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Multi-indicators of environmental pollution in the Olaria system, Cananéia, São Paulo (SP), Brazil

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Abstract

The assessment of water quality using multiple abiotic and biological indicators is very important for understanding anthropogenic impacts and the health of the ecosystem. The Olaria is a short river system that flows into the Cananéia estuary. This aquatic system crosses the urban center of the city of Cananéia, where it eventually receives untreated domestic wastewater. This study aims to apply multi-indicators of environmental pollution to understand the influence of the Olaria system on the Cananéia estuary. For this purpose, indicators of water quality and genotoxic and pathological responses in fish were used. Data on salinity, pH, and silicates in surface water indicate a more accentuated continental influence in the inner part of the Olaria river system. Moreover, the data recorded on the inner area of the Olaria system, which presents lower hydrodynamic characteristics and the largest urban population, indicated the presence of dissolved oxygen and nutrients (phosphate [$P\text{-}PO_4^{-3}$], nitrite [$N\text{-}NO_2^-$], and ammonium [$N\text{-}NH_4^+$]) at levels that exceed the established limits for water quality by the Brazilian environmental legislation (Conselho Nacional do Meio Ambiente - CONAMA). The presence of *Escherichia coli* in all analyzed water samples indicates a local or point source of domestic wastewater contamination near the Olaria system. Fish species such as *Centropomus undecimalis* (Robalo Flexa) and *Sphoeroides testudineus* (Baiacu Pintado) showed toxicogenetic damage, indicating clastogenic and/or aneugenic exposure in the aquatic environment. Hepatic pathologies such as pyknosis nuclei, inflammation, hepatocyte swelling, and necrosis were found in all specimens evaluated, and *C. undecimallis* exhibited all these pathological changes. These results highlight the importance of biomonitoring the effects of anthropogenic disturbance on the aquatic biota that frequent the Olaria system and are dependent on the water quality.

Keywords: Water quality, Biomarkers, Fish, estuary, *Escherichia coli*

The city of Cananéia is located at the southern end of the coast of São Paulo state, covering an area of approximately 1,237 km² (Brazilian Institute of Geography and Statistics [IBGE], 2020), of which around 90% (1,113 km²) are protected areas. The Cananéia-Iguape Estuarine-Lagoon Complex (CIELC), located between latitudes 24º50' to 25°10'S and longitudes 47°25' to 48°00'W, is part of the Mosaic of Conservation Units of Lagamar (Cananéia-Iguape-Peruibe Environmental Protection Area), recognized since 1992 as part of the Atlantic Forest Biosphere Reserve and since 1999 as a Natural Heritage of Humanity

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(Instituto Chico Mendes de Conservação da Biodiversidade, 2016). Since 2017, the CIELC has been an international priority area for conservation, as it is a Ramsar site (https://rsis.ramsar.org/ris/2310).

Cananéia presents an extensive hydrographic network, much in the form of protected areas, as the one in the CIELC (Mishima et al., 1985). Olaria River, for example, is part of the hydrographic network located there: it crosses the urban center of Cananéia (Barcellos et al., 2005) and flows between São João Hill and the urban environment (Almeida, 1961) into the sea, where it influences the local hydrodynamics. Although the region is part of a mosaic of protected areas, Cananéia shows some urban areas (e.g., Cananéia/Sede, Porto Cubatão, Itapitangui, Arujá, and Marujá), and some of these neighborhoods (e.g., Carijo) release domestic wastewater into the aquatic system without treatment (Barcellos et al., 2005), affecting water quality and local biodiversity and endangering human health.

According to the Brazilian National System of Sanitation Statistics (SNIS), the population of Cananéia was around 12,542 inhabitants in 2020. From this number, 88% and 72% had access to water supply services and sanitary sewage services, respectively. However, 28% (3,461 inhabitants) did not have any access to sanitary sewage services and, therefore, there is uncertainty about where this domestic sewage is discarded. In this context, the great number of protected areas in Cananéia, -- a wet zone of national and international importance for conservation (i.e., a Ramsar site) -- the paucity of data on domestic sewage disposal, and the fact that the entire population lacks sanitary sewage services show risks to human health and local biodiversity, such as ichthyofauna. Therefore, this study aims to apply multi-indicators of environmental pollution to understand the influence of the Olaria riverine system on the Cananéia estuary. For this purpose, indicators of water quality and genotoxic and pathological responses in fish were used.

Surface water and fish were collected in August 2022 (winter period) from the Olaria river system of the CIELC (São Paulo, Brazil) (Figure 1). Tidal data for the sampling period were obtained from the Laboratory of Tides and Ocean Processes (MAPTOLAB) of the Oceanographic Institute of São Paulo University (IOUSP) for the Cananéia estuary (Harari, 2022). The surface water was sampled with a bucket before the fish were collected. Prior to sampling, the bucket was washed three times with local water. The local depth in meters was obtained using a graduated Secchi disc cable. Temperature (°C) was measured using a Hanna multiparameter probe (HI 9835). Salinity was determined in aliquots of water by an inductive method using a Beckman salinometer (RS-10) (± 0.001) calibrated with standard seawater. The pH values were obtained using a potentiometer (Orion®) with a combined glass electrode (± 0.01), following the recommendations of Aminot and Chaussepied (1983). Dissolved oxygen (DO) concentrations were determined by the Winkler method, as described by Grasshoff et al. (1983), using a potentiometric determination with a Metrohm® Titrando titrator (± 0.02 mL L^{-1}). Dissolved inorganic silicon (DISi) and dissolved inorganic phosphorous (DIP) were determined spectrophotometrically, following the method described by Grasshoff et al. (1983), using a Genesys II® spectrophotometer with an accuracy of ±0.02 µmol L^{-1} and ±0.01µmol L^{-1}, respectively. Nitrate ($N\text{-}NO_3^-$) and nitrite ($N\text{-}NO_2^-$) were determined following Grasshoff et al. (1983), using a BranLuebbe® AutoAnalyzer II with an accuracy of ± 0.02 µmol L^{-1}. N-ammonium ($N\text{-}NH_4^+$) was determined following Solórzano (1969), with a precision of ± 0.02 µmol L^{-1}. Dissolved inorganic nitrogen (DIN) was calculated as the sum of $N\text{-}NO_3^-$, $N\text{-}NO_2^-$, and $N\text{-}NH_4^+$. Total dissolved nitrogen (TDN) and total dissolved phosphorus (TDP) were obtained by the photo-oxidation method using a UV lamp following the recommendations of Armstrong and Tibbitts (1968), with modifications according to Braga (2020). The precision was ± 0.02 µmol L^{-1}. The dissolved organic nitrogen (DON) was obtained by calculating the difference between TDN and DIN, whereas the dissolved organic phosphorus (DOP) was obtained by calculating the difference between TDP and DIP. Moreover, the urea contents were determined by a colorimetric method following Aminot and Kerouel (1982), with a precision of ±0.05 µmol L^{-1} N-urea. An aliquot of the surface water (~1,000 mL to 2,000 mL) was filtered through pre-cleaned 0.7µm membrane filters. In the laboratory,

the organic matter (OM) was determined by weight loss on ignition at 450 °C for 4 hours. The %OM was calculated as: $\%OM=((w_i-w_f)/w_i)*100$, where w=weight, i=initial, f=final. Suspended particulate matter (SPM) and suspended particulate organic matter (SPOM) were determined, both with a precision of ±0.01 mg L^{-1}, and the chlorophyll-a content was determined with an accuracy of ±0.01 mg L^{-1}; these analyses followed the method described by Strickland and Parsons (1968). The presence of total coliforms and *Escherichia coli* was determined using the COLI test®, which contains substances in the culture medium that support the growth of bacteria of the Coliform group and facilitate the identification of *E. coli*. Consequently, in the presence of these bacteria, the pH of the culture medium changes and the initial color of the sample (purple) changes to a range of yellow tones. The variation in the color gradient of samples, from the lowest to the highest concentration, follows the sequence: yellow-green, brown, camel, light yellow, and golden yellow. The COLI test® culture medium contains a MUG reagent that, if positive for *E. coli*, gives blue fluorescence visible under ultraviolet light. After reading the fluorescence, 0.2 mL of Kovacs reagent was added to the medium, which forms a red ring on the surface of the medium if positive for *E. coli*.

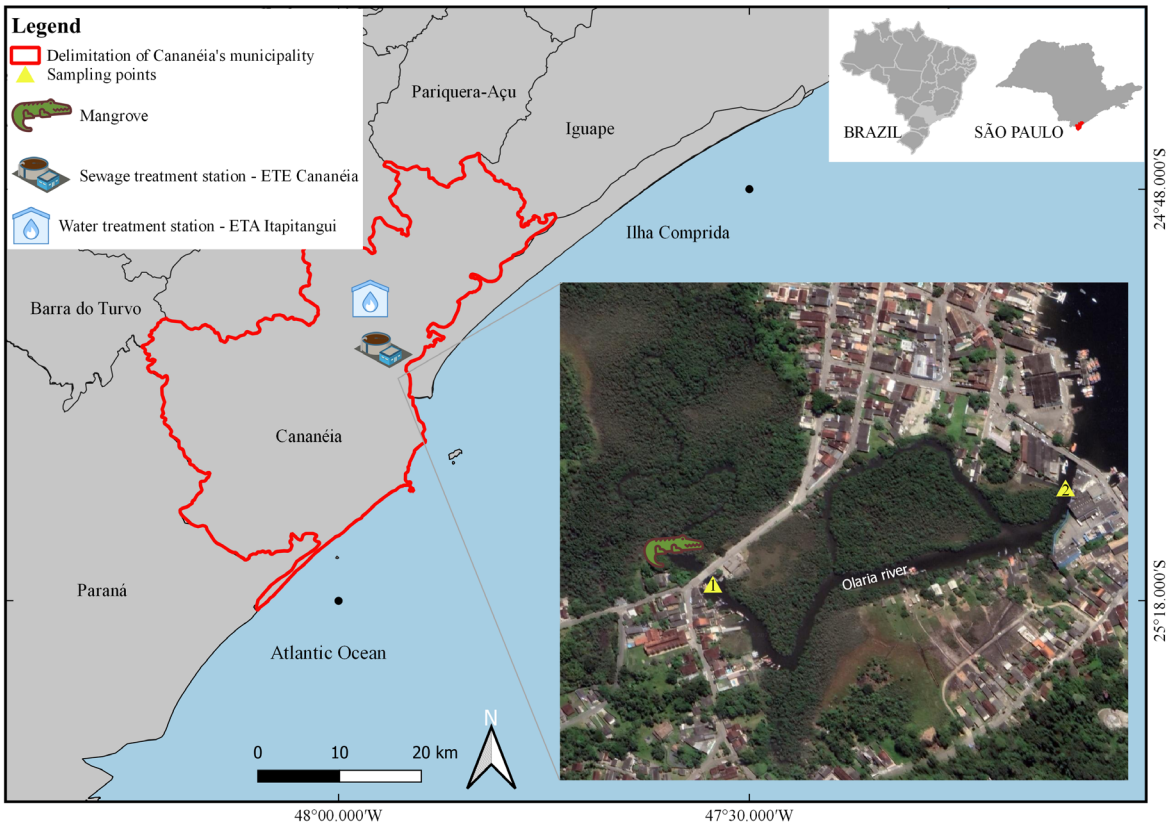

Figure 1. Map of the sampling sites indicating the inner station (1) and the point where the water flows into the estuary (2) in the Olaria system, Cananéia, Brazil.

Fish sampling was performed using a cast net (40 mm mesh) and eight fish of four species (*Diapterus rhombeus*, *Centropomus undecimalis, Mugil curema*, and *Sphoeroides testudineus*) were caught. The collected fish were immediately placed in a box with water from the site and transported to the laboratory Dr João de Paiva Carvalho Research Base at IOUSP. At the laboratory, blood was taken from the live fish by caudal punction; then they were dissected for liver extraction. The analysis and collection of fish species was authorized by ICMBio (Sisbio process nº 80258-1) and approved by IOUSP Research Ethics Committee on the Use of Animals (CEUA process nº 017pesq.).

Fish were dissected and biometric data recorded on total body size, measured as the length from the tip of the snout to the end of the caudal fin using an ichthyometer (± 0.01 cm). The total weight of each fish was obtained using a precision scale (± 0.01g). Species identification was based on the keys by Figueiredo and Menezes (1978) and Carpenter (2002a, 2002b).

Toxicogenetic damage was assessed by the identification and quantification of nuclear abnormalities in erythrocytes (NAE) and micronuclei, according to Carrasco et al. (1990) and Amaral et al. (2021). Peripheral blood of each live fish was collected, and a sample drop was obtained from the caudal vessel of each fish with 1-mL syringe, smeared on a microscope slide, and air-dried. The obtained blood smear was fixed with 80% methanol for 10 minutes, air dried, and the slides were stained with 10% Giemsa solution for 45 minutes (Azevedo et al., 2012). The counting of micronuclei and NAE on the microscope slides was performed using microscopy-immersion light. The slides were observed with optical 1,000-fold amplification, scoring an average of 2,000 mature mononucleated erythrocytes, and the analysis was conducted following Amaral et al. (2021). NAE was calculated as the sum of all five classifications: binucleated, vacuolated, notched, lobed, and blebbed. For histopathological examination, the obtained liver samples were processed and stained for routine histology. Still in the field, immediately after fish sampling, the tissues were preserved in Alfac fixative solution (ethanol, formaldehyde, and glacial acetic acid) for 16 hours and stored in 70% ethanol for future procedures in the laboratory after being dehydrated in a graded series of ethanol baths and embedded in Paraplast-Plus resin (Sigma©); slides with five-micrometer-thick sections were obtained. For histological examinations, one slide of each tissue was stained with haematoxilin and eosin. Sub-sets of slides were scored independently to check for observer bias. Slides were also processed in batches containing controls and treatments to eliminate staining artifacts. All the pathological analyses were conducted using an optical microscope (400x magnification), and photographs were taken using ZEN2011 software in the Zeiss-Axiophot photomicroscope.

In August 2022, the mean rainfall was 140.6 mm, with 4.0 mm on the first day of the sampling (August 10, 2022) and 33.2 mm on the second day (August 11, 2022) (Instituto Nacional de Meteorologia, 2022), characteristic of a dry season. Data about salinity, pH, DIP, DIN, and silicate in the surface waters indicate a more accentuated continental influence in the inner area of the Olaria riverine system (St. 1) (Table 1). Elevated N-urea values (10.56 µmol L^{-1}) also suggest a possible influence of domestic sewage in this area. High N-ammonium (161.69 µmol L^{-1}) and low DO (1.92 mg L^{-1}) values may indicate organic matter degradation processes occurring mainly in flood tide conditions at that location. In general, the Olaria system discharges a large amount of nutrients, especially inorganic, into the CIELC, showing eutrophic or mesotrophic conditions depending on tidal characteristics (flood/ebb) at the time of sampling. On the other hand, station 2, which is more external, shows higher levels of organic forms of N and P, indicating that, in addition to the process of dilution process of the river's nutrient load by the more saline waters of the estuary, an important process of biogeochemical transformation also occurs in the region, which has its productivity increased towards the estuary. This is supported by the increase in chlorophyll-*a* contents in the same direction.

In accordance with CONAMA resolution no. 357 of 2005, the waters of the Olaria system are classified as Class 1 since they can be used for artisanal and local fishing and can attend the aquatic communities' protection. The inner area of the Olaria system, which presents a lower hydrodynamic influence and is located in a characteristically urban area, demonstrated amounts of DO, DIP, $N\text{-}NO_2^-$, and $N\text{-}NH_4^+$ that exceed the established limits by the Brazilian Environmental Law (CONAMA, n° 357/2005), which sets out acceptable standards for unpolluted water concentrations of DO $\geq$ 5.00 mg L^{-1} and a maximum of 1.15 µmol L^{-1} for $N\text{-}NO_2^-$, 22.22 µmol L^{-1} for $N\text{-}NH_4^+$, and 1.30 µmol L^{-1} for DIP. The worst conditions were observed in the innermost internal area of the Olaria system (St. 1). In general, in the sampling undertaken in the more hydrodynamic site that flowed into the estuary, the indicators of water quality were below the established

limits set by Brazilian environmental law, both on the sampling day -- which presented the lowest rainfall -- and on the day of collection in surging seas (Table 1). These data show the influence and importance of tidal and estuarine fluxes in depuration and dispersion processes. The detection of *E. coli* is another indication of low water quality in this system. Thermotolerant coliforms and *E. coli* were found in all samples (Table 1). These results indicate the presence of some local or point source contamination by domestic sewage near the Olaria system.

Table 1. Hydrochemical data of surface water in the Olaria riverine system in CIELC, winter-2022.

	Water indicators			
	St. 1 (1st day)	St. 2 (1st day)	St. 1 (2nd day)	St. 2 (2nd day)
Tidal	Syzygy/Flood	Syzygy/Flood	Syzygy/Ebb	Syzygy/Ebb
T (ºC)	22.00	22.50	22.10	21.30
S	6.673	23.467	26.270	31.940
pH	7.11	7.88	7.98	8.16
DO (mg L^{-1})	1.92	6.47	6.65	7.21
DISi (μmol L^{-1})	106.67	7.69	307.69	208.72
DIP (μmol L^{-1})	13.95	0.62	0.67	0.48
N-NH_4^+ (μmol L^{-1})	161.69	2.11	5.69	1.60
N-NO_2^- (μmol L^{-1})	3.19	0.22	0.33	0.25
N-NO_3^- (μmol L^{-1})	6.09	0.77	1.25	0.92
DIN (μmol L^{-1})	170.97	3.11	7.27	2.77
DIN:DIP	12.26	5.00	10.89	5.77
DTP (μmol L^{-1})	13.98	1.18	1.01	0.72
DOP (μmol L^{-1})	0.03	0.56	0.35	0.24
DTN (μmol L^{-1})	183.32	33.06	31.16	29.38
DON (μmol L^{-1})	12.35	29.95	23.90	26.61
N-Urea (μmol L^{-1})	10.56	2.52	1.46	0.43
SPM (mg L^{-1})	27.0	36.7	89.3	82.5
SPOM (mg L^{-1})	4.0	3.2	14.7	12.25
%OM	14.8	8.6	16.4	14.8
Chl-a (mg m^{-3})	1.83	7.84	2.55	5.32
Total coliforms	Golden yellow	Light yellow	Golden yellow	Light yellow
Escherichia coli	Presence	Presence	Presence	Presence
∑ANEs				
D. rhombeus	-	34	-	-
C. undecimalis	75	-	254	-
M. curema,	65	-	-	-
S. testudines	-	266	-	-
Micronuclei				
D. rhombeus	-	2	-	-
C. undecimalis	1	-	6	-
M. curema,	4	-	-	-
S. testudines	-	6	-	-

St.: station; T: Temperature; S: Salinity; DO: Dissolved Oxygen; DISi: Dissolved Inorganic Silicon; DIP: Phosphorus in Phosphate; N-NH_4^+: N-Nitrogen in Ammonium; N-NO_2^-: Nitrogen in Nitrite; N-NO_3^- : Nitrogen in Nitrate; DIN: Dissolved Inorganic Nitrogen; SPM: Suspended Particulate Matter; SPOM: Suspended Particulate Organic Matter; %OM: % Organic Matter; Chl-*a*.: Chlorophyll-a.

Concerning the aquatic biota that uses the Olaria system, fish species such as *Centropomus undecimalis* (Bloch, 1792) and *Sphoeroides testudineus* (Linnaeus, 1758) showed more toxicogenetic damage, as indicated by their highest frequency of nuclear abnormalities or/and micronucleus detection (Table 1). Therefore, these fish had been exposed to some clastogenic or/and aneugenic compounds in the aquatic environment (WHO (World Health Organization), 1993). It is important to consider the migration capacity of fish such as *C. undecimalis* since they may have contact with xenobiotic compounds in the Olaria system or in other areas. Overall, these results indicate damage to important economic and ecological species for the region. The most frequent pathological changes found in the livers of the analyzed fish were pyknosis nuclei (67%), inflammation (50%), hepatocyte swelling (17%), and necrosis (17%). All *D. rhombeus* and *C. undecimalis* specimens showed pyknosis. Additionally, *C. undecimalis* was the only species in which all the forms of hepatic damage were identified. Hepatic changes such as pyknosis nuclei and inflammation are less severe and may be associated with an increase in the metabolic activity of fish cells in response to exposure to stressors (Gomes et al., 1990). However, necrosis and hepatocyte swelling are of major importance since they are irreversible lesions that can be caused by several abiotic factors such as the presence of chemical compounds (van der Oost et al., 2003; Azevedo et al., 2013; Yancheva, 2016). Therefore, these results reinforce the importance of biomonitoring anthropogenic effects on the aquatic biota that frequent the Olaria system.

AUTHOR CONTRIBUTIONS

G.L.S.: Methodology; Formal analysis; Writing – original draft.
V.G.C.: Methodology; Formal analysis; Writing – review & editing.
E.S.B.: Resources; Supervision; Writing – review & editing.
J.S.A.: Conceptualization; Formal analysis; Data curation; Resources; Supervision Writing – original draft; Writing – review & editing;.

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