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THE EFFECTS OF ALGAE AND THEIR PRODUCTS ON HEAVY METAL BINDING

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Thesis submitted to the School of Graduate Studies, University of Ottawa, in partial fulfillment of the requirements for the degree of Master of Science in Biology.

Candidate

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Supervisor

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The work reported here was made possible because of the guidance and help, so kindly offered to me.

Dr. B.G.Oliver analyzed natural water samples for Total Inorganic Carbon and Total Organic Carbon and Dr. D. Shindler provided analyses for nitrogen compounds and silica.

Dr. F. Briand not only located algal blooms but also identified the predominant algae and determined their cell numbers and biomass during peak bloom periods.

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ABSTRACT

Using ion-specific electrode and atomic absorption spectroscopy the heavy metal (HM) binding capacities of four Ottawa area natural water systems supporting algal blooms were examined, and the effects of HM ions and Anabaena 7120 on each other were studied.

The HM binding capacities of the natural water systems studied during 1975 were much higher than those reported previously for Ottawa River water. The HM binding capacities of at least two systems had not decreased several months after the blooms disappeared.

Ultrafiltration of the water did not decrease the HM binding capacities of the waters indicating that the HM binding substrate had a molecular weight of less than 500. Complete ashing of the water samples removed the Hg^{2+} binding capacity, indicating that organic carbon compounds formed the predominant HM binding substrate in the four water systems.

Concentrations of 10^{-3}M and 10^{-4}M $Cu(NO_3)_2$ and $Cd(NO_3)_2$ completely inhibited growth of Anabaena 7120 in GO medium. Copper and cadmium concentrations below 10^{-4}M produced an elongated lag phase compared to cultures with no HM ions present in the medium. When 10^{-5}M nitrilotriacetic acid (NTA) was added to the medium with equal concentrations of $Cu(NO_3)_2$ or $Cd(NO_3)_2$ the lag phase was further elongated.

An elongated lag phase in Anabaena 7120 cultures was produced by 10^{-5}M to 10^{-7}M $Pb(NO_3)_2$. Growth was not inhibited by 10^{-3}, 10^{-4} or 10^{-8}M $Pb(NO_3)_2$

ABSTRACT (Cont'd)

in GO medium. A heavy white precipitate formed in GO medium containing 10^{-3}M and 10^{-4}M $Pb(NO_3)_2$.

Atomic absorption analysis of Anabaena 7120 culture grown in GO medium containing 10^{-5} $Cd(NO_3)_2$ had the same cadmium concentration associated with the cell fraction after the lag phase as at the beginning. In contrast, copper was almost completely released from the cells at the end of the lag phase.

INTRODUCTION

Heavy metals (HM) have been known for a long time to have antimicrobial activity, and it is thought that the ability of HM ions to bind sulphydryl groups may affect enzyme activity.[31] More recently, the interest in HM's has developed into a concern for the environment and for ourselves. As a result, the transport of HM's, their availability to biota in natural waters and their entry into food chains have become important areas of research.

Since toxic amounts of HM's have been entering the soil, air and waters of this planet for some time, solving this pollution problem requires not only control of the HM's entering the environment but also the study of their interactions under conditions to which they are subjected in nature.

Background Levels of Heavy Metals and Their Sources of Pollution

The information about HM background levels and sources of pollution was taken primarily from the following reviews: The Environmental Mercury Problem[10], Environmental Mercury Contamination[29], Advances in Environmental Science and Technology[13], Lead in Soils and Plants: A Literature Review[59], and Cadmium in the Environment[19]. Further reference will only be made to sources other than these.

When starting a study concerned with the pollution of the environment, the first question that naturally comes to mind is: what is the source of this pollution? Or to put it more loosely, how did we get into this mess? Heavy metals enter the environment from natural sources and as a result of man's ingenious ability to release them as industrial waste products. Since this study included Hg^{2+}, Pb^{2+}, Cu^{2+} and Cd^{2+} ions, the sources of these heavy metals to the environment will be described.

Cinnabar (HgS), the red ore, and metacinnabar, a black dimorphous form of HgS, are the important forms of mercury mineral deposits found in nature. The largest deposits are present in Spain where mining first began around 700 B.C. Only three other areas in the world, Yugoslavia, Italy, and California have become important producers. Because of its volatile nature and the extent of its release to the environment, it is difficult to provide true background counts for mercury. Although natural background values for mercury in the soil range from 10 to 920 ppb, D'Itri concludes that a reasonable range would be between 10 and 150 ppb depending on the pH and the organic content of the soil. Klein reported values averaging 30 ppb in soil samples distant from industrial areas, while in industrial regions mercury concentrations were as high as 350 ppb. The atmospheric mercury values given by Williston ranged from 1 to 50 ng/m^3 while in 1967, Ericksson estimated the average world concentration to be about 20 ng/m^3. After presenting the results of other workers D'Itri concluded that a reasonable range for the "normal background levels" of mercury in ground waters might be 0.02 to 0.7 ppb. He also concluded that ocean waters though varying greatly in mercury content, probably have background levels ranging from 0.03 to 0.3 ppb.

Although mercury is used widely, major consumption is limited to a relatively small number of industries. In 1968, the electrical apparatus industry accounted for over a quarter of the total mercury consumed in the United States. A million pounds of mercury were used in the manufacture of mercury batteries and alkaline energy cells alone, which are easily disposed of, releasing mercury into the environment. Chlorine and caustic soda production caused the second major consumption of mercury in 1968, using 23.1 percent of the total in the United States. Because of the purer grade of caustic soda formed, an increasing proportion is being manufactured using a continuous flow mercury cathode cell, resulting in the production of over 8.4 million tons of chlorine, with 0.45 pounds of mercury being lost for each ton of chlorine produced. Besides this loss during manufacture, trace quantities of mercury can be introduced to the human food cycle if caustic soda containing trace amounts of mercury is used in the preparation of foods. Of the total United States mercury consumption in 1968, 14.4 percent was used to prevent bacterial and fungal damage of paints and 10.6 percent was used in the manufacture of industrial control instruments such as mercury switchs, relays, gauges, pump seals and valves. Mercury used in agriculture, dental preparations, the pulp and paper industry, pharmaceuticals, cosmetics and in the extraction of gold by amalgamation accounted for the remaining 25 percent of United States mercury consumption in 1968. On the average, the mercury content of sewage effluent is one order of magnitude greater than that of the aquatic system which it enters, although it appears that treatment plants prevent substantial amounts from entering water systems. However, if sludge is ashed and released to the atmosphere, or used as fertilizer, mercury could reach the environment indirectly.

Lead occurs naturally in the environment in the form of minerals such as $PbCO_3$, $PbSO_4$ and mainly as PbS. The average value for the lead content in the earth's crust is 16 μg/g of soil, with the highest lead concentrations in the upper layers. Normal background levels of lead in surface waters have been reported to be in the area of 0.55 ppb.[15] Lead is released as a result of volcanic eruptions and forest fires, but these contributions are small compared to contributions resulting from man's activities.

Organic lead added to gasoline accounts for about half of the lead introduced to the environment each year. During combustion the lead is converted to inorganic compounds, and emitted to the atmosphere as lead halides, hydroxides and oxides. Automobiles, like smelters, act as point sources of lead contamination, the concentration of lead in the air and soil decreasing rapidly with distance from the source as particles greater than 5 μ in diameter settle rapidly. Considerable amounts of lead from these point sources may enter surface waters as run-off as a result of washing dust from streets. Lead compounds are also used as pigments in cosmetics and paint products and it is estimated that about 16 percent of lead used annually is introduced to the environment by these products. In fact, lead chromate is used to paint the inside of water storage tanks.[15] Lead is spread on the soil as the insecticide lead arsenate, and as an impurity in fertilizers. Combustion of fossil fuels releases lead into the environment, but this source represents a minute amount of contamination. The use of lead to manufacture storage batteries has tripled since 1934. However, since approximately 90 percent of the lead used in the batteries is recycled after 3 or 4 years, they contribute very little to the massive pollution by other sources.

Governments interested in the safety of waters as drinking supplies have provided normal background levels for copper. The United States Department of the Interior analyzed 1577 surface waters between 1962 and 1967. Out of the 1577 samples, copper was detected in 1173, in concentrations ranging from 1 to 280 ppb with an average concentration of 15 ppb.[30] Warren et al reported concentrations of copper in polluted soils to be as high as 1000 ppm with normal concentrations ranging from 5-70 ppm.[57]

Cupric sulfate has been used since 1904 to control algal blooms.[20] Moderate use of insecticides began in the latter part of the nineteenth century in the form of arsenic and cupric oxide.[47] Copper used in agriculture as a non-degradable, general toxicant can accumulate in soils, particularly in those of vineyards[53] and orchards[25]. Also, copper is found in the effluent of brass and wire mills, foundaries and in factories involved with copper plating and cuprammonium-rayon manufacture.[2,4] There is an increase of copper content in water which has travelled through copper pipes. When unsoftened water remained in contact with copper tubing for 24 hours, the copper concentration increased from 37 ug/l to 245 ug/l.[30]

Cadmium is usually found together with zinc, the ratio of cadmium to zinc being 1:100 to 1:1000 in most soils. Kneip et al have reported a yearly average cadmium concentration in non-urban air to be about 0.003 ug/m^3. Values of cadmium concentrations in unpolluted waters are less than 1 ng/g.

Although there has been a sharp increase in cadmium pollution recently, man has released cadmium to the environment ever since he started using other metals such as zinc, copper and lead. Cadmium is introduced to the environment by mines, lead, copper and zinc smelters and by incineration of cadmium-containing products such as rubber tires and plastic containers. Industrially, over 10 million tons of cadmium are used in the United States in photographic, plating, rubber, motor, metal and battery manufacture. Cadmium emitted to the air is sometimes deposited up to 10 Km from the source in significant quantities, making it, together with lead, the most important metallic air pollutant. In water, near a pollution source, relatively low concentrations of cadmium may be found in solution, while large amounts are associated with suspended particles and sediment. Cadmium is present in insecticides, fertilizer, and sludge from sewage treatment plants.[15] Such sludge in England and Wales was reported to have 100 μg Cd/g (dry weight). This sludge if used as fertilizer could act as a high source of cadmium for plants, thereby causing cadmium to enter the food cycle.

Heavy Metals in Aquatic Systems

Heavy metals or other pollutants entering aquatic systems are exposed to various compartments of the systems. Aquatic systems may be considered as three compartments: water, biota and sediment. The sediment acts as a sink in binding of heavy metals. The metals are found in the sediment in biologically unavailable forms bound to sulfide, organic matter and clays, in higher concentrations than those in water.[44] For example, in Sweden, 500 m downstream from a cadmium emitting

factory, 4 ng/ml of cadmium were found in the water while 80 μg/g (dry weight) were found in the mud.[19] Binding to the sediment depends to a large extent on its organic content. Sandy sediments have a lower metal binding capacity than clay-type sediments high in organic content.[44] This gives an indication of the importance of organic substances in natural systems with regard to the binding of heavy metal ions.

The physico-chemical conditions of water can affect the status of HM ions. The redox potential, pH and the presence of soluble chelators and particulates can affect the chemical and biological cycles in which these ions are involved. Williams found that 5-28 percent of the total soluble copper in sea water was associated with organic matter[58] and others have reported the presence of copper in both soluble and particulate forms in natural waters.[11,51] Binding of HM ions to particulates can be important in the physical transport of the ions in the water system or in their settling to the bottom sediment. Chelation of the HM ions by soluble material can also have widespread effects. Humic acids are known to leach cations from soils.[46] It has been demonstrated experimentally that nitrilotriacetic acid (NTA), a strong complexing agent used in detergents to replace phosphate, increases the corrosion of HM's in sewage system pipes. Some HM ions are released at NTA concentrations as low as 1 μg/ml.[60] Chelators, natural or synthetic, present in the water systems not only release HM ions by solubilization from their sinks but also enhance their mobility. Chelating substances present in aquatic systems may also change the toxicity of HM ions.[21]

While methylmercury is more toxic than free mercuric ion, bound HM ions are generally considered to be less toxic than free HM ions. For example, Lloyd and Herbert reported that Cu^{2+} ions were less toxic to fish in hard water than in soft water, presumably because binding of the Cu^{2+} ions by the carbonate reduced the concentration of free Cu^{2+} ions in solution.[34] Therefore, the total HM ion concentration in solution does not give an accurate indication of HM toxicity to the biota. Instead, the chelating capacity of the water system as well as the free metal ion concentration is critical when studying effects of HM ions.

The first compartment for which man developed a concern in relation to pollution was the biota because of the retention of HM's in fish and seafood which resulted in human disease and death. The biota is made up of microorganisms, plants, invertebrates and fish. While the presence of HM's in various aquatic organisms has been well documented, the molecular mechanisms by which HM ions disrupt biological functions are still a source of much research.

Effect of free and bound ions on algae

Copper sulphate has been used since the beginning of this century to control algal blooms.[20] Heavy metals are generally considered to be inhibitors of enzymes.[31,56] Reports indicate that heavy metal ions adsorb to the surface of the cell wall and the cell membrane.[22,50,54] A recent paper showed the presence of lead inside vacuoles of the alga, Stigeoclonium tenue. The

author suggested that part of the cells' defence mechanism against the lead was to trap the lead inside the vacuole making it unavailable for binding to functional sites.[48]

An important effect of HM ions is their inhibition of algal photosynthesis. While some consider this to be a result of HM ions affecting enzymes directly involved in photosynthesis,[41] others feel that the effect of HM ions is an indirect one, due to the build-up of photosynthetic products inhibiting other metabolic processes.[50] Various workers have noted a release of potassium ions by algal cultures in the presence of Cu^{2+}, Hg^{2+} or Cd^{2+} ions, suggesting the disruption of a diffusion barrier.[37,41] Also, in some algae an increased lag phase is induced by the presence of Hg^{2+} or Pb^{2+} ions.[3,8]

A critical factor in relation to the sensitivity of algae to HM ions is the presence of metal-sequestering substances, called chelators. Concentrations of HM's used to achieve comparable results in different algal experiments or in bloom control vary widely.[20] Much of this apparent inconsistency is due to the presence of different chelating agents in water systems or culture media. The HM ions bind to these agents to varying degrees depending upon the nature of the metal ion and the type of complexing agent. These equilibria determine the amount of free HM's available in natural waters or culture media. It is essential, therefore, that the complexing capacity of such media be determined, to assess the amount of free HM ions in such systems.

Excretion of Extracellular Products by Algae

The addition of chelating agents in algal culture media to control the availability of essential elements has become common practice. Algae themselves excrete extracellular products which achieve the same purpose.[32] These extracellular products may consist of polysaccharides, organic acids, amino acids or polypeptides, depending on the algal species and growth conditions.[17,24,27] The brown alga, Pilayella littoralis, excrete two large fractions of polysaccharides, one consisting of alginic acid and the other having the properties of α-cellulose.[33] Glycollate is excreted by many algae, and Fogg et al reported its presence in many natural waters.[18] Amino acids and polypeptides are usually released in small amounts. However, blue-green algae excrete large amounts of nitrogenous material[24] not necessarily related to nitrogen-fixing activity.[28] Anabaena cylindrica releases about 30 percent of its organic nitrogen in rapidly growing cultures[16] while Calothrix scapulorum releases 20-60 percent of its assimilated nitrogen when transferred to unfavourable conditions.[28] Some blue-green algae may excrete large amounts of nitrogenous compounds during the early stages of growth and, during senescence, large amounts of mucilaginous or colloidal substances mainly made up of polysaccharides similar to those found on the surface waters just before a bloom disappears.[32]

The ability of algal extracellular products to chelate essential elements required for nutrition, makes them eligible to chelate HM ions. The question that arises then is: can substances excreted by algae increase the metal binding capacity of natural waters significantly? One of the objectives of this study was to find the answer. Also studied were some effects of various heavy metals on blue-green algae in laboratory cultures.

EXPERIMENTAL METHODS

A. Binding of HM's by Natural Water Systems

1. Principle of the Experimental Method

The metal binding capacity of a water sample is expressed as the amount of heavy metal (HM) ion bound under equilibrium conditions. This is determined by metal ion titrations using solid state ion-specific electrodes which respond reversibly to the amount of free metal ion present in solution. For each point in the titration, the amount of metal ion bound is $\left[M^{2+}_{total}\right] - \left[M^{2+}_{free}\right]$.

The ion-specific electrodes contain a solid state sensing element mounted in a very durable plastic body and are designed to be used like a conventional pH electrode. The electrodes develop a potential proportional to the logarithm of the activity of the metal ion in solution. This relationship is described by the Nernst equation:

$$E = E_o + \frac{2.303RT}{nF} \times \log A_{M2+}$$

where: E = measured total potential of the system

E_o = potential due to the reference electrodes and internal solutions.

R = gas constant

T = temperature in degrees Kelvin - this was kept constant at 20 C in the experiments of this study

n = valence of the metal ion

F = Faraday's constant

A_{M2+} = metal ion activity in the sample

The free, divalent metal ion concentration $[M^{2+}]$, and the metal ion activity, A_{M2+}, are related by a factor called the ionic activity coefficient, α, in the following equation:

$$A_{M2+} = \alpha [M^{2+}]$$

Since at free metal ion concentrations below 10^{-3}M, the activity coefficient is approximately equal to unity, the activity of the metal ion and the free metal ion concentration become equal. Therefore, the potential developed by the electrodes is proportional to the logarithm of the concentration of the free metal ion in solution.

For methods relating to use of ion-specific electrodes see Ramamoorthy and Kushner, 1975.[42]

2. Calculations Related to Metal Binding Characteristics of a Solution

(i) Calculation of Metal Bound

The metal ion-specific electrodes measure the concentration of free, divalent metal ion, M^{2+}_{free}, in solution. Since the total metal ion concentration, T_M, present in the solution is known, the concentration bound, M_{bound}, can be calculated using the following equation:

$$[M_{bound}] = [T_M] - [M^{2+}_{free}] \qquad (1)$$

(ii) Metal Binding Capacity

When M^{2+} ion binding sites of a solution become saturated, the amount of metal ion bound remains constant over a wide range of T_M added. The average concentration of metal ion bound over this range of T_M represents the metal binding capacity of a solution.

(iii) α, Fraction of Metal Ion Bound

Equation (1) provides a method of determining the absolute concentration of metal ion bound by components in the solution. Often, the fraction of metal ion bound, α, is a more convenient parameter to use when studying the ability of a solution to bind HM ions where:

$$\text{Fraction Bound} = \alpha = \frac{[M_{bound}]}{[T_M]} \qquad (2)$$

(iv) $K_{Cond.}$, Conditional Stability Constant

Because the composition of the binding component is not known the true equilibrium constants for the metal complexation, as expressed in the following equation, cannot be calculated.

$$K = \frac{[\text{Metal ion bound}]}{[\text{Metal ion unbound}][\text{Water Component unbound}]} \qquad (3)$$

However, if we arbitrarily assume the formation of 1:1 complexes between the metal ions and water component(s), a conditional stability constant can be calculated because equation (3) becomes:

$$K_{Cond.} = \frac{[\text{Metal ion bound}]}{[\text{Metal ion unbound}]^2} \qquad (4)$$

This conditional constant does not indicate the number of binding sites on each component but does give the relative binding strength of the water for the metal ions.

3. The Metal Binding Capacity of a Solution

The M^{2+} ion binding ability of various solutions was studied over a wide range of metal ion concentrations. In the laboratory, the appropriate set of electrodes were equilibrated in a specified volume (25 or 50 ml) of the solution being studied. The initial potential was recorded in millivolts and then small volumes of the appropriate 0.01M metal ion solution were added, gradually increasing the concentration of the metal ion in the sample from 2×10^{-6}M to 9.1×10^{-4}M. The potential readings were allowed to equilibrate after each increment of metal ion was added and the millivolt values were recorded. The millivolt values were then converted to free, divalent metal ion concentration by using the appropriate standard curve. By using this procedure, the metal ion binding capacity of a solution could be studied after each increment of metal ion was added. The metal ion solutions were added in small volumes so that the total volume of the sample would not be affected significantly. Corrections were made in the calculations to allow for volume changes from 1 to 5 percent.

4. Sample Collection and Storage

All the collecting bottles and glassware involved in the binding experiments were washed with No Chromix-Sulphuric acid and then thoroughly rinsed with distilled-deionized water. Previously, Ramamoorthy and Kushner[43] have shown that adsorption to glass by the metal ions was insignificant over the experimental period of approximately one-half hour to one hour.

The method of Lund et al[36] was used to identify and count the number of cells of the predominant algal species present during peak bloom periods.

Surface samples were collected in one gallon Nalgene bottles from four fresh water systems (Fig. 1). The most thorough study was accomplished on Leamy Lake where a blue-green algal bloom of Aphanizomenon flos-aquae and Anabaena spiroides had formed (Fig. 2). The sampling stations were at the end of the dock near the beach area, directly across the lake where a building was being erected, which we called the Construction Site and at the former outlet of the lake which we called the X outlet because it had been blocked off while a bridge was being built. Samples were taken, starting August 6, 1975, once a week for the first three weeks and then every two weeks until October 1, 1975 when we found that the bloom had disappeared.

Samples were collected from the Rideau River on August 15, 1975 which contained a green algal bloom consisting mainly of Ulothrix aequalis (Figs 3 and 4). Water was collected at Hog's Back above the falls, at the Minto Bridge, just above the Rideau Falls Dam where a mat approximately 8 cm thick had collected and just below the Dam over the edge of the falls.

In mid-November samples were taken at Lac Beauchamps where a chrysophyte, Dinobryons divergens was predominant and at Lac Carriere where a diatom, Asterionella formosa, predominated.

The samples were stored at 20 C in the light with the caps set loosely on top. Initial binding experiments and analyses were carried out on the samples within two weeks from the time they were collected.

FIGURE 1

Map of major water systems in Ottawa area.
Solid black dots indicate sampling sites.

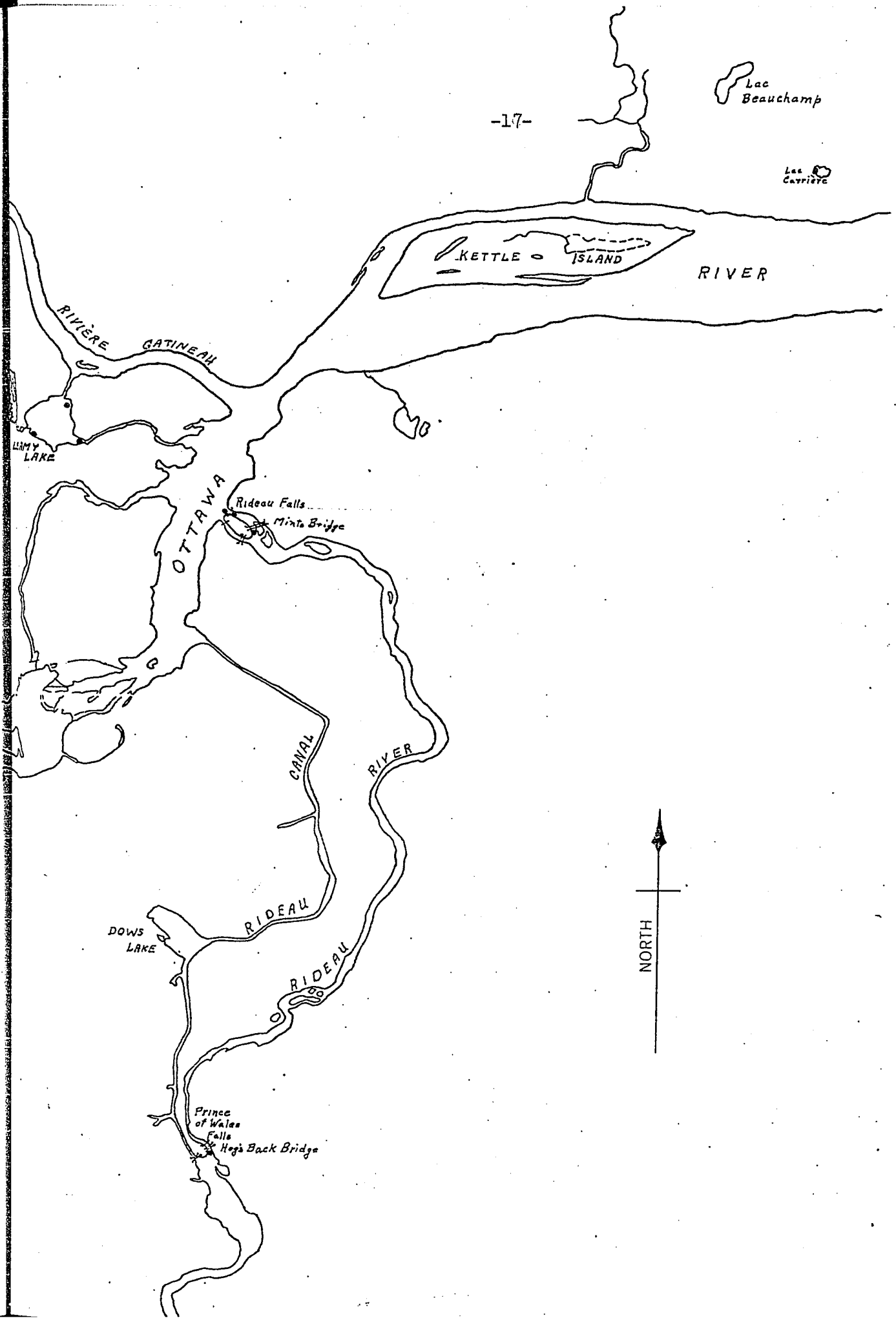
Lac Beauchamp
Lac Carrière
KETTLE ISLAND
RIVER
RIVIÈRE GATINEAU
LEMY LAKE
OTTAWA
Rideau Falls
Minto Bridge
CANAL
RIVER
RIDEAU
DOWS LAKE
RIDEAU
Prince of Wales Falls
Hog's Back Bridge
NORTH

FIGURE 2

Leamy Lake - water samples containing Aphanizomenon flos-aquae and Anabaena spiroides being collected at the Construction Site. The End of the Dock sampling site is across the lake to the left of the picture and the X outlet is across the lake to the right side.

FIGURE 3

Rideau River - a water sample being collected from the Minto Bridge

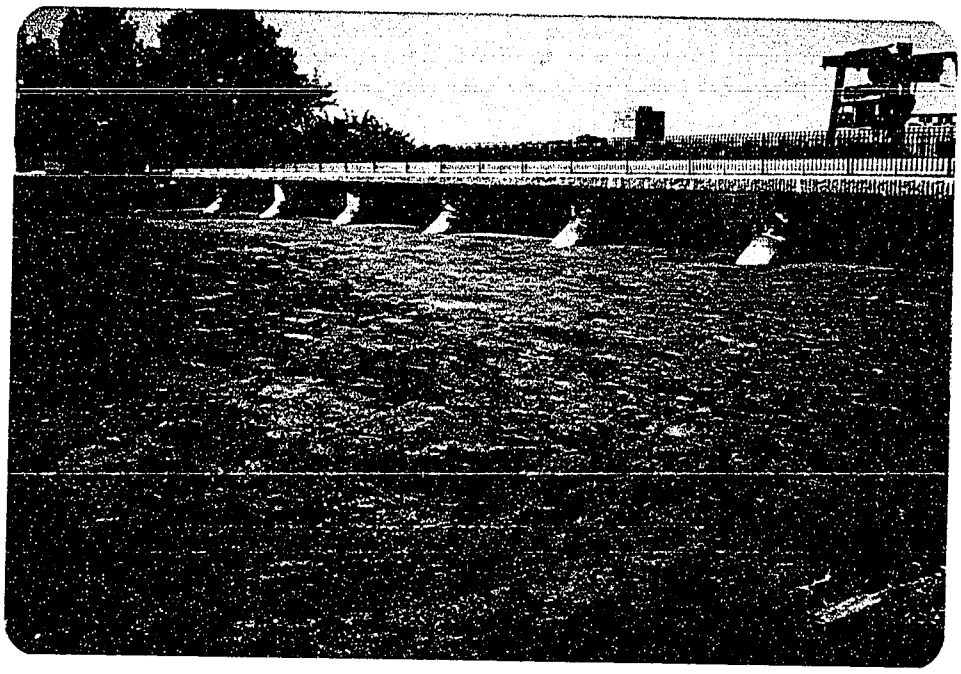

FIGURE 4

Rideau River - a picture of the thick mat of Ulothrix aequalis which collected above the Rideau Falls dam.

The binding capacity of Leamy Lake water was determined on unfiltered water and on samples filtered through a Nalgene 0.2 μm filter and various Amicon ultrafilters (Amicon Corp., Lexington, Mass.). The 76 mm Amicon Diaflo ultrafiltration membranes used were the PM30 filter which retains molecules having a molecular weight greater than 45,000, the UM 10 filter retaining material greater than 16,000 M.W., the UM2 filter which retains material greater than 1400 M.W., and the UM 05 filter which retains molecules with a molecular weight of greater than 500. Ultrafiltration was carried out in an Amicon cell Model 402 under 50 psi of nitrogen. Filtration of the samples began immediately upon return to the laboratory and was usually completed within three days.

The binding capacity was determined for unfiltered water from the Rideau River, Lac Beauchamps and Lac Carriere and for 0.2 μm filtrates of these waters.

5. Physico-Chemical Analysis of Water Samples

Besides undergoing the binding studies, the samples were also analyzed physico-chemically in an attempt to discern the nature of the binding component. Such values as temperature, pH, redox potential, ionic background, conductivity, total organic carbon, total inorganic carbon, chloride, orthophosphate, a variety of nitrogens and silica were determined.

The temperature was recorded at the sampling station. On the first field trip, the pH and redox potential were determined at the sampling stations using an Orion Model 407 portable pH/mv meter and the appropriate electrodes. These values were determined again upon arrival back at the laboratory. Since there was no significant change in the values over that period of time, subsequent determinations of pH and redox potential were made upon return to the laboratory.

(i) pH

The equipment and method for determining pH has been described by Ramamoorthy and Kushner, 1975.[42]

(ii) Redox Potential

The Orion Platinum redox electrode, Model 96-78, connected to the Orion pH/mv meter Model 801, was used to make direct measurements of redox potentials in samples. When the electrode is filled with 90-00-01 filling solution, its potential characteristics match those of a conventional calomel reference electrode.[40]

Since it is customary to report redox potential readings relative to the normal hydrogen electrode (NHE)[6], the potential developed by the reference electrode relative to the NHE is added to the potential developed by the platinum redox electrode. This is described in the following equation

$$E_{NHE} = E_o + C$$

where E_{NHE} = oxidation reduction potential of the sample relative to the NHE following the international sign convention.

E_o = potential developed by the platinum redox electrode.

C = potential developed by the reference electrode portion relative to the NHE.

At 25 C the value of C is 241 mv, which was added to all the mv readings recorded by the platinum redox electrode so the redox potential values reported in this study are relative to the NHE.

(iii) Conductivity

Conductivity of water samples was determined at 25 C by a Leeds and Northrup conductivity bridge, using a glass conductivity cell electrode coated with platinum black, having a cell constant of 0.3358. The lower limit of conductance measurement using this cell is 3 μmho ± 5 percent. At 50 μmho, the reliability of the conductance measurement is ± 0.01 percent.

(iv) Ionic Background

Ionic background was calculated from the conductivity measurements using calibration values reported by Orion Research Inc.[39] relating the two values.

(v) Chloride Ion

Chloride ion concentration was determined using an Orion combination chloride electrode Model 96-17 with 90-00-17 filling solution, connected to the Orion Model 801 pH/mv meter. At 25 C the limit of detection of chloride ion concentration is 1.4×10^{-5}M. The electrode must not be placed in strongly reducing solutions containing oxidizing agents such as Cu^{2+}, Fe^{3+} and MnO_4^-. If The surface of the sensing element becomes contaminated, response can be restored by moistening it and polishing it with Orion Polishing Strips.

(vi) Orthophosphate

Inorganic phosphorus content was measured in surface water samples using a modification of the method of Eibl and Lands.[12]

(vii) Sulphate

Water samples were analyzed for the presence of sulphate concentrations of greater than 2.4 ppm. Lead nitrate was added to aliquots of the water samples to a concentration of 5 ppm. The formation of a precipitate indicated the presence of 2.4 ppm or more sulphate in the water.

(viii) Total Carbon (Total Organic, Dissolved Organic, Total Inorganic, Dissolved Inorganic)

The carbon content of 1 to 100 mg carbon/litre may be determined in surface waters, wastes and saline waters by infrared analysis. Total Inorganic Carbon (TIC) and Total Organic Carbon (TOC) were determined in surface water samples using the method of the Federal Water Pollution Administration[14] as described by Traversy.[55]

Loss can occur from the failure of large carbon-containing particles in the sample to enter the hypodermic needle used for injection. Part of each sample was filtered through a 0.2 μm filter and the results termed "Dissolved Organic Carbon" (DOC) and "Dissolved Inorganic Carbon" (DIC).

(ix) Nitrogen

(a) Ammonia

Ammonia in surface waters was determined by the Automated O-Toluidine Method of Sawyer and Grisley[45] as described by Traversy.[55]

(b) Nitrate, Nitrite

Nitrate and/or nitrite were determined by the Automated Cadmium Reduction method of Brewer and Riley[5] as described by Traversy.[55]

(ix) Nitrogen (Cont'd)

(c) Total Kjeldahl Nitrogen

Total Kjeldahl nitrogen, the sum of the free ammonia and organic nitrogen compounds in the sample, was determined using the method described by the Federal Water Pollution Control Administration.[14]

(x) Silica, Reactive

Silica may be determined in surface, waste and saline waters in the concentration range of 0.5 to 20 mg/l SiO_2 using the Automated Heteropoly Blue Method.[1,55]

6. Determination of Binding Sites by Elimination of Individual Components of Water Samples

(i) Elimination of Inorganic Carbon

To remove the inorganic carbon present in the water samples, the sample was acidified to pH 1.0 with concentrated HNO_3. The carbon dioxide formed was driven off by gassing the sample with nitrogen for 30 minutes. The pH of the sample was raised to pH 6.0 with 1M NaOH before the HM binding capacity was measured.

(ii) Elimination of Organic Carbon

The organic carbon content of the water samples was removed by evaporating a specific volume of a sample to dryness and then ashing the sample at 900 C for 16 hours, thus volatilizing the organic carbon. After cooling, the ashed samples were reconstituted to the original volume with distilled-deionized water.

B. Effect of Metal Ions on Blue-green Alga Anabaena 7120

1. Growth Experiments

A filamentous, nitrogen fixing blue-green alga was used in the growth experiments of this study. An axenic culture of Anabaena species (strain 7120) was obtained from Dr. R. Y. Stanier at the Pasteur Institute.

Cultures were grown in the BG-11 medium described by Stanier et al[49] without any sodium nitrate, which will be referred to as GO medium. Sodium nitrate was intentionally eliminated from the medium because under conditions of nitrogen fixation, the pH was kept near neutrality, whereas it rose if sodium nitrate was used as a nitrogen source.

In all the culture experiments the algae were incubated at 25 C and were shaken on a New Brunswick Scientific Co. Gyratory Shaker at 100 cycles per minute. Illumination of 1300Lux intensity was provided continuously by fluorescent lights. Growth was measured by monitoring the optical density of the culture at 650nm on a Coleman Junior II spectrophotometer using 18mm cuvettes or 18mm sidearms on the flasks. The protein content of algal cultures was measured by the method of Lowry et al[35] to relate growth to optical density at 650nm.

2. Effects of HMs on the Growth of Blue-Green Algae

To determine effects of the HM ions Pb^{2+}, Cu^{2+} and Cd^{2+} on algal growth, the algae were inoculated into GO medium containing concentrations of these metals varying from 10^{-8} to 10^{-3}M. In a parallel set of experiments, nitrilotriacetic acid (NTA), a low molecular weight, strong complexing agent was included in a 1:1 molar ratio with the HM ions. Since these experiments required many samples, cultures were grown in 18 X 150 mm Pyrex tubes containing 7 milliliters of GO medium. The tubes were placed on the shaker at approximately a 20° angle from the horizontal to insure mixing and aeration of the culture. The optical density was read directly in the tubes. These growth experiments were repeated twice.

3. Metal Ion Binding by Batch-Grown Cultures of Anabaena 7120

Anabaena 7120 was grown in 500 ml flasks containing 100 ml of GO medium. The flasks were fitted with 18 mm sidearms so that the optical density could be measured without removing samples. The control cultures had no HM ions added to the medium while the other cultures had 10^{-5}M$Cu(NO_3)_2$ or $Cd(NO_3)_2$ present in the medium.

At Day 0, the GO medium was inoculated with 2 ml of early log phase cells and then 25 millilitre samples of each culture were removed under sterile conditions. The Cu^{2+} and Cd^{2+} ion binding capacities were determined for the control cultures and the cultures containing 10^{-5}M$Cu(NO_3)_2$ and $Cd(NO_3)_2$ respectively.

At the end of the lag phase, 25 millilitre samples were removed from the flasks and the Cu^{2+} and Cd^{2+} binding capacities determined for the respective cultures, using the ion-specific electrodes.

Atomic absorption analyses were also conducted on Day 0 and at the end of the lag phase. On both days, 25 ml samples of the GO medium, the control and the cultures containing the HM's were removed from the flasks using sterile techniques. These samples were placed in 30 ml centrifuge tubes and centrifuged in a Sorval RC 2-B centrifuge with an SS-34 head at 4500G for 12 minutes at room temperature. The supernatant was removed, put in glass beakers and 1.25 ml of concentrated nitric acid was added. The pellet was placed in a beaker, evaporated to dryness and then ashed in a muffle furnace at 450 C overnight. After cooling, the ash was acidified with 25 mls. of 5 percent HNO_3 and boiled to dissolve the HM's. The supernatant and the pellet (cells) were then analyzed on a Model 810 Jarrell-Ash atomic absorption spectrophotometer to measure the total Cu or Cd content present in each.

Fresh Cu and Cd standard solutions were made on the day of the analysis from 10^{-2}M stock $Cu(NO_3)_2$ or $Cd(NO_3)_2$ solutions. The stock solutions were diluted using distilled-deionized water with 5 percent nitric acid. At least three different concentration standards of each metal were made to calibrate the instrument. The blank and control cultures were subjected to the same treatment as the cultures containing the HM's, in order to monitor any interference which might produce false results in the atomic absorption analysis. No interference was observed.

RESULTS AND DISCUSSION

Binding of HM's by Natural Water Systems

1. Algal Populations and Biomass Calculations

The water systems sampled are located within a ten mile radius of the university campus (Fig. 1). The study included flowing and non-flowing water systems of different chemical compositions. The lakes are used mainly for recreational activities, whereas the Rideau River is subject to pollution by surface runoffs with high chloride concentrations, sewage inlets contributing nitrogen and phosphorus, and other municipal and industrial activities. Each water system also harboured different species of algae.

In order to establish any relationship between the algal population and the metal binding capacity of a water system, the major types and number of algae present must be known. Table 1 presents the names of the predominant algae in each system with the estimated number of cells observed per cubic meter of water. Unfortunately these values only represent the peak periods of the blooms. Cell volumes calculated from size measurements were converted to carbon content using published relationships.[38,54] In terms of cell volume per cubic metre, the order followed is the Rideau River > Lac Carriere > Leamy Lake > Lac Beauchamps. When biomass is expressed in terms of grams of carbon per cubic metre, the order is slightly altered to Lac Carriere > Rideau River > Leamy Lake > Lac Beauchamps. However, in terms of actual figures, the differences in algal biomass of the four water systems are relatively small. The largest difference was noted in Lac Beauchamps which has one-tenth the biomass (expressed as

TABLE 1

LOCATION	ALGAL SPECIES	NO. OF CELLS/m^3	CELL VOLUME(ml/m^3)	GRAMS OF CARBON/m^3
Leamy Lake	Aphanizomenon flos-aquae	4.4×10^8	0.13	0.10
	Anabaena spiroides	1.4×10^8	0.30	
Lac Carriere	Asterionella formosa	2.4×10^8	0.52	0.22
Lac Beauchamps	Dinobryons divergens	1.6×10^8	0.09	0.02
Rideau River	Ulothrix aequalis	2.7×10^8	0.86	0.20

TABLE 1 - The names and cell numbers of the predominant algal species in each water system are presented. Cell volumes calculated from size measurements were converted to carbon content using published relationships.[38,52]

carbon content) of Lac Carriere.

2. Metal Binding Capacity of the Water Samples

The metal binding capacity of a solution is expressed, in this report, as the maximum concentration of M^{2+} ion which can be held in the bound form in the solution. The metal binding capacities of the unfiltered water samples studied are shown in Table 2. The metal binding capacity, in relation to each metal, followed a different order in each system. The orders of binding for Leamy Lake and Lac Carriere waters were $Hg^{2+} > Cu^{2+} > Cd^{2+}$. Lac Beauchamps and the Rideau River waters fit into the order of $Cu^{2+} > Hg^{2+} > Cd^{2+}$.

The binding capacity of Pb^{2+} ions cannot be compared with that of the three other HM's studied because a precipitate formed in all the water samples when they were titrated with $Pb(NO_3)_2$. The precipitate formed at very high $Pb(NO_3)_2$ concentrations but before the end point was reached in each case. The Pb^{2+} binding values displayed in Tables 2 and 3 represent the concentrations of Pb^{2+} bound just before the precipitate formed.

Precipitates introduce error when metal binding capacities of solutions are being assessed, since, in addition to HM complexation by the solution, the precipitate may sorb HM ions. During HM titration, some compounds bind HM ions and the concentration of the HM-complex exceeds the solubility product, forming a precipitate. However, this

TABLE 2

Location	Hg^{2+} Ions	Pb^{2+} Ions*	Cu^{2+} Ions	Cd^{2+} Ions	Average metal binding capacity**
Leamy Lake	896	174	309	241	482
Lac Carriere	893	175	749	85	576
Lac Beauchamps	378	175	449	66	298
Rideau River					
- Above Hog's Back Falls	341	187	589	21	317
- Minto Bridge	376	186	649	9	345
- Above Dam	391	185	649	7	349
- Below Dam	378	186	569	16	321

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TABLE 2 - Values for the metal binding capacity in terms μ moles of M^{2+} ions bound per litre of unfiltered natural waters sampled in the Ottawa area during the summer and fall of 1975.

* Precipitate formed during titration. The values presented only indicate the concentration of Pb^{2+} bound before precipitation occurred.

** Average metal binding capacity does not include Pb^{2+} binding values.

TABLE 3

Location	Hg^{2+} Ions	Pb^{2+} Ions	Cu^{2+} Ions	Cd^{2+} Ions
Leamy Lake	1.4×10^8	5.3×10^5	2.5×10^5	6.0×10^3
Lac Carriere	7.8×10^8	$\sim 10^6$*	6.1×10^6	7.1×10^3
Lac Beauchamps	9.5×10^6	4.0×10^5	1.6×10^5	3.9×10^3
Rideau River				
- Above Hog's Back Falls	2.1×10^6	2.4×10^6	1.0×10^6	6.9×10^2
- Minto Bridge	1.1×10^7	1.9×10^6	1.4×10^6	1.6×10^2
- Above Dam	1.2×10^7	2.2×10^6	9.3×10^5	1.6×10^2
- Below Dam	5.5×10^6	2.0×10^6	8.0×10^5	5.0×10^2

TABLE 3 - Values of the conditional stability constants** for the binding of unfiltered natural waters in the Ottawa area to Hg^{2+}, Pb^{2+}, Cu^{2+} or Cd^{2+} ions.

* Value is approximate due to precipitation and consequent inaccuracy in measurement of unbound Pb^{2+} ions.

** Conditional Stability Constant, $K_{Cond.} = \frac{\text{Metal ion bound}}{\text{Metal ion unbound}^2}$

It is assumed that the metal ion-water component binding is in 1:1 molar ratio. Calculations were made in equilibrium regions of the binding curves.

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is not necessarily the saturation point of the HM binding capacity of the solution. Other soluble compounds may yet be available to bind the free HM ions. Thus, there is a chance of underestimating the HM binding capacity of a water sample in the event of precipitate formation, if the titration is stopped when the precipitate first forms. There is an equal possibility of overestimating the HM binding capacity, if the titration is continued, due to sorption of HM ions onto the precipitate. Therefore, the Pb^{2+} binding values could neither be compared to other HM binding capacities determined nor be included as part of the average metal binding capacities of the water samples.

3. $K_{Cond.}$ - Conditional Stability Constants

Table 3 lists the conditional stability constants calculated for the water samples collected between August 6, 1975 and November 15, 1975. The method for calculating the conditional stability constant, $K_{Cond.}$, is given in Experimental Methods, Section 2(iv) and in Table 3 as a footnote. For all the samples, with the exception of the Rideau River samples, the magnitude of $K_{Cond.}$ followed the order of $Hg^{2+} > Pb^{2+} > Cu^{2+} > Cd^{2+}$. The order of $K_{Cond.}$ for the Rideau River samples was $Hg^{2+} \geqslant Pb^{2+} > Cu^{2+} > Cd^{2+}$.

The $K_{Cond.}$ values for the Pb^{2+} complex are valid because $K_{Cond.}$ was determined before precipitation occurred over equilibrium regions of the binding curves. The value for Lac Carriere is approximate since precipitation occurred before many points on the curve could be determined.

4. Metal Binding Capacity of Leamy Lake water as a Function of Time

Table 4 indicates that the Hg^{2+} and Cu^{2+} binding capacities of Leamy Lake water did not change markedly over a period of two months.

Determination of the Hg^{2+} binding capacity values produced the most precise results of the four HM's studied. Little variation occurred between sampling dates or sampling locations. Because of the reproducibility of the data with relation to time and location, it appears that the Hg^{2+} binding capacity is the most suitable parameter for studying the HM binding compound(s) in Leamy Lake water.

Precipitation during Pb^{2+} ion titrations prevented determination of the Pb^{2+} binding capacity of Leamy Lake water. It was hoped, however, that the Pb^{2+} binding capacity would decrease to a value below the point of precipitation after the bloom disappeared. As seen in Table 4, when the bloom disappeared the precipitate formed at a slightly lower concentration than in previous samples. As a result, comparison of October 1, 1975 Pb^{2+} binding values with those of previous samples is impossible. However, the Pb^{2+} binding values of Table 5 determined from samples collected March 11, 1976 (5 months after the bloom disappeared) are comparable to those determined during the bloom and indicate that the Pb^{2+} binding capacity did not decrease to below the point of precipitation.

The Cu^{2+} binding capacity remained stable over the two month sampling period (Table 4), except for the X Outlet sample of August 6, 1975 and the End of Dock sample of August 19, 1975. Cu^{2+} binding capacity values determined for all sixteen samples collected over the two month period

TABLE 4

DATE	M^{2+} ION	SAMPLING LOCATION		
		End of Dock	X Outlet	Construction Site
Aug. 6/75	Hg	885	889	890
Aug. 19/75	Hg	890	892	906
Oct. 1/75	Hg	908	907	___
Aug. 6/75	Pb	186	174	176
Aug. 19/75	Pb	166	172	172
Oct. 1/75	Pb	94*	95*	___
Aug. 6/75	Cu	291	226	301
Aug. 19/75	Cu	361	316	326
Oct. 1/75	Cu	315	315	___
Aug. 6/75	Cd	207	209	165
Aug. 19/75	Cd	---	---	---
Oct. 1/75	Cd	379	245	___

TABLE 4 - The effect of time on the metal binding capacity of Leamy Lake water in terms of μmoles of M^{2+} ions bound per litre of unfiltered water.

___ Sample not collected.

* Precipitation at lower $Pb(NO_3)_2$ concentration than for previous samples.

--- Highly unreliable data was computed for Cd^{2+} binding in the August 19, 1975 samples and therefore is not included in the Table.

TABLE 5

Location	Hg^{2+} Ions	Pb^{2+} Ions*	Cu^{2+} Ions	Cd^{2+} Ions
Leamy Lake	904	170	361	379
Lac Carriere	904	170	763	96

TABLE 5 - Values for the Metal Binding Capacity in terms of μmoles of M^{2+} ions bound per litre of unfiltered natural waters sampled in the Ottawa area on March 11, 1976.

* The remarks relating to precipitation in Table 2 apply to these values.

indicated that the two values which did vary markedly are not representative of the metal binding capacity of the water.

Values obtained for the Cd^{2+} binding capacity of Leamy Lake water were not precise. They varied from one sampling period to another and from one sampling site to another. However, the Cd^{2+} binding capacity did not change by an order of magnitude which might be expected if, for example, the concentration of the Cd^{2+} binding components in the water increased or decreased drastically. Therefore, the variation observed in the Cd^{2+} binding capacity probably does not reflect a significant change in the HM binding capacity of Leamy Lake water.

Further evidence for the stability of the HM binding capacity of Leamy Lake water was obtained when we found that the HM binding capacity values of samples collected March 11, 1976 had not changed markedly (Table 5). The same phenomemon was observed in Lac Carriere water, indicating that the metal binding component was present for long periods of time in these two water systems.

5. The Relation of Algal Populations to Metal Binding Capacities and Conditional Stability Constants

The HM binding capacities and conditional stability constants of the samples collected from the three lakes and the Rideau River during this study were noticeably higher than those values for the Ottawa

River in 1974. The average HM binding capacities in Table 2 were from 15 to 20 times greater and the conditional stability constants in Table 3 for Hg^{2+}, Pb^{2+} and Cu^{2+} were approximately 100 times greater than those of the Ottawa River as determined by Ramamoorthy and Kushner.[42] Reference to other work[9] showed that the period of sampling for the 1974 HM binding experiments occurred at a time when the algal population in the Ottawa River was at a low level. Thus the coincidence in 1975 of the algal blooms with the high HM binding capacities and conditional stability constants encouraged the hypothesis that these biological and chemical parameters might be related.

Comparison of the biomass of the predominant algae in the water systems with their respective HM binding capacities demonstrated that factors other than biomass affect the contribution that algal blooms have on the HM binding capacities, if algae do contribute. For example, the algal biomass of Lac Carriere and the Rideau River were approximately equal (Table 1) while the HM binding capacities (Table 2) were considerably different. In contrast, the biomass of Dinobryons divergens in Lac Beauchamps was about one-tenth that of Asterionella formosa in Lac Carriere and the HM binding capacity of Lac Beauchamps was markedly lower than that of Lac Carriere. Therefore, at first glance, it would appear that large differences in algal biomass might be paralleled by noticeable differences in HM binding capacities.

However, the importance of the admittedly rough estimate of a single season's algal biomass became further overshadowed when the HM binding capacity of Leamy Lake water did not change markedly over a period of at least 7 months (Tables 4 and 5). Likewise, the HM binding capacity of Lac Carriere water did not change noticeably over a period of at least 4 months (Tables 2 and 5). If the algae did affect the HM binding capacity of these waters, it is not unlikely that many successions of blooms had already contributed to the total HM binding capacity of each water system, especially in non-flowing systems.

More important to the HM binding capacities of water systems than algal biomass may be the algal species, the conditions which induce excretion of various kinds of algal extracellular products, and/or the resistance of the HM binding compounds to decomposition.

6. Characteristics of the HM Binding Component

Since one aim of the project was to determine the effect of algae and their extracellular products on the HM binding capacity of natural waters, the nature of the HM binding components was of interest.

(i) Effects of Filtration

To estimate the size or molecular weight of the HM binding components, filtration of the water samples was followed by HM binding capacity determinations of the filtrates. As shown in Table 6, removal of particulate matter by filtration through 0.2 micron pore size filters

TABLE 6

M^{2+} Ion	Unfiltered Water	0.2µm Filtrate	UM05 Filtrate
Hg	896	890	905
Pb	174*	136*	60*
Cu	309	298*	135*
Cd	241	233	248

TABLE 6 - The effect of filtration on the metal binding capacity of Leamy Lake water in terms of µmoles of M^{2+} ions bound per litre.

* Titration not completed due to precipitate formation.

did not decrease the Hg^{2+} or Cd^{2+} binding capacities of Leamy Lake water. Nor did filtration of the water through an Amicon UM05 filter lower those HM binding capacities, indicating that the Hg^{2+} and Cd^{2+} binding components in Leamy Lake water had a molecular weight of less than 500.

Precipitate formation during Pb^{2+} and Cu^{2+} titrations of the filtrates prevented determination of their Pb^{2+} and Cu^{2+} binding capacities. The values presented merely represent the concentrations of Pb^{2+} and Cu^{2+} bound before precipitation occurred. The effects of precipitation on Pb^{2+} binding capacities which were discussed with relation to Table 2 also apply to the Pb^{2+} and Cu^{2+} titrations of these filtrates.

The HM binding capacities of Rideau River, Lac Carriere and Lac Beauchamps 0.2 micron filtrates were also determined (Table 7). The Hg^{2+} binding capacity was only affected in the Above the Dam sample where the very thick mat of Ulothrix aequalis had collected. The Hg^{2+} binding capacity of this sample was higher than that of the Minto Bridge and the Below the Dam samples. Upon filtration the HM binding capacity decreased about 20 percent.

The only other change in HM binding capacity due to filtration was noted by the Cu^{2+} ion specific electrode.

TABLE 7

Location	Hg^{2+} Ions	Pb^{2+} Ions*	Cu^{2+} Ions	Cd^{2+} Ions
Rideau River				
- Above Hog's Back Falls				
- Unfiltered water	341	187	589	21
- 0.2µm filtrate	356	186	559	6
- Minto Bridge				
- Unfiltered water	376	186	649	9
- 0.2µm filtrate	371	187	584	10
- Above Dam				
- Unfiltered water	391	185	649	7
- 0.2µm filtrate	319	186	589	6
- Below Dam				
- Unfiltered water	378	186	569	16
- 0.2µm filtrate	376	188	569	16
Lac Carriere				
- Unfiltered water	893	175	749	85
- 0.2µm filtrate	896	169	759	81
Lac Beauchamps				
- Unfiltered water	378	175	449	31
- 0.2µm filtrate	376	——	449	36

TABLE 7 - The effects of filtration of the metal binding capacity in terms of µmoles of M^{2+} ion bound per litre of Natural Waters sampled in the Ottawa area during the summer and fall of 1975.

* The remarks relating to precipitation in Table 2 apply to these values.

In this case Rideau River water sampled at locations above the dam experienced approximately a 10 percent decrease in Cu^{2+} binding capacity after filtration. The sample collected below the dam appeared to have been "filtered" while passing under the dam. The Cu^{2+} binding capacity of this sample was lower than that of the other samples and did not decrease as a result of filtration. Thus in the Rideau River samples, particulate matter did increase the HM binding capacity slightly. However, particulate matter could not be considered a major HM binding component.

Since the HM binding capacity of Leamy Lake water did not change after being passed through a UM05 filter, it appears that the major HM binding component have a molecular weight of less than 500. These results are in contrast to those of Ramamoorthy and Kushner[42], and Stiff[51] which suggested that particulate matter bound substantial amounts of Cu^{2+} ions. It would appear in this study that because of the high affinity of the HM ions for the soluble HM binding components (Tables 6 and 7) that particulate matter does not contribute to the HM binding capacity significantly.

(ii) Physico-Chemical Analysis

To further study the nature of the HM binding components, the samples were analyzed physico-chemically (Table 8). The pH of the waters varied from 7.70 to 9.45, with that of Leamy Lake decreasing from 9.45 to 8.06 over the eight week sampling period.

TABLE 8A

	pH	Ionic Background (10^{-3}M)	Conductivity (μmhos)	Cl (10^{-3}M)	Pi (mg/l)	Silica (mg/l)
Leamy Lake - Aug. 6/75						
- End of Dock	9.42	6.74	543.8	2.2	0.045	2.0
- Construction Site	9.25	6.76	545.6	2.1	0.045	0.5
- X Outlet	9.45	6.67	538.0	2.2	0.066	2.0
Leamy Lake - Aug. 19/75						
- End of Dock	8.76	6.74	547.4	4.0	0.040	-
- Construction Site	8.67	6.79	552.0	4.5	0.058	-
- X Outlet	8.67	6.87	558.5	4.8	0.080	3.0
Leamy Lake - Oct. 1/75						
- End of Dock	8.09	7.44	616.8	2.1	0.066	-
- X Outlet	8.06	7.41	606.4	2.1	0.050	-
Leamy Lake - Mar. 11/76						
- End of Dock	7.70	2.62	220.0	2.5	--	-
Lac Beauchamps - Nov 15/75	7.88	--	--	0.4	0.015	-
Lac Carriere - Nov 15/75	8.15	--	--	1.3	ND	-
- Mar 11/76	8.07	3.30	280.0	1.8	--	-
Rideau River - Aug 18/75						
- Above Hog's Back Falls	8.80	2.29	191.3	0.4	ND	1.0
- Minto Bridge	8.44	2.82	234.9	0.5	0.047	1.4
- Above Dam	8.09	3.01	250.9	0.6	ND	0.8
- Below Falls	8.59	2.84	236.6	0.8	0.117	< 1.0

TABLE 8A - Physico-chemical data of Natural Waters sampled in the Ottawa area.

* ND = Not detectable

TABLE 8B

		TEMP. (°C)	Eh (mv)	TIC (mg/l)	DIC (mg/l)	TOC (mg/l)	DOC (mg/l)
Leamy Lake -	Aug. 6/75						
- End of Dock		23 C	315	21.0	20.4	6.0	6.0
- Construction Site		23 C	313	22.8	22.4	6.1	6.1
- X outlet		23 C	312	22.4	20.8	5.9	5.9
Leamy Lake -	Aug. 19/75						
- End of Dock		21 C	316	19.0	18.4	4.9	4.9
- Construction Site		21 C	308	20.0	18.8	4.7	4.7
- X Outlet		22 C	273	18.8	17.6	4.7	4.7
Leamy Lake -	Oct. 1/75						
- End of Dock		14 C	432	19.0	18.0	5.3	5.3
- X Outlet		14 C	396	19.0	19.0	5.3	5.3
Leamy Lake -	Mar. 11/76						
- End of Dock			293	20.5	20.0	5.8	5.8
Lac Beauchamps -	Nov. 15/75	4 C	216	16.5	16.5	8.1	8.1
Lac Carriere -	Nov. 15/75	4 C	215	33.5	33.5	2.5	2.5
-	Mar. 11/76		268	40.0	39.0	4.0	4.0
Rideau River -	Aug. 18/75						
- Above Hog's Back Falls			322	21.6	22.0	8.1	8.1
- Minto Bridge			338	24.0	24.0	8.1	8.1
- Above Dam			339	27.6	25.4	8.5	8.5
- Below Falls			348	23.6	23.6	8.0	8.0

TABLE 8B - Physico-chemical data of Natural Waters sampled in the Ottawa area.

TABLE 8C

		AMMONIA (mg/l)	TKN (mg/l)	NITRATE (mg/l)	NITRITE (mg/l)
Leamy Lake -	Aug. 6/75				
- End of Dock		0.13	1.5	ND	ND
- Construction Site		0.19	1.3	0.03	ND
- X Outlet		0.15	1.1	ND	ND
Leamy Lake -	Aug. 19/75				
- End of Dock		0.22	1.2	0.02	ND
- Construction Site		0.13	1.0	0.02	0.01
- X Outlet		0.22	1.0	0.01	ND
Leamy Lake -	Oct. 1/75				
- End of Dock		0.16	0.5	ND	ND
- X Outlet		0.09	0.5	ND	ND
Lac Beauchamps -	Nov. 15/75	ND	0.5	ND	ND
Lac Carriere -	Nov. 15/75	ND	0.3	0.01	ND
Rideau River -	Aug. 18/75				
- Above Hog's Back Falls		0.11	1.5	ND	ND
- Minto Bridge		0.05	1.0	0.05	0.03
- Above Dam		0.06	1.2	0.17	0.15
- Below Falls		0.04	1.0	0.01	ND

TABLE 8C - Physico-chemical data of Natural Waters sampled in the Ottawa area.

However, despite the change in hydrogen ion concentration in Leamy Lake water, there was no noticeable change in the HM binding capacity.

The redox potentials of Lac Carriere and Lac Beauchamps were the lowest at +215mv and +216mv respectively, while those of the Rideau River ranged from +322mv to +348mv. The redox potential of the Leamy Lake samples increased from + 312mv on August 6,1975 to + 432mv on October 1,1975. While the redox potential of the waters sampled did vary by as much as a factor of two, the magnitude or change in redox potentials observed did not appear to be related to the difference in HM binding capacities. It should be noted though, that at the redox potentials exhibited by these waters with such high chelating capacities, the free heavy metal ions in solution should be present in the M^{2+} state. This is important, since it is the free HM ions in the M^{2+} state which the electrodes detect.

Since Table 8 showed that nitrate, nitrite, Kjeldahl - nitrogen, ammonia, inorganic phosphorus and silica were present at very low concentrations in the samples, they were eliminated as important metal binding sites.

The parameters which did appear to be noticeably high in value were the inorganic carbon, organic carbon, chloride and ionic background. The concentrations of chloride ranged

from 3.5 X 10^{-4}M to 4.8 X 10^{-3}M, while the ionic background ranged from 2.29 X 10^{-3}M to 7.44 X 10^{-3}M. There was no significant difference between the values of Total Inorganic Carbon (TIC) and Dissolved Inorganic Carbon (DIC) or between Total Organic Carbon (TOC) and Dissolved Organic Carbon (DOC). The inorganic carbon content was lowest in Lac Beauchamps at 16.5 mg/l and highest in Lac Carriere at 33.5 mg/l, while the organic carbon content ranged from 2.5 mg/l to 8.5 mg/l.

(iii) Elimination of Inorganic Carbon

In order to examine the significance of a particular class of compounds in relation to its metal binding capacity, it is important to compare the metal binding capacity of a sample with and without the compounds. The inorganic carbon contents of the water samples collected for this study were comparable to those of the Rideau River and Rideau Canal in 1974. Ramamoorthy and Kushner showed that much of the metal binding by Rideau Canal water was due to the carbonates and bicarbonates present.[42] In contrast to these results, the elimination of inorganic carbon (Table 9) did not reduce the Hg^{2+} binding capacity of any of the 1975 samples. It appears that the Hg^{2+} ions must have a very strong affinity to some other metal binding component.

TABLE 9

(Hg^{2+}) added	Untreated Water	TIC Eliminated	TOC Eliminated
Leamy Lake			
2μM	2	2	1
20μM	20	20	14
100μM	100	100	42
200μM	200	200	78
909μM	896	877	Precipitate
Rideau River - Above Dam			
2μM	2	2	0
20μM	20	19	2
100μM	99	99	17
200μM	198	197	47
909μM	714	710	Precipitate
Lac Beauchamps			
2μM	2	2	0
20μM	19	19	1
100μM	98	98	16
200μM	195	180	57
909μM	378	366	Precipitate
Lac Carriere			
2μM	2	2	1
20μM	20	20	2
100μM	100	100	19
200μM	200	200	62
909μM	893	819	Precipitate

TABLE 9 - Hg^{2+} binding capacities of Natural Waters* after the removal of Total Inorganic Carbon (TIC) and Total Organic Carbon (TOC). The Hg^{2+} binding capacity is expressed as the μM concentration of Hg^{2+} ions bound for various total Hg^{2+} ion concentrations added to the solution.

* Leamy Lake water was passed through a filter retaining molecules greater than 500 molecular weight while other samples were passed through a 0.2μm filter.

(iv) Elimination of Organic Carbon

Ashing of the water samples at 900 C reduced the Hg^{2+} binding capacity drastically (Table 9). The purpose of the ashing was to remove organic carbon. However, this treatment also volatilizes other constituents such as silica, phosphate, nitrogen compounds, inorganic carbon and chloride. The contribution of phosphate, silica, nitrogen compounds and inorganic carbon has already been discussed and is thought to be very small.

Analysis for sulphate indicated that there was less than 5mg SO_4^{2-}/l in the water samples collected. This concentration of sulphate ions is not high enough to bind significant amounts of the HM ions. Furthermore, Ramamoorthy and Kushner have shown that after ashing of pure Na_2SO_4 solution at 900 C and reconstitution with distilled water, the metal binding capacity was not reduced.[42] Since the HM binding capacities of the waters sampled in this study did decrease after ashing, it appears that sulphate ions were not the main HM binding sites.

Before concluding that the high metal binding capacity of those waters is due to organic carbon, the contribution of chloride ions to the metal binding capacity must be considered. When the metal binding capacities of distilled water containing

2.2 X 10^{-3}M Cl^- ions (Fig. 5) are compared to those of unfiltered Leamy Lake water collected on August 6, 1975 at the Construction Site which had 2.2 X 10^{-3}M Cl^- ions present, it is obvious that the magnitude and order of metal binding are completely different for the two systems. Figure 5 shows that in the sodium chloride solution less than 21 percent of the Pb^{2+}, Cu^{2+} or Cd^{2+} ions were bound, while in Leamy Lake water (Fig. 6), Cu^{2+} and Pb^{2+} were almost completely in the bound form and bound Cd^{2+} ions ranged from approximately 60 to 35 percent of the total Cd^{2+} ions added. Also, the Hg^{2+} ions were in the bound form at all concentrations in Leamy Lake water while in the sodium chloride solution, Hg^{2+} ions were ionized at concentrations below 10μM Hg^{2+} and greater than 100μM Hg^{2+} even though there was a large excess of chloride ions present. Furthermore, the order of metal binding by the chloride solution was different from the order of metal binding by the Leamy Lake water.

Reference to Table 8A shows a five-fold difference between the chloride concentrations of Leamy Lake and Lac Beauchamps but only a two-fold difference in their metal binding capacities. Table 8A also indicates that chloride ions only account for one-third of the total ionic background. Moreover, acidification, as described in Methods Section 8(i), volatilized 50 percent of the chloride in

FIGURE 5

The fraction of M^{2+} ions bound versus the total M^{2+} ions added. To a solution of 2.2 X 10^{-3}M sodium chloride, Hg^{2+}, Pb^{2+}, Cu^{2+} or Cd^{2+} ions were added. Using ion-specific electrodes the amount of these heavy metal ions bound was determined.

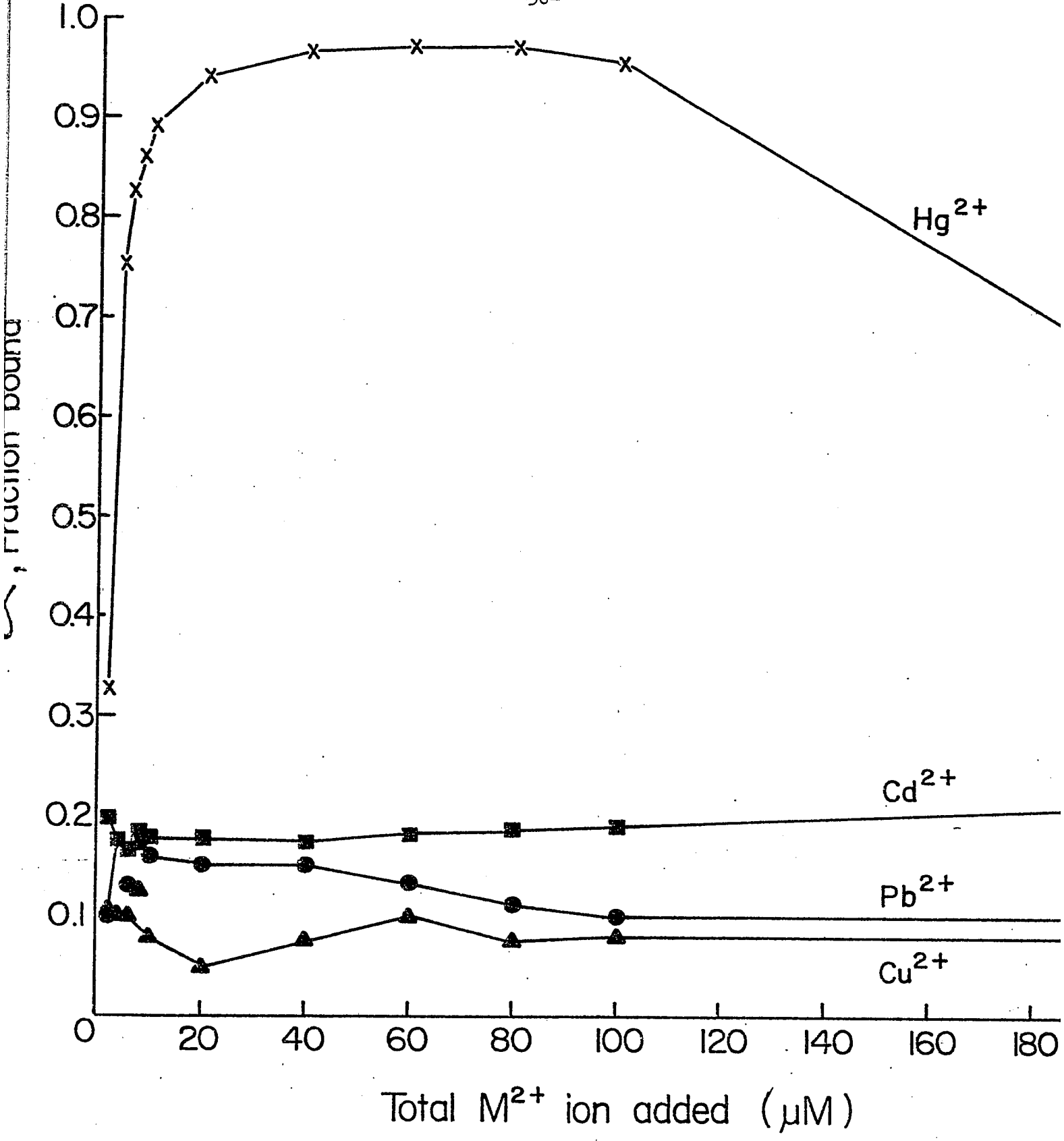
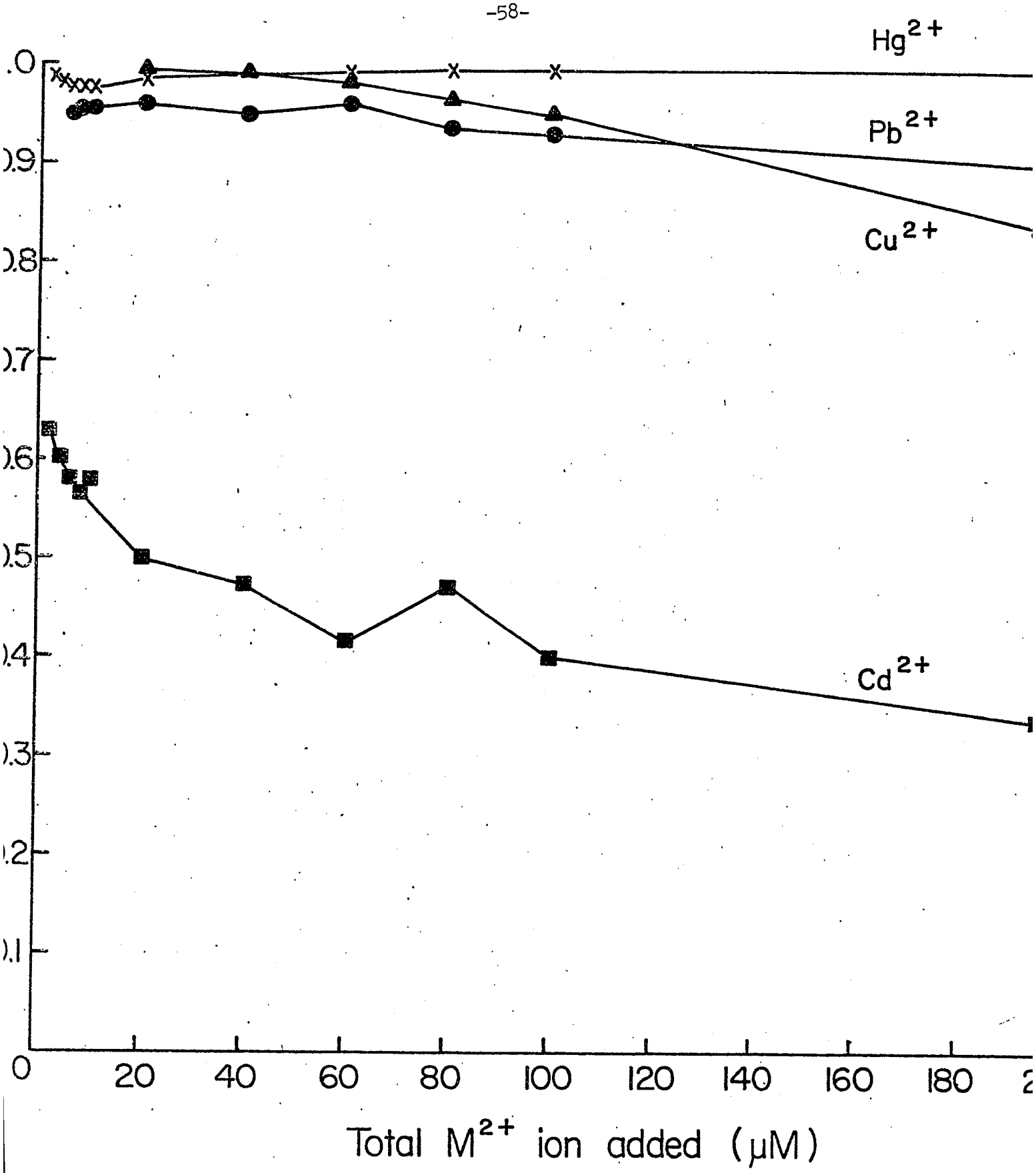

1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
20
40
60
80
100
120
140
160
180
Hg2+
Cd2+
Pb2+
Cu2+
Total M2+ ion added (μM)

FIGURE 6

The fraction of M^{2+} ions bound versus the total M^{2+} ions added. To Leamy Lake water collected on August 6, 1975, Hg^{2+}, Pb^{2+}, Cu^{2+} or Cd^{2+} ions were added. Using ion-specific electrodes the amount of these heavy metal ions bound was determined.


Hg2+
Pb2+
Cu2+
Cd2+
.0
.9
.8
.7
.6
.5
.4
.3
.2
.1
0
20
40
60
80
100
120
140
160
180
Total M2+ ion added (μM)

solution while Table 9 shows that there was no decrease in the Hg^{2+} binding capacity. These observations indicate that chloride ions do not contribute significantly to the metal binding capacities of the water systems studied.

The metal binding capacity of Leamy Lake water did not decrease after ashing as much as the other water samples. This relatively high binding capacity may be due to adsorption to undissolved oxides in the reconstituted sample, possibly aluminum oxide due to the proximity of a cement plant. No analysis for aluminum was conducted.

Having considered the major anionic species and their qualifications as metal binding substances, it appears, by the process of elimination, that organic carbon is responsible for the high metal binding capacities observed in most of the water systems studied.

While the increase in the HM-binding capacity coincides with the presence of algal blooms, the experiments in this study have not shown conclusively the organic material, apparently responsible for the high HM binding capacities, is derived from the algae. Thus it is possible that other organisms, as a result of metabolic processes or decomposition could be producing this organic carbon. Furthermore, since the HM binding component is soluble, it is possible that it may enter the water systems as a fraction of the run-off

from the surrounding area. And finally, since certain types of sediment are able to bind large quantities of HM ions, the possibility exists that the sediments of the water systems studied may release HM binding organic material into the water. Thus it is obvious that further study is required to show a direct contribution by the algae to the high metal binding capacities of these waters.

Observation of the metal binding capacities of Leamy Lake and Lac Carriere waters (Tables 2 and 5) showed that the metal binding substrate persisted for at least 5-7 months. Thus, assuming that algae produce the metal binding substrate, it would appear that the organic matter may continue to build up from one bloom to the next. To confirm that the metal binding organic matter does indeed come from the algae, it will be important to determine how much each algal cycle contributes to the total metal binding capacity of the water. Also, further chemical characterization of the metal binding substrate would be important in establishing a direct relationship between the substrate and the algae.

B. Culture Experiments

1. Effect of HM Ions on Growth of Anabaena 7120

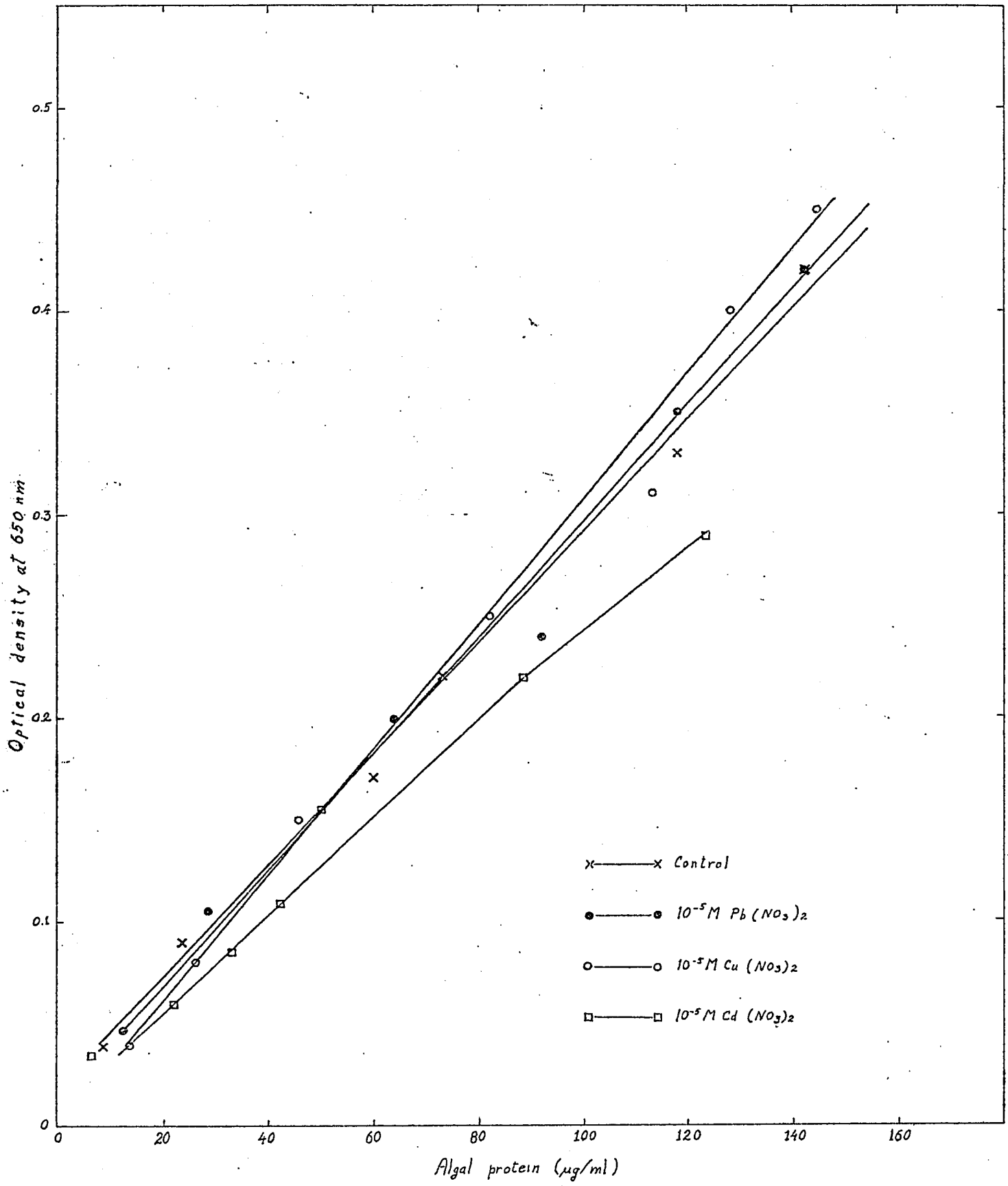
The second part of the study involved culture experiments to assess some effects of HM ions on a blue-green alga grown in batch cultures. The culture experiments were initiated by trying to find the concentrations of copper nitrate, cadmium nitrate or lead nitrate which in a particular medium produced observable changes in the growth of the blue-green alga, Anabaena 7120.

(i) Optical Density at 650nm as Indication of Growth

Growth of the batch cultures was observed by measuring their optical density at 650nm. Since some algal studies have shown that HM's inhibit photosynthesis,[41,50] it was essential to determine whether the optical density being measured actually represented algal growth or merely changes in density of pigments. Consequently, the optical density at 650nm was recorded periodically and at the same time aliquots were removed and the protein content of the cultures determined. The optical density was plotted versus the protein content of the cultures. Figure 7 shows that optical densities recorded for control cultures and for cultures containing 10^{-5}M $Pb(NO_3)_2$ or $Cu(NO_3)_2$ represent the same protein concentration for each culture. When 10^{-5}M

FIGURE 7

Optical densities versus the protein content of cultures of Anabaena 7120. The cultures were inoculated into GO medium with or without 10^{-5}M $Cu(NO_3)_2$ or $Cd(NO_3)_2$. The points on the graph were obtained by recording the optical density of each culture and then removing an aliquot of the culture to determine its protein content.


Optical density at 650 nm
0.5
0.4
0.3
0.2
0.1
0
0
20
40
60
80
100
120
140
160
Algal protein (µg/ml)
Control
10⁻⁵M Pb (NO₃)₂
10⁻⁵M Cu (NO₃)₂
10⁻⁵M Cd (NO₃)₂

$Cd(NO_3)_2$ was present in the GO medium the ratio of optical density to protein concentration was very slightly but consistently lower than that of the control cultures. However, this difference was quite small, especially during the lag period. Therefore, the optical densities recorded at 650nm for all of the cultures were assumed to be an indication of growth or lack of growth of Anabaena 7120.

(ii) Effect of Cu, with and without NTA

Figure 8 presents the growth curves of Anabaena 7120 when cultured in GO medium containing various concentrations of copper nitrate. Growth was inhibited completely by 10^{-3}M and 10^{-4}M $Cu(NO_3)_2$. In concentrations of $Cu(NO_3)_2$ from 10^{-5}M to 10^{-8}M, cultures underwent a lag phase which was approximately twice as long as that of the control, and then grew at a rate comparable to that of the control. When NTA was included in the medium at 1:1 molar concentrations with $Cu(NO_3)_2$ (Fig. 9), similar growth curves were produced, except that at 10^{-5}M concentrations of $Cu(NO_3)_2$ and NTA, the lag phase was further extended.

(iii) Effect of Cd, with and without NTA

Concentrations of 10^{-3}M and 10^{-4}M $Cd(NO_3)_2$ (Fig. 10) in GO medium inhibited Anabaena 7120 growth.

FIGURE 8

Growth curves of the blue-green alga Anabaena 7120 cultured in GO medium containing different concentrations of copper nitrate. Growth has been plotted in terms of optical density at 650nm. These growth experiments were done three times. Typical results as shown.

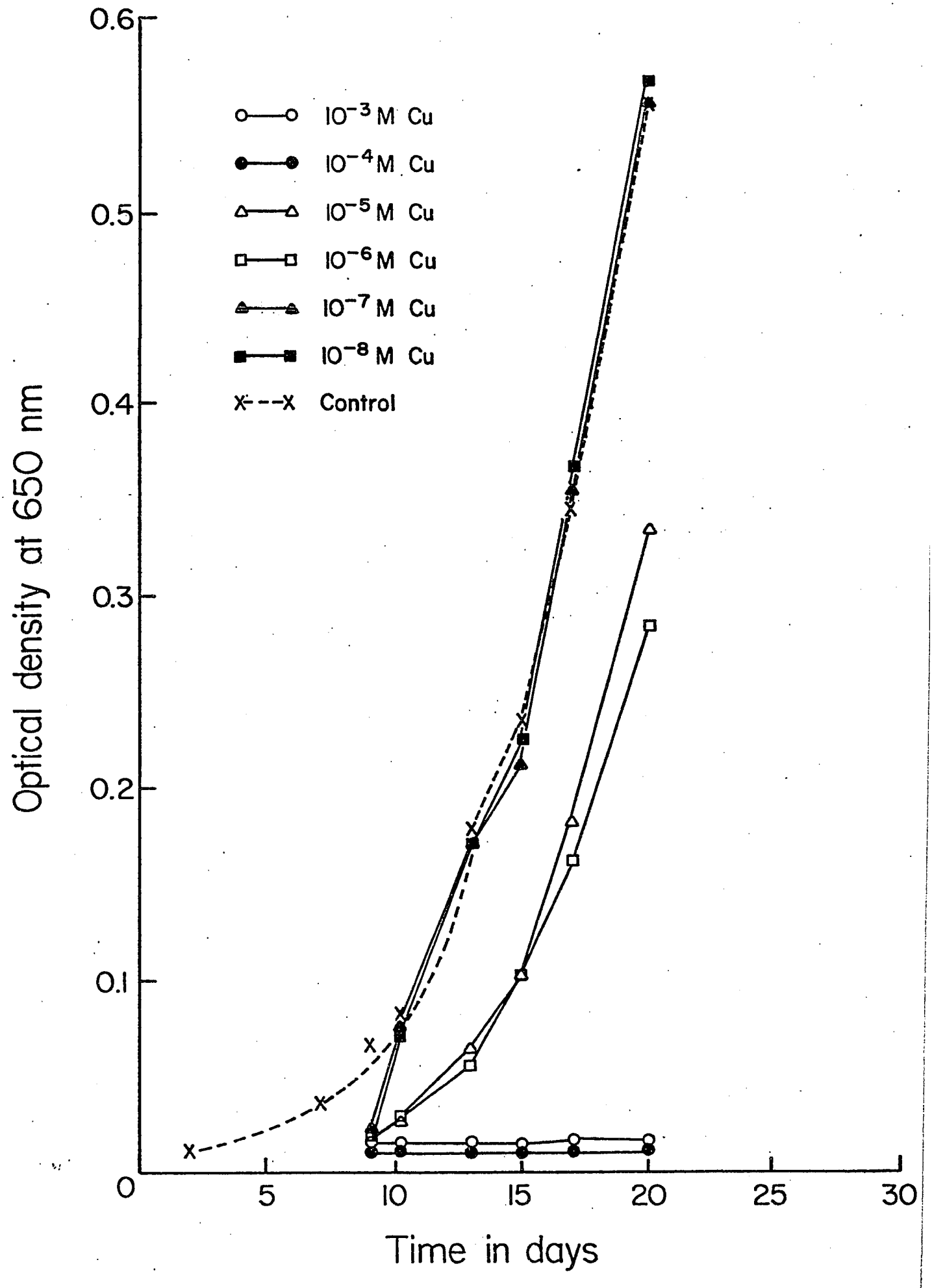

0.6
0.5
0.4
0.3
0.2
0.1
0
Optical density at 650 nm
10⁻³ M Cu
10⁻⁴ M Cu
10⁻⁵ M Cu
10⁻⁶ M Cu
10⁻⁷ M Cu
10⁻⁸ M Cu
Control
5
10
15
20
25
30
Time in days

FIGURE 9

Growth curves of the blue-green alga Anabaena 7120 cultured in GO medium containing different 1:1 molar concentrations of copper nitrate and nitrilotriacetic acid (NTA). Growth has been plotted in terms of optical density at 650nm. These growth experiments were done three times. Typical results are shown.

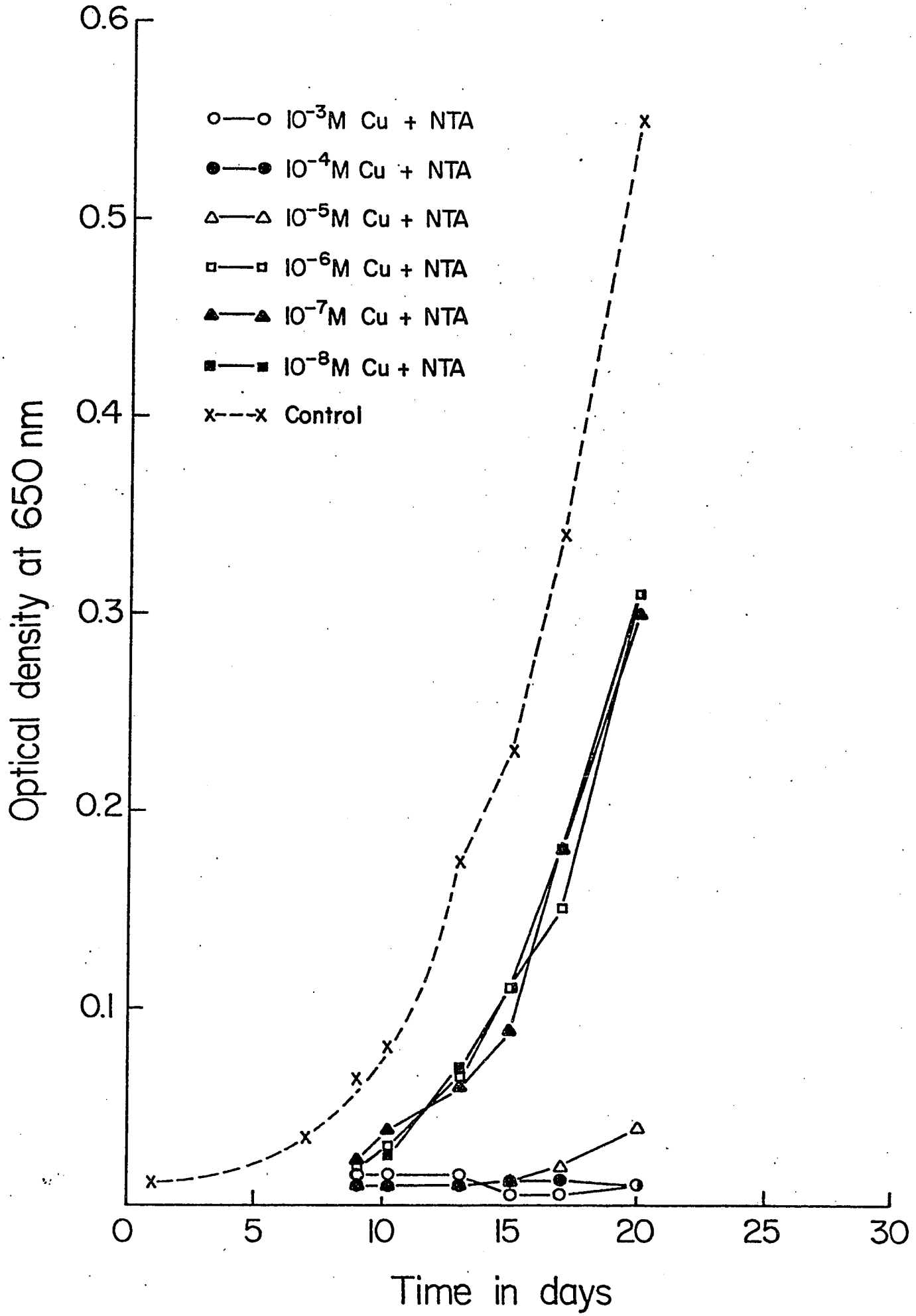
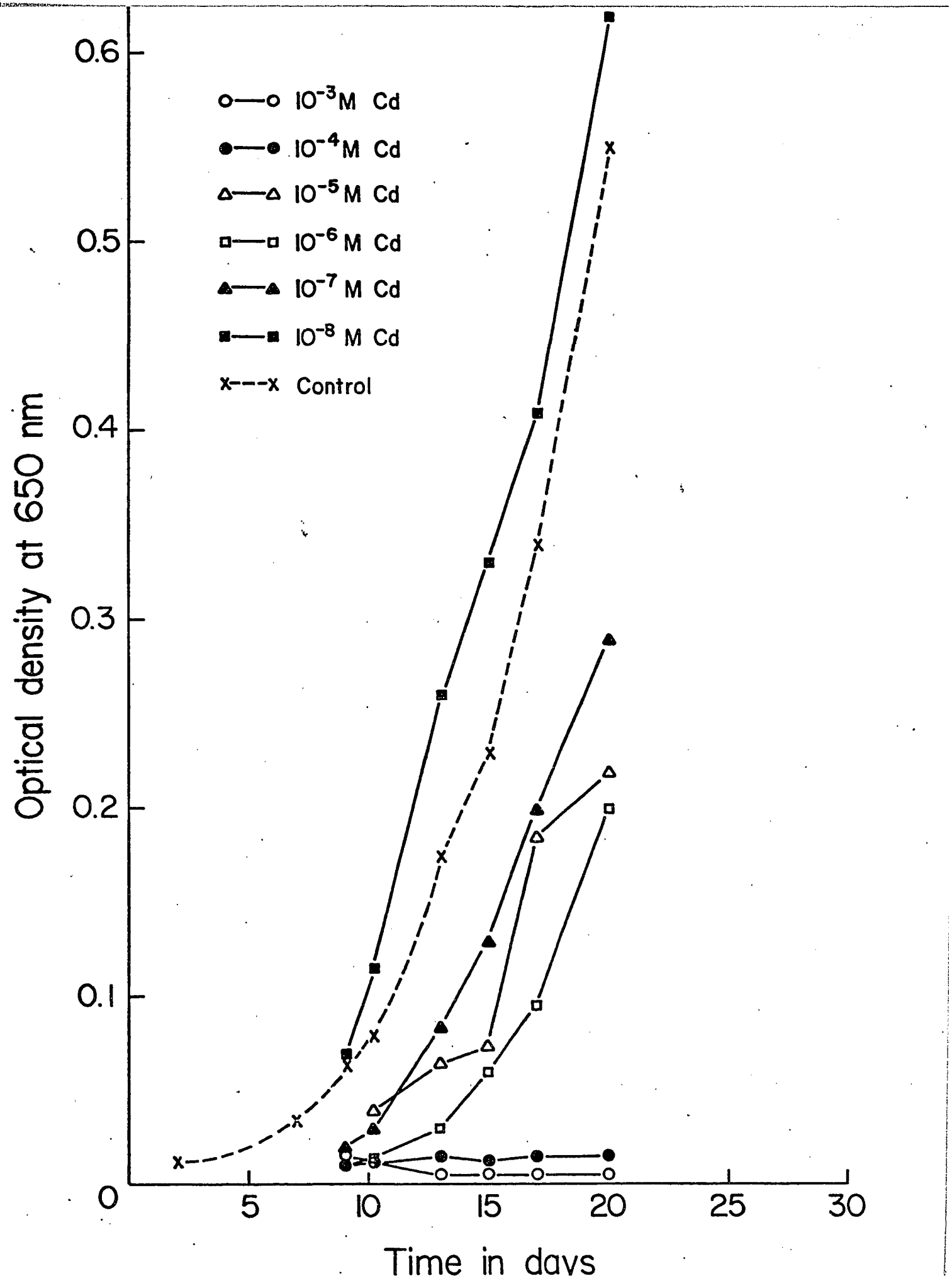

0.6
0.5
0.4
0.3
0.2
0.1
Optical density at 650 nm
10^{-3}M Cu + NTA
10^{-4}M Cu + NTA
10^{-5}M Cu + NTA
10^{-6}M Cu + NTA
10^{-7}M Cu + NTA
10^{-8}M Cu + NTA
Control
0
5
10
15
20
25
30
Time in days

FIGURE 10

Growth curves of the blue-green alga Anabaena 7120 cultured in GO medium containing different concentrations of cadmium nitrate. Growth was plotted in terms of optical density at 650nm. These growth experiments were done three times. Typical results are shown.


0.6
0.5
0.4
0.3
0.2
0.1
0
Optical density at 650 nm
10⁻³M Cd
10⁻⁴M Cd
10⁻⁵M Cd
10⁻⁶M Cd
10⁻⁷M Cd
10⁻⁸M Cd
Control
5
10
15
20
25
30
Time in days

completely, while concentrations in the range of 10^{-5}M to 10^{-7}M $Cd(NO_3)_2$ resulted in an extended lag phase. With 10^{-8}M $Cd(NO_3)_2$ present, growth was not affected. The combination of 10^{-5}M $Cd(NO_3)_2$ and NTA (Fig. 11) caused more growth inhibition than 10^{-5}M $Cd(NO_3)_2$ alone. At other concentrations, growth was the same as in the absence of NTA.

(iv) Effect of Pb, with and without NTA

Figure 12 shows the growth curves of Anabaena 7120 cultures with $Pb(NO_3)_2$ in the medium. At a concentration of 10^{-3}M $Pb(NO_3)_2$, growth was inhibited completely, whereas in 10^{-4}M, 10^{-5}M and 10^{-8}M $Pb(NO_3)_2$, the cultures appeared to grow at the same rate as the control culture. However, when 10^{-6}M or 10^{-7}M $Pb(NO_3)_2$ were present, growth began only after an extended lag phase. Growth curves with $Pb(NO_3)_2$ and NTA in the medium (Fig. 13) were similar to those produced without NTA except that the elongated lag phase was also produced with 10^{-8}M $Pb(NO_3)_2$ and NTA.

2. Discussion of the Effects on HM's with and without NTA on the Growth of Anabaena 7120

The presence of Cu^{2+} or Cd^{2+} ions in the GO medium produced similar effects on Anabaena 7120. Ben-Bassat also observed an extended lag phase when culturing Chlamydomonas in the presence of

FIGURE 11

Growth curves of the blue-green alga Anabaena 7120 cultured in GO medium containing different 1:1 molar concentrations of $Cd(NO_3)_2$ and NTA. Growth was plotted in terms of optical density at 650nm. These growth experiments were done three times. Typical results are shown.

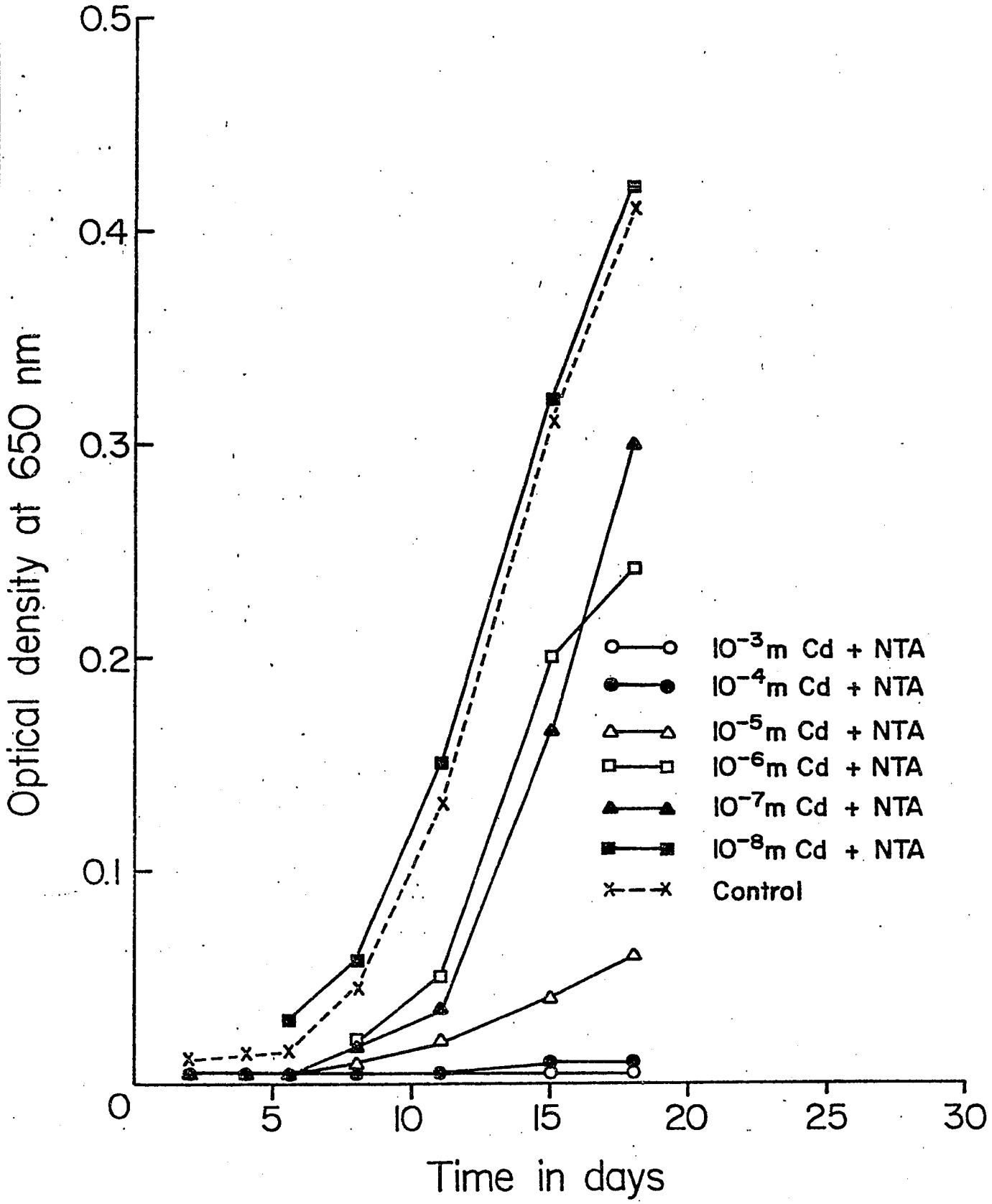

Optical density at 650 nm
0.5
0.4
0.3
0.2
0.1
0
5
10
15
20
25
30
Time in days
10^{-3}m Cd + NTA
10^{-4}m Cd + NTA
10^{-5}m Cd + NTA
10^{-6}m Cd + NTA
10^{-7}m Cd + NTA
10^{-8}m Cd + NTA
Control

FIGURE 12

Growth curves of the blue-green alga Anabaena 7120 cultured in GO medium containing different concentrations of lead nitrate. Growth was plotted in terms of optical density at 650nm. These growth experiments were done three times. Typical results are shown.

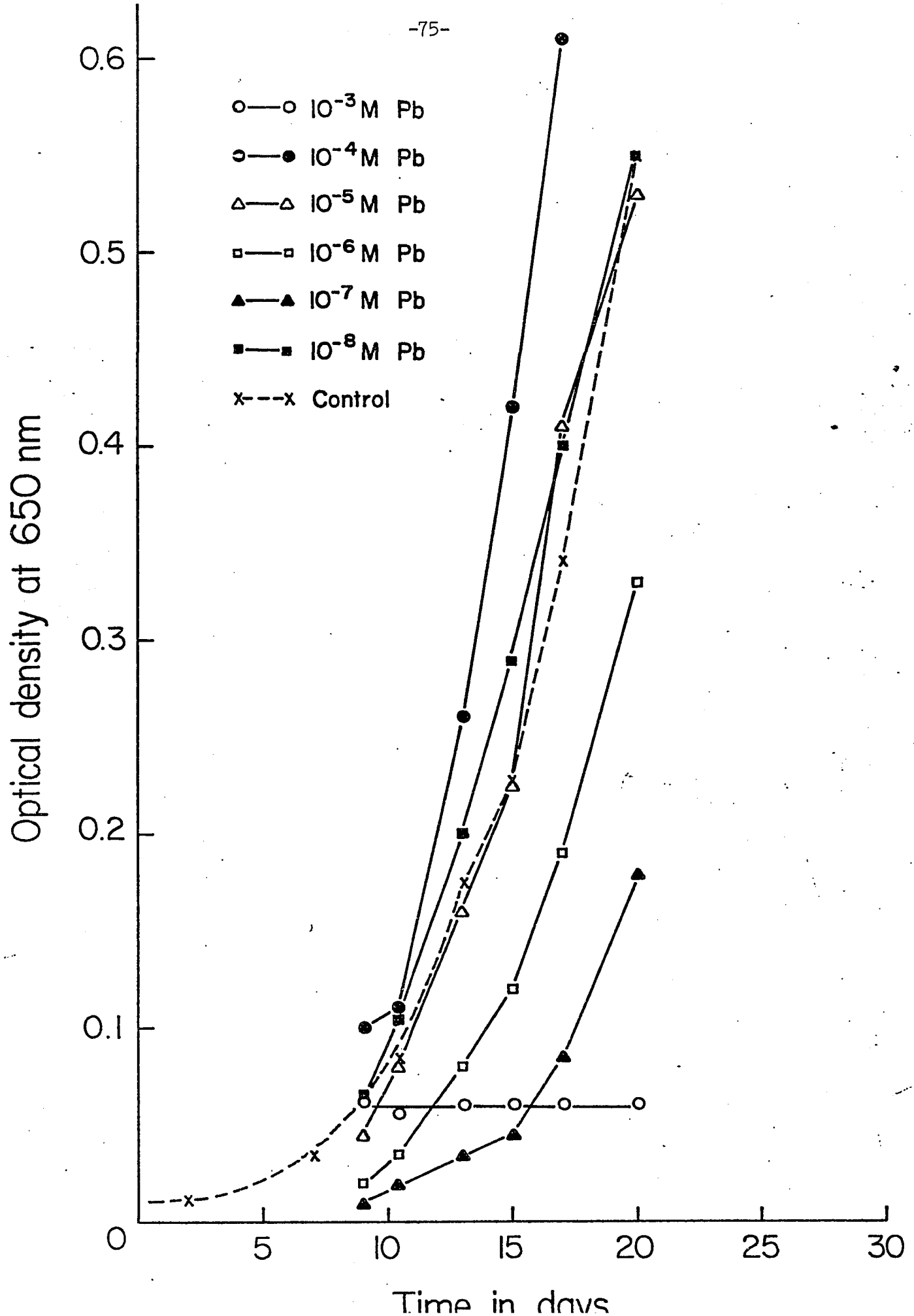
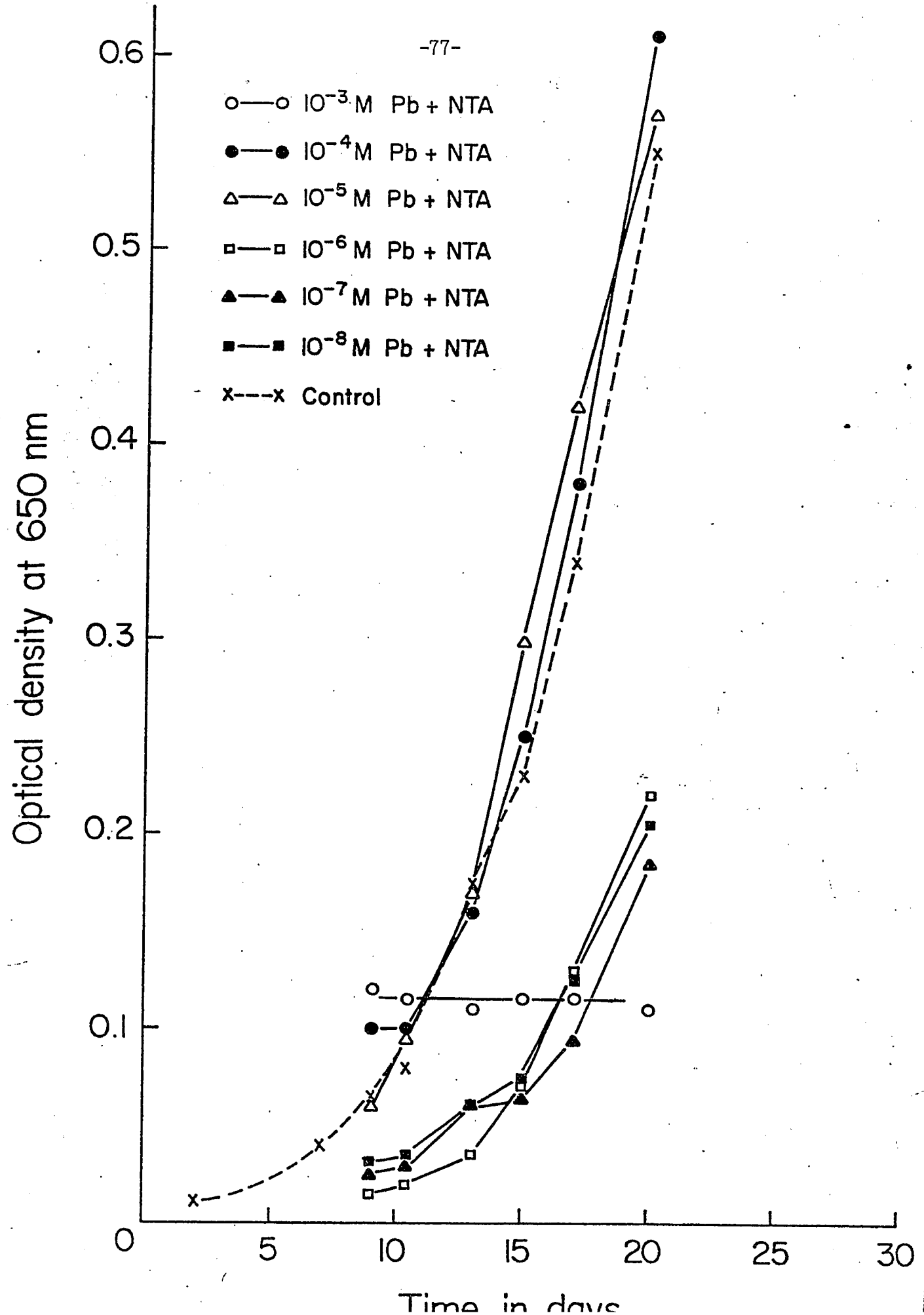

0.6
0.5
0.4
0.3
0.2
0.1
0
Optical density at 650 nm
10^-3 M Pb
10^-4 M Pb
10^-5 M Pb
10^-6 M Pb
10^-7 M Pb
10^-8 M Pb
Control
5
10
15
20
25
30
Time in days

FIGURE 13

Growth curves of the blue-green alga Anabaena 7120 cultured in GO medium containing different 1:1 molar concentrations of $Pb(NO_3)_2$ and NTA. Growth was plotted in terms of optical density at 650nm. These growth experiments were done three times. Typical results are shown.


o—o 10^{-3} M Pb + NTA
●—● 10^{-4} M Pb + NTA
△—△ 10^{-5} M Pb + NTA
□—□ 10^{-6} M Pb + NTA
▲—▲ 10^{-7} M Pb + NTA
■—■ 10^{-8} M Pb + NTA
x---x Control
Optical density at 650 nm
0
0.1
0.2
0.3
0.4
0.5
0.6
5
10
15
20
25
30
Time in days

Hg^{2+} ions.[3] A possible explanation for the initial inhibition observed, might be that the Cu^{2+} or Cd^{2+} ions bind to functional sites inhibiting some cellular process(es) and during the lag phase the cells remove Cu^{2+} or Cd^{2+} ions from those functional sites.* The algae are then able to grow at a rate similar to cultures with only trace amounts of these HM ions in the medium.

When NTA was present at 1:1 molar concentrations with $Cu(NO_3)_2$ or $Cd(NO_3)_2$ (Figs 9 and 11), enhancement of the HM inhibition was noted at 10^{-5}M concentrations as a further elongation of the lag phase. Two obvious possibilities should be considered in relation to this observation. First, the extra inhibitory effect might have been due to some direct surfactant action of NTA, itself not dependent on the presence of Cu^{2+} or Cd^{2+} ions. This possibility was eliminated when it was shown that cultures of Anabaena 7120 grew as well in GO medium without NTA and with NTA concentrations as high as 10^{-4}M. It seems more probable that the NTA-HM combination is more toxic to the cells. This is in sharp contrast to the thought that chelated HM's are less toxic than free HM ions.[7, 26, 50] Since NTA is a low molecular weight complexing agent, it would be interesting to see if NTA increases the amount of HM ions inside the algal cells.

The cultures containing lead nitrate or lead nitrate and NTA produced a series of confusing curves (Figs. 12 and 13). While moderate concentrations of the inhibitors produced longer lag phases,

* Another possible explanation, that there was extensive cell lysis, was not examined.

higher concentrations apparently had no effect on the growth of these cultures. The media with concentrations of $> 10^{-5}$M $Pb(NO_3)_2$ formed precipitates. The solubility constants of $Pb_3(PO_4)_2$ and $Pb(HSO_4)_2H_2O$ are 1.2×10^{-21} and 4.6×10^{-15}, respectively.[23] It would appear that the effective lead ion concentration was reduced to a non-inhibitory level as a result of the precipitate formation and by adsorption of the Pb^{2+} ions to the precipitate, thus producing the apparent inconsistency in results.

3. Effect of Growth of Anabaena 7120 on HM binding

If blue-green algae excrete large amounts of extracellular material capable of binding HM ions, one would expect an increase in the HM binding capacity of a culture after a period of growth. Anabaena 7120 appeared to overcome the inhibitory effect of 10^{-5}M $Cu(NO_3)_2$ and $Cd(NO_3)_2$ (Figures 8 and 10). Therefore, the HM binding capacities of cultures with and without Cu^{2+} or Cd^{2+} ions, were determined on Day 0 and at the end of the lag phase on two occassions. The results of Table 10 show that on Day 0 the Cu^{2+} binding capacity of the inoculated cultures was slightly greater than that of uninoculated medium. After the lag phase the Cu^{2+} binding capacity of the control and of the culture with 10^{-5}M $Cu(NO_3)_2$ had increased approximately 15-20 percent. This marked increase in the Cu^{2+} binding capacity of copper-containing cultures is certainly compatible with the idea that, by excreting materials, the algae overcome inhibition imposed by the $Cu(NO_3)_2$.

TABLE 10

	Experiment #1	Experiment #2
GO Medium	364	419
Control Culture		
- Day 0	379	439
- After Lag Phase	429	479
Culture with 10^{-5}M $Cu(NO_3)_2$		
- Day 0	379	419
- After Lag Phase	439	499

TABLE 10 - The Cu^{2+} binding capacity of Anabaena 7120 cultures before and after growth in GO medium. The Cu^{2+} binding capacity is expressed in terms of μmoles of Cu^{2+} ions bound per litre of culture.

Experiments with $Cd(NO_3)_2$ produced different results. The Cd binding capacity of freshly inoculated cultures were not markedly greater than uninoculated GO medium (Table 11). Even more striking, by the end of the lag phase there was a decrease in the Cd^{2+} binding capacity, both for the control culture and for the Cd-containing culture. This does not, however, exclude the possibility that Anabaena 7120 overcame the HM inhibition by excreting Cd^{2+} binding substances.

The multicomponent nature of the culture makes it difficult to assign HM binding ability to any one component. During the incubation period, the following changes could occur: (i) a decrease in HM binding capacity due to consumption of the GO medium which contains HM binding substances such as sulphate, phosphate and carbonate; (ii) an increase in HM binding capacity due to the release of extracellular products by the algae; and (iii) an increase in the HM binding capacity due to increased cell numbers. The Cd^{2+} binding experiments appear to indicate that the algal consumption of GO medium not only nullified the effects of the extracellular products released and the increase in cell number, but actually produced a net decrease in the HM binding capacity of the cultures.

In an attempt to determine if any of the above possibilities, relating to the Cu^{2+} and Cd^{2+} binding capacities, were valid, the problem was approached using a different technique.

TABLE 11

	Experiment #1	Experiment #2
GO Medium	469	519
Control Culture		
- Day 0	469	519
- After Lag Phase	439	459
Culture with 10^{-5}M $Cd(NO_3)_2$		
- Day 0	489	529
- After Lag Phase	429	449

TABLE 11 - The Cd^{2+} binding capacity of Anabaena 7120 cultures before and after growth in GO medium. The Cd^{2+} binding capacity is expressed in terms of μmoles of Cd^{2+} ions bound per litre of culture.

Atomic absorption analyses of these cultures showed clearly that the Cu ions were released from the cells during the lag phase. The data of Table 12 (experiments done in duplicate on two separate occassions) show that at Day 0 approximately 30 percent of the Cu was associated with the cells, while at the end of the lag phase (Fig. 8) almost 100 percent of the Cu was present in the cell-free fraction. The Cu^{2+} ion-selective electrode indicated that the released copper ions were in the bound form.

As mentioned in the introduction, algae are known to excrete materials capable of increasing the availability of essential nutrients when growth conditions are unfavourable.[16,24,26,27,32] Furthermore, the observation that some algae release exceptionally large amounts of extracellular products in unfavourable environments and during senescence, [27,32] makes the hypothesis that algae may have mechanisms which protect them against the unfavourable conditions presented by HM toxicity even more plausible.

Now, consider the following observations together:

(i) The atomic absorption analyses (Table 12) indicated that Anabaena 7120 is capable of releasing large amounts of copper.

(ii) The measurement of free Cu^{2+} ions indicated that the copper is released in the bound form.

TABLE 12

	Cells	Cell-free Fraction
Cultures containing 10^{-5}M $Cu(NO_3)_2$		
(Cu) at Day 0	3.2 ± 0.6μM*	7.0 ± 0.5μM
(Cu) After Lag Phase	1.4 ± 0.3μM*	9.8 ± 0.3μM
Cultures containing 10^{-5}M $Cd(NO_3)_2$		
(Cd) at Day 0	5.6 ± 1.8μM*	4.4 ± 0.3μM
(Cd) After Lag Phase	5.3 ± 0.2μM*	5.3 ± 0.3μM

TABLE 12 - Binding of Cu^{2+} and Cd^{2+} ions to Anabaena 7120 cells and cell-free fractions with relation to time. Measurements of total Cu and Cd were made by atomic absorption spectroscopy.

* Expressed as the concentration of metal that would be in an equivalent volume as the Cell-free Fraction. See page 30 for a description of the experimental procedure. The cells originally in 25 millilitres of growth medium were dissolved, after centrifugation and ashing, in 25 millilitres of 5 percent HNO_3. Concentrations are those found in 25 millilitres. Any exchange of Cu or Cd between the cells and the medium could be detected. Changes in the total Cu or Cd concentration originally added to the medium could also be detected.

(iii) The Cu^{2+} binding experiments (Table 10) indicated that the Cu^{2+} binding capacity of the Anabaena 7120 cultures containing 10^{-5}M $Cu(NO_3)_2$ increased approximately 15-20 percent by the end of the lag phase.

It would appear possible, therefore, that Anabaena 7120 may have released material during the lag phase which bound to the Cu^{2+} ions, removing them from the cells.

In contrast to the movement of Cu in the cultures, atomic absorption analyses of Cd (Table 12) showed that the concentration of Cd associated with the cells did not change. These results could be explained if Cd^{2+} ions bind first to functional sites causing inhibition, and then become bound more strongly by sites on or in the cell which are non-functional and which are not excreted. If this is so, the mechanisms of Anabaena 7120 for reducing the toxic effects during the lag phase are different for copper and cadmium ions.

GENERAL DISCUSSION AND CONCLUSIONS

Specific ion electrode technology has provided a convenient, precise and quick method of measuring free and bound forms of metal ions in natural waters. This study has shown, by the use of ion-specific electrodes, the degree to which toxic metal ions such as Hg^{2+}, Pb^{2+}, Cu^{2+} and Cd^{2+} may be present in the free or bound forms in waters (both flowing and non-flowing) that support algal growth. By measuring the total metal concentration during culture experiments using atomic absorption spectroscopy in conjunction with free metal ion measurements, we were able to confirm that the loss of $(M^{2+})_{free}$ in solution was due to the formation of bound M^{2+}. Therefore, metal ion complexation within the systems was being measured.

All of the water systems studied, supported algal blooms when they were sampled. The water systems sampled also exhibited very high HM binding capacities.

One aim of this study was to determine whether or not there was any relationship between algal excretion and the high HM binding capacities of water systems containing algal blooms. Therefore, the water samples were analyzed in an attempt to determine the nature of the HM binding compounds. Chemical analysis and correlation of various components with their respective HM binding capacities eliminated several possible components including silica, phosphate and nitrogen compounds. Inorganic carbon was eliminated as a major binding component when volatilization of carbonates and bicarbonates did not reduce the HM binding capacities of the samples. Chloride was excluded as a major binding site when HM binding experiments in distilled-deionized water and in Leamy Lake water, each solution containing equal concentrations of chloride ions, indicated differences in the magnitude and order of binding by Hg^{2+}, Pb^{2+}, Cu^{2+} and

Cd^{2+} ions. From these observations and because of the loss in the HM binding capacities after volatilization of the organic carbon, it was concluded that organic carbon-containing compounds are responsible for the high HM binding capacities of the water systems studied.

Ultrafiltration, using diaflo membranes, followed by HM binding experiments has shown that the HM binding of Leamy Lake water is concentrated in the fraction of less than 500 molecular weight (size range $\leq$ 1nm). Particulate matter in Rideau River water was responsible for a small amount of the Cu^{2+} binding capacity. However, the HM binding components were almost entirely present in the soluble fraction of Rideau River, Lac Beauchamps and Lac Carriere waters.

Determination of HM binding capacities over extended periods indicated the persistence and stability of the HM binding substrate. If algal extracellular products do contribute to the HM binding capacities of these waters, the long term presence of the substrate would tend to result in its continuous accumulation with time.

While the coincidence of the algal blooms with the high HM binding capacities of the water systems studied implies that these two factors are related, more work is required to arrive at a firm conclusion.

The persistence of the HM binding substrate and further detailed chemical analyses may be keys to producing the necessary information. Chemical analyses should be designed to show <u>qualitatively</u> whether or not the HM-binding substrate is related to algal blooms and/or extracellular products. If the qualitative analyses indicate that there is a relationship and the HM-binding substrate

continues to persist, HM binding experiments and quantitative chemical analyses might show increases in substrate concentration as the total number of blooms increases.

Batch cultures, while not producing the same environmental conditions as aquatic ecosystems, can be useful as model systems which supply information for certain controlled conditions. The growth of batch cultures of the blue-green alga, Anabaena 7120, in GO medium was inhibited completely by concentrations of $Cu(NO_3)_2$ or $Cd(NO_3)_2$ greater than 10^{-4}M. At concentrations of these HM ions less than 10^{-4}M, the cultures exhibited a prolonged lag phase, after which they appeared to grow at a rate comparable to cultures without HM ions present. These results may suggest that Anabaena 7120 cells have a mechanism(s) for releasing the Cu^{2+} or Cd^{2+} ions from functional sites and that this release is required before algal growth is able to continue.

Precipitates which formed, upon addition of $Pb(NO_3)_2$ to GO medium, produced apparent inconsistencies in the inhibition of Anabaena 7120 growth. Therefore, further work with Pb^{2+} ions will have to be manipulated in order to avoid erroneous interpretation.

Nitrilotriacetic acid (NTA), a synthetic organic chelating agent, increased the toxicity of Cu^{2+} and Cd^{2+} ions toward Anabaena 7120. The increased toxicity is not due to NTA itself, nor to the unavailability of nutrients in the presence of NTA. It would appear instead that the metal bound NTA enhances the inhibitory effects of the Cu^{2+} and Cd^{2+} ions.

Cu^{2+} binding experiments and atomic absorption spectroscopy provided information about possible mechanisms by which Anabaena 7120 overcomes Cu^{2+} ion inhibition. The Cu^{2+} binding experiments indicated that by the end of the lag phase there was an increase in the Cu^{2+} binding capacity of the cultures with 10^{-5}M $Cu(NO_3)_2$ added. Atomic absorption analysis of the pellet and supernatant of centrifuged cultures showed that almost all of the Cu^{2+} ions initially associated with the cells were released into the supernatant by the end of the lag phase. These data seems to support the hypothesis that Anabaena 7120 cells release extracellular products which bind the Cu^{2+} ions.

Just as the results mentioned in the preceding paragraph suggest the release of bound Cu^{2+} ions from Anabaena 7120, similar analytical techniques used to study Cd^{2+} ions show clearly that cadmium is not released by the end of the lag phase. Consequently, it appears that Annabaena 7120 overcomes cadmium inhibition by a mechanism different from that of copper inhibition for the culture conditions stipulated.

Experiments aimed at monitoring and identifying the release of extracellular products in the presence and absence of $Cu(NO_3)_2$ should be undertaken for the following reasons: (1) to confirm that the results displayed by the Cu^{2+} ion-specific electrode were due to the release of extracellular products by Anabaena 7120; (2) to establish that the release of extracellular products by Anabaena 7120 is related to overcoming copper inhibition; and (3) to identify the compound(s) which increase the Cu^{2+} binding capacity of the Anabaena 7120 cultures.

Such an extension of this study might be helpful in identifying (i) the inhibitory mechanisms of Cu^{2+} ions, (ii) the mechanisms by which Anabaena 7120 overcomes Cu inhibition and (iii) possibly the nature of the HM binding components which increase the HM binding capacity of natural waters which support algal blooms.

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