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

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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 similis 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

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 similis) and chronic (Ceriodaphnia dubia) toxicities were however recorded.
- Results suggest potential risk to the environmental and public health.
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 polychlorobiphenyls (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 similis 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. similis 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.
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
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 performed 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)):

$$MI = \frac{\text{number of revertants per plate (test compound)}}{\text{number of revertants per plate (negative control)}}$$  \hspace{1cm} (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 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 used. It was made up by adding specific amounts of reagents of recognised analytical grade to distilled water. Final pH ranged between 7.0 and 7.6, and hardness between 40 and 48 mg/L (as CaCO₃). For more details, see ecotoxicological guidelines ABNT NBR 12773 (2016) and ABNT NBR 13373 (2017). Both tests adopted a level of significance ($\alpha$) 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
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 organisms 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
Responsible for introducing a mixture of conventional and emerging pollutants into the coastal waters, as a 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 2018; 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 2018; 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). Therefore, the introduction of harmful bacteria into coastal waters can result in potential toxic effects (synergistic, antagonistic, and/or additives) on the aquatic ecosystems (Kalmykova et al. 2013; Gosset et al. 2016; Khallef et al. 2019). It is expected that 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, 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).

Table 2 | Acute and chronic ecotoxicity tests results with Daphnia similis (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 Total | % toxicity classification | Immobility Total | % toxicity classification | Immobility Total | % toxicity classification | Immobility Total | % toxicity classification |
| Control | 0 0 Non-toxic | - | 0 0 Non-toxic | - | 0 0 Non-toxic | - |
| Tombo  | 0 0 Non-toxic | - | 1 5 Non-toxic | - | 0 0 Non-toxic | - |
| Enseada | 20 100 Very toxic | 12 | 30 Very toxic | 2 | 0 0 Non-toxic | - |
| Perequê | 20 100 Very toxic | 12 | 30 Very toxic | 2 | 0 0 Non-toxic | - |
| Iporanga | 0 0 Non-toxic | - | 0 0 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.

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| Beach | January (rainy season) | March (rainy season) | May (dry season) | July (dry season) |
|-------|------------------------|----------------------|------------------|------------------|
|       | Immobility Total | % toxicity classification | Immobility Total | % toxicity classification | Immobility Total | % toxicity classification | Immobility Total | % toxicity classification |
| Control | 0 0 Non-toxic | - | 0 0 Non-toxic | - | 0 0 Non-toxic | - |
| Tombo  | 10 100 Very toxic | 8 | 30 Toxic | 3 | 0 0 Non-toxic | - |
| Enseada | 10 100 Very toxic | 8 | 30 Toxic | 3 | 0 0 Non-toxic | - |
| Perequê | 10 100 Very toxic | 8 | 30 Toxic | 3 | 0 0 Non-toxic | - |
| Iporanga | 0 0 Non-toxic | - | 0 0 Non-toxic | - | 0 0 Non-toxic | - |

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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. similis*, the immobility rate in the control was \( \leq 5\% \) 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 that 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; Ceteb 2017; Roveri et al. 2020a). Conversely, samples collected from Enseada and Perequê channels revealed a severe toxic effect on *D. similis* (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. similis* 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 \( \leq 10\% \) 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. similis*) (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 (Musolf 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 as 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
biological) is warmly supported by the European Water Framework Directive 2000/60/CE (EC 2000) and by the Nonpoint Source Monitoring Program (NNPSMP, Section 519) 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

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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.

REFERENCES

Abdel-Shafy, H. I. & Mansour, M. S. M. 2016 A review on polycyclic aromatic hydrocarbons: source, environmental impact, effect on human health and remediation. *Egyptian Journal of Petroleum* 5 (1), 107–123. doi: 10.1016/j.ejpe.2015.03.011.

ABNT – Associação Brasileira de Normas Técnicas. NBR 12713:2016 2016 Aquatic Ecotoxicology - Acute Toxicity – Test with Daphnia spp. (Crustacea, Cladocera).

ABNT – Associação Brasileira de Normas Técnicas NBR 13373:2017 Aquatic Ecotoxicology – Chronic Toxicity – Test Method with Ceriodaphnia spp. (Crustacea, Cladocera). Ambrosevicius, A. P. & Abessa, D. M. S. 2008 Acute toxicity of waters from the urban drainage channels of Santos (São Paulo, Brazil). *Pan-American Journal of Aquatic Sciences* 3 (2), 108–115.

Barišene, J., Rybakovas, A., Lang, T., Grygiel, W., Andreikenaite & L. & Michailovas, A. 2012 Risk of environmental genotoxicity in the Baltic Sea over the period of 2009–2011 assessed by micronuclei frequencies in blood erythrocytes of flounder (*Platichthys flesus*), herring (*Clupea harengus*) and eelpout (*Zoarces viviparus*). *Marine Environmental Research* 77, 35–42. doi:10.1016/j.marenvres.2012.01.004.

Casadio, A., Maglionico, M., Bolognesi, A. & Artina, S. 2010 Toxicity and pollutant impact analysis in an urban river due to combined sewer overflows loads. *Water Science and Technology* 61 (1), 207–215. doi:10.2166/wst.2010.809.

Cetesb – Companhia Estadual de Tecnologia e Saneamento ambiental. Guia Nacional de Coleta e Preservação de Amostras – Água, Sedimento, Comunidades Aquáticas e Efluentes Líquidos 2011 Cia. Ambient. do Estado São Paulo. São Paulo, Brazil, pp. 326. https://doi.org/10.5772/12371.

Cetesb – Companhia Estadual de Tecnologia e Saneamento ambiental. Relatório de qualidade das praias litorâneas do Estado de São Paulo 2017 Série Relatórios/Cetesb. pp190. ISSN 0103-4103.

Dutka, B. J., Marsalek, J., Jurkovic, A., Kwan, K. K. & McInnis, R. 1994 A seasonal ecotoxicological study of stormwater ponds. *Zeitschrift fuer angewandte Zoologie* 80, 361–381.

European Commission (EC) 2000 Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for community action in the field of water policy. *Official Journal of the European Union* 327 (1), 73.

Gooré, B. E., Monette, F. & Gasperi, J. 2015 Assessment of the ecotoxicological risk of combined sewer overflows for an aquatic system using a coupled ‘substance and bioassay’ approach. *Environmental Science and Pollution Research* 22, 4460–4474. https://doi.org/10.1007/s11356-014-3650-9.

Gosset, A., Ferro, Y. & Durrieu, C. 2016 Methods for evaluating the pollution impact of urban wet weather discharges on...
biocenosis: a review. Water Research 89, 330–354. doi:10.1016/j.watres.2015.11.020.

Ibge – Instituto brasileiro de Geografia e Estatística 2018 Estimativa da população brasileira. Rio de Janeiro, Brazil. Available from: http://ibge.gov.br/Estimativas_de_Populacao/Estimativas_2019 (accessed 11 November 2020).

Kalmykova, Y., Björklund, K., Strömwall, A. M. & Blom, L. 2013 Partitioning of polycyclic aromatic hydrocarbons, alkylphenols, bisphenol A and phthalates in landfill leachates and stormwater. Water Research 47 (3), 1317–1328. doi:10.1016/j.watres.2012.11.054.

Khalef, M., Benouareth, D. E., Konuk, M., Liman, R., Bouchelaghem, S., Hazzem, S. & Kerdouci, K. 2019 The effect of silver nanoparticles on the mutagenic and the genotoxic properties of the urban wastewater liquid sludges. Environmental Science and Technology Pollution Research, 1–8. doi:10.1007/s11356-019-05225-8.

Lamparelli, C. C., Pogreba-brown, K., Verhougstraete, M. & Marsalek, J., Rochfort, Q., Brownlee, B., Mayer, T. & Servos, M. –– Mortelmans, K. & Zeiger, E. Ministério do desenvolvimento urbano e meio ambiente. Brazil. –– Musolff, A., Leschik, S., Möder, M., Strauch, G., Reinstorf, F. & V. Roveri. Instituto brasileiro de Geografia e Estatística. Streams: biodegradability and molecular composition studies.

Publicada no DOU nº 14 de 2013. 1007/s11356-019-05225-8.

Rastetter, N. & Gerhardt, A. 2017 Toxic potential of different types of sewage sludge as fertiliser in agriculture: ecotoxicological effects on aquatic, sediment and soil indicator species. Journal of Soils and Sediments 17 (1), 106–121.

R Core Team 2017 R. A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna. Available from: http://www.R-project.org/ (accessed 20 November 2020).

Ribeiro, A. L. P. M. & Oliveira, R. C. (Orgs) 2015 Baixada Santista: uma contribuição à análise geobambiental, 1st edn. UNESP, São Paulo, Brazil, p. 255. ISBN 978-85-68334-55-3. Available from: http://editoraunesp.com.br/catalogo?criterio=978-85-68334-55-3 (accessed 10 November 2020).

Roberta, B., Benedetta, P. & Silvia, Q. 2014 An ecotoxicological approach to assess the environmental quality of freshwater basins: a possible implementation of the EU water framework directive? Environments 1 (1), 92–106. doi:10.3390/environments1010092.

Roveri, V., Guimarães, L. L. & Correia, A. T. 2020a Spatial and temporal evaluation of the urban runoff water flowing into recreational areas of Guarujá, São Paulo State, Brazil. International Journal of River Basin Management 1–0. doi:10.1080/15715124.2020.1776304.

Roveri, V., Guimarães, L. L., Correa, A. T., Demoliner, M. & Spiliki, F. R. 2020b Occurrence of human adenosiviruses in a beach area of Guarujá, São Paulo, Brazil. Water Environment Research doi:10.1002/wer.1338.

Roveri, V., Guimarães, L. L., Toma, W. & Correa, A. T. 2020c Occurrence and ecological risk assessment of pharmaceuticals and cocaine in a beach area of Guarujá, São Paulo State, Brazil, under the influence of urban surface runoff. Environmental Science and Pollution Research doi:10.1007/s11356-020-10316-y.

Souza, I. S., Araujo, G. S., Cruz, A. C. F., Fonseca, T. G., Camargo, J. B. D. A., Medeiros, G. F. & Abessa, D. M. S. 2016 Using an integrated approach to assess the sediment quality of an estuary from the semi-arid coast of Brazil. Marine Pollution Bulletin 104 (1-2), 70–82. doi:10.1016/j.marpolbul.2016.02.009.

USEPA (United States Environmental Protection Agency) 2002 Section 319 Success Stories Volume III: The Successful Implementation of the Clean Water Act's 319 Nonpoint Source Pollution Program. EPA 841-S-01-0001. EPA, Office of Water, Washington, DC.

Xiang, C., Wang, Y. & Liu, H. 2017 A scientometrics review on nonpoint source pollution research. Ecological Engineering 99, 400–408. doi:10.1016/j.ecoleng.2016.11.028.

Yang, Y. Y. & Toor, G. S. 2017 Sources and mechanisms of nitrate and orthophosphate transport in urban stormwater runoff from residential catchments. Water Research 112, 176–184. doi:10.1016/j.watres.2017.01.039.

Zheng, Y., Luo, X., Zhang, W., Wu, B., Han, F., Lin, Z. & Wang, X. 2012 Enrichment behavior and transport mechanism of soil-bound PAHs during rainfall runoff events. Environmental Pollution 171, 85–92. doi:10.1016/j.envpol.2012.07.030.

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