Observations on Algal Populations in an Experimental Maturation Pond System

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Abstract

A dense algal population is of primary importance in sewage maturation ponds for the removal of plant nutrients, especially ammonia and orthophosphate. In the Republic of South Africa and in South West Africa sudden algal population declines have occurred at times in maturation ponds. These declines cause serious problems in the reclamation of purified sewage effluents for re-use. The possible causes of these declines are considered here from the physical, biological and chemical observations of an experimental maturation pond system. The algal populations of the system fluctuated from very high to very low cell concentrations. High algal cell concentrations were only maintained for short periods of time. Zooplankton grazing was responsible for removing algae from the system at times but this was not the primary cause of all the algal population declines. It is very likely that sudden increases in ammonia nitrogen concentration when the pH was high, caused the algal population declines. Carbon deficiencies could have occurred during peak algal cell concentrations which would have resulted in population declines. A possible means of preventing the algal population declines could be by increasing the retention period of the pond system. The results confirmed that dense algal populations removed plant nutrients efficiently from the pond system.

Introduction

Maturation ponds in Southern Africa receive secondary treated effluent from conventional sewage works. They are used to reduce settleable solids, faecal organisms and dissolved plant nutrients in the water. Normally the effluents from maturation ponds are released into streams and rivers. However, at Windhoek (South West Africa), maturation pond effluent was reclaimed to produce water for domestic use. The reclamation process relies partly on algal metabolism in the maturation ponds to reduce ammonia nitrogen concentrations in the reclamation plant intake to levels suitable for subsequent breakpoint chlorination of the product water (Van der Post and Toerien, 1974).

Recently there have been reports of algal population declines at certain times of the year in the Windhoek maturation ponds and in other ponds throughout South West Africa and the Republic of South Africa. These declines obviously cause serious problems in the water reclamation process.

Previous research on algal population declines in maturation ponds, reviewed by Pieterse and Shillinglaw (1977) has resulted in the following observations: The dramatic reductions in algal populations appeared to occur only during the summer and autumn months. Hence climate was the most obvious factor to consider as a possible cause of the declines. However, previous research indicated that climatic conditions were probably not a direct cause of the declines. Various biological factors have been mentioned as possible suppressors of the algal populations in maturation ponds. A bacterial film has been observed on the surface of experimental maturation ponds at the time of algal decline but it is not certain whether this was the cause or result of the declines. Predation by zooplankton could cause the algal population declines. However, previous research workers (Van der Post and Toerien, 1974) did not consider predation rate significant as no abnormal increases in zooplankton concentrations in the maturation ponds were observed immediately before or during the algal declines. Data on the inorganic chemical composition of the maturation pond water showed that the declines were not due to plant nutrient deficiencies as these nutrients were present in high concentrations. Rather the high concentrations have been considered to be a possible cause of the algal population declines. High ammonia concentration in particular has been mentioned as an inhibitor of algal growth in sewage oxidation ponds (Abeliovich and Azov, 1976). Research has indicated that an organic inhibitor might suppress the algal populations in the ponds. Attempts to isolate and identify the organic inhibitor have been unsuccessful to date. Auto- and hetero- inhibition by algal cells could also be a cause of the algal population declines. However, no definite results have been obtained to support this idea.

This paper deals with detailed observations on the algal populations in an experimental maturation pond system situated at Pretoria. The algal population dynamics are

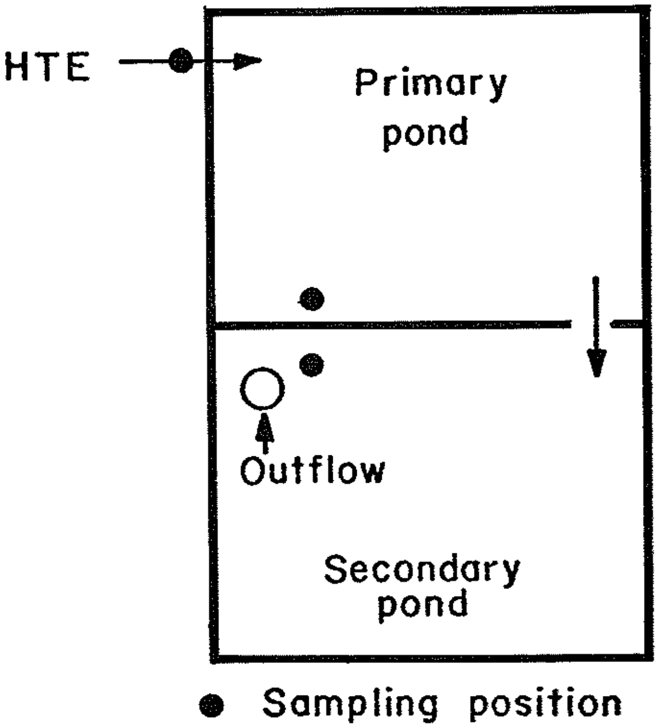

Figure 1
Diagram of experimental pond system showing sampling positions

compared with other biological, physical and chemical parameters in an attempt to find one or more parameters which change when the algal populations decline, thus identifying the possible cause of the declines.

Materials and Methods

The experimental maturation pond system consisted of a primary and secondary pond of equal volume and shape, in series, with a total volume of 107,4 m^3. The ponds were one metre deep and were lined with cement. The influent water of the system was humus tank effluent (HTE) which is the conventional secondary treated effluent from biological filters of sewage purification plants. The outflow from the secondary pond was a funnel at the surface of the water. Water retention time for the system was approximately five days. The study period extended from 4th August 1975 to 29th July 1976.

The sampling sites in the system were kept constant for the entire study and are shown in the diagram (Fig. 1) of the pond system. The system was sampled daily between 14h00 and 15h00. From August 1975 to February 1976, the ponds were sampled five times a week while samples were collected twice a week for the remainder of the study period.

Dissolved oxygen (DO) and temperature were measured with a portable YSI oxygen and temperature meter. DO was measured at a depth of 0,1 m while temperature was measured at the surface of the water. Measurements of pH were made with a glass electrode.

The chlorophyll *a* concentrations of the water samples taken with a hose-pipe sampler (18 mm – inside diameter) were determined by the method described by Marker (1972), using 95 per cent methanol as the extraction solvent.

The suspended particle counts were performed with an electronic Coulter counter (Model B). The thresholds were set to count particles above 5 μm in size. The diameter of the orifice was 100 μm, so that particles between 5 and 100 μm were counted.

Zooplankton densities were determined by making vertical hauls with a 100 μm mesh net in the ponds and the dry mass of the samples was used to estimate the zooplankton biomass per unit volume.

The algal species present were determined from hose-pipe water samples fixed with a 2 per cent acid Lugol's solution (Vollenweider, 1971). Algal cell counting was done according to the method of Utermöhl (1958).

Automated techniques were used for all the chemical analyses of the water samples. Alkalinity was measured as total alkalinity (mg $CaCO_3\ \ell^{-1}$) by colorimetry employing bromocresol green and a titration endpoint of pH 4,2. Ammonia nitrogen (NH_3-N) was measured by a technique involving phenol and hypochlorite (Harwood and Huyser, 1970). Nitrate nitrogen (NO_3^- -N) was determined by a method in which nitrate was reduced to nitrite in an alkaline hydrazine sulphate solution in the presence of a copper II catalyst. Nitrite was then determined by a colorimetric technique which employed sulphanyl amide and naphthylethylene diamine dihydrochloride (or naphthylamine) under acid conditions (Kamphake, *et al.* 1967). Orthophosphate phosphorus (PO_4^{3-} -P) was determined by a technique involving a molybdenum blue complex formed under acidic conditions and in the presence of ascorbic acid (Murphy and Riley, 1962).

Total plate counts for bacteria were performed on samples using the pour plate method with Oxoid Yeast Extract Agar and incubated at 37°C for 48 hours. For short periods of time total coliform counts (Mc Conkey Agar; incubation at 37°C for 24 hours) and presumptive *E. coli* counts (Mc Conkey Agar; incubation at 44,5°C for 24 hours) were made.

Results

Chlorophyll *a* concentration

The chlorophyll *a* concentrations of the pond water were assumed to be representative of the algal cell concentrations. This assumption was partly substantiated by the relationship which existed between the chlorophyll *a* concentrations (Fig. 2) and the suspended particle counts (Fig. 3). It should be emphasized that the Coulter counter would count both dead and living cells, hence the chlorophyll *a* results should not be expected to match the particle counts exactly.

The results (Fig. 2) clearly show periods of increase in algal cell concentrations followed by periods of fairly sudden decrease in cell concentrations. On some occasions the algal cell concentration declines occurred simultaneously in both ponds of the system, while on other occasions the declines were not synchronized in the two ponds (see Table 1). The results (Fig. 2) indicated that there were eleven periods when significant algal population declines occurred. These periods are indicated in Figure 2 and are summarized in Table 1.

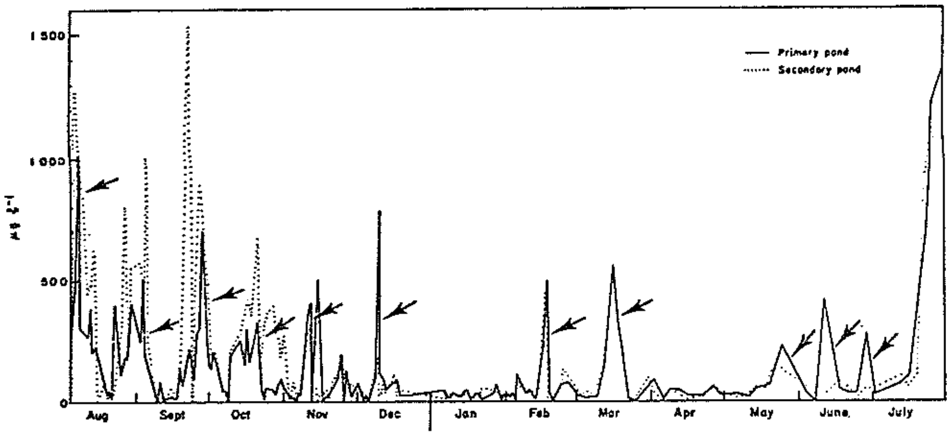

Figure 2
Chlorophyll a *concentrations (in $\mu g\ \ell^{-1}$) of pond system. Severe algal concentration declines are indicated by arrows.*

TABLE 1

PERIODS AND DURATION OF SIGNIFICANT ALGAL CONCENTRATION DECLINES AS WELL AS THE POND IN WHICH THE DECLINE OCCURRED. DURATION OF DECLINE IS THE PERIOD FROM THE FIRST DAY THE DECLINE WAS NOTED TO THE DAY THE ALGAL POPULATION REACHED ITS LOWEST CONCENTRATION.

Decline period	Duration of decline	Pond of occurrence
7 to 17 Aug.	10 days	Primary and secondary
4 to 8 Sept.	4 days	Primary and secondary
26 to 30 Sept.	4 days	Primary and secondary
20 to 23 Oct.	3 days	Primary and secondary
11 to 12 Nov.	1 day	Primary and secondary
9 to 10 Dec.	1 day	Primary and secondary
17 to 20 Feb.	3 days	Primary and secondary
15 to 22 Mar.	7 days	Primary and secondary
24 May to 7 June	11 days	Primary and secondary
10 to 17 June	7 days	Primary
28 June to 1 July	3 days	Primary

From the results (Fig. 2), it is evident that the algal populations could reach extremely high cell concentrations (i.e. 1 500 μg chlorophyll *a* ℓ^{-1}) but these high concentrations did not last long and were often followed by population declines to very low concentrations (sometimes almost zero μg chlorophyll *a* ℓ^{-1}).

Periods of algal population declines occurred during the summer and winter months of the study period. The highest values of chlorophyll *a* were reached during July, August and September which corresponded with the periods of increasing water temperature (Fig. 4) after the cold winter months. The chlorophyll *a* concentrations were never very high between January and June 1976.

Suspended particle counts

The particle counts (Fig. 3) for the system showed very marked fluctuations ranging from 2 000 to over 200 000 particles $m\ell^{-1}$. These fluctuations occurred over short periods of time which coincided generally with large changes in the chlorophyll *a* concentration.

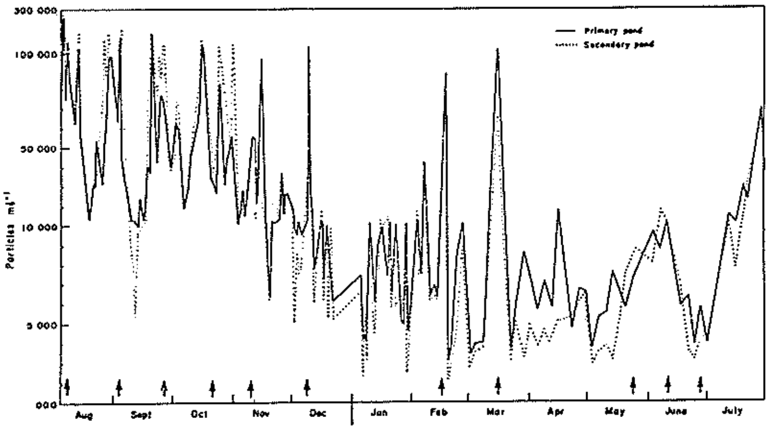

Figure 3
Suspended particle counts in pond system. The arrows indicate the beginning of the noted algal concentration declines.

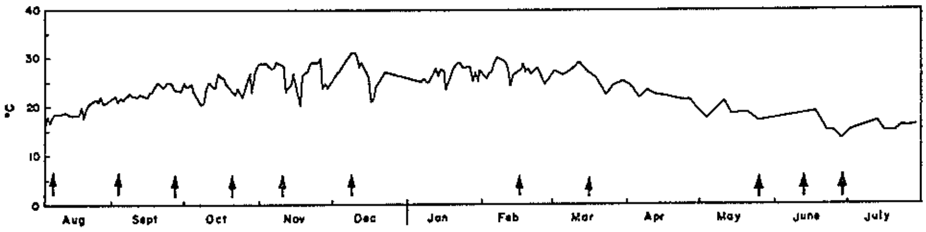

Figure 4
Average surface water temperature (in °C) of the two ponds. The arrows indicate the beginning of the noted algal concentration declines.

Quantitative estimation of algal populations

Microscopical studies of the water samples indicated that different species of algae were dominant immediately before the various population declines. Table 2 lists the dominant algal species present prior to each recorded algal concentration decline.

While the Chlorophyceae were involved in most of the population declines, members of the Cryptophyceae, Euglenophyceae and Cyanophyceae were occasionally involved. The most frequently dominant algae in the system were the unicellular flagellate forms of the Chlorophyceae.

From Table 2 a seasonal succession of dominant algal species appears to be evident: *Chlorella sp.* was dominant during mid-winter, but as temperatures increased in spring it was replaced by *Chlamydomonas globosa* which in turn was followed by *Micractinium pusillum* in early summer. *Euglena sanguinea* and *Cryptomonas ovata* together with *Chlamydomonas globosa* were dominant during mid-summer. *Chlamydomonas globosa* again became dominant during late summer and autumn.

Animal populations

Zooplankton concentration was not regularly monitored. Nevertheless periods of high and low concentrations were evident, but on a number of occasions the concentrations were not synchronized in both ponds of the experimental system.

TABLE 2

LIST OF THE DOMINANT ALGAL SPECIES PRESENT AT THE BEGINNING OF THE OBSERVED POPULATION DECLINES WHICH OCCURRED IN THE SYSTEM DURING THE STUDY PERIOD

Decline period	Dominant algal species
7 to 17 Aug.	*Chlamydomonas globosa* Snow
4 to 8 Sept.	*Chlamydomonas globosa* Snow
26 to 30 Sept.	*Micractinium pusillum* Fresenius
20 to 23 Oct.	*Micractinium pusillum* Fresenius
11 to 12 Nov.	*Euglena sanguinea* Ehrenberg and *Chlamydomonas globosa* Snow
9 to 10 Dec.	*Cryptomonas ovata* Ehrenberg and *Chlamydomonas globosa* Snow
17 to 20 Feb.	*Chlamydomonas globosa* Snow
15 to 22 Mar.	*Chlamydomonas globosa* Snow
24 May to 7 June	*Chlorella* sp.
10 June to 17 June	*Chlorella* sp.
28 June to 1 July	*Chlorella* sp. and *Anabaena* sp.

Table 3 shows the chlorophyll *a* and zooplankton concentrations immediately before and during the algal population declines for which zooplankton data are available. The first two algal population declines were co-incident with considerable increases in the zooplankton concentrations. However this relationship was not evident during the latter four algal population declines.

The dominant zooplankton species were *Daphnia* sp., *Moina* sp., *Asplanchna* sp., and *Branchionus calyciflorus* Pallas.

Large populations of tadpoles and adults of the toad, *Xenopus laevis* (Daudin) inhabited the ponds. The tadpoles feed mainly on algae which they filter from the water. They could therefore have had an effect on the algal concentrations. Likewise the adults, which are carnivorous, could have had an effect on zooplankton and other animal populations in the ponds. *Xenopus laevis* populations were unfortunately not monitored.

Temperature

There was a seasonal increase in water temperature from August to the beginning of November (Fig. 4). The temperature remained above 20°C for most of the study period and was below 20°C only during May, June and July. The lowest temperature was recorded on June 29 (13°C). The temperature gradually increased during July. The highest temperature of 29,6°C was recorded on March 11.

The results presented in Figs. 2 and 4 indicated that generally higher algal concentrations occurred together with higher summer temperatures, while in the winter months lower algal concentrations were associated with lower temperatures.

No discernable relationship existed between the water temperature and the occurrence of algal population declines (cf. Figs. 2 and 4). Very large populations never occurred when the temperatures were low.

Dissolved oxygen (DO)

The DO concentrations of the water (Fig. 5) were generally high during periods of peak algal cell concentrations (Fig. 2) and low during periods of low algal cell concentrations. This indicated that the biological activity of the algae contributed the major portion of the DO to the system. The DO concentrations were above 10 mg ℓ^{-1} and the algal cell concentrations

TABLE 3

CHLOROPHYLL *a* AND ZOOPLANKTON (DRY MASS) CONCENTRATIONS IMMEDIATELY BEFORE AND DURING SOME OF THE ALGAL POPULATION DECLINES

Decline period	Date	Primary pond		Secondary pond	
		Chlorophyll *a* ($\mu g\ \ell^{-1}$)	Zooplankton ($g\ m^{-3}$)	Chlorophyll *a* ($\mu g\ \ell^{-1}$)	Zooplankton ($g\ m^{-3}$)
4-8 Sept.	3 Sept.	507	1,35	417	1,63
	10 Sept.	78	51,25	3	31,78
26-30 Sept.	17 Sept.	12	4,96	9	8,41
	26 Sept.	696	0,65	885	0,87
	1 Oct.	132	7,82	180	1,45
11-12 Nov.	5 Nov.	0	78,43	24	4,69
	12 Nov.	0	5,78	66	21,64
24 May – 7 June	20 May	69	0,57	129	0,26
	24 May	225	1,03	129	0,69
	3 June	36	0,37	60	0,27
	7 June	6	0,13	30	0,06
10-17 June	10 June	414	0,27		
	17 June	48	0,45		
18 June – 1 July	24 June	36	0,01		
	28 June	276	0,03		
	1 July	30	0,07		

were high for major portions of the first five months of study, while the DO concentrations were often below 10 mg ℓ^{-1} and the algal cell concentrations low during the last seven months. Only towards the end of July 1976 did marked increases occur in the DO and algal cell concentrations.

The ponds were aerobic at all times. The surface water was supersaturated with oxygen for large portions of the first five months of study due to the high algal cell concentrations during this period.

pH

The pH (Fig. 6) of the HTE varied between 7,0 and 8,0 for most of the study period. The pH of the secondary pond was always higher than that of HTE indicating that algal photosynthesis was probably removing carbon dioxide thus raising the pH in the pond system.

High pond pH values generally coincided with periods of large algal cell concentration in the system (cf. Figs. 6 and 2). Substantial decreases in pond pH were associated with the algal population declines.

A comparison between the pH and chlorophyll *a* concentrations of the secondary pond gave the following correlation coefficients: r = 0,545 (n=74) for the period August to November and r = 0,705 (n=72) for January to July. This result indicates that a highly significant and positive correlation existed between the pH of the pond water and the chlorophyll *a* concentration. Hence the algal metabolism in the pond system primarily determined the pH of the water. The fact that there were instances where the pH was relatively high

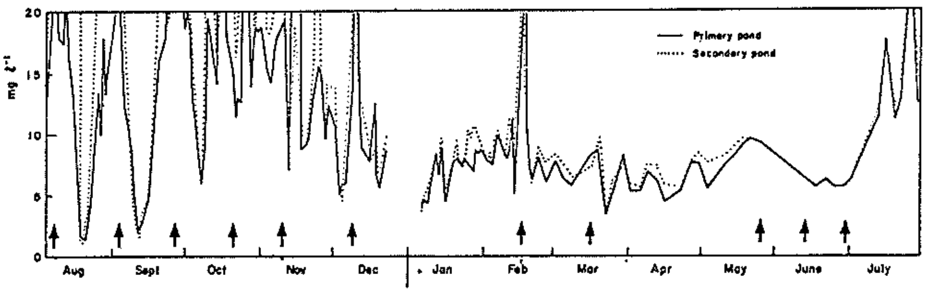

Figure 5
Dissolved oxygen concentrations (in mg ℓ^{-1}) for pond system. The arrows indicate the beginning of the noted algal concentration declines.

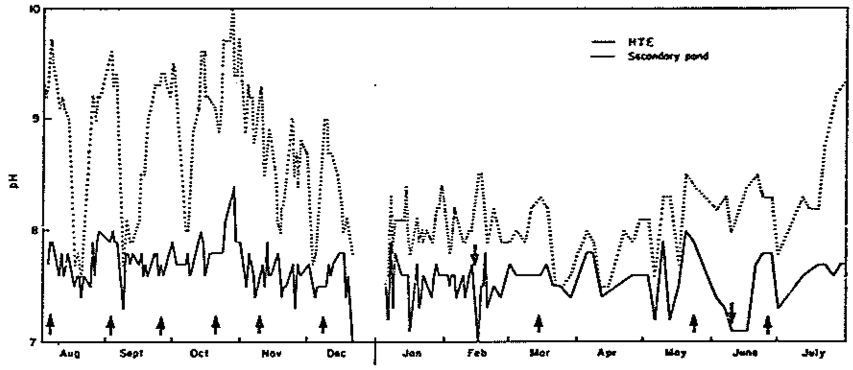

Figure 6
pH of influent (HTE) and secondary pond. The arrows indicate the beginning of the noted algal concentration declines.

while the chlorophyll *a* concentration was low indicated that either the cells at these low concentrations were metabolically extremely active or that some other factor also influenced the pH.

In general the pH values of the pond water were lower between January and July 1976 when algal cell concentrations were lower (cf. Figs. 6 and 2).

Total alkalinity

The total alkalinity (Fig. 7) of the pond system and the HTE fluctuated between 50 and 260 mg $CaCO_3$ ℓ^{-1}. The alkalinity of the HTE had an overriding influence on the pond's alkalinity. Only at times of large algal cell concentrations were the pond alkalinity values substantially lower than the HTE values (cf. Figs. 7 and 2). Changes in alkalinity values of the HTE or ponds could not be related to the observed algal population declines.

Ammonia nitrogen

The ammonia concentrations of HTE were generally higher and more variable than that of the maturation pond system (Fig. 8). The HTE ammonia concentrations were lower during the last seven months of the study than during the first five months. This was due to reduced loading of the secondary treatment bio-filters.

On a few occasions the ammonia concentrations (Fig. 8) were the same in the HTE and the pond system indicating that the biological activities in the ponds were not reducing the ammonia concentration of the influent water (HTE). The periods when the ammonia concentrations of the pond water were considerably lower than the HTE concentrations, indicated that biological activity in the ponds was reducing the ammonia concentrations fairly efficiently. These periods normally corresponded with periods of large algal cell concentrations (cf. Figs. 8 and 2). The percentage ammonia reduction in the ponds when algal cell concentrations were high was usually over 80 per cent, and was as high as 95 per cent between February 12 and 16. When the algal cell concentrations were at their lowest, virtually no reduction in ammonia concentrations occurred in the ponds. This indicates that a high algal concentration was necessary in the ponds for an efficient removal of ammonia from the system. An exception to this generalization occurred during January 1976 when the percentage ammonia reduction was 80 per cent while the algal cell concentration was relatively low. The only possible explanation which could be given for this discrepancy from the normal trend was that a dense growth of a filamentous alga, *Mougeotia* sp., occurred on the surface water of the system during this period. This alga could have removed large quantities of ammonia. The long *Mougeotia* filaments formed dense mats on the surface of the water and it is quite possible that the small diameter (18 mm) hose-pipe sampler might have passed through the filamentous mat without breaking any of the filaments. Therefore, probably no or very few filaments were collected in the samples. Hence the measured chlorophyll *a*

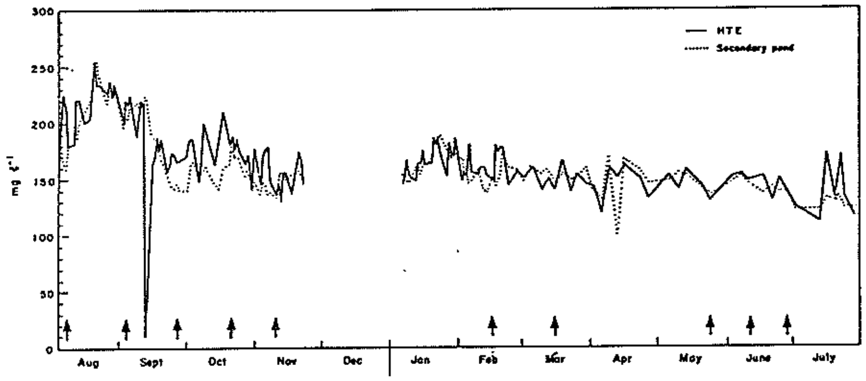

Figure 7
Total alkalinity (as mg $CaCO_3\ell^{-1}$) of influent (HTE) and secondary pond. The arrows indicate the beginning of the noted algal concentration declines.

concentration would not have increased during the period of dense *Mougeotia* growth.

From the results presented in Table 4, it is evident that during the algal population declines in late September, November and early June, the algal cell concentration decreased as the ammonia concentration of the HTE increased. This would seem to indicate that the increase in ammonia concentration of the HTE by influencing the ammonia concentration of the pond water caused a decline in the algal concentration. It is furthermore noteworthy that during the algal population declines in late September, November, February and early June the pH of the pond water was high at the onset of the decline in algal concentration. However, during the algal population decline in March, the ammonia concentration of HTE decreased with the algal cell concentration. Absolute ammonia concentrations of HTE were not available for the other algal population declines observed.

A statistical comparison between the ammonia concentration of the HTE and the chlorophyll *a* concentration of the secondary pond resulted in the following correlation coefficients: $r = 0{,}253$ ($n=74$) for the period August to November and $r = -0{,}248$ ($n=72$) for the period January to July. These results indicate that the ammonia concentration of the HTE is correlated with the chlorophyll *a* concentration of the secondary pond of the system. However the first period of August to November gave a positive correlation while the second period of January to July gave a negative correlation. Hence it is confusing whether the correlation between the ammonia concentration of HTE and the chlorophyll *a* concentration of the secondary pond is positive or negative.

The correlation coefficients of the comparison between the pH and ammonia concentrations of the secondary pond of the system were as follows: $r = 0{,}068$ ($n=74$) for the period August to November and $r = -0{,}306$ ($n=72$) for January to July. Hence the pH and ammonia concentrations of the secondary pond were not correlated during the period August to November, and were negatively correlated between January and July.

TABLE 4

A COMPARISON OF THE HTE AMMONIA CONCENTRATION WITH THE CHLOROPHYLL *a* AND pH OF THE SECONDARY POND FOR SOME OF THE OBSERVED ALGAL POPULATION DECLINES

Decline period	Date	HTE-NH_3-N mg ℓ^{-1}	pH	Chlorophyll $\mu g\ \ell^{-1}$
26 to 30 Sept.	24 Sept.	5,5	9,3	576
	26 Sept.	5,5	9,4	885
	29 Sept.	6,5	9,2	363
	30 Sept.	7,0	9,4	189
11 to 12 Nov.	10 Nov.	1,5	9,3	279
	11 Nov.	2,4	8,7	306
	12 Nov.	3,6	8,5	66
	13 Nov.	5,1	8,7	51
17 to 20 Feb.	16 Feb.	1,9	8,5	438
	17 Feb.	3,1	8,5	357
	18 Feb.	3,6	8,4	15
	19 Feb.	4,5	8,1	9
	20 Feb.	4,9	7,9	6
15 to 22 Mar.	11 Mar.	2,8	8,2	135
	15 Mar.	2,3	8,3	498
	18 Mar.	1,3	8,2	294
	22 Mar.	0,2	7,5	18
24 May to 7 June	20 May	2,0	8,5	129
	24 'May	1,2	8,4	129
	3 June	2,4	8,2	60
	7 June	1,7	8,3	30
	10 June	3,9	8,0	21

Nitrate nitrogen

The nitrate concentrations (Fig. 9) of the pond water tended to follow those of the HTE. The concentration of nitrate in the HTE fluctuated between 0,2 and 10 mg ℓ^{-1}.

Efficient nitrate removal in the ponds corresponded with high algal cell concentrations only to a certain extent (cf. Figs. 9 and 2). Periods during January, March and April exhibited quite efficient nitrate removal (54% during mid January) while the algal populations were relatively low. The nitrate removal in the ponds appeared to be never greater than 55 per cent.

Since no observations were made on the extent of nitrification in the ponds, the above statement must be viewed with caution keeping in mind that nitrification could have contributed to the nitrate nitrogen present in the pond water.

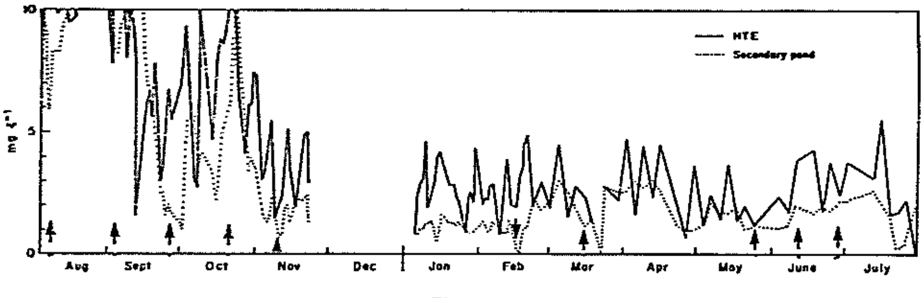

Figure 8
Ammonia-nitrogen concentrations (in mg ℓ^{-1}) of influent (HTE) and secondary pond. The arrows indicate the beginning of the noted algal concentration declines.

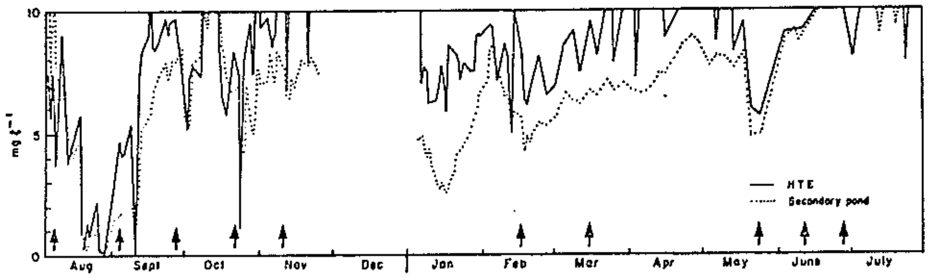

Figure 9
Nitrate-nitrogen concentrations (in mg ℓ^{-1}) of influent (HTE) and secondary pond. The arrows indicate the beginning of the noted algal concentration declines.

The nitrate concentrations were higher during the second half of the study period which corresponded with a lower ammonia concentration. This suggests that because the loading was less to the biological filters, more ammonia had been oxidized to nitrate by the time the effluent (HTE) reached the pond system.

Orthophosphate phosphorus

The orthophosphate concentrations of the HTE never rose above 10 mg ℓ^{-1} or fell below 1 mg ℓ^{-1} (Fig. 10). The phosphate removal from the system was particularly efficient at times during the first five months of study which corresponded to periods of high algal cell concentrations (cf. Figs. 10 and 2). The highest recorded orthophosphate removal (84%) occurred between September 19 and 25. During the second half of the study period the orthophosphate removal was exceptionally poor even in January when the nitrogen removal was high. On many occasions during the second half of the study period the HTE had orthophosphate concentrations lower than those of the pond system which indicated that firstly organic decay in the ponds was increasing the dissolved orthophosphate concentrations of the system, or secondly that phosphate precipitated due to alkaline pH conditions could again become soluble under different conditions.

Other parameters

Superficial observations on bacterial cell concentrations indicated that increases in bacterial plate counts did not coincide with declines in algal cell concentrations.

The concentration of methylene blue active substances fluctuated in a range of 300 to 1 800 $\mu g\ \ell^{-1}$. These fluctuations showed no patterns coinciding with the algal cell concentration patterns.

The calcium concentration of the HTE and pond system was below 50 mgℓ^{-1} for the entire study period. For most of the study period the sulphate concentration of the HTE and pond system varied between 30 and 45 mg ℓ^{-1}.

The magnesium concentration of the system was remarkably stable between 14 and 23 mg ℓ^{-1}. The zinc concentration was less than 25 $\mu g\ \ell^{-1}$ for most of the study period. However on one occasion the concentration of zinc in the HTE was 145 $\mu g\ \ell^{-1}$. This high zinc concentration could not be related to any changes in the algal cell concentration. The copper concentrations were rarely above 25 $\mu g\ \ell^{-1}$.

Discussion

The algal cell concentrations of the experimental ponds exhibited a general pattern of wax and wane which is indicative of populations subjected to changing environmental conditions. The pond system had the potential to support large concentrations of algal cells but these concentrations could only be sustained for brief periods. The high algal cell concentrations reached were ultimately followed by declines to very low concentrations. The wax and wane pattern continued throughout the twelve months of study contrary to Van der Post and Toerien's (1974) results which suggested that the algal population declines to low concentrations only occurred during the warmer months of the year. This evidence points to the

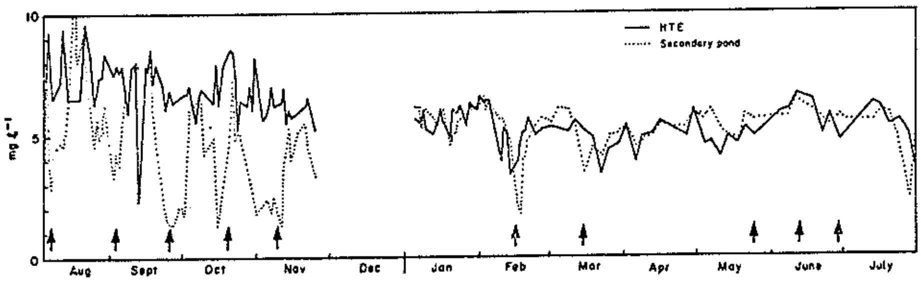

Figure 10
Orthophosphate-phosphorus concentrations (in mg ℓ^{-1}) of influent (HTE) and secondary pond. The arrows indicate the beginning of the noted algal concentration declines.

intermittent presence of some factor which suppresses algal growth and/or removes algal cells from the system at a very rapid rate. Another possibility is that an algal growth suppressor is almost continuously present and only when the suppressing factor is intermittently absent, do the algal concentrations exhibit a peak. Based on the results presented in this paper it seems unlikely that an intermittently present algal growth stimulant is responsible for the onset of increased algal growth.

The relationship between the suspended particle counts and the chlorophyll *a* concentration indicated that the declines in chlorophyll *a* were primarily due to a reduction in the number of viable algal cells present and not merely to a reduction in the chlorophyll *a* concentration due to dead cells. This implies that algal cells were actively removed from suspension during periods of algal population declines, either by sedimentation or by removal through the outflow. Because algal cells were the dominant form of suspended particles in the system, it may be concluded that any increase in turbidity was due to an increase in the algal cell concentration. Therefore light limitation would only be likely to occur during peak algal cell concentrations. Nevertheless, were light limitation to cause a decline of peak algal cell concentrations, it is difficult to conceive why such declines should continue to the point where algal populations became very sparse.

The list of dominant algal species associated with the population declines (Table 2) indicates that members of the Chlorophyceae and other classes of algae were involved. This result does not agree with previous observations (reviewed by Pieterse and Shillinglaw, 1977) which suggested that only the Chlorophyceae were dominant at the beginning of the population declines.

De Noyelles (1967) and Raschki (1970) have reported that severe reductions in algal numbers occurred in sewage ponds due to heavy grazing by cladocerans and rotifers. Therefore with this evidence and the results of Table 3, it can be postulated that the grazing pressure by zooplankton could have accounted for some of the algal population declines but not for all of them. It is important to consider that the grazing pressure could be of even greater importance if the algal growth rate is suppressed by some other factor.

The temperature conditions of the pond water were probably not the direct causes of the algal population declines. Nevertheless, temperature could have had an indirect influence on the algal populations as the highest algal cell concentrations occurred during periods of increasing and higher temperatures and the algal population declines were most frequent during the warmer months of the study period.

The results presented in this paper indicated that high algal cell concentrations generally resulted in high pH conditions which are according to Talling (1976) associated with free CO_2 depletion. According to King (1972) the high demand for CO_2 by sewage lagoon algae maintains the free CO_2 concentration during daylight hours below atmospheric equilibrium for most of the summer so that under specific pH and alkalinity conditions algae may suffer a marked CO_2 limitation for significant periods of the day. In enriched waters (like maturation pond water) with low alkalinity carbon uptake by algae can reduce CO_2 species to rate limiting concentrations that exogenous and endogenous fluxes of CO_2 may not circumvent (Talling, 1976). Thus carbon dioxide deficiency could occur during peak algal cell concentrations in maturation pond water. Goldman *et al.* (1972) in fact stated the possibility that carbon could become growth rate limiting in sewage lagoons. However, the possible algal growth rate limitation by carbon in maturation ponds must be investigated thoroughly since Goldman *et al.* (1972, 1974) argued that the availability of inorganic carbon from the carbonate-bicarbonate system is sufficient in most natural waters to ensure that some other nutrient is growth limiting, and also since Schindler *et al.* (1972) showed that even the slow transport of atmospheric carbon dioxide to the aqueous phase in a Canadian Shield lake is fast enough under conditions of low alkalinity and high pH that sufficient quantities of inorganic carbon are available for algal growth.

The results indicate that efficient removal of ammonia from a maturation pond system is possible if an actively growing algal population is present in the system. Either the high pH conditions generated by algal metabolism cause ammonia to volatilize or else the algae use ammonia directly in their metabolism. It does not appear that bacterial activity was responsible for the efficient ammonia removal as no significant increases occurred in the bacterial concentration (total plate counts) during periods of efficient ammonia removal. However, this observation must be treated with extreme caution since plate count numbers depend largely upon the culture medium used. Obviously more observations using different media would seem to be in order. It is also evident that the algal populations could be capable of removing nitrate simultaneously with ammonia as the nitrate concentrations were also reduced during periods of high algal cell concentrations. The orthophosphate removal was also efficient during periods of dense algal populations. This evidence indicates that high algal cell concentrations are an efficient and inexpensive method for removing plant nutrients from a hypereutrophic pond system. The only problem is to maintain the dense algal populations necessary for the nutrient removal.

The fact that algal population declines where experienced in the system at times of high pH and increased ammonia concentrations in the HTE (Table 4), indicated that these conditions might be inhibitory to algal growth. Work from Israel (Abeliovich and Azov, 1976) indicated that ammonia, at concentrations greater than 2,0 mM and at pH values over 8,0 inhibits photosynthesis and growth of *Scenedesmus obliquus*, a dominant species in high-rate sewage oxidation ponds. Photosynthesis of other species of algae was also susceptible to ammonia inhibition. The results presented in the present study are confusing as the highest algal cell concentrations and most of the algal population declines occurred during the first five months of study which corresponds to the period of highest ammonia concentrations. Moreover algal population declines still occurred during the last seven months of study which corresponds to the period of much lower ammonia concentrations. Hence it is possible that the concentration of ammonia is not of paramount importance, but rather a sudden increase in ammonia during high pH conditions, might be the cause of algal population declines. Possibly the algal populations can adapt to high ammonia concentrations but they cannot adapt to sudden increases in ammonia at high pH.

The work of Abeliovich and Azov (1976) indicated also that a high-rate oxidation pond operated at 120 hours retention time, maintained a steady state with respect to algal growth and oxygen concentration, and the concentration of ammonia did not exceed 1,0 mM. Shifting the pond to 48 hour retention time caused an increase in ammonia concentration in the pond water to 2,5 mM, and the pond gradually turned anaerobic. This suggests that by lengthening the retention time of a pond system, the ammonia concentration in the system is reduced and any changes in the concentration will be gradual thus allowing the algal population time to adapt to the changes. Therefore, longer retention periods could possibly prevent the occurrence of severe algal population declines in maturation pond systems.

The oxidation ponds (Abeliovich and Azov, 1976) functioned well at 120 hours (5 days) retention time while the maturation pond system in the present study did not function well at 5 days retention period. One possible means of explaining this is that carbon dioxide deficiency would be more severe in maturation ponds than in oxidation ponds owing to the lower concentration of organic material entering maturation ponds. By lengthening the retention time of maturation ponds, more carbon dioxide diffusion per unit volume would occur at the air-water interphase thus preventing severe carbon dioxide deficiency.

Conclusion

The algal populations of the experimental pond system exhibited a wax and wane pattern extending over the entire twelve month study period. Members of different classes of algae were dominant during periods of population declines. Zooplankton grazing was capable of reducing the algal cell concentrations but this was not the cause of all the dramatic algal population declines observed. The water temperature conditions of the ponds were not a direct cause of the algal population declines. The pH and DO conditions in the ponds were influenced by the algal metabolism in the ponds. The algae present during peak cell concentrations were probably obtaining most of their carbon dioxide from the bicarbonate-carbonate system owing to the high pH conditions produced by the metabolism of the algae. Therefore, carbon dioxide deficiency might have occurred during peak algal cell concentrations which could have resulted in the population declines. This aspect of carbon dioxide deficiency must be investigated thoroughly. Dense algal populations were capable of removing directly and/or indirectly large amounts of dissolved ammonia, nitrate and orthophosphate from the pond system. One possible cause of the algal population declines was the sudden increases in ammonia concentration of the HTE at times of high pH conditions in the ponds. The results from this work indicated that bacteria, heavy metal toxicity and high concentrations of detergents can probably all be excluded as possible causes of the observed algal population declines. A possible means of preventing the algal population declines, which is being investigated, is to lengthen the retention period of the system thus allowing the algal populations time to adapt to any increases in ammonia concentration of the influent or to alleviate acute carbon deficiency through extended absorption of atmospheric carbon dioxide.

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References

ABELIOVICH, A. and AZOV, Y. (1976): Toxicity of ammonia to algae in sewage oxidation ponds. *Appl. Environ. Microbiol.* **31** 801-806.

DE NOYELLES, Jr. F. (1967): Factors affecting phytoplankton distribution in a double-cell sewage lagoon. *J. Phycol.* **31** 174-181.

GOLDMAN, J.C. *et al.* (1972): The effect of carbon on algal growth – its relationship to eutrophication. *Wat. Res.* **6** 637-679.

GOLDMAN, J.C. *et al.* (1974): The kinetics of inorganic carbon limited algal growth. *J. Wat. Poll. Control Fed.* **46** 554-574.

HARWOOD, J. E. and HUYSER, D. J. (1970): Automated analyses of ammonia in water. *Wat. Res.* **4** 695-704.

KAMPHAKE, L.J., HANNA, S.A. and COHEN, J.M. (1967): Automated analyses for nitrate by hydrazine reduction. *Wat. Res.* **1** 205-216.

KING, D.L. (1972): Carbon limitation in sewage lagoons. *Nutrients and Eutrophication: The limiting nutrient controversy.* (Ed. by G.E. Likens), pp 98-110. Spec. Symp. Am. Soc. Limnol. Oceanogr. 1.

MARKER, A.F.H. (1972): The use of acetone and methanol in the estimation of chlorophyll in the presence of phaeophytin. *Freshwat. Biol.* **2** 361-385.

MURPHY, J. and RILEY, J.P.A. (1962): Modified single solution method for the determination of soluble phosphate in sea water. *J. mar. biol. Ass. U.K.* **37** 9-14.

PIETERSE, A.J.H. and SHILLINGLAW, S.N. (1977): Die vertraging van alggroei in verouderingsdamme. In the press.

RASCHKI, R.L. (1970): Algal periodicity and waste reclamation in a stabilization pond ecosystem. *J. Wat. Poll. Control Fed.* **42** 518-530.

SCHINDLER, D.W. *et al.* (1972): Atmospheric carbon dioxide: its role in maintaining phytoplankton standing crops. *Science, N.Y.* **177** 1192-1194.

TALLING, J.F. (1976): The depletion of carbon dioxide from lake water by phytoplankton. *J. Ecol.* **64** 79-121.

UTERMÖHL, H. (1958): Zur Vervollkommnung der quantitativen Phytoplankton – Methodik. *Mitt. Int. Verein. Limnol.* **9** 1-38.

VAN DER POST D.C. and TOERIEN, D.F. (1974): The retardment of algal growth in maturation ponds. *Wat. Res.* **8** 593-600.

VOLLENWEIDER, R.A. (1971): A manual on methods for measuring primary production in aquatic environments. *IBP handbook No. 12.* Blackwell Scientific Publications.