INFLUENCE OF NUTRIENTS ON THE GROWTH AND PHYSIOLOGY OF BLUE-GREEN ALGAE AND COLIFORM BACTERIA (IN A CLOSED LAKE WATER)

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CERTIFICATE

This dissertation entitled "Influence of nutrients on the growth and physiology of blue-green algae and coliform bacteria in a closed lake water" embodies the work carried out at the School of Life Sciences, Jawaharlal Nehru University, New Delhi. This work has not been submitted in part or in full for any degree or diploma of any university.

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CONTENTS

INTRODUCTION

"Waste is Pollution"

Man has been polluting his environment since he began to live in large settlements, to burn fuels, and to apply technology to the needs of city dwellers. The process accelerated during the Industrial and Agricultural Revolution with the introduction of steam power for factories, with greater concentrations of population in the manufacturing centres and large scale application of chemical fertilizers to the soils. The number of variety of pollutants also increased markedly with the development of modern chemical technology in the last century.

Lake and River waters are subject to physical, chemical and biological processes that will eventually result in their extinction. These processes, however, are being accelerated by human activities. Water can purify itself upto a point, by natural processes, but there is limit to the pollution load that a lake can handle. Self purification of lake water, a complicated process, is brought about by a combination of physical, chemical and biological factors. The process is the same in all bodies of water, but its intensity is governed by varying environmental conditions.

The natural aging of a lake results from a process called 'Eutrophication', which means biological enrichment

of its waters. A new formed lake begins on a body of cold, clear, non-smelling and nearly sterile water. Gradually streams from its drainage basin bring-in nutrient substances, such as phosphorous and nitrogen, and the lake water's increasing fertility gives rise to an accumulating growth of aquatic organisms, plants, animals and microorganisms. As the living matter increases and organic deposits pile up on the lake bottom the lake becomes smaller and shallower, its water become warmer, plants take root in the bottom and gradually take over more and more space and their remains accelerate the filling of the basin. Eventually the lake becomes a marsh, is over run by vegetation from the surrounding area and thus disappears.

The new campus of Jawaharlal Nehru University shows evidence of human habitation dating back to 14th and 15th century. Except for the Mehrauli village nearby area around the campus upto Palam village does not show evidence of continuous human habitation since the period referred above and upto mid-fifties of the present century. We can treat this area as virtually untouched by civilization. In this back ground human population is entering the area for the first time. Past five years evidence shows that with human habitation in this area, the flora

is undergoing a change through chance and systematic introduction. Increased vegetation is leading to diversity in the fauna as well.

This visible biological change on the New Campus may also in due course of time lead to a change in the microflora. I am not aware of any systematic record of the dynamics of ecological change in water consequent to human introduction.

The present study aims to study the blue-green algae and pathogenic microflora of a lake water on the J.N.U. Campus, in relation to physical and chemical changes of water. There is evidence to suggest that biological components play an important but as yet poorly understood role in regulation in aquatic environment.

An introduction to the study of certain aspects of the biology of lake during one season does not intend to be in any sense conclusive. It is expected that this proliminary work will throw some light on the seasonal and bigger cycles in nature and explore the effect of introduction factor and this work could be expanded in future on larger scales for critical biological studies.

REVIEW LITERATURE

The recent concern over environmental deterioration has spread from professional ecologists to wider public. A knowledge, which is as accurate as possible of microbial ecology is indispensible for ecosystem research and environmental protection. This is particularly true for lake waters, wherby pathogenic bacteria and blue-green algae (cyanobacteria) occupy a special position (Dykyjova and Kvet, 1978). Phytoplankton are the living autotrophic which float in water and are primary producers of organic matter in aquatic habitats. The phytoplanktons thus stand on the baseline of food webs in aquatic environments and in inturn dependent on the activities of other microbial organisms mainly bacteria which convert organic material into inorganic nutrients required by phytoplanktons and plants. In sea, Diatoms or Dinoflagellates are the more obvious representatives of the phytoplankton, in terms of both the cell size and availability when water samples are examined under lowpowered microscope. Certain blue-green algae also give rise to periodic summer blooms in fresh water lakes in tropical countries. The members of this group are distinguished from all other algae in being prokaryote, in common with bacteria i.e. the cells are characterized by the absence of organized nuclei, lacking nuclear membranes and chromosomes, they are however, capable of genetic replication.

Planktonic blue-green algae are either unicellular, colonial and filamentous in habit. Both the cell, colonies and the filaments cause extensive 'blooms' under certain fresh water conditions. Filamentous blue-green algae possess specialized cells called 'heterocyst', these are thought to be concerned with nitrogen fixation Nielsen and MacDonald,(1978a,b). The cells of planktonic blue-green algae contain conspicuous gas vacuoles presumably as aid to floatation.

Phytoplanktons make varying contributions to primary productivity depending on their abundance at the time of measurement. The contribution to energy turnover on the earth is considerable. Annual production of all plant life is estimated as 100×10^8 metric tons of fixed carbon. The major contribution to this organic matter production comes from phytoplankton. The oxygen liberated by phytoplankton photosynthesis via vital part of the life "Life support" system on the earth. Whilst production is measured as fixed carbon, the essential role of phyto-plankton in food web is to supply proteins, carbohydrates, fats, vitamins and mineral salt to primary consumers.

The chloroplast in a phytoplankton cells traps and utilize light energy to convert carbon dioxide to carbo-hydrate. Of the phytoplankton organisms only the blue-

green algae lack discrete cells organelles of a chloroplasts per cell. Chlorophyll a is the one pigment common to all phytoplankton. In all chlorophyll a is the primary pigment involved in photosynthesis and is the one way by which the radiation energy is converted to chemical energy.

Many authors have commented that blue-green algae are more prominent in the plankton of tropical fresh waters than in temperate ones (Fritsch, 1907a,b; Singh, 1955). It is not yet clear to what extent this is a direct effect of the higher temperatures or an indirect one associated with chemical differences in the waters. Most of the genera and many species are the same as in temperate regions, but there are some differences. The genera Anabaenopsis and Spirulina have been recorded much more frequently from tropical than temperate waters, while Aphanizomenon is apparently less common. In Desikachary's (1959) flora of India there are many species not recorded from temperate waters, while Oscillatoria redekei, relatively common in the latter, is not listed. Singh (1955) made an attempt to draw up a list of bloom-forming species in Indian fresh waters. These are in decreasing orders of abundance: Microcystis aeruginosa, M. flos-aquae, anabaenopsis circularis, A. arnoldii, A. milleri, A. raciborskii, Raphidiopsis indica, Anabaena aphanizomenoides, A. spirulinoides, Wollea bharadwajae and species of

Oscillatoria and Spirulina. Hill (1970) reported that among planktonic species spore production is confined largely to those genera some of whose species have been shown to be capable of fixing nitrogen (Anabaena, Aphanizomenon, Gloeotrichia).

Geitler (1932) and Desikachary (1959) indicate that several hundred species of fresh water blue-green algae were described as predominantly planktonic. Nevertheless, the ecological literature on planktonic blue-green algae is concerned largely with only about twenty species, the ones which sometimes form population dense enough to be termed "water blooms".

With the rapid increase of nutrient levels in the fresh waters of the highly populated regions in the world, the nuisance situations have become more and more abundant. It is now well documented that a variety of changes in the environment may tend to favour the growth of planktonic blue-green algae. However, it is much less clear to what extent bloom forming species may be favoured by them in comparison with other planktonic blue-green algae, or of blue-green algae as a whole in comparison with other plankton algae.

One obvious factor which will lead to selection for particular species is the availability of combined nitrogen.

In situation where the level of combined nitrogen is relatively low as compared with other essential elements like phosphorus. Those species of Anabaena, Aphanizomenon and Gloeotrichia, which can fix nitrogen will be at a selective advantage as compared with Coelosphaerium, Microcystis and Oscillatoria, none of which have been shown to be capable of doing so (Stewart et al., 1968; Horne and Fogg, 1970).

There have also been observations which indicate differences in the ability of bloom-forming species to use different phosphorus sources and levels, although attempts to correlate population densities and environmental phosphate levels are complicated by the ability of these species to accumulate large amount of reserve polyphosphate (Gerloff and Skoog, 1954; Stewart and Alexander, 1971).

Fogg (1969) reported that even low concentrations of inorganic phosphate in artificial media appear to be inhibitory for some blue-green algae such as Gloeotrichia. He suggested that if such algae utilize organic phosphates, or if the decomposition of organic matter yields a continuous supply of inorganic phosphate at a non-inhibitory concentration, the apparent paradox that blooms develop when concentrations of inorganic phosphate are minimal might

be exalained. Studies with seven blue-green algae in media with various sources of phosphorus, showed that Pseudoanabaena sp., Oscillatoria rubescens, Anabaena sp., Oscillatoria agardhi var. isothrix, Aphanocapsa grevillei and Aphanothece sp. grew best with inorganic orthophosphate, with Aphanizomenon preferred phytin.

A direct comparison of the extent to which various organisms in lake Monona, U.S.A. were being influenced by the levels of phosphate and combined nitrogen present was carried out by Fitzgerald (1968, 1969). Phosphate analysis and measurements of alkaline phosphate activity on the components of a mixed bloom of Microcystis sp. and Anabaena sp. occurring indicated that the Microcystis had surplus available phosphorus, where as Anabaena was phosphorus limited.

In natural waters phosphorus occurs in solution in both inorganic and organic forms, in lakes, orthophosphate appear to be the main source. Phytoplankton cells seems able to accumulate phosphorus reserves well in excess of immediate requirements which nutrient levels are high in the so-called phase of 'luxury consumption', and to utilize these reserves during periods of low phosphate concentration in the natural medium. These reserves enable cell growth to continue for some time after the level of nutrients in

the water has been significantly reduced. Organic forms of phosphorus also occur in fresh water, and may serve as a source of the element for some phytoplankton during periods of deficiency. Enzymes able to breakdown organo-phosphorus compounds have been detected in lake waters probably after autolysis of phytoplankton cells.

Filamentous blue-green algae, Calothrix growing in lake make significant contribution to the nitrogen budget by fixation of atmospheric nitrogen. Many of the prominent blue-green representatives of the fresh water phytoplankton lack heterocyst and whilst some planktonic heterocyst bearing species also occur. The view that nitrogen fixation does occur in fresh water lakes is based on indirect evidence from field observations, notably the very high concentrations of nitrogen associated with 'blooms' of blue-green algae compared with that available in combined form in water.

Phosphate is an absolute necessity for life, yet it is primary concern to-day as a water pollutant when it is a growth limiting nutritional factor, because excessive amounts which have been deposited in the environment promote excessive growths of algal cells in lakes. Water pollutants for the most part are chemicals dissolved or suspended in water. Some pollutants are physical factors

and not chemicals, e.g. heat, radiation are physical factors and a marked effect on biochemical reactions.

Many investigators believed that nitrogen and phosphorus are the principal limiting nutrients which govern algal growth in most lakes. This is apparently because agricultural run-off water and treated sewage have a comparatively high content of these two nutrients. Provasoli (1969) has reviewed algal nutrition in relation to "eutrophication" and pointed out that agricultural run-off and sewage effluents also add sodium potassium and other trace metal ions in the water. Dugan (1972) has shown that filamentous blue-green algae grew at the bottom mud in water surface underlow dissolved oxygen (< 3 ppm) at 15°C with minimal light. The filament break, loose, en masse and tend to rise to the surface, giving the appearance of a sudden bloom.

Dugan et al. (1970) and Küentzel (1969) have presented evidence suggestive of CO_2 stimulating the growth of bacterial blooms. The role of bacteria could be one of providing CO_2, vitamins, phosphate, amino acids, organic acid and other stimulatory nutrients, or bacteria could be removing an algal inhibitor or triggering release of a bottom growth of algae. Algae are known to photo-assimilate 18 to 32 per cent of their cell weight from organic compounds such as acetate,

glucose, amino acids, urea, casein and certain other compounds which could originate from pollution or from excretory product by bacteria and other organisms in the lake. Once an algal bloom has formed, the algae are also capable of excreting substantial amounts of amino acids, peptides and polysaccharides into the surrounding water (Holm-Hansen, 1968).

Most immediate effects of blooms are production of off tastes and odours in the water, potential clogging of water supplying intake filters, the depletion of oxygen in water as the algal cells are decomposed by aerobic bacteria, and the potential toxicity of metabolic by-products, by some species of blue-green algae e.g. Anabaena. Algal toxins production and its lethal effect on animals has been reviewed by Shilo (1967) and Gorham (1964).

There have been few detailed studies of seasonal changes in the phytoplankton of small lakes in Britain. Preliminary observations on the phytoplankton of the lake (Whitton, 1969) indicated that it has an interesting microflora. The great bulk of the phytoplankton consisted of species from the phyla Myxophyta, Chlorophyta, Bacillariophyta and Cryptophyta. Algae mostly consisted of Oscillatoria redekei, Raphidonema longieta, Nitzschia acicularis and Cryptamonas erosa.

Nielsen and Jørgensen (1968) investigated an 'oligotrophic' and 'eutrophic' lake in Alberta for 14 months to elucidate relationship between physicochemical environment, and the species of composition, seasonal succession, vertical distribution and seasonal cycles of their phytoplankton. One hundred and seven algal species were found in Muir lake and 63 species in Hastings lake. Increases in the numbers of Anabaena flos-aquae accompanied decreases in the numbers of several green algae, viz., Pediastrum and Scenedesmus sp., suggesting antagonistic effect.

Olga and Owens (1976) have reviewed four major factors of the aquatic environment-phosphorus, nitrogen, carbon and light which exert influence on the physiological responses of phytoplankton. It has been very clear that phytoplanktons are subjected to a continually changing environment and must therefore, be able to adopt to short-term e.g. changes as well as changes that are seasonal. The dynamic aspect, especially the lake of equilibrium in the relation of the algae to their environment was brought by Hutchinson (1969). Rao (1953,1955) studied the distribution of algae in a group of six small ponds situated on Hadley common in Southern Hortfordshire and relation to chemical composition of water.

In India, Gonzalves and Joshi (1946) and Ganpati (1940) made an attempt to correlate the distribution and periodicity

of the algae with chemical factors like pH, silica, nitrate, phosphate, oxidizable organic matter, ammonia, C/N ratio, oxygen, CO_2, Iron and total dissolved solid as well as water temperature, air, rainfall and sunshine.

Jayangoudar (1964) studied for one complete year the bio-ecological conditions of a tropical lake called Nuggi Kari lake in Mysore from the view point of regional limnology of South India. The inter-relationship existing between the physicochemical variables and the biological conditions were highlighted.

Seenayya (1972, 1973) has examined the distribution and periodicity of blue-green algae and diatoms in three ponds in Hyderabad. Blue-green algae showed thick growth in ponds having high nitrate content and pH around 9.0. Many of the species appeared in summer. The sustained growth of blue-green enriched the water in oxidizable and nitrogenous organic materials and rendered it more alkaline.

Several investigators e.g. Cairns (1974); Patrick, (1973); Kumar et al. (1974); Rai, 1976, 1978) have studied the algal communities of Ganga river and correlated it with the physicochemical characteristics of water. The blue-green algal communities mostly contained of Oscillatoria, lyngbya, Spirulina, Anabaena and Anacystis.

The greatest danger associated with drinking water is the possibility of its recent contamination by sewage or by human excrement; and the danger of animal pollution. If faecal contamination has occurred sufficiently recently and if among the contributors there are cases or carriers of such infections diseases as enteric fever or dysentery, the water may contain the living organisms of these diseases, and the drinking of such water may result of fresh cases of disease. Although modern bacteriological methods have made it possible to detect these pathogenic bacteria in sewage and sewage effluents, it is not practicable to attempt to isolate them as a routine procedure from samples of drinking water. When pathogenic organisms are present in faeces or sewage they are almost always greatly outnumbered by the normal excremental organisms and these normal intestinal organisms are easier to detect in water. If these organisms are not found in the water it can, in general, be inferred that disease-producing organisms are also absent and the use of normal excremental organisms as an indicator of faecal pollution in itself introduces a margin of safety.

The coliforms include Escherichia coli, E. freundii and Aerobacter aerogenes. As a faecal coliforms do not normally propagate outside the digestive tract, their presence in water is a reliable source of sewage pollution. There are

organisms which are not themselves normally pathogenic but they indicate the probably presence of much less numerous pathogens of faecal origin as Shigella, Salmonella, Proteus, Vibrio, Entamoeba histolytica and virus causing infections hepatitis. The organisms commonly used as indicators of pollution are E. coli and the coliform group as a whole.

Quite apart from the question of their being indicative of faecal pollution, organisms of coliform group as a whole are foreign to water and must atleast be regarded an indicative of pollution in its widest sense. The search for faecal streptococci, of which the most characteristic type is Streptococcus faecalis may well be of value in confirming the faecal nature of pollution in doubtful cases. Faecal streptococci regularly occur in faeces in varying numbers, which are usually considerably smaller than those of E. coli. In water they probably die and disappear at approximately the same rate as E. coli and usually more rapid than other members of the coliform group. When therefore, organisms of coliform group, but not E. coli, are found in a water sample, the finding of faecal stroptococci is important confirmatory evidence of the faecal nature of the pollution.

Anaerobic spore forming organisms of which the most characteristic is Clostridium perfringens, are also

regularly present in faeces, though generally in much smaller numbers than *E. coli*. The spores are capable of surviving in water for a longer time than organisms of the coliform group and usually resist chlorination at the doses normally used in water works practice. The presence of spores of *Cl. perfringens* in a natural waters suggested that faecal contamination has occurred, and their presence in the absence of organisms of the coliform group, suggest that contamination occurred at some remote time.

In India, Health and Sewage disposal department routinely carry out the periodical check of coliform organisms by multiple tube method or membrane, Filteration method. As far as this survey goes only a few systematic study on coliform bacteria has been carried in Indian Lake Water (Varma, 1977). No attempt has been made to correlate it's presence with the physicochemical or biological factors.

MATERIALS AND METHODS

The material used for the present investigation was water samples from a lake, situated on East-North of New Campus of Jawaharlal Nehru University, New Delhi. The lake is surrounded by red stony rocks and it forms a deep depression at the centre of the region. The area surrounding it gently slopes towards the lake (Fig. 1). The rain water that drains into the lake from the surrounding area during monsoon season contains nutritive substances of biological significance, which adds to the fertility of the underneath waterlogged soil. Several types of aquatic vegetation and planktonic algae are seen in abundance in the lake. The water surface is about 2-3 acres in area and maximum depth in the month of August is about 5 meters. While minimum depth is about 2 meters in the summer months (May-June). There is no outlet through which the lake water is driven out. But as the village Masudpur is situated near the lake, its cattles are daily washed in the lake. Also water is used by villagers for bathing, washing and building construction etc.

In order to get a comprehensive data of the conditions throughout the lake, six spots of observations were arbitrarily choosen and were named as spots, I, II, III, IV, V and VI, respectively. The spot I lies at shallow end and spot VI

Fig. 1: A topographical view of the lake. Arrow indicates the Life Sciences building.

Fig. 1

is situated at the deep end of the lake while spots II to V lie along the middle region of the lake.

Water samples were taken from the lake on the following dates: 16th April, 4th May, 25th May, 20th June and 14th August in between 7 A.M. and 8 A.M. Water samples were taken by a water sampler made up of stainless jacket as proposed in standard methods (APHA standard methods, 12th edition). Estimation for pH, conductivity, Mg-hardness, Ca-hardness, temperature and ammonia, nitrite, nitrate, phosphate and sulphate were done just after collection. The results are expressed in parts per million except for pH and conductivity.

PHYSICAL ESTIMATION:

Temperature : Measurement of temperature was done immediately after collection. Temperature was measured with a Clinical thermometer and was noted in ^{o}C.

pH : Measurement of pH was also done on the spot using a portable pH meter (Philips, India).

Electrical conductivity: Measurement of electrical conductivity of the lake water was completed immediately on the spot with a portable direct reading conductivity meter (303 Systronics, India) and was noted as MHO.

Hardness:

Principle : The EDTA titrametric method followed here

for hardness determination (Standard Methods, 12th edition) is based on principle that in alkaline condition EDTA reacts with Calcium and Magnesium to form a soluble chelate complex. Ca and Mg ions develop red colour with eriochrome black T. Under alkaline condition when EDTA is added as a titrant the Ca and Mg divalent ions get complexed resulting in sharp change from red to blue which indicates end point of the reaction. The pH for this titration has to be maintained at 10.0 ± 0.1. At the higher pH i.e. about 12, ion precipitates and only Ca ion remains in the solution. At pH 7.3 Nurex indicator form a pink colour with Ca^{++}, when EDTA is added Ca^{++} gets complexed resulting in a change from pink to purple, which indicates end point of the reaction.

Reagents:

i. Buffer solution - 16.9 gm NH_4Cl was dissolved in 143 ml NH_4OH and added 1.25 gm EDTA salt to obtain sharp change in indicator and diluted to 250 ml.

ii. Inhibitor - 4.5 gm hydroxylamine hydrochloride was dissolved in 100 ml 95% ethyl alkohol.

iii. Eriochrome black T - 0.5 gm dye was mixed with 100 gm NaCl to prepare dry powder.

iv. Nurex indicator - dry powder.

v. Sodium hydroxide 2N - 80 gm NaOH was dissolved and diluted to 1000 ml.

vi. Standard EDTA solution - 3.723 gm EDTA sodium salt was dissolved and diluted to 1000 ml.

vii. Standard calcium solution - 1 gm A.R. grade calcium carbonate was weighed accurately and transferred to 250 ml conical flask, a funnel was placed in the neck of a flask and 1+1 HCl was added till $CaCO_3$ dissolved completely. Then 200 ml distilled water was added and boiled for 20-30 min to expell CO_2, cooled and added methyl red indicator. After this 2 normal NH_4OH was added dropwise till intermediate colour, develops. It was lastly diluted to 1000 ml to obtain 1 ml = 1 mg $CaCO_3$.

Procedure - Total Hardness:

50 ml well shaked and filtered sample was taken in a conical flask. 2 ml buffer solution followed by 1 ml inhibitor were added to flask containing sample. A pinch of eriochrome black T was added and titrated with standard EDTA, till red colour changed to blue. Volume of EDTA required was noted down as (A). A reagent blank was run to check buffer properly. Volume of EDTA required by blank was noted down as (B). Then volume of EDTA required by sample was calculated as C = (A ± B). Calculation for total hardness was done as follows:

$$\text{Total Hardness mg/l} = \frac{C \times D \times 1000}{\text{ml. sample}}$$

where C = vol. in ml of EDTA required by sample,

D = mg $CaCO_3$ = 1.0 ml EDTA used as titrant.

Procedure - Calcium Hardness:

50 ml filtered sample was taken in a conical flask and added 1 ml of NaOH to raise pH to 12.0. Then a pinch of Nurex indicator was added to it. It was titrated with EDTA till pink colour changed to purple. The volume of EDTA used was noted as (A). Calculations were done as follows:

$$\text{Calcium Hardness mg/l} = \frac{A \times D \times 1000}{\text{ml. sample}}$$

where A = Volume of EDTA used by sample for titration.

Procedure - Magnesium Hardness:

Magnesium Hardness was calculated considering total hardness and calcium hardness indirectly as follows:

Magnesium Hardness = Total Hardness - Ca Hardness.

CHEMICAL ESTIMATION:

PHOSPHATE (PO_4):

Principle - The aminonaphthosulfonic acid method used here for phosphate determination (Standard Methods, 12th ed.) is based on the principle that under acid condition, soluble phosphate react with ammonium molybdate and produce molybdophosphoric acid which in turn, gets reduced to molybdenum blue after adding a reducing reagent such as ANSA (1-amino-2-

naphthyl 4-sulphonic acid) or stanous chloride. Colour developed is directly proportional to phosphate concentration.

Reagents:

i. Strong acid reagent - 300 ml concentrated H_2SO_4 was added to 600 ml distilled water and 4 ml of concentrated HNO_3 was added to it. It was cooled and diluted to 1000 ml.

ii. Sodium hydroxide approximately 3N - 12.0 gm of NaOH was dissolved and diluted to 100 ml.

iii. Phenolphthalein indicator - 5 gm phenolphthalein was dissolved in 500 ml 95% ethyl alcohol and 500 ml distilled water was added. 0.02N NaOH was dropwise added till faint colour appeared.

iv. Ammonium molybdate - 31.4 gm ammonium molybdate was dissolved in about 200 ml distilled water. 252 ml concentrated H_2SO_4 was added carefully to 500 ml distilled water, cooled and 3.4 ml con. HNO_3 was added in it. To this solution molybdate solution was added and diluted to 1000 ml.

v. 1-amino 2-naphthyl 4-sulphonic acid (ANSA) - 0.75 gm ANSA, 42 gm Na_2SO_3 and 70 gm $Na_2S_2O_3$ were weighed separately. ANSA was grind with small portion of $Na_2S_2O_3$ in water. Solution of remaining salts (Na_2SO_3 and $Na_2S_2O_3$) was prepared in small quantity of water.

above mixture was dissolved in this solution and diluted to 1000 ml. It was then filtered through Whatman's filter paper No. 42.

vi. Stock phosphate solution - 0.7165 gm anhydrous KH_2PO_4 was dissolved to 1000 ml to get 1 ml = 0.5 gm PO_4.

vii. Standard phosphate solution - 100 ml stock phosphate solution was diluted to 1000 ml to get 1 ml = 0.05 gm PO_4.

PROCEDURE:

A well mixed sample was filtered and 50 ml was taken into 250 ml conical flask. 1 ml of phenolphthalein indicator was added to it. Then strong acid was added dropwise till pink colour disappeared, 1 ml added in excess. It was boiled for 45 minutes, cooled and neutralized the faint pink colour with NaOH. Then filtered and developed colour for orthophosphate as follows:

Filtered sample after pretreatment as above was taken in volumetric (100 ml) flask and diluted to 50 ml. 2 ml ammonium molybdate was added and mixed well. Then 2.0 ml of ANSA was added and mixed well and diluted to 100 ml by making the volume in volumetric flask upto mark. A blank was prepared using distilled water. Colour developed was measured at 690 nm and 1 cm light path. A standard graph using standard PO_4 solution in the range 5-30 mg/l for ready reference was prepared. Calculations

were made as follows:

$$PO_4 \text{ mg/l} = \frac{\text{mg. sample} \times 1000}{\text{ml. sample}}$$

Sulphate (SO_4):

Principle - The turbidiometric method was followed here for the determination of sulphate (standard methods, 12th edition) which is based on principle that sulphate ions are precipitated in HCl medium with $BaCl_2$, so as to form an uniform suspension of $BaSO_4$ crystals. The absorbance of suspension is measured by spectrophotometer.

Reagents:

i. Conditioning reagent - 50 ml glycerol was mixed with solution containing 30 ml concentrated HCl, 300 ml distilled water, 100 ml 95% ethyl alcohol and 75 gm NaCl.

ii. Barium chloride - Crystal form.

iii. Standard sulphate solution - 147.9mg anhydrous Na_2SO_4 was dissolved and diluted to 1000 ml to get 1 ml = 100 µg SO_4.

Procedure:

The sample was filtered and 50 ml of it was taken in 250 ml conical flask. 5 ml conditioning reagent was added accurately to it and mixed well. The flask was then kept constantly stirred with help of magnetic stirrer and $BaCl_2$, crystals were added while stirring. Stirring was continued for 1 min after addition of $BaCl_2$. Turbidity developed was

measured at 420 nm and 1 cm light path by spectronic 20 (Bausch, Lamb). A standard curve was prepared by using standard sulphate solution and from the standard curve mgSO_4 present in the sample was calculated. Calculations were made as follows:

$$mg/l\ SO_4 = \frac{mg.\ SO_4 \times 1000}{ml.\ sample}$$

Ammonia (NH_3):

Principle - The direct nesslerization method which was employed here (standard methods, 12th edition) is based on the principle that ammonia produces a yellow colour compound when reacted with alkaline Nessler's reagent. Pretreatment with $ZnSO_4$ and NaOH precipitates Ca, Fe, Mn and sulfide and causes turbidity and apparent colour. Addition of EDTA or Rochelle salt solution prevents precipitation of residual Ca and Mg in the presence of alkaline Nessler's reagent.

Reagents:

i. Zinc sulphate - 10 gm $ZnSO_4.7H_2O$ was dissolved in distilled water and diluted 100 ml.

ii. Sodium hydroxide (6N) - 24 mg NaOH was dissolved and diluted to 100 ml.

iii. EDTA - 50 gm EDTA was dissolved in 60 ml distilled water containing 10 gm NaOH. Then cooled and diluted to 100 ml.

iv. Rochelle salt solution - 50 gm potassium, sodium tartarate was dissolved in 100 ml distilled water. 30 ml of it was boiled then to remove ammonia, cooled and diluted to 100 ml.

v. Nessler's reagent - 100 gm HgI_2 and 70 gm KI were mixed well and dissolved in small quantity of water. This mixture was added to a cool solution of 160 gm NaOH in 500 ml and diluted to 1000 ml. It was kept overnight and supernatant was stored in coloured bottles.

vi. Standard ammonia solution - 3.819 gm NH_4Cl dried at 100°C was dissolved in distilled water and diluted to 1000 ml. 10 ml of this solution was diluted to 1000 ml to get concentration of 1 ml = 10 µg N or 12.2 µg NH_3.

Procedure:

50 ml filtered sample was taken in 250 ml conical flask and 1 ml of $ZnSO_4$ was added followed by 0.4 - 0.5 ml NaOH to obtain a pH of 10.5 and allowed to settle, filtered the supernatant through Whatman's No. 42 filter paper. The filterate of sample was then diluted to 50 ml and 3 drops of Rochelle salt solution was added and mixed well. 1 ml of Nesslor's reagent was added to it and was transferred to volumetric flask and made upto 100 ml. It was mixed

well and transmission was read after 10 minutes at 410 nm using a blank prepared in the same way using double distilled water instead of sample. A calibration curve was prepared using standard solution in the range of 5 to 120 µg/100 ml for reference following the same procedure as above, but using the standard NH_3 solution.

Nitrite (NO_2):

Principle - The method used here for NO_2 determination was modified method of Barnes, and Folkard (1951) (standard methods, 12th edition) is based on principle that under acid (pH 2-2.5) condition nitrate ion as nitrous acid reacts with sulfinilic acid and forms diazonium salt which combines with 1 naphthylamine hydro-chloride to form pinkish red azodye. Colour produced is proportional to the concentration of NO_2 and obeys Bear's law in the range of 5 µg to 180 µg/l.

Reagents:

i. EDTA - 500 g EDTA sodium salt was dissolved in distilled water and diluted to 100 ml.

ii. Sulfinilic acid - 600 mg sulfinilic acid was dissolved in 70 ml hot water, cooled and added 20 ml concentrated HCl and diluted to 100 ml.

iii. 1-naphthylamine hydrochloride - 600 mg 1-naphthylamine hydrochloride was dissolved in distilled water containing

1 ml concentrated HCl. It was then diluted to 100 ml and stored at low temperature.

iv. Sodium acetate buffer 2N - 16.9 gm sodium acetate was dissolved and diluted to 100 ml.

v. Stock Nitrite solution - 1.2320 gm sodium nitrite was dissolved and diluted to 1000 ml to get 1 ml = 250 mg N, It was standardized against 0.05 $KMnO_4$.

vi. Standard Nitrite solution - The appropriate aliquot of stock solution was diluted to 1000 ml to get 1 ml = 0.5 µg N in the solution.

Procedure:

50 ml filtered sample was taken in 250 ml conical flask, neutralized to pH 7.0. 1 ml of EDTA followed by 1 ml of sulfinilic acid were added, mixed well and pH was adjusted to 1.4. After 2-3 min 1 ml of 1-naphthylamine hydrochloride and 0.1 ml sodium acetate were added and transferred to 100 ml volumetric flask and diluted upto mark on volumetric flask. At this stage pH was around 2 to 2.5. A blank was prepared in the same way by substituting distilled water for the sample. Colour developed was measured after 10 min at 520 nm and 1 cm light path. A calibration curve using standard NO_2 solution in the range of 5-50 mg/l was prepared for ready reference. Calculations were made and recorded as NO_2-N mg/l:

$$NO_2 - N = \frac{mgNO_2 - N \times 1000}{Ml.sample\ taken}$$

Nitrate (NO_3):

Principle - The phenol disulfonic acid method followed here for Nitrate determination (standard methods, 12th edition) encounters the principles that nitrate reacts with phenol-disulphonic acid and produces a nitro derivation which is alkaline solution, develops yellow colour due to rearrangement of its structure. The colour produced follows Bear's law and is proportional to the concentration of nitrate present in the sample.

Reagents:

i. Standard silver sulphate - 4.40 gm $AgSO_4$ was dissolved in distilled water and diluted to 1000 ml to get 1 ml = 1 mg.

ii. Phenol disulphonic acid - 25 gm white phenol was dissolved in 150 ml, concentrated H_2SO_4 and 75 ml fuming H_2SO_3 (15% free SO_3) was added to it, stirred well and heated for 2 hours on waterbath. Stored in volumetric flask.

iii. Ammonium hydroxide concentrated - (ready made). B.D.H product

iv. Potassium hydroxide (12N) - 673 gm KOH was dissolved in distilled water and diluted to 1000 ml.

v. Stock Nitrate solution - 721.8 mg unhydrous potassium nitrate was dissolved in distilled water and diluted to 1000 ml.

vi. Standard nitrate solution - 50 ml stock NO_3 solution was evaporated to dryness on water bath. Residue was dissolved

in 2 ml phenol disulphonic acid reagent and diluted to 500 ml so as to get 1 ml = 10 mg N.

vii. EDTA - 50 gm EDTA was rubed with 20 ml distilled water to form a paste 60 ml NH_4OH was added to it and mixed well.

viii. Aluminium hydroxide - 125 gm potash-alum was dissolved in 100 ml distilled water, warmed to 60°C and 55-60 ml NH_4OH was added and allowed to stand for 1 hour. Then supernatant was decanted and precipitate was washed a number of times till it was free from Cl^- or NO_2^-.

Procedure:

50 ml well shaked and filtered sample was taken in 250 ml conical flask and to remove excess colour, 1 ml of aluminium hydroxide was added to it. It was then stirred well and filterate was used decanting first portion of filterate. To oxidize NO_2^- to NO_3^- under acid condition, $KMnO_4$ was used. Also, to suppress NO_2^- interference, sulfinilic acid was added. For chloride removal Ag_2SO_4 was added carefully to precipitate out-chloride as $AgCl_2$. After above pretreatment of sample, colour was developed as follows:

Clarified sample as above was neutralized to pH 9.0 and evaporated to dryness on waterbath. The residue was dissolved using glass rod with 2 ml phenol disulphonic acid reagent,

diluted and transferred to volumetric flask. After this 10 ml of KOH was added. EDTA reagent was then added dropwise till turbidity developed was dissolved completely. It was filtered and made upto 100 ml. A blank was prepared in the same way using distilled water instead of sample. Colour developed was measured at 410 nm with a light path of 1 cm. Calibration curve was prepared using standard NO_3 solution in the range 5 to 300 µg N/L following above procedure. NO_3 was recorded as mg/l:

$$NO_3 - N \text{ mg/l} = \frac{\text{mg in sample} \times 1000}{\text{ml. sample taken}}$$

Culture media for blue-green algae:

The enrichment cultures were prepared with De's (De, 1939) and Beneck's (Benecke, 1898) media. A parallel series of cultures were also maintained by adding only sterile 'Pyrex' distilled water to the lake water. The composition of the nutrient media were also follows:

De's medium : (Per liter of 'Pyrex' double distilled water)

KNO_3	0.2 gm
K_2HPO_4	0.2 gm
$MgSO_4.7H_2O$	0.2 gm
$CaCl_2$	0.1 gm
$FeCl_3$	trace

Beneck's medium: (Per liter of 'Pyrex' double distilled water)

NH_4NO_3	0.2 gm
K_2HPO_4	0.2 gm
$MgSO_4.7H_2O$	0.2 gm
$CaCl_2$	0.1 gm
$FeCl_3$	trace

The pH of the media was adjusted to 7.5 in all the series. After isolation, the algae were grown on solid media in which $FeCl_3$ was replaced by Hutner's trace element solution (Hutner *et al.*, 1950) in which $CaCl_2$ was omitted. The trace element solution contained per litre of distilled water: 1 gm H_3BO_3; 0.15 gm $CuSO_4.5H_2O$; 5 gm ethylene diamine tetra-acetic acid (EDTA); 2.2. gm $ZnSO_4.7H_2O$; 0.5 gm $FeSO_4$; 0.15 gm $CaCl_2$; 1.1 gm $(NH_4)_6MO_7O_{24}.H_2O$.

Culture methods:

The enrichment cultures were maintained by transferring 10 ml of lake water to sterile petridishes containing 20 ml of culture medium with a periodic addition of the medium (1/4 diluted) to compensate the loss by evaporation. The petridishes were placed in a culture chamber provided with light arrangement supplying a light of 200 lux. Periodic examination of the algae that came up in the cultures were carried out at weekly intervals. Most of the forms were carefully isolated in unialgal cultures.

Isolation - The colonies were picked up under a binocular stereoscopic microscope, washed several times with sterile distilled water and streaked on nutrient agar. Unialgal cultures were isolated by repeated sub-culturing (Pringsheim, 1945) and maintained in cultures tubes on agar slant (Pandey, 1965; Varma, 1966 and Varma and Mitra, 1968).

The taxonomic consideration of blue-green algae is based on monograph on blue-green algae by Desikachary (1959).

ESTIMATION OF CHLOROPHYLL:

This is based on the method described by Staples (1973).

Reagents:

i. 90% aqueous acetone solution.

Procedure - The millipore filter was placed in the filteration apparatus and the $MgCO_3$ powder was dispersed over the surface of the filter. A 100 ml volume of water sample was filtered under suction of about 2/3 atmosphere. After filteration, the filter with $MgSO_4$ and phytoplankton retained on the filter were placed in a small glass pestle type homo-genizor and 2/3 ml of 90% acetone was added. The homogenizer was set at 500 revs./min and run for about 1 min. The material was then transferred to a centrifuge tube and the pestle and homogenizer were rinsed 2/3 times with 90% acetone and washes were added to the centrifuge tube. The final volume in the centrifuge tube was about 10.0 ml. The centrifuge tube was allowed to stand for 10 min in the dark

at room temperature. The mixture was then centrifuged at 5000 g for 10 min. The supernatant was then carefully decanted. The spectrophotometer with a band width of 3.0 nm was used. The cuvette to be used was cleaned appropriately. All readings were taken against a 90% acetone blank. The optical density was recorded at 750, 663, 645 and 630 nm. The calculations were made as follows:

The optical density at 750 nm was substracted from that at 663, 645 and 630 nm. Then data were divided by the light path in centimeters of cells i.e. 1 cm. Keeping their respective values in the following equations concentrations of chlorophylla, chlorophyll b and chlorophyll c were found out:

$$\text{Chlorophyll a} = 11.64\ e_{663} - 2.16\ e_{645} + 0.10\ e_{630}$$

$$\text{Chlorophyll b} = 20.97\ e_{645} - 3.94\ e_{663} - 3.66\ e_{645}$$

$$\text{Chlorophyll c} = 54.22\ e_{630} - 5.53\ e_{663} - 14.81\ e_{645}$$

where e presents the final tabulated numbers by substracting the optical density at 750 nm from that at 663, 645 and 630 nm. The solutions are in micrograms per millilitre. The solutions were multiplied to the equations given above by the total volume of the water sample in liters i.e. 1/10 to obtain the final concentrations of the chlorophylls in micrograms per liter of water sample.

Enumeration of pathogenic (Coliform) bacteria in water:

The method employed here (APHA standard methods 14th edition) was multiple tube method. It determined the presence of bacteria as well most probable number of coliforms by planting a series of measured quantities of water into test tubes containing favourable culture media. The method is based on the laws of probability and can be used to obtain an estimate of the number of bacteria in a sample as most probable number (MPN). For the determination of coliform bacteria, water samples were taken in sterile glass bottles by means of a modified Zo Bell sampling apparatus, and were immediately prepared for analysis (Gocke, 1975). Immediately after the sampling vessel were filled under sterile conditions with necessary amounts of water needed for the various investigations.

Composition and preparation of different growth media:

1. Lactose Broth medium (1 litre medium)

Beef extract	3.0 gm
Peptone	5.0 gm
Lactose	5.0 gm
Agar	10.0 gm
(for solid medium)	

Preparation - To rehydrate the medium, 13 gms of above chemicals were added and dissolved one by one in a conical flask, containing 1000 ml distilled water. pH was adjusted

to 6.8, flask was then tightly corked with cotton and aluminium foil over it. Medium was autoclaved for 15 min at 15 lbs/sq.inch pressure.

ii. Eosine Methylene Blue Medium (1 litre)

Peptone	1.0 gm
Lactose	5.0 gm
Sucrose	5.0 gm
K_2HPO_4	2.0 gm
Eosine	044 gm
Methylene blue	0.065 gm
Agar	13.5 gm
(for solid medium)	

Preparation - Above components were dissolved one after the other in a conical flask of 1 litre capacity containing 1000 ml distilled water. pH was adjusted to 7.3-7.5. Flask was thon tightly corked with cotton and aluminium foil was covered over it. Medium was autoclaved for 15 min at 15 lbs/sq.inch pressure.

Procedure - The tests were carried through 3 distinct stages.

i. Presumptive test -The lactose broth medium prepared as above was poured in 1/3 protion of test tubes arranged in cultures tube rack. About 90 tubes were prepared, corked with cotton and were allowed to solidify. Culture tubes were labelled as I_1, I_2 and I_3. For sample No.I. For each five tubes of I_1 five of I_2 and five tubes of I_3 were labelled. In the same way II_1, II_2, II_3 upto VI_3 labelled

tubes were arranged in a tube rack. The sample was shaked vigorously and in I_1 five tubes 10 ml of sample. in I_2 tubes 1 ml of sample and in I_3 tubes 0.1 ml of sample was added from sample No. I. In the same manner all samples were added to their respective tubes. Then melted wax of melting point 56-58°C was added to each tube just sufficient to cover surface of medium in the tube to avoid communication of outer gaseous environment to inner gas envirnment in the tube. The tubes were shaken gently, but vigorous shaking was avoided as it may introduce air bubbles. All tubes in racks were incubated at constant 37°C temperature for 24 ± 2 hours.

After incubation racks of culture tubes were removed from the incubator, shaken gently and positive or negative test for gas production in each tube was reported. In the tube showing positive gas production, wax layer seems to be raised from surface of culture medium in the tube. Gas negative tubes were returned to incubator for next 24 ± 2 hours incubation. After incubation again gas positive tubes were reported and noted down. Then from each set of five tubes, gas positive tubes for confirmed test were selected. Not more than 3 gas positivo tubes were selected from each set of tubes. Gas negative tubes were discarded.

ii. Confirmed test - Eosine methylene blue agar petriplates were prepared for confirmed test by pouring medium in the

petriplates. Petriplates were marked at the bottom. Flame sterilized needle was then dipped in gas positive tube, little inoculum was taken on tip of the needle and inoculation was done with streaking method. Tearing of the surface of culture medium with needle was avoided. Inoculation was done by touching the end of the needle to the surface of the medium. After resterilizing and cooling the inoculation needle, again inoculation was done. This way inoculums from all tubes were inoculated to their respective petriplates. After subculturing plates were kept in an incubator in an inverted position at 37°C for 24 ± 2 hours.

After 24 hours incubation, eosin methylene blue agar plates were removed from the incubator and colonies formed were observed. Large colonies with metallic green-sheen colour were confirmed E. coli and small pink colonies were confirmed Aerobacter aerogens. No colonies means no coliform bacteria was confirmed. Plates for completed test were selected from these plates and other plates were discarded.

iii. Completed test - To produce further for completed test, test tubes of lactose broth medium were prepared and kept ready as prepared for presumptive test. Agar plate having typical green sheen coloured colony atleast 0.5 cm away from another was transferred with a flame

sterilized and cooled needle on the surface of lactose broth medium in the tube. All tubes were inoculated by inoculum from their respective petriplates. Eosin methylene blue agar plates were discarded after use. Test tubes in rack were kept in the incubator for 20-24 hours incubation. Longer incubation was avoided as it may result in irregular staining for gram test.

Gas production in 20-24 hours again confirmed the presence of E. coli. Microscopic examination showed gram negative, non spore forming bacteria. Completed test thus confirmed presence of enteric bacteria in the sample. Calculations for most probable number of bacteria in the samples were made using MPN standard Table I.

Determination of MPN of Bacteria:

i. Results were codified first. Suppose out of five tubes in which 10 ml portion of a sample was planted, all five showed positive test for coliform bacteria; 3 out of five in which 1 ml portion of sample was planted, and 2 out of five in which 0.1 ml portion of sample was planted showed positive test for coliform bacteria; then coded result was noted as 5-3-2.

ii. Most probable number was found out from standard MPN Table I for coded result 5-3-2 by considering code 5 from

TABLE I

(for 5-10 ml, 5 - 1 ml; 5 - 0.1 ml tubes planted)

No.of tubes giving positive reaction out of				No.of tubes giving positive reaction out of			
5-10 ml portion	5-1 ml portion	5-0.1 ml portion 100 ml	MPN Index/ 100 ml	5-10 ml portion	5- 1ml portion	5-01 ml portion	MPN Index/ 100 ml
0	0	0	2	3	5	0	25
0	0	2	4	4	0	0	13
0	1	0	2	4	0	1	17
0	1	1	4	4	0	2	21
0	1	2	6	4	0	3	25
0	2	0	4	4	1	0	17
0	2	1	6	4	1	1	21
0	3	0	6	4	1	2	26
1	0	0	2	4	2	0	22
1	0	1	4	4	2	1	26
1	0	2	4	4	2	2	32
1	0	3	8	4	3	0	27
1	1	0	4	4	3	1	33
1	1	1	6	4	3	2	39
1	1	2	8	4	4	0	34
1	2	0	6	4	4	1	40
1	2	1	8	4	5	0	41
1	2	2	10	4	5	1	48
1	3	0	8	5	0	0	23
1	3	1	10	5	0	1	31
1	4	0	11	5	0	2	43
2	0	0	5	5	0	3	58
2	0	1	7	5	0	4	76
2	0	2	9	5	1	0	33
2	0	3	12	5	1	1	46
2	1	0	7	5	1	2	63
2	1	1	9	5	1	3	84
2	1	2	12	5	2	0	49
2	2	0	9	5	2	1	70
2	2	1	12	5	2	2	94
2	2	2	14	5	2	3	120
2	3	0	12	5	2	4	148
2	3	1	14	5	2	5	177
2	4	0	15	5	3	0	79
3	0	0	8	5	3	1	109
3	0	1	11	5	3	2	141
3	0	2	13	5	3	3	175
3	1	0	11	5	3	4	212
3	1	1	14	5	3	5	253
3	1	2	17	5	4	0	130
3	1	3	20	5	4	1	172
3	2	0	14	5	4	3	221
3	3	0	21	5	5	0	240
3	4	0	21	5	5	1	348
3	4	1	24	5	5	2	542
				5	5	3	918
				5	5	4	1609

column headed 5-10 ml portion, 3 from column headed 5-1 ml portion and 2 in column headed 5-0.1ml portion. Following line to the right 141 were read in the column headed by MPN index. Thus, knowing most probable number of coliform bacteria in each sample separate table of results was prepared.

RESULTS

PHYSICAL PROPERTIES OF LAKE WATER:

i. pH and electrical conductivity of the water - Over all H-ion concentration of the water varied at different locations of the lake. This was almost consistent with every sampling collection. pH in the month of April and early May was between 7.5 to 8.0 which slightly changed to alkalinity towards the end of May with a pH of 3.2 to 8.4 and this sharply declined to neutral pH in the month of August (Fig. 2).

A more of less similar pattern was seen for the electrical conductivity (Fig. 2). The electrical conductivity of the lake water ranged from 0.37-0.64 μ mho. Interestingly, in the month of August all the water samples have shown an abrupt drop (0.37-0.48 mho) in the electrical conductivity.

ii. Temperature - Temperature of the lake water ranged from 21°C-32°C. The minimum temperature (21°C) was recorded in the month of April. On on-set of summer, there was seen a steady increase in water temperature and the maximum was attained in August. This is almost true for all the samples analysed (Fig. 3).

iii. Hardness - Hardness of water was estimated both for calcium and magnesium hardness. Calcium hardness varied

Fig. 2: Hydrogen ion-concentration (pH) and electrical conductivity of the water.

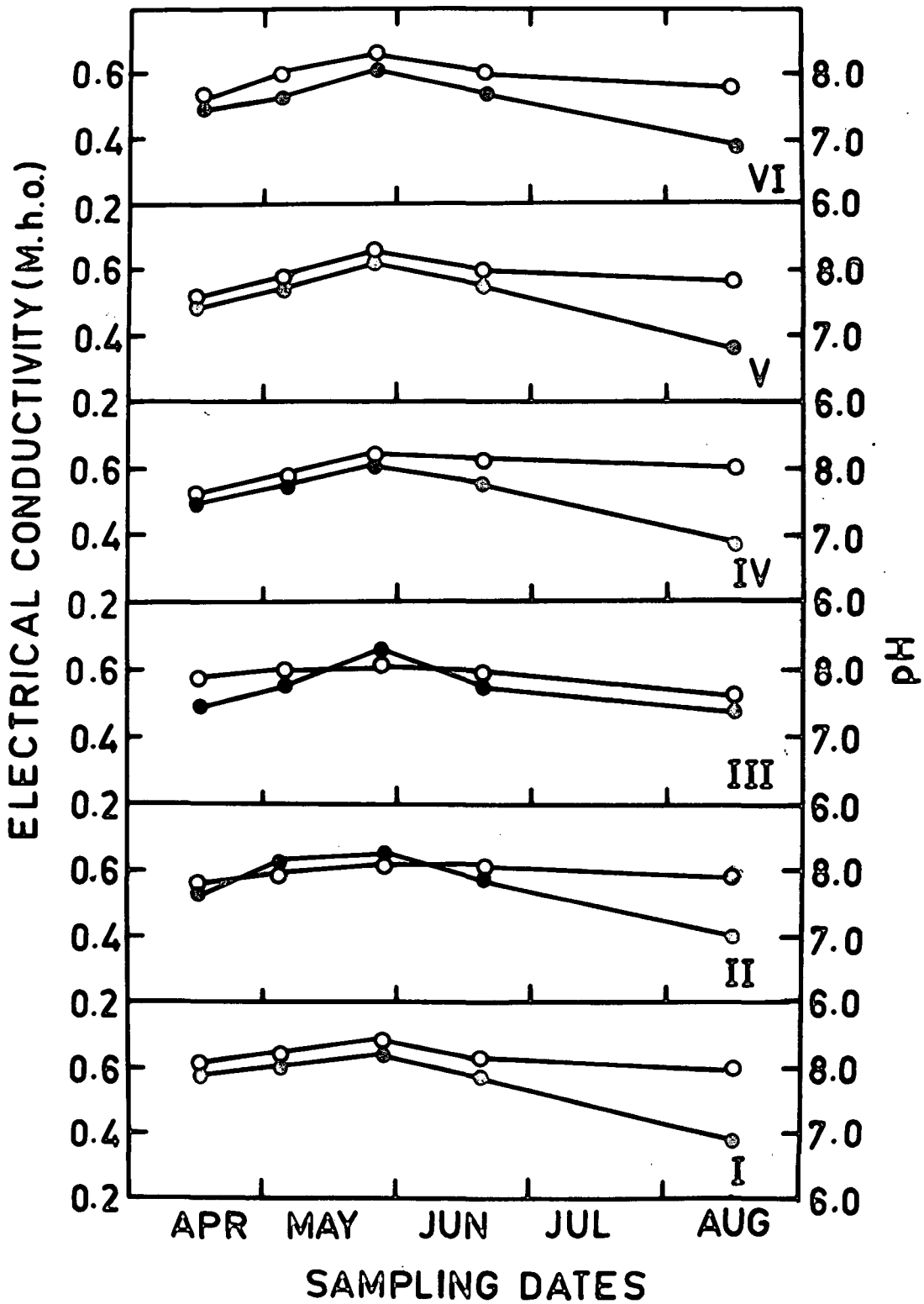

ELECTRICAL CONDUCTIVITY (M.h.o.)
0.6
0.4
0.2
8.0
7.0
6.0
VI
V
IV
III
II
I
pH
APR
MAY
JUN
JUL
AUG
SAMPLING DATES

Fig. 2

Fig. 3 : Temperature of the water.

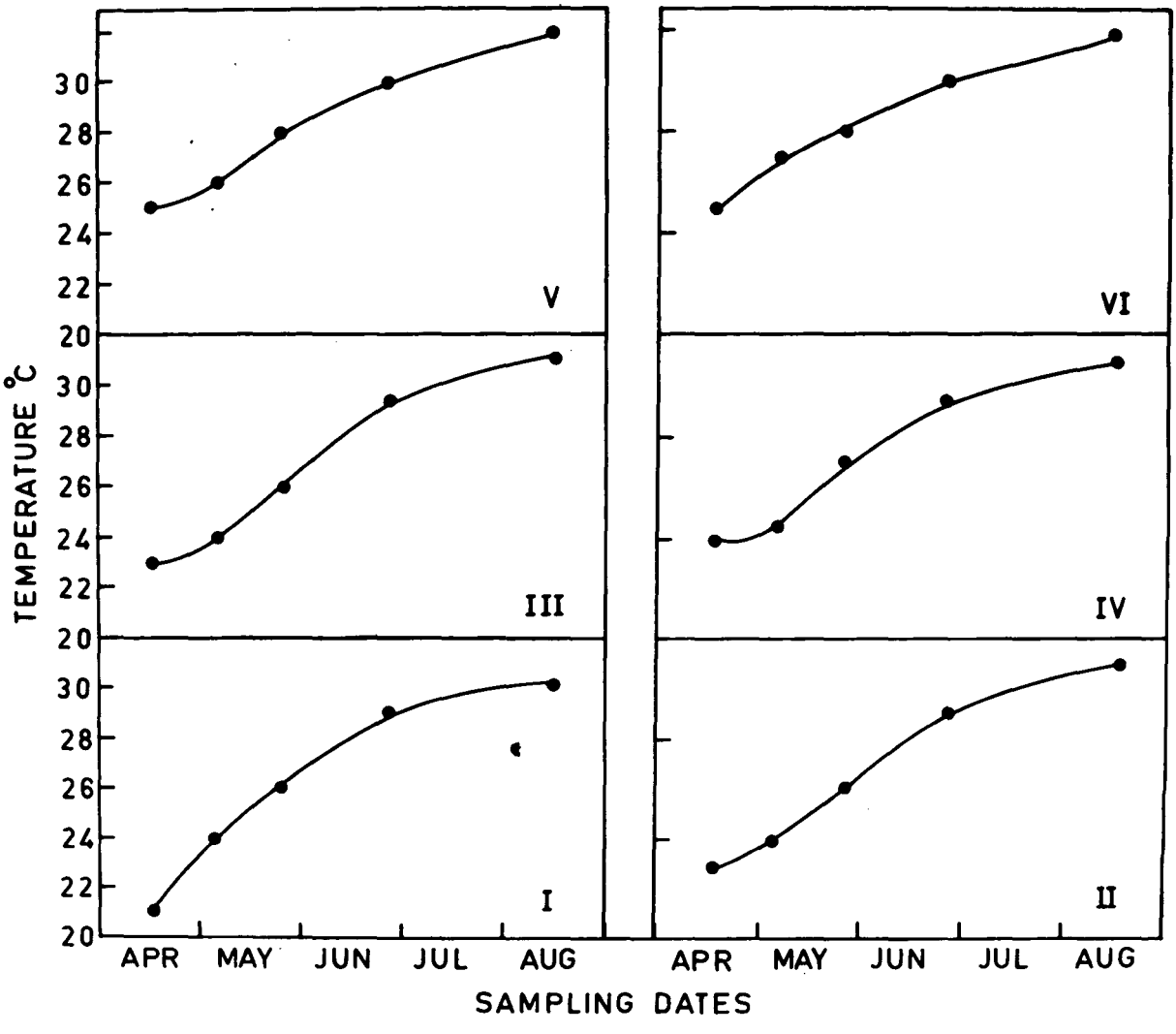

TEMPERATURE °C
30
28
26
24
22
20
V
VI
III
IV
I
II
APR
MAY
JUN
JUL
AUG
SAMPLING DATES

Fig. 3

from 0.200-0.640 ppm. It was interesting to note that the samples I, II and V had the lowest calcium hardness (0.2-0.28 ppm) while the samples III and VI had the maximum hardness (0.320-0.460 ppm) in the month of April. In the month of June, the hardness was seen highest all over the lake water which again declined on the onset of monsoon i.e. in the month of August (Fig. 4).

Magnesium hardness had a wide variation from one sampling place to other and ranged from 0.2-0.64 ppm. Like calcium hardness it was lowest in month of April which gradually increased in the months, May and June and was higher in earlier June and attained the highest hardness towards the end of June followed by sharp decline in August (Fig. 5). Samples II and V attained a very distinct peaks in the month of June followed by a rapid decline. Samples I and VI did not show any radical change throughout the course of the present investigation and the magnesium hardness varied from 0.2-0.5 ppm.

CHEMICAL PROPERTIES OF LAKE WATER:

1. Ammonical nitrogen - Ammonical nitrogen of the water ranged from 0.39-0.79 ppm. Samples III, IV, V had the lowest concentration of ammonia at end of June-which rapidly improved in August. The highest value recorded was 0.79 ppm. Samples III and V did not show any marked

Fig. 4 : Ca-hardness of the water.

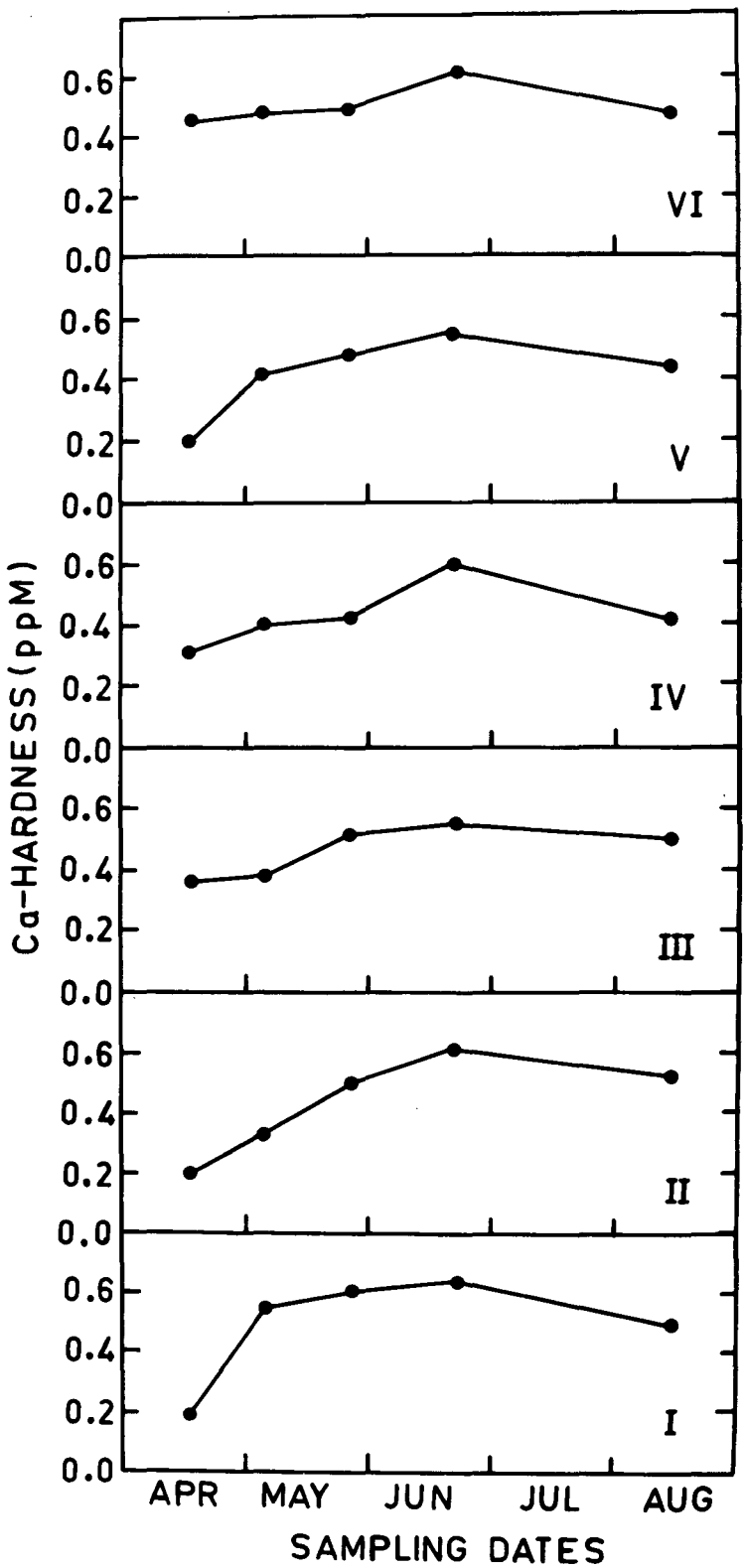

Ca-HARDNESS (ppM)
0.6
0.4
0.2
0.0
VI
V
IV
III
II
I
APR
MAY
JUN
JUL
AUG
SAMPLING DATES

Fig. 4

Fig. 5 : Mg-hardness of the water.

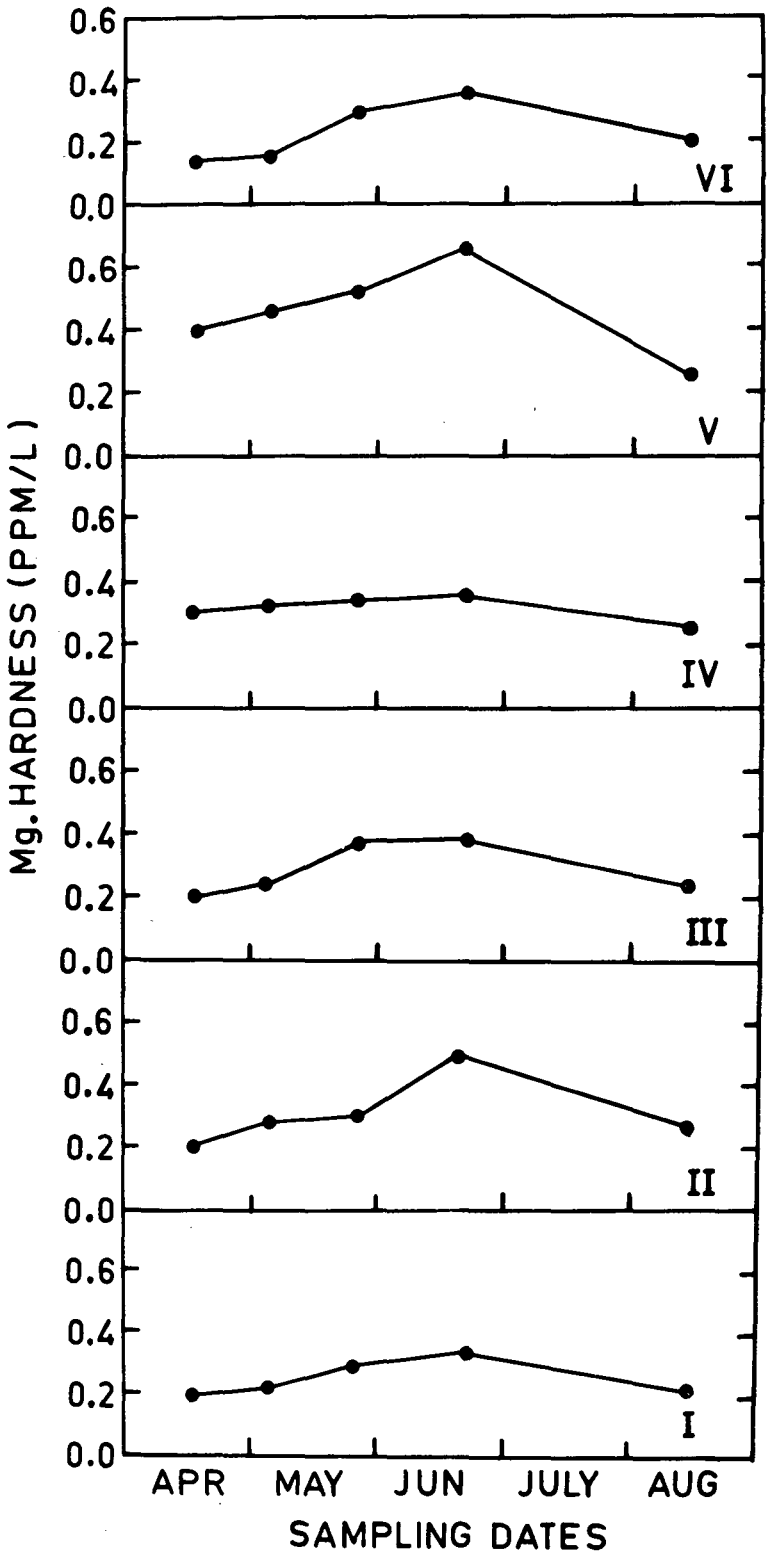

Mg. HARDNESS (PPM/L)
0.6
0.4
0.2
0.0
VI
V
IV
III
II
I
APR
MAY
JUN
JULY
AUG
SAMPLING DATES

Fig. 5

variation in ammonical nitrogen concentration (Fig. 6).

ii. Nitrite nitrogen - Nitrite varied from 0.1-0.6 ppm. The value of this nitrogen was high on the on-set of summer (April) which rapidly declined towards the end of June and on the onset of monsoon nitrite further improved in its concentration. Contrast to concentration of ammonia, the nitrite in all the water samples showed this characteristics (Fig. 7).

iii. Nitrate nitrogen - More or less nitrate was running parallel to that of nitrite nitrogen in its concentration. The overall variations seen were from 0.02 - 0.38 ppm. Characteristically, like the ammonia and nitrite nitrogen the nitrate nitrogen concentrations were at its lowest value in the end of June which again improved to great extent in August. Like the other nitrogen factors, nitrate nitrogen values were considerably high in the month of April, gradually reducing in the end of June and again recovered in August. This observation was almost consistent in all the samples. However, extremes of variations were noted in samples I, II and III (Fig. 8).

iv. Phosphate - All the samples tested showed a high value of phosphate in the month of April, which declined to its lowest concentrations in June and again recovered in August. The overall values ranged from 0.015-0.45 ppm (Fig. 9).

Fig. 6 : Ammonical nitrogen (NH_4^+) in the water.

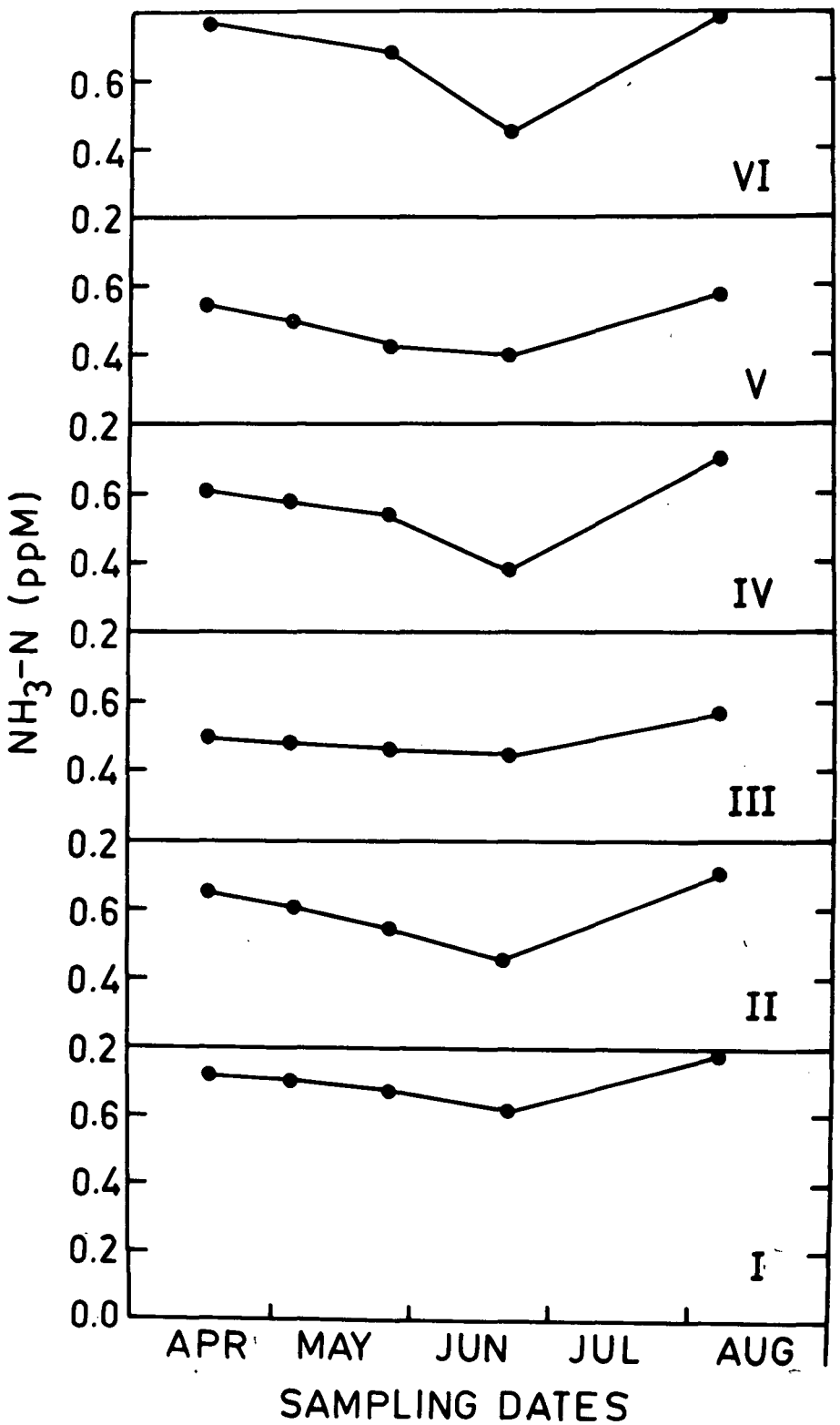

0.6
0.4
0.2
VI
V
IV
III
II
I
0.0
APR
MAY
JUN
JUL
AUG
SAMPLING DATES
NH3-N (ppM)

Fig.6

Fig. 7 : Nitrite nitrogen (NO_2^-) in the water.

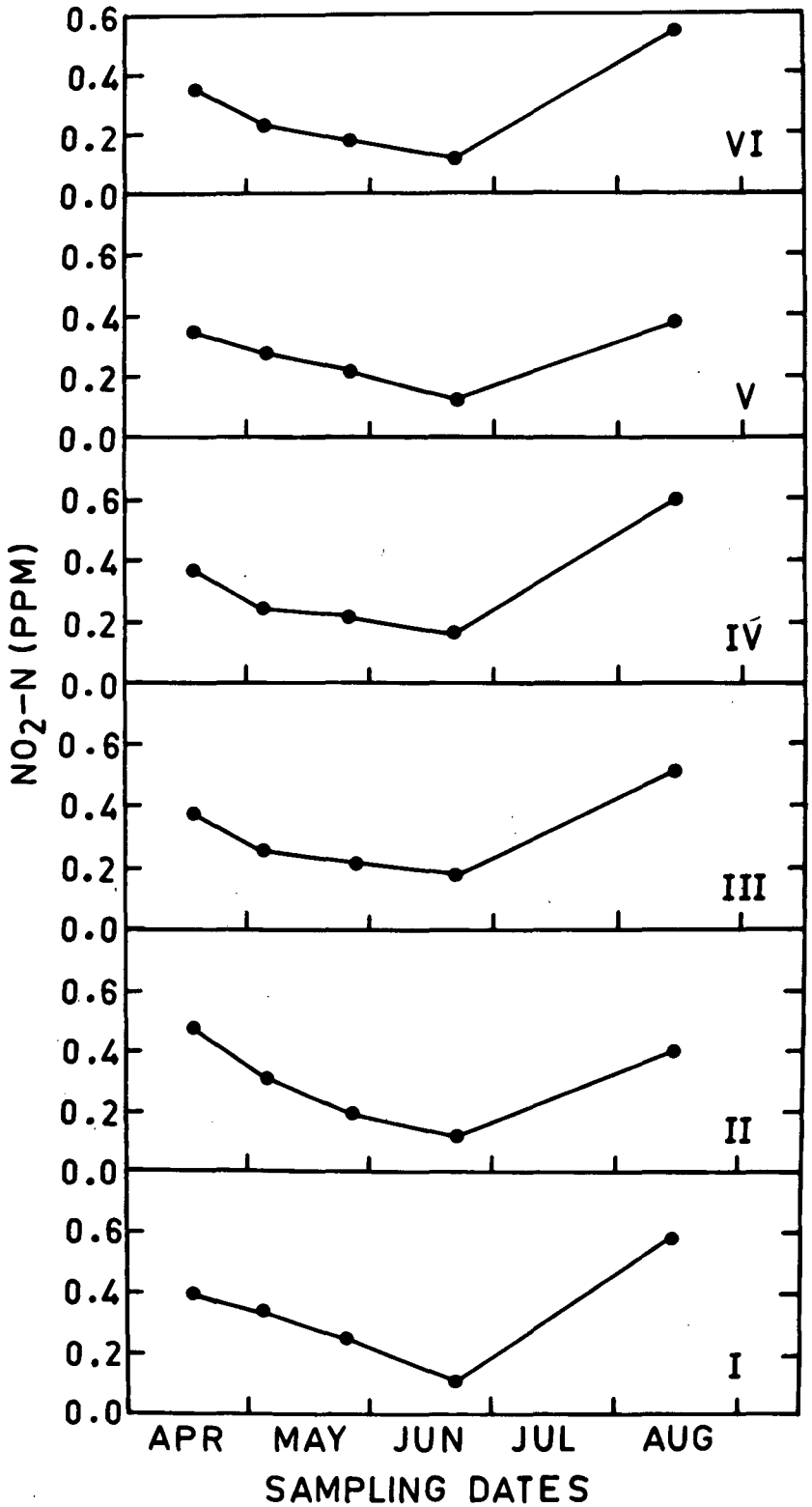

0.6
0.4
0.2
0.0
VI
V
IV
III
II
I
NO_2-N (PPM)
APR
MAY
JUN
JUL
AUG
SAMPLING DATES

Fig.7

Fig. 8 : Nitrate nitrogen (NO_3^-) in the water.

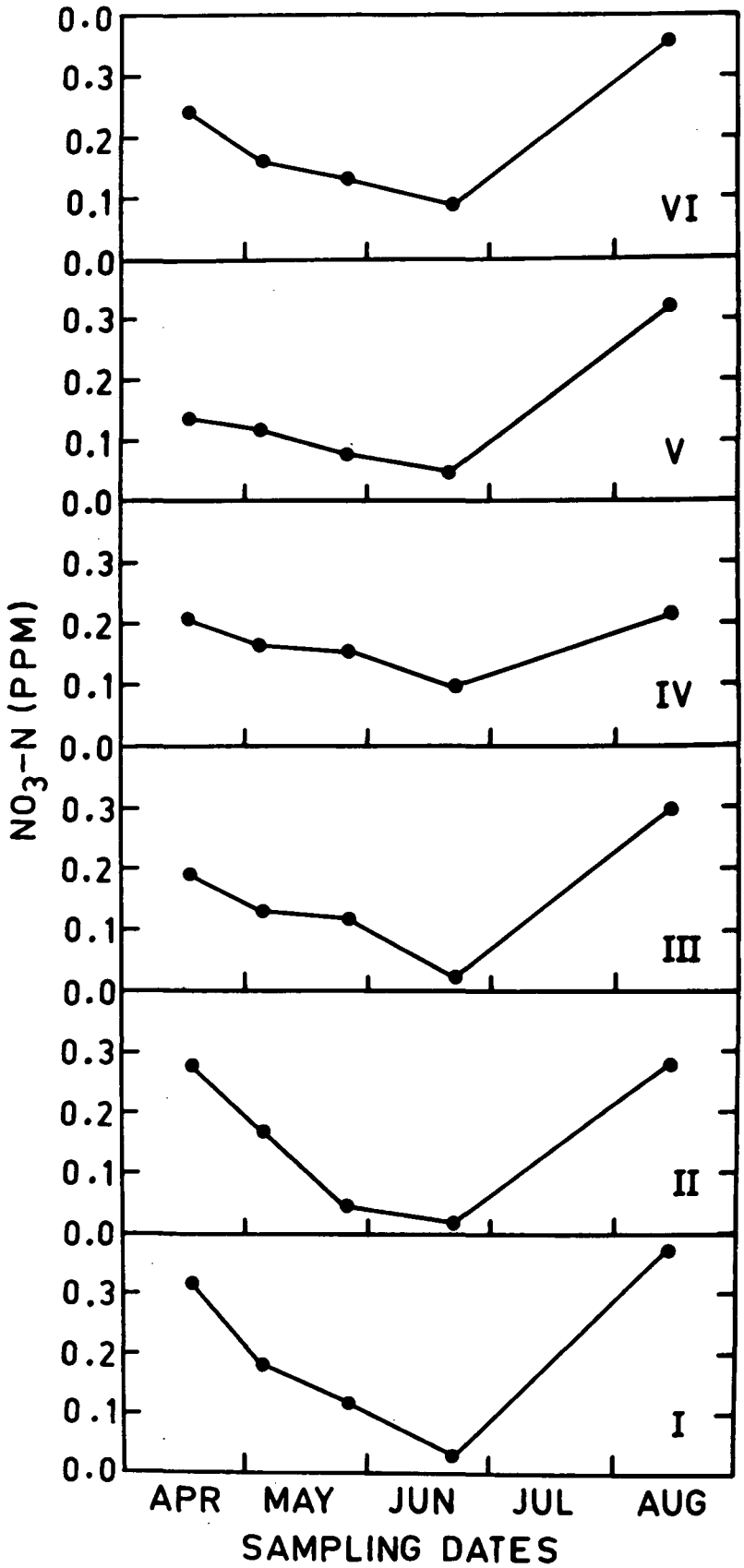

0.0
0.3
0.2
0.1
0.0
VI
V
IV
III
II
I
NO_3-N (PPM)
APR
MAY
JUN
JUL
AUG
SAMPLING DATES

Fig.8

Fig. 9: Phosphate ion (PO_4^{-}) in the water.

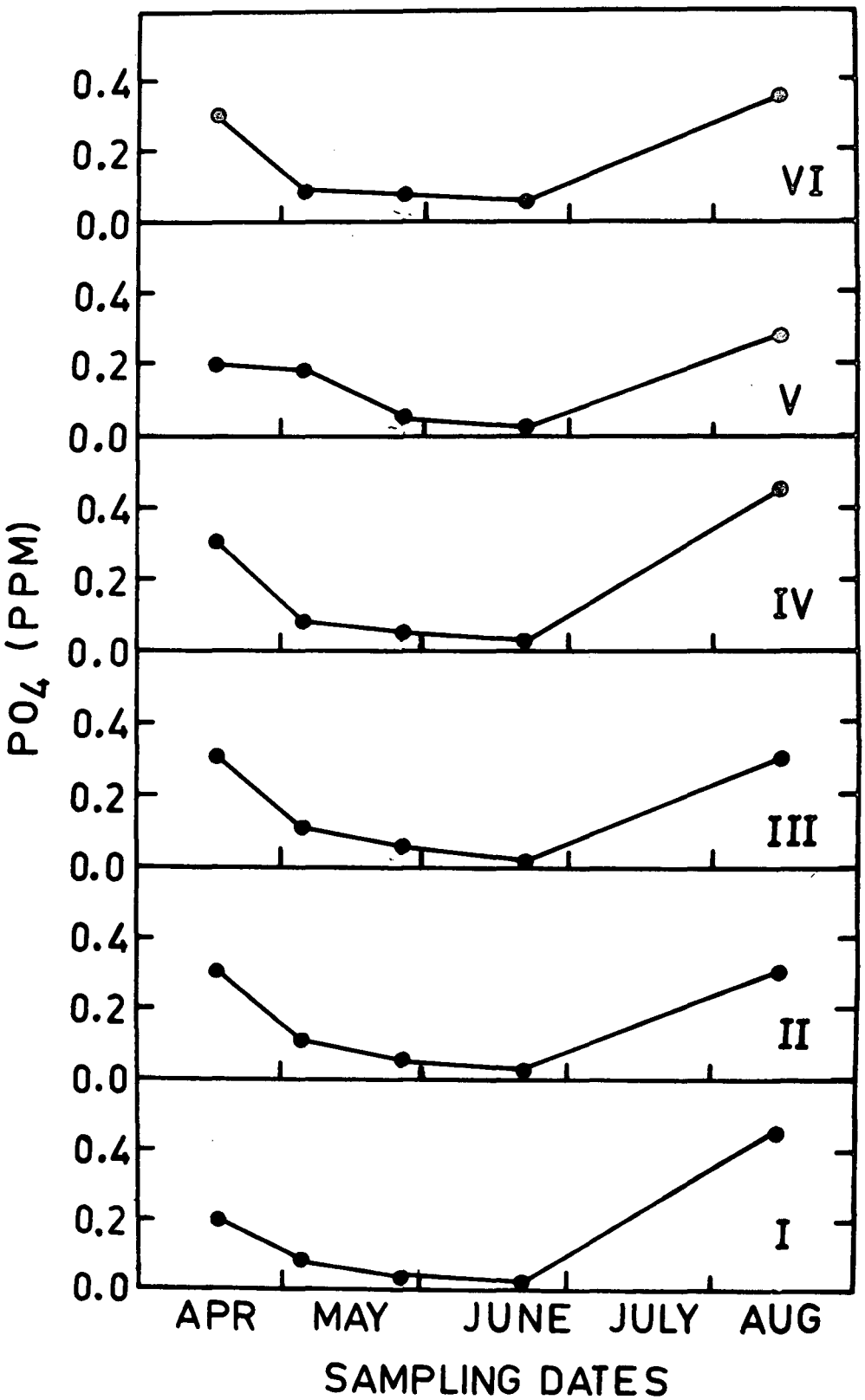

0.4
0.2
0.0
VI
V
IV
III
II
I
PO4 (PPM)
APR
MAY
JUNE
JULY
AUG
SAMPLING DATES

Fig.9

v. Sulphate - The sulphate concentration of the lake water varied from 0.37-1.3 ppm. Like the other chemical factors, there were seen a high values in April (0.85 - 1.1 ppm) with a quick fall in its value in the summer times (June). The lowest concentration recorded was 0.3 ppm. Interestingly, sulphate concentration improved considerably to a highest peak (1.3 ppm) on the onset of monsoon (Fig. 10).

INTERACTION BETWEEN PHYSICAL AND CHEMICAL FACTORS:

i. pH and Ca-hardness, Mg-hardness - pH, calcium and magnesium-hardness were running parallel. It was evident from the fact that when the pH was lowest, the calcium and magnesium-hardness were also lowest and while pH changed towards alkalinity, the hardness factor also correspondingly increased (Fig. 11). It is interesting to note that the pH was at its highest peak in the month of May, however, calcium, magnesium-hardness attained the highest peak in the month of June and again when the pH of the lake water declined both the calcium and magnesium factors correspondingly decreased.

ii. pH and nitrate, nitrite and ammonical nitrogen - The correlation of pH against NO_3, NO_2 and ammonical nitrogen concentrations did not indicate any distinct trend, but for the observation that at pH 8.0-8.2 all the chemical

Fig. 10 : Sulphate ion (SO_4^{2-}) in the water.

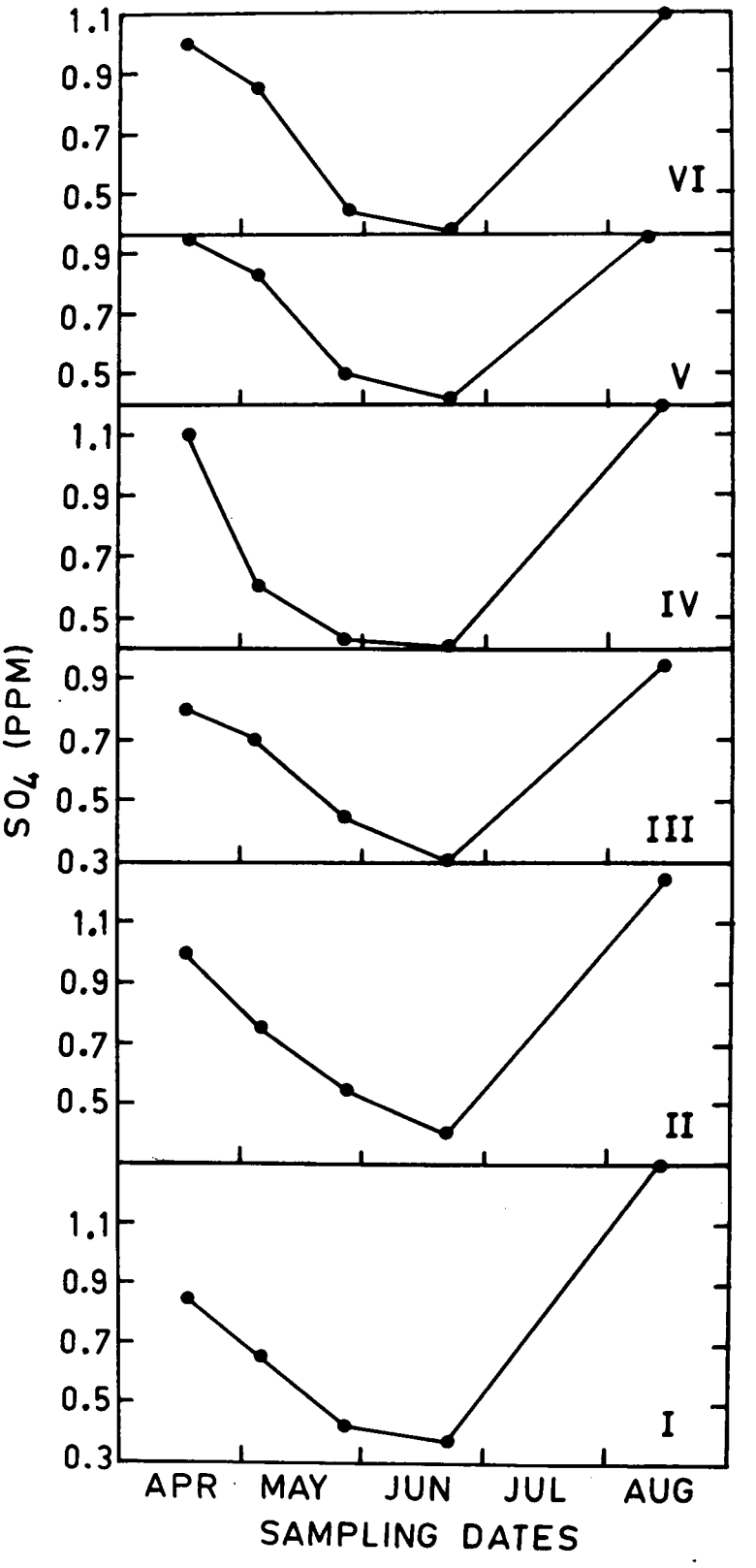

1.1
0.9
0.7
0.5
VI
0.9
0.7
0.5
V
1.1
0.9
0.7
0.5
IV
0.9
0.7
0.5
0.3
III
1.1
0.9
0.7
0.5
II
1.1
0.9
0.7
0.5
0.3
I
SO4 (PPM)
APR
MAY
JUN
JUL
AUG
SAMPLING DATES

Fig.10

Fig. 11 : An interrelationship between Ca and Mg-hardness against pH.

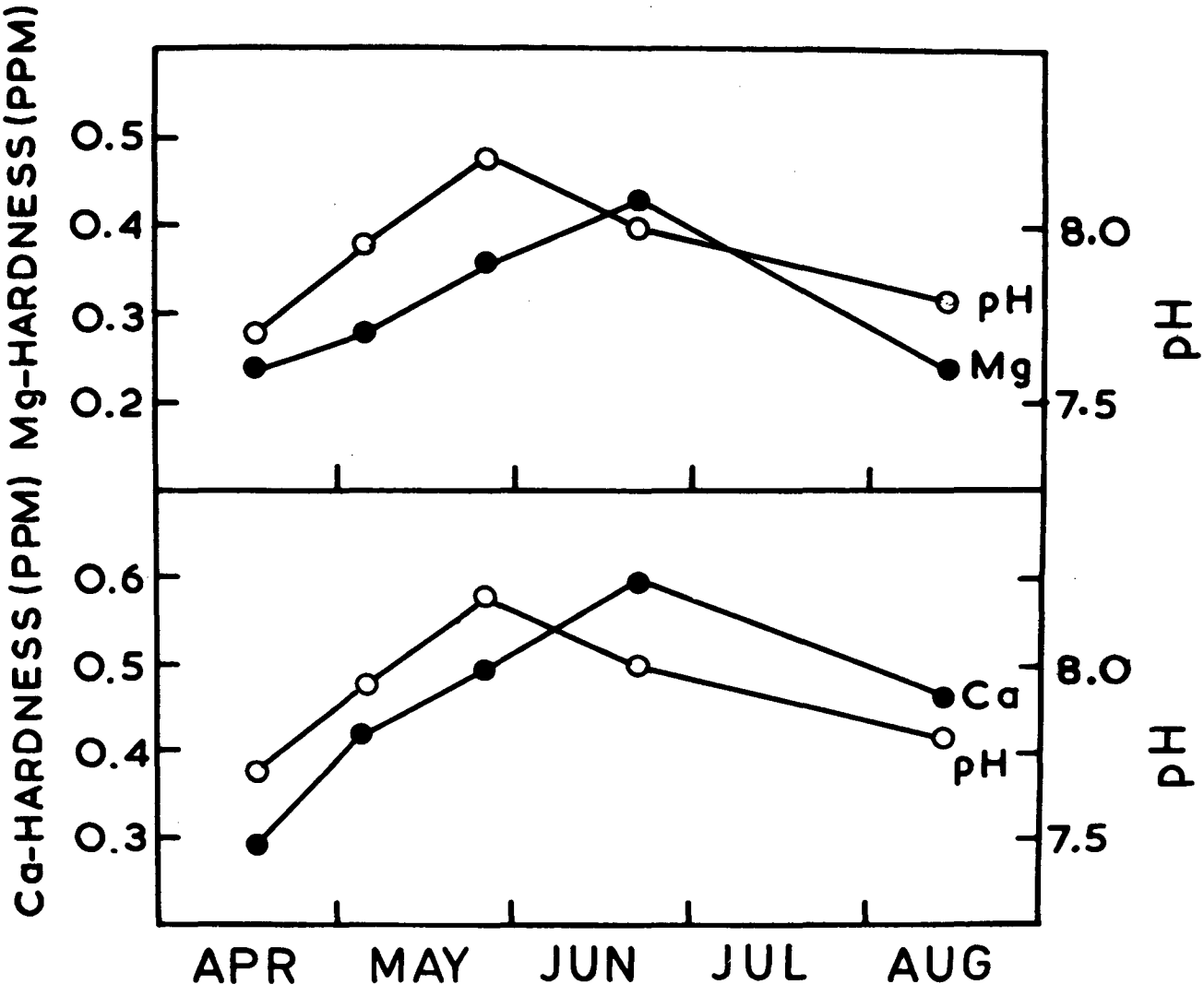

Ca-HARDNESS (PPM) Mg-HARDNESS (PPM)
0.5
0.4
0.3
0.2
8.0
7.5
pH
Mg
pH
0.6
0.5
0.4
0.3
8.0
7.5
Ca
pH
pH
APR
MAY
JUN
JUL
AUG

Fig. 11

factors were at its lowest value. Similarly, there were seen a high value at pH 7.7. All the nitrogen compounds at pH 7.8 attained a peak with a characteristic decline at pH 8.0. There was seen minor alteration in all chemical figures with corresponding change at variable pH value (Fig. 12).

iii. pH and PO_4, SO_4 - Phosphate and sulphate showed a direct correlation with H-ion concentration (Fig. 12). PO_4 and SO_4 were at its lowest value at pH 8.0-8.2. There was seen a high value of PO_4 and SO_4 at pH 7.7. However, at pH 7.8 the maximum values were recorded.

iv. Ca, Mg, hardness and nitrate, nitrite and ammonical nitrogen - A critical appraisal of Fig. 13 indicates that the calcium hardness of the water has got a definite and direct correlation with nitrogen compounds. This correlation is more marked when values for calcium hardness is compared with that of NO_3 and NO_2 nitrogen compounds. At the end of June while the total nitrogen concentration was lowest the Ca-hardness in contrast, was at its highest peak. Like the calcium-hardness, the magnesium-hardness also showed a relation to different nitrogenous compounds. A close examination of the curves (Fig. 13) shows a situation like 'ping-pong game'. A more or less similar situation was seen in relation to Mg-hardness. The relative separation peaks between magnesium hardness and different nitrogen

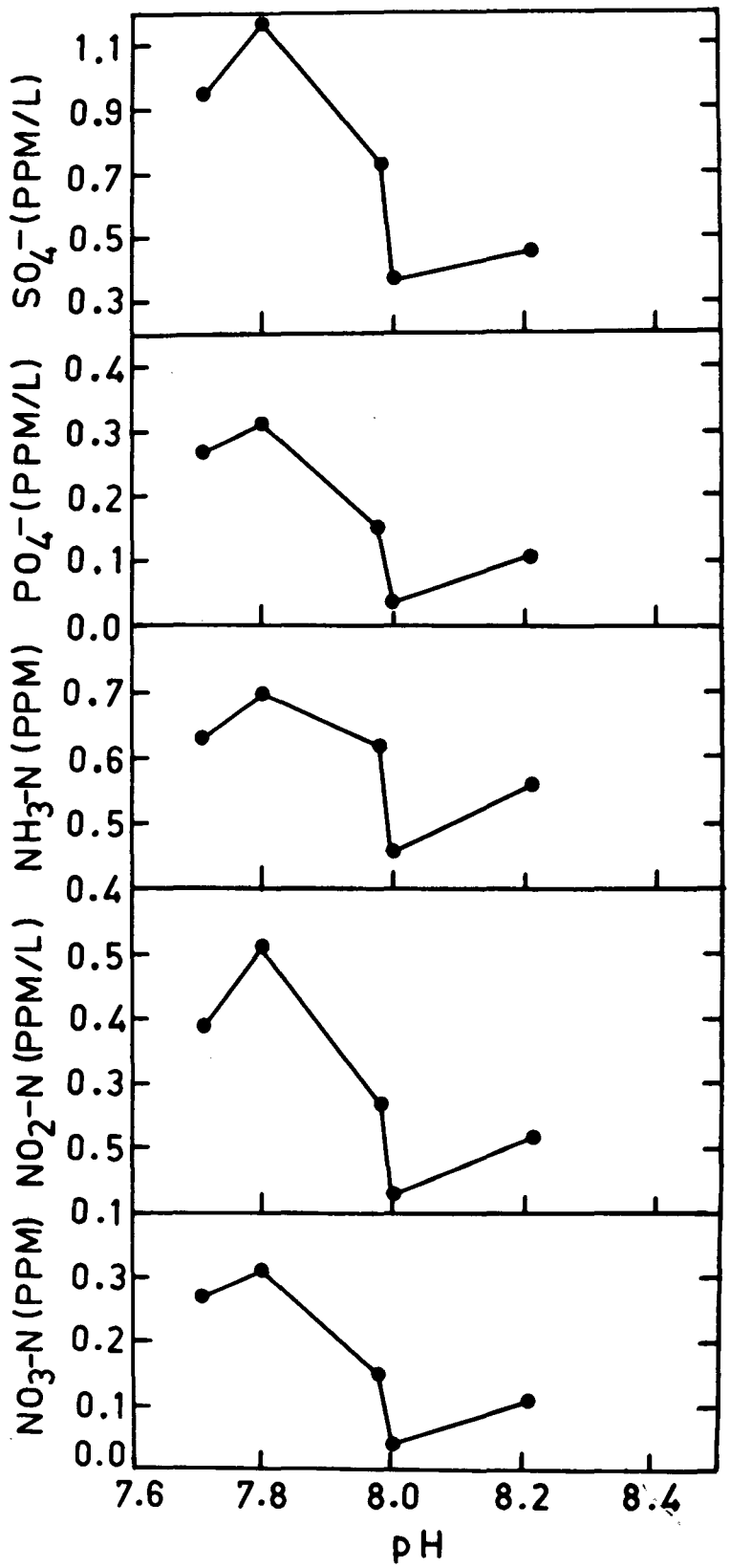

SO_4^-(PPM/L)
1.1
0.9
0.7
0.5
0.3
PO_4^-(PPM/L)
0.4
0.3
0.2
0.1
0.0
NH_3-N (PPM)
0.7
0.6
0.5
0.4
NO_2-N (PPM/L)
0.5
0.4
0.3
0.5
0.1
NO_3-N (PPM)
0.3
0.2
0.1
0.0
7.6
7.8
8.0
8.2
8.4
pH

Fig 12

Fig. 13 : Interrelationships between Ca, Mg-hardness and different inorganic nitrogen sources.

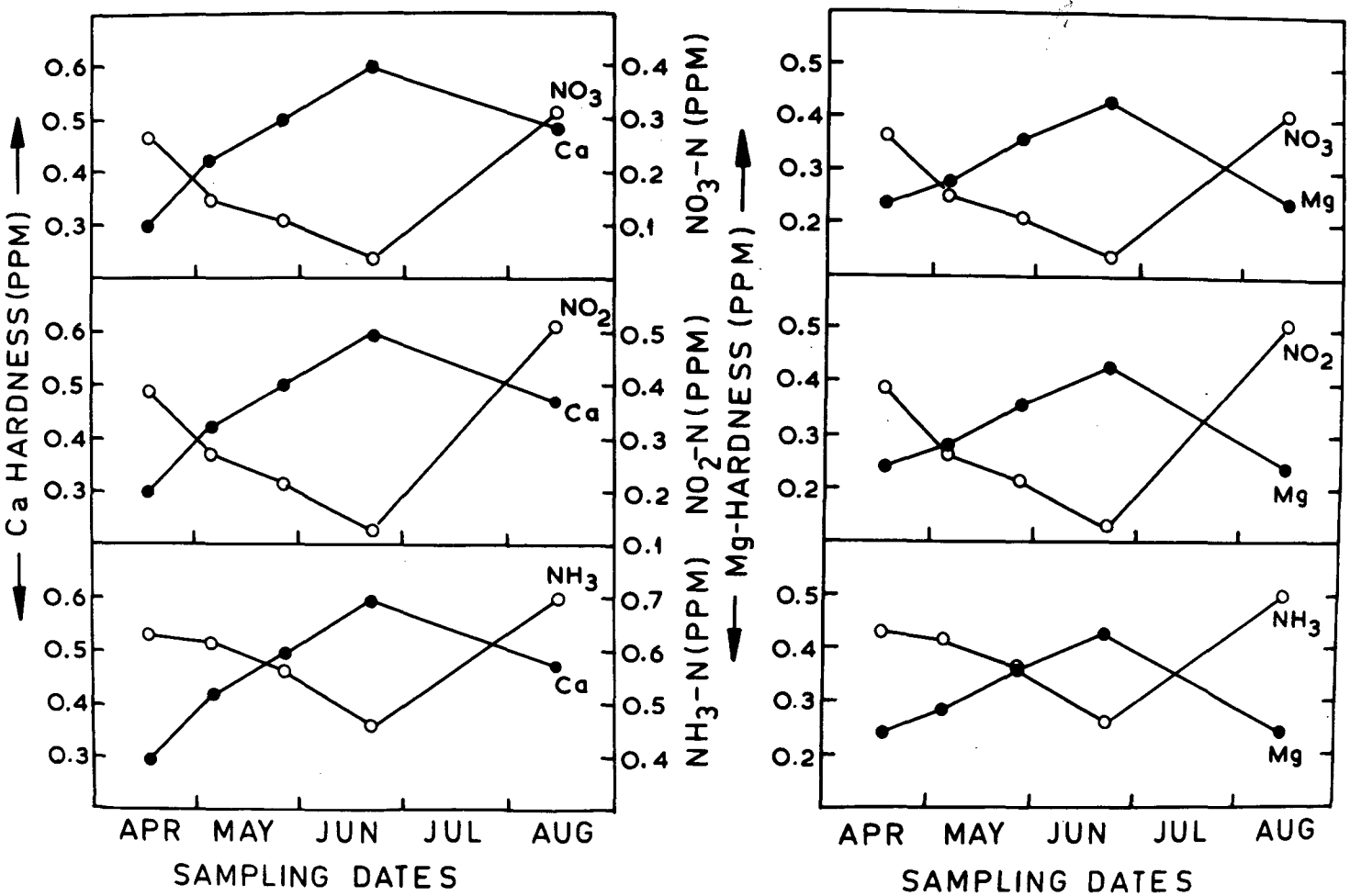

Ca HARDNESS (PPM)
NO3-N (PPM)
NO2-N (PPM)
NH3-N (PPM)
Mg-HARDNESS (PPM)
NO3
Ca
NO2
Ca
NH3
Ca
NO3
Mg
NO2
Mg
NH3
Mg
APR MAY JUN JUL AUG
SAMPLING DATES
APR MAY JUN JUL AUG
SAMPLING DATES

Fig.13

Fig. 14 : Interrelationships between different nitrogenous sources (NH_3^+, NO_3^- and NO_2^-).

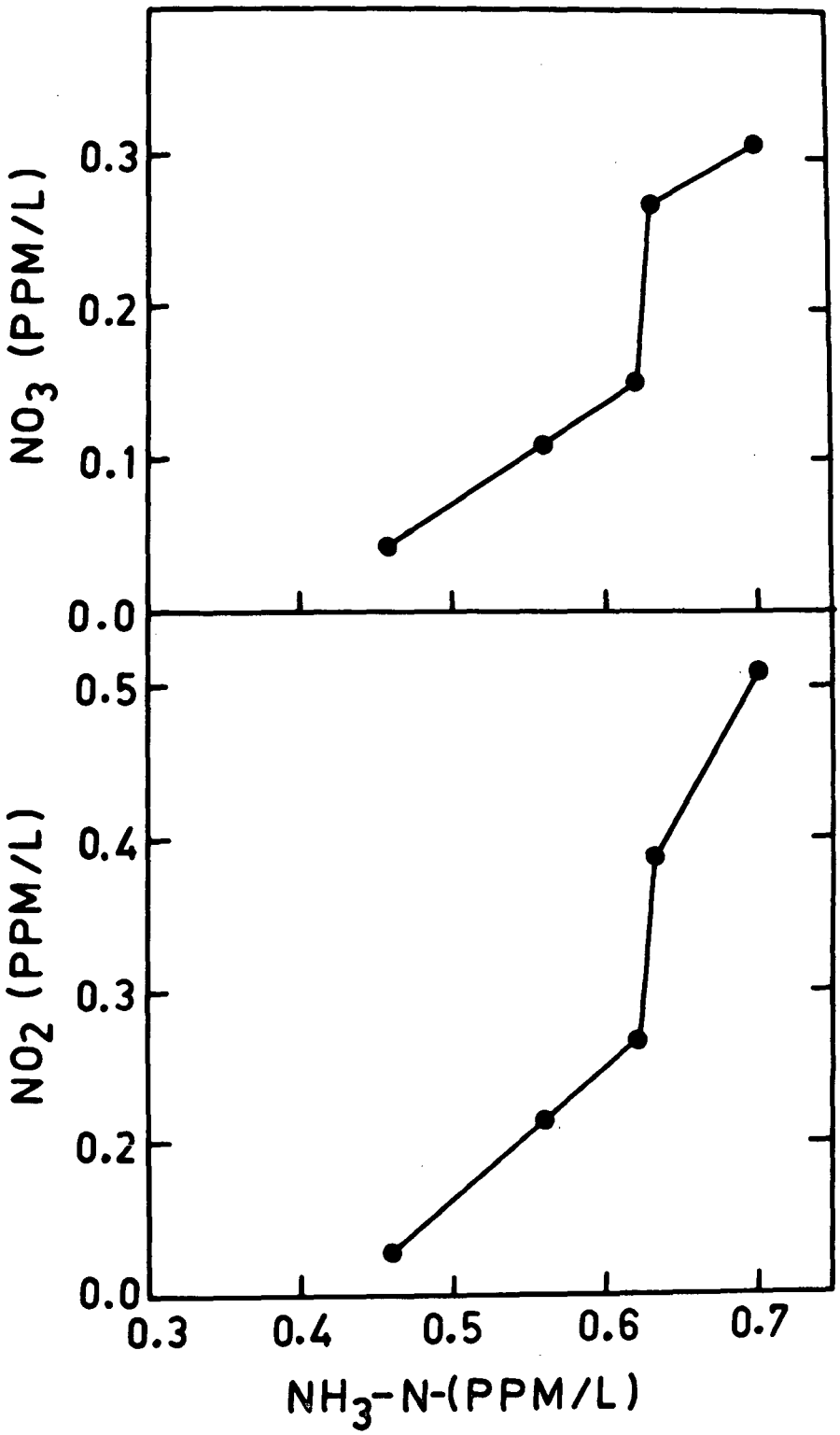

0.3
0.2
0.1
0.0
NO_3 (PPM/L)
0.5
0.4
0.3
0.2
0.0
NO_2 (PPM/L)
0.3
0.4
0.5
0.6
0.7
NH_3-N-(PPM/L)

Fig.14

concentrations were not distinctly apart and once again while the nitrogen factors were lowest in the end of June the magnesium-hardness was highest. Characteristically, the trend was quite different in April and August when the concentration of calcium, magnesium hardness were lowest, where as the NO_3, NO_2 and NH_3 factors were on higher side.

v. Ammonia, nitrate and nitrite nitrogein - An attempt was made to correlate the ammonical nitrogen against NO_2^- and NO_3^- nitrogen . It was found that NO_2^- and NO_3^- concentrations had a positive correlation with that of ammonical nitrogen. All the three nitrogen factors were either lowest or highest at the same time. There were seen absolutely no variation between three factors and they almost ran parallel (Fig. 14).

BIOLOGICAL PROPERTIES OF LAKE WATER:

i. Table II gives the list of algae encountered in the water. The taxonomic consideration of the blue-green algae is given below:

Oscillatoria chalybea (Mertens) Gomont
(Plate I, Fig. 7)

Mertens in Jurgens, Alg. aquaticae, Decas, 12, nr. 4, 1822; Gomont, Monogr. Oscillariees, 232, pl. 7. fig. 19, 1892; Fremy, Myxo, d'Afr. equat. franc., 224, fig. 196, 1929; Forti in De Toni, Sylloge Algarum, 5 : 185, 1907;

TABLE II

LIST OF BLUE-GREEN ALGAE ENCOUNTERED IN ENRICHMENT CULTURES

Oscillatoria chalybea	*Nostoc spongiaeforme*
Lyngbya nigra	*Nostoc piscinale*
Phormidium tenue	*Anabaena oryzae*
Phormidium africanum	*Anabaena fertilissima*
Phormidium molle	*Anabaena naviculoides*
Phormidium foveolarum	*Anabaena variabilis*
Phormidium laminosum	*Anabaena vaginicola*
Nostoc commune	
Nostoc punctiforme	
Nostoc linckia	

Plate I : Camera leucida sketch of filamentous blue-green algae.

Fig. (1) *Phormidium tenue*, (2) *P. molle*, (3) *P. africanum*; (4) *P. foveolarum*, (5) *P. laminosum*; (6) *Lyngbya nigra*; (7) *Oscillatoria chalybya*.

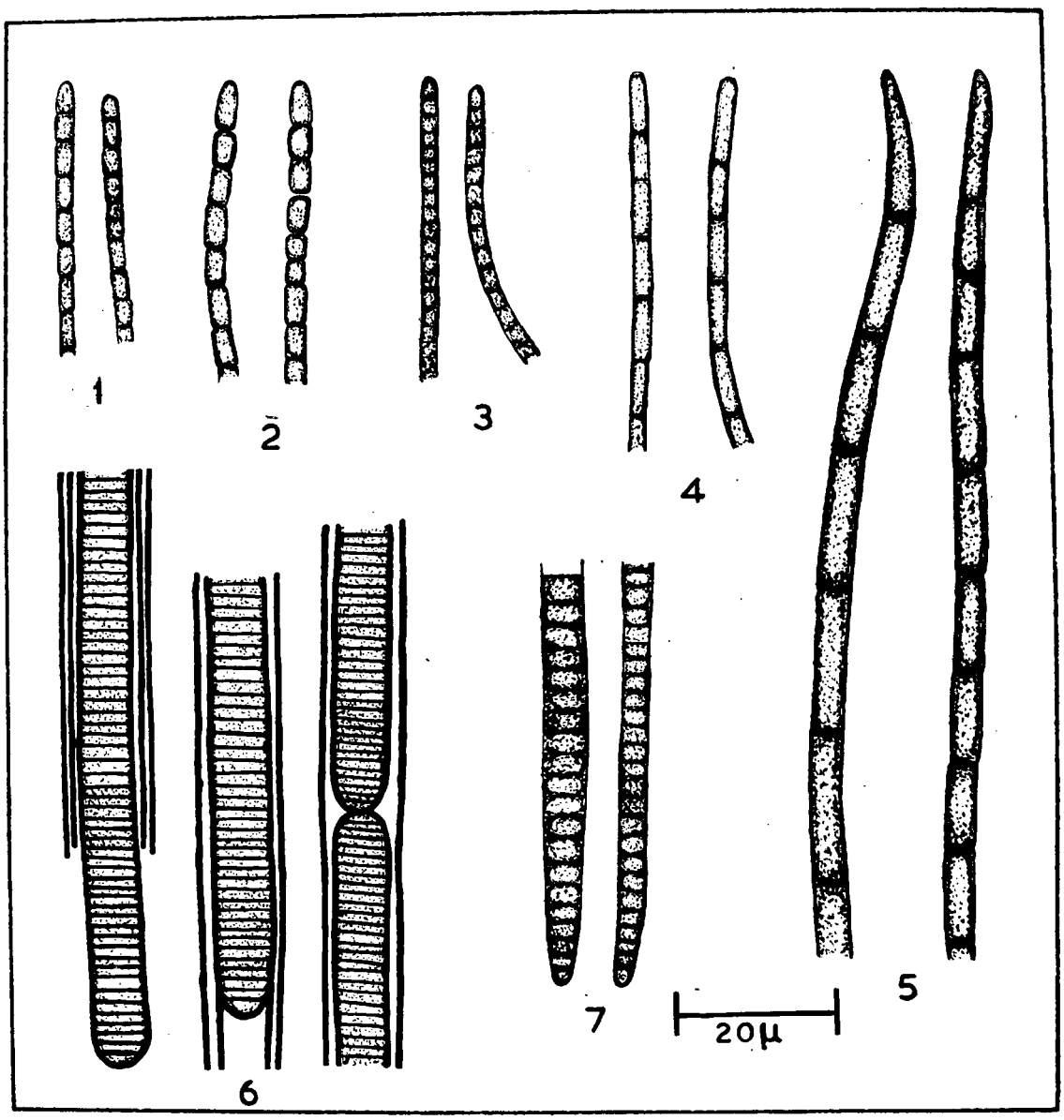


Plate I

Geitler, Kryptogamenflora, 956, fig. 608b, 1932; Fremy, Cyano, cotes d'Fur., 127, 1933. incl. Oscillatoria subsalsa Ag., Sp. Alg., 66, 1925.

Trichome straight or lightly coiled, slightly constricted at the cross walls, slightly attenuated at the apex and some what bent, 3.6 to 4.4 μ broad and 3.6 to 8 μ long, septa not granulated and end cell obuse, not capitate; without calyptra.

Lyngbya nigra C. Ag. ex Gomont
(Plate I, Fig. 6)

Agardh. C., Syst. Alg., 312, 1824; Gomont, Monogr. Oscillariees, 145, pl. 3, fig. 16, 1892; Geitler, Kryptogamenflora, 1063, Fig. 675a, 1932.

Filaments long, straight sheath thin, colourless, not lamellated; trichome 6 to 8 μ broad, not constricted at the cross walls, septa not granulated, yellowish green, ends attenuated, cells 2-4 μ long, content faintly granular, apical cell round.

Phormidium tenue (Menegh.) Gomont
(Plate I, Fig. 1)

Monogr. Oscillariees, 169, pl.4, figs. 23-25, 1892; Forti in De Toni, Sylloge Algarum, 5: 227, 1907; Fremy, Myxo, d'Afr. equat. franc., 146, fig. 131, 1929; Geitler, Kryptogamenflora, 1004, fig. 642d,e. 1932; Fremy, Cyano, cotes d'Eur., 88, pl. 23, fig. 4, 1933.

Trichome straight densely entangled, slightly constricted at the cross walls, attenuated at the ends; 1.5 to 2 μ broad, pale blue green cell upto 3 times longer than broad, 2.5 to 5 μ long septa not granulated, end cells truncated; calyptra absent.

Phormidium africanum Lemm.
(Plate I, Fig. 3)

Deutsche Zentr. Afr. Exped. 2: 89, 1911; Fremy, Myxo, d'Afr. equat. franc., 138, 1929; Geitler, Krytogamenflora, 999, 1932.

Filaments straight slightly bent, sheath diffluent; trichome pale blue green, slightly constricted at the cross wall, not attenuated at the end. 1.5-2 μ broad cells, quadrate, or rectangular, 2-3 μ long and the cells truncated; calyptra absent.

Phormidium molle (Kutz.) Gomont
(Plate I, Fig. 2)

Monogr. Oscillariees, 163, pl.4, fig. 12, 1892; Forti in De Toni, Sylloge, Algraum, 5: 219, 1907; Fremy, Myxo, d'Afr. equat. franc., 138, fig. 119, 1929; Geitler, Krypto-gamenflora, 1000, 1932; Fremy, Cyano, cotes d'Eur, 85, pl, 22, fig. 5, 1933.

Trichome straight or variously bent, distinctly constricted at the cross walls, not attenuated at the ends, 1 - 1.6 u (-2.5 μ) shorter or longer than broad. 2.4-5.4 μ long end cell rounded; calyptra absent.

Phormidium foveolarum (Mont.) Gomont
(Plate I, Fig. 4)

Monogr. Oscillariees, 164, pl. 4, fig. 16, 1892; Forti in De Toni, Sylloge Algarum, 5: 221, 1907; Fremy, Myxo, d'Afr. equat. franc., 139, fig. 121, 1929; Geitler, Kryptogamenflora, 999, fig. 636, c,d, 1932.

Trichome highly flexuous, constricted at cross walls, ends not attenuated, about 2-2.4 μ broad, light blue green; sheath colourless diffluent in an amorphos gelatinous mucilage; cells nearly quadrate or somewhat shorter than broad; septa not granulated; end cell rounded; calyptra absent.

Phormidium laminosum Gomont
(Plate I, Fig. 5)

Essai class, Nostoc. hom., J. de Bot., 4: 355, 1890. Monogr. Oscillariees, 167, Pl. 4, figs 21,22, 1892., Forti in De Toni Sylloge Algarum, 5: 225, 1907, Fremy, Myxo, d'Afr. equat. franc., 146, fig. 130, 1929, Geitler, Kryptogamenflora, 1005, fig. 642c, 1932, Fremy, Cyano, Cotes d'Ear., 88, Pl. 23, fig. 3, 1933.

Filaments flexuous, densely entangled, sheath thin, diffluent, trichome pale blue green slightly constricted at cross walls, cross walls with fine granules, 1.5-3 μ broad; cells longer than broad 3-5 μ long; end cells conical; without calyptra.

Plate II : Camera leucida sketch of *Nostoc* species:

Fig. (1) *N. commune* ; (2) *N. spongiaeforme*;

(3) *N. linckia*.

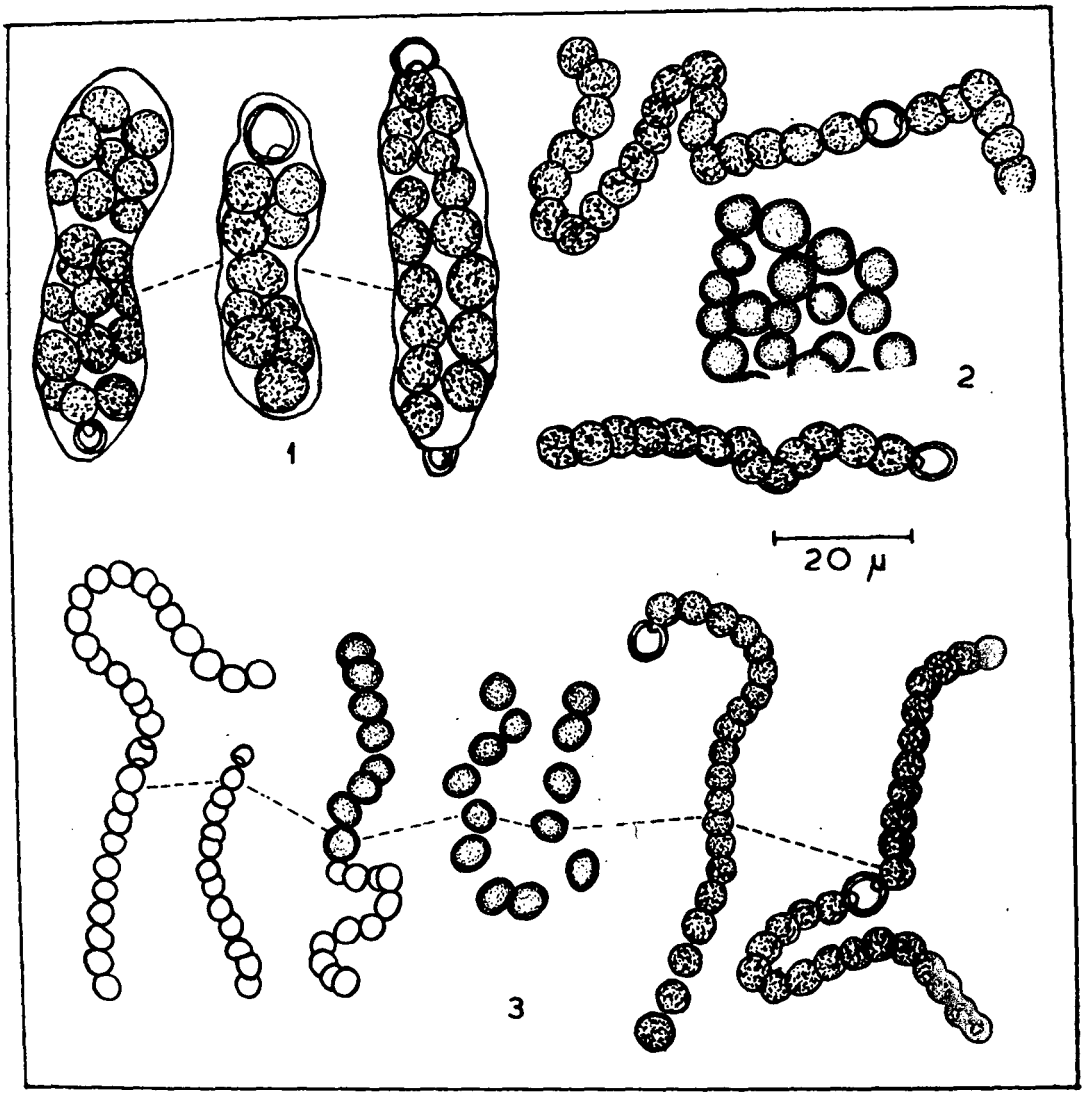


Plate II

Nostoc commune Vaucher ex Born. et Flah.
(Plate II, Fig. 1)

Vaucher, Histoire des Conferves d'eau douce, 222, pl. 16, fig. 1 (mala), 1803; Bornet et Flahault, Revision des Nostocacees heterocystees, 203, 1888; Forti in De Toni, Sylloge Algarum, 5, 404; 1907; Fremy, Myxo. d'Afr. equat. franc; 342, fig. 283, 1929; Geitler, Kryptogamenflora, 845, fig. 536, 537, 1932; Fremy, Cyano, cotes d'Eur., 177, Pl. 58, fig. 3, 1933.

Thallus firm, gelatinous, at first globose, later flattened, many centimeters in diameter; blue-green turning brown when cultures were old; filaments flexuous, highly entangled; sheath distinct, thick, yellowish brown, homogenous, hyaline; trichome 3.8 μ - 9.6 μ broad; cells short spherical or short barrel shaped; heterocysts nearly spherical, 3.8-7 μ broad and 3.6-6.0 μ long; spores not seen.

Nostoc punctiforme (Kutz.) Hariot
(Plate III, Fig. 2)

J. de Bot., 5: 31, 1891; Forti in De Toni, Sylloge Algarum, 388, 1907; Fremy, Myxo. d'Afr. equat. franc., 331, fig. 274, 1929; Geitler, Kryptogamen-flora, 834, 1932.

Thallus forming irregular blue green lobed colonies; filaments flexuous densely packed into loose net like masses; sheath not clearly seen; trichome 3.6-4.4 μ broad; cells oblong to spherical, heterocysts 2.4-3.8 μ broad; spores globose or spherical 4.6-6.2 μ broad and 4.6-7.4 μ long, mostly in cutinate chain; epispore thick smooth and brown.

Plate III : Camera leucida sketch of Nostoc species:

Fig. (1) N. piscinale; (2) N. punctiforme;.

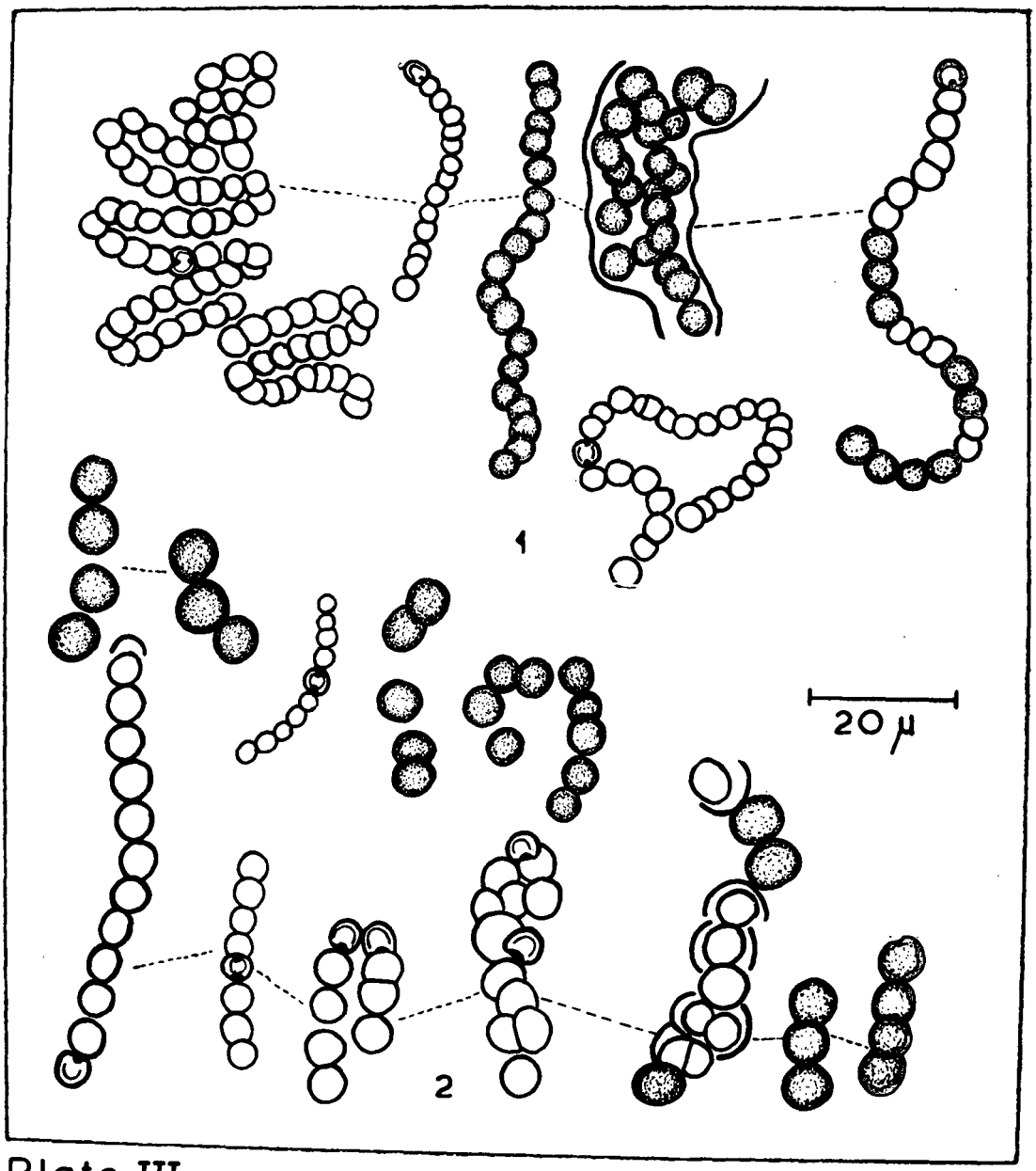


Plate III

Nostoc linckia (Roth) Bornet ex Born. et Flah.
(Plate II, Fig. 3)

Bornet in Bornet and Thuret, Notes algologiques, II., 86, Pl. 18, figs 1-12, 1880; Born et Flah., Revision des Nostocacees heterocystees, 192, 1888; Forti in De Toni, Sylloge Algarum, 5: 391, 1907; Fremy, Myxo, d. Afr. equat. franc., 332, fig. 276, 1929; Geitler, Kryptogamenflora, 838, fig. 528b, 1932; Fremy, Cyano, cotes d'Eur., 175, Pl. 58, fig. 1, 1933.

Thallus gelatinous varying in size when young blue green but turning yellow on maturity; filaments numerous, highly flexuous; trichome 3.8 -4.4 μ broad, sometimes embedded in a light yellow brown hyaline mucilaginous sheath; heterocyst almost spherical, 4.4-6 μ broad; spores in short chains, spherical 5.4-6.6 μ broad, outer wall brown.

Nostoc spongiaefome Agardh ex Born. et Flah.
(Plate II, Fig. 2)

C. Ag., Syst., Alg. 22, 1824; Bornet and Flahault, Revision des Nostocaceos heterocystees, 197, 1888; Forti in De Toni, Sylloge Algarum, 5: 397, 1907; Fremy, Myxo, d'Afr. equat. franc., 337, fig. 279, 1929; Geitler, Kryptogamenflora, 839, fig. 531, 1932 (non Tilden).

Thallus gelatinous brown black, sheath hyaline turning yellow on maturity usually diffluent; trichomes densely entageled 4-5.2 μ broad cells spherical or ellipsoidal 4.8-5.6 μ long; heterocyst spherical sometimes ellipsoidal 4-5.6 μ broad and 4.6-5.8 μ long; spores spherical or semispherical sometime ellipsoidal 4.9-6.2 μ broad and

4.6 - 6.6 μ long, outer membrane smooth and hyaline.

Nostoc piscinale Kutzing ex Born. et Flah.
(Plate II, Fig. 1)

Kützing, Phyc. gene. 208, 1843; Bornet and Flahault, Revision des Nostocacees heterocystees, 194, 1888; Forti in De Toni, Sylloge Algarum, 5: 393, 1907; Fremy, Myxo, d'Afr. Equat. franc., 335, fig. 277, 1929; Geitler, Kryptogamenflora, 838, fig. 529, 1932.

Thallus globose later flattened mucilaginous, light blue green, turning brown when old; filaments highly fluxuous loosely entangled; sheath distinct at the periphery of the thallus, brownish diffluent in inner portions, hyaline; trichome 3-7 μ broad, cells shorter or longer than broad; heterocyst spherical or sub-spherical 5.2-7 μ broad; spores globose 6-10 μ broad; epispore smooth and hyaline with a sheath.

Anabaena oryzae Fritsch
(Plate IV, Fig. 2)

The genus Anabaena, J. Indian bot. Soc., 28: 135, figs. 1-16, 1949; = A. gelatinosa Fritsch ex De 1939 (nomen nudum) non Reinsch non Wood,

Thallus soft, green, gelatinous; trichomes straight and densely aggregated 2.8-3 μ broad, more or less barrel shaped 3-6.2 μ long; heterocysts both terminal and intercalary 4-4.4 μ broad, 5-5.6 μ long, terminal ones conical and

Plate IV : Camera leucida sketch of *Anabaena* species :
(1) *A. variabilis*; (2) *A. oryzae*.

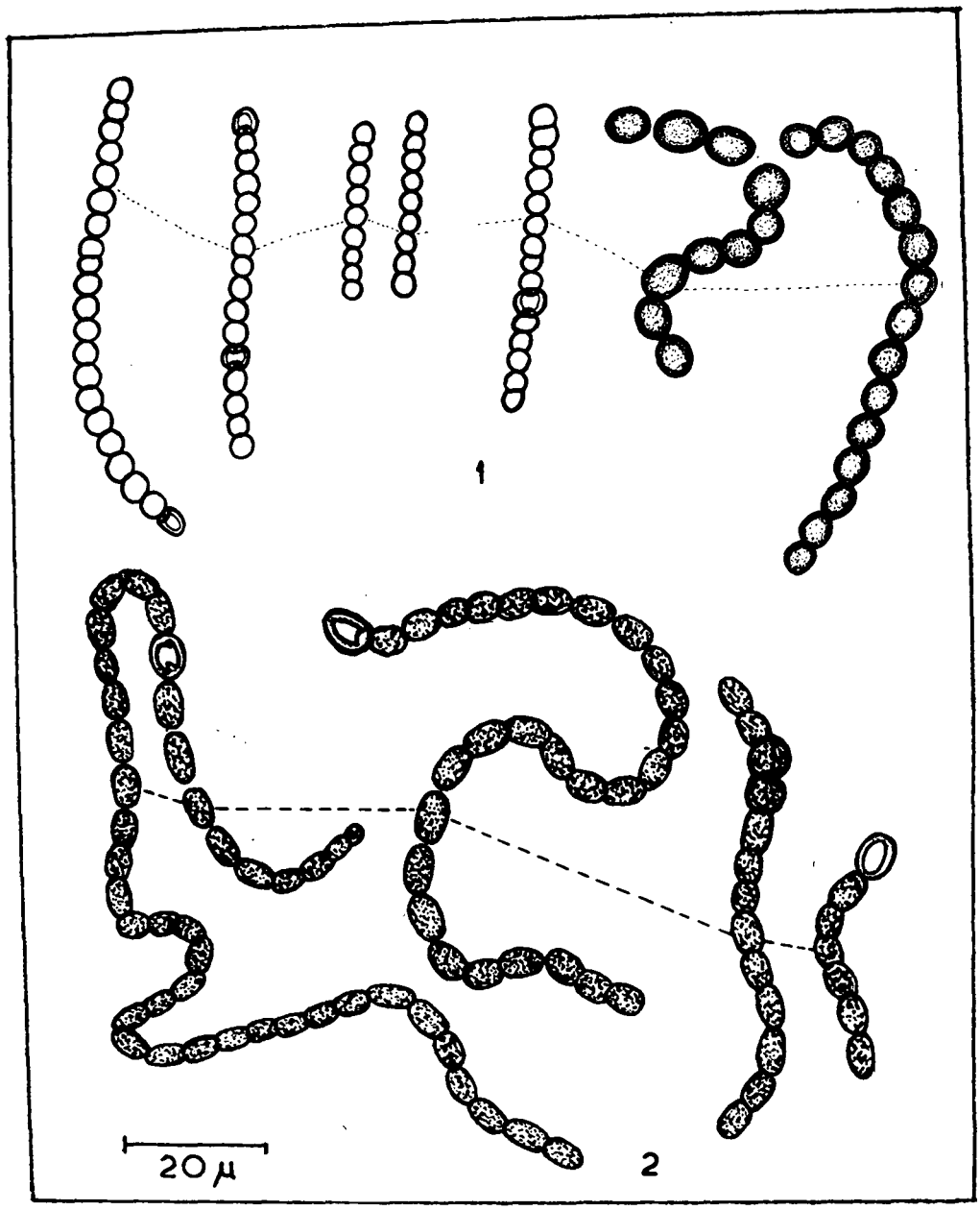


Plate IV

intercalary ones single or 2-3 in series generally barrel shaped very rarely spherical and single, spores rarely seen either singly or in short chain of 2-3; sub-spherical or ellipsoidal 4.6-5.2 μ broad and 4.8-5.4 μ long, exospore light brown.

Anabaena fertilissima Rao, C.B.
(Plate V, Fig. 3)

The Mysophyceae of the United Provinces, III, Proc. Indian Acad. Sci., B.6: 363, fig. 6A-C, 1937b.

Trichome single densely bent, with almost rounded end cells, 3.8-4.4 μ broad; cells mostly globular, sometimes barrel shaped; heterocyst almost spherical rarely barrel shaped 4.0-6.0 μ broad; spores in long chains often making the whole trichome sporogenous, almost spherical with smooth, hyaline outer wall, 4.4-8 μ broad and 3.2-8 μ long.

Anabaena naviculoides Fritsch
(Plate V, Fig. 1)

The genus Anabaena etc., J. Indian bot. Soc., 28: 138, figs. 17-39, 1949.

Thallus slightly geltinous deep blue green, trichome short more or less coiled; cells 2.8-3.2 μ broad, as long as broad or slightly longer 2.4-4.4 μ long; apical cell obtuse conical or acute; heterocyst on both the terminal ends, rarely intercalary, barrel shaped 4 to 6 μ broad as long as or slightly longer than broad; spores not seen.

Plate V : Camera leucida sketch of *Anabaena* species:

Fig. (1) *A. naviculoides*; (2) *A. vaginicola*;

(3) *A. fertilissima*.

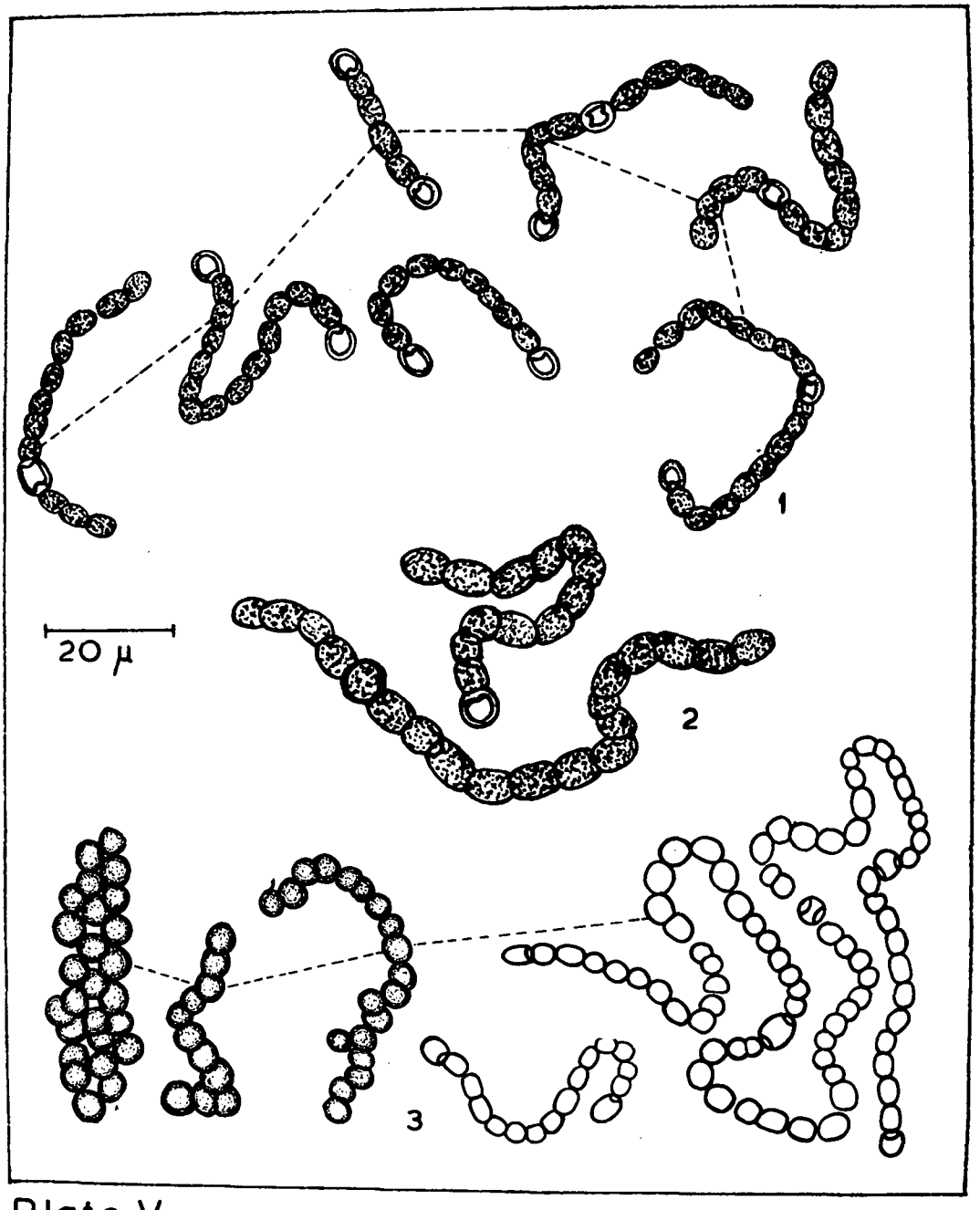


Plate V

Anabaena variabilis Kutzing ex Born. et Flah.
(Plate IV, Fig. 1)

Kützing, Phys. gene, 210, 1843; Bornet and Flahault, Revision des Nostocacees heterocystees, 226, 1888; Forti in De Toni, Sylloge Algarum, 5, 437, 1907; Fremy, Myxo. d'Afr. equat. franc. 360, fig. 294, 1929; Geitler, Kryptogamenflora, 876, fig. 558, 1932.

Trichome without any definite sheath, flexous 3.8-4.2 μ broad, slightly constricted at the cross walls; cells conical or obtuse very rarely with gas vacuoles; heterocyst spherical, sometimes oval, 6 μ broad and upto 8 μ long; spores formed centrifugally, not contiguous with the heterocyst barrel shaped, spherical 5-6 μ broad 6-7 long, epispores smooth colourless or very light brown.

Anabaena vaginicola Fritsch et Rich fertilissima prasad
(Plate V, Fig. 2)

Contributions to our knowledge of the fresh water algae of Africa, 7, Fresh water algae from Girqualand West, Trans. roy. soc. s. Afr., 18(1): 87, 1929.

Thallus pale green, mucilaginous, trichomes blue green, rarely free, commonly one or several entangled in a diffluent mucilaginous sheath, cells barrel shaped 4.5-6.5 μ broad, 4-6 μ long, constricted at cross walls, heterocyst conspicuous spherical or barrel shaped. 6-7.5 μ broad and 5-6.5 μ long, spores rare, ellipsoidal 6.4-8 μ broad, Sporulation commencing away from the heterocyst.

ii. GENERIC VARIATIONS IN BLUE-GREEN ALGAE:

Filamentous blue green alga Phormidium had a distinct preponderence over other blue green algae. Phormidium, Anabaena and Nostoc were found throughout the course of this investigation. As many as 5 species each of Phormidium, Anabaena and Nostoc were recorded under laboratory conditions. One species each of Oscillatoria and Lyngbya were isolated on synthetic medium.

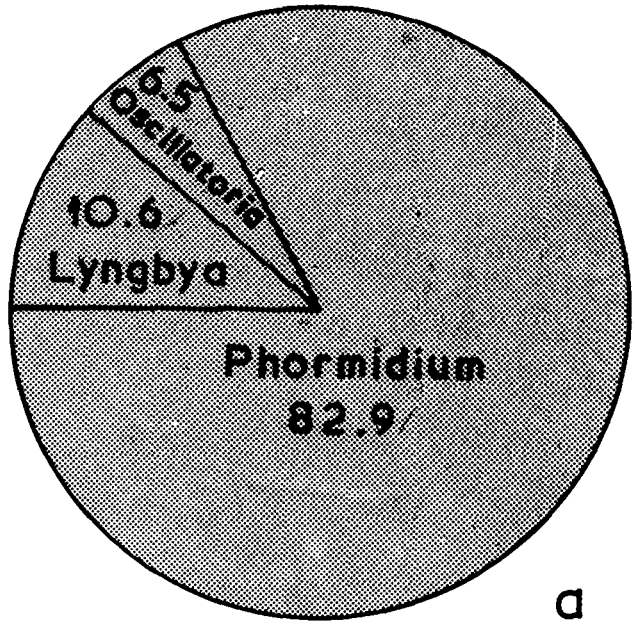
The total mass of Phormidium was 82.9% where as 10.6 and 6.5% respectively were recorded for Lyngbya and Oscillatoria (Fig. 15a). A comparison of the per cent occurrence of the established dinitrogen fixing and non-dinitrogen fixing blue green algae showed that they were almost equal in proportions. A rough calculation indicated the established nitrogen fixing forms were 50.05% while those of non-nitrogen fixing strains were 49.95% (Fig. 15b).

iii. CHLOROPHYLL-a ESTIMATIONS IN BLUE-GREEN ALGAE:

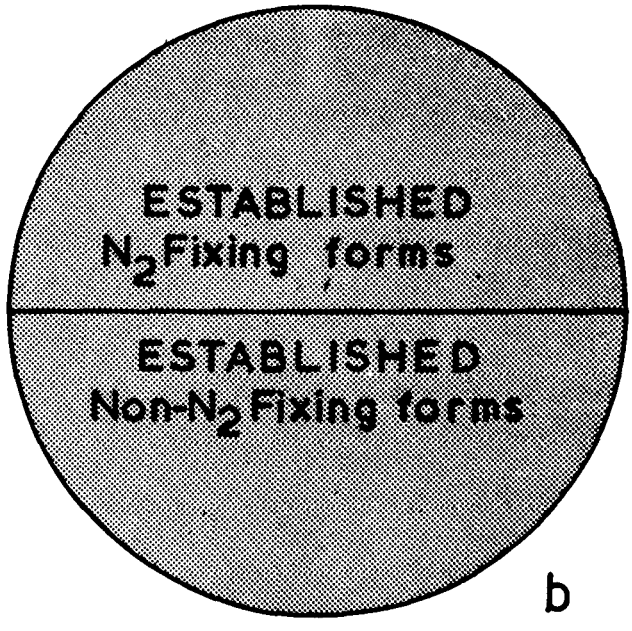
Fig. 16 shows that the total chlorophyll a contents were almost steady in the peak summer months (May and June), however, there were seen an increase in the month of August. The overall values of chlorophyll a varied from 12.44 to 417.62 µg/l. A critical study of Fig. 16a indicates a great deal of variation in the chlorophyll contents in samples collected at different placed within the lake. Analysis of the chlorophylla contents showed a great deal of variations

Fig. 15(a): A diagrammatic representation of percent occurrence of non-nitrogen fixing blue-green algae.

(b): Percent occurrence of nitrogen fixing and non-nitrogen fixing blue-green algae.


6.5
Oscillatoria
10.6
Lyngbya
Phormidium
82.9%

a


ESTABLISHED
N_2Fixing forms
ESTABLISHED
Non-N_2Fixing forms

b

Fig.15

Fig. 16(a) : Chlorophylla contents of blue-green algal cells in the water samples from different location of lake.

(b) : A diagrammatic view to depict the total chlorophyll a contents in algal mat during summer; premonsoon and monsoon periods (Chlorophyll a, contents were calculated on 6 litre basis).

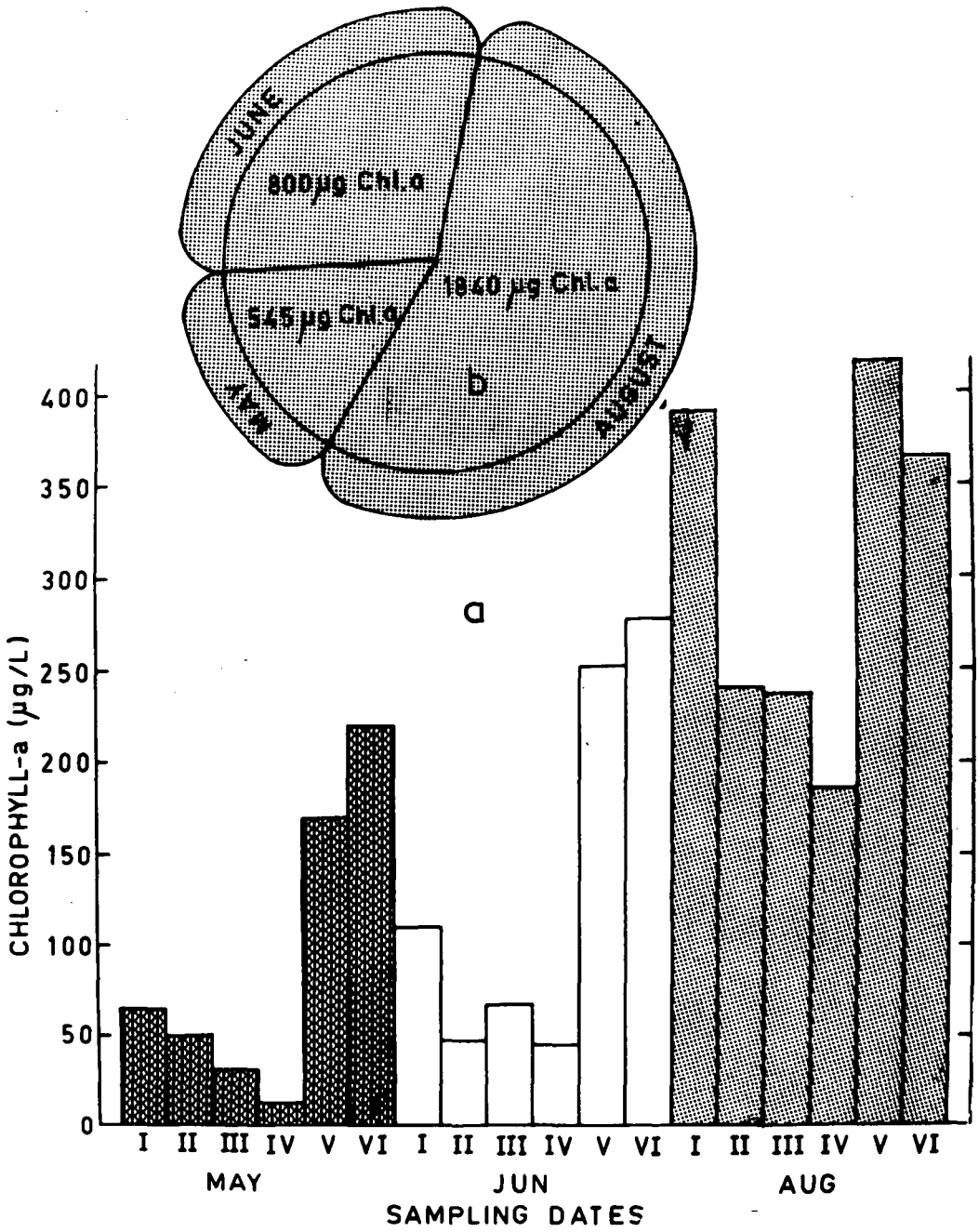

JUNE
800µg Chl.a
545 µg Chl.a
1840 µg Chl.a
MAY
AUGUST
b
a
CHLOROPHYLL-a (µg/L)
400
350
300
250
200
150
100
50
0
I II III IV V VI I II III IV V VI I II III IV V VI
MAY
JUN
AUG
SAMPLING DATES

Fig.16

in the samples collected even the same day. In the month of May, the high values of chlorophyll a were recorded in sample V (169.13 µg/l) and sample VI (220.98 µg/l). Sample IV showed the lowest value of chlorophyll a (12.44 µg/l). Samples collected in the month of June showed an overall increase in chlorophyll a contents. During this period the values for V and VI were 417.62, 364.35 µg/l, respectively. Quite contrast to previous observations the water samples collected during August showed significant increase in the overall chlorophyll a contents which is a direct reflection on the growth and development of blue-green algae, in lake water. Samples I, V and VI showed a values of 390.8, 417.62 and 364.35 µg/l respectively.

Fig. 16b indicates an estimation of chlorophyll a one of the important pigment constituents of blue-green algae. This directly reflects the cell population of blue-green algal cells in the lake water at a given time. This observation indicates that the maximum concentration of healthy cells occurs during the monsoon time i.e. during the month of August.

An attempt to compare to average chlorophyll a contents against H-ion concentration of lake waters revealed an interesting observation. When H-ion concentration was on alkaline side the chlorophyll a content was minimum and as the value of pH changed to acidic condition, chlorophyll a

concentration attained a highest peak (Fig. 17). Interestingly, the temperature and the chlorophyll-a content had a direct correlation. There was observed an increase in the chlorophyll a content with a rise in temperature of the lake waters (Fig. 17). In the month of May when the temperature was low the chlorophyll a content was also low and both attained a new high peaks in the month of August.

A comparison of chlorophyll a contents with that of ammonia, nitrate and nitrite concentrations in the lake water revealed a direct correlation. With the increase in nitrogenous compounds there were seen an increase in chlorophyll a content and the highest values for NH_3, NO_2, NO_3 against the average chlorophyll a contents were observed in the month of August (Fig. 17).

A more or less similar observation was recorded while analysing the chlorophyll a contents against the PO_4 and SO_4 concentrations of the lake water (Fig. 18).

COLIFORM BACTERIA:

The total counts for the coliform bacteria were estimated in the water collected from six different sampling areas of the lake in early May, June and in the month of August. Fig. 19 indicates that the counts varied from

Fig. 17 : A correlation between chlorophyll a dontent in blue-green algal mat with that of pH, different nitrogen sources and temperatures.

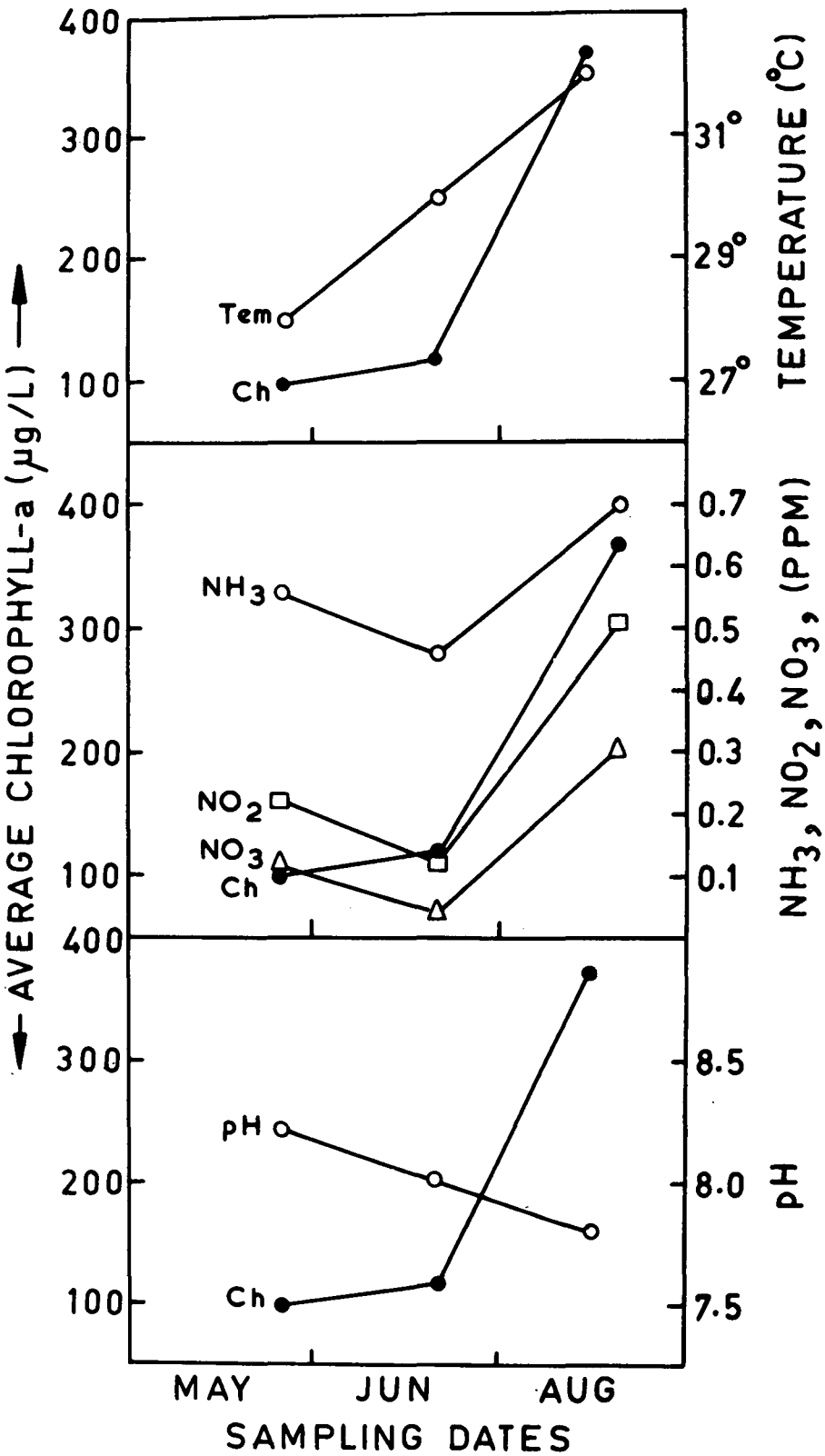

400
300
200
100
Tem
Ch
31°
29°
27°
TEMPERATURE (°C)
AVERAGE CHLOROPHYLL-a (µg/L)
NH3
NO2
NO3
Ch
0.7
0.6
0.5
0.4
0.3
0.2
0.1
NH3, NO2, NO3, (PPM)
pH
8.5
8.0
7.5
MAY
JUN
AUG
SAMPLING DATES

Fig.17

Fig. 18 : A comparison between chlorophyll a contents in the blue-green algal mat with that of PO_4 and SO_4^{--} ion concentrations.

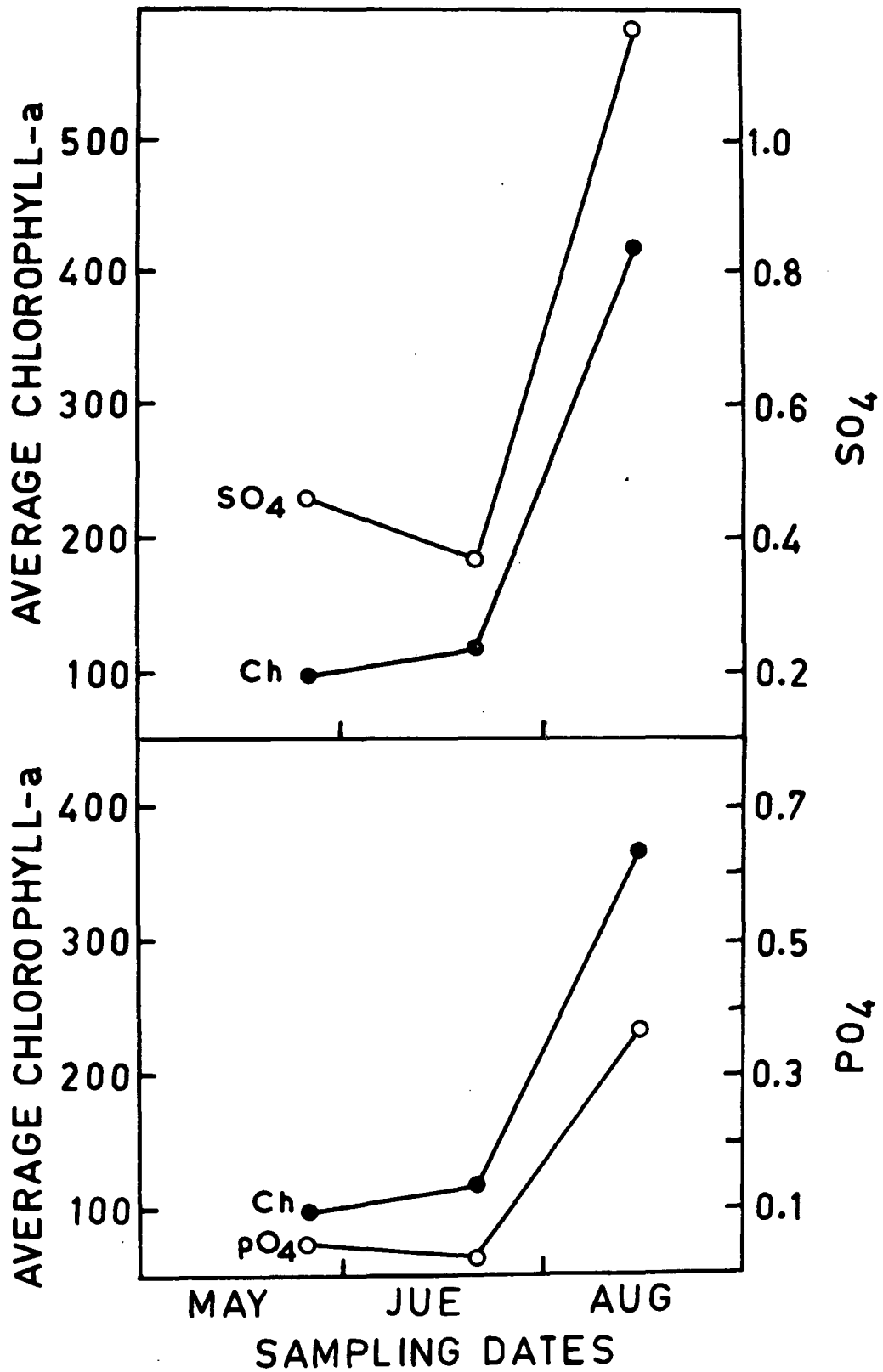

AVERAGE CHLOROPHYLL-a
500
400
300
200
100
1.0
0.8
0.6
0.4
0.2
SO4
SO4
Ch
AVERAGE CHLOROPHYLL-a
400
300
200
100
0.7
0.5
0.3
0.1
PO4
Ch
PO4
MAY
JUE
AUG
SAMPLING DATES

Fig.18

Fig. 19 : Counts of coliform bacteria in water samples.

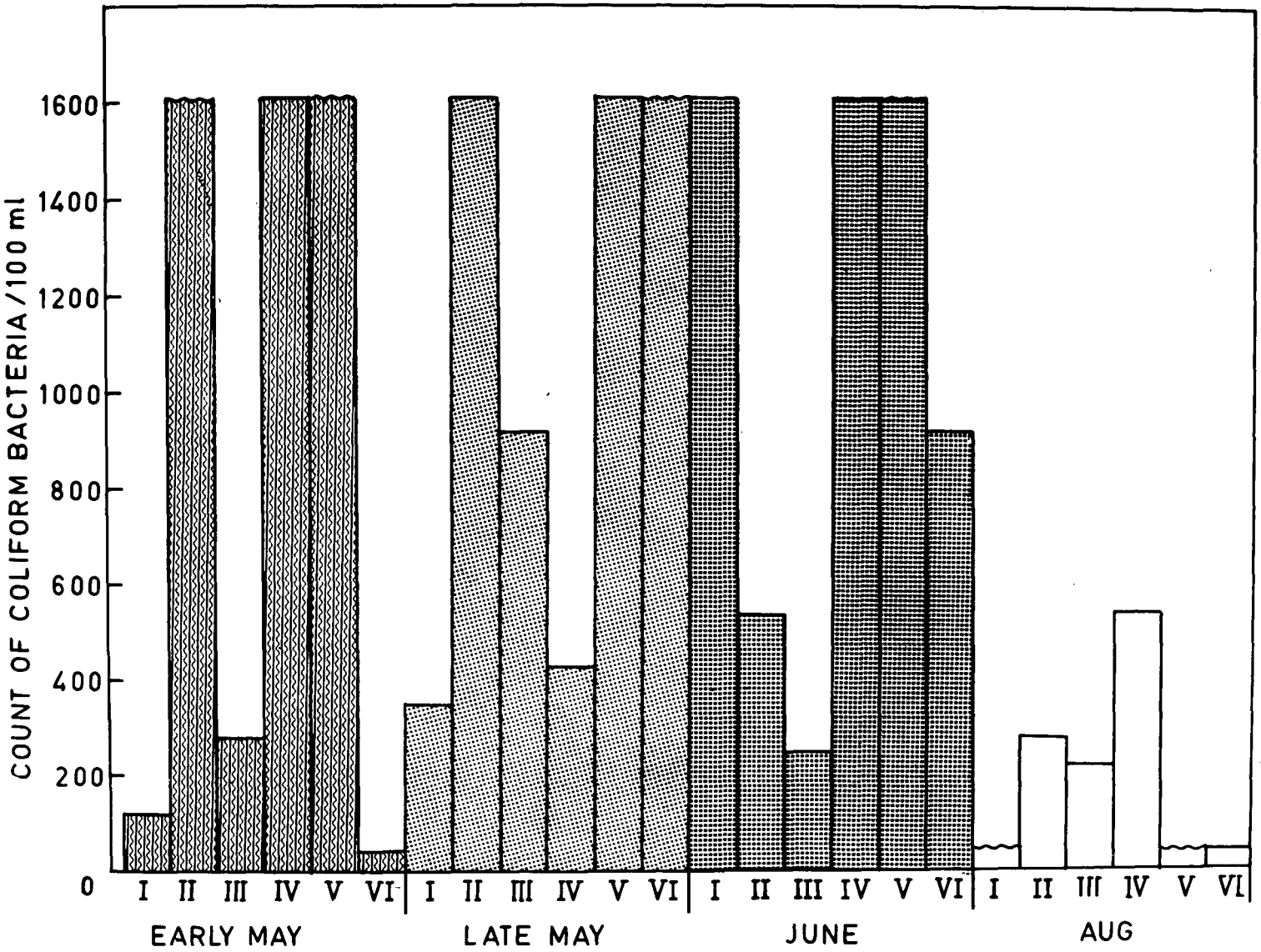

COUNT OF COLIFORM BACTERIA /100 ml
1600
1400
1200
1000
800
600
400
200
0
I II III IV V VI I II III IV V VI I II III IV V VI I II III IV V VI
EARLY MAY
LATE MAY
JUNE
AUG

Fig.19

39-1609/100 ml water in May; 253-1609/100 ml water in June, and 39-542/100 ml water in August. Interestingly, there were seen an extreme variations in the bacterial counts in different samples collected from the lake water at the same time. This observation clearly reflects the presence of variable population of coliform bacteria at different locations of the lake water at one time of sampling. The maximum bacterial counts were seen during the periods of late May and June, 39-1609, 253-1609/100 ml, respectively. In August i.e. during the monsoon time there were recorded a very low bacterial counts, 39-542/100 ml.

An attempt was made to compare the Ca, Mg-hardness of the water to that of total coliform bacterial counts. One very striking observation is that the maximum counts of coliform bacteria coincided with the maximum values of the water hardness. Similarly, the counts were low when the hardness were also low as noted in early May and August (Fig. 20).

An effort was made to correlate the effect of different sources of nitrogen with that of coliform bacterial counts. Characteristically, NH_3, NO_2, NO_3 nitrogen, all indicated a more less similar pattern. There were seen relative increase in counts with a consequent decrease in nitrogenous compounds. In the month of June, there were seen the lowest

Fig. 20 : Correlation between coliform counts and Ca, Mg-hardness of the water.

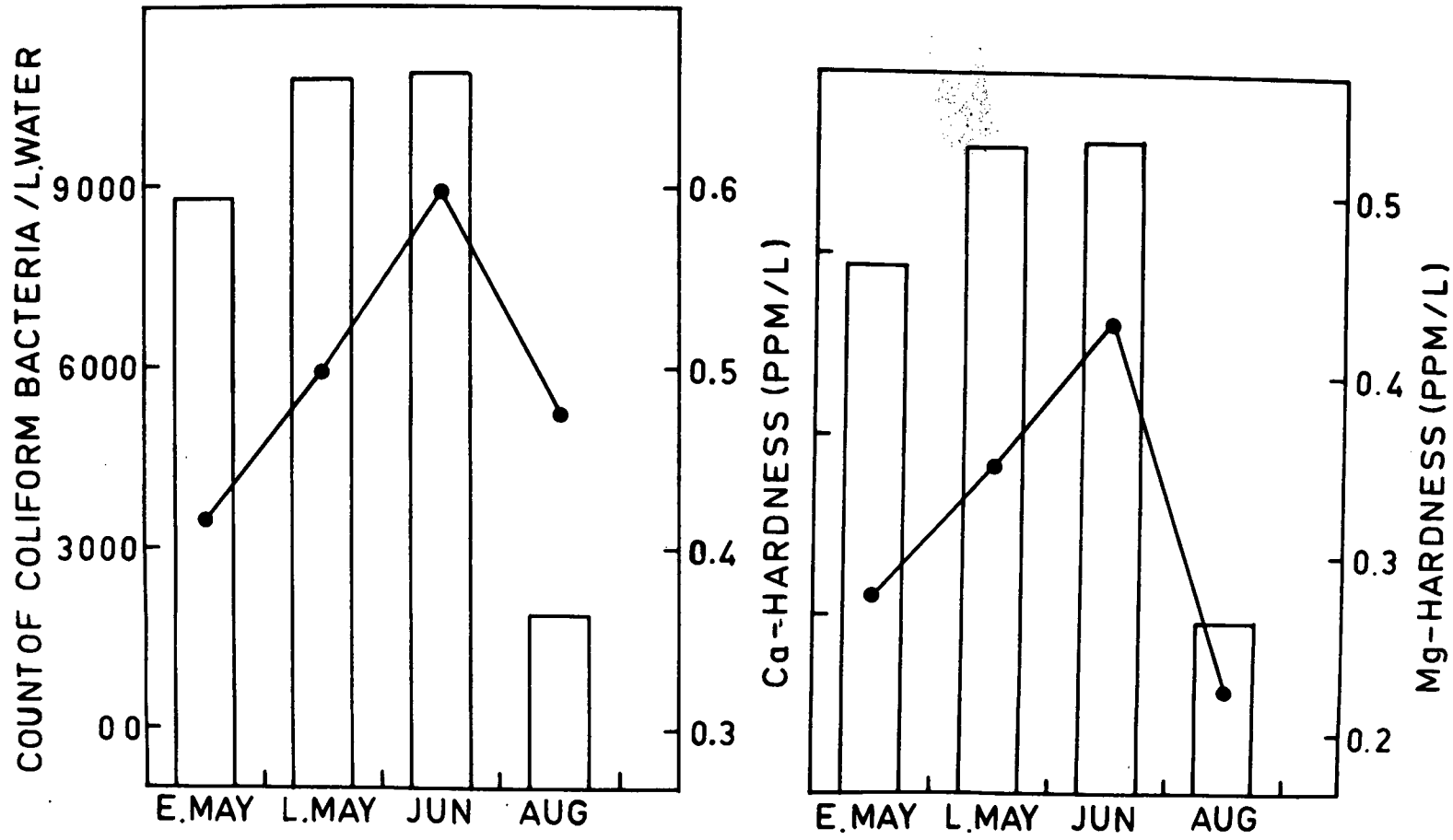

COUNT OF COLIFORM BACTERIA /L.WATER
9000
6000
3000
0 0
0.6
0.5
0.4
0.3
E.MAY
L.MAY
JUN
AUG
Ca-HARDNESS (PPM/L)
Mg-HARDNESS (PPM/L)
0.5
0.4
0.3
0.2
E.MAY
L.MAY
JUN
AUG

Fig. 20

nitrogen source in lake water while bacterial counts were at the highest value. Similarly, in August when counts were low the concentrations of NH_3 (Fig. 21) NO_3 (Fig. 22), and NO_2 (Fig. 22) had the maximum values. Fig. 23 indicates a distinct correlation of phosphate concentrations with that of coliform bacteria. The concentration of the phosphate was inversely proportional to the bacterial counts. During late May and June there were recorded the highest bacterial counts while during this time phosphate concentration was minimum. Similarly, the samples collected in the month of August revealed the maximum PO_4 concentration in the lake water where as the bacterial counts were lowest.

An almost similar pattern was seen while comparing the coliform counts with that of sulphate (SO_4^{2-}) concentration. Although the pattern of the graph was not that distinct as seen for phosphate ions but nevertheless the bacterial counts were highest while SO_4 concentrations were lowest. When sulphate concentration was highest i.e. in month of August, the bacterial counts were lowest (Fig. 23).

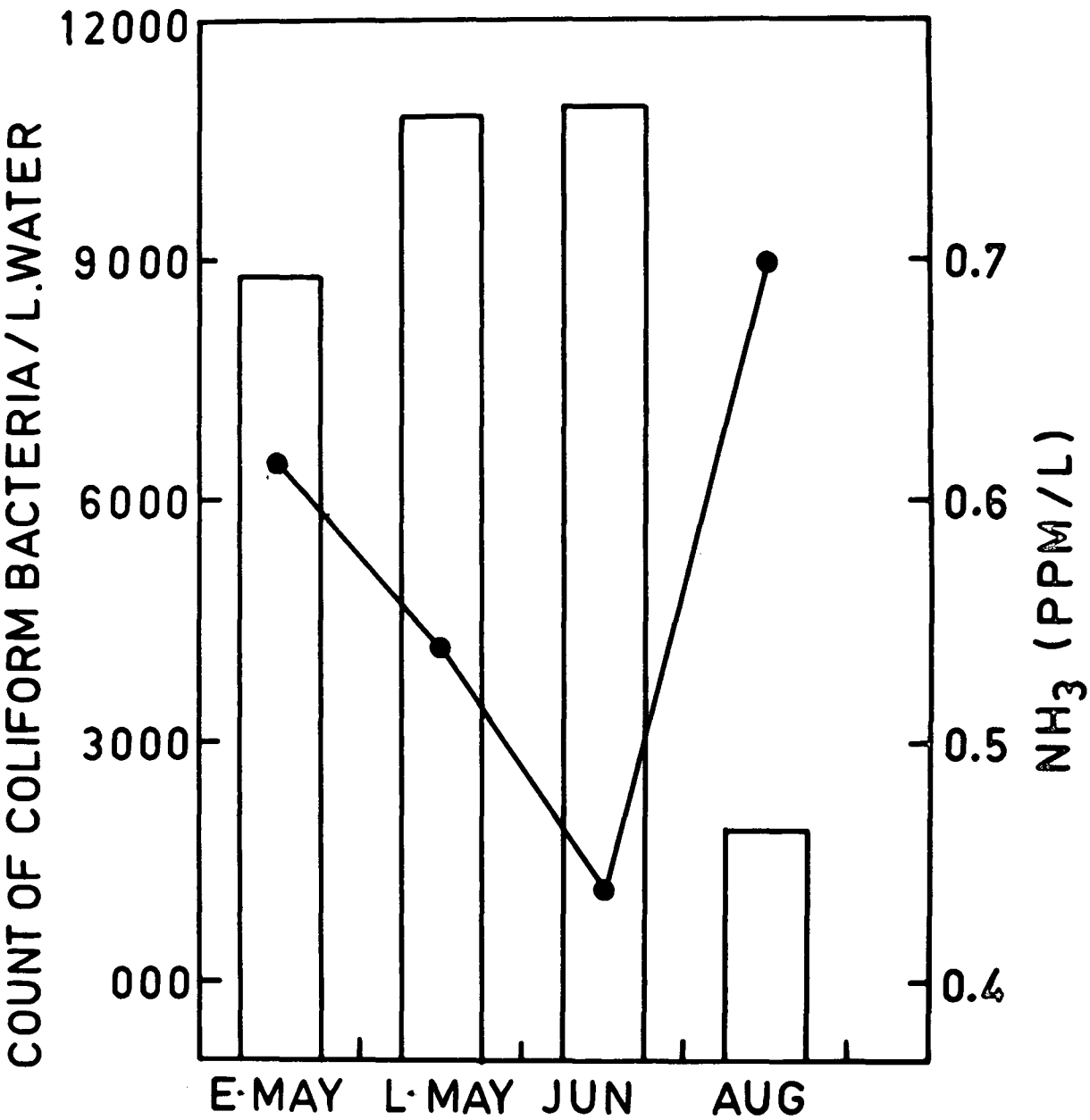

12000
9000
6000
3000
000
COUNT OF COLIFORM BACTERIA / L.WATER
0.7
0.6
0.5
0.4
NH3 (PPM/L)
E·MAY
L·MAY
JUN
AUG

Fig. 21

Fig. 22 : Relationship between NO_2, NO_3 nitrogen sources and coliform bacteria .

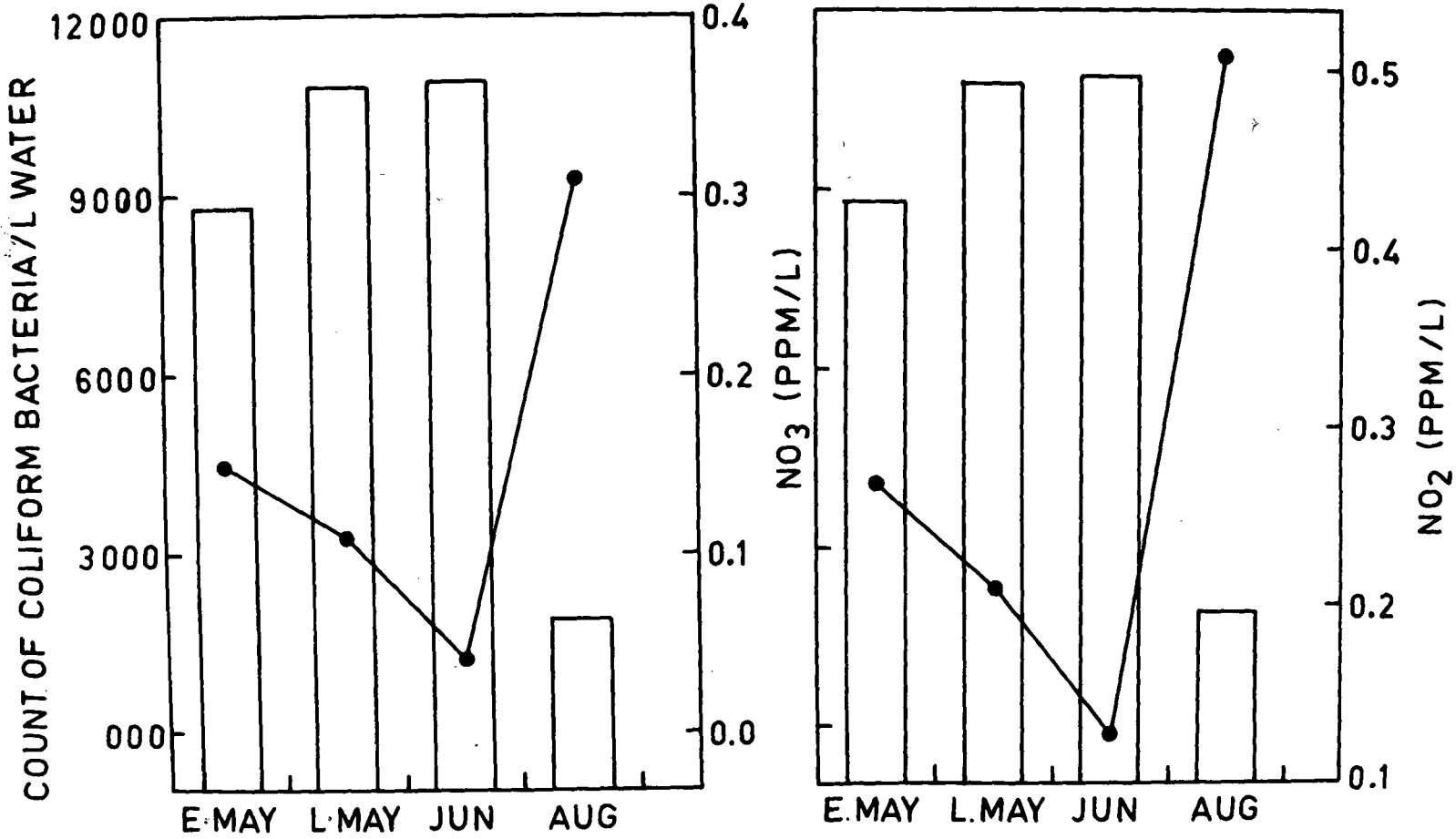

12000
9000
6000
3000
000
COUNT OF COLIFORM BACTERIA/L WATER
0.4
0.3
0.2
0.1
0.0
E.MAY
L.MAY
JUN
AUG
NO3 (PPM/L)
NO2 (PPM/L)
0.5
0.4
0.3
0.2
0.1
E.MAY
L.MAY
JUN
AUG

Fig. 22

Fig. 23 : Relationship between SO_4^-, PO_4^- and coliform bacteria.

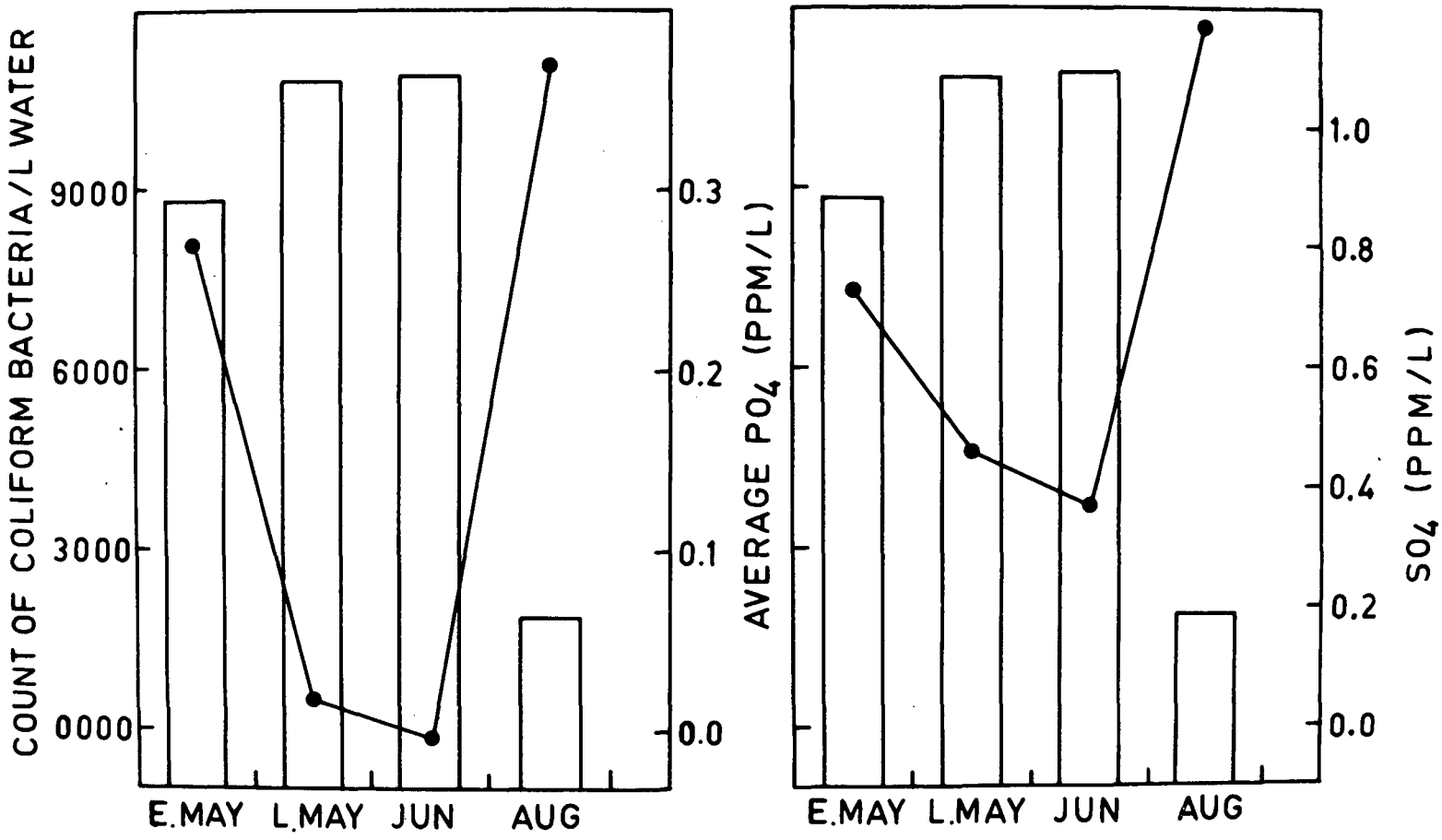

COUNT OF COLIFORM BACTERIA/L WATER
9000
6000
3000
0000
0.3
0.2
0.1
0.0
E.MAY
L.MAY
JUN
AUG
AVERAGE PO4 (PPM/L)
SO4 (PPM/L)
1.0
0.8
0.6
0.4
0.2
0.0
E.MAY
L.MAY
JUN
AUG

Fig. 23

DISCUSSION

In contrast to most micro-ecosystem investigation carried out upto the present in the lake, this study is mainly concerned, with blue-green algae (phytoplankton) and the heterotrophic faecal bacteria. The importance of bacteria and blue-green algae for the cycle of matter and for the food chain is well known for a long time (Rheinheimer, 1978). However, correlation of physico-chemical parameters with that of biological factors have not been sufficiently included in most research programmes.

Natural self-purification functions, however, only under conditions where composition and quantity of pollutants do not overtax the power of self-purification of receiving body of water. The growth of aquatic micro-organisms is affected by a great variety of physical and chemical factors which in a multitude of ways, may also act with or against one another. The life process of all microorganisms are affected by the temperature of water. Temperature exceeding the maximum, cause quick death, as the cytoplasm suffers irreversible damage (Hutchinson, 1969; Rheinheimer, 1971). The water temperature in this investigation varied from 21^{o}C to

32°C (Fig. 3) and blue-green algal phytoplankton grew the most at higher temperature depicting a typical 'mesophilic' characteristics.

The growth and reproduction of microorganisms is much affected by Hydrogen-ion concentration (pH) of the environment. Most microorganisms can grow only within the range of pH 4 to 9 (Thimann, 1964). The optimum for most aquatic microbes is between 6.5 and 8.5. This corresponds to the pH range of most of the larger bodies of water, and a similar situation was recorded in the present lake water (Fig. 2).

The life of microorganisms in water is affected by inorganic substances like nitrogen and phosphorus compounds, which in the productive zone of many water, represents the limiting factor for microbial life (Staples, 1973). In oligotrophic lakes, ammonia, nitrate, nitrite, orthophosphate and sulphate can hardly be demonstrated because as soon as they are released, they are immediately bound again by phytoplankton. Nitrogen appears to be the primary major nutrient limiting primary production in certain fresh waters (Likens, 1972). Most phytoplankton species are capable of utilizing many commonly occurring combined nitrogen to satisfy their requirements including nitrate, nitrite and ammonium. The ranges of concentration for

these compounds are 0.01 - 50 µM for NO_3^-; 0.01 - 5 µM for NO_2^- and 0.1 - 10 µM for NH_4^+ (Parson and Takahashi, 1973). Ammonium is the most energetically favourable source of inorganic nitrogen because of the reduced state; ammonium is taken up inpreference to nitrate and nitrite when all forms of nitrogen are present (McCarthy and Epply, 1972; Eppley et al., 1969), however, lake water under investigation recorded a high concentration of NO_3^-, NO_2^- and NH_4^+ (Figs. 6, 7 and 8).

The summer decrease in nitrite and nitrate, however, is much greater than ammonia. Therefore, its percentage of the inorganic bound nitrogen increases greatly in the summer (Fig.. 14). This is mainly on effect of phytoplankton grazing and excretion of Zooplankton and Zoobenthos (Rheinheimer, 1978). These observations are in confirmity with the present investigation.

The fact that phytoplankton cells can survive and grow at concentrations below the detectable limit of phosphorus (about 0.03 µg/l) has stimulated studies relating the rate of supply of phosphorus under natural conditions to its rate of utilization (Strickland et al., 1972). Edmondson (1970) stated that during summer algal crop was related to the surface phosphorus concentration present during the previous winter. Reports by Schindler and

coworkers (1970, 1971) showed conclusively that in a small lake phosphorus was the nutrient which could most often be called the 'limiting nutrient' for the growth of phytoplankton and thus for eutrophication. The present studies are in agreement with earlier observations. With the deficiency of phosphorus availability the blue-green algal growth were also reduced.

Singh, 1955; Rai, 1976, 1978; Das, 1977; Das and Pandey, 1978 made several attempts to draw up a list of bloom forming species in Indian waters. Their list included Microcystis, Oscillatoria, Spirulina, Phormidium, lyngbya, Anabaena, Anabaenopsis, Anacystis, Raphidiopsis and Wollea. This investigation also report the presence of Oscillatoria, Phormidium lyngbya and Anabaena, but does not report the occurrence of Anacystis, Raphidiopsis, Spirulina and Wollea. Several species of Nostoc have been reported throughout the period of the investigation. Like the earlier finding the the maximum growth of blue-green algae was recorded in this lake during summer (April to June) characterized by high temperature.

Hutchinson (1969) whilst describing the observation of algal blooms in eutrophic lakes, pointed out that the blooms actually appear during periods of nutrient deficiency in the water. Rigler (1964) demonstrated that ionic phosphorus

had a turnover time of approximately 1 min in the epilimnion of a lake in the summer. It is evident that a eutrophic system will support water blooms of algae and that the frequency of blooms is likely to be related to sediment fixation.

Filamentous blue-green algae (Nostoc, Anabaena) growing in lake make a significant contribution to the nitrogen budget by fixation of atmospheric nitrogen (Nielsen and MacDonald, 1978a,b; Döbereiner, 1977). Many ofthe prominent blue-green algae of the fresh water phytoplankton lack heterocyst and whilst some planktonic hetercyst-bearing species occur. The view that nitrogen fixation does occur in fresh water lakes is based on indirect evidence from field observation, notably the high concentration of nitrogen associated with blooms of blue-green algae compared with that available in the combined form in water. Present investigation records both established (Heterocystous forms) and non-established nitrogen fixing blue-green algae in almost equal proportions (Fig. 15b). Quite contrast to earlier observations, there has been dominance of species of Nostoc and Anabaena in this lake waters.

Oligotrophic lakes vary markedly in the extent to which their flora includes planktonic blue-green algae.

Often they are almost absent, but sometimes (Vallentyne, 1971), the blue-green algal flora may be quite rich. It is evident that as a result of eutrophication, environments turn favourable to the growth of dense populations of blue-green algae and they have become wide spread in recent years, and it is hard to imagine that same types of habit at ever occurred prior to man's activities. Eutrophication has often been reported to lead also to changes in the blue-green algal species composition, and there are apparently no exception to the generalization that all records of invasion by Oscillatoria rubescens have been associated with increased water pollution. Oscillatoria rubescens, Aphanizomenon flos-aquae and Microcystis aeruginosa have been regarded as indicators of lake eutrophication (Skinner and Carr, 1976). Oscillatoria chalybea, Phormidium tenue, P. africanum, P. molle, P. foveolarum, P. laminosum and lyngbya nigra have been recorded from the present lake, which normally do not form algal blooms in oligotrophic lake (Table II).

Chlorophyll a content of phytoplankton gives an index for the amount of chlorophylls, i.e. the primary producers in the pelagic zone. A direct correlation between chlorophyll a concentrations and algal bloom has been established (Fig. 16a,16b) in the investigation.

Human pathogenic bacteria get into waters mainly with domestic sewage. They cannot grow permanently there and die-off eventually in inland waters; but depending on the kind of water and prevailing conditions various pathogens can survive for a longer period. In tropical countries Vibrio comma, the causative agent of cholera occurs epidemically and is commonly spread by water contamination.

Like the earlier observations (William and Frans, 1972; Aggrwal et al., 1976; Miakhan, 1971; and Varma and Dalella, 1975), the present study also reports the variation in the bacterial counts with the time of collection and the sampling place (Fig. 19). The total bacterial number decreased from summer towards monsoon at all places and the highest bacterial numbers were found at several pollulated placed.

SUMMARY

Many of our oligotrophic lakes are becoming increasingly murky, smelly and choked with excessive growth of algae and coliform bacteria. Pure water is one of natures greatest gifts to mankind and clean water stream, lakes and reservoirs not only appeal aesthetically but are vital to the vary functioning and continuance of life.

1. The lake under investigation is situated on the East-North of New J.N.U. Campus, it is surrounded by hillocks so that lake forms a deep depression at the centre of region and falls into category of shallow tropical lakes with typical phytoplankton content.

2. The dominant blue-green algal phytoplankton were species of Phormidium, Oscillatoria, Lyngbya, Anabaena and Nostoc.

3. An attempt was made to correlate the blue-green algal periodicity with physico-chemical factors of water like pH, electrical conductivity, temperature, water hardness, ammonia , nitrite and nitrate nitrogen, phosphate and sulphate.

4. The temperature of the water is between 21°C and 32°C.

The blue-green algal phytoplankton vary with the change of temperature.

5. This periodicity is correlated with periodicity of Hydrogen-ion-concentration. The pH ranging from 7.5 to 8.5, appears as an index of the relative phytoplankton content of the lake; the maximum pH coinciding with a maximum distribution of phytoplankton and the minimum pH with the phytoplankton minimum.

6. The minimum pH and phytoplankton distribution occurs during the period of greatest dilution of water i.e. during the maximum rain fall of the season (July-August).

7. A definite correlation between phytoplankton periodicities and the level of different nutrients like, Ammonia, Nitrite, Nitrate, Phosphate, Sulphate in the lake waters has been established.

8. It has become evident that blue-green algal phytoplankton are subjected to a continually changing environment and must therefore be able to short term, e.g., diel, changes as well as changes that are seasonal or longer. The dynamic aspects especially the lack of equilibrium in relation of the algae to their environment has been discussed.

9. Chlorophyll a content of algal bloom was analysed and it was correlated with the algal periodicity.

10. The present investigation is concerned with examination of bacteriological tests, a pollution; organism of the coliform group as a whole are foreign to water and has regarded as indicative of water pollution in its widest sense.

11. The coliform count vary with seasonal variation and the changes in physico-chemical nature of the water bodies.

12. The lake water under investigation has shown characteristics of a typical oligotrophic lake but it has given certain parameters indicative of Eutrophic conditions.

13. Although such a time limited investigation, cannot clarify all the question on the complex relationships between microorganisms and their biotope. However, the results of this investigation led not only to new knowledge of the role of the microorganisms in the closed lake water ecosystem, but also to a revision of earlier conceptions, especially on the influence of wastes on the microflora and its role in the self purification of lake water.

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