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Mutagenic and ecotoxicological assessment of urban surface runoff flowing to the beaches of Guarujá, State of São Paulo, Brazil

Vinicius Roveri, Luciana Lopes Guimarães and Alberto Teodorico Correia

ABSTRACT

Along the coast of the State of São Paulo, Brazil, urban drainage channels introduce a complex mixture of pollutants into the South Atlantic Ocean, that may cause deleterious effects to the aquatic biota. The objective of this study was to analyse, for the first time, the mutagenicity (Ames *Salmonella*/microsome test) and ecotoxicity (acute and chronic tests, with *Daphnia simillis* and *Ceriodaphnia dubia*, respectively) exerted by the diffuse loads discharged in Guarujá, São Paulo coast, Brazil. Water sampling occurred bimonthly between January and July 2018 (rainy season: January through March; dry season: May through July) at four beaches with different profiles of use and land occupation: Tombo (Blue Flag certification), Enseada (high use by tourists), Perequê (fishing community) and Iporanga (conservation unit). No mutagenic potential was detected in the complex mixtures flowing to the study beaches. However, 30 and 80% of the analyses showed acute and chronic toxicities, respectively, mainly in the Enseada and Perequê channels during the rainy season. To improve the environmental quality of these coastal waters and to reduce the ecological risks posed to the aquatic organisms and public health, several actions are imperative, such as the amelioration of the basic sanitation facilities and land regularisation actions.

Key words | bioassay, domestic sewage, non-point source pollution, pollution effect, subtropical zone, urban drainage channel

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HIGHLIGHTS

- Evaluation of the genotoxicity and ecotoxicity of urban surface runoff of Guarujá (State of São Paulo, Brazil).
- No mutagenicity (Ames *Salmonella*) was detected in the water samples.
- Acute (*Daphnia simillis*) and chronic (*Ceriodaphnia dubia*) toxicities were however recorded.
- Results suggest potential risk to the environmental and public health.

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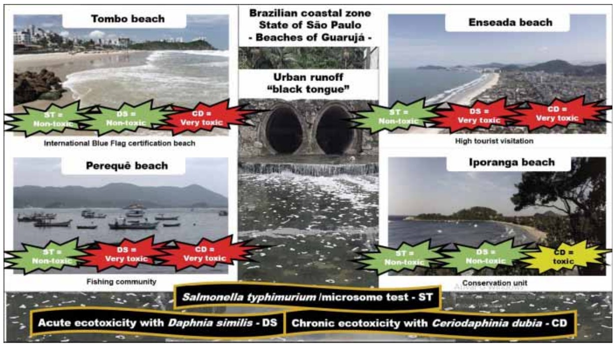
GRAPHICAL ABSTRACT



INTRODUCTION

As urbanisation progresses, especially in coastal areas, diffuse pollution increases and introduces a complex mixture of pollutants into estuaries and oceans, resulting in a systematic decline in the environmental quality of the aquatic ecosystems (Lamparelli *et al.* 2015; Lusk & Toor 2016; Yang & Toor 2017). In South America, namely Brazil, the management of these diffuse loads in the vast coastline (8,500 km) is challenging due to the complexity in the identification, delimitation and control of these non-point pollution sources (Xiang *et al.* 2017). This is the current scenario along the coast of São Paulo, Brazil, a region that includes 16 municipalities and represents 10% of the Brazilian coast with over 600 urban drainage channels, whose waters flow over or through 290 touristic beaches (Lamparelli *et al.* 2015; Cetesb 2017; Ibge 2018). One of these touristic municipalities is Guarujá, one of the largest cities of the São Paulo coast, with an estimated population of 316,000 residents, that almost doubles during the high tourist season, between December and March (Cetesb 2017; Ibge 2018).

Recent studies have demonstrated that urban drainage channels in Guarujá transport conventional (e.g. physical, chemical and microbiological) (Roveri *et al.* 2020a, 2020b) and emerging pollutants (e.g. pharmaceuticals and illicit drugs) (Roveri *et al.* 2020c) to the Atlantic Ocean. Therefore, these complex mixtures may contain compounds with genotoxicity (Baršiene *et al.* 2012) and ecotoxicological (Gosset *et al.* 2016) potential, which could cause a detrimental effect on the aquatic biota (Kalmykova *et al.* 2013). A way to assess the mutagenic potential of chemical compounds is through the use of bioassays (*in vitro*), such as the Ames (*Salmonella*/microsome) test (Khallef *et al.* 2019). The Ames test is a quick and convenient assay specifically designed to detect a wide range of chemical substances that can produce gene mutations, such as polychlorobiphenols (PCBs) and polycyclic aromatic hydrocarbons (PAH) (Baršiene *et al.* 2012; Kalmykova *et al.* 2013; Khallef *et al.* 2019). The risk screening from pollutant exposure can be also assessed with standardised acute and chronic toxicity tests (*in vivo*) using microcrustaceans, such as *Daphnia simillis* and *Ceriodaphnia dubia*, as test organisms. These daphnids are often used for toxicity tests due to their short life cycles, high reproductive rates and sensitivity to a broad range of toxicants (Gosset *et al.* 2016; Roveri *et al.* 2020a, 2020c). These assays are easy to implement and require few financial resources, therefore they are suitable for developing countries like Brazil (Baršiene *et al.* 2012; Gosset *et al.* 2016; Khallef *et al.* 2019).

In addition to the scarcity of studies focusing the urban surface runoff of the São Paulo coastal area (Roveri *et al.* 2020a, 2020b, 2020c), none of the existent works have been dedicated to detect the mutagenic and ecotoxic potential of these complex mixtures, that carry the diffuse load into the ocean, along areas of intense human recreation (beaches). In this context, the objective of this study was to characterise, for the first time, the mutagenicity (Ames *Salmonella*/microsome test) and ecotoxicity (acute and chronic tests, with *D. simillis* and *C. dubia*, respectively) of the diffuse loads with origin in the urban drainage channels that flow to four beaches in Guarujá (Tombo, Enseada, Perequê and Iporanga), São Paulo, Brazil. This new knowledge will allow us to understand the potential risk for the human and environmental health arising from anthropic activities that take place along the Brazilian coastline.

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MATERIALS AND METHODS

Study area

The study area comprised the coastal communities of Guarujá, São Paulo State, Brazil (23°59′34″S, 46°15′21″W) (Ribeiro & Oliveira 2015). The economy of the municipality is mainly driven by the seasonal tourism occurring along eight touristic beaches (Ribeiro & Oliveira 2015; Cetesb 2017). These beaches receive daily contributions of urban runoff from 43 rainwater drainage channels (Cetesb 2017; Roveri *et al.* 2020a, 2020b, 2020c). These channels are made of concrete, and none of them has a grating system, so all of their content is discharged directly onto these beaches without previous treatment (Cetesb 2017). In this study, four beaches were selected: (i) Tombo (Blue Flag certification at 24°00′53″S, 46°16′23″W), (ii) Enseada (high tourist visitation at 23°59′12″S, 46°13′38″W), (iii) Perequê (fishing community at 23°56′05″S, 46°10′51″W), and (iv) Iporanga (conservation unit at 23°42′02″S, 46°08′26″W) (Ribeiro & Oliveira 2015). Figure 1 presents the main characteristics regarding the use and land occupation of the sampling beaches.

Water sampling

In Guarujá, the mean annual precipitation and temperature are approximately 3,000 mm and 22 °C, respectively. Two quite distinct periods are observed in the municipality: a rainy season (November to March) and a dry (April to October) season (Ribeiro & Oliveira 2015; Cetesb 2017). Therefore, sampling occurred bimonthly between January and July 2018 (rainy season: January and March, dry season: May and July). At each beach, water samples were collected in the supralittoral region, at the mouth of the drainage channels, without interference of the tidal regime. The methodology used to collect samples was based on the National Guide for Collection and Preservation of Samples (Cetesb 2011). Water

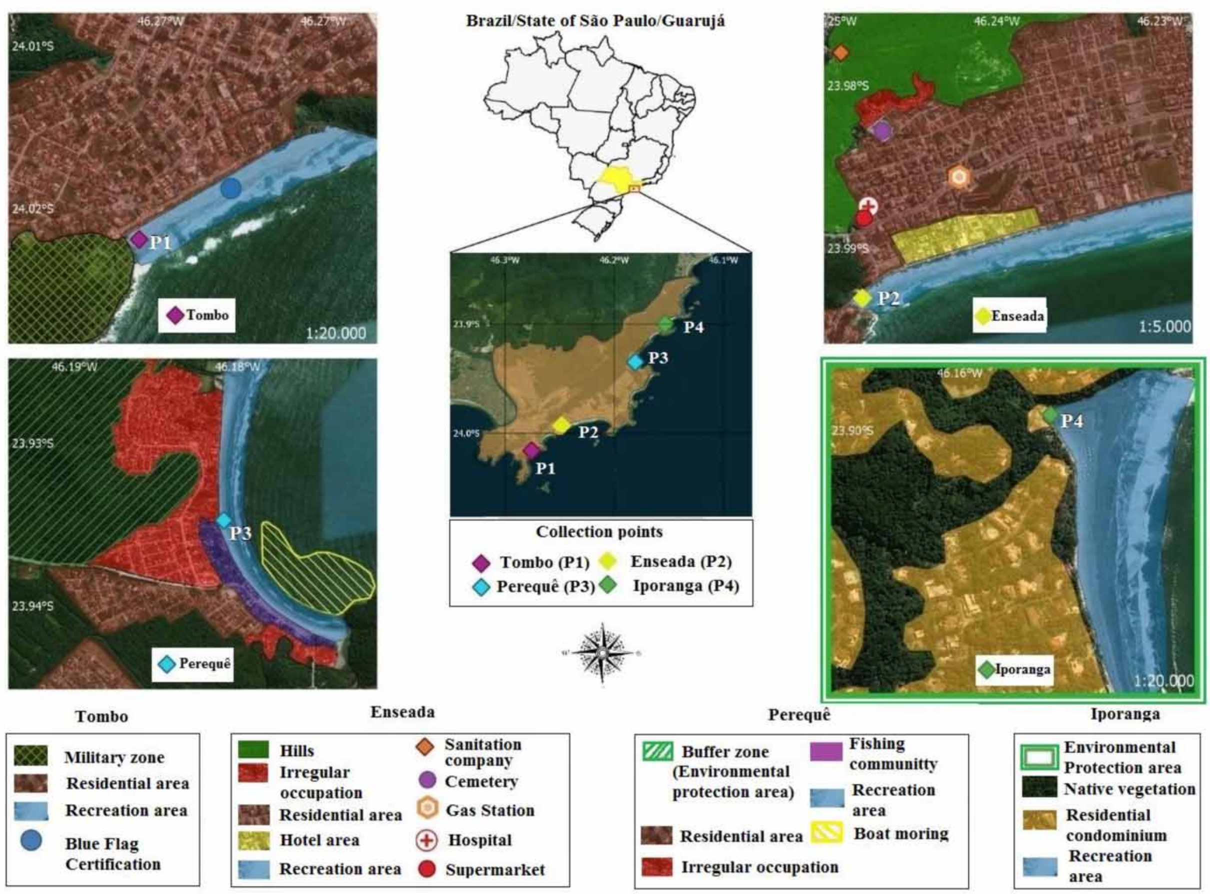

Figure 1 | Map of the study area showing the city of Guarujá, São Paulo State, Brazil. Beaches [Tombo (P1), Enseada (P2), Perequê (P3), Iporanga (P4)] here water samples were collected for the genotoxicity and ecotoxicity assessment of urban surface runoff. The figure also presents the main characteristics regarding the use and land occupation of the selected beaches. Please refer to the online version of this paper to see this figure in colour: http://dx.doi.org/10.2166/wst.2021.175.

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samples (2 L and 1 L for mutagenic and ecotoxicological tests, respectively) were collected from each location and packed into pre-cleaned amber glass bottles. All samples were kept in dark at 4°C, and the laboratorial analyses were preformed within 2 days of collection (Cetesb 2011). For the rainy season (January and March) rainfall was recorded in the last 24 hours prior to sample collection. There was no record of rain during the dry season (May and July).

Bioassays

The samples were sent to the NSF bioassay laboratory, Viamão, Rio Grande do Sul, Brazil (Accreditation of Inmetro/ABNT ISO/IEC17025), where three bioassays were performed:

(i) The Ames test (*Salmonella*/microsome), to assess the mutagenic potential of chemical compounds, according to OECD (Organisation for Economic Co-operation and Development) Method 471 'Bacterial Reverse Mutation Test' (adopted: 21 July 1997). The sample was tested for induction of reverse mutation to his *locus* in two strains of *Salmonella typhimurium*, TA98 and TA100, using the preincubation method in the absence and presence of 8% of the metabolic activation mixture (S9) (OECD 1997). The test was carried out with increasing amounts of sample: 100, 200, 500, 1,000, 1,500, and 2,000 μL/plate. Additionally, the mutagenicity index (MI) was calculated for each concentration according to the following formula (Equation (1)):

$$MI = \frac{number\ of\ revertants\ per\ plate\ (test\ compound)}{number\ of\ revertants\ per\ plate\ (negative\ control)} \quad (1)$$

The sample is considered positive when it presents a statistically significant difference from the negative control through ANOVA ($p \leq 0.05$); for a significantly positive dose–response effect, $p \leq 0.05$ and MI is ≥ 2 (Mortelmans & Zeiger 2000).

(ii) An acute toxicity test with *D. simillis*, according to ABNT (Brazilian Association of Technical Standards) NBR 12713: 2016. This method consisted of exposing young organisms of *D. simillis* (neonates age 6–24 h) to the water samples, including a negative control group, for a period of 48 h (ABNT 2016).

(iii) A chronic ecotoxicological assay with *C. dubia*, according to ABNT NBR 13373: 2017. This method consisted of exposing young organisms of *C. dubia* (neonates age 6–24 h) to the water samples, including a negative control group, for a period of 7 days (ABNT 2017).

In both ecotoxicological tests (ii) and (iii), the results were considered valid when the percentage of stationary organisms in the control treatment did not exceed 10%. For the controls reconstituted water was use. It was made up by adding specific amounts of reagents of recognised analytical grade to distilled water. Final pH ranged between of 7.0 and 7.6, and hardness between 40 and 48 mg/L (as $CaCO_3$). For more details, see ecotoxicological guidelines ABNT NBR 12713 (2016) and ABNT NBR 13373 (2017). Both tests adopted a level of significance (α) of 0.05. All analyses were carried out using R statistical software version 3.6.1 (R Core Team 2017).

Toxicity classification

This classification was based on the work of Souza *et al.* (2016), which enables a qualitative response of the genotoxicity and ecotoxicity tests as a whole. The Ames test and the acute and chronic tests with *D. simillis* and *C. dubia*, respectively, are classified into three classes: (i) non-toxic (no significant differences for the control), (ii) toxic (significant difference to the control <50%) and (iii) very toxic (significant difference with control ≥50%).

RESULTS

Regarding Ames test (Table 1), the results obtained in the channels of the Tombo (Table 1(a)), Enseada (Table 1(b)), Perequê (Table 1(c)) and Iporanga (Table 1(d)) indicate that the all samples (January through July) were not able to induce reverse mutations in *his* for both strains (TA98 and TA100), both in the presence and absence of the metabolic system (S9). In addition, for TA98 and TA100, at any concentration tested, the values of MI were always <2; therefore, no toxicity was observed.

Table 2(a) presents the results of the acute toxicity test for the microcrustacean *D. simillis* and the respective toxicity classifications. The immobility rate in the controls was 0% (January), 5% (March), and 0% (May and July). Only 26.7% of the analyses showed acute toxicity for the rainwater channels. In January, only Enseada and Perequê samples showed acute toxicity (with 100% immobility of organisms and classification of 'very toxic'). In March, acute toxicity was observed only for Enseada where immobility was recorded in 60% of the organisms (classification of 'very toxic'). In May, none of the samples showed any toxicity, because no immobility

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Table 1 | Results of the Ames test (*Salmonella typhimurium*/microsome test), performed in urban runoff waters flowing into the beaches of Tombo (A); Enseada (B); Perequê (C) and Iporanga (D), of Guarujá Municipality, State of São Paulo, Brazil

Beach	Concentration (μL/plate)	Ames Test (*Salmonella typhimurium* /microsome test)																															
		January (rainy season)								March (rainy season)								May (dry season)								July (dry season)							
		TA98				TA100				TA98				TA100				TA98				TA100				TA98				TA100			
		S9 (-)	MI	S9 (+)	MI	S9 (-)	MI	S9 (+)	MI	S9 (-)	MI	S9 (+)	MI	S9 (-)	MI	S9 (+)	MI	S9 (-)	MI	S9 (+)	MI	S9 (-)	MI	S9 (+)	MI	S9 (-)	MI	S9 (+)	MI	S9 (-)	MI	S9 (+)	MI
A																																	
Tombo	100	Negative	1.2	Negative	1.1	Negative	1.2	Negative	1.3	Negative	1.1	Negative	1.1	Negative	0.9	Negative	1.0	Negative	0.7	Negative	0.9	Negative	0.9	Negative	0.9		-		-		-		-
	200		1.1		1.0		0.9		1.2		1.1		1.0		1.2		1.0		0.9		0.9		0.7		0.7		-		-		-		-
	500		0.9		1.1		1.2		0.9		0.9		0.9		1.2		1.2		0.9		1.0		0.7		1.0		-		-		-		-
	1000		1.3		1.3		1.0		0.9		0.9		1.3		1.1		1.2		0.9		0.8		0.9		0.8		-		-		-		-
	1500		1.2		1.1		0.7		0.7		0.7		0.9		1.2		1.2		0.8		0.7		0.9		0.8		-		-		-		-
	2000		0.8		1.8		0.9		0.8		0.9		1.1		1.1		1.0		1.0		0.7		0.9		0.7		-		-		-		-
Toxicity classification		Non-toxic				Non-toxic				Non-toxic				Non-toxic				Non-toxic				Non-toxic				Not Determined				Not Determined			
B																																	
Enseada	100	Negative	1.3	Negative	1.4	Negative	1.2	Negative	1.3	Negative	1.4	Negative	1.5	Negative	1.3	Negative	1.5	Negative	1.3	Negative	1.4	Negative	1.3	Negative	1.3	Negative	1.2	Negative	1.3	Negative	0.9	Negative	1.1
	200		1.5		1.3		1.3		1.4		1.3		1.4		1.4		1.4		1.2		1.3		1.3		1.2		1.2		1.2		1.2		1.2
	500		1.3		1.4		1.2		1.5		1.4		1.3		1.3		1.3		1.4		1.4		1.2		1.3		1.3		1.0		1.1		1.0
	1000		1.4		1.4		1.5		1.5		1.5		1.4		1.4		1.4		1.4		1.3		1.4		1.4		1.2		1.2		1.3		1.1
	1500		1.3		1.4		1.4		1.3		1.4		1.4		1.3		1.3		1.2		1.4		1.3		1.2		1.1		0.9		0.9		0.9
	2000		1.4		1.4		1.4		1.4		1.5		1.5		1.4		1.6		1.2		1.4		1.3		1.3		0.9		1.1		1.2		0.8
Toxicity classification		Non-toxic				Non-toxic				Non-toxic				Non-toxic				Non-toxic				Non-toxic				Non-toxic				Non-toxic			
C																																	
Perequê	100	Negative	1.3	Negative	1.3	Negative	1.2	Negative	1.3	Negative	1.2	Negative	1.2	Negative	1.2	Negative	1.2	Negative	0.8	Negative	0.8	Negative	0.9	Negative	0.8	Negative	1.2	Negative	1.3	Negative	1.1	Negative	0.9
	200		1.3		1.2		1.3		1.3		1.4		1.3		1.4		1.4		1.1		1.0		0.9		1.0		1.1		1.3		1.3		1.3
	500		1.4		1.4		1.3		1.3		1.4		1.3		1.5		1.3		1.2		1.0		1.1		1.2		1.2		1.3		1.2		1.2
	1000		1.3		1.3		1.5		1.4		1.2		1.5		1.4		1.5		1.1		1.2		1.2		1.2		1.3		1.2		1.3		1.2
	1500		1.4		1.4		1.4		1.3		1.4		1.4		1.4		1.3		1.2		1.3		1.1		1.2		1.2		1.3		1.2		1.2
	2000		1.3		1.4		1.4		1.3		1.3		1.4		1.3		1.5		1.3		1.1		1.3		1.2		1.1		1.1		1.1		1.2
Toxicity classification		Non-toxic				Non-toxic				Non-toxic				Non-toxic				Non-toxic				Non-toxic				Non-toxic				Non-toxic			
D																																	
Iporanga	100	Negative	0.9	Negative	0.6	Negative	0.9	Negative	0.8	Negative	0.8	Negative	0.8	Negative	0.8	Negative	0.9	Negative	0.9	Negative	0.8	Negative	0.8	Negative	0.8	Negative	0.7	Negative	0.7	Negative	0.8	Negative	0.8
	200		0.6		0.7		0.9		0.8		0.8		0.7		0.7		0.8		0.9		0.8		0.8		0.8		0.7		0.6		0.6		0.6
	500		0.9		0.8		0.7		0.8		0.9		1.0		0.8		0.8		0.8		0.7		0.8		0.8		0.9		0.7		0.9		0.8
	1000		0.9		0.9		1.0		0.8		0.9		0.8		0.8		0.8		0.8		0.9		0.8		0.8		0.9		0.9		0.9		0.9
	1500		0.8		0.8		0.8		0.9		0.7		0.9		0.7		0.7		0.7		0.8		0.7		0.7		0.8		0.8		0.7		0.8
	2000		0.9		0.9		0.9		0.9		0.9		1.0		0.9		0.8		0.9		0.9		0.9		0.8		0.8		0.8		0.8		0.8
Toxicity classification		Non-toxic				Non-toxic				Non-toxic				Non-toxic				Non-toxic				Non-toxic				Non-toxic				Non-toxic			

The results are presented considering two different seasonal periods: rainy season (January and March of 2018); and dry season: (May and July of 2018). These tables also show: (i) different concentrations tested (expressed as μL/plate); (ii) results of lineages *of Salmonella typhimurium* (TA98 and TA100) performed by in the absence (−S9) and presence (+S9) of the metabolic activation mixture (S9) (result of mutagenic activity expressed as positive or negative); (iii) results of mutagenicity index (MI) and (iv) toxicity classification for the mutagenic tests (i.e. non-toxic, signalled in green. For more details, see item 2. Materials and Method. *Note:* *Tombo channel could not be sampled in the July campaign (dry season) because the rainwater course was dry.

was recorded for the microcrustaceans. In July, only the Enseada sample showed acute toxicity (with 60% immobility of organisms and classification of 'very toxic').

Table 2(b) presents data on the chronic toxicity of samples for *C. dubia* and its respective toxicity classification. The immobility rate in the control was 10% in January and March and 0% in May and July. Most of the analyses (80%) showed chronic toxicity for channel waters. In January, samples collected from Tombo, Enseada, and Perequê channels showed 100% immobility of organism and therefore did not show neonatal production due to high toxicity (each of these three channels had an individual toxicity classification of *very toxic*). In March, all samples presented toxicity. The channels of Tombo, Perequê and Iporanga were classified as *toxic* and the channel of Enseada was classified as *very toxic*. In May, the highest immobility rate occurred in the Enseada sample, with 80% immobility (*very toxic*), followed by the Tombo and Perequê samples, with 40% and 30% immobility, respectively (*toxic*). As noted in January, the Iporanga sample showed no toxicity in May. In July, only the Enseada and Perequê samples showed a toxic effect as evidenced by immobility of 40% and 60% for the trial replicates, respectively (toxicity classification for Enseada and Perequê were toxic and very toxic, respectively).

DISCUSSION

The urban surface runoff flowing to the beaches of Guarujá, São Paulo, Brazil, popularly known as 'black tongues,' is

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Table 2 | Acute and chronic ecotoxicity testes results with *Daphnia simillis* (A) and *Ceriodaphnia dubia* (B) respectively, applied to water samples obtained from four urban drainage channels (Tombo, Enseada, Perequê and Iporanga) in the city of Guarujá, São Paulo, Brazil

Beach	January (rainy season)			March (rainy season)			May (dry season)			July (dry season)		
	Immobility		toxicity classification	Immobility		toxicity classification	Immobility		toxicity classification	Immobility		toxicity classification
	Total	%		Total	%		Total	%		Total	%	
A	**Acute ecotoxicity with *Daphnia similis***											
Control	0	0	-	1	5	-	0	0	-	0	0	-
Tombo	0	0	Non-toxic	0	0	Non-toxic	0	0	Non-toxic	-	-	*Not determined
Enseada	20	100	Very toxic	12	60	Very toxic	0	0	Non-toxic	12	60	Very toxic
Perequê	20	100	Very toxic	2	10	Non-toxic	0	0	Non-toxic	1	5	Non-toxic
Iporanga	0	0	Non-toxic	0	0	Non-toxic	0	0	Non-toxic	0	0	Non-toxic
B	**Chronic ecotoxicity with *Ceriodaphnia dubia***											
Control	1	10	-	1	10	-	0	0	-	0	0	-
Tombo	10	100	Very toxic	4	40	Toxic	4	40	Toxic	-	-	*Not determined
Enseada	10	100	Very toxic	8	80	Very toxic	8	80	Very toxic	4	40	Toxic
Perequê	10	100	Very toxic	3	30	Toxic	3	30	Toxic	6	60	Very toxic
Iporanga	0	0	Non-toxic	3	30	Toxic	1	10	Non-toxic	0	0	Non-toxic

The results are presented considering two different seasonal periods: rainy season (January and March of 2018); and dry season: (May and July of 2018). These tables also show: (i) rates of immobility of controls and beaches (total of immovable organisms and their percentages); (ii) toxicity classification for the acute and chronic tests (i.e. non-toxic, signalled in green; toxic: signalled in yellow; and very toxic, signalled in red. For more details, see Materials and Methods). *Note*: *Tombo channel could not be sampled in the July campaign (dry season) because the water course was dry.

responsible for introducing a mixture of conventional and emerging pollutants into the coastal waters, as result of numerous anthropic activities that take place along this hydrographic basin (Roveri *et al.* 2020a, 2020b, 2020c). Tombo, Enseada and Perequê are considered to be the most impacted beaches in the study area, as they receive the clandestine domestic sewage from regular and irregular occupations; according with physical, chemical and microbiological analyses performed on drainage channels that flow to these beaches, only 34% to 43% were in compliance with current Brazilian legislation (Ribeiro & Oliveira 2015; Cetesb 2017; Roveri *et al.* 2020a). Moreover, these channels have already been identified as a potential threat to the public health, as they are responsible for the introduction of allochthonous pathogenic microorganisms, related to disease outbreaks, in areas of intense recreation (Ribeiro & Oliveira 2015; Cetesb 2017; Roveri *et al.* 2020a). For example, the concentrations of bacteria (*Escherichia coli* and *enterococci*) detected in these three channels are alarming, as they are higher than the maximum concentrations detected in different coastal areas around the world (Roveri *et al.* 2020a). Furthermore, in Enseada and Perequê, the viral loads (species HAdV-C, D and F) found in the channels were similar to those commonly found in wastewater (Roveri *et al.* 2020b). In addition, these waters are eutrophic (low levels of dissolved oxygen, high biochemical oxygen demand, excess of nutrients such as phosphorus, phosphates, and ammonia were reported) and show high levels of aluminium contamination (Roveri *et al.* 2020a). Regarding emerging pollutants, a total of 16 pharmaceuticals (among them: caffeine, acetaminophen, diclofenac, losartan and valsartan) and illicit drugs (cocaine and its metabolite benzoylecgonine), were also detected in these channels (mainly in the Brazilian summer/rainy season) (Roveri *et al.* 2020c). However, these physical, chemical and microbiological datasets only represent a first snapshot of the water quality in Guarujá. It should be complemented with biological tests (e.g., Ames test and acute and chronic ecotoxicity tests, with *D. simillis* and *C. dubia*, respectively), which will allow an integrated assessment of the water quality (Kalmykova *et al.* 2013; Gosset *et al.* 2016; Khallef *et al.* 2019).

It is expected than this mixture of compounds previously detected in the urban surface runoff of Guarujá can cause potential toxic effects (synergistic, antagonistic, and/or additives) on the aquatic ecosystems (Kalmykova *et al.* 2013; Gosset *et al.* 2016; Khallef *et al.* 2019). However, the genotoxicity assays performed with the Ames test for both strains (TA98 and TA100 with and without metabolic activation by S9) did not recorded mutagenic activity for any of the samples collected in Guarujá, including Tombo, Enseada and Perequê. TA98 and TA100 strains are used to detect frameshift mutations and base pair substitutions, respectively (Mortelmans & Zeiger 2000; Okunola *et al.* 2016; Khallef *et al.* 2019). This lack of genotoxicity in Guarujá may simply indicate the absence or the low concentration of a few organic substances (e.g. PCBs and PAH), known to induce damage to genetic material (Kalmykova *et al.* 2013; Khallef *et al.* 2019). To confirm this hypothesis, further studies are need and should include three other strains of

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bacteria (TA97a, TA102, and TA1535), which are more sensitive to genotoxicity (Mortelmans & Zeiger 2000; Okunola et al. 2016; Khallef et al. 2019).

Regarding the ecotoxicological acute tests with *D. simillis*, the immobility rate in the control was ≤5% for all samples, which attests to the viability of the bioassays performed (Ambrozevicius & Abessa 2008; Gosset et al. 2016). Samples collected in Tombo and Iporanga channels did not cause any toxicity to the freshwater microcrustaceans in any of the campaigns. Tombo (a channel with intermittent flow regime) could not be sampled in the July campaign (Brazilian winter/dry season) because the water-rain course was dry. In the case of Iporanga, the negative result was expected because it is a conservation unit. The physical, chemical and microbiological analyses carried out on this channel, showed than 90% complied with current Brazilian legislation, reflecting the good environmental quality of the area due to its restricted access to tourists and to the existence of an adequate sewage treatment system (Ribeiro & Oliveira 2015; Cetesb 2017; Roveri et al. 2020a). Conversely, samples collected from Enseada and Perequê channels revealed a severe toxic effect on *D. simillis* (mainly in January through March, Brazilian summer/rainy season). Similar studies that took place in the channels of Santos (Brazil) (Ambrozevicius & Abessa 2008) and in the Navile Channel (Italy) (Casadio et al. 2010), which analysed the spatiotemporal characteristics of diffuse pollution, also found high toxicity for microcrustacean, especially in urbanised areas and during the rainy season.

Although short-term toxicity tests with *D. simillis* are widely used to test severe acute effects of complex mixtures (Dutka et al. 1994; Ambrozevicius & Abessa 2008; Gosset et al. 2016), sub-lethal effects resulting from long-term exposure to urban runoff could be also evaluated using chronic tests (Marsalek et al. 1999; Rastetter & Gerhardt 2017). Indeed, the obtained results from the chronic toxicity tests with *C. dubia* regarding the Guarujá water samples recorded more severe outcomes than those from the acute toxicity tests. Chronic exposure revealed toxicity effects in 12 of the 15 water samples, with the toxicity levels being higher during the rainy season compared to the dry season, mainly in the Tombo, Enseada and Perequê channels (the immobility rate in the control was ≤10% for all samples, which attest to the viability of the chronic tests performed). The prevalence of chronic toxicity for urban drainage has been already observed by other authors. In the city of Longueuil, (heavily affected by runoff and urbanisation), Montreal, Canada, while chronic ecotoxicity tests revealed a potential impact of urban runoff in *C. dubia*, acute toxicity tests showed less expressive effects on *D. magna* (species with sensitivity similar to *D. simillis*) (Gooré et al. 2015). In the southwestern basin of Lake Como, Italy, a typically urban basin with a high anthropogenic impact, none of the exposures to the water samples affected the mobility of *D. magna*, excluding direct acute effects (Roberta et al. 2014). However, the authors suggest that serious effects can be expected after a chronic exposure, mainly due to the interactions between micropollutants (Musolff et al. 2009; Roberta et al. 2014). Indeed, multiple categories of contaminants have been found in the Tombo, Enseada and Perequê channels, such as (i) heavy metals (aluminum, cadmium, copper, chrome, nickel), (ii) organic substance (surfactants) and (iii) pharmaceuticals products (antihypertensives, stimulants, analgesics/anti-inflammatory, antiepileptic, antidepressant, anticholesteremic, diuretic, antiplatelet drug, illicit drugs) (Roveri et al. 2020a, 2020c). Therefore, this complex mixture of compounds, even at low concentrations, may explain the greater chronic toxicity of the urban runoff in Guarujá. However, the effect of PAHs cannot be ruled out, especially in the rainy season. It is well known that during rainfall–runoff events there is an enrichment of PAHs, consequence of the local atmospheric deposition (Menzie et al. 2002) and soil erosion processes (Zheng et al. 2012). Moreover, many PAHs are highly toxic, mutagenic and carcinogenic to different types of organisms, including humans (Abdel-Shafy & Mansour 2016).

This study confirms that urban runoff from Guarujá, in addition to being recognised as an important transport mechanism for conventional and emerging pollutants into receiving water bodies (seawater) (Roveri et al. 2020a, 2020b, 2020c), has the potential to cause deleterious effects in the aquatic biota and, therefore, deserves further attention.

CONCLUSION

As far we know, this is the first study to evaluate the genotoxicity and ecotoxicity of the urban surface runoff in Guarujá, São Paulo, Brazil. This work confirmed the toxicity of the waters that flow continuously to the beaches from the municipality creating potential ecological risks for the aquatic ecosystem. Although, it is not requested by the Brazilian Water Resources Policy (Law No. 9433/1997) (Brazil 1997), it is important to regularly include ecotoxicological (acute and chronic) bioassays in national environmental monitoring programs for water control quality, allowing evaluation of the impact of the diffuse loads into the coastal areas. This approach is already implemented in Europe and the USA, where integrated water assessment (physical, chemical and

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biological) is warmly supported by the European Water Framework Directive 2000/60/CE (EC 2000) and by the Nonpoint Source Monitoring Program (NNPSMP, Section 319) of the Environmental Protection Agency National, respectively (USEPA 2002). To improve the environmental quality of these coastal waters and to reduce ecological risks to the aquatic biota and public health, best management practices (BMPs) are required, such as the installation of a floodgate system in the urban drainage channels, connection of the urban surface drainage to the sewage collection network, amelioration of basic sanitation facilities and land regularisation actions, mainly at Enseada and Perequê beaches. Finally, a broad characterisation of the pollutants (including PAHs), and further ecotoxicological bioassays (by testing other trophic levels, such as algae and fish, in water samples collected throughout the year) must be carried out on the urban runoff in Guarujá.

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DISCLOSURE STATEMENT

There are no conflicts of interest.

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COMPLIANCE WITH ETHICAL STANDARDS

Statement of animal rights: all applicable international and/or national guidelines for the care and use of animals were followed.

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

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