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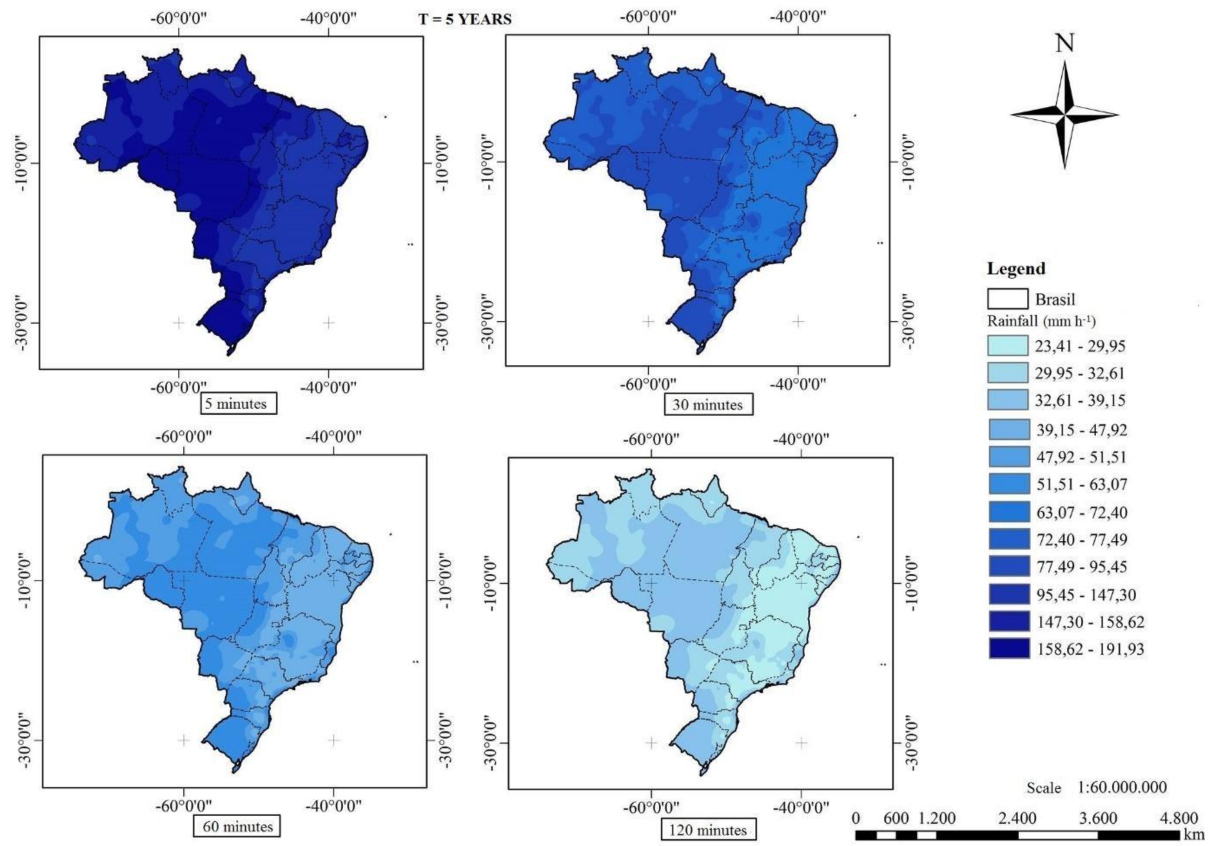

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Land use and water quality in watersheds in the State of São Paulo, based on GIS and SWAT data

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ABSTRACT

Land use influences the quality and availability of water resources, but Brazil has made little progress in integrated watershed management. This study therefore applied geoprocessing for land-use classification and evaluated the impact on the hydrological balance in order to contribute to the integrated management of water resources. Using GIS tools, two drainage areas from the water catchment points of two municipalities, Santa Cruz das Palmeiras and Piedade, were delimited; land-use mapping was carried out using the supervised classification method of satellite images, and the SWAT model was applied for hydrological simulation. The methods used were appropriate. The surface runoff was related to the absence of vegetation and the predominance of exposed soil. The relationship between land use/land cover and the hydrological balance was evidenced, especially the impact of agricultural activities and the lack of natural vegetation in the surface runoff.

Keywords: land use/land cover, SWAT, watersheds management.

Uso do solo e qualidade da água em bacias hidrográficas do Estado de São Paulo, utilizando GIS e SWAT

RESUMO

O uso e ocupação do solo influenciam a qualidade e a disponibilidade de água, devendo ser considerados numa gestão integrada dos recursos hídricos e no planejamento das bacias hidrográficas. Ferramentas de geoprocessamento têm sido utilizadas para classificação do uso do solo e combinadas com modelos hidrológicos para avaliação do impacto de diferentes cenários de uso no balanço hidrológico visando contribuir para uma gestão integrada dos recursos hídricos. Por meio do SIG, foram delimitadas duas áreas de drenagem dos pontos de captação de água para abastecimento dos municípios de Santa Cruz das Palmeiras e Piedade; foi realizado o mapeamento do uso do solo pelo método de classificação supervisionada de imagens de satélite e aplicado o modelo SWAT para simulação hidrológica. As ferramentas utilizadas se mostraram adequadas. O escoamento superficial esteve relacionado à ausência de vegetação e predomínio de solo exposto. Foi evidenciada relação entre o uso e ocupação do solo e o balanço hidrológico, em especial o impacto das atividades agrícolas e da falta de vegetação natural no escoamento superficial.

Palavras-chave: bacias hidrográficas, SWAT, uso do solo.

1. INTRODUCTION

In the state of São Paulo, Brazil, water resource management came to be more participatory only in 1991, when the State Policy on Water Resources and Watershed Committees was introduced, initiating a process involving discussions among representatives of various sectors, such as sanitation, environment, and industry. Prior to that time, the energy and industrial sectors had been given priority access to water resources (Eça *et al.*, 2013; Jacobi *et al.*, 2015).

The state of São Paulo has more than 40 million inhabitants, a high (96%) degree of urbanization, and intense agro-industrial production, anchored especially in sugarcane, and involving intensive use of chemical products, factors that have exerted significant anthropogenic pressure on local water resources (SEADE, 2017). Aggravating this scenario, between the years of 2014 and 2016, the state experienced a major water crisis, with a drastic reduction in the public water supply, especially in the metropolitan area of São Paulo. In addition to generating great concern on the part of public agencies and society, that crisis had a considerable socioeconomic impact in the state (Marengo *et al.*, 2015).

According to the São Paulo State Environmental Protection Agency (CETESB, 2017), the state collected 87% of the sewage generated in 2016 and treated only 62% of that sewage, all of the remaining (untreated) sewage being released directly into bodies of water, many of which are used as reservoirs for the public water supply. The indicators showed a decrease in water quality during the rainy season of 2016, with elevated trihalomethane formation potential, as well as high levels of iron, aluminum, and manganese, together with leaching from soil during heavy precipitation events, a process that is intensified in the absence of riparian forest.

Although several studies have addressed the importance of water management in the state of São Paulo, there is still a lack of tools to facilitate integrated water management, reconciling the marked regional diversity with ecosystem protection and the interdependence between the often conflicting uses, such as public water supply, industry, agriculture, as well as the producer and receiver channels of the sewage generated in urban areas (Porto and Kelman, 2000). Therefore, the use of geographic information systems (GIS), involving geoprocessing, remote sensing, and the development of hydrological models, has been considered an important strategy, allowing not only the identification of vulnerabilities in the watersheds, but also the simulation of different scenarios of land use, application of pesticides, and even climatic events, providing support for environmental monitoring and zoning plans aimed at the preservation of water resources (Ward *et al.*, 2000; Lari *et al.*, 2014; Taylor *et al.*, 2016).

The influence of land use on surface water quality has been widely studied in northern hemisphere countries (Lalancette *et al.*, 2014; Zhou *et al.*, 2016; Salmoral *et al.*, 2017), although not in countries in tropical or subtropical regions, such as Brazil. Through searches of the Scopus database, we found that, among studies using the Soil and Water Assessment Tool (SWAT) in the 2014-2018 period, only 34 had been conducted in Brazil, whereas 523 had been conducted in the United States. The majority of studies using the SWAT model for environmental analyses in Brazil have focused on watersheds in the southern or southeastern parts of the Atlantic Forest biome and have presented results indicating that the hydrological dynamics are linked to changes in land use/land cover (LULC); that type of analysis is often prioritized due to the scarcity of observed watershed monitoring data (Bressiani *et al.*, 2015).

Changes in LULC and management not only affect the vegetation cover but can also degrade the quality of the soil and of water resources (Smith *et al.*, 2016). The quality and quantity of surface waters are directly related to economic activities in the watershed and the level of preservation. Watersheds that have riparian forests along their riverbanks and preserved areas of natural vegetation have better water quality than do those in which there is intensive agricultural activity and degraded areas (Connolly *et al.*, 2015). Therefore, this study evaluated the use of image processing for LULC classification in two watersheds for public water supply in the state of São Paulo, in which there is intensive agricultural activity of various patterns, as well as to evaluate the impact of land use on the water balance in these watersheds, through the use of the SWAT model, with the ultimate goal of contributing to the integrated management of the quality of the water resources destined for public supply.

2. MATERIALS AND METHODS

2.1. Description of the watersheds

The two watersheds selected, both of which are used for public water supply, are located in the municipalities of Santa Cruz das Palmeiras and Piedade, respectively, in the state of São Paulo (Figure 1). In the Ribeirão da Prata River watershed (RPRWS), in the municipality of Santa Cruz das Palmeiras, sugarcane monoculture predominates. In the Pirapora River watershed (PRWS), in the municipality of Piedade, there are a variety of fruit and vegetable crops on small- and medium-sized properties. Those watersheds were chosen as study areas for the continuation of a research project funded by the São Paulo Research Foundation and the Brazilian Unified Health Care System Research Program,[1] in which pesticides in the water supplies of several municipalities in the state of São Paulo were monitored during 2014. The results showed elevated levels of pesticide residues in the catchment water sources within the RPRWS and PRWS.

Santa Cruz das Palmeiras has a population of 29,932 inhabitants, is within Water Resources Management Zone 9 (WRMZ - Mogi-Guaçú), and presents a landscape of flat hills with elevations of 20-50 m, red Oxisol being the predominant soil type. According to the Köppen climate classification system, the climate of the region is type Aw (tropical savanna), with dry winters and an average annual rainfall of 1500 mm. The main economic activity in Santa Cruz das Palmeiras is the production of sugarcane. Piedade has a population of 52,123 inhabitants, is mostly within Water Resources Management Zone 10 (WRMZ - Sorocaba/Middle Tiete), and presents a type of landscape known in Brazil as *mares de morros* (“seas of hills”) with elevations between 80 m and 200 m, red-yellow Ultisol being the predominant soil type. According to the Köppen climate classification system, the climate of the region is type Cwa (humid subtropical), with dry winters and an average annual rainfall of 1300 mm. The main economic driver in Piedade is the service industry, followed by agriculture (production of vegetables and legumes).

[1]*Fundação de Amparo à Pesquisa do Estado de São Paulo/Programa de Pesquisa para o Sistema Único de Saúde* (FAPESP/PPSUS, São Paulo Research Foundation/Brazilian Unified Health Care System Research Program; Grant no. 2014/50016-3; project title: Evaluation of pesticide residues and pathogenic protozoa in the public water supply in the state of São Paulo).

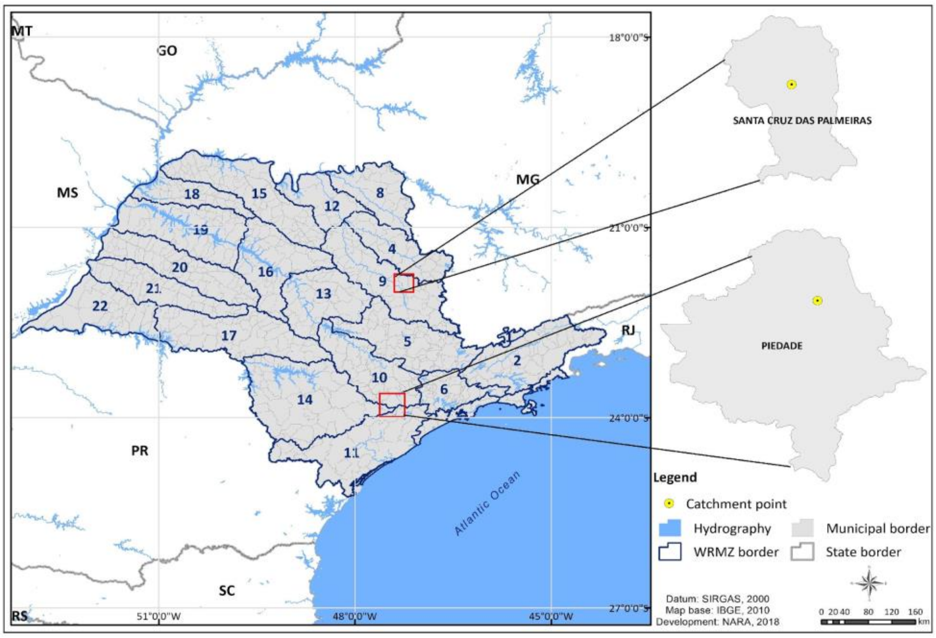

Figure 1. Location of the studied municipalities and the Water Resources Management Zones (WRMZs) within the state of São Paulo.
SIRGAS, *Sistema de Referência Geocêntrico para as Américas* (Geocentric Reference System for the Americas); IBGE, *Instituto Brasileiro de Geografia e Estatística* (Brazilian Institute of Geography and Statistics); CVS, *Centro de Vigilância Sanitária* (Center for Health Surveillance); NARA, *Núcleo de Pesquisas em Avaliação de Riscos Ambientais* (Center for Research in the Evaluation of Environmental Risk).

For the delimitation of the drainage area of the water catchment area of the municipalities, the hydrographic network was extracted from the Hydrology tool of the ArcGIS software, Version 10.1. Digital Elevation Models (DEMs) were obtained from the TOPODATA project of the *Instituto Nacional de Pesquisas Espaciais* (INPE, Brazilian National Institute for Space Research), which were derived from Shuttle Radar Topography Mission data (SRTM) provided by the United States Geological Service, with a resolution of 30 m and 16 bits. We thus generated the files for flow direction, a process that defines the flow of the watercourse, pixel by pixel, in eight directions, sending each flow in one of those directions. The algorithm for calculating the discrete aspect (flow direction) was derived by Jenson *et al.* (1988), the flow direction being made in a 3 × 3 moving window that traverses the DEM and assigns to each cell the direction of one of its eight neighbors. The attribution is made by determining the direction with the steepest slope: the slope is calculated as dZ/dS, where dZ is the difference between the elevations in the cell of the considered direction and the central cell, and dS has a value of 1 in the perpendicular directions and a root of 2 in the diagonal directions (Mendes and Cirilo, 2001). The pixels are given values from 1 to 255, and the values from each direction to the center respect the following distribution:

The next step was the identification of flow accumulation, the pixels with accumulated flow represent areas with greater flow concentration; from those data, together with the previous file, the drainage network was extracted and later ordered according to the Strahler method (Christofoletti, 1980). The area of water contribution to the catchment area for the public water supply was then calculated for both municipalities with the ArcGIS Watershed tool, which uses data related to flow direction, drainage networks, and points of interest. That delimitation corresponds to the basin of water contribution of the catchment area, which was considered in the following stages of this study.

2.2. Classification of land use

For the classification of land use/land cover, RapidEye satellite images, dated October 2014 and made available by the Brazilian Ministry of the Environment, were submitted to the Supervised Maximum Likelihood Classification method. The RapidEye is a system composed of five identical remote sensing satellites in the same sun-synchronous orbit, at an altitude of 630 km. The image collection path is 77 km wide and 1500 km long. The satellite image is composed of five spectral bands (blue, green, red, red edge, and near-infrared), with a 5-m resolution in the Horizontal Datum WGS84 (Antunes *et al.*, 2014).

We classified the images with ArcGIS software, Version 10.1, using the maximum likelihood method, because it presents better accuracy and global accuracy values than do other classification methods (Cattani *et al.*, 2013). The method involves pixel-by-pixel multispectral analysis, considers the weighting of the distances between means of the gray levels of the classes, and uses the training samples to calculate the probability of a pixel belonging to a certain class (IBGE, 2001). Google Earth Pro images were used in order to facilitate the visual interpretation process, and field work was used in order to quantify agricultural land use within the catchment area.

2.3. Water balance analysis

To analyze the impact of land use on the hydrological cycle of the areas, the SWAT model was developed to predict the long-term effects of water management and agricultural practices on the hydrological cycle of watersheds (Arnold *et al.*, 1998). The SWAT employs the modified universal soil loss equation, which uses the amount of runoff to simulate erosion and sediment production (Arnold *et al.*, 2012). The model performs the simulation after dividing the area into subwatersheds, grouping them by the characteristics they have in common, their specificities, and their contributions to the hydrological cycle. The subwatersheds are further divided into hydrological response units (HRUs), which correspond to smaller divisions within the subwatersheds, with unique land use, soil, and management features (Neitsch *et al.*, 2011).

The process of water balance analysis discriminates surface runoff, infiltration, evapotranspiration, lateral flow, drainage, tributary channels, and water redistribution, according to the soil profile. The SWAT simulates the soil hydrological cycle based on the following Equation 1 (Neitsch *et al.*, 2011):

$$SW_1 = SW_0 + \sum^t (R_{day} - Q_{surf} - E_a - W_{seep} - Q_{gw}) \tag{1}$$

Where SW_1 is the final amount of water in the soil (mmH_2O), SW_0 is the initial soil moisture (in mmH_2O) on day *i*, *t* is the time in days, R_{day} is the amount of precipitation (in mmH_2O) on day *i*, Q_{surf} is the surface runoff (in mmH_2O) on day *i*, E_a is the evapotranspiration (in mmH_2O) on day *i*, W_{seep} is the amount of water (in mmH_2O) entering the aeration zone of the soil profile on day *i*, and Q_{gw} is the amount of flow return (in mmH_2O) on day *i*.

This water balance analysis considers the characteristics of the soil, land use/land cover, slope, and climate (Arnold *et al.*, 2012). The daily data for precipitation, temperature, relative humidity, solar radiation, and wind speed in the 2008-2015 period were obtained from the Brazilian National Meteorological Institute for the stations closest to the study areas (INMET, 2016). The monthly precipitation data for the Piedade and Santa Cruz das Palmeiras were obtained from the Center for the Promotion of Agriculture of the São Paulo State University at Campinas (CEPAGRI, 2016). As previously mentioned, the DEMs with a resolution of 30 m and 16 bits were obtained from the INPE. Soil data, at a scale of 1:500,000, were obtained from the Brazilian Agency for Agricultural Research (EMBRAPA, 2006).

3. RESULTS AND DISCUSSION

For the RPRWS, the area of water contribution, calculated from the catchment area, was 11.6 km^2 divided into 23 subwatersheds or HRUs, with slopes of 3-8% and for PRWS the area of contribution of the catchment area was 93.59 km^2 divided the PRWS into 25 subwatersheds or HRUs, with slopes of 20-45%. The results of primary simulation show that surface runoff and flow have behaviors that are similar and are directly related to the pattern of precipitation distribution (Figure 2). The period of high surface runoff corresponded to the rainy months (October through March), for these months the SWAT simulation produced the greater differences between observed values; similar results were obtained by Sousa *et al.* (2018) even when the authors compared two methods of rainfall simulation.

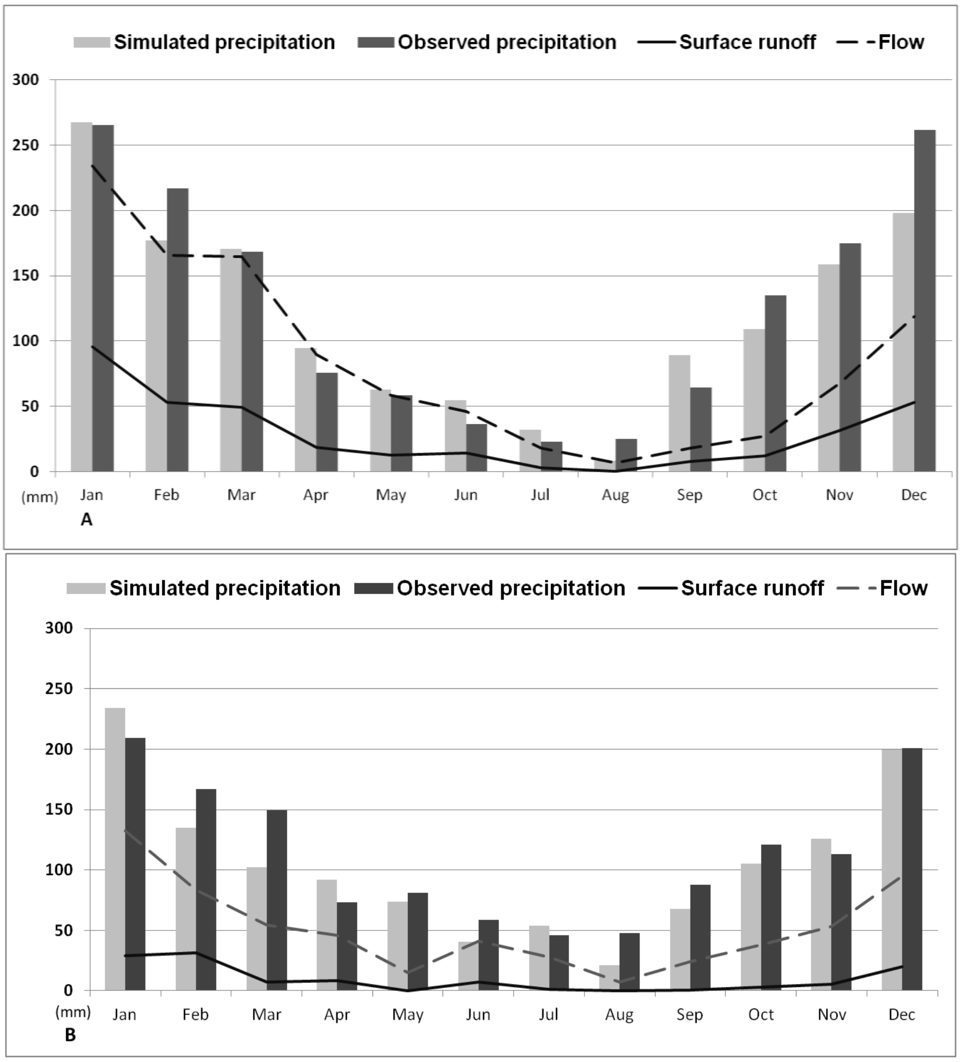

Figure 2. Mean values for observed precipitation, simulated precipitation, surface runoff, and flow, in Santa Cruz das Palmeiras (A) and Piedade (B). 2008-2015.

The hydrological results tend to be better when there is more than one rainfall station in the basin, and the results also depend on the size of the drainage area. Even in simulations calibrated using Nash-Sutcliffe efficiency (NSE), the model tends to underestimate periods of higher rainfall as demonstrated by Pereira *et al.* (2016). Despite the limitations of the data, this simulation was satisfactory, especially when analyzing other hydrological parameters (Table 1).

For the RPRWS, the observed evapotranspiration was estimated at 60% of the evapotranspiration potential and 51% of the total precipitation. Soil percolation and surface runoff corresponded to the greatest amount of water in the terrestrial process, being 47% of the volume precipitated. That can be associated with the permeability of Oxisol (the predominant soil type in the area), as well as with the type of vegetation cover. The curve number was 77.9, a value considered high and that can describe a situation of reduced vegetation cover or intensive agriculture (Tucci, 1993).

Table 1. Water balance estimated with the SWAT model.

Parameter	RPRWS	PRWS
Precipitation (mm)	1424.5	1261.9
Potential evapotranspiration (mm)	1217.2	1253.3
Observed evapotranspiration (mm)	734.0	621.3
Surface runoff (mm)	353.9	113.4
Subsurface flow (mm)	21.5	469.93
Percolation (mm)	313.9	52.5
Return flow (mm)	272.9	32.3
Other* (mm)	40.04	27.7
Curve number	77.9	64.4

RPRWS, Ribeirão da Prata River watershed (in the town of Santa Cruz das Palmeiras); PRWS, Pirapora River watershed (in the town of Piedade).
*Recharge of the deep aquifer and return of the shallow aquifer.

For the PRWS, the simulation estimated the observed evapotranspiration at 49.7% of the evapotranspiration potential and 49.2% of the total precipitation, and the subsurface (lateral) flow accounted for 37.2% of the precipitation, those being the main destinations of the water entering the system. Percolation and surface runoff were considered low and collectively corresponded to only 13% of the total rainfall. The estimated curve number was significantly lower for the PRWS than for the RPRWS, reflecting greater preservation in the former, and consequently the surface runoff, since they are directly related processes.

The results of the hydrological simulation were considered satisfactory, because there is coherence between the values of precipitation, surface runoff, number curve, soil type and LULC. The curve number is related to the LULC and soil type is considered one of the most sensitive parameters in hydrological modeling (Ficklin *et al.*, 2013), as demonstrated by Fukunaga *et al.* (2015), the result of the SWAT simulation in a Brazilian Southeastern basin presented values of simulation higher than those observed for precipitation and surface runoff, which increased the value of the Curve Number. Nevertheless, in the calibration process the authors concluded that this difference is within the expected range, and in accordance with other simulations in tropical watersheds.

According to Wallace *et al.* (2018), the curve number is often used because it is simple, stable and does not require a long historical series of data. The authors evaluated the influence of basin size on the variation of several parameters in the SWAT hydrologic simulation process. The authors concluded that although there was a difference between the observed and simulated values, the modeling was satisfactory and indicated as a watershed planning and management tool.

The classification of LULC (Figure 3) indicated that the conditions of water source preservation differed between the two watersheds. In the RPRWS, more than 80% of the area was occupied by agricultural activities, whereas forest occupied only 6.28%, well below the 20% established by legislation (Brasil, 2006). In contrast, the PRWS presented 53.29% forest and 35.82% agricultural areas. The proportion of land used for agricultural purposes within the two watersheds corresponded to that observed for the respective municipalities.

The poor preservation in the RPRWS was attributable to the agricultural production method adopted by the municipality of Santa Cruz das Palmeiras, which is dependent on sugarcane monoculture, as well as coffee and oranges, which are grown as permanent crops (IBGE, 2015). Likewise, the degree of preservation in the PRWS reflects the allocation of land in the municipality of Piedade, where there are small plots and variety of crops, including maize, onions, sweet potatoes, and beans, persimmons, avocados, and grapes being grown as permanent crops (IBGE, 2015) (Table 2).

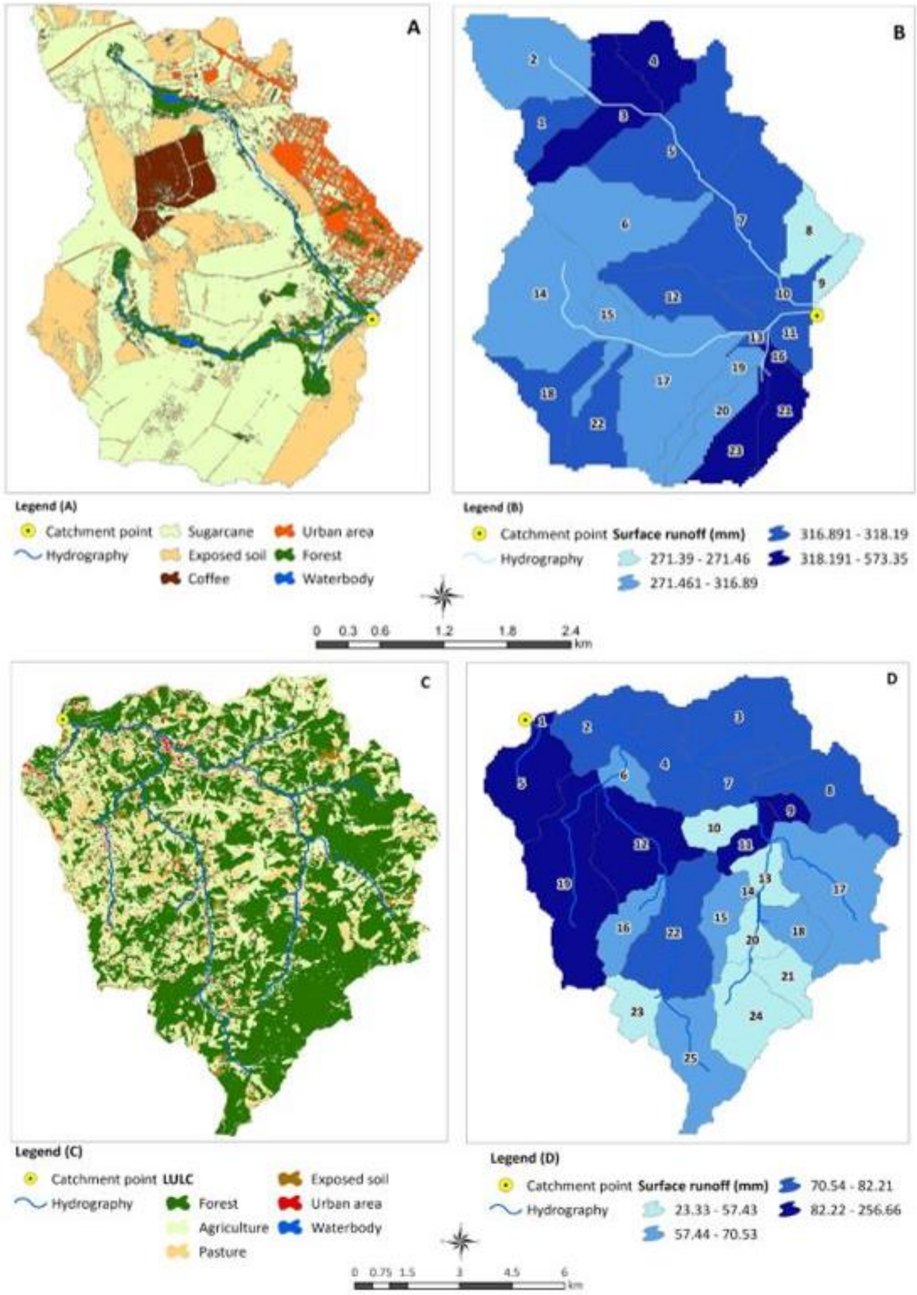

Figure 3. Classification of land use/occupation and simulation of surface runoff in the watersheds.
RPRWS: (A) Classification of land use/occupation; and B) estimated annual surface runoff (mm), by subwatershed. PRWS: (C) Classification of land use/occupation; and (D) estimated annual surface runoff (mm), by subwatershed.

Table 2. Classification of land use/occupation in the two study areas.

RPRWS		PRWS	
Land use	*(%)*	*Land use*	*(%)*
Sugarcane	57.42	Agricultural	32.65
Coffee	4.63	Pasture	8.26
Forest	6.28	Forest	53.29
Water	0.23	Water	0.41
Urban area	6.66	Urban area	2.22
Bare soil	24.78	Bare soil	3.17

RPRWS, Ribeirão da Prata River watershed (in the town of Santa Cruz das Palmeiras); PRWS, Pirapora River watershed (in the town of Piedade).

Applying the Supervised Maximum Likelihood Classification method to the RapidEye satellite images allowed us to observe stretches, throughout the catchment areas, where there was no riparian forest and there was exposure to agricultural activities, aspects that potentiate the transport of sediment and pesticides. Similar results were obtained by Twesigye *et al.* (2011), based on the supervised classification of a historical series of Landsat 5 images of Lake Victoria, in Africa. The authors correlated the reduction in natural vegetation with the expansion of agriculture and the presence of pesticides in the watershed.

For the RPRWS, the surface runoff was highest in the subwatersheds with a preponderance of exposed soil, followed by those with a preponderance of sugarcane (Figure 3, A and B). We also observed a scarcity of natural vegetation, especially in the Permanent Preservation Areas, which correspond to the preservation of the riparian forests. Within the PRWS, the surface runoff values were highest in the subwatersheds with a predominance of agriculture (Figure 3, C and D) and lowest in those with the most well preserved vegetation cover.

The results of our water balance evaluation were satisfactory for analysis of the behavior of the main variables of the hydrological cycle and the relationship with LULC. The rates of percolation and surface runoff were significantly higher in the RPRWS than in the PRWS, correlating with soil permeability, as well as with the degree and type of vegetation cover. Oxisol, the predominant soil type in the RPRWS, is a soil that is deep, well drained, and permeable. The absence of vegetation cover and the cultivation of sugarcane, which has short roots, tend to contribute to the increase of surface runoff (Armas *et al.*, 2007).

The PRWS presented results consistent with greater preservation of natural vegetation and therefore greater water storage capacity of the soil. Although Ultisol, a shallow soil type, predominates in the PRWS, the preserved vegetation cover favors the greater subsurface flow. Because the PRWS is in a region with a “sea of hills” landscape, the loss of natural vegetation in the area would increase the risk of soil instability and silting of the water sources. In both areas, the surface runoff rates were higher in the subwatersheds in which there was a predominance of exposed soil and agricultural activity. A similar result was obtained by Oliveira *et al*. (2018a) which identified that deforestation and agricultural use increase peak flows at the same time as percolation decreases; these alterations can result in degradation of source water quality.

Armas *et al.* (2007) found that the presence of pesticides in surface waters in the Piracicaba River watershed was related to aspects of land use, mainly to the predominance of sugarcane cultivation. In the Corumbataí River and its tributaries, the authors found high levels of atrazine, ametryn and glyphosate. In addition to contaminant loading, different land uses can also influence the amount of soil lost and the water balance within the watershed, as demonstrated by Silva *et al.* (2011). Evaluating the degree of contamination of water sources in the Jacaré

River watershed, within Water Resources Management Zone 13, Souza *et al.* (2013) concluded that the level of ammonia was high in the subwatersheds that did not contain Permanent Preservation Areas or that presented only grass cover. The authors found that preservation of riparian forests along the river also reduced the levels of dissolved oxygen, nitrates, and fine sediment in the water sources.

A similar result was reported by Mello *et al.* (2017), who used the SWAT to analyze the influence of land use on water quality in the Sarapuí River, near the city of Sorocaba, also in the state of São Paulo. The authors concluded that there were high levels of sediment, decreasing water quality, in the subwatershed areas in which there was agricultural activity. In addition to the spatial variation, those authors identified a temporal variation in the concentrations of sediments and substances, which were found to be higher during the rainy months.

The use of SWAT to predict impacts on the water management has been recommended as decision support in different countries, including studies on climate and land-use change, cross-boundary water transfers, nitrogen loads and others (Abbaspour *et al.*, 2015). In addition, it is possible to prioritize areas of the basin to monitor and control pollutants, and thus to prevent or improve water quality (Conolly *et al.*, 2015). The lack of continuous monitoring data on flow and sediment in watersheds has been a limiting factor for the validation of the results obtained and for their use in the formal processes of water resource management in the watersheds. Nevertheless, the SWAT has been considered an important tool for the prediction of maximum annual concentrations of pollutants even in unmonitored basins and without modeling calibration (Winchell *et al.*, 2018).

At the beginning of the GIS application, the mapping LULC contributed to differentiate and quantify the main activities in a watershed, in the last few years this analysis has become more sophisticated, it is possible to relate the LULC with the hydrological dynamics, emission of pollutants, to identify patterns of alteration in the spatial ordering. From this information, it is also possible to predict costs to the management of natural resources, using it as a decision-support system (Shao *et al.*, 2017).

It is understood that integrated management of watersheds should consider, in an interconnected way, the physical processes of water and the hydrological cycle, as well as their relationships with other natural strata, such as soil, relief, flora, and fauna, together with the interests of the multiple uses of water sources, and participatory management relationships at different administrative levels (Machado, 2003). However, more than 20 years after the introduction of the National Water Resources Policy and the passage of São Paulo State Water Protection Law No. 9866/97, the use of water for economic pursuits continues to be prioritized, resulting in the degradation of various water sources, especially by agro-industrial sector. The state of São Paulo has several water sources that are at high risk, requiring conservation and restoration measures (IDS and LABGEO, 2017; Oliveira *et al.*, 2018b) to ensure the supply and quality of water for human consumption.

The determination of areas with greater surface runoff can support the actions to prevent contamination of water in the basin. Increased surface runoff caused by agricultural occupation of the watershed may lead to water contamination, requiring additional and costly treatment to ensure safe water. With integrated management, managers can prioritize these areas for control and reduction of the use of pesticides, collection and treatment of domestic effluents, incentives for the preservation of forested areas on agricultural property, among others. The knowledge of hydrology may be used to improve environmental studies and the management of natural resources.

This work corroborates the importance of applying geotechnologies as decision support for water-resource management, not only for analyses of water availability, but mainly to emphasize the relevance of its application to the management of water quality and the risks to which the source of supply is submitted.

4. CONCLUSION

The present study identified alterations in the hydrological cycle according to land use/land cover. We also showed that agricultural activities and a lack of natural vegetation increase surface runoff, which is a concern for water quality. The remote-sensing tools employed in our study represent an efficient, fast and low-cost means of classifying land use/occupation in watersheds. Using those tools in combination with ecosystem modeling tools, such as the SWAT, allow land use patterns to be correlated with the quality of the water supply and areas of greater concern in terms of the impact of water sources to be identified. This favors the collection of information and the development of plans to facilitate the integrated management of watersheds by the different actors involved, the ultimate goal being, above all, to maintain water quality, to protect the sources of the public water supply.

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Chemical attributes of soil irrigated with treated sewage effluent and cultivated with bell pepper

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ABSTRACT

The use of treated sewage effluents (TSE) for irrigation purposes is a viable alternative for wastewater reuse and nutrient supply to the soil, which represent the two main environmental benefits. This work therefore evaluated the effect of the application of TSE on the quantity of nutrients added to the soil and changes in the chemical attributes of a Red-Yellow Latosol in the layer from 0.0 to 0.2 m cultivated with the bell pepper F1 Canary hybrid and surface drip irrigated. The experiment was carried out under field conditions from September 2015 to January 2016 in a randomized block statistical design with four replications, including the following treatments: river water, TSE, river water with base fertilization, TSE with base fertilization, TSE with cover fertilization, and TSE with cover and base fertilization. Irrigation of the bell pepper cultivation with TSE increased the level of potassium but did not affect the levels of phosphorus, organic matter and pH of the soil at the end of the growing cycle. The following amounts of nutrients were added to the soil by irrigation with TSE: 10 kg ha^{-1} of total nitrogen, 10 kg ha^{-1} of total potassium and 0.5 kg ha^{-1} of total phosphate. In general, TSE induced greater productivity of the peppers than those irrigated with river water, even when including mineral fertilization.

Keywords: soil nutrients, wastewater reuse, water quality.

Atributos químicos do solo irrigado com efluente de esgoto tratado e cultivado com pimentão

RESUMO

O uso de efluentes de esgoto tratado (EET) para fins de irrigação se destaca pelo reaproveitamento da água e pelo aporte de nutrientes ao solo, representando os dois principais benefícios ambientais. Assim, o objetivo deste trabalho foi avaliar o efeito da aplicação de EET sobre a quantidade de nutriente adicionado ao solo e alteração nos atributos químicos de um Latossolo Vermelho Amarelo na camada de 0,0 a 0,2 m, sob cultivo de pimentão, híbrido Canário F1, irrigado por gotejamento superficial. O experimento foi desenvolvido em condições de campo no período de setembro de 2015 a janeiro de 2016. O delineamento estatístico foi em blocos casualizados, com quatro repetições, com os seguintes tratamentos:

água de córrego, EET, água de córrego com adubação de base, EET com adubação de base, EET com adubação de cobertura e EET com adubação de cobertura e de base, com quatro repetições. A irrigação com EET no cultivo de pimentão aumentou os teores de potássio, mas não afetou os teores de fósforo, matéria orgânica e o pH do solo ao final do ciclo de cultivo. Foram adicionados ao solo, por meio de irrigação com EET, 10 kg ha^{-1} de nitrogênio total, 10 kg ha^{-1} de potássio total e 0,5 kg ha^{-1} de fosfato total. De maneira geral, a aplicação de EET promoveu maior produtividade de pimentão do que o uso de água de córrego, mesmo sob complementação de adubação mineral.

Palavras-chave: nutrientes no solo, qualidade da água, reúso de água.

1. INTRODUCTION

With the growing awareness that water is a limited natural resource and has significant economic value, sustainable and rational use techniques are being increasingly implemented, including the use of treated sewage effluents (TSE), which is important in many regions, as in the Federal District, Brazil. Oliveira *et al.* (2016) pointed out that irrigation with TSE is a promising practice in areas facing water scarcity and pollution of water resources by the release of untreated sewage. At the same time, the scarcity of freshwater in semi-arid regions generates social and environmental impacts, which is likely to be intensified in the coming years due to high rates of population growth and increased water demand by the agricultural sector (Bedbabis *et al.*, 2014).

According to Sapkota (2019), climate change and population growth are contributing to a growing global freshwater crisis that is exacerbating agricultural water scarcity and compromising food security and public health. In light of these challenges, the increased reliance on nontraditional irrigation water sources, such as reclaimed or recycled water, is emerging as a potentially viable strategy to address water and food insecurity worldwide.

Thus, according to Rahav *et al.* (2017), there is a clear expansion of the use of treated effluents (TSEs) as an alternative resource in regions with fresh water shortage. In Israel, for example, most orchards are irrigated with TSE, and although the benefits to irrigation are apparent, there is evidence of its cumulative negative effects on soil, plants and productivity. However, the study of the movement of salts and metals in the soil is complex and is subject to multifactorial effects as well as to the soil physical-chemical properties. In view of this, additional studies are necessary to understand salt and nutrient concentrations influence on relationships with plants (Papaioannou *et al.*, 2018). Although agricultural irrigation is an important destination for the TSE, its management must be environmentally sustainable (Musazura *et al.*, 2019).

Effluents generally contain several types of salts that originate from houses, restaurants, etc., which, when ionized, are converted into cations such as Na^+, K^+, Ca^{2+} and anions such as Cl^-, HCO_3^-, CO_3^{2-} and SO_4^{2-}. These ions behave differently and are required by plants, animals, and humans in varying amounts. Therefore, it is necessary to determine the composition of effluents for cations and/or anions before their application to the soil.

According to Oteng-Peprah *et al.* (2018), the generation rates of domestic effluents are mostly influenced by lifestyle, types of installations used and climatic conditions. The contaminants found in greywater, for example, are largely associated with the type of detergent used and influenced by other household practices. At the same time, the treatment system interferes greatly with the final composition of the water.

On the other hand, Sandri and Rosa (2017) emphasized that the practice of reusing treated sewage effluent allows water resources with better composition to be used for more noble purposes, providing environmental, social and economic benefits, as well as reducing costs of agricultural production due to its great potential to provide nutrients to the plants, helping to promote sustainable agriculture and rural development. According to Monteiro *et al.* (2014), Lavrnié *et al.* (2017), Soothar *et al.* (2018) and Papaioannou *et al.* (2018), wastewater has been widely used as fertigation due to its contribution of nutrients that benefit the development of crops, its potential to improve the soil chemical properties, and because it effectively contributes to environmental protection, in addition to being a viable alternative for controlling water demand in regions with low availability of water resources. In addition, it increases crop yields and farmer profit.

Nonetheless, problems may be observed, which according to Vasudevan *et al.* (2010) are influenced not only by the total amount of salts present in the water but also by the types of salts, and the potential severity of the problems may vary depending on soil type, climate and crop. For irrigation purposes, TSE is highlighted for wastewater reuse, nutrient supply and the fact that it can be applied in agriculture, an activity that represents about 70% of total water consumption around the world (Almeida, 2010). According to Leuthe *et al.* (2019), secondary treated wastewater, a commonly used water resource in agriculture in (semi-)arid areas, often contains salts, sodium, and organic matter which may affect soil structure and hydraulic properties.

Reuse of treated domestic effluent has become a very common technique in agricultural irrigation and industrial application in many countries, such as the United States, Saudi Arabia, Egypt, Israel, Syria, Spain, Mexico, Chile and others. According to Kramer (2016), Israel stands out globally by reusing 85% of domestic sewage for irrigation after treatment, while Spain, which occupies second place in wastewater reuse, reuses only 20%.

Although wastewater reuse is not a new practice and has well-proven benefits by scientific research, economic, social and environmental factors are often obstacles to wider adoption of this technology. Furthermore, the low adoption of wastewater reuse is hampered by the limitation of its integration into conventional urban treatment systems, as well as by the absence of standardization, as in the case of Brazil. This is fundamental to avoid endangering public health due to waterborne diseases and to prevent environmental damage due to its polluting potential when improperly utilized. Therefore, technical references such as that described by Ayers and Westcot (1991) have been implemented, which characterizes water used in irrigation according to the restriction potential as follows: none, mild, moderate and severe.

Numerous studies have been carried out to verify updates to crop yields and various attributes of soil irrigated with TSE, such as the electrical conductivity (EC), sodium level (Na), pH, organic matter and others (Duarte *et al.*, 2008; Sandri *et al.*, 2009; Oliveira *et al.*, 2016; Urbano *et al.*, 2015; Silva *et al.*, 2016; Malafaia *et al.*, 2016; Sandri and Rosa, 2017; Soothar *et al.*, 2018). Thus, studies indicate that one must be careful when using TSE in plant cultivation, especially with plants sensitive to saline soils. Despite this, improvement in the physical and chemical properties of the soil has been reported after the application of TSE in agriculture (Silva *et al.*, 2014) and swine wastewater (Medeiros *et al.*, 2011; Cabral *et al.*, 2014), where it is affirmed that these are water sources that favor improved soil fertility.

Despite the great potential of TSE for agricultural use, if used improperly it can be harmful to the irrigation system, soil, plant, water resources and users. For example, Silva *et al.* (2016) found that when using treated sewage in irrigation there was a greater accumulation of micronutrients, potassium and sodium in the soil in relation to water of the public supply system, increasing the risks of sodification. Tunc and Sahin (2015) reported that soil chemical, physical and hydraulic properties can be significantly affected by irrigation with wastewater. Thus, when using TSE and groundwater for cauliflower and purple cabbage cultivation during two years in a semi-arid region with a cold climate, they observed that TSE increased the stability of aggregates, density, porosity, organic carbon and electrical conductivity of the soil, and reduced the percentage of exchangeable sodium (< 2.25%).

Therefore, it is essential to monitor and indicate appropriate management techniques based on several aspects, including composition of the effluent and effects on the physical and chemical attributes of the soil, and when appropriate the operation of the irrigation system.

Therefore, the objective of this work was to evaluate the amount of nutrients added to Red-Yellow Latosol and the effect of drip irrigation with TSE on the chemical attributes of soil cultivated with bell pepper (*Capsicum annuum* L.), F1 Canary hybrid.

2. MATERIAL AND METHODS

The study was developed at the Água Limpa Farm (FAL), belonging to the University of Brasilia (UnB), located at 15°46′46″ S and 7°55′46″ W, with an elevation of 1100 m. According to the Köppen's climate classification, the region presents an Aw climate, characterized by two well-defined seasons, one hot and rainy from October to April, and another cold and dry from May to September (Álvares *et al.*, 2013).

The average temperature from 1993 to 2013 was 22.1°C with average annual precipitation of 1,469 mm. The soil was classified as a typical dystrophic Red-Yellow Latosol (Embrapa, 2013), Oxisol (Typic Haplustox) (Soil Survey Staff, 1998) or Gibbsic Ferralsol (FAO, 2015). It presents a sandy texture, containing 14.2% silt, 15.0% clay and 70.8% sand, and has a soil bulk density of 1 g cm^{-3}.

The TSE comes from the FAL/UnB refectory and toilets, characterized as essentially domestic, receiving primary treatment in three septic tanks in series, which are constructed of polyvinyl chloride (PVC) boxes with individual volume of 5,100 L. For secondary treatment, four constructed wetlands (CW) were used, arranged in parallel with dimensions of 6.5 x 2.5 x 0.5 m, respectively, in length, width and height, filled with gravel #2, which had porosity of 50%, resulting in an individual useful volume of 3.82 m^3. One CW was cultivated with taboa (*Typha spp*), one with Brazilian Papyrus (*Cyperus Giganteus*), another with white ginger lily (*Hedychium coronarium Koehne*), and the last was unplanted. The TSE was conducted to a vessel with a useful volume of 4,750 L and pumped to another of 2,000 L, from which it was then pumped into the irrigation system for the pepper plants.

Bell pepper (*Capsicum annuum* L.), F1 Canary hybrid, was cultivated from September 4th, 2015 to January 10th, 2016. The experimental design consisted of randomized blocks, with four replications for the following treatments: soil without fertilization and irrigated with river water-control (Ic); soil without fertilization and irrigated with TSE (Ie); soil with cover fertilization and irrigated with TSE (AcIe); soil with base fertilization and irrigated with river water (AbIc); soil with base fertilization and irrigated with TSE (AbIe); soil with base and cover fertilization and irrigated with TSE (AbAcIe).

Each experimental plot was planted with four rows of pepper with 5 plants each, where the plants of the two central rows were considered useful, excluding those along the border, resulting in 6 useful plants and 24 plants per treatment, with a density of 23.800 plants ha^{-1}. Spacing between plants in the row was 0.50 m and 0.80 m between planting rows, with each plot measuring 3.0 m x 2.8 m (8.4 m^2).

Irrigation was carried out in a two-day cycle using drip tapes, arranged on the soil surface at 0.05 m from the plants. In-line turbulent flow emitters were used, spaced 0.30 m from the lateral line, with a 12 mm internal diameter and a flow rate of 1.3 L h^{-1} at a pressure of 147 kPa.

Reference evapotranspiration (ETo) was obtained by the Penman-Monteith Equation (Allen *et al.*, 1998), estimated from climatic data provided by a meteorological station located 300 m from the experiment. The crop evapotranspiration (Kc) was adjusted considering the wet area percentage of 68.5% and Kc values of 0.4 during the establishment of seedlings/vegetative, 0.7 during flowering/fruiting, 1.05 during full production and 0.85 during decline in production (Marouelli and Silva, 2012).

During the pepper cycle adverse conditions were observed, both at low temperatures (during 46 days of the cycle the minimum temperatures were lower than 15°C), as well as at temperatures above 35°C during 7 days of the cycle. During the flowering and full production periods, an average relative humidity of the air below the appropriate range (50 to 70%) was recorded over the course of 62 days. Thus, these factors interfered with pepper cultivation. Although there was no etiolation, some flowers fell and there was a failure in the establishment of seedlings, reducing the development of plants and prolonging the crop cycle. The total precipitation throughout the pepper cycle was 641.55 mm, but was more frequent as of November 2015.

TSE and river water (Ic) were analyzed at the beginning, middle and end of the experiment for the attributes presented in Table 1, according to the APHA *et al.* (2005) methodologies.

Before beginning the experiment, soil samples were collected at six points in the experimental area at depths of 0 to 0.2 m. From the soil chemical analysis results, the need for liming was determined with the objective of increasing base saturation to 70%. For this, 510 kg ha^{-1} of dolomitic limestone with 100% PRNT containing 47% CaO and 7% MgO were applied 60 days before transplanting (DAT).

Table 1. Physical-chemical attributes of the treated sewage effluent (Ie) and river water (Ic) used in irrigation of the yellow pepper crop.

Attributes	Unit	Ic	Ie
Hydrogen Potential (pH)	Units	8.30	7.05
Electrical Conductivity (EC)	dS m^{-1}	0.003	0.530
Temperature	°C	26.10	26.70
Suspended Solids (SS)	mg L^{-1}	0.00	68.50
Total Dissolved Solids (TDS)	mg L^{-1}	1.92	339.2
Turbidity	NTU	4.88	29.005
Nitrite ($N\text{-}NO_2^-$)	mg L^{-1}	0.002	0.034
Ammonia ($N\text{-}NH_3$)	mg L^{-1}	0.035	3.695
Nitrate (NO_3^-)	mg L^{-1}	0.33	1.79
Total Nitrogen (N total)	mg L^{-1}	7.00	37.80
Potassium (K)	mg L^{-1}	0.00	38.87
Iron (Fe)	mg L^{-1}	0.26	0.26
Total Phosphate (P)	mg L^{-1}	1.10	1.80
Manganese (Mn)	mg L^{-1}	0.05	0.05
Boron (B)	mg L^{-1}	0.31	0.49
Sulfur (S)	mg L^{-1}	1.00	1.00
Sodium (Na)	mg L^{-1}	0.06	2.97
Calcium (Ca)	mg L^{-1}	0.68	0.98
Magnesium (Mg)	mg L^{-1}	0.11	0.12
SAR[1]	$(mmol_c\ L^{-1})^{0.5}$	0.10	4.01

[1]SAR, sodium adsorption ratio, obtained by SAR = $[Na^+]/([Ca^{2+}]+[Mg^{2+}])^{0.5}$.

After liming, new soil sampling was performed at depths of 0.0 to 0.2 m and the results of the chemical analysis (Table 2) were used to calculate the base and cover fertilization needs. In base fertilization, 80 kg of P_2O_5 and 50 kg of K_2O ha^{-1} were applied, in the form of simple superphosphate and potassium chloride, respectively. These fertilizers were applied throughout the area and incorporated into the soil.

Table 2. Values of soil chemical attributes in the 0 to 0.2 m layer at the beginning of the experiment with pepper cultivation.

pH	OM	K^+	Ca^{2}+	Mg^{2+}	Al^{3+}	H^+Al	CECe	CECt	P	m	V
H_2O	g kg^{-1}			cmol$_c$ dm^{-3}					mg dm^{-3}	%	%
5.9	11.25	0.12	5.18	3.25	0.15	7.7	8.68	16.23	0.88	0.95	52.3

pH: pH in water; OM: Organic matter; K: Extractable Potassium; Ca: Extractable Calcium; Mg: Extractable Magnesium; Al^{3+}: Exchangeable Aluminum; H+Al: Potential Acidity; CECe: Effective cation exchange capacity; CECt: total cation exchange capacity; m: Aluminum saturation; V: Base Saturation and P: Extractable Phosphorus.

As cover fertilizer, 165 kg ha^{-1} of N and 180 kg ha^{-1} of K_2O in the form of urea and potassium chloride were applied, respectively. These amounts were divided into 7 applications throughout the crop cycle. The first application took place at planting, the second 20 days after transplanting and the others at every 15 days. In addition, 300 kg ha^{-1} of P_2O_5, in the form of single superphosphate, were applied in a single application 10 days before transplanting. All applications followed the recommendation of Ribeiro *et al.* (1999).

At the end of the experiment, 24 soil samples were collected, one from each plot, in the 0 to 0.2 m layer and located in the center of the wet bulb formed by the dripper. Three sub samples were collected in the plot to make up the sample.

The samples were homogenized, air-dried and passed through a 2 mm mesh sieve to perform soil chemical analyses. The soil chemical properties were determined according to Silva (1999). In brief, the pH was determined in H_2O using a 1:2.5 (w/v) soil: solution ratio suspension; 0.5 mol L^{-1} potassium chloride was used as an extractor in the determination of the calcium (Ca^{2+}), magnesium (Mg^{2+}) and exchangeable aluminum (Al^{3+}) contents. This solution was left for overnight equilibration. Subsequently, Ca^{2+} and Mg^{2+} were determined by atomic absorption spectrometry (Shimadzu AA-6300) and Al^{3+} by titration with a 0.01 mol L^{-1} sodium hydroxide solution. The potassium and phosphorus contents were determined using the Mehlich-1 extractor and analyzed by flame spectrophotometry (K^+). Aluminum saturation was calculated as follows: m, % = $(Ca^{2+} + Mg^{2+} + K^+) / Al^{3+}$.

Data were submitted to analysis of variance (one-way ANOVA) followed by the Duncan test ($P < 0.05$) as a post-hoc to detect statistical significant differences among all treatments. Normality and homoscedasticity of the residues were evaluated by the Lilliefors and Cochran's C tests, respectively. The XLSTAT 2015 software was used for statistical analyses (Addinsoft, 2016).

3. RESULTS AND DISCUSSION

3.1. Attributes of the river water and treated sewage effluent

In irrigation with the treated sewage effluent (Ie), the pH and magnesium were generally lower than in river water (Ic), while electrical conductivity, suspended and total solids, turbidity, nitrite, ammonia, nitrate, sodium, potassium, total nitrogen and total phosphate were higher than in river water (Ic) (Table 1).

Ic showed no restriction of irrigation use in relation to boron, EC, sodium and SAR; however, total nitrogen and magnesium showed a slight or moderate restriction according to

Ayers and Westcot (1991) classification. On the other hand, Almeida (2010) reported that all attributes evaluated in the Ic are within a normal range for irrigation application. The EC value obtained in the effluent of 0.53 dS m^{-1} has no restriction for use in irrigation (Ayers and Westcot, 1991), possibly due to low solids loading in the sewage because it is mainly from the refectory. The content of diluted salts decreased in the effluent, due to the lower availability of organic matter for mineralization. This value is lower than that obtained by Urbano *et al.* (2015) of 0.83 dS m^{-1} and Sandri and Rosa (2017) of 2.01 dS m^{-1}, whereas Oliveira *et al.* (2016) observed EC of 1.55 dS m^{-1}, where in these cases the restriction is slight or moderate.

The level of total Fe was 0.26 mg L^{-1} in both the river and Ie water, in agreement with Metcalf and Eddy (1991) who recommend irrigation values up to 5.0 mg L^{-1}, because higher values begin to present toxicity problems for crops. According to these authors, this value is not toxic to soils with good aeration, although it contributes to make phosphorus and molybdenum unavailable to plants. The level of iron obtained in Ie was lower than that observed by Sandri *et al.* (2009), who verified a maximum value of 1.8 mg L^{-1} in effluent from a university unit, treated with septic tanks followed by constructed wetlands filled with gravel #2 and cultivated with macrophyte *Typha sp* ("taboa").

Likewise, Ie did not present restrictions regarding the levels of B, pH, Mg, NO_3, EC and RAS according to Almeida (2010); but according to Ayers and Westcot (1991), when EC and RAS are analyzed together, they express a slight or moderate restriction. The same occurred for total N and Na, where NO_3, for example, can reach concentrations of up to 5 mg L^{-1}, significantly higher than that observed in Ie which was 0.034 mg L^{-1}. In turn, the average levels of Mg were 0.05 mg L^{-1} for Ic and Ie (Table 1), also lower than those recommended by Metcalf and Eddy (1991) for irrigation water. When the wastewater has a high concentration of nitrogen, this nutrient can be retained in the soil at the time of reuse. This was demonstrated in a study carried out by Sun *et al.* (2018), that when evaluating the NO_3^- removal efficiencies in soil column under laboratory conditions filled with sterilized soil (SS), silty clay (SC), soil with submerged plant (SSP) and soil mixed with biochar (BCS) for constant concentration of NO_3^- of 15 mg L^{-1} and flow rate of 0.6 ± 0.1 m d^{-1}, concluded that both SSP and BCS were able to provide levels of NO_3^- <0.2 mg L^{-1} in the wastewater percolated in the column.

The values of Na and Ca were considered high, which is justified by the fact that the sewages were composed predominantly of food and cleaning product residues produced in the refectory, reaching 2.97 $mmol_c$ L^{-1} and 0.98 $mmol_c$ L^{-1}, respectively, lower than those obtained by Oliveira *et al.* (2016) for domestic sewage. As a consequence, the obtained SAR value was 4.01 $(mmol_c\ L^{-1})^{0.5}$, lower than those obtained by the authors above, who observed a value of 7.8 $(mmol_c\ L^{-1})^{0.5}$, but higher than that of Sandri and Rosa (2017) with 0.87 $(mmol_c\ L^{-1})^{0.5}$. The SAR obtained in this work is considered of slight or moderate restriction for irrigation use. However, because the irrigation time was short and the soil presented a high infiltration velocity (192 mm h^{-1}), no significant accumulation of these attributes in the soil was observed.

3.2. Chemical attributes of the soil

Regarding the chemical attributes present in the soil at the end of the experiment, it is observed that only the K^+ content was affected by the different treatments (P <0.05), where the AbAcIe treatment resulted in a higher value when compared to the treatments Ie and AcIe (Table 3). In the soil layer evaluated from 0 to 0.20 m, the highest values were 0.12 $cmol_c$ dm^3 and 0.09 $cmol_c$ dm^{-3}, respectively, for the treatments AbAcIe and Ie, while Oliveira *et al.* (2016) observed a value of 0.3 to 0.4 $cmol_c$ dm^{-3}. Sandri and Rosa (2017) also observed variation with the use of well water between the micro sprinkler and drip irrigation and between the beginning and the end of the experiment in the layer from 0 to 0.20 m. According to these authors, in the soil layer of 0.2 to 0.4, except for the well water treatment applied by a micro sprinkler, every other treatment using well water and treated effluent presented significant elevations.

This behavior, according to Doblinski *et al.* (2010), is attributed to the mobility of K^+ in the soil profile, which is much greater than that of phosphorus. It is favored by irrigation water, but also by natural precipitation during the experiment. This also reinforces the authors' report that when applying swine wastewater in the bean crop greater mobility in the soil profile is found for potassium, followed by nitrogen and phosphorus.

Considering the irrigation application of 253.1 mm during the crop cycle, 9.57 kg ha^{-1} of total N (composed of approximately 10% of ammonia, 5% of nitrate, 1% of nitrite and 84% of organic N), 9.84 kg ha^{-1} of total potassium (11.85 kg ha^{-1} of K_2O) and 0.46 kg ha^{-1} of total phosphate (1.04 kg ha^{-1} of P_2O_5) were applied. While for Ic, the increase in total N was only 1.77 kg ha^{-1} and 0.28 kg ha^{-1} of total phosphate, with no increase in total potassium.

Table 3. Chemical attributes of the soil evaluated at the end of the pepper experiment for different treatments in the soil layer from 0 to 0.2 m.

Soil Attributes					
Treatments	Al^{3+} ($cmol_c$ dm^{-3})	H + Al ($cmol_c$ dm^{-3})	CEC	V (%)	TOM (g kg^{-1})
AbAcIe	0.018 a	3.3 a	7.3 a	54.8 a	61.4 a
Ie	0.010 a	3.1 a	7.4 a	58.4 a	57.4 a
Ic	0.015 a	2.9 a	7.4 a	60.5 a	58.8 a
AcIe	0.013 a	3.0 a	7.4 a	59.3 a	50.8 a
AbIc	0.015 a	3.0 a	7.1 a	57.4 a	53.9 a
AbIe	0.013 a	3.2 a	7.2 a	53.8 a	57.7 a
Treatments	pH ($CaCl_2$)	P (mg dm^{-3})	K^+ ($cmol_c$ dm^{-3})	Ca^{2+} ($cmol_c$ dm^{-3})	Mg^{2+} ($cmol_c$ dm^{-3})
AbAcIe	5.9 a	6.1 a	0.120 a	3.0 a	0.9 a
Ie	6.0 a	5.4 a	0.09 ab	3.2 a	1.0 a
Ic	6.0 a	5.4 a	0.07 b	3.3 a	1.0 a
AcIe	6.0 a	6.0 a	0.08 b	3.4 a	0.9 a
AbIc	6.0 a	4.4 a	0.09 ab	3.0 a	1.0 a
AbIe	5.9 a	5.7 a	0.09 ab	2.9 a	0.9 a

Soil without fertilization and irrigated with river water (Ic), soil without fertilization and irrigated with TSE (Ie), soil with cover fertilization and irrigated with TSE (AcIe), soil with base fertilization and irrigated with river water (AbIc), soil with base fertilization and irrigated with TSE (AbIe), soil with base and cover fertilization and irrigated with TSE (AbAcIe). Total organic matter (TOM), base saturation (V)

Although the salt concentrations of practically all attributes evaluated in the effluent (Ie) were higher than in the river water (Ic), no toxicity symptoms were verified in the plants and no changes in the chemical properties in the soil that could compromise the development of the plants were found. Contrarily, there was an increase in salts in the soil, although not significant, except for $K^{+,}$ which is beneficial for the plants. Cunha *et al.* (2014) reported that the amounts of nitrogen, phosphorus and potassium in treated sewage effluent represent savings in the acquisition of synthetic nutrient salts, which improves the cost-benefit ratio compared to irrigation with natural water. These authors obtained savings ranging from 65 to 100% of calcium, sulfate, copper and molybdenum.

When comparing the quantities of nutrients in the effluent with the amounts in synthetic formulations to meet crop demand, the values are generally very small, since the quantities demanded by plants are high, especially macronutrients. At the same time, effects of using the effluent on chemical attributes of the soil are very variable among works published in literature (Sandri *et al.*, 2009; Andrade Filho *et al.*, 2013; Oliveira *et al.*, 2016; Silva *et al.*, 2016; Malafaia *et al.*, 2016; Sandri and Rosa, 2017), influenced by several factors, such as irrigation system, soil water distribution, pH and soil organic matter. As demonstrated by Kalavrouziotis *et al.* (2018), both pH and organic matter were significantly related to the level of immobilization and adsorption of heavy metals in the soil. Rahav *et al.* (2017) evaluated the spatial and temporal distribution of water content and chemical properties of the soil in the root zone after application of 700 mm of TSE and natural water by surface drip. These authors verified the existence of preferential flow, which led to the uneven distribution of soil chemical properties, with substantially high concentrations at the driest and lowest points in the wet points.

Rahav *et al.* (2017), after application of TSE with high concentrations of salts and nutrients, verified high salinity, SAR and nutrient concentrations in the root zone of citrus plants. Silva *et al.* (2016), for example, found that irrigation with treated sewage provides a greater accumulation of micronutrients, potassium and sodium in the soil, increasing the risks of sodification in irrigated areas when compared to water from the public supply system, when testing five doses of nitrogenous fertilization (0, 45, 90, 135 and 180 kg ha^{-1}). In turn, Urbano *et al.* (2015) concluded that the reuse of domestic effluents did not cause damage to the physical properties of the soil, but resulted in a tendency for salinization when irrigation was performed in undisturbed soil samples arranged in a constant load permeameter to simulate irrigation equivalent to 5 cycles of lettuce cultivation. Barreto *et al.* (2013), similar to other authors, verified that after the application of wastewater to soil the levels of phosphorus, calcium, potassium and organic matter, mainly in the superficial layer (0 to 10 cm), and sodium, at depth (up to 50 cm), increased significantly compared with the application of water from public supply. In turn, Andrade Filho *et al.* (2013), when applying different doses of the domestic effluent in Cambisol, observed that the attributes pH, MO, P, K^+, Na^+, Mg^{2+}, Al^{3+} and H+Al were significantly altered.

Different from the results obtained in this experiment, in the study performed by Duarte *et al.* (2008) the use of TSE in a soil cultivated with pepper promoted an increase only in the levels of organic matter of the soil when compared with the application of well water. On the other hand, Sandri *et al.* (2009) reported that after two cultivation cycles of Elisa lettuce, TSE irrigation promoted higher levels of potassium, manganese, copper, sulfur and electrical conductivity, but there was no observed difference in CEC, organic matter, magnesium, calcium, total nitrogen, sodium and phosphorus when compared to dam water. Sandri and Rosa (2017), when irrigating banana trees with surface drip irrigation and micro sprinkler using treated sewage effluent (Ie), conventional fertigation and well water, observed that the levels of Na and B declined or remained stable in the soil, but drip application of the effluent raises the levels of phosphorus, sulfur, total acidity and base saturation in the soil layer from 0 to 0.2 m and when using the micro sprinkler in the layer of 0.2 to 0.4 m.

Soothar *et al.* (2018) concluded that the use of untreated sewage had unacceptable levels of EC, pH, K^+, Na^+ and Cl^-, which resulted in excessive concentrations of these ions in both rice plant (*Oryza sativa*) and irrigated soil. In view of the conclusion of these authors, it is verified that the type of sewage treatment can be a differential, thus, the use of SZR can be an alternative in the sense of assisting in the previous removal of part of the fertilizers present in the effluent.

Phosphorus is commonly present at high concentrations in TSE due to its presence in soaps that are easily conducted by water. Therefore, it is common to find significant differences in its concentration when compared to natural water. This did not occur in the present study, possibly because soap was not used to clean the facilities of the refectory. Cabral *et al.* (2014), when applying the doses of 0, 150, 300, 450, 600 and 750 m^3 ha^{-1} of swine wastewater to an area of elephant grass cultivation, concluded that in the first stage there was an increase but in the second stage there was a reduction of P in the soil, and the same occurred for K^+ and Ca^{2+}.

Musazura *et al.* (2019), when using TSE and natural water in banana and taro irrigation, did not find significant differences in N and P uptake by the two crops; however, irrigation with treated effluent increased significantly inorganic N and P, especially at the depth of 0.3 m of the soil. The authors also concluded that there was no difference between the types of irrigation related to phosphorus leaching, but obtained a higher concentration of inorganic N in TSE leachates up to 0.3 m of soil depth.

Other attributes, such as organic matter and nitrogen, also showed differences in this type of experiment; however the differences are dependent on concentrations in the TSE, the application period and the respective climatic and soil conditions. Garcia *et al.* (2012), cultivating fertirrigated forages with TSE confirmed increases in the levels of phosphorus, potassium and base saturation, a fact that in the present study only occurred with potassium.

In general, the average levels of magnesium in Ie were below those considered adequate. However, in Ic they were slightly higher than those in Ie (Table 1), but not enough to generate a statistical difference in the soil between treatments (Table 3), and with no indication of leaching. This was also observed by Medeiros *et al.* (2011) when analyzing the soil profile to a depth of 1.0 m with collections every 0.2 m for different dosages of swine effluent. Although aluminum was not evaluated in the irrigation water, in the soil this element was not affected by the different treatments applied.

Although Ie presented a higher concentration of calcium than Ic (Table 1), the soil that received this effluent showed no change in relation to the beginning of the experiment (Table 3). This may have occurred due to high consumption by the plant which may not have reached its maximum potential in treatments without Ie due to possible deficiencies of other nutrients, causing the plant to not require high calcium absorption. Another possible factor is the fact that the increase in sodium from Ie displaced calcium, making it more susceptible to leaching. Despite the huge increase in TOM levels, there was no statistical difference between the treatments, with levels varying from 9 to 14 g kg^{-1} and an average value of 11.25 g kg^{-1} before beginning the experiment. At the end of the experiment, the TOM varied from 50.9 to 61.4 g kg^{-1} between treatments. The fact that Ie did not cause the accumulation of TOM in the soil may be due to the rapid mineralization of this attribute, promoted by the environment considered favorable for microbial activity in the soil (Table 3).

These results reinforce the conclusions of Duarte *et al.* (2008), who in a study evaluating the application of TSE in the soil observed lower concentrations of TOM in these treatments compared to natural water, justified as a result of the easy decomposition of the organic matter existing in the effluent. Another factor is that the TOM levels in the TSE were not that high when compared to Ic, possibly due to the fact that the sewage is mostly generated by the refectory (low concentration of solids). Finally, removal of organic matter is remarkably efficient in the cultivated wetlands sewage treatment system, and also because the effluent is filtered through a 125 micron disk filter before being applied via drip irrigation.

The absence of significant increases in soil nutrients in this experiment, commonly cited in other works, may be associated with the short application period and the rainy period occurring at the end of the cycle. At the same time, there may have been leaching of salts in the soil profile, since the local soil presents a high rate of basic infiltration (RBI), which on average is 192 mm h^{-1}.

Therefore, additional studies should be carried out with a larger number of crop cycles aiming to extend the period of application, considering the climatic conditions of the site, soil types, sewage treatment system and irrigation system.

3.3. Plant height, fresh mass of fruits and productivity of the bell pepper

There was no difference in the plant height between the treatments, a behavior also verified by Sousa *et al.* (2006) when irrigating the bell pepper with the effluent from an anaerobic decomposition reactor and well water. In addition, in the present study the plant height was lower than that indicated by the company which developed the F1 Canary pepper (120 cm), ranging from 30% (Ie) to 38.3% (AcIe) of the expected height. This can be explained by the absence of competition for luminosity, thus allowing greater horizontal growth than vertical growth.

The application of TSE, exclusively or combined with mineral fertilization, promoted higher pepper productivity than the use of river water, reaching 5.85 t ha^{-1} in Ie (Table 4). Furthermore, in the treatments irrigated with TSE (AbAcIe, Ie, AcIe and AbIe), the yields obtained were higher than those predicted by the company which developed the cultivar. However, when irrigation was performed with river water (Ic and AbIc), the yield was lower than expected.

Table 4. Plant height, fresh mass of fruits and productivity of the bell pepper, F1 Canary hybrid, at 140 DAT and variation, in percentage, in relation to that expected for the cultivar according to the company which developed the hybrid.

Treatment	PH (cm)	PH (% of expected)	FMV (g $fruit^{-1}$)	Prod. (t ha^{-1})	Prod. (% of expected)
AbAcIe	43	35.8	230.1 a	5.67 a	115.7
Ie	36	30.0	195.9 ab	5.85 a	119.3
Ic	42	35.0	160.0 b	4.44 b	90.6
AcIe	46	38.3	187.9 ab	5.62 a	114.7
AbIc	43	35.8	165.7 b	4.16 b	84.9
AbIe	39	32.5	202.7 ab	5.76 a	117.6

Soil with base and cover fertilization and irrigated with TSE (AbAcIe), soil without fertilization and irrigated with TSE (Ie), soil without fertilization and irrigated with river water (Ic), soil with cover fertilization and irrigated with TSE (AcIe), soil with base fertilization and irrigated with river water (AbIc), soil with base fertilization and irrigated with TSE (AbIe). Plant height (PH), Fresh mass of the fruits (FMV) and productivity (Prod.).

The fresh mass of the fruits in AbAcIe (230.1 g $fruit^{-1}$) was higher than in the treatments that used river water (Ic - 160.0 g $fruit^{-1}$ and AbIc - 165.7 g $fruit^{-1}$), and similar to those which used TSE. This shows that the TSE provides better conditions for the growth of the bell peppers, corroborating the results obtained by Sousa *et al.* (2006).

4. CONCLUSIONS

During the pepper crop cycle, 10 kg ha^{-1} of total nitrogen, 10 kg ha^{-1} of total potassium and 0.5 kg ha^{-1} of total phosphate were added to the soil by means of irrigation with TSE. Irrigation performed exclusively with the effluent (Ie) in the pepper crop increased the levels of potassium, but did not affect phosphorus, organic matter and soil pH at the end of the growing cycle. In general, TSE application, exclusive or combined with mineral fertilization, promoted higher pepper productivity than the use of river water, even when it was supplemented with mineral fertilizer. TSE irrigation combined with mineral fertilization (base and cover) also promoted a higher mass of pepper fruits in relation to irrigation with river water. However, in order to identify changes in soil chemical attributes due to the application of domestic sewage effluent, additional studies should be carried out with a larger number of crop cycles aiming to extend the period of application, considering the climatic conditions of the site, soil types, sewage treatment system and irrigation system.

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Multivariate analysis in the evaluation of soil attributes in areas under different uses in the region of Humaitá, AM

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ABSTRACT

The recognition of the influence of management practices on soil physical and chemical conditions is substantial for sustainable agriculture. For this reason, this study was developed for the purpose of evaluating the behavior of soil attributes under different uses in the region of Humaitá, AM, using multivariate statistical methods. The study was developed in 8 rural properties producing bananas, grassland, maize, coffee, cassava, vegetables, agroforestry system and a forest fragment. Samples of soils with preserved structure in the 0.0 - 0.10 and 0.10 - 0.20 m layers were randomly collected in 5 small trenches per area, totaling 32 samples in the management systems, to determine the physical and chemical attributes. The data were then submitted to univariate and multivariate statistical analysis. Exploratory data analysis (principal components and dendrogram) and frequency of environmental covariates was efficient in distinguishing production environments, so multivariate classification based on physical and chemical attributes of the soil can help in the proper planning of land use. The analysis of the principal components indicates that the BD presents direct dependence with the SPR, signaling the use of the soil with grassland the only one in the process of compaction. Soil acidity is the main limiting factor for crop development, requiring the adoption of pH corrective practices with improvements in nutrient supply. The conversion of the forest to grassland maintained the structural characteristics of the soil, while the other uses increased improvements in physical quality and soil fertility.

Keywords: environmental covariates, physical and chemical attributes, soil management.

Análise multivariada na avaliação de atributos do solo em áreas sob diferentes usos na região de Humaitá, AM

RESUMO

O reconhecimento da influência das práticas de manejo nas condições físicas e químicas do solo é substancial para a agricultura sustentável. Por essa razão, o estudo foi desenvolvido com o objetivo de avaliar o comportamento de atributos do solo sob diferentes usos na região de Humaitá, AM, utilizando métodos estatísticos multivariados. O estudo foi desenvolvido em 8 propriedades rurais produtoras de banana, pastagem, milho, café, mandioca, hortaliças, sistema agroflorestal e fragmento florestal. Amostras de solos com estrutura preservada nas camadas de 0,0 - 0,10 e 0,10 - 0,20 m foram coletadas aleatoriamente em 5 pequenas trincheiras por área, totalizando 32 amostras nos sistemas de manejo, para determinar os atributos físicos e químicos. Os dados foram então submetidos à análise estatística univariada e multivariada. Análises exploratórias de dados (componentes principais e dendrogramas) e frequência de covariáveis ambientais foram eficientes em distinguir ambientes de produção, portanto a classificação multivariada baseada em atributos físicos e químicos do solo pode auxiliar no planejamento adequado do uso da terra. A análise dos componentes principais indica que a DS apresenta dependência direta com o RSP, sinalizando o uso do solo com pastagem o único no processo de compactação. A acidez do solo é o principal fator limitante para o desenvolvimento da cultura, exigindo a adoção de práticas corretivas de pH do solo com melhorias na oferta de nutrientes. A conversão da floresta para pastagem manteve a característica estrutural do solo, enquanto os demais usos incrementaram melhorias na qualidade física e fertilidade do solo.

Palavras-chave: atributos físicos e químicos, covariáveis ambientais, manejo do solo.

1. INTRODUCTION

The different systems of land use and management aim to create favorable conditions for crop development and yield (Costa *et al.*, 2013). As soil undergoes interventions in use, changes occur in its physical attributes, such as increased bulk density, decreased total porosity, pore diameter distribution, alteration in aggregation and organic matter content (Oliveira *et al.*, 2013). Thus, inadequate management leads to changes in soil properties, which, if maintained, leads to irreversible soil degradation, making agricultural practices unusable (Vasconcelos *et al.*, 2014; Gomes *et al.*, 2019).

The occupation and replacement of previously forested areas by agricultural areas without due technical criteria is one of the main problems caused by the anthropic action in the Amazon region. Problems that directly affect the preservation of natural resources, the soil attributes being the main physical and chemical indicators of these changes (Oliveira *et al.*, 2015). Studying the influence of different land uses in the southern Amazon, Gomes *et al.* (2017) reported that the use of grassland soil increased bulk density with reduction of total pore space and aggregate stability when compared to soil management with cocoa and coffee. In this same alignment, Aquino *et al.* (2014) found that conversion of forest areas to grassland increased soil resistance to penetration. In all of these studies, soil degradation was related to the decay of organic matter, due to its low specific density ranging from 0.9 to 1.3 g cm^{-3} (Reichert *et al.*, 2007), which gives it absorbing function or dissipating of compaction energies of the soil.

Recognition of soil changes imposed by changes in use is often not an easy task. Thus, soil variables, mainly structure-related, are used with indicators of physical and chemical quality. However, conventional and univariate statistical methods make it difficult to interpret the results when there are many variables involved in the process, hence the need to use multivariate analysis (Silva *et al.*, 2006; Oliveira *et al.*, 2018). Multivariate statistics allows the extraction from a set of original data of only the variables capable of explaining a significant part of the total variance of the data, through linear combinations (Silva *et al.*, 2016). Thus, fewer variables are required to be interpreted, summarized in only two dimensions (Freitas *et al.*, 2015).

In the Amazon region, some studies have focused efforts to evaluate the transformations occurring in the soil after the replacement of forest ecosystems for agricultural use (Aquino *et al.*, 2014; Oliveira *et al.*, 2015; Gomes *et al.*, 2018). From this point of view, knowledge of the damages caused by different management systems is essential to improve the physical quality of the soil, since the conversion of forest to agricultural areas or grassland areas has been causing serious problems due to improper management (Soares *et al.*, 2016). Thus, aiming at the recognition of physical and chemical indicators of soil quality, this study was developed with the purpose of evaluating the behavior of soil attributes under different uses in the region of Humaitá, AM, using multivariate statistical methods.

2. MATERIAL AND METHODS

The sample collection was carried out in two communities in the rural area of Humaitá - AM, where soil samples were collected at five properties in the Realidade community, located at BR - 319, Km 100 of Humaitá - Manaus, and at three properties in Alto in BR - 230. The region presents relief similar to the type "tray", with very small differences and slightly bulging edges. These higher lands constitute the topographic dividers of water between the rivers of the region. The gap between these higher zones and the valleys of the igarapés is of the order of 15 to 29 meters; however, it occurs suddenly (Braun and Ramos, 1959).

Regarding the geology, the studied areas are located under an area formed from undifferentiated alluvial sediments, which are chronologically derived from the Holocene. The region has contact vegetation between field and forest, which is characterized by areas that include various formations, where the predominant vegetation is grassy, low woodland and alternates with small isolated trees and forest galleries along the rivers (Braun and Ramos, 1959).

All areas are located in the same climatic zone, according to the Köppen classification, belonging to group A (Tropical Rainy Weather) and climatic type Am (monsoon rainfall), presenting a short dry season (Brasil, 1978). The rainfall is limited between 2,250 and 2,750 mm, with the rainy season beginning in October and extending until June. The average annual temperatures alternate between 25 and 27ºC and the relative humidity remains between 85 and 90% (Brasil, 1978). The soil of the study areas was classified as Cambissolo Haplico Alitico Plintico - Brazilian Soil Classification (Embrapa, 2013; Campos *et al.*, 2011) equivalent to Inceptisol in the Soil Taxonomy (Soil Survey Staff, 1999).

Eight systems of land use were selected, typical of the Amazon region: a) Banana (*Musa spp*.): area with banana plantation with 4 years, without fertilization and correction, with spacing 3x3 m; b) Coffee (*Coffea canephora*): with 3 years of cultivation, with only soil correction and spacing 3x3,5 m; c) Native forest; d) Vegetables: area used for at least 8 years, without fertilization and correction; e) Cassava (*Manihot esculenta Crantz*) with spacing of 0.80 x 0.60 m, without liming and fertilization; f) Maize: approximately 120 days after planting in a conventional system; g) grassland: area cultivated with Brachiaria (*Brachiaria* brizantha) with approximately 10 years of use in extensive grazing; h) Agroforestry System: the area has been used for about 20 years, with coffee (*Coffea canephora*), cocoa (*Theobroma cacao*), palm trees (*Attalea speciosa*), andiroba (*Carapa guianensis*) among others for commercial and subsistence purposes.

In each system of use, an area of 80 × 80 m, with 16 sample blocks, was demarcated, the soils were collected in 5 small trenches per area, in the layers of 0.00-0.10 and 0.10-0.20 m, in soil-core with preserved structure, totaling 32 samples per management system.

The granulometric analysis of the soil was determined using the pipette method, with 1 mol L^{-1} NaOH solution as chemical dispersant and mechanical stirring in a high rotation apparatus for 15 minutes (Embrapa, 2011). The total porosity (TP) was obtained by the difference between the mass of the saturated soil and the mass of the dry soil in an oven at 105ºC for 24h (Embrapa, 2011). The microporosity of the soil was determined by the tension table method, according to Embrapa methodology (2011). By the difference between total porosity and microporosity, macroporosity was obtained. The bulk density (BD) was calculated by the relation between the dry mass in the greenhouse at 105°C for 24 h of the soil sample of the volumetric cylinder and the volume of the same (Embrapa, 2011). Volumetric moisture was obtained by the difference between the wet soil mass and the dry soil mass in an oven at 105°C for 24 h (Embrapa, 2011).

For the determination of soil penetration resistance (SPR), the same samples were collected for bulk density (BD) and soil porosity, and the same were determined in the laboratory using an electronic penetrometer with a constant velocity of 0.1667 mm s^{-1}, equipped with a 200 N load cell, 4 mm diameter base cone and 30° semiangle, receiver and interface coupled to a microcomputer to record the readings using the equipment's own software. The determinations were performed in a sample with preserved structure, with water tension in the soil near the field capacity (Dalchiavon *et al.*, 2011). For each sample, 290 values were obtained, eliminating the 30 initial and 30 final values.

The determination of the stability of the soil aggregates was carried out by the wet sieving method. The separation and stability of the aggregates were determined according to Kemper and Chepil (1965), which was performed by placing the samples on a set of sieves with 2.0 mesh; 1.0; 0.5; 0.25; 0.125; and 0.063 mm and subjecting them to vertical oscillations for 15 minutes. The geometric mean diameter (GMD) and the weighted mean diameter (WMD) was adopted as stability index.

The pH was determined potentiometrically using a 1:2.5 ratio of soil in KCl. For the determination of exchangeable aluminum (Al^{3+}), 1 mol L^{-1} KCl was used as the extractor and 0.025 mol L^{-1} NaOH was used as titrant in the presence of bromothymol blue as a colorimetric indicator (Embrapa, 2011). The potential acidity (H+Al) was determined volumetrically by titration of NaOH in calcium acetate at pH 7.0 as a reagent, in addition to phenolphthalein as an indicator (Embrapa, 2011). The chemical extractor used for the analysis of phosphorus (P) and potassium (K) is called Mehlich-1 (Embrapa, 2011).

The organic carbon (OC) was determined according to Walkley and Black methodology (1934) and modified by Yoemans and Bremner (1988), with organic matter being estimated based on organic carbon (Embrapa, 2011). The carbon stock (CS) was determined in all areas studied, and was calculated by the expression: $CS = (OC \times BD \times e) / 10$, where: CS = organic carbon stock of the soil (Mg ha^{-1}); OC = total organic carbon content (g kg^{-1}); BD= bulk density (Mg m^{-3}); e = thickness of the layer considered (cm) (Costa *et al.*, 2009a).

A variance analysis was performed, and, when significant, by the f test, the data were analyzed by the Tukey test ($p < 0.05$). Multivariate statistical analysis was also performed, using hierarchical cluster analysis and principal component analysis (PCA) techniques. The analysis of hierarchical groupings was performed by calculating the Euclidean distance in the set of 13 original variables. This analysis allowed grouping the use of the soil (handling) by its similarities graphically represented in a structure called dendrogram of similarity. Then, the original sets of variables were subjected to factor analysis for a pre-selection of the variables with greater discriminatory power of the environments. The selected and non colinearized variables were submitted to PCA analysis, according to the criterion recommended by Hair *et al.* (2005). Thus, it was possible to construct a data dispersion graph (biplot*)* associative of the influence of the management on the alteration of the soil attributes, complementing the clustering analysis. All multivariate statistical analyses were processed in STATISTICA® software version 7.0 (Statsoft, 2004).

3. RESULTS AND DISCUSSION

In both evaluated layers the soil belonged to the same textural class, clay-loam (Table 1), dominating the silt fraction, which did not differ to 5% of significance in the 0.00-0.10m layer. Although there was no significant difference, the silt values increase in the following order: Forest (694 g kg^{-1}) > grassland (692 g kg^{-1}) > Banana (681 g kg^{-1}) > maize (674 g kg^{-1}) > coffee

(634 g kg^{-1}) > vegetable (633 g kg^{-1}) > AS > cassava (597 g kg^{-1}) at 0.0-0.10 m. Similarly, it occurred in the 0.10-0.20 m depth only for forest (674 g kg^{-1}), grassland (651 g kg^{-1}) and cassava (540 g kg^{-1}). These results are similar to other investigations carried out on soils under different uses in the Humaitá, AM region (Oliveira *et al.*, 2013; Mantovanelli *et al.*, 2015; Soares *et al.*, 2016).

It was verified that the bulk density (BD) was sensitive to the land-use practices, differing between cultivation and depth, which altered the others covariate attributes. The highest value of bulk density (BD= 1.50 Mg m^{-3}) was found in grassland soil, which did not differ significantly between the layers. On the other hand, the cultivation of cassava in the layer 0.0 to 0.20 m caused a lower value of BD (1.04 Mg m^{-3}), an increase of 16.13% to 0.10 – 0.20 m depth. The tilling and weeding of the soil prior to planting, as well as the practice of weeding and covering the ridges with the weeding material, justify the lower values of BD.

The management of soil did not significantly affect the soil properties microporosity (MiP) and the geometric mean diameter (GMD) in two layers with average diameter (WMD) and volumetric water content (Uws), differing only in layer 0.0 to 0.10 m. Several studies concerned with the mystification of the effect of management on the reorganization of the porous space of the soil conclude that the MiP does not change much in depth, and its increase reflects the deformations occurred in the MaP during the management (Giarola *et al.*, 2007; Vasconcelos *et al.*, 2014). Therefore, no significant difference was observed for MaP in the grassland, coffee, forest and vegetables areas, for both layers. However, it was the areas with lower values of MaP, showing in common similar values of BD, suggesting deformation of the macropores or clogging of these by the silt fraction, establishing soil compaction (Soares *et al.*, 2016).

The total porosity (TP) ranged from 0.44 to 0.58 cm^3 cm^{-3} and did not differ for grassland system, the forest and AS layer from 0.0 to 0.10 m. However, for all types of soil use, the amplitude of TP values ranging from 0.44-0.58 cm^3 cm^{-3}, is below that recommended as ideal, which is 10%, indicating aeration conditions unsatisfactory for crop development (Baver *et al.*, 1972). However, it is worth noting that this criterion cannot be generalized, since there are plants tolerant to low levels of aeration.

Soil penetration resistance (SPR) presented high values of ≥ 2.00 kPa in forest and grassland soils in the 0.0-0.10 m layers, with decreases of 44.92% and 70%, respectively on the 0.10-0.20 m depth (Table 1). In fact, the highest SPR value found on the soil surface under grassland was due to animal trampling, which produces in the area in contact with hull a force greater than the soil can withstand (Gomes *et al.*, 2017; Debiasi and Franchini; 2012) (Table 1), which is consistent with the high values of BD (>1.50 Mg m-3), lower MaP (0.06 m^3 m^{-3}) and TP (0.44 cm^3 cm^{-3}). For Giarola *et al.* (2007), the reduction of TP in the grassland areas is due to the reduction of the MaP, since the MiP is little influenced by the soil management, as was verified in the present study.

The stability of aggregates expressed by WMD and GMD in the 0.10 – 0.20 m layer presented a significant difference among them, attributed to management specificity, growth habit, density and root thickness (Pedra et al., 2012). The soil under forest presented higher WMD and GMD due to the greater input of vegetal material, which mixed with the soil matrix acts as a natural polymer aggregating or cementing the soil particles. A similar thing occurred with GMD in soils under grassland, motivated by the extensive development of the grass root system, considered the main particle aggregation agent in tropical soils, both by the release of exudates and by interlacing small clods and, consequently, forming larger structures (Salton and Tomazi et al., 2014). This result corroborates those of Gomes et al. (2017) when assessing spatial variability of aggregates and organic carbon in different land uses in southern Amazonia. According to Campos et al. (2013), there is a highly significant correlation between the increase in organic matter content and the increase in aggregate stability; however, Alho et al. (2014) points out that a high WMD aggregate does not always have adequate pore-size distribution in the interior.

Table 1. Size and physical attributes of the soil of banana, grassland, maize, coffee, cassava, forest, Agroforestry system and vegetables in the layers 0.0-0.10 m and 0.10-0.20 m, in the region of Humaitá, AM.

Cultivation	Sand	Silt	Clay	SPR	MaP	MiP	Uws	TP	BD	GMD	WMD
		g kg^{-1}		kPa		m^3 m^{-3}		cm^3 cm^{-3}	Mg m^{-3}	mm	
					0.00–0.10 m						
Banana	97.89 b	681.97 a	138.12 c	1.20 bc	0.15 a	0.45 a	0.45 a	0.58 a	1.19 bcd	2.52 a	3.07 a
Grassland	109.07 b	692.42 a	139.33 c	3.00 a	0.06 c	0.37 a	0.37 a	0.44 c	1.50 a	2.68 a	3.15 a
Maize	139.16 b	674.94 a	166.45 bc	1.37 bc	0.16 a	0.41 a	0.41 a	0.56 ab	1.10 cd	2.65 a	3.16 a
Coffee	122.93 b	634.69 a	245.73 a	1.51 bc	0.10 bc	0.39 a	0.39 a	0.51 abc	1.21 bcd	2.42 a	2.98 a
Cassava	243.70 a	597.17 a	204.25 ab	0.85 c	0.13 ab	0.38 a	0.38 a	0.51 abc	1.04 d	2.50 a	3.11 a
Forest	111.32 b	694.42 a	138.58 c	3.05 a	0.06 c	0.37 a	0.36 a	0.45 c	1.36 ab	2.75 a	3.21 a
Agroforestry system	130.78 b	613.57 a	213.73ab	1.83 b	0.10 bc	0.38 a	0.38 a	0.48 c	1.32 abc	2.41 a	3.00 a
Vegetables	133.11 b	633.29 a	263.97 a	1.46 bc	0.08 bc	0.43 a	0.43 a	0.49 bc	1.31 abc	2.32 a	2.92 a
CV (%)	13.14	9.34	14.14	16.31	20.21	9.02	9.14	6.74	8.48	13.80	5.64
					0.10–0.20 m						
Banana	72.29 c	616.71 ab	253.82 ab	1.59 b	0.09 bc	0.41 a	0.54 ab	0.52 ab	1.21 ab	2.57 a	3.07 a
Grassland	113.41 bc	651.71 ab	265.48 ab	2.11 a	0.06 c	0.39 a	0.56 a	0.45 c	1.56 a	2.42 a	3.03 ab
Maize	125.62 bc	645.54 ab	205.04 bc	1.50 b	0.09 bc	0.41 a	0.48 abc	0.49 bc	1.30 ab	1.83 a	2.62 b
Coffee	122.53 bc	619.15 ab	288.69 a	1.28 b	0.09 bc	0.42 a	0.42 c	0.50 abc	1.31 ab	2.54 a	3.08 ab
Cassava	190.58 a	540.06 b	280.98 ab	1.15 b	0.09 bc	0.41 a	0.37 c	0.50 abc	1.24 ab	2.57 a	3.08 ab
Forest	139.16 ab	674.94 a	166.45 ab	1.37 b	0.16 a	0.41 a	0.41 c	0.56 a	1.10 b	2.65 a	3.16 a
Agroforestry system	122.93 bc	634.69 ab	245.73 abc	1.51 b	0.10 b	0.39 a	0.39 c	0.51 abc	1.32 ab	2.42 a	2.98 a
Vegetables	149.72 ab	629.63 ab	251.01 ab	1.18 b	0.09 bc	0.43 a	0.43 bc	0.51 abc	1.34 ab	2.51 a	3.08 ab
CV (%)	19.90	7.90	13.99	15.08	15.06	5.40	10.73	5.81	9.44	17.65	7.05

SPR (Soil Penetration Resistance), MaP (Macro Porosity of soil), MiP (Micro Porosity of soil), TP (Total Porosity), Uws (volumetric water content), BD), GMD (Mean Geometric Diameter), WMD (Weighted Mean Diameter) CV (%): coefficient of variation. Means followed by the same letter do not differ from each other (Tukey $p \leq 0.05$).

The results for the chemical analyses are presented in Table 2. The pH values (4.29-4.95) classify the soil in all cultivation systems as acid to moderately acid (Embrapa, 2011), not differentiating between them. The low pH values favored the increase of the potential acidity (H+Al), which varied from 3 to 6 $cmol_c\ dm^{-3}$. In the different cultivation systems, especially in the maize and cassava areas, the potential acidity is considered strong and very strong, limiting soil fertility (Embrapa, 2011), due to the high Al^{3+} concentration available. According to Ernani (2008), pH values lower than 5.5 decrease organic matter decomposition, increasing the exchangeable Al^{3+} and the solubility of the iron and aluminum compounds. Therefore, in the pH values of the evaluated areas, the Al^{3+} readily available in the medium may lead to reduced growth and development of roots and reduced absorption of nutrients (Rampim *et al.*, 2013).

Table 2. Chemical soil attributes under different uses in the 0.0-0.10 m and 0.10-0.20 m layers in the Humaitá-Am region.

Cultivation System	pH	OC	CS	H+ Al	Al^{3+}	K	P
	H_2O	g kg^{-1}	Mg ha^{-1}		$cmol_c\ dm^{-3}$		mg dm^{-3}
			0.0–0.10 m				
Banana	4.52 a	12.19 ab	64.92 a	16.17 a	5.27 a	9.88 a	2.37 ab
Grassland	4.82 a	7.61 c	19.32 c	10.60 a	2.95 b	5.60 a	1.93 ab
Maize	4.39 a	10.61 abc	39.25 bc	16.17 a	3.77 ab	10.46 a	3.21 a
Coffee	4.51 a	10.34 abc	53.50 ab	15.88 a	5.25 a	4.70 a	1.86 ab
Cassava	4.29 a	10.76 abc	47.15 ab	17.49 a	4.40 ab	5.17 a	2.42 ab
Forest	4.77 a	9.53 abc	19.06 b	10.64 a	2.94 b	7.03 a	1.63 ab
Agroforestry system	4.65 a	10.34 abc	40.08 abc	12.25 a	4.55 ab	5.67 a	1.21 b
Vegetables	4.64 a	13.03 a	65.26 a	15.01 a	5.07 a	4.72 a	1.63
CV (%)	5.70	17.90	24.99	21.14	17.72	42.29	33,08
			0.10–0.20 m				
Banana	4.62 a	10.78 ab	25.84 a	15.13 ab	6.20 a	4.02 a	1.23 a
Grassland	4.51 a	7.17 b	20.98 a	9.65 c	4.10 ab	1.62 a	1.18 a
Maize	4.70 a	10.85 ab	28.26 a	11.50 bc	4.72 ab	3.87 a	1.27 a
Coffee	4.61 a	12.77 a	33.73 a	14.23 ab	5.70 ab	2.94 a	1.41 a
Cassava	4.42 a	11.58 a	28.76 a	12.62 abc	5.02 ab	4.32 a	1.35 a
Forest	4.39 a	6.61 b	23.26 a	16.17 a	3.77 b	4.17 a	1.27 a
Agroforestry system	4.95 a	8.67 b	24.90 a	15.88 a	5.25 ab	2.79 a	1.63 a
Vegetables	4.63 a	12.86 a	32.14 a	15.09 ab	4.82 ab	3.04 a	1.21 a
CV (%)	5.14	16.86	20.19	13.07	18.68	59.84	33.66

CV (%): coefficient of variation. Means followed by the same letter do not differ from each other (Tukey $p \leq 0.05$).

The carbon content (OC) in the soil in the different systems studied presented similar behavior, with the highest levels in the depth 0.0-0.10 m decreasing with depth increase (Table 2). The highest OC contents were found in the cultivated areas in the occurrence of the deposition of the cultural residues under the soil. The soil under cultivation of vegetables is highlighted, assuming the highest OC contents of 13.03 to 12.86 in the layer 0.0-0.10 and 0.10-0.20 m, respectively. This was due mainly to the short cycle of vegetables combined with the high C/N ratio, which conditions the easy decomposition (Silva *et al.*, 2016).

On the other hand, the practice of fires for grassland formation responds to the lower levels of OC in the soil under grassland, varying from 7.17 to 7.61 g kg^{-1}. In addition, Silva *et al.* (2004) argue that intensive grazing results in degradation of OC; added to this, we have the low input of OM by the grass. In turn, Campos *et al.* (2016) studying carbon stock and aggregates in a Cambisol under different managements in southern Amazonas, found OC content

(16.13 g kg^{-1}) higher than that found in the present study. The study by Campos *et al.* (2016) allows us to conclude that the increment of OC in grassland system depends on the grass species and the grazing system adopted.

The carbon stock (CS) followed the observed behavior for OC with higher content in the superficial layer of 0.0-0.10 m. Due to the characteristics of each crop, there was a significant difference in the supply of organic matter and, consequently, in the CS, with bananas, vegetables and coffee crops contributing the most to soil CS at 0.0-0.10 m depth. In the 0.10-0.20 m layer, there was a significant similarity between the cultivation systems, with the highest content being promoted in the soil with coffee (CS = 33.73 g kg^{-1}) and vegetables (CS = 32, 14 g kg^{-1}). Although in all cultivations the CS decreased with depth, the decay in the order of 63.05% g kg^{-1} for banana, 50.75% g kg^{-1} for coffee and vegetables with 39% g kg^{-1} is related the contribution of the root system, an important contributor to the CS in depth. Behavior, which explains the increment in 7.9% and 18.05% in the soil with grassland and forest, respectively, at depths of 0.10 – 0.20 m.

The available phosphorus levels did not differ significantly between the cultivation systems, with similar behavior at all depths, varying from 1.21 to 3.21 mg kg^{-1}. A small decrease in depth, according to Silva *et al.* (2006), phosphorus remains stable in depth due to its low mobility and its compounds. Richter *et al.* (2011) observed an increase of the surface concentration (0.00-0.10 m) of phosphorus and other attributes of the soil. The decrease in OM content and the increase in iron and aluminum content reduce P availability in depth. Behavior was also noted for K levels that did not differ between cultivation systems, with higher values in the soil with bananas, maize and forest in the 0.0-0.10 m layer.

The dendrogram obtained by the cluster analysis allowed the formation of groups with similarity between the management systems and physical and chemical characteristics in the soil (Figure 1). The variation of the Euclidean distance in function of the similarity and non-similarity of the management allowed an exact division of the system of use of the soil in three groups (I, II and III) in the layer 0.00-0.10 and four to 0.10-0.20 m (IV, V, VI, and VII). Positioned at the end, the GI, consisting of forest and grassland, indicates that the characteristics imposed on the soil are very different from the other groups, mainly the GIII. The bifurcation that separates the GII and GIII groups evidences that the cultivation of cassava, vegetables, AS and coffee promote closer characteristics in the soil, and are far more similar to the use of maize and banana, GIII.

Considering the group formed in the layer 0.0-0.10 m (Figure 1A), there was a reorganization of the groups in the layer 0.10-0.20 m (Figure 1B). It is observed an isolated formation of the soil characteristics of GVI and GIII, indicating that there is no similarity of the forest and maize soil with the other groups, corroborating the results presented in Tables 1 and 2. Also consistent results are those of Freitas et al (2015), studying the changes in the chemical and physical attributes of the soil submitted to sugarcane, forest and reforestation, with the use of dendrogram and other multivariate statistical techniques. The distinct behavior between the depths studied reinforces that the use of the soil changes its physical and chemical properties, due to the peculiarities of the handling and the capacity that each type of use entails.

The attributes of the soils evaluated by factor analysis (Table 3), allowed us to evaluate the attributes that presented higher factor loads by the varimax method. This procedure defines which attributes presented discriminatory power in common for the studied management, selecting attributes that can be considered as potential indicators of the original changes of the soil. The first two factors explained 78.00% and 57.69% of the total data variance in the two layers, revealing that only the sand attribute does not have a high factorial load. The SPR, MaP, MiP, TP, Uws, WMD, GMD, OC, CS, H+Al, Al3+ and pH were the most relevant attributes for the determination of Factor 1 that explained 52.14% and 35.39% of the total variance in the two studied layers, respectively. The attributes clay, silt, K and P were explained in Factor 2 with 25.86% and 22.29% of the variance in the two studied layers, respectively.

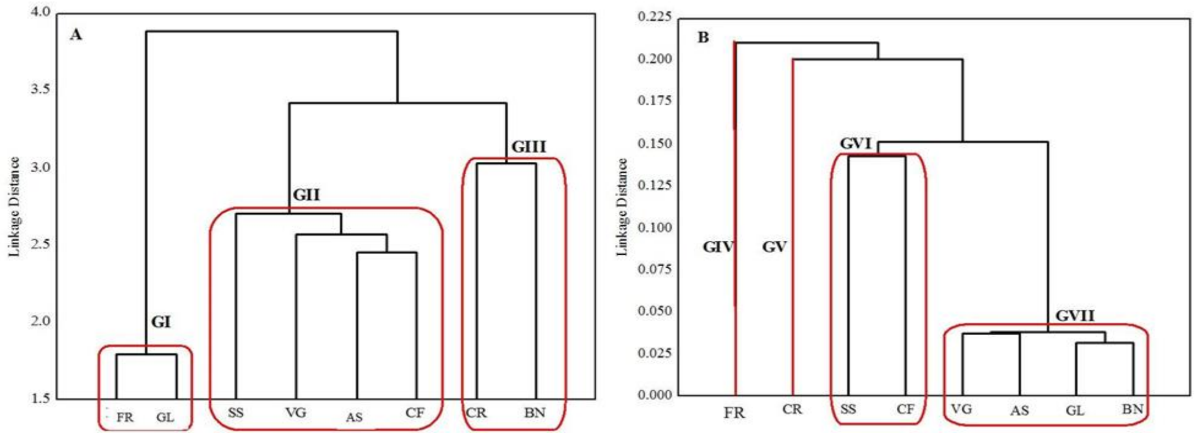

Figure 1. Dendrogram resulting from the hierarchical analysis of clusters showing the formation of groups (I, II, III, IV, V, VI, VII) according to the type of soil use (BN = bananas, GL = grassland, CR = maize, CF = coffee, SS = cassava, FR = forest, AS = agroforestry system, VG = vegetables) for layers A (0.0-0.10 m) and B (0.10-0.20 m) respectively.

Table 3. Factors extracted by principal components, highlighting attributes with loads higher than 0.7 (modulus) for soils under different management.

Attributes	CP 1	CP 2	CP 1	CP 2
	Layer 0.0 – 0.10 m		Layer 0.10 – 0.20 m	
Soil penetration resistance	**0.973982**	0.014616	**-0.851957**	-0.433365
Macroporosity	**-0.785679**	0.549132	**0.778896**	**-0.601862**
Microporosity	**-0.727773**	0.115549	0.589004	0.442441
Total porosity	**-0.838293**	0.474199	**0.915874**	-0.348526
Volumetric soil moisture	**-0.759118**	0.091025	**-0.691887**	-0.190896
Bulk density	**0.811004**	-0.334664	**-0.904685**	0.197828
Geometric Mean Diameter	**0.634630**	**0.753223**	0.414200	-0.033960
Weighted mean diameter	0.512307	**0.801038**	0.371930	-0.069967
Sand	-0.368536	-0.045991	0.078420	-0.018064
Silt	0.500849	0.595218	-0.152352	**-0.719980**
Clay	-0.468387	**-0.794624**	-0.333917	**0.814386**
pH on water	**0.815923**	-0.287279	-0.165962	0.233384
Organic carbon	**-0.831751**	-0.221428	0.251466	**0.915309**
Carbon stock	**-0.737664**	-0.476332	0.403844	**0.802689**
Potential Acidity	**-0.942766**	0.133433	**0.824352**	-0.091562
Aluminum	**-0.827362**	-0.416827	0.131536	**0.707473**
Potassium	-0.223108	**0.866237**	**0.751332**	-0.011715
Phosphor	-0.441862	**0.806093**	0.204458	0.185252
Eigenvalues	**9.907264**	**4.913975**	**6.725936**	**4.236814**
% of total variance	**52.14349**	**25.86302**	**35.39966**	**22.29902**
Cumulative eigenvalues	9.90726	14.82124	6.72594	10.96275
Cumulative %	**52.14349**	**78.00652**	**35.39966**	**57.69868**

The graphical representation and the correlation of the variables in the principal components (Figure 2AB) allowed to characterize the variables that more discriminated the formation of groups I, II, III, IV, V, VI, VII. With potential for explanation of 86.38% and 79.74 of the total data variance, CP1 revealed that grassland and forest environments (GII) were characterized by BD and SPR attributes, indicating a soil compaction process. However, this affirmation is only possible for the soil under grassland, since at both depths the grassland area (GVII) was related to the physical parameters BD and SPR indicators of soil compaction

(Aquino *et al.*, 2014; Vasconcelos *et al.*, 2014). It is possible that the content very close to the silt fraction in the 0.00 - 0.10 m layer has characterized grassland and forest soils as similar, because the small size of this particle obstructs the soil pores (Resende *et al.*, 2002), raising the values of BD and the SPR. In addition, it indicates that area under grassland is not in the process of intense compaction.

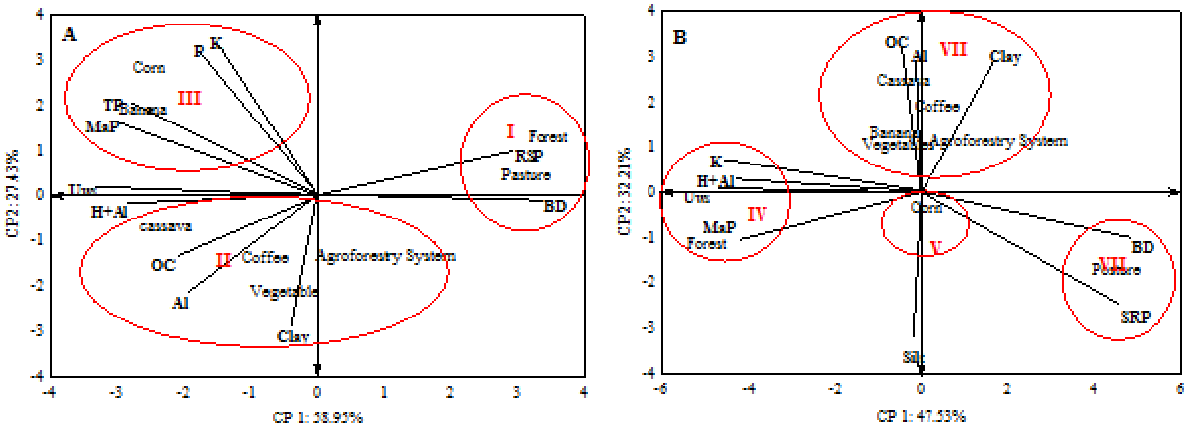

Figure 2. Dispersion (biplot plot) of the physical and chemical attributes in the different land uses, for layers a (0.0 - 0.10 m) and b (0.10 - 0.20 m). Indications I, II, III, IV, V, VI, VII are the groupings obtained in the cluster analysis.

The attributes H+Al, MaP, Uws and K defined the natural and conservative conditions of the forest area (GIV), consistent with the observations of Freitas *et al.* 2015 (Oliveira *et al.*, 2018), potential acidity and macroporosity strongly correlated with forest environments in relation to agricultural soils. As found here, Campos *et al.* (2012) report that the potential acidity in Amazonian forest soils results from the leaching process promoted by the intense water regime associated with the best drainage conditions in the region. No anthropic interference, without the use of agricultural implements and cultural treatments, does not degrade the stability of soil aggregates and allows soil moisture. Incorporation of OM complexed with K in the soil matrix under the activity of the microbiota provide chemical structural improvements and soil improved physics (Gomes *et al.*, 2018).

Among the evaluated soil attributes, the attributes K and Uws were more influenced by land use as observed in the biplot plot (Figure 2AB). The diversity of land use promotes more heterogeneous environments, with a reflection on the most sensitive physical-water attributes, soil moisture (Stefanoski et al., 2013), which is inversely proportional to BD and SPR (Machado et al., 2008). In addition to this, it can be stated that K variability was attributed to the diffusive flux of K influenced by soil moisture and soil compaction. For Costa et al. (2009b) and Ohland et al. (2014), the elevation of BD intensifies the aggregation force and reduces the macroporosity, thus hindering the mobility and absorption of K by the plants, due to the higher degree of hardness of the aggregates.

Only in the 0.0-0.10 m layer was the formation of a specific crop group such as maize and banana differentiated from the other crops by high levels of K and P and improvement in physical quality, verified by TP, contributing to the larger values of MaP. The formation of this group was also verified in the GIII cluster analysis (Figure 1A), probably as a consequence of the highest root volume in the first 0.1 m. The two CPs (0.0-0.10 and 0.10-0.20 m) revealed that the attributes clay, OC and Al were responsible for discriminating the GII and GVII groups, corresponding to the use of the soil with cassava, vegetables, coffee and agroforestry system.

The frequency histograms of the environmental covariates used and the descriptive statistics of the continuous covariates are shown in Figure 3. Most BD values are distributed in the class between 1.10-1.40, with frequencies of 30%, 24% and 22% for areas 5, 7 and 1, respectively. Low BD values group areas 3 and 2 with a percentage of occurrence of 10% and 5% in the class 1.00-1.10 respectively. The other areas presented non significant values. For the SPR was (Figure 3B) larger frequency between classes from 0.0 to 2.0 with a frequency of 25%, 17%, 15% and 12% for the areas 2, 3, 4 and 6, respectively. Thus, it was possible to classify most studied fields, such as low - SPR points exist even in areas that are higher than the class of 2.50 considered critical for root development of the plants (Tormena and Rollof, 1996).

The frequency distribution of MaP (Figure 3C) presented higher frequency between the ranges 0.40-1.00, with values of 20%, 18%, 15% and 10%, for areas 2, 4, 1 and 8, respectively. The respective areas presented frequencies lower than 6%. As shown in Figure 3C, maize and AS areas were the ones with the lowest percentage of macroporosity.

According to Pereira *et al.* (2011), the volume of macropores is expressively decreased when the pressure exerted on the soil is greater than it can withstand, contributing to microporosity. These reasons have raised the percentage of MiP (Figure 3D), with higher frequency of classes 3.50-4.20 and 4.00-4.50, corresponding to 30%, 20%, 18% and 13% respectively for areas IV, V, VIII, II and I. The lower frequencies found in areas II and VI translate the good physical conditions for maize and forest areas, behavior also portrayed in previous analyses.

Two significant peaks in areas III and VI were observed for WMD (Figure 3E), with a percentage of occurrence of 16% and 28% for the intervals of classes 2.80 - 3.00 and 3.00 - 3.20. In general, most of the areas had a higher frequency in the range of 2.60 classes until the interval of class 3.20. Although the GMD presents frequency distribution similar to the WMD (Figure 3F), for most areas aggregate stability is grouped in class 2.50-3.00, with a minimum occurrence of 7%, except for areas IV and VIII that respond with frequencies above 10% and 15%. Given that GMD represents an estimate of the size of the highest occurrence aggregate class and the WMD the percentage of large aggregates, it can be stated that stable and larger aggregates are dominant in the evaluated areas, important in soil resistance to erosion (Aquino *et al.*, 2014; Vasconcelos *et al.*, 2014; Gomes *et al.*, 2019).

In Figure 3G, the pH values with the highest frequency are distributed in the class 4.5-4.6 with frequencies of 50%, the other areas presented frequencies equal to or less than 20%, from the class 4.30 to the class 4.90. This result corroborates those of Cunha *et al.* (2017), which state that most of the soils of the cultivated areas of the study region are acidic, with low cation exchange capacity, which translates into low natural fertility. In the VI environment, favorable conditions for OM accumulation and maintenance contributed to high potential acidity (Figure 3I), according to Silva *et al.* (2016) the abundance of organic acids in the forest soil releases H^+ ions that will compose the potential acidity. This justifies the frequency percentage equal to or greater than 35% in forest areas and in their production environments VI, I, II and V.

Soil organic carbon presented values of frequency above 20% in the range of Class 10 (Figure 3H). The areas VI and II presented higher frequency, reaching values of 50%, 30% of frequency, respectively, due to the higher OM production and maintenance. In fact, the forest environment is an important CO_2 mitigating reservoir. In the case of soil under maize cultivation, the practice of leaving straw on the soil culminated in the increase of OC in area III. Summarizing, frequency histograms of environmental covariates based on soil attributes can assist in the identification and monitoring of natural areas being converted to agricultural activities, thus leading to proper management of agricultural crops and safety of chemical and physical quality from soil.

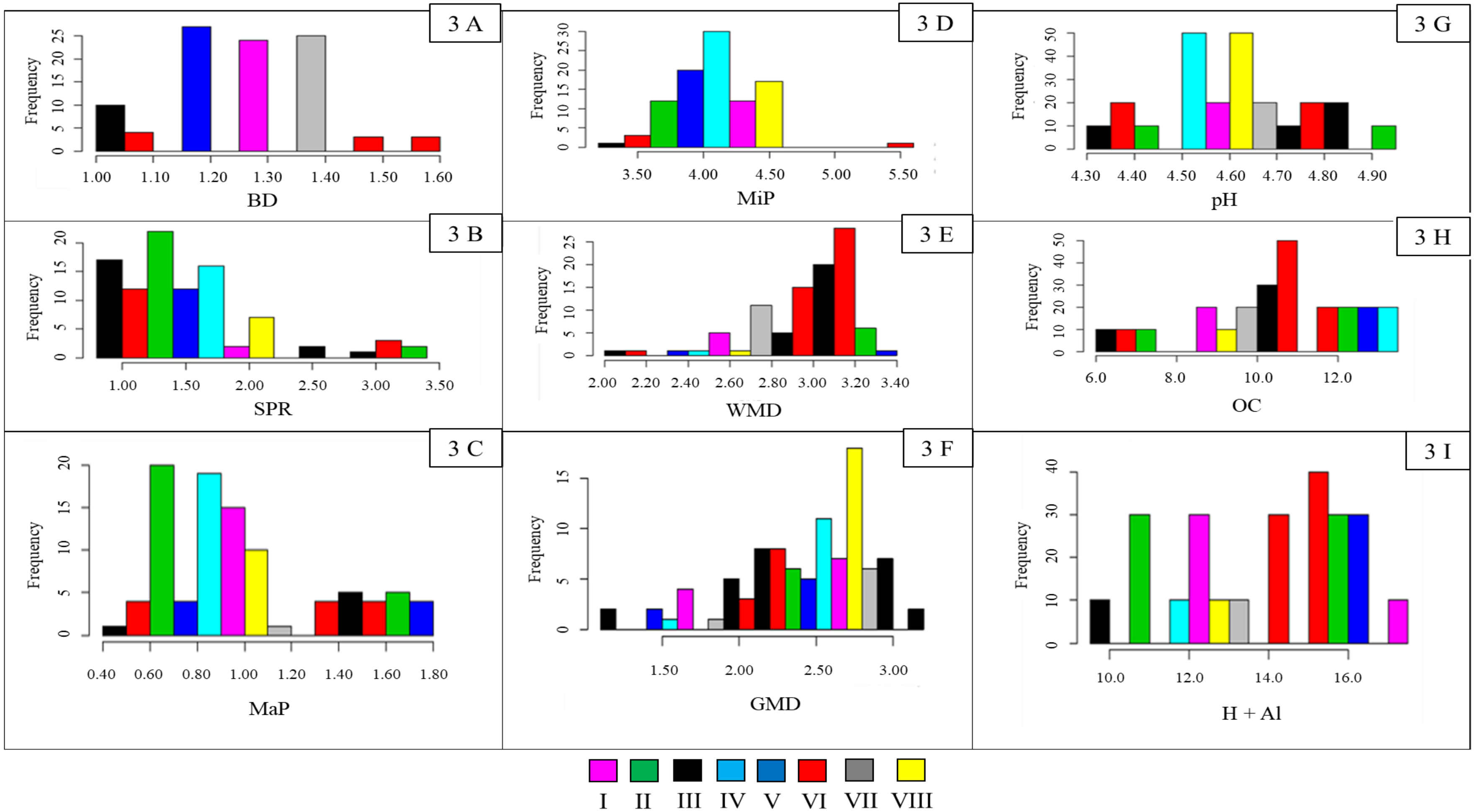

Figure 3. Distribution of the Bulk density Frequency 3 A; Soil penetration resistance 3 B; Macroporosity 3 C; Microporosity 3 D; Weighted average diameter 3 E; Geometric mean diameter 3 F; pH 3 G; Organic Carbon 3 H; Potential Acidity 3 I. I: Banana; II: Grassland; III: Maize; IV: Coffee; V: Cassava; VI: Forest; VII: AS; VIII: Vegetables.

4. CONCLUSIONS

The exploratory data analysis (principal components and dendrogram) and frequency of environmental covariates were efficient in distinguishing the production environments; thus, multivariate classification based on the physical and chemical attributes of the soil can aid in the proper planning of soil use.

The analysis of the principal components indicates greater variability for the attributes K and Uws, showing sensitive pedoindicators of land use.

Soil acidity is the main limiting factor for crop development, requiring the adoption of corrective pH practices with improvements in nutrient supply.

The conversion of the forest to grassland maintained the structural characteristics of the soil, while the other uses increased improvements in physical quality and soil fertility.

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Determination of the junction angle in fluvial channels from georeferenced aerial images from Google Earth Pro and UAV

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ABSTRACT

The junction angles in fluvial channels are determined from complex erosion and deposition processes, resulting from river-flow dynamics, bed and margin morphology, and so on. Knowledge regarding these angles is important in order to better understand the existing conditions in a basin. In this sense, the objective of the present study was to determine the junction angles on fluvial channels, called α, β and γ, applying the law of cosines. Georeferenced Google Earth Pro images and UAV images were used. Then, the values calculated from the georeferenced aerial images were compared with the values calculated from the minimum energy principle. To visualize and understand the obtained angles, the Junction Angles Diagram was used. The obtained result shows that the methodology using georeferenced aerial images have good performance for determining junction angles on fluvial channels.

Keywords: georeferencing, junction angle, law of cosines.

Determinação do ângulo de junção em canais fluviais a partir de imagens aéreas georreferenciadas obtidas do Google Earth Pro e com VANT

RESUMO

Os ângulos de junção em canais fluviais são determinados a partir de complexos processos de erosão e deposição, resultantes da dinâmica do fluxo da vazão, da morfologia do leito e das margens, das feições hidrogeomorfológicas da bacia, de sua ordem e do uso e da ocupação do solo. Conhecer os valores desses ângulos é importante para melhor entender as condições atuantes na bacia em estudo. Nesse sentido, o presente trabalho teve por objetivo determinar os ângulos de junção em canais fluviais, denominados α, β e γ, aplicando a lei dos cossenos. Foram utilizadas imagens aéreas do Google Earth Pro e coletadas VANT. Então, determinou-se os valores dos ângulos obtidos a partir dessas imagens, comparando-as aos valores calculados a partir do princípio da energia mínima. Para visualização e entendimento dos ângulos obtidos, foi utilizado o diagrama de ângulos de junção, sendo possível afirmar que a metodologia utilizando a lei dos cossenos, apresentou bons resultados para a determinação de ângulos de junção em canais fluviais.

Palavras-chave: ângulo de junção, imagens aéreas, lei dos cossenos.

1. INTRODUCTION

The understanding of hydrodynamics and morphodynamics at the confluence of fluvial channels is of a great interest for researchers in hydrology, fluvial geomorphology and sedimentology. Junctions' angles are an important fluvial feature, as well as a critical component of the drainage network, which, in turn, makes up the river basin. Small (1978) stated that drainage systems had been always highlighted both for men and for the mechanisms of landscape transformation, because they are linked to the dissection processes and landforms (re)modeling, being correlated with the slope and discharge at their junctions. Small (1978) and Beven *et al.* (1988) reported that the present landforms result from the sum of the processes related to the rivers and the hillslopes, variation of magnitude and frequency of such processes along geological time.

According to Santos and Stevaux (2017), scientific interest in geomorphic processes occurring at channel confluences has increased in the last decades, due to the growing awareness that the river processes and the confluence morphology reflect the characteristics of the upstream basin. Such geomorphic processes also play an important role in the regulation of water and sediment delivers in the downstream drainage network, because the interaction between the hydrological, geological and geomorphic mechanisms seeks to promote equilibrium between the natural processes that occur in the basin (Leopold and Maddock Jr., 1953). Although many studies on junction angles have been done up until now (Best and Rhoads, 2008; Santos and Stevaux, 2017), knowledge about its formation and determination still maintains strong interest (Hooshyar *et al.*, 2017; Alomari *et al.*, 2018; Yukawa *et al.*, 2019). However, the majority of the studies in this topic is directed to the understanding of the flow regime, of the interactions that occur inside the channel, and of the sediment mobility of the channels (Park and Latrubesse, 2015; Penna *et al.*, 2018; Herrero *et al.*, 2018; Dixon *et al.*, 2018).

There are still gaps in methodologies to analytically determine the values of these angles (Roy, 1983; Woldenderg and Horsfield, 1983). Here it must be noted that the angle value reflects the minimum energy principle. The application of these methodologies normally requires the data collection in the field, such as slope determination of the waterline and measurements of water and sediment discharge. This collecting activity needs specialized technical teams and adequate equipment, which makes their acquisition very expensive.

Therefore, the objective of the present study was to determine the junction angles: angle of confluence between the channels upstream (α), angle between the main river upstream and downstream main river (β), and angle between the tributary and the downstream main river (γ), which are shown in Figure 1A, using the law of cosines. The procedure proposed in the present study presents a faster, more accurate, safer and cheaper way to determine the junction angles in fluvial channels, applying the law of cosines in georeferenced aerial images. Then, we used Google Earth Pro and UAV (Unmanned Aerial Vehicle) images, supported in georeferenced points. For the visualization and the comparison of the angles, the Junction Angles Diagram proposed by Kobiyama *et al.* (2016) (Figure 1B) was adopted.

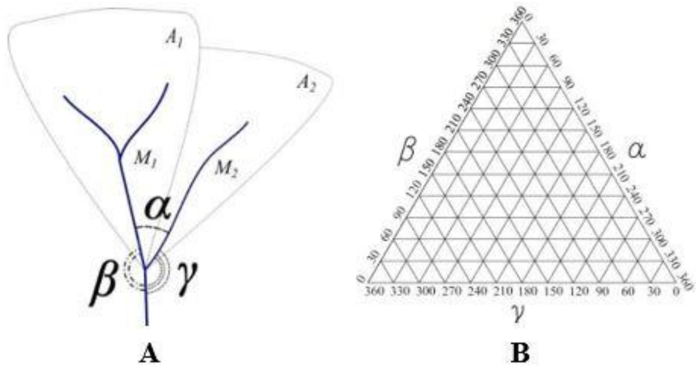


Figure 1. Fundamental concept of junction angles: (A) Angles on map; (B) Junction Angles Diagram proposed by Kobiyama *et al.* (2016).

2. BASIC THEORY

2.1. Confluence on fluvial channels

At the channel junctions (or confluences) in the drainage network, complex interactions between matter (water and sediment) and energy (channel power) provided by the combination of two different streams are observed (Best, 1987; 1988; Roy, 2008; Santos and Stevaux, 2017). When two channels join, materials (especially water and sediments) change the downstream channel morphology, and sometimes river islands can be temporarily or permanently formed at the main channel confluence site (Benda *et al.*, 2004a). Improving a work of Mosley (1976), Best (1987) proposed a general flow model for confluence zones in open channels, which consists of six different zones (flow stagnation region, flow deflection, flow separation, maximum velocity, flow recovery, and shear layers (Figure 2). The location and extent of these zones respond to variations in both the junction angle and the discharge ratio between the two rivers (tributary and receptor) (Best, 1987; 1988; Biron *et al.*, 1996). This set of zones with distinct characteristics was denominated hydrodynamic zone of confluence by Kenworthy and Rhoads (1995). Benda *et al.* (2004b) considered the drainage area to define the tributary and the main channel in which the tributary is the one with the smaller area and the main channel is the one with the larger area. Specifically, a tributary junction is defined at a point where two different channels unite, forming a single channel.

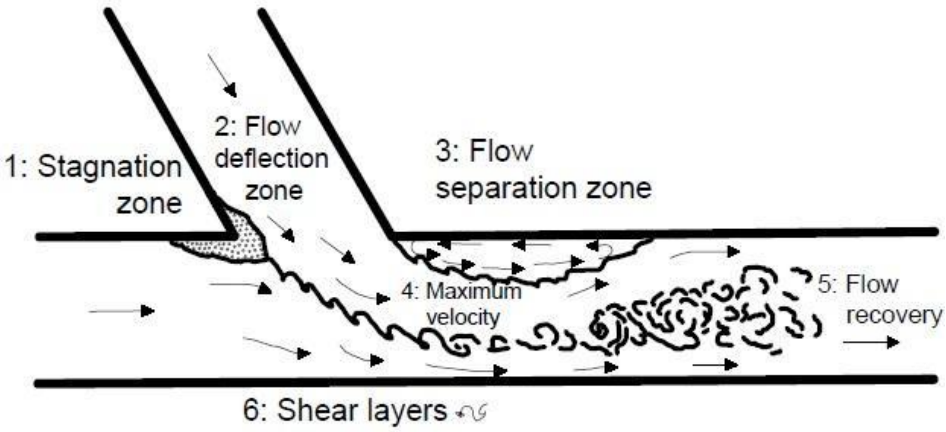


Figure 2. Flow dynamics in open confluent channels.
Source: Adapted from Best (1987).

According to Santos and Stevaux (2017), these zones were controlled by the confluence angle and the discharge ratio (Equation 1):

$$Qr = \frac{Q_t}{Q_p} \tag{1}$$

Where Q_r is the net discharge ratio [m^3 s^{-1}]; and Q_t and Q_p refer to the discharge of the tributary and the main channel, respectively. However, there are other factors that influence, such as symmetry, the momentum ratio (Equation 2).

$$Mr = \frac{\rho tQtVt}{\rho pQpVp} \tag{2}$$

Where *Mr* is the momentum ratio; ρ is the fluid density [g cm^{-3}] and *V* is the main velocity [m s^{-1}].

Furthermore, symmetry, bed dislocation, transported load (suspended and solid), any differences in density between input streams, and also other local aspects affect junction zones (Biron and Lane, 2008; Riley *et al.*, 2015). In evaluating the 14 studies carried out in the western United States and Canada, Benda *et al.* (2004b) concluded that the confluences' effects on river morphology are conditioned by the basin shape, by the drainage network patterns and by its density. Other modification factors are local geology because of rock porosity, precipitation intensity, and frequency and magnitude of flood events. The confluence-related hydrogeomorphic forms (e.g., benches, terraces and bars) are dominated by older features at headwaters and by younger characteristics downstream. These forms are derived from flood frequency and magnitude, the sedimentary contribution, and the basin characteristics and size.

2.2. Determination of the junction angle

When two channels unite, stemming from them a single downstream channel, the angular relationship of the junction is given by the three-segment gradient, regardless of which are the two angles between the flows (Howard, 1971). The first postulates were that junction angles were controlled by erosion and sediment transport at the confluence (Schumm, 1956) and by the flow gradients of the tributary and the receiving channels (Horton, 1945). Posteriorly, it was evidenced that the angle is directly proportional to the basin order (Lubowe, 1964) and that it varies with the net discharge of the two tributaries (Howard, 1971) and with the discharge per unit width (Mosley, 1976). Discharge, channel width, channel depth, contribution area and slope gradient are important factors for the junction angle formation (Shit and Maiti, 2013). In addition to these factors, junction angles are influenced by variations of land use and occupation in the upstream basin. In order to mathematically determine the junction angle, Horton (1932) assumed that the flow lines follow the channel with a larger slope (Equation 3):

$$cos\alpha = \frac{S_c}{S_g} \tag{3}$$

Where S_c and S_g are the slope of the channel and inclination of the hillslope, respectively.

Horton (1945) adapted his model by inferring that the junction angle is a product of the main channel and tributary flow (Equation 4):

$$cos\alpha = \frac{S_0}{S_1} \tag{4}$$

Where S_0 and S_1 denote the gradients of the receiving and the tributary channels, respectively.

Modifying this model, Howard (1971) determined two input angles α_1 and α_2 from the axial extension of the receiving channel on the tributary channel (Equation 5) (Figure 3):

$$cos\alpha_1 = \frac{S_0}{S_1}; \quad cos\alpha_2 = \frac{S_0}{S_2} \tag{5}$$

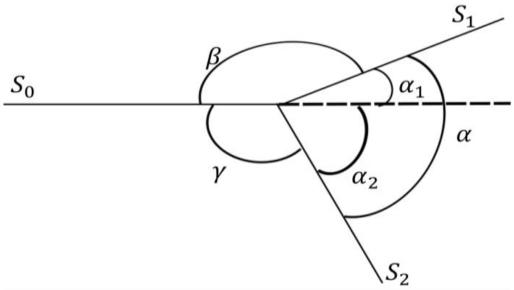


Figure 3. Representation of the junction scheme.
Source: Howard (1971).

Howard (1971) argued that the junction angle in channels can be examined in relation to the working rate (energy) given by gravity and the flow of both inlet and outlet at the junction, and suggested that this connection occurs in a local (Ω), which can be calculated by summing the costs per unit of length (Ci) in three connections multiplied by the length of the segment (Li) (Equations 6 and 7):

$$\Omega = \sum_{i=1}^{3} C_i . L_i \tag{6}$$

$$C_i L_i = \rho g Q_i S_i \tag{7}$$

Where g is the gravitational acceleration [m s²]; Q_i is the flow in the channel [m^3 s^{-1}]; and S_i is the channel gradient (energy line slope) [m/m].

Hack (1973) proposed that the gradient index of the channel is closely connected to the slope of the channel energy line (Equation 8):

$$S = K = \frac{dH}{(lnL_2 - lnL_1)} \tag{8}$$

Where dH is the height difference between the points of interest; and L is the length of the channel between two points.

Roy (1983) and Woldenderg and Horsfield (1983), based on the work of Zamir (1976), affirmed that the junction angle is independent of the channel length (L_i) and emphasized that this is a function of the minimum energy and can be expressed by the law of cosines (Equations 9, 10, 11, 12, 13 and 14):

$$cos\alpha_1 = \frac{S_0^2 + S_1^2 - S_2^2}{2.S_0.S_1} \tag{9}$$

$$cos\alpha_2 = \frac{S_0^2 + S_2^2 - S_1^2}{2.S_0.S_2} \tag{10}$$

$$cos\ \alpha = \frac{S_0^2 - S_1^2 - S_2^2}{2.S_1.S_2} \tag{11}$$

$$\alpha = \alpha_1 + \alpha_2 \tag{12}$$

$$\beta = 180 - \alpha_1 \tag{13}$$

$$\gamma = 180 - \alpha_2 \tag{14}$$

However, Figure 3 demonstrates that the determination of β and γ is conditioned to have an alignment at an angle of 180° from S_0. The fact that it is not very common to have fluvial channels with the Y type format at a natural junction proves the limitation of this methodology.

3. MATERIALS AND METHODS

3.1. Study Area

The Sinos River Basin is located in the northeast of Rio Grande do Sul state, Brazil, between the geographic coordinates 29°20' and 30°10'S, and 50°15' and 51°20'W (Figure 4). The basin has an area of 3,746.68 km² and a total population estimated at 1,249,100 inhabitants. The drainage network of the basin is composed of the Sinos River, Rolante River, Ilha River and Paranhana River. The latter has its sources in the cities of Gramado and Canela, at an altitude of approximately 900 m, and its outlet is located between the cities of Parobé and Taquara.

The Paranhana River basin approximately has an area of 574 km² and an extension of 50 km in its main channel, with several cascades. The Sinos River has its headwaters in Caraá municipality, at an altitude of approximately 800 m, and its outlet is located at the border between the municipalities of Canoas and São Leopoldo, at an altitude of approximately 4 m. This river has an approximate extension of 190 km, covering 13 municipalities. The upper part of the Sinos River, which is determined from the junction point that the present study selected, has a drainage area of 1,791 km².

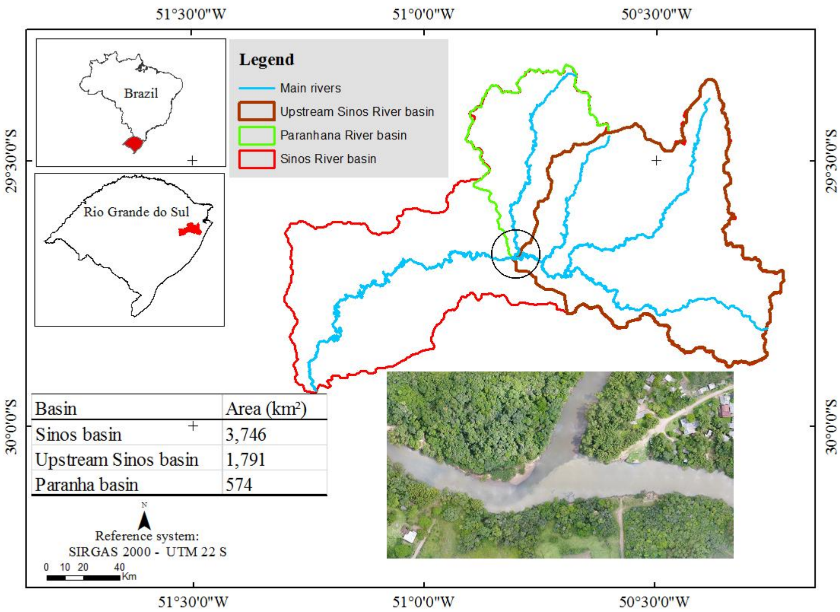


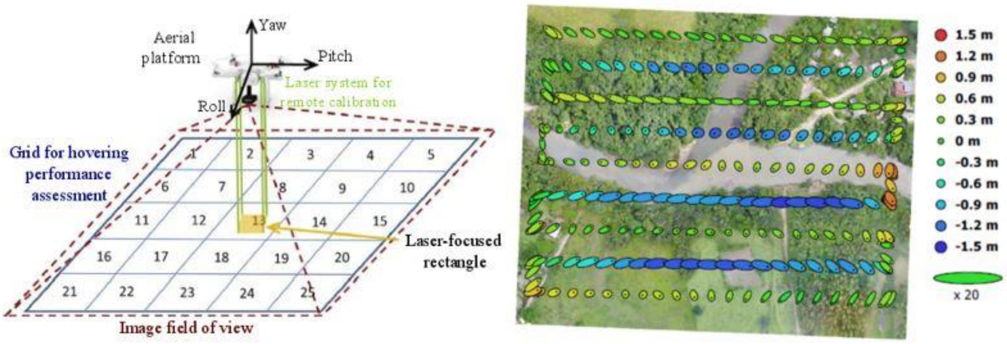
Figure 4. Location of the confluence point between the Sinos River and the Paranhana River.

3.2. Obtaining aerial images

Google Earth Pro images is formed from a mixture of images from sensors of diverse resolutions, arranged to form a continuous image of the whole terrestrial globe. A detailed description of the procedures for obtaining and making these images available can be found in Lopes (2009). The image used to perform the present study came from a flight conducted on August 20th, 2017. The image determined from the UAV was made on November 16th, 2017. For the acquisition of this image, the present study used a multi-rotor UAV of four propellers, Model Phantum 4, with a digital camera Model FC6310, with a resolution of 5472 x 3648 pixels, focal length of 8.8 mm, pixel of 2.41 x 2.41 cm and a Memory Stick storage card with a capacity of 1024Mb. Table 1 shows a summary of flight specifications and conditions. Figure 5A schematically shows the flight plan. The errors in altitude (Z) are represented by the color of the ellipse and the errors in east orientation (X) and north orientation (Y) are represented by the shape of the ellipse (Figure 5B). The main error was 46.35 cm, 24.37 cm and 68.55 cm, in the X, Y and Z directions, respectively.

Table 1. Parameters of the flight mission.

Number of photos	Covered area	Weather conditions	Flight speed	Estimated flight time	Flight altitude	Time between two photos	Overlap
258	0.236 km²	Partly cloudy	8 m s^{-1}	6 min and 41 sec.	108 m	0.2 sec.	80%



A B

Figure 5. (A) Scheme of the flight plan; (B) possible errors in X, Y and Z axes.
Source: Adapted from Taurus (2016).

3.3. Georeferencing of images

For the determination of the coordinates in the field, a reference frame was implanted, where the equipment base was installed. Subsequently spatial targets were distributed at strategic points (points of support) in order to georeference the images. The coordinates of the base were determined by post-processing of the base, that is, precise point positioning (PPP). This system is free and available online for the GNSS (Global Navigation Satellite System) data processing, which makes use of the GPS Precise Point Positioning (GPS) program developed by the Geodetic Survey Division of Natural Resources of Canada (NRCan). It allows users with GPS and/or GLONASS receivers to obtain precision coordinates in the Geocentric Reference System for the Americas (SIRGAS2000) and the International Terrestrial Reference Frame (ITRF) (IBGE, 2017). After determining the correct coordinate of the base, based on the PPP, the coordinates of the strategic points were translated. For this procedure, the software GNSS solutions was used. The field survey for georeferencing as well as the flight with the UAV was carried out on November 16th, 2017 from 4h20pm to 5h30pm. The coordinate of the base UTM (m) MC -51: 6,716,0149.363 m (N) and 518,400.842 m (E), obtained by the static mode and orbits of fast satellites.

3.4. Determination of the junction point (*p*)

Woldenberg and Horsfield (1983) commented that the problem of the three points, point of junction, has been debated over 200 years, being approached predominantly in three ways.

First, the found junction-point is at a lower cost location that is identical to the equilibrium point of the forces. In the second, at the lowest point to the point of equilibrium. And the third at a point that minimizes the sum of the costs of the channels by an interactive process of moving the junction point to the minimum point. These authors presented an analytical solution for the determination of the point (*p*); however this solution is an alignment at an angle of 180º from S_0 (Figure 3). Since the dispersion of the channels mostly did not follow this standard, the present study determined the point (*p*) taking into account the alignments of the receiving and affluent channel margins (Figure 6A).

For this, an extension (red line) of the right-margin alignment of the tributary channel was made, upstream to downstream. Subsequently, an alignment of the right margin of the receiving channel was made, from downstream to upstream, until the alignment previously made, thus defining point (*p*) (Figure 6B). It should be noted that the determination of (*p*), is due to the researcher's perception to define the alignment that best represents the alignment of the channels. Moreover, the dynamics and mobility of the channels (Leopold and Maddock Jr., 1953), the point (*p*) varies according to the conditions in the channels. In this sense, its determination will represent the current conditions of field collections. From point (*p*), the segment of interest was aligned for the definition of the coordinates (X_0, Y_0; X_1, Y_1; and X_2, Y_2), (Figure 6C). This alignment occurred from point (*p*) to the middle of the channel of the given location. The definition of the length of this section was 4 times larger than the channel width, near point (*p*). Figure 6C represents the alignments in the image obtained with the UAV, and Figure 6D represents the alignments defined in the Google Earth Pro image.

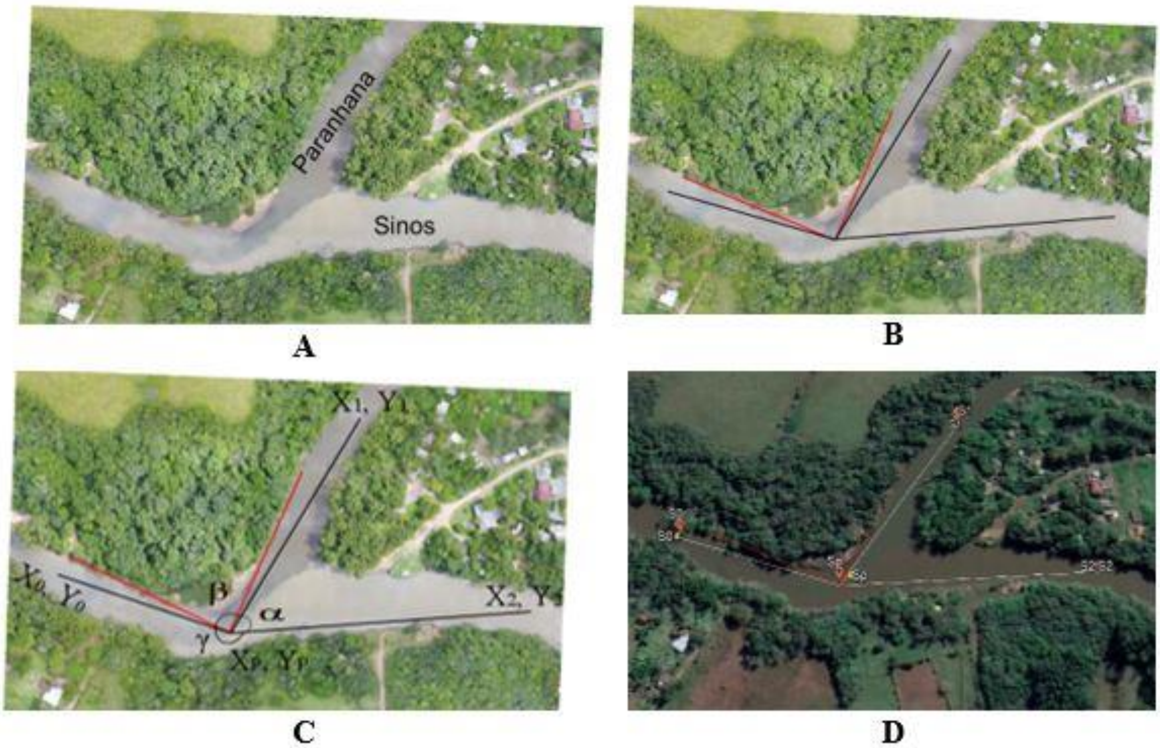


Figure 6. Representative scheme for determining the intersection point of the channels and the channel gradient of the study area: (A) visualization at the junction of the channels; (B) definition of the alignment for the determined point *p*; (C) definition of the length of the study area from images collected UAV and (D) definition of the length of the study area from Google Earth Pro images.

3.5. Determination of junction angles from georeferenced data

Based on the assumptions presented by Zamir (1976), Roy (1983), and Woldenderg and Horsfield (1983), the present study determined the angles α, β and γ (Figure 7) by using georeferenced aerial images collected with UAV and DGPS (Differential Global Positioning Systems). Determination of the respective angles are expressed as (Equations 15, 16 and 17):

$$\alpha = cos^{-1} \frac{(b^2+c^2-a^2)}{(2*b*c)} \tag{15}$$

$$\beta = cos^{-1} \frac{(a^2+c^2-b^2)}{(2*a*c)} \tag{16}$$

$$\gamma = cos^{-1} \frac{(a^2+b^2-c^2)}{(2*a*b)} \tag{17}$$

The horizontal distance between the points S_0, S1 and S_2 are determined from the Euclidean distance (Equation 18):

$$D(m) = \sqrt{(X_{n+1} - X_n)^2 + (Y_{n+1} - Y_n)^2 + (Z_{n+1} - Z_n)^2} \tag{18}$$

Where *D* is the distance between the ($\underline{S_0\, S_1}$, $\underline{S_1 S_2}$, $\underline{S_0 S_2}$).

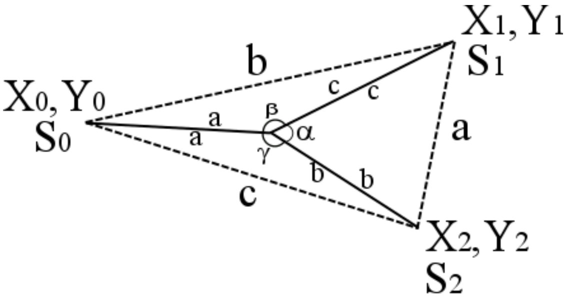


Figure 7. Determination scheme of the junction angle based on georeferenced data.

4. RESULTS AND DISCUSSION

Adopting Equations (9) to (14), and based on the minimum energy, the values of the angles α, β and γ were determined (Table 2). To determine the height difference between the points (S0, S1 and S2) used in determining the channel energy line (Equation 8), DGPS-RTK equipment was used. The used flow data were from the discharge stations Foz do Paranhana (87376000) and Taquara Montante (87374000), which are located upstream and downstream of the confluence point, respectively. These data were obtained from the Hydrological Information System (HidroWeb) on the ANA (National Water Agency) website. This electronic system freely provides the database of information collected by the National Hydrometeorological Network (RHN).

Table 2. Values found as a function of the minimum energy.

Basin	Slope (S) (m/m)	Angle	(minimum energy)
Sinos downstream (S0)	0.0023	α	154°23’13”
Paranhana (S1)	0.0051	β	97°45’46”
Sinos upstream (S2)	0.0053	γ	107°51’01”

For determining the junction angles in fluvial channels by applying the law of cosines in georeferenced aerial images, Table 3 shows the values referring to the aerial images collected with the UAV and the values from the Google Earth Pro image.

Table 3. Values of junction angles obtained: (a) with the georeferenced image from UAV, and (b) from Google Earth Pro image.

(a)

Point	Coordinate E (m)	Coordinate N (m)	Angle	From UAV	*a* (m)	*b* (m)	*c* (m)
SP	518,281.014	6,716,006.668	α	57°55'14"	219.008	237.658	212.326
S0	518,110.357	6,716,049.113	β	106°36'57"	175.856	312.029	212.326
S1	518,389.042	6,716,189.459	γ	195°27'49"	175.856	237.658	409.839
S2	518,518.591	6,716,012.875					

(b)

Point	Coordinate E (m)	Coordinate N (m)	Angle	From Google Earth Pro	*a* (m)	*b* (m)	*c* (m)
SP	518,272.47	6,716,001.190	α	54° 14' 46"	213.663	246.398	219.256
S0	518,114.00	6,716,053.000	β	104° 55' 52"	166.724	307.746	219.256
S1	518,392.00	6,716,185.000	γ	200° 49' 22"	166.724	246.398	406.575
S2	518,518.59	6,716,012.875					

Comparing the calculated values from the minimum energy with the values measured from Google Earth PRO and UAV images, the difference values of α are 100º08'27"and 96º27'59", respectively. When comparing the Google Earth Pro and UAV images, the difference of α is 2°19'32. For β, the differences between the methodology of the minimum energy and from Google Earth PRO and UAV images are 7º10’6” and 8º51’11”, respectively. When comparing Google Earth Pro and UAV images, the difference of β is 1°1'5". For γ, the difference between the minimum energy methodology and from Google Earth PRO and UAV images are 92º58'21 "and 87º36'48", respectively. When comparing Google Earth Pro and UAV images, the difference of γ is 5°21'33".

This evaluation of the values obtained as a function of the minimum energy confirms that this methodology was not suitable to the study area. This assertion is supported by Figure 3 where the determination of α, β and γ was conditioned to an alignment of 180° from S_0. It did not match with the channels of the present study. In addition, the occurrence of the “Y” shaped junctions in fluvial channels is not very common.

Limitation of the method based on the minimum energy also results from the difficulty to determine the slope between the points of interest. In the case of river banks, the slope determination of the water line by topographic survey becomes very laborious. And when the GNSS system is adopted, there is a limitation of satellite signal interference due to the riparian vegetation.

As observed in Table 3, there is a difference, for each point, between the coordinates obtained from the Google Earth Pro and with UAV images. Here it must be noted that the coordinates obtained by the UAV flight and georeferenced in the field with the DGPS-RTK are more accurate than the ones from Google Earth Pro image. Thus, it may be said that the values obtained with the UAV flight better represent the angles α, β and γ. The technique’s limitations (disadvantages) include the need to perform the precision georeferencing concomitant to the flight with UAV as well as the need to know how to use the described equipment.

On the other hand, the satellite image has the advantage of being more easily obtained for the citizen user. Furthermore, it provides the advantage of applying the methodology, cosine law in georeferenced aerial images, in a wider range of places.

In order to better visualize and compare the obtained angles, the α, β and γ values of the georeferenced aerial images with the minimum-energy values were plotted in the Junction Angles Diagram (adapted from Kobiyama *et al.*, 2016) (Figure 8). The plotted points in the diagram demonstrate a good coherence between the values obtained from the georeferenced image and a discrepancy with the values obtained from the minimum energy.

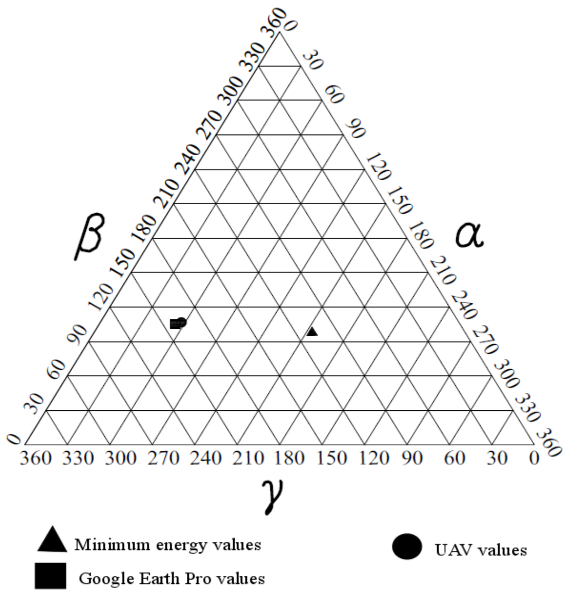


Figure 8. Junction Angles Diagram with the obtained values of α, β and γ.

The comparison between the two methods (georeferenced images and minimum energy) allows us to observe that the use of the first one is easier to obtain the values of the junction angles, because it does not require to measure flow and the energy line slope. Thus, this method is cheaper, faster, safer and more accurate. Another vantage of this method is its mathematical simplicity, just using the law of cosines. Furthermore, it may possibly be applied in aerial images obtained from high-resolution satellites. Then the evaluation of the dynamics of the junction angles can be possible using previous images. Thus, the present study is one example of the efficiency of the georeferenced-images method.

5. CONCLUSION

Aiming to contribute to a better understanding of the fluvial and geomorphological processes occurring in a river basin, the present study had the objective of determining the junction angles in fluvial channels, applying the law of cosines in georeferenced aerial images from Google Earth Pro images and collected with UAV. For better visualization and comparison between the obtained values of the angles α, β and γ, they were plotted on the Junction Angles Diagram (adapted from Kobiyama *et al.*, 2016).

In this sense, the importance of the insertion of new technologies in environmental studies is highlighted. The use of aerial images, collected with UAV, provides a detailed view on small scales, thus providing a better understanding of the complexity and integration of the involved processes. In addition, the use of the UAV has the advantage of acquiring data in a faster and more practical way, even enabling studies in places of difficult access. The high accuracy provided by georeferencing contributes significantly to the environmental studies, because the measured data certainly has better accuracy, and consequently provides a better understanding of the measured variables.

The use of aerial imagery obtained by satellite, Google Earth Pro image, has the great advantage of its ease of access and the high degree of applicability in various parts of the Earth. Furthermore, evaluation of the dynamics of the junction angles can be possible in past times, which allows the understanding of hydrodynamic and morphodynamic processes in basins

It was verified that the values determined from georeferenced images presented values more coherent with the field reality, than the values obtained from minimum energy. It confirms the efficiency of the proposed methodology. The use of the Law of Cosines, using georeferenced aerial images, was shown to be simple and efficient for the study to determine the angles α, β and γ in fluvial channels. In this sense, the proposed methodology presents great potential for its applicability. Its further applicability should be tested in various cases in Brazil and other countries.

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Preparation and application of Zero Valent Iron immobilized in Activated Carbon for removal of hexavalent Chromium from synthetic effluent

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ABSTRACT

Unlike organic contaminants, heavy metals are not biodegradable and tend to accumulate in living organisms; they are also recognized for being toxic or carcinogenic. The use of nanoparticles of zero-valent iron (nZVI) is reported as an alternative technique with high potential for in situ and ex situ remediation of contaminated matrices with this metal, mainly due to its large active surface area and significant adsorption capacity to consolidate into a simple and efficient method of treatment. In this study, ZVI particles were synthesized by the chemical reduction method using hydrated ferrous sulfate ($FeSO_4.7H_2O$) and sodium borohydride ($NaBH_4$) with subsequent aggregation to powdered activated carbon (PAC), forming the adsorbent PAC-ZVI, which was characterized by the techniques of XRD and SEM, which revealed the integration of the catalyst to the activated carbon matrix. Finally, developed kinetic studies revealed that the adsorption kinetics was better adapted to a pseudo second order model, the isotherms were better represented by the Freundlich model and the thermodynamic results showed that the adsorption reaction occurred through a spontaneous process with endothermic interaction between Cr (VI) and PAC-ZVI with increase in the randomness of the system.

Keywords: activated carbon, hexavalent chromium, zero valent iron.

Preparação e aplicação de Ferro Valência Zero imobilizado em Carvão Ativado para a remoção de Cromo hexavalente de efluente sintético

RESUMO

Diferentemente dos contaminantes orgânicos, os metais pesados não são biodegradáveis e tendem a se acumular nos organismos vivos e também são reconhecidos por serem tóxicos ou carcinogênicos. A utilização de nanopartículas de ferro zero valente (nFVZ) é relatada como uma técnica alternativa e de elevado potencial para remediação *in situ* e *ex situ* de matrizes contaminadas com este metal, principalmente devido à sua grande área superficial ativa e significativa capacidade de adsorção, podendo se consolidar em um método simples e eficiente de tratamento. Neste estudo foram sintetizadas partículas de Ferro Valencia Zero (FVZ) pelo método de redução química utilizando o sulfato ferroso hexa hidratado ($FeSO_4.7H_2O$) e Borohidreto de Sódio ($NaBH_4$) com posterior agregação a partículas de carvão ativado em pó (PAC), formando o adsorvente PAC-FVZ, o qual foi caracterizado pelas técnicas de DRX e MEV, as quais revelaram a integração do catalisador à matriz de PAC. Finalmente, estudos cinéticos desenvolvidos revelaram que a cinética de adsorção se adaptou melhor a um modelo de pseudo segunda ordem, sendo que as isotermas foram melhor representadas pelo modelo de Freundlich e os resultados termodinâmicos demonstraram que a reação de adsorção ocorreu através de um processo espontâneo com interação endotérmica entre o Cr (VI) e o PAC-FVZ com aumento na aleatoriedade do sistema.

Palavras-chave: carvão ativado, cromo hexavalente, ferro valência zero.

1. INTRODUCTION

Toxic heavy metals of particular concern in sewage treatment include chromium, copper, nickel, mercury, cadmium and lead. Besides its recognized ecotoxicity, hexavalent chromium gathers other characteristics that require intensification of its environmental monitoring, such as its high oxidizing strength, persistence and significant carcinogenic potential. In general, the main sources of Cr (VI) contamination are from industrial processes, and the conventional treatments used for its remediation have not been able to reduce their compatible concentration levels with the established limits in Brazilian or International environmental legislation. Cr (VI) is known for being very toxic to plants and animals, because it is a strong oxidizing agent with carcinogenic potential (Cao *et al.*, 2019). The main sources of Cr (VI) contamination come from industrial processes in the electroplating, mining, leather processing, paint, dye, explosive and other industries (Park *et al.*, 2006; Lin *et al.*, 2019; Bavaresco *et al.*, 2019).

Although conventional treatments are able to achieve, in optimized conditions, significant hexavalent chromium removal efficiencies, in many cases they are not efficient in reducing the concentration of the contaminant to compatible levels with the established limits in environmental legislation or which do not present ecotoxicological significant effect. In response to this problem, alternatives that can be used in substitution or as a complement to conventional processes (tertiary treatment) have been proposed. Studies with emerging technologies for the removal of heavy metals from waters and soils were developed, including adsorption, reverse osmosis, ion exchange, solvent extraction, biological reduction, biosorption and zero valent iron (Fu *et al.*, 2014). Among various available technologies, the use of zero valent iron nanoparticles (nZVI) is reported as an ideal technique for in situ and ex situ remediation due to its active and large surface area and its high adsorption capacity of these metals and because it is a simple and efficient method (Khatoon *et al.*, 2013). During the last few years, zero valent iron (nZVI) has been reported as capable of promoting effective removal of different types of metallic ions, including Cr^{6+}, Ni^{2+}, Pb^{2+}, Cu^{2+} and Zn^{2+}. In most studies, the removal of heavy metals by ZVI focused on the removal of hexavalent chromium (Mortazavian *et al.*, 2018).

Synthesized nZVI particles tend to agglomerate rapidly in water through Van der Waals forces and magnetic attraction and form particles with diameters variation from microns to several millimeters. In addition, nZVI particles can react with the surrounding environment (dissolved oxygen, water and other oxidizing agents), leading to rapid loss of reactivity (Barreto-Rodrigues *et al.*, 2017; Fu *et al.*, 2014).

In previous studies, several adsorbents such as activated carbon granulates, activated carbon particles, carbon nanotubes, mesoporous carbon, biomass of biological and agricultural residues were studied in the removal of Cr (VI) (Huang *et al.*, 2014; Lv *et al.*, 2011;

Mortazavian *et al.*, 2018). Among these adsorbents, activated carbon particles has aroused significant interest mainly due to its high porosity, large surface area and high efficiency (Kakavandi *et al.*, 2014). Since the immobilized zero valent iron has higher activity and greater flexibility for environmental remediation applications compared to free nanoparticles, its immobilization in an activated carbon matrix could serve to preconcentrate reagents, mediate electron transfer reactions, and promote the growth of the product phases (Zhang *et al.*, 2006). For this reason, the objective of this work was the preparation, characterization and application of zero valence iron immobilized in powdered activated carbon (PAC) to remove hexavalent chromium from synthetic effluent, as well as obtaining useful kinetic and thermodynamic parameters to aid the understanding of the adsorption process.

2. MATERIAL AND METHODS

2.1. Materials and reagents

$NaBH_4$ (Purity: 98%) was used as a reducing agent and FeSO4.7H2O (Purity: 99%) as source of Fe^{2+} ions. Activated powdered carbon was used without previous treatment. The organic solvents used were the ethanol with analytical grade and methanol of chromatographic pattern. A 1000 mg L^{-1} stock solution of Cr (VI) was prepared by dissolving 2.829 g of $K_2Cr_2O_7$ in 1000 ml of water. For preparing all the solutions, ultrapure water was used; they were processed in *Mili-Q direct 8 millipore* water purification equipment.

2.2. Preparation of PAC-ZVI

The preparation of PAC-ZVI was carried out by means of the reduction of Fe^{2+} ion in zero iron by $NaBH_4$ reduction method according to the conditions described by Barreto-Rodrigues *et al.* (2017), according to a [Fe^{2+}] molar ratio: [BH_4^-] of 3:1, according to Equation 1:

$$6\,Fe^{2+} + 2\,BH_4^- + 6\,H_2O \rightarrow 6\,Fe^0 + 2\,H_2BO_3^- + 12\,H^+ + 2\,H_2 \qquad (1)$$

In a typical synthesis, 25 mL of the 1.5 mol L^{-1} $NaBH_4$ solution was added to 200 mL of $FeSO_4.7H_2O$ 0.125 mol L^{-1} prepared with methanol:water solution (30:70) at pH 3.0 contained in a 500 mL kitassate. The solution was homogenized using magnetic stirrer. After complete addition of $NaBH_4$, 1g of Synth-activated powdered carbon (PAC) was added, stirring for 45 minutes. After the synthesis the purification was carried out by washing with ethanol with analytical pattern purity (200 mL in triple wash) and then drying on a heating plate with magnetic stirrer and inert atmosphere using nitrogen. The temperature was maintained at 75°C until drying was complete.

2.3. Characterization of adsorbents

X-ray diffraction (XRD) tests: Were performed with a Rigaku® MiniFlex 600 Model, with CuKα copper radiation, with a wavelength 1.54 Ǻ, step of 0.020, speed of 2°/min and interval of 2θ = 10° to 90°.

Scanning Electron Microscopy (SEM): The morphological characteristics of the ZVI, PAC nd PAC-ZVI surfaces were obtained by Scanning Electron Microscopy (SEM) (Model: Hitachi TM3000), and the image amplifications were 1500 times.

2.4. Adsorption study

The studies were conducted using 250 ml Erlenmeyers Flasks (28 units), each containing 50 ml of the synthetic solution with 50 mg L^{-1} Cr (VI) and 0.5 g of the PAC-ZVI adsorbent. The effect of pH on the adsorption process was evaluated according to the range of 3-8.5 (3, 5, 7 and 8.5) pH units. The study was carried out at room temperature (25 ± 2ºC) with 130 rpm of rotational speed in Benchtop Incubator (Model: Shaker Mod MAQL-200), at different intervals

(0, 2, 4, 6, 8, 10, 15, 20, 25, 30, 45, 60, 90 and 120 minutes). At each time interval, two erlenmeyers (duplicate) of the Benchtop Incubator for ion determination were removed. In order to calculate the adsorption capacity and the percentage of chromium removal, Equations 2 and 3 were used:

$$\%\ \text{Removal} = (C_i - C_e)\,.\,100/C_i \quad (2)$$

$$Q_e = (C_i - C_e)\,.\,V/W \quad (3)$$

Where C is the initial concentration of chromium (mg L^{-1}), and final chromium concentration (mg L^{-1}), with removal capacity (Q_e, mg g^{-1}), V(L) of chromium, and W (mg) represents the mass of adsorbent used.

The experimental data were adjusted to the kinetic models of pseudo-first order and pseudo-second order according to Equations 4 and 5 in linear form:

$$log\ log\ (q_1 - q_t) = log\ log\ q_m - \left(\frac{k_1}{2.303}\right) t \quad (4)$$

$$\frac{t}{q_t} = \left(\frac{1}{(k_2 * q_2^2)}\right) + \left(\frac{1}{q_2}\right) t \quad (5)$$

Being that: k_1 is constant of the adsorption rate of the pseudo first order model (min^{-1}); q1 adsorbed amount of adsorbate at equilibrium ($mg.g^{-1}$); qt adsorbed amount of adsorbate at time t (mg g^{-1}); qm calculated adsorbed amount of adsorbate at equilibrium (mg g^{-1}). In order to obtain adsorption isotherms, different concentrations of Cr (VI) (5, 10, 25, 50, 75 and 100 mg L^{-1}) were used at pH 3 at 25 ± 2°C in a period of 90 minutes at 130 rpm. With the experimental adsorption data, the Langmuir and Freundlich isotherms models were applied, using Equations 6 and 7 in linear form:

$$\frac{C_e}{q_e} = \frac{1}{q_{max} K_L} + \frac{C_e}{q_{max}} \quad (6)$$

$$log\ log\ q_e = log\ log\ K_f + \frac{1}{n} log\ log\ C_e \quad (7)$$

Where: qe is the amount of solute adsorbed per gram of adsorbent at equilibrium (mg g^{-1}); C_e is the adsorbate concentration at equilibrium (mg L^{-1}); qmax maximum adsorption capacity (mg g^{-1}); KL adsorbate / adsorbent interaction constant (L mg^{-1}); Kf constant Freundlich adsorption capacity (mg^{-1}-(l/n) (g^{-1}) L/n); n is the Freundlich constant related to the heterogeneity of the energy system and the size of the adsorbed (dimensionless) molecule.

In order to determine the thermodynamic parameters, different temperature ranges of 25, 40 and 45°C were studied, and different initial concentrations of Cr (VI) 50, 75 and 100 mg L^{-1} were used, with a volume of 50 mL and a constant mass of adsorbent (0.5 g) at 130 rpm at pH 3 for 90 minutes. These parameters were obtained from Equations 8 and 9:

$$\Delta G = -RT\ ln\ ln\ K_b \quad (8)$$

$$ln\ ln\ K_b = \frac{\Delta S}{R} - \frac{\Delta H}{RT} \quad \frac{1}{T} \quad (9)$$

Being that: ΔG Gibbs free energy; T a Temperature of solution (K); R the ideal gas constant (8.314 J $mol^{-1}K^{-1}$); K_b equilibrium constant of the adsorption process at determined temperatures.

3. RESULTS AND DISCUSSION

3.1. Characterization of the materials obtained

Through the analysis of X-ray diffraction of the obtained materials it was possible to characterize the incorporation of the zero valent iron in the activated carbon particles. The diffractogram of Figure 1 (a) shows that the ZVI shows a major peak at $2\theta = 44.8°$, typical of iron zero valence, some smaller peaks and dispersed noises can be justified by the presence of synthetic precursor residues and oxides formed during synthesis or drying of ZVI. In the diffractogram of the activated carbon particle sample of Figure 1 (b), the peak at $2\theta = 24.5°$ is attributed to the high concentrations in carbon. Finally, the diffractogram of Figure 1 (c) shows typical peaks of the FVZ and PAC precursors suggesting that the FVZ particles were coated with activated carbon powder (PAC).

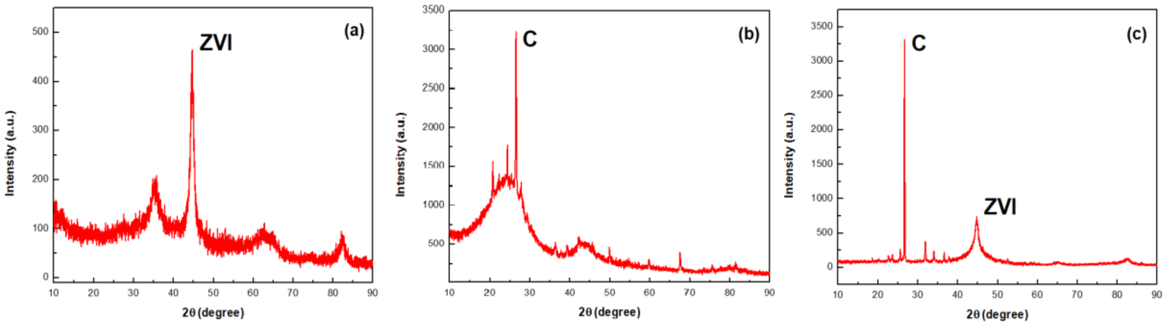

Figure 1. XRD of the zero valent iron - ZVI (a), powdered activated carbon - PAC (b) and adsorbent PAC-ZVI (c).

Morphologies (Figure 2) of the ZVI, PAC and PAC-ZVI were analyzed through the SEM.

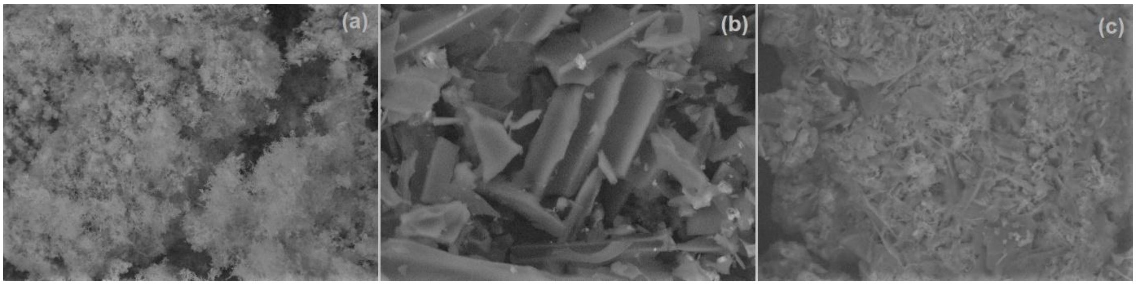

Figure 2. Micrographs of the zero valent iron - ZVI (a), powdered activated carbon - PAC (b) and adsorbent PAC-ZVI (c).

Figure 2 (a) shows the ZVI image before being immobilized with activated carbon. An aggregation is observed between the particles; according to Niu *et al.* (2005), this aggregation is attributed to the magnetic forces between the iron particles. Figure 2 (b) shows the activated carbon image without being incorporated into the ZVI. The structure of the coal used is similar to the structure obtained by the carbonization of other materials the cellulose base (Cherifi *et al.*, 2013). Finally, Figure 2 (c) shows the activated carbon already incorporated into the ZVI. The activated carbon particles appear interspersed with the ZVI particles, limiting the self-aggregation.

3.2. Kinetic Adsorption Modeling

In order to evaluate the kinetic behavior of the Cr (VI) removal process through PCA-ZVI, the experimental data collected under set conditions at pH 3.0 and 120 minutes of treatment were adjusted to the kinetic models of pseudo-first order and pseudo-second order in linear form. These results are presented in Table 1.

Table 1. Adsorption kinetic data for Cr (VI) in PAC-ZVI and Parameters obtained from the Langmuir and Freundlich isotherm models.

	Pseudo-first-order				Pseudo-second-order		
pH inicial	**qe,exp ($mg\ g^{-1}$)**	**qe,cal ($mg\ g^{-1}$)**	**k_1 (min^{-1})**	**R^2**	**qe,cal ($mg\ g^{-1}$)**	**k_2 (min^{-1})**	**R^2**
3	3.292	3.738	0.0375	0.961	4.246	0.0139	0.989
5	2.784	3.294	0.0320	0.948	4.118	0.0090	0.957
7	1.943	3.131	0.0377	0.231	3.489	0.0781	0.970
8.5	1.966	2.917	0.0204	0.264	3.294	0.0541	0.966

Langmuir		Freundlich	
q_{max} ($mg\ g^{-1}$)	2.604	**K_F ($mg\ g^{-1}$)**	0.917
K_L ($mg\ L^{-1}$)	0.147	**N**	3.905
R^2	0.968	**R^2**	0.976

It can be seen that the determination coefficient of the pseudo-first-order and pseudo-second-order models for Cr (VI) differs in large part; the pseudo-second order model presented a better correlation for Cr adsorption on the PAC-ZVI. This result also confirms that adsorption rather than reduction is the predominant mechanism most likely (Mortazavian *et al.*, 2018).

The pseudo-second order model assumes that the adsorption speed is directly proportional to the square of available adsorption sites, and suggests that surface reaction controls the process. Thus, the PAC, in addition to stabilizing the nanoparticles of ZVI, may have better adsorption capacity.

3.3. Adsorption Isotherms

The ability of the PAC-ZVI to adsorb the Cr (VI) was evaluated by the experimental data obtained for the equilibrium, which were adjusted to the Langmuir and Freundlich models in different concentrations, according to Figure 3.

Observing Table 1, it can be inferred that the isotherms were better represented by the Freundlich model. The coefficients of determination (R2) were better for the Freundlich model compared to the Langmuir model. These results demonstrate that multilayer formation occurs due to the adsorbate-adsorbent interaction. The Freundlich isotherm assumes multilayer adsorption and considers that the adsorbent has sites with different adsorption potentials and heterogeneous surface, presenting a favorable adsorption process (n> 1).

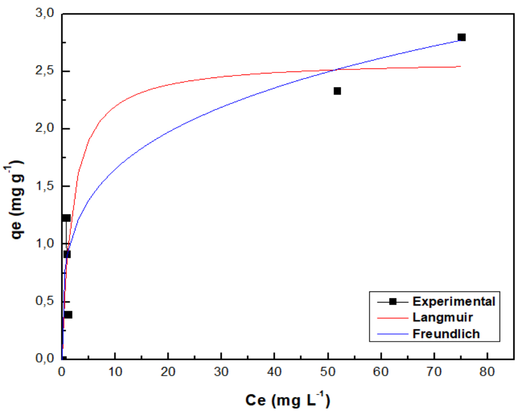

Figure 3. Adjustments of the experimental data on the Langmuir and Freundlich isotherms at 25°C, pH 3, at Cr (VI) concentrations 5, 10, 25, 50, 75 and 100 mg L^{-1}.

3.4. Thermodynamic Parameters

In the thermodynamic tests as shown in Table 2, different temperature ranges were selected, from which can be obtained energy free of Gibbs, Enthalpy and Entropy. It can be observed that with the increase of temperature, the constant equilibrium and the energy free of Gibbs did not present variation. The energy free of Gibbs showed negative values, indicating that the adsorption reaction is a spontaneous process, and positive enthalpy values implies that an endothermic interaction occurred between Cr (VI) and the PAC-ZVI. According to authors Dawodu and Akpomie (2014), in endothermic processes, an increase in temperature will lead to an increase in the thermodynamic constant equilibrium (K) and an increase in adsorption efficiency.

Table 2. Thermodynamic Parameters of Cr (VI) adsorption in PAC-ZVI.

Temperatura, K	*K*	ΔG **(kJ mol^{-1})**	ΔH **(kJ mol^{-1})**	ΔS **(kJ mol^{-1})**
298	5.049	-4.011		
313	5.848	-4.595	6.641	0.036
318	5.934	-4.708		

However, the positive values of Entropy indicate an increase in the randomness at the solid-liquid interface (adsorvent/solution) that occurs during the Cr (VI) adsorption process in the PAC-ZVI, which is in agreement with a study done by authors Araújo *et al.* (2009), which infers that the entropy variation is related to the order-disorder variation of the adsorption system and the higher the ΔS value is, the more random the system.

In the study by Kakavandi *et al.* (2014), using Ag / Fe bimetallic nanoparticles activated in activated carbon to remove Cr (VI), in a period of 60 minutes, with pH 3, obtained a maximum adsorption of 91.95% Cr (VI). Huang *et al.* (2014), used zero valent iron immobilized on activated carbon fibers to remove Cr (VI) and had a removal efficiency of 67%. In the present study, the removal of Cr (VI) by the PAC-ZVI adsorbent is strongly affected by pH, the best removal condition occurred at pH 3, with a removal efficiency of 71%. Although Cr (VI) removal efficiency has not been as significant compared to free activated carbon (Mortazavian *et al.*, 2018) applications, in future studies the ZVI synthesis and immobilization process may be better optimized. As for the potential of PAC-ZVI, it goes beyond removal, and it is possible to promote the reduction of Cr (VI) in Cr (III) on the surface of the adsorbent material, stabilizing the metal in its safest form.

4. CONCLUSIONS

In this study, ZVI was synthesized by the chemical reduction method and immobilized with activated carbon powder. The compound formed PAC-ZVI was used as adsorbent to remove Cr (VI) from the synthetic solutions prepared with potassium dichromate. The results showed that the synthesized adsorbent had a good efficiency in Cr (VI) adsorption. The efficiency of Cr (VI) reduction is strongly affected by pH, the best removal condition occurred at pH 3 at a contact time of 60 minutes. This is because the Cr (VI) ion at acid pH is positively charged, favoring the access of the metal complexes on the surface of the adsorbent. The data from the kinetic studies showed that Cr (VI) adsorption kinetics in PAC-ZVI were better adapted to the kinetic model of pseudo second order. The isotherms were better represented by the Freundlich model, with multilayer formation occurring due to the adsorbate-adsorbent interaction. The thermodynamic results demonstrated that the adsorption reaction is a spontaneous process, with an endothermic interaction between Cr (VI) and the PAC-ZVI with increasing randomness of the adsorvent/solution. Thus, the present study suggests that the PAC-ZVI material has potential to be used in the treatment of synthetic and industrial effluents as an efficient adsorbent.

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Distribution of major and trace elements in bottom sediments of the Taquari River Basin, Caldas municipality (Brazil)

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ABSTRACT

The Taquari River Basin, located in Poços de Caldas Alkaline Complex, in the southern portion of Minas Gerais state, Brazil, is situated in an old volcanic caldera. Due to its chemical and radiological characteristics, it is an area of economic and mineral interest, and is also home to diverse flora and fauna systems. In its surroundings, there are agricultural areas, industries (active and inactive) and urban and rural centers. This work investigated the total and potentially bioavailable concentrations of major and trace elements for the evaluation of geogenic and anthropogenic contamination potentials in the water bodies. The results show that there is an anthropogenic contribution (fertilizers and mining tailings) in some sectors of the Taquari River Basin, generating possible concerns regarding the quantity of elements that may be transferred to the water bodies. Furthermore, there is the striking geogenic contribution from naturally enriched areas, presenting distinct situations that generate an increase in the concentration of chemical elements in the water bodies.

Keywords: fertilizers, sediments quality, uranium mine wastes.

Distribuição de elementos maiores e traços em sedimentos de fundo da Bacia do Rio Taquari, município de Caldas (Brasil)

RESUMO

A Bacia do Rio Taquari, localizada no Complexo Alcalino de Poços de Caldas, na porção sul de Minas Gerais, está situada em uma antiga caldeira vulcânica. Devido às suas características químicas e radiológicas, é uma área de interesse econômico e mineral, além de abrigar diversos sistemas de flora e fauna. Nos seus arredores existem áreas agrícolas, indústrias (ativas e inativas) e centros urbanos e rurais. O objetivo deste trabalho foi investigar as concentrações totais e potencialmente biodisponíveis de elementos principais e traços para a avaliação de potenciais de contaminação geogênica e antropogênica em corpos d'água. Os resultados mostram que há uma contribuição antrópica (fertilizantes e rejeitos de mineração) em alguns setores da bacia do rio Taquari, gerando possíveis preocupações quanto à quantidade de elementos que podem ser transferidos para os corpos hídricos. Além disso, há contribuição geogênica de áreas naturalmente enriquecidas, apresentando situações distintas que geram um aumento na concentração de elementos químicos nos corpos d'água.

Palavras-chave: fertilizantes, qualidade dos sedimentos, rejeitos de mina de urânio.

1. INTRODUCTION

Sites that present areas contaminated by agrochemicals (EMBRAPA, 2004) or by industrial effluents can have, for the most part, soils with high metal contents, affecting sustainability, biodiversity and productivity, and offering a high degree of danger to the population (Sun *et al.*, 2001). Mining activities raise various environmental impacts depending on their size, exploitation and processing methods employed. These potential impacts directly or indirectly affect the local environment and communities and, in some cases, also the surrounding area (Marnika *et al.*, 2015). In the mineral industry generally, a large amount of tailings is generated, mainly in the steps of mining and the chemical processing of the ore, generally deposited in piles and tailing dams, respectively. Effluents from both tailings can carry toxic substances and seriously impact the environment. The presence of sulfides in tailings can lead to acid mine drainage, considered a serious problem in mining projects, due to the degradation of the quality of groundwater and surface waters, as well as soils and sediments (USEPA, 1994)

The systematic and often inadequate use of compounds for soil correction can result in increased accumulation of metals and toxic substances in soils, which may migrate to groundwater, surface water and river beds, often in concentrations that can cause severe impacts to the environment. The river bottom sediments are fundamental components of the river environment, which provide nutrients for living organisms and act as receptors of anthropogenic contaminants (Reis *et al.*, 2010). Studies on the evaluation of environmental impacts due to the use of agricultural correctives have been developed throughout the world and in Brazil (Savci, 2012; Yousaf *et al.*, 2017). One aspect to consider in agricultural/industrial activities is rock phosphates. These constitute the main raw material for manufacture of phosphate fertilizers. During the production process, most metals remain in fertilizers (Freitas *et al.*, 2009; Valle, 2012).

It is known that these phosphates naturally contain several metals, which may be of concern with regards to environmental contamination (Agbenin, 2002). It is important to mention some studies that indicate uranium (U) and thorium (Th) contents and their decay products in phosphate fertilizers, which, depending on the origin of the phosphate rock, may be significant (Saueia and Mazzilli, 2006; Jacomino *et al.*, 2009). The main objective of the present study was to evaluate if anthropogenic and/or geogenic agents are contributing to the increase of the concentration of certain metals in the fluvial sediments of the study area.

The study area extends for 102 km^2 and is located in the municipality of Caldas, in the central-southeast region of the Poços de Caldas Alkaline Complex (PCAC) (Figure 1). The area is located in the Taquari River Basin, consisting of the Consulta and Soberbo Streams and the Taquari River itself. The main types of soil use and occupation of the Taquari River Basin are agriculture and the Caldas Uranium Mine (CUM), which is currently in the decommissioning process (Figure 1). Although no longer in production, previous works (Fernandes *et al.*, 1995; Carvalho Filho *et al.*, 2016; 2017) in the area surrounding this former mine indicate possibilities for the generation of liabilities and contamination of the external environment, mainly as a consequence of acid mine drainage (AMD).

The agricultural sector is represented in the study area by vegetables, dairy and cattle farming, ornamental plants (mainly roses) and silviculture (with greatest expansion in the last 20 years). This plantation growth has generated a change in the local soil chemistry, resulting from agrochemicals, fertilizers and soil correctives. Continuous application together with the high turnover can generate undue accumulations of non-essential elements in the soil and later in water bodies.

The local geology comprises a set of plutonic, volcanic and subvolcanic rocks belonging to the PCAC (Figure 1). Nepheline syenites, phonolites, volcanic breccias and pyroclastic rocks predominate in the area (Schorscher and Shea, 1992; Valeton *et al.*, 1997). The CUM uranium deposit is defined as a mineralization of U-Th-Zr-Mo-REE (rare earth elements), composed mainly of uranium black oxides (uraninite and pitchblende - UO_2), which is occasionally accompanied by cofinite $(U^{4+},Th)(SiO_4)_{1-x}(OH)_{4x}$. The mineralogy of the ore consists of: sulfide minerals, mainly pyrite FeS_2 and rarely galena PbS and sphalerite ZnS; zirconium minerals, generally zircon-Zr SiO_4; minerals containing Mo, mainly jordisite MoS_2 and ilsemanite $Mo_3O_8.nH_2O$; fluorite CaF_2 and REE phases (Schorscher and Shea, 1992; Waber *et al.*, 1992).

The Morro do Taquari U-Zr-anomaly is found in the study area (Figure 1) and was characterized by the presence of caldasite (CT), a complex ore of zircon ($ZrSiO_4$) and badeleite (ZrO_2) containing uranium inclusions (Schorscher and Shea, 1992). The mining waste, consisting of barren rocks and low-grade ore, were added to dump deposits or bota-fora (BF), among which bota-fora 4 (BF4; Figure 1) is the most important from an environmental point of view. The wastes from the chemical treatment and acid neutralization were deposited in the tailings dam (BR; Figure 1), and after the addition of $BaCl_2$ to force radio precipitation, the effluent from the BR pour into the Soberbo Stream. The oxidation of pyrite in the wastes of BF4 and BR produces AMD, which when percolating through the waste can dissolve uranium and other toxic metals present.

Studies performed in the region of the uranium mine (Fernandes *et al.*, 1995; 1998; Carvalho Filho *et al.*, 2016; 2017) show a migration of acid mine drainage to water bodies, which can result in surface water and groundwater contamination.

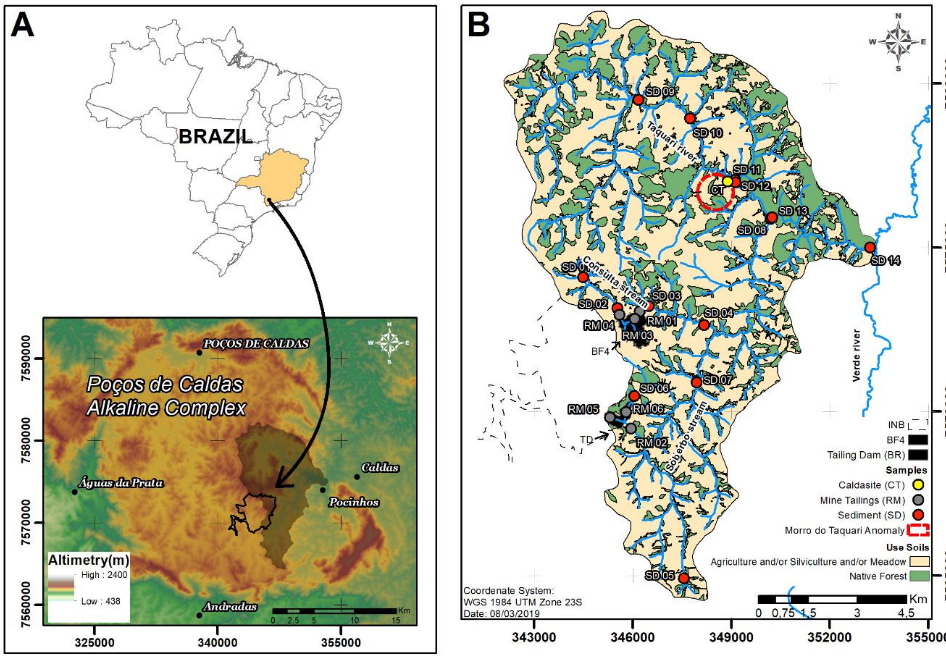

Figure 1. A) Location map of the study area. Source: Modified from Carvalho Filho *et al.* (2016); B) the sampling stations and soil use and occupation map.
Source: research data.

2. MATERIALS AND METHODS

The methodology of the study was structured considering the following: a) the potential anthropogenic sources of contamination are represented by fertilizers and CUM tailings, classified as diffuse and point sources, respectively; b) The caldasite ore was considered as a geogenic source; c) Fluvial sediments from the Taquari River Basin represent the matrix under investigation.

A total of 14 fluvial sediment samples (SD) were collected (Figure 1): six upstream of the CUM discharges - SD01, SD05, SD09, SD10 SD11 and SD12. The last two are a few meters downstream of the Morro do Taquari U-Zr-anomaly; the others are downstream of CUM. All fluvial samples are in places where there may be fertilizer application. A total of six mining tailings samples were collected (RM, Figure 1): 3 in BF4 (RM01, RM03 and RM04) and 3 in BR (RM02, RM05 and RM06).

The procedure for collecting the sediment and mining tailing samples was performed by using a shovel or a device for removing material from the river bottom, called a "rock-islander". Selection of fertilizers was done in conjunction with the "Company of Technical Assistance and Rural Extension of the State of Minas Gerais" (EMATER), where seven samples (FT01 to FT07) were collected from farms near the sediment sampling points. For the geogenic species, a composite sample of caldasite ores (CT) was prepared from 10 single random samples collected in outcrops at the Morro do Taquari anomaly (Figure 1).

The sediment and tailing samples were dried at a temperature of approximately 80ºC, then disaggregated and sieved, obtaining an approximate fraction of <63μm for identification and quantification of the elements present. Caldasite was crushed and pulverized (63 μm). The fertilizer samples were only pulverized (63 μm).

To quantify the elements Al, Ca, Fe, K, Mg, Mn, Na, P, Pb, Rb, Si, Sr, Ti, Zn and Zr, the powdered samples underwent a press and compaction process with boric acid. Measurements of this material were performed using an X-ray fluorescence spectrometer, brand Rigaku, model ZSX Primus II, at the Mineral Technology Service of the Nuclear Technology Development Center (CDTN).

Uranium and thorium were analyzed at Analytical Chemistry and Radiochemistry Service of the CDTN, by the neutron activation technique, thorium by the k0-AAN method (Menezes and Jacimovic, 2006) and uranium by the delayed fission neutrons method (Jacomino *et al.*, 2009). The samples were irradiated using the CDTN's nuclear research reactor TRIGA MARK I IPR-R1 and measured by a Canberra gamma spectrometry system, coaxial model 5019 HPGe detector with 50% nominal efficiency.

The Enrichment Factor (EF) is an index that evaluates the influence of metal sources of anthropogenic and geogenic origin in sediments (Selvaraj *et al.*, 2004), and is defined as Equation 1:

$$EF = \frac{\left[\frac{C_M\,sample}{C_{NE}\,sample}\right]}{\left[\frac{C_M\ reference}{C_{NE}\ reference}\right]} \tag{1}$$

C_M sample is the total concentration of metal M in the sample; C_{NE} sample is the total concentration of the normalizing element in the sample; C_M reference is the total concentration of element M in the reference standard; C_{NE} reference is the total concentration of the normalizing element in the reference standard. For calculating the enrichment factor, the elements Fe, Al and Sc are usually adopted as normalizing elements (NE) (Schropp *et al.*, 1990; Din, 1992). In the present study, iron was selected as NE, and as reference standard were used the mean values of elemental concentrations found by Valeton *et al.* (1997) for the PCAC rocks.

EF values of approximately 1.0 indicate that the metal in the analyzed sediment is predominantly of geogenic origin, contrary to values greater than 1 which indicates that the metal is of anthropogenic origin. Chen *et al.* (2007) proposed a degree of enrichment based on EF values: EF<1, no enrichment; 1<EF≤3, little enrichment; 3<EF≤5, moderate enrichment; 5<EF≤10, moderately severe enrichment; 10<EF≤25, severe enrichment, 25<EF≤50 very severe enrichment, >50, extremely severe enrichment.

3. RESULTS AND DISCUSSION

Table 1 shows the elemental concentrations found in sediment (research data) and local rock (Valeton et al., 1997).

Table 1. Fluvial sediment concentrations (SD) and reference standard local rock (LR).

ELEMENTS (%)										
Samples	Al	Ca	Fe	K	Mg	Mn	Na	P	Si	Ti
SD01	20.01±0.10	0.06±3x10^{-4}	3.85±0.01	3.03±0.01	0.07±3x10^{-4}	0.04±2x10^{-5}	0.01±5x10^{-6}	0.31±1x10^{-4}	22.81±0.11	1.08±0.005
SD02	19.85±0.09	0.04±2x10^{-4}	4.20±0.02	5.56±0.02	0.08±4x10^{-4}	0.27±1x10^{-4}	0.04±2x10^{-5}	0.18±9x10^{-5}	21.50±0.10	0.78±0.003
SD03	18.68±0.09	0.03±2x10^{-4}	4.34±0.02	6.72±0.03	0.07±3x10^{-4}	0.39±2x10^{-4}	0.03±1x10^{-5}	0.11±5x10^{-5}	21.46±0.10	0.60±0.003
SD04	18.56±0.09	0.24±1x10^{-4}	1.92±0.01	3.72±0.01	0.08±4x10^{-4}	0.06±3x10^{-5}	0.50±2x10^{-4}	0.06±3x10^{-5}	20.04±0.10	0.60±0.003
SD05	21.59±0.10	0.20±1x10^{-4}	7.55±0.03	1.49±0.01	0.21±1x10^{-4}	0.19±9x10^{-5}	0.02±2x10^{-5}	0.18±9x10^{-5}	19.02±0.09	2.04±0.10
SD06	21.70±0.10	0.12±6x10^{-5}	4.76±0.02	4.32±0.02	0.10±5x10^{-5}	0.08±4x10^{-5}	0.05±2x10^{-5}	0.19±9x10^{-5}	20.05±0.10	1.20±0.006
SD07	18.03±0.09	0.25±1x10^{-4}	4.85±0.02	2.22±0.01	0.17±1x10^{-4}	0.15±7x10^{-5}	1.33±6x10^{-4}	0.06±3x10^{-5}	16.54±0.08	1.00±0.005
SD08	17.65±0.08	0.34±1x10^{-4}	4.57±0.02	3.42±0.01	0.63±3x10^{-4}	0.15±7x10^{-5}	0.16±1x10^{-4}	0.07±3x10^{-5}	17.85±0.08	1.09±0.005
SD09	19.16±0.09	0.19±2x10^{-4}	4.83±0.02	5.81±0.02	0.10±5x10^{-5}	0.46±2x10^{-4}	0.11±5x10^{-5}	0.11±5x10^{-5}	21.27±0.10	1.08±0.005
SD10	23.39±0.11	0.07±3x10^{-5}	4.90±0.02	3.82±0.01	0.11±5x10^{-5}	0.26±1x10^{-4}	0.03±1x10^{-5}	0.09±4x10^{-5}	18.74±0.09	1.14±0.005
SD11	19.58±0.09	0.24±1x10^{-4}	4.34±0.02	5.73±0.02	0.11±5x10^{-5}	0.66±3x10^{-4}	0.10±5x10^{-5}	0.12±6x10^{-5}	20.89±0.10	1.14±0.005
SD12	21.12±0.10	0.08±4x10^{-5}	5.81±0.02	4.81±0.02	0.11±5x10^{-5}	0.40±2x10^{-4}	0.04±2x10^{-5}	0.12±6x10^{-5}	19.73±0.09	0.78±0.003
SD13	17.65±0.08	0.34±1x10^{-4}	4.56±0.02	3.41±0.01	0.14±5x10^{-5}	0.15±7x10^{-5}	0.23±1x10^{-4}	0.07±3x10^{-5}	17.85±0.08	1.09±0.005
SD14	17.56±0.08	0.34±1x10^{-4}	4.52±0.02	3.39±0.01	0.27±1x10^{-4}	0.15±7x10^{-5}	0.24±1x10^{-4}	0.03±2x10^{-5}	17.73±0.08	1.08±0.005
LR	17.36±0.08	0.46±2x10^{-4}	3.42±0.01	4.21±0.02	0.09±4x10^{-5}	0.10±5x10^{-5}	2.22±1x10^{-3}	0.03±1x10^{-5}	18.24±0.09	0.44±0.002
ELEMENTS (mg.kg^{-1})										
Samples	Ba	Cu	Ni	Pb	Rb	Sr	Th	U	Zn	Zr
SD01	<1	<1	<1	176.4±0.88	128.0±0.64	803.3±4.01	66.18±0.33	10±0.05	273.2±1.36	1111±5.55
SD02	447.8±2.24	15.98±0.08	<1	557.0±2,78	210.3±1.05	1015±5.07	118.7±0.59	68±0.34	128.5±0.64	1851±9.25
SD03	223.9±1.12	<1	<1	185.7±0.92	274.3±1.37	651.1±3.25	106.6±0.53	210±1.05	514.2±2.67	2147±10.73
SD04	1692±84.6.	19.15±0.09	<1	30.2±0.15	126.3±0.63	595.2±2.97	100±0.50	47±0.28	118.5±0.59	1339±6.69
SD05	<1	31.95±0.15	133.6±0.66	185.7±0.92	91.4±0.45	761.0±3.80	101.8±0.51	10±0.05	401.7±2.05	2517±12.58
SD06	474.7±2.37	31.95±0.15	39.29±0.19	83.6±0.41	210.3±1.21	532.7±2.66	50.54±0.25	8±0.04	321.4±1.60	962±4.81
SD07	2856±14.28	21.53±0.11	<1	57.3±0.28	99.7±0.49	438.1±2.19	106±0.53	16±0.08	176.1±0.88	1886±9.43
SD08	3094±15.47	21.07±0.10	<1	49.9±0.25	137.9±0.68	603.5±3.01	107±0.53	33±0.16	220.0±1.10	2788±13.94
SD09	209.7±1.05	23.97±0.12	<1	120.7±0.60	181.8±0.90	813.8±4.06	55.72±0.27	10±0.05	273.2±1.36	1890±9.45
SD10	447.4±2.24	<1	<1	185.7±0.93	181.8±0.90	292.6±1.46	64.69±0.32	36±0.18	192.8±0.96	4095±20.47
SD11	251.7±1.21	47.93±0.24	47.15±0.23	148.5±0.74	189.4±0.94	786.4±3.93	76.41±0.38	27±0.14	249.1±1.24	2913±14.56
SD12	321.6±1.61	15.98±0.08	<1	371.3±1.85	212.1±1.06	640.1±3.20	57.4±0.28	22±0.11	257.1±1.28	2205±11.02
SD13	2414±12.07	21.05±0.10	<1	49.9±0.25	114.1±0.57	652.2±3.26	95±0.47	24±0.12	219.8±1.09	2962±14.81
SD14	3066±15.33	20.83±0.10	<1	49.4±0.25	136.3±0.68	596.7±3.98	84±0.42	31±0.15	217.5±1.08	2755±13.77
LR	243.6±1.21	5.49±0.02	5.66±0.03	91.3±0.45	138.8±0.69	783.8±3.41	52.26±0.26	18.59±0.09	139.8±0.69	1571±7.85

SD=Sediment.

Source: research data. LR – Local Rock. Geochemistry data reported by (Valeton *et al.*,1997).

The elemental concentrations found in potential sources of contamination-PSC (fertilizers, wastes and caldasite) are shown in Table 2.

Table 2. Potential sources of contamination concentrations.

						ELEMENTS (%)					
	Samples	Al	Ca	Fe	Mg	Mn	Na	K	P	Si	Ti
BF4	RM-03	15.56±0.07	0.03±1x10^{-4}	4.55±0.02	0.07±3x10^{-4}	0.19±9x10^{-4}	0.07±3x10^{-4}	10.04±0.05	0.11±5x10^{-4}	23.00±0.11	0.44
	RM-04	14.71±0.07	0.09±4x10^{-4}	3.92±0.02	0.06±3x10^{-4}	0.15±7x10^{-4}	0.10±5x10^{-4}	10.63±0.05	0.11±5x10^{-4}	23.75±0.12	0.44
	RM-01	16.37±0.08	0.29±1x10^{-3}	5.15±0.02	0.11±5x10^{-4}	0.08±4x10^{-4}	0.22±1x10^{-3}	3.56±0.01	0.07±3x10^{-4}	17.78±0.08	0.49
	BF4-Mean	15.55±0.07	0.14±7x10^{-4}	4.54±0.07	0.08±4x10^{-4}	0.14±7x10^{-4}	0.13±6x10^{-4}	8.08±0.04	0.10±5x10^{-4}	21.51±0.11	0.46
BR	RM-05	13.97±0.07	0.04±2x10^{-4}	1.47±7x10^{-3}	0.04±2x10^{-4}	0.01±5x10^{-5}	0.06±3x10^{-4}	12.45±0.06	0.08±4x10^{-4}	25.15±0.12	0.50
	RM-06	14.55±0.07	0.02±1x10^{-4}	2.59±0.01	0.04±2x10^{-4}	0.01±5x10^{-5}	0.06±3x10^{-4}	11.54±0.05	0.07±3x10^{-4}	23.98±0.12	0.66
	RM-02	13.64±0.06	1.66±8x10^{-3}	5.22±0.02	0.27±1x10^{-3}	5.35±0.02	0.21±1x10^{-3}	2.19±0.01	0.10±5x10^{-4}	10.52±0.05	2.71
	BR-Mean	14.06±0.07	0.57±3x10^{-4}	3.09±0.01	0.12±6x10^{-4}	1.79±0.01	0.11±5x10^{-4}	8.73±0.04	0.09±4x10^{-4}	19.88±0.10	1.29
FT	FT-01	0.21±1x10^{-4}	31.73±0.15	2.03±0.01	0.23±1x10^{-3}	0.13±6x10^{-4}	0.13±6x10^{-4}	0.70±3x10^{-3}	7.64±0.03	0.65±3x10^{-4}	0.30±1x10^{-4}
	FT-02	<0.1	0.31±1x10^{-3}	0.02±1x10^{-5}	0.00±1x10^{-5}	0.00±2x10^{-5}	0.02±1x10^{-4}	0.13±6x10^{-4}	0.88±4x10^{-3}	0.35±1x10^{-4}	<0.01
	FT-03	<0.1	9.91±0.49	1.56±7x10^{-3}	0.00±1x10^{-5}	0.07±3x10^{-5}	4.01±0.02	24.65±0.12	2.40±0.01	0.41±2x10^{-4}	<0.01
	FT-04	0.26±1x10^{-4}	24.01±0.12	1.54±7x10^{-3}	0.38±1x10^{-3}	0.09±4x10^{-5}	0.38±1x10^{-3}	7.80±0.03	6.20±0.03	0.79±4x10^{-4}	0.22±1x10^{-4}
	FT-05	1.33±0,06	51.82±0.25	0.63±3x10^{-3}	9.17±0.04	0.15±7x10^{-4}	0.02±1x10^{-4}	0.37±1x10^{-3}	0.10±5x10^{-4}	3.01±0.01	0.07±3x10^{-5}
	FT-06	<0.1	25.03±0.12	0.23±1x10^{-3}	1.50±7x10^{-3}	0.00±2x10^{-5}	4.89±0.02	24.18±0.12	0.03±1x10^{-4}	0.56±3x10^{-4}	<0.01
	FT-07	3.97±0,02	18.15±0.09	5.95±0.02	6.87±0.03	0.77±3x10^{-3}	1.19±5x10^{-3}	1.58±7x10^{-3}	6.15±0.03	10.24±0.05	0.35±1x10^{-4}
	FT-Mean	1.44±7x10^{-3}	23.00±0.12	1.71±8x10^{-3}	2.59±0.01	0.18±9x10^{-4}	1.52±6x10^{-3}	8.49±0.04	3.34±0.01	2.29±0.01	0.24±1x10^{-4}
CT	CT	7.41±0.03	0.09±4x10^{-4}	27.98±0.13	0.03±1x10^{-4}	3.18±0.01	0.02±1x10^{-4}	1.99±0,01	0.65±3x10^{-4}	6.26±0.03	0.31±6x10^{-4}
						ELEMENTS (mg kg^{-1})					
	Samples	Ba	Cu	Ni	Pb	Rb	Sr	Th	U	Zn	Zr
BF4	RM-03	895.7±4.42	<1	<1	380.6±1.90	365.8±1.32	710.3±3.55	99.8±0.49	121±0.65	136.6±0.68	2073±10.36
	RM-04	1075±5.32	<1	<1	157.8±7.89	384.1±1.92	676.5±3.38	103.4±0.51	169±0.84	120.5±0.60	2443±12.21
	RM-01	1373±6.31	18.38±0,09	<1	74.5±0.37	129.6±0.64	380.2±1.90	430.0±2.10	480±2.40	928.7±4.64	1988±9.94
	BF4-Mean	1115±5.57	<1	-	204.3±1.02	293.1±1.46	589.0±2.94	211.1±1.05	256.7±1.28	395.3±1.97	2168±10.84
BR	RM-05	985.2±4.92	<1	<1	176.4±0.88	457.2±2.26	456.6±2.28	39.4±0.19	81±0.40	24.1±0.12	2591±12.95
	RM-06	1344±6.72	<1	<1	232.1±1.16	457.2±2.26	431.3±2.15	56.7±0.28	95±0.47	32.1±0.16	3924±14.62
	RM-02	3633±13.16	22.10±0.11	<1	16.1±0.08	94.1±0.47	4807.9±24.03	277.0±1.38	100±0.50	1052±5.26	971.1±4.85
	BR-Mean	1987±9.93	22.10±0.11	-	141.5±0.71	336.2±1.68	1898.6±9.49	124.3±0.62	92±0.46	369.4±1.84	2495±12.47
FT	FT-01	4210±21.05	143.8±0.71	102.2±0.51	<1	<1	11838±59.19	65.8±0.32	72±0.36	257.1±1.28	37.0±0.16
	FT-02	<1	79.89±0.39	<1	<1	<1	42.3±0.21	0.3±1x10^{-3}	1±5x10^{-3}	40.2±0.20	<1
	FT-03	<1	159.8±0.79	<1	<1	<1	2537±12.68	53.6±0.26	12±0.06	200.9±1.01	<1
	FT-04	42010±210.05	95.86±0.47	70.72±0.35	120.7±0.60	<1	5835±29.16	48.8±0.24	48±0.24	160.7±0.80	266.5±1.33
	FT-05	<1	<1	78.58±0.39	<1	<1	2368±11.84	0.3±1x10^{-3}	2±0.01	112.5±0.56	<1
	FT-06	<1	207.7±1.03	<1	<1	<1	338.2±1.69	33.0±0.16	16±0.08	40.2±0.02	<1
	FT-07	26870±134.35	591.2±2.95	722.9±3.61	102.1±0.60	45.7±0.22	5666±28.33	1.3±0.06	1±5x10^{-3}	5865±29.32	177.7±0.88
	FT-Mean	11763±56.81	213.0±1.06	243.6±1.21	111.4±0.55	45.7±0.22	4089±20.44	29.0±0.14	21.64±0.11	953.8±4.76	160.4±0.80
CT	CT	1701.7±6.00	<1	<1	529.1±2.14	<1	2114.0±10.57	611.1±30.55	1200	498.1±2.49	139921±699.60

FT=fertilizers; BF4-mean= mean of samples RM-03, RM-04 and RM-01; BR-mean= mean of samples RM-05, RM-06 and RM-02; CT=Caldasite.
Source: research data.

After calculating the enrichment factor (EF), the nine elements that obtained FE> 3 in at least one (1) sediment sample are represented in Table 3: Ba, Cu, Mg, Mn, Ni, P, Pb and U. The EF values calculated from the mean concentration of each PSC are shown in Table 3.

Table 3. Enrichment Factor for elements with at least 1 sediment sample with EF> 3. No colour (EF≤3), green (3<EF≤5), blue (5<EF≤10), orange (10<EF≤25), red (EF>50).

	Ba	Cu	Mg	Mn	Ni	P	Pb	Th	U	Total	WR	WE
SD01	-	-	0.68	0.35	-	10.72	1.72	1.13	0.48	1	10.72	P
SD02	1.50	2.55	0.73	2.11	-	5.89	4.97	1.85	2.98	2	5.89	P
SD03	0.73	-	0.65	2.97	-	3.40	1.60	1.61	8.91	1	3.40	U
SD04	12.40	3.26	1.60	1.02	-	4.38	0.59	3.42	4.51	4	12.40	Ba
SD05	-	4.68	1.10	0.80	-	3.27	0.92	0.88	0.24	2	4.68	Cu
SD06	1.40	4.66	0.85	0.52	5.56	5.45	0.66	0.70	0.31	3	5.56	Ni
SD07	8.26	3.78	1.36	1.04	-	1.76	0.44	1.43	0.61	2	8.26	Ba
SD08	9.52	3.77	5.44	1.07	-	2.01	0.41	1.53	1.33	3	9.52	Ba
SD09	0.61	-	0.83	3.09	-	3.05	0.94	0.76	0.38	2	3.09	Mn
SD10	1.28	-	0.87	1.70	-	2.41	1.42	0.87	1.35	0	2.41	P
SD11	0.82	-	0.98	4.96	-	3.80	1.28	1.15	1.15	2	4.96	Mn
SD12	0.78	2.39	0.77	2.27	-	2.84	2.40	0.65	0.70	0	2.84	P
SD13	7.43	3.77	1.24	1.07	-	2.01	0.41	1.36	0.97	2	7.43	Ba
SD14	9.53	3.75	2.30	1.07	-	2.01	0.41	1.22	1.26	2	9.53	Ba
EF>3 (SD)	5	7	1	2	1	8	1	1	2			
BF4-Mean	3.45	2.52	0.69	1.01	-	2.97	1.69	3.04	10.41	2	3.45	U
BR-Mean	9.03	4.46	1.52	18.91	-	3.93	1.72	2.64	5.48	4	18.91	Mn
FT-Mean	96.62	77.67	59.41	3.44	86.18	263.60	2.44	1.11	2.33	6	263.60	P
CT	0.85	-	0.04	3.71	-	3.14	0.71	1.43	7.89	2	3.71	U
EF>3 (souces)	3	2	1	3	1	3	0	1	3			

HE= Highest Enrichment; EE=Most Enriched Element; SD=sediment; CT=caldasite; FT=fertilizers; BR and BF4= wastes.
Source: research data.

The distribution of EF for sediments and PSC is shown in Figure 2. Phosphorus is an important macronutrient for plants and is very present in the analyzed fertilizers (Table 3 and Figure 2), with an extremely severe enrichment, while only moderately enriched in BR and CT. It is the element with the highest occurrence of results (8) with EF> 3 in the fluvial sediments: moderately enriched (SD03, SD04, SD05, SD09 and SD11), moderately severe enrichment (SD02 and SD06) to severe enrichment (SD01). Phosphorus is the most enriched element (Figure 3) in samples SD01, SD02, SD06, SD09, SD10 and SD12. The samples SD01, SD02, SD09 and SD10 are located upstream of the CUM and the caldasite ore. In these samples, fertilizers are probably the source of phosphorus enrichment.

In the SD06 sample, phosphorus and nickel (present only in SD06) are the most enriched elements. Nickel is a micronutrient for plants, and its occurrence in PSC is restricted to the analyzed fertilizers (Table 3 and Figure 2). As will be seen later, this sample is moderately enriched in copper. This P-Ni-Cu association has a severe enrichment in the evaluated fertilizers, it strengthens them as the cause of the P enrichment verified in the SD06 sample. The SD12 sample occurs in the agricultural area downstream of the CT. In this sample, the phosphorus must be related to fertilizers, but the contribution of the CT cannot be ruled out.

Copper is a micronutrient for plants; therefore it occurs in fertilizers with extremely severe enrichment, while it is moderately enriched in BR. In the sediments it is moderately enriched in 7 samples: SD04, SD05, SD06, SD07, SD08, SD13 and SD14. This copper enrichment, although moderate, is a strong indication of fertilizer input. Cu occurs as the most enriched element in sample SD05 (Figure 3).

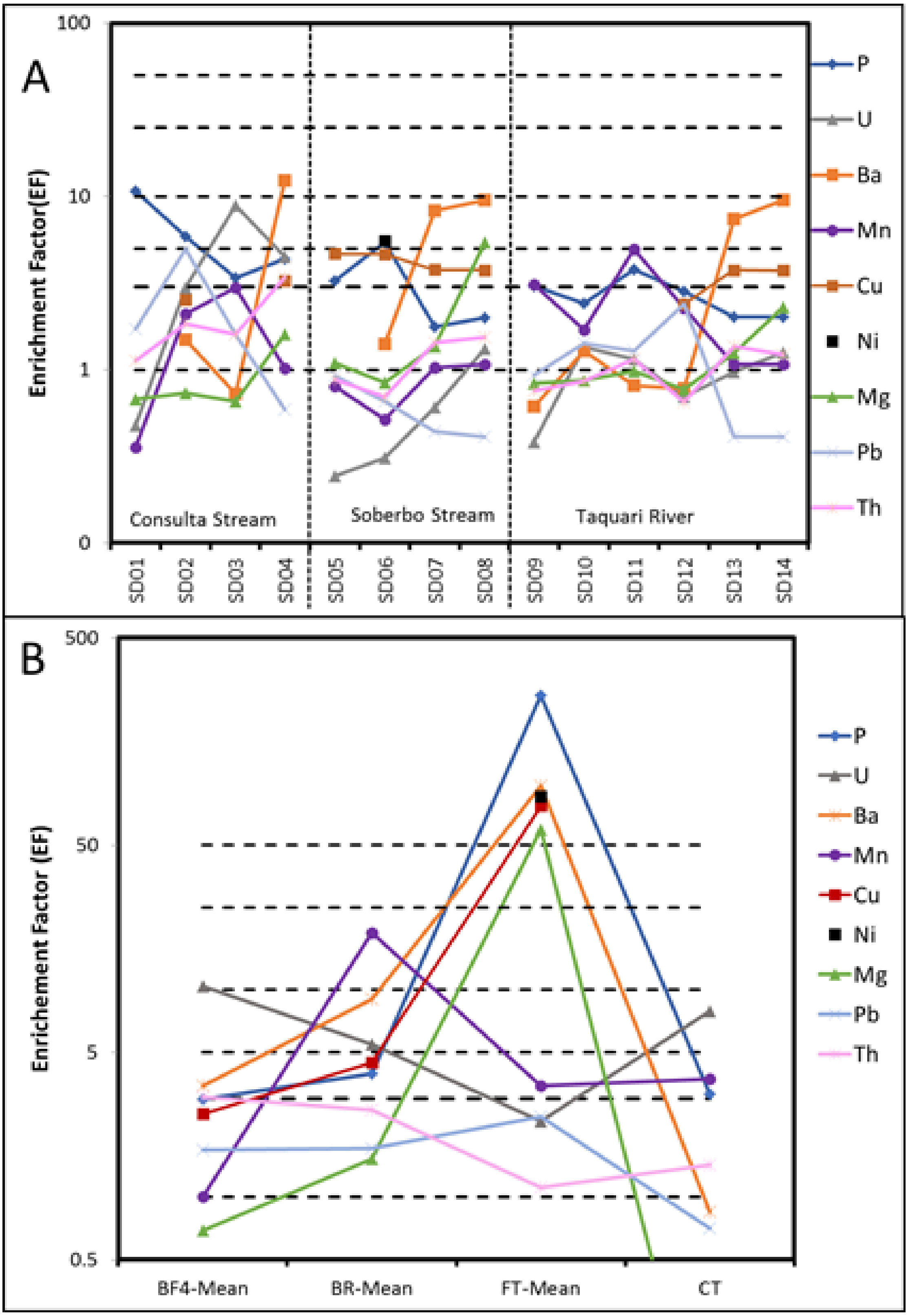

Figure 2. Distribution of EF values in A) sediments and in B) sources of contamination.
Source: research data.

Barium is moderately enriched in BR tailings, but presents extremely severe enrichment in the analyzed fertilizers. In BR, the enrichment in Ba certainly arises from the addition of $BaCl_2$ to treat BR effluents. Ba is not a plant nutrient, but is present associated with calcium and phosphorus in fertilizers. In the evaluated sediments, barium presents moderate enrichment (SD07, SD08, SD13 and SD14) to severe enrichment (SD04). In SD04 sample certainly the fertilizer application is the cause of the enrichment. In the other samples, the enrichment must come from fertilizers and/or from BR effluent discharges, since all the samples are in the drainage line downstream of this dam.

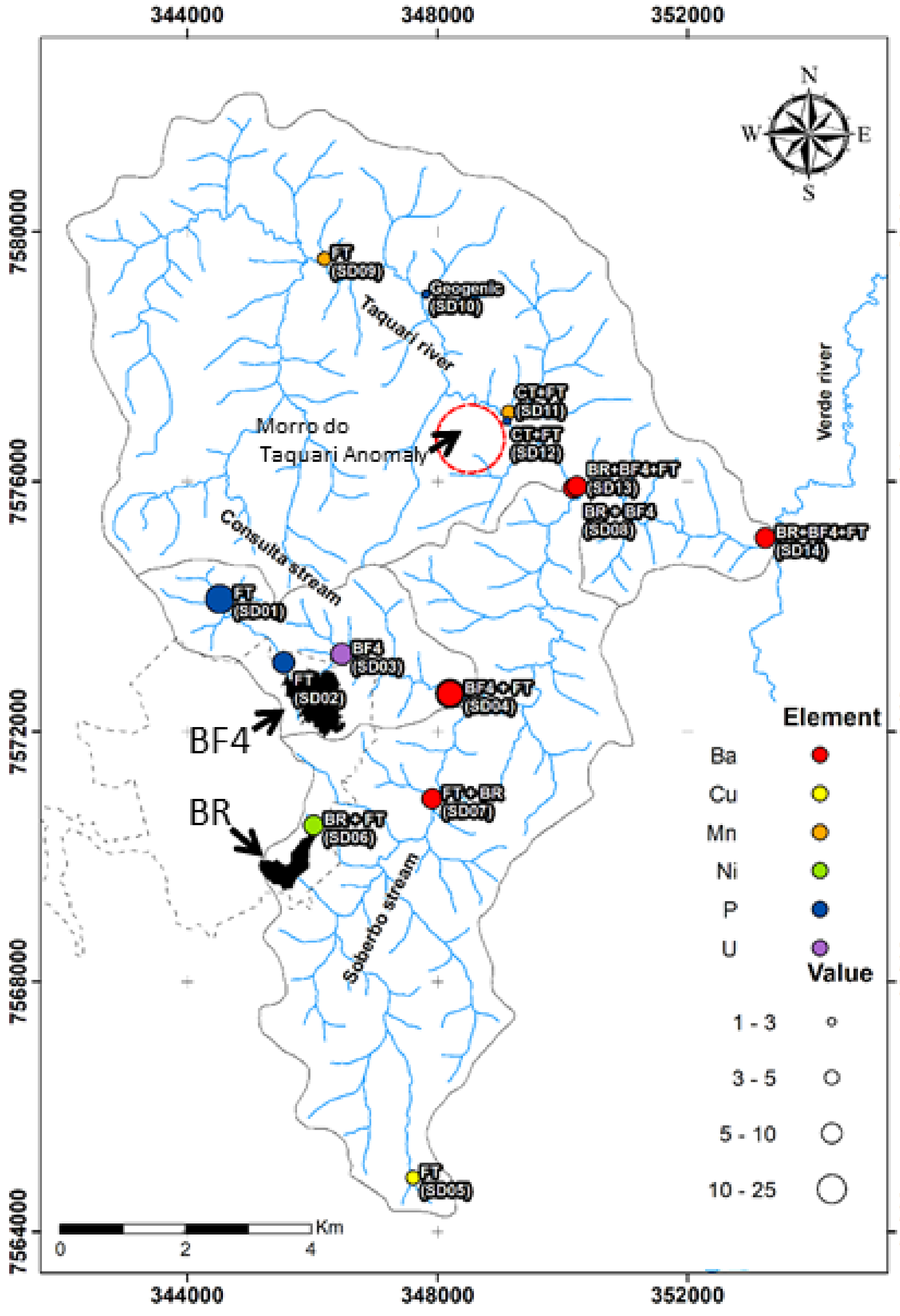

Figure 3. Distribution of the most enriched elements in each of the sediment samples and an indication of the likely source (s) for enrichment.
Source: research data.

Uranium has EF ranging from moderately severe enrichment (CT and BR) to severe enrichment (BF4). In the sediments it is enriched in samples SD04 (moderately enriched) and

SD03 (moderately severe enrichment), being the element more enriched in the latter. BF4 effluents are certainly responsible for this uranium enrichment. In the potential sources of contamination Mn has a moderately enriched (CT and FT) to moderately severe enrichment (BR). In the mineral treatment process crushed pyrolusite (MnO_2) was added to act as a uranium ore oxidizing agent (Golder, 2012). This source of exogenous Mn must be the main cause for the enrichment in Mn in the wastes deposited in BR. Mn is a micronutrient so it is found in the analyzed fertilizers. In the fluvial sediments Mn occurs moderately enriched in samples SD09 and SD11, in which it is the most enriched element. In sample SD09, located in an area with exclusively agricultural occupation, the presence of Mn (together with P) indicates the fertilizers as cause of the enrichment. In the SD11 sample, Mn may come from the fertilizers and/or from the caldasite.

Magnesium (macronutrient for plants) occurs only in fertilizers with EF> 50 (extremely severe enrichment). In the sediments it is enriched only in sample SD08 (moderately severe enrichment), probably due to the application of fertilizers. The Pb is not enriched in any of the PSC, whereas in the sediments it occurs moderately enriched in SD02 sample. It is difficult to associate this enrichment with any source, but since this sample was collected upstream of BF4, Pb must come from fertilizers. Thorium is enriched (moderately) only in BF4, which should be the thorium source for sample SD04.

4. CONCLUSION

The results obtained in this study provide an overview of the concentrations of the selected chemical elements in sediments of water bodies in the Taquari River Basin. These results indicate relevance for this region, considering the large presence of areas with fertilizer application, mining areas and natural anomaly. The application of the enrichment factor showed that the fluvial sediments present moderate to severe enrichment in P, Cu, Ba, U, Mn, Ni, Mg, Pb and Th, indicating the predominant influences of anthropogenic material and natural anomalies.

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Coliform removal in a constructed wetland system used in post-swine effluent treatment

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ABSTRACT

This study evaluated the efficiency of a constructed wetland system (CWS) in removing total coliforms (TC) and thermotolerant coliforms (ThC) of swine wastewater, as a complementary treatment to an anaerobic system. At Stage 1, the experimental system was combined using a vertical flow constructed wetland system (VFCWS) cultivated with Tifton 85 grass in series with a horizontal subsurface flow constructed wetland system (HFCWS1) cultivated with Taboa. In HFCWS1, the hydraulic detention times (HDT) were 4.7, 3.1 and 2.3 days and the surface application rates (SAR) were 294, 319 and 397 kg ha^{-1} d^{-1} of COD, in Phases I, II and III, respectively. At Stage 2, the experimental system was combined using a horizontal subsurface flow constructed wetland system (HFCWS2) cultivated with Tifton 85 grass, HDT were 6.1, 2.0 and 0.5 days and the SAR were 850, 656 and 6.34 kg ha^{-1} d^{-1} of COD, in Phases I, II and III, respectively. In Stage 1, it was verified that the VFCWS was more efficient in coliform removal when compared to HFCWS1. When only HFCWS were compared, coliform removal in Stage 1 was between 1 and 2 log units in HFCWS1. In the stage 2, the HFCWS2 was more limited, with the highest removal efficiencies during Phase I of 1.6 and 0.8 log units for TC and ThC, respectively. In general, the association resulted in efficiencies that ranged from 96.4 to 99.0% for TC, 94.2 and 97.6% for ThC, equivalent to the average removal of 1.2 to 2 log units, considered satisfactory.

Keywords: agricultural reuse, *Cynodon* spp., sanitary risk, tertiary treatment, *Typha* sp.

Remoção de coliformes em sistema alagado construído utilizado no pós-tratamento de efluentes de suinocultura

RESUMO

O objetivo deste estudo foi avaliar o desempenho de um sistema alagado construído (CWS) na remoção de coliformes totais (TC) e coliformes termotolerantes (ThC) de água residuária da suinocultura, como tratamento complementar a um sistema anaeróbio. Na etapa 1, o sistema experimental foi composto por um sistema alagado construído de escoamento vertical (VFCWS) cultivado com capim Tifton 85 em série com um sistema alagado construído de escoamento subsuperficial horizontal (HFCWS1) cultivado com Taboa. No HFCWS1 os tempos de detenção hidráulica foram de 4,7; 3,1 e 2,3 dias e as taxas de aplicação superficial foram 294, 319 e 397 kg ha^{-1} d^{-1} de DQO, nas fases I, II e III, respectivamente. Na etapa 2, o sistema experimental foi composto por um sistema alagado construído de escoamento subsuperficial horizontal (HFCWS2) cultivado com capim Tifton 85, os tempos de detenção hidráulica foram 6,1; 2,0 e 0,5 d e as taxas de aplicação superficial foram 850, 656 e 6,34 kg ha^{-1} d^{-1} de DQO, nas fases I, II e III, respectivamente. Na etapa 1, verificou-se que o VFCWS foi mais eficiente na remoção de coliformes quando comparado ao HFCWS1. Quando comparados apenas os HFCWS, verificou-se que a remoção de coliformes na etapa 1 variou de 1 a 2 unidades log no HFCWS1. Na etapa 2, o HFCWS2 mostrou-se mais limitado, apresentando maiores eficiências de remoção na fase I, de 1,6 a 0,8 unidades log para TC e ThC, respectivamente. Em geral, a associação resultou em eficiências que variaram de 96,4 a 99,0% para TC e de 94,2 e 97,6% para ThC, equivalente a remoção média de 1,2 a 2 unidades log, considerada satisfatória.

Palavras-chave: *Cynodon* spp., reúso agrícola, risco sanitário, tratamento terciário, *Typha* sp.

1. INTRODUCTION

Agricultural business is the largest consumer of water in its various stages of production, and the scarcity of water resources in quantity and quality warns of the necessity to improve processes of treatment and reuse of water. It is estimated that 50% of the world's population will live in regions with water shortages by 2025, which highlights the importance of appropriate treatment and management of water (WHO, 2015).

The biggest challenge to the use of wastewater in agriculture is to propose techniques of treatment and management that reduce the possibility of crop contamination by pathogenic microorganisms and heavy metals.

Wastewater from pig farming has some of its components (organic matter, nitrogen, phosphorus, copper, etc.) in concentrations that are sufficiently high to constitute a risk of ecological imbalance when disposed inappropriately in watercourses. However, once it is well monitored, the use of this type of wastewater in agricultural arises as an alternative to its disposal, with the benefit of recycling nutrients for crops (Cavalett *et al*., 2006).

In order to do that, the fertigation of pastures and fodder has been recommended, according to Matos (2007), as an alternative for the use of these effluents due to the rapid growth and the formation of large root mass of these cultures. Therefore, it must consider the desired levels of purification for reuse or disposal final destination, in accordance with the established conditions for the quality of water bodies receptors (Conama, 2011) or further uses, defining from these, respective processes and treatment systems.

Standards are based on the values established in the guidelines of the World Health Organization (WHO, 2006), and also in resolution N°. 430 of the National Council of Environment (Conama, 2011), which define the limits of thermotolerant coliforms by 2×10^2, x 1×10^3 and 4×10^3 MPN 100 mL^{-1} in bodies of fresh water of Classes 1, 2 and 3, respectively, which can be captured water for irrigation of different crops.

Wastewater from pig farming contains large amount of coliforms, and their disposal in the environment without proper management puts the sustainability and the expansion of pig farming at risk.

According to Fia *et al.* (2010), the use of built reactors cultivated with plant species, also called constructed wetlands systems (CWSs), as post-treatment of these effluents allows to obtain a better quality effluent that meets current environmental legislation for disposal in watercourses.

The CWSs are artificial systems consisting of ponds or shallow vegetative channels used for the treatment of wastewater rich in organic material susceptible to biodegradation, enabling the improvement of landscape aesthetics and increasing habitat for wildlife (Kadlec and Wallace, 2008). The CWSs have long been used due to their simple technology, moderate installation cost, reduced energy consumption, easy operation and maintenance (Brasil *et al.*, 2007).

In the CWSs, the removal of pathogen microorganisms occurs through a combination of physical, chemical and biological processes. Also through mechanisms of sedimentation, filtration, ultraviolet radiation, oxidation, adsorption to organic matter, exposure to biocides excreted by macrophytes, predation and natural decay (Lin *et al.*, 2005; Kadlec and Wallace, 2008; Morató *et al.*, 2014; Zurita and Carreón-Álvarez, 2014; Rachmadi *et al.*, 2016; Wu *et al* 2016; Machado *et al.*, 2017).

Plant species being cultured in CWSs, Tifton 85 grass (*Cynodon* sp.), proved to be suitable due to its high productivity and nutrient extraction capacity reached due to the fast recovery after cutting, with good coverage of the soil and preventing the development of invasive species (Queiroz *et al.*, 2004). Matos *et al*. (2009) verified satisfactory removal of ThC (average values measured at the influent and effluent were, respectively: 1.70×10^7 and 7.93×10^5 MPN 100 mL^{-1}) in CWSs treating swine wastewater pretreated in organic filters. In addition, Tifton 85 grass can tolerate wide temperature variations and shows interesting nutritional characteristics for livestock (Sanches *et al.*, 2016; Pereira *et al.*, 2012; Matos *et al.*, 2013; Amorim *et al*., 2015a).

Thus, this work aimed to evaluate the removal of total coliforms (TC) and thermotolerant coliforms (ThC) of swine wastewater treated in a constructed wetland system (CWS) as a complementary treatment to the anaerobic system, and to verify the contamination of the aerial part of the plant grown in the CWS.

2. MATERIAL AND METHODS

The experiment was conducted in the area of wastewater treatment in the Lavras Federal University (UFLA) Department of Animal Science (DZO), under the responsibility of the UFLA Engineering Department (DEG), Minas Gerais, Brazil, with geographic coordinates 21°13'55" South latitude and 44º58'12" West longitude, and the average elevation of 895 m.

The system was installed in a protected environment (greenhouse), with a plastic structure of transparent polyethylene of 150 mm. The structure dimensions were: 12 m long by 10 m wide, 3 m ceiling height and 1.5 m arches. On the sides of the greenhouse were installed black mesh shading (Sombrite®50%). The experiment was conducted in two stages.

2.1. Stage 1

The swine wastewater was from the DZO Pig Sector. The swine wastewater treatment is composed of a static sieve pretreatment and primary treatment compound of a decanter, a secondary treatment compound Anaerobic Baffled Reactor (ABR) followed by UASB reactor (Pereira *et al.*, 2012). In this way, the swine wastewater used in this stage was the anaerobic treatment system effluent.

The experimental arrangement was composed of two constructed wetlands systems. A vertical flow constructed wetland system (VFCWS) and a horizontal subsurface flow construct system (HFCWS1) (Figure 1).

The VFCWS consisted of fiberglass tanks, with total volume of 100 L, with 0.54 m tall and 0.86 m in diameter, filled with gravel (diameter D-60 = 7.0 mm and initial porosity of 0.494 $m^3\ m^{-3}$).

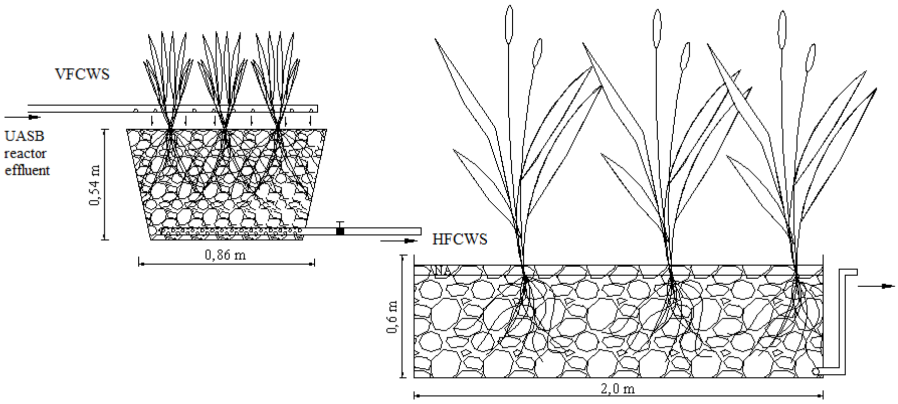

Figure 1. Schematic diagram of vertical flow constructed wetland system (VFCWS) and horizontal subsurface flow constructed wetland system (HFCWS1).

In HFCWS1, 4, chicanes were equally spaced installed along the structure in order to improve the distribution of the flow inside the unit. The dimensions of HFCWS1 were 2.0 m long by 0.5 m wide and 0.70 m in height. The HFCWS1 was filled with same the gravel, as a support, up to the height of 0.55 m, comprising a porosity of 0.494 m^3 m^{-3}. The disposal of the flow occurred at 0.05 m below the surface, totaling a volume of 237 L, more details can be obtained from the work of Fia *et al*. (2014).

The VFCWS was cultivated with Tifton 85 grass (*Cynodon* spp.) from DZO Forage Sector. The density of planting was 20 seedlings per m^2. The specie cultivated in the HFCWS was “Taboa” (*Typha* sp.). The seedlings were obtained in natural wetlands in the UFLA Fish Farming Sector. The density of planting was 14 seedlings per m^2.

This stage was composed of three phases (September 2011 to February 2012). Phase 1 was carried out to adapt the systems to the swine wastewater, it lasted 80 days. In Phase 2 and Phase 3, the surface application rates were increased, with a duration of 60 days each.

The differentiation in the surface application rates was made through the variation of the VFCWS affluent. VFCWS feeds were made through a solenoid metering pump and hoses of PVC, which pumped from a container that held swine wastewater. The pumped wastewater came from an existing effluent originated from a previous treatment system (ABR and UASB reactors and decanter). The HFCWS1 was conducted by gravity from the VFCWS. The operational characteristics of monitoring were obtained from the affluent COD concentration multiplied by the input flow and dividing by the surface area of the VFCWS and HFCWS1. The hydraulic retention time values were obtained by dividing the flow by HFCWS1 volume. The operational characteristics of wetlands systems are presented in Table 1.

Table 1. Operational average characteristics observed in vertical flow constructed wetland system (VFCWS) and horizontal subsurface flow constructed wetland system (HFCWS) at the phases of operation of the treatment system in Stage 1.

System	Phase I (80 d)			Phase II (60 d)			Phase III (60 d)		
	HDT^{51}	Q^{51}	SAR^{13}	HDT^{29}	Q^{29}	SAR^{9}	HDT^{24}	Q^{24}	SAR^{8}
VFCWS	-	0.064	763	-	0.095	828	-	0.129	1,032
HFCWS	4.7	-	294	3.1	-	319	2.3	-	397

Legend: HDT - hydraulic detention time (days); Q - flow (m^3 d^{-1}); SAR - surface application rate (kg ha^{-1} d^{-1} of COD); superscript the number of sampling considered in the calculation of the average.

2.2. Stage 2

The constructive characteristics were kept identical to HFCWS1 (Stage 1). However, the HFCWS2 was cultivated with Tifton 85 grass (*Cynodon* spp.). Parts of the stem of the plant, from DZO Forage Sector, were planted in plastic containers, containing sand and a mixture of water and swine wastewater in proportion of 1:1 (v/v), for the development of the root system. After 15 days, it was planted in the HFCWS2. This procedure was performed during 40 days before placing the swine wastewater in the treatment system, using the density of 25 plants per m^2. Every 2 days, in this initial stage, water and swine wastewater in proportion of 1:1 (v/v) was applied, according to the CWS evapotranspiration.

The swine wastewater applied in HFCWS2 was also from an anaerobic system (Amorim *et al*., 2015b). The effluent was conducted by continuous-flow pipe, with the aid of a metering pump for UASB and subsequently by gravity to the CWS.

This stage had three phases during the monitoring (February to July 2014), being the duration periods determined by forage cutting, which were carried out when the first inflorescence happened. This fact occurred 60 days after the beginning of the monitoring system, 32 days after the first cut of the plants and 50 days after the second cutting of plants, coinciding with the completion of Phases I, II and III, respectively. From the affluent concentration of COD, the flow, and the surface area of the HFCWS2, were obtained the operational characteristics of monitoring. The hydraulic detention time values were obtained by dividing the flow by the HFCWS2 volume. The operational characteristics of HFCWS2 are presented in Table 2.

Table 2. Operational characteristics observed in horizontal subsurface flow constructed wetland system (HFCWS2) at the phases of operation of the treatment system in Stage 2.

Variables	Phase I (47 d)	Phase II (32 d)	Phase III (52 d)
HDT	6.1 $^{(20)}$	2.0 $^{(20)}$	0.5 $^{(18)}$
Q	0.0439	0.1175	0.4766
SAR	850 $^{(18)}$	656 $^{(9)}$	6,335 $^{(14)}$

Legend: HDT - hydraulic detention time (days); Q - flow (m^3 d^{-1}); SAR - surface application rate (kg ha^{-1} d^{-1} of COD); superscript the number of sampling considered in the calculation of the average.

At the end of each phase, which coincided with the emergence of the first stems, the grass with 5 to 7 cm height above the middle support was mowed. Fresh biomass collected, 10 g every 0.40 m along the CWS, was placed in a plastic bag with 90 mL of peptone (0.1%) and taken to 3,500 rpm in a Stomacher Homogenizer for 3 minutes for immediate microbiological analysis. Therefore, the quantification of total coliforms was carried out using the method of multiple tubes (APHA *et al.*, 2005). Three repetitions were made and the assessment was done in triplicate for each sampling.

In two phases, the quantification of coliforms presented in the influent was held weekly, also by the method of multiple tubes (APHA *et al.*, 2005). The instantaneous air and liquid temperature in treatment was measured daily, at 07h00 AM by a *thermo-hygrometer*.

3. RESULTS AND DISCUSSION

During the monitoring system, the temperature in the greenhouse ranged from 14.9°C to 36.5°C in the first stage, and from 8.0°C to 49.0°C in the second stage. However, swine wastewater in treatment showed lower temperature variation, with average values of 23.8°C, 23.5°C and 23.8°C; 26.5°C, 20.8°C and 18.0°C, in Phases I, II and III, of Stages 1 and 2, respectively. Temperature is extremely important in biological treatment processes, being related to the speed of biochemical reactions and enzymes of microorganisms responsible for pollutant removal, reducing the temperature values along the phases may interfere in the removal efficiency of substrates and microorganisms present in the swine wastewater (Olson *et al.*, 2004). Winward *et al.* (2008) reported that seasonal changes in temperature could strongly influence the coliform removal in CWS, in which was a positive correlation between the removal of bacteria and the temperature rise.

In this way, the reduction of the temperature of the liquid, especially in the Phase III of Stage 2, may have contributed to a minor reduction in the number of indicators of fecal contamination, compared to previous phases. The constants of the first order for decay of *E. coli* in CWS obtained by Boutilier *et al.* (2009) were 0.09 d^{-1} at 7.6°C and 0.18 d^{-1} at 22.8°C.

The amount of TC and influent concentration to the experimental units varied according to the composition of the wastewater, which was dependent on the cleaning procedure and handling of swine breeding facilities (Figures 2 and 3). This variation reflected in effluent concentrations of the systems, as the secondary treatment units, such as the constructed wetlands systems feature, are less efficient to removal these microorganisms. Von Sperling (2005) reports that anaerobic reactors and CWS are able to remove between one and three and one and four log units of resolutions, respectively.

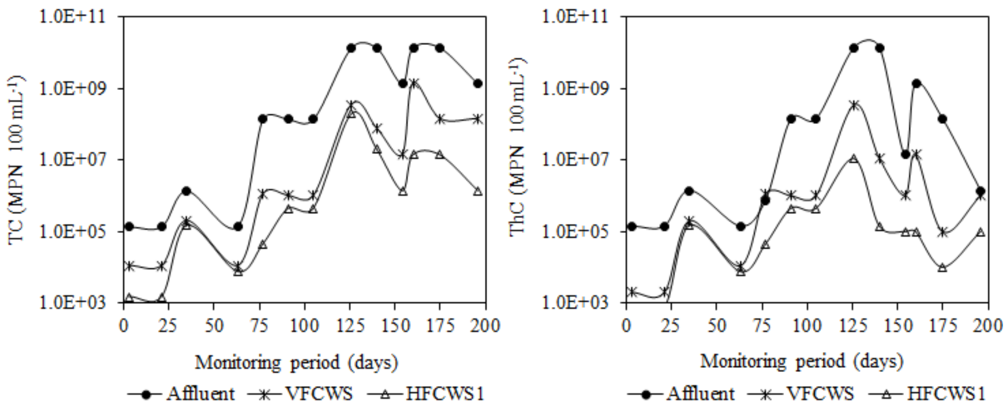

Figure 2. Variation in most probable number of total coliforms (TC) and thermotolerant coliforms (ThC) in the affluent and effluent from vertical flow constructed wetland system (VFCWS) and horizontal subsurface flow constructed wetland system (HFCWS1) at the phases of operation of the treatment system in Stage 1.

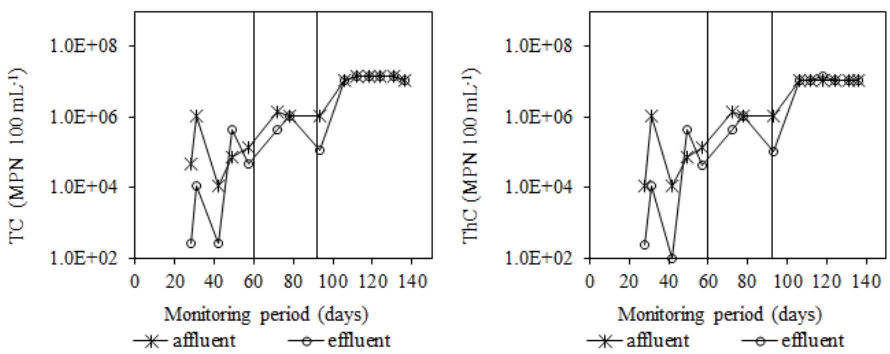

Figure 3. Variation in most probable number of total coliforms (TC) and thermotolerant coliforms (ThC) in the affluent and effluent of horizontal subsurface flow constructed wetland system (HFCWS2) at the phases of operation of the treatment system in Stage 2. Vertical lines indicate the changes in the monitoring phase.

In general, in Stage 1, it was found that the VFCWS was more efficient in the removal of coliforms compared to HFCWS1. This fact is related to the operation manner of the systems. The VFCWS was not kept saturated with effluent, increasing the possibility of removing the microorganisms by humidity reducing (Kadam *et al.*, 2008; Tunçsiper *et al.*, 2012). Abou-Elela and Hellal (2012) achieved in domestic sewage treated with VFCWS $1,25 \times 10^3$ MPN 100 mL^{-1}, which resulted in an efficiency of removal between 94.0% and 99.9%. Authors attributed the best efficiencies observed, when compared to literature, to the highest rate of oxygenation in vertical systems, in addition to the highest temperature observed (25 to 30°C) during the experiment. Aerobic conditions reduce the length of survival for pathogens in constructed wetlands systems.

Increasing the organic load and the consequent reduction in the hydraulic detention time, in different phases of the two stages, resulted that the effluent concentrations of the VFCWS, HFCWS1 and HFCWS2 also followed an increase in the number of microorganisms to the system. This fact was also verified by Hill and Sobsey (2001) in constructed wetland system.

In Stage 1, it was verified that, independent of the concentration of coliforms and the applied, there was a higher coliform removal, compared to Stage 2 (Table 3). Although in Stage 1 higher values of coliforms were verified in the influent, mainly in the third stage, it seems that the hydraulic detention time and organic concentration are the most affected factors in coliform removal efficiency in constructed wetlands systems.

Table 3. Average removal of total coliforms (TC) and thermotolerant coliforms (ThC), in %, in vertical flow constructed wetland system (VFCWS) and horizontal subsurface flow constructed wetland system (HFCWS1) in Stage 1, and horizontal subsurface flow constructed wetland system (HFCWS2) in Stage 2 at the phases of operation of the treatment system.

Stage	Phase	TC			ThC		
		VFCWS	HFCWS1/HFCWS2	Total	VFCWS	HFCWS1/HFCWS2	Total
1	I	92.2 (2.0)	65.1 (1.8)	96.3 (2.0)	75.0 (1.6)	55.5 (1.7)	95.4 (2.0)
	II	98.9 (2.0)	56.6 (1.7)	99.5 (2.0)	99.0 (2.0)	76.4 (1.9)	99.8 (2.0)
	III	94.5 (2.0)	94.5 (2.0)	99.9 (2.0)	80.1 (1.9)	92.3 (2.0)	98.1 (2.0)
2	I	-	73.0 (1.6)	-	-	73.0 (0.8)	-
	II	-	53.0 (0.7)	-	-	53.0 (0.0)	-
	III	-	0.0 (0.2)	-	-	0.0 (0.2)	-

The values in parentheses correspond to the log units removed.

When only the HFCWS were compared, it was verified that removal of coliform in the first phase ranged from 1 to 2 log units in HFCWS1. In Stage 2, the HFCWS2 showed to be more limited, being the highest removal efficiencies in Phase I, 1.6 log units for TC and 0.8 log units for ThC. The smallest removal in Phases II and III may be related to an increase in the load rates and, consequently, to the reduction of hydraulic detention time, besides the lowest temperatures.

Highest values of hydraulic detention time increase the exposure of bacteria to removal processes such as sedimentation, adsorption, predation, and exposure to toxins excreted by microorganisms and plants (Díaz *et al.*, 2010). The reduction of hydraulic detention time, due to the increase in the flow rate, influence on the hydraulic regime, potentially leading to short hydraulic circuits and reduced removal efficiency (Jasper *et al.*, 2013; Weerakoon *et al.*, 2013).

From the data compilation presented by Wu *et al*. (2016), it is possible to verify that the removal of TC in horizontal flow constructed wetland increased from 72.5 to 99.7% when the hydraulic detention time was increased from 2 to 8 days, while the removal of ThC increased from 63.5 to 99.2%.

Chagas *et al.* (2012) managed to remove from domestic sewage treated in CWS, 1 to 4 and 2 to 4 log units of TC and *E. coli*, respectively. Such removals have been directly associated with the increase of HDT and consequent decrease of organic load rates in the CWS.

According to Calijuri *et al*. (2009), 2 log units removals of TC and ThC (efficiencies exceeding 99.0%) were verified in CWS with hydraulic detention time between 4.5 and 5 days. Gonzales *et al*. (2009) achieved the removal of 3.3 to 4.2 log units in CWS with hydraulic detention time in 3 days used in the treatment of swine wastwwater with 10^8 to 10^{10} MPN 100 mL^{-1} of total coliforms.

Matos *et al.* (2009) used a CWS cultivated with Tifton 85 grass, with 4.8 days of hydraulic detention time for treatment of swine wastewater pre-treated in organic filters. An average removal efficiency of 98.3% of *E. coli. was verified.* The effluent showed from 10^4 to 10^7 x 100 mL^{-1} MPN of total coliforms, and from 10^4 to 10^6 MPN 100 mL^{-1} of *E. coli*, with an average reduction of 2 log units.

In this study, although there are stages subject to different conditions (Stage 1 and Stage 2), it was found that higher efficiencies are obtained with the application in vertical-horizontal systems; better performance occurred in vertical systems, as reported by Vymazal (2005) and Haghshenas-Adarmanabadi *et al*. (2016). In general, the VFCWS associated to HFCWS resulted in efficiencies that ranged from 96.4 to 99.0% for TC and from 94.2 to 97.6% to ThC, equal to an average of 2 to 3 log units of removal, considered as satisfactory. Vymazal (2009) reports that the CWS can remove between 1 and 4 log units of ThC. Certifying the efficiency of CWS, Elfanssi *et al.* (2018) obtained remove over 4 units log in a hybrid constructed wetland system (vertical-horizontal) in domestic effluent treatment.

Observed concentration of coliforms in the effluent in this work remained high, which could restrict the application of treated swine wastewater in fertirrigation, mainly for some plant species. The effluent values recommended by the World Health Organization, depending on the type of crop being irrigated, vary from 10^3 to 10^6 x 100 mL^{-1} MPN ThC (*E. coli)* (WHO, 2006). The standard used, less than or equal to 10^3 x 100 mL^{-1} of MPN ThC (ABNT, 1997). It is noted that for unrestricted irrigation a safety margin is applied, more restrictive than World Health Organization guidelines.

Bastos *et al.* (2008) showed that the irrigation of vegetables that grow low to the ground with effluent from stabilization ponds resulted in acceptable products for consumption, according to the Brazilian legislation criteria for the microbiological quality of food (ANA, 2001). This also was verified with the irrigation of vegetables that grow far from the soil with wastewater containing around 10^4 100 mL^{-1} MPN of *E. Coli.*

Bevilacqua *et al*. (2014) evaluated the health effects on livestock consuming forage fertirrigated by the method of flood and sprinkling system, with biological reactors, whose effluent number of coliforms was greater than recommended by the World Health Organization and found that there was no contamination injurious to health. They stated that this practice did not cause disease (or death) in animals.

Regarding the microbiological analysis of species, coliform contamination has not been verified in Tifton 85 grass (the values obtained in the analysis were equal to zero), although it is believed that the subsurface runoff may contribute by the lack of direct contact of effluent with the aerial parts of the plant.

4. CONCLUSIONS

The decrease in hydraulic detention time during the stages reduced coliform removal efficiency.

The increase in organic loads concomitantly with the low temperatures influenced the decrease in coliform removal efficiency.

Effluent concentration of coliforms remained above the World Health Organization recommendations, however, there was no contamination by coliforms on the shoots of Tifton 85 grass.

The results obtained indicate the necessity of a detailed study of other variables, in order to better understand the removal mechanisms of bacteria coliform groups in constructed wetlands systems.

5. ACKNOWLEDGMENTS

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Composition and floral diversity in Andean grasslands in natural post-harvest restoration with *Lepidium meyenii* Walpers

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ABSTRACT

The Andean grassland ecosystems undergo natural and anthropogenic degradation processes. The change of land use for agricultural use is the greatest threat, with a great loss of biodiversity followed by a very slow process of revegetalization. The objective was to assess the richness, abundance and diversity, alpha and beta, in areas of two-, three-, five-, six- and eight years of post-harvest abandonment of *Lepidium meyenni* Walpers. Ten affected areas were selected for agrostological evaluation, through four linear transects of interception points with 100 records each, applied on the edge and inside the affected area, inside and outside the affected areas, as well as samples composed of soils for each area. Composite soil samples were collected from each transect and study area to analyze their physical and chemical properties. The data were analyzed using the generalized linear mixed model with Rstudio v 5.3.2, and the multivariate analysis of canonical correspondence between biological and environmental variables, using CANOCO v.1.4 software. A significant difference was found between floral composition and alpha diversity according to abandonment time and seasonal periods. The greatest richness and abundance was observed between five- and eight years of abandonment, due to the greater presence of perennial species. Linear correspondence of alpha richness and diversity with organic matter, nitrogen, soil phosphorus and abandonment time was observed.

Keywords: alpha diversity, Andean grassland, beta diversity, dominance richness.

Composição e diversidade florística em campos andinos em restauração pós-colheita natural com *Lepidium meyenii* Walpers

RESUMO

Os ecossistemas de prados andinos sofrem processos de degradação natural e antropogênica. A mudança de uso da terra para uso agrícola é a maior ameaça com grande perda de biodiversidade, seguido por um processo muito lento de revegetação. O objetivo do estudo foi avaliar a riqueza, abundância e diversidade alfa e beta em áreas de dois, três, cinco, seis e oito anos pós-colheita *Lepidium meyenni* Walpers. Foram estabelecidas dez áreas afetadas para avaliação agrostológica por meio de quatro transectos lineares de pontos de interceptação com 100 registros aplicados na borda e no interior da área afetada. Amostras compostas de solo foram coletadas de cada transecto e da área de estudo para analisar suas propriedades físicas e químicas. Os dados foram analisados por meio do modelo linear generalizado misturado com

Rstudio v 5.3.2 e da análise multivariada de correspondência canônica entre variáveis biológicas e ambientais, utilizando o software CANOCO v.1.4. Uma diferença significativa foi encontrada entre composição florística e diversidade alfa de acordo com o tempo de abandono e períodos sazonais. A maior riqueza e abundância foi observada entre cinco e oito anos de abandono, devido à maior presença de espécies perenes. Observou-se correspondência linear de riqueza e diversidade alfa com matéria orgânica, azoto, fósforo do solo e tempo de abandono.

Palavras-chave: diversidade alfa, diversidade beta, pastagem Andina, riqueza florística e dominância.

1. INTRODUCTION

Natural grassland ecosystems include a diversity of plant species and provide habitat for diverse wildlife species, as well as important resources for humanity and the environment (Rolando *et al.*, 2017). The floral diversity of the grassland ecosystems of these high Andean ecoregions (Britto, 2017) is similar in complexity and heterogeneity to the patterns of floral variation determined by gradients (Cuesta and Becerra, 2012). Species richness generally declines as elevation increases (McCain and Grytnes, 2010). Whittaker (1960) demonstrated a positive relationship between temperature and precipitation and species richness for many diversity patterns (Hawkins *et al.*, 2003) and as the study area increases, the number of species found increases (Rosenzweig, 1995). On the other hand, the history of agricultural-livestock use (Mudongo *et al.*, 2016) and the feeding ofanimal by grazing (Yaranga *et al.*, 2018), the strategy of grasslands' adaptation to climate change and the economic factors of the herders (Hinojosa *et al.*, 2016) determine the diversity of species.

These ecosystems are being severely disturbed in their structure and function by the change of pasture land use for agricultural purposes, with a great loss of biodiversity and the generation of a secondary succession process (Schmidt, 2018). Revegetation begins with the establishment of pioneering invasive species unrelated to the original composition (Wiesmair *et al.*, 2017), generating important changes in physiognomic and physiological properties (Gartzia *et al.*, 2016; Silva Mota *et al.*, 2018) and causing the decrease of multiple environmental services according to the variation of spatiotemporal scenarios (Tang *et al.*, 2018). In different parts of the world faced with these problems, strategies have been developed to reverse these processes through conservation and restoration (Jurado *et al.*, 2013; Caro *et al.*, 2014).

In the central Andes of Peru, the change in the use of Andean grasslands through the cultivation of *Lepidium meyenii* Walpers, due to the removal of the soil with loss of vegetation cover, constitutes the greatest regional concern. This is due to the fact that the recovery of plant communities is slow during the four years of rest, and their capacity to shelter diverse species is diminished (Cao *et al.*, 2017), and they are gradually being repopulated by herbaceous communities very different from the original ecosystem, substituting the original plant community for another (Cárdenas, 2013).

The quantification of species richness, abundance and similarity through alpha and beta diversity indices allows studying changes in the plant community. For this, it is important to identify the species with the highest importance (Silva Mota *et al.*, 2018) based on the relative abundance and community structure of species (Moreno, 2001; Moreno *et al.*, 2011; Barragán Solís *et al.*, 2011), since these determine the characteristics of the plant community and the function of the ecosystem according to environmental gradient and limiting factors referred to by Whittaker and Clements (Hagen, 2012), in addition to being an indication of the level of relevance among the other species and the level of spatial occupation (Enriquez *et al.*, 2016). Alpha diversity, also known as specific diversity, varies in time from one site to another, in relation to the spatial and temporal gradient between communities or habitats (Halffter *et al.*, 2005), and is used in the ecological evaluation of conservation and restoration of ecosystems

(Caballero-Vázquez and Vega-Cendejas, 2012). Beta diversity, on the other hand, implies knowing the degree of replacement of species between communities or the number of species that share two or more communities (Moreno, 2001).

This study assessed the richness, abundance and floral diversity of vascular plants in Andean grassland areas with land-use change, in space-time gradients of two-, three-, five-, six- and eight years of post-harvest abandonment of *Lepidium meyenii* Walpers and two seasonal periods.

2. MATERIAL AND METHODS

2.1. Study area

The study was conducted in "Lomo Largo", in the district of Pomacancha, province of Jauja and department of Junín, between coordinates 11º33'37.50" and 11º32'02.35" and between altitudes 3998 and 4262 meters (Figure 1). The study area is covered with natural grass vegetation, mostly poaceae and asteraceae, with a scarce presence of shrubs such as *Margiricarpus pinnatus* and *Chuquiraga spinosa*, which are generally observed in rocky areas and rocky outcrops. The soils are of colluvial origin with parental material formed mainly by rocks: sandstones, shales, slates and quartzite; of very variable depth between 10 and 30 cm, and generally poor in nutrients. The soil is classified as “entisol” according to the "Soil Taxonomy" of the USA, containing less than 30% of rock fragments (USDA, 2018). However, due to the high physiographic heterogeneity, other soils such as inceptisols and mollisols are observed, due to the altitude of over 3800 meters, cold temperatures, grass cover, and slopes differentiated between 2% and 15%.

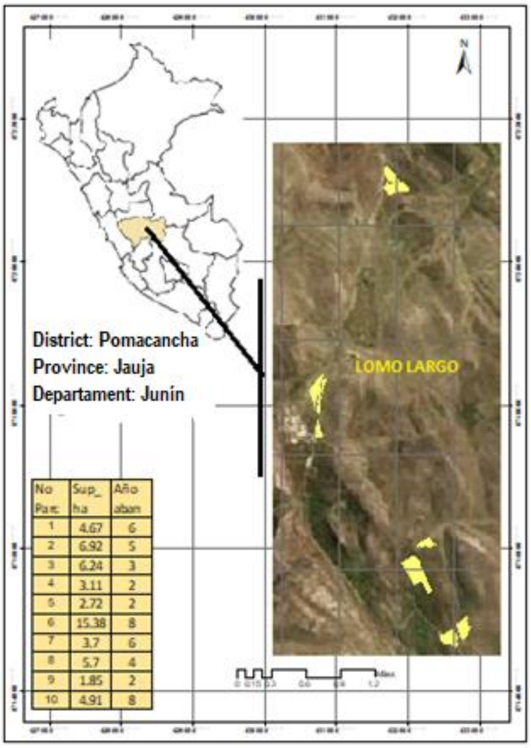


Figure 1. Location map of the study. Área 1 starts on the north side.

The rainy season occurs from October to April, with a peak of greater precipitation between January and March, with accumulated precipitation between 106 and 118 mm per month. The dry season occurs from May to September with minimum precipitation of 8.9 to 28 mm per month. The average maximum daytime temperature fluctuates between 12.87 and 14.63°C, the minimum nighttime and early morning temperature fluctuates between 0.8 and 5.4°C, according to data obtained from the Ricran Meteorological Station.

2.2. Methods

2.2.1. Agrostological evaluation and soils

Ten areas affected by land-use change were identified and established with two-, three-, five-, six- and eight years of post-harvest abandonment with *L. meyenii*, based on cultivation history provided by the ranchers at the study site. The agrostological evaluation was carried out using the "point interception line" method proposed by the Gloria Method (Enriquez *et al.*, 2016), which consisted of reading 100 points at intervals of 10 cm, in which the following were recorded: vascular plant species, mulch, moss, eroded soil and rock. In each affected area, four transects on the outer edge (native vegetation) and four transects inside the affected area (revegetation) were carried out in May (rainy season) and August (dry season) in 2017, according to the protocol of the GLORIA methodology (Cuesta and Becerra, 2012), used by the Network to monitor the impacts of climate change on biodiversity in the countries of the Andean region (Halloy, 2011). Species recognition was carried out by experts from the Faculty of Zootechnics and the Faculty of Forestry and Environmental Sciences (depository of the constructed herbarium) of the National University of Central Peru, authorized by Resolution No. 095-2018-MINAGRI-SERFOR/DGGSPFF of the Forestry and Wildlife Service.

Soil sampling was carried out according to the technique recommended by the Ministry of the Environment [MINAM] (Perú, 2014) at a depth of 30 cm. Three soil samples were collected per transect, which were mixed to obtain a sample composed of native vegetation and another from the revegetation area, which were sent to the soil laboratory of the National Agricultural Research Institute (INIA) Santa Ana de Huancayo, for the analysis of soil texture, pH, organic matter (OM), nitrogen (N), phosphorus (P) and potassium (K).

2.2.2. Data analysis

The data obtained in the field and laboratory were systematized in a double entry matrix in Excel sheet, adopting the method of the species list presentation used by Uchida *et al.* (2018) for each area, separated between native vegetation and revegetalization area. The agrostological sampling quality test was performed using the cumulative curve method recommended by Kindt and Coe (2011). The floral data was transformed into abundance using the formula: number of individuals of the species divided by the total number of individuals recorded for each transect. The procedure performed by Ali *et al.* (2017) in the Significant Minimum Difference (LSD) comparison test was adopted to separate the different means ($p \leq 0.05$) using the generalized mixed linear model, with the use of RStudio software version 3.5.2. in the analysis of richness and abundance, Shannon Wiener alpha diversity index (H'), and Jaccard (Ij) beta diversity index (Moreno, 2001). Multivariate analysis was also carried out between biological variables and environmental variables (revegetation time, seasonal period and soil characteristics) as explanatory variables, by means of the canonical correspondence analysis (CCA) that allows for visualization in a geometric figure, the proximities between the set of biological data with certain predictor variables (Cayuela, 2011) in a final biplot graph (Montanero, 2018), using CANOCO software version 4.5.

3. RESULTS AND DISCUSSION

3.1. Richness and abundance

In the native vegetation around the affected areas, 24 families with 57 genera and 79 species were found, of which the family Poaceae contributes with 28 species, the family Asteraceae with 16 species, followed by Fabaceae with four species. In the areas in post-harvest revegetation of *L. meyenii*, abandoned after the only harvest of the product, 23 families with 55 genera and 73 species were found, including 23 species of the family Poaceae and 16 species of the family Asteraceae. The composition of genera and species in both vegetations varied according to seasonal periods and post-harvest abandonment time. The greatest importance of the Poaceae family is a fundamental characteristic of natural grass ecosystems of any environmental condition in the world (Fiallos *et al.*, 2015); it diminishes or loses its composition due to anthropogenic intervention, and does not show a complete recovery, despite several years of revegetation (Sedlar *et al.*, 2017). Significant differences were observed in the number and abundance of species between seasonal periods, mainly in revegetation areas. Richness varied according to seasonal periods and post-harvest abandonment time with *L. meyenii*.

The small variation in the number of families could be due to the environmental conditions of location within the humid Subalpine Tropical paramo life zone, where the 10 evaluated areas are located. However, the variation in the number of genera and species was significant after five years of post-harvest abandonment, due to the rugged microclimatic condition of the soil and the scarcity of rainfall (Uchida *et al.*, 2018). The composition and floristic structure continued until the age of 15, since the process of revegetation in the areas affected by land-use change follows a sequential pattern, first entering temporary species with great capacity to adapt to hostile environmental situations, by the existence of free spaces that allow them to take direct advantage of solar radiation, mainly the infrared that enhances their photosynthetic capacity (Silva Mota *et al.*, 2018), then they are gradually replaced by perennial species more tolerant to competition for light and space. This information is valid for decision making in research processes and the planning of directed restoration actions in grassland ecosystems.

The variation of the richness in the areas of revegetalization according to seasonal period was significant, with p = 0.001 and at post-harvest abandonment time level with *L. meyenii*. The areas of five-, six- and eight years were superior to the first years with p = 0.01, according to the ANOVA analysis using the generalized mixed linear model (Figure 2), the most common species in years two and three post-harvest with *L. meyenii* were: *Paspalum pygmaeum* Hack, *Alchemilla pinnata* Ruiz & Pav, *Bidens andícola* Kunt *and Oxalis sp*, while from the age of five they were: *P. pilgerianum* Hack, *A. pinnata*, *Calamagrostis heterophylla* Wedd, *Stipa depauperata*, *B. andícola.*

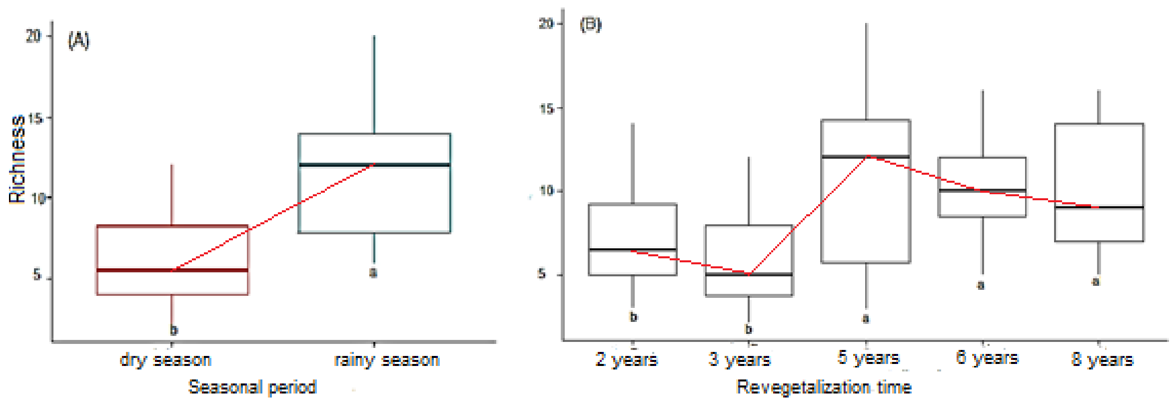


Figure 2. variation of the richness in plots in revegetalization, according to **(A)** seasonal period and **(B)** postharvest time of *L. meyeni*.

The abundance in revegetation plots also varied significantly between seasonal periods with p = 0.0001, while at the post-harvest time level no significant difference was found (Figure 3). The 10 species that showed the greatest abundance were: *P. pilgerianum*, *P. pygmaeum*, *Trifolium amabili* Lojac, *A. pinnata*, *Oxalis andicolae* Knut, *sp*, *Calamagrostis heterophylla*, *Paronychia sp*, *Rumex asetocella*, *S. depauperata* Pilg and *B. andícola*; of which Alchemilla, Calamagrostis and Stipa are perennial plants, which make a notable presence in the fifth year so that the peak of the curve is also observed.

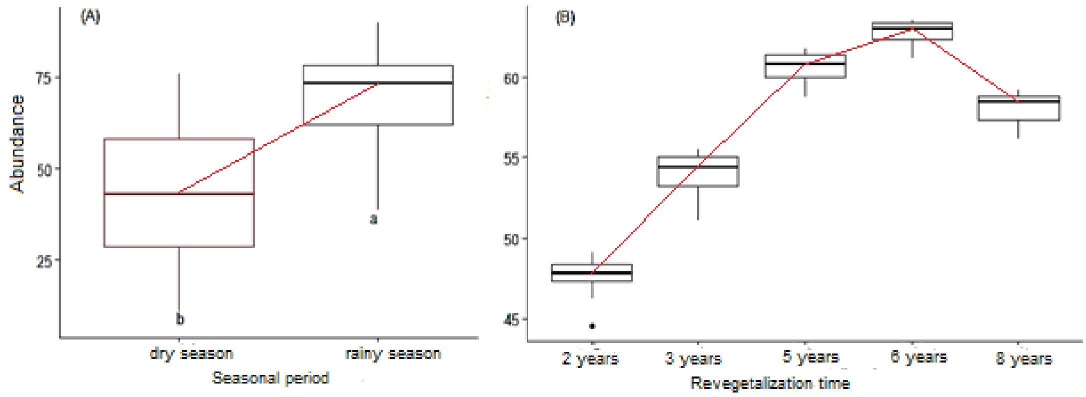


Figure 3. Upper limit of means of abundance, in areas in revegetation according to **(A)** seasonal period and **(B)** time of post-harvest abandonment of *L. meyenii*.

The richness and scarce abundance in the first post-harvest years with *L. meyenii* leaves soils with very little cover, affecting soil conditions mainly by loss of organic matter and nutrients (Mirazadi *et al.*, 2017). The perennial species found in this study are similar to those found in Puno by Cutimbo *et al.* (2016), and the decrease in species coincides with the results in fragmented areas of Condori (2012), which reaffirms that the floral composition in high mountain ecosystems is somewhat similar.

3.2. Floristic diversity

3.2.1. Alpha Diversity Index

The relationship between the number of species and their abundance expressed by H' in native vegetation (Table 1) ranged from 2.6 to 3.0 in the seasonal rainy season and from 1.95 to 2.4 in the dry season. In the revegetation areas, it ranged from 2.37 to 2.89 in the rainy season and 1.35 to 2.46 in the dry season. According to the classification range of the index, these values correspond to mean diversity in native vegetation and low diversity in areas with revegetation (Campo and Duval, 2014; Uchida *et al.*, 2018).

Table 1. Alpha diversity indices according to years of abandonment in two seasonal periods.

Transects	2 years	3 years	5 years	6 years	8 years
VN - rainy season	2.655	2.638	2.771	2.787	3.057
VN - dry season	2.212	2.015	1.947	2.356	2.111
PR - rainy season	2.374	2.538	2.893	2.811	2.886
PR - dry season	1.713	1.353	2.37	2.449	2.461

Legend: 2 years ... 8 years = years of post-harvest abandonment; VN= native vegetation, PR= areas in revegetalization.

A significant variation of the H' index was observed between seasonal periods with higher value in the rainy period with p = 0.00003. Also a significant increase of the index was observed according to the increase of the post-harvest abandonment time, with more than five-, six- and eight years with p = 0.001 (Figure 4), as opposed to the times of two- and three years. This indicates that pasture land use change has affected floral diversity for at least five years, and by the relationship between biodiversity and environmental services, particularly the production of useful fodder for livestock (Wehn *et al.*, 2018) which is the economic base of the rural population (Plieninger and Huntsinger, 2018). This behavior of the H' index over time makes it clear that it is important to know the change in species composition over the time of revegetalization in order to understand the effect of anthropogenic action on land-use change in grassland ecosystems (Uchida *et al.*, 2018).

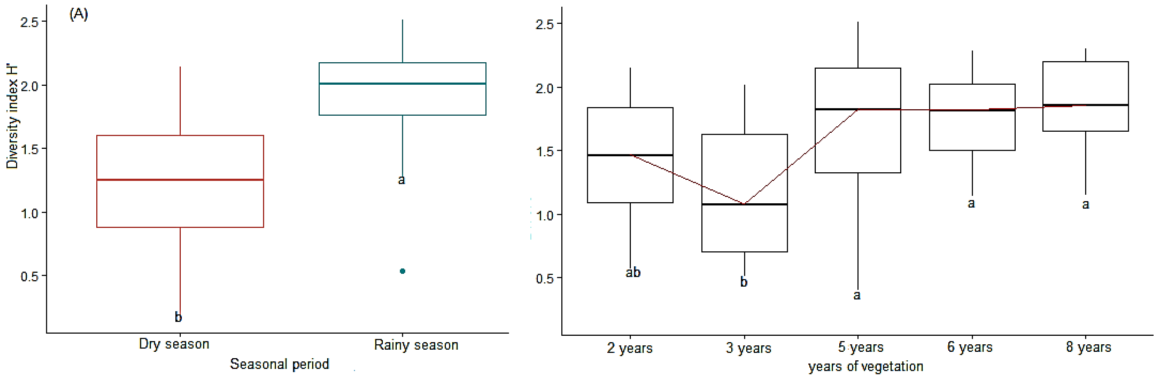


Figure 4. Upper limit of means of diversity of Shannon Wiener and in plots in revegetation according to seasonal period and time of post-harvest abandonment of *L. meyenii*

Several results have been obtained on alpha diversity indices, for the case of H' they vary between 2.4 and 3.36 in Mexico (Álvarez-Lopeztello *et al.*, 2016), 2.56 in Colombia (González-Pinto, 2017), 2.7 to 3.47 in the páramos of Ecuador (Fiallos *et al.*, 2015), in the central Andes of Peru between 2.75 and 3.41 (Yaranga *et al.*, 2018). These values coincide in the classification of medium to low diversity in high mountain pasture conditions, despite its different geographical location on the planet

3.2.2. Beta diversity index

The similarity in the presence of species between pairs of areas in times of abandonment was measured by the Jaccard Index (Table 2), the areas with different post-harvest abandonment times of two-, three-, five-, six- and eight years, compared by pairs, in native vegetation share a greater number of species (12 to 18) in the rainy season than in the dry season (9 to 18); in both cases the degree of similarity is intermediate, with values from 0.31 to 0.5. The areas in revegetation share between 7 to 14 species in the rainy season and 3 to 8 species in the dry season; the degree of similarity varied from intermediate to low. The indices obtained in two-, three- and five- years of post-harvest abandonment showed intermediate levels around 0.5, while in the sixth and eighth years the degree of similarity was low, with values ranging from 0.31 to 0.37 in the rainy season and from 0.29 to 0.46 in the dry season. These results indicated that native vegetation shares between 50% and 38.2% of common species in the seasonal rainy period and between 43.8% and 31% in low water; while in areas with revegetation they share between 61.3% and 38.7% of species in the rainy period between 61.5% and 29.7% in the low-water period.

Beta diversity is a very important ecological indicator that allows us to know the dynamics of the pattern of shared species between areas in recovery according to the years of abandonment, and this is very variable according to the environmental conditions of each place (Condori, 2012; Zhou *et al.*, 2014; Mota *et al.*, 2018). Differences were observed between sampling sites in native vegetation. It is useful to interpret the dynamics of dissimilarity in areas affected by land-use change. The Jaccard index, which measures the capacity to share common species by areas in revegetation, showed higher values than native vegetation, especially in the first years of revegetation, which was due to the entry of exotic or invasive species adapted to disturbance conditions in the soil (Sedlar *et al.*, 2017), which decreased as the years of post-harvest recovery increased, due to the entry of perennial species that replaced the pioneer species. This influenced the observed medium- to low similarity (Uchida *et al.*, 2018). The value of J = 0.79 shown by González-Pinto (2017) is high, compared to the lower indices found in the study, due to the fact that in arid regions the richness of grassland communities is lower than in humid regions (Enriquez *et al.*, 2016; Uchida *et al.*, 2018).

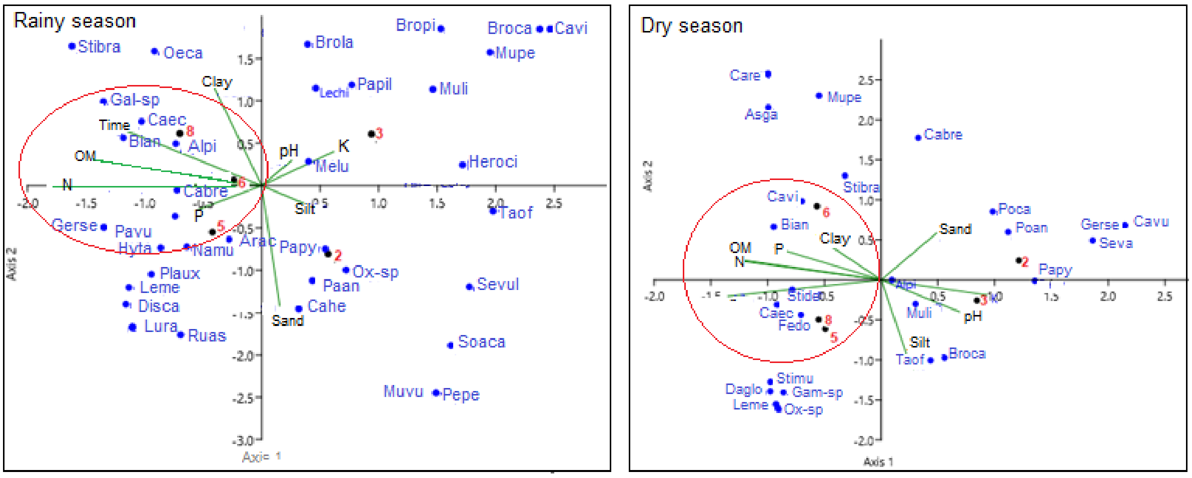
Table 2. Jaccard similarity index of native vegetation and plots with revegetation, number of common species in parentheses.

Transects	Years of abandonment	2 years	3 years	5 years	6 years
Native vegitation - rainy season	3	0.500 (16)			
	5	0.532(14)	0.511(13)		
	6	0.444(18)	0.577(14)	0.382(16)	
	8	0.418(12)	0.396(12)	0.464(11)	0.429(13)
Native vegitation – dry season	3	0.438(9)			
	5	0.385(9)	0.375(9)		
	6	0.379(18)	0.486(9)	0.310(10)	
	8	0.375(11)	0.158(11)	0.375(10)	0.371(10)
Revegetation – rainy season	3	0.529(8)			
	5	0.544(7)	0.531(8)		
	6	0.448(10)	0.446(9)	0.408(14)	
	8	0.613(7)	0.420(9)	0.387(13)	0.474(12)
Revegetation – dry season	3	0.615(3)			
	5	0.471(5)	0.500(3)		
	6	0.421(6)	0.500(4)	0.400(7)	
	8	0.600(5)	0.379(5)	0.297(8)	0.463(8)

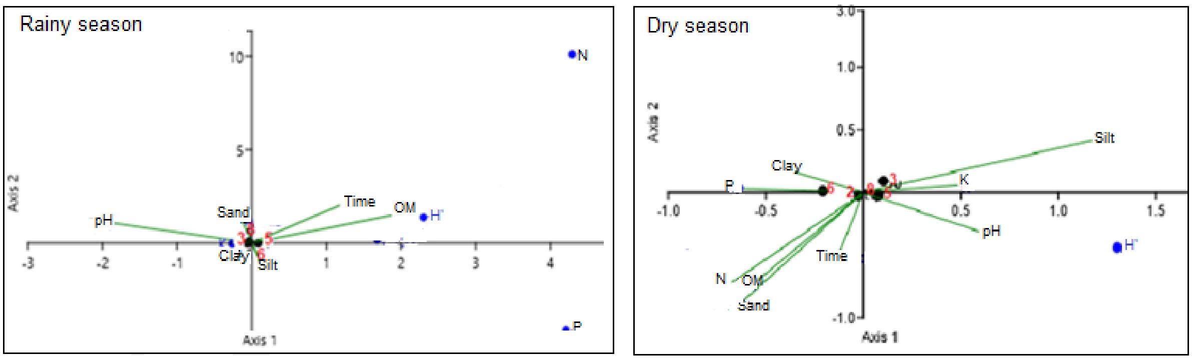
3.3. Canonical correspondence (CCA) between species dominance and floristic diversity with soil characteristics, according to post-harvest abandonment time, in plots in natural revegetalization

The analysis of the canonical correspondence between the dominance of species in areas in natural revegetation with the physical-chemical characteristics of the soil, the post-harvest abandonment time of L. meyenii, showed the sensitivity among the species of greatest presence in the five-, six- and eight years of post-harvest abandonment of L. meyenii, with organic matter, nitrogen and phosphorus, both in the seasonal rainy period and in the dry season (Figure 5A). The interest of knowing, in simplified way, the relation of the complex set of environmental data that explain the variation of biological indicators (Cayuela, 2011) has taken into account the diversity of grassland ecosystem species as a key component (Fattahi *et al.*, 2017), which varies according to the sensitivity of the species to the variables of the temporal gradient (years of abandonment) and soil factors, which was the interest of our analysis.

This correspondence confirmed the close relationship between soil and vegetation (Fattahi *et al.*, 2017), which, as a result of the increasingly prolonged abandonment time, shows the correspondence of perennial species with organic matter and nitrogen, which are the most important elements in the quality of soils that energize microbial activity (Ali *et al.*, 2017), and plant nutrition (Wang *et al.*, 2017), favored by the increase in biomass produced, litterfall, root death and the increase in microorganisms, arthropods and others, which improve soil quality (Acevedo and Delibes-Mateos, 2013; Niemandt and Greve, 2016). This indicated that the post-harvest vegetative recovery time is a determining factor in the variation and correspondence of soil characteristics with the richness and abundance of species. This correspondence behavior explained the variability of invasive and temporary species in recently abandoned areas that are not demanding in soil quality (Andrade *et al.*, 2015; Sedlar *et al.*, 2017) and the stability of perennial species in the medium- and long term (Gartzia *et al.*, 2016; Mota *et al.*, 2018).



(A) canonical correspondence of the abundance of richness with the physical-chemical characteristics of the soil and the time of abandonment



(B) Canonical correspondence of the alpha diversity with the physical-chemical characteristics of the soil and the time of abandonment

Figure 5. Canonical correspondence between A) richness and abundance, B) alpha diversity, in revegetation plots, according to seasonal period and post-harvest abandonment time of *L. meyenii*.

In the analysis of the canonical correspondence of alpha diversity (Shannon Wiener index - H') with the physical-chemical characteristics of the soil, the post-harvest abandonment time of L. meyenii showed a weak linear relationship of the H' index with the organic matter and the abandonment time in the seasonal rainy period, which was not maintained in the low-water period; on the other hand, the affected areas in different abandonment times were concentrated very close to the axes centroid, which indicates that they maintain some independence of the correspondence with other variables. This is due to the fact that the index comes from the application of algorithms to the number of species found (Halffter *et al.*, 2005), so searching for correspondence of the diversity index will not be of practical use.

4. CONCLUSION

It is the Poaceae and Asteraceae families that characterized the high Andean grassland ecosystem that maintained dominance in revegetalization areas, despite the secondary succession process with invasive species. The post-harvest abandonment time of *L. meyenii* and the seasonal period affected the behavior of composition, richness and dominance, which determined the variation of alpha and beta diversity. The visible recovery time of areas under revegetalization was evidenced after five years of abandonment, which indicates that the effect of land-use change was very severe in the loss of floral diversity; however, the linear canonical correspondence between floral composition and physical-chemical factors of the soil indicate the possibility of accelerating the process of replacement of temporary species by perennials, through the replacement of organic matter and possible correction of pH.

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Performance of colored cotton under irrigation water salinity and organic matter dosages

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ABSTRACT

This work evaluated the development of colored cotton submitted to irrigation water of different salinity levels and organic matter doses from tanned manure. The experimental design was completely randomized in a 4 x 4 factorial scheme with 3 replications, totaling 48 experimental plots. The factors studied were 4 doses of organic matter (1, 4, 7 and 10%) and four levels of irrigation water salinity (0.26; 1, 2 and 4 dS m^{-1}). The variables analyzed were plant height, stem diameter, number of leaves, number of fruits and fresh shoot mass. Significant effect of organic matter was observed on all studied variables, with a positive response on the increase of all variables as a function of increasing doses of organic matter. The isolated effect of salinity was significant for all parameters evaluated, except for the number of leaves, with significant reductions of 6.03; 3.27; 5.23; 6.94% in the parameters: plant height, stem diameter, number of fruits and shoot fresh weight respectively, for each unit increase of irrigation water salinity. The interaction between the variation sources studied had a significant effect only for fresh shoot mass, where the highest average for this parameter was observed at 10% organic matter dosage at irrigation water salinity level of 1.0 dS m^{-1}.

Keywords: fertilization, *Gossypium hirsutum*, saline stress.

Desempenho do algodoeiro colorido sob salinidades da água de irrigação e dosagens de matéria orgânica

RESUMO

Este trabalho foi realizado com o objetivo de avaliar o desenvolvimento do algodoeiro colorido, submetido a diferentes níveis de salinidade da água de irrigação e doses de matéria orgânica proveniente de esterco bovino curtido. O delineamento experimental utilizado foi inteiramente casualizado em esquema fatorial 4 x 4, com 3 repetições, totalizando 48 parcelas experimentais. Os fatores estudados foram 4 doses de matéria orgânica (1; 4; 7 e 10%) e quatro níveis de salinidade da água de irrigação (0,26; 1; 2 e 4 dS m^{-1}). As variáveis analisadas foram: altura da planta, diâmetro do caule, número de folhas, número de frutos e massa fresca da parte aérea. Notou-se efeito significativo da matéria orgânica sobre todas as variáveis estudadas, com uma resposta positiva no aumento de todas as variáveis em função das doses crescentes de matéria orgânica. O efeito isolado da salinidade foi significativo para todos os parâmetros avaliados, com exceção para o número de folhas, sendo verificadas reduções significativas de 6,03; 3,27; 5,23; 6,94% nos parâmetros: altura de plantas, diâmetro do caule, número de frutos e massa fresca da parte aérea respectivamente, para cada acréscimo unitário da salinidade da água de irrigação. A interação entre as fontes de variação estudadas ocasionou efeito significativo somente para a massa fresca da parte aérea, onde a maior média para esse parâmetro foi observada na dosagem de 10% de matéria orgânica no nível de salinidade da água de irrigação de 1,0 dS m^{-1}.

Palavras-chave: adubação, estresse salino, *Gossypium hirsutum.*

1. INTRODUCTION

Herbaceous cotton (*Gossypium hirsutum* L.) is one of the main crops in Brazil (Costa *et al.*, 2008). Its production is concentrated in the midwest and northeast, where cotton is a socio economically important crop, cultivated under rainfed or irrigated conditions (Carvalho *et al.*, 2015).

Cotton cultivation with the naturally colored fiber has been gaining prominence in the national scenario, especially due to the environmentally correct character of its cultivation, which dispenses with the need for artificial dyeing. In this context, many studies have been developed to better understand the productive aspects and conditions of culture (Souza *et al.*, 2018; Pedro *et al.*, 2016).

Thus, cotton farming in the northeast region has been highlighted as one of the agricultural activities of great value for Brazilian agribusiness, as well as being a means of fixing man in the countryside and strengthening family agriculture. However, research is needed to better manage the crop and improve productive efficiency.

Salinity is one of the main environmental factors that limit plant growth and productivity. This limitation occurs because, under saline conditions, there is a reduction in the availability of water to the plants, due to the decrease in the osmotic potential of the soil solution; Thus, the plant tends to expend more energy to absorb water and nutrients (Leonardo *et al.*, 2003).

The high concentrations of salts in soil and water, besides reducing soil water potential, are reflected in the inhibition and unevenness of growth, decline in production capacity and quality of the products obtained from plants grown due to the direct effects on the osmotic potential, nutritional imbalance and toxic effect of ions, especially chloride and sodium (Lacerda *et al.*, 2003).

Cotton is considered a moderately tolerant crop to the presence of salts in the soil (Taiz and Zeiger, 2009). However, the authors point out that, despite this tolerance, the culture can suffer substantial reductions in its growth, production and quality of the product obtained when exposed to the saline stress condition (Oliveira *et al.*, 2008). Thus, one aspect that has been studied is the use of fertilizers in the plants under salinity conditions, demonstrating that the use of biofertilizers in saline environments may reduce the effects of salinity on plant growth (Dias *et al.*, 2016; Lima *et al.*, 2014; Santos *et al.*, 2014).

This work evaluated the development of the colored cotton submitted to irrigation water of different salinity levels and organic matter doses.

2. MATERIALS AND METHODS

The experiment was conducted in a greenhouse belonging to the Postgraduate Program in Agricultural Engineering of the Federal University of Recôncavo da Bahia (PPGEA / UFRB), in the municipality of Cruz das Almas, BA, at 12º40'19' latitude south, 39º06 '23' west longitude and average altitude of 220 m.

Seeds of the cultivar BRS Topázio, of brown color fiber, were used. The experimental unit was represented by a plastic vessel with a capacity of 20 L of soil, which was filled with 0.5 kg of gravel (Nº 0), which covered the base of the vessel, followed by a bidim blanket cut in the shape of the base of the vessel, in the sequence was added 18 kg of soil from the municipality of Cruz das Almas, BA. Each of the vessels contained a hole in the base, which allowed the soil to drain.

The experimental design was completely randomized in a 4 x 4 factorial scheme, with three replications, totaling 48 experimental plots. The treatments were the result of the combination of four levels of irrigation water salinity: water supply (0.26 - control); 1, 2 and 4 dS m^{-1} with four doses of organic matter: 1, 4, 7 and 10% of the weight of the soil contained in the vessel. The organic matter used in this experimental conduction was cattle manure, being the only nutritional source for the development of the crop during the cycle. The manure was inserted in the vessels in the assembly phase of the same as a function of the proposed treatments. The salinity of the irrigation water was obtained by adding sodium chloride (NaCl) to the local water supply (0.26 dS m^{-1}), in order to obtain water with different levels of salinity. Irrigation management was carried out based on the tensiometry, associated to the information of the characteristic curve of the soil in question. A tensiometer was installed at a depth of 15 cm in each of the treatments and replicates tested, the respective irrigation slides were calculated based on the matric potential of the soil, so as to adjust soil moisture to the field capacity, such replacement was manually performed as needed. The application of salinity treatments occurred at 8 days after sowing and lasted until the end of the crop cycle (114 days after sowing) in the different treatments proposed.

The following biometric parameters of the cotton plant were measured during the development cycle: plant height (AP), stem diameter (DC), number of leaves (NFOL), number of fruits (NFRU) and at the end of the cycle the mass was evaluated (MFPA) in order to verify the effect of the treatments tested.

The results were submitted to analysis of variance by the F test and regression, which were adjusted to linear and polynomial models of first and second degree. For this, the Tukey test was applied at a 5% probability level. For the significant interactions, the analysis of variance was performed considering the factors salinity levels and organic matter levels.

3. RESULTS AND DISCUSSION

The results of the analysis of variance shown in Table 1 show that there was a significant effect of the treatments tested for the parameters: plant height (AP), stem diameter (DC), fruit number (NFRU) and fresh shoot mass MFPA); only the number of leaves (NFOL) did not show a significant effect as a function of irrigation water salinity. In the interaction between the sources of variation, the increases in organic matter dosages together with irrigation water salinity levels interfered significantly ($p<0,01$) in fresh shoot mass. In a study by Souza *et al.* (2018) in the cotton crop, only the stem diameter parameter presented significant interaction when evaluating salinity levels and organic matter doses.

The height of plants, stem diameter, fruit number and number of leaves of the cotton was not influenced by the interaction between salinity and organic matter doses, corroborating the results observed by Santos *et al.* (2014) and Nobre *et al.* (2010) when evaluating irrigation water salinity levels and nitrogen fertilization on the growth of colored cotton and sunflower plants, respectively.

Table 1. Summary of analysis of variance for plant height (AP), stem diameter (DC), leaf number (NFOL), number of fruits (NFRU) and fresh shoot mass (MFPA) of the colored cotton submitted to doses of organic matter and increasing levels of salinity of irrigation water.

Sources of Variation	GL	Average Squares				
		AP	DC	NFOL	NFRU	MFPA
Organic Matter (MO)	3	5288.22**	25.45**	1342.40**	302.02**	2878.23**
Salinity (S)	3	1737.50**	4.70**	46.18 ns	16.52*	1246.48**
MO x S	9	121.16 ns	0.79 ns	66.15 ns	2.52 ns	374.19**
Error	32	158.37	0.55	54.29	5,43	111.55
CV (%)		11.83	7.29	19.44	19,95	22.25

**,* represent significant effect at 0,01 e 0,05 probability; ns - not significant by F test; GL - degree of freedom; CV - coefficient of variation

By analyzing the isolated effects of the sources of variation, it is observed that, in general, the increase of the organic matter doses stimulated the cotton growth (Figures 1A, B, C, D and E). Thus, the highest values of AP, DC, NFOL, NFRU and MFPA, respectively, were found in the highest dosages of organic matter, respectively. These results demonstrate the contribution of nitrogen to the physiological metabolism of plants and may be directly related to the formation of proteins, which are essential for the plant to express its agronomic potential (Nascimento *et al.*, 2017a).

The organic matter yields from 4% increased plant height, such biometric expressiveness was favored by fertilizer supply, which may have contributed to soil fertility and structuring (Costa *et al.*, 2008). Pereira *et al.* (2012), when evaluating the influence of cattle manure doses on the agronomic characteristics of the herbaceous cotton cv. BRS Rubi, observed a linear increase in plant height. The trend of growth as a function of the applied organic matter was also verified for the parameter diameter of the stem, similar to that observed by Dias *et al.* (2016) in cotton fiber cultivation.

Regarding the number of leaves and fruits, the evolution was similar to the growth of the plants in height and diameter of the stem, it is observed that the organic matter doses from 4% provided increases in these variables with the emission of leaves and fruits by the cotton plants. For Souza *et al.* (2018) the addition of organic matter promotes increased growth and cotton production.

The application of the organic matter doses promoted an increase of 33.95 g of the fresh mass of the aerial part with the dose of 10% of organic matter, a value greater than twice that found in the control treatment (26.52 g). This behavior is justified by the addition of nitrogen and its influence in most physiological processes that occur in plants, such as protein synthesis and photosynthesis, defining it as the nutrient that most limits the production of plant biomass (Yong *et al.*, 2010).

As shown in Figures 2 A, B, C and D, the increase in electrical conductivity of irrigation water from 0.26 to 4.0 dS m^{-1} provided a significant reduction in plant height (AP), diameter of stem (DC), number of fruits (NFRU) and fresh shoot mass (MFPA), with reductions of 6.03; 3.27; 5.23; 6.94% respectively, for each unit increase in the salinity of the irrigation water.

The increase in salinity of irrigation water promoted a reduction in water availability due to the high concentration of salts in the root zone, which may have caused a reduction in the plant metabolism, evidenced by the reduction of height of plants, stem diameter, number of fruits and fresh mass of the aerial part. These results corroborate those observed by Oliveira *et al.* (2008) and Santos *et al.* (2014), who observed that, when evaluating saline water levels in cotton cultivars, the fresh phytomass presented linear declining behavior.

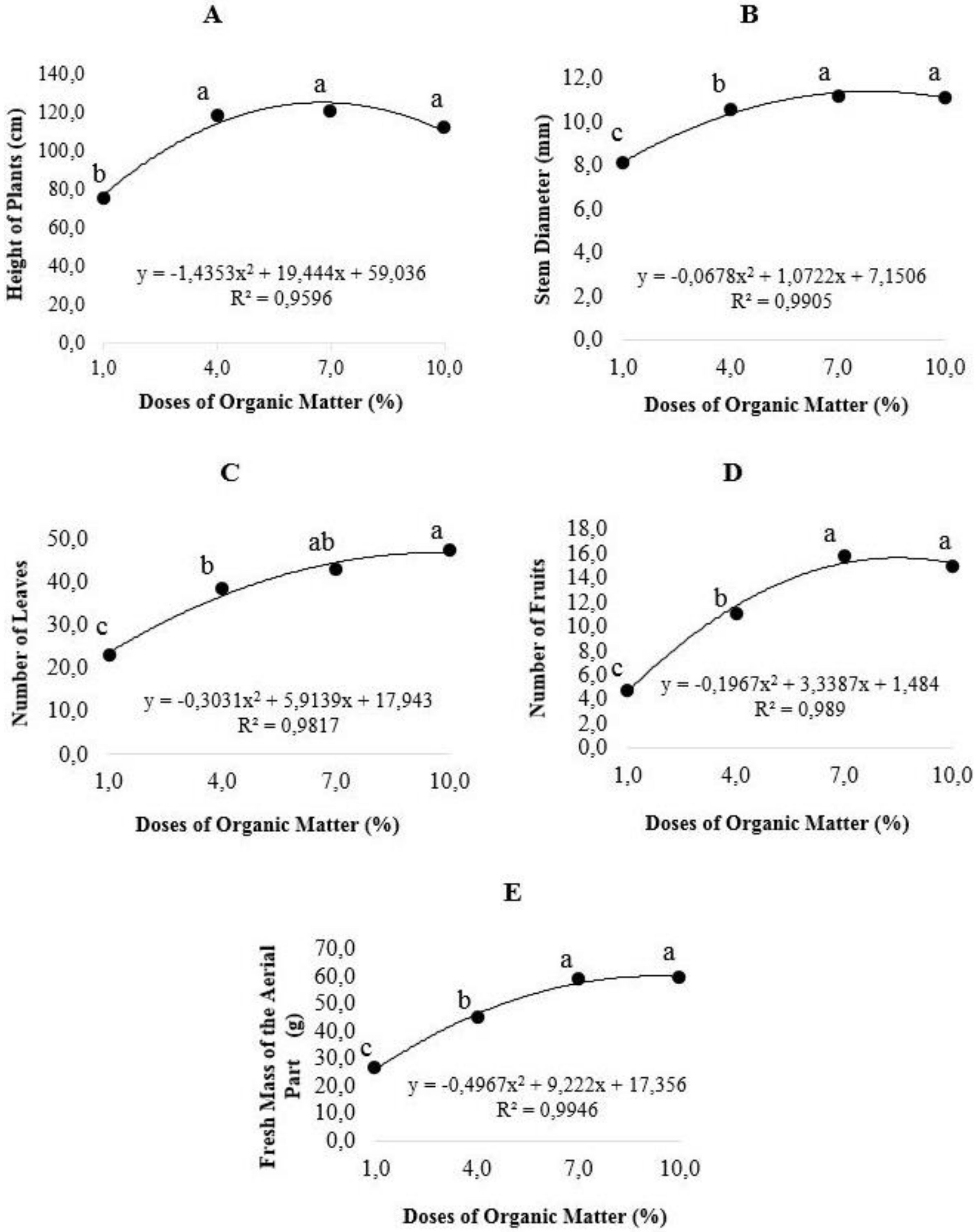

Figure 1. Height of Plants (A), Stem Diameter (B), Number of Leaves (C), Number of Fruits (D) and Fresh Mass of the Aerial Part (E) as a function of organic matter doses; Means followed by different lower case letters differ statistically from each other by the 0,05 probability Tukey test.

The reduction in the expression of biometric characteristics with the increase of irrigation water salinity levels was also observed by Nascimento *et al.* (2017b) in okra cultivation, Araújo *et al.* (2016) in melon cultivation, Nobre *et al.* (2014) in castor bean cultivation and Sá *et al.* (2013) in papaya cultivation.

According to the F test, no significant difference was observed for the number of leaves observed in the salinity treatment; such results agree with those observed by Ferreira *et al.* (2012) and Nascimento *et al.* (2017b) when studying the effect of salinity on the production of okra grains.

For plant height and stem diameter, treatments with salinity levels of 0.26 and 1.00 dS m^{-1} did not differ statistically, presenting higher results than treatments with salinity levels 2 and 4 dS m^{-1}.

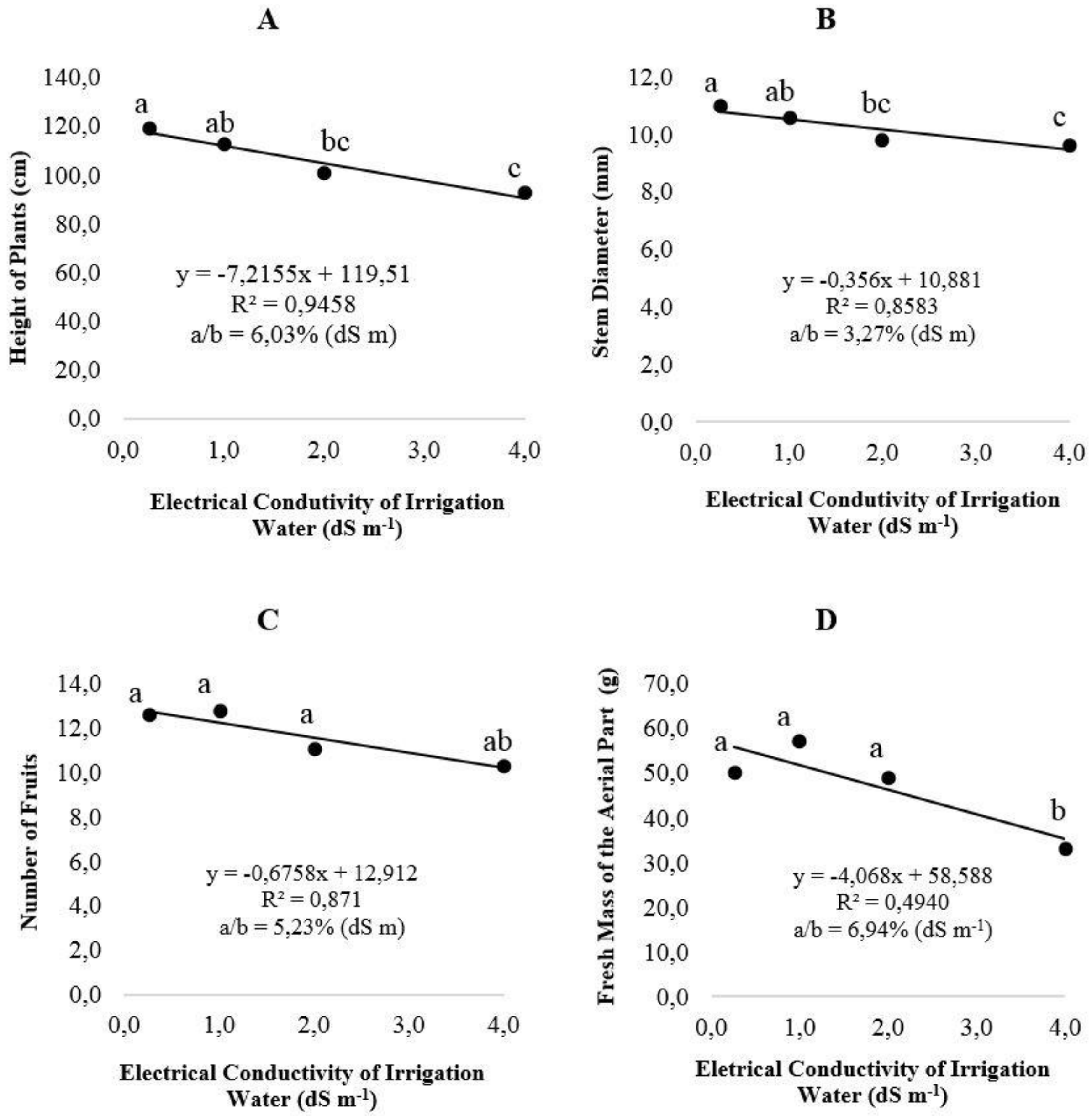

Figure 2. Height of Plants (A), Stem Diameter (B), Number of Fruits (C) and Fresh Mass of the Aerial Part (D) as a function of salinity of irrigation water; Means followed by different lower case letters differ statistically from each other by the 0.05 probability Tukey test.

From the significance verified for the parameter shoot fresh mass (MFPA) in the interaction between organic matter doses and salinity levels, the organic matter doses were split within the irrigation water salinity levels, verifying that the highest MFPA averages were obtained at a 10% dose within the 1.0 dS m^{-1} salinity level (Table 2).

Table 2. Interaction split means (Organic Matter x Salinity) for shoot fresh matter (MFPA).

Medium				
Variation source	**Salinity**			
	0,26 dS m^{-1}	**1,0 dS m^{-1}**	**2,0 dS m^{-1}**	**4,0 dS m^{-1}**
Organic Matter				
1%	21.94 bA	31.37 bA	31.31 bA	21.46 bA
4%	54.30 aA	45.89 bA	39.88 bA	39.86 abA
7%	66.57 aAB	69.34 aA	54.38 abAB	45.27 aB
10%	58.46 aB	83.20 aA	69.80 aAB	26.43 abC

Lower case letters in the columns compare the average dosages of organic matter within the salinity levels of irrigation water; capital letters in the lines compare the average salinity levels of irrigation water within the organic matter dosages by the Tukey test at a 0.05 probability level.

In general, the increase in the organic matter dosage to the same salinity level provides an increase in the average values found for the MFPA parameter, except for the 10% organic matter dosage at 4.0 dS m$^{-1,}$ salinity presented a reduction in the mean values of the MFPA. It was also observed that there was no significant difference in the 1% dose of organic matter, regardless of the salinity level tested (0.26-4.0 dS m^{-1}).

Alves *et al.* (2009), evaluating the interaction of nitrogen fertilization versus water type in brown cotton cultivation, also found significant effect only for biomass.

4. CONCLUSIONS

The organic matter doses tested had a significant effect on plant height, stem diameter, number of leaves and fruits and fresh shoot mass.

The increase in salinity levels of irrigation water had a negative influence on the expression of the following biometric characteristics: plant height, stem diameter, number of fruits and fresh shoot mass, with mean decreases of 6.03; 3.27; 5.23 and 6.94%, respectively, for each unit increase in the salinity of the irrigation water.

The interaction between the organic matter and the salinity levels were significant for the parameter fresh mass of the aerial part.

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Sustainability assessment of sludge and biogas management in wastewater treatment plants using the LCA technique

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ABSTRACT

Upflow anaerobic sludge blanket reactors (UASBs) used in sewage treatment generate two useful byproducts: sludge and biogas. This study evaluated the sustainability of four different scenarios for the treatment and final destination of biological sludge and biogas in a medium-sized wastewater treatment plant (WWTP) in South Brazil. At this plant, the sludge is sanitized by Prolonged Alkaline Stabilization and applied to agriculture (base scenario). Scenario 1 is about biogas use to dry sludge, which is taken to be used in agriculture. In Scenarios 2 and 3 the heat of the sludge burning is used for drying and sanitation. Finally, in Scenario 3 the ashes are destined to landfills. An environmental impact assessment was performed through life-cycle assessment using the ReCiPe 2016 evaluation method. Social life-cycle assessment indicators, adapted and developed for WWTPs, were used for social assessment. Economic assessment was performed through the analysis of life-cycle costs. The *dashboard of sustainability* (DoS) method was used for global assessment of sustainability. For overall sustainability assessment, Scenario 1 had the highest score (678 points) (best scenario) in the DoS. The environmental dimension was what facilitated this scenario. For this dimension, the following indicators presented the highest points when compared to the other scenarios: soil acidification, ozone formation, terrestrial ecosystem. The base scenario had the lowest score (worst case scenario) (375 points).

Keywords: anaerobic reactor, life cycle assessment, wastewater treatment.

Avaliação da sustentabilidade no gerenciamento do lodo e biogás, em estação de tratamento de esgotos, utilizando a técnica de ACV

RESUMO

Reatores de manta de lodo anaeróbio de fluxo ascendente (UASB), usados no tratamento de esgoto, geram dois subprodutos que podem ser utilizados: lodo e biogás. O objetivo do presente estudo foi realizar a avaliação da sustentabilidade de quatro diferentes cenários de tratamento e destinação final do lodo biológico e biogás, numa Estação de Tratamento de Esgoto (ETE), de porte médio, localizada no Sul do Brasil. Nesta Estação o lodo é higienizado, pela Estabilização Alcalina Prolongada, e disposto na agricultura (cenário base). O cenário 1 corresponde a utilização do biogás para a secagem do lodo e este destinado na agricultura. Nos cenários 2 e 3, o calor da combustão do lodo é utilizado para a secagem e higienização, sendo no cenário 3, as cinzas destinadas para aterro. A avaliação dos impactos ambientais foi realizada através da avaliação do ciclo de vida, utilizando-se como método de avaliação o ReCiPe 2016. Para a avaliação social foram utilizados os indicadores da avaliação de ciclo de vida social, adaptados e desenvolvidos para ETEs, a avaliação econômica foi realizada através da análise dos custos do ciclo de vida. Para a avaliação global da sustentabilidade, foi utilizado o método *Dashboard of sustainability*. Com relação à avaliação global da sustentabilidade, o cenário 1 obteve a maior pontuação (678 pontos) (melhor cenário) no DoS. A dimensão que favoreceu esse cenário foi a ambiental, onde os indicadores de acidificação terrestre e formação de ozônio, ecossistema terrestre foram os indicadores que apresentaram uma pontuação mais elevada, em comparação com os demais cenários. O cenário base apresentou a menor pontuação (pior cenário) (375 pontos).

Palavras-chave: avaliação do ciclo de vida, estação de tratamento de esgotos, reatores anaeróbios.

1. INTRODUCTION

Population growth in Latin America and the Caribbean has exceeded the capacity of national and local governments to meet demand for water supply and sanitation (Noyola *et al.*, 2012). New WWTPs, and the expansion of existing capacity and treatment level, should be designed to meet the needs of the sanitary sewage thus generated. As a consequence of this expansion, there has been increased generation of biological sludge and biogas. Approximately 30 kg of dry sludge/inhabitant.year (Hospido *et al.*, 2010) and 10 to 28 NL of biogas/inhabitant.year (Jesus Netto, 1936; Azevedo Netto, 1977; Duarte *et al.*, 2018) is generated, with approximately 40% of biogas being lost together with effluent in stations using UASB anaerobic reactors (Nelting *et al.*, 2017).

Treatment systems have the function of minimizing the environmental impacts of the release of effluents into the environment, but they themselves are an impacting element in the three dimensions of sustainability throughout their life cycle (Sanches, 2009). The adoption of sustainability criteria in the choice of the best technology for treatment and disposal of sludge should be taken into consideration (Hernandez-Padilla *et al.*, 2017). A sustainable waste management system should be environmentally effective, economically accessible and socially acceptable (Noyola *et al.*, 2013). In addition, it should be safe for workers and the community involved, with particular attention to the possibility of affecting the stakeholders involved (Padilla-Rivera *et al.*, 2016).

Depending on the final destination adopted, biological sludge needs to be sanitized, with prolonged alkaline stabilization (PAS) being one of the techniques adopted in Brazil. In *Paraná*, *Curitiba* Metropolitan Region (*RMC*) is the main producer of sludge and it has been primarily destined for agriculture use (Bittencourt *et al.*, 2014). According to Gutierrez *et al.* (2015), the potential impact related of using virgin lime for sanitizing sludge, in the route studied (sludge being sanitized by PAS and prepared for agriculture), signaled a demand for research on alternatives to lime in order to increase the credits of systems that choose to use sludge for agriculture use as a final destination.

Upflow sludge blanket reactor technology (UASB/RALF) is the second most-used in terms of the number of WWTPs in Brazil (Noyola *et al.*, 2012; ANA, 2017), and represents 94.6% of the stations in existence in the state of Paraná in South Brazil (Ross *et al.*, 2014). A characteristic of this technology is the generation of stabilized biological sludge and the generation of biogas rich in methane, the latter of which is still under-used for energy purposes in Brazil - currently most biogas generated in Brazilian WWTPs is burned by *flare* and emitted into the atmosphere.

Preliminary studies indicate that the treatment and management of biological sludge and biogas have a significant contribution in the calculation of environmental impact. In this sense, Amaral *et al.* (2016) carried out an environmental life-cycle assessment (LCA-environmental) of a medium-sized WWTP equipped with UASB reactors, and demonstrated that the processes of treatment and disposal of sludge and burning biogas in open *flare* together account for 44% of the contribution to the climate change category, 36% of the contribution to the depletion of the ozone layer category, 55% to human toxicity and 86% to terrestrial acidification.

Methodologies for the assessment of social impacts are still in the research phase or exist only as proposals in scientific articles. In 2004, the United Nations Environmental Programme (UNEP) recognized the need for social criteria in LCA and established a working group, which created the guide *"Guidelines for Social Life Cycle Assessment of Products"* (UNEP; SETAC, 2009). Another guide was released in 2010, divided by impact subcategories, to provide methodological help on how to create indicators and how to obtain data by source (UNEP; SETAC, 2013). The two documents are aimed at LCA-S for companies producing consumer goods. Recently, the Universidade Autônoma do México published an article suggesting social indicators linked to WWTPs (Padilla-Rivera *et al.*, 2016). These authors used the stakeholders present in the guide and added five more indicators that they consider specific to WWTPs.

Regarding biogas and sludge sustainable managing, no studies were found that evaluate the integrated management of both sub products generated through UASBs reactors, using the technique of life cycle assessment. The studies are focused on environmental LCA and economic evaluation, or contemplate only biologic sludge management (Xu *et al.*, 2014; Mills *et al.*, 2014; Garrido-Baserba *et al.*, 2015). Studies differ from each other when analyzing economic and environmental best options. In addition, none of the studies evaluated social dimension.

This study evaluates the sustainability (environmental, social and economic assessment) of different destination routes for biological sludge and biogas coming from anaerobic WWTPs, to support the selection of technologies to be used in future WWTPs and to assure the adequacy of those in existence.

2. MATERIALS AND METHODS

The investigated WWTP is located in South Brazil, has a capacity to treat 440 L s^{-1} of domestic sewage, and serves a population of 235,000 inhabitants. Preliminary treatment of sewage is provided by two mechanized screens, with 3 mm spacing, and a Dorr-Oliver type grit trap. For the biological stage of sewage treatment, the STS has six UASB reactors (secondary treatment) and two aerated facultative ponds (post-treatment). The biological sludge produced in the UASB reactors and in the aerated ponds is periodically discarded. The sludge is thickened (gravity thickener), dewatered in a centrifuge, subjected to prolonged alkaline stabilization (PAS) in the so-called Sludge Management Unit (SMU), and then destined for agriculture. The biogas generated by the UASB reactors is burned in open *flare* with an efficiency of approximately 50% (Kaminski *et al.*, 2018).

The functional unit of the study is the management of byproducts - biological sludge and biogas — generated by treating 1 m^3 of domestic effluent, and encompasses the phases of treatment and final destination. The reference flows are 0.052 Nm^3 of biogas captured at the top of the UASB reactor and 2.51 kg biological sludge obtained from the UASB anaerobic reactor (TS = 2.6%). Methane dissolved in treated effluent (8.5 mg L^{-1}) was also considered. The flows were obtained through a survey carried out in the studied wastewater treatment plant (WWTP) (between 2015 and 2017), which can treat up to 420 L s^{-1}, and a pilot sludge drying system that processed 100 kg h^{-1} of wet sludge during 62 hours. The limits of the system, highlighting the case study and proposed scenarios, are presented in Figure 1.

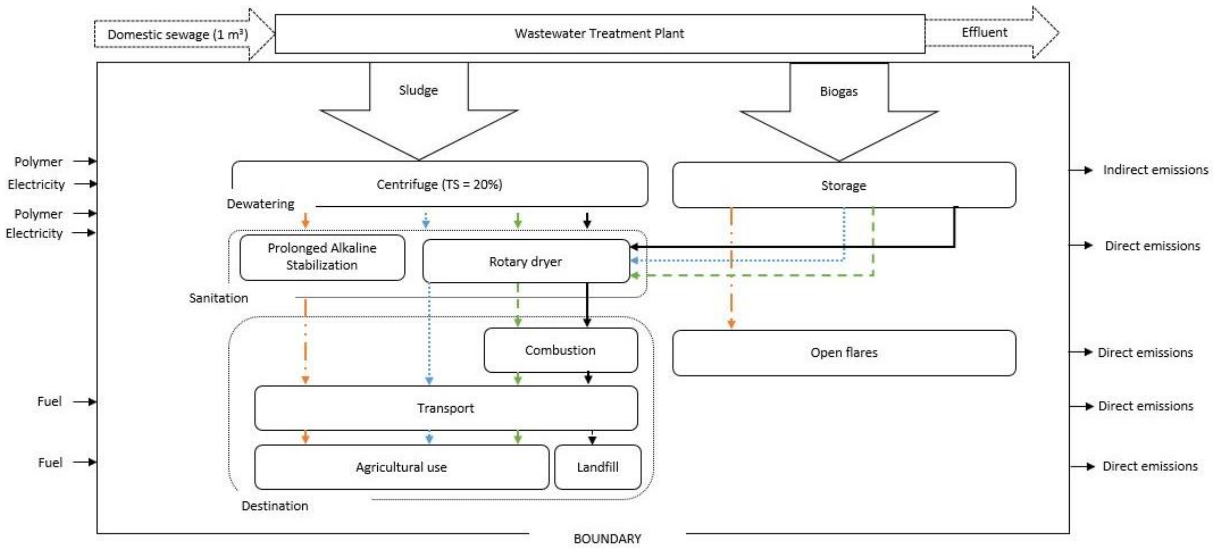

Figure 1. Scenarios for treatment and final disposal of sludge and biogas from WWTP.
Legend: Baseline Scenario ( ): sludge is dewatered in the centrifuge, undergoes prolonged alkaline stabilization (PAS), and is destined for agricultural use, while biogas is destroyed in an open flare. Scenario 1 ( ): Biogas is used as a heat source to dry the sludge in a rotary dryer, and the dried sludge is used in agriculture. Scenario 2 ( ): Sludge is combusted and the heat generated is used to dry the dewatered sludge, while ashes are directed toward agriculture. Scenario 3 ( ): Identical to Scenario 2, but ashes are discarded in the sanitary landfill.

The base scenario corresponds to the case study of studied WWTPs, wherein stabilized sludge is dewatered in a centrifuge and then submitted to PAS with virgin lime. The sanitized sludge is then sent to an agricultural destination while the biogas generated by the reactors is destroyed with low-efficiency open burners. Scenario 1 corresponds to the route where in generated biogas is used as a fuel source for drying and sanitizing sludge in a rotary dryer; the dried sanitized sludge is then sent to an agricultural destination. Scenario 2 corresponds to the route wherein sludge is combusted to produce heat used in the drying of sludge. Since the caloric value of sludge is not sufficient, a percentage of generated biogas is used. The resultant ashes are destined for agriculture. Scenario 3 is similar to Scenario 2, except the ashes are disposed in a sanitary landfill.

The methodology used to perform this work was based on the LCA and associated evaluations (LCC, S - LCA and LCSA).

The flow chart with the descriptions of the study steps is presented in Figure 2.

2.1. Environmental assessment

To develop the environmental inventory, the mass flow of the WWTP for the years 2015 and 2016 was determined. Energy flow was mapped by surveying equipment potentials and hours of operation. Emissions due to the transportation of products consumed in the treatment of sludge and the destination of the sludge in agricultural areas were reported as a function of the tkm unit, which considers the amount transported (in tons) and the distance traveled round trip (in kilometers). Distances between chemical manufacturers and the plant and the average distance to receiving agricultural areas for the last four years were used. Emissions for the application of virgin lime for sanitization (losses of N and C expressed in NH_3 and CO_2) were calculated by the N and C contents in the sludge before applying the lime, adopting a value of 2.81% for N (Andreoli *et al.*, 2014; Aisse *et al.*, 2001) and 15% for C (Ross *et al.*, 2014). Emissions due to the application in agriculture followed the models presented by Nemecek and Schnetzer (2011).

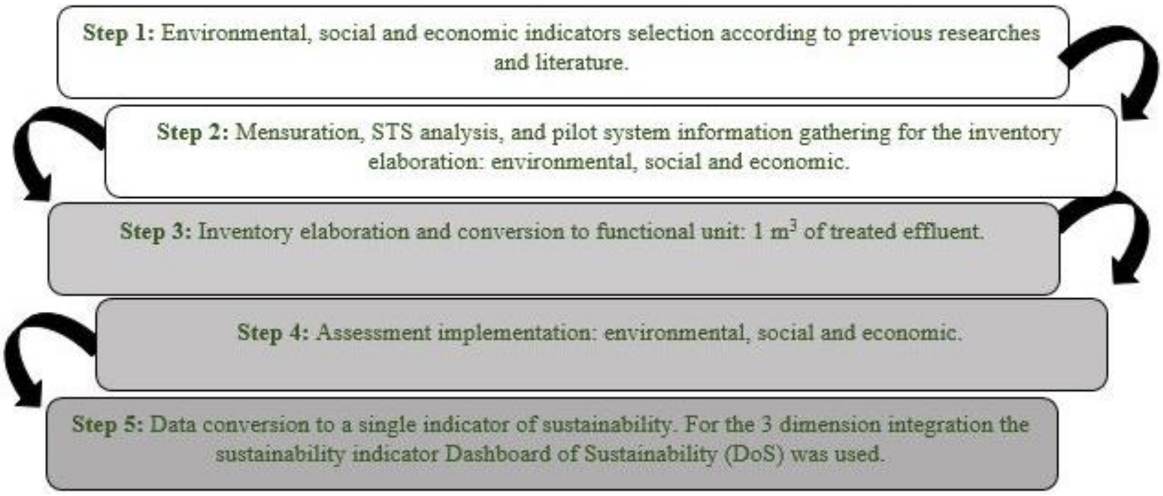

Figure 2. Steps of the research.

The environmental inventory for Scenario 1 was developed with data of the rotary dryer installed in a WWTP in South Brazil and presented by Possetti *et al.* (2015). The methodology for developing the inventory for the stages of centrifugation and agricultural application were the same as presented for the base scenario. In Scenario 2, the sludge is combusted with the ash destined for agriculture. The calorific value of dry biological sludge is 2,497.84 kcal kg^{-1} (Possetti *et al.*, 2015). Scenario 3 is similar to Scenario 2 except that the ashes are destined for a sanitary landfill.

Calculation of the environmental impact used the Life-Cycle Assessment methodology and its two phases, by means of SimaPro 8.4 software. The ReCiPe 2016 Midpoint (H) method was used in the present study. The categories of impacts evaluated were: global warming (GWP), stratospheric ozone depletion (ODP), ozone formation, terrestrial ecosystems (OF_TE), terrestrial acidification (TAP), freshwater eutrophication (FEP), terrestrial ecotoxicity (TETP), freshwater ecotoxicity (FETP) and human non-carcinogenic toxicity (HTPnc).

A pedigree matrix was used for analysis of uncertainty using a Monte Carlo simulation in the software. For this simulation, the standard deviation (SD) of each entry of the inventoried life-cycle was obtained by combining the pedigree matrix, a basic uncertainty matrix and the pedigree vector. The matrix considers two types of uncertainty parameters: intrinsic variability and uncertainty due to imperfect data usage. This is due to the use of estimated results without time and spatial extrapolation verification or to the use of different technologies (Muller *et al.*, 2014). The considered indicators are reliability, completeness, temporal correlation, geographical correlation and further technological correlation.

Details of the environmental assessment and its results are presented in Amaral *et al.* (2018a).

2.2. Social assessment

The following categories of stakeholders were considered in the development of the social inventory: workers, consumers and the local community and society (UNEP; SETAC, 2009). Indicators were selected for each subcategory as suggested by UNEP and SETAC (2013) and the study of Padilla-Rivera *et al.* (2016), who presents the main social aspects associated with wastewater treatment. In order to produce a consistent method for all subcategories the methodology establishes a baseline for assessing the organization's profile called the basic requirement (BR).

For monitoring and mapping odor, the concentration of H_2S was measured at certain points of the WWTP and in its vicinity using a portable meter. Measurements of NH_3 were made on the day that the anaerobic sludge was being limed in the SMU. Occupation noise values were provided by the sanitation company that operates the studied STS in relation to the base scenario. For the other scenarios, measurements were taken in the pilot system of the rotary dryer installed. An adaptation of the methodologies described by Ramirez *et al.* (2014), Zortea *et al.* (2017) and Padilla-Rivera *et al.* (2016) was used for the assessment of social impacts.

Details of the social assessment methodology are presented in Amaral *et al.* (2017).

2.3. Life-cycle cost analysis – LCC

The economic inventory was performed by the life-cycle cost analysis (LCC) method. The method is based on the life-cycle assessment, but considers economic costs rather than environmental impacts. Costs related to the consumption of energy, chemicals, workers, equipment acquisition and maintenance were applied to the WWTP described in the base scenario and the other scenarios.

Total Cost (TC) was calculated for each alternative process analyzed as the sum of all annual acquisition, operation and maintenance costs. The cost of transport and the fuel used in the final destination of biological sludge was also considered in TC.

After the annual cost survey, the values were converted to the functional unit — the treatment and management of by-products to treat 1 m^3 of effluent.

Details of the economic analysis and its results are presented in Amaral *et al.* (2018b).

2.4. Assessment of the three dimensions of sustainability

The next step was to convert the data into a single sustainability indicator. The sustainability indicator *Dashboard of Sustainability* (DoS), introduced by Traverso *et al.* (2012) (UNEP; SETAC, 2011) was used to integrate the three dimensions of sustainability. The model weights all indicators for the same scale and represents them in mathematical or graphical form. From this a score between 0 and 1000 points is determined, with zero points for the worst case possible and 1000 points for the best. Intermediate cases are then calculated using linear interpolation between these two delimitations, as presented in the Equation 1:

$$(\text{DoS score})_i = 1000 \times \frac{[(\text{value})_i - (\text{value})_0\,]}{[(\text{value})_{1000} - (\text{value})_0\,]} \tag{1}$$

In which:

(DoS score) = the DoS score assigned to the indicator in a context I;

(value) i = indicator value for context i (intermediate);

(value) 0 = indicator with the worst value among all contexts;

(value) 1000 = indicator with the best value among all contexts.

This indicator works with a scale of 7 to 9 colors that correspond to different levels of sustainability. This color scale ranges from dark green (excellent) to deep red (critical) (Figure 3). In the final presentation, a ring is visualized where the external three circles present the indicators of the dimensions (environmental, social and economic) in a single measurement while the central circle presents the final sustainability index (SI or SID).

Environmental, social and economic dimensions were used for this work for a total of 23 indicators, selected according to previous studies (Amaral et al., 2016) and literature (Padilla-Rivera et al., 2016; Anabestani e Zareie, 2017; Iftekhar et al., 2018; Xu et al., 2014; Mills et al., 2014; Garrido-Baserba et al., 2015) of which eight were environmental, 10 social and five economic (Table 1). Inventory details (environmental, social and economic) with the considered variables for each indicator is in an additional material.

Color Scale	Degree of Sustainability	Point interval
	Excellent	889 - 1000
	Very good	778 - 888
	Good	667 - 777
	Reasonable	556 - 666
	Medium	445 - 555
	Bad	334 - 444
	Very bad	223 - 333
	Severe attention	111 - 222
	Critical state	0 - 110
	No data	----

Figure 3. Color scale used in DoS software.

Table 1. Indicators considered in the present study for the evaluation of sustainability.

Environmental	Social	Economic
Global warming	Wages paid to workers	Cost of dewatering stage (centrifuge)
Stratospheric ozone depletion	Noise level (workers)	Cost of sanitization system
Terrestrial ecosystem ozone formation	Use of hazardous chemicals	Cost of sludge disposal
Terrestrial acidification	Odor emission (H_2S and NH_3) (workers)	Cost of biogas disposal
Aquatic eutrophication	Biological risks (bacteria, fungi, viruses)	Maintenance cost
Terrestrial ecotoxicity	Sludge N and P content	
Freshwater ecotoxicity	Values of pathogens present in sludge	
Non-carcinogenic human toxicity	Noise level (community and society)	
	Odor emission (community and society)	
	Capacity to generate employment	

After the environmental, social and economic evaluation, the DoS model was used to interpret the three dimensions for the four scenarios.

3. RESULTS AND DISCUSSION

The results for the environmental, social and economic indicators are presented in Table 2.

Table 3 and Figure 4 present the results of the sustainability indicators. The arrow shows the position of the sustainability index in the color scale. The degree of sustainability of each dimension is also demonstrated through the color scale.

On the base scenario, the environmental dimension was the worst with the categories of climate change, terrestrial ecotoxicity, stratospheric ozone depletion, freshwater ecotoxicity and non-carcinogenic human toxicity being "very poor" on the scale. The steps that most contributed to the climatic change category was the emission of methane and carbon dioxide through biogas destruction in open burners (56.13%), and by the emission of dissolved methane on the effluent (36.75%). Houillon and Jolliet (2005) did en environmental LCA (Life Cycle

Assessment) (climatic changes category) to destine sludge and concluded that agriculture and landfill destination were the most outrageous for the category of climatic changes. Out of these, incineration was the best option. The high value of human toxicity is due to heavy metals existing in sludge and spread by agriculture use, also evidenced by Tarantini et al. (2007). Hospido et al. (2010) studied the reuse of anaerobic reactor sludge in agriculture in Spain. The results suggest that emergent pollutants' contribution is less important than heavy metals. The indicators that presented the worst scale for the economic dimension were: maintenance cost, disposal of sludge cost and disposal of biogas cost. Biological risk was the worst indicator for the social dimension, as a result of thermo tolerant coliforms presence in biologic sludge and the biologic risk intrinsic in manual sludge manipulation.

Table 2. Results for the indicators considered in the present study for sustainability assessment.

	BS	S1	S2	S3
Environmental Indicators				
GWP (kg de CO2eq.)	0.7864	0.4409	0.4325	0.4598
ODP (kg de CFC-11eq.)	7.94×10^{-08}	3.88×10^{-08}	3.43×10^{-08}	3.09×10^{-08}
OF_TE (kg NOx eq.)	1.39×10^{-04}	7.58×10^{-05}	2.89×10^{-04}	2.96×10^{-04}
TAP (kg de SO_2)	0.0033	0.0007	0.0059	0.0048
FEP (kg de Peq.)	1.34×10^{-05}	1.41×10^{-05}	1.39×10^{-05}	1.43×10^{-05}
TETP (kg de 1,4 DBeq.)	5.3×10^{-05}	1.44×10^{-05}	2.47×10^{-07}	3.69×10^{-06}
FETP (kg de 1,4 DBeq)	9.65×10^{-05}	1.62×10^{-05}	4.06×10^{-06}	1.12×10^{-05}
HTPnc (kg de 1,4 DBeq.)	14.3482	0.1029	0.0642	0.0715
Social Indicators				
Wages paid to workers	4	4	4	4
Noise level (workers)	3	3	3	3
Use of hazardous chemicals	3	3	3	3
Odor emission (H_2S and NH_3) (workers)	3	3	3	3
Biological risks (bacteria, fungi, viruses)	2	3	3	3
Sludge N and P content	3	3	4	0
Values of pathogens present in sludge	3	3	4	0
Noise level (community and society)	2	2	2	2
Odor emission (community and society)	3	3	3	3
Capacity to generate employment	4	3	3	3
Economic Indicators				
Dewatering stage cost	0.0237	0.0237	0.0237	0.0237
Sanitization system cost	0.021254	0.075414	0.085121	0.085121
Sludge disposal cost	0.006539	0.003024	0.001754	0.002135
Biogas disposal cost	0.000125	0	0	0
Maintenance cost	0.010007	0.004528	0.006153	0.006153

Table 3. Score for each dimension and the sustainability index for each of the studied scenarios.

Scenarios/Dimension	Environmental	Social	Economic	Sustainability index
Base Scenario	277	550	300	375
Scenario 1	813	550	673	678
Scenario 2	656	600	633	629
Scenario 3	624	400	619	547

NOTE: Base scenario = sludge sanitized by PAS and destined for agriculture, biogas destroyed by low efficiency burning; S1 = Scenario 1 sludge sanitized in rotary dryer using biogas and destined for agriculture; S2 = Scenario 2 = sludge sanitized in rotary dryer through the heat from sludge combustion, with the ashes destined for agriculture; S3 = Scenario 3 = similar to Scenario 2 but ashes destined for sanitary landfill.

On the ozone formation category (terrestrial ecosystems), Scenarios 2 (2.89×10^{-04} kg NO_x eq) and 3 (2.96×10^{-04} kg NO_x eq) have a high impact potential when compared to the base scenario (1.39×10^{-04} kg NO_x eq). These scenarios were higher because of the increase of NO_x emissions during sludge combustion; in Scenario 2, this step was responsible for 99% and 97% in Scenario 3.

Scenario 1 (6.78×10^{-04} kg SO_2 eq) presented a reduction of 79% of the potential environmental impact for the category of soil acidification when compared to the base scenario (3.26×10^{-03} kg SO_2 eq). Sludge sanitation is the step that most contributed to this category. In the base scenario, this step was responsible for 72% of the total impact. NH_3 emissions during sludge liming had the greatest impact on this category, representing 70% of the total impact. In Scenarios 2 and 3, sludge combustion represented 78% and 95% of the total impact, respectively. The contributing elementary flows were nitrogen emissions and sulfur oxide combustion. Within it were SO_x emissions, representing 57% of environmental impact for this category in Scenario 2 and 70% in Scenario 3.

Wang *et al.* (2013) studied options for sludge destination and treatment and the conclusion was that the best environmental option was combustion followed by co-incineration and landfill, although heavy metal emissions were not considered.

The bad performance of the category of employment-generation capacity in the social dimension, in Scenarios 1, 2, and 3, is by reason of using a rotating dryer for the sanitation and drying of biological sludge, increasing the level of mechanization and generating fewer jobs.

Regarding the economic evaluation, the elevated cost in Scenarios 1, 2, and 3 is due to the biologic sludge sanitation step, with the drying equipment acquisition being more than 50%.

As shown in Table 3 and Figure 4, Scenario 1 had the best score (darker green coloration). The environmental dimension favored this scenario, with the indicators of terrestrial acidification and terrestrial ecosystem ozone formation having higher scores than in the other scenarios. The lowest score was for the base scenario for the category of terrestrial acidification due to the emission of NH_3 during the process of sludge liming, and Scenario 2 and Scenario 3 due to the emission of SO_x from the combustion of anaerobic sludge. Scenario 2 and Scenario 3 also had the lowest scores for terrestrial ecosystem ozone formation due to increased NO_x emissions during sludge combustion.

The sustainability index for Scenario 1 was 678 points ("good" on the DoS scale). None of the three dimensions were "bad" on the scale. The social dimension scored 550 points ("average" on the scale), with the worst indicator being the capacity to generate employment. The worst indicator for the economic dimension was the sanitization stage, while for the environmental dimension it was freshwater eutrophication. The best indicators in the environmental dimension were the categories of climate change, terrestrial ecosystem ozone formation, human toxicity and terrestrial acidification. The best indicators for the economic dimension were maintenance cost and biogas disposal cost, while the best indicator for the social dimension was biological risks.

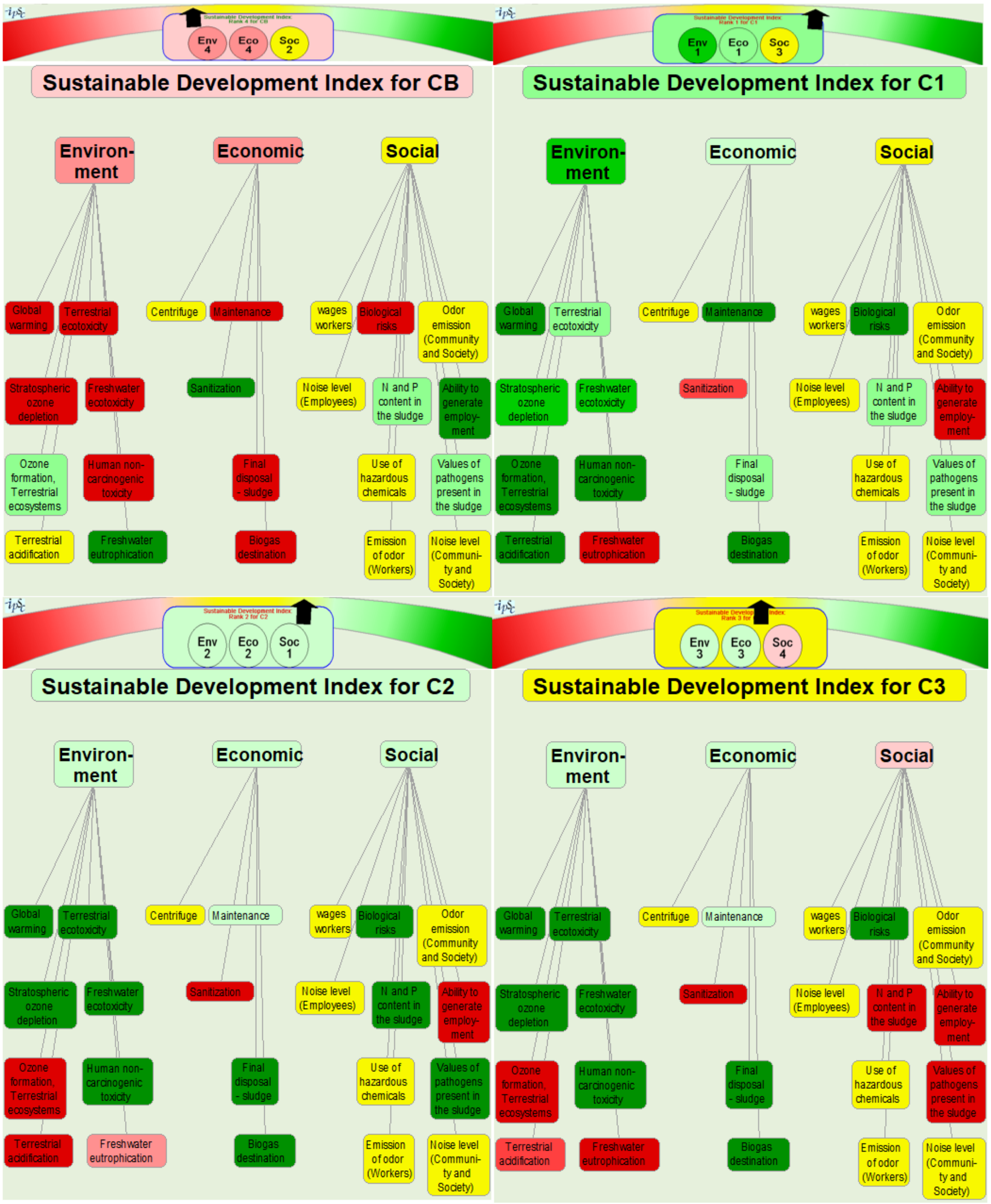

Figure 4. Results for sustainability indicators for the different scenarios *.
*NOTE: CB = base scenario (sludge sanitized by PAS and destined for agriculture, biogas destroyed by low efficiency burning). C1 = Scenario 1 (sludge sanitized in rotary dryer using biogas and destined for agriculture). C2 = Scenario 2 (sludge sanitized in rotary dryer through heat from sludge combustion, with the ashes destined for agriculture. C3 = Scenario 3 (similar to Scenario 2 but ashes destined for landfill).

Scenario 2 had a sustainability index of 629 points ("reasonable" on the DoS scale). The level of sustainability was the same for the three dimensions. The indicators with the lowest indexes in the environmental dimension were: terrestrial ecosystem ozone formation and terrestrial acidification. The stage with the lowest index in the economic dimension was sanitization system cost while the capacity to generate employment was the lowest for the social dimension.

Scenario 3 had a sustainability index of 547 points. The social dimension had the lowest score (400 points), where the indicators of sludge N and P content, capacity to generate employment and values of pathogens present in the sludge were the indicators that were “bad” on DoS scale. The worst indicators for the environmental dimension were terrestrial ecosystem ozone formation, terrestrial acidification and aquatic eutrophication. For the economic dimension the sanitization system cost had the lowest score. The best indicators for the environmental dimension were the categories of climate change, terrestrial ecotoxicity, stratospheric ozone depletion, freshwater ecotoxicity and human toxicity. The best indicators for the economic dimension were sludge disposal cost and biogas disposal cost, while the best indicator for the social dimension was biological risk.

Scenario 2 had the second-best score, being scaled as “reasonable”. This scenario had a better index in the social dimension than did Scenario 1, due to it having higher N and P concentrations in the ashes of the biological sludge.

Scenario 3 had a lower score than the other scenarios because of the social dimension, which was due to non-utilization of the agronomic potential of the biological sludge. Thus, the final “rank” for the sustainability index was (from best to worst): Scenario 1, Scenario 2, Scenario 3 and Base Scenario.

4. CONCLUSIONS

The sustainability indicator Dashboard of Sustainability (DoS), used to integrate the three dimensions of sustainability, proved to be an important instrument for full display of the dimensions used.

Scenario 1, in which biogas is used for drying sludge that is destined for agriculture, obtained the highest score (best scenario) in the DoS. The environmental dimension favored this scenario, with the indicators of terrestrial acidification and terrestrial ecosystem ozone formation being the indicators that had higher scores compared to the other scenarios. Scenario 2 (sanitization of sludge by rotary dryer, through sludge combustion, and ashes used in agriculture) was the second best, with a better index in the social dimension than Scenario 1 due to higher N and P content in the ashes of the biological sludge. Scenario 3 (similar to Scenario 2, but ashes are destined for landfill) had a lower score in comparison to the other scenarios because of the social dimension due to non-utilization of the agronomic potential of biological sludge.

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Antibiotic resistance in surface waters from a coastal lagoon of Southern Brazil under the impact of anthropogenic activities

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ABSTRACT

Wastes arising from human activities can reach water bodies and contribute significantly to the presence of antibiotic resistant bacterial populations in aquatic environments. The objective of this study was to evaluate the cultivable antibiotic resistant bacterial populations from a coastal lagoon impacted by agriculture and urbanization activities. Water samples were collected in low and peak season and characterized regarding physicochemical variables, microbiological indicators and the presence of antimicrobial residues. In order to analyze the presence of resistant bacterial populations, the samples were grown in the presence of nalidixic acid, ceftazidime, imipenem and tetracycline. Genes associated with β-lactamic resistance (bla_{CTX-M}-like, bla_{GES}-like, bla_{OXA-51}, bla_{OXA-23}-like, bla_{SHV}-like, bla_{TEM}-like and bla_{SPM-1}), class I integron and efflux systems (*tet*A, *tet*B, *acr*A, *acr*B, *tol*C, *ade*A, *ade*B, *ade*R, *ade*S, *mex*B, *mex*D, *mex*F and *mex*Y) were analyzed by conventional *in vitro* amplification. Although antimicrobials residues were below the detection limit, resistant bacteria and resistance determinants - bla_{GES}, class I integron, *ade*S, *acr*A, *acr*B, *tol*C, *mex*B, *mex*F - were present at almost all points, in both seasons and for all antimicrobials assessed. The high numbers of resistant bacteria counts observed after the antibiotic treatment were positively correlated to the urbanization effects on the Lagoon. Some resistant populations were even higher in the low season samples, indicating the importance of a systematic evaluation of antibiotic resistance on water resources.

Keywords: anthropic impact, antibiotic resistance, aquatic bacteria, estuary.

Resistência antibiótica nas águas superfíciais de uma lagoa costeira do Sul do Brasil sob impacto de atividades antropogênicas

RESUMO

Resíduos oriundos de atividades humanas podem atingir corpos d'água e contribuir significativamente para a presença de populações bacterianas resistentes a antibióticos. O objetivo deste estudo foi avaliar populações de bactérias cultiváveis resistentes a antibióticos em uma lagoa costeira impactada por atividades como agricultura e urbanização. Amostras de água foram coletadas nos períodos de baixa e alta temporada e caracterizadas quanto a variáveis físico-químicas, indicadores microbiológicos e presença de resíduos de antimicrobianos. Para analisar a presença de populações bacterianas resistentes, as amostras foram crescidas na presença de ácido nalidíxico, ceftazidima, imipenem e tetraciclina. Genes de resistencia a β-lactamicos (*bla*$_{CTX-M}$-like, *bla*$_{GES}$-like, *bla*$_{OXA-51}$, *bla*$_{OXA-23}$-like,*bla*$_{SHV}$-like, *bla*$_{TEM}$-like and *bla*$_{SPM-1}$), de classe I integron e sistemas de efluxo (*tet*A, *tet*B, *acr*A, *acr*B, *tol*C, *ade*A, *ade*B, *ade*R, *ade*S, *mex*B, *mex*D, *mex*F and *mex*Y) foram analisados por amplificação *in vitro*. Embora não tenham sido encontrados níveis detectáveis de residuos de antimicrobianos nas amostras, bactérias resistentes estavam presentes em quase todos os pontos, em ambas as estações e para todos os antimicrobianos avaliados. A presença de populações bacterianas resistentes observada foi positivamente correlacionada aos efeitos da urbanização na Lagoa. Algumas populações resistentes foram ainda maiores na baixa temporada, indicando a importância de avaliar sistematicamente a resistência a antibióticos em corpos d'água.

Palavras-chave: bactérias aquáticas, estuário, impacto antrópico, resistência antimicrobiana.

1. INTRODUCTION

Wastes arising from human activities are rich in recalcitrant compounds such as antimicrobials, metals and biocides, which can reach water bodies and contribute significantly to the selection of resistant bacteria phenotypes (Petit *et al.*, 2014). Although water courses seem to play a key role in the maintenance of resistance phenotypes in nature, little attention has been paid to the influence of human activities on the prevalence of resistance in these resources (Pruden *et al.*, 2012; Czekalski *et al.*, 2015; Yang *et al.*, 2017).

Bacteria can be intrinsically resistant to chemicals or acquire resistance determinants via DNA mutations, transformation by foreign DNA incorporation or phage-mediated transduction, or by conjugation (Blair *et al.*, 2015). Antimicrobial-resistant bacteria represent a severe public health problem on a global scale. This has been studied from the clinical perspective over time but the aquatic environment has also been source of resistance determinants with clinical relevance, e. g. *bla*$_{GES-5}$ gene - a carbapenem resistance determinant found in different species of water streams (Manageiro *et al.*, 2014). The *qnrA* and *bla*$_{OXA-181}$ genes - involved in resistance to quinolones and carbapenems, were found in *Shewanella algae* from marine water samples and in *Shewanella xiamenensis* from swabs of seepage water, respectively (Poirel *et al.*, 2005; Potron *et al.*, 2011).

Around 39% of the global population lives less than 100 km from the coastline (Kummu *et al.*, 2016). Some beaches from southern Brazil attract many travellers, particularly during the summer (from December to February), when populations from some seaside locations increase up to five times (Zuanazzi and Bartels, 2016). The area of Tramandaí Hydrographic River Basin, e. g., has a population of 220,296 inhabitants; however, it can reach up to 580,000 inhabitants during summer (Rio Grande do Sul, 2017). This area is composed of a set of many coastal lagoons which flow into the Tramandaí Lagoon before flowing to the Atlantic Ocean.

The increased human activities along the coastal might directly imply in waste overproduction, raising the risk of exposure the water resources to pollutant inputs, which constitutes a premise for constant environmental monitoring.

Levels of antimicrobial contamination have already been detected in water bodies of Brazil (Quadra *et al.*, 2017), but bacterial resistance has been poorly studied in these environments (Nascimento and Araújo, 2014; da Costa Andrade *et al.*, 2015; Monteiro *et al.*, 2016; Oliveira *et al.*, 2016). In this way, this study aims to evaluate the presence of antibiotic resistant bacteria (ARB) in surface waters of Tramandaí Lagoon and whether it varies according to the presence of different anthropic disturbances. For this purpose, water samples were characterized for physicochemical and biological variables, the resistance to the antimicrobial classes quinolone, cephalosporin, carbapenem and tetracycline and presence of antibiotic resistance genes (ARGs) by conventional PCR. The study was designed to infer whether the presence of resistant bacteria in the Lagoon may vary according to 1) the presence of antibiotic residues in the environment, 2) human activities present *in loco*, and 3) increase in local population during peak season.

2. MATERIAL AND METHODS

The Tramandaí Lagoon (29º55’49” S, 30º00’56” S; 50º06’21” W, 50º11’20” W) lies between the Tramandaí and Imbé cities, on the Northern coastal region of Rio Grande do Sul, Southern Brazil (Figure 1). The Lagoon has about 18.8 km^2 of area with 1.4 m of maximum depth, presenting an estuarine channel with a length of 1.5 km, maximum width of 300 m and maximum depth of 5 m. This estuary is responsible for draining to waters from Tramandaí Hydrographic Basin into the Atlantic Ocean (Figure 1B), a system which occupies an area of approximately 2700 km^2 and supplies water to 17 different cities. The Tramandaí Lagoon water uses include irrigation of rice crops areas (mainly from November to February), recreational activities, public supply and the dilution of domestic and industrial wastes, particularly near urban areas (Loitzenbauer and Mendes, 2012). Fishing and tourism are the main regional economic activities.

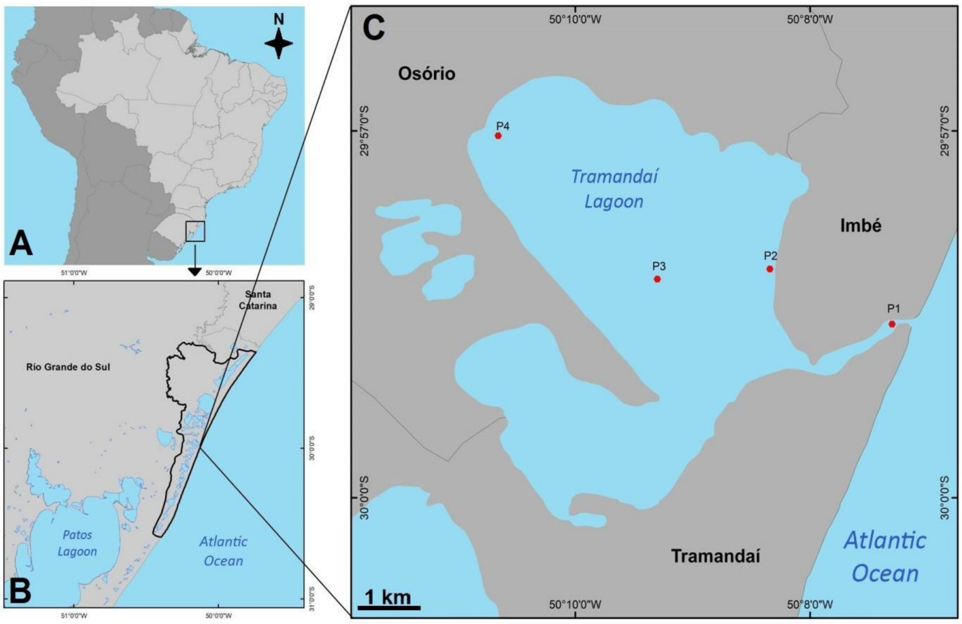


Figure 1. Study site. (A) Rio Grande do Sul state in Southern Brazil. (B) Tramandaí Hydrographic River Basin in Rio Grande do Sul. (C) Tramandaí Lagoon position and the sampling points (P1: Point 1; P2: Point 2; P3: Point 3; P4: Point 4). At the south is Tramandaí city and at the north is Imbé city.

The surrounding beaches are classified as intermediate and dissipative, being dominated by waves of 1.5 m on average. They are governed by a micro-tidal regime; the astronomical tide in this area has annual average amplitude of 0.47 m. South storms induce meteorological tides that can reach 1.3 m, significantly amplifying the erosion effects over the shoreline (Calliari *et al.*, 2009). Tidal oscillation causes the ingression of marine waters by the estuarine channel, salinising not only portions of the Tramandaí Lagoon, but also distant water bodies. Ocean water circulation patterns are driven by the confluence of Tropical Waters - transported by Brazilian Current, with a North-South direction and higher prevalence in the summer, and Subtropical and Subantarctic Waters - transported by Malvinas Current, with South-North direction and higher intensity in winter. Northeast and east winds are prevalent. The annual average is about 1000-1200 mm of precipitation and surface water temperature of 18°C (24.6°C in January and 13.1°C in July) (Medeanic, 2006).

Since the population varies considerably among the seasons in the study area, samples were collected in two samplings, one in the “low season” (August 2014) and another one in the “peak season” (January 2015). The sampling points were selected according the different anthropogenic pressures acting on the environment. The location of points is shown in Figure 1C, while their respective landscapes are illustrated in Figure 1. Point 1 (29°58'34.7" S; 50°07'18.8" W) (Figure 1C), the closest one to the sea, is marked by accentuated urbanization (Figure 2A) and presents a maritime terminal for oil exploration (Almirante Dutra Maritime Terminal) (Figure 2B). Point 2 (29°58'10.4" S; 50°08'20.7" W) (Figure 1C) is in a densely urbanized zone, adjoining the exit of a residential horizontal condominium at Imbé city (Figures 2C and 2D). It is most protected from the marine currents, located closest to a connection with Tramandaí River, by which water flows from other locations in the Basin enter the Tramandaí Lagoon. Point 3 (29°58'12.2" S; 50°09'19.7" W) (Figure 1C) is sited at the center of the lagoon, representing the waters confluence of the whole Lagoon (Figure 2E). Point 4 (29°57' 05.8 S, 50°10'41.7" W) (Figure 1C) is not urbanized, and is surrounded by a native forest (Figure 2F) with nearby rice fields. These points were reached with the assistance of a small watercraft. Samples of surface water (less than 1m deep) were collected with sterile flasks (capacity of two liters) and kept at 4°C.

The water samples were characterized for the following physicochemical variables: temperature, turbidity (by Secchi disk, in the collecting), salinity (by electric conductivity), total solid particles (by gravimetric method), dissolved oxygen (by Winkler method), total nitrogen (by colorimetric method), ammoniacal nitrogen (by Nessler method) and total phosphorus, nitrate, nitrite (both by ion chromatography). Total heterotrophic counts were determined by serial dilution directly from the water samples, followed by spread plate cultivation of 0.1 ml in PCA media (Plate Counting Agar) (incubation at 35°C, until 72h). Total and thermotolerant coliform counts were determined by membrane filtering method: aliquots of water (100 mL) were filtered through nitrocellulose membranes (0.2 μm porosity), which were subsequently deposited on plates containing LES-ENDO Agar. Colonies were counted after 24h of incubation at 35°C for total coliforms and 45°C for thermotolerant coliforms.

The presence of antimicrobials was analyzed on samples according to Jank *et al.* (2014) (technique able to detect residues in concentration above 400 $ng.L^{-1}$ in water). Forty-five types of antimicrobials, from eight different classes, were investigated in samples: sulphadoxazole, sulphadiazine, sulphaclorpyridazine, sulphadoxine, sulphadimetoxin, sulphisoxazole and sulphamethoxazole (sulphonamides); tetracycline, oxytetracycline, chlortetracycline and doxycycline (tetracyclines); norfloxacin, sarafloxacin, difloxacin, danofloxacin, ciprofloxacin, enrofloxacin, norfloxacin, nalidixic acid and flumequine (quinolones); trimethoprim (pyrimidines); penicillin G, ampicillin, cloxacillin, oxacillin and dicloxacillin (penicillins); cephalexin, cephapirin, cephalone, ceftiofur, cephoperazone, cefquinome (cephalosporins);

lincomycin (lincosamines); erythromycin, azithromycin, tylosin, clindamycin, tilmicosin, spiramycin and nafcillin (macrolides). Additionally, 171 organic compounds (pesticides) were monitored on samples by the qualitative analytical methodology (Diaz *et al.*, 2013). Both these analyses were carried using equipment from the National Agricultural Laboratory (LANAGRO): a liquid chromatography 1100 Series (Agilent Technologies) coupled to a tandem triple quadrupole mass spectrometer API 5000 (Applied Biosystems) using an electrospray probe (ESI) in positive mode as an ionisation source.

Figure 2. Different landscapes from sampling points. (A) Buildings surrounding Point 1. (B) Maritime terminal for oil exploration next to Point 1. (C) (D) Houses from residential condominium adjacent to Point 2. (E) Landscape from Point 3, in the center of Tramandaí Lagoon. Densely urbanized margins are represented in the background. (F) Native forest in Point 4, where there are no buildings.

The water samples were concentrated by filtration through membranes with 0.2μm of porosity and the total DNA was extracted by UltraClean Soil DNA Kit (MoBio Laboratories, Inc.), according to manufacturer's instructions. The DNA was quantified using Quantus Fluorometer (Promega) with the Quantifluor DNA System kit (Promega) according to manufacturer´s recommendations. The presence of antimicrobial resistance genes was assessed by conventional PCR, according to Poirel *et al.* (2001) ($bla_{\text{CTX-M}}$-like genes); Jeong *et al.* (2006) (bla_{GES}-like genes); Woodford *et al.* (2006) ($bla_{\text{OXA-51}}$,$bla_{\text{OXA-23}}$-like genes); Talukdar *et al.* (2013) (bla_{SHV}-like and bla_{TEM}-like genes); Fuentefria *et al.* (2009) ($bla_{\text{SPM-1}}$ gene); Ng *et al.* (2001) (*tet*A and *tet*B); Lévesque *et al.* (1995) (Class I integron using primers to the 5' and 3' conserved segments); Swick *et al.* (2011) (*acr*A, *acr*B and *tol*C); Beheshti *et al.* (2014) (adeA, *ade*B, *ade*R and *ade*S); Yoneda *et al.* (2005) (*mex*B and *mex*Y); Xavier *et al.* (2010) (*mex*D) and Oh *et al.* (2003) (*mex*F). Positive controls of the genes were included in all reactions. The primers and PCR conditions are listed in Supplemental material S1. The amplifications were performed with 10ng of genomic DNA in a final volume of 25 μL. The primers were able to amplify from 2ng to 0.01 ng of control DNA. Amplification of the 16SrRNA gene was performed for all the DNA samples, to ensure the negative result.

Antibiotic resistant bacterial populations from Tramandaí Lagoon waters samples were determined by counts of colony-forming units able to growth in the presence of nalidixic acid, ceftazidime, imipenem and tetracycline in a final concentration of 20 $mg.L^{-1}$ (chosen according to MIC breakpoints) (CLSI, 2014). Water samples were cultured with nutrient broth (volume 1:1, by 24h to 35°C) with antibiotic supplementation and after were grown in Plate Counting Agar (PCA) also supplemented with the corresponding antibiotic (24h at 35°C). Control samples for all sites were grown in nutrient broth and PCA without antibiotic supplementation.

All bacterial counts (total heterotrophic, total and thermotolerant coliforms and antibiotic resistant) were reported as log_{10}-transformed counts and weighted by analysis of variance (ANOVA). Tukey's post hoc test was applied to compare the data between seasons and lagoon points. Dunnett's post hoc test was used to compare the data of different antimicrobial treatments with the control. Statistical significance was assigned to $P<0.05$. Correlations between the environmental variables, microbiological variables and resistant bacteria counts were appraised by Pearson's correlation coefficient. A multivariate statistical projection method (Principal Component Analysis - PCA) was also applied to reduce the dimensionality and analyze the data.

3. RESULTS AND DISCUSSION

Tramandaí Lagoon was under the ebbing tide in "low season" sampling, while in "peak season" it was under the flooding tide. Although the lagoon is a highly dynamic environment, the environmental variables assessed allow inferences about the *in situ* conditions at sampling time. The tide seems to have influenced especially the higher total solids content and the lower salinity and visibility values in "low season" (Table 1), since river inflows can reduce the salinity and carry particles arising from upstream erosion processes in addition to resuspending the bottom sediment.

Independent of the season and tide, the Points 1 and 2 showed some characteristics attributed to greater urbanization, like higher nutrient concentrations (nitrate was detectable just in Point 2) and higher bacterial counts, especially for thermotolerant coliforms (Table 1). Under acceptable conditions by the Brazilian government environmental law (CONAMA Resolution n°357) (CONAMA, 2005), natural brackish waters have levels of phosphorus below 0.124 $mg.L^{-1}$ and ammoniacal nitrogen below 0.40 mg.L-1, higher values indicate environmental imbalance in the lagoon at points marked by urbanization. Therefore, the human activities impact present in the Points 1 and 2 might represent a punctual disturbance, even in the periods of population decline ("low season"). On the other hand, Points 3 and 4, not urbanized, generally exhibited the lower nutrient levels and the higher values for oxygen dissolved and visibility (Table 1).

The total heterotrophic counts in Point 3 differed between samplings (Table 1), probably influenced by the position in the center of the lagoon and by inputs of nutrient-enriched waters due to the river ebbing. Point 3 has also shown the lower thermotolerant coliforms counts in the "low season" (Table 1), which can be connected to a lower punctual impact or even to the dilution of faecal pollution. Point 2 was the only point where all microbiological variables varied among the seasons (Table 1). Though has presented the highest thermotolerant coliforms counts in both seasons (Table 1), this point was more affected by faecal pollution in the "peak season".

Despite the anthropogenic activities observed locally, antibiotic residues were not observed in water samples at any season or point from Tramandaí Lagoon, which does not exclude the possibility of contamination in lower levels than the limit of detection (400$ng.L^{-1}$). Antibiotics rarely occur in lethal concentrations outside clinical environments, mainly in highly diluted environments such as water courses (Kummerer, 2009). Even though, resistant bacteria were present in Tramandaí Lagoon samples in both seasons, in almost all points and for almost all antibiotic treatments tested (Table 2). Therefore, low antibiotic concentrations can also contribute to the selection of resistance in wild-type susceptible populations (Gullberg *et al.*, 2011).

Table 1. Characterization of Lagoon samples to physicochemical and microbiological variables, in "low" and "peak" seasons (respectively on August 2014 and January 2015).

Variables	Seasons	Point 1	Point 2	Point 3	Point 4
Temperature (°C)	Low	15.7	16.2	15.5	17.2
	Peak	24.8	26.5	24.6	25.1
Salinity (ppt)	Low	9.22	0.1	10.05	12.11
	Peak	64.98	31.42	22.36	11.97
Dissolved oxygen (O_2) ($mg.L^{-1}$)	Low	10.32	9.16	9.99	10.44
	Peak	7.9	7.5	7.3	7.1
Total phosphorus (P) ($mg.L^{-1}$)	Low	0.08	0.12	0.06	0.06
	Peak	0.08	0.07	0.07	0.06
Total nitrogen (N) ($mg.L^{-1}$)	Low	5.3	3.99	1.37	1.44
	Peak	2.08	9.63	2.6	2.08
Nitrite (NO_2) ($mg.L^{-1}$)	Low	ND	1,02	ND	ND
	Peak	ND	ND	ND	ND
Nitrate (NO_3) ($mg.L^{-1}$)	Low	ND	ND	ND	ND
	Peak	ND	ND	ND	ND
Ammoniacal nitrogen (NH_4) ($mg.L^{-1}$)	Low	4.3	3.7	0.07	0.16
	Peak	0.78	2.34	2.21	1.56
Total solids ($mg.L^{-1}$)	Low	5018	122	5548	6319
	Peak	37.99	17.69	13.03	6.98
Visibility (cm)	Low	50	40	40	70
	Peak	60	60	80	60
Total heterotrophic (mean CFU count + SD)	Low	3.74 ± 0.17^{A}	**$2.73 \pm 0.46^{A, C}$**	**$4.37 \pm 0.05^{A,B}$**	$2.91 \pm 0.44^{A,C}$
	Peak	2.82 ± 1.04^{B}	**4.45 ± 0.25^{A}**	**$2.25 \pm 0.24^{B,C}$**	$3.49 \pm 0.88^{A,B,C}$
Total coliforms (mean CFU count + SD)	Low	3 ± 0.14 A	**2.4 ± 0.46^{A}**	3.02 ± 0.04^{A}	3.03 ± 0.48^{A}
	Peak	2.45 ± 0.59^{B}	**4.29 ± 0.25^{A}**	2.43 ± 0.1^{B}	2.4 ± 0.1^{B}
Thermotolerant coliforms (mean CFU count + SD)	Low	**2.47 ± 0.45^{A}**	**2.5 ± 0.24^{A}**	2 ± 0.1^{B}	**2.64 ± 0.19^{A}**
	Peak	**0.88 ± 0.32^{C}**	**4.24 ± 0.21^{A}**	1.84 ± 0.25^{B}	**$1.44 \pm 0.18^{B, C}$**

ppt: *parts per thousand.* **ND**: *not detected.* **Limits of detection:** *nitrate (0.03 $mg.L^{-1}$), nitrite (0.04 $mg.L^{-1}$). Counts were expressed in colony-forming unit mean ± standard deviation (log_{10}-transformed). Significantly different values are bold marked (seasons comparison) or present different letters (points comparison) ($p < 0.05$, Two-way ANOVA followed by Tukey's post hoc test).*

Table 2. Characterization of Lagoon water samples according to the presence of resistance genes in the DNA samples and total counts of resistant bacteria after growth in the presence of antibiotic, in “low” and “peak” seasons (respectively, on August 2014 and January 2015).

	Season	Point 1	Point 2	Point 3	Point 4
Resistance genes amplified	Low	Class I integron, *bla*$_{GES}$, *acr*A, *acr*B, *tol*C, *mex*B, *mex*F.	*bla*$_{GES}$, *tet*B, *acr*A, *acr*B, *ade*S, *mex*B, *mex*F.	Class I integron.	*bla*$_{GES}$, *acr*B, *tol*C.
	Peak	Class I integron, *bla*$_{GES}$, *acr*A, *tol*C, *mex*B, *mex*F.	Class I integron, *bla*$_{GES}$, *ade*S, *mex*B, *mex*F.	Class I integron, *bla*$_{GES}$, *acr*A, *acr*B, *tol*C, *mex*B.	Class I integron, *bla*$_{GES}$, *acr*A, *acr*B, *ade*S, *mex*B, *mex*F.
Control	Low	**> 14** $^{\mathbf{A}}$	> 14 ^{A}	> 14 ^{A}	> 14 ^{A}
	Peak	**5.52 ± 0.10** $^{\mathbf{B}}$	11.61 ± 1.91 ^{A}	11.79 ± 1.90 ^{A}	> 14 ^{A}
Nalidixic acid treatment	Low	**7.56 ± 0.02** $^{\mathbf{B}}$	8.00 ± 1.36 ^{B}	**> 14** $^{\mathbf{A}}$ $*$	> 14 ^{A} $*$
	Peak	**> 14** $^{\mathbf{A}}$ $**$	11.21 ± 2.28 ^{A} $*$	**9.32 ± 0.07** $^{\mathbf{B}}$ $*$	13.41 ± 0.51 ^{A} $*$
Ceftazidime treatment	Low	**8.43 ± 0.71** $^{\mathbf{B}}$	9.71 ± 0.03 ^{B}	**1.02 ± 0.01** $^{\mathbf{C}}$	**> 14** $^{\mathbf{A}}$
	Peak	**5.11 ± 0.14** $^{\mathbf{B}}$ $*$	9.49 ± 2.27 ^{A} $*$	**4.84 ± 0.21** $^{\mathbf{B}}$	**5.41 ± 0.18** $^{\mathbf{AB}}$
Imipenem treatment	Low	**10.09 ± 0.03** $^{\mathbf{B}}$	9.79 ± 0.05 ^{B}	**8.93 ± 1.12** $^{\mathbf{B}}$	**> 14** $^{\mathbf{A}}$
	Peak	**ND**	7.73 ± 4.21 ^{A}	**3.13 ± 1.02** $^{\mathbf{B}}$	**2.72 ± 0.33** $^{\mathbf{B}}$
Tetracycline treatment	Low	13.02 ± 2.53 ^{A} $*$	> 14 ^{A} $*$	8.93 ± 1.12 ^{B}	**> 14** $^{\mathbf{A}}$ $*$
	Peak	11.24 ± 1.86 ^{A} $**$	12.86 ± 0.02 ^{A} $**$	8.66 ± 0.45 ^{A}$*$	**2.3 ± 0.01** $^{\mathbf{B}}$

Bacterial counts expressed as Colony-forming unit mean ± standard deviation (values $\log_{10}$-transformed).
Significantly different values are bold marked (seasons comparison) or present different letters (points comparison in the same season) ($p < 0.05$, Two-way ANOVA followed by Tukey’s post hoc test).
$*$ Resistant bacteria count is equivalent to control bacteria count ($p > 0.05$, Two-way ANOVA followed by Dunnett’s post hoc test). $**$ Resistant bacteria quantity higher than those present in control.
ND: not detected.

Comparing the colony-forming unit counts, those from Points 2 and 4 highlighted by the higher scores in both seasons and for almost all types of antimicrobial supplementation (Table 2). Counts from controls samples refers to total cultivable bacteria under non selective enrichment and might include both native resistant and sensitive bacteria. Then, ARB counts from samples with antimicrobials which were equivalent to the respective control counts (values marked with * in Table 2) attracted attention. Although the counts were usually higher in the “low season”, counts equivalent to controls were observed in 56.25% of samples (9/16) in the “peak season”, when anthropogenic activities have increased in the region (Table 2). Additionally, Point 2 was the only one in which the ARB counts did not vary between seasons (Table 2), reinforcing the likely influence of local factors to antibiotic resistance maintenance.

Nalidixic acid and tetracycline selected higher counts of ARB than ceftazidime and imipenem (Table 2). The tetracycline was the only antimicrobial evaluated which has a biological origin. Hatosy and Martiny (2015) drew attention to the fact of the resistance to synthetic antimicrobials can be a result of contaminated effluents, since microbes would not experience these molecules in natural microbe-microbe interactions. Then, the prevalence of nalidixic acid resistant bacteria might be a reflection of local anthropic disturbances. Higher counts of nalidixic acid resistance were observed at Points 3 and 4 in low season and at Point 1 in peak season (Table 2). This shows that even the microbiota from non-urbanized regions (Points 3 and 4) can be impacted for antimicrobial resistance, which has already been reported around the world (Bhullar *et al.*, 2012; Chen *et al.*, 2013).

The increase in tetracycline resistance on water courses has also been attributed to the possible overuse in aquaculture (Young *et al.*, 2013) or agriculture (Winkworth-Lawrence and Lange, 2016). A large part of the Tramandaí River Basin area is composed of crops (Loitzenbauer and Mendes, 2012) which probably impact the adjoining waters. Waters from Point 4, next to rice crop areas, revealed high resistant counts in the “low season”. These results suggest that the agriculture management seemed to impact the ARB as well as the presence of urbanization, and that the “low season” represents a period that is as critical to the resistance expression as the “peak season”. Residues of Tiamethoxan, Triciclazole and Propoxur (out of the 171 pesticides evaluated) were detected in all sampling sites, probably due to influence of the cropping areas nearby Point 4. Triciclazole is a broad spectrum fungicide used to control plant diseases in cereals and fruits. Tiametoxan is a neoticotinoid and Propoxur is a carbamate, both groups of drugs are used to control agricultural pests, acting on the central nervous system of insects. Albeit the action on bacterial cells of the pesticides found is unknown, it is a consensus that metals as well as organic compounds can influence the antibiotic resistance genomic display of bacteria (Pal *et al.*, 2017).

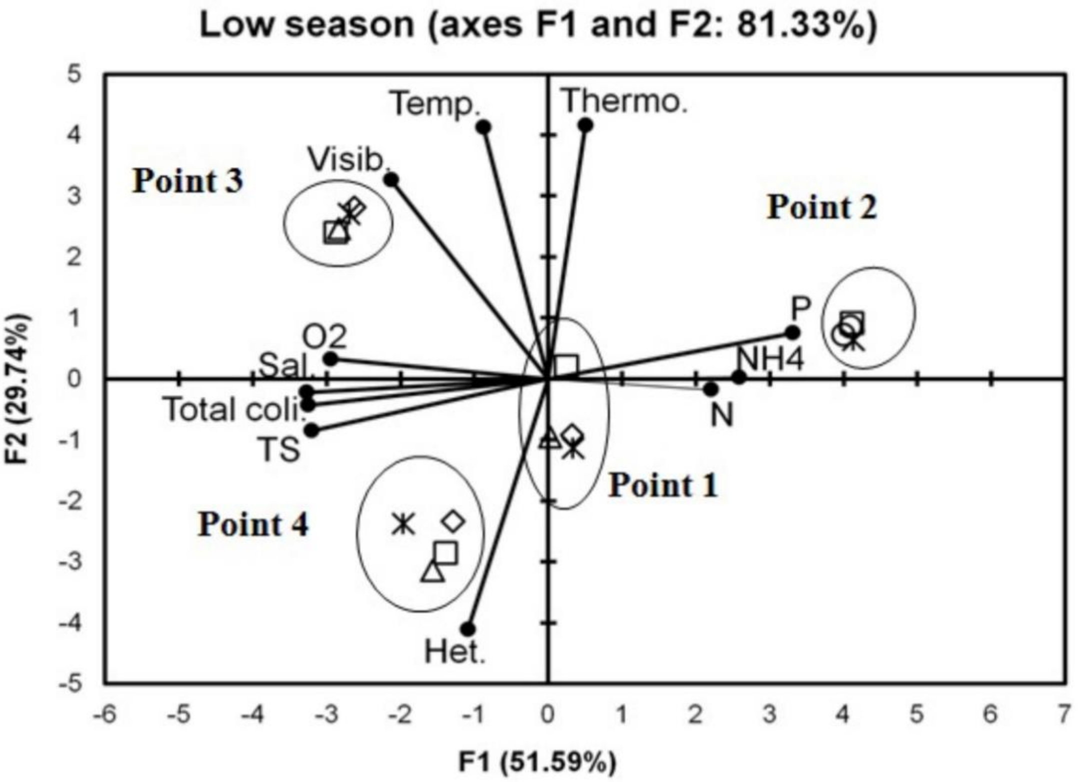
The ceftazidime and imipenem resistant populations seemed more sensitive to seasonal variation (different values among seasons are bold marked in Table 2). Even though these populations have been less represented, the selection of imipenem and ceftazidime antimicrobial resistances has been rarely documented in natural environments (Kittinger et al., 2016). The low prevalence of imipenem-resistant bacteria (Table 2) corroborates with previous reports (Montezzi et al., 2015; Malik et al., 2015). The imipenem resistance could probably be related with the presence of blaGES gene on samples - the only bla gene found in Tramandaí Lagoon waters (Table 2). The carbapenemase blaGES-5 has been commonly reported in aquatic isolates of Enterobacteriaceae, considered ubiquitous bacteria in these environments (Manageiro et al., 2014). This gene has also been detected in isolates of *Klebsiella sp.* and *Enterobacter sp.* in recreational waters (Paschoal et al., 2017) and aquatic environments (de Araújo et al., 2016).

The Class I integron amplification observed in both groups of samples has been associated with water sources under anthropic impact (Abella *et al.*, 2015; Gillings *et al.*, 2015) and its importance in the assembly of ARGs cassettes and its maintenance in the environment has been consistently demonstrated (Stalder *et al.*, 2012). The tetracycline resistance is commonly disseminated in aquatic environments (Harnisz *et al.*, 2015), but despite this we have detected this phenotype throughout the Lagoon area; the *tet*B gene was identified only in the low season, at Point 2. Although the genes of the Mex and AcrAB-TolC systems are constitutively expressed, their overexpression has been associated with resistance to fluoroquinolones and carbapenems in isolates of clinical origin (Neves *et al.*, 2011; Singh *et al.*, 2012). In aquatic ecosystems, these efflux pump genes may be associated with distinct resistant phenotypes, mainly to organic molecules (the earlier mentioned pesticides) and metals. Because antibiotics can also be a substrate for them, they also have an important role in the maintenance of resistance phenotypes in oligotrophic environments.

The clustering of resistant counts (including the respective controls) with environmental and microbiological characters by the principal component analysis (PCA) showed some relevant correlations (Figure 3). The variation observed was better explained in the low season (81.33% of the variability) than in the peak season (76.92% of the variability) (Figure 3). Since the counts were grouped by the sampling points, the environmental conditions of the points seemed to be more important to assess the total resistance in the cultivable bacteria from the Lagoon than the selective pressure of the antimicrobial class used in the *in vitro* experiment (Figure 3). In the low season, the resistant counts at Point 1 were related to the same variables from Points 2 and 3, which did not present similar interactions with each other (Figure 3). Point 4 counts were weakly correlated with those from other sampling points (Figure 3), evidencing the effect of environmental conditioners in this point. On the other hand, in the peak season, the resistant counts from non-urbanized points (3 and 4) were strongly correlated with each other, while those from the urbanized points (1 and 2) were weakly correlated and influenced by different variables (Figure 3). In both seasons, the resistant counts from Point 2 were explained by some of the nutrients analyzed as well as by the thermotolerant coliform amounts (whose correlation is even higher in peak season) (Figure 3). Thus, the ARB count variance in this point appeared to be influenced by faecal pollution, especially in the peak season, as a possible effect of the intense anthropogenic activities observed in this period. Landscape changes as a consequence of urbanization have been associated to impact on bacterial communities modifications, including the increasing in both thermotolerant coliforms (Garbossa *et al.*, 2017; Oliveira *et al.*, 2016) and ARB abundance (Wang *et al.*, 2016; Yang *et al.*, 2017).

The quantification of ARB on culture medium supplemented with antimicrobials usually supports studies focused on resistance analysis in water bodies around the world (Harnisz *et al.*, 2015; Mao *et al.*, 2015). However, antimicrobial resistance has been notoriously underestimated in aquatic environments, since both microbial cells and nutrients are highly diluted (Xu *et al.*, 2016). Although present limitations (such as considering just the cultivable fraction of populations, estimating resistance indirectly, not distinguishing between intrinsic and acquired resistance mechanisms or not appraising the variation of ecological attributes like diversity or richness), this approach is a low-cost alternative which allows the resistance in nature to be assessed, even under little environmental representativeness (for example, the ceftazidime and imipenem resistance).

Brazilian studies involving antimicrobial resistance in water sources have focused on freshwater systems, mainly in aquaculture, being based on the disk diffusion technique (Nascimento and Araújo, 2014). This indicates a demand for additional studies on natural and saline environments. The methodology adopted in this paper allows inferences about the resistance in nature, contributing to reduce the analysis time and costs associated with the isolation of pure cultures or antibiotic disks. Antimicrobial resistance has been poorly prospected in water reservoirs from the Brazilian south coast. As estuaries are highly dynamic and complex, evaluating the resistance variability using a larger time scale study is essential to prevent, detect or even mitigate possible resistance outbreaks in these environments.



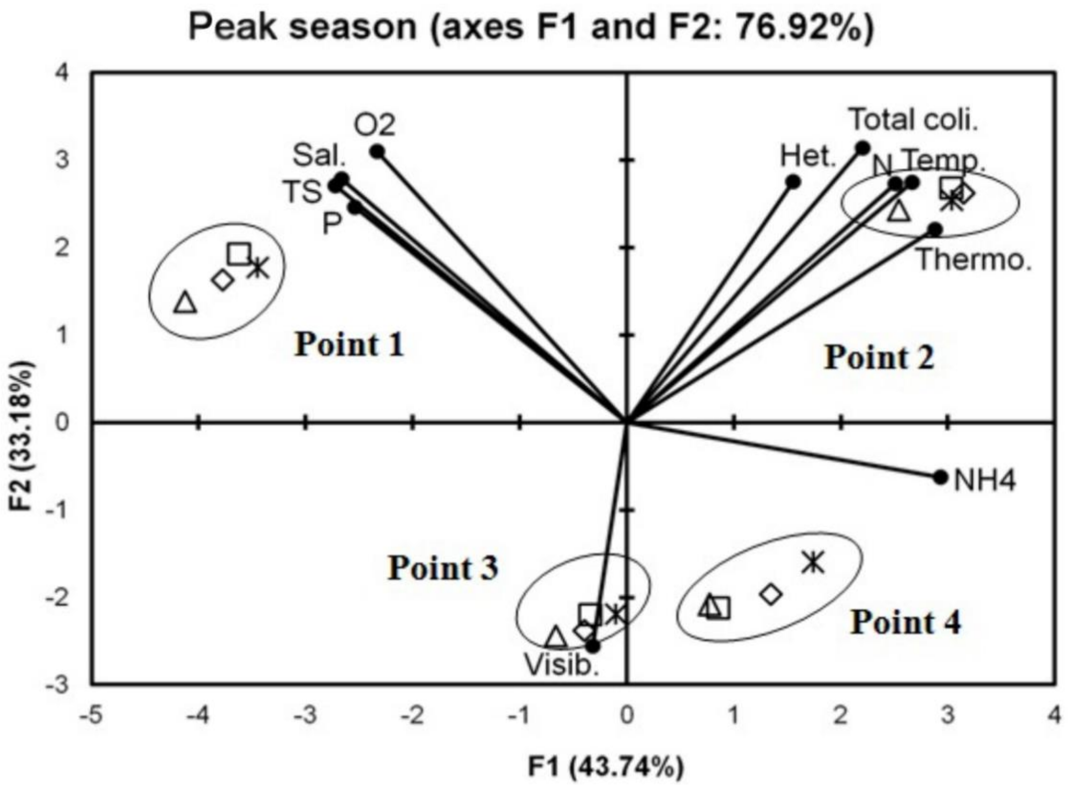


Figure 3. Principal component analysis (PCA) of environment and resistant populations interactions in Tramandaí Lagoon, according to different antimicrobial treatments, sampling points and seasons. ⚹: nalidixic acid treatment. □: ceftazidime treatment. △: imipenem treatment. ◇: tetracycline treatment. *Temp.*: temperature. *Sal.*: salinity. *O2*: dissolved oxygen. *P*: total phosphorus. *N*: total nitrogen. *NH4*: ammoniacal nitrogen. *TS*: total solids. *Visib.*: visibility. *Het.*: total heterotrophic. *Total coli*: total coliforms. *Thermo.*: thermotolerant coliforms.

4. CONCLUSION

The present study was able to identify a coastal Lagoon from Southern Brazil as a reservoir of ARGs, even in the absence of detectable antimicrobial molecules. Although the aquatic system assessed has substantial ecological and economic importance for the region, the resistance types detected (especially ceftazidime and imipenem resistance) are clinically relevant in an international overview. Since ARB populations were identified even under low population season, we draw attention to the need to monitor systematically these environments. These insights reinforce the importance of carrying out more studies on natural habitats, in order to identify sources of contamination as well as the response of native bacteria to stresses of anthropogenic origin. Since the coastline zone is worldwide pressured by urbanization, analysis of the impact of human activities on persistent resistance in coastal environments is critical to adopting mitigating actions on a global scale.

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ABSTRACT

Changes in land use and land cover in watersheds, together with population increases and urbanization of these areas, have caused negative impacts on surface water quality. Based on land-use types, we conducted a comprehensive water quality study of the Rio do Peixe watershed, a tributary of the Rio Doce River, located in the Iron Quadrangle, with different land uses/land covers. For this study, the relationship between water quality variables and land use types were examined according to the water quality index and principal components analysis. The water samples were collected from twelve stations located along the basin in two different seasons. The results of metals/semimetals concentrations reflected regional lithology, and in the case of iron, it was anthropic activities. Also, the computed water quality index values were between 26.8 and 74.9. The water quality was poor in four stations of the river basin in both seasons. Furthermore, the variables that influence water quality the most were *E. coli*, DBO, turbidity, nitrate, and total phosphorus. The multivariate statistics gave five principal components that together accounted for 58.3%, whereas analysis of score plots identified the formation of two groups with more perceptible anthropic influence. Finally, environmental impacts of the river basin, such as deforestation, erosion, domestic sewage, and iron ore tailings, were the main factors that interfered with water quality.

Keywords: iron mining, multivariate statistics, Rio Doce.

Relação entre o uso e ocupação do solo e qualidade de água numa bacia hidrográfica impactada por rejeitos de mineração de ferro e esgoto doméstico

RESUMO

O uso e ocupação do solo, o aumento populacional e a urbanização de áreas próximas a bacias hidrográficas causam diversos impactos negativos na qualidade das águas superficiais. Diante essa assertiva, foi realizado um estudo abrangente sobre a qualidade da água na bacia hidrográfica do Rio do Peixe, tributário do Rio Doce, localizado no Quadrilátero Ferrífero e que possui diferentes usos. Para a compreensão e discussão da relação entre as variáveis de qualidade da água e os tipos de uso foi calculado o índice de qualidade da água e realizada a análise de componentes principais. Para tal, foram coletadas amostras de água em doze estações amostrais localizadas ao longo da bacia em diferentes períodos sazonais. Os resultados da concentração de metais/semimetais refletiram a litologia regional, no caso das concentrações de ferro também por atividades antrópicas. Os valores do índice de qualidade da água calculados estão entre 26,8 e 74,9 e que a qualidade da água foi considerada ruim em quatro estações em ambos os períodos sazonais. Sendo que as variáveis de maior influência na qualidade da água foram *E. coli*, DBO, turbidez, nitrato e fósforo total. Os resultados da estatística multivariada obtida permitiram diminuir as dez variáveis iniciais em cinco componentes principais que juntos representaram 58,3% da variância dos dados. A análise do gráfico dos escores permitiu identificar dois grupos formados onde a interferência antrópica era mais perceptível. Os impactos ambientais da bacia hidrográfica, como desmatamento, erosão, esgoto doméstico e rejeitos de mineração de ferro foram os principais fatores que interferiram na qualidade da água.

Palavras-chave: estatística multivariada, mineração de ferro, Rio Doce.

1. INTRODUCTION

Water quality suffers constant environmental degradation in many watersheds around the world and remains one of the major ecological concerns of contemporary society. Furthermore, the scarcity of studies that correlate land uses and water resources to the intensity of anthropogenically active regions makes it difficult to implement effective measures.

A typical example is found in the rivers within the Iron Quadrangle (IQ). The region is an important Brazilian geological geomorphological domain of mountainous relief with a significant history of gold, iron, and bauxite mining (De Vicq *et al.*, 2015). In spite of the geological diversity, studies in the IQ have verified the contents of metals in fluvial systems derived from anthropogenic activities (Parra *et al.*, 2012, Nascimento *et al.*, 2018, Dos Reis *et al.* 2019). Several water bodies presently are faced with a decrease in water quality and quantity due to the activities of territorial occupations, the application of inappropriate farming techniques, industrialization without adequate environmental management, and the increase in population.

This type of water contamination is directly related to land use. For example, agricultural activities have a strong influence on nutrient concentrations, such as nitrogen and phosphorus levels (Arheimer and Liden, 2000). Residential areas usually contribute to urban wastewater effluent, high loads of organic matter and many contaminants of emerging concern, such as pharmaceutical wastes and personal care products, especially when the municipalities do not have wastewater treatment plants (Kilunga *et al.*, 2017). Industrial activities are point sources of pollution that may have a large relevance. Regarding mining activities, the large amount of residues that are generated and the potential presence of trace elements in them may influence on water quality even greater.

Considering this assertion, many studies used multivariate approaches to analyze the interference of anthropogenic and natural processes in the water quality of different regions (Reis *et al.*, 2017; Kändler *et al.*, 2017; Khaledian *et al.*, 2018). In these studies, the techniques of multivariate analysis were important to obtain the relevant information, aiding in the understanding of the same.

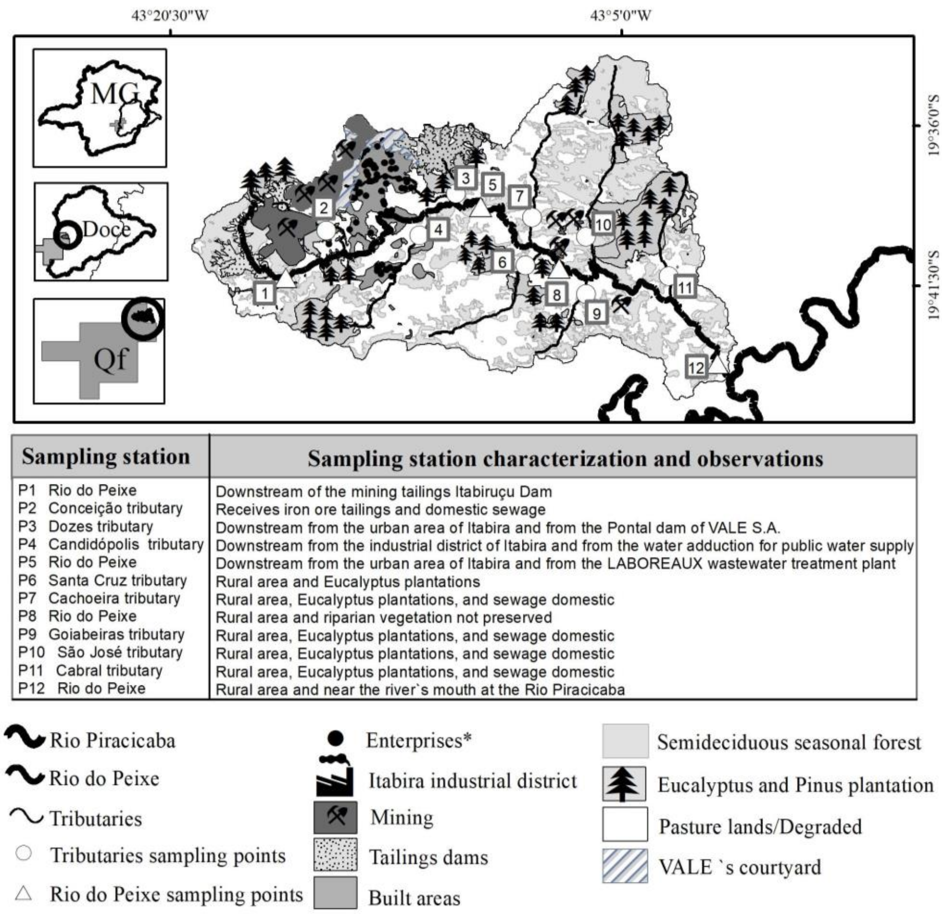
Among the exploratory analytical techniques, Principal Component Analysis (PCA) tool has been used to analyze environmental data sets, which are generally complex, due to the large number of variables involved and the strong relationship between them (Khaledian *et al.*, 2018).

Therefore, this study evaluated possible anthropogenic interference relationships in the quality of surface waters in the Rio do Peixe watershed, an important tributary of the Rio Doce, which has made international headlines because of the mine-tailings dam disaster in Mariana in 2015.

2. MATERIALS AND METHODS

2.1. Region of study

The study area includes the catchment area of Rio do Peixe, located in the State of Minas Gerais, between the municipalities of Itabira and Nova Era (Figure 1). The basin is part of the Itabira Complex, a geological unit located at the northeast end of the IQ. Geologically, the study area is one of the classic areas of the Pre-Cambrian, situated in the southern end of the San Francisco Craton. Since gold exploitation around the 18th century until the present, the region has attracted large immigration movements due to its mineral potentials. Land use in the basin is characterized by 44.5% grassland areas, 27.9% forest areas, 13.8% reforested areas (pine and eucalyptus), 6.4% of mining, 5.2% urban industrial activities.



Sampling station	Sampling station characterization and observations
P1 Rio do Peixe	Downstream of the mining tailings Itabiruçu Dam
P2 Conceição tributary	Receives iron ore tailings and domestic sewage
P3 Dozes tributary	Downstream from the urban area of Itabira and from the Pontal dam of VALE S.A.
P4 Candidópolis tributary	Downstream from the industrial district of Itabira and from the water adduction for public water supply
P5 Rio do Peixe	Downstream from the urban area of Itabira and from the LABOREAUX wastewater treatment plant
P6 Santa Cruz tributary	Rural area and Eucalyptus plantations
P7 Cachoeira tributary	Rural area, Eucalyptus plantations, and sewage domestic
P8 Rio do Peixe	Rural area and riparian vegetation not preserved
P9 Goiabeiras tributary	Rural area, Eucalyptus plantations, and sewage domestic
P10 São José tributary	Rural area, Eucalyptus plantations, and sewage domestic
P11 Cabral tributary	Rural area, Eucalyptus plantations, and sewage domestic
P12 Rio do Peixe	Rural area and near the river`s mouth at the Rio Piracicaba

Figure 1. Locations of the sampling stations in the Rio do Peixe Basin in the Iron Quadrangle, Brazil.

The region has a population of approximately 110,000, of which 93% are located in the urban area of the city of Itabira and 7% rural areas de Itabira and Nova Era (IBGE, 2013). Considered for its importance as the mineral production hub of Brazil, the Rio do Peixe watershed is one of the main regions for iron ore and emerald exploitation in the world. Approximately 39 million tons of iron ore and 80 kg of emeralds have been mined in the region (Brasil, 2015).

2.2. Sampling stations

A total of 12 sampling stations were established along the waterways of the basin, of which four were situated in the main channel of the Rio do Peixe and eight in its tributaries (Figure 1). Samples were collected in the rainy (March) and dry (July) seasons in 2015, for a total of 24 water samples.

2.3. Chemical analysis of water

2.3.1. Reagents and material

All solutions prepared were analytical grade reagents and the final volumes adjusted with deionized water from the Milli-Q® system (Millipore Corporation). All glassware were previously washed with 10% v/v nitric acid (Synth, Brazil) for 24 h. The reagents used for chloride and total phosphorus analyses were of the Synth (Brazil) brand. Hach™ reagents were used for nitrate analysis. The analyses of total phosphorus and nitrate were done in a spectrophotometer (DR6000™ UV VIS, Hach™). Equipment used *in situ* was a dissolved oxygen meter (Model HQ40D with an LDO101 electrode, Hach™, Germany) and a portable digital turbidimeter (Model 2100Q, Hach™).

For the analyses of the metal and semimetals (As, Ca, Cu, Fe, K, Mn, Ni, and Zn), the following were used: a membrane (Millex HP syringe filter with Millipore Express® membrane, USA), nitric acid (Merck, Germany), inductively coupled plasma mass spectrometry (ICP MS, Agilent 7700 series, Agilent Technologies, Germany), and Standard Reference Material (SRM) 1643e for Trace Elements in Water, The National Institute of Standards and Technology (NIST), USA. The reference material used (Nist 1643e) showed a recovery rate between 90.7 and 98.1% for most evaluated elements as follows: As (95.5%); Ca (97.6%); Cu (95.6%); Fe (94.9%); K (91.9%); Mn (93.7%); Ni (90.7%), and Zn (98.1%).

2.3.2. Analysis

Several physicochemical variables were measured in the laboratory following the methods listed in Table 1. Also, dissolved oxygen (DO), pH, temperature (T), and turbidity (Turb) were analyzed using portable digital probe.

For the analyses of the metal and semimetal concentrations, the water samples were filtered with a 0.45 μm membrane, acidified with concentrated nitric acid and preserved at 4°C. Subsequently, the chemical concentrations were determined using inductively coupled plasma mass spectrometry. The results were validated using a certified reference material SRM 1643e.

Table 1. Standard methods adopted for the analysis of samples.

Variables	**Method used**
Biochemical Oxygen Demand (BOD)	5-Day BOD Test, 5210B*
Chloride (Cl^-)	Titulometric 4500-ClB*
Escherichia coli (E. coli)	Colilert® 9223B Enzyme Substrate Coliform Test
Nitrate (NO_3^-)	Colorimetric (Hach™ 8171)
Total phosphorus (TP)	Colorimetric 4500PE*
Total solids (TS)	Gravimetric 2340*

***Source:** APHA *et al.* (2012).

2.4. Data analysis

The obtained data was used to determine the water quality index (WQI) as proposed by Instituto Mineiro de Gestão das Águas (1997). WQI is defined as a rating of that reflects the composite influence of different water quality parameters as BOD, DO, *E. coli*, NO_3^-, pH, T, TP, TS, and Turb. To calculate WQI was in accordance with IGAM instructions (http://portalinfohidro.igam.mg.gov.br/sem-categoria/319-indice-de-qualidade-das-aguas-iqa. Access in July 2019). The computed WQI values are classified into five categories as follows: 90<WQI≤100 (Excellent); 70<WQI≤90 (Good); 50<WQI≤70 (Medium); 25<WQI≤50 (Poor); WQI≤25 (Very poor). The multivariate statistical procedures used was the FactorMineR by the software R©, version 3.1.3, developed by the R Foundation for Statistical. The variables used in the PCA were: BOD, Cl^-, DO, *E. coli*, NO_3^-, pH, T, TP, TS, Turb, and Fe.

3. RESULTS AND DISCUSSION

As shown in Figure 2, concentrations of the metals and semimetals measured in this study were above the limits recommended by the national regulatory agency Conselho Nacional do Meio Ambiente (CONAMA, 2005) 357/05 for arsenic (0.008 to 0.0150 mg L^{-1}); copper (0.014 to 0.155 mg L^{-1}); manganese (0.02 to 13.21 mg L^{-1}), nickel (0.012 to 2.63 mg L^{-1}), and zinc (0.106 to 0.445 mg L^{-1}). The occurrence of these chemical elements is probably from regional geology. However, mining activities in the region intensify the mobilization and availability of metals to the watershed. Iron was detected in all the samples and concentrations ranged from 0.5 to 10.2 mg L^{-1} far higher than the recommended limit of 0.3 mg L^{-1} in all the samples (n = 24). The occurrence of iron in the Rio do Peixe watershed is due to local geology, since iron-rich rocks are typical in the IQ. However, these elevated values exhibited a direct relationship with the tailings retention and the practice of iron ore beneficiation that have been taking place in the Rio do Peixe (Nascimento *et al.*, 2018). Iron concentrations in the bottom sediments of the Rio do Peixe exhibit a direct relationship with the IQ geology and with anthropogenic activities. De Vicq *et al.* (2015), when studying basins in the state of Minas Gerais, Brazil, with similar characteristics and mining activities, also found iron enrichment in both water and sediment. According to Nascimento *et al.* (2018), the other metals and semimetals measured in the watershed probably originate in regional lithography, but anthropogenic activities carried out in the basin led to the mobilization and the availability of the metals for the river systems.

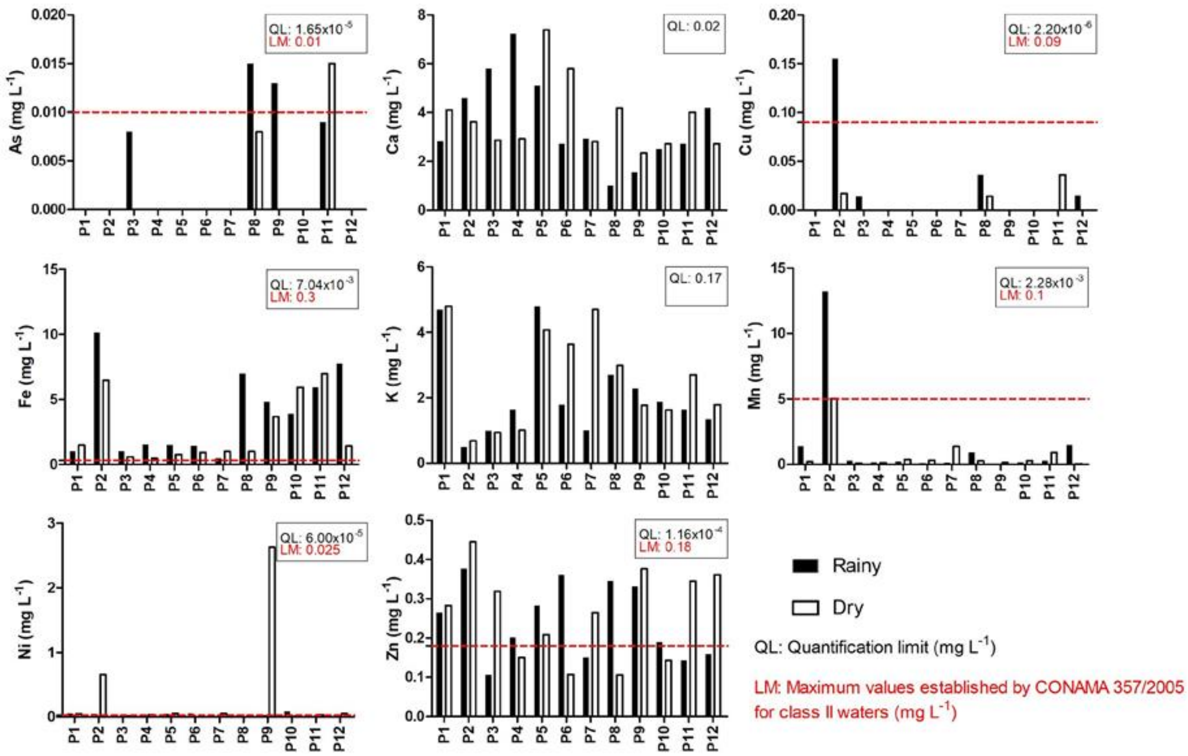


Figure 2. Concentrations of metals and semimetals in Rio do Peixe Basin.

The concentrations of the variables in the water quality measured in this study did not exceed the limits recommended by CONAMA 357/05 with the exception of biochemical oxygen demand, *E. coli*, dissolved oxygen, and turbidity, where values were above the limit in some sample stations (Figure 3). The lowest BOD was observed in stations P1, P4, P6, P7, P9, P10, and P11 (mean = 2.2±1.2 mg L^{-1}). As for *E. coli*, the maximum was observed at stations P2, P3, P5, P6, P8, P10, and (mean = 2.2±1.2 mg L^{-1}). As for *E. coli*, the maximum was observed at stations P2, P3, P5, P6, P8, P10, and P12 (mean = 32.157±44.634 MPN 100^{-} mL^{-1}). Stations P3 (dry), P5 (both periods), and P8 in rainy period showed the minimum value of DO (mean = 3.5±0.3 mg L^{-1}). Finally, the highest concentration of turbidity was recorded at station P2 in both periods, and in stations P8 to P12 in rainy period (mean = 928.7±260.6 NTU). It was observed that concentrations were higher at stations that have anthropogenic influence, such as deforestation, erosion, domestic sewage, and mining waste.

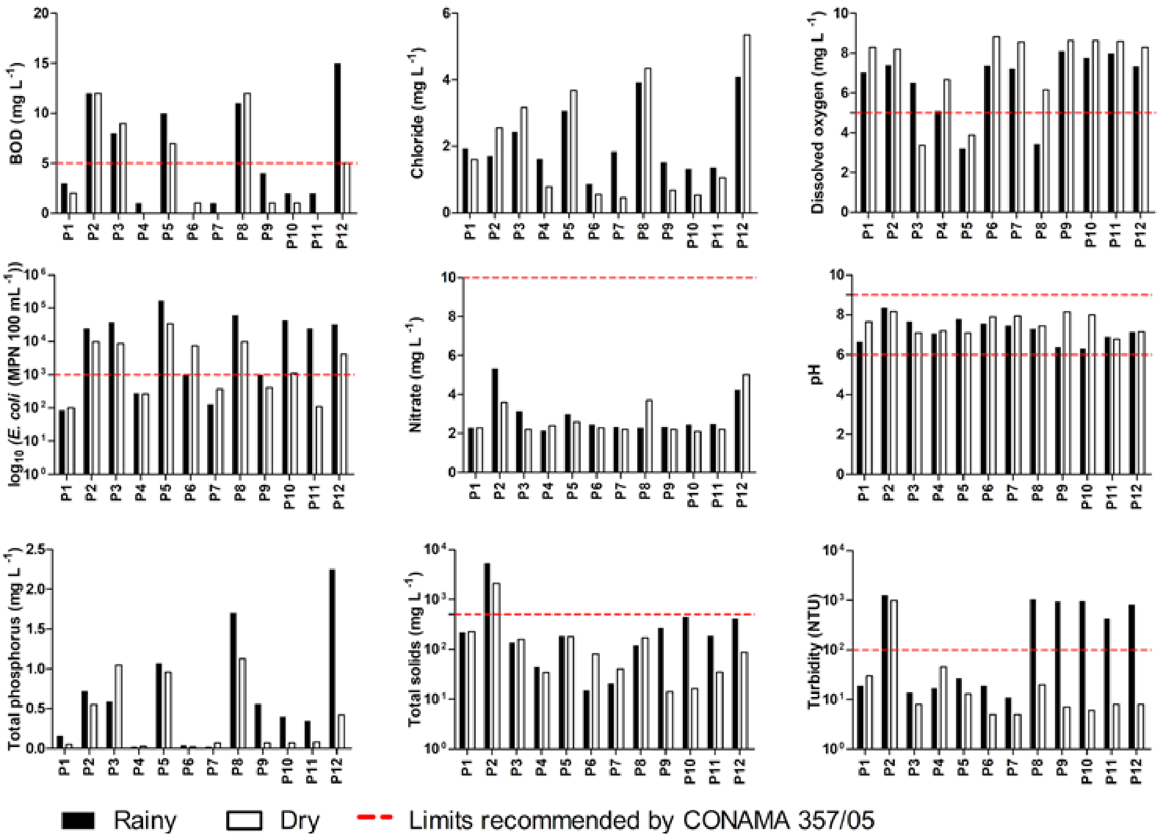


Figure 3. Concentrations of measured variables in waters of the Rio do Peixe Basin.

The computed WQI values are 26.8 to 74.9 in the rainy period; and 33.2 to 74.2 in the dry period. The water quality of Rio do Peixe is characterized as “poor water” in sampling stations P2, P3, P5, and P8 in both periods (Figure 4). The maximum value of WQI (“good water”) was recorded at station P7 (mean = 73.1), which had the lowest pollution. We believe that the decrease of the WQI on the water bodies were mainly caused by the significant presence of the bacteria due to contamination by domestic sewage, especially *E. coli* that is the most common indicator of fecal contamination. Therefore, treatment is required before use as a water supply resource.

In the urban area, *E. coli* bacteria persists, being a domestic wastewater characteristic from residential areas with high population density, which is the case of Itabira. These results can be explained by the dumping of domestic waste in nature of approximately 60% of the resident population of the studied watershed and this reflects in the water quality (Brasil, 2013). In the rural area, the presence of bacteria in surface water indicates that it has been contaminated by farm animals and other point sources, such as those reported by Al-*Badaii et al.* (2015). During seasons with high precipitation, the contamination of water bodies by these bacteria is increased, since the degradation situation of the watershed favors a higher load of fecal material dragged from grassland areas and rural residences. The BOD variable values negatively influenced the WQI indicates that the increased consumption of dissolved oxygen by bacteria is related to oxidative processes of degradation of high concentrations of organic matter. Menezes *et al.* (2016) have also reported high values of BOD when studying an urbanized watershed.

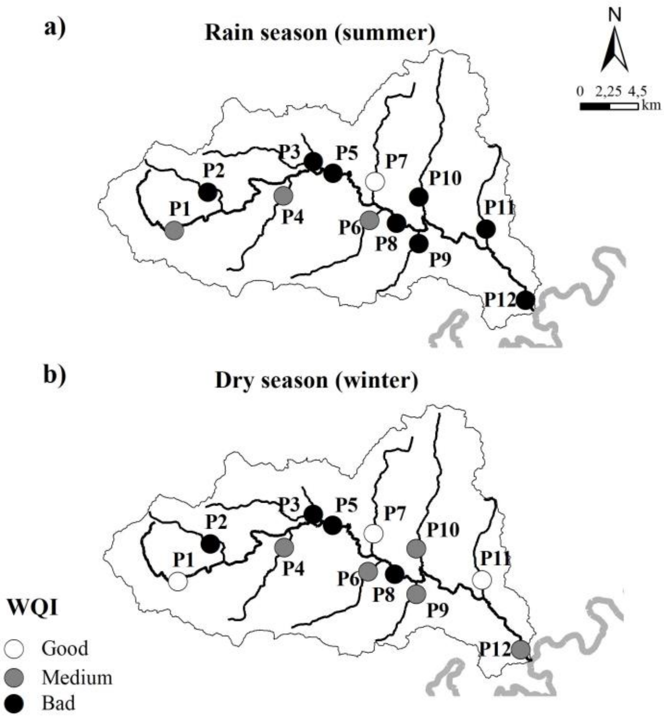


Figure 4. The water quality index of the Rio do Peixe Basin (a) rainy (b) dry.

Other variables that contributed to the decrease in WQI in the water bodies of the watershed in most sampling stations were nitrate (mean = 2.8±0.9 mg L^{-1}) and total phosphorus (mean = 0.5±0.2 mg L^{-1}). In urban areas the values are related to the domestic sewage. In rural areas, the occurrence of nitrate and total phosphorus in water bodies during the rainy season is associated with the lack of conservation and adequate land handling, which favors leaching and transport of fertilizers from land crops (Reis *et al.*, 2017). According to Bu *et al.* (2014), the use of fertilizers based on nitrate and total phosphorus on agricultural lands with steep relief and degraded soils favors a greater contribution and enrichment of surface waters. Beef cattle and dairy livestock were frequently observed around sampling stations. We believe that their presence may have caused total phosphorus influence values to be found in these samples, as animal manure may have been carried into the river by rainwater. Similar results of total phosphorus were found in tributaries of the Doce River Oratórios River by Lacerda and Roeser (2017) and the Matipó River in Minas Gerais– Brazil by Reis *et al.* (2017). In both studies, the authors attributed water quality impairment to untreated sewage disposal.

The turbidity variable also influenced the drop in the WQI levels for most of the sampling stations, especially during the rainy season. This behavior could be the result of erosive processes which occurred in urban and rural areas of grasslands, soil exposure, eucalyptus cultivation, and mining activities near the watershed. During the rainy season, there is much undissolved matter arising from runoffs, erosion and weathering, which contributes to the high turbidity of the river. Studies in the Iron Quadrangle also found an increase of solids during the rainy season and differences in the seasons were due to impacts of mining activity (Raposo *et al.*, 2011; Parra *et al.*, 2012). While in urban industrial (mining operation) areas, the high turbidity values for the different seasons of the year were directly associated with the residues escaping from the dams and containment basin of the mining companies located upstream of the stations (Nascimento *et al.*, 2018). Particularly, sampling station P2 experienced more mining influences, hence it showed high turbidity values: 1281 NTU in the rainy period and 990 NTU in the dry season.

The application of PCA to the initial ten variables and iron used for the calculation of WQI, gave two principal components (PC) that together accounted for 58.30% of the total data variation. The PC1 accounted for 35.65% and PC2 22.65% of the data set, respectively (Table 2). The distribution of the samples by principal component analysis (PCA) is presented in Figure 5, where we have the graph of the values for PC_1 *vs* PC_2. The stations with the letter "R" refer to the rainy season and the letter "D" the dry season.

Table 2. Component loadings of the monitored variables for the principal components (PC) for surface water.

Variable	PC1	PC2
BOD	0.81	-0.26
Cl^-	0.52	-0.61
DO	-0.23	0.83
E. coli	0.43	-0.61
NO_3^-	0.80	0.09
pH	0.25	0.28
T	0.33	-0.28
TP	0.65	0.41
TS	0.75	0.46
Turb	0.72	0.33
Fe	0.63	0.53
% of variation explained	35.65	22.65
% accumulated	35.65	58.30

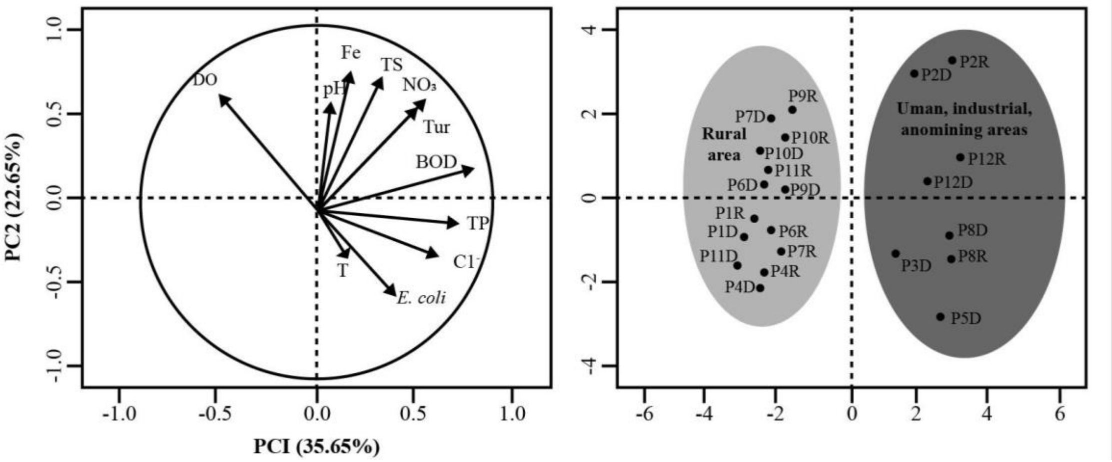


Figure 5. Principal component analysis of water quality variables.

The PCA separated the data into two groups. Group 1, formed by the sampling stations in tributaries and located in rural areas, such as stations P1, P4, P6, P7, P9, P10, and P11, was positively influenced by the presence of dissolved oxygen (Figure 5). This group can be considered an ecological response to some changes introduced in the system caused by natural factors or anthropogenic contamination, such as grassland, agricultural, and sewage.

Group 2 is composed of the sampling stations that receive contributions from urban industrial activities and mining areas such as: P2, P3, P5, P8, and P12. These stations were most influenced by the variables: temperature, Cl^-, *E. coli*, pH, BOD, NO_3^-, TP, Tur, and TS. The occurrence of such variables is related to the degradation of soils, domestic sewage and iron ore mining. The sampling stations P3, P5, and P8 were directly affected by the sewage from the main urban area of Itabira, while sampling station P2 received substantial contributions of ore tailings leaked from mining tailings contention dams. These caused the elevated values for turbidity and total solids. Besides the tailings, the sampling station P2 showed high contents of *E. coli*.

This could be related to the constant loads of sewage received from residential areas. The analysis carried out for the Rio do Peixe Basin stations demonstrates severe fecal contamination of the water. The problem had been reported for the Doce River watershed since the 1990s (IGAM, 1997). In 2018, Drummond *et al.* (2018) attributed the bacterial contamination in the Rio Xopotó, tributary of the Rio Doce, during the spring to the introduction of untreated domestic effluents from the riparian residences and the presence of animals (cattle and pigs), even within the river.

4. CONCLUSIONS

In this research, the potential of the multivariate approach for the understanding of water quality dynamics in watersheds was verified. It is important to note that, even with the use of fewer variables, it was possible to identify important influences of anthropic activities on the results of biodegradable oxygen demand, dissolved oxygen, *E. coli*, iron, and turbidity. The results of WQI showed that the river can be classified between "poor water" and "good water".

The PCA application to 10 variables analyzed for surface waters showed two major components that together accounted for 57.3%, whereas the analysis of score plots identified the formation of two groups with anthropic interference being more perceptible.

Finally, the environmental impacts of Rio do Peixe watershed, including deforestation, erosion, domestic sewage, and iron ore tailings were the main factors that affected the river’s water quality.

5. ACKNOWLEDGEMENTS

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Heavy rainfall maps in Brazil to 5 year return period

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ABSTRACT

This study created thematic heavy rainfall maps for Brazil, with durations of 5-, 30-, 60- and 120 minutes and 5 years of return period (T). The intensity-duration-frequency (IDF) relationships used were compiled from studies found in the literature (798 locations) and derived for 4411 rainfall gauges available in the *Hidroweb* information system, totaling 5209 rainfall data collected. To derive IDF relationships, Gumbel's probability distributions were used, with parameters estimated by the method of moments. Distribution adequacy was verified by the Kolmogorov-Smirnov test. Rainfall-intensity values obtained by IDF relationships were spatialized in Geographic Information Systems, allowing elaboration of the thematic maps. Thematic maps enable obtain rainfall intensities for places without rain gauge data and/or precarious time-series data. Therefore, these maps are a great tool for the design of hydraulic structures related to urban and rural micro-drainage.

Keywords: Gumbel frequency distribution, intensity-duration-frequency relationships, kriging.

Mapas de Chuvas Intensas no Brasil com Tempo de Retorno de 5 Anos

RESUMO

Este estudo tem como objetivo criar mapas temáticos de chuvas intensas para o Brasil para durações de 5, 30, 60 e 120 minutos e período de retorno de 5 anos (T). As relações intensidade-duração-frequência (IDF) utilizadas foram compiladas a partir de estudos encontrados na literatura (798 locais) e estimadas para 4411 estações pluviométricos disponíveis no sistema de informações *Hidroweb*, totalizando dados de 5209 estações. Para a derivação das relações IDF, se utilizou a distribuição de probabilidade de Gumbel com parâmetros estimados pelo método dos momentos e aderência da distribuição verificada pelo teste de Kolmogorov-Smirnov. Os valores das intensidades de precipitação obtidos pelas relações IDF foram espacializados em um Sistema de Informações Geográficas, permitindo a criação de mapas temáticos. Os mapas temáticos permitem obter intensidades de precipitação para locais sem dados de pluviometria e/ou dados de séries temporais precários. Portanto, esses mapas são uma ótima ferramenta para o projeto de construções hidráulicas relacionadas à drenagem urbana e rural.

Palavras-chave: distribuição de frequência de Gumbel, krigagem, relações intensidade-duração-frequência.

1. INTRODUCTION

Heavy or extreme rainfalls are those that present large rain depth in short time intervals, which can cause damage both in urban and agricultural areas, such as flooding, soil erosion, nutrient loss and water body silting (Campos *et al.*, 2014).

Studies related to heavy rainfall are of great importance to the knowledge of hydrological watersheds behavior for flood management. Maximum rainfall intensities are used to design hydraulic structures, such as agricultural and urban flooding control measures and water storage and supply for irrigation, industry, domestic and/or animals (Caldeira *et al.*, 2015; Silva Miranda *et al.*, 2017).

For hydraulic structure design, a rainfall intensity is always associated with a duration and a certain risk of it being equaled or exceeded, defined by the return period (T). Therefore, it is necessary to know the intensity-duration-frequency relation (IDF) of heavy rain, allowing practical and adequate rainfall data use (Xavier *et al.*, 2014). In Brazil, pioneering studies were developed by Pfafstetter (1957) and Denardin and Freitas (1982), in which were adjusted the IDF relationships of 80 gauging stations distributed throughout the country.

Among the most recent studies related to IDF relationships derivation in Brazil are in the states of Goiás and Federal District (Oliveira *et al.*, 2005); Mato Grosso do Sul (Santos *et al.*, 2009); Mato Grosso (Oliveira *et al.*, 2011); Pará (Souza *et al.*, 2012); Sergipe (Aragão *et al.*, 2013); Piauí and Maranhão (Campos *et al.*, 2014; 2015); Paraíba (Campos *et al.*, 2017).

Heavy rainfall modeling using IDF relationships can be performed with data from rain gauges and recording gauges that are able to provide continuous records of rainfall. Souza *et al.* (2012) emphasize that the IDF relationship determination presents great difficulties due to the low number of recording gauges and short periods of observation available. Another complication is the exhaustive tabulation, analysis and interpretation work of many data from recording gauges (Silva and Oliveira, 2017).

An alternative to construct IDF curves is to use methodologies that permit the expression of IDF relationships from time-series of annual maximum rainfall events. These data are easily obtained from rain gauges (Aragão *et al.*, 2013). The estimate of precipitation intensities associated with the occurrence frequencies can be obtained by applying probability distributions (Franco *et al.*, 2018). The Gumbel's distribution has been shown adequate to describe extreme events, mainly maximum precipitations (Penner and Lima, 2016).

Even so, there are places lacking rainfall data records. In these cases, one of the techniques employed is the rainfall spatial interpolation. Spatial interpolation is performed in Geographic Information Systems (GIS) environments by means of interpolation methods, which generate distributed surfaces of a given variable from point data (Righi and Basso, 2016).

According to Almeida (2017), the interpolators can be divided into deterministic and geostatistical, these last being applied more in spatial interpolation of heavy rainfall (Mello *et al.*, 2013; Louvet *et al.*, 2016). As reported by Carvalho *et al.* (2009), the geostatistical kriging interpolator has the capacity to produce better estimates, since it is based on two premises: no biased estimator and minimum estimate variance. In addition, random errors associated with spatial dependence can be reduced (Bachir *et al.*, 2016).

In view of the above, this study aimed to estimate the IDF relationships of gauge stations in the Brazilian territory in order to generate thematic maps of heavy rainfalls of 5 years return period that are important to the design of micro-drainage structures.

2. MATERIALS AND METHODS

There are 5209 rain data stations in Brazil. Of these, 798 stations are equipped with recording gauges and the IDF relationships are available in the literature. For the other 4411 rain gauge stations, the IDF curves were derived using the method of least squares.

Gauge stations that presented at least 15 years of daily observations were selected in the information system *Hidroweb* of *Agência Nacional das Águas* (National Water Agency), and the months missing rain data in the historical series were not considered. Then the annual maximum rainfall time-series were elaborated for each station. The maximum rainfall associated with return periods of 2-, 5-, 10-, 20-, 50- and 100 years were estimated by the Gumbel's distribution, since this probability distribution is applied to analyze maximum rainfall frequencies (Mello and Silva, 2005). The Gumbel's distribution parameters were adjusted by the method of moments and theirs adequacy were verified by the Kolmogorov-Smirnov test to a 5% significance level.

Subsequently, through the methodology proposed by DAEE and CETESB (1980) were obtained the heavy rainfall of 5-, 10-, 15-, 20-, 25- and 30 minutes durations, as well as 1-, 6-, 8-, 10-, 12- and 24 hours durations, associated with each return period. In this methodology, a rainfall depth of lower duration time is estimated by multiplying a rainfall depth of higher duration by a derivation factor (Equation 1). The derivation factor values proposed by DAEE-CETESB are: h_{24h}/h_{daily}=1.14; h_{12h}/h_{24h}=0.85; h_{10h}/h_{24h}=0.82; h_{8h}/h_{24h}=0.78; h_{6h}/h_{24h}=0.72; h_{1h}/h_{24h}=0.42; h_{30min}/h_{1h}=0.74; h_{25min}/h_{30min}=0.91; h_{20min}/h_{30min}=0.81; h_{15min}/h_{30min} = 0.70; h_{10min}/h_{30min} = 0.54; h_{5min}/h_{30min} = 0.34.

$$P_t = \frac{h_t}{h_{td}} \times P_{td} \quad (1)$$

In which: P_t = rainfall depth of lower duration (mm); P_{td} = rainfall depth of higher duration; h_t/h_{td} = derivation factor.

Finally, the IDF relationship where determined to each gauge station, using the model described by Equation 2:

$$i = (aT^b)(t + c)^{-d} \quad (2)$$

In which: i = average maximum rainfall intensity (mm h^{-1}); T = return period (year); t = rainfall duration (minutes) e a, b, c e d = local adjustment parameters.

The add-in program Solver (Microsoft Excel®) was used to adjust the parameters in order to minimize the sum of residuals between the values of average rainfall intensities estimated by the model and those observed. The fit quality was verified by the Nash-Sutcliffe coefficient and Camargo and Sentelhas (1997) performance coefficient.

Then, in possession of IDF relationships available in the literature and those adjusted in this study, the maximum average rainfall intensities were estimated for rain durations of 5-, 30-, 60- and 120 minutes and 5 years return period. The estimated rainfall intensity values were spatially interpolated using *ArcGIS* software, applying the ordinary kriging interpolator.

Initially, an exploratory data analysis was performed, to verify data normality and the outliers. According to Naghettini and Pinto (2007), for an observation sample, an element is considered an outlier when it deviates significantly from all other points. To verify the outliers, the statistical tests Z-score, Grubbs and Beck and boxplot analysis were used.

After the exploratory analysis and removal of the outlier data, the theoretical semivariograms were adjusted using the Spherical, Exponential and Gaussian models. By means of cross-validation technique, the semivariogram model that provided the lowest standard errors among the values predicted by the model was verified, and it was employed in the rainfall intensity spatial interpolation.

3. RESULTS AND DISCUSSION

The Gumbel's distribution fitted to the observed frequencies by the Kolmogorov-Smirnov test to 5% significance for all the rainfall data employed in this study. Figure 1 shows the INMET-Lavras station IDF curves and the dispersion of the average rainfall intensities estimated by the Gumbel's distribution around the 1:1 line. A satisfactory adjustment of the IDF curve to the values estimated by the Gumbel's distribution is observed, with determination coefficients (r^2), Nash-Sutcliffe coefficient (C_{NS}) and Camargo and Sentelhas performance coefficient (c) near to 1.0 and a low dispersion around the 1:1 line. This same behavior was verified in the IDF adjustments for the 4411 gauge stations used in this study.

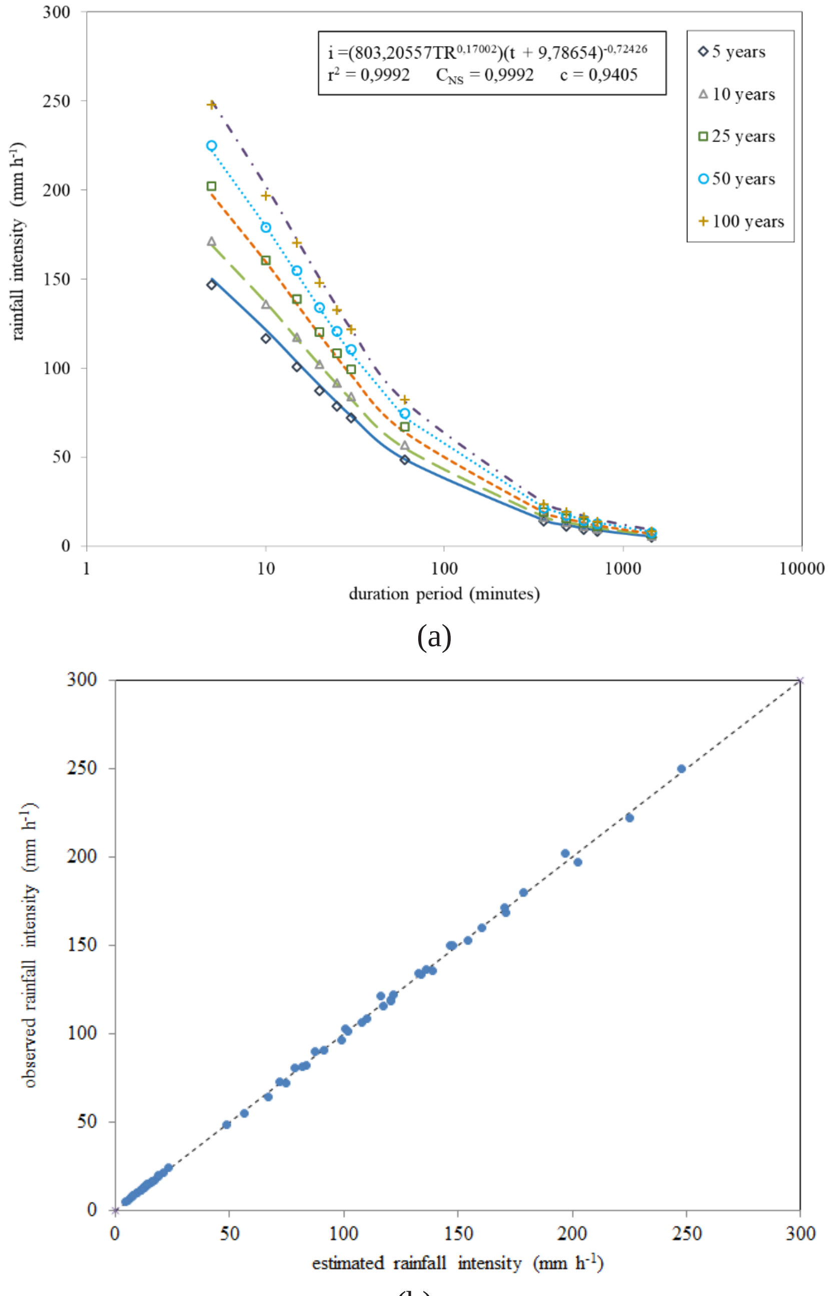
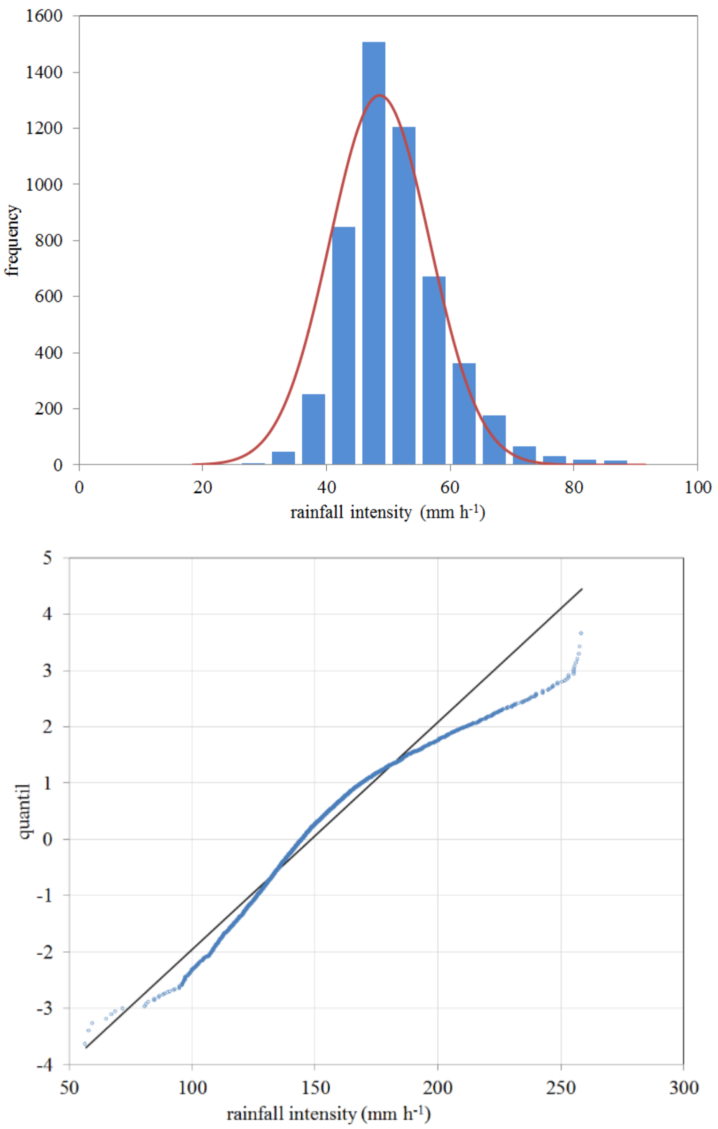


Figure 1. IDF curves for Lavras (a) and rainfall intensities dispersion estimated by Gumbel's distribution and predicted by the IDF relationship around the 1: 1 line (b).

Analyzing the mean rainfall maximum intensities as a latitude and longitude function was verified a good data distribution around the average, characterizing unbiased data. Therefore, the conditions of the intrinsic hypothesis of geostatistics are met, so that rainfall intensities can be spatially interpolated.

The frequency histogram and the Quantil-Quantil (QQ) graphic of the maximum estimated precipitation for the 5-minute duration rainfall and 5-year return period are presented in Figure 2. According to Torman *et al.* (2012), the QQ chart can be used to evaluate the variable normality, and there will be a good data fitting to the normal distribution if the points are close to the reference line shown in the graph. Analyzing the histogram and the QQ graph, data normality was verified, and according to Mello *et al.* (2013) it is a desirable condition in geostatistics application in the data spatial interpolation. The rainfall intensity normality was also verified by the Anderson-Darling test at a 5% significance level.



(b)

Figure 2. Frequency distribution for the duration of 60 minutes and the 5-year return period (a) and Quantil-Quantil graph (b) of the maximum average rainfall intensities estimated for the duration of 5 minutes and the 5-year return period.

In Figure 2, it is observed that some rainfall intensity values are distanced from the theoretical normal distribution line. These points can represent observations results with gross errors or simply the manifestation of very rare events, which can be considered as outliers. Verifying the presence of outliers, the boxplot analysis was more sensitive in comparison to the other tests, with 194 stations (3.6%), on average, classified with outliers for different rainfall durations.

Once the outliers were removed, the adjustments of the theoretical semivariograms models to the observed ones were analyzed. All the theoretical semivariogram models presented low values of the standardized mean errors (SME). For the Spherical model the value of SME was -0.061%; for the Exponential -0.042% and for the Gaussian -0.125% (Figure 3). In general, the Exponential model was the one that provided the lowest values of SME.

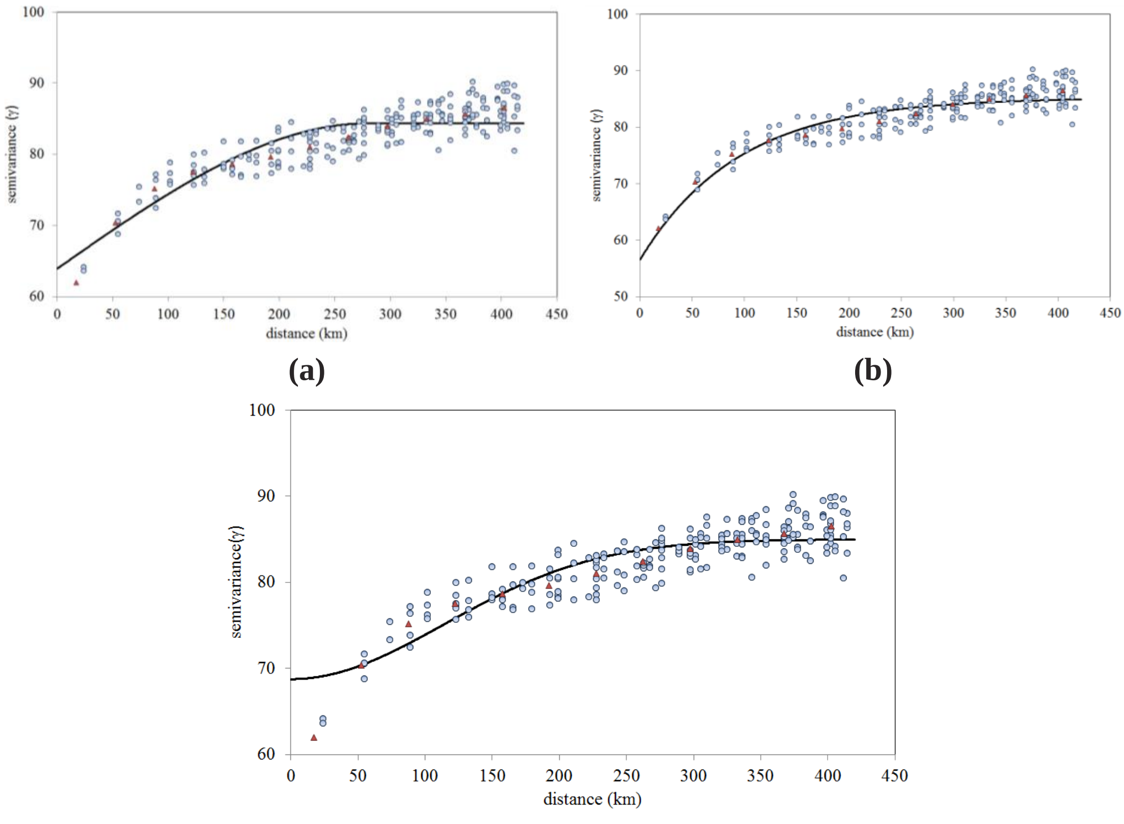
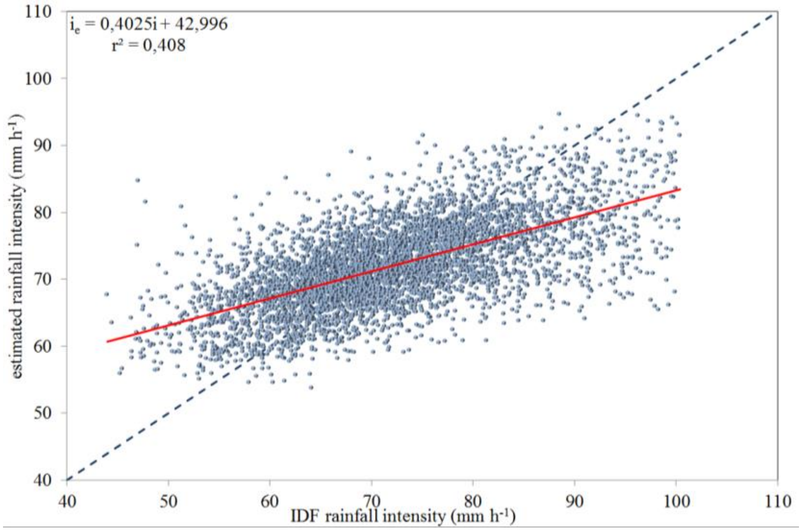


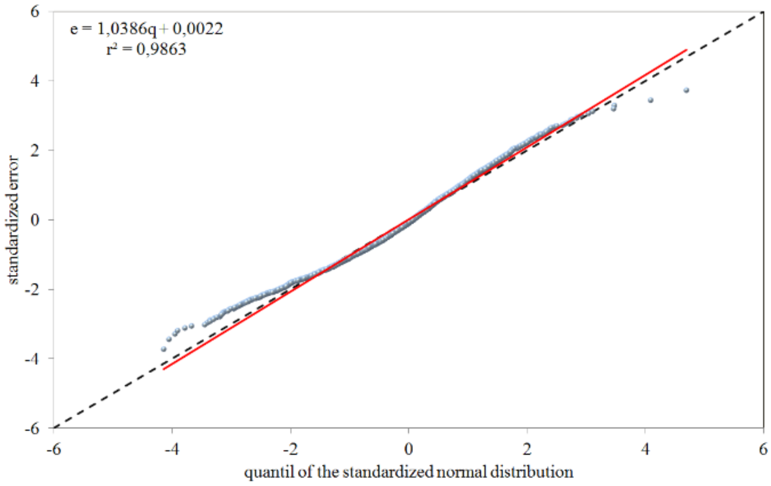
Figure 3. Experimental and theoretical semivariograms obtained by fitting the models (a) Spherical, (b) Exponential and (c) Gaussian for the duration of 30 minutes and 5 years return period.

Therefore, the thematic maps were generated by interpolation applying the ordinary kriging method and using the theoretical semivariogram adjusted by the Exponential model. In order to verify the quality values predicted by the interpolation, the dispersion of the predicted values in relation to the 1:1 line was analyzed. In the same way, the standard errors normality between rainfall intensities predicted by cross-validation and those estimated by the adjusted IDF curves were verified (Figure 4).

A linear trend of rainfall intensities predicted by cross-validation is observed in relation to those estimated by the adjusted IDF curves, with a reasonable data mass dispersion around the 1:1 line. The standardized errors among the rainfall intensity values predicted by cross-validation and those estimated by the adjusted IDF relationships are normally distributed, which according to Mello *et al.* (2013) is a desirable condition in geostatistics.



(a)



(b)

Figure 4. Maximum mean precipitation intensities estimated by cross-validation and obtained by IDF (a) and standard error obtained by using the exponential theoretical semivariogram model (b) for the duration of 5 minutes and 5 years return period.

Finally, the maximum average rainfall intensities thematic maps obtained for the durations of 5-, 30-, 60- and 120 minutes and 5-year return period are shown in Figure 5. According to the maps, there is a great variability of heavy rainfall in the Brazilian territory, with the highest values concentrated in the Amazon states and the southern region of the country. The lowest intensity rainfall values are concentrated in the semi-arid regions. This behavior is due to the atmospheric movement responsible for the formation of the convergence zones of the South Atlantic and Intertropical, which promote a contribution of air masses with high humidity favoring the rainfall formation.

The results obtained from the thematic maps are consistent with the work of Basso *et al.* (2016). The authors divided the Brazilian territory into homogeneous areas related to the heavy rainfalls, denominated isozones that consider the regional climatic behavior. These isozones obtained by Basso *et al.* (2016) have been preserved in the thematic maps created in this work, except for the Continental Isozone that involves a large part of the Amazon, the south of Piauí state and part of Goiás and Mato Grosso do Sul states.

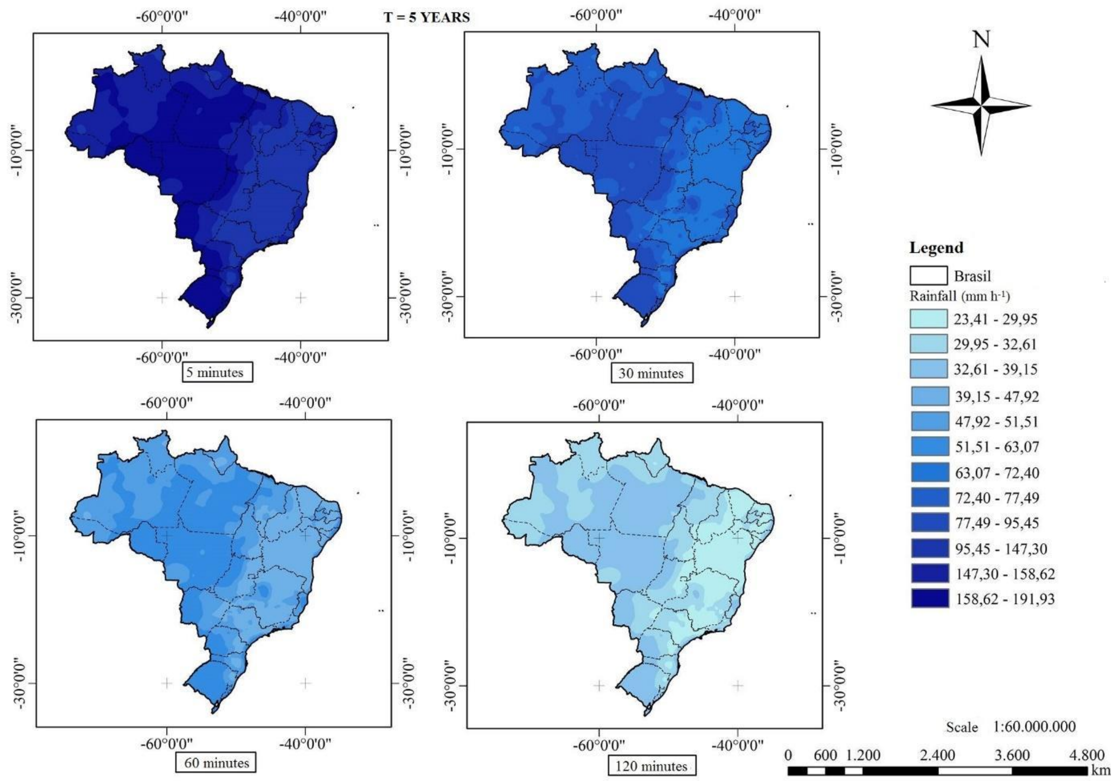


Figure 5. Heavy rainfall spatial distribution for the durations of 5-, 30-, 60- and 120 minutes and 5 years return period.

4. CONCLUSIONS

The methodology adopted to obtain IDF relationships was adequate, providing the creation of thematic maps of maximum rainfall intensities for durations of 5-, 30-, 60- and 120 minutes and return period of 5 years. Such maps allow obtaining rainfall values to design hydraulic structures such as flood control measures, representing a great tool for designers, mainly in locations lacking rainfall data.

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Phytoplankton, Trophic State and Ecological Potential in reservoirs in the State of São Paulo, Brazil

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ABSTRACT

This study evaluated the ecological potential of reservoirs in the Brazilian state of São Paulo, having phytoplankton as a biological quality element. Integrated water column sampling was carried out in the dam, and in the intermediate and fluvial zones of the Igaratá, Atibainha, Paiva Castro, Rio Grande, Itupararanga, Broa, Barra Bonita, Guarapiranga and Salto Grande reservoirs in July 2015. Physico-chemical and biological parameters were analyzed in all environments. The phytoplankton was analyzed under an inverted microscope, and measurements of density, diversity, equitability and dominance were determined. The data was ordered using PCA and CCA analysis. The ecological potential of the reservoirs was determined through the evenness index. The electrical conductivity, nitrate, nitrite and orthophosphate were higher in the more eutrophic reservoirs: Salto Grande, Barra Bonita, Guarapiranga and Rio Grande. A trophic gradient was observed among the sampling points, suggesting a conservation spectrum. There was dominance of cyanobacteria in the eutrophic reservoirs associated with low diversity and high dominance. The total density was correlated with TP, TN, and pH. A divergent relationship between the trophic state index and the evenness index was observed. The Atibainha, Itupararanga, Broa, Barra Bonita and Salto Grande reservoirs were classified as water bodies of very poor ecological quality (Bad). The evenness index seems to be a good alternative to the biomonitoring of the studied reservoirs.

Keywords: eutrophication, pollution, water quality.

Fitoplâncton, Estado Trófico e Potencial Ecológico em Reservatórios do Estado de São Paulo, Brasil

RESUMO

O objetivo deste trabalho foi estabelecer Potencial Ecológico de alguns reservatórios do estado de São Paulo, tendo como elemento de qualidade biológica o fitoplâncton. Amostras de água integrando a coluna d'água foram tomadas nas zonas lótica, central e de barragem dos reservatórios Igaratá, Atibainha, Paiva Castro, Rio Grande, Itupararanga, Broa, Barra Bonita, Guarapiranga e Salto Grande em julho de 2015. Paramentos físico-químicos e biológicos da água foram analisados em todos os ambientes. O fitoplâncton foi analisado sob microscópio invertido e medidas de densidade, diversidade, equitabilidade e dominância foram determinadas. A ordenação dos dados ocorreu através de uma PCA e uma CCA. O Potencial Ecológico dos reservatórios foi determinado por meio do *Evenness E2* Index. A condutividade elétrica, nitrato, nitrito e ortofosfato foram maiores nos reservatórios mais eutrofizados, ou seja, Salto Grande, Barra Bonita, Guarapiranga e Rio Grande. Um gradiente trófico foi observado entre os pontos amostrais o que sugere um espectro de conservação. Houve dominância de cianobactérias em reservatórios eutrofizados associada a baixa diversidade e elevada dominância. A densidade total foi relacionada aos PT, NT e pH. Uma relação divergente entre os Índice do Estado Trófico e o Evenness E2 Index foi observada. Os reservatórios Atibainha, Itupararanga, Broa, Barra Bonita e Salto Grande foram classificados integralmente como corpos d'água de péssima qualidade ecológica (Bad). O Evenness E2 Index apresentou-se como uma boa alternativa ao biomonitoramento dos reservatórios investigados.

Palavras-chave: eutrofização, poluição, qualidade da água.

1. INTRODUCTION

In recent years the water quality of reservoirs has been significantly affected by human activities, increasing the frequency of impacts related to pollution and contamination. These impacts lead to eutrophication, resulting in loss of water quality and permanent change in the trophic condition of these environments, making them eutrophic. The effects of eutrophication are well known in the scientific world and affect mainly the productivity of aquatic environments. In eutrophic environments, phytoplankton is among the most-affected organisms, undergoing changes in their dynamics and ecological structure. These organisms represent an important element of aquatic ecosystems, playing a central role in their structure and functioning. Phytoplankton responds rapidly to any change in the environmental characteristics of an ecosystem, mainly to nutrient concentrations, and has been widely used in water monitoring studies (Vicentin *et al.*, 2018; Batista and Fonseca, 2018; Moschini-Carlos *et al.*, 2017; Tucci *et al.*, 2009).

The quality of the water bodies in Brazil is evaluated according to standards established by the National Policy of Water Resources through Law Nº 9.433/1997 (Brasil, 1997), which promotes the management of national water resources. According to this law, a diagnosis of the current state of water resources must be carried out by monitoring the quality of the water bodies. This monitoring is based on the parameter quality limits according to CONAMA Resolution 357/2005 (CONAMA, 2005). According to this resolution, the phytoplankton is analyzed considering the density of cyanobacteria and the concentration of chlorophyll a in the water. In the member states of the European Union (EU), the process of management of water resources, river basins and monitoring of water bodies follows the European Water Framework Directive (WFD) (EC, 2000). The WFD adopts an ecosystem approach in a way that the water bodies will reach a state of minimum degradation. To do so, monitoring of inland water bodies using biological groups is required, and the phytoplankton represents one of these groups. When the physical, chemical, hydromorphological and biological conditions are good, they have a slight deviation from the conditions of the water body without anthropic pressures (Acreeman and Ferguson, 2010). The objective of the WFD is to achieve good ecological potential and good chemical status of surface waters, i.e., good condition, taking into account the uses for which these water bodies were created. Due to the operation and complexity of the uses, reservoir studies are necessary for a better understanding of the structure of the phytoplankton community, which is subject to the effects of eutrophication. This study established the ecological potential of nine reservoirs in the Brazilian state of São Paulo, using phytoplankton as a biological quality element, through the application of the evenness index.

2. MATERIALS AND METHODS

Nine reservoirs (Table 1) were studied in different regions of the Brazilian state of São Paulo. These reservoirs are formed by different water-producing systems and have multiple uses such as recreation, electricity generation, irrigation, nautical uses and public water supply. Sampling occurred in July 2015 during winter, in the dry season. In each reservoir three sampling points were chosen. The sampling points were distributed between the dam, intermediate and fluvial zones. For all the parameters, including phytoplankton, a single integrated water column sample was collected, considering 2.7 times the depth of Secchi (m) (Cole, 1994). Besides depth (m), the following parameters were determined in situ with the aid of a multiparameter probe YSI 556: SPM, surface temperature (ºC), dissolved oxygen (mg L-1), electrical conductivity (µS cm-1) and the pH of the water. Concentrations of nitrite (NO2-) and nitrate (NO3-) (Mackereth *et al.*, 1978), ammonium (NH4+) (Koroleff, 1976), total nitrogen (TN) and total phosphorus (TP) (Valderrama, 1981), orthophosphate (P-orto) (Strickland and Parsons, 1960) and chlorophyll-a and phaeopigments (Lorenzen, 1967; Wetzel and Likens, 1991) were determined in the laboratory.

Table 1. Hydrological characterization of the nine reservoirs studied in the state of São Paulo. Area: Lake area in km^2; Prof.: average depth (m); Vol. Volume (m3); R.T. residence time (days), Alt. Altitude (m).

Reservoirs	Area	Depth	Vol.	R.T.	Alt.	Source
Broa	6.8	3	22×10^6	20-40	712	Cervi *et al.* (2017);
Barra Bonita	324.84	25	3160×10^6	100	451	Cunha *et al.* (2013); Tundisi *et al.* (2008)
Atibainha	21.8	12.5	$95{,}26 \times 10^6$	105,8	793	Barros (2010)
Paiva Castro	5.1	13	$7{,}0 \times 10^6$	1	753	Pires *et al.* (2015)
Rio Grande	7.4	10	1,1E+08	319	752	Cunha *et al.* (2013); Beyruth and Pereira (2002)
Itupararanga	29.9	7.8	286×10^6	250	841	Smith and Barrella, 2000
Igaratá*	60.5	14.2	1,4E+06	306	621	Soares *et al.* (2015)
Guarapiranga	33	3.5	194×10^6	145	738	Cunha *et al.* (2013); Pires *et al.* (2015)
Salto Grande	11.5	19	106×10^6	30	538	Martins *et al.* (2011); Hofling *et al.* (2000); Zanata and Espindola (2002)

2.1. Phytoplankton Analysis and Ecological Potential

Regarding the qualitative analysis, the samples were collected with a 20µm mesh net by horizontal trawls at the surface. The material was identified following specialized bibliography: (Ramos *et al.*, 2015a; 2015b; Alves-Da-Silva *et al.*, 2013; Tucci *et al.*, 2006; Sant'anna and Azevedo, 2000). The organisms were counted using the method described by Utermöhl (1958). Equitability (J '), specific richness (S), and specific diversity were determined using the software PAST (Hammer, 2001). Dominant species were determined according to Lobo and Leighton (1986). The ecological potential of the reservoirs was determined through the evenness index (Spatharis and Tsirtsis, 2010). Evenness Index formula (Equation 1):

$$Evenness\ Index = \frac{EXP(H')}{S} \quad (1)$$

Where: H' is the Shannon-Weaver Index, and S is the number of species in a sample or population. The limits for the different ecological status classes according to the WFD were: Bad (0.0 – 0.2); Poor; (0.2 – 0.4) Moderate (0.4 – 0.6), Good (0.6 – 0.8) and High (0.8 – 1.0).

2.2. Data analysis

The trophic state index (TSI) was determined according to Lampareli (2004). The following classification was considered: ultraoligotrophic (TSI ≤ 47); oligotrophic (47 < TSI ≤ 52); mesotrophic (52 < TSI ≤ 59), eutrophic (59 < TSI ≤ 63); supereutrophic (63 < TSI ≤ 67) and hypereutrophic (TSI > 67). A Principal Component Analysis (PCA) was performed using PAST software (Hammer, 2001). The PCA aimed to verify the ordering of the sampling points regarding the phytoplankton and the environmental variables for the reservoirs. Abiotic and biotic limnological variables were standardized through Z-score transformation. The ordering of the sampling points, through canonical correspondence analysis (CCA) was performed using the dominant species and environmental variables. For the CCA, the transformation was applied through the square root of the data of the species.

3. RESULTS AND DISCUSSION

A total of 196 species distributed into 99 genera were identified in this study, which suggests a very diversified phytoplankton community. Although the specific richness shows the predominance of green algae (26%), the dominant group in terms of abundance was Cyanophyta. This result is similar to that found by other authors in Brazilian reservoirs: Adloff *et al.* (2018); Batista and Fonseca (2018); Silva *et al.* (2014); Soares *et al.* (2012); Cunha and Calijuri, (2011); Souza and Fernandes (2009). In addition, the highest values of density were observed where the concentration of nutrients was higher and where there was a high degree of eutrophication (Table 2).

Table 2. Total Density (x10³ cel.l⁻¹), of the phytoplankton in the different zones in reservoirs in the state of São Paulo. IGA (Igaratá), ATB (Atibainha), PC (Paiva Castro), RG (Rio Grande), ITUP (Itupararanga), BR (Broa/ Carlos Botelho), BB (Barra Bonita), GUA (Guarapiranga), SG (Salto Grande).

Zone	IGA	ATI	PC	RG	ITUP	BR	BB	GUA	SG
River	1.167	55.024	616.243	5.077	116.204	345.181	211.246	37.699	406.250
Cent	1.414	42.438	602.982	8.047	35.746	239.298	1.251.650	79.446	518.047
Dam	1.212	55.923	885.949	10.027	86.595	150.699	758.509	396.396	36.550

The variation of diversity, equitability and dominance indexes followed the degree of conservation of the reservoirs, with the lowest values of diversity and equitability being recorded in the most eutrophic reservoirs (Figure 1). The dominance was higher in eutrophic reservoirs reflecting the impact caused by the share of cyanobacteria in phytoplankton. The low diversity values observed in Broa, Barra Bonita, Itupararanga and Salto Grande reservoirs may be directly related to the dominance of *Microcystis aeruginosa*. In these same reservoirs, a lower richness was also observed, and high values of density (Figure 1).

The dominance of cyanobacteria forming dense blooms has been particularly useful as an indicator of high nutritional status (Bellinger and Sigee, 2010). Our results have suggested the formation of monospecific blooms of Microcystis aeruginosa in Broa (supereutrophic to eutrophic), Barra Bonita (supereutrophic to eutrophic) and Salto Grande (supereutrophic to hypereutrophic) reservoirs. These results have already been presented in previous studies, including records of other species blooms, such as Cylindrospermopsis raciborskii, Microcystis spp and Coelosphaerium evidenter-maginatum in Broa (Tundisi et al., 2015), Barra Bonita (Bittencourt-Oliveira, 2003) and Salto Grande (Fonseca, 2014) reservoirs, respectively. The high density of cyanobacteria may lead to problems of water quality since several genera can produce potent cyanotoxins, which are harmful to human health (Fotiou et al., 2016). The following genera with toxic potential were registered in this study: Aphanizomenon, Cylindrospermopsis, Microcystis, Dolichosperum (Anabaena) and Oscillatoria.

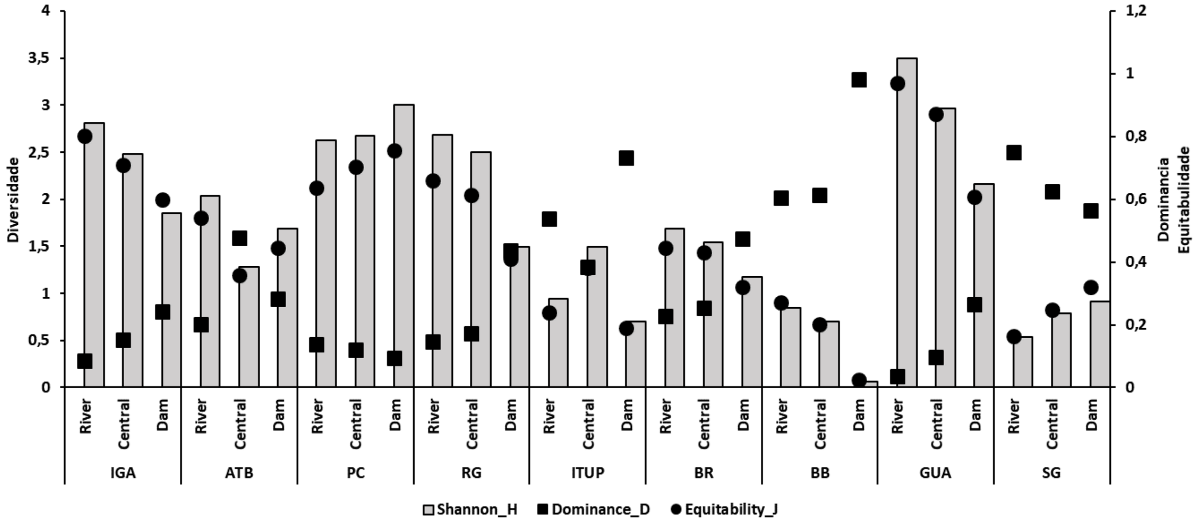

Figure 1. Diversity (bits.cel^{-1}), equitability and dominance of phytoplankton in reservoirs in the state of São Paulo. IGA (Igaratá), ATB (Atibainha), PC (Paiva Castro), RG (Rio Grande), ITUP (Ituparanga), BR (Broa/ Carlos Botelho), BB (Barra Bonita), GUA (Guarapiranga), SG (Salto Grande).

The clustering of points with the same trophic state presented in the PCA (Figure 2), showed a trophic gradient, gathering environments with trophic status ranging from oligotrophic to hypereutrophic. The parameters most correlated to the first two axes are the same parameters associated in the determination of the trophic state of the investigated environments, which validates the PCA results. The total density of the phytoplankton regarding the Barra Bonita reservoir highlight the importance of this parameter in that environment, since some of the highest density values and inorganic nutrient concentrations (mainly NH4) were recorded in this reservoir (Table 1). The points located in the reservoirs classified as supereutrophic and hypereutrophic were correlated with the parameters DO (dissolved oxygen), Dens (density), pH, TP (total phosphorus), TN (total nitrogen), SS (suspended solids), and EC (electrical conductivity) (Figure 2). The Igaratá, Atibainha, Itupararanga, Paiva Castro and Rio Grande reservoirs were grouped together; these reservoirs were correlated with depth and transparency, which is confirmed by the hydrological characteristics of these environments. The sampling points in the Barra Bonita, Salto Grande, Guarapiranga and Broa reservoirs were located close to each other. These environments were correlated with phytoplankton density, water temperature, pH, dissolved oxygen concentration, total nitrogen and phosphorus, suspended solids and electrical conductivity. The sampling points located in the Paiva Castro, Rio Grande and Itupararanga reservoirs are located in a position that indicates a transition between mesotrophic, eutrophic and super eutrophic environments; this position is intermediate between oligo-mesotrophic and super-hypereutrophic reservoirs. Axis 01 was correlated with the parameters TN (0.48), TP (0.43), SS (0.45), SD (-0.36) pH (0.23) while Axis 02 was more correlated with TEMP (0.56), DO (0.64), EC (-0.43), and DENS (0.16) (Figure 2).

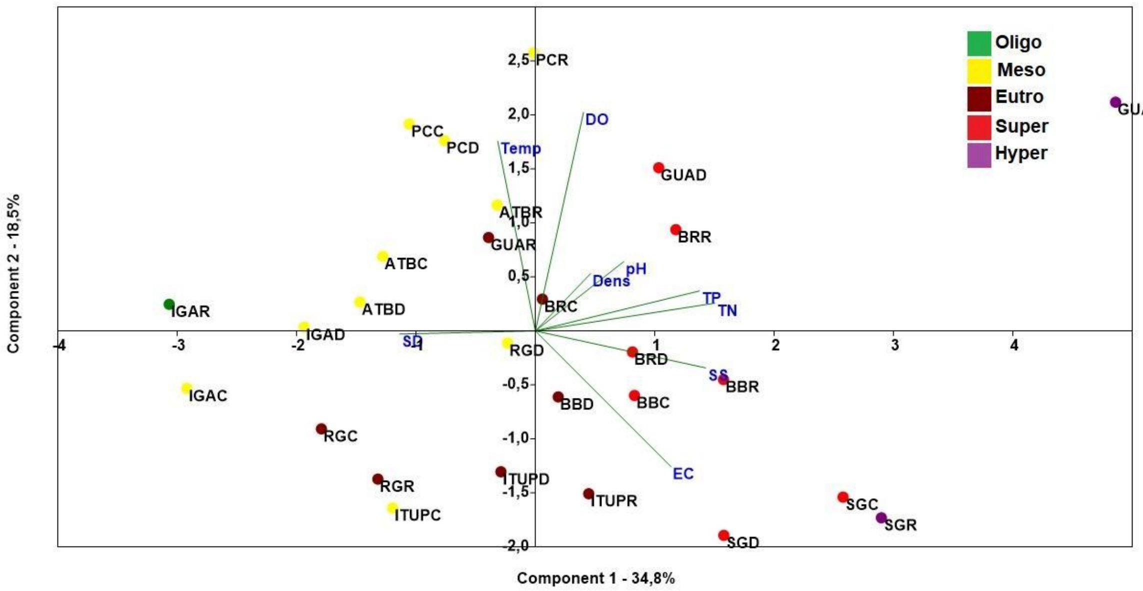

Figure 2. Principal Component Analysis Diagram (PCA), with the ordering of the sampling points in the different reservoirs, density scores and environmental variables. Limnological variables: Temp., temperature; pH, TN, total nitrogen; TP, total phosphorus, SS suspended solids; DO Dissolved oxygen; SD, Secchi; EC, Electric conductivity.

The Canonical Correspondence Analysis (CCA), performed with the environmental parameters and the species density of the 12 dominant species, presented an eigenvalue of 0.99, explaining 20.7% of the variation, while in the second axis it has an eigenvalue of 0.97, explaining 40.9% of the variation (Table 2). The correlation between density and the environmental parameters was considered strong, 0.99 for the first axis, and 0.98 for the second axis. The first axis was correlated with transparency, temperature, nutrients (TP and TN), suspended solids (SS), electrical conductivity (EC) and dissolved oxygen concentration (OD) (Figure 3a). The second axis was more correlated with pH. The association with the parameters and the sampling points in each reservoir evidenced by the CCA corroborated with the limnological characteristics of each environment investigated. A massive clustering of sample points from all reservoirs can be observed in the central part of the CCA diagram. Some dispersed points were observed: Igaratá (2), Rio Grande (10-11) and Guarapiranga (23) (Figure 3a). The species were distributed according to their prominent position in the studied reservoirs (Figure 3b), showing similar ordering to sampling points. According to the CCA, SD (transparency) seems to be the most important variable for variability of dominant species in the studied environments. *Pseudanabaena galeata* Böcher was correlated with the reservoir that had the highest trophy level (Rio Grande), where it was dominant. *Eudorina sp* was correlated with Igaratá Reservoir. *Synechocystis aquatilis* Sauvageau and *Planktolyngbya limnetica* (Lemmermann) Komárková-Legnerová & Cronberg were correlated to Guarapiranga Reservoir.

The cyanobacteria *Konvophorum sp*, *Pseudanabaena galeata*, *Cylindrospermopsis raciborskii* and *Merismopedia glauca* and the green seaweed *Eudorina illinoisensis* were more correlated with the reservoirs classified as oligo-mesotrophic (Figure 3b). The correlation of the dinoflagellate *Ceratium furcoides* with the lower trophic state reservoirs was due to the dominance of this organism in Igaratá and Atibainha Reservoirs. The cyanobacteria *Microcystis aeruginosa*, *Microcystis panniformis*, *Synechocystis aquatilis*, *Planktothrix agardhii*, *Aphanizomenon gracile* were correlated with the most eutrophic reservoirs: Broa, Barra Bonita, Guarapiranga and Salto Grande (Figure 3b).

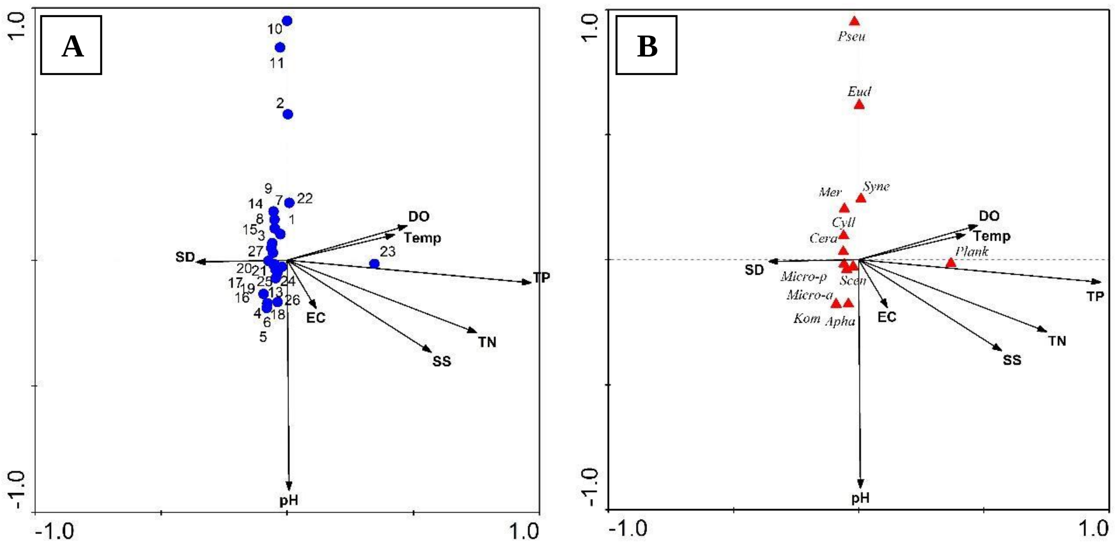

Figure 3. Ordering the samples corresponding to the different zones and reservoirs studied through biplots of the Canonical Correspondence Analysis (CCA) (A) biplot between variables and sampling points reservoirs (B) biplot between species and environmental variables.

The classification of the ecological potential of the reservoirs, evaluated through the evenness index, directly affected the environmental quality of these environments. In general, the reservoirs characterized by high trophy levels obtained the worst classifications, while the less eutrophic reservoirs had better classifications (Figure 4). Overall, the limnological variables indicate that the studied reservoirs are impacted or in the process of degradation, corroborating the scenario described for these environments in Figure 4. The results of the present study showed that the Ituraranga, Broa, Barra Bonita and Salto Grande reservoirs are among the most impacted environments, with high concentrations of inorganic nutrients and chlorophyll a, as well as a high eutrophic level. These reservoirs are located in basins with high level of anthropic impacts, and this directly affects the limnological characteristics of the reservoirs; for example, in Bonita Barra, reservoirs blooms of *Microcystis sp* and other potentially toxic cyanobacteria are common (Tundisi *et al.*, 2008; Sotero-Santos *et al.*, 2006). The Salto Grande reservoir, located in the central-eastern region of the State of São Paulo, is located in the Atibaia River basin, that has high urban and industrial density (Zanata and Espíndola, 2002), and there are also contributions from several small tributaries draining the urban centers (Rietzler *et al.,* 2018). In the region where Barra Bonita Reservoir is located, there is a strong anthropogenic pressure due to intense industrial activities (Petesse *et al.*, 2007), being considered one of the most populous and industrialized areas in the state of São Paulo (Rietzler *et al.,* 2018).

The evenness index was a good response to the reality of the Brazilian reservoirs, but a divergence between the TSI and the evenness index can be observed in the Atibainha (mesotrophic) and Guarapiranga (super-hypereutrophic) reservoirs. That was probably motivated by modifications in the phytoplankton community structure caused by different phenomena, of natural or anthropic origin. In Guarapiranga Reservoir the changes may be related to the management measures regarding the use of raw water, while in Atibainha, changes in the reservoir level during the atypical drought of 2013-2015 may also have affected phytoplankton structure in this environment.

In reservoirs, water level fluctuations, as well as changes in hydraulic conditions, are factors that affect phytoplankton structure (Tucci et al., 2009). The reduction of the water volume affects the concentration of nutrients and material, and there is also a decrease in the current velocity (Granado et al., 2009). These changes may be the main factors controlling the development and composition of algae (Martinet et al., 2014), as well as the density and richness of phytoplankton. In our studies, this may have been directly reflected in the ecological potential, since these metrics (richness and density) are important for the determination of the evenness index. In the case of the Guarapiranga Reservoir, raw water management measures, with application of algicides, may have influenced the divergence between the trophic state indices and the ecological potential. Due to the high eutrophic level, Guarapiranga has frequent blooms (Ogashawara et al., 2014), which led to the application of algicide, decreasing the density of phytoplankton, mainly cyanobacteria, as the index represents a direct relation between richness and density. The increase or decrease in density implied significant changes in the index response in this environment. However the trophic condition of this reservoir remains the same, which could imply in new cases of blooms, therefore increasing the application of algicides.

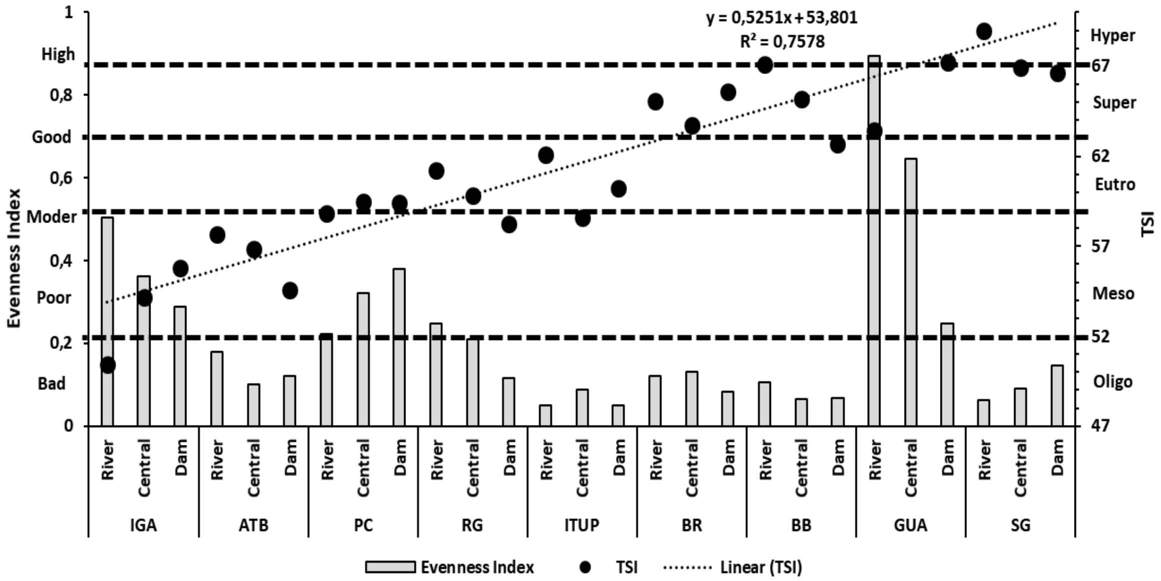

Figure 4. Variation of the evenness index and ecological potential in reservoirs in the state of São Paulo. IGA (Igaratá), ATB (Atibainha), PC (Paiva Castro), RG (Rio Grande), ITUP (Ituparanga), BR (Broa/ Carlos Botelho), BB (Barra Bonita), GUA (Guarapiranga), SG (Salto Grande).

4. CONCLUSIONS

The trophic gradient observed among the studied reservoirs reflected directly on the biological quality of these environments, affecting the phytoplankton structure (density, richness, diversity and evenness), this result was accompanied by the scenario shown by the evenness index, which uses diversity (H ') as the main metric. This gradient suggests a scenario of the conservation status of these environments and what can be anticipated regarding the limnological characteristics of these reservoirs. In our study, diversity was negatively affected by the eutrophication process. In this sense, this index has become a particularly useful tool for assessing the ecological potential of reservoirs, since the ecological quality of the aquatic environment is a consequence of its conservation status, which in turn is affected by the eutrophication process. The Evenness Index responded well to the reservoirs and works as a complementary measure to the biomonitoring of these reservoirs; however, other components are still needed. The use of means of evaluation is suggested for a better understanding of ecological information and monitoring in reservoirs. The Evenness Index is a tool that could help the decision makers with better management measures.

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