AN INVESTIGATION OF THE EFFECT OF CD, HG AND ZN ON THE GROWTH OF TWO SPECIES OF BLUE GREEN ALGAE

by

Zeynep Ağlarca

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ABSTRACT

In this research, effects of three metals, cadmium, mercury and zinc were investigated on two species of blue green algae, *Anabaena flos aquae* and *Gloeocapsa*. Cells were grown in sterile medium free of combined nitrogen and under continuous illumination. Metal ions were introduced as chloride salts and different concentrations of the metal ion solutions were used. Absorbance and cell counts were determined for each concentration of the metal ion solution and results were evaluated in terms of these two criteria.

Zinc was found to be the most toxic metal for both *Anabaena flos aquae* and *Gloeocapsa* whereas the other metals showed inhibitory and stimulatory effects depending on the concentration of the metal ion solution. This showed that effect of the metal whether inhibitory or stimulatory is dependent on the species used and the metal ion itself.

Ö Z E T

Bu çalışmada *Anabaena flos aquae* ve *Gloeocapsa* isimli mavi yeşil alg türlerinde kadmiyum, civa ve çinko metallerinin etkileri araştırıldı. Gerekli azotsuz vasat hazırlandıktan sonra hücreler bu vasatta ve sürekli bir aydınlatma tertibatı altında büyütüldü. Değişik konsantrasyonlardaki metal iyonları ortama klorür tuzları halinde verildi ve her konsantrasyon için soğurum değerleri ve hücre sayıları belirlendi. Sonuçlar bu iki nokta hesaba katılarak karşılaştırıldı ve değerlendirildi.

Çinko metalinin her iki tür için de en toksik metal olduğu görüldü. Buna karşılık diğer iki metal konsantrasyona bağlı olarak bir türde büyümeyi engelledi, diğerinde ise hızlandırdı. Bu çalışma metalin büyüme üzerindeki etkisinin kullanılan türe ve metal iyonuna bağlı olduğunu ispatladı.

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CHAPTER I
INTRODUCTION

I. INTRODUCTION

1.1 IMPORTANCE OF ALGAE

The term "algae" derived from the Latin name for sea weed, has come to be applied to all relatively simple, marine and fresh-water vegetation. Included under "algae", are the smallest and most simple of chlorophyll bearing organisms, the entire plant being a single cell. Some of these may be less than 1 micron in diameter whereas others may be as much as 700 ft in diameter.

Algae are very ancient organisms. It is believed that they have been existing on earth for more than 3000 million years. It is likely that they have been major sources of oxygen.

Algae are found in different environments, including soil, fresh and sea water. Blue green algae, subject of this research, are quite important because of the role they play in nitrogen fixation.

Algae play a small but important part in the direct economy of many countries. It is impossible to assess the full economic importance of algal growth; in aquatic habitats, algae are part of the food chain leading to crustacea and fish; on agricultural land, they are an important constituent of the soil flora, and in water supply reservoirs, purification plants and in sewage disposal plants, they play an important role in oxygenation and filtration.

Algae are important indicators of pollution in aquatic habitats, while under other circumstances, they themselves become polluting agents, for example, of chemical plants, fish ponds and so on. In scientific work they are used as assay organisms for vitamins and in the dating of sedimentary rocks in oil prospecting.

Uses of algae are so many in number that it might be hard to list all of them. As commercial uses, one can list the production of certain substances like alginic acid derivatives, carrageenin, diatomite, agar, and funori. Alginic acid derivatives are extracted from Phaeophyta (Brown Algae) and use of alginates in industry depends on the chemical and physical properties of these compounds, e.g. they are non-toxic, highly viscous and readily form gels. Carrageenin and agar are extracted from Rhodohpyta (Red Algae) and carrageenin is used in textile, pharmaceutical and leather industries whereas agar is used as a medium in the culture of bacteria, fungi and other algae. Diatomite on the other hand, is extracted from diatoms which contain siliceous cell walls, and is used in the recycling of water and as an industrial catalyst.

Algae are also used as fertilizers where the larger brown and red algae are the commonly used classes. Interestingly enough, in Japan, a glue is made from the marine algae *Gloppeltis furcate* and is called funori.

Even though it is only in the Far East, algae have been regularly used for human food. In the Pacific Islands, the raw algae, usually species of red algae, but also green algae and brown algae are added to some dishes.

In addition to these uses, algae also have medicinal uses. The Rhodophycaen algae *Digenia simplex* is made into a drug in South China and is of some importance as an antihelmitic. It has been also shown that products obtained from certain algae have antibiotic action and are used as antibiotics.

Algae play an important role in sewage disposal which is usually an aerobic process and the presence of algae greatly facilitates oxygenation and filtration.

However, the most important role of algae is found in ecology, through photosynthesis and nitrogen fixation.. Although one usually thinks of trees and other green plants as the most important sites of photosynthesis, it is estimated that over half the carbohydrates on earth is produced by the aquatic microorganisms like blue green algae. Algae also add chemicals to the soil either on their death or by diffusion from the cells. Well known examples are Cyanophyta which fix atmospheric nitrogen and thus increase the nitrogen content of the soil. It has been shown by Watanable [1] that nitrogen fixing Cyanophyta supply nitrogen to rice fields.

1.2 CLASSIFICATION OF ALGAE

The organisms which constitute what are commonly known as "algae" are extremely diverse in form color, habit and in their habitats. Because of this great diversity, a classification of algae is needed [2,3]. The phyla of fresh water algae and their characteristics are as follows:

1. Chlorophyta (Green algae)

Plants unicellular, colonial, or filamentous; floating, swimming or attached and stationary cells containing plastids in which chlorophyll is dominant, and in which there is a starch storing body called the pyrenoid; nucleus definite, cell wall composed of cellulose and pectose; sexual reproduction.

2. Cyanophyta (Blue green algae)

Plants unicellular, colonial or in simple or branched filaments, cell wall thin, a membrane with a gelatinous outer sheath, contents often with false vacuoles which refract light and obscure the true color of the cells, definite nucleus lacking; asexual reproduction by cell division or by spores; food storage glycogen.

3. Chrysophyta (Yellow green or yellow brown algae)

Plants unicellular or colonial, rarely filamentous, food storage in the form of oil or leucosin, wall relatively thick, made up of pectin with silicon.

4. Euglenophyta (Euglenoids)

Cells solitary, food reserve in the form of an insoluble starch and fatty substances, nucleus large and centrally located, rigid cell membrane, vegetative reproduction.

5. Cryptophyta (Crypotomonads)

Cells solitary, food reserve in the form of solid starch, or starch like substances; reproduction by longitudinal cell division.

6. Pyrrophyta (Dinoflagellates)

Cells solitary, firm cell wall, food reserve as starch, starch like substances and oil; reproduction by longitudinal cell division, and asexual zoospores formed in some genera.

7. Rhodophyta (Red algae)

Plants simple or branched, filaments, food reserve in the form of floridean starch, thick walls containing pores, sexual reproduction and asexual reproduction in some genera.

8. Chloromonodophyta (Chloromonads)

Little understood,group composed of a few genera, cells solitary, food reserve in the form of oils or fats, vacuoles present.

9. Phaeophyta (Brown algae)

A phylum almost entirely marine, including the brown sea weeds; essentially filamentous (some microscopic) but mostly macroscopic; food reserve in the form of soluble carbohydrates; pyrenoids sometimes present.

Despite the extreme morphological, cytological and reproductive variation in the groups of algae, the basic biochemical mechanisms and pathways appear to be similar to those of other plants. For example, they all possess chlorophyll and have a photosynthetic system working via this pigment. The basic nutrient requirements and the end products of assimilation, carbohydrates and proteins, are similar to higher plants even though the range of carbohydrates is greater. Both because of this similarity and because of the interest in investigating plants forming unfamiliar carbohydrates, the algae have been used in many basic physiological problems where the growth of the organisms is facilitated by liquid culture. They are now proving equally valuable tools in studying metabolic pathways since some of the species can be induced to form biochemical mutants.

1.3 PLACES WHERE ALGAE CAN BE FOUND

Ecologically the algae are a world wide group occurring on the land surface, on all types of soil, and on permanent ice and snowfields but having their major center of distribution in the waters which cover seventy percent of the earth's surface. Here they are the major and primary organic producers occurring in the form of microscopic phytoplankton and as both microscopic and macroscopic growths along the boundary between land and water.

Algae may also be classified according to the places where they can be found.

1. Kryoflora (Snow and ice flora)

Algae are found in these habitats only where the surface is stable for some length of time, e.g. on polar ice caps and on permanent snow fields in mountains. Both freshwater and marine algae exist. The species are usually unicellular and at least in the freshwater habitat are confined to a few genera, mainly of Chlorophyta. The marine part is found on the permanent ice around ice caps, often coating the undersurface of the ice with a brown film due to abundance of diatoms.

2. Aerial epiphytic algae

These are algal communities dependent directly on rain water or high humidity for their water supply. This habitat is characterized by the absence of nutrients other than those in rain and those obtained by solution of dust and material or from the plant tissues.

3. Aerial epilithic algae

These algae are found on rock and store surfaces which are relatively stable. On rocks, which receive only atmospheric moisture, the two species *Desmococcus* and *Trentepohlia* are quite common. However on rock surfaces receiving seepage water from the soils, diatoms and blackish Cyanophyta consisting of *Chroococcus, Gloeocapsa, Stigonema, Calothrix* are observed. In general, the four groups of algae found in the epilithic flora are Cyanophyta, Chlorophyta, Rhodophyta and Bacillariophyta.

4. Soil flora

As the name implies, the surface of most soils supports a rich algal flora. Fragments of algae are washed to greater depths and remain there for long periods, but actively growing algae are probably bound to within a mm or so of the surface. Soil algae not only derive nutrients from the soil water, but also add chemicals to the soil either on their death or by diffusion from the cells.

5. Epipelic algae

These algae are found on the sediments which are deposited along the length of rivers and streams. This epipelic flora is mainly composed of diatoms, blue green algae, green algae and euglenoids. These algae may be growing in streams, rivers, lakes or ponds, each habitat having some common and different characteristics, due to different factors affecting the growth in static and running waters.

1.4 HISTORICAL BACKGROUND

It is found out that all algae need certain elements for normal growth and proper functioning. However, a distinction must be made between absolute requirements, i.e. essentiality of a nutrient for growth, reproduction or photosynthesis and normal requirements, i.e. the quantity of each nutrient in the cells during active growth. Thus for *Chlorella* for every 100 parts by weight of carbon the following are normally required: nitrogen 15, phosphorous 5, magnesium 2.5, potassium 1.8, sulfur 1.6. The minimum and optimal requirements seem

to show some variations depending on the species. It has been previously concluded that the elements required by algae were the same for higher plants. To a certain extent this is true but some points are characteristic of the algae. The basic minerals required are similar, with the addition of silica for some groups. For instance, sodium is not generally regarded as an absolute requirement for the majority of algae but *Arabaena cylindirica* was found to require sodium and this could not be substituted by potassium, lithium, rubidium or cesium. In other words, sodium is found to be an essential element for growth of Cyanophyta. In addition to the major elements carbon, nitrogen, phosphorous, sulfur, potassium, magnesium and calcium, trace elements such as iron, manganase, silicon, zinc, copper, cobalt, molybdenum, boron and vanadium are required at least for some algae.

Even though certain elements are needed in small amounts for algal growth, they may inhibit algal growth if present above a certain concentration and unfortunately concentration of metal ions has increased a great deal due to pollution of waters. Discharge of industrial wastes to lakes and rivers has increased the concentration of metal ions a great deal and these ions tend to be accumulated in the algae and other marine organisms which then affects the higher organisms in the food chain.

For instance, as part of a larger study of toxic *chromium* in natural waters, an investigation was made of the effects of chromium on several species of algae and duckweeds from the upper Susquehama River, New York [4] cultures were made from the material collected and pure cultures of the following were obtained:

Palmella mucosa (motile unicellular chlorophyta)

Oedogonium (filamentous chlorophyta)

Hydrodictyton reticulatum (filamentous colonial chlorophyta)

Palmellococcus protethecoides (non-motile unicellular chlorophyta)

Lemna minor L (Duckweed)

Spirodela polygorhize (Duckweed)

Chromium was introduced as K_2CrO_4 and the concentration of chromium used in the toxicity and uptake laboratory studies ranged from 0.003 ppm to 10 ppm for the duckweed and 0.01 ppm to 10 ppm for the algae. The growth of all plants was inhibited by 10 ppm chromium. The unicells declined in cell numbers after two weeks; the filamentous forms showed a loss of weight. Yet a percentage of healthy looking cells and filaments, remained among the distorted ones. The duckweeds grew during the two weeks but not as much as the chromium free controls. The effects of lower doses of chromium were mixed. At higher levels of chromium, the ratio taken up by the plants to the concentration in the initial medium was not as great as at lower levels. In all cases a large amount of chromium was picked up by the plants. Varying responses to chromium were observed by a range of algae. The duckweeds showed greater resistance. The plants had a significant capacity to remove low doses of chromium from solution through adsorption which might allow some surviving cells to grow and re-establish the population.

Cadmium uptake by the unicellular green algae *Chlorella salina Cu 1* was studied by Wong and Chan [5]. It has been reported that

considerable inhibition of growth of *Chlorella ellipsoidea* C27 was observed when the cells were grown in fresh water medium containing 25 ppm Cd [6]. However, no significant retardation of growth was observed for *Chlorella salina Cu 1* in 5 ppm cadmium contaminated medium with zero salinity. The cadmium tolerance of *Chlorella salina* was surprisingly high so that even in high salt and cadmium contaminated media, this alga could still survive and grow well. The cadmium content in cells which have been cultured in 0.001 ppm cadmium contaminated medium was 3.9 ppm and in 0.1 ppm contaminated medium, it was 142 ppm. However, the amount of cadmium accumulated was found to be quite different in the presence of sodium chloride. In 0.1 ppm cadmium contaminated medium, the contents of cadmium in the algal cells were found to be 124 ppm in medium without added sodium chloride, 25 ppm in medium with 5 ppt sodium chloride, 18 ppm in medium with 10 ppt sodium chloride and only 7.6 ppm in medium with 15 ppt sodium chloride. Similar declines were observed for media containing 0.001 ppm, 0.005 ppm, 0.01 ppm and 0.05 ppm Cd with different salinities. This shows that the alga *Chlorella salina* can uptake cadmium to a certain extent from the environment containing low cadmium concentrations. However the presence of sodium chloride which is a major component of the sewage effluent of the area where the study is carried out (Hong Kong) can suppress the cadmium uptake by the algal cells and therefore greatly reduce the amount of cadmium being accumulated in algal cells.

Mercury may also accumulate in many organisms. Klein and Goldberg [7] reported average concentrations in animals from near La Jolla. California of 0.9 ppm dry weight with a maximum of 21 ppm

in a cowry. Jones and Stewart have done some studies to determine the mercury content of samples taken from the Tay Region of Great Britain. The mercury content of certain species of Chlorophyceae, Rhodophyceae and Phaeophyceae are reported [8].

Mercury was found to be toxic not only to algae but also to freshwater fish. Panigrahi and Misra have observed the toxicological effects of mercury on a freshwater fish, *Anabas scandens* [9].

Anabas scandens exposed to mercuric nitrate died in concentrations greater than 5 mg/L. However at a 3 mg/L concentration they survived although they showed a variety of pathological and biochemical disorders. The main clinical disorders such as inappetence and ataxia appeared after five days' exposure. After three weeks' exposure blindness was noted in twenty-nine percent of the fish and respiration rate was considerably reduced. After four weeks a total of seventy-one percent of fish had become blind. Partial recovery of respiration rate occurred in all treated fish when they were transferred to fresh mercury free water.

Although *copper* is known to be an essential micronutrient for algae, most algae are extremely sensitive to excess copper. An increase of copper concentration from 9 to 24-28 µg/L was found to prevent a bloom of blue green algae, but the green algae developed normally [10]. The photosynthetic production was reduced to eighty percent of the control by the addition of 0.1 mg/L Cu and fifty percent by the addition of 0.15-0.20 mg/L Cu. The growth rate of *Spirulina platensis*, blue green alga , was more affected by copper than the photosynthesis of Phytoplankton. Addition of 0.05 mg/L Cu reduced the growth rate

to about forty percent of the control. The rotifers were found to be less sensitive to copper than the algae, but after eight days exposure to 0.5 mg/L Cu or more the population was greatly affected.

In another study done to determine the effects of copper, *Spirogyra, Oedogonium, Microspora, Mougeotia, Ulothrix* and *Draparnadia* were used as algal species [11]. Copper was added in the form of $CuSO_4.5H_2O$ in such a way that the final concentration of the metal would be 10 mM and 0.001 mM. The overall effect of increased copper levels in the medium was to cause a species specific decrease in growth at levels above 0.001 mM. Higher copper concentrations in the growth medium coincided with lower dry weights and higher copper uptakes. Among the algal species, *Mougeotia, Microspora* tolerated higher copper concentrations up to 0.1 mM Cu than the other species and accumulated less of it. The distribution of copper within the cell wall also varied betwen the species. In all species with the exception of *Ulothrix*, more than half of the copper was accumulated in the cell walls.

Lead at levels found in most sea water does not appear to have toxic effects in short terms. However it is easily concentrated by marine plants and animals and may affect mortality rates of animals when accumulated over longer periods of time. The effect of lead was investigated on three species of small regularly branched marine red algae, *Platythamnion pectinatum, Platysiphonia decumbens, Pleonosporium squarrulosum* [12]. These species were grown in medium containing concentration of lead up to 10 mg/L Pb. A larger amount of growth was obtained in controls and at lower levels of lead than in dishes with

greater amounts of lead. No visually observed effects of the added lead on vegetative morphology or development of reproductive structures were observed, although in medium with added lead, these algae grew more slowly. At concentrations higher than 10 mg/L Pb, cells became colorless, and growth stopped within a few days. To more precisely evaluate the results of adding lead, cell division and cell elongation were measured in *Tiffaniella spermothamnion synderae*, another species of red algae.

Both of these measures of growth were affected in proportion to the amount of lead present.

Nickel has been reported to have both a stimulatory and inhibitory effect on algal growth. Bertrand and DeWolf [13] have suggested that nickel may be required for plant growth. This was based on their observation that cultures of *Chlorella* species exposed to 3 µg/L Ni produced greater yields than nickel free controls. However, nickel has also been found very toxic to algae, though some species are more tolerant than others. Studies on nickel toxicity indicate that nickel inhibition occurs at relatively low levels and is variable. Nickel has also been shown to inhibit algal growth when 0.05 to 0.1 mg/L solution is present. For some species 0.3 to 0.5 g/L Ni reduces growth by eighty to ninty percent. Effects of nickel on growth of some laboratory algal cultures are summarized by Spencer [14]. Spencer reported that green algae and diatoms were inhibited at lower divalent nickel, Ni^{+2} concentrations than blue green algae. Growth of *Ankistrodesmus falcatus, Ankistrodesmus falcatus var. acicularis, Pediastrum tetras, Scenedesmus quadricauda* and *Navicula pelliculosa* was significantly

reduced by 0.1 mg/L Ni. *S. dimorphous* grew at 0.1 mg/L solution, but growth was significantly reduced by 0.3 mg/L and 0.6 mg/L solutions. *Anabaena flos aquae* was not inhibited until divalent nickel reached a concentration of 0.6 mg/L and *Anabaena cylindirica* was not significantly affected by nickel levels up to 0.6 mg/L. Nickel toxicity appeared to be related to other environmental factors including the presence of other metals.

As far as *zinc* is considered, tolerance levels to zinc of three diatoms, *Skeletonema costatum, Thalassiosira pseudonana* and *Phaeodactylum tricornutum* grown in culture in the local fyord water were studied [15]. Declining relative growth rates were observed by the addition of 50, 250 and 25000 µg/L of zinc ions, respectively, for the three algae. Reduced final cell concentrations were found at lower zinc levels. The data obtained demonstrated the large differences in zinc tolerances of the algae. *Skeletonema costatum* tolerated no more than 25 µg/L Zn in addition to the normal content of the sea water. While additions of 100 µg/L Zn seemed to have no effect on the growth rate of *Thalassiosira pseudonana* which also grew well in sea water with 250 µg Zn. Cultures of *Phaeodactylum tricornutum* grew well in sea water containing 10 mg/L Zn and still divided quite rapidly upon exposure of 25000 µg/L Zn. Large differences in the zinc tolerance were also observed for the two dones of *Skeletonema costatum* which demonstrated that this diatom showed significant intraspecific differences.

It should be emphasized not only the type of metal is important, but also the species play important roles in determining whether the

effect will be toxic or not. Certain metals may be toxic for certain species, but may not show any toxic effect for others.

Ecologists are also interested in the effects of mixtures of metals since wastewaters contain a combination of metals rather than one single metal ion.

P.V. Devi Prasad and P.S. Devi Prasad have collected three species of green algae, mainly *Ankistrodesmus falcatus, Scenedesmus obliquus* and *Chlorococcum spp.* and have studied the effects of *Cadmium, lead and nickel* [16]. $CdSO_4$, $PbCl_2$ and $NiCl_2$ were used so that the final concentrations of the metals were 0.1, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 5.0 and 10 ppm. Of the three metals tested, cadmium was more toxic than lead and nickel. Cadmium concentrations of 5 ppm and above were lethal to *Scenedesmus obliquus* and *Ankistrodesmus falcatus* while 10 ppm was lethal to *Chlorococcum spp.* At lower concentrations, there was a slightly stimulation in the growth of the three algae. Lead did not show any stimulatory effect, at lower concentrations. At 10 ppm, lead killed *Ankistrodesmus falcatus* and greatly reduced the growth of the other two algae. Nickel was not lethal to any of the algae even at the highest concentration used though the growth was reduced considerably.

Nickel and cadmium, when used together at 0.5 ppm each, gave more increased growth than when they were used individually. This trend was reflected in the higher concentration and also as 5 ppm cadmium was not lethal, when used along with nickel, to *Ankistrodesmus falcatus.* A combination of cadmium and lead at 0.5 ppm each also gave considerable increase in growth of *Ankistrodesmus falcatus* when compared

to the control as well as when they were used individually. There was not much change in the effects of lead and nickel when used together from their individual effects. *Chlorococcum spp.* was found to be more tolerant to cadmium than *Scenedesmus obliquus* and *Ankistrodesmus falcatus*, while lower concentrations of cadmium were stimulatory to the growth of these algae. Nickel was not lethal even at 10 ppm to any of the algae studied. The observed effects of lead and cadmium and cadmium and nickel were important while no interaction between lead and nickel was seen.

The effect of a mixture of metals containing *arsenic, cadmium, chromium, copper, iron, lead, mercury, nickel, selenium and zinc* was investigated on the species *Scenedesmus quadricauda, Chloralla pyrenoidosa, Anabaena flos aquae* and *Navicula pelliculosa* [17]. Primary productivity and cell number were the parameters used to test the effects of metal on algae. The metal mixture inhibited about seventy percent of the primary productivity of *Scenedesmus quadricauda* when compared with the control. In fact, even one tenth of the concentration decreased forty percent of the primary productivity of the algae. Similar results were obtained when cell number was used as another indicator. The metal toxicity was found to be more pronounced at $20^{o}C$ than $10^{o}C$ or $5^{o}C$. The relative metal toxicity also varied with different algal species with the limited species, the diatom *(Navicula pelluculosa)* was the most sensitive, followed by *Anabaena flos aquae* and a green alga *Scenedesmus quadricauda* and *Chlorella pyrenoidosa.*

In other study by Trollope and Evans, the concentrations of *copper, iron, lead, nickel and zinc* in algal blooms from freshwater

areas near zinc smelting wastes in the lower Swansea Valley have been compared with levels of metals in algae and waters from areas outside the valley [18]. The metal concentrations of the blooms were considered with respect to: a) Their nature and origin (b) Metal levels in the water (c) Algal uptake and concentration factors. Three areas, mainly areas adjacent to, near and distant from zinc smelting wastes were chosen and the algae were composed of species of Chlorophyta, Xanthophyta and Cyanophyta. The samples were analyzed for copper, iron, nickel, lead and zinc. For the three groups, mean metal concentrations in the algae were ordered Fe > Zn > Pb > Cu > Ni whereas the metal concentrations in the water were ordered differently: distant Fe > Zn > Ni Pb > Cu; near Zn > Ni > Pb > Fe > Cu; adjacent Zn > Pb > Fe > Ni > Cu. As far as the concentration factors were considered; iron showed the greatest concentration factor and nickel the lowest. Large differences in the range of concentration factors exhibited within each algal group were apparent for metals except lead.

Wong et.al reported that a mixture of ten metals, *arsenic, cadmium, chromium, copper, iron, lead, mercury, nickel, selenium and zinc,* were not toxic to algae if present individually but strongly inhibited primary production when present together [19]. The primary productivity of four cultured freshwater algae, *Scenedesmus, Chlorella, Anabaena* and *Navicula* as well as natural phytoplankton from Lake Ontario water was reduced by this mixture and the species as well as the natural phytoplankton was found to be equally sensitive. The primary production of another common alga, *Ankistrodesmus falcatus*, was also reduced by the metal mixture. Apart from the primary production, other

physiological and biochemical indicators such as reproduction, transport of a non-metabolizable amino acid analogue and nitrogenase activity were also affected by the metal mixture. Since the primary production technique involved a short incubation time of four hours, the effect of the metal mixture on a longer incubation time such as reproduction was examined over 28 days on *Ankistrodesmus falcatus*. The reproduction of the algae was found to be negatively affected by the presence of the mixture. As far as nitrogenase activity was considered, nitrogenase activity of *Anabaena flos aquae* was investigated and it was also sensitive to the metal mixture. However, in comparison with photosynthesis, the nitrogenase activity of *Anabaena flos aquae* was found to be less sensitive to the metal inhibition.

In another study by Foster, concentrations and concentration factors of *zinc, copper, manganese, nickel and iron* in two species of brown algae, *Fucus vesiculosus* and *Ascophyllum nodosum* collected from an environment in which the temporal variations in dissolved trace metal concentrations have been intensively studied over several years [20]. The range of element concentrations varied for both species. The concentrations of zinc in *Fucus vesiculosus* and *Ascophyllum nodosum* were found to be very similar as were those of copper and nickel. In contrast, iron and manganese concentrations were much greater in *Fucus vesiculosus*. Concentration factors of zinc, copper and nickel for the two species sampled during this work had similar magnitudes while manganese was more concentrated in *Fucus vesiculosus*. There were differences in the degree to which the two species of algae reflected the environmental concentrations of heavy metals. The concentration

of copper in both species taken from the Straits were of similar magnitude as were the elevated concentration in Dulas Bay samples. This observation suggested that either *Fucus vesiculosus* or *Ascophyllum nodosum* could be used as an environmental indicator to copper with equal sensitivity. In the case of zinc, the available concentration in Dulas water caused a much greater relative increase on the content of *Fucus vesiculosus* than *Ascophyllum nodosum*, suggesting the former as the more sensitive biological indicator for this element.

It should be noted that the actual effects observed upon treatment with heavy metal combinations depend on both the algal species and the metal combination involved. For instance, combinations of copper and nickel interact synergistically towards the growth of *Chlorella* [21] while mixture of copper and cadmium act antagonistically towards the growth of *Selenastrum* [22]. The response of *Anabaera inequalis* towards the combination of mercuric, cadmium and nickel ion was found to be dependent on the order of metal addition and the actual metal concentration involved [23]. The effects of mercuric, cadmium and nickel ions, individually on the growth, photosynthesis and nitrogenase activity of *Anabaera inequalis* have been considered by Stratton and Corke [24,25,26]. The response of *Anabaena inequalis* towards combination of mercuric, cadmium and nickel ion was found to be dependent on the order of the metal. Mercuric and cadmium ions interacted synergistically towards photosynthesis and nitrogenase activity, but resulted in mixed synergism and antagonism towards growth, depending on the actual metal concentrations used. Mercuric and nickel ions interacted in both a synergistic and antagonistic manner, depending on the metal concentrations

used, towards growth and acetylene reductions but evidenced an additive response to photosynthesis. Nickel and cadmium ions interacted antagonistically towards all three assay criteria. Tri-metallic ion combinations resulted in antagonism towards growth and synergism towards photosynthesis and acetylene reduction. The pre-treatment of cells with either cadmium or nickel ion protected against the toxicity of subsequently added mercuric ion. Similar results were obtained by pre-treating cells with either mercuric or nickel ions prior to the addition of high levels of cadmium ion.

CHAPTER II
MATERIALS AND METHODS

II. MATERIALS AND METHODS

2.1 MATERIALS

a) Cultures

Original sample of *Anabaena flos aquae* was obtained from U.S. Environmental Protection Agency, Research Laboratory, Corvallis, Oregon. *Gloeocapsa sp. LB 795* was a kind gift from Dr. J.R. Gallon, University College of Swansea, Great Britain.

b) Chemicals

All the chemicals used in this experiment were obtained from:

Fisher,
Baker,
Merck,
Riedel-de Haën .

2.2 METHODS

a) Growth of Cultures

Anabaena flos aquae and *Gloeocapsa* were grown in 4.5 L of sterile medium free of combined nitrogen. The medium which was originally

described by Tözüm and Gallon [27] had the following composition (μmol/L). NaCl, 2000; $MgSO_4$, 150; $MgCl_2$, 145; $CaCl_2$, 190; K_2HPO_4, 100; Na_2HPO_4, 100; $FeCl_3$, 9; H_3BO_3, 6; $MnCl_2$, 6; $ZnCl_2$, 3; $CoCl_2$, 0.08; $CuCl_2$, 0.0008; MoO_3, 0.1;Na_2EDTA, 20 and its pH was adjusted to 7.5 by the addition of solid $NaHCO_3$.

Cells were grown in 6 L Erlenmeyer flasks under continuous illumination of 2500 lx at 25°C. The cells were bubbled continously with air at a flow rate of 0.3l /min/L of cells.

b) Metal Assays With Intact Cells of *Anabaera flos aquae* and *Gloeocapsa*

375 mL of medium was introudced into each of 5 500 mL Erlenmeyer flasks. Then aqueous solutions of $CdCl_2$, $HgCl_2$ and $ZnCl_2$ were added in such a way that the final metal concentrations would be 0.2 M; 0.02 M, 0.002 M and 0.0002 M for Cd and Zn and 0.02 M, 0.002 M, 0.0002 M for Hg. Flasks were inoculated with either *Anabaena flos aqua* or *Gloeocapsa* after being sterilized in the autoclave by Precision Scientific Co., at 121°C and 15 lbs/sq.in.

Then flasks were maintained at 25°C and air was bubbled through each flask. Experiments were run for 17 days with samples being taken from the fifth day on, considering the day of inoculation as zeroth day.

5 mL samples were taken from each flask and % Transmittance values were measured at Carl-Zeiss Spectrophotometer and Bosch-Lomb Spectrophotometer at 600 nm. These transmittance values were then

converted to absorption and ln A values were plotted against time (days).

c) Cell Count

The number of cells in samples removed from cultures of Gloeocapsa was determined by counting in a Thoma haemocytometer obtained from Great Britain. The values obtained are the means of at least two determinations.

CHAPTER III
RESULTS

III. RESULTS

3.1 INTRODUCTION

Studies done so far show that metal ions affect the growth of organisms if they are present above a threshold concentration. Taking this into consideration, we decided to investigate the effects of Cd, Hg and Zn on *Anabaena flos aquae* and *Gloeocapsa*. These two species were specifically chosen because no similar studies have been done on them. So effects of three metals, cadmium, mercury and zinc were tested on these two species. Each metal tested had different effects on the species tested. This was expected because the effect is very much dependent on the nature of the species and the type of metal used.

3.2 STUDIES ON *ANABAENA FLOS AQUAE*

Taking *Anabaena flos aquae* into consideration, cadmium was found to be the least toxic. Stimulation was observed with cadmium except for 0.2 M cadmium solution. Refer to Figure 3.2.1. The curve for 0.2 M cadmium solution is below that of the blank except for the last 2 days which shows cadmium present in this amount inhibits the growth of *Anabaena flos aquae*. Although an inhibition

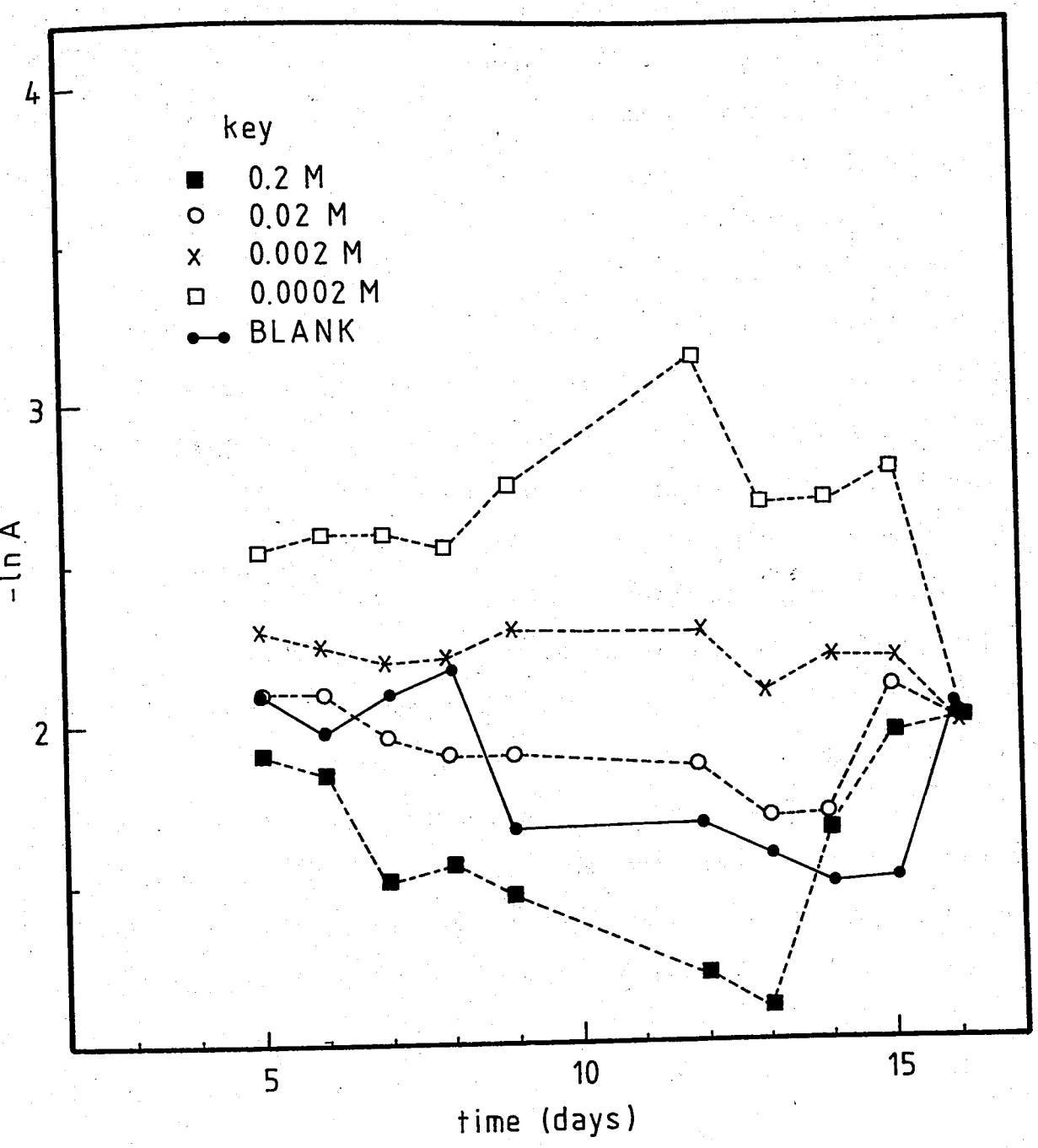

Figure 3.2.1 Effect of different concentrations of Cd metal on Anabaena flos aquae.

Each pt is the average of four sets.

was observed with 0.2 M cadmium solution, the other concentrations showed a different effect. Curve for 0.02 M cadmium solution is almost the same as the curve for the blank and curves for 0.002 M and 0.0002 M cadmium solutions are above the curve for the blank. This shows that cadmium when present at a 0.02 M concentration has no effect on the growth of *Anabaera flos aquae*, but when present at 0.002 M and 0.0002 M concentrations, stimulates the growth of cells.

More interesting results were obtained with mercury and zinc. All concentrations of mercury exhibited inhibition upto the 12^{th} day, but inhibition stopped after this day. Results are shown in Figure 3.2.2. It is clear from the graph that curves for all concentrations of mercury are below the curve for the blank upto the 12^{th} day, but after the 12^{th} day, they become similar to that of the blank. This implies that inhibition ceases after a certain time.

As far as zinc was considered, all different concentrations used had toxic effects on *Anabaera flos aquae*. As it is clear from Figure 3.2.3, 0.2 M zinc solution was found to be the most inhibitory whereas 0.0002 M zinc solution the least inhibitory. The other concentrations lie in between these two extremes.

3.3 STUDIES ON *GLOEOCAPSA*

When these metals were tested on *Gloeocapsa* the results were different. Cadmium, when tested on *Gloeocapsa*, for instance, showed slightly different effects. Results for different concentrations of cadmium are shown in Figure 3.3.1. Curves for all concentrations of

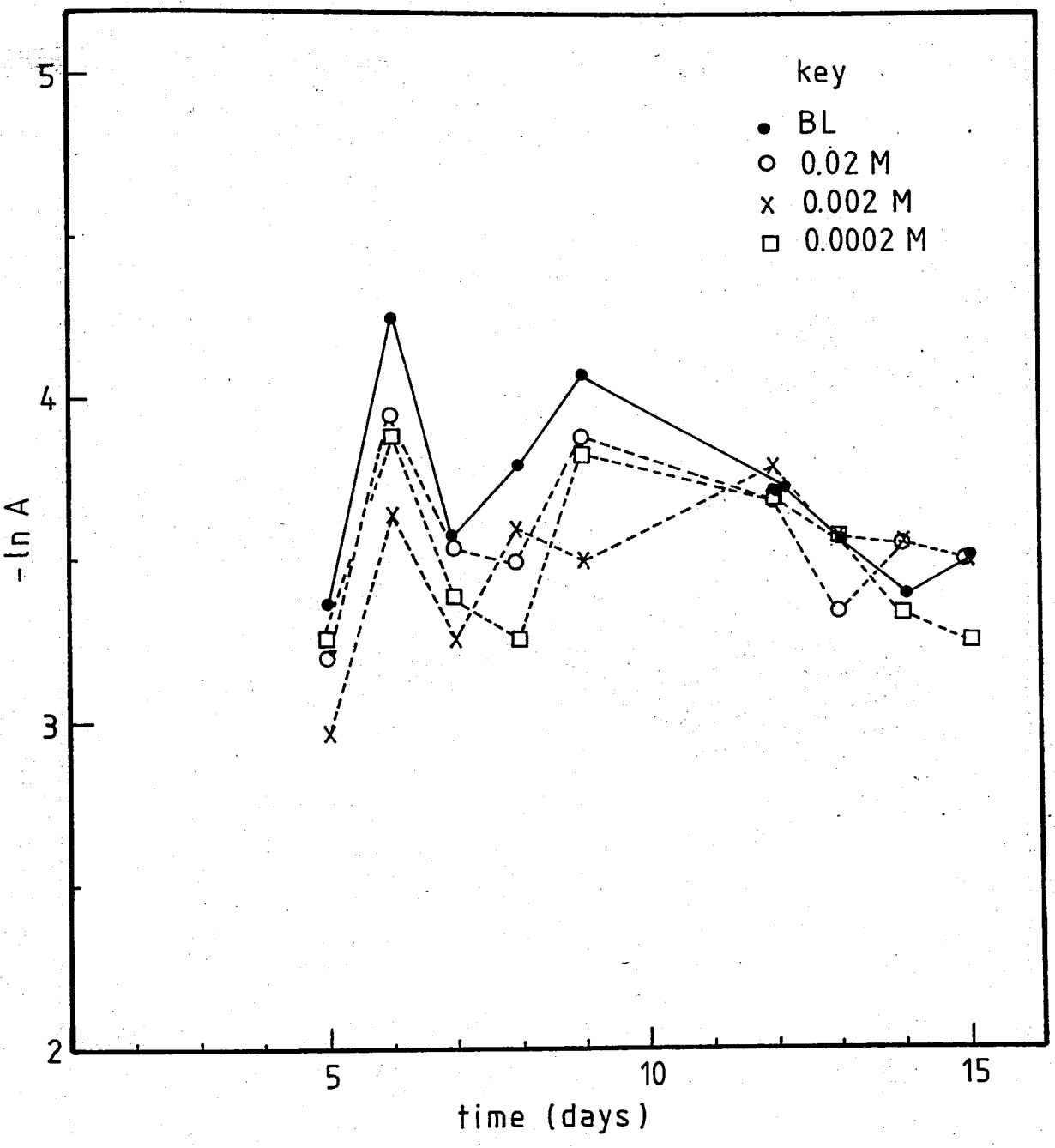

Figure 3.2.2 Effect of different concentrations of Hg metal on Anabaena flos aquae.

Each pt is the average of four sets.

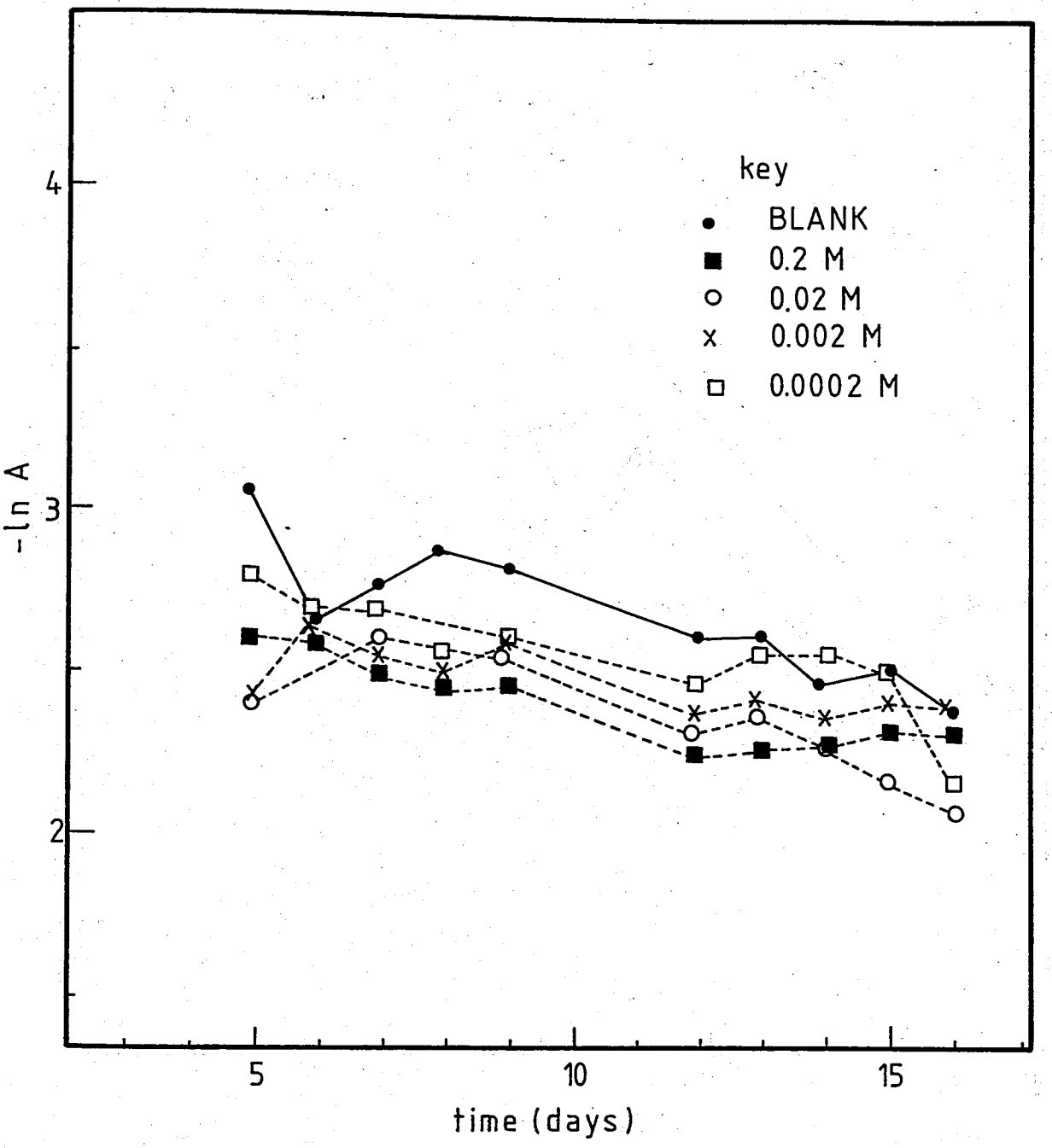

Figure 3.2.3 Effect of different concentrations of Zn metal on Anabaena flos aquae.

Each pt is the average of four sets.

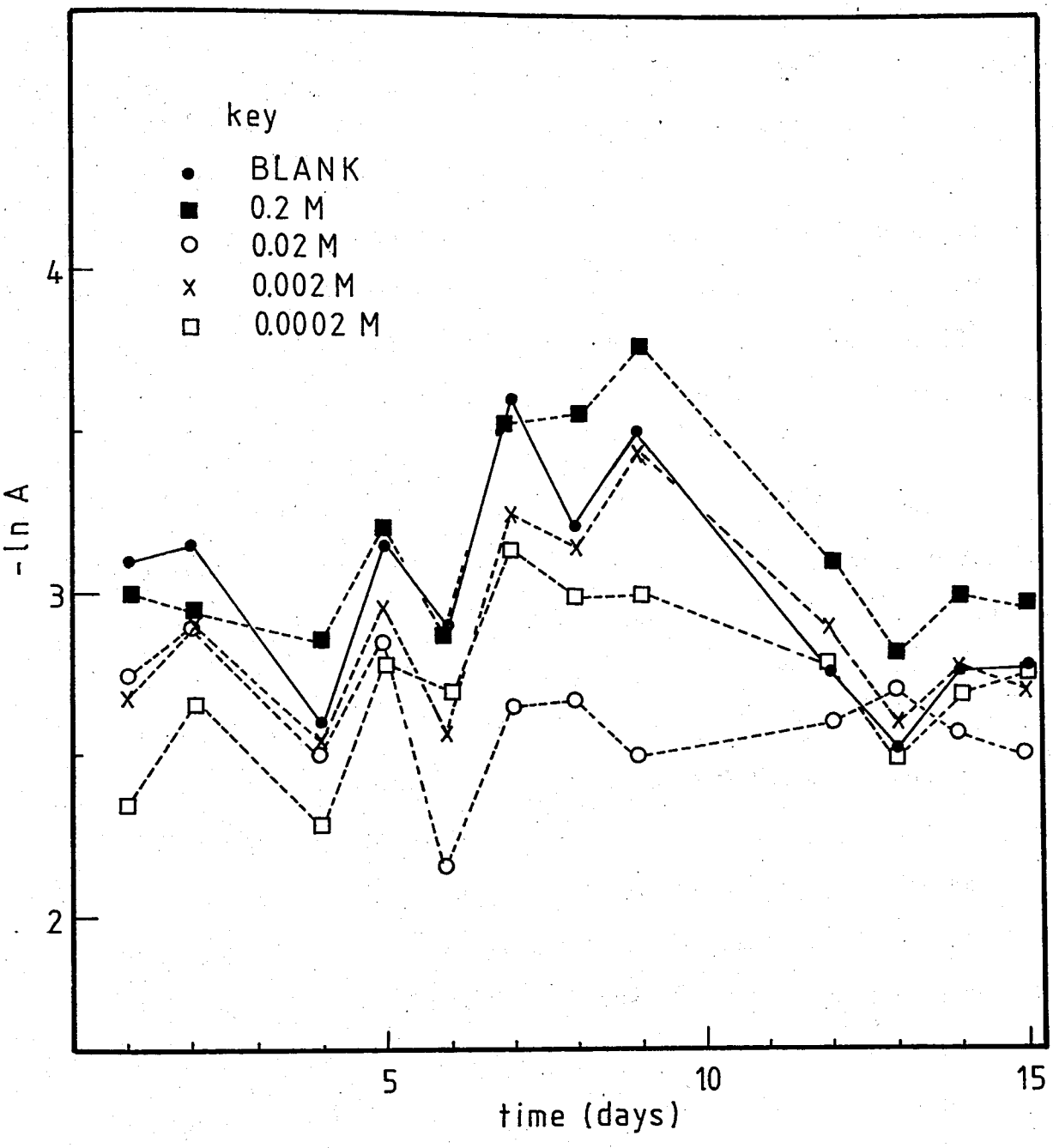

Figure 3.3.1 Effect of different concentrations of Cd metal on Gloeocapsa.

Each point is the average of four sets.

cadmium are below the curve for the blank upto a certain day, but go above it after this day. 0.2 M cadmium solution inhibited upto the fourth day and started stimulating on the fifth day whereas the other concentrations started stimulating on the 12^{th} day. This shows that all concentrations of cadmium except, 0.2 M showed inhitory effect on the growth upto the 12^{th} day, but this inhibition stopped and the metal turned out to be stimulatory after the 12^{th} day. However, 0.2 M cadmium solution had the most stimulatory effect all throughout the experiment.

No inhibition was observed with mercury. Results obtained for different concentrations of mercury are plotted in Figure 3.3.2. As it can be seen from the graph, the curves for all different concentrations of mercury are above the curve for the blank which is an indication of stimulation by the metal. By looking at the graph, one can say that mercury, when present at a 0.0002 M concentration seems to have the greatest stimulation.

Zinc, on the other hand, showed inhibitory and stimulatory effects at the same time. Results for different concentrations of zinc are plotted in Figure 3.3.3. Curves for all concentrations tested are below the curve for the blank upto the 12^{th} day, but they become similar to it after this except 0.2 M zinc solution. This shows that when zinc is present in these amounts, it inhibits the growth upto the 12^{th} day, but inhibition stops after the 12^{th} day, except for 0.2 M zinc solution. 0.2 M zinc solution however, always inhibits the growth. 0.2 M zinc solution seems to have inhibited the least upto the eighth day, and after the ninth day, 0.2 M zinc solution inhibits the most, as expected.

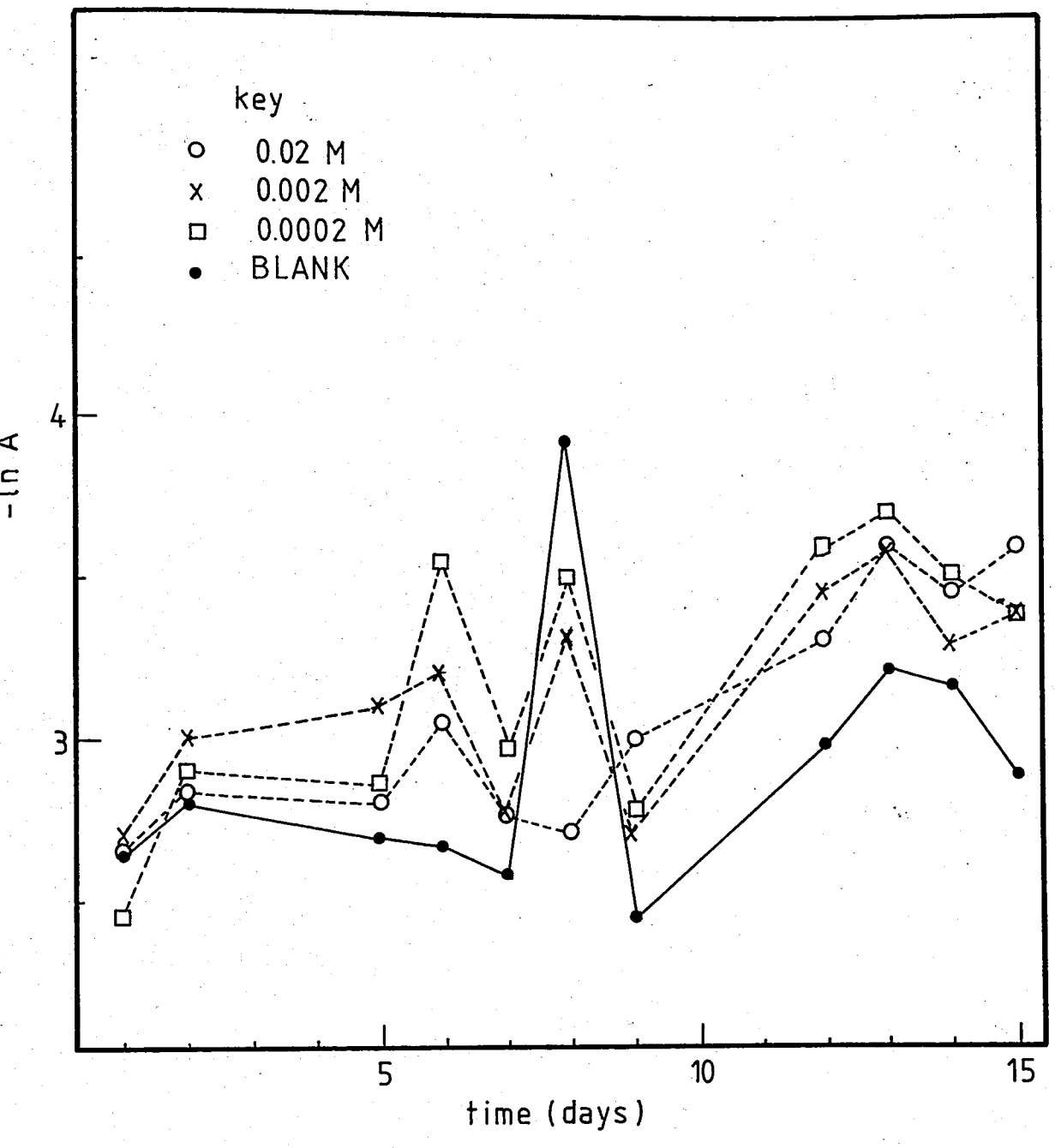

Figure 3.3.2 Effect of different concentrations of Hg metal on Gloeocapsa.

Each point is the average of four sets.

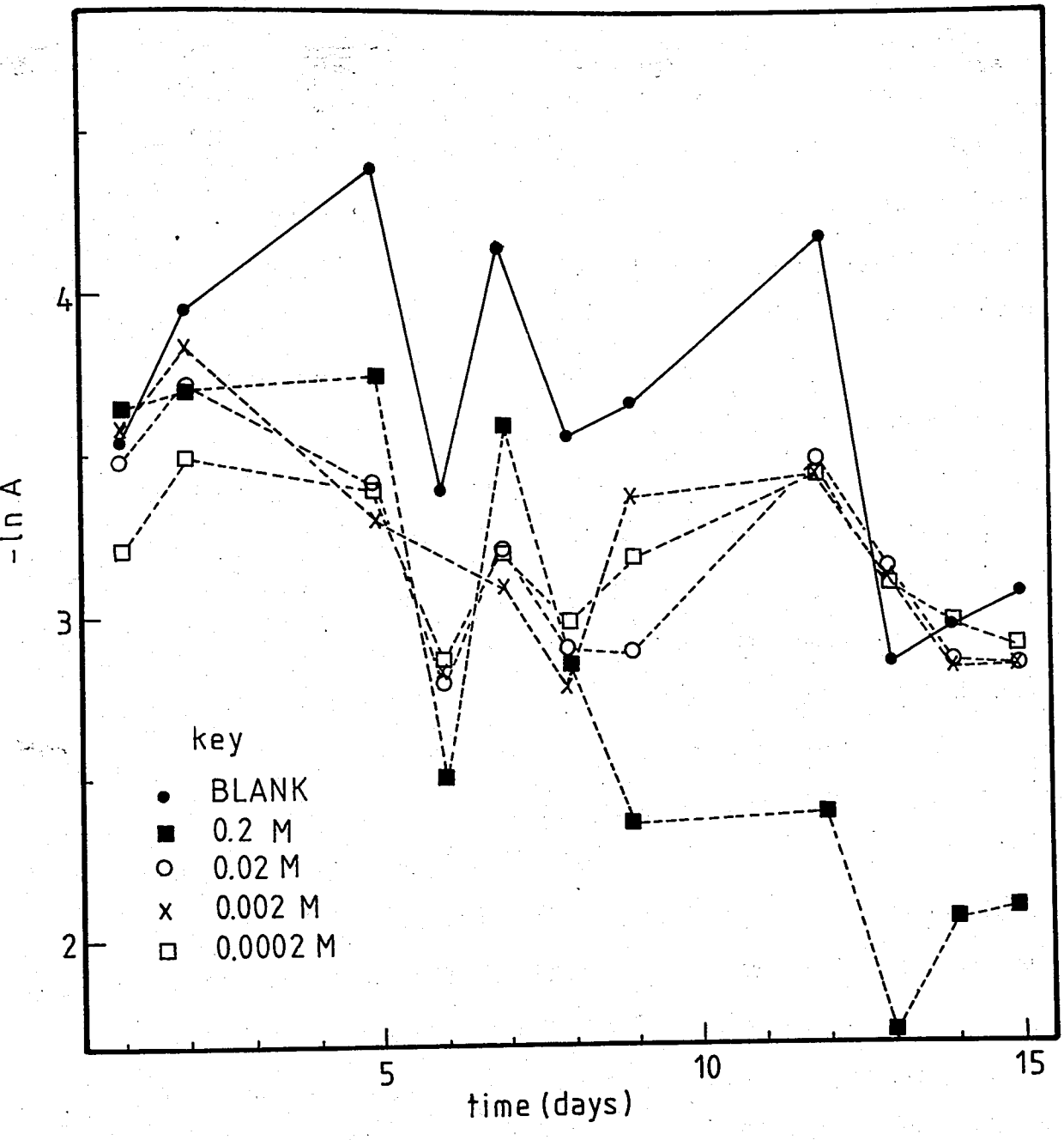

Figure 3.3.3 Effect of different concentrations of Zn metal on Gleocapsa.

Each point is the average of four sets.

Besides absorbance values cell count was determined for the growth of *Gloeocapsa* in the presence of mercury and zinc metals. Even though there are no cell counts for any of the metals on *Anabaena flos aquae*, results using cell counts are quite similar to those obtained using ln A values. For mercury and zinc in *Gloeocapsa* it should be pointed out that even though there are data of cell counts starting with *Gloeocapsa*, there are no data available for cadmium on *Gloeocapsa* because of experimental reasons.

Results using cell counts for mercury are shown in Figure 3.3.4, when compared with the blank it is observed that there is an increase in cell number, 0.02 M mercury solution gives a curve which is very similar to that of the blank with very slight inhibition, though. However, the curves for the other two concentrations, mainly for 0.002 M and 0.0002 M mercury solutions are above the blank curve which again shows that there is stimulation of growth. 0.0002 M mercury solution seems to be stimulating more which is also true for the results obtained using ln A values.

Results again using cell count for zinc are shown in Figure 3.3.5. 0.2 M zinc solution inhibited the growth which is also true for ln A case. Curves for 0.02 M and 0.002 M zinc solutions are below the blank curve upto the 12^{th} and seventh days, respectively, but then they go above the blank. This shows that 0.02 M and 0.002 M zinc solutions inhibit the growth up to a certain day, and then start stimulating it. Curve for 0.0002 M zinc solution is always above the blank curve. So this implies that the metal stimulates when it is present at a 0.0002 M concentration.

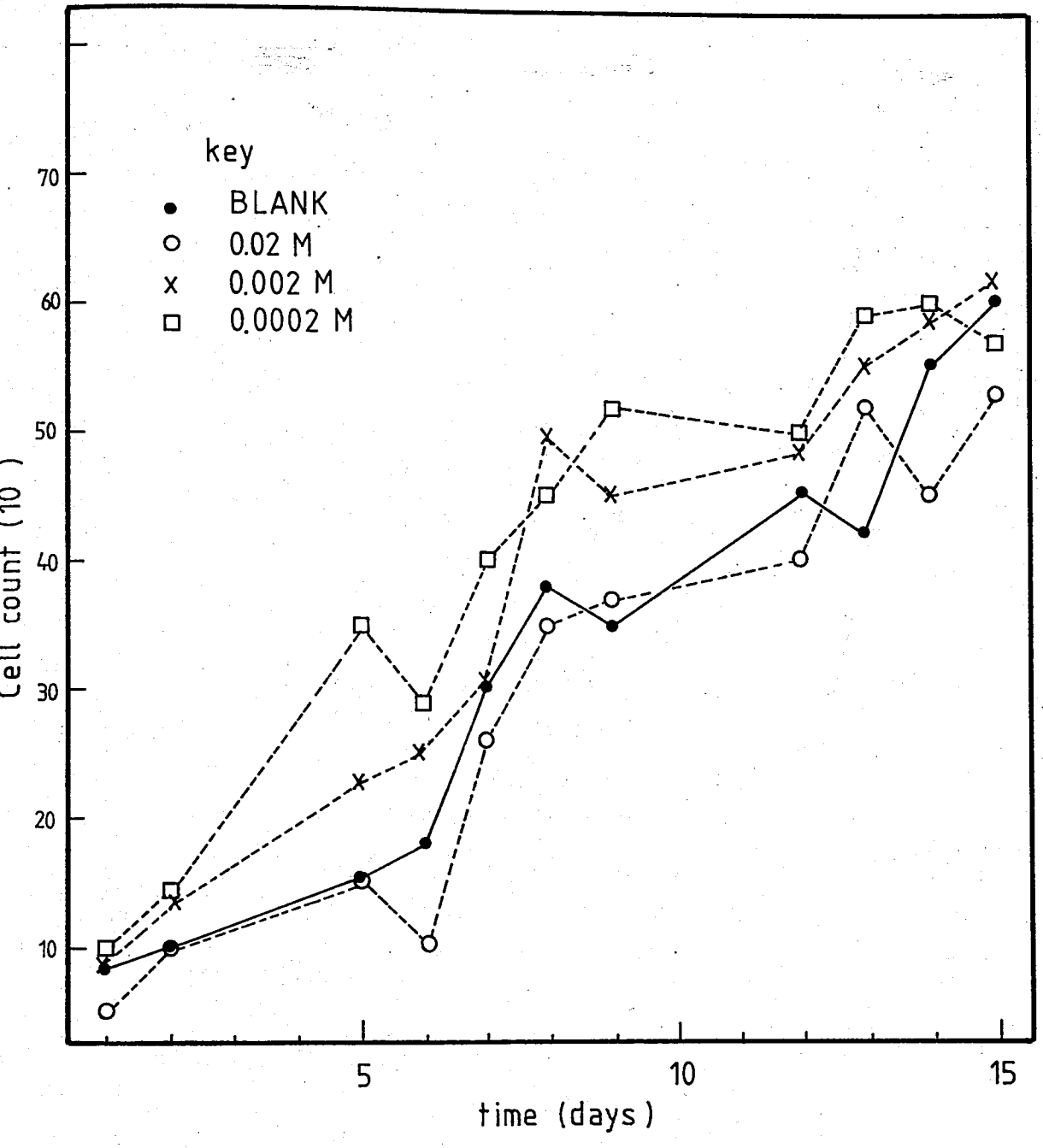

Figure 3.3.4 Effect of different concentrations of Hg metal on Gloeocapsa.

Each point is the average of two sets.

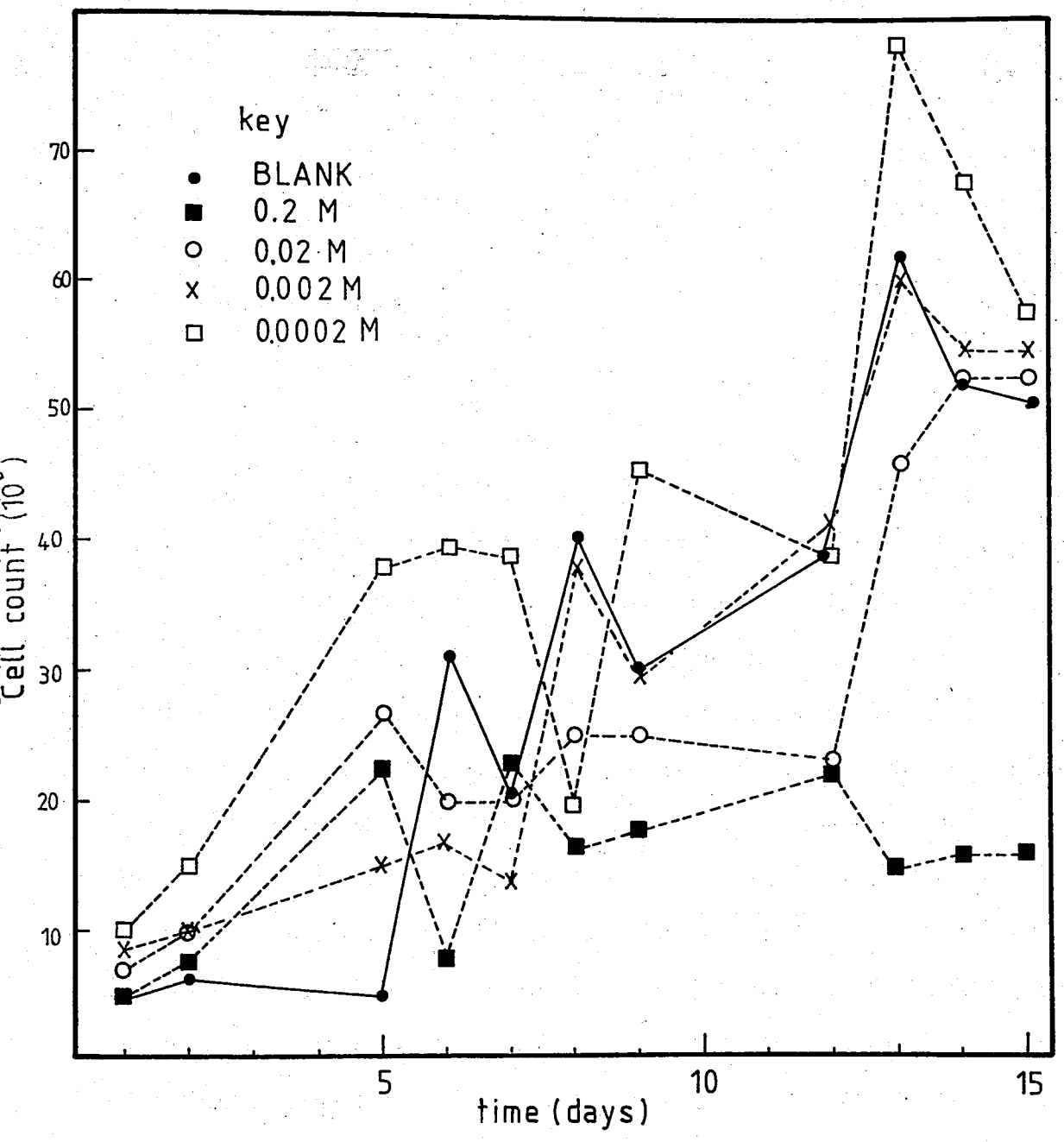

Figure 3.3.5 Effect of different concentrations of Zn metal on Gloeocapsa.

Each point is the average of two sets.

3.4 EVALUATION OF THE DATA FOR *ANABAENA FLOS AQUAE*

In addition to ln A values and cell counts, results were also compared using specific growth rates. Specific growth rates (μ) were determined by substituting growth measurements (absorbance or cell count) in the formula [28]

$$\mu = \frac{\ln(x_1/x_0)}{t_1 - t_0}$$

where x_0 = growth measurement at time t_0

x_1 = growth measurement at time t_1.

In spite of a few contradictions, results obtained using specific growth rates are quite similar to those using ln A and cell count. For instance, when the specific growth rates of cadmium on *Anabaena flos aquae* (Table 3.4.1*) are considered, it can be observed that specific growth rates for different concentrations between the 15^{th} and 16^{th} days are all higher than the specific growth rate of the blank and this shows that there is stimulation after the 15^{th} day. 0.0002 M cadmium solution has the highest specific growth rate, 0.002 M cadmium solution next highest and so on. All of these are quite valid for ln A case because the curves for different concentrations of cadmium were in the same order as the specific growth rate values.

When the specific growth rates of mercury are considered, (Table 3.4.2), it is observed that all the specific growth rates for different concentrations are lower than that of the blank between the 12^{th} and ninth days. However, the growth rates increase for all concentrations between the 12^{th} and 13^{th} days. This shows that there is

*All the values in the tables are the average of four values except the last two tables.

Table 3.4.1 - Specific growth rates using absorbance values. Cadmium metal on Anabaena flos aquae.

Day	Blank	0.2 M Cd	0.02 M Cd	0.002 M Cd	0.0002 M Cd
5	-0.0451	-0.0742	0.0876	0.1166	0.1525
6	0.0305	0.3512	0.1670	0.0689	0.0016
7	-0.0314	-0.0727	0.0458	-0.0073	-0.1474
8	0.4612	0.1149	-0.0311	-0.0813	0.0089
9	-0.1193	0.0824	0.0252	-0.0086	-0.1382
12	0.1444	0.1527	0.1596	0.2231	0.4467
13	0.0506	0.1941	-0.0011	-0.1206	-0.0032
14	0.0023	0.0095	-0.4394	0.1195	-0.0722
15	-0.1378	-0.0186	0.0831	0.1181	0.4127
16					
μ_{max} =	0.4612	0.3512	0.1670	0.2231	0.4467

Table 3.4.2 - Specific growth rates using absorbance values. Mercury metal on Anabaena flos aquae.

Day	Blank	0.02 M Hg	0.002 M Hg	0.0002 M Hg
5				
	-0.1531	-0.4585	-0.3572	-0.2148
6				
	-0.1062	-0.6001	-0.5611	0.1137
7				
	-0.2180	0.4155	-0.3252	0.2917
8				
	-0.2624	-0.1076	0.0923	-0.2654
9				
	0.1319	0.0737	-0.1018	0.0691
12				
	0.0824	0.3147	0.1807	0.1136
13				
	0.1947	-0.1948	0.0427	0.2252
14				
	-0.1241	0.0805	0.0509	0.0600
15				
	0.0209	0.1774	0.4981	0.3396
16				
μ_{max} =	0.1947	0.4155	0.4981	0.3396

inhibition by all concentrations of mercury upto the 12^{th} day, but this inhibition stops and even stimulation starts after this day. These results are again consistent with the ones obtained with ln A values. In ln A case, inhibition was observed for all concentrations upto the 12^{th} day, and curves had become similar to that of the blank after this day.

Specific growth rates of zinc metal are given in Table 3.4.3. It should be pointed out that no general trend is observed with the results obtained from specific growth rates. The growth rates are sometimes higher and sometimes lower than the blank. Considering ln A values, all the concentrations had toxic effects compared to the blank 0.2 M zinc solution was the most inhibitory and 0.0002 M zinc solution the least inhibitory, but such a regular trend is not observed in specific growth rates.

3.5 EVALUATION OF THE DATA FOR *GLOEOCAPSA*

Specific growth rates were also calculated for *Gloeocapsa*, using absorbance and cell counts separately. Growth rates for cadmium are given in Table 3.5.1. By looking at the specific growth rates, one observes that the rate for 0.2 M cadmium solution is lower than the blank between the fifth and sixth days, but is higher between the sixth and seventh days.

Specific growth rates for other concentrations are found to increase after the 14^{th} day. This shows that 0.2 M cadmium inhibits the growth upto the sixth day and then starts stimulating on the seventh day. Other concentrations start stimulating after the 14^{th}

Table 3.4.3 - Specific growth rates using absorbance values. Zinc metal on Anabaena flos aquae.

Day	Blank	0.2 M Zn	0.02 M Zn	0.002 M Zn	0.0002 M Zn
5	-	0.1042	-0.4427	0.0167	-0.2846
6	0.0541	0.3931	0.2119	0.3067	0.6197
7	0.3825	0.0927	0.0967	0.1955	0.4015
8	-0.0191	0.0695	0.1060	0.0221	-0.1258
9	0.0508	0.0343	0.0864	0.0577	0.0732
12	-0.0042	-0.0204	-0.0467	-0.0374	-0.0796
13	-0.0951	-0.2757	-0.2974	-0.4691	-0.7672
14	0.2739	0.3175	0.4609	0.6467	0.9199
15	-0.0195	-0.0010	0.0155	0.0884	0.1387
16					
μ_{max} =	0.3825	0.3931	0.4609	0.6467	0.9199

Table 3.5.1 - Specific growth rates using absorbance values. Cadmium metal on Gloeocapsa.

Day	Blank	0.2 M Cd	0.02 M Cd	0.002 M Cd	0.0002 M Cd
1	0.0879	0.0306	-0.1045	-0.2236	-0.3289
2	-0.0590	-0.0711	0.0214	-0.0009	-0.0491
5	0.4510	0.3839	0.8477	0.4492	0.1679
6	-1.1055	-1.0148	-0.5083	-1.2066	-0.9572
7	0.0416	-0.0125	0.0766	0.1205	0.0531
8	0.0948	0.3578	0.0638	0.0374	-0.0203
9	0.2165	0.1558	0.0288	0.1322	0.1737
12	0.1254	0.1519	0.0633	0.1233	0.0857
13	-0.1268	-0.0957	-0.0399	0.0237	0.0401
14	0.0031	0.0847	0.0823	0.0478	-0.0474
15					
μ_{max} =	0.4510	0.3839	0.8477	0.4492	0.1737

day. These results are quite consistent with ln A case because results obtained using ln A values showed that 0.2 M cadmium stimulated after the fifth day and other concentrations stimulated after the 12^{th} day. So both of these results show that 0.2 M cadmium solution inhibits at the very beginning, but it then stimulates whereas others start stimulating after a longer time.

Logical results are obtained for mercury only when maximum specific growth rates are considered rather than specific growth rates (Table 3.5.2). All the maximum growth rates are higher than the maximum growth rate for the blank except for 0.02 M mercury solution. This shows that mercury when present at 0.002 M and 0.0002 M concentrations respectively, does not inhibit the growth, but in fact stimulates it. When the results obtained using growth rates are compared with the ones using ln A, it is found out that they are in good agreement with each other except for 0.02 M mercury solution. In ln A case, no inhibition was observed for any of the concentrations. The only contradiction,though, comes from the 0.2M solution. The maximum specific growth rate for the 0.02 M solution is lower than the blank, but it is not found to be inhibitory in the ln A case.

Specific growth rates for zinc on Gloeocapsa are listed in Table 3.5.3. Comparing maximum specific growth rates rather than specific growth rates, one observes that the maximum specific growth rates for all concentrations are higher than the blank except for 0.2 M zinc solution. This shows that there is inhibition by all concentrations except for 0.2 M zinc solution. This is consistent and contradictory at the same time with ln A values. Lower maximum specific growth rates

Table 3.5.2 - Specific growth rates using absorbance values. Mercury metal on Gloeocapsa.

Day	Blank	0.02 M Hg	0.002 M Hg	0.0002 M Hg
1	-0.0779	-0.1282	-0.2065	-0.0865
2	0.0164	-0.0203	-0.0837	-0.0029
5	-0.0744	-0.2467	-0.0992	-0.6639
6	0.1206	0.2983	0.4315	0.5710
7	-0.3061	0.1198	-0.4291	-0.6453
8	0.4799	-0.2832	0.5807	0.7419
9	-0.2261	-0.1273	-0.2077	0.2310
12	-0.1469	-0.2929	-0.1629	-0.0934
13	0.0371	0.1439	0.3298	0.1890
14	0.0997	0.1224	-0.1851	0.0991
15	0.0924	0.0820	-0.2203	0.1080
16				
μ_{max} =	0.4799	0.2983	0.5807	0.7419

Table 3.5.3 - Specific growth rates using absorbance values. Zinc metal on Gloeocapsa.

Day	Blank	0.2 M Zn	0.02 M Zn	0.002 M Zn	0.0002 M Zn
1	-0.1318	0.1266	-0.1697	-0.2334	-0.2005
2	-0.2757	-0.0884	0.0919	0.1199	0.0507
5	1.1181	1.2405	0.5866	0.5074	0.5192
6	-0.4240	-0.7683	-0.2969	-0.2276	-0.2045
7	0.4672	0.7403	0.2821	0.3139	0.2004
8	-0.3114	0.1248	0.0845	-0.1437	0.0182
9	-0.1719	-0.0223	-0.1871	-0.0555	-0.0762
12	0.2645	0.2341	0.3841	0.4709	0.3535
13	0.2292	0.2107	0.2714	0.3598	0.2698
14	-0.0950	-0.0399	0.0100	-0.0078	0.0542
15	-0.0378	0.0010	-0.0353	0.0620	0.0623
16					
μ_{max} =	1.1188	1.2405	0.5866	0.5074	0.5192

imply that there is inhibition which is also true for ln A case. Also ln A curves and specific growth rates are quite close to blank between the 12^{th} and 13^{th} days. However, 0.2 M solution is found to be inhibitory in ln A case, but it has stimulatory effect as far as maximum specific growth rates are considered.

Specific growth rates were also calculated using cell counts wherever cell counts were available. For instance Table 3.5.4 shows the specific growth rates using cell counts for mercury on *Gloeocapsa*. Results are not very consistent with cell count curves, though comparing maximum specific growth rates, 0.02 M mercury solution has a maximum specific growth rate higher than the blank, 0.002 M solution equal to the blank and 0.0002 M solution lower than blank. According to these results 0.02 M mercury solution seems to be stimulatory and 0.0002 M solution seems to be inhibitory and 0.002 M solution is not affected at all. However, cell count curve for 0.02 M solution was close to the blank with slight inhibition and the cell count curves for other concentrations were above the blank curve.

Specific growth rates using cell counts for zinc on *Gloeocapsa* are given in Table 3.5.5. Maximum specific growth rates for all concentrations of zinc are lower than the maximum specific growth rate for the blank. Comparing the results with the cell count curves, one sees that they are quite consistent except 0.0002 M solution. The curve for the 0.02 M solution is below the blank curve upto the 12^{th} day, but it goes above the blank after the 12^{th} day. Specific growth rates for 0.02 M solution are higher than the blank between the 12^{th} and 13^{th} days. Curve for 0.002 M solution is below the blank upto the seventh day, but is above afterwards.

Table 3.5.4 - Specific growth rates using cell counts. Mercury metal on Gloeocapsa.

Day	Blank	0.02 M Hg	0.002 M Hg	0.0002 M Hg
1	0.2231	0.6931	0.4855	0.3365
2	0.1351	0.1351	0.1902	0.3054
5	0.1823	-0.4055	0.0834	-0.1880
6	0.5110	0.9555	0.1823	0.3216
7	0.2364	0.2972	0.5110	0.1178
8	-0.0822	0.0556	-0.1054	0.1636
9	0.0838	0.0260	0.0284	-0.0194
12	-0.0689	0.2624	0.1155	0.1655
13	0.2696	-0.1446	0.0702	0.0168
14	0.0870	0.1636	0.0496	0.0513
15				
μ_{max} =	0.5110	0.9555	0.5110	0.3365

Table 3.5.5 - Specific growth rates using cell counts. Zinc metal on Gloecapsa.

Day	Blank	0.2 M Zn	0.02 M Zn	0.002 M Zn	0.0002 M Zn
1	0.2027	0.3789	0.2499	0.2154	0.2887
2	0.1351	0.2169	0.1959	0.1095	0.2310
5	0	-1.0560	0.2877	0.2744	0.2744
6	-0.3965	1.0805	0.4852	-0.0265	-0.0620
7	1.2910	-0.5232	0.2744	0.8044	-0.2231
8	-0.2549	0.1178	0.1133	-0.2364	0.8329
9	-0.0717	0.0669	-0.0378	-0.0744	-0.1315
12	0.1568	-0.3829	0.8329	0.0504	0.5534
13	0.2423	0.1252	0.2144	0.0938	-0.0253
14	0.1624	0.0345	-0.0291	-0.0115	-0.1545
15	-	-	-	-	-
16					
μ_{max} =	1.2910	1.0805	0.8329	0.8044	0.8329

CHAPTER IV
DISCUSSION

IV. DISCUSSION

Of these three metals tested, zinc was found to be the most toxic. This can be inferred from the fact that there is inhibition for all concentrations of zinc whereas there is inhibition for only one concentration of cadmium all throughout the experiment and inhibition for all concentrations of mercury upto the 12^{th} day, and stimulation after the 12^{th} day. The order of toxicity for *Anabaena flos aquae* is as follows.

Zn > Hg > Cd
(the most toxic)

of the three metals tested on *Gloeocapsa*, mercury was found to be the least toxic.

Zn ~ Cd > Hg

No inhibition was observed for the different concentrations of mercury. Cadmium and zinc are almost similar in toxicity strength with zinc being a little more toxic. Since there is no inhibition by any of the concentrations of mercury it can be considered the least toxic. Comparison of cadmium and zinc shows that both metals inhibit at the different concentrations upto the 12^{th} day, but the difference in

toxicities comes from the 0.2 M solution. Even though there is inhibition upto the 12^{th} day for both metals, 0.2 M cadmium solution stimulates all throughout the experiment whereas 0.2 M zinc solution inhibits all throughout the experiment. This is why zinc is a little more toxic than cadmium.

However, it should be pointed out that the order of toxicity for both species is based on different concentrations used, but excluding 0.2 M mercury solution. This specific concentration had to be omitted because of solubility problem. Preparation of a stock solution from which 0.2 M solution would be prepared required large amounts of water and chemical which was thought to be not very practical. If 0.2 M mercury solution had been tested, slightly different results might have been obtained.

The results obtained are in quite good agreement with the results of studies done so far, but of course there are slight variations due to different species and different metals used. It is really hard to obtain exactly the same results because different species react differently to the same metal.

In spite of all these differences in species zinc was found to be the most toxic for both species. Order of toxicity for *Anabaena flos aquae*: (most toxic) Zn > Hg > Cd. Order of toxicity for *Gloeocapsa*: Zn > Cd > Hg (least toxic).

Previous studies show that on an average, the relative growth rates of the algae tested decreased upon increased zinc content in the sea water [15], reduced relative growth rate being observed at and above 50, 250, and 25000 µg/L zinc for *Skeletonema costatum, Thalassiosira*

pseudonana and *Phaeodactylum tricornutum*. The effect of zinc on the algae becomes clearer when not only the growth rate in the early exponential phase, but also the total development of the cultures are considered. The long term effect of the exposure to increased levels of zinc was, therefore, more serious than that expressed by the growth rate.

Another study also shows that zinc is accumulated in the greatest amount in a freshwater algae [18]. Mean metal concentrations, in the algae are given as:

$$\underline{Zn} > Pb > Fe > Ni > Cu$$

for the blooms.

Mercury was found to be of medium strength in *Anabaena flos aquae*, but the least toxic in *Gloeocapsa*. Mercury inhibited the growth of *Anabaena flos aquae* upto the 12^{th} day, but then started stimulating it, but it did not inhibit the growth of *Gloeocapsa* at all. Inhibition upto a certain day and then stimulation is observed for certain concentrations of metals all throughout the experiment. This might be explained by the fact that growth reaches a maximum around the 10^{th} day and then stays constant. So if the metal is to have a stimulatory effect on the growth, it starts affecting the growth when it is considered as constant.

The reason why mercury is of medium strength in toxicity might be related to its volatility. Since it is volatile, it might have evaporated and the amount of actual metal ion to be tested might have been reduced.

Cadmium was found to be the least toxic for *Anabaena flos aquae* whereas it was quite toxic (almost of equal toxicity as zinc) for *Gloeocapsa*. Cadmium is found to be less toxic in the presence of sodium chloride [5] but this concept can not be used to explain the toxicity of cadmium in this experiment since the medium for *Anabaena flos aquae* and also for *Gloeocapsa* contained appreciable amounts of sodium chloride. This different behaviour is again related to the fact that certain species are more tolerant to certain metals [16]. This shows that *Anabaena flos aquae* was more tolerant to cadmium compared to *Gloeocapsa*.

Although some conclusions can be made based on the results of individual metals used, the effect of a mixture of these metals might have also been investigated because the effect of the same metal might be very different when used together with other metals. Certain metals might have antagonistic or synergistic effects when used together. For instance when cadmium and nickel are used in combination, they seem to have interacted antagonistically as the stimulation of growth at lower concentrations was more and the toxicity was reduced at higher concentrations when compared to their individual effects [16].

Experiments could have also been carried out to determine the effect of the metal ions not only added as a mixture, but also in some order because order of metal addition is also found to be an important factor [23].

Interestingly enough, when cadmium was added prior to mercury, marked antagonism was observed between the two metals as judged by all growth criteria [23]. This effect might be explained by a competition for binding sites. So metal combination (mercury and cadmium in the

same order) might have been tried on both *Anabaena flos aquae* and *Gloeocapsa* and results might have been compared. Whether or not the same results will be obtained, is of course, not predetermined, but is open to further research and investigation.

Effect of different metals might have also been determined for different pH ranges and temperatures, but of course this requires more sensitive experimental techniques and might be more difficult.

Based on these studies, several important conclusions can be made. Firstly, studies on metal interactions should take into account the fact that the response obtained depends on the order of metal addition. Conclusions are better if a mixture of metals is considered. Secondly, the test criterion chosen is very important. In order for studies to be conclusive, metal combinations should be tested for their toxicity towards as many criteria as possible. Thirdly, the concentration of metals used and the species chosen must be given importance. In other words, it should be emphasized that different species will behave differently in the presence of a certain metal or a combination of metals.

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