DOI:10.21715/GB2358-2812.202236005

Endocrine disruptors, estrogenic activity by the YES bioassay, and acute toxicity in Southeastern Brazil metropolitan surface waters

Marilia Teresa Lima do Nascimento[1]
Ana Dalva de Oliveira Santos[1]
Danieli Lima da Cunha[1]*
Louise Cruz Felix[2]
Giselle Gomes[2]
Carlos Marclei Arruda Rangel[1]
Rachel Ann Hauser-Davis[3]
Estefan Monteiro da Fonseca[1]
Daniele Maia Bila[2]
José Antônio Baptista Neto[1]

[1] Universidade Federal Fluminense
Instituto de Geociências
Departamento de Geologia
Avenida General Milton Tavares de Souza, s/nº
Boa Viagem
Niterói RJ Brasil.
CEP 24210-340.

[2] Universidade do Estado do Rio de Janeiro
Departamento de Engenharia Sanitária e Ambiental
Rua São Francisco Xavier, n. 524
Maracanã
Rio de Janeiro RJ Brasil
CEP 20550-900

[3] Fundação Oswaldo Cruz
Instituto Oswaldo Cruz
Avenida Brasil, n. 4.365
Manguinhos
Rio de Janeiro RJ Brasil
CEP 21040-360

* Corresponding author:
danielicunha@hotmail.com

RESUMO

Este estudo tem como objetivo determinar os desreguladores endócrinos nos rios urbanizados Maracanã e Canal do Mangue, no Rio de Janeiro, a segunda cidade mais populosa do Brasil. Bisfenol A, 17β-estradiol, estriol e 17α-etinilestradiol foram determinados por cromatografia líquida de alta eficiência com detector de matriz de diodo e detector de fluorescência. Além disso, foi realizada a avaliação da atividade estrogênica pelo bioensaio YES (*Yeast estrogen screen*) com a levedura *Saccharomyces cerevisiae* e ensaios de toxicidade aguda utilizando *Daphnia similis* e *Vibrio fischeri*. A atividade estrogênica nas amostras de água variou de abaixo do limite de detecção (<LD) a 1,6 ng L^{-1}, enquanto o bisfenol A variou de 22,3 a 1325,2 ng L^{-1} e os estrogênios 17β-estradiol de <LD a 55,2 ng L^{-1}, estriol de <LD a 313,7 ng L^{-1} e 17α-etinilestradiol de <LD a 409,4 ng L^{-1}. A toxicidade aguda não foi detectada em ambos os organismos analisados. O presente estudo indica um sério nível de poluição pelos compostos avaliados com riscos significativos para o ecossistema aquático, principalmente para a Baía de Guanabara que recebe descarga de rios urbanizados. Recomenda-se, portanto, a necessidade de ações sistemáticas de monitoramento e mitigação nesses corpos d'água.

Palavras-chave: Atividade estrogênica, desreguladores endócrinos, bioensaio YES, ensaios de toxicidade.

ABSTRACT

This study aimed to determine endocrine disruptors in urbanized rivers Maracanã and Mangue Channel in Rio de Janeiro the second most populated city in Brazil. Bisphenol A, 17β-estradiol, estriol, and 17α-ethinylestradiol were determined by high-performance liquid chromatography with a diode array detector and fluorescence detector In addition, the evaluation of estrogenic activity was performed by the YES bioassay (Yeast estrogen screen) with the *Saccharomyces cerevisiae* yeast, and acute toxicity assays were performed using *Daphnia similis* and *Vibrio fischeri*. Estrogenic activity in the water samples ranged from below the limit of detection (<LD) to 1.6 ng L^{-1}, while bisphenol A ranged from 22.3 to 1325.2 ng L^{-1} and estrogens 17β-estradiol from <LD to 55.2 ng L^{-1}, estriol from <LD to 313.7 ng L^{-1} and 17α-ethinylestradiol, from <LD to 409.4 ng L^{-1}. The acute toxicity was not detected in both analyzed organisms. The present study indicates a serious level of pollution by the compounds evaluated with significant risks to the aquatic ecosystem, mainly for Guanabara Bay which receives urbanized river discharge. Therefore, recommending the need for systematic monitoring and mitigating actions in these water bodies.

Keywords: Estrogenic activity, endocrine disrupters, YES bioassay, toxicity assays.

1 INTRODUCTION

Historically a significant portion of the human population lives in coastal areas, and anthropogenic activities in these regions have contributed considerably to continuous pollutant discharges into rivers, seas, and lakes (LAPWORTH *et al*., 2012; SILVA *et al*., 2015; KÖCK-SCHULMYER *et al*., 2019; CORRÊA *et al*., 2021). The endocrine disruptors (EDs), a heterogeneous group of chemicals and by-products, are of significant concern because may result in harmful endocrine system effects in both humans and animals (VALDÉZ *et al*., 2015; GONG *et al*., 2019; NG *et al*., 2021). In addition, they may present biological activity at very low concentrations (μg L^{-1} and ng L^{-1}) in the aquatic environment (BILA & DEZOTTI, 2007; NEGINTAJI *et al*., 2018). EDs are introduced into the aquatic environment through different routes, such as the incorrect disposal of pharmaceuticals, untreated domestic and industrial sewage, complex mixtures of agrochemicals, landfill leachate, among others (SINGARE, 2016; HUANG *et al*., 2019; DEICH *et al*., 2021).

Water quality concerns have resulted in a significant amount of research in this regard in the last decades in several countries. Water bodies continuously act as receptors for different types of pollutants, and this environmental degradation significantly affects ecosystems through contaminant exposure (TERNES *et al*., 1999; SHI *et al*., 2014; ISMAIL *et al*., 2019; SOLAUN *et al*., 2021). In Brazil, as well as in countries with deficient sanitation, water bodies reflect alarming concerns regarding risks associated to micro-contaminants (KUSTER *et al*., 2009; MONTAGNER *et al*., 2014; PESSOA *et al*., 2014). Research worldwide has, thus, increasingly advanced with the aim of understanding potential deleterious effects and impacts on Brazilian ecosystems (PETEFFI *et al*., 2018; PUSCEDDU *et al*., 2019; MONTAGNER *et al*., 2019; CHAVES *et al*., 2021; BRANCO *et al*., 2021).

Effects attributed to EDs include chronic changes in the cardiovascular, reproductive, endocrine, and immune systems (WAYE *et al*., 2011; PORSERYD *et al*., 2017; ABDEL-KHALEK *et al*., 2017; WEI *et al*., 2018). In animals, decreased bird, fish, and turtle egg hatching rates have been reported, as well feminization or masculinization (IWANO-WICZ *et al*., 2019; FORBES *et al*., 2019) which may lead to the decline of entire populations (WANG *et al*., 2018). In humans, studies have reported increased cancer incidence, fertility problems, as well as metabolic, cardiovascular, pulmonary, neuropsychiatric, and neurodegenerative diseases (BIRKETT & LESTER, 2003; MELO *et al*., 2009; ROCHA *et al*., 2018; PONTELLI *et al*., 2019).

Assessing the impacts and behavior of EDs compounds in water bodies is an increasingly complex and environmentally relevant issue (SHAO *et al*., 2019), reflecting the urgency of ED discussion and management (LUO *et al*., 2019). In this context, water bodies in metropolitan regions, lacking basic sanitation infrastructure and poor environmental legislation, are at the most risk for EDs contamination (MEYER *et al*., 1999; SODRÉ *et al*., 2010). This is the case of two water bodies, the Mangue Channel and your main tributary, the Maracanã River. Both flow through the metropolitan area of Rio de Janeiro, Southeastern Brazil, and it flows into the bay Guanabara Bay, one of the most anthropogenically impacted estuaries in Brazil which display significant social, economic and tourist importance (ROSMAN, 2011; HAUSER-DAVIS *et al*., 2019).

The Maracanã River is highly deteriorated, due to the disorderly human population growth associated with a lack of basic sanitation in the region. The river runs through three neighborhoods, Maracanã, Vila Isabel, and Tijuca and approximately 393,000 in habitants live in surrounding areas, with dozens of slums located on neighboring Tijuca Mountain Range slopes, where 13% of this total population lives (IBGE, 2010). The sewage network connects this area to the Alegria Sewage Treatment Station, located in the Caju neighborhood, where the sewage is treated and subsequently disposed of into Guanabara Bay (PSAM, 2015). However, this sewage network does not serve the entire population of this area. Thus, clandestine sewer pipes from different slums, such as the Indiana and Borel complexes, are directly linked to the Maracanã River, and crude sewage is directly disposed of into this water body. In addition, solid waste collection is not

regular in the upper part of the neighborhood, being routinely disposed in the Maracanã River.

The presence of EDs in the environment represents a potential threat to ecosystems and human health, due to evidence of toxicity, genotoxicity and endocrine disruption at very low concentrations (YOU and SONG, 2021). More studies are needed to understand the real impact of these micropollutants on rivers in Brazil.

In this context, the aim of this study was to investigate the presence of EDs such as bisphenol A (BPA), 17β-estradiol (E2), estriol (E3) and 17α-ethinylestradiol (EE2) by high-performance liquid chromatography (HPLC), estrogenic activity by the yeast estrogen screen (YES) bioassay and acute toxicity assays using *Daphnia similis* and *Vibrio fischeri*. Data on how these micropollutants are discharged into Guanabara Bay may aid in environmental conservation and decision-making.

2 MATERIALS AND METHODS

Eight sampling stations were chosen in the study area, covering three points in the Maracanã River (stations 1, 2, and 3), one in the Trapicheiro River (station 4), one in the Comprido River (station 5), and three in the Mangue Channel (stations 6, 7, and 8). Figure 1 shows the sampling stations assessed herein.

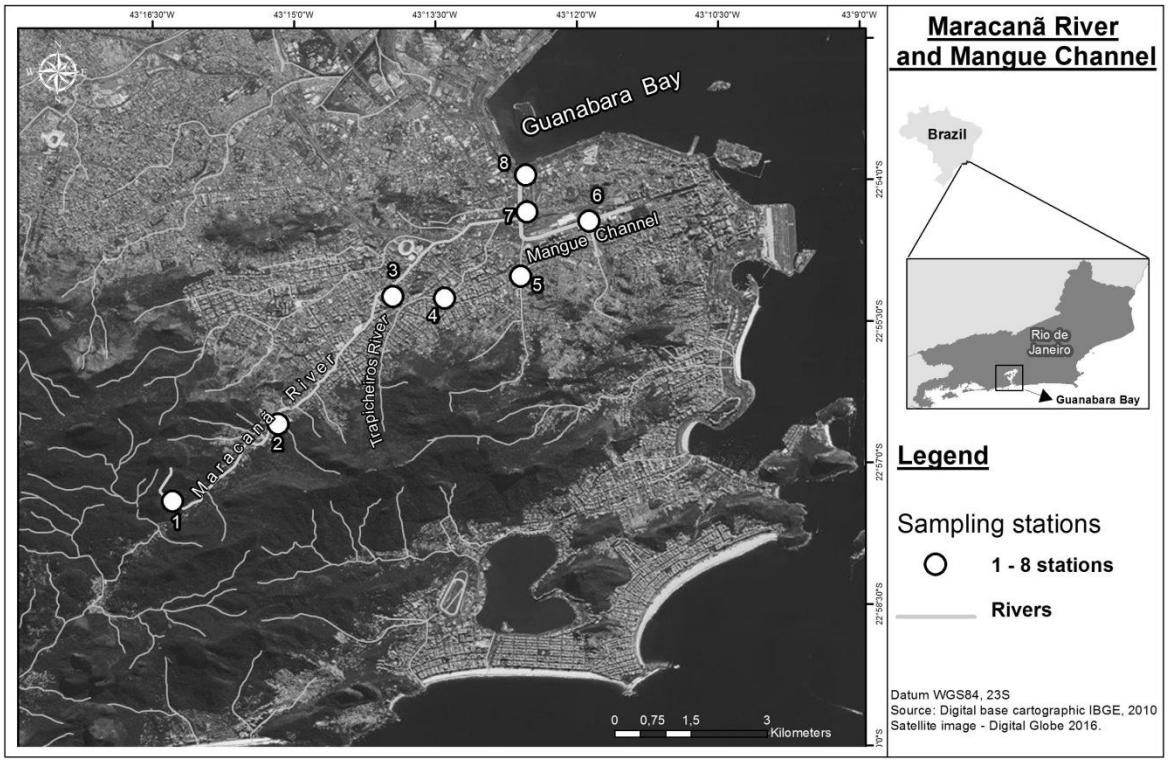

Figure 1
Surface water sampling stations on the Maracanã River and Mangue Channel (IBGE, 2010; Satelite image - Digitalglobe 2016).

Water samples were collected using a Van Dorn probe (AFK 34) and transferred to 1 L amber glass bottles for the chromatographic analyses and the YES bioassay. 50 mL aliquot was collected in polypropylene tubes for physicochemical parameter evaluations and toxicity assays. To prevent microbiological degradation of the BPA, E2, E3, and EE2 10 mL methanol (1% v/v) were added to each amber glass bottle. Samples were stored at 4 °C until they arrived at the laboratory.

All materials and glasswares used for sample collection and preparation were thoroughly decontaminated with 10% nitric acid solution to avoid interference from potential contaminants in the final results.

After collection and within 48 h, 1 L of each sample was filtered through a 0.45 μm cellulose

membrane (Merck®) by a vacuum pump and acidified to pH 3 with HCl (3 mol L^{-1}). Analytes of interest were isolated by solid-phase extraction (SPE) being used Strata X cartridges (500 mg per 6 mL, Phenomenex®) with the aid of a manifold (Agilent Technologies®). The cartridges were preconditioned with 3 x 2 mL hexane, 2 mL acetone, 3 x 2 mL methanol, and 5 x 2 mL ultrapure water at a flow rate of 5-10 mL min^{-1}. Samples were percolated through the cartridges at the same flow rate. A cleaning up was then performed with 10 mL methanol and ultrapure water at a 1:9 ratio. Subsequently, the cartridges were dried under vacuum for 10 min and eluted with 4 mL of acetone. The extracts were evaporated under a nitrogen stream, reconstituted with 2 mL ethanol for the YES bioassays and 500 µL acetonitrile (ACN) for the HPLC analyses. The analyses were performed in triplicate.

The EDs detection and quantification were performed by a previously validated methodology, reported by Silva *et al.* (2016) based on the methodology described by Brazil National Institute of Metrology, Standar-dization and Industrial Quality (INMETRO, 2010). Liquid chromatography (Waters Corporation®) with a Diode Array Detector (DAD), and Fluorescence Detector (FLU) were used, with a mobile phase of 60% ACN and 40% ultrapure water, and an injection volume of 20 µL. The eluent flow to BPA was 1 mL min^{-1}, λexc = 223 nm and λem = 300 nm, and to E2, E3, and EE2 was 1 mL min-1, λexc = 230 nm and λem = 306 nm. The stationary phase consisted of a Novapak PAH chromatographic column (4.6 x 250 mm, 5 microns). Compounds were identified by comparing retention times and peaks area with the corresponding standard solution.

The limits of detection (LD) and quantification (LQ), linearity, and recovery parameters were determined according to INMETRO (2010). Linearity was determined as a function of the standard curve evaluating R and R^2 values. The LQ was obtained by the lowest concentration of the analytical curve and the LD was determined as LD = SD × 6.965. Analytical curve correlation coefficient (R^2) values were > 0.99 for all compounds. For BPA, the LD was 1.899 µg L^{-1} and the LQ was 62.5 µg L^{-1}. LD values for E2, E3, and EE2 were 0.002, 0.002 and 0.005 µg L^{-1} and LQ values were 0.098, 0.013, and 0.074 µg L^{-1} respectively, with recoveries ranging from 97 to 106%.

For the YES bioassay, dilutions of the extracts and the E2 standard were performed in 96-well sterile flat-bottom microplates. 17β-estradiol dose-response curves (2724 at 1.3301 ng L^{-1}) were prepared by successive dilutions of E2 and ethanol from a stock solution at 54.48 µg L^{-1}. In each assay, ethanol and E2 were diluted in series, and used as negative and positive control, respectively. A 10 µL aliquot of each dilution was then transferred to duplicate microplates and evaporated to dryness, followed by the addition of 200 µL of the analysis medium (growth medium, yeast, and CPRG) to each well. The microplates were then shaken on a plate shaker (IKA MS3®) and maintained for 72 h at 30 °C in an incubator (New Ethics 410®). After incubation, sample absorbances were determined at 575 nm for color and 620 nm for turbidity on a plate reader (Spectramax M3, Molecular Devices®). The dose-response curves for the E2 standard followed a concentration versus corrected absorbance behavior resulting in sigmoidal curves, adjusted by the Source 6.0 software (Microsoft®). The estrogenic activity of each sample was determined in 17β-estradiol equivalents (E2-EQ) by interpolation from the standard E2 curve, and divided by the SPE concentration factor, obtaining the E2-EQ concentrations for each water sample. The mean EC50 value of the E2 dose-response curve was 38 ± 10 ng L^{-1}. The LD was 9 ± 3 ng L^{-1} and the LQ was 28 ± 10 ng L^{-1}.

Cytotoxicity may occur due to the presence of toxic compounds in samples that inhibit *Saccharomyces cerevisiae* yeast growth. This inhibition is observed by the absence of turbidity at the bottom of the sample wells. Thus, a 620 nm absorbance control is used as a tool to quantify yeast growth inhibition due to sample toxicity (FRISCHE *et al*., 2009) (Equation 1).

$$\text{Toxicity} = 1 - \left(\frac{\text{ABS620 sample}}{\text{ABS620 negative control}}\right) \quad [1]$$

Based on the study by Kunz *et al.* (2015), which uses the described methodology by the EU Water Framework Directive (EU WFD), the ecotoxicological risk estimation was performed through the risk quotient (RQ). Where the RQ is expressed as the analytical relationship between measured environmental concentrations (MEC) or E2-EQ concentrations and the annual average environmental quality standard (AA EQS), as shown in Equation 2:

$$RQ = \frac{MEC \text{ or } E2 - EQ}{AA\ EQS} \quad [2]$$

According to the AA EQS, the concentration of E2 to protect the aquatic environment in the surface water is 0.4 ng L^{-1} (SCHER, 2011; DIRECTIVE 2013/39/EC). For data interpretation, the environmental risk varies between none (< 1), at-risk (1 to 10), and high risk (> 10), in which harmful effects can be expected due to the presence of estrogenic compounds in the water (KUNZ *et al*., 2015).

The *Daphnia similis* microcrustacean assay was performed according to the Brazilian NBR 12713 standard (ABNT, 2009). Each sample was diluted 4, 2, 1, 0.5 and 0.25 times in cultured water, followed by 10 mL addition of each solution to test tubes containing five microcrustaceans each. Assays were conducted in quadruplicate, totaling 20 individuals per dilution. The temperature was maintained (20 ± 2 ºC) under a 48 h photoperiod system (16 h light/8 h dark) in an incubator (Ethik Tecnology®). The EC50 was determined by the Spearman-LCPIN software.

The *Vibrio fischeri* marine bacterium assay was performed by exposing the bioluminescent bacteria to the samples for 0, 15 and 30 min and determining reduced luminescence, according to the Brazilian NBR 15411-3 standard (ABNT, 2005), on a Microtox 500® SDI analyzer using the MICROTOX® Omni Software, version 4.1 (New Castle, USA). The initial sample concentration was 81.9% with four serial dilutions with 2% NaCl solution.

Water quality was evaluated by determining physicochemical parameters established by Brazilian legislation, pH (APHA method 4500-H^+ B), turbidity (APHA method 2130 B), conductivity (APHA method 2510 B), ammonia nitrogen (APHA method 4500-NH_3 D), chloride (APHA method 4500-Cl^- B), dissolved organic carbon (DOC) (APHA method 5310 B) and total suspended solids (TSS) (APHA method 2540 C) (APHA, 2012).

3 RESULTS AND DISCUSSION

Bisphenol A concentrations ranged from 22.3 to 1325.2 ng L^{-1}, with higher concentrations at stations 7, 4, and 8 (Table 1). These areas coexist with expressive transport and individual fluxes, leading to a huge daily waste contribution to this water body. The area also reflects severe flooding problems during the rains, aggravated due to the inefficiency of the collecting network (IBGE, 2014). The detected BPA values are high when compared to other studies, for example, an at Mondego River surface water in Portugal (2.4 ng L^{-1}), Yangtze River in China (0.98 to 43.8 ng L^{-1}), in three rivers in South India (2.8 to 136 ng L^{-1}) and in Poland (5.0 to 95 ng L^{-1}) (ROCHA *et al*., 2014; SHI *et al*., 2014; SELVARAJ *et al*., 2014; CZARZYÑSKA-GOŚLIŃSKA *et al*., 2017). However, the values were lower compared to those detected at the Yong River (15 to 1415 ng L^{-1}) and Bahe River (1573.1 ng L^{-1}), both in China (CHENG *et al*., 2018; WANG *et al*., 2018), and in Brazil, BPA values in surface waters from rivers in the state of São Paulo ranged from 2.8 to 39860 ng L^{-1} (MONTAGNER *et al*., 2017).

Regarding estrogens, E2 values ranged from <LD to 55.2 ng L^{-1} with higher concentrations at stations 8, 6, 7 and 1 (Table 1). Samples from stations 2, 3, 4, and 5 were below the LD. 17β-estradiol results were higher herein compared to surface water studies in South Korea, which reported values from 1.1 to 10.1 ng L^{-1} (KIM *et al*., 2009), the Mondego River in Portugal, at 2.8 ng L^{-1} (ROCHA *et al*., 2014) and in the USA, ranging from 0.7 to 1.7 ng L^{-1} (KOLODZIEJ & SEDLAK, 2007). In the Bahe River in China, high concentrations of 23.9 ng L^{-1} (WANG *et al*., 2018) were detected, while a WWTP tributary presented values from 7.4 to 32.7 ng L^{-1} (YE *et al*., 2014). An important caveat regarding risk assessments for E2 is that concentrations ≥ 1.0 ng L^{-1} already pose risks to the reproductive capacity of fish (SCHER, 2011). Thus, a maximum limit of 0.4 ng L^{-1} for E2 in water bodies has been adopted (KUNZ *et al*., 2017).

Estriol levels ranged from <LD to 313.7 ng L^{-1} at stations 1, 2, 4, 7, and 8, while the highest values were observed at stations 4, 2, and 8 (Table 1). Concentrations at stations 3, 5, and 6 were below the LD. These values are higher than detected at three rivers in Tianjin China, at 37.20 L^{-1} (RAO *et al*., 2013) and the Bahe River, also in China, at 5.2 ng L^{-1} (WANG *et al*., 2018), surface water and wastewater in Italy values have been reported as 0.95 L^{-1} (CIOFI *et al*., 2013), surface water in Taiwan, from <LD to 210 ng L^{-1} in (CHEN *et al*., 2010) and the

Tubarão stream in São Paulo, Brazil, from <LD to 60 ng L^{-1} (DANIEL & LIMA, 2014).

17α-ethinylestradiol results ranged from <LD to 409.4 ng L^{-1}, with the highest detections at stations 4 and 8, while only station 2 presented values <LD (Table 1). Again, the values reported herein are significantly higher than those reported in other studies, such as in South Korea (1.9 ng L^{-1}) (KIM *et al*., 2009), at the Bahe River, also in China (31.5 ng L^{-1}) (WANG *et al*., 2018) and at the Mondego River, in Portugal (4.4 ng L^{-1}) (ROCHA *et al*., 2014). Considering the potential adverse effects of EE2 on aquatic environments, the European Commission proposed a maximum limit of 0.035 ng L^{-1} for this estrogen through Directive 2008/105/EC (GILBERT, 2012; CUNHA *et al*. 2016). Another important contribution in this regard was Directive 2013/39/EC which proposed the first list for observation and monitoring of certain substances, published in Decision 2015/495/EC (CUNHA *et al*. 2016; BARBOSA *et al*., 2019).

Table 1 - BPA, E2, E3, and EE2 concentrations in water samples from the Maracanã River and Mangue Channel.

Sampling stations	Description	BPA (ng L^{-1})	E2 (ng L^{-1})	E3 (ng L^{-1})	EE2 (ng L^{-1})
1	Alto da Boa Vista	22.3	6.0	2.9	9.7
2	Conde Bonfim	46.2	< 0.002	129.3	< 0.005
3	Maracanã stadium	42.8	< 0.002	< 0.002	8.6
4	Trapicheiro River	567.6	< 0.002	313.7	409.4
5	Comprido River	20.4	< 0.002	< 0.002	16.3
6	Presidente Vargas Avenue	46.1	16.2	< 0.002	21.7
7	Train station Leopoldina	1325.2	6.1	45	13.9
8	Novo Rio bus terminal	560.3	55.2	54.8	225.9

BPA: bisphenol A, E2: 17β-estradiol, E3: estriol, EE2: 17α-ethinylestradiol.

Most samples displayed estrogenic activity (<LD at 1.6 ng L^{-1}), except for station 1, at Alto da Boa Vista, the Maracanã River source The highest estrogenic responses were observed in samples from stations 7, 5, 6, 4, 8, 2, and 3, respectively in increasing order. This is probably due to different inputs, such as domestic and industrial sewage, solid waste and substances displaying estrogenic potential as pharmaceutical products, pesticides, hormones and illicit drugs, among others, as the study areas are subject to intense anthropogenic action and suffer from inefficient basic sanitation (OTTONI, 2010; LUNELLI, 2011).

Cytotoxicity was observed in the YES bioassay for samples from stations 3, 4, 5, 6, and 7. Therefore, serial dilutions were performed and cytotoxicity was reduced until it no longer interfered with E2-EQ quantification. Samples from stations 1, 2, and 8 were not cytotoxic. Figure 2 displays the E2-EQ values of the sampled surface waters.

The surface water samples displayed lower estrogenic potential compared to similar studies. For example, in one study carried out at Pearl River in southern China, values ranged from 0.23 to 324 ng L^{-1}, with higher concentrations in surface waters and only minor variations in sediment (ZHAO *et al*., 2011), while concentrations ranging from 5.72 to 59.06 ng L^{-1} were found in samples from three rivers in Tianjin, China (RAO *et al*., 2013). In Brazil, Paraíba do Sul River samples filtered through 1.2 μm membranes have been reported as ranging from 0 to 16 ng L^{-1}, while samples filtered through 0.45 μm membrane ranged from 0 to 3.1 ng L^{-1} (DIAS *et al*., 2015). A study carried out in two streams of the state Rio de Janeiro reported E2-EQ concentrations of 10.4 and 23 ng L^{-1} (CUNHA *et al*., 2021). Regarding saline water from Jurujuba Cove, in the city of Niterói, Rio de Janeiro, concentrations ranged from 0.5 to 3.2 ng L^{-1} (NASCIMENTO *et al*., 2018). Only one study has reported lower concentrations, in Langat River in Malaysia, with a LD < 1 ng L^{-1}. However, even values below 1 ng L^{-1} may pose risks to the aquatic environment (PRAVEENA *et al*., 2016). Thus, the values at the assessed sited (<LD to 1.6 ng L^{-1}) still represent potential concerns and indicate a polluting source resulting in contaminant transport to Guanabara Bay (BAPTISTA NETO *et al*., 2013; RANGEL *et al*., 2013).

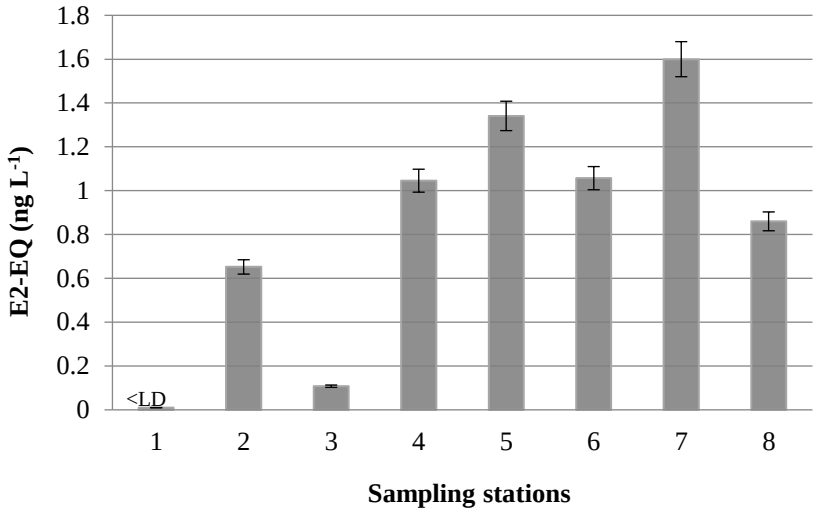

Figure 2
17β- estradiol equivalents (E2-EQ) concentrations in surface water samples from the Maracanã River and Mangue. <LD: below the limit of detection.

The micropollutants detected in the Maracanã River and Mangue Channel pose a significant risk to both water bodies, especially considering the proposed E2 (0.4 ng L^{-1}) and EE2 (0.035 ng L^{-1}) limits for water bodies according to the Directive 2013/39/EC (GILBERT, 2012; CUNHA *et al*., 2016). In addition, other factors concerning micropollutant contamination in Brazil must also be taken into account, such as territorial dimensions, population contingents, types of developed economy and land use and occupation (MONTAGNER *et al*., 2017). Therefore, further incentives for further research on this topic are paramount (COTRIM, *et al*., 2016). The water body monitoring should, thus, be considered an environmental priority, especially concerning estrogenic exposure, in the Rio de Janeiro metropolitan region, which is surrounded by huge urban densities and suffers disorderly occupation processes, sewage networks that do not meet population demands, clandestine sewage pipes and irregular garbage collection (VON SPERLING, 2004).

The ecotoxicological risk was estimated considering the RQ indicated in Table 2. Points 1 and 3 indicated no risk, as the RQ were 0.03 and 0.27, respectively. Therefore, these points comply with the environmental quality standards for estrogenic substances set by Directive 2013/39/EC. However, the RQ value at the other points ranged from 1.63 to 4.00, which should be considered ecologically relevant due to the possibility of adverse effects on aquatic species.

In the case of the city of Rio de Janeiro, only 78% of all households are connected to the sewage system (IBGE, 2014), although this number is actually much higher, as official data only consider "permanent" households, i.e., regular legal households, and not slums, which house a significantly high population and discharge crude sewage into the Guanabara Bay Basin rivers and into the bay itself. Thus, it is not surprising that all determined parameter values increased (Table 3) towards the mouth of the Mangue Channel and its confluence between rivers and streams, where a greater accumulation of polluting agents is probable, as the metropolitan region of Rio de Janeiro receives tons of solid waste daily from different polluting sources that reach the aquatic environment (COUDERC *et al*., 2016).

Table 2 - Ecotoxicological risk estimation through the risk quotient (RQ) in surface water from the Maracanã River and Mangue Channel.

Sampling stations	E2 EQ / AA EQS	RQ
1	0.03	none
2	1.63	at-risk
3	0.27	none
4	2.61	at-risk
5	3.35	at-risk
6	2.64	at-risk
7	4.00	at-risk
8	2.15	at-risk

E2-EQ: 17β- estradiol equivalents, AA EQS: annual average environmental quality standard.

Table 3 - Physicochemical parameters determined in surface water from the Maracanã River and Mangue Channel.

Sampling Stations	Turbidity (NTU)	pH	Chloride (mg L^{-1})	N-NH_3 (mg L^{-1})	Conductivity (mS cm^{-1})	DOC (mg L^{-1})	TSS (mg L^{-1})
1	5.2	7.9	8	0.3	75.5	3.8	64
2	8.6	7.8	16	0.5	80	5.1	78
3	8.4	7.8	16	0.6	87.4	8.8	76
4	9.1	7.0	16	0.9	78	10.2	85
5	12.2	7.9	14	0.7	98	10.5	97
6	14.4	7.1	17	1.1	37.7	9.6	126
7	16.0	7.3	20	1.6	97.3	9.9	182
8	18.3	7.2	20	1.9	95.6	14.7	220

N-NH_3: ammonia nitrogen, DOC: dissolved organic carbon, TSS: total suspended solids.

4 CONCLUSIONS

In sum, estrogenic activity was detected in almost all evaluated stations, except for the first, located upstream of the Maracanã River. The cytotoxicity observed was reduced until it no longer interfered with the quantification of E2-EQ. The highest micropollutant concentrations were observed for BPA, followed by estrogens EE2, E3, and E2. No toxicity was noted for *Daphnia similis* and *Vibrio fischeri*. The present study indicates severe pollution by the assessed compounds at the Maracanã River and Mangue Channel, with serious consequences for Guanabara Bay. Thus, mitigating actions concerning the risks associated with ED in complex environmental matrices are paramount.

5 ACKNOWLEDGMENTS

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) – Finance Code 001, FAPERJ, CNPq, and the National Secretary of Ports.

.

6 REFERENCES

ABDEL-KHALEK, A. A. Chronic exposure to water of lake qaroun induced metal-related testicular damage and endocrine disruption in male fish. **Biol. Trace Elem. Res**. 18:1-8, 2017. https://doi.org/10.1007/s12011-017-1220-y

ABESSA, D. M. S.; MORAIS, L. G.; PERINA, F. C.; DAVANSO, M. B.; BURUAEM, L. M.; MARTINS, L. M. P.; SÍGOLO, J. B.; RODRIGUES, V. G. S. Water and sediment toxicity in a river affected by former mining residues. **Mundo da Saúde**. 36(4):610-618, 2012.

ABNT - Associação Brasileira de Normas Técnicas. Ecotoxicologia Aquática. **Determinação do efeito inibitório de amostras de água sobre a emissão de luz de *Vibrio fischeri* (Ensaio de bactéria luminescente)**. NBR 15411, 2005.

ABNT - Associação Brasileira de Normas Técnicas. Ecotoxicologia aquática. **Toxicidade aguda - Método de ensaio com *Daphnia spp* (Crustadea, Cladocera).** NBR 12713, 2009.

APHA; AWWA; WEF. **Standard methods for the examination of water and wastewater**. 22th Edition. 1360 p. 2012.

BAPTISTA NETO, J. A.; PEIXOTO, T. C. S.; SMITH, B. J.; MCALLISTER, J. J.; PATCHINEELAM, S. M.; PATCHINEELAM, S. R.; FONSECA, E. M. Geochronology and heavy metal flux to Guanabara Bay, Rio de Janeiro state: a preliminary study. **An. Acad. Bras. Ciênc**. 85:1317-1327, 2013. https://doi.org/10.1590/0001-3765201394612

BARBOSA, M. O.; MOREIRA, F. F.; RIBEIRO, A. R.; PEREIRA, M. F. R.; SILVA, A. M. T. Occurrence and removal of organic micropollutants: An overview of the watch list of EU Decision 2015/495. **Water Res**. 94:257-279, 2019. https://doi.org/10.1016/j.watres.2016.02.047

BILA, D. M.; DEZOTTI, M. Desreguladores endócrinos no meio ambiente: efeitos e consequências. **Quím. Nova**. 30:651-666, 2007. https://doi.org/10.1590/S0100-40422007000300027

BIRKETT, J. W & LESTER, J. N. **Endocrine Disrupters in Wastewater and Sludge Treatment Process**. Lewis Publisher. 1st Edition. Washington D.C. 259 p. 2003.

CHAVES, M. D. J. S.; BARBOSA, S. C. & PRIMEL, E.G. Emerging contaminants in Brazilian aquatic environment: identifying targets of potential concern based on occurrence and ecological risk. **Environ Sci Pollut Res**, 2021. https://doi.org/10.1007/s11356-021-15245-y

CHEN, T. F.; CHEN, T. C.; YEH, K. J. C.; CHAO, H. R.; LIAW, E. T.; HSIEH, C. Y.; CHEN, K. C.; HSIEH, L. T.; YEH, Y. L. High estrogen concentrations in receiving river discharge from a concentrated livestock feedlot. **Sci. Total Environ**. 408 (16):3223-3230, 2010. https://doi.org/10.1016/j.scitotenv.2010.03.054

CHENG, J. R.; WANG, K.; YU, J.; ZHEN, X. Y.; YU, X. B.; ZHANG, Z. Z. Distribution and fate modeling of 4-nonylphenol, 4-t-octylphenol, and bisphenol A in the Yong River of China. **Chemosphere**. 195:594-605, 2018. https://doi.org/10.1016/j.chemosphere.2017.12.085

CIOFI, L.; FIBBI, D.; CHIUMINATTO, U.; COPPINI, E.; CHECCHINI, L.; BUBBA, M. D. Fully-automated on-line solid phase extraction coupled to high-performance liquid chromatography–tandem mass spectrometric analysis at sub-ng/L levels of selected estrogens in surface water and wastewater. **J. Chromatogr. A**. 1283:53-61, 2013. https://doi.org/10.1016/j.chroma.2013.01.084

CORRÊA, J. M.; SANSON, A. L.; MACHADO, C. F.; AQUINO, S. F.; AFONSO, R. J. Occurrence of contaminants of emerging concern in surface waters from Paraopeba River Basin in Brazil: seasonal changes and risk assessment. Environ. Sci. Pollut. Res. Int. 1-13, 2021. https://doi.org/10.1007/s11356-021-12787-z

COTRIM, G.; FAHNINGB, C. S.; ROCHA, G. O.; HATJE, V. Endocrine disruptors: strategies for determination and occurrence in marine environments. **J. Integr. Coast. Zone Manag**. 16, 299-326, 2016. https://doi.org/10.5894/rgci669

COUDERC, M.; MARCHAND, J.; ZALOUK-VERGNOUX, A.; KAMARI, A.; MOREAU, B.; BLANCHETL-LETROUVÉ; LE BIZEC, B.; MOUNEYRAC, C.; POIRIER, L. Thyroid endocrine status of wild European eels (*Anguilla anguilla*) in the Loire (France). Relationships with organic contaminant body burdens. **Sci. Total Environ**. 550:391-405, 2016. https://doi.org/10.1016/j.scitotenv.2015.12.136

CUNHA, D. L..; SILVA, S. M. C.; BILA, D. M.; OLIVEIRA, J. L.; SARCINELLI, P. N.; LARENTIS, A. L. Regulamentação do estrogênio sintético 17α-etinilestradiol em matrizes aquáticas na Europa, Estados Unidos e Brasil. **Cad. de Saúde Pública**. 32(3), 2016. https://doi.org/10.1590/0102-311X00056715

CUNHA, D. L.; MUYLAERT, S.; NASCIMENTO, M. T. L.; GOMES, G.; FONSECA, E. M.; BILA, D. M.; SARCINELLI, P. N.; LARENTIS, A. L. Atividade estrogênica de desreguladores endócrinos em águas superficiais do município de Santa Maria Madalena,

Sudeste do Brasil. Eng. Sanit. e Ambient. 26(1):21-28, 2021. https://doi.org/10.1590/s1413-415220180133

CZARZYÑSKA-GOŚLIŃSKA; BZGOŁA-GRZEŚKOWIAK, A.; JESZKA-SKOWRON, M.; FRANKOWSKI, R,; GRZEŚKOWIAK, G. Detection of bisphenol A, cumylphenol and parabens in surface waters of Greater Poland Voivodeship. **J. Environ. Manage**. 204,1:50-60, 2017. https://doi.org/10.1016/j.jenvman.2017.08.034

DEICH, C.; FRAZÃO, H. C.; APPELT, J. S.; LI, W.; POHLMANN, T.; WANIEK, J. J. Occurrence and distribution of estrogenic substances in the northern South China Sea. **Sci. Total Environ**. 770, 145239, 2021. https://doi.org/10.1016/j.scitotenv.2021.145239

DANIEL, M. S. & LIMA, E. C. Determinação simultânea de estriol, 17β-estradiol, 17α-etinilestradiol e estrona empregando-se extração em fase sólida (SPE) e cromatografia líquida de alta eficiência (HPLC). **Rev. Ambient. Água**. 9(4), 2014. https://doi.org/10.4136/ambi-agua.1346

DIAS, C.V.; GOMES, F. W.; BILA, D. M.; SANT'ANNA JÚNIOR, G. L.; DEZOTTI, M. Analysis of estrogenic activity in environmental waters in Rio de Janeiro state (Brazil) using the yeast estrogen screen. **Ecotoxicol. Environ. Saf**. 120:41–47, 2015. https://doi.org/10.1016/j.ecoenv.2015.05.013

DIRECTIVE 2013/39/EC 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. Official Journal of the European Union. Available at http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2013:226:0001:0017:PT:PDF. Accessed on 21 May 2018.

DECISION 2015/495/EC of the European Parliament and of the Council of of 20 March 2015 establishing a watch list of substances for Union-wide monitoring in the field of water policy pursuant to Directive 2008/105/EC. Official Journal of the European Union. Available at https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=uriserv%3AOJ.L_.2015.078.01.0040.01.ENG. Accessed on 21 May 2018.

FORBES, V. E.; RAILSBACK, S; ACCOLLA, C.; BIRNIR, B.; BRUINS, R. J. F.; DUCROT, V.; GALIC, N.; GARBER, K.; HARVEY, B. C.; JAGER, H. I.; KANAREK, A.; PASTOROK, R.;REBARBER, R.; THORBEK, P., SALICE, C. J. Predicting impacts of chemicals from organisms to ecosystem service delivery: A case study of endocrine disruptor effects on trout. **Sci. Total Environ**. 649:949–959, 2019. https://doi.org/10.1016/j.scitotenv.2018.08.344

FRISCHE, T.; FAUST, M.; MEYER, W.; BACKHAUS, T. Toxic masking and synergistic modulation of the estrogenic activity of chemical mixtures in a yeast estrogen screen (YES). Environ. Sci. Pollut. 16:593-603, 2009. https://doi.org/10.1007/s11356-009-0184-7

GARCIA, V. S. G.; MATSUSHITA, E.T.D.; MESQUITA, L.C. A.; FÁVARO, D. I. T.; BORRELY, S. I. Water Quality and Ecotoxicity Assessment in Surface Waters from Cubatão River and Surroundings, São Paulo, Brazil. **J Water Resour**. Prot. 9:1510-1525, 2017. https://doi.org/10.4236/jwarp.2017.912096

GILBERT, N. Drug-pollution law all washed up. **Nature**. 491(7425):503–504, 2012. https://doi.org/10.1038/491503a

GONG, J.; RAN, Y.; ZHANG, D.; CHEN, D.; LI, H.; HUANG, Y. Vertical profiles and distributions of aqueous endocrine-disrupting chemicals in different matrices from the Pearl River Delta and the influence of environmental factors. **Environ. Pollut**. 246:328-335, 2019. https://doi.org/10.1016/j.envpol.2018.12.015

HAUSER-DAVIS, R. A.; SILVA-JUNIOR, D. R.; LINDE-ARIAS, A. R.; VIANNA, M. Hepatic Metal and Metallothionein Levels in a Potential Sentinel Teleost, Dules auriga, from a Southeastern Brazilian Estuary. **Bull. Environ. Contam. Toxicol**. 103:538–543, 2019. https://doi.org/10.1007/s00128-019-02654-6

HUANG, Y.; LI, W.; QIN, L.; XIE, S.; GAO, B.; SUN, J.; LI, A. Distribution of endocrine-disrupting chemicals in colloidal and soluble phases in municipal secondary effluents and their removal by different advanced treatment processes. **Chemosphere**. 219:730-739, 2019. https://doi.org/10.1016/j.chemosphere.2018.11.201

IBGE - Instituto Brasileiro de Geografia e Estatística. Censo 2010 – Sinopse por setores. Governo Federal. Available at

http://www.censo2010.ibge.gov.br/sinopsep orsetores/. Accessed on 11 April 2018.

IBGE - Instituto Brasileiro de Geografia e Estatística. **Pesquisa Nacional de Saneamento Básico (PNSB)**, Rio de Janeiro, Brasil. 2014.

INMETRO - Instituto Nacional de Metrologia, Qualidade e Tecnologia. **Orientation about analytical methody validation, overal coordination of accreditation**. 20 p. 2010.

ISMAIL, N.A.H; WEE, S.Y; KAMARULZAMAN, N. H; ARIS, A. Z. Quantification of multi-classes of endocrine-disrupting compounds in estuarine water. **Environ. Pollut.** 249:1019:1028, 2019. https://doi.org/10.1016/j.envpol.2019.03.089

IWANOWICZ, L. R.; PINKNEY, A. E.; GUY, C. P.; MAJOR, A. M.; MUNNEY, K.; BLAZER, V. S.; ALVAREZ , D. A.; WALSH, H. L.; SPERRY, A.; BRAHAM, R.; SANDERS, L. R.; SMITH, D. R. Temporal evaluation of estrogenic endocrine disruption markers in smallmouth bass (*Micropterus dolomieu*) reveals seasonal variability in intersex. **Sci. Total Environ**. 646:245–256, 2019. https://doi.org/10.1016/j.scitotenv.2018.07.167

KIM, S. D.; CHO, J; KIM, I. S.; VANDERFORD, B. J.; SNYDER, S. A. Occurrence and removal of pharmaceuticals and endocrine disruptors in South Korean surface, drinking, and waste waters. **Water Res**. 41(5):1013-21, 2009. https://doi.org/10.1016/j.watres.2006.06.034

KÖCK-SCHULMEYER, M.; POSTIGO, C.; FARRÉ, M.; BARCELÓ, M.; ALDA, M. L. Medium to highly polar pesticides in seawater: Analysis and fate in coastal areas of Catalonia (NE Spain). **Chemosphere**. 215:515-523, 2019. https://doi.org/10.1016/j.chemosphere.2018.10.049

KOLODZIEJ, E. P; SEDLAK, D. L. Rangeland grazing as a source of steroid hormones to surface waters. **Environ. Sci. Technol**. 41:3514-3520, 2007. https://doi.org/10.1021/es063050y

KUNZ, P. Y.; KIENLE, C.; CARERE, M.; HOMAZAVA, N.; KASE, R. In vitro bioassays to screen for endocrine active pharmaceuticals in surface and waste waters. **J. Pharm. Biomed**. 106:107-115, 2015. https://doi.org/10.1016/j.jpba.2014.11.018

KUNZ, P.Y.; SIMON, E.; CREUSOT, N.; JAYASINGHE, B. S.; KIENLE, C.; MALETZ, S.; SCHIFFERL, A.; SCHÖNLAU, C.; AÏT-AÏSSA, S.; DENSLOW, N. D.; HOLLERT, H.; WERNER, I.; VERMEIRSSEN, E. L. M. Effect-based tools for monitoring estrogenic mixtures: Evaluation of five in vitro bioassays. **Water Res**. 110:378–88, 2017. https://doi.org/10.1016/j.watres.2016.10.062

KUSTER, D. A.; LÓPEZ DE ALDA M. J.; AQUINO NETO, F. R.; BARCELÓ, D. Analysis of phytoestrogens, progestogens and estrogens in environmental waters from Rio de Janeiro (Brazil). **Environ. Int**. 35(7):997-1003, 2009. https://doi.org/10.1016/j.envint.2009.04.006

LAPWORTH, D. J.; BARAN, N.; STUART, M. E.; WARD, R. S. Emerging organic contaminants in groundwater: A review of sources, fate and occurrence. **Environ. Pollut**. 163:287-303, 2012. https://doi.org/10.1016/j.envpol.2011.12.034

LUNELLI, C. A. **Direito, ambiente e políticas públicas**. Curitiba, Juruá. 226 p. 2011.

LUO, Z.; TU, Y.; LI, H.; QIU, B.; LIU, Y.; YANG, Z. Endocrine-disrupting compounds in the Xiangjiang River of China: Spatio-temporal distribution, source apportionment, and risk assessment. **Ecotoxicol. Environ. Saf**. 167:476–484, 2019. https://doi.org/10.1016/j.ecoenv.2018.10.053

MELO, S. A. S.; TROVO, A. G.; BAUTITZ, I. R; NOGUEIRA, R. F. P. Degradation of Residual Pharmaceuticals by Advanced Oxidation Processes. **Quím. Nova**. 32(1):88-197, 2009. https://doi.org/10.1590/S0100-40422009000100034

MEYER, A.; SARCINELLI, P. N.; MOREIRA, J. C. Are some Brazilian population groups subject to endocrine disrupters? **Cad. Saúde Pública**. 15(4):845-850, 1999. https://doi.org/10.1590/S0102-311X1999000400018

MONTAGNER, C. C.; VIDAL, C.; ACAYABA, R. D. Emerging contaminats in aquatic matrices from Brazil: current scenario and analytical, ecotoxicological and legislational aspects. **Quim. Nova**. 40(9):1094-1110, 2017. https://doi.org/10.21577/0100-4042.20170091

MONTAGNER,C. G. A. UMBUZEIRO, C. PASQUINI and W. F. JARDIM. Caffeine as an indicator of estrogenic activity in

source water. **Environmental Science: Processes & Impacts**. 16(8): 1866-1869, 2014. https://doi.org/10.1039/C4EM00058G

MONTAGNER, C. C.; SODRÉ, F. F.; ACAYABA, R. D.; VIDAL, C.; CAMPESTRINI, I.; LOCATELLI, M. A.; PESCARA, I. C.; ALBUQUERQUE, A. F.; UMBUZEIRO, G. A.; JARDIM, W. F. Ten Years-Snapshot of the Occurrence of Emerging Contaminants in Drinking, Surface and Ground Waters and Wastewaters from São Paulo State, Brazil. **J. Braz. Chem. Soc**. 30(3):614-636, 2019. http://dx.doi.org/10.21577/0103-5053.20180232

NASCIMENTO, M. T. L.; SANTOS, A. D. O.; FELIX, L. C.; GOMES, G.; OLIVEIRA E SÁ, M.; CUNHA, D. L.; VIEIRA, N.; HAUSER-DAVIS, R. A.; BAPTISTA NETO, J. A.; BILA, D. M. Determination of water quality, toxicity and estrogenic activity in a nearshore marine environment in Rio de Janeiro, Southeastern Brazil. **Ecotoxicol. Environ. Saf**. 149:197–202, 2018. https://doi.org/10.1016/j.ecoenv.2017.11.045

NEGINTAJI, A.; SAFAHIEH, A.; ZOLGHARNEIN, H.; MATROODI, S. Short-term induction of vitellogenesis in the immature male yellowfin seabream (*Acanthopagrus latus*) exposed to bisphenol A and 17 β-estradiol. **Toxicol Ind Health**. 34(2):119–2, 2018. https://doi.org/10.1177/0748233717748099

NG, B.; QUINETE, N.; MALDONADO, S.; LUGO, K.; PURRINOS, J.; BRICEÑO, H.; GARDINALI, P. Understanding the occurrence and distribution of emerging pollutants and endocrine disruptors in sensitive coastal South Florida Ecosystems. **Sci. Total Environ**. 757:143720, 2021. https://doi.org/10.1016/j.scitotenv.2020.143720

NOGUEIRA NETO, A. C.; BORRELY, S. I.; CAVALCANTE, P.S. Avaliação de toxicidade aguda e crônica em águas do rio Jundiaí e em afluentes e efluentes da ETE Novo Horizonte, Jundiaí, São Paulo. **Rev. Saberes UNIJIPA**, 8(1), 2018. https://doi.org/10.11606/D.85.2009.tde-05112009-150922

OTTONI, A. B. Diagnóstico Preliminar sobre as Enchentes Ocorridas na Praça da Bandeira com as Chuvas de Abril/2010. ANAP Brasil. Rio de Janeiro, RJ, 2010.

PETEFFI, G. P.; FLECK, J. D.; KAEL, I. M.; ROSA, D. C.; ANTUNES,M. V.; LINDEN, R. Ecotoxicological risk assessment due to the presence of bisphenol A and caffeine in surface waters in the Sinos River Basin - Rio Grande do Sul – Brazil. **Braz. J. Biol**. 79(4):712-721, 2019. https://doi.org/10.1590/1519-6984.189752

PESSOA, G. P.; SOUZA, N. C.; VIDAL, C. B.; ALVES, J. A. C.; FIRMINO, P. I. M..; NASCIMENTO, R. F.; SANTOS, A. B. Occurrence and removal of estrogens in Brazilian wastewater treatment plants. **Sci. Total Environ**. 490:288-295, 2014. https://doi.org/10.1016/j.scitotenv.2014.05.008

POMPÊO, M.; PADIAL, P. R.; MARIANI, C. F.; SILVA, S. C.; CARLOS, V. M.; SILVA, D. C. V. R.; PAIVA, T. C. B.; BRANDIMARTE, A. L. Biodisponibilidade de metais no sedimento de um reservatório tropical urbano (reservatório Guarapiranga - São Paulo (SP), Brasil): há toxicidade potencial e heterogeneidade espacial? **Geochim. Bras**. 27(2):104-119, 2013. https://doi.org/10.21715

PONTELLI, R. C. N.; SOUZA, M. C. O.; FANTUCCIA, M. Z; ANDRADE, M.; ROCHA, E. M. The role of endocrine disruptors in ocular surface diseases. **Med. Hypotheses**. 122:157–164, 2019. https://doi.org/10.1016/j.mehy.2018.11.009

PORSERYD, T.; KELLNER, M.; REYHANIAN CASPILLO, N.; VOLKOVA, K.; ELABBAS, L.; ULLAH, S. Combinatory effects of low concentrations of 17α-etinylestradiol and citalopram on non-reproductive behavior in adult zebrafish (*Danio rerio*). **Aquat. Toxicol**. 193:9–17, 2017. https://doi.org/10.1016/j.aquatox.2017.10.001

PRAVEENA, S. M.; LUI, T. S.; HAMIN, N.; RAZAK, S. Q.; ARIS, A. Z. Occurrence of selected estrogenic compounds and estrogenic activity in surface water and sediment of Langat River (Malaysia). **Environ. Monit. Assess**. 188(7):442, 2016. https://doi.org/10.1007/s10661-016-5438-5

PSAM - Programa de Saneamento Ambiental dos Municípios do Entorno da Baía de Guanabara 2015. Diagnóstico de Sistemas de Esgotamento Sanitário. Secretaria de Estado do Ambiente (SEA). Available at https://www.iets.org.br/IMG/pdf/apresentac

ao_saneamento_correa.pdf. Accessed on 10 April 2018.

PUSCEDDU, F. H.; SUGAUARA, L. E.; MARCHI, M. R.; CHOUERI, R. B.; CASTRO, I. B. Estrogen levels in surface sediments from a multi-impacted Brazilian estuarine system. **Mar. Pollut. Bull**. 142:576-580, 2019. https://doi.org/10.1016/j.marpolbul.2019.03.052

RANGEL, C. M. A.; BAPTISTA NETO, J. A.; LIMA, L. G.; OLIVEIRA, P. S. Speciation of Phosphorus in an estuarine system inside Guanabara Bay, RJ – SE Brazil. **J. Coast. Res**. 65(sp2):1194-1199, 2013. https://doi.org/10.2112/SI65-202.1

RAO, K.; LEI, B.; LI, N.; MA, M.; WANG Z. Determination of estrogens and estrogenic activities in water from three rivers in Tianjin, China. **J. Environ. Sci**. 25(6) 1164–1171, 2013. https://doi.org/10.1016/s1001-0742(12)60149-1

ROCHA, B. A.; ASIMAKOPOULOS, A. G.; HONDA, M.; NATTANE L. DA COSTA, N. L.; BARBOSA, R. M.; BARBOSA JR, F.; KURUNTHACHALAM, K. Advanced data mining approaches in the assessment of urinary concentrations of bisphenols, chlorophenols, parabens and benzophenones in Brazilian children and their association to DNA damage. **Environ. Internat**. 116:269–277, 2018. https://doi.org/1010.1016/j.envint.2018.04.023.

ROCHA, M. J.; CRUZEIRO, C.; REIS, M.; PARDAL, M. A.; ROCHA, E. Spatial and seasonal distribution of 17 endocrine disruptor compounds in an urban estuary (Mondego River, Portugal): evaluation of the estrogenic load of the area. **Environ. Monit. Assess**. 186:3337-3350, 2014. https://doi.org/10.1007/s10661-014-3621-0

RODRIGUES, D.O.; SILVA, S. L. R; DA SILVA, M. S. R. Avaliação Ecotoxicológica Preliminar das Águas das Bacias Hidrográficas dos rios Tarumã, São Raimundo e Educandos. **Acta Amazon**. 39(4):935–942, 2009. https://doi.org/10.1590/S0044-59672009000400021

ROSMAN, P. C. Relatório de Modelagem de Processos Sedimentológicos na Região do Canal do Mangue Porto do Rio de Janeiro - RJ. Rio de Janeiro: Fundação COPPETEC in

Galicia (NW Spain). **Chemosphere**. 86(10):1040-9, 2011.

SCHER - Scientific Committee on Health and Environmental Risks. Environmental Quality Standards - 17β-estradiol (E2). Available at https://ec.europa.eu/health/scientific_committees/environmental_risks/docs/scher_o_13.pdf. Accessed on 8 May 2018.

SELVARAJ, K. K.; SHANMUGAM, G.; SAMPATH, S.; LARSSON, D. G. J.; RAMASWAMY, B. R. GC–MS determination of bisphenol A and alkylphenol ethoxylates in river water from India and their ecotoxicological risk assessment. **Ecotoxicol. Environ. Saf**. 99:13-20, 2014. https://doi.org/10.1016/j.ecoenv.2013.09.006

SHAO, Y.; CHEN, Z.; HOLLERT, H.; ZHOU, S.; DEUTSCHMANN, B.; SEILER, T. B. Toxicity of 10 organic micropollutants and their mixture: Implications for aquatic risk assessment. **Sci. Total Environ**. 666:1273-1282, 2019. https://doi.org/10.1016/j.scitotenv.2019.02.047

SHI, J.; LIU, X.; CHEN, Q.; ZHANG, H. Spatial and seasonal distributions of estrogens and bisphenol A in the Yangtze River Estuary and the adjacent East China Sea. **Chemosphere**. 111: 336-343, 2014. https://doi.org/10.1016/j.chemosphere.2014.04.046

SILVA, F. S.; BITENCOURT, J. A. P.; SAVERGNINI, F.; GUERRA, L. V.; BAPTISTA NETO J. A.; CRAPEZ, M. A. C. Bioavailability of Organic Matter in the Superficial Sediment of Guanabara Bay, Rio de Janeiro, Brazil. **Anu. Inst. Geociência**. 34(1):52-63, 2015. https://doi.org/10.11137/2011_1_52-63

SILVA, L. L. S.; SALES, J. C. S.; FONSECA, F. V.; CAMPOS, J. C.; BILA, D. M. Advanced oxidative processes and membrane separation for micropollutant removal from biotreated domestic wastewater. **Environ. Sci. Pollut. Res. Int**. 16:1-10, 2016. https://doi.org/10.1007/s11356-016-7312-y

SINGARE, P.U. Distribution and risk assessment of suspected encocrine-disrupting pesticides in creek water of Munbai, India. **Mar. Pollut. Bull**. 102:72-83, 2016.

https://doi.org/10.1016/j.marpolbul.2015.11.055

SODRÉ, F. F.; PESCARA, I. C.; MONTAGNER, C. C.; JARDIM, W. F. Assessing selected estrogens and xenoestrogens in Brazilian surface waters by liquid chromatography–tandem mass spectrometry. **Microchem. J**. 96:92–98, 2010. https://doi.org/10.1016/j.microc.2010.02.012

SOLAUN, O.; RODRÍGUEZ, J. G.; MENCHACA, I.; LÓPEZ-GARCÍA, E.; MARTÍNEZ, E.; ZONJA, B.; LARRETA, J. Contaminants of emerging concern in the Basque coast (N Spain): Occurrence and risk assessment for a better monitoring and management decisions. Sci. Total Environ. 765:142765, 2021. https://doi.org/10.1016/j.scitotenv.2020.142765

TERNES, T. A.; STUMPF, M.; MUELLER, J.; HABERER, K.; WILKEN, R.D.; SERVOS, M. Behavior and occurrence of estrogens in municipal sewage treatment plants — I. Investigations in Germany, Canada and Brazil, **Sci. Total Environ.** 225(1–2):81-90, 1999. https://doi.org/10.1016/S0048-9697(98)00334-9

VALDÉZ, M. E.; MARINO, D. J.; WUNDERLIN, D. A.; SONOZA, G.; RONCO, A. E., CARRIGUIRIBORDE, P. Screening Concentration of E1, E2 and EE2 in Sewage Effluents and Surface Waters of the ‘‘Pampas’’ Region and the ‘‘Río de la Plata’’ Estuary (Argentina). Bull Environ Contam Toxicol B. 94(1), 2015. https://doi.org/10.1007/s00128-014-1417-0

VON SPERLING, M. Constraints to improving water and sanitation services. In: GRABOW, W. (Ed.). **Water and Public Health**. Developed under the Auspices of Unesco. Oxford, UK: EOLSS Publishers, 2004.

WANG, S.; ZHU, Z.; HE, J.; YUE, X.; PAN, J; WANG, Z. Steroidal and phenolic endocrine disrupting chemicals (EDCs) in surface water of Bahe River, China: Distribution, bioaccumulation, risk assessment and estrogenic effect on *Hemiculter leucisculus*. **Environ. Pollut**. 243:103-114, 2018. https://doi.org/10.1016/j.envpol.2018.08.063

WAYE, A. & TRUDEAU, V. L. Neuroendocrine disruption: more than hormones are upset. **J. Toxicol. Environ. Health Part A**. Critical reviews. 14(5-7):270-91, 2011. https://doi.org/10.1080/10937404.2011.578273

WEI, P.; ZHAO, F.; ZHANG, X.; LIU, W.; JIANG, G.; WANG, H.; RU, S. Transgenerational thyroid endocrine disruption induced by bisphenol S affects the early development of zebrafish offspring. Environ. Pollut. 243:800-808, 2018. https://doi.org/10.1016/j.envpol.2018.09.042

YE, T.; KANG M.; HUANG, Q.; FANG, C.; CHEN, Y.; LIU, L.; DONG, S. Accumulation of di(2-ethylhexyl) phthalate causes endocrine-disruptive effects in marine medaka (*Oryzias melastigma*) embryos. **Environ. Toxicol**. 31(1):116–127, 2014. https://doi.org/10.1002/tox.22028

YOU, H.H; SONG, G. Review of endocrine disruptors on male and female reproductive systems. **Comp. Biochem. Physiol. Part - C: Toxicol. Pharmacol**. 244, 109002, 2021. https://doi.org/10.1016/j.cbpc.2021.109002

ZHAO, J. L.; YING, G. G.; CHEN, F.; LIU; Y. S.; WANG, L.; YANG, B.; LIU, S.; TAO, R. Estrogenic activity profiles and risks in surface waters and sediments of the Pearl River system in South China assessed by chemical analysis and in vitro bioassay. **J. Environ. Monit**. 13:813-821, 2011. https://doi.org/10.1039/c0em00473a