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UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
Instituto de Biociências
Câmpus do Litoral Paulista

ESTUDO ECOTOXICOLÓGICO E AVALIAÇÃO DO RISCO AMBIENTAL DA COCAÍNA EM ECOSSISTEMAS MARINHOS

MAYANA KAROLINE FONTES

São Vicente, SP.

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Mayana Karoline Fontes

Orientador: Camilo Dias Seabra Pereira

Coorientadora: Luciane Alves Maranho

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Dedico esse trabalho a todos aqueles que acreditam na educação e na Ciência como principais ferramentas na busca de uma sociedade justa, desenvolvida e próspera.

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“Gostaria de te desejar tantas coisas.
Mas nada seria suficiente.
Então, desejo apenas que você tenha muitos desejos.
Desejos grandes!
E que eles possam te mover a cada minuto ao rumo da sua felicidade!”

(Autor desconhecido)

Sumário

Lista de abreviações

6-ACM: 6-monoacetyl morphine

AChE: acetylcholinesterase

AMP: amphetamines

B: bottom

BAF: bioaccumulation factor

BE: Benzoilecgonine

CNS: Central Nervous System

COC: Cocaine

COD: codeine

COX: Cicloxygenase

DBF: dibenzilfluoresceína

DOPA: dopamine

EC: Emerging contaminants

EDDP: 2-ethylidene-1,5-dimethyl-3,3-diphenylpy

EME: Ecgonine metil ester

EPH: Ephedrine

EROD: etoxiresorufin O-deetilase

ETE: estação de tratamento de esgoto

GPx: Glutathione Peroxidase

GST: Glutathione S- Transferase

HER: heroin

ID: illicit drugs

LMS: lysosomal membrane stability

LOD: limit of detection

LOQ: limits of quantification

LPO: lipoperoxidation

LSD: lysergic acid diethylamide

MAMP: Methamphetamines

MAO: Monoamine oxidase

MDA: 3,4-methyledioxy amphetamine

MDMA: 3,4- methylediozy methamphetamine

MET: Mitochondrial electron transportation

MOR: morphine

MRM: multiple reaction monitoring

NOR-BZE: nor-benzoilecgonine

NOR-COC: norcocaine

NRRT: Neutral Red Retention Time

OCDE: Organização para a Cooperação e Desenvolvimento Econômico

PEPH: pseudoephedrine

PLANASA: Plano Nacional de Saneamento

S: surface

SSO: Sewage Submarine Outfall

STP: sewage treatment

THC: Δ9 -tetrahydrocannabinol

THC-COO: 11- Nor-Δ9 -carboxy-tetrahydrocannabinol

TLP: Total lipids

TRA: tramadol

UN: United Nations

UNODC: United Nations Office on Drugs and Crime

WWTPs: wastewater treatment plants

17β-HSD: 17β-hydroxysteroid dehydrogenase

3β-HSD: 3β-hydroxysteroid dehydrogenase

5-HT: serotonin

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concentrations.

Fig 2. Mean (standard deviation) biomarker activity EROD, DBF, GST, GPX, DNA strand break and LPO) in divestive gland tissue of Perna perna exposed to COC (200 ng.l-1 and 2000 ng. l-1) and controls; *represents significant differences (ANOVA, Dunnett's test, p<0.05) between the controls and COC concentrations

Fig 3. NRRT in hemocytes of Perna perna exposed to COC (ANOVA- Dunnet's test, p< 0.05). Asterisk indicate significance compared to control. Error bars indicate the standard errors.

Fig 4. Integrated biomarker response (IBR) and COC star plots in gills tissue. IBR index for biomarker response includes LMS, EROD, DBF, GST, GPx, DNA strand break and LPO.

Fig 5. Integrated biomarker response (IBR) and COC star plots in digestive gland tissue. IBR index for biomarker response includes LMS, EROD, DBF, GST, GPx, DNA strand break and LPO.

Morphological and hormonal responses in ovaries of *Anguilla anguilla* exposed to environmental cocaine concentrations

Fig. 1. Light micrographs of *Anguilla anguilla* ovaries with Mallory staining. (A-D): control specimens. The ovary shows numerous fully vitellogenic oocytes (fvOo) and early vitellogenic oocytes (evOo), along with few previtellogenic oocytes (pvOo) and connective interfollicular cells (arrowheads). (E-H): Ovary tissue from animals exposed to cocaine, containing more connective tissue (arrows) and previtellogenic oocytes (pvOo) than the control specimens. (FC): follicle cells; (asterisk): theca cells. Scale bars: A, E=100 μm; B=50 μm; C, D, F, G, H=5 μm.

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Fig. 4: Light micrographs of *Anguilla anguilla* ovary specimens from control animals (A, B, C) and eels exposed to cocaine (D, E, F) immunolabeled with anti-3β-HSD antibody. (A, B, C) A wide and strong positive reaction (arrow) was visible at the level of the fully vitellogenic oocytes (fvOo) and early vitellogenic oocytes (evOo); a weak signal was evident in the follicular (FC) and theca cells (asterisk) as well as in the connective tissue (arrowheads). (D, E, F) Previtellogenic oocytes (pvOo) and early vitellogenic oocytes (evOo) were immunolabeled. In these cells, the immunohistochemical signal was located in the cytoplasm as spots (arrow). A weak signal was evident in the connective cells (arrowheads). No positivity was found in the follicular (FC) or theca (asterisk) cells. (F) (insert) Negative control: no labeling was evident in the ovary. Bars: A=20 μm; B, C, E, F=5 μm, D=50 μm, F inset=100 μm.

Fig. 5: Light micrographs of *Anguilla anguilla* ovaries. Control (A, B, C) and exposed specimens (D, E, F) immunolabeled with anti-17β-HSD antibody (A, B, C). A wide and strong positive reaction was present in the fully vitellogenic oocytes (fvOo) and early vitellogenic oocytes (evOo); a weak signal was evident in the follicular (FC) and theca (asterisk) cells as well as in the connective cells (arrowheads). (D, E, F) Early vitellogenic oocytes (evOo) were immunolabeled and the signal was located in some places of the cytoplasm as spots (arrow); meanwhile, weak positivity (arrow) was localized within previtellogenic oocytes (pvOo), follicular (FC), and connective cells (arrowheads). No signal was evident in the theca cells (asterisk). (E) (inset) Negative control: no labeling was evident in the ovary. Bars: A=20 μm; B, C, E, F, G=5 μm, D=50 μm, E inset=100 μm.

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Fig.7. Values presented as mean±SD for serum cortisol, FSH, and LH levels in *Anguilla anguilla* specimens exposed to cocaine and control animals. Note that cortisol levels were lower in the exposed eels than in the controls. In contrast, FSH and LH concentrations were higher in the control specimens. (*) Significantly different ($p<0.05$) than the control values.

Resumo

Os esgotos urbanos representam uma importante fonte de poluição em ecossistemas marinhos, devido, principalmente, ao aumento populacional em regiões metropolitanas costeiras e à ineficiência dos processos de coleta, tratamento e disposição de efluentes domésticos. Entre as principais substâncias que podem ser encontradas nos efluentes estão os produtos farmacêuticos, de cuidados pessoais e drogas ilícitas, tais como a cocaína, que é considerada como um sério problema de saúde pública. Os emissários submarinos representam o principal mecanismo de liberação de contaminantes de preocupação emergente (como as drogas ilícitas) no ambiente marinho. Estudos anteriores identificaram a presença de cocaína (COC) em água superficial continental e costeira, bem como efeitos biológicos em concentrações ambientalmente relevantes. Dessa forma, o presente trabalho tem como objetivo a realização de uma avaliação do risco ambiental de cocaína em ambientes costeiros através de uma metodologia escalonada que contemple a quantificação em matrizes ambientais na Baía de Santos (água superficial, sedimento e mexilhões) e ensaios ecotoxicológicos empregando como modelos o mexilhão *Perna perna* e o peixe *Anguilla anguilla*. Os mexilhões foram expostos a duas diferentes concentrações de cocaína (200 $ng.l^{-1}$ e 2000 $ng.l^{-1}$), a um controle de água do mar eum controle de solvente, por 168h. Ao todo, foram 21 mexilhões divididos em 2 aquários por tratamento. Após os períodos de exposição (48h, 96h e 168h), 7 mexilhões eram aleatoriamenteretirados dos aquários e submetidos à avaliação de citotoxicidade (pelo ensaio do Tempo de Retenção do Vermelho Neutro) e posteriormente eram excisados para retirada de tecidos (músculo adutor, glândula digestiva, brânquia e gônadas) para análise de biomarcadores (dopamina- DOPA; 5-hydroxytryptamine- 5-HT – serotonina; acetilcholinesterase- AChE; monoamina oxidase- MAO; ciclooxigenase- COX; transporte mitocrondrial de elétrons- MET; lipidios totais- TLP; 7-ethoxiresorufin O-deetilase – EROD; dibenzilfluoresceina dealkilase – DBF; glutationa S-transferase – GST; glutationa peroxidase – GPX; DNA danos em DNA; e peroxidação lipídica – LPO). A capacidade da cocaína bioacumular nos mexilhões também foi verificada. Além disso, peixes (*Anguilla anguilla*) também foram expostos à cocaína (20 $ng.l^{-1}$) para investigar potenciais danos histopatológicos, imunohistoquímicos (3β-hidroxisteroide dehidrogenase- 3β-HSD; 17β- hidroxisteroid dehidrogenase- 17β-HSD type 3; e P450 aromatase) e endócrinos (hormonônio folículo estimulante- FHS; hormônio luteinizante- LH and cortisol) da cocaína em vertebrados.Os resultados demonstram que a cocaína foi encontrada na Baía de Santos em concentrações variando de 1.91 – 203.6 $ng.l^{-1}$, sendo as maiores concentrações detectadas na primavera/verão, período que coincide com o aumento populacional na Baixada Santista devido ao início da alta temporada. No sedimento, as concentrações quantificadas variaram de 0.94 – 46.85 $ng.g^{-1}$. A cocaína também foi detectada no tecido de mexilhões coletados na Baía em concentrações variando de 0.914-4.58 $\mu g \cdot kg^{-1}$ (peso úmido). O fator de bioacumulação calculado variou de 163 – 1454 $l \cdot kg^{-1}$. Quanto às vias metabólicas investigadas, foram observadas alterações nas atividades de EROD e DBF, além de GST e GPx, indicando comprometimento na metabolização e resposta antioxidante. A exposição à cocaína provocou citotoxicidade nos mexilhões, que foi observada pela reduzida estabilidade da membrana lisossomica dos hemócitos, além do aumento significativo de peroxidação lipídica e danos em DNA. A cocaína aumentou os níveis de neurotransmissores (dopamine e serotonina) nos mexilhões em todas as concentrações testadas. Já após 168h foi observada uma redução nos níveis de AChE e COX. Além disso, foi observada um aumento nos níveis de MET e TLP em ambas as concentrações. Os peixes expostos à cocaína apresentaram um atraso no desenvolvimento e maturação dos folículos ovarianos, fato que pode comprometer a reprodução desses organismos. Isso também foi demonstrado pela menor expressão de 3β-HSD, e P450 aromatase, bem como menores níveis de FHS e LH. Os resultados indicam que concentrações ambientalmente relevantes de cocaína, mesmo na ordem de $ng.l^{-1}$ são capazes de afetar negativamente a saúde dos animais marinhos a partir de perturbações no sistema reprodutivo e citogenotoxicidade. A avaliação de risco realizada apontou para quocientes situados entre os níveis moderado e alto para Baía de Santos. É preciso melhorar a estrutura de coleta e tratamento de esgoto em regiões costeiras a fim de minimizar o descarte de efluentes contaminados com drogas ilícitas em ambientes marinhos. Além disso, é importante que essas substâncias sejam incluídas em programas de monitoramento da qualidade de água, a fim de se estabelecerem parâmetros de segurança que permitam a preservação dos recursos naturais e da saúde humana.

Palavras-chave: Cocaína, ecossistemas marinhos, concentrações ambientais, mexilhões *Perna perna*, enguia europeia, biomarcadores.

Abstract

Urban sewage represents an important source of pollution to marine ecosystems, mainly due to the population growth on coastal metropolitan regions and the inefficiency of the processes of collection, treatment and disposal of domestic effluents. Pharmaceutical, persoanl care products and illicit drugs, such as cocaine, constitute some of the main substances found in domestic effluents representing serious problem to the public health. Submarine sewage outfalls represent the main source of contaminants of emerging concern (such as illicit drugs) into the marine environment. Previous studies have identified the presence of cocaine (COC) in inland and coastal surface water, as well as biological effects at environmentally relevant concentrations. The present work aimed to assess of the environmental risk of cocaine in coastal environments through a tiered approach that included the COC (and by products) quantification in environmental matrices of Santos Bay (surface water, sediment and mussels) and ecotoxicological tests using as models the mussel *Perna perna* and the fish *Anguilla anguilla*. Mussels were exposed to two different concentrations of cocaine (200 $ng.l^{-1}$ and 2000 $ng.l^{-1}$), a seawater control and a solvent control, for 168h. The mussels (21 specimens) were divided into 2 aquariums per treatment. After the exposure periods (48h, 96h and 168h), 7 mussels were randomly removed from the aquariums and submitted to cytotoxicity evaluation (by the Neutral Red Retention Time assay) and later they were excised for tissue removal (adductor muscle, digestive tract, gills and gonads) for the analysis of biomarkers (dopamine-DOPA; 5-hydroxytryptamine- 5-HT - serotonin; acetylcholinesterase-AChE; monoamine oxidase-MAO; cyclooxygenase-COX; mitochondrial electron transport-MET; total lipids- TLP; 7-ethoxyresorufin O-deethylase – EROD; dibenzylfluorescein dealkylase – DBF; glutathione S-transferase – GST; glutathione peroxidase – GPX; DNA damage to DNA; and lipid peroxidation – LPO). The bioaccumulation of COC in mussels soft tissues was also verified. In addition, fish (*Anguilla anguilla*) were also exposed to cocaine (20 $ng.l^{-1}$) to investigate potential histopathological, immunohistochemical (3β-hydroxysteroid dehydrogenase-3β-HSD; 17β-hydroxysteroid dehydrogenase-17β-HSD type 3; and P450 aromatase) and endocrine (follicle stimulating hormone-FHS); luteinizing hormone- LH and cortisol) damages caused by cocaine in marine vertebrates. Cocaine was found in Santos Bay at concentrations ranging from 1.9 – 203.6 $ng.l^{-1}$, with the highest concentrations detected in spring/summer, a period that coincides with the population increase in Baixada Santista due to the beginning of the warmer high season. In the sediment, the quantified concentrations ranged from 0.94 – 46.85 $ng.g^{-1}$.

Cocaine was also detected in the soft tissue of mussels collected in the Bay at concentrations ranging from 0.914-4.58 $\mu g \cdot kg^{-1}$ (wet weight). The calculated bioaccumulation factor ranged from 163 – 1454 $l \cdot kg^{-1}$. As for the metabolic pathways investigated, changes were observed in the activities of EROD and DBF, in addition to GST and GPx, indicating impairment in metabolismand antioxidant response. Cocaine exposure caused cytotoxicity in mussels, which was observed by the reduced stability of the lysosomal membrane of hemocytes, and the significant increase in lipid peroxidation and DNA damage. Cocaine increased the levels of neurotransmitters (dopamine and serotonin) in mussels at all concentrations tested. After 168h, a reduction in AChE and COX levels was observed. In addition, an increase in MET and TLP levels was observed at both concentrations. Fish exposed to cocaine showed a delay in the development and maturation of ovarian follicles, a fact that can compromise the reproduction of these organisms. This was also demonstrated by lower expression of 3β-HSD, and P450 aromatase, as well as lower levels of FHS and LH. The results indicated that environmentally relevant concentrations of cocaine, even in the order of $ng.l^{-1}$, are capable of negatively affecting the health of marine animals through disturbances in their reproductive system and cytogenotoxicity. The risk assessment carried out pointed to moderate to high risks in Santos Bay.It is necessary to improve the structure of sewage collection and treatment in coastal regions in order to minimize the disposal of effluents contaminated with illicit drugs in marine environments. Furthermore, it is important that these substances are included in water quality monitoring programs, in order to establish safety parameters that allow the preservation of natural resources and human health.

Keywords: Cocaine, marine ecosystems, environmental concentrations, *Perna perna*, European eels, biomarkers

General introduction, aims and structure of the thesis

1. Brazilian Sanitation

The basic sanitation can be defined as a set of services that aims to provide potable water supply, sewage collection and treatment, urban and cleaning services, solid waste management and urban rainwater drainage and management (Campos, 2017). The access to improved water and sanitation are recognized by the United Nations as human rights (UN, 2020) and are essentials to guarantee the environment quality, human health, and economic development (Kresh and Schneider, 2020). The supply of water in adequate quantity and quality is of extreme importance for the prevention of diseases, reduction of infant mortality and helps in the expansion of tourism and real estate appreciation mainly in areas with high population density (Alemu, 2017; Choumert et al. 2015; Yindong et al. 2017; Shidhar & Adejumo, 2020; Ali et al. 2018; O'Gorman, 2020; Ferreira et al. 2021). Furthermore, sanitation is also related to environmental protection, depollution of rivers and preservation of water resources (Trata Brasil, 2021).

In 1971 the "Banco Nacional da Habitação"launched the Water and Sewage National Plan (PLANASA) aiming to increase geographical coverage in urban centers, allocatingresources for municipalities to create their own sanitation companies (Barbosa & Brusca, 2015).In 1992, with the dissolution of Planasa, the Federal Government made new investments through the creation of programs such as Sanitation for Urban Centers, Pro-Sanitation and the Social Action Program in Sanitation, defined the universalization of sanitation services (Ramos et al. 2020).

In 2007 the Basic Sanitation Law N. 11,445 was enacted and defined the principles of national operation and established the general bases and guidelines for a State policy to be developed such as universalization of access, as well as the prevention the abuse of economic power by the definition of tariffs to ensure the economic-financial stability of the contracts and general framework for each state to carry out its specific implementation strategy (UN, 2018). Newly, the Federal Senate approved the Basic Sanitation Legal Framework (Bill Law N. 4,162/2019) with the aim of universalizing of sanitation and water supply ("Services") in Brazil until the end of 2033, changing the way such Services are provided in Brazil and its regulation, to open the sector to private initiative (Sampaio and Sampaio, 2020).

Brazil experienced an early urban transition compared to other developing countries of Asia and Africa, however it occurred unevenly between the different regions of the country (Martine and McGranahan, 2012). Due to its vast territory population, Brazil presents regional diversities which are reflected in the access to basic sanitation (von Sperling, 2016). The North and Northeast regions of the country had the lowest coverage (27.4% and 47.2%, respectively), while the highest coverage is observed in the southeastern region (88.9%); followed by the South and Midwest regions (68.7%) (Gomes, et al. 2020).

According to Brazilian Institute of Geography and Statistic, about 9 million households (12.6%) had a rudimentary cesspit, a ditch, in addition to other forms of waste disposal, being these conditions prevalent in the North region (29.6% of households- 1.6 million), exceeding the estimated 27.4% of households connected to the general network (IBGE, 2019). Regarding treatment, the Midwest has a treatment rate of 52.62% against only 18.3% in the North. In terms of national average, only 10 of the 100 largest Brazilian cities are able to treat over 80% of sewage and only 44.92% of all sewage in the country receives treatment (SNIS, 2021).

Coastal zonas are areas densely populated and exhibit higher rates of population growth and urbanization, that produced several economic benefits such as marine trade and transport, industrial and urban development, tourism and food production (Neumann et al. 2015). During holidays, the population in coastal areas may duplicate due to an increase in the number of tourists, overloading the water supply as well as sewage collection and treatment (Chili et al. 2017). However, the booming population growth on coastal areas occurs faster than the increase in supply of sanitation facilities, promoting the continuous disposal of contaminants that affect water quality, coastal biodiversity and consequently, human health (Feitosa et al. 2017).

2.Marine ecosystems

The oceans cover more than 70% of the Earth's surface and contain more than 90% of the living species, being deeply related to the evolution and development of humanity, because oceans provide food for billions of people worldwide, play an important role in the renewable energy (wind, wave, currents and tides) and have large oil and gas reserves (Sevilla & Le Bail, 2017). In addition to the ecological importance, the ocean asset value is estimated in US$24,4 trillion/year and are responsible for two thirds of gross primary marine productivity, that would make the ocean the world's 7th largest economy (Hoegh-Guldberg *et al*. 2010). It is also estimated that the oceans produce about 50-80% of the oxygen production on Earth and captures

2.4 Gt carbon dioxide per year. Also, the ocean plays an important role in climate regulation (Rackley, 2009; NOAA, 2022a; 2022b).

Brazil has one of the largest coastal areas in the world (7, 491 km), with a total area covering about 3.5 million km^2 with coastal zones extraordinarily diverse, composed of subtropical to temperate waters (South andSoutheast regions) and warm waters (North and Northeast regions), supporting a wide variety of marine ecosystems that include mangroves, coral reefs, dunes, sandbanks, sandy beaches, rocky shores, lagoons and estuaries (MMA, 2010; Obraczka *et al*. 2017). Seas and oceans standout for hosting unique ecosystems and rich in biodiversity and have almost twice the number of animal phyla compared to terrestrial ecosystems, such as cnidarians, crustaceans, fish, birds,reptiles and mammals, many of which are endemic and endangered (Jefferson et al. 2021).

Marine ecosystems provide some goods (fish harvests, wild plant and animal resources, genetic material) and services (breeding and nursery habitats, shoreline stabilization anderosion control, carbon sequestration, biogeochemical cycling, flow energy and others) (Barbier*et al*. 2017). Despite their importance, marine ecosystems are threatened by several human activities directly related to population growth, urban and industrial, exploratory fish, climate change, habitat loss, nutrient enrichment, introduction of exotic species and pollution (Partelow*et al*. 2015; Santos & Schiavetti, 2014; Wang *et al*. 2021).

3.Marine pollution

The United Convention on the Law of the Sea defines pollution of the marine environment as the introduction by humans, directly or indirectly, of substances or energy into marine environment, resulting in deleterious effects to the living resources, quality water, marine activities, and hazards to human health (UN, 1982). Then, pollutants are defined as anthropogenically introduced substances that is present in concentrations that may harm several organisms or exceed an environment quality standard (OECD, 2005). Contaminants can be defined as substances (chemical elements or compounds) potentially toxic, persistent, and liable to bioaccumulate (Tornero & d'Alcalà, 2014).

According to Chapman (2007), contamination is the presence of a substance where it should not be or in concentrations above background and pollution is contamination that provokes or may provoke adverse biological effects. So, all pollutants are contaminants, but not all contaminants are pollutants. The distinction between pollutant and contaminant is not always clear, as the concentrations at which contaminants become pollutants cannot always be defined and some negative effects are only observed in the long-term exposure (Stengel *et al*. 2006).

For a long time, the oceans were considered as a great reservoir for the safe elimination of contaminants, due to its high dilution and rapid dispersal capacity of contaminants (Feitosa *et al*. 2017). However, despite efforts to reduce the release of pollutants from anthropogenic sources into the environment, direct and indirect discharge of waste into coastal waters is a major environmental problem (Priya et al. 2021).

The marine environment is constantly threatened by various contaminants from various sources commonly found in the surrounding area (Kumar & Prasannamedha, 2021). Oil spills, nutrient enrichment, plastic waste, metals, pharmaceuticals, personal care products and even illicit drugs are recognized as the main contaminants that threat to the marine ecosystem (Vikas & Dwarakish, 2015; Pereira *et al*. 2016; Fontes *et al*. 2017; Thushari & Senevirathna, 2020).

Domestic sewage is a major contamination vector for marine environment (Araújo *et al*. 2021). The dumping of sewage untreated or without primary treatment is a great concern because the effluents may carry several substances with potential contaminant (Roth *et al*. 2016). In Brazil, only 60.2% of the urban areas have sewage collection and 73.3% of these have adequate treatment (Brazil, 2019). In coastal areas the Sewage Submarine Outfalls (SSO) are proposed as an efficient alternative for the final destination of wastewater in densely populated coastal, mainly due to economic aspects (low cost) and high dispersal capacity compared with other forms of treatment (Ortiz et al. 2016; Birocchi *et al*. 2021).

The SSO is constituted from a pipeline or tunnel, or combination of the two, that collect the effluents produced in coastal cities for a final discharge in the ocean, considering the encompass population, geographic, hydraulic, oceanographic and microbiologic aspects (Feitosa et al. 2017). However, the preliminary or primary treatments applied to the effluents before being sent to the outfall is insufficient to treat or remove some contaminants, such as drugs that are continuously discharge into the environment, representing a potential risk to the marine ecosystem (Pereira *et al*. 2016).

4. Emerging contaminants

According to Environmental Protec Agency (EPA), an emerging contaminant (EC) is a chemical or material characterized by a perceived, potential, or real threat to human health or the environment or by a lack of published health standards (EPA, 2021). The ECs are natural or anthropogenic substances including pharmaceuticals, personal care products, endocrine disruption compounds, flame retardants andillicit drugs (Maranho *et al.* 2015; Pereira *et al*.

2016; Fontes *et al*. 2017; Kasonga *et al*. 2021).The term "emergent" does not necessarily refer to substances that have recently emerged, but to compounds that were synthesized or discovered a long time ago, but only recently recognizedas potentially dangerous (Valbonesi *et al*. 2021). The ECs are currently not included in monitoring programs, but are candidates for future regulations depending on researching of potential health effects, ecotoxicity and data regarding monitoring and occurrence in the environment (Barcelò et al. 2005; Yadav *et al*. 2021).

The presence of ECs in the aquatic environment was first observed in 1970, but the studies have intensified since the 90s due to the development of new analytical methodologies that allowed the detection of these substances at very low concentrations (Vélez- et al. 2019). The ECs have a wide variety of chemical structure, mode of action and toxicity to the environment and human health (Raies and Bajic, 2016). Over the past 20 years, the global ECs presence in surface waters, wastewater effluents, groundwater and drinking water has been well documented (Luo *et al*. 2014).

ECs occur in several environments matrices in concentrations ranging from $ng.l^{-1}$ to $mg.l^{-1}$ and are continuously discharged into the environment due to inefficient treatment processes of WWTPs (Castiglioni et al. 2018). However, even in low concentrations ECs can affect the ecosystems and human health, causing bacterial resistance, reproductive abnormalities, oxidative stress and carcinogenic effects (Martini et al. 2021). Although there are several studies in the literature regarding the effects of pharmaceuticals and personal care products in freshwater environments, data on the occurrence and effects of illicit drugs in the aquatic environment are still scarce, especially in marine ecosystems.

According UNODC (2020), the number of drug users around the world was estimated at 269 million people in 2018: 192 million (cannabis), 58 million (opioids), and the use drugs is 27 million (amphetamines and prescription stimulants), 21 million (ecstasy) and 19 million (cocaine) users. The drug use is more widespread in developed countries than in developing countries (UNODC, 2020). Among the drugs mentioned, cocaine (COC) is considered one of the most potent and addictive illicit drug. In mammals, COC enhances monoamine neurotransmitter (dopamine,norepinephrine, and serotonin) activity in the central and peripheral nervous systems, blockingits reuptake into the nerve terminal (Volkow et al. 2000). Hence, cocaine addiction is recognized as the brain's dopamine reward system. Cocaine can also block sodium ion channelsin the plasma membrane, which causes a local anesthetic effect and can contribute to cardiac arrhythmias (Cubo, 2014).

Illicit drugs are highly bioactive substances, able to interact with cellular receptors, even at low concentrations, therefore, the occurrence of these substances in aquatic ecosystems is a concern as they can cause adverse effects on non-target organisms (Baker and Kaspzryk-Horder, 2013; Capaldo et al. 2019).

COC has been frequently detected in the aquatic environment (Sulej- Suchomska *et al*. 2020). Several studies have been reporting that COC and its metabolites (Benzoilecgonine-BE; Ecgonine metil ester- EME) affect freshwater organisms, causing oxidative stress, cytotoxicity, behavior changes, morphological damage (Binelli *et al*. 2013; Capaldo *et al*. 2018; De Felice and Parolini, 2019). However, ecotoxicological studies regarding effects of illicit drugs on marine organisms are still limited.

5 Aims and structure of the thesis

With the background presented by this introduction, we can hypothesize that COC and its metabolites are present in São Paulo coastal zone (Santos Bay) in concentrations able to bioaccumulate and affect marine organisms.

In this context, the main objective of the thesis is to assess the occurrence of cocaine in environmental matrices of Santos Bay as well as its biological effects leading to environmental risk. The specific objectives are:

1. To quantify the concentrations of COC and BE around the Santossubmarine sewage outfall during the four seasons of the year;
2. To quantify the concentrations of COC and BE in surface water,sediment and mussels of the Santos Bay;
3. To determine the Field-measured Bioaccumulation Factor in *P. perna* mussels from Santos Bay;
4. To elucidate metabolic pathways involved in biotransformation, conjugation and excretion of cocaine in *Perna perna*
5. To assess sublethal effects related to oxidative stress and cytogenotoxicity in mussels exposed to COC (*in vivo*)
6. To assess disturbances on neurotransmitter and energy balance in mussels exposed to COC (*in vivo*)
7. To assess histopathological effects in fishes (*Anguilla anguilla*) exposed to COC (*in vivo*)
8. To perform a preliminary environmental risk of cocaine based on the adaptation of protocolsalready established for drugs;

9. To provide data for the inclusion of cocaine in water quality monitoring programs

To achieve what was proposed, this thesis was structured in five chapters:

Chapter 1 presents a review of the main groups of illicit drugs found in the aquatic ecosystem (opioids, cannabinoids, amphetamines and cocaine) and theirs effects on biota.

Chapter 2 presents the contamination of cocaine and benzoylecgonine in a subtropical coastal zone (Santos Bay), considering a seasonal monitoring (Article 1) and different environmental matrices (seawater, sediment and mussel *Perna perna*) collected from Santos Bay (Article 2)**.**

Chapter 3 provides the assessment of cocaine`s effects at environmentally realistic concentration on mussels *Perna perna* and fishes *Anguilla Anguilla.* Disturbances on neurotransmitters and energy balance in mussels exposed to COC (*in vivo*) are presented in Article 1. Metabolic pathways involved in biotransformation, conjugation and excretion of cocaine in *Perna perna*, as such as sublethal effects are assessed in mussels exposed to COC (*Article 2*), whereas histopathological effects in fishes (*Anguilla anguilla*) exposed to COC are shown in Article 3.

Chapter 4 presents an environmental risk assessment of COC in marine ecosystems**.**

Chapter 5 brings the conclusions and suggestions for future research.

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