CHAPTER VIII

Behaviour of a few fresh water ciliates to increased concentrations of phosphate and nitrate in the culture medium.
Behaviour of a few fresh water ciliates to increased concentrations of Phosphates and Nitrates in the Culture medium.

INTRODUCTION

Sewage, sludges and effluents contain appreciable amounts of chemicals which affect aquatic life. Natural bodies of water contain a large number of free living Protozoans. Sewage water normally contains high concentrations of nutrients especially phosphorus. Phosphorus was discovered under somewhat curious circumstances in 1669 by Brandt. In 1844 Leibig (16) considered phosphorus to be an essential element in plant nutrition. In lakes and rivers addition of this phosphorus increases the photosynthesis by plants with undesirable effects - heavy algal bloom in summer, oxygen deficiency in winter (4). The prolific growth of weeds and algal blooming as a result of fertilizing with sewage containing PO₄ have given rise to biological problems. The algal blooms are known to kill fish in inland waters apparently through anoxia, they secondarily kill people along the California Coast by producing a poison which accumulates in edible mussels to toxic amounts, they kill, by some not understood mechanism, cattle which drink from inland ponds, and clog filters in water plants. These algae include the blue green, yellow brown algae and a few dinoflagellates and diatoms (15). The oxygen deficiency is caused by the oxidation of ammonia and nitrite forms of nitrogen, oxygen is withdrawn from the resources of the water body (33).

The phosphorus content of waste liquids has been found to be the most important factor causing the extraordinary growth of algae. It is known from the investigations of the last few years by different authors (2, 25, 17, 18, 10, 7, 34) that the most important factor for eutrophication is the total inflow of phosphorus and nitrogen, especially at the productive time of the year (spring and summer). The nitrogen of nitrates and the phosphorus of soluble phosphates have long been considered as the main fertilizing elements connected with the development of human activities causing the proliferation of algae, and in the main entrophication and premature aging of the lakes (15).

Since Protozoans form a part of the zooplankton found in the natural bodies of water, an attempt has been made to study the effects of different concentrations of phosphates and nitrates on the behaviour and population density of five fresh water ciliates.

MATERIAL AND METHODS

Cultures of Spirostomum ambiguum major, Spirostomum ambiguum minor, Blepharisma intermedium, Blepharisma seshachari and Frontonia leucaes were grown in hay infusion (as described in chapter 6) and fortified with horlicks malt at room temperature 28°C±10°C.
The behaviour of the five ciliates to different concentrations of phosphates and nitrates was studied by inoculating them into culture medium supplemented with the salts separately. The culture medium fortified with horlicks was supplemented with 2 gms/100 ml, 1gm/100 ml, 500 mg/100 ml, 250 mg/100 ml, 200 mg/100 ml, 150 mg/100 ml, 100 mg/100 ml and 50 mg/100 ml of NH₄ NO₃ and KH₂ PO₄ separately. The immediate reaction of the ciliates to these concentrations was recorded.

As 50 mg/100 ml of KH₂ PO₄ and NH₄ NO₃ were not very lethal, the population density of the five ciliates at this concentration was studied. For the population study, 50 organisms of each of the five species were inoculated into 100 ml of the culture medium with 10 mg of horlicks in 125 ml capacity bottles made of hard glass. 50 mg/100 ml of KH₂ PO₄ and 50 mg/100 ml of NH₄ NO₃ were added separately to three bottles and the unsupplemented bottles served as controls. The number of organisms in three aliquots of 1 ml of the medium in the controls and the treated was counted daily for a week and the average taken.

**OBSERVATIONS**

I. Effect of different concentrations of NH₄ NO₃

50 mg/100 ml was not very toxic to Blepharisma species and Spirostomum species. These ciliates had reduced in size. The number of Frontonia in the culture medium were depleted. The population density at this concentration was studied for a week (Fig. 1).

After 24 hours in 100 mg/100 ml there were a few normal B. intermedium, B. seshachari which were elongated and pale, most of the Spirostomum species had burst, the remaining few were reduced in size while Frontonia were completely eliminated.

Concentration of 200 mg/100 ml and 150 mg/100 ml eliminated Spirostomum species, B. seshachari and Frontonia within 24 hours, while there were a few B. intermedium.

250 mg/100 ml brought about encystment of all the five species within 5 to 6 hours. When the cysts were transferred to fresh culture medium after 24 hours, there was rapid movement of the cysts. Cysts of Frontonia and Blepharisma species excysted within 1 and 3 hours respectively. Cysts of Spirostomum species failed to excyst.

All the five species burst within 8 hours, 6 hours and immediately with 500 mg/100 ml, 1 gm/100 ml and 2 gms/100 ml respectively.
II. Effect of different concentration of \( \text{KH}_2\text{PO}_4 \)

In 50mg/100 ml and 100 mg/100 ml the five species of ciliates were normal both in morphology and behaviour. The population density of the five ciliates was studied for a week at a concentration of 50mg/100 ml (Fig. II).

A concentration of 150 mg/100 ml was lethal. Immediately after inoculation, Spirostomum major burst, Spirostomum minor contracted and reduced in size. Frontonia burst immediately. Blepharisma species struggled, elongated and a few burst immediately.

All the five species burst within 6 hours and 5 hours with 200mg/100ml and 250mg/100 ml respectively. With 500mg/100 ml, 1gm/100 ml and 2gms/100 ml the ciliates burst immediately.

**DISCUSSION**

The discharge of nitrogenous wastes from municipalities, industries and land run-off causes two important changes in estuarine water quality. First, during oxidation of the ammonia and nitrite forms of nitrogen, oxygen is withdrawn from the water body. Second, both ammonia and nitrate serve as important nutrient sources for phytoplankton growth (33),

From a water pollution control view point, there are two broad areas of concern in the nitrogen cycle: a) the discharge of large amounts of nitrogenous wastes results in a dominance of bacterial nitrification and contributes to the decrease of dissolved oxygen. Under anaerobic conditions, nitrate reduction may occur. b) in more favourable relatively unpolluted estuaries, ammonia and nitrate — nitrogen discharges may result in in undesirable proliferation of aquatic plants (33).

The problem of preventing sea water pollution by industrial and domestic discharges, is one of importance since progressive pollution of sea water can result in a sharp deterioration of sanitary conditions and may cause considerable damage to the fishing industry.

Since ammonia is the intermediate stage of complex oxidation of organic nitrogen to nitrates, it can be assumed that the condition of the sea is inadequate to cope with the complete mineralization process (29).

Sewage is known to contain fertilizers such as nitrogen, phosphorus and potassium, which are beneficial if the sewage is intended for irrigation. On the other hand, these
fertilizers would be harmful if sewage was disposed of in a lake, which serves as a water source for domestic or recreational purposes, since they may enhance eutrophication. Nitrates in potable water in concentrations exceeding a permissible value are objectionable from health aspects (9).

Condensed phosphates from detergents in waste water are a major supply of phosphorus to surface waters. They hydrolyze to orthophosphate, the form most readily available to plants and organisms.

Problems caused by the presence of condensed phosphates in water and waste water have been reviewed by a Task group of the American water works association (3). They have been summarized as follows:

1) Interferences with water and waste water treatment.

2) Eutrophicatlon problem caused by increased phosphorus available to plants and organisms, either as condensed phosphates directly or after hydrolysis to orthophosphate.

Condensed phosphates from detergents are the largest contributors of phosphorus to waste water, about twice as much as the human waste contribution. It hydrolyzes extensively during activated sludge treatment with about 15% condensed phosphate remaining in the effluent (12).

The influence of photosynthetic and respiratory processes on the composition of natural waters is frequently reflected in a correlation of concentration of soluble phosphate and nitrates.

Many references have been cited concerning the ill-effects resulting from the release of nitrogenous compounds into receiving waters. These obnoxious consequences ranging from eutrophication of lakes to toxicity to humans and stream life have been reported (20). The fertilizer waste contains nitrogen in the form of ammonium nitrate, ammonia, nitric acid and very small amounts of urea. The second stream of the plant effluent has been called the ammonium nitrate waste which has a flow of 50,000 gpd. (gallons per dap) with ammonia, nitrogen and nitrate-nitrogen concentrations of 1800 and 600 mg/ml respectively (1).

In another investigation it has been found that the average waste water flow of 340 million gallons per day (mgd) had approximately 86,000 pounds of phosphates, 51,000 pounds of ammonia and 102,000 pounds of organic nitrogen (5).
Nitrogen if converted to nitrates was of physiological consequence (21) but phosphorus in the amount found in waste water presented no such problems.

In the present investigation it is seen that NH₄NO₃ even at a concentration of 50mg/100 ml affected the five ciliates bringing about reduction in the size of Blepharisma and Spirostomum species while large numbers of Frontonia were eliminated. The same concentration of KH₂PO₄ did not show any adverse effect.

High concentrations of NH₄NO₃ (2gms/100 ml to 150mg/100 ml) was toxic to the ciliates, as they were eliminated within 24 hours. 100 mg/100 ml was sublethal, 24 hours after the ciliates were inoculated, four of the five species were surviving, though there was reduction in their size, activity and number.

Nitrate-nitrogen constituted the growth limiting nutrient for strains of Chlorella pyrenoidosa and a mixed indigenous algae population (28). Phosphorous in an aquatic ecosystem is often present as a growth limiting factor (32).

Wiebe (36) has studied the soluble phosphorus and inorganic nitrogen in the waters of the Mississippi River and has observed that phosphorus could be a limiting factor in the growth of plankton. Algae populations utilize the phosphate and silicate in streams and rivers and thus reduce the concentrations of these elements in the waters (11). The amount of plankton in Lac Leman at Geneva was closely related to the concentration of phosphates (22).

The occurrence of a "red tide" (produced by Gymnodium, a flagellate) in the streams of Florida was related to the high concentration of phosphates (24).

Chu (8) studied the influence of mineral composition of the medium on the growth of a few forms of planktonic algae and observed a marked inhibition on their growth when the concentration of nitrogen or phosphorus exceed 45 ppm. Concentration of nitrogen below 0.1 ppm. and phosphorus below 0.009 ppm. also had an inhibitory effect on the algal growth. Benoit (6) has found it difficult to determine the critical concentration of phosphates below which the algal blooms did not develop. Under certain conditions there could be abundant plankton even with a very small amount of phosphate (18). It was observed that 60–70% of the organic phosphorus in the dead plankton was converted to phosphate during decomposition (30).

Margief (19) reported on the liberation of inorganic phosphates from organic phosphorus compounds by the action of crustacea (Daphnia, Pulex and Chydorus
sphaericus). This observation suggests that the presence of algae may stimulate the production of phosphates by crustacea and that marine animals may play an important part in the phosphorus cycle in some lakes and seas.

Rideal (28) pointed out the value of the phosphate test in assessing drinking water for sewage contamination. According to a report from the USSR on the importance of phosphorus in the sanitary evaluation of water, an amount of phosphorus exceeding 0.2 mg/l of phosphorus indicates fecal pollution of surface waters and a concentration of soluble phosphate greater than 0.05 mg/l of water would indicate recent contamination (33).

Stangenberg (31) reported that the highest amounts of phosphates combined in unpolluted surface waters were 1 mg/l in lakes, 2mg/l in ponds and 1.5 mg/l in rivers. Sewage and industrial waste waters that contained phosphates, if discharged into a stream would cause excessive growth of algae. The algal material would decompose rapidly, causing a lack of dissolved oxygen in the water, production of H₂S and death of fish.

The enrichment of surface with plant nutrients is receiving widespread attention by engineers and scientists concerned with water quality. Although this complex phenomenon is not well understood, the evidence indicates that nitrogen and phosphorus are two of the most important biological nutrients. Phosphorus therefore appears to be the "Achilles heel" at which to strike in order to overcome the problem of excessive growth in receiving waters (27).


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Fig. II Population density of the five ciliates treated with 50 mg/100 ml KH$_2$PO$_4$.

BL—Blepharisma intermedium
BS—Blepharisma seshachari
SMA—Spirostomum ambiguum major
SMI—Spirostomum ambiguum minor
FR—Frontonia leucas
Fig. 1 Population density of the five ciliates treated with 50 mg/100 ml NH₄NO₃.

BI—Blepharisma intermedium
BS—Blepharisma seshachari
SMA—Spirostomum ambiguum major
SMI—Spirostomum ambiguum minor
FR—Frontonia leucas