Anuário do Instituto de Geociências
Universidade Federal do Rio de Janeiro
https://revistas.ufrj.br/index.php/aigeo/

ISSN 0101-9759
e-ISSN 1982-3908

ENVIRONMENTAL SCIENCES

Estrogenic Sediment Activity and Toxicity at the Cunha Channel Watershed, a Major Micropollutant Contributor to Guanabara Bay, the Most Polluted Estuarine System in Southeastern Brazil

Atividade Estrogênica e Toxicidade de Sedimentos na Bacia Hidrográfica do Canal do Cunha, um dos Principais Contribuintes de Micropoluentes para a Baía de Guanabara, o Sistema Estuarino Mais Poluído do Sudeste do Brasil

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Abstract

Disruptors cause hormonal function disturbances and adverse effects in animals and humans, even at low concentrations in the micro and nanogram range, and have become a serious environmental concern. In this sense, this study aimed to determine the estrogenic activity and toxicity of sediment samples from the Cunha Channel watershed, a major contributor of micropollutants to Guanabara Bay, the most polluted but most productive estuarine system in Rio de Janeiro, southeastern Brazil. Estrogenic activities were determined by the in vitro Yeast Assay Estrogen Screen (YES) and acute toxicity was determined using the *Vibrio fischeri* bacteria assay, in samples processed with protocols for wet and dry sediments. The wet test was the most efficient, with values ranging from 3.4 to 17 $ng.g^{-1}$,while sample drying led to no detectable estrogenic activity and with toxicity in all samples except one (0.16 $ng.g^{-1}$). All samples were categorized as very toxic or toxic in the acute toxicity assessment, suggesting high Ethylene Dichloride contamination in the Cunha Channel. This is due to anthropogenic and *Vibrio fischeri* the lack of local sanitation policies, indicating major environmental concerns in one of the most productive estuarine sites in the state of Rio de Janeiro, southeastern Brazil. This assessment contributes to studies in estuaries estrogenic compounds, which are still extremely scarce worldwide.

Keywords: Endocrine disruptor; YES; *Vibrio fischeri*

Received: 28 January 2025; Accepted: 23 June 2025

Resumo

Disruptores causam distúrbios na função hormonal e efeitos adversos em animais e humanos, mesmo em baixas concentrações na faixa de micro e nanogramas, e se tornaram uma séria preocupação ambiental. Nesse sentido, este estudo teve como objetivo determinar a atividade estrogênica e a toxicidade de amostras de sedimento da bacia hidrográfica do Canal do Cunha, um dos principais contribuintes de micropoluentes para a Baía de Guanabara, o sistema estuarino mais poluído, mas mais produtivo, no Rio de Janeiro, sudeste do Brasil. As atividades estrogênicas foram determinadas pelo Yeast Assay Estrogen Screen (YES) *in vitro* e a toxicidade aguda foi determinada usando o ensaio da bactéria *Vibrio fischeri*, em amostras processadas com protocolos para sedimentos úmidos e secos. O teste úmido foi o mais eficiente, com valores variando de 3,4 a 17 $ng.g^{-1}$, enquanto a secagem da amostra não levou a nenhuma atividade estrogênica detectável e com toxicidade em todas as amostras, exceto uma (0,16 $ng.g^{-1}$). Todas as amostras foram categorizadas como muito tóxicas ou tóxicas na avaliação de toxicidade aguda, sugerindo alta contaminação por EDC no Canal do Cunha. Isso se deve à pesca antropogênica e à falta de políticas locais de saneamento, indicando grandes preocupações ambientais em um dos estuários mais produtivos do estado do Rio de Janeiro, sudeste do Brasil. Esta avaliação contribui para estudos sobre compostos estrogênicos em estuários, ainda extremamente escassos em todo o mundo.

Palavras-chave: Desregulador endócrino; YES; *Vibrio fischeri*

1 Introduction

The significant pressures on coastal areas worldwide due to high human population densities and resulting anthropogenic activities have led to continuous and enormous discharges of pollutants. The sources of these pollutants are untreated domestic and industrial effluents, solid waste, animal manure and leachate, among others (Chi et al. 2016; Huang et al. 2021; Xu et al. 2021), which are discharged into coastal aquatic systems (Lapworth et al. 2012; Köck-schulmyer et al. 2019; Corrêa et al. 2021).

Disrupting compounds (EDCs), also called emerging microcontaminants or micropollutants, have become a significant environmental concern as they exhibit mutagenic, genotoxic, and carcinogenic effects in animals and humans (Solaun et al. 2021), even at very low concentrations, in the micro and nanogram range (Bila & Dezotti 2007; You & Song 2021). The effects of EDCs include reduced egg hatching and alterations in the reproductive immune system in reptiles and importex, hermaphroditism, masculinization, and feminization in mussels, fish, turtles, birds, and mammals (Janex-Habibi et al. 2009; Li et al. 2019). Furthermore, these compounds have been reported to be capable of bioaccumulating and biomagnifying along food chains, leading to serious ecological concerns (Salgueiro-González et al. 2015; Wang et al. 2018; Peng et al. 2018). In humans, the effects range from fertility problems to metabolic, cardiovascular, pulmonary, psychiatric, and degenerative diseases, as well as several types of cancer (Halem et al. 2014; Rocha et al. 2018; Miret et al. 2019; Li et al. 2021).

These compounds have been detected in different types of aquatic environments, such as estuaries, rivers, lakes, and oceans (Ismail et al. 2019; Nascimento et al. 2022), both in water and in sediment. Sediments, especially fine sediments due to their high organic matter content), constitute a significant sink for pollutants (Ali et al. 2018; Poleto & Mertem 2006), capable of accumulating contaminants for long periods of time (Aragão et al. 2006; Esteves 1988). In addition to plastic waste, such as fishing products with nets made of plastics and synthetic fibers considered responsible for huge amounts of microplastics, as well as weights made of lead that have estrogenic activity and can affect environmental quality (Rocha et al. 1985; Zanella 2013; Baptista Neto et al. 2019; Wood et al. 2024). This, in turn, leads to considerable ecological risks, especially for benthic organisms that inhabit the substrate-water interface, such as many crustaceans (shrimp, crabs) and fish, both bony and cartilaginous (Kim et al. 2019; Santos et al. 2022). In this scenario, EDC efforts become even more paramount (Zhao & Lung 2022; Müller et al. 2021). Although estuarine environments are significantly important areas worldwide, both in ecological and public health scenarios (León-Silva et al. 2016; Pinho et al. 2023), EDC assessments in estuaries, especially in the tropics, are still scarce. Therefore, this study aimed to determine the activities and toxicity of estrogenic compounds in sediment samples from the Cunha Channel watershed, a major contributor of micropollutants to Guanabara Bay, the most polluted but most productive estuarine system in Rio de Janeiro, southeastern Brazil.

2 Methodology

2.1 Study area

The Cunha Channel watershed, within the Cunha sub-basin, in the municipality of Rio de Janeiro, Rio de Janeiro, southeastern Brazil, is located in a highly urbanized area in the metropolitan area of Rio de Janeiro, crossing totally or partially 36 districts in the city of Rio de Janeiro (Amador 2012) to the main estuary of the state, Guanabara Bay. Guanabara Bay is one of the most polluted estuarine systems, but still one of the most productive in the state, exhibiting high socioeconomic importance for the entire state of Rio de Janeiro. The Cunha Channel sub-basin occupies 7,015.99 ha, with only 415.43 ha still comprising natural areas, and a massive absence of vegetation forms a huge heat island over the region, which is alleviated by the constant heat exchanges with the neighboring Guanabara Bay. This The sub-basin is considered one of the most polluted in Brazil, encompassing 133 favelas and 24 clandestine or irregular subdivisions, leading to poor water quality due to solid waste and raw sewage inputs (Amaral 2006). This is aggravated by the presence of a nearby landfill, several refineries and intense occupation of riverbanks, as well as the presence of many solid waste and raw sewage inputs. The sub-basin is also crossed by many roads, leading to high levels of pollution associated with the burning of fossil fuels (Amaral 2006). Thus, the areas surrounding the sub-basin are categorized by a high degree of environmental and social degradation, including precarious employment, low levels of education and health below adequate levels, and inadequate sanitation (Pereira 2012).

2.2 Reagents and glassware

All glassware was previously washed with analytical grade alcohol and acetone (HPLC grade, Tedia Brasil®). All solvents (hexane, methanol, ethanol, acetone, acetonitrile) were HPLC grade from Tedia Brasil® and the reagents used to perform the YES assay were purchased from Sigma Aldrich®. Ultrapure water was obtained from a Milli-Q Biocell system (Millipore). Chlorophenol red-β-D-galactose (CPRG) was purchased from Merk®. Two different YES assays were performed, in dry sediment (EMBRAPA 1999) and wet (Lorenzen et al. 2004), to evaluate the thermal decomposition or volatilization of estrogenic compounds and considering that more diluted samples may contain very low levels of estrogenic substances (Lorenzen et al. 2004).

2.3 Sediment sampling

Ten surface sediment samples were collected along the Cunha Channel (Figure 1) in the dry season of (June, 2015) with the aid of a Van Veen dredge. The samples were stored in amber bottles (300g) previously sterilized with HNO3 and Milli -Q water and taken to the laboratory where they were kept at 4°C until analysis. The sampling points were as follows: Point 1 is where the Cunha Channel meets Guanabara Bay; point 2 is located near a densely populated favela; point 3 is below a high-traffic area; point 4 is where the Cunha Channel meets the Fundão Channel, a significant source of pollution; point 5 is near a sewage treatment plant; point 6 is where the Cunha Channel meets the Faria Timbó River near an inactive oil refinery; point 7 is near a second high-traffic area and the inactive oil refinery; point 8 is the Faria Timbó River near a second favela in the second high-traffic area; point 9 is the Faria Timbó River near a third favela; point 10 is the Cunha Channel near a second high-traffic area. These sampling points are presented in more detail in Figure 1.

2.4 Sample processing

Two protocols tests were with the ediment samples, one wet and one dry, for comparison of protocols. Regarding the protocol Dry sediment, 100 g of each sediment sample was placed in a 250 mL beaker and mixed with 100 mL of Milli -Q water and left to stand for 24 h, with the pH measured at the beginning and end of this period. After 24 h, the water was discarded and the suspension was placed in an oven at 60º for 48 h until completely dry. The samples were then homogenized with the aid of a pestle and mortar until a fine sediment was obtained. A 10 g aliquot was used for extraction by sonication with 10 mL of methanol for 5 min. The liquid phase was then separated by centrifugation at 2,500 rpm for 5 minutes. After repeating this procedure three times, the supernatants were combined, obtaining a final volume of 30 mL, which was then mixed with 170 mL of Milli -Q water and acidified with H2SO4 to pH 2.

Regarding the protocol wet sediment test, applied only at points 1, 2, 5 and 10, considering the highest incidence of contaminant inputs, 30 g of wet sediment were separated and stored for 5 h in crucibles in a desiccator. Ten-gram aliquots of each sample were then mixed with 10 mL of methanol and sonicated for 5 min. The liquids were separated by centrifugation and pooled following the same procedure described above, and the final volume was then mixed with 170 mL of Milli-Q water and acidified with H2SO4 to pH 2. Another 10-g aliquot was oven-dried for 24 h to determine the dry weights of the samples.

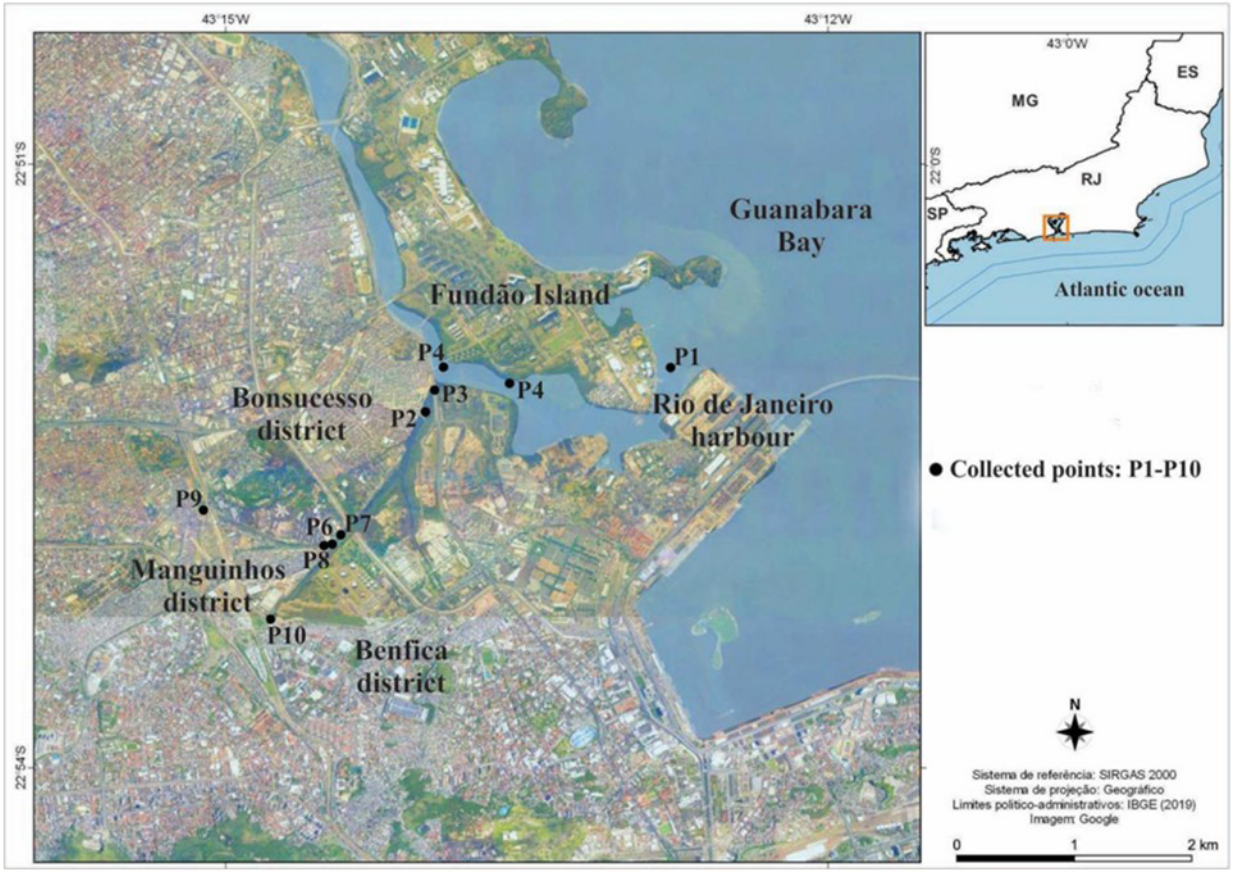

Figure 1 Map of surface sediment sample collection points along the Cunha Channel, in city of Rio de Janeiro, southeastern Brazil.

2.5 Estrogenic activity assay

Estrogenic activity was determined by the in vitro Yeast assay Estrogen Screen (YES). A standard solution of 17β-estradiol (E2) and sample extracts were serially diluted in ethanol and 10 µL of each dilution was transferred in duplicate to a flat plate and evaporated. Then, 200 µL of the assay medium (growth medium, yeast and CPRG) was pipetted into 96-well test plates and each E2 dilution series was used as a calibration curve. The plates were sealed with tape and shaken vigorously on a plate shaker for 2 min, incubated in the dark at 30 °C for 72 h and absorbances were determined at 540 nm for color development and estrogenic activity determinations and 620 nm for turbidity correction (Coleman et al. 2004, Equation 1) using a Softmax Pro 5 Spectra Max M3 plate reader. The quantification limit (LQ) was determined according to INMETRO (2010) at 0.035 ng g^{-1}.

$$Abs_{sample} = Abs575_{sample} - (Abs620_{sample} - Abs620_{negative\ control}) \quad (1)$$

Estrogenic activity was calculated in estradiol equivalents (E2-EQ) by interpolating the sample curve with the dose-response curve of 17β-estradiol, expressed in ng L^{-1} and transformed into ng.g^{-1} considering the amount of sediment used in the extraction. The toxicity effects were determined by a yeast cell growth inhibition assay, determined by the decreased absorbance values at 620 nm compared to the negative control (Bistan et al. 2011; Beck et al. 2006; Beresford et al. 2000), using Equation 2 (Frische et al. 2009).

$$Toxicity=1-(\llbracket ABS \rrbracket\_(620\ sample)/\llbracket ABS\_(620\ negative\ control)) \quad (2)$$

2.6 Acute toxicity test

Acute toxicity levels of sediments were determined using a strain of *Vibrio fischeri* bacteria freeze-dried that decreases its light emissions after exposure to estrogenic compounds. Different devices and agitation times were compared to determine the optimal sediment extraction procedure, the first according to the Microtox solid phase protocol of the Microtox software Omni 4.1 (Pérez et al. 2012) and the second, according to the wet phase protocol, Environmental described by AZUR (2015).

Microtox Solid Phase Protocol consists of using 7 g of dried sediments at 60º C mixed with 100 mL of 3.5% NaCl and magnetically stirred at 500 rpm for 2 hours, followed by separation of the supernatant after a 1-hour rest. The wet phase protocol, Environmental AZUR (2015) used 10 g of oven-dried sediment at 60 oC and resuspended with 100 mL of 35% NaCl followed by orbital shaking at 200 rpm for 24 h, followed by separation of the supernatant after a 1-hour rest. Subsequently, the supernatants of both protocols were mixed with 0.02 g of EDTA to remove metallic interferences (Pérez et al. 2012).

Each sediment sample was serially diluted in 3.5% NaCl solution and 900 µL of each dilution was added to 10 µL of the diluted bacterial solution in borosilicate cuvettes. The luminescence of a 10 µL solution containing the pure bacteria was determined at the beginning of the assay using an SDI 500 Microtox system, a precision photometer specially developed for this bioassay, according to the Brazilian standard NBR 15411-3 (ABNT 2005). Then, 900 µL of each sample was transferred to the cuvettes, homogenized manually, incubated at 15º C for 30 minutes and the emitted light was then determined. A positive control (blank) was prepared containing only 3.5% NaCl and five dilutions, in duplicate. The Microtox software Omni 4.1 was used to determine the 50% effect concentration of the test organisms (EC50), and the analyzed results were compared with the toxicity ranges described by Bulich (1982) and modified by Coleman and Quereshi (1985), described in Table 1.

Table 1 Toxicity ranges for the Microtox® system, adapted from Bulich (1982).

EC_{50} – $\%_{v/v}$	Classification
< 25	Very toxic
25-50	Toxic
51-75	Moderately toxic
>75	Slightly toxic

3 Results and discussion

3.1 Estrogenic activity

The detections of estrogenic compounds in the protocol with dry sediments were much lower than those obtained with wet sediments. In dry sediment, detection was possible only at sampling point 1 (0.16 $ng.g^{-1}$). According to Lorenzen et al. 2004, this occurs due to heating of the sediment, thermal decomposition and volatilization of the compound that can influence detection. Research by Grund et al. 2011, carried out in the Upper Danube, Germany, analyzed with dry sediments, had results that corroborate this study. Of the nine samples analyzed, only five presented estrogenic activity, ranging from 0.03 to 1.3 ng g^{-1} (Grund et al. 2011).

On the other hand, the wet sediment test detected higher values of estrogenic activity, with results of 3.4 $ng.g^{-1}$ and 17 $ng.g^{-1}$ at sampling points 1 and 2, respectively. Thus, comparing the protocols, the wet sediment test proved to bemore efficient for determining estrogenic activity. Similar results have been reported worldwide. In the Haihe and Dagu Rivers in China, estrogenic activity ranged from 8.24 to 95.28 $ng.g^{-1}$ (Song et al. 2006). In the Rhine River in Germany, it ranged from 1.03 to 5.14 $ng.g^{-1}$ (Schulze-Sylvester et al. 2016). Also in Germany, estrogenic activities in sediments from the Elbe River and along the Luppe River ranged from 20 ± 2.4 µ gg^{-1} (Müller et al. 2019).

The study by Wang et al. (2011) evaluated the estrogenic activity of water and sediment samples from the Liao River in northeastern China. Values ranged from not detected to 4.76 $ng.g^{-1}$ (wet) and from not detected to 6.04 $ng.g^{-1}$ (dry). Still in China, in analysis of water and sediment samples from the Yellow River, estrogenic activity was observed at higher levels in the rainy (wet) season (1.29 $ng.g^{-1}$) compared to the dry season (0.45 $ng.g^{-1}$) (Wang et al. 2012). In the Santa Lucia Basin, Uruguay, values ranged from not detected to 8.49 $ng.g^{-1}$ (Griffero et al. 2018), in the Po River, Italy around 15.6 $ng.g^{-1}$ (Viganò et al. 2008), and from not detected to 101 $ng.g^{-1}$ in the Pearl River System in southern China (Zhao et al. 2011).

Cytotoxicity, which can cause decreased yeast growth or death (Frische et al. 2009), was observed in samples from stations 3, 5 and 10, even after serial dilutions (Comprehend 2002). This indicates very high concentrations of estrogenic compounds. Site 3 is located below a high-traffic area, site 5 is located near a sewage treatment plant and site 10 near a second high-traffic area. High concentrations of micropollutants, and consequently elevated estrogenic activity levels, near sewage treatment plants are expected. This is because most sewage treatment technologies are unable to remove micropollutants from raw sewage (Margot et al. 2015), which end up being discharged into water bodies. The findings reported in this assessment indicate the high toxic estrogenic potential of the Cunha Channel and serious potential ecological and public health risks, made even more likely by the continued inputs of multiple micropollutant sources along this tributary of Guanabara Bay.

Effective actions regarding basic sanitation and adequate infrastructure are therefore Paramount (Kuster et al. 2009; Montagner et al. 2017). However, Brazilian environmental legislation is considered quite precarious in this regard (Meyer et al. 1999; Sodré et al. 2007), generating

social, economic and environmental impacts (Rosman 2011; Hauser-Davis et al. 2019) due to environmental degradation (Ternes et al. 1999; Ismail et al. 2019). This becomes even more worrying due to the high socioeconomic importance of Guanabara Bay, which provides many important ecosystem services for southeastern Brazil. The bay, in fact, supports the most productive estuarine fishery in Rio de Janeiro, supporting a significant number of fishermen and providing a large quantity of fishery products consumed in the state (Jablonski et al. 2006; Prestrelo & Vianna 2016). In addition, it is also home to several species of invertebrates and fish and encompasses several ecosystems, with high historical, environmental, cultural, touristic and landscape importance, comprising ecosystem resources of extreme importance and considered a priority area for conservation according to the criteria defined by the Federal Government (Teixeira-Leite et al. 2018). It is also important to highlight that studies on estrogenic activity in estuarine systems are also severely lacking worldwide, especially in Brazil, making this type of evaluation essential (Pusceddu et al. 2019; Montagner et al. 2019; Branco et al. 2021; Shao et al. 2019; Luo et al. 2019).

3.2 Acute toxicity test

The two protocols tested, according to Pérez et al. (2012) (Microtox solid phase protocol) and AZUR (2015) (The Environmental protocol), were relatively similar except for sampling points 1 and 2 (Figure 2). The toxicity results were <25 in all samples prepared according to Pérez et al. (2012). These samples were categorized as very toxic, except for Point 2, which was considered moderately toxic. This was probably due to the concentration of micropollutants during the drying process. The samples prepared according to the AZUR (2015) protocol were considered toxic (<19) in all samples. Figure 2 presents the acute toxicity results according to both preparation methods.

All sediments in the Cunha Channel were classified as very toxic, similar to other reports around the world. For example, water sediment samples from the Çorlu and Ergene rivers in Turkey were classified as toxic, as it is a highly contaminated area (Günesf et al. 2008), while sediments from the Po River in Italy (Viganò et al. 2003) were considered moderate to low toxic. In contrast, sediments from the Juqueri River (São Paulo, Brazil) were not classified as toxic (Hwang et al. 2009). Which can be attributed to the synergistic and suppressive effect and the different methodology used. Similarly, Sousa et al. (2014) who evaluated 217 water and sediment samples from Baixada Santista stations in São Paulo state, Brazil, did not report acute toxicity, similar to water from the Maracanã River and the Mangue Channel in the city of Rio de Janeiro, Brazil, both located in highly degraded areas (Nascimento et al. 2022). In this sense, although some studies report low toxicity values, it is important to consider the processes of accumulation and biomagnification of estrogenic compounds in the trophic chain (Brigante & Espíndola, 2003) and the effects at higher trophic levels.

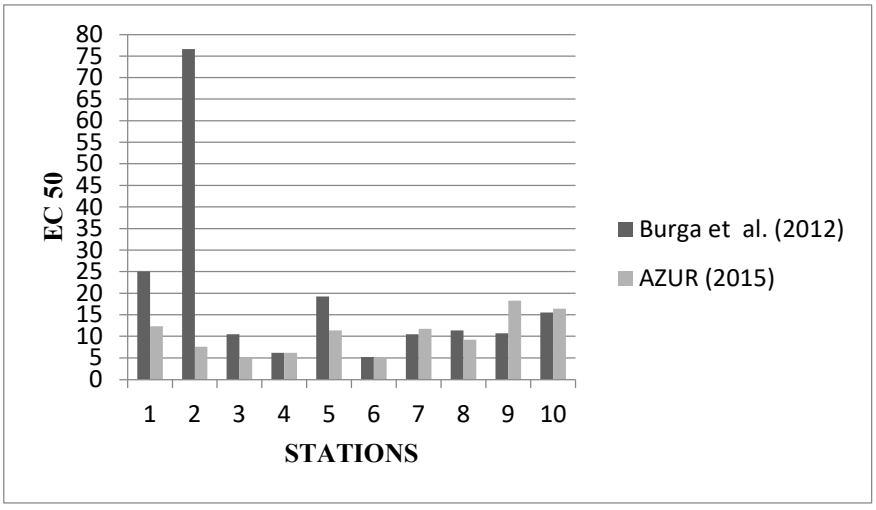

Figure 2 Acute toxicity detections in the wet and dry assays according to Pérez et al. (2012) and AZUR (2015) in surface sediments collected in Cunha Channel, Rio de Janeiro, Brazil.

4 Conclusions

The estrogenic activity in dry sediment samples from the Cunha Channel was low, with only one sample presenting a detectable value of 0.16 $ng.g^{-1}$. The wet sediment assay was more efficient, with activity detected at sampling points 1 and 2 (3.4 $ng.g^{-1}$ and 17 $ng.g^{-1}$, respectively). All sediment samples were considered very toxic in the acute toxicity assay, except for one sample from station 2, categorized as moderately toxic. Comparisons between the protocols applied in relation to acute toxicity were similar, being high for both. This study contributes to efforts in a highly degraded estuarine area, extensible to other estuarine areas worldwide, which still lack assessments of estrogenic activity of micropollutants and acute toxicity. Future studies should include seasonality assessments and investigations using organisms belonging to different trophic levels, due to the potential for biomagnification of EDCs.

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Conflict of interest

The authors declare no conflict of interest.

Data availability statement

All data included in this study are publicly available in the literature.

Funding information

The authors would also like to thank the Rio de Janeiro State Research Foundation (FAPERJ) (process number E-26/203.040/2017) and the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for their financial support.

How to cite:

Santos, R.F., Santos, A.D.O., Nascimento, M.T.L., Freitas, A.S., Melo, G.V., Felix, L.C., Silva, G.G.M., Carvalho, D.G., Hauser-Davis, R.A., Bila, D.M. & Baptista Neto, J.A. 2025, 'Estrogenic Sediment Activity and Toxicity at the Cunha Channel Watershed, a Major Micropollutant Contributor to Guanabara Bay, the Most Polluted Estuarine System in outheastern Brazil', *Anuário do Instituto de Geociências,* 48:66992. https://doi.org/10.11137/1982-3908_2025_48_66992