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Research Article

Occurrence of Pharmaceutical Products, Female Sex Hormones and Caffeine in a Subtropical Region in Brazil

Water quality is currently a subject of increasing concern, especially because of the emerging pollutants contaminating aquatic environment. The aim of this study was to verify the presence of emerging pollutants in the Iguaçu River, from source to mouth, such as: pharmaceuticals, including acetylsalicylic acid (ASA), salicylic acid, ketoprofen (KET), naproxen (NAP), gemfibrozil and fenofibrate; female sex hormones, including estradiol (E1), ethinylestradiol (EET) and estrone (E2); UV filters, including 4-methylbenzylidene camphor and octylmethoxycinnamate; and caffeine (CAF). The extracted pollutants were purified using solid phase extraction and then analyzed by liquid chromatography with diode array detection and gas chromatography-tandem mass spectrometry. All analyzed compounds were detected in at least one site in one sample. The analysis revealed maximum concentrations of 27.0 $\mu g\,L^{-1}$ CAF, 5.17 $\mu g\,L^{-1}$ ASA, 0.34 $\mu g\,L^{-1}$ NAP, 0.62 $\mu g\,L^{-1}$ KET, 1.42 $\mu g\,L^{-1}$ E1, 1.48 $\mu g\,L^{-1}$ EET, and 0.94 $\mu g\,L^{-1}$ E2. The results indicated that Iguaçu river water is contaminated with emerging pollutants and nutrients and that this may be due to domestic sewage and water from tributary rivers mixing with Iguaçu river water. Statistical analysis demonstrated that the study of emerging environmental contaminants is a useful tool to determine if contamination is due to domestic sewage input once traditional physical, chemical and spectroscopic analyses have been insufficient to prove an anthropogenic influence.

Keywords: Emerging contaminants; Iguaçu River; Multivariate statistical analysis; Water quality

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1 Introduction

Emerging pollutants such as pharmaceuticals, personal care products, pesticides, anthropogenic markers, industrial by-products, endocrine disruptors, illicit drugs, female sex hormones and UV filters are found in different environmental matrices such as water, soil, and air. Even at trace levels, these emerging contaminants are causing adverse effects on humans and non-target organism biota, as well as causing food contamination [1–3]. Although these compounds have become the focus of studies in the last 15 years, their presence in the environment is not necessarily recent, as they may be present once they are part of the composition of many widely consumed products. However, the synthesis of new compounds and changes in their use and disposal may generate new sources of environmental contaminants [4].

Domestic sewage is the main source of pharmaceuticals and personal care products, which may contain metabolites or parent compounds excreted through urine and faeces [5]. The widespread occurrence of emerging contaminants in river and sea water [6, 7], groundwater [4], tap water [8, 9], hospital wastewater [10] and final effluents of wastewater treatment plants (WWTPs) [11] displays the deficiency in the removal of these compounds, contributing to their persistence in the environment. Conventional WWTPs use biological treatment systems to remove organic matter and nutrients. However, these systems may not be effectively removing the emerging contaminants because of their low biodegradability [12].

Ecotoxicological tests are performed to assess the potential risk of these contaminants, but they often use much higher concentrations than those found in the environment. The compounds are also studied individually, while in nature they are found in mixtures. Therefore, the real effects of the emerging contaminants are still unknown [13].

Although several studies have addressed the presence of emerging contaminants in various parts of the world [4, 6–9], few studies exist for Brazil. More research investigating the occurrence of these

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Abbreviations: **ASA**, acetylsalicylic acid; **CAF**, caffeine; **DO**, dissolved oxygen; **DOC**, dissolved organic carbon; **E1**, estradiol; **E2**, estrone; **EET**, ethinylestradiol; **FEN**, fenofibrate; **GC-MS/MS**, gas chromatography mass spectrometry; **GEM**, gemfibrozil; **GIHC**, general index of human contamination; **HCA**, hierarchical cluster analysis; **HPLC-DAD**, high performance liquid chromatography with diode array detector; **KET**, ketoprofen; **LOD**, limit of detection; **LOQ**, limit of quantification; **4-MBC**, 4-methylbenzylidene camphor; **MRC**, Metropolitan Region of Curitiba; **NAP**, naproxen; OMC, octylmethoxycinnamate; **PCA**, principal component analysis; **SA**, salicylic acid; **SL**, single-linkage; **SPE**, solid phase extraction; **SRM**, selected reaction monitoring; **TDS**, total dissolved solids; **WWTP**, wastewater treatment plant.

compounds in the environment is necessary, as they can negatively affect human health and the non-targeted biota [14].

The aim of the present work was to assess the quality of the water and evaluate the pollution status of the Iguaçu River by studying the occurrence and distribution of selected emerging pollutants in the river, such as: Pharmaceuticals, including salicylic acid (SA), acetylsalicylic acid (ASA), naproxen (NAP), ketoprofen (KET), gemfibrozil (GEM) and fenofibrate (FEN); female sex hormones, including estradiol (E1), ethinylestradiol (EET) and estrone (E2); UV filters, including methylbenzylidene camphor (4-MBC) and octyl methoxycinnamate (OMC); and caffeine (CAF).

2 Materials and methods

2.1 Study sites and sample collection

The Iguaçu River is located in the south of Brazil and drains a total area of 70 799 km^2 (Fig. 1). At its source, the Iguaçu River is subject to strong anthropogenic influences of the Metropolitan Region of Curitiba (MRC), due to improper occupation along its banks and problems related to the water supply and wastewater treatment systems. Near its mouth are the Iguaçu Falls, which are the largest waterfalls in the world by volume of water. The Iguaçu River is 910 km long and borders three countries: Brazil, Argentina and Paraguay.

River water samples were collected at 16 sampling sites (IG1 to IG16) along the banks of the Iguaçu River from source to mouth in Jul 2012 (C1), Nov 2012 (C2), Feb 2013 (C3) and Sep 2013 (C4). The water samples were collected in prewashed polyethylene terephthalate bottles with distilled water containing 5% hydrochloric acid for analysis of inorganic compounds and prewashed amber glass bottles for analysis of organic compounds. All samples were stored at 4°C.

Water quality parameters included pH, dissolved oxygen (DO) (mg L^{-1}), electrical conductivity (μS cm^{-1}), redox potential (mV), salinity (PSU), resistivity (Ωm) and total dissolved solids (TDS) (mg L^{-1}) and were measured in situ by using a multiparameter probe (Hanna, USA), following the manufacturer's guidelines for calibration. The turbidity (NTU) (mg L^{-1}) was determined by a digital turbidimeter (Hanna).

2.2 Sample preparation and instrumental method for the determination of emerging contaminants

An extraction method for the twelve selected emerging contaminants was developed using solid phase extraction (SPE) and performed on the same day as the sampling. One liter of each sample was filtered through 0.45 μm cellulose acetate filters, after which the pH was adjusted to 3. 1 L of the filtered water sample was eluted through a preconditioned C18 SPE column (6 mL of hexane, 6 mL of ethyl acetate, 6 mL of methanol and 6 mL of ultrapure water) at a constant elution rate (10 mL min^{-1}). Finally, the C18 SPE column was eluted with 12 mL of acetonitrile, the eluate was evaporated to dryness in a rotary evaporator, and 1 mL of acetonitrile was added to re-dissolve the extract. The concentrated samples were used for the analysis of selected emerging pollutants by gas chromatography mass spectrometry (GC-MS/MS) and high performance liquid chromatography with diode array detector (HPLC-DAD).

The following emergent contaminants were studied and analyzed by HPLC: CAF (2.54 min; 273 nm), ASA (3.68 min; 230 nm), SA (4.24 min, 230 nm), E1 (6.99 min; 280 nm), KET (8.01 min; 254 nm), NAP (8.39 min; 230 nm), EET (8.94 min; 280 nm) and E2 (10.4 min; 280 nm). HPLC analyses were carried out with an LC chromatographic system 1200 series (Agilent Technologies, USA) equipped with a quaternary pump, a C18 column (250 mm × 4.6 mm × 5 μm) and a diode array detector. The mobile phase consisted of acidified water (pH 3.5) and acetonitrile (1:1, v/v) in isocratic mode in a flow rate of 1.0 mL min^{-1}. The injection volume was 5 μL. Figure 2 shows a chromatogram obtained for a mixture of standard solutions of 1 mg L^{-1} of each compound analyzed by HPLC-DAD under optimized conditions.

The analytes studied by GC-MS/MS were GEM, FEN, 4-MBC and OMC. Separation was performed on an HP-5msi silica capillary column (30 m × 0.25 mm × 0.25 μm) by gas chromatography (model 7890A, Agilent Technologies) coupled with a model 7000 triple quadrupole mass detector equipped with a PAL autosampler. The

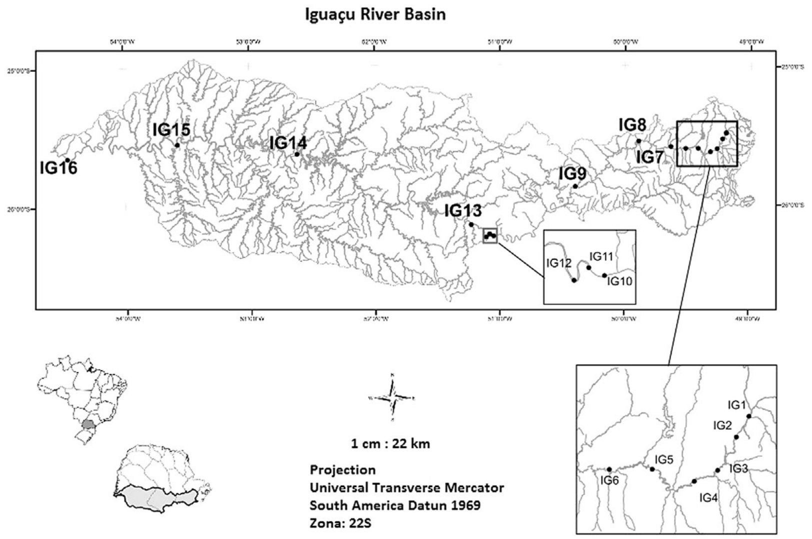

Figure 1. Monitoring sites in the Iguaçu River (IG1 to IG16)

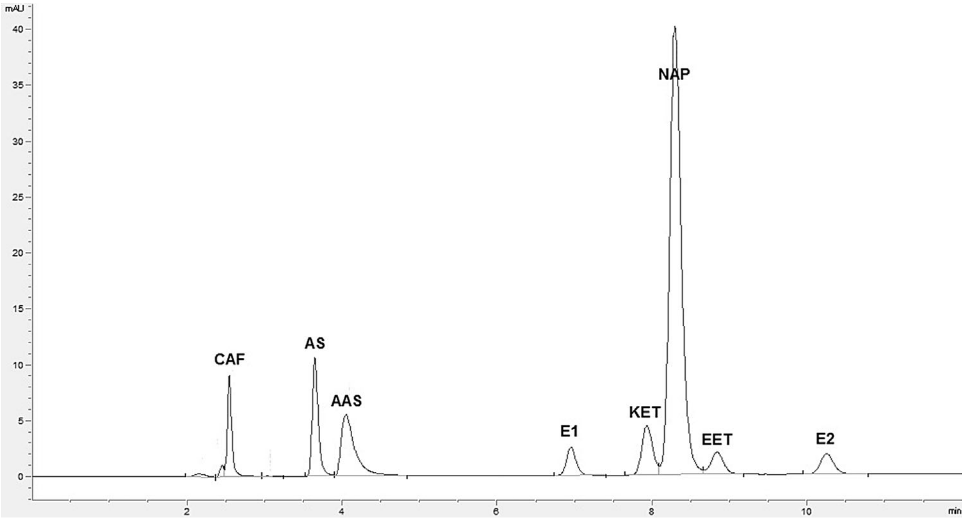

Figure 2. Chromatogram obtained for a mixed standard solution of 1 mg L^{-1} of each compound analyzed by HPLC-DAD under optimized conditions

temperature programme was set to 80°C for 2 min, increased to 280°C at 15°C min^{-1}, and held for 3 min. The injector port and transfer line temperatures were 280°C and the ion source temperature was 230°C. Helium was used as the carrier gas at a constant flow rate of 1 mL min^{-1}. One microliter volume of the sample was injected in splitless mode. The GC-MS/MS analysis was conducted using the electron ionization mode at 70 eV. Optimum conditions for each compound were applied in selected reaction monitoring (SRM) mode. The SRM transitions (*m*/*z*) used for each compound were: $194 \rightarrow 105.1$ for GEM, $273.2 \rightarrow 138.9$ for FEN, $211.1 \rightarrow 169.1$ for 4-MBC and $178 \rightarrow 161.1$ for OMC. Figure 3 shows a chromatogram obtained for a mixture standard solution of 1 mg L^{-1} of each compound analyzed by GC-MS/MS under optimized conditions.

2.3 Chemical and spectrophotometric parameters

The ammoniacal nitrogen (N-NH_3, phenate method), nitrite (N-NO_2, colourimetric method), nitrate (N-NO_3^-, cadmium reduction method), total nitrogen (N-total, persulfate digestion), orthophosphate (P-PO_4^{3-}, ascorbic acid method) and total phosphate (P-total, acid digestion) assays were carried out according to the American Public Health Association, APHA, standard methods (2005). The dissolved organic carbon (DOC) was determined with HiperToc Thermo Scientific equipment, using the high temperature oxidation method. UV-vis absorbance was measured using a Cary 50 spectrometer (Varian), with 1 cm quartz cells and ultrapure water as a blank, in the range of 200 to 700 nm. Fluorescence measurements were performed using a Cary Eclipse (Varian) fluorescence spectrometer. Emission spectra were obtained with excitation at 370 nm. Synchronized spectra were obtained with an excitation wavelength in the range of 268–618 nm in 18 nm intervals. The emission and excitation slits were fixed at 5 nm, the scanning speed was 240 nm min^{-1}, and the xenon lamp voltage was set at 900 V. Ultrapure water was used as both a reference and a blank, in 1 cm quartz cells.

2.4 Statistical analysis

To avoid the effects of scale, all variables were normalized (centred and scaled) based on their mean and standard deviation. To obtain a ranking (top ten major and ten minor), variables were reduced [0–1] using their maximum and minimum extremes.

Different groups were used to perform the statistical analysis: Physical parameters (temperature, pH, dissolved oxygen, electrical conductivity, turbidity, redox potential, salinity, resistivity, and

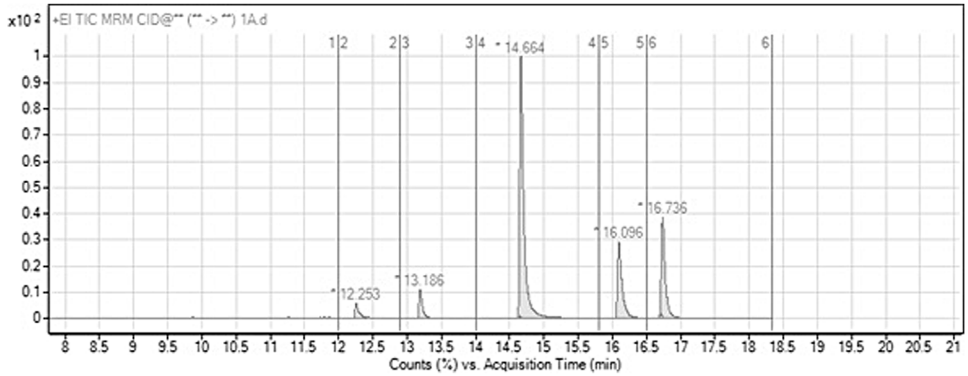

Figure 3. Chromatogram obtained for a mixed standard solution of 1 mg L^{-1} of each compound analyzed by GC-MS/MS under optimized conditions

Table 1. Maximum concentrations and frequency of detection of the emerging contaminants

Compound	Maximum ($\mu g\,L^{-1}$)	Frequency (%)
Caffeine (CAF)	27.0	58
Salicylic acid (SA)	5.17	22
Acetylsalicylic acid (ASA)	0.93	20
Naproxen (NAP)	0.34	34
Ketoprofen (KET)	0.62	18
Estradiol (E1)	1.42	24
Ethinylestradiol (EET)	1.48	7
Estrone (E2)	0.94	2
Fenofibrate (FEN)	0.04	25
Gemfibrozil (GEM)	0.07	40
4-Methylbenzylidene camphor (4-MBC)	0.05	20
Octylmethoxycinnamate (OMC)	0.20	5

total dissolved solids), chemical parameters (ammoniacal nitrogen, nitrite, nitrate, chloride, dissolved organic carbon, orthophosphate, sulphate and sulphide), spectrophotometric parameters (absorbance at 254, 280, and 285 nm, fluorescence emission at 450 nm with excitation at 370 nm, and emission at 298, 468 and 520 nm of the synchronized spectra, with $\Delta = 18$ nm) and emerging contaminants (CAF, ASA, SA, NAP, KET, FEN, GEM, E1, EET, E2, 4-MBC and OMC). Based on these groups, the following statistical analyses were performed:

(1) Separate group analyses;
(2) Globalized statistical analyses using all data separated by group;
(3) Global analyses, involving all groups.

The following analytic procedures were evaluated:

i. Direct correlation analysis to evaluate the interdependencies among the variables;
ii. Hierarchical analysis and ANOVA (Fisher test) for pattern recognition among groups (groups of close similarity);
iii. Principal component analysis (PCA) to represent and summarize the dimensionality of the data and sort results. PCA was performed separately on each group (physical, chemical, spectrophotometric, and emerging contaminants) and globalized on combinations between the groups.

Table 2. Limits of detection (LOD) and limits of quantification (LOQ) for the compounds under study

Compound	LOD ($ng\,L^{-1}$)	LOQ ($ng\,L^{-1}$)
Caffeine (CAF)	8.2	27.4
Salicylic acid (SA)	33.7	112.5
Acetylsalicylic acid (ASA)	36.1	120.4
Naproxen (NAP)	9.5	31.6
Ketoprofen (KET)	5.0	16.6
Estradiol (E1)	25.4	84.8
Ethinylestradiol (EET)	48.2	160.7
Estrone (E2)	26.7	89.0
Fenofibrate (FEN)	0.77	2.6
Gemfibrozil (GEM)	0.92	3.0
4-Methylbenzylidene camphor (4-MBC)	0.33	1.1
Octylmethoxycinnamate (OMC)	2.0	7.0

For each set of values the following strategy was adopted:

(1) Correlations were calculated to study the interdependence of variables and to verify whether there were interdependent parameters. The limit values estimated were of significant correlation ($p\ [r = 1]$) and of zero correlation ($p\ [r = 0]$). To estimate the correlation between two variables, 64 data points were used ($N = 64$), which corresponds to 62 degrees of freedom. Two extreme correlation values were calculated, corresponding to the test value 0.03: in the case of the zero correlation test (H0: corr $= 0$), the value found was 0.271, while for significant correlation (H0: corr $= 1$) it was 0.853;
(2) Degree of contamination of the samples based on the reduced variable [0–1]. A global value was estimated from the general index of human contamination of the sample (GIHC). This global value was sorted to generate pollution rankings – the ten lowest and ten highest, related to the 15 and 85% percentiles;
(3) Recognition of groups by the supervised hierarchical analysis (HCA) based on the single-linkage (SL) direct association, the minimum object distances, and recognition of structures based on the evolution of internal variability. An *F*-test was carried out for evolutionary sequence of internal variability to detect when it was no longer similar ($p\ [H0] < 0.03$); (H0: $s^2\ (n + 1) \leq s^2$ (n); H1: $s^2\ (n + 1) > s^2\ (n)$);
(4) Representation using PCA to display the arrangement obtained in each case and identify the location of extreme contamination values (GIHC).
(5) For cluster analysis and PCA, considering each group separately (physical, chemical, spectrophotometric and emerging contaminants) and the combined groups, the sample positions with higher and lower contaminations were verified, taking into account the GIHC of the other sampled sites. The GIHC was determined by the sum of the normalized emerging contaminant concentrations. These contaminants were considered due to their anthropogenic origin.

3 Results and discussion

3.1 Emerging contaminants

Several authors have reported the occurrence of emergent contaminants in water collected in Brazil [14–16], but there has been no systematic study on the levels of emerging contaminants in Brazilian water sources. To date, no studies have been published assessing the contamination of the Iguaçu River, which presented varying concentrations of CAF, ASA, NAP and other compounds analyzed in this work.

Table 1 shows the maximum concentrations and frequency of detection of the contaminants under study, over all sample sites and different seasonal periods ($N = 64$). The minimum concentrations were below the limits of detection (Tab. 2), mainly in sites downstream of the MRC, where the effects of anthropogenic activity were less pronounced.

The February 2013 sampling was characterized by a long period of rain in the days preceding the sampling and the emerging contaminants were consequently detected at lower levels, probably

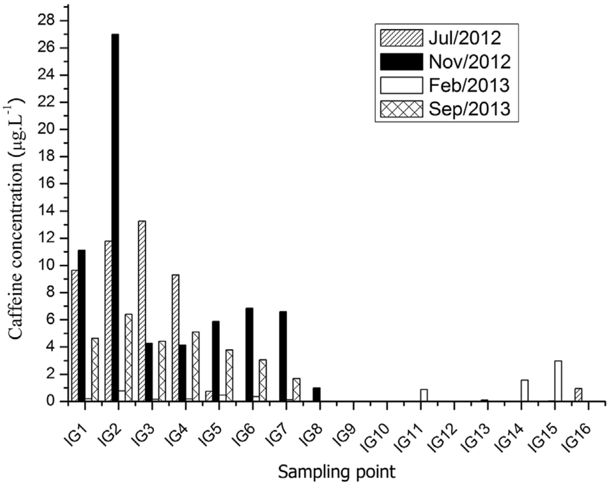

Figure 4. Variation of CAF concentrations along the Iguaçu River (IG1 to IG16) during all samplings (C1 to C4)

due to their dilution. Conversely, during the September 2013 sampling, the Iguaçu River was at lower than normal levels, due to a dry season, contributing to an increase of the contaminant concentrations.

The CAF distribution pattern along the river was also observed for the other emerging contaminants. Concentrations were higher close to the source of the river (IG1 to IG4) due to the large human influence of the MRC. Concentrations decreased along the Iguaçu River, especially from site IG9 and onwards, where concentrations of the compounds were usually below detection limits. Decreasing in the population density, self-purification and effects of dilution along the river caused contamination to decrease farther away from the MRC. Thus, towards its mouth, the Iguaçu River showed good recovery capacity from the high contamination levels caused by the MRC.

Among all the compounds analyzed, CAF had the highest concentration and frequency of detection (Tab. 1, Fig. 4), being observed in both the dry and rainy seasons, especially in the MRC. This is likely due to the widespread consumption of products containing caffeine, such as food, drinks and medicine. Caffeine is commonly used as a human activity indicator in natural environments, because its presence is directly related to the input of untreated domestic sewage. In alignment with this, a strong presence of sewage was observed in the Iguaçu River until the site IG8, located in the municipality of Porto Amazonas.

Table 3. General Index of Human Contamination ranking considering only the extremes of the sampling sites

	Lower contamination			Higher contamination		
RANK	Site	Sampling	GIHC	Site	Sampling	GIHC
1	IG7	C1	0.0	IG2	C4	6.9
2	IG8	C1	0.0	IG2	C2	5.9
3	IG9	C1	0.0	IG1	C4	5.2
4	IG10	C1	0.0	IG3	C4	4.8
5	IG11	C1	0.0	IG2	C1	4.3
6	IG12	C1	0.0	IG5	C4	3.9
7	IG13	C1	0.0	IG6	C4	3.7
8	IG14	C1	0.0	IG1	C1	3.2
9	IG15	C1	0.0	IG4	C4	3.2
10	IG16	C1	0.0	IG1	C2	3.1

Similar data have been obtained in other studies conducted in Brazil. Sodré et al. found CAF in all samples collected in the Atibaia River, with a maximum concentration of 32.4 $\mu g\,L^{-1}$ [17]. Higher concentrations were also detected in the dry season. These values are very high when compared to studies in other countries: Petrie et al., in their study in the UK, found a maximum CAF concentration of 1.72 $\mu g\,L^{-1}$ [18] and Esteban et al., in Spain, reported 5.93 $\mu g\,L^{-1}$ as the highest CAF concentration [19].

Of the pharmaceutical products, ASA was detected in the highest concentrations and GEM and NAP were detected most frequently. Among the female sex hormones, E1 was the most commonly detected, likely because it has two environmental sources as it is produced both naturally and synthetically, while EET is synthetic only and E2 only natural.

These results are in agreement with data obtained by Montaner and Jardim, wherein ASA was also the anti-inflammatory with the highest concentrations (20.9 $\mu g\,L^{-1}$) and E2 the female sex hormone most frequently detected (6.8 $\mu g\,L^{-1}$) [20]. Studies conducted in other countries showed lower concentrations of these compounds: Esteban and collaborators found a maximum concentration of 17.0 $ng\,L^{-1}$ of E2 in Spain and Gavrilescu et al. showed the presence of NAP in the USA at a maximum concentration of 0.14 $ng\,L^{-1}$ [21].

Assuming that emerging contaminants reveal direct human intervention in the environment, these quantification data were used to create the GIHC, calculated from the sum at each site and sampling period. The extremes of the ten least contaminated and the ten most contaminated sites (Tab. 3) were considered in the analysis of the groups studied. The GIHC values showed that in the first sampling, sites close to the mouth of the Iguaçu River, presented the lowest levels of contamination and the sites located in the MRC (IG1 to IG6), over different samplings, had the highest concentrations of several contaminants.

During data analysis, none of the correlations found were very strong, but there were some relatively high correlations, according to the adopted criteria. Positive correlations were observed between KET and FEN ($R = 0.811$, $p < 0.001$), CAF and GEM ($R = 0.785$, $p < 0.001$), E1 and FEN ($R = 0.777$, $p < 0.001$), KET and GEM ($R = 0\ 754$, $p < 0.001$), E2 and 4-MBC ($R = 0\ 744$, $p < 0.001$), ASA and SA ($R = 0\ 729$, $p < 0.001$), KET and CAF ($R = 0\ 712$, $p < 0.001$) and E2 and CAF ($R = 0.710$, $p < 0.001$), confirming that these compounds are probably from the same anthropogenic source, wastewater.

The cluster analysis generated a dendrogram, grouping almost all the objects with only three distinct values. PCA required the first four principal components to capture 83.4% of the original variance, the first component with 48.4%, the second with 16.3%, the third with 10.1% and the fourth with 8.6%.

The samples collected from several sites along the Iguaçu River showed that wastewater treatment plants exert a strong influence on contamination levels. The sites IG1, IG2, and IG3 are located downstream of three important WWTPs and presented, in most samplings, high concentrations of all contaminants studied.

Therefore, the lack of better sanitation conditions in the region directly affects the water quality of the Iguaçu River, an important water body for the entire state of Paraná. In addition to the inefficient treatment used in WWTPs, the presence of irregular habitation on the banks of rivers contributes to the worsening of the problem.

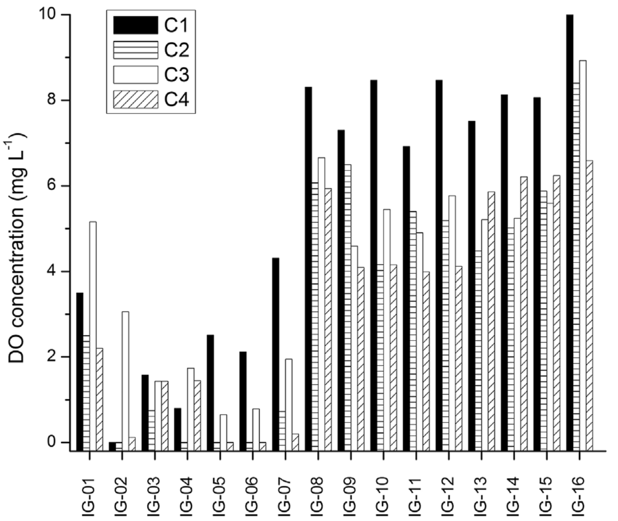

Figure 5. Variation of dissolved oxygen concentration (DO) in the Iguaçu River (IG) along all samplings (C1 to C4)

3.2 Physical parameters

Dissolved oxygen concentrations $<5\,\mathrm{mg\,L^{-1}}$ were observed in different seasonal periods in all sites of the Iguaçu River (Fig. 5) located in the MRC (IG1 to IG7). The decrease of DO concentration in aquatic environments is probably related to the input of labile material from wastewater, which is degraded by the microorganisms that consume DO.

Beyond site IG8 in Porto Amazonas, the DO concentration gets higher because of the increased river flow and lessened influence of urbanization. Another aspect that may be contributing to the increase of the DO concentration is the existence of small rock falls in this region that aid in oxygenation.

Among the physical parameters (DO, pH, salinity, turbidity, redox potential, electrical conductivity, resistivity, total dissolved solids and temperature), a significant correlation was observed only between the electrical conductivity and salinity ($R = 0.945$), which was expected. No other significant correlations were observed, using ($|\mathrm{Corr}| > 0.853$) as the adopted significant correlation value.

The cluster analysis (dendrogram) showed the existence of a very diffuse population of similar samples. This similarity was confirmed via performing an *F*-test of the variability of the internal transitions, which indicated no large variation.

PCA of the physical parameters was used to sort the samples according to the GIHC. 75.1% of the original variance was recovered in the first three principal components −42.0% in the first component, 20.5% in the second component and 12.6% in the third component. PCA revealed the formation of six groups. The places with lower rates of contamination formed two groups (negative axis of PC1) and the most contaminated sites also formed two groups, but on the positive axis of PC1. The physical parameters analyzed were not enough to indicate which environments were more or less contaminated.

3.3 Chemical parameters

Ammoniacal nitrogen and orthophosphate are important parameters to classify natural water and are used in quality index calculations, mainly with respect to their relation to sewage and eutrophication of aquatic environments. The variation of N-NH_3 and P-PO_4^{3-} concentrations along the river is shown in Fig. 6. There was a positive and significant correlation ($R = 0.911$) between the N-ammoniacal and orthophosphate concentrations, indicating that they are probably derived from domestic sewage. There was also a positive correlation between N-nitrite and sulphide ion concentrations ($R = 0.801$), which is related to the anoxia of the medium, as was observed by the low DO concentrations, especially at the source of the Iguaçu River (Fig. 5).

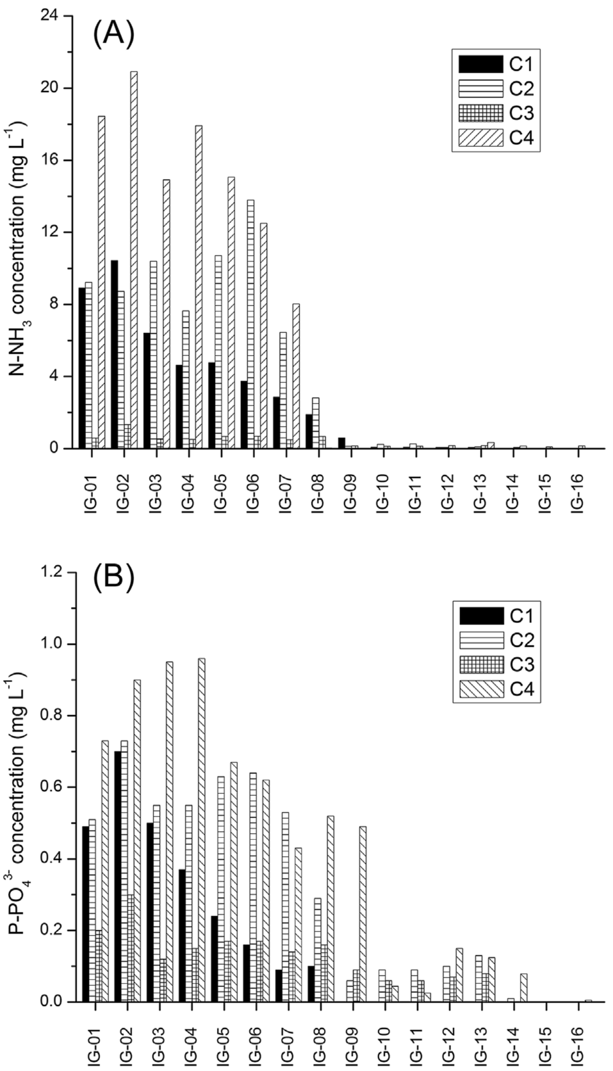

Figure 6. Variation of N-NH_3 and P-PO_4^{3-}concentration in the Iguaçu River (IG) along all samplings (C1 to C4)

The supervised hierarchical cluster analysis (HCA) based on direct single-linkage membership (SL) (dendrogram) yielded six groups, one of which comprised 25 samples. No statistically significant transition (*F*-test) was detected by assessing the evolution of variability.

Two components from PCA contained 70% of the variance, and including the third PC yielded 82.9% of the original variance. The first component contained 40.5% of the data variation and the second contained 29.6%. Seven groups were formed in PCA. The sampling sites with lower GIHC formed two groups (negative PC1 axis) and the ones with higher GIHC formed another two groups, but

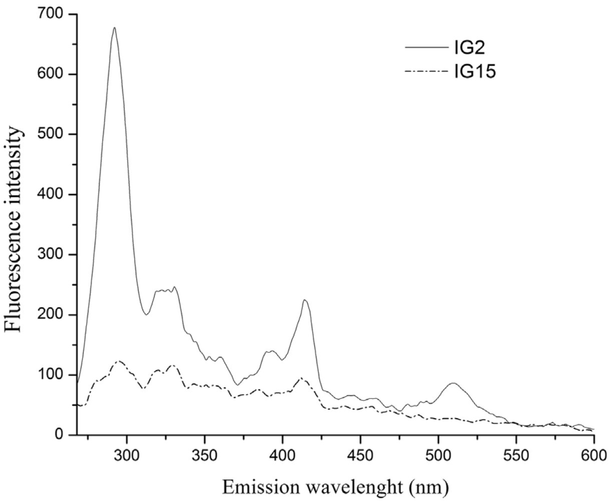

Figure 7. Synchronized spectrum ($\Delta\lambda = 18$ nm) of samples from the Iguaçu River (IG2 and IG15) in the second sampling (C2)

in the positive axis. The first component was related to the ranking of contamination of the sampled environments, but because of the variation of other parameters, this effect was not very evident, mainly because some unpolluted sites presented variations in the nitrate, chloride, ammoniacal nitrogen and orthophosphate concentrations.

3.4 Spectrophotometric parameters

Considering that the presence of ammoniacal nitrogen and orthophosphate at the mouth of the Iguaçu River represents the input of sewage, the levels of dissolved organic carbon were studied via spectrophotometry. Figure 7 shows the fluorescence emission at 298 nm, which characterizes labile organic matter that could originate from domestic sewage [22–25]. Several authors employ fluorescence emission analysis to identify and characterize natural organic matter from different sources or to differentiate the organic material of natural origin from the domestic effluent [22–24, 26].

The correlation studies of this peak (298 nm) with DOC ($R = 0.6766$, $p < 0.001$), P-PO_4^{3-} ($R = 0.5077$, $p < 0.001$), N-NH_3 ($R = 0.5042$, $p < 0.001$) and DO ($R = -0.6444$; $p < 0.001$) corroborate this aspect, showing that in this aquatic environment, this band is likely related to labile organic matter from domestic sewage.

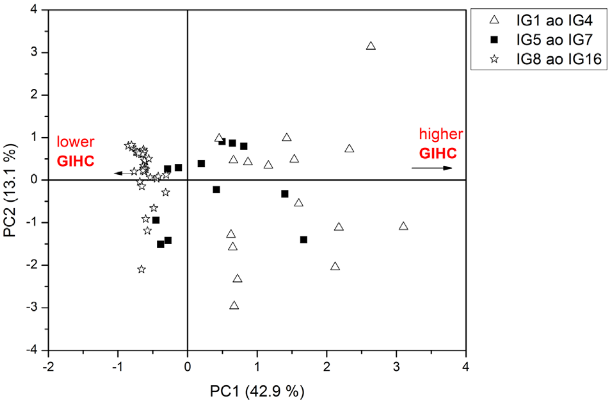

Figure 8. Principal component analysis of chemical parameters and emerging contaminants determined along the Iguaçu River during four sampling periods

The supervised hierarchical analysis based on direct SL presented a dendrogram with a very large group with 54 members, another small group (three members) and four outliers. In this case, the statistical test for the direct comparison of the evolution of internal variability detected two significant transitions.

PCA showed that two components contained 82.9% of the original variance. The first axis contained 53.6% of the variability and the second axis contained 29.2%. Six groups formed, wherein some sites with lower GIHC mixed with sites with higher GICH, showing that spectroscopic analysis alone is insufficient to characterize the environments.

Cluster analysis was used to recognize the formation of possible groups represented through PCA. Cluster analysis and PCA did not verify the similarity or dissimilarity of the sampled sites in terms of pollution, considering only the analysis of in situ, chemical and spectroscopic parameters.

3.5 Global analysis of the data

When using statistical analysis to assess the contaminant concentrations together with the spectrometric analysis, no significant correlations were observed between the contaminant concentrations and spectral data for each sample. In the cluster analysis, using a cut-off of half of the overall diffuse distance, three individual objects and a group of 61 members were observed. During PCA, five components were necessary to recover 80% of the variance. The first component contained 39.3% of the total variance and the second contained 16.7%. The river sites with lower GIHC values were located in the second quadrant. The remaining sites with higher GIHC (most contaminated) and the sampled sites with intermediate contamination were dispersed in the first and third quadrants.

All statistical analyses involving the groups of chemical and spectral parameters also revealed no significant correlations. In the cluster analysis, using the cut-off of half the distance, three groups were formed, one with 43 members, where Fisher's test did not detect any accordance fault in the evolution of the variability. PCA required four components to contain 83.9% of the variance, the first of which had 38.9% of the variance, the second had 27.8% and the third 10.2%. When drawing these PCs, the sites of the river that had lower GIHC were in the second quadrant. The remaining sites with higher GIHC (most contaminated) and the sampled sites with intermediate contamination had scattered data but were concentrated in the first quadrant.

In the statistical analysis of chemical parameters and emerging contaminant concentrations, significant correlations were observed between the concentrations of N-NH_3 and NAP ($R = 0.887$, $p < 0.001$); N-NH_3 and GEM ($R = 0.808$, $p < 0.001$), P-PO_4^{3-} and GEM ($R = 0.836$, $p < 0.001$); P-PO_4^{3-} and NAP ($R = 0.780$, $p < 0.001$) and DO and FEN ($R = -0.728$, $p < 0.001$). Analysing the dendrogram halfway up the distance, a very large group was observed (60 elements) and only four individual objects, where the evolution of the internal variability of groups presented a discontinuity. In the PCA, five components contained 81.0% of the variance, the first of which had 42.9% of the variance and the second had 13.0%. In this PCA (Fig. 8), it was observed that the sites of the river with lower GIHC were situated in the second quadrant. The remaining sites with higher GIHC (most contaminated) and the sampled sites with intermediate contamination were located in the first quadrant.

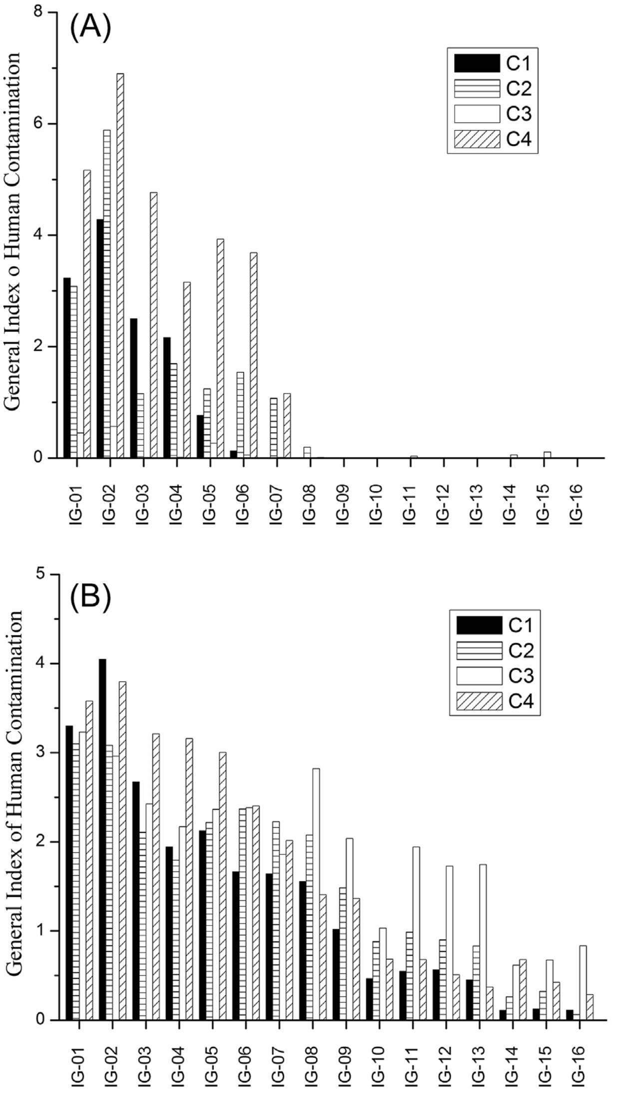

Figure 9. Variation of the GIHC calculated from the emerging contaminants (A) and chemical parameters (B) analyzed from samples collected along the Iguaçu River during four sampling periods (C1, C2, C3 and C4)

To summarize, observing only the results of chemical analysis and emerging contaminants by statistical means, a discontinuity was observed in the physical state of the samples (fine structure), where three components contained 66.4% of the variance. These may be a good prospect for the identification and classification of the sample contamination. The first axis (PC1) indicated the water quality of the Iguaçu River, wherein the most relevant positive information was related to N-NH$_3$, P-PO$_4^{3-}$, environmental anoxia and emerging contaminants (CAF, ASA, NAP, KET, E1, FEN, and GEM), indicating the degradation of the Iguaçu River by domestic sewage from its source until a point close to Porto Amazonas.

PCA of chemical parameters, spectroscopic analysis and emerging contaminants required six components to contain over 80% of the original variance. With only three components it was possible to obtain approximately two-thirds of this information. This approach allowed us to detect cohesive groups (low contaminated samples), highlight anomalies (sparse sampling) and detect contamination levels based on PCA—the most contaminated samples appeared on the positive side of the axis while the less contaminated were in the negative axis. PC1 conveyed information related to the degree of contamination: N-NH$_3$, P-PO$_4^{3-}$, anoxia, the fluorescence intensities emitted at 298 and 520 nm of the synchronized spectra and of emerging contaminants (CAF, ASA, NAP, KET, E1, FEN, and GEM).

PCA involving all groups (physical, chemical, spectrometric and emerging contaminants) indicated that seven components were necessary to contain 80% of the total variance. Using only three components, the variance captured was 61.4%. In summary, in the global analysis it is more difficult to distinguish states of aggregation of the samples.

The GIHC was calculated using the data from the emerging contaminant and chemical parameter groups. The GIHC obtained from the emerging contaminants (Fig. 9A) indicated better recovery of the river, because the pollutants degrade and/or dilute at the sites towards the mouth of the river. The contaminants were only sparingly detected after site IG8. Examining the GIHC from the chemical parameters (Fig. 9B), even with decreasing contaminant concentrations after IG8, some sites still presented relevant values, which may be related to the fact that N-NO$_3^-$, sulphide, N-NO$_2$–, N-NH$_3$ and P-PO$_4^{3-}$exist in different environments and cannot be strictly tied to anthropogenic sources, making the PCA plot more dispersed. In this case, the chemical parameters alone could not be used to conclude whether the environment is affected by domestic sewage input, while the emerging contaminants, due to their anthropogenic origins, contribute to a better characterization of the environment in terms of pollution.

4 Concluding remarks

This work aimed to evaluate the occurrence of emerging contaminants in the Iguaçu River, an important water body in the southern region of Brazil, to better understand a topic not yet addressed in this country. The concentrations of the contaminants studied were comparable to those reported in other studies conducted in Brazil. However, when compared to other countries, the concentrations are uniformly higher in Brazil. It was also observed that wastewater treatment plants greatly influence the contamination levels of the emerging contaminants. The sites IG1, IG2, and IG3, which are located downstream of the discharge of three large WWTPs, presented peak concentrations of all contaminants in most samples. Statistical analysis revealed that conventional analysis of physical, chemical or spectroscopic parameters alone is insufficient to affirm that the contamination in the Iguaçu River is caused by the input of domestic sewage. However, the presence of emerging contaminants in the river indicates the anthropogenic influence and is therefore a more useful analytic method to investigate the presence of domestic sewage in the environment. The GIHC showed a strong influence of anthropogenic activity in the Iguaçu River, especially close to its source, which is the region with higher population density. These results indicate that the lack of basic sanitation, such as sewage collection and more effective treatments, is a serious problem in our country, requiring greater attention.

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