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Occurrence of female sexual hormones in the Iguazu river basin, Curitiba, Paraná State, Brazil

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ABSTRACT. Female sexual hormones have attracted the attention of the scientific community due to the effects that they cause by interfering in the endocrine system. Many contemporary studies have sought to monitor some of the main female sexual hormones in surface waters in Brazil. Current article evaluates the presence of 17β-estradiol, 17α-ethinylestradiol, estrone and progesterone in the surface waters of Curitiba and the surrounding metropolitan area in the state of Paraná, Brazil, by high-performance liquid chromatography (HPLC), performed at 7 different sites. The study revealed that a range of concentrations between 0.07 and 13.45 μg L^{-1} of female sexual hormones was extant; higher values than these were found in other regions of Brazil and in other countries. Higher concentrations have been attributed to the region´s sanitation due to large sewage amounts. Sewage discharge has also been confirmed by results of limnological parameters.

Keywords: contraceptives, water quality, liquid chromatography.

Ocorrência de hormônios sexuais femininos na bacia do Rio Iguaçu, Curitiba, Estado do Paraná, Brasil

RESUMO. A presença de hormônios sexuais femininos é um assunto que tem atraído a atenção da comunidade científica pelos efeitos que eles causam na interferência no sistema endócrino. Muitos estudos atuais têm buscado monitorar alguns dos principais hormônios sexuais femininos em águas superficiais no Brasil. Assim, o objetivo deste artigo é a avaliação da presença de 17β estradiol, 17α-etinilestradiol, estrona e progesterona em águas superficiais da cidade de Curitiba e região metropolitana, no Estado do Paraná, Brasil, utilizando cromatografia líquida de alta eficiência. O estudo revelou uma faixa de concentração, de 0,07 a 13,45 μg L^{-1} de hormônios sexuais femininos, valores superiores a estes foram encontrados em outras regiões do Brasil e outros países. As altas concentrações têm sido atribuídas às condições sanitárias da região estudada, pelas grandes quantidades de esgoto. O descarte de esgoto foi também confirmado pelos resultados de parâmetros limnológicos.

Palavras-chave: contraceptivos, qualidade da água, cromatografia líquida.

Introduction

Growing consumption of contraceptives by the female population worldwide has given rise to a new environmental concern: the contamination of the environment by female sexual hormones (FSHs). Some of these hormones, such as the estrogens oestradiol, oestriol and oestrone, and the progestogen progesterone, are produced naturally by the human and animal organism in small quantities by various endocrinal glands under the command of protein hormones released into the bloodstream (CAI, 2011). Others, such as ethinylestradiol, are produced synthetically (SIAH et al., 2003). When hormones are synthetically produced, they make up most of the oral and injected contraceptives, where they are found in concentrations ranging between 30 and 300 μg per pill (GOLDEFIEN, 1995).

Although the main function of contraceptives is to inhibit ovulation, they are also used to combat menopause symptoms, physiological disorders and in prostate and breast cancer treatments (BOSCO et al., 2004). Both natural and synthetic FSHs are rapidly absorbed by the organism and are metabolized in the liver (CAI, 2011). Once metabolized, they are eliminated daily in the urine and, to a lesser extent, in the feces (BELFROID et al., 1999). Several organisms excrete different amounts of sexual hormones, depending on age, state of health, diet or pregnancy condition. The amount of hormones excreted by a pregnant woman may be up to a thousand times higher than a woman who is not (2 to 20 μg estrone day^{-1}, 3 to 65 μg estriol day^{-1} and 0.3 to 5 μg estradiol day^{-1}), depending on the former´s stage of pregnancy (ELF et al., 2002).

Although their presence has been suspected in the environment for over twenty years, FSHs in surface waters were first reported by Purdom et al. (1994) and Fent and Gies (1996) in England, where fish were being contaminated by estrogens originating from a sewage treatment plant (STP). Even at low concentrations, between μg L^{-1} and ng L^{-1}, FSHs may interfere in the endocrinal glands of animals and human beings, affecting the normal functioning of the endocrinal system and influencing development, growth and reproduction stages (WEN et al., 2006).

Several studies have shown the interference of FSHs in fish, fowl, reptiles and mammals (HAHLBECK et al., 2004; PARROT; BLANT, 2005; TRAINOR et al., 2006). Hahlbeck et al. (2004) reported gonadal sex reversal in a species of wild fish *Gasterosteus aculeatus* following treatment with 17α ethinylestradiol (estrogen). Parrot and Blank (2005) reported the feminization of fish of the *Pimephales promelas* (minnow) species by synthetic estrogens from birth-control pills. Trainor et al. (2006) observed increasing aggressiveness in birds due to an increase in estrogen. According to Takeshi et al. (2003), FSHs cause disorders in the maturing of female sexual gonads in salmon, whereas Fry et al. (2006) reduced anxiety in rats after administering specific doses of progesterone.

Coupled to other causes (smoking, stress, etc.) in humans, the presence of female hormones in drinking water may be related to masculine infertility diseases, such as varicocele (enlargement of the vein in the scrotum), cryptorchidism (undescended testicles), hydrocele (accumulation of liquid in the scrotum), and other deformities in the penis and testicles, low sperm count and cancer (BECK et al., 2005; PONEZI et al., 2007).

Research in the USA, Spain, Germany, Japan, Israel, the Netherlands and other countries has detected considerable concentrations of these compounds in surface waters, especially those close to STP (BELFROID et al., 1999; KUCH; BALLSCHMITER, 2001; BAREL-COHEN et al., 2006).

In addition to the harmful effects of the presence of FSHs in surface waters, there is also the fact that the demand for contraceptives continues to grow all over Brazil as they are one of the most popular alternatives when it comes to family planning (PONEZI et al., 2007). Thus, it is highly important to detect these compounds in water systems and domestic sewage, since their removal or destruction after passing through a STP is inefficient because they are resistant to most sewage treatment processes and are constantly found in surface waters (CARBALLA et al., 2004; YAMAMOTO et al., 2006; WATABE et al., 2004) and even in drinking water (KUCH; BALLSCHMITER, 2001; LOPEZ DE ALDA; BARCELO, 2000).

Knowing where FSHs are finally disposed of in the environment is a fundamental issue when it comes to comprehending the potential of human pollution. Furthermore, these compounds may be related to local contamination levels caused by domestic sewage, be it as a result of treated effluent or discharge without any treatment (CARBALLA et al., 2004; WATABE et al., 2004). Therefore, regions with poor sanitation may be considered the source of FSHs and tend to have limnological parameters of water quality that are indicative of sewage discharges containing these compounds. In this context, current paper determines the level of concentration of the following FSHs: estrone (E1), 17 β estradiol (E2), 17 α ethinylestradiol (EE2) and progesterone (Pg) in the Iguazu River Basin in Curitiba, south Brazil, and relate them to limnological parameters that indicate contamination by domestic sewage discharges.

Area under study

The area under study is located in the Iguazu Basin in Curitiba and the surrounding metropolitan district in the state of Paraná, Brazil, as shown in Figure 1. The source of the Iguazu Basin is near the Serra do Mar (Atlantic Rainforest); the Iguazu, the main river, is approximately 90 km long and stretches to the Curitiba Metropolitan District. The drainage area of this basin is around 3,000 km^2 and the population is about three million people in 14 districts and towns. About a quarter of the population of the state of Paraná lives in this basin, with low sewage treatment rates. Considering the size of the basin, the results in current study are mainly related to the upper part of the Iguazu river Basin which includes the southern region of Curitiba and the Metropolitan District.

Material and methods

In current study, the upper part of the Iguazu Basin, comprising the sub-basins of the rivers Atuba, Palmital, Itaqui, Pequeno and Piraquara, was monitored. This densely populated region has a considerable water supply potential for humans. Figure 1 shows the seven sampling stations monitored on the Iguazu (IG-01 and IG-02), Atuba (AT-01, AT-02 & AT-03), Itaqui (IT-01) and Canal Paralelo (CP-01).

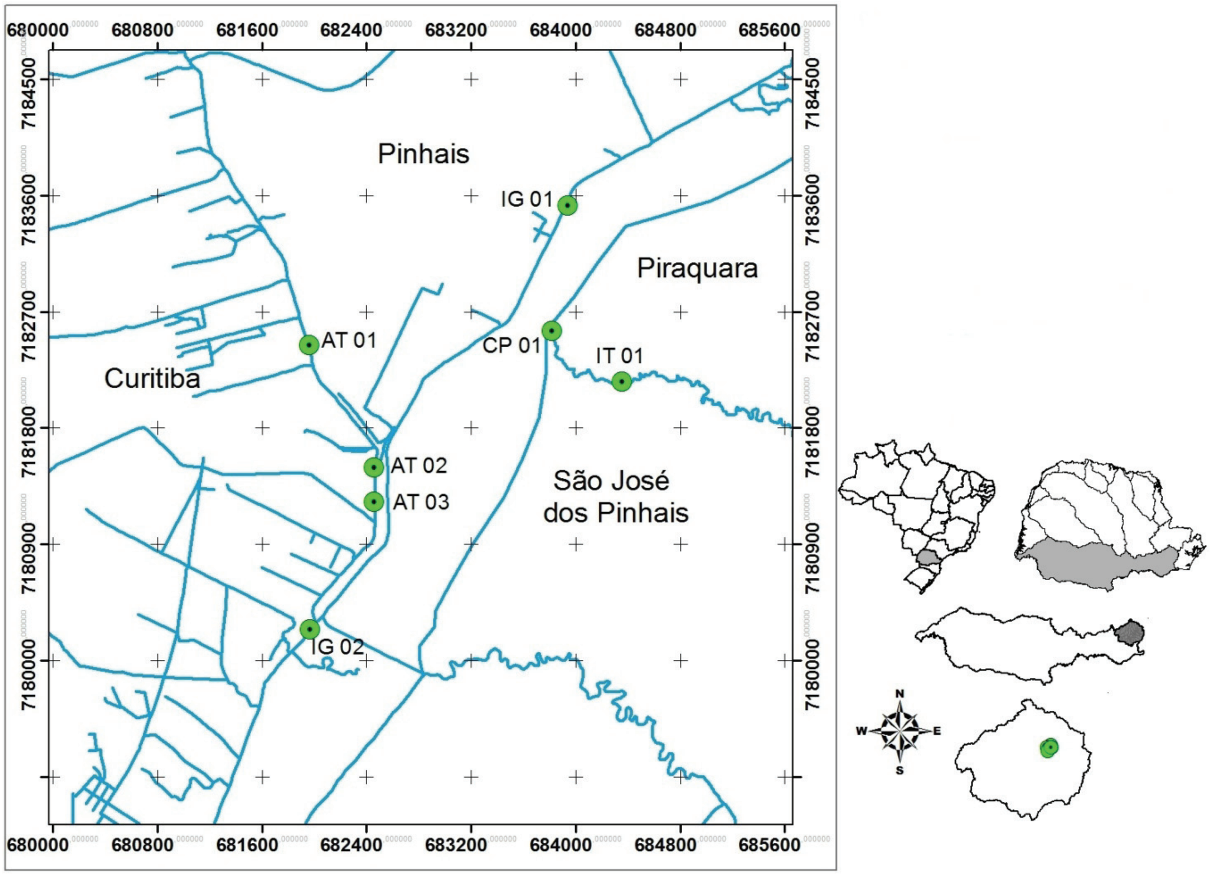

Figure 1. Location of the sampling stations in the Iguazu basin, Curitiba Metropolitan District, Brazil. Source: IAP (2012).

The sites of sampling stations were defined according to the characteristics of the surrounding area so that all pollution levels in the basin could be covered. Stations IG-01 and IG-02 are located on the River Iguazu downstream the confluence with the rivers Palmital and Atuba. The two rivers run through the urban area of Colombo and Curitiba and receive considerable domestic sewage discharges. Sampling stations on the river Atuba were located around the sewage treatment plant. Station AT-01 lay upstream, AT-02 immediately upstream and AT-03 downstream the STP. Water samples in station IT-01 represent the Itaqui river water quality prior to the confluence with the Canal Paralelo; however this river receives upstream domestic sewage discharges from a small STP. The water samples in station CP-01 represent the Canal Paralelo after confluence with the river Itaqui; this channel of the river Iguazu passes through an environmental protection area. To make matter worse, water quality is also affected by unauthorized settlements.

Analysis

Sampling, consisting of five liters of water samples collected with a Van Dorn-type bottle, was carried out between February and October 2009. Samples were kept at 4°C, transported to the laboratory and refrigerated. The samples were preserved for nitrogen analyses by adding 1 mL of concentrated sulfuric acid, as established by APHA (1998). Amber glass bottles were used to store the samples, except for preservation with sulfuric acid when plastic bottles were used. The water samples were collected at a maximum depth of 50 cm. All the reagents used in the limnological analysis were of analytical grade.

HPLC solvents (J. T. Baker®), with over 98% purity, were used to quantify FSHs. Shimadzu HPLC was equipped with a peristaltic pump model LC 20AT, a model DGU-20A degasser and an ultraviolet detector with an SPD M20A model diode-array detector. The injection volume was 20 μL and the chromatography column to separate the FSHs was a 4.66 mm x 15 cm Shimadzu ODS C8.

Due to the influence of estrogens on the anthropogenic activities of the region under analysis, some limnological parameters were studied in order to obtain a scenario of the water quality levels during sample collection and to see whether any correlations existed. Dissolved oxygen concentration, pH, electrical conductivity (μS cm^{-1}), turbidity (NTU) and temperature were determined at each site by portable equipments. Water samples were collected in a Van Dorn-type bottle.

Phosphorous concentrations (total and dissolved orthophosphate) were determined by applying the spectrophotometric method of the reaction with molybdate/ascorbic acid (APHA, 1998). All the forms of nitrogen were analyzed by spectrophotometry in filtered samples (0.45 μm). Ammonia nitrogen concentration was determined using the phenol-nitroprusside method. Nitrite, nitrate (following reduction to nitrate by the Cd column) and total nitrogen (persulfate digestion) were determined by sulfanilamide/N-naftil reagents (APHA, 1998).

The methodology for determining FSHs was based on Lopez de Alda and Barceló (2000). First, one liter of the sample was filtered in a membrane with 0.45 μm porosity and its pH corrected to 3.0 with phosphorous acid. The sample passed through a Stracta C18 solid phase extraction cartridge (1.0 g 6 mL^{-1}) at a flow of 8-10 mL $min.^{-1}$, after conditioning with 5 mL acetonitrile, 5 mL methanol and 5 mL pH 3.0 water. After passing the sample through the cartridge, FSHs were eluted with 10 mL of acetonitrile, reduced under a nitrogen atmosphere and re-dissolved in 1 mL of methanol.

The mobile phase used in the analysis was acetonitrile and water at a ratio of 50:50 for the estrogens and 90:10 for the progesterone, at a flow of 1.4 mL $min.^{-1}$. The wavelength used to detect the estrogens: estrone (E1), 17β estradiol (E2), 17α ethinylestradiol (EE2) was 280 nm, while the progesterone (Pg) was detected at 241 nm. The retention time of the compounds analyzed was 7.56 min. for E2; 8.88 min. for EE2; 10.31 min. for E1; 4.60 min. for Pg.

Results and discussion

Since the main source of FSHs in the environment was related to the discharge of domestic sewage, a strong link between the limnological features of the environment under study and the concentration of these compounds could be surmised. Results of the limnological parameters are demonstrated in Table 1.

In the Iguazu basin, water evaluation resulted by observing the great human influence on the region. High concentrations of ammonia nitrogen were detected (between 0.05 and 33.1 mg L^{-1}) (Table 1), with highest concentrations occurring in the river Atuba, downstream from the outlet of the Atuba Sul STP (AT-03), featuring an average of 24.82 ± 8.12 mg L^{-1} of ammonia nitrogen (Table 1). Total phosphorous varied from 0.08 to 5.47 mg L^{-1}, with the highest concentrations once again in the river Atuba (Table 1), indicating contamination by domestic sewage in the region.

In addition to sewage discharge, there were indications of the influence of surface runoff, which, following rainfall, transports to water bodies considerable amounts of organic material originating from soil, plants and sewage produced in homes without sanitation and remobilized from soil pore spaces. Indications of such contamination are DOC values, which were as high as 91.0 mg L^{-1} in the river Atuba (AT-02), and organic nitrogen, which reached concentrations as high as 160.83 mg L^{-1} (Table 1) in the same region.

In the case of dissolved oxygen, with the exception of the stations on the Itaqui (IT-01) and the Canal Paralelo (CP-01), which reached rates as high as 5.70 mg L^{-1}, the remainder of the stations had low DO rates, with a minimum of 1.25 mg L^{-1} and a maximum of 4.94 mg L^{-1} (Table 1). These data demonstrate the low water quality in the region. Chloride concentrations in all sampling stations ranged between 11.18 and 27.08 mg L^{-1} (Table 1) and confirmed contamination by domestic sewage, with influence on the region´s water quality. Considering sewage discharge as the main source of FSHs in the region under study, as confirmed by the results of the water quality mentioned above, the presence of these compounds is evident. Sampling was carried out in a period which covered all seasons of the year. However, it should be underscored that hydrological variability and discharge variability of sewage effluents were not considered in the analysis of the results. Table 2 shows the concentrations of FSHs found in the five samplings conducted.

Among the hormones under analysis, 17β-estradiol (E2) was the least concentrated and frequent, varying between < 0.10 and 13.45 μg L^{-1} (Table 2). This is one of the main estrogens produced by the human body, with a fundamental role in the menstrual cycle. Furthermore, it is a commonly used estrogen in contraceptives. Since it may be of a natural and synthetic origin, its detection in surface waters is a strong sign of contamination by domestic sewage discharges.

Considering data on excretion provided by Johnson et al. (2000), approximately half a ton of E2 is discharged into the sewage all over Brazil every day, which explains its concentration in certain bodies of water, resulting from human activities.

Table 1. Results of limnological parameters observed in the rivers Iguazu (IG), Atuba (AT) and Itaqui (IT) and in the Canal Paralelo (CP).

	IG-01	IG-02	AT-01	AT-02	AT-03	IT-01	CP-01
N.tot	4,99 ± 3,99	5,17 ± 8,35	19,63 ± 20,83	36,79 ± 69,57	25,43 ± 31,72	1,39 ± 1,07	0,61 ± 0,48
(mg L^{-1})	0.31 - 8.59	1.62 - 20.03	0.43 - 48.47	0.15 - 160.83	3.35 - 80.14	0.40 - 2.90	0.08 - 1.16
NH4-N	3,58 ± 2,20	8,62 ± 6,72	4,48 ± 2,51	16,45 ± 8,55	24,82 ± 8,12	0.32 ± 0.23	0.19 ± 0.12
(mg L^{-1})	0.44 - 5.34	1.18 - 17.85	2.38 - 8.10	5.29 - 23.64	0.05 - 33.10	0.05 - 0.54	0.08 - 0.30
pH	6,91 ± 0.27	6,62 ± 0.79	7,10 ± 0.50	7,18 ± 0.14	7,01 ± 0.14	6,24 ± 1.25	6,64 ± 1.20
(un. pH)	6.58 - 7.17	5.70 - 7.26	6.45 - 7.45	6.93 - 7.36	6.82 - 7.20	4.67 - 7.72	4.83 - 7.52
DO	3.98 ± 1.12	3.70 ± 1.41	4.40 ± 0.56	3,75 ± 1.37	3.85 ± 1.61	5.51 ± 0.31	5.05 ± 5.95
(mg L^{-1})	2.47 - 4.93	1.83 - 4.94	3.75 - 4.94	1.83 - 4.92	1.25 - 4.92	5.05 - 5.70	3.63 - 5.70
P. tot	1,35 ± 0.65	1,94 ± 0.91	1,35 ± 0,38	3.12 ± 1.98	2,70 ± 1.86	0.64 ± 0.51	0.66 ± 0.87
(mg L^{-1})	0.60 - 2.03	0.57 - 2.90	0.98 - 1.86	0.33 - 5.30	0.99 - 5.47	0.31 - 1.90	0.08 - 1.37
Cl. tot	13.65 ± 2.69	16.50 ± 6.78	17,07 ± 6.24	19,59 ± 6.49	20.51 ± 6.14	16.76 ± 6.00	14.53 ± 1.79
(mg L^{-1})	11.68 - 17.56	11.08 - 25.99	11.00 - 24.99	11.08 - 26.49	12.05 - 27.08	11.50 - 25.00	11.88 - 15.75
DOC	8.47 ± 2.68	10.23 ± 3.08	8.28 ± 3.23	37.65 ± 35.57	8.32 ± 1.40	7.48 ± 1.02	8.12 ± 3.84
(mg L^{-1})	5.5 - 10.72	5.93 - 62.16	4.56 - 45.82	16.24 - 91.00	6.58 - 9.61	6.31 - 8.23	4.54 - 12.18

In the case of 17 α-ethinylestradiol (EE2), the second most frequently found hormone, concentrations ranged between < 0.12 and 5.90 μg L^{-1} (Table 2).

Table 2. Concentrations of FSHs (μg L^{-1}) detected in the rivers Iguazu (IG), Atuba (AT) and Itaqui (IT) and in the Canal Paralelo (CP).

Hormone	Collected	IG-01	AT-01	AT-02	AT-03	IG-02	IT-01	CP -01
	02/09	2.09	NA	0.33	13.45	5.92	1.78	1.10
	04/09	NA	4.55	4.33	8.93	5.39	NA	NA
E2	06/09	5.85	5.51	11.13	4.41	6.28	1.97	2.15
	08/09	4.42	4.03	5.83	9.77	3.67	0.17	3.21
	10/09	ND	1.96	ND	2.66	ND	ND	ND
	02/09	ND	NA	0.70	5.90	4.53	0.17	0.67
	04/09	NA	1.26	ND	ND	ND	NA	NA
EET	06/09	2.82	ND	0.98	4.53	0.85	ND	ND
	08/09	2.36	2.28	3.52	2.94	0.63	ND	3.98
	10/09	ND	0.16	ND	0.60	ND	0.16	ND
	02/09	ND	NA	0.83	1.80	ND	0.14	0.65
	04/09	NA	0.34	ND	ND	ND	NA	NA
E1	06/09	ND	ND	ND	ND	0.29	ND	ND
	08/09	ND	ND	0.92	0.25	0.76	ND	1.04
	10/09	ND	0.12	0.20	0.45	ND	ND	ND
	02/09	ND	NA	ND	ND	ND	ND	ND
	04/09	NA	ND	ND	ND	ND	NA	NA
PG	06/09	NA	0.15	0.22	0.12	0.35	ND	ND
	08/09	0.14	0.28	ND	0.45	0.14	0.28	0.31
	10/09	ND	0.35	ND	0.15	ND	ND	0.07

ND: not detectable, NA: not analyzed.

The above data are related to the fact that the compound is a synthetic estrogen used in contraceptives, with only 15% absorption by the human organism, whereas the remaining percentage is eliminated with urine (JOHNSON; WILLIAMS, 2004). In addition to human beings, cattle and swine also excrete natural FSHs. Although the use and occupation of the soil in the region under analysis showed no signs of large quantities of livestock, even if these animals are in the area under study, the presence of E2 confirms contamination by human sewage since it is a synthetic hormone.

On the other hand, estrone (E1) had the lowest concentrations among the estrogens, varying between < 0.10 and 1.80 μg L^{-1}. E1 originates only from the human body, or rather, it derives from an exclusively natural human source and it is twelve times less active than E2. Progesterone (Pg) was the least concentrated of the compounds, varying between < 0.06 and 0.45 μg L^{-1} (Table 2). Since it is a pregnancy-related hormone and released throughout the ovarian cycle, it is during pregnancy that higher concentrations of this hormone are released (GOLDEFIEN, 1995).

Highest rates were found at station AT-03, downstream the Atuba Sul STP, and prove that the sewage plant was not efficient in removing FSHs. It was only at this station that a positive correlation was found between DOC and estradiol (R = 0.9309) and DOC and ethinylestradiol (R = 0.9562). Therefore, the concentration of FSHs in this location may be estimated by DOC concentration. It may also be an indication that when there is high DOC degradation at STP, the hormones are also degraded.

In general, FSHs concentrations in surface waters found in current study were higher than those found in other studies both in Brazil and in other countries, as shown in Table 3.

According to the region´s sanitation data, the percentage of sewage collection in Curitiba is 80.2%, while in neighboring towns, such as Pinhais, Piraquara

Table 3. Concentrations of FSHs found in other studies.

Origin / FSHs (ng L^{-1})	E1	E2	EET	PG	Source
Netherlands	0.3 – 3.4	0.6 – 5.5	0.3 – 4.3	--	Belfroid et al. (1999)
Germany	4.1	3.6	5.1	--	Kuch and Ballschmiter (2001)
Spain	22	< 2.5	< 2.5	--	Mozaz et al. (2004)
Israel	--	--	6.1	--	Barel-Cohen et al. (2006)
Japan	51	--	--	--	Yamamoto et al. (2006)
Brazil-Campinas	1200 - 1700	1900 – 3000	--	--	Ghiselli (2007)
Brazil-Campinas	<16	106 – 6806	501 - 4390	87 - 195	Montagner and Jardim (2011)
Brazil-Curitiba	120 – 1040	170 – 13450	160 - 5900	70 - 450	Current study

and São José dos Pinhais (Figure 1), percentage are 38.4, 45 and 40.5%, respectively. One should also take into account that not all sewage collected in the region was treated and that consumption of antibiotics increased in Brazil from 2007 to 2009. Further, according to Araujo and Costa (2009), the consumption of contraceptives in the country had a 23% yearly increase.

Among the studies analyzed, the highest levels of FSHs were obtained by Montagner and Jardim (2011) in Campinas, São Palo State, Brazil, with 6806 ng L^{-1} of E2 (Table 3). In current assay, even higher concentrations were found, reaching as high as 13450 ng L^{-1} of E2 (Table 2), precisely at a location directly affected by the outlet of the aerobic sewage plant (AT-03). The other stations also showed hormone concentrations, although their rates were lower than those at AT-03. The above data may be linked to the contamination level of the station under study, especially on the outskirts of Curitiba.

In accordance with FSHs results and the limnological parameters obtained, it was observed that the location of the sampling stations has a strong influence on the spatial variability of FSHs. On the river Iguazu, at station IG-01, it is likely that part of the pollution is due to its proximity to the confluence with the Palmital river, which is used for the discharge of a large amount of untreated domestic sewage from the town of Colombo. The main characteristic of station IG-02, also on the river Iguazu, is that it is connected to all the sampled stations. Located downstream from the area under study, it comprises a section of the Iguazu Basin, including Curitiba, Pinhais, Piraquara and São Jose dos Pinhais (Figure 1). Stations AT-02 and AT-03 in the river Atuba are directly reached by the Atuba Sul STP, and AT-03 is downstream from the plant's outlet. These features of the sampling stations are part of a scenario of human influence that corroborates the presence of high concentrations of FSHs. In the case of river Itaqui and Canal Paralelo, stations IT-01 and CP-01 have the lowest contamination levels, but the highest concentration levels of ammonia nitrogen, organic nitrogen, total phosphorus, chloride and DOC were found in the river Itaqui over the Canal Paralelo. In this case, results show that the Canal Paralelo, also known as the Canal Extravasor, is affected by contamination that stems from the river Itaqui, as the sampling station is located after the confluence with the Canal Paralelo.

Conclusion

FSHs contamination levels were evaluated. The river with the highest contamination was river Atuba river, followed by Iguazu , Itaqui and Canal Paralelo, with concentrations up to 1.98 times higher than other places in Brazil (MONTAGNER; JARDIM, 2011), and other countries (Japan, Israel and Spain), due to low sanitation rates, inefficiency to remove FSHs by sewage treatment and yearly increase in Brazilian contraceptive consumption. E2 was the FSHs present in most samples and in highest concentrations, due to human excretion and high contraceptives usage.

Current assay contributes towards a better understanding of FSHs in Brazilian surface water and their main sources.

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