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Biology-Ecology

FREE-LIVING PROTOZOA (CILIOPHORA, EXCAVATA AND AMOEBOZOA) IN TWO WATER SOURCES FOR HUMAN SUPPLY IN THE MUNICIPALITY OF BLUMENAU, SC

Protozoários de vida livre (Ciliofora, Excavate e Amoebozoa) em duas fontes de água utilizadas para o abastecimento humano no município de Blumenau, SC

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ABSTRACT

The knowledge of the diversity of free-living protozoa in aquatic ecosystems represents an important tool in environmental quality management. It has been widely used in studies evaluating the water quality of fresh-water sources with environmental variables. Therefore, this study aimed to verify the diversity of free-living protozoa in two water sources used as a public supply for human consumption in the municipality of Blumenau, SC, for 12 months. Water samples were collected monthly for analysis of environmental variables and identification of organisms. In total, 39 taxa were recorded in P1 and P2, both in the readings performed on the day of collection and in the cultivation. In P1, 11 taxa were identified in the readings of the day of collection, and after reading the cultivation, 20 new taxa were recorded. P2 presented 12 taxa in the readings performed on the day of collection and 10 new taxa in the cultivations' readings. Ciliates were the most expressive group in the period, with 69.5% frequency in P1 and 66.1% in P2. The results of the environmental variables in conjunction with the survey of the free-living protozoa of P1 and P2 did not show marked differences between the points. Still, they contributed to the knowledge of the diversity of protozoa in aquatic environments.

Keywords: free-living protozoa; water source; water quality indicators; Itajaí –Açu river; Itoupava Rega stream

RESUMO

O conhecimento da diversidade de protozoários de vida livre em ecossistemas aquáticos representa uma importante ferramenta na gestão da qualidade ambiental. Tem sido amplamente utilizado em estudos de avaliação da qualidade da água de mananciais de água doce com variáveis ambientais. Portanto, este estudo teve como objetivo verificar a diversidade de protozoários de vida livre em dois mananciais utilizados como abastecimento público para consumo humano no município de Blumenau, SC, durante 12 meses. Amostras de água foram coletadas mensalmente para análise de variáveis ambientais e identificação de organismos. No total, 39 táxons foram registrados em P1 e P2, tanto nas leituras realizadas no dia da coleta quanto no cultivo. Em P1, 11 táxons foram identificados nas leituras do dia da coleta, e após a leitura do cultivo, 20 novos táxons foram registrados. P2 apresentou 12 táxons nas leituras realizadas no dia da coleta e 10 novos táxons nas leituras dos cultivos. Os ciliados foram o grupo mais expressivo no período, com frequência de 69,5% em P1 e 66,1% em P2. Os resultados das variáveis ambientais em conjunto com o levantamento dos protozoários de vida livre de P1 e P2 não mostraram diferenças marcantes entre os pontos. Ainda assim, contribuem para o conhecimento da diversidade de protozoários em ambientes aquáticos.

Palavras-chave: protozoários de vida livre; manancial; indicadores de qualidade da água; rio Itajaí – Açu; córrego Itoupava Rega.

INTRODUCTION

Pollution of surface water is a global environmental problem. From the impacted environments, rivers and streams represent ecosystems intensely altered by the advancing demographic growth of populations, the release of high amounts of effluents of industrial and domestic origin, destruction of habitats, and the introduction of exotic species (SMITH; LAMP, 2008; GRAZZIOTTI; SILVA; LIMA, 2018). This contamination has generated an imbalance in fresh-water ecosystems, altering trophic activities and influencing the diversity of organisms that inhabit them (VANDEWALLE *et al*., 2010).

The assessment of water quality in the natural environment can be performed using physical, chemical, and biological methods (LOBO; CALLEGARO, 2000), and the joint use of the analysis of these three proceduresrepresent an important instrument for assessing the conditions of these sources (MADONI; BASSANINI, 1999; MADONI, 2005; MADONI; BRAGHIROLI, 2007). Most aquatic organisms can respond directly to changes in water's physical, chemical, and biological profile (COSTA *et al*., 2008; ZHONG *et al*., 2017).

Among the aquatic organisms used as bioindicators, free-living protozoa have received attention in recent decades (BONATTI *et al.*, 2016; REGALI-SELEGHIM *et al.*, 2011). These organisms are unicellular, eukaryotic, heterotrophic, or mixotrophic (GODINHO; REGALI-SELEGHIM, 1999), with a short life cycle, being sensitive to environmental changes (NORF *et al.*, 2009; XU et al., 2015; SIKDER; XU, 2020). In addition, they represent the main organisms of the microbial communities and are responsible for the functioning of the food webs, serving as food for the upper trophic levels (XU *et al.*, 2014; SIKDER *et al.*, 2019).

Free-living protozoa are abundant in the most diverse aquatic and edaphic environments, in free-swimming form or associated with biotic and abiotic surfaces (ROCHA, 2005), and have a high capacity to adapt to different environmental conditions. According to FINLAY (2002) and FINLAY *et al.* (2006), some species can respond quickly to changes that occur through the formation of resistance cysts, which act as a form of protection and dispersion, which possibly justifies its wide geographic distribution, making its use viable in the biomonitoring of aquatic ecosystems (SHI *et al.*, 2012; CABRAL *et al.*, 2017).

In addition, due to their global distribution, high abundance, rapid growth rates, short generation time, ease of collection, and the fact that they are separated from their environment only by the cell membrane, protozoa have been used to evaluate the quality and ecological status of water in a variety of aquatic ecosystems (LIU *et al.*, 2014).

The diversity of protozoa is still underestimated in ecosystems (ADL *et al.*, 2007); however, in recent years, advances in morphological and molecular analyzes, and consequent changes in the proposals for the phylogenetic systematics of eukaryotes, have contributed to a better knowledge of the group (ADL *et al.*, 2012; RODRIGUES, 2011). In the new classification proposed by Adl et al. (2012), protozoa are inserted in the groups Amoebozoa, Excavata, and SAR (Stramenopiles, Alveolata, and Rhizaria). Ciliates (Alveolata: Ciliophora), with a diversity of about 8,000 described species, are heterotrophic organisms, exhibiting a wide variety of eating behaviors and wide geographical distribution (LYNN, 2008). However, it is estimated that about 89% of the existing diversity has not yet been identified (FOISSNER; HAWKSWORTH, 2009), emphasizing the importance of carrying out studies, mainly due to the great importance of these organisms in aquatic ecosystems (FENCHEL, 1987; FOISSNER, 2006; DOPHEIDE et al., 2009; RODRÍGUEZ, 2011, KUMAR; FOISSNER, 2016).

In Brazil, in the 1920 the first studies on the identification of free-living protozoa began with the works of Provazek (1910) and Cunha (1913; 1914; 1916; 1918). There are few studies on protozoa in freshwater environments (BAGATINI et al., 2013), mainly in lotic environments.

Among the studies on freshwater protozoa in the country, which stand out for presenting surveys of species, we can mention the studies by Dias *et al*. (2008), Pauleto *et al*., (2009), Regali-Seleghim *et al*., (1992), Colzani; Alves (2013), Velho *et al*., (2013), Bonatti *et al*., (2016), Debastiani *et al*., (2016), Segovia *et al*., (2016) and Souza *et al*., (2019). According to Regali-Seleghin *et al*., (2011), large surveys involving the protozoofauna have been carried out in Europe and North America; however, few studies focus on the Neotropical region.

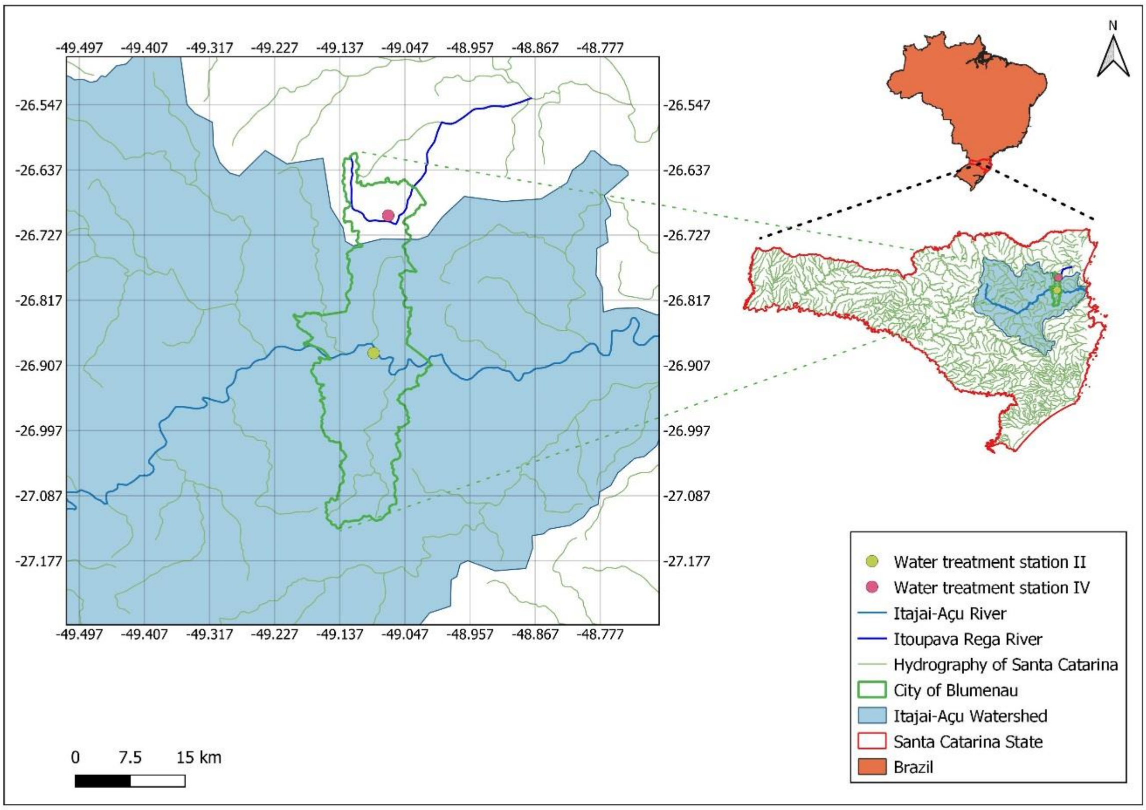
Considering the importance and the need to expand studies on the diversity of free-living protozoa in Brazil, the present study aims to document the diversity and taxonomic composition of free-living protozoa in two water sources used to collect water for public supply in the municipality of Blumenau, SC and to verify the possibility of using these organisms as bioindicators of water quality.

2 MATERIALS AND METHODS

2.1 Study area

Raw water samples were collected from the Itajaí-Açu river (26º 53 '85' 'S and 49º 05' 46 '' W) and the Itoupava Rega stream (26º 42 '03' 'S and 49º 4' 75 '' W), which are two important water sources in the municipality of Blumenau, located in the state of Santa Catarina (Figure 1). The municipality has a total area of 518,619 km2 (IBGE, 2019), with a population of 361,855 inhabitants, of which 95.5% live in urban areas and 4.5% in rural areas (IBGE, 2020). Its relief is rugged, with great differences in altitudes and slopes, being at an altitude of 21 meters above sea level. The region's climate is classified as humid subtropical, for all seasons, characterized by hot and rainy summers. The seasons are well defined, with drier winters (IBGE, 2010).

Figure 1 - Map of the municipality of Blumenau, SC. The yellow circle indicates ETA II's location (P1), and the pink circle indicates ETA IV's location (P2).



Source: Authors (2021)

The sampling points were designated as P1 and P2, respectively, for the Itajaí-Açu Riversand Itoupava Rega streams. The choice of the collection points occurred due to its environmental characteristics and the easy access to the river and to the stream. In these locations are installed two of the four water treatment plants operated by the Municipal Autonomous Water and Sewage Service - SAMAE in the municipality of Blumenau, ETA II and IV.

The P1 (ETA II) is located in the central region of the municipality of Blumenau, on the Itajaí-Açú river (Figure 2A), the largest basin in the state's Atlantic coastal zone (16.5% of the territorial area) operating since 1970 with a capacity 840 Ls-1, working 24 hours a day (SAMAE, 2020). In the municipality, the Itajaí-Açu River receives effluent releases from five streams, namely: Garcia stream, Velha stream, Itoupava Rega stream, Testo stream, and Salto do Norte stream. According to Piazza et al., (2017), ETA II is the most impacted Wastewater Treatment Plant, in Blumenau, by anthropic action, mainly due to its extension in uses and drainage area. Upstream of this ETA, there are approximately 45 municipalities, and many of them have municipal centers and extensive agricultural areas.

P2 (ETA IV) is located in a small basin in the north of the municipality of Blumenau (Figure 2B), on the Itoupava Rega stream (belonging to the Itapocú basin), with a drainage area of 31 km^2.The Itoupava Rega stream together with the Sarmento, Saxônia, Fundo Sete, Areia, Braço do Sul Central and Tifa da Banana streams flow towards the Massaranduba River (Blumenau, 2020). ETA IV has been operating since 1995 and has a production capacity of 20 Ls^{-1} and a 6-hour daily operation (SAMAE, 2020). There is no city upstream of this basin, as it is entirely within the municipality of Blumenau. The region is characterized by hills and narrow valleys, with a dense area of vegetation, and the water produced supplies the neighborhood in which it is located - Vila Itoupava.

Figure 2 - Water collection point. A: P1 - ETA II (Itajaí-Açu river) and, B: P2- ETA IV (Itoupava Rega River), Blumenau, SC.

Source: Authors (2021)

2.2 Experimental Procedure

Monthly, between September 2013 and August 2014, 250 mL of raw water was collected in glass bottles with a wide opening and previously autoclaved during the morning period. The samples were collected at the margins of points P1 and P2, at a depth of approximately 10 cm. The collections were always carried out at the same collection point and at the same time. The collected material was conditioned and transported in a thermal box (15º C) to the Parasitology laboratory of theRegional University Foundation of Blumenau (FURB) for: (i) the quantification of organisms right after collection, (ii) the cultivation of protozoa allowing the visualization of species not active on the day of collection and (iii) fixing the protozoa for later identification.

To identify the organisms on the day of the water collection, 10 slides (slide covered with a coverslip) containing 10 μL of water from each point's samples were assembled, observed under optical microscopy, with magnification from 400 to 1000 X, within 4 hours after the collection.

The samples were observed without the use of fixatives to avoid changes in protozoa's morphological characteristics. After identifying the species, the total count of the organisms present in each slide was carried out to calculate frequency and abundance later. The frequency was expressed in% of organisms per mL, and the total density was expressed in individuals / L and was calculated according to Bush et al., (1997).

To recover encysted species at the time of initial reading (FOISSNER et al., 1999; FOISSNER et al., 2002), collected water samples were kept in simple cultivation (in duplicate). For this, the water samples from each collection point were gently homogenized by hand. A 30 ml aliquot of water was distributed in Petri dishes at room temperature, adding 2 grains of brown rice previously autoclaved and macerated. Rice is used as a carbon source to promote bacterial populations' growth that serves as initial food for bacterivore organisms (FOISSNER et al., 2002, PAIVA; SILVA-NETO, 2007). To accompany the succession of species and excystment of dormant protozoa, the assembly and reading of 10 slides (slides with coverslips) containing 10 μL of water from the cultivation and observed under optical microscopy were performed, with a magnification of 400 to 1000 X. Cultivation readings occurred on the 3rd, 7th, 14th, 21st and 28th days after the beginning of the cultivations. After this period, sporadic readings were performed until the second month, and the material was discarded afterward.

For the species in which the identification was not possible in the fresh material and the cultures, permanent slides were assembled out. The organisms were placed in an embryo dish with the aid of glass micropipettes of their manufacture. They were fixed in Bouin's solution for subsequent impregnation with silver proteinate (protargol), according to the protocol proposed by Dieckmann (1995).

The organisms found in the fresh samples and the cultures were identified according to identification guides (FOISSNER et al., 1991, 1992, 1994, 1995; FOISSNER, 2002; FOISSNER; BERGER, 1996). Then, the identified species were categorized according to their food sources (FOISSNER; BERGER 1996). Namely: Ba: bacteria, O: omnivorous, Al: algae, R: predator, Ki: diatoms, Fl: heterotrophic flagellates, Sb: sulfur-feeding bacteria. Finally, ciliated protozoa were classified according to the level of sabrobity, with p = polisaprobity;i = isosaprobity; a = alpha-mesosaprobity; b = beta-mesosaprobability.

The registration of morphospecies was performed using photo documentation and filming, with the aid of a scientific digital camera model Olympus® U-CMAD3, coupled with the Olympus® CX 31 optical microscope.

2.3 Environmental variables

For the characterization of each sampling point, physical and chemical variables of the water were analyzed, with the aid of a multiparameter probe (Hidrolab DS5X®), which measures the following parameters: water temperature (Cº), turbidity (UNT), pH, dissolved oxygen (mg $O_2.L^{-1}$) and conductivity (μS / cm $^{-1}$). In addition to these parameters, at each monitoring point, water samples were collected in polypropylene flasks, as recommended by APHA (1998), to determine concentrations in the laboratory of nitrate (NO_3-), nitrate (NO_2–), phosphate (PO_4^{3}-) and ammonium (NH_4+). The samples were processed within 24 hours. The analyses were analyzed by Ion Exchange Chromatography (IC) to determine cations and anions and followed the methodologies proposed by DIONEX (2010a) for anions and DIONEX (2010b) for cations.

Precipitation data was provided by the Alert System Operation Center - CEOPS / FURB. This information makes up the Pluviometric Bulletin of the station located at the Regional University Foundation of Blumenau, Campus I, and the monthly average was considered in the analyzes. Due to the lack of a pluviometric data collection station close to P2, the study's values were considered valid for the two sample points.

RESULTS AND DISCUSSION

3.1 Environmental variables

The environmental variables of the two sampling sites over the period of 12months are summarized in Table 1. The data are presented as mean, standard deviation, maximum and minimum.

P1 presented higher mean values in practically all parameters analyzed in relation to P2. Among them, the pH that presented a higher average, in addition to a greater variation between the analyzed months (maximum of 8.9 and minimum of 5.2).

Dissolved oxygen presented the lowest values in all analyzed months, with a maximum of 6.3 $mg.L^{-1}$ and a minimum of 3.02 $mg.L^{-1}$, with an average below the standard value established by CONAMA Resolution nº 357/ 05 for Class 2 (5 $mg.L^{-1}$), is the class in which the river is classified. P2 had an average of 7.6 $mg.L^{-1}$, with a maximum value of 9.5 $mg.L^{-1}$ and a minimum of 5.4 $mg.L^{-1}$ (Table 1). These results suggest that, possibly, the waters of P1 present higher amounts of pollutants in relation to P2, mainly due to the dumping of organic matter, since the degradation of this matter consumes dissolved oxygen and increases the concentration of some nitrogen compounds (ESTEVES, 1998; VON SPERLING, 1996).

P1 showed higher turbidity values than P2 (average of 418.6 NTU for P1 and 198.1 NTU for P2). The highest turbidity values occurred in October 2013, with 865.87 NTU and January 2014, with 934 NTU — coinciding with periods of high rainfall close to the collection dates, with 120.6 mm and 204.9 mm, respectively. In part, high turbidity is a characteristic of the Itajaí-Açú River, which normally presents higher values for this parameter, mainly due to soil characteristics (Fluvic Neosols), with darker colors and different granule textures (GROTT et al., 2016).

The electrical conductivity at the sampling points was lower at P2 (average 21.1 μS. cm^{-1}) and higher at P1 (average 149 μS. cm^{-1}). Wetzel; Likens, (1991) consider that the more polluted the water, the greater the conductivity, due to the increase in the mineral content present in the water.

According to Liu; Leff (2002), changes in dissolved oxygen, electrical conductivity and turbidity are important parameters and can strongly interfere in the dynamics of aquatic microorganisms, including free-living protozoa.

Table 01 - Environmental variables monitored on the Itajaí Açu rRiver (P1) and the Itoupava Rega stream (P2) in the municipality of Blumenau, SC, from September 2013 to August 2014.

Variables	**P1**			**P2**		
	Average(DP)	**Maximum**	**Minimum**	**Average(DP)**	**Maximum**	**Minimum**
pH	7.22±1.15	8.90	5.20	7.09±0.27	7.7	6.8
OD (mg L^{-1})	4.52±1.11	6.35	3.02	7.69±1.57	9.78	5.42
EC* (μS. $cm^{-1)}$	149±33.45	195.5	98.87	21.1±18.17	54.69	2.58
NO_3^- (μg L^{-1})	5.82±11.94	57.43	0.02	6.46±10.75	44.45	0.207
NO_2^- (μg L^{-1})	0.04±0.05	0.16	0.0011	0.027±0.02	0.10	0.0023
PO_4^{3-} (μg L^{-1})	0.23±0.24	0.70	0.0014	0.23±0.26	0.80	0.021
NH_4^+ (μg L^{-1})	0.75±1.08	4.22	0.0015	1.43±1.87	7.24	0.025
Turbidity (NTU)	418.6±297.5	985.5	125.5	198.1±175.3	555.1	15.78
Water Temperature (ºC)	19.78±2.83	24.91	15.98	18.46±2.59	23.40	14.98

*EC = electrical conductivity.
Source: Authors (2021)

3.2 Taxonomy and composition of free-living protozoa in vivo

The inventory of free-living protozoan species identified at points P1 and P2 during the study and each species' occurrence over twelve months of sampling are shown in table 2. At the end of the study, 39 taxa registered at points P1 and P2 were identified, considering the organisms observed on the day of collection and under cultivation conditions (figures 3 to 9).

In fresh readings and from slides made and impregnated by protargol, 16 taxa were identified in P1 and P2. This value is below the range found by researchers in different aquatic environments. Studies on the species richness of several groups of free-living protozoa in lotic environments have identified between 20 and 200 species (MADONI; BRAGHIROLI, 2007; DIAS et al., 2008; BRADLEY et al., 2010; PEREIRA et al., 2014; LOBATO JUNIOR; ARAÚJO, 2015; BERNARDO et al., 2010; GOMES; GODINHO. 2003; PAULETO et al., 2009; VELHO et al., 2013, COLZANI; ALVES, 2013, KÜPPERS, CLAPS, 2012, MEDEIROS et al., 2013, BONATTI et al., 2016).

Environmental conditions, such as temperature, humidity, pH, food availability, biological and geomorphological factors are most responsible for this diversity (FOISSNER et al., 1999). The protozoa, in general, form a morphologically and physiologically diverse group and can be regulated both by the availability of food resources and by predation (ARNDT, 1993), thus changing patterns of abundance and biomass (GASOL et al., 1995; WEISSE, 2002). Foissner (2014), studying the ciliate fauna of four clear-water rivers in Germany, attributed the low abundance of ciliate found to a possible shortage of nutrients in these environments. In this study, however, some factors that were not analyzed may have contributed to the low number of taxa found in the readings taken on the day of the collection. This fact indicates the need for more in-depth studies to verify the existence of possible environmental variables that cause the low amount of free-living protozoa in the city's springs — or whether this low amount is a natural feature in these environments.

In P1, in the readings on the day of collection, 11 taxa were identified. The ciliated protozoa observed were *Colpidium colpoda* Ehrenberg, 1833, *Frontonia leucas* Ehrenberg, 1833, *Lembadion lucens* Maskell, 1887, *Loxodes* and *Vorticella microstoma* Ehrenberg, 1830. In addition to the ciliates, other protozoa were observed in the samples, with the occurrence of *Entosiphon sulcatum* (Dujardin) Stein 1878*, Euglena* sp., *Peranema* sp. and *Phacus* sp., *Amoeba radiosa* Ehrenberg, 1838, *Arcella hemisphaerica* Perty, 1852.

In P2, 12 taxa were recorded in the readings on the day of collection, six of which were ciliated (*Aspidisca* sp., *Coleps* sp., *C. colpoda*, *L. lucens*, *Litonotus* sp., *Loxodes* sp.), the flagellates *Ansionema* sp., *Euglena* sp., *Peranema* sp., naked amoebas, and *A.hemisphaerica*.

In a study by Gomes; Godinho (2003), the authors identified 28 species of protozooplankton in an eutrophic lake in Monte Alegre, SP. Jian; Shen (2005), in a study, carried out on surface water sources in China, revealed the existence of 488 species distributed in 16 collection points. Dias et al. (2008) in a study of benthic ciliate recorded 42 species in surface waters in Minas Gerais. Bonatti et al. (2016), in a study, carried out on the Atibaia River, SP identified 66 taxa belonging to 55 genera occurring in samples of raw water and sediment.

Studies indicate that the greater the protozoa diversity, the better the water quality (XU et al., 2008; JIANG et al., 2011). However, at both points (P1 and P2), despite the differences in environmental variables, the points showed similar amounts of protozoa, which may mean that these species coexist without dominance from the sites' nutrients. Shi et al., (2012) suggest that when environmental stress situations are minimal, species diversity is reduced due to competitive exclusion between them, while in a small increase in environmental stress competition decreases, increasing diversity.

The morphospecies that had the highest occurrence in P1 were the flagellate *Peranema* sp. (33.3%), and the amoebae *A. radiosa* (25%) and *A. hemisphaerica* (25%). In P2, besides the morphospecies reported in P1, the ciliate, *Loxodes* sp. (16.6%), and *Litonotus* sp. (16.6%) were the most frequently recorded morphospecies at this sampling point.

Studies indicate that the protozoan *Peranema* sp. has a wide distribution in places with polluted wastewater (KACHIENG'A; MOMBA, 2015) and shows its potential for removing nitrate and phosphate in wastewater systems containing nickel and other metals (KAMIKA; MOMBA, 2015). In addition, flagellates have a wide range of adaptations, especially concerning their food versatility (MEDEIROS, 2013), and can adapt to different food availability environments.

The *A. radiosa* species identified in this analysis may indicate a location with a higher degree of pollution. In the study by Júnior; Araújo, 2015, the species was found only at the most impacted point of the Pium River, RN. In contrast, the species *A. hemisphaerica* was not registered in the same place; only at the collection point located before the beach towns, with low water flow and with several banks of macrophytes; that is, it had better environmental conditions.

Several studies have recorded the genera *Amoeba* sp. and *Arcella* sp. in Brazilian water sources (LANSAC-TÔHA et al., 2000; VELHO et al., 2000; MEDEIROS et al., 2013). Araújo; Godinho (2008) and Gomes; Godinho (2003), in studies carried out in tropical environments, reported the association relationships between Thecamoebae and aquatic macrophytes, which could justify the presence of these organisms in P1 and P2 since in both places there is the presence of vegetation in the water sources margins.

The greatest occurrence of *Litonotus* sp. and *Loxodes* sp. ciliates in P2 was unexpected since both species have a preference for environments with a lower concentration of dissolved oxygen and relationships with affected areas, including the occurrence of raw sewage from wastewater treatment plants (SIQUEIRA-CASTRO et al., 2016; PAIVA; SILVA -NETO, 2004). We hypothesize that even though it is an area with a higher degree of environmental preservation, some rural propertiesare located close to the collection site, with the possibility of pesticides and other compounds not analyzed in the study that may be modifying the biodiversity of species of free-living protozoa.

The protozoa *F. leucas*, *V. microstoma*, and *E. sulcatum* were the only species in P1. The species *Aspidisca* sp., *Coleps* sp., *Litonotus* sp., *Anisonema* sp.,and the naked amoebae occurred only in P2.

Table 2 - Free-living protozoa recorded in samples of raw water from the Itajaí-Açú river (P1) and the Itoupava Rega stream (P2), in the municipality of Blumenau, SC, Saprobity level and food source. The organisms were observed on the day of collection (V) and cultivated (C).

	Occurrence (%)					
TAXON	**P1 (V)**	**P2 (V)**	**P1 (C)**	**P2 (C)**	**S**	**FS**
CILIOPHORA						
Aspidisca sp.	-	8,3	16,6	25	a-b	Ba
Coleps sp.	-	8,3	16,6	41,6		O
Colpidium colpoda Ehrenberg, 1833	8,3	8,3	58,3	25	p-i	Ba, Fl, AL
Cyclidium glaucoma Müller, 1786	-	-	8,3	8,3	a	Ba
Campanella umbellaria Linnaeus, 1758	-	-	8,3	8,3	a-b	Ba
Chilodonella sp.	-	-	8,3	-	a	Ba
Euplotes aediculatus Ehrenberg, 1830	-	-	25	-	a	O
Frontonia leucas Ehrenberg, 1833	8,3	-	16,6	25	b-a	O
Glaucoma frontata Stokes, 1886	-	-	16,6	8,3		
Glaucoma sp.	-	-	8,3	16,6		
Lembadion lucens Maskell, 1887	8,3	8,3	25	33,3	b-a	O
Loxodes sp.	8,3	16,6	25	16,6	p	
Litonotus sp.	-	16,6	16,6	33,3		

Table 2 – continue

Table 2 – continuation

Lacrymaria olor Müller, 1786	-	-	16,6	8,3	b	R
Oxytricha sp.	-	-	-	8,34		
Paramecium caudatum Ehrenberg, 1833	-	-	25	33,3	p-a	Ba, Al
Paramecium bursaria Ehrenberg,1831	-	-	8,3	-	b-a	Ba, Al, Ki
Prorodon sp.	-	-	8,34	-		
Spirostomum teres Claparède & Lachmann, 1859	-	-	16,6	-	-	Sb, Ba,Al, Ki
Spathidium sp.	-	-	-	8,3	-	
Tetmemena pustulata Müller, 1786			16,64	8,3		
Urocentrum turbo Müller, 1786	-	-	-	8,3	a-b	Ba, Ki
Uronema sp.	-	-	8,3	33,3	a-p	
Vorticella microstoma Ehrenberg, 1830	8,34	-	-	-	p-a	Ba, Al
Vorticella sp. (1)	-	-	33,3	25		
Vorticella sp. (2)	-	-	16,6	16,6		
Vorticella sp. (3)	-	-	-	8,3		
Vorticella sp. (4)	-	-	-	8,3		
Vaginicola sp.	-	-	8,3	-		
EXCAVATA						
Anisonema sp.	-	8,3	-	-	-	
Entosiphon sulcatum (Dujardin) Stein 1878	8,3	-	33,34	25	-	
Euglena sp.	8,3	8,3	16,6	41,6		
Peranema sp.	33,3	16,6	50	41,6	-	
Phacus sp.	8,3	-	8,3	8,3		

Table 2 – Continue

Table 2 - conclusion

AMOEBOZOA					
Amoeba radiosa Ehrenberg, 1838	25	16,6	41,6	50	-
Naked amoeba	-	8,3	8,3	8,3	
Arcella hemisphaerica Perty, 1852.	25	16,6	41,6	25	-
Centropyxis sp.	-	-	-	8,3	-
Euglypha sp.	-	-	-	8,3	-

P1 and P2 (V) = Observations on the day of collection;
P1 and P2 (C) = Culture;
S = Saprobity level according to Foissner; Berger (1996), p = polisaprobity;i = isosaprobity; a = alpha-mesosaprobity; b = beta-mesosaprobity
FS = Food source: Ba: bacteria, O: omnivore, Al: algae, R: predator, Ki: diatoms, Fl: heterotrophic flagellates, Sb: sulfur-feeding bactéria
Source: Authors (2021)

Among the analyzed group, the Ciliophora was the most abundant and diversified in the study period, with 69.5% of frequency in P1 and 66.1% in P2 (Table 3), with taxa distribution occurring similarly in both sampling points. Santos et al., 2013 and Medeiros et al., 2013 reported equal superiority of the ciliate concerning the other microorganisms in a study carried out in an urban stream in São Paulo and in a hydrographic basin in Rio Grande do Norte, respectively. The observed differences indicated the presence of the taxa *C. colpoda, L. lucens, Loxodes* sp. at both collection points, which suggests that organisms are important in the composition of the trophic network (ARAÚJO; COSTA, 2007).

Ciliated protozoa have been widely used as bioindicators through the saprobic system to assess and monitor lotic and lentic environments under different levels of anthropic impact. The saprobic index is based on the degree of tolerance of ciliate species to organic pollution (SLADECEK, 1973; FOISSNER, 1996). Among the morphospecies of ciliates found in the study, four are included in the saprobic system. They are considered bioindicators of the environment withpolisaprobity (*V. microstoma, C. colpoda*) and beta-mesosaprobe (*F. leucas, L.*

lucens). Only *V. microstoma* and *F. leucas* were recorded in P1, while *C. colpoda* and *L. lucens* were found in both sample points (Table 2).

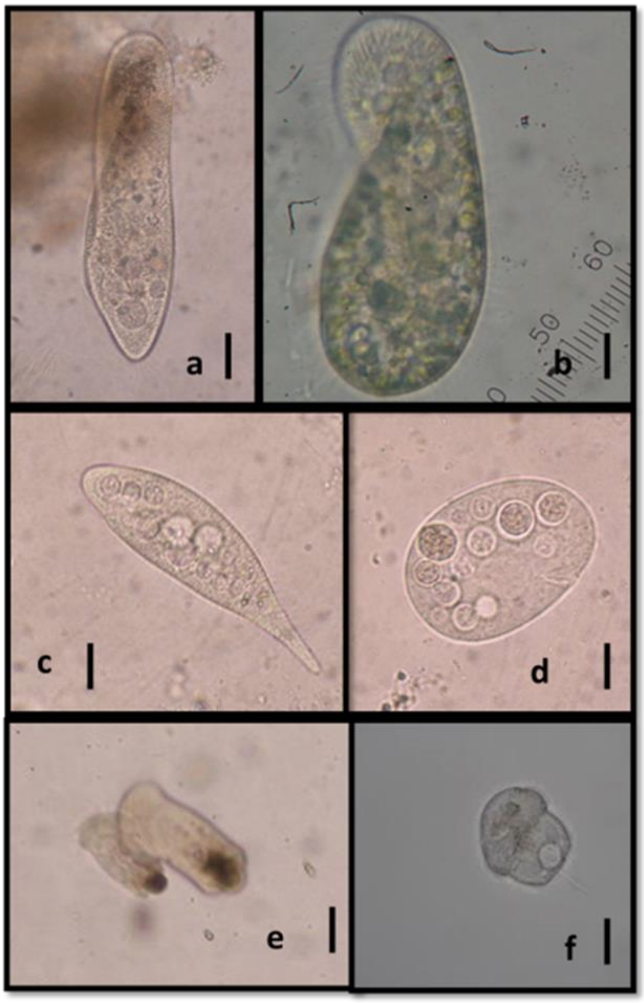
The species F. leucas and L. lucens are omnivorous organisms and suggest a continuous supply of organic matter of allochthonous origin in the river (ZINGEL, 2005), as occurs in P1. These species, being omnivorous, can be found in different environments and tolerate major changes in physical and chemical variables (FOISSNER et al., 1999).

Excavata showed a frequency of 19.1% in P1 and 10.8% in P2, emphasizing morphospecies *Euglena* sp. and *Peranem*a sp., recorded at both points. Possibly, the occurrence of phytoflagellates that make up the peripheral community is related to their dietary diversity. Studies show that these organisms can feed on viruses and even cyanobacteria (GASOL et al., 1995).

Amoebozoas, represented by the genres *Amoeba* and *Arcella*, had high occurrences in both P1 and P2. This result corroborates the studies by Lansac-Tôha et al., (2000) and Velho et al., (2000), which identified the genera in different aquatic environments, both natural and eutrophic, as previously mentioned.

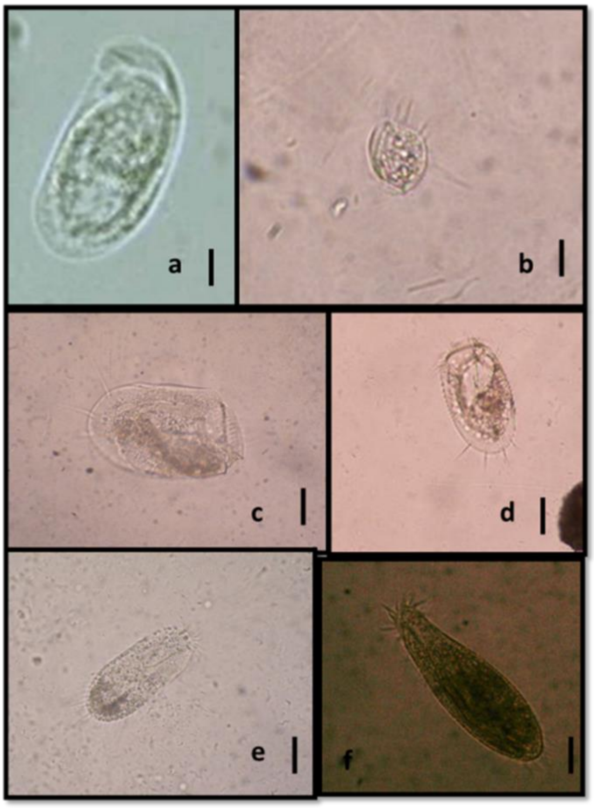
Regarding the abundance of individuals, it can be seen that the flagellates obtained a higher value in P1 (102 ind. mL) and the ciliate in P2 (85.5 ind. mL). This superiority of flagellates in P1 maybe because they play an important role in the food chains because they consume autotrophic and heterotrophic fractions of plankton (bacteria and picophytoplankton) (WEISSE, 2002), thus constituting an important source of matter and energy among microbial food webs and upper trophic levels (SHERR; SHERR, 1994). With greater abundance in P2, ciliates may be related to a period with greater dumping of waste in the stream, as the species found are indicators of a polluted environment.

Figure 3 - Photomicrographs of free-swimming ciliated protozoa identified at the sampling points (P1- Itajaí Açu river and P2- Itoupava Rega stream), on the day of the collections as well in the cultures (Sept. 2013 to Aug. 2014) in the municipality of Blumenau, SC. (a) *Paramecium caudatum*; (b) *Paramecium bursaria*; (c) *Glaucoma frontata*; (d) *Glaucoma* sp.; (e) *Spathidium* sp.;(f) *Urocentrum turbo*; (g) *Cyclidium glaucoma;* (h) *Prorodon* sp. Bar = 10 μm.



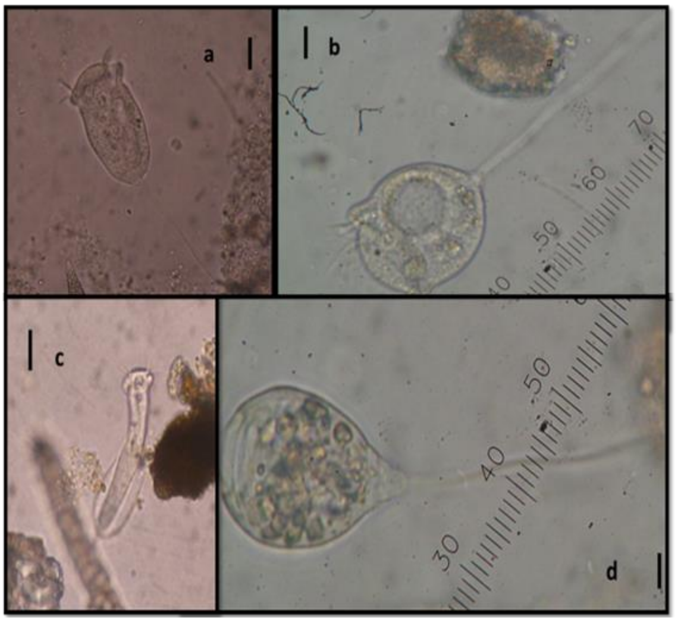
Source: Authors (2021)

Figure 4 - Photomicrographs of crawling bacterivorous protozoa identified at the sampling points (P1- Itajaí Açu river and P2- Itoupava Rega stream), in the readings on the day of the collections as well as in the cultures (Sept. 2013 to Aug. 2014) in the municipality of Blumenau, SC.(a) *Chilodonella* sp.; (b) *Aspidisca* sp.; (c-d) *Euplotes aediculatus*; (e) *Tetmemena postulata*; (f) *Oxytricha* sp. Bar = 30 μm.



Source: Authors (2021)

Figure 5 - Photomicrographs of sessile bacterivorous protozoa identified in the sampling points (P1- Itajaí Açu river and P2- Itoupava Rega stream), in the readings taken on the day of the collection and in the cultures (Sept. 2013 to Aug. 2014) in the municipality of Blumenau, SC. (a)*Vorticella microstoma*; (b)*Vorticella* sp. (1); (c)*Vaginicola* sp.; (d)*Vorticella* sp. (2). Bar = 20 μm.

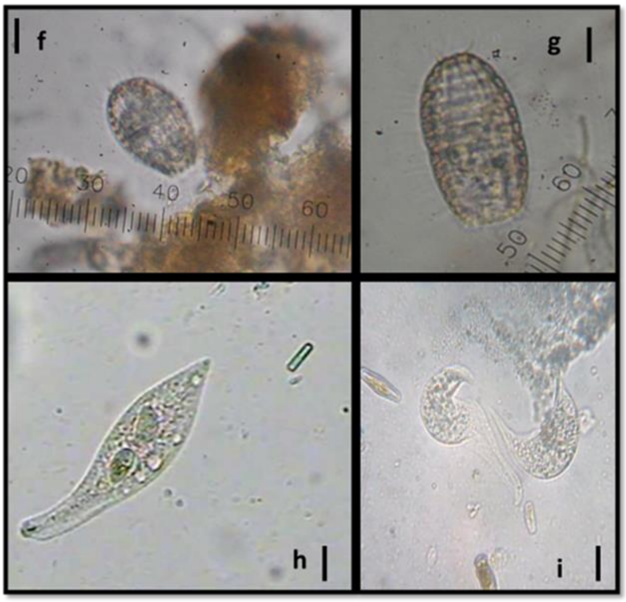


Source: Authors (2021)

Figure 6 - Photomicrographs of free-swimming ciliated protozoa with different eating habits. The specimens were identified at the sampling points (P1- Itajaí Açu river and P2- Itoupava Rega stream), in the readings taken on the day of the collection and in the cultures during the collection period (Sept. 2013 to Aug. 2014) in the municipality of Blumenau, SC (a)*Frontonia leucas* (omnivorous); (b)*Colpidium colpoda*(bacterivore, algivore and flagellate); (c)*Lembadion lucens* (omnivorous); (d)*Spirostomum teres* (bacterivore, algivore); (e)*Loxodes* sp.(no information). Bar = 20 μm.

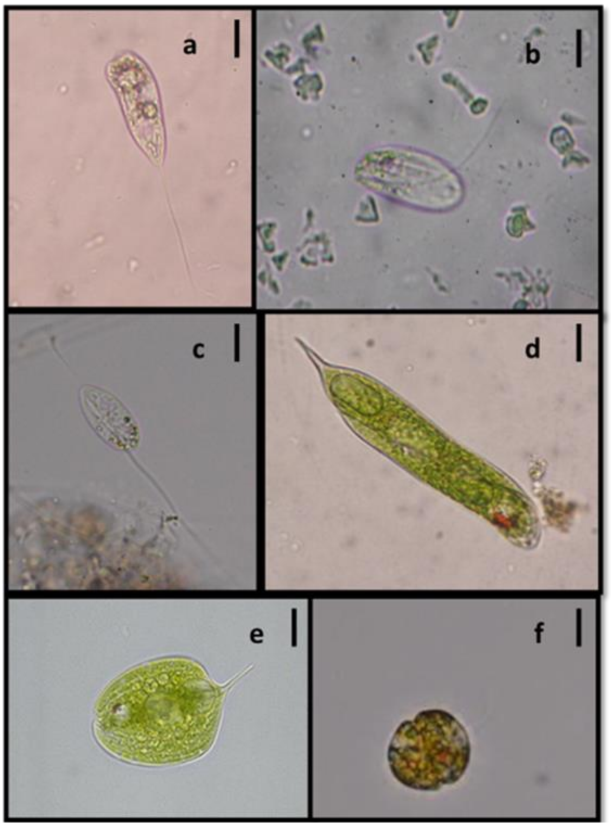
Source: Authors (2021)

Figure 7 - Photomicrographs of free-swimming native carnivorous ciliated protozoa identified at the sampling points (P1- Itajaí Açu river and P2- Vila Itoupava stream), both *in vivo* and in cultures during the collection period (Sept. 2013 to Aug.2014) in the municipality from Blumenau, SC. (f-g)*Coleps* sp.; (h)*Litonotus* sp.;(i)*Lacrymaria olor*. Bar = 20 μm.



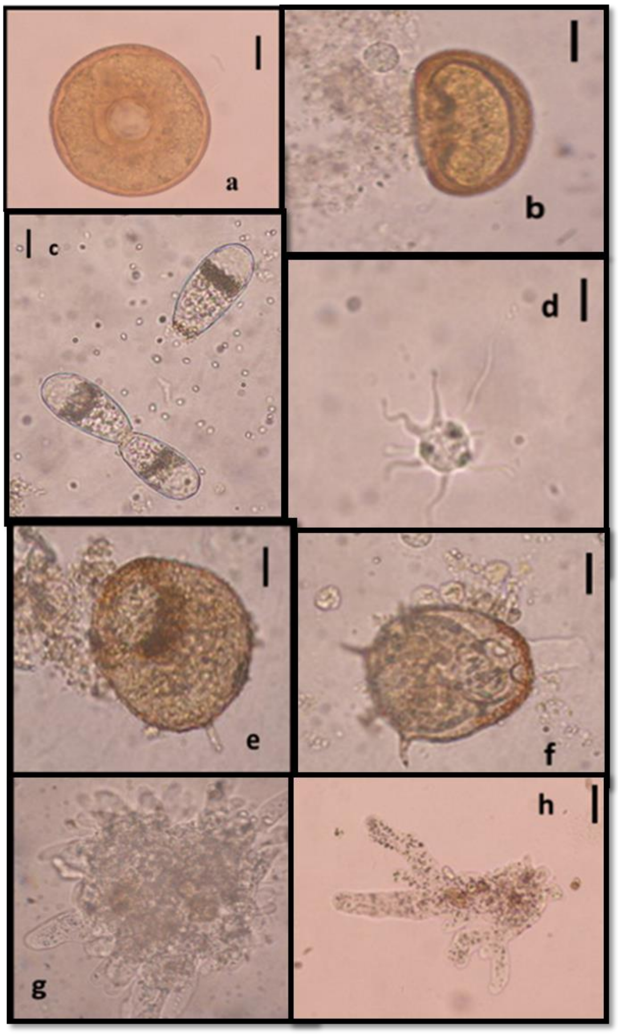
Source: Authors (2021)

Figure 8 - Photomicrographs of flagellated protozoa identified at the sampling points (P1- Itajaí Açu river and P2- Vila Itoupava stream), both in vivo and in cultures during the collection period (Sep. 2013 to Aug.2014) in the municipality of Blumenau, SC. (a)*Peranema* sp.; (b)*Entosiphon sulcatum*; (c)*Anisonema* sp.; (d) *Euglena* sp.; (e)*Phacus* sp.; (f)*Gymnodinium* sp. Bar = 20 µm.



Source: Authors (2021)

Figure 9 - Photomicrographs of amoeboid protozoa identified at the sampling points (P1- Itajaí Açu river and P2- Itoupava Rega stream), in the readings taken on the day of the collections and in the cultures (Sept. 2013 to Aug. 2014), in the municipality of Blumenau, SC. (a-b)*Arcella hemisphaerica*; (b)*Euglypha* sp.; (c)*Centropixys* sp .; (d)*Amoeba radiosa*; g-h: naked amoebas. Bar = 20 µm (pictures a / b / d / e / f / g / h), 40 µm (picture d).



Source: Authors (2021)

Table 3 - Relative frequency and mean abundance of free-living protozoa at two sample points (P1- Itajaí Açu river and P2- Itoupava Rega stream) during the collection period (September 2013 to August 2014), *in vivo* readings in the municipality of Blumenau, SC.

Sampling point	**Frequency (%)**		**Average Abundance (Individuals / mL)**	
Group	P1	P2	P1	P2
Ciliates	69.5	66.1	36.3	85.5
Naked amoebas	9.8	7.4	5.1	9.5
Thecamoebae	0.7	14.3	0.4	18.5
Flagellate	19.1	10.8	102	14

Source: Authors (2021)

3.3 Succession of species in cultivation condition

The cultures' analysis revealed the occurrence of morphospecies present in both sampling points, which were not detected on the day of collection. Of these, it was possible to identify 20 new taxain P1 and 10 taxa in P2 (Table 2). These values corroborate with those observed in other studies (PAULETO et al., 2009; VELHO et al., 2013, COLZANI, ALVES, 2013, KÜPPERS, CLAPS, 2012, MEDEIROS et al., 2013), which registered values between 22 and 65 species in cultivations. The difference found between the numbers of taxa identified in the readings taken on the day of collection concerning the cultures can be explained by the fact that the protozoa are encysted at the time of collection. In natural conditions, protozoa may be in the form of cysts, becoming active under favorable conditions, with the preparation of cultures being the most accessible form of recovery and registration of this morphospecies (FOISSNER et al., 2002). Taylor (1981) emphasizes the importance of encystment mechanismsthat allow many species of ciliates to withstand predation, physical and chemical stress, and increased food availability. These species' presence can be useful in understanding possible changes in the physical and chemical conditions of ecosystems caused by anthropic impacts.

The morphospecies of ciliates that had their occurrence recorded only once during the study period were: *Chilodonella* sp., *Euplotes aediculatus*, *Oxytricha* sp., *Paramecium bursaria*, *Prorodon* sp., *Spirostomus teres*, *Spathidium* sp., *Urocentrum turbo*, *Vaginicola* sp., *Vorticella microstoma*, *Vorticella* sp. (3), *Vorticella* sp. (4). For flagellates, the single-occurring morphospecies was *Ansionema* sp. and among amoebas*, Centropyxis* sp. and *Euglypha* sp. were recorded in a single collection during the study.

Among these species, the record of the genus *Chilodonella* sp. in P1 — which are reported as opportunistic parasites in fresh-water fish (BASTOS GOMES et al., 2016) — occurs when fish are affected by stressors, such as changes in environmental conditions (temperature, sunlight, levels of oxygen and pH).

4 CONCLUSIONS

This study represents the first survey of free-living protozoa in two aquatic environments in the municipality of Blumenau, SC, being an important contribution to the knowledge of the diversity and distribution of these organisms. Although the results obtained do not show wide differentiation between the sampling points, the free-living protozoa represent important tools for analyzing environments affected by different degrees of pollution. However, the importance of conducting further studies on the dynamics and distribution of aquatic microorganism communities must be emphasized to expand knowledge of species' taxonomic diversity and ecology, especially in impacted environments. In addition, the need to include different environmental variables is essential. Together with free-living protozoa, these variables can help classify water sources and the use of these organisms as possible bioindicators of environmental pollution.

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