Phytoplankton and nutrient dynamics in two ponds of the Esmoriz wastewater treatment plant (Northern Portugal).

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ABSTRACT

Phytoplankton dynamics in two ponds of the Esmoriz wastewater treatment plant (Northern Portugal), were studied between December 1998 and July 1999. Ponds were characterized by a wide range of water temperatures (i.e. between 9.1 and 25.3 ºC), and dissolved oxygen concentrations (i.e. 0.9 - 10.8 mg l^{-1} in the facultative pond, and 2.8 - 18.2 mg l^{-1} in the maturation one). The maximum chlorophyll *a* concentration was reached in July in the facultative pond (i.e. 2054.6 mg m^{-3}) and in February in the maturation pond (i.e. 2042.6 mg m^{-3}). Mean chlorophyll *a* concentrations varied between 694.1 mg m^{-3} in the facultative pond and 942.1 mg m^{-3} in the maturation pond. Nutrient concentrations were 1.6 mgl^{-1} NH_4^+-N, 0.1 mgl^{-1} NO_2—N, 10.2 mgl-' NO_3—N and 1.6 mgl^{-1} PO_4^{3}—P. Chlorophytes and euglenophytes dominated the phytoplankton in the facultative pond; the first group represented almost 85 % of total phytoplankton density in January, May and June, whereas euglenophytes reached 96 % of total phytoplankton density in April. The genera *Oocystis* and *Pandorina* were dominant within the chlorophytes, while the genus *Euglena* was dominant within euglenophytes. Cyanobacteria in the maturation pond represented more than 95 % of total phytoplankton density in April, May and July. Dominant cyanobacteria were *Planktothrix mougeotii* (April and May), *Microcystis aeruginosa* and *Pseudoanabaena mucicola* (July). In the maturation pond, euglenophytes were less than 6 % of total phytoplankton abundance, except in March, when *Euglena* accounted for *67* % of total phytoplankton density. These densities indicate that the wastewater treatment plant (WWTP) may be an optimal habitat for cyanobacteria, and consequently, a possible source of cyanobacterial toxins.

Key words: phytoplankton, cyanobacteria, nutrients, waste water treatment plant

RESUMEN

Desde Diciembre de 1998 a Julio de 1999 se ha estudiado la dinamica del fitoplancton en dos lagunas de la planta de tratamiento de aguas residuales de Esmoriz. Las lagunas se caracterizaron por un amplio rango de temperaturas (p.e. entre 9.1 y 25.3 ºC), y concentraciones de oxigeno disuelto (p.e. 0.9- 10.8 mg l^{-1} en la laguna facultativa, y 2.8 – 18.2 mg l^{-1} en la de maduración)- La concentracidn maxima de clorofila a se alcanzó en Julio en la laguna facultativa (2054.6 mg m^{-3}) y en Febrero en la de maduracidn (2042.6 mg m^{-3}). Las concentraciones medias de clorofila a variaron entre 694.1 mg m^{-3} en la laguna facultativa y 942.1 mg m^{-3} en la de maduracidn. Las concentraciones de nutrientes fueron 1.6 mg l-1 NH_4-N, 0.1 mg l^{-1}, NO_2-N, 10.2 mg l^{-1}, NO_3-N y 1.6 mg l^{-1} PO_4^{3}-P. Las cloroficeas y las euglenoficeas dominaron el fitoplancton en la laguna facultativa; el primer grupo representa casi el 85% de la densidad total del fitoplancton en Enero, Mayo y Junio, mientras que los euglenófitos alcanzaron el 96% de la densidad del fitoplancton en Abril. Los géneros Oocystis y Pandorina fueron dominantes entre las cloroficeas, mientras que el género Euglena fue dominantes entre las euglenófitas. Las cianobactkrias en Abril, Mayo y Julio representaron más del 95% de la densidad del fitoplancton en la laguna de maduracidn. Entre las cianofíceas dominantes estaban Planktothrix mougeotii (Abril y Mayo), Microcystis aeruginosa y Pseudobaena mucicola (Julio. En la laguna de maduracidn los euglenófitos alcanzaron densidades menores del 6% deljtoplancton. Estas densidades indican que la planta de tratamiento de aguas residuales (WWTP) pueden ser un hábitat optimo para las cianobacterias, y consecuentemente, una posible fuente de toxinas cianobacterianas.

Palabras clave: fitoplancton, cianobacteria, nutrientes, planta de tratamiento de aguas residuales

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INTRODUCTION

Lagooning is one of the most common wastewater treatment systems in Portugal (Mendes *et al.,* 1995; Oliveira, 1995). The mutualistic relationship existing between bacteria and the dominant algal community in stabilization ponds of wastewater treatment plants and the dominance of the algal community, promotes the removal of nutrients, organic matter and pathogenic organisms (Troussellier *et al.,* 1986; Curtis & Mara, 1994; Nurdogan & Oswald, 1995).

Microalgae play multiple and complex roles in the wastewater treatment. Microalgae release molecular oxygen during photosynthesis, which in conjunction with oxygen at the air-water interface, promotes the aerobic conditions necessary for organic matter degradation by the aerobic bacteria. Also, small algae predominant in WWTP stabilization lagoons transform and accumulate organic matter degraded by the aerobic bacteria (Rodrigues & Santana, 1993).

The phytoplankton community is often dominated by cyanobacteria in eutrophic ecosystems. At high densities, cyanobacteria may produce toxins, endangering public health (Vasconcelos & Araújo, 1994). Common algae in wastewater stabilization ponds belong to Chlorophyceae, Euglenophyceae, Bacillariophyceae and Cyanophyceae (Silva, 1998). Cyanobacteria have been reported to dominate in some WWTP (e.g. in Morocco; Oudra, 1990). Among them, *Phormidium* is a typical genus in wastewater treatments (Canizares-Villanueva *et al.,* 1994). Silva (1998) recorded the presence of the cyanobacterium *Planktothrix* sp. in the Parada WWTP (Portugal), although chlorophytes were dominant.

Few studies are available examining the relationship between phytoplankton and nutrient concentration changes in wastewater treatment plants in the Iberian Peninsula and elsewhere (Oudra, 1990). The present paper contributes results of an investigation on the dynamics of the phytoplankton community in a WWTP in Portugal, and their relationship to the main algal nutrients (i.e. phosphorus and nitrogen).

Table 1. Analyses of physical and chemical variables in samples taken from the Esmoriz wastewater treatment plant (WWTP), Northern Portugal. *Análisis de las variables físicas y químicas en las muestras tomadas de la planta de tratamiento de aguas residuales de Esmoriz (WWTP)*

PARAMETER	METHOD OF ANALYSES	REFERENCE
Water temperature / Dissolved oxygen	*in situ*, using a multiparameter device	
pH	*in situ*, using a multiparameter device	
Nitrates	Spectrophotometry	StricKland & Parsons (1972)
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Ammonia	Spectrophotometry	KOROLEFF (1970)
Soluble Reactive phosphorus	Spectrophotometry	GOLTERMAN *et al.* (1978)
Photosynthetic pigments	Spectrophotometry with acidification (HCl IN)	NP 4327 -1996

MATERIAL AND METHODS

The WWTP at Esmoriz is situated in the North of Portugal (40" 56' N and 8" 38' W), practically at sea level, and covers a total area of 12 ha. It is a system of two sets of ponds, each set being independent and composed by three ponds in series (anaerobic, facultative and maturation). The final effluent is discharged into the river Lambo (3.5 m wide, 0.3 to 0.6 m deep, and annual mean flow of 0.3 m^3s^{-1}). Sampling was monthly between December 1998 and July 1999 in the facultative and maturation ponds. In the maturation pond, samples were taken from the banks and at the outlet. All samples were stored in plastic bottles and immediately preserved with Lugol's solution, for later identification and counting of phytoplankton species. Samples for physical and chemical analyses were kept refrigerated until they were analysed in the laboratory, within 24 hours of collection. Analyses and methods used are described in table 1.

Identification and quantification of phytoplanktonic forms

Different algal species and groups were identified and counted under an inverted microscope (400x magnification) using with Utermohl sedimentation chambers of 5 and 25 ml. Transects of each sample were counted to an error of less than 20 % (Lund *et al.*, 1958). Results are expressed in cells per millilitre.

Toxin analyses and quantification

The toxicity of ***Planktothrix*** *mougeotii* was measured with a mouse bioassay. Male Charles River mice (I: Gulbenkian Ciencia, Lisboa; weighing 20-25 g) were used. Freeze-dried cyanobacteria were suspended in 0.9% NaCl solution. A maximum dose of 1500 mg Kg^{-1} was used. Survival time and symptoms after ingestion of this solution by mice, were observed during a 48-hour period and *post-mortem*. Toxicity of the water in the different ponds was estimated with an ELISA test specific for microcystins (Envirogard).

Statistical analyses

Oxygen concentrations, algae-related and chlorophyll *a* were log-transformed to stabilize variances, before analysis of correlations. Pearson Product-Moment Correlation coefficients were considered as significant at a p= 0.05.

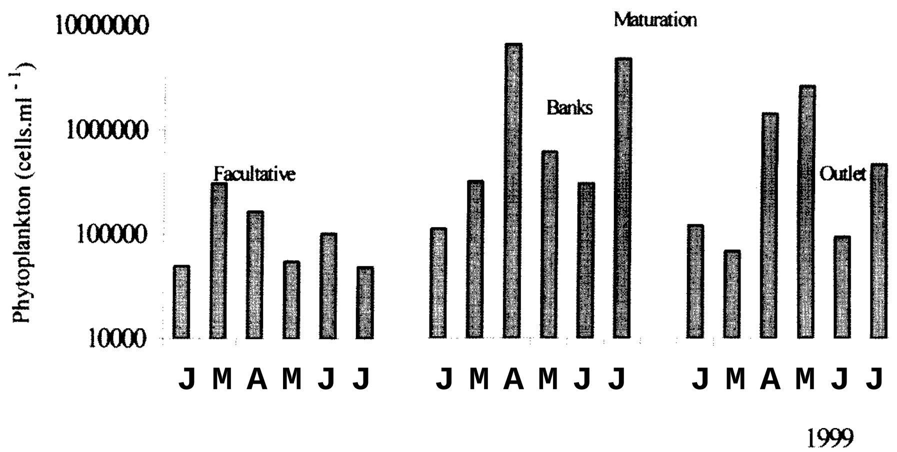

Figure 1. Changes in total phytoplankton abundance in the facultative and maturation (banks and outlet) ponds of Esmoriz WWTP. *Cambios en la abundancia total del fitoplancton* ***en*** *la laguna* ***de*** *maduracion de la EDAR* ***de*** *Esmoriz.*

RESULTS

Phytoplankton dynamics in the facultative pond

Chlorophytes and euglenophytes were the dominant algal group throughout the sampling period in the facultative pond (Fig. 2A). Chlorophytes represented 80-85 % of total phytoplankton abundance in January, March, May and June, while euglenophytes dominated (i.e. 60-96 % of total phytoplankton abundance) in April and July. The genera *Oocystis* and *Pandorina* were dominant among the chlorophytes, and *Euglena* within euglenophytes.

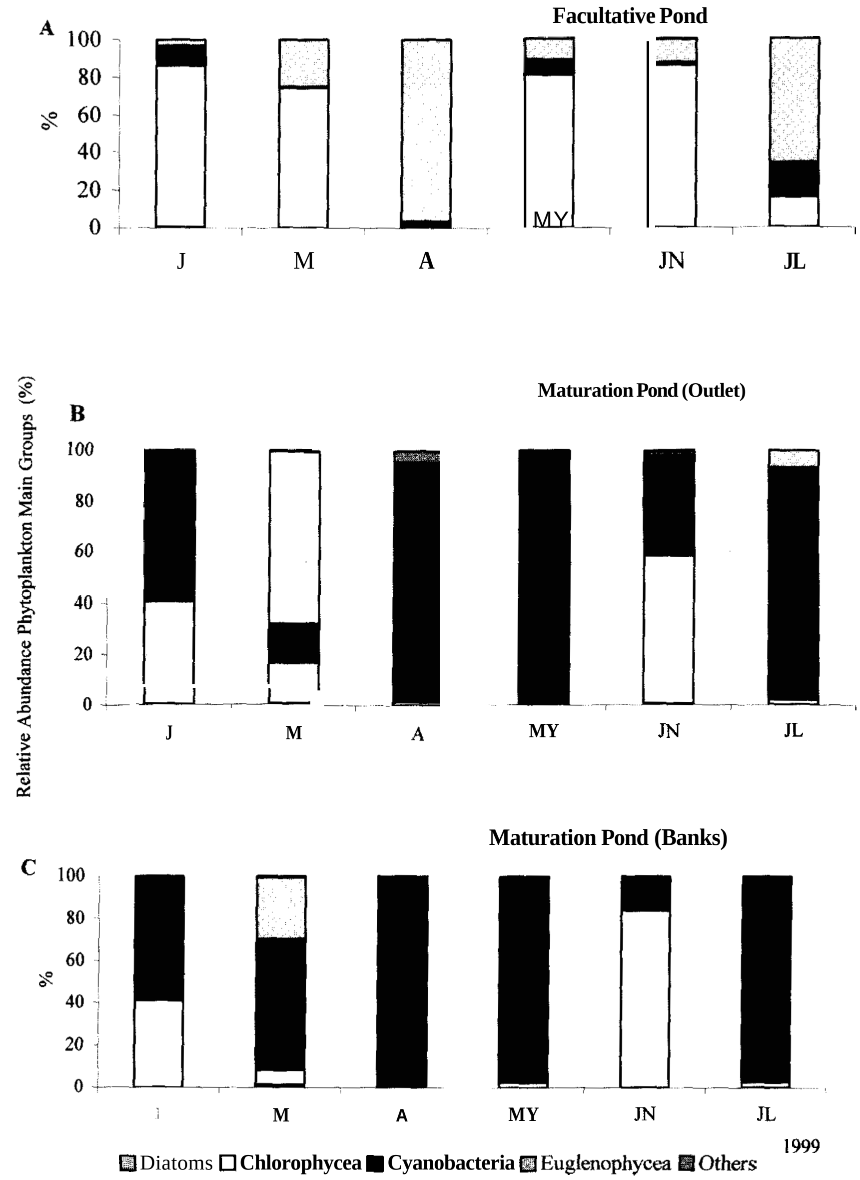

Figure 2. Changes in the relative abundance of phytoplankton main groups in **A,** the facultative pond and B, the maturation pond at the outlet and C, the maturation pond near the banks *Cumbios en la abundancia relativa de los principales grupos del fitoplancton en A, la laguna facultativa y B, la laguna de maduración a la salida y C la laguna de maduración cerca de las entradas*

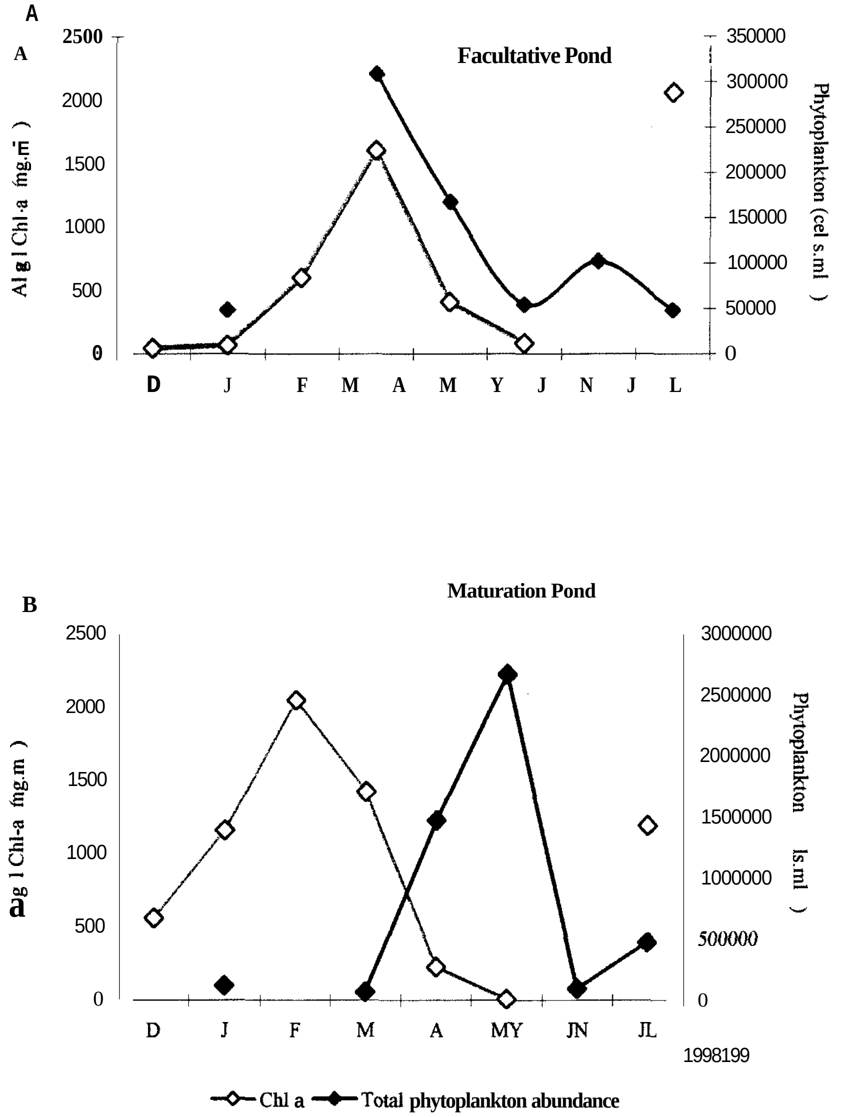

Figure 3. Changes in phytoplankton abundance and chlorophyll a concentrations in **A,-** the facultative pond and B, in the maturation pond, at the outlet. *Cambios en la abundáncia del fitoplancton y en las concentraciones de clorofila a* ***en*** *A.- la laguna facultativa y B,* ***en*** *la laguna de maduracion a la salida.*

Phytoplankton dynamics in the maturation pond

In the maturation pond, the highest abundances were found, at the banks in April and July. Maximum algae abundance was attained in April (banks, 6.7 -·10^6 cells ml^{-1}) and in May (outlet, 2.7 -·10^6 cells ml^{-1}). In the facultative pond, the maximum algal density was 0.3 -·10^6 cells ml^{-1}, attained in March. Cyanobacteria were present in all samples (Figs. 2B & 2C), and represented over 95 % of total phytoplankton abundance in April, May and July. *Planktothrix mougeotii* (April and May), *Microcystis aeruginosa* and

Table 2. Pearson Product-Moment Correlation coefficients between the analysed physical and chemical variables and the different algal groups in the facultative and maturation ponds of the Esmoriz (WWTP). *Coeficientes* ***de*** *correlacidn producto-momento de Pearson entre las variables físicas y químicas analizadas y los diferentes grupos de algas* ***en*** *las lagunas facultativa y* ***de*** *muduracidn* ***de*** *Esmoriz (WWTP).*

Variables	Facultative pond r	P	Maturation pond r	P
Chl/ Diatoms	0.30	ns	0.58	ns
Chl/ Chlorophytes	0.19	ns	0.76	ns
Chl/ Cyanobacteria	-0.96	0.04	-0.74	ns
Chl/ Euglenophytes	0.85	ns	0.23	ns
Oxygenl Diatoms	0,48	ns	0.19	ns
Oxygenl Chlorophytes	-0.41	ns	0.21	ns
Oxygenl Cyanobacteria	0.08	ns	-0.05	ns
Oxygen1 Euglenophytes	0.44	ns	0.57	ns
pH/ Diatoms	0.09	ns	-0.86	0.03
pH/ Chlorophytes	-0.80	ns	-0.40	ns
pH/ Cyanobacteria	0.33	ns	0.71	ns
pH/ Euglenophytes	0.49	ns	0.54	ns

ns: not significant

Variable	Ammonia	Nitrates	SRP*
DIATOMS	0.27 (ns)	0.72 (ns)	-0.44 (ns)
Chlorophytes	0.21 (ns)	-0.06 (ns)	0.57 (ns)
Cyanobacteria	-0,58 (ns)	**0.13** (ns)	0.12 (ns)
Euglenophytes	-0,78 (ns)	**0.10** (ns)	-0.77 (ns)

Pseudanabaena mucicola (July) were dominant species. Chlorophytes were the main algal group in June (60-80 %) and in January (41 %). *Oocystis* sp. in June and *Sphaerolopsis* sp. in January were the main chlorophyte species during these periods. Euglenophytes represented less than 6% of total phytoplankton abundance, except March (67 %), when *Euglena* sp. was the dominant species.

In the facultative pond there was a good relationship between chlorophyll *a* concentration and phytoplankton density, while this was not true for the maturation pond (Figs. *3* & 4). Highest oxygen concentrations were found in April in both sampling locations. Pearson Product-Moment Correlation coefficients are shown in Tables 2 & ***3***.

Changes in ammonia, nitrate and soluble reactive phosphorus concentrations against chlorophyll *a* in the maturation pond are shown in Figure *5*.

DISCUSSION

The two ponds are clearly different, in both phytoplankton abundance and algal species composition and proportions of each algal group. These differences are related to nutrient concentrations (nitrogen and phosphorus), hydrodynamics of the ponds, dissolved oxygen concentrations and organic matter in both ponds.

Facultative pond

In the facultative pond, phytoplankton was mainly composed by chlorophytes and euglenophytes (Figs. 2 & *3)*, in agreement with data reported by Oliveira (1995). In March, the maximum algae abundance coincided with the maximum concentration of chlorophyll *a*. Chlorophyll *a* concentration reached 2054.6 mg m^{-3} in July. Dissolved oxygen concentrations ranged between 0.9 and 10.8 mgl^{-1} (Fig. **3A** & **4A).**

Cyanobacteria apparently made up less of the chlorophyll *a* than diatoms, chlorophytes and, especially, euglenophytes. Increases in chloro-

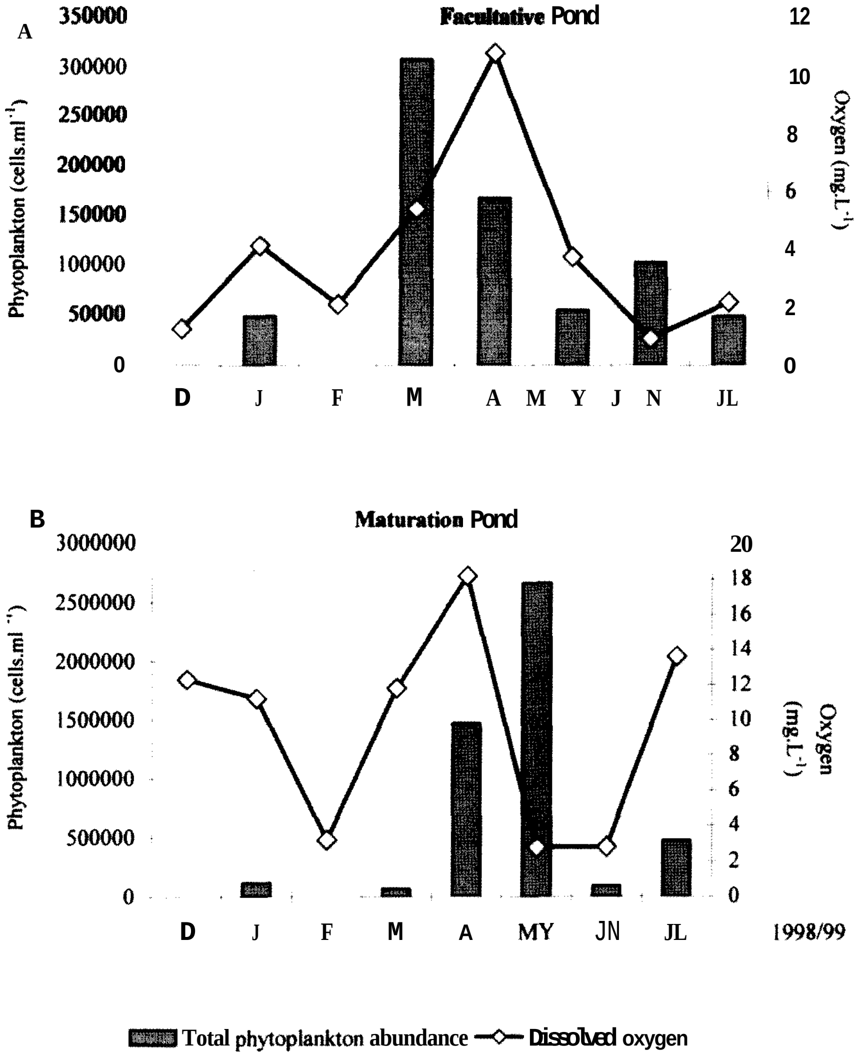

Figure 4. Total phytoplankton dynamics and dissolved oxygen concentration changes in **A,** the facultative pond and B, in the maturation pond, at the outlet. *Dinámica del fitoplancton y de los cumbios en la concentración de oxigeno en A, la laguna facultativa y B, en la laguna de maduración a la salida.*

phyll *a* concentration in the water and pH were related to euglenophyte density, whereas oxygen concentration changes were related to changes in density of both diatoms and euglenophytes.

Maturation pond

In the maturation pond, *Planktothrix mougeotii* (April and May), *Microcystis aeruginosa* and *Pseudanabaena mucicola* (July) were the dominant algal species. These results are similar to those observed during cyanobacteria blooms in fi-eshwaters ecosystems (Vasconcelos, 1994), but contrast with those of Oliveira (1995), who reported dominance of chlorophytes in wastewater stabilization ponds. *Microcystis aeruginosa* is one of the most common species in freshwaters ecosystems (Vasconcelos, 1994). However, it is not common in WWTP ponds. Other cyanobacterial species

Maturation Pond

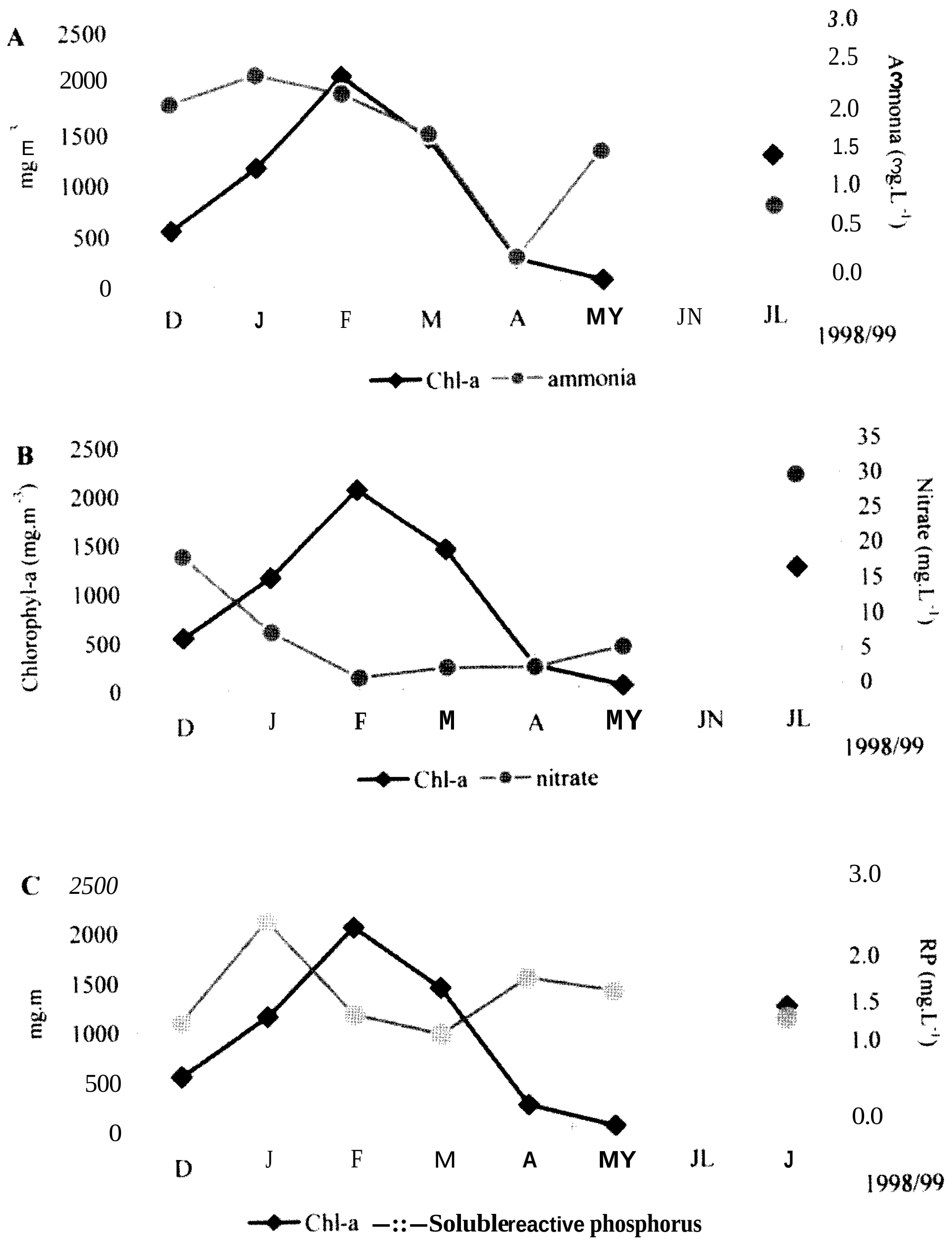

Figure 5. Changes in chlorophyll a concentrations (mg m-3) in the maturation pond at the outlet versus **A,** dissolved ammonia concentrations (mg l-1); B, dissolved nitrate concentrations (mg 1-1) and C, soluble reactive phosphorus concentrations (mg 1-1). *Cambios en las concentraciones de clorofila a (mg m-3) en el estanque de maduracidn a la salida frente* **A,** *las concentraciones de amonio (mg l-1); B, concentraciones de nitrato (mg l-1) y C, concentracion de fósforo reactivo soluble (mg l-1).*

common in WWTP are *Synechoccocus* and *Synechocystis* (Oudra, 1990).

The lowest chlorophyll *a* concentration was registered in April and May, when cyanobacteria represented 95% of total phytoplankton density. Thus, the density peak observed in May was mainly the result of a cyanobacterial bloom. Chlorophyll *a* concentrations in July were high, with a predominance of cyanobacteria, and small amounts of chlorophytes. This could be explained by the varying chlorophyll *a* content in different algal groups. Chlorophyll *a* content in cyanobacteria is less than in chlorophytes and euglenophytes (Reynolds, 1984). Also, chlorophyll *a* content is less in *Planktothrix mougeotii* than in *Microcystis aeruginosa* and *Pseudanabaena mucicola.* Chlorophyll *a* concentration reached 2042.6 mg m^{-3} in February. Dissolved oxygen concentrations ranged between 2.8 and 18.2 mg l^{-1} (Fig. 3B & 4B).

Increases in chlorophyll *a* content were correlated to chlorophyte and diatom cell abundance, whereas increases in oxygen concentrations were correlated to density of euglenophytes. Oxygen concentrations and pH were generally high in WSP due to high photosynthetic activity of phytoplankton. Euglenophytes showed a modest positive correlation to both oxygen concentration and pH, whereas cyanobacteria were found regardless of oxygen concentrations and were strongly correlated with pH. This suggests that Euglenophytes and Cyanobacteria were better adapted to the environment represented by the maturation pond than diatoms and chlorophytes were.

Toxin analyses and quantification

Negative results for cyanobacterial toxicity were obtained during the *Planktothrix mougeotii* bloom, using the mouse bioassay. However, ELISA assays specific for hepatotoxic microcystins revealed the presence of these toxins. (Vasconcelos & Pereira, 2001). The maximum value recorded was of 56.0 µg MCYST-LR equiv l^{-1} in the maturation pond (samples taken from its banks) in July.

Chlorophyll *a* concentrations showed a marked tendency to follow nutrient concentrations changes, especially at the time of the largest algal bloom, recorded in February. Nitrate, nitrite and soluble reactive phosphorus concentrations registered a strong decrease during this bloom. The decrease in ammonia, however, only began at the end of the algal peak (Fig.5).

Nutrients in the water apparently accumulated before the algal bloom (end of winter-beginning of spring; air temperature was 11°C). An increase in pH and in dissolved oxygen concentration followed, during or immediately after the algal blooms. These seasonal algal blooms (at least three during the whole year) are typical in lagoons (Surampalli *et al.*, 1995).

Large cyanobacteria densities were measured at the banks of the maturation pond and in the effluent leaving the pond to be discharged into the receiving river Lambo. The Esmoriz wastewater treatment plant may be considered as an optimal habitat for the development of cyanobacteria, and consequently as a potential source of toxins produced by the cyanobacteria and released into the environment.

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