



ARTÍCULO DE INVESTIGACIÓN / RESEARCH ARTICLE

# DYNAMICS OF THE PLANKTONIC COMMUNITY IN A SANITARY SEWAGE TREATMENT SYSTEM THROUGH POLISHING PONDS

## Dinámica de la comunidad planctónica en un sistema de tratamiento de efluentes sanitarios mediante lagunas de pulimento

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**Received:** 23rd February 2023. **Returned for revision:** 16th February 2024. **Accepted:** 30th April 2024

**Associate Editor:** Rosa Angélica Plata Rueda

**Citation/ citar este artículo como:** Sales, A.B., Bastos, R.K.X., Sánchez-Ortiz, I.A., y Magalhães, M.A. (2024). Dynamics of the Planktonic Community in a Sanitary Sewage Treatment System Through Polishing Ponds. *Acta Biol. Colomb.*, 29(3), 106-116. <https://doi.org/10.15446/abc.v29n3.106871>

### ABSTRACT

For a better understanding of the factors involved in biological sewage treatment and the importance of the planktonic community in stabilization ponds, this research evaluated the functioning of a domestic sewage treatment system, considering pollutant removal, with special attention to the planktonic community. The system was composed of an up-flow anaerobic sludge blanket reactor and a full-scale submerged aerated biofilter, followed by a series of four pilot-scale polishing ponds. The following variables were studied: transparency, temperature, dissolved oxygen, pH, alkalinity, biochemical oxygen demand, chemical oxygen demand, total suspended solids, and chlorophyll-*a*, besides precipitation and air temperature. During the study period, the planktonic community was represented by 103 taxa, divided into seven classes: Chlorophyceae (51.5 %), Euglenophyceae (20.4 %), Cyanobacteria (17.5 %), Cryptophyceae (5.8 %), Zygnemaphyceae (1.9 %), Chrysophyceae (1.9 %) and Dinophyceae (1 %). The minimum and maximum values of the total planktonic count were 2647 cells/mL in Pond three and 151 357 cells/mL in Pond one. The high concentrations of microalgae and cyanobacteria increased the total suspended solids. The zooplankton community was represented by Rotifers, Copepod, and Cladocera, which were found at low densities, with total values ranging from 310 org/mL in Pond one to 2436 org/mL in Pond two.

**Keywords:** plankton, sewage treatment, waste stabilization ponds, water quality.

### RESUMEN

Ante la necesidad de un mejor entendimiento de los factores involucrados en el tratamiento biológico de las aguas residuales y la importancia de la comunidad planctónica en lagunas de estabilización, esta investigación evaluó el funcionamiento de un sistema de tratamiento de efluentes domésticos, considerando la remoción de contaminantes, con especial atención a la comunidad planctónica. El sistema estuvo compuesto por un reactor anaerobio de manto de lodo y flujo ascendente y un biofiltro aireado en escala real, seguidos de cuatro lagunas de pulimento en serie en escala piloto. Se estudiaron las siguientes variables: transparencia, temperatura, oxígeno disuelto, pH, alcalinidad, demanda bioquímica de oxígeno, demanda química de oxígeno, sólidos suspendidos totales y clorofila-*a*, además de la precipitación y la temperatura del aire. Durante el periodo de estudio, la comunidad planctónica estuvo

representada por 103 taxones, divididos en siete clases: Chlorophyceae (51,5 %), Euglenophyceae (20,4 %), Cyanobacteria (17,5 %), Cryptophyceae (5,8 %), Zygnemaphyceae (1,9 %), Chrysophyceae (1,9 %) y Dinophyceae (1 %). Los valores mínimos y máximos del conteo de plancton fueron 2647 células/mL en la Laguna tres y 151 357 células/mL en la Laguna uno. Las altas concentraciones de microalgas y cianobacterias incrementaron los sólidos suspendidos totales. La comunidad zooplanctónica estuvo representada por Rotíferos, Copépodos y Cladóceros, los cuales se encontraron en bajas densidades, con valores totales variando entre 310 org/mL en la Laguna uno hasta 2436 org/mL en la Laguna dos.

**Palabras clave:** calidad del agua, lagunas de estabilización, plancton, tratamiento de efluentes

## INTRODUCTION

Urbanization, industrial development and agricultural production carried out without environmental knowledge can have a great impact on water resources. The over-exploitation of water bodies and the deterioration of water quality are global trends (Gutterer et al. 2009).

According to Peña and Mara (2004), waste stabilization ponds (WSP) are usually the most appropriate method for domestic and municipal wastewater treatment in developing countries, where the weather conditions are favorable for the operation of the ponds. These ponds present high efficiency and totally natural and highly sustainable operations. They require low investment, low operating and maintenance costs, and minimal energy consumption (Sah et al. 2012).

WSPs are typically shallow ponds and include aerobic, facultative, and anaerobic zones, allowing for the settling of suspended solids, degradation of organic matter, and pathogen removal by natural processes (Ingliš et al. 2021). Although polishing ponds also carry out complementary organic matter removal, they receive very low organic loads. However, based on the pretreatment performance, the main role of the ponds is the removal of pathogens (referred to here as maturation ponds) and nitrogen; design criteria for polishing ponds are very similar to those used for maturation ponds (von Sperling 2007).

Microalgae play a fundamental role in wastewater treatment, due to the production of oxygen through photosynthesis, which becomes available to bacteria for organic matter decomposition; microalgae also contribute, directly and indirectly, to nutrient removal processes (volatilization, precipitation, and assimilation) and pathogen reduction (Bento et al. 2005).

According to Barthel (2007) and Uehara and Vidal (1989), zooplankton is also an important component in the dynamics of the aquatic environment. Rotifers, cladocerans and copepods mainly occur in secondary and tertiary ponds; their presence contribute to the treatment process, once they can clarify the effluent when feeding on bacteria, microalgae, protozoa and organic waste. At high densities, these organisms impact the oxygen balance, due their herbivory, once the algae content decreases as the respiratory demand increases.

Studies on the structure and dynamics of phytoplankton are important, as organisms that respond promptly to environmental change, working as ecological indicators and increasing knowledge about existing interactions between

physical processes, on the one hand, and biological responses, on the other. Knowledge about planktonic organisms in stabilization ponds can improve system management. The present research mainly sought to conduct biomass analysis and investigate the time variations and composition of the planktonic communities (phytoplankton and zooplankton) in four polishing ponds used for wastewater treatment, through the qualitative and quantitative analysis of these organisms.

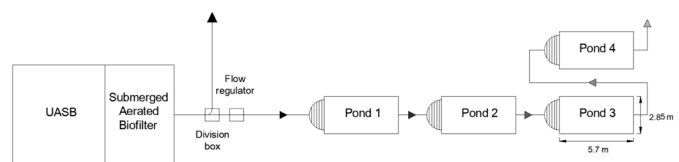
## MATERIALS AND METHODS

### STUDY AREA

The study was performed in the Integrated Unit for Sewage Treatment and Effluent Usage, located in the city of Viçosa, Minas Gerais, geographical coordinates 20°45'14" S and 42°52'54" W.

Pretreatment was performed by an upflow anaerobic sludge blanket (UASB) reactor and a submerged aerated biofilter, in full-scale (115 m<sup>3</sup>/d), made up of prefabricated steel units, followed by a series of four polishing ponds (P1, P2, P3 and P4), all in pilot-scale prefabricated fiberglass, with 16.3 m<sup>2</sup> individual surface area, 0.90 m average useful depth and 2.0 length/width ratio (Fig. 1).

The study was conducted from February 2009 to June 2010, interrupted in September, November and December, due to operational problems. Throughout the study, the pond system was operated at two different hydraulic retention times (HRT), in two operational periods: from February to October 2009 – Period I – the pond flow rate was 3.5 m<sup>3</sup>/d, with HRT of four days for each pond; from January to June 2010 – Period II – the flow rate was 2 m<sup>3</sup>/d, with a HRT of seven days for each pond.



**Figure 1.** Schematic illustration of the experimental series of polishing ponds

## SAMPLING AND DATA ANALYSIS

Sampling was carried out in both operational periods. To determine the abiotic and biotic variables, the water was collected from polishing ponds P1, P2, P3 and P4.

Precipitation data (mm) and air temperature (°C) were provided by the Universidade Federal de Viçosa (UFV) Weather Station, located in the state of Minas Gerais, Brazil, geographical coordinates 20°25' S, 42°52' W, and 657 m high.

The following physical-chemical water variables were analyzed in situ: temperature, measured in °C, using a bulb thermometer; dissolved oxygen (DO), measured by an oximeter device (model DM-4- DIGIMED) on the surface (15 cm), in the middle of the water column (45 cm) and at the bottom (90 cm) of the ponds. The water transparency was determined and estimated in terms of the depth of the Secchi disk disappearance.

Measurements of on-site water quality parameters and collections for laboratory analysis were simultaneously carried out between 8 a.m. and 10 a.m.

In the Water Quality Control Laboratory of the Water and Sewage Division at UFV, the following parameters were quantified: biochemical oxygen demand (BOD), chemical oxygen demand (COD), total suspended solids (TSS), alkalinity and chlorophyll-*a*, following standardized methodologies set by the Standard Methods for the Examination of Water and Wastewater (APHA et al. 2005).

The phytoplankton composition was analyzed, after samples were collected using a plankton net (20 µm mesh size). Horizontal drags were made on the surface. The collected samples were subdivided into two groups. One was fixed in 4 % formaldehyde, and the other was kept alive for the observation of the morphological characteristics, indispensable to taxonomic identification, such as movement, locomotion, metabolism, shape and number of chloroplasts, and disposition of pyramid grains in the protoplast. A Zeiss microscope, model Axioscope, was used for taxonomic analysis, in combination with a photographic camera, phase-contrast, and epifluorescence.

The samplings for the phytoplankton density study were performed close to the outlet pipe of each pond, using a depth collector. The samples were preserved in 5 % acetic lugol, and the density of the phytoplankton was estimated by cell counting, in Sedgwick-Rafter chambers (APHA et al. 2005), using a Zeiss microscope, model Axioskop, with 400 times magnification, in the long-range lens.

The zooplankton samples were collected using a plankton net (68 µm mesh size), kept in glass containers, and preserved in 4 % formaldehyde. For taxonomic analysis and the counting of organisms, a stereomicroscope and an optical microscope with up to 1000 times magnification were used. An acrylic checkered bottom bucket was used to count the organisms, and population density was expressed in individuals per liter (ind/L).

The data were analyzed using descriptive statistics. The Pearson correlation coefficient was used to measure the association between the variables analyzed. All results were interpreted using a 5 % significance level as a reference.

Principal Component Analysis (PCA) was performed on each polishing pond for the two seasons of the research. The water quality variables involved in the first type of principal component analysis were: total phytoplankton, total zooplankton, water transparency, temperature, DO, and pH of the water at the surface level of the ponds, since phytoplankton and zooplankton were sampled at that depth; BOD, as an indicator of the amount of organic matter present in the treatment units; TSS and chlorophyll-*a*, as indicators of the presence of both particulate organic matter and planktonic organisms. Statistical analyses were performed using Statistica software, version 10.0, developed by StatSoft®.

Additionally, PCA was performed for the four maturation ponds involving the phytoplankton classes that registered the highest abundance: Chlorophyceae, Euglenophyceae, Cyanophyceae, and Cryptophyceae, as well as the water quality parameters monitored during the research, for which Jamovi software, Version 2.3 was used.

## RESULTS

### CLIMATE VARIABLES

During the research, the highest monthly precipitation rate was registered in Viçosa in December 2009 (393.5 mm); in the two monitoring periods, the highest precipitation rates occurred in March of Period I and Period II (273.4 mm and 184.8 mm, respectively). The lowest precipitation rates were registered (0.8, 1.0, and 0.9 mm/month, each) in May and July of Period I, and June of Period II.

### PHYSICAL AND CHEMICAL WATER VARIABLES

#### Water transparency

Water transparency increased in the series of ponds. The mean values and standard deviation calculated for P1, P2, P3, and P4 were, respectively,  $40 \pm 9$ ,  $45 \pm 19$ ,  $48 \pm 16$  and  $63 \pm 20$  cm. During the experiment, the highest values measured in polishing ponds P1 to P4 were 61.5, 88.5, 78.0, and 96.0 cm, while the respective minimum values were 27.0, 15.0, 24.0, and 28.5 cm.

#### Temperature, DO, pH, and alkalinity

Water temperature presented negligible variation between ponds during the study period; in general, as expected, higher temperatures were observed in superficial layers.

During the research period, water temperature behavior followed the same dynamics as environmental temperature,

with increased and decreased values, according to the seasonal periods of drought and rain. The highest temperatures registered on the surface of the ponds (15 cm) were 28.2, 28.6, 28.5, and 29.0°C, respectively, for ponds P1, P2, P3, and P4; in the same sequence, the lowest temperatures registered at the bottom (90 cm) were 18.6, 17.3, 17.6 and 17.5°C, respectively.

The average values of DO concentrations calculated according to the medium depth (45 cm) were 5.0 mg/L in ponds P1, P2, and P3, and 6.0 mg/L in pond P4. The lowest levels registered at the bottom of the ponds for P1, P2, P3, and P4 were, respectively, 0.0, 0.5, 0.8, and 0.3 mg/L. The maximum levels of gas were measured on the surface of the treatment units, whose values were 15.0, 18.1, 15.5 and 21.0 mg/L at the sequence order of the ponds.

The maximum pH values, registered on the surface of the ponds were 9.1 in P1, 10.2 in P2 and P3, and 11.0 in P4; the mean values, for the middle of the water column, were 7.4 in P1 and 8.0 in ponds P2, P3 and P4. The minimum values measured at the bottom of the ponds according to the treatment sequence were 6.5, 6.6, 6.3, and 6.0 pH units.

### BOD, COD, and TSS

The calculated values of average and respective standard deviations of BOD calculated for ponds P1, P2, P3, and P4 were, respectively,  $30.1 \pm 14.1$ ,  $32.6 \pm 15.6$ ,  $23.9 \pm 11.6$  and  $20.2 \pm 9.8$  mg/L.

In domestic sewage, COD concentrations are usually higher than BOD values, since chemical oxidation decomposes biodegradable and non-biodegradable organic matter. The calculated values of means and standard deviations for ponds P1, P2, P3, and P4 were, respectively,  $162.6 \pm 75.3$ ,  $169.3 \pm 80.9$ ,  $150.1 \pm 35.6$ , and  $119.5 \pm 40.2$  mg/L.

Suspended solids and microalgae may have undesirable effects on the receiving body, including BOD increases. In general terms, an increasing tendency in TSS in P1 towards P2 and a reduction in P3 and P4 were observed. The average values and the standard deviations of TSS, calculated for

the ponds P1, P2, P3, and P4, were  $87.9 \pm 46.4$ ,  $101.0 \pm 51.2$ ,  $74.4 \pm 28.8$ , and  $62.4 \pm 29.1$  mg/L, respectively.

## BIOLOGICAL VARIABLES

### Chlorophyll-a

The concentrations measured for chlorophyll-*a* during the two research periods showed higher values for Period II (Fig. 2)

The maximum measured concentrations of chlorophyll-*a* were 554.5, 453.9, 465.8 and 385.0 µg/L, respectively, in ponds P1, P2, P3 and P4; the mean values and the standard deviations were  $200.3 \pm 150.5$ ,  $222.2 \pm 123.5$ ,  $149.8 \pm 115.4$  and  $99.8 \pm 120.6$  µg/L; the minimum concentration values were 30.0, 69.7, 39.3 and 4.6 µg/L (Fig. 2). A pattern of variation similar to that found in TSS was observed, which corroborated the effect of microalgae on the variation of TSS values.

### Phytoplankton

Throughout the study period, 103 taxa, divided into seven classes were found, namely, Chlorophyceae (53), Euglenophyceae (21), Cyanobacteria (18), Cryptophyceae (6), Zygnemaphyceae (2), Chrysophyceae (2) and Dinophyceae (1). (Table 1) presents the taxa count in the ponds during the study period.

Total cell density in the series of ponds reported wide variation (2647 cell/mL to 151678 cell/mL), represented by four numerically representative classes: Chlorophyceae, Euglenophyceae, Cyanophyceae, and Cryptophyceae.

### Zooplankton

During the research, the zooplankton community was composed of ten genera, and group Rotifer was the richest, with six identified genera. Cladoceran and Copepod were also inventoried. In addition to the organisms, young stage Copepod individuals were observed (nauplii and copepodites), besides Insecta larvae. (Table 2) presents the

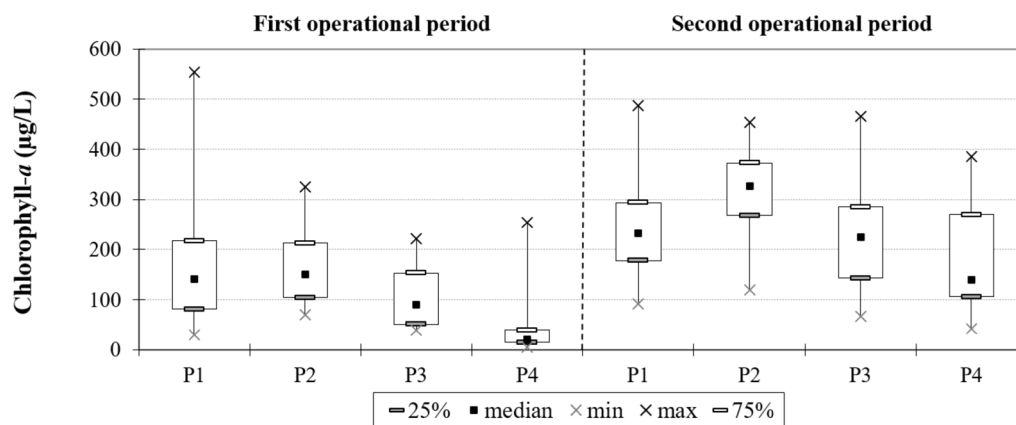


Figure 2. Variation of chlorophyll-*a* values in the series of ponds for the two operational periods.

zooplankton community found in the series of ponds during the research.

From February of the first year to June of the second year, Nauplii occurred more frequently, followed by the genus *Thermocyclops* and insect larvae. *Colurella*, *Mesocyclops* and *Lecane* were the least frequent genera, and the latter was only found in P4 (Table 3).

**Table 1.** Total taxa count in the ponds during the study period, divided into phytoplankton classes.

Classes	Polishing Ponds			
	P 1	P 2	P 3	P 4
Chlorophyceae	33	48	48	43
Euglenophyceae	19	17	16	13
Cyanophyceae	13	13	16	16
Cryptophyceae	6	6	6	5
Zygnemaphyceae	1	2	2	2
Crysofhyceae	0	0	2	1
Dinophyceae	1	1	1	1
TOTAL	73	87	91	81

**Table 2.** Zooplankton community found in the series of ponds.

Zooplankton	Polishing ponds			
	P1	P2	P3	P4
Nauplii	x	x	x	x
CLADOCERAN				
<i>Moina</i>	x	x	x	x
COPEPOD				
Copepodite	x	x	x	x
<i>Mesocyclops</i>		x	x	x
<i>Microcyclops</i>		x	x	x
<i>Thermocyclops</i>	x	x	x	x
ROTIFER				
<i>Brachionus</i>	x	x	x	x
<i>Colurella</i>		x	x	x
<i>Euchlanis</i>		x	x	x
<i>Lecane</i>				x
<i>Lepadella</i>		x	x	x
<i>Proales</i>	x	x	x	x
Insecta larvae	x	x	x	x

**Table 3.** Abundance (%) of zooplankton, identified in each polishing pond, during the research.

ZOOPLANKTON	P1	P2	P3	P4
	Occurrence frequency (%)			
Nauplii	15	55	50	65
CLADOCERAN				
<i>Moina</i>	15	10	15	10
COPEPOD				
Copepodite	5	30	15	20
<i>Mesocyclops</i>	0	10	10	5
<i>Microcyclops</i>	0	30	25	25
<i>Thermocyclops</i>	40	90	75	60
ROTIFER				
<i>Brachionus</i>	20	40	45	50
<i>Colurella</i>	0	5	5	5
<i>Euchlanis</i>	0	15	10	10
<i>Lecane</i>	0	0	0	5
<i>Lepadella</i>	0	5	20	25
<i>Proales</i>	25	25	37	25
Insecta larvae	95	55	45	50

### PRINCIPAL COMPONENTS ANALYSIS

In the PCA for the ponds, the number of factors with eigenvalues > 1 that were identified during the first operational period was two, three, four, and three respectively for maturation ponds P1, P2, P3, and P4; for the second operational period, the number of factors was three, three, two and three for ponds P1, P2, P3 and P4.

In the eight principal component analyses performed, it was observed that the first two eigenvalues explained a high percentage of the total variability of the data. For the first operational period, the first two eigenvalues explained 65.7 %, 67.9 %, 57.9 %, and 61.1 % of the variability for the P1, P2, P3, and P4 ponds, respectively. By the second operational period, these values accounted for 68.8 %, 60.6 %, 74.4 %, and 76.8 % of the variability for maturation ponds P1, P2, P3, and P4, respectively.

For the first operational period, the PCA indicated that there was a high positive correlation between the variables: total phytoplankton, BOD, TSS, and chlorophyll-*a* in every one of the polishing ponds in the series. On the other hand, there was a high correlation between the variables: total zooplankton, transparency, DO, pH, and temperature;

however, for the P3 and P4 ponds, there was also a slight correlation of total zooplankton concerning the first group of variables mentioned above.

The PCA for the second operational period again revealed that there was a high correlation between the variables in total phytoplankton, BOD, TSS, and chlorophyll-*a* in all polishing ponds. In addition to this, there was a high correlation between the variables of total zooplankton, transparency, DO, pH, and temperature; however, for the P3 pond, there was also a slight correlation of total phytoplankton with transparency.

The PCA with emphasis on phytoplanktonic organisms yielded the following results: in the P1 pond for the class Euglenophyceae, a positive correlation was recorded concerning BOD and alkalinity, as well as a negative correlation with relation to temperature and DO; for the classes Cyanophyceae and Cryptophyceae there was no correlation with the parameters analyzed; for the class Chlorophyceae a positive correlation was recorded about TSS, COD and chlorophyll-*a*, as well as a negative correlation with transparency. In the P2 for the class Chlorophyceae, a positive correlation was observed with TSS and COD, as well as a negative correlation with temperature; for the classes Euglenophyceae and Cyanophyceae, no correlation was recorded with the parameters analyzed; for the Cryptophyceae class, there was a positive correlation with alkalinity, BOD, chlorophyll-*a*, pH, as well as a negative correlation concerning DO. In the P3 for the classes Cyanophyceae, Cryptophyceae and Chlorophyceae, a positive correlation was recorded concerning BOD, chlorophyll-*a*, TSS, pH and alkalinity, as well as a negative correlation with temperature and transparency; for the class Euglenophyceae there was no correlation concerning the parameters analyzed. In the P4 for the classes Chlorophyceae and Cryptophyceae, a positive correlation was observed about chlorophyll-*a*, BOD, alkalinity, TSS, and a negative correlation concerning transparency; for the class Euglenophyceae there was a positive correlation concerning COD; finally, for the class Cyanophyceae, a positive correlation was recorded concerning pH and DO, as well as a negative correlation with COD.

## DISCUSSION

According to Nogueira and Matsumura-Tundisi (1996), climate factors, such as precipitation and wind, greatly affect plankton composition and biomass. In this study, a significant inverse correlation between precipitation and the total density of phytoplankton was verified only in P2.

In this study, the lowest Secchi transparency levels were associated with a great amount of suspended material in the water column, including phytoplankton. It was evidenced by the negative correlation between water transparency and TSS in the four studied ponds, namely, -0.646 in P1, -0.651 in P2, -0.648 in P3, and -0.629 in P4. The higher the transparency, the greater the light penetration in the entire

water column, which increases photosynthetic activity, oxygen production and pH.

The water quality results revealed an alkaline condition in the water column, with a slight tendency to acidification at the bottom. Broadly speaking, the pH variation pattern was similar to the DO variation pattern, with an increasing tendency from the first to the last pond, in sequence. High concentrations of chlorophyll-*a* and TSS, as well as lower transparency in the water, are often the result of high densities of phytoplanktonic organisms, which have a greater capacity to survive in environments with high concentrations of BOD, while many zooplanktonic organisms do not. Some extreme values of DO and temperature are due to the timing of measurement of the parameters in situ, early in the morning, due to lower night temperatures, the absence of photosynthetic processes, and nocturnal respiration by planktonic organisms was observed. Likewise, as reported by Pham et al. (2020), organic matter exerts a strong influence on DO concentrations, since the higher the loads of this pollutant, the greater the consumption of the dissolved gas by the heterotrophic bacteria of the stabilization ponds.

The photosynthesis performed by microalgae in stabilization ponds contributes to pH elevation, which can reach values over 9.0 in the event of high photosynthetic activity (von Sperling, 2007).

Decreased alkalinity was generally observed during the treatment process. The mean values and standard deviations of alkalinity, calculated for ponds P1, P2, P3, and P4 were, respectively,  $135.0 \pm 53.3$ ,  $104.6 \pm 53.9$ ,  $83.6 \pm 40.2$  and  $70.9 \pm 33.5$  mg CaCO<sub>3</sub>/L.

Increased chlorophyll-*a* concentrations indicate higher biomass density of green algae, confirming the photosynthetic activity of microalgae. The high primary productivity also contributes to the increase in suspended solids, as well as to the presence of organic debris, to the resuspension of sediments from the ponds.

During the study period, the highest number of taxa was found in P3 (91), followed by P2 (87), P4 (81) and P1 (73). Most organisms belonged to the class Chlorophyceae, which reveals the highest number of taxa in P2 and P3 (48 taxa each). The Euglenophyceae class presented the highest number of taxa in P1: 19 records, gradually decreasing towards P4, where 13 taxa were registered (Table 1). Classes Zygnemaphyceae, Dinophyceae, and Chrysophyceae provided the lowest number of taxa, from which organisms of class Chrysophyceae were only registered in P3 and P4.

In a phytoplankton survey conducted in a stabilization pond located in São José dos Campos, São Paulo-Brazil, Cardoso (1979) found that classes Chlorophyceae and Euglenophyceae were the most common, while the genera with the highest densities were: *Chlorella*, *Golenkinia*, *Euglena*, *Oscillatoria*, *Merismopedia*, *Chlamydomonas*, *Scenedesmus* and *Monoraphidium*. All of them were observed at the polishing pond system evaluated in this research. *Merismopedia* and *Oscillatoria* were identified by Cruz et al. (2003) in facultative

ponds subjected to high organic load in Espírito Santo-Brazil. The main microalgae and cyanobacteria genera detected by Branco (1986) in stabilization ponds include *Chlorella*, *Chlorococcum*, *Euglena*, *Golenkinia*, *Lepocinlis*, *Phacus*, *Scenedesmus*, *Dolichospermum*, *Phormidium* and *Oscillatoria*; additionally, the genera found were similar to those reported by Wrigley and Toerien (1990) in small stabilization ponds in Bloemfontein – Australia, and by Florentino (1992) in a series of stabilization ponds in Paraíba state-Brazil. According to Molinuevo-Salces et al. (2019), some of the main microalgae present in photobioreactors used for municipal wastewater bioremediation are *Coelastrum microporum*, registered in P1, P3, and P4, and *Scenedesmus obliquus*, registered in the four ponds of this study.

Bastos et al. (2010) evaluated different values of affluent flow and water depths in the same polishing pond system studied in this research, during the years 2004, 2005, and 2006. They observed, in three distinct periods, the genera *Chlorella*, *Chlamydomonas*, *Euglena*, *Scenedesmus*, and the cyanobacteria genus *Oscillatoria*, all of which were present in the three periods. In this study, many taxa of the aforementioned genera were found in the four polishing ponds during the research period.

In Belo Horizonte-Brazil, von Sperling and Oliveira (2010) investigated the effect of HRT and surface application rate on the composition of the phytoplankton community present in four polishing ponds, while treating the effluent of an UASB reactor. During the whole study period, the classes Chlorophyceae and Euglenophyceae were prevalent. At the facultative and maturation ponds of wastewater treatment plants (WWTP) in Trindade, State of Goiás-Brazil (D'alessandro et al. 2020) and in the city of Cuenca, Equator (Pham et al. 2014), the highest count of species was found for the classes Chlorophyceae and Euglenophyceae. The same was observed in the polishing ponds analyzed in this study, which suggests that such classes can adapt in stabilization ponds in different treatment system configurations.

The class Chlorophyceae exhibited the highest number of taxa registered in the polishing ponds. These microalgae are opportunistic and develop well under extreme conditions, especially in waters with a high level of eutrophication. This class was predominant in a secondary facultative pond of the WWTP in Guarabira, state of Paraíba, Brazil (König et al. 1999); two facultative ponds in a WWTP conformed by Australian system in Novo Horizonte, state of São Paulo, Brazil, with HRT from eight to ten days (Granado 2004); a secondary facultative pond, with HRT of 4.8 days, in Vitória Espírito Santo, Brazil (Cruz 2005); a polishing pond with HRT of 10.5 days in Venda Nova do Imigrante, state of Espírito Santo, Brazil (Delazari-Barroso et al. 2009); a primary facultative pond in Barbalha, state of Ceará, Brazil (Aquino et al. 2011).

In our study, the Chlorophyceae counts were mostly represented by the species *Chlorella vulgaris*; several studies performed in stabilization ponds point out this species as

dominant. In the Grand Canal Jing-Han, in China, Shi et al. (2012) studied the composition of phytoplankton communities in six points of this polluted aquatic environment and observed that class Chlorophyceae presented the highest number of species (38 %) and relative abundance (40 %).

The species *Desmodesmus communis*, *Menoidium gracile* and *Phacus dangeardii* were also present during the whole study period, while the genera *Phacus* and *Euglena* were found by Reynolds (1998) in hypereutrophic environments.

For class Chlorophyceae, the results shown in the Spearman statistical test presented a positive and significant correlation with COD, BOD (P1), and TSS (P1 and P2), and a negative correlation with water transparency (P1 and P2). According to Reynolds (1996), the genus *Chlorella* presents excellent growth in waters rich in phosphorus and inorganic carbon. Therefore, the predominance of Chlorophyceae in the polishing ponds may be related to the adaptive advantage of these organisms for survival in hypereutrophic habitats.

During the period of this study, the class Euglenophyceae presented maximum densities in P1 and P2 and much lower concentrations than the class Chlorophyceae, reaching a maximum of 24633 cell/mL. In the series of ponds, it was observed an inverse growth behavior of Euglenophyceae, compared to Chlorophyceae, which indicates, as expected, the efficiency of the treatment. The presence of Euglenophyceae in polluted environments agrees with Palmer (1969), who classified Euglenophyceae and Cyanophyceae as the most tolerant to pollution, once they are mixotrophic. This condition was observed in the studied polishing ponds that presented higher counts in P1 and P2, which received the highest organic loads. The relation of Euglenophyceae with the amount of organic matter may explain its presence in high densities from July to October of Period I, and March of Period II (P1), when the highest values of BOD were measured. The Spearman test presented significant and positive relation between BOD (0.540) and class Euglenophyceae. Euglenoids proliferate in environments with high organic matter (high BOD), using it as an energy source (Wetzel, 2001; Rohini and Manikya, 2023). Euglenoids are more sensitive to elevated temperatures (Reynolds, 1984; Bellinger and Sigee, 2010), and their growth can be reduced for temperatures above 25°C (Wetzel, 2001).

While monitoring a polishing pond system in Paraíba-Brazil, Cavalcanti (2003) observed the predominance of the genera *Euglena*, *Chlorella*, and *Phacus*, whose taxa were counted in the four ponds studied in this research.

The highest densities of individuals of class Cyanophyceae were registered in P1, in February of Period II; and in P4, in August of Period I. Organisms of class Cyanophyceae were not found in samples from February of Period I in P3, nor in August in the same period, nor in March or May of Period II in P2. Cyanobacteria can grow abundantly in environments with high concentrations of organic charge in the form of BOD; in this sense, Chorus and Bartram (1999) observed

a positive correlation between cyanobacterial density with BOD, chlorophyll-*a*, and temperature.

The class Cyanophyceae is represented by organisms that are well-adapted to extreme conditions. Therefore, they are highly proliferative in eutrophic ponds. Eight abundant species were found, and only *Aphanocapsa delicatissima* was dominant in the count of May of Period I (7784 cell/mL). Reynolds (1996) described this genus as well adapted to calm waters, with moderate concentrations of nutrients. That might explain the fact that *Aphanocapsa delicatissima* has contributed the highest density in low precipitation periods. Rain might be a disturbing agent of the structure of the phytoplankton community, thus contributing to the reduced density of this class in the rainy season due to dilution, as reported by D'alessandro et al. (2020). For the class Cyanophyceae, unlike studies such as those by Chorus and Bartram (1999) and Komárek and Anagnostidis (2008), no correlation was observed in the P1 concerning BOD, chlorophyll-*a*, temperature, DO, pH or COD, according to Paerl and Otten (2013), this could have been due to specific conditions of the pond such as pH variation, temperature, nutrients, as well as predation, competition with other algae or specific characteristics of the cyanobacterial community.

Gonçalves (2008) characterized the phytoplankton community in maturation ponds of sanitary sewage treatment system in Petrolândia, Pernambuco state, Brazil. The phytoplankton was composed of 20 taxa, mainly Cyanophyceae *Oscillatoria* sp. and Chlorophyceae *Chlosteriopsis acicularis*, both present in most samples; in this research, these taxa were recorded in ponds P2, P3 and P4.

The class Cryptophyceae presented important variations in the densities during the sampling period, from a minimum of 23 cell/mL in P3 (February of Period I) to a maximum of 14814 cell/mL in P1. There was a clear time separation, with low densities in the months of the first period of the research, and higher numbers over the second period. Cryptophyceae use available organic matter as an energy source and are abundant in environments with high organic load; Im et al. (2023) stated that Cryptophyceae showed significant correlations with BOD and total organic carbon in the artificial Lake Uiam, South Korea.

While investigating the effect of HRT and surface application rate on the composition of phytoplankton community in polishing ponds on a pilot scale, von Sperling and Oliveira (2010) verified the dominance of classes Chlorophyceae and Euglenophyceae. A decrease was observed in order Chlorococcales and genus *Cryptomonas*, due to reduced HRT. In the present study, statistical results pointed to significant differences in the density of class Cryptophyceae during the research. Low density was coincident with the period when the system was operated with a shorter hydraulic retention time (four days).

The lowest densities were observed in the classes Dinophyceae (38 to 506 cell/mL) and Zignemaphyceae (37

to 1502 cell/mL). Organisms from class Dinophyceae were only found in three collections (October of Period I and January and March of Period II). Class Chrysophyceae was only found in qualitative sampling, represented by two taxa (*Dinobryon sertularia* and *Synura* sp.).

To achieve greater accuracy in the comparison of the ponds, the density per class data were submitted to ANOVA, followed by the Tukey mean comparison test. The results were interpreted taking a 5 % significance level reference. The density values of classes Cyanophyceae and Cryptophyceae differed during the two periods of the study; no significant differences were observed between classes Chlorophyceae and Euglenophyceae. The difference was observed between the four ponds only in class Euglenophyceae. No other class expressed significant differences from one pond to the other.

The zooplankton community plays an essential role in the aquatic system dynamics due to its characteristic of being a link in the food chain, once they feed on algae and serve as food for more complex organisms, such as fish.

In general terms, the zooplankton found in the series of ponds presented low density, with total numbers of 310 org/mL in P1, 2436 org/mL in P2, 1077 org/mL in P3, and 513 org/mL in P4.

The zooplankton community presented time variation over the study period, with the disappearance of some organisms. In P1, zooplankton was detected in 55 % of the samples. In August of Period I, there was a peak in P2, mainly due to high Rotifer density (1049 org/mL).

The absence of cladocerans throughout the sampling period was indicative of the eutrophic environment.

Eutrophication is an event capable of producing deep transformation in the zooplankton community, which is associated with the replacement of palatable forms of phytoplankton by less-savoring ones, such as filamentous cyanobacteria. Thus, changes in the food chain cause the "bottom-up" effect on zooplankton and modify the community composition.

The study conducted by Silva and Matsumura-Tundisi (2002) demonstrated the sensitivity of the Copepod community to changes in water quality, which helps to explain the lower densities of these organisms in P1 (operated with higher organic loads).

The life cycle of Copepods is known to be long if compared to other zooplankton components (Rotifer and Cladoceran), which makes them susceptible to a high mortality rate before reaching the adult stage. In this research, Copepod exhibited low density, which ranged from 0.1 to 74.1 org/mL.

Moscoso et al. (1992) observed intense proliferation of ciliate, rotifers, copepods, and cladocerans in stabilization ponds in Peru; Guerrin (1988) and Nandini (1999) registered populations of Rotifer and Cladoceran with respective values of 3000 and 300 organisms/L density in stabilization ponds.



The predominance of Rotifer could be related to its ability to reach maturity earlier, and to have a faster replacement rate if compared to microcrustaceans (Nogueira and Matsumura-Tundisi 1996).

In the studied polishing ponds, the Rotifer density ranged from 0.1 to 1028.6 org/mL; the maximum density was registered in August of the first year in P2.

Rotifer and Cladoceran are particularly able to grow at high densities, feeding on organic waste and bacteria (Roche, 1998, Arevalo-Stevenson et al. 1998).

While studying the same polishing pond system, Bastos et al. (2010) found five genera composing the zooplankton community, four of which were identified in this research: *Brachionus*, *Moina*, *Mesocyclops*, and *Thermocyclops*. Only *Daphnia* was not observed.

In the present study, no pattern was found to explain the behavior of zooplankton in the series of ponds. As these organisms depend mainly on DO in water for its maintenance, this may have probably been one of the limiting factors in the community, especially in P1. However, as the distribution of zooplankton is affected by several other factors, additional research is needed to elucidate doubts and help to understand the dynamics of these organisms in WSP; furthermore, knowledge of planktonic community fluctuations can assist in operation management and maintenance of the ponds through the anticipation of certain harmful phases, such as the cyanobacteria domain (Amengual-Morro et al. 2012).

## CONCLUSIONS

In conclusion, the evaluated polishing pond system was efficient in reducing both particulate and organic matter, which resulted in effluents with average concentrations of 62.4 mg/L (TSS), and BOD concentrations ranging from 4.1 to 37.5 mg/L. Most of the taxa identified in phytoplankton belong to classes Chlorophyceae (53), Euglenophyceae (21) and Cyanophyceae (18), besides Cryptophyceae (6), Zygnemaphyceae (2), Chrysophyceae (2) and Dinophyceae (1). The total cell density of phytoplankton in the series of ponds presented a wide range (2647 cell/mL to 151678 cell/mL), with four numerically representative classes: Chlorophyceae, Euglenophyceae, Cyanophyceae, and Cryptophyceae. Class Euglenophyceae was associated with high levels of organic matter, mostly present in P1 and P2, and only one species of class Cyanophyceae was dominant during the study period (*Aphanocapsa delicatissima*), in the sampling from May of Period I. The zooplankton community presented low densities, and the group Rotifer was the most representative. No correlations were found between phytoplankton and zooplankton.

## AUTHOR'S PARTICIPATION

Conceptualization of the work, Sales, A.B. and Bastos, R.K.X.; collection and processing of biological material,

Sales, A.B.; supervision, Bastos, R.K.X.; data analysis, Sales, A.B., Bastos, R.K.X., Sánchez-Ortiz, I.A., Magalhães, M.A.; writing and reviewing of the document, Sánchez-Ortiz, I.A.

## ACKNOWLEDGEMENTS

The authors express their gratitude to Professors Hernán Abdón García, director of the Center for Studies and Advice in Statistics at the University of Nariño UDENAR (Pasto, Nariño, Colombia) and Joildo Fernandes Costa Júnior, from the Fundação educacional de Caratinga -FUNEC- (Caratinga, Minas Gerais, Brazil) for their valuable guidance on the processing and statistical analysis of the results.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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