

I. INTRODUCTION

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Phytoplankton, 'the grass' of the surface waters of rivers, lakes and oceans are responsible for the process of primary production over much of the outface of the earth. The vast majority of phytoplankton are algae and belong to diverse group of lower, non flowering plants. It has been estimated that 90% of the world's photosynthesis is confined to these aquatic plants of oceans and freshwater. Thus the algae form the fundamental, but yet only incompletely investigated part of food chain in most of the aquatic environments. In the absence of combatable morphological mechanisms these organisms especially the unicellular forms, possess rather flexible physiological characteristics as an adaptation to the vagaries of the aquatic ecosystem. This property raises them to the status of 'good model' organisms to be used to answer most of the questions of

biological significance. The small, simple, single-celled construction of most of the phytoplankters along with their rapid growth rate further establish the relevance of these organisms in various physiological and ecological studies.

The species composition of phytoplankters in the aquatic environment is greatly influenced by the biological, physical and chemical factors operating directly or indirectly in the habitat. There are significant differences between the dominant groups of phytoplankton in the marine and freshwater systems, in that, the dinoflagellates are more diverse in the oceans, the diversity of green and blue-green algae is higher in freshwater and diatoms are common in both habitats.

Huge volumes of literature are available regarding the responses of algae to different environmental perturbations. Important factors that control the growth rate of dominant and subdominant species in the phytoplankton community have been identified as nutrients, chelators that regulate the solubility and availability of trace metals, inorganic and organic substances which may be inhibitory or stimulatory, light intensity and quality, temperature, pH, mixing and bacterial and fungal infections (Talling, 1976; Kalff and Knoechel, 1978).

The phytoplankters are in general, photoautotrophic prokaryotes and eukaryotes. Some species have rather complex nutritional requirements and may be partially dependent on organic substrates (heterotrophic) (Droop, 1973). However, most species of phytoplankters being autotrophic

use light energy to build macromolecules from simple precursors so that the major ecological factors of concern are those which influence all photosynthetic organisms : light, temperature and the supply of major nutrient ions.

The microalgae acquire nutrients for growth and multiplication from their environment. According to Arnon(1950), an element is considered essential for an organism when the organism can neither grow nor complete its life cycle in its absence or it can not be replaced by any other element and has a direct influence on the metabolism of the organism. Earlier works performed on the mineral nutrition have revealed that the nutritional requirements of algae are fairly similar to those for the complex phanerogams (Venkataraman, 1969). Thus, the essential elements needed by microalgae include carbon, nitrogen, phosphorus, sulphur, silicon, calcium, magnesium, sodium, potassium, iron, manganese, copper, vanadium, molybdenum *etc.*

Based on the quantitative requirements, these nutrients have been classified into two groups.

1. Macronutrients:- These are required in large quantities and include elements like C, N, P, S, Si, Mg, Ca, Na and K.

2. Micronutrients or trace or oligo elements:- These are needed only in very small amounts for the normal growth and division of these organisms and include Fe, Cu, Co, Mn, Zn, *etc.*

According to Ketchum (1954), the requirements of these nutrients by the algae may be absolute, normal, minimum or optimum. When an

alga cannot grow and carry out its life processes in the absence of a nutrient and that cannot be replaced or substituted by any other, then the alga is said to have an absolute requirement for that particular element. The normal requirement is the quantity of each nutrient contained in cells produced during active growth of a population, while no nutrient is limiting. The minimum requirement is the quantity of a nutrient in the cell when it is limiting the growth of the population, all other nutrients being present in excess. The concentration which permits the maximum growth and other metabolic processes is known as the optimum concentration.

The absolute, normal, minimum or optimum requirements of nutrients vary with the species and the studies have shown that the nutrient composition of a given water body has a profound influence on the composition of flora it contains (Jordan and Bender, 1973; Riegman *et al.*, 1992; Peeters *et al.*, 1993; Lebo *et al.*, 1994; Blomqvist *et al.*, 1995; Monteiro *et al.*, 1995; Holz and Hoagland, 1996; Pandey *et al.*, 2000; Shyamalendu Bikash Saha *et al.*, 2000). Role of nutrients in determining the species composition of phytoplankton in natural waters has been confirmed by the nutrient enrichment experiments conducted by Gloosechenko and Alvis (1973), Jordan and Bender (1973), Stoermer (1978), Oivind Lovstad (1983), Pappas and Stoermer (1995) and Levich (1996). The importance of algal bioassays to study the biological responses of these organisms to water quality and to determine growth-limiting nutrients has been documented by the earlier works performed by US

Environmental Protection Agency (1975), Fitzgerald (1972), Miller *et al.* (1974) and Maloney and Miller (1975).

A comparison of the chemical composition of most freshwater with that of algal cells suggest that silicon, nitrogen and phosphorus are among the elements most likely to be depleted by algal growth. Silicon is an absolute requirement for diatoms and for some species of Chrysophyceae and Xanthophyceae, but probably not for other groups. This element constitutes an important and major component of cell walls in diatoms and has additional metabolic functions in these cells. Silicon as the growth limiting nutrient for diatoms has been established by the enrichment experiments of Schelske and Stoermer (1971), Gloosechenko and Alvis (1973), Schelske (1984) and by the field studies of Oivind Lovstad (1983).

Next to carbon and oxygen, the element present in greatest amounts in algal cells is nitrogen. It is an important element in the general metabolism of algae. In natural habitats the main sources of nitrogen are nitrates and ammonium salts. In artificial culture, nitrogen is usually supplied as ammonium salts or nitrates. Both these forms can be assimilated by most algae, although varied preferences have been reported (Proctor, 1957; Provasoli, 1958). Elemental nitrogen is in abundant supply both in the air and water; but only a few algae of Cyanophyta can utilize it under normal conditions. The assimilation of this element is indirectly

connected with photosynthesis since the energy source, hydrogen donors and carbon skeletons are ultimately derived from the latter process (Round, 1973).

Phosphorus is one of the major nutrient elements required for normal growth of any organism including algae. In many freshwater bodies, growth of phytoplankters tends to be limited by the supply of inorganic phosphate (Talling, 1962; Halmann, 1972; Dillon and Rigler, 1974b; Vollenweider, 1975; Gernot Falkner *et al.*, 1984; Axier *et al.*, 1991; Whitton *et al.*, 1991; and Harvey *et al.*, 1998). Investigations of Fogg (1973) on the relationship between algae and phosphorus indicate that the requirements of phosphorus is species dependent and the prevailing concentrations of phosphate in a water body have a determining influence on its floral composition. The role of phosphorus in regulating the species constitution of phytoplankters in water bodies has been further substantiated by the explorations of Condit (1972), Gloosechenko and Alvis (1973) and Wang *et al.* (1995).

Levich (1996) suggests that N : P ratio in an aquatic ecosystem governs the dominance of planktonic communities by blue-green or green micro-organisms. A decrease in N : P ratio leads to cyanobacteria blooming while its increase results in the predominance of green algae. However, *in situ* experiments carried out by Bachmann *et al.* (1995) at lake Okeechobee failed to exhibit a shift in the phytoplankton community with the changing N : P ratio. The observations of Levine *et al.* (1997) on the algal population of Lake Champlain elucidate that enclosure is a

more powerful determinant of species composition than nutrient inputs and that seasonal variations have a significant influence on algal growth. Bachmann *et al.* (1995) and Levine *et al.* (1997) independently proposed that the addition of N and P in combination produced greater algal biomass and productivity than the singular addition of either of these elements. The discrepancies in the observations of different authors emphasise the necessity of detailed investigations in the field of phytoplankton responses towards various nutrient inputs.

In addition to Si, N and P, other major ions of concern include sulphur, calcium, magnesium, sodium and potassium and these are found to be essential for phytoplankton growth. Sulphur is an important constituent of living matter and is required by all groups of algae in relatively large amounts. The requirements of Ca, Mg, Na and K vary with species. While some algae require these elements in macroquantities others respond to them in microquantities.

The influence of these major inorganic ions in the formation of algal flora of a broad range of African lakes has been noticed by Gasse *et al.* in 1983. According to Margalef and Mir (1979) and Margalef *et al.* (1982), the assemblage of phytoplankton species in surface waters of a number of Spanish reservoirs could be correlated with the total mineral content of the waters. From the results of the inquiries, Shoemith and Brook (1983) and Brugam and Patterson (1983) suggest the use of monovalent to divalent ion ratio as a predictor of species

composition of an aquatic ecosystem. The concentration of major ions in the water can therefore be considered as an imprecise indicator of algal biomass and productivity.

For any organism to grow and reproduce normally, micronutrients or trace elements are necessary in at least very low concentrations and these elements cannot be replaced by any other mineral factors. The micronutrients needed by various algal species are iron, manganese, zinc, copper, molybdenum, cobalt, vanadium, boron *etc.* Most of these elements belong to the class heavy metals.

Many of the trace elements are normal constituents of aquatic organisms and are essential for their metabolism by having definite functional roles. Each of the trace metals either singly or in combination, along with the major environmental parameters can affect the biota as a whole and the food chain in particular. Apart from the existence of these elements in natural conditions, there is considerable input from land run-off especially if there is a discharge point from man made sources. These trace metals which form essential nutrients enhancing the growth at threshold levels can pose problems of toxicity when there is a subtle increase in concentrations resulting in impairment of growth kinetics, physiological activity as well as intracellular changes.

In natural concentrations, trace elements constitute the prosthetic group of enzymes or function as enzyme activators. However, at elevated

levels they act as inactivators of enzyme system and as protein precipitants. Studies of De Filippis and Ziegler (1993) on the effect of sublethal concentrations of zinc, cadmium and mercury have shown that these metals retarded the activities of four enzymes involved in the fixation of CO₂. Some of the possible modes of toxic action of metals as outlined by Aberg (1948) are i) as antimetabolites ii) as substances forming stable precipitates or chelates or as substances catalysing the decomposition of essential metabolites iii) combining with cell membrane and affecting its permeability iv) structural replacement of electrochemically important elements.

Phytoplankton species vary in their tolerance to trace metals. Investigators like Gibson (1972), Nelson *et al.* (1991), Ithack and Gopinathan (1995), Angadi *et al.* (1996), Graham *et al.* (1996), Knauer (1996) and Bhattacharyya *et al.* (2000) found out the tolerance limit of various phytoplankton to different trace metals like Cu, Zn, Pb, Ni, Mn and Cd. Differential response of marine diatoms to trace metals has been studied by Tadros *et al.* (1990).

In spite of the importance of these trace elements in algal growth and metabolism, a little attention has been paid on them on the nutritional point of view (Lee *et al.*, 1994; Lin *et al.*, 1994; Moreau *et al.*, 1994; Chow *et al.*, 1998; Katiyar and Katiyar, 2000). Most of the reports available focus on the toxicological impact of trace elements

especially those belonging to the class heavy metals (Malanchuk and Greundling, 1973; Jampani, 1938; Ahluwalia and Kaur, 1989; Maeda *et al.*, 1990; Ning-Zheng *et al.*, 1990; Srivastava *et al.*, 1991; Andrade *et al.*, 1994; Fargasova, 1994; Voloshko and Gavrilova, 1994; Angadi *et al.*, 1996; and Bhattacharyya *et al.*, 2000). They furnish a meagre information regarding the significance of trace elements in algal nutrition.

In addition to the above mentioned essential elements, certain organic compounds including vitamin B₁, B₁₂ and biotin are found to be essential for the growth of some algal species in culture system.

Microalgae perform much diversified and vital functions in an aquatic ecosystem like incorporation of solar energy into biomass, production of oxygen by photosynthesis that get dissolved into water, cycling and mineralisation of chemical elements and as the food source for herbaceous and omnivorous animals. When they die they sink to the bottom where their chemical constituents are transformed, solubilised and recycled into the water. These functions depend on phytoplankton population dynamics which in turn is determined by the behaviour of individual species. The latter can be understood only by the laboratory studies in unialgal cultures.

Miquel (1890) and Schreiber (1927) were among the pioneers to initiate the culturing of microalgae. Filtered sea water with adequate amount of nitrates and phosphates was used as the basic growth medium

for these organisms. Pringsheim (1949) summarised the history and procedure of phytoplankton culture. In the 1950s Provasoli, Droop and others modified the culture medium by adding trace metals, chelating compounds and vitamins (Provasoli *et al.*, 1954; Provasoli, 1958; Droop, 1954, 1968). The mineral requirements of microalgae were further reviewed by Lewin (1962), Stein (1973), Stewart (1974), Walne (1974), Ward and Parish (1982).

Popularisation and commercial application of photosynthetic biomass production systems like cultivation of algae are more relevant now than ever before in the international context of energy shortage, water disposal problems, environmental protection, alternative food additives and cheaper sources of feed proteins (Venkataraman, 1990). Production of microalgae for more varied and newer applications like aquacultural practices has come of age. Since the nutritional needs of algae differ with species, a large scale cultivation of any economically important algal species demands a thorough knowledge of its nutritional requirements.

There has been very few attempts in the past to study the nutritional requirements of microalgae under laboratory conditions. Further more, none of the reviews available places adequate emphasis on the impact of these nutrients on the synthesis of various biochemical compounds like protein and carbohydrate. With this view, the present work was undertaken with an objective to elucidate the influence of selected macronutrients and micronutrients on the growth and physiological activities

of three unicellular Chlorophycean freshwater microalgae, *Chlorococcum humicola*, (Naeg.) Rabenhorst, *Chlorella ellipsoidea*, Gerneck and *Scenedesmus bijuga*, (Turp.) Lagerheim.

Chlorococcum is a common genus of soil algae which reproduces by means of zoospores. The species *Chlorococcum humicola*, (Naeg.) Rabenhorst is also found in freshwater. Its cells are spherical, solitary or in small clumps and are variable in size within the same plant mass. Cells are 8 - 20 μ in diameter. It often forms green films in association with *Scenedesmus* and *Euglena* species.

The genus *Chlorella* (Beyerinck, 1890) represents one of the simplest and commonest green alga with cosmopolitan distribution. *Chlorella* forms ideal experimental material for research in various basic problems of plant physiology and biochemistry. In addition, it has got variety of practical applications in agriculture and technology, as protein rich food or feed, for the production of biomass for conversion to energy, for sewage oxidation and for gas exchange in space travel (Golueke and Oswald, 1964; Fogg, 1971; Shelef *et al.*, 1976; Krasnovsky, 1979; Soeder, 1976, 1980).

The species *Chlorella ellipsoidea*, Gerneck is generally distributed in many lakes and ponds. Cells are ellipsoidal, sometimes unsymmetrical with chloroplast as a folded plate over part of the cell wall. It produces as many as 32 autospores during reproduction. Vegetative cells are 7-8 μ in diameter, 9-9.5 μ long.

The genus *Scenedesmus*, a colonial green alga is extremely common in nature and plays a very important role in basic applied phycolgical research. They are rich in protein and can also be used for purifying sewage effluent. Due to the tendency of the coenobia of some species to disintegrate into unicells under certain conditions, there exists a high degree of morphological variability within the same species of *Scenedesmus* (Komarek, 1964).

The colony of the species *Scenedesmus bijuga*, (Turp.) Lagerheim is composed of 2-8 cells in a single flat series (rarely alternate), cells ovate or oblong, without teeth or spines. Cells are 4-8 μ in diameter and 8-16 μ long. The species is widely distributed, often appearing as a prominent component of the littoral plankton.

The nutrients selected for the study were phosphorus (P), magnesium (Mg) and calcium (Ca) (macronutrients) and iron (Fe), manganese (Mn) and zinc (Zn) (micronutrients). From the observations, the optimum concentrations of these elements which would possibly accelerate growth resulting in larger biomass and sustained period of exponential phase for economically viable harvest were determined.

