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TABLE OF CONTENTS

TABLE OF CONTENTS (cont'd)

TABLE OF CONTENTS (cont'd)

LIST OF TABLES

LIST OF TABLES (Cont'd)

LIST OF FIGURES

LIST OF FIGURES (Cont'd)

ACKNOWLEDGEMENTS

I would like to express my gratitude to the people who helped and encouraged me in the course of doing this work.

Special thanks are extended to Dr. D.J. Kushner and Dr. S. Ramamoorthy for their guidance, to Dr. C. Breuil for her helpful comments and discussion and to my husband, Jan, for his patience and understanding.

ABBREVIATIONS

A. braunii	Ankistrodesmus braunii
A. flos-aquae	Anabaena flos-aquae
AAS	atomic absorption spectroscopy
Cu	copper
Cd	cadmium
g	grams or times gravity
HM(s)	heavy metal(s)
ppm	parts per million
OD/ 650	absorbance measured at a wavelength of 650 nanometers

ABSTRACT

The experiments described in this study examine the effects of HMs on two algae, Anabaena flos-aquae and Ankistrodesmus braunii and the competition for HMs between these algae and clay sediment. Cell lysis followed by normal growth, was observed when 10^{-5}M $Cu(NO_3)_2$ was added to growing cultures of A. flos-aquae. Time course AAS studies during these additions showed that all of the added Cu, some of which had been associated with A. flos-aquae cells prior to and during lysis, was present in the supernatant when normal growth resumed. 10^{-5}M $(Cu(NO_3)_2$ additions had no effect on late log phase cultures, whereas 10^{-4}M additions in all cases resulted in cellular lysis and no growth resumption. In the former case no Cu was found associated with the cells.

Microscopic examination of the above cultures revealed that a disruption in the algal chain structure and cellular clumping, as well as cellular lysis, occurred in response to the Cu^{2+} additions.

'Cu^{2+} specific electrode' studies during A. flos-aquae growth in a nutritionally diluted medium showed that the alga released Cu^{2+} complexing substances during the late log phase of growth.

In the presence of increasing amounts of Cu and Cd, A. braunii displayed progressively decreasing growth rates. No growth occurred in 10^{-4}M or 10^{-3}M $Cd(NO_3)_2$ and 10^{-3}M $Cu(NO_3)_2$.

Additions of 10^{-5}M $Cu(NO_3)_2$ to growing A. braunii cultures also resulted in decreased growth rates.

AAS analyses showed that progressively more Cu was associated with the algal cells grown in the presence of increasing Cu concentrations. At higher concentrations, the amount of Cu associated per cell remained the same throughout growth. In the presence of 10^{-5}M Cu, 1.47 $\pm$ 0.08 x 10^{-8} µg Cu was found per cell. With 10^{-4}M Cu present, 6.81 $\pm$ 0.17 x 10^{-8} µg Cu was associated per cell. On the other hand the HM per cell decreased during A. braunii growth in the presence of 10^{-6}M or 10^{-5}M Cd.

The cell associated HM in A. braunii cultures grown in the presence of 10^{-5}M or 10^{-4}M Cu or 10^{-5}M Cd was distributed between the cell wall and cytoplasmic fractions. About two-thirds of the HM was associated with the cellular contents and one-third with the cell envelope.

In a simulated aquatic system, the A. braunii compartment competed well with the sediment in accumulating Cu^{2+} and Cd^{2+} from the water compartment. On the other hand, cellular lysis occurred when A. flos-aquae was exposed to 1 ppm Cu^{2+} or Cd^{2+} in the water column. Initial accumulation by the algal compartment, prior to massive lysis, can be attributed to A. flos-aquae cells and/or their extracellular products.

Further experiments in the simulated ecosystem showed

mobilization and accumulation of sediment bound Cu and Cd by the two algal compartments. Such mobilization was also found to occur during growth of the two algae. A. braunii and A. flos-aquae cells tended to accumulate the mobilized Cd. The former alga also accumulated Cu. On the other hand, Cu was found predominantly in the supernatant of A. flos-aquae cultures.

Growth was unaffected when A. braunii was cultured in the presence of natural or HM loaded sediment. In contrast, a decrease in the growth rate of A. flos-aquae was seen in all cases with maximum inhibition occurring during growth in the presence of Cd-loaded sediment.

INTRODUCTION

The economic and technical benefits derived from using heavy metals (HMs) has made them virtually indispensible to our economy. The advantages, however, are accompanied by drawbacks. One such drawback which has become blatantly obvious in the last few years is the generation of waste materials containing HMs and HM compounds. These wastes are invariably discharged into the environment.

Cadmium (Cd) and copper (Cu) are two HMs whose extensive use has made them major environmental contaminants.

Copper*

i) Utilization

Initially utilized in the production of tools (SMITH, 1959) Cu is now being used in a wide variety of ways. Its properties of high electrical and thermal conductivity, ability to enter into alloys, maleability and tensile strength, corrosion resistance and good electro-deposition make it invaluable in many industrial processes.

Since it is the most practical material for the generation and transmission of electricity, half of the total Cu consumed goes into the electrical industry. Subdivisions of this industry

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*The main references used for Cu information were: BOWEN and GUNATILAKA, 1977; VALLEE, 1975, and SCHEINBERG et al., 1977.

include cables, wires, motor, generators and electrodes, just to mention a few.

Utilization of Cu in the engineering, building and transportation industries accounts for approximately 40% of the total Cu used. The remaining 10% is used in domestic consumer products such as refrigerators, washing machines, pots and pans, etc.

Cu is also consumed in the production of ammunition as well as money. Cu salts are found useful as fungicides in agriculture and in algal control operations.

ii) Environmental contamination

The production of Cu in Canada is substantial. For example, 7.47×10^5 metric tons of Cu were mined and smelted in Canada in 1976. Since these processes are reported to be approximately 90% efficient (RICKARD, 1970), production in 1976 was accompanied by a loss amounting to around 7.47×10^4 metric tons. The stress placed on the environment by such a loss cannot be considered negligible.

Most of the Cu lost to the environment during production is in the form of sulfides and oxides in mine waters, mill tailings (WHITE and RULE, 1971) and air emissions (VANDERGRIFT et al., 1971). Such losses, however, are being controlled to some extent by mandatory pollution abatement devices as well as the increasing economical capture of Cu.

The manufacturing of Cu bearing materials also results in the discharge of Cu into the environment. One statistic, although somewhat dated, is nonetheless noteworthy. Vaughan and Harlow (1970) reported that in 1968 the Ford Motor Company factory at Monroe, Michigan was depositing 700 lbs of Cu into the Raisin River daily! This gives a rough idea of the possible release of Cu during manufacturing processes.

Currently, Cu reclamation from industrial wastes is being attempted. If moderate success is achieved, potential Cu pollution will be considerably reduced. However, because the total amount of Cu being used in manufacturing is increasing, losses will increase proportionally.

Corrosion of Cu containing wastes as well as of Cu containing materials in use, is also a contributing factor in the contamination of the environment.

Cadmium*

i) Utilization

Cd is used in a wide variety of materials. The greatest proportion of Cd is consumed in electroplating (55-60%) and pigment production (30-35%). These pigments are used in paints, printing inks and plastics. Cd is used extensively as a stabilizer for polyvinyl chloride plastics and as a component of low-

*The main references used for Cd information were: FLEISCHER et al., 1974; FRIBERG et al., 1974; McCAULL,1971; and MALIN, 1971.

melting alloys and batteries. It is also used in nuclear control rods, fluorescent and TV tubes and ceramics.

ii) Environmental contamination

Canada produced 2.986×10^6 lbs of Cd and imported 5.01×10^4 lbs of the refined metal in 1976; no ore was imported. 5.8×10^4 lbs of Cd were used in electroplating and 6.1×10^4 lbs were consumed in the manufacturing of pigments, alloys, etc. in the same year.* Assuming that the losses during mining and production are similar in Canada and the USA and that these did not change markedly between 1974 and 1976, the relative order of magnitude of the environmental discharges of Cd can be calculated using figure 1. The figure, a revised version of the original, has the above-mentioned Canadian data incorporated into it.

Major Cd losses tend to occur in the overburden during the concentration of ores (9.4×10^6 lbs in 1976) and in the atmospheric emissions during ore processing (3.0×10^6 lbs in 1976). The latter losses are attributed to the high vapor pressure of Cd (16 mm at 500°C). In fact, because the vapor pressure of Cd is considerably higher than that of Zn (1 mm at 500°C), major recovery of Cd as a by-product of the Zn-reduction process is carried out. The actual magnitude of emissions is, of course, dependent on a number of factors, the most significant being the particulate collection devices of the plant and the

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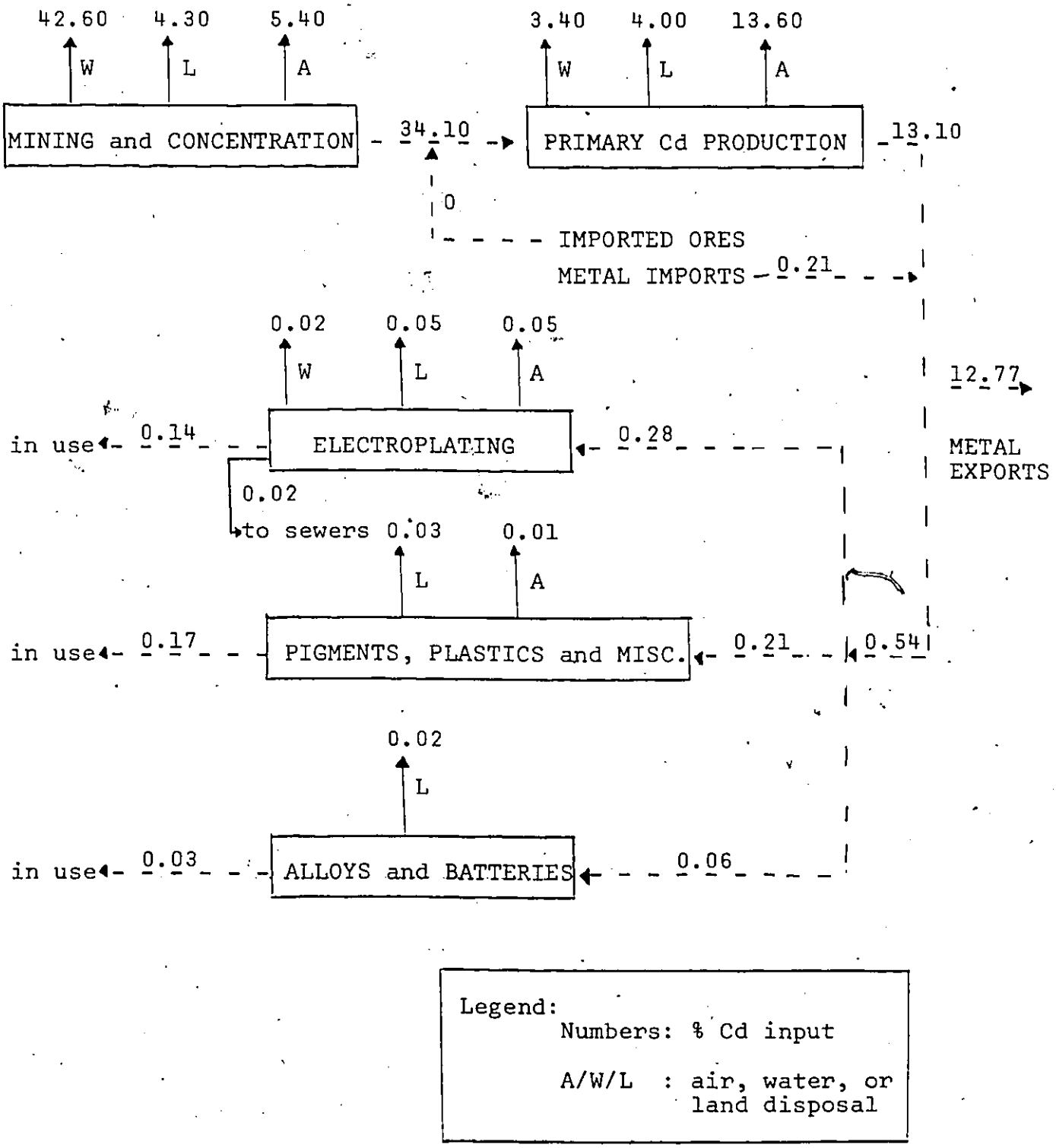
*Data was obtained from Statistics Canada.

FIGURE 1

Cadmium release into the environment*



*Taken and revised from Fleischer et al. (1974)

initial Cd concentration in the parent ore.

The industrial utilization of Cd in electroplating as well as pigment, plastic, alloy and battery production also results in environmental losses of Cd (Figure 1). For example, land disposal and atmospheric emissions are major Cd releasing routes in the electroplating industry. Rough calculations indicate that such discharges were in the range of 1.1×10^4 lbs in 1976.

The use of Cd containing materials such as the wear of rubber tires results in environmental Cd loss (LANGERWERFF and SPRECHT, 1970). Greater losses, however, are attributed to the disposal of materials after use as, for example, in the incineration of plastics. The recycling of scrap metals can also be placed in the latter category. Because it is not economically feasible to recycle Cd, atmospheric loss is reported to be quite extensive. This occurs, for example, when Cd-coated ferrous scrap is reprocessed.

Fossil fuel combustion (BERTINE and GOLDBERG, 1971), phosphate fertilization of soils and sewage disposal (REGAN and PETERS, 1970) all add to the Cd content of the environment.

Man-inspired activities are the major source of Cu and Cd environmental contamination. However, it should be mentioned that natural geochemical erosion and weathering can also increase the levels of these HMs in the environment. This is a more

common phenomenon with Cu than with Cd due to the higher concentration of Cu in the earth's crust (Cu: 55 ppm/ Cd: 0.2 ppm).

These environmental contaminants cause concern because of their non-biodegradable nature and toxicity. Consequently, questions are being asked regarding their persistence in the environment, their accumulation and availability in environmental sinks and their concentration in and effect upon components of the food chain.

Atmospheric Cu and Cd

Atmospheric concentrations of Cu and Cd vary considerably. Nevertheless, a general trend is evident. Due to the concentration of emission sources in urban areas, the air above such centres contains considerably more HMs than the air above rural districts. Average Cd concentrations ranging from 0.002 to 0.37 $\mu g/m^3$ and from 0.004 to 0.026 $\mu g/m^3$ have been recorded for a number of US cities and rural areas respectively. The atmospheric Cu content of urban districts ranges from 0.01 to 0.57 $\mu g/m^3$ whereas rural air concentrations are somewhat lower, averaging between 0.01 and 0.25 $\mu g/m^3$ (SCHROEDER, 1970).

HMs in aerosol format may be accumulated by living biological material (BUCHAUER, 1973). The more common fate of atmospheric HMs, however, tends to be deposition. Deposition

of Cu and Cd emissions follows the general pattern of suspended matter in atmospheric discharges. Thus, a large percentage of the emissions is quickly deposited after being released. Undoubtedly, this is a contributing factor to the high Cu and Cd concentrations of the vegetation, soil and water in the vicinity of point emission sources (COSTESQUE and HUTCHINSON, 1972).

Cu and Cd in soils

Uncontaminated soils have been reported to have a Cd level of between 0.01 and 0.7 ppm (HEM, 1972) and a Cu level of around 20 ppm (COSTESQUE and HUTCHINSON, 1972). Polluted counterparts may contain as much as 1750 ppm Cd and 2000 ppm Cu (BUCHAUER, 1973).

The fate of HMs in soils is dependent on a number of physico-chemical properties of the soil, i.e. pH, O-R potential, organic content, and on the properties of the HM. Both Cu and Cd tend to be more mobile under acidic conditions (LANGERWERFF and SPRECHT, 1970). This type of mobility in fact has become evident in the last few years as a result of the increase in the acidity of rainfall and snow (LIKENS and BORMANN, 1974; JONASSON, 1973). The trend, undoubtedly, has resulted in much leaching of Cu and Cd from soils as well as in an increase of the HM content of run-off waters. Such run-off may be instrumental in contaminating larger ground areas as well as

natural waters.

In impeded drainage conditions, mobilized HMs remain in the soil and may be readily available to plants. When excessively high levels are available, plants may accumulate the HMs and show symptoms of toxicity (DAS GUPTA and MUKHERJI, 1977). A major consequence of such HM accumulation was reported to have occurred in Japan. The human consumption of rice grown in soils polluted with Cd was supposedly one of the major factors which led to the outbreak of the infamous Itai-Itai epidemic (KOBAYASHI, 1972). In fact, high concentrations of Cd were present in soils because the land was irrigated with heavily polluted waters.

HMs in aquatic systems

i) Cu and Cd in water and sediment

The chemical state of HMs in water is dependent on the Eh-pH conditions, the amount of suspended matter, and the complexing capacity of the water. For example, at the pH of most fresh waters, between 6 and 8.5, little Cu is present in the cupric ion form. It tends to be complexed, predominantly with carbonate and organic substances. In addition, malachite ($Cu(OH)_2CO_3$) may be found in equilibrium with the soluble Cu (STIFF, 1971; SYLVA, 1976). In contrast, a large fraction of the total Cd found in uncontaminated as well as polluted fresh waters is present in the free Cd ion form (GARDINER, 1974).

Complexation, either organic or inorganic, does not play a major role in Cd speciation in natural waters. However, the precipitated greenockite form of Cd (CdS) may be present to some extent (HEM, 1972).

Irrespective of the speciation, very little Cu or Cd is present in natural waters when compared to the absolute holding capacity of the water for the two HMs. The average background levels of Cu and Cd in freshwaters are 7 ppb (RICKARD, 1970) and less than 1 ppb respectively. Even under conditions of pollution, the values are not much higher. These low concentrations are due to the fact that the vast majority of the HMs entering waters are accumulated by sedimentary sinks. The results obtained by DeGroot and Allersma (1975) clearly illustrate this point. 600 ppm Cu and 45 ppm Cd were present in Rhine River sediments compared to low ppb values of the same HMs present in the water column.

A variety of HM accumulating mechanisms have been suggested for sediment. Cationic sorption, irreversible sorption by sulfide surfaces, coordination complex formation with organic matter, i.e. humic substances and porphyrins, and formation of organometallic compounds are all possibilities. The actual means and effectiveness of sedimentary accumulation depend on the sediment's characteristics. Surface area, organic content and cation exchange properties of sediment have been shown to be valid indices of HM sorption (RAMAMOORTHY and RUST, 1976).

The properties of the HMs being accumulated must, however, also be considered. Cd and Cu, being soft acids, tend to associate with soft bases (AHRLAND, 1966). Consequently, sediments rich in organic nitrogeneous compounds and sulfides will tend to concentrate these two HMs. In fact, Timperley and Allan (1974) demonstrated that Cu^{2+} was accumulated by such sediments.

ii) Cu and Cd interactions with algae

HMs in aquatic systems, besides interacting with the water and sediment, also interact with the biota. Experiments have shown that excessive amounts of Cu^{2+} and Cd^{2+} may adversely affect the components of the aquatic biota (BAUDOUIN and SCOPPA, 1974; BRUNGS et al., 1976) including algae. Algal exposure to Cu^{2+}, or growth in the presence of Cu^{2+} may affect photosynthesis (STEEMANN NIELSEN et al., 1969), respiration (McBRIEN and HASSALL, 1967) and nitrogen fixation (HORNE and GOLDMAN, 1974). Changes in algal growth patterns in response to Cu^{2+}, such as decreased growth rates (STOKES et al., 1973) and extended lag periods (STEEMANN NIELSEN and WIUM-ANDERSEN, 1971) have also been observed. Cd^{2+} likewise has been shown to affect algal photosynthesis (OVERNELL, 1975) and growth (CONWAY, 1978).

Field studies have provided ecological insight into the effects of HMs on the aquatic biota. Changes in fauna and flora have been shown to occur in response to high concentrations of HMs (WHITTON, 1970). Changes in the predominant algal species

downstream from a Cu factory, occurred in the Churit River in England (BUTCHER, 1955) whereas a more drastic algal response to Cu^{2+} - low species diversity and numbers - was observed in lakes in the Sudbury region (STOKES et al., 1973).

HMs may also affect the aquatic ecosystem in a more subtle way. Algae, being primary producers, form a low rung on the food-chain ladder. Consequently, algal accumulation of HMs, known to occur with Cu^{2+} (HASSALL, 1963) and Cd^{2+} (COSSA, 1976), may lead to biomagnification of the HMs (KING, 1977). Such a transfer through the food chain to higher organisms constitutes a potential danger to man.

Besides affecting the ecosystem, HMs may themselves be acted upon. It has been proposed that ionic Cu is the biologically toxic form of the HM whereas organic complexes are virtually innocuous (STEEMANN NIELSEN and WIUM-ANDERSEN, 1970). Normally occurring algal extracellular secretions such as polypeptides (FOGG, 1966), polysaccharides (WANG and TISCHER, 1973) and hydroxamates (MURPHY et al., 1976) may play a role in HM detoxification by complexing Cd^{2+} and Cu^{2+} (FOGG and WESTLAKE, 1955). An organic release from algal cells in response to Cu^{2+} and subsequent Cu detoxification has also been suggested (STEEMANN NIELSEN and WIUM-ANDERSEN, 1971).

iii) Objectives of the research

Experiments were carried out in the laboratory to

investigate the interaction of two different algal species with HMs. One of the major objectives of these studies was to determine whether a release of organic substances constitutes a defense mechanism for these algae.

In addition, competition studies involving algae, sediment and water were conducted in a simulated aquatic system. Although, as mentioned previously, both algae and sediment accumulate HMs, it is not clear how well algae compete with sediment in accumulating HMs from water and if algae can withdraw bound HMs from sediment. Consequently, these questions were investigated.

EXPERIMENTAL METHODS AND MATERIALS

1. Principle Analytical Technique: Atomic Absorption Spectroscopy

i) General theory and introduction

Atomic absorption spectroscopy (AAS), used in this research for the detection of Cu and Cd in water, sediment and algal samples, is based on light absorption by atoms. Electromagnetic radiation of an appropriate wave-length provides the correct amount of energy to promote electrons of the element under consideration, from their ground state to their first excited state. The amount of light absorbed is directly proportional to the number of atoms in the free, unexcited state. Since the resonance lines of Cu and Cd fall within the high energy UV spectrum, the vast majority of the atoms present in the vapor phrase, even at high temperatures, fulfill the ground state requirement (FELL and SMITH, 1976). Thus, AAS is a useful technique for Cu and Cd determinations.

The application of the technique is straightforward. The element of interest is introduced in solution into the flame of the spectrophotometer. The flame functions to produce a vapor of free atoms. Those atoms present in their ground state absorb light of the resonance wavelength which is produced by a specially designed low pressure gas discharge tube. Any decrease in the intensity of the light of the given wavelength

is recorded as the amount of light absorbed by the atoms and can be correlated to the concentration of the element in the sample via a calibration curve. Typical Cu and Cd calibration curves are presented in Figures 2 and 3.

ii) Glassware cleaning

It is imperative that the glassware used be thoroughly cleaned in order to minimize sample contamination by extraneous HMs. Therefore, glassware was thoroughly washed with a solution of sulfuric acid and No-Chromix cleaner (Godax Laboratories) and profusely rinsed with deionized water. This was considered standard procedure with the glassware used in all the experiments and the analyses.

iii) Sample preparation

Samples were treated in following manner, prior to analysis, to insure that the metal of concern was present in solution:

They were deposited into 50 ml test tubes to which a 1:1 perchloric acid to nitric acid (Baker Reagent Grade) solution was added as a digest (STOKES et al., 1973; KOBAYASHI, 1972). The amount added was dependent on the final analysable volume of the sample.* Water and biological samples were then heated

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*The acid digest concentration in samples ready for AA analysis was 5%.

FIGURE 2

Typical calibration curve for Cadmium determination by AAS

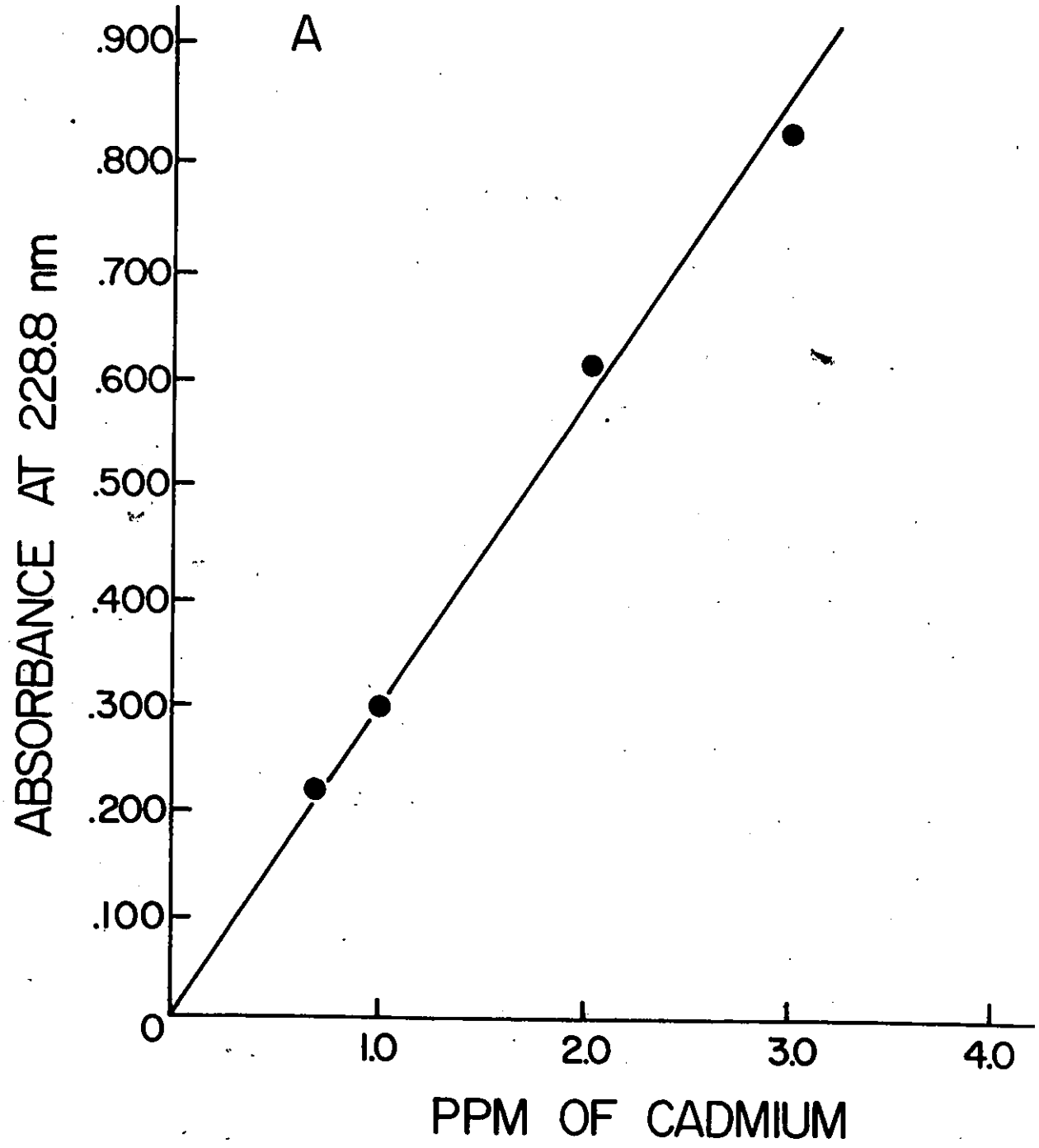
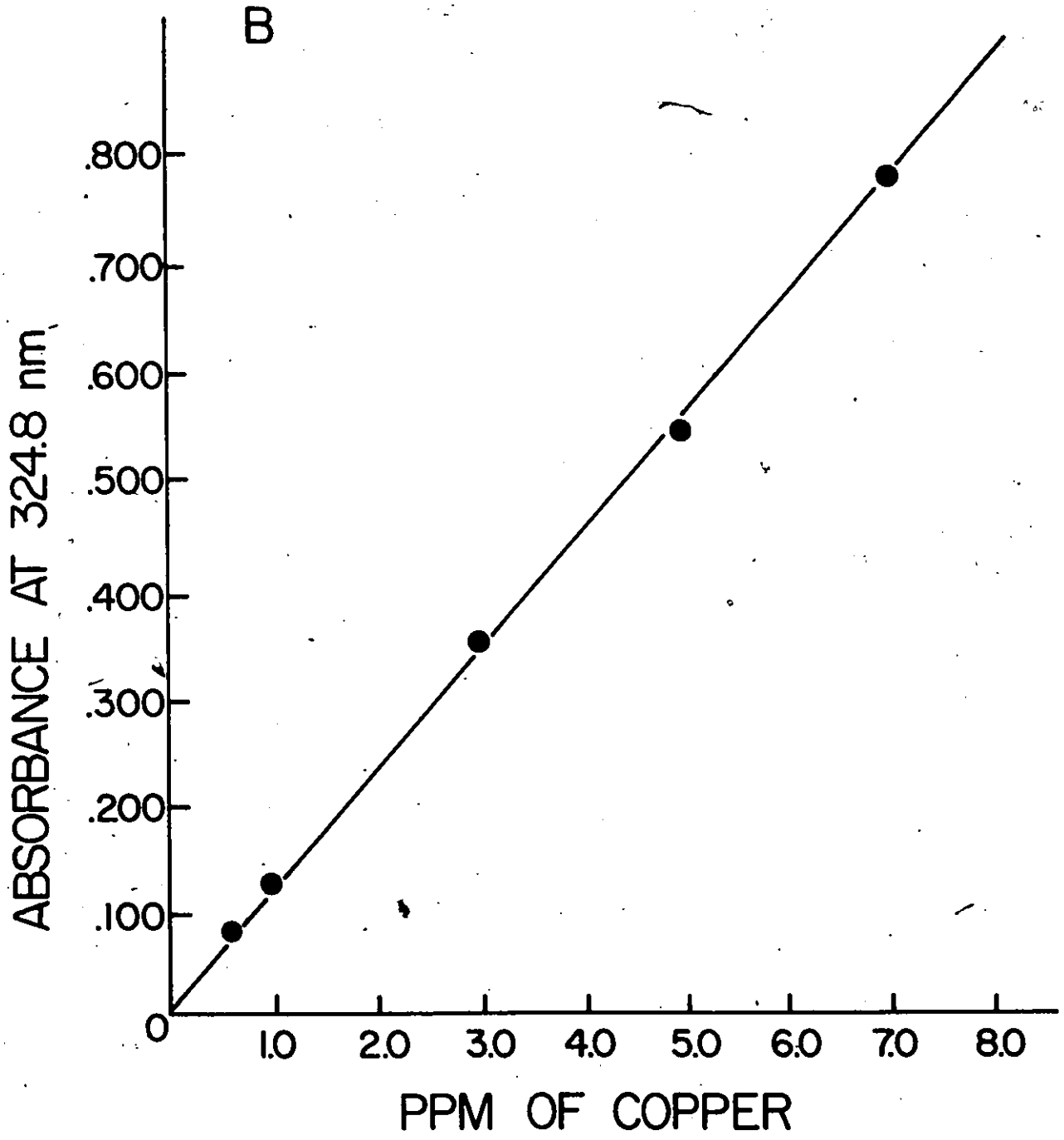

A
.900
.800
.700
.600
.500
.400
.300
.200
.100
0
ABSORBANCE AT 228.8 nm
1.0
2.0
3.0
4.0
PPM OF CADMIUM

FIGURE 3

Typical calibration curve for Copper determination by AAS


B
ABSORBANCE AT 324.8 nm
.800
.700
.600
.500
.400
.300
.200
.100
0
1.0
2.0
3.0
4.0
5.0
6.0
7.0
8.0
PPM OF COPPER

in a 160°C oven for at least 2.5 hrs. After this treatment, a fine, light coloured residue was always observed in the biological samples indicating that complete digestion of the organic matter had occurred (SCHEINBERG et al., 1977). After cooling to room temperature, deionized water was added to the samples to make up the analysable volume.

Sediments were treated similarly although the heating time was increased to at least 5 hrs. This seemed adequate since further heating did not result in any appreciable increase in the metal content of samples. Also, the values obtained for the Cu and Cd content of the Ottawa River clay sediment were in close agreement with the values from other analyses (OLIVER and KINRADE, 1972).

After the above treatment, particulate matter was allowed to settle out of solution. The supernatant was then aspirated into the flame.

Samples could be stored for at least one week after digestion without any loss in their HM content.

iv) Preparation of standards

Commercially available AAS standard stock solutions (Fisher Scientific Company - 1000 ppm Cu or Cd in dilute nitric acid) were used in preparing working standards. The concentrated solutions were diluted with deionized water and an appropriate volume of acid digest. The latter was added to maintain the

acid concentration in the standards comparable to the acid concentration in the samples.

Standards prepared with algal growth media or river water gave the same results as those prepared with deionized water.

Unheated standards were used since heating the standards in the same manner as the samples did not affect the metal content.

The acidified working standards could be stored, tightly stoppered, over extended periods of time without any decreases in their HM concentrations.

Blanks were prepared in a similar manner - with deionized water and the acid digest.

v) Analytical parameters and procedure

Cu and Cd analyses were done using either the Jarrell Ash Model 810 or Model 850 AA spectrophotometer. The operating parameters for these two HMs are outlined in table 1.

Unknown samples were analyzed in triplicate with blanks and standards being repeated very often to ensure reproducibility in the instruments performance.

Within their optimum range, AAS measurements have an accuracy of approximately 2% (SCHEINBERG et al., 1977).

TABLE 1

Operating parameters for Cu and Cd analysis by AAS.

Element	COPPER	CADMIUM
Wavelength (nm)	324.8	228.8
Hollow Cathode Lamp	Varian Techtron-Cu Westinghouse- Cu, Zn, Pb, Cd	Varian Techtron-Cd Westinghouse- Cu, Zn, Pb,Cd
Spectral slit size (A^{o})	4	4
Electrical supply (milliamperes)	10	12
Flame	Air: 10 psi Acetylene: 4 psi	Air: 10 psi Acetylene: 4 psi
Optimum range (µg/ml)	1.0-10.0@ 0.5-7.0 @@	0.5-3.0@ 0.3-2.5@@
*Detection limit (µg/ml)	.003	.003
**Sensitivity (µg/ml)	.04	.02

*Considered to be, in practical terms, the smallest concentration that can be reliably detected under optimum conditions.

**The concentration obtained for 1% absorbance (0.004 absorbance units). This value is nebuliser dependent and can vary up to 30% with different nebulizers.

@Atomic Absorption Analytical Methods, Fisher Scientific Co., 1972, Model 810

@@Atomic Absorption, Fisher Scientific Co., 1976, Model 850

2. Algae

i) Description of algae

The two algae of concern in this study were Anabaena flos-aquae strain no. 7120 and Ankistrodesmus braunii. The former alga, initially obtained from Dr. R.Y. Stanier, is a common filamentous Cyanophyta, often found in blooms in Canadian lakes and rivers. The latter is a Chlorophyta which was isolated from the Ottawa River. Species of this genus -Ankistrodesmus- are found widely (KRAUSS and HUTCHINSON, 1975) and at times, very abundantly (STOERMER, 1978) in Canadian freshwaters.

Microscopic and plate examinations showed no bacteria associated with the A. flos-aquae cultures. Similarly, no bacteria were discovered by the two techniques, in purified cultures of A. braunii.

ii) Culture conditions

The algae were maintained and grown at 20°C ± 1°C under cool white light (Westinghouse) of 1300 Lux, on for 16 hrs and off for 8 hrs.

Chlamydomonas medium (ONAD et al., 1967) was the culture medium used for A. braunii. A. flos-aquae, on the other hand, was maintained in BG II medium (STANIER et al., 1971), also termed GN, but grown for or during experiments in GO medium (GN minus $NaNO_3$). The pH during growth in the latter medium remained around 7, whereas in GN medium, the pH would rise

during growth and at times reach as high as 10.

Growing cultures were aerated by continuous agitation on a gyrating shaker (New Brunswick Co. - 100 cycles/min.) unless otherwise indicated.

iii) Growth monitoring

Growth was monitored by optical density measurements using a Coleman Junior II Spectrophotometer. The path length was 18 mm and the wavelength was set at 650 nm.

For convenient growth monitoring without the removal of culture aliquots, algae were grown in flasks with 18 mm side arm test tubes. Aliquot removal and culture dilution, however, were necessary when measurements of high density cultures were required.

3. Particulars of the experiments

A. Culture experiments

i) Addition of Cu to growing cultures

Sterile $Cu(NO_3)_2$ solutions (10^{-5} or 10^{-4}M final concentration) were added to growing cultures of either A. flos-aquae or A. braunii. The growth response was monitored and AAS analyses were carried out on aliquots from the cultures.

ii) Growth in the presence of HMs

These experiments were performed only with A. braunii.

Flasks with Chlamydomonas medium containing $Cu(NO_3)_2$ or $Cd(NO_3)_2$ (10^{-7} to 10^{-3}M) were innoculated with 0.5 ml of a mid-log preculture.* The cultures were allowed to grow and the OD/650 was monitored.

Once reproducible growth curves were obtained, the 10^{-4}, 10^{-5} and 10^{-6}M Cu and 10^{-5} and 10^{-6}M Cd experiments were repeated and aliquots were removed, at various stages of growth, for AAS analysis.

iii) HM solutions

The HM solutions used in the culture experiments were made with Cu or Cd nitrate (Baker Reagent Grade) and deionized water. The solutions were of a range of concentrations so that a 0.5 ml addition to 100 mls of medium or culture resulted in the desired overall concentration. In this way no substantial pH or volume changes were produced.

The Cu solutions added during algal growth were filter sterilized. No changes in the Cu concentration after filtration were detected by AAS.

iv) AAS analysis of algal cultures

The procedure used for obtaining the fractions analysed is outlined in figure 4.

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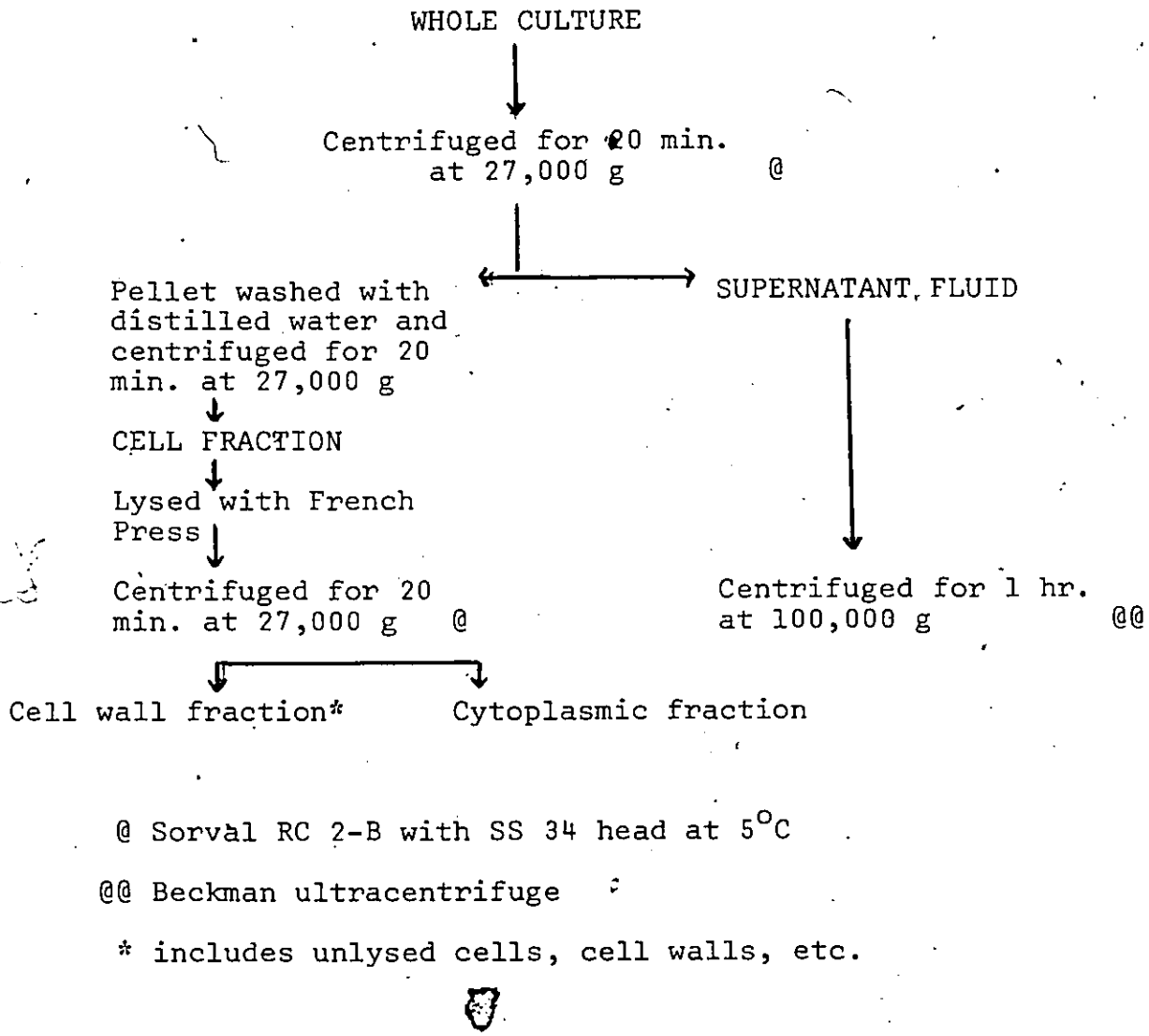
*Standard innoculum

FIGURE 4

Procedure followed in obtaining various fractions of algal cultures



@ Sorval RC 2-B with SS 34 head at 5°C

@@ Beckman ultracentrifuge

* includes unlysed cells, cell walls, etc.

v) Counting algae

Algal cultures were counted in order to relate cell numbers to optical density readings at 650 nm. Cultures were grown up to various densities as determined by OD/650 measurements. Aliquots from these cultures were removed, preserved with Lugol's solution, placed in counting chambers and allowed to settle. The algae were then counted using a Wild-40 inverted microscope (400 x magnification) and calculations of cell numbers per ml of culture were made according to the method of Lund et al. (1958).

vi) 'Cupric ion electrode' studies

a) General theory:

A cupric ion electrode - Orion Model 94-29 - in conjunction with a reference electrode, was used to determine the Cu^{2+} complexing capacity of supernatants of growing A. flos-aquae cultures. The electrode responds in a Nernstian manner to divalent cupric ions:

$$E = E_a + \frac{2.3\ RT}{2F} \log A_{cu}{}^{2+}$$

where E : measured total potential of the system

E_a : constant term incorporating various junction potentials, references and internal solutions

$\frac{2.3\ RT}{2F}$: Nernst factor (29.58 at 25°C)

$A_{cu}{}^{2+}$: activity of the cupric ion in the sample

Thus, the potential increases 30 mv in response to a 10 fold increase in the cupric ion activity (Figure 5). At Cu^{2+} concentrations of 10^{-3}M or lower, the electrode potential responds in the same manner to concentration as it does to activity. However, at higher concentrations, activity and concentration responses diverge. The amount of divergence is dependent on the activity coefficient Y which in turn is dependent on the total ionic strength of the solution being measured (ORION IONALYZER INSTRUCTION MANUAL, 1968).

Under optimum conditions, Cu^{2+} measurements can be made of concentration as low as 10^{-8}M. The most accurate (2%) measurements, however,are made between 10^{-6} and 10^{-4}M Cu^{2+}.

b) Standards, calibration curve and stock solutions

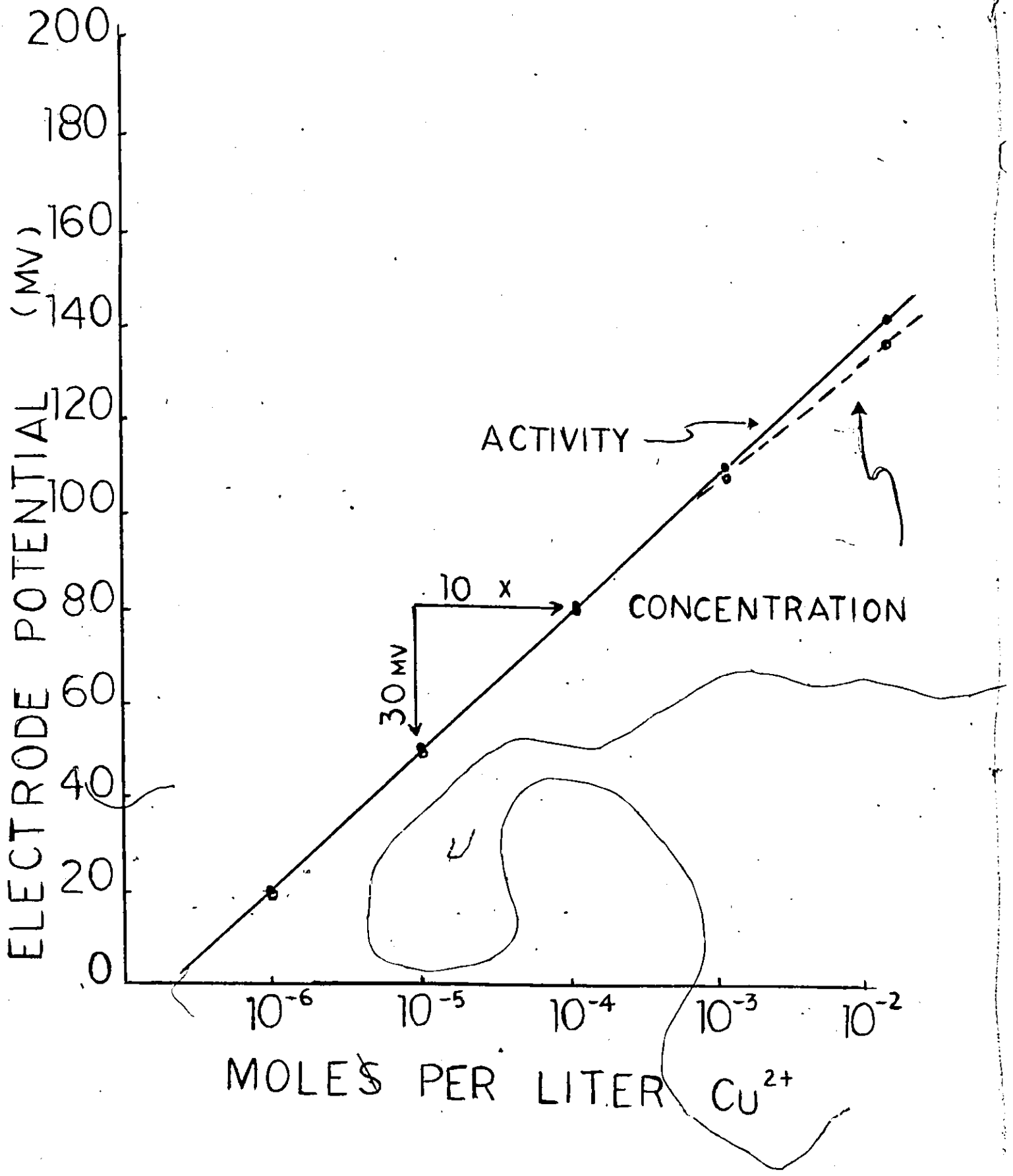
Standards in the range of 10^{-6} to 10^{-4}M Cu^{2+} were prepared from a 0.01 M stock solution with deionized water as the diluent. These standards were used in making up a calibration curve, similar to figure 5, relating the recorded potential of the standards to the log of their concentrations.

The 0.01 M stock solution (used as indicated above as well as added to samples under investigation) was prepared using deionized water and $Cu(NO_3)_2$ (Baker Reagent Grade). Its exact concentration was determined by H^+- exchange and titration with phenolphthalein. Such a stock was never stored more than two months whereas standards were prepared fresh daily.

FIGURE 5

Typical electrode response to changes in cupric ion activity and concentration in pure $Cu(NO_3)_2$ solutions*

*Figure was taken from Orion Ionalyzer/Cu^{2+} -electrode instruction manual, 1968.


200
180
160
140
120
100
80
60
40
20
0
ELECTRODE POTENTIAL (MV)
ACTIVITY
10 x
CONCENTRATION
30 MV
10^{-6}
10^{-5}
10^{-4}
10^{-3}
10^{-2}
MOLES PER LITER Cu^{2+}

c) Analytical procedure

Known increments of a 0.01 M stock solution of $Cu(NO_3)_2$ were added to 25 ml samples of A. flos-aquae supernatant. The samples were allowed to equilibrate and the potentials were recorded. The potentials were then converted to the concentration of the Cu^{2+} via the standard curve.

B. Competition studies

i) General procedure

A. flos-aquae and A. braunii were grown up to mid-log phase. The cells were harvested by spinning in a desk model centrifuge (International Clinical Centrifuge) set at 5 (800 rpm) for 3 minutes and washed with distilled water. Seven dialysis sacs were prepared into each of which the alga of concern was placed in amounts equivalent to 0.008 to 0.040 g dry weight of A. flos-aquae or 0.031 to 0.094 g dry weight of A. braunii in 6 ml of distilled water. Seven dialysis sacs were also prepared for containing the Ottawa River sediment. 0.3 g dry weight of sediment along with 6 ml of distilled water was put into each of the sacs.

All the dialysis sacs were suspended in 5.5 l of Ottawa River water. They were then mechanically rotated in a multiple dialyser (Pope Scientific Co.) and the water was agitated with a magnetic stirrer.

Sediment and algal sacs, as well as water aliquots, were taken out at specified time intervals. After digestion, AAS was used to analyze the samples for Cd or Cu. In this way, with Cd or Cu initially present in one compartment, either in the water or loaded onto the sediment, the accumulation by the other two compartments could be monitored.

Microscopic examination of the algal cells was carried out during the experiments to detect possible cell lysis or major cellular perturbations.

All experiments were performed at $22^{\circ}C \pm 1^{\circ}C$.

ii) Algal dry weight determinations

Nine algal pellets, having been similarly harvested and washed, were weighed. Seven were used in the experiment and two were placed in an $80^{\circ}C$ oven and dried to constant weight. The relationship of wet to dry weight was thus established and used to calculate the dry weight of the experimental samples.

iii) Characteristics and treatment of sediment

Ottawa River sediment was used in these studies. Previous analysis (RAMAMOORTHY and RUST, 1976) showed that it was of a clay consistency, composed of poorly crystallized kaolite and illite and possessing the following characteristics: 25.5 m^2/g dry weight surface area; 10.1% organic content; 0.35 mm mean grain size; 18.58 mEq/100 g dry wt. cation exchange capacity.

The sediment was gently dried in an 80°C oven and then stored in an air tight container until needed.

iv) HM loading of sediment

Loading was done in a beaker with 10 g of sediment. Ten mls of deionized water containing 1 ppm of Cu^{2+} or 100 ppm of Cd^{2+} (Baker Reagent Grade Cu and $Cd(NO_3)_2$) were poured over the sediment. This mixture was magnetically agitated for 24 hrs and then carefully rinsed with deionized water. The sediment was dried at 80°C, ground up with a mortar and pestle and stored in the same manner as the regular sediment.

v) Water characterization and treatment

The Ottawa River water was collected in thoroughly cleaned and rinsed 20 l polyethylene containers, from a number of different locales. The general characteristics of the water were determined in the laboratory, after collection. Since all the water collected could not be used immediately, it was stored at 3°C and used within two weeks of collection. The water characteristics did not change markedly during this period.

The pH of the water samples was determined using a standard pH electrode and meter (Fisher Scientific Co.) whereas the conductivity measurements were taken with a glass conductivity cell coated with platinum black (Leeds and Northrop conductivity bridge). The redox potential was determined with an Orion

platinum redox electrode - Model 96-78 and the measurements obtained were adjusted to be relative to the normal hydrogen electrode. The chloride ion content of the water was determined with another Orion electrode - Model 96-17. In addition, analyses for orthophosphate were conducted (EIBL and LANDS, 1969).

vi) Growth-competition studies

a) Procedure

500 ml side-arm flasks, each contained 100 ml of growth medium and a dialysis sac with 2 g of sediment* (either unloaded or loaded with 100 ppm Cu^{2+} or Cd^{2+}) were innoculated with the standard algal innoculum. Those flasks destined to be 'no-growth' controls were left uninnoculated.

All flasks were incubated under growth conditions and agitated by hand, once a day. The latter was done to minimize sediment sac breakage.

Growth was monitored and the cultures and controls were removed at various stages of growth for Cu or Cd analysis. Cell and supernatant fractions were obtained according to figure 4.

b) Algal dry weight determinations

Ten ml aliquots removed from cultures ready for AAS

---

*The sediment used here was the same as that used in the competition studies.

analysis, were treated according to figure 4 to obtain the cell fraction. The cells were then dried at 80°C to constant weight. The dry weights, thus determined, were used in calculating the HMs per g dry weight of algae.

RESULTS AND DISCUSSION

A. CULTURE EXPERIMENTS

Anabaena flos-aquae

1. Issues arising from growth and Cu distribution studies

Growth studies dealing with the effect of Cu on A. flos-aquae were previously carried out (McKENZIE, 1977). The experiments revealed that cultures of A. flos-aquae, when grown in the presence of 10^{-5}M to 10^{-8}M $Cu(NO_3)_2$, initially underwent an extended lag phase. Following this lag, the cultures grew at a rate comparable to that of the control. No growth was evident when 10^{-4}M or more Cu was present in the medium.

The distribution of 10^{-5}M of Cu in A. flos-aquae cultures at the start of the experiment and when normal growth commenced was also examined (McKENZIE, 1977). At day 0, 30% of the Cu was associated with the cells whereas at the end of the lag period, almost all of the Cu was found in the supernatant.

The results of the above Cu studies led to the suggestion that possibly A. flos-aquae releases organic substances during its extended lag phase when grown in the presence of 10^{-5}M $Cu(NO_3)_2$. These compounds, released in response to Cu^{2+} stimulation, would remove Cu from the cells and allow normal growth to proceed. In this case, a Cu addition during growth

would probably halt the growth for a time. During this interval, binding substances would be produced and would sequester the Cu away from the cells. Normal growth would eventually resume.

If Cu-binding substances are released during normal growth, the response of the alga to a sublethal Cu^{2+} addition during growth would be somewhat different from the response outlined above. Additions of Cu^{2+} to cultures at advanced stages of growth would probably have a smaller effect on the lag response than additions to early growth-stage cultures.

Algal lysis was also considered compatible with the results obtained by McKenzie (1977). If a population of cells exhibits a range of sensitivities towards HMs, a sublethal Cu^{2+} exposure could result in the lysis of some of the cells of the population. Those cells remaining would probably be only the most Cu resistant. These would eventually grow - perhaps aided to some extent by the complexation of the cupric ion with the lysate. In this case, if Cu was added to growing cultures, lysis would occur. The amount of lysis would be dependent on whether in fact the lysate plays a major role in the eventual growth of cells or whether a selection of a more resistant population is occurring.

In order to determine the most probable response of A. flos-aquae to Cu^{2+}, $Cu(NO_3)_2$ additions were made to growing cultures of the alga.

2. $Cu(NO_3)_2$ additions to growing cultures

The addition of 10^{-5}M $Cu(NO_3)_2$ to growing cultures of A. flos-aquae resulted in a drop in the OD/ 650 of the cultures with the actual drop being greater at the higher levels of growth (Figure 6). Although all the cultures resumed normal growth, the recovery period was somewhat shorter in the denser cultures.

The association of Cu with the algal cells following the Cu^{-5}M $Cu(NO_3)_2$ addition, was determined in the above cultures. The four cultures showed a similar trend (Table 2). Cu became associated with the algal cells almost immediately after it was added to the cultures. The maximum Cu associated with the cell fraction tended to occur just prior to cell lysis and ranged between 46 and 50 percent of the added Cu. A decrease in the cell-associated Cu was observed as the OD/650 dropped. When normal growth resumed, almost all of the added Cu was present in the supernatant.

Microscopic examinations were also carried out on the cultures following the Cu additions. No changes in cellular structure were immediately visible when 10^{-5}M $Cu(NO_3)_2$ was added to the cultures. However, when the OD began to drop, clumping of the alga became evident along with the disruption of the A. flos-aquae chains (Figure 7). As the OD proceeded to drop, whole cells became scarcer and cellular

FIGURE 6

The effect of the addition of 10^{-5}M $Cu(NO_3)_2$ to growing cultures of A. flos-aquae

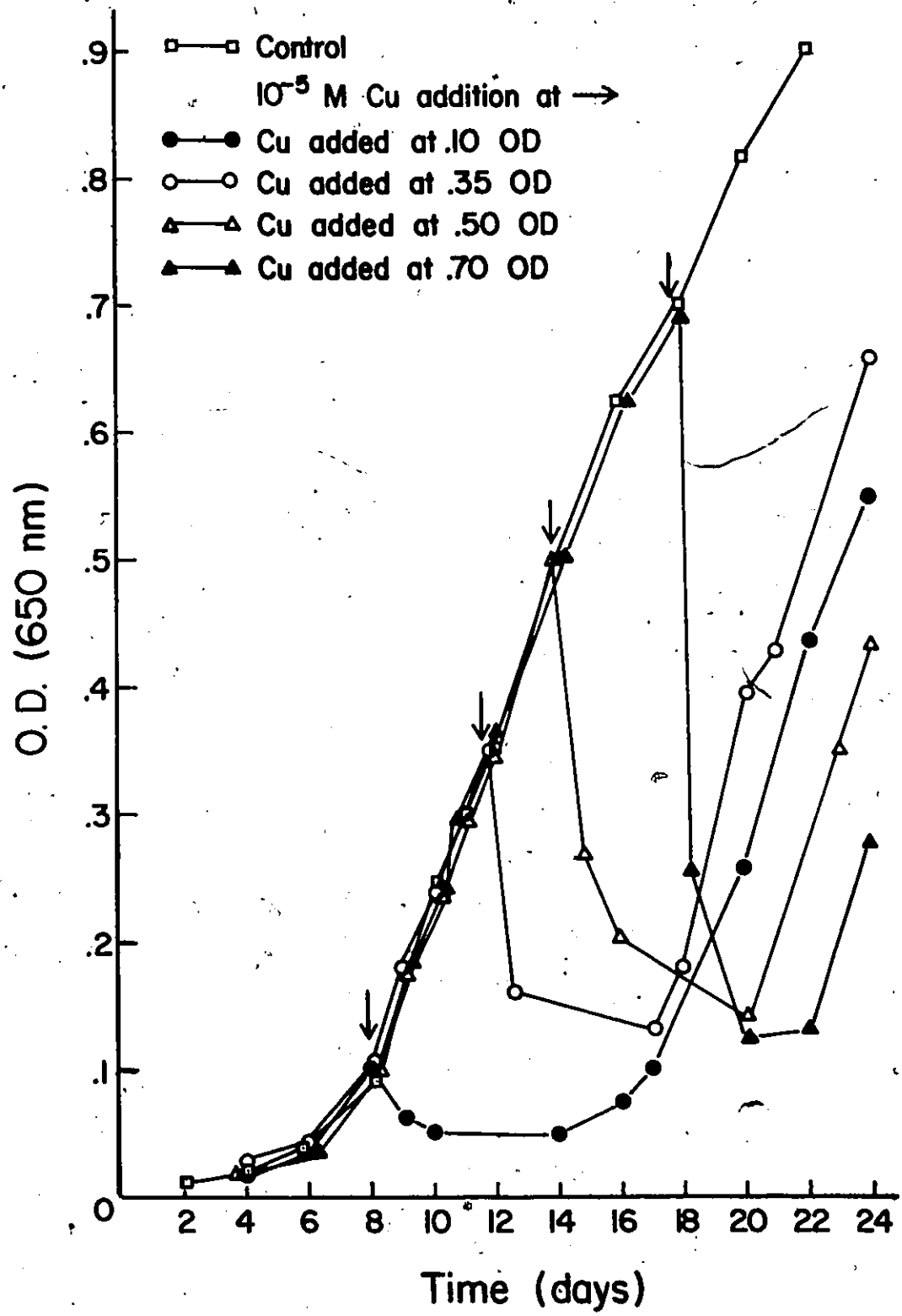

Control
10^{-5} M Cu addition at →
Cu added at .10 OD
Cu added at .35 OD
Cu added at .50 OD
Cu added at .70 OD
O.D. (650 nm)
0
.1
.2
.3
.4
.5
.6
.7
.8
.9
2
4
6
8
10
12
14
16
18
20
22
24
Time (days)

TABLE 2

The distribution of Cu between the algal cells and the supernatant of cultures of A. flos-aquae to which 10^{-5} $Cu(NO_3)_2$ was added

OD (650 nm)	Time after addition	Amount of Cu in mg/l		Amount of Cu as %		Total Cu Recovery as %
		CELLS	SUPERNATANT	CELLS	SUPERNATANT	
.100	0	.28	.33	46	54	95
.100	1 hr	.31	.30	51	49	95
.050	2 days	.0	.59	0	100	92
.075	8 days	.01	.65	2	98	103
.350	0	.23	.44	35	65	105
.350	1 hr	.31	.37	46	54	106
.170	2.5 hrs	.14	.48	23	77	97
.130	5 days	.06	.65	9	91	111
.175	6 days	.03	.60	5	95	98
.400	8 days	.0	.62	0	100	97
.660	12 days	.04	.56	7	93	94
.500	0	.16	.42	28	72	91
.490	1 hr	.25	.30	45	55	87
.200	2 days	.12	.52	19	81	101
.170	4 days	.05	.59	8	92	100
.140	6 days	.01	.61	2	98	97
.700	0	.26	.36	42	58	97
.700	1 hr	.31	.36	46	54	105
.175	1 day	.02	.67	3	97	108
.125	4 days	.01	.65	2	98	103
1.100	0	.0	.69	0	100	108
1.340	4 days	.04	.58	6	94	97
1.395	12 days	.02	.67	3	97	108

FIGURE 7

The cytological effect of a 10^{-5}M $Cu(NO_3)_2$ addition to logarithmically growing cultures of A. flos-aquae as observed by light microscopy

a) Typical chain formations

b) Change accompanying OD drop

a

b

debris became more pronounced. When normal growth resumed, small chains of algal cells initially predominated. Eventually, the cultures became indistinguishable from the control.

A 10^{-5}M $Cu(NO_3)_2$ addition to A. flos-aquae cultures at an advanced stage in growth did not result in an OD drop and growth continued (Table 3). No lag period was evident following the addition and little if any Cu was associated with the cells (Table 2).

Disruption of algal chains, cellular clumping, and decreases in OD/650 were also observed when 10^{-4}M $Cu(NO_3)_2$ additions were made to logarithmically growing as well as to older cultures of A. flos-aquae (Figure 8 and Table 3). Growth, however, did not resume after the OD dropped.

Thus, cellular lysis occurred when 10^{-4}M or 10^{-5}M $Cu(NO_3)_2$ was added to growing cultures of A. flos-aquae. In the former case, all the cells lysed. In the latter case, because some cells remained intact and viable, normal growth eventually resumed. Part of the extended lag period when A. flos-aquae is grown in the presence of 10^{-5}M $Cu(NO_3)_2$ is possibly also due to lysis. The similar Cu-cell association and subsequent Cu dislodgement from the cell fraction when Cu is initially present in the growth medium and when Cu is added during algal growth, tends to support this.

TABLE 3

The effect of the addition of $Cu(NO_3)_2$ on older cultures of *A. flos-aquae* (Monitored by OD/650)

Time after HM addition (days)	Control growth	10^{-4}M $Cu(NO_3)_2$ addition	10^{-5}M $Cu(NO_3)_2$ addition
0	1.100	1.100	1.100
4	1.265	1.090	1.220
8	1.360	0.945	1.340
12	1.425	0.705	1.395
16	1.500	0.420	1.420
20	1.620	0.360*	1.490

*OD continued dropping and eventually levelled off at 0.250

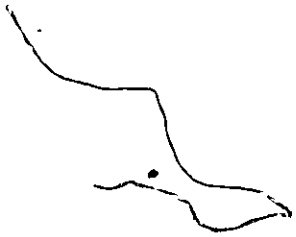
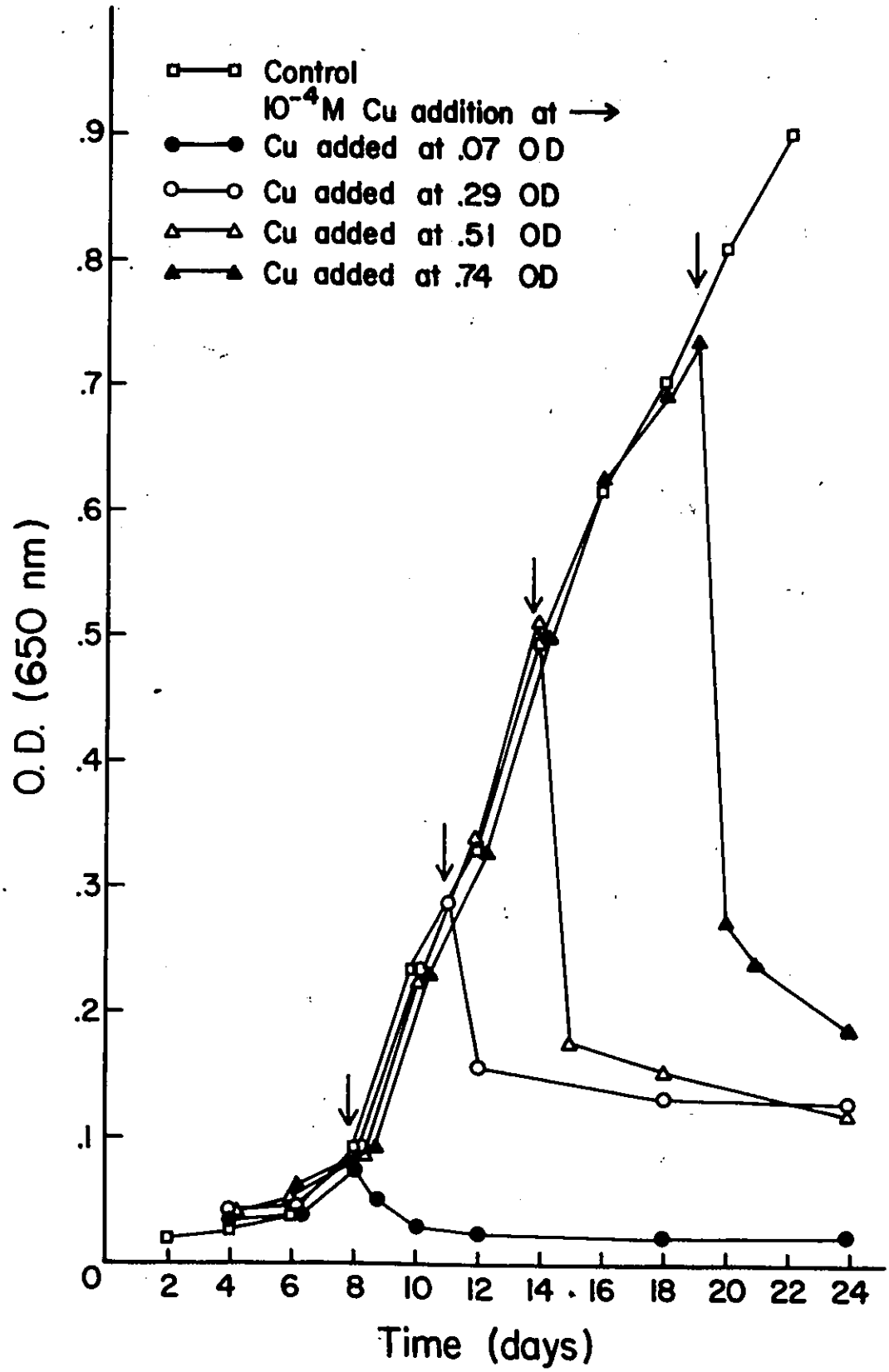


FIGURE 8

The effect of the addition of 10^{-4}M $Cu(NO_3)_2$ to growing cultures of A. flos-aquae


Control
10^{-4}M Cu addition at →
Cu added at .07 OD
Cu added at .29 OD
Cu added at .51 OD
Cu added at .74 OD
O.D. (650 nm)
0
.1
.2
.3
.4
.5
.6
.7
.8
.9
2
4
6
8
10
12
14
16
18
20
22
24
Time (days)

Since more lysis and a shorter recovery time was evident when Cu was added to the more advanced cultures, it seems plausible that the more resistant cells of the population remain and eventually grow. If this were true, the new population would be somewhat more Cu resistant than the initial population. However, preliminary experiments tend to indicate that this is not the case (Personal communications with G. Murray).

The same lytic response was not seen in older cultures of A. flos-aquae. In fact, the cultures did not seem to be affected by sublethal Cu additions. These results are understandable if extracellular compounds are being produced by the alga. It is not an uncommon phenomenon for algae to produce such substances only at certain stages of growth (FOGG, 1966).

In an attempt to show that A. flos-aquae secretes HM-binding material sometime during normal growth, studies were carried out using a cupric-ion specific electrode.

3. Cu^{2+} binding capacity of supernatant fractions from growing cultures

The first problem encountered when attempting to do binding studies in microbial growth media is that the media tend to bind substantial amounts of HM ions (RAMAMOORTHY and KUSHNER, 1975). This is evident even in a defined algal medium containing low concentrations of nutrients. No free

Cu^{2+} was detectable in G0 medium when 10^{-4}M $Cu(NO_3)_2$ was added (Table 4). It was thus necessary to devise a growth medium which bound little Cu^{2+} but which still allowed for good algal growth.

Since both citrate and EDTA are known HM chelators, an attempt was made to lower the concentration of these substances in the G0 medium. A five-fold decrease of both allowed for good algal growth. However, only a slight decrease in the medium's binding capacity was observed. A ten-fold decrease gave the same results. A deletion of phosphate was then attempted and some success in decreasing the binding capacity was achieved. Eventually, all the components of the G0 medium were diluted except for the trace elements (Table 5). This modified medium, termed 'dilute G0 medium', bound only 20 percent of the added 10^{-4}M $Cu(NO_3)_2$ (Table 4). Furthermore, A. flos-aquae grew moderately well in it (Figure 9).

Complications may also arise in interpreting HM binding results obtained with growing cultures. In determining the HM-binding capacity of a culture, it is necessary to consider the decreased binding capacity of the medium due to solute utilization by the alga during growth, the possible increased binding capacity due to algal secretions as well as the possible increased binding due to increased algal numbers (McKENZIE, 1977). The situation with respect to algal secretions can be

TABLE 4

Cu^{2+} binding capacity of the supernatant of A. flos-aquae cultures grown in dilute GO medium

Sample	OD	pH	µM Cu^{2+} bound*
GO medium	-	7.05	100
**Dilute GO medium	-	6.98	20
Dilute GO medium plus A. flos-aquae	0 time: .005	6.94	31
	.195	7.08	22
	.305	7.26	34
	.540	7.35	43
	.550	7.40	66
	.590	7.51	78

*10^{-4}M $Cu(NO_3)_2$ was added to 25 ml samples of supernatant

** Refer to Figure 5 for composition of this Medium.

TABLE 5

Solute concentrations in dilute GO medium

Solute	Concentration (mM) dilute GO	GO
$K_2HPO_4 \cdot 3H_2O$	.004	.175
$MgSO_4 \cdot 7H_2O$	.031	.305
$CaCl_2$	.024	.243
Na_2CO_3	0	.189
Na EDTA	.003	.030
Citric acid/Ferric citrate	.003 / .003	.029 / .028
Trace metals	standard	standard*

*According to Stanier et al., 1971.

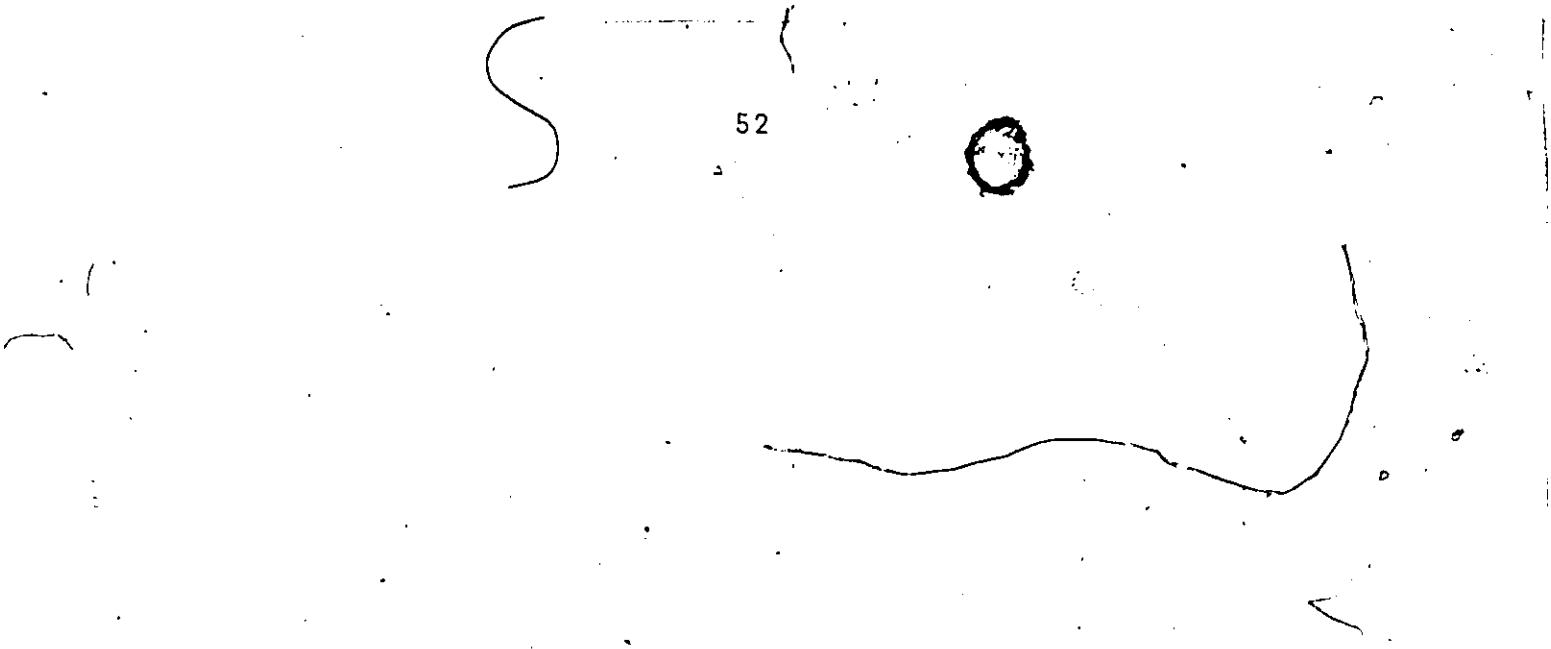
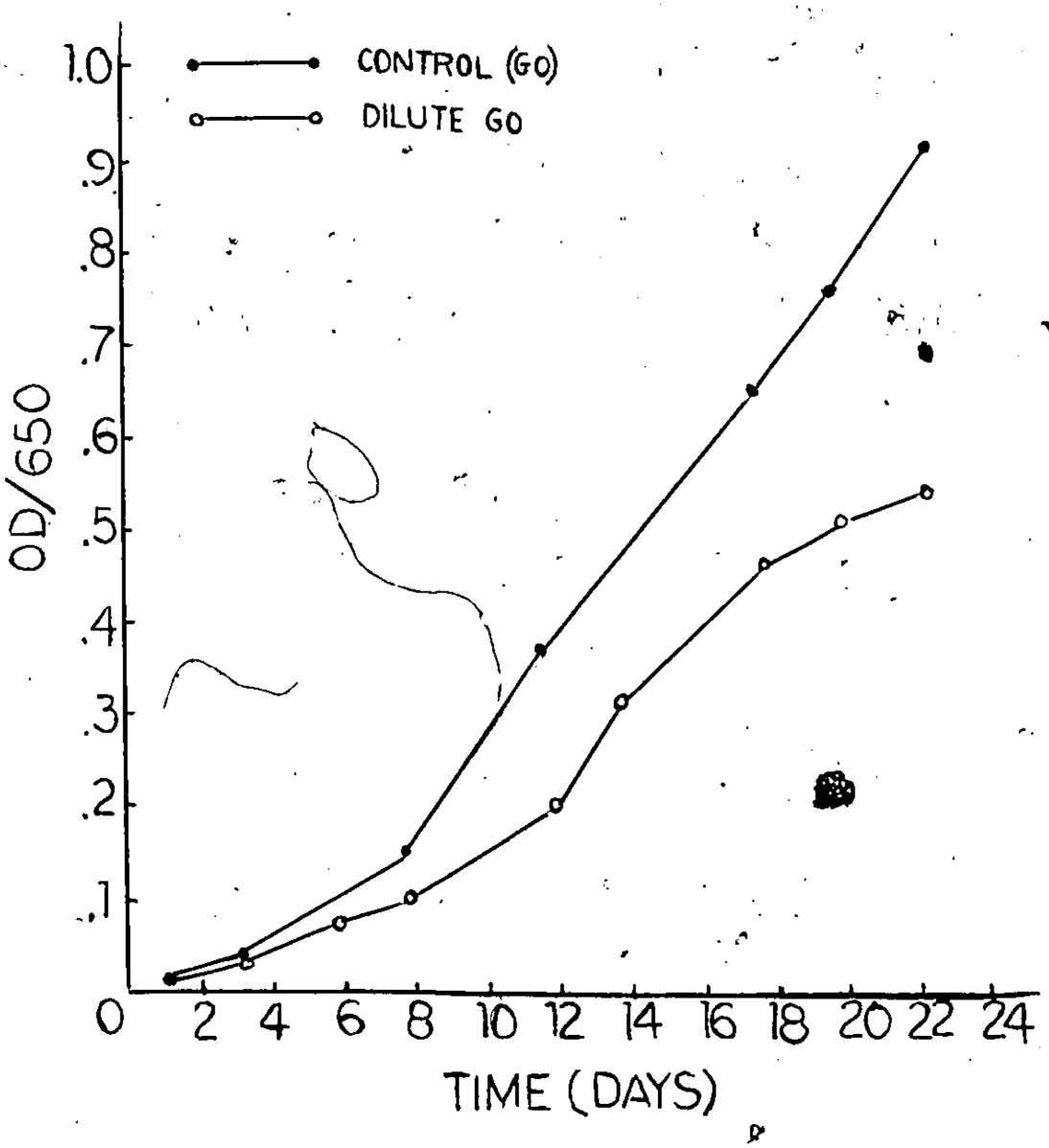


FIGURE 9

Growth of A. flos-aquae in dilute GO medium


CONTROL (GO)
DILUTE GO
OD/650
1.0
.9
.8
.7
.6
.5
.4
.3
.2
.1
0
2
4
6
8
10
12
14
16
18
20
22
24
TIME (DAYS)

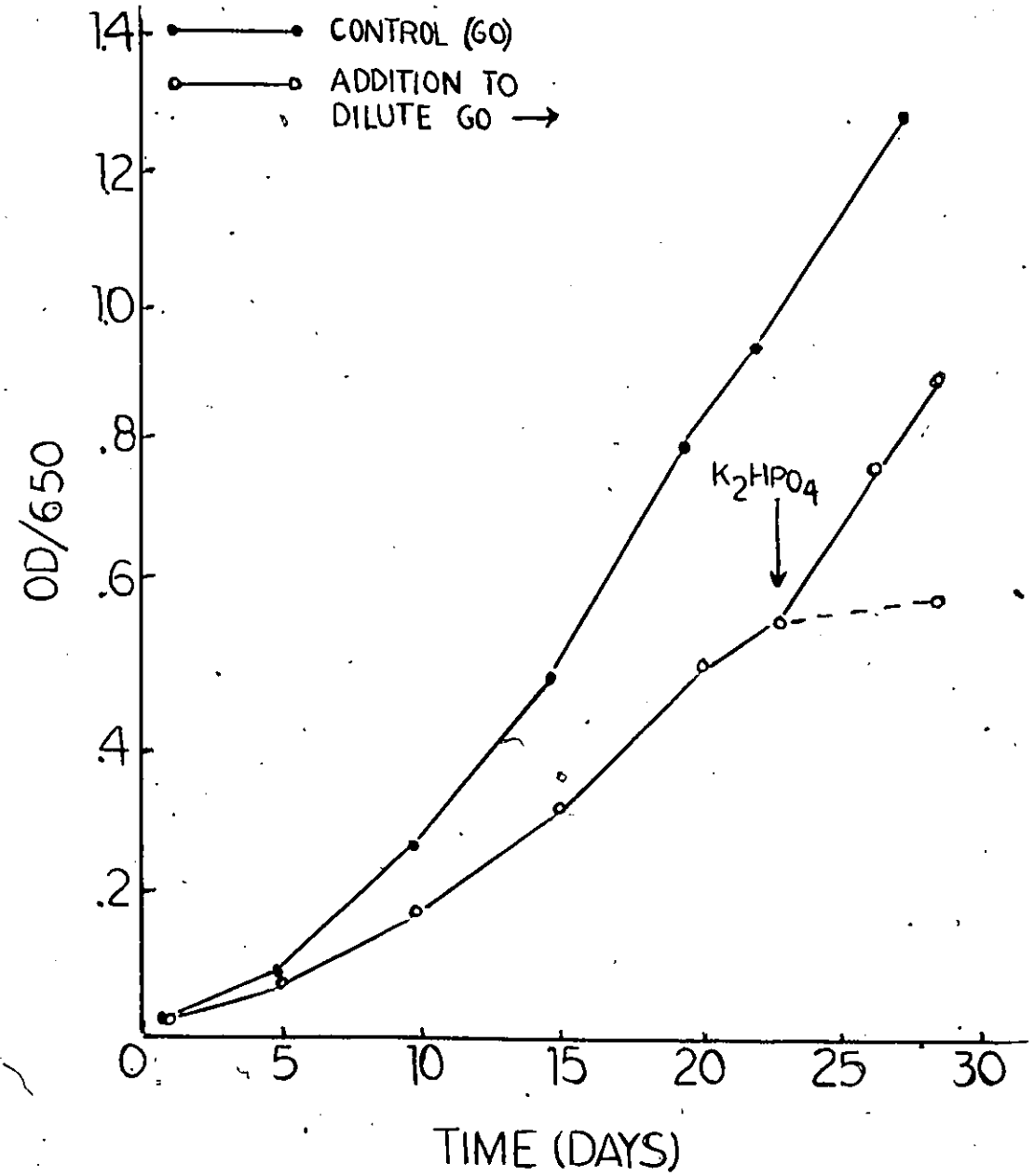
made clearer if the last variable is eliminated, i.e. the binding capacity of the supernatant is measured as opposed to the binding capacity of the whole culture. Definite conclusions in this case, however, can only be drawn when the binding capacity of the supernatant under consideration is equal to or higher than that of the uninnoculated medium.

The binding capacity of the supernatant of A. flos-aquae cultures growing in dilute GO medium fluctuated in the initial stages of log growth (Table 4). No definite trend was observed, however, until the late log phase was reached (OD/ 650 = 0.540), when the binding increased substantially over that of the control.

Thus, it seems that A. flos-aquae produced extracellular HM-binding materials during the late stages of growth in dilute GO medium. Quite probably a similar phenomenon occurs during natural growth. However, this cannot be stated conclusively since growth stops in dilute GO medium due to a nutritional limitation, i.e. a phosphate addition to stationary cultures in dilute GO medium stimulated growth (Figure 10). Whitton (1966) suggests that nutritional limitations may be a possible, but as yet unproven, factor influencing the production of extracellular substances.

FIGURE 10

The addition of 10^{-5}M K_2HPO_4 ,to stationary cultures of A. flos-aquae grown in dilute GO medium


CONTROL (GO)
ADDITION TO DILUTE GO →
K_2HPO_4
OD/650
1.4
1.2
1.0
.8
.6
.4
.2
0
5
10
15
20
25
30
TIME (DAYS)

Ankistrodesmus braunii

1. The effect of Cu and Cd on growth

When A. braunii was grown in the presence of increasing concentrations of Cu or Cd, its growth rate progressively decreased (Figure 11 and 12). Although 10^{-7}M of either HM appeared to have little effect on growth, higher concentrations were inhibitory. In 10^{-4}M Cd or 10^{-3}M Cu growth ceased.

Growth inhibition also occurred when 10^{-5}M $Cu(NO_3)_2$ was added to growing cultures of A. braunii (Figure 13). It is noteworthy here that in contrast to A. flos-aquae cultures, cellular lysis was not observed, and inhibition seemed to be the same at all levels of growth. Thus, culture age is not a factor affecting the algal response to 10^{-5}M Cu.

Aliquots of the cultures grown in the presence of $Cu(NO_3)_2$ or $Cd(NO_3)_2$ and then transferred into fresh medium, without any HM additions, showed normal growth. The alga did not incur any permanent damage when grown in the presence of the HMs. Aliquots from those HM cultures in which no growth was evident did not grow in the fresh medium when transferred after 11 days of exposure.

HM association with the algal cells was probably responsible for the pattern of growth exhibited by A. braunii cultures exposed to Cu and Cd. Consequently, the association of HMs with the cell and supernatant fractions of cultures grown in different HM concentrations was examined.

FIGURE 11

Growth of A. braunii in media containing different concentrations of $Cu(NO_3)_2$

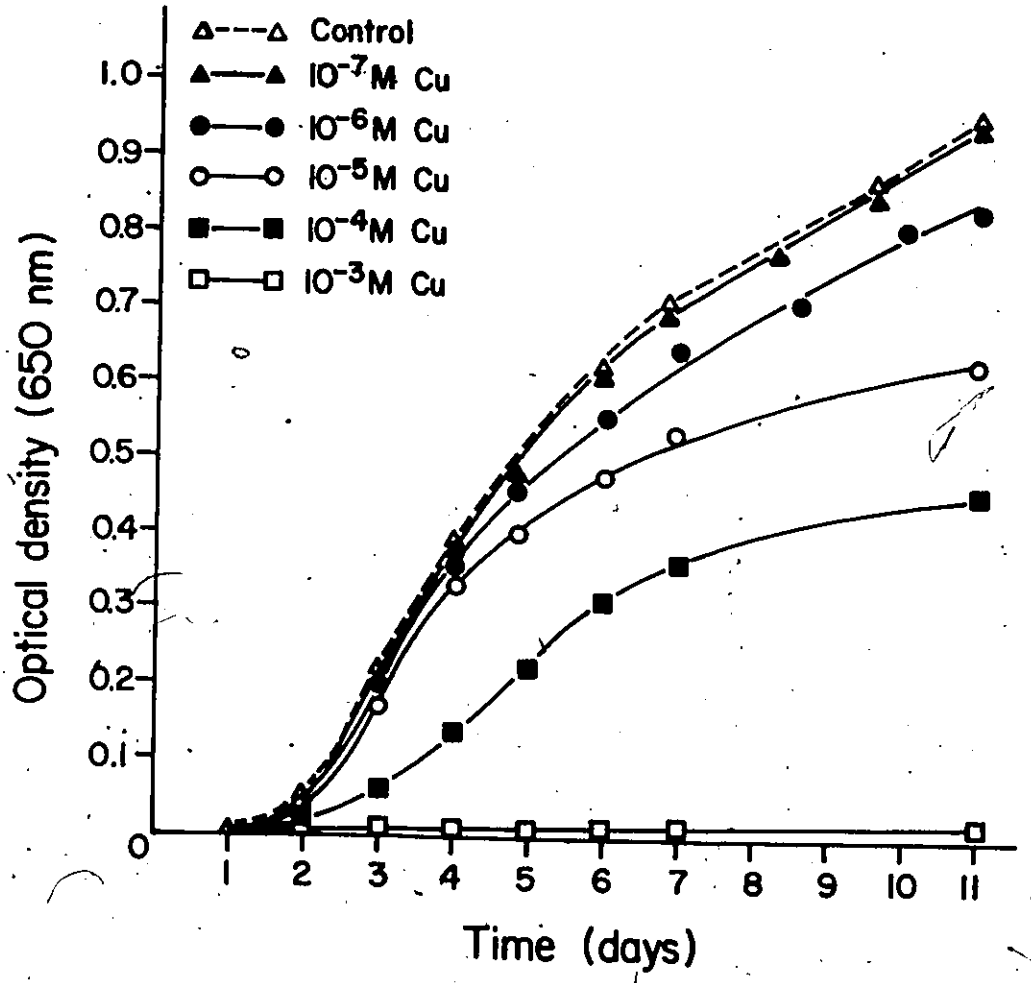

Control
10⁻⁷M Cu
10⁻⁶M Cu
10⁻⁵M Cu
10⁻⁴M Cu
10⁻³M Cu
Optical density (650 nm)
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1
2
3
4
5
6
7
8
9
10
11
Time (days)

FIGURE 12

Growth of A. braunii in media containing different concentrations of $Cd(NO_3)_2$

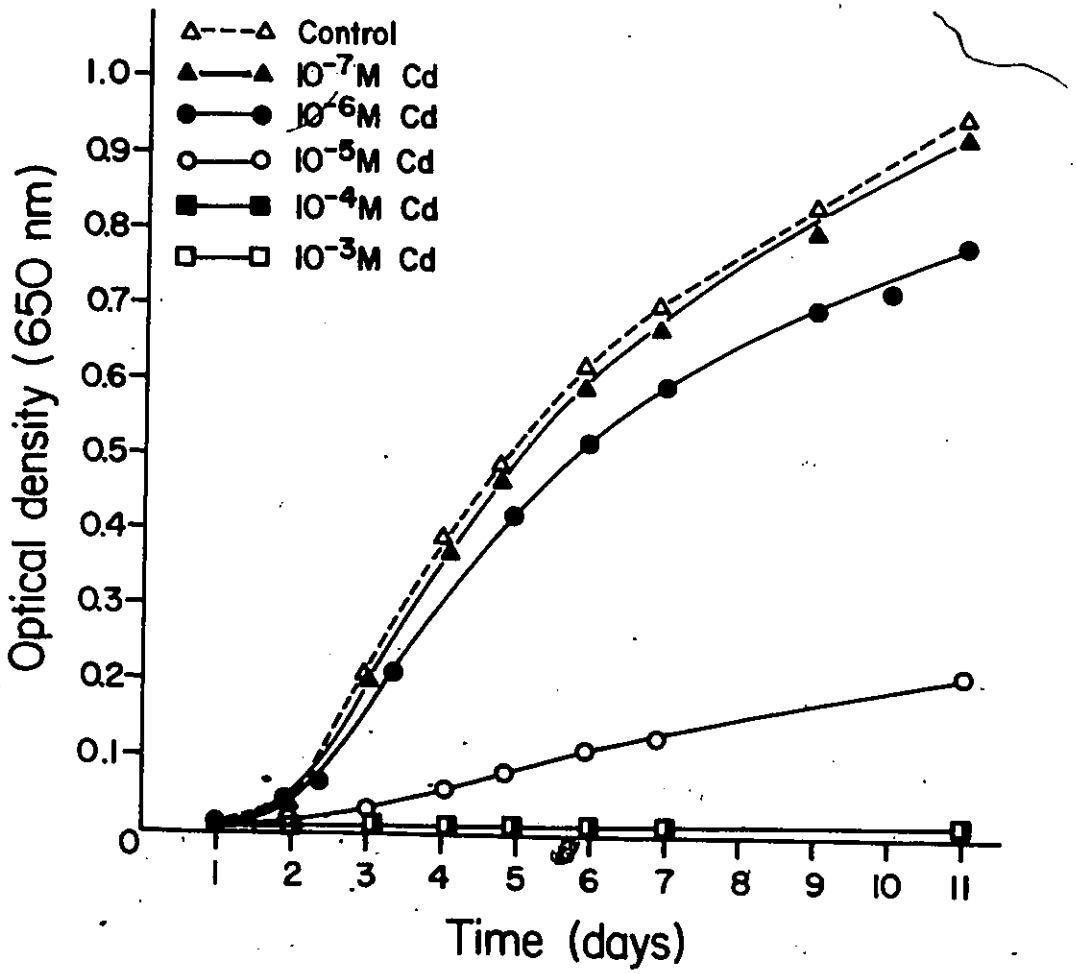
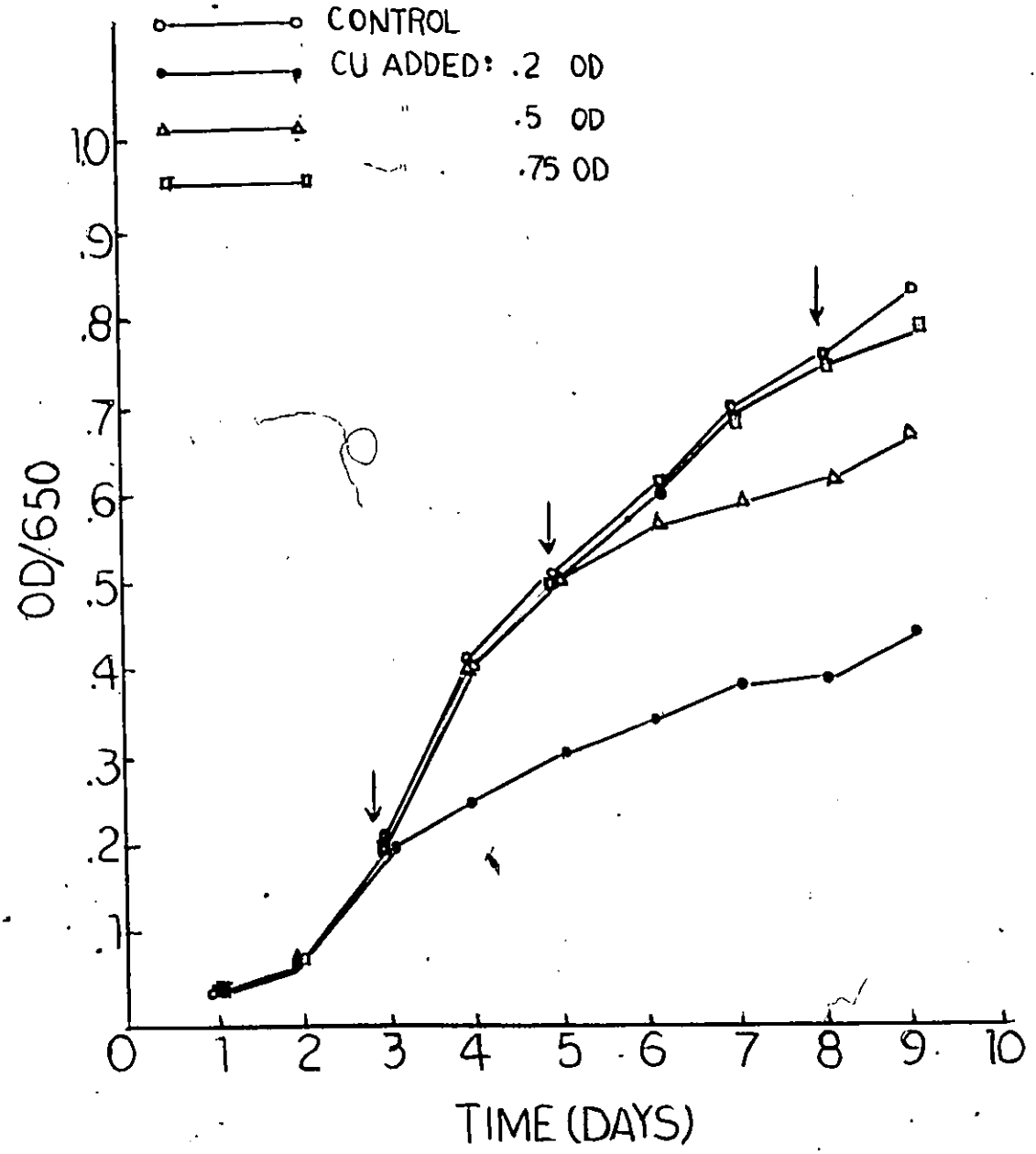

Control
10⁻⁷M Cd
10⁻⁶M Cd
10⁻⁵M Cd
10⁻⁴M Cd
10⁻³M Cd
Optical density (650 nm)
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
1
2
3
4
5
6
7
8
9
10
11
Time (days)

FIGURE 13

The effect of a 10^{-5}M $Cu(NO_3)_2$ addition to growing cultures of A. braunii


CONTROL
CU ADDED: .2 OD
.5 OD
.75 OD
OD/650
1.0
.9
.8
.7
.6
.5
.4
.3
.2
.1
0
1
2
3
4
5
6
7
8
9
10
TIME (DAYS)

2. The distribution of Cu and Cd between the cell and supernatant fractions

The amount of Cu associated with the cells was determined during algal growth in the presence of 10^{-6}M, 10^{-5}M and 10^{-4}M $Cu(NO_3)_2$ (0.06, 0.64, and 6.35 ppm Cu respectively).

As the number of cells of A. braunii increased during growth in the presence of 0.64 and 6.35 ppm of Cu^{2+}, the amount of Cu associated with the cells increased (Table 6). More Cu^{2+} was associated with the same number of cells when the alga was grown in medium containing 6.35 ppm Cu than when the alga was grown in the presence of 0.64 ppm Cu. The percentage of the Cu associated with the cells, however, was approximately twice as great when growth occurred at the lower concentration of Cu.

When the alga grew in the presence of 0.06 ppm Cu, the Cu found in the cells initially increased (Table 6). Eventually, all of the Cu became associated with the cells.

Using the relationship between OD/ 650 and cell number (Figure 14), the amount of Cu present in the cells was converted to the amount of Cu present per cell at each OD. The same amount of Cu was found to be associated per cell - 6.81×10^{-8}µg, throughout the growth of A. braunii in

TABLE 6

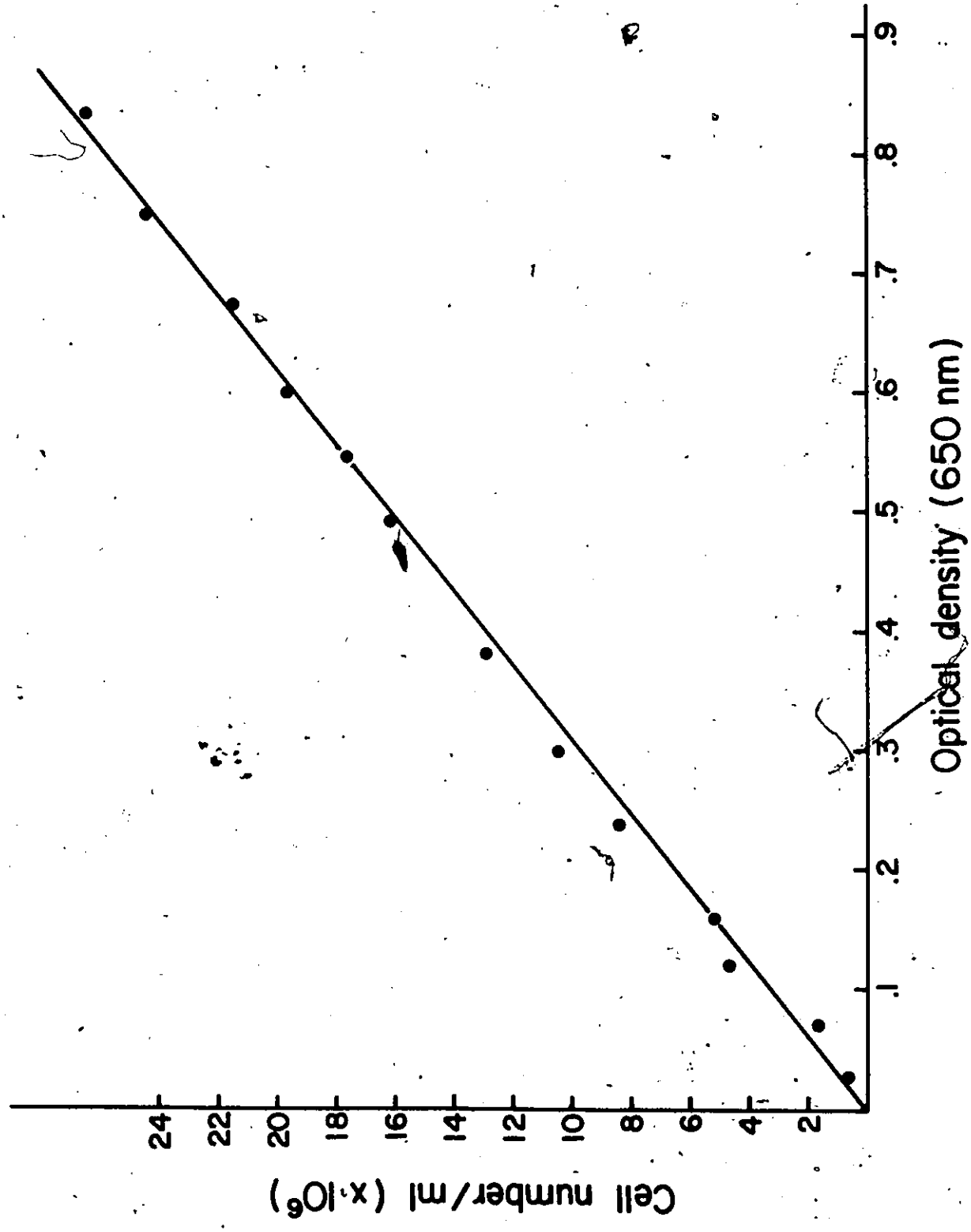
The distribution of Cu between the algal cells and the supernatant of cultures of A. braunii grown in the presence of 0.06, 0.64 and 6.35 ppm Cu

Initial Cu concentration in growth medium	OD (650 nm)	Amount of Cu in mg/l		Amount of Cu as %		Total Cu recovery as %
		CELL	SUPERNATANT	CELLS	SUPERNATANT	
0.06 ppm	.075	.02	.03	40	60	83
	.105	.03	.03	50	50	100
	.490	.06	0	100	0	100
	.570	.06	0	100	0	100
	.640	.06	0	100	0	100
	.960	.07	0	100	0	100
0.64 ppm	.055	.03	.62	4	96	102
	.100	.05	.55	8	92	94
	.195	.10	.55	15	85	102
	.400	.20	.40	33	67	94
	.490	.23	.41	36	64	100
	.610	.27	.40	40	60	105
	.840	.36	.34	51	49	109
	.950	.44	.24	65	35	106
6.35 ppm	.022	0. 05	6.20	0.5	99.5	98
	.030	0.07	6.00	1	99	96
	.450	0.99	5.13	16	84	96
	.580	1.28	4.90	21	79	97
	.650	1.31	4.46	22	78	91
	.800	1.80	3.55	28	72	100

FIGURE 14

The relationship between the cell number and the OD/650 of growing cultures of A. braunii*

*The same relationship was obtained for A. braunii cultures grown in the presence of (6.35, .64 or .06 ppm) Cu or (1.12 or .11 ppm) Cd.


Cell number/ml (x·10⁶)
24
22
20
18
16
14
12
10
8
6
4
2
Optical density (650 nm)
.1
.2
.3
.4
.5
.6
.7
.8
.9

medium containing 6.35 ppm Cu (Table 7). A similar phenomenon was observed when A. braunii was grown in the presence of 0.64 ppm Cu. The absolute value per cell in this case, however, was lower - being 1.47×10^{-8} µg (Table 7).

Initially, a value around 0.90×10^{-8} ug Cu per cell was obtained for the alga grown in the presence of 0.06 ppm Cu (Table 7). However, as the cell number increased without a concomitant increase in the Cu associated with the cells, the Cu was diluted out.

These results tend to point to a relationship between the initial amount of Cu present in the growth medium and the amount of Cu per cell. In other words, the Cu binding capacity (absorption and/or adsorption) of A. braunii cells seems to be dependent on the total Cu concentration in the culture.

An initial increase in the cell associated Cd, followed by a decrease, was evident in A. braunii cultures growing in the presence of 0.11 ppm Cd (Table 8). The concentration of the Cd per cell, however, progressively decreased as growth proceeded (Table 9). When the concentration of Cd in cells of A. braunii cultures growing in the presence of 1.12 ppm Cd was determined, an increase accompanying growth was observed (Table 8). However, once again on a per cell basis,

TABLE 7

The amount of Cu present per cell during growth of *A. braunii* in the presence of 0.06, 0.64 and 6.35 ppm Cu.

Initial Cu concentration in growth medium	OD (650 nm)	µg Cu ($x \cdot 10^{-8}$) per cell	Means ±	Standard error
0.06 ppm	.075	0.83		
	.105	0.90		
	.490	0.38		
	.570	0.33		
	.640	0.29		
	.960	0.02		
0.64 ppm	.055	1.42		
	.100	1.56		
	.195	1.59		
	.400	1.56		
	.490	1.47		
	.610	1.38		
	.840	1.34		
	.950	1.45	1.47	0.08
6.35 ppm	.022	6.86		
	.030	6.88		
	.450	6.88		
	.580	6.90		
	.650	6.30		
	.800	7.03	6.81	0.17

TABLE 8

The distribution of Cd between the algal cells and the supernatant of cultures of A. braunii grown in the presence of 0.11 and 1.12 ppm Cd.

Initial Cd concentration in growth medium	OD (650 nm)	Amount of Cd in mg/l		Amount of Cd as %		Total Cd Recovery as %
		CELL	SUPERNATANT	CELL	SUPERNATANT	
0.11 ppm	.075	.04	.09	31	69	118
	.210	.10	.02	83	17	109
	.360	.12	.01	92	8	118
	.490	.08	.02	80	20	91
	.650	.07	.05	58	42	109
	1.050	.07	.03	70	30	100
1.12 ppm	.205	.35	.53	40	60	79
	.385	.35	.55	39	61	81
	.860	.47	.53	52	48	81
	1.100	.76	.15	84	16	89

Cd decreased (Table 9). Such a tendency has previously been observed with Cd in algal cultures (COSSA, 1976; CONWAY, 1978). A suggestion was made that the trend may be a result of chelation by extracellular metabolites.

Perhaps HM-binding metabolites are released by A. braunii. In an attempt to determine whether this alga secreted extracellular colloidal substances when grown in the presence of HMs, the supernatant fractions of such algal cultures were examined further.

3. The distribution of Cu and Cd between colloidal, soluble, cell wall and cytoplasmic fractions

When the supernatants of algal cultures grown in the presence of HMs were centrifuged for 1 hr at 100,000 g, no pellets were observed. Furthermore, the same amount of Cu and Cd was present in the soluble fractions as in the initial supernatants of the cultures. This tends to indicate that no substantial amount of extracellular HM binding particles were produced by A. braunii at the growth stages examined.

The HMs associated with algal cells during growth in the presence of HMs are found in both the cell wall and cytoplasmic fractions of lysed cells (Table 10). Since the cell wall and cytoplasmic fraction distribution is similar

TABLE 9

The amount of Cd present per cell during growth of A. braunii in the presence of 0.11 and 1.12 ppm of Cd

Initial Cd concentration in growth medium	OD (650 nm)	µg Cd ($\times 10^{-8}$) per cell
0.11 ppm	.075	1.67
	.210	1.49
	.360	1.04
	.490	0.51
	.650	0.34
	1.050	0.21
1.12 ppm	.205	5.30
	.385	2.85
	.860	1.71
	1.100	2.16

TABLE 10

The distribution of HMs in the cytoplasmic and cell wall fractions* of A. braunii cells grown in the presence of Cu or Cd

HM	OD (650 nm)	HM concentration in whole culture (mg/l)	Total amount of HM present in cell fraction (mg/l)	Amount of HM present in: cell wall fraction in mg/l	as %	cytoplasmic fraction in mg/l	as %
Cu	0.610	0.64	0.27	.08	33	.16	67
	0.465	6.35	0.99	.25	32	.54	68
	0.480	6.35	1.09	.21	26	.60	74
	0.570	6.35	1.27	.56	45	.68	55
Cd	0.195	1.12	0.35	.11	34	.22	66
	0.235	1.12	0.40	.14	29	.26	71
	0.800	1.12	0.45	.15	33	.30	67
			Mean ± standard error:		33±4		67±4

*Fractions were obtained by the procedure outlined in Figure 4.

regardless of the HM used, the concentration of the HM and the stage of algal growth, it is possible that some type of cellular equilibrium process is functioning.

B. COMPETITION STUDIES

It is known from the numerous studies done on HM pollution in aquatic systems (OTTAWA RIVER PROJECT REPORT NO. 2, 1974; KRENKEL, 1975) that HMs are partitioned among the three major compartments of such ecosystems - namely among the water, sediment and biota. Most of these investigations, however, concentrated on only one or two compartments at a time. Recently, two multi-compartmental studies were reported (STØKES and SZOKALO, 1977; RAMAMOORTHY et al., 1977). The former study investigated the mobilization of Cu and Ni from sediment by a floating plant whereas the latter dealt with the distribution of Hg in bacteria, sediment and water. Using a similar method, as that outlined by Ramamoorthy et al. (1977), the distribution and interaction of Cd and Cu in river water, sediment and algae were examined.

1. HMs added to river water

When either Cd^{2+} or Cu^{2+} was added to Ottawa River water (characteristics shown in Table 11), it was taken up by both the algal and sediment compartments. In the case of Cd^{2+} the A. flos-aquae compartment steadily accumulated the metal ion over a 72 hr period. A steady state, as determined by the ratio of the HM ion accumulated by the algal compartment to the HM ion accumulated by the sediment, was reached by 24 hrs (Table 12 - I). The A. braunii compartment also accumulated

TABLE 11

Characteristics of Ottawa River water used in competition studies

pH	Conductivity	Eh	pCl	Orthophosphate @
7.30-7.63	52-80 umhos	+303- +333 mv	3.43-3.60	< 1 nM

@ Methods used in determining the various parameters are described in the methods section.

TABLE 12

The distribution of Cd^{2+} in a multicomponent system after the addition of Cd^{2+} to the water compartment

	Time (hr)	pH	AQUEOUS CONC. (mg) per litre	AQUEOUS CONC. (mg) total	CONC. IN CLAY (µg) per bag	CONC. IN CLAY (µg) total	CONC. IN ALGAE* (µg) per bag	CONC. IN ALGAE* (µg) total	TOTAL RECOVERY OF ADDED Cd in mg	TOTAL RECOVERY OF ADDED Cd as %
I	0	-	1.02	5.61	.40	2.80	0	0 (0) **	5.61	100
	1	7.40	.96	5.27	11.50	69.40	29.10	165.90 (2.40)	5.51	98
	6	7.61	.89	4.89	32.10	172.40	76.20	387.20 (2.20)	5.45	97
	12	7.63	.79	4.32	41.60	210.40	92.30	460.70 (2.19)	4.99	89
	24	7.64	.74	4.03	91.50	360.10	133.00	591.30 (1.60)	4.98	89
	48	7.64	.65	3.53	121.00	419.10	168.00	659.90 (1.57)	4.61	82
	72	7.63	.70	3.79	94.00	392.10	224.00	722.60 (1.84)	4.91	88
II	0	7.12	1.05	5.72	.20	1.40	0	0 (0)	5.72	100
	1	7.03	.96	5.27	7.50	46.40	16.50	107.25 (2.30)	5.42	94
	6	7.12	.88	4.82	32.70	171.20	60.60	349.80 (2.04)	5.34	93
	12	7.13	.73	3.99	56.80	267.60	112.50	527.10 (1.97)	4.78	84
	24	7.15	.73	3.98	74.40	320.40	193.60	770.40 (2.40)	5.07	89
	48	-	.66	3.58	133.00	437.60	284.00	922.80 (2.11)	4.94	86
	72	-	.66	3.57	148.00	452.60	304.00	971.20 (2.15)	4.99	87

* Algae I = Anabaena 7120, II = Ankistrodesmus braunii
Cd^{2+} added to water compartment as $Cd(NO_3)^2$: 15 mg/5.5 l ≈ 1 ppm Cd^{2+}
** Cd^{2+} accumulated by alga/Cd^{2+} accumulated by sediment

Cd^{2+} and reached a steady state within the same time interval (Table 12 - II).

When accumulation was calculated on a dry weight basis, the A. flos-aquae compartment accumulated 31 times more Cd^{2+} than the sediment whereas the A. braunii compartment accumulated 13 times more Cd^{2+} than the sediment (Figure 15).

Similar results were obtained when $Cu(NO_3)_2$ was added to the water compartment. Accumulation of Cu^{2+} by the algal and sediment compartments reached a steady state after 12 hrs (Table 13). On a dry weight basis, the A. flos-aquae compartment accumulated 16 times and the A. braunii compartment 8.5 times more Cu^{2+} than the sediment (Figure 16).

Some HM losses were observed during the experiments (Table 12 and 13). Since no HMs were found associated with the dialysis sacs, HM adsorption to the walls of the 'river water' container probably accounted for the losses.

Because the sacs containing either the sediment or the algae also contained distilled water, sacs containing only distilled water were included, as controls, in the experiments. These controls, removed after 72 hrs, showed insignificant accumulation of HMs.

Prior to HM analysis, routine microscopic examinations were performed on algal samples removed from the dialysing system. In samples of A. flos-aquae examined after 12 hrs of exposure to river water containing 1 ppm Cu^{2+} or Cd^{2+},

FIGURE 15

The accumulation of Cd^{2+} from river water, by algal and sediment compartments

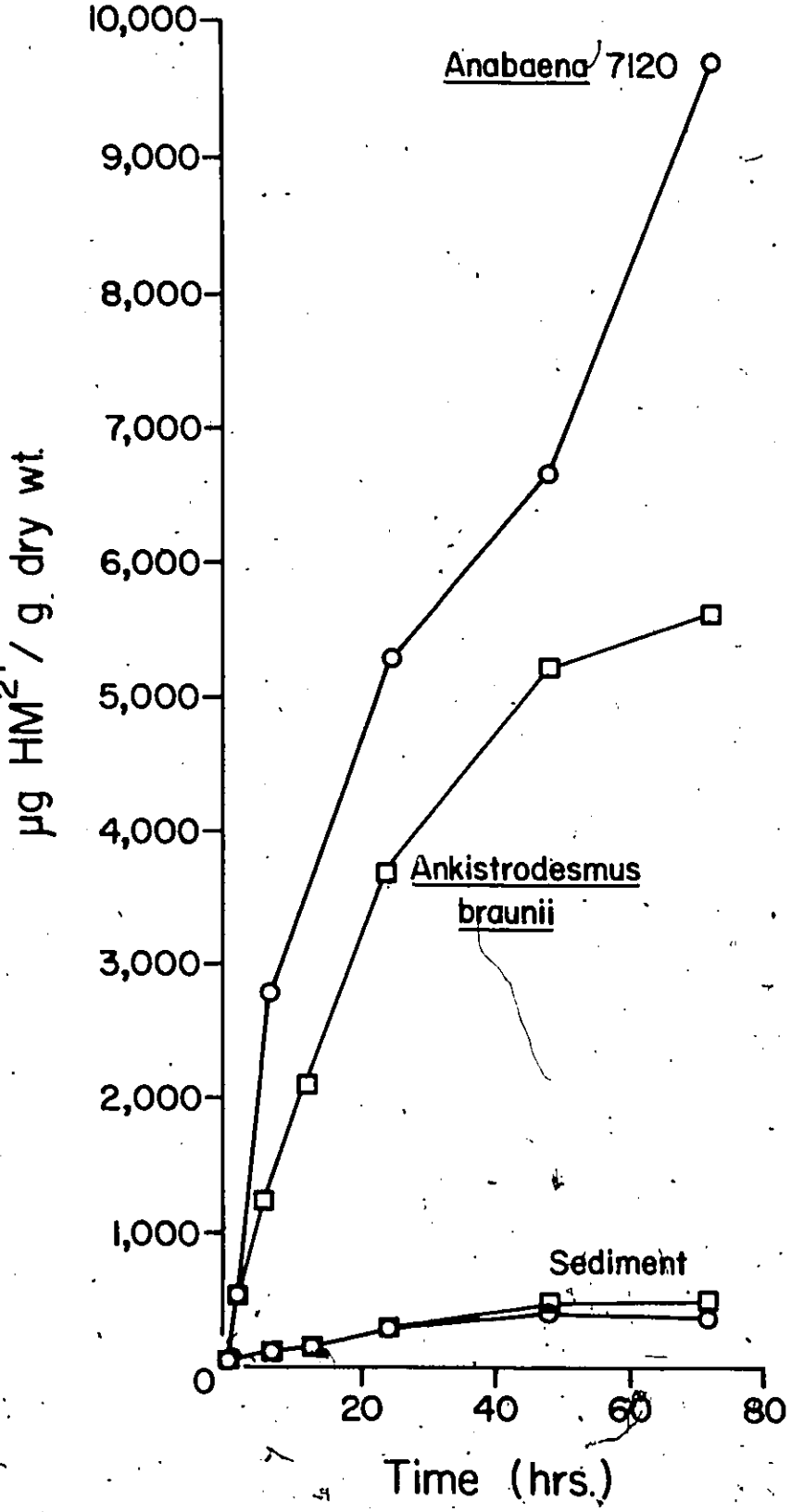

10,000
9,000
8,000
7,000
6,000
5,000
4,000
3,000
2,000
1,000
0
μg HM2+ / g. dry wt
Anabaena 7120
Ankistrodesmus braunii
Sediment
20
40
60
80
Time (hrs.)

TABLE 13

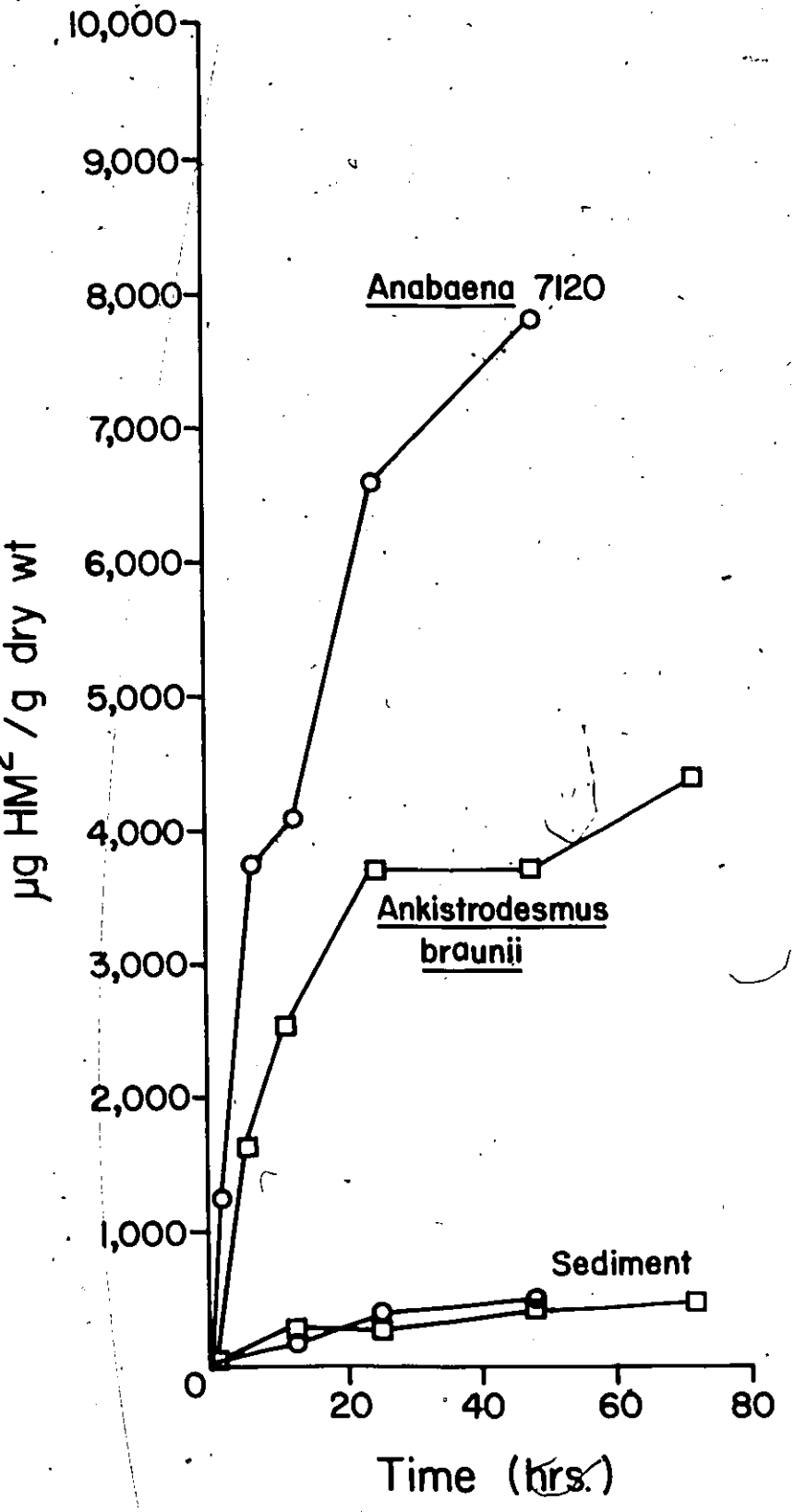
The distribution of Cu^{2+} in a multicomponent system after the addition of Cu^{2+} to the water compartment

	Time (hr)	pH	AQUEOUS CONC. (mg) per litre	AQUEOUS CONC. (mg) total	CONC. IN CLAY (µg) per bag	CONC. IN CLAY (µg) total	CONC. IN ALGAE* (µg) per bag	CONC. IN ALGAE* (µg) total	TOTAL RECOVERY OF ADDED Cu in mg	TOTAL RECOVERY OF ADDED Cu as %
I	0	7.01	1.02	5.61	16.25	97.50	1.25	7.1 ** (0.07)	5.71	100
	1	6.98	.92	5.05	30.75	170.00	8.75	43.43 (0.26)	5.26	92
	6	7.40	.85	4.66	66.25	312.00	45.00	163.45 (0.52)	5.14	90
	12	6.96	.82	4.48	80.00	353.25	63.75	232.22 (0.66)	5.07	89
	24	6.60	.77	4.23	101.25	395.75	69.76	256.16 (0.65)	4.88	85
	48	6.40	.70	3.79	153.00	447.50	110.25	298.75 (0.67)	4.54	80
II	0	7.07	1.04	5.72	19.20	134.40	1.20	8.28 (0.06)	5.86	100
	1	7.08	.83	4.56	30.00	199.20	13.20	80.00 (0.40)	4.84	83
	6	7.06	.83	4.55	63.00	364.20	50.70	287.17 (0.79)	5.20	89
	12	6.82	.83	4.54	77.28	421.32	78.28	408.74 (0.97)	5.37	92
	24	7.03	.73	4.00	99.96	489.36	118.44	553.05 (1.09)	5.02	86
	48	6.80	.73	4.00	123.00	535.44	140.40	532.79 (0.99)	5.07	87
	72	6.20	.63	3.35	140.40	552.84	156.60	558.82 (1.01)	4.46	76

* Algae I = *Anabaena* 7120, II = *Ankistrodesmus braunii*
Cu^{2+} added to water compartment as $Cu(NO_3)_2$: 21 mg/5.5 l ≃ 1 ppm Cu^{2+}
** Cu^{2+} accumulated by alga/Cu^{2+} accumulated by sediment

FIGURE 16

The accumulation of Cu^{2+} from river water, by algal and sediment compartments


10,000
9,000
8,000
7,000
6,000
5,000
4,000
3,000
2,000
1,000
0
μg HM2+/g dry wt
Anabaena 7120
Ankistrodesmus braunii
Sediment
20
40
60
80
Time (hrs.)

cellular debris was interspersed with single cells and cellular clumps. Few chains were evident. Subsequent examinations revealed more debris and progressively fewer whole cells. Furthermore, between 12 and 24 hrs, a blue-green color appeared in the algal dialysis sacs. This pigmentation was probably due to phycocyanin release upon cell lysis. Although a quantative examination of lysis was not carried out, it was noted that complete lysis of A. flos-aquae samples did not occur within 72 hrs. Since 72 hr control sacs of A. flos-aquae placed into river water without any HM additions did not lyse, the presence of the Cu^{2+} and Cd^{2+} was responsible for the phenomenon.

No lysis was observed in microscopic examinations of A. braunii cells. In fact, such light microscopic investigations at 1000 x magnification, did not reveal any cellular perturbations whatsoever.

These results tend to indicate that A. braunii and/or extracellular substances produced by this alga, competed very well with the sediment in accumulating Cu^{2+} and Cd^{2+} from the water column. The A. flos-aquae results, however, point to a somewhat different situation. Initial accumulation of Cu^{2+} and Cd^{2+} by the algal compartment was probably due to the cells and/or extracellular compounds. Once lysis began, the wide variety of released substances remaining in the 6,000 mwt

cut-off dialysis sacs were probably also responsible for HM accumulation. HMs are known to interact with electron donating atoms (i.e. N,O, S,P) as well as with π - electron donating systems (i.e. aromatic rings). Thus, proteins, nucleic acids - and prophyrins - just to mention a few organic substances - are candidates for complex formation (SAXBY, 1969; MARTELL, 1971).

2. HM loaded onto the sediment

The sediment from the Ottawa River bed contained no detectable Cd and 50.6 $\pm$ 0.5 ppm Cu (an average of five samples). It was loaded with 100 ppm Cd^{2+} - as opposed to a lower concentration of the HM - in order to have high Cd detection during the experiments. On the other hand, as a substantial amount of Cu was naturally present in the sediment, emphasis was placed on maintaining the natural Cu concentration. The possibility, however, existed that the natural Cu was irreversibly bound and/or sorbed to crystalline lattices, sulfides and/or organic compounds (JONASSON, 1970). Therefore, 1 ppm Cu^{2+} was loaded onto the sediment.

Samples of the loaded sediment were analysed. The average Cu concentration of five loaded samples was 51.5 $\pm$ 0.6 ppm whereas five Cd-loaded samples averaged 87.5 $\pm$ 1.0 ppm Cd. Sorption to the glass container during loading probably accounted for the Cd loss since no significant amounts of Cd

were found in the water of the post-loading wash.

The algal compartments accumulated substantial amounts of the HMs when the source compartment of the metal ion was the sediment. The A. flos-aquae compartment accumulated 20 µg of Cu per g dry weight whereas the A.braunii compartment accumulated 7 µg per g dry weight over a 72 hr period (Figure 17). Cd accumulation was similar to Cu accumulation - 20 µg per g dry weight of A. flos-aquae and 9 µg per g dry weight of A. braunii(Figure 18).

Since the amount of HM lost was within the experimental error of the amount initially present in the sediment, the sediment did not show any progressive decrease in its HM content. The water showed a negligible accumulation of Cu and Cd during the experiments (Figure 16 and 17) as well as during control studies when only loaded sediment samples were placed in the water column.

No algal lysis was observed colorimetrically or microscopically throughout these experiments.

These experiments showed that the two algae and/or extracellular compounds produced by them, mobilized sediment bound HMs. In terms of efficiency, however, the A. flos-aquae compartment accumulated more HMs. One physical factor which could account for this observation is the larger surface area of A. flos-aquae per g dry weight.

Since large masses of A. flos-aquae and A. braunii

FIGURE 17

The accumulation of sediment-bound Cu by algal compartments and river water

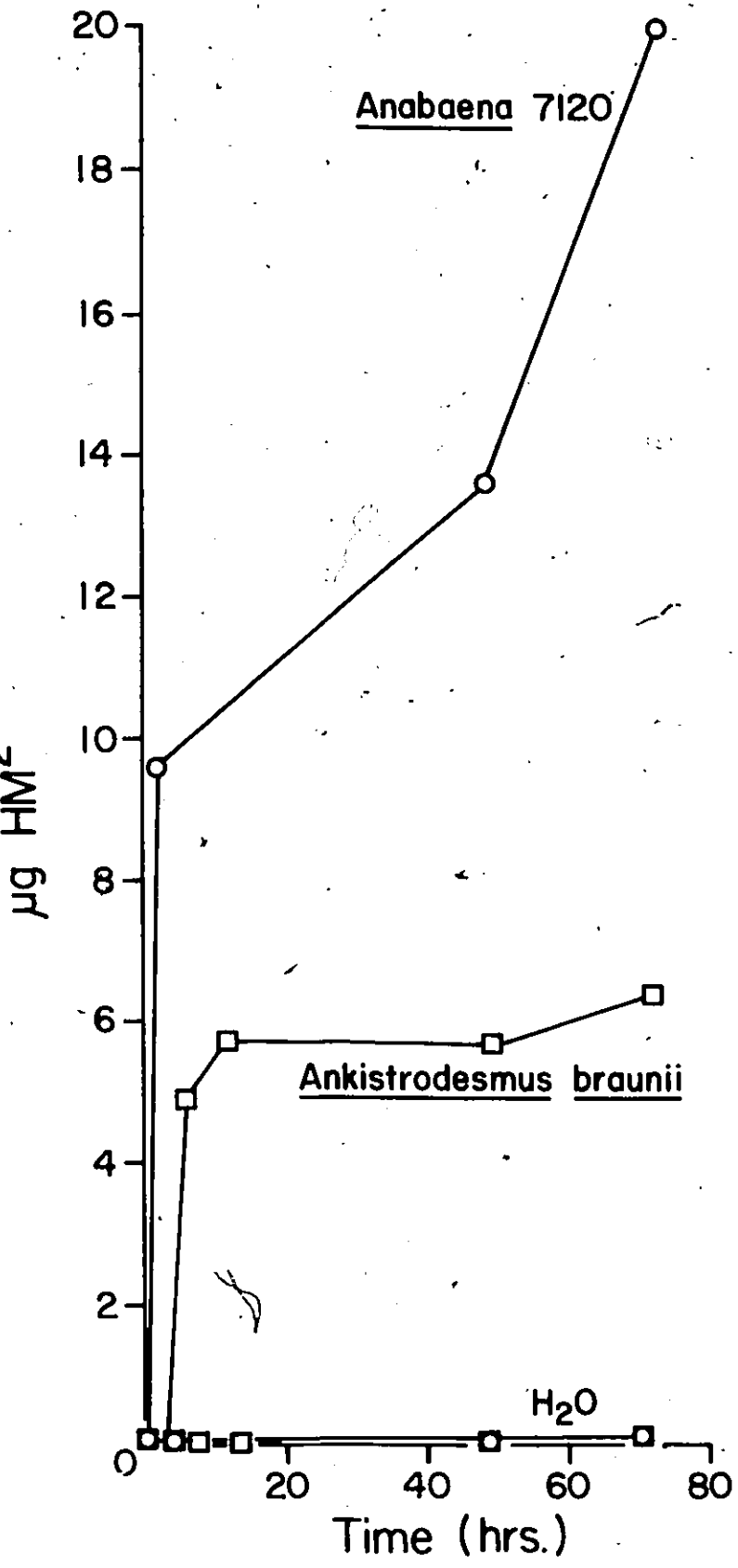
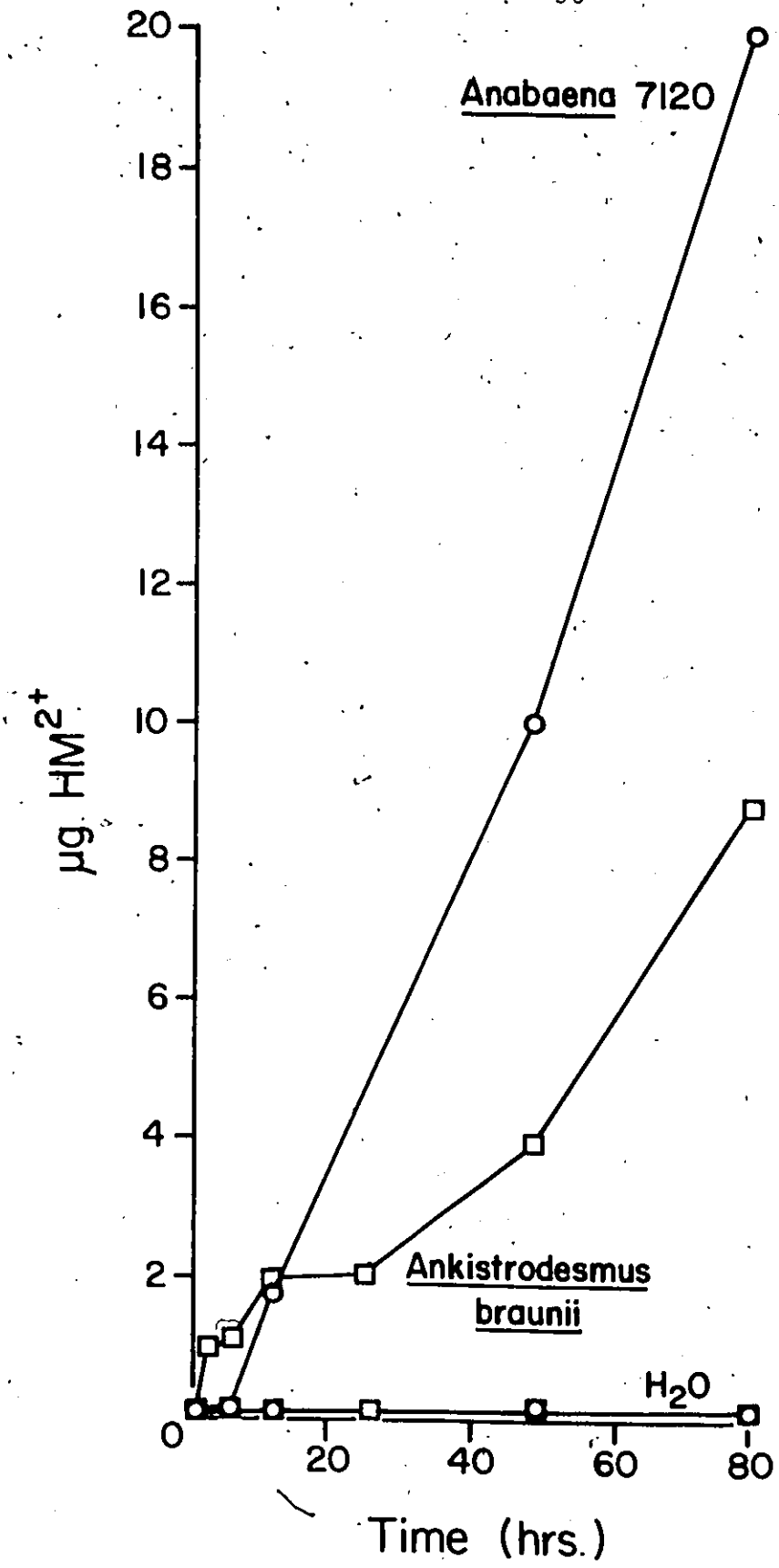

20
18
16
14
12
10
8
6
4
2
0
μg HM2+
Anabaena 7120
Ankistrodesmus braunii
H2O
20
40
60
80
Time (hrs.)

FIGURE 18

The accumulation of sediment-bound Cd by algal compartments and river water


Anabaena 7120
Ankistrodesmus braunii
H_2O
μg. HM^{2+}
Time (hrs.)
0
2
4
6
8
10
12
14
16
18
20
20
40
60
80

(and/or extracellular substances produced by these algae) mobilized sediment-bound HMs, the question was raised of whether this type of mobilization could occur during algal growth.

3. Growth of algae in the presence of sediment

Both A. braunii and A. flos-aquae grew when 2 g of natural or loaded (100 ppm Cu^{2+} or Cd^{2+}) sediment were present in the culture medium. The growth rate of the former alga was the same as the control in all cases (Figure 19) whereas that of the latter was always lower (Figure 20). The slowest rate was observed when A. flos-aquae was grown with Cd-loaded sediment.

Although one cannot conclude that growth inhibition in the presence of non-loaded sediment was a result of the naturally present Cu in the sediment, the further decreases in the growth rate when A. flos-aquae was cultured in the presence of loaded sediment can be attributed to the HMs.

4. Competition during growth

AAS analyses at various stages of A. braunii growth in the presence of non-loaded and Cu-loaded sediment revealed that Cu was mobilized from the sediment. A substantial amount of this Cu was found to be associated with the algal cells (Table 14). Although a progressive increase was seen in

FIGURE 19

Growth of A. braunii in the presence of sediment.

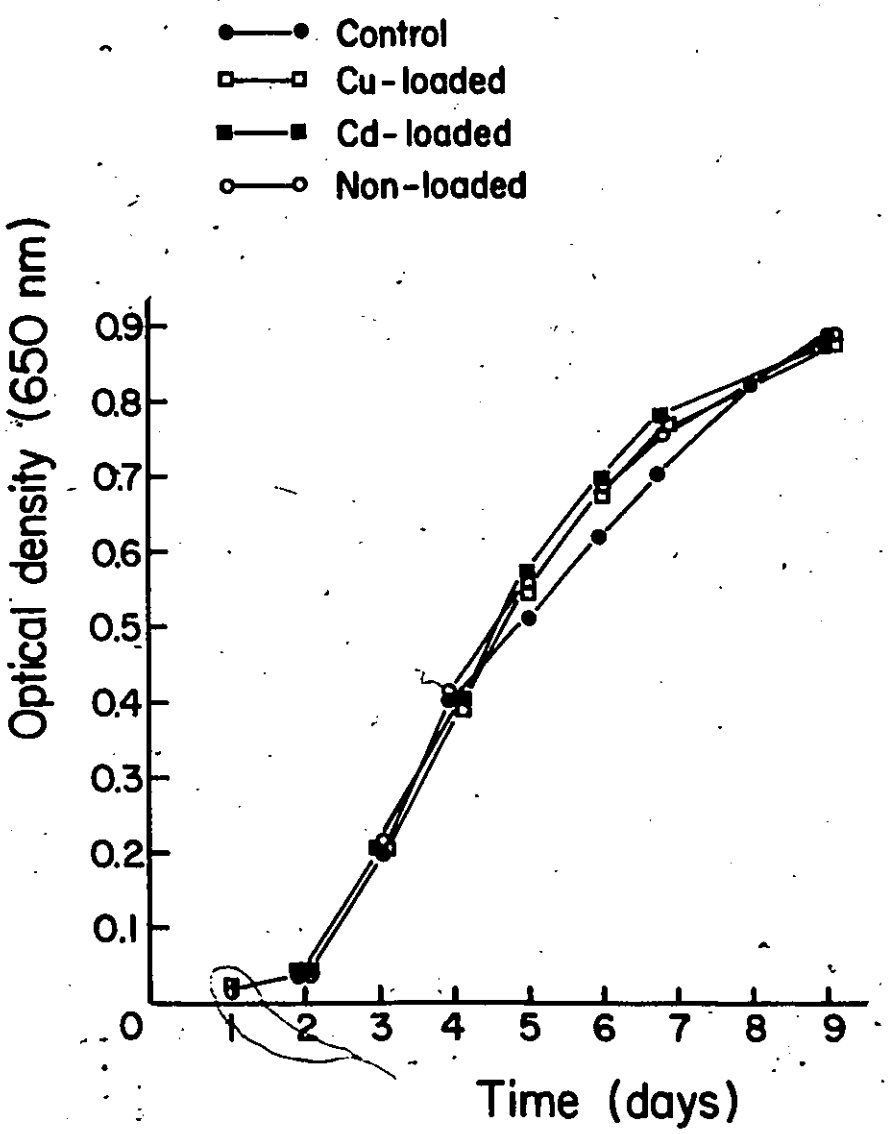

Control
Cu-loaded
Cd-loaded
Non-loaded
Optical density (650 nm)
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1
2
3
4
5
6
7
8
9
Time (days)

FIGURE 20

Growth of A. flos-aquae in the presence of sediment.

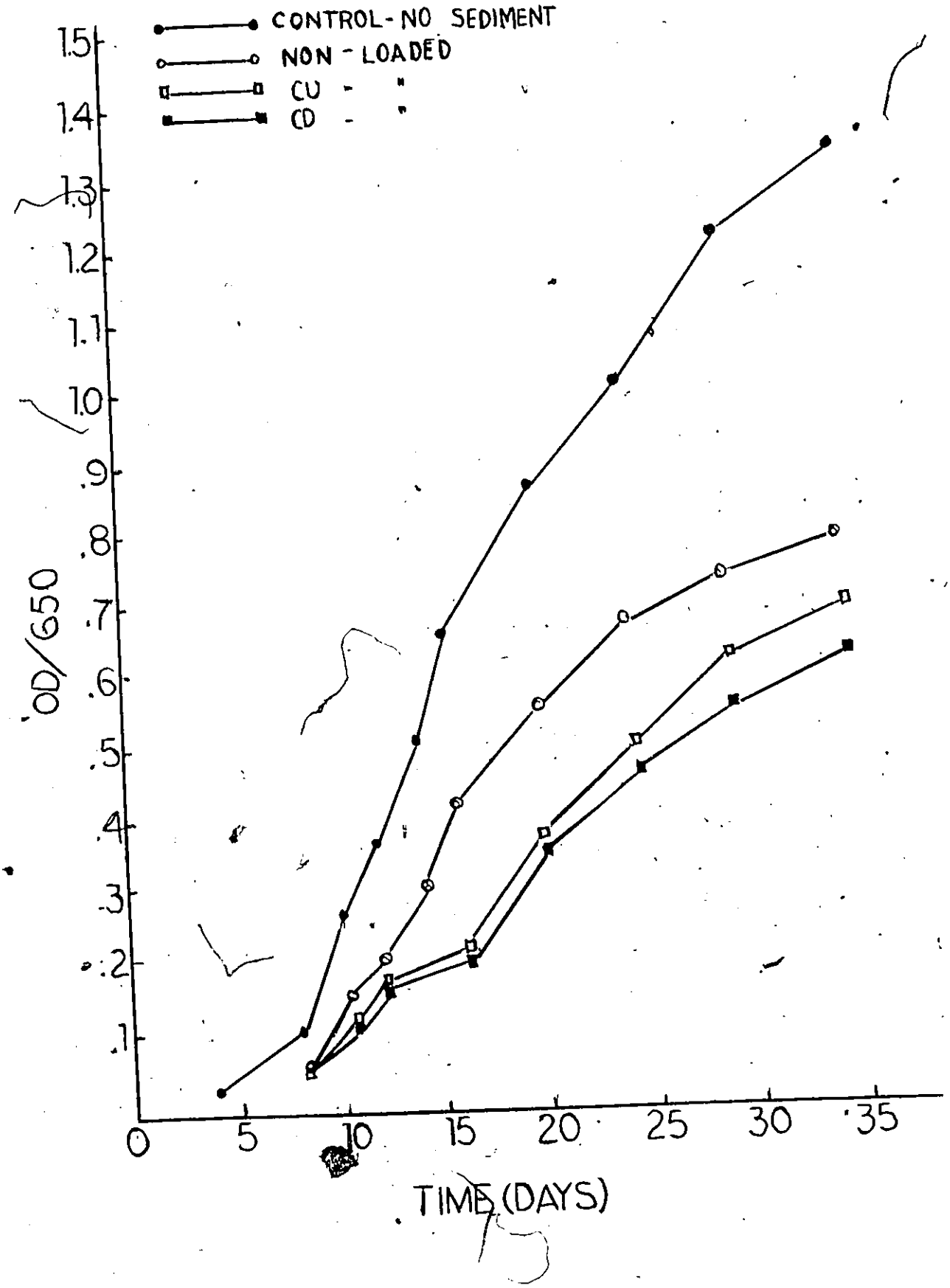

CONTROL - NO SEDIMENT
NON - LOADED
CU - "
CD - "
1.5
1.4
1.3
1.2
1.1
1.0
.9
.8
.7
.6
.5
.4
.3
.2
.1
OD/650
0
5
10
15
20
25
30
35
TIME (DAYS)

TABLE 14

HMs in the supernatant and associated with A. braunii cells during algal growth in the presence of sediment.

Sediment	HM analysed	Time (days)	OD/ 650	HM associated with* Supernatant µg/ 100 ml culture	A. braunii µg/ 100 ml culture	A. braunii µg/ g dry weight
Non-loaded (natural)	Cu	2	.050	0.10	0	0
		5	.500	0.30	0.15	4.80
		7	.720	0.50	0.20	4.45
		13	1.020	0.50	1.00	15.72
		17	1.150	0.50	1.20	16.74
Cu-loaded	Cu	1	.005	0.05	0	0
		3	.220	0.05	0.30	21.89
		5	.550	0.20	1.20	27.65
		12	.990	0.70	2.30	37.30
		17	1.105	1.00	2.80	40.64
Cd-loaded	Cd	3	.215	0	0.05	3.73
		5	.545	0	0.70	20.59
		7	.790	0	1.80	36.51
		10	.960	0	1.40	23.37
		17	1.155	0	1.80	25.00

* Values were corrected for controls (Table 16).

both cases, as growth proceeded, more Cu was cell associated when growth occurred in the presence of Cu-loaded sediments - 40.64 µg per g dry weight was accumulated in comparison to 16.74 µg per g dry weight in non-loaded sediment. More Cu was also present in the supernatant (1.00 µg per culture compared to 0.50 µg per culture).

Mobilization of Cd during A. braunii growth also occurred. The cells accumulated Cd until a maximum of 36.51 µg/g dry weight was reached. This was followed by a desorption. Cd was not detected in the supernatant during algal growth in the presence of Cd-loaded sediment.

When A. flos-aquae was grown in the presence of Cd-loaded sediment, mobilized Cd was also accumulated by the alga (Table 15). However, some Cd was found in the supernatant. On the other hand, mobilized Cu was located predominantly in the supernatant. Furthermore, substantial amounts became evident only late in the growth of the algal cultures. As with A. braunii cultures, more Cu was mobilized from the Cu-loaded sediment than the unloaded sediment - 3.40 and 1.60 µg/ culture respectively.

No significant decreases were observed in the HM content of the sediment during these experiments. This is understandable since the values of the HM losses were within the experimental error of the initial values - 153 $\pm$ 1.5 ppm Cu and 87.5 $\pm$ 1.0 ppm Cd.

TABLE 15

HMs in the supernatant and associated with A. flos-aquae cells during algal growth in the presence of sediment.

Sediment	HM analysed	Time (days)	OD/ 650	HM associated with* Supernatant µg/ 100 ml culture	A. flos-aquae µg/ 100 ml culture	A. flos-aquae µg/ g dry weight
Non-loaded (natural)	Cu	9	.100	0.10	0	0
		12	.180	0.50	0	0
		20	.545	0.50	0.05	2.26
		32	.760	1.50	0.10	3.25
Cu-loaded	Cu	10	.120	0.05	0	0
		19	.330	0.10	0.05	3.73
		25	.500	2.50	0.10	4.93
		35	.650	3.30	0.10	3.80
Cd-loaded	Cd	10	.110	0.05	0	0
		15	.200	0.10	0.15	18.52
		20	.340	0.40	0.50	36.23
		25	.450	0.50	0.90	49.45
		35	.635	0.80	1.50	58.37

*Values were corrected for controls (Table 16).

TABLE 16

HMs present in Chlamydomonas and G0 growth media after exposure to sediment.

Medium	Sediment	HM analysed	Exposure time (days)	HM present in: µg/100 ml medium
Chlamydomonas	Non-loaded	Cu	2	0.05
			5	0.05
			7	0.05
			13	0.10
			17	0.10
	Cu-loaded	Cu	1	0.25
			3	0.25
			5	0.35
			12	0.60
			17	0.55
	Cd-loaded	Cd	3	0.50
			5	0.50
			7	0.50
			10	0.45
			17	0.60
G0	Non-loaded	Cu	9	0.25
			12	0.30
			20	0.35
			32	0.30
	Cu-loaded	Cu	10	0.45
			19	0.55
			25	0.50
			35	0.65
	Cd-loaded	Cd	10	0.25
			15	0.30
			20	0.50
			25	0.35
			35	0.70

Thus, algal growth in the presence of sediment instigated HM mobilization. A. braunii was able to accumulate Cu and Cd. Cells of A. flos-aquae accumulated Cd but little Cu. Since substantial amounts of Cu were located in the supernatant of A. flos-aquae cultures at late stages of growth, it is possible that extracellular secretions are accumulating the HM and playing a significant role in the mobilization of Cu.

GENERAL DISCUSSION AND CONCLUSIONS

Both accumulation of HMs and decreases in growth rates are commonly reported responses of algae to HMs (HUTCHINSON and STOKES, 1975; CONWAY, 1978; SUNDA and GUILLARD, 1976). These responses were observed when A. braunii was grown in the presence of Cu and Cd nitrate. The growth rate of the alga progressively decreased as the Cu concentration in the medium increased from 10^{-7}M to 10^{-4}M. At a 10^{-3}M concentration of Cu, growth was completely inhibited. Similarly a decrease in growth rate accompanied increases in the Cd concentration between 10^{-7}M to 10^{-5}M whereas 10^{-4}M and 10^{-3}M of the HM stopped growth.

Although accumulation of both Cu and Cd occurred under culture conditions, a different pattern was exhibited for each HM. With A. braunii an initial accumulation of Cd per cell, followed by a decrease occurred, a pattern previously documented in the literature for Cd accumulation by Asterionella formosa (CONWAY, 1978) and Phaeodactylum tricornutum (COSSA, 1976). On the other hand, the pattern of Cu accumulation by A. braunii seems to be peculiar to this alga. When grown in the presence of 10^{-4}M or 10^{-5}M $Cu(NO_3)_2$, the amount of Cu per cell remained constant throughout growth. The absolute amount bound, however, increased when the cells were grown with the higher Cu concentration. At a 10^{-6}M

concentration of Cu, the Cu associated per cell seemed to be constant. Once all the Cu was accumulated from the medium, a concomitant decrease per cell was, of course, observed as growth proceeded.

In spite of the different pattern of accumulation displayed by A. braunii for Cu and Cd, the distribution of the cell associated HM between the cell envelope and its contents was the same for both HMs. Approximately one-third of the cell-associated HM was bound to the cell wall fraction whereas two-thirds were associated with the cytoplasmic fraction. It is noteworthy here that this distribution ratio has been previously reported for Cd in another alga - Asterionella formosa (CONWAY, 1978). Thus, the distribution does not seem to be a species-specific phenomenon. It should also be mentioned that even though Cu and Cd show the same distribution, this cannot be generalized to include all HMs. For example, although Cd was distributed in a 30:60 ratio in A. formosa cells, the distribution of arsenic was completely different (CONWAY, 1978).

When experiments were performed in which 10^{-5}M $Cu(NO_3)_2$ was added to growing cultures of A. braunii, the algae responded in the same manner as it did when Cu was present throughout growth, i.e. a decrease in growth rate. In contrast, lysis was observed when Cu additions were made to logarithmically growing cultures of A. flos-aquae. Lysis was followed by normal growth when 10^{-5}M $Cu(NO_3)_2$ additions were made whereas

growth did not resume after 10^{-4}M additions. Precisely what is happening in the former case is not clear. Since a selection of more resistant vegetative cells does not seem to be occurring, it is possible that akinetes or spores are responsible for the phenomenon observed. Akinetes are known to be present in a member of blue green algae, including Anabaena cylindrica. Furthermore, akinetes have been shown to be very resistant although the exact nature and extent of their resistance is not, as yet, clear (WOLK, 1973).

Even though the presence of akinetes in A. flos-aquae has not been documented in the literature, it is quite possible that this alga produces spores when nitrogen is not directly available in the growth medium. These may have been overlooked under the light microscope and noted as slightly larger vegetative cells. This is not unlikely since Anabaena cylindrica spores possess the same pigmentation as the vegetative cells (WOLK and SIMON, 1969).

AAS studies revealed that no Cu was associated with A. flos-aquae cells when normal growth resumed after 10^{-5}M Cu additions. No Cu became cell associated when the same additions were made to late log phase cultures. Thus, A. flos-aquae does not accumulate Cu present in the growth medium. Furthermore, the Cu is probably complexed with organic compounds, i.e. perhaps the lysate in the former case and extracellular secretions in the latter case, thus making it

non-toxic and allowing for normal growth.

It is generally believed that HM organic complexes are less toxic to phytoplankton than the ionic forms of HMs. For example, it is demonstrated by Sunda and Guillard (1976) that the growth rates of Thalassiosira pseudonana and Nannochloris atomus were adversely affected by increases in cupric ion activity as opposed to increases in the total Cu concentration in the growth media. Cupric ion activity was varied during these experiments by the use of the chelator trishydroxymethylamino methane and by pH adjustments.

Naturally produced chelators have also been shown to reduce HM toxicity to algae. Organic matter excreted by Nitzschia palea in response to the presence of Cu in its growth medium, was thought to complex with the HM. This, supposedly, allowed for the resumption of normal growth after an extended lag period (STEEMAN NIELSEN and WIUM-ANDERSEN, 1971). Extracellular polypeptides of Anabaena cylindrica, produced concomitantly with the growth of healthy cells, have been shown to reduce the toxicity of Cu sulfate to the alga. The presence of these excretions allowed for algal growth in the presence of higher Cu concentrations (FOGG and WESTLAKE, 1955).

Using a Cu^{2+} specific electrode, Cu-binding substances were shown to be produced by A. flos-aquae during late stages of growth in dilute medium. Since it is likely that this alga secretes abundant polysaccharides (WANG and TISCHER, 1973)

and probably polypeptides (FOGG and WESTLAKE, 1955) and other organic compounds (FOGG, 1966) it is still uncertain which substance plays the major role in HM binding. Studies of natural waters have revealed that hydroxamates can be major Fe (and HM) binding substances (MURPHY et al., 1976) whereas electrode studies have implicated a substrate of less than 500 mwt (McKENZIE, 1977).

Further studies should be undertaken to determine, in more detail, the morphology and/or morphological changes in A. flos-aquae cells exposed to HMs. Although preliminary light microscopy studies have revealed that 10^{-5}M or 10^{-4}M Cu additions to A. flos-aquae cultures tend to disrupt algal chains, induce clumping, and cause lysis, electron microscopic obervations would be more revealing.

In this study, pure culture experiments were succeeded by more environmentally oriented work. Although information from these simulated aquatic system studies is not directly applicable to nature, the results can be useful in predicting outcomes of HM stresses imposed on natural systems.

One aim of these studies was to determine the competition between algae and sediment for Cu and Cd present in water. A. braunii competed extremely well with clay sediment in accumulating both Cu^{2+} and Cd^{2+} from river water. (From the result of the culture studies, it seems reasonable to assume

that most, if not all, accumulation was carried out by the alga itself as opposed to secreted substances). The algal accumulation was substantially larger than that of the sediment even though the surface to volume ratio of the sediment was greater. Thus, surface adsorption was probably not the only mode of accumulation by the alga. In fact, it was shown in the culture studies that HMs are found in the cytoplasmic fraction of cells grown in the presence of HMs.

In contrast to accumulation, lysis was seen when A. flos-aquae was exposed to 1 ppm Cu or Cd in the water compartment. Since lysis was not evident when the alga was exposed to HMs from loaded sediment (low HM levels in the water compartment), it was probably caused by the high concentration and direct availability of the HM in the water compartment during the above experiment.

Since the conditions of the above competition experiments were the same for both algae, one can conclude that this strain of A. braunii is more resistant to HMs than A. flos-aquae. This could be due to the fact that A. flos-aquae has been kept in culture in the laboratory for a number of years whereas A. braunii was freshly isolated from the polluted Ottawa River. Laboratory cultures of Scenedesmus acuminatus and Chlorella vulgaris have been shown to be less Cu resistant than currently isolated (from polluted Sudbury lakes) counterparts -Scenedesmus acutoformis and Chlorella fusca (STOKES et

al, 1973). On the other hand, the difference in sensitivity could be due to the inherent characteristics of A. braunii and A. flos-aquae. A suggestion has been made by Whitton (1973) after evaluating a number of studies, that blue-green algae may be generally more sensitive to HMs than green algae.

Competition between algae and sediment for HMs was also examined from a different angle. Once accumulated by the sediment, HMs cannot be considered totally immobilized. Physical factors, such as acidification of waters by, for example, sulfur dioxide (SO_2) solution from industrial sources, can induce HM solubilization. In fact, HM extractions from sediment rely on this principle. In addition, to acid-mediated release of HMs from sediment, other types of mobilization can also occur. In the simulated aquatic system A. braunii and A. flos-aquae compartments were able to mobilize Cu and Cd from the sediment and accumulate the HMs. This was not carried out by direct algal-sediment contact since the two compartments were spatially separated during the experiments. Consequently, water was the medium of transport even though it did not accumulate substantial amounts of the HMs.

Mobilization and accumulation of HMs by biological material from sediment in laboratory aquaria has been shown to occur. The bacterium, Pseudomonas fluorescens, accumulated substantial amounts of Hg from sediment (RAMAMOORTHY et al., 1977). Salvina natans, a floating aquatic fern, also accumulated

HMs from sediment, i.e. Ni and Cu. Furthermore, the accumulation tended to increase the movement of the HMs into the water compartment from the sediment (STOKES and SZOKALO, 1977).

Mobilization and accumulation of Cu and Cd was also observed during A. braunii growth in the presence of sediment (HM loaded and natural). AAS studies on the algal fraction of the culture showed that most of the mobilized Cu and all of the Cd was associated with the algal cells. Similarly, A. flos-aquae cells mobilized and accumulated some Cd from the sediment. However, the supernatant accumulated most of the mobilized Cu from natural and Cu loaded sediments. This was particularly evident during late growth and stationary phases. It would seem that in the case of A. flos-aquae growth extracellular organic chelators produced by the alga are facilitating HM mobilization from the sediment. It has previously been shown that organic chelators, i.e. NTA and EDTA, can influence the mobilization of HMs such as Pb and Zn into water from contaminated sediments (BARCIA et al., 1973).

Possible implications for higher trophic levels exist as a consequence of HM accumulation by algal cells and their extracellular compounds. Accumulation of Cu and Cd from natural waters and/or from sediments may occur in edible algae, such as A. braunii. (Generally, algae, except Cyanophyta, smaller than 50 µm are considered readily edible BRIAND and McCAULEY, 1978). This initial accumulation could

then result in the biological magnification of these HMs up the food chain. Filter feeders such as small crustacea and larvae of invertebrates and fishes may be initially involved in the magnification. Such zooplankton in turn may be consumed by surface fish. Eventually, if the HM concentrations do not result in the mortality of the consumer organism (i.e. HMs have been shown to be extremely toxic for some fresh water fish species such as trout (BALL, 1967) at least, under laboratory conditions), higher levels may reach man. For example, Cd levels above the average value termed acceptable in foodstuffs (0.05 µg/g wet wt. - FRIBERG et al., 1974) have been found in some edible fish of the Laurentian Great Lakes (LUCAS et al, 1970), i.e. 0.094 µg/g wet wt.

Accumulation of HMs by extracellular compounds of algae may possibly also result in biomagnification. Fogg (1966) mentions that organic matter released by algae has been shown to be an adequate food source for Artemia salina (BAYLOR and SUTCLIFFE, 1963). The brine shrimp, in turn, is a food source of many zooplankton.

The question of whether Cd biomagnification occurs is still controversial. Friberg et al. (1974) contend that there is little evidence in support of Cd concentration in aquatic food chains when data from various studies on different trophic levels is considered. However, King (1977) has shown that in fact a biomagnification can occur. Little information

is available on Cu biomagnification with the exception of one long term study (TING and DEVEGA, 1967). Consequently, studies in a simulated aquatic system involving a number of different trophic levels could be useful in determining to what extent Cu and Cd biomagnification can occur.

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