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An integrated environmental assessment of the water and sediments from the coastal areas of Guarujá, São Paulo, Brazil: a physico-chemical, biological and ecotoxicological approach

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Tese apresentada à Universidade Fernando Pessoa como parte dos requisitos para obtenção do grau de Doutor em Ecologia e Saúde Ambiental, sob a orientação científica do Prof. Doutor Alberto Teodorico Correia da Universidade Fernando Pessoa e coorientação da Profa. Doutora Luciana Lopes Guimarães da Universidade Santa Cecília.

Previous Note: This thesis integrates in the main body severeal articles published, accepted or submitted to international peer-reviewed scientific journals. Some of the results have been also partially presented in some congresses. The candidate also states that he conceived the ideas, compiled the data, analyzed and discussed the results, and led the writing of the different chapters.

(i) Papers

Roveri, V., Guimarães, L. L., Correia, A. T., 2020. 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

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(ii) Congresses

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RESUMO

VINICIUS ROVERI: Avaliação ambiental integrada da água e dos sedimentos nas zonas costeiras de Guarujá, São Paulo, Brasil: uma abordagem físico-química, biológica e ecotoxicológica

(Sob orientação do Prof. Doutor Alberto Teodorico Correia)

O município de Guarujá, litoral do Estado de São Paulo, Brasil, considerado um dos principais destinos turísticos brasileiros, possui uma sub-bacia hidrográfica sob forte pressão antrópica. Isto ocorre porque embora possua uma área de drenagem de aproximadamente 143 km², cerca de 107 km² são áreas ambientalmente protegidas e, portanto, 316.000 habitantes se concentram em uma pequena fração territorial (correspondente a 36 km^2). Durante o verão, quando o turismo intensifica, a população chega a duplicar. Em decorrência deste forte adensamento populacional e da ausência de planeamento urbano, Guarujá sofre há décadas com problemas socioambientais e de infraestrutura urbana, principalmente porque mais de 64.000 moradores habitam áreas de favelas (principalmente nos bairros da Enseada e do Perequê) e, portanto, não possuem saneamento básico. Desta forma, embora a rede de coleta e tratamento de esgotos municipal esteja dimensionada para uma população de 450.000 habitantes, o destino dos esgotos clandestinos destas favelas acaba sendo os 43 canais artificiais de drenagem urbana, que descarregam uma mistura complexa (composta por esgoto doméstico e carga difusa urbana) diretamente para as praias do município e, portanto, podendo comprometer a saúde pública e ambiental de Guarujá. Embora avaliar a qualidade balnear, se mostre emergencial no Guarujá, estes estudos ainda são escassos no município. Diante deste cenário, o objetivo geral desta tese foi avaliar a qualidade da água e do sedimento nos canais de drenagem urbana localizados em quatro diferentes praias do Guarujá: Tombo (certificada com Bandeira Azul), Enseada (com alta visitação turística), Perequê (habitada por uma comunidade pesqueira) e Iporanga (localizada numa unidade de conservação). Além destes quatro canais, foi investigada a coluna de água e o sedimento da zona de mistura do emissário oceânico submarino, cujo esgoto municipal passa por um tratamento preliminar em terra. Para atender a estes objetivos, amostras mensais foram obtidas no período entre outubro de 2017 a agosto de 2018, e trinta e oito variáveis ambientais selecionadas (físicas, químicas, biológicas e ecotóxicológicas), juntamente com vinte e três compostos farmacêuticos de diferentes classes terapêuticas, foram analisados utilizando testes estatísticos padrão e/ou diferentes índices de qualidade da água. Nos canais de drenagem urbana, os resultados mais expressivos obtidos desta tese, foram: (i) há evidenciais de que os canais do Guarujá são importantes veículos de transporte de poluentes convencionais e emergentes para o Oceano Atlântico Sul; (ii) áreas com déficit de saneamento (principalmente na Enseada e Perequê), juntamente com o período de maior afluência turística (verão brasileiro/período chuvoso), foram fatores que potencializaram a piora da qualidade da água; (iii) de maneira geral, a melhor qualidade da água foi registrada em Iporanga (mais de 90% em conformidade com a legislação brasileira); (iv) os canais do Tombo, Enseada e Perequê foram classificados como muito ruins (apenas 34-43% em conformidade com a legislação vigente) e, como consequência, altas concentrações das bactérias *Escherichia coli* e *Enterococcus ssp* foram detectadas nestes canais. Além disso, a presença de mastadenovírus humanos (espécies C, D e F) na Enseada e Perequê, também foram importantes descobertas, potencialmente preocupantes para a saúde pública local; (v) táxons da macrofauna bentônica, tolerantes à poluição orgânica, foram inventariados no sedimento adjacente aos canais (por exemplo, Oligochaeta, Ceratopogonidae, Chironomidae e *Chironomus*); (vi) dezasseis compostos farmacêuticos foram detectados, sendo que cinco deles (cafeína, acetaminofeno, diclofenaco, valsartan e losartan), indicaram riscos escológicos moderados a altos para algas, crustáceos e/ou peixes; e (vii) toda esta complexa mistura de poluentes dos canais, indicaram uma alta toxicidade aguda e crônica para os microcrustáceos *Daphnia simillis* e *Ceriodaphnia dúbia*, respectivamente. Já na descarga marinha de esgotos, os resultados mais importantes, na coluna de água, foram: (i) das trinta e três variáveis fisicoquímicas e bacteriológicas analisadas, nove não atenderam à legislação vigente e, portanto, a água foi classificada como de qualidade regular; (ii) dez compostos farmacêuticos foram detectados, sendo que três deles (cafeína, diclofenaco e acetaminofeno), indicaram riscos baixos a moderados para algas, crustáceos e/ou peixes. Já nos sedimentos, as baixas concentrações de seis metais pesados, a boa representatividade de anfípodes e poliquetas (dentre os vinte e cinco táxons bentônicos inventariados), além da ausência de toxicidade aguda para o tanaidaceo *Kalliapseudes* *schubartii*, indicaram sedimentos pouco poluídos. Com a evidência da forte interferência antrópica na qualidade da água do Guarujá, melhorias em benefício da saúde pública e da proteção dos sistemas ecológicos (fundamentadas em boas práticas internacionais e na legislação brasileira vigente) foram sugeridas, como sejam: (i) programas de educação e conscientização ambiental; (ii) programas de recolhimento de resíduos de medicamentos; (iii) manutenção de vias públicas; (iv) fiscalização das ligações clandestinas de esgotos no bairro do Tombo; (v) regularização fundiária das favelas da Enseada e do Perequê; (vi) instalação de um sistema de comporta, interceptor oceânico e barreiras de conteção nos canais de drenagem. E por fim, foi sugerida a modernização do sistema de tratamento de esgotos do município, através da instalação de um tratamento, no mínimo, com um nível secundário.

Palavras-chave: Zona subtropical, Águas balneares, Ecologia marinha, Canais de drenagem urbana, Fonte difusa de poluição, Tratamento de esgoto, Esgoto doméstico, Descarga oceânica, Produtos farmacêuticos, Drogas ilícitas, Bioensaios, Indicador ecológico, Indicador microbiológico, Efeitos da poluição.

ABSTRACT

VINICIUS ROVERI: An integrated environmental assessment of the water and
sediments from the coastal areas of Guarujá, São Paulo, Brazil: a physico-chemical,
biological and ecotoxicological approach

(Under the supervision of Professor Alberto Teodorico Correia)

The municipality of Guarujá, on the coast of the State of São Paulo, Brazil, considered one of the main tourist destinations in Brazil, has a sub-basin under strong anthropic pressure. This occurs because although it has a drainage area of approximately 143 km², about 107 km² are environmentally protected areas and, therefore, 316,000 inhabitants are concentrated in a small territorial fraction (corresponding to 36 km^2). During the summer, when tourism intensifies, the population almost doubles. As a result of this high-population density and absence of urban planning, Guarujá has suffered for decades from socio-environmental and urban infrastructure problems, mainly because more than 64,000 residents live in slum areas (locally named "favelas", mainly in the Enseada and Perequê neighbourhoods) and therefore do not have basic sanitation. Thus, although the municipal sewage collection and treatment network is sized for a population of 450,000 inhabitants, the destination of clandestine sewage from the "favelas" ends up on the 43 artificial urban drainage channels, which drain a complex mixture (composed of domestic sewage and urban diffuse load) directly to the beaches of the municipality and, therefore, may compromise the public and environmental health of Guarujá. Although the assessment of the bathing water quality is a priority in Guarujá, these studies are scarce in the municipality. In this scenario, the general objective of this thesis was to evaluate the water and sediment quality in urban drainage channels located in four different beaches of Guarujá: Tombo (Blue Flag certified), Enseada (high tourist visitation), Perequê (inhabited by a fishing community) and Iporanga (located in a conservation unit).

In addition to these four channels, the water column and the sediment of the mixing zone of the submarine ocean outfall, whose municipal sewage undergoes preliminary treatment on land, were investigated. To meet these objectives, monthly samples were obtained during the period from October 2017 to August 2018, and thirty-eight selected environmental variables (physical, chemical, biological and ecotoxicological), together with the twenty-three pharmaceutical compounds of different therapeutic classes, were analysed using standard statistical tests and/or different water quality indices. In the urban drainage channels, the most expressive results obtained from this thesis, were: (i) there is evidence that the channels of Guarujá are important vehicles for the transport of conventional and emerging pollutants to the South Atlantic Ocean; (ii) areas with sanitation deficit (mainly in Enseada and Perequê), together with the period of greatest tourist affluence (Brazilian summer/rainy season), were factors that potentiated the worsening of water quality; (iii) in general, the best water quality was recorded at Iporanga (more than 90% of compliance with the Brazilian legislation); (iv) the Tombo, Enseada and Perequê channels were classified as very bad (only 34-43% of compliance with the current legislation) and, as a consequence, high concentrations of the bacteria *Escherichia coli* and *Enterococcus ssp* were detected in these channels. Furthermore, the presence of human mastadenovirus (species C, D and F) in Enseada and Perequê were also important findings, and raises great concern for local public health; (v) benthic macrofauna taxa, tolerant to organic pollution, were inventoried in the sediment adjacent to the channels (e.g. Oligochaeta, Ceratopogonidae, Chironomidae and *Chironomus*); (vi) sixteen pharmaceutical compounds were detected, five of which (caffeine, acetaminophen, diclofenac, valsartan and losartan), indicated a moderate to high ecological risks to algae, crustaceans and/or fish; and (vii) this whole complex mixture of pollutants from the channels, indicated high acute and chronic toxicity to the microcrustaceans *Daphnia simillis* and *Ceriodaphnia dubia*, respectively. As for the marine sewage discharge, the most important results, in the water column, were: (i) of the thirty-three physicochemical and bacteriological variables analysed, nine did not comply with the current legislation and, therefore, the water was classified as regular; (ii) ten pharmaceutical compounds were detected, three of them (caffeine, diclofenac and acetaminophen), indicating low to moderate risks for algae, crustaceans and/or fish. In the sediments, the low concentrations of six heavy metals, the good representation of amphipods and polychaetes (among the twenty-five benthic taxa inventoried), besides the absence of acute toxicity for the tanaidaceous *Kalliapseudes schubartii*, indicated slightly polluted sediments. With the evidence of the strong anthropic interference in the quality of the water of Guarujá, improvements for the benefit of public health and the protection of the ecological systems (based on good international practices and on the Brazilian legislation in force) were suggested, such as: (i) community awareness and educational programs; (ii) drug disposal programs; (iii) public road maintenance; (iv) inspection of clandestine sewage connections in the Tombo neighbourhood; (v) land regularization of the Enseada and Perequê favelas; (vi) installation of a floodgate system, ocean interceptor and containment barriers in the drainage channels. Finally, it was suggested that the municipal sewage system should be modernised by installing, at least, a secondary level of treatment.

Keywords: Subtropical zone, Bathing water, Marine ecology, Urban drainage channels, Diffuse source of pollution, Sewage treatment, Domestic sewage, Oceanic discharge, Pharmaceutical products, Illicit drugs, Bioassays, Ecological indicator, Microbiological indicator, Pollution effects.

RÉSUMÉ

VINICIUS ROVERI: Une évaluation environnementale intégrée de l'eau et des sédiments dans les zones côtières de Guarujá, São Paulo, Brésil: une approche physico-chimique, biologique et écotoxicologique

(Sous l´orientation du Professeur Alberto Teodorico Correia)

La municipalité de Guarujá, sur la côte de l'État de São Paulo, au Brésil, considérée comme l'une des principales destinations touristiques du Brésil, possède un sous-bassin soumis à une forte pression anthropique. En effet, bien qu'elle ait une zone de drainage d'environ 143 km^2, 107 km^2 sont des zones protégées sur le plan environnemental et, par conséquent, 316 000 habitants sont concentrés dans une petite fraction territoriale (correspondant à 36 km^2). Pendant l'été, lorsque le tourisme s'intensifie, la population presque double. En raison de cette forte densité de la population et de l'absence de planification urbaine, Guarujá souffre depuis des décennies de problèmes socio-environnementaux et d'infrastructure urbaine, principalement parce que plus de 64 000 habitants habitent les bidonvilles (localement appelés « favelas », principalement dans les quartiers d'Enseada et de Perequê) sans aucun système sanitaire de base. Ainsi, bien que le système municipal de collecte et de traitement des eaux usées soit dimensionné pour une population de 450 000 habitants, la destination des effluents clandestines des « favelas » est les 43 canaux artificiels de drainage urbain, qui drainent un mélange complexe (composé d'eaux usées domestiques et de charge urbaine diffuse) directement vers les plages de la municipalité et, par conséquent, peuvent compromettre la santé publique et environnementale de Guarujá. Bien que l'évaluation de la qualité des eaux balnéaires soit une urgence à Guarujá, ces études sont encore rares dans la municipalité. Compte tenu de ce scénario, l'objectif général de cette thèse était d'évaluer la qualité de l'eau et des sédiments dans les canaux de drainage urbains situés sur quatre plages

différentes de Guarujá: Tombo (certifié Pavillon Bleu), Enseada (forte fréquentation touristique), Perequê (habité par une communauté de pêcheurs) et Iporanga (situé dans une unité de conservation). En plus de ces quatre canaux, la colonne d'eau et les sédiments de la zone de mélange de l'émissaire sous-marin, dont les eaux usées municipales subissent un traitement préliminaire à terre, ont été étudiés. Pour atteindre ces objectifs, des échantillons mensuels ont été obtenus entre octobre 2017 et août 2018, et trente-huit variables environnementales sélectionnées (physiques, chimiques, biologiques et écotoxicologiques), ainsi que vingt-trois composés pharmaceutiques de différentes classes thérapeutiques, ont été analysés en utilisant des tests statistiques standard et/ou différents indices de qualité de l'eau. Dans les canaux de drainage urbains, les résultats les plus expressifs obtenus dans le cadre de cette thèse, ont été: (i) il est prouvé que les canaux de Guarujá sont d'importants véhicules de transport de polluants conventionnels et émergents vers l'océan Atlantique Sud; (ii) les plages présentant un déficit sanitaire (principalement à Enseada et Perequê), ainsi que la période de plus grande affluence touristique (été brésilien/saison des pluies), sont des facteurs qui ont potentialisé la détérioration de la qualité de l'eau; (iii) en général, la meilleure qualité d'eau a été enregistrée à Iporanga (plus de 90% de conformité avec la législation brésilienne); (iv) les canaux Tombo, Enseada et Perequê ont été classés comme très mauvais (seulement 34-43% de conformité avec la législation en vigueur) et, en conséquence, de fortes concentrations des bactéries *Escherichia coli* et *Enterococcus ssp* ont été détectées dans ces canaux. En outre, la présence de mastadenovirus humains (espèces C, D et F) à Enseada et Perequê a également été constatée, ce qui a suscité une grande inquiétude pour la santé publique locale; (v) des taxons de la macrofaune benthique, tolérante à la pollution organique, ont été inventoriés dans les sédiments adjacents aux canaux (par exemple Oligochaeta, Ceratopogonidae, Chironomidae et *Chironomus*); (vi) seize composés pharmaceutiques ont été détectés, et cinq d'entre eux (caféine, acétaminophène, diclofénac, valsartan et losartan), ont indiqué des risques escologiques modérés et élevés pour les algues, les crustacés et/ou les poissons; et (vii) tout ce mélange complexe de polluants provenant des canaux, a indiqué une toxicité aiguë et chronique élevée pour les microcrustacés *Daphnia simillis* et *Ceriodaphnia dubia*, respectivement. En ce qui concerne le rejet des eaux usées en mer, les résultats les plus importants dans la colonne d'eau ont été obtenus: (i) sur les trente-trois variables physico-chimiques et bactériologiques analysées, neuf ne répondaient pas à la législation en vigueur et, par conséquent, l'eau a été classée comme régulière; (ii) dix composés pharmaceutiques ont

été détectés, dont trois (caféine, diclofénac et acétaminophène), indiquant des risques faibles et modérés pour les algues, les crustacés et/ou les poissons. Dans les sédiments, les faibles concentrations de six métaux lourds, la bonne représentation des amphipodes et des polychètes (parmi les vingt-cinq taxons benthiques inventoriés), outre l'absence de toxicité aiguë pour le tanaidacé *Kalliapseudes schubartii*, indiquaient des sédiments peu pollués. Compte tenu des preuves de la forte interférence anthropique dans la qualité de l'eau de Guarujá, des améliorations au profit de la santé publique et de la protection des systèmes écologiques (basées sur les bonnes pratiques internationales et sur la législation brésilienne en vigueur) ont été suggérées, parmi lesquelles : (i) programmes de sensibilisation environnementale et d'information de la communauté ; (ii) programmes de collecte des déchets médicaux; (iii) amélioration de la voie publique; (iv) surveillance des branchements clandestins d'eaux usées dans le quartier de Tombo; (v) régularisation foncière des favelas d'Enseada et de Perequê; (vi) installation d'un système de vannes, d'un intercepteur océanique et de barrières de confinement dans les canaux de drainage. Finalement, il a été suggéré de moderniser le réseau d'égouts municipal en installant, au moins, un système de traitement secondaire.

Mots-clés: Zone subtropicale, Eaux de baignade, Ecologie marine, Canaux de drainage urbains, Source diffuse de pollution, Traitement des eaux usées, Eaux usées domestiques, Rejet en mer, Produits pharmaceutiques, Drogues illicites, Essais biologiques, Indicateur écologique, Indicateur microbiologique, Effets de la pollution.

DEDICATÓRIA (Dedicatory, Portuguese only)

Dedico especialmente este trabalho, à minha querida e inesquecível mãe **Alice** “*in memorian*”, e ao meu padrasto **Hans** “*in memorian*”. Tenho certeza que continuam iluminando os caminhos dos seus filhos, além de comemorar todas as nossas vitórias.

Dedico, também, aos meus irmãos, **Rodrigo e Samanta**, e ao meu sobrinho **Kron**. Vocês são inspiração para todos os meus dias!

Por último, gostaria de fazer uma dedicatória mais que especial, para a minha esposa **Cyntia**. Muito obrigado pelo apoio, ajuda, incentivo e “paciência” durante a realização desta tese. Com a sua inteligência emocional e positividade frente às adversidades, as coisas se tornaram mais fáceis!

Amo vocês!!

AGRADECIMENTOS (Acknowledgements, Portuguese only)

Uma tese, independente do fato de sua autoria ser individual (resultado de uma escrita exaustiva e isolada), é uma construção fruto do esforço de muitos que contribuem indiretamente ou, no caso desta investigação de caráter multidisciplinar, diretamente com a sua construção. Eis que chegou o momento de expressar meus sinceros agradecimentos a muitos amigos, profissionais e instituições que estiveram ao meu lado durante este importante ciclo da minha vida pessoal e profissional.

Em primeiro lugar, e não haveria de ser diferente, meu agradecimento especial vai para o Prof. Doutor **Alberto Teodorico Correia**. Sou extremamente grato por ter aceitado orientar esta tese e, desde as nossas primeiras conversas, eu percebi que o desafio seria grande. Com altíssimo rigor científico: orientou, exigiu, revisou e extraiu o máximo de mim, para que fosse desenvolvido um trabalho de qualidade. Confesso que foram inúmeras noites de insônia e incontáveis horas de dedicação e concentração neste trabalho, tentando encontrar soluções para os desafios que me eram impostos. Sinto que evolui muito, tanto pessoalmente quanto profissionalmente, após todo este ciclo de aprendizagem. Muitíssimo obrigado, Alberto!!

Agradecimento especial, também, para a minha co-orientadora, a farmacêutica Profa. Doutora **Luciana Lopes Guimarães**, que me acompanha desde o mestrado. Novamente, com sua dedicação e paciência, me introduziu às análises fisicoquímicas e de compostos farmacêuticos e contribuiu com excelência para o fechamento de mais este ciclo. Muito obrigado, Luciana, por estar novamente ao meu lado e me ajudar a superar mais este desafio!

Queria agradecer, também, a um profissional que sempre admirei, e que tive o prazer de contar com a sua presença durante o planejamento e desenvolvimento desta tese. Acompanhava o trabalho do veterinário, Prof. Doutor **Fernando Rosado Spilki** (presidente da Sociedade Brasileira de Virologia), desde a pesquisa realizada por ele e sua equipe nas águas da baía de Guanabara, no Rio de Janeiro, local onde ocorreu as competições aquáticas dos Jogos Olímpicos 2016. Em 2017, quando iniciei o planejamento desta pesquisa, decidi convidá-lo para realizar estudo semelhante em minha tese. Enviei um “email institucional, domingo, às 21hs”, esperando que ele fosse responder (com sorte!) na segunda pela manhã, mas 10 minutos depois ele respondeu: “pode contar comigo no que for preciso!”. E assim foi durante todo o desenvolvimento deste trabalho. Muito obrigado prof. Spilki, pela confiança, pronta disponibilidade e auxílio nesta tese. Minha admiração só aumentou!

Agradeço aos meus ex-alunos “guerreiros” e, agora colegas de profissão, **Camila Santos Garcia** e **Thiago Henrique Toscano** (engenheiros ambientais) e **Flávio Távora Campi** (gestor ambiental), pelo auxílio nos 11 meses de trabalho de coleta de amostras ao longo das praias do Guarujá. Foram longas manhãs, debaixo de sol forte, expostos às águas contaminadas, subindo as escadarias do condomínio Iporanga e carregando pesadas caixas térmicas. Além disso, continuamente éramos perturbardos pelos turistas mais curiosos e exaltados. Estendo meu agradecimento, também, ao ex-aluno e agora engenheiro ambiental **Gustavo Santos**, que posterior às nossas coletas, realizava o transporte das amostras para os diferentes laboratórios, garantido que as mesmas chegassem dentro dos prazos de validade determinados pelos diferentes padrões normativos. Meu muito obrigado, amigos! Sem vocês eu não teria conseguido!

Agradeço ao grupo de profissionais do **laboratório Econsult**, Guarujá, São Paulo, Brasil: **Gilberto Rivero Chang** (biólogo e capitão de embarcação), **Taiane Pires Farias** (bióloga) e **Edson Wilmsen Ferreira** (gestor ambiental), pelo suporte técnico nas coletas de amostras na descarga de esgotos do emissário submarino do Guarujá. Obrigado por me ajudarem nos procedimentos de coleta e demais análises *in loco*, já que fiquei mareado nas duas campanhas de coleta. Ainda na Econsult, agradeço, também, as biólogas mestras **Vilma Maria Cavinatto Rivero** (responsável técnica pelo laboratório) e **Jaquline Cristiane Siquitelli** pelo suporte técnico nas análises da macrofauna bentônica. Querida Vilma, muito obrigado por ter me introduzido, em 2007, no universo da “qualidade da água”.

Agradeço ao **Centro Interdisciplinar de Investigação Marinha e Ambiental (CIIMAR/CIMAR)**, Matosinhos, Portugal, representado aqui pelo Prof. Doutor **Alberto Teodorico Correia**, pelo apoio no desenvolvimento desta tese.

Agradeço a **Universidade Santa Cecília de Santos (UNISANTA),** São Paulo, Brasil, representada aqui pela Profa. Doutora **Luciana Lopes Guimarães**. Na UNISANTA, estendo o meu agradecimento ao farmacêutico Prof. Doutor **Walber Toma**, por ter me acolhido no Laboratório de Pesquisa em Produtos Naturais, para a realização de parte das análises desta tese.

Agraceço a **Universidade Feevale**, Novo Hamburgo, Rio Grande do Sul, Brasil, representada aqui pelo Prof. Doutor **Fernando Rosado Spilki**. Na Feevale, estendo meus agradecimentos a biomédica mestre, **Meriane Demoliner**, do Laboratório de Microbiologia Molecular, pelo suporte técnico nas análises virológicas.

Agradeço ao **Centro de Espectrometria de Massa Aplicada - Instituto de Pesquisas Energéticas e Nucleares (CEMSA - IPEN)**, São Paulo, Brasil, representado aqui pelo químico mestre, **Daniel Temponi Lebre**, pelo suporte técnico nas análises LC–MS/MS para a detecção de compostos farmacêuticos.

Agradeço a **Universidade Metropolitana de Santos (UNIMES)**, São Paulo, Brasil, representada aqui pela Profa. Doutora **Elisabeth dos Santos Tavares**, por todo o apoio ao longo da realização deste programa de Doutoramento. Na UNIMES, estendo meus agradecimentos a geógrafa Prof^{a}. Mestre **Técia Regiane Bérgamo**, pela amizade e pela parceria na produção e apresentação de trabalhos em diferentes eventos científicos (Congressos, Simpósios e Encontros).

Agradeço ao biólogo, Prof. Doutor **Walter Barrella**, da Universidade Paulista, Sorocaba, São Paulo, Brasil, pelas conversas e importante direcionamento em um momento de indecisão sobre como prosseguir com a pesquisa.

Agradeço a equipe técnica do **laboratório NSF Brasil** (antiga NSF Bioensaios), Viamão, Rio Grande do Sul, Brasil, pelo suporte técnico nas análises biológicas.

Agradeço a equipe técnica do **laboratório Controle Analítico Análises Técnicas**, Osasco, São Paulo, Brasil, pelo suporte técnico nas análises fisicoquímicas e microbiológicas.

Agradeço a **Pulsar Imagens** Ltda, São Paulo, Brasil, pela licença para reproduzir as imagens das praias do Guarujá (contrato nº 27918).

Agradeço a todos os revisores "anônimos", das diferentes revistas internacionais, que de forma ética, crítica e construtiva, contribuíram para a construção desta tese.

Por último, reservei este espaço especialmente para agradecer a toda a comunidade Pessoana. Agradeço a **Universidade Fernando Pessoa (UFP)**, representada aqui pelo Magnífico Reitor Prof. Doutor **Salvato Trigo**, que com excelência, criou condições para que fosse possível a continuidade de meus estudos e aperfeiçoamento pessoal e profissional. Na UFP, estendo os meus agradecimentos a todos os professores do Programa de Doutoramento em Ecologia e Saúde Ambiental, em especial: Prof. Doutor **Alberto Teodorico Correia**, Prof. Doutor **Álvaro Monteiro**, Profª. Doutora **Alzira Dinis**, Profª. Doutora **Ana Fonseca,** Profª. Doutora **Cláudia Silva,** Profª. Doutora **Conceição Manso**, Profª. Doutora **Márcia Carvalho**, Profª. Doutora **Maria João Guerreiro** e Prof. Doutor **Nelson Barros** (coordenador do Programa), pelo profissionalismo e competência profissional. Ainda na UFP, agradeço todos os meus colegas do Programa de Doutoramento: **Lilian Monteiro Ferrari Viterbo, Maria Luisa Souto Porto, Diana Mônica da Silva Furtado, Carlos Tadashi Kunioka, Ana Paula Alves Camardo, Diogo Guedes Vidal, Evailton Arantes de Oliveira, Maria do Perpetuo Socorro Lamego Oliveira, Maria Goretti Poliparco Barreto, Carmem**

Sulinete Suliano da Costa Lima, Maria do Rosário Rabelo do Amaral, Jocelia Maria de Azevedo Bringel e João Batista Silva, pela amizade e bons momentos em terras portuguesas (país que aprendi a adorar e respeitar!).

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LIST OF ABBREVIATIONS

A

ABNT – **A**ssociação **B**rasileira de **N**ormas **T**écnicas

Abrafarma – **A**ssociação **B**rasileira de **F**armácias

AdV – **Ad**eno**v**iruses

AF – **A**ssessment **F**actor

Al – **Al**uminium

ANVISA–**A**gência de **V**igilância **S**anitária

APHA – **A**merican **P**ublic **H**ealth **A**ssociation

ASPT – **A**verage **S**core **p**er **T**axon

B

BMP – **B**est **M**anagement **P**ractices

BMWP – **B**iological **M**onitoring **W**orking **P**arty

BOD – **B**iochemical **O**xygen **D**emand

BPWM – **B**est **P**ractices in **W**ater **M**anagement

C

CAS – **C**hemical **A**bstracts **S**ervice

CCME – **C**anadian **C**ouncil of **M**inisters of the **E**nvironment

CCMEWQI – **C**anadian **C**ouncil of **M**inisters of the **E**nvironment **W**ater **Q**uality **I**ndex

Cd – **C**a**d**mium

CETESB – **C**ompanhia **E**stadual de **T**ecnologia e **S**aneamento ambiental

CMED – Câmara de Regulação do Mercado de Medicamentos

CONAMA – **C**onselho **N**acional do **M**eio **A**mbiente

Cr – **Chr**ome

CTSI – **C**oastal **T**rophic **S**tate **I**ndex

Cu – **C**opper

CWQI – **C**oastal **W**ater **Q**uality **I**ndex

D

DAEE – **D**epartamento de **Á**guas e **E**nergia **E**létrica do Estado de São Paulo

E

EC – **E**ffective **C**oncentration

ECB – **E**uropean **C**hemical **B**ureau

ECHA – **E**uropean **C**hemicals **A**gency

E.coli – ***E**scherichia **coli***

ECOSAR – **E**cological **S**tructure **A**ctivity **R**elationships

EMA – **E**uropean **M**edicines **A**gency

EMBRAPA – **E**mpresa **B**rasileira de **P**esquisa **A**gropecuária

EPA – **E**nvironmental **P**rotection **A**gency

F

FDA – **F**ood and **D**rug **A**dministration

FEE – **F**oundation for **E**nvironmental **E**ducation

G

GC/L – **G**enomic **c**opies per **L**

Geol – **G**eoaccumulation **I**ndex

GPS – **G**lobal **P**osition **S**ystem

GST–**G**lutathione **S-t**ransferase

GVS&DD–**G**reater **V**ancouver **S**ewerage and **D**rainage **D**istrict

H

HAdV – **H**uman **ad**eno**v**irus

HCA – **H**ierarchy **C**luster **A**nalysis

I

IBGE – **I**nstituto **B**rasileiro de **G**eografia e **E**statística

IGAM – **I**nstituto de **G**estão das **Á**guas **M**ineiro

IMVPAL – **I**ndex of **M**inimum **V**ariables for the **P**reservation of **A**quatic **L**ife

INMETRO – **I**nstituto **N**acional de **Metro**logia, Normalização e Qualidade Industrial

L

LAS – Linear **a**lkyl **s**ulfonate

LC – **L**iquid **C**hromatography

LC–MS/MS - Liquid chromatography coupled with tandem mass spectrometry

LC50 – 50% **L**ethal **C**oncentration

LID – **L**ow **I**mpact **D**evelopment

LOD – **L**imit **o**f Detection

LOEC – **L**owest **O**bserved **E**ffect **C**oncentration

LOQ – **L**imit **o**f Quantification

M

MBAS – **M**ethylene-**b**lue-**a**ctive **s**ubstances

MEC – **M**easured **E**nvironmental **C**oncentration

MET–**M**itochondrial **E**lectron **T**ransport

MI – **M**utagenicity **I**ndex

MMA – **M**inistério do **M**eio **A**mbiente

MRBS – **M**etropolitan **R**egion of **B**aixada **S**antista

MRM – **M**ultiple **R**eaction **M**onitoring

MUPSCPI – **M**unicipal **U**rban **P**opulation **S**ewage **C**ollection and **P**rocessing **I**ndex

N

NCDENR–**N**orth **C**arolina **D**epartment of **E**nvironment and **N**atural **R**esources

Nested PCR – **Nested P**olymerase **C**hain **R**eaction

Ni – **Ni**ckel

NOEC – **N**o **O**bserved **E**ffect **C**oncentration

NZWERF–**N**ew **Z**ealand **W**ater **E**nvironment **R**esearch **F**oundation

O

OECD – **O**rganisation for **E**conomic **C**o-operation and **D**evelopment

OG – **O**ils and **g**reases

OSM–**O**n-site **S**tormwater **M**anagement

P

Pb – Lead

PCA – **P**rincipal **C**omponent **A**nalysis

PEL – **P**robable **E**ffect **L**evel

PNEC – **P**redicted **N**o **E**ffect **C**oncentration

POLI– Escola Politécnica da Universidade de São Paulo

PPCP – **P**harmaceuticals and **P**ersonal **C**are **P**roducts

Q

qPCR – **q**uantitative **P**olymerase **C**hain **R**eaction

S

SENAD–Secretaria Nacional de Políticas Sobre Drogas

SMA/CPLA – **S**ecretaria de **M**eio **A**mbiente do Estado de São Paulo/**C**oordenação de **Pl**anejamento **A**mbiental

SMA/CPLEA – **S**ecretaria do **M**eio **A**mbiente do Estado de São Paulo/**C**oordenadoria de **Pl**anejamento e **E**ducação **A**mbiental

SMDU–**S**ecretaria **M**unicipal de **D**esenvolvimento **U**rbano de São Paulo

SPE – **S**olid **P**hase **E**xtraction

SPS – **S**ewage Pre-Conditioning **S**tation

SS – **S**edimented **S**olids

T

TC – **T**otal **c**oliforms

TCI – **T**oxic **C**ontamination **I**ndex

TDS – **T**otal **D**issolved **S**olids

TEL – **T**hreshold **E**ffect **L**evel

TLP–**T**otal **L**ipid **C**ontent

TP – **T**otal **p**hosphorus

TSI – **T**rophic **S**tate **I**ndex

U

UDFCD–**U**rban **D**rainage and **F**lood **C**ontrol **D**istrict

UGRHI – **U**nidades de **G**erenciamento de **R**ecursos **H**ídricos

UNDESA – **U**nited **N**ations **D**epartment of **E**conomic and **S**ocial **A**ffairs

UNIFESP – **U**niversidade **F**ederal de **S**ão **P**aulo

UNODC–**U**nited **N**ations **O**ffice on **D**rugs and **C**rime

USEPA – **U**nited **S**tates **E**nvironmental **P**rotection **A**gency

USW – **U**rban **S**olid **W**aste

W

WHO – **W**orld **H**ealth **O**rganization

WQI –**W**ater **Q**uality **I**ndex

WQI-PALAC – **W**ater **Q**uality **I**ndex for the **P**rotection of **A**quatic **L**ife and **A**quatic **C**ommunities

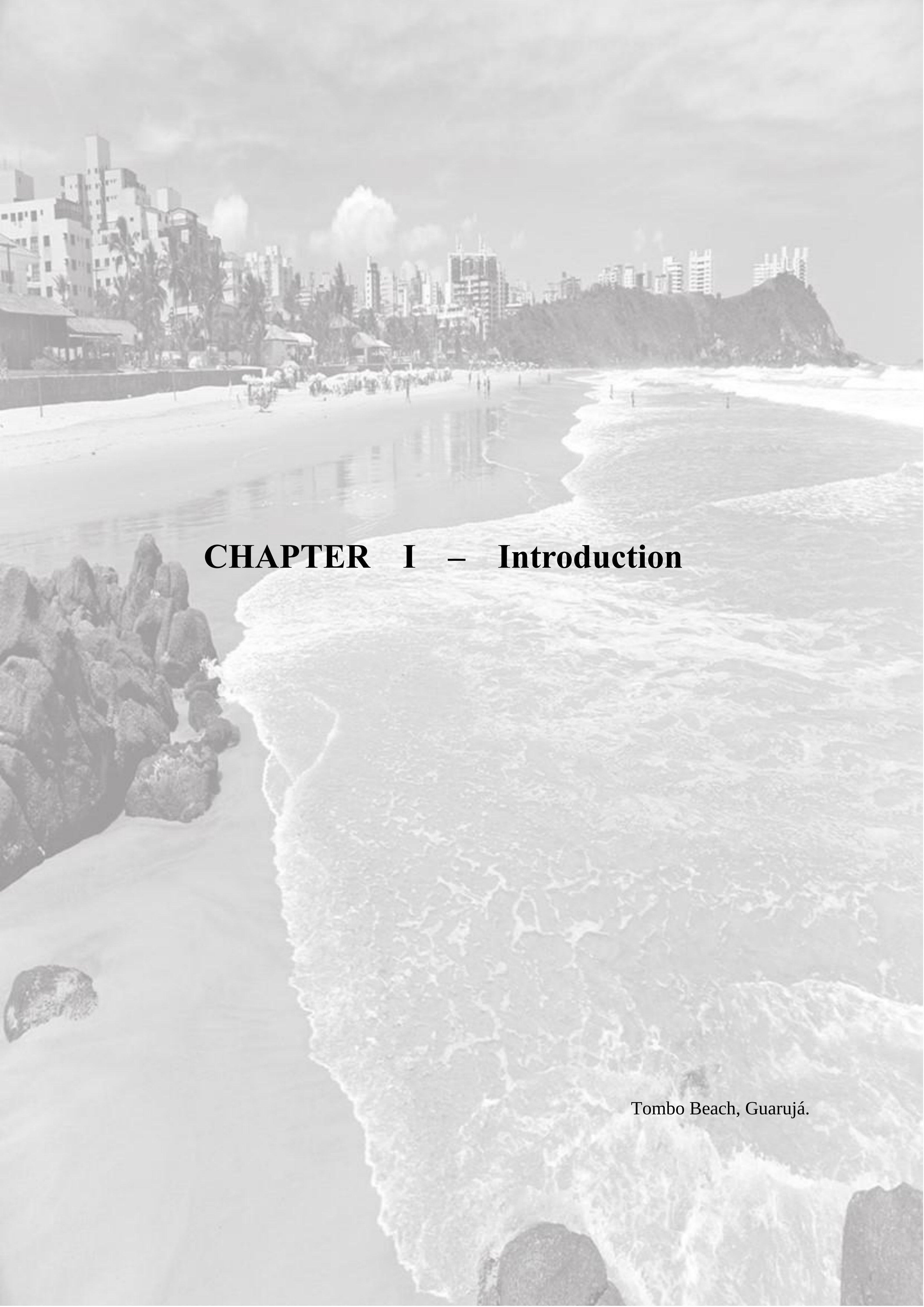
WRMUs – Water **R**esource **M**anagement **U**nits

WWTP – **W**astewater **T**reatment **P**lants

Z

Zn – **Z**inc

CHAPTER I – Introduction



Tombo Beach, Guarujá.

1. General introduction

1.1 Brazilian coastal area

Currently 40% of the world's population (estimated at 7.2 billion people) lives in about 2100 coastal cities (Pelling and Blackburn, 2013; Undesa, 2017; Blackburn et al., 2019). According to geographic delimitation criteria, coastal cities can be considered those located up to 100 km from the coast and up to 50 meters in altitude (Barragán and Andrés, 2015; Undesa, 2017; Blackburn et al., 2019). The high concentration of population in the world's coastal cities raises some concern because there are many people living in this small area of the world's land surface (estimated at between 4 and 8%), thus causing intensive antropic use of the narrow coastal areas (Costanza et al., 1997; Undesa, 2017; Blackburn et al., 2019). As a consequence, countless economic activities, mainly related to the port, industry, transportation, civil construction and tourism, cause several economic and socio-environmental conflicts, with direct consequences on the coastal marine ecosystems (Federigi et al., 2016; Lusk and Toor, 2016; Yang and Toor, 2017).

Many of the coastal occupation processes observed worldwide are closely related with the rate of population growth in developing countries, such as China (Asia) and Brazil (Latin America) (Von Glasow et al., 2012; Barragán and Andrés, 2015; Blackburn et al., 2019). In Brazil, the process of urbanization, followed by an extensive occupation of the coastal areas, became evident mainly between the 1960s and 1980s, when there was a massive rural exodus throughout the country, which contributed to about 20% of the country's urbanization. Between 1990 and 2010, there were also small records of this exodus, when an increase of 3.5% of the population in the urban area was observed (Alves et al., 2011). Figure 1.1 shows the urban growth observed in Latin America coastal zone from 1954 to 2012.

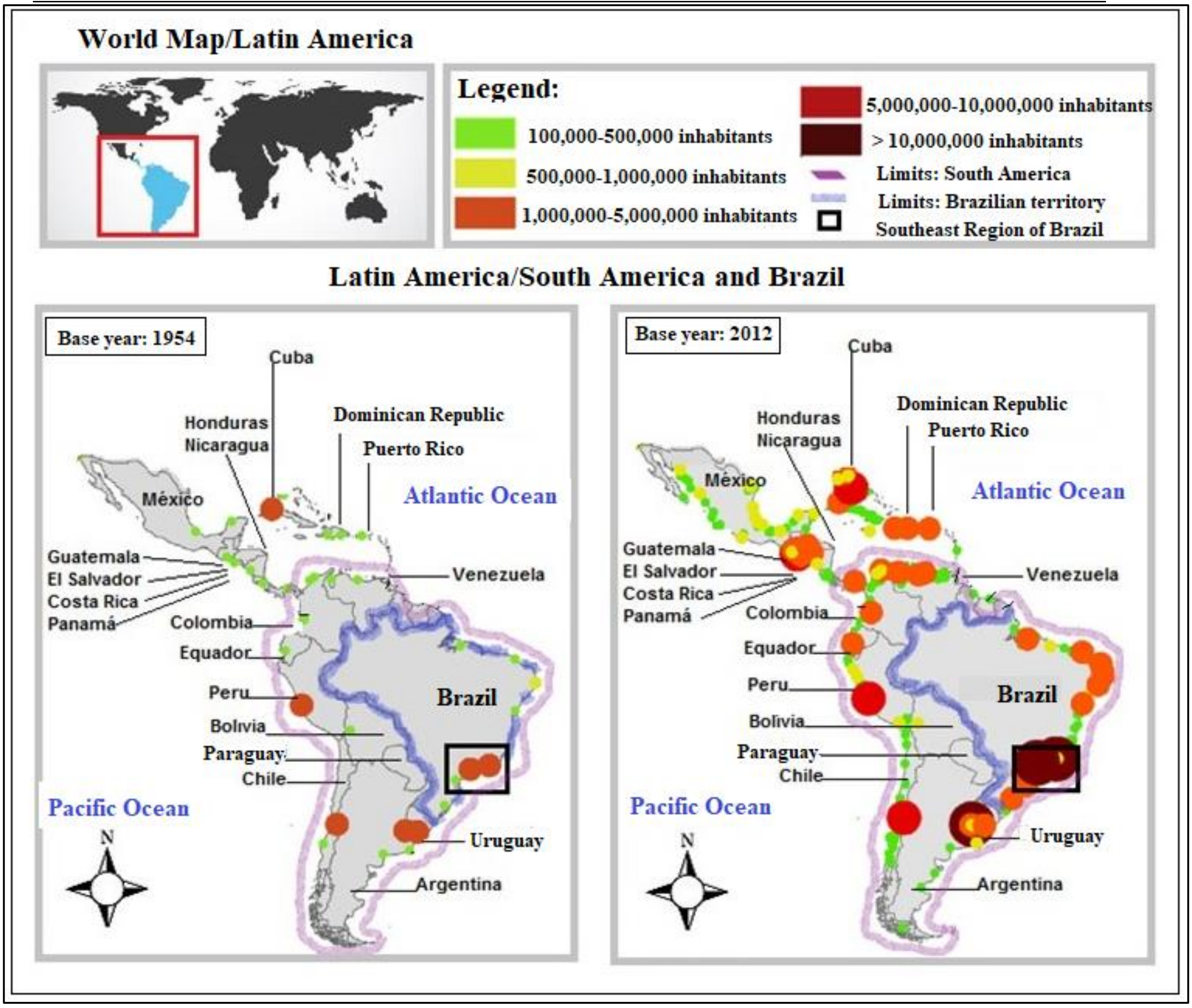


Figure – 1.1: Latin America, South America, Brazil: evolution of urban occupation in coastal area between 1954 and 2012.
Source: Adapted from Barragán and Andrés (2015).

According to IBGE (Brazilian Institute of Geography and Statistics), Brazil is the largest country in South America whose territory occupies almost half of the South American continent (47.3%), with an area that exceeds 8.5 million km². Due to its size, it is the 5th largest country in the world (Ibge, 2018). In the lastest census, the Brazilian government estimated its population around 210 million inhabitants, 26.6% of which inhabit the coastal zone of the country, which is a territorial unit distributed from the equatorial North to the temperate South, that covers 17 states, 463 municipalities and 17.4 million homes, 9.2% of which are used occasionally (used on weekends, vacations or holidays) (SMA/CPLA, 2012; Ibge, 2018).

One of the Brazilian states is São Paulo (Southeast region), which has about 880 km of coastline and covers 16 municipalities (about 2.2 million inhabitants), with a total area of 7,759 km^2. The São Paulo municipalities are subdivided into 3 WRMUs (Water Resource Management Units). Besides WRMU-3 - North Coast (which covers 15% of the population) and WRMU-11 - Ribeira do Iguape/South Coast (which covers 3% of the population), there is also WRMU-7 - MRBS: Metropolitan Region of Baixada Santista (focus region of this thesis), which houses 82% of the coastal population and encompasses 9 municipalities: Mongaguá (approximately 55,000 inhabitants), Bertioga (62,000 inhabitants), Peruíbe (67,000 inhabitants), Itanhaém (100,000 inhabitants), Cubatão (129,000 inhabitants), Guarujá (316,000 inhabitants - which will be the city studied in this thesis), Praia Grande (319,000 inhabitants), São Vicente (363,000 inhabitants) and Santos (432,000 inhabitants) (SMA/CPLA, 2012; Cetesb, 2018a; Ibge, 2018).

These nine municipalities of MRBS have a great socioeconomic interdependence (mainly in terms of job generation and basic public services offers) (SMA/CPLA, 2012). The MRBS has a geographic strategic position, serving as a link between the industrial hub of Cubatão and the Port Complex Santos-Guarujá, and the city of São Paulo (the state capital, and the country's main economic center), which is home to around 12 million inhabitants (SMA/CPLA, 2012; Ibge, 2018). Moreover, MRBS has great socio-environmental relevance for Brazil because this coast is located in a transition area between humid tropical and subtropical climates (SMA/CPLEA, 2005; SMA/CPLA, 2012). Favored by these climate conditions, and endowed with an ecosystem diversity, it is possible to find along this coast mangroves, rocky shorelines, sandbanks and estuaries, besides beaches which provide good conditions for tourism throughout the year (SMA/CPLEA, 2005; SMA/CPLA, 2012; Ribeiro and Oliveira, 2015). During the Brazilian summer (between December and March), when tourism intensifies, the population of MRBS doubles (SMA/CPLA, 2012; Cetesb, 2018a; Ibge, 2018). Figure 1.2 presents the main characteristics of the São Paulo coastal area (WRMU-7 – MRBS).

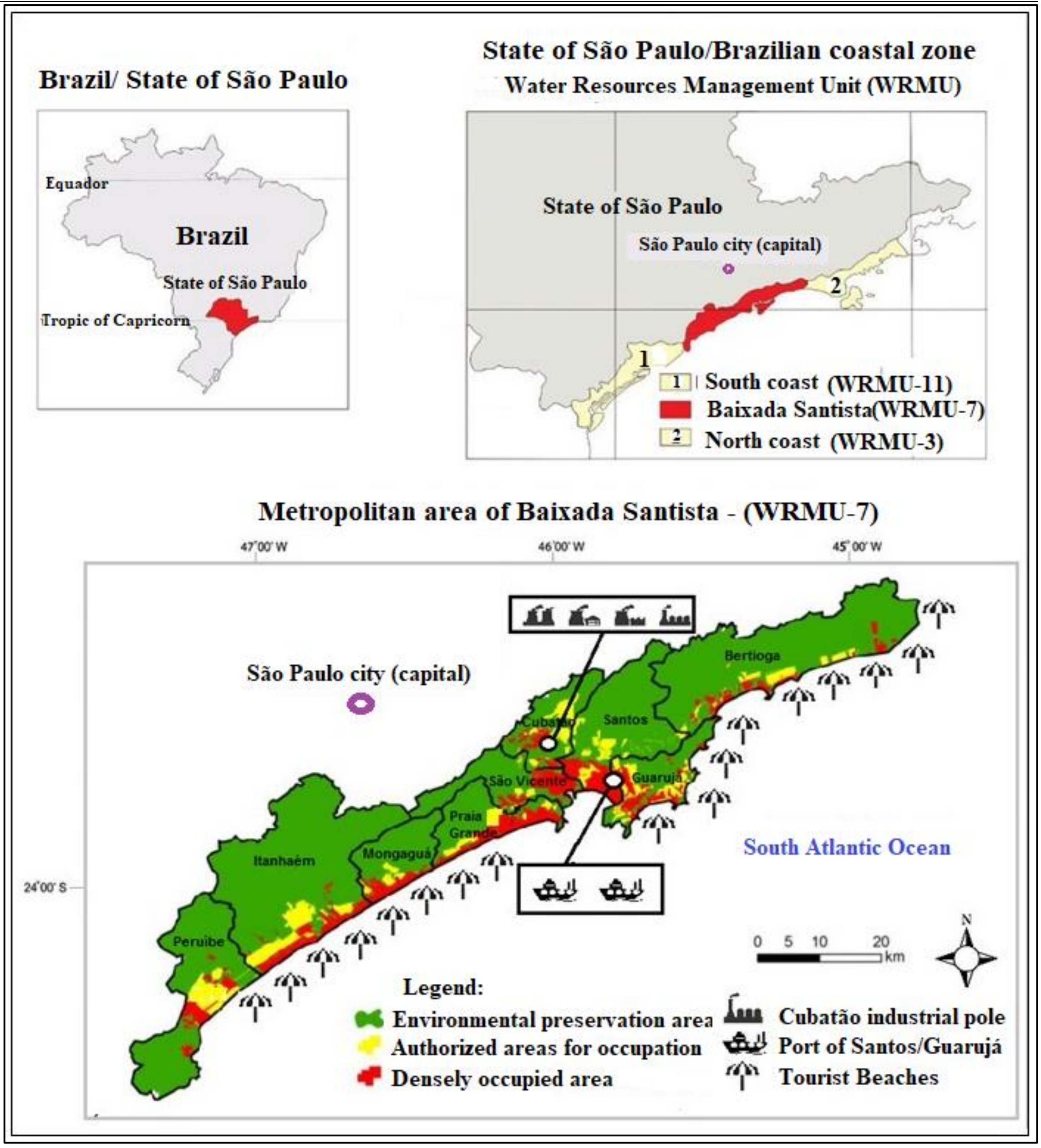


Figure – 1.2: Brazil, State of São Paulo - São Paulo coastal area: main characteristics of the nine municipalities of the Metropolitan Region of Baixada Santista.

Source: Adapted from SMA /CPLA (2012).

1.2 History of occupation and anthropic pressure in Guarujá

The municipality of Guarujá is located on the island of Santo Amaro (latitude S 23º 59' 18" and longitude W 46º 14' 32"), which is considered the third largest coastal island in São Paulo coast, with an area of 143 km^2. Due to its natural richness, beautiful beaches and historical constructions, in the middle of the 20th century, the city received the status of " Balneary Resort” (State Law No. 163 of 1948), guaranteeing to Guarujá an additional budget from the State for the promotion of regional tourism (São Paulo, 1948; De Oliveira et al., 2007; SMA/CPLA, 2012). As result from this new status, and with the construction of Anchieta highway in 1947, which allowed the interconnection between the capital of São Paulo and the cities of MRBS, the anthropic pressure began in Guarujá, namely on the beaches that started to be more visited, such as the Enseada and the Perequê (Vieira, 2004; Vaz, 2010; Mele, 2015). With the economy of the municipality heated up, the city began to attract numerous investments, mainly in the area of civil construction, which were applied in the process of urbanization of the Guarujá shoreline. With the generation of these new jobs, a first wave of migration was registered in the city, with the arrival of a working class coming mainly from the northeast region of the country. However, due to the high cost of the new properties on the shore, this working class started to migrate to a previously uninhabited area, in the northwest of the island, where the municipal district of Vicente de Carvalho began. Guarujá, which had a population of about 7,000 inhabitants in 1940, has risen to about 40,000 inhabitants in mid-1960 (Vieira, 2004; Vaz, 2010; Mele, 2015).

In the early 1970s, with the construction of Piaçaguera-Guarujá highway and its interconnection with Anchieta highway, the access to Guarujá was made even easier, as tourists began to arrive in town directly by car, which before was only possible by a Ferry Boat via the city of Santos. As result, from this exponential increase in visits to Guarujá, jointly with the arrival of new residents, a significant disorderly growth of the municipality occurred between 1970 and 1980. The entire city edge was occupied, and numerous buildings were constructed. In this period, requests for building approval increased from 30,000 m^2 in 1971 to 1,000,000 m^2 in 1975, when the population reached about 95,000 inhabitants. In 1980s, Guarujá was already recognized as one of the main tourist destinations of the State of São Paulo and, by the end of this decade, the population had already reached an impressive 150,000 inhabitants. With inflation of land prices, part of the impoverished population began to occupy environmentally protected and/or disaster-sensitive areas, leading to precarious occupations, both on the shoreline of Perequê Beach and on the beach hills of Enseada (areas with energy and/or sanitation deficits: water, sewage and/or garbage collection). On the beach hills of Enseada, exist eight poor communities (popularly known as *favelas*), namely: Vila Baiana, Jardim 3 Marias, Vale da Morte, Vila Júlia, Jardim Boa Vista, Vila Edna, Engenho and Cachoeira. Figure 1.3 presents the main characteristics regarding the land use and occupation of Guarujá (Vieira, 2004; Vaz, 2010; Mele, 2015).

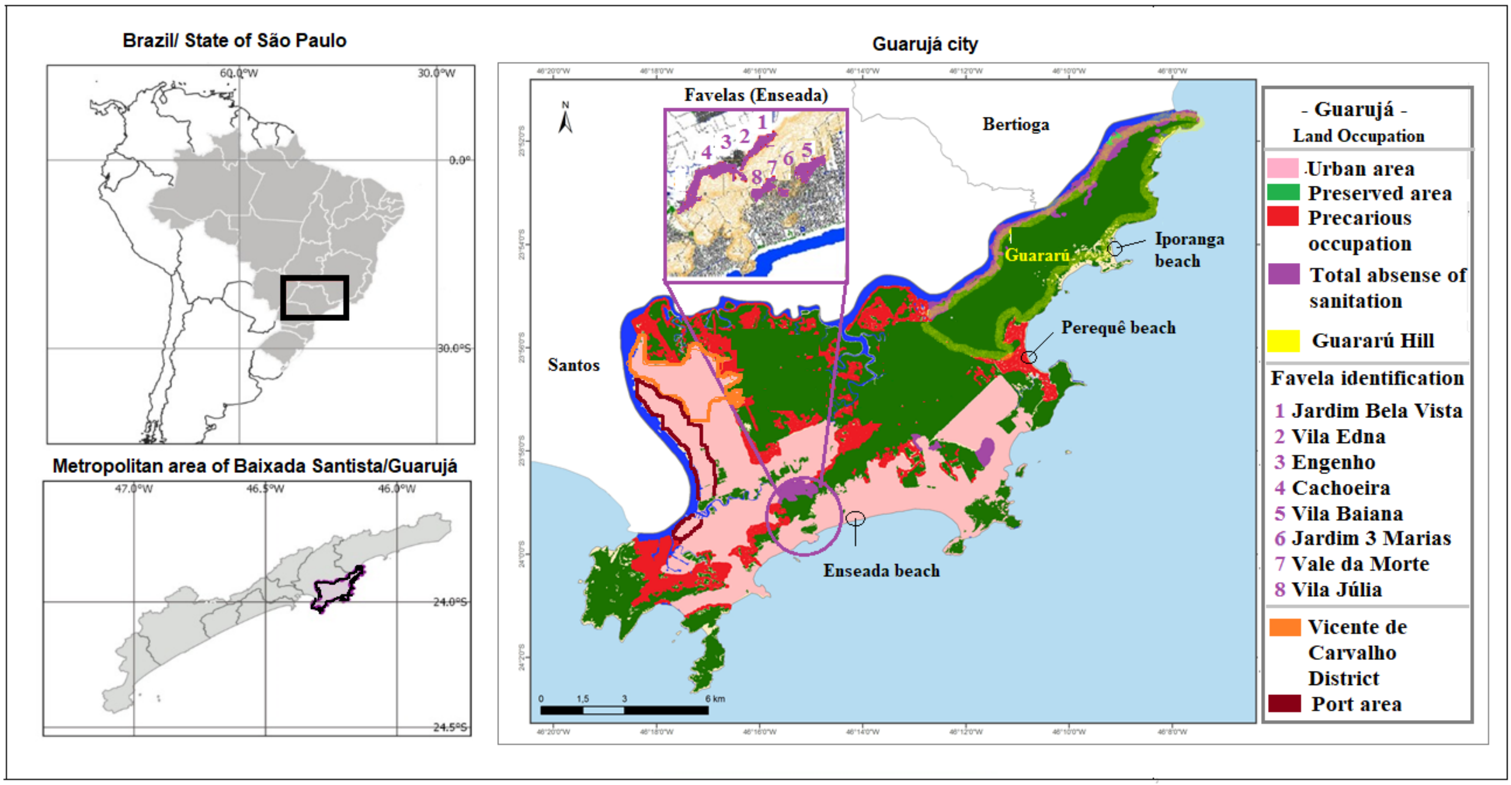


Figure – 1.3: Brazil, State of São Paulo, Metropolitan Region of Baixada Santista, Guarujá: main characteristics regarding the land use and occupation of the territory. Detail for occupations in *favela* areas at Enseada beach, precarious occupations at Perequê beach and environmentally protected areas at Guararú Hill (Iporanga beach).

Source: Adapted from SMA /CPLA (2012) and Mele (2015).

The 1990s were marked by a collapse in the public services of Guarujá, coincidental with the increase in the precarious occupations, growth of slums ("favelas") and rise of crime in the municipality, causing numerous conflicts of economic and socio-environmental nature. The city that used to be the favorite lazer zone of the citizens of São Paulo, started to suffer latent problems, leading to the departure of most tourists, that changed their preferences to the beaches located on the northen area of the State of São Paulo (WRMU-3 – see location in Figure 1.2) (Vieira, 2004; Vaz, 2010; Mele, 2015).

At the beginning of 21st century, the public managers of Guarujá, in partnership with the private initiative, started a process of progressive socio-environmental recovery of the municipality, receiving new public and private investments in the areas of sanitation, housing and tourism (Vieira, 2004; Vaz, 2010, Mele, 2015). A first action involved a partnership between the public authorities and the private sector, to protect part of the 107 km^2 of environmentally protected areas in the municipality, against new deforestation and invasions, which resulted in the creation of the EPA (Environmental Protection Area) of Guararú Hill, according to Municipal Decree 9.948, 2012 (see the location of Guararú Hill in Figure 1.3). The region of Guararú Hill, presented below in Figure 1.4, has great environmental, aesthetic, landscape, and historical relevance for the municipality (Guarujá, 2012).

Figure – 1.4: Municipality of Guarujá, State of São Paulo, Brazil: view of Iporanga beach, located in the EPA of Guararú Hill.

Source: Pulsar imagens (2016).

With regard to tourism, despite all the difficulties faced by municipal public management in past decades, in 2016, a survey conducted by the Ministry of Tourism showed that the beaches of Guarujá still appeared as one of the main destinations of the domestic tourist. Of the twenty most visited beaches in Brazil, Guarujá appeared in the 12th position in 2017 (SMA/CPLA, 2012; Brazil, 2016). Figure 1.5 shows an image of Enseada beach, one of the most visited by national tourists.

Figure – 1.5: Municipality of Guarujá, State of São Paulo, Brazil: 2016 image of Enseada beach, one of the most visited by Brazilian tourists.
Source: Pulsar imagens (2016).

At present, the population of Guarujá is approximately 316,000 inhabitants, where it is estimated that about 64,000 inhabitants live in *favelas* and/or in precarious occupations (Mele, 2015; Ribeiro and Oliveira, 2015; Cetesb, 2018a). This entire population contingent is concentrated in a small area of about 36 km^2, which represents an upsetting demographic density of over 2,000 inhabitants per km^2 (SMA/CPLEA, 2005; SMA/CPLA, 2012; Ribeiro and Oliveira, 2015).

1.3 Characteristics of the hydrographic sub-basin of Guarujá

WRMU -7 occupies an area of about 3,000 km² and extends for about 160 km along the entire coastline of São Paulo (SMA/CPLEA, 2005; SMA/CPLA, 2012; Ribeiro and Oliveira, 2015), including fresh (salinity equal to or less than 0.5 ‰), brackish (salinity greater than 0.5 ‰ and less than 30 ‰) and salt (salinity equal to or greater than 30 ‰) waters, as defined by Conama Resolution (National Environmental Council) nº 357/2005

(Brazil, 2005). In total, the WRMU-7 integrates 21 hydrographic sub-basins, highlighting sub-basin 13 (Santo Amaro Island), which corresponds to the municipal territory of Guarujá, a micro-region of Santos (SMA/CPLEA, 2005; SMA/CPLA, 2012; Ribeiro and Oliveira, 2015). Sub-basin 13 has a drainage area of about 143 km² and 64 km long, and, as described above, about 36 km^2 are already largely urbanized, with the remaining 107 km^2 being environmental preservation areas (SMA/CPLEA, 2005; SMA/CPLA, 2012; Ribeiro and Oliveira, 2015).

The precipitation and average annual temperature of sub-basin 13 reach approximately 3,000 mm and 22°C, respectively. Two very distinct sazonal periods are observed in the municipality, one being rainy (November to March) and the other being dry (April to October) (SMA/CPLEA, 2005; Ribeiro and Oliveira, 2015, Cetesb, 2018a). In sub-basin 13, there are a total of 13 rivers registered by the National Water Agency, with their springs on the hills or highest points of the island of Santo Amaro. The rivers Acaraú, Caipira, Maratauá, Crumaú, Emboabas and Do Pote flow into the Bertioga Channel (northern portion of the island). The Ichanhema, Do Meio, Santo Amaro and Pouca Saúde rivers flow into the Santos estuary (western portion of the island). The Santo Amaro River is the only river in the sub-basin 13 that is monitored annually by Cetesb (State Environmental Agency). According to the WQI (Water Quality Index), this river has a bad environmental classification. The main underlying cause appears to be the disposal of clandestine domestic sewage from the irregular occupations (SMA/CPLEA, 2005; SMA/CPLA, 2012; Cetesb, 2018a). The rivers Peixe, Ponte Grande and Perequê Mirim are the only ones that flow towards the South Atlantic Ocean (Eastern portion of the island). Figure 1.6 shows the Perequê Mirim River, located on Perequê beach, which suffers from the disposal of raw sewage resulting from precarious and irregular occupations.

Figure – 1.6: Municipality of Guarujá, State of São Paulo, Brazil: fishing community that inhabits the banks of the Perequê Mirim River and discharges domestic sewage directly into this river.
Source: Pulsar imagens (2016).

In addition to these 13 rivers, there are 43 urban drainage channels on the beach edge, responsible for draining the diffuse loads arising from the rainwater. These channels have a concrete structure, and none of them has a sluice gate system, so they flow daily to the beaches of the city (South Atlantic Ocean - southern and eastern portion of the island), in an area of intense recreational activities (SMA/CPLEA, 2005; SMA/CPLA, 2012; Ribeiro and Oliveira, 2015). Figure 1.7 presents the sub-basin hydrographic network 13 (Guarujá).

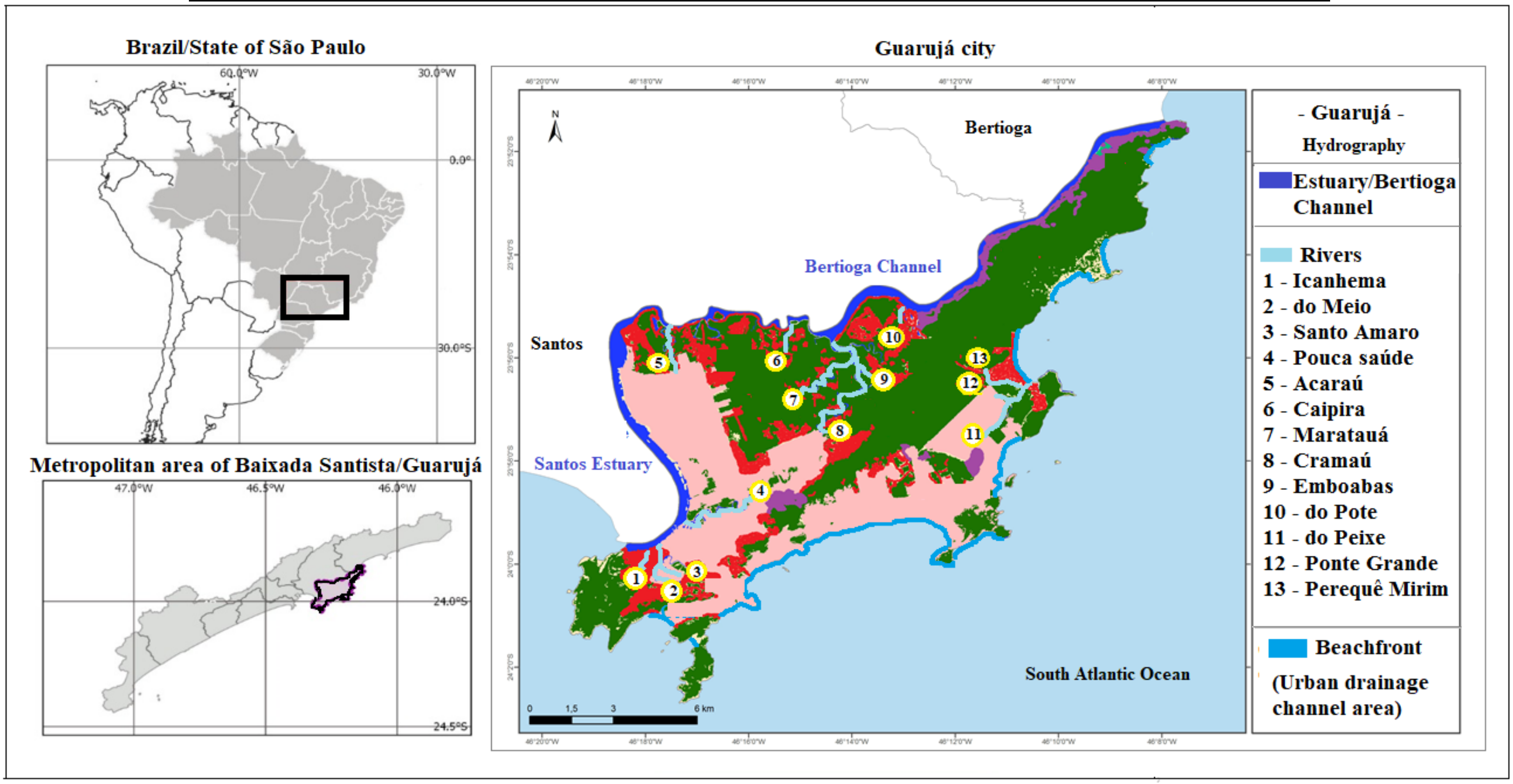


Figure – 1.7: Brazil, State of São Paulo, Metropolitan Region of Baixada Santista, Guarujá: main characteristics of the hydrographic network of the municipality (sub-basin 13). Detail for the location of the 13 main rivers and also the 43 urban drainage channels located along the edge of the municipality.

Source: Adapted from SMA /CPLEA (2005); SMA/CPLA (2012); Cetesb (2018a).

Due to the lack of basic sanitation in the slums and the precarious occupation of the municipality's land (mainly on the Enseada and Perequê beaches), the sewage is discharged into the rivers and drainage channels, which flow, most of the time, to the municipality's beaches (South Atlantic Ocean), compromising the public and environmental health of Guarujá (Mele, 2015; Cetesb, 2018a). Figure 1.8 shows the urban runoff (mixture of clandestine sewage and urban drainage), popularly known as "black tongue" (Rocha et al., 2011), which occurs daily on the beaches of Enseada and Perequê.

Figure – 1.8: Municipality of Guarujá, State of São Paulo, Brazil: diffuse pollution in the urban drainage channels that flow to the beaches of Enseada (A) and (B) and Perequê (C) and (D).
Source: Personal Photograph (2018).

1.4 Sewage processing system of Guarujá

The sewage of Guarujá is treated through a combined system, where the largest portion, covering the tourist beaches and the neighborhoods of the shoreline, is served by the SPS (Sewage Pre-Conditioning Station) of Vila Zilda, where the sewage goes through a preliminary simplified system (railing and screening for the removal of solids), which is followed by chlorination for the elimination of pathogenic microorganisms (De Souza Abessa et al., 2012; Baptistelli and Marcellino, 2016; Ortiz et al., 2016). The final destination of this pre-conditioned sewer is a 4,500-meter-long and 14-meter-deep submarine outfall that disposes the sewers daily in the marine environment (Enseada beach, South Atlantic Ocean), with a flow of 1.45 m^3/s. The other parcel, covering the district of Vicente de Carvalho, is served by a WWTP (Wastewater Treatment Plant) with a secondary level of treatment (removal of organic load/BOD: Biological Oxygen Demand), located at the other end of the island. WWTP has the sewers in the Acaraú River, whose final contribution is the Bertioga Channel. Together, both systems are sized for a population of approximately 450,000 inhabitants (Lamparelli and Ortiz, 2007;

Baptistelli and Marcellino, 2016; Cetesb, 2018b). Figure 1.9 shows the location of WWTP Vicente de Carvalho and SPS Vila Zilda in Guarujá.

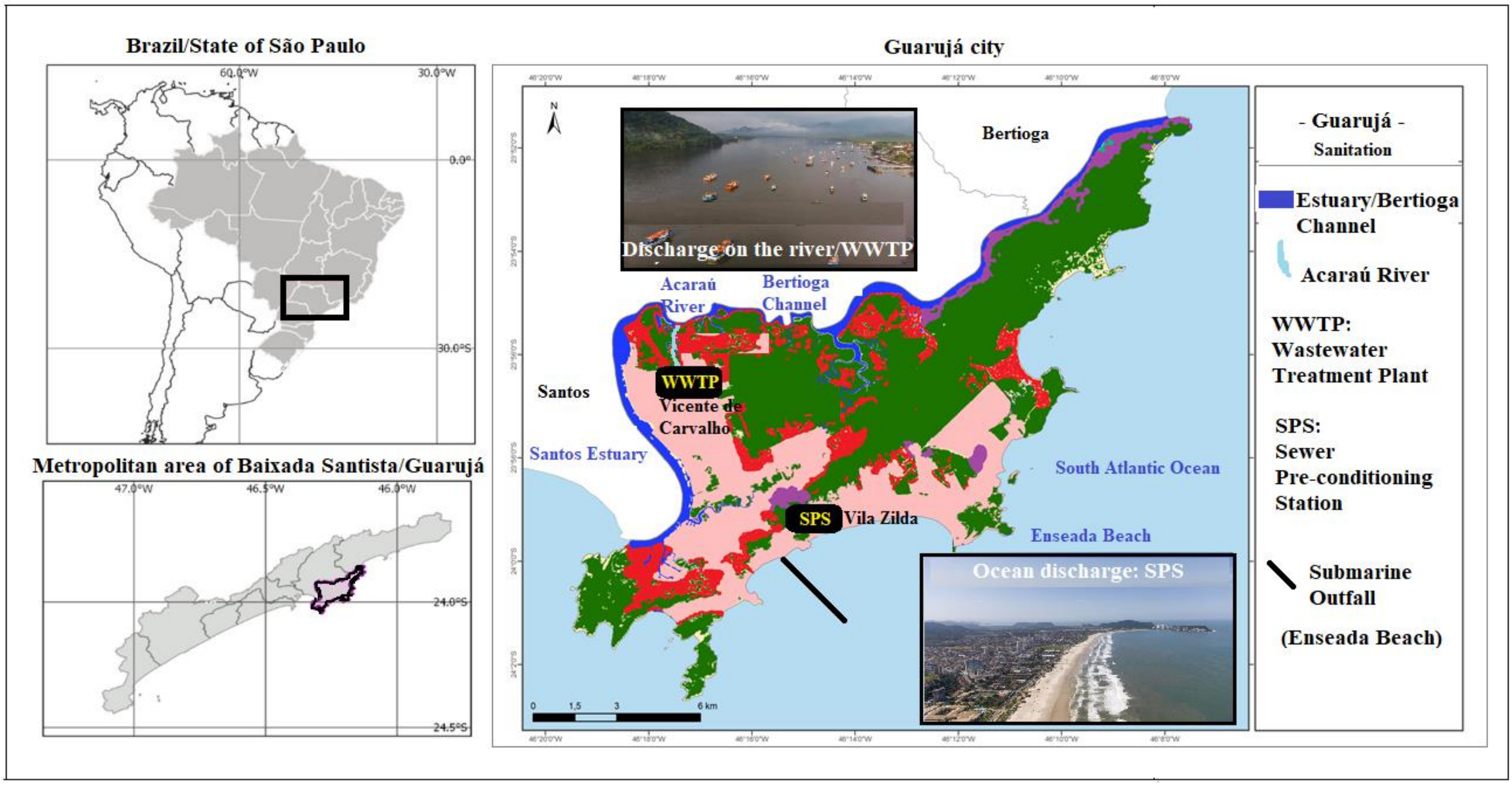


Figure – 1.9: Brazil, State of São Paulo, Metropolitan Region of Baixada Santista, Guarujá: location of WWTP (Wastewater Treatment Plant) of Vicente de Carvalho and SPS (Sewage Pre-Conditioning Station) of Vila Zilda. Highlights include the sewage discharge areas of WWTP (Acaraú River/Bertioga Channel) and SPS (Enseada beach).

Source: Adapted from Cetesb (2018b).

In total, Guarujá collects 62% of the sewage, but only treats 6% (Cetesb, 2018b). In this context, and according to the report of Instituto Trata Brasil (Instituto Trata Brasil, 2018), which ranks the 100 best sanitation cities in Brazil (out of a total of about 5,500 municipalities), Guarujá appears in 60th place. This quantitative ranking system takes into account the amount collected and treated from the sewers of the municipality (Instituto Trata Brasil, 2018). However, from a qualitative point of vue these numbers show a quite different picture. Cetesb considers in its sanitation quality analyses the MUPSCPI (Municipal Urban Population Sewage Collection and Processing Index) whose objective is to obtain an effective measure on the removal of the produced organic load (BOD), which should be greater than 80%. When analyzing from the MUPSCPI perspective, it can be observed that the municipality of Guarujá, which appears in the 60th position in the national ranking, has a very low MUPSCPI (1.75 on a scale that goes from 1 to 10) (Cetesb, 2018b). This occurs because the SPS plus the submarine outfall system does not have organic load removal and, therefore, although the submarine outfall moves the sewage away from the shoreline, the BOD released into the sea may bring great environmental impacts (De Souza Abessa et al., 2012; Baptistelli and Marcellino, 2016; Bleninger et al., 2016).

The submarine outfall of Guarujá was installed in 1998 and, since 2010, Cetesb monitors the area of influence of this discharge (water and sediment), through two annual campaigns. In 2018, the outflow of the submarine outfall presented some water samples that did not comply with the standards established by CONAMA Resolution 357/2005, such as Dissolved Oxygen (67%), phosphorus (46%) and chlorophyll (38%). The annual average of CWQI (Coastal Water Quality Index) was "regular" and the annual average of CTSI (Coastal Trophic State Index) was classified as "mesotrophic" (moderate enrichment by chlorophyll) (Brazil, 2005; Cetesb, 2018b). The sediments presented a quite negative redox potential, a result of the submarine outfall's influence in the area. Even so, the ecotoxicological evaluation (acute assay with the amphipod *Grandidierella bonnieroides*) was considered "non-toxic" (Cetesb, 2018b).

1.5 Monitoring of Guarujá beaches

The CONAMA Resolution nº 274 of 2000 has as its main objective to monitor the bathing water quality, in view of public health protection, water quality management and creation of information systems for the community (Brazil, 2000). The bathing water quality can be understood as being an instrument to control the quality of the waters of the beaches, as it allows a more detailed verification of the waters destined to primary contact recreation, including swimming, diving and water sports activities. Among the factors that can interfere with the balneability of beaches are the occurrence of domestic sewage, the presence of tourism during public holidays and long vacations, the occurrence of rainfall, tidal conditions, as well as streams and channels flowing into the sea (Lamparelli et al., 2015; Cetesb, 2018a).

Guarujá has 26 beaches, and since 2005, 8 are regularly monitored, namely Guaiúba, Tombo, Astúrias, Pitangueiras, Enseada, Pernambuco, Perequê and Iporanga. The sampling scheme tooks into consideration the frequency of bathers, the physiography of beaches and the risks of pollution. The frequency of analysis of these beaches is weekly (on Sundays) throughout the year (Cetesb, 2018a). Figure 1.10 presents Tombo Beach, the only beach in the State of São Paulo (from a total of approximately 290 beaches) with international Blue Flag certification, supplied by FEE (Foundation for Environmental Education) (Fee, 2018).

Figure – 1.10: Municipality of Guarujá, State of São Paulo, Brazil: Tombo beach, the only beach in the State with international Blue Flag certification.
Source: Pulsar imagens (2016).

In addition, Cetesb also monitors the 43 urban drainage channels that flow to these different beaches once every semester (therefore, twice a year) (Cetesb, 2013; 2018a).

In the State of São Paulo, according to the criteria established by CONAMA nº 274/2000, the beaches are classified in two categories: appropriate and inappropriate, and the first classification gathers three distinct categories: excellent, very good and satisfactory (Brazil, 2000). This classification is made according to the densities of faecal bacteria in seawater, resulting from analyses made on samples of five consecutive weeks. Cetesb uses *Enterecocci* and *E. coli* (*Escherichia coli*) for monitoring the seawater and the drainage channels affluent to the beaches, respectively (Cetesb, 2013; 2018a). The beach with improper classification, therefore, indicates a decline in the quality of the waters, implying an increase in the risk to the health of the bather and making its use for bathing inadvisable. Even when the density of coliforms is low, the beach can be classified as improper if the presence of oil, occurrence of red tides, blooming of potentially toxic algae or outbreaks of water diseases are identified (Brazil, 2000; Cetesb, 2018a). Figure 1.11 shows images of an accidental municipal sewage leak on Pitangueiras beach (in 2018), which was conducted to the sea through urban drainage channels. At that time, Cetesb banned the use of beaches (Cetesb, 2018a).

Figure – 1.11: Municipality of Guarujá, State of São Paulo: accidental sewage leak in Pitangueiras beach (2018), conducted to the sea through urban drainage channels.
Source: Adapted from Cetesb (2018a).

Cetesb publishes the conditions of balneability by issuing a weekly bulletin (Cetesb, 2018a). In order to show the quality trend of the beaches in an integrated way, Cetesb has developed an annual beach classification. This classification is a synthesis of the results obtained in the weekly monitoring and expresses the quality that the beach presents with more constancy in that year. The criteria are: (i) "great": excellent in 100% of the year; (ii) "good": proper in 100% of the year except when classified as excellent; (iii) "regular": improper in 25% of the year; (iv) "bad": improper between 25% and 50% of the year; and (v) "terrible": improper over 50% of the year (Cetesb, 2018a). In 2018, Iporanga beach was the only one that presented a "great" annual rating. The beaches of Pernambuco and Tombo, presented a "good" classification. With the exception of Enseada beach (on Chile street and Santa Maria avenue monitoring points), which presented a "bad" rating, and Perequê which obtained a "terrible" rating, all the other 6 monitoring points obtained a "regular" annual rating (Figure 1.12) (Cetesb, 2018a). In relation to the 43 urban drainage channels, in 2018, the microbiological analysis revealed that only 13% of them complied with the legislation according to the standard adopted by Cetesb [600 CFU (Colônia

Forming Unit) *E. coli* /100mL], This result is 9% lower than in 2017 (Cetesb, 2013; 2018a). Figure 1.12 shows the history of Cetesb's monitoring (between 2009 and 2018) on the 8 beaches of the municipality.

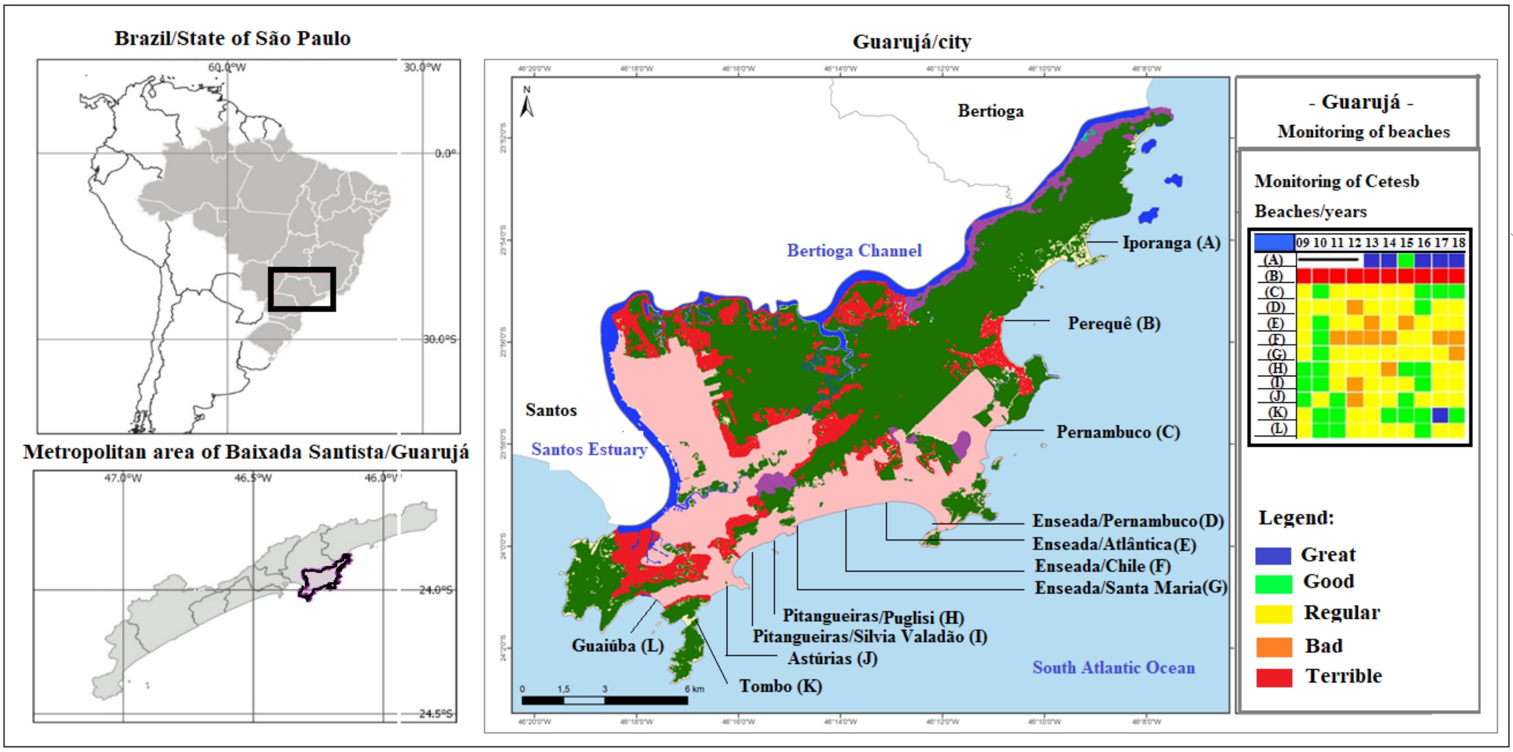


Figure – 1.12: Brazil, State of São Paulo, Metropolitan Region of Baixada Santista, Guarujá: location of Cetesb's 12 monitoring points on the 8 beaches of the city, and the evolution of the bathing ability results between 2009 and 2018.

Source: Adapted from Cetesb (2018a).

It is also important to highlight that the presence of urban drainage channels flowing to beaches, in addition to the presence of submarine outfall with preliminary level of handling, is a systemic problem along the entire Brazilian coast. For example, there are 600 urban drainage channels (registered by Cetesb) in São Paulo coastal area, with characteristics similar to those of Guarujá (Cetesb, 2018a). In Brazilian coast, there are 20 submarine outfalls that daily dispose of the sewers in the Atlantic Ocean (De Abessa Souza et al., 2012; Baptistelli and Marcellino, 2016).

1.6 Past studies on water and sediment quality in Guarujá

In order to obtain an overview of what has been studied about water and sediment quality in the different environmental matrices of Guarujá, a literature review was made using the Google Scholar (in the case of the search for diverse academic works) but, above all, prioritizing the indexed journals, according to the databases (i) Science Direct; (ii) SciELO; (iii) Scopus and (iv) PubMed and using the keywords: Brazil, Guarujá, surface water, marine sediment, submarine outfall, ocean layout, urban drainage channel, urban runoff, sewage, marine pollution, both in Portuguese and English languages, and published until the year 2020. The results showed that in the last 35 years, few studies have been conducted. Only 25 studies were identified, 3 of them in rivers of Guarujá, 14 in beaches of the city (sea water), 2 in urban drainage channels and 6 in the areas adjacent of submarine outfall. Of this total, only 6 were published in indexed journals (4 international and 2 Brazilian) (Table 1.1).

Table – 1.1: Municipality of Guarujá, State of São Paulo, Brazil: synthesis of studies conducted in different environmental matrices of the sub-basin - 13, in the period between 1985 and 2020. Note: * Indexed journals.

Sub-basin 13	Location	Author (year)	Study characteristics	Variables analyzed	Environmental matrix
River	Bertioga Channel	Rodrigues et al. (2002)	Congress proceedings	Benthic community	Estuarine water
	Perequê River	Ferreira (2015)	Master`s dissertation	Secondary data from Cetesb (Bacteriological)	River water
	Bertioga Channel	Sutti et al. (2015)	Brazilian Academic Journal (Bioscience)	Physicochemical	Estuarine water
Beaches	Tombo, Pitangueiras and Pernambuco	Sanchez et al. (1986)	*International Journal (Water Science & Technology)	Bacteriological	Seawater
	Perequê Beach	Mastroti et al. (1993)	*Brazilian Journal (Revista Brasileira de Oceanografia)	Physicochemical	Seawater
	Perequê Beach	Hollnagel (1994)	Master`s dissertation	Physicochemical and Bacteriological	Sediment
	Pernambuco Beach	David et al. (2003)	Symposium Proceedings	Benthic community	Seawater
	Tombo Beach	De Oliveira et al. (2007)	* International Journal (Marine Pollution Bulletin)	Physicochemical and Bacteriological	Sediment
	Tombo, Enseada, Perequê and Iporanga Beaches	Medeiros et al. (2009)	Brazilian Academic journal (Ceciliana)	Toxicity (sea urchin: *Lytechinus variegatus*).	Seawater
	Pitangueiras Beach	Pinto (2010)	Master`s dissertation	Bacteriological	Seawater

	Enseada Bach	Brunholi et al. (2015)	Congress proceedings	Secondary data from Cetesb (Bacteriological)	Seawater
	Enseada and Perequê Beaches	Ferreira (2015)	Master`s dissertation	Secondary data from Cetesb (Bacteriological)	Seawater
	Astúrias, Pitangueiras and Enseada Beaches	Lamparelli et al. (2015)	* International Journal (Water Reserach)	Bacteriological	Seawater
	Perequê Beach	Prypchan and Dalmas (2015)	Brazilian Academic Journal (UNG – SER)	Secondary data from Cetesb (Bacteriological)	Seawater
	Pernambuco Beach	Ribeiro and Dalmas (2016)	Brazilian Academic Journal (UNG – SER)	Secondary data from Cetesb (Bacteriological)	Seawater
	Astúrias, Pitangueiras and Enseada Beaches	Piffer and Arruda (2017)	Brazilian Academic Journal (UNG – SER)	Secondary data from Cetesb (Bacteriological)	Seawater
	Guaiúba Beach	Tiusso et al. (2018)	Congress proceedings	Bacteriological	Seawater
Urban drainage channel	Enseada Beach	Roveri (2013)	Master`s dissertation	Physicochemical, bacteriological and ecotoxicological	Seawater
	Enseada and Perequê Beaches	Ferreira (2015)	Master`s dissertation	Secondary data from Cetesb (Bacteriological)	Drainage channel
Submarine outfall	Enseada Beach	De Souza Abessa and Souza (2002)	Congress proceedings	Toxicity: amphipoda *Tiburonella*)	Sediment
	Enseada Beach	Gianesella and Saldanha-Corrêa (2002)	Congress proceedings	Phytoplankton community	Seawater
	Enseada Beach	Moser et al. (2004)	* International Journal (Aquatic Ecosystem Health & Management)	Physicochemical	Seawater
	Enseada Beach	Duleba (2006)	Master`s dissertation	Phytoplankton community	Seawater
	Enseada Beach	De Souza Abessa et al. (2012)	* Brazilian Journal (Mundo da Saúde)	Review (Submarine outfall)	Seawater
	Enseada Beach	Dos Santos et al. (2018)	* International Journal (Marine Polluttion Bulletin	Chemical products (endocrine disrupters)	Sediment

In view of this scenario of land occupation and anthropic pressure under the sub-basin 13 of Guarujá, in Chapter 2 will be presented the rational, objectives and structure of this thesis.

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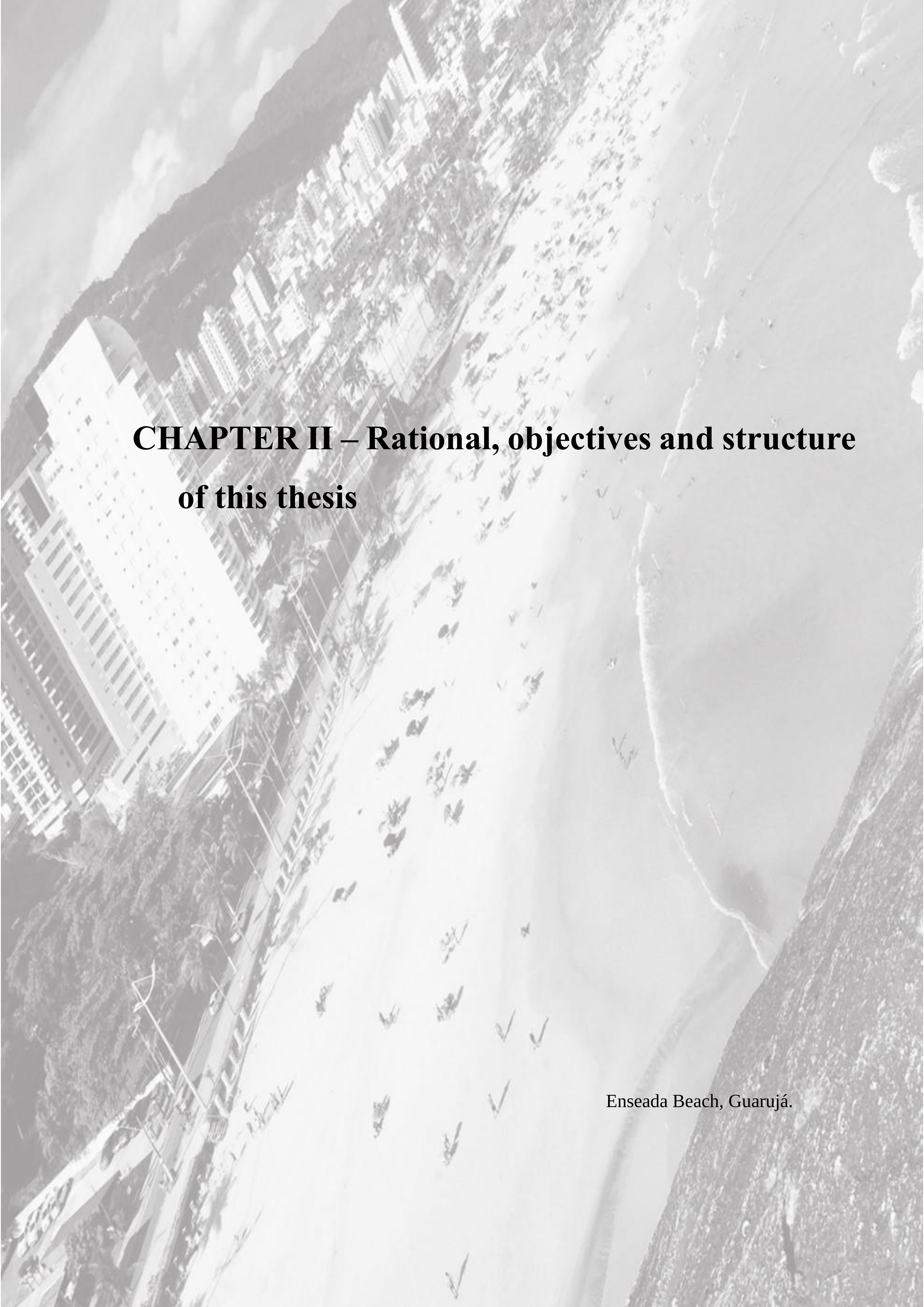

CHAPTER II – Rational, objectives and structure of this thesis

Enseada Beach, Guarujá.

2.1 Rational

In view of what was presented in Chapter 1 - General Introduction, some issues raise serious concerns and justify the importance and urgency of a study on water and sediment quality in the sub-basin 13, for the benefit of public health and the protection of the ecological systems of Guarujá:

(i) Strong anthropic pressure: the sub-basin of Guarujá is under hard human pressures, because, although it has a drainage area of about 143 km^2, approximately 316,000 inhabitants are concentrated in a small area of about 36 km^2, which represents a demographic density of over 2,000 inhabitants per km^2 (SMA/CPLEA, 2005; SMA/CPLA, 2012; Ribeiro and Oliveira, 2015). This ~~s~~ituation is exacerbated during the summer season (between December and March), when beach tourism intensifies and the population doubles (SMA/CPLA, 2012; Cetesb, 2018a; Ibge, 2018).

(ii) Deficit in environmental sanitation: it is estimated that about 64,000 inhabitants of Guarujá live in slums and/or in precarious constructions (which have a deficit in basic public services), mainly on the beaches of Enseada and Perequê (Mele, 2015; Ribeiro and Oliveira, 2015; Cetesb, 2018a). Due to the environmental sanitation deficit of these occupations, the destination of the clandestine sewers is, in most cases, the 43 urban drainage channels of the city (Mele, 2015; Cetesb, 2018a).

(iii) Presence of drainage channels flowing into the beaches: these artificial channels have a concrete structure, but none of them has a floodgate system; thus, all of them discharge their waters, daily on the beaches of the municipality, in a place of wide occurrence of recreational activities, and may thus compromise the public and environmental health of Guarujá (SMA/CPLEA, 2005; SMA/CPLA, 2012; Ribeiro and Oliveira, 2015).

(iv) Quality of sewage processing: although Guarujá is ranked 60th in the 100 best sanitation cities in Brazil, the municipality has an MUPSCPI equal to 1.75 and therefore quite low (Cetesb, 2018b; Instituto Trata Brasil, 2018). This occurs because the SPS plus the submarine outfall system of Guarujá does not have processes to remove the organic load and, therefore, all pollutants flow into the sea, which may compromise the public health and ecological systems of Guarujá (Cetesb, 2018b; Instituto Trata Brasil, 2018).

(v) Limitation of available studies on water and sediment quality in Guarujá: although Guarujá has a socioeconomic, environmental and tourism relevance in São Paulo coastal area, and the marine coastal ecosystems of the municipality are constantly under risk of contamination and/or pollution, scientific data on the quality of water and sediment in the municipality are scarce. In the last 35 years, only 25 studies have been carried out, and the monitoring of this sub-basin is restricted to the work of Cetesb (Cetesb, 2018b).

2.2 Study area

As described in the previous chapter (Chapter 1 - General Introduction), Guarujá has eight urbanized beaches that are effectively monitored by Cetesb. The criterion used to choose the beaches of Tombo, Enseada, Perequê and Iporanga in this thesis, was the representativeness of these four beaches in the Waterfront Project. This project is a joint action of the Ministry of the Environment, the Ministry of Planning and the city halls of the Brazilian municipalities (Brazil, 2006). The “Guarujá Waterfront Project" was developed based on surveys about the current conditions of the land use and occupation of the city's waterfront, and integrates strategic tools such as the State Plan for Coastal Management (Law nº 10.019/1998) (São Paulo, 1998), the Economic Ecological Zoning of Baixada Santista (State Decree nº 58.996/2013) (São Paulo, 2013), besides the Guarujá Master Plan and the respective criteria regarding the land use and occupation of the city (Complementary Law nº 156/2013) (Guarujá, 2013; 2019).

The Guarujá Waterfront Project has the following objectives: prevent, correct and/or mitigate the impacts on the natural environment caused by the human intervention, generally disorganized, illegal or improper, having as consequences the pollution of watercourses, launching of untreated sewers, mangrove landfills, deforestation of protected areas, improper use of public goods, among other damages. It also aims to contribute to the reduction of conflicts related to the land use and occupation of the seashore, establishing strategies to rescue the attractiveness of this space as a democratic place of leisure, and, based on the possible agreements to be signed between the City Hall and the Union Patrimony Secretariat, enabling a more effective action of the municipal government in the management of the coastal areas, making its proper planning possible (Guarujá, 2019).

Thus, the Guarujá Waterfront Project established for management purposes six sectors, as follows:

(i) Santos/Guarujá Sector;
(ii) Cabeça do Dragão Sector (Tombo Beach location area);
(iii) Enseada Sector (Enseada Beach location area);
(iv) Pernambuco/Perequê Sector (Perequê Beach location area),
(v) Rabo do Dragão Sector (Iporanga Beach location area),
(iv) Bertioga Channel Sector (Guarujá, 2013; 2019).

Figure 2.1 presents the geographic delimitation of the six sectors of the Guarujá Waterfront Project:

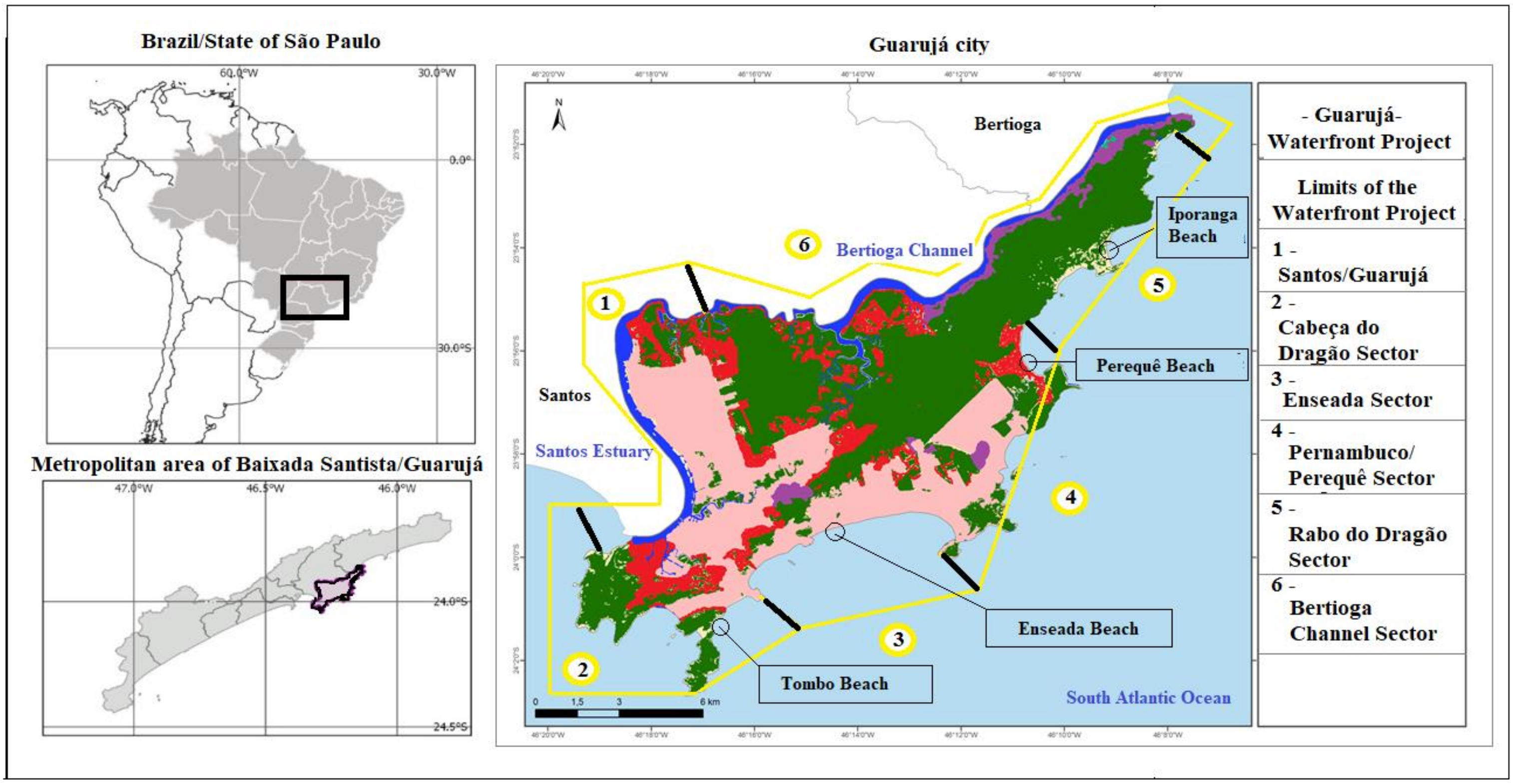

Figure – 2.1: Brazil, São Paulo State, Metropolitan Region of Baixada Santista, Guarujá: Boundaries of the Guarujá Waterfront Project and the respective locations of Tombo, Enseada, Perequê and Iporanga Beaches.

Source: Adapted from Guarujá (2019).

However, despite its socioeconomic and environmental importance, the Guarujá Waterfront Project still lacks primary data on water and sediment quality (Guarujá, 2013; 2019).

2.3 Sampling points

The selection of the collection points on the beaches of Tombo, Enseada, Perequê and Iporanga was preceded by an on-site inspection (on 20th, 21st and 22th of September 2017), with the aim of: (i) perform the geographic characterization of the site and georeferencing it with GPS (Global Position System) followed by the location on maps (use of ArcGis and Google Earth); (ii) identify possible interference with land use and occupation around the points; (iii) check the accessibility and safety conditions of the collection points (to carry out water and sediment collections over one or more years); (iv) obtain information to collect the samples from the Iporanga condominium (authorization granted by the environment manager, Ronaldo Justo); (v) inform the community of Perequê and the residents of Enseada and Tombo (located near the collection points) about the objectives of the work; (vi) assist in choosing a set of the most representative water quality variables to characterize the study area. Figure 2.2 and Table 2.1 present the different characteristics regarding the land use and occupation of the beaches of Tombo, Enseada, Perequê and Iporanga selected as study areas in this thesis.

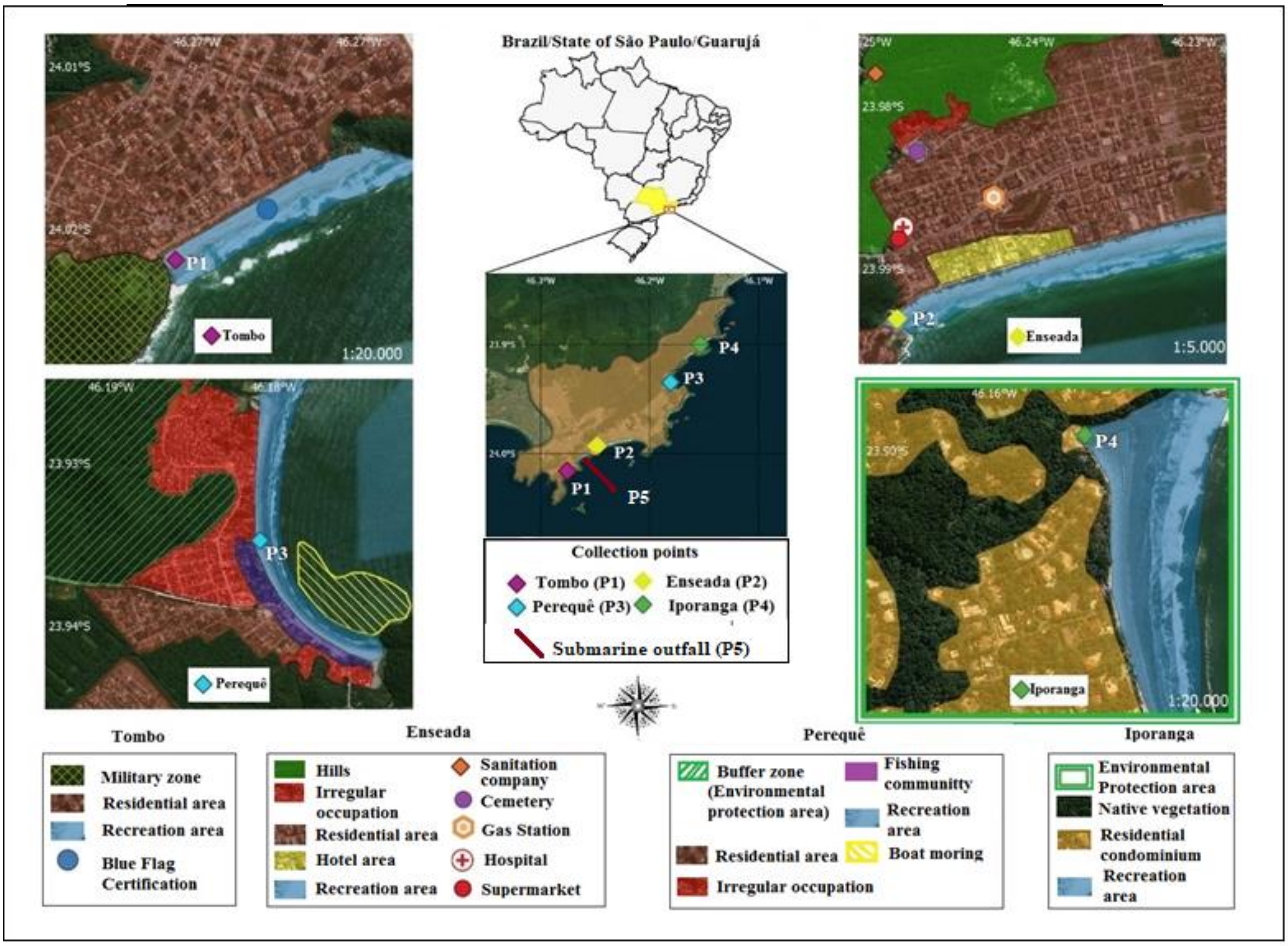

Figure – 2.2: Brazil, State of São Paulo, Guarujá: location and main characteristics regarding the using and occupation of the soil of the beaches of Tombo (sampling point - P1), Enseada (sampling point - P2), Perequê (sampling point - P3), Iporanga (sampling point - P4) and submarine outfall located in the beach of Enseada (sampling point - P5).

Source: Personal Archive.

Table – 2.1: Description of the main characteristics of the selected sampling points on the coast of the city of Guarujá, São Paulo, Brazil. The sampling points are located at the beaches of Tombo, Enseada, Perequê and Iporanga. The characteristics presented include location (geographical coordinates); type and extent of beaches; description of the different characteristics related to land use and occupation, and photographs of the locations. For more details, see Figure 2.2

Beach	Location	Type	Extension	Characteristics	Photographs
Tombo	24° 00′ 53″ S; 46° 16′ 23″W	oceanic	800 meters	(i) Located in a predominantly residential neighbourhood of medium and high standard. The main attraction of this beach is the international Blue Flag certification (Ribeiro and Oliveira, 2015; Fee, 2018). (ii) Orla Project Location: Sector 2 - Cabeça do Dragão (Guarujá, 2019). (iii) Annual classification of beaches (Cetesb): good (Cetesb, 2018a).	
Enseada	23° 59′ 12″ S; 46° 13′ 38″W	oceanic	6,000 meters	(i) It is a very touristic beach (Ribeiro and Oliveira, 2015). The system of oceanic disposal of sanitary sewage of the municipality of Guarujá (preliminary treatment) is located on this beach (Cetesb, 2018b). Although it has medium and high standard hotels and residences, this beach contrasts with the presence of illegally occupations (sanitation deficiency) on the slope of Enseada's hill (Ribeiro and Oliveira, 2015). (ii) Orla Project Location: Sector 3 – Enseada (Guarujá, 2019). (iii) Annual classification of beaches (Cetesb): bad (Cetesb, 2018a).	
Pererquê	23° 56′ 05″ S; 46° 10′ 51″W	oceanic	2,400 meters	(i) Located in a region with sanitation deficit, it hosts the largest fishing community in Guarujá (about 10,000 residents), which settled illegally along the edge of the beach in an environmentally protected area (Ribeiro and Oliveira, 2015). (ii) Orla Project Location: Sector 4 – Perequê (Guarujá, 2019). (iii) Annual classification of beaches (Cetesb): terrible (Cetesb, 2018a).	
Iporanga	23° 42′ 02″ S; 46° 08′ 26″W	oceanic	800 meters	(i) Located in the Environmental Protection Area (EPA) of Serra do Guararu. This conservation unit houses a condominium with about 380 high standard residences that owns a secondary level sewage treatment. This beach has restricted tourist access (Ribeiro and Oliveira, 2015). (ii) Orla Project Location: Sector 5 – Rabo do Dragão (Guarujá, 2019). (iii) Annual classification of beaches (Cetesb): excellent (Cetesb, 2018a).	

2.4 Environmental variables

Besides the scarcity of studies in the sub-basin 13, there is a limitation about the environmental variables already analyzed, for example, in urban drainage channels and in the adjacencies of the submarine outfall (see details in Table 1.1 - Chapter 1). Specifically, on the urban drainage channels of Guarujá, there are no previous studies dedicated to measuring the potential risks to public health and, especially, the ecological risks of these discharges into the sea (during the period of 1 year) in areas of intense recreation. In the hereby study the following variables were analysed:

(i) Physicochemical: e.g. water temperature, salinity, conductivity, total dissolved solids, pH, dissolved oxygen, ammonia, nitrites, nitrates, phosphate, total phosphorus, anionic surfactants, sedimented solids, biochemical oxygen demand, oils and greases, plus heavy metals: aluminium, cadmium, lead, copper, chromium, nickel, and zinc. As an example, studies on urban runoff in different coastal regions of the world have already detected a high presence of pollutant load, containing dissolved inorganic nitrogen (ammonia, nitrites, and nitrates) (Ballo et al., 2009; Yu et al., 2011), phosphorus (phosphate and total phosphorus) (Badruzzaman et al., 2012; Yang and Toor, 2017), surfactants (Ghose et al., 2009; Renzi et al., 2012) and heavy metals (Suresh et al., 2012; Gunawardena et al., 2013).

(ii) Microbiological: for example, the protozoa (*Cryptosporidium ssp* and *Giardia ssp*) and viruses (human mastadenovirus - species C, D and F), in addition to other species of adenovirus. Pathogenic microorganisms such as bacteria (*Enterococci* and *E. coli*) (Tilburg et al., 2015; Federigi et al., 2016), protozoa (*Cryptosporidium ssp* e *Giardia ssp*) (Cetesb, 2013; Pinto, 2016) and the human mastadenovirus (species C, D and F) (Rigotto et al., 2010; Staggemeier et al., 2017), have also been widely detected in urban runoff to beaches subject to intense recreational activities.

(iii) Benthic macrofauna and ecotoxicological tests: impacts of non-point source pollution on the benthic macrofauna, in addition to genotoxic (*Salmonella*/ microsomic) and ecotoxic effects (acute and chronic with microcrustaceans *Daphnia simillis* and *Ceriodaphnia dubia*) were evaluated. Urban diffuse loads, containing a mixture of different pollutants, have already proved capable of causing severe ecological changes, reflecting the change in the structure of the benthonic community (McQueen et al., 2010; Tang et al., 2013) and ecotoxicological effects at different trophic levels (Tabet et al., 2015; Gosset et al., 2016).

(iv) Pharmaceuticals and personal care products (PPCP): to date there are no studies on these compounds in Guarujá. PPCPs constitute a group of a large number of chemical compounds, including pharmaceuticals of different therapeutic classes (e.g. anti-epileptics, stimulants, analgesics / anti-inflammatory drugs and antihypertensives) (Klosterhaus et al., 2013; Celle-Jeanton et al., 2014; Lolić et al., 2015) and illicit drugs (for example, cocaine) (Borova et al., 2014; Parolini et al., 2016; Pereira et al., 2016). Guarujá's public agencies have shown great concern about the possible occurrence of these drugs in the municipality's marine coastal ecosystems and its potential ecological risks. In 2017, the city hall even planned an agreement with UNIFESP (Federal University of São Paulo) to perform a diagnosis on PPCPs, but by the end of this thesis, no study had been performed yet (Guarujá, 2017).

(v) Multiple-variable analysis: The submarine outfall of Guarujá is in operation since 1998 (De Souza Abessa et al., 2012; Baptistelli and Marcellino, 2016), but also there are no past data on the presence of anionic surfactants and also protozoans (*Cryptosporidium ssp* and *Giardia ssp*), human mastadenovirus (species C, D and F) and other species of adenovirus. Furthermore, there are no reports on the impact of this sewage discharge on the aquatic biota (benthic macrofauna) and its genotoxic effects (*Salmonella*/microsomics). There is also no information on the occurrence of PPCPs and illicit drugs in the vicinity of this submarine outfall.

Table 2.2 presents the 38 environmental variables selected in this thesis and, in Table 2.3, the 23 PPCPs that were selected for the inventory in the urban drainage channels, sea water and/or the mixing zone of the Guarujá submarine outfall:

Table – 2.2: The table presents the 38 variables (with the definition of the LOQ - limit of quantification and the standard and/or analytical procedure), being them: (i) physical, (ii) chemical (including heavy metals), (iii) microbiological (including bacteria, protozoa and viruses), (iv) genotoxic, (v) ecotoxic, (vi) hydrobiological (benthic macrofauna), in addition to the particle size characterization of water and sediment samples selected for the urban runoff inventory at the beaches of Tombo, Enseada, Perequê and Iporanga, as well as the mixing zone of the submarine outfall of Guarujá, São Paulo, Brazil. Note: (1) analyses that will be performed in the channel waters; (2) analyses that will be performed in the sea water (beach); (3) analyses that will be performed in the mixing zone of the submarine outfall; (4) analyses that will be performed in all the collection points mentioned above.

Variables		Method	Limit of Quantification (LOQ)	Standard reference
Physico and chemical	Air temperature (4)	Multiparameter probe (HANNA HI 98184).	-	-
	Water teperature (4)			
	Water density (3)			
	Salinity (4)			
	Conductivity (4)			
	Absolute conductivity (4)			
	Dissolved oxygen (DO) (4)			
	pH (4)			
	Color (4)	Photometer (Hanna - HI 83200/01)	-	-
	Turbidity (4)	Turbidimeter (Hanna - HI 98703/01)	-	-
	Sedimented solids (SS) (4)	Imhoff Cone	0.10 mg/L	2540F (APHA, 2012)
	biochemical oxygen demand (BOD) (4)	Electrometric	2.00 mg/L	5210B (APHA, 2012)
	oils and greases (OG) (4)	Liquid-liquid gravimetric	5.00 mg/L	5520D (APHA, 2012)
	ammonia (NH_3)(4)	Spectrophotometry	0.001 mg/L	4500D (APHA, 1999)
	nitrite (NO_2-)(4)		0.001 mg/L	4500B (APHA, 2005)
	nitrate (NO_3-)(4)		0.001 mg/L	
	phosphate ($PO4^{3}$-) (4)		0.001 mg/L	4500E (APHA, 2012)
	anionic surfactants (MBAS) (4)		0.01 mg/L	3500B (APHA, 2005)

Heavy metals	Aluminum (Al)(4)	Atomic absorption spectrophotometry	0.025 mg/L	3120B (APHA, 2017)
	Cadmium (Cd)(4)		0.001 mg/L	
	Lead (Pb)(4)		0.005 mg/L	
	Copper (Cu)(4)		0.001 mg/L	
	Chrome (Cr)(4)		0.005 mg/L	
	Nickel (Ni)(4)		0.005 mg/L	
	Zinc (Zn)(4)		0.05 mg/L	
Microbiological	*Escherichia Coli (E.coli)* (4)	Filter membrane technique	1 CFU/mL	9222B (APHA, 2012)
	Enterococci (4)	Filter membrane technique	1 CFU/mL	9230C (APHA, 2012)
	Salmonella (3)	Presence or absence technique	-	9260B (APHA,2012)
	Cryptosporidium ssp (4)	Immunomagnetic separation and immunofluorescence microscopy	1 Oocisto/L	1623.1 (USEPA, 2012)
	Giardia ssp (4)		1 cisto/L	
	Human mastadenovirus (species C, D and F) (4)	Real-time polymerase chain reaction (qPCR) Nested polymerase chain reaction (Nested PCR)	-	protocol described by Dalla Vecchia et al. (2015)
	Other adenovirus species (4)		-	protocol described by Li et al. (2010)
Genotoxicicity	*Salmonella* / microsomal assay (1,3)	Ames test, lineages TA98 and TA100 Method 471 - Adopted: 21st July 1997	-	OECD (1997)
Ecotoxicity	Acute toxicity (1)	Integral sediment ($CE_{50;96h}$) – tanaidáceo *Kalliapseudes schubartii*	-	Zamboni and Costa (2002)

		Daphnia spp (Crustacea, Cladocera)	0 – 100%	ABNT/NBR 12713: 2016
	Chronic toxicity (1)	*Ceriodaphnia spp* (Crustacea, Cladocera)	0 – 100%	ABNT NBR 13373: 2017
Aquatic communitie	Benthic macroinvertebrates (1,3)	Identification and quantification	1 org./m^2	10500 (APHA, 2012)
Granulometric	Sediment analysis (1,3)	Fractionation of the sediment in sand, silt and clay	-	Embrapa (2011)

Table –2.3: The table presents the name of the 23 pharmaceutical compounds and their respective CAS (Chemical Abstracts Service) numbers, in addition to LOD (Limit of detection, in ng/L) and LOQ (Limit of quantification, also in ng/L) that were selected for the urban runoff inventory of the beaches of Tombo, Enseada, Perequê and Iporanga, as well as the mixing zone of the submarine outfall of Guarujá, São Paulo, Brazil. Note: Analytical method adopted: chromatography coupled with tandem mass spectrometry (LC-MS/MS) (Method validated by Shihomatzu et al., 2015).

	Compound	**CAS Number**	**LOD (ng/L)**	**LOQ (ng/L)**
Antiepileptic	Carbamazepine	298-46-4	0.003	0.01
	Clonazepam	1622-61-3	0.0013	0.01
Stimulants	Caffeine	58-08-2	0.0001	0.0085
	Cocaine	50-36-2	0.003	0.0012
	Benzoylecgonine	519-09-5	0.0012	0.0077
Antidepressant	Citalopram	59729-33-8	0.0006	0.0059
	Paroxetine	61869-08-7	0.004	0.031
Analgesic/ Anti-inflammatory	Acetaminophen	103-90-2	0.0014	0.0084
	Diclofenac	15307-86-5	0.0001	0.0074

	Orphenadrine	83-98-7	0.0009	0.0034
Antihypertensive	Atenolol	29122-68-7	0.0016	0.0069
	Propranolol	525-66-6	0.0013	0.0072
	Enalapril	75847-73-3	0.003	0.009
	Losartan	114798-26-4	0.0007	0.0061
	Valsartan	137862-53-4	0.0014	0.0077
Anticholesteremic	Rosuvastatin	287714-41-4	0.0008	0.0069
Anxiolytic	Bromazepam	1812-30-2	0.005	0.0281
	Midazolam	59467-70-8	0.0006	0.0059
Contraceptive	Cyproterone	2098-66-0	0.0015	0.0075

Diuretic	Chlortalidone	77-36-1	0.0023	0.0088
Antiplatelet	Clopidogrel	113665-84-2	0.0004	0.0003
Antihistamine	Loratadine	79794-75-5	0.0014	0.0126
Sexual stimulant	Sildenafil	171599-83-0	0.006	0.043

2.5 Main objective

In view of the above, the general objective of this thesis was to evaluate the water quality and sediment of the urban drainage channels located at the beaches of Tombo, Enseada, Perequê and Iporanga, in addition to the adjacent area to the discharge of marine sewage on the beach of Enseada, Guarujá, São Paulo, Brazil. The ultimate goal of this thesis is to contribute to the reduction of conflicts related to the land use and occupation of the seashore and assist the city hall of Guarujá in properly managing the Waterfront Project.

2.5.1 Specific objectives

The specific objectives of this thesis were:

- Perform the physical-chemical and bacteriological evaluation of the waters of the urban drainage channels and of the sea water of the beaches of Tombo, Enseada, Perequê and Iporanga;

- Verify the occurrence of protozoa and enteric viruses in the waters of the channels that flow to the beaches of Enseada and Perequê;

- Characterize the structure of the benthic assembly near the urban drainage channels of Tombo, Enseada, Perequê and Iporanga beaches;

- Verify the occurrence and evaluate the ecological risk of PPCPs and illicit drugs in the waters of the urban drainage channels and in the sea water of the beaches of Tombo, Enseada, Perequê and Iporanga;

- Analyze the genotoxicity and ecotoxicity (acute and chronic) of diffuse loads that drain to the beaches of Tombo, Enseada, Perequê and Iporanga;

- Evaluate the quality of the water column and sediments in the mixing zone of the submarine outfall, through physicochemical (including heavy metals), microbiological (bacteria, protozoa and viruses), hydrobiological (benthic macrofauna), genotoxic and ecotoxic variables;

- And to verify the occurrence and evaluate the ecological risk of PPCPs and illicit drugs in the water column of the mixing zone of the submarine outfall of Guarujá.

2.6 Structure of the thesis

In order to meet these specific objectives and with the purpose of allowing a better understanding of the data obtained, the thesis was organized in nine distinct, but sequential chapters. Chapters 1 and 2 contextualize the work (state of art) and justify the choice of the subject (rationale), respectively. The main body of the thesis (i.e., Chapters 3, 4, 5, 6, 7, 8 and 9) are results already published, accepted or submitted to peer-reviewed international journals, which will be described in more detail. Chapter 10, is the final chapter, a general discussion of the thesis which integrates and discusses the main findings.

Chapter 3: "Spatial and temporal evaluation of the urban runoff water flowing into recreational areas of Guarujá, São Paulo State, Brazil". At this stage of the research, an evaluation of the water quality of the urban drainage channels that drain to the beaches of Tombo, Enseada, Perequê and Iporanga was carried out. Monthly samples were obtained from October 2017 to August 2018 and 28 environmental variables (physical, chemical, and bacteriological) were analyzed using standard statistical tests and different quality indexes. This chapter was published in the International Journal of River Basin Management from Taylor & Frances Online.

Chapter 4: "Occurrence of human adenoviruses in a beach area of Guarujá, São Paulo, Brazil". At this stage of the research the presence of protozoans was evaluated (*Cryptosporidium ssp* e *Giardia ssp*), as well as human mastadenovirus (species C, D and F) and other species of adenovirus in the urban drainage channels of the Enseada and the Perequê. Water collections took place between October 2017 and August 2018. This chapter was published in Water Environment Research from Wiley Online Library.

Chapter 5: "Temporal and spatial variation of benthic macroinvertebrates on the shoreline of Guarujá, São Paulo, Brazil, under the influence of urban surface runoff". In this stage of the research a characterization of the structure of the benthic assembly near the channels of the beaches of Tombo, Enseada, Perequê and Iporanga was carried out. The collection of sediments and the screening of macroinvertebrates also took place between October 2017 and August 2018. To verify the water pollution level of these channels, the identified macroinvertebrates were analyzed through standard statistical tests and different quality indexes. This chapter was published in Regional Studies in Marine Science from Elsevier.

Chapter 6: "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*"*. At this stage of the research, PPCPs from different therapeutic classes were screened, including illicit drugs such as cocaine and its metabolite benzoilecgonine (total of 23 investigated drugs), in the urban drainage channels and sea water of Tombo, Enseada, Perequê and Iporanga. A unique collection was carried out in January 2018 (Brazilian summer, high tourist season). After the identification of the different PPCPs, a risk assessment analysis of these compounds in the different trophic levels of the aquatic ecosystem (algae, crustaceans and fish) was done. This chapter was published in Environmental Science and Pollution Research from Springer.

Chapter 7: "Mutagenic and ecotoxicological assessment of urban surface runoff onto the beaches of Guarujá, State of São Paulo, Brazil". In this stage of the research, genotoxicity (through *Salmonella*/microsomics) and ecotoxicity (acute and chronic through the microcrustaceans *Daphnia simillis* and *Ceriodaphnia dubia*, respectively) of the diffuse loads that drain to the beaches of Tombo, Enseada, Perequê and Iporanga were verified. Toxicity collections and analyses were bimonthly, and occurred in January, March, May and July 2018. This chapter is under review in Water Science and Technology from IWA Publishing.

Chapter 8: "Assessment of the water and sediments quality around the coastal submarine sewage outfall in Guarujá, São Paulo, Brazil". In this stage an evaluation of the quality of the water and sediments in the mixing zone of the submarine outfall of Guarujá was carried out. Water collection (obtained at two distinct depths, surface:1 meter; and bottom: 10 meters) and sediment collection, was performed in January (during the high tourist season/ Brazilian summer) and in April (during the low tourist season/ autumn) of 2018. With the exception of microcrustaceans (*Daphnia simillis* and *Ceriodaphnia dúbia*) all other variables investigated in the urban drainage channels (indicated in Chapters 3, 4, 5 and 7) were also investigated in the mixing zone of the submarine outfall. This chapter is under review in Thalassas: An International Journal of Marine Sciences from Springer.

Chapter 9: “Occurrence and risk assessment of pharmaceuticals and cocaine around the coastal submarine sewage outfall in Guarujá, São Paulo State, Brazil”. The same analytical methodology used in Chapter 6 was also applied in the mixing zone of the submarine outfall. The collections (according to the period described in Chapter 8), in this chapter, were made only in the water column (surface:1 meter; and bottom: 10 meters). Ths chapter is published in Environmental Science and Pollution Research from Springer.

In addition to the Chapters 6 and 9, a third study on these emerging pollutants (PPCPs) has been developed, but in the seven urban drainage channels of Santos city, São Paulo (neighbouring city of Guarujá). This chapter although related appears as an appendix of this thesis, since it was not the main focus of the study. This article ["Occurrence and ecological risk assessment of pharmaceuticals and cocaine in the urban drainage channels of the beaches of Santos (São Paulo, Brazil): a neglected but sensitive issue"] is under review in Environmental Science and Pollution Research from Springer.

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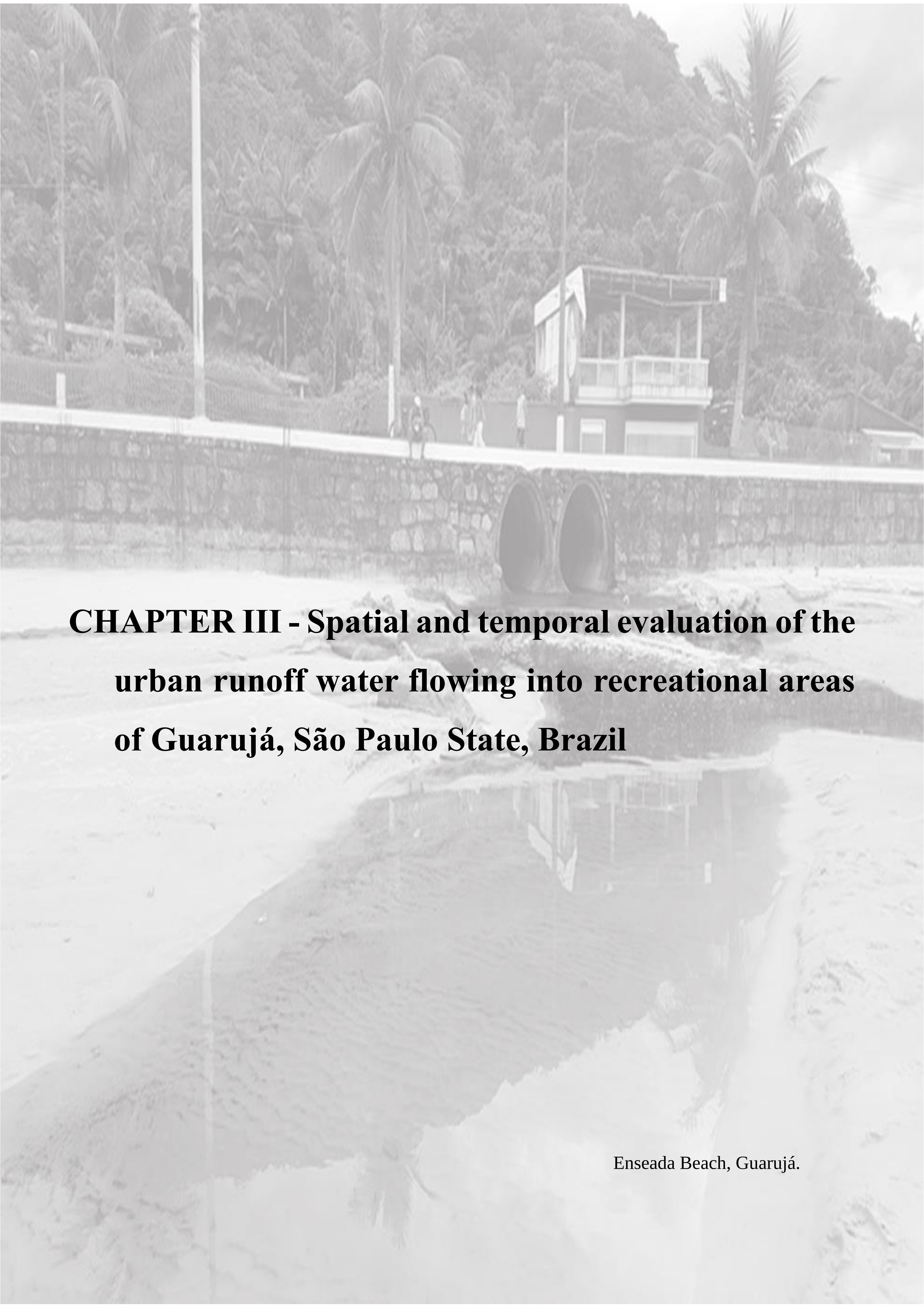

CHAPTER III - Spatial and temporal evaluation of the urban runoff water flowing into recreational areas of Guarujá, São Paulo State, Brazil

Enseada Beach, Guarujá.

Spatial and temporal evaluation of urban surface runoff in a beach area of Guarujá, São Paulo State, Brazil

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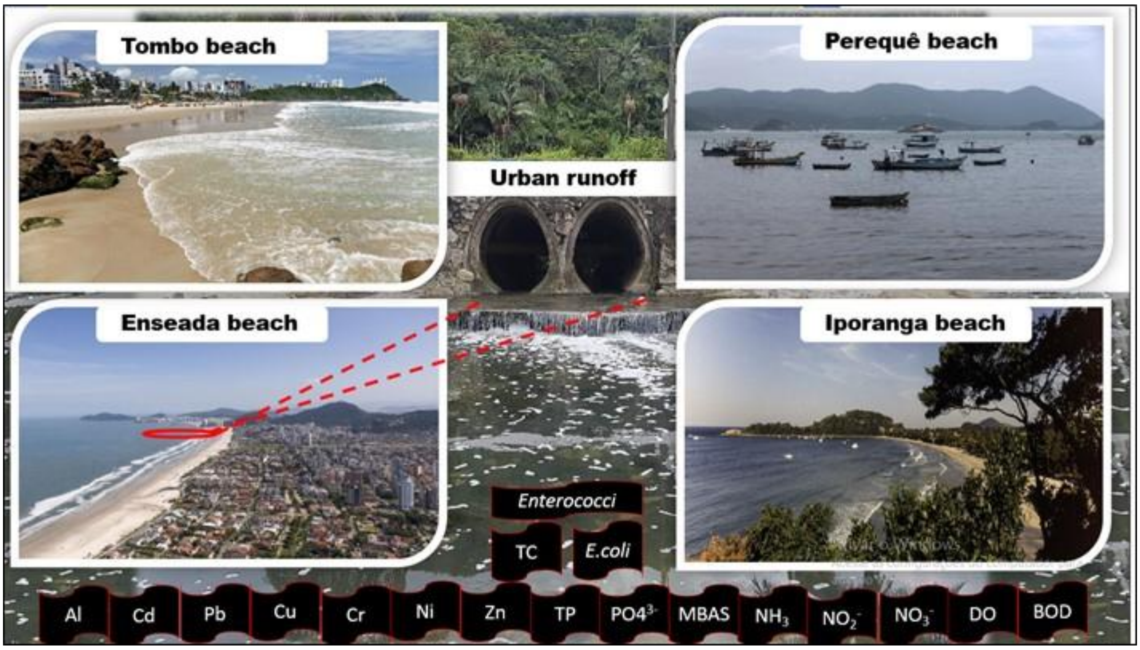
Published in: International Journal of River Basin Management (2020).

Graphical Abstract



Abstract

This study evaluated the water quality of the urban runoff flowing into the public beaches and recreational waters of Guarujá, São Paulo State, Brazil. Monthly samples were obtained from October 2017 to August 2018 in four beaches: Tombo (Blue Flag certification), Enseada (high-used by tourist), Perequê (fishing community) and Iporanga (conservation unit). Twenty-eight environmental variables (physical, chemical and microbiological) were analysed using standard statistical tests, and different quality indexes were obtained. Data showed that urban drainage channels are the main responsible for pollutant transport to the Guarujá Sea, mainly during the rainy season. Moreover, the different occupations and land uses appear to interfere with the water quality of the urban runoff. The best water quality was recorded at Iporanga (environmental compliance over 90% according to the Brazilian law, with low total phosphorus enrichment, but medium aluminium toxicity). The quality ratings for the other beaches were classified as very bad (34–43% compliance with current legislation). Moreover, these waters were eutrophic showing high aluminium toxicity. This study highlights the environmental and public health risks arising from the urban runoff flowing into the beaches of Guarujá. Better water management practices, improvement of basic sanitation and population awareness, is highly recommended to prevent potential socio-environmental risks in this tourist area.

Keywords: Subtropical zone, Non-point source pollution, Domestic sewage, Bathing waters, Pollution effects.

3.1 Introduction

The world population has recently reached about 7.6 billion inhabitants, and more than half live in urban areas (Undesa, 2017). This high population density has led to numerous anthropogenic activities in cities, resulting in point (e.g. domestic sewage) and diffuse (e.g. urban runoff) sources of water pollution (Choi et al., 2011; Xue et al., 2015; Zotou et al., 2018). Managing urban runoff is currently a challenge because its identification, delimitation and control are complex processes (Yu et al., 2011; Saraswat et al., 2016; Yang and Toor, 2017). The water quality of the urban runoff is linked with the level of urbanization (e.g. population densification, reduction of green areas, and high asphalt waterproofing) (Yang and Toor, 2017; Zotou et al., 2018; Moreno et al., 2019). In addition, the environmental sanitation infrastructures (e.g. sewage treatment and urban drainage) (Ferraz et al., 2012; Larrea-Murrel et al, 2013; Lamparelli et al., 2015) and the hydrological regime (e.g. dry or rainy period, intensity and duration of precipitation), are issues that also interfere with urban diffuse load generation (Wang et al., 2013; Xiang et al., 2017). The final contribution of the urban surface runoff to different receiving bodies, has been considered one of the main world`s threats to water quality, as it causes numerous economic and socio-environmental problems (Federigi et al., 2016; Lusk and Toor, 2016; Yang and Toor, 2017). These impacts include the pollution of public water sources, the decline of fishing economic activity and the loss of aquatic biodiversity (Federigi et al., 2016; Lusk and Toor, 2016; Yang and Toor, 2017). Asia, South Korea (Choi et al., 2011), Japan, Vietnam, Thailand (Saraswat et al., 2016) and China (Xiang et al., 2017) have expanded their efforts over the last decade in an attempt to manage these diffuse loads throughout cities.

Several studies conducted in México (Curiel-Ayala, 2012), China (Zhang et al., 2013), Cuba (Larrea-Murreal et al., 2103), Columbia (Botero et al., 2015), United States (Tilburg et al., 2015) and Italy (Federigi et al., 2016), have already identified urban runoff as a potential threat to public and environmental health, as it is responsible for introducing chemical and biological pollutants directly into estuaries and coastal areas oceans, known as regions of intense recreation (Zhang et al., 2013; Tilburg et al., 2015; Federigi et al., 2016). Among the various pollutants detected in the urban runoff waters, dissolved inorganic nitrogen (Ballo et al., 2009; Yu et al., 2011), phosphorus (Badruzzaman et al., 2012; Yang and Toor, 2017), detergents (Ghose et al., 2009; Renzi et al., 2012), heavy metals (Suresh et al., 2012; Gunawardena et al., 2013), and pathogenic microorganisms (Tilburg et al., 2015; Federigi et al., 2016), are the most dangerous.

However, in Brazil (the fifth largest country in the world), where approximately 50 million people live in coastal municipalities (along 8,500 km of coastline) (Ibge, 2018), although studies on the control of diffuse pollution are of great importance, they are scarce (Ferraz et al., 2012; Martins et al., 2014; Lamparelli et al., 2015). Interestingly, the Brazilian Water Resources Policy (Law No. 9433/1997) makes no reference to the control of this source of pollution (Brazil, 1997). The state of São Paulo is among the main Brazilian coastal zones. With an area of 7,759 km^2 and a coastline of 880 km, this region encompasses 16 municipalities that house about 2,200 inhabitants (which doubles during the high tourist season) (Cetesb, 2017; Ibge, 2018). Historically, measuring the contribution of diffuse charges to urban pollution has proven to be very difficult on the coast of São Paulo, because urban runoff waters are usually mixed with clandestine domestic sewage before they even reach the receiving seas (Ferraz et al., 2012; Lamparelli et al., 2015; Cetesb 2017). In some cities of São Paulo, urban runoff is popularly known as “black tongues” (runoff of black water with a strong odour) (Rocha et al., 2011). Guarujá city (the focus of this study), which is one of these municipalities from São Paulo, and also one of the leading Brazilian tourist destinations, has already suffered from a lack of urban and environmental planning (SMA/CPLEA, 2005). Between 1950 and 1990, the population increased from 13,000 to 210,000, and the current resident population is about 316,000 (Ibge, 2018). As a result, the unplanned growth of the municipality and the lack of investments in sanitation infrastructures (e.g. sewage treatment and urban drainage) have caused intense economic and socio-environmental conflicts that persist today (SMA/CPLEA, 2005; Cetesb 2017). In Guarujá, the São Paulo State Environmental Agency has registered about 43 drainage channels, receivers of urban diffuse loads, whose waters flow directly into eight tourist beaches before reaching the South Atlantic Ocean (Cetesb, 2017). Despite this scenario, no previous studies exist in Guarujá dedicated to measuring the potential risks to public and environmental health of these discharges at inshore recreational areas (i.e. beaches).

The objective of this study was to evaluate, for the first time, the water quality (physical, chemical, and microbiological variables) of four urban drainage channels, whose waters flow directly to the beaches of Tombo, Enseada, Perequê, and Iporanga, in the city of Guarujá, São Paulo, Brazil.

3.2 Material and Methods

3.2.1 Sampling sites and characterization of the study area

This study was carried out in sub-basin 13, Santo Amaro Island, Guarujá city, a micro-region of Santos municipality, São Paulo State, Brazil. The mean annual precipitation and temperature of Guarujá reach 3,000 mm and 22°C, respectively. Two quite distinct periods are observed in the municipality: a rainy (November to March) and dry (April to October) seasons. Guarujá has an area of 143 km^2 and 64 km of extension, 36 km^2 of which is already completely urbanized (Ribeiro and Oliveira, 2015). The remaining 107 km^2 are made up of environmental protection areas (Ribeiro and Oliveira, 2015). This coastal island contains a resident population of approximately 316,000 inhabitants (Ibge, 2018). During the high tourist season, in summer, between December and February, this population practically doubles (Cetesb, 2017). It is estimated that about 20% of this population lives in irregular residential buildings, commonly known as “favelas”, occupying areas of relevant ecological interest (hills, restingas, and mangroves) (Ribeiro and Oliveira, 2015).

The municipality’s economy is mainly driven by the port of Santos (the largest in Latin America), located in the western portion of the island, but also by the seasonal tourism that takes place along the eight main beaches, located in the eastern and southern portions of the island (Ribeiro and Oliveira, 2015). These beaches receive the input of rainwater from superficial runoff, through 43 urban drainage channels. These channels are made of concrete, and none of them has a grating system, so all of their content is discharged directly into the beaches, on a daily basis, in an area of ample occurrence of primary and secondary contact recreation activities (Cetesb, 2017).

The Environmental Agency of the State of São Paulo (Cetesb) monitors these beaches weekly (on Sundays) and uses the microbiological indicator *Enterococci* as the main water quality parameter. This monitoring network takes into consideration the frequency of bathers, the physiography of beaches, and the risks of pollution (Cetesb, 2017). Taking into account this information, four of the eight monitored beaches were selected for this study based on the different characteristics regarding their use and soil occupation: Tombo (Blue Flag certification), Enseada (high-used by tourist), Perequê (fishing community) and Iporanga (conservation unit). At each beach, two sampling points were selected. A first point was defined at the mouth of the channels (beach sand), but without the interference of the tidal regime (identified with the letter A). The second sample was collected at the mouth of this drainage channel, but directly in seawater (30 cm deep) at a primary and secondary contact recreation point (identified with the letter B) (Figure 3.1).

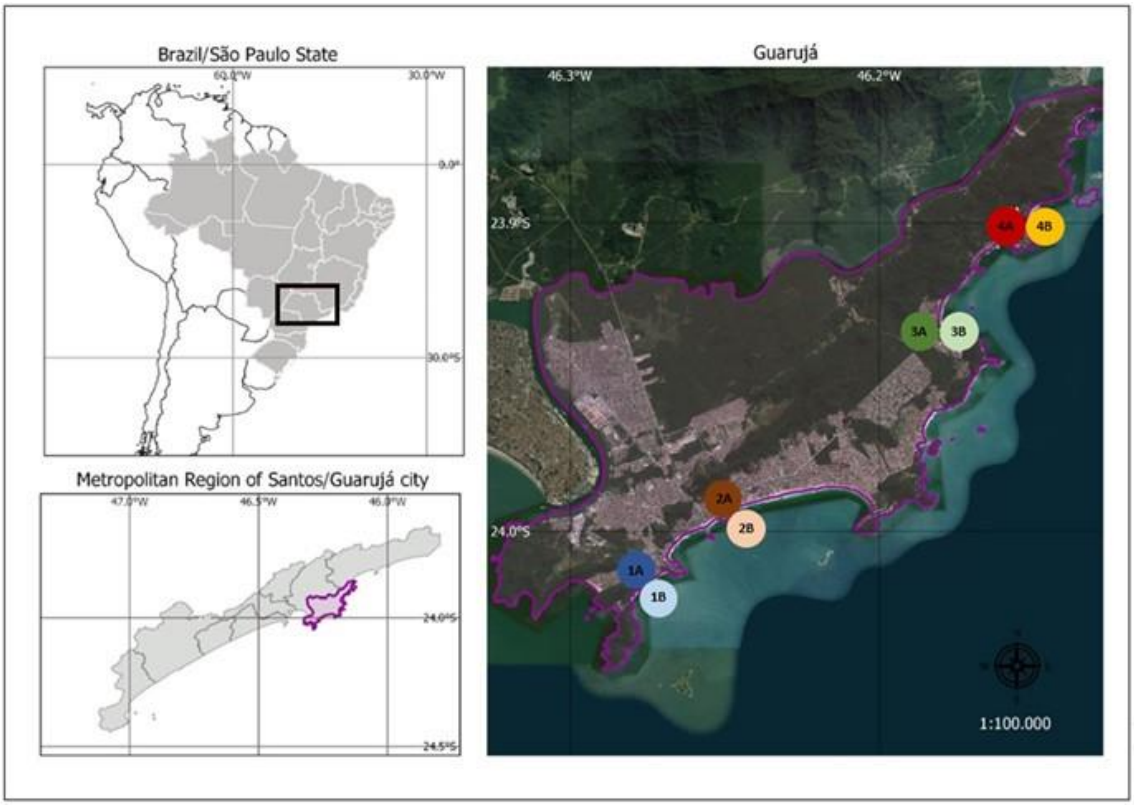


Figure – 3.1: Map of the study area showing the Brazil, São Paulo State, Metropolitan Region of Santos. Water sampling stations on four beaches in the municipality of Guarujá: i) Tombo beach (point 1A: urban drainage channel; point 1B: seawater); ii) Enseada beach (point 2A: urban drainage channel; point 2B: seawater); iii) Perequê beach (point 3A: urban drainage channel; point 3B: seawater); iv) Iporanga beach (point 4A: urban drainage channel; point 4B: seawater).

3.2.2 Sample preparation and analysis

The monthly collections were carried out over a period of 11 months (October 2017–August 2018), allowing us to observe some environmental (rainy season: November to

March vs dry season: April to October) and anthropogenic interferences (high tourist season: December to February vs low season: March to August) in the urban runoff water quality.

Prior to the water collections, several measurements were made *in loco* using a multiparameter probe (HANNA HI 98184, calibrated with standard solutions prior to start of each campaign); these measurements included air and water temperature (ºC), salinity, conductivity (Ec), total dissolved solids (TDS), pH and dissolved oxygen (DO). The water colour (photometer Hanna HI 83200/01) and turbidity (turbidimeter Hanna HI 98703/01) were also obtained in the field. Thereafter, water samples were collected according to the National Guide for Collection and Preservation of Samples (Cetesb, 2011). All samples were labelled, conditioned under refrigeration (≤6°C) in collection bottles, previously sterilized, and properly identified with the sampling data.

Ammonia (NH_3), nitrites (NO_2-), nitrates (NO_3-), phosphate (PO_4^{3}-), total phosphorus (TP), and anionic surfactants (MBAS) in water samples were analysed in the Research Laboratory of Natural Products of Santa Cecília University in Santos, São Paulo State, Brazil.

In the Laboratory of Analytical Control Analysis Techniques, in Osasco, São Paulo State, Brazil (accreditation of Inmetro/ABNT ISO/IEC17025), the following water analyses were performed: (i) physico-chemical analyses: sedimented solids (SS); biochemical oxygen demand (BOD); and oils and greases (OG); (ii) heavy metals: aluminium (Al), cadmium (Cd), lead (Pb), copper (Cu), chrome (Cr), nickel (Ni), and zinc (Zn); and (iii) microbiological analyses: total coliforms (TC), *Escherichia coli* (*E. coli*), and *Enterococci*.

All variables and analytical methods are detailed in Table 3.1. All analyses were carried out in triplicate.

Table –3.1: Analytical methods (with definition of the quantification limit and standard reference protocol) applied to physical and chemical (including heavy metals and microbiological) variables of water samples collected in the surface urban runoff in a beach area of Guarujá city, São Paulo State, Brazil.

	Variables	Analytical Method	Limit of Quantification (L.Q)	Standard reference protocol
Physical and chemical	ammonia (NH_3)	Spectrophotometry	0.001 mg/L	4500D (APHA, 1999)
	nitrite (NO_2-)		0.001 mg/L	4500B (APHA, 2005)
	nitrate (NO_3-)		0.001 mg/L	
	phosphate (PO_4^{3}-)		0.001 mg/L	4500E (APHA, 2012)
	total phosphorus (TP)		0.001 mg/L	4500E (APHA, 2012)
	anionic surfactants (MBAS)		0.01 mg/L	3500B (APHA, 2005)
	sedimented solids (SS)	Imhoff Cone	0.10 mg/L	2540F (APHA, 2012)
	biochemical oxygen demand (BOD)	Electrometric	2.00 mg/L	5210B (APHA, 2012)
	oils and greases (OG)	Liquid-liquid gravimetric	5.00 mg/L	5520D (APHA, 2012)
Heavy metals	aluminum (Al)	Atomic absorption spetrophotometry	0.025 mg/L	3120B (APHA, 2017)
	cadmium (Cd)		0.001 mg/L	
	lead (Pb)		0.005 mg/L	
	copper (Cu)		0.001 mg/L	
	chrome (Cr)		0.005 mg/L	
	nickel (Ni)		0.005 mg/L	
	zinc (Zn)		0.05 mg/L	
Microbiological	total coliforms (TC)	Filter membrane technique	1 CFU/mL	9222B (APHA, 2012)
	Escherichia Coli (E.coli)			
	Enterococci	Filter membrane technique	1 CFU/mL	9230C (APHA, 2012)

3.2.3 Statistical analysis

A Mann-Whitney (nonparametric) test was performed (Hollander and Wolfe, 1999) to compare the numerical variables among the sampling locations (Tombo, Enseada, Perequê, and Iporanga: for both channels and seawater sampling points) and seasonal periods (rainy and dry season). To verify the association among numerical variables, a Spearman correlation was used (Hollander and Wolfe, 1999). To visualize the correlations among the variables, perceptual maps were constructed via PCA (principal component analysis) after data normalization. Moreover, HCA (Hierarchy Cluster Analysis) dendograms were obtained using the Euclidean distance and Ward's linkage method (Mingoti, 2005). In all cases, only physicochemical and microbiological variables that were quantified in all campaigns and sampling points were selected for correlation. The significance level was $p < 0.05$ in all cases. All analyses were carried out using R statistical software (version 3.6.1) (R Core Team, 2017).

3.2.4 Water quality indexes

3.2.4.1 Canadian Council of Ministers of the Environment Water Quality Index (CCMEWQI)

CCMEWQI was used to evaluate whether the physico-chemical and microbiological results are within the Brazilian normative standard. The Brazilian waters of urban drainage channels and beaches do not have yet any quality criteria to separate them into classes. However, regarding the guidelines of Conama Resolution n° 357 of 2005, article 42, “while their classifications are not approved, fresh waters should be classified in class 2 and saline waters in class 1” (Brazil, 2005). After this definition, three guidelines were adopted in this study: for the physico-chemical analysis, Conama Resolution nº 357/2005 (Brazil, 2005); for the microbiological analyses, Conama Resolution nº 274/2000, that establishes criteria for bathing Brazilian beaches (*Enterococci* in seawater) (Brazil, 2000) and Board Decision nº 112/2013/E of 2013 of the Environmental Agency of São Paulo (*Escherichia Coli* in urban drainage channels) (Cetesb, 2013). The calculation of index scores in the CCMEWQI method can be obtained by using the following relation:

$$\mathrm{CCMEWQI} = 100 - \frac{\sqrt{\mathrm{F1}^2 + \mathrm{F2}^2 + \mathrm{F3}^2}}{1.732}$$

where range (F1) evaluates the number of variables that show nonconformity; frequency (F2) evaluates the amount of nonconformities as a whole and does not differentiate within variables; and amplitude (F3) refers to noncompliance with the current legislation. Using the index, waters are classified as optimal (≥ 95), good (< 95 to ≥ 80), regular (< 80 to ≥ 65), bad (< 65 to ≥ 45), and very poor (< 45) (CCME, 2001).

3.2.4.2 Trophic state index (TSI)

The trophic degree of the drainage channels (points 1A to 4A) was verified through the use of TSI, from the values of TP (µg/L) (Lamparelli, 2004). The TSI is given by the equation:

$$\mathrm{TSI\ (TP)} = 10.[6 - ((0.42 - 0.36 \text{ x } (\ln \mathrm{TP}))/\ln 2)] - 20$$

At the end, the index is obtained, whose classifications are: ultra-oligotrophic (TSI ≤ 47); oligotrophic (TSI 47–52); mesotrophic (TSI 52–59); eutrophic (TSI 59–63); supereutrophic (TSI 63– 67), and hypereutrophic (TSI > 67).

3.2.4.3 Toxic contamination index (TCI)

The TCI classifies the monitoring points according to the concentration of the toxic heavy metals Al, Cd, Pb, Cu, Cr, Ni, and Zn (Igam, 2013) and is based on the limits recommended by Conama nº 357/2005 (Brazil, 2005). TCI may be “low” if the concentrations of the variables are ≤ 20% of the established limits, “average” for values 20%–100%, or “high” if ≥ 100% of the limits. The worst-case scenario in the results set defines the contamination range. Thus, if one of the variables has a value > 100% (twice the limit concentration) in at least one of the analyses, the contamination at the sampling point will be classified as high (Igam, 2013).

3.3 Results

3.3.1 Multiple comparisons between variables

Figure 3.2 shows a perceptual map via PCA according to source variables [(channel or seawater) (Figure 3.2(a)], site [(points 1A to 4A and points 1B to 4B) (Figure 3.2(b)], and hydrological period [(rainy and dry) (Figure 3.2(c) and (d)] (Mann-Whitney test; p < 0.05).

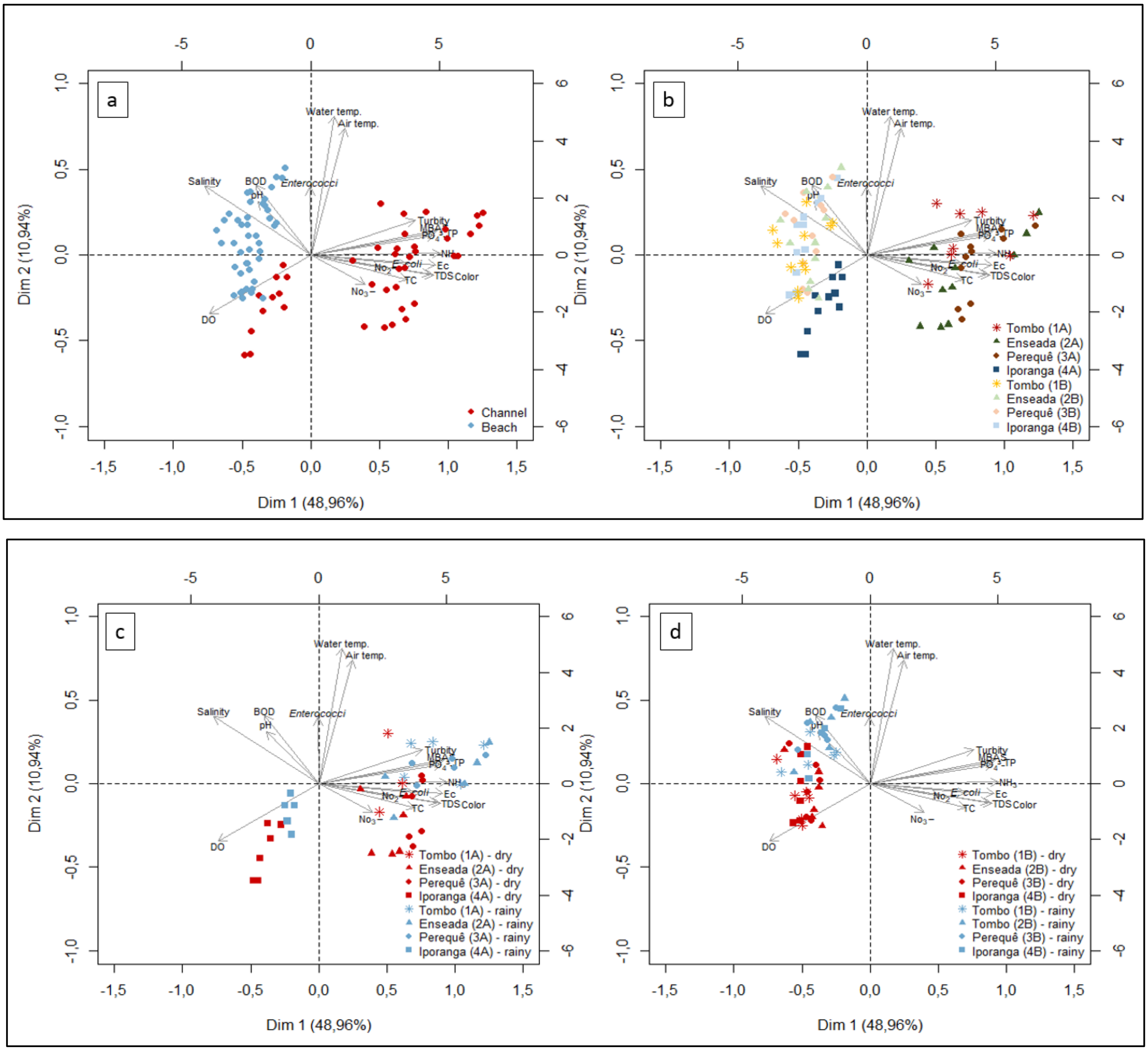


Figure – 3.2: Perceptual map via principal components analysis (PCA), applied to water variables: physical, chemical, and microbiological (including air temperature) of samples collected in the surface urban runoff in a beach area of Guarujá city, São Paulo State, Brazil. All tests were carried with the Mann-Whitney test (nonparametric; p < 0.05). Four distinct scenarios were considered (a, b, c, and d). In all cases, only the physicochemical and microbiological variables that were quantified in all campaigns and points were selected for correlation.

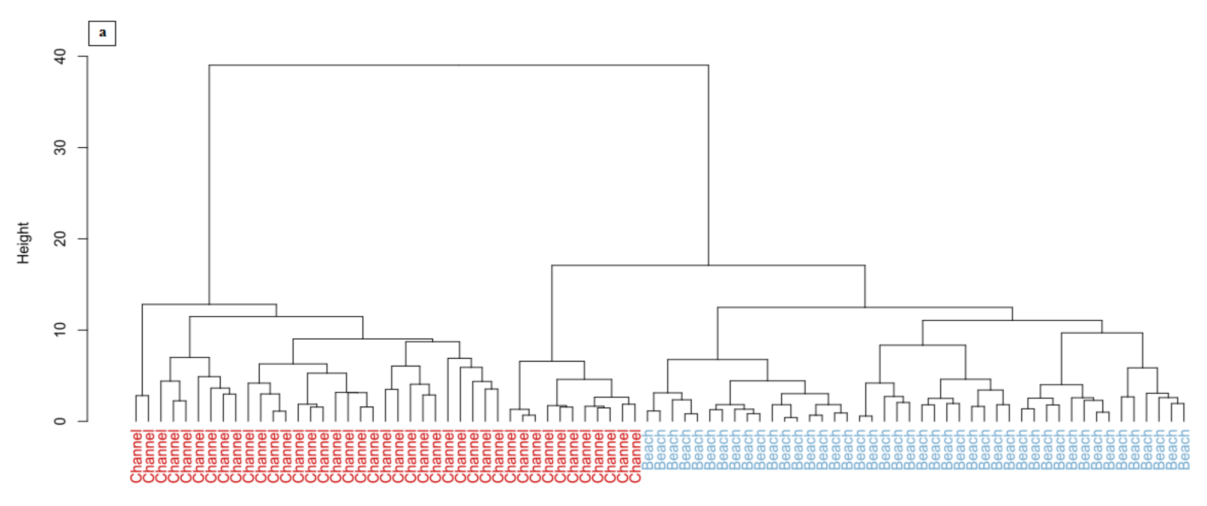
(a): PCA, means of the 18 variables (water) and 1 of air (temperature) to verify in which site, channel (red diamond) or beach/seawater (blue diamond), the environmental conditions are worse. At these two sites, the following combined analyses were carried out between points: channel [(points: Tombo (1A) + Enseada (2A) + Perequê (3A) + Iporanga (4A)] and in beach/seawater [(points: Tombo (1B) + Enseada (2B) + Perequê (3B) + Iporanga (4B)].

(b): PCA, means of the 18 variables (water) and 1 of air (temperature) to verify in which sample site, channel [(points: Tombo (1A); Enseada (2A); Perequê (3A) or Iporanga (4A)] and/or seawater [(points: Tombo (1B); Enseada (2B); Perequê (3B) or Iporanga (4B)], the environmental conditions are worse.

(c): PCA, means of the 18 variables (water) and 1 of air (temperature) to verify in which seasonal period (rainy or dry) the points of channels [(points: Tombo (1A), Enseada (2A), Perequê (3A) or Iporanga (4A)] have worse environmental conditions.

(d): PCA, means of the 18 variables (water) and 1 of air (temperature) to verify in which seasonal period (rainy or dry) the points of seawater [(points: Tombo (1B), Enseada (2B), Perequê (3B) or Iporanga (4B)] have worse environmental conditions.

Thus, the set of variables was able to discriminate the sources and sites. Variables such as turbidity, Ec, TDS, colour, TP, MBAS, PO_4^{3-}, NH_3, NO_2^- , NO_3, TC, and *E.coli* showed the highest values for all the channels (points 1A to 4A), whereas the variables DO, BOD, pH, and salinity showed the highest values at seawater (points 1B to 4B). Meanwhile, the set of variables was not able to discriminate the periods (rainy and dry). The groups formed with the analysis of the PCA, were later confirmed through the HCA dendrograms [Figure 3.3(a-c)].



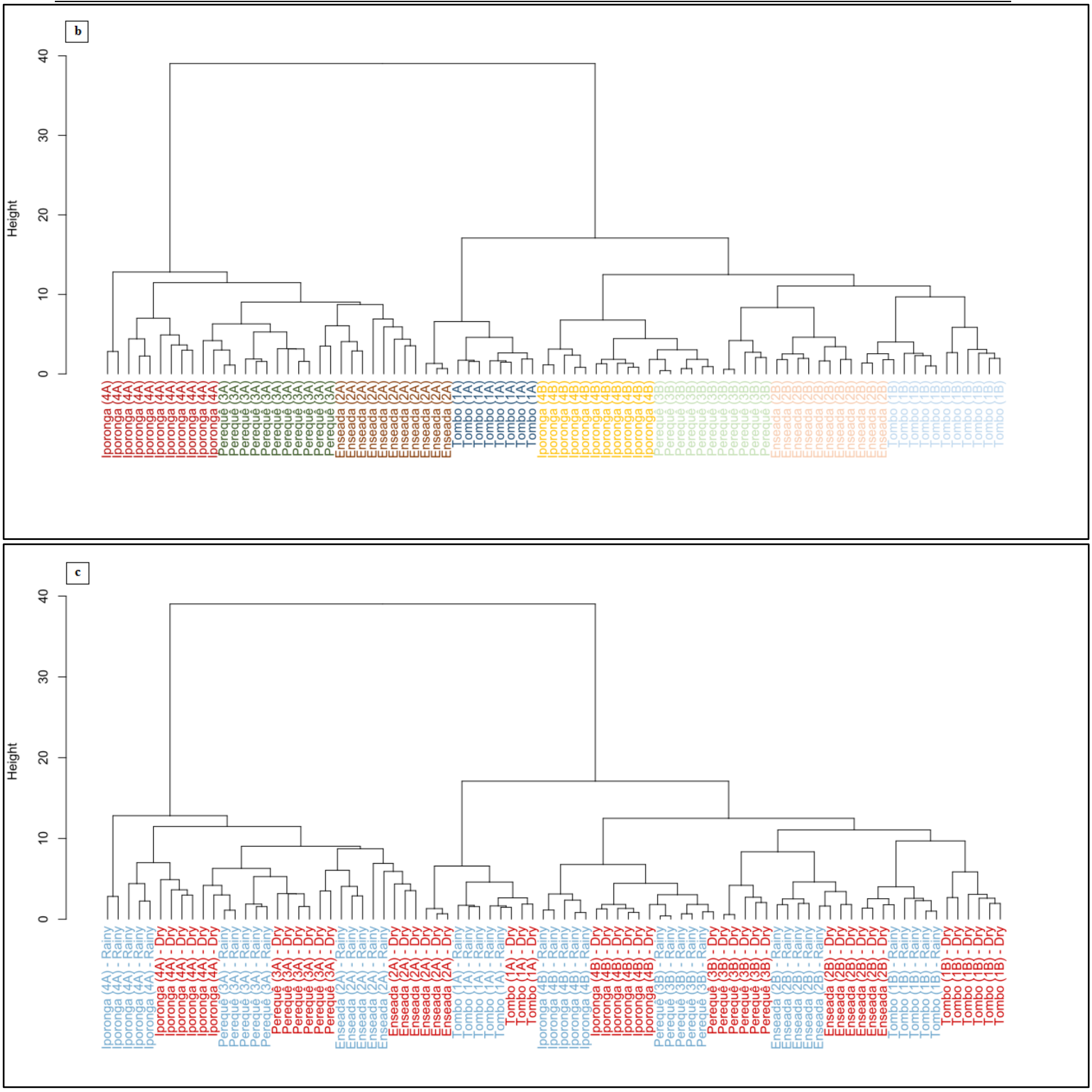


Figure – 3.3: Dendrogram of Hierarquical Cluster Analysis - (HCA) showing cluster of variables (water quality parameters) of samples collected in the urban surface runoff in a beach area of Guarujá city, São Paulo State, Brazil. To obtain the HCA dendrogram, Ward's method and Euclidean distance were used. Four distinct scenarios were considered, as described in Perceptual map via principal components analysis (PCA) [Figure 3.2(a)(b)(c) and (d)].

(a): shows a dendrogram of HCA according to source variables [(channel or seawater) (Figure 3.2(a)].

(b): shows a dendrogram of HCA according to site [(points 1A to 4A and points 1B to 4B) (Figure 3.2(b)].

(c): show a dendrogram of HCA according to hydrological period [(rainy and dry) (Figure 3.2(c) and (d)].

3.3.2 Results of physicochemical and microbiological variables

The spatial and temporal evaluation carried out from October 2017 to August 2018 showed that the mean air temperature was 23.5ºC [Figures 3.4(a) and 3.5(a)] and the mean water temperature was 24.5ºC (in the channels and seawater) [Figures 3.4(b) and 3.5(b)].

In 100% of the samples from the four channels, salinity was < 0.5%. [Figure 4(c)]. At sea, a salinity >30% was recorded in all sampling sites [Figure 3.5(c)]. In the channels, turbidity (< 100.00 NTU) [Figure 3.4(d)], pH (6.16-7.62) [Figure 3.4(e)], and TDS (< 500.00 mg/L) [Figure 3.4(f)] were recorded throughout the year. Lower turbidity values were observed at seawater sampling sites (in general < 15.00 NTU) [Figure 3.5(d)]. In overall, the pH at sea sampling sites ranged mainly between 6.70 and 8.04, with exception of Tombo which recorded waters slightly acidic (pH range between 7.62 and 6.16) [Figure 3.5(e)]. TDS values at sea were also low at all seawater sampling sites (< 26.03 mg/L throughout the study) [Figure 3.5(f)].

The colour showed values of 18.00-85.00 mgPt/L (in the channels). The highest concentrations were observed in Perequê [Figure 3.4(g)]. Seawater showed values of 2.00 to 42.00 mgPt/L [Figure 3.5(g)].

In all four channels, Ec was higher than 100.00 µS/cm in 100% of samples [Figure 3.4(h)]. For sea sites, values ranged between 30.01 and 52.03 mS/cm [Figure 3.5(h)].

In the channels there was a predominance of NH_3 in relation to NOx (NO_2- + NO_3-) [Figure 3.4(i)(j)(k)] and, at sea, a predominance of NO_3- in relation to NO_2- and NH_3 [Figure 3.5(i)(j)(k)].

The other nutrients—TP [0.15 to 2.32 mg/L (channels) and 0.49 to 0.95 mg/L (seawater)] [Figures 3.4(i) and 3.5(m)] and PO_4^3 [0.03 to 1.06 mg/L (channels) and 0.13 to 0.41 mg/L (seawater)] [Figures 3.4(m) and 3.5(m)]—presented high mean concentrations.

MBAS also had high mean concentrations in the channels (0.12 to 1.18 mg/L) and in seawater (0.29 to 0.45 mg/L) [Figures 3.4(n) and 3.5(n)].

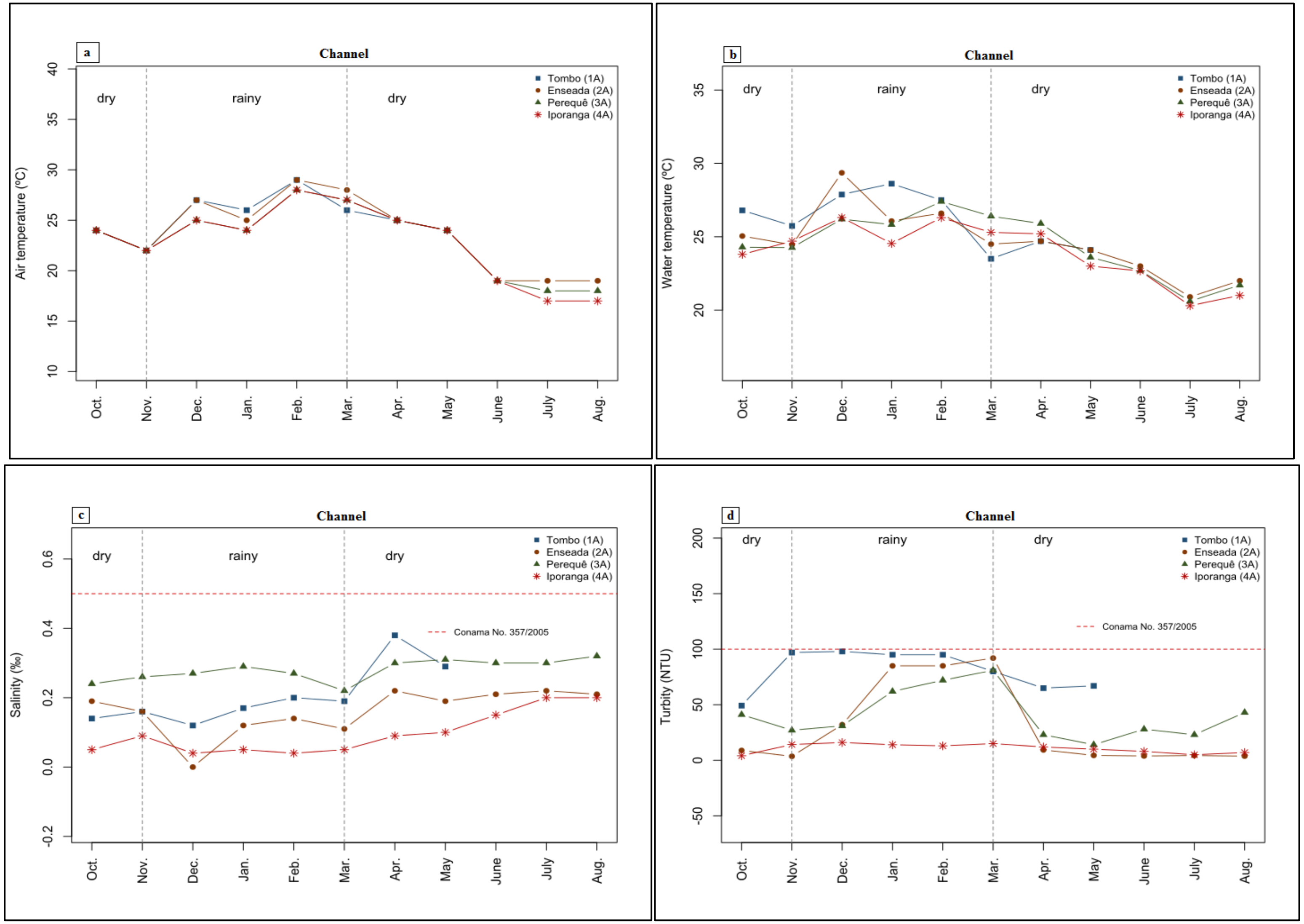
Low mean concentrations of DO were observed in both the channels and seawater. DO in the channel was 1.18 mg/L (Tombo), 0.35 mg/L (Perequê), 2.16 mg/L (Enseada), and 5.39 mg/L (Iporanga); and in seawater was 2.91 mg/L (Tombo), 4.03 mg/L (Perequê), 3.04 mg/L (Enseada), and 3.23 mg/L (Iporanga) [Figures 3.4(o) and 3.5(o)]. Conversely, mean concentrations of BOD were high [Figures 3.4(p) and 3.5(p)].

The OG was below the quantification limit (L.Q < 5mg/L) (Table 3.2) in 100% of the samples (channels and seawater).

The coliforms presented high mean concentrations throughout the study: TC [5.910,73 x 10^3 to 268.318,2 x 10^3 CFU/mL (channels) and 159.272,73 x 10^3 to 5.284,55 x 10^3 CFU/mL (seawater), Figures 3.4(q) and 3.5(q)]; *E. coli* [0,186 x 10^3 to 33.272,73 x 10^3 CFU/mL (channels) and 0,298 x 10^3 to 22.181,82 x 10^3 CFU/mL (seawater), Figure 3.4(r) and 3.5(r)]; and *Enterococci* [0,105 x 10^3 to 9.748,82 x 10^3 CFU/mL (channels) and 0,118 x 10^3 to 8.570,9 x 10^3 CFU/mL (seawater) Figures 3.4(s) and 3.5(s)].

The SS were quantified only in the channels and during the rainy period (November to March 2018), in concentrations of 1.00 mg/L.

Metals were detected only in the channel samples (November–March rainy season) and were not quantified at any site at sea. Al was quantified in high concentrations in three channels: Tombo (between 0.21 and 0.26 mg/L), Perequê (0.23 mg/L), and Enseada (between 0.10 to 0.21 mg/L). In Iporanga the lowest concentrations were detected (0.05 mg/L Al). Cd was detected at Enseada, only in February (< 0.001 mg/L). Pb was detected at Iporanga (January) and Enseada (February to March), at concentrations < 0.01 mg/L. Cu was detected January–March at Tombo (concentrations between 0.02 and 0.05 mg/L) and also at Iporanga (January–February, < 0.05 mg/L) was detected January–March at Tombo, Enseada, and Perequê. Cr was detected only at Iporanga in February (0.014 mg/L). Ni was detected only at Tombo (January to March), with high concentrations (0.07 mg/L) recorded in January. Zn (< 0.05 mg/L) was detected January–March at Tombo, Enseada, and Perequê. For more details on the physicochemical and microbiological data set (raw data), see the supplementary material (channel data and seawater data).


a
Channel
Air temperature (°C)
dry
rainy
dry
Tombo (1A)
Enseada (2A)
Perequê (3A)
Iporanga (4A)
10
15
20
25
30
35
40
Oct.
Nov.
Dec.
Jan.
Feb.
Mar.
Apr.
May
June
July
Aug.
b
Channel
Water temperature (°C)
dry
rainy
dry
Tombo (1A)
Enseada (2A)
Perequê (3A)
Iporanga (4A)
20
25
30
35
Oct.
Nov.
Dec.
Jan.
Feb.
Mar.
Apr.
May
June
July
Aug.
c
Channel
Salinity (‰)
dry
rainy
dry
Tombo (1A)
Enseada (2A)
Perequê (3A)
Iporanga (4A)
Conama No. 357/2005
-0.2
0.0
0.2
0.4
0.6
Oct.
Nov.
Dec.
Jan.
Feb.
Mar.
Apr.
May
June
July
Aug.
d
Channel
Turbity (NTU)
dry
rainy
dry
Tombo (1A)
Enseada (2A)
Perequê (3A)
Iporanga (4A)
Conama No. 357/2005
-50
0
50
100
150
200
Oct.
Nov.
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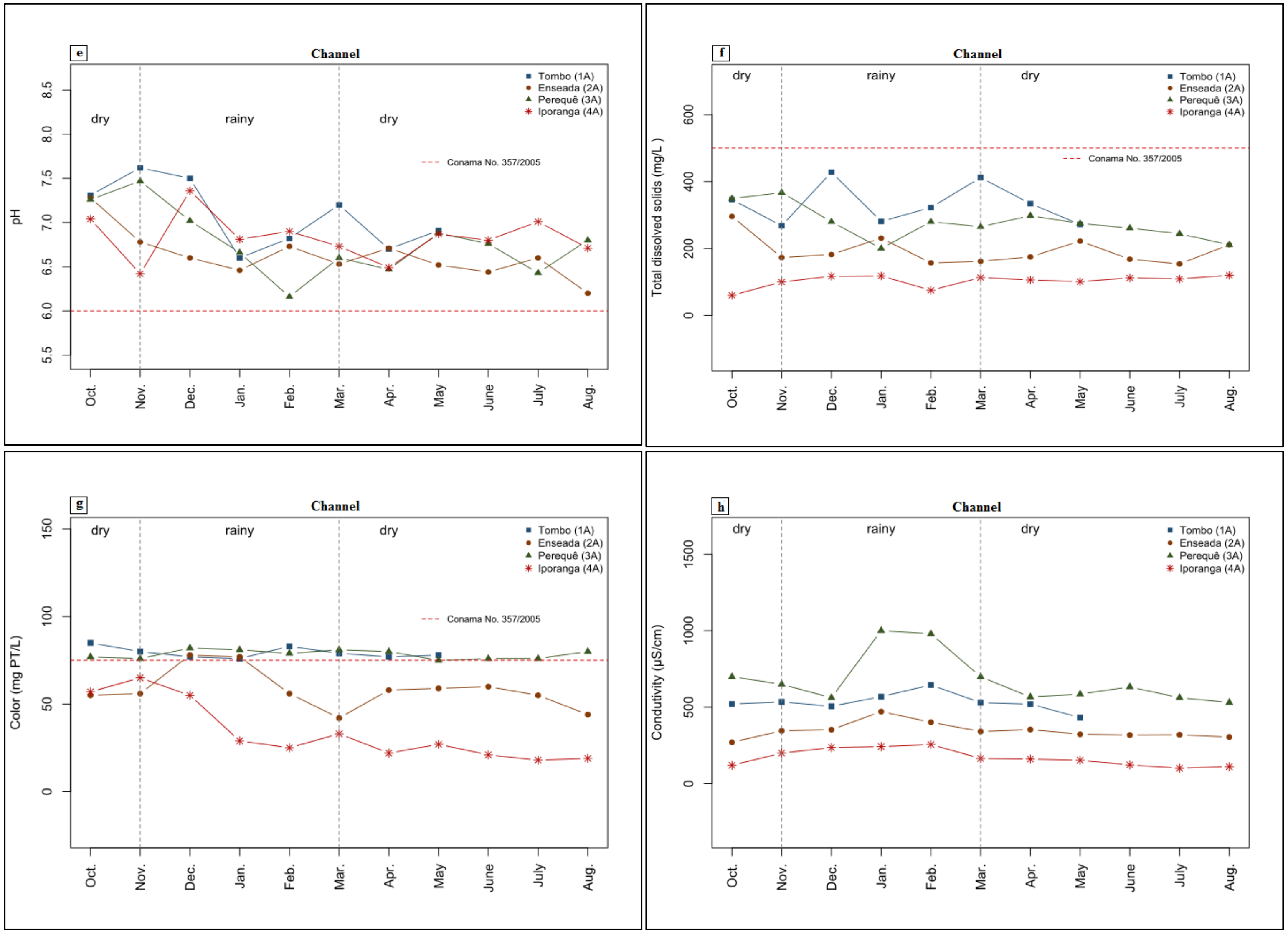
May
June
July
Aug.


e
Channel
pH
5.5
6.0
6.5
7.0
7.5
8.0
8.5
dry
rainy
dry
Conama No. 357/2005
f
Channel
Total dissolved solids (mg/L)
0
200
400
600
g
Channel
Color (mg PT/L)
0
50
100
150
h
Channel
Condutivity (µS/cm)
0
500
1000
1500
Tombo (1A)
Enseada (2A)
Perequê (3A)
Iporanga (4A)
Oct.
Nov.
Dec.
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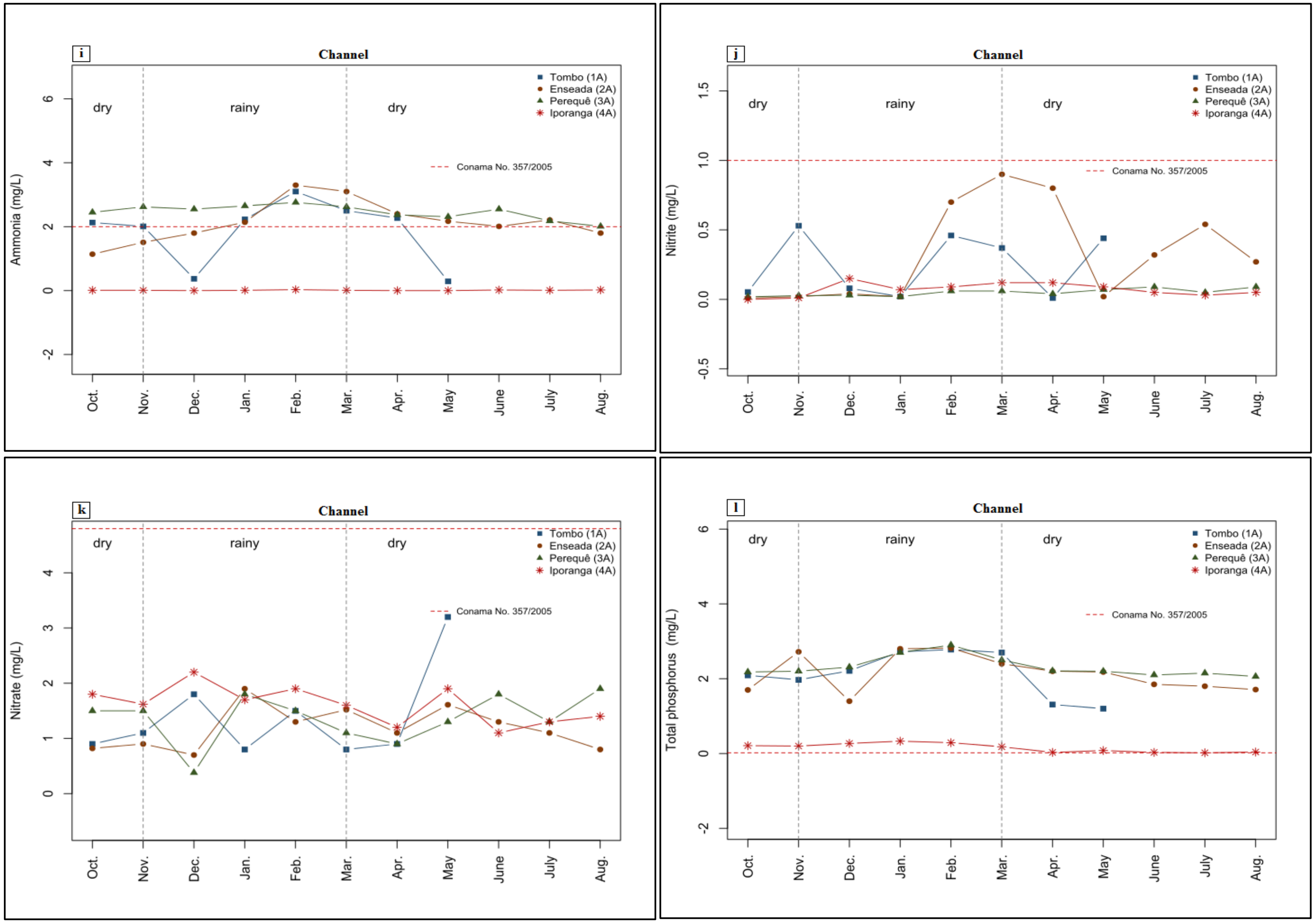
i
Channel
Ammonia (mg/L)
dry
rainy
dry
Conama No. 357/2005
j
Channel
Nitrite (mg/L)
k
Channel
Nitrate (mg/L)
l
Channel
Total phosphorus (mg/L)
■ Tombo (1A)
● Enseada (2A)
▲ Perequê (3A)
✱ Iporanga (4A)
Oct.
Nov.
Dec.
Jan.
Feb.
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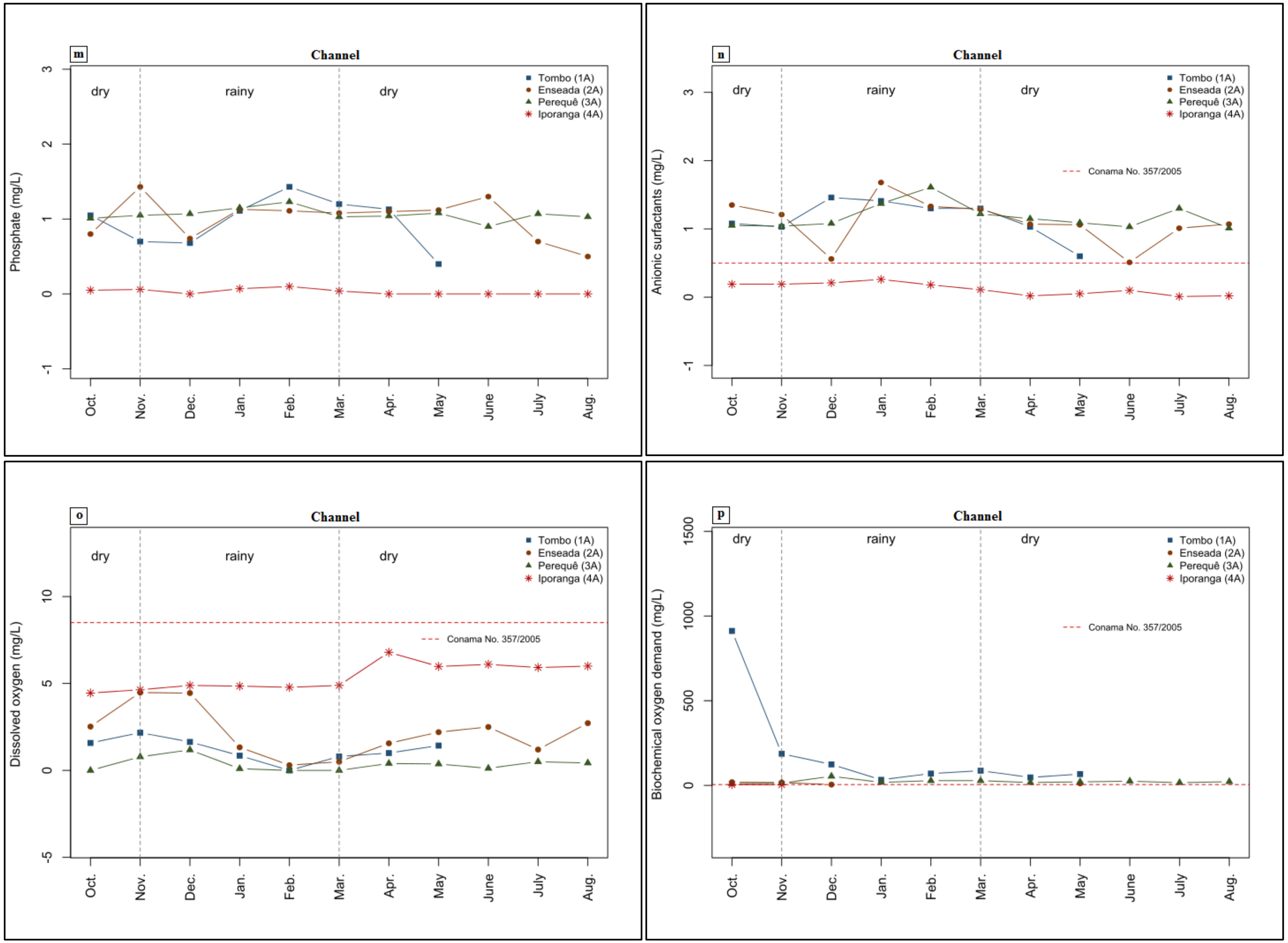
Aug.


m
Channel
Phosphate (mg/L)
dry
rainy
dry
Tombo (1A)
Enseada (2A)
Perequê (3A)
Iporanga (4A)
-1
0
1
2
3
Oct.
Nov.
Dec.
Jan.
Feb.
Mar.
Apr.
May
June
July
Aug.
n
Channel
Anionic surfactants (mg/L)
dry
rainy
dry
Tombo (1A)
Enseada (2A)
Perequê (3A)
Iporanga (4A)
Conama No. 357/2005
-1
0
1
2
3
Oct.
Nov.
Dec.
Jan.
Feb.
Mar.
Apr.
May
June
July
Aug.
o
Channel
Dissolved oxygen (mg/L)
dry
rainy
dry
Tombo (1A)
Enseada (2A)
Perequê (3A)
Iporanga (4A)
Conama No. 357/2005
-5
0
5
10
Oct.
Nov.
Dec.
Jan.
Feb.
Mar.
Apr.
May
June
July
Aug.
p
Channel
Biochemical oxygen demand (mg/L)
dry
rainy
dry
Tombo (1A)
Enseada (2A)
Perequê (3A)
Iporanga (4A)
Conama No. 357/2005
0
500
1000
1500
Oct.
Nov.
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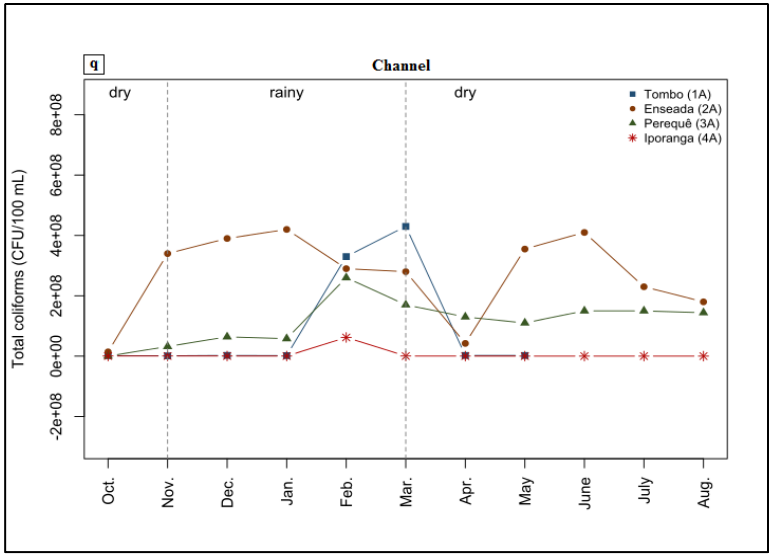
May
June
July
Aug.


q
Channel
dry
rainy
dry
Tombo (1A)
Enseada (2A)
Perequê (3A)
Iporanga (4A)
Total coliforms (CFU/100 mL)
-2e+08
0e+00
2e+08
4e+08
6e+08
8e+08
Oct.
Nov.
Dec.
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May
June
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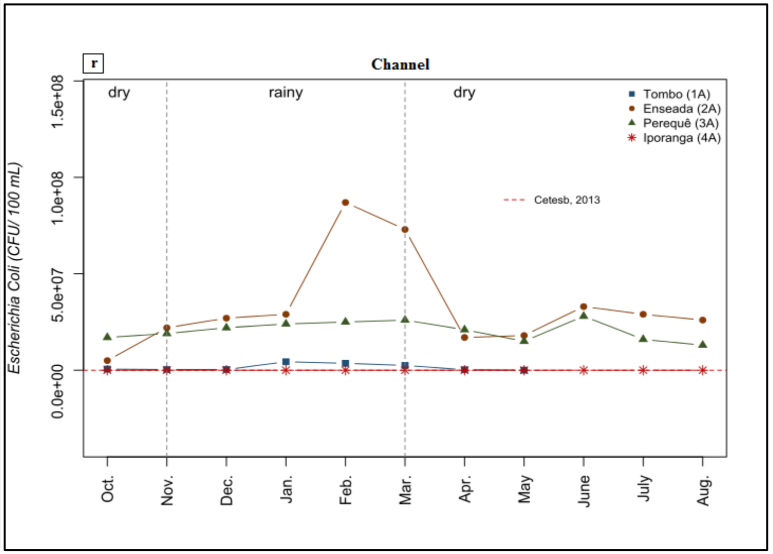

r
Channel
dry
rainy
dry
Tombo (1A)
Enseada (2A)
Perequê (3A)
Iporanga (4A)
Cetesb, 2013
Escherichia Coli (CFU/ 100 mL)
0.0e+00
5.0e+07
1.0e+08
1.5e+08
Oct.
Nov.
Dec.
Jan.
Feb.
Mar.
Apr.
May
June
July
Aug.

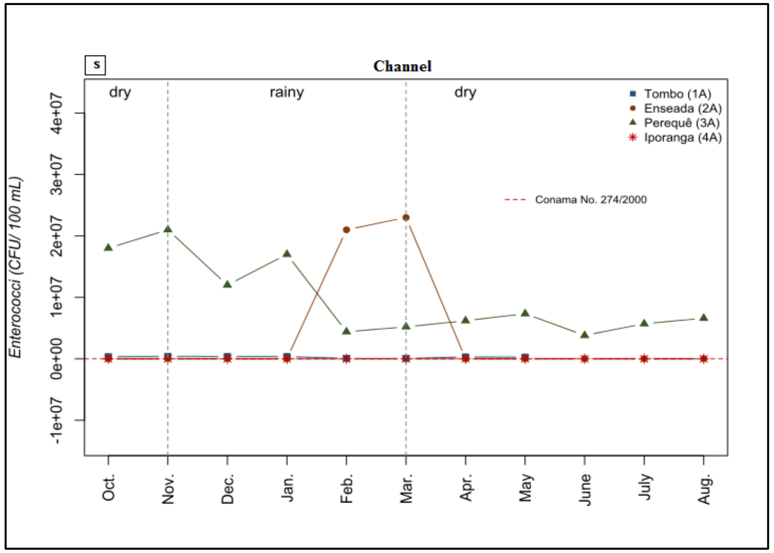
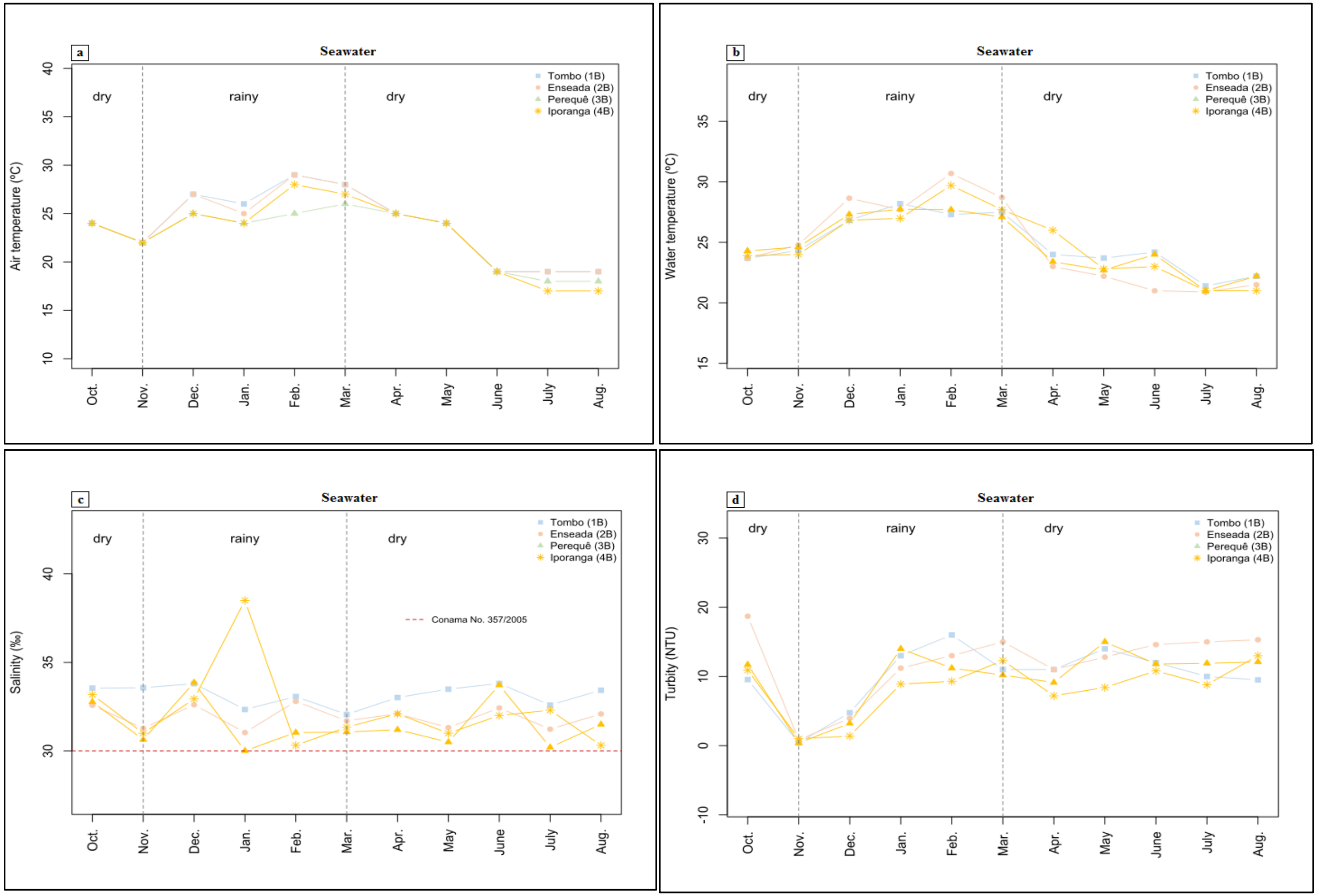
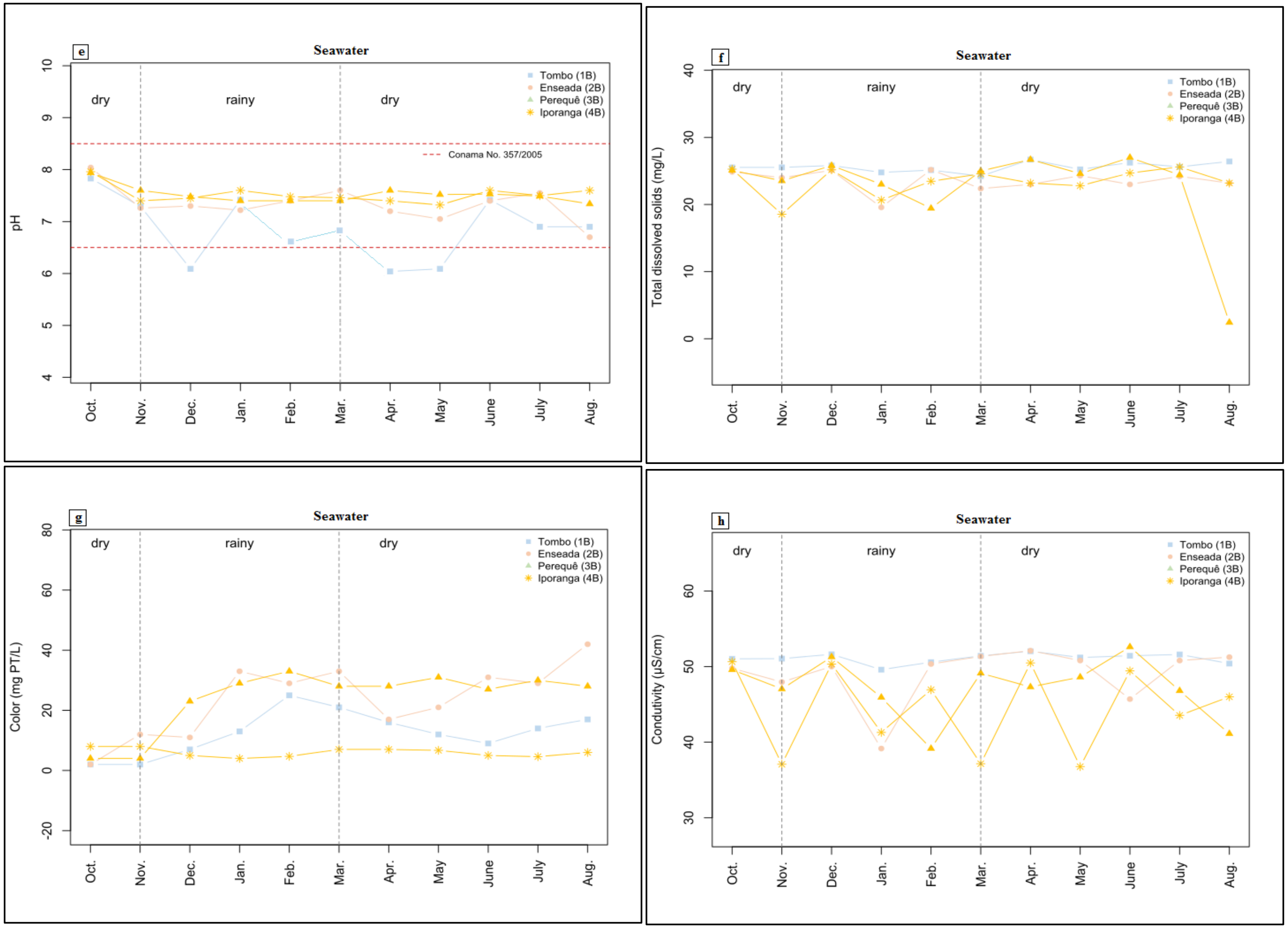
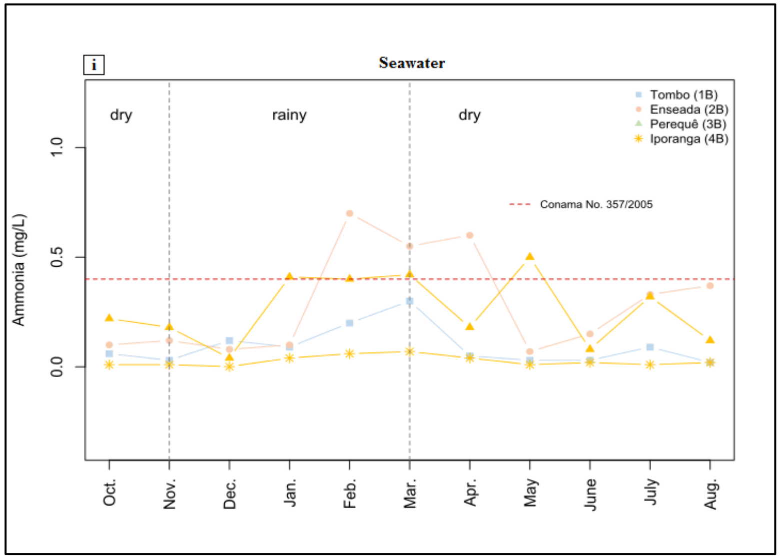
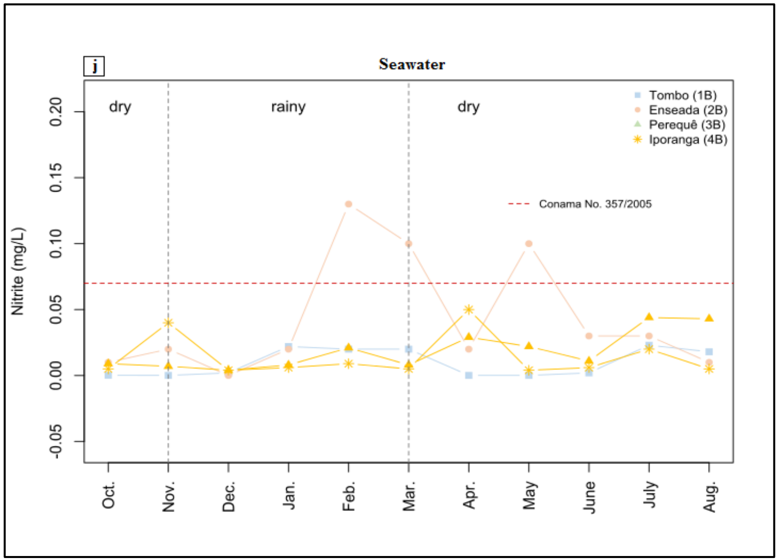

s
Channel
dry
rainy
dry
Tombo (1A)
Enseada (2A)
Perequê (3A)
Iporanga (4A)
Conama No. 274/2000
Enterococci (CFU/ 100 mL)
-1e+07
0e+00
1e+07
2e+07
3e+07
4e+07
Oct.
Nov.
Dec.
Jan.
Feb.
Mar.
Apr.
May
June
July
Aug.

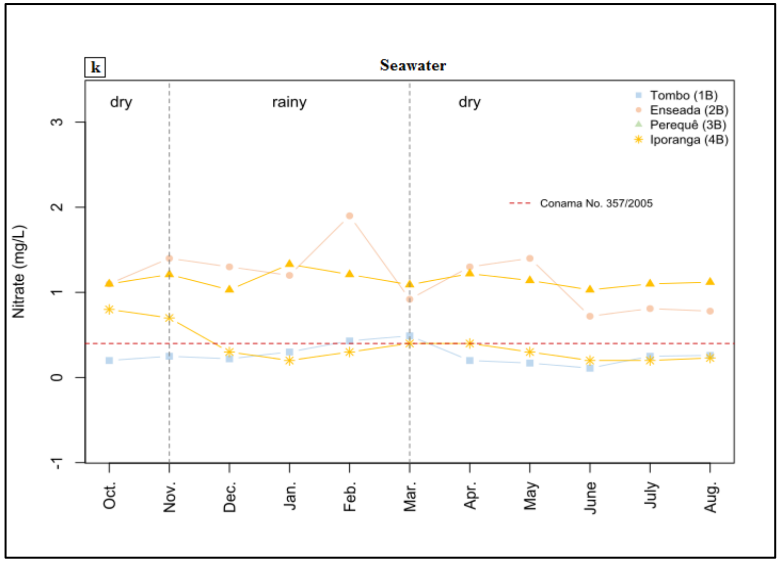
Figure – 3.4: Graphs of temporal analysis applied to physical, chemical, and microbiological variables of water samples collected in the surface urban runoff in a beach area of Guarujá city, São Paulo State, Brazil. Identification of water sampling stations on four beaches in the Guarujá: i) Tombo beach (point 1A, urban drainage channel); ii) Enseada beach (point 2A, urban drainage channel); iii) Perequê beach (point 3A, urban drainage channel); iv) Iporanga beach (point 4A, urban drainage channel). The graphs show the results of the environmental analyses over an 11-month period (October 2017–August 2018), allowing observation of different environmental conditions and seasonal variations (rainy season, November–March, vs dry season, April–October).The results ware compared with the Brazilian normative standard (dashed line in red): a) Conama Resolution nº 357/2005: physico-chemical analysis; b) Conama Resolution nº 274/2000: *Enterococci* and *E. coli* in seawater; c) Board Decision nº 112/2013/E of 2013 of the Environmental Agency of São Paulo (Cetesb, 2013) (*Escherichia Coli* in urban drainage channels).

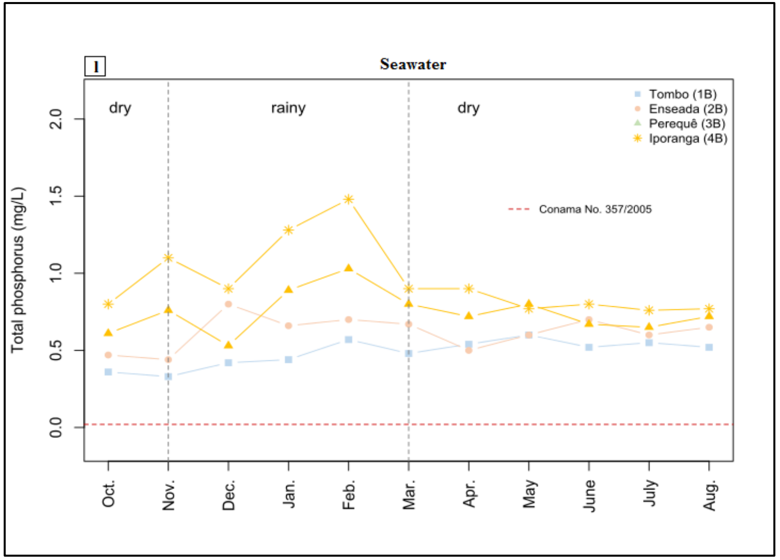

a
Seawater
Air temperature (°C)
10
15
20
25
30
35
40
dry
rainy
dry
Tombo (1B)
Enseada (2B)
Perequê (3B)
Iporanga (4B)
Oct.
Nov.
Dec.
Jan.
Feb.
Mar.
Apr.
May
June
July
Aug.
b
Seawater
Water temperature (°C)
15
20
25
30
35
c
Seawater
Salinity (‰)
30
35
40
Conama No. 357/2005
d
Seawater
Turbity (NTU)
-10
0
10
20
30

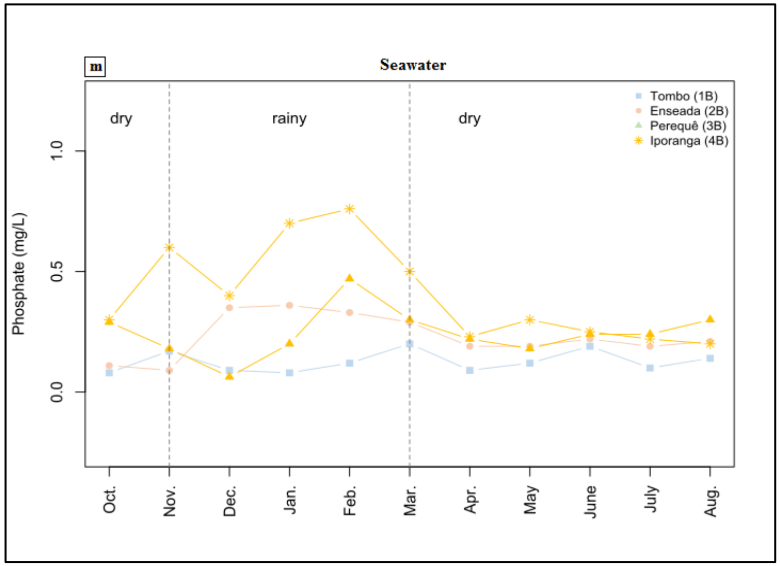

e
Seawater
pH
4
5
6
7
8
9
10
dry
rainy
dry
Conama No. 357/2005
Tombo (1B)
Enseada (2B)
Perequê (3B)
Iporanga (4B)
Oct.
Nov.
Dec.
Jan.
Feb.
Mar.
Apr.
May
June
July
Aug.
f
Seawater
Total dissolved solids (mg/L)
0
10
20
30
40
g
Seawater
Color (mg PT/L)
-20
0
20
40
60
80
h
Seawater
Condutivity (µS/cm)
30
40
50
60

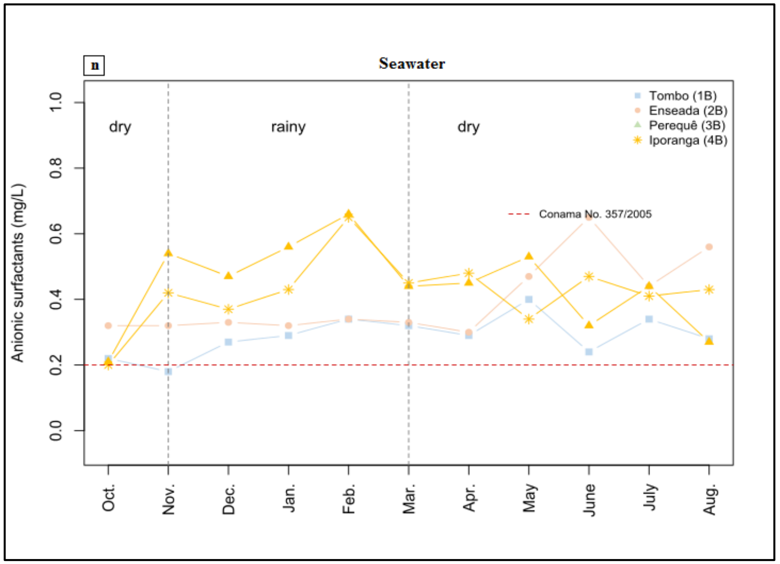

i
Seawater
dry
rainy
dry
Tombo (1B)
Enseada (2B)
Perequê (3B)
Iporanga (4B)
Conama No. 357/2005
Ammonia (mg/L)
0.0
0.5
1.0
Oct.
Nov.
Dec.
Jan.
Feb.
Mar.
Apr.
May
June
July
Aug.

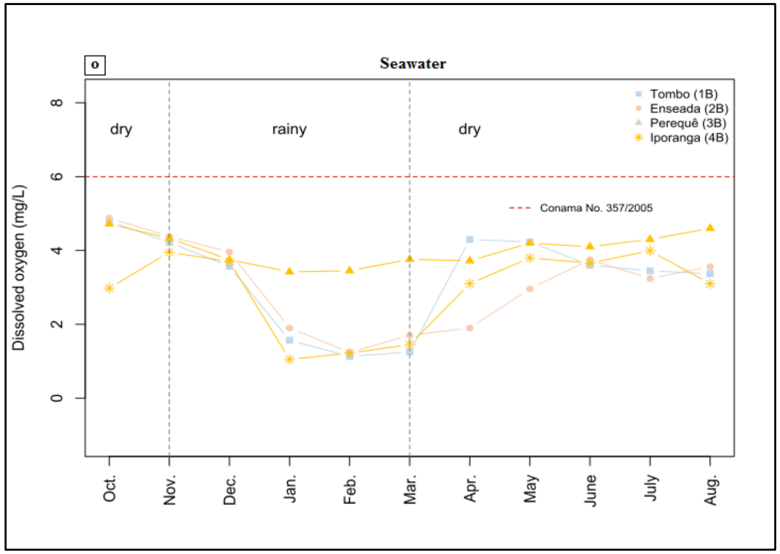

j
Seawater
dry
rainy
dry
Tombo (1B)
Enseada (2B)
Perequê (3B)
Iporanga (4B)
Conama No. 357/2005
Nitrite (mg/L)
-0.05
0.00
0.05
0.10
0.15
0.20
Oct.
Nov.
Dec.
Jan.
Feb.
Mar.
Apr.
May
June
July
Aug.

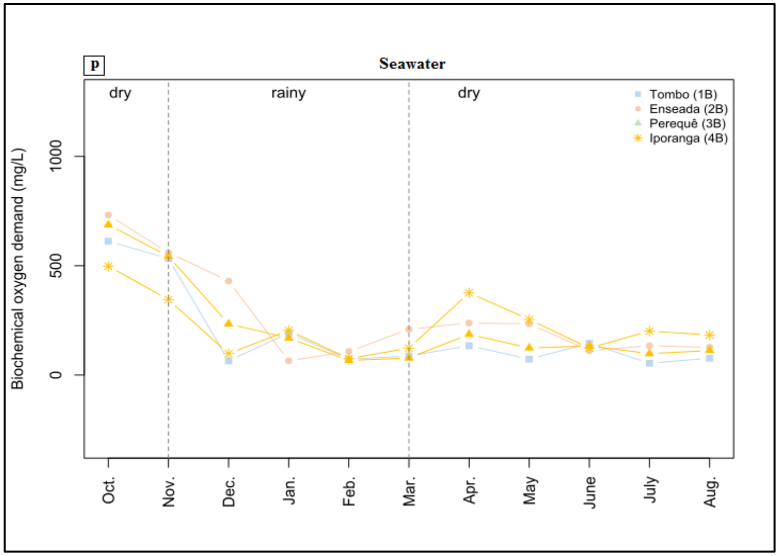

k
Seawater
dry
rainy
dry
Tombo (1B)
Enseada (2B)
Perequê (3B)
Iporanga (4B)
Conama No. 357/2005
Nitrate (mg/L)
-1
0
1
2
3
Oct.
Nov.
Dec.
Jan.
Feb.
Mar.
Apr.
May
June
July
Aug.

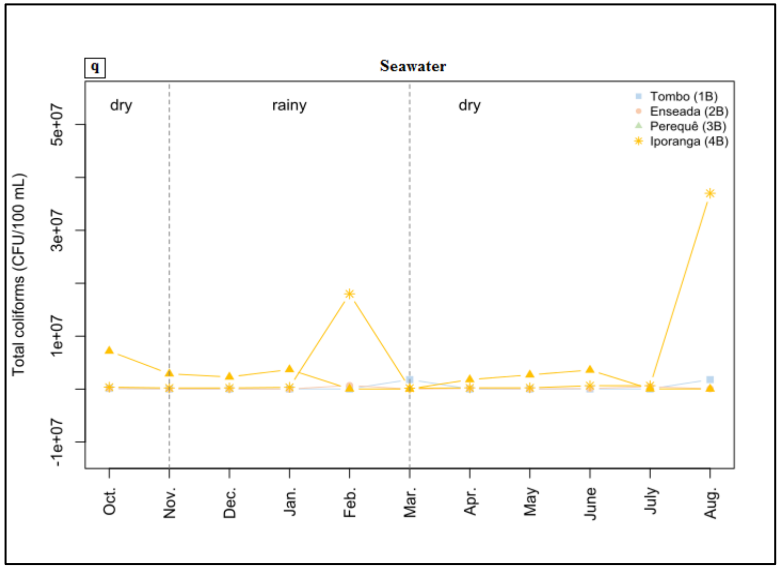

l
Seawater
dry
rainy
dry
Tombo (1B)
Enseada (2B)
Perequê (3B)
Iporanga (4B)
Conama No. 357/2005
Total phosphorus (mg/L)
0.0
0.5
1.0
1.5
2.0
Oct.
Nov.
Dec.
Jan.
Feb.
Mar.
Apr.
May
June
July
Aug.

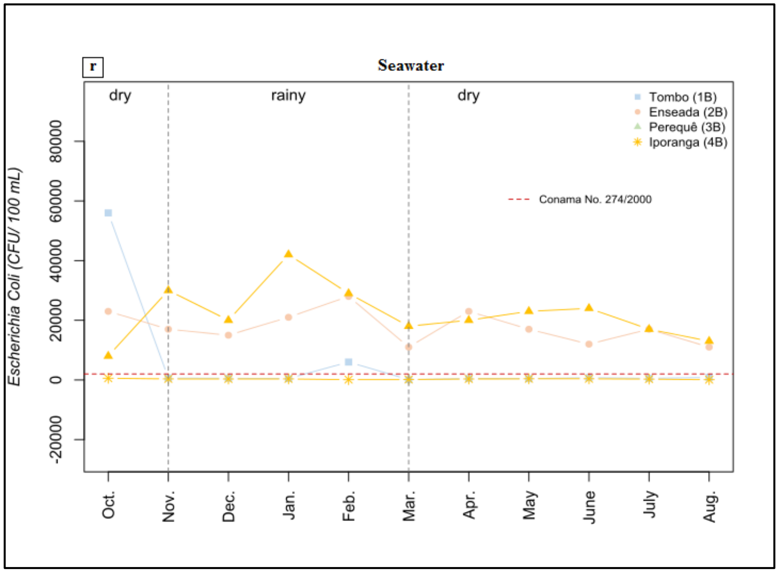

m
Seawater
dry
rainy
dry
Tombo (1B)
Enseada (2B)
Perequê (3B)
Iporanga (4B)
Phosphate (mg/L)
0.0
0.5
1.0
Oct.
Nov.
Dec.
Jan.
Feb.
Mar.
Apr.
May
June
July
Aug.


n
Seawater
dry
rainy
dry
Tombo (1B)
Enseada (2B)
Perequê (3B)
Iporanga (4B)
Conama No. 357/2005
Anionic surfactants (mg/L)
0.0
0.2
0.4
0.6
0.8
1.0
Oct.
Nov.
Dec.
Jan.
Feb.
Mar.
Apr.
May
June
July
Aug.


o
Seawater
dry
rainy
dry
Tombo (1B)
Enseada (2B)
Perequê (3B)
Iporanga (4B)
Conama No. 357/2005
Dissolved oxygen (mg/L)
0
2
4
6
8
Oct.
Nov.
Dec.
Jan.
Feb.
Mar.
Apr.
May
June
July
Aug.


p
Seawater
dry
rainy
dry
Tombo (1B)
Enseada (2B)
Perequê (3B)
Iporanga (4B)
Biochemical oxygen demand (mg/L)
0
500
1000
Oct.
Nov.
Dec.
Jan.
Feb.
Mar.
Apr.
May
June
July
Aug.


q
Seawater
dry
rainy
dry
Tombo (1B)
Enseada (2B)
Perequê (3B)
Iporanga (4B)
Total coliforms (CFU/100 mL)
5e+07
3e+07
1e+07
-1e+07
Oct.
Nov.
Dec.
Jan.
Feb.
Mar.
Apr.
May
June
July
Aug.


r
Seawater
dry
rainy
dry
Tombo (1B)
Enseada (2B)
Perequê (3B)
Iporanga (4B)
Conama No. 274/2000
Escherichia Coli (CFU/ 100 mL)
80000
60000
40000
20000
0
-20000
Oct.
Nov.
Dec.
Jan.
Feb.
Mar.
Apr.
May
June
July
Aug.

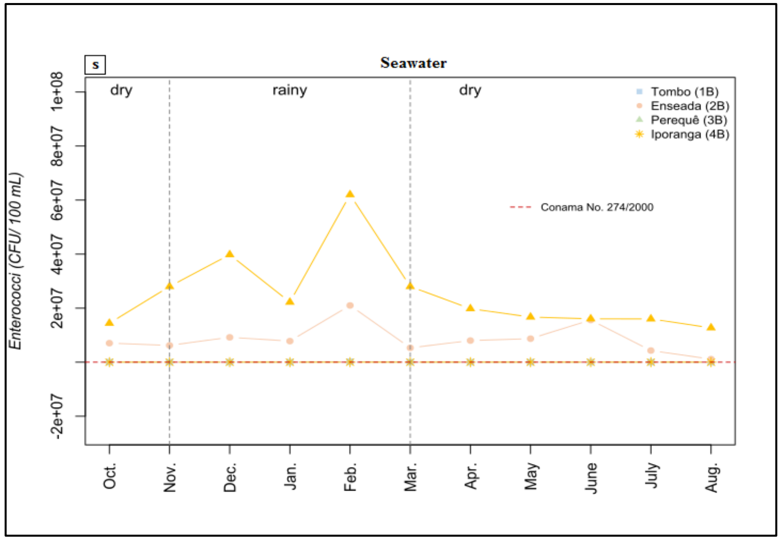

s
Seawater
dry
rainy
dry
Tombo (1B)
Enseada (2B)
Perequê (3B)
Iporanga (4B)
Conama No. 274/2000
Enterococci (CFU/ 100 mL)
1e+08
8e+07
6e+07
4e+07
2e+07
-2e+07
Oct.
Nov.
Dec.
Jan.
Feb.
Mar.
Apr.
May
June
July
Aug.

Figure – 3.5: Graphs of temporal analysis applied to physical, chemical, and microbiological variables of water samples collected in the urban surface runoff in a beach area of Guarujá city, São Paulo State, Brazil. Identification of water sampling stations on four beaches in the Guarujá: i) Tombo beach (point 1B, seawater); ii) Enseada beach (point 2B, seawater); iii) Perequê beach (point 3B, seawater); iv) Iporanga beach (point 4B, seawater). The graphs show the results of the environmental analyses over an 11-month period (October 2017–August 2018) allowing observations of different environmental conditions and seasonal variations (rainy season, November–March vs dry season, April–October). The results ware compared with the Brazilian normative standard (dashed line in red): a) Conama Resolution nº 357/2005: physico-chemical analysis; b) Conama Resolution nº 274/2000: *Enterococci* and *Escherichia coli* in seawater.

3.3.3 Water quality indexes

The CCMEWQI for the drainage were as follows: Iporanga (CCMEWQI = 85) > Enseada (CCMEWQI = 34) > Perequê (CCMEWQI = 33) > Tombo (CCMEWQI = 32) (Table 3.2). For the seawater points, the results obtained for the CCMEWQI were: Iporanga (CCMEWQI = 78) > Tombo (CCMEWQI = 36) > Enseada and Perequê (both locations with CCMEWQI = 34) (Table 3.2).

The classification obtained for the TSI for the water of the Iporanga channel was average (47). A similar average TSI classification (60) was obtained for the other channels (Tombo, Enseada, and Perequê) (Table 3.2).

After applying the TCI, the data showed that Al was responsible for a TCI of 20%–100% in Iporanga and > 100% in the other channels (Tombo, Enseada, and Perequê) (Table 3.2).

Table –3.2: Results of the three water quality indices applied to the physical-chemical and microbiological analyses of water samples collected in urban drainage channels that flow to beaches in the city of Guarujá, São Paulo, Brazil. The table presents the four beaches (Tombo, Enseada, Perequê, and Iporanga), and their respective sampling location (urban drainage channel or seawater). The study was conducted October 2017–August 2018. The CCMEWQI[1]: Canadian Council of Ministers of the Environment Water Quality Index was used to evaluate whether the physico-chemical and microbiological (channels and seawater) results are within the Brazilian standard: a) Conama Resolution nº 357/2005: (physico-chemical analysis); b) Conama Resolution nº 274/2000: (*Enterococci* and *Escherichia coli* in seawater); c) Board Decision nº 112/2013/E of 2013 of the Environmental Agency of São Paulo (*Escherichia Coli* in urban drainage channels). The trophic degree was verified through the use of the TSI (Trophic State Index)[2], from the values of total phosphorus (TP) (µg/L). The TCI (Toxic Contamination Index)[3] classifies the monitoring points according to the concentration of the toxic heavy metals—aluminium (Al), cadmium (Cd), lead (Pb), cooper (Cu), chrome (Cr), nickel (Ni), and zinc (Zn)—and is based on the limits recommended by Conama nº 357/2005. Note: (*): TSI method developed for fresh water (not applied to seawater). (**): heavy metals not detected in seawater (TCI not calculated).

Sampling sites		**CCMEWQI[1]** (Canadian Council of Ministers of the Environment Water Quality Index)	**TSI[2]** (Trophic State Index)	**TCI[3]** (Toxic Contamination Index)
Channel	Tombo (1A)	very poor (score: 32)	eutrophic characteristics (score: 60)	high (≥100%) – indicator: Al
	Enseada (2A)	very poor (score: 34)	eutrophic characteristics (score: 60)	high (≥100%) – indicator: Al
	Perequê (3A)	very poor (score: 33)	eutrophic characteristics (score: 60)	high (≥100%) – indicator: Al
	Iporanga (4A)	good (score: 85)	oligotrophic characteristics (score: 47)	Average (20% ≤ TCI ≤ 100%) – indicator: Al
Seawater	Tombo (1B)	very poor (score: 36)	*	**
	Enseada (2B)	very poor (score: 34)	*	**
	Perequê (3B)	very poor (score: 34)	*	**
	Iporanga (4B)	regular (score: 78)	*	**

3.4. Discussion

Urban runoff water flowing into tourist beaches poses a significant threat to environmental and public health (Rocha et al., 2011). This runoff is responsible for introducing chemical and biological pollutants directly into the sea, in areas of intense recreation (Zhang et al., 2013; Tilburg et al., 2015; Federigi et al., 2016). This condition was observed in the urban drainage channels of Guarujá (Tombo, Enseada, Perequê, and Iporanga), where the variables of turbidity, Ec, TDS, colour, TP, MBAS, PO_4^3-, NH_3, NO_2- , NO_3- , TC, and *E. coli* showed significantly higher concentrations in channel waters when compared to seawater concentrations. This is probably the result of higher dilution and dispersion of pollutants in seawater (Cetesb, 2017). These results support the findings of other researchers, who have identified urban surface water runoff as the primary mechanism for transporting pollutants from land to estuaries and receiving seas (e.g. Guarujá) (Galloway et al., 2003; Yang and Toor, 2017; Zotou et al., 2018).

3.4.1 Compliance with Brazilian water legislation

The different characteristics regarding the land use and occupation are also factors that interfere with the quality of urban runoff water (Yu et al., 2011; Wang et al., 2013; Saraswat et al., 2016). The Guarujá beaches have different characteristics - Tombo (a Blue Flag beach), Enseada (a high tourist visitation beach), Perequê (a fishing community), and Iporanga (considered a conservation unit) (Ribeiro and Oliveira, 2015; Fee, 2018) - and therefore different levels of pollution were observed in the urban drainage channels. The CCMEWQI values showed that the Iporanga beach presented the best environmental classification (channel: “good”; seawater: “regular”) (CCME, 2001). According to CCME (2001), a good quality index indicates that there is some level of deterioration observed; however, the observed conditions rarely differ from natural conditions or desirable normative levels. Of the 231 environmental analyses performed in Iporanga, more than 90% complied with the current Brazilian legislation (Brazil, 2000; 2005; Cetesb, 2013). The beaches of Tombo, Enseada, and Perequê were rated “very bad” at all sampling points (channel and seawater), indicating that the quality of these waters is always almost deteriorated and the variables often deviate from desirable natural conditions or normative levels (CCME, 2001). Of the 231 environmental analyses performed, 34% (Enseada) and 39% (Perequê) did not comply with current legislation

(Brazil, 2000; 2005; Cetesb, 2013). The Tombo (channel with intermittent flow regime) could not be sampled in the June, July and August 2018 (Brazilian winter/dry season) because the drainage channel was fully dry. Therefore, in the Tombo channel, of the 168 environmental analyses performed, 43% did not comply with Brazilian legislation (Brazil 2000; 2005, Cetesb, 2013).

3.4.2 Urban surface runoff and potential impacts on marine ecosystems

Urban surface water runoff (e.g. Guarujá channels) is responsible for introducing different physical, chemical, and biological pollutants into the receiving bodies, as result from the numerous anthropogenic activities developed along the watershed (Choi et al., 2011; Saraswat et al., 2016; Xiang et al., 2017). According to the São Paulo Environmental Agency, the presence of Ec > 100.00 µS/cm in all channels can be the result of high concentrations of pollutants, since they have many dissolved ions (Cetesb, 2017). In seawater, the detected Ec is within an expected range for continental waters (relative to salinity > 30% and average temperature of 24.5°C obtained in this study) (Cetesb, 2017). One of the possible explanations for the high Ec values in the channels is the presence of nutrients (e.g. N and P). Historically, the discharge of these nutrients into coastal streams in Asia, Oceania, and North America has compromised the ecological integrity of estuaries and receiving seas (Abal et al., 2001; Lusk and Toor, 2016; Yang and Toor, 2017). In the case of dissolved inorganic nitrogen (NH_3, NO_2- and NO_3-), it has a higher ecological impact on water bodies because it is readily available for absorption by aquatic organisms (Badruzzaman et al., 2012; Lusk and Toor, 2016). Its bioavailability can cause hypoxia and anoxia in oceans, with direct impacts on the structure of aquatic communities (Lusk and Toor, 2016; Yang and Toor, 2017). In San Andres, Colombia, and in the island of Culebra, Puerto Rico, where sewage discharge is the main source of pollution in coastal waters, the anthropogenic pressure in the coastal system is high, as result of the nutrient’s water enrichment, posing at risk seagrass and coral ecosystems (Gavio et al., 2010; Hernandez-Delgado et al., 2017). Increased waterproofing and channelling of urban streams (e.g. Guarujá) can significantly reduce N retention functions in urban watersheds, especially during the rainy season (Brush, 2009). In Tombo, Enseada, and Perequê channels, where urbanization is highest, there was a predominance of NH_3 over NOx (e.g. February 2018, rainy season), suggesting recent pollution under anaerobic conditions and/or organic sewage pollution (Wollheim et al., 2005). This condition corroborates the studies by Yu et al. (2011) that have analysed the spatiotemporal characteristics of diffuse N pollution in the Lean River basin (China) and found that NH_3 concentrations were high during the rainy season, especially in highly urbanized areas. N loads in urban runoff are variable but generally higher than those found in runoff from preserved and/or less disturbed areas (e.g. Iporanga) (Groffman et al., 2004). In the Guarujá channels (except Iporanga), the average summed concentrations of dissolved inorganic nitrogen at Tombo (3.20 mg/L), Enseada (3.60 mg/L), and Perequê (3.80 mg/L) exceeded the average nitrogen concentrations (TN) detected in the studies of urban runoff in Alabama (US) (2.00 mg/L TN) (Pitt et al., 2005) and Shanghai (China) (1.30 mg/L TN) (Ballo et al., 2009). At the sea sampling points, NO_3- predominated over NH_3, suggesting possible pollution from ancient or distant sources (e.g. February 2018, Tombo, Enseada, and Perequê) (Zotou et al., 2018). In Guarujá there are a total of 43 urban drainage channels with similar characteristics to those being analysed in this study (Cetesb, 2017). In this way, it is possible that the other channels (more distant sources) are also contributing to the N input in the Guarujá seawaters.

As with dissolved inorganic nitrogen, TP concentrations were also higher in the Tombo, Enseada, and Perequê channels. The TSI calculation allowed us to conclude that the waters of these three channels presented a eutrophic characteristic (high TP enrichment) (Zotou et al., 2018). The blooming of toxic cyanobacteria, caused by the high enrichment of P, has already been responsible for the extinction of aquatic species in two Australian cities - Phillip Bay, Melbourne (Harris et al., 1996) and Moreton Bay, Queensland (Abal et al., 2001) - and at Chesapeake Bay (US) (Galloway et al., 2003). Because the waters of the Tombo, Enseada, and Perequê channels flow into tourist beaches, these high concentrations of detected TP may also affect recreational activities, as in more extreme cases, some toxins produced may contribute to neurological or respiratory problems in humans (Kirkpatrick et al., 2004). In the Iporanga channel, the TSI calculation indicated an oligotrophic characteristic (low TP enrichment) (Zotou et al., 2018). The low concentrations of TP in Iporanga are very similar to those found in two studies on urban runoff in Florida (US): 0.26 mg/L TP (Badruzzaman et al., 2012) and 0.43 mg/L TP (Yang and Toor, 2017). In Florida there are conditions similar to Iporanga (proper sewage treatment system, but P-enriched soil) (Khare et al., 2012; Ribeiro and Oliveira, 2015). Thus, in both locations, it is likely that the contributions of TP are natural and due to soil leaching during rain episodes (Miguntanna et al., 2013). In affected water bodies, TP occurs mainly in the form of PO_4^3, originated by MBAS (such as linear alkyl sulfonate, LAS), which is used worldwide in household cleaning and personal care products. Concentrations of these substances can represent up to 50% of the concentration of TP (León et al., 2006; Renzi et al., 2012). The presence of PO_4^3- and, mainly, MBAS in the channels and sea of Tombo, Enseada, and Perequê demonstrate the sanitation deficiency of the municipality, because surfactants are good indicators of sewage (León et al., 2006; Ghose et al., 2009; Renzi et al. 2012). The mean concentrations detected in the Tombo, Enseada, and Perequê channels are higher than those reported in other studies in coastal waters receiving urban runoff, such as in Kolkata (India) (0.08–0.42 mg/L LAS) (Ghose et al., 2009) and southern Tuscany (Italy) (0.07– 0.53 mg/L LAS) (Renzi et al., 2012). The contribution of MBAS in the Guarujá Sea warns, mainly, about the existing ecological risks, as studies have already shown its toxicity in crustaceans (Ole Kusk and Petersen, 1999) and fish (Nunes et al., 2016). Average MBAS concentrations detected in the Guarujá Sea are, for example, above safe limits (LC50: 50% lethal concentration) for marine organisms that are typical of the North Sea: sole (*Platichthys flesus*, LC50, 0.02 mg/L LAS) (Stalmans et al., 1991) and tiger shrimp (*Panaeus monodon*, LC50, 0.06 mg/L LAS) (Hwang et al., 1993).

The high BOD load (channels and seawater) showed typical mean concentrations of raw domestic sewage (100-300 mg/L) (Cromey et al., 1998). A possible interpretation is the occurrence of an active decomposition process taking place at the Tombo, Enseada and Perequê channels, suggesting a recent effluent disposal process (domestic sewage mix and diffuse charge), where low levels of DO, high BOD, presence of color (in addition to the aforementioned: Ec > 100 μS/cm and predominance of NH_3 in relation to NOx) can be observed (Xue et al., 2015; Zotou et al., 2018). This organic enrichment observed in the Tombo, Enseada, and Perequê Sea can cause impacts on benthic communities. Studies have already shown that organic enrichment was responsible for changes in species numbers and abundance of marine benthic organisms (Cromey et al., 1998; Elías et al., 2005).

3.4.3 Urban surface runoff and potential impacts on human health

The quality of beach waters, which often receive urban runoff, is also of concern due to the introduction of allochthonous pathogenic microorganisms (Lamparelli et al., 2015;

Tilburg et al., 2015; Federigi et al., 2016). Studies carried out in coastal environments in Mexico (Curiel-Ayala et al., 2012), China (Zhang et al., 2013), Cuba (Larrea-Murrel et al., 2013), Colombia (Botero et al., 2015), Brazil (Lamparelli et al., 2015), US (Tilburg et al., 2015), and Italy (Federigi et al., 2016) have shown that these pathogens, when carried to the sea via urban runoff (conditions aggravated during rain episodes and an increase of tourists), are responsible for the systematic loss of bathing water quality standards. In Latin America, namely in Mexico (Orozco-Borbón et al., 2006), Cuba (Larrea-Murrel et al., 2013), Puerto Rico (Hernandez-Delgado et al., 2017) and Colombia (Sánchez Moreno et al., 2019), the microbiological quality of sea water has raised public health concerns, with serious repercussions in the tourism and other economy sectors. *E. coli* was detected at high concentrations in the Tombo, Enseada, and Perequê channels, and this indicates poor sanitation, as this bacterium is an important marker of domestic sewage (present in human faeces in percentages between 96% and 99%) (Rozen and Belkin, 2001). At the sea sampling points (Tombo, Enseada, and Perequê), *E. coli* was detected, which suggested recent faecal pollution, as this bacterium has low resistance to marine environment conditions (e.g. solar radiation and high salinity) (Rozen and Belkin, 2001). The seawater concentrations of *E. coli* detected in the Enseada and Perequê are higher, for example, than those found at eleven beach water points along the coast of the Caribbean Sea of Colombia, where concentrations were found to be between 16 and 572 CFU/100 mL (Sánchez Moreno et al., 2019). *Enterococci* have also been detected at high concentrations in the channels and seawaters of Tombo, Enseada, and Perequê, and this reinforces the risks to public health, as these bacteria are related to gastrointestinal diseases (Zhang et al., 2013; Lamparelli et al., 2015; Tilburg et al., 2015). The seawater mean concentrations of *Enterococci* detected in the Tombo, Enseada, and Perequê were higher, for example, than those found in the Mexican beaches of Caletilla (157 CFU / 100mL), Hornos (153 CFU / 100mL) and Papagayo (153 CFU / 100mL) (Curiel-Ayala et al., 2012). In addition to public health risks, some studies have shown that the presence of *E. coli* and *Enterococci* in the marine environment can also cause contamination in edible mussels (*Mytilus edulis*) (Roslev et al., 2009) and oysters (*Crassostrea gigas*) (Olalemi et al., 2016), with deleterious impacts on fishing economic activity (Roslev et al., 2009; Olalemi et al., 2016).

Urban runoff is known to be a significant source of heavy metals to water bodies (Suresh et al., 2012; Gunawardena et al., 2013). The presence of Pb, Cr, Cd, Zn, Ni, Cu, and Al in the waters of Guarujá channels may be related to the improper disposal of detergents, cosmetics, fragrances, antacids, pesticides, paints, varnishes, and pharmaceuticals (Suresh et al., 2012), and also due to vehicle traffic (Gunawardena et al., 2013). Studies have shown that there is a strong correlation between SS and heavy metals (adsorption process) during urban runoff (Djukićet et al., 2016). Thus, this can explain why both metals (Pb, Cr, Cd, Zn, Ni, and Cu) and SS were quantified in Guarujá (only in the channels) during the same period (rainy period, between November and March 2018). After calculating the TCI, Al was responsible for the “medium” TCI in the Iporanga channel and by the “high” TCI in the Tombo, Enseada, and Perequê channels (Brazil, 2005; Igam, 2013). At high exposures, Al poses risks to public health, causing osteoporosis, rickets, and Alzheimer’s and Parkinson’s disease (Wang, 2013). The other metals found in Guarujá also present potential risks to public health. Human contact with Pb-contaminated water - considered a teratogenic agent- can cause headaches, seizures, and nauseas (Wang, 2013). High Cr concentration may cause contact dermatitis and skin ulcer (Wang, 2013). Exposures to Zn can cause fever, nausea, and vomiting, among other consequences (Wang, 2013). Cd and Ni (in high concentrations), which are considered carcinogens, can cause a drop in immunity and contact dermatitis, respectively (Wang, 2013). Although metals have not been detected in the Guarujá Sea, they deserve attention in coastal waters as they present environmental hazards due to their toxicity, persistence and bioaccumulation in marine species (Türkmen et al., 2006; Wan et al., 2008; Stofberg et al., 2011). For example, Al may bioaccumulate in marine species (Gonzalez et al., 1999; Türkmen et al., 2006). In regions strongly affected by domestic sewage discharge, Al concentrations have been detected in sea urchin (*Echinometra lucunter*) in Havana (Cuba) (Gonzalez et al., 1999) and in the blue crab (*Callinectes sapidus*) in Skenderun Bay (Turkey) (Türkmen et al., 2006). In the case of Cu, it is highly persistent in the environment and can be toxic even at low concentrations (Wan et al., 2008). Stofberg et al. (2011) reported a significant reduction in survival of *Haliotis midae* (marine gastropod mollusk) larvae after exposure to environmentally realistic Cu concentrations. Heavy metals contamination of marine edible seafood is a matter of public concern.

3.5 Conclusion

This was the first study on the spatial and temporal characterization of the urban runoff that flows into the beaches of Guarujá city, São Paulo State, Brazil. This work also confirms the interference of urban drainage channels, precipitation events, and environmental sanitation deficiency in the worsening of the quality of the beaches. It can be concluded, therefore, that in order to maintain the quality of these beaches and, above all, to prevent risks to the environment and public health, urgent actions are required. Among these actions, the following are highly recommended: installation of a floodgate system in the urban drainage channels of the municipality and connection of the urban surface drainage to the sewage collection network; land regularization of irregularly occupied areas (Perequê beach and Enseada hills), aiming the correction of environmental sanitation; supervision and control of the commercial and residential properties of the municipality, requesting its connection to the sewage collection network already established (Tombo, Enseada, and Perequê beaches); awareness, *in loco*, of beach users (especially during the summer tourist season), because during the field work it was possible to observe that people constantly come into contact with the waters of these channels, demonstrating total ignorance about the health risks of this behaviour; and establishment of a broader microbiological analysis design, as established in Conama Resolution nº 274 of 2000, which recommends that research on pathogenic organisms [e.g. viruses (Human mastadenovirus and other species of adenovirus) and protozoa (*Cryptosporidium ssp* and *Giardia ssp*)] should be carried out systematically in beaches (such as Perequê and Enseada beaches).

Acknowledgements

The authors thank the environmental engineers Camila Santos Garcia, Thiago Henrique Toscano, and the environmental manager Flávio Távora Campi for their support in the field work. The authors are also grateful to biologist Ronaldo Justo, environmental manager of the Iporanga/Guarujá condominium, for the authorization of the collection works. Vinicius Roveri also thanks Pulsar Pictures Ltda, Apiacás St., 934, São Paulo, Brazil, for the license to reproduce the images of the beaches of Guarujá (contract nº 27918). Correia A.T. I&D activities are supported by national funds through FCT - Foundation for Science and Technology within the scope of UIDB/04423/2020 and UIDP/04423/2020.

Disclosure statement

No potential conflict of interest was reported by the authors.

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Supplementary material (Chanel data)

Tombo (1A - channel)														
Variables and standards: CONAMA 357/2005 (classs 2) and Cetesb (2013)			Unit	October 22 (2017) 1 st campaign	November 20 (2017) 2 st campaign	December 10 (2017) 3 st campaign	January14 (2018) 4 st campaign	February 25 (2018) 5 st campaign	March 25 (2018) 6 st campaign	April 22 (2018) 7 st campaign	May 27 (2018) 8 st campaign	June 24 (2018) 9 st campaign	July 29 (2018) 10 st campaign	August 19 (2018) 11 st campaign
Physicochemical	Seasons			spring		summer				autumn			winter	
	tourist season			low tourist season		hight tourist season			low tourist season					
	climatic period			dry	rainy					dry				
	has it rained in the last 24 hours before the collections?			yes	yes	yes	yes	yes	yes	no	no	no	no	no
	air temperature	–	ºC	24.00	22.00	27.00	26.00	29.00	26.00	25.00	24.00	dry***	dry***	dry***
	water temperature	−	ºC	26.80	25.74	27.88	28.62	27.50	23.50	24.70	24.10	dry***	dry***	dry***
	Salinity	< 0.5 ‰	‰	0.14	0.16	0.12	0.17	0.20	0.19	0.38	0.29	dry***	dry***	dry***
	conductivity (Ec)	–	µS/cm	521.00	535.00	506.00	569.00	646.00	530.00	520.00	432.00	dry***	dry***	dry***
	total dissolved solids (TDS)	500	mg/L	346.00	268.00	428.00	281.00	322.00	412.00	334.00	272.00	dry***	dry***	dry***
	dissolved oxygen (DO)	≥ 5.0	mg/L	1.58*	2.17*	1.64*	0.85*	0*	0.80*	1.00*	1.43*	dry***	dry***	dry***
	pH	6-9	pH	7.31	7.62	7.50	6.60	6.82	7.20	6.70	6.91	dry***	dry***	dry***
	Turbity	Up to 100	NTU	49.00	97.00	98.00	95.00	95.00	80.00	65.00	67.00	dry***	dry***	dry***
	Color	75	mg Pt/L	85.00*	80.00*	77.00*	76.00*	83.00*	79.00*	77.00*	78.00*	dry***	dry***	dry***
	sedimented solids (SS)	−	ml/L	<0.10	0.10	0.10	0.10	0.10	0.10	<0.10	<0.10	dry***	dry***	dry***
	biochemical oxygen demand (BOD)	Up to 5	mg/L	912.00*	187.00*	42.00*	33.00*	70.00*	87.00*	47.00*	67.00*	dry***	dry***	dry***

	oils and greases (OG)	_	mg/L	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	dry***	dry***	dry***
	ammonia (NH$_3$)	2	mg/L	2.13*	2.01*	0.37	2.23*	3.10*	2.50*	2.27*	0.29	dry***	dry***	dry***
	nitrite (NO$_2^-$)	1	mg/L	0.05	0.53	0.08	0.02	0.46	0.37	0.01	0.44	dry***	dry***	dry***
	nitrate (NO$_3^-$)	10	mg/L	0.90	1.10	1.80	0.80	1.50	0.80	0.90	3.20	dry***	dry***	dry***
	phosphate (PO$_4^{3}$-)	–	mg/L	1.05	0.70	0.68	1.11	1.43	1.20	1.13	0.40	dry***	dry***	dry***
	anionic surfactants (MBAS)	0.5	mg/L	1.08*	1.03*	1.46*	1.41*	1.30*	1.30*	1.03*	0.60*	dry***	dry***	dry***
	total phosphorus (TP)	0.02	mg/L	2.09*	1.97*	2.21*	2.72*	2.78*	2.70*	1.31*	1.20*	dry***	dry***	dry***
heavy metals	aluminum (Al)	0.1	mg/L	0.24*	0	0	0.27*	0.25*	0.26*	0.21*	<LQ	dry***	dry***	dry***
	cadmium (Cd)	0.001	mg/L	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	dry***	dry***	dry***
	lead (Pb)	0.01	mg/L	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	dry***	dry***	dry***
	copper (Cu)	0.009	mg/L	0.005	0	0	0.019*	0.05*	0.05*	0.04*	<LQ	dry***	dry***	dry***
	chrome (Cr)	0.05	mg/L	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	dry***	dry***	dry***
	nickel (Ni)	0.025	mg/L	0	0	0	0.07*	0.01	0.02	<LQ	<LQ	dry***	dry***	dry***
	zinc (Zn)	0.18	mg/L	<LQ	<LQ	<LQ	<LQ	<0.05	<0.05	<0.05	<LQ	dry***	dry***	dry***
Microbiologic	total coliforms (TC)	–	CFU/mL	1800000	1100000	2500000	1800000	330000000	430000000	2300000	2200000	dry***	dry***	dry***
	Escherichia Coli (E.coli)	0.06 x 10^4	CFU/mL	580000 **	360000 **	370000 **	4400000 **	3600000 **	2500000 **	280000 **	25000 **	dry***	dry***	dry***
	Enterococci	0.04 x 10^4	CFU/mL	360000*	410000*	390000*	380000*	74000*	64000*	320000*	280000*	dry***	dry***	dry***

Legend: *Does not meet standards (Conama 357/2005)/ **Does not meet standards Cetesb (2013). <LQ: Limit of Quantification.
*** Intermittent flow regime, could not be sampled in the June, July and August 2018 campaigns, because your course was dry.

Enseada (2A - channel)

Variables and standards: CONAMA 357/2005 (classs 2) and Cetesb (2013)			Unit	October 22 (2017) 1 st campaign	November 20 (2017) 2 st campaign	December 10 (2017) 3 st campaign	January14 (2018) 4 st campaign	February 25 (2018) 5 st campaign	March 25 (2018) 6 st campaign	April 22 (2018) 7 st campaign	May 27 (2018) 8 st campaign	June 24 (2018) 9 st campaign	July 29 (2018) 10 st campaign	August 19 (2018) 11 st campaign
Physicochemical	Seasons			spring		summer				autumn			winter	
	tourist season			low tourist season		hight tourist season			low tourist season					
	climatic period			dry	rainy					dry				
	has it rained in the last 24 hours before the collections?			yes	yes	yes	yes	yes	yes	no	no	no	no	no
	air temperature	–	ºC	24.00	22.00	27.00	25.00	29.00	28.00	25.00	24.00	19.00	19.00	19.00
	water temperature	–	ºC	25.05	24.45	29.36	26.07	26.60	24.50	24.70	24.10	23.00	20.90	22.00
	Salinity	< 0.5 ‰	‰	0.19	0.16	0	0.12	0.14	0.11	0.22	0.19	0.21	0.22	0.21
	conductivity (Ec)	–	µS/cm	270.00	346.00	353.00	471.00	402.00	341.00	354.00	323.00	318.00	320.00	305.00
	total dissolved solids (TDS)	500	mg/L	296.00	173.00	182.00	231.00	157.00	162.00	175.00	222.00	168.00	154.00	211.00
	dissolved oxygen (DO)	≥ 5.0	mg/L	2.52*	4.48*	4.45*	1.33*	0.30*	0.50*	1.56*	2.20*	2.50*	1.20*	2.72*
	pH	6-9	pH	7.28	6.78	6.60	6.46	6.73	6.53	6.71	6.52	6.44	6.60	6.20
	Turbity	Up to 100	NTU	8.86	3.59	32.00	85.00	85.00	92.00	9.32	4.45	3.90	4.30	3.76
	Color	75	mg Pt/L	55.00	56.00	78.00	77.00	56.00	42.00	58.00	59.00	60.00	55.00	44.00
	sedimented solids (SS)	–	ml/L	<0.10	<0.10	0.10	0.10	0.10	0.10	<0.10	<0.10	<0.10	<0.10	<0.10
	biochemical oxygen demand (BOD)	Up to 5	mg/L	19.00*	17.00*	5.00*	5.40*	8.60*	9.90*	11.20*	12.00*	9.20*	7.50*	8.20*
	oils and greases (OG)	_	mg/L	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ

	ammonia (NH_3)	2	mg/L	1.14	1.51	1.80	2.14*	3.30*	3.10*	2.40*	2.17	2.01	2.21	1.80
	nitrite (NO_2^-)	1	mg/L	0.01	0.02	0.04	0.02	0.70	0.90	0.80	0.02	0.32	0.54	0.27
	nitrate (NO_3^-)	10	mg/L	0.82	0.90	0.70	1.90	1.30	1.52	1.10	1.61	1.30	1.10	0.80
	phosphate (PO_4^{3}-)	–	mg/L	0.80	1.43	0.74	1.13	1.11	1.08	1.10	1.12	1.30	0.70	0.50
	anionic surfactants (MBAS)	0.5	mg/L	1.35*	1.21*	0.56*	1.68*	1.33*	1.29*	1.07*	1.06*	0.51*	1.01*	1.07*
	total phosphorus (TP)	0.02	mg/L	1.70*	2.72*	1.40*	2.80*	2.82*	2.40*	2.20*	2.18*	1.85*	1.80*	1.71*
heavy metals	aluminum (Al)	0.1	mg/L	0.20*	0	0	0.06	0.10*	0.10*	0.20*	0.10*	0.20*	0	0
	cadmium (Cd)	0.001	mg/L	<LQ	<LQ	<LQ	<0.001	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ
	lead (Pb)	0.01	mg/L	<LQ	<LQ	<LQ	<LQ	<0.01	<0.01	<0.01	<LQ	<LQ	<LQ	<LQ
	copper (Cu)	0.009	mg/L	0.007	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ
	chrome (Cr)	0.05	mg/L	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ
	nickel (Ni)	0.025	mg/L	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ
	zinc (Zn)	0.18	mg/L	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ
Microbiologic	total coliforms (TC)	–	CFU/100mL	14200000	340000000	390000000	420000000	290000000	280000000	42300000	355000000	410000000	230000000	180000000
	Escherichia Coli (E.coli)	0.06 x 10^4	CFU/100mL	5000000**	22000000**	27000000**	29000000**	87000000**	73000000**	17000000**	18000000**	33000000**	29000000**	26000000**
	Enterococci	0.04 x 10^4	CFU/100mL	11000*	31000*	29000*	16000*	21000000*	23000000*	12000*	32000*	32000*	18000*	34000*

Legend: *Does not meet standards (Conama 357/2005)/ **Does not meet standards Cetesb (2013). <LQ: Limit of Quantification.

Perequê (3A - channel)

Variables and standards: CONAMA 357/2005 (classs 2) and Cetesb (2013)			Unit	October 22 (2017) 1 st campaign	November 20 (2017) 2 st campaign	December 10 (2017) 3 st campaign	January14 (2018) 4 st campaign	February 25 (2018) 5 st campaign	March 25 (2018) 6 st campaign	April 22 (2018) 7 st campaign	May 27 (2018) 8 st campaign	June 24 (2018) 9 st campaign	July 29 (2018) 10 st campaign	August 19 (2018) 11 st campaign
Physicochemical	Seasons			spring		summer				autumn			winter	
	tourist season			low tourist season		hight tourist season			low tourist season					
	climatic period			dry	rainy					dry				
	has it rained in the last 24 hours before the collections?			yes	yes	yes	yes	yes	yes	no	no	no	no	no
	air temperature	–	ºC	24.00	22.00	25.00	24.00	28.00	27.00	25.00	24.00	19.00	18.00	18.00
	water temperature	–	ºC	24.29	24.26	26.20	25.83	27.40	26.40	25.90	23.60	22.70	20.60	21.70
	Salinity	< 0.5 ‰	‰	0.24	0.26	0.27	0.29	0.27	0.22	0.30	0.31	0.30	0.30	0.32
	conductivity (Ec)	–	µS/cm	699.00	650.00	562.00	1001.00	980.00	699.00	567.00	586.00	633.00	562.00	531.00
	total dissolved solids (TDS)	500	mg/L	349.00	367.00	280.00	200.00	280.00	265.00	298.00	275.00	261.00	244.00	211.00
	dissolved oxygen (DO)	≥ 5.0	mg/L	0*	0.78*	1.18*	0.10*	0*	0*	0.40*	0.37*	0.12*	0.50*	0.43*
	pH	6-9	pH	7.26	7.47	7.02	6.66	6.16	6.60	6.47	6.88	6.76	6.43	6.80
	Turbity	Up to 100	NTU	41.10	27.00	31.00	62.00	72.00	81.00	23.00	14.00	28.00	23.00	43.00
	Color	75	mg Pt/L	77.00*	76.00*	82.00*	81.00*	79.00*	81.00*	80.00*	75.00*	76.00*	76.00*	80.00*
	sedimented solids (SS)	–	ml/L	<0.10	<0.10	0.10	0.10	0.10	0.10	0.10	<0.10	<0.10	<0.10	<0.10
	biochemical oxygen demand (BOD)	Up to 5	mg/L	10.00*	13.00*	54.00*	18.00*	28.00*	28.00*	17.00*	21.00*	25.00*	16.00*	22.00*
	oils and greases (OG)	_	mg/L	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ

	ammonia (NH_3)	2	mg/L	2.45*	2.62*	2.55*	2.65*	2.76*	2.62*	2.37*	2.31*	2.55*	2.18*	2.01*
	nitrite (NO_2^-)	1	mg/L	0.02	0.03	0.03	0.02	0.06	0.06	0.04	0.07	0.09	0.05	0.09
	nitrate (NO_3^-)	10	mg/L	1.50	1.50	0.38	1.80	1.50	1.10	0.90	1.30	1.80	1.30	1.90
	phosphate (PO_4^{3}-)	–	mg/L	1.01	1.05	1.07	1.15	1.23	1.03	1.04	1.08	0.90	1.07	1.03
	anionic surfactants (MBAS)	0.5	mg/L	1.05*	1.04*	1.08*	1.37*	1.61*	1.22*	1.15*	1.09*	1.03*	1.3*	1.01*
	total phosphorus (TP)	0.02	mg/L	2.18*	2.20*	2.31*	2.70*	2.90*	2.50*	2.21*	2.20*	2.10*	2.15*	2.06*
heavy metals	aluminum (Al)	0.1	mg/L	0.15*	<LQ	<LQ	0.07	0.23	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ
	cadmium (Cd)	0.001	mg/L	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ
	lead (Pb)	0.01	mg/L	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ
	copper (Cu)	0.009	mg/L	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ
	chrome (Cr)	0.05	mg/L	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ
	nickel (Ni)	0.025	mg/L	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ
	zinc (Zn)	0.18	mg/L	<LQ	<LQ	<LQ	<LQ	<0.05	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ
Microbiologic	total coliforms (TC)	–	CFU/mL	330000	32000000	64000000	58000000	260000000	170000000	130000000	110000000	150000000	150000000	144000000
	Escherichia Coli (E.coli)	0.06 x 10^4	CFU/mL	17000000 **	19000000 **	22000000 **	24000000 **	25000000 **	26000000 **	21000000 **	15000000 **	28000000 **	16000000 **	13000000 **
	Enterococci	0.04 x 10^4	CFU/mL	18000000*	21000000*	12000000*	17000000*	4400000*	5200000*	6200000*	7337000*	3800000*	5700000*	6600000*

Legend: *Does not meet standards (Conama 357/2005)/ **Does not meet standards Cetesb (2013). <LQ: Limit of Quantification.

Iporanga (4A - channel)														
Variables and standards: CONAMA 357/2005 (classs 2) and Cetesb (2013)			Unit	October 22 (2017) 1 st campaign	November 20 (2017) 2 st campaign	December 10 (2017) 3 st campaign	January14 (2018) 4 st campaign	February 25 (2018) 5 st campaign	March 25 (2018) 6 st campaign	April 22 (2018) 7 st campaign	May 27 (2018) 8 st campaign	June 24 (2018) 9 st campaign	July 29 (2018) 10 st campaign	August 19 (2018) 11 st campaign
	Seasons			spring		summer				autumn			winter	
	tourist season			low tourist season		hight tourist season			low tourist season					
	climatic period			dry	rainy					dry				
	has it rained in the last 24 hours before the collections?			yes	yes	yes	yes	yes	yes	no	no	no	no	no
Physicochemical	air temperature	–	ºC	24.00	22.00	25.00	24.00	28.00	27.00	25.00	24.00	19.00	17.00	17.00
	water temperature	–	ºC	23.80	24.70	26.31	24.54	26.30	25.30	25.20	23.00	22,67	20.30	21.00
	Salinity	< 0.5 ‰	‰	0.05	0.09	0.04	0.05	0.04	0.05	0.09	0.10	0.15	0.20	0.20
	conductivity (Ec)	–	µS/cm	120.00	200.00	236.00	242.00	256.00	165.00	161.00	153.00	123.00	101.00	111.00
	total dissolved solids (TDS)	500	mg/L	60.00	100.00	117.00	118.00	75.00	113.00	106.00	101.00	112.00	109.00	120.00
	dissolved oxygen (DO)	≥ 5.0	mg/L	4.45*	4.64*	4.89*	4.85*	4.78*	4.89*	6.79	5.98	6.10	5.92	6.00
	pH	6-9	pH	7.04	6.42	7.36	6.81	6.90	6.73	6.49	6.87	6.80	7.01	6.71
	Turbity	Up to 100	NTU	4.10	14.20	16.00	14.00	13.00	15.00	12.00	10.00	8.00	5.00	7.00
	Color	75	mg Pt/L	57.00	65.00	55.00	29.00	25.00	33.00	22.00	27.00	21.00	18.00	19.00
	sedimented solids (SS)	–	ml/L	<0.10	<0.10	0.10	0.10	0.10	0.10	<0.10	<0.10	<0.10	<0.10	<0.10
	biochemical oxygen demand (BOD)	Up to 5	mg/L	4.90	4.20	< LQ	< LQ	< LQ	< LQ	< LQ	< LQ	< LQ	< LQ	< LQ
	oils and greases (OG)	_	mg/L	< LQ	< LQ	< LQ	< LQ	< LQ	< LQ	< LQ	< LQ	< LQ	< LQ	< LQ

	ammonia (NH_3)	2	mg/L	0.01	0.01	0.002	0.01	0.03	0.01	0.002	0.001	0.02	0.01	0.02
	nitrite (NO_2^-)	1	mg/L	0.004	0.006	0.15	0.07	0.09	0.12	0.12	0.09	0.05	0.03	0.05
	nitrate (NO_3^-)	10	mg/L	1.80	1.62	2.20	1.70	1.90	1.60	1.20	1.90	1.10	1.30	1.40
	phosphate (PO_4^{3}-)	–	mg/L	0.05	0.06	0.001	0.07	0.10	0.04	0.001	0.002	0.002	0.003	0.002
	anionic surfactants (MBAS)	0.5	mg/L	0.19	0.19	0.21	0.26	0.18	0.11	0.02	0.05	0.10	0.01	0.02
	total phosphorus (TP)	0.02	mg/L	0.21*	0.20*	0.27*	0.33*	0.29*	0.18*	0.03*	0.08*	0.03*	0.02*	0.04*
heavy metals	aluminum (Al)	0.1	mg/L	0.05	< LQ	< LQ	0.05	0.05	< LQ	< LQ	< LQ	< LQ	< LQ	< LQ
	cadmium (Cd)	0.001	mg/L	< LQ	< LQ	< LQ	< LQ	< LQ	< LQ	< LQ	< LQ	< LQ	< LQ	< LQ
	lead (Pb)	0.01	mg/L	< LQ	< LQ	< LQ	<0.01	< LQ	< LQ	< LQ	< LQ	< LQ	< LQ	< LQ
	copper (Cu)	0.009	mg/L	< LQ	< LQ	< LQ	<0.005	<0.005	< LQ	< LQ	< LQ	< LQ	< LQ	< LQ
	chrome (Cr)	0.05	mg/L	< LQ	< LQ	< LQ	< LQ	0.014	< LQ	< LQ	< LQ	< LQ	< LQ	< LQ
	nickel (Ni)	0.025	mg/L	< LQ	< LQ	< LQ	< LQ	< LQ	< LQ	< LQ	< LQ	< LQ	< LQ	< LQ
	zinc (Zn)	0.18	mg/L	< LQ	< LQ	< LQ	< LQ	< LQ	< LQ	< LQ	< LQ	< LQ	< LQ	< LQ
Microbiologic	total coliforms (TC)	–	CFU/ mL	430000	520000	410000	370000	62000000	440000	220000	188000	175000	137000	128000
	Escherichia Coli (E.coli)	0.06 x 10^4	CFU/ mL	340	460	230	340	88	140	83	101	97	88	79
	Enterococci	0.04 x 10^4	CFU/ mL	67	96	58	67	490*	67	56	76	55	65	59

Legend: *Does not meet standards (Conama 357/2005). <LQ: Limit of Quantification.

Supplementary material (Beach data)

Tombo beach (1B - seawater)

Variables and standards: CONAMA 357/2005 (class 1) and CONAMA 274/2000			Unit	October 22 (2017) 1 st campaign	November 20 (2017) 2 st campaign	December 10 (2017) 3 st campaign	January14 (2018) 4 st campaign	February 25 (2018) 5 st campaign	March 25 (2018) 6 st campaign	April 22 (2018) 7 st campaign	May 27 (2018) 8 st campaign	June 24 (2018) 9 st campaign	July 29 (2018) 10 st campaign	August 19 (2018) 11 st campaign
	Seasons			spring		summer				autumn			winter	
	tourist season			low tourist season		hight tourist season			low tourist season					
	climatic period			dry	rainy					dry				
	has it rained in the last 24 hours before the collections?			yes	yes	yes	yes	yes	yes	no	no	no	no	no
physicochemical	air temperature	–	ºC	24.00	22.00	27.00	26.00	29.00	28.00	25.00	24.00	19.00	19.00	19.00
	water temperature	–	ºC	23.68	24.33	26.83	28.20	27.30	27.50	24.00	23.70	24.20	21.40	22.20
	Salinity	> 30 ‰	‰	33.55	33.57	33.80	32.34	33.07	32.06	33.02	33.49	33.81	32.59	33.43
	conductivity (Ec)	–	mS/cm	51.02	51.05	51.62	49.59	50.58	51.42	52.03	51.00	51.43	51.60	50.40
	total dissolved solids (TDS)	–	mg/L	25.53	25.54	25.81	24.79	25.13	24.23	26.70	25.24	26.24	25.62	26.42
	dissolved oxygen (DO)	≥ 6.0	mg/L	4.79*	4.22*	3.57*	1.57*	1.13*	1.25*	4.30*	4.23*	3.60*	3.45*	3.37*
	pH	6.5 - 8.5	pH	7.83	7.29	6.09*	7.42	5.31*	5.44*	6.04*	6.09*	7.42	6.90	6.90
	Turbity	–	NTU	9.55	0.40	4.77	13.00	16.00	11.00	11.00	14.00	12.00	10.00	9.50
	Color	–	mg Pt/L	2.00	2.00	7.00	13.00	25.00	21.00	16.00	12.00	9.00	14.00	17.00
	ammonia (NH3)	0.40	mg/L	0.06	0.03	0.12	0.09	0.20	0.30	0.05	0.03	0.03	0.09	0.02
	nitrite (NO2-)	0.07	mg/L	0.002	0.001	0.002	0.022	0.020	0.020	0.001	0.001	0.002	0.023	0.018

	nitrate (NO3-)	0.40	mg/L	0.20	0.25	0.22	0.30	0.43*	0.49*	0.20	0.17	0.11	0.25	0.26
	phosphate (PO43-)	–	mg/L	0.08	0.17	0.09	0.08	0.12	0.20	0.09	0.12	0.19	0.10	0.14
	total phosphorus (TP)	0.062	mg/L	0.36*	0.33*	0.42*	0.44*	0.57*	0.48*	0.54*	0.60*	0.52*	0.55*	0.52*
	anionic surfactants (MBAS)	0.2	mg/L	0.22*	0.18*	0.27*	0.29*	0.34*	0.32*	0.29*	0.40*	0.24*	0.34*	0.28*
	sedimented solids (SS)	–	ml/L	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
	biochemical oxygen demand (BOD)	–	mg/L	612.00	533.00	65.00	191.00	73.00	87.00	134.00	73.00	145.00	54.00	77.00
	oils and greases (OG)	–	mg/L	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ
heavy metals	aluminum (Al)	1.5	mg/L	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ
	cadmium (Cd)	0.005	mg/L	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ
	lead (Pb)	0.01	mg/L	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ
	copper (Cu)	0.005	mg/L	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ
	chrome (Cr)	0.05	mg/L	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ
	nickel (Ni)	0.025	mg/L	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ
	zinc (Zn)	0.09	mg/L	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ
microbiological	total coliforms (TC)	–	CFU/mL	124000	13000	18000	27000	22000	1800000	12000	23000	34000	22000	1800000
	Escherichia Coli (E.coli)	0.2 x 104	CFU/mL	56000*	640	567	453	6000*	87	487	435	765	546	873
	Enterococci	0.04 x 104	CFU/mL	39600**	180	165	2310	170000**	231	187	154	345	234	394

Legend: *Does not meet standards (Conama 357/2005)/ **Does not meet standards (Conama 274/2000)/ <LQ: Limit of Quantification.

Enseada beach (2B - seawater)														
Variables and standards: CONAMA 357/2005 (class 1) and CONAMA 274/2000			Unit	October 22 (2017) 1 st campaign	November 20 (2017) 2 st campaign	December 10 (2017) 3 st campaign	January14 (2018) 4 st campaign	February 25 (2018) 5 st campaign	March 25 (2018) 6 st campaign	April 22 (2018) 7 st campaign	May 27 (2018) 8 st campaign	June 24 (2018) 9 st campaign	July 29 (2018) 10 st campaign	August 19 (2018) 11 st campaign
	Seasons			spring		summer				autumn			winter	
	tourist season			low tourist season		hight tourist season			low tourist season					
	climatic period			dry	rainy					dry				
	has it rained in the last 24 hours before the collections?			yes	yes	yes	yes	yes	yes	no	no	no	no	no
Physicochemical	air temperature	–	ºC	24.00	22.00	27.00	25.00	29.00	28.00	25.00	24.00	19.00	19.00	19.00
	water temperature	–	ºC	23.68	24.79	28.65	27.70	30.70	28.70	23.00	22.20	21.00	20.90	21.50
	Salinity	> 30 ‰	‰	32.58	31.28	32.61	31.04	32.80	31.70	32.10	31.32	32.43	31.23	32.09
	conductivity (Ec)	–	mS/cm	49.77	47.96	50.00	39.16	50.34	51.34	52.10	50.80	45.70	50.80	51.25
	total dissolved solids (TDS)	–	mg/L	24.87	24.00	25.10	19.58	25.18	22.38	23.00	24.30	23.00	24.20	23.20
	dissolved oxygen (DO)	≥ 6.0	mg/L	4.88*	4.38*	3.96*	1.90*	1.24*	1.70*	1.90*	2.96*	3.75*	3.24*	3. 56*
	pH	6.5 - 8.5	pH	8.04	7.26	7.30	7.22	7.40	7.60	7.20	7.05	7.40	7.55	6.70
	Turbity	–	UNT	18.70	0.84	3.96	11.20	13.00	15.00	11.00	12.80	14.60	15.00	15.30
	Color	–	mg Pt/L	2.00	12.00	11.00	33.00	29.00	33.00	17.00	21.00	31.00	29.00	42.00
	ammonia (NH_3)	0.40	mg/L	0.10	0.12	0.08	0.10	0.70*	0.55*	0.60*	0.07	0.15	0.33	0.37
	nitrite (NO_2^-)	0.07	mg/L	0.01	0.02	0	0.02	0.13	0.10	0.02	0.10	0.02	0.03	0.01
	nitrate (NO_3^-)	0.40	mg/L	1.10*	1.40*	1.30*	1.20*	1.90*	0.92*	1.30*	1.40*	0.72	0.81	0.78

	phosphate (PO_4^{3-})	–	mg/L	0.11	0.09	0.35	0.36	0.33	0.29	0.19	0.19	0.22	0.19	0.21
	total phosphorus (TP)	0.062	mg/L	0.47*	0.44*	0.80*	0.66*	0.70*	0.67*	0.50*	0.60*	0.70*	0.60*	0.65*
	anionic surfactants (MBAS)	0.2	mg/L	0.32*	0.32*	0.33*	0.32*	0.34*	0.33*	0.30*	0.47*	0.65*	0.44*	0.56*
	sedimented solids (SS)	–	ml/L	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
	biochemical oxygen demand (BOD)	–	mg/L	732.00	560.00	430.00	65.00	108.00	209.00	238.00	234.00	112.00	134.00	127.00
	oils and greases (OG)	–	mg/L	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ
heavy metals	aluminum (Al)	1.5	mg/L	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ
	cadmium (Cd)	0.005	mg/L	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ
	lead (Pb)	0.01	mg/L	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ
	copper (Cu)	0.005	mg/L	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ
	chrome (Cr)	0.05	mg/L	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ
	nickel (Ni)	0.025	mg/L	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ
	zinc (Zn)	0.09	mg/L	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ
Microbiological	total coliforms (TC)	–	CFU/mL	138000	28000	47000	23000	690000	9000	123000	41000	124000	470000	59000
	Escherichia Coli (E.coli)	0.2×10^4	CFU/mL	23000*	17000*	15000*	21000*	28000*	11000*	23000*	17000*	12000*	17000*	11000*
	Enterococci	0.04×10^4	CFU/mL	7040000**	6200000**	9200000**	7800000**	21000000**	5300000**	8000000**	8700000**	15600000**	4300000**	1140000**

Legend: *Does not meet standards (Conama 357/2005)/ **Does not meet standards (Conama 274/2000)/ <LQ: Limit of Quantification.

Perequê beach (3B - seawater)														
Variables and standards: CONAMA 357/2005 (class 1) and CONAMA 274/2000			Unit	October 22 (2017) 1 st campaign	November 20 (2017) 2 st campaign	December 10 (2017) 3 st campaign	January14 (2018) 4 st campaign	February 25 (2018) 5 st campaign	March 25 (2018) 6 st campaign	April 22 (2018) 7 st campaign	May 27 (2018) 8 st campaign	June 24 (2018) 9 st campaign	July 29 (2018) 10 st campaign	August 19 (2018) 11 st campaign
Physicochemical	Seasons			spring		summer				autumn			winter	
	tourist season			low tourist season		hight tourist season			low tourist season					
	climatic period			dry	rainy					dry				
	has it rained in the last 24 hours before the collections?			yes	yes	yes	yes	yes	yes	no	no	no	no	no
	air temperature	–	ºC	24.00	22.00	25.00	24.00	25.00	26.00	25.00	24.00	19.00	18.00	18.00
	water temperature	–	ºC	24.29	24.62	27.31	27.74	27.70	27.10	23.40	22.70	24.00	21.00	22.20
	Salinity	> 30 ‰	‰	32.77	30.64	33.86	30.01	31.04	31.07	31.20	30.50	33.72	30.20	31.50
	conductivity (Ec)	–	mS/cm	49.58	47.03	51.27	45.92	39.16	49.12	47.30	48.60	52.60	46.80	41.12
	total dissolved solids (TDS)	–	mg/L	25.10	23.53	25.80	23.01	19.43	25.00	26.70	24.60	27.00	24.40	2.43
	dissolved oxygen (DO)	≥ 6.0	mg/L	4.72*	4.33*	3.75*	3.42*	3.45*	3.76*	3.72*	4.20*	4.10*	4.30*	4.60*
	pH	6.5 - 8.5	pH	7.94	7.60	7.48	7.40	7.40	7.40	7.60	7.52	7.53	7.49	7.34
	Turbity	–	UNT	11.70	0.40	3.22	14.00	11.20	10.20	9.12	15.00	11.80	11.90	12.10
	Color	–	mg Pt/L	4.00	4.00	23.00	29.00	33.00	28.00	28.00	31.00	27.00	30.00	28.00
	ammonia (NH_3)	0.40	mg/L	0.22	0.18	0.04	0.41*	0.40*	0.42*	0.18	0.50	0.08	0.32	0.12
	nitrite (NO_2^-)	0.07	mg/L	0.009	0.007	0.004	0.008	0.021	0.008	0.029	0.022	0.011	0.044	0.043
	nitrate (NO_3^-)	0.40	mg/L	1.10*	1.21*	1.03*	1.33*	1.21*	1.09*	1.22*	1.14*	1.03*	1.10*	1.12*

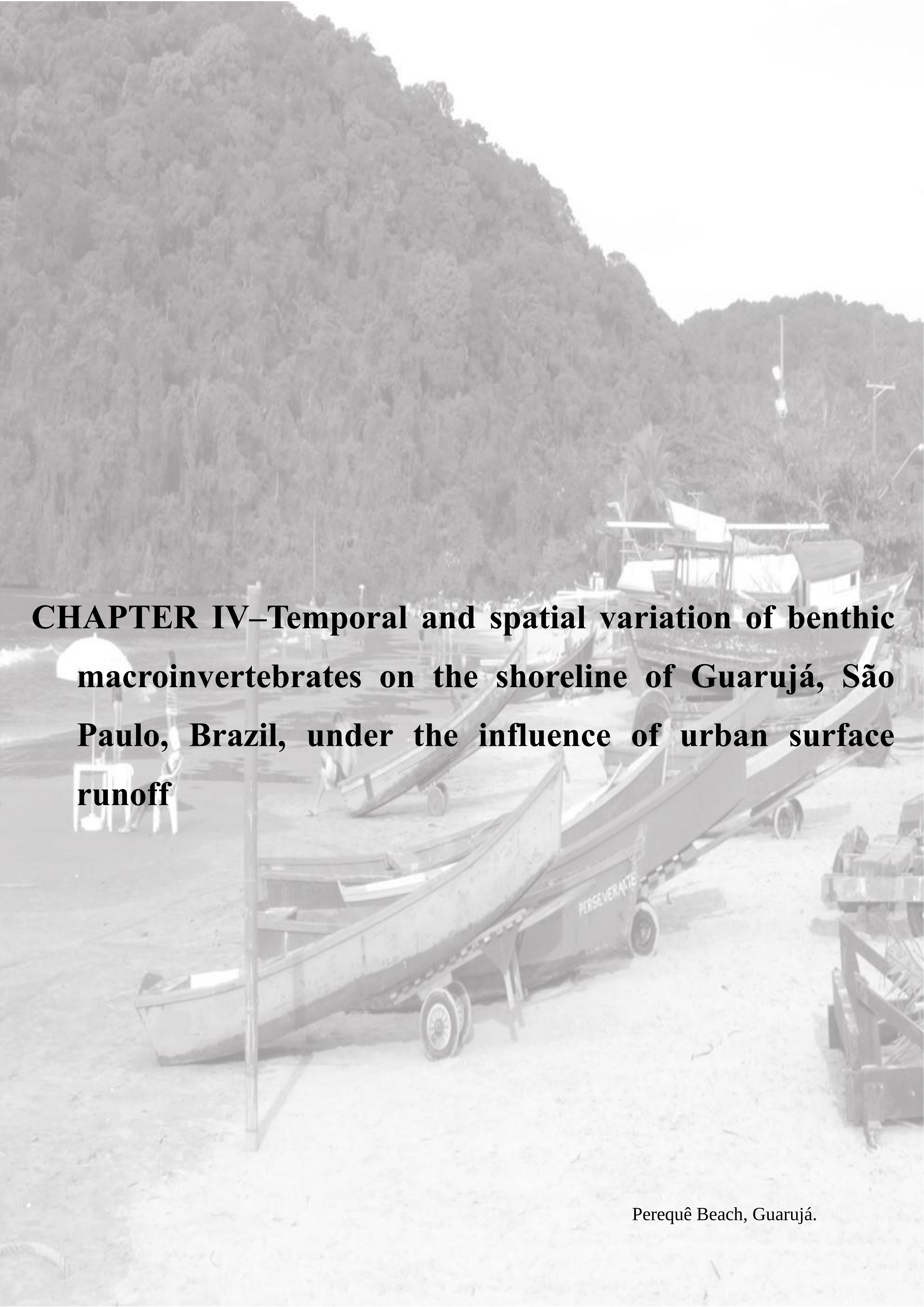
	phosphate (PO_4^{3-})	–	mg/L	0.29	0.18	0.06	0.20	0.47	0.30	0.22	0.18	0.24	0.24	0.30
	total phosphorus (TP)	0.062	mg/L	0.61*	0.76*	0.53*	0.89*	1.03*	0.80*	0.72*	0.80*	0.67*	0.65*	0.72*
	anionic surfactants (MBAS)	0.2	mg/L	0.21*	0.54*	0.47*	0.56*	0.66*	0.44*	0.45*	0.53*	0.32*	0.44*	0.27*
	sedimented solids (SS)	–	ml/L	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
	biochemical oxygen demand (BOD)	–	mg/L	687.00	543.00	234.00	169.00	67.00	78.00	186.00	124.00	133.00	98.00	112.00
	oils and greases (OG)	–	mg/L	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ
heavy metals	aluminum (Al)	1.5	mg/L	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ
	cadmium (Cd)	0.005	mg/L	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ
	lead (Pb)	0.01	mg/L	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ
	copper (Cu)	0.005	mg/L	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ
	chrome (Cr)	0.05	mg/L	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ
	nickel (Ni)	0.025	mg/L	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ
	zinc (Zn)	0.09	mg/L	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ
Microbiological	total coliforms (TC)	–	CFU/mL	7200000	2900000	2300000	3700000	39000	27000	1800000	2700000	3600000	44000	28000
	Escherichia Coli (E.coli)	0.2×10^4	CFU/mL	8000*	30000*	20000*	42000*	29000*	18000*	20000*	23000*	24000*	17000*	13000*
	Enterococci	0.04×10^4	CFU/mL	14400000**	28000000**	39800000**	22200000**	62000000**	28000000**	19800000**	16700000**	16050000**	16000000**	12700000**

Legend: *Does not meet standards (Conama 357/2005)/ **Does not meet standards (Conama 274/2000)/ <LQ: Limit of Quantification.

Iporanga beach (4B - seawater)														
Variables and standards: CONAMA 357/2005 (class 1) and CONAMA 274/2000			Unit	October 22 (2017) 1 st campaign	November 20 (2017) 2 st campaign	December 10 (2017) 3 st campaign	January14 (2018) 4 st campaign	February 25 (2018) 5 st campaign	March 25 (2018) 6 st campaign	April 22 (2018) 7 st campaign	May 27 (2018) 8 st campaign	June 24 (2018) 9 st campaign	July 29 (2018) 10 st campaign	August 19 (2018) 11 st campaign
Physicochemical	Seasons			spring		summer				autumn			winter	
	tourist season			low tourist season		hight tourist season			low tourist season					
	climatic period			dry	rainy					dry				
	has it rained in the last 24 hours before the collections?			yes	yes	yes	yes	yes	yes	no	no	no	no	no
	air temperature	–	ºC	24.00	22.00	25.00	24.00	28.00	27.00	25.00	24.00	19.00	17,00	17,00
	water temperature	–	ºC	23.9	24,00	2.6.83	26.99	29.7	27.7	26,00	22.8	23,00	21,00	21,00
	Salinity	> 30 ‰	‰	33.18	31,00	32.94	38.5	30.32	31.34	32.1	31,00	32,00	32.3	30.32
	conductivity (Ec)	–	mS/cm	50.64	37.08	50.33	41.29	46.93	37.15	50.48	36.76	49.43	43.54	46,00
	total dissolved solids (TDS)	–	mg/L	25.27	18.55	25.17	20.66	23.47	24.56	23.2	22.8	24.7	25.6	23.2
	dissolved oxygen (DO)	≥ 6.0	mg/L	2.98*	3.95*	3.70*	1.05*	1.22*	1,.45*	3.10*	3.80*	3.66*	3.99*	3.10*
	pH	6.5 a 8.5	pH	7.96	7.40	7.45	7.60	7.48	7.46	7.40	7.32	7.60	7.50	7.60
	Turbity	–	NTU	10.90	1.01	1.39	8.90	9.30	12.30	7.20	8.40	10.80	8.80	13.00
	Color	–	mg Pt/L	8.00	8.00	5.00	4.00	4.70	7.00	7.00	6.70	5.00	4.60	6.00
	ammonia (NH_3)	0.40	mg/L	0.01	0.01	0.001	0.04	0.06	0.07	0.04	0.01	0.02	0.01	0.02

	nitrite (NO_2^-)	0.07	mg/L	0.005	0.04	0.004	0.006	0.009	0.005	0.05	0.004	0.006	0.02	0.005
	nitrate (NO_3^-)	0.40	mg/L	0.80*	0.70*	0.30	0.20	0.30	0.40*	0.40*	0.30	0.20	0.20	0.23
	phosphate (PO_4^{3-})	–	mg/L	0.30	0.60	0.40	0.70	0.76	0.50	0.23	0.30	0.25	0.22	0.20
	total phosphorus (TP)	0.062	mg/L	0.80*	1.10*	0.90*	1.28*	1.48*	0.90*	0.90*	0.77*	0.80*	0.76*	0.77*
	anionic surfactants (MBAS)	0.2	mg/L	0.20*	0.42*	0.37*	0.43*	0.65*	0.45*	0.48*	0.34*	0.47*	0.41*	0.43*
	sedimented solids (SS)	–	ml/L	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
	biochemical oxygen demand (BOD)	–	mg/L	498.00	345.00	98.00	204.00	75.00	123.00	376.00	254.00	123.00	201.00	183.00
	oils and greases (OG)	–	mg/L	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ
heavy metals	aluminum (Al)	1.5	mg/L	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ
	cadmium (Cd)	0.005	mg/L	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ
	lead (Pb)	0.01	mg/L	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ
	copper (Cu)	0.005	mg/L	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ
	chrome (Cr)	0.05	mg/L	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ
	nickel (Ni)	0.025	mg/L	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ
	zinc (Zn)	0.09	mg/L	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ
Microbiological	total coliforms (TC)	–	CFU/mL	370000	220000	230000	340000	18000000	180000	240000	250000	650000	650000	37000000
	Escherichia Coli (E.coli)	0.2×10^4	CFU/mL	565	345	321	344	98	145	325	387	387	276	87
	Enterococci	0.04×10^4	CFU/mL	69	78	66	65	555	88	72	86	71	87	65

Legend: *Does not meet standards (Conama 357/2005)/ <LQ: Limit of Quantification.



CHAPTER IV–Temporal and spatial variation of benthic macroinvertebrates on the shoreline of Guarujá, São Paulo, Brazil, under the influence of urban surface runoff

Perequê Beach, Guarujá.

Temporal and spatial variation of benthic macroinvertebrates on the shoreline of Guarujá, São Paulo, Brazil, under the influence of urban surface runoff

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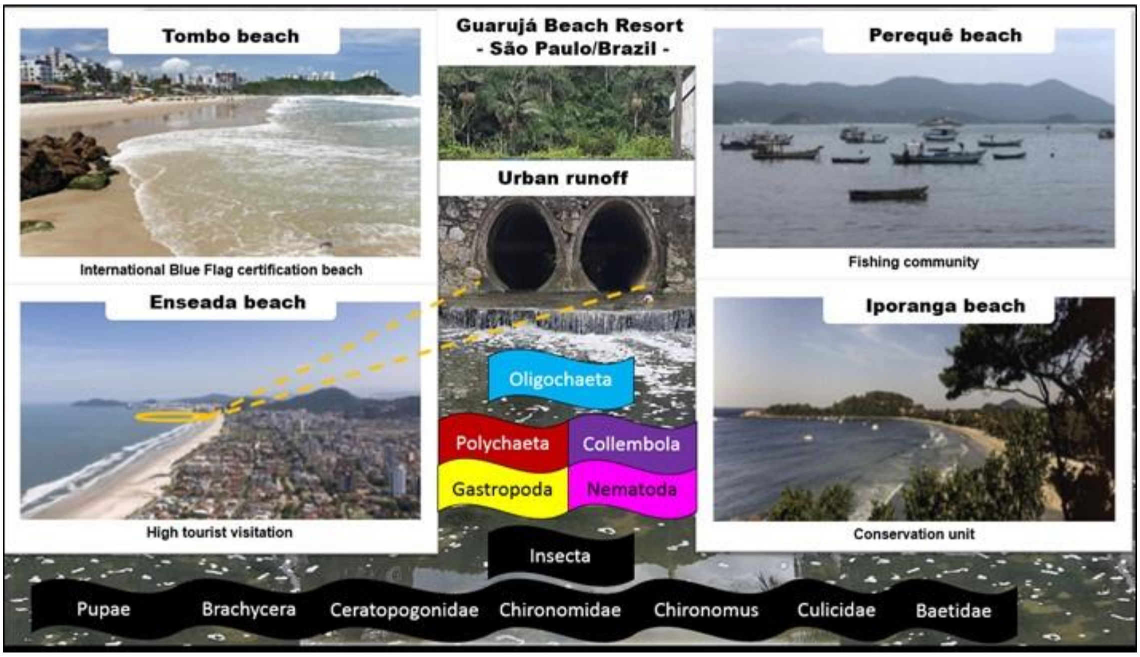
Published in: Regional Studies in Marine Science (2020).

Graphical Abstract



Abstract

In recent decades, the management of urban surface runoff in coastal regions has become a global priority. However, in Brazil, whose coastline is 8,500 km, these actions are still scarce. This study aimed to characterise, for the first time, the structure of the benthic assemblages near urban drainage channels, whose waters flow permanently to four beaches of Guarujá, São Paulo, Brazil. Collection of sediments took place between October 2017 and August 2018. Macroinvertebrate fauna were identified in a certified laboratory using international standard methodologies. The data showed that although the species richness was in general low, a great abundance and density of taxa tolerant to organic pollution was observed (e.g. Oligochaeta, Gastropoda and, mainly Insecta: Ceratopogonidae, Chironomidae, *Chironomus* and Culicidae) during the period of greatest tourist influx (rainy season/summer). Furthermore, the results of water quality indexes (in all channels) suggested heavily polluted environments: (i) Shannon-Wiener: $H' < 1$; (ii) Biological Monitoring Working Party: BMWP’ < 12 and (iii) Average Score per Taxon: ASPT < 2. Therefore, it is urgent to adopt management measures to improve the environmental water quality and to prevent human health risks associated with tourism activities taking place in the recreational coastal waters of Guarujá municipality.

Keywords: Brazil, Non-point source pollution, Domestic sewage, Ecological indicator, Bathing water.

4.1 Introduction

The different anthropic activities developed in urban areas around the world have been responsible for the intense removal of green areas (greater pavement and waterproofing of terrestrial routes) (Yang et al., 2011), which consequently has led to an increase in the volume and intensity of diffuse pollution flowing into different receptor bodies (Angrill et al., 2017; Yang and Toor, 2017). In order to evaluate the effects of these diffuse loads, traditional physical–chemical and microbiological analyses of water and sediments are used to verify the safety of aquatic ecosystems and predict potential human health risks; however, it is also necessary to assess the effects of these urban flows on aquatic biota, since all trophic levels may be affected (McQueen et al., 2010; Tang et al., 2013). These effects can be evaluated, for example, through the characterisation of benthic macrofauna, which can be used as an ecological indicator of environmental quality, reflecting physical, chemical and biological changes (both of natural and anthropogenic origin), as well as their synergistic effects (Bruno et al., 2012; Sánchez et al., 2013).

Communities of benthic organisms are particularly sensitive to pollutant exposure and can be an efficient tool to assess aquatic ecosystems that receive environmental stressors from point (sewage discharge) and diffuse (urban storm water) sources (Morrisey et al., 2003; Schiff and Bay, 2003; Ferraz et al., 2012). Moreover, benthic macroinvertebrates are easy to collect, unable to escape from sources of urban pollution since they are generally sessile organisms or have reduced mobility, and because they have a relatively long life, they integrate a historical set of ecological conditions (Arimoro et al., 2007; Lin and Yo, 2008; Daief et al., 2014). Thus, macroinvertebrate taxa can serve as indicators of environmental conditions (Moreno and Callisto, 2006; Bruno et al., 2012; Ferraz et al., 2012). Benthic macrofauna, namely pollution-tolerant species, such as Insecta and Oligochaeta classes (Meyer et al., 2005; Arimoro et al., 2007; Lin and Yo, 2008) and those that are intolerant to pollution, such as *Ephemeroptera, Plecoptera* and *Trichoptera* orders (Dohet et al., 2002; Adakole and Anunne, 2003; Lin and Yo, 2008), have been used successfully worldwide to characterise ecological conditions in different water bodies (Arimoro et al., 2007; Bruno et al., 2012; Arslan et al., 2016a).

The high population density in coastal zones worldwide (Pelling and Blackburn, 2013) results in intense anthropic pressures, such as wastewater discharge, urban surface runoff and tourism (Defeo et al., 2009; Ferraz et al., 2012; Daief et al., 2014). This condition can be observed in the coastal zone of the State of São Paulo, Brazil, an area with approximately 880 km of coastline, and a resident population of more than two million inhabitants, where the 16 coastal municipalities are a major tourist destination in the country (SMA/CPLEA, 2005; SMA/CPLA, 2012; Ibge, 2018). Guarujá is one of these municipalities, where good climatic conditions favour the use of its beaches throughout the year (De Oliveira et al., 2007; SMA/CPLA, 2012). The municipality of Guarujá, besides being one of the main Brazilian tourist destinations, also attracts a large migratory contingent of new residents (SMA/CPLEA, 2005). Between 1950 and 2018, the population increased from 13,000 to around 316,000 inhabitants (Ibge, 2018). As a result, the disorderly growth of the municipality and the lack of investment in sanitation infrastructure caused intense economic and socio-environmental conflicts, which persist today (SMA/CPLEA, 2005; Cetesb, 2017).

Additional studies on macrofauna are necessary, because: (i) the Guarujá drainage channels do not have a gate system, thus spreading their waters continuously on to the beaches (Cetesb, 2017); (ii) urbanisation (type of land use) interferes with the quality of water flowing from urban drainage channels (Lee et al., 2009; Onderka et al., 2012); (iii) during precipitation episodes, there is greater transport of inorganic and organic solid compounds to water bodies (Chebbo and Gromaire, 2004; McLellan, 2004); (iv) in summer, when the number of tourists increases significantly, sewage discharge into the ocean (via drainage channels) intensifies (Sato et al., 2005); and (v) recent water-quality monitoring programmes in the coastal zone of the State of São Paulo have been solely based on the determination of microbiological parameters (*Escherichia coli* and *Enterococci*) (Cetesb, 2017), additional studies on the impact of these diffuse loads on the Guarujá benthic macrofauna is mandatory.

The objective of this study was to characterise, for the first time, the structure of the benthic assemblages of four beaches in Guarujá (Tombo, Enseada, Perequê and Iporanga) that receive diffuse loads from nearby neighbourhoods through urban drainage channels. This data allows us to understand the ecological changes derived from this particular anthropogenic activity, and to evaluate the potential environmental and human risks associated with the discharge of urban runoff into marine recreational waters.

4.2 Material and methods

4.2.1 Characterisation of the sampling area

This study was carried out in Santo Amaro Island, Guarujá city, a micro-region of Santos municipality, São Paulo State, Brazil (Ribeiro and Oliveira, 2015). Guarujá has a total area of 14,000 ha and 316,000 inhabitants (Ibge, 2018). Most of its territory comprises environmental protection areas (10,700 ha), which remain uninhabited. The other 3,300 ha (urban perimeter) are highly developed (Ribeiro and Oliveira, 2015; Ibge, 2018). Twenty percent of the population illegally inhabits protected areas (e.g. mangroves, sandbanks and hills), which leads the municipality to have one of the highest slum indexes in the state of São Paulo (31%) (SMA/CPLA, 2012). Due to the lack of land regulations, these areas are not served by a sanitation network (SMA/CPLA, 2012), and therefore a portion of this sewage is discharged into urban drainage channels that flow into the nearshore areas of Guarujá city (Cetesb, 2017).

The municipality's economy is mainly driven by the port of Santos, the largest in Latin America and 42nd largest worldwide, located in the western portion of the island. But the commercial activities associated with tourism, developed along the 26 beaches located in the eastern and southern portions of the island, are important sources of employment (SMA/CPLA, 2012; Ribeiro and Oliveira, 2015). In Guarujá, the climate favours the use of beaches throughout the year. The mean annual precipitation and temperature are approximately 3000 mm and 22 °C, respectively (Ribeiro and Oliveira, 2015; Cetesb, 2017). About 18 beaches are isolated and preserved, accessible only by trail or boat. The remaining eight beaches are located in the urbanised area of the municipality and are monitored for *Enterococci* weekly by the state environmental agency (Cetesb, 2017). Taking into consideration the different characteristics regarding land use and occupation, four of these eight urbanised beaches were selected for this study: Tombo (an international Blue Flag certification beach), Enseada (high tourist visitation), Perequê (a fishing community) and Iporanga (a conservation unit). For further details, see Figure 4.1.

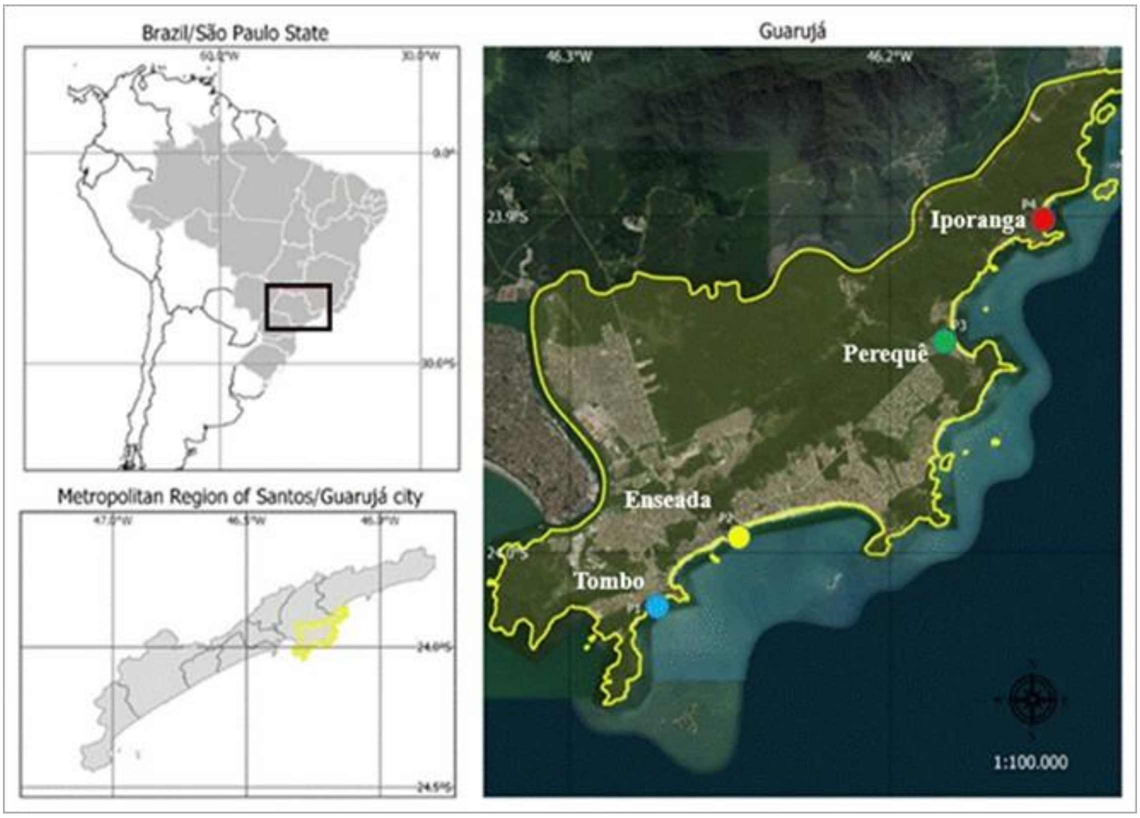


Figure – 4.1: Map of the study area showing the Metropolitan Region of Santos, in São Paulo State, Brazil. Sampling stations of the benthic macrofauna collected on the shoreline of Guarujá municipality susceptible to urban surface runoff. Identification of sampling points: Tombo beach (blue solid circle, P1), Enseada beach (yellow solid circle, P2), Perequê beach (green solid circle, P3) and Iporanga beach (red solid circle, P4).

4.2.2 Sample preparation and analysis

At each beach, sediments were collected in the supralittoral region, at the mouth of the drainage channels, without interference of the tidal regime. The sampling was carried out monthly between October 2017 and August 2018 (rainy season: November through March—equivalent to the high tourist season; and dry season: October and April through August—equivalent to the low tourist season).

The methodology used for the collection of the benthic macrofauna in the channels was based on the National Guide of Collection and Preservation of Samples (Cetesb, 2011). At each collection point, samples of benthic invertebrates were taken using a Corer sampler (area = 0.002 m^2), standardising the sampling effort in five footprints. The collected sediment was sieved *in situ* with the aid of a 250µm mesh sieve (Cetesb, 2011). The sediments were placed in labelled bottles, preserved in 70% ethanol-rose Bengal solution (Cetesb, 2011), and thereafter sent for analysis to the laboratory Econsult, Guarujá, São Paulo, Brazil (accreditation by Inmetro/ABNT ISO/IEC17025).

In the laboratory, the analyses were performed with reference to the Standard Methods for the Examination of Water and Wastewater (APHA, 2012). Initially, the organisms were screened in Petri dishes with the aid of a stereomicroscope. Later, taxonomic identification was made according to the benthic invertebrate group detected in the sample, using identification keys and descriptions available in specialised literature (e.g. Amaral and Nonato, 1996; Amaral et al., 2005; Trivinho-Strixino, 2011). The sediment organic matter (%) was determined by the modified Walkley–Black method by the Econsult Laboratory. The method consists of oxidising organic matter with potassium dichromate ($K_2Cr_2O_7$) in a sulphuric medium (Embrapa, 2011).

4.2.3 Data analysis

4.2.3.1 Density (N) of the benthic community

N expressed in organisms per square metre (org./ m^2) was obtained using the following formula (Welch, 1948): $N = \frac{X}{A \cdot S}$ where: X = number of organisms counted in the sample; A = area of the sampler (m^2); S = number of samples collected.

4.2.3.2 Shannon-Wiener diversity (*H'*) index

H' was calculated through the following equation (Magurran, 1988): $H' = \sum_{i=1}^{S} pi \ln pi$ where: pi = fraction of the entire population made up of species I; S = numbers of species encountered. Diversity tends to be higher as H' increases. $H' > 4$, H' 3–4 and $H' < 2$, indicates a clean, moderately-polluted and strongly-polluted water, respectively (Magurran, 1988; Mason, 2002; Shekhar et al., 2008).

4.2.3.3 Equitability index (J)

J was obtained through the following equation (Magurran, 1988): $J = \frac{H'}{H'\,max}$ where H' is the Shannon's index, and H' max the maximum diversity.

4.2.3.4 Biological monitoring working party (BMWP) index

BMWP followed a methodology adapted to the environmental characteristics of Brazil (Junqueira and Campos, 1998). Each family is allocated a score ranging between 1 and 10, according to their sensitivity to environmental disturbance. The highest scores were assigned to species most sensitive to organic pollution. The scores for each family represented in the sample are then summed to give the BMWP index. BMWP could be classified as excellent (BMWP′ > 81: water very clean), good (BMWP′ 61–80: water slightly polluted), acceptable (BMWP′ 41–60: water moderately polluted), bad (BMWP′ 26–40: water severely polluted) and very bad (BMWP′ < 25: highly polluted water).

4.2.3.5 Average score per taxon (ASPT) index

ASPT was obtained from the value of BMWP′ divided by the total number of identified families in each sample point. According to Junqueira and Campos (1998) a high ASPT score (ASPT > 6) is indicative of a clean site containing large numbers of high-scoring taxa; ASPT of 5–6 indicates doubtful water quality; ASPT 4–5, moderately polluted water; and ASPT < 4, highly polluted water.

4.2.4 Statistics

Data analyses were performed (N, *H′* and J) using the software Past (PAleontological STatistics), version 2.17c (Hammer et al., 2001). A chi-square analysis (contingency table) was used to test whether organism density (N) depends on the sampling site (four levels: Tombo, Enseada, Perequê and Iporanga) and period (two levels: rainy and dry seasons). To test the relationship between the concentrations of organic matter on organism density, a simple linear regression was also performed. All statistics adopted a level of significance (α) of 0.05.

4.3 Results

Sediments collected near the four Guarajá drainage channels recorded a total of 12 taxa of benthic invertebrates, namely Insecta (7), Oligochaeta (1), Polychaeta (1), Collembola (1), Mollusca (1) and Nematoda (1) (Table 4.1; Figure 4.2A).

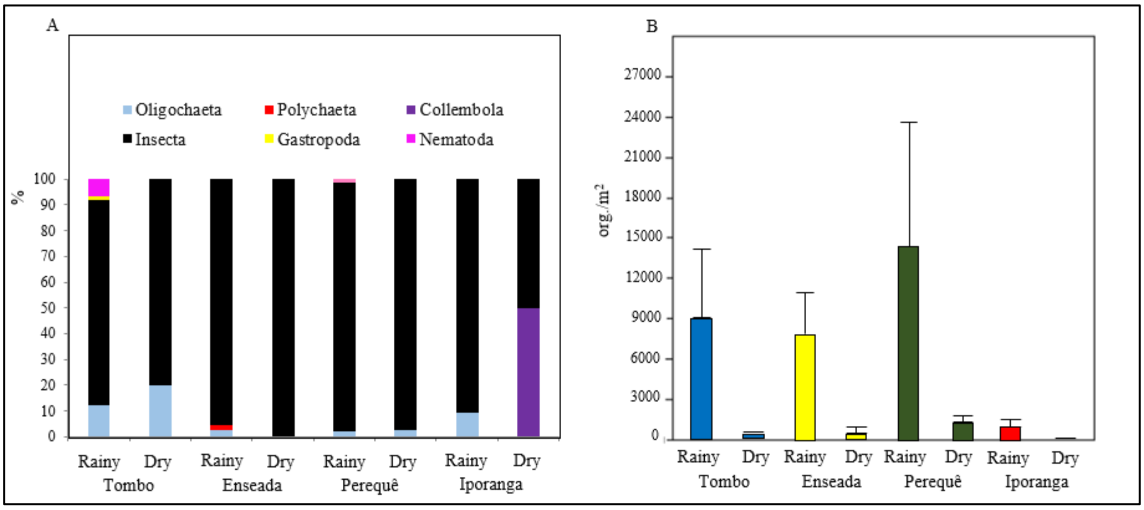


Figure – 4.2: Abundance of benthic macrofauna groups (%) (A) and total mean of benthic organism density (org./m^2) (B) collected on the shoreline of Guarujá, São Paulo, Brazil, under the influence of urban surface runoff. The results are presented considering two different periods: rainy season (November through March: n = 5) and dry season (October 2017 and April through August 2018: n = 6). Identification of the four sampling points: Tombo beach, Enseada beach, Perequê beach and Iporanga beach. Data represent the mean values ± standard errors.

Table – 4.1: Results of the taxonomic composition, frequency of occurrence (percentage) and mean density (org./m^2) of the benthic macrofauna collected in the four urban drainage channels (Tombo, Enseada, Perequê and Iporanga) on the shoreline of Guarujá, São Paulo. The sediment was collected in the supralittoral region, without the interference of the tidal regime. The sample collections were carried out monthly between October 2017 and August 2018. The results of the macrofauna are presented considering two different seasonal periods: rainy season (November until March: n = 5) and dry season (October until April: n = 6). The table also shows the results of total richness (n), total density (org./m^2), mean results of organic matter percentages and the water quality indexes.

Taxonomic composition	Iporanga - 1C				Perequê - 2C				Enseada - 3C				Tombo - 4C			
	Rainy		Dry		Rainy		Dry		Rainy		Dry		Rainy		Dry	
	%	org./m^2	%	org./m^2	%	org./m^2	%	org./m^2	%	org./m^2	%	org./m^2	%	org./m^2	%	org./m^2
Phylum ANNELIDA																
Class Clitellata																
Subclass Oligochaeta	25.0	75	0.0	0	50.0	250	25.0	25	50.0	175	0.0	0	100.0	950	25.0	25
Class Polychaeta																
Subclass Canalipalpata																
Order Spionida																
Family Spionidae	0.0	0	0.0	0	0.0	0	0.0	0	25.0	25	0.0	0	0.0	0	0.0	0
Phylum ARTHROPODA																
Subphylum HEXAPODA																
Class Collembola	0.0	0	25.0	25	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0
Class Insecta																
Order Diptera																
Pupae	0.0	0	0.0	0	50.0	775	25.0	50	0.0	0	0.0	0	25.0	25	0.0	0
Suborder Brachycera	0.0	0	0.0	0	0.0	0	25.0	25	0.0	0	0.0	0	0.0	0	0.0	0
Suborder Nematocera																
Family Ceratopogonidae	0.0	0	0.0	0	50.0	11000	50.0	975	0.0	0	0.0	0	0.0	0	0.0	0
Family Chironomidae	25.0	750	25.0	25	50.0	825	0.0	0	75.0	6500	50.0	225	75.0	6150	25.0	75
Chironomus sp.	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	25.0	25	0.0	0
Family Culicidae	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	25.0	25
Ordem Ephemeroptera																
Family Baetidae	0.0	0	0.0	0	25.0	50	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0

Phylum MOLLUSCA																
Class Gastropoda	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	25.0	25	0.0	0
Phylum NEMATODA	0.0	0	0.0	0	25.0	25	0.0	0	0.0	0	0.0	0	50.0	550	0.0	0
Total richness (n) / Density (org./m^2)	**2**	**825**	**2**	**50**	**6**	**12925**	**4**	**1075**	**3**	**6700**	**1**	**225**	**6**	**7725**	**3**	**125**

There were a significant variations in benthic community richness (n) and density (org./m^2) among beaches (Tombo, Enseada, Perequê and Iporanga) and between seasonal periods (rainy and dry) (chi-square, d.f. = 3, $\chi 2$=1804.5, $p < 0.01$). The highest richness of the benthic macrofauna among the sampling sites were obtained during the rainy season in Tombo and Perequê (with 6 taxa each). Enseada and Iporanga recorded a total of 3 and 2 taxa, respectively (Table 4.1).

In general, insects and oligochaetes were frequently observed (highest percent occurrence) in all channels (Table 4.1; Figure 4.2A). In relation to other taxa, distributions were spatially restricted. Polychaetes (family *Spionidae*) were only found in Enseada, Collembola only in Iporanga, mollusks (class Gastropoda) only in Tombo, and Nematodes only in Perequê and Tombo (Table 4.1). Furthermore, the highest density of benthic invertebrates was observed during the rainy season for all sampling points. The locations with the highest amount of benthic invertebrates was Perequê, followed by Tombo, Enseada and Iporanga, respectively (Table 4.1; Figure 4.2B). In general, dipteran insects of the Ceratopogonidae and Chironomidae families were the taxa with the highest densities during the rainy season (Table 4.1). A significant, although weak, relationship was found (linear regression, F1,6=4.95, $p < 0.01$] between the total density of the benthic macrofauna and the percentage of organic matter, with a r^2 of 0.36 (Table 4.1).

The obtained H' and J indexes, showed that all studied channels from both seasonal periods had scores < 1. The BMWP′ index showed a score < 11 for all studied channels and seasonal periods. The ASTP index scored was < 2 for all sites and seasons. For further details, see Table 4.1.

4.4 Discussion

Urban drainage channels are vulnerable to various impacts, such as discharge of domestic effluents and non-point source pollution (Yang et al., 2011; Angrill et al., 2017), that could change the structure of the benthic communities in nearby areas (McQueen et al., 2010; Tang et al., 2013). The main benthic taxa (e.g. Insecta and Olioghochaeta) recorded in the four channels of Guarujá are typically associated with fresh and brackish ecosystems (Hooper et al., 2003; Lin and Yo, 2008), reflecting the contribution of urban surface runoff in these locations (supralittoral region), without the interference of the tidal regime.

In general, the richness (n) and density (org./m^2) of macroinvertebrates of Guarujá were higher during the rainy season, coincidental with the high summer tourist season (Ribeiro and Oliveira, 2015). During precipitation episodes, there is a greater transport of organic and inorganic pollutants (Chebbo and Gromaire, 2004; McLellan, 2004) via urban drainage channels (Sato et al., 2005). Consequently, this organic enrichment can affect the richness and density of pollution-tolerant benthic species and promote their colonisation (Hooper et al., 2003; Moreno and Callisto, 2006). This pattern was observed in the present study since urban channels are potential receptors of organic pollutants, mainly during the rainy season (Cetesb, 2017). Furthermore, a positive trend between density of the benthic community and organic matter was found. In general, the benthic assemblages detected in Guarujá are tolerant to organic pollution and consequently considered indicators of polluted environments (Sánchez et al., 2013): e.g. Gastropoda (Trauben and Olive, 1983), Culicidae (Forattini and Massad, 1998), Spionidae (Méndez et al., 1998), Nematoda (Johnson and Kleve, 2000), *Chironomus* (Hooper et al., 2003), Chironomidae (Meyer et al., 2005), Ceratopogonidae (Arimoro et al., 2007) and Oligochaeta (Lin and Yo, 2008). With the exception of Ephemeroptera of the family Baetidae recorded in Perequê during the rainy season, which are also found in clean water environments (Dohet et al., 2002; Adakole and Anunne, 2003), no other invertebrate macro-indicator of good water quality (e.g. sensitive species such as Plecoptera and Trichoptera groups: Dohet et al., 2002; Adakole and Anunne, 2003) were inventoried throughout the study period in Guarujá. The prevalence of benthic taxa tolerant to organic pollution has been reported in urban rivers and streams (Couceiro et al., 2006; Rodil et al., 2007; Rodil et al., 2008) and in urban surface runoff receiving systems (like Guarujá) (Ferraz et al., 2012; Tixier et al., 2012). Similar to the study of Adakole and Anunne (2003), the organic pollution of Guarujá channels resulted in a decrease in benthic macroinvertebrate species richness and an increase in species density (mainly during the rainy season). The location with the lower species richness and higher species density was Perequê, followed by Tombo, Enseada and Iporanga. This pattern demonstrates that different levels of urbanisation and sanitation facilities are reflected in the quality and quantity of pollutants that flow into urban drainage channels (Carle et al., 2005; Lee et al., 2009; Onderka et al., 2012). In the case of Iporanga (lower richness and density of indicator species of polluted environments), this could be related to the fact that this channel is a receptor of the diffuse loads generated along a conservation unit of sustainable use, with less urbanisation, restricted access of vehicles and tourists, and extensive environmental sanitation (private sewage treatment) (Ribeiro and Oliveira, 2015). Since Perequê and Enseada are receivers of domestic clandestine sewage from irregular occupations (Ribeiro and Oliveira, 2015) and receive the contribution of diffuse loads from urban surface runoff (Cetesb, 2017), this poor condition at both points was expected. However, the conditions found in Tombo were different, since the neighbourhood is widely served by the municipal sanitation network (Ribeiro and Oliveira, 2015), and also because this beach is the only one of the 300 beaches in the state which holds the international Blue Flag certification (Fee, 2018).

Diptera was the main benthic taxa recorded in Guarujá. The high abundance of Diptera collected at the four sampling points was mainly due to the Chironomidae family. This group of insects is quite abundant in environments with lentic features (such as the Guarujá channels) and generally makes up more than 80% of the entomofauna (Merrit and Cummins, 1996). The presence of Chironomidae can be explained by the fact that this group develops resistance and adapts to different environmental conditions (tolerance to different levels of pollution) (Johnson and Kleve, 2000; Bubinas and Jagminiene, 2001). Chironomidae species are abundant in urban stream environments enriched with nutrients and organic matter, but oxygen-depleted waters (Gafner and Robinson, 2007). The presence of Nematoda (Perequê and Tombo) can be explained by the fact that this group constitutes the most common parasites of the Chironomidae family (Johnson and Kleve, 2000). Among the genera Chironomidae, *Chironomus* (detected only at Tombo during the rainy season) is considered an excellent indicator of low-quality water (survival at low dissolved-oxygen conditions) (Hooper et al., 2003). Their presence can be probably explained by the availability of food at this sampling location, since these organisms feed on fine organic matter (Moreno and Callisto, 2006). *Chironomus* are not bloodsuckers, but cause dense swarms. When they emerge in abundance in residential or recreational areas (e.g. the tourist beach of Tombo), they may impair visibility (traffic) or be inhaled by humans and domestic animals (Ali, 1991). The increase in the density of *Chironomus* larvae in environments strongly affected by anthropic activities and with eutrophic characteristics has already been reported (Tate and Heiny, 1995).

The presence of Ceratopogonidae in Perequê (greater abundance during the rainy season), in addition to being a good indicator of poor water quality, also suggests potential public health risks (Arimoro et al., 2007). This family, popularly known as the biting mosquito, has hematophagous females that feed on human and animal blood (Linley et al., 1983). Their bite may cause discomfort, insomnia, irritability and allergic reactions in their hosts (Strickman et al., 1995). The presence of larval habitats on beaches has already been reported as a significant problem for tourism (Strickman et al., 1995). Some Ceratopogonidae are recognised virus vectors for humans and for ruminants (wild and domestic) and may, in some cases, cause veterinary diseases (e.g. encephalitis and equine onchocerciasis) (Mellor et al., 2000).

The presence of Diptera Culicidae (dry season at Tombo), in addition to being a good indicator of polluted water, is a danger to the population living near Tombo beach (Blue Flag certification) (Fee, 2018), since these insects are vectors of diseases (Forattini et al., 1995; Forattini and Massad, 1998). One of the vectors is *Aedes aegypti* (which presents synanthropic and anthropogenic behaviour) that is now one of the main problems for public health in Brazil due to its role as a transmitter of dengue, yellow fever, Chikungunya and Zika (Cardoso et al., 2015). Although this species prefers to reproduce in clean water, it also develops in open-air sewers (situation observed in the Tombo channel) (Tilak et al., 2005).

Oligochaetes have been widely used as bioindicators to reflect organic pollution in rivers and streams (Lin and Yo, 2008), because they include species tolerant to pollution and with opportunistic behaviour (Meyer et al., 2005). Generally, eutrophic water bodies with a dissolved oxygen deficit (Chapman, 2001; Schenková and Helešic, 2006) favour the increase of oligochaetes in comparison to other macroinvertebrates (Schenková and Helešic, 2006). The observed abundance pattern of oligochaetes in this study reflects, once again, the deficit of local sanitation, especially at Tombo beach (Ribeiro and Oliveira, 2015; Cetesb, 2017).

The previous findings are somewhat supported by the results regarding the H', BMWP′ and ASPT indexes. In this study, these three indexes are in concordance with one another, giving more information about the ecological structure of the channels. In the case of the four Guarujá channels, all sampling points had index $H' < 1$ indicating strongly-polluted waters (Magurran, 1988; Mason, 2002; Shekhar et al., 2008). For the BMWP′ and ASPT indexes, the waters of the four Guarujá channels are also highly polluted (BMWP′ < 12 and ASPT < 2) (Junqueira and Campos, 1998; Hammer et al., 2001; Mason, 2002). A situation similar to Guarujá has been observed in other rivers, lakes and urban streams that suffer strong anthropic interference, such as Thachin in Thailand (H' < 2; BMWP′ < 12 and ASPT < 3) (Sripongpun, 2003), Sapanca Lake (H' < 2; BMWP′ < 25 and ASPT < 3) (Arslan et al., 2016a) and Kucuk Menderes River in Turkey (H' < 2; BMWP′ = 8 and ASPT = 3.5) (Arslan et al., 2016b), which are rated as highly polluted water bodies.

4.5 Conclusion

The use of benthic macroinvertebrates as bioindicators proved to be an efficient tool to assess the quality of the water released in the urban drainage channels of Guarujá. This study indicates that the waters of the Guarujá channels are highly polluted, as suggested by the different environmental quality indexes, with the predominance of organisms tolerant to organic pollution and indicators of human health risks. Moreover, the observed temporal variation of the macrofauna was related to the frequency of rainfall, coincidental with the high tourism season. The present study confirms that the biological indices based on macroinvertebrates are good monitoring tools for water-quality assessment. The obtained information is crucial to inform public agencies about the poor environmental conditions of the municipality, allowing them to adopt urgent management measures to improve water quality, and thus minimising the potentials human health risks of the beachgoers.

CRediT authorship contribution statement

Vinicius Roveri: Conceptualization, Data curation, Formal analysis, Methodology, Writing - original draft. Luciana Lopes Guimarães: Supervision, Writing - review & editing. Alberto Teodorico Correia: Funding acquisition, Supervision, Writing - original draft, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors thank Camila Santos Garcia, Thiago Henrique Toscano, Taiane Pires Farias and Flávio Távora Campi for their support in the field work. The authors are grateful to Ronaldo Justo, for the sampling collection authorisation in the Iporanga/Guarujá condominium. Statement of animal rights: All applicable international and/or national guidelines for the care and use of animals were followed. ATC is supported by national funds through FCT — Foundation for Science and Technology, Portugal within the scope of UIDB/04423/2020 and UIDP/04423/2020.

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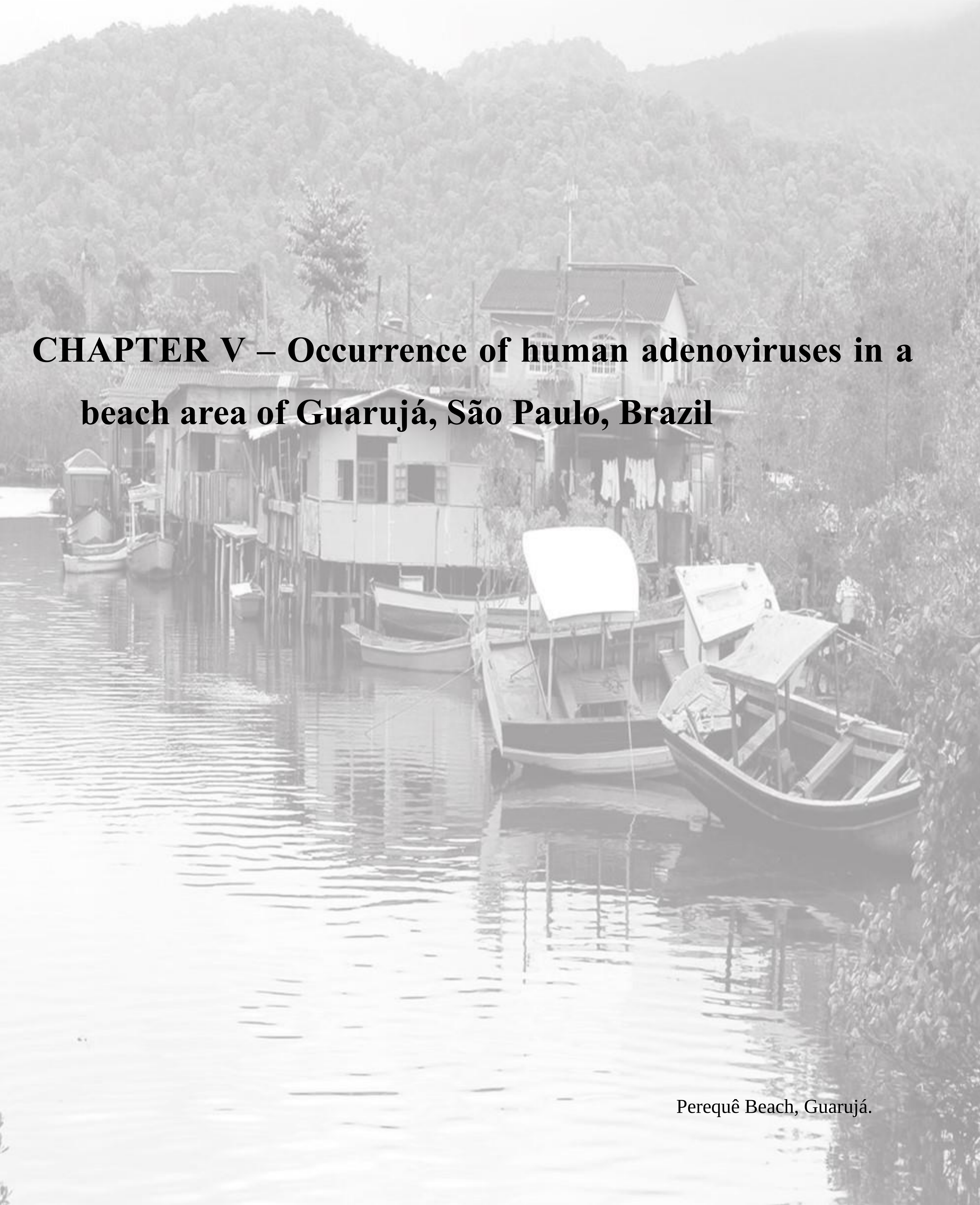

CHAPTER V – Occurrence of human adenoviruses in a beach area of Guarujá, São Paulo, Brazil

Perequê Beach, Guarujá.

Occurrence of human adenoviruses in a beach area of Guarujá, São Paulo, Brazil

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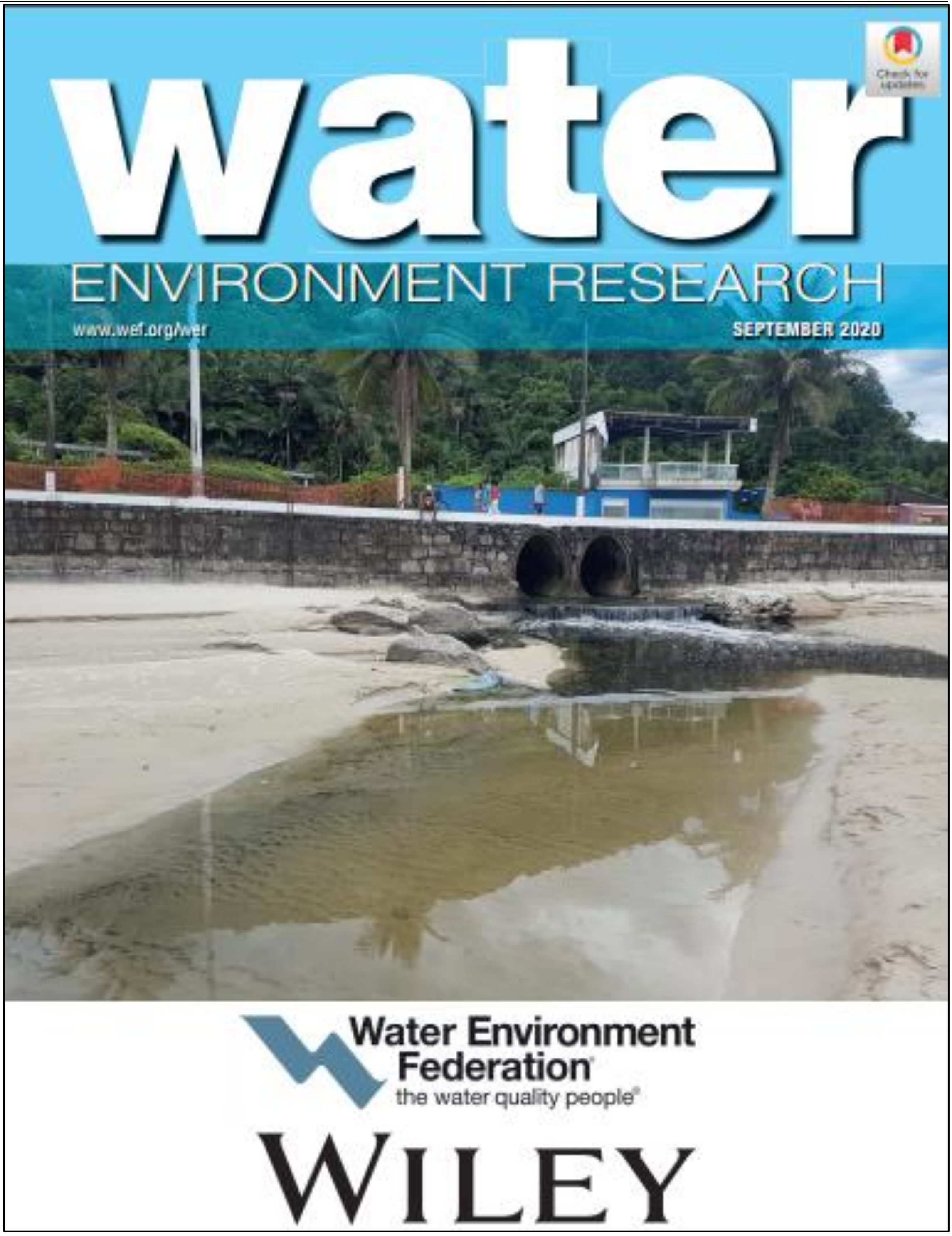
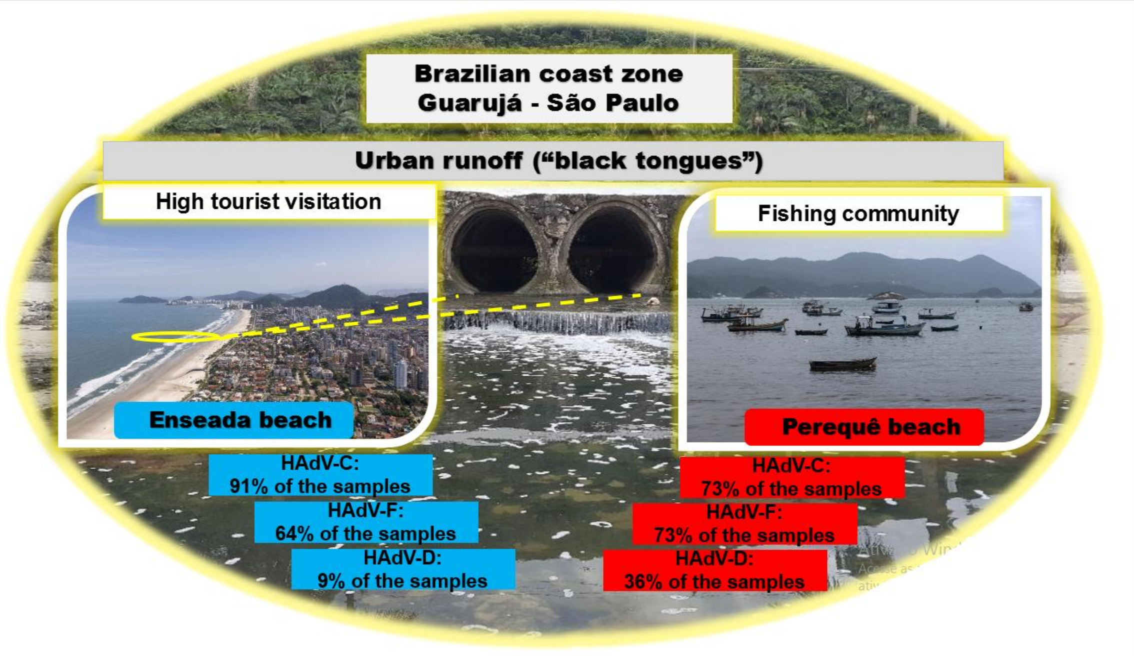
Published in: Water Environment Research (2020).



Editor`s choice. On the cover: Urban runoff water flowing into recreational areas of Enseada Beach, Guarujá, São Paulo State, Brazil. See related article, “Occurrence of human adenoviruses in a beach area of Guarujá, São Paulo, Brazil” by Vinicius Roveri, Luciana Lopes Guimarães, Alberto Teodorico Correia, Meriane Demoliner and Fernando Rosado Spilki. Image courtesy by Vinicius Roveri.

Graphical Abstract



Highlights

- First report on the presence of human adenoviruses in urban drainage channels in the coast of São Paulo State, Brazil.

- Urban surface water runoff is responsible for introducing human adenoviruses linked to disease outbreaks in areas of intense recreation.

- *Cryptosporidium* oocysts and *Giardia* cysts were below the limit of detection for all analysed samples.

- However, all sites were positive for at least one of the viral species (HAdV–C, HAdV–D, or HAdV–F).

- Viral loads found in the water were similar to those commonly found in the wastewater.

Abstract

Along the coastal zone of the State of São Paulo, Brazil, diffuse discharges that flow directly to tourist beaches are responsible for introducing various pathogens into recreational waters. The objective of this study was to analyze, for the first time, the presence of protozoa (*Cryptosporidium ssp* and *Giardia ssp*), as well as human mastadenoviruses (HAdV–species C and F) and other species of adenoviruses (AdV) in beach drainage channels of Enseada and Perequê, municipality of Guarujá, São Paulo, Brazil. Protozoa were not detected in any sample over the course of the 11-month study. In relation to HAdVs, 100% (n=22) of the water samples presented contamination by at least one type of virus (C, D or F species), suggesting potential risks to the public health following recreational exposure of beach users to these waters.

Keywords: Bathing waters, domestic wastewater, microbial indicator, non-point source pollution, subtropical zone.

5.1 Introduction

Urban surface water runoff on tourist beaches, popularly known as "black tongues" (Rocha et al., 2011), has been identified as a potential threat to public health since it is responsible for introducing pathogens linked to disease outbreaks (e.g. protozoa and viruses) in areas of intense recreation (Parker et al., 2010; Lamparelli et al., 2015). Globally, exposure to unsuitable recreational waters causes seasonally several human diseases (Shuval, 2003), such as gastrointestinal and respiratory illnesses (DeFlorio-Barker et al., 2018). Although the risks to this exposure are well documented (Parker et al., 2010; Lamparelli et al., 2015; Staggemeier et al., 2017), no information exists for the coastal area of the State of São Paulo, Brazil. This region represents 10% of the Brazilian coast and has about 600 urban drainage channels, whose waters flow into 290 tourist beaches (Lamparelli et al., 2015; Cetesb, 2017; Ibge, 2018). In Brazil, the recreational water quality criteria are defined by the identification and quantification of thermotolerant coliforms, *Escherichia coli* and *Enterococci* (Brazil, 2000). Although water resource recovery facilities may effectively reduce the level of fecal indicator bacteria, some pathogenic microorganisms, such as protozoa and enteroviruses, are often more resistant to disinfection than indicator bacteria and can cause infections at very low doses (Bonilla et al., 2015). The objective of this study was to analyze, for the first time, the presence of protozoa (*Cryptosporidium ssp* and *Giardia ssp*) and human mastadenoviruses (HAdV–species C and F), and other species of adenoviruses (AdV) in the drainage channels of the beaches of Enseada and Perequê, in the municipality of Guarujá, São Paulo, Brazil. The dataset on the occurrence of these microbiological contaminants will also allow a broad discussion about their potential public health risks.

5.2 Material and Methods

5.2.1 Characterisation of the study area

This study took place in Guarujá, coast of Sao Paulo, Brazil (23° 59' 34" S; 46° 15' 21" W) (Ribeiro and Oliveira, 2015), a region that concentrates a permanent population of approximately 316,000 inhabitants (Ibge, 2018). Precipitation and average annual temperatures in Guarujá reach approximately 3,000 mm and 22°C, respectively. Two quite distinct seasonal periods are observed, one rainy (November through March) and one dry (April through October) (Ribeiro and Oliveira, 2015). The economy of the municipality is driven by the port of Santos (the largest in Latin America) and also by the tourism that takes place along the eight main tourist beaches (Ribeiro and Oliveira, 2015; Cetesb, 2017). These beaches receive daily contributions from urban runoff waters from 43 channels (Cetesb, 2017). Since most of these beaches are located in environmental protection areas and/or have good sanitation facilities, they are regularly classified as "good" and "excellent" in terms of water quality according to monitoring data of the state environmental agency (Cetesb, 2017). However, Enseada (high tourist visitation: 23° 59′ 12″ S; 46° 13′ 38″ W) and Perequê (fishing community: 23° 56′ 05″ S; 46° 10′ 51″W) (Ribeiro and Oliveira, 2015), were rated respectively as "fair" (improper for bathing 25% of the year) and "bad" (improper more than 50% of the year), recommending that pathogens (including protozoa and viruses) should be systematically screened in these beaches (Brazil, 2000). Moreover, these beaches suffer from the disposal of clandestine domestic wastewater (Ribeiro and Oliveira, 2015; Cetesb, 2017).

5.2.2 Sample preparation and analysis

At each beach, a sampling point was selected at the mouth of the channels, in the supralittoral region, without the interference of the tidal regime (Figure 5.1).

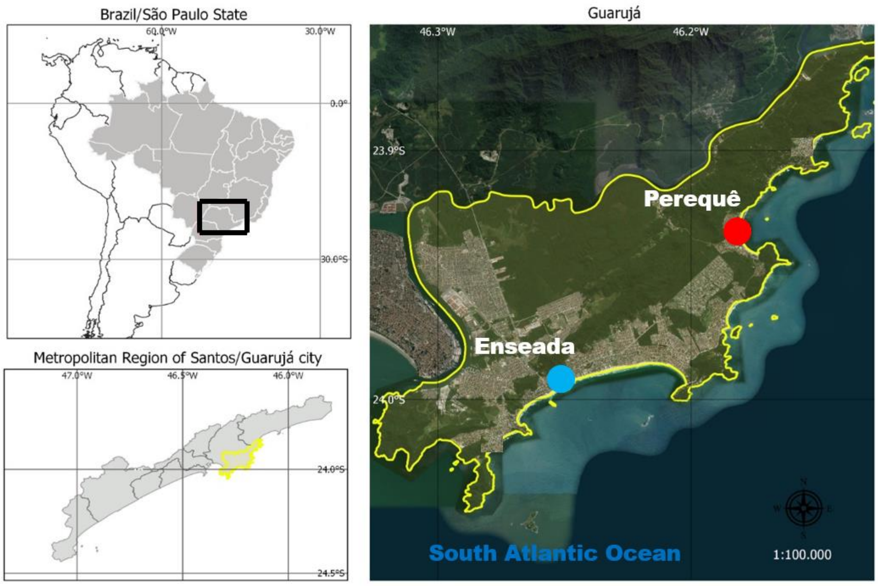


Figure – 5.1: Map of the study area: Brazil/São Paulo State/Metropolitan Region of Santos. Sampling stations of protozoa and enteric viruses collected from urban drainage channels in

Guarujá municipality. Identification of sampling points: Enseada beach (P1) and Perequê beach (P2).

A 10-L water samples were collected monthly in each sampling point between October 2017 and August 2018 (n=22) following a standard methodology (Cetesb, 2011). *Cryptosporidium spp* oocysts and *Giardia spp* cysts analyses were performed in a certified laboratory (accreditation of Inmetro/ABNT ISO/ IEC17025) by immunomagnetic separation (IMS) and immunofluorescence assay (IFA) (USEPA, 2012). Water samples were filtered using a nitrocellulose membrane (1.2 µm pore size). Thereafter, the accumulated sediment on the membrane filter was scraped by using an adequate amount of 0.1% Tween-80 and was aspirated to 10 mL upon centrifugation at 3000 × g for 15 min. The 10 mL eluate was stored at 4°C for further analysis. A commercial kit (Dynabeads GC-Combo, Invitrogen) was utilized to isolate *Cryptosporidium and Giardia* by using an IMS method. The attachment and detachment of (oo) cysts with magnetic beads were made according to an existent protocol (Method 1623: USEPA, 2012). The 50 µL of purified (oo) cysts was then subjected to IFA through Crypto/Giardia cell (CeLLabs). Slides were evaluated using epifluorescence microscopy (Olympus BX51, Japan), and round, oval, or ellipse bright green fluorescence shapes (4–6 µm for *Cryptosporidium* and 8 to 18 µm × 5 to 15 µm for *Giardia*) were identified (USEPA, 2012). All samples that were found to be positive through fluorescein isothiocyanate examination under 400X magnification were subsequently examined through a 4′,6-diamidino-2-phenylindole filter to confirm significant characteristics (light blue/intense blue internal staining and distinct sky blue nuclei). A Crypto/Giardia positive control slide (CeLLabs) was used as positive control. Negative control was prepared by using a phosphate buffered saline solution. The presence of *Cryptosporidium* and *Giardia* were enumerated and calculated to obtain the quantity of (oo) cysts per liter, as follows: Number of (oo)cysts per liter = Number of (oo)cysts on slide (contained by 50 µL)/10 L (USEPA, 2012).

Enteric virus analysis HAdV–C and HAdV–F and other species of AdV, were performed in the Laboratory of Molecular Microbiology, Institute of Health Sciences, Feevale University, Novo Hamburgo, Brazil. All water samples were concentrated by ultracentrifugation method (Girardi et al., 2019). Aliquots of 36 mL were centrifuged, and the precipitates were resuspended with 1mL of Tris-EDTA buffer (pH 8.0). The DNA was extracted from concentrated samples by BioPur® kit, following the instructions of the manufacturer. The qPCR reactions were performed using the VTB1 and VTB2 oligonucleotides pairs (Wolf et al., 2010), and following the volumes and reaction steps previously established (Gularte et al., 2019). For detecting the presence of the AdV genome of different hosts, it was used the region that codifies the DNA-polymerase using pan-adenovirus oligonucleotides external pair (Pol-F and Pol-R) and oligonucleotides internal pair (Pol-nF and Pol-nR) (Li et al., 2010), through of a nested-PCR (Gularte et al., 2019). For the phylogenetic analysis, the DNA sequencing of amplicons obtained from nested-PCR was performed by the Sanger Method. The obtained sequences were aligned with other nucleotide fragments available in GenBank using the BioEdit 7.0.5 software. The phylogenetic tree was elaborated using the Neighbor-Joining method (Saitou and Nei, 1987) whit a bootstrap test (500 replicates). The evolutionary distances were computed using the Kimura 2-parameter method (Kimura, 1980). The evolutionary analyses were conducted in MEGA X (Kumar et al., 2018). The concentration of viruses detected was expressed as genomic copies per L (GC/L).

5.3 Results

Protozoa *Cryptosporidium* and *Giardia* were not detected in any water sample over the 11 months of study. However, for the enteric viruses, all water samples regardless the sampling location showed contamination by, at least, one type of virus. For HAdV–C occurrence ranged from 73% (8/11) to 91% (10/11) for Perequê and Enseada, respectively. For HAdV–F occurrence varied from 64% (7/11) to 73% (8/11) for Enseada and Perequê, respectively. HAdV-D recorded 36% (4/11) and 9% (1/11) of occurrence for Perequê and Enseada, respectively. The overall virus concentration was 10^6–10^8 GC/L for HAdV–C (Figure 5.2A) and 10^3–10^8 GC/L for HAdV–F (Figure 5.2B).

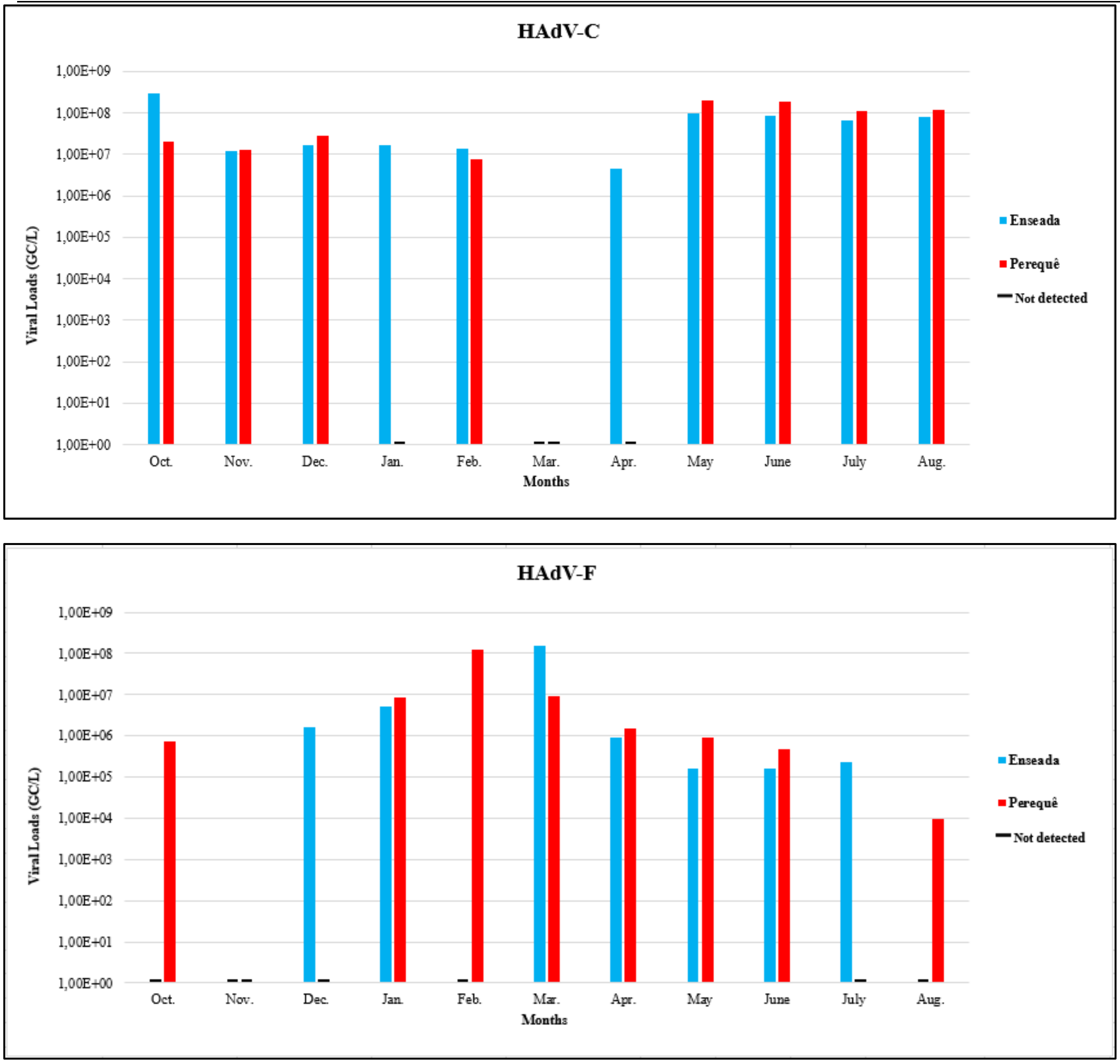


Figure – 5.2: Genomic copies per L (GC/L) quantification of C (HAdV–C) (A) and F (HAdV–F) (B) viruses species detected in the drainage channels of Enseada and Perequê beaches (Guarujá, São Paulo State, Brazil).

HAdV-D was only detected, not quantified. Phylogenetic analysis confirmed the presence of three different species of HAdVs. Of the sequenced samples, 5 were identified as HAdV–C, five as HAdV–D and five HAdV–F (Figure 5.3).

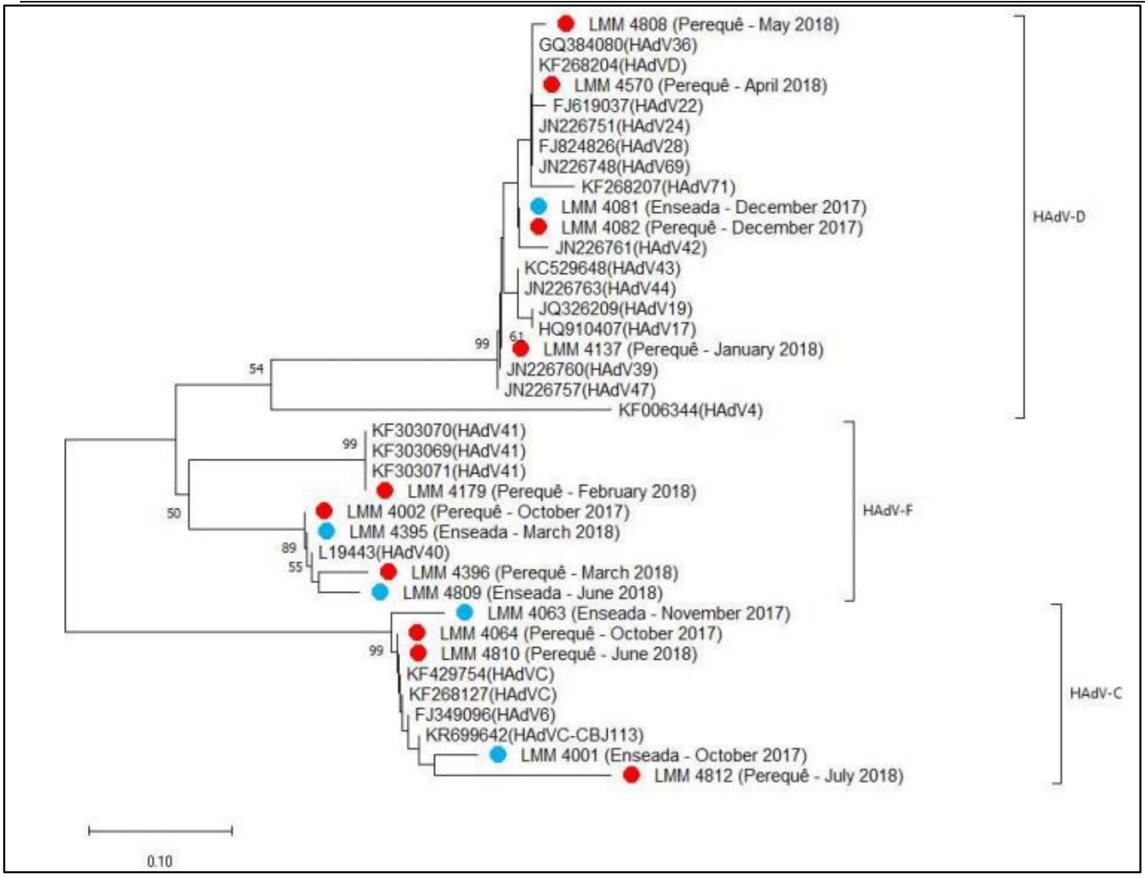


Figure – 5.3: Phylogenetic analysis of HAdVs using the Neighbor-Joining method based on the Kimura 2-parameter model. The tree was constructed using AdV sequences obtained in water samples and reference strains from the NCBI GenBank database. Bootstrap values are indicated at each tree root (percentage of 500 bootstrap replicates that support the branch).

5.4 Discussion

The presence of HAdVs (C, D and F) in the Enseada and Perequê channels proves the deficiency of the environmental sanitation in these beaches (Cetesb, 2017), since these species are important markers of human fecal contamination (Rigotto et al., 2010; Rames et al., 2016). Moreover, a high number of HAdV positive samples were recorded, contrarily to *Giardia* cysts and *Cryptosporidium* oocysts (which are also excreted by human feces) not recorded in the hereby study (Cetesb, 2013). A similar finding was observed in a 12-month study in the coastal waters of Pitangueiras beach, also in Guarujá, where viruses were prevalent compared to protozoa (e.g. Enterovirus, *Giardia* and *Cryptosporidium* were prevalent in 42%, 25% and 0% of the samples, respectively) (Cetesb, 2013). Previous studies also reported HAdVs in environmental samples with a frequency higher than 50% (Rigotto et al., 2010; Dalla Vecchia et al., 2015; Staggemeier et al., 2016). HAdV-C, which recorded a high concentration in the hereby study, seems to a ubiquitous virus in Brazil and has been reported in several coastal areas associated to the lack of sanitation, such as in Rio de Janeiro (93%: Staggemeier et al., 2017) and Rio Grande do Sul (26%) (21%: Girardi et al., 2017; 26%: Gularte et al., 2019). The concentrations detected in Guarujá of HAdVs (C, but also F) are alarming as they are higher, for example, than the maximum concentrations detected in wastewaters of Michigan, USA (HAdV–C and F, viral load range from: 10^6–10^7 GC/L) (Kuo et al., 2010). However, in Brazil, similar viral loads were found in recreational waters in Florianopolis (HAdV: 10^4–10^7 GC/L) (Rigotto et al., 2010) and in Rio de Janeiro (HAdV: 10^6–10^9 GC/L) (Staggemeier et al., 2017). Once in the environment, HAdVs are resistant to environmental stressors (e.g. UV radiation, water temperature and pH) (Rigotto et al., 2010; Girardi et al., 2017) and, therefore, permanent exposure of humans to these viruses (both in beach sand and in seawater) can significantly affect the public health of the municipality (Li et al., 2010; Staggemeier et al., 2017). HAdVs are transmitted via the fecal-oral route and possibly airborne, which can cause asymptomatic (e.g. species D) and symptomatic (e.g. species C and F) infections; HAdV–C, for instance, mainly affects the respiratory tract (Ghebremedhin, 2014). Species C was the most frequently detected viral load in this study, similar to other studies that reported domestic wastewater input into recreational waters (Staggemeier et al., 2017) and urban rivers (Osuolale et al., 2015). HAdVs D and F, on the other hand, cause gastroenteritis. Specifically, species F is considered one of the main etiological agents of childhood gastroenteric infections (Ghebremedhin, 2014). The association between fecal contamination of recreational waters and detection of gastroenteritis indicator viruses is well established (Rigotto et al., 2010; Girardi et al., 2017; Staggemeier et al., 2017). In Guarujá, an outbreak of viral gastroenteritis was reported in the summer of 2010 (between January and March), where 6,300 cases were reported. Curiously, both Enseada and Perequê were among the neighbourhoods with the highest incidence rates. The most commonly detected etiological agents in these patients were noroviruses, transmitted via the consumption of contaminated food and drinking water according to the competent public institutes (Morillo et al., 2011). At that time, no research on adenoviruses was conducted in the drainage channels or recreational waters of Enseada or Perequê. The situation of Guarujá deserves attention, because while noroviruses are frequently detected, although seasonally (situation observed in 2010 in Guarujá), HAdVs are prevalent in environmental waters throughout the year (situation observed in this study) (Rames et al., 2016). Moreover, there is a strong evidence that HAdVs may contribute to gastroenteritis outbreaks. Indeed, it was shown that the number of reported of cases of diarrheal diseases coincides with the presence of HAdV-F in recreational freshwaters (Girardi et al, 2019).

5.5 Conclusion

The present study showed the urgency of conducting further research on viral loads in recreational waters off the coast of São Paulo. In this region, around two million people attend approximately 290 tourist beaches throughout the year, which are continuously exposed to daily discharges from 600 drainage channels. In order to improve the environmental quality of these recreational coastal waters and to reduce the risk to beach users, several actions are urgently required, such as the amelioration of the basic sanitation facilities, land regularization actions and enhancement of the public awareness about the health hazards associated with the use of recreational-water environments.

Acknowledgment

Authors want to acknowledge of Pulsar pictures Ltda, Apiacás St., 934, São Paulo, Brazil, for the license to reproduce images of the beaches of Guarujá (contract nº 27918). Alberto Teodorico Correia is supported by national funds through FCT - Foundation for Science and Technology within the scope of UIDB/04423/2020 and UIDP/04423/2020.

Conflict of interest

The authors declare no conflict of interest.

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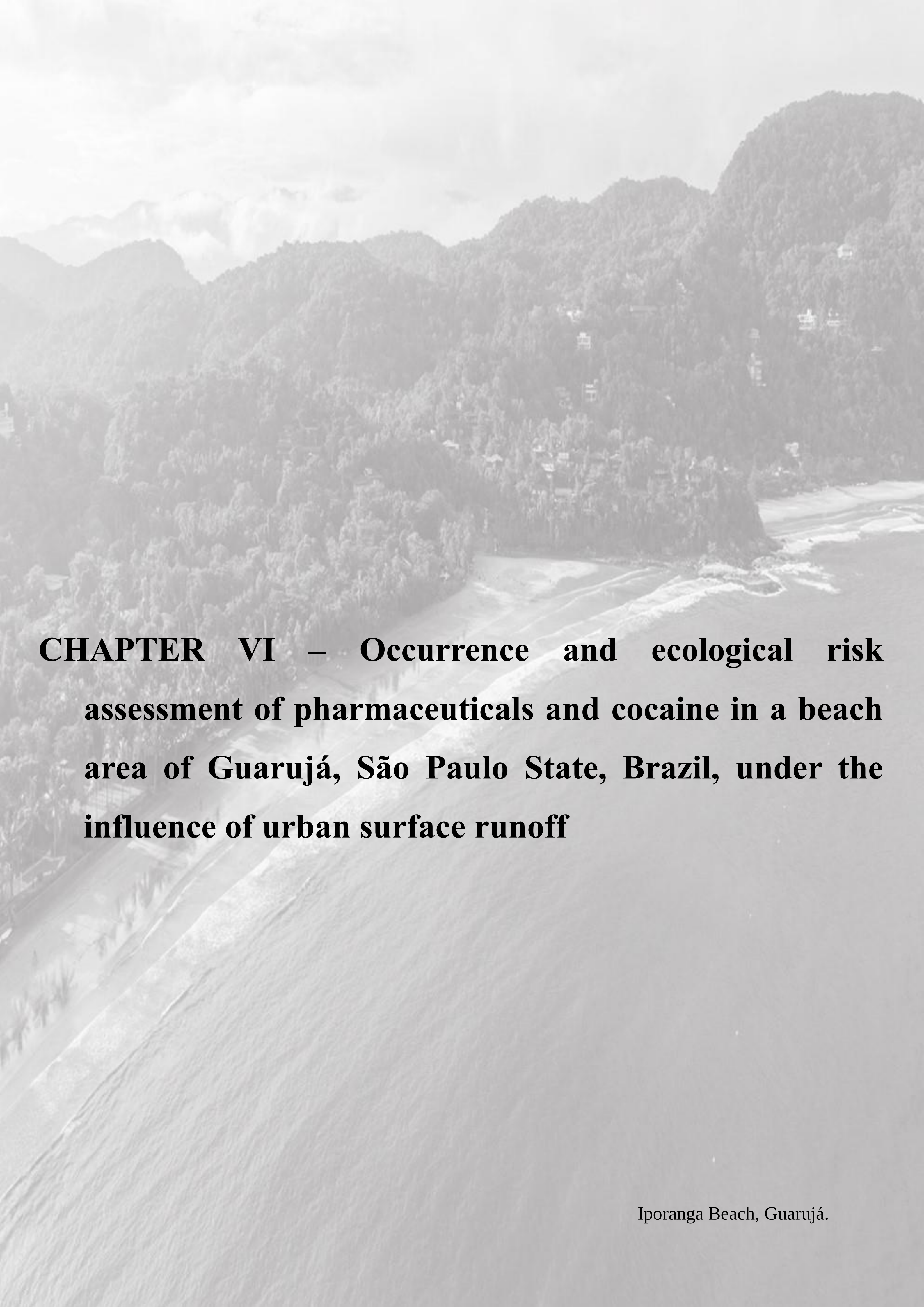

CHAPTER VI – Occurrence and ecological risk assessment of pharmaceuticals and cocaine in a beach area of Guarujá, São Paulo State, Brazil, under the influence of urban surface runoff

Iporanga Beach, Guarujá.

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

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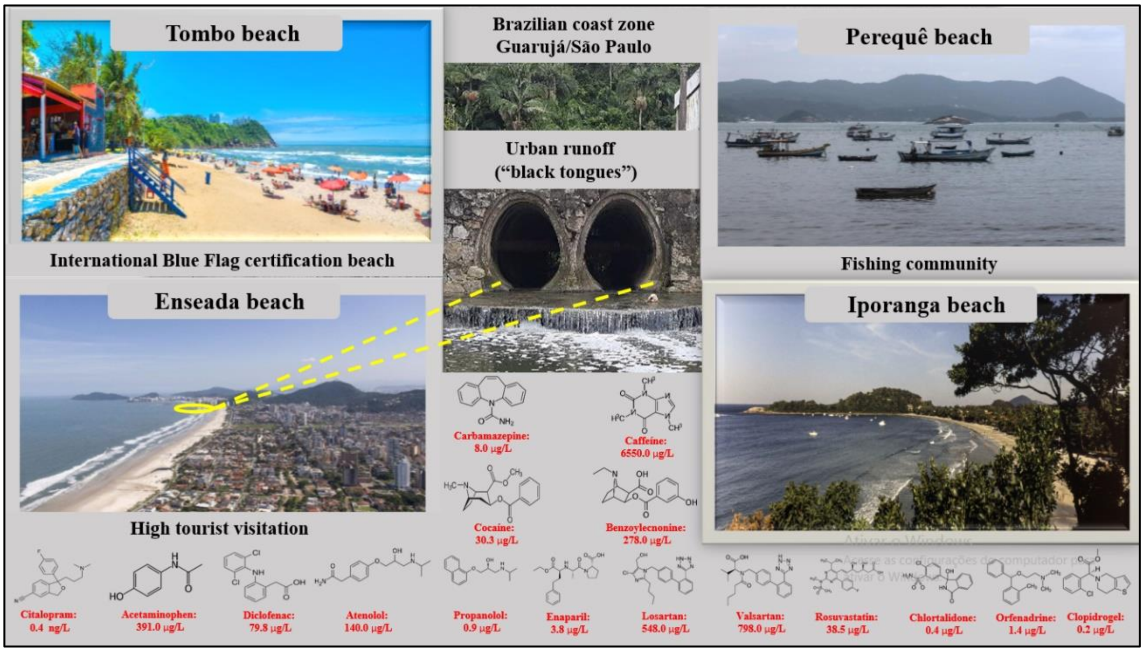
Published in: Environmental Science and Pollution Research (2020).

Graphical Abstract



Abstract

The occurrence of pharmaceuticals and illicit drugs in water resources is widely documented in Europe, North America and Asia. However, in South America, these studies are still incipient. The objective of this study was to screen and identify the presence of pharmaceuticals of various therapeutic classes, including illicit drugs such as cocaine and its metabolite benzoylecgonine, in urban drainage channels that flow into the bathing waters of Guarujá city, State of São Paulo, Brazil. Moreover, the ecological potential risks to the aquatic biota were also assessed. The water samples were collected from four beaches of Guarujá in two different points: in the urban drainage channels and in the nearby coast line. A total of 16 compounds were detected using liquid chromatography coupled with tandem mass spectrometry: carbamazepine (0.1–8.0 ng/L), caffeine (33.5–6550.0 ng/L), cocaine (0.2–30.3 ng/L), benzoylecgonine (0.9–278.0 ng/L), citalopram (0.2–0.4 ng/L), acetaminophen (18.3–391.0 ng/L), diclofenac (0.9–79.8 ng/L), orphenadrine (0.2–1.5 ng/L), atenolol (0.1–140.0 ng/L), propranolol (limit of detection: LOD—0.9 ng/L), enalapril (2.2–3.8 ng/L), losartan (3.6–548.0 ng/L), valsartan (19.8–798.0 ng/L), rosuvastatin (2.5–38.5 ng/L), chlortalidone (0.1–0.4 ng/L) and clopidogrel (0.1–0.2 ng/L). The hereby data also showed that five of these compounds, namely caffeine, acetaminophen, diclofenac, losartan and valsartan, could raise moderate to severe risks to aquatic organisms (algae, crustaceans and fishes). This study is the first report of the occurrence of several pharmaceuticals and illicit drugs in urban drainage channels that flow to the bathing waters in South America, and it is the first quantification of rosuvastatin, chlortalidone and clopidogrel in environmental marine waters of Latin America.

Keywords: Non-point source pollution, Domestic sewage, Pharmaceuticals, Illicit drugs, risk assessment.

6.1 Introduction

Municipal wastewater is being discharged into marine water bodies as world population grows and concentrates in coastal areas (Lolić et al., 2015; Dafouz et al., 2018; Roveri et al., 2020b). These sewers can contain thousands of different chemicals which could compromise the quality of these receiving waters (Quadra et al., 2016; Biel-Maeso et al., 2018; Dafouz et al., 2018). Among these compounds, in the last decade, there has been a growing interest in pharmaceuticals and personal care products (PPCPs), which constitute a group of large numbers of chemical compounds from different therapeutic classes, including pharmaceuticals (e.g. antiepileptics, stimulants, analgesics/anti inflammatory and antihypertensive drugs) (Celle-Jeanton et al., 2014; Lolić et al., 2015; Peña-Guzmán et al., 2019) and illicit drugs (e.g. cocaine) (Pereira et al., 2016; Parolini et al., 2017; Capaldo et al., 2018). These emerging compounds are considered potentially hazardous to the environment because they are ubiquitous, not easily removed by conventional sewage treatment plants and pseudo-persistent (Archana et al., 2017; Diamanti et al., 2019; Fontes et al., 2019), since they enter continuously into the aquatic compartment, affecting water, sediment and biota (Fabbri and Franzellitti, 2015; Cortez et al., 2018; Dafouz et al., 2018). PPCPs are biologically active compounds, and therefore, upon reaching the aquatic ecosystem, they can cause deleterious effects in non-target organisms (Parolini et al., 2016; Pires et al., 2016; Cortez et al., 2018), such as algae (Mano and Okamoto, 2016; Watanabe et al., 2016; Tamura et al., 2017), crustacean (Nunes et al., 2014; Beiras and Tao, 2018; Chen et al., 2019), mollusks (McEneff et al., 2014; Parolini et al., 2016; Cortez et al., 2018) and fishes (Nunes et al. 2015; Parolini et al., 2017; Capaldo et a., 2019). Although concentrations of PPCPs are generally at the ng/L or µg/L levels in surface waters, they can induce severe effects, such as endocrine disruption, inhibition of primary productivity and reduced survival or reproductive success (Fabbri and Franzellitti, 2015; Godoy et al., 2015; Godoy and Kummrow, 2017). All these features have made PPCPs a management priority among leading environmental protection agencies, such as the European Commission (EMA, 2006) and the US Environmental Protection Agency (USEPA, 2017).

Globally, research on the detection of PPCPs in freshwater surface waters under intense anthropogenic pressure is already well documented in Europe (e.g. Milione et al., 2016; Pereira et al., 2017; Burns et al., 2018), North America (e.g. Klosterhaus et al., 2013;

Lara-Martín et al., 2014; Anumol and Snyder, 2015) and Asia (e.g. Hossain et al., 2018; Praveena et al., 2018; Yang et al., 2018). However, in the marine environment, the detection of PPCPs has been neglected for many years under the assumption that dilution would represent a safety factor (Biel-Maeso et al., 2018; Desbiolles et al., 2018). Still, several worldwide monitoring studies to detect the presence of PPCPs have been done in marine environments (e.g. Lolić et al., 2015; Pereira et al., 2016; Dafouz et al., 2018). In South America, specifically in Brazil where the disposal of PPCPs is not regulated, studies aiming to detect the occurrence of PPCPs in coastal waters are however limited (Quadra et al., 2016; Godoy and Kummrow, 2017; Peña-Guzmán et al., 2019).

In addition to the scarcity of studies on pharmaceuticals and illicit drugs in South America, as far it is known, none of these have been dedicated to detect the presence of these emerging pollutants in urban drainage channels that carry the diffuse load into the ocean, along tourist beaches (areas of intense recreation) (Quadra et al., 2016; Starling et al., 2018; Peña-Guzmán et al., 2019). The runoff of these urban surface waters to beaches (popularly known as “black tongues”) (Rocha et al., 2011) has already been identified as a potential threat to the environmental and public health and can be responsible for introducing chemical and biological pollutants related to disease outbreaks in bathing waters (Roveri et al., 2020a; 2020b).

The aim of this study was to investigate, for the first time, the environmental occurrence and toxicity risk assessment of 23 emerging pollutants from different pharmaceutical and illicit drugs classes in water samples from the urban drainage channels that flow to four tourist beaches (Tombo, Enseada, Perequê and Iporanga), located in the municipality of Guarujá, coast of São Paulo, Brazil.

6.2 Material and Methods

6.2.1 Study site description and sample collection

This study was carried out in Guarujá city, a micro-region of Santos municipality, São Paulo State, Brazil. The mean annual precipitation and temperature of the sub-basin reach approximately 3,000 mm and 22°C, respectively. Two quite distinct seasonal periods are observed in the municipality: rainy (November to March) and dry (April to October) seasons. The Guarujá city has an area of 143 km^2 and 64 km of extension, 36 km^2 of which are already completely urbanized. The remaining 107 km^2 are made up of environmental protection areas (SMA/CPLEA, 2016). Guarujá contains a resident population of approximately 316,000 inhabitants (Ibge, 2018). This municipality is one of the main Brazilian tourist destinations (MMA, 2017). In the high tourist season during summer, between December and February, this population practically doubles (Cetesb, 2017). It is estimated that about 20% of this resident population lives in irregular occupations, commonly known as favela, in areas of relevant ecological interest (such as hills and mangroves) (SMA/SPLA, 2018).

The municipality's economy is mainly driven by the port of Santos (the largest in Latin America), located in the western portion of the island, and also by seasonal tourism along the eight main beaches, located in the eastern and southern portions of the island (Ribeiro and Oliveira, 2015). These beaches receive the input of rainwater from superficial runoff, through 43 urban drainage channels. These channels are made of concrete, and none of them has a grated system, meaning that all of their content is discharged directly onto the beaches, on a daily basis, in an area of recreational activities (bathing waters) (Cetesb, 2017). Taking into consideration the different characteristics regarding land use and occupation, four of these urbanised beaches were selected for this study: Tombo (an international Blue Flag certification beach), Enseada (a high tourist visitation), Perequê (a fishing community) and Iporanga (a conservation unit).

At each beach, two sampling points were selected. One point was located at the mouth of the urban drainage channel (beach sand), without the influence of the tidal regime (identified with the letter A). The second water sample was collected at the mouth of this drainage channel, but in seawater (30 cm deep) at a recreation point (identified with the letter B) (Figure 6.1).

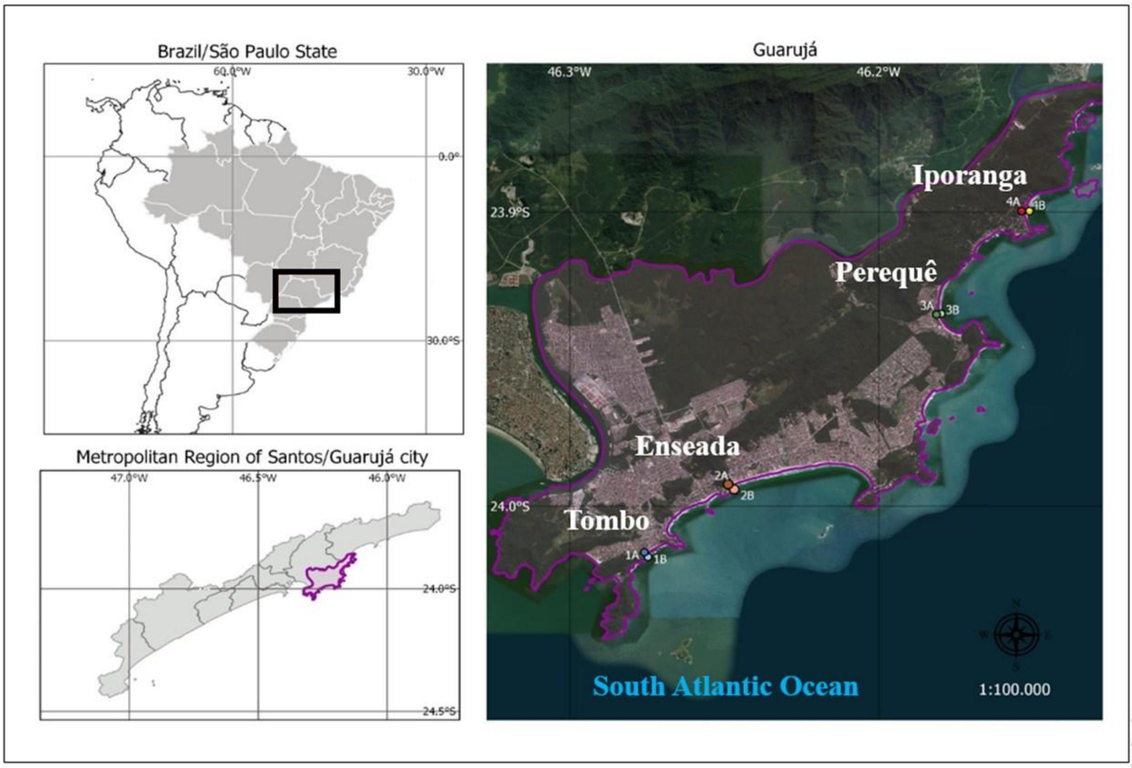


Figure – 6.1: Map of the study area showing Brazil, São Paulo State and the metropolitan region of Santos city. Water sampling locations of the current study in the municipality of Guarujá, namely Tombo (point 1), Enseada (point 2), Perequê (point 3) and Iporanga (point 4) beaches. Letters A and B represent the urban drainage channels and the nearby seawater areas, respectively.

Because it is known that cultural and festive events occurring in different regions may influence water quality by increasing the presence of sewers containing pharmaceuticals and illicit drugs in the environment (Pereira et al., 2016), it was decided to collect samples on Monday, January 15, 2018, during the Brazilian summer, on the day of the municipal holiday of Santo Amaro. No rainfall was recorded 48 hours prior to collection.

Water sampling collection followed a standard procedure (USEPA, 2007). Water samples were packaged into 1-L amber glass bottles previously cleaned, transported to the laboratory in an insulated box with ice (<6°C), filtered with 0.45 µm pore size membrane to remove suspended solids and kept under refrigeration (-20ºC). Further chemical processing occurred within 7 days after filtration.

6.2.2 Preparation and analysis of pharmaceutical compounds

6.2.2.1 Chemical and standards

High purity reagents such as nitric acid and sulphuric acid were purchased from Merck. Organic solvents used in HPLC grade solid phase extraction and LC-MS grade such as acetonitrile, methanol and isopropanol were acquired from Sigma -Aldrich. Mobile phase additives, namely LC-MS grade formic acid and ammonium acetate, were acquired from Sigma-Aldrich and Merck, respectively. Analytical standards of acetaminophen, atenolol, bromazepam, caffeine, carbamazepine, cyproterone, clonazepam, clopidogrel, diclofenac, enalapril, loratadine, losartan, midazolam, orphenadrine, propranolol, sildenafil, and valsartan were acquired from Sigma-Aldrich. Cocaine and benzoylecgonine were acquired from Cerillant. The other pharmaceuticals were bought in several suppliers: Citalopram (Alcytam®, Torrent by Brazil Limited), Clortalidona (Higroton®, Novarts), rosuvastatin (Crestor®, AstraZeneca), and generic paroxetine medication (Meddley).

6.2.2.2 Sample preparation

In this study, the extraction technique was adapted from Wille et al. (2010). Before extraction, the pH of each sample (channel and seawaters) was adjusted to 7.0 using a hydrochloric acid solution (1M). Next, 1-L samples were filtered through Whatman® filter paper (GF/C particle retention 1.2 µm, diameter 47 mm; Merck KGaA, Darmstadt, Germany), and to prevent the loss of the compounds of interest, the filters were washed with 2 mL of methanol (Sigma-Aldrich, St. Louis, USA). The methanol extract collected was then combined to the filtered sample. Subsequently, the SPE (solid phase extraction) procedure using spherical, hydrophobic polystyrene-divinylbenzene resin for SPE cartridges Chromabond ® 163 HR-X, (200 mg, 3 mL, Macherey-Nagel GmbH & Co. KG, Düren, Germany) was accomplished as described by Wille et al. (2010) and Ghoshdastidar et al. (2015). The cartridges were preconditioned with methanol (5 mL) and Milli-Q-Water (5 mL) (Milli-Q® -Merck KGaA). Thereafter, they were loaded with 1-L of the filtered sample and the cartridges were rinsed with 5 mL of Milli-Q water (procedure adopted twice). The cartridges were then dried under vacuum for 30 min. The elution was performed using 2 × 5 mL of methanol and 5 mL of acetone. Prior to the analysis, the concentrated eluate was evaporated to dryness under a nitrogen flow (at 50°C), re-suspended in 1 mL with a solution of water/acetonitrile (95:5, v/v) and then filtered through a 0.45µm pore size membrane (Merck Millipore). Each resuspension was analysed in triplicate using liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS). A concentration factor (1/1000) was used to obtain the measured concentration following LC-MS/MS quantification.

6.2.2.3 LC–MS/MS analysis

Based on the reported annual consumption, expected toxicity and environmental persistence (Cmed, 2017), a total of 23 chemical compounds, namely pharmaceuticals and illicit drugs, were analysed using liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS) (for further analytical details, see the Table S6.1 - supplementary material- online recourse). LC-MS/MS methodology was described and validated by Shihomatzu et al. (2015). The validation was performed using the parameters of selectivity, matrix effect, dynamic range, linearity, limit of detection (LOD), limit of quantification (LOQ), precision (% RSD), accuracy (% CV), recovery and robustness. An aliquot (10 µL) of each sample was analysed using an Agilent 1260 Infinity HPLC (Agilent™, Germany) combined with a hybrid triple quadrupole/LIT instrument (3200QTRAP® - linear ion trap) mass spectrometer (ABSciex, Ontario, Canada). The conditions used for the liquid chromatography (LC) separation were as follows: an injection volume of 10 µL of each sample was loaded in an Agilent Zorbax Eclipse XDB – C18 column (50 × 4.6 mm ID, 1.8 µm column at 25°C). The eluent flow rate was 0.7 mL/min, and the mobile phase for positive mode analysis was 0.1% formic acid (Sigma Aldrich; LC–MS Grade) in solvent A (water) and solvent B (acetonitrile) (J.T. Baker, Philipsburg, NJ, USA). For negative mode analysis, the mobile phase was a 5 mM ammonium acetate buffer (Sigma Aldrich) with a pH of 4.6 (solvent A) and acetonitrile (solvent B). For both modes of ionisation (negative and positive), a linear gradient of 0.7 mL/min was used, starting with a mixture of solvent A (95%) and solvent (5%). The solvent A percentage was decreased linearly from 95% to 5% over the course of 5 min and this condition was maintained for 1 min. Over the course of 2 min, the mixture was then returned to the initial conditions. Using electrospray ionisation (ESI: positive and negative modes) and Multiple Reaction Monitoring (MRM mode) analytes were detected and quantified. This procedure was performed with the selection of a precursor ion and two ion products to quantify and qualify each compound. MRM parameters for the positive and negative modes for each chemical compound, LOD and LOQ are shown in Table S6.1. A sweater matrix-matched external calibration curve was employed, as described by Shihomatzu et al. (2015). LOD and LOQ values were determined, using spiked matrix samples and obtained from seven measurements of the lowest detectable concentration of the calibration curves (with signal-to-noise ratio of at least 10), following the Brazilian Institute of Metrology, Quality and Technology procedures (INMETRO, 2011). Both field and laboratory blanks were below LOD. Data analysis was performed with Analyst® 1.5.2 software (ABsciex). Results, expressed in ng/L, were the average value of the three technical replicates for each concentrated water sample (Table 6.1).

6.2.3 Ecological risk assessment

The ecological risk assessment was performed calculating the risk quotient (RQ) for 3 different aquatic organisms (algae, crustaceans and fishes) following the equation RQ = MEC/PNEC, in which MEC is the maximum Measured Environmental Concentration, and PNEC the Predicted No Effect Concentration, both expressed in ng/L. PNEC values were obtained from base-set reliable ecotoxicity data available for the aquatic compartment regarding short-term [Lethal Concentration 50 (LC50) or median Effective Concentration (EC50)] and long-term [No Observed Effect Concentration (NOEC)] toxicological endpoints. In the absence of NOEC, the Lowest Observed Effect Concentration (LOEC) or, in alternative, the 10% Effective Concentration (EC10) were used, when available. Since urban drainage channels are already recognised as an important transport mechanism of conventional pollutants (chemical and biological) to Ocean Atlantic (Roveri et al., 2020a; 2020b), it was decided to measure ecological risk through marine species. According to the existent studies and current marine risk assessment practices, a reasonable correlation exists between the ecotoxicological responses of freshwater and saltwater biota, at least for the usual aquatic taxa (i.e., acute and chronic toxicity to algae, crustaceans and fishes) (EMA, 2006; Li et al., 2012; Thomaidi et al., 2015). In this context, an attempt was made to compile specifically PNEC data for marine and coastal species. When these data were not available, data from freshwater communities were used. In order to collect available ecotoxicity test endpoints, an extensive search was carried out in the Ecotoxicology Database (ECOTOX) from the United States Environmental Protection Agency (USEPA, 2019), as well as in other literature sources using the PubMed database. When ecotoxicity laboratory experimentally derived data were not available, short [L(E)C50] and long toxicological endpoint [Chv, geometric mean of NOEC and LOEC, ChV=10^([log(NOECxLOEC)]/2)] were estimated using the Ecological Structure Activity Relationships Program

(ECOSAR, v 2.0) (USEPA, 2017). The derived PNEC values for the acute and chronic toxicity data were thereafter calculated by dividing each toxicological endpoint by an assessment factor (AF). For saltwater environments, an AF of 10 000 and 100 should be considered in short and long-term data sets, respectively. For further details, see the European Chemical Bureau (ECB, 2003) and the European Chemicals Agency (ECHA, 2008) guidelines. The toxic data that is selected as the toxicological benchmarks for the calculation of the PNECs is shown in Table 2 and Table S6.2. Finally, the risk ($RQ=MEC/PNEC$) was categorized into four levels: no ($RQ<0.01$), low ($0.01\leq RQ<0.1$), moderate ($0.1\leq RQ<1.0$) and high ecological risk ($RQ\geq 1.0$) to aquatic organisms (Hernando et al., 2006).

6.3 Results and Discussion

6.3.1 Occurrence of PPCPs in Guarujá

In light of the lack of data on the occurrence of pharmaceuticals and illicit drugs in tropical coastal zones (Pereira et al., 2016), this study screened and identified, for the first time, the occurrence of PPCPs of various therapeutic classes, including illicit drugs such as cocaine and its metabolite benzoylecgonine, in urban drainage channels that flow into bathing waters of Guarujá City, São Paulo State, Brazil. These channels receive the input of rainwater from urban surface runoff, and none of them has a grating system, meaning that all of their content is daily discharged directly onto the beaches, in an area of extensive recreational activities (Cetesb, 2017). In the present study, 16 PPCPs were successfully detected in the beaches of Guarujá, almost covering all the target compounds screened (excluding anxiolytics, contraceptives, antihistamines and sexual stimulants): five antihypertensives, three stimulants and three analgesics/anti-inflammatory, one antiepileptic, one antidepressant, one anticholesteremic, one diuretic and one antiplatelet drug (Table 6.1).

- **Table - 6.1:** Results of the occurrence and concentrations of 16 pharmaceutical therapeutic classes and illicit drugs (cocaine and its metabolite benzoylecgonine) on the shoreline of Guarujá, São Paulo, Brazil. At each selected beach (Tombo, Enseada, Perequê and Iporanga), samples were collected in the urban drainage channels (urban surface runoff) and in nearby seawater (bathing waters). For more details about these beaches, see Figure 6.1. Concentrations (average) are expressed in ng/L. * and ** mean below limits of detection (LOD) and quantification (LOQ), respectively. For more details about the analytical methodology see MS, "Preparation and analysis of pharmaceutical compounds" and Table S6.1. Italicised values represent the maximum measured environmental concentrations (MEC) for each compound.

Compound	**Tombo**		**Enseada**		**Perequê**		**Iporanga**	
	(channel –1A)	(seawater – 1B)	(channel - 2A)	(seawater - 2B)	(channel - 3A)	(seawater - 3B)	(channel - 4A)	(seawater - 4B)
Antiepileptic								
Carbamazepine	0.2	0.1	5.0	1.8	8.0	1.0	0.1	< 0.01**
Stimulants								
Caffeine	2690.0	358.0	6550.0	1300.0	2960.0	372.0	33.5	350.0
Cocaíne	1.4	3.1	30.3	13.6	17.6	4.3	0.2	2.3
Benzoylecgonine	7.4	7.7	278.0	67.0	242.0	23.5	0.9	7.6
Antidepressant								
Citalopram	< 0.6*	< 0.6*	0.4	0.2	< 0.6*	< 0.6*	< 0.6*	< 0.6*
Analgesic/anti-inflammatory								
Acetaminophen	79.9	< 1.4*	142.0	17.5	391.0	18.3	< 1.4*	< 1.4*
Diclofenac	0.9	1.7	11.3	12.1	79.8	17.4	4.1	8.4
Orphenadrine	< 0.9*	0.4	1.5	1.4	< 0.9*	0.2	< 3.4**	0.1
Antihypertensive								
Atenolol	2.3	0.1	75.9	2.2	140.0	10.7	< 1.6*	< 1.6*
Propranolol	< 1.3*	< 1.3*	0.9	< 1.3*	< 1.3*	< 1.3*	< 1.3*	< 1.3*
Enalapril	< 3.0*	< 3.0*	3.8	2.7	< 3.0*	2.2	< 3.0*	< 3.0*
Losartan	25.8	3.6	260.0	70.2	548.0	72.0	< 0.7*	< 0.7*
Valsartan	221.0	20.2	458.0	103.0	798.0	217.0	19.8	23.1
Anticholesteremic								
Rosuvastatin	38.5	< 0.8*	10.3	< 0.8*	2.5	< 0.8*	< 0.8*	< 0.8*
Diuretic								

Chlorthalidone	< 2.3*	< 2.3*	0.2	0.1	0.4	0.1	< 2.3*	< 2.3*
Antiplatelet								
Clopidogrel	0.2	< 0.04*	0.1	0.1	0.1	< 0.04*	< 0.04*	< 0.04*

For the water sampling points located directly at the mouth of the urban drainage channels (1A-4A), the most frequently detected compounds were carbamazepine (4/4: 100%) caffeine (4/4: 100%), cocaíne (4/4: 100%), benzoylecgonine (4/4: 100%), diclofenac (4/4: 100%), valsartan (4/4: 100%), acetaminophen (3/4: 75%), atenolol (3/4: 75%), losartan (3/4: 75%), rosuvastatin (3/4: 75%), clopidogrel (3/4: 75%), citalopram (1/4: 25%), orphenadrine (1/4: 25%), propranolol (1/4: 25%), enalapril (1/4: 25%) and chlorthalidone (2/4: 50%). MEC ranged between 0.2 and 6550 ng/L, such as summarized in Table 6.1. The concentrations of the PPCPs were typically below 1000 ng/L. However, 50% of the caffeine samples exceeded 1000 ng/L (Table 6.1 and Figure 6.2).

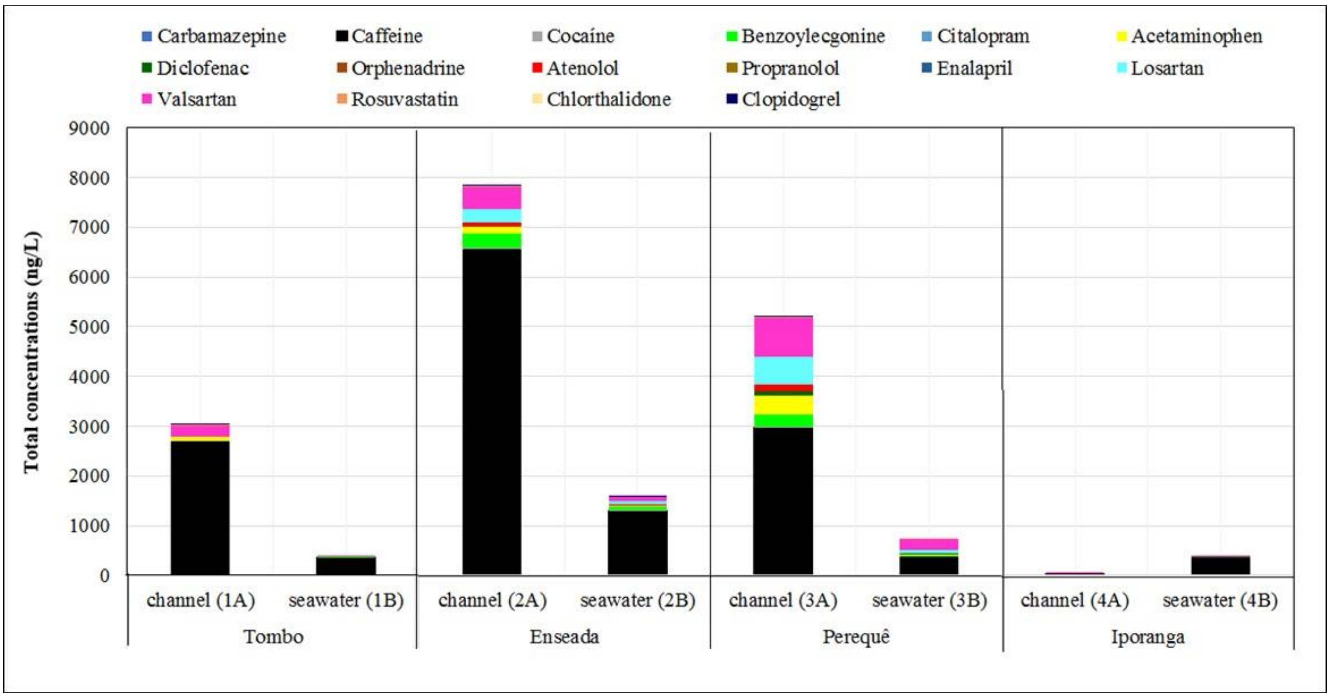


Figure – 6.2: Total concentrations of 16 pharmaceutical and personal care products (PPCPs) and illicit drugs (cocaine and its metabolite benzoylecgonine) detected on the shoreline of Guarujá, São Paulo, Brazil. At each selected beach (Tombo, Enseada, Perequê and Iporanga), samples were collected in the urban drainage channels (urban surface runoff) and in nearby seawater (bathing waters). Letters A and B represent the urban drainage channels and the nearby seawater areas, respectively.

Caffeine is found in many consumer products, such as coffee, tea, soft drinks, chocolate and painkillers, and has been reported worldwide with high occurrence (Dafouz et al., 2018; Yang et al., 2018; Griffero et al., 2019). In Guarujá, this product is probably coffee, because of the long tradition of Brazil in the coffee industry (Quadra et al., 2016). In overall, these results were consistent with most of the worldwide published studies on pharmaceuticals and illicit drugs found in surface waters: USA (Klosterhaus et al., 2013;

Lara-Martín et al., 2014; Anumol and Snyder, 2015), Europe (Milione et al., 2016; Pereira et al., 2017; Burns et al., 2018), Asia (Hossain et al., 2018; Praveena et al., 2018; Yang et al., 2018) and Latin America (Pereira et al., 2016; Rivera-Jaimes et al. 2018; Griffero et al., 2019). In some cases, MEC detected in Guarujá, were much higher than those reported in different environmental matrices around the world, with exception of enalapril, chlorthalidone and clopidogrel, (Table S6.3). In Guarujá, the consumption and disposal of these pharmaceuticals and illicit drugs may have been motivated by tourism, since collecting water samples took place in the high summer season. During the summer season, holidays and weekends, the population increases, as well as the consumption of pharmaceuticals and illicit drugs in the cities (Pereira et al., 2016; Fontes et al., 2019).

The occurrence and concentration of the PPCPs found in the seawater sampling points (1B-4B) nearby the drainage channels, will depended on the physicochemical properties of the target compounds, degradation processes and water dispersal processes (Biel-Maeso et al., 2018). Ten PPCPs did not show changes in the frequency of occurrence in relation to the respective sampling points of the channels: caffeine (100%), cocaine (100%), benzoylecgonine (100%), diclofenac (100%), valsartan (100%), atenolol (75%), losartan (75%), chlorthalidone (50%), citalopram (25%), orphenadrine (25%). These findings suggest therefore that they are ubiquitous and environmentally pseudo-persistent (Diamanti et al., 2019; Fontes et al., 2019). However, six PPCPs showed changes in the frequency of occurrence after the dilution in seawater: carbamazepine (reduction observed in Iporanga: 3/4 - 75%), acetaminophen (reduction observed in Tombo: 2/4 - 50%), rosuvastatin (was not detected at any point: 0/4 - 0%), clopidogrel (reduction observed in Tombo and Enseada: 1/4 - 25% in both points) and propranolol (reduction observed in Perequê - 0/4: 0%) (Table 6.1 and Figure 6.2). Regarding enalapril, unexpectedly, there was an increase in its frequency of occurrence. At Enseada beach, enalapril was not detected in the channels (3A), but was detected at seawater (3B) [enalapril (2/2: 50%)] (Table 6.1). In this way, it is possible that other similar channels (there are 39 in Guarujá with similar characteristics: Cetesb, 2017), are also contributing to the PPCPs (e.g. enalapril) input in the Guarujá seawaters. Moreover, from the eight PPCPs detected in Guarujá seawaters (e.g. caffeine, cocaine, benzoylecgonine, valsartan, losartan, acetaminophen, atenolol and diclofenac), some of them considered bestselling drugs in Brazil (Cmed, 2017), many recorded concentrations above the surface water safety limits (10 ng/L) (Table 6.1) (EMA, 2006). These high concentrations prove that the supposed dilution of PPCPs into the marine environment does not always represent a safety factor and therefore deserves attention (Fabbri and Franzellitti, 2015).

In addition, even some PPCPs that have been detected in low concentrations (≤ 10 ng/L, Table 6.1), six of them (e.g. diclofenac, losartan, clopidogrel, citalopram, orphenadrine and valsartan) deserve attention because of their high n-octanol/water partition coefficients (log Kow ≥ 3: Table S6.3), which indicate that they could bioaccumulate and exert toxicity (European Commission, 2003; Mendoza et al., 2015; Pereira et al., 2016). The remaining ten PPCPs (e.g. propranolol, rosuvastatin, enalapril, carbamazepine, cocaine, chlorthalidone, benzoylecgonine, acetaminophen, caffeine and atenolol), have low potential for bioaccumulation (log Kow < 3: Table S6.3) (Mendoza et al., 2015; ECOSAR, 2017; Fontes et al., 2019).

The discharge of domestic sewage, which includes waste water containing pharmaceuticals and illicit drugs (original, metabolised or conjugated forms), is the main route by which these emerging pollutants reach the aquatic environment (Fabbri and Franzellitti, 2015; Biel-Maeso et al., 2018; Dafouz et al., 2018). Indeed, the highest concentrations (87% of the MEC) were detected mainly in the beaches of Perequê (7 occurrences) and Enseada (6 occurrences) (Table 6.1 and Figure 6.2), both with sanitation deficiencies. Historically, sewage discharge stowaways in drainage channels usually occur in these neighbourhoods, which lead to diffuse loads ("black tongues") flowing directly into the marine environment (Roveri et al., 2020a). However, although in lower concentrations, PPCPs have been also recorded in channels that cross the city's neighbourhoods and that are served by sanitation networks, such as Tombo (a Blue Flag certified beach served by the municipal sanitation network) and Iporanga (a conservation unit area with its own sewage treatment). Thus, considering the presence of two recognized sewage markers (carbamazepine and caffeine) (Aguirre-Martínez et al., 2015; Dafouz et al., 2018), it is evident that there is a clandestine discharge of sewage into the Tombo and Iporanga channels. This study confirms that urban runoff is a potential driver of emerging pollutants (e.g. pharmaceuticals and illicit drugs) into receiving water bodies (seawater), besides being recognised as an important transport mechanism of conventional pollutants (chemical and biological) (Roveri et al., 2020a; 2020b).

Although the literature suggests that the presence of compounds can be detected in coastal environments due to discharge of sewage or terrestrial input, their concentrations are generally below the limits of detection in the seawater owing to the degradation processes effect and/or the dilution (Biel-Maeso et al., 2018), which was not the present situation. Moreover, and based on the recent reviews (Starling et al., 2018; Peña-Guzmán et al., 2019), the hereby work seem to be the first report on the occurrence of rosuvastatin, chlortalidone and clopidogrel in the urban water cycle in Latin America, and therefore, studies on the risks of these PPCPs to humans and aquatic biota are required.

6.3.2 Environmental risk assessment

The ecological risk assessment of pharmaceuticals and illicit drugs released in the aquatic compartment is of great importance to protect the environmental and public health. Thus, considering a worst case scenario in accordance with the Technical Guidance Document on Risk Assessment of the European Union (ECB, 2003), a screening-level of environmental risk assessment (RQ) was conducted for the hereby reported PPCPs. PNEC were estimated from data available in the scientific peer-reviewed literature or estimated by the Ecosar program, as described in Table 6.2 and Table S6.2. In fact, the shortfall of toxicity data for marine organisms was a major hindrance to the effective risk assessment of these PPCPs resulting in only 30% of the RQ calculations. It means that the present study also adopted toxicity data from freshwater species to calculate the PNEC (Borecka et al., 2015). Moreover, the chronic toxicity data for most of the PPCPs are still scarce (Borecka et al., 2015; Archana et al., 2017). Thus, 60% of hereby chronic PNEC were estimated using ECOSAR (Table 6.2 and Table S6.2) (USEPA, 2017). Therefore, the present study reinforces the need for further ecotoxicological studies (especially with tropical marine organisms) to assess the acute and chronic toxicity of these bioactive compounds (Borecka et al., 2015; Biel-Maeso et al., 2018)

Table - 6.2: Results from the ecological risk assessment tests (RQ) regarding the pharmaceuticals of the different therapeutic classes and illicit drugs) detected on the shoreline of Guarujá, São Paulo, Brazil, that pose some risk for the aquatic biota. The table presents the name of each compound; MEC: maximum measured environmental concentration in the Guarujá water body (ng/L); acute and chronic toxicity data: [(trophic level; organism's test, toxicological endpoint and concentration (ng/L)], assessment factor (AF), predicted no-effect concentration (PNEC, ng/L) and risk quotients (RQ, signalled in white, green, yellow and red for no, low, moderate and high risk, respectively). Data from the toxicological endpoints was obtained from several published works (reference) available from the Ecotoxicology Database (ECOTOX), or, in the absence of derived experimentally data, estimated from the ECOSAR program. Note: 1 freshwater; 2 seawater; EC10: 10% effective concentration; EC50: 50% effective concentration; LC50: 50% lethal concentration; NOEC: no observed effect. concentration; LOEC: lowest observed effect concentration. For more details, see MS, "Ecological risk assessment". For all screened compounds, see Table S6.2 (online material).

		Toxicity data							
Compound	**MEC** (ng/L)	**Trophic level**		**Organism/Species**	**Endpoint** (ng/L)	**Reference**	**AF**	**PNEC** (ng/L)	**RQ**
Carbamazepine	8.0	Acute	Algae	*Skeletonema marinoi* $^{(2)}$	72h EC50 (1.00E+08)	Minguez et al. (2014)	10^4	1.00E+04	<0.01
			Crustacea	*Artemia salina* $^{(2)}$	48h EC50 (1.00E+08)	Minguez et al. (2014)		1.00E+04	<0.01
			Fish	*Oryzias latipes* $^{(1)}$	48h EC50 (3.52E+07)	Kim et al. (2007)		3.52E+03	<0.01
		Chronic	Algae	*Lemna gibba* $^{(1)}$	LOEC/2 (5.00E+05)	Brain et al. (2004)	10^2	5.00E+03	<0.01
			Crustacea	*Ceriodaphnia dubia* $^{(1)}$	NOEC (2.50E+04)	Ferrari et al. (2003)		2.50E+02	0.03
			Fish	*Danio rerio* $^{(1)}$	NOEC (2.50E+07)	Ferrari et al. (2003)		2.50E+05	<0.01
Caffeine	6550.0	Acute	Algae	*Pseudokirchneriella subcapitata* $^{(1)}$	72h LC50 (3.39E+08)	Blaise et al. (2006)	10^4	3.39E+04	0.19
			Crustacea	*Daphnia dubia* $^{(1)}$	48h LC50 (5.00E+07)	Moore et al. (2008)		5.00E+03	1.31
			Fish	*Pimephales promelas* $^{(1)}$	48h LC50 (8.00E+07)	Moore et al. (2008)		8.00E+03	0.82
		Chronic	Algae	*Lemna gibba* $^{(1)}$	LOEC/2 (5.00E+05)	Brain et al. (2004)	10^2	5.00E+03	1.31
			Crustacea	*Ceriodaphnia dubia* $^{(1)}$	NOEC (2.00E+07)	Brain et al. (2004)		2.00E+05	0.03
			Fish	*Pimephales promelas* $^{(1)}$	NOEC (3.00E+07)	Brain et al. (2004)		3.00E+05	0.02
Cocaine	30.3	Acute	Algae	Green algae $^{(1)}$	96h EC50 (4.35E+06)	ECOSAR	10^4	4.35E+02	0.07
			Crustacea	Daphnid $^{(1)}$	48h LC50 (5.48E+06)	ECOSAR		5.48E+02	0.06
			Fish	Fish $^{(2)}$	96h LC50 (4.86E+07)	ECOSAR		4.86E+03	<0.01
		Chronic	Algae	Green algae $^{(1)}$	ChV (1.46E+06)	ECOSAR	10^2	1.46E+04	<0.01
			Crustacea	Mysid $^{(2)}$	ChV (2.29E+09)	ECOSAR		2.29E+07	<0.01
			Fish	Fish $^{(2)}$	ChV (7.18E+06)	ECOSAR		7.18E+04	<0.01

Acetaminophen	391.0	Acute	Algae	*Phaeodactylum tricornutum* (2)	72h EC50 (2.39E+08)	Claessens et al. (2013)	10^4	2.39E+04	0.02
			Crustacea	*Artemia salina* (2)	48h EC50 (1.00E+08)	Minguez et al. (2014)		1.00E+04	0.04
			Fish	*Oryzias latipes* (1)	48h EC50 (2.66E+08)	Kim et al. (2007)		2.66E+04	0.02
		Chronic	Algae	*Phaeodactylum tricornutum* (2)	72h EC10 (7.21E+07)	Claessens et al. (2013)	10^2	7.21E+05	<0,01
			Crustacea	*Daphnia magna* (1)	NOEC (4.03E+05)	Kim et al. (2007)		4.03E+03	0.10
			Fish	*Danio rerio* (1)	LOEC/2 (5.00E+03)	Galus et al. (2013)		5.00E+01	7.82
Diclofenac	79.8	Acute	Algae	*Dunaliella tertiolecta* (2)	96h EC50 (1.86E+08)	DeLorenzo and Fleming (2007)	10^4	1.86E+04	<0,01
			Crustacea	*Artemia salina* (2)	48h EC50 (1.00E+08)	Minguez et al. (2014)		1.00E+04	<0,01
			Fish	*Danio rerio* (1)	72h LC50 (7.80E+06)	Van den Brandof and Montforts (2010)		7.80E+02	0.10
		Chronic	Algae	*Lemna minor* (1)	NOEC (3.75E+06)	Cleuvers (2003)	10^2	3.75E+04	<0,01
			Crustacea	*Ceriodaphnia dubia* (1)	NOEC (1.00E+06)	Ferrari et al. (2003)		1.00E+04	<0,01
			Fish	*Danio rerio* (1)	NOEC (4.00E+06)	Ferrari et al. (2003)		4.00E+04	<0,01
Atenolol	140.0	Acute	Algae	*Phaeodactylum tricornutum* (2)	72h EC50 (2.62E+08)	Claessens et al. (2013)	10^4	2.62E+04	<0.01
			Crustacea	*Artemia salina* (2)	48h EC50 (1.00E+08)	Minguez et al. (2014)		1.00E+04	0.01
			Fish	*Oryzias latipes* (1)	96h LC50 (1.00E+08)	Kim et al. (2009)		1.00E+04	0.01
		Chronic	Algae	*Phaeodactylum tricornutum* (2)	72h EC10 (3.30E+06)	Claessens et al. (2013)	10^2	3.30E+04	<0.01
			Crustacea	*Daphnia magna* (1)	NOEC (1.48E+06)	Küster et al. (2010)		1.48E+04	<0.01
			Fish	*Pimephales promelas* (1)	NOEC (1.00E+06)	Winter et al. (2008)		1.00E+04	0.01
Propranolol	0.9	Acute	Algae	*Pseudokirchneriella subcapitata* (1)	96h EC50 (5.00E+05)	Ferrari et al. (2003)	10^4	5.00E+01	0.02
			Crustacea	*Daphnia magna* (1)	48h EC50 (7.50E+06)	Cleuvers (2003)		7.50E+02	<0.01
			Fish	*Pimephales promelas* (1)	48h LC50 (1.20E+06)	Huggett et al. (2002)		1.20E+02	<0.01

		Chronic	Algae	*Lemna minor* (1)	LOEC/2 (5.70E+07)	Cleuvers (2003)	10^2	5.70E+05	<0.01
			Crustacea	*Ceriodaphnia dubia* (1)	NOEC (9.00E+06)	Ferrari et al. (2003)		9.00E+04	<0.01
			Fish	Fish (1)	ChV (9.51E+05)	ECOSAR		9.51E+03	<0.01
Losartan	548.0	Acute	Algae	*Lemna minor* (1)	96h EC50 (6.46E+07)	Godoy et al. (2015)	10^4	6.46E+03	0.08
			Crustacea	*Daphnia magna* (1)	48h LC50 (3.31E+05)	FDA (2012)		3.31E+01	16.56
			Fish	*Pimephales promelas* (1)	48h LC50 (1.00E+09)	FDA (2012)		1.00E+05	<0.01
		Chronic	Algae	Green algae (1)	ChV (1.64E+06)	ECOSAR	10^2	1.64E+04	0.03
			Crustacea	Daphnid (1)	ChV (5.55E+05)	ECOSAR		5.55E+03	0.10
			Fish	Fish (1)	ChV (2.94E+05)	ECOSAR		2.94E+03	0.19
Valsartan	798.0	Acute	Algae	Green algae (1)	96h EC50 (1.39E+07)	ECOSAR	10^4	1.39E+03	0.57
			Crustacea	*Artemia salina* (2)	48h EC50 (1.00E+08)	Minguez et al. (2014)		1.00E+04	0.08
			Fish	Fish (2)	96h LC50 (7.73E+07)	ECOSAR		7.73E+03	0.10
		Chronic	Algae	Green algae (1)	ChV (1.84E+07)	ECOSAR	10^2	1.84E+05	<0.01
			Crustacea	Mysid (2)	ChV (2.12E+05)	ECOSAR		2.12E+03	0.38
			Fish	Fish (1)	ChV (1.69E+06)	ECOSAR		1.69E+04	0.05
Rosuvastatin	38.5	Acute	Algae	Green algae (1)	96h EC50 (4.21E+06)	ECOSAR	10^4	4.21E+02	0.09
			Crustacea	Mysid (2)	96h EC50 (4.29E+07)	ECOSAR		4.29E+03	<0.01
			Fish	Fish (2)	96h LC50 (5.84E+08)	ECOSAR		5.84E+04	<0.01
		Chronic	Algae	Green algae (1)	ChV (2.78E+07)	ECOSAR	10^2	2.78E+05	<0.01
			Crustacea	Mysid (2)	ChV (3.15E+06)	ECOSAR		3.15E+04	<0.01
			Fish	Fish (1)	ChV (9.21E+06)	ECOSAR		9.21E+04	<0.01

Table S6.2 lists in detail the final RQ values of the 16 PPCPs detected. The results showed the following trend: (i) regarding the acute toxicity, more than 80% of PPCPs showed no or low toxicity for algae, crustaceans and fishes. However, some PPCPs raised great concern. For example, caffeine and valsartan showed moderate toxicity for algae. For crustaceans, caffeine and losartan represented high toxicity. For fishes, diclofenac, together with caffeine and losartan, indicated moderate toxicity; (ii) concerning the chronic toxicity, 93.8%, 81.3% and 87.5% of PPCPs also are non or low toxic for algae, crustaceans and fishes, respectively. However, caffeine recorded high toxicity for algae. For crustaceans, acetaminophen, losartan and valsartan indicated moderate toxicity, and for fish, losartan and acetaminophen showed moderate and high toxicity, respectively.

Thus, the evidences presented here, suggest that PPCPs potentially dangerous in Guarujá were especially caffeine, acetaminophen, diclofenac, valsartan and losartan. The risks of these PPCPs were already supported by previous literature. For example, chronic exposure to 500 ng/L of caffeine (less than the concentration found in Guarujá) could alter the regenerative capacity of the annelid *Diopatra neapolitan* (Pires et al., 2016). Similarly, other authors found that, in the aquatic environment, acetaminophen may be toxic to crustaceans and fishes (in general, toxicity results from oxidative stress) (Nunes et al., 2014, 2015; Ramos et al., 2014). Previous studies shown that diclofenac, at environmentally realistic concentrations, could cause bioaccumulation in zebra mussel (*Dreissena polymorpha*) (Daniele et al., 2016) and in Mediterranean mussel (*Mytilus galloprovincialis*) (Bonnefille et al., 2017). In relation to losartan and valsartan, these antihypertensives also deserve attention because their consumption has increased dramatically in many parts of the world, although studies on the toxicity of these substances are poorly documented (Godoy et al., 2015; Pereira et al., 2016; Desbiolles et al., 2018). A recent study detected cytotoxic effects on gills and hemocytes of the mussel *Perna perna* exposed to environmental concentrations of up to 300 ng/L of losartan (Cortez et al., 2018), less than the MEC detected in Guarujá.

Although cocaine and benzoylecgonine presented, respectively, no to low toxicity in the detected levels, a precautionary approach is recommended, because of the concentrations found in Guarujá (MEC of 30.3 and 278.0, respectively) (Table 6.1). The low toxicity can be explained by the high PNEC estimated by ECOSAR, due to the scarcity of toxicity data from marine organisms reported in the peer reviewed literature (Table S6.2)

(USEPA, 2017). However, environmentally realistic concentrations of cocaine (20 ng/L) have the potential to cause adverse effects on the European eel (*Anguilla anguilla*) (Capaldo et al., 2018; 2019). Moreover, cocaine and benzoylecgonine may interact with other therapeutic substances, leading to unexpected pharmacological interactions (Parolini et al., 2016; 2017).

Meanwhile, citalopram, orphenadrine, enalapril and chlorthalidone (100% of RQ <0.01 for all trophic levels), and carbamazepine, propanolol, rosuvastatin and clopidogrel (more than 75% of RQ <0.01 for all trophic levels) appear as the less relevant PPCPs in terms of aquatic toxicity effects, due to their high PNEC values and/or low concentrations in the investigated area (Table S6.2). However, these RQs were estimated for individual compounds, and therefore, one should consider that these PPCPs were detected in the environment usually as chemical mixtures.

Indeed, some studies have shown that toxicity of pharmaceuticals to non-target organisms may occur even at very low environmentally realistic concentrations due to additive or synergic effects (Desbiolles et al., 2018; Sathishkumar et al., 2019). In this regard, a study who analysed the single and combined effect of four drugs at environmental concentrations (including carbamazepine, caffeine and diclofenac) on the metabolism of marine bacteria *Aliivibrio fischeri*, showed that a mixture of pharmaceutical compounds could be more severe than each drug individually (Di Nica et al., 2016). However, the combined toxicity of pharmaceuticals is still poorly known (Biel-Maeso et al., 2018; Desbiolles et al., 2018).

6.4 Conclusion

The hereby study showed that according to the ecological risk assessment performed, caffeine, acetaminophen, diclofenac, valsartan and losartan can potentially exert deleterious effects on the aquatic biota. However, the dilution effect of the seawater and the synergetic and/or additive effect of a mixture of chemical compounds cannot be ignored. This research showed that in addition to the sewage disposal (generally over 2 km from the coast) being recognised as vehicles for the transport of PPCPs and illicit drugs to the marine environment, urban drainage channels (which may receive input from underground sewers) are also potential anthropogenic sources of these compounds.

Moreover, the present study suggests that these emerging pollutants are ubiquitous in beach areas around the world, which receive urban runoff (e.g. the coast of the state of São Paulo in Brazil has 600 drainage channels flowing to 290 tourist beaches). Thus, in order to maintain the quality of Guarujá beaches and, therefore to prevent environmental and public risks, it is necessary to promote the installation of basic sanitation facilities. It is highly recommended the installation of gate systems in the 43 urban drainage channels of the municipality and interconnection of the urban surface drainage to the sewage collection network (reduction of the first flush effect on the beaches of the municipality). Furthermore, land regularisation of irregularly occupied areas (Perequê beach and Enseada hills) is also recommended. Additional actions need to be taken such as supervision of the trade and residences of the municipality, requiring that they be interconnected to the sewage collection network already established (Tombo, Enseada and Perequê beaches), and increasing awareness, *in loco*, of users (especially during the summer tourist season), because, during the field work, it was observed that people constantly come into contact with the waters of these channels, demonstrating total ignorance about the health risks of this behaviour.

Acknowledgments

Authors want to acknowledge Daniel Temponi Lebre from the Centro de Espectrometria de Massa Aplicada - Instituto de Pesquisas Energéticas e Nucleares (CEMSA, IPEN, São Paulo, Brazil) for technical support regarding the LC–MS/MS analyses. Thanks also to Pulsar pictures Ltda, Apiacás St., 934, São Paulo, Brazil, for the license to reproduce the images of the beaches of Guarujá (contract nº 27918). Correia AT was supported by national funds through FCT - Foundation for Science and Technology within the scope of UIDB/04423/2020 and UIDP/04423/2020.

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Supplementary material

Table – S6.1: Multiple reactions for positive and negative ion modes. The table presents: name of compound and its respective CAS (Chemical Abstracts Service) number; Q1: mass to charge ratio of the mother ion in the first quadrupole (m/z); Q3: mass to charge ratio of the most intensive daughter ion in the third quadrupole (m/z); DP: declustering potential (V); CE: collision energy (V); CXP: collision cell exit potential (V); LOD: Limits of detection (ng/L); LOQ: Limits of quantification (ng/L); and RT: Retention time. Note: It was assumed hereby the sample concentration factor to be 1,000 times in water matrix.

Therapeutic Class	**CAS number**	**Q1** (m/z)	**Q3** (m/z)	**DP** (V)	**CE** (V)	**CXP** (V)	**LOD** (ng/L)	**LOQ** (ng/L)	**RT** (min)
Antiepiletic									
Carbamazepine	298-46-4	237.1	194.2 179.1	36 36	43 25	4 4	0.003	0.01	4.7
Clonazepam	1622-61-3	316.1	270.0 214.2	51 51	31 47	4 4	0.0013	0.01	5.1
Stimulants									
Caffeine	58-08-2	195.2	138.3 110.1	26 26	19 29	4 4	0.0001	0.0085	3.4
Cocaine	50-36-2	304.2	182.2 105.1	36 36	39 25	4 4	0.003	0.0012	3.9
Benzoylecgonine	519-09-5	290.2	168.2 105.1	31 31	25 37	4 4	0.0012	0.0077	3.6
Antidepressant									
Citalopram	59729-33-8	325.2	109.2 262.1	41 41	37 25	4 4	0.0006	0.0059	4.3
Paroxetine	61869-08-7	330.2	192.2 135.1	41 41	27 54	4 4	0.004	0.031	4.6
Analgesic/ Anti-inflammatory									
Acetaminophen	103-90-2	152.1	109.9 93.1	26 26	19 29	4 4	0.0014	0.0084	3.0
Diclofenac	15307-86-5	296.1	214.1 250.0	21 21	39 25	4 4	1.0	0.0074	5.8

Orphenadrine	83-98-7	270.2	181.1 165.0	16	19 53	4 4	0.0009	0.0034	4.4
Antihypertensive									
Atenolol	29122-68-7	267.3	145.2 190.3	31 31	37 25	4 4	0.0016	0.0069	2.9
Propranolol	525-66-6	260.2	116.0 183.0	41 41	23 23	4 4	0.0013	0.0072	4.4
Enalapril	75847-73-3	377.3	234.2 303.3	36 36	27 25	4 4	0.003	0.009	4.4
Losartan	114798-26-4	423.2	207.2 405.2	21 21	31 17	4 6	0.0007	0.0061	4.8
Valsartan	137862-53-4	436.3	235.1 207.1	21 21	25 33	4 4	0.0014	0.0077	5.3
Anticholesteremic									
Rosuvastatin	287714-41-4	482.2	258.2 270.2	61 61	41 47	4 4	0.0008	0.0069	4.9
Anxiolytic									
Bromazepam	1812-30-2	316.0	182.2 209.2	51 51	41 33	4 4	0.005	0.0281	4.3
Midazolam	59467-70-8	326.1	291.2 249.1	51 51	33 44	4 4	0.0006	0.0059	4.5
Contraceptive									
Cyproterone	2098-66-0	417.3	357.2 279.3	41	25 41	6 4	0.0015	0.0075	6.4
Diuretic									
Chlortalidone	77-36-1	336.9	189.9 146.2	−35 −35	−22 −28	−2 −2	0.0023	0.0088	4.1
Antiplatelet									
Clopidogrel	113665-84-2	322.2	212.2 155.0	31 31	23 51	4 4	0.00004	0.0003	6.2

Antihistamine									
Loratadine	79794-75-5	383.3	337.3 267.1	41 41	33 41	6 4	0.0014	0.0126	5.2
Sexual stimulant									
Sidenafil	171599-83-0	475.3	100.0 283.2	51 51	37 47	4 4	0.006	0.043	4.2

Reference (Table S6.1)

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Table - S6.2: Results from the ecological risk assessment tests (RQ) regarding the pharmaceuticals of the different therapeutic classes and illicit drugs (cocaine and its metabolite benzoylecgonine) detected on the shoreline of Guarujá, São Paulo, Brazil. The table presents: name of each compound; MEC: measured environmental concentration in the Guarujá water body (ng/L); acute and chronic toxicity data: [(trophic level; organism's test, toxicological endpoint and concentration (ng/L)], Assessment Factor (AF), Predicted No-Effect Concentration (PNEC, ng /L) and risk quotients (RQ, signalled in white, green, yellow and red for no, low, moderate and high risk, respectively). Data from the toxicological endpoints was obtained from several published works (References) available from the Ecotoxicology Database (ECOTOX), or, in the absence of derived experimentally data, estimated from the ECOSAR program. Note: 1 Freshwater; 2 Seawater; EC10: 10% Effective Concentration; EC50: 50% Effective Concentration; LC50: 50% Lethal Concentration; NOEC: No Observed Effect. Concentration; LOEC: Lowest Observed Effect Concentration.

		Toxicity data							
Compound	**MEC** (ng/L)	**Trophic level**		**Organism/Species**	**Endpoint** (ng/L)	**Reference**	**AF**	**PNEC** (ng/L)	**RQ**
Carbamazepine	8.0	Acute	Algae	*Skeletonema marinoi* (2)	72h EC50 (1.00E+08)	Minguez et al. (2014)	10^4	1.00E+04	<0.01
			Crustacea	*Artemia salina* (2)	48h EC50 (1.00E+08)	Minguez et al. (2014)		1.00E+04	<0.01
			Fish	*Oryzias latipes* (1)	48h EC50 (3.52E+07)	Kim et al. (2007)		3.52E+03	<0.01
		Chronic	Algae	*Lemna gibba* (1)	LOEC/2 (5.00E+05)	Brain et al. (2004)	10^2	5.00E+03	<0.01
			Crustacea	*Ceriodaphnia dubia* (1)	NOEC (2.50E+04)	Ferrari et al. (2003)		2.50E+02	0.03
			Fish	*Danio rerio* (1)	NOEC (2.50E+07)	Ferrari et al. (2003)		2.50E+05	<0.01
Caffeine	6550.0	Acute	Algae	*Pseudokirchneriella subcapitata* (1)	72h LC50 (3.39E+08)	Blaise et al. (2006)	10^4	3.39E+04	0.19
			Crustacea	*Daphnia dubia* (1)	48h LC50 (5.00E+07)	Moore et al. (2008)		5.00E+03	1.31
			Fish	*Pimephales promelas* (1)	48h LC50 (8.00E+07)	Moore et al. (2008)		8.00E+03	0.82
		Chronic	Algae	*Lemna gibba* (1)	LOEC/2 (5.00E+05)	Brain et al. (2004)	10^2	5.00E+03	1.31
			Crustacea	*Ceriodaphnia dubia* (1)	NOEC (2.00E+07)	Brain et al. (2004)		2.00E+05	0.03
			Fish	*Pimephales promelas* (1)	NOEC (3.00E+07)	Brain et al. (2004)		3.00E+05	0.02
Cocaine	30.3	Acute	Algae	Green algae (1)	96h EC50 (4.35E+06)	ECOSAR	10^4	4.35E+02	0.07
			Crustacea	Daphnid (1)	48h LC50 (5.48E+06)	ECOSAR		5.48E+02	0.06
			Fish	Fish (2)	96h LC50 (4.86E+07)	ECOSAR		4.86E+03	<0.01
		Chronic	Algae	Green algae (1)	ChV (1.46E+06)	ECOSAR	10^2	1.46E+04	<0.01

			Crustacea	Mysid (2)	ChV (2.29E+09)	ECOSAR		2.29E+07	<0.01
			Fish	Fish (2)	ChV (7.18E+06)	ECOSAR		7.18E+04	<0.01
Benzoylecgonine	278.0	Acute	Algae	Green algae (1)	96h EC50 (1.20E+10)	ECOSAR	10^4	1.20E+06	<0.01
			Crustacea	Mysid (2)	96h LC50 (3.14E+12)	ECOSAR		3.14E+08	<0.01
			Fish	Fish (2)	96h LC50 (6.24E+11)	ECOSAR		6.24E+07	<0.01
		Chronic	Algae	Green algae (1)	ChV (3.03E+09)	ECOSAR	10^2	3.03E+07	<0.01
			Crustacea	Mysid (2)	ChV (2.00E+13)	ECOSAR		2.00E+11	<0.01
			Fish	Fish (1)	ChV (4.92E+09)	ECOSAR		4.92E+07	<0.01
Citalopram	0.4	Acute	Algae	*Pseudokirchneriella subcapitata* (1)	48h EC50 (1.60E+06)	Christensen et al. (2007)	10^4	1.60E+02	<0.01
			Crustacea	*Ceriodaphnia dubia* (1)	48h LC50 (4.00E+06)	Henry et al. (2004)		4.00E+02	<0.01
			Fish	Fish (1)	96h LC50 (4.47E+06)	ECOSAR		4.47E+02	<0.01
		Chronic	Algae	Green algae (1)	ChV (1.38E+05)	ECOSAR	10^2	1.38E+03	<0.01
			Crustacea	*Ceriodaphnia dubia* (1)	LOEC/2 (4.00E+08)	Henry et al. (2004)		4.00E+06	<0.01
			Fish	Fish (1)	ChV (1.40E+05)	ECOSAR		1.40E+03	<0.01
Acetaminophen	391.0	Acute	Algae	*Phaeodactylum tricornutum* (2)	72h EC50 (2.39E+08)	Claessens et al. (2013)	10^4	2.39E+04	0.02
			Crustacea	*Artemia salina* (2)	48h EC50 (1.00E+08)	Minguez et al. (2014)		1.00E+04	0.04
			Fish	*Oryzias latipes* (1)	48h EC50 (2.66E+08)	Kim et al. (2007)		2.66E+04	0.02
		Chronic	Algae	*Phaeodactylum tricornutum* (2)	72h EC10 (7.21E+07)	Claessens et al. (2013)	10^2	7.21E+05	<0,01
			Crustacea	*Daphnia magna* (1)	NOEC (4.03E+05)	Kim et al. (2007)		4.03E+03	0.10
			Fish	*Danio rerio* (1)	LOEC/2 (5.00E+03)	Galus et al. (2013)		5.00E+01	7.82
Diclofenac	79.8	Acute	Algae	*Dunaliella tertiolecta* (2)	96h EC50 (1.86E+08)	DeLorenzo and Fleming (2007)	10^4	1.86E+04	<0,01
			Crustacea	*Artemia salina* (2)	48h EC50 (1.00E+08)	Minguez et al. (2014)		1.00E+04	<0,01

			Fish	*Danio rerio* (1)	72h LC50 (7.80E+06)	Van den Brandof and Montforts (2010)		7.80E+02	0.10
		Chronic	Algae	*Lemna minor* (1)	NOEC (3.75E+06)	Cleuvers (2003)	10^2	3.75E+04	<0,01
			Crustacea	*Ceriodaphnia dubia* (1)	NOEC (1.00E+06)	Ferrari et al. (2003)		1.00E+04	<0,01
			Fish	*Danio rerio* (1)	NOEC (4.00E+06)	Ferrari et al. (2003)		4.00E+04	<0,01
Orphenadrine	1.5	Acute	Algae	*Lemna minor* (1)	168h EC50 (1.20E+07)	Kaza et al. (2007)	10^4	1.20E+03	<0,01
			Crustacea	*Artemia salina* (2)	24h EC50 (4.50E+07)	Calleja et al. (1994)		4.50E+03	<0,01
			Fish	Fish (1)	96h LC50 (4.24E+07)	ECOSAR		4.24E+03	<0,01
		Chronic	Algae	Green algae (1)	ChV (1.32E+05)	ECOSAR	10^2	1.32E+03	<0,01
			Crustacea	Daphnid (1)	ChV (6.10E+04)	ECOSAR		6.10E+02	<0,01
			Fish	Fish (1)	ChV (1.37E+05)	ECOSAR		1.37E+03	<0,01
Atenolol	140.0	Acute	Algae	*Phaeodactylum tricornutum* (2)	72h EC50 (2.62E+08)	Claessens et al. (2013)	10^4	2.62E+04	<0.01
			Crustacea	*Artemia salina* (2)	48h EC50 (1.00E+08)	Minguez et al. (2014)		1.00E+04	0.01
			Fish	*Oryzias latipes* (1)	96h LC50 (1.00E+08)	Kim et al. (2009)		1.00E+04	0.01
		Chronic	Algae	*Phaeodactylum tricornutum* (2)	72h EC10 (3.30E+06)	Claessens et al. (2013)	10^2	3.30E+04	<0.01
			Crustacea	*Daphnia magna* (1)	NOEC (1.48E+06)	Küster et al. (2010)		1.48E+04	<0.01
			Fish	*Pimephales promelas* (1)	NOEC (1.00E+06)	Winter et al. (2008)		1.00E+04	0.01
Propranolol	0.9	Acute	Algae	*Pseudokirchneriella subcapitata* (1)	96h EC50 (5.00E+05)	Ferrari et al. (2003)	10^4	5.00E+01	0.02
			Crustacea	*Daphnia magna* (1)	48h EC50 (7.50E+06)	Cleuvers (2003)		7.50E+02	<0.01
			Fish	*Pimephales promelas* (1)	48h LC50 (1.20E+06)	Huggett et al. (2002)		1.20E+02	<0.01
		Chronic	Algae	*Lemna minor* (1)	LOEC/2 (5.70E+07)	Cleuvers (2003)	10^2	5.70E+05	<0.01
			Crustacea	*Ceriodaphnia dubia* (1)	NOEC (9.00E+06)	Ferrari et al. (2003)		9.00E+04	<0.01
			Fish	Fish (1)	ChV (9.51E+05)	ECOSAR		9.51E+03	<0.01
Enalapril	3.8	Acute	Algae	Green algae (1)	96h EC50 (3.45E+07)	ECOSAR	10^4	3.45E+03	<0.01
			Crustacea	Mysid (2)	96h LC50 (3.49E+07)	ECOSAR		3.40E+03	<0.01

			Fish	Fish (2)	96h LC50 (4.79E+08)	ECOSAR		4.79E+04	<0.01
		Chronic	Algae	Green algae (1)	ChV (5.24E+07)	ECOSAR	10^2	5.24E+05	<0.01
			Crustacea	Mysid (2)	ChV (2.63E+06)	ECOSAR		2.63E+04	<0.01
			Fish	Fish (2)	ChV (6.36E+07)	ECOSAR		6.36E+05	<0.01
Losartan	548.0	Acute	Algae	*Lemna minor* (1)	96h EC50 (6.46E+07)	Godoy et al. (2015)	10^4	6.46E+03	0.08
			Crustacea	*Daphnia magna* (1)	48h LC50 (3.31E+05)	FDA (2012)		3.31E+01	16.56
			Fish	*Pimephales promelas* (1)	48h LC50 (1.00E+09)	FDA (2012)		1.00E+05	<0.01
		Chronic	Algae	Green algae (1)	ChV (1.64E+06)	ECOSAR	10^2	1.64E+04	0.03
			Crustacea	Daphnid (1)	ChV (5.55E+05)	ECOSAR		5.55E+03	0.10
			Fish	Fish (1)	ChV (2.94E+05)	ECOSAR		2.94E+03	0.19
Valsartan	798.0	Acute	Algae	Green algae (1)	96h EC50 (1.39E+07)	ECOSAR	10^4	1.39E+03	0.57
			Crustacea	*Artemia salina* (2)	48h EC50 (1.00E+08)	Minguez et al. (2014)		1.00E+04	0.08
			Fish	Fish (2)	96h LC50 (7.73E+07)	ECOSAR		7.73E+03	0.10
		Chronic	Algae	Green algae (1)	ChV (1.84E+07)	ECOSAR	10^2	1.84E+05	<0.01
			Crustacea	Mysid (2)	ChV (2.12E+05)	ECOSAR		2.12E+03	0.38
			Fish	Fish (1)	ChV (1.69E+06)	ECOSAR		1.69E+04	0.05
Rosuvastatin	38.5	Acute	Algae	Green algae (1)	96h EC50 (4.21E+06)	ECOSAR	10^4	4.21E+02	0.09
			Crustacea	Mysid (2)	96h EC50 (4.29E+07)	ECOSAR		4.29E+03	<0.01
			Fish	Fish (2)	96h LC50 (5.84E+08)	ECOSAR		5.84E+04	<0.01
		Chronic	Algae	Green algae (1)	ChV (2.78E+07)	ECOSAR	10^2	2.78E+05	<0.01
			Crustacea	Mysid (2)	ChV (3.15E+06)	ECOSAR		3.15E+04	<0.01
			Fish	Fish (1)	ChV (9.21E+06)	ECOSAR		9.21E+04	<0.01
Chlorthalidone	0.4	Acute	Algae	Green algae (1)	96h EC50 (1.69E+08)	ECOSAR	10^4	1.69E+04	<0.01
			Crustacea	Daphnid (1)	48h LC50 (5.66E+08)	ECOSAR		5.66E+04	<0.01

			Fish	Fish (1)	96h LC50 (7.71E+08)	ECOSAR		7.71E+04	<0.01
		Chronic	Algae	Green algae (1)	ChV (6.54E+07)	ECOSAR	10^2	6.54E+05	<0.01
			Crustacea	Daphnid (1)	ChV (7.33E+07)	ECOSAR		7.33E+05	<0.01
			Fish	Fish (1)	ChV (5.52E+07)	ECOSAR		5.52E+05	<0.01
Clopidogrel	0.2	Acute	Algae	Green algae (1)	96h EC50 (3.15E+05)	ECOSAR	10^4	3.15E+01	<0.01
			Crustacea	Daphnid (1)	48h LC50 (5.79E+05)	ECOSAR		5.79E+01	<0.01
			Fish	Fish (1)	96h LC50 (5.06E+06)	ECOSAR		5.06E+02	<0.01
		Chronic	Algae	Green algae (1)	ChV (1.22E+05)	ECOSAR	10^2	1.22E+03	<0.01
			Crustacea	Mysid (2)	ChV (3.65E+06)	ECOSAR		3.65E+04	<0.01
			Fish	Fish (2)	ChV (1.01E+06)	ECOSAR		1.01E+04	<0.01

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Table - S6.3: Name, n-octanol/water partition coefficient (Log Kow) and maximum measured concentrations (in bold) of pharmaceuticals and illicit drugs recorded in the urban drainage channels of Guarujá, São Paulo, Brazil, compared to the concentrations reported by other studies in several aquatic compartments worldwide. For more details about the Log Kow see manuscript.

Compound	Log Kow	Environmental Matrix	Concentration (ng/L)	Country	Reference
Carbamazepine	2.45	river	5.8	USA	Anumol et al. (2015)
		river	8.8	Bangladesh	Hossain et al. (2018)
		river	3.5	China	Yang et al. (2018)
		urban drainage channel	**8.0**	**Brazil**	**This study**
Caffeine	-0.07	estuary water	1389.0	Singapore	Bayen et al. (2016)
		river	430.0	China	Yang et al. (2018)
		coastal lagoon	1120.0	Uruguay	Griffero et al. (2019)
		urban drainage channel	**6550.0**	**Brazil**	**This study**
Cocaine	2.30	seawater	6.6	Greece	Borova et al. (2014)
		seawater	7.8	Greece	Borova et al. (2014)
		coastal lagoon	<LOQ	Uruguay	Griffero et al. (2019)
		urban drainage channel	**30.3**	**Brazil**	**This study**
Benzoylecgonine	1.32	seawater	5.2	USA	Klosterhaus et al. (2013)
		seawater	6.6	Greece	Borova et al. (2014)
		coastal lagoon	<LOQ	Uruguay	Griffero et al. (2019)
		urban drainage channel	**278.0**	**Brazil**	**This study**
Citalopram	3.74	sewage	197.0	Germany	Gurke et al. (2015)
		river	0.11	Sweden	Lindim et al. (2016)
		river	28.9	Portugal	Paiga er al. (2016)
		urban drainage channel	**0.4**	**Brazil**	**This study**
Acetaminophen	-0.27	river	200.0	France	Celle-Jeanton et al. (2014)
		river	518.0	Mexico	Rivera-Jaimes et al. (2018)
		river	9.6	China	Yang et al. (2018)
		urban drainage channel	**391.0**	**Brazil**	**This study**

Diclofenac	4.51	river	1.49	Sweden	Lindim et al. (2016)
		river	51.24	Portugal	Pereira et al. (2017)
		river	15.49	Malaysia	Praveena et al. (2018)
		urban drainage channel	**79.8**	**Brazil**	**This study**
Orphenadrine	3.65	river	not reported	Italy	Milione et al. (2016)
		urban drainage channel	**1.5**	**Brazil**	**This study**
Atenolol	- 0.03	river	13.0	France	Celle-Jeanton et al. (2014)
		seawater	0.9	USA	Lara-Martín et al. (2014)
		river	95.0	Mexico	Rivera-Jaimes et al. (2018)
		urban drainage channel	**140.0**	**Brazil**	**This study**
Propranolol	2.60	river	39.2	Spain	Serna et al. (2011)
		river	1.3	USA	Anumol et al. (2015)
		river	64.9	United Kingdom	Burns et al. (2018)
		urban drainage channel	**0.9**	**Brazil**	**This study**
Enalapril	2.45	river	0.5	Spain	Gros et al. (2009)
		river	3.1	Spain	Silva et al. (2011)
		seawater	40.0	USA	Klosterhaus et al. (2013)
		urban drainage channel	**3.8**	**Brazil**	**This study**
Losartan	4.01	river	1.2	Serbia	Lv et al. (2014)
		river	154.0	Serbia	Petrovic et al. (2014)
		sewage	417.0	Germany	Subedi et al. (2017)
		urban drainage channel	**548.0**	**Brazil**	**This study**
Valsartan	3.65	river	89.6	Serbia	Petrovic et al. (2014)
		coastal lagoon	4.7	Spain	Moreno-González et al. (2015)
		seawater	75.0	Brazil	Pereira et al. (2016)
		urban drainage channel	**798.0**	**Brazil**	**This study**
Rosuvastatin	2.48	seawater	20.8	Malaysia	Praveena et al. (2018)
		urban drainage channel	**38.5**	**Brazil**	**This study**
Chlorthalidone	1.45	stream	18.9	Brazil	Shihomatsu et al. (2017)

		urban drainage channel	**0.4**	**Brazil**	**This study**
Clopidogrel	3.82	sewage	99.0	Spain	Gros et al. (2012)
		sewage hospital	16.8	Portugal	Santos et al. (2013)
		urban drainage channel	**0.2**	**Brazil**	**This study**

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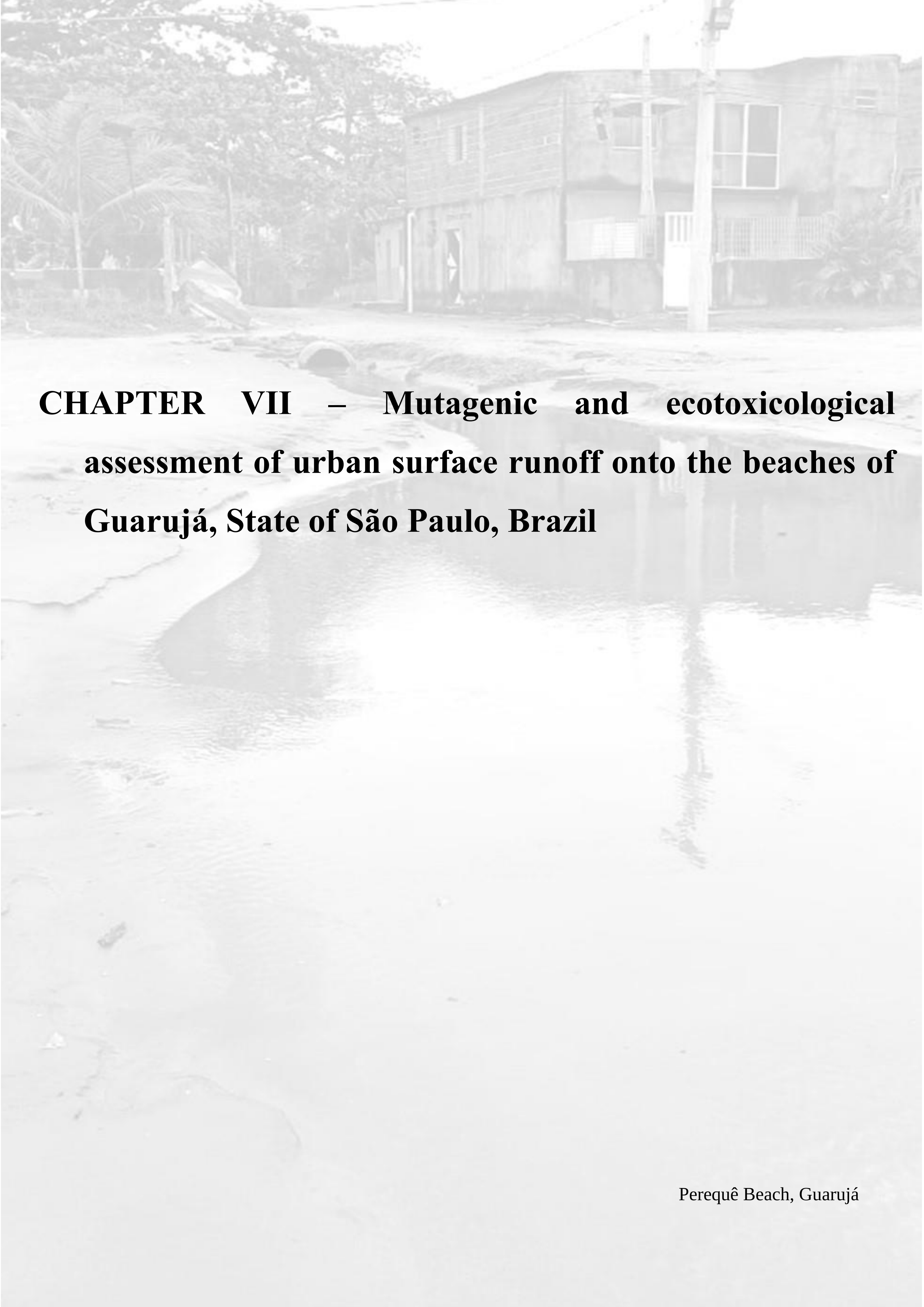

CHAPTER VII – Mutagenic and ecotoxicological assessment of urban surface runoff onto the beaches of Guarujá, State of São Paulo, Brazil

Perequê Beach, Guarujá

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

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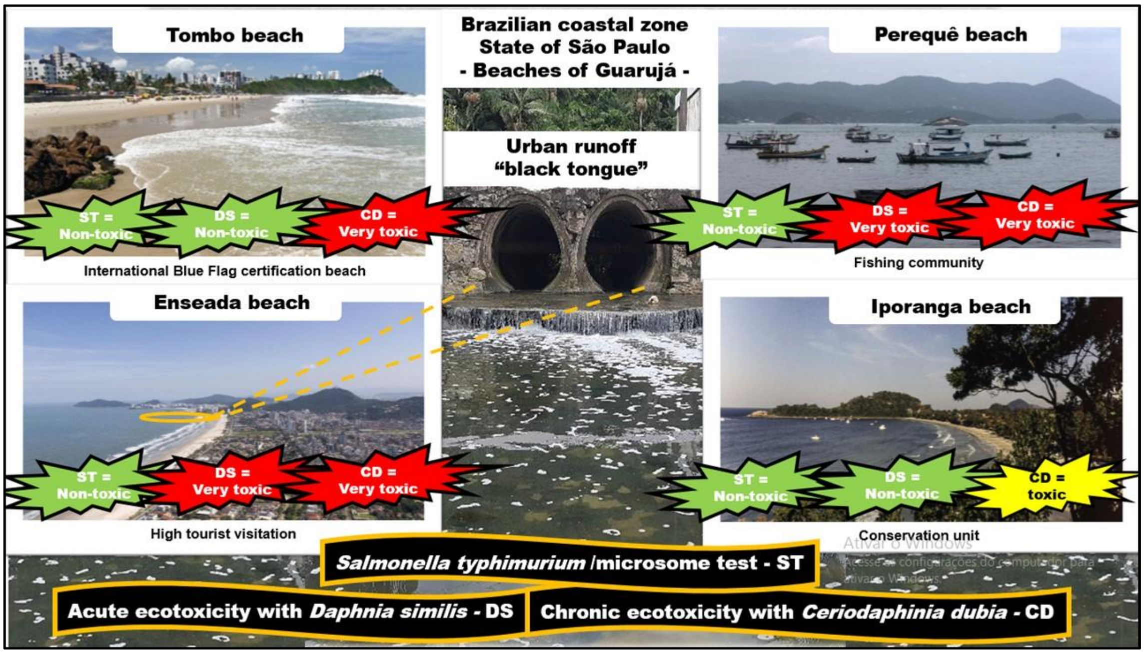
Under review: Water Science and Technology (2021).

Graphical Abstract



Highlights

- Evaluation of the genotoxicity and ecotoxicity of urban surface runoff of Guaruja (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.

Abstract

Along the coast of the State of São Paulo, Brazil, urban 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 land use and 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 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 of these beaches.

Keywords: Bioassay; Domestic sewage; Non-point source pollution; Pollution effect; Subtropical zone; Urban drainage channel.

7.1 Introduction

As urbanisation progresses, especially in coastal areas, the 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 and Toor, 2016; Yang and Toor, 2017). In South America, namely Brazil, the management of these diffuse loads in the vast coastline (8,500 km of coastline) 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, 2019). One of these touristic municipalities is Guarujá, one of the largest cities of São Paulo coast, with an estimated population of 316,000 residents, that almost doubles during the high tourist season (Cetesb, 2017; Ibge, 2019).

Recent studies 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 screening of risk 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 cycle, high reproductive rates and sensitivity to a broad range of toxicants (Gosset et al., 2016; Roveri et al., 2020a; 2020c). These tests 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 São Paulo coastal area (Roveri et al., 2020a, 2020b, 2020c), none of these 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.

7.2 Material and Methods

7.2.1 Study area

The study area comprised the coastal communities of Guarujá, São Paulo State, Brazil (23°59'34"S, 46°15'21"W) (Ribeiro and Oliveira, 2015). The economy of the municipality is mainly driven by the seasonal tourism occurring along eight touristic beaches (Ribeiro and Oliveira, 2015; Cetesb et al., 2017). These beaches receive daily contributions of urban runoff from 43 rainwater drainage channels (Cetesb et al., 2017). 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 et al., 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 and Oliveira, 2015). Figure 7.1 presents the main characteristics regarding the use and land occupation of the sampling beaches.

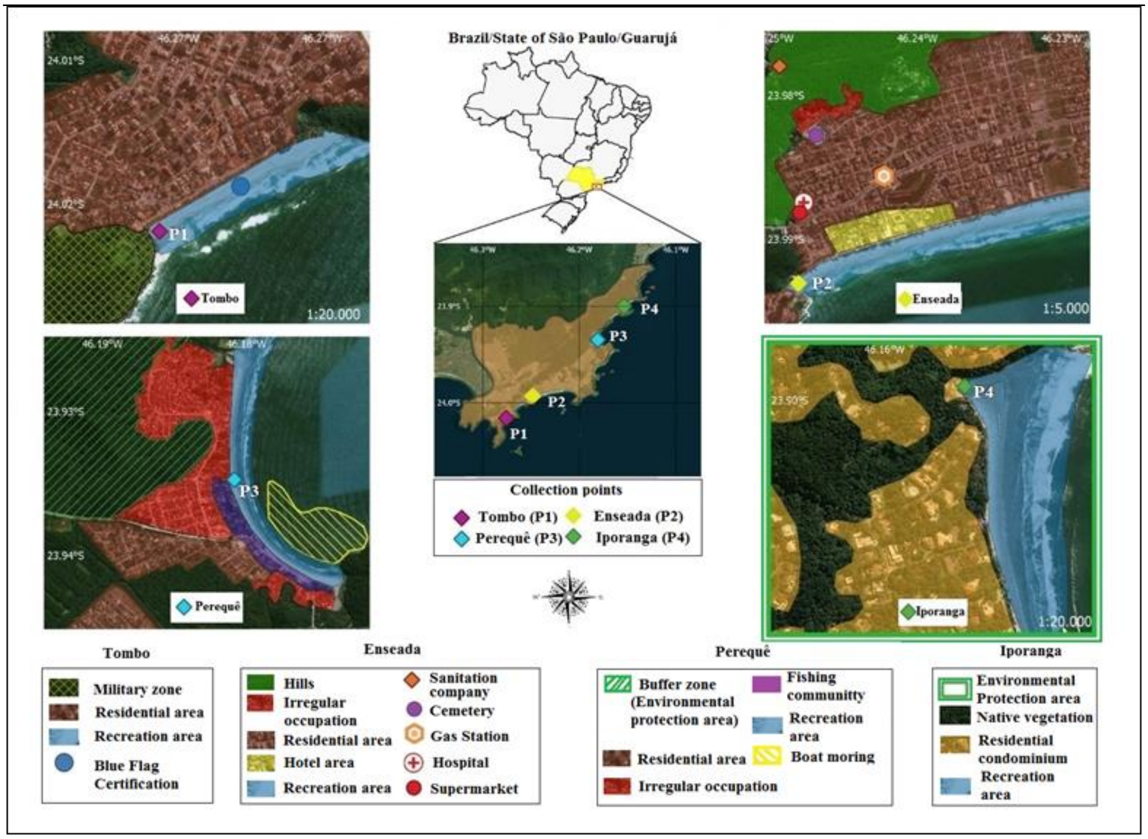


Figure 7.1: Map of the study area showing the city of Guarujá, São Paulo State, Brazil. Beaches [Tombo (P1), Enseada (P2), Perequê (P3), Iporanga (P4)] here water samples were collected for the genotoxicity and ecotoxicity assessment of urban surface runoff. The figure also presents the main characteristics regarding the use and land occupation of the selected beaches.

7.2.2 Water Sampling

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). For the rainy season (January and March) rainfall was recorded in the last 24 hours prior to sample collection.

7.2.3 Bioassays

The analyses were performed within 2 days after sample collection. 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, 1000, 1500, and 2000 µL/plate. Additionally, the mutagenicity index (MI) was calculated for each concentration according to the following formula (Eq. 7.1):

$$MI = \frac{number\ of\ revertants\ per\ plate\ (test\ compound)}{number\ of\ revertants\ per\ plate\ (negative\ control)} \qquad (7.1)$$

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 and 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) and 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%. 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).

7.2.4 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* 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%).

7.3 Results

Regarding Ames test (Table 7.1), the results obtained in the channels of the Tombo (Table 7.1a), Enseada (Table 7.1b), Perequê (Table 7.1c) and Iporanga (Table 7.1d) 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 7.1. 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. 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 shows: (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 7.2 Material and Method. Note: *Tombo channel could not be sampled in the July campaign (dry season) because the rainwater course was dry.

Beach	Concentration (µL/plate)	Ames Test (*Salmonella typhimurium* /microsome test)															
		January (rainy season)								March (rainy season)							
		TA98				TA100				TA98				TA100			
		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
	200		1.1		1.0		0.9		1.2		1.1		1.0		1.2		1.0
	500		0.9		1.1		1.2		0.9		0.9		0.9		1.2		1.2
	1000		1.3		1.3		1.0		0.9		0.9		1.3		1.1		1.2
	1500		1.2		1.1		0.7		0.7		0.7		0.9		1.2		1.2
	2000		0.8		1.8		0.9		0.8		0.9		1.1		1.1		1.0
Toxicity classification		Non-toxic				Non-toxic				Non-toxic				Non-toxic			
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
	200		1.5		1.3		1.3		1.4		1.3		1.4		1.4		1.4
	500		1.3		1.4		1.2		1.5		1.4		1.3		1.3		1.3
	1000		1.4		1.4		1.5		1.5		1.5		1.4		1.4		1.4
	1500		1.3		1.4		1.4		1.3		1.4		1.4		1.3		1.3
	2000		1.4		1.4		1.4		1.4		1.5		1.5		1.4		1.6
Toxicity classification		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
	200		1.3		1.2		1.3		1.3		1.4		1.3		1.4		1.4
	500		1.4		1.4		1.3		1.3		1.4		1.3		1.5		1.3

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

Table 7.2a 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 7.2b 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).

Table 7.2: 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 in the city of Guarujá, São Paulo, Brazil (Tombo, Enseada, Perequê and Iporanga). 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 shows: (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 item 7.2 Materials and Method). Note: *Tombo channel could not be sampled in the July campaign (dry season) because the water course was dry.

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	-	1	0	-	0	0	-
Tombo	10	100	Very toxic	10	40	Toxic	4	40	Toxic	-	-	*Not determined
Enseada	10	100	Very toxic	10	80	Very toxic	8	80	Very toxic	4	40	Toxic
Perequê	10	100	Very toxic	10	30	Toxic	3	30	Toxic	6	60	Very toxic
Iporanga	0	0	Non-toxic	10	30	Toxic	1	10	Non-toxic	0	0	Non-toxic

7.4 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 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 and 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 and 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 Brazilian summer/rainy season) (Roveri et al., 2020c). However, these physical, chemical and microbiological characterizations 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 and Zeiger, 2000; Okunola et al., 2016; Khallef et al., 2019). This lack of genotoxicity in Guarujá may simply indicates 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 bacteria (TA97a, TA102, and TA1535), which are more sensitive to genotoxicity (Mortelmans and 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 acute tests performed (Ambrozevicius and 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 and Oliveira, 2015; Cetesb, 2017; Roveri et al., 2020a). On the other hand, 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 in the channels of Santos (Brazil) (Ambrozevicius and 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 and 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 and 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 urbanization), 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 exposure 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 chronic exposure, mainly due to the interactions between micropollutants (Musolff et al., 2009; Roberta et al., 2014). In this context, different families of micropollutants have been successfully detected 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 micropollutants may also explain the greater chronic toxicity of the urban runoff in Guarujá.

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.

7.5 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 to evaluate the impact of the diffuse loads into the coastal areas. This approach is already implemented in Europe and US, 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 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.

Acknowledgements

The authors thank environmental engineers Camila Santos Garcia, Thiago Henrique Toscano, and the environmental manager Flávio Távora Campi for their support in the field work. The authors are also grateful to the biologist Ronaldo Justo, environmental manager of Iporanga/Guarujá condominiums for authorisation of the collection works. The authors thanks Pulsar Pictures Ltda, Apiacás St., 934, São Paulo, Brazil, for the license to reproduce the images of the beaches of Guarujá (contract n° 27918).

Disclosure statement

There are no conflicts of interest.

Funding

Correia AT was supported by national funds through FCT-Foundation for Science and Technology within the scope of UIDB/04423/2020 and UIDP/04423/2020.

Compliance with ethical standards

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

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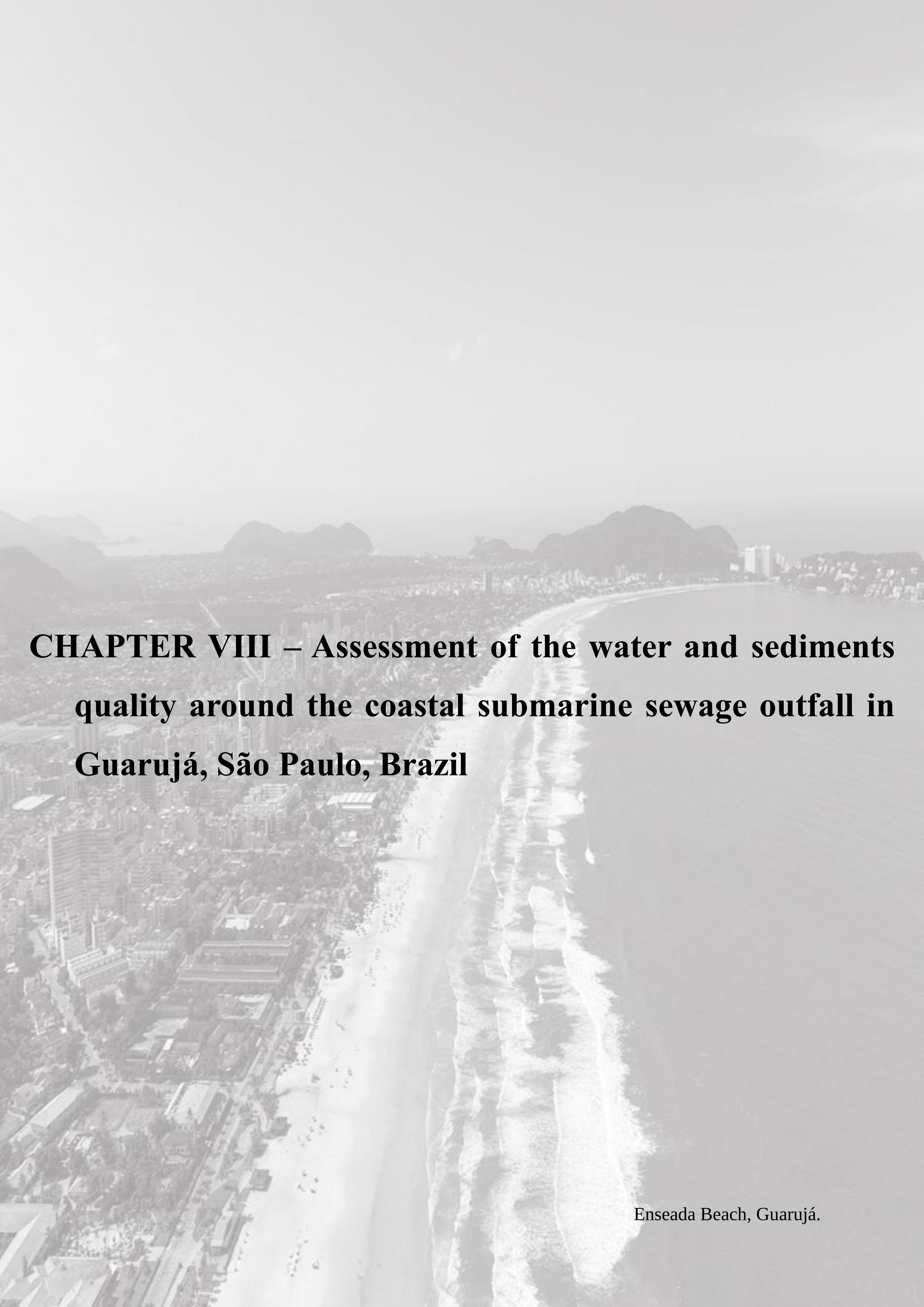

CHAPTER VIII – Assessment of the water and sediments quality around the coastal submarine sewage outfall in Guarujá, São Paulo, Brazil

Enseada Beach, Guarujá.

Assessment of the water and sediments quality around the coastal submarine sewage outfall in Guarujá, São Paulo, Brazil

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Under review: Thalassas: An International Journal of Marine Sciences (2021).

Graphical Abstract

Abstract

The discharge of domestic sewage is one of the most common types of marine pollution, namely through submarine outfalls. In this study, water and sediments of the coastal submarine sewage outfall in Guarujá, São Paulo, Brazil were assessed during the high (January) and low (April) tourist seasons in 2018. The Canadian Council of Ministers of the Environmental Water Quality Index (CCMEWQI) showed a “marginal” water quality, in both seasons, where dissolved oxygen, biochemical oxygen demand, oil and greases, ammonia, surfactants, aluminium, lead, copper, nickel, *Escherichia coli* and *Enterococci* showed potential ecological risks. However, no mutagenic potential was detected in the complex mixture (Ames *Salmonella*/microsome test: MI<2), and no adenoviruses, protozoa and *Salmonella* bacteria were found. In the sediment, a total of 25 benthic taxa were inventoried, suggesting that the macrofauna is not under contamination stress. Cadmium, lead, copper, chromium, nickel and zinc were below the Threshold Effect Level and the Geoaccumulation Index was <0. Furthermore, the absence of acute toxicity to the test organism *Kalliapseudes schubartii* (EC50: 96h) and the Shannon-Wiener diversity index (H’: 2.5 to 3.5 bits/ind) suggests healthy or unpolluted environments. However, the deviation of some environmental indicators suggests the need of continuous monitoring based on field measurements.

Keywords: Subtropical zone, Waste treatment, Ocean dumping, Marine ecology, Pollution effects.

8.1 Introduction

Worldwide population density in coastal areas (Pelling and Blackburn, 2013) has been highly responsible for the alteration of the marine ecosystems environment (Libhaber, 2016), namely due to untreated municipal sewage discharges (Cesar et al., 2007; Soto-Jiménez et al., 2011; De La Ossa Carretero et al., 2012). Sewage disposal, especially in the marine environment, is still a cause of great public concern (Baptistelli an Marcellino, 2016; Ortiz et al., 2016) and has been reported in several studies around the world, such as Havana/Cuba (Gonzalez et al., 1999), Castellon Coast/Spain (De La Ossa Carretero et al., 2008; 2012), Jinzhou/China (Wan et al., 2008), Gulf of Thermaikos/Greece (Christophoridis et al., 2009), Mazatlan Bay/México (Soto-Jiménez et al., 2011) and Darwin Harbor/Australia (Padovan et al., 2012). Although the average composition of sewage is 99.9% water and only 0.1% contaminants (Libhaber, 2016), this former fraction represents a complex physical, chemical and biological mixture, which may lead to the depletion of oxygen levels (Bleninger et al., 2016), eutrophication (N and P enrichment) (Heisler et al., 2008; Howarth, 2008), introduction of pathogenic organisms (Olalemi et al., 2016) and heavy metal contamination (Christophoridis et al., 2009). This complex mixture can cause severe changes in the environmental quality of waters (Christophoridis et al., 2009; Tabrez et al., 2011) and sediments (Gonzalez et al., 1999; Santi and Tavares, 2009) in areas adjacent to oceanic sewage disposals (Cesar et al., 2007; Soto-Jiménez et al., 2011; De La Ossa Carretero et al., 2012).

In Brazil (the fifth largest country in the world), where approximately 50 million people live in coastal municipalities (along 8,500 km of coastline) (Ibge, 2018), this situation is particularly evident. Here, the 17 states are served by 20 wastewater treatment plants (WWTP) with preliminary levels of treatment (Lamparelli and Ortiz, 2007; De Souza Abessa et al., 2012), including the WWTP of the municipality of Guarujá, São Paulo State (Baptistelli and Marcellino, 2016). Each WWTP performs only a mechanical treatment (railing and screening for the removal of solids) that is followed by chlorination. The final destination of the preconditioned sewage is a submarine outfall that disposes the sewage daily into the marine environment (South Atlantic Ocean) (Cesar et al., 2007; Lamparelli and Ortiz, 2007; De Souza Abessa et al., 2012). These systems are chosen mainly due to their lower maintenance and operation costs (10 to 20 times lower than a secondary system) (Roberts, 2016) and minimum space requirements (Libhaber, 2016), in addition to being considered cheap and safe systems, which makes them an ideal alternative for developing countries such as Brazil (Bleninger et al., 2016).

Submarine outfall plans are projected with a timeline of more than 20 years, which means that the cumulative effects in the water and sediment matrices are not fully evident in the initial years of the operation (Lamparelli and Ortiz, 2007). Several studies on the discharge of Submarine outfall have been carried out on the coast of São Paulo, but the main focus of the scientific community has been the submarine outfall of Santos (municipality located to the east of Guarujá), which is the oldest in the region, having been built in the 1970s (e.g. Moser et al., 2004; Cesar et al., 2007; De Souza Abessa et al., 2012). However, there are few studies dedicated to the Guarujá submarine outfall, with the exception of the annual monitoring program (water and sediment) carried out since 2010 by the Environmental Agency of the State of São Paulo (Cetesb) (Cetesb, 2017). The Guarujá submarine outfall has been operating for the last 20 years (Baptistelli and Marcellino, 2016) but, as far as it is known, no data exists about the impact of its discharges on the aquatic biota (benthic macrofauna) and/or on the presence of bacteria (*Salmonella*), protozoa (*Cryptosporidium* and *Giardia*), Human mastadenovirus (HAdV) (species C and F) and other species of Adenovirus (AdV). Moreover, environmental monitoring in the adjacent areas of the oceanic route of this submarine outfall is extremely important in order to propose any improvement to this obsolete system. Recently, our group evaluated the water quality of the urban runoff flowing into the public beaches and recreational waters of Guarujá, São Paulo State, Brazil using a similar approach highlighting the need of better water management practices, improvement of basic sanitation and population awareness (Roveri et al., 2020).

The present study was intended to evaluate the water (physical, chemical and microbiological parameters, including mutagenicity tests) and sediment quality (heavy metals, ecotoxicological tests and benthic macroinvertebrates) in the vicinity of the submarine outfall of Guarujá, in São Paulo, Brazil.

8.2 Material and Methods

8.2.1 Study area

This study was carried out in sub basin—13, island of Santo Amaro, Guarujá, microregion of Santos, São Paulo State, Brazil. It is an area of 143 km^2 and 64 km of extension, of which 107 km^2 is made up of environmental protection areas and 36 km^2 of urbanised areas (Ribeiro and Oliveira, 2015), with 316,000 inhabitants (Ibge, 2018).

On the island of Santo Amaro, there are two distinct seasonal periods: one rainy season from November to March, and a dry season that occurs from April to October (Cetesb, 2017). The annual precipitation of the region ranges between 2,500 and 3,000 mm, and the annual mean temperature is 22°C (Cetesb, 2017). The municipality's economy is mainly driven by three activities; two of which are non-seasonal, occurring in the western portion of the island and characterised by trade in the district of Vicente de Carvalho and by port-related activities in the port of Santos (the largest in Latin America). A third activity, which takes place in the eastern and southern portions of the island, is tourism, which provides economic benefits to 26 beaches and almost double the number of inhabitants, especially during the high summer season (between December and February) (Ribeiro and Oliveira, 2015; Cetesb, 2017).

The Municipal sewage is treated through a combined system, where the largest portion, covering six tourist beaches and four neighbourhoods, is served by a WWTP using a preliminary treatment (Cetesb, 2017). This WWTP consists of a set of crates, sandbox and sieve, which aims to remove only the solid and floating material from the sewage, followed by a chlorination step, in order to eliminate pathogenic microorganisms (Baptistelli and Marcellino, 2016; Ortiz et al., 2016). The final destination of this pre-conditioned sewer is a 4,500-meter-long and 14-meter-deep submarine outfall that disposes the sewers daily in the marine environment (Enseada beach, South Atlantic Ocean), with a flow of 1.45 m^3/s. The other parcel, covering the neighbourhood of the district of Vicente de Carvalho, is served by a WWTP characterised by a secondary level of treatment, which empties into the Acaraú River, located at the other end of the island. Together, both systems are sized for a population of approximately 450,000 inhabitants (Cetesb, 2017).

8.2.2 Sampling procedures

The municipality has an environmental agency monitoring program in the vicinity of the oceanic layout of the submarine outfall, which samples, twice a year, water and sediment matrices (Cetesb, 2017). Two field campaigns were carried out: one on the 12nd January 2018 during the high tourist season (summer/rainy season), and another on the 13th April 2018 in the low tourist season (dry/fall season). This study adopted two of the already existent environmental agency sampling points: P1: 24° 01' 39, 7"S; 46° 13' 27, 5"W and P2: 146 24° 01' 34, 3"S; 46° 13' 21, 3"W. Both points: P1 (Southwest) and P2 (Northeast) are located about 100 meters from the discharge point (Figure 8.1). During the sampling work, no rainfall was recorded in the last 48 hours prior to both campaigns.

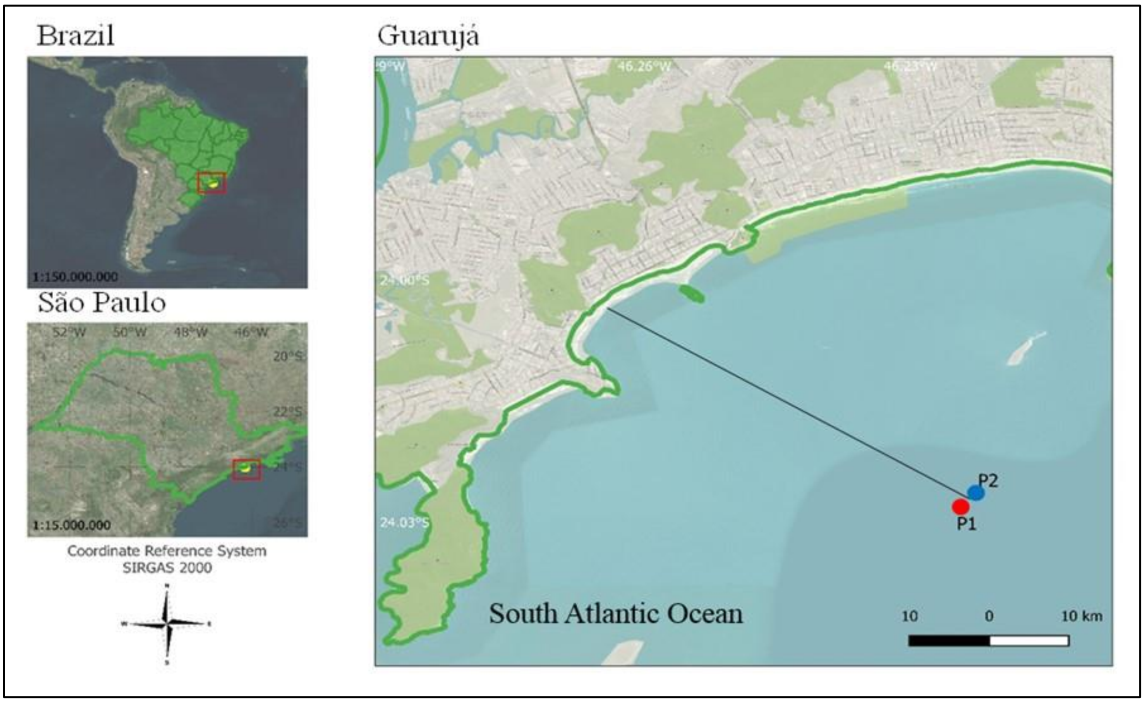

Figure – 8.1: Map showing the location of the Guarujá municipality, in the state of São Paulo, Brazil. The two sampling points (P1: red circle and P2: blue circle) are located around the coastal submarine sewage outfall in Guarujá (Enseada beach, South Atlantic Ocean).

Prior to sample collection, measurements were made *in loco* for the following variables: air and water temperatures, water density, salinity, conductivity (Ec), absolute conductivity, dissolved oxygen (DO), and pH. These measurements were performed with the aid of a multiparameter probe (HANNA HI 98184, calibrated with standard solution prior to start of each campaign). The colour of the water (Hanna photometer – HI 83200/01) and its turbidity (Hanna – HI 98703/01 turbidimeter) were also obtained in the field.

Thereafter, different environmental matrices (e.g. water and sediment) were obtained following the national guidelines for the collection and preservation of samples (Cetesb, 2011). The water was collected using a Van Dorn bottle at two different depths: surface (1 meter) and bottom (10 meters). The sediment, obtained at a depth of 14 meters, was collected with a Ponar dredge with an area of $0.037m^2$. Samples were labelled according to the sampling points (P1 and P2), collection seasons [high (H) and low (L) tourist seasons], and water depths [surface (A), bottom (B), or sediment (C)]. Samples were kept in refrigerated conditions (≤ 6°C) and the respective collection flasks, previously sterilized, were sent for laboratory to further analysis.

Ammonia (NH_3), nitrites (NO_2-) and nitrates (NO_3-), phosphate ($PO_4{}^3$-), and anionic surfactants (MBAS) in the water samples were analyzed in the Research Laboratory of Natural Products of the Santa Cecília University - Unisanta/Santos/São Paulo.

In the laboratory Analytical Control Analyzes Techniques, in Osasco/SP (accreditation of Inmetro/ABNT ISO/ IEC17025), the following water analyzes were performed: i) physico chemical analyses: sedimented solids (SS); biochemical oxygen demand (BOD), and oils and greases (OG); ii) heavy metals (also in sediments): aluminium (Al), cadmium (Cd), lead (Pb), copper (Cu), chromium (Cr), nickel (Ni), and zinc (Zn); and iii) microbiological analyses: *Escherichia Coli* (*E.coli*), *Enterococci*, *Salmonella*, *Cryptosporidium*, and *Giardia*.

The HAdV-C, HAdV-F and other species of AdV were analyzed in the Laboratory of Molecular Microbiology, Institute of Health Sciences, Feevale University, Novo Hamburgo, Brazil.

The Ames *Salmonella*/microsome mutagenicity assay [(Ames test: lineages TA98 and TA100, with and without metabolism system (S9)] was sent to the NSF Bioassays laboratory, Viamão/Rio Grande do Sul (accreditation of Inmetro/ABNT ISO/ IEC17025). In the present study, the Ames test was performed through a composite sample (low season only) added to a 2-liter flask: point P1 (P1-LA: 500 mL and P1-LB: 500 mL) and P2 (P2-LA: 500 mL and P2-LB: 500 mL).

The acute toxicity test (integral sediment - CE50; 96h) with the Tanaidacean *Kalliapseudes schubartii* was performed by the NSF Bioassays laboratory, Viamão/Rio Grande do Sul.

The identification and quantification of benthic macroinvertebrates and the granulometric analysis (fractionation of the sediment in sand, silt and clay), was performed by the Econsult Laboratory, in Guarujá, São Paulo (accreditation of Inmetro/ABNT ISO/ IEC17025).

All variables (in water and sediment matrices) and analytical methods adopted in this study are fully detailed in Table 8.1. All analyzes were carried out in triplicate.

The obtained results from water (physicochemical and microbiological) were assessed according to the current national legislation [(guidelines of the Conama Resolution – National Environmental Council No. 357/ 2005, marine waters/class 1 (Brazil, 2005)]. Due to the lack of quality standards for sediments in Brazilian legislation, the results of heavy metals were compared to the quality criteria established by the Canadian Environmental Assessment Agency (CCME, 2001a), which establishes two distinct limits: one for the threshold effect (TEL – Threshold Effect Level) and another, above which severe effects are expected (PEL – Probable Effect Level).

Table – 8.1: Analytical methods (with definition of the quantification limit and standard reference) applied to water (physical, chemical - including heavy metals, microbiological and mutagenicity test) and sediment variables (heavy metals, ecotoxicological test, benthic macroinvertebrates including granulometric analysis) of samples collected around the coastal submarine sewage outfall in Guarujá, São Paulo, Brazil.

Variables		Method	Limit of Quantification (LOQ)	Standard reference
physico chemical	sedimented solids (SS)	Imhoff Cone	0.10 mg/L	2540F (APHA, 2012)
	biochemical oxygen demand (BOD)	Electrometric	2.00 mg/L	5210B (APHA, 2012)
	oils and greases (OG)	Liquid-liquid gravimetric	5.00 mg/L	5520D (APHA, 2012)
	ammonia (NH_3)	Spectrophotometry	0.001 mg/L	4500D (APHA, 1999)
	nitrite (NO_2-)		0.001 mg/L	4500B (APHA, 2005)
	nitrate (NO_3-)		0.001 mg/L	
	phosphate (PO4^{3}-)		0.001 mg/L	4500E (APHA, 2012)
	anionic surfactants (MBAS)		0.01 mg/L	3500B (APHA, 2005)
heavy metals	aluminum (Al)	Atomic absorption spetrophotometry	0.025 mg/L	3120B (APHA, 2017)
	cadmium (Cd)		0.001 mg/L	
	lead (Pb)		0.005 mg/L	
	copper (Cu)		0.001 mg/L	
	chrome (Cr)		0.005 mg/L	
	nickel (Ni)		0.005 mg/L	
	zinc (Zn)		0.05 mg/L	
microbiological	*Escherichia Coli (E.coli)*	Filter membrane technique	1 CFU/mL	9222B (APHA, 2012)
	Enterococci	Filter membrane technique	1 CFU/mL	9230C (APHA, 2012)
	Salmonella	Presence or absence technique	-	9260B (APHA,2012)
	Cryptosporidium		1 Oocisto/L	1623.1 (EPA, 2012)

	Giardia	Immunomagnetic separation and immunofluorescence microscopy	1 cisto/L	
	HAdV-C and HAdV-F	Real-time polymerase chain reaction (qPCR) Nested polymerase chain reaction (Nested PCR)	-	protocol described by Dalla Vecchia et al. (2015)
	AdV of different hosts		-	protocol described by Li et al. (2010)
mutagenicity	mutagenicity assay	Ames test, lineages TA98 and TA100 Method 471 - Adopted: 21st July 1997	-	OECD (1997)
ecotoxicity	acute toxicity	integral sediment ($CE_{50;96h}$) – tanaidáceo *Kalliapseudes schubartii*	-	Zamboni and Costa (2002)
aquatic communitie	benthic macroinvertebrates	Identification and quantification	1 org./m²	10500 (APHA, 2012)
granulometric analysis	sediment analysis	fractionation of the sediment in sand, silt and clay	-	Embrapa (2011)

8.2.3 Quality indexes

8.2.3.1 Canadian Council of Ministers of the Environment Water Quality Index (CCMEWQI)

In addition to the individual analyses of each abiotic variable, the following quality indexes were also adopted. The CCMEWQI was developed by the Canadian Council of Ministers of the Environment: Water Quality Guidelines (CCME, 2001b), with the purpose of assessing the compliance of water quality goals (Conama No. 357/2005, class 1). The calculation of index scores using the CCMEWQI method can be obtained by using the following formula (Eq.1):

$$WQI = 100 - \frac{\sqrt{F1^2 + F2^2 + F3^2}}{1.732}\ (Eq.1)$$

Where: The range (F1): which evaluates the number of variables that show non-conformity; the frequency (F2): which evaluates the amount of non-conformities as a whole and does not differentiate within variables; and the amplitude (F3), that refers to non compliance with the current legislation (Conama No. 357/2005, class 1). This index allows the classification of the water as: excellent (95-100), good (80-94), fair (65-79), marginal (45-64), or poor (0-44) (CCME, 2001b).

8.2.3.2 Mutagenicity Index (MI)

The mutagenicity index (MI) was calculated for each concentration tested according to the following formula (Eq.2):

$$\mathrm{MI} = \frac{\text{number of revertants per plate (test compound)}}{\text{number of revertants per plate (negative control)}}\ (Eq.2)$$

The sample is considered mutagenic when the MI is ≥ 2 in at least one of the concentrations tested (Mortelmans and Zeiger, 2000).

8.2.3.3 Geoaccumulation Index (GeoI)

The GeoI was determined as a quantitative measure of pollution in sediments caused by heavy metals (Moreira and Boaventura, 2003), and can be obtained through the following equation (Eq.3):

$$\mathrm{GeoI} = \mathrm{Log2}\ [\mathrm{Cn}\ (1.5\ \mathrm{Bn}) - 1]\ (Eq.3)$$

Where: Cn is the concentration of metal “n” in the sediment and “Bn” the metallic contents indicated by the TEL (Thresholds Effects Levels) (CCME, 2001a). The 1.5 factor in the equation is used to compensate for possible variations of background data caused by lithological differences, which allows the degree of contamination of different areas to be compared. According to this index, sediments are classified as: basal level (GeoI <0), uncontaminated (0≤GeoI ≤1), uncontaminated to moderately contaminated (1≤GeoI ≤2), moderately contaminated (2≤ GeoI ≤3), moderately to heavily contaminated (3≤ GeoI ≤4), heavily contaminated (4≤GeoI ≤5), or highly contaminated (GeoI > 5).

8.2.3.4 Shannon's diversity index (*H*')

Shannon's Index (*H*'), applied to benthic macroinvertebrates, measures the degree of uncertainty in predicting which species a randomly chosen individual will belong to from a sample with S species and N individuals. The lower the value of Shannon's index, the lower the degree of uncertainty and, therefore, the sample diversity is low. Diversity tends to be higher as the index value increases. The *H*' is calculated through the following equation (Eq.4):

$$H' = \sum_{i=1}^{S} pi \ln pi \quad (Eq.4)$$

Where: pi is the frequency of each species, for i ranging from 1 to S (Wealth) (Magurran, 1988).

8.2.3.5 Statistical tests

The Mann-Whitney U test, a nonparametric statistic, was used to compare the physicochemical and microbiological variables of water between seasons [high (H) and low (L)] and depths [surface (A) and bottom (B)]. The correlation between (H x L) and (A x B) was evaluated using Spearman's correlation coefficient (Hollander and Wolfe, 2013). Only physicochemical and microbiological variables that were quantified in all campaigns and points were selected for correlation. Perceptual maps were developed, via Principal Component Analysis (PCA), in order to visualize the correlations between variables. For sediment, PCA was also applied to order the results of physicochemical variables of the bottom portion of the waters (specifically organic indicators BOD and DO), sediments (granulometry and heavy metal content), and the most representative benthic taxa in the quantitative analysis, as well as the total density of this community (Mingoti, 2005). The software Past (PAleontological STatistics), version 2.17c (Hammer, et al., 2001), was used to perform these analyses. In the mutagenicity test, the dose-response curves were evaluated by the SALANAL (*Salmonella typhimurium* Assay Analysis - statistical method developed by Environmental Monitoring Systems Laboratory, EPA – Software version 1,0 – ILS, NC, 275 USA), which includes analysis of variance and linear regression. A sample is considered positive when it presents a statistically significant difference to the negative control through ANOVA ($p \leq 0.05$); significantly positive dose-response effect ($p \leq 0.05$). The results of the acute toxicity test (integral sediment - CE50; 96h), using the Tanaidacean *Kalliapseudes schubartii*, were compared to the control with Student’s t-test for independent samples to determine the differences with the respective controls. All statistics adopted the level of significance (α) of 0.05 (Zamboni and Costa, 2002).

8.3 Results

8.3.1 Water

Although the field work occurred in two distinct climatic periods, onsite measurements showed that the air temperature was very similar in both seasons (27ºC and 26ºC for summer and autumn, respectively). Water density was lower at the surface in comparison to the bottom, showing inverse conditions when compared to water temperature. At all points, the salinity levels were within the expected range for the sampled water body (salinity $\geq$ 30%). The results of both campaigns showed Ec between 51.04 and 52.37 mS/cm and absolute conductivity (without interference of temperature) between 53.85 and 55.67 mS/cm (Table 8.2).

The maximum concentration for turbidity was 4.30 NTU recorded in point P2-LB, and the pH values obtained in both campaigns (7.30 to 7.49) revealed the water to be slightly alkaline (Table 8.2). The colour showed maximum values of 34.00 mgPt/L (point P2-HB), and the SS were <0.10 mg/L throughout the study (Table 8.2). The DO values were low in all campaigns, with sampling points ranging from 2.29 to 4.64 mg/L. Conversely, BOD values (with the exception of point P1-LA) were high in both campaigns (159.00 to 456.00 mg/L) (Table 8.2). Organic substances such as OG (5.20 to 5.80 mg/L) were detected only in surface samples (points P1-HA, P2-HA, P1-LA, and P2-LA) (Table 8.2). The results demonstrate a predominance of NH_3 (0.18 to 0.44 mg/L) in relation to NO_x (NO_2 - + NO_3 -), in both seasons (Table 8.2). $PO_4{}^{3}$- (0.06 to 0.31 mg/L) and MBAS (1.32 to 2.71 mg/L) were detected in both campaigns and in all sampling points (Table 8.2). Bacteria, such as *E. coli* (187 to 887 305 CFU/mL) and *Enterococci* (112 to 450 CFU/mL), were also detected in both campaigns and at all sampling points (Table 8.2). Other pathogenic organisms, such as *Salmonella*, were absent in all analyzes. The protozoa were below the limit of quantification (LOQ) (*Cryptosporidium*: LOQ < 1 Ocisto/L and *Giardia*: LOQ < 1 cisto/L). Adenoviruses HAdV (genogroups C and F) and DNApol were not detected (Table 8.2). Al (1.02 to 1.77 mg/L), Pb (0.01 to 0.02 mg/L), Cu (0.038 to 0.088 mg/L), and Ni metals (0.036 to 0.038 mg/L) were detected only in water samples from the low season collected from the bottom (points P1-LB and P2-LB) (Table 8.2). The CCMEWQI results showed a “marginal” water quality, in both seasons: 64.01 (P1) and 63.06 (P2) (Table 8.2).

Table – 8.2: Results of the physical, chemical (total of 24 variables), and microbiological (total of 8 variables) analyses of water samples collected around the coastal submarine sewage outfall in Guarujá, São Paulo, Brazil. Two field campaigns were carried out: one during the high tourist season (summer/rainy season: pontos: P1-HA e P2-HA) and another in the low tourist season (dry/fall season: points P1-LA and P2-LA). The water was collected at two different depths: surface (1 meter) and bottom (10 meters). The results were compared to the standard of the CONAMA (Brazilian National Environmental Council, No. 357/2005 – Class 1). The final column of the table shows the CCMEWQI index results (Canadian Council of Ministers of the Environment Water Quality Index). Notes: (------) = without of the CONAMA standard reference. (*) = Does not meet the CONAMA reference standard. (<LOQ) = Limit of Quantification. (ND) = Not detected. (P/N) = Positive/Negative. (A/P) = Absence/Presence.

Variables		Unit	High season				Low season				Standard Reference (Brazil, 2005)
			P1-HA	P1-HB	P2-HA	P2-HB	P1-LA	P1-LB	P2-LA	P2-LB	
physical and chemical	water temperature	ºC	27.89	27.41	27.92	27.33	28.48	28.06	28.30	28.07	------
	density	σ t	21.32	21.41	21.39	21.55	20.93	21.87	21.82	21.92	------
	salinity	‰	33.52	33.43	33.63	33.59	34.26	34.33	34.37	34.40	------
	conductivity (Ec)	mS/cm	51.17	51.04	51.32	51.28	52.21	52.26	52.36	52.37	------
	absolute conductivity	mS/cmA	53.98	53.85	54.17	54.12	55.66	55.40	55.54	55.67	------
	dissolved oxygen (DO)	mg/L	4.52*	4.49*	4.64*	4.58*	4.31*	2.29*	4.33*	2.22*	≥ 6.00
	turbidity	NTU	1.00	1.30	1.80	3.40	1.00	1.40	2.40	4.30	------
	pH	pH	7.45	7.33	7.49	7.34	7.49	7.42	7.46	7.30	6.50 to 8.50
	color	mgPt/L	16.00	26.00	28.00	34.00	21.00	29.00	30.00	33.00	------
	sediment solids (SS)	mg/L	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	------
	biochemical oxygen demand (BOD)	mg/L	159.00	239.00	182.00	238.00	2.90	321.00	456.00	402.00	------
	oil and grease (OG)	mg/L	5.80*	< LOQ	5.70*	< LOQ	5.20*	< LOQ	5.20*	< LOQ	virtually absent
	ammonia (NH_3)	mg/L	0.18	0.27	0.19	0.20	0.28	0.41*	0.44*	0.80*	0.40
	nitrites (NO_2^-)	mg/L	0.002	0.001	0.004	0.004	0.003	0.004	0.004	0.007	0.07
	nitrates (NO_3^-)	mg/L	0.06	0.07	0.05	0.06	0.12	0.13	0.09	0.18	0.40
	phosphate ($PO4^{3-}$)	mg/L	0.07	0.31	0.14	0.07	0.06	0.29	0.22	0.29	------
	anionic surfactants (MBAS)	mg/L	1.44*	1.66*	2.71*	2.08*	1.32*	1.78*	2.17*	2.34*	0.20
	aluminum (Al)	mg/L	ND	ND	ND	ND	ND	1.77*	ND	1.02	1.50
	cadmium (Cd)	mg/L	ND	ND	ND	ND	ND	ND	ND	ND	0.005
	lead (Pb)	mg/L	ND	ND	ND	ND	ND	0.01	ND	0.02*	0.01
	cooper (Cu)	mg/L	ND	ND	ND	ND	ND	0.038*	ND	0.088*	0.005

	chrome (Cr)	mg/L	ND	ND	ND	ND	ND	ND	ND	ND	0.05
	nickel (Ni)	mg/L	ND	ND	ND	ND	ND	0.038*	ND	0.036*	0.025
	zinc (Zn)	mg/L	ND	ND	ND	ND	ND	ND	ND	ND	0.09
microbiological	*Escherichia Coli (E. coli)*	CFU/mL	444	560	187	339	612	666	887	665	------
	Enterococci	CFU/mL	122*	112*	221*	115*	220*	287*	450*	338*	100
	Salmonella	A/P	A	A	A	A	A	A	A	A	------
	Cryptosporidium	Oocisto/L	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	------
	Giardia	cisto/L	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	------
	HAdV – C	cg/L	ND	ND	ND	ND	ND	ND	ND	ND	------
	HAdV- F	cg/L	ND	ND	ND	ND	ND	ND	ND	ND	------
	AdV of different hosts	P/N	ND	ND	ND	ND	ND	ND	ND	ND	------
CCMEWQI index			64.01	64.01	63.06	63.06	64.01	64.01	63.06	63.06	

Figure 8.2 shows the perceptual map (PCA) considering the variables that were quantified in all campaigns and collection points (water and air temperature, density, salinity, Ec, absolute conductivity, DO, turbidity, pH, colour, BOD, NH_3, NO_2-, NO_3-, $PO_4{}^{3}$ -, MBAS, *E. coli* and *Enterococci*) (Table 8.2), between seasons: high (H) and low (L) (Figure 8.2a) and collection points surface: (A) and bottom (B) (Figure 8.2b). This set of variables was able to discriminate the two seasons very well, and with the exception of DO, all variables showed the highest values during the low season (L). However, the set of variables was unable to discriminate between surface (A) and bottom (B).

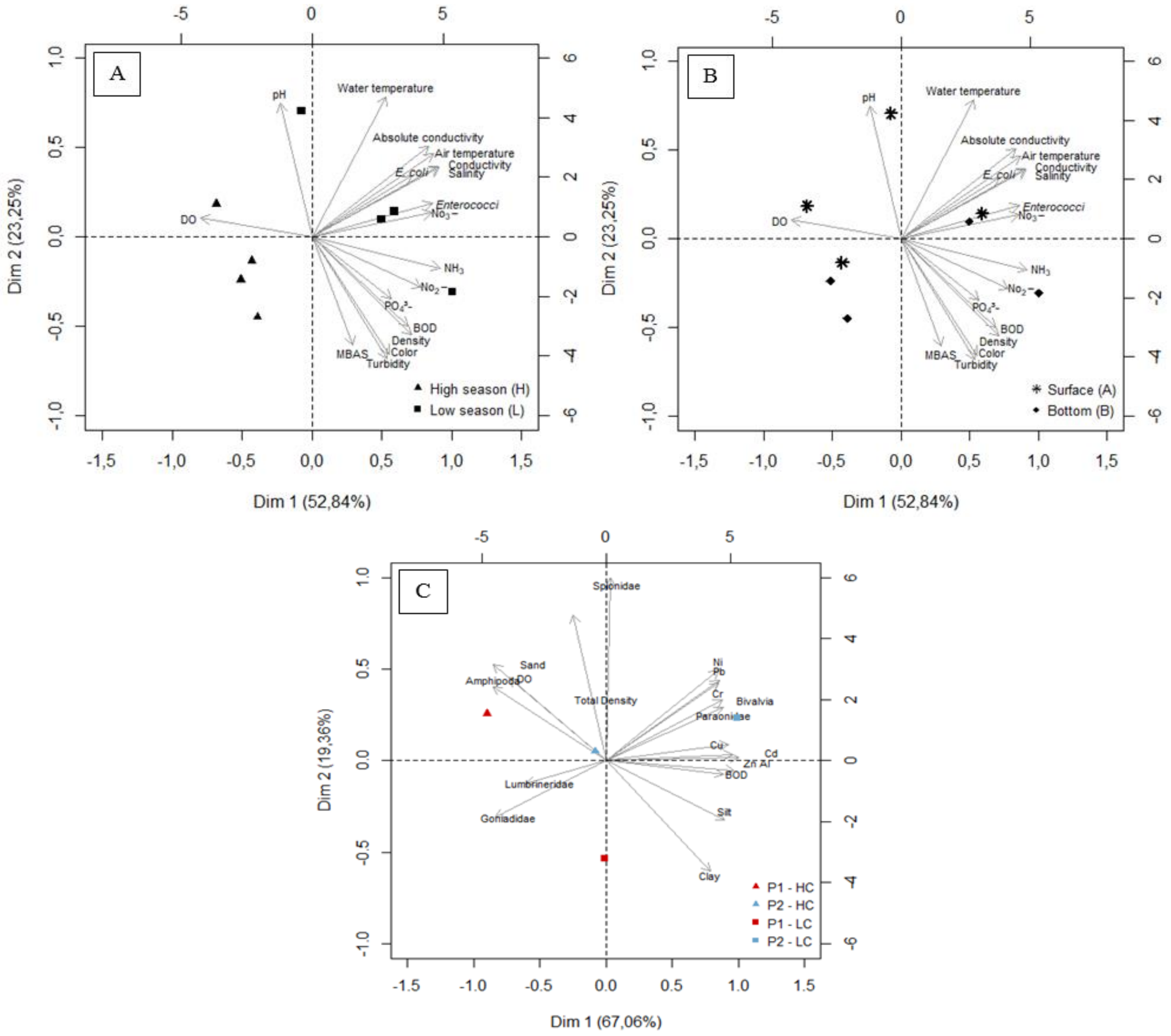
A
Dim 2 (23,25%)
Dim 1 (52,84%)
pH
Water temperature
Absolute conductivity
Air temperature
E. coli
Conductivity
Salinity
Enterococci
No_3^-
DO
NH_3
No_2^-
PO_4^{3-}
BOD
Density
Color
MBAS
Turbidity
High season (H)
Low season (L)
B
Surface (A)
Bottom (B)
C
Dim 2 (19,36%)
Dim 1 (67,06%)
Spionidae
Sand
Amphipoda
DO
Total Density
Ni
Pb
Cr
Bivalvia
Paraonidae
Cu
Cd
Zn Al
BOD
Lumbrineridae
Goniadidae
Silt
Clay
P1 - HC
P2 - HC
P1 - LC
P2 - LC

Figure 8.2. Perceptual map via Principal Component Analysis (PCA), applied to water variables: physical, chemical, and microbiological (including air temperature) and sediment (heavy metals, benthic macroinvertebrates, and granulometric analysis) of samples collected in the mix zone of the coastal submarine sewage outfall in Guarujá, São Paulo, Brazil. Three distinct scenarios were considered: (A): Principal Component Analysis (PCA) of comparison between the means of the 17 variables (water) and 1 of air (temperature) to verify in which period: high season [(H) (identification: black triangle)] or low season [(L) (identification: black square)] there was worse environmental conditions. At these two stations, the following combined analyses were carried out between collection points: high season (H): [(point P1- HA) + (point P1 - HB) + (point P2 - HA) + (point P2 - HB)] and in low season (L): [(points P1- LA) + (points P1 - LB) + (points P2 - LA) + (points P2 - LB)]. (B): Principal Component Analysis (PCA) of comparison between the means of the 17 variables (water) and 1 of air (temperature) to verify at what depth: surface [(A) (identification: black star)] or bottom [(B) (identification: black diamond)] there was worse environmental conditions. At these two depths, the following combined analyses were carried out between collection points: surface (A): [(point P1- HA) + (point P2 - HA) + (point P1 - LA) + (point P2 - LA)] and in bottom (B): [(points P1- HB) + (points P2 - HB) + (points P1 - LB) + (points P2 - LB)]. (C): Principal Component Analysis (PCA) of abiotic water variables (DO and BOD - obtained in the bottom portion), sediment (seven heavy metals: Al, Zn, Cr, Pb, Cu, Ni, Cd), grain size fraction (silt, clay and sand) together with benthic macrofauna collected during the high (H) and low season (L). On the positive axis, the abiotic variables (BOD and Al, Zn, Cr, Pb, Cu, Ni, Cd) were collected together with the grain size fractions (silt and clay), polychaetes (Paraonidae) and bivalves. These variables presented higher contributions in the low season. On the negative axis, the abiotic variables (DO), the sand fraction, the polychaetes (Lumbrineridae and Goniadidae), and the amphipods were collected. This group of variables presented greater contributions in the high season. Points of reference in the sediment: in high season (H) [(point P1-HC: identification: red triangle) and (P2 - HC: identification: blue triangle)] and in the low season (L) [(point P1-LC: identification: red square) and (point P2 - LC: identification: blue square)].

Regarding the mutagenicity assay, the mean number of revertant colonies on the negative control plates for all strains was within the spontaneous mutation frequency range of the laboratory history (period: July, to February, 2018) (Tables 8.3a, 8.3b, 8.3c, 8.3d, 8.3e and 8.3f). The results also demonstrate that the number of revertant colonies on the positive control plaques confirms the reversal property and specificity of each test line and the efficiency of the metabolic activation system. The statistical analysis of the results presented in Tables 3a and 3b (TA98) and Tables 3c and 3d (TA100) demonstrate that the sample was not able to induce reverse mutations His+ (ANOVA P Variance > 0.05 and P dose-response > 0.05) for both strains, both in the presence and absence of the metabolic system (S9). In addition, for these strains, at any concentration of the tested sample, the frequency of revertant colonies His+ was always less than twice the frequency of revertant colonies in the negative control, that is, the values of the mutagenicity index (MI) were always < 2 (Tables 8.3a, 8.3b, 8.3c and 8.3d).

Table – 8.3: Results of the Ames Salmonella/microsome test (Ames test) performed through a composite sample of water collected around the coastal submarine sewage outfall in Guarujá, São Paulo, Brazil. The field campaign was conducted only during the low tourist season (dry season / fall season). The composite sample (total of 2 liters) was sterilised by filtration membrane (0.22 µm) and tested for induction of reverse mutation for locus His in two lineages of Salmonella typhimurium: [TA98 (Tables 8.3a and 8.3b)] and [TA100 (Tables 8.3c and 8.3d)]. The Ames tests were performed by modified the pre-incubation method, in the absence [(-S9) (Tables 8.3a and 8.3c)] and presence [(+S9) (Tables 8.3b and 8.3d)] of the metabolic activation mixture (S9). The assays were performed in increasing amounts of sample (triplicates). The doses tested were 100, 200, 500, 1000, 1500, and 2000 µL/plate. All experiments included a negative control (100 µL H2O / plate). The positive control was applied according to that indicated for each lineage: [TA98 (-S9): 4 nitroquinoline 1-oxide (0,5 µg/plate) (Table 8.3a)]; [TA98 (+S9): 2 Aminofluorene (10 µg/plate) (Table 8.3b)]; [TA100 (-S9): Sodium azide (1 µg/plate) (Table 8.3c)]; [TA100 (+S9): 2 Aminofluorene (10 µg/plate) (Tables 8.3d)]. The results (average ± standard desviation) were expressed in Revertants His+/plate, and the Mutagenicity Index was calculated (MI) (Tables 8.3a to 8.3d). Tables 8.3e and 8.3f shown the historical Ames test [(TA98 and TA100 with (-S) and without (+S9)] performed by the NSF Bioassays laboratory, Viamão/Rio Grande do Sul/Brazil (test period: July, 2003 to February, 2018). The historical laboratory results are expressed in average ± standard desviation; n = sample size and minimum and maximum values.

(a)

TA98: Without metabolzation (-S9)

Substance	Concentration (µL/plate)	Revertants *His*+/plate (- S9)			Average ± standard desviation	Mutagenicity Index (MI)
		Replic 1	Replic 2	Replic 3		
Negative control (100 µL H2O / plate)	0	34	46	39	39.67 ± 6.03	
Salmonella typhimurium	100	42	36	50	42.67 ± 7.02	1.08
Salmonella typhimurium	200	43	40	42	41.67 ± 1.53	1.05
Salmonella typhimurium	500	32	46	46	41.33 ± 8.08	1.04
Salmonella typhimurium	1000	36	36	45	39.00 ± 5.20	0.98
Salmonella typhimurium	1500	38	41	41	40.00 ± 1.73	1.01
Salmonella typhimurium	2000	32	36	41	36.33 ± 4.51	0.92
Positive control (4 nitroquinoline 1-oxide)	0,5 µg	634	628		631.00 ± 4.24	15.91

(b)

TA98: With metabolzation (+S9)

Substance	Concentration (µL/plate)	Revertants *His*+/plate (+ S9)			Average ± standard desviation	Mutagenicity Index (MI)
		Replic 1	Replic 2	Replic 3		
Negative control (100 µL H2O / plate)	0	42	46	49	45.67 ± 3.51	
Salmonella typhimurium	100	47	48	49	48.00 ± 1.00	1.05
Salmonella typhimurium	200	44	42	47	44.33 ± 2.52	0.97
Salmonella typhimurium	500	42	49	51	47.33 ± 4.73	1.04
Salmonella typhimurium	1000	44	39	34	39.00 ± 5.00	0.85
Salmonella typhimurium	1500	41	44	39	41.33 ± 2.52	0.91

Salmonella typhimurium	2000	47	47	35	43.00 ± 6.93	0.94
Positive control (2 Aminofluorene)	10 µg	1065	728		896.50 ± 238.29	19.63

(c)

TA100: Without metabolzation (-S9)

Substance	Concentration (µL/plate)	Revertants *His*+/plate (- S9)			Average ± standard desviation	Mutagenicity Index (MI)
		Replic 1	Replic 2	Replic 3		
Negative control (100 µL H2O / plate)	0	163	121	138	140.67 ± 21.13	
Salmonella typhimurium	100	144	137	141	140.67 ± 3.51	1.00
Salmonella typhimurium	200	140	131	138	136.33 ± 4.73	0.97
Salmonella typhimurium	500	133	145	163	147.00 ± 15.10	1.05
Salmonella typhimurium	1000	140	141	109	130.00 ± 18.19	0.92
Salmonella typhimurium	1500	113	129	109	117.00 ± 10.58	0.83
Salmonella typhimurium	2000	98	129	112	113.00 ± 15.52	0.80
Positive control (Sodium azide))	1 µg	1064	1176		1120.00 ±79.20	7.96

(d)

TA100: With metabolzation (+S9)

Substance	Concentration (µL/plate)	Revertants *His*+/plate (+ S9)			Average ± standard desviation	Mutagenicity Index (MI)
		Replic 1	Replic 2	Replic 3		
Negative control (100 µL H2O / plate)	0	97	107	121	108.33 ± 12.06	
Salmonella typhimurium	100	96	89	109	98.00 ± 10.15	0.90
Salmonella typhimurium	200	109	108	106	107.67 ± 1.53	0.99
Salmonella typhimurium	500	105	101	114	106.67 ± 6.66	0.98
Salmonella typhimurium	1000	97	98	93	96.00 ± 2.65	0.89
Salmonella typhimurium	1500	108	100	90	99.33 ± 9.02	0.92
Salmonella typhimurium	2000	100	90	109	99.67 ± 9.50	0.92

Positive control (2 Aminofluorene)	10 µg	644	860		752.00 ± 152.74	6.94

(e)

	TA98: Without metabolzation (-S9)		TA98: With metabolzation (+S9)	
TA98	Control/ solvent	0.5 µg 4 nitroquinoline 1-oxide.	Control/ solvent	10 µg 2 Aminofluorene
Average ± standard desviation	28.32 ± 5.60	651.49 ± 218.56	29.64 ± 6.09	902.35 ± 354.44
N	513	513	502	502
Minimum	15.00	119.00	13.00	139.00
Maximum	50.00	1764.00	52.00	2390.00

(f)

	TA100: Without metabolzation (-S9)		TA100: Witht metabolzation (+S9)	
TA100	Control/ solvent	1 µg Sodium azide	Control/ solvent	10 µg 2 -AF
Average ± standard desviation	150.88 ± 24.63	1476.34± 698.16	151.79 ± 24.20	1064,09 ± 374,13
N	586	586	515	515
Minimum	88.00	292.00	82.00	313.00
Maximum	224.00	6636.00	218.00	4148.00

8.3.2 Sediment

The concentration of heavy metals in sediments was Al>Zn>Cr>Pb>Cu>Ni>Cd, at both sampling points, with a tendency to be higher during the low season, when a higher proportion of fine grains was also recorded (silt and clay). The Geoaccumulation Index was classified at a basal level (GeoI < 0) (Table 8.4). An absence of acute toxicity to the test organism *Kalliapseudes schubartii* (Crustacea: Tanaidacean) was observed, which had a survival rate ≥ 80% (method limit) that was statistically equal to the control.

The list of taxa sampled at the mouth of the Guarujá submarine outfall, in addition to their respective quantitative results, density, and relative abundance, are shown in Table 8.5. A total of 25 benthic macroinvertebrates taxa were recorded being distributed among the groups Polychaeta (11), Mollusca (6), Crustacea (5), Hemichordata (1), Nematoda (1), and Nemertea (1) for both sampling points and field campaigns. In the Guarujá submarine outfall, part of the collected taxa was recorded in the two evaluated periods, with six unique taxa identified in the high season and ten taxa verified only in the low season (Table 8.5). A density of 1215 org./m^2 (point P1-HC) and 810 org./m^2 (point P2-HC) were obtained for the high season, and 756 org./m^2 (point P1-LC) and 1053 org./m^2 (point P2-LC), in the low season (Table 8.5). Polychaeta were dominant in most of the samples obtained in both collections, with a density of up to 594 org. /m^2 at P1-HC, during the high season. However, P2-HC is an exception for this sampling, because crustaceans were more numerous (351 org./m^2), although the maximum result for this group was also obtained in P1-HC, with org./m^2. In addition to Polychaeta and crustaceans, molluscs were also observed, reaching a density of 351 org./m^2 (point P2-LC) in the low season, while other groups were less abundant in the samples (Table 8.5). Among crustaceans, the order Amphipoda was more representative, with 378 org. /m^2 (point P1-HC) during the high season. Bivalves accounted for the highest densities of molluscs, with 270 org. /m^2 in low season (point P2-LC).

The PCA showed that axes 1 and 2 accounted for 86.42% of the data variability, 67.06% for the first axis and 19.36% for the second axis (Figure 8.2c). The metals present in the sediments were placed on the positive axis 1 along with the silt and clay particle size fractions, the BOD, the depositivore polychaetes of the family Paraonidae, and also the bivalves. To this set, the samples obtained during the low season were added, in which these elements occurred in relatively higher amounts. In the negative portion of this axis, the sand fraction, DO, the most carnivorous polychaetes of the families Lumbrineridae and Goniadidae and the amphipods were correlated, which tended to present higher contributions during the high season. Data from the benthic community were synthesized through the diversity (*H´*) index. The low season recorded a greater diversity, with 3.26 bits/ind (point P1-LC) and 3.33 bits/ind (point P2-LC), while in the high season, *H*' remained between 2.84 bits/ind (point P1- 372 HC) and 2.95 bits/ind (point P2-HC) (Table 8.5).

Table - 8.4: Results of heavy metals (Al, Cd, Pb, Cu, Cr, Ni and Zn) and granulometrics (sand, silt and clay) analysis of samples collected around the coastal submarine sewage outfall in Guarujá, São Paulo, Brazil. Two field campaigns were carried out: one during the high tourist season (summer/rainy season: points P1-LC and P2-LC) and another in the low tourist season (dry/fall season: points P1-LC and P2-LC). The sediment was collected at 14 meters deep. Results of the heavy metals analysis was compared to the CCME (Canadian Council of Ministers of the Environment) standard of reference, which establishes two distinct limits: one for the threshold effect (TEL – Threshold Effect Level) and another, above which severe effects are expected (PEL– Probable Effect Level), and, in the last column last, results of the Geoaccumulation Index (GeoI) analysis calculated for the heavy metals. Note: (------) without CCME normative reference. (NA) GeoI not applicable.

Variables	Unit	High season		Low season		Standard reference (CCME, 2001a)		Geoacumulation Index (GeoI)
		P1-HC (sediment)	P2-HC (sediment)	P1-LC (sediment)	P2-LC (sediment)	Threshold Effect Level (TEL)	Probable Effect Level (PEL)	
aluminium (Al)	mg/Kg	13600	15455	15555	17899	------	------	<0 (basal level)
cadmium (Cd)	mg/Kg	0.30	0.50	0.50	0.60	0.70	4.20	<0 (basal level)
lead (Pb)	mg/Kg	15.70	19.00	16.10	21.30	30.20	112.00	<0 (basal level)
cooper (Cu)	mg/Kg	11.10	13.30	12.30	14.10	18.70	108.00	<0 (basal level)
chrome (Cr)	mg/Kg	23.10	23.90	23.30	36.30	52.30	160.00	<0 (basal level)
nickel (Ni)	mg/Kg	7.20	10.60	7.10	14.10	15.90	42.80	<0 (basal level)
zinc (Zn)	mg/Kg	58.00	66.00	76.00	94.00	124.00	271.00	<0 (basal level)
Sand	%	67	56	48	48	------	------	NA
Silt	%	25	31	31	32	------	------	NA
Clay	%	7	13	21	19	------	------	NA

Table – 8.5: Results of the taxonomic composition, total density (org./m²), relative abundance, and total richness of the benthic community of samples collected around the coastal submarine sewage outfall in Guarujá, São Paulo, Brazil. Two field campaigns were carried out: one during the high tourist season (summer/rainy season: points P1-HC and P2-HC) and another in the low tourist season (dry/fall season: points P1-LC and P2-LC). The benthic macrofauna were collected at 14 meters deep. In the final column of the table are the results of Shannon's diversity index (*H*') (bits/ind).

Taxonomic composition	High season				Low season			
	P1-HC		P2-HC		P1-LC		P2-LC	
	org./m²	%	org./m²	%	org./m²	%	org./m²	%
Phylum ANNELIDA								
Class Polychaeta								
Subclass Aciculata								
Order Eunicida								
Family Lumbrineridae	243	20.0	54	6.7	189	25.0	108	10.3
Family Onuphidae	-	-	-	-	-	-	27	2.6
Order Phyllodocida								
Family Glyceridae	-	-	27	3.3	-	-	-	-
Family Goniadidae	216	17.8	108	13.3	189	25.0	81	7.7
Family Hesionidae	-	-	-	-	-	-	27	2.6
Family Pilargidae	-	-	-	-	27	3.6	54	5.1
Family Polynoidae	27	2.2	-	-	-	-	-	-
Subclass Canalipalpata								
Order Sabellida								
Family Oweniidae	-	-	-	-	27	3.6	-	-
Order Spionida								
Family Magelonidae	27	2.2	-	-	-	-	-	-
Family Spionidae	81	6.7	54	6.7	-	-	81	7.7
Subclass Scolecida								
Family Paraonidae	-	-	-	-	27	3.6	189	17.9
Subtotal	**594**	**48.9**	**243**	**30.0**	**459**	**60.7**	**567**	**53.8**
Phylum ARTHROPODA								
Subphylum CRUSTACEA								

Class Ostracoda	-	-	-	-	-	-	27	2.6
Class Malacostraca								
Subclass Eumalacostraca								
Superorder Eucarida								
Order Decapoda								
Suborder Pleocyemata								
Infraorder Brachyura	-	-	-	-	27	3.6	27	2.6
Infraorder Caridea	54	4.4	27	3.3	27	3.6	-	-
Superorder Peracarida								
Order Amphipoda	378	31.1	297	36.7	54	7.1	-	-
Order Cumacea	-	-	27	3.3	27	3.6	27	2.6
Subtotal	**432**	**35.6**	**351**	**43.3**	**135**	**17.9**	**81**	**7.7**
Phylum HEMICHORDATA								
Class Enteropneusta	27	2.2	-	-	-	-	-	-
Subtotal	**27**	**2.2**	**-**	**-**	**-**	**-**	**-**	**-**
Phylum MOLLUSCA								
Class Bivalvia	-	-	-	-	27	3.6	270	25.6
Subclass Heterodonta								
Order Veneroida								
Family Tellinidae	81	6.7	81	10.0	-	-	-	-
Family Veneridae	54	4.4	54	6.7	27	3.6	-	-
Subclass Protobranchia								
Order Nuculida								
Family Nuculidae	-	-	54	6.7	-	-	54	5.1
Class Gastropoda								
Subclass Caenogastropoda								
Order Neogastropoda								

Family Columbellidae	-	-	-	-	54	7.1	27	2.6
Family Olividae	-	-	-	-	27	3.6	-	-
Subtotal	**135**	**11.1**	**189**	**23.3**	**135**	**17.9**	**351**	**33.3**
Phylum NEMATODA	-	-	27	3.3	-	-	-	-
Subtotal	**-**	**-**	**27**	**3.3**	**-**	**-**	**-**	**-**
Phylum NEMERTEA	27	2.2	-	-	27	3.6	54	5.1
Subtotal	**27**	**2.2**	**-**	**-**	**27**	**3.6**	**54**	**5.1**
Total Density	**1.215**	**100**	**810**	**100**	**756**	**100**	**1.053**	**100**
Total Richness	**11**		**11**		**14**		**14**	
Shannon's diversity index (*H'*)	**2.84 bits/ind**		**2.95 bits/ind**		**3.26 bits/ind**		**3.33 bits/ind**	

8.4 Discussion

8.4.1 Water

The main purpose of a WWTP preliminary treatment, such as Guarujá's WWTP, is the removal of floating materials and suspended solids often found in raw wastewater (Libhaber, 2016). The low solid content suspension and turbidity of waters recorded here show that the Guarujá's WWTP is fulfilling its main function. However, organic substances such as OG and MBAS, high BOD load, nutrients (e.g. NH_3 and PO_4^{3-}), and heavy metals (e.g. Al, Pb, Cu and Ni) that are usually not removed during this treatment step (Bleninger et al., 2016) were detected at the mouth of this submarine outfall. As a consequence, the physicochemical and microbiological variables indicate environmental/human health potential risks. From the 33 variables analyzed in this study OG, DO, NH_3, MBAS, Al, Pb, Cu, Ni, and *Enterococci* were not compliant, at least once, with national legislation. From these 9 variables, a total of 14 and 23 environmental analyzes did not meet the normative standard for the high and low seasons, respectively. As a consequence, the quality of the coastal waters in the vicinity of the Guarujá submarine outfall (in both P1 and P2 stations) was classified as “marginal” (CCME, 2001b).

The CCMEWQI index indicates that the water quality is threatened or compromised (CCME, 2001b), since the observed conditions deviate from the normative standards and, thus, from the expected environmental quality (salt water, class 1) (Brazil, 2005). Marine waters with classification 1 (e.g. Guarujá) should provide safe conditions for recreation, ensure the protection of aquatic communities, and favour fishing and aquaculture activities (Brazil, 2005).

OG, in addition to causing aesthetic problems (as well as SS, turbidity, and colour), can interfere with the exchange of oxygen between water surface and the atmosphere (lowering the DO) compromising the equilibrium of the ecosystem (Baptistelli and Marcellino, 2016). Low concentrations of DO (e.g. P1-LB and P2-LB points) may be harmful to bottom marine species (e.g. macrobenthos). Changes in amphipod assemblages were related to a hypoxia situation in stations close to sewer emissaries (De La Ossa Carretero et al., 2012). Moreover, hypoxia causes mortality in many invertebrates, and crustaceans are especially sensitive to this lack of oxygen (Gray et al., 2002). The BOD load, mainly at P1-LB, P2-LA, and P2-LB points, was higher than 300.00 mg/L, which is a typical concentration for crude domestic sewage and promotes organic enrichment of the marine environment (Cromey et al., 1998). It has been pointed out by many authors that excess organic matter may cause changes in the number of species, abundance of organisms, and in the biomass of benthic communities (Cromey et al., 1998; Elías et al., 2005).

Nitrogen and phosphorus, as well as DO, are prerequisite for primary production in aquatic ecosystems (Heisler et al., 2008). However, discharges with excess organic material, as observed here, can promote a eutrophication processes due to nutrient enrichment (Moser et al., 2004; Howarth, 2008). Several studies have shown high concentration of nutrients in the vicinity of submarine outfall worldwide, namely in Santos and Niterói (Brazil), Mumbai (India) and Boston (USA) (Moser et al., 2004). In more extreme scenarios, eutrophication can trigger proliferation of phytoplankton potentially toxic to both aquatic communities and public health (Heisler et al., 2008; Howarth, 2008).

Eutrophication caused by sewage discharges was responsible for the creation of anoxic zones on the seafloor, causing mortality of benthic organisms in the Black Sea (Gray et al., 2002; Surugiu, 2005). The predominance of NH_3 in relation to NO_X in Guarujá indicates a recent or near source of pollution (Baptistelli and Marcellino, 2016; Ortiz et al., 2016). In addition, NH_3 (a typical form of untreated domestic sewage), together with the low DO and high BOD, at the outlet of this submarine outfall suggest the occurrence of an active microbiological decomposition process, something that is common in a mixing zone (Libhaber, 2016; Ortiz et al., 2016).

In water bodies impacted by sewage, total phosphorus (TP), almost all in the form of PO_4^{3-} , is mainly originated by MBAS (up to 50%), such as linear alkyl sulphonate, that is used worldwide in household cleaning and personal hygiene products (León et al., 2006). MBAS detected in the submarine outfall mixing zone, in conjunction with OG, can impair the exchange of oxygen between water and the atmosphere, causing damage to aquatic biota (Stalmans et al.,1991; Stoll et al., 1997). In addition to remaining on the water surface, detergents also tend to settle on the bottom (Stoll et al., 1997), as observed in this study. Unfortunately, MBAS have not been a priority in coastal zone monitoring (De Souza Abessa et al., 2012), but there are studies showing their toxicity to aquatic organism such as crustaceans (Ole Kusk and Petersen, 1999) and fish (Nunes et al., 2016). Only ineffective sewage treatments (e.g. Guarujá submarine outfall) can cause high concentrations of MBAS in the surrounding environment, capable of causing toxic effects on sensitive marine organisms (e.g. *Acartia tonsa* – Crustacea) (Ole Kusk and Petersen, 1999). The concentrations of MBAS detected in the Guarujá oceanic layout, at both sampling points and seasons, are above the safety limits (LC50: 50% Lethal Concentration) for marine organisms already found in the North Sea: cod (*Gadus morrhua* – LC50: 1 mg/L) and sole (*Platichthys flesus* – LC50: 0.02 mg/L) (Stalmans et al., 1991). Using aerobic treatment systems with biological degradation and through chemical adsorption/absorption, MBAS removal is largely effective with a removal rate between 98.8 and 99.9% (León et al., 2006).

The release of sewage with an excess of organic matter is also alarming due to the introduction of pathogenic microorganisms such as bacteria, viruses, and protozoa (Ortiz et al., 2016; Roberts, 2016). In this study the presence of *E. coli* (present in human faeces in percentages ranging between 96 to 99%) (Rozen and Belkin, 2001; Selegean et al., 2001), indicated recent faecal contamination, since these bacteria are poorly resistant to the conditions of the marine environment, namely to the levels of solar radiation and salinity (Rozen and Belkin, 2001). *Enterococci* were also detected in this mixture zone. Unlike *E. coli,* it is characterized by high tolerance to the adverse conditions of the marine environment, which makes it a good indicator of human faecal contamination (Roslev et al., 2009). It is known that in the vicinity of a submarine outfall, some environmental conditions, such as UV radiation, can cause bacterial decay by up to 90% in less than one hour (Libhaber, 2016).

This factor is why preliminary systems combined with submarine outfalls are considered an adequate alternative for public health, as they remove sewers from the beach area (Roberts, 2016). However, some studies have demonstrated that the presence of *E. coli* and *Enterococci* related to domestic sewage in marine environment can also cause contamination of shellfish species, namely mussels: *Dreissena polymorpha* (Selegean et al., 2001), *Mytilus edulis* (Roslev et al., 2009), and oysters (*Crassostrea gigas*) (Olalemi et al., 2016) with deleterious impacts on fishing activity (Selegean et al., 2001; Roslev et al., 2009; Olalemi et al., 2016).

In regions where there is an oceanic discharge of domestic sewage, studies have already demonstrated the ability of heavy metals to remain in the water column (Wan et al., 2008; Christophoridis et al. 2009), which can be bioaccumulated and biomagnified by aquatic organisms (Soto-Jiménez et al., 2011), and to be adsorbed into the sediment (Gonzalez et al., 1999). Concentrations of Pb (0.01 and 0.02 mg/L) and Cu (0.038 and 0.088 mg/L) in the Guarujá mixing zone (e.g. points P1-LB and P2-LB), exceeded the limits allowed by national legislation (Brazil, 2005) and the maximum concentrations detected near the submarine outfall of Jinzhou Bay/China (Pb: 0.001 mg/L and Cu: 0.002 mg/L), with these concentrations exceeded the allowed standards of China (Wan et al., 2008). Jinzhou Bay has been identified as one of the main heavy metal–polluted coastal regions along the Chinese coastline (Wan et al., 2008). In the areas surrounding the submarine outfall of the Gulf of Thermaikos, which is the second largest metropolitan centre in Greece with a population of over 1,000,000 inhabitants, concentrations lower than in Guarujá (Cu: 0.003 mg/L) were detected (Christophoridis et al., 2009). Trace metals (e.g. Cu) are highly persistent in the environment and may be toxic even at low concentrations (Wan et al., 2008). Stofberg et al. (2011) quantified the effect of Cu on the survival of larvae of *Haliotis midae* (a marine gastropod mollusc) and reported a significant reduction in survival (LC50 = 5.58 μg/L). Thus, the concentrations of Cu detected in the Guarujá water (38.00 and 88.00 μg/L) could have the potential to interfere with the survival of some aquatic species, at least during the early life stages.

Besides the traditional chemical and microbiological tests, the use of *in vitro* assays (such as the Ames test) is a useful methodology to assess water quality (Tabrez et al., 2011). This assay is often used as a screening for *in vivo* testing to detect the mutagenic potential of environmental samples that often constitute complex mixtures (e.g. sewage discharges) (Mortelmans and Zeiger, 2000; Tabrez et al., 2011). The TA98 (displacement of the reading frame) and TA100 (substitution of base pairs) strains, with and without metabolic activation (S9), are complementary and therefore allow the characterisation of different types of mutations (Mortelmans and Zeiger, 2000; Tabrez et al., 2011). However, the composite sample (points P1 + P2) obtained in the mixing zone of the submarine oufall of Guarujá did not present mutagenic activity for the two lines tested (MI were always < 2).

8.4.2 Sediment

The pollutant load from WWT with preliminary treatments, still containing heavy metals, tends to precipitate and settle in the area surrounding the submarine outfalls (Matthai and Birch, 2000). Thus, due to the persistence of the metals in these sediments, this area is the most suitable compartment for environmental monitoring (Matthai and Birch, 2000; Cesar et al., 2007). In this study, the concentrations of Al detected in the Guarujá sediment (13600 to 17899 mg/Kg) was the largest in relation of the other six metals (Cd, Pb, Cu, Cr, Ni and Zn). Domestic sewage is an important source of Al for the marine environment (Gonzalez et al., 1999). However, its higher concentration in the Guarujá sediment can be explained by the fact that this metal is naturally abundant in the soils and sediments of the Brazilian coast (Moreira and Boaventura, 2003). Nevertheless, environmental monitoring is still needed for this metal, since it can bioaccumulate in marine species (Türkmen et al., 2006). Al concentrations of 1.23–10.65 µg/g were detected in the blue crab (*Callinectes sapidus*) in Skenderun Bay/Turkey, a region heavily impacted by sewage (Türkmen et al., 2006). In the discharge zone of the submarine sewage outfall of Havana city, Cuba, Al concentrations of 8.60 – 25.00 µg/g were also detected in the sea urchin *Echinometra lucunter* (Gonzalez et al., 1999).

Benthic macroinvertebrates are considered bioindicators of environmental quality, responding to changes caused by anthropic processes in aquatic systems, such as heavy metal contamination (Brown et al., 2000; Surugiu, 2005). At high concentrations in the sediments, metals are potentially toxic to the benthic community in the vicinity of the submarine outfalls (Padovan et al., 2012). However, the conditions observed in the sediment of Guarujá at both sampling points and seasonal campaigns [(concentrations of metals Cd, Pb, Cu, Cr, Ni and Zn < TEL (Threshold Effect Level); GeoI = 0: basal level) (CCME, 2001a)] were not able to cause acute toxicity to the test organism *Kalliapseudes schubartii* (Crustacea: Tanaidacean).

Although no toxicity was detected, it is important to mention that similar concentrations of metals (below TEL) such as Cd, Pb, Zn, and Ni were also detected in the sediment in areas adjacent to the submarine outfalls of Darwin Harbour (Australia) and Mazatlán Bay (Mexico) (Soto-Jiménez et al., 2011; Padovan et al., 2012). However, a high concentration of these four metals was detected in the tissues of the wandering sponge *Spheciospongia* (typical of Oceania) (Padovan et al., 2012) and iridescent oysters *Crassostrea* (typical species of the Gulf of California) (Soto-Jiménez et al., 2011). These examples show that the trace metal concentrations detected in the Guarujá sediment may be potential deleterious for the aquatic biota at long term through bioaccumulation phenomena. Furthermore, only tertiary treatments, not existent in Guarujá, such as chemical precipitation, active carbon adsorption, ion exchange, and reverse osmosis are able to remove heavy metals from sewage systems (Blomqvist et al., 1992).

In the benthic community of marine environments, as observed here, polychaetes are generally the group with the highest taxonomic richness. Polychaetes were also reported to be relevant in marine environments where domestic sewage is introduced (Anderlini and Wear, 1992; Santi and Tavares, 2009). The highest densities of these species, both during the high and low season and for both collection sites, in the area of the Guarujá submarine outfall were mainly attributed to the families Lumbrineridae and Goniadidae (both carnivores) (Fauchald and Jumars, 1979). The high density Lumbrineridae in Guarujá can be explained by the fact that this family is tolerant to environmental disturbance and resistant to organic and heavy metal contaminations from domestic sewage discharges (a condition observed in Guarujá) (Tabatabaie et al., 2009). The family Paraonidae (depositivores) (Fauchald and Jumars, 1979) also stood out in the low season (points P1-LC and P2-LC). However, due to the plasticity of polychaete species (containing both sensitive and pollution-tolerant taxa), their presence is not always synonymous with polluted areas (Belan, 2003). The polychaete families inventoried in the Guarujá sediments, such as the Lumbrineridae and Glyceridae (Pearson, 1975; Rosenberg, 1976), Polynoidae (Rygg, 1985), and Spionidae (Hily and Glémarec, 1990), have already been detected in organic matter and metal enriched sediments. However, the presence of these families was also indicative of healthy environments and unpolluted sediments (Rygg, 1985; Hily and Glémarec, 1990; Belan, 2003).

In this study, the Amphipoda order was well represented within the Crustacea class, especially during the high season (points P1-HC and P2-HC), where there was a lower level of pollution. Most species of crustaceans are subsurface inhabitants and therefore sensitive to hypoxic/anoxic conditions resulting from the microbial decomposition of organic matter (Smith and Shackley, 2006). This finding makes our results consistent with the published literature, which states that amphipods, due to their higher sensitivity to sediment polluted by sewers (Dauvin and Ruellet, 2007), tend to decrease in abundance and diversity under the influence of high organic debris from an submarine outfall (e.g. point P2-LC) (Anderlini and Wear, 1992). The sensitivity of the Amphipoda order was also observed in studies carried out in submarine outfall of the Spanish coast. Amphipods showed greater sensitivity (decrease of abundance and diversity) in the areas adjacent to submarine sewage outfall whose discharges were from WWTPs with lower level of treatment (preliminary and primary). Conversely, this decrease was not observed in the adjacent areas of the submarine outfall whose WWTP had a biological activated sludge treatment (De La Ossa Carretero et al., 2008; 2012). Given that amphipods are more sensitive to pollution than other marine species (De La Ossa Carretero et al., 2008), the good representation of these individuals in the adjacent areas of the Guarujá submarine outfall, could reflect a low level sediment pollution (Dauvin and Ruellet, 2007). In this way, the good representation of amphipods in association with the other conditions already mentioned (plasticity of the polychaeta species, concentrations of six metals < TEL; GeoI < 0 = uncontaminated sediment, and absence of toxicity to *Kalliapseudes schubartii*), suggest that the macrofauna of the region surrounding the Guarujá submarine outfall is not under strong contamination stress. These results are somewhat corroborated by the Shannon-Wiener index found here (*H';* 2.5-3.5 bits/ind), which indicates healthy environments and/or unpolluted conditions (Zettler et al., 2007; Al-Farraj et al., 2012).

8.5 Conclusion

This study added new information to databases on water and sediment of the Guarujá municipality. This information had not yet been inventoried in the oceanic mixing zone of the Guarujá submarine outfall. Furthermore, the fact that some variables do not meet the quality standards in the water column demonstrates the importance of expanding the monitoring of the dispersion of the plume of this sewer, to certify that these contaminants are not reaching the tourist beaches of the municipality and causing health public issues. Regarding the occurrence of oils, greases, detergents, nutrients, and heavy metals, these conditions will not improve without installing a secondary and tertiary treatment level on land. This work also points to a need to revise Conama Resolution No. 430 of 2011 (conditions and standards for effluent release in Brazil), so that more sustainable operational criteria for the submarine outfalls installed along the Brazilian coastal zone can be adopted. The data from this work also contributes towards a reflection of stakeholders (civil society, public authorities, and environmental agencies) on the extent to which marine dispersal and self-purification capacity must be relied upon, and the acceptance of future impacts on the environmental effects of strategy. Although the physicochemical and microbiological data indicate a worsening in some water quality variables (notably during the low season), the benthic community (in the submarine outfall's adjacent areas) was not negatively affected by these conditions. However, it is important to mention that interpretations of the temporal changes of communities associated with a pollution gradient should be cautious in a short-term study (Ferraro et al., 1991). This study covered a relatively short period; therefore, monitoring should continue in order to evidence at larger time scale the possible effects of the discharge of Guarujá sewage on the composition and structure of the benthic community. Finally, it is suggested that further studies must be carried out on the occurrence of other emerging pollutants, such as pharmaceuticals, since there is no record on the inventory of these substances in the Guarujá, São Paulo, Brazil.

Acknowledgments

The authors wish to thank Gilberto Rivero Chang, Edson Wilmsen Ferreira, and Taiane Pires Farias for their support during the field campaigns.

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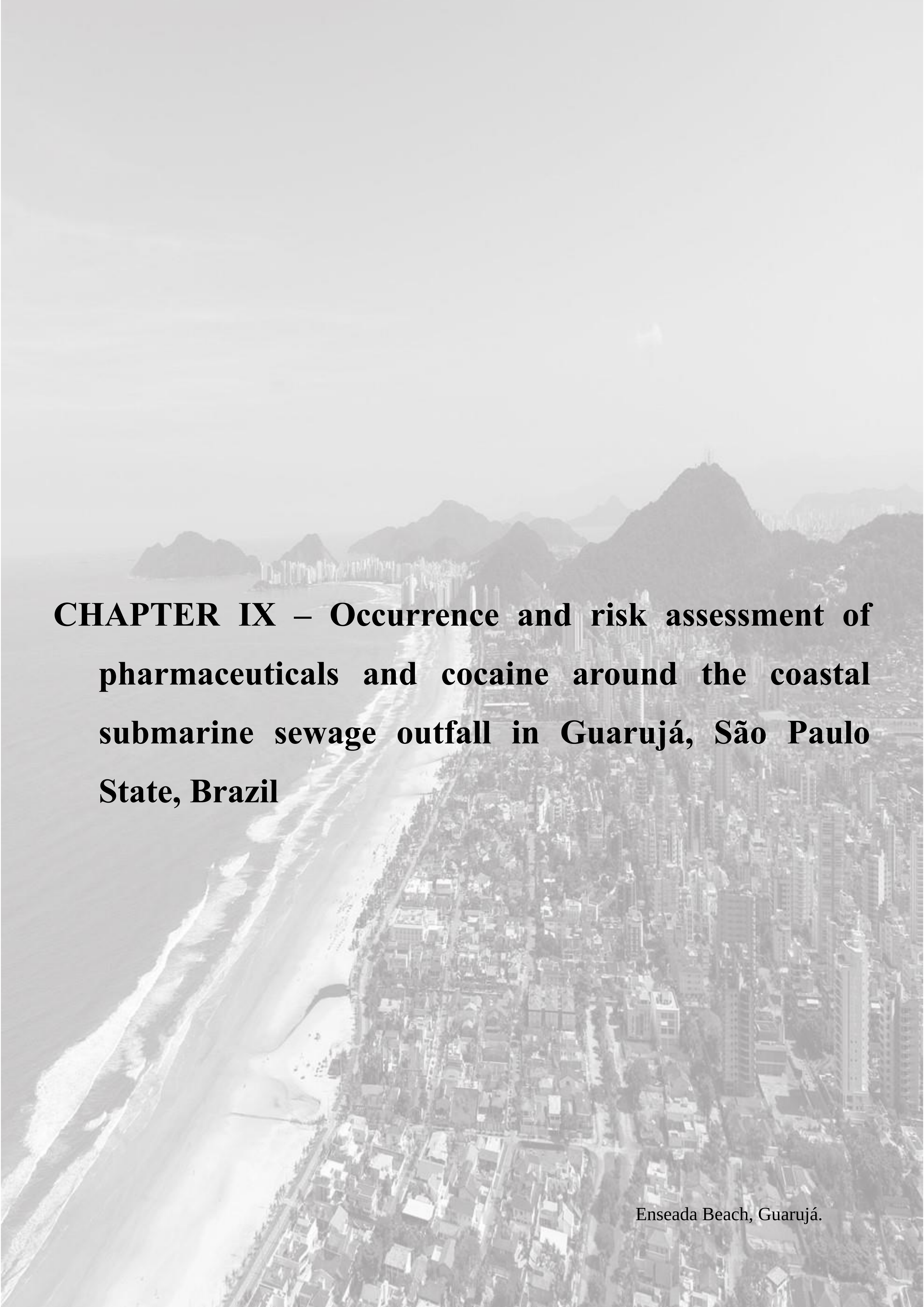

CHAPTER IX – Occurrence and risk assessment of pharmaceuticals and cocaine around the coastal submarine sewage outfall in Guarujá, São Paulo State, Brazil

Enseada Beach, Guarujá.

Occurrence and risk assessment of pharmaceuticals and cocaine around the coastal submarine sewage outfall in Guarujá, São Paulo State, Brazil

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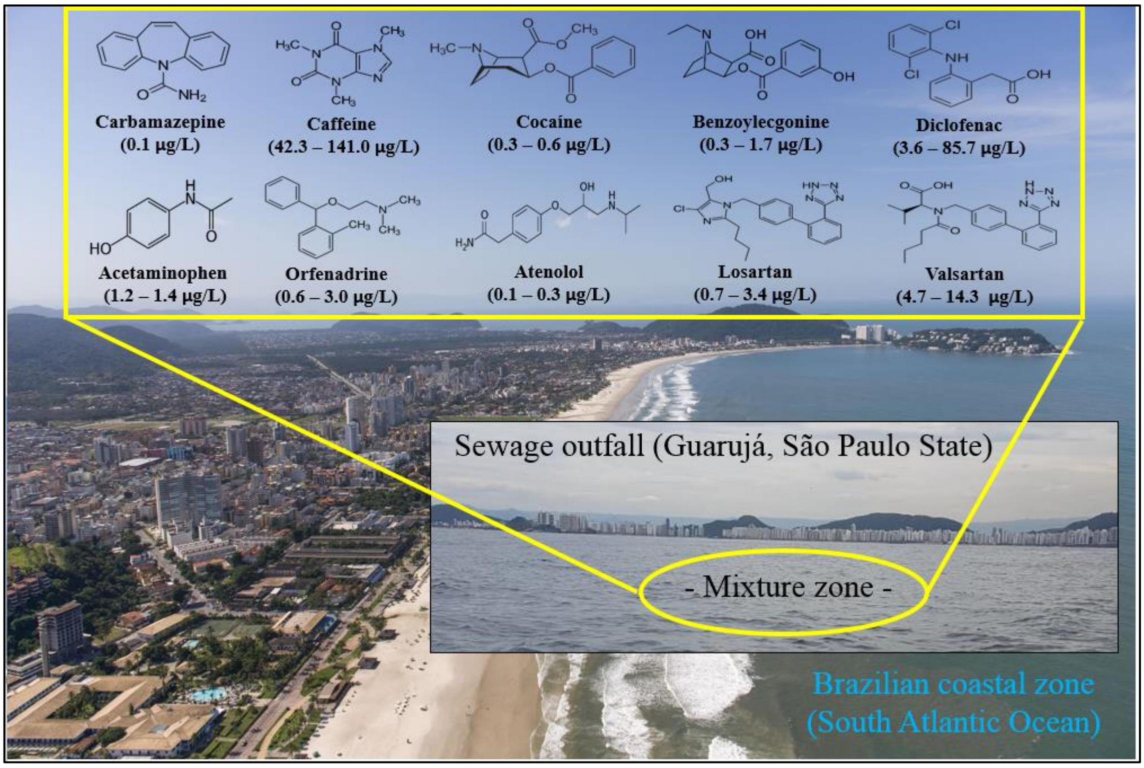
Published in: Environmental Science and Pollution Research (2020).

Graphical Abstract



Highlights

- First report of the presence of pharmaceutical compounds and illicit drugs in the mixture zone of the Guarujá submarine outfall (State of São Paulo, Brazil).

- First record of the occurrence of orphenadrine near a Latin American submarine sewage outfall.

- All detected compounds presented concentrations below the surface water safety limits (0.01 µg/L), except for caffeine.

- For almost all compounds, the observed concentrations indicate nonenvironmental risk for the aquatic biota.

- Only acetaminophen, diclofenac, and caffeine showed low to moderate ecological risk.

Abstract

The aim of this study was to screen and quantify 23 pharmaceutical compounds (including illicit drugs), at two sampling points near the diffusers of the Guarujá submarine outfall, State of São Paulo, Brazil. Samples were collected in triplicate during the high (January 2018) and low (April 2018) seasons at two different water column depths (surface and bottom). A total of 10 compounds were detected using liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS). Caffeine (42.3–141.0 ng/L), diclofenac (3.6–85.7 ng/L), valsartan (4.7–14.3 ng/L), benzoylecgonine (0.3–1.7 ng/L), and cocaine (0.3–0.6 ng/L) were frequently detected (75% occurrence). Orphenadrine (0.6–3.0 ng/L) and atenolol (0.1–0.3 ng/L), and acetaminophen (1.2–1.4 ng/L) and losartan (0.7–3.4 ng/L), were detected in 50% and 25% of the samples, respectively. Only one sample (12.5%) detected the presence of carbamazepine (< 0.001–0.1 ng/L). Unexpectedly a lower frequency of occurrence and concentration of these compounds occurred during the summer season, suggesting that other factors, such as the oceanographic and hydrodynamic regimes of the study area, besides the population rise, should be taken into account. Caffeine presented concentrations above the surface water safety limits (0.01 μg/L). For almost all compounds, the observed concentrations indicate nonenvironmental risk for the aquatic biota, except for caffeine, diclofenac, and acetaminophen that showed low to moderate ecological risk for the three trophic levels tested.

Keywords: Subtropical zone, Waste treatment, Ocean discharge, Pharmaceuticals, Illicit drugs, Marine ecology, Pollution effects.

9.1 Introduction

Coastal areas are of great economic and socio-environmental importance because 50% of the world’s population live within 60 km of a coastline (Roberts et al., 2010). This high concentration of people exposes coastal ecosystems to different anthropogenic pressures, such as the disposal of untreated sewage in the marine environment (Rodgers-Gray et al., 2000). This sewage can contain thousands of chemical substances, such as pharmaceuticals and personal care products (PPCPs) (Moreno-González et al., 2015; Brumovský et al., 2017; Fontes et al., 2019). PPCPs are a vast group of emerging environmental contaminants, such as pharmaceuticals from different therapeutic classes (e.g. antiepileptics, stimulants, analgesics/anti-inflammatory, and antihypertensive drugs) (Moreno-González et al., 2015; Brumovský et al., 2017; Comtois-Marotte et al., 2017), and illicit drugs (e.g. cocaine) (Pereira et al., 2016; Löve et al., 2018; Fontes et al., 2019).

Currently, there is no worldwide regulatory legislation that sets safety limits for these emerging compounds in the environment (Beretta et al., 2014; Machado et al., 2016; Pereira et al., 2016). Consequently, they end up in wastewater treatment plants (WWTPs), which are often inefficient in removing these pollutants (Santos et al., 2009; Behera et al., 2011; Pereira et al., 2016). Pharmaceuticals and illicit drugs are ubiquitous in coastal marine environments at concentrations ranging from ng/L to μg/L (Pereira et al., 2016; Diamanti et al., 2019; Fontes et al., 2019) and could cause harmful effects on the aquatic biota at different trophic levels, namely in molluscs (Aguirre-Martínez et al., 2013b; Almeida et al., 2015; Capolupo et al., 2016), crustaceans (Aguirre-Martínez et al., 2013a; Binelli et al., 2013; Imeh-Nathaniel et al., 2017), and fishes (Ramos et al., 2014; Nunes et al., 2015; Capaldo et al., 2019). Among the various harmful effects, some physiological, biochemical, and behavioural changes at different trophic levels are frequently observed when individuals are chronically exposed to environmentally realistic concentrations (Fabbri and Franzellitti, 2015; Godoy et al., 2015a, 2015b; Godoy and Kummrow, 2017). Under this scenario, caffeine can cause induction of oxidative stress or metabolism disturbances in molluscs (Binelli et al., 2013; Almeida et al., 2014, 2015); carbamazepine can alter the stability of the lysosomal membrane in crabs (Aguirre-Martínez et al., 2013a); and acetaminophen can cause oxidative stress in fish (Ramos et al., 2014; Nunes et al., 2015), among other effects.

The detection of PPCPs in the coastal and marine environment has been neglected for many years under the assumption that ocean dilution would represent a safety factor (Fabbri and Franzellitti, 2015; Desbiolles et al., 2018). Meanwhile, the number of studies on underwater sewage discharge is increasing around the world (Nodler et al., 2010; Afonso-Olivares et al., 2013; Alygizakis et al., 2016). In Brazil (the fifth largest country in the world), where approximately 50 million people live in coastal municipalities (along 8,500 km of coastline) (Quadra et al., 2016; Ibge, 2018), only a limited number of' studies have been dedicated to detecting the presence of PPCPs and/or illicit drugs in coastal and marine ecosystems in the last 20 years (Beretta et al., 2014; Pereira et al., 2016; Dos Santos et al., 2018; Fontes et al., 2019). In Brazilian coastal cities, WWTPs with only preliminary levels of treatment (20 systems along the coast) are predominant (Abessa et al., 2012; Ortiz et al., 2016). Each WWTP consists of a series of tanks including a sand filter and a screen, which aims to remove only the solid and floating material from the sewage, followed by a chlorination step in order to eliminate pathogenic microorganisms; but they are not specifically designed to remove PPCPs (Abessa et al., 2012; Ortiz et al., 2016; Pereira et al., 2016). The final destination of the preconditioned sewage is a submarine outfall that disposes the sewage daily into the marine environment (South Atlantic Ocean) (Abessa et al., 2012; Ortiz et al., 2016; Pereira et al., 2016). One such WWTP, which completed 20 years in 2018, is located in the Guarujá municipality (Abessa et al., 2012; Ortiz et al., 2016). Guarujá is a microregion of Santos, São Paulo State, Brazil, where the good climatic conditions (average annual temperature of 22 °C) favour the use of its beaches throughout the year, making the municipality one of the main Brazilian tourist destinations, doubling the population in summer (Ribeiro and Oliveira, 2015; Cetesb, 2017). However, to the best of the author’s knowledge, no data exist about the occurrence of pharmaceuticals and illicit drugs around this coastal submarine sewage outfall. Therefore, considering that (i) pharmaceuticals and illicit drugs pose a growing risk to marine species and ecosystems (Desbiolles et al., 2018); (ii) marine organisms are exposed to these environmental stressors, mainly in highly urbanised coastal areas and at recreational sites (Fabbri and Franzellitti, 2015); (iii) data on marine pollution by these compounds is scarce, namely, in South America, where the consumption of these substances is rapidly increasing (Quadra et al., 2016); and (iv) after the report of PPCPs and illicit drugs in Santos Bay (Pereira et al., 2016), Brazilian environmental agencies showed great concern about their occurrence and associated ecological risks, additional studies on the occurrence and risk assessment of these compounds in the marine environment around the Guarujá sewage outfall are of extreme relevance.

In this context, the objective of this study was, for the first time, to screen and identify the occurrence of 23 pharmaceuticals of various therapeutic classes (including cocaine and its primary metabolite, benzoylecgonine), near the discharge of the outfall of Guarujá on the coast of São Paulo, Brazil. This work also discusses the potential acute and chronic aquatic toxicology and adverse effects of each chemical compound reported hereby to ecologically relevant aquatic species.

9.2 Material and Methods

9.2.1 Study site description and sample collection

This study was carried out in Guarujá municipality, a microregion of Santos, São Paulo State, Brazil. It is an area of 143 km^2 and 64 km of extension, in which 107 km^2 are made up of environmental protection areas and 36 km^2 of urbanised area (Ribeiro and Oliveira, 2015), with 318,000 inhabitants (Ibge, 2018). In Guarujá, there are two quite distinct seasonal periods: a rainy season that occurs from November through March and a dry season that occurs from April through October (Cetesb, 2017). The annual precipitation of the region ranges between 2500 and 3000 mm and the annual mean temperature is 22 °C (Cetesb, 2017). The municipality's economy is mainly driven by three activities. Two of these activities are non-seasonal and occur in the western portion of the island: trade in the district of Vicente de Carvalho and port-related activities in the port of Santos (the largest port in Latin America). A third activity, which takes place in the eastern and southern parts of the island, is tourism that benefits of the existent 26 beaches. The number of inhabitants almost doubles in the high summer season (between December and February) (Ribeiro and Oliveira, 2015; Cetesb, 2017).

The municipal sewage of Guarujá is treated through a WWTP with a preliminary treatment (Cetesb, 2017). This WWTP consists of a series of tanks including a sand filter and a screen, followed by a chlorination step in order to eliminate pathogenic microorganisms (Ortiz et al., 2016). The final destination of the preconditioned sewage is a submarine outfall 4500 m long and 14 m deep that daily disposes sewage (1.45 m^3/s) into the marine environment (Enseada beach) (Cetesb, 2017).

Two field campaigns were carried out: one on 12 January 2018 during the high tourist season (summer/rainy season) and another on 13 April 2018 in the low tourist season (fall/dry season). This study adopted two existing environmental agency (Cetesb) sampling points: P1: 24° 01′ 39, 7″ S; 46° 13′ 27, 5″ W and P2: 24° 01′ 34, 3″ S; 46° 13′ 21, 3″ W. Both points, P1 (southwest) and P2 (northeast), are located about 100 m from the discharge point (Figure 9.1). During the sampling work, no rainfall was recorded in the 48 h prior to water collection. The water was collected using a Van Dorn bottle at two different depths: surface (1 m) and bottom (10 m). Samples were labelled according to the sampling points (P1 and P2), collection seasons [high (HS) and low (LS) tourist seasons], and water depths [(surface (S) and bottom (B)]. Thus, a total of eight samples were collected (2 sampling points × 2 water depths × 2 seasons).

Water samples were stored in 1-L amber glass bottles previously cleaned, transported to the laboratory in an insulated box with ice (< 6 °C), filtered with 0.45 μm pore size membrane to remove suspended solids, and kept under refrigeration (−20 °C) until further processing. The extraction, concentration, and purification of the drugs of interest were performed within 7 days after filtration (USEPA, 2007).

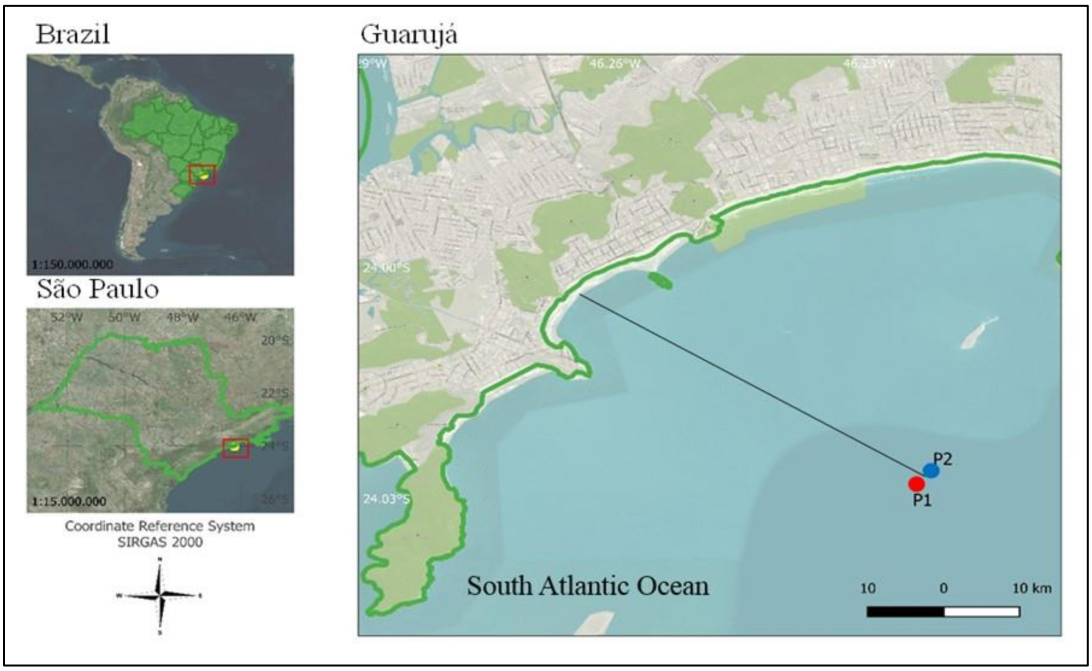


Figure – 9.1: Map showing the location of the Guarujá municipality, in the state of São Paulo, Brazil. The two sampling points (P1: red circle and P2: blue circle) are located around the coastal submarine sewage outfall in Guarujá (Enseada beach, South Atlantic Ocean).

9.2.2 Preparation and analysis of pharmaceutical compounds

9.2.2.1 Chemical and standards

High purity reagents such as nitric acid and sulphuric acid were purchased from Merck. Organic solvents of HPLC or LCMS grade, including acetonitrile, methanol and isopropanol, were acquired from Sigma-Aldrich. Mobile phase additives, namely LC-MS grade formic acid and ammonium acetate were acquired from Sigma-Aldrich and Merck, respectively. Analytical standards of acetaminophen, atenolol, bromazepam, caffeine, carbamazepine, cyproterone, clonazepam, clopidogrel, diclofenac, enalapril, loratadine, losartan, midazolam orphenadrine, propranolol, sildenafil, and valsartan were acquired from Sigma-Aldrich. Cocaine and benzoylecgonine from acquired from Cerillant. Other targeted pharmaceuticals were purchased from several providers: Citalopram (Alcytam®, Torrent by Brazil Limited), Clortalidona (Higroton®, Novarts), rosuvastatin (Crestor®, AstraZeneca), and generic paroxetine medication (Meddley).

9.2.2.2 Sample preparation

In this study, the extraction technique was adapted from Wille et al. (2010). Before extraction, the pH of each sample (channel and seawaters) was adjusted to 7.0 using a hydrochloric acid solution (1M). Next, 1 L samples were filtered through Whatman® filter paper (GF/C particle retention 1.2 μm, diameter 47 mm; Merck KGaA, Darmstadt, Germany) and to prevent the loss of the compounds of interest, the filters were washed with 2 mL of methanol (CH_3OH) (Sigma-Aldrich, St. Louis, USA). The methanol extract collected was then combined to the filtered sample. Subsequently, the SPE (solid phase extraction) procedure using spherical, hydrophobic polystyrene-divinylbenzene resin for SPE cartridges (Chromabond ® HR-X, 200 mg, 3 mL, Macherey-Nagel GmbH & Co. KG, Düren, Germany) was accomplished as described by Wille et al. (2010) and Ghoshdastidar et al. (2015). The cartridges were preconditioned with methanol (5 mL) and Milli-Q-Water (5 mL) (Milli-Q®-Merck KGaA). They were loaded with 1 L of the filtered sample and the cartridges were rinsed twice with 5 mL of Milli-Q-Water. The cartridges were then dried under vacuum for 30 min. The elution was performed using 2 × 5 mL of methanol and 5 mL of acetone. Prior to the analysis, the concentrated eluate was evaporated to dryness under a nitrogen flow (at 50 °C), re-suspended in 1 mL with a solution of water/acetonitrile (C_2H_3N) (95:5, v/v) and then filtered through a 0.45 μm pore size membrane (Merck Millipore).

9.2.2.3 LC–MS/MS analysis

Based on the reported annual consumption, expected toxicity and environmental persistence (Abrafarma, 2013), a total of 23 chemical compounds, namely pharmaceutical and illicit drugs, were analysed using liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS) (Table 9.1). LC-MS/MS methodology was described and validated by Shihomatzu et al. (2015). The validation was performed using the parameters of selectivity, matrix effect, dynamic range, linearity, limit of detection (LOD), limit of quantification (LOQ), precision (% Relative Standard Deviation), accuracy (% Coefficient of Variation), recovery and robustness. An aliquot (10 μL) of each sample was analysed using an Agilent 1260 Infinity HPLC (Agilent™, Germany) combined with a hybrid triple quadrupole/LIT instrument (3200QTRAP®- linear ion trap) mass spectrometer (ABSciex, Ontario, Canada). The conditions used for the liquid chromatography (LC) separation were as follows: an injection volume of 10 μL of each sample was loaded in an Agilent Zorbax Eclipse XDB – C18 column (50 × 4.6 mm ID, 1.8 μm column at 25°C). The eluent flow rate was 0.7 mL/min, and the mobile phase for positive mode analysis was 0.1% formic acid (Sigma-Aldrich; LC–MS Grade) in solvent A (water – H_2O) and solvent B (acetonitrile – C_2H_3N) (J.T. Baker, Philipsburg, NJ, USA). For negative mode analysis, the mobile phase was a 5 mM ammonium acetate buffer (Sigma-Aldrich) with a pH of 4.6 (solvent A) and acetonitrile (solvent B). For both modes of ionisation (negative and positive), a linear gradient of 0.7 mL/min was used, starting with a mixture of solvent A (95%) and solvent B (5%). The solvent A percentage was decreased linearly from 95% to 5% over the course of 5 min and this condition was maintained for 1 min. Over the course of 2 min, the mixture was then returned to the initial conditions. Using electrospray ionisation (ESI: positive and negative modes) and Multiple Reaction Monitoring (MRM mode) analytes were detected and quantified. This procedure was performed with the selection of a precursor ion and two ion products to quantify and qualify each compound. MRM parameters for the positive and negative modes for each chemical compound, LOD and LOQ are shown in Table 9.1. A seawater matrix-matched external calibration curve was employed, as described by Shihomatzu et al. (2015). LOD and LOQ values were determined, using spiked matrix samples and obtained from seven measurements of the lowest detectable concentration of the calibration curves (with signal-to-noise ratio of at least 10), following the Brazilian Institute of Metrology, Quality and Technology procedures (INMETRO, 2011). Both field and laboratory blanks were below LOD. Data analysis was performed with Analyst® 1.5.2 software (ABsciex). A concentration factor (1/1000) was used to obtain the measured concentration (ng/L) following LC-MS/MS quantification (Table 9.2).

Table –9.1: Multiple reactions for positive and negative ion modes. The table presents: name of compound and its respective CAS (Chemical Abstracts Service) number; Q1: mass to charge ratio of the mother ion in the first quadrupole (m/z); Q3: mass to charge ratio of the most intensive daughter ion in the third quadrupole (m/z); DP: declustering potential (V); CE: collision energy (V); LOD: Limits of detection (µg/L); LOQ: Limits of quantification (ng/L); and RT: Retention time. Note: It was assumed hereby the sample concentration factor to be 1,000 times in water matrix.

Compounds	CAS number	Q1 (m/z)	Q3 (m/z)	DP (V)	CE (V)	CXP (V)	LOD (µg/L)	LOQ (µg/L)	RT (min)
Antiepileptic									
Carbamazepine	298-46-4	237.1	194.2	36	43	4	0.003	0.01	4.7
			179.1	36	25	4			
Clonazepam	1622-61-3	316.1	270.0	51	31	4	0.0013	0.01	5.1
			214.2	51	47	4			
Stimulants									
Caffeine	58-08-2	195.2	138.3	26	19	4	0.0001	0.0085	3.4
			110.1	26	29	4			
Cocaine	50-36-2	304.2	182.2	36	39	4	0.003	0.012	3.9
			105.1	36	25	4			
Benzoylecgonine	519-09-5	290.2	168.2	31	25	4	0.0012	0.0077	3.6
			105.1	31	37	4			
Analgesics and anti-inflammatory Drugs									
Diclofenac	15307-86-5	296.1	214.1	21	39	4	0.001	0.0074	5.8
			250.0	21	25	4			
Acetaminophen	103-90-2	152.1	109.9	26	19	4	0.0014	0.0084	3.0
			93.1	26	29	4			
Orphenadrine	83-98-7	270.2	181.1	16	19	4	0.0009	0.0034	4.4
			165.0		53	4			
Antihypertensives									
Atenolol	29122-68-7	267.3	145.2	31	37	4	0.0016	0.0069	2.9
			190.3	31	25	4			
Losartan	114798-26-4	423.2	207.2	21	31	4	0.0007	0.0061	4.8
			405.2	21	17	6			

Valsartan	137862-53-4	436.3	235.1 207.1	21 21	25 33	4 4	0.0014	0.0077	5.3
Propranolol	525-66-6	260.2	116.0 183.0	41 41	23 23	4 4	0.0013	0.0072	4.4
Enalapril	75847-73-3	377.3	234.2 303.3	36 36	27 25	4 4	0.003	0.009	4.4
Antidepressants									
Citalopram	59729-33-8	325.2	109.2 262.1	41 41	37 25	4 4	0.0006	0.0059	4.3
Paroxetine	61869-08-7	330.2	192.2 135.1	41 41	27 54	4 4	0.004	0.031	4.6
Anxiolytics									
Bromazepam	1812-30-2	316.0	182.2 209.2	51 51	41 33	4 4	0.005	0.0281	4.3
Midazolam	59467-70-8	326.1	291.2 249.1	51 51	33 44	4 4	0.0006	0.0059	4.5
Antiplatelets									
Clopidrogel	113665-84-2	322.2	212.2 155.0	31 31	23 51	4 4	0.00004	0.0003	6.2
Contraceptives									
Cyproterone	2098-66-0	417.3	357.2 279.3	41	25 41	6 4	0.0015	0.0075	6.4
Diuretics									
Chlortalidone	77-36-1	336.9	189.9 146.2	−35 −35	−22 −28	−2 −2	0.0023	0.0088	4.1
Anticholesteremic Agents									
Rosuvastatin	287714-41-4	482.2	258.2 270.2	61 61	41 47	4 4	0.0008	0.0069	4.9

Antihistamines									
Loratadine	79794-75-5	383.3	337.3 267.1	41 41	33 41	6 4	0.0014	0.0126	5.2
Sexual Stimulants									
Sildenafil	171599-83-0	475.3	100.0 283.2	51 51	37 47	4 4	0.006	0.043	4.2

9.2.2.4 Ecological risk assessment

The ecological risk assessment for aquatic organisms was performed calculating the risk quotient (RQ) for 3 different trophic levels (algae, crustacean, and fish) following the equation RQ = MEC/PNEC, in which MEC is the maximum measured environmental concentration and PNEC the predicted no effect concentration, both expressed in µg/L. PNEC values were obtained from base-set reliable ecotoxicity data available for the aquatic compartment regarding short-term [lethal concentration 50 (LC50) or median effective concentration (EC50)] and long-term [no observed effect concentration (NOEC)] toxicological endpoints. In the absence of NOEC, the lowest observed effect concentration (LOEC) or, in alternative, the 10% effective concentration (EC10) were used, when available. In the present study, an attempt was made to compile specifically data for coastal marine species. When this information was not available, data from freshwater species were used instead, since a reasonable correlation exists between the ecotoxicological responses of freshwater and saltwater biota, at least for the usual aquatic taxa (i.e. acute and chronic toxicity to algae, crustacean, and fish) (EMA, 2006; Li et al., 2012; Thomaidi et al., 2015). In order to collect available aquatic ecotoxicity test endpoints, an extensive search was carried out in the Ecotoxicology Database (ECOTOX) from the United States Environmental Protection Agency (USEPA, 2019), as well as in other literature sources using the PubMed database. When ecotoxicity laboratory experimentally derived data were not available, short [L(E)C50] and long toxicological endpoints [Chv, geometric mean of NOEC and LOEC, ChV=10^([log(NOEC × LOEC)]/2)] were estimated using the Ecological Structure Activity Relationships Program (ECOSAR, v 2.0) (USEPA, 2017). The derived PNEC values for the acute and chronic toxicity data were thereafter calculated by dividing each toxicological endpoint by an assessment factor (AF). For saltwater environments, an AF of 10,000 and 100 should be considered in short-and long-term data sets. For further details, see the European Chemical Bureau (ECB, 2003) and the European Chemicals Agency (ECHA, 2008) guidelines. Finally, the risk (RQ = MEC/PNEC) was categorised into four levels: no ($RQ < 0.01$), low ($0.01 \leq RQ < 0.1$), moderate ($0.1 \leq RQ < 1$), and high ecological risk ($RQ \geq 1.0$) to aquatic organisms (Hernando et al., 2006).

9.3 Results and Discussion

In the vicinity of Guarujá sewage outfall, from the 23 compounds surveyed, 13 were below the LOD (e.g. clonazepam, propranolol, enalapril, citalopram, paroxetine, bromazepam, midazolam, clopidogrel, cyproterone, chlortalidone, rosuvastatin, loratadine, and sildenafil). The other 10 compounds were detected, at least once, according to the different therapeutic classes (Table 9.2). The detection of PPCPs in the Guarujá outfall mixing zone is a consequence of the significant production (Brazil is the ninth largest producer of medicines in the world) and high consumption of pharmaceuticals in Brazil (CMED, 2017). Among the drugs detected in Guarujá, losartan was the second best-selling drug in Brazil in 2017, followed by acetaminophen (sixth), atenolol (twelfth), and diclofenac (fifteenth) (Cmed, 2017). In addition, there are problems related to exaggerated drug consumption (self-medication is a common habit among the Brazilian population) (de Loyola Filho et al., 2004) and to the inappropriate disposal of expired and/or unusable drugs into environmental matrices (e.g. household sinks, toilets, and garbage) (WHO, 2011). Consequently, these PPCPs and illicit drugs (in parental, metabolised or conjugated forms in human excreta) (Fabbri and Franzellitti, 2015; Dafouz et al., 2018) are released indiscriminately into the receiving waters because most of the conventional WWTPs, such as Guarujá, are not efficient in removing these emerging pollutants (Petrie et al., 2015; Dos Santos et al., 2018). Removal of compounds with low octanol-water partition coefficient (i.e. log Kow values < 3.00) generally only occurs in secondary level treatment systems (Behera et al., 2011). This could explain the presence of these PPCPs and illicit drugs in the Guarujá Sea [e.g. carbamazepine (log Kow =1.51), caffeine (log Kow = − 0.07), cocaine and benzoylecgonine (log Kow < 3.00), diclofenac (log Kow = 0.70), acetaminophen (log Kow = 0.46), and atenolol (log Kow = 0.16) (Behera et al., 2011; Benotti et al., 2012; Fontes et al., 2019).

During the summer season, holidays, and weekends, the population increases such as the consumption of pharmaceuticals and illicit drugs in the cities (Fontes et al., 2019). However, this situation appears to have no interference in the disposal of these compounds in Guarujá, since a higher frequency of occurrence and also the highest concentrations occurred during the low season. It is important to mention that the present study covered a relatively short time period, as the focus was a preliminary assessment occurrence of PPCPs and illicit drugs in Guarujá. Moreover, several factors can influence the occurrence and spatial distribution of these compounds in the marine environment, such as the rain regime, oceanographic conditions, and complex hydrodynamics of the marine environment in coastal areas (Vidal-Dorsch et al., 2012; Arpin-Pont et al., 2014). It is not possible to test these hypotheses with the hereby data. Therefore, monitoring should continue in order to evidence, at a larger time scale, the possible effects of the discharge of Guarujá sewage.

Table – 9.2: Results of the occurrence of eight pharmaceuticals of different therapeutic classes and two illicit drugs (cocaine and its metabolite benzoylecgonine) collected around the coastal submarine sewage outfall in Guarujá, São Paulo, Brazil (P1 and P2 represents the sampling sites). Two field campaigns were carried out: one during the high tourist season (summer/rainy season: HS) and another in the low tourist season (fall/dry season: LS). The water was collected at two different depths: surface (S) (1 m) and bottom (B) (10 m). Concentrations are expressed in ng/L. * and ** means below limits of detection (LOD) and quantification (LOQ), respectively. Bold values represent the maximum measured concentration for each compound.

	P1-HS-S	P1-HS-B	P2-HS-S	P2-HS-B	P1-LS-S	P1-LS-B	P2-LS-S	P2-LS-B
Antiepileptics								
Carbamazepine	<0.01**	<0.01**	**0.1**	<0.01**	<0.01**	<0.01**	<0.01**	<0.01**
Stimulants								
Caffeine	<0.0001*	**141.0**	82.6	<0.0001*	90.3	42.3	54.9	71.5
Cocaíne	0.4	<0.003*	0.3	<0.003*	**0.6**	0.3	0.3	0.4
Benzoylecgonine	0.4	<0.0012*	0.4	<0.0012*	0.3	0.9	0.5	**1.7**
Analgesics and anti-inflammatory drugs								
Diclofenac	9.9	3.6	<0.001*	<0.001*	57.4	29.7	80.0	**85.7**
Acetaminophen	<0.0014*	<0.0014*	<0.0014*	<0.0014*	<0.0014*	<0.0014*	**1.4**	1.2
Orphenadrine	<0.0034**	<0.0034**	<0.0034**	<0.0034**	2.0	0.6	**3.0**	2.8
Antihypertensives								
Atenolol	<0.0069**	<0.0069**	<0.0069**	<0.0069**	0.1	**0.3**	0.1	0.1
Losartan	<0.0061**	<0.0061**	<0.0061**	<0.0061**	0.7	**3.4**	<0.0061**	<0.0061**
Valsartan	<0.0014*	<0.0014*	12.0	10.6	4.7	9.0	8.0	**14.3**

9.3.1 Antiepileptics

In this study, a frequency of occurrence of carbamazepine was observed in only 12.5% of the samples (1/8), being quantified only during the high season at sampling point P2-HS-S (Table 9.2). Carbamazepine is an anticonvulsant used to treat schizophrenia, epilepsy, and neuralgia (Almeida and Cruciol, 2013). It is an anthropogenic indicator and allows us to confirm the presence of sewage (e.g. Guarujá mixing zone) (Donner et al., 2013). Carbamazepine is persistent and resistant to degradation and adsorption, so even secondary and tertiary treatment levels WWTPs (inexistent in Guarujá) show a low rate of carbamazepine depuration (ranging from 5 to 30%, respectively) (Lin et al., 2009; Sui et al., 2010). However, the concentrations found in Guarujá (< 0.01 × 10-3–0.1 × 10-3 µg/L) were much lower than the levels detected in oceanic sewage disposal in the Baltic Sea, Germany (0.026 µg/L) (Nodler et al., 2010), and in San Francisco Bay, USA (0.004 µg/L) (Klosterhaus et al., 2013). Therefore, carbamazepine presented no ecological risk (RQ) regarding the acute and chronic exposures for all trophic levels tested (Table 9.3). Indeed, reported concentrations of carbamazepine capable of causing toxic effects in different species of marine invertebrates are higher. For example, the mollusks *Venerupis decussata*, *Venerupis philippinarum*, and *Ruditapes philippinarum*, exposed to concentrations between 0.03 and 9.0 µg/L, suffered a dose-related reduction in health status due to the induction of an oxidative stress scenario (Almeida et al., 2014, 2015). The crab *Carcinus maenas*, after a 28-day exposure to increasing concentrations of carbamazepine (0.1, 1.0, 10.0, and 50.0 µg/L), showed alteration in the stability of the lysosomal membrane (LMS) and activation of glutathione S-transferase (GST) (Aguirre-Martínez et al., 2013a). The polychaeta *Hediste diversicolor* (Maranho et al., 2015a) and microalgae *Isochrysis galbana* and *Tetraselmis chuii* (Maranho et al., 2015b) exposed for 14 days to sediments containing a concentration of 50.0 µg/kg carbamazepine, increased the total lipid content (TLP) and the activity of mitochondrial electron transport (MET) of the polychaeta, in addition to inhibiting the growth of both microalgae.

Table – 9.3: Results from the ecological risk assessment tests regarding the pharmaceuticals of the different therapeutic classes and illicit drugs (cocaine and its metabolite benzoylecgonine) detected around the coastal submarine sewage outfall in Guarujá, São Paulo, Brazil. The table presents the name of each compound, maximum measured concentration (MEC, µg/L), acute and chronic toxicity data [(trophic level, organism's test, toxicological endpoint and concentration (µg/L)], Assessment Factor (AF), Predicted No-Effect Concentration (PNEC, µg /L) and Risk Quotients (RQ, signalled in white, green, yellow and red for no, low, moderate and high risk, respectively). Data from the toxicological endpoints was obtained from several published works (References) available from the Ecotoxicology Database (ECOTOX), or, in the absence of derived experimentally data, estimated from the ECOSAR program. Note: FW Freshwater; SW Seawater; EC10: 10% Effective Concentration; EC50: 50% Effective Concentration; LC50: 50% Lethal Concentration; NOEC: No Observed Effect. Concentration; LOEC: Lowest Observed Effect Concentration. For more details, see item 2.3.

Toxicity data

Compound	**MEC** (µg/L)	**Trophic Level**		**Organisms/Species**	**Toxicological Endpoint**	**Concentration** (µg/L)	**AF**	**PNEC** (µg/L)	**References**	**RQ**
Carbamazepine	0.0001	Acute	Algae	*Skeletonema marinoi* $^{(SW)}$	72h EC50	100000	10000	10.00	Minguez et al. (2014)	<0.01
			Crustacea	*Artemia salina* $^{(SW)}$	48h EC50	100000		10.00	Minguez et al. (2014)	<0.01
			Fish	*Oryzias latipes* $^{(FW)}$	48h EC50	35200		3.52	Kim et al. (2007)	<0.01
		Chronic	Algae	*Lemna gibba* $^{(FW)}$	LOEC/2	500	100	5.00	Brain et al. (2004)	<0.01
			Crustacea	*Ceriodaphnia dubia* $^{(FW)}$	NOEC	25		0.25	Ferrari et al. (2003)	<0.01
			Fish	*Danio rerio* $^{(FW)}$	NOEC	25000		250.00	Ferrari et al. (2003)	<0.01
Caffeine	0.141	Acute	Algae	*Pseudokirchneriella subcapitata* $^{(FW)}$	72h LC50	339300	10000	33.93	Blaise et al. (2006)	<0.01
			Crustacea	*Daphnia dubia* $^{(FW)}$	48h LC50	50000		5.00	Moore et al. (2008)	0.03
			Fish	*Pimephales promelas* $^{(FW)}$	48h LC50	80000		8.00	Moore et al. (2008)	0.02
		Chronic	Algae	*Lemna gibba* $^{(FW)}$	LOEC/2	500	100	5.00	Brain et al. (2004)	0.03
			Crustacea	Daphnid $^{(FW)}$	10^([log (LOEC x NOEC)]/2)	2800		28.00	ECOSAR	<0.01
			Fish	*Pimephales promelas* $^{(FW)}$	LOEC/2	10000		100.00	Moore et al. (2008)	<0.01
Cocaine	0.0006	Acute	Algae	Green algae $^{(FW)}$	96h EC50	4350	10000	0.44	ECOSAR	<0.01
			Crustacea	Daphnid $^{(FW)}$	48h LC50	5480		0.55	ECOSAR	<0.01
			Fish	Fish $^{(SW)}$	96h LC50	48600		4.86	ECOSAR	<0.01
		Chronic	Algae	Green algae $^{(FW)}$	10^([log (LOEC x NOEC)]/2)	1460	100	14.60	ECOSAR	<0.01
			Crustacea	Mysid $^{(SW)}$	10^([log (LOEC x NOEC)]/2)	2290000		22900.00	ECOSAR	<0.01
			Fish	Fish $^{(SW)}$	10^([log (LOEC x NOEC)]/2)	7180		71.80	ECOSAR	<0.01
Benzoylecgonine	0.0017	Acute	Algae	Green algae $^{(FW)}$	96h EC50	12000000	10000	1200.00	ECOSAR	<0.01

			Crustacea	Mysid $^{(SW)}$	96h LC50	314000000		31400.00	ECOSAR	<0.01
			Fish	Fish $^{(SW)}$	96h LC50	62400000		624.00	ECOSAR	<0.01
		Chronic	Algae	Green algae $^{(FW)}$	10^([log (LOEC x NOEC)]/2)	3030000	100	3.0E+04	ECOSAR	<0.01
			Crustacea	Mysid $^{(SW)}$	10^([log (LOEC x NOEC)]/2)	2,00E+13		2.00E+11	ECOSAR	<0.01
			Fish	Fish $^{(FW)}$	10^([log (LOEC x NOEC)]/2)	4920000		4.9E+04	ECOSAR	<0.01
Diclofenac	0.0857	Acute	Algae	*Dunaliella tertiolecta* $^{(SW)}$	96h EC50	185690	10000	18.57	DeLorenzo and Fleming (2007)	<0.01
			Crustacea	*Artemia salina* $^{(SW)}$	48h EC50	100000		10.00	Minguez et al. (2014)	<0.01
			Fish	*Danio rerio* $^{(FW)}$	72h LC50	7800		0.78	Van den Brandof and Montforts (2010)	0.11
		Chronic	Algae	*Lemna minor* $^{(FW)}$	NOEC	3750	100	37.50	Cleuvers (2003)	<0.01
			Crustacea	*Ceriodaphnia dubia* $^{(FW)}$	NOEC	1000		10.00	Ferrari et al. (2003)	<0.01
			Fish	*Danio rerio* $^{(FW)}$	NOEC	4000		40.00	Ferrari et al. (2003	<0.01
Acetaminophen	0.0014	Acute	Algae	*Phaeodactylum tricornutum* $^{(SW)}$	72h EC50	239400	10000	23.94	Claessens et al. (2013)	<0.01
			Crustacea	*Artemia salina* $^{(SW)}$	48h EC50	100000		10.00	Minguez et al. (2014)	<0.01
			Fish	*Oryzias latipes* $^{(FW)}$	48h EC50	26600		2.66	Kim et al. (2007)	<0.01
		Chronic	Algae	*Phaeodactylum tricornutum* $^{(SW)}$	72h EC10	72100	100	721.00	Claessens et al. (2013)	<0.01
			Crustacea	*Daphnia magna* $^{(FW)}$	NOEC	403		4.03	Kim et al. (2007)	<0.01
			Fish	*Danio rerio* $^{(FW)}$	LOEC/2	5		0.05	Galus et al. (2013)	0.03
Orphenadrine	0.003	Acute	Algae	*Lemna minor* $^{(FW)}$	168h EC50	12000	10000	1.20	Kaza et al. 2007)	<0.01
			Crustacea	*Artemia salina* $^{(SW)}$	24h EC50	45000		4.50	Calleja et al. (1994)	<0.01
			Fish	Fish $^{(FW)}$	96h LC50	4240		0.42	ECOSAR	<0.01
		Chronic	Algae	Green algae $^{(FW)}$	10^([log (LOEC x NOEC)]/2)	132	100	1.32	ECOSAR	<0.01
			Crustacea	Daphnid $^{(FW)}$	10^([log (LOEC x NOEC)]/2)	61		0.61	ECOSAR	<0.01

			Fish	Fish $^{(FW)}$	10^([log (LOEC x NOEC)]/2)	137		1.37	ECOSAR	<0.01
Atenolol	0.0003	Acute	Algae	*Phaeodactylum tricornutum* $^{(SW)}$	72h EC50	262400	10000	26.24	Claessens et al. (2013)	<0.01
			Crustacea	*Artemia salina* $^{(SW)}$	48h EC50	100000		10.00	Minguez et al. (2014)	<0.01
			Fish	*Oryzias latipes* $^{(FW)}$	96h LC50	100000		10.00	Kim et al. (2009)	<0.01
		Chronic	Algae	*Phaeodactylum tricornutum* $^{(SW)}$	72h EC10	3300	100	33.00	Claessens et al. (2013)	<0.01
			Crustacea	*Daphnia magna* $^{(FW)}$	NOEC	1480		14.80	Küster et al. (2007)	<0.01
			Fish	*Pimephales promelas* $^{(FW)}$	NOEC	1000		10.00	Winter et al. (2008)	<0.01
Losartan	0.0034	Acute	Algae	*Lemna minor* $^{(FW)}$	96h EC50	64600	10000	6.46	Godoy et al. (2015)	<0.01
			Crustacea	*Daphnia magna* $^{(FW)}$	48h LC50	331000		31.10	FDA (2002)	<0.01
			Fish	*Pimephales promelas* $^{(FW)}$	48h LC50	1,00E+06		100.00	FDA (2002)	<0.01
		Chronic	Algae	Green algae $^{(FW)}$	10^([log (LOEC x NOEC)]/2)	1640	100	16.40	ECOSAR	<0.01
			Crustacea	Daphnid $^{(FW)}$	10^([log (LOEC x NOEC)]/2)	555		5.55	ECOSAR	<0.01
			Fish	Fish $^{(FW)}$	10^([log (LOEC x NOEC)]/2)	294		2.94	ECOSAR	<0.01
Valsartan	0.0143	Acute	Algae	*Pseudokirchneriella subcapitata* $^{(FW)}$	72h EC50	100000	10000	10.00	Minguez et al. (2014)	<0.01
			Crustacea	*Artemia salina* $^{(SW)}$	48h EC50	100000		10.00	Minguez et al. (2014)	<0.01
			Fish	*Oncorhynchus mykiss* $^{(FW)}$	96h LC50	100000		10.00	Bayer et al. (2014)	<0.01
		Chronic	Algae	*Desmodesmus subspicatus* $^{(FW)}$	NOEC	85000	100	850.00	Perrodin and Orias 2017	<0.01
			Crustacea	Mysid $^{(SW)}$	10^([log (LOEC x NOEC)]/2)	212		2.12	ECOSAR	<0.01
			Fish	Fish $^{(FW)}$	10^([log (LOEC x NOEC)]/2)	1690		16.90	ECOSAR	<0.01

9.3.2 Stimulants

Caffeine was detected during the high season only at sampling points P1-HS-B and P2-HS-S. During the low season, it was present at all sampling points (frequency of occurrence in 75% of the samples: 6/8) (Table 9.2). Caffeine is another critical marker of domestic sewage, as its presence is related to the disposal of food and drugs used exclusively by humans. After consumption, caffeine is rapidly metabolised by the liver and converted into one or more metabolites (e.g. paraxanthine) (Machado et al., 2016). Paraxanthine, is the main metabolite in humans, accounting for 80% of the total caffeine excretion in urine and faeces (Almeida and Cruciol, 2013; Machado et al., 2016). When submitted to a secondary treatment WWTP, caffeine is rapidly biodegraded through biochemical reactions, with a removal rate of 72 to 98% (Lin et al., 2009; Bueno et al., 2011). Because the Guarujá WWTP is only a primary level system, caffeine was detected at sea (up to 0.141 μg/L) in higher concentrations than those found in discharges to the Baltic Sea, Germany (0.058 μg/L) (Nodler et al., 2010), in San Francisco Bay, USA (0.040 μg/L) (Klosterhaus et al., 2013), and in the Gulf of Saronikos, Greece (0.078 μg/L) (a region that concentrates a population 10 times larger than Guarujá) (Alygizakis et al., 2016). In Guarujá, the RQ for acute (crustaceans and fish) and chronic (algae) tests was between 0.02 and 0.03 (Table 9.3), signalling a low risk of caffeine for these species. Caffeine usually causes harmful effects in different marine species in slightly higher concentrations. For example, Del Rey et al. (2011) showed that caffeine concentrations of 0.2 μg/L may have an effect in the gill tissue of the mussel *Mytilus californianus* at a molecular level (positive regulation of Hsp70). Studies have also shown that the crab *Carcinus maenas* (Aguirre-Martínez et al., 2013a) and mollusks *Ruditapes philippinarum* (Aguirre-Martínez et al., 2013b) and *Mytilus galloprovincialis* (Capolupo et al., 2016) have suffered destabilisation of the LMS after exposure to caffeine concentrations of 50.0 μg/L (in the case of Carcinus maenas and *Ruditapes philippinarum*) and 0.5 μg/L (in the case of *Mytilus galloprovincialis*). Regarding *Ruditapes philippinarum*, after the exposure of this mollusk to caffeine for 28 days (0.5, 3.0, and 18.0 μg/L), it was observed that as the concentrations increased, the mollusk lost the ability to prevent lipid peroxidation of cells and also to combat oxidative stress (Cruz et al., 2016).

Cocaine and benzoylecgonine were detected during the high season only at surface sampling points P1-HS-S and P2-HS-S (Table 9.2). During the low season, both substances were detected at all sampling points at higher concentrations when compared to the high season (both compounds were present in 75% of the samples: 6/8) (Table 9.2). Some environmental and public health concerns exist regarding cocaine and its metabolite in Brazil (Brazil, 2009). It is well-known that Brazil is the main transit route for the cocaine produced in South America, whose final destination is Europe and Asia (Unodc, 2016). Five million Brazilians aged 18 and over have used cocaine at least once during their lifetime (Laranjeira et al., 2012). It is known that even WWTP with secondary treatment levels only partially remove cocaine (40–93%) and benzoylecgonine (12–92%) (Zuccato et al., 2008; Domènech et al., 2009). Since the Guarujá WWTP does not serve this purpose, both compounds were detected in the present study raising significant concerns due to their potential effects on biota (Baker and Kasprzyk-Hordern, 2013). The concentrations of cocaine (0.0003–0.0006 µg/L) and benzoylecgonine (0.002–0.0003 µg/L) detected in Guarujá are however lower than other studies worldwide. In Santos Bay, Brazil, concentrations of cocaine and benzoylecgonine respectively of 0.537 µg/L and 0.038 µg/L were reported (Pereira et al., 2016; Fontes et al., 2019). In San Francisco Bay, USA, which receives municipal sewage discharge, benzoylecgonine (0.007 µg/L) was also detected (Klosterhaus et al., 2013). Cocaine has a strong pharmacological effect, and its presence in a body of water can produce unpredictable interactions (Zuccato et al., 2008), such as that with chlorine used in sewage treatment. In this sense, the chlorination process performed in a WWTP (e.g., Guarujá) (Ortiz et al., 2016) may interact with cocaine and benzoylecgonine and might generate unwanted transformation products (TPs) (e.g., cocaine – TP:$C_{18}H_{24}NO_4$; and benzoylecgonine – TP:$C_{16}H_{18}NO_5$). The toxicity of these TPs is still unknown (Bijlsma et al., 2013). The RQ for the acute and chronic exposures to cocaine or benzoylecgonine were < 0.01 for all three trophic levels tested (Table 9.3). These data suggest no environmental risk of these compounds for local aquatic species. However, some studies have already shown that environmentally realistic concentrations of cocaine are capable of producing changes in shellfish metabolism (*Dreissena polymorpha*) (Binelli et al., 2013), behavioural changes in crustaceans (*Orconectes rusticus*) (Imeh-Nathaniel et al., 2017), and bioaccumulation in the tissue of the eel (*Anguilla anguilla*) (Capaldo et al., 2019).

9.3.3 Analgesics and anti-inflammatory drugs

Diclofenac was detected during the high season at sampling points P1-HS-S and P1-HS-B (Table 9.2). During the low season, it was detected at all sampling points at higher concentrations when compared to the high season (frequency of occurrence in 75% of the samples: 6/8) (Table 9.2). Diclofenac is an analgesic and anti-inflammatory drug (Almeida and Cruciol, 2013). Due to its high worldwide consumption and sale (usually without a prescription), it has become one of the most commonly detected PPCPs in aquatic ecosystems and therefore has been rated as high management priority (Sotelo et al., 2014). When submitted to a secondary treatment level WWTP, the percentage of diclofenac removal rate is in the range of 60% (Jelic et al., 2011). However, in Guarujá, the Enseada mixing zone recorded higher concentrations (0.086 μg/L) than those found in other sewage outfalls, namely, in Spain: Gran Canaria Island (0.048 μg/L) and Jinámar (0.028 μg/L) (Afonso-Olivares et al., 2013), Greece: Gulf of Saronikos (0.016 μg/L) (Alygizakis et al., 2016), and Brazil: Santos (0.019 μg/L) (Pereira et al., 2016). Diclofenac is a biologically active compound with a low biodegradation rate and has a rapid photo transformation into new by-products after disposal in the aquatic environment, which can cause deleterious effects in biota at different trophic levels (Lee et al., 2011; Toufexi et al., 2016; Bonnefille et al., 2018). The RQ of diclofenac in the sea of Guarujá was 0.11 for fish acutely exposed (Table 9.3), indicating a moderate environmental risk. However, the recorded low concentrations are unlikely to cause damage to aquatic biota. For example, diclofenac concentrations of 1 μg/L strongly affected the development of the larvae of this mollusk (after 48-h exposure) (Fabbri et al., 2014) and caused an increase in the DNA breakages of this species (after 1-h exposure) (Mezzelani et al., 2016). Higher concentrations of diclofenac have also caused cytotoxic and genotoxic effects (after exposure to a concentration of 25 μg/L for 14 days) (Toufexi et al., 2016), molecular effects on specific targets (inhibition of prostaglandin E2 synthesis) after exposure to a concentration of 100 μg/L for 3 days (Courant et al., 2017), and potential effects on osmoregulation and reproduction of the mollusk *Mytilus galloprovincialis* (after exposure to a concentration of 100 μg/L for 7 days) (Bonnefille et al., 2018).

Acetaminophen was not detected at any sampling point during the high season, whereas during the low season, it was detected only at sampling points P2-LS-S and P2-LS-B (frequency of occurrence in 25% of the samples: 2/8) (Table 9.2). Acetaminophen is a drug with antipyretic and analgesic action (Almeida and Cruciol, 2013). Because of its high worldwide consumption (generally without medical prescription), environmental persistence, and significant toxicity to aquatic species, acetaminophen is included in the class II priority pollutants list, requiring future monitoring and the development of specific ecotoxicological studies to address their toxic effects, and therefore must be given priority management (Antunes et al., 2013). When submitted to a secondary treatment level WWTP, the percentage of acetaminophen removal rate is around 90% (Sun et al., 2014). Guarujá concentrations (0.0012–0.0014 µg/L) are well below those detected in the sewage outfall of the island of Gran Canaria, Spain (0.297 µg/L) (Afonso-Olivares et al., 2013), in the Gulf of Saronikos, Greece (0.040 µg/L) (Alygizakis et al., 2016), and in Santos Bay, Brazil (0.035 µg/L) (Pereira et al., 2016). The RQ for acetaminophen was in general < 0.01 (Table 9.3), with exception of fish chronically exposed showing a low environmental risk. Studies have already demonstrated the ability of environmentally realistic concentrations of acetaminophen (e.g. Guarujá) to bioaccumulate in blue mussels (*Mytilus edulis*) (Wille et al., 2011) and cause oxidative stress in three species of bivalves, namely, *Corbicula fluminea* (Brandão et al. 2011), Venerupis decussata, and Venerupis philippinarum (Antunes et al., 2013), and in two species of fish, namely *Oncorhynchus mykiss* (Ramos et al., 2014) and *Anguilla anguilla* (Nunes et al., 2015).

Orphenadrine was not quantified at any sampling point during the high season. During the low season, it was detected at all sampling points (frequency of occurrence in 75% of the samples: 4/8) (Table 9.2). Orphenadrine is a psychoactive drug used as a muscle relaxant anticholinergic drug with low antihistamine activity and is also prescribed to treat Parkinson’s disease (Almeida and Cruciol, 2013). Taking into consideration recent reviews (Fabbri and Franzellitti, 2015; Quadra et al., 2016; Godoy and Kummrow, 2017; Starling et al., 2018), this study seems be the first to report the occurrence of this PPCP in a Latin American submarine sewage outfall. Orphenadrine was reported in Psyttalia Island, Athens, Greece, a country where the drug was widely consumed in 2018 (1.7 mg/day/1000 people) (Diamanti et al., 2019). It was also reported in the Tiber River, Perugia, Italy, but concentrations were not detailed (Milione et al., 2016). The RQ of orphenadrine in Guarujá was < 0.01 for all exposures times and trophic levels (Table 9.3), thus indicating no risk for aquatic species. Studies have shown that concentrations of 0.014 µg/L orphenadrine (higher than those detected in Guarujá, up to 0.003 µg/L) are capable of bioconcentrating in the blood plasma of rainbow trout (*Oncorhynchus mykiss*) (Fick et al., 2010). A reduced growth of *Lemna minor* (duckweed) after exposure to orphenadrine "non-relevant" environmental concentrations of 12.0 mg/L was also found (Kaza et al., 2007).

9.3.4 Antihypertensives

Atenolol was not quantified at any sampling point during the high season. During the low season, it was detected at all sampling points (frequency of occurrence in 50% of the samples: 4/8) (Table 9.2). The β-blocker atenolol has several therapeutic indications, but it is particularly indicated as an antiarrhythmic and antihypertensive drug in cardiac protection after myocardial infarction (Almeida and Cruciol, 2013). In secondary treatment level WWTPs, the atenolol removal rate was reported to be approximately 40% (Papageorgiou et al., 2016). Atenolol is one of the most frequently detected antihypertensives in fresh surface waters in the world (Godoy et al., 2015a, 2015b). In Brazil, there are only a few reports of its presence in fresh surface water (e.g. Billings Reservoir, São Paulo: 0.016 μg/L and São Domingos Stream, Rio de Janeiro: 0.821 μg/L) (Quadra et al., 2016). In the Guarujá sewage outfall, concentrations were very low (up to 0.0003 μg/L). For other similar studies conducted in marine waters, for example, sewage outfalls in Gran Canaria, Spain (Afonso-Olivares et al., 2013), in the Adriatic Sea, Italy (Loos et al., 2013), in Santos, Brazil (Pereira et al., 2016), and on the west coast of the Mediterranean Sea (Brumovský et al., 2017), atenolol was below the detection limit in all samples. The RQ of atenolol in Guarujá was < 0.01 for all trophic levels and both exposure tests (Table 9.3), thus indicating absence of risk for the aquatic species. However, it has shown that atenolol has an effect on prokaryotic and eukaryotic cells at environmentally relevant exposure levels (ng/L to μg/L) (Pomati et al., 2007). Other studies have also demonstrated that atenolol causes toxic effects in "non-relevant" environmental concentrations. In this context, atenolol concentrations of 2.5, 10.0, and 33.4 mg/L caused larval mortality (LC50-96h) of the fish *Danio rerio* (Küster et al., 2007) and growth inhibition in the fish larvae of *Pimephales promelas* (after a 28-day exposure to atenolol) (Winter et al., 2008) and of the crustacean *Ceriodaphnia dubia* (EC50-48 h) (Fraysse and Garric, 2005), respectively. However, Massarsky et al. (2011) argue that new (long-term) experiments are needed with aquatic species (e.g. fish and crustaceans), specifically to test the hypothesis that atenolol is an endocrine disruptor.

Losartan was not quantified at any sampling point during the high season. During the low season, it was quantified only at sampling points P1-LS-S and P1-LS-B (frequency of occurrence in 25% of the samples: 2/8) (Table 9.2). Valsartan was detected, during the high season, only at sampling points P2-HS-S and P2-HS-B. During the low season, it was detected at all sampling points (frequency of occurrence in 75% of the samples: 6/8) (Table 9.2). Losartan and valsartan are prescribed drugs to treat hypertension and are generally consumed by the elderly population (Almeida and Cruciol, 2013). Guarujá has an estimated population of 316,000 (about 27,000 elderly) (Ibge, 2018). When submitted to a WWTP (activated sludge system), the percentage of losartan and valsartan removed is as high as 90% (Oosterhuis et al., 2013). Losartan concentrations (up to 0.0034 μg/L) were lower than those found in the Mediterranean Sea, Spain (0.004 μg/L) (Gros et al., 2012), and in Santos Bay, Brazil (0.032 μg/L) (Pereira et al., 2016). RQ < 0.01 was recorded for losartan (Table 9.3). Valsartan concentrations detected in Guarujá (up to 0.0143 μg/L) were higher than those found in the Lesser Sea lagoon, Spain (0.004 μg/L) (Moreno-González et al., 2015), and in the Saronikos Gulf, Greece (0.003 μg/L) (Alygizakis et al., 2016) but lower than those reported in Santos Bay, Brazil (0.075 μg/L) (Pereira et al., 2016). For valsartan, the RQ was < 0.01 for all trophic levels and exposures times (Table 9.3). These results indicate that losartan and valsartan does not represent any risk for the aquatic species in the hereby reported seawater concentrations. However, these antihypertensives deserve attention, as their consumption has increased in many parts of the world, and because studies on the toxicity of these substances are still poorly documented (Godoy et al., 2015a, 2015b; Pereira et al., 2016; Desbiolles et al., 2018). Bayer et al. (2014) did not observe inhibition of *Desmodesmus subspicatus* algae growth after 72-h exposure at 120.0 mg/L of valsartan; Yamamoto et al. (2014) found embryo/larval alteration of the sea urchin *Lytechinus variegatus* exposed to concentrations of valsartan 25.0 mg/L; and Cortez et al. (2018) detected cytotoxic effects on gills and haemocytes of *Perna perna mussel*, exposed at environmental realistic concentrations of up to 0.3 μg/L of losartan. However, the previous tested concentrations of both antihypertensives were much higher than the concentrations found in Guarujá.

9.4 Conclusion

The detection of emerging pollutants such as carbamazepine, caffeine, cocaine, benzoylecgonine, diclofenac, acetaminophen, orphenadrine, atenolol, losartan, and valsartan in the Guarujá outfall reinforces the worldwide concern about the disposal of pharmaceuticals and illicit drugs via primary-level treatment sewage outlets in the marine coastal areas. More rigorous standards for oceanic sewage disposal in Brazil need to be imposed. There is also evidence for the need to resize sewage treatment along the Brazilian coastal zone (total of 20 outfalls), including a level of treatment capable of removing, at least partially, PPCPs and illicit drugs. In order to understand what happens to these PPCPs after ocean disposal, a long-term monitoring programme would be necessary because Guarujá Island has strong hydrodynamic conditions, and therefore, these contaminants are likely to be dispersed along the coastal zone. Although most of the screened drugs do not present environmental risks in the hereby reported concentrations, the present study reinforces the need for further ecotoxicological studies (especially with tropical marine organisms) to assess the long-term toxicity of these bioactive compounds.

Acknowledgments

Authors want to acknowledge Daniel Temponi Lebre from the Centro de Espectrometria de Massa Aplicada - Instituto de Pesquisas Energéticas e Nucleares (CEMSA, IPEN, São Paulo, Brazil) for technical support regarding the LC–MS/MS analyses. Thanks also to Vinicius Roveri of Pulsar pictures Ltda, Apiacás St., 934, São Paulo, Brazil, for the license to reproduce images of the beaches of Guarujá (contract nº 27918). This research was supported by national funds through FCT - Foundation for Science and Technology within the scope of UIDB/04423/2020 and UIDP/04423/2020.

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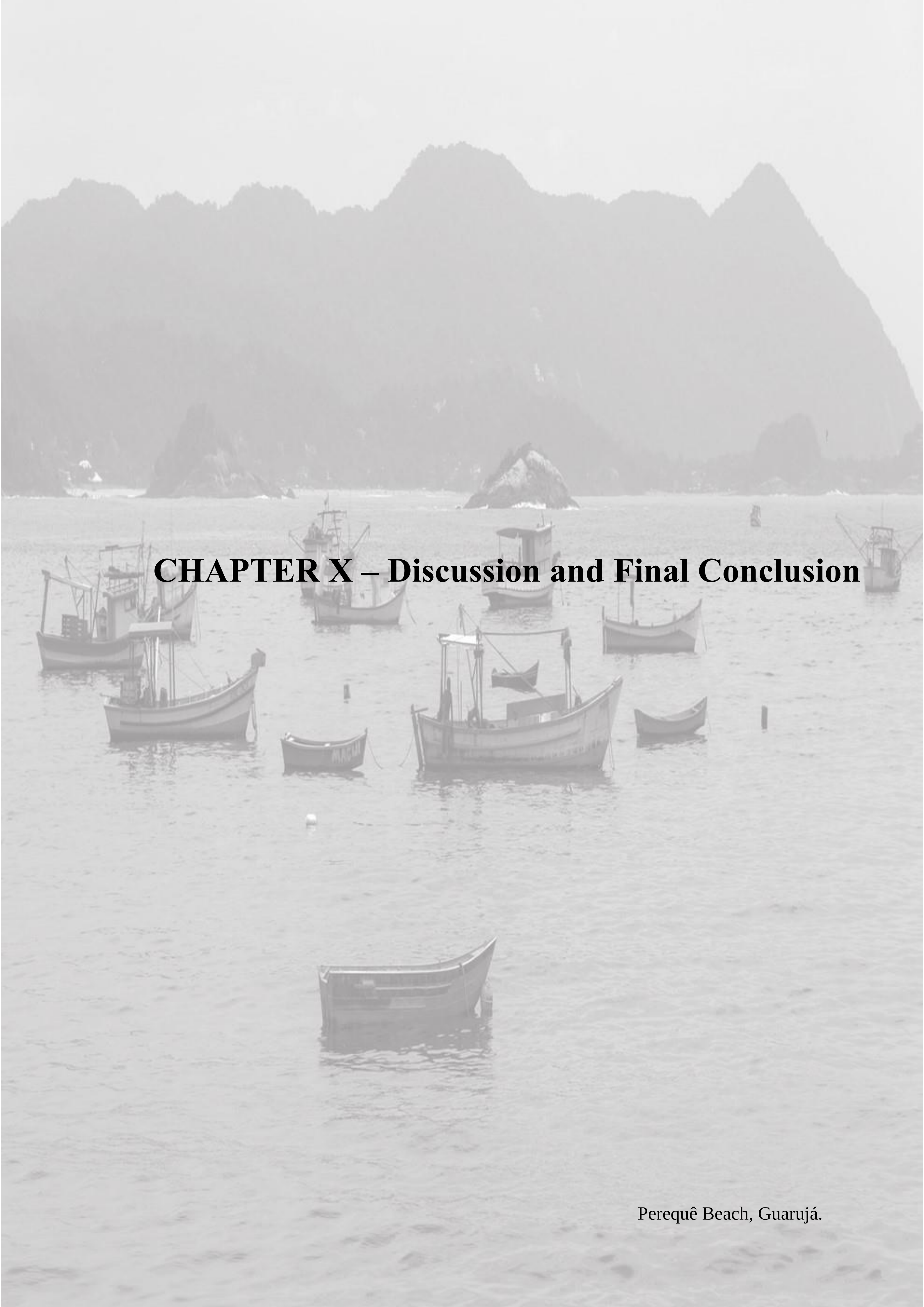
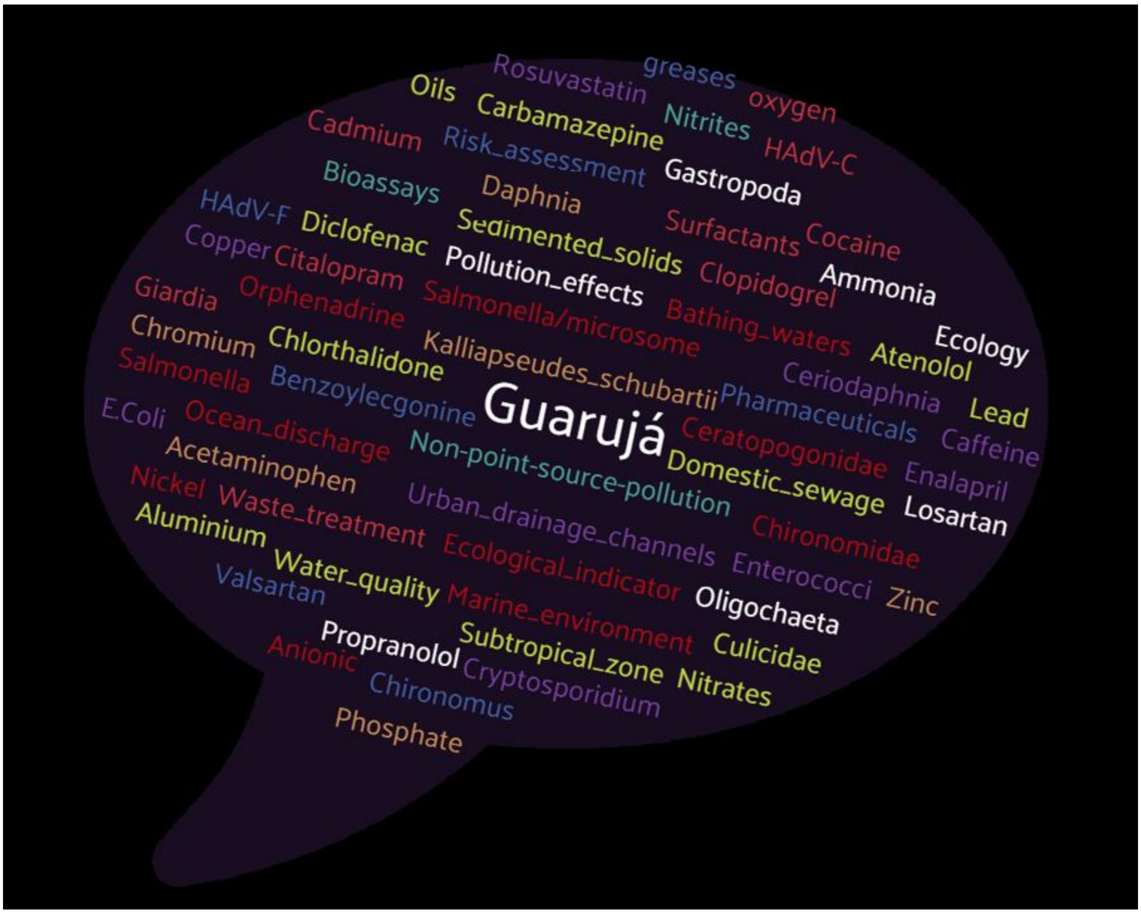


CHAPTER X – Discussion and Final Conclusion

Perequê Beach, Guarujá.

Graphical Abstract



10.1 General discussion

10.1.1 Rational and objectives of the thesis: a synthesis

As described in **Chapter 1**, a disordered urban growth of Guarujá, occurred mainly between the 1950s and the beginning of the 21st century, resulting in a strong anthropic pressure in the sub-basin of the municipality (SMA/CPLEA, 2005; SMA/CPLA, 2012; Ribeiro and Oliveira, 2015). Although the sub-basin of Guarujá has a drainage area of about 143 km², about 107 km² are made up of areas of environmental preservation. Thus, currently about 316,000 inhabitants are concentrated in a small urbanized area of 36 km^2, which represents a demographic density of more than 2,000 inhabitants per km^2 (SMA/CPLA, 2012; Cetesb, 2018a; Ibge, 2018). As a consequence, Guarujá has been suffering for decades from socio-environmental problems, inclunding the collapse of the city urban infrastructure. For instance, more than 64,000 residents of slums and/or precarious constructions in the municipality (one of the highest slums growth annual rate in the State of São Paulo - 31%) who do not have access to sewage collection and processing (SMA/CPLA, 2012; Cetesb, 2018a; Instituto Trata Brasil, 2018). The situation is further exarcebated by the fact that 500 precarious buildings still do not have sanitary facilities, including bathrooms for their residents (Instituto Trata Brasil, 2018). This sanitary deficiency was reflected in the public health. In 2010, an outbreak of viral gastroenteritis, mainly due to contaminated waterborne, occurred in Guarujá and affected the health of 6,300 residents (Morillo et al., 2011). In 2018, 40 hospitalizations due to different water diseases, 4 from dengue fever and 5 from leptospirosis were registered, generating a total health expense for the municipality of around R$ 30,000 (5000 €) (SMA/CPLA, 2012; Ibge, 2018; Instituto Trata Brasil, 2018). Thus, although the Guarujá sewage treatment is composed by a combined system (SPS of Vila Zilda plus WWTP of Vicente de Carvalho) sized for a population of approximately 450,000 inhabitants (De Souza Abessa et al., 2012; Baptistelli and Marcellino 2016; Ortiz et al. 2016), due to the environmental sanitation deficit of the slums and precarious occupations, this untreated sewage is mostly discharged into the 43 urban drainage channels of the city leading to a complex mixture (urban sewage plus diffuse load), popularly known as "black tongue", which flows into the beaches of the city (Rocha et al., 2011; Ribeiro and Oliveira, 2015; Cetesb, 2018a). Moreover, the portion of the municipal sewage pre-conditioned in the SPS and the submarine outfall system have also been a cause for great concern. This occurs because this system does not have a stage for the removal of the organic load and, therefore, is being launched in the Guarujá marine environment (South Atlantic Ocean) (Baptistelli and Marcellino 2016; Ortiz et al., 2016; Cetesb, 2018b). In addition, these municipal sewers may contain emerging pollutants, such as PPCPs and, therefore, the complex pollutant load may compromise the public health and ecological systems of Guarujá (Moreno-González et al., 2015; Brumovský et al., 2017; Fontes et al., 2019).

In this scenario, although actions to control water pollution in Guarujá are critical, studies on the causes and consequences of these sources of pollution are limited in the municipality (Ferraz et al., 2012; Martins et al., 2013; Lamparelli et al., 2015). This lack of scientific research on the municipality's water resources was detailed in **Chapter 1 (Table 1.1)**. In the case of diffuse pollution that occurs in urban drainage channels, the scarcity of studies is mainly due to the absence of normative imposition, since the National Policy on Water Resources (Law 9433/1997) makes no reference to the control of this source of pollution (Brazil, 1997). In the case of PPCPs (both for urban drainage channels and for marine sewage discharges) their presence has always been neglected, under the justification that there is no global regulatory legislation that establishes safety limits for PPCPs in the environment (Beretta et al., 2014; Machado et al., 2016; Pereira et al., 2016).

Thus, in order to improve the knowledge about the pollution levels in Guarujá water resources, the present thesis established some criteria (detailed in **Chapter 2; Figure 2.2** and **Table 2.1**), to select the beaches of Tombo, Enseada, Perequê and Iporanga as study areas:

(i) Different characteristics regarding land using and occupation: (i) Tombo beach, besides being widely served by the municipal sewage network, has international Blue Flag certification; (ii) The beach of Enseada, has a sanitation deficit in the neighborhood but is still highly visited by tourists. Furthermore, the submarine outfall of Guarujá is located in Enseada; (iii) Perequê beach was irregularly occupied by a fishing community and, as a consequence, also presents a deficit in environmental sanitation; and (iv) Iporanga beach, was selected for being located in the EPA of Guararu Hill, for having a private sewage treatment and also for having restricted tourist access (Ribeiro and Oliveira, 2015; Cetesb, 2018b; Fee, 2018).

(ii) Annual beach classification by Cetesb: in 2018, Iporanga beach was the only one that presented an "optimal" annual classification. Tombo beach presented a "good" classification. The beach of Enseada presented a "bad" classification and, the Perequê, obtained a " terrible " classification. (Cetesb, 2018a).

(iii) Representativeness of these four beaches in the Guarujá Waterfront Project: among the six study sectors, delimited by the City Hall for the Guarujá Waterfront Project, the Tombo is located in sector 2, the Enseada (sector 3), the Perequê (sector 4) and the Iporanga (sector 5) (Brazil, 2006; São Paulo, 2013; Guarujá, 2013; 2019).

In view of the above, the main goal of this thesis was to evaluate the quality of the water and sediment of the urban drainage channels located at the beaches of Tombo, Enseada, Perequê and Iporanga, besides the discharge of marine sewage at the beach of Enseada, Guarujá, São Paulo, Brazil. The final purpose is to provide the municipality with data enabling a rational and sustained management of the coastal environment in Guarujá.

For this, as described in **Chapter 2 (Tables 2.2** and **2.3)**, multiple environmental variables (total of 38) were considered, in addition to 23 PPCPs of different therapeutic classes, which were inventoried in the urban drainage channels, sea water (recreation area) and/or in the mixing zone of the submarine outfall of Guarujá. Thus, the specific objectives of this thesis were accomplished:

- The physical-chemical and bacteriological evaluation of the waters of the urban drainage channels and of the sea water of the beaches of Tombo, Enseada, Perequê and Iporanga (described in **Chapter 3**).

- The occurrence of protozoa and enteric viruses in the waters of the channels that flow to the beaches of Enseada and Perequê (**Chapter 4**).

- The structure of the benthic assembly near the urban drainage channels of Tombo, Enseada, Perequê and Iporanga beaches was characterized (**Chapter 5**).

- The occurrence and evaluation of the ecological risk of PPCPs and illicit drugs in the waters of the drainage channels and in the sea water of the beaches of Tombo, Enseada, Perequê and Iporanga was done (**Chapter 6**).

- The genotoxicity and ecotoxicity (acute and chronic) of diffuse loads that drain to the beaches of Tombo, Enseada, Perequê and Iporanga were analyzed (**Chapter 7**).

- The quality of the water column and sediment in the mixing zone of the submarine outfall, through physicochemical, microbiological, hydrobiological, genotoxic and ecotoxic variables was assessed (**Chapter 8**).

- The occurrence and evaluation of the ecological risk of PPCPs and illicit drugs in the water column, mixing zone of the submarine outfall of Guarujá was done (**Chapter 9**).

As a corollary of this thesis, a series of scientific results have resulted, from which we highlight the 7 published accepted or submitted papers, which fill a gap about the water quality and sediment in the marine costal area of the Guarujá municipality. One of the important contributions of these datasets, will be the assistance to the municipal government in the management of the Guarujá Waterfront Project, because despite its socioeconomic and environmental importance, the Guarujá Waterfront Project still lacks primary data on water and sediment quality (Brazil, 2006; São Paulo, 2013; Guarujá, 2013; 2019).

10.1.2 Public health and ecological risks: urban drainage channels

The problem of the diffuse pollution flowing into the coastal areas has been viewed with concern in the last decade, as several studies conducted in coastal cities of South Korea (Choi et al., 2011), Mexico (Curiel-Ayala, 2012), China (Zhang et al., 2013), Cuba (Larrea-Murreal et al., 2103), Colombia (Botero et al., 2015), United States (Tilburg et al., 2015) and Italy (Federigi et al., 2016), have warned that urban runoff is a potential threat to the public and environmental health, as it is responsible for introducing chemical and biological pollutants directly into estuaries and oceans (areas of intense recreation) (Zhang et al., 2013; Tilburg et al., 2015; Federigi et al. 2016). The hereby results showed, for the first time, that the Guarujá urban drainage channels (mainly from Tombo, Enseada and Perequê) are important vehicles for transporting conventional and emerging pollutants to the South Atlantic Ocean, raising potential risks to the public health and to the aquatic environment, as shortly described below:

(i) **Chapter 3** (physical-chemical variables): the waters of these channels presented important markers for the presence of domestic sewers: high conductivity and high concentrations of total dissolved solids; high organic load (low levels of dissolved oxygen and high biochemical demand for oxygen); high load of nutrients (ammonia, nitrites, nitrates, phosphate, and total phosphorus); presence of anionic surfactants, besides heavy metals, such as aluminium, cadmium, lead, copper, chromium, nickel, and zinc (Roveri et al., 2020a).

(ii) **Chapters 3 and 4** (microbiological variables): the diffuse load of these channels also indicated potential risks to public health, as they showed high concentrations of *Enterococci* and *E. coli* bacteria (namely in the channels of the Tombo, Enseada and Perequê) and human mastadenovirus - species C, D and F (inventoried only in the Enseada and Perequê). In the case of viruses, this was the first detection in urban drainage channels of the State of São Paulo coast (a region that has 16 municipalities and more than 600 drainage channels registered by Cetesb). As many of these channels have similar characteristics to the Enseada and the Perequê, special attention should be paid to the millions of beach users of the São Paulo coast (Roveri et al., 2020a; 2020b).

(iii) **Chapter 5** (hydrobiological variables): this diffuse load of Guarujá, with typical characteristics of domestic sewage, allowed the colonization of different taxons of the benthic macrofauna tolerant to organic pollution (for example, Oligochaeta, Gastropoda and, especially Insecta: Ceratopogonidae, Chironomidae and *Chironomus*) (Roveri et al., 2020c).

(iv) **Chapter 6** (PPCPs): regarding the pharmaceutical compounds (carbamazepine, caffeine, citalopram, acetaminophen, diclofenac, orphenadrine, atenolol, propranolol, enalapril, losartan and valsartan) and illicit drugs (cocaine and benzoilecgonine), this is the first report of the occurrence of these emerging pollutants in urban drainage channels that flow into South American bathing waters. It is also the first quantification of rosuvastatin, chloralidone, and clopidogrel in Latin American marine waters. The environmental risk assessment, at screening level, showed that caffeine, acetaminophen, diclofenac, valsartan and losartan, if not mitigated on their way to the South Atlantic Ocean, may cause moderate and high risks to aquatic organisms (e.g. algae, crustaceans and/or fish) (Roveri et al., 2020d).

(v) **Chapter 7** (toxicity): this complex mixture of the channels of Guarujá also indicated a significant acute and chronic toxicity for the microcrustaceans *Daphnia simillis* and *Ceriodaphnia dubia*, respectively [Roveri et al., Submitted (a)].

Regarding the poor conditions of the sanitation facilities, past studies had also indicated that the different characteristics regarding land use and occupation (Ferraz et al., 2012; Larrea-Murrel et al., 2013; Lamparelli et al., 2015) and the hydrological regime (rainy or dry season) (Wang et al., 2013; Xiang et al., 2017), are also factors that can interfere with the quality of the diffuse loads. This condition has also been corroborated in the present thesis, since physical-chemical disturbances and microbiological pollutants (e.g. *Enterococcus* and *E. coli* bacteria) (**Chapter 3**) (Roveri et al., 2020a), total density of benthic macrofauna taxons tolerant to organic pollution (**Chapter 5**) (Roveri et al., 2020c), maximum concentrations of PPCPs (**Chapter 6**) (Roveri et al., 2020d) and effluents with acute and chronic toxicity (**Chapter 7**) [Roveri et al., Submitted (a)], were observed, in neighborhoods that historically suffer from anthropic interference (Enseada and Perequê) (Roveri et al., 2020a; 2020c; 2020d). Unexpectedly, Tombo, a blue flag certification beach, also presented poor water and sediment quality, showing that in this neighborhood there is also clandestine disposal of domestic sewage in the drainage channels (Roveri et al., 2020a; 2020c; 2020d).

Table 10.1 presents a synthesis of the quality of the water and sediment of the four channels of Guarujá. Table 10.2, on the other hand, shows that during the rainy period (mainly in the Brazilian summer, between December and March), a worse water quality was observed in these four channels. Additionally, Table 10.3 presents a compilation of the different water quality indexes that were applied throughout this thesis, reinforcing the poor quality of these diffuse loads that flow through these channels to the beaches.

Table - 10.1: Synthesis of water and sediment quality results obtained in the urban drainage channels of Tombo, Enseada, Perequê and Iporanga beaches, Guarujá, Brazil. The table describes: names of the beaches; synthesis of the data obtained in Chapters 3 to 7 of the present thesis, besides the images of the drainage channels.

Beach	Synthesis of the results of water quality and sediment in the Guarujá channels	Photograph of sampled channels
Tombo	(i) Of the 168 physico-chemical and bacteriological analyzes carried out, only 43% complied with the current Brazilian legislation (**Chapter 3**) (Roveri et al., 2020a). (ii) Total density of 6 taxa of inventoried benthic macroinvertebrates (tolerant to organic pollution): 7,725 org./m^2 (**Chapter 5**) (Roveri et al., 2020c). (iii) Maximum concentrations of 11 PPCPs: 3,067 ng / L (**Chapter 6**) (Roveri et al., 2020d). (iv)Toxicity classification: acute: non-toxic; chronic: very toxic (**Chapter 7**) [Roveri et al., Submitted (a)].	
Enseada	(i) Of the 231 physical-chemical and bacteriological analyzes carried out, only 34% complied with the current Brazilian legislation (**Chapter 3**) (Roveri et al., 2020a). (ii) For mastadenoviruses - C, D and F, the occurrence varied from 73% (8/11), 36% (4/11) and 64% (7/11), respectively (**Chapter 4**) (Roveri et al., 2020b). (iii) Total density of 3 taxa of inventoried benthic macroinvertebrates (tolerant to organic pollution): 6,700 org./m^2 (**Chapter 5**) (Roveri et al., 2020c). (iv) Maximum concentrations of 16 PPCPs: 7,828 ng / L (**Chapter 6**) (Roveri et al., 2020d). (v)Classification of toxicity, acute: very toxic; chronic: very toxic (**Chapter 7**) [Roveri et al., Submitted (a)].	
Pererquê	(i)Of the 231 physico-chemical and bacteriological analyzes carried out, only 39% complied with current Brazilian legislation (**Chapter 3**) (Roveri et al., 2020a). (ii) For mastadenoviruses - C, D and F, the occurrence varied from 91% (10/11), 9% (1/11) and 73% (8/11), respectively (**Chapter 4)** (Roveri et al., 2020b). (iii) Total density of 6 taxa of inventoried benthic macroinvertebrates (tolerant to organic pollution): 12,925 org./m^2 (**Chapter 5**) (Roveri et al., 2020c). (iv) Maximum concentrations of 12 PPCPs: 5,179 ng / L (**Chapter 6**) (Roveri et al., 2020d). (v)Classification of toxicity, acute: very toxic; chronic: very toxic (**Chapter 7**) [Roveri et al., Submitted (a)].	
Iporanga	(i)Of the 231 physico-chemical and bacteriological analyzes carried out, more than 90% complied with the current Brazilian legislation (**Chapter 3**) (Roveri et al., 2020a). (ii)Total density of 2 taxa of inventoried benthic macroinvertebrates (tolerant to organic pollution): 825 org./m^2 (**Chapter 5**) (Roveri et al., 2020c). (iii)Maximum concentrations of 6 PPCPs: 58.6 ng / L (**Chapter 6**) (Roveri et al., 2020d). (iv)Classification of toxicity, acute: non-toxic; chronic: toxic (**Chapter 7**) [Roveri et al., Submitted (a)].	

Table - 10.2: Synthesis of water and sediment quality results obtained in the urban drainage channels of Tombo, Enseada, Perequê and Iporanga beaches, Guarujá, Brazil. The table describes the 14 variables analyzed (water or sediment), the data obtained in each of the four drainage channels (rainy or dry period) and the references (Chapters 3,5 or 7 of this thesis). Note: Tombo channel was not sampled during the winter (dry season), because its course was dry.

Variables		Unit	Tombo		Enseada		Perequê		Iporanga		Refererence
			Rainy	Dry	Rainy	Dry	Rainy	Dry	Rainy	Dry	
Water	Dissolved oxygen	mg/L	0.82	Dry	1.64	1.86	0.3	0.35	4.85	6.19	**(Chapter3)**
	Anionic surfactants	mg/L	1.26	Dry	1.22	0.91	1.32	1.14	0.19	0.04	**(Chapter3)**
	Ammonia	mg/L	2.05	Dry	2.58	2.19	2.64	2.35	0.013	0.008	**(Chapter3)**
	Phosphate	mg/L	1.11	Dry	1.02	1.05	1.12	1.02	0.053	0.002	**(Chapter3)**
	Escherichia Coli	CFU/mL	2,72E+06	Dry	5,40E+07	2,43E+07	2,43E+07	2,00E+07	199	92	**(Chapter3)**
	Enterococci	CFU/mL	2,27E+05	Dry	1,10E+07	2,35E+04	9,65E+06	5,76E+06	170	63	**(Chapter3)**
Sediment	Oligochaeta	org./m^2	950	25	175	0	250	25	75	0	**(Chapter 5)**
	Ceratopogonidae	org./m^2	0	0	0	0	11000	975	0	0	**(Chapter 5)**
	Chironomidae	org./m^2	6150	75	6500	225	825	0	750	25	**(Chapter 5)**
	Chironomus	org./m^2	25	0	0	0	0	0	0	0	**(Chapter 5)**
	Gastropoda	org./m^2	25	0	0	0	0	0	0	0	**(Chapter 5)**
	Organic matter	%	4.0	2.3	3.4	3.1	4.9	4.3	0.5	0.4	**(Chapter 5)**
Water	Acute toxicity	----	Non-toxic	Non-toxic	Very toxic	Toxic	Toxic	Non-toxic	Non-toxic	Non-toxic	**(Chapter 7)**
	Chronic toxicity	----	Toxic	Toxic	Very toxic	Toxic	Toxic	Toxic	Potentially toxic	Toxic	**(Chapter 7)**

Table - 10.3: Synthesis of the water quality and sediment indexes calculated in the urban drainage channels of Tombo, Enseada, Perequê and Iporanga beaches, Guarujá, Brazil. The table describes the indexes (calculated in water or sediment), the results obtained in each one of the four drainage channels, and also the references (Chapters 3 or 5 of this thesis).

Quality Indexes		Tombo	Enseada	Perequê	Iporanga	Reference
Water	Canadian Council of Ministers of theEnvironment Water Quality Index (CCMEWQI)	very poor	very poor	very poor	good	**(Chapter 3)**
	Trophic State Index (TSI)	eutrophic	eutrophic	eutrophic	oligotrophic	**(Chapter 3)**
	Toxic Contamination Index (TCI)	high aluminum contamination	high aluminum contamination	high aluminum contamination	average aluminum contamination	**(Chapter 3)**
Sediment	Shannon index (H')	highly polluted	highly polluted	highly polluted	highly polluted	**(Chapter 5)**
	Biological Monitoring Working Party index (BMWP')	highly polluted	highly polluted	highly polluted	highly polluted	**(Chapter 5)**
	Average Score per Taxon index (ASPT)	highly polluted	highly polluted	highly polluted	highly polluted	**(Chapter 5)**

10.1.2.1 WQI-PALAC – Water Quality Index for the Protection of Aquatic Life and Aquatic Communities

In addition to the indexes described in Table 10.3, and to integrate the different results obtained in **Chapters 3 and 7**, the WQI-PALAC index was calculatedin this section. The WQI-PALAC has the objective of evaluating water quality for the purpose of protecting fauna and flora. This index performs an integrated analysis of water quality, through a combination of different environmental variables (Zagatto et al., 1999). First the IMVPAL (Index of Minimum Variables for the Preservation of Aquatic Life) was calculated. In IMVPAL two groups of variables are considered: (i) essential variables (dissolved oxygen, pH and acute and chronic toxicity) and (ii) toxic variables (anionic surfactants and heavy metals: cadmium, lead, copper, chromium, nickel and zinc). The essential and toxic variables were obtained in **Chapters 3 and 7** [Roveri et al., 2020a; Roveri et al., Submitted (a)]. According to IMVPAL, three levels could be established for the water masses:

(i) Level A (weighting 1): waters with desirable characteristics to maintain the survival and reproduction of aquatic organisms. It meets the quality standards of the CONAMA Resolution nº 357/2005 (class 2) (Brazil, 2005).

(ii) Level B (weighting 2): waters with desirable characteristics for the survival of aquatic organisms, but reproduction may be affected in the long term.

(iii) Level C (weighting 3): waters with characteristics that may compromise the survival of aquatic organisms.

According to Zagatto et al. (1999), the limits of levels B and C are obtained from the French (Code Permanent: Environnement et Nuisances, 1986) and American (USEPA, 1991) legislations that establish maximum permissible limits of chemicals in water, with the purpose of avoiding acute and chronic toxicity effects to aquatic biota.

The IMVPAL was calculated using the following formula:

$$\mathrm{IMVPAL} = \mathrm{VE} \times \mathrm{ST}$$

where:

VE: Value of the highest weighting of the group of essential variables.
ST: Average value of the three highest weightings of the group of toxic variables.

After the calculation, the following classification is obtained: good = 1 (indicated with the green color); regular = 2 (indicated with the yellow color); bad = 3 and 4 (indicated with the orange color); terrible ≥ 6 (indicated with red color).

In a second step, the TSI (Trophic State Index) was calculated according to Lamparelli et al. (2004) (this index is already calculated in **Chapter 3** (Roveri et al., 2020a). After the calculation, the following classification is obtained: ultraoligotrophic = 0.5 (indicated with the blue color); oligotrophic = 1 (indicated with the green color); mesotrophic = 2 (indicated with the yellow color); eutrophic = 3 (indicated with the orange color); superutrophic = 4 (indicated with the red color); hyperutrophic = 5 (indicated with the purple color).

Finally, the WQI-PALAC (considering the crossing of the IMVPAL and TSI results) was calculated using the following formula:

$$\mathrm{WQI} - \mathrm{PALAC} = (\mathrm{IMVPAL} \text{ x } 1.2) + \mathrm{TSI}$$

After the calculation, the following final rating is obtained: optimal = WQI-PALAC ≤ 2.5 (indicated with the blue color); good = 2.6 ≤ WQI-PALAC ≤ 3.3 (indicated with the green color); regular = 3.4 ≤ WQI-PALAC ≤ 4.5 (indicated with the yellow color); bad = 4.6 ≤ WQI-PALAC ≤ 6.7 (indicated with the orange color) and bad = 6.8 ≤ WQI-PALAC (indicated with the red color).

Table 10.4 presents the results of the IMVPAL, the TSI and finally the WQI-PALAC calculated for the Guarujá channels, corroborating with the analyses of the other water quality indexes of the present thesis (Table 10.3), where Tombo, Enseada and Perequê channels presented the worst quality (water classified as bad) and the Iporanga, the best quality, being the water classified as great.

Table - 10.4: Results of WQI-PALAC (Water Quality Index for the Protection of Aquatic Life and Aquatic Communities) calculated for the urban drainage channels of Tombo, Enseada, Perequê and Iporanga beaches, Guarujá, Brazil. The table describes the groups of variables (essential or toxic), the levels and the respective ranges of variation and weighting of these variables, as well as the results of the weighting obtained in each of the four drainage channels. The last lines of the table present the final ratings of the IMVPAL (Index of Minimum Variables for the Preservation of Aquatic Life), the TSI (Trophic State Index) and the WQI-PALAC.

Groups	Variables	Levels	Variation range	Weighting	Tombo	Enseada	Perequê	Iporanga
Essential variables	DO	A	≥ 5.0	1	--	--	--	1
		B	3.0 to < 5.0	2	--	--	--	--
		C	< 3.0	3	3	3	3	--
	pH	A	6.0 to 9.0	1	1	1	1	1
		B	5.0 to < 6.0 and > 9.0 to 9.5	2	--	--	--	--
		C	< 5.0 and > 9.5	3	--	--	--	--
	Toxicity	A	Non-toxic	1	--	--	--	--
		B	Chronic effect	2	2	2	2	2
		C	Acute effect	3	--	3	3	
Toxicity variables	Cadmium	A	≤0.001	1	1	1	1	1
		B	> 0.001 to 0.005	2	--	--	--	--
		C	> 0.005	3	--	--	--	--
	Chrome	A	≤0.05	1	1	1	1	1
		B	> 0.05 to 1.0	2	--	--	--	--
		C	> 1.0	3	--	--	--	--
	Copper	A	≤0.009	1	--	1	1	1
		B	> 0.009 to 0.05	2	2	--	--	--
		C	> 0.05	3	--	--	--	--
	Lead	A	≤0.01	1	1	1	1	1
		B	> 0.01to 0.08	2	--	--	--	--
		C	> 0.08	3	--	--	--	--

	Nickel	A	≤0.025	1	1	1	1	1
		B	> 0.025 a 0.16	2	--	--	--	--
		C	> 0.16	3	--	--	--	--
	Zinc	A	≤0.18	1	1	1	1	1
		B	> 0.18 a 1.0	2	--	--	--	--
		C	> 1.0	3	--	--	--	--
	Surfactants	A	≤0.5	1	--	--	--	1
		B	> 0.5 a 1.0	2	--	--	--	
		C	> 1.0	3	3	3	3	
IMVPA					6 (terrible)	6 (terrible)	6 (terrible)	1 (good)
TSI					3(eutrophic)	3(eutrophic)	3(eutrophic)	1(oligotrophic)
WQI-PALAC					10 (terrible)	10 (terrible)	10 (terrible)	2 (great)

10.1.2.2 Set of environmental variables that have the greatest potential to cause acute and chronic toxicity

In this last chapter, we also sought to verify the set of environmental variables that had the greatest potential to cause the acute and chronic toxicity in the waters of the drainage channels of Guarujá. This evaluation was carried out by cross-checking physical and bacteriological variables [**Chapter 3** - available in the supplementary material from Roveri et al. (2020a)] and the results of acute and chronic toxicity [obtained in **Chapter 7:** Roveri et al., Submitted (a)]. The physicochemical and bacteriological variables (**Chapter 3** were collected and analyzed concomitantly with the **Chapter 7** toxicity samples (Tombo, Enseada, Perequê and Iporanga channels during February, March, May and July 2018). Of the 28 physicochemical and bacteriological variables present in this database, only 19 were selected (i.e., all the variables that had results above the limit of quantification: LOQ). Initially, the average and standard deviation of these 19 variables were calculated. After obtaining the average, the "Cohen D test" was calculated in order to verify the size of the effect of toxicity of each of these 19 variables. After the calculation, the following classification was obtained: Cohen d test < 0.50 = low effect (indicated with green coloration); 0.50 ≤ Cohen test d < 0.80 = medium effect (indicated with yellow coloration); Cohen test d > 0.80 = high effect (indicated with red coloration) (Cohen, 1988). Finally, for the variables that presented the highest effects (Cohen's d test > 0.80), the Standardized Ratio of Chance and its respective lower and upper limits were calculated, from a Logistic Regression (Agresti, 2002). From the Standardized Chance Ratio, it was possible to highlight the variables that present the greatest evidence of causing the acute (Table 10.5) and chronic (Table 10.6) toxicity in the Guarujá channels waters. All analyses were performed in the statistical software R (version 3.6.1) (R Core Team, 2017).

Table - 10.5: Results from the set of environmental variables that have the greatest potential to cause acute toxicity in the urban drainage channels of the beaches of Tombo, Enseada, Perequê and Iporanga, Guarujá, Brazil. The table presents: (i) the set of 19 environmental variables used for the calculations (N, mean and standard deviation), in two distinct conditions (without and with acute toxicity); (ii) result of Cohen's D Test; (iii) result of the Standardized Chance Ratio (and their respective lower and upper limits) calculated only for those variables that indicated a high toxicity effect (Cohen's d test > 0.80, flagged in red).

Variables	Unit	Without acute toxicity			With acute toxicity			Cohen's D test	Standardized Chance Ratio	Lower limit	Upper limit
		N	Average	Standard deviation	N	Average	Standard deviation				
Water temperature	ºC	11	24.10	2.36	4	24.08	2.41	0.01	-	-	-
Conductivity	µS/cm	11	379.36	203.75	4	580.50	293.95	0.85	2.61	0.65	10.54
Total Dissolved Solids	mg/L	11	196.55	75.96	4	249.25	113.02	0.61	-	-	-
DO	mg/L	11	2.5	2.40	4	0.86	0.55	0.75	-	-	-
pH	pH	11	6.72	0.19	4	6.73	0.32	0.06	-	-	-
Turbidity	NTU	11	38.22	37.09	4	57.83	37.03	0.53	-	-	-
Color	mg Pt/L	11	54.00	24.44	4	73.00	12.11	0.62	-	-	-
Sedimented solids	ml/L	5	0.10	-	3	0.10	-	-	-	-	-
BOD	mg/L	7	26.70	19.62	4	29.48	38.74	0.11	-	-	-
Ammonia	mg/L	11	1.36	1.27	4	2.38	0.24	0.87	4.21	0.44	40.14
Nitrite	mg/L	11	0.17	0.27	4	0.24	0.26	0.26	-	-	-
Nitrate	mg/L	11	1.8	0.62	4	1.40	0.54	0.30	-	-	-
Phosphate	mg/L	11	0.64	0.52	4	1.05	0.23	0.83	4.00	0.42	37.74
Surfactants	mg/L	11	0.76	0.56	4	1.34	0.28	1.03	10.35	0.34	320.03
Total Phosphorus	mg/L	11	1.45	1.10	4	2.50	0.47	0.98	9.63	0.28	334.80
Aluminum	mg/L	4	0.13	0.10	4	0.10	0.11	0.33	-	-	-
Total coliforms	CFU/mL	11	9,73E+07	1,28E+08	4	2,85E+08	1,77E+08	1.17	3.90	0.90	16.91
Escherichia Coli (*E.coli*)	CFU/mL	11	1,39E+07	2,17E+07	4	2,11E+07	1,26E+07	0.37	-	-	-
Enterococci	CFU/mL	11	3,81E+06	6,95E+06	4	4,27E+06	8,48E+06	0.07	-	-	-

Table - 10.6: Results from the set of environmental variables that have the greatest potential to cause chronic toxicity in the urban drainage channels of the beaches of Tombo, Enseada, Perequê and Iporanga, Guarujá, Brazil. The table presents: (i) the set of 19 environmental variables used for the calculations (N, mean and standard deviation), in two distinct conditions (without and with chronic toxicity); (ii) Cohen D test result; (iii) Standardized odds ratio result (and their respective lower and upper limits) calculated only for those variables that indicated high toxicity effect (Cohen d test > 0.80, indicated in red).

Variables	Unit	Without acute toxicity			With acute toxicity			Cohen's D test	Standardized Chance Ratio	Lower limit	Upper limit
		N	Average	Standard deviation	N	Average	Standard deviation				
Water temperature	ºC	3	22.81	1.83	12	24.41	2.34	0.70	-	-	-
Conductivity	µS/cm	3	238.33	83.56	12	481.67	240.64	1.02	6.27	0.57	68.54
Total Dissolved Solids	mg/L	3	124.33	27.06	12	232.17	82.46	1.25	16.27	0.51	519.,92
DO	mg/L	3	4.01	2.5	12	1.57	1.90	1.12	0.32	0.08	1.28
pH	pH	3	6.76	0.14	12	6.71	0.24	0.22	-	-	-
Turbidity	NTU	3	9.43	4.87	12	51.95	36.45	0.45	-	-	-
Color	mg Pt/L	3	37.00	15.62	12	64.58	21.66	0.69	-	-	-
Sedimented solids	ml/L	1	0.10	-	7	0.10	-	-	-	-	-
BOD	mg/L	1	7.50	-	10	29.73	26.63	0.55	-	-	-
Ammonia	mg/L	3	0.74	1.27	12	1.85	1.09	0.95	2.76	0.67	11.4
Nitrite	mg/L	3	0.30	0.27	12	0.18	0.27	0.22	-	-	-
Nitrate	mg/L	3	1.57	0.42	12	1.52	0.63	0.08	-	-	-
Phosphate	mg/L	3	0.26	0.38	12	0.87	0.45	1.24	4.04	0.81	20.04
Surfactants	mg/L	3	0.44	0.50	12	1.04	0.52	1.07	3.21	0.73	14.05
Total Phosphorus	mg/L	3	0.74	0.93	12	1.98	0.98	1.16	3.56	0.79	16.07
Aluminum	mg/L	2	0.03	0.04	6	0.14	0.10	0.40	-	-	-
Total coliforms	CFU/mL	3	7,69E+07	1,33E+08	12	1,65E+08	1,67E+08	0.55		-	-
Escherichia Coli (*E.coli*)	CFU/mL	3	9,67E+06	1,67E+07	12	1,73E+07	2,06E+07	0.39	-	-	-
Enterococci	CFU/mL	3	6,05E+03	1,04E+04	12	4,92E+06	7,64E+06	0.70	-	-	-

After the application of Cohen's D test followed by the Standardized Ratio of Chance, it was possible to identify the most prevalent families of pollutants responsible for the acute and chronic toxicity of the urban runoff of Guarujá. Thus, these pollutants deserve attention, as they can have a great deleterious impact on the aquatic biota. For example, (as described in **Chapter 3**) in the case of nutrients (ammonia, phosphate and total phosphorus), their bioavailability can cause the proliferation of toxic cyanobacteria and hypoxia in urban water bodies, with direct impacts on the structure of aquatic communities (Lusk and Toor, 2016; Yang and Toor, 2017). The presence of surfactants also warns about the potential ecological risks, because studies have already demonstrated their toxicity on crustaceans and fish (Ole Kusk and Petersen, 1999; Renzi et al., 2012). The quality of urban surface waters, which often receive diffuse pollution, is also of concern due to the introduction of allochthonous pathogenic microorganisms (e.g. total coliforms) (Lamparelli et al., 2015). In addition, the indication of the most prevalent pollutants could contribute to a better targeting of future studies in urban drainage channels along of São Paulo coastal zone, because there will be a better selection of representative environmental variables to monitor these waters, i.e., a smaller number of variables and therefore lower costs (ideal condition for developing countries such as Brazil).

10.1.3 Marine discharge from domestic sewers

Worldwide, several studies have proven the impacts of conventional and emerging pollutants on the marine environment discharged untreated by obsolete WWTPs. (De La Ossa Carretero et al., 2012; Moreno-González et al., 2015; Alygizakis et al., 2016). Regarding conventional pollutants there are reports, for example, in Castellon, Spain (De La Ossa Carretero et al., 2012), Mazatlan Bay, México (Soto-Jiménez et al., 2011) and Darwin Harbour, Australia (Padovan et al., 2012). As for PPCPs, studies were conducted in San Francisco Bay, USA (Klosterhaus et al., 2013), Laguna del Mar Menor, Spain (Moreno-González et al., 2015) and the Saronikos Gulf, Greece (Alygizakis et al., 2016). In Guarujá, the analyses carried out at the mouth of the submarine outfall (**Chapters 8 and 9**) presented the following results:

(i) **Chapter 8** (conventional pollutants - water column and sediment). Although the submarine outfall of Guarujá was installed in 1998 and the surroundings of this system are monitored since 2010 by Cetesb (Baptistelli and Marcellino 2016; Cetesb, 2018b), for the first time, the presence of surfactants, *Salmonella* bacteria, protozoa (*Cryptosporidium ssp* and *Giardia ssp*) and human mastadenovirus (C and F species) were studied in the water column. In addition, and also for the first time the mutagenic potential was verified in this mixing zone, and no risk was observed (Ames Test: Mutagenic Index < 2) [Roveri et al., Submitted (b)]. In the water column, of the 33 physicochemical and bacteriological analyses performed, 9 presented concentrations above the permissible limits by Conama legislation nº 357/2005 (saline waters - class 1): oil and grease, dissolved oxygen, ammonia, surfactants, aluminium, lead, copper, nickel and *Enterococcus*, therefore, signalized attention due to potential ecological risks. [Roveri et al., Submitted (b)]. However, CCMEWQI index showed that the waters of the Guarujá mixing zone presented a regular classification, which corroborates with Cetesb's monitoring (2013, 2016 and 2018) the CWQI index of Cetesb was also regular. In 2012, 2014, 2015 and 2017, a significant improvement was observed, and Cetesb's CWQI obtained an excellent rating [Cetesb, 2018b; Roveri et al., Submitted (b)]. The sediments in the surroundings of the submarine outfall, also indicated lightly polluted environments. The indicators were the low concentrations of the metals, cadmium, lead, copper, chromium, nickel, and zinc, which were below the Threshold Effect Level and also showed a Geoaccumulation Index < 0 (uncontaminated sediment). In addition, the 25 benthic taxons inventoried for the first time in the surroundings of this submarine outfall, showed good representation of amphipods and polychaetes, indicating a H' between 2.5 and 3.5 (moderately clean environment) (Dauvin and Ruellet, 2007; Zettler et al., 2007; Al-Farraj et al., 2012). Another indicator of good quality was the absence of acute toxicity for the *Kalliapseudes schubartii* test organism (EC50: 96h) corroborating, once again, with Cetesb's data, since the results of its monitoring (carried out between 2015 and 2018), also indicated the absence of acute toxicity of sediments [Cetesb, 2018b; Roveri et al., Submitted (b)]

(ii) **Chapter 9** (emerging pollutants - water column). Regarding PPCPs, for the first time 23 compounds were researched in the Guarujá mixture zone, and 10 were detected (at least once): carbamazepine, caffeine, diclofenac, valsartan, benzoilecgonine, cocaine, orphenadrine, atenolol, acetaminophen and losartan.

Regarding orphenadrin, this was the first account of the presence of this compound near a submarine outfall in Latin America. Of these 10 compounds detected, only 3 indicated ecological risk (low to moderate) for the three evaluated trophic levels (algae, crustaceans and/or fish): caffeine (which presented concentrations above the safety limits for surface waters: 10 ng/L), diclofenac and acetaminophen (Roveri et al., 2020e).

In a complementary way, the statistical tests carried out in **Chapter 8** were performed again in this subsection. However, the data of the PPCPs obtained in **Chapter 9** were added [Roveri et al., 2020e; Roveri et al., Submitted (b)]. The Mann-Whitney U-test was used to compare the physical-chemical, microbiological and pharmaceutical compounds variables, analyzed in the water column, taking into account the tourist seasons (high and low) and water depth (surface and bottom). The correlation between (high season x low season) and (surface x bottom) was evaluated using the Spearman's correlation coefficient (Hollander and Wolfe, 2013). A total of 22 variables were selected. Only the physical-chemical and microbiological variables that were quantified in all campaigns and points were selected. As for pharmaceutical compounds: carbamazepine, acetaminophen, orphenadrine, atenolol and losartan, they were not selected for the correlation, due to the large number of results below the limit of detection (<LOD, over 25%). Perceptual maps were developed via PCA (Principal Component Analysis) in order to visualize the correlations between the variables (Figure 10.1) (Mingoti, 2005). All the analyses were performed using the statistical software R (version 3.6.1) (R Core Team, 2017).

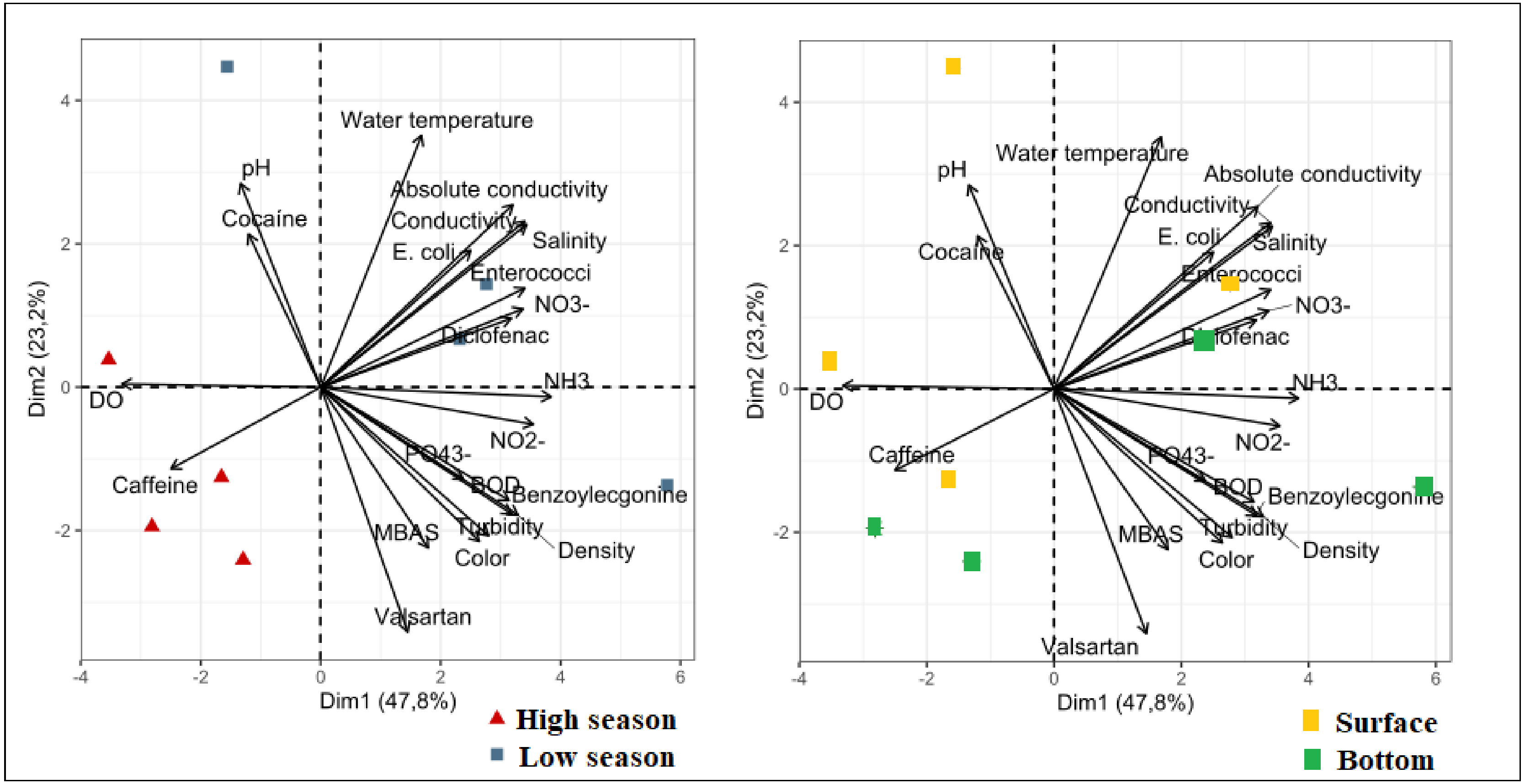


Figure – 10.1: Perceptual map via PCA (Principal Component Analysis), applied to physical, chemical, microbiological and pharmaceutical compound variables of samples collected in the mixing zone of the submarine outfall in Guarujá, São Paulo, Brazil. Two distinct scenarios were considered: (i) PCA of comparison between the averages of 22 environmental variables (16 physicochemical and microbiological variables, 1 of air and another 5 of pharmaceutical compounds), to verify in which period: high season (identification: red triangle) or low season (identification: blue square) there were worse environmental conditions; (ii) PCA of comparison between the averages of the same 22 environmental variables, to check at what depth: surface (identification: yellow square) or background (identification: green square) there were worse environmental conditions.

Figure 10.1 shows the PCA plots considering the physicochemical and microbiological variables that were quantified in all campaigns and collection points (water and air temperature, density, salinity, Ec, absolute conductivity, DO, turbidity, pH, color, BOD, NH_3, NO_2- , NO_3-, PO_4^{3}-, MBAS, *E.coli* and *Enterococci*) and, in the case of pharmaceutical compounds, those that presented 25% of the results above detection limits (caffeine, cocaine, benzoilecgonine, diclofenac and valartan), between seasons (high and low) (Figure 10.1a) and also between the two depths (surface and bottom) (Figure 10.1b). As already observed in **Chapter 8**, this set of variables was able to discriminate very well between the two seasons and, with the exception of DO (and now also caffeine), all the other variables presented the highest concentrations during the low season. However, the set of physicochemical and microbiological variables (as also observed in **Chapter 8**), together with the set of five pharmaceutical compounds **(Chapter 9**), were not able to discriminate between depths (surface or bottom). It is important to mention that the present study covered a relatively short period of time window because the main focus was a preliminary screening on variables that had not yet been study in Guarujá (e.g., surfactants and PPCPs). In addition, several factors may influence the occurrence and spatial distribution of these compounds in the marine environment, such as rainfall regime, oceanographic conditions, and the complex hydrodynamics of the marine environment in coastal areas (Vidal-Dorsch et al., 2012; Arpin-Pont et al., 2014). It was not possible to test these hypotheses with the data presented here. Therefore, the monitoring should continue in order to highlight, in a larger scale of time and space, the possible effects of the discharge of sewage from Guarujá in the marine coastal environments.

10.1.4 Recommendations for the improvement of the social and environmental quality of Guarujá

With the evidence of the environmental impacts detected along the urban drainage channels and the disposal of the submarine outfall of Guarujá, it became relevant to mention possible recommendations for social and environmental management actions plans for the region. The BPWM (Best Practices in Water Management) programs have been adopted in recent years by several countries. For example, in the United States (one of the developers of this program) the Environmental Agency has adopted two programs: BMP (Best Management Practices) and LID (Low Impact Development) (USEPA, 2004;

NCDENR, 2007; UDFCD, 2007). In Canada, the Environmental Agency has adopted the GVS&DD program (Greater Vancouver Sewerage and Drainage District) (GVS&DD, 1999). In New Zealand, the Environmental Water Research Foundation uses the program OSM (On-site Stormwater Management) (NZWERF, 2004). In Brazil, the most recent and innovative actions come from the program entitled "Optimal measures for the management of diffuse loads", developed by SMDU (Municipal Secretary of Urban Development of São Paulo), in partnership with DAEE (Department of Water and Electricity of the State of São Paulo) and POLI (Polytechnic School of the University of São Paulo) (SMDU, 2012a; 2012b). All these programs are based on holistic practices and therefore adopt "non-structural" and "structural" management measures (NOVOTNY, 2003; USEPA, 2004; SMDU, 2012a; 2012b). The "non-structural" measures are related to community education programs; recycling actions; maintenance practices; control of pollutants at source; planning of land use and occupation; control the waste disposal; urban cleaning; maintenance of public roads and drainage systems; control of irregular sewage connections, among other practices. The “structural" measures are related to engineering works and other physical interventions related to the control of water flow in urban channels (e.g. adoption of porous paving; installation of infiltration wells; installation of infiltration and/or retention basins, etc.) (NOVOTNY, 2003; USEPA, 2004; SMDU, 2012a; 2012b). The following are some recommendations based on the BMP, LID, GVS&DD and OSM programs and supported by the current Brazilian legislation, which can subsidize action planning for the Guarujá Waterfront Project.

(i) Environmental education programs: it is recommended that the City Hall of Guarujá, through the Secretariats of Environment and Public Health, promotes the environmental information programs oriented to the community, discussing the problems related to the environmental and public health due to improper disposal of USW (urban solid waste) in the environment. During the eleven months of sample collection work, inadequate USW disposal was constantly observed in the streets, drainage channels and beaches, which can be transported to the sea (especially during the rainy season). As provided in the Complementary Law 44/1998 - articles: 62 to 66; 67 to 72 and 95 to 99 (Code of Guarujá Positions), the irregular disposal of USW on beaches and other public roads of the municipality is prohibited (Guarujá, 1998). In addition, it is important to stress that the irregular disposal of waste on beaches and public roads is considered an environmental crime, according to Federal Law No. 9,605/1998 - Article 54 (Brazil, 1998). In addition, a program of awareness, in loco, targeted to the beach users (mainly during the summer tourist season) should be pondered, because during the fieldwork it was possible to observe that people constantly come into contact with the waters of these channels, demonstrating total ignorance about the health risks due to this behavior (Figure 10.2).

Figure - 10.2: Irregular garbage disposal at Enseada beach, Guarujá, Brazil. In the foreground of the image, child lying along the urban runoff of that beach.
Source: Pulsar imagens (2016).

(ii) Awareness program on the collection of drug residues: in Brazil, the National Policy for Solid Waste (Federal Law No. 12,305/2010) requires the correct disposal of expired drugs (Brazil, 2010). For this, Anvisa (Health Surveillance Agency) through the ABNT NBR standard 16457:2016, established a reverse logistics program, where pharmacies and drugstores throughout the country became accredited collection points (ABNT, 2016). These establishments receive the expired and unused drugs to forward them to their final destination, thus minimizing the risks of environmental contamination. This way, the city hall of Guarujá needs to carry out a program of awareness of the residents, so that they can carry out the reverse logistics of medicines and avoid the inappropriate disposal of these residues in the environment.

(iii) New studies about PPCPs and illicit drugs in urban drainage channels on the São Paulo coastal zone are needed: chapter 3 of this thesis presented a preliminary assessment (in January 2018) of the occurrence of 16 PPCPs in the urban drainage channels of Guarujá, indicating that these pollutants were ubiquitous in the study area. This work was the first report about the occurrence of these compounds in urban drainage channels that flow to the bathing waters in South America, demonstrating that studies in this environment matrix are still limited (Roveri et al., 2020d). Therefore, to improve the knowledge about the risks of these PPCPs in Metropolitan Region of Baixada Santista, new studies are urgent. In this context, recently (in October 2020), our group evaluated the occurrence and ecological risk of 21 PPCPs in the urban drainage channels of Santos, São Paulo (neighbouring city of Guarujá) reinforcing the need of better PPCPs management practices in Metropolitan Region of Baixada Santista. This article can be read in the "Appendix" to this thesis [Roveri et al., Submitted (c)].

(iv) Correction of the sanitation infrastucures: inspection and control of commercial and residential buildings in the municipality, mainly at Tombo Beach, requiring the connection of these real estate to the existing municipal sewage collection system, as provided for in Supplementary Law No. 44/1998 - Articles: 62 to 66 and 67 to 72 (Guarujá, 1998). On the beaches of Enseada and Perequê, it is necessary to carry out the land regularization of the irregular occupations on the hills of Enseada and on the edge of Perequê, since according to article 38 of Federal Law No. 9,605/1998, it is prohibited to degrade areas of permanent preservation. The land regularization of these areas (a partnership between the Federal, State and Municipal governments) is provided for in Article 14 of Federal Law No. 13.465/2017 (Brazil, 2017). Without this regularization, the State Sanitation Agency is prevented, due to legal issues, from installing the sewage collection system in these neighborhoods.

(v) Public cleaning: the city hall needs to intensify the cleaning of public roads in order to reduce the carrying of USW into the channels (especially during the period of greater rainfall in the region). In addition, a program of cleaning and silting of the channels needs to be intensified, as provided in Complementary Law 44/1998 - articles: 3 to 7 and 73 to 89 (Guarujá, 1998) [Figures 10.3(a) and (b)].

Figure - 10.3: Services of silting (a) and cleaning (b) performed by the city hall of Guarujá in the urban drainage channels of Enseada, Guarujá, Brazil.

Source: Personal Archive.

(vi) Installation of floodgate system: similar to what occurs in the urban drainage channels of the city of Santos, São Paulo it is essential that the Guarujá government install a floodgate system in the city channels [Figures 10.4 (a) and (b)]. In this way, this physical barrier will prevent the diffuse load of the channels from coming into continuous contact with the sea water, thus reducing the flow of pollutants to the beaches of the municipality. For more details on the floodgate system of Santos, see the "Appendix" of this thesis.

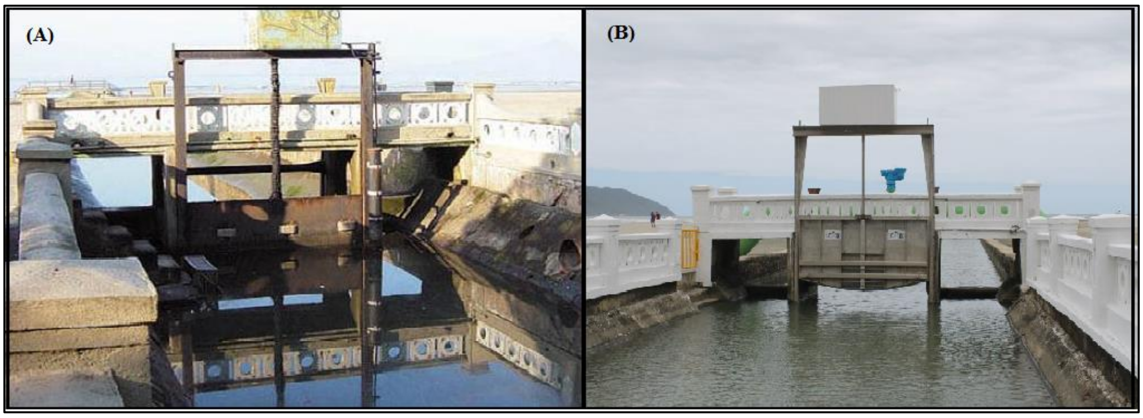


Figure - 10.4: Floodgate system installed in the urban drainage channels of Santos, Brazil. The Figure (a) presents the closed floodgates, preventing urban drainage from coming into contact with sea water. In Figure (b), the locks are opened by the city hall when there is rain and, this way, floods in the city are avoided.

Source: Personal Archive.

(vii) Installation of an oceanic interceptor upstream of the floodgates: in addition to the installation of the floodgate system, it is recommended that the Guarujá government install an oceanic interceptor (also similar to the one existing in Santos) [Figures 10.5(a) e (b)] that interconnects the waters of the channels to the SPS of Vila Zilda. In this way, the channel waters will be conducted to the pre-conditioning station, together with the city's sewers and, soon after, this complex mixture will be launched into the sea, via submarine outfall. With the removal of this diffuse load from the beach area, there will certainly be an improvement in bathing waters with direct effects on public health, tourism and the economy of the city. For more details on the Santos oceanic interceptor, see the "Appendix" of this thesis.

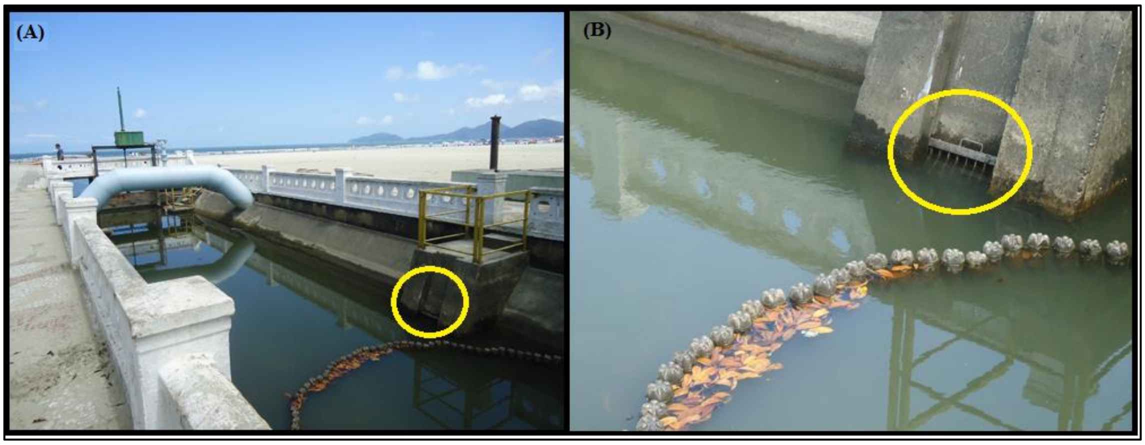


Figure - 10.5: Oceanic interceptor, installed upstream of the floodgate and signaled in yellow, conducts the waters from urban drainage (by gravity) to the SPS of the city of Santos, avoiding the contribution of this diffuse cargo directly to the beaches of the municipality.

Source: Personal Archive.

(viii) Installation of containment barriers: to reduce the risks of accidental spillage of chemical and/or oily substances generated along the cities, and which generally flow through drainage channels, it is necessary to install containment barriers, upstream of the oceanic interceptor, avoiding that these pollutants are discharged to the sea [Figures 10.6(a) e (b)]. It is important to emphasize that the disposal of oily substances is considered an environmental crime according to the Federal Laws No. 9605/1998 – (Article 54) and No. 9966/2000 – (Articles 15 to 21) (Brazil, 1998; 2000).

Figure - 10.6: Content barrier system (configured in "U" format), used to retain solid waste and other chemical and/or oily pollutants that are conducted into the urban drainage channels in Santos, Brazil.

Source: Personal Archive.

(ix) Improvements in the treatment and monitoring of the Guarujá sewage: the quality of the coastal waters and, especially, of the beaches, is greatly influenced by the conditions of basic sanitation. The greater the coverage of the collection network and the better the level of sewage treatment, the lower are the chances that the contribution of pollutants will occur in these water resources, which contributes to the maintenance of good ecological and sanitary conditions of the waters (De Souza Abessa et al., 2012; Baptistelli e Marcellino 2016). Although the present study indicates punctual and concentrated deleterious effects around the launching of sewers, the dispersion of these pollutants can make the impacts of the submarine outfall of Guarujá occur in a larger area. Thus, although the SPS plus submarine outfall operation present benefits for the quality of Guarujá's beaches (because it keeps the sewage away from the recreation area), **Chapters 8 and 9** presented countless indications that the disposal of pre-conditioned sewage in the marine environment does not represent the best solution for environmental sanitation for this coastal region. In this sense, it is possible to enumerate that: (i) due to the presence of several pollutants above the permissible limits by the Brazilian legislation; (ii) due to the presence of pollutants that are not removed by the preliminary treatment of Guarujá (example: oils, greases, surfactants, nutrients, heavy metals and pharmaceutical compounds); and (iii) due to the capacity of these pollutants to cause eutrophication and to be toxic, bioaccumulative and persistent, it is recommended the modernization of SPS of Guarujá. For this, the revision of Conama Resolution no. 430/2011 (conditions and standards for discharge of effluents in Brazil) becomes fundamental, so that more sustainable operational criteria for the SPS of Guarujá can be adopted, such as the installation of a system with secondary and/or tertiary level of treatment (Brazil, 2011; De Souza Abessa et al., 2012). Moreover, due to the scarcity of studies along the Guarujá submarine outfall, it is urgent that new studies of water and sediment monitoring be conducted. For this, besides new studies in the vicinity of the mixing zone, an extended monitoring is recommended to control the dispersion of these pollutants along the coast of Guarujá, aiming at the analysis of the cumulative and/or synergistic effects of the pollutants, so that the maintenance of the quality of human health and the protection of ecological processes are ensured.

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APPENDIX:

Urban drainage channel, Santos city.

Occurrence and ecological risk assessment of pharmaceuticals and cocaine in the urban drainage channels of Santos beaches (São Paulo, Brazil): a neglected, but sensitive issue

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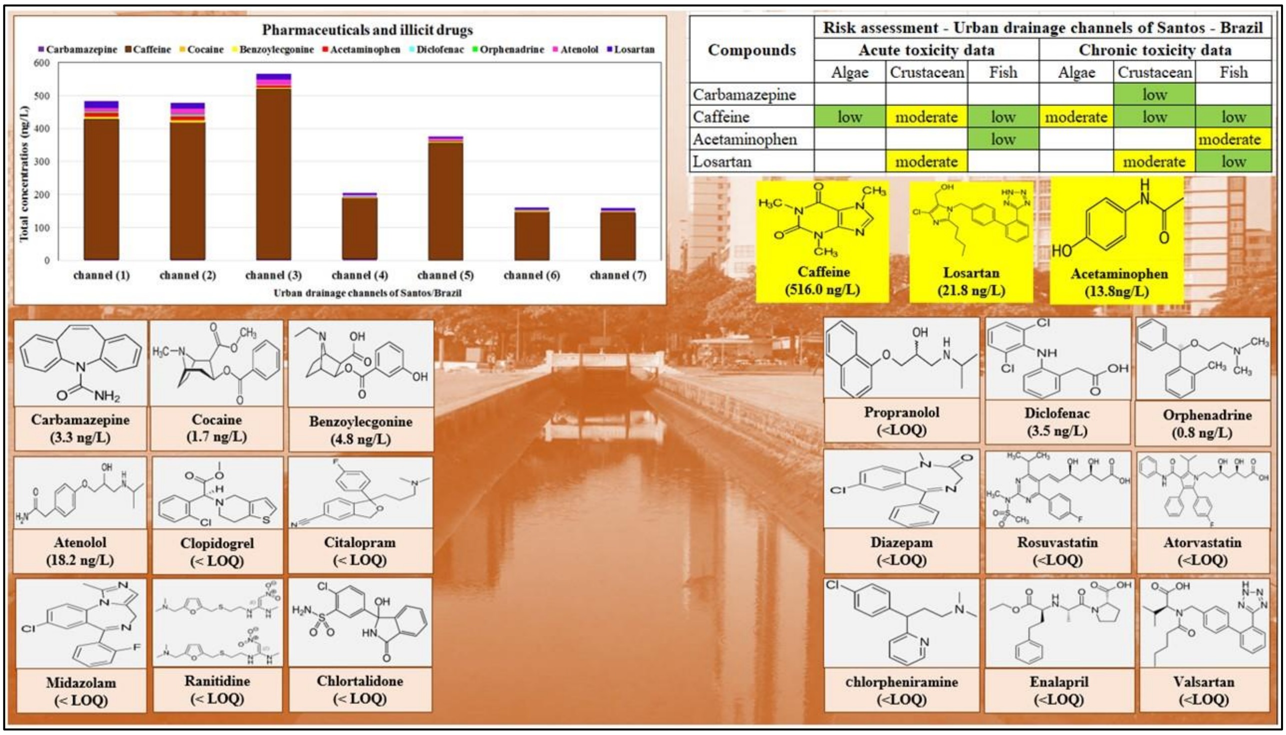
Under review: Environmental Science and Pollution Research (2021).

Graphical Abstract



Compounds	Risk assessment - Urban drainage channels of Santos - Brazil					
	Acute toxicity data			Chronic toxicity data		
	Algae	Crustacean	Fish	Algae	Crustacean	Fish
Carbamazepine					low	
Caffeine	low	moderate	low	moderate	low	low
Acetaminophen			low			moderate
Losartan		moderate			moderate	low

Highlights

First detection of 21 PPCPs, including illicit drugs, in the urban drainage channels of Santos city, São Paulo, Brazil.

First report on the occurrence of midazolam, ranitidine and chlorpheniramine in surface waters in Latin America.

The stimulants (caffeine, cocaine and benzoylecgonine) were the dominant drugs in all urban drainage channels.

Caffeine was the most critical drug in terms of overall contribution and environmental risk.

Caffeine, acetaminophen and losartan in the urban surface runoff indicated moderate ecological risk for the aquatic biota.

Abstract

In some Brazilian coastal cities, it is common to observe 'black tongues' in beaches, i.e. a mixture of urban runoff and untreated domestic sewage containing pollutants of emerging concern (e.g., PPCPs: pharmaceutical and personal care products) flowing into the South Atlantic Ocean. Such diffuse loads of pollutants might expose non-target aquatic organisms to harmful compounds. In this work, the occurrence and preliminary ecological risk of 27 PPCPs of various therapeutic classes (including cocaine and its primary metabolite, benzoylecgonine) were investigated, for the first time, in seven urban drainage channels whose diffuse loads flow permanently to the beaches of Santos Bay, São Paulo, Brazil. Of these, 21 compounds were detected using chromatography coupled with tandem mass spectrometry (LC-MS/MS), and nine of them were consistently quantified in all urban channels of Santos, suggesting that those pollutants are ubiquitous in this region: caffeine (143.4–516.0 ng/L), losartan (4.2–21.8 ng/L), atenolol (1.1–18.2 ng/L), acetaminophen (1.5–13.8 ng/L), benzoylecgonine (1.0–4.8 ng/L), carbamazepine (1.1–4.0 ng/L), diclofenac (1.9–3.5 ng/L), cocaine (0.5–1.7 ng/L) and orphenadrine (0.1–0.8 ng/L). Moreover, twelve were found to be below the limit of quantification (<LOQ): citalopram, propranolol, diazepam, rosuvastatin, atorvastatin, midazolam, ranitidine, chlortalidone, clopidogrel, chlorpheniramine, enalapril and valsartan. This work seems to be the first report on the occurrence of midazolam, ranitidine and chlorpheniramine in surface waters in Latin America and, therefore, these compounds should be considered environmental warning signs. A preliminary ecological risk assessment revealed that caffeine, acetaminophen and losartan presented a moderate risk, and carbamazepine a low risk to sensitive aquatic organisms at maximum measured concentrations. This study provides valuable information to reinforce the importance of a continuous monitoring of the diffuse loads (containing PPCPs and illicit drugs) flowing to the coastal zones, namely in developing countries.

Keywords: Subtropical ecosystem; Non-point source pollution; Ocean discharge; Pharmaceuticals; Illicit drugs; Risk assessment; Pollution effects.

11.1 Introduction

The occurrence of emerging pollutants, such as pharmaceutical and personal care products (PPCPs), in the aquatic compartment, and their potential detrimental effects on these ecosystems, have raised concerns in the coastal zones worldwide (Dafouz et al., 2018; Desbiolles et al., 2018; Ojemaye and Petrik, 2018). The high urban pressure in the coastline and associated anthropogenic activities, leaded to the unregulated disposal of domestic sewage and urban surface runoff in different marine environmental matrices (e.g., water, sediment and/or biota) (Cortez et al., 2018; Dos Santos et al., 2018; Blackburn et al., 2019). PPCPs are being continuously reported in several coastal zones worldwide, although they are not routinely monitored and not legally regulated (Beretta et al., 2014; Pereira et al., 2016; Quadra et al., 2017). However, PPCPs (e.g., antiepileptics, stimulants, analgesics/anti-inflammatories and antihypertensive drugs) and illicit drugs (e.g., cocaine) are ubiquitous, potentially hazardous and environmentally pseudo-persistent in marine areas (Wang et al., 2019; Fontes et al., 2020; Valdez-Carrillo et al., 2020). Although most of these compounds are often detected at low concentrations (generally at the ng/L or µg/L levels), they are still of major concern because they may exert deleterious effects on aquatic non-target organisms, such as algae, crustacean, molluscs and fishes (Cortez et al., 2018; Capaldo et al., 2019; Chen et al., 2019). Some of the reported harmful effects of low concentrations of PPCPs and illicit drugs includes mortality, immobilization, growth and reproduction inhibition, endocrine disruption, genotoxicity and carcinogenicity (Godoy and Kummrow, 2017; Radwan et al., 2020; Valdez-Carrillo et al., 2020).

At present, most studies concerning the occurrence and risk assessment of PPCPs in coastal zones took place on Europe (Wilkinson et al., 2017; Mijangos et al., 2018; Maasz et al., 2019), North America (Klosterhaus et al., 2013; Lara-Martín et al., 2014; Anumol et al., 2015) and Asia (Bayen et al., 2016; Li et al., 2016; Hossain et al., 2018). This kind of information regarding the coastal zones of the Latin America is however scarce (Starling et al., 2018; Griffero et al., 2019; Peña-Guzmán et al., 2019). Although Brazil is the ninth largest producer of pharmaceuticals in the world and approximately 50 million people live in coastal municipalities (along 8,500 km of coastline) (Quadra et al., 2017; Ibge 2019; Roveri et al., 2020b), studies reporting the detection of PPCPs in its coastline are relatively new and still limited (Beretta et al., 2014; Pereira et al., 2016; Cortez et al., 2018; Dos Santos et al., 2018; Pusceddu et al., 2018; Fontes et al., 2019; Fontes et al., 2020; Roveri et al., 2020a and 2020b).

Most of the studies conducted in coastal zones across the world, showed that the discharge of wastewater treatment plants (WWTPs) is one of the major entry sources of PPCPs and illicit drugs into the marine environment (Desbiolles et al., 2018; Ojemaye and Petrik, 2018; Fontes et al., 2020). However, the works regarding the detection of PPCPs and illicit drugs in urban drainage channels in coastal zones across the world (e.g. Brazil) are almost inexistent (Roveri et al., 2020a and 2020b). This is the case of Santos, one of the main coastal cities of São Paulo, Brazil, homeland for 430,000 people, which suffers long ago from environmental deterioration due to several anthropogenic stressors (e.g., strong urbanisation, port activities and seasonal tourism) (Moreira et al., 2017; SMA/CPLA, 2018; Ibge, 2019). Historically, the channels of Santos are responsible for carrying the diffuse load of the city (popularly known as ‘black tongues’: mixture of urban runoff and untreated domestic sewage) (Rocha et al., 2011) onto the tourist beaches (Ambrozevicius and Abessa, 2008; Ferraz et al., 2012; Lamparelli et al., 2015). Some studies conducted in the channels of Santos have already identified urban runoff as a potential threat to public and environmental health, as it is responsible for introducing chemical and biological pollutants directly onto the beaches, which are areas of intense human recreation (Ferraz et al., 2012; Lamparelli et al., 2015; Gandra et al., 2020).

Recently, the occurrence of a vast mixture of 16 PPCPs and illicit drugs in urban drainage channels of Guarujá, a neighbouring city of Santos, showed that five of these compounds, namely caffeine, acetaminophen, diclofenac, losartan and valsartan, presented moderate to severe risks to the aquatic biota (Roveri et al., 2020b). This emphasises the need to screen and identify the presence of PPCs in the urban drainage channels of Santos (a city with substantial urban development) and to determine the potential associated ecological risks.

In this context, the purpose of this study was to assess, for the first time, the occurrence and the ecological risk potential of 27 PPCPs of various therapeutic classes (including cocaine and its primary metabolite, benzoylecgonine) along the beaches of Santos, which daily receive diffuse loads from nearby neighbourhoods through seven urban drainage channels. The gathered information is crucial to monitor and to manage the diffuse loads that flow to this important Brazilian recreational area.

11.2Material and methods

11.2.1 Study site description and sample collection

This study took place in Santos city (23°57′52″S 46°20′0″W), located within the metropolitan area of Baixada Santista, São Paulo, Brazil. Santos has a tropical climate, characterized by a mean annual precipitation and temperature of 3000 mm and 22°C, respectively. Two main annual seasons are observed in the region: a rainy (November to March) and a dry (April to October) season (SMA/CPLEA, 2016; SMA/SPLA, 2018). Santos has an area of 280.6 km^2 of which 39.8 km^2 are already completely urbanised (island area). Santos has a permanent population of approximately 430,000 inhabitants (Ibge, 2019), which practically doubles during the summer, between December and February (Cetesb, 2019a; 2019b). It is estimated that about 99.3% of the population lives in the island area (SMA/CPLEA, 2016; SMA/SPLA, 2018; Ibge, 2019). The remaining 240.8 km^2 are located on the mainland, of which about 150 km^2 comprises environmental preservation areas, which therefore remain uninhabited (SMA/CPLEA, 2016; SMA/SPLA, 2018; Ibge, 2019). Santos city holds the largest marine port in Latin America, but the commerce, fishing and tourism occurring along the sandy beaches are also economically important (SMA/CPLEA, 2016; SMA/SPLA, 2018). These beaches include seven urban drainage channels across the city edge, which are the natural boundaries of different neighbourhoods (José Menino; Gonzaga; Boqueirão; Embaré; Aparecida and Ponta da Praia) (SMA/CPLEA, 2016; SMA/SPLA, 2018).

The field study was conducted along these urban drainage channels which are part of the city's basic sanitation infrastructures (Ambrozevicius and Abessa, 2008; Cetesb, 2019b; Gandra et al., 2020). Six of these channels are made of concrete and have a floodgate system with automatic mechanisms for their opening and closure. A seventh artificial channel, without floodgates, which flows continuously onto the beach also exists (Ambrozevicius and Abessa, 2008; Cetesb, 2019b; Gandra et al., 2020). During storm events or periods of high rainfall, the floodgates are opened, and the diffuse loads are discharged directly onto the beaches, in an area of intense recreational activities (bathing waters) (Rocha et al., 2011; Cetesb, 2019b). During the dry season or periods of low rainfall (condition observed in this study), the channel floodgates are closed and the diffuse loads drain towards the WWTP, through an underground oceanic interceptor located at the edge of the beaches (Ambrozevicius and Abessa, 2008; Cetesb, 2019b; Gandra et al., 2020). However, this WWTP only performs a mechanical treatment (railing and screening for the removal of solids), followed by a chlorination step (De Souza Abessa et al., 2012; Baptistelli and Marcellino, 2016; Ortiz et al., 2016). The final destination of this preconditioned sewage is a submarine outfall 4500 m long and 10 m deep that daily disposes sewage into a low-wave energy semi-closed bay (De Souza Abessa et al., 2012; Baptistelli and Marcellino, 2016; Ortiz et al., 2016). For further details, see Fig.11.1.

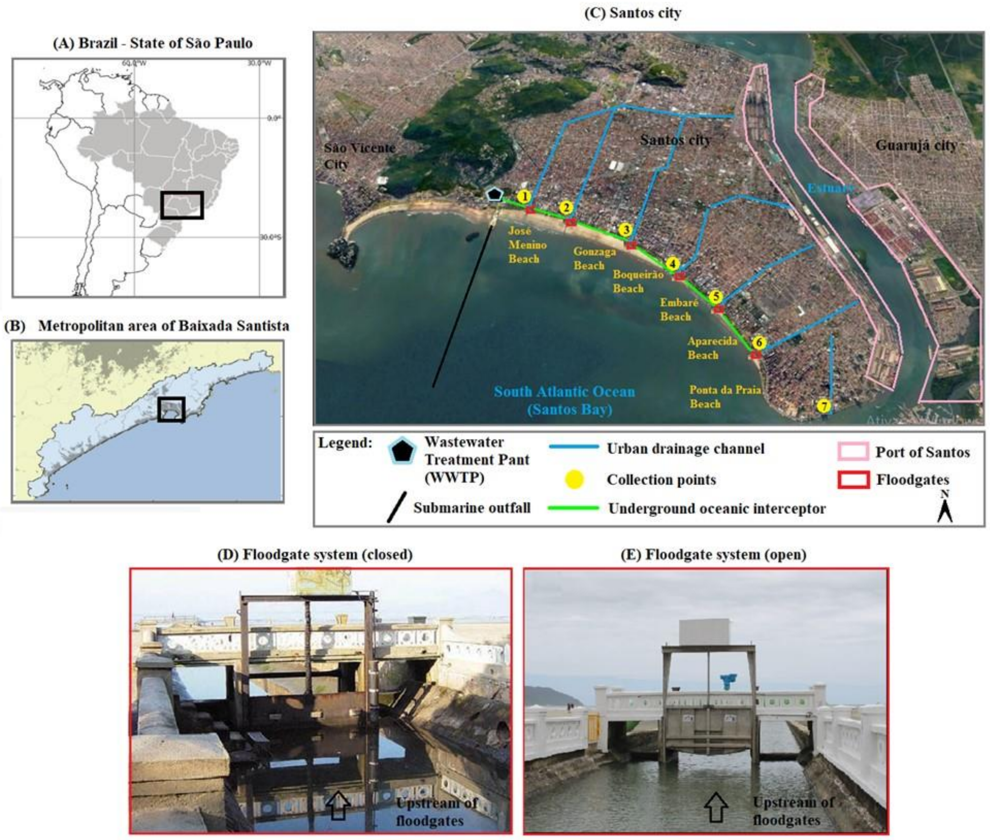


Figure -11.1. Map of the study area showing Brazil (in grey colour), São Paulo State (A), the Metropolitan area of Baixada Santista (B) and Santos city (C). Water sampling locations (urban surface runoff) on the seven urban drainage channels of the municipality of Santos were shown in numbered yellow circles. These channels are made of concrete, and six of them (channels 1 to 6) have a floodgate system with an automatic closure (D) and opening (E) mechanism. During the dry period, the floodgates are closed and the diffuse load flows to the Santos wastewater treatment plant (WWTP), through an underground oceanic interceptor located on the edge of the beaches. However, during intense rain and floods events, the floodgates are opened and the diffuse loads are discharged directly into the bathing waters of Santos. The channel 7 does not have a floodgate system and therefore all the diffuse loads flow directly to Santos Bay (South Atlantic Ocean) throughout the year. All water samples were collected upstream the floodgates.

A sampling point was selected at each channel, located at the mouth of the channel (upstream of the gate system) and therefore, unaffected by the tidal regime. The water samples were collected during the dry season and low tourist season, and weekends are avoided, to include only the disposal of PPCPs and illicit drugs by the resident population of Santos (Pereira et al., 2016; Fontes et al., 2019; Roveri et al., 2020b). Water (1L) was collected on Wednesday, 21st October of 2020, from each location and packed into pre-cleaned amber glass bottles. All samples were kept at 4°C, and target PPCPs were extracted from water samples within four days of collection (USEPA, 2007).

11.2.2 Preparation and analysis of pharmaceutical compounds

11.2.2.1 Chemical and standards

Chemicals and analytical reagents such as nitric acid and sulphuric acid were purchased from Merck (Darmstadt, Germany). Grade solvents used in HPLC and LC-MS, such as acetonitrile, methanol and isopropanol, were acquired from Sigma-Aldrich (Massachusetts, USA). Mobile phase additives, namely LC-MS grade formic acid and ammonium acetate, were acquired from Sigma-Aldrich and Merck, respectively. Analytical standards of acetaminophen, atenolol, bromazepam, caffeine, carbamazepine, cyproterone, clonazepam, clopidogrel, diclofenac, enalapril, loratadine, losartan, midazolam orphenadrine, propranolol, sildenafil, atorvastatin, ranitidine, diazepam, chlorpheniramine and valsartan were acquired from Sigma-Aldrich. Cocaine and benzoylecgonine were acquired from Cerillant (Texas, USA). The other pharmaceuticals were bought in several suppliers: citalopram (Alcytam®: Torrent, Brazil), chlortalidone (Higroton®: Novartis, Swiss), rosuvastatin (Crestor®: AstraZeneca, UK) and generic paroxetine medication (Medley, Brazil).

11.2.2.2 Sample preparation

The extraction technique used in the hereby study was modified from Wille et al. (2010). Firstly, the pH of each samples of channels were adjusted to 7.0 using a 1M hydrochloric acid solution. Thereafter, 1-L samples were filtered using a 1.2 µm pore size cellulose paper (Whatman® Filter Paper, Merck KGaA, Darmstadt, German) and at the end the filters were washed with 2 mL of methanol (Sigma-Aldrich, St. Louis, USA). The methanol extract collected was then combined to the filtered sample. Subsequently, the solid phase extraction (SPE) took place using spherical, hydrophobic polystyrene-divinylbenzene resin for SPE cartridges Chromabond ® HR-X, (200 mg, 3 mL, Macherey-Nagel GmbH & Co. KG, Düren, Germany) as described by Wille et al. (2010) and Ghoshdastidar et al. (2015). The cartridges were preconditioned with methanol (5 mL) and ultrapure water (5 mL) (Milli-Q®-Merck KGaA). Thereafter, they were load with 1 L of the filtered sample and the cartridges were rinsed twice with 5 mL of ultrapure water. The cartridges were then dried under vacuum for 30 min. The elution was performed twice using 5 mL of methanol and 5 mL of acetone. Prior to the analysis, the concentrated eluate was evaporated to dryness under a nitrogen flow (at 50°C), re-suspended in 1 mL with a solution of water/acetonitrile (95:5, v/v) and then filtered through a 0.45 µm pore size membrane (Merck Millipore). Each resuspension was analysed in triplicate using liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS). A concentration factor (1/1000) was used to obtain the measured concentration following LC–MS/MS quantification.

11.2.2.3 LC–MS/MS analysis

Taking into account the annual consumption, expected toxicity and environmental persistence (Cmed, 2019), a total of 27 chemical compounds, namely pharmaceuticals and illicit drugs, were analysed using LC–MS/MS (for further details, see the Table S1-supplementary material- online resource). The validation was performed using the parameters of selectivity, matrix effect, dynamic range, linearity, limit of detection (LOD), limit of quantification (LOQ), precision (% RSD), accuracy (% CV), recovery and robustness (Shihomatzu, 2015). Each water sample was analysed using the Agilent 1260 Infinity HPLC (Agilent™, Germany) combined with a hybrid triple quadrupole/LIT instrument (3200QTRAP®-linear ion trap) mass spectrometer (ABSciex, Ontario, Canada). Water samples (an aliquot of 10 µL) were injected in an Agilent Zorbax Eclipse XDB–C18 column (50 × 4.6 mm ID, 1.8 µm column at 25°C). The eluent flow rate was

0.7 mL/min, and the mobile phase for positive mode analysis was 0.1% formic acid (Sigma-Aldrich; LC–MS Grade) in water (solvent A) and acetonitrile (solvent B) (J.T. Baker, Philipsburg, NJ, USA). For negative mode analysis, the mobile phase was a 5 mM ammonium acetate buffer (Sigma-Aldrich) with a pH of 4.6 (solvent A) and acetonitrile (solvent B). For both modes of ionisation (negative and positive), a linear gradient of 0.7 mL/min was used, starting with a mixture of solvent A (95%) and solvent B (5%). The solvent A percentage was decreased linearly from 95% to 5% over the course of 5 min and this condition was maintained for 1 min. Over the course of 2 min, the mixture was then returned to the initial conditions. Using electrospray ionisation (ESI: positive and negative modes) and multiple reaction monitoring (MRM mode), analyses were detected and quantified. This procedure was performed with the selection of a precursor ion and two ion products to quantify and qualify each compound. MRM parameters for the positive and negative modes for each chemical compound, LOD and LOQ are shown in Table S1. A water matrix-matched external calibration curve was used (Shihomatzu , 2015). LOD and LOQ values were determined, using spiked matrix samples and obtained from seven measurements of the lowest detectable concentration of the calibration curves (with signal-to-noise ratio of at least 10), following the Brazilian Institute of Metrology, Quality and Technology procedures (INMETRO, 2011). Both field and laboratory blanks were below LOD. Data analysis was performed using the Analyst® 1.5.2 software (ABsciex). Results, expressed in ng/L, were the average value of the three technical replicates for each concentrated water sample (Table 11.1).

11.2.2.4 Ecological risk assessment

The ecological risk assessment followed the works of Roveri et al. (2020a, 2020b). The risk quotient (RQ) for three different aquatic organisms (algae, crustaceans and fishes) was calculated following the equation RQ = MEC/PNEC, in which MEC is the maximum Measured Environmental Concentration, and PNEC the Predicted No Effect Concentration, both expressed in ng/L. PNEC values were obtained from the existent literature ecotoxicity data for the aquatic compartment regarding short-term [lethal concentration 50 (LC50) or median effective concentration (EC50)] and long-term [no observed effect concentration (NOEC)] toxicological endpoints. In the absence of NOEC, the lowest observed effect concentration (LOEC) or, in alternative, the 10% effective concentration (EC10) were used, when available. Since urban drainage channels are already recognised as an important transport mechanism of conventional pollutants that flow to the sea (Lamparelli et al., 2015; Grandra et al., 2020; Roveri et al., 2020b), it was decided to measure ecological risk through marine species. In this context, an attempt was made to compile specifically PNEC data for marine coastal species. When these data were not available, data from freshwater communities were used, since the existent studies and current marine risk assessment practices, show a reasonable correlation between the ecotoxicological responses of freshwater and saltwater biota, at least for the usual aquatic taxa (i.e., acute and chronic toxicity to algae, crustaceans and fishes) (EMA, 2006; Li et al., 2012; Thomaidi et al., 2015). In order to collect available ecotoxicity test endpoints, an extensive search was carried out in the Ecotoxicology Database (ECOTOX) from the United States Environmental Protection Agency (USEPA, 2019), as well as in other literature sources using the PubMed database. When ecotoxicity laboratory experimentally derived data were not available, short [L(E)C50] and long toxicological endpoints [ChV, geometric mean of NOEC and LOEC, ChV = 10^([log (NOECxLOEC)]/2)] were estimated using the Ecological Structure Activity Relationships Program (ECOSAR, v 2.0) (USEPA, 2017). The PNEC values for the acute and chronic toxicity data were thereafter calculated by dividing each toxicological endpoint by an assessment factor (AF). For saltwater environments, an AF of 10,000 and 100 should be considered in short- and long-term data sets, respectively. For further details, see the European Chemical Bureau (ECB, 2003) and the European Chemicals Agency (ECHA, 2008) guidelines. The toxicological endpoints selected for the calculation of the PNECs are shown in Table S3. Finally, RQ was categorised into four levels: no ($RQ < 0.01$), low ($0.01 \leq RQ < 0.1$), moderate ($0.1 \leq RQ < 1.0$) and high ecological risk ($RQ \geq 1.0$) to aquatic organisms (Hernando et al., 2006).

11.3 Results and discussion

11.3.1 Occurrence of PPCPs and illicit drugs in channels of Santos

This study screened and identified, for the first time, the occurrence of 21 PPCPs of various therapeutic classes, including illicit drugs such as cocaine and its metabolite benzoylecgonine, in seven urban drainage channels that flow onto Santos beaches (Santos

Bay), in the Brazilian coastal zone. The occurrence of these chemical compounds in the channels of Santos is shown in Table 11.1.

Table 11.1 - Results of the occurrence, concentrations and detection frequency (%) of 21 pharmaceuticals of various therapeutic classes (including cocaine and its primary metabolite, benzoylecgonine) screened in the seven urban drainage channels (urban surface runoff) on the shoreline of Santos, São Paulo, Brazil. At the mouth of each channel, one water sampling point was selected (upstream of the gate system for channels 1 to 6) and therefore, without the influence of the tidal regime. For further details, see Fig.1. Note: (i) concentrations are expressed in ng/L; (ii) bold values represent the maximum measured environmental concentrations (MEC) for each compound; (iii) <LOD and <LOQ means below limits of detection and quantification, respectively.

Compound	**Urban drainage channels of Santos**							
	Channel 1	Channel 2	Channel 3	Channel 4	Channel 5	Channel 6	Channel 7	Detection rate (%)
Concentration (ng/L)								
Antiepiletic								
Carbamazepine	2.4	2.6	3.3	**4.0**	1.5	1.1	1.2	100.0
Diazepam	<LOQ	<LOQ	<LOD	<LOD	<LOD	<LOD	<LOD	28.6
Stimulants								
Caffeine	426.0	416.0	**516.0**	185.2	354.0	146.4	143.4	100.0
Cocaíne	**1.7**	1.3	1.0	0.5	1.4	1.1	1.0	100.0
Benzoylecgonine	**4.8**	4.3	2.5	1.3	3.5	1.1	1.0	100.0
Antidepressant								
Citalopram	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOD	<LOQ	85.7
Analgesic/ Anti-inflammatory								
Acetaminophen	12.6	**13.8**	7.2	2.2	3.2	1.5	1.5	100.0
Diclofenac	2.9	**3.5**	2.6	2.6	1.9	1.9	2.9	100.0
Orfenadrine	0.4	**0.8**	0.5	0.2	0.1	0.2	0.1	100.0
Antihypertensive								
Atenolol	11.0	**18.2**	15.2	4.1	4.7	1.1	1.2	100.0
Propranolol	<LOQ	<LOQ	<LOD	<LOQ	<LOD	<LOD	<LOQ	57.1
Enalapril	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	100.0
Losartan	**21.8**	17.8	18.2	4.2	6.2	7.2	7.4	100.0
Valsartan	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	100.0
Anticholesteremic								

Rosuvastatin	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	100.0
Anxiolytic								
Midazolam	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOD	<LOD	28.6
Diuretic								
Chlortalidone	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOD	<LOD	71.4
Antiplatelet								
Clopidogrel	<LOQ	<LOQ	<LOQ	<LOD	<LOD	<LOD	<LOD	42.9
Antihistamine								
Chlorpheniramine	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	100.0
Statin								
Atorvastatin	<LOQ	<LOQ	<LOQ	<LOD	<LOD	<LOD	<LOD	42.9
Antiulcerous								
Ranitidine	<LOD	<LOQ	<LOQ	<LOD	<LOD	<LOD	<LOD	28.6

Of the 21 PPCPs detected (>LOD), 13 compounds were detected in all channels (100.0% frequency), indicating that those pollutants were ubiquitous in the environment of Santos: antiepileptics (carbamazepine); stimulants (caffeine, cocaine and benzoylecgonine); analgesics/anti-inflammatories (acetaminophen, diclofenac and orphenadrine); antihypertensives (atenolol, enalapril, losartan and valsartan); an anticholesteremic (rosuvastatin) and an antihistamine (chlorpheniramine). An antidepressant (citalopram: 85.7%); diuretic (chlortalidone: 71.4%) and antihypertensive (propranolol: 57.1%) drugs were also detected. Five other compounds were detected at a frequency below 50%: antiplatelets (clopidogrel) and statins (atorvastatin) (both at 42.9%); and anxiolytics (midazolam), antiulcerous drugs (ranitidine) and antiepileptics (diazepam) (all at 28.6%).

This occurrence profile corresponds somewhat to the Brazilian pattern of PPCPs production and consumption. Brazil is the ninth largest producer of pharmaceutical drugs in the world; moreover, the pharmacies and drugstores, namely in the southeast region, are responsible for the majority of the sales in the country (Locatelli et al., 2010; Quadra et al., 2017; Cmed, 2019). These establishments sell non-prescription drugs, which means that the consumption of acetaminophen, diclofenac and orphenadrine is not controlled (Locatelli et al., 2010; Quadra et al., 2017; Cmed, 2019). The human excretion of PPCPs and illicit drugs in their parental, metabolised or conjugated forms trough urine and faeces, explains their occurrence in different environmental matrices (e.g., urban channels and marine environment) in the coastal zone of São Paulo (Pereira et al., 2016; Fontes et al., 2019; Roveri et al., 2020a; 2020b).

Overall, the concentrations of 21 PPCPs and illicit drugs detected in the seven urban drainage channels of Santos ranged from <LOQ to 516.0 ng/L (Table 11.1). This study also considers as warning signs even the twelve drugs with concentrations at levels <LOQ, but >LOD (e.g. citalopram, propranolol, diazepam, rosuvastatin, atorvastatin, midazolam, ranitidine, chlortalidone, clopidogrel, chlorpheniramine, enalapril and valsartan), because: (i) six these PPCPs (citalopram, atorvastatin, midazolam, clopidogrel, chlorpheniramine and valsartan) have high n-octanol/water partition coefficients (log Kow ≥ 3), which indicate that they could bioaccumulate and exert toxicity (see Log Kow in Table S11.1)(EMA, 2006; Mendoza et al., 2015; USEPA, 2017); (ii) although the samples were collected during low season, and therefore, only involved the disposal of PPCPs and illicit drugs by the resident population of Santos, tourism increases the population in Santos during the summer season, holidays and weekends, and so also the consumption of pharmaceuticals and illicit drugs (Pereira et al., 2016; Fontes et al., 2019; Molnar et al, 2020); (iii) according to recent reviews (Starling et al., 2018; Peña-Guzmán et al., 2019; Valdez-Carrillo et al., 2020), and to the best of our knowledge, this study seems to be the first report on the occurrence of midazolam, ranitidine and chlorpheniramine in surface waters in Latin America, which thus deserve more attention and further investigation.

The nine PPCPs with concentrations above LOQ in the urban channels of Santos were caffeine (MEC = 516.0 ng/L) > losartan (MEC =21.8 ng/L) > atenolol (MEC =18.2 ng/L) > acetaminophen (MEC =13.8 ng/L) > benzoylecgonine (MEC =4.8 ng/L) > carbamazepine (MEC =4.0 ng/L), diclofenac (MEC =3.5 ng/L) > cocaine (MEC =1.7 ng/L) and orphenadrine (MEC =0.8 ng/L) (Table 11.1; Fig. 11.2). These seven channels pass through six crowded neighbourhoods in Santos (e.g., José Menino; Gonzaga; Boqueirão; Embaré; Aparecida and Ponta da Praia), here several commercial establishments, including restaurants, bars, supermarkets, pharmacies, drugstores, numerous hotel establishments and also medical clinics and hospitals could be found. Consequently, these channels receive the daily input of urban runoff waters, which are usually mixed with unregulated domestic sewage, before they even reach the beaches of Santos (Lamparelli et al., 2015; Cetesb, 2019b; Gandra et al., 2020). Moreover, sample collection was performed during the dry season and no rainfall was recorded for 72 hours prior to collection. This means that channel floodgates 1 to 6 were closed, so that these diffuse loads drained towards the WWTP (for further information about the floodgates system, see Fig.11.1) (Ambrozevicius and Abessa, 2008; Ferraz et al., 2012; Cetesb, 2019b). Because the Santos WWTP is only a primary level system, and therefore inefficient in removing PPCPs and illicit drugs, the final destination of the preconditioned sewage (containing these 21 compounds) was a submarine outfall that disposes the sewage daily into the Santos Bay (a semi-closed and low energy coastal system) (Pereira et al., 2016; Ortiz et al., 2016; Fontes et al., 2019). Channel 7 does not have a floodgate system, and therefore all the diffuse load containing PPCPs and illicit drugs flow directly into Santos Bay without passing by the WWTP (South Atlantic Ocean) (Ambrozevicius and Abessa, 2008; Ferraz et al., 2012; Cetesb, 2019b).

The individual PPCPs concentrations quantified were generally below 50.0 ng/L for most of the target chemicals, except for caffeine (predominant compound in this study) which was present in considerably high concentrations in all channels (143.0-516.0 ng/L) (Fig. 11.2). Caffeine consumption is very high in Brazil, mainly due to the national consumption of various foods containing caffeine, such as beverages, stimulants, energy drinks, tea, coffee, soft drinks and painkillers (e.g., use in the formulation of acetaminophen) (Quadra et al., 2017). A study conducted in the stormwater pipes of Montreal, Canada, showed that caffeine concentrations above 400 ng/L are strongly correlated with water samples including at least 200 CFU/100 mL of faecal coliforms (Potera et al., 2012; Sauvé et al 2012). Moreover, some studies conducted by the São Paulo Environmental Agency (between 2010 and 2019) detected *Escherichia coli* (*E. coli*) at high concentrations in the Santos channels (> 600 CFU/100 mL *E. coli* in 87.0% of samples). This indicates poor sanitation conditions, as this bacterium is an important marker of domestic sewage (present in human faeces in percentages between 96.0% and 99.0%) (Lamparelli et al., 2015; Cetesb, 2019b). In this context, the concentrations of caffeine detected in this study (range: 143.4–516.0 ng/L) (Table 11.1) may also indicate faulty sanitation. The high concentration of caffeine in urban surface waters has also been found in other countries, such as Singapore (1389.0 ng/L) (Bayen et al., 2016), China (430.0 ng/L) (Yang et al., 2018) and Uruguay (1120.0 ng/L) (Griffero et al., 2019). In short, the caffeine detected in the channels of Santos is a suitable marker for sewage contamination, due to its widespread occurrence, environmental persistence, and high concentration.

In addition to caffeine, other psychoactive stimulants, such as cocaine and its metabolite benzoylecgonine, were also found in all channels (Fig.11.2).

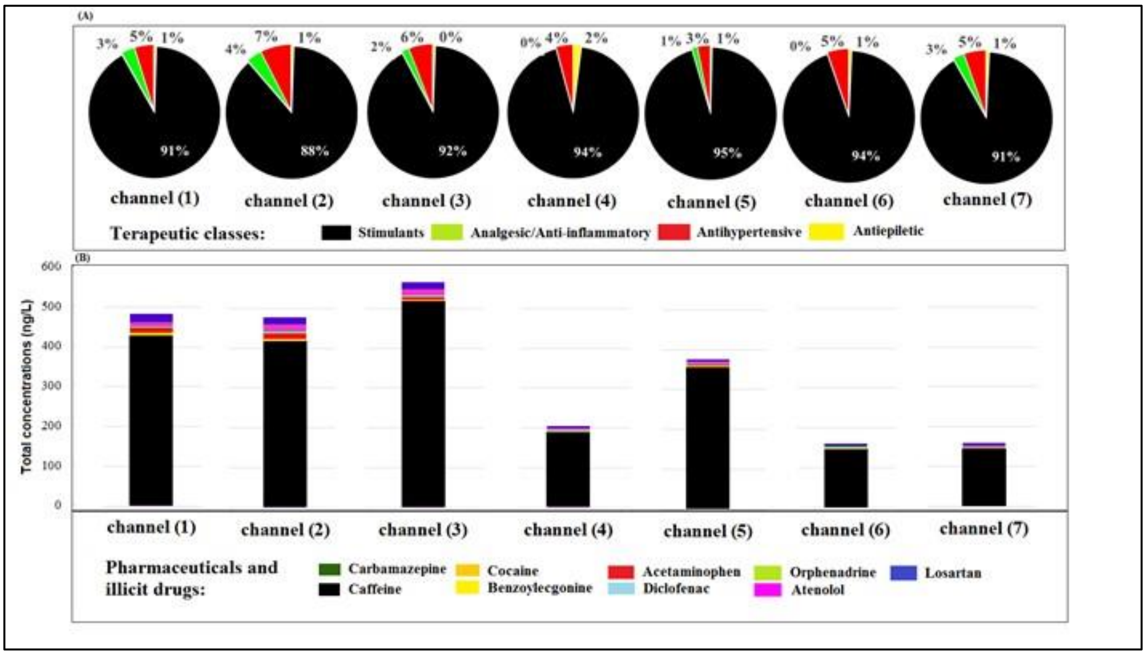


Figure-11.2. Total concentrations (in ng/L) of the nine pharmaceutical and personal care products (PPCPs) and illicit drugs (cocaine and its metabolite benzoylecgonine) detected and quantified on the seven urban drainage channels of Santos, São Paulo coast, Brazil, by therapeutic class (A) and by particular PPCP or illicit drug (B).

South America is almost responsible for all the world's cocaine production, and specifically in Brazil, 1.75% of the young adult population of both sexes frequently uses it (Abdalla et al., 2014; Fontes et al., 2020; UNODC, 2020). This data raises concerns, because the use of this illicit drug may have negative effects on the health and social life of the population of Santos (Pereira et al., 2016; Campestrini and Jardim, 2017; Fontes et al., 2019). Cocaine is also a threat to the aquatic ecosystem, since it can interact biologically with non-target organisms exerting deleterious effects (Fontes et al., 2019; 2020). The combined presence of cocaine and benzoylecgonine provides a reliable indication of human consumption of cocaine in Santos city, since cocaine is readily metabolised into benzoylecgonine, and higher quantities of benzoylecgonine are expected in the water, compared to those of cocaine (Van Nuijs et al., 2009; Li et al., 2016; Maasz et al., 2019). In this context, the hereby results are similar to those obtained by other studies performed in surface waters of other countries, where the levels of benzoylecgonine (BE) detected were higher than those of cocaine (COC), such as in Belgium (8.6 ng/L BE; 4.1 ng/L COC) (Van Nuijs et al., 2009), China (1.4 ng/L BE; 0.7 ng/L COC) (Li et al., 2016) and Hungary (2.3 ng/L BE; 1.2 ng/L COC) (Maasz et al., 2019). Other studies have already shown the widespread contamination of Santos Bay by cocaine and benzoylecgonine in different environmental matrices (water, marine sediments and mussels), mainly due to discharge from the WWTP (Pereira et al., 2016; Fontes et al., 2019; 2020). However, the present study also shows that the urban channels of Santos are also a source of PPCPs and illicit drugs for the marine ecosystem.

Another important factor that leads to a high consumption of pharmaceuticals drugs worldwide, is the aging population, as the immune system is weaker, and the incidence of chronic diseases normally increases (Linjakumpu et al., 2002; Tummala et al., 2010). It is estimated that the population over 65 years of age usually consumes 5–10 pills/patient/day (Lacorte et al., 2018). Atenolol and losartan, for instance, are antihypertensives commonly taken by elderly people (Pereira et al., 2016). Even diclofenac, an anti-inflammatory broad spectrum drug, can be used to treat osteoarthritis and rheumatoid arthritis (Locatelli et al., 2010; Cmed, 2019). Studies conducted in Germany (Herrmann et al., 2015), France, Spain, and Portugal (Lacorte et al., 2018), showed that the most commonly administered pharmaceuticals in senior residences are carbamazepine, acetaminophen and diclofenac (Herrmann et al., 2015; Lacorte et al., 2018). The elderly people of Santos is approximately 19.0% of the population, one of the largest values in the country (Ibge, 2019). This context could explain the occurrence of these five PPCPs in Santos, which were detected in all channels in relatively high concentrations (losartan: 4.2–21.8 ng/L, atenolol: 1.1–18.2 ng/L, acetaminophen: 1.5–13.8 ng/L, carbamazepine:1.1–4.0 ng/L and diclofenac: 1.9–3.5 ng/L) (Table 11.1; Fig. 11.2).

The concentrations of the nine PPCPs quantified in the channels in this study were within those values reported in Europe (Wilkinson et al., 2017; Mijangos et al., 2018; Maasz et al., 2019), North America (Klosterhaus et al., 2013; Lara-Martín et al., 2014; Anumol et al., 2015), Latin America (Spongberg et al., 2011; Rivera-Jaimes et al., 2018; Griffero et al., 2019) and Asia (Bayen et al., 2016; Li et al., 2016; Hossain et al., 2018). For more details, see Table S11.2. Again, these results also reflect the pattern of consumption on the coast of São Paulo and were already expected. Of the nine PPCPs quantified (including illicit drugs), seven compounds (caffeine, cocaine, benzoylecgonine, acetaminophen, diclofenac, atenolol and losartan) were also detected in the sewage discharge from Santos Bay (Pereira et al., 2016; Fontes et al., 2019; Fontes et al., 2020) and urban channels, tourist beaches and the sewage discharge from Guarujá (Roveri et al., 2020a;2020b). Therefore, these PPCPs could be considered environmental tracers of wastewater on the coast of São Paulo. It is necessary to understand the risks of these PPCPs to aquatic biota, because the MEC for caffeine (530.0 ng/L), losartan (21.8 ng/L), atenolol (18.2 ng/L) and acetaminophen (13.8 ng/L) were higher than 10.0 ng/L (Table 11.1), which is considered the threshold for the risk evaluation of pharmaceuticals in surface waters according to the European Medicines Agency (EMA, 2006).

11.3.2 Risk assessment of PPCPs and illicit drugs

Using the PNEC from data available in the scientific peer-reviewed literature or estimated by the ECOSARr program, the RQ in the channels of Santos were calculated using the maximum MEC to evaluate the worst case of environmental risk for the aquatic biota. For further details, see Table 11.2 (summary data with the four compounds that indicated ecological potential risks) and Table S11.3 (complete data for the nine detected and quantified PPCPs).

Table 11.2 - Ecological risk assessment results regarding the pharmaceuticals and illicit drugs detected on the urban drainage channel of Santos, São Paulo, Brazil. This summary table presents: name of each compound; measured environmental concentration (MEC, ng/L) in the Santos water body; acute and chronic toxicity data: [(trophic level; organism's test, toxicological endpoint and concentration (ng/L)], Assessment Factor (AF), Predicted No-Effect Concentration (PNEC, ng /L). Data from the toxicological endpoints was obtained from several published works (References) available from the Ecotoxicology Database (ECOTOX), or, in the absence of derived experimentally data, estimated from the ECOSAR program. Note: Freshwater (1); Seawater (2); EC10: 10% Effective Concentration; EC50: 50% Effective Concentration; LC50: 50% Lethal Concentration; NOEC: No Observed Effect. Concentration; LOEC: Lowest Observed Effect Concentration. For further details, see item 2.3 and Table S3.

		Toxicity data								
Compound	MEC (ng/L)	Trophic level		Organisms/species	Endpoint	Concentrations (ng/L)	AF	PNEC (ng/L)	Reference	RQ
Carbamazepine	4.0	Acute	Algae	*Skeletonema marinoi* (2)	72h EC50	1,00E+08	10000	1,00E+04	Minguez et al. (2014)	<0.01
			Crustacea	*Artemia salina* (2)	48h EC50	1,00E+08		1,00E+04	Minguez et al. (2014)	<0.01
			Fish	*Oryzias latipes* (1)	48h EC50	3,52E+07		3,52E+02	Kim et al. (2009)	<0.01
		Chronic	Algae	*Lemna gibba* (1)	LOEC/2	5,00E+05	100	5,00E+03	Brain et al. (2004)	<0.01
			Crustacea	*Ceriodaphnia dubia* (1)	NOEC	2,50E+04		2,50E+02	Ferrari et al. (2003)	0.01
			Fish	*Danio rerio* (1)	NOEC	2,50E+07		2,50E+05	Ferrari et al. (2003)	<0.01
Caffeine	516.0	Acute	Algae	*Pseudokirchneriella subcapitata* (1)	72h LC50	3,39E+08	10000	3,39E+05	Blaise et al. (2006)	0.02
			Crustacea	*Daphnia dubia* (1)	48h LC50	5,00E+07		5,00E+03	Moore et al. (2008)	0.10
			Fish	*Pimephales promelas* (1)	48h LC50	8,00E+07		8,00E+03	Moore et al. (2008)	0.06
		Chronic	Algae	*Lemna gibba* (1)	LOEC/2	5,00E+05	100	5,00E+03	Brain et al. (2004)	0.10
			Crustacea	*Ceriodaphnia dubia* (1)	NOEC	2,00E+07		2,00E+05	Brain et al. (2004)	0.02
			Fish	*Pimephales promelas* (1)	NOEC	3,00E+07		3,00E+05	Brain et al. (2004)	0.01
Acetaminophen	13.8	Acute	Algae	*Phaeodactylum tricornutum* (2)	72h EC50	2,39E+08	10000	2,39E+04	Claessens et al. (2013)	<0.01
			Crustacea	*Artemia salina* (2)	48h EC50	1,00E+08		1,00E+04	Minguez et al. (2014)	<0.01
			Fish	*Oryzias latipes* (1)	48h EC50	2,66E+08		2,66E+04	Kim et al. (2009)	0.01
		Chronic	Algae	*Phaeodactylum tricornutum* (2)	72h EC10	7,21E+07	100	7,21E+05	Claessens et al. (2013)	<0.01
			Crustacea	*Daphnia magna* (1)	NOEC	4,03E+05		4,03E+03	Kim et al. (2009)	<0.01
			Fish	*Danio rerio* (1)	LOEC/2	5,00E+03		5,00E+01	Galus et al. (2013)	0.28
Losartan	21.8	Acute	Algae	*Lemna minor* (1)	96h EC50	6,46E+07	10000	6,46E+03	Godoy et al. (2015)	<0.01
			Crustacea	*Daphnia magna* (1)	48h LC50	331000,00		3,31E+01	FDA (2012)	0.66
			Fish	*Pimephales promelas* (1)	48h LC50	1,00E+09		1,00E+06	FDA (2012)	<0.01

		Chronic	Algae	Green algae [(1)]	10^([log (LOEC x NOEC)]/2)	1,64E+06	100	1,64E+04	ECOSAR	<0.01
			Crustacea	Daphnid [(1)]		5,55E+05		5,55E+03	ECOSAR	<0.01
			Fish	Fish [(1)]		2,94E+05		2,94E+03	ECOSAR	0.01

The acute and chronic RQs for most individual PPCPs (e.g., carbamazepine, cocaine, benzoylecgonine, diclofenac, orphenadrine and atenolol) were equal or below 0.01, demonstrating a low to none ecological risk. However, even to some compounds which showed no ecological risk, other environmental issues, such as a high lipophilic character, low biodegradability and/or environmental pseudo-persistence cannot be neglected. Cocaine/carbamazepine and orphenadrine/diclofenac have moderate (log Kow > 2.3) to high (log Kow > 3.0) hydrophobicity, respectively, and therefore can potentially bioaccumulate (Table S11.1) (EMA, 2006; Mendoza et al., 2015; USEPA, 2017), such as demonstrated for algae (*Pseudokirchneriella subcapitata*) and crustacean (*Thamnocephalus platyurus*) (Vernouillet et al., 2010). Moreover, the tendency of a substance for biological degradation (biodegradability) can be estimated by its kinetic reaction rate (k biol), based on grams of suspended solids (ss) and days (L/gss day). In this context, diclofenac and carbamazepine are barely degradable (k biol < 0.5 L/gss day) and atenolol is considered moderately degradable: (0.5 < k biol < 1.0 L/gss day) (Schröder et al., 2016; Arola et al., 2017). Finally, PPCPs and illicit drugs have typical environmental pseudo-persistence (e.g. carbamazepine: half-life > 100 days in seawater) (USEPA, 2017) due to continuous input through these channels, and are likely to exert long-term effects which cannot usually be detected after short-term exposure (condition observed in this study, Table S11.3) (Di Poi et al., 2018). It means that toxic effects may therefore still be generated after a certain concentration is long-term accumulated *in vivo* (Wang et al., 2019; Radwan et al., 2020; Valdez-Carrillo et al., 2020).

Another important issue is that these compounds, even at very low concentrations, can have exerted toxicity in a mixture because of their combined effects (e.g., non-interactive, additive action, antagonism or synergism) (Di Poi et al., 2018). PPCPs and illicit drugs found in the Santos channels are in a complex mixture of various therapeutic classes which may lead to an increase of the overall ecological risk (Wang et al., 2019; Radwan et al., 2020; Valdez-Carrillo et al., 2020). In this context, some studies have already showed that: (i) the combined effects of cocaine and benzoylecgonine induced genotoxicity and apoptotic cells in zebra mussels (*Dreissena polymorpha*) and in zebrafish (*Danio rerio*) embryos (Parolini et al., 2015; 2017); (ii) the sea urchin *Paracentrotus lividus* experienced developmental abnormalities when exposed to environmental combined concentrations of carbamazepine and ibuprofen (Aguirre-Martínez et al., 2015); and (iii) adult *zebrafish* (*Danio rerio*) showed significantly decreased embryo production after a six week exposure to environmental concentrations of acetaminophen, carbamazepine and gemfibrozil (Galus et al., 2013).

The occurrence of caffeine, acetaminophen and losartan are a cause for environmental concern because the RQ were high suggesting low to moderate ecological risk (for both acute and chronic exposures) for algae, crustacean and/or fishes (Table 11.2). The risks of these PPCPs have already been reported in previous works. For example, the MEC of caffeine detected in different Spanish freshwater bodies (similar to that obtained in Santos), including the Jarama River (MEC=410.0 ng/L) (Ferdandéz et al., 2010), Henares River (MEC=670.0 ng/L) (Valcárcel et al., 2011) and Guadalquivir River (MEC=230.0 ng/L) (Robles-Molina et al., 2014), indicated a moderate ecological risk for the aquatic biota. Caffeine was also identified as high risk in the priority list of pharmaceuticals in European surface waters (Zhou et al., 2019). Previous studies also identified a moderate risk from acetaminophen to *Daphnia magna* in surface waters of the Pego–Oliva Marshlands, Valencia, Spain (RQ=0.3: Vazquez-Roig et al., 2012), Sindian river, Taiwan (RQ=0.9: Yu-Chen Lin et al., 2010) and Lahore channel, Pakistan (RQ=0.4: Ashfaq et al., 2019). Although studies on the toxicity of losartan are poorly documented (Pereira et al., 2016; Desbiolles et al., 2018), a recent study detected cytotoxic effects on the gills and hemocytes of the mussel *Perna perna* exposed to environmental concentrations of up to 300.0 ng/L of losartan (Cortez et al., 2018).

Another issue to be considered in the present study is that the concentrations of PPCPs vary seasonally as result of the land occupation (low or high tourist seasons) and climate effects (e.g., rainfall, wind and temperature), which will affect the risk assessment results (Zhang et al., 2017; Wang et al., 2019; Molnar et al., 2020). The present study was however a preliminary assessment, it means that diffuse load samples were collected in a single day during the dry season and low tourist season. Therefore, in order to clearly understand the risk of PPCPs to the aquatic environment, further studies (including water collection the rainy season) are needed. A recent study conducted in the urban drainage channels of Guarujá city, Brazil (during the rainy season and high tourist season) also found moderate to high ecological risks from caffeine, acetaminophen and losartan. However, the MEC of these three PPCs were much higher than those recorded in the hereby study (Roveri et al., 2020a).

11.4 Conclusion

This study reported, for the first time, the occurrence of 21 PPCPs in the urban drainage channels of Santos city, Brazil, during the dry season and low tourist season in late October 2020. The results showed that although the concentrations of twelve detected PPCPs were below the LOQ, the other nine (e.g. carbamazepine, caffeine, cocaine, benzoylecgonine, acetaminophen, diclofenac, orphenadrine, atenolol and losartan) were quantified in all urban channels of Santos, indicating that those pollutants were ubiquitous in the study area. This preliminary ecological risk assessment revealed that caffeine, acetaminophen and losartan were of moderate risk, and carbamazepine of low risk to sensitive aquatic organisms in the maximum measured environmental concentrations. It is therefore of the utmost importance to the environmental quality of Santos Bay, and thus also for all the Brazilian coastal areas, that stakeholders (e.g. civil society, public authorities and environmental agencies) took substantial efforts to: (i) educate the public about the use and correct disposal of pharmaceutical waste in the environment; (ii) review the laws on the proper disposal of pharmaceutical waste in the environment; (iii) include PPCPs and illicit drugs in Brazilian environmental legislation as priority pollutants; (iv) define more rigorous guidelines for oceanic sewage disposal along the Brazilian coastal zone, namely where there is insufficient wastewater treatment plants; (v) implement continuous environmental monitoring programs of PPCPs and illicit drugs in Brazilian coastal zone; and finally (vi) to conduct further research about the occurrence and the potential ecological risk of PPCPs and illicit drugs along the Brazilian coastal zone.

Acknowledgments

Authors want to acknowledge Daniel Temponi Lebre from the Centro de Espectrometria de Massa Aplicada - Instituto de Pesquisas Energéticas e Nucleares (CEMSA, IPEN, São Paulo, Brazil) for technical support regarding the LC–MS/MS analyses. Thanks also to Professor Ítalo Braga de Castro, from Instituto do Mar, Universidade Federal de São Paulo (IMAR-UNIFESP, São Paulo, Brazil) for technical support with the Manifold.

Competing interests

The authors declare that they have no competing interests.

Funding

Correia AT was supported by national funds through FCT - Foundation for Science and Technology within the scope of UIDB/04423/2020 and UIDP/04423/2020.

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Supplementary material

Table S11.1 - Multiple reactions for positive and negative ion modes. The table presents: name of compound and its respective CAS (Chemical Abstracts Service); Log Kow; number; Q1: mass to charge ratio of the mother ion in the first quadrupole (m/z); Q3: mass to charge ratio of the most intensive daughter ion in the third quadrupole (m/z); DP: declustering potential (V); CE: collision energy (V); CXP: collision cell exit potential (V); LOD: Limits of detection (ng/L); LOQ: Limits of quantification (ng/L); and RT: Retention time. Note: It was assumed hereby the sample concentration factor to be 1,000 times in water matrix.

Therapeutic Class	**CAS number**	**Log Kow**	**Q1** (m/z)	**Q3** (m/z)	**DP** (V)	**CE** (V)	**CXP** (V)	**LOD** (ng/L)	**LOQ** (ng/L)	**RT** (min)
Antiepileptic										
Carbamazepine	298-46-4	2.25	237.1	194.2 179.1	36 36	43 25	4 4	0.003	0.01	4.7
Clonazepam	1622-61-3	2.53	316.1	270.0 214.2	51 51	31 47	4 4	0.0013	0.01	5.1
Diazepam	439-14-5	2.70	285.2	105.1 154.1	51 51	33 39	4 4	0.0004	0.0015	4.8
Stimulants										
Caffeine	58-08-2	-0.07	195.2	138.3 110.1	26 26	19 29	4 4	0.0001	0.0085	3.4
Cocaine	50-36-2	2.30	304.2	182.2 105.1	36 36	39 25	4 4	0.003	0.0012	3.9
Benzoylecgonine	519-09-5	-1.31	290.2	168.2 105.1	31 31	25 37	4 4	0.0012	0.0077	3.6
Antidepressant										
Citalopram	59729-33-8	3.74	325.2	109.2 262.1	41 41	37 25	4 4	0.0006	0.0059	4.3
Paroxetine	61869-08-7	3.95	330.2	192.2 135.1	41 41	27 54	4 4	0.004	0.031	4.6
Analgesic/ Anti-inflammatory										
Acetaminophen	103-90-2	0.27	152.1	109.9 93.1	26 26	19 29	4 4	0.0014	0.0084	3.0
Diclofenac	15307-86-5	4.02	296.1	214.1 250.0	21 21	39 25	4 4	1.0	0.0074	5.8
Orphenadrine	83-98-7	3.65	270.2	181.1 165.0	16	19 53	4 4	0.0009	0.0034	4.4
Antihypertensive										

Atenolol	29122-68-7	-0.03	267.3	145.2	31	37	4	0.0016	0.0069	2.9
				190.3	31	25	4			
Propranolol	525-66-6	2.60	260.2	116.0	41	23	4	0.0013	0.0072	4.4
				183.0	41	23	4			
Enalapril	75847-73-3	2.45	377.3	234.2	36	27	4	0.003	0.009	4.4
				303.3	36	25	4			
Losartan	114798-26-4	4.01	423.2	207.2	21	31	4	0.0007	0.0061	4.8
				405.2	21	17	6			
Valsartan	137862-53-4	3.65	436.3	235.1	21	25	4	0.0014	0.0077	5.3
				207.1	21	33	4			
Anticholesteremic										
Rosuvastatin	287714-41-4	2.46	482.2	258.2	61	41	4	0.0008	0.0069	4.9
				270.2	61	47	4			
Anxiolytic										
Bromazepam	1812-30-2	1.93	316.0	182.2	51	41	4	0.005	0.0281	4.3
				209.2	51	33	4			
Midazolam	59467-70-8	4.33	326.1	291.2	51	33	4	0.0006	0.0059	4.5
				249.1	51	44	4			
Contraceptive										
Cyproterone	427-51-0	4.18	417.3	357.2	41	25	6	0.0015	0.0075	6.4
				279.3		41	4			
Diuretic										
Chlortalidone	77-36-1	1.01	336.9	189.9	−35	−22	−2	0.0023	0.0088	4.1
				146.2	−35	−28	−2			
Antiplatelet										
Clopidogrel	113665-84-2	3.82	322.2	212.2	31	23	4	0.00004	0.0003	6.2
				155.0	31	51	4			
Antihistamine										
Chlorpheniramine	132-22-9	3.81	275.2	230.1	21	21	4	0.0003	0.0011	3.4
				167.2	21	53	4			

Loratadine	79794-75-5	5.65	383.3	337.3	41	33	6	0.0014	0.0126	5.2
				267.1	41	41	4			
Sexual stimulant										
Sildenafil	171599-83-0	-1.75	475.3	100.0	51	37	4	0.006	0.043	4.2
				283.2	51	47	4			
Statin										
Atorvastatin	134523-00-5	6.35	559.3	440.2	41	31	6	0.0092	0,0308	5.8
				250.2	41	57	4			
Antiulcerous										
Ranitidine	66357-35-5	0.29	315.2	176.1	21	23	4	0.0011	0.0037	3.3
				130.1	21	21	4			

Reference (Table S11.1)

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Table S11.2 – Name and maximum measured environmental concentrations (MEC, in bold) of 9 pharmaceuticals and illicit drugs detected in the urban drainage channels of Santos, São Paulo, Brazil, compared to the concentrations reported by other studies in several aquatic compartments worldwide.

Compound	Concentration (ng/L)	Environmental Matrix	Country	Reference
Carbamazepine	**4.0**	**urban drainage channel**	**Brazil**	**This study**
	5.8	river	USA	Anumol et al. (2015)
	8.8	river	Bangladesh	Hossain et al. (2018)
	3.5	river	China	Yang et al. (2018)
	5.8	river	France	Celle-Jeanton et al. (2014)
	82	Urban surface	Costa Rican	Spongberg et al. (2011)
Caffeine	**516.0**	**urban drainage channel**	**Brazil**	**This study**
	1389.0	estuary water	Singapore	Bayen et al. (2016)
	430.0	river	China	Yang et al. (2018)
	1120.0	coastal lagoon	Uruguay	Griffero et al. (2019)
	699.0	estuary water	Spain	Mijangos et al. (2018)
	138.7	lake	Hungary	Maasz et al. (2019)
Cocaine	**1.7**	**urban drainage channel**	**Brazil**	**This study**
	6.6	seawater	Greece	Borova et al. (2014)
	4.1	river	Belgium	Van Nuijs et al. (2009)
	<LOQ	coastal lagoon	Uruguay	Griffero et al. (2019)
	1.2	lake	Hungary	Maasz et al. (2019)
	0.7	lake	China	Li et al. (2016)
Benzoylecgonine	**4.8**	**urban drainage channel**	**Brazil**	**This study**
	5.2	seawater	USA	Klosterhaus et al. (2013)
	8.6	river	Belgium	Van Nuijs et al. (2009)
	<LOQ	coastal lagoon	Uruguay	Griffero et al. (2019)
	2.3	lake	Hungary	Maasz et al. (2019)
	1.4	lake	China	Li et al. (2016)
Acetaminophen	**13.8**	**urban drainage channel**	**Brazil**	**This study**
	200.0	river	France	Celle-Jeanton et al. (2014)
	518.0	river	Mexico	Rivera-Jaimes et al. (2018)
	9.6	river	China	Yang et al. (2018)

	20.8	river	England	Wilkinson et al. (2017)
	49.0	estuary water	Spain	Mijangos et al. (2018)
Diclofenac	**3.5**	**urban drainage channel**	**Brazil**	**This study**
	1.49	river	Sweden	Lindim et al. (2016)
	51.24	river	Portugal	Pereira et al. (2017)
	15.49	river	Malaysia	Praveena et al. (2018)
	52.0	river	Spain	Gros et al. (2012)
	51.8	estuary water	Portugal	Reis-Santos et al. (2018)
Orphenadrine	**0.8**	**urban drainage channel**	**Brazil**	**This study**
	1.5	urban drainage channel	Brazil	Roveri et al. (2020)
	not reported	river	Italy	Milione et al. (2016)
Atenolol	**18.2**	**urban drainage channel**	**Brazil**	**This study**
	13.0	river	France	Celle-Jeanton et al. (2014)
	0.9	seawater	USA	Lara-Martín et al. (2014)
	95.0	river	Mexico	Rivera-Jaimes et al. (2018)
	0.5	estuary water	Portugal	Reis-Santos et al. (2018)
	< LOD	river	Greece	Nannou et al. (2015)
Losartan	**21.8**	**urban drainage channel**	**Brazil**	**This study**
	1.2	river	Serbia	Lv et al. (2014)
	154.0	river	Serbia	Petrovic et al. (2014)
	417.0	sewage	Germany	Subedi et al. (2017)
	183.0	estuary water	Spain	Mijangos et al. (2018)
	24.8	lake	Hungary	Maasz et al. (2019)

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Table S11.3 - Results from the ecological risk assessment tests regarding the pharmaceuticals of the different therapeutic classes and illicit drugs (cocaine and its metabolite benzoylecgonine) detected on the urban drainage channel of Santos, São Paulo, Brazil. The table presents: name of each compound; MEC: measured environmental concentration in the Santos water body (ng/L); acute and chronic toxicity data: [(trophic level; organism's test, toxicological endpoint and concentration (ng/L)], Assessment Factor (AF), Predicted No-Effect Concentration (PNEC, ng /L). Data from the toxicological endpoints was obtained from several published works (References) available from the Ecotoxicology Database (ECOTOX), or, in the absence of derived experimentally data, estimated from the ECOSAR program. Note: Freshwater (1); Seawater (2); EC10: 10% Effective Concentration; EC50: 50% Effective Concentration; LC50: 50% Lethal Concentration; NOEC: No Observed Effect. Concentration; LOEC: Lowest Observed Effect Concentration. For more details, see item 2.3.

		Toxicity data								
Compound	MEC (ng/L)	Trophic level		Organisms/species	Endpoint	Concentrations (ng/L)	AF	PNEC (ng/L)	Reference	RQ
Carbamazepine	3.3	Acute	Algae	*Skeletonema marinoi* (2)	72h EC50	1,00E+08	10000	1,00E+04	Minguez et al. (2014)	<0.01
			Crustacea	*Artemia salina* (2)	48h EC50	1,00E+08		1,00E+04	Minguez et al. (2014)	<0.01
			Fish	*Oryzias latipes* (1)	48h EC50	3,52E+07		3,52E+02	Kim et al. (2009)	<0.01
		Chronic	Algae	*Lemna gibba* (1)	LOEC/2	5,00E+05	100	5,00E+03	Brain et al. (2004)	<0.01
			Crustacea	*Ceriodaphnia dubia* (1)	NOEC	2,50E+04		2,50E+02	Ferrari et al. (2003)	0.01
			Fish	*Danio rerio* (1)	NOEC	2,50E+07		2,50E+05	Ferrari et al. (2003)	<0.01
Caffeine	516.0	Acute	Algae	*Pseudokirchneriella subcapitata* (1)	72h LC50	3,39E+08	10000	3,39E+05	Blaise et al. (2006)	0.02
			Crustacea	*Daphnia dubia* (1)	48h LC50	5,00E+07		5,00E+03	Moore et al. (2008)	0.10
			Fish	*Pimephales promelas* (1)	48h LC50	8,00E+07		8,00E+03	Moore et al. (2008)	0.06
		Chronic	Algae	*Lemna gibba* (1)	LOEC/2	5,00E+05	100	5,00E+03	Brain et al. (2004)	0.10
			Crustacea	*Ceriodaphnia dubia* (1)	NOEC	2,00E+07		2,00E+05	Brain et al. (2004)	0.02
			Fish	*Pimephales promelas* (1)	NOEC	3,00E+07		3,00E+05	Brain et al. (2004)	0.01
Cocaine	1.7	Acute	Algae	Green algae (1)	96h EC50	4,35E+06	10000	4,35E+02	ECOSAR	<0.01
			Crustacea	Daphnid (1)	48h LC50	5,48E+06		5,48E+02	ECOSAR	<0.01
			Fish	Fish (2)	96h LC50	4,86E+07		4,86E+03	ECOSAR	<0.01
		Chronic	Algae	Green algae (1)	10^([log (LOEC x NOEC)]/2)	1,46E+06	100	1,46E+04	ECOSAR	<0.01
			Crustacea	Mysid (2)		2,29E+09		2,29E+07	ECOSAR	<0.01
			Fish	Fish (2)		7,18E+06		7,18E+04	ECOSAR	<0.01
Benzoylecgonine	4.8	Acute	Algae	Green algae (1)	96h EC50	1,20E+10	10000	1,20E+06	ECOSAR	<0.01
			Crustacea	Mysid (2)	96h LC50	3,14E+12		3,14E+08	ECOSAR	<0.01
			Fish	Fish (2)	96h LC50	6,24E+11		6,24E+07	ECOSAR	<0.01
		Chronic	Algae	Green algae (1)	10^([log (LOEC x NOEC)]/2)	3,03E+09	100	3,03E+05	ECOSAR	<0.01
			Crustacea	Mysid (2)		2,00E+13		2.00E+11	ECOSAR	<0.01

			Fish	Fish (1)		4,92E+09		4,92E+05	ECOSAR	<0.01
Acetaminophen	13.8	Acute	Algae	*Phaeodactylum tricornutum* (2)	72h EC50	2,39E+08	10000	2,39E+04	Claessens et al. (2013)	<0.01
			Crustacea	*Artemia salina* (2)	48h EC50	1,00E+08		1,00E+04	Minguez et al. (2014)	<0.01
			Fish	*Oryzias latipes* (1)	48h EC50	2,66E+08		2,66E+04	Kim et al. (2007)	0.01
		Chronic	Algae	*Phaeodactylum tricornutum* (2)	72h EC10	7,21E+07	100	7,21E+05	Claessens et al. (2013)	<0.01
			Crustacea	*Daphnia magna* (1)	NOEC	4,03E+05		4,03E+03	Kim et al. (2009)	<0.01
			Fish	*Danio rerio* (1)	LOEC/2	5,00E+03		5,00E+01	Galus et al. (2013)	0.28
Diclofenac	3.5	Acute	Algae	*Dunaliella tertiolecta* (2)	96h EC50	1,86E+08	10000	1,86E+04	DeLorenzo and Fleming (2007)	<0.01
			Crustacea	*Artemia salina* (2)	48h EC50	1,00E+08		1,00E+04	Minguez et al. (2014)	<0.01
			Fish	*Danio rerio* (1)	72h LC50	7,80E+06		7,80E+02	Van den Brandof and Montforts (2010)	<0.01
		Chronic	Algae	*Lemna minor* (1)	NOEC	3,75E+06	100	3,75E+04	Cleuvers (2003)	<0.01
			Crustacea	*Ceriodaphnia dubia* (1)	NOEC	1,00E+06		1,00E+04	Ferrari et al. (2003)	<0.01
			Fish	*Danio rerio* (1)	NOEC	4,00E+06		4,00E+04	Ferrari et al. (2003)	<0.01
Orphenadrine	0.8	Acute	Algae	*Lemna minor* (1)	168h EC50	1,20E+07	10000	1,20E+03	Kaza et al. (2009)	<0.01
			Crustacea	*Artemia salina* (2)	24h EC50	4,50E+07		4,50E+03	Calleja et al. (1994)	<0.01
			Fish	Fish (1)	96h LC50	4,24E+07		4,24E+03	ECOSAR	<0.01
		Chronic	Algae	Green algae (1)	10^([log (LOEC x NOEC)]/2)	1,32E+05	100	1,32E+03	ECOSAR	<0.01
			Crustacea	Daphnid (1)		6,10E+04		6,10E+02	ECOSAR	<0.01
			Fish	Fish (1)		1,37E+05		1,37E+03	ECOSAR	<0.01
Atenolol	18.2	Acute	Algae	*Phaeodactylum tricornutum* (2)	72h EC50	2,62E+08	10000	2,62E+04	Claessens et al. (2013)	<0.01
			Crustacea	*Artemia salina* (2)	48h EC50	1,00E+08		1,00E+04	Minguez et al. (2014)	<0.01
			Fish	*Oryzias latipes* (1)	96h LC50	1,00E+08		1,00E+04	Kim et al. (2009)	<0.01
		Chronic	Algae	*Phaeodactylum tricornutum* (2)	72h EC10	3,30E+06	100	3,30E+04	Claessens et al. (2013)	<0.01
			Crustacea	*Daphnia magna* (1)	NOEC	1,48E+06		1,48E+04	Küster et al. (2010)	<0.01

			Fish	*Pimephales promelas* (1)	NOEC	1,00E+06		1,00E+04	Winter et al. (2008)	<0.01
Losartan	21.8	Acute	Algae	*Lemna minor* (1)	96h EC50	6,46E+07	10000	6,46E+03	Godoy et al. (2015)	<0.01
			Crustacea	*Daphnia magna* (1)	48h LC50	331000,00		3,31E+01	FDA (2012)	0.66
			Fish	*Pimephales promelas* (1)	48h LC50	1,00E+09		1,00E+06	FDA (2012)	<0.01
		Chronic	Algae	Green algae (1)	10^([log (LOEC x NOEC)]/2)	1,64E+06	100	1,64E+04	ECOSAR	<0.01
			Crustacea	Daphnid (1)		5,55E+05		5,55E+03	ECOSAR	<0.01
			Fish	Fish (1)		2,94E+05		2,94E+03	ECOSAR	0.01

Reference (Table S11.3)

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ONOMASTIC INDEX

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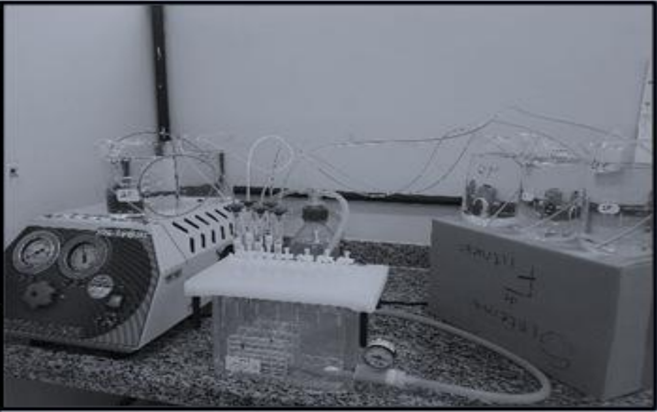






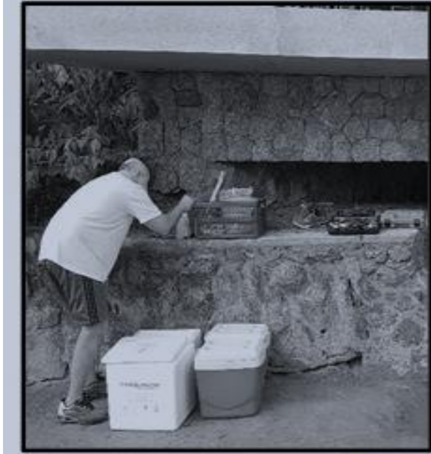



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