Estrogenic Hormones in São Paulo Waters (Brazil) and Their Relationship with Environmental Variables and *Sinapis alba* Phytotoxicity

Lucia Helena Gomes Coelho ⬩ Tatiane Araújo de Jesus ⬩ Marcio Yukihiro Kohatsu ⬩
Geovana Tognella Poccia ⬩ Vitor Chicarolli ⬩ Karin Helwig ⬩ Colin Hunter ⬩
Joanne Roberts ⬩ Paul Teedon ⬩ Ole Pahl

Abstract The present study evaluated the relationship between estrogenic hormone concentrations (17α-ethinylestradiol and 17β-estradiol) in surface waters in the Metropolitan Area of São Paulo (Brazil) and environmental variables. Four sampling stations were monitored ranging from a protected area to streams discharging human effluent in and around Billings Reservoir. Four sampling campaigns were carried out in each seasonal period: dry and wet. Samples for hormone analysis (in ng L^{-1}) were concentrated ($1000\times$) using solid-phase extraction C_{18} cartridges and analyzed by liquid chromatography coupled to quadrupole mass spectrometry detection, with 100 ng L^{-1} limit of quantification. Water temperature, pH, electrical conductivity (EC), and total dissolved solids were determined *in situ*; total phosphorus and *Sinapis alba* bioassays were performed subsequently. Reservoir active capacity (AC) and precipitation were also obtained. Estrogenic hormone concentrations were always below limit of quantification at pristine site; at the other sampling stations, 17β-estradiol concentrations varied from below limit of quantification to 1720 ng L^{-1} and 17α-ethinylestradiol from below limit of quantification to 1200 ng L^{-1}, with the highest concentrations found in the streams discharging into the reservoir. These streams showed higher Pearson's correlation between 17α-ethinylestradiol, total phosphorus, and electrical conductivity when compared with reservoir stations. Ger-

L. H. G. Coelho (✉) ⬩ T. A. de Jesus ⬩ M. Y. Kohatsu ⬩
G. T. Poccia ⬩ V. Chicarolli
Federal University of ABC, Avenida dos Estados, 5001, Santo
Andre, SP 09210-580, Brazil
e-mail: lucia.coelho@ufabc.edu.br

T. A. de Jesus
e-mail: tatiane.jesus@ufabc.edu.br

M. Y. Kohatsu
e-mail: mykohatsu@gmail.com

G. T. Poccia
e-mail: geovana.poccia@gmail.com

V. Chicarolli
e-mail: vitor_chica@hotmail.com

K. Helwig ⬩ C. Hunter ⬩ J. Roberts ⬩ P. Teedon ⬩ O. Pahl
Glasgow Caledonian University, Cowcaddens Road, Glasgow G4
0BA, UK

K. Helwig
e-mail: Karin.Helwig@gcu.ac.uk

C. Hunter
e-mail: Colin.Hunter@gcu.ac.uk

J. Roberts
e-mail: Joanne.Roberts@gcu.ac.uk

P. Teedon
e-mail: Paul.Teedon@gcu.ac.uk

O. Pahl
e-mail: Ole.Pahl@gcu.ac.uk

mination index and EC presented negative correlation (Pearson's $r = -0.61$), denoting a phytotoxicity increase with EC increment. AC influenced the dilution of pollutants and showed negative correlations with total phosphorus (Pearson's $r = -0.56$). These results highlight the relevance of including streams in water-monitoring programs, since they are important pollutants loads into watersheds.

Keywords 17α-Ethinylestradiol · 17β-Estradiol · Electrical conductivity · Endocrine disruptors · Stream monitoring · Water pollution

1 Introduction

In the last decades, an emerging issue in the area of environmental management is the contamination of the water sources with micropollutants (or so-called pollutants of emerging concern), defined as organic and inorganic compounds that, even at low concentrations (in the order of $\mu g\ L^{-1}$ and $ng\ L^{-1}$), present ecotoxicological risks. These contaminants comprehend a wide range of natural or synthetic chemical compounds, including pharmaceuticals, personal care products (PCPs), hormones, surfactants, flame retardants, pesticides and nanoparticles (Barceló and Petrovic 2008; Quadra et al. 2017). Some chemicals such as phthalates and polychlorinated biphenyls (PCBs) have industrial effluents as their main source of discharge, while pharmaceuticals and personal hygiene products commonly originate from domestic effluents (Ebele et al. 2017).

Among these micropollutants, the group known as endocrine disrupters (EDs) includes exogenous substances that have the capacity to alter organisms' endocrine functions, thus causing adverse effects on human health (if ingested) and the aquatic ecosystem (Johns et al. 2011; Rani and Karthikeyan 2016). Although the concentration of these contaminants in the environment is typically low (ng range), adverse effects due to chronic exposure cannot be excluded (Adeel et al. 2017).

Estrogenic hormones are considered responsible for the majority of endocrine effects triggered by the disposal of effluents contaminated with these compounds, since they are very active biologically and are related to the etiology of various types of cancers (Aris et al. 2014; Adeel et al. 2017). Estrogens introduced into the environment may be natural, such as 17β-estradiol (E2), estriol, estrone, or synthetic, such as 17α-ethinylestradiol (EE2) and levonorgestrel, developed for use in hormone replacement therapies and contraceptive methods (Reis-Filho et al. 2006; Pereira et al. 2015). These contaminants may occur as parent compounds or in a partially metabolized form. In general, 50–80% of the total parent compounds are excreted in the urine and partly in the animal feces as a mixture of metabolized conjugated compounds (Lienert et al. 2007). Women who do not use contraceptives excrete between 10 and 100 μg of estrogenic hormones daily, whereas women who are pregnant may excrete up to 30 mg of estrogens per day (Baronti et al. 2000).

Once released into water bodies, compound's physicochemical properties govern its partition between the water, sediment, and biomass matrices in an ecosystem. Compounds with low water solubility and high organic phase partition coefficient (k_{ow}) are generally more present in the adipose tissue of organisms, promoting bioaccumulation along the trophic levels. For example, estrogenic hormones have log k_{ow} values ranging from 2.8 to 4.1, denoting that they are lipophilic (affinity for lipids), poorly soluble in water and more likely to be present in higher concentrations in the biomass, the organic matter that makes up the sediment, or adhered to suspended solids present in the liquid phase of a waterbody (Ebele et al. 2017). However, when excreted by mammals, estrogenic hormones are not in their parental form, but conjugated (i.e., glucuronic acid or sulfate conjugates) which makes them 10 to 50 times more soluble in water (Birkett and Lester 2002; Ebele et al. 2017).

In the European Union (EU), these substances are currently on a watch list in order to gather monitoring data for the purpose of facilitating the determination of appropriate measures to address the risk posed by those substances (Directive 2013/39/EU n.d.). The United States Environmental Protection Agency (USEPA) included these substances in their Third Unregulated Contaminant Monitoring Rule (USEPA 2012). In Brazil, there are still no national regulations that define concentration limits for these compounds in environmental matrices (Padhye and Tezel 2013).

Electrical conductivity (EC) is a normalized measure of the ability of water to conduct an electric current and is directly related to the concentration of dissolved salts in water (Hem 2012). According to Su et al. (2017), the monitoring of in-stream EC is a feasible alternative to multi-sampling of hydrogeochemical parameters. EC has been widely investigated as a marker of pollution from wastewater discharges (Chalupová et al. 2012; Thompson et al. 2012). EC measurements are therefore

useful as a screening tool of pollution levels, indicating loads of anthropogenic contribution. Bonvin et al. (2011) found a strong correlation between EC and concentrations of wastewater-derived micropollutants in Lake Geneva. Total dissolved solids (TDSs) measure the combination between all inorganic and organic dissolved substances contained in the water and directly correlate with EC values.

Ecotoxicological bioassays for water quality assessment are an environmental monitoring tool that relate the concentration of xenobiotics to a response in the test organisms (Magalhães and Ferrão-Filho 2008; Silva et al. 2015). Belo (2011) recommends that ecotoxicological bioassays with seeds should be used in an integrated way with other chemical parameters for a better understanding. As an example, Dash (2012) verified the toxicity level of raw sewage samples from Bhubaneswar, India, using rice seeds. The germination index (GI) of rice seeds was 70% after 3-day incubation, which could be considered as moderately phytotoxic.

Seasonality may affect the water bodies' quality through greater dilution in rainy periods (Girardi et al. 2016; Ling et al. 2017). However, Gomes et al. (2019) found inferior water quality in wet season, elucidating impact of catchment laden pollutant runoff. This was in contrast to the common local perception that rainy season would flush out pollutants.

This study is part of the project Water Environmental Micropollutant Scientific Initiative (WEMSI), a collaborative partnership between Brazil and Scotland. The work objectives were to (i) evaluate the concentrations of selected estrogenic hormones in surface waters of Rio Grande, a Billings Reservoir branch, an important drinking water source for Metropolitan Area of São Paulo (MASP) and in streams which inflows this waterbody; (ii) examine correlations between the concentrations of these hormones and environmental variables, as well as with *Sinapis alba* bioassays; (iii) evaluate the influence of seasonality and reservoir active capacity (AC) in pollutant dilution; and (iv) evaluate the relevance of stream monitoring as source of pollutants to the reservoir.

2 Material and Methods

2.1 Study Area

Billings Reservoir (Fig. 1), located in the Tietê River Basin (Cardoso-Silva et al. 2014), is the largest reservoir in MASP with a surface area of 127 million m^2 and a maximum depth of 19 m. One of the Billings Reservoir branches, the Rio Grande, the focus of the present study, is used to supply water to 1.2 million inhabitants of the Greater São Paulo ABC Region (Cardoso-Silva et al. 2014). The waters of Rio Grande branch are also valuable for amateur fishing, recreation (swimming and boating), landscape esthetics, and agriculture. Unfortunately, this branch also receives raw and treated sewage effluents from legal and illegal surrounding settlements. Preliminary EC monitoring along Rio Grande branch allowed the selection of four sampling stations reported in Table 1.
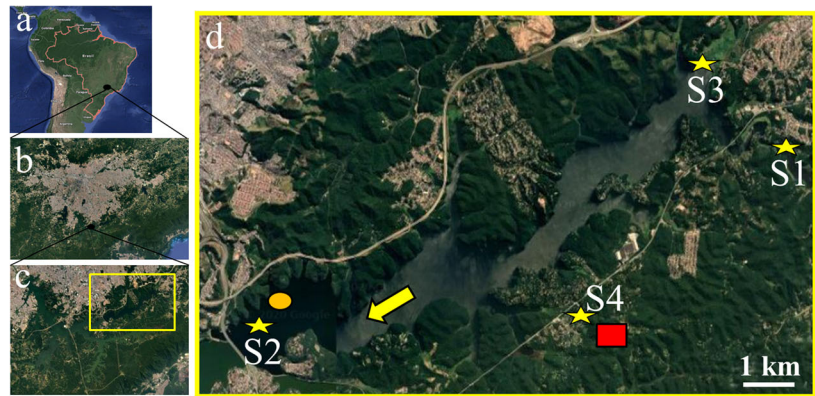
2.2 Sampling

The climate in MASP is typically dry in winter and wet in summer (Lima and Rueda 2018). Thus, to evaluate the influence of seasonality in pollutants dilution, eight sampling campaigns were undertaken from June 2017 to February 2018. The dry period (April to August) was identified with "D" and wet period (September to March) was identified with "W." The dry period sampling campaigns were conducted: DI on June 13, 2017, DII on June 19, 2017, DIII on July 28, 2017, and DIV on August 23, 2017. The wet period sampling campaigns were conducted: WI on October 27, 2017, WII on November 10, 2017, WIII on December 7, 2017, and WIV on February 22, 2017. Rainfall days were avoided.

Subsurface water samples were collected in triplicate ($n = 3$) using a Van Dorn bottle and stored in 5-L capacity glass bottles previously cleaned with 1 mol L^{-1} HCl (10% v/v) and rinsed with deionized water. Field blank containing free water poured into the container in the field was also preserved and shipped to the laboratory. All samples were refrigerated during transportation to the Laboratory of Environmental Analyses of Federal University of ABC for estrogenic hormones analysis, total phosphorus, and *Sinapis alba* bioassays.

2.3 Reagents

Analytical standards (EE2 and E2) with purity levels greater than 98% obtained from Sigma Aldrich were used for the chromatographic analyses. Organic solvents of high-performance liquid chromatography (HPLC) grade with purity > 99.9% (JT Baker) were used for extraction and elution and American Chemical Society

Fig. 1 Study area location: **a** São Paulo state in Brazil, **b** metropolitan area of São Paulo and Billings Reservoir, **c** and **d** sampling stations on the Rio Grande branch. Faltou:Include information. Source: Adapted from Google Maps, 2020. Pictures from authors, 2018

reagent grades (Sigma-Aldrich) for total phosphorus analysis and phytotoxicity bioassays.

2.4 Estrogenic Hormone Analysis

Analytical determination of estrogenic hormones was based in EPA Method 539 (USEPA 2010). The glassware used during the analysis was washed with Extran detergent in tap water (8% v/v) and then rinsed with deionized water. The concentration of the estrogenic hormones (E2 and EE2) in water samples was performed using solid-phase extraction (SPE) as described

by Machado et al. (2014). Briefly, the collected samples were filtered through a cellulose acetate membrane (0.45-μm porosity) to remove particulate material, and the pH adjusted to 3.0. SPE cartridges (C_{18}, 500 mg, 6 mL, Strata Phenomenex) was conditioned with solvents of increasing polarity (methanol and ultra-pure water) at a flow rate of 5 mL min^{-1}.

After conditioning, 1-L of filtered aqueous sample was passed through the cartridge to extract the target compounds. The cartridges were stored in a refrigerator prior to liquid chromatography-mass spectrometry (LC-MS) analysis where the retained compounds

Table 1 Sampling station coordinates and description (IBGE 2010; Trata Brasil 2018)

Sampling station	Coordinates	Description
S1	− 23, 7150 S − 46, 4294 W	A pristine area of the Rio Grande branch considered as reference at present study. There is no direct disposal of effluents, and it is used for bathing and amateur fishing by nearby resident. Average depth is 10 m. Presence of native vegetation and aquatic macrophytes in some periods.
S2	− 23, 7511 S − 46, 4694 W	Water abstraction point for drinking water supply located in São Bernardo do Campo municipality. Densely occupied area with precarious urbanization. Application of copper-based algaecides to avoid algal bloom.
S3	− 23, 7714 S − 46, 4750 W	Ribeirão Pires stream that receives raw sewage from the municipality of Ribeirão Pires with a population of around 122,000 inhabitants of which only approximately 14% is connected to a sewer. This stream has about 10-m width and 3-m depth and enters the Rio Grande branch from the northeast. The sampling spot was on a bridge that crosses the stream next to a residential neighborhood that dumps sewage directly into the water body. Presence of solid waste at the borders.
S4	− 23, 7692 S − 46, 5322 W	Tubarão stream that receives treated effluent from Parque Andreense wastewater treatment plant (WWTP). This WWTP uses activated sludge technology and treats around 80% of the wastewater from the Parque Andreense neighborhood in Santo André, SP, amounting to a population of around 5000 inhabitants. WWTP do not include tertiary treatment step, thus disposing high amounts of phosphorus into the water body. The sampling spot was in a water column of about 20-cm depth close to a community with 20 residences that directly dispose wastewater on the stream.

were eluted with acetonitrile. The solvent was evaporated in an inert atmosphere at 40 °C and residual content suspended in 1 mL of methanol (achieving a 1000× concentration factor).

To assure and control data quality, a QA/QC protocol was planned and systematically implemented along the analytical processes. For SPE blanks were used to assess potential contamination as well as spikes of hormone standards with two-level concentrations used to obtain recovery and accuracy. All experiments were conducted in duplicates to determine analytical precision.

The concentrations of estrogenic hormones were determined using liquid chromatography (Agilent 1200 Infinity, USA) coupled to a triple quadrupole type mass spectrometer (Agilent 6130, USA).

The simultaneous determination of EE2 and E2 by LC-MS was performed utilizing a C_{18} column (Zorbax Eclipse Plus, 100 mm × 4.6 mm × 3.5 μm, Agilent, USA) at 40 °C with a mobile phase flow of 0.3 mL min^{-1}. The mobile phases consisted of 0.02% NH_4OH in water and 0.02% NH_4OH in methanol under gradient elution (USEPA 2010). The injection volume was 20 μL and detection wavelength at $\lambda = 281$ nm. After the chromatographic separation, the mobile phase containing the analytes was subjected to the electrospray ionization stage in the mass spectrometer. The ionization source was operated in the negative mode at a voltage of 4000 V. Dry nitrogen gas was used as the carrier at a flow rate of 10 L min^{-1} and a temperature of 350 °C.

Estrogenic hormones were identified and quantified using an external calibration method over a concentration range of 100 to 5000 μg L^{-1}. Mass spectra quantification and confirmation were acquired in multiple reaction monitoring (MRM) mode using the m/z transitions 295 > 145 for EE2 and 271 > 159 for E2.

The limits of detection (LOD) and limits of quantification (LOQ) were determined by injecting progressively lower concentrations of the standard solutions of E2 and EE2 under the chromatographic condition described above. LOD and LOQ were calculated directly from the calibration plot, considering LOD and LOQ as 3 and 10 ρ/S, respectively, where ρ is the standard deviation (SD) of intercept and S is the slope of the calibration curve. For repeatability evaluation, 10 replicate of both compounds were analyzed at three concentration levels (100, 500, and 1000 μg L^{-1}).

LC-MS linear calibration curves resulted in Pearson $r = 0.999$ for E2 and 0.997 for EE2. For both compounds, the LOD and LOQ were 30 μg L^{-1} and 100 μg L^{-1}, respectively. Considering the concentration factor of 1000, the LC-MS method enabled quantification of 100 ng L^{-1} of each hormone in water samples. The LC-MS analysis of the 10 replicates at the three selected concentrations showed accuracies between 90 and 97%, demonstrating good repeatability of the chromatographic method.

The mean recovery for E2 using the Strata SPE cartridges was 89.3 ± 4.1%, and for EE2, it was 75.7 ±

4.5%. EPA method 539 (USEPA 2010) outlined the acceptance recovery efficiencies for steroid hormones in water samples. The acceptance criteria are generous, for example, for analysis of E2, the EPA acceptance criteria ranged from 55 to 108% with relative standard deviations (RSDs) of 30% and from 55 to 110% with a RSD of 30% for EE2. Results indicate little sample matrix effect on the extraction procedure and the non-necessity of further sample clean-up steps which would be more laborious, expensive, and time-consuming, besides being an extra source of sample contamination.

2.5 Environmental Variables

Daily precipitation and reservoir active capacity data were obtained from Sabesp (2020). Water temperature, pH, EC, and TDS were evaluated in the field using a pre-calibrated multiparameter probe (Horiba U50, Japan). Total phosphorus (TP) analysis was conducted at UFABC, with all glassware previously decontaminated with 10% (v/v) HCl and rinsed with deionized water. Analytical blanks and calibration curve was performed (0.01 - 1.0 mg L^{-1}). Water samples were digested in an autoclave using oxidation reagents and the concentrations determined spectrophotometrically (Hach DR 5000, USA) in the visible ultraviolet region (APHA 2012).

2.6 Sinapis alba Bioassays

Sinapis alba bioassays were performed to assess the phytotoxicity of water samples and followed an adapted methodology based on Belo (2011), ISO 11269-1 2012, Vieira (2016) and Kohatsu et al. (2018). The experiment consisted of placing a filter paper in a 90-mm-diameter glass petri dish and moistening with sample (3 mL) to be tested. Mustard seeds were placed centrally and uniformly (in-line) on the wetted filter paper in each petri dish. Subsequently, the petri dishes were capped and wrapped in plastic film to avoid evaporative loss of the sample. The petri dishes were placed in an upright position with no exposure to light and at (21 ± 2) °C for 3 days. International Standard Organization (ISO) water (ISO 11269-1 2012) was used as positive control to determine normal root lengths under optimum conditions and evaluate the seeds quality. The experiments were performed in triplicate.

After the 3-day period, germination was determined and root length measured with a digital caliper (Mtx,

150 mm, 0.01-mm resolution). Any potential toxic effect was assessed as relative percentage of seed germination (%RSG), relative percentage of root growth (%RRG), and the germination index (GI).

To calculate the %RSG for each plate, Eq. 1 was used, where Sg_s is the arithmetic mean of the number of germinated seeds in the sample and Sg_c is the arithmetic mean of the number of germinated seeds in the control.

$$\%RSG = \frac{\bar{\bar{S}}g_s}{\bar{\bar{S}}g_c} \times 100\% \tag{1}$$

The relative percentage of root growth (%RRG) for each plate was estimated by Eq. 2, where $\bar{\bar{L}}r_s$ is the arithmetic mean of the root length in the sample and $\bar{\bar{L}}r_c$ is the arithmetic mean of the root length in the control.

$$\%RRG = \frac{\bar{\bar{L}}r_s}{\bar{\bar{L}}r_c} \times 100\% \tag{2}$$

After determining %RSG and %RRG, the GI was determined by Eq. 3:

$$GI = \frac{\%RSG \times \%RRG}{100} \tag{3}$$

The phytotoxicity of the samples was rated according to Belo (2011): GI > 100%—material enhances germination and root growth of seeds; 80 > GI < 100%—no phytotoxic, mature compound; 60 > GI < 80%—moderately phytotoxic; 30 > GI < 60%—phytotoxic; GI < 30%—strongly phytotoxic.

2.7 Statistical Analysis

Analyses of Pearson product moment correlation coefficients were used to determine significant associations between limnological parameters (EC, pH, TDS, and TP), hormone concentrations, and phytotoxicity results. Strong correlation for Pearson coefficient value lies between ± 0.50 and ± 1, for moderate degree between ± 0.30 and ± 0.49, and low degree below ± 0.29 (Statstutor 2020). For analysis of variance (ANOVA), a p value < 0.05 was considered for a significant statistical difference between the evaluated groups. The Tukey's test results were shown by letters above boxplot representations. Levels that are not significantly different from each other are represented with the same letter (e.g., a, b, or c). Pearson correlation analyses, ANOVA

statistics, and Tukey's tests were done using Microcal Origin 8.1 software.

Principal component analysis (PCA) was done to facilitate interpretation of multivariate results and was performed using PAST 3.20 software (Hammer et al. 2001). Limnological data were treated by PCA to identify possible temporal or spatial patterns among the sampling stations. Factor loadings > 0.7 are typically regarded as excellent and < 0.3 very poor. In this study, all principal factors extracted from the variables were retained with eigenvalues > 1.0 (Yongming et al. 2006).

3 Results

3.1 Estrogenic Hormone Concentration

E2 and EE2 levels during the eight sampling campaigns are shown in Table 2. Concentrations of both compounds at station S1 (reference) were below LOQ (100 ng L^{-1}) in all the sampling campaigns. Station S3 (Ribeirão Pires stream) measurable E2 was found in all campaigns (110–1700 ng L^{-1}) and EE2 in six (210–

1200 ng L^{-1}). The S4 sampling station, Tubarão stream, yielded E2 in five campaigns (300–1720 ng L^{-1}) and EE2 in seven (120–650 ng L^{-1}).
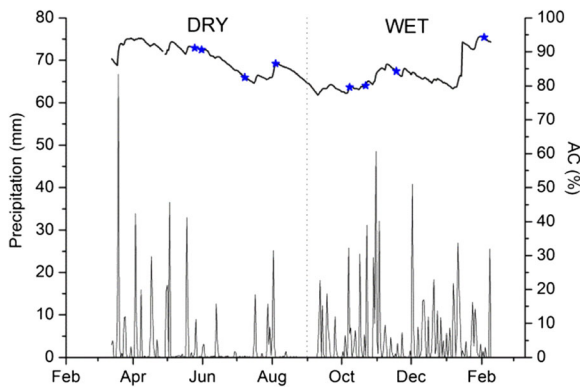
3.2 Environmental Variable Monitoring

Precipitation data (mm) within the studied area, together with the Rio Grande active capacity (AC, %) and sampling campaign dates, are presented in Fig. 2. Total rainfall during the study period was 1157 mm, with 39% in dry (April–August) and 61% in wet (September–March) periods, respectively. The AC (%) showed a different pattern, with a greater capacity (> 70%) at sampling DI, DII, and WIV. Whereas sampling campaigns WI, WII, and DIII occurred when the reservoir was operating at around 65% capacity. This indicates that environmental dilution during the dry sampling campaigns could be typically greater than that in the wet sampling campaigns.

Figure 3 shows environmental variable boxplots and ANOVA results with Tukey's test. Water temperature was higher in the wet than in the dry season, but no statistical difference was observed seasonally or

Table 2 Estrogenic hormone concentrations and standard deviation (ng L^{-1}) in surface waters of Metropolitan Area of São Paulo, MASP, Brazil (n = 3)

Sampling stations	Dry period			Wet period		
	Sampling campaigns	E2 (ng L^{-1})	EE2 (ng L^{-1})	Sampling campaigns	E2 (ng L^{-1})	EE2 (ng L^{-1})
S1	DI	< LOQ	< LOQ	WI	< LOQ	< LOQ
S2		860 ± 120	630 ± 80		< LOQ	< LOQ
S3		1700 ± 300	1200 ± 140		180 ± 50	300 ± 90
S4		< LOQ	200 ± 80		< LOQ	250 ± 100
S1	DII	< LOQ	< LOQ	WII	< LOQ	< LOQ
S2		< LOQ	< LOQ		890 ± 240	< LOQ
S3		160 ± 30	460 ± 100		250 ± 80	< LOQ
S4		710 ± 160	340 ± 80		630 ± 130	320 ± 120
S1	DIII	< LOQ	< LOQ	WIII	< LOQ	< LOQ
S2		< LOQ	< LOQ		900 ± 110	210 ± 90
S3		120 ± 30	< LOQ		140 ± 50	210 ± 80
S4		300 ± 80	120 ± 30		1720 ± 560	250 ± 100
S1	DIV	< LOQ	< LOQ	WIV	< LOQ	< LOQ
S2		< LOQ	< LOQ		< LOQ	< LOQ
S3		360 ± 90	450 ± 30		450 ± 130	240 ± 90
S4		< LOQ	650 ± 120		870 ± 150	< LOQ
S1	Number of occurrences	0	0	Number of occurrences	0	0
S2		1	1		2	1
S3		4	3		4	3
S4		2	4		3	3

Fig. 2 Precipitation (mm) at the studied area and Rio Grande active capacity (AC, %) in dry and wet seasons. Stars on the AC plot indicate sampling dates

spatially. Considering seasonal variation, no statistical difference was observed within EC, TDS, and TP, which might have been influenced by AC variability. However, for these parameters, ANOVA results showed spatial significant differences among stations located in the reservoir (S1 and S2) and in the streams (S3 and S4), evidencing the contribution of the streams to pollution loads in the reservoir with subsequent dilution effect.

The mean GI value for samples from the dry period was $83.16 \pm 0.32\%$ and the wet season $82.74 \pm 0.37\%$ (both no phytotoxic). S3 was the only station where the GI value in both periods was classified as moderately phytotoxic (GI between 60 and 80%). Overall, no seasonal difference among GI values was observed.

3.3 PCA of Reservoir Stations

Figure 4 shows PCA results obtained using the general indicators of anthropogenic pollution to reservoir stations (S1 and S2). The first two components explained 97.7% of the total variance. The PC1 represents 78.4% of the data variability, and the most influential variables are EC, TDS, pH, and GI. PC2 explained 19.2% of the variance, where EC, TDS, and TP are the variables with more influence. Temperature (T) is the most important factor in the PC3, which explains 1.8%. This PCA analysis shows clearly that TDS and EC are highly correlated.

The score graph does not show the separation of sites S1 and S2 into different groups, which may not mean that there is no distinction, and could simply mean that largest sources of variation are similar in both groups. Water samples from sampling campaigns D1-S1, D1-S2, and WII-S2 showed the highest values of the EC,

TDS, and TP suggesting no existence of spatial and seasonal trends in the pollutant concentrations in the reservoir. The loading graphic permits to observe that EC and TDS showed high positive correlation among themselves and strong negative with TP.

Figure 5 a–c show the Pearson's correlation coefficients for AC ($r_{S1} = -0.59$ and $r_{S2} = -0.89$), TP ($r = -0.56$), and GI ($r = -0.61$) with respect to EC values obtained to reservoir stations (S1 and S2).

3.4 PCA of Stream Stations

Figure 6 shows PCA with estrogenic hormone concentration, GI, and limnological parameters for the stream stations (S3 and S4). The first two components explained 91.9% of the total variance. The PC1 represents 63.4% of the data variability, and the most influential variables are E2 and EE2 concentrations and PC2 explained 28.7% of the variance, where TP most influenced the results.

The score graph does not show spatial distinction between streams S3 and S4 which might indicate that largest sources of variation are similar in both groups. Neither seasonal group distinction was observed within data. The loading graphic permits to observe that E2 levels most influenced PCA with no significant correlation to common indicators of anthropogenic pollution (e.g., EC and TP).

4 Discussion

4.1 Presence of Hormones in Rio Grande Waters and Adjacent Streams

The development of more selective and sensitive analytical techniques has allowed the identification and quantification of these active estrogenic compounds in low concentrations in aquatic environments from different parts of the world (Zuccato et al. 2005; Nakada et al. 2006; Kim et al. 2007; Benoti et al. 2009; Kummerer 2009; Sui et al. 2010; Gracia-Lor et al. 2012; Tran et al. 2018).

At present study, estrogenic hormone levels were not detected at station S1, reflecting the preservation status of this area, which is less impacted by anthropogenic sources. Sousa et al. (2014) evaluated different emerging pollutants in river water from metropolitan region of São Paulo and Campinas city and did not find detectable
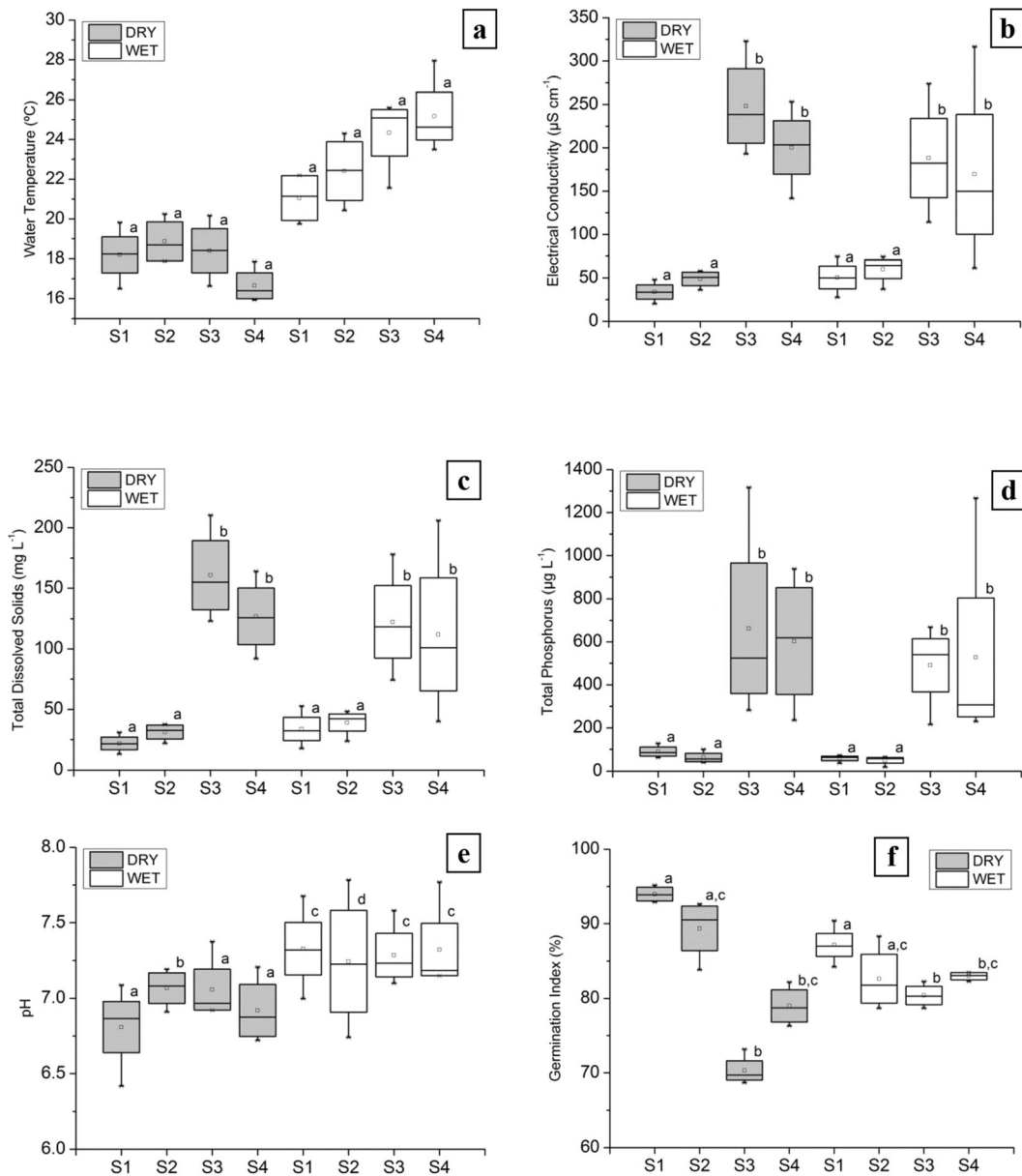
Fig. 3 Boxplot of: **a** water temperature (°C), **b** EC (μS cm^{-1}), **c** TDS (mg L^{-1}), **d** TP (μg L^{-1}), **e** pH, and **f** GI (%) in dry and wet periods, over the eight sampling campaigns

E2 and EE2. Even in very clear water, hormones have been reported in the literature, for example, Scala-Benuzzi et al. (2018) found 8.2 ng L^{-1} of EE2 in pristine river waters in Argentina. Caldwell et al. (2012) noted that reported concentrations in the USA and Europe reach about 0.2 or 0.3 ng L^{-1} in low flow conditions, and caution against studies that report higher concentrations as unrepresentative.

At present work, station S2, closer to precarious urbanization with inadequate wastewater disposal and a drinking water abstraction point, yielded measurable E2 levels in three campaigns (860–900 ng L^{-1}) and EE2 in two campaigns (210 and 630 ng L^{-1}). This is possibly due to input of raw or inefficiently treated wastewater into the ecosystem. Ghiselli (2006) reported E2 at 1800 to 6000 ng L^{-1} and EE2 at 1000 to 3500 ng L^{-1} in

**Fig. 4** PCA scores (**a**) and loadings (**b**) for samples and variables studied at reservoir stations (S1 and S2)
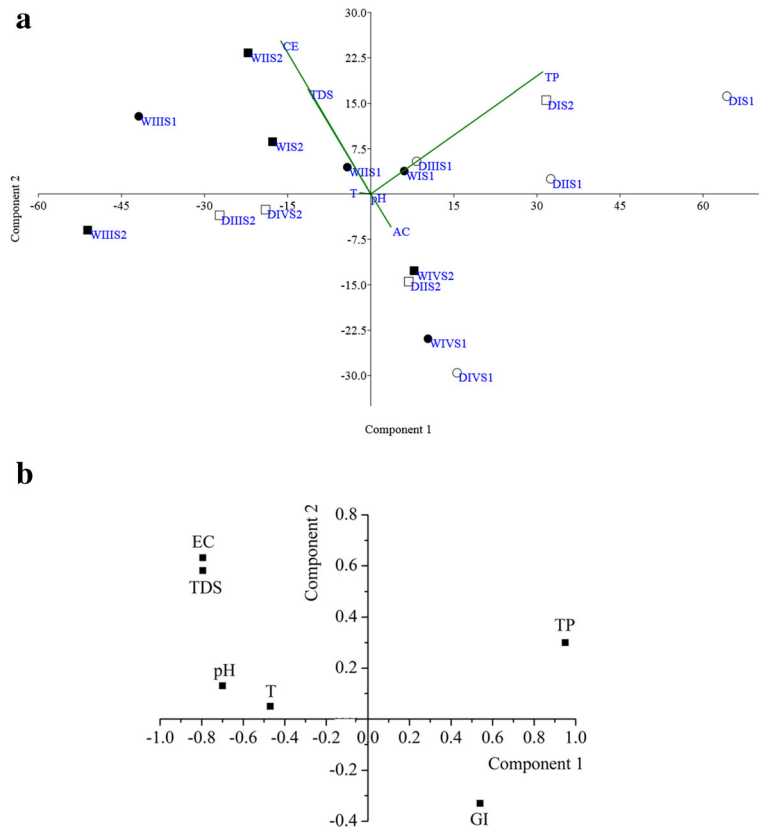
surface water in Campinas, Brazil. Other Brazilian studies have indicated levels in the order of 500 ng L^{-1} of estrogenic hormones in the Atibaia River, which supplies 92% of Campinas city (Ghiselli and Jardim 2007; Sodré et al. 2010; Maldaner and Jardim 2012). Sodré et al. (2010) identified E2 and EE2 levels, which reached 2510 and 310 ng L^{-1}, respectively, at places located along the Atibaia River watershed.

Levels of estrogen hormones in effluents might be higher even after wastewater treatment by conventional technologies. Pessoa et al. (2014) found estrogen levels in the effluent of a Brazilian WWTP between < LOD to 776 ng L^{-1} and 3180 ng L^{-1} for raw wastewater and 397 and 176 ng L^{-1} for activated sludge–treated effluent (for E2 and EE2, respectively). These values are of similar magnitude to the concentrations detected in the present study at locations S3 and S4.

Ashfaq et al. (2018) quantified these hormones in influent wastewater in China and reported EE2 concentrations from < LOD to 4.04 ng L^{-1}, but much higher levels of E2 of 46 to 150 ng L^{-1}. In Europe, concentrations reported in a review paper by Schröder et al. (2016) remain below 97 ng L^{-1} (E2) and 106 ng L^{-1}

(EE2) in WWTP influent. Vymazal et al. (2015) in Czech Republic did not detect EE2 in influent wastewater but found levels of E2 from < LOD to 199 ng L^{-1}. Adeel et al. (2017), in a global review paper, comment on the particularly high levels of some estrogenic hormones in Brazil. Thus, in the Brazilian context of inadequate sewage treatment, high concentrations should not be dismissed out of hand and may indeed be cause for concern.

All mammals excrete estrogenic hormones, which end up in the environment through direct excretion or through animal waste disposal. Multiple biological effects resulting from the exposure to E2 and EE2 have been described in the literature. In humans, the binding affinity of EE2 to the estrogen receptor is higher than for E2, and has been shown to be up to five times higher in some fish species (Aris et al. 2014). This higher affinity indicates that EE2 can be a more potent estrogenic compound in terms of endocrine disrupting effect compared with the naturally produced E2 (Tomšíková et al. 2012). For example, when early life stages of the zebrafish (*Danio rerio*) were exposed to 54 ng L^{-1} of E2, sex ratio was significantly altered (Holbech et al.
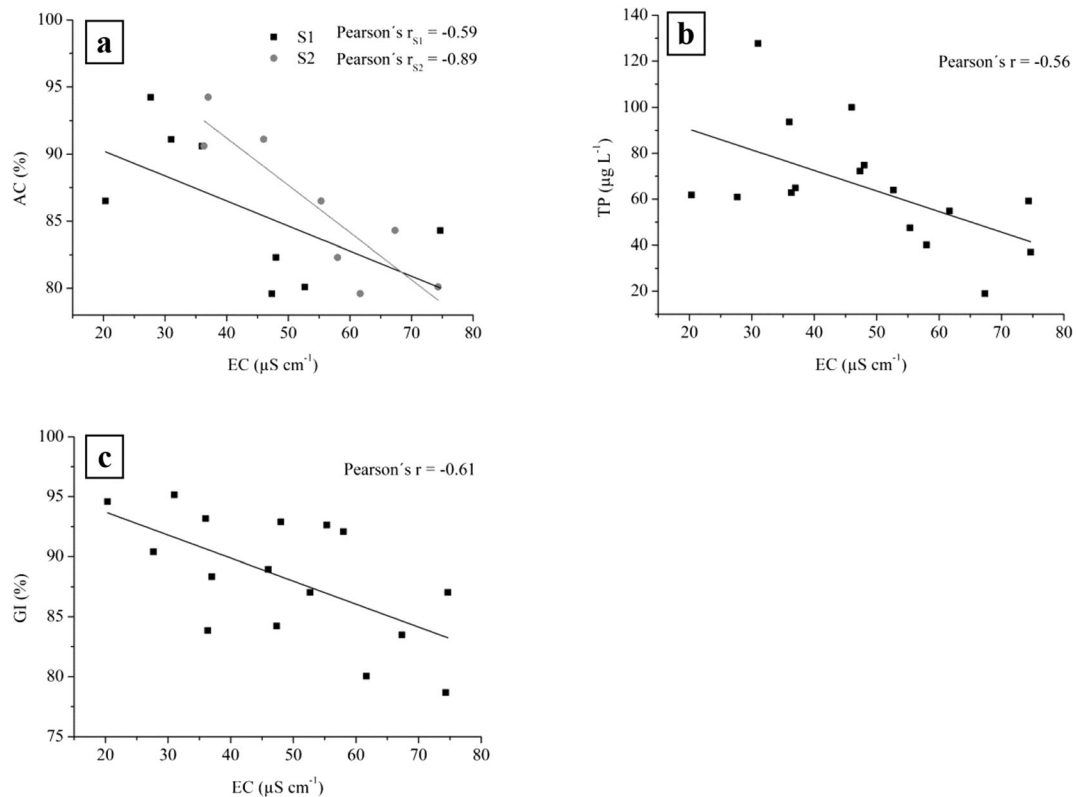
Fig. 5 Pearson's correlations of EC (μs cm^{-1}) with: **a** AC (%), **b** TP (μg L^{-1}), and **c** GI (%) in reservoir stations (S1 and S2)

2006). EE2 determined in water courses is cause for concern as its estrogenic potency is 25 times higher *in vitro* tests using Zebra fish compared with E2 (van den Belt et al. 2004; Andrew et al. 2010).

E2 and EC had a weak correlation (Pearson's r = 0.21) (Fig. 7c). This can be explained by E2 sources and biodegradation rates in the environment. Ma and Yates (2018) evaluated E2 degradation in batch assays using river and sediment samples from Santa Ana River (USA). The study reported higher E2 degradation rates in the presence of microorganisms, especially in assays with sediment samples where microbiological density was higher: E2 degradation in water took 10 days and in sediment about 2 days. E2 is typically excreted in conjugated forms such as sulfate and glucuronide which the microorganisms de-conjugate back to parental estradiol. These results support the low correlation between E2 and EC in the present study, due to their excretion in the conjugated form, which is not detected by the LC-MS method and also by the faster degradation rates of this compound in polluted water containing microorganisms from wastewater, such as *Escherichia coli*. However, as

expected, EE2 and EC had a strong correlation (Pearson's r = 0.71). EE2 is more resistant to biodegradation than E2 and remains stable even after wastewater treatment by activated sludge process (Panter et al. 1999; Aris et al. 2014). This support the higher correlation between EE2 and EC in the present study that involved sampling stations with raw wastewater disposal (S4) and treated effluent from WWTP (S3), reinforcing the persistence and detectability of EE2.

At the present study, given that half of the sampling sites were locations where human pollutants would be well diluted (reservoir), the overall average E2 (650 ng L^{-1}) and EE2 (720 ng L^{-1}) are of environmental concern. These are 10× and 3000× the reported EC$_{50}$ values to zebra fish for E2 and EE2, respectively (Fig. 8) and are likely to occur in association with other emerging pollutants (van den Belt et al. 2004). This clearly demonstrates the need to eliminate discharge of raw sewage into Brazilian watercourses and to improve existing wastewater treatment procedures and plants, as well include streams in environmental monitoring to avoid pollution of waterbodies used to water supply.

Fig. 6 PCA for stream stations (S3 and S4): **a** biplot scores and **b** loadings
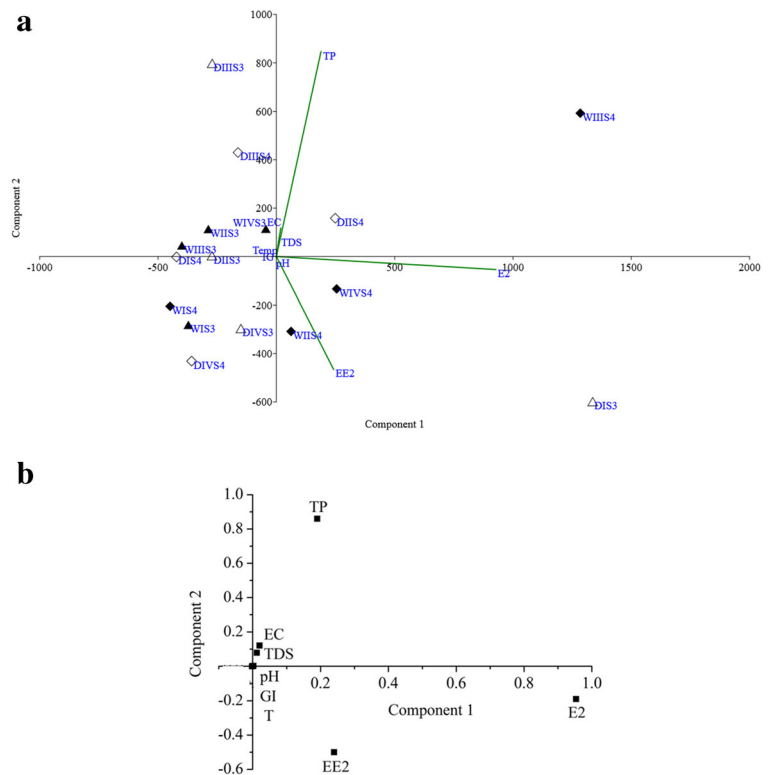
4.2 Spatial Distribution of Pollutants

During this study, EC and TDS values were one order of magnitude higher in the two streams compared with sampling stations in the reservoir, indicating the influence of anthropogenic sources on the streams. In fact, EC is pointed as a domestic sewage marker (Sousa et al. 2014), since each person consumes an average of 6 g of chloride per day (WHO 2003), which increases chloride ion concentrations in effluents. In opposite, undisturbed catchments are characterized by very low in-stream ionic concentrations and by $EC < 10$ μS cm^{-1} (Markewitz et al. 2006). Yet, EC can be used to assess water discharge, since these parameters have an inverse relationship due to the fact that new water contributing to runoff has shorter residence time than old water and hence lower ionic content (Weijs et al. 2013; Cano-Paoli et al. 2019).

Unexpected, S3 and S4 showed similar pollution levels, despite their difference in discharge content. S3 is a water body which receives huge quantity of raw sewage from Ribeirão Pires municipality. S4 receives effluent from a secondary level WWTP, rich in P. This can be explained by the dilution effect in S3, which has a higher flow capacity, which reduces pollution effects (Gomes et al. 2014, 2019). However, hydrological data of the studied streams are not available, and this is a reality in tropical areas, where hydrological data use to be very scarce (Hartemink et al. 2008; Rodrigues et al. 2018).

For all the monitoring stations, the concentration of TP exceeded the Brazilian Standards limit value (Brasil 2005), even at the reference station (S1), reinforcing the anthropogenic contribution by sewage in the reservoir and necessity of improving sanitation in the Rio Grande basin, aiming the eutrophication control (Mariani et al. 2006; Moschini-Carlos et al. 2010; Wengrat and Bicudo 2011).

4.3 Seasonal Variation of Pollutant Concentration

Rangel-Peraza et al. (2009) showed that water quality parameters have seasonal responses in large tropical reservoirs, especially for temperature, electrical conductivity, biochemical oxygen demand, and total coliforms.
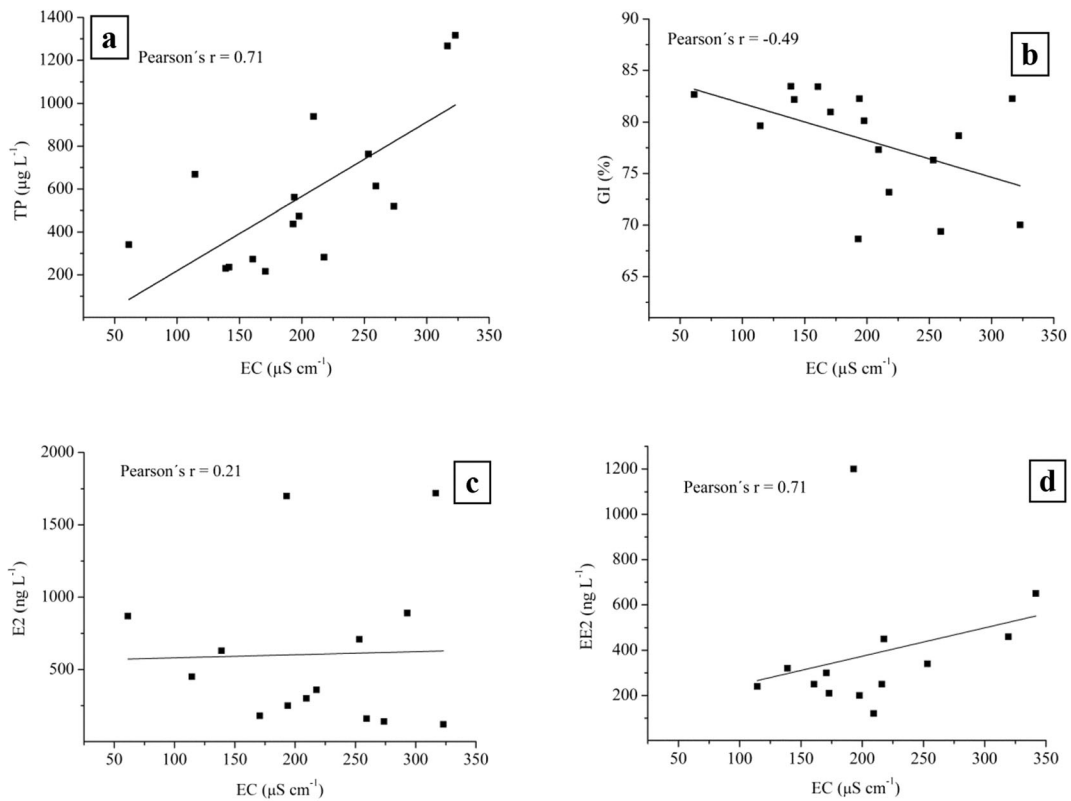
Fig. 7 Pearson's correlations of electrical conductivity with environmental data to stream stations (S3 and S4): **a** TP (μg L^{-1}), **b** GI (%), **c** E2 (ng L^{-1}), and **d** EE2 (ng L^{-1})

In general, wet season may cause soil erosion and bring eroded material to the water body. The dry season may cause river water lost through evaporation, increasing concentrations of suspended materials and dissolved elements (Nguyen et al. 2020).

Rio Grande basin presents two distinct rainfall seasons: wet and dry. Wet season is characterized by higher temperatures. AC presented a strong negative correlation with EC, especially for S2 station; thus, the lower volume of the reservoir concentrated chemical species enhancing electrical conductivity. For the estrogenic hormones, the weak correlations might be related to their lipophilicity (log $k_{ow,\ E2}$ = 4.0 and log $k_{ow,\ EE2}$ = 3.7), partitioning mainly into particle and sediment phases rather than being present in water column, thus not presenting a direct correlation with the dissolved
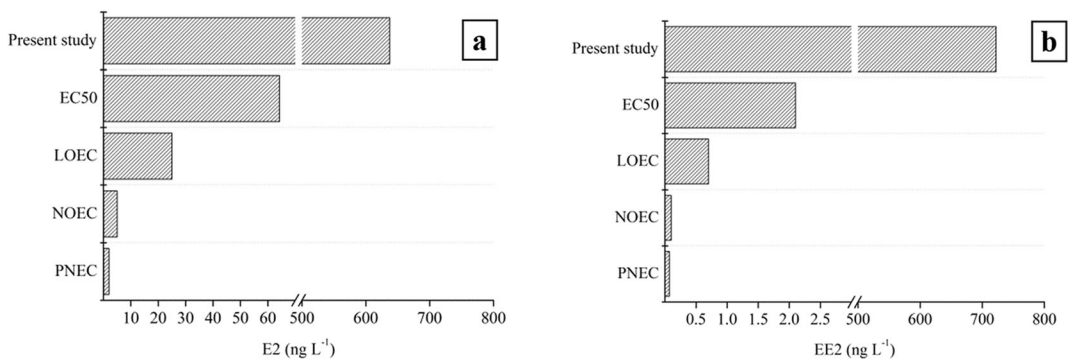
Fig. 8 E2 (**a**) and EE2 (**b**) levels and risk assessment for PNEC (Caldwell et al. 2012), NOEC (Aris et al. 2014), LOEC (Aris et al. 2014), and EC$_{50}$ (van den Belt et al. 2004)

ions responsible for EC. Regarding GI, a negative strong correlation with EC indicates that higher levels of pollution can increase water toxicity, reflecting in lower GI values. As expected, both in dry and wet seasons, GI was less affected at control station S1.

Our findings indicated higher mean values of EC, TDS, TP, and low GI (more phytotoxic) to streams (S3 and S4) during the dry season, but without a statistical difference (Fig. 3). This can be explained by low rain volume to dilute pollutants in the dry season in the streams. These findings are in according to Coulibaly et al. (2012) who found higher metal (Hg, Cd, Pb, and Zn) concentrations in the dry season than in the rainy season in sediments from Biétri Bay in Ebrié Lagoon, Ivory Coast. These authors attributed this to "reduced water volume during the dry season." Strong correlation between TP and EC and a medium one between GI and EC were observed, as expected, since streams receive domestic wastewater and treated effluent from WWTP loads and are more affected by precipitation dilution. It is noteworthy that only two abundant rainfalls (greater than 20 mm) occurred in the 2 days prior to sampling (DIV and WI). Rodrigues et al. (2018) found higher discharge values in the rainy season. To these authors, pH and EC were higher probably because sewers can discharge via combined sewer overflows an input of water flow to the main stream channel during intense rainstorms which was not observed in the study.

The pH had a statistical difference between stations and seasonality (Fig. 3). This could be due to eutrophication process. Algal blooms in Rio Grande branch mainly occur in summer, between the months of December and March (wet period), due to increased runoff, but this also depends on reservoir active capacity, which might dilute these effects (Leal et al. 2018). These blooms tend to consume CO_2 from water during photosynthesis and thus contribute to pH increase (Tailing 2010; Brasil et al. 2016). Bicudo et al. (2007) obtained a strong positive correlation between CO_2 and water transparency, as well as strong negative correlations of these variables with chlorophyll-a and pH, when evaluating data from a hypereutrophic lentic aquatic ecosystem in Brazil before and after removal of aquatic macrophytes and subsequent dominance of cyanobacteria. Before removal of the macrophytes, the pH was always neutral to slightly acid and free CO_2 was around 30 mg L^{-1}. After removal of the macrophytes and with the subsequent dominance of the cyanobacteria, the pH was always above 7, reaching almost 11 and free CO_2 decreased dramatically.

Algal blooms frequently occur around station S2 which is close to the drinking water abstraction location; therefore, the Sanitation Company of the state of São Paulo applies copper sulfate and hydrogen peroxide for phytoplankton and cyanobacteria control in this area (Sabesp 2011). Cyanobacteria lead to socioeconomic problems once toxins released degrades organoleptic water characteristics such as odor and taste, enhancing the cost of water treatment (Leal et al. 2018). Chronic ingestion to levels of $CuSO_4$ may cause respiratory inflammation, hepatotoxicity, and loss of fertility in humans (Huh and Ahn 2017). In developed countries, the use of $CuSO_4$ as an algaecide was abolished (Codd et al. 2005). However, in Brazil, it is still one of the most used cyanobacteria control methods due to its low cost and effectiveness (Leal et al. 2018). Additionally, pH might increase due to residual copper hydrolysis (Souza and Wasserman 2015).

GI values for reservoir stations S1 and S2 were lower (i.e., more toxic) in the wet season than in the dry period. In the wet season, it was expected that the dilution factor would decrease the toxicity, given that the rainfall volume was generally higher, despite that this AC was typically higher in dry period. Thus, the dilution factor was not the dominant factor in GI of the samples. The opposite was observed with samples taken from the streams S3 and S4 where, as expected, the dry season samples were more toxic.

4.4 Stream Monitoring Relevance

Stream monitoring supports water resource management in recognized segments severely impaired requiring remediation; identify priority pollutants that might achieve watersheds; prioritize watersheds for restoration; assess effectiveness of storm water management retrofits to capture, slow down, and filter water runoff; track progress in meeting water quality goals and legally mandated water quality standards; and assess and track the health of the watershed ecological functions (US Forest Service 1995; Ziemer 1998).

Freshwater environments offer many ecosystem services for the society such as water, food, water purification, genetic resources, regulation of water flow, recreation, and esthetic aspects (Harrison et al. 2010;

Schäefer et al. 2012). In this context, streams have hydrological functions in the environment once they receive direct discharge of wastewater. These pollutant loads are diluted by stream flow rates and are biochemically transformed by the microorganisms present in these ecosystems (Thompson et al. 2018). However, environmental degradation has been threatening these ecosystems functions, especially by the provision of pollutants that change natural characteristics of the environment, as well as presenting some toxicity level to the biota (MEA 2005). For example, the release of treated sewage (Pereda et al. 2020) and nanoparticles (Du et al. 2019) may affect ecosystem functions. Pereda et al. (2020) carried out an experiment in a stream, evaluating the influence of effluent discharge from a WWTP on the biota and stream's function. They concluded that structure and functioning of the ecosystem were affected even with well-treated and highly diluted discharges. Du et al. (2019) concluded that after a certain dosage of nanoparticles (from 10 to 1000 mg L^{-1} of ZnO), there was a reduction in fungi diversity, which affects the decomposition and the environment ecological functions.

These functions highlight the stream importance as attenuators to ingress of pollutants into reservoirs. The present study found hormone levels in the same order of magnitude of raw wastewater, which can cause hazards to biota. This reinforces the importance of the present study of considering streams in water quality monitoring programs aiming reservoir management and protection especially those directly receiving pollution from its drainage basin.

5 Conclusions

This leads us to following main conclusions:

- E2 concentrations varied from < LOQ to 1720 ng L^{-1} and EE2 from < LOQ to 1200 ng L^{-1}, with the highest levels being present in the two streams, contaminated with raw and treated domestic wastewater, that feed into Billings Reservoir. For both of these compounds, this exceeds PNEC and NOEC levels by at least an order of magnitude, indicating a very high ecotoxicological risk in the investigated area and giving rise to possible concern for concentrations in drinking water.

- Stream monitoring stations showed higher correlations between TP, GI, and EE2 with EC, which indicates wastewater discharge into the streams. Thus, EC is a good marker for these variables in streams and reinforces their relevance for inclusion in water quality monitoring programs. Seasonality influences precipitation and active capacity of the reservoir. These variables influenced the pollutant dilution in the present study. This reinforces the necessity of including hydraulic and hydrological data in environmental monitoring. Unfortunately, this kind of data is very scarce in tropical regions yet.

- The findings in this paper emphasize the importance of including streams in water quality monitoring programs, not only because of ecotoxicological impacts in these water bodies themselves, but also because they are important as pathways for pollutants to reservoirs, which are used for fishing, bathing, and drinking water abstraction.

Compliance of Ethical Standards

Conflict of Interest   The authors declare that they have no conflict of interest.

References

Adeel, M., Song, X., Wang, Y., Francis, D., & Yang, Y. (2017). Environmental impact of estrogens on human, animal and plant life: a critical review. *Environmental International, 99*, 107–119.

Andrew, M. N., O'Connor, W. A., Dunstan, R. H., & MacFarlane, G. R. (2010). Exposure to 17α-ethynylestradiol causes dose ad temporally dependent changes in intersex, females and vitellogenin production in the Sydney rock oyster. *Ecotoxicology, 19*, 1440–1451.

APHA. (2012). *Standard methods for the examination of water and wastewater* (22nd ed.). Washington: American Public Health Association.

Aris, A. Z., Shamsuddin, A. S., & Praveena, S. M. (2014). Occurrence of 17α-ethynylestradiol (EE2) in the

environment and effect on exposed biota: a review. *Environmental International, 69*, 104–119.

Ashfaq, M., Li, Y., Wang, Y., Qin, D., Rehman, M. S. U., Rashid, A., Yu, C. P., & Sun, Q. (2018). Monitoring and mass balance analysis of endocrine disrupting compounds and their transformation products in an anaerobic-anoxic-oxic wastewater treatment system in Xiamen, China. *Chemosphere, 204*, 170–177.

Barceló, D., & Petrovic, M. (2008). *Emerging contaminants from industrial and municipal waste: occurrence, analysis and effects*. Berlin: Springer 192 p.

Baronti, C., Curini, R., D'Ascenzo, G., Corcia, A., Gentilli, A., & Samperi, R. (2000). Monitoring natural and synthetic estrogens at activated sludge sewage treatment plants and in a receiving river water. *Environmental Science and Technology, 34*, 5059–5066.

Belo, S. R. (2011). Avaliação de fitotoxicidade através de *Lepidium sativum* no âmbito de processos de compostagem. *[Evaluation of phytotoxicity through Lepidium sativum in the context of composting processes]* Masters Dissertation, Universidade de Coimbra, Portugal, 79 p.

Benoti, M. J., Trenholm, R. A., Vanderford, B. J., Holady, J. C., Stanford, B. D., & Snyder, S. A. (2009). Pharmaceuticals and endocrine disrupting compounds in U.S. drinking water. *Environmental Science and Technology, 43*(3), 597–603.

Bicudo, D. C., Fonseca, B. M., Bini, L. M., Crossetti, L. O., Bicudo, C. E. M., & Jesus, T. A. (2007). Undesirable side-effects of water hyacinth control in a shallow tropical reservoir. *Freshwater Biology, 52*, 1120–1133.

Birkett, J. W., & Lester, J. N. (Eds.). (2002). *Endocrine disruptors in wastewater and sludge treatment processes* (312p). Boca Raton: Lewis Publishers.

Bonvin, F., Rutler, R., Chevre, N., Halder, J., & Kohn, T. (2011). Spatial and temporal presence of a wastewater-derived micropollutant plume in Lake Geneva. *Environmental Science and Technology, 45*, 4702–4709.

Brasil. (2005). CONAMA: Conselho Nacional do Meio Ambiente [*National Council for the Environment*]. Resolution n.° 357. Brasília, DF. Brazil. 27 p. Available in: http://www.mma.gov.br/port/conama/legiabre.cfm?codlegi=459. Accessed in 30 July 2018.

Brasil, J., Attayde, J. L., Vasconcelos, F. R., Dantas, D. D. F., & Huszar, V. L. M. (2016). Drought-induced water-level reduction favors cyanobacteria blooms in tropical shallow lakes. *Hydrobiologia, 770*, 145–164.

Caldwell, D. J., Mastrocco, F., Anderson, P. D., Länge, R., & Sumpter, J. P. (2012). Predicted-no-effect concentrations for the steroid estrogens estrone, 17β-estradiol, estriol, and 17α-ethinylestradiol. *Environmental Toxicology Chemistry, 31*(6), 1396–1406.

Cano-Paoli, K., Chiogna, G., & Bellin, A. (2019). Convenient use of electrical conductivity measurements to investigate hydrological process in Alpine headwaters. *Science of the Total Environment, 685*, 37–49.

Cardoso-Silva, S., Nishimura, P. Y., Padial, P. R., Mariani, C. F., Moschini-Carlos, V., & Pompêo, M. L. M. (2014). Compartimentalização e qualidade da água: o caso da Represa Billings. *Bioikos Campinas, 28*(1), 31–43.

Chalupová, D., Havlíková, P., & Janský, B. (2012). Water quality of selected fluvial lakes in the context of the Elbe River pollution and anthropogenic activities in the floodplain. *Environmental Monitoring Assessment, 184*, 6283–6295.

Codd, G. A., Azevedo, S. M. F. O., Bagchi, S. N., Burch, M. D., Carmichael, W. W., Harding, W. R., Kaya, K., & Utkilen, H. C. (2005). *CYANONET: a global network for cyanobacterial bloom and toxin risk management. Initial situation assessment and recommendations*. Paris: International Hydrological Programme.

Coulibaly, S., Atse, B. C., Koffi, K. M., Sylla, S., Konan, K. J., & Kouassi, N. G. J. (2012). Seasonal accumulations of some heavy metal in water, sediment and tissues of black-chinned tilapia Sarotherodon melanotheron from Biétri Bay in Ebrié lagoon, Ivory Coast. *Bulletin of Environmental Contamination and Toxicology, 88*, 571–576.

Dash, A. K. (2012). Impact of domestic wastewater on seed germination and physiological parameters of rice and wheat. *International Journal of Recent Research and Applied Studies, 12*(2), 280–286.

Directive 2013/39/EU of the European Parliament and of the Council of 12 August 2013 amending Directives 2000/60/EC and 2008/105/EC as regards priority substances in the field of water policy. https://eur-lex.europa.eu/eli/dir/2013/39/oj. Accessed 19 December 2018.

Du, J., Zhang, Y., Guo, R., Meng, F., Gao, Y., Ma, C., & Zhang, H. (2019). Harmful effect of nanoparticles on the functions of freshwater ecosystems: insight into nanoZnO-polluted stream. *Chemosphere, 214*, 830–838.

Ebele, A. J., Abdallah, M. A., & Harrad, S. (2017). Pharmaceuticals and personal care products (PPCPs) in the freshwater aquatic environment. *Emerging Contaminants, 3*, 1–16.

Ghiselli, G. (2006). Avaliação das águas destinadas ao abastecimento público na região de Campinas: ocorrência e determinação dos interferentes endócrinos (IE) e produtos farmacêuticos e de higiene pessoal (PFHP). [*Potable water quality in the Campinas region: occurrence and determination of endocrine disruptors (EDs) and pharmaceuticals and personal care products (PPCPs)*]. pHD Thesis, UNICAMP, São Paulo, 181 p.

Ghiselli, G., & Jardim, W. F. (2007). Endocrine disruptors in the environment. *Química Nova, 30*, 695–706.

Girardi, R., Pinheiro, A., Garbossa, L. H. P., & Torres, E. (2016). Water quality change of rivers during rainy events in a watershed with different land uses in southern Brazil. *Brazilian Journal of Water Resources, 21*, 514–524.

Gomes, P. I. A., Wai, O. W. H., Kularatne, R. K. A., Priyankara, T. D. P., Anojika, K. G. M. S., & Kumari, G. M. N. R. (2014). Relationships among anthropogenic disturbances representative riparian and non-riparian herbaceous indicators (biomass and diversity), land use, and lotic water quality: implications on rehabilitation of lotic waters. *Water, Air, and Soil Pollution, 225*, 2060.

Gomes, P. I. A., Fernando, B. A. V. W., & Dehini, G. K. (2019). Assessment of pollution sources, fate of pollutants, and potential instream interventions to mitigate pollution of earthen canals of urban to rural-urban fringe. *Water, Air, and Soil Pollution, 230*, 262.

Gracia-Lor, E., Sancho, J. V., Serrano, R., & Hernández, F. (2012). Occurrence and removal of pharmaceuticals in wastewater treatment plants at the Spanish Mediterranean area of Valencia. *Chemosphere, 87*(5), 453–462.

Hammer, Ø., Harper, D. A. T., & Ryan, P. D. (2001). PAST: paleontological statistics software package for education and data analysis. *Palaeontologia Electronica*, 4 9 p. http://palaeo-electronica.org/2001_1/past/issue1_01.htm.

Harrison, P. A., Vandewalle, M., Sykes, M. T., Berry, P. M., Bugter, R., Bello, F., Feld, C. K., Grandin, U., Harrington, R., Haslett, J. R., Jongman, R. H. G., Luck, G. W., Silva, P. M., Moora, M., Settele, J., Sousa, J. P., & Zobel, M. (2010). Identifying and prioritising services in European terrestrial and freshwater ecosystems. *Biodiversity and Conservation, 19*, 2791–2821.

Hartemink, A. E., Veldkamp, A., & Bai, Z. (2008). Land cover change and soil fertility decline in tropical regions. *Turkish Journal of Agriculture and Forestry, 32*, 195–213.

Hem, J. D. (2012). Conductance: a collective measure of dissolved ions. Water analysis: Inorganic species, 1, 137–161.

Holbech, H., Kinnberg, K., Petersen, G. I., Jackson, P., Hylland, K., Norrgren, L., & Bjerregaard, P. (2006). Detection of endocrine disrupters: evaluation of a fish sexual development test (FSDT). *Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology, 144*, 57–66.

Huh, J. H., & Ahn, J. W. (2017). A perspective of chemical treatment for cyanobacteria control toward sustainable freshwater development. *Environmental Engineering Research, 22*, 1–11.

IBGE - Instituto Brasileiro de Geografia e Estatística. (2010). Pesquisa Nacional de Saneamento Básico - PNSB https://www.ibge.gov.br/estatisticas/multidominio/meio-ambiente/9073-pesquisa-nacional-de-saneamento-basico.html?=&t=o-que-e. Accessed 30 January 2020.

ISO - International Organization for Standardization. (2012). Method n° 11269. *Soil Quality – Determination of the effect of pollutants on soil flora – Part 1: Method for the measurement of inhibition of root growth*, 2nd Edition. Geneva, 19 p.

Johns, S. M., Denslow, N. D., Kane, M. D., Watanabe, K. H., Orlando, E. F., & Sepúlveda, M. S. (2011). Effects of estrogens and antiestrogens on gene expression of fated minnow (*Pimephales promelas*) early life stages. *Environmental Toxicology, 26*, 195–206.

Kim, S. D., Cho, J., Kim, I. S., Vanderford, B. J., & Snyder, S. A. (2007). Occurrence and removal of pharmaceuticals and endocrine disruptors in south Korean surface, drinking, and wastewaters. *Water Research, 41*, 1013–1021.

Kohatsu, M. Y., Jesus, T. A., Coelho, L. H. G., Peixoto, D. C., Poccia, G. T., & Hunter, C. (2018). Fitotoxicidade de água superficial da Região Metropolitana de São Paulo utilizando bioensaio com *Sinapis alba*. *Acta Brasiliensis, 2*(2), 58–62.

Kummerer, K. (2009). The presence of pharmaceuticals in the environment due to human use—present knowledge and future challenges. *Journal of Environmental Management, 90*, 2354–2366.

Leal, P. R., Moschini-Carlos, V., López-Doval, J. C., Cintra, J. P., Yamamoto, J. K., Bitencourt, M. D., Santos, R. F., Abreu, G. C., & Pompêo, M. L. M. (2018). Impact of copper sulfate application at an urban Brazilian reservoir: a geostatistical and ecotoxicological approach. *Science of the Total Environment, 618*, 621–634.

Lienert, J., Burki, T., & Escher, B. I. (2007). Reducing micropollutants with source control: substance flow analysis of 212 Pharmaceuticals in Faeces and Urine. *Water Science and Technology, 56*, 87–96.

Lima, G. N., & Rueda, V. O. M. (2018). The urban growth of the metropolitan área of São Paulo and its impacto n the climate. *Weather and Climate Extremes, 21*, 17–26.

Ling, T. Y., Gerunsin, N., Soo, C. L., Nyanti, L., Sim, S. F., & Grinang, J. (2017). Seasonal changes and spatial variation in water quality of a large young tropical reservoir and its Downstream River. *Journal of Chemistry, 2017*, 16 p.

Ma, L., & Yates, S. R. (2018). Degradation and metabolite formation of 17ß-estradiol-3-glucuronide and 17ß-estradiol-3-sulphate in river water and sediment. *Water Research, 139*, 1–9.

Machado, K. S., Cardoso, F. D., & Azevedo, J. C. R. (2014). Occurrence of female sexual hormones in the Iguazu river basin, Curitiba, Parana state, Brazil. *Acta Scientiarum Technology, 36*, 421–427.

Magalhães, D. P., & Ferrão-Filho, A. S. (2008). A Ecotoxicologia como ferramenta no biomonitoramento de ecossistemas aquáticos. *Oecologia brasiliensis, 12*, 355–381.

Maldaner, L., & Jardim, I. C. S. F. (2012). Determination of some organic contaminants in water samples by solid-phase extraction and liquid chromatography tandem mass spectrometry. *Talanta, 100*, 38–44.

Mariani, C. F., Moschini-Carlos, V., Brandimarte, A. L., Nishimura, P. Y., Tófoli, C. F., Duran, D. S., Lourenço, E. M., Braidotti, J. C., Almeida, L. P., Fidalgo, V. H., & Pompêo, M. L. M. (2006). Biota and water quality in the Riacho Grande reservoir, Billings complex (São Paulo, Brazil). *Acta Limnologica Brasiliensia, 18*(3), 267–280.

Markewitz, D., Resende, J. C. F., Parron, L., Bustamante, M., Klink, C. A., Figuereido, R. O., & Davidson, E. A. (2006). Dissolved rainfall inputs and streamwater outputs in an undisturbed watershed on highly weathered soils in the Brazilian cerrado. *Hydrological Processes, 20*, 2615–2639.

MEA - Millenium Ecosystem Assessment. (2005). *Ecosystems and human well-being: synthesis* (p. 137). Washington, DC: Island Press.

Moschini-Carlos, V., Freitas, L. G., & Pompêo, M. (2010). Limnological evaluation of water in the Rio Grande and Taquacetuba branches of the Billings Complex (São Paulo, Brazil) and management implications. *Ambiente e Água - An Interdisciplinary Journal of Applied Science, 5*(3), 47–59.

Nakada, N., Tanishima, T., Shinohara, H., Kiri, K., & Takada, H. (2006). Pharmaceutical chemicals and endocrine disrupters in municipal wastewater in Tokyo and their removal during activated sludge treatment. *Water Research, 40*, 3297–3303.

Nguyen, B. T., Do, D. D., Nguyen, T. X., Nguyen, V. N., Nguyen, D. T. P., Nguyen, M. H., Truong, H. T. T., Dong, H. P., Le, A. H., & Bach, Q. V. (2020). Seasonal, spatial variation, and pollution sources of heavy metals in the sediment of the Saigon River, Vietnam. *Environmental Pollution, 256*, 113412.

Padhye, L. P., & Tezel, U. (2013). Fate of environmental pollutants. *Water Environmental Research, 85*, 1734–1785.

Panter, G. H., Thompson, R. S., Beresford, N., & Sumpter, J. P. (1999). Transformation of a non-oestrogenic steroid metabolite to an oestrogenically active substance by minimal bacterial activity. *Chemosphere, 38*, 3579–3596.

Pereda, O., Solagaistua, L., Atristain, M., Guzmán, I., Larrañaga, A., von Schiller, D., & Elosegi, A. (2020). Impact of wastewater effluent pollution on stream functioning: a whole-ecosystem manipulation experiment. *Environmental Pollution, 258*, 113719.

Pereira, L. C., Souza, A. O., Bernardes, M. F. F., Pazin, M., Tasso, M. J., Pereira, P. H., & Dorta, D. J. (2015). A perspective on the potential risks of emerging contaminants to human and environmental health. *Environmental Science and Pollution, 22*(18), 13800–13823.

Pessoa, G. P., Souza, N. C., Vidal, C. B., Alves, J. A. C., Firmino, P. I. M., Nascimento, R. F., & Santos, A. B. S. (2014). Occurrence and removal of estrogens in Brazilian wastewater treatment plants. *Science of the Total Environment, 490*, 288–295.

Quadra, G. R., Souza, H. O., Costa, R. D., & Fernandez, M. A. D. (2017). Do pharmaceuticals reach and affect the aquatic ecosystems in Brazil? A critical review of current studies in a developing country. *Environmental Science and Pollution, 24*(2), 1200–1218.

Rangel-Peraza, J. G., Anda, J., González-Farias, F., & Erickson, D. (2009). Statistical assessment of water quality seasonality in large tropical reservoirs. *Lakes & Reservoirs Research & Management, 14*, 315–323.

Rani, C. N., & Karthikeyan, S. (2016). Endocrine disrupting compounds in water and wastewater and their treatment options—a review. *International Journal of Environmental Technology Management, 19*, 392–431.

Reis-Filho, R. W., Araújo, J. C., & Vieira, E. M. (2006). Hormônios sexuais estrógenos: contaminantes bioativos. *Química Nova, 29*, 817–822.

Rodrigues, V., Estrany, J., Ranzini, M., Cicco, V., Martín-Benito, J. M. T., Hedo, J., & Lucas-Borja, M. E. (2018). Effects of land use and seasonality on stream water quality in a small tropical catchment: the headwater of Córrego Água Limpa, São Paulo (Brazil). *Science of the Total Environment, 622-623*, 1553–1561.

Sabesp - Companhia de Saneamento Básico do Estado de São Paulo. (2011). *Ata da Sessão Pública 58749/10*. Objeto: Fornecimento de Sulfato de Cobre para Tratamento de Água – Compra Estratégica.

Sabesp - Companhia de Saneamento Básico do Estado de São Paulo (Sabesp). (2020). Dados dos sistemas produtores. http://mananciais.sabesp.com.br/HistoricoSistemas?SistemaId=0. Accessed 30 January 2020.

Scala-Benuzzi, M. L., Takara, E. A., Alderete, M., Soler-Illia, G. J. A. A., Schneider, R. J., Raba, J., & Messina, G. A. (2018). Ethinylestradiol quantification in drinking water sources using a fluorescent paper based immunosensor. *Microchemical Journal, 141*, 287–293.

Schäefer, R. B., Bundschuh, M., Rouch, D. A., Szöcs, E., von der Ohe, P. C., Pettigrove, V., Schulz, R., Nugegoda, D., & Kefford, B. J. (2012). Effects of pesticide toxicity, salinity and other environmental variables on selected ecosystem functions in streams and the relevance for ecosystem services. *Science of the Total Environment, 415*, 69–78.

Schröder, P., Helmreich, B., Škrbić, B., Carballa, M., Papa, M., Pastore, C., Emre, Z., Oehmen, A., Langenhoff, A., Molinos, M., Dvarioniene, J., Huber, C., Tsagarakis, K. P., Martinez-Lopez, E., Meric-Pagano, S., Vogelsang, C., & Mascolo, G. (2016). Status of hormones and painkillers in wastewater effluents across several European states—Considerations for the EU watch list concerning estradiols and diclofenac. *Environmental Science and Pollution Research (international), 23*, 12835–12866.

Silva, E., Daam, M. A., & Cerejeira, M. J. (2015). Aquatic risk assessment of priority and other river basin specific pesticides in surface waters of Mediterranean river basins. *Chemosphere, 135*, 394–402.

Sodré, F. F., Pescara, I. C., Montagner, C. C., & Jardim, W. F. (2010). Assessing selected estrogens and xenoestrogens in Brazilian surface waters by liquid chromatography-tandem mass spectrometry. *Microchemical Journal, 96*, 92–98.

Sousa, D. N. R., Mozeto, A. A., Carneiro, R. L., & Fadini, P. S. (2014). Electrical conductivity and emerging contaminants as markers of surface freshwater contamination by wastewater. *Science of the Total Environment, 484*, 19–26.

Souza, V. A., & Wasserman, J. C. (2015). Distribution of heavy metals in sediments of a tropical reservoir in Brazil: sources and fate. *Journal of South American Earth Sciences, 63*, 208–216.

Statstutor (2020). Pearson's correlation. http://www.statstutor.ac.uk/resources/uploaded/pearsons.pdf. Accessed 23 Jan 2020.

Su, Y., Langhammer, J., & Jarsjö, J. (2017). Geochemical responses of forested catchments to bark beetle infestation: evidence from high frequency in-stream electrical conductivity monitoring. *Journal of Hydrology, 550*, 635–649.

Sui, Q., Huang, J., Deng, S., Yu, G., & Fan, Q. (2010). Occurrence and removal of pharmaceuticals, caffeine and DEET in wastewater treatment plants of Beijing, China. *Water Research, 44*, 417–426.

Tailing, J. F. (2010). pH, the CO_2 system and freshwater science. *Freshwater Reviews, 3*, 133–146.

Thompson, M. Y., Brandes, D., & Kney, A. D. (2012). Using electronic conductivity and hardness data for rapid assessment of stream water quality. *Journal of Environmental Management, 104*, 152–157.

Thompson, J., Pelc, C. E., Brogan, W. R., & Jordan, T. E. (2018). The multiscale effects of stream restoration on water quality. *Ecological Engineering, 124*, 7–18.

Tomšíková, H., Aufartová, J., Solich, P., Nováková, L., Sosa-Ferrera, Z., & Santana-Rodríguez, J. J. (2012). High-sensitivity analysis of female-steroid hormones in environmental samples. *Trends in Analytical Chemistry, 34*, 35–58.

Tran, N. H., Reinhard, M., & Gin, K. Y. H. (2018). Occurrence and fate of emerging contaminants in municipal wastewater treatment plants from different geographical regions – A review. *Water Research, 133*, 182–207.

Trata Brasil. (2018). http://www.tratabrasil.org.br/saneamento/principais-estatisticas. Accessed in 13 November 2018.

US Forest Service. (1995). *Ecosystem analysis at the watershed scale: federal guide for watershed analysis. watershed scale: federal guide for watershed analysis.* Version 2.2, Regional Interagency Executive Committee: Regional Ecosystem Office, U.S. Government; 26 p. https://www.fs.fed.us/r6/reo/library/docs/watershd.pdf. Accessed in 30 January 2020.

USEPA - United States Environmental Protection Agency. (2010). *Method 539: Determination of Hormones in Drinking Water by Solid Phase Extraction (SPE) and Liquid Chromatography Electrospray Ionization Tandem Mass Spectrometry (LC-ESI-MS/MS).* 37 p. Accessed in 30 January 2020.

USEPA - United States Environmental Protection Agency. (2012). *Monitoring Unregulated Drinking Water Contaminants.* https://www.epa.gov/dwucmr/third-unregulated-

contaminant-monitoring-rule. Accessed in 17 December 2018.

van den Belt, K., Berckmans, P., Vangenechten, C., Verheyen, R., & Witters, H. (2004). Comparative study on the in vitro/in vivo estrogenic potencies of 17-estradiol, estrone, 17-ethynylestradiol and nonylphenol. *Aquatic Toxicology, 66*, 183–195.

Vieira, L. A. (2016). Compostagem de biossólido de estação de tratamento de efluentes de frigorífico com serragem e cama de aves. [*Composting of biosolids from wastewater treatment plant with sawdust and poultry litter*]. Master Dissertation, Universidade Federal de Pelotas, Rio Grande do Sul, 66 p.

Vymazal, J., Brezinová, T., & Kozeluh, M. (2015). Occurrence and removal of estrogens, progesterone and testeosterone in three constructed wetlands treating municipal sewage in the Czech Republic. *Science of the Total Environment, 536*, 625–631.

Weijs, S. V., Mutzner, R., & Parlange, M. B. (2013). Could electrical conductivity replace water level in rating curves for alpine streams? *Water Resource Research, 49*(1), 343–351.

Wengrat, S., & Bicudo, D. C. (2011). Spatial evaluation of water quality in an urban reservoir (Billings complex, southeastern Brazil). *Acta Limnologica Brasiliensia, 23*(2), 200–216.

WHO – World Health Organization. (2003). *Chloride in drinking-water: background document for development.* WHO Guidelines for Drinking-water Quality.

Yongming, H., Peixuan, D., Junji, C., & Posmentier, E. S. (2006). Multivariate analysis of heavy metal contamination in urban dusts of Xi'an, Central China. *Science of the Total Environment, 355*, 176–186.

Ziemer, R.R. (1998). Monitoring watersheds and streams. USDA Forest Service gen. Tech. Rep. PSW-GTR-168, 6 p. https://www.fs.fed.us/psw/publications/documents/gtr-168/15ziemer.pdf. Accessed in 30 January 2020.

Zuccato, E., Castiglioni, S., Fanelli, R., Reitano, G., Bagnati, R., Chiabrando, C., Pomati, F., Rossetti, C., & Calamari, D. (2005). Pharmaceuticals in the Environment in Italy: causes, occurrence, effects and control. *Environmental Science Pollution Research (International), 13*(1), 15–21.