Farming and soil urban occupation in the water quality of Jaboticabal and Cerradinho streams

Agricultura e ocupação urbana do solo na qualidade da água dos córregos Jaboticabal e Cerradinho

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ABSTRACT

Since the end of the twentieth century, 100% of the urban sewage from the city of Jaboticabal has been collected by interceptors and routed to a treatment plant. Between 1999 and 2000, studies on the effect of this environmental care showed that it did not efficiently clean up the water from the two streams that run through the city and flow into an agricultural area. This paper focuses on assessing the influence of soil use on the water quality of surface waters from these two streams. The study was conducted 15 years after the implementation of sewage interceptors. The sampling dates were bimonthly at eight points (P1 to P8) in the Cerradinho and Jaboticabal streams, in Jaboticabal (São Paulo State, Brazil). P1 was located at the source of the Jaboticabal stream, P2 was in a farming area, P3, P4 and P5 were in an urban area, and P6, P7 and P8 were in a farming area. The physical and chemical variables of the water were assessed. We compared the ability of microorganisms to metabolize different sources of carbon using the EcoPlate (Biolog). The total phosphorus (TP) concentration exceeded the limit set by the Brazilian legislation as well as values found in previous studies, which was also observed for the chemical oxygen demand. However, the bacterial metabolic profile had no association with urban or farming practices. The results of the analysis indicated the possibility of clandestine discharge of wastewater in the streams studied and the influence of the agricultural soil.

Index terms: Leaching; EcoPlate; metabolic profile; water resources.

RESUMO

Desde o final do século vinte, 100% do esgoto urbano da cidade de Jaboticabal é recolhido por interceptores e conduzido a uma estação de tratamento. Entre 1999 e 2000, estudos sobre o efeito desse cuidado ambiental, mostraram que a coleta de esgoto ainda não tinha sido eficiente para despoluir as águas dos dois córregos que atravessam a cidade e fluem para uma área agrícola. Neste trabalho avalia-se a influência do uso do solo na qualidade da água destes dois córregos. O estudo foi realizado 15 anos após a implantação dos interceptores de esgoto. Durante o ano de 2014, bimestralmente, coletaram-se amostras de água em oito pontos (P1 a P8), dos córregos Cerradinho e Jaboticabal, em Jaboticabal (SP, Brasil). P1 estava localizado na nascente do córrego Jaboticabal; P2 em área agrícola; P3, P4 e P5 em área urbana; P6, P7 e P8 em área agrícola. Foram determinadas variáveis físicas e químicas e a comparação metabólica da população microbiana em diversas fontes de carbono pelo método EcoPlate (Biolog). Em todos os pontos e coletas, o teor de fósforo total excedeu o limite estabelecido pela legislação brasileira, assim como valores encontrados em estudos anteriores, o que também foi observado em relação à demanda química de oxigênio. Entretanto, a população microbiana da água parece não estar sendo alterada pelas atividades urbanas e agrícolas. Os resultados das análises sinalizam à possibilidade de descarga clandestina de águas residuárias nos córregos estudados e à influência do uso agrícola do solo.

Termos para indexação: Lixiviação; EcoPlate; perfil metabólico; recursos hídricos.

INTRODUCTION

Many Brazilian municipalities capture water from surface water sources to supply and launch domestic and industrial effluents in the river basin, resulting in potentially negative impacts on the water quality. At the same time, these water bodies, which are important for the development of agriculture, are negatively impacted by these activities, including contamination by agrochemicals and increased suspension materials. Considering the importance of water in our society and the need to maintain the environmental balance, the study of anthropogenic influences on water quality in water bodies are critical to environmental planning studies for the protection and restoration of areas with great human rural or urban pressure.

Studies on water quality based on physical, chemical and microbiological variables enable quality evolution monitoring in a water body as well as over an

entire watershed (Medihala et al., 2012; Sahu et al., 2013). Furthermore, water analysis plays an important role in assessing chemicals with concentrations greater than the maximum values established by environmental law, which can cause health problems and environmental impacts (Jabben et al., 2014). Several physical (temperature, dissolved and suspended solid contents, color, transparency and turbidity), chemical (chloride, nitrate, nitrite, ammonia, total phosphorus, soluble reactive phosphorus, pH, electric conductivity, dissolved oxygen, etc.) and microbiological attributes may be used as indicators of water quality.

The traditional methodology for microbial studies has changed because of methods that can show the behavior of a microbial population. EcoPlate (Biolog) is a rapid and convenient method to mainly investigate and estimate the impact of stressors on microbial communities from several environmental sources: soil (Muniz et al., 2014; Flores-Rentería et al., 2016), water (Patel et al., 2014; Xiao; Tinglin; Haihan, 2015), wastewater from different technologies (Jałowiecki et al., 2016) and many other sources.

Starting from the premise that land use and occupation change the physico-chemical and natural biological processes, resulting in potentially negative impacts on water quality, the aim of this study was to evaluate the influence of human activity on water quality by analyzing the physical and chemical variables as well as the microbial community metabolic profile.

The water bodies studied in this work (Cerradinho and Jaboticabal streams) run through the city of Jaboticabal (São Paulo, Brazil) and move to an agricultural region. Thus, these water sources are areas subjected to pressure by urban activities and agriculture. Historically the streams were sites used to launch domestic raw sewage. At the end of the 1990s, 100% of the city sewage started to be collected by receivers along streams and was treated. Borges, Galbiati and Ferraudo (2003) found that sewage interceptors by that time were not enough to de-pollute the streams in urban areas. Consequently, this pollution came to agricultural areas that were located downstream from the urban area. Studies of the riparian forests and areas occupying the banks of streams were carried out over 10 years (Pissarra et al., 2009) and it was observed that the Jaboticabal stream preserved its riparian forests better (47.6% of APP area) than the Cerradinho stream (0.7% of APP with vegetation). Thus, further studies on the water quality of these water resources and the current influences of the urban and rural human activities were the goals of this work. The results observed after 15 years from the addition of the sewage interceptor can show that the pollution potential of the activities in the running

water can also help with planning protection processes and environmental recovery in the region.

MATERIAL AND METHODS

The study was performed using water samples taken from the Cerradinho and Jaboticabal streams, which are located within the city of Jaboticabal, Northeastern São Paulo State, in Brazil. Both streams belong to the Córrego Rico subwatershed within the drainage area of Mogi-Guaçu and are tributaries of the Mogi-Guaçu River (Pissarra et al., 2009). Six sampling points were set along the Cerradinho and Jaboticabal streams. These water sources run through Jaboticabal city and intersect each other near the city hall, where it is called the Jaboticabal stream, which goes toward the College of Agricultural and Veterinarian Sciences (FCAV/UNESP) downstream of the city. Figure 1 shows the Jaboticabal stream subwatershed. The sampling points (P1 to P8) were distributed as following: P1 was at the Jaboticabal stream source; P2 was at the entrance to Jaboticabal city in the Cerradinho stream; P3, P4 and P5 were located within the urban area after the Cerradinho and Jaboticabal streams intersect; and P6, P7 and P8 were within the agricultural area (FEPE, Education, Research and Extension Farm at FCAV/UNESP). Agricultural practices in this area are from soybean, corn and other crops and have been carried out for 80 years through conventional management. The local soil is classified as Eutroferric Red Latosol - LVef (Oxisol) with a clayey texture.

The samples were collected bimonthly throughout 2014, for a total of six sampling dates in February, April, June, August, October and December represented by D1, D2, D3, D4, D5 and D6, respectively. Monthly rainfall data were obtained from a weather station located in the FCAV (Rolim, 2014). The water samples were collected up to 30 cm below the surface of the water by adopting the procedures for sampling, storage and preservation of inorganic nonmetallic constituents and aggregate organic constituents (Baker, 2005) described in the Standard Methods for the Examination of Water and Wastewater. The water samples that were collected for microbial activity analysis were collected and stored in amber glass vials, sterilized in an autoclave, transported at 4 to 8 °C and used immediately.

Physical and chemical variables

We measured the following variables: pH, electric conductivity (COND), temperature (T), dissolved oxygen (DO), turbidity (TURB), total dissolved solids (TDS) and oxidation-reduction potential or redox potential (ORP)

at the sampling points with the aid of a multiparameter probe (origin Horiba U52). The chloride content (Cl⁻) was determined by titrimetric precipitation, which is an argentometric method (Askew; Smith, 2005). Ammonia nitrogen (NH_4^+), nitrate (NO_3^-), nitrite (NO_2^-), total phosphorus (TP), soluble reactive phosphorus (SRP) and chemical oxygen demand (COD) were determined by molecular spectroscopy in the visible region with a spectrophotometer SPEC 20D. All analyses were performed in triplicate for each variable. The phenate method was used for NH_4^+; the colorimetric method was used for NO_2^- using N-(1-naphthyl)-ethylenediamine dihydrochloride (NED dihydrochloride) to react with the analyte; the reduction method was used for NO_3^-; the ascorbic acid method was used for TP after digesting the water samples with sulfuric and nitric acids; SRP was also analyzed using the ascorbic acid method after the sample was percolated through a 0.45 µm membrane (Askew; Smith, 2005). The closed reflux colorimetric method was used for COD (Baird, 2005).
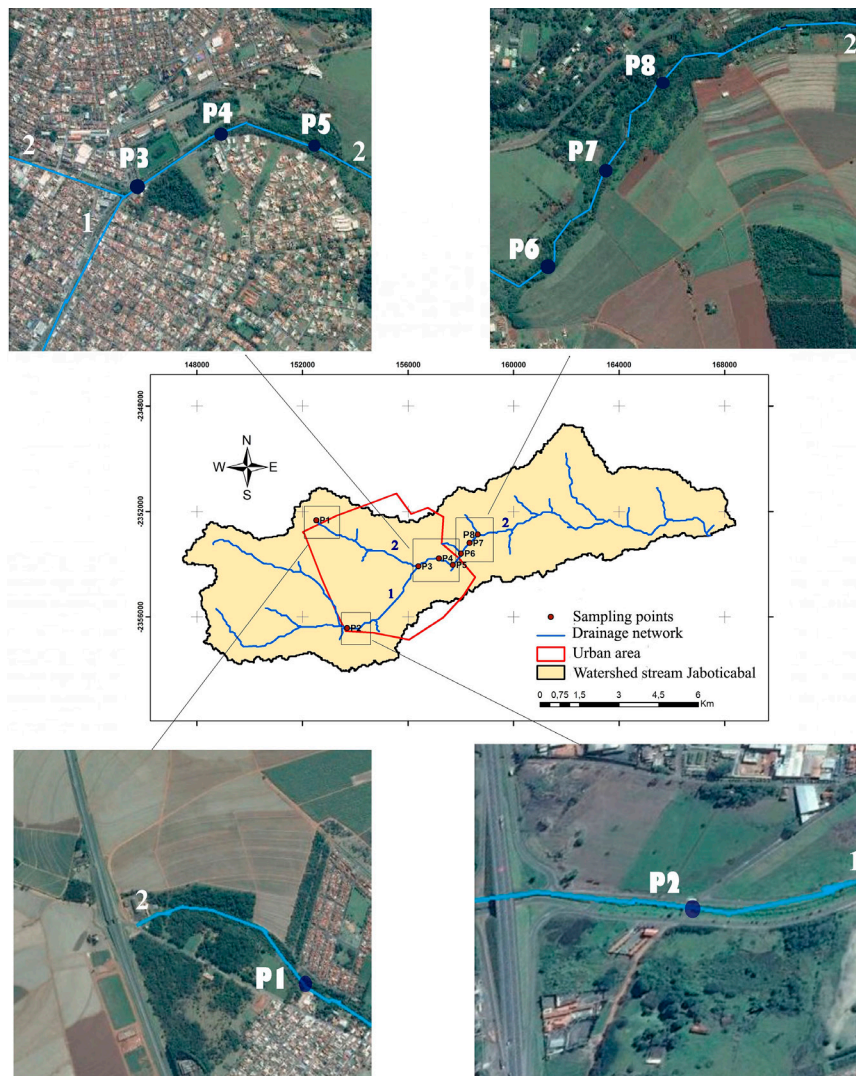
Figure 1: Description of the sampling points on the Jaboticabal stream subwatershed showing the urban and farming area. The blue lines represent tributaries that compose the subwatershed (1 - Cerradinho Stream and 2 - Jaboticabal Stream). The geographical coordinates are: P1 (S - 21.276.88, O - 48.335.39); P2 (S - 21.244.10, O – 48.244. 30); P3 (S - 21.256.08, O - 48.295.59); P4 (S - 21.253.31, O - 48.304.77); P5 (S - 21.255.45, O - 48.300.27); P6 (S - 21.249.05, O - 48.295.59); P7 (S - 21.246.38, O - 48.290.10); and P8 (S - 21.245.65, O - 48.289.53).

Metabolic profile

The microbial communities in the samples were compared by the type of carbon source that was metabolized using a Biolog EcoPlateTM (Garland; Mills, 1991). This plate contained 31 of the most useful carbon sources for soil community analysis. These 31 carbon sources were repeated 3 times to provide replicates of the data. A purple color formed when the microbes utilized the carbon source. The respiration of the cells in the community reduced a tetrazolium dye that was included with the carbon source. The water samples were distributed on plates under a laminar flux as soon as they arrived at the laboratory. Each of the 96 plate wells were filled with a 130 µL aliquot of the sample, which remained at 27 ºC in a biological oxygen demand incubator (BOD incubator) for 5 days. Carbon source consumption was measured in a Molecular Devices Spectramax M2 plate reader at an emission of 590 nm. The readings were taken immediately after the addition of the water samples on the plate (T0) and every 24 h for 5 consecutive days. Control readings (without the substrate, only water) were subtracted from each substrate reading. The generated data enabled the comparison of the microbial population for each sample with respect to the number of substrates used or the substrate index richness (S), average well color development (AWCD) after 120 h of incubation, Shannon diversity index (H) and Shannon evenness index (E). AWCD was used as an indicator of the general microbial activity and was assessed as the average optical density across all of the wells in the plate (Zhang et al., 2014). The H index was calculated from the sum of all of the incubation times and the Shannon Evenness (E) index was calculated from the Shannon-Weiner diversity index (H) and substrate richness (S) index.

Statistical Analysis

The results of the physical and chemical variables at each water sampling point are presented in the boxplot graphics, which enabled the evaluation of the spatial variability of each studied parameter and a comparison of the different sampling sites. The boxplot of each sampling site was built using the following numbers: minimum value, first quartile (Q1), second quartile (or median, Q2), third quartile (Q3) and maximum value. The rule adopted for identifying outliers based on the interquartile range (IQR) was defined as the distance between the first and third quartiles. Thus, any values below Q1 or above Q3 by more than 1.5xIQR were considered outliers. Furthermore, the results of the physical and chemical parameters underwent multivariate principal

component analysis (PCA), which is classified as an interdependence technique in which no variable is defined as independent or dependent because the process involves the simultaneous analysis of all variables. This analysis was applied to evaluate the water quality in relation to all variables and identify the possible temporal variations and the most impacted sampling sites. The means of all variables were used, which allowed for the determination of standard values, with a zero mean and variance equal to one (Guedes et al., 2012). The principal component analysis consisted of calculating the eigenvalues and corresponding eigenvectors of a correlation matrix. PCA was performed by selecting the eigenvectors above 1.00, according to Kaiser-Guttman (Jackson, 1993), with the aid of Statistica Software, Version 7 (Statsoft Inc., Tulsa, USA). In this work, as in other studies reported in the literature (Guedes et al., 2012; Pereira-Silva et al., 2011), we adopted eigenvalues above 0.5. The first two principal components (PC1 and PC2) were used because they had a higher total variation than the original data.

For information on the microbial metabolic profile, primarily by having five absorbance reading times, we performed a mathematical calculation for each substrate using the formula proposed by Hackett and Griffiths (1997), which proposed the use of an equation using data from all of the time points, thereby determining the metabolism and consumption during the entire incubation period. Then, they were submitted to the normalization proposed by Weber et al. (2007). Afterwards, hierarchical clustering heatmaps were generated using "R" software (The R Project for Statistical Computing, 2014) through both the Ward's Method linking method and Euclidean distance between sampling points, as well as for the consumption of the carbon sources.

RESULTS AND DISCUSSION

Physical and chemical variables

The results of the physical and chemical parameters were compared to the local conditions and class 2 freshwater quality standards established by the National Council of the Environment (CONAMA) at a resolution of 357/2005 (Brasil, 2005). Figures 2 and 3 show the spatial variation of TP, SRP, Cl^-, NH_4^+, NO_2^-, NO_3^-, COD, pH, ORP, electric conductivity, turbidity, DO, TDS and T. Regarding the phosphorus content, TP exceeded the limit established by CONAMA for lotic environments (0.1 mg L^{-1}) at concentrations up to 12 times higher than the limit within a range of 0.27 to 1.25 mg L^{-1} (Figure 2). There was an increase in the concentration of TP at the sampling points (P6, P7 and P8) near farming areas. The

location of these sampling points means that the water quality is affected by agricultural activities, due to the proximity of P6 to sugarcane farming, and also to urban activities upstream. Domestic and industrial effluents, fertilizers and leaching from animal farms are the main anthropogenic sources of phosphorus (Libânio, 2010). According Jian et al. (2016), phosphorus (P) in the soil can be lost to water through two main transportation pathways, overland in surface runoff or eroded soils and vertical leaching with drainage. In most fields, P loss is predominantly through surface runoff and erosion.
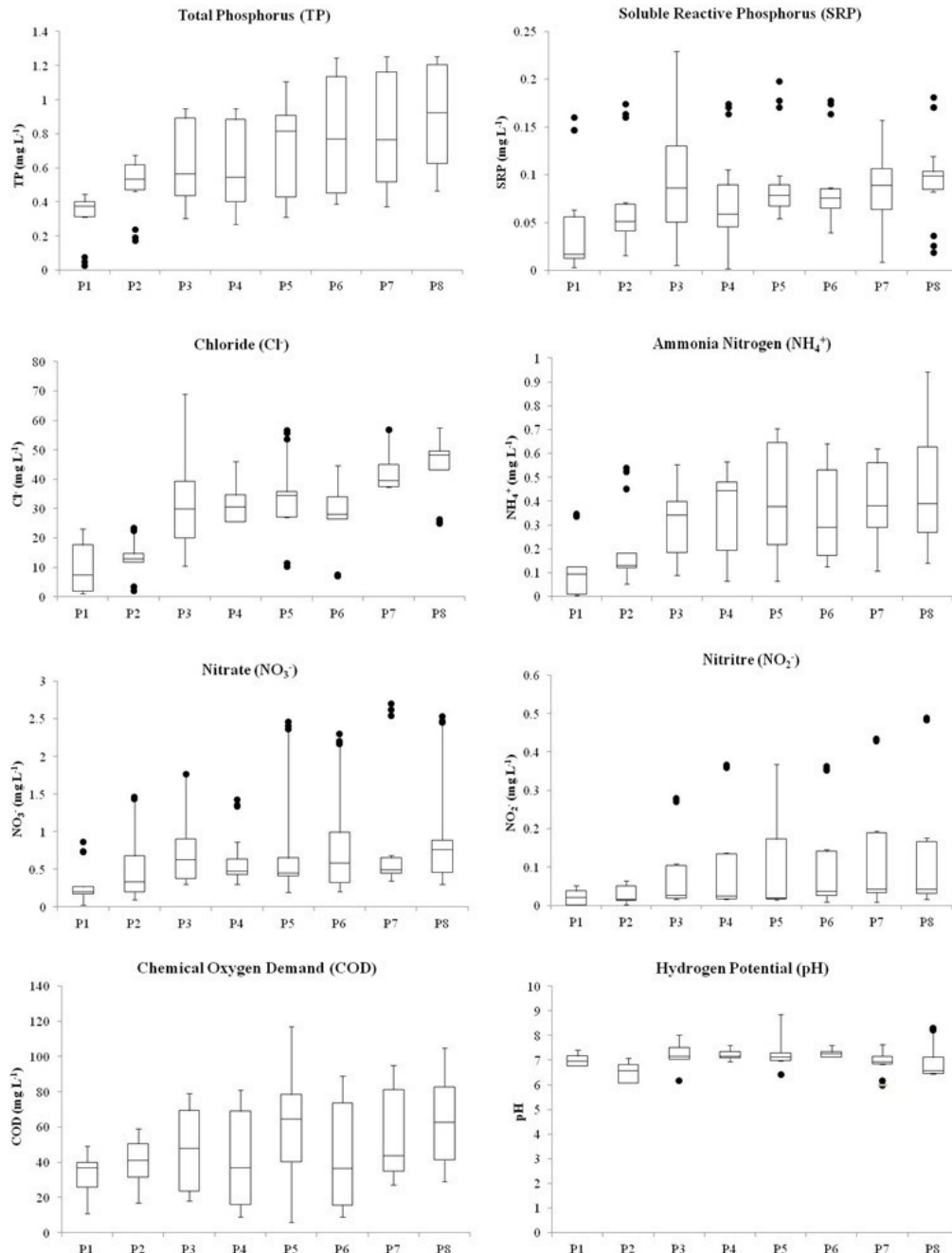
Figure 2: Spatial variation of total phosphorus (TP), soluble reactive phosphorus (SRP), Cl⁻, NH₄⁺, NO₂⁻, NO₃⁻, chemical oxygen demand (COD) and pH.
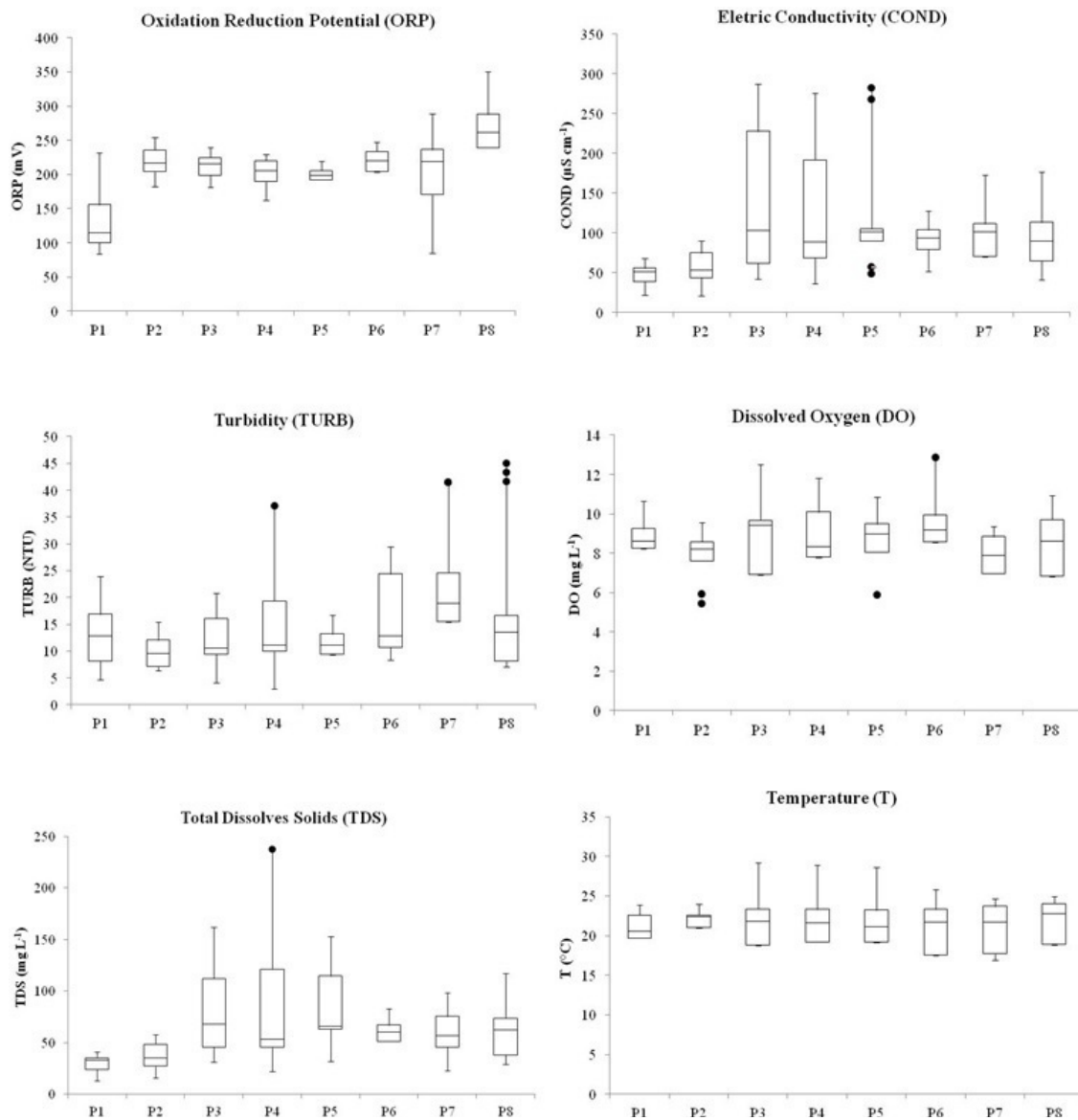
Figure 3: Spatial variation of oxidation-reduction or redox potential (ORP), electric conductivity, turbidity, dissolved oxygen (DO), total dissolved solids (TDS) and temperature (T).

A study conducted in the Cerradinho and Jaboticabal streams between 1999 and 2000 evaluated the effect of the implantation of sewage interceptors as a remediation technique in the urban area of Jaboticabal. In this study the TP concentrations were observed in the range of 0 to 0.243 mg L^{-1} and were attributed to the discharge of effluents into the sample sites because they were located in areas where phosphate was used to prevent scale formation, inhibit corrosion and improve the efficiency of washing and cleaning (Borges; Galbiati; Ferraudo, 2003).

It should be emphasized that, in the present study, the TP concentrations found at the sampling sites exceeded the concentrations reported by Borges, Galbiati and Ferraudo (2003), indicating that the studied streams continue to be influenced by discharged effluents.

Figure 2 shows that the SRP concentrations increased from P3, and although the CONAMA resolution of 357/2005 did not establish values for this variable, the limnological point of view, which is the main storage form of phosphorus assimilated by micro-organisms and aquatic plants, made their

quantification indispensable (Dias; Sipauba-Tavares, 2012). Surplus phosphate in water can propitiate the uncontrolled growth of algae and aquatic plants, which restricts its use for fishing, recreation, industry and drinking consumption due to eutrophication (Sharpley; Wang, 2014). Whereas SRP is the form that is more easily assimilated by algae and macrophytes, which incorporate SRP into their biomass, in unpolluted waters, the ability of phytoplankton and aquatic plants to incorporate SRP results in SRP concentrations less than 0.02 mg L^{-1} near the surface of the body of water (Libânio, 2010). However, in this study, the median concentration of SRP exceeded 0.02 mg L^{-1}, except at P1, which was located at the source of the Jaboticabal stream in a permanently protected preservation area.

For the Cl^- concentrations, P7 and P8 reached maximum values of 56.45 and 57.46 mg L^{-1}, respectively (Figure 2). Nevertheless, these values did not exceed the limit established by CONAMA (250 mg L^{-1}). Low chloride concentrations are essential for normal cell function. However, high concentrations contaminate freshwater streams and lakes causing damage to aquatic life (Sulaiman et al., 2014). The major anthropogenic sources of Cl^- are domestic sewage and the use of agricultural inputs, such as KCl, which is used as a potassium source. Thus, the highest chloride concentrations were observed at points P3, P4 and P5, which was due to those locations receiving urban influences, and P6, P7 and P8, which were subjected to urban and rural influences.

Regarding the nitrogen, NH_4^+ reached concentrations of 0.94 mg L^{-1} at P8 (Figure 2) but the ammonia nitrogen concentration never exceeded the maximum limit set by CONAMA (3.7 mg L^{-1} at pH $\leq$ 7.5). The main nitrogen anthropogenic sources, are the domestic sewage, where almost all of the nitrogen is in the organic form (40%) and as ammonia (60%), as well as industrial sewage, farm animal sewage and fertilizers (as nitrate) applied to arable land, result in nitrogen being leached by the rain for the watercourses (Libânio, 2010). We observed NO_2^- concentrations below 0.1 mg L^{-1} at P1, which receives an urban influence because it is located at the source of the Jaboticabal stream, which is on the border between an urban area and an area where sugarcane is planted, but this source is protected because it is a permanent preservation area. The concentrations at P2 were below 0.1 mg L^{-1} too. The maximum concentration of NO_2^- was observed at P8 (0.49 mg L^{-1}) and there were high concentrations at P7 (0.43 mg L^{-1}) and at P5 (0.37 mg L^{-1}). The high levels of NO_3^- were observed at P2 (1.38 mg L^{-1}) and at P3 (1.69 mg L^{-1}), which were located just after the junction of the two streams in a region devoid of a permanent preservation area and prone to

flooding. Given the location, these points receive an urban influence and a large volume of rainwater. However, higher concentrations were observed at P7 (2.48 mg L^{-1}) and at P8 (2.62 mg L^{-1}), which are near farming areas (Figure 2). There was an increase in the concentration of NO_3^- at points near agricultural areas. However, the concentrations of NO_3^- and NO_2^- were lower than the maximum values set by legislation (10.0 and 1.0 mg L^{-1}, respectively). Thus, among the forms of nitrogen studied, there was a predominance of nitrate (an oxidized nitrogen form and essential nutrient for most aquatic organisms) at the expense of nitrite and ammonia nitrogen (a reduced form, present in anaerobic conditions). The nitrite occurs at low concentrations under aerobic conditions such as those found in this study, where we observed DO concentrations close to saturation.

With respect to COD (Figure 2), the maximum values were found at P5 (117 mg L^{-1}) and at P8 (105 mg L^{-1}). The median values for COD were 37 (at P1), 41 (at P2), 48 (at P3), 37 (at P4), 64.5 (at P5), 36.5 (at P6), 43.5 (at P7) and 62.5 mg L^{-1} (at P8). Borges, Galbiati and Ferraudo (2003) observed a tendency for COD, TP and total nitrogen to increase from the sources to the confluence of the Jaboticabal and Cerradinho streams mainly in the period in which the sewage collectors were not in operation. They obtained the following annual averages for COD: 7.6 and 25.3 mg L^{-1} at the Cerradinho stream, located 4.7 km from the source (in a rural) area and 10.5 km from the source (in an urban area), respectively. They observed at Jaboticabal stream average for COD of 5.4, 22.9, 90.3 and 62.3 mg L^{-1} in a rural area, at 1.3 km from the source (in a rural/urban area), at 4.5 km from the source (in an urban area) and at the confluence of the two streams, respectively. The COD values obtained in the present study compared with the results observed by Borges, Galbiati and Ferraudo (2003) indicated that, despite the installation of the sewage collection system approximately 15 years ago, it is still possible to illegally discharge wastewater into the studied streams.

The pH of the evaluated points ranged between 6.0 and 8.5, except at P2, which had a pH of 5.12 (Figure 2), thus the pH was below the lower limit set by CONAMA (pH between 6 and 9). The median pH values were between 6.56 and 7.27, indicating a small variation for this parameter. The pH of the continental waters can vary from 6.7 to 8.6 depending on factors such as temperature, dissolved oxygen, cation and anion concentrations, among other factors (Pereira-Silva et al., 2011). A small variation in pH, as observed in this study, may be due to the good buffering capacity of the ecosystem. For the oxidation-reduction potential (ORP, Figure 3), the maximum values that were obtained ranged from 232 (at P1) to 350 mV (at P8).

ORP influences the water quality by interfering with the respiration of aquatic organisms (Chang; Ku; Yeh, 2014). Our results for the redox potential showed that the different sites of the studied streams had oxidizing conditions because the ORP values were positive.

The median values of the electric conductivity (Figure 3) ranged from 51 (at P1) to 104 µS cm^{-1} (at P3). Maximum values were observed at P3 (287 µS cm^{-1}) and at P4 (276 µS cm^{-1}). This variable is related to the total concentration of ions and dissolved solids. The highest values were observed at P3 and P4, which can be explained by these points being located after the junction of the streams, so there is a greater movement of particles. CONAMA sets no limits on this variable. However, according to the Environmental Company of São Paulo (CETESB, 2008), values higher than 100 µS cm^{-1} denote an environmental impact.

The highest values for turbidity (Figure 3) were found at points near farms, such as at P7 and P8, reaching vales of 41.52 and 45.10 NTU, respectively. Soil misuse and inappropriate occupation and management may increase the water turbidity due to erosion that carries sediment to the water bodies (Lollo; Vivanco, 2014).

The DO concentration should not be below 5.0 mg L^{-1} according to CONAMA and none of the sampling sites had DO concentrations below 5.0 mg L^{-1} (Figure 3). DO is an important parameter for the assessment of water quality because many chemical and biological reactions depend directly or indirectly on the DO content, and values below 5 mg L^{-1} may harm aquatic life (Camas-Anzueto et al., 2015). Maximum values of TDS (Figure 3) were observed at P3 (162 mg L^{-1}), P4 (214 mg L^{-1}) and P5 (153 mg L^{-1}). According to Libânio (2010), as TDS increases, the conductivity also increases. The temperature values showed variations but remained in the range of 17 to 30 °C (Figure 3), which is coherent with averages taken within the city during the year, where the average air temperature ranged from 19.1 to 25.6 °C (Rolim, 2014).

Multivariate analysis of chemical and physical variables

For physical and chemical variables, the principal components resulted in a variable loss from 12 to 4 variables. Temperature and pH had no significant effects on the total variance explanation, so they were not used. Table 1 shows the eigenvectors that are the newly acquired variables that correspond to the four principal components, obtained in growing order of maximum variance, with greater variability from the original data retained by the first components (PC1 and PC2).

Table 1: Eigenvectors and the correlation between each principal component and the physical and chemical variables studied.

Variables	PC1	PC2	PC3	PC4
TP	0.887*	-0.318	-0.05	0.059
COD	0.832*	-0.293	-0.015	0.142
NO$_2^-$	0.798*	0.043	-0.267	0.219
ORP	0.527*	-0.242	0.338	-0.209
NO$_3^-$	0.553*	-0.589*	0.223	0.193
COND	0.466	0.764*	0.264	0.155
TDS	0.509	0.764*	0.154	0.235
TURB	0.01	0.113	-0.711*	-0.188
DO	-0.118	-0.349	0.661*	-0.213
SRP	0.029	0.233	0.637*	-0.455
Cl-	0.576*	0.307	0.016	-0.588*
NH$_4^+$	0.336	-0.081	-0.523	-0.613*

*Discriminatory values; PC = principal components; total phosphorus (TP); chemical oxygen demand (COD); oxidation-reduction potential (ORP); electric conductivity (COND); total dissolved solids (TDS); turbidity (TURB); dissolved oxygen (DO); soluble reactive phosphorus (SRP).

The first principal component explains most of the total variability data; the second accounts for the largest remaining data variability and it is not correlated with the first. The third component explains the greater variability in the total remaining data not correlated with the first and second components and so on, until the number of principal components obtained in the analysis was reached (Guedes et al., 2012).

The eigenvectors are considered to be a measure of the importance of each variable, and the positive or negative signs indicate that the relationships are directly or inversely proportional, respectively. Coefficients with values above 0.5 were assigned to attributes, whether positive or negative. It is noteworthy that all variables have correlations higher than 0.5 or lower than -0.5 for any of the four main components, and TP and COD showed correlations above 0.8, which is important to represent water quality. Figure 4 displays the plane formed by two principal components (PC1 and PC2), whereby it is noted that the sum of the retained variability of these components explains 47.54% of the original variability, while PC1 and PC2 were retained individually at 30.41% and 17.13%, respectively.

A biplot (Figure 4), which is the projection on a two-dimensional plane, aims to identify the behavior of variables, observe associations between the sample points

and identify variables that are strongly related to each sampling date and point. Samples toward the direction of the variables exhibit higher values, while in the opposite direction the values are smaller. Samples on the ends of the plane have specific characteristics that are different from the other samples and samples that are closer to the center are more similar.

Thus, D4P3 and D4P5 are related to higher COND and TDS. D5 and D6 are different from the others because they are away from each other within the plane. In this way, the variables more closely related to D6, except at P1 and P2, were COND, TDS, Cl⁻ and NO_2^-. The variables closely related to D5, as well as at P1 and P2, were TP, COD, ORP and NO_3^-. The highest concentrations of NO_2^- were found for D6 and the highest values for TP, COD and NO_3^- were for D5. The lowest concentrations were found for TP on D3, which was in the opposite direction of this variable. Therefore, it is noteworthy that the D5 and D6 samplings were the most distant from the other samplings because they had the highest values for the variables studied. This difference may have occurred because the D5 (in October, rainfall of 46.8 mm) and D6 (in December, rainfall of 150.3 mm) samplings were carried out in months of high rainfall and after severe drought. Thus, the rain may have washed the surface of the urban and rural areas, dragging compounds and particles to the streams through leaching or erosion processes.

P1 and P2 are in opposite directions from the studied variables, presenting the lowest values. However, these points are less impacted. Upstream of P1 there is an industry and sugarcane farm, but P1 is located at the source of the Jaboticabal stream, which has the protection of being a permanent preservation area. Upstream of P2 there is a sugarcane farm, but there is a large drainage network too, so P2 receives a high volume of water, so it has high water flow and suffers dilution. In this sense, based on principal component analysis, we can state that there was a separation of sampling dates, and P1 and P2 had less influence. This is shown in Figure 4, where P1 and P2 are located opposite of the direction of the arrows for the variables.

This disparity among points can also be observed on D3P1, which was at the end, far from the D3P8 sample, making them distinct and showing a drastic rise in the concentration values in the direction of P8. This change of location in the biplot projection shows that from P1 (located at the source of the Jaboticabal stream) until the last sampled point (P8) there was an increase in the values of the variables. These results suggest that the last point was influenced by the points upstream and also by the activities carried out in its surroundings, from which it receives urban and rural influences. Thus, we can also assert that anthropogenic activities can affect water quality by increasing the concentration of some variables.
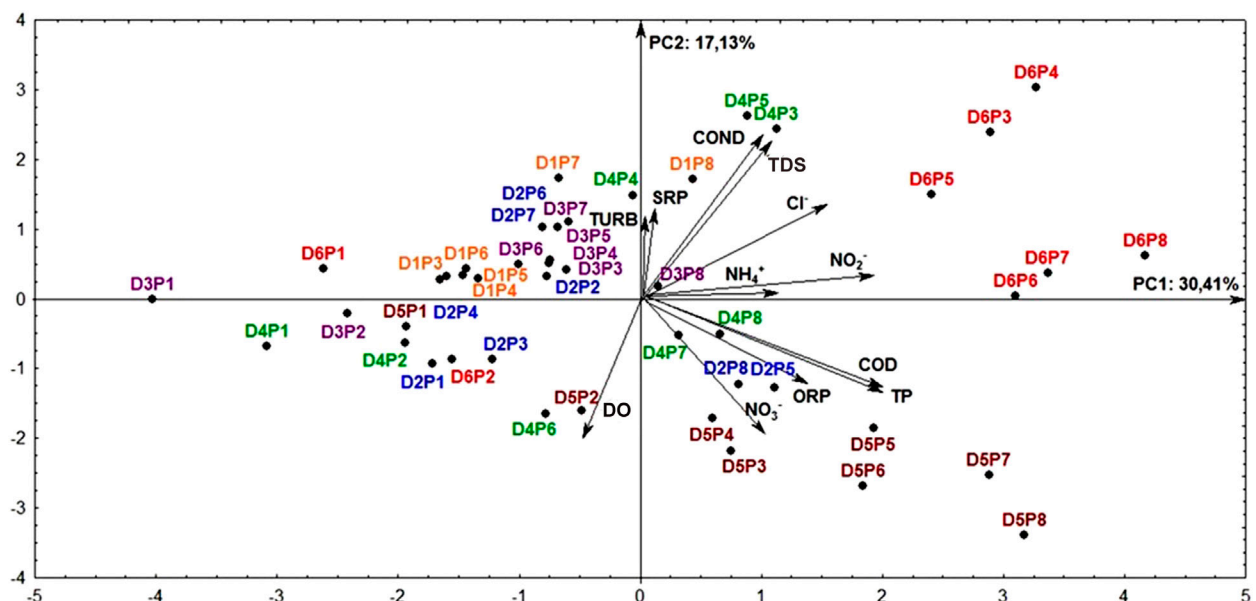
Figure 4: Spatial projection of the first two principal components with a biplot related to the physical and chemical variables of the sampled dates and sites.

Microbial metabolic profile

EcoPlate Biolog™ analysis was first described by Garland and Mills (1991). This methodology compares the use of different carbon sources and is generally used in studies on bacterial communities in various environments such as soil, sludge, fresh water, sediments, biofilms and several other environments (Oliveira et al., 2009; Salomo; Munch; Roske, 2009; Medihala et al., 2012; Gryta; Frac; Oszust, 2014; Patel et al, 2014; Flores-Rentería et al., 2016). By knowing the potential of this method to compare microbial communities in various environments, we opted to use it to measure the ability of bacteria to metabolize different sources of carbon as a comparison tool for water samples.

The calculated values of S, AWCD, H and E are useful to describe the activity and diversity of microbial populations and the results are presented in Table 2. In light of these values, we can infer that the microbial population appears not to be altered significantly in the streams when analyzed under the metabolic aspect. Most of the samples can metabolize 28 to 31 carbon sources (S) and the AWCD ranged from 1.12 to 1.62, which shows good behavior on the EcoPlate. The D3P4, D4P3 and D6P3 points had different behaviors, in these samples the values of S and AWCD were approximately 50% smaller than for the other points. However, when we analyzed the diversity index, we noted similarities among all of the points. This index ranged from 3.23 to 3.42 and E changed only in the points

Table 2: Substrate richness (S), Average Well Color Development (AWCD), Shannon diversity index (H) and Shannon Evenness index (E) calculated using the EcoPlate data at the sampling times (D1 to D6) and at the sampling points (P1 to P8) that were studied.

Collecting	Attributes	P1	P2	P3	P4	P5	P6	P7	P8
D1	S	*	*	30	30	30	30	30	30
	AWCD	*	*	1.5	1.5	1.59	1.49	1.62	1.48
	H	*	*	3.37	3.37	3.35	3.37	3.37	3.34
	E	*	*	0.99	0.99	0.98	0.99	0.99	0.98
D2	S	31	30	28	29	31	30	29	28
	AWCD	1.48	1.54	1.33	1.48	1.45	1.4	1.4	1.24
	H	3.42	3.37	3.34	3.32	3.41	3.34	3.38	3.35
	E	0.99	0.99	1.0	0.99	0.99	0.98	1.0	1.01
D3	S	30	28	30	15	29	29	29	29
	AWCD	1.49	1.12	1.48	0.65	1.29	1.15	1.36	1.32
	H	3.33	3.23	3.33	3.26	3.28	3.27	3.31	3.28
	E	0.98	0.97	0.98	1.2	0.97	0.98	0.98	0.97
D4	S	29	28	13	30	31	31	21	30
	AWCD	1.26	1.16	0.93	1.37	1.34	1.16	1.0	1.41
	H	3.33	3.30	3.40	3.37	3.40	3.41	3.33	3.37
	E	0.99	0.99	1.33	0.99	0.99	0.99	1.1	0.99
D5	S	28	30	31	31	30	30	30	29
	AWCD	1.22	1.37	1.33	1.38	1.18	1.32	1.57	1.38
	H	3.35	3.30	3.39	3.37	3.39	3.39	3.38	3.35
	E	1.01	0.97	0.99	0.98	1.0	1.0	0.99	1.0
D6	S	30	29	16	29	29	30	31	31
	AWCD	1.59	1.37	1.01	1.21	1.24	1.55	1.56	1.51
	H	3.35	3.25	3.35	3.36	3.35	3.36	3.36	3.38
	E	0.99	0.97	1.21	1.0	0.99	0.99	0.98	0.98

*Have not been conducted.

where S was low. These results suggested a great and conserved diversity at the study sites.

Recent studies in a water reservoir in China (Yang; Huang; Zhang, 2015) showed the effect of thermal stratification on bacterial communities. The authors explored the functional diversity and bacterial community composition using the Biolog method and 16S rRNA-based 454 pyrosequencing. The results showed a variation (0.3 to 0.6) in the color development index (AWCD) and a small variation in the Shannon diversity index (approximately 3) for the studied stratification. Molecular studies of bacterial diversity were consistent with the carbon consumption data, confirming the interference of thermal stratification in the bacterial community in the water.

The functional diversity, understood in our study to be the utilization of carbon sources in the Biolog EcoPlates, showed no differences among samples. In general, the investigated samples were characterized by the different utilization pattern of the five guilds. The five guilds of the carbon substrates proposed by Weber and Legge (2009) were used: 1) carbohydrates, 2) carboxylic and acetic acids, 3) amino acids, 4) polymers, and 5) amines and amides. To compare the water population in the samples, the corrected absorbance values of the substrates were summarized and expressed as a percentage of the total absorbance value of the plate for each guild. All of the water samples showed the same results for the use of different carbon sources. This relationship was similar for all of the data collection points. Furthermore, the insignificant differences among the AWCDs (Table 2) manifested the equivalent potential of microbes to use a set of naturally relevant carbon sources in all of the studied samples.

Heterotrophic bacteria more intensively utilized carboxylic and acetic acids and carbohydrates, which are intermediates for organic matter degradation (Li and Yu, 2010). The high or low catabolic capacity that was observed in a sample may refer to a high number of heterotrophic bacteria, and change in the community composition. The physical and chemical parameters studied in the samples were not so different, and there were nutrients in adequate concentrations in the environment. Therefore, it was not difficult to suggest that the bacterial population of the studied streams was not undergoing changes due to anthropogenic activities. On the other hand, we noted a slight increase in the metabolic capacity of polymers (data not shown). This increase in activity appeared at points 3 to 8, referring to the urban (3-5) and agricultural (6-8) areas where the Jaboticabal stream flows.

These data can be important considering the results presented by Freixa and Romani (2014). Based on the chemical analysis results in our work, we cannot say that

we studied a highly polluted stream. However, we noticed a suggestive rise in some of the chemical components along the watercourse during the analyzed period. This may suggest the interference of human activities on water quality in urban and rural environments even with basic sanitation care being applied in the area. These authors showed that the EcoPlate was useful to detect changes in the microbial functional fingerprint in fresh sediment along a river pollution gradient. The microbial community from the upstream site was able to metabolize more labile substrates than the downstream sediment community. The functional fingerprint analysis clearly distinguished the sediment microbial communities from the most polluted sites downstream, which showed a potential capacity to use more complex carbon substrates such as polymers.

The Biolog EcoPlates were proven to effectively differentiate between the microbial communities based on the carbon metabolism, and this methodology can be used as an important ecological indicator of changes in the ecosystem. Thus, in our work we decided to compare the metabolic profile of the microbial populations of each sample based on the consumption of several carbon sources. This comparative study was conducted using a cluster analysis and the construction of a heatmap (Figure 5). The score in this analysis represented the difference between the consumption of each sample for the same substrate. The biggest consumption was shown by the red color and the lowest consumption was represented by the yellow color. The black color represented a similar use of the substrate. Through the heatmap shown in Figure 5, we observed the formation of two hierarchical groups for all of the sampled times and points. One of these groups comprised the sampled points (A) and the other was from the consumption of the substrates (B).

For the first group (A), we noted that the samples were grouped into three clusters (a, b, c) and they were not correlated among the point or date collections. The other group (B) showed four clusters (1, 2, 3 and 4). Cluster 1 was formed by the least consumed substrates group. In cluster 2, we noted the highest amount of consumed carbon sources followed by cluster 4. Cluster 3 comprises a number of substrates that were most differentiated from the water samples that were studied. This group was composed of three carboxylic and acetic acids (4-hydroxy Benzoic acid, D-Malic acid, and D-Glucosaminic acid), one polymer (Tween 40), one amino acid (L-Arginine), and the Putrecine; however, the metabolic profile was similar for all of the points and collection times. The default in the metabolic profiles showed similar functionality (based on the consumption of different sources of carbon) among the sampled points along the streams.
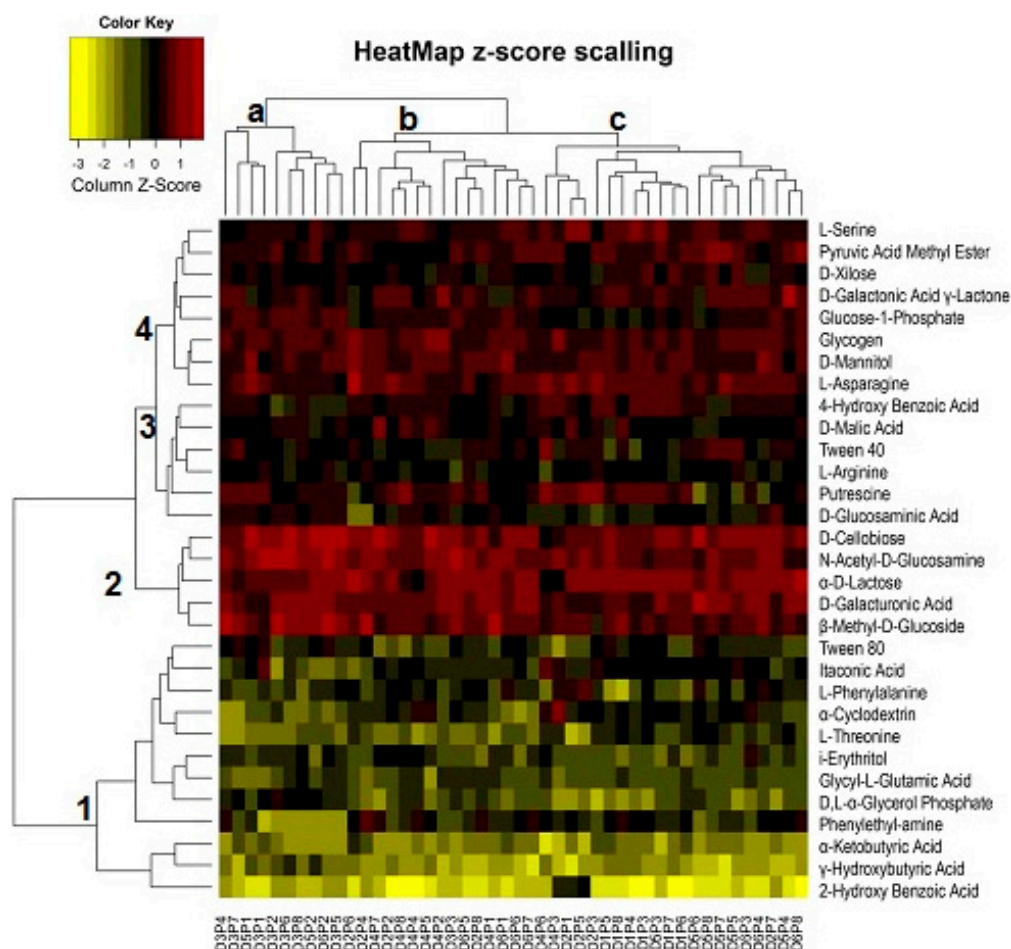
Figure 5: Heatmap for the water samples using 31 carbon sources by the Biolog EcoPlate.

CONCLUSIONS

Our results showed elevated values of TP and COD in the water bodies with higher values at sites (P6, P7 and P8) near agricultural areas compared to urban areas. The last three sampling sites were downstream of the urban area where, despite the existence of a sewage disposal system, the results of the chemical analyses indicated the possibility of the clandestine discharge of wastewater in the studied streams and the influence of soil agricultural. For the metabolic analysis of the microbial population in the different samples from our study, we observed that there was no relationship for the EcoPlate methodology results regarding farming or urban activities. The hierarchical clustering method enabled the observation of differences among the sampling dates regardless of the sample point or collection time. The diversity index was similar at the different sampling points depending on the type of substrate that was consumed. Our results did not allow us to state that the urban and agricultural areas were or were not interfering significantly with the functionality of the microbial population of the water sources that were studied, but there was strong evidence for this interference. To address these issues, further studies need to be performed at these locations, such as the use of molecular techniques for identification and quantification of the constituent organisms at the different study sites as well as the microbial population in the stream sediments.

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