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DIURNAL VARIATION IN POPULATION OF VIABLE HETEROTROPHIC MICROORGANISMS, ALKALINE PHOSPHATASE AND HETEROTROPHIC ACTIVITIES OF TWO SMALL PONDS

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Two small ponds were sampled for heterotrophic microbial population, alkaline phosphatases and coefficient of heterotrophic activity measurements at an interval of three hr. The values for these parameters showed significant variation with sampling period. Microbial population and total enzymatic activity was maximum at noon sampling but the soluble enzyme increased in the evening. The coefficient of heterotrophic activity showed an independent behaviour. Terrestrial fungi were dominant in both the ponds. Interestingly enough the fungal and yeast populations also showed a diurnal variation. The results indicate that the shallow systems show a marked diurnal variation in microbial populations and their activity.

INTRODUCTION

The bacterial and other heterotrophic microbial population of natural waters are subjected to various environmental changes in light radiation, quality and quantity of available nutrients, temperature, oxygen saturation and biological activity of phyto and zooplanktons. All these variables vary with time in 24 hr, as a consequence of which the microbial populations and their activity should be quite prone to short time changes in the environment. Most of the measurements of microbial activities are based on the quantitative estimation of microbial populations. The reliability of numerical count as a measure of bioactivity has been criticised at length (Stevenson, 1978; Strickland, 1971). Utilization of substrates (Godlewska-Lipowa, 1974) and enzyme activities (Reichardt *et al.*, 1967) are two most widely used methods to understand the involvement of microbial community in the functioning of various ecosystems. First demonstrated by Steiner (1938), phosphatases have engaged the attention of various workers in recent years and it has now been established that phosphatases play a key role in short and long time phosphorus cycle in aquatic systems (Jansson, 1976; Stevens and Parr, 1977).

The present communication reports investigations of the diurnal variation in heterotrophic microbial populations, free and seston associated alkaline phosphatases, heterotrophic activity, dissolved oxygen concentration, temperature, conductivity and pH in two biotypes of differing biological characters.

MATERIALS AND METHODS

The present study has been done in two shallow ponds at N. E Hill University Campus, Shillong which has an altitude of 1500 m at 25° 34' N latitude and 91° 52' E longitude. Pond1 has a surface area of 175 m^2 with almost uniform mean depth of 0·5 m The pond bottom is covered with profuse growth of filamentous algae predominantly composed of *Oscillatoria* sp. The algae are present in various stages of decomposition as depicted by the presence of brown to black bundles of filaments floating in and on the surface of water. The pond is situated in a low lying land on one side of which is a hillock while the other side is further down. The pond is a permanent system which receives water from the seapage from the hillock side and it maintains a uniform water level due to seapage of water to downward side. It is also provided with margin macrophyte vegetation predominantly composed of *Rotala rotundifolia*, *Polygonum hydropiper*, *Juncus prismatocarpus* and *Scirpus* sp. The system is autotrophic, with higher primary production estimates than respiration estimates (P/R ratio more than 1). Pond 2 is a temporary man-made water reservoir, with 10m^2 surface area and it accumulates water from a spring and maintains a depth of 1 m when full. The reservoir has cemented walls. The pond bottom, however, is clay mud with decaying leaves and twig as one of the major components of the system. The system is predominantly heterotrophic with lower primary production estimate than respiration estimates (P/R ratio less than 1). These ponds are 20 m apart, situated in a pine woodland with scattered pine (*Pinus kesiya* Royle) trees. Canopy is comparatively open near pond 1 and it receives sunlight in forenoon hours. Pond 2 is in shaded area and receives only diffused light radiations.

Samples for microbiological and enzymatic study were collected in sterile 500 ml conical flask avoiding the surface film from the same point every time. BOD bottles were used for samples collected for oxygen estimations. Polythene bottles were used for the samples of heterotropaic activity measurement. Immediately after collection, the samples were inoculated for estimating viable bacteria and fungi by spread plate method. For bacteria peptone yeast extract agar (Bacteriological peptone BDH 0·1% and yeast extract (Difco) 0·05% in distilled water and pH adjusted to 7·20 before sterilization) and Martins Rose Bengal Streptomycin Agar (Martin, 1950) was used for fungi. The samples were diluted thousand fold in the case of bacteria and were directly inoculated for fungi. 0 05 ml aliquot was used as inoculum and three replicates were maintained in each case. Plates were incubated at 20°C for 5 and 7 days for bacteria and fungi respectively and the visible colonies were counted. Taxonomic identification was done for filamentous fungi. Yeasts were counted from the Martin's rose bengal agar plates.

Alkaline phosphatase assay was done following the methods of Verstraete *et al.* (1976). The only modification was that the reagents and distilled water were filter sterilized and aseptic precautions were observed. Free alkaline phos-

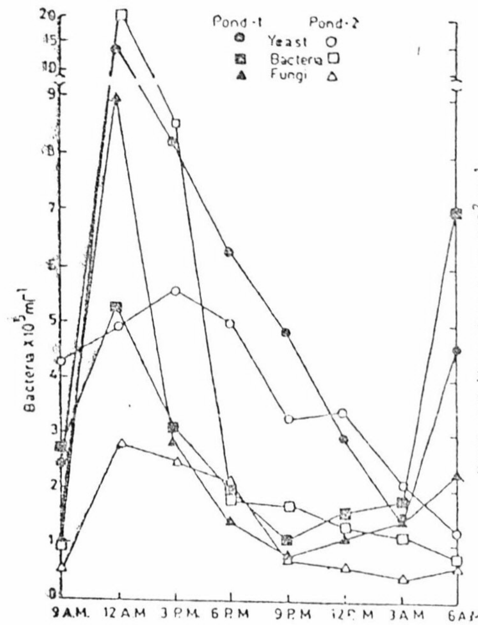

Fig. 1 Diurnal fluctuation in the population of bacteria, filamentous fungi and yeast, in the two ponds (all points represent mean of four determinations).

phatases were measured in water filtered through 0·2μ filter (Millipore Corporation, Mass., USA). Heterotrophic activity was determined following the method of Godlewska-Lipowa (1974). The determination of dissolved oxygen concentration was done by Winkler's method. The pH was measured be Systronic degital pH meter and conductivity was measured by Elico conductivity bridge. Whole processing of the sample was done within an hour after collection. Results presented in the figures are mean values of four diurnal cycles conducted on March 6–7, 13–14, 20–21 and 27–28, 1979.

RESULTS

A definite trend of microbial population change has been noticed. The maximum, viable heterotrophic bacteria was found at noon sampling which decreased at subsequent sampling periods. There was, however, slight increase in bacterial population in each hour. Same general trend was depicted by viable filamentous fungi and yeasts also (Fig. 1).

The coefficient of heterotrophic activity in autotrophic pond ranged from 1·1 to 1·69 while in saprobic it varied between 1 19 to 3·71. While oxygen consumption was related to the dissolved oxygen and microbial activity in the autotrophic pond, the saprobic pond showed a fluctuating behaviour. The

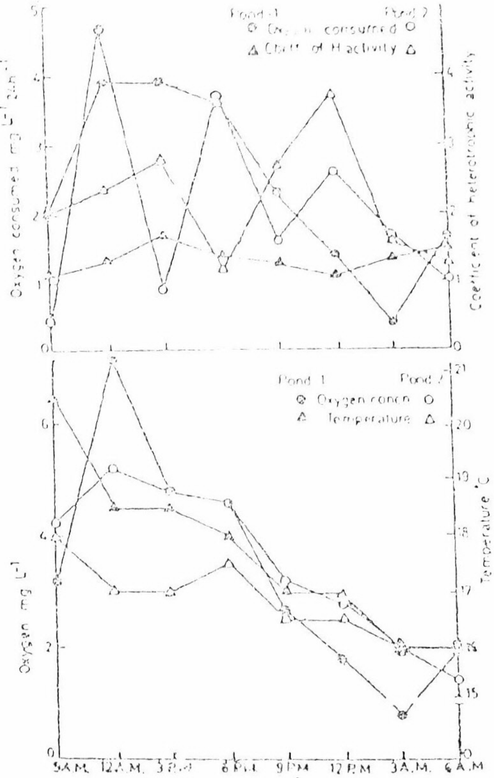

Fig. 2. Diurnal fluctuation oxygen consumed, coefficient of heterotrophic activity, oxygen concentration and temperature in the two ponds (all points represent mean of four determinations).

saprobic pond showed two maxima of heterotrophic activity, one at afternoon and the other at midnight sampling (Fig. 2). Dissolved oxygen concentration showed maxima at noon hour and thereafter it decreased gradually. Almost same pattern is followed by both systems except the autotrophic pond which showed an increase at sunrise while this was not shown by the saprobic pond (Fig. 2).

Maximum total alkaline phosphatase activity was found at the noon hour sampling in the saprobic pond. It was soluble free enzyme that showed interesting pattern. Autotrophic pond showed an increase in soluble free enzyme with the onset of dark, which shows a dark induced excretion of enzyme. The soluble free enzyme activity decreases in late night hours. The ratio of unfiltered to filtered sample enzyme decreases with onset of dark and reaches to minimum at 9 p.m. (Fig. 3). Saprobic pond exhibits less enzyme activity than the autotrophic pond. Significantly high content of soluble free enzyme was estimated only at 12 a.m., 3 p.m. and 6 p.m. Other samples either did not show any soluble free enzyme or the quantity was very meagre (Fig. 3).

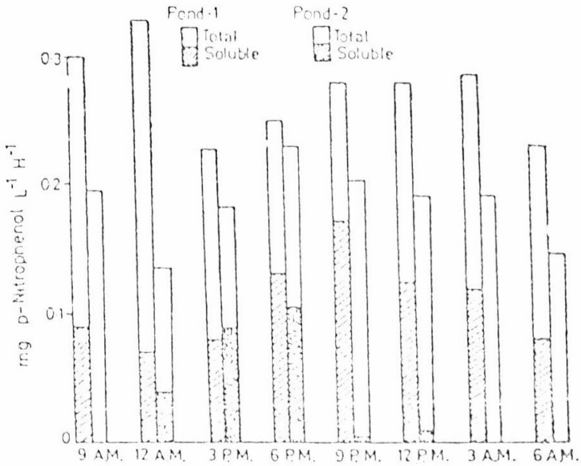

Fig. 3. Diurnal fluctuations in alkaline phosphatase activity in the two ponds (all points represent mean of four determinations).

The pH of autotrophic pond ranged from 6·09 to 8·02 and in saprobic pond it varied between 6·27 and 7·10. The conductivity of autotrophic pond ranged, between 36 and 88 and in saprobic pond it ranged from 30 to 75 umho cm^{-1} (Table 1).

TABLE 1. Conductivity umho cm-1 and pH values of two ponds (mean of four diurnal cycles).

	9 AM	12 AM	3 PM	6 PM	9 PM	12 PM	3AM	6AM
Conductivity Pond–1	53	36	48	88	46	48	47	43
Pond–2	37	46	48	75	47	71	30	35
Pond–1	7·06	6·91	8·02	7·44	6·09	6·55	6·54	6·54
Pond–2	6·55	6·35	6·80	7·10	6·95	6·34	6·30	6·27
Ambient Temperature (°C)	28	28·5	22	18	17·5	15·5	17·0	19

Cladosporium herbarum, *Trichoderma viride*, *Mucor racemosus*, *M. circinelloides*, *Penicillium* spp., *Geotrichum candidum*, *Alternaria alternata* and *Aspergillus niger* were dominant fungi isolated at various sampling periods. Most of these were common terrestrial fungi. No marked difference was noted in taxonomic composition of these fungi at various sampling times.

DISCUSSION

In present investigation the magnitude of change in various parameters under study was noted to be quite high. This may probably be due to the shallowness and very small size of the system. As pointed out under introduction, most of the studies made earlier are concerned with the seasonality of various hydro-microbiological characters. The present study emphasizes that there may be a wide difference in the values even at various times of the sampling. Dark induced excretion of organic matter was reported by Watt (1966). This study also shows that the onset of dark hours has increased the soluble free fraction of enzyme. It may, therefore, be pointed out that the storage of water samples in dark while transporting to the laboratory for enzymatic study may cause error in enzymatic estimation also as already pointed out by Sharp (1977) in the case of soluble organic matter determinations.

Reichardt *et al.* (1967) and Jones (1972 a) while studying depthwise distribution of alkaline phosphatases and their association with algal cell number, found a definite relation between them and concluded that the phytoplankton mineralization is accompanied with increased amount of soluble alkaline phosphatases. But the decrease in the ratio here in the first three dark hours is probably the result of light. Jones (1972 b) found significant correlation between total phosphatase activity and the microbial biomass. Although high phosphatase activity is coupled with the maximum microbial population in the autotrophic pond, the same does not hold good in all the cases and in both the systems. The heterotrophic activity measurements correspond well with the microbial number in the autotrophic system. This pattern, however, is not followed in the saprobic system. The rythmic behaviour in the oxygen consumption of the saprobic pond shows that probably some chemical or biological feedback is operating there.

The marked variation in fungal population can not be explained but probably it is clear from this observation that the fungi of terrestrial origin also reproduce and multiply in aquatic systems although the mode of their reproduction and growth behaviour may be 'atypical' as pointed out by Cooke (1976).

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