Water Quality and the Impact by Contaminants of Emerging Concern: The Case of Verde River in Campos Gerais

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Abstract

In this article, we report the results of the work carried out on the Verde River, a predominantly urban river located in the city of Ponta Grossa, Paraná, Brazil. Developed in three stages, it aimed to evaluate the water quality through physical-chemical parameters, the presence of caffeine as a chemical tracer, and the compound Triclosan (TCS), considered a pollutant of emerging concern. An attempt was made to identify possible polluting sources along the river. Samples were collected at 4 points from the spring region, with sampling upstream and downstream of a Sewage Treatment Plant (STP).

The physical-chemical parameters were determined in situ, through portable analysers, as well as in the laboratory, in this case, through analyses using molecular absorption spectroscopy. For the determination of TCS and Caffeine, high-performance liquid chromatography (HPLC) was used, preceded by a pre-concentration step in the solid phase (SPE), and the techniques were implemented after validation. The physicochemical evaluations of the surface samples showed a decline in water quality after the STP. Mean total phosphorus concentrations of 1.0 mg·L−1 (±0.4), as well as dissolved oxygen (DO) levels below 5.0 mg·L−1 were verified downstream of the STP. Caffeine concentrations for Verde River were between 49.0 to 299.0 µg·L−1, verified upstream and downstream of the STP. The results demonstrated the relationship between the presence of caffeine and impaction, either by the input of raw sewage or by treated effluent. TCS was detected by chromatographic analysis, downstream of the STP, with an average concentration of 27.6 (±1.7) µg·L−1, noting that its presence was associated with the reactor maintenance.

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procedure, with the STP in the process called bypass. Obtaining quality data from Verde River is important for the protection of the environment and affects the health of the population.

Keywords
Surface Water, Micropollutants, Triclosan

1. Introduction

The intensification of urbanization processes has generated worldwide, unavoidable impacts on water bodies. In Brazil, in urban areas, the loss of raw water quality is mainly caused by the irregular discharge of untreated domestic sewage directly into water bodies or by effluents resulting from inefficient sewage treatment. According to IBGE data [1], only 62.8% of Brazilian municipalities treated sewage in 2017. The Midwest (94.4%) and South (71.7%) regions had the highest percentages of municipalities with STP in operation. As for the volume, only 67% of the generated sewage was collected by the network, that is, away from homes and commercial and business establishments through closed pipes, and of this total, only 77% was taken to the STP for proper treatment.

Through sewage, numerous substances contribute to water bodies without any knowledge of their real impact. Among the types of contaminants, the so-called contaminants of emerging concern have been highlighted, substances so called because their concentrations in the environment have not yet been regulated, which previously were not detected in analyses and were not considered as a risk, or capable of causing adverse health effects [2] [3] [4] [5]. Such contaminants are associated with substances present mainly in a variety of commercial products such as personal care products, suntan lotions, repellents, fragrances, anticorrosive, cleaning products, gasoline additives, pesticides, drugs in general: anti-inflammatory, analgesics, antiseptics, hormones and steroids, antibiotics, more recently including nanoparticles [3] [6] [7] [8] [9] [10].

Among the emerging contaminants, the Triclosan compound (TCS) deserves to be highlighted due to its wide use and the important consequences of its disposal in the environment. Studies indicate the toxicity of TCS to aquatic organisms (such as algae, fish and invertebrates), which may contribute, in the long term, to bacterial resistance and represent possible impacts, including on human health.

TCS is chemically characterised as 2,4,4’-trichloro-2’-hydroxydiphenyl ether (Figure 1); it has a bacteriostatic function at low concentrations and a bactericidal function at higher concentrations, with a broad spectrum of action on Gram positive and negative bacteria [11].

It is a preservative compound, and due to its antimicrobial action, used increasingly in personal care products such as bactericidal soaps, mouthwashes, deodorants, and toothpastes, and incorporated in various consumer items such
Figure 1. Molecular formula of the TCS [12].

as toys, kitchen utensils, socks, and bags of trash. It is among the preservatives added to cosmetics to increase the shelf life of products. According to the cosmetics industry, it is important that the product has a good preservative system formed by one or more substances to prevent changes in color, odor, and consistency, to ensure consumer safety and maintain product stability [13] [14] [15].

The direct identification and quantification of compounds of emerging concern in the environment have become possible through the evolution of equipment, sampling, extraction, and analysis techniques, as they are present in concentrations that are difficult to detect. The monitoring of residual drugs in the environment, including the TCS, has frequently demonstrated the presence of these substances in effluents from ETEs and natural waters in concentrations in the range of ng∙L⁻¹ to µg∙L⁻¹ [16] [17] [18].

On the other hand, the prediction of the presence of emerging contaminants in raw water can be done indirectly through the so-called chemical markers. These substances are exclusively present in human metabolism or result from human activity, with no natural sources of production [19].

Caffeine is an important example of a chemical marker. It is among the most consumed substances in the world and, due to the similarity of origin, its presence in surface waters signals the existence of other contaminants, which enter through sanitary sewage in springs. [3] [20] [21] [22] [23] [24]. It belongs to the group of soluble lipid compounds called purines, chemically known as 1,3,7, trimethyl xanthine (Figure 2). It is classified as an alkaloid and can be found naturally associated with two other compounds of the same group: theophylline and theobromine, differentiated by the position of the methyl group [19] [25] [26].

The classic methods for determining caffeine in food samples or biological fluids initially include the use of gravimetric techniques officially adopted by the “Association of Official Analytical Chemists” (AOAC). Later, they were replaced by the method called “Bailey-Andrew”, which, although consuming less time than gravimetry, was based on the determination of total nitrogen, and thus, accounted for other nitrogen compounds, causing an overestimation of caffeine values. The same question of quantification of interferents computed together with caffeine was verified using molecular absorption spectroscopy. From the 1970s onwards, there was a need to develop methods that offered greater reproducibility and sensitivity and could be applied for analysis in small sample volumes. Thus, chromatography starts to represent the main methodology used, initially in a traditional way, evolving until HPLC, was used in this work.
Figure 2. Molecular formula of caffeine [26].

In the present work, we sought to determine TCS and caffeine, through chromatographic analysis, and to evaluate physico-chemical parameters for all samples in the years 2014 (10 collections), 2016 (8 collections) and 2017 (12 collections). The physico-chemical parameters reveal the characteristics of Verde River, and thus, represent the scenario in which we seek to evaluate the presence of substances of interest in this study.

Characterization of the Study Area

The Verde River, whose waters are the object of study in this work, has its source in the rural area of the municipality of Ponta Grossa, where agricultural and livestock activities take place. It follows its path, cutting through the urban area, and in some stretches, its margins are occupied by houses and other enterprises. The city of Ponta Grossa, in the Campos Gerais region of Paraná, had its urban development, as well as most Brazilian municipalities, accompanied by disorderly population density, a fact that has direct consequences for the quality of the water sources.

The Verde River, despite being widely used for recreation, receives a significant load of pollution from urban streams [27], and at the end of the route, before emptying into the Pitangui River, it receives the effluent from the ETE Verde River, of the Paraná Sanitation Company (SANEPAR).

It is impacted throughout its path, either by the disposal of untreated domestic sewage, urban waste, or even the discharge of effluent from the ETE. In the present research, the water quality was studied through aquatic parameters in 4 points from its source, analysing the presence of caffeine and TCS in the samples collected upstream and downstream from the ETE.

2. Materials and Methods

2.1. Materials

Ultrapure water obtained in one system was used: a Milli-Q system (Millipore); Sigma Aldrich® brand caffeine with a minimum purity of 99.0%, methanol (MtOH) Merck®, HPLC grade; acetonitrile (ACN) Merck®, HPLC grade; other reagent salts and Biotec brand buffer solution. Samples were pre-concentrated in Applied Separation. The pre-concentration of the samples was carried out in Applied Separation Cartridges for C18 solid phase extraction, containing 18% octadecyl and
500 mg of silica, with an extractor volume of 6.0 mL. Portable analyzers were used to analyze the samples in situ: Instrutherm CD-860 conductivity meter; LT Lutron DO-5519 analyser for pH, DO and Temperature. In the analysis of physicochemical parameters, a UV/VIS VARIAN spectrophotometer, model CARY 50, quartz cells with a capacity of 3.0 mL and the optical path of 5 mm. For caffeine and triclosan determination, Allcrom YL9100 chromatograph, C18 column (550 mm × 4.6 mm × 5.0 mm) equipped with YL9120 UV/Vis detector.

2.2. Sample Collection and Preparation

All collection flasks and glassware used in the analysis were previously washed with neutral detergent and then rinsed with running water and distilled water, placed in an acid bath in containers containing 1.0 mol∙L⁻¹ HNO₃ nitric acid solution, leaving the material immersed for at least 12 hours. Before use, rinse again with ultrapure water at least five times. Disposable polyethylene gloves and plastic trays were used so that there was no direct contact of any material with the bench. At the collection site, the bottles were rinsed at least twice, with the water to be collected, for ambiance. Information about the collection, such as location, time, number of samples, ambient and sample temperature, observations on the environmental conditions of the sampling site, among others, were recorded. After collection, the bottles were labeled and packaged in plastic bags, and the samples were kept at low-temperature for transport, in a thermos box, to the laboratory. In the laboratory, aliquots of the samples were filtered under reduced pressure in 0.45 µm porosity cellulose acetate membranes. The filtered and natural samples were preserved at 4.0°C, in a refrigerator until the completion of all analyses.

2.3. Pre-Concentration Step

To pre-concentrate, the samples in relation to TCS and caffeine analytes, the solid phase extraction technique (SPE) was used. For this step, 1.0 L of each sample was filtered in C18/18 Applied Separations cartridges under negative pressure and subsequently eluted in 6 mL of ACN. The aliquots submitted to pre-concentration were previously filtered in three stages: 1) qualitative filter paper; 2) 0.45 µm cellulose acetate membrane; 3) polypropylene coated 0.22 µm cellulose membrane. Studies to validate the efficiency of the C18/18 cartridge, used in the pre-concentration of samples in surface water, were carried out through standard solution recovery tests in triplicate.

2.4. Methodology for Determination of TCS and Caffeine

The analytical methodology used is based on the specialised literature [28] [29] [30] [31] [32] on the use of HPLC in the determination of emerging pollutants at dash levels for natural administrators. A careful evaluation of the experimental conditions was carried out: mobile phase (FM), flow rate, temperature, and injection volume. All chromatographic analyses were performed in triplicate. In-
Initially, a C-18 column (250 mm × 4.6 mm × 5 m) was driven, due to its high degree of affinity for the analytes and coupled to the chromatograph. The temperature was programmed at 25.0˚C and the flow at 1 mL/min for both substances, according to the literature.

Solvent units were also evaluated. For TCS, it was observed that the best retention times were 10.21 min for ACN and water (65:35). For caffeine, the proposal according to the literature [19] was observed, being a mobile phase MeOH: Water, in a proportion of 78:22 v/v, operating in isocratic mode.

In the step of implementing the methodology and analytical validation, the selection of the ideal wavelength for the quantification of TCS (Figure 3) and Caffeine (Figure 4) was performed, that is, the selection of the region of the electromagnetic spectrum in which the higher coefficient of molar absorptivity for the analytes. Being 280 nm for TCS and 273 nm for Caffeine, under the mentioned experimental conditions.

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**Figure 3.** Chromatogram obtained from the analysis of TCS standard solution at wavelengths of 260 and 280 nm.

**Figure 4.** Chromatogram obtained from the analysis of standard caffeine solution at wavelengths of 273 nm.
2.5. Analyte Recovery Rates

Real samples were subjected to the standard addition test [33]. These were analysed by HPLC, with and without fortification, under the following conditions: blank and in low, medium, and high concentrations. For TCS, they were fortified with a standard solution: 1.6 mg·L⁻¹, 3.1 mg·L⁻¹ and 6.2 mg·L⁻¹. For caffeine in concentrations: 0.1 mg·L⁻¹; 0.7 mg·L⁻¹ and 2.0 mg·L⁻¹. After chromatographic analysis, the peak area for standard solutions with known concentrations was obtained, and thus, the recovery rate of real samples, calculating the recovery index (RI) in the chromatographic analysis. It was found that RI ranged from 99.5% to 116.5% for both analytes, in the three concentration levels evaluated, corroborating the validation of the analytical methodology.

3. Results and Discussion

3.1. Physical-Chemical Characteristics of the Verde River Waters

The data presented in this work include results for 14 determined physicochemical parameters in 4 collection points (Figure 5). The sampling points were as follows: P1 near the source of the river, in a tourist site called Capão da Onça; P2 in a region of rural properties, in a place called Curva do Cemitério; P3 in the urban area under a bridge; P4 downstream of STP Verde River.

It was found that the variation in water quality, represented through the various parameters, is related to the studied reference area, with increasing impact as the source moves towards the mouth. To analyze the results, CONAMA Resolution [35] was observed, which provides for the classification of water bodies and provides guidelines for their classification [35].

According to historical monitoring data, Ponta Grossa has a temperate climate, with significant rainfall throughout the year and an average temperature of 17.6°C [36]. These data corroborate what was verified in this work, where the ambient temperature on the days of collection varied between a minimum of
17.0°C (June) and a maximum of 27.0°C (January), with the average temperature being equal to 17.5°C (±0.5). The water temperature did not change significantly between the points and in the sequence of collections; that is, there is no thermal pollution, a fact that does not restrict its influence on the variation of the results. The average water temperatures were 17.2°C (±0.4) noting that the temperature is slightly increasing in the source-mouth direction, influenced, in part, by the presence of riparian vegetation in the source region.

As for pH, in some measurements, values below 6.0 were verified. There is no significant difference between the sampled locations, with the mean pH found equal to 6.4 (±0.2), indicating results in accordance with CONAMA Resolution [35], according to which the pH can fluctuate between 6.0 and 9.0 in Class 2 watercourses.

The presence of heterotrophic organisms tends to make the pH of the liquid mass more acidic through the metabolic processes of respiration and decomposition, which release CO₂ into the environment. Heterotrophic bacteria are widely used as indicators of water quality, and in the case of portability, they can indicate an impediment to its consumption or the need for water disinfection. The term heterotrophic bacteria include all bacteria that use organic nutrients for their growth, including thermotolerant coliforms, determined in the present study. It was verified, only for P4, concentration above the limit established in the CONAMA legislation [35], that is, greater than 1000 thermotolerant coliforms per 100 mL of water, in 100% of the collections.

The total alkalinity is also higher at point 4, which is located after the Verde River STP, since while for P1 and P2, the values obtained do not exceed 24.0 mg\text{CaCO₃} L⁻¹, in P4 they are greater than 50.0 mg\text{CaCO₃} L⁻¹, with a value maximum 130.0 mg\text{CaCO₃} L⁻¹, pointing to the presence of a higher concentration of inorganic carbon, which may result from organic matter decomposition processes. Alkalinity is an indicator of anthropogenic activities, and the analysis of the results obtained must be comparative between the points studied, as there is no reference value in current legislation. Likewise, the conductivity measurements indicated changes in the composition of water in P4, especially in the concentration of ions. In general, values above 100 µS·cm⁻¹ may indicate impacted environments [37], and only for P4, the conductivity exceeded this value in 50% of the collections.

As for dissolved oxygen (DO), it is a variable whose concentration reduction can significantly affect aquatic biota and which, in surface waters, is influenced by factors such as temperature and natural oxygenation of water through rapids, as well as the impact mainly by organic matter. CONAMA Resolution [35] establishes that the minimum recommended value is 5.0 mg·L⁻¹ for class 2 water bodies. For Verde River, the DO concentrations were above this limit at P1 and at P2 in all collections; at critical concentration (3.8 mg·L⁻¹) only in the third collection for P3; and below 5.0 mg·L⁻¹ in most collections (60%) for P4. This river has many falls and rapids and runs over a bed of extensively exposed rock slabs, which spontaneously increases the concentration of DO. Thus, concentra-
tions below 5.0 mg·L$^{-1}$ show the consumption of DO in the degradation reactions of the organic matter that contributes to the river from clandestine connections verified in loco, and from the STP effluent, whose flow seems to exceed the capacity of dissolution of the water body.

It was also determined the concentration of dissolved chloride in the collected samples, which is associated with the introduction of domestic and wastewater into water bodies. For public water supply, the concentration of chloride is a potability standard, according to Ordinance 518/2004 of the Ministry of Health, as its presence can cause a salty taste in the water, with 250 mg·L$^{-1}$ being the maximum concentration allowed. For Verde River, the concentrations are much lower than the established maximum value, but the comparison between the points can differentiate the most impacted places. It is verified for P1 and P2 that the average is around 7.0 mg·L$^{-1}$ (±0.7); for P3, it is 10.3 mg·L$^{-1}$ (±3.3), and again, he observes the highest concentrations are P4 with a mean of 17.0 mg·L$^{-1}$ (±3.1). Inefficient effluent treatment systems or operating above the flow capacity for the receiving body, can contribute to the increase in chloride concentration [38].

When it comes to turbidity and total solids concentration in this river, the values are low when compared to those established in CONAMA Resolution [35]: turbidity up to 100 UNT and Total Solids up to 500 mg·L$^{-1}$, and show a reduced presence of suspended solids, and this is due to the geological type of the riverbed, characterised by rock formation, with little sediment at the bottom. The turbidity of the water did not show a direct relationship with the concentration of TSS and may be associated with the presence of suspended colloidal material, consisting mainly of organic material of low molecular weight, which may be related to the presence of compounds from living organisms and their processes biological resources, as well as the external input of effluents.

With the concern about the nutrient load present, the concentrations of Total Nitrogen and Ammoniacal Nitrogen were determined. The concentrations of total nitrogen maintained a certain homogeneity for the samples at the four collection points, being higher in all collections for the P4 samples, ranging from 0.56 to 1.76 mg·L$^{-1}$. However, current legislation does not include this parameter. Total nitrogen encompasses the various species of nitrogen transported by rivers, whether in the form of nitrate, nitrite, or ammonia (or ammoniacal nitrogen), which can be released by biochemical or physical processes. The nitrogen species called ammonia nitrogen is established in CONAMA Resolution [35], and for Class 2 rivers, the maximum concentration must be 3.7 mg·L$^{-1}$, at pH ≤ 7.5. All values found in the analyzed samples were below 1.3 mg·L$^{-1}$, but the highest concentrations, comparatively, always occurred at Point 4, downstream of the STP Verde River. However, even at low concentrations, this compound can impact aquatic life, in terms of chronic toxicity with effects on reproductive capacity, growth and biochemical alterations [39]. In this work, ammonia nitrogen increased along the river, mainly after the sewage treatment plant, which can be verified through the maximum values obtained: 0.03 mg·L$^{-1}$ for P1; 0.04 mg·L$^{-1}$ for P2; 0.17 mg·L$^{-1}$ for P3 and 1.16 mg·L$^{-1}$ for P4.
In the case of total phosphorus, almost all (96%) of the analyzed samples were found, with concentrations higher than the limit for class 2, which is 0.1 mg·L⁻¹, considering that it is an environment lotic [35]. The main source of phosphorus in urban surface waters is the discharge of sanitary sewage containing detergents; however, in areas with agricultural activity, there is a contribution from the surface drainage of organic matter rich in phosphorus and products applied in crops [38].

In the case of P3, the mean concentration was 0.6 mg·L⁻¹ (±0.1). It should be noted that in this area, the river runs through an urbanised region, receiving input from the Pilão de Pedra Stream, the mainstream in the central area of the city. As for P4, with an average concentration of 1.0 mg·L⁻¹ (±0.4), its impact is mainly due to the effluent from the STP Verde River, as already mentioned for other parameters. But what was surprising was the phosphorus concentration was slightly above the limit of 0.1 mg·L⁻¹, also for P1 and P2, in which all other parameters were within the current Brazilian legislation. Even though the Verde River exhibits, in these points, apparently preserved margins, the surrounding areas present agricultural and livestock activities, and surface drainage may promote the contribution of phosphorus to the source [40] [41].

The physicochemical evaluation of the collected samples showed, therefore, enrichment of the aquatic body by phosphorus, but mainly a decline in water quality after disposal of the effluent by the STP, in all collections. In addition, during the study period, a process known as by-pass occurred twice, when effluent disposal occurs without complete treatment, due to equipment maintenance. In these two cases, the process at the STP can be described as a pre-concentration of sewage, bringing even more impact to the quality of the receiving water and to the biota.

3.2. Chromatographic Analysis: Determination of Caffeine and TCS

3.2.1. Analytical Validation Process

The analytical performance parameters determined in the validation of the chromatographic method were linearity and application range, accuracy, precision, selectivity, and detection limit [42] for both analytes.

For TCS, the linearity was determined through the analysis in triplicate of 5 different concentrations (1.0 mg·L⁻¹, 2.0 mg·L⁻¹, 3.0 mg·L⁻¹, 4.0 mg·L⁻¹ and 5.0 mg·L⁻¹), obtaining an average correlation coefficient (R²) of three analytical curves of 0.99847 and reaching 80% to 120% of the theoretical concentration of the test. For the calculation of accuracy, a reference standard was used, where a linear range was established by determining 3 concentrations, low (1.0 mg·L⁻¹), medium (3.0 mg·L⁻¹) and high (5.0 mg·L⁻¹), all analysed in replicas, obtaining an accuracy of 88.0%; 95.3% and 88.0%, respectively. The intra-run precision, that is, the repeatability, was evaluated through the results obtained in a series of measurements from multiple sampling, resulting in a relative standard deviation (RPD) of all measurements equal to 4.7%. As for selectivity, the influence of ma-
trix components was evaluated, comparing the chromatographic analysis of the standard, the surface water sample with and without the addition of standard, verifying that there was no interference of the matrix in the TCS peak. As for the detection limit or sensitivity of the method, analyses of TCS patterns were carried out in increasingly smaller concentrations, comparing the corresponding peak in its retention time to noise. For a sample with a TCS concentration equal to 1.0 mg·L−1, the lowest concentration in the analytical curve, a 2- to 3-fold relationship between the chromatographic peak and the noise presented by the equipment was obtained. The signal/noise ratio was equal to 0.34, that is, well below the value 3 considered acceptable to estimate the LD [43].

In validating caffeine, linearity was determined by analyzing in triplicate 7 different concentrations (0.1 mg·L−1; 0.2 mg·L−1; 0.5 mg·L−1; 0.7 mg·L−1; 1 mg·L−1; 1.5 mg·L−1 and 2.0 mg·L−1) obtaining an average correlation coefficient (R²) of three analytical curves of 0.9986 and range from 86% to 93% of the theoretical concentration of the test.

For the calculation of accuracy, a reference standard was used, where a linear range was established by determining 3 concentrations, low (0.1 mg·L−1), medium (0.7 mg·L−1) and high (2.0 mg·L−1), all analysed in replicas, obtaining an accuracy of 119.1%; 94.7% and 101.4%, respectively. The intra-run precision, that is, the repeatability, was evaluated through the results obtained in a series of measurements from multiple sampling, resulting in a relative standard deviation (RPD) of all measurements equal to 4.43%. As for selectivity, the influence of matrix components was evaluated. A comparison was made between the chromatographic analysis of the standard, the surface water sample with and without standard addition. It was verified in the obtained chromatograms that there was no interference of the matrix in the caffeine peak.

To calculate the limit of detection (LD), three analytical curves obtained on different days were used, and the slope of the analytical curve and the standard deviation of the intercept with the y-axis of these curves were calculated. The LD obtained was equal to 10.0 µg·L−1.

3.2.2. Determination of Analytes in Real Samples

In the first collection, TCS was detected at a concentration of 28.8 µg·L−1 only in the sample collected downstream of the STP, which was not repeated in the second collection. Comparing the two samples, it was found that in the first collection, the STP was under maintenance, in bypass operation; that is, the effluent discarded in the Verde River had not undergone adequate treatment, as the reactor was not in operation. The fact was repeated in the 5th collection, in which TCS was detected at a concentration of 26.4 µg·L−1 and again there was a reactor shutdown due to technical reasons. Thus, the chromatographic analyses allowed detecting TCS in samples collected just downstream of the STP, in the 1st and 5th collections, and in both the STP was under maintenance, discarding untreated effluent. There are indications that the reduction of TCS in the effluent after the reactor may be related to the high hydraulic retention time inside
Thus, the effluent treatment system using RALF seems to be efficient in the TCS degradation process, but it is interesting to emphasize that the metabolites generated by the TCS may be present. According to the literature [50], chlorophenols such as TCS have high toxicity, since they are considered the largest sources of dioxins, which can persist in the environment. Thus, it is possible that the TCS has suffered decomposition, thus causing a reduction in its concentration, which may justify not having been detected at concentration levels within the LD limit of the analytical method used, in the other collections downstream of the STP. Degradation of TCS generates metabolites such as MTCS and 2,4-dichlorophenol [30] [51].

Chromatographic analyses allowed detecting and quantifying caffeine upstream and downstream from the STP (Table 1). For the determination of caffeine in the samples collected in Verde River, HPLC with a UV-Vis detector was used, as previously described. All chromatographic analyses were performed in triplicate, and after SPE.

In reports in the literature [19] [52] [53], there are similar concentrations of caffeine in urban rivers, which corroborate the concentrations found here. In this work, caffeine concentrations were between 48.6 to 299.1 µg·L⁻¹ upstream of the STP, and between 52.4 and 270.6 µg·L⁻¹, downstream of the STP. Although the minimum and maximum values are similar, caffeine concentrations were higher downstream of the STP in seven of the collections carried out, and practically equivalent to twice the upstream concentrations, in some of them, showing the impact of the STP effluent on the Verde River again. It is also observed that in the last two collections, caffeine concentrations are higher, especially in collection 7. The period in question was very rainy, and the concentrations may be related to the greater input of sewage through irregular connections in the river network. With the increase in the flow of water in the river network due to

| COLLECTIONS | CAFFEINE CONCENTRATION (µg·L⁻¹) |
|------------|---------------------------------|
|            | Amount to STP | Downstream to STP |
| Collection 1 | 70.7           | 149.6           |
| Collection 2 | 48.6           | 78.2            |
| Collection 3 | 56.7           | 74.2            |
| Collection 4 | 52.4           | 52.4            |
| Collection 5 | 149.0          | 170.2           |
| Collection 6 | 75.4           | 128.0           |
| Collection 7 | 299.1          | 270.6           |
| Collection 8 | 217.0          | 227.8           |

Table 1. Caffeine concentrations (µg·L⁻¹) upstream and downstream of STP Verde river.
the rains, there is also a more intense transport of domestic sewage, increasing the concentrations of caffeine, which explains what was detected.

4. Final Considerations

This research allowed us to verify the loss of water quality in the Verde River, mainly after receiving the effluent from the STP, at Point 4, as can be seen through the physical-chemical parameters, outside the conditions established in the legislation for the main parameters analysed, DO and phosphorus, specially. The detection of TCS downstream of the STP, although in just two collections, and justified by the by-pass process, is also an indication of impactation. Furthermore, the presence of the chemical marker, caffeine, upstream and downstream of the STP, suggests the presence of other emerging contaminants, including the TCS, whose absence in the other samples can only indicate the non-detection by the applied method.

The legislation governing the emission of effluents in Brazil is fundamentally based on CONAMA Resolution [54], according to which the company responsible for the collection network and sewage treatment must maintain control over the quality of the effluents produced, carrying out self-monitoring. Pursuant to Article 3: “Effluents from any polluting source may only be discharged directly into receiving bodies after proper treatment and provided they comply with the standard conditions and requirements set forth in the same Resolution and in other applicable norms” [54].

However, numerous substances, particularly those of emerging concern, are not covered by standards or legislation, and thus are not monitored. Even though there are self-monitoring reports indicating that the effluent is in conditions of supply, there is an important aspect to be considered: the flow of effluent generated in the STP. This flow cannot be greater than the volume and flow of the river that receives it. According to Art 12 of CONAMA Resolution [54]:

The discharge of effluents into water bodies, except for those classified in the special class (where discharge is not allowed), cannot exceed the conditions and water quality standards established for the respective classes under the conditions of the reference flow or volume available in addition to meeting other applicable requirements. [54]

The importance of the residual finding of TCS in the environment is due not only to its antimicrobial action and the possibility of resistance caused to humans but also to damage to aquatic biota, with high toxicity for some species, and the production of derived compounds, also more toxic, from its photodegradation, mainly in STP. [51]

In the case of Verde River, in addition to the contribution of effluent from the STP, the urban streams, which are its tributaries, contribute to the impact, as well as the use of land and occupation of the banks, which occur at points along its course. Basic sanitation and inspection measures, without a doubt, are essential to recover and maintain the quality of raw water and the preservation of aq-
aquatic biota. Such measures, if adopted, reflect on the entire community, health and quality of life.

**Authors’ Contributions**

- Renato I. D. da Fonseca (Ph.D. student in science); Juliana A. Silva (Ph.D. student in chemistry); Karine A. Costa (Ph.D. student in chemistry); Gabrielle Delfrate (Ph.D. student in science); Egon C. de Castro (master in chemistry): Responsible for the collection and preparation of samples, *in situ* and laboratory analysis, in addition to the preparation of written material on their results and conclusions.
- Rosilene Prestes (Ph.D. in chemistry): Full Professor at Department of Chemistry at Federal University of Technology of Paraná (UTFPR): Responsible for the chromatography laboratory in UTFPR and for the chromatographic analyses performed.
- Sheisa Fin Dantas Sierpinski (Ph.D. in Chemistry), Adriano Gonçalves Viana (Ph.D. in Biochemistry) and Elizabeth W. O. Scheffer (Ph.D. in Chemistry): Advisors and co-advisors in the present research work, within the Graduate Program in Chemistry at the State University of Ponta Grossa.

**Conflicts of Interest**

The authors declare no conflicts of interest regarding the publication of this paper.

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