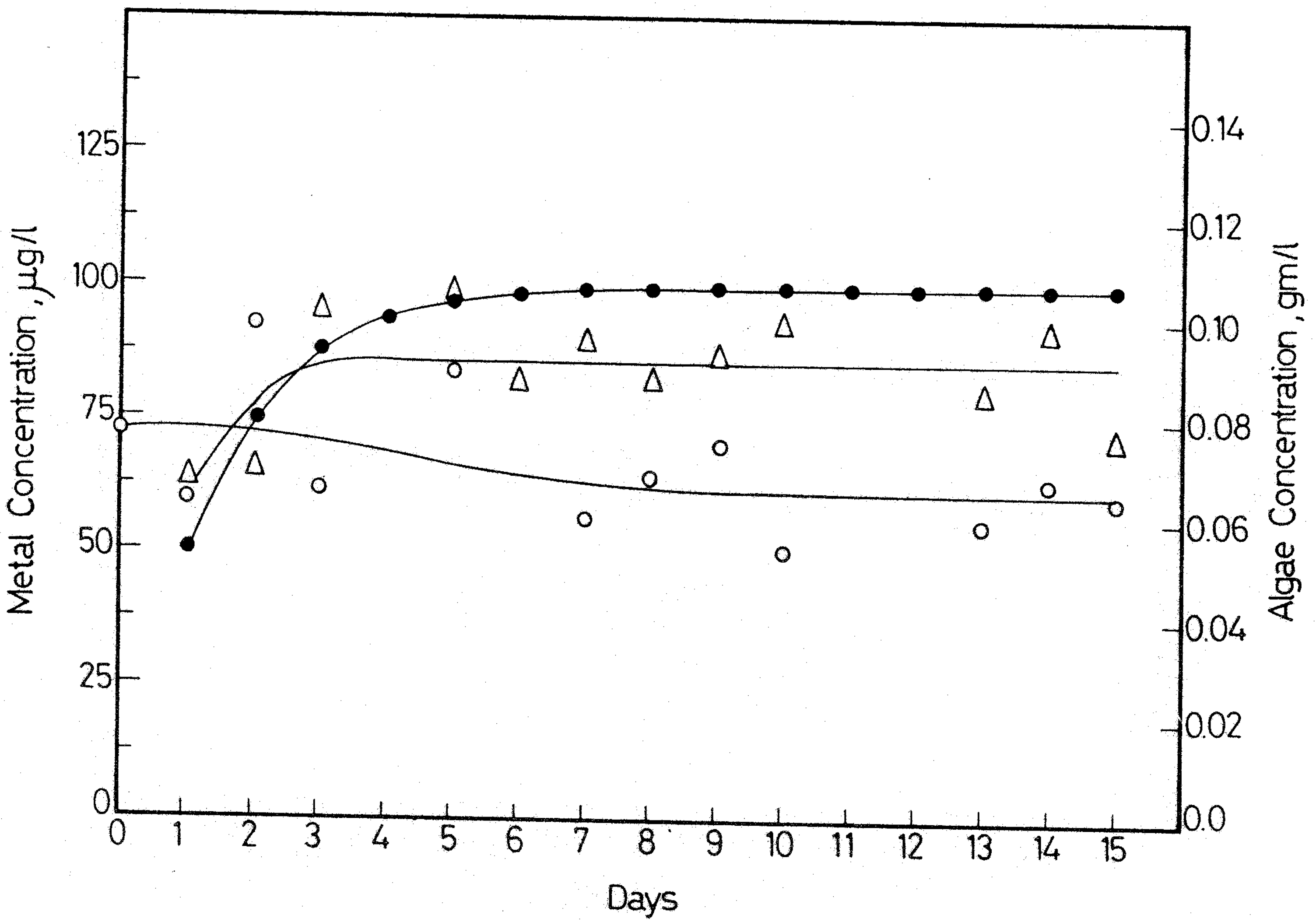


Fig. (10) Changes in Algae Concentration o——o ; Lead Concentration in the Reactor ●——● and Accumulated Lead in Algae Δ——Δ at the Flow Rate 0.25 l/Day .

Steady State
Exposure Ratio : 4.92 mg/gm

Metal Accumulation per gram
Algae : 2.64 mg



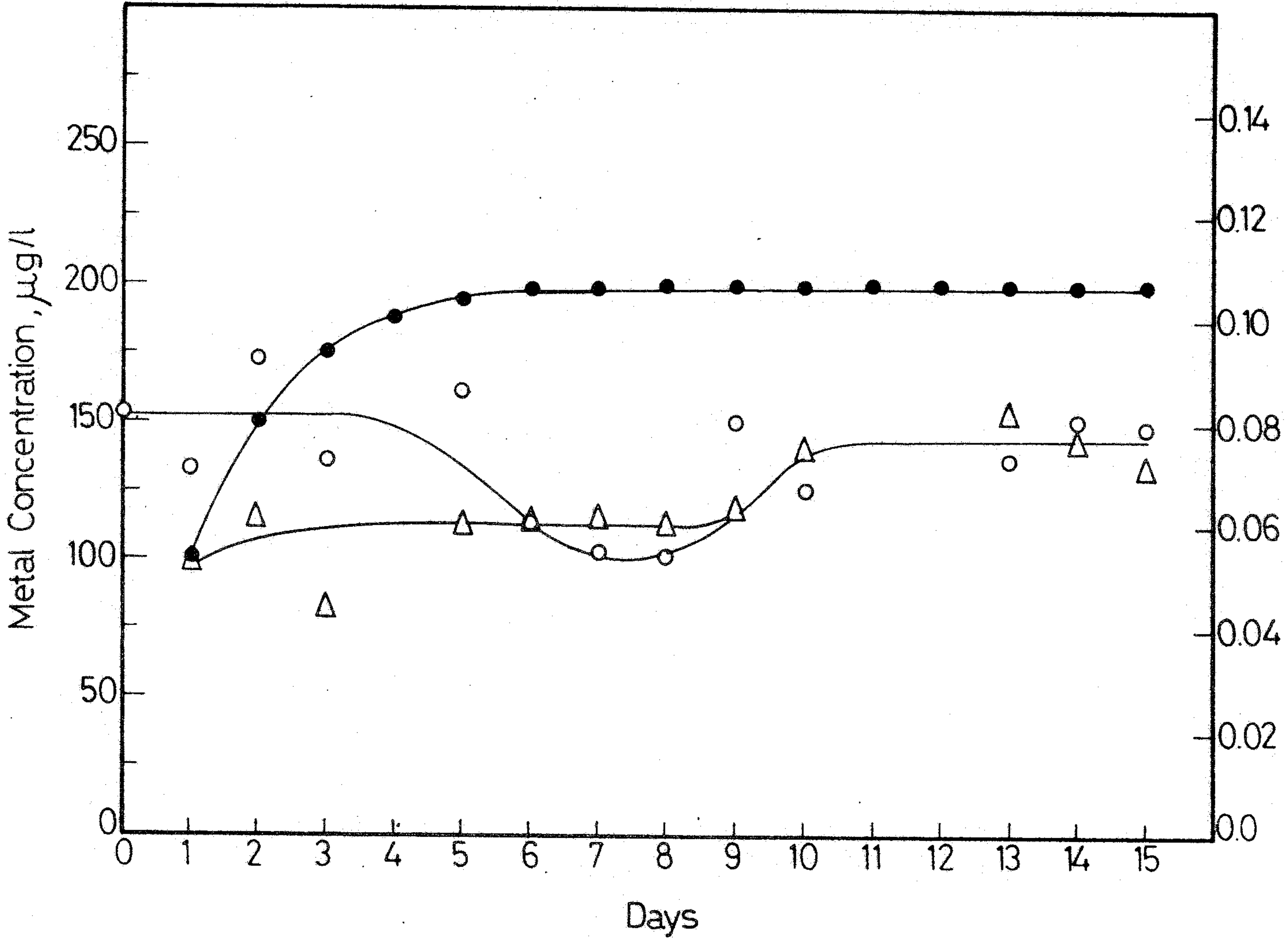
Steady State

Exposure Ratio : 1.53 mg/gm

Metal Accumulation per gram

Algae : 1.32 mg/gm

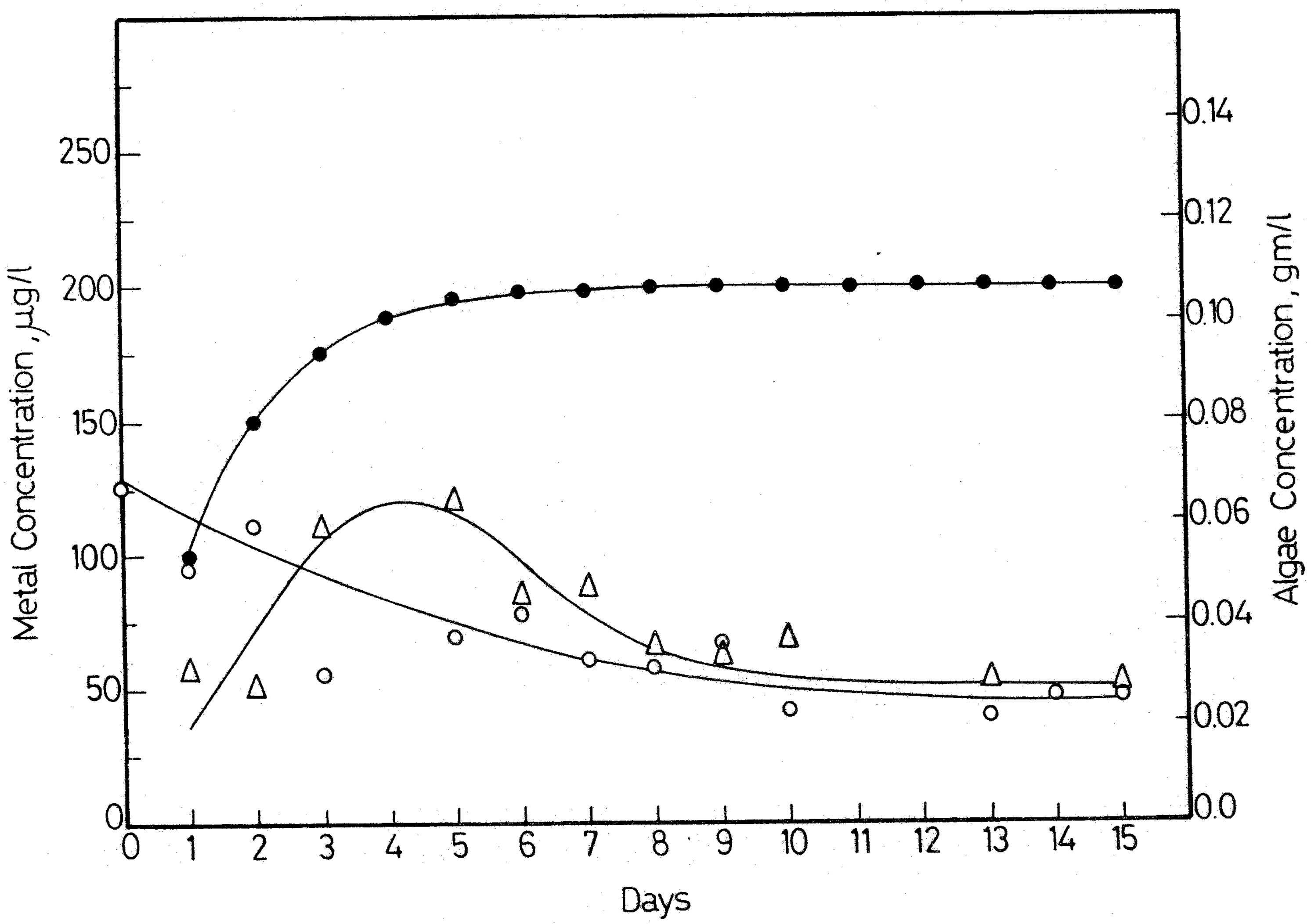
Fig.(11) Changes in Algae Concentration o——o ; Copper Concentration in the Reactor ●——● and Determined Accumulated Copper in Algae Δ——Δ at the Flow Rate 0.50 l/Day.



Steady State
Exposur Ratio : 2.59 mg/gm

Metal Accumulation per gram
Algae : 1.8 mg/gm

Fig. (12) Changes in Algae Concentration o——o ; Zinc Concentration in the Reactor ●——● and Determined Accumulated Zinc in Algae Δ——Δ at the Flow Rate 0.50 l/Day.



Steady State
Exposure Ratio : 8.3 mg/gm

Metal Accumulation per gram
Algae : 2.1 mg

Fig. (13) Changes in Algae Concentration o——o ; Cadmium Concentration in the Reactor ●——● and Determined Accumulated Cadmium in Algae Δ——Δ at the Flow Rate 0.50 l/Day.

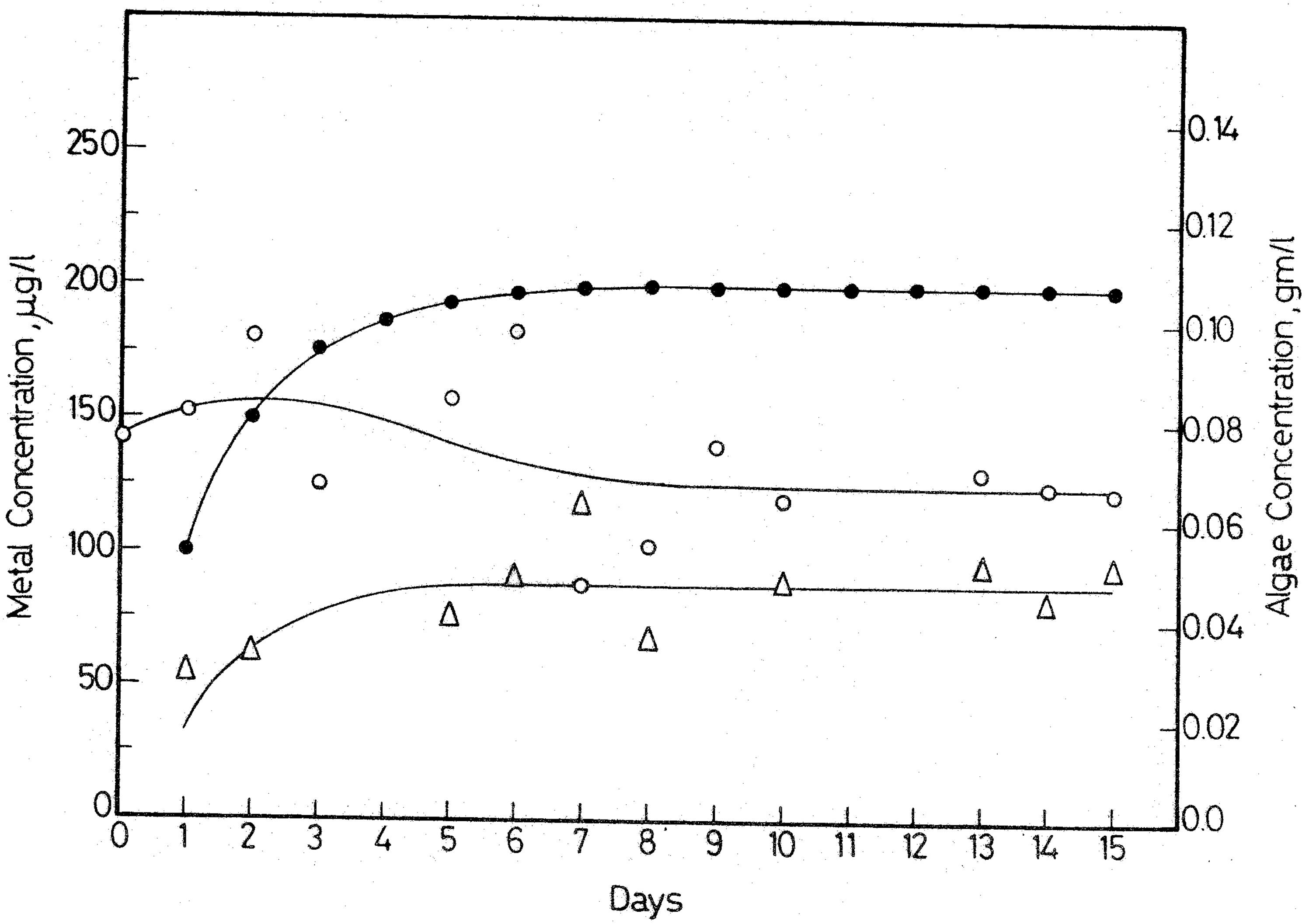


Fig.(14) Changes in Algae Concentration o——o; Lead Concentration in the Reactor ●——● and Determined Accumulated Lead in Algae Δ——Δ at the Flow Rate 0.50 l/Day.

Steady State
Exposure Ratio : 3.0 mg/gm

Metal Accumulation per gram
Algae : 1.3 mg

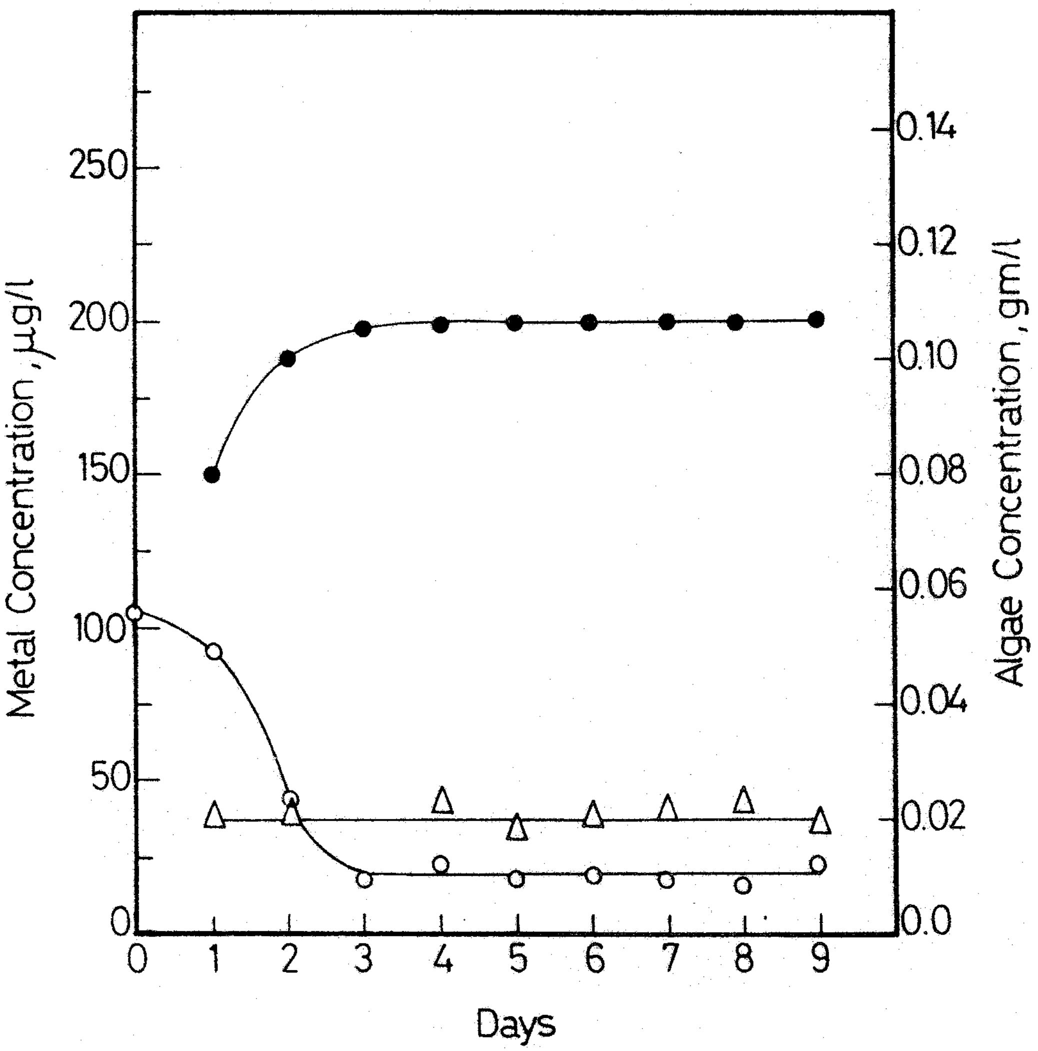


Fig. (15) Changes in Algae Concentration o—o ;
Copper Concentration in the Reactor ●—●
and Determined Accumulated Copper
in Algae Δ—Δ at the Flow Rate 0.75 l/Days.

Steady State
Exposure Ratio : 18.87 mg/gm
Metal Accumulation per gram
Algae : 3.5 mg

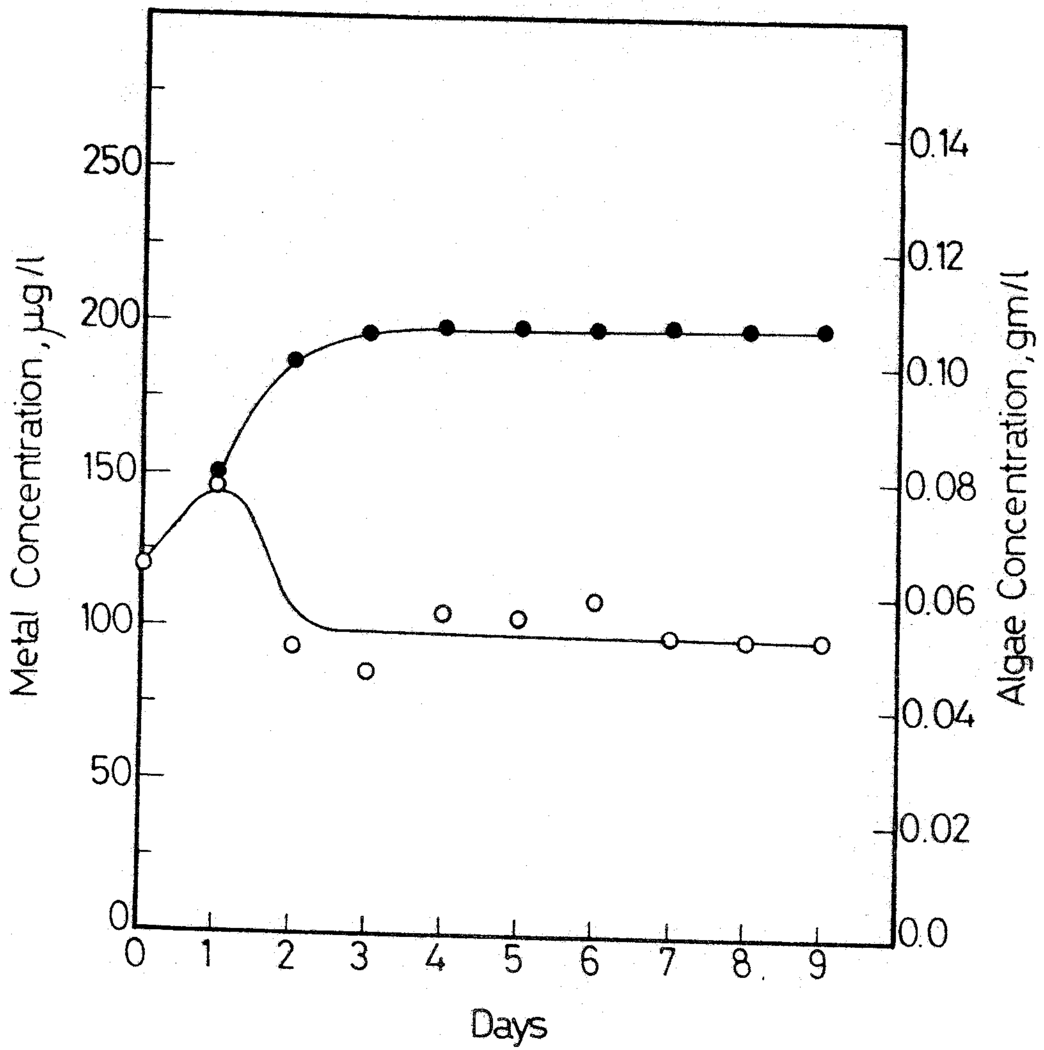


Fig. (16) Changes in Algae Concentration ○——○ ; Zinc Concentration in the Reactor ●——● and Determined Accumulated Zinc in Algae Δ——Δ at the Flow Rate 0.75 l/Day.

Steady State

Exposure Ratio : 3.85 mg/gm

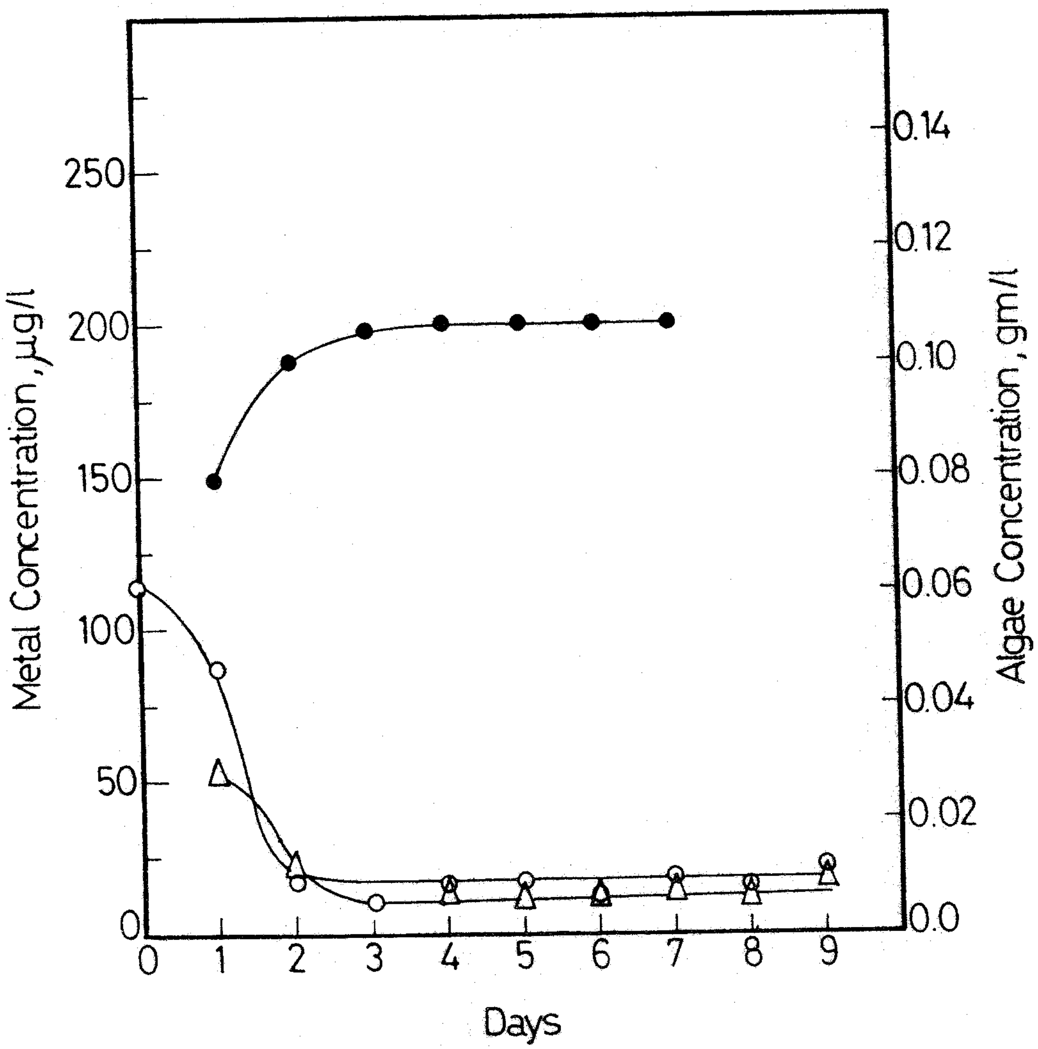


Fig. (17) Changes in Algae Concentration ○——○ ; Cadmium Concentration in the Reactor ●——● and Determined Accumulated Cadmium in Algae Δ——Δ at the Flow Rate 0.75 l/Day.

Steady State :
Exposure Ratio : 22.2 mg/gm
Metal Accumulation per gram
Algae : 1.39 mg

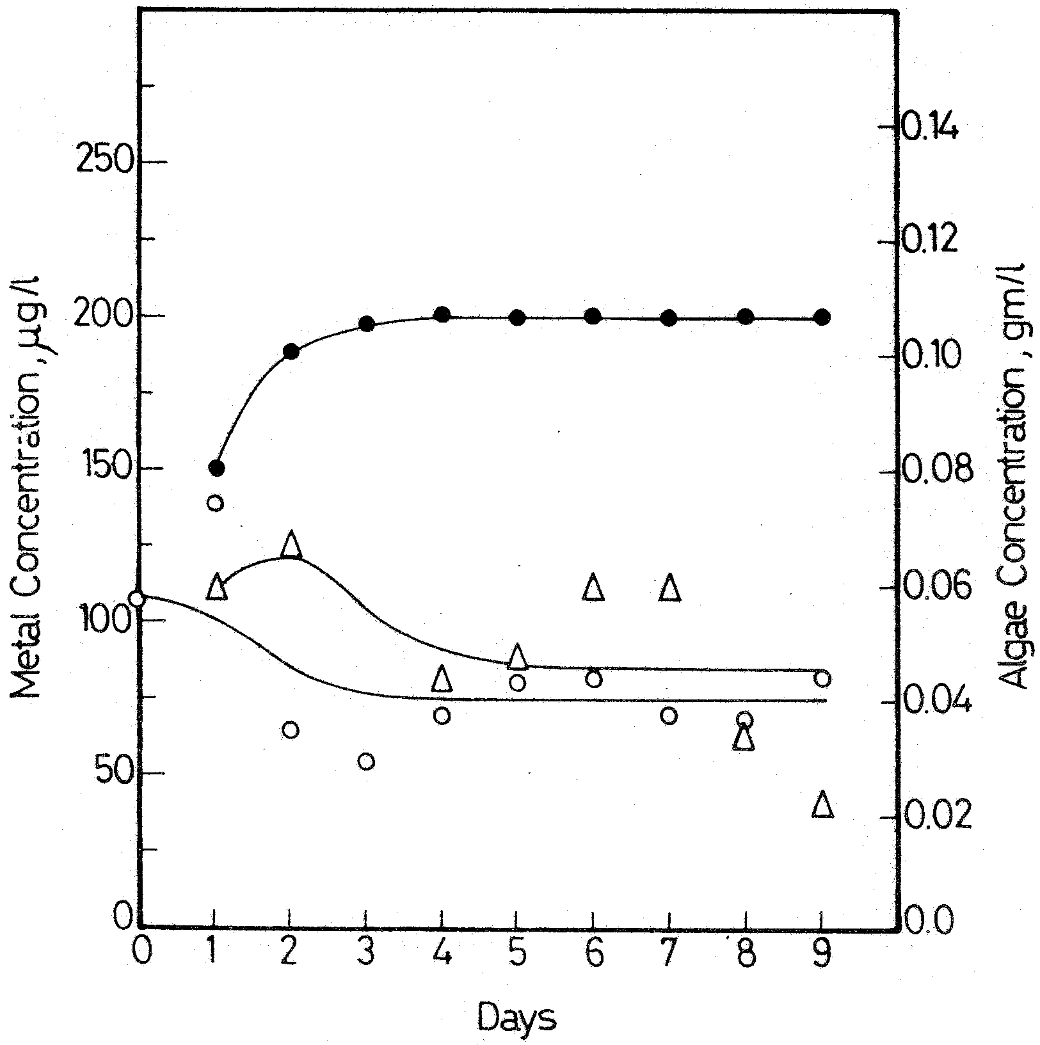
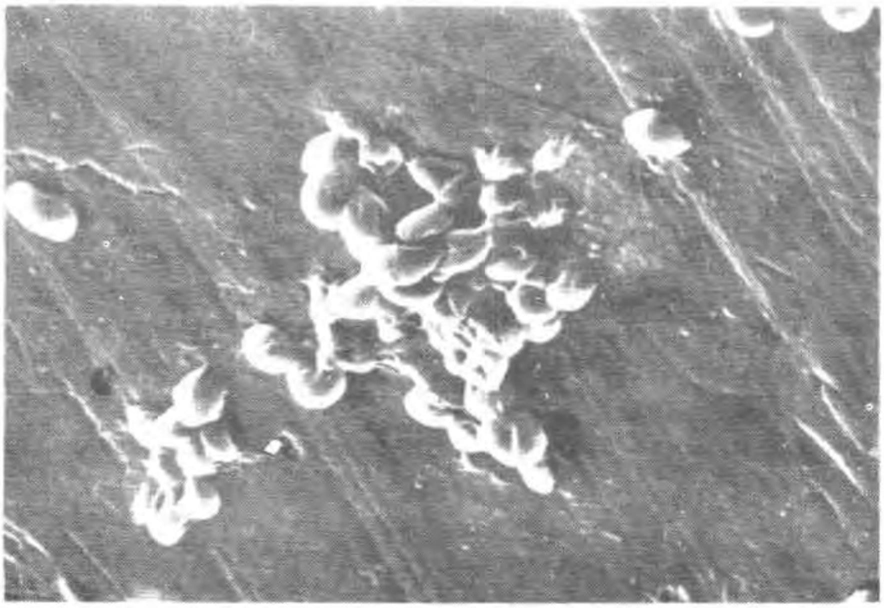


Fig. (18) Changes in Algae Concentration o——o ; Lead Concentration in the Reactor ●——● and Determined Accumulated Lead in Algae Δ——Δ at the Flow Rate 0.75 l/Day.

Steady State
Exposure Ratio : 4.4 mg/gm
Metal Accumulation per gram
Algae : 1.92 mg



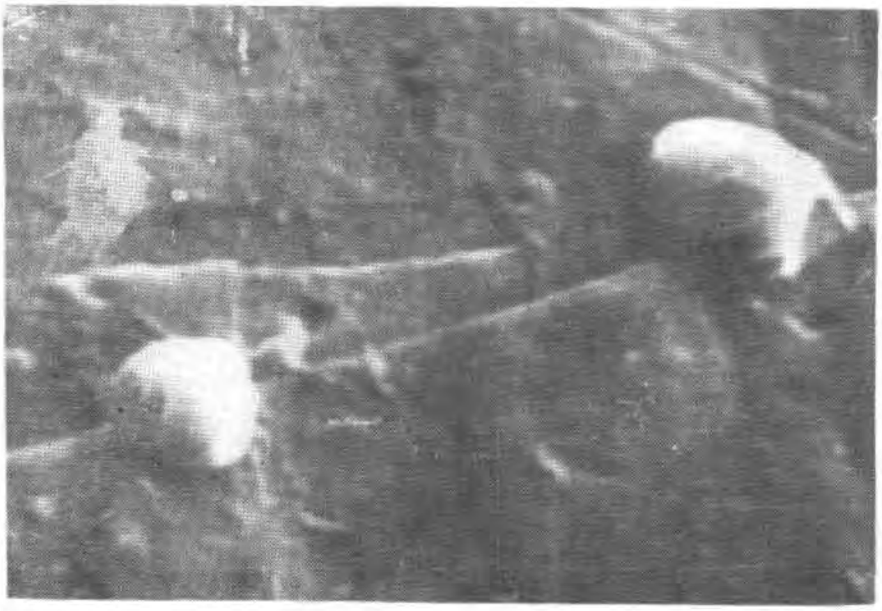


Fig. (19): Scanning Electron Micrographs of Scenedesmus Obliquus Grown Semicontinuously in AAM Medium Containing 200 ug/l Copper (the upper: X 1500; the lower: X 5000).

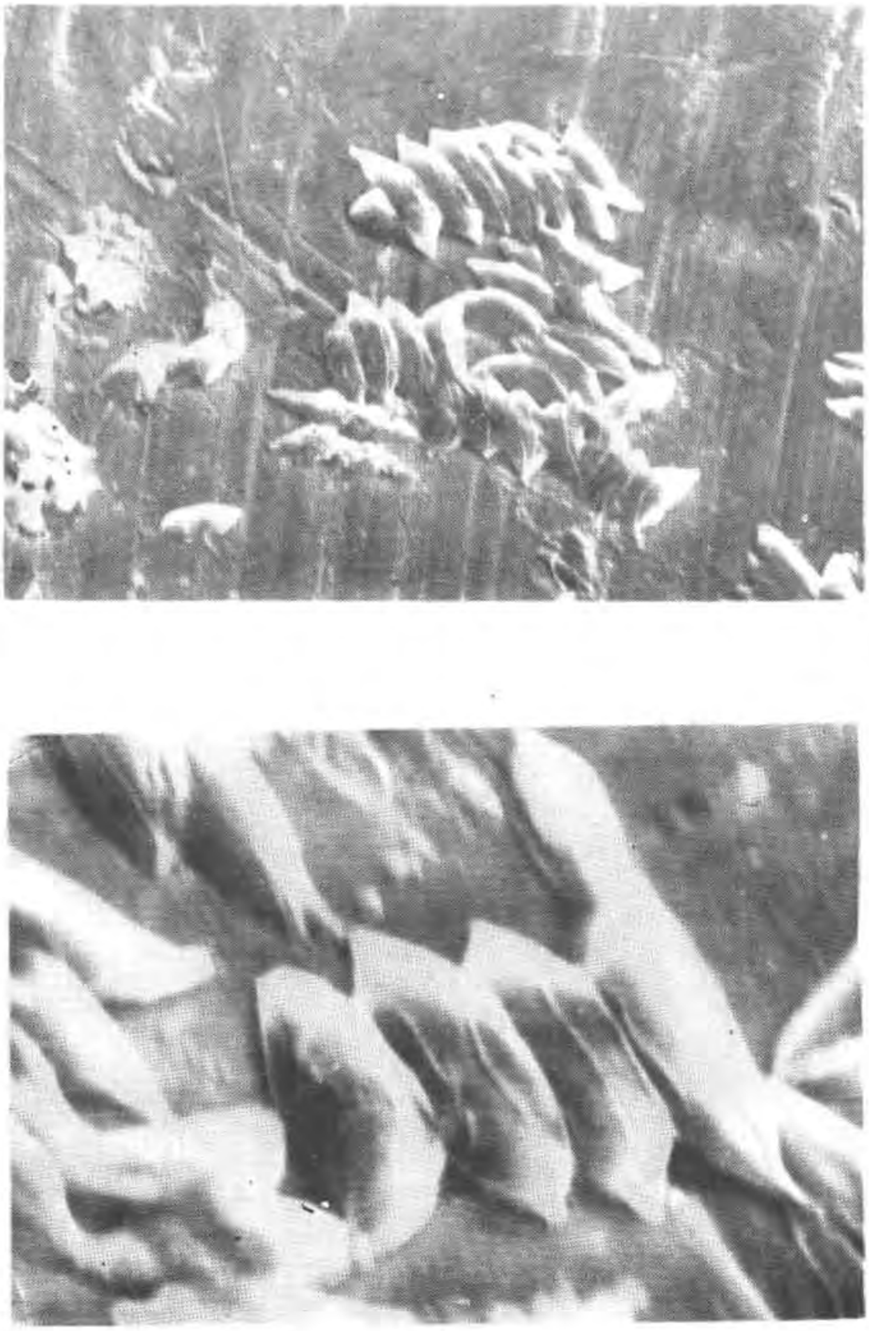


Fig. (20): Scanning Electron Micrographs of Scenedesmus Obliquus Grown Semicontinuously in AAM Medium Containing 400 ug/L Zinc (the upper: X 1500 the lower: X 5000).

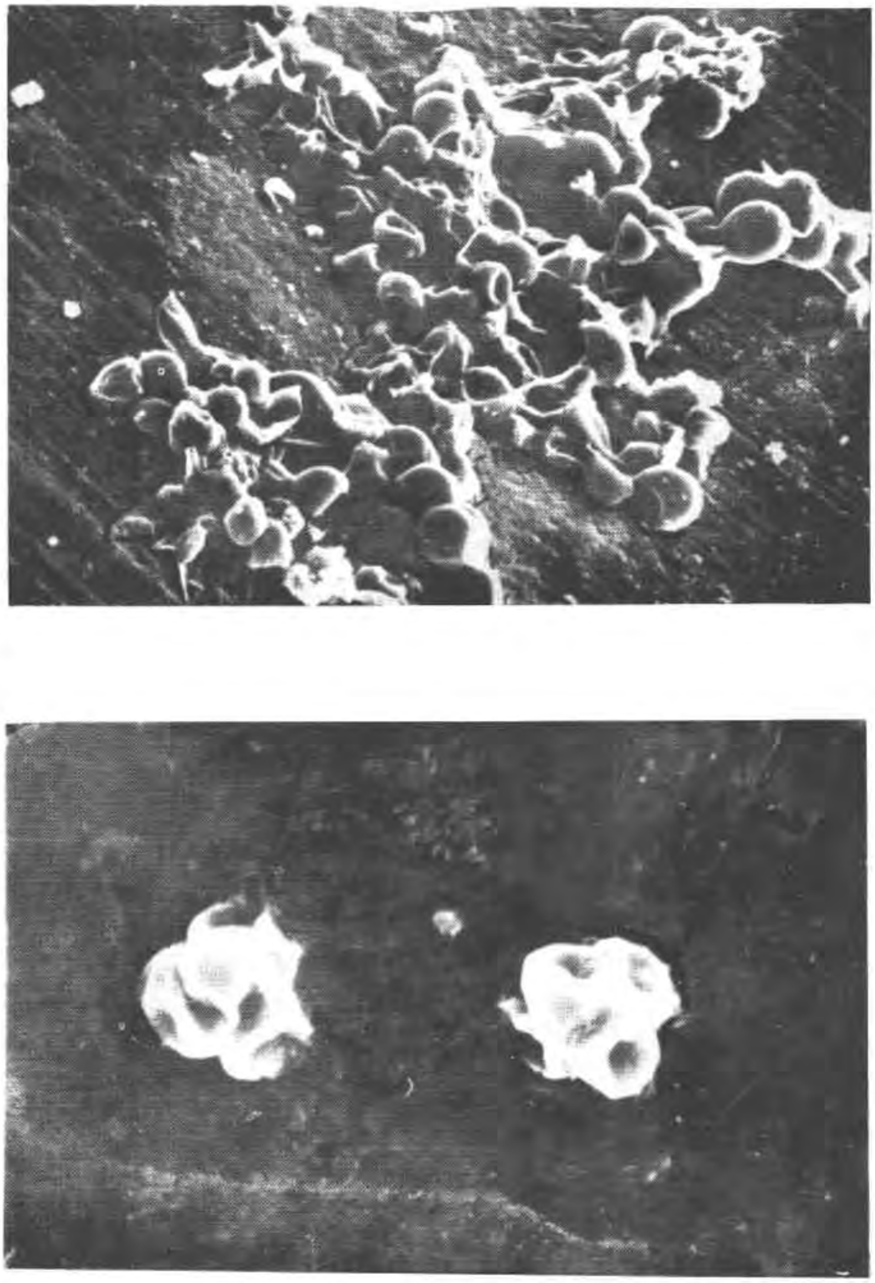


Fig. (21) Scanning Electron Micrographs of *Scenedesmus* Obliquus Grown Semicontinuously in AAM Medium Containing 400 ug/l Cadmium. (the upper: X 1500 ; the lower: X 3000).

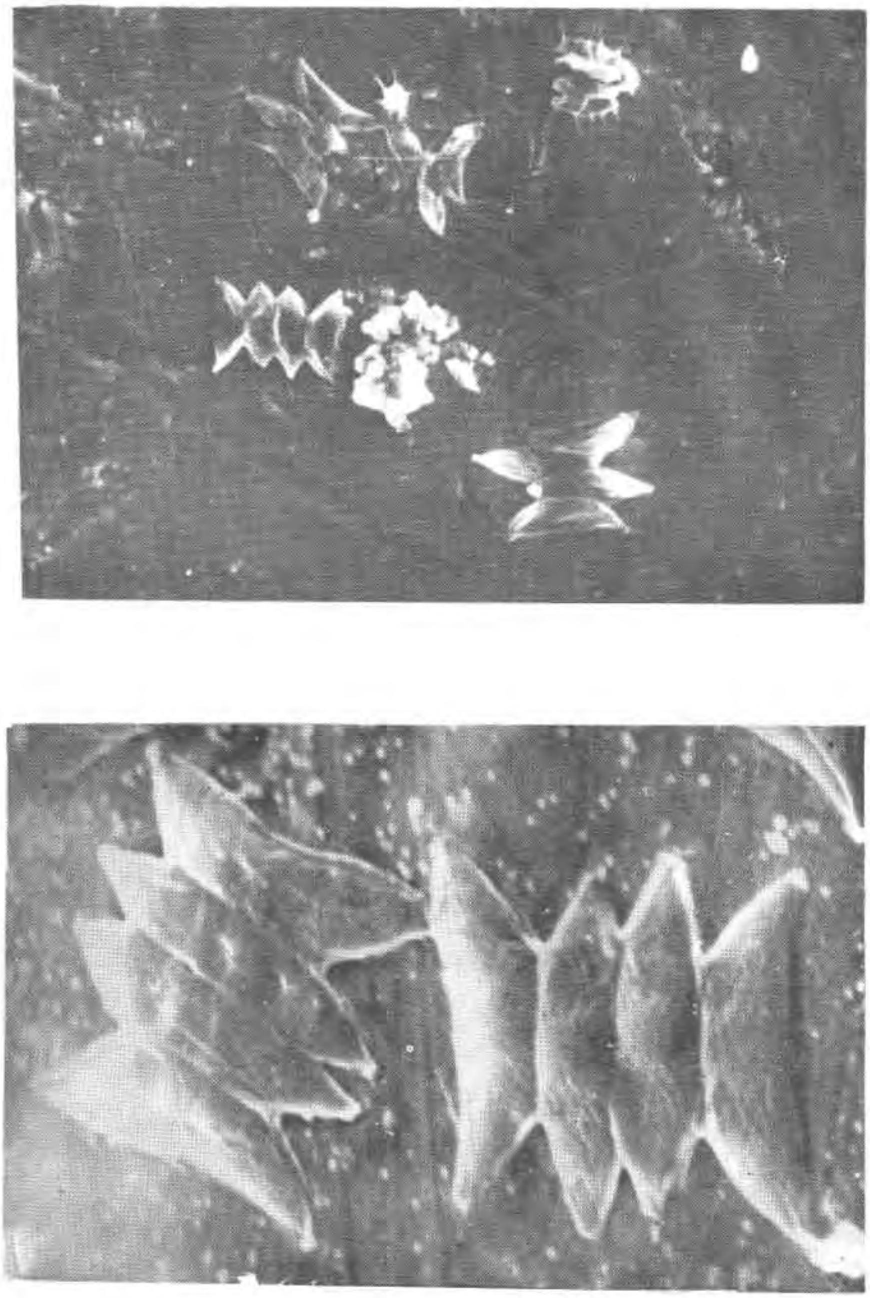
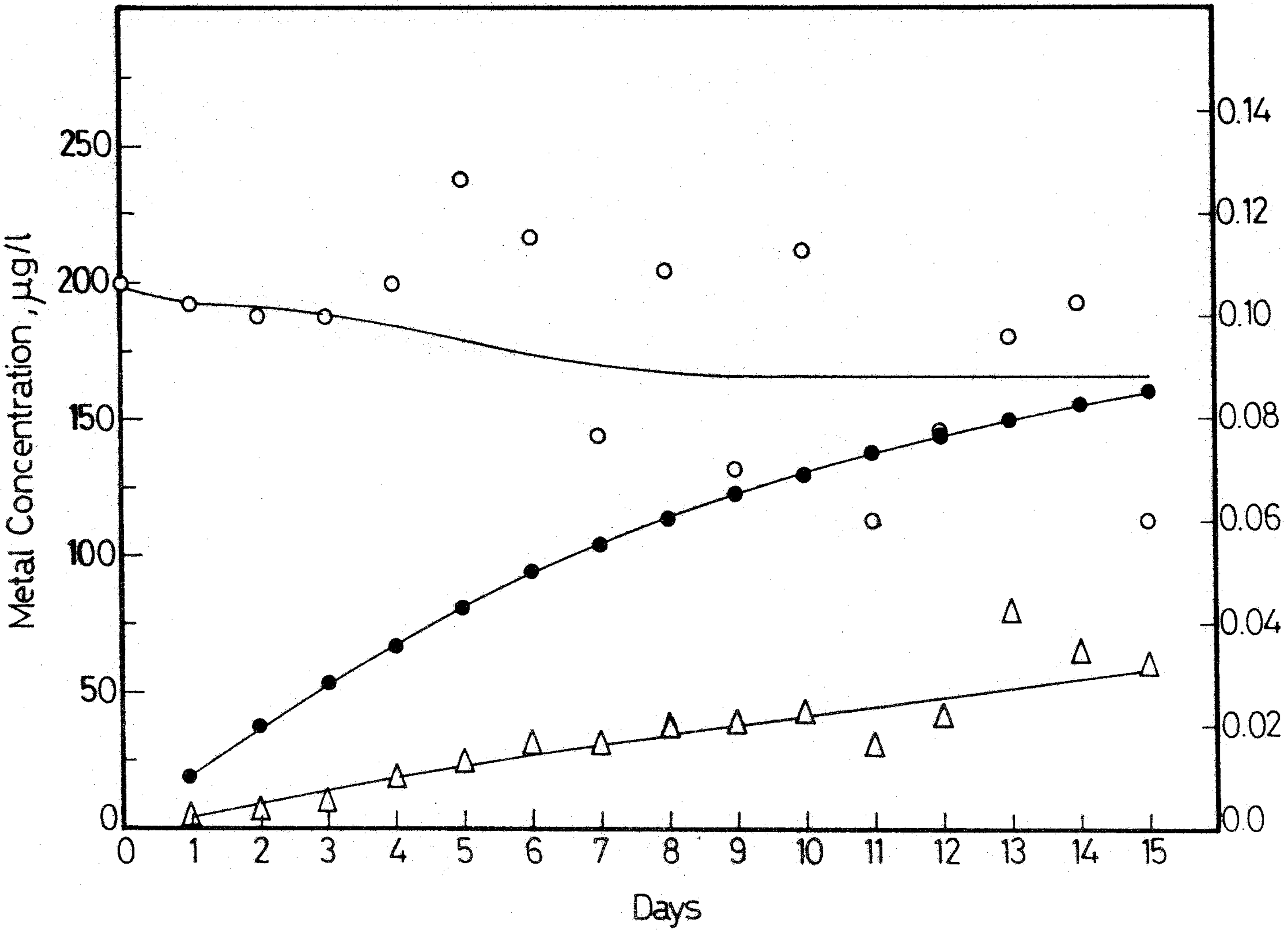


Fig. (22): Scanning Electron Micrographs of <u>Scenedesmus</u> Obliquus Grown Semicontinuously in AAM Medium (the upper: X 1500) and in the Medium containing 400 ug/l Lead (the lower: X 5000).

separate. Specific changes in the morphology of microorganisms have been doccumented in several articles (9, 26, 39, 43, 58).

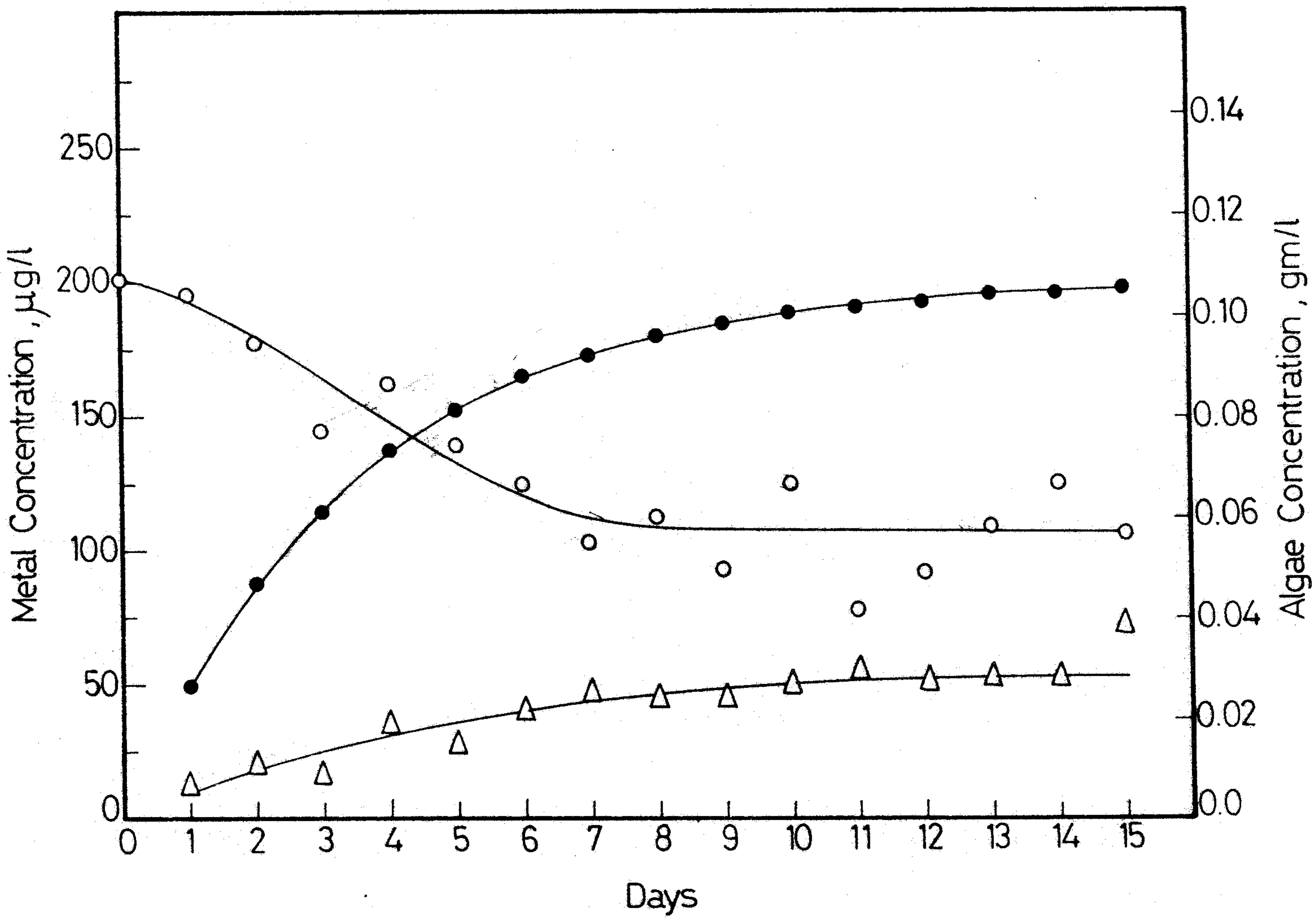
VII.1.4.2. Effect of Dilution Rate on Cadmium Accumulation.

The semicontinuous flow system was employed to assess the effect of dilution rate on the steady state algae concentration into the reactor and metal accumulation by these algae. The results given in tables 32 and shown in Fig. 23-26 were summarized in Fig 27 which tells us that the steady state algae concentration in absence of cadmium decreased with the increase in dilution rate and consequently the growth rate. Lower algal concentrations were recorded in presence of 200 ug Cd per liter of the feed medium and a steeper curve expressing the correlation between steady state algae concentration and growth rate was obtained. In the same time, the figure shows that cadmium accumulation in algae increased with the increase in dilution rate. Moreover, by extrapolation of the curves it appeared that under the experimental growth conditions, namely, available nutrients, adequate illumination and optimum temperature and in absence of cadmium the maximum algal (Scenedesmus) growth rate would have not exceeded 2.4 day^{-1} while in the presence of 200 ug Cd per liter medium this rate would have not exceeded 1.6 day^{-1} and the maximum cadmium accumulation would have been 2.75 mg Cd per gm algae on dry weight basis.



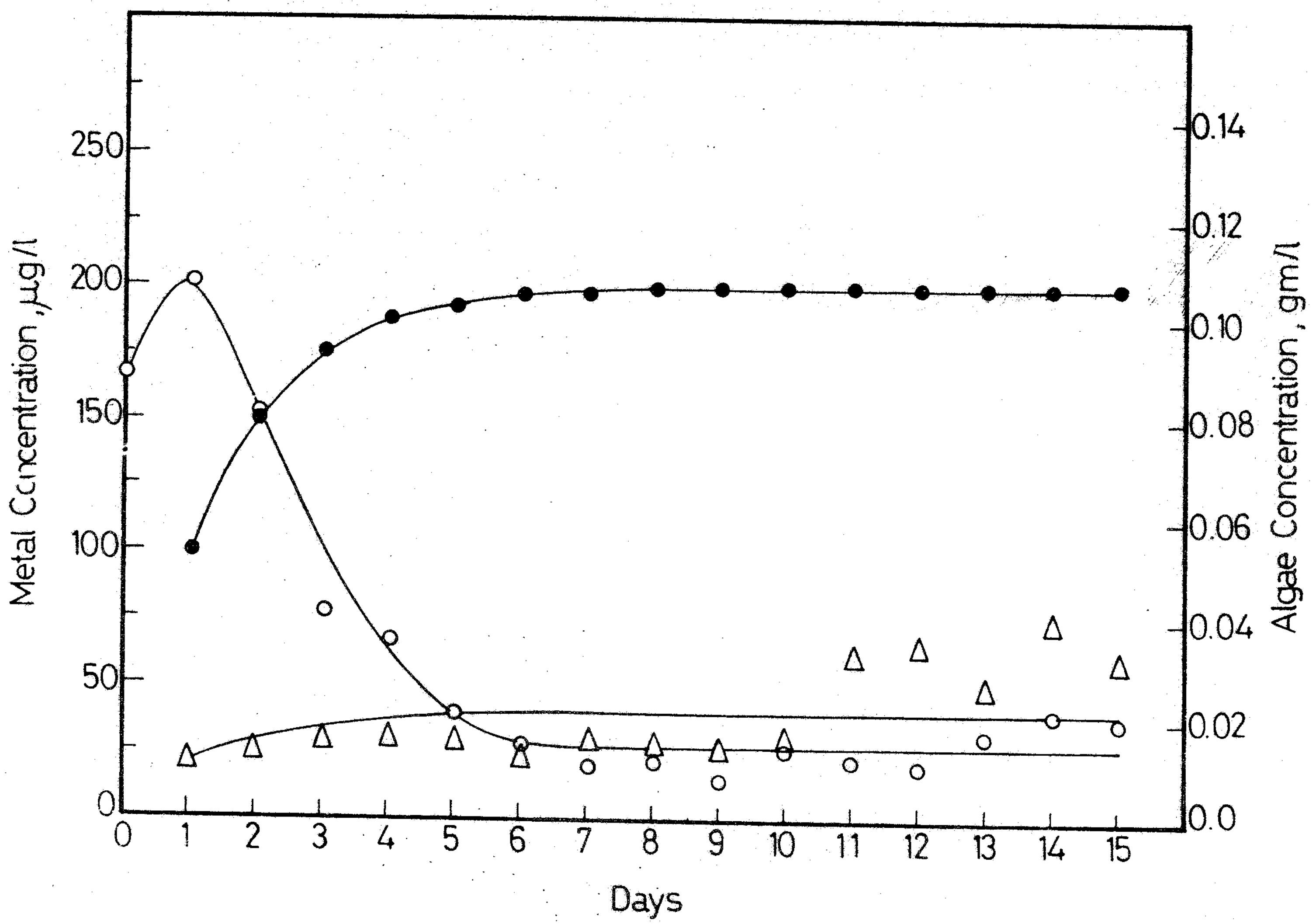
Algae concentration at steady state : 0.088 gm/l
Steady state exposure ratio : 2.27 mg/gm
Metal accumulated in algae : 0.045 mg/l
Metal accumulation per gm algae : 0.51 mg
Dilution rate : 0.1 day^{-1}
Growth rate : 0.1036 day^{-1}

Fig. (23) Changes in Algae Concentration ○—○ ; Cadmium Concentration in the Reactor ●—● and Determined Accumulated Cadmium in Algae △—△ at the Flow Rate 0.10 l/Day.



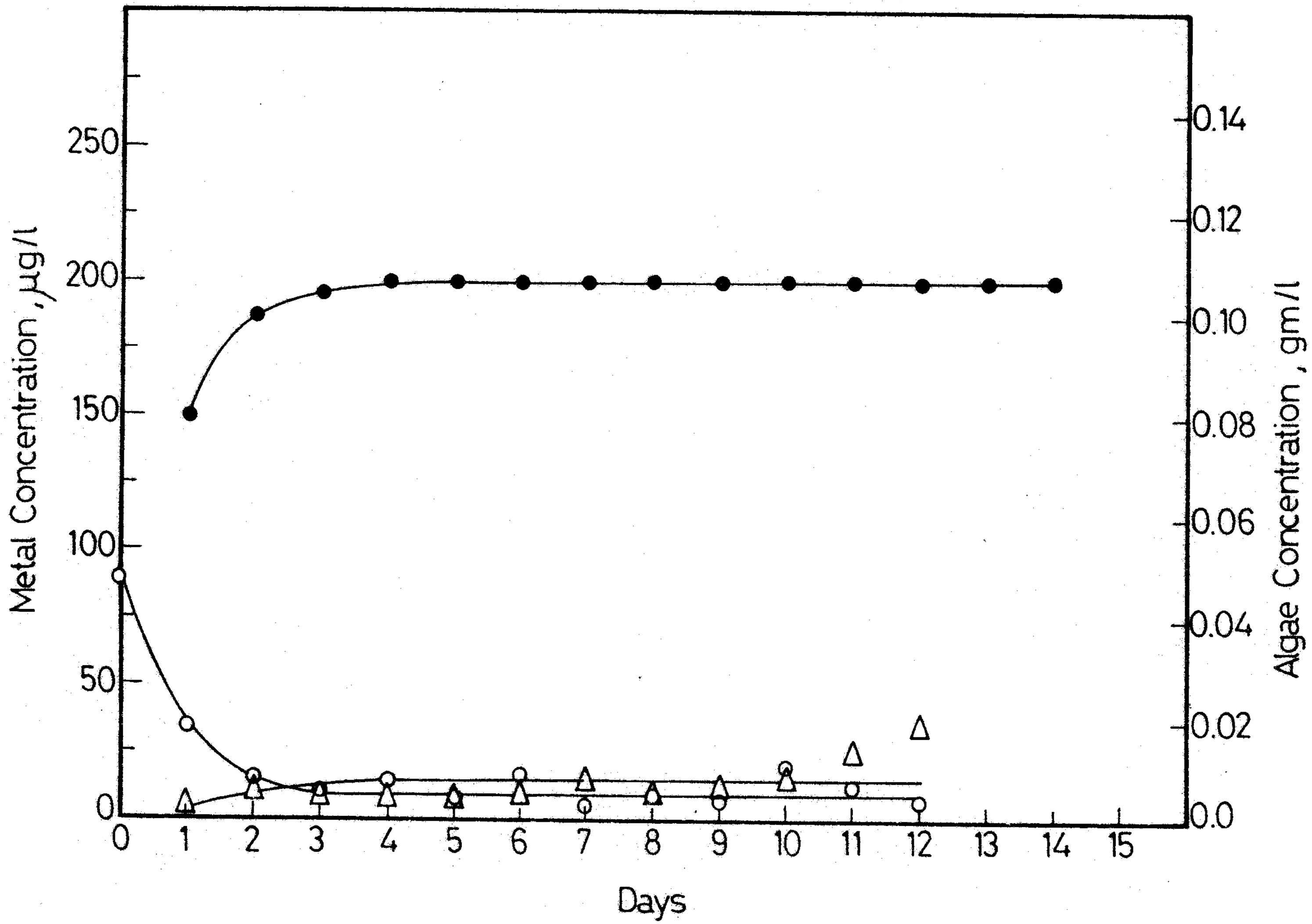
Algae concentration at steady state : 0.057 gm/l
Steady state exposure ratio : 3.51 mg/gm
Metal accumulated in algae : 0.052 mg/l
Metal accumulation per gm algae : 0.91 mg
Dilution rate : 0.25 day^{-1}
Growth rate : 0.285 day^{-1}

Fig. (24) Changes in Algae Concentration ○—○ ; Cadmium Concentration in the Reactor ●—● and Determined Accumulated Cadmium in Algae △—△ at the Flow Rate 0.25 l/Day.



Algae concentration at steady state : 0.015 gm/l
Steady state exposure ratio : 13.3 mg/gm
Metal accumulated in algae : 0.043 mg/l
Metal accumulation per gm algae : 2.87 mg
Dilution rate : 0.5 , day^{-1}
Growth rate : 0.691 day^{-1}

Fig. (25) Changes in Algae Concentration o——o ; Cadmium Concentration in the Reactor ●——● and Determined Accumulated Cadmium in Algae Δ——Δ at the Flow Rate 0.50 l/Day.



Algae concentration at steady state : 0.005 gm/l
Steady state exposure ratio : 40 mg/gm
Metal accumulated in algae : 0.014 mg/l
Metal accumulation per gm algae : 2.8 mg
Dilution rate : 0.75 day^{-1}
Growth rate : 1.386 day^{-1}

Fig.(26) Changes in Algae Concentration o——o ; Cadmium Concentration in the Reactor ●——● and Determined Accumulated Cadmium in Algae Δ——Δ at the Flow Rate 0.75 l/Day.

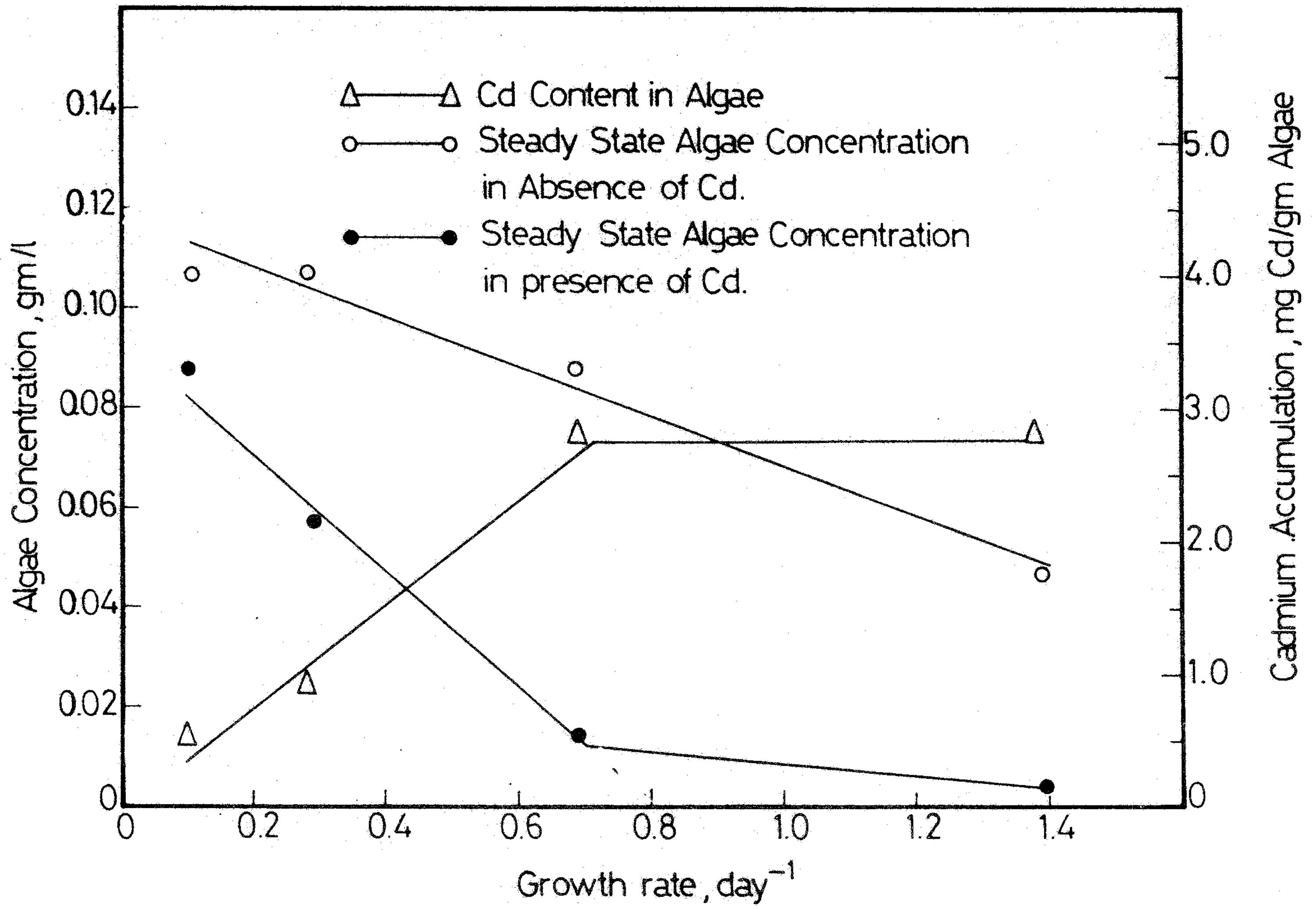


Fig.(27) Change in Algae Concentration with Change in Dilution Rate in Absence and presence of Cadmium and Change in Metal Content.

VII.2. Metals Accumulation by Aquatic Macrophytes

VII.2.1. Field Investigation of Metals Content of Aquatic Macrophytes.

Four procedures for heavy metal determination in aquatic macrophytes were applied on one of the samples. The procedure that resulted in the highest values for cadmium and lead concentrations was chosen to be applied on all the natural samples collected. This was the method of ashing the dried plant samples and dissolution in 1:1 $HNO_3 + H_2O_2$.

The results obtained are presented in table 33 . Analysis of water and sediment samples taken in the same time from specified locations, namely Helwan and Kafr El Zayaat, two heavily industrialized areas on the river, are shown in table 34. The concentration factors of Cd, Cu, Pb, and Zn in the sediment were much higher in most cases than those in aquatic plants table 35. This can be attributed to the larger surface area of sediment particulates and also to longer contact time with the sediment. In this respect analysis of plants such as Eichhornia can be a better tool for assessing recent pollution by metals than do the analysis of sediment. In most of the cases, metals contents of the sediment and the plants picked up from downstream the waste discharge point were higher than those determined in the samples taken from the upstream points.

Table (33) Concentration of Heavy Metals (ug/gm dry plant) in Plants Isolated From Different Locations in The Nile and Its Branches

Location	Plant	Part	Metal concentration ug/gm			
			Cu	Zn	Cd	Pb
1- The Lake	Ceratophyllum	whole	2.7	13.8	0.0	2.2
2- The Nile at Helwan						
U.S. Indust. complex	Eichhornia	whole	21.0	52.5	0.3	12.2
	Panicum	whole	3.2	30.5	0.2	11.4
	Ceratophyllum	whole	15.5	47.8	0.5	11.8
D.S. Indust. complex	Eichhornia	whole	89.7	34.2	0.3	10.2
	Panicum	whole	33.8	194.8	0.3	12.1
3- The Nile at mid Cairo	Eichhornia	whole	18.8	82.3	0.3	10.8
	Ceratophyllum	whole	36.4	117.0	0.3	22.2
	Panicum	whole	10.3	90.0	0.2	12.4
4- Tawfiki Canal at Banha						
U.S. Indust. complex	Eichhornia	whole	42.8	51.2	-	18.0
	Panicum	whole	7.0	63.0	0.5	12.8
D.S. Indust. complex	Eichhornia	whole	25.2	76.8	0.5	11.4
	Panicum	whole	13,0	42.3	0.6	-

U.S. = Upstream

D.S. = Downstream

Table (33) Continued

			Metal concentration, ug/gm			
Location	Plant	Part	Cu	Zn	Cd	Pb
5- Rosetta branch at Kafer Zayat						
U.S. Indust. complex	Eichhornia	whole	20.3	64.6	0.1	6.5
	Panicum	whole	4.3	31.3	0.1	4.8
In front of Indust complex	Eichhornia	Root	44.1	105.8	0.3	5.2
		Shoot	20.3	74.5	0.6	1.7
	Panicum	Root	47.8	105.4	0.3	7.8
		Stem	3.8	35.5	0.5	6.1
		Leaf	5.7	39.9	0.4	14.8
D.S. Indust. complex	Eichhornia	whole	22.5	68.4	0.6	13.2
6- Mahmodia Canal						
at Moharem press	Eichhornia	whole	47.9	127.7	0.4	6.0
	Ceratophyllum	whole	44.4	127.7	0.5	6.2
at Soap and Soda Factory	Eichhornia	whole	34.1	125.2	0.4	4.6
	Ceratophyllum	whole	41.9	140.7	0.6	5.0
at Weaving and Spining Fact.	Eichhornia	whole	57.2	171.3	0.8	6.8
	Ceratophyllum	whole	65.8	324.7	0.4	9.6
at end of canal	Eichhornia	whole	165.1	468.5	4.6	38.5
7- Smouha Drain at the end	Eichhornia	whole	57.3	273.6	0.3	28.8

Table (34) Concentrations of Heavy Metals in Water (ug/l) and Sediment (ug/gm) Samples Taken from Selected Locations

Water

Location	Metal concentration					
	Cd	Cu	Ni	Pb	Zn	Mn
1. Helwan						
1.1. U.S. Factories	1.0	4.2	4.0	6.7	1.4	34
1.2. D.S. "	1.0	4.8	7.0	7.7	1.7	34
2. Kafr. Zayat						
2.1. U.S. Factories	1.1	4.5	6.2	9.5	11.5	73
2.2. At discharge point	1.6	3.9	5.3	9.0	12.1	59
2.3. D.S. discharge point	1.1	5.1	6.4	9.0	6.6	89

Sediment

1. Helwan						
1.1. U.S. Factories	5.8	72	96	56	290	1496
1.2. D.S. "	4.7	53	83	73	180	925
2. Kafr Zayat						
2.1. U.S. Factories	9.8	80	80	147	170	2159
2.2. At discharge point	4.0	61	76	96	140	1062
2.3. D.S. discharge point	17.7	494	107	1800	480	966

Table (35) Concentration Factors (CF) Of Metals in Water, Aquatic Plants and Sediments of the River Nile System at Helwan and Kafr Zayat

Location	Ecophase	Metal, ppb			
		Cd	Cu	Zn	Pb
Helwan, upstream Indust. Complex	Water	1.0	4.2	1.4	6.7
	Plant				
	Eichhornia	300	21000	52500	12200
	CF	300	5000	37500	1820
	Panicum	200	3200	30500	11400
	CF	200	762	21785	1701
	Ceratophyllum	500	15500	47800	11800
	CF	500	3690	34142	1761
	Sediment	5800	72000	290000	56000
	CF	5800	17142	207142	8358
Helwan, Down-Stream Indust. Complex	Water	1.0	4.8	1.7	7.7
	Plant				
	Eichhornia	300	89700	34200	10200
	CF	300	18687	20117	1324
	Panicum	300	33800	194800	12100
	CF	300	7041	114588	1571
	Sediment	4700	53000	180000	7300
	CF	4700	11042	105882	948

Table (35) Continued

Location	Ecophase	Metal, ppb			
		Cd	Cu	Zn	Pb
Kafr Zayat upstream	Water	1.1	4.5	11.5	9.5
	Plant				
	Eichhornia	100	20300	64600	6500
	CF	91	4511	5617	684
	Panicum	100	4300	31300	4800
	CF	91	955	27200	505
	Sediment	9800	80000	17000	147000
	CF	8909	17777	14782	15473
Kafr Zayaat Downstream Indust. Complex.	Water	1.1	5.1	6.6	9.0
	Plant				
	Eichhornia	600	22500	68400	13200
	CF	545	4412	10364	1466
	Sediment	17700	494000	480000	1800000
	CF	16090	96862	72727	200000

The results indicated that aquatic plants can act as scavengers for heavy metals from flowing waters and in the same time can maintain healthy status.

Analysis of aquatic plants showed that the aquatic ecosystem of the river Nile at Helwan was heavily polluted by copper; at mid Cairo by zinc and at Kafr-El-Zayaat by zinc too. In Mahmoudia Canal at Alexandria it appeared that the aquatic ecosystem was heavily polluted by zinc while at the end of the canal there was an indication of heavy pollution by zinc copper, lead and cadmium.

VII.2.2. Heavy Metal Accumulation by Eichhornia Crassipes in a Static System.

VII.2.2.1. Effect of exposure ratio on Metal Accumulation

From the results obtained from this experiment it appeared that metal accumulation depends on the amount of metal in a confined body of water and the amount of plant material in it or more precisely the surface area of plant material exposed to the metal. In other words the exposure ratio of metal to plant and not the concentration of metal in water determined the metal accumulation by plant in mg metal/gm plant. Fig 28 (a-c) illustrates the relationship between metal: plant exposure ratio and metal accumulation in mg metal per gram dry plant material. This relationship could be described as linear especially at low exposure ratios indicating that metal accumulation be plant material is a surface phenomenon. Hulchinson and Czyrska (1975) also reported that the concentration factor of heavy metals by aquatic plants increased at higher levels of metal in solution.

Table (36) Chemical Characteristics of Nile Water (average)

Electric Conductance, umho cm^{-1}	320	
pH	7.8 - 8.2	
Total Hardness, as $CaCO_3$	102	mg/l
Total Alkalinity, as $CaCO_3$	112	mg/l
Chlorides, as Cl^-	18	mg/l
Sulfates, as SO_4^-	16	mg/l
Total phosphorous, as P	0.098	mg/l
Ortho phosphate, as P	0.052	mg/l
Nitrate, as N	0.054	mg/l

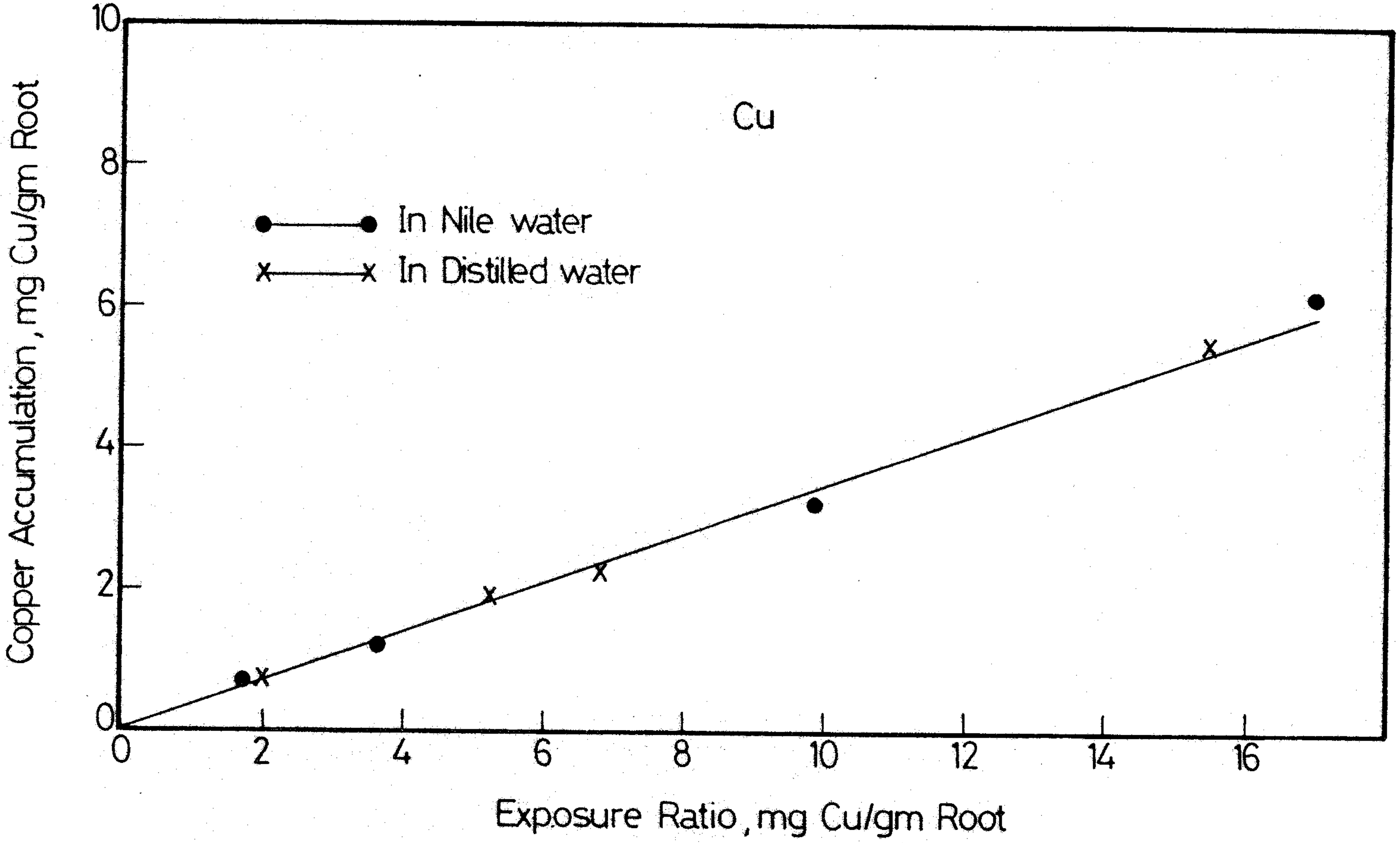


Fig. (28a) Effect of Exposure Ratio on Metal Accumulation by Eichhornia Crassipes.

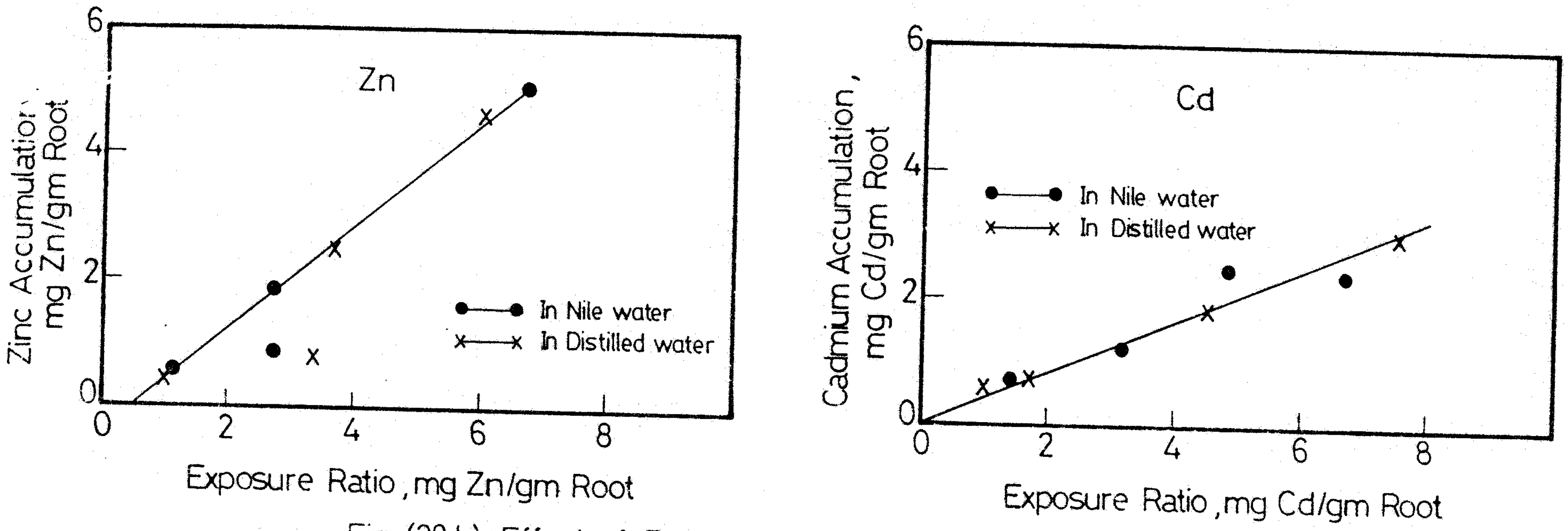


Fig. (28 b) Effect of Exposure Ratio on Metal Accumulation by Eichhornia Crassipes.

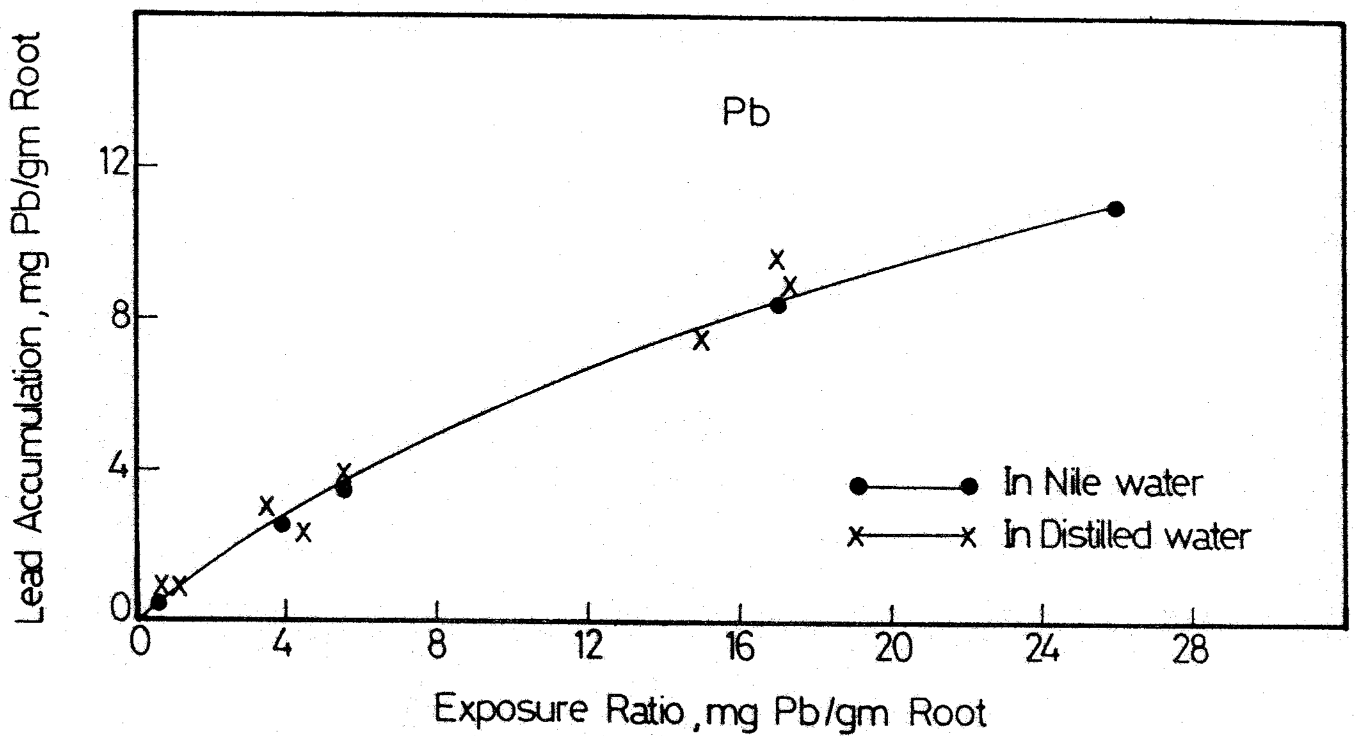


Fig. (28 c) Effect of Exposure Ratio on Metal Accumulation by Eichhornia Crassipes.

Table (37) Accumulation of Copper in Eichhorina Crassipes After 4 Days Exposure in a Static System

Medium	Cu added mg/l	Shoot			Root			Total Plant		
		Dry wt. gm	E.R. mg Cu/gm	Cu Acc. mg/gm	Dry wt. gm	E.R. mg Cu/gm	Cu Acc. mg/gm	Dry wt. gm	E.R. mg Cu/gm	Cu Acc. mg/gm
Nile Water	0.0	2.4515	0.0	0.0020	0.9735	0.0	0.0033	3.4250	0.0	0.005
	1.0	2.1344	0.703	0.0120	0.8900	1.685	0.6845	3.0244	0.496	0.210
	2.5	3.5330	1.061	0.0370	1.0391	3.609	1.1906	4.5721	0.820	0.299
	5.0	3.1137	2.409	0.0870	0.7577	9.898	3.200	3.8714	1.937	0.696
	10.0	2.9322	5.116	0.1592	0.8808	17.030	6.1264	3.8130	3.934	1.538
Distilled Water + $NaHCO_3$	0.0	2.0800	0.0	0.0031	0.7200	0.0	0.0057	2.800	0.0	0.009
	1.0	3.5961	0.417	0.0087	0.7613	1.970	0.7271	4.3574	0.0344	0.134
	2.5	2.1343	1.757	0.0413	0.1733	5.257	1.9049	2.8476	1.317	0.508
	5.0	3.6700	2.044	0.0348	1.1563	6.786	2.2323	4.8263	1.557	0.561
	10.0	3.6916	4.063	0.1516	0.9700	15.464	5.4668	4.6616	3.218	1.257

Test water volume : 1.5 liters
E.R. = Exposure Ratio, mg Cu/gm dry weight plant material
At the doses of Cu over 1.0 mg/l yellowing of the shoot started on one day exposure and shrinkage of leaves was observed further on.
pH distilled water + $Na HCO_3$ = 8.0
pH Nile water = 8.0

Table (38) Accumulation of Zinc in <u>Eichhorina</u> crassipes After 4 days Exposure in a Static System

Medium	Zn added mg/l	Shoot			Root			Total Plant		
		Dry Wt. gm	E.R. mg Zn/gm	Zn Acc. mg/gm	Dry Wt. gm	E.R. mg Zn/gm	Zn Acc. mg/gm	Dry Wt. gm	E.R. mg Zn/gm	Zn Acc. mg/gm
Nile Water	0.0	3.659	0.0	0.1025	1.280	0.0	0.110	4.939	0.0	
	1.0	2.427	0.62	0.1800	1.338	1.12	0.560	3.765	0.40	0.315
	2.5	2.870	1.31	0.2350	1.395	2.69	0.850	4.625	0.88	0.436
	5.0	2.829	2.65	0.3235	2.773	2.70	1.922	5.602	1.34	1.115
	10.0	2.726	5.50	0.5441	2.255	6.65	5.075	4.981	3.01	2.596
Distilled Water + $NaHCO_3$	0.0	3.2100	0.0	0.0670	1.303	0.0	0.040	4.513	0.0	
	1.0	3.054	0.49	0.1750	1.568	0.96	0.410	4.622	0.32	0.522
	2.5	3.117	1.20	0.3000	1.117	3.36	0.800	4.234	0.89	0.408
	5.0	4.645	1.61	0.2794	2.054	3.65	2.481	6.699	1.12	0.955
	10.0	5.170	2.90	0.3823	2.475	6.06	4.576	7.645	1.96	1.740

Test water volume: 1.5 liters

E.R.: Exposure Ratio, mg Zn / gm dry weight plant material

pH Nile water = 8.3

pH distilled water + Na HCO_3 = 8.0

Table (39) Accumulation of Cadmium by *Eichhornia* crassipes After 4 days Exposure in a Static System

Medium	Cd added mg/l	Shoot			Root			Total Plant		
		Dry wt. gm	E.R. mg Cd/gm	Cd Acc. mg/gm	Dry wt. gm	E.R. mg Cd/gm	Cd Acc. mg/gm	Dry wt. gm	E.R. mg Cd/gm	Cd Acc. mg/gm
	0.0	1.0476	0.0	0.0015	2.0565	0.0	0.0014	3.104	0.0	
Nile	1.0	1.6141	0.93	0.048	1.0600	1.42	0.715	2.675	1.80	0.312
Water	2.5	1.9400	1.93	0.088	1.1700	3.21	1.180	3.110	2.42	0.499
	5.0	1.1024	6.29	0.116	1.5500	4.84	2.457	2.724	1.90	1.449
	10.0	1.3400	11.79	0.500	2.2300	6.73	2.445	3.570	2.45	1.715
	0.0	1.1800	0.0	0.0014	2.060	0.0	0.003	3.24	0.0	
Distilled	1.0	0.9800	0.65	0.063	1.5250	0.98	0.566	2.505	1.62	0.369
Water	2.5	1.335	2.81	0.100	2.2556	1.66	0.724	3.541	1.26	0.838
+Na HCO_3	5.0	1.4445	5.19	0.756	1.6600	4.52	1.814	3.105	1.83	1.321
	10.0	1.0500	14.29	0.767	2.0055	7.48	3.030	3.056	2.18	2.252

Test Water volume: 1.5 liters

E.R.: Exposure Ratio, mg Cd/gm dry weight plant material

pH Nile water = 8.1

pH distilled water + Na HCO_3 = 8.1

Table (40) Accumulation of Lead in Eichhornia crassipes After 4 Days Exposure in a **Static System**

Medium	Pb added mg/l	Shoot			Root			Total Plant		
		Dry Wt. gm	E.R. mg Pb/gm	Pb Acc. mg/gm	Dry Wt. gm	E.R. mg Pb/gm	Pb Acc. mg/gm	Dry Wt. gm	E.R. mg Pb/gm	Pb Acc. mg/gm
Nile water	1.0	3.2017	0.4685	0.0532	2.3017	0.6517	0.3359	5.5034	0.273	0.1714
		2.6465	0.5668	0.0605	2.2800	0.6579	0.5059	4.9265	0.304	0.2666
	5.0	2.5834	2.9032	0.1025	1.9126	3.9213	2.3735	4.4960	1.668	1.0686
		1.9330	3.880	0.3024	1.3645	5.4961	3.5937	3.2976	2.274	1.5788
	20.0	2.7357	10.9661	0.1328	1.7622	17.0242	8.2786	4.4974	6.670	3.3246
		2.7357	18.1083	0.3969	1.1323	26.4924	11.000	2.7891	10.756	4.7019
Distilled Water + $NaHCO_3$	1.0	2.2748	0.6594	0.2621	1.4709	1.0198	0.9754	3.7457	0.400	0.5422
		2.3800	0.6303	0.2772	1.8329	0.8184	0.8234	4.2129	0.356	0.5105
		2.4120	0.6219	0.2605	1.9100	0.7853	0.9387	4.3220	0.347	0.5602
	5.0	2.5310	2.9633	0.2634	1.7085	4.3898	2.2178	4.2395	1.769	1.0510
		2.1757	3.4472	0.1876	2.1526	3.4842	3.0552	4.3283	1.733	1.6640
		1.6713	4.4875	0.3456	1.3754	5.4530	3.8806	3.0467	2.462	1.9414
	20.0	2.7276	10.9987	0.3619	1.7300	17.3410	8.8876	4.4576	6.730	3.6707
		2.9321	10.2316	0.2819	2.004	14.9970	7.4651	4.9325	6.082	3.1951
		2.0519	14.6206	0.1323	1.7580	17.0048	9.5600	3.8099	7.874	2.5805

Test water volume : 1.5 liters
E.R. : Exposure Ratio, mg Pb/gm dry weight plant material
pH Nile water : 8.1
pH Distilled water + $NaHCO_3$: 7.8

The results of analysis of exposed plants showed (tables 37-40) that the shoots of exposed Eichhornia contained metals in relatively low concentrations compared to the roots. Also, these results indicated translocation of metals to the shoots even with cadmium and lead which are not plant nutrients. Englande(1980) also noticed little translocation of cadmium in water hyacynth.

The figures 28a - 28c revealed also that the test plants had similar capacities for metal accumulation from the two types of waters namely the Nile water (moderately hard) of natural composition (table 36) and the dilute $NaHCO_3$ solution.

The roots of Eichhornia were able to accumulate from test Nile water up to 6.126 mg Cu, 5.075 mg Zn, 2.445 mg Cd or 6.000 mg Pb per one gram plant meterial on a dry weight basis. However, it was noticed that at the doses over one mg Cu/liter yellowing of the shoot started on one day exposure, while this did not occur with the other metals.

VII.2.2.2. Effect of Chemical Composition of Water on Metal Accumulation

It appeared from the results presented in table 42 that the presence of EDTA-Na_2 in water greatly reduced lead accumulation by the root of the test plant. This effect of EDTA was much less displayed on cadmium accumulation (table 41). Comparing metal content of the shoots of exposed plants at the similar exposure ratios - in

Table (41) Accumulation of Cd by Eichhornia crassipes After One Day Exposure in Media of Different Compositions Contained One mg/l Cd

Medium	Final pH	Shoot				Root			
		Dry Wt. gm	Exposure ratio, mg/gm	Cd accum., mg	Cd accum., mg/gm	Dry Wt. gm	Exposure ratio, mg/gm	Cd accum., mg	Cd accum., mg/gm
Dist. water	7.1	2.1714	0.691	0.056	0.026	1.4704	1.020	1.190	0.809
+ $NaHCO_3$	7.1	2.0342	0.737	0.096	0.047	1.3856	1.121	1.097	0.792
	7.0	1.9439	0.772	0.071	0.036	0.9814	1.528	1.026	1.045
Dist. water	7.2	2.6038	0.576	0.068	0.026	1.7106	0.877	1.040	0.608
+ $NaHCO_3$	7.2	1.6112	0.931	0.127	0.079	0.6344	2.364	0.878	1.385
+ Ca & Mg salts	7.2	1.9844	0.756	0.057	0.029	1.1229	1.336	0.720	0.642
Dist. water	7.1	2.4200	0.620	0.032	0.013	1.2950	1.158	0.492	0.380
+ $NaHCO_3$	7.1	2.5752	0.582	0.030	0.012	2.0747	0.723	0.852	0.410
+ EDTA	7.4	1.3427	1.117	0.064	0.048	0.5749	2.609	0.451	0.785
Dist. water	7.2	3.0132	0.498	0.048	0.016	1.8000	1.2	0.872	0.485
+ $NaHCO_3$	7.3	1.9314	0.777	0.058	0.030	0.6700	2.239	0.581	0.867
+ EDTA+Ca & Mg Salts	7.3	2.2861	0.656	0.071	0.031	1.0072	1.489	0.664	0.660

Test water volume = 1.5 liters

Table (41) Continued

Medium	Final pH	Total Plant		Removal of Cd, % of initial		
		Dry Wt. gm	Cd accum., mg	by Shoot	by Root	by Total plant
Dis. water	7.1	3.6418	1.264	3.73	79.33	83.07
+ NaHCO$_3$	7.1	3.4198	1.193	6.40	73.13	79.53
	7.0	2.9253	1.097	4.73	68.40	73.13
Dist. water	7.2	4.3144	1.108	4.53	69.33	73.87
+ NaHCO$_3$	7.2	2.2456	1.005	8.47	58.53	67.00
+ Ca & Mg salts	7.2	3.1073	0.777	3.80	48.00	51.00
Dist. water	7.1	3.715	0.524	2.13	32.80	34.93
+ NaHCO$_3$	7.1	4.6499	0.882	2.00	56.80	58.80
+ EDTA	7.4	1.9176	0.515	4.27	30.07	34.33
Dist. water	7.2	4.8132	0.920	3.20	58.13	61.33
+ NaHCO$_3$	7.3	2.6014	0.639	3.87	38.73	42.60
+ EDTA + Ca & Mg	7.3	3.2933	0.735	4.73	44.27	49.00

Table (42) Accumulation of Pb by Eichhornia crassipes After One Day Exposure in Media of Different Composition Contained One mg/l Pb.

Medium	Shoot				Root			
	Dry wt. gm	Exposure ratio, mg/gm	Pb accum. mg	Pb accum. mg/gm	Dry Wt. gm	Exposure ratio mg/gm	Pb accum. mg	Pb accum. mg/gm
Dist-water	1.5400	0.972	0.0631	0.0410	0.9837	1.525	0.7756	0.7884
+ $NaHCO_3$	1.1564	0.771	0.0380	0.0329	1.4541	1.032	1.0800	0.7289
	2.2785	0.658	0.0406	0.0178	2.4622	0.609	1.0059	0.4085
Dist. water	1.3220	1.135	0.0424	0.0320	0.9700	1.545	1.1816	1.2181
+ $NaHCO_3$	0.9266	1.619	0.0288	0.0311	1.1428	1.313	0.9648	0.8442
+ Ca, Mg salts	1.5900	0.943	0.0338	0.0212	1.1668	1.286	1.0914	0.9354
Dist. water	2.1700	0.691	0.0215	0.0099	1.3922	1.077	0.0744	0.0534
+ $NaHCO_3$	1.5700	0.955	0.0335	0.0214	1.6350	0.917	0.0755	0.0462
+ EDTA	2.1951	0.683	0.0270	0.0123	1.7963	0.835	0.0957	0.0533
Dist. Water	3.2807	0.457	0.0269	0.0082	2.4050	0.634	0.1327	0.0552
+ $NaHCO_3$	1.7779	0.844	0.0104	0.0059	1.9158	0.783	0.1086	0.0567
+ EDTA	2.6271	0.571	0.0277	0.0106	1.3208	1.136	0.0844	0.0639
+ Ca, Mg salts								

Test water volume : 1.5 liters.

Table (42) Continued

Medium	Total Plant		Final pH	Removal of Pb, % of initial		
	Dry Wt. gm	Pb accum. mg		by shoot	by Root	by Total Plant
Dist. water	2.5237	0.8387	6.7	4.207	51.707	55.91
+ $NaHCO_3$	2.6105	1.0980	6.7	2.533	70.667	73.20
	4.7407	1.0465	6.7	2.707	67.060	69.77
Dist. water	2.2920	1.2240	6.9	2.827	78.773	81.60
+ $NaHCO_3$	4.3614	0.9936	7.0	1.920	64.320	66.24
+ Ca, Mg salts	2.7568	1.1252	7.0	2.253	72.760	75.01
Dist. water	3.5622	0.1013	6.9	1.433	4.960	6.75
+ $NaHCO_3$	3.2050	0.1090	6.8	2.233	5.033	7.27
+ EDTA	3.9914	0.1227	6.8	1.800	6.380	8.18
Dist. water	5.6857	0.1596	6.8	1.793	8.847	10.64
+ $NaHCO_3$	3.6937	0.1190	6.9	0.693	77.240	7.93
+ EDTA	3.9479	0.1121	6.8	1.847	5.627	7.47
+ Ca, Mg salts						

presence and absence of EDTA revealed that the presence of this organic ligand at the concentration applied in this experiment did not show a clear influence on metal translocation to the shoot. This matter will be elaborated in the next experiment under VII.2.2.3.

From the results it seemed that at similar exposure ratios calcium and magnesium enhanced lead accumulation in the roots but inhibited cadmium accumulation. Kinkade and Erdman (1975) also reported that cadmium uptake by aquatic plants was inhibited in hard water.

On the other hand the presence of calcium and magnesium in such applied concentrations did not show evident competition with lead or cadmium on the chelating action of EDTA added to the test water.

VII.2.2.3. Effect of EDTA on Metal Accumulation

This experiment was carried out on two of the non-nutrient heavy metals, namely cadmium and lead. The results of EDTA presence in increasing concentrations on metals accumulation by Eichhornia crassipes are compiled in tables 43 and 44. Metal accumulations within narrow ranges of metal: plant exposure ratios were compared at the different EDTA concentrations(Figs 29 and 30). It appears from these figures that with one mg metal per liter metal accumulation was inhibited to a great extent in presence of 6 mg EDTA per liter, in both root and shoot, indicating that organic ligands in natural waters could be good competitor with plant surface on metallic pollutants. Figs 31 and 32 showing the effect of contact time on metal accumulation revealed that in a narrow range

Table (43) Accumulation of Cd by Eichhornia crassipes from $NaHCO_3$ Solution (60 mg/l) Contained One mg/l Cd and Varied Concentrations of EDTA.

EDTA concent., mg/l	Exposure time, h	Final pH	Shoot				Root			
			Dry Wt., gm	Exposure ratio, mg/gm	Cd accum., mg	Cd accum., mg/gm	Dry Wt. gm	Exposure ratio, mg/gm	Cd accum., mg	Cd accum., mg/gm
1 mg/l	one h	7.0	3.7832	0.3965 /	0.031	0.008	3.8676	0.3878	0.780	0.202
		7.0	3.2700	0.4587	0.023	0.007	3.1683	0.4734 /	0.838	0.264
	2 h	6.8	4.8409	0.3099	0.079	0.016	2.4558	0.6108 /	0.448	0.182
		6.8	3.8758	0.3870 /	0.052	0.013	1.5681	0.704	0.360	0.360
	24 h	7.2	3.1323	0.4789 x	0.112	0.036	1.7876	0.8391	0.850	0.476
		7.3	3.5800	0.4190 /	0.078	0.022	2.4146	0.6212 /	0.910	0.377
		7.2	3.2138	0.4667	0.084	0.026	1.1090	1.3526 x	0.650	0.586
6 mg/l		7.2	2.9182	0.5140 x	0.025	0.008	0.7242	2.0713	0.014	0.020
		7.1	2.8441	0.5274	0.035	0.012	1.6979	0.8834 x	0.146	0.086
		7.1	3.6277	0.4135	0.023	0.006	1.8418	0.8144	0.072	0.039
12 mg/l		7.1	3.6526	0.4107	0.017	0.005	1.0644	0.8045	0.050	0.027
		7.3	2.1989	0.6822	0.014	0.006	0.4778	3.1394	0.018	0.038
		7.1	2.9531	0.5079 x	0.012	0.004	1.1923	1.2581 x	0.011	0.009
24 mg/l		7.	2.9967	0.5006 x	0.029	0.010	1.2609	1.1896 x	0.029	0.023
		7.2	1.5862	0.9457	0.015	0.010	0.8358	1.7947	0.007	0.008
		7.2	2.1438	0.6997	0.016	0.008	0.6564	2.2851	0.017	0.017

Volume of test water: 1.5 liters

/ Presented on Fig. 31 x Presented on Fig. 29

Table (43) Continued

EDTA concent., mg/l	Exposure time, h	Final pH	Total Plant: Dry Wt. gm	Total Plant: Cd accum., mg	% Removal: by Shoot	% Removal: by Root	% Removal: by Total Plant
1 mg/l	one h	7.0	7.6508	0.811	2.067	52.000	54.067
		7.0	6.4383	0.861	1.533	55.867	57.400
	2 h	6.8	7.2967	0.527	5.267	29.867	35.133
		6.8	5.8324	0.756	3.467	46.933	50.400
	24 h	7.2	4.9199	0.962	7.467	56.667	64.133
		7.3	5.9946	0.988	5.200	60.667	65.867
		7.2	4.3228	0.734	5.600	43.333	48.933
6 mg/l		7.2	3.6424	0.039	1.667	0.933	2.600
		7.1	4.5420	0.181	2.333	9.733	12.067
		7.1	5.4695	0.095	1.533	4.800	6.333
12 mg/l		7.1	5.5170	0.067	1.133	3.333	4.467
		7.3	2.6767	0.032	0.933	1.200	2.133
		7.1	4.1454	0.023	0.800	0.733	1.533
24 mg/l		7.0	4.2576	0.058	1.933	1933	3.867
		7.2	2.4220	0.022	1.000	0.466	1.466
		7.2	2.8002	0.027	1.067	0.733	1.800

Table (44) . Accumulation of Pb by Eichhornia crassipes from $NaHCO_3$ Solution (60 mg/l) Contained One mg/l Pb and Varied Concentrations of EDTA

EDTA concentration	Exposure time, h	Shoot				Root			
		Dry Wt. gm	Exposure ratio mg/gm	Pb accum., mg	Pb accum. mg/gm	Dry Wt., gm	Exposure ratio, mg/gm	Pb accum. mg	Pb accum. mg/gm
1 mg/l	one h	2.9521	0.508	0.0634	0.0215	0.8871	1.691	0.2455	0.2768
		4.2611	0.352 /	0.0472	0.0111	1.8700	0.902 /	0.3153	0.1686
	2 h	5.3080	0.283	0.0529	0.0100	1.3484	1.112	0.4343	0.3220
		3.8766	0.387 /	0.0343	0.0089	1.7300	0.867 /	0.3676	0.2125
		4.0688	0.369 /x	0.0773	0.0190	1.9080	0.786 /x	0.5642	0.2957
	24 h	5.3088	0.283	0.0901	0.0170	1.9361	0.775	0.5406	0.2792
		5.5040	0.273	0.0841	0.0153	2.0311	0.739	0.5730	0.2821
6 mg/l		4.2535	0.353 x	0.0344	0.0081	2.1375	0.702	0.0871	0.0407
		2.9978	0.500	0.0149	0.0050	1.6143	0.929 x	0.0959	0.0594
		3.7878	0.396	0.0503	0.0133	1.1840	1.267	0.1310	0.1110
12 mg/l		4.2470	0.353 x	0.0329	0.0077	1.7543	0.855	0.0298	0.0170
		3.4767	0.431	0.0268	0.0077	1.4339	1.046	0.0322	0.0224
		4.7251	0.317	0.0392	0.0083	1.4527	1.033 x	0.0305	0.0210
24 mg/l		3.7105	0.404	0.0370	0.0100	1.3976	1.077	0.0139	0.0099
		3.9300	0.382 x	0.0480	0.0122	1.4515	1.033 x	0.0387	0.0267
		2.4892	0.603			1.2680	1.183		

Volume of test water : 1.5 liters

/ Presented in Fig. 32 x Presented in Fig. 30

Table (44) Continued

EDTA Concentration mg/l	Exposure time, h	Total Plant		Final PH	% Removal of Cd		
		Dry Wt. gm	Pb accum. gm		by Shoot	by Root	by Total Plant
1 mg/l	One h	3.8392	0.3089	7.1	4.2267	16.3667	20.593
		6.1311	0.3625	7.1	3.1457	21.0200	24.167
	2 h	6.6564	0.4872	6.6	3.5267	28.9533	32.480
		5.6066	0.4019	6.8	2.2867	24.5087	26.793
	24 h	5.9768	0.6046	6.5	2.7	37.6133	40.313
		7.2449	0.6307	6.5	6.0067	36.0400	42.050
		7.5351	0.6571	6.4	5.6067	38.2000	43.810
6 mg/l		6.391	0.2215	6.6	8.96	5.8067	14.770
		4.621	0.1108	6.6	0.9933	6.3933	7.390
		4.9718	0.0634	6.7	3.3533	0.8733	
12 mg/l		6.0013	0.0627	6.6	2.1933	1.9866	4.180
		4.9106	0.059	6.6	1.7867	2.1487	3.930
		6.1778	0.0697	6.6	2.6133	2.0333	4.647
24 mg/l		5.1031	0.0509	6.[illegible]	2.4667	0.9287	3.393
		5.3815	0.0867	6.6	3.2000	2.5800	5.780
		3.7572		6.6			

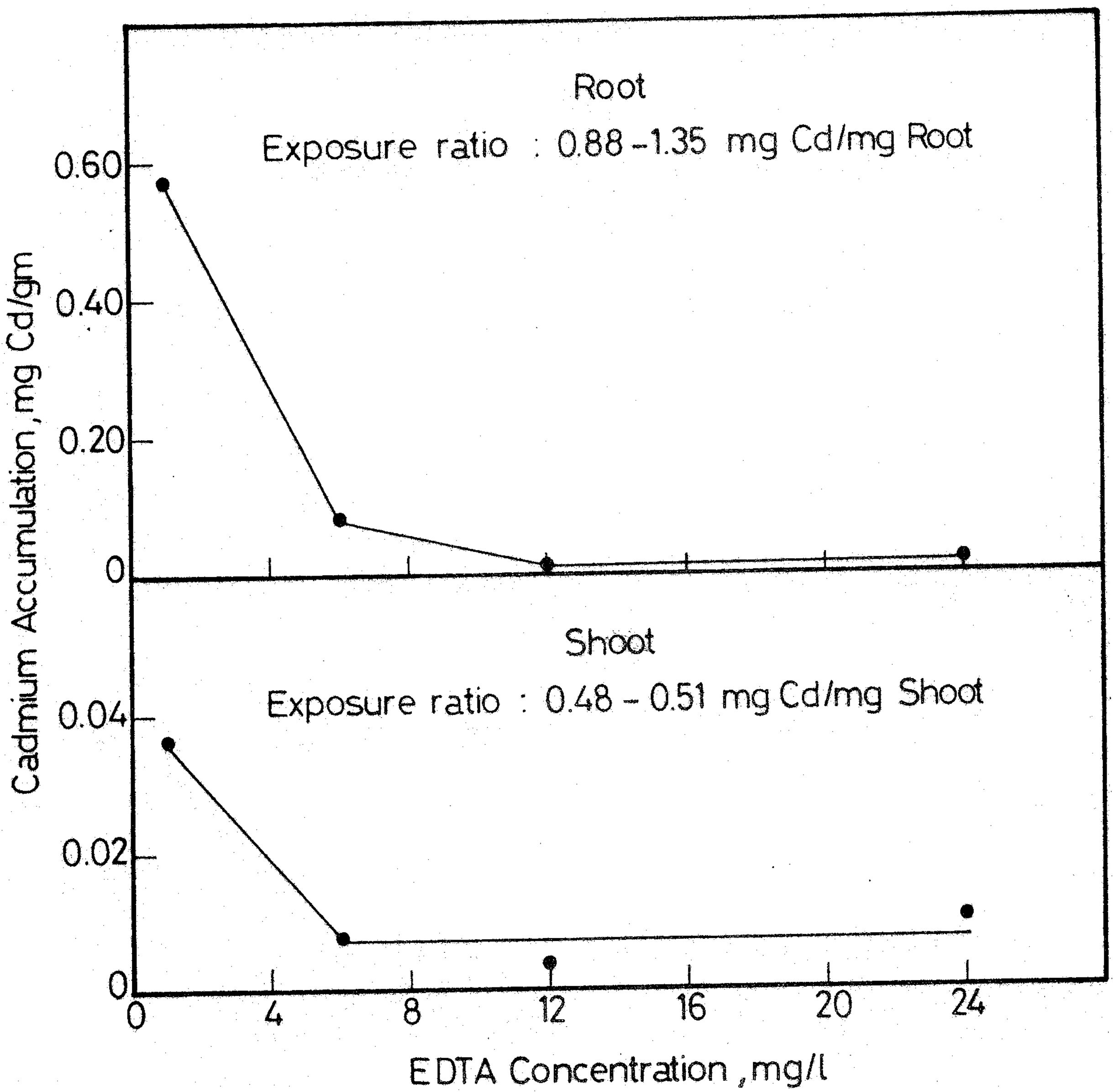


Fig. (29) Effect of EDTA Concentration on Cadmium Accumulation by Eichhornia Crassipes.

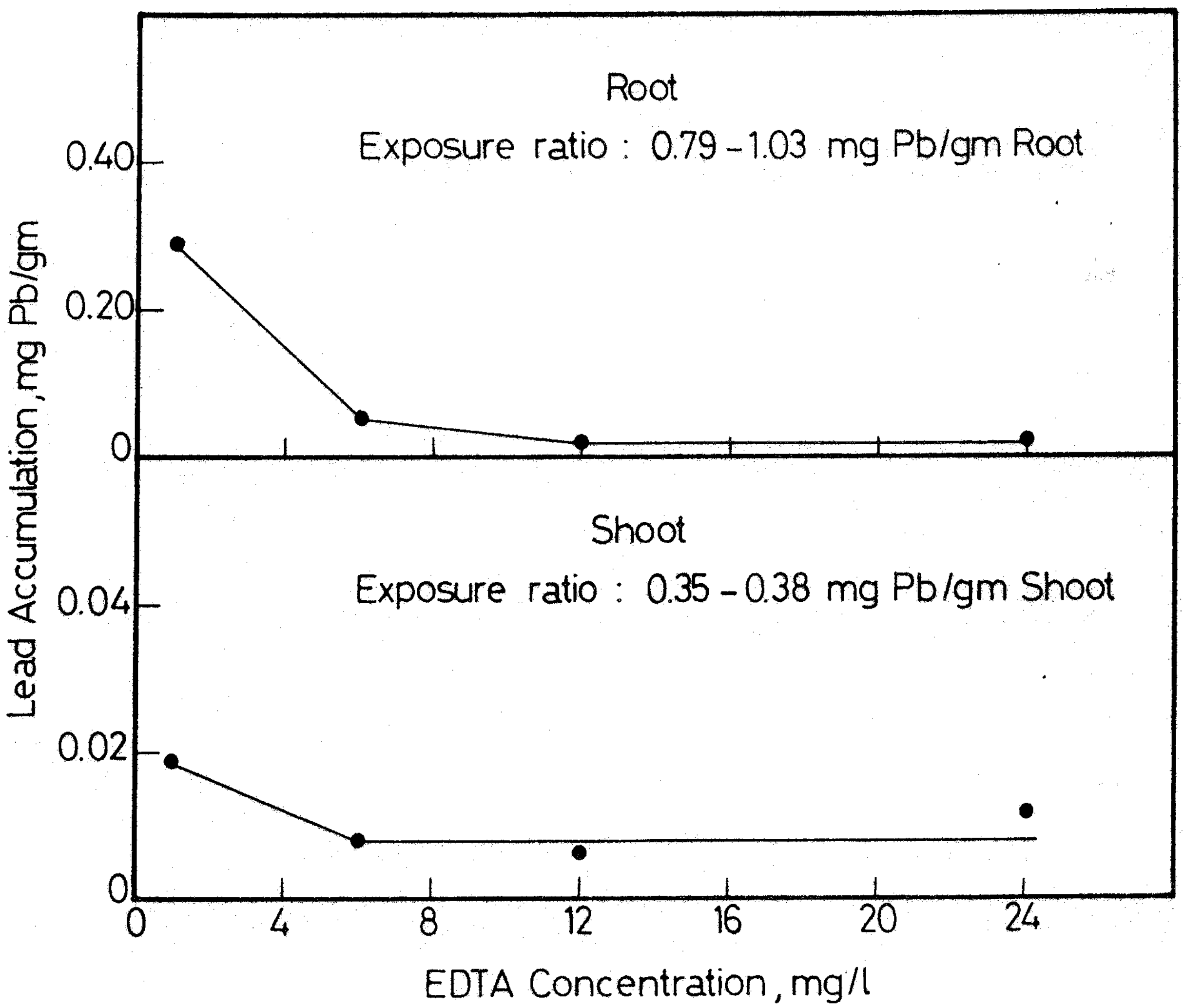


Fig. (30) Effect of EDTA Concentration on Lead Accumulation by Eichhornia Crassipes.

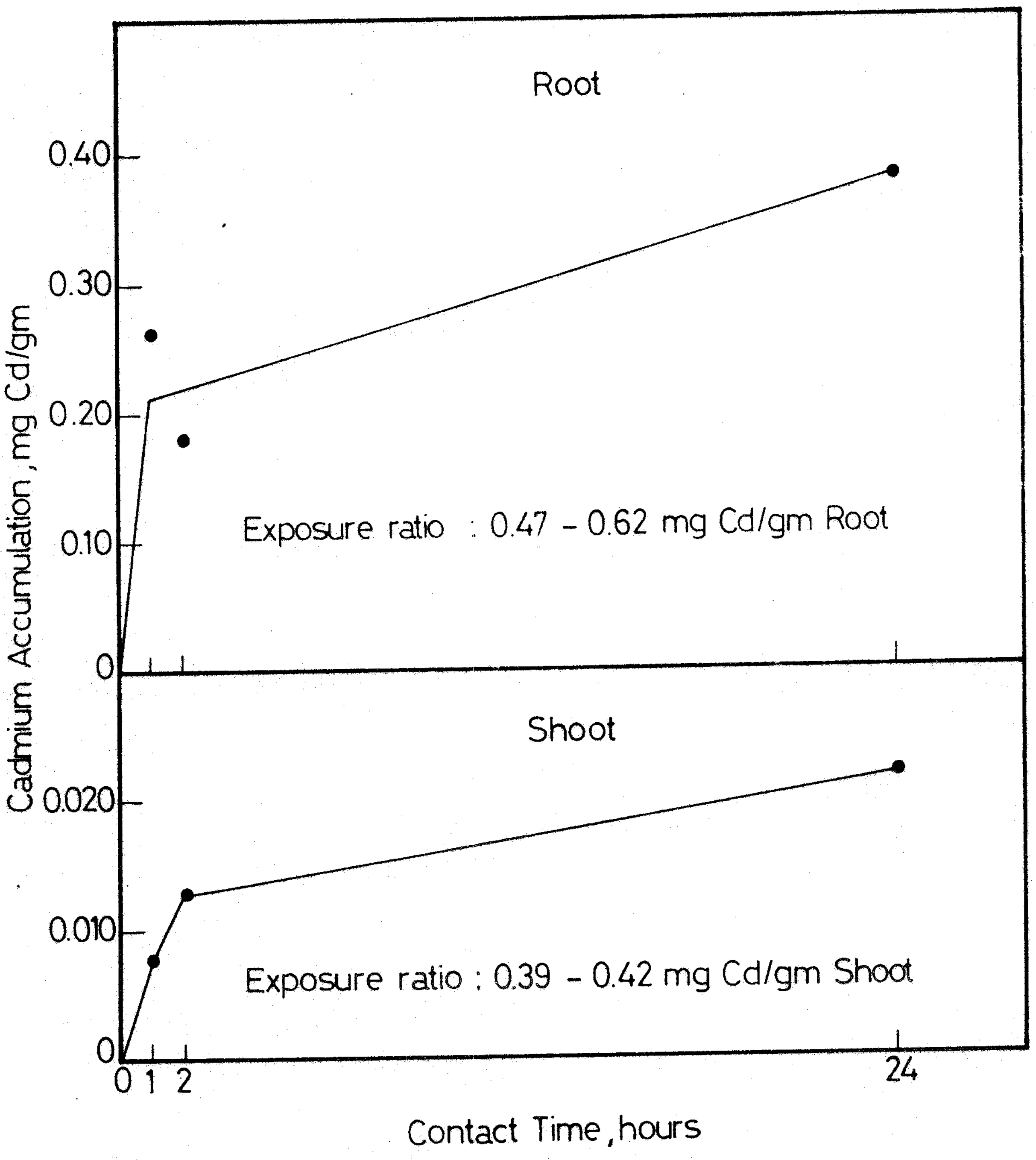


Fig. (31) Effect of Contact Time on Cadmium Accumulation by Eichhornia Crassipes.

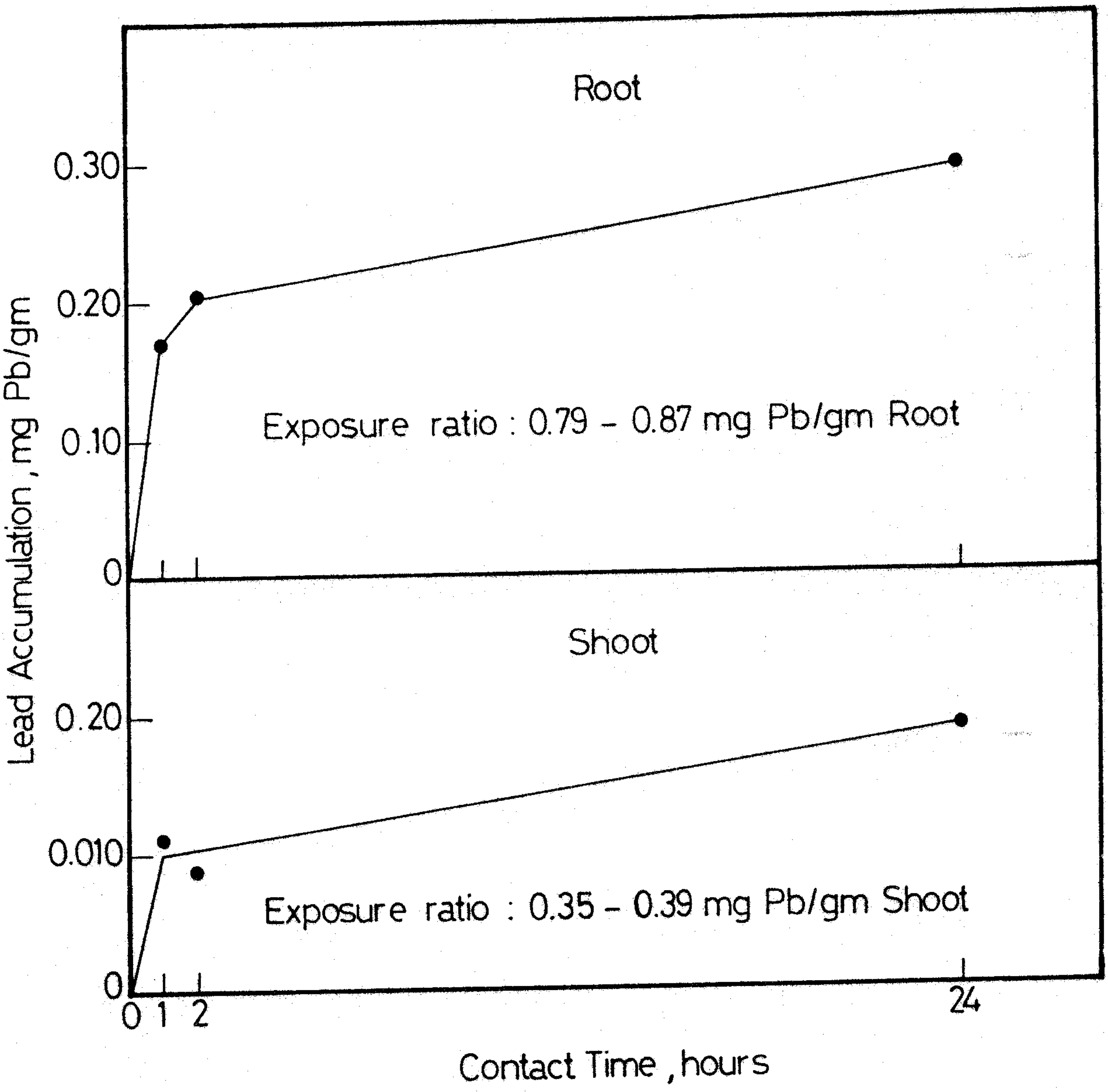


Fig. (32) Effect of Contact Time on Lead Accumulation by Eichhornia Crassipes.

of metal: plant exposure ratios about 56% of cadmium and 67% of lead accumulation in 24 hours took place in the first two hours, in both shoot and root, indicating that the theme of metal accumulation in the root was associated with a theme of metal translocation to the shoot of very similar trend.

VII.2.2.4. Accumulation of Copper, Cadmium, Zinc and Lead in Combination

Accumulation of Cu, Cd, Zn and Pb by Eichhornia crassipes from solutions containing equimolar concentrations of these metals in combination was assessed and the results obtained were compiled in table 45. Because the experimental plants differed in weights of both shoot and root metals accumulations were compared for the same metal: plant exposure ratio. Figure 33 illustrates the dependance of a metal accumulation in roots on the metal : root exposure ratio. The figure shows that there was no clear preferential accumulation among the metals in contact water though two of these metals namely Cu and Zn are nutrient elements and the other two are not.

Metal accumulations determined in this experiment were compared with those determined separtely (VII.2.2.1, tables 37-40 and Fig. 28a-c) assuming that the test plants used in the two experiment were of similar physiological state since they were taken from the same environment and in the same period. This comparison was made on same exposure ratio basis (table 46). This comparison revealed that accumulation of both copper and cadmium by the root was enhanced by the presence of the other metals while Zinc and lead accumulations

Table (45) Accumulation of Cu, Zn Cd and Pb by Eichhornia crassipes from $NaHCO_3$ Solutions (60 mg/l) Contained Varied Equimolar Concentrations of Heavy Metals

M* added mg/l	Final pH	Root									
		Dry wt gm	Expos. ratio uM/gm	Metal Accumulated							
				Cu		Zn		Cd		Pb	
				mg	uM/gm	mg	uM/gm	mg	uM/gm	mg	uM/gm
A	6.5	0.8090	3.71	0.149	2.895	0.251	4.740	0.225	2.447	0.369	2.206
	6.5	1.5900	1.89	0.201	2.030	0.333	3.196	0.272	1.521	0.395	1.197
	6.5	1.0721	2.80	0.163	2.565	0.310	4.419	0.253	2.100	0.374	1.684
B	6.5	0.9042	6.64	0.257	4.469	0.315	5.321	0.482	4.742	0.744	3.972
	6.6	0.8891	6.75	0.262	4.642	0.328	5.642	0.466	4.662	0.701	3.803
	6.6	1.1400	5.26	0.267	3.698	0.323	4.327	0.485	3.781	0.813	3.441
C	6.5	0.9134	13.14	0.426	7.333	0.473	7.905	0.834	8.123	1.272	6.723
	6.6	0.5.65	23.69	0.442	13.737	0.380	11.483	0.732	12.865	1.226	11.680
	6.5	1.4868	8.07	0.474	5.020	0.586	6.024	0.87[illegible]	5.224	1.289	4.184
D	6.5	0.8600	27.91	0.703	12.856	0.697	12.385	1.559	16.130	2.504	14.049
	6.6	0.6313	38.02	0.784	19.544	0.786	19.0[illegible]	2.621	23.318	2.714	20.743
	6.6	0.3811	62.98	0.618	25.492	0.524	21.024	1.160	27.082	2.266	28.697

*	Cu	Zn	Cd	Pb			Total M	
A:	0.1271	0.1308	0.2248	0.4144	mg/l	($2x10^{-6}$M)	0.8971	mg/l
B:	0.2542	0.2616	0.4496	0.8288	"	($4x10^{-6}$M)	1.7942	"
C:	0.5084	0.5232	0.8992	1.6576	"	($8x10^{-6}$M)	3.5883	"
D:	1.0168	1.0464	1.7984	3.3152	"	($16x10^{-6}$M)	7.1766	"

Exposure ratio = amount of metal in test water (1.5 liters) in uM devided by dry weight of exposed plant material.

M = Metal

Table (45) Continued

M* added mg/l	Dry Wt., gm	Expos. ratio, uM/gm	Shoot							
			Metal accumulated							
			Cu		Zn		Cd		Pb	
			mg	uM/gm	mg	uM/gm	mg	uM/gm	mg	uM/gm
A	1.4825	2.02	0.049	0.519	0.190	1.957	0.043	0.0258	0.037	0.121
	2.1683	1.38	0.062	0.441	0.168	1.177	0.041	0.169	0.021	0.048
	1.8739	1.60	0.049	0.409	0.126	1.024	0.026	0.124	0.038	0.096
B	2.1982	2.73	0.074	0.535	0.177	1.223	0.054	0.222	0.061	0.135
	2.2411	2.68	0.105	0.739	0.151	1.040	0.050	0.196	0.062	0.135
	2.4640	2.44	0.101	0.645	0.233	1.453	0.101	0.365	0.096	0.188
C	2.1467	5.59	0.102	0.739	0.157	1.116	0.142	0.587	0.177	0.396
	1.5151	7.92	0.146	1.603	0.147	1.483	0.135	0.792	0.176	0.560
	2.0029	5.99	0.086	0.677	0.144	1.101	0.127	0.569	0.144	0.347
D	2.3538	10.20	0.148	0.991	0.254	1.651	0.250	1.059	0.402	0.825
	1.6248	14.77	0.100	0.960	0.147	1.376	0.185	1.014	0.243	0.719
	1.2325	19.47	0.121	1.574	0.230	2.859	0.220	1.584	0.313	1.226

Table (46) Comparison Between Metal Accumulation in Combimation (1) and Separate (2)

Cu			Zn			Cd			Pb		
E.R,* mg/gm	Accumulation m g/gm		E.R. mg/gm	Accumulation mg/gm		E.R. mg/gm	Accumulation mg/gm		E.R. mg/gm	Accumulation mg/gm	
	1**	2***		1	2		1	2		1	2
0.12	0.13	0.05	0.12	0.11	0.08	0.21	0.17	0.09	0.39	0.25	0.24
0.18	0.16	0.08	0.18	0.19	0.12	0.31	0.24	0.13	0.58	0.35	0.36
0.24	0.18	0.10	0.24	0.21	0.16	0.42	0.28	0.18	0.77	0.46	0.48
0.33	0.23	0.15	0.34	0.18	0.23	0.59	0.43	0.25	1.09	0.71	0.68
0.42	0.28	0.18	0.43	0.25	0.29	0.75	0.53	0.31	1.38	0.82	0.86
0.43	0.29	0.18	0.44	0.27	0.29	0.76	0.52	0.31	1.40	0.79	0.88
0.51	0.32	0.20	0.53	0.29	0.36	0.91	0.59	0.38	1.67	0.87	1.04
0.83	0.46	0.30	0.86	0.42	0.58	1.48	0.91	0.62	2.72	1.39	1.70
1.51	0.87	0.50	1.55	0.65	1.04	2.66	1.45	1.11	4.91	1.42	3.07
1.77	0.82	0.60	1.82	0.71	1.22	3.14	1.81	1.31	5.78	2.91	3.61
2.42	1.24	0.82	2.49	1.14	1.67	4.27	2.62	1.78	7.88	4.30	4.92
4.00	1.62	1.40	4.12	1.27	2.76	7.08	3.04	2.95	13.05	5.95	7.20

* From table 45 transfered from uM metal /gm into mg/gm

** From table 45 " " " " "

*** From figure 28

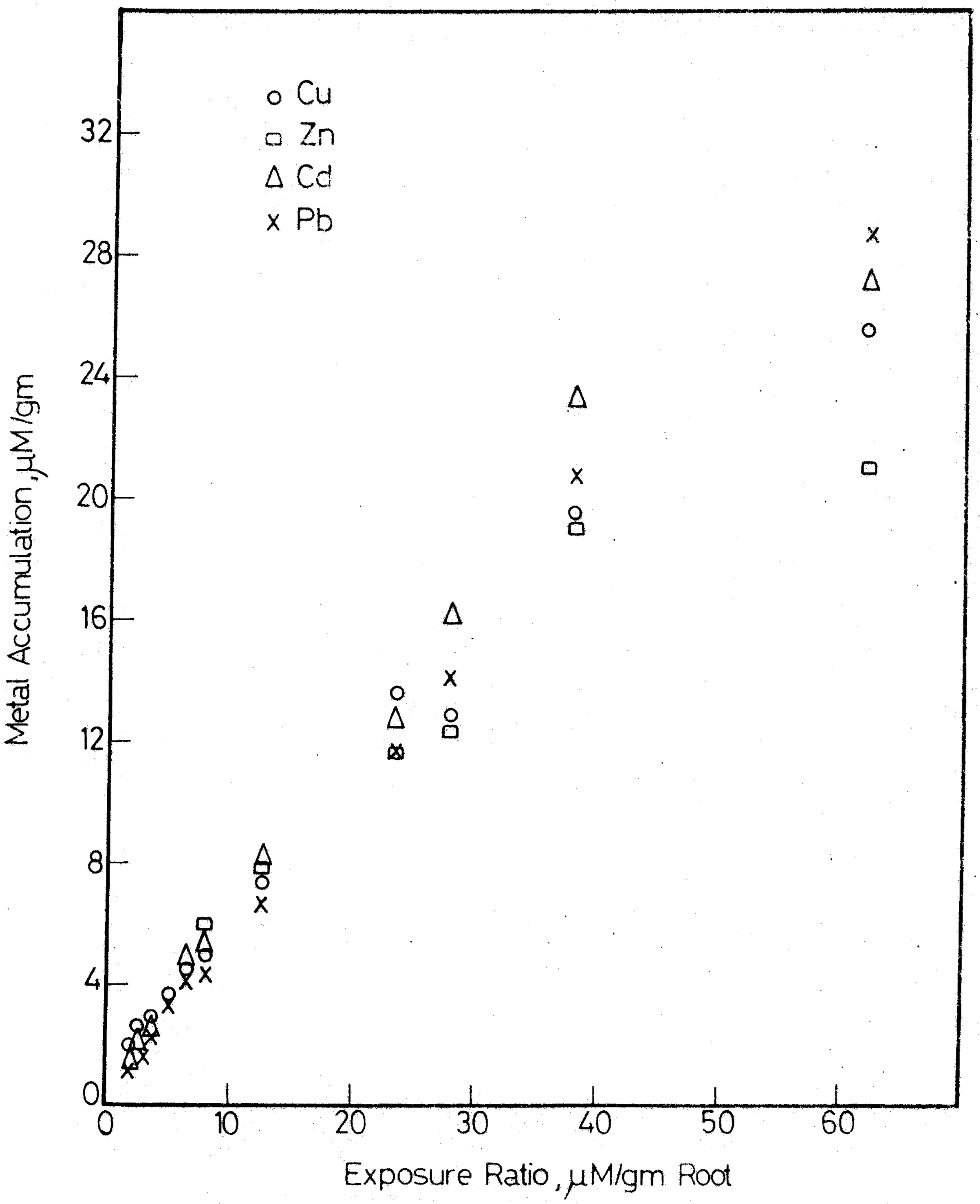


Fig. (33) Metals Accumulation in Eichhornia Root at Different Exposure Ratios.

were retatded. Also, Hutchinson and Czyrska(1975) found that both cobalt and nickel uptake were increased by the presence of other metals and they believe that part of the synergestic effects are related directly to increased metal uptake.

VII.2.3. Metal Accumulation by Eichhornia Crassipes in Continuous Plug Flow System.

During 15 days and before metals addition to the feed water, the plants showed flourishing appearance and displayed growth activities giving offsprings but in a relatively slow rate. The plants could keep their healthy appearance further on for three weeks during feeding the gutter with the settled Nile water to which heavy metals were added in equimolar concentrations. Later on, symptoms of unhealthy conditions were observed in allover the gutter. Examination of the plants showed that they were infected with the red spider Tetranychus sp. The fungi Sclerotium sp. Penicillium sp., Phycomyces sp. and Asperigullus flavus were isolated from the shoots and the roots. Chemical analysis of settled Nile water used as feed water compared with that of the effluent water that came out of the gutter after five weeks water flow is given in table 47 which shows that an increase in total dissolved salts, expressed as electric conductivity, E.C., occurred as the water passed through the gutter (detention time about 12 days). This increase in E.C. could be attributed to evaporation in addition to transpiration of the plants in gutter No. 1. It may be noticed from table 48 that the E.C. was higher by 70 and 60 umho cm^{-1} over the E.C. of the feed water in zone 1 of the gutters 1 and 2 respectively. The rise

Table (47) Physico-chemical Characteristics of Nile Water Before and After Metals Addition Compared With The Characteristics of Effluent of The Gutters After 5 Weaks Water Flow.

Characteristics	Nile Water		Effluent of	
	Raw	After Metals Addition	Gutter 1	Gutter 2
pH	7.7	7.6	7.8	7.6
E.C., u mho cm^{-1}	350	330	470	405
Total alkalinity Ca CO_3 mg/l	128	132	156	140
Chlorides, mg Cl/l	24	20	28	28
Total hardness, $CaCO_3$ mg/l	112	112	140	130
Ca hardness, $CaCO_3$ mg/l	72	70	82	82
Mg hardness, $CaCO_3$ mg/l	40	42	58	48
Nitrate, mg N/l	0.150	0.150	0.110	0.056
Phoshate, mg P/l	0.200	0.170	0.100	0.100
Sulfate, mg 50_4/l	18	18	27	24

Table (48) Changes In Electric Conductivity (u mho Cm^{-1}) of Water Along the Two Gutters After 5 Weaks Water Flow.

Gutter	Feed	Zone 1	Zone 2	Zone 3	Zone 4
1	330	400	410	435	470
2	330	390	390	400	405

in E.C. from zone 1 to zone 2 was 70 umho cm^{-1} in gutter 1 and only 15 umho cm^{-1} in gutter 2 indicating water losses by the plants.

It may be noticed also from table 47 that nitrate nitrogen was less in the effluents from the two gutters especially in gutter No. 2. This could be attributed to algal growths observed in this gutter. Table 49 and 50 present the data of temporal changes in metals concentrations in water and in plants respectively in gutter 1. It appeared that copper and lead were rapidly removed from the test water while cadmium removal was at lower rate. Copper and cadmium were not detected in water samples taken from zone 4 up to the flow of 465 litres of feed water while cadmium was detected in water samples taken from the same zone after the flow of 243 litres of feed water. This was reflected in the metal content of the roots of Eichhornia plants isolated from the gutter as shown in Fig. 34 where it appears that lead and copper content were higher than cadmium in the plants isolated from zone 1. In zone 4 where the ratio of metals concentrations changed due to differences in metal removal in the preceding zones, metal content of the roots of plants isolated from this zone was of different order of magnitude and of lower levels. Fig. 35 shows that metal translocation in the shoot followed the order Cd > Cu > Pb when the plants were exposed to equimolar concentrations of these metals in zone 1 while in zone 4 where these concentrations changed the order of translocation also changed with much lower metal contents close to the initial levels.

Table 51 shows that the surface of gutter 2 with the biological growths on it had metal removal

Table (49) Temporal and Spacial Changes in Metals Concentrations in Water Samples taken from Gutter 1

Date	Flow after Metals add.	Conc. of Metals, uM/l											
		Zone 1			Zone 2			Zone 3			Zone 4		
	L	Cd	Cu	Pb	Cd	Cu	Pb	Cd	Cu	Pb	Cd	Cu	Pb
5.27	0	0.0	0.0	0.0									
5.30	40	0.2	0.0	0.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
6.03	94	0.4	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
6.08	195	0.6	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
6.12	243	1.1	0.2	0.0	0.6	0.0	0.0	0.1	0.0	0.0	0.0	0.09	0.0
6.20	327	0.9	0.3	0.5	0.6	0.0	0.0	0.1	0.0	0.0	0.1	0.0	0.0
6.25	405	0.9	0.5	0.3							0.2	0.1	0.0
6.30	465	1.4	0.5	0.2							1.4	0.0	0.0

Table (50) Temporal and Spacial Changes in Metals Concentrations in Plants Isolated from Gutter 1

Date	Flow After metal add. L		Metal Concentration, uM/gm plant material					
			Zone 1			Zone 4		
			Cd	Cu	Pb	Cd	Cu	Pb
5.27	0	L	0.00	0.18	0.02			
		R	0.02	0.18	0.05			
5.30	40							
6.03	94							
6.08	195	L	0.41	0.34	0.20	0.03	0.23	0.07
		R	2.63	5.70	6.71	0.09	0.56	0.24
6.12	143	L	0.40	0.51	0.19	0.02	0.25	0.07
		R	3.92	6.96	8.15	0.25	0.70	0.22
6.20	327	L	0.89	0.57	0.28	0.08	0.31	0.06
		R	5.14	12.09	14.53	0.93	1.58	0.48
6.30	465	L	0.99	0.84	0.64	0.19	0.35	0.09
		R	5.58	16.19	24.67	3.81	1.92	0.60

L : Shoot R : Root

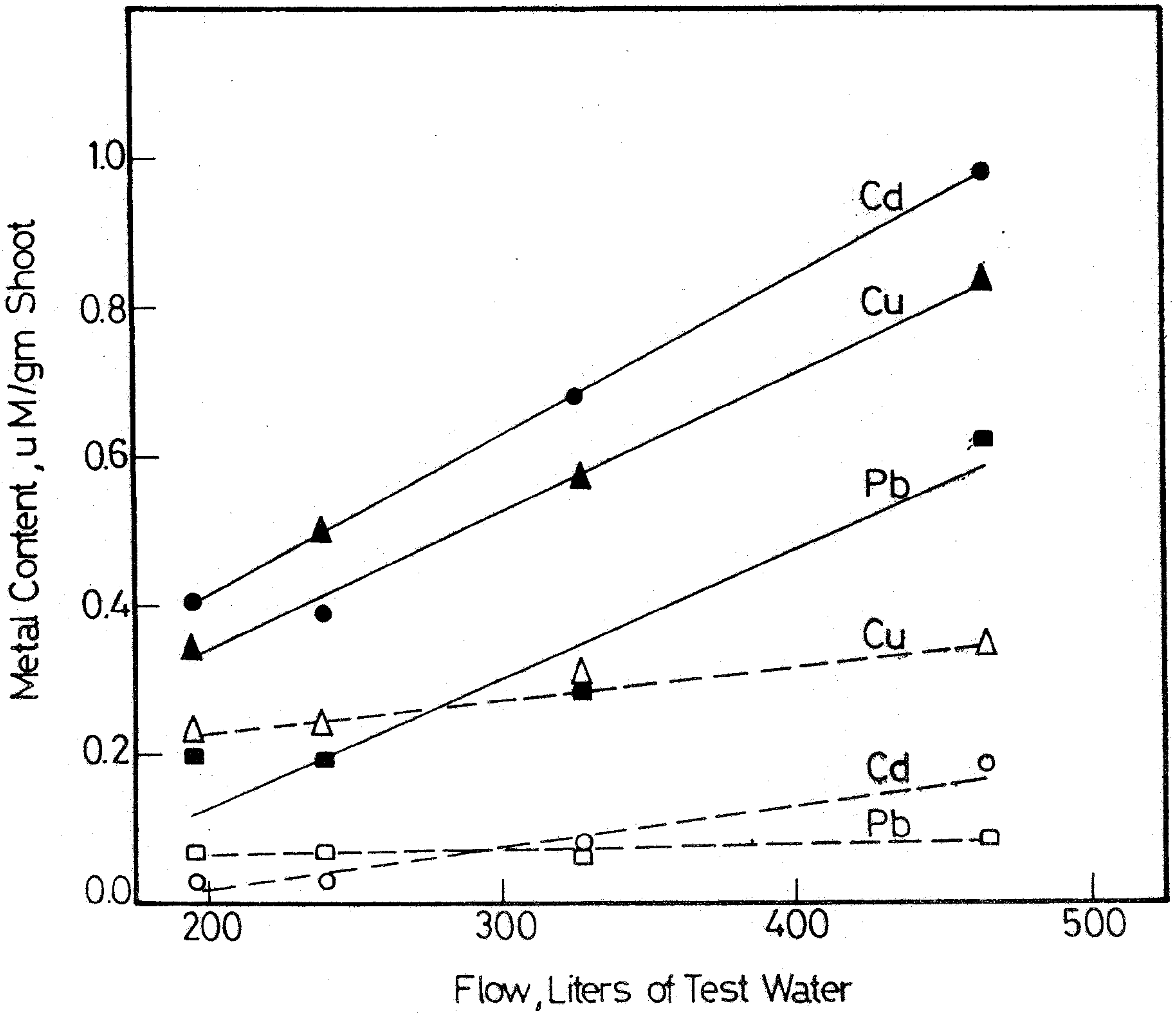


Fig. (34) Metal Content of Eichhornia Shoots Isolated from the Ends of Zone 1 (●, ▲ and ■) and Zone 4 (○, △ and □) During Test Water Flow.

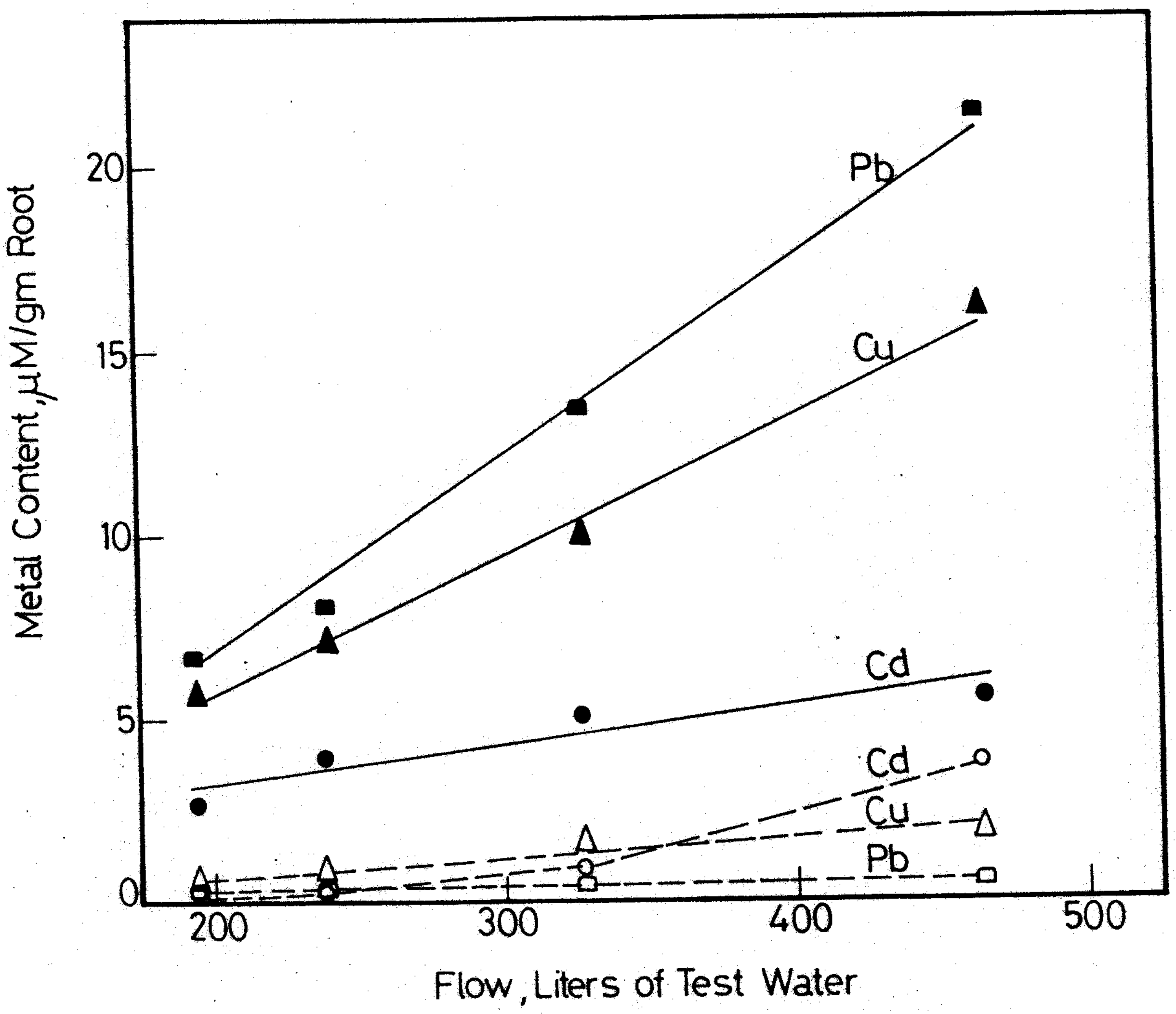


Fig. (35) Metal Content of Eichhornia Roots Isolated from the Ends of Zone 1 (● , ▲ and ■) and Zone 4 (○, Δ and □) During Test Water Flow.

Teble (51) Temporal and Spacial Changes in Metal Concentration in Water in Gutter 2

Date	Flow after metal add. L	Metals Concentration, uM/l											
		Zone 1			Zone 2			Zone 3			Zone 4		
		Cd	Cu	Pb	Cd	Cu	Pb	Cd	Cu	Pb	Cd	Cu	Pb
4.27		0.0	0.0	0.0	-	-	-	-	-	-	-	-	-
5.30	40	0.1	0.0	0.0	0.1	0.0	0.0	0.1	0.0	0.0	0.1	0.0	0.0
6.03	94	0.5	0.0	0.0	0.4	0.0	0.0	0.4	0.0	0.0	0.4	0.0	0.0
6.08	195	0.6	0.0	0.0	0.6	0.0	0.0	0.6	0.0	0.0	0.5	0.0	0.0
6.12	243	1.3	0.9	0.0	1.0	1.1	0.0	0.9	0.8	0.0	1.0	0.8	0.0
6.20	327	1.0	0.6	0.6	1.0	0.6	0.5	0.9	0.5	0.5	1.0	0.7	0.9
6.25	405	0.9	0.7	0.6							0.9	0.6	0.9
6.30	465	1.1	1.0	0.7							1.3	0.8	0.9

capacity too, however, the results obtained from gutter 1 indicated that metal accumulation by the test plants in gutter 1 was overwhelming.

VII.2.4. Metals Accumulation by *Eichhornia* Crassipes in a Continuous Flow Completely Mixed System

In this system detention time was varying between 6.45 and 6.67 days. As in the plug flow system (VII.2.3.) cadmium was the first metal to appear in the experimental container. On the date 6.03 (table 52) the water in the container must have been completely replaced since 48.2 liters flowed through it. Five days later cadmium started to appear in water samples from the containter at 38% of the initial concentration of the feed water. Meanwhile the plants isolated from the container in that date contained the three metals. When the experiment was terminated the order of magnitude of metal content in the roots of exposed plants was as follows: Pb$\rangle$ Cu$\rangle$ Cd. which was similar to that occurred in the plug flow system. It appears from the table that metal translocation took place in the shoots of the plants and the concentration of translocated metals increased with the increase in the amount of test water that flowed through the container.

From the results obtained from VII.2.3. and VII.2.4. it appeared that both the two systems can be efficient in metals removal from water and reduction of metal content to zero concentration in the effluent can be reached by taking into consideration the following factors:

1. metal: plant exposure ratio
2. water flow rate.

Tables (52) Temporal Changes in Metals Concentrations in Water and in Plant in the Continuous Flow Mixed System.

Date	Flow (L) after metal add.	Conc. of Metals in dish water, uM/l				Conc. of Metals in plant uM/gm		
		Cd	Cu	Pb		Cd	Cu	Pb
4.27	0	0.00	0.03	0.00	L	0.00	0.16	0.02
					R	0.02	0.16	0.05
5.30	19.8	0.00	0.00	0.00				
6.03	48.2	0.00	0.00	0.00				
6.08	80.2	0.19	0.00	0.00	L	0.23	0.39	0.10
					R	2.36	2.35	1.99
6.14	116.8	0.61	0.52	-	L	0.29	0.39	0.12
					R	4.31	5.28	6.14
6.20	153.4	0.85	0.42	0.45	L	0.99	0.63	0.27
					R	7.14	12.03	13.15

L : Shoot

R : Root

Concentrations of metals in feed water was 0.5 x 10^{-6} M raised to 10^{-6} M on 6.08.

VIII. CONCLUSIONS AND RECOMMENDATIONS

The outcome from this work provides indications that lead to the following conclusions and recommendations:

1. Heavy metals complexes with inorganic ligands are readily accumulated in algae.

2. Metal accumulation by algae can take place,without involvement of biological processes,as a physical phenomenon. Accumulated metal is tightly bound to algal surface and are not released easily, but chelating agents can mobilize metals accumulated by algae.

3. Chelating agents can be good competitors with algal surface for metal ions. Metal complexes with chelating compounds do not have such affinity to algal surface as do complexes with inorganic ligands. Therefore, algae are not expected to be good accumulators for metals in the ponds that are designed to receive organic wastewaters of a metal chelating power.

4. Calcium and magnesium do not compete with heavy metals on algal surface. Their presence in the soft water level does not retard metal chelation.

5. Metal accumulation is a function of metal: algae exposure ratio. Algae can carry as much as 1.2 and 1.6% of its dry weight cadmium and lead respectively. This makes algae a potential pollutant carrier and the limits of their number in drinking water should be revised.

6. The physiological state and metabolic activity of algae are important factors in metal accumulation

by algae. It is recommended to keep algae in an active growth state in order to achieve maximum metal removal.

7. Metabolites released in growing algal suspensions interfere with metal accumulation by algae. For the removal of metals in a mixed continuous flow system it is recommended to select the dilution rate that results into the maximum algal concentration with the least concentration of algal metabolites. However failure of the algal system could be expected due to toxicity especially in case of high cadmium algae:exposure ratios.

8. The root of water hyacinth is a good accumulator for heavy metals and accumulation is a function of metal: plant material exposure ratio.

9. As with algae, metal complexes with inorganic ligands are readily accumulated on exposed plant surfaces.

10. In presence of chelating agents in water metal accumulation by the plant is retarded. Therefore, aquatic plants are not expected to be a good accumulator for heavy metals in ponds designed to receive wastewaters carrying organic chelators.

11. Translocation of the investigated metals from the root of <u>Eichhornia</u> crassipes to the shoot is very weak. The root can carry up to 0.3 and 1.1% of its dry weight cadmium and lead respectively. Therefore, it is recommended not to use the roots of such plants grown in water bodies polluted by heavy metals as a fodder for animals.

12. Presence of calcium and magnesium in as much as the level of moderate hard waters (e.g. Nile water) does not interfere with metal accumulation.

13. Analysis of aquatic plants for heavy metal content is a good mean for assessing recent pollution of an aquatic ecosystem by heavy metals.

14. Heavy metal content of a water can be reduced to zero in continuous flow systems, the plug flow and the completely mixed systems, taking into consideration the metal: plant ratio and the detention time. It is easier to manage the plug flow system where the plants adjacent to the point of wastewater discharge will be removed periodically. However, cadmium among the tested metals will be the first to appear in the effluent water.

15. Aquatic weeds existing around the point of discharge of a wastewater into a water supply play beneficial role in the aquatic ecosystem. They act as scavengers for dangerous pollutants such as heavy metals. These plants should not be eradicated from these places but can be controlled. The exceeding masses might be removed and disposed by inceneration in a controlled area or utilized in a useful way taking into consideration their high metal content.

Water ways authorities should not consider aquatic plants as foes but as an essential element for a balanced ecosystem. They should be controlled where they are really troublesome. Its control should be limited to mechanical removal with the purpose of clearing the flow in water ways.

16. The fate of heavy metals discharged into a water supply is determined primarily by suspended and submerged matter of which phytoplankton and macrophytes are of special importance since they constitute food for the successive tropic levels.

For example a permissible level of 0.5 ug Cd/l in a water supply may give rise to 0.135 gm cadmium per Kg dry fish caught from this water supposing concentration factors of 270000 through the food chain. Therefore permissible limits of toxic substances in water supplies should be revised taking into consideration their affinity to be accumulated in the food chain.

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