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Caffeine as a chemical tracer for contamination of urban rivers

Utilização da cafeína como traçador antrópico em rios urbanos

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ABSTRACT

The growing urbanization in urban centers have continuously contributed to the deterioration of water quality in rivers. The use of caffeine as a chemical tracer for anthropic activities is an approach to the environmental monitoring of urban waterbodies, as its use is limited to humans and less susceptible to sampling error comparing to other traditional parameters for anthropic pollution. To analyze the possibility of using caffeine as a water quality parameter, the anthropic influence over three watersheds (Atuba, Belem and Palmital) from the Greater Curitiba was observed over five sampling campaigns. The caffeine was analyzed by liquid chromatography tandem diode array detection, after the solid phase extraction. Traditional parameters of water quality monitoring, such as ammoniacal nitrogen, thermotolerant coliforms, biochemical oxygen demand and orthophosphate, were measured for comparison. The results indicate anthropic influence over the basins, probably due to the lack of infrastructure, thus leading to the assumption of untreated domestic wastewater being discharged. The most degraded river was the Belem river (caffeine concentration of 23.08 $\mu g.L^{-1}$). Caffeine presented itself as an appropriate approach for environmental monitoring, presenting a good correlation with the traditional parameters, such as for thermotolerant coliforms (R = 0.7375).

Keywords: Iguassu watershed; HPLC-DAD; Environmental monitoring.

RESUMO

A crescente urbanização nos grandes centros urbanos têm contribuído de forma contínua na deterioração da qualidade das águas dos corpos hídricos receptores. Uma nova abordagem para monitoramento da qualidade d'água é o uso de cafeína como traçador de atividade antrópica em rios urbanos, uma vez que seu uso é limitado a humanos e sua metodologia de análise é menos susceptível a erros quando comparado com parâmetro tradicionais de poluição antrópica. Para analisar a possibilidade do uso da cafeína como parâmetro de qualidade da água, foram monitoradas três bacias hidrográficas (Atuba, Belém e Palmital) na grande Curitiba em 5 campanhas amostrais. A cafeína foi analisada em cromatografia líquida de alta eficiência com detector de arraste de diodo após extração em fase sólida. Parâmetros tradicionais de monitoramento de qualidade da água, como N-amoniacal, coliformes termotolerantes, demanda bioquímica de oxigênio e Ortofosfato foram analisados como comparação. Os resultados indicam que a área de estudo apresenta grande influência antrópica, provavelmente pela falta de infraestrutura, que indica a presença de efluentes domésticos não tratados. O rio mais degradado foi o rio Belém (concentrações de 23,08 $\mu g.L^{-1}$ de cafeína). A cafeína mostrou-se uma abordagem apropriada para monitoramento ambiental, correlacionando-se com os parâmetros tradicionais, como Coliformes Termotolerantes (R = 0,7375).

Palavras-chave: Bacia hidrográfica do Iguaçu; CLAE-DAD; Monitoramento ambiental.

INTRODUCTION

Currently, environmental problems caused by the population growth, along with the advancement of agricultural and industrial activities, have raised great concern not only for governmental authorities but for society. The uncontrolled development of some cities led to the degradation of basic natural resources, such as the water. Contaminants tend to find their way into bodies of water, mainly, through the discharge of wastewater.

One approach used to determine the sources and magnitudes of the anthropic influence in aquatic environments is through the use of appropriate chemical tracers. The presence of caffeine in aquatic ecosystems indicates that such an environment suffered from some sort of human contribution. Caffeine (1,3,7 – trimethylxanthine) is used as a stimulant, commonly found in coffee, tea, sodas and chocolate-based products. It is also present in a variety of medicaments and appetite modulators. Due to this substance's broad use, as well as being a stable compound under different environmental conditions, presenting high solubility (21.7 $g.L^{-1}$), pka=0.7, molecular mass of 194.191, low octanol/water partition coefficient (-0.07) along with insignificant volatility, a high persistence in aquatic environments for caffeine is expected (KURISSERY et al., 2012). Also, the biodegradation is the main mechanism for its elimination of caffeine (BUERGE et al., 2003).

Caffeine can be easily eliminated from wastewater treatment plants (WWTP), as shown by Froehner et al. (2011), where the results revealed a removal rate close to 100%. In aquatic environments, the main source of caffeine is sewage, this implies that caffeine found in rivers and lakes certainly comes from untreated sewage.

Due to its properties, caffeine has been used worldwide as a chemical tracer (DANESHVAR et al., 2012; PAÍGA; DELERUE-MATOS, 2017; IDE et al., 2013; KURISSERY et al., 2012; MACHADO et al., 2016; NARA et al., 2014), replacing traditional microbiological tracers (KNEE et al., 2010).

The current Brazilian legislation concerning the quality of water does not address, systematically, pollutants related to human consuming habits, such as caffeine, present in domestic wastewater. Therefore, the lack of knowledge over the sources and fate of contaminants prevents advancements in water protection policies, as the identification of the origins of pollution plays a fundamental role in the conception and adoption of proper measures (KURISSERY et al., 2012). The constant improvement in analytical techniques has allowed for the detection of low concentrations of new contaminants in watercourses, thus granting new technical approaches for environmental monitoring.

The Iguassu River is in a sub-tropical region in southern Brazil with its spring situated in the metropolitan region of Curitiba (MRC). It is also the biggest river of the state it is located at, Paraná. The Iguassu river is formed by the confluence of the Atuba and Iraí rivers. It extends through 1,275 kilometers and its basin spreads over 70,799 km^2, including 98 tributaries. The Iguassu river watershed has a subdivision called the Alto Iguassu sub-basin, which comprehends important streams that serve as water suppliers to the MRC. Approximately 3 million people, spread over 14 municipalities, inhabit the Alto Iguassu sub-basin (IBGE, 2010). This watershed has a drainage area of 2700 km^2 and it is divided in 26 main subdivisions. Approximately one quarter of the Paraná State's population lives within the Alto Iguassu basin, which, then, concentrates over 30% of the State's urban population, being that many of these lack proper sanitation or treated water supply (PORTO et al., 2007). The dense human population, along with the on-going activities within the area, resulted in a substantial drop in the quality of water of the streams confined within this basin. The biggest contribution of pollution in the region is from WWTP that are not capable of depurating all the affluent pollution charge, thus discharging sewage without proper treatment.

This paper aims to present an alternative tool for water quality monitoring by the analysis of caffeine in subtropical urban aquatic environments as an anthropic tracer, followed by a comparison of its concentration to traditional chemical and biological parameters, for then be able to map the tendency of contamination of the Alto Iguassu basin, through the mapping of the contamination present in the tributaries of the Iguassu river.

EXPERIMENTAL

Study area and data collection

Three sub-basins from the Alto Iguassu watershed were chosen as the study area, the Atuba, Belem and Palmital river basins (Figure 1). These areas were chosen due to the number of streams with potential to urban water supply, that compose each one, along with the scenario of irregular housing and uncontrolled growth which would lead to degraded water quality.

The Belem river, which extends through 21 km, is located within the municipality of Curitiba. Its basin spreads over 87.77 km^2, with an average flow of 1.3 $m^3.s^{-1}$, which comprehends a population of 690,684 inhabitants (PORTO et al., 2007). In this study, three sampling sites were defined. The site BL1 was the one which presented the lowest amount of pollution impact, being located 3.7 km from the river's spring, with a drainage area of 3.7 km^2.

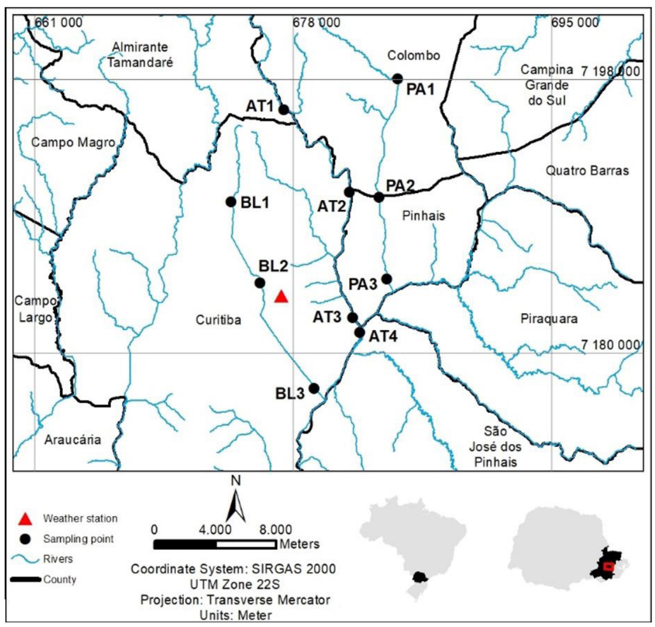

Figure 1. Sampling sites on the Alto Iguassu Basin.

The site BL2 is within a densely populated, low income region at 10.35 km from the spring, with a drainage area of 43 km^2. The site BL3 is in a densely populated area, in the Parque Náutico's vicinity. The drainage area of this last sampling site is of 86.0 km^2.

The Palmital River has its basin situated within the MRC. Its spring is located on the municipality of Colombo, and sheds into the Iraí River, in the city of Pinhais, its basin spans over 95.27 km^2 and encompass over 220,000 inhabitants, its main watercourse runs through 23 km and has an average flow of 1.4 $m^3.s^{-1}$. On the area close to this river's spring, agricultural activities are predominant, yet there are some sparse urban centers, which differs from the rest of the basin, in which urban activities are most common. In this study, three sampling sites were determined within the Palmital river. The site PA1, located in a rural area of Colombo, is situated 8.48 km from its spring and its drainage area spans over 27.2 km^2. The PA2 site is located 16.34 km from the river's basin, in the city of Pinhais, near a housing complex that gathers more than two thousand residences that lack sanitation infrastructure. The PA3 site has a drainage area of 89.2 km^2, it is also located in Pinhais, near a large urbanized area.

The Atuba river, along with the Iraí river, forms the Iguassu river in the city of Curitiba. Its basin spreads over 128.6 km^2, encompassing over 500 thousand residents, its main watercourse has an extent of 29.5 km, and average flow of 1.9 $m^3.s^{-1}$. Four sampling sites were defined within this watershed. The AT1 site is in Colombo, at 7.59 km from the spring. The AT2 site is on the border of Curitiba and Colombo, at a mark of 14.87 km from its spring. The AT3 site is on the border of Curitiba and Pinhais, right before the river receives the discharge from the WWTP Atuba Sul, within a densely populated, low income area. The AT4 site is located right after the same WWTP.

Sampling

Surface water samples were collected in five sampling campaigns: April/2014, June/2014, October/2014, March/2015 and June/2015. Van Dorne water samplers (5 L) were used for the sampling. The samples that would be used for the caffeine analyses were stored in 1 L amber bottles, that were previously decontaminated using Extran detergent solution (5% v/v.). The samples that would be destined for the physical and chemical parameters analyses were stored in 1 L Polyethylene terephthalate bottles, that were previously decontaminated using an acidic solution (HCl 5% v/v.). After the samples were obtained, they were preserved at a temperature of 4 °C, in thermally isolated boxes, and immediately transported to the laboratory.

Caffeine analysis

The method presented by Ide et al. (2013) was used for the caffeine analyses. One liter of each sample was filtered through a cellulose acetate membrane (45 μm) and acidified using an HCl solution (6.0 $mol.L^{-1}$) until the pH ≤ 3.The samples were passed through (at speeds that spanned from 6 to 8 $mL.min^{-1}$) HLB 12 mL cartridges, which were previously conditioned with hexane, ethyl acetate, and ultrapure water (pH = 3), then vacuum dried. Afterwards, the cartridges were eluted with a mixture of 10 mL of acetonitrile and acetone (v/v 1:1), the extract was retroevaporated at 40 °C and its contents were redissolved in 1 mL of acetonitrile and transferred into 2 mL vials, resulting in a 1000 times pre-concentration.

For the chromatographic analyses, the liquid chromatographer Agilent (model 1260) was equipped with a 600 bar quaternary pump, along with a octadecylsilane column (Eclipse Plus C18) with 5 μm of particle diameter, 250 mm of length and 4.6 mm of internal diameter. A detector with a photodiode array, model 1260, was used for the detection. The sample injection was of 5 μL, at a 1.0 $mL.min^{-1}$ flow on the isocratic mode with a composition of 1:1 of acetonitrile and ultrapurified water (the pH was adjusted to 3.0 on the mobile phase). The monitored wavelength was 273 nm.

The quality control parameters of caffeine analyses showed a limit of detection (LOD) of 8.0 $ng.L^{-1}$, limit of quantification (LOQ) of 27 $ng.L^{-1}$ was within the 0.1-2.0 $mg.L^{-1}$ range in a wide linear range with good regression coefficient (R^2 = 0.9974). Reproducibility and repeatability of 0.8 and 4.0, respectively, expressed as coefficients of variation, had satisfactory values (<15%).

Physical, chemical and biological parameters analyses

The concentration of dissolved oxygen (DO) and the pH were promptly obtained, on site, through the multiparameter from Hanna HI9828.

The physical and chemical analyses of the water samples were performed in both filtered and unfiltered samples. The filtered samples were passed through in cellulose ester membranes (0.45 μm). All the materials used were previously decontaminated, according to each analysis to be performed.

The orthophosphate concentration was determined after the reaction between the filtered sample and a molybdate/ascorbic acid solution, following the method presented on the Standard Methods for the examination of water and wastewater (APHA, 1998). The different nitrogen forms were analyzed through the spectrophotometric method on filtered samples. The concentration of ammoniacal nitrogen ($mg.L^{-1}$) was determined through the nitroprusside/phenol colorimetric method. The nitrate ($mg.L^{-1}$) was degraded into nitrite, through a cadmium column, and determined through the sulfanilamide/N-naphthyl colorimetric method (APHA, 1998). The chemical oxygen demand (COD) ($mg.L^{-1}$) was determined through the closed reflux colorimetric method (APHA, 1998). The biochemical oxygen demand (BOD) was determined by reflux colorimetric method (APHA, 1998). For the analyses of the thermotolerant coliforms (*E. colli*) the defined substrate technology method, with the Colilert brand substrate, was used.

Spatial distribution maps

The spatial distribution maps aim to represent the behavior of chemical compounds in the environment. These can be used as tools to support better decision making for policy makers, when associated with socio-environmental data from the region.

In this study, the software ArcGis 10.2.2 (Geostatistical Analyst) was used to perform the spatial estimative for the analyzed compound in the Alto Iguassu basin. The data for caffeine concentration, along with the traditional physical and chemical parameters, was interpolated. The averages of all concentration data were used to represent the annual variability of occurrence.

The maps that presented spatial dependency, after semivariogram analyses, went through the ordinary kriging interpolation method. It was not possible to generate the spatial distribution maps for all the compounds, as some of the semivariograms exhibited nugget effects, which would indicate inexistence of spatial correlation (ANDRIOTTI, 2000).

RESULT AND DISCUSSION

Physical, chemical and biological parameters determination and the relation with caffeine

DO within water is essential to the metabolism of all aerobic aquatic organisms and its distribution on the aquatic environment affects greatly the solubility of several inorganic nutrients. The variations of the concentrations of DO through the five sampling campaigns are illustrated on Figure 2. The low concentrations of DO, along with its tendencies (Figure 3), were, probably, a consequence of the influx of organic matter, mainly due to the discharge of domestic wastewater. This is confirmed by the concentrations of ammoniacal nitrogen (Figure 4) and caffeine. To degrade this organic matter, microorganisms consume DO, which cause its concentration to drop. The spatial distribution of the annual averages of DO concentration, in the region, is presented in Figure 3. The BL2 and BL3 sites had low DO concentration (lower than 2.5 mg.L^{-1}), which indicates that the region near the river's outfall presented a greater degradation. The sites AT1 and PA1 exhibited the greatest DO concentrations (both greater than 5 mg.L^{-1}), which indicates that even though these sites have still suffered some sort of anthropic influence, they are the least impacted.

The ammoniacal nitrogen can be originated through the hydrolysis of urea present in the water. The latter compound is deeply related to the discharge of untreated domestic wastewater in aquatic environments. Therefore, the concentration of ammoniacal nitrogen is an important parameter to be established, to properly classify the water quality of a body of water. According to the current Brazilian legislation, the maximum concentration of ammoniacal nitrogen (pH lower than 7.5) for rivers of Class 2 and 3 of rivers is of 3.7 mg.L^{-1} and 13.3 mg.L^{-1}, respectively (BRASIL, 2005). The site AT4, directly downstream of the WWTP Atuba Sul, had the greatest concentrations of ammoniacal nitrogen (46.54 mg.L^{-1}), where the concentrations of the AT3 site (upstream from said WWTP) were no greater than 15.48 mg.L^{-1}. This increase in concentration was expected, as the WWTP is based upon anaerobic treatment. The WWTP Atuba Sul is designed with anaerobic reactors with upflow anaerobic sludge blanket digestion, this layout produces an increase in the concentration of ammoniacal nitrogen in the plant's outflow. The Belem river, especially sites BL2 and BL3, also exhibited high concentrations of ammoniacal nitrogen, in

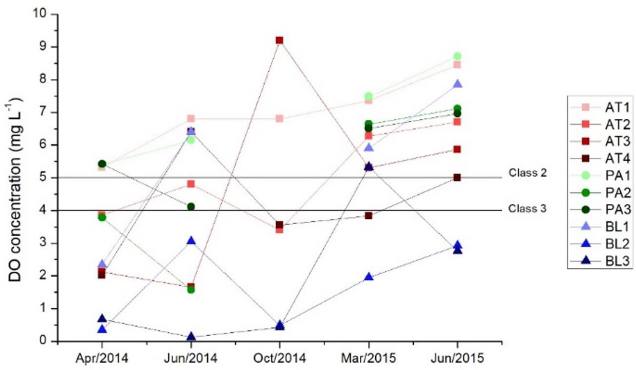

Figure 2. Dissolved oxygen concentrations (mg.L^{-1}) during the five sampling campaigns. AT = Atuba river; PA = Palmital river; BL = Belem river.

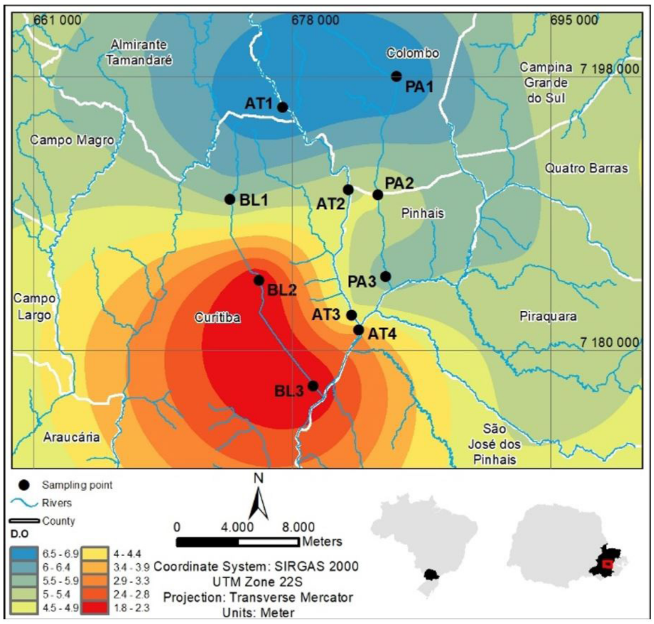

Figure 3. Spatial distribution map of the estimatives of the average concentrations of dissolved oxygen on the alto Iguassu basin – ordinary kriging and spherical method.

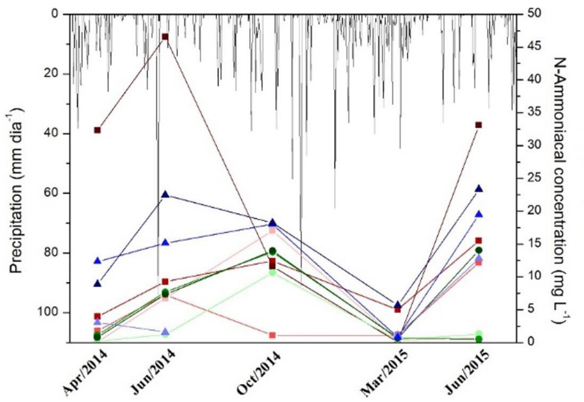

Figure 4. Ammoniacal nitrogen concentrations (mg.L^{-1}) during the five sampling campaigns.

this case, the greater populational density could indicate the discharge of untreated domestic wastewater onto the river. During the campaign April/2014 (C1) and March/2015(C4) the lower concentrations of ammoniacal nitrogen detected may be related to the high precipitation intensity that preceded the campaigns, in contrast, of the other collections that occurred during the dry season. The precipitation influences the river flow and hence compound dilution.

The COD is a parameter used to quantify the organic matter in bodies of water. It has the same behavior as the ammoniacal nitrogen. The COD values on sites AT4, BL2 and BL3 were higher than those observed on other sites (Figure 5). The increase in the concentration of COD, in a natural environment, is mainly due to the discharge of wastewater. As previously discussed, the concentration of nutrients indicates that the environments which are most degraded are the ones that received the outflow from inefficient WWTPs (site AT4) or where there was the discharge of raw sewage (sites BL2 and BL3).

The BOD is a parameter used to quantify the biodegradable organic matter in bodies of water. It has a strict relation with the COD parameter. The greatest concentrations of BOD (Figure 6) were exhibited on the sites BL2 and BL3 (especially during the second and third sampling campaign), which indicates the discharge of domestic wastewater onto the river. During the first sampling campaign, a peculiar behavior was observed at the samples collected from the site AT4 (downstream from the WWTP): Even over a period of intense precipitation (13 mm of accumulated rainfall from the last 5 days before the campaign), the concentrations of BOD and ammoniacal nitrogen were high, such a behavior could indicate the discharge of untreated wastewater onto the river. The problem involving the discharge of high concentrations of BOD onto the environment, especially labile organic matter, is the complete extinction of dissolved oxygen, due to the decomposition of organic matter.

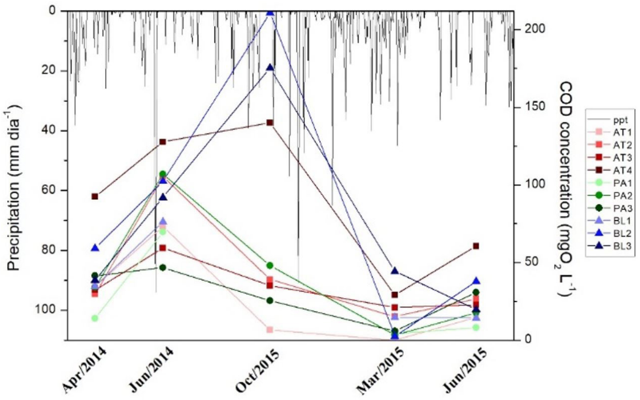

Figure 5. Chemical oxygen demand concentrations (mg.L^{-1}) during the five sampling campaigns.

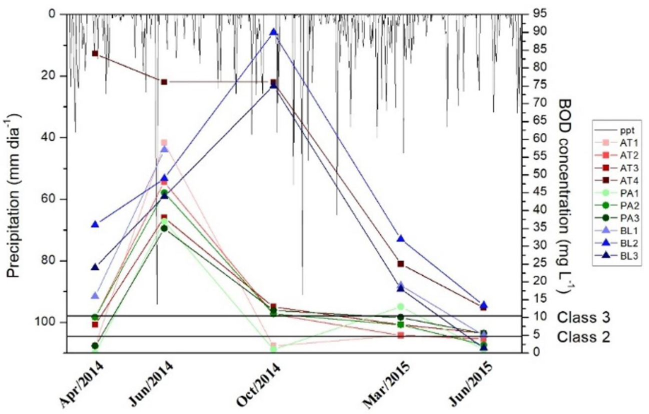

Figure 6. Biochemical oxygen demand concentrations (mg.L^{-1}) during the five sampling campaigns.

From all the sampling campaigns, the third, performed in October/2014 (C3), and second, performed in June/2014 (C2), were the ones that presented the highest concentrations of BOD. During the five days that preceded both these campaigns, there was no rainfall. In this campaign, the highest concentration of BOD were above the values suggested by the norm. According to the Brazilian legislation, the maximum concentration BOD for rivers of Class 2 and 3 is of 5.0 mg.L^{-1} and 10.0 mg.L^{-1}, respectively (BRASIL, 2005). Yet, on the first (C1, performed in April/2014) and fourth (C4, performed in March/2015) sampling campaigns, the accumulated precipitation was of 13.0 and 7.4 mm, respectively. This indicates that the contribution from rainfall and runoff lowered the concentrations of BOD, which would characterize the dilution of contaminants in the rivers.

The subtropical climate, characterized by the high precipitation regime, may influence seasonally on the quality of water. Even though the region does not suffer from designated periods of drought, the seasonal rainfall fluctuation interferes on the concentrations of pollutants on the rivers (MIZUKAWA et al., 2018).

Caffeine on the aquatic environment

The analyses on the physical, chemical and biological parameters were performed to support the understanding of the sources of pollution, as well as the current state of the quality of water, to use caffeine as a chemical tracer for the anthropic activities on the natural environment (GARDINALI; ZHAO, 2002). The quantification of caffeine on samples of aquatic environments is, usually, directly related to the discharge of domestic wastewater, as the caffeine's presence is related exclusively to humans. Even though it has a half-life of just below 5 hours (MORET; HIDALGO; SANCHEZ, 2012), its continuous release on the environment renders it a persistent contaminant.

Caffeine was detected in 98% of the analyzed samples from the Atuba and Belem rivers. The concentrations observed ranged from 0.07 μg.L^{-1} (first campaign on PA1 site), to 23.08 μg.L^{-1} (also on the first campaign, but on BL2 site).

The variations on the concentrations of caffeine (as well as the amount of rainfall observed during the period) in the Atuba, Palmital and Belem rivers are illustrated on Figure 7.

The lowest concentrations of caffeine were exhibited through the Palmital river. The PA1 site, located near the river's spring, in a predominantly rural area, was the most preserved site

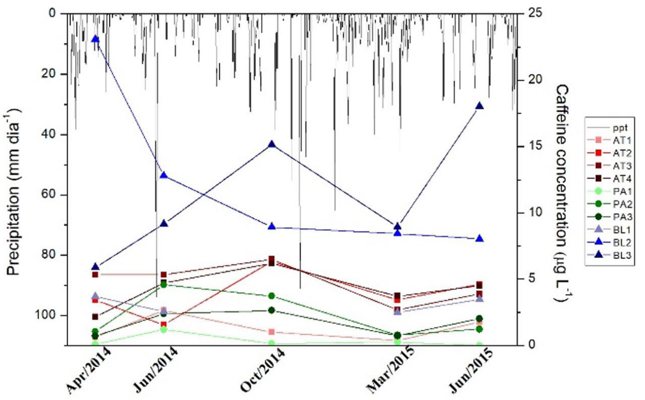

Figure 7. Caffeine concentrations and rainfall intensity during the period that encompasses the five sampling campaigns.

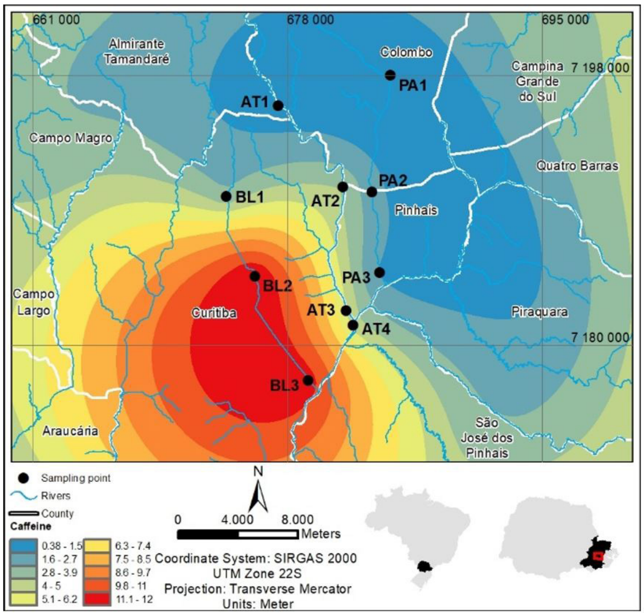

Figure 8. Spatial distribution map of the average caffeine concentrations within the Alto Iguassu basin – ordinary kriging/ spherical method.

on this river, with caffeine concentrations that were inferior to 1.2 µg L^{-1}. Due to the proximity to an urban gathering that lacks proper sanitation infrastructure (Vila Zumbi), the PA2 site was the one that had the highest concentrations on this watercourse. The PA3 site also presented some degree of anthropic influence, as the caffeine concentrations varied from 0.71 to 2.64 µg L^{-1}, being it the closest site to the river's outfall, it accumulated the contaminants discharged in the populated regions upstream.

On the Atuba river, the AT3 and AT4 sites were observed as the most degraded. Though the AT4 site is downstream from a WWTP, there was not an increase in the caffeine concentrations, when compared to the concentrations of the AT3 site, just upstream from the same WWTP. This may indicate that the WWTP is able to partially remove the caffeine, confirming the proposition of Ide et al. (2013). Caffeine's half-life in a WWTP varies from 0.8 to 5 hours, depending on the amount of biological activity (MORET; HIDALGO; SANCHEZ, 2012), suggesting that biodegradation is an important fate of this substance. The AT1 site, near the river's spring, was the least impacted.

The greatest concentrations on all waterbodies were observed on the Belem river. On the BL1 site, the least impacted, the concentrations fluctuated from 2.49 to 3.69 µg L^{-1} of caffeine through the sampling campaigns. As the river flows through more urbanized area (such as Vila Torres), this compound's concentrations rise. At the BL2 site, the concentrations vary from 8.03 to 23.08 µg L^{-1}. At the BL3 site, the high concentrations perdured, being its maximum, at this site, of 18.04 µg L^{-1}, observed on the last sampling campaign (C5).

A map containing the average annual concentrations was generated to observe the spatial distribution of caffeine concentration (Figure 8). The Belem river's detected concentrations were superior to those of other rivers. Also, the regions close to the rivers' springs presented a tendency to suffer less anthropic influences. The Belem river basin has a greater populational density than those of the other analyzed rivers (7,867.7 residents/km^2), making it more susceptible to contamination. The region that encompasses the sites BL2 and BL3 has an extensive low-income population, which may indicate lack of proper sanitation infrastructure, which, in consequence, awards it a greater vulnerability to contamination. Also, the Belem river has the lowest average flow (1.3 $m^3.s^{-1}$), this may indicate higher concentrations.

To be able to determine the degree of contamination of the rivers present in the Alto Iguassu basin, a comparison among rivers from different regions was performed (Table 1). The concentrations observed for the Alto Iguassu basin are compatible to those found by previous studies on the same region or on other regions of Brazil (IDE et al., 2017; MONTAGNER; JARDIM, 2011). Nevertheless, when comparing it to the Iguassu river, the concentrations observed in its tributaries are considerably higher, this may be due its bigger flow rate that dilutes the contaminants that arrive into the river from its tributaries.

When comparing to the results observed in different parts of the world, the results of this study are significantly higher, which could be a consequence of the better sanitation conditions present on more developed countries.

Caffeine versus traditional parameters of water quality

To analyze the behavioral relationship between caffeine and traditional water quality parameters and verify the validity of the former as a chemical tracer for the anthropic influence over aquatic environments graphs of each sample were plotted. Figure 9 illustrates the comparison of variations of caffeine and ammoniacal nitrogen. Figure 10 compared the variations of caffeine and thermotolerant coliforms. The similar behaviors between caffeine and these indicators indicate the same source of contamination. Also, the resemblance observed (especially over thermotolerant coliforms) confirms that caffeine may be used as a water quality parameter, as well as a chemical tracer that indicates anthropic influence over aquatic environments, especially from the discharge of domestic wastewater. This is confirmed by the

Table 1. Caffeine concentrations in surface waters worldwide.

Local	Country	Concentration ($\mu g\ L^{-1}$)	Reference
Alto Iguassu basin, Curitiba	Brazil	0.07-23.08	Present study
Alto Iguassu basin, Curitiba	Brazil	0.07-59.81	Ide et al. (2017)
Iguassu river, Curitiba	Brail	0.02-9.37	Scipioni (2018)
Atibaia basin, São Carlos	Brazil	0.17-127.09	Montagner and Jardim (2011)
West Coast, Vancouver	Canada	0.02-1.59	Verenitch, Lowe and Mazumder (2006)
Hérault basin, Marseilles	France	0.01-0.10	Togola and Budzinski (2008)
Ebro river	Spain	0.41	Gorga, Petrovic and Barcelo (2013)
Water reservoir and tributaries	Singapore	0.03 - 2.98	You et al. (2015)
Dalong Lake, Xuzhou	China	0.03-0.07	Yu e Cao (2016)
Zhujiang River	China	0.01-0.86	Yang et al. (2013)
Jiulong River estuary	China	0.008-3.06	Sun et al. (2016)
Umgeni River	South Africa	0.4-9.25	Matongo et al. (2015)

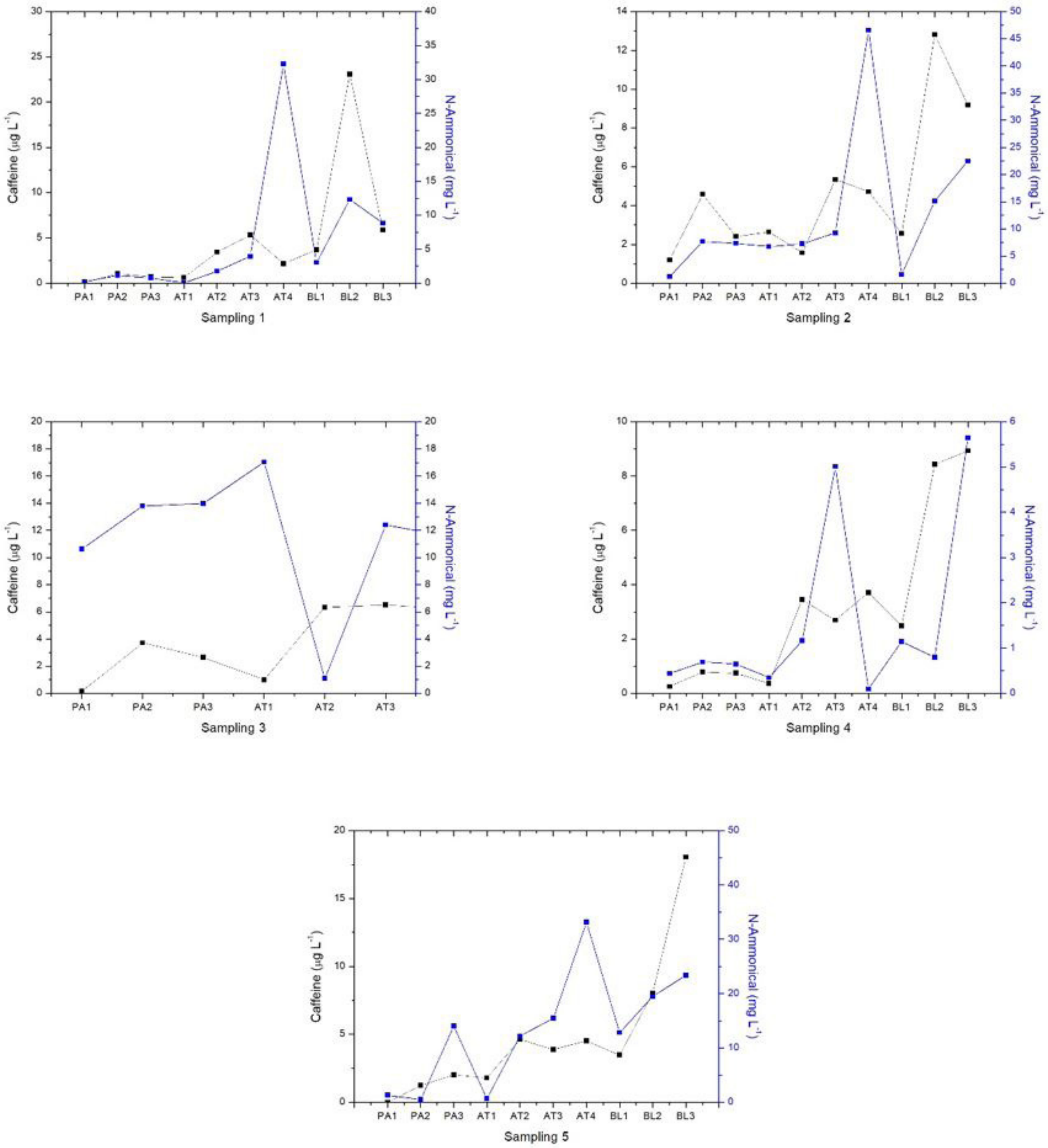

Figure 9. Correlations between caffeine and ammoniacal nitrogen during the five sampling campaigns.

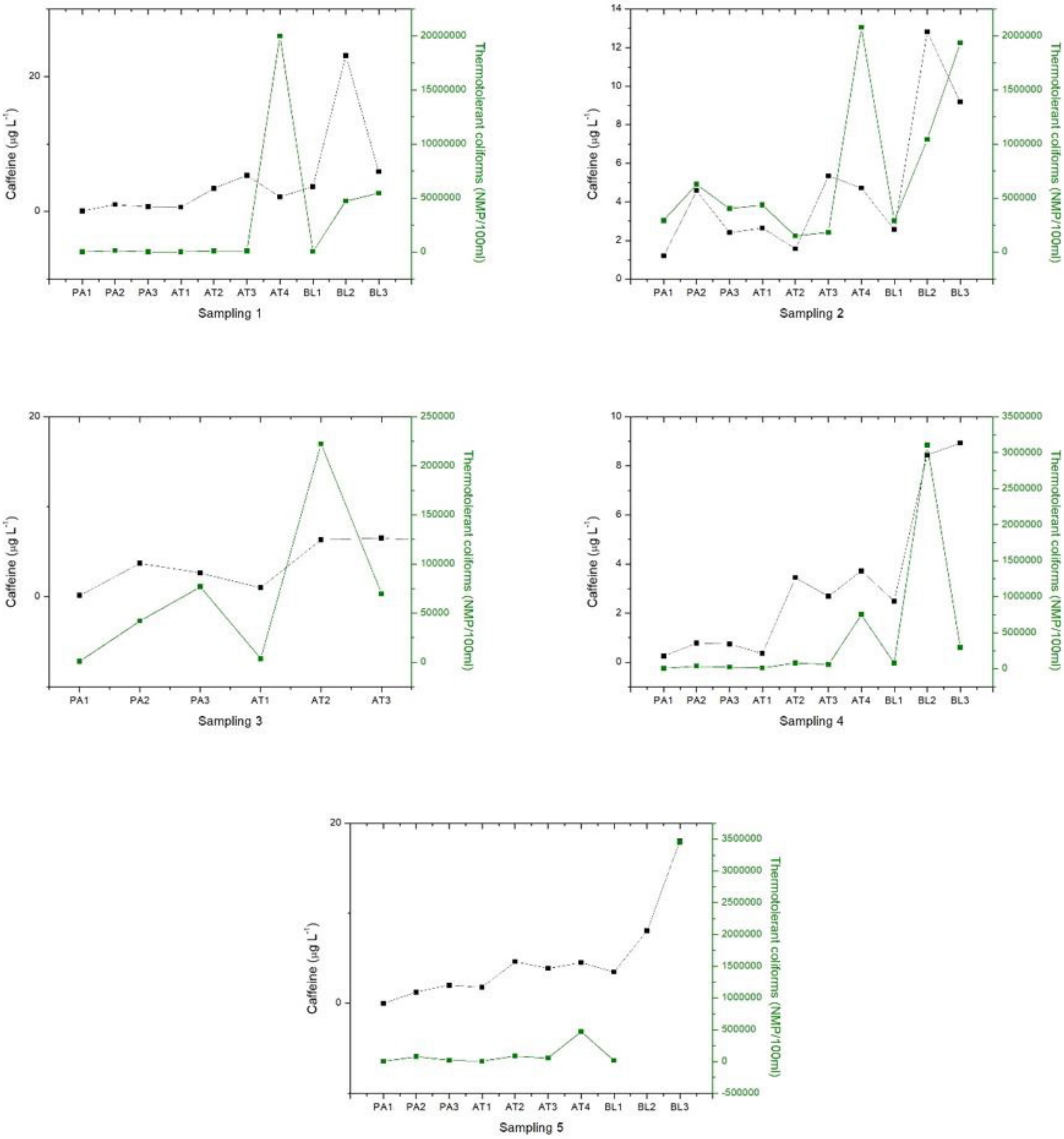

Figure 10. Correlations between caffeine and thermotolerant coliforms during the five sampling campaigns.

Pearson correlation between caffeine and ammoniacal nitrogen (R = 0.6482), as well as between caffeine and thermotolerant coliforms (R = 0.7375). The site AT4 was excluded from this analysis due to the interference of the WWTP on the observed concentrations.

Due to being less susceptible to sampling errors, the caffeine analysis presents itself as more reliable than biological parameters, yet, on the downside, it is still costlier and demands qualified equipment and specialists to run the experiments. Advances on analytical methods could render caffeine as a more competitive tool for supporting water quality policy making.

The presence of caffeine in aquatic environments may indicate the presence of contaminants of emerging concern, such as pharmaceuticals, personal care products and estrogens, that are currently being detected on waterbodies due to improvements on analytical techniques. The caffeine has low toxicity in aquatic organisms but is known that many of these contaminants could interfere with the endocrine system in humans, being called endocrine disruptors, yet, little is known of the risks involved in the long-term exposures to these compounds on the environment and on the human life. Even some of these contaminants are included in the watch list of substances for prioritization monitoring of the European Union due their actual risk posed (example: Estrone, diclofenac and some antibiotics) (EUROPEAN COMMISSION, 2015).

CONCLUSION

The results indicate that the sampled rivers suffered great anthropic influence, probably due the discharge of treated or untreated wastewater. The most impacted river was the Belem, especially the BL2 site (23.08 μg L^{-1} of caffeine), this is due the lack of sanitation on the area. The Atuba river exhibited great influence from a WWTP. The AT4 site, located just downstream from a WWTP, presented a concentration eight times higher of ammoniacal nitrogen than the AT3 site located just upstream from the outflow of the WWTP.

The caffeine analysis is an appropriate approach to environmental monitoring, presenting a solid correlation to the traditional parameters. As it indicates the influence of humankind over an aquatic environment, the caffeine analysis might be taken as a fundamental tool for the better understanding of the anthropogenic effects over a water system. Spatial distribution maps are good alternatives to understand the degrees of contamination on a basin, as a whole, and to easily visualize the most degraded areas, making it a concise tool for environmental decision making.

The study area is of economic, social and environmental significance once it is one of the biggest cities in Brazil. Besides, the metropolitan region of Curitiba is an area of natural water reservoirs used to supply potable water to the population. The concentrations found in this study were remarkably high, especially when compared to data of other countries.

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