Estrogenic Activity and Endocrine Disruptor Compounds Determined in Guanabara Bay (Brazil) by Yeast Estrogen Screen Assays and Chemical Analyses

Atividade Estrogênica e Desreguladores Endócrinos Determinados na Baía de Guanabara (Brasil) por Ensaios de Leveduras e Análises Químicas

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Abstract

Studies assessing the presence of endocrine disrupting compounds in marine environments have increased in the last decades. In Brazil, the combination of poor sanitation conditions and low investment in sewage treatment plants leads to significant contamination of receiving waters. The risks of these micropollutants in the aquatic biota include biochemical and histopathological alterations of the liver, gonads, and kidneys, as well as, reproductive process and development modifications, and behavioral changes, among others. The aim of this study was to evaluate the quality of the surface and deep waters of Guanabara Bay, southeastern Brazil, regarding the presence of estrogenic substances. Acute toxicity assays were also conducted employing *Vibrio fischeri*. The estrogenic activity of the water samples was determined by Yeast Estrogen Screen assay and the quantification of the Bisphenol A, estriol, 17β-estradiol, and 17α-ethinylestradiol by high-performance liquid chromatography, using fluorescence and diode array detectors. Estrogenic activity ranged from 9 to 77 ng L^{-1} of estradiol equivalents. The highest micropollutants concentrations were detected for bisphenol A (298.5 and 465.5 ng L^{-1}), followed by 17α-ethinylestradiol (248 and 256.9 ng L^{-1}), estriol (70.7 and 179.6 ng L^{-1}), and 17β-estradiol (167 and 174.8 ng L^{-1}) for surface and deep waters, respectively. The findings indicate significant risks for the Guanabara Bay ecosystem. No acute toxicity effects were observed in the *V. fisheri* assay. These data reflect the current environmental degradation situation of the bay's waters and highlight the need for the systematic monitoring of this important estuary.

Keywords: Micropollutants; HPLC; Acute toxicity

Resumo

Os estudos que avaliam a presença de compostos desreguladores endócrinos em ambientes marinhos têm aumentado nas últimas décadas. No Brasil, a combinação de péssimas condições de saneamento e baixo investimento em estações de tratamento de esgoto leva à contaminação significativa das águas receptoras. Os riscos desses micropoluentes na biota aquática incluem alterações bioquímicas e histopatológicas do fígado, gônadas e rins, por exemplo, bem como modificações no processo reprodutivo e no desenvolvimento, alterações comportamentais, entre outros. O objetivo deste estudo foi avaliar a qualidade das águas superficiais e profundas da Baía de Guanabara, sudeste do Brasil, quanto à presença de substâncias estrogênicas. Ensaios de toxicidade aguda também foram realizados usando *Vibrio fischeri*. A atividade estrogênica das amostras de água foi determinada pelo ensaio Yeast Estrogen Screen e a quantificação do Bisfenol A, estriol, 17β-estradiol e 17α-etinilestradiol por cromatografia líquida de alta eficiência, usando detectores fluorescência e arranjo de diodos. A atividade estrogênica variou de 9 a 77 ng L^{-1} de equivalente estradiol. Entre os micropoluentes, as maiores concentrações foram detectadas para bisfenol A (298,5 e 465,5 ng L^{-1}) seguido por 17α-etinilestradiol (248 e 256,9 ng L^{-1}), estriol (70,7 e 179,6 ng L^{-1}) e 17β-estradiol (167 e 174,8 ng L^{-1}) para águas superficiais e profundas, respectivamente. Os resultados indicam riscos significativos para o ecossistema da Baía de Guanabara. Nenhum efeito de toxicidade aguda foi observado no ensaio com *V. fisheri*. Esses dados refletem a atual situação de degradação ambiental das águas da baía e destacam a necessidade de monitoramento sistemático deste importante estuário.

Palavras-chave: Micropoluentes; HPLC; Toxicidade aguda

1 Introduction

Environmental degradation by anthropic activities strongly affects ecosystems, mainly due to exposure to various contaminants (Solaun et al. 2021). Even when present in concentrations in the order of µg L^{-1} and ng L^{-1}, several substances can cause risks to both human and animal health (Liu et al. 2018; Negintaji et al. 2018). Concerns regarding the quality of water resources are extremely relevant, due to significant increases in emerging micropollutants, as well as their by-products and metabolites originating from the chemical and biological degradation of the original compounds (Locatelli et al. 2016; Zhong et al. 2021). Factors associated with demographic expansion and industrial development, especially in the coastal areas of large industrial centers, result in serious environmental risks for estuarine ecosystems (Silva et al. 2011).

Micropollutants have been reported in several different environmental matrices (Cunha et al. 2020; Ismail et al. 2019; Shi et al. 2014), due to the various point and diffuse sources such as domestic and industrial effluents, solid waste, sewage sludge drainage, animal manure, leachate, landfills, among others (Chi et al. 2016; Huang, Karu & Campos 2021). According to Porseryd et al. (2017) and Abdel-Khalek (2018), exposure to micropollutants as endocrine disruptors lead to several adverse effects for a variety of species, such as reduced egg hatching, reproductive system issues, and immune system alterations in reptiles, birds, mammals, and fish (Li et al. 2019; Ismail et al. 2019). In humans, metabolic, cardiovascular, pulmonary, neuropsychiatric, neurodegenerative diseases, fertility problems, and various types of cancer have been reported (Li et al. 2021; Miret et al. 2019; Rocha et al. 2018).

Guanabara Bay, an estuary of significant importance located on the Southeastern Brazilian coastline, is considered one of the most polluted estuaries in Brazil (Baptista Neto et al. 2006). Located in the state of Rio de Janeiro and presenting high ecological and socio-economic relevance, it is exposed to several pollutant sources concerning numerous contaminants displaying toxic potential (Baptista Neto et al. 2016; Carreira, Wagener & Readman 2004; Fernandez et al. 2005). Studies in this estuary are relevant due to its enormous richness, productivity, biodiversity, and, especially, its importance in the maintenance and balance of other ecosystems. The impacts that reach this bay can result in serious consequences, both for the environment and for the surrounding human population (Carvalho et al. 2016).

In this context, the aim of this study was to investigate the presence of estrogenic substances using the *in vitro* Yeast Estrogen Screen (YES) assay and detection of endocrine disruptors, such as bisphenol A (BPA),

17β-estradiol (E2), estriol (E3) and 17α-ethinylestradiol (EE2) in certain Guanabara Bay areas, at two water column depths. In addition, the physicochemical parameters foresaw in Brazilian legislation and acute toxicity assay for the organism *Vibrio fischeri* was also determined carried out.

2 Methods

2.1 Study Area

Guanabara Bay, located in Rio de Janeiro, Southeastern Brazil (Figure 1), between 22º40'S and 23º00'S latitude and 043º00'- 043º18'W longitude, is one of the largest Brazilian coastline bays, comprising about 384 km^2. In the last 100 years, its surrounding area has been strongly modified by human activities, increasing the number of contaminants introduced mainly by sewage effluents industrial and domestics, urban and agricultural runoff, as well as atmospheric fallout (Amador 2012; Baptista Neto et al. 2006; Soares-Gomes et al. 2016).

The bay's coastline is 131 km long, with a mean water volume of 1.87x10^9 m^3 (Amador 2012). It measures 28 km from west to east and 30 km from south to north, but its narrow entrance is only 1.6 km wide (Soares-Gomes et al. 2016). A complex bathymetry with a relatively flat central channel is noted. The channel is 400 m wide, stretching from the mouth more than 5 km into the bay, and is defined by the 30 m isobath. The deepest point of the bay measures 58 m and is located within the main channel (Melo et al. 2015). According to these same authors, the channel loses its characteristics north of Rio de Janeiro-Niterói Bridge, as the bay rapidly becomes shallower, with an average depth of 5.7 m, due to high sedimentation rates, accelerated in the past century by anthropogenic activities in the catchment area. Guanabara Bay lies within the tropics of southeastern Brazil, but because of its coastal location, a humid subtropical climate with 2,500 mm (high altitudes) and 1,500 mm (low altitudes) of rainfall prevails between December and April. The mean annual temperature ranges between 20º and 25ºC (Nimer 1989). Bay receives untreated agricultural runoffs and urban and industrial sewage from surrounding rivers from the Rio de Janeiro metropolitan area, from two harbors, refineries, and over 12,000 industries located throughout the drainage basin, which account for 25% of the organic pollution released in this area (Baptista Neto et al. 2006; Soares-Gomes et al. 2016).

Bay's hydrographic basin measures approximately 4,000 km^2, drained by a total of 45 rivers, which contribute with an average annual flow of 100 m^3 s^{-1}. Of this total, six rivers are responsible for 85% of the mean annual freshwater
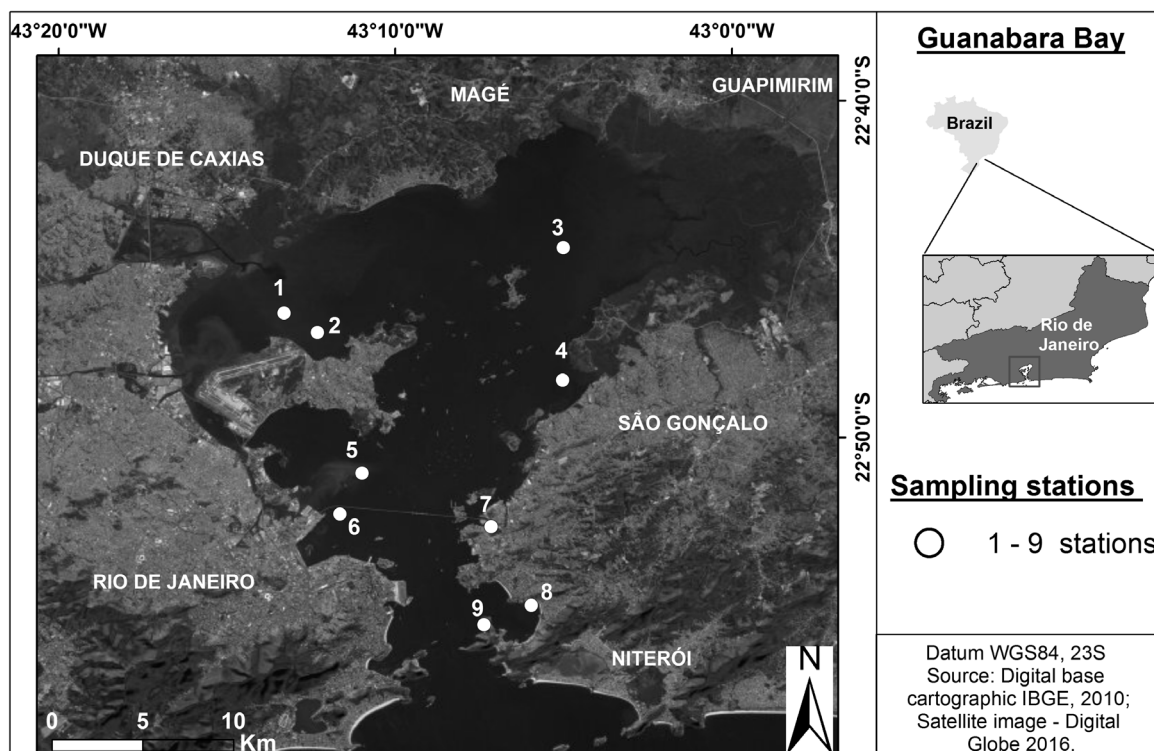
Figure 1 Location Guanabara Bay (Rio de Janeiro, Brazil) with sampling stations.

discharge, namely Guapimirim River (20.8%), Iguaçu River (16.7%), Caceribu River (13.7%), Estrela River (12.7%), Meriti River (12.3%) and Sarapui River (9.3%) (Baptista Neto et al. 2006; Soares-Gomes et al. 2016).

During the 2016 Brazil Olympics, Guanabara Bay gained international prominence, mainly due to serious criticism concerning its water conditions. The current environmental degradation of the bay has promoted socio-environmental consequences, such as serious damage to fishing activities and decreased productivity. These factors led to the departure of fishers from this activity, generating a social problem for several communities that used to basically survive on fishing. In addition, tourism, which before the industrial and population expansion of the metropolitan region was a relatively important sector, is currently practically non-existent (Soares-Gomes et al. 2016).

The release of untreated domestic sewage in the bay and in its tributaries is one of the main obstacles to be solved, as this type of pollution can cause environmental conservation and biota survival damage (Carreira, Wagener & Readman 2004). Therefore, multidisciplinary studies that integrate different conditioning factors and, above all, joint actions that provide a holistic view concerning contributions to minimizing the risks to this important water body, have become paramount (Baptista Neto et al. 2013).

2.2 Sampling

In December 2014, during the rainy period (summer), a total of 2 L of water samples were collected, in the surface and deep layer, at nine sampling stations in the Guanabara Bay. The sampling stations were chosen to provide a general view of the anthropic actions that contribute to the current pollution levels in this estuarine system. The location and description of each sampling station are displayed in Table 1.

Water samples were collected using a Van Dorn probe (AFK 34) and transferred to amber glass bottles. In order to avoid microbiological degradation of the compounds of interest, 10 mL of methanol (1% v/v) were added.

The samples were then transported in coolers and maintained at 4 °C in the laboratory until the analysis procedures. Analyses were carried out in triplicate for the chromatography analyses and in duplicate for the YES assay.

A total of 1 L of water was collected in amber glass bottles for the physicochemical parameters and 50 mL of water was collected in sterile polypropylene tubes for acute toxicity assay. Both samples did not receive preservatives and they were also kept refrigerated at 4 °C until the moment of analysis.

Table 1 Description, depth (m), and coordinates of the sampling stations in the Guanabara Bay.

Sampling stations	Depth (m) (surface – deep)	Description	Geographic coordinates
1	0.55 - 3.79	Governador Island	22°46'19"/43°13'17,9"
2	0.52 - 2.88	Governador Island - Southeast	22°46'53,9"/43°12'18,6"
3	0.46 - 4.37	Paquetá Island	22°44'22,7"/43°5'00,1"
4	0.49 - 2.39	São Gonçalo	22°48'19,0"/43°5'1,2"
5	0.62 - 4.09	Fundão Island	22°51'4,6"/43°10'59,0"
6	0.59 - 5.93	Portuary zone – Rio de Janeiro	22°52'17,3"/43°11'38,4"
7	0.53 - 3.64	Portuary zone - Niterói	22°52'39,9"/43°0,7'9,2'
8	0.46 - 2.41	Jurujuba Sound – Cachoeiras River	22°55'00,3"/43°5'57,6"
9	0.52 - 4.63	Jurujuba Sound – Fishing Boats	22°55'30,13"/43°6'55,91"

2.3 Reagents

All reagents used in the YES assay were obtained from Sigma-Aldrich®. The glasswares were cleaned with Merck® neutral Extran® detergent. Chromatography solvents HPLC grade were used, obtained from Tedia® Brazil. BPA, E2, E3, and EE2 standards (99.8% purity) were purchased from Sigma-Aldrich®. Ultrapure water was obtained by Milli-Q Biocell (Millipore®) and chlorophenolred-β-D-galactopyranoside (CPRG) by Merck®. The solid-phase extraction (SPE) of the samples was performed using Merck® branded hexane, methanol, ethanol, acetone, and acetonitrile. The ethyl acetate by J. T. Baker®, hydrochloric acid by Merck®, and ethylenediaminetetraacetic acid (EDTA) by Fluka®.

2.4 Sample Preparation and Solid-Phase Extraction (SPE)

Aliquots of 1 L were filtered through a 0.45 μm cellulose membrane (Merck®), with a vacuum pump (Manifold Agilent Technologies), and acidified at pH 3 with HCl (3 mol L^{-1}) to increase the interaction between the analytes and the stationary phase of the SPE cartridge. The cartridges (Strata X, 500mg per 6 mL, Phenomenex®) were preconditioned with 3 x (2 mL hexane, 1 mL acetone, 2 mL methanol) and 5 x 2 mL ultrapure water at pH 3. The samples were percolated through the cartridges at a flow rate of approximately 10 mL min^{-1} and subsequently kept under vacuum for 30 minutes. After which the analytes retained on the cartridges were eluted with 4mL of acetone at a flow rate of 5–10 mL min^{-1}. Finally, the solvent was evaporated under a gentle nitrogen flow to dryness. The extracts were then reconstituted with 2 mL of ethanol for the YES assay and 500 μL of acetonitrile for the HPLC analyses and stored at 4 °C.

2.4.1. *In vitro* YES Assay

The genetically modified *Saccharomyces cerevisiae* yeast, which contains a human estrogen receptor (ERh) DNA sequence, developed by Routledge and Sumpter (1996), was used for the YES assay. In the presence of estrogenic compounds, occurs an interaction with the human receptor, resulting in the expression of the Lac-Z receptor gene producing the β-galactosidase enzyme excreted in the medium, which in turn metabolizes the chromogenic substrate CPRG.

The assay was performed in 96-well microplates employing serial dilutions of sample extracts in ethanol. E2 was used as the positive control (standard curve from 2724 to 1.33 ng L^{-1}) and ethanol was used as the negative control. In a test plate, 10 μL of each sample dilution were transferred and allowed to evaporate. Subsequently, a 200 μL aliquot of the culture medium containing yeast and CPRG was added. The microplates were then sealed with adhesive tape, shaken vigorously on a plate shaker (IKA® MS3), and maintained for 72 hours at 30 ºC in an incubator (New Ethics, 410). The absorbance determinations at 575 nm (for color) and 620 nm (for turbidity) on a Spectramax® M3 plate reader (Molecular Devices).

The dose-response curves for the E2 standard were constructed using the concentration versus corrected absorbance, resulting in sigmoidal curves adjusted by the Source 6.0 software (Microsoft®). The estrogenic activity of the sample extracts was determined as estradiol equivalents (E2-EQ), by interpolation of the standard E2 curve in the assay. These values were divided by the SPE concentration factor, resulting in the final E2-EQ concentrations in the water samples. The mean EC50 value of the 17β-estradiol dose-response curve in the test period was 38 ± 10 ng L^{-1}. The limit of detection (LD) and limit of quantification (LQ) were 9 ± 3 ng L^{-1} and 28 ± 10 ng L^{-1}, respectively.

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Cytotoxicity may occur during the YES assay due to the presence of toxic compounds in the samples, which inhibit the growth of the *Saccharomyces cerevisiae* yeast. This inhibition can be visualized by the absence of turbidity at the bottom of the wells. According to Frische et al. (2009), the absorbance control at 620 nm is used as a tool to quantify yeast growth inhibition as a function of sample toxicity, according to Equation 1.

$$Toxicity = 1 - \left(\frac{ABS_{620\,sample}}{ABS_{620\,negativecontrol}} \right)$$

2.4.2. High-performance Liquid Chromatography (HPLC)

Chromatographic analyses were carried out on a liquid chromatograph (Waters Corporation®) with fluorescence (FLU) and diode array (DAD) detectors. The mobile phase consisted of 60% to 40% of acetonitrile and ultrapure water, with an extract injection volume of 20 μL, and the stationary phase used was a chromatographic column C18 Nova-pak® (4.6 mm x 250 mm x 5 μm).

Based on the analytical curves of the compounds, the linearity, recovery, LD, and LQ were calculated. Linearity was determined according to the standard curve by evaluating R and R^2 values. Thus, the LQ was obtained by the lowest concentration of the analytical curve and the LD, using Equation 2, with the standard deviation (SD) referring to three blank injections (standard with the lowest acceptable analyte condition). The recoveries of the target compounds ranged from 89% to 107%.

$$LD = SD \times 6.965$$

The correlation coefficients (R^2) of the curves were above 0.99 for all compounds. Concerning BPA, the LD was 1.889 μg L^{-1} and LQ of 62.5 μg L^{-1}. For the assessed estrogens, the LD values for E2, E3, and EE2 were 0.002, 0.002, and 0.005 μg L^{-1}, respectively, and the LQ values were 0.098, 0.013, and 0.074 μg L^{-1}, respectively.

2.5 Analytical Water Quality Determinations

Surface and deep water quality was evaluated based on physicochemical parameters established in the Brazilian legislation, on the methods described by the American Public Health Association (APHA, AWWA & WEF 2012): pH (4500 - H + B), turbidity (2130 B), conductivity (2510 B), ammoniacal nitrogen (4500 - NH3 D), chloride (4500 - Cl - B), dissolved organic carbon (DOC) (5310 B), and total suspended solids (TSS) (2540 C).

2.6 Acute Toxicity

Acute toxicity assay was performed using *Vibrio fischeri* (luminescent marine gram-negative bacterium) and Microtox SDI 500 analyzer (Microtox Omni® 4.1), according to NBR 15411-3 standard (Associação Brasileira de Normas Técnicas 2005). This toxicity test requires small amounts of samples and has been found suitable for evaluating the toxicity of Guanabara Bay waters, as *Vibrio fischeri* is a saltwater organism (Kahru, Kurvet & Külm 1996). Toxicity was determined by decreased bacteria luminescence, comparing initial levels with recorded values after 0, 15, and 30 minutes of exposure. Bacteria were exposed to 81.9% dilution water samples in four serial dilutions using 2% NaCl as the diluent control (Nascimento et al. 2018).

3 Results and Discussion

3.1 Estrogenic Activity

The estrogenic activity assay detected E2-EQ values of 8.96 to 76.85 ng L^{-1}, considering surface and deep waters. The highest detections were observed at stations 8 and 9, located in Jurujuba Sound, which may be associated with the fact that Jurujuba Sound is a restricted environment with lower hydrodynamics compared to the other sampling stations (Baptista Neto et al. 2006). Cytotoxicity was observed during the YES assay for samples from stations 3, 4, 7, 8, and 9. After serial dilutions, cytotoxicity was reduced until it did not interfere with E2-EQ determinations. Figure 2 exhibits the E2-EQ values of the water samples collected at the nine Guanabara Bay stations.
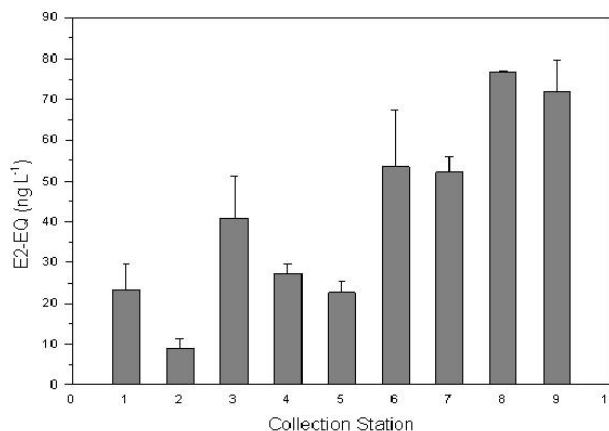

Figure 2 Estradiol equivalent (E2-EQ) values for water samples obtained from nine water sampling stations located throughout Guanabara Bay.

Several studies have assessed estrogenic activity in environmental samples. For example, one study was conducted in three large tributaries of the Bohai Sea in Tianjin, China, reporting E2-EQ results ranging between 5.72 to 59.06 ng L^{-1} (Rao et al. 2013). In southern China, values reported for surface waters of the Pearl River ranged between 0.23 to 324 ng L^{-1}, and in sediments, between 0 to 101 ng L^{-1} (Zhao et al. 2011). In saline waters, such as the Baltic Sea, values have been reported as ranging from 0.01 to 0.82 ng L^{-1} (Beck, Bruhn & Gandrass 2006). Truter, Wyk & Newman (2015) observed high concentrations at the mouth of the Sal River in Cape Town, of 20.96 ng L^{-1} which, according to the authors, are higher than the lowest concentrations reported in the literature, suggesting possible adverse effects on aquatic organisms.

In Brazil, values detected in Jurujuba Sound (saline waters) in previous assessments ranged from 0.5 to 3.2 ng L^{-1} (Nascimento et al. 2018). In surface waters of the Guandu River, in Rio de Janeiro, results ranged from 0 to 16 ng L^{-1}, which, after filtering through 1.2 μm membranes, ranged from 0 to 3.1 ng L^{-1} (Dias et al. 2015). Recently, a study carried out in different environmental matrices, in two lagoons near Guanabara Bay, reported high estrogenic activity in suspended particulate matter and surface water, 67.06 ng L^{-1} and 70.39 ng L^{-1}, respectively (Cunha et al. 2020). According to the same authors, untreated domestic wastewater contains high concentrations of estrogenic compounds (both natural estrogens and synthetic) and, consequently, high estrogenic activities (Cunha et al. 2020).

Studies carried out at Guanabara Bay report differential environmental contamination levels of anthropogenic origin, reflecting different contamination sources (Baptista Neto et al. 2006; Baptista Neto et al. 2013; De Carvalho & Baptista Neto 2016; Fistarol et al. 2015; Fonseca et al. 2013; Francioni et al. 2005; Silva et al. 2003, Soares-Gomes et al. 2016). Regarding estrogenic activity, some stations located in Jurujuba Sound, have been reported as exhibiting significant increases in estrogenic activity levels (Nascimento et al. 2018) corroborating with the present study, especially for stations 8 and 9 (Figure 2).

It is important to highlight that studies on endocrine disrupters, especially in saline environments, are still scarce for organisms, sediments, and water (Fernandez et al. 2005; Silva et al. 2003; Xavier, De Andrade & Moreira 2002). Thus, the identification of endocrine disrupters in Guanabara Bay waters is an important baseline assessment for this degraded environment.

3.2 BPA and E2, EE2, and E3 Concentrations

The frequency of BPA, E2, E3, and EE2 evaluated in surface and deep water samples for the nine Guanabara Bay sampling stations are displayed in Figure 3. The compounds frequency was BPA>E2>E3>EE2 and the highest concentrations of contaminants were noted for the Jurujuba Sound samples, where BPA>EE2>E3>E2. These results are probably due to the local pollution sources, which include intense slum processes towards cliff slopes, luxury houses, two hospitals (maternity and psychiatric), a graveyard, a waterway terminal, several restaurants, and intense vehicle circulation in the area. In addition, Jurujuba Sound is a restricted area undergoing lower wave effects, which can increase pollutant concentrations (Silva et al. 2016).
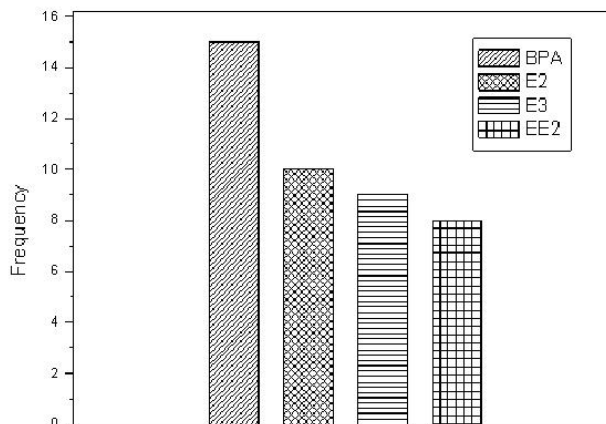

Figure 3 Compounds frequency for water samples obtained in the nine sampling stations located throughout Guanabara Bay.

BPA concentrations in surface water samples varied from 14 to 298.5 ng L^{-1} with the highest values detected at stations 3, 6, 8, and 9. These areas are highly degraded by the continuous input of several types of pollutants. In deeper water, BPA values ranged from 51.2 to 465.5 ng L^{-1}, with higher concentrations noted at stations 8 and 9. These BPA results are higher than the other evaluated micropollutants, mainly in deeper water at the two stations of the Jurujuba Sound. This may be indicative of higher concentrations nearer bottom sediments (Viganò et al. 2008; Ferreira, Horta & Cunha 2010). The BPA values observed herein are lower compared to surface waters reported by Yamazaki et al. (2015) ranging from 54 to 1950 ng L^{-1};

Montagner, Vidal and Acayaba (2017) from 2.8 to 39860 ng L^{-1} and Cheng et al. (2018) between 15-1415 ng L^{-1}. Other studies, however, indicate higher or similar values than in the present study, i.e. Caldwell et al. (2010) from 29.2 to 124 ng L^{-1}; Selvaraj et al. (2014) between 2.8 to 136 ng L^{-1}; Shi et al. (2014) between 0.98 to 43.8 ng L^{-1} and Czarczyńska-Goślińska et al. (2017) 5.0 to 95 ng L^{-1}.

Differential marine and freshwater BPA degradation processes have been reported (Kang & Kondo 2005), suggesting that BPA contamination in marine organisms may be higher than in freshwater organisms. Gu et al. (2016) reported BPA ranging from the < LQ to 13.06 ng g^{-1} in 95 samples of wild-marine biota and in 88 samples from the East China Sea (Yangtze River Delta), and highlighted that, depending on the species distribution, the trophic transfer may occur, leading to potential health risks, especially concerning seafood consumption.

Regarding estrogen results, E2 concentrations in surface water ranged from 33.5 to 167 ng L^{-1}, with the highest concentrations observed at stations 3 and 6. For the deep water samples, values ranged from 34.5 to 174.8 ng L^{-1}, with higher concentrations detected at stations 6, 8, and 9. For E3, values ranged from 29.7 to 70.7 ng L^{-1}, with a higher concentration noted at station 7, while deep water samples ranged between 28.4 and 179.6 ng L^{-1}, with higher concentrations observed at stations 6 and 9. EE2 in surface water ranged between 110.6 and 248 ng L^{-1}, with the highest concentrations detected at stations 1, 5 and 7 while in the deep water samples ranged between 42.2 to 256.9 ng L^{-1}, with higher concentrations observed at stations 6, 8, and 9.

In a data survey concerning 44 published articles evaluating 193 compounds from different classes of Brazilian surface waters (in about 75 water bodies), high endocrine disruptor values were reported associated to sanitation conditions, population density and types of economy developed in different states (Montagner, Vidal & Acayaba 2017). Ecotoxicological effect assessments have established a limit of 0.4 ng L^{-1} for E2 in water bodies (Kunz et al. 2017). According to the Scientific Committee on Risks to Health and the Environment (SCHER 2011), E2 concentrations around $\geq$ 1.0 ng L^{-1} may already represent risks for the reproductive capacity of fish, for example.

Likewise, the proposal for EE2 was set at 0.035 L^{-1} for water bodies (Cunha et al. 2016; European Commission Directive 2013; Gilbert 2012; Kunz et al. 2017). Therefore, the concentrations detected in the Guanabara Bay indicate potential risks for this important estuary, reinforcing the need to perform further studies and carry out monitoring programs in the study area and implement actions that reduce potential adverse effects on coastal ecosystems (Cotrim et al. 2016). It is also important to take into account additive, synergistic and antagonistic effects that may occur in the aquatic environment (Costa et al. 2008).

3.3 Physicochemical Analyses

Physicochemical parameters (Table 2) were assessed according to maximum reference values for class I water bodies (saltwater). Salinity varied between 30.6 and 35.2, pH between 7.0 and 8.0, turbidity from 6.2 to 15.2 NTU, N-NH$_3$ from 1.3 to 2.5 mg L^{-1}. TSS values ranged from 63 to 97 mg L^{-1}, conductivity from 60.0 to 99.2 mS cm^{-1} and DOC between 9.4 and 16.7 mg L^{-1} (APHA, AWWA & WEF 2012).

It is important to highlight that the samplings were carried out at high tide and during a high rainfall period. Therefore, the results may be related to factors that contribute to water quality alterations, such as tidal influence, circulation, winds and rainfall patterns, among others (Melo et al. 2015). Water quality assessments are paramount since the challenge of sanitation in Brazil is still far from being overcome. Likewise, pollution source control is required in order to provide integrated subsidies for water quality assessment (Soares-Gomes et al. 2016).

3.4 Acute Toxicity

The acute toxicity assay employing the *Vibrio fischeri* bacterium is performed by evaluating the bacteria's decrease in luminescence. In the present study, no decreases in the bioluminescence of the surface and deep water samples were noted compared to the control, so the samples were considered non-toxic. However, assays conducted with organisms belonging to other trophic levels are also required.

Table 2 Physicochemical parameters determined in water surface and deep water samples in the Guanabara Bay (S - surface water/ D - deep water).

Samples	Turbidity (NTU)	pH –	Salinity ‰	N-NH$_3$ (mg L^{-1})	Conductivity (mS cm^{-1})	DOC (mg L^{-1})	TSS (mg L^{-1})
1S	15.2	7.9	30.6	1.6	65.1	9.4	96
1D	13.0	8.0	34.5	1.7	60.0	9.7	94
2S	11.6	7.6	30.8	2.5	70.0	11.6	88
2D	9.5	7.2	34.7	2.2	71.1	14.1	91
3S	10.4	7.5	32.3	1.5	77.4	14.3	66
3D	8.0	7.2	33.2	1.3	75.6	14.6	74
4S	9.7	7.0	33.0	1.9	83.9	14.9	82
4D	8.2	7.2	34.0	2.0	80.5	15.0	89
5S	11.2	7.4	34.5	1.3	99.2	15.2	89
5D	9.1	7.0	33.5	1.5	96.7	15.4	97
6S	10.4	7.1	34.0	1.9	87.8	15.6	86
6D	8.4	7.5	32.5	2.1	84.9	16.2	92
7S	8.9	7.3	34.1	1.7	94.4	16.6	72
7D	6.2	7.9	32.5	1.7	89.9	14.9	85
8S	10.3	7.2	34.0	1.1	84.6	13.4	67
8D	9.1	7.0	34.6	1.2	81.4	14.7	72
9S	11.1	7.0	34.6	1.7	91.3	15.3	63
9D	9.9	7.3	35.2	1.9	90.6	15.7	80

*Ammoniacal nitrogen (N-NH$_3$); dissolved organic carbon (DOC); total suspended solids (TSS).

4 Conclusion

The combination of chemical tests and bioassay is important to assess the presence of micropollutants in environmental matrices, in addition to investigating the real harmful effects of these pollutants on the environment. *In vitro* bioassays detect the global (combination) effect of complex mixtures as a result of the sum of substances displaying the same mechanism of action and not neglecting unknown substances. Thus, the *in vitro* YES assay allowed for the determination of the estrogenic activity of the investigated samples.

Estrogenic activity and endocrine disruptor levels indicate risks for the Guanabara Bay estuary environment. Although acute toxicity assay was not observed, further tests with organisms belonging to other trophic levels and chronic tests should be performed. Water quality values were within the reference limits established in the Brazilian legislation for class I saltwater bodies. Further assessments and continuous monitoring are required to advance quantitative and qualitative research concerning estrogenic activity and endocrine disruptors in water bodies, as a lack of general assessments in this regard is noted.

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6 References

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