

Universidade Federal do Rio Grande – FURG

Instituto de Oceanografia

Programa de Pós-Graduação em Oceanologia

**RELEVÂNCIA DA CONTAMINAÇÃO POR
BIOCIDAS ANTI-INCRUSTANTES NA COSTA
BRASILEIRA**

Fiamma Eugênia Lemos Abreu

Tese apresentada ao Programa
de Pós-Graduação em
Oceanologia, como parte dos
requisitos para a obtenção do
Título de Doutor.

Orientador: *Prof. Dr.* Gilberto Fillmann

Universidade Federal do Rio Grande (FURG), Brasil

Rio Grande, RS, Brasil

Dezembro 2020

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Abreu, Fiamma Eugênia Lemos Abreu

Relevância da contaminação por biocidas anti-incrustantes na costa brasileira. Fiamma E L Abreu – Rio Grande: FURG, 2020.

178 p.

Tese (Doutorado) – Universidade Federal do Rio Grande. Doutorado em Oceanologia.

Área de Concentração: Biogeoquímica, Poluição e Ecossistemas Marinhos.

1. Biocidas anti-incrustantes. 2. Sedimento. 3. Avaliação de Risco.



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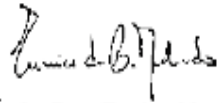


ATA ESPECIAL DE DEFESA DE TESE DE DOUTORADO – 10/2020

Às 14h do dia 17 de dezembro do ano de dois mil e vinte, por videoconferência, reuniu-se a Comissão Examinadora da Tese de **DOUTORADO** intitulada "**Relevância da contaminação por biocidas anti-incrustantes na Costa Brasileira**", da **Acad. Fiamma Eugênia Lemos Abreu**. A Comissão Examinadora foi composta pelos seguintes membros: Prof. Dr. Gilberto Fillmann – Orientador – IO/FURG, Profa. Dra. Grasiela Lopes L. Pinho – IO/FURG, Profa. Dra. Eunice Machado – IO/FURG, Profa. Dra. Samantha E. Martins. – NIVA/Noruega e Prof. Dr. Carlos Miguez Barroso – Univ. de Aveiro/Portugal. Dando início à reunião, o Orientador e Presidente da sessão, Prof. Dr. Gilberto Fillmann, agradeceu a presença de todos e fez a apresentação da Comissão Examinadora. Logo após esclareceu que a Candidata teria um tempo de 45 a 60 min para explanação do tema, e cada membro da Comissão Examinadora, um tempo máximo de 30 min para perguntas. A seguir, passou à palavra a Candidata que apresentou o tema e respondeu às perguntas formuladas. Após ampla explanação, a Comissão Examinadora reuniu-se em reservado para discussão do conceito a ser atribuído a Candidata. Foi estabelecido que as sugestões de todos os membros da Comissão Examinadora, que seguem em pareceres em anexo, foram aceitas pelo Orientador/Candidata para incorporação na versão final da Tese. Finalmente, a Comissão Examinadora considerou a candidata APROVADA, por unanimidade. Nada mais havendo a tratar, foi lavrada a presente ATA que após lida e aprovada, será assinada pela Comissão Examinadora, pela Candidata e pelo Coordenador do Programa de Pós-Graduação em Oceanologia.


Prof. Dr. Gilberto Fillmann
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Prof. Dr. Carlos Miguez Barroso


Prof. Dr. Rodrigo Kerr
Coordenador PPGO


Acad. Fiamma Eugênia Lemos Abreu

Aos meus pais, Felipe e Auxiliadora, por serem
o meu porto de segurança e de felicidade.

*De novo andar: as distâncias,
as cores, posse das ruas.
Tudo que à noite perdemos
se nos confia outra vez.
Obrigado, coisas fiéis!
Saber que ainda há florestas,
sinos, palavras; que a terra
prosegue seu giro, e o tempo
não murchou; não nos diluímos.
Chupar o gosto do dia!
Clara manhã, obrigado,
o essencial é viver!
**Passagem da noite –
Carlos Drummond de Andrade***

Trecho de um poema como exemplo e lembrança da bravura do povo brasileiro.

Agradecimentos

À Deus por ser sempre me dá esperanças e força para vencer cada obstáculo, mas principalmente por cuidar daqueles que amo.

Ao Prof. Dr. Gilberto Fillmann, por cada explicação, por ensinar muito mais do que escrever um artigo, por cada puxão de orelha e por cada voto de confiança ao longo da minha formação. Obrigada por tudo!

Ao Prof Dr. Italo Castro, por transmitir sua paixão e empolgação pelos anti-incrustantes. Te agradeço pela companhia nas coletas, nos congressos e por está nessa caminhada desde minha graduação. Você sempre será uma inspiração.

Ao Prof. Dr. Carlos Miguez, por ter me aceitado no estágio doutoral na Universidade de Aveiro, Portugal, e que embora tenhamos o inconveniente de uma pandemia, não deixou de me acolher, ensinar e confiar no meu trabalho. Muito Obrigada.

À Dra. Samantha Martins por ter me apresentando o maravilhoso e novo mundo da Avaliação de Risco. Agradeço por todos os treinamentos, aulas e por cada conhecimento compartilhado. Muito Obrigada!

À Prof. Dra. Grasiela Pinho pelas valiosas sugestões e apontamentos desde a banca de acompanhamento e por aceitar continuar a jornada na banca de avaliação da Tese.

À Prof. Dra. Eunice Machado por sempre ter me acompanhado nos Seminários e sempre contribuir com uma nova visão do trabalho. Obrigada por também continuar a jornada na minha banca de avaliação da Tese.

À todos pesquisadores da Rede Nacional de Estudos em Anti-incrustantes que estiveram dispostos a coletar amostras e esclarecer todas as dúvidas ao longo desse quatro anos. Obrigada por toda disponibilidade.

À Dra. Sanye Soroldoni que tanto me escutou sobre as eternas dúvidas de partículas de tinta e sobre as incertezas da vida. Eu lhe agradeço por me escutar quando dentro de mim estava um caos de sentimentos. Obrigada por sua amizade de sempre.

Ao MSc. Rodrigo Moço, por sempre está disposto à me acalmar quando o cromatógrafo criava vida própria mas também por sempre está disposto a escutar meus lamentos diários. Obrigada por me ajudar a apertar parafusos e a enfrentar medos da vida acadêmica. Muito Obrigada pela construção dessa amizade.

Ao Dr. Amaro Azevedo por tanta ajuda logística no laboratório, mas principalmente pela companhia durante o horário extra, sábado, domingos e feriados que passamos no laboratório no meio de inúmeras análises. Obrigada por toda amizade.

Ao alunos de Iniciação Científica, Pablo Teófilo e Eduarda Garcia, por me acompanharem nas loucuras das análises infinitas. Obrigada por toda ajuda em qualquer momento que eu precisasse e por nunca reclamarem do quanto eu explorei vocês. Vocês foram mais do que mãos e pernas extras. Muito Obrigada!

Aos companheiros de laboratório CONECO, Carol, Cesar, Daiana, Ivna, Jerusa, Juliane, Karina, Lara, Lenon, Nilson, Russell e Vanessa que compartilharam momentos deliciosos na mesinha do café assim como compartilharam diversos momentos felizes.

Em meio ao caos de uma pandemia global devido à um vírus desconhecido e as inseguranças de viver em um país no exterior, encontrei duas pessoas que trouxeram calma na mais recente jornada da minha vida: Joana Fonseca e Alexandra Lobão. Muito obrigada por terem sido as melhores companheiras de casa (ainda acho que deviam abrir uma padaria)! À Joana devo um agradecimento especial por ter me mostrado o melhor de Aveiro (principalmente de bike) e por ter me acolhido nesse coração tão grande.

Agradeço à Andrea Franco, Camila Máximo, Joseane Marques, Maria Isabel e Willton Aguiar pelas experiências vividas em Rio Grande e por me oferecerem o conforto de uma amizade nesses últimos anos. Espero sempre encontrá-los ao longo da vida.

Às minhas amigas de longa data que estão sempre juntas de mim, não importando a distância. Crislane, Jessica B., Jessika T., Yris vocês tornam a vida numa completa aventura!

Ao Felipe Santos por me mostrar que era possível cuidar de mim sem desistir dos meus sonhos. Você aguentou choros, inseguranças, pesadelos e permitiu que pudéssemos crescer juntos. Passamos por diversos obstáculos da vida e descobrimos que podemos ir muito mais além do que já sonhamos. Obrigada pela parceria de vida!

E aos meus pais, Felipe e Auxiliadora, o meu mais profundo e carinhoso agradecimento. Eles permitiram que eu voasse, que eu escolhesse o caminho da minha vida e que independente de onde fosse, eles sempre estariam comigo. Eles são minha inspiração de vida e à eles eu quero dar o mundo!

Aos órgãos de fomento CAPES pela bolsa de estudo, pelo formento ao projeto Ciências do Mar (CIMAR II) e à FINEP pelo financiamento do projeto (AIBRASIL II).

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Lista de Acrônimos e Abreviações

A – B – C

AFS – Sigla da Convenção Internacional sobre o Controle de Sistemas Anti-incrustantes Nocivos em Navios (International Convention on the Control of Harmful Anti-fouling Systems in Ships)

BTs- Butilestânicos

CENO - Concentração de Efeito Não Observado (NOEC)

CG-DCE – Cromatografia gasosa com detector por captura de elétrons (GC-ECD)

CL-EM/EM - Cromatografia de fase líquida com detector de espectrometria de massas em tandem (LC-MS/MS)

COEs – Compostos Organoestânicos

CONECO - Laboratório de Microcontaminantes Orgânicos e Ecotoxicologia Aquática

COT - Carbono Orgânico Total (TOC)

D – E

DBT - Dibutilestano

DCOIT - 4,5-Dicloro-2-n-octil-4-isotiazolin-3-ona

ECHA - European Chemicals Agency

EFSA - European Food Safety Authority

ELP - Estuário da Lagoa dos Patos

EPA - United States Environmental Protection Agency

ETOX - Database for Ecotoxicological Effect Data and

Quality Targets (Base de dados Alemã)

I - K - L - M

IDB – Índice de Degradação dos Butilestânicos (DBI)

K_{oc} - Coeficiente de partição entre a água e o carbono orgânico

K_{ow} - Coeficiente de partição entre a água e o octanol

LANUV- Conservation and Consumer Protection of the State of North Rhine-Westphalia

LD - Limite de detecção do método

LQ - Limite de quantificação do método

MBT – Monobutilestano

MEC – Concentração Ambiental Medida

N - O - P - Q

NITE - National Institute of Technology and Evaluation

NOEL - Nível de Efeito Não Observado (No Observed Effect Level)

NORMAN - Network of reference laboratories, research centres and related organisations for monitoring of emerging environmental substances

OMI – Organização Marítima Internacional (IMO)

PAN - Pesticide Action Network

PNEC – Concentração Sem Efeito Previsível (Predicted No Effect Concentration)

PNEC_a – PNEC na matriz água (PNEC_w)

PNEC_{sed} – PNEC na matriz sedimento (PNEC_{sed})

PPDB - Pesticide Properties Database

PTA – Partícula de Tinta Anti-Incrustante (APP)

QR – Quociente de Risco (RQ)

S – T

SESS - Sistema Estuarino de Santos e São Vicente (SSES)

EFS – Extração em fase sólida (SPE)

TBT - Tributilestanho

TGD - Documento de Orientação Técnica (Technical Guidance Document)

TPrT - Tripropilestanho

TPT – Trifenilestanho

Resumo

A fim de conhecer os níveis atuais da contaminação por biocidas anti-incrustantes de 2ª geração e preencher lacunas sobre o possível impacto dos biocidas de reforço de tintas anti-incrustantes, a presente Tese teve como objetivo geral avaliar a efetiva relevância ambiental dos principais biocidas anti-incrustantes nas zonas costeiras do Brasil. Análises de butilestânicos (tributilestanho, dibutilestanho, monobutilestanho), biocidas de reforço (diuron, Irgarol, clorotalonil, diclofluanida, DCOIT) e partículas de tintas anti-incrustantes (PTAs) foram analisadas em sedimentos coletados ao longo de todo Brasil. Embora, a contaminação tenha sido relacionada diretamente com as atividades marítimas locais em um estuário de São Paulo (butilestânicos próximos à áreas de tráfego e estaleiros de barcos de pesca enquanto biocidas de reforço foram mais evidentes em áreas de barcos de lazer), o mesmo não foi observado no restante das áreas brasileiras. De forma geral, os butilestânicos apresentaram maiores concentrações em áreas de estaleiros do que áreas próximas à portos, marinas ou de zonas de tráfego. Adicionalmente, a contaminação desse grupo ocorreu preferencialmente pelos produtos de degradação. Dentre os biocidas de reforço, DCOIT foi amplamente detectado ao longo da costa do Brasil seguido pelo diuron sugerindo o uso atual de tintas anti-incrustantes compostas por esses biocidas. Possivelmente, pelas baixas concentrações encontradas e fontes difusas dos biocidas de reforço, não foi possível observar diferenças de contaminação entre as principais atividades marítimas. Além disso, os resultados mostraram um aporte secundário dos biocidas anti-incrustantes provenientes das PTAs. Por fim, a Avaliação de Risco Ecológico para os biocidas de reforços nos sedimentos de toda costa do Brasil mostrou que DCOIT, diuron, clorotalonil e diclofluanida representam alto risco para biota em pelo menos um local estudado. Assim, algumas regiões de São Paulo, Pernambuco e Santa Catarina devem ser melhor investigadas quanto aos possíveis impactos ambientais. Portanto, o presente trabalho fornece os primeiros subsídios para atualização e implementação de medidas regulatórias para uso dos biocidas anti-incrustantes e consequentemente proteção dos ecossistemas aquáticos costeiros.

Palavras chave: biocidas de reforço; butilestânicos; sedimento; Avaliação de Risco

Abstract

In order to assess the current levels of butyltins and to fill data gaps about likely impacts of booster biocides, the present Thesis had as the main objective evaluate the environmental relevance of the main antifouling biocides in coastal areas of Brazil. Analysis of butyltins (tributyltin, dibutyltin, monobutyltin), booster biocides (diuron, Irgarol, chlorothalonil, dichlofluanid DCOIT), and antifouling paint particles (APPs) were performed in sediments collected along to Brazilian coast. Although a contamination profile has been related directly to local maritime activities in an estuary on São Paulo (butyltins close to trafficking areas and fishing boat yards while booster biocides were evident in recreational boat areas), a pattern similar was not observed in the remaining Brazilian coastal areas. In general, butyltins showed higher concentrations in shipyard areas than areas close to ports, marinas, or traffic areas. Additionally, the contamination of this group takes place preferentially by degradation products. Among the booster biocides, DCOIT was widely detected along the coast of Brazil, followed by diuron, suggesting the current use of antifouling paints composed of these biocides. Possibly, due to the low concentration and diffuse sources of booster biocides, it was not possible to observe differences in contamination among the marine activities. In addition, the results showed a secondary source of antifouling biocides from APPs. Finally, the Ecological Risk Assessment for booster biocides in sediments in the Brazil coastal area showed that DCOIT, diuron, chlorothalonil and diclofluanid pose a high risk for biota in at least one studied site. Thus, some areas of São Paulo, Pernambuco, and Santa Catarina must be better investigated for possible environmental impacts. Therefore, the present work provides the first subsidies for updating and implementing regulatory measures for the use of antifouling biocides and, consequently, the protection of aquatic ecosystems.

Keywords: booster biocides; butyltins; sediments; Risk Assessment

Desde as civilizações mais antigas, o ser humano tem relação direta com o oceano para navegar em busca de novos territórios ou explorar recursos naturais para alimentação. Porém, simultaneamente, o homem sempre teve a bioincrustação com um dos principais obstáculos. Esse é um fenômeno onde há acumulação de micro e macro organismos (bactérias, algas, moluscos, crustáceos) em estruturas submersas (Yebra et al., 2004) e está relacionado com danos ecológicos aos ambientes aquáticos. A bioincrustação em navios comerciais, por exemplo, pode ocasionar o transporte de espécies exóticas que pode vir a desequilibrar os ecossistemas incluindo competição com organismos que são importantes fontes de alimento para o homem (Almeida et al., 2007). A bioincrustação também pode causar danos econômicos, principalmente pelo aumento no consumo de combustível (maior fricção da embarcação com a água) e deterioração dos revestimentos externos, o que aumenta a necessidade de manutenção e docagens (Schultz et al., 2011). Além disso, especialmente na época atual do Antropoceno, a prevenção da bioincrustação é essencial uma vez que esse fenômeno pode causar um aumento de até 40% do consumo de combustíveis fósseis pelas embarcações em todo o mundo (Buskens et al., 2013)

Essa prevenção é feita principalmente pelo uso de sistemas anti-incrustantes, tintas para revestimento superficial com biocidas de propriedades anti-incrustantes e polímeros de ligação que alteram a performance desse conjunto de materiais (Xie et al., 2019). Quanto aos biocidas utilizados nas tintas, diferentes compostos químicos foram usados ao longo da história. No século XIX, as embarcações eram revestidas com uma mistura de óleo e tintas com elementos metálicos como cobre, zinco e arsênio (tintas anti-incrustantes de 1ª geração). Porém, essa mistura foi abandonada devido à baixa durabilidade e risco à estrutura da embarcação devido a eventos de corrosão (Almeida et al., 2007). Embora o cobre continuasse a ser a base das versões seguintes de tintas anti-incrustantes devido a sua ampla toxicidade a organismos marinhos, foi apenas com o conhecimento das propriedades dos compostos organoestânicos (COEs) que a problemática das inscrustações teria sido moderada.

Alguns organometálicos já eram conhecidos por suas propriedades biocidas, mas a eficácia contra ostras, mariscos e crustáceos, aliada à descoberta também da tecnologia de tintas de autopolimento na década de 1970, tornaram o Tributilestanho-TBT e Trifenilestanho-TPT os princípios ativos de tintas anti-incrustantes (tintas anti-incrustantes de 2º geração) mais usados no mundo (Almeida et al., 2007). O TBT é um

composto lipofílico ($\log K_{ow} = 4,7$), com baixa solubilidade ($5 - 50 \text{ mg L}^{-1}$) e com elevada meia vida nos sedimentos (até 30 anos em sedimentos anóxicos) (Rüdel and Rudel, 2003). No entanto, a elevada toxicidade (efeitos subletais em concentrações a partir de 1 ng L^{-1}) e persistência ambiental, causou vários impactos à vida aquática desde moluscos a mamíferos marinhos (Tanabe, 1999). Além disso, quando degradado por ação de microorganismos ou por fotólise, o TBT gera dibutilestanho (DBT) e monobutilestanho (MBT) como produtos de degradação, que ainda apresentam toxicidade a organismos aquáticos (Fent, 1996). Assim, em 2001, a Organização Marítima Internacional (OMI) propôs, através da Convenção sobre Sistemas Anti-incrustantes, o banimento mundial do uso de organoestânicos em tintas anti-incrustantes em embarcações (maior que 400 de arqueação bruta e maior que 24 metros). Em 2003, quando assinado por 25% da frota comercial, novas pinturas à base de TBT em embarcações se tornaram proibidas. Porém, apenas em setembro de 2008, a convenção entrou efetivamente em vigor com assinatura de 26 países (Sonak et al., 2009). A partir de então, pinturas antigas ainda à base de organoestânicos deveriam ser removidas das embarcações. Atualmente, a Convenção conta com 89 países signatários (IMO, 2020).

Concomitante ao reconhecimento dos danos ecológicos do TBT ao ambiente, outros biocidas de menor impacto ambiental (baixa permanência ambiental e menor potencial de bioacumulação e biomagnificação) eram avaliados sobre o potencial anti-incrustante para uso nas tintas (Price and Readman, 2013). Associado principalmente ao óxido de cobre ou zinco, alguns biocidas orgânicos e organometálicos como diuron, Irgarol, clorotalonil, diclofluanida, 4,5-Dicloro-2-n-octil-4- isotiazolin- 3-ona (DCOIT), medetomidina, ziram, zinco piritona, e tolilfluanida são usados para potencializar o efeito do cobre e do zinco nas tintas anti-incrustantes (Thomas and Brooks, 2010; Tornero and Hanke, 2016). Esses co-biocidas ou biocidas de reforço (*booster biocides*, em inglês) são reconhecidos como a terceira geração de tintas anti-incrustantes (Fernandez and Pinheiro, 2007). São contaminantes pertencentes a diferentes grupos químicos (triazinas, isotiazolinona, ureia, entre outros) que configuram uma diversidade de propriedades físico-químicas nas formulações das tintas (Omae, 2003). Assim, além de um recente apontamento dos principais biocidas usados em tintas anti-incrustantes baseado em suas fichas técnicas disponíveis no mercado atual (Paz-Villarraga, submetido), diversas pesquisas têm identificado a ocorrência dos biocidas diuron, Irgarol, DCOIT, clorotalonil e diclofluanida em áreas de atividades pesqueiras, náuticas e portuárias por todo mundo (Batista-Andrade et al., 2018; Chen and Lam, 2017; Koning et al., 2020). O Brasil ganha destaque em áreas com tais atividades pelo tamanho da malha portuária, com mais de 1 bilhão de toneladas de cargas

movimentadas pelos portos brasileiros (ANTAQ, 2019), e o consequente impacto potencial do uso de tintas anti-incrustantes nos ecossistemas aquáticos. Nesse sentido, a criação da Rede Nacional de Estudos em Anti-incrustantes (RNEA) tem a finalidade de avaliar a contaminação, o comportamento e possíveis efeitos do biocidas anti-incrustantes (presentes em tintas de 2ª e 3ª geração) ao longo de toda costa brasileira. No Brasil, a ocorrência e os efeitos ambientais dos butilestânicos (TBT, DBT e MBT) vem sendo estudados desde 1998 (Fernandez et al., 2002) e monitorados ao longo do tempo devido ao seu uso e elevada persistência ambiental (Artifon et al., 2016; Maciel et al., 2018). A elaboração e execução de projetos como AIBRASIL I (2011-2014, FINEP) permitiu uma avaliação mais sistemática da ocorrência desses contaminantes nos ecossistemas brasileiros, tendo apontado áreas de atenção quanto ao impacto ambiental. Entre 2014 e 2019, com o Projeto AIBRASIL II (FINEP), foi dado continuidade ao monitoramento dos compostos butilestânicos (BTs), assim como uma avaliação do efetivo impacto da utilização e presença dos biocidas anti-incrustantes de 3ª geração sobre os ecossistemas da costa do Brasil. Assim, ao longo dos últimos 20 anos, vem sendo construída uma importante base de dados de informações ambientais sobre esses contaminantes químicos no meio aquático brasileiro.

No entanto, além de informações básicas sobre o uso, níveis ambientais e efeitos dos biocidas, é necessário fornecer subsídios claros para a aplicação em políticas ambientais. Uma das ferramentas para mediar tais políticas públicas é a Avaliação de Risco Ecológico (ARE) usada para verificar a probabilidade de efeitos adversos e danosos em decorrência da exposição a um ou mais estressores ambientais (ECB, 2003). Por meio da formulação de um problema, caracterização do cenário de exposição e dos efeitos ecológicos causados pelo estressor e, finalmente, pela caracterização do risco, estima-se a probabilidade da ocorrência de efeitos (risco ambiental) em função da contaminação no ambiente. Tal metodologia é aplicada para criação de regulamentações rígidas quanto ao uso e comércio de biocidas em países desenvolvidos como Nova Zelândia e da União Europeia (Carvalho et al., 2015; Peijnenburg, 2020). Portanto, considerando a ausência de regulamentação no Brasil para os biocidas de 3ª geração, torna-se fundamental um entendimento mais abrangente sobre esta problemática visando a elaboração de uma Avaliação do Risco que identifique a existência ou não do impacto decorrente do uso destes biocidas. A partir disso, ações concretas como o estabelecimento de políticas públicas eficazes, poderão ser elaboradas para proteger e conservar ambientes marinhos e costeiros frente a forças antrópicas.

Desse modo, minha tese foi motivada pela construção de uma robusta base de dados sobre ocorrência e distribuição de biocidas anti-incrustantes de 2ª e 3ª geração,

bem como avaliar sistematicamente o seu impacto na costa do Brasil. A figura 1 ilustra o panorama atual do status da contaminação que contribuiu para definir as motivações da tese. E tais motivações estão de acordo com o cumprimento das metas dos Objetivos de Desenvolvimento Sustentável (ODS) propostos pela ONU na Agenda 2030, especialmente dentro do ODS 14 (Vida na água) sobre a “Conservação e uso sustentável dos oceanos, dos mares e dos recursos marinhos para o desenvolvimento sustentável”.

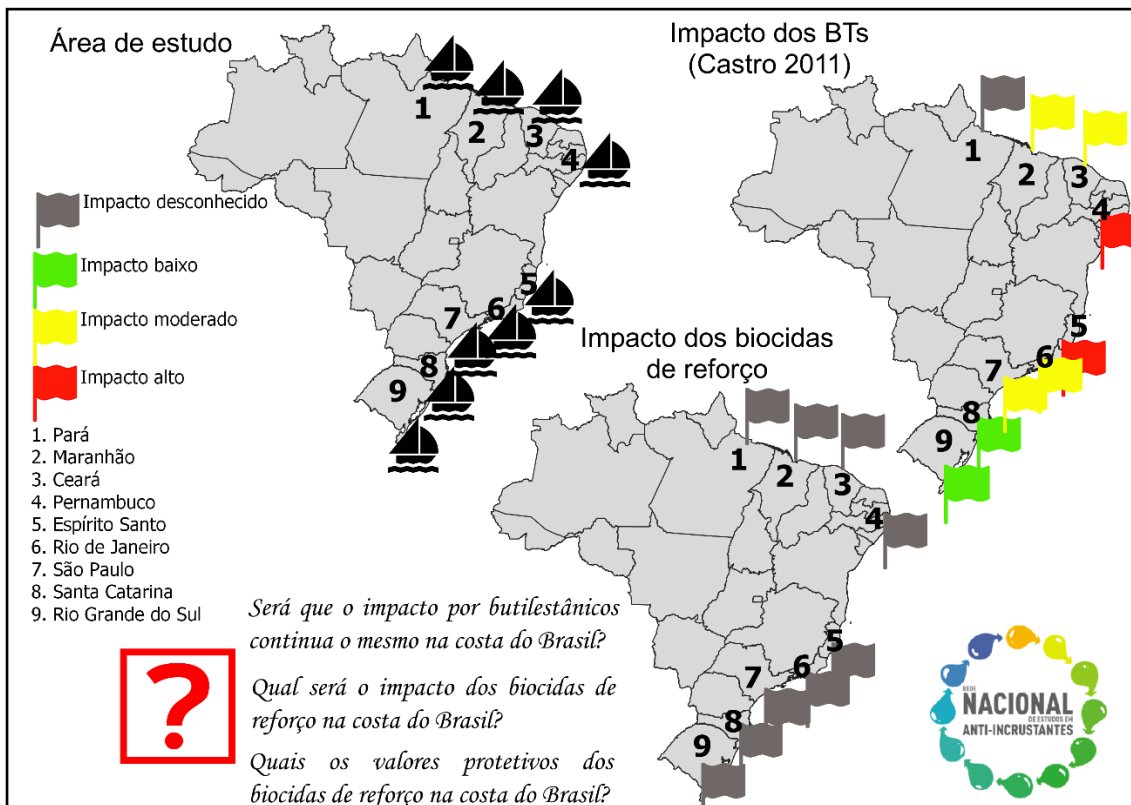


Figura 1: Panorama atual do status do impacto (efeitos adversos à biota) por biocidas anti-incrustantes na costa do Brasil e as motivações (perguntas) da presente Tese de Doutorado. Os barcos indicam as áreas portuárias avaliada na presente Tese.

Os níveis de tributilestanho mantiveram a tendência de redução e não mais representam um risco significativo para os organismos bentônicos dos ambientes costeiros brasileiros sob a influência de atividades marítimas, enquanto os níveis de biocidas de reforço (diuron, Irgarol, diclofluanida, clorotalonil e DCOIT) atualmente detectados também não representam um risco significativo, excetuando sedimentos com a presença de partículas de tintas anti-incrustantes.

Objetivo Geral:

Avaliar a efetiva relevância ambiental dos principais biocidas anti-incrustantes nas zonas costeiras do Brasil sob influência de atividades marítimas.

Objetivos Específicos:

1. Avaliar a contaminação ambiental por biocidas e co-biocidas anti-incrustantes (butilestânicos, diuron, Irgarol, clorotalonil, diclofluanida e DCOIT) em sedimentos costeiros da Baía de Marajó e Guajará (Pará), Baía de São Marcos (Maranhão), Fortaleza (Ceará), Estuário de Suape e Capibaribe (Pernambuco), Sistema Estuarino de Vitória (Espírito Santo), Baía de Guanabara (Rio de Janeiro), Sistema Estuarino de Santos-SãoVicente (São Paulo), Rio Itajaí-Açu (Santa Catarina) e Estuário da Lagoa dos Patos (Rio Grande do Sul);
2. Verificar a presença de partículas de tintas anti-incrustantes nestes sedimentos e a contaminação por biocidas anti-incrustantes associada à essas partículas;
3. Estimar valores padrões de qualidade através da Concentração Sem Efeito Previsível (*PNECs*, em inglês) para a caracterização do risco ambiental dos biocidas anti-incrustantes nos sedimentos da costa do Brasil;
4. Realizar a Avaliação de Risco Ecológico (ARE) dos biocidas anti-incrustantes de 3º geração com base na sua ocorrência ambiental, características físico-químicas e dados de toxicidade produzidos pelo projeto AIBRASIL II.

A zona costeira brasileira que inclui toda planície costeira e bacias hidrográficas, estende-se por 8500 km desde a zona norte equatorial até a zona sul de clima temperado. Essa ampla extensão climática e territorial permite a presença dos mais diversos ecossistemas de importância ecológica e comercial como manguezais, estuários, lagoas costeiras, praias, restingas, ilhas, marismas e recifes de corais (Scherer et al., 2010). A ampla extensão dessa zona também favoreceu o crescimento econômico do país através do desenvolvimento de atividades náuticas e instalação de diversos portos e estaleiros por toda costa. Assim, considerando a logística, os parceiros de trabalho dentro da Rede Nacional de Estudos em Anti-incrustantes (RNEA) e importantes zonas portuárias/neuicas do Brasil, a presente Tese teve como foco 9 áreas costeiras brasileiras do norte ao sul do país (Figura 2).

1. **Baía de Guajará e Marajó, Pará (PA):** A costa Atlântica Paraense perfaz uma extensão de 500 km (linha reta, entre a foz dos rios Amazonas e Gurupi). O sistema estuarino do rio Pará compreende a baía de Marajó na parte oeste, onde se concentram os rios Tocantins e a baía de Guajará que deságua no Oceano Atlântico, estando localizado na foz do delta do Amazonas (Muehe, 2018). No estuário, a circulação da água é altamente dinâmica com regime de macromaré (amplitude média de 3,6 m). A deposição e dispersão dos sedimentos são controladas por intenso regime fluvial e correntes de maré, com predomínio dos depósitos de lama (Gregório e Mendes, 2009). Esse sistema estuarino abriga o Porto de Belém (Porto de Vila do Conde) que movimentava cerca de 4 milhões de toneladas e abastece o maior mercado aberto da América Latina (Ver-o-Peso) (ANTAQ, 2020). A região no entorno também se destaca pela pesca artesanal, sendo uma das principais atividades econômicas do estado (Paz et al., 2011).

2. **Baía de São Marcos, Maranhão (MA):** Localizada entre a região amazônica e o semi-árido nordestino, a região possui uma das maiores áreas de manguezais do mundo (Menezes et al., 2008). A zona estuarina é regida por marés semidiurnas com amplitudes de variação macromaré, variando entre 4 e 7 metros (González-Gorbeña et al., 2015). A região portuária possui uma hidrovia navegável para instalações portuárias, incluindo o terminal de Ponta da Madeira, o maior porto

privado do Brasil (exportação de 190 milhões de toneladas por ano) (ANTAQ, 2020).

3. *Costa litorânea de Fortaleza, Ceará (CE)*: Essa área é localizada no nordeste brasileiro onde o clima é influenciado pela Zona de Convergência Intertropical (ZCIT) e pelo El-Niño Oscilação Sul (ENOS). A linha de costa de Fortaleza cobre 194 km, incluindo municípios da Região Metropolitana da capital e suas respectivas bacias hidrográficas (Muehe, 2018). Os rios Ceará, Cocó e Pacoti nascem em uma área rural e suburbana e cruzam uma das maiores áreas urbanizadas do Brasil até chegar ao Oceano Atlântico (Maia, 1998). Com exceção das regiões de deságue desses rios e da área entre os molhes do Porto do Mucuripe, os sedimentos da costa de Fortaleza são predominantemente grosseiros (tamanhos de partículas > 63 µm), resultado de um costa exposta (Marino et al., 2013). As correntes costeiras normalmente oeste criam um padrão circular dentro da área do porto do Mucuripe que aprisiona os sedimentos e permite a formação de arcos praias (Morais et al., 2006) . A infraestrutura do Porto de Mucuripe inclui canal de acesso, bacias de evolução e um longo cais (1900m de extensão), sendo considerado um porto estratégico de comércio internacional devido sua localização (Docas, 2016).

4. *Estuário de Suape e Capibaribe, Pernambuco (PE)*: Os estuários de Capibaribe e Suape situam-se no estado de Pernambuco, litoral nordeste do Brasil, uma área tropical também sob influência da zona de convergência intertropical (ZCIT). Os Portos de Recife e Suape têm cerca de 40 km de distância entre si (Maciel et al., 2018). O porto de Suape foi construído em 1977 pela interrupção do fluxo de quatro rios. A inundação da paisagem circundante, florestas de manguezais e grande quantidade de material suspenso exige eventos de dragagem constantes (Neumann-Leitão and Matsumura-Tundisi, 1998). Já o Porto do Recife está inserido no sistema estuarino de Capibaribe, onde a circulação local é determinada pelo regime de marés e vazão de água doce. Este estuário inclui a bacia do Pina que recebe esgoto doméstico e industrial da cidade do Recife, mas ainda preserva uma estreita faixa de manguezal. As profundidades do Porto do Recife variam de 8 a 12 m devido à dragagem (Schettini et al., 2016b).

5. *Sistema Estuarino de Vitória, Espírito Santo (ES)*: O Sistema Estuarino de Vitória (SEV) está localizado no sudeste do Brasil, onde a conexão com o Oceano Atlântico se faz pelo Canal do Porto e pelo Canal de Passagem, e inclui a zona da Praia de Camburí na Baía do Espírito Santo (Figura 2). É um estuário de micromaré, classificado como semi-diurno e caracterizado como bem misturado a parcialmente

misto durante as marés vazante (Bastos et al., 2010). A hidrodinâmica também é influenciada por áreas de manguezais que cobrem aproximadamente 18 km² (Zamprogno, 2016). Além do porto de Vitória, a região abriga diversas marinas de apoio à pesca, recreação e atividades de manutenção de pequenas embarcações (Costa et al., 2017). O porto de Tubarão, localizado na Baía de Vitória, também está inserido nesse sistema estuarino, sendo um dos mais importantes portos no transporte de minérios de ferro do país (Vale, 2015).

6. **Baía de Guanabara, Rio de Janeiro (RJ):** Essa é uma baía costeira eutrófica localizada no coração da cidade do Rio de Janeiro. Internacionalmente, ela é reconhecida por níveis severos de contaminação devido a descargas industriais e domésticas provenientes de vários municípios. A profundidade média é 5,7 m e com uma dinâmica governada, principalmente, por correntes de maré semi-diurnas mistas (amplitude da maré: 0,7 m) (Kjerve et al., 1997). A baía de Guanabara é parcialmente estratificada com ampla distribuição de sedimentos anóxicos com alto teor de areia fina e lama (Quaresma et al., 2001). A baía de Guanabara abriga o Porto do Rio de Janeiro que realiza embarque de automóveis, contêineres e transporte de turistas. Estaleiros e áreas de navegação para esportes náuticos também são comuns nesta região (Fernandez et al., 2005).

7. **Sistema Estuarino de Santos – São Vicente (São Paulo, SP):** Em uma área de 835 km² que está inserida em cinco municípios da região (São Vicente, Santos, Cubatão, Guarujá e Praia Grande), o sistema estuarino de Santos e São Vicente (SESS) tem extensas áreas alagáveis com formação de manguezais e bancos de lama (Roversi et al., 2016). O sistema é formado pelo Estuário de Santos, Estuário de São Vicente e o canal da Bertoga mais à nordeste. De forma geral, o sistema estuarino é classificado como moderadamente estratificado, com um ciclo de maré diurno (amplitude de 0,4 m na quadratura a 1,85 m na sizígia) e velocidade média de corrente de 50 cm s⁻¹ (Roversi et al., 2016). O Porto de Santos, maior porto da América do Sul, está situado no Estuário homônimo, sendo responsável por 30% do comércio de importação do Brasil e uma movimentação de cargas equivalente a mais de 130 milhões de toneladas por ano (Porto de Santos, 2019). Nas áreas adjacentes há intensa atividades de marinas e estaleiros que fazem a manutenção desde barcos de pesca e grandes iates de luxo. Além disso, a presença de manguezais tem função retentora e contribui para a carga de matéria orgânica e sedimentos finos (Buruam et al., 2013).

8. ***Estuário do Rio Itajaí-Açu, Santa Catarina (SC)***: O sistema estuarino Itajaí-Açu apresenta uma extensão aproximada de 70 km, onde o baixo estuário é formado por meandros bem definidos, com uma orientação geral de noroeste-sudoeste (Schettini, 2002). Essa região do baixo estuário é dominada por influência marinha e assim como o médio estuário é classificado do tipo cunha salina (Schettini and Toldo, 2006). O estuário inferior consiste principalmente de sedimentos de tamanho de argila (> 70%), maré local semi-diurna com uma amplitude média de 0,8 m (Schettini, 2002). O aporte fluvial é proveniente do rio Itajaí-Mirim que junto ao rio Itajaí-Açu (Figura 2), são responsáveis pela maior parte da afluência à foz formando uma bacia hidrográfica de 15.500 km² (Frena et al., 2016). As atividades de dragagens continuam em desenvolvimento para manter o aprofundamento do canal em torno de 14 metros e permitir a entrada de navios maiores (Naval, 2020). Além disso, o rio Itajaí-Açu concentra diversos estaleiros e locais de manutenção de barcos, bem como o maior terminal de pescado do Brasil e diversas fábricas de processamento (Pereira-Filho et al., 2010).

9. ***Estuário da Lagoa dos Patos, Rio Grande do Sul (RG)***: A Lagoa dos Patos é a maior laguna estrangulada do mundo com 250 km de extensão (Kjerfve et al., 1997), onde o estuário cobre cerca de 10% da área total da superfície. O vento e a descarga dos rios são os principais impulsionadores da circulação (Fernandes et al., 2002). Os ventos de noroeste favorecem a descarga de água doce da lagoa, enquanto os ventos de sul e sudoeste favorecem a entrada de água do mar no estuário (Moller et al., 2001). As características morfológicas da lagoa mostram uma predominância de sedimentos grosseiros em áreas rasas e finos em áreas mais profundas (Calliari et al., 2009). A atividade portuária do estuário da Lagoa dos Patos é uma das atividades econômicas da região abrigando três portos (Super Porto, Porto Velho e Porto Novo) que movimentam mais de 7 milhões de toneladas de mercadorias (ANTAQ, 2020). Além disso, o tráfego marítimo é intenso na região do estuário, contando com estaleiros de médio e grande porte e instalações de manutenção de embarcações de pesca e lazer (Soroldoni et al., 2018).

5.1 Amostragem de Sedimentos

Os sedimentos superficiais (camada superior de 2 cm) foram coletados com dragas tipo Eckman ou Van Veen nas 9 áreas de estudo ao longo da costa brasileira entre setembro de 2015 e abril de 2018 (Figura 2). A amostragem foi feita por parceiros da Rede Nacional de Estudos em Anti-incrustantes (RNEA). De modo a possibilitar uma visão da distribuição espacial dos contaminantes, bem como estabelecer uma relação com possíveis fontes, 113 pontos foram amostrados em áreas sob influência direta de atividades marítimas (portuárias, marinas, estaleiros, colônia de pescadores ou apenas áreas de tráfego de barcos) e ocorrência de sedimentos finos.

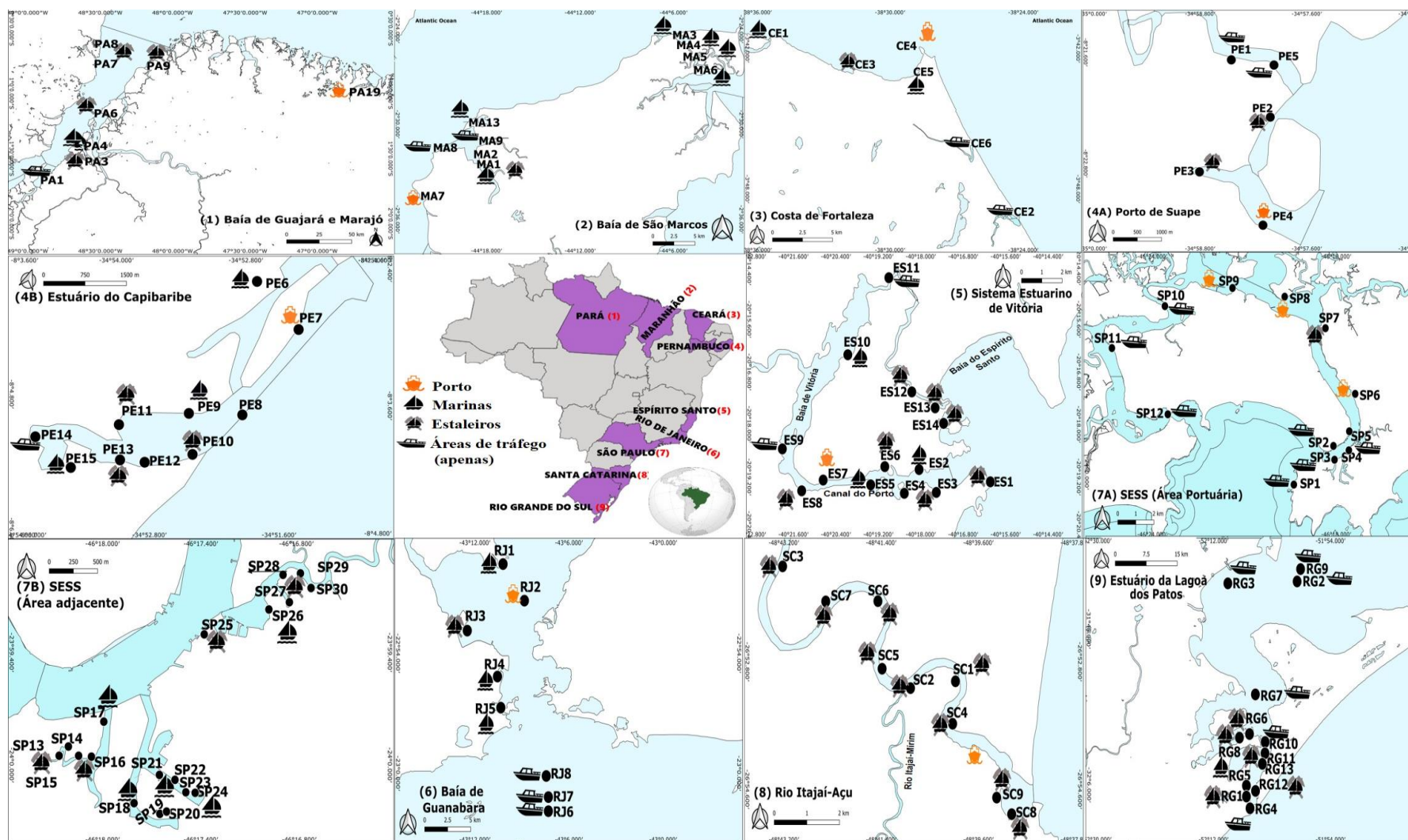


Figura 2: Pontos de amostragem (sigla para o estado e o número da estação) de sedimentos ao longo da costa do Brasil. Cada quadrado tem representado uma área de estudo com os pontos amostrados e a indicação da principal atividade marítima que ocorre nesse sítio. Em laranja, estão destacados as regiões dos portos que foram amostrados.

Em campo, cerca de 500 a 1000 g de sedimentos foram coletadas, acondicionadas em potes de alumínio previamente calcinados (450 °C por 8 horas), refrigerados e transportados para o laboratório. Em sequência, as amostras de sedimentos foram congeladas a -20 °C e transportadas até o Laboratório de Microcontaminantes Orgânicos e Ecotoxicologia Aquática (CONECO) da FURG, onde toda caracterização e procedimento analítico foi realizado. Na tabela 2 estão os detalhes sobre o período amostrado e a quantidade de pontos amostrados por região.

Tabela 1: Região, data de coleta e quantidade de pontos amostrados ao longo da costa do Brasil.

Estado do Brasil	Região amostrada	Data de coleta	Número de pontos amostrados
Pará (PA)	Baía de Guajará e Marajó	Julho/2017	8
Maranhão (MA)	Baía de São Marcos	Abril/2018	10
Ceará (CE)	Costa de Fortaleza	Janeiro/2018	6
Espírito Santo (ES)	Sistema Estuarino de Vitória	Novembro/2017	14
Pernambuco (PE)	Porto de Suape	Março/2018	5
	Estuário do Capibaribe	Abril/2018	10
Rio de Janeiro (RJ)	Baía de Guanabara	Agosto/2017	8
São Paulo (SP)	Sistema Estuarino de Santo e São Vicente	Outubro/2015	30
Santa Catarina (SC)	Rio Itajaí-Açu	Dezembro/2017	9
Rio Grande do Sul (RG)	Estuário da Lagoa dos Patos	Setembro/2015	13

5.2 Caracterização dos sedimentos e identificação de Partículas de Tintas Anti-incrustantes (PTAs)

Em laboratório, os sedimentos foram liofilizados, homogeneizados e separados em duas alíquotas: uma para caracterização da granulometria e das PTAs (Fração 1) e outra para análise química e análise do teor de carbono orgânico (Fração 2). A fração 1 foi pesada (50 g) e peneirada em malha 0,063 mm (previamente descontaminada com acetona e hexano) sob fluxo lento de água para retirada dos grãos de sais e desagregação do silte/argila dos grãos mais grossos. O restante sobre a peneira foi seco em estufa à 40 °C. Após seco, o restante da amostra foi fracionado em um conjunto de peneiras com

diferentes tamanhos de malhas (1000, 500, 250, 125 e 63 μm). A porcentagem de finos foi então calculada e expressa em porcentagem da amostra (Gray and Elliott, 2014). Sedimentos maiores que 500 μm foram separados e congelados ($-20\text{ }^\circ\text{C}$) para posterior triagem das PTAs, conforme descrito por Soroldoni et al. (2018). A triagem das PTAs foi feita em microscópio com lente de aumento de 40x, onde foram separadas de acordo com a coloração e forma para posterior análise química dos biocidas anti-incrustantes.

A fração 2 foi desagregada em grau e pistilo até completa homogeneização da amostra. Para análise do Carbono Orgânico Total (COT) foi seguido o protocolo descrito por Kristensen and Andersen (1987), onde 1 g desse sedimento foi previamente descarboxado sob vapor de ácido clorídrico HCl (10%) por 24 h e posteriormente seco em estufa à $60\text{ }^\circ\text{C}$. Após a secagem, o sedimento descarboxado foi pesado (20 mg) e analisado por detecção por infravermelho não dispersivo (Shimadzu TOC-L CPH / CPN) equipado com módulo para amostras sólidas (Shimadzu SSM-5000A). A quantificação foi feita com uma curva analítica de glicose e os resultados expressos em percentual de COT. Material certificado (SRM 1941b, NIST[®]) foi periodicamente analisado como controle de qualidade.

5.3 Análises Químicas

5.3.1 Butilestânicos em sedimentos e PTAs

Os butilestânicos Tributiestanho (TBT), Dibutilestanho (DBT) e Monobutiestanho (MBT) foram analisados de acordo com Moço et al. (em preparo). Resumidamente, 2 g de sedimento liofilizado e homogeneizado ou 0,01 g de PTAs (apenas partículas de São Paulo e Rio Grande do Sul devido à disponibilidade) foram fortificados com padrão de recuperação (100 ng de tripropilestano - TPrT) e deixados para equilíbrio (30 min). A extração foi realizada com tropolona 0,03% em metanol (m v^{-1}) (9 mL) e ácido glacial acético (1 mL). As amostras foram misturadas em vórtice por 1 min e sonicadas (40 kHz, 132 W) por 30 min. Cinco mL do sobrenadante foram coletados e misturados com tampão de acetato de sódio (pH 4,5) (1 mL), sendo 100 μL de solução de Tetraetilborato de Sódio (NaBEt_4 - 10%) usados para derivação. A limpeza da amostra foi realizada em coluna de sílica (2,5 g) e uma eluição com n-hexano (10 mL). Posteriormente, 100 μL da solução de tetrabutilestano (1000 ng Sn mL^{-1}) foram adicionados como padrão interno. Os extratos foram analisados por cromatografia gasosa (CG) usando um Perkin Elmer Clarus 500EM acoplado a um

espectrômetro de massa (EM) e equipado com uma coluna capilar Elite-5MS (difenildimetilpolissiloxano 5%) (30 m × 0,25 mm × 0,25 µm).

A garantia e controle de qualidade foram baseados em análises regulares de brancos, matrizes fortificadas e material de referência certificado (CRM - PACS-3 / NIRST, Ottawa, Canadá). As recuperações obtidas no CRM estiveram de acordo com as concentrações certificadas (89 ± 2,3% para TBT; 80 ± 9,7% para DBT e 114 ± 27,6% para MBT). As recuperações para o padrão de recuperação variaram entre 68% e 130%, N = 4) enquanto o RSD (desvio padrão relativo) foi inferior a 20%. Limites de detecção (LD) e quantificação (LQ), calculados pela relação sinal-ruído (S / N) ("pico a pico") obtida pelo software Turbo Mass (S / N = 3 para LD e S / N = 10 para LQ, respectivamente), foram de 1 e 5 ng Sn g⁻¹ em sedimentos e 250 e 500 ng Sn g⁻¹ em PTAs, respectivamente, para todos os butilestânicos. Uma curva analítica de matriz em solvente e sedimento foi usada para quantificação de PTAs e sedimentos, respectivamente. Os resultados foram relatados como ng Sn g⁻¹.

A avaliação da recentidade do aporte dos butilestânicos foi feita pelo Índice de Degradação de Butilestanho (IDB) (Equação 1) (Díez et al., 2002a). Quando obtido valores inferiores a 1 (IDB < 1) infere-se que houve um aporte recente de TBT no local amostrado, enquanto valores maiores que 1 (IDB > 1) indicam aportes antigos e predominância dos produtos de degradação do TBT.

$$IDB = \frac{\text{Concentração do DBT} + \text{Concentração do MBT}}{\text{Concentração do TBT}} \quad \text{Eq. (1)}$$

5.3.2 Biocidas de reforço em sedimentos e PTAs

Biocidas de reforço (Irgarol, diuron, clorotalonil, diclofluanida e DCOIT) foram extraídos de sedimentos e PTAs de acordo com Abreu et al. (2020). Resumidamente, 1 g de sedimento liofilizado ou 0,01 g de PTAs (provenientes das amostras do Maranhão, Ceará, Espírito Santo, Rio de Janeiro, São Paulo, Santa Catarina e Rio Grande do Sul) foram enriquecidos com atrazina-d5 (20 ng L⁻¹, padrão de recuperação para análises em cromatografia líquida) e PCB112 (20 ng L⁻¹, padrão de recuperação para cromatografia gasosa) e deixados para equilibrar por 30 min. Em seguida, acetonitrila (15 mL) foi utilizada como solvente extrator, sendo sonicado por 30 min (duas vezes). Os sobrenadantes foram misturados e concentrados até 1 mL. O extrato foi deixado durante a noite em contato com cobre ativado. Posteriormente o

extrato foi diluído em 50 mL de água ultrapura, sendo purificado por extração em fase sólida (SPE) com cartuchos C18 eluída com 2 x 2 mL de acetato de etila: hexano (1:1). O eluato foi dividido em duas frações, sendo o solvente evaporado e resuspenso em metanol (1ª fração) para análise de Irgarol, diuron e DCOIT por Cromatografia de fase líquida com Ionização por eletrospray e detector de espectrometria de massas em tandem (CL-IES–EM/EM) (Alliance Separations, modelo 2695, Waters - Milford, MA, EUA). A 2ª fração foi resuspensa em hexano, fortificada com PCB30 (10 ng mL⁻¹) como padrão interno para análise de clorotalonil e diclofluanida por Cromatografia gasosa com detector por captura de elétrons (CG-DCE) (Perkin Elmer Clarus 500; Waltham, MA, EUA) usando duas colunas capilares diferentes (30m x 25mm x 0,25µm) para confirmação: ZB-5MS (Phenomenex, Alcobendas, ES) e DB-1701 (Agilent, CA, EUA).

O controle de qualidade analítico foi baseado em análises regulares de branco e de matrizes fortificadas. As recuperações variaram entre 59% e 140%, enquanto o RSD se manteve abaixo de 20%. Para sedimentos, LOD e LOQ foram 0,5 e 1,4 ng g⁻¹ para diuron, 0,4 e 1,2 ng g⁻¹ para Irgarol, 0,1 e 0,4 ng g⁻¹ para clorotalonil, 0,7 e 2,1 ng g⁻¹ para diclofluanida e 0,2 e 0,7 ng g⁻¹ para DCOIT, respectivamente. Para PTAs, LOD e LOQ foram 100 vezes maiores do que aqueles relatados para sedimentos. Os resultados foram expressos em ng g⁻¹.

5.4 Avaliação de Risco Ecológico dos co-biocidas em sedimentos

5.4.1 Base de dados ecotoxicológicos

Os dados de ecotoxicidade de cada biocida em sedimentos (Irgarol, diuron, clorotalonil, diclofluanida e DCOIT) foram obtidos por meio de uma revisão sistemática de dois tipos de conjunto de dados: 1) banco de dados disponível online de grupos de redes e agências ambientais (NITE, PubChem, EPA, LANUV, PPDB, NORMAN, ECHA, EnviroTox, Pan, ETOX, EFSA) e 2) artigos publicados em revistas científicas indexadas disponíveis no Google Academic, Web of Science e PubMed (Adriaanse e Rensleigh, 2011). As palavras-chave usadas para pesquisar os dados de ecotoxicidade para cada biocida foram “cas number” e uma combinação de “nome químico + sedimento + toxicidade ou ecotoxicidade” e “sinônimo de nome químico + sedimento + toxicidade OU ecotoxicidade”.

A partir dessa pesquisa, os estudos ecotoxicológicos com informações substanciais (organismo teste, duração do teste, endpoint, valor de toxicidade e outros)

foram avaliados quanto à confiabilidade usando a ferramenta Science in Risk Assessment and Policy (SciRAP) (<http://www.scirap.org/>). Esta ferramenta segue o método Criteria for Reporting and Evaluating Ecotoxicity Data (CRED) proposto por Moermond et al. (2016) para verificar a confiabilidade e relevância dos estudos de ecotoxicidade. Os documentos que alcançaram confiabilidade $\geq 70\%$ na soma dos campos “preenchido” e “parcialmente preenchido” foram selecionados para derivar a Concentração Sem Efeito Previsível (PNEC). Resultados da Concentração de Efeito Não Observado (CENO) foram usados preferencialmente devido à representatividade na proteção ambiental (exposição de longo prazo a níveis baixos). Dados de órgãos ambientais foram considerados confiáveis e robustos devido à reconhecida qualidade das informações. Uma planilha Excel foi então organizada com as informações adquiridas e relevantes sobre a ecotoxicidade dos biocidas em sedimentos.

5.4.2 Derivação da Concentração Sem Efeito Previsível (PNEC)

Na avaliação dos efeitos ecológicos, um valor de proteção deve ser calculado ou utilizado para garantir a proteção do meio ambiente representado por aquele compartimento (no caso, sedimentos). Uma Concentração Sem Efeito Previsível (PNEC, do inglês *predicted no effect concentration*) é considerada como uma concentração abaixo da qual um efeito adverso provavelmente não ocorrerá (ECB, 2003). No entanto, para a maioria dos compostos químicos, o número de dados de toxicidade para organismos bentônicos é muito limitado e requer a derivação PNEC pela abordagem determinística. A abordagem determinística consiste em dividir o menor valor de limite de toxicidade (ou seja, o organismo mais sensível) por um fator de segurança (AF, em inglês Assessment factor).

Para Irgarol, diuron, clorotalonil e DCOIT, os PNECs foram derivados de acordo com o Documento de Orientação Técnica (TGD, em inglês *Technical Guidance Document*) da Comissão Europeia para Biocidas (ECB, 2017). Os meios de exposição dos organismos-teste ao contaminantes, seu regime de alimentação e o uso de testes de sedimento total usando organismos bentônicos foram levados em consideração para a aplicação do AF. Além disso, foi aplicada uma AF adequado (dependendo do número de testes de longa duração e do hábito alimentar das espécies testadas) sobre o menor valor de toxicidade, de acordo com os critérios estabelecidos para o compartimento sedimento (ECB, 2017).

Para a diclofluanida, não foram encontrados dados de testes ecotoxicológicos realizados na matriz sedimento. Neste caso, o valor do PNEC para sedimentos ($PNEC_{sed}$) foi derivado do valor de $PNEC_{água}$ ($PNEC_a$) mais baixo disponível no site da rede NORMAN (<https://www.norman-network.net/>). O $PNEC_{sed}$ então representa a concentração de um determinado contaminante no sedimento, equivalente à sua concentração na coluna d'água quando o sistema está em equilíbrio (Dulio and Ohe, 2013). Primeiramente, o menor $PNEC_a$ é calculado usando a abordagem de particionamento de equilíbrio (EqP), aplicada em dados de toxicidade previstos por modelos de relação quantitativa estrutura/atividade (QSAR) ou obtidos experimentalmente, e que estão disponíveis no banco de dados NORMAN. A rede NORMAN também julga vários critérios (relevância e confiabilidade do estudo-chave) para a derivação do $PNEC_a$ mais baixo e robusto (Dulio and Ohe, 2013). Assim, o $PNEC_{sed}$ para diclofluanida foi derivado através da equação 2 (Dulio and Ohe, 2013). Esta equação resulta de pressupostos de cálculo a partir das disposições do TGD para derivação de valores padrão de qualidade (EQS, em inglês *Environmental Quality Standard*) (ECB, 2011).

$$PNEC_{sed} = PNEC_a * 2.6 * (0.615 + 0.019 * K_{oc}) \quad \text{Eq. (2)}$$

Onde $PNEC_{sed}$ é expresso em peso seco ($\mu\text{g kg}^{-1}$); $PNEC_a$ é o menor PNEC para água ($\mu\text{g l}^{-1}$) disponível no site NORMAN e K_{oc} (<https://pubchem.ncbi.nlm.nih.gov/>) é o coeficiente de partição entre o carbono orgânico e a água ($l \text{ kg}^{-1}$). A fim de comparar com o método determinístico aplicado no estudo, esta equação também foi aplicada para Irgarol, diuron, clorotalonil e DCOIT.

5.4.3 Caracterização do Risco Ecológico

O Quociente de Risco (QR) é calculado através da razão entre a concentração de exposição medida (MEC, em inglês *measured environmental concentration*) obtida pela quantificação dos biocidas nos sedimentos amostrados ao longo da costa do Brasil e os respectivos valores PNEC (para cada biocida) (Equação 3) (ECB, 2003):

$$QR = \frac{MEC}{PNEC} \quad \text{Eq. (2)}$$

Quando a MEC estava abaixo do LD e LQ, foram considerados os valores de $\frac{1}{2}$ do LD ou $\frac{1}{2}$ do LQ para efeito de cálculo. Os QRs foram calculados para cada estação de amostragem e classificados de acordo com a Comissão Europeia (ECB, 2003) com adaptações devido à limitação do LD e LQ obtidos para os co-biocidas. Assim, propusemos a seguinte classificação para clorotalonil, diclofluanida e Irgarol: “baixo risco” / sem prováveis efeitos adversos à biota ($QR \leq 0,1$); “Risco moderado” ($0,1 < QR < 1$) ou “alto risco” / potencialmente causa efeitos adversos à biota ($QR \geq 1$). Dado os LDs / LQs obtidos para DCOIT, adicionamos a categoria de risco Baixo-Moderado (L-M) quando o DCOIT medido foi $<LQ$. O diuron, por sua vez, apresentou LDs / LQs acima dos valores do PNEC. Portanto, quando as amostras eram $<LD$ ou $<LQ$, o risco era indeterminado e qualquer valor acima do LQ já significava “alto risco” ($RQ \geq 1$). Uma exceção foi feita para as amostras do estuário da Lagoa dos Patos, devido aos valores de LDs / LQs obtidos para Diuron por Soroldoni et al. (2018), às quais foi possível adicionar a categoria L-M quando o diuron era detectado, mas estava abaixo do LQ.

5.5 Análise dos dados

Normalidade e homogeneidade foram verificadas usando ShapiroWilk e Testes de Levene, respectivamente. A análise de correlação não paramétrica de Spearman foi usada para investigar as relações entre as concentrações de biocidas e os parâmetros do sedimento, bem como os níveis de concentração de PTAs e dos biocidas. Uma análise de similaridade comparando locais de amostragem sob diferentes atividades navais no SESS (São Paulo) foi realizada usando matrizes de similaridade Bray-Curtis por escalonamento multidimensional não métrico (nMDS). Grupos formados por nMDS foram examinados posteriormente usando um PERMANOVA unilateral para análise de significância 95%. Considerando a amostragem realizada nos outros estados, foi analisada a relação entre a atividade marítima (portos, marina, estaleiro e zona de tráfego) e os níveis dos biocidas através da correlação de Spearman suportada por gráficos Box plot. A metade dos valores do LD e LQ foram usados para análise estatística, cálculos de IDB e somatórios de BTs e co-biocidas. Todas as análises estatísticas foram realizadas usando PAST para Windows (versão 3.25) com um nível de significância de 0,05 (Hammer et al., 2001).

Capítulo VI: Artigos Científicos

Para a obtenção do título de Doutor pelo Programa de Pós-Graduação em Oceanologia, é requerido que o discente realize a submissão de pelo menos dois artigos científicos como primeiro autor em periódico com corpo indexado, sendo um deles aceito. Desse modo, os resultados da pesquisa desenvolvida durante o período do doutorado e a discussão dos resultados serão apresentados em forma de artigos neste Capítulo. O primeiro manuscrito, de autoria de Fiamma Eugênia Lemos Abreu, Juliane Natália Lima da Silva, Ítalo Braga Castro, e Gilberto Fillmann é intitulado “*Are antifouling residues a matter of concern in the largest South American port?*” e foi publicado no periódico *Journal of Hazardous Materials*. O segundo manuscrito, de autoria de Fiamma Eugenia Lemos Abreu, Rodrigo Moço Batista, Ítalo Braga Castro, Gilberto Fillmann é intitulado “*Legacy and emerging antifouling biocide residues in a tropical estuarine system (Vitória state, SE, Brazil)*” e foi publicado periódico *Marine Pollution Bulletin*. O terceiro manuscrito, de autoria de Fiamma Eugênia Lemos Abreu; Rodrigo Moço Batista; Lílian Lund Amado; Danielle Ribeiro Brasil; Teresa Cristina Rodrigues dos Santos Franco; José Lucas Martins Viana; Eliete Zanardi-Lamardo; Gilvan Takeshi Yogui; Marcos Antônio Fernandez; Italo Braga Castro; Gilberto Fillmann é intitulado “*Antifouling biocides in sediments along the Brazilian coast*” e será submetido ao periódico *Science of the Total Environment*. O quarto manuscrito, de autoria de Fiamma Eugênia Lemos Abreu; Samantha Eslava Martins, Gilberto Fillmann, é intitulado *Tier-1 Ecological Risk Assessment of booster biocides in sediments from Brazil Coastal Areas* e foi publicado no periódico *Chemosphere*.

ARTIGO 1

Are antifouling residues a matter of concern in the largest South American port?

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ABSTRACT

In the present study, levels of booster biocides (diuron, Irgarol, chlorothalonil, dichlofluanid and DCOIT), butyltin compounds (TBT, DBT and MBT) and antifouling paint particles (APPs) were assessed in sediments of areas under the influence of the largest Latin American port, marinas, boat traffic and ship/boat maintenance facilities located within Santos-São Vicente Estuarine System (SSES). Contamination profile was directly related to local maritime activities, where sediments from the main navigation channel (MNC) presented low levels of antifouling residues while adjacent areas (AA), characterized by the presence of boats and boatyards, showed higher contamination considering all analyzed residues. Moreover, areas under the influence of fishing boats/yards presented relevant levels of butyltins (Σ BTs $> 300 \text{ ng g}^{-1}$) and APPs ($>100 \mu\text{g g}^{-1}$), while marinas dominated by recreational boats showed higher booster biocides occurrence. Sites located nearby shipyards in the MNC and boatyards in the AA presented expressive amounts of APPs ($>200 \mu\text{g g}^{-1}$). These APPs represent an important long-term source of biocides to the SSES. Thus, the profile of maritime activities in association to local oceanographic conditions drive the spatial distribution of antifouling residues within SESS, which in some case presented levels above sediment guidelines for TBT, DCOIT and diuron.

Keywords: Antifouling biocides, paint particles, sediment, fresh TBT input, sources

1. Introduction

Antifouling systems are used to prevent marine biofouling, that is a biological event characterized by the settling and growing of organisms on the surfaces exposed to natural waters (Almeida et al., 2007). This process occurs in aquatic environments and should be avoided in naval structures, especially vessels, since biofouling increases corrosion rates, frequency of dry-docking maintenance procedures and fuel consumption (Almeida et al., 2007). To meet this demand, naval industry developed antifouling paints containing chemical biocides used as protective coating in boat and ship hulls. However, these biocides are hazardous substances which are released to the water column and may cause unwanted environmental effects on aquatic biota (Castro et al., 2011). Although widely used as biocide in antifouling paints since 1960's, tributyltin (TBT) is highly toxic to non-target organisms, being considered an

endocrine and metabolic disruptor (Fent, 1996). Its deleterious effects on aquatic biota, including imposex in gastropods and malformation in bivalves, is seen at environmental concentrations as low as 1 ng TBT L⁻¹ (Horiguchi, 2017). Thus, due to negative impacts onto aquatic environments, the use of TBT-based antifouling paints was gradually constrained at country levels and, finally, globally by the International Maritime Organization in 2008 (IMO, 2008a). Nevertheless, contamination by TBT and its degradation products (dibutyltin (DBT) and monobutyltin (MBT)) is still been detected, representing a serious environmental issue along coastal areas under the influence of maritime activities, such as Asia (Z. Chen et al., 2017), Europe (Abraham et al., 2017), Africa (van Gesselten et al., 2018) and South America (Mattos et al., 2017).

The new antifouling paints developed to comply with the increasing restrictions on TBT use slowly reintroduced metals (such as copper and zinc) in combination with up to 4 booster biocides as active ingredients (Omae, 2003). Nowadays, at least 23 chemical compounds have been used in TBT-free antifouling paints due to their lower persistency and, in some cases, a more environmental friendly behavior (Thomas and Brooks, 2010). However, compounds such as Irgarol, diuron, chlorothalonil, dichlofluanid and 4,5-dichloro-2-octylisothiazol- 3(2H)-one (DCOIT) have been detected in water and sediment samples from ship/boat traffic areas of Asia (Lam et al., 2017), South America (Batista-Andrade et al., 2018), Africa (Sánchez-Rodríguez et al., 2011a) and Europe (Ansanelli et al., 2017). In fact, some have been reported (in water and sediment samples) at the same levels they produced toxicity in marine life (Martins et al., 2018). Deleterious effects of antifouling booster biocides have been detected through classical and microcosm bioassays, evaluating from molecular endpoints to damages in meiofaunal community structure (Gallucci et al., 2015; Martins et al., 2018). Furthermore, antifouling paint particles (APPs), that are generated during repair, cleaning and painting procedures of vessel hulls, have also been recognized as a hazardous waste (Soroldoni et al., 2018). These APPs, usually generated in shipyards, boatyards and marinas, have been identified as a highly toxic residue, acting as secondary sources of metals and biocides to aquatic environments (Soroldoni et al., 2017).

Although butyltin compounds (BTs) were previously detected at Santos-São Vicente Estuarine System (SSES) (Buruaem et al., 2013; Santos et al., 2016), booster biocides and APPs have never been assessed in this area. SESS holds the biggest port of South America (Santos port), which is responsible for 30% of importation commerce in

Brazil and has a cargo-handling equivalent to more than 130 million tons per year (Porto de Santos, 2019). Thus, the present study assessed the environmental occurrence of antifouling residues (Irgarol, diuron, chlorothalonil, dichlofluanid, DCOIT, BTs and APPs) in surface sediments of SSES in association to the profile of maritime activities and hydrodynamics.

2. Materials and methods

2.1. Study area and sampling

The Santos-São Vicente Estuarine System (SSES) has an intense naval traffic characterized by commercial ships and small vessels (pleasure and fishing boats) distributed along different areas inside the estuary (Figure 1; Table S1). Surface sediments (upper 2 cm) were sampled in 1 site in Santos bay (SP1), 11 sites along the main channel that circulates the SSES island and under the influence of ship traffic (Santos port; SP2, SP3, SP5, SP6, SP8, SP9), ship yard (SP 7 and SP25) and boat traffic (SP10 – SP12), and 18 sites on an area adjacent to the main navigation channel (MNC) and under the influence of marinas, fishing boats and boat maintenance facilities (SP4, SP13 - SP24, SP26 - SP30) (Table 1). Samples were collected in December 2015 using a stainless steel “Ekman” grab. Sediments were frozen (-20 °C) and stored for subsequent analysis. In stations SP2, SP5, SP17 and SP26, according with availability, were collected manually up to 30 adult specimens of gastropod *Stramonita brasiliensis* (20 – 30 mm-long) to imposex assessment.

2.2. Imposex characterization

Imposex determination was evaluated according to Rossato et al. (2018) in 4 sampling sites (SP2, SP5, SP17 and SP26) (Figure 1). Imposex levels were assessed using the % of imposex in females (I%) and Relative Penis Length Index (RPLI = [mean penis length in females/mean penis length in males] x 100), according to Stroben et al. (1992). The Vas Deferens Sequence Index (VDSI), based on the development of male sexual characters (particularly the vas deferens) by female, was evaluated according to Gibbs et al. (1987).

2.3. Sediment characterization

Sediment granulometry was determined according to Gray and Elliott (2014) and results were expressed in percentage of fine sediments (grain size lower than 0.0063mm). Total organic carbon (TOC %) was measured, after decarbonation of

sediment samples, using a TOC-L SSM 5000 A (Shimadzu) (Kristensen and Andersen, 1987).

2.4. Antifouling Paint Particles (APP) characterization

The paint particles quantification was according to Takahashi et al. (2012). Aliquots of each sediment sample (100 g wet) were sieved (500 μm) using distilled water, oven dried (40°C), and examined under a stereo microscope (5x magnification). Subsequently, the isolated APPs were weighed and stored until chemical analyzes.

2.5. Chemical Analyses

2.5.1. Butyltins

Butyltins (TBT, DBT and MBT) were analyzed according to Castro et al. (2015). Five grams of freeze-dried and homogenized sediments or 0.01 g of APPs (due to limited availability of APPs, BTs were analyzed only on sites SP8, SP14, SP15 and SP25) were spiked with tripropyltin (TPrT, 100 ng) as surrogate standard. After equilibration (30 min.), 15 mL of tropolone (0.05%) in methanol and 1 mL of concentrated hydrochloric acid were added. Samples were ultra-sonicated for 15 min (40 kHz, 132 W, 40° C) and centrifuged at 3000 rpm for 10 min (in triplicate). Supernatants were collected in funnels with 150 mL of sodium chloride (10%) and the analytes extracted using 3x20 mL of dichloromethane. The organic phase was collected and evaporated down to 1 mL in a Syncore® system. Derivatization was performed with 2 mL of Grignard reagent and, subsequently, a liquid extraction using 3x5 mL of hexane was done. Extracts were evaporated down to 1 mL and cleaned up using silica and 15 mL of hexane/toluene (1:1). Then, extracts were concentrated, fortified with tetrabutyltin (1000 ng Sn mL⁻¹) as internal standard and analyzed by gas chromatography using a Perkin Elmer Clarus 500MS equipped with mass spectrometer detector.

QA & QC was based on regular analysis of blanks, spiked matrices and certified reference material (CRM – PACS-3/National Research Council of Canada, Ottawa, Canada). Recoveries obtained from the CRM were in adequate agreement with the certified concentrations (85% for TBT; 70% for DBT and 76% for MBT). Recoveries for the surrogate standard varied between 75% and 144%, while RSD (relative standard deviation) was below 20%. Limits of detection (LOD) and quantification (LOQ), calculated by the signal to noise (S/N) ratio (“peak to peak”) obtained by the Turbo Mass software (S/N = 3 for LOD and S/N = 10 for LOQ, respectively), were <0.5 and <1 ng Sn g⁻¹ (sediments), and <250 and <500 ng Sn g⁻¹ (APPs), respectively, for all

butyltins. An analytical curve equation in sediment matrix-matched (Castro et al., 2015) and solvent was used for quantification of sediment and APPs samples, respectively (Table S2). All concentrations were reported as ng Sn g⁻¹ (dry weight).

2.5.2. Booster biocides

Sediment samples and APPs were extracted by ultra-sonication and cleaned-up by Solid Phase Extraction (SPE), as proposed by Soroldoni et al. (2017). One gram of freeze-dried sediment samples or 0.01 g of APPs were spiked with 10 ng L⁻¹ of Atrazine d5 (surrogate used for LC-MS/MS analyses) and 20 ng L⁻¹ of PCB112 (surrogate used for GC-ECD analyses). Afterwards, samples were mixed with 15 mL of acetonitrile, sonicated and centrifuged twice. Supernatants were combined, evaporated (Syncore®) down to 1 mL, diluted with 50 mL of ultrapure water and cleaned-up by SPE using C18 cartridges. Analytes were eluted with 2 x 2 mL of ethyl acetate:hexane (1:1). Eluates were divided and one fraction was solvent exchanged to methanol (LC-MS/MS) and another to hexane (GC-ECD), using PCB30 (10 ng mL⁻¹) as internal standard. Irgarol, diuron, dichlofluanid and DCOIT (sediment samples and APPs) were analyzed by LC-ESI-MS/MS (Alliance Separations, model 2695, Waters – Milford, MA, USA) and chlorothalonil (sediment samples only) by GC-ECD (Perkin Elmer Clarus 500; Waltham, MA, USA).

QA & QC was based on regular analyses of blanks and spiked matrices. Recoveries for the surrogate standard ranged between 90% and 117%, while RSD was below 20%. For sediments, LOD and LOQ were 0.5 and 1.0 ng g⁻¹ for diuron and Irgarol, 0.1 and 0.4 ng g⁻¹ for chlorothalonil, 0.7 and 2.1 for dichlofluanid, and 0.2 and 0.7 ng g⁻¹ for DCOIT, respectively. For APPs, LOD and LOQ were 100 times higher than those reported for sediments. An analytical curve equation in sediment (matrix-matched) and solvent was used for quantification of sediment and APPs samples, respectively (Table S2). All concentrations were reported as ng g⁻¹ (dry weight).

2.6. Butyltin Degradation Index

Butyltin Degradation Index (BDI) was calculated based on the equation: $BDI = \frac{[DBT] + [MBT]}{[TBT]}$, where values of $BDI < 1$ indicate fresh inputs of TBT (Díez et al., 2002).

2.7. Estimation of amounts of biocides stored in the APPs found in sediments

The amounts of Irgarol, diuron, DCOIT and dichlofluanid that are stored in the APPs found in sediments of each of the 4 regions assessed in the present study (Main Navigation Channel, Pouca Farinha river, Meio river and Santo Amaro river) were

estimated based on a method adapted from (Turra et al., 2014). The surface of each 4 regions (m^2) was obtained using GoogleEarth software and the corresponding sediment volume (m^3) was calculated considering the top sediment layer only (2 cm), since this was the thickness of the layer analyzed. The average amount of each biocide stored in this sediment volume was calculated considering the average concentration of APPs found in those sites sampled inside each region and their corresponding measured levels of biocides in these particles. Results were expressed as g of each biocide stored in the total sediment volume (m^3) of each region and μg of each biocide stored per square meter of sediment ($\mu g m^{-2}$).

2.8. Statistical analysis

Normality and homogeneity were verified using ShapiroWilk and Levene tests, respectively. Spearman non-parametric correlation analysis was used to investigate the relationships between concentrations of biocides and sediment parameters. A similarity analysis comparing sample sites under different naval activities (with and without maintenance facilities) was performed using Bray-Curtis similarity matrices by non-metric multidimensional scaling (nMDS). Groups formed by nMDS were further examined using a one-way PERMANOVA to significance analysis (Clarke et al., 2014). All statistical analyses were performed using Statistica® (version 12.0 (Statsoft)) with a significance level of 0.05.

3. Results and Discussion

3.1 Total carbon and granulometry

Sediments sampled in Santos-São Vicente Estuarine System (SP1 at SP30) presented fine sediments (silt + clay) ranging between 10.2% (SP25) and 73.6% (SP21) and total organic carbon (%TOC) between 0.5% (SP25) and 6.7% (SP15) (Table 1). Fine sediments and total organic carbon showed a significant correlation ($r=0.53$, $p<0.05$), as reported in other studies (Batista et al., 2016; Laitano et al., 2016), since organic matter tends to concentrate in the fine fraction of sediments (Gray and Elliott, 2014). The content of organic matter and sediment particle size influence the sorption capacity of sediments and, thus, the partitioning of organic contaminants (including organotins and booster biocides) (Pinochet et al., 2009).

3.2 Butyltin levels in sediments and imposex occurrence in gastropods

Butyltin residues (TBT, DBT and MBT) were detected in all sites with concentrations ranging from <0.8 to $1416 ng Sn g^{-1}$ (Table 1). Total organic carbon

contents (TOC %) correlated well with TBT ($r=0.61$, $p < 0.05$), DBT ($r=0.69$, $p < 0.05$) and MBT ($r=0.73$, $p < 0.05$) levels, while fine sediments (Fine %) correlated moderately with TBT ($r=0.38$, $p < 0.05$), DBT ($r=0.38$, $p < 0.05$) and MBT ($r=0.37$, $p < 0.05$) levels. In addition, there are a straightforward correlation between TBT and DBT levels (0.94 , $p < 0.05$), DBT and MBT levels (0.95 , $p < 0.05$) and TBT and MBT levels (0.9 , $p < 0.05$), indicating all analytes were originated from same sources, as also seen by Mattos et al. (2017).

In general, the highest concentrations of BTs were found along those areas adjacent to the main navigation channel, especially Pouca Farinha river (SP13-SP17) and Santo Amaro river (SP26 – SP30) where Σ BTs levels ranged from 266 to 1416 ng Sn g⁻¹ and 739 to 1394 ng Sn g⁻¹, respectively. Although not exclusively, the predominant activities in these areas are boat traffic (fishing boats) and boatyards (maintenance of small (<25m) fishing and commercial boats). In this regard, areas under the influence of marinas and fishing boats have been highlighted as important recent source of butyltins to coastal ecosystems presenting similar high butyltin levels (Batista-Andrade et al., 2018; Maciel et al., 2018). The highest contaminated samples (>200 ng Sn g⁻¹ of TBT and >700 ng Sn g⁻¹ of Σ BTs) presented carbon contents >4% associated to the >40% of fine sediments. In addition, these relatively sheltered areas present a limited capacity of dilution due to low hydrodynamics and water exchange rates. As seen in other studies, this combination of factors are often associated with high contamination levels hindering dilution and dispersion processes in coastal zones (Castro et al., 2012; Mattos et al., 2017). Intermediate BT levels ranging from 19 to 412 ng Sn g⁻¹ were found at Meio river (sites SP4; SP18 – SP24), an area dominated by marinas (pleasure boats), and SP25 (Σ BTs – 282 ng Sn g⁻¹), a shipyard by the main channel. On the other hand, other sites at the main navigation channel (SP1 – SP3; SP5-SP12) presented the lowest levels of BTs, between <0.8 and 76 ng Sn g⁻¹ (Table 1). Lower concentrations are consistent with areas of higher hydrodynamic which, in general, promote a fast dispersion of local BT inputs (Castro et al., 2018).

Although most sites have shown BDI > 1 (22 sites), indicating old inputs, levels >60 ng Sn g⁻¹ for TBT suggest that a combination of fresh and old (chronic) inputs were taking place in, at least, 12 of these sites located in those areas adjacent to the MNC. This pattern was also seen for sites SP13, SP17, SP22 and SP25, which presented BDI < 1. This may possibly happen due to recent and chronic local emission from a shipyard (SP25), and boatyards (fishing and commercial boats) and boat maintenance facilities at

marinas (other sites). Indeed, shipyards and boat maintenance facilities has been pointed out as important local sources of TBT and other antifouling residues to aquatic environments (Castro et al., 2012; Filipkowska and Kowalewska, 2019).

Based on the European, Australian and Brazilian sediment guidelines, the aquatic wildlife of SSES is under threat by butyltins contamination. The Brazilian least restrictive guideline indicate indicates that only 4 sites located in areas under the influence of boat traffic/boatyards (SP13, SP14, SP27 and SP28) showed levels of TBT above the “lowest-observed-effect concentration (LOEC)” ($\geq 410 \text{ ng Sn g}^{-1}$) (Brasil, 2012a). However, according to the classification proposed by OSPAR for TBT levels in sediments, the sites SP1, SP5, SP6 and SP10 (in MNC) were categorized in the class B (TBT levels $< 2 \text{ ng g}^{-1}$) whereas 12 (SP2-SP4, SP7 - SP9, SP11, SP12, SP18, SP20, SP22 and SP24) out of 30 were within class C and D. The highest contaminated sites (SP13-SP17, SP19, SP21, SP23 and SP25 – SP30) were inside categories E and F (OSPAR, 2011). These higher assessment classes (C, D, E and F) indicate levels able to cause reproductive effects on the sensitive gastropod species. In fact, 100% imposex incidence was detected in adult specimens of *Stramonita brasiliensis* collected at SP2 (RPLI = 26 and VDSI= 2.8), SP17 (RPLI = 18 and VDSI= 1.3) and SP26 (RPLI = 49 and VDSI= 3.4). However, imposex incidence was also detected in SP5 (38%, RPLI = 9.1 and VDSI= 0.4), a site classified as category B. This finding is probably result of old TBT inputs in this area, since imposex is an irreversible phenomenon as reported by several studies (Castro et al., 2012; Castro and Fillmann, 2012). In addition, TOC content and fines were relatively low at SP2 and SP5, indicating that higher levels of BTs might be circulating in these areas but were not properly registered in the sediments. Based on the Australian sediment quality guidelines (SQG), 17 sites showed TBT levels that may induce deleterious effects (possible adverse effects) on benthic organisms, once they exceeded the threshold value (SQGV) of 9 ng Sn g^{-1} (normalized to 1% organic carbon), while 6 sites exceeded the high trigger value (SQG High) of 70 ng Sn g^{-1} (significant adverse effects) (Simpson et al., 2013) (Table S3).

Due to the high variability in TOC levels (0.5 to 6.7 %), which may influenced sedimentary BT levels and, thus, biased data interpretation, data was normalized to 1% organic carbon (1% OC) (Simpson et al., 2013) (Table S3). Even presenting the highest TOC contents, Pouca Farinha river (SP13-SP17) and Santo Amaro (SP 26-SP30) river still presented the highest TOC normalized concentrations (Table S3). The highest TOC-normalized concentration was seen at SP25 (340 ng Sn g^{-1}), the shipyard located

at the MNC, indicating significant sources of TBT at this area since sediments with low TOC value (0.5%) and fines (10.2%) are less likely to accumulate BTs residues (Artifon et al., 2016). Although showing slightly lower TOC normalized concentrations, Meio river is still a relevant source of BTs, while data confirmed that Santos Port is not acting as a relevant source. Thus, areas under the influence of the shipyard (SP25) located at the MNC, and the boatyards, marinas (with maintenance facilities) and fishing boats located at the adjacent areas must be treated as hotspots of fresh and chronic contamination by TBT and, as such, subject to continuous monitoring and management aiming at the environmental mitigation of TBT antifouling residues and wastes.

3.3 *Booster biocides levels in sediments*

DCOIT followed by diuron, chlorothalonil and dichlofluanid were the most frequently detected booster biocides, seen in 77%, 57%, 50% and 40% of the SSES sediments, respectively, while Irgarol was <LOD for all sites (Figure 3). Contamination levels of DCOIT, diuron, chlorothalonil and dichlofluanid ranged from <0.2 to 75 ng g⁻¹, <0.5 to 9.9 ng g⁻¹, <0.1 to 9.2 ng g⁻¹, and <0.7 to 16 ng g⁻¹, respectively. To our knowledge, the present study is the first reporting the occurrence of chlorothalonil and dichlofluanid in estuarine sediments of Latin America. The intermediate log K_{oc} (partition coefficient related to the organic carbon content) suggests diuron (2.3), Irgarol (3.3), chlorothalonil (2.9) and dichlofluanid (3.1) residues partition less into particulate phases than DCOIT (4.2) and TBT (4.7) (Chen et al., 2015a; Konstantinou and Albanis, 2004). The lack of correlation between booster biocide levels presenting log K_{oc} ≈ 3 and TOC % and granulometry is an indication that their partitioning into water phases can be more relevant than sediment accumulation (Chen and Lam, 2017).

Contamination by DCOIT was evenly distributed in SSES, including areas along the main navigation channel (ship traffic and shipyard) and adjacent areas of Meio river (SP4, SP18 - SP24) and Pouca Farinha river (SP14 – SP17) and Santo Amaro river (SP26) under the influence of marinas, boat traffic and boatyards (fishing), indicating its widespread use in the region. Even so, DCOIT contamination was consistent (3.2 – 74.6 ng g⁻¹) and presented the highest levels at Meio river, which might be due to the predominance of yachts and huge pleasure boats probably using modern DCOIT-based formulation paints. Although only a couple of studies have assessed DCOIT in sediments of Latin America, the range of concentrations are comparable. Batista-Andrade et al. (2018) found levels between < 0.4 and 82 ng g⁻¹ in sediments of Panamá, while Soroldoni et al. (2018) reported concentrations ranging from <0.5 to 274

ng g⁻¹ in sediments of an estuary in Southern Brazil. For both studies, the highest levels were associated to the occurrence of antifouling paint particles (APPs), which could explain the high DCOIT levels at sites SP8 - International terminal (63.7 ng g⁻¹), located in the main channel nearby a large shipyards, and SP22 (74.6 ng g⁻¹), located in Meio river nearby a large boatyard (see Section 3.4).

DCOIT was recognized as an environment safer alternative in comparison to TBT (Jacobson and Willingham, 2000) and it is approved for use (1st January 2016) by the European Chemicals Agency (ECHA) based on its fast degradation (< 24h in sediment) and low bioaccumulation factor (ECHA, 2019). Biocides used in antifouling products are categorized by ECHA as product type 21 (PT 21), which are products used to control the growth and settlement of fouling organisms on vessels, aquaculture equipment or other structures used in water (Wezenbeek et al., 2018). As a consequence, DCOIT is one of the main biocides registered as active ingredient in formulation of many antifouling paints currently in use (Paz-Villarraga et al., submitted.; Tornero and Hanke, 2016; Wezenbeek et al., 2018). However, due to the increase in its frequency of use and continuous inputs, even with short half-life (Chen and Lam, 2017; Soroldoni et al., 2018), DCOIT has been regularly detected around the world in areas under the influence of maritime activities (Lee et al., 2015; Mukhtar et al., 2019) and at levels capable of causing toxic effects onto non-target aquatic organisms (Ito et al., 2013; Onduka et al., 2013). In addition, new uses of DCOIT as fungicides in plastics materials (Schoknecht et al., 2012) and silicone rubbers (Akbar et al., 2019) may contribute as future sources nearby highly urbanized areas.

Onduka et al. (2013), for instance, set a NOEC (no observed effect concentration) of 9.7 ng g⁻¹ based on toxicity tests assessing growth of polychaeta *Perinereis nuntia* exposed to DCOIT in sediment. For assays in water column, LOEC (lowest observed effect concentration) was set at 0.07 µg L⁻¹ using diatom (Onduka et al., 2013) and 0.76 µg L⁻¹ using fish (Chen et al., 2015b), respectively. Since no other toxicity test for sediment is available, no robust environmental quality standard (EQS), based on predicted no effect concentrations (PNECs), can be established (Martins et al., 2018). However, considering the threshold level of Onduka et al. (2013), most sediments located in Meio river (SP4, SP19-SP22, SP24) and main navigation channel under direct influence of Santos port (SP5-SP6, SP8) are likely to cause toxicity to the biota. Therefore, an environmental risk assessment for DCOIT is urgent to support decision-makers on regulating this biocide since there are no laws to control it in Brazil.

Chlorothalonil was mainly detected in the main navigation channel (7 out of 12 sediments) and less often in the areas adjacent to the main channel and under the influence of marinas and boatyards (7 out of 18 sites). However, 60% of Pouca Farinha river sites were contaminated, presenting the 2nd highest level at SP16 (5.3 ng g⁻¹), while the highest level of 9.2 ng g⁻¹ was at Terminal 3 (SP8), one of the main mooring areas of Santos Port. On contrary, although one of the highest levels (15.1 ng g⁻¹) was also detected at Terminal 3 (SP8), dichlofluanid was mainly detected in the adjacent areas (50% of sites), specially at Santo Amaro river (5 out of 5 sites), and only at 3 out of 12 sediments from the main navigation channel. Bearing in mind that release rates are likely to be distinct, this dissimilar distribution pattern may indicate distinct sources of contamination for these biocides. Although these booster biocides had been associated to maritime activities, their sources may additionally be related to agricultural runoff. However, only 1.9% and 0.4% of antifouling paint formulations registered for use worldwide (none in Brazil) utilize dichlofluanid and chlorothalonil, respectively, as active ingredients (Paz-Villarraga et al., submitted). In addition, almost no agriculture activities have been detected in the drainage basin of SSES (Oliveira, 2013), and only chlorothalonil is registered for pesticide use in Brazil (Ministério da Agricultura, 2019).

Levels of chlorothalonil ranging from <4.1 to 47 and <LOD to 56 ng g⁻¹ were found in coastal sediments of UK and Greece, respectively. However, the highest concentrations were associated to antifouling paint particles that preserve biocides longer in sediments (Albanis et al., 2002). A degradation product of dichlofluanid (DMSA) and chlorothalonil were detected in sediments along the coastal area of Korea (< LOD – 81.5 ng g⁻¹) and (1.2 – 99 ng g⁻¹), respectively. Furthermore, higher levels were detected in areas with intense maritime activity (ports and shipyards) such as, for example, Busan bay (21.5 – 1123 ng g⁻¹ for DMSA and 22 – 1065 ng g⁻¹ for chlorothalonil) (Lee et al., 2015). However, almost no information is available on sedimentary levels of chlorothalonil and dichlofluanid along the American continent. Vieira et al. (2016) detected chlorothalonil and dichlofluanid (0.004 ng g⁻¹ and 0.02 ng g⁻¹, respectively) in tributaries of Tibagi river basin in Southern Brazil, while Sapozhnikova et al. (2004) detected chlorothalonil (<0.1 – 8.9 ng g⁻¹) in Salton Sea lake in the USA (dichlofluanid was not analyzed). Dichlofluanid was not detected (<LOD) in sediments along the coast of Panama (chlorothalonil was not analyzed) (Batista-Andrade et al., 2018).

In the European Union, chlorothalonil has not been registered for use as biocide in antifouling products (ECHA product type PT 21) (Wezenbeek et al., 2018) and is no longer used as fungicide due, especially, to its carcinogenic potential (European Commission, 2019). On other hand, dichlofluanid has its use as antifouling biocide approved on 1st November 2018 (Wezenbeek et al., 2018). In Brazil, there is no specific regulation for use of chlorothalonil and dichlofluanid in antifouling paint formulations. Chlorothalonil acts in inactivation of cell sulfhydryl enzymes, presents moderate toxicity to aquatic organisms (Haque et al., 2019) and has the potential to bioaccumulate in aquatic species (Scoy and Tjeerdema, 2007). Barreto et al. (2018) has observed that short-term exposure to relatively high chlorothalonil levels ($70 \mu\text{g L}^{-1}$) induced oxidative stress and changes in the cholinergic system of resistant polychaeta species (*Laeonereis acuta*). As for dichlofluanid, even acting as inhibitor of electron transport, it has a lower water toxicity compared to other antifouling biocides (Amara et al., 2018b). However, since no information about toxicity of chlorothalonil and dichlofluanid to benthic organisms is available, it is not possible to establish a robust environmental quality standard (EQS) (Martins et al., 2018). Considering these compounds have been detected in sediments, despite their short half-lives (8 days and <1 day in sediments, respectively) (Cassi et al., 2008), their toxicity to benthic organisms should be better assessed.

Diuron and Irgarol are more persistent than DCOIT, chlorothalonil and dichlofluanid in the environment (half-lives of 14 and 100 days in sediment, respectively), and their intermediate log K_{oc} (2.3 and 3.3, respectively) indicate partition in both sediments and water column (Thomas and Brooks, 2010). Due to the toxicity for primary producers, their use as antifouling biocides was restricted in many countries (Lambert et al., 2006). For example, New Zealand has banned diuron, Australia prohibited Irgarol and both has been banned in the European Union (Price and Readman, 2013; Wezenbeek et al., 2018). Therefore, reductions in the environmental levels following the effectiveness of regulations issued worldwide are expected, especially for areas receiving ships mainly from international flags, such as Santos Port. Levels of Irgarol and diuron in sediments of the main navigation channel were below detection limit ($<0.5 \text{ ng g}^{-1}$), except for diuron at SP3 (by the entrance of Pouca Farinha river). However, although Irgarol was not detected in any sediments of those areas adjacent to the main channel, diuron was detected in 16 out of 18 sites with the highest concentrations in areas directly under the influence of marinas (with expensive leisure

boats) at Pouca Farinha river (SP3-SP17) and Meio River (SP4, SP18 – SP24), as seen for DCOIT. This may suggest the current use of modern antifouling paints in these areas. Micron® (Soroldoni et al., 2017), for instance, indicates the simultaneous use of diuron and DCOIT in the Material Safety Data Sheet of some formulations, composition that was analytically confirmed by our research group (unpublished data). Baptista-Andrade et al. (2018) have also identified marinas and shipyards as current sources of diuron to coastal areas of Panama.

Regarding toxicity, level of diuron measured in SP20 (9.9 ng g^{-1}) was above the threshold limit (Maximum Permissible Concentration – MPC) proposed by Dutch authorities (9 ng g^{-1}) (Crommentuijn et al., 2000). Thus, organisms from this area may present toxic effects following short-term exposure to diuron. In addition, sites SP18 to SP24 (Meio river) and SP3 (Pouca Farinha river) (Table S3) can be classified as class III “moderate” (0.7 and 6.4 ng g^{-1} , normalized to 1% OC) by the Norwegian sediment quality criteria (Bakke et al., 2010), and organisms from these areas may present toxic effects following chronic exposure to diuron.

3.4 Paint Particles in sediments

APPs are residues of current environmental concern because they can behave as local “hot spots”, becoming a long-term repository and a continuous source of contemporary and legacy antifouling biocides to aquatic environments (Tolhurst et al., 2007). Once associated to paint particles, dichlofluanid and DCOIT increased their half-lives and, thus, persistence in the environment (Thomas et al., 2003). APPs act as capsule and protect biocides from degradation factors (Soroldoni et al., 2017). APPs pose a threat to filter-feeding organisms by enhancing concentrations of these contaminants in interstitial waters and, by desorption or during sediment resuspension or dredging, to the overlying water column (Turner, 2010). At the same time, deposit-feeding organisms can be contaminated by feeding on particles directly from sediments (Soroldoni et al., 2017; Gallucci et al., 2015).

Antifouling paint particles (APPs) levels ranging between 4.8 and $5,248 \text{ } \mu\text{g g}^{-1}$ were observed in 29 out of 30 sediments of SSES (Table 2). The highest amounts of APPs ($>1500 \text{ } \mu\text{g g}^{-1}$) were observed nearby shipyards (SP7 and SP25) located at the main navigation channel of Santos port. Indeed, the prevalence of APPs in sediments seems to be related to boat/ship maintenance activities, as demonstrated by Soroldoni et al. (2017), with relevant amounts ($>100 \text{ } \mu\text{g g}^{-1}$) occurring also in sites located within

Pouca Farinha (SP3, SP13 – SP16), Meio (SP4, SP19) and Santo Amaro (SP26 – SP28, SP30) rivers.

The detection of booster biocides (diuron, Irgarol, DCOIT and dichlofluanid) and BTs (in select particles) collected within sediments of SSES confirm their origin associated to antifouling paints (Table 2). Levels of booster biocides in APPs ranged from <50 to 5,200 ng g⁻¹ for diuron, <50 to 37,600 ng g⁻¹ for Irgarol, <20 to 110,000 ng g⁻¹ for DCOIT and <70 to 46,700 ng g⁻¹ for dichlofluanid, while BTs varied from <250 to 4,890 ng Sn g⁻¹ for TBT, <500 to 2,540 ng Sn g⁻¹ for DBT and <500 to 6,470 ng Sn g⁻¹ for MBT. The significant levels, especially at sites from the main navigation channel and Meio river (SP22-SP24), confirmed APPs as a relevant secondary source of DCOIT (>1 – 110 µg g⁻¹ APPs), but also Irgarol (0.3 – 37 µg g⁻¹ APPs) and diuron (0.3 – 5 µg g⁻¹ APPs), in spite of the relatively low levels of APPs (except SP7 and SP25). This pattern coincides with the general distribution of DCOIT in sediments, confirming, as seen by Paz-Villarraga et al. (submitted), its prevalence in modern antifouling paint formulations currently used by ships and recreational boats from marinas. The high concentrations of DCOIT in sediments with low levels of APPs are possibly explained by the occurrence of a fraction of paint particles that was not detectable by the method applied. Methods that wear out the coating (i.e. sanding, dry or wet blasting) instead of removing the whole coating (i.e. scraping off) for instance, are more likely to generate APPs smaller than 500 µm. And significant levels of biocide are present in a wider size range of APPs (30 – 2000 µm) (Wu et al., 2016). Conversely, in spite of relatively more abundant, APPs from Pouca Farinha and Santo Amaro rivers showed no DCOIT in their composition which, in combination with levels of this biocide in sediments of these two areas, indicate a recent use of DCOIT-based paints and/or chronic use of cheap versions of antifouling paints (booster biocides free and/or TBT-based) and/or APPs originated from old and leached out coatings.

The high levels of BTs in APPs from SP14 and SP15, together with the correspondent BTs levels in sediments, reinforce the idea that TBT is still in use as antifouling biocide. It is also possible that maintenance of quite old vessels may be remobilizing ancient coating containing significant levels of BTs, whose APPs may act as source of contamination to the surrounding sediments, as seen by Lagerström et al. (2017). APPs from the main channel (SP8) where BTs levels were <LOD/<LOQ, however, confirm that activities directly related to the largest port in South America itself are unlikely to be related to current sources of TBT. An exception is the high

levels of BTs in APPs and sediment of site SP25 (shipyard) that are probably associated to the operation and maintenance of ferryboats in that specific area.

Although not very often, the occurrence of booster biocides associated to APPs has already been reported. Irgarol has been found in APPs (up to 60 ng g⁻¹) obtained in a marina from southern England (Zhou, 2008), while dichlofluanid (<0.01 and 22.1x10⁶ ng g⁻¹), Irgarol (<0.01 and 1.15x10⁶ ng g⁻¹) and chlorothalonil (<0.01 and 1.17x10⁶ ng g⁻¹) were found in APPs from marinas and boatyards from southwest England and Channel Islands (Parks et al., 2010). Recently, Soroldoni et al. (2018) found diuron (<0.1 – 25.3 ng g⁻¹), Irgarol (<0.1 – 3.2 ng g⁻¹) and, specially, DCOIT (<1.5 – 67,100 ng g⁻¹) in APPs from an estuary in Southern Brazil. These analyses in paint particles reflect the biocides currently used in antifouling paints formulations. During the last decade, for example, some studies have related sedimentary and water levels of Irgarol to the environmental occurrence of paint particles (Thomas et al., 2003). However, in addition to release rate from APP, Irgarol concentrations found in these studies may be related to common use of other Irgarol-based products (Bowman et al., 2003). On the other hand, the biocide amounts associated to APPs in the present study were not consistent to levels measured in sediment samples. This distortion might be explained by the low release rates of these biocides, occurrence of old particles and/or distribution heterogeneity of APP in analyzed samples (Hasan et al., 2014). In this regard, bias may emerge from the occurrence of APP in sediments samples during monitoring studies of antifouling biocides, as seen by Lagerström et al. (2017).

Since APPs can act as a long-term repository and a continuous source of contemporary and legacy antifouling biocides to aquatic environments (Tolhurst et al., 2007), the amounts of Irgarol, diuron, DCOIT and dichlofluanid that are likely to be stored in the APPs found in the first 2 cm of sediment layer were estimated for each of the 4 regions assessed in the present study (MNC, Pouca Farinha river, Meio river and Santo Amaro river). The higher biocide load was found in the MNC region (Irgarol – 252 g, diuron – 153 g, dichlofluanid – 24 g and DCOIT - 3,876 g), which was influenced by high levels of APP in both shipyards, its large surface area and volume of sediment (367,336 m³) involved. Although the load of biocides stored in sediment from other regions seemed to be proportionally lower (≤ 2.4 g), the amount of diuron, Irgarol and dichlofluanid per m² in Santo Amaro river (8.0, 10.9 and 2.5 $\mu\text{g m}^{-2}$, respectively) is similar to those in the MNC (8.3, 13.7 and 1.3 $\mu\text{g m}^{-2}$, respectively), while DCOIT may reach 211 $\mu\text{g m}^{-2}$ in the MNC (Table 3). However, since the APPs were assessed using

only the > 500 μm fraction, which underestimate the amount of paint particles in the sediment (Lagerström et al., 2017), the amounts of biocides stored in SSES may be even higher than those estimated here. This hypothesis is corroborated considering that blasting techniques used to remove old coatings from commercial ships and recreational boats may lead to APPs fragmentation at sizes below 500 μm , which could not be quantified based on the method used in the present study (Mills and Twigg, 2017). Thus, it is possible that APPs as well as biocides levels (released to sediments and water phase) in Meio river and MNC are underestimated because the heterogeneity (size, biocide load, age, shape, etc.) of these residues. Similar situation has already been seen for organotin compounds in Sweden, Finland and Germany (Lagerström et al., 2017).

Either in normal leaching, or under dredging and storm events, for example, particles and APP-associate biocides are likely to be bioavailable in the environment (Singh and Turner, 2009a), which clearly represents a risk to aquatic organisms. In addition, even though the biocide leaching from these APPs is reduced along time, paint particles still denotes a threat to benthic invertebrates, as demonstrated by Soroldoni et al. (2020) on organism from different habitats and feeding modes (a tanaid and an amphipod).

In addition to high booster biocide concentrations, APPs are heterogeneous residues constituted by multiple layers of paint that contain many other chemicals, such as additives, solvents, pigments, primers, characterizing a very complex matrix and, therefore, an analytical challenge to be overcome (Turner et al., 2015). Besides that, chemical determinations in APPs should be done carefully due to the heterogeneity of formulations used over the years and their differential degrees of leaching (or ages since deposition) (Muller-Karanassos et al., 2019). Although the present study represents an advance regarding the assessment of spatial distribution and chemical contamination by APPs, additional efforts should be made to include other currently used booster biocides. Cu and Zn pyrithione, for instance, are widely used in antifouling paints (Paz-Villarraga et al., submitted), but little information is available since requires a complex analytical procedure due to the transchelation reaction (Thomas and Brooks, 2010).

4. Data Integration

The SSES circulation is strongly influenced by mixed tides, cold fronts, intense rainfall regime and variation of local mean sea level (Harari and Camargo, 1998). Thus, in addition to contamination sources, biocide residues in sedimentary layers can be

modulated by hydrodynamic features, since high flows observed in the MNC (SP1, SP2, SP3, SP5, SP6, SP7, SP8, SP9, SP10, SP11, SP12 and SP25) promote mobilization and consequent dilution, as seen by Santos et al. (2016). A higher energy of superficial currents in MN channel and the consequent dispersion of floating litter toward Santos bay was already pointed out by Fernandino et al. (2016). Similarly, a hydrodynamic model evaluating estrogen levels in surface waters showed low residence times (seven days) in Santos estuary, including the MNC (Gimiliani et al., 2016). These alterations may also emerge from changes in the depositional dynamics of contaminants or dredging operations performed at the MNC, which can remobilize the contaminants historically deposited, as seen by Sousa et al. (2018) for PCBs.

Accordingly, the contamination by antifouling biocides measured in SSES along the MNC and adjacent areas showed different profiles. These differences, confirmed by dissimilarity matrix using Bray-Curtis distance (Figure 4), are probably results of the presence of different sources associated to local hydrodynamics, since the current fluxes are less intense in sheltered areas (adjacent areas). The main aspects evidenced by dissimilarity matrix are the differential releases of antifouling biocides in each region of SSES. The majority of sites located in the MNC (Figure 4A), for instance, presented lower levels for BTs, Irgarol, diuron, chlorothalonil and dichlofluanid, in association to moderate concentrations of DCOIT, which is due probably to the influence of commercial ships serviced with modern and more expensive paints (Tornero and Hanke, 2016). The opposite pattern was observed in most sites inside Pouca Farinha river (SP13, SP14 and SP15) and in all samples obtained in Santo Amaro river (SP26, SP27, SP28, SP 29 and SP30) (Figure 4C), where higher butyltins concentrations, moderate to low levels of DCOIT and low levels for all other booster biocides were found. The maritime activities in these areas are dominated by fishing boats probably using TBT-based antifouling paints or ancient coatings. In addition, inside Santo Amaro and Pouca Farinha rivers operates very precarious boatyards facilities (maintenance of small (<25m) fishing and commercial boats), which contributed locally with large inputs of APPs. In Meio river, sampling sites were under the influence of marinas used by recreational (more expensive) boats (Figure 4B), where moderate to high levels of DCOIT and diuron, and moderate BTs concentrations were found (except SP18). In this regard, the multi-dimensional scale plot grouped SP7 and SP25 (shipyards) together with other sites from Meio River ($p < 0.05$) due to the high levels of BTs (SP25) or DCOIT (SP7).

5. Conclusions

Impacts associated to antifouling biocides along the sediments of Santos-São Vicente Estuarine System were directly related to the profile of local maritime activities, where sediments from the main navigation channel (except those under the influence of shipyards) presented relatively lower levels of antifouling residues while adjacent areas, characterized by the presence of boats and boatyards, showed much higher contamination levels. The relatively high levels ($>60 \text{ ng Sn g}^{-1}$) suggest that a combination of fresh and old (chronic) inputs of TBT were taking place. DCOIT followed by diuron, chlorothalonil and dichlofluanid were the most frequently detected booster biocides, while Irgarol was $<\text{LOD}$. Although ubiquitously distributed, DCOIT was mainly detected in sediments under the influence of Santos port (MNC) and recreational boats (adjacent areas), while diuron was mainly detected in areas under the direct influence of marinas.

Sites located nearby shipyards in the main navigation channel and boatyards in the adjacent areas presented expressive amounts of APPs ($>200 \mu\text{g g}^{-1}$). The detection of booster biocides and BTs confirm their origin associated to antifouling paints, highlighting the relevance of APPs as long-term repository and a continuous source of contemporary and legacy antifouling biocides to SSES.

Although it was not possible to assess the threat associated to the sedimentary levels of chlorothalonil and dichlofluanid, some levels of TBT, DCOIT and diuron exceeded international threshold limits and are likely to cause sublethal effects to the local aquatic biota. Thus, new effective regulations must be implemented (since only TBT-based antifouling paints are regulated) to protect organisms and functionalities of aquatic ecosystems exposed to antifouling residues originated from different maritime activities.

Acknowledgment

The present research was sponsored by FINEP – *Financiadora de Estudos e Projetos* (Proc. No 1111/13 – 01.14.0141.00) and CAPES - *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior* (CIMAR II No 1988/2014). F. E. L. Abreu (PhD grant) and J.N. Lima da Silva (MSc grant) were sponsored by the CAPES and *Programa de Monitoramento Ambiental da Superintendência do Porto do Rio*

Grande (FURG-FAURG-SUPRG 816/2017). I.B. Castro (PQ 302713/2018-2) and G. Fillmann (PQ 312341/2013-0 and 314202/2018-8) are research fellows of CNPq. Authors thank the LACOM - *Laboratório de Análises de Compostos Orgânicos e Metais* (FURG) for LC-ESIMS/MS analyses.

Tabela 1: Total organic carbon (% TOC), percentage of fines, levels of TBT, DBT, MBT and sum of BTs (Σ BTs) (ng Sn g⁻¹), butyltin degradation index (BDI), and levels of diuron, Irgarol, chlorothalonil (Chlor), dichlofluanid (Dichlo) and DCOIT (ng g⁻¹) for sediment samples of SSES.

Site Code	Site Location	Main Activity	TOC (%)	Fine (%)	TBT	DBT (ng Sn g ⁻¹)	MBT	Σ BTs*	BDI*	Diuron	Irgarol	Chlor (ng g ⁻¹)	Dichlo	DCOIT
SP1	Santos Bay	Ship traffic/boat traffic	1.5	19.3	<0.5	<0.5	<0.5	<0.8	2	<0.5	<0.5	4.8	<0.7	6.5
SP2	MN Channel	Ship traffic/boat traffic	0.8	17.3	1.1	<0.5	2.2	3.3	2.5	<0.5	<0.5	<0.1	<0.7	<0.2
SP3	MNC/AA-PFR	Ship traffic/boat traffic	3.5	38.8	8.1	10.8	14.9	33.8	3.2	5.9	<0.5	2.4	<2.1	5.7
SP4	AA-MR	Ship traffic/boat traffic	3.2	33.9	23.5	18	35.5	77	2.3	1.3	<0.5	4.7	<0.7	20.2
SP5	MN Channel	Ship traffic	1.3	22.2	<0.5	4.2	3.0	7.5	28	<0.5	<0.5	1.4	<0.7	12.2
SP6	MN Channel	Ship traffic	3.0	37.2	<0.5	1.8	<0.5	2.3	8.2	<0.5	<0.5	0.6	<0.7	11.3
SP7	MN Channel	Ship traffic / shipyard	2.7	32.5	2.5	9.0	64.8	76.3	30	<0.5	<0.5	<0.4	<2.1	5.4
SP8	MN Channel	Ship traffic	2.8	39.4	16.9	8.1	2.8	27.8	0.6	<0.5	<0.5	9.2	15.1	63.7
SP9	MN Channel	Ship traffic	3.4	65.1	23.6	4.4	3.3	31.3	0.3	<0.5	<0.5	<0.1	<0.7	8.5
SP10	MN Channel	Boat traffic	3.5	33.5	<0.5	8.6	5.2	14.1	55	<0.5	<0.5	<0.1	<0.7	<0.2
SP11	MN Channel	Boat traffic	1.1	14.8	1.4	<0.5	<0.5	1.9	0.4	<0.5	<0.5	3.0	<0.7	7.7
SP12	MN Channel	Boat traffic	1.0	11.2	7.9	4.0	<0.5	12.4	0.5	<0.5	<0.5	2.0	<0.7	<0.7
SP13	AA(PFR)	Boat traffic/ boatyard	5.4	59.2	688	304	306	1298	0.9	<1	<0.5	0.6	<2.1	<0.2
SP14	AA(PFR)	Boat traffic/ boatyard	6.3	48.9	444	292	381	1118	1.5	<1	<0.5	<0.1	<0.7	8.5
SP15	AA(PFR)	Boat traffic/ boatyard	6.7	64.6	383	224	809	1416	2.7	<0.5	<0.5	<0.1	<0.7	1.0
SP16	AA(PFR)	Boat traffic/ boatyard	3.7	36.3	104	60.0	164	328	2.2	<1	<0.5	5.3	2.7	1.2
SP17	AA(PFR)	Boat traffic/ marina	2.5	52.6	140	65.2	61	266	0.9	1.6	<0.5	<0.4	<0.7	9.4
SP18	AA(MR)	Boat traffic/ marina	1.7	53.5	5.0	9.0	5.2	19.2	2.8	2.3	<0.5	<0.1	<0.7	3.2
SP19	AA(MR)	Boat traffic/ marina	2.8	62.7	90.9	41.3	97	229	1.5	4.9	<0.5	<0.1	4.7	11.9
SP20	AA(MR)	Boat traffic/ marina	3.3	53.9	65.2	42.4	84.6	192	1.9	9.9	<0.5	2.1	<0.7	17.3
SP21	AA(MR)	Boat traffic/ marina	3.2	73.6	169	73.7	123	365	1.2	6.5	<0.5	<0.1	<0.7	15.6
SP22	AA(MR)	Boat traffic/ marina	3.3	63.9	68.4	32.4	22.2	123	0.8	7.3	<0.5	<0.1	<0.7	74.6
SP23	AA(MR)	Boat traffic/ marina	2.8	58.2	118	126	168	412	2.5	3.4	<0.5	<0.1	3.0	6.4
SP24	AA(MR)	Boat traffic/ marina	4.1	53.3	80.4	41.4	109.2	231	1.9	5.8	<0.5	<0.1	<0.7	27.4

SP25	MN Channel	Ship traffic/ shipyard	0.5	10.2	160	56.3	65.5	282	0.8	<0.5	<0.5	<0.1	<0.7	<0.2
SP26	AA(SAR)	Boat traffic/ marina	5.7	47.1	214	104	420	739	2.5	<1	<0.5	<0.4	<2.1	6.5
SP27	AA(SAR)	Boat traffic/ boatyard	5.3	35.3	465	202	727	1394	2	<1	<0.5	<0.4	<2.1	<0.2
SP28	AA(SAR)	Boat traffic/ boatyard	4.7	32.1	462	158	637	1257	1.7	<0.5	<0.5	<0.1	<2.1	<0.2
SP29	AA(SAR)	Boat traffic/ boatyard	4.3	50.2	297	140	322	759	1.6	<1	<0.5	<0.1	16.0	2.7
SP30	AA(SAR)	Boat traffic/ boatyard	3.3	71.6	347	130	266	743	1.1	<1	<0.5	<0.1	<2.1	<0.2

* Whenever results were <LOD or < LOQ, one-half of the detection or quantification limit, respectively, was used for calculating ΣBTs and BDI.

MN Channel – main navigation channel, AA(PFR) – adjacent area (Pouca Farinha river); AA(MR) – adjacent area (Meio river); AA(SAR) – adjacent area (Santo Amaro river)

Table 2: Occurrence (ng g^{-1}) of antifouling paint particles (APPs) in sediments collected along the SSES and concentrations of diuron, Irgarol, DCOIT, dichlofluanid and BTs (ng g^{-1}) in the correspondent APPs.

Site Code	APPs ($\mu\text{g g}^{-1}$)	Diuron (ng g^{-1})	Irgarol (ng g^{-1})	DCOIT (ng g^{-1})	Dichlo. (ng g^{-1})	TBT (ng Sn g^{-1})	DBT (ng Sn g^{-1})	MBT (ng Sn g^{-1})
SP1	<0.01	n.a	n.a	n.a	n.a	n.a	n.a	n.a
SP2	219	500	430	2,350	<70	n.a.	n.a.	n.a.
SP3	268	320	390	3,250	1,100	n.a.	n.a.	n.a.
SP4	210	<50	310	2,150	860	n.a.	n.a.	n.a.
SP5	5.0	450	280	1,600	<70	n.a.	n.a.	n.a.
SP6	4.8	630	390	2,500	<70	n.a.	n.a.	n.a.
SP7	5248	<50	520	3,100	<70	n.a.	n.a.	n.a.
SP8	6.5	<50	300	1,600	<70	<250	<500	<500
SP9	7.2	1,150	580	3,800	<70	n.a.	n.a.	n.a.
SP10	31.8	<50	520	4,450	<70	n.a.	n.a.	n.a.
SP11	7.2	<50	720	28,750	1,370	n.a.	n.a.	n.a.
SP12	260	<50	310	4,050	820	n.a.	n.a.	n.a.
SP13	164	<50	<50	<20	<70	n.a.	n.a.	n.a.
SP14	664	<50	<50	<20	<70	4890	<500	<500
SP15	1496	<50	<50	<20	<70	3590	2540	4900
SP16	267	<50	<50	<20	<70	n.a.	n.a.	n.a.
SP17	11	<50	<50	<20	<70	n.a.	n.a.	n.a.
SP18	98	<50	<50	<20	<70	n.a.	n.a.	n.a.
SP19	300	<50	<50	<20	<70	n.a.	n.a.	n.a.
SP20	46	<50	<50	3,350	18,500	n.a.	n.a.	n.a.
SP21	4.9	<50	<50	1,100	1,500	n.a.	n.a.	n.a.
SP22	67	1,350	1,200	7,300	46,700	n.a.	n.a.	n.a.
SP23	47	5,200	3,050	28,800	<70	n.a.	n.a.	n.a.
SP24	58	5,150	37,600	110,000	<70	n.a.	n.a.	n.a.
SP25	1851	1,700	1,300	35,250	<70	3730	2040	6470
SP26	210	<50	<50	550	<70	n.a.	n.a.	n.a.
SP27	586	<50	<50	<20	710	n.a.	n.a.	n.a.
SP28	609	2,100	2,400	<20	<70	n.a.	n.a.	n.a.
SP29	53	1,050	1,050	<20	<70	n.a.	n.a.	n.a.
SP30	156	<50	1,800	<20	<70	n.a.	n.a.	n.a.

* Dichlo – dichlofluanid, n.a. – APP was no longer available for this analysis, MN Channel – main navigation channel, AA(PFR) – adjacent area (Pouca Farinha river); AA(MR) – adjacent area (Meio river); AA(SAR) – adjacent area (Santo Amaro river)

Table 3 – Amounts of biocides stored in the antifouling paint particles found in the total volume of sediments (g) and per area ($\mu\text{g m}^{-2}$) of different regions of Santos-São Vicente Estuarine System. * m^3 - considering 2 cm of the top sediment layer only. <LOD- below limit of detection.

Region	Sediment volume (m^3) in the region*	Amounts of biocides stored in the total volume of sediments (g) and per area ($\mu\text{g m}^{-2}$)			
		Diuron	Irgarol	DCOIT	Dichlofluanid
Main navig. channel	367,336	153 / 8.3	252 / 13.7	3,876 / 211	23.7 / 1.3
Pouca Farinha river	1,452	<LOD/<LOD	<LOD/<LOD	<LOD/<LOD	<LOD/<LOD
Meio river	4,800	0.2 / 2.3	0.7 / 9.3	2.4 / 33.1	1.1 / 15.5
Santo Amaro river	4,200	1.7 / 8.0	2.3 / 10.9	0.5 / 0.7	0.1 / 2.5

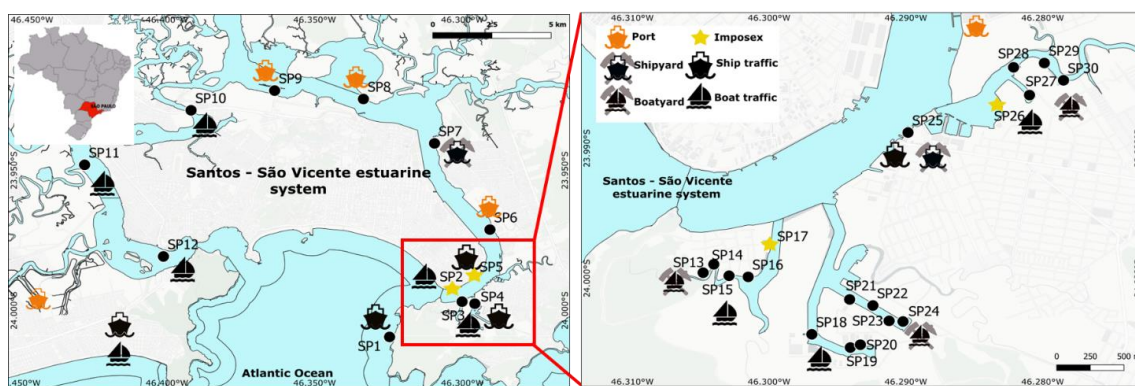


Fig. 1. Sampling sites of surface sediments and imposex in the Santos-São Vicente Estuarine system (SSES)

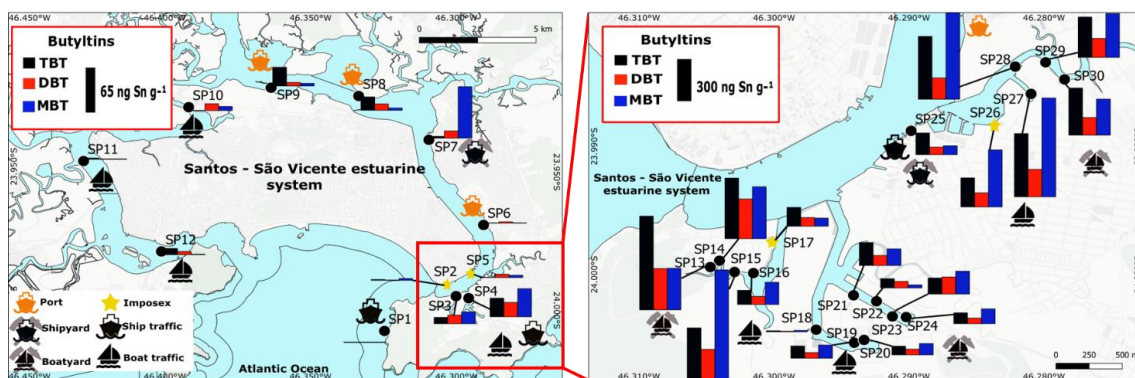


Fig. 2. Concentrations of butyltins (ng Sn g^{-1}) in sediments of Santos-São Vicente Estuarine system (SSES).

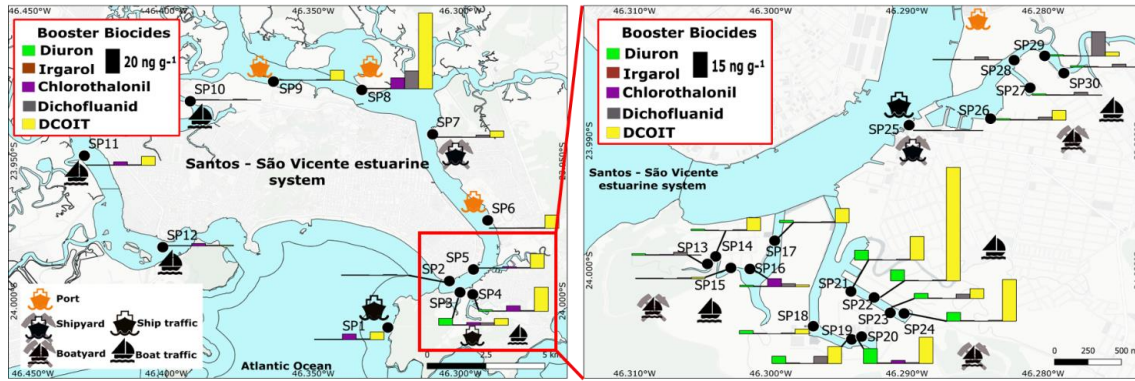


Fig. 3. Concentrations of booster biocides (ng g⁻¹) in sediments of Santos-São Vicente Estuarine system (SSES).

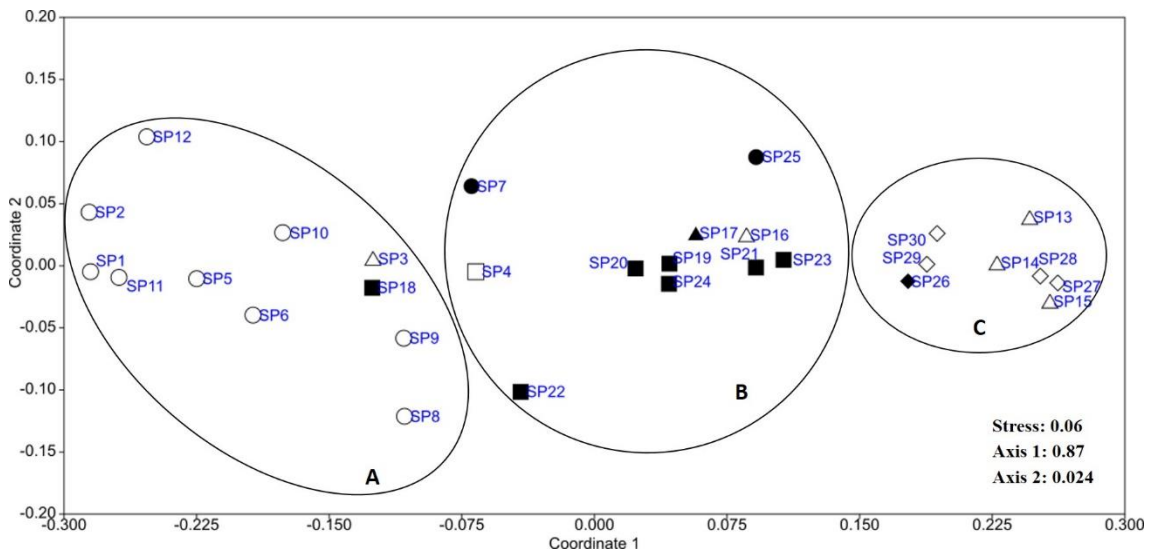


Fig. 4. Non-metric multi-dimensional scale plot of distances by Bray-Curtis dissimilarity matrix discriminating sediments with contamination profile characteristic of sites under the influence of (A) main navigation channel with ship/boat traffic (B) leisure boats (traffic and boatyard) and (C) fishing boats (traffic and boatyard). O – sites located at the main navigation channel; □ – sites located at Meio river; Δ – sites located at Pouca Farinha river and ◇ – sites located at Santo Amaro river. Solid circle indicates shipyard area, while solid square, triangle and diamond indicates marina region.

ARTIGO 2

Legacy and emerging antifouling biocide residues in a tropical estuarine system (Vitória state, SE, Brazil)

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ABSTRACT

The contamination by antifouling biocide residues (booster biocides - diuron, Irgarol, chlorothalonil, dichlofluanid and DCOIT; butyltin compounds-BTs (TBT, DBT and MBT); and antifouling paint particles-APPs) was appraised in sediments of Vitoria Estuarine System (VES). Even at its historical lower ($\Sigma\text{BTs} \leq 113 \text{ ng Sn g}^{-1} \text{ dry wt}$), the current environmental levels of BTs in areas with a predominance of boatyards still pose a risk to the local biota and human population. DCOIT, among booster biocides, was the most frequently detected, especially in boatyards ($\leq 40 \text{ ng g}^{-1} \text{ dry wt}$) and Vitoria Port ($64 \text{ ng g}^{-1} \text{ dry wt}$), while APPs were also detected mainly in sediments of boatyards ($\leq 5,969 \mu\text{g g}^{-1} \text{ dry wt}$). Since levels of diuron and DCOIT in APPs were as high as 1,670,000 and 899,000 $\text{ng g}^{-1} \text{ dry wt}$, respectively, they are acting as secondary sources of these antifouling biocides. Therefore, VES is threatened by antifouling biocide residues due to the multiple diffuse sources of contamination, showing the need for more efforts on public policies (including temporal trend monitoring studies).

1. Introduction

Biofouling, defined as the accumulation of micro- and macro-organisms, such as the settlement of bacteria, algae, slime, weed, or barnacles on man-made structures (Lindholdt et al., 2015), can cause ecological damages by transporting invasive species, and/or economic losses by increasing maintenance costs and fuel consumption (Almeida et al., 2007). Chemicals have been used since ancient civilizations to avoid or reduce this natural phenomenon, making the current use of antifouling coatings mandatory to protected underwater equipment and vessel hulls (Yebra et al., 2004). The environment also benefits from a reduction in carbon emissions to the atmosphere (about 12,5000 ton of CO₂ emissions along 15 years) by decreasing frictional resistance of ship and boat hulls due to consequent reduction in fuel consumption (Buskens et al., 2013).

Due to their durability and effectiveness against fouling organisms, organotin compounds, especially tributyltin (TBT) and triphenyltin (TPT), were widely used in antifouling paint since the early 1960s. Although their durability in boat/ship hulls has been further improved by the self-polishing paint technology (Almeida et al., 2007), the TBT released from the coatings preferentially partition into particulate compartments, bioaccumulate and persist in the aquatic environments for up to 30 years (in anoxic sediments) (Fent, 1996). Its high partition coefficients Log K_{oc} (4.5) and Log K_{ow} (3.85) indicate, respectively, the trends to adsorb to the organic carbon associated to sediments and suspended particles, and to partition to the lipid fraction (Hoch, 2001).

TBT is highly toxic to non-target organisms from bacteria to mammals (Antizar-Ladislao, 2008). Environmental concentrations as low as 1 ng TBT L⁻¹ have been associated with imposex induction in gastropods and malformation in bivalve shells. For this reason, the International Maritime Organization (IMO) through the Antifouling Systems (AFS) Convention proposed, in 2001, a global ban of TBT-based antifouling systems to all IMO registered vessels. The AFS Convention entered into force on 17th September 2008 (IMO, 2008). Furthermore, TBT degradation products dibutyltin (DBT) and monobutyltin (MBT), produced by photolysis or biological activity, although less toxic may still cause environmental impacts (Hoch, 2001). After the TBT IMO banning, up to thirty booster biocides, including Irgarol 1051, diuron, dichlofluanid, chlorothalonil, DCOIT, have been reported to be in use in antifouling

formulations associated with Cu and Zn (Amara et al., 2018a; Thomas and Brooks, 2010).

In comparison to TBT, booster biocides tend to be less persistent in the environment (Konstantinou and Albanis, 2004). Chlorothalonil, dichlofluanid, and DCOIT (4,5-dichloro-2-n-octyl-4-isothiazolin-3-one) degrade within days, while Irgarol and diuron may remain in sediments for about a year (Thomas and Brooks, 2010). Even so, deleterious effects were observed during experimental exposure of Irgarol and diuron (Gallucci et al., 2015), chlorothalonil (Lopes et al., 2020) and DCOIT (Fonseca et al., 2020) to native Brazilian species. In addition, antifouling paint particles (APPs) generated during maintenance of vessel hulls act as secondary sources of antifouling biocides (Abreu et al., 2020), being toxic to benthic organisms (Soroldoni et al., 2020). Thus, due to inadequate disposal and consequent release of organic and metal contaminants, APPs have turned punctually contaminated sites into “hot spots” (Soroldoni et al., 2018).

Although butyltins, booster biocides and APPs have been assessed in sediments (Lahbib et al., 2011; Muller-Karanassos et al., 2019; Sánchez-Rodríguez et al., 2011; Viana et al., 2020; Wang et al., 2019), just a few studies have appraised them simultaneously. In Latin America, the simultaneous assessment of different residues from antifouling systems was only performed in sediments of Panamá (Batista-Andrade et al., 2018) and Santos-São Vicente estuarine system (Southeastern Brazil) (Abreu et al., 2020). The lack of integrated information about antifouling biocides makes it difficult to fill the gaps on the current use of antifouling biocides. Therefore, the present study investigated the occurrence and spatial distribution of butyltins (TBT, DBT and MBT), booster biocides (Irgarol, diuron, chlorothalonil, dichlofluanid and DCOIT) and APPs in sediment samples from Vitoria Estuarine System (VES). In addition, insights were provided on the influence of distinct types of maritime activities (shipyards, ports, boat traffic only, marinas) in the sedimentary levels of these legacy and emerging antifouling residues. Despite relevant and intense maritime activities and showing previous evidences of contamination and effects caused by exposure to TBT (Costa et al., 2017), VES has no contemporaneous information about the contamination related to residues of antifouling systems (Costa et al., 2014).

2. Materials and methods

2.1. Study area and sampling

Vitoria Estuarine System (VES), located in South-eastern Brazil, consists of Espírito Santo bay, Port channel, Vitoria bay and Passagem channel (Figure 1). It is a microtidal estuary, classified as semi-diurnal, and characterized as well-mixed to partially mixed during neap tides (Bastos et al., 2010). Local hydrodynamic is also influenced by approximately 18 km² of mangroves (Zamprogno, 2016). VES shelters an important private port (Tubarão port) on exportation of commodities (Vale, 2015), the Port of Vitoria with a cargo-handling equivalent to more than 6.5 million tons per year (CODESA, 2019), and several fishing ports, marinas, shipyards and boat maintenance facilities (Table S1) (Costa et al., 2017),

Samples were collected in November 2017 using a stainless steel “Ekman” grab. Surface sediments (upper 2 cm) were sampled along the VES in areas under the influence of boatyards (ES1, ES2, ES3, ES4, ES6, ES8, ES12, ES13, ES14), marinas (ES1, ES6, ES12, ES14), port (ES5, ES7), fishing port (ES1, ES2, ES3, ES10, ES13) and boat traffic only (ES9, ES11) (Table 1). After sampling, sediments were frozen (-20 °C) and stored for analysis. Prior the analyses, sediments were freeze-dried and homogenized.

2.2. Sediment and Antifouling Paint Particles characterization

One g of each sample was acidified using HCl (37%, w/w) in a desiccator for removal of inorganic carbon and the Total Organic Carbon (TOC %) content was analyzed in a TOC-L SSM 5000 A (Shimadzu) (Kristensen and Andersen, 1987). Granulometry was determined using 50 g of homogenized sediments and results were presented as % of fine fraction (<63 µm) (Gray and Elliott, 2014). The fraction above 500 µm was separated to quantify Antifouling Paint Particles (APPs) using a stereo microscope (5x magnification), as described by Soroldoni et al. (2018). APPs were weighed and stored until chemical analyses.

2.3. Chemical Analyses

2.3.1. Butyltins

Butyltins (TBT, DBT and MBT) were analyzed according Batista et al. (submitted). Two g of freeze-dried and homogenized sediments were spiked with surrogate standard (100 ng of tripropyltin - TPrT). After equilibration (30 min.), the extraction was performed using 9 mL of tropolone 0.03% in methanol (w/v) and 1 mL of glacial acid acetic. Samples were mixed on vortex for 1 min and sonicated (40 kHz, 132 W) for 30 min. After resting for 30 min, five mL of supernatant was collected and mixed with 1 mL of sodium acetate buffer (pH 4.5). For derivatization, 100 µL of

NaBEt₄ solution (10%, w/w) was added and sonicated for 10 min. Extracts were cleaned-up using silica column (2.5 g, activated at 160°C for 4h). The extraction was eluted with hexane (10 mL), concentrated down to 0.5 mL under gentle nitrogen flow, and fortified with tetrabutyltin (100 µL, 1000 ng Sn mL⁻¹) as internal standard. Extracts were injected in a Perkin Elmer Clarus 500MS gas chromatographer coupled to a mass spectrometer (GC-MS) and equipped with an Elite-5MS (5% diphenyldimethylpolysiloxane) capillary column (30 m × 0.25 mm × 0.25 µm).

Quality assurance (QA) and quality control (QC) was based on regular analysis of blanks, spiked matrices and certified reference material (CRM – PACS-3/National Research Council of Canada, Ottawa, Canada). Recoveries obtained from the CRM were in adequate agreement with the certified concentrations (88% for TBT; 85% for DBT and 114% for MBT). Recoveries for the surrogate standard varied between 69% and 118%, while RSD (relative standard deviation) was below 20%. Limits of detection (LOD) and quantification (LOQ), calculated by the signal to noise (S/N) ratio (“peak to peak”) obtained by the Turbo Mass software (S/N = 3 for LOD and S/N = 10 for LOQ, respectively), were 1 and 5 ng Sn g⁻¹ for all butyltins, respectively. An analytical curve equation in sediment matrix-matched was used for quantification. All concentrations were reported as ng Sn g⁻¹ (dry weight).

Butyltin Degradation Index (BDI) was calculated based on the equation: $BDI = \frac{[DBT] + [MBT]}{[TBT]}$, where values of BDI < 1 indicate fresh inputs of TBT (Díez et al., 2002b).

2.3.2. Booster biocides

Sediment and APPs samples were analyzed according to Abreu et al. (2020). One g of freeze-dried sediment or 0.01 g of APP were spiked with 20 ng L⁻¹ of Atrazine-d₅ (surrogate std used for LC-MS/MS analyses) and 20 ng L⁻¹ of PCB112 (surrogate std used for GC-ECD analyses). Afterwards, samples were mixed with 15 mL of acetonitrile, sonicated, and centrifuged twice. Supernatants were combined, evaporated (Syncore®) down to 1 mL and left over night in contact with activated copper (for remove the natural sulphur of sediments). Posteriorly, extracts were diluted with 50 mL of ultrapure water and cleaned-up by SPE using C18 cartridges. Analytes were eluted with 2 x 2 mL of ethyl acetate:hexane (1:1). Eluates were split and one fraction was solvent exchanged to methanol (LC-MS/MS) and another to hexane (GC-ECD). PCB30 (10 ng mL⁻¹) was used as internal standard for GC analyses. Irgarol, diuron, and DCOIT were analyzed by LC-ESI-MS/MS (Alliance Separations, model

2695, Waters – Milford, MA, USA). Chlorothalonil and dichlofluanid were determined by GC- ECD (Perkin Elmer Clarus 500; Waltham, MA, USA) using two different capillary columns (30mx25mm0.25 μ m) for confirmation: ZB-5MS (Phenomex, Alcobendas, ES) and DB-1701 (Agilent, CA, USA).

QA and QC was based on regular analyses of blanks and spiked matrices. Recoveries for the surrogate standards in LC-ESI-MS/MS ranged between 95% and 130% (RSD < 20%), while in GC-ECD ranged between 65% and 108% (RSD < 20%). For sediments, LOD and LOQ were 0.5 and 1.4 ng g⁻¹ for diuron, 0.4 and 1.2 ng g⁻¹ for Irgarol, 0.1 and 0.4 ng g⁻¹ for chlorothalonil, 0.7 and 2.1 ng g⁻¹ for dichlofluanid, and 0.2 and 0.7 ng g⁻¹ for DCOIT, respectively. For APPs, LOD and LOQ were 100 times higher than those reported for sediments. All concentrations were reported as ng g⁻¹ (dry weight).

2.4. Statistical analysis

A simple linear regression was used to investigate the relationships between biocide concentrations and sediment parameters. In addition, a Principal Component Analysis (PCA) was used to identify associations among the biocide levels and main activity in each site sampled. All statistical analyses were performed using the software PAST for Windows (version 3.25) with a significant level of 0.05 (Clarke et al., 2014). Whenever results were <LOD or < LOQ, one-half of the detection or quantification limit, respectively, was used for calculating Σ BTs, BDI and statistics.

3. Results

3.1 Sediment characteristics

Except for site ES3 (nearby the Port channel entrance), total organic carbon (% TOC) and % of fines were relatively high (Table 1) in sediments of VES. TOC content varied between 0.5 (ES3) and 11.1 % (ES11), while fines (<63 μ m) ranged from 20.1 (ES3) to 66.5 % (ES10). No significant statistical correlation was seen between % fine and TOC (p>0.05).

3.2 Butyltins in sediments

Butyltin residues were detected in all sites with levels ranging from <1 to 21.1 ng g⁻¹ for TBT, <1 to 24 ng g⁻¹ for DBT, <1 to 79.5 ng g⁻¹, for MBT, and <3.5 to 113 ng g⁻¹ for Σ BTs (Table 1 and Figure 2B). Although a significant negative correlation was found between TOC content with DBT (r= -0.55, p < 0.05) and MBT (r = -0.78, p < 0.05) levels, this is more likely related to the location of each site in relation to the

sources (better discussed ahead). No significant correlation was found between TOC and TBT levels ($r = -0.33$, $p > 0.05$). However, the significant correlations between TBT and DBT (0.48, $p < 0.05$), and DBT and MBT (0.65, $p < 0.05$) levels inferred the common source of these analytes (Castro et al., 2012). No correlation was found between % of fines with butyltins levels (Σ BTs; $p > 0.05$).

3.3 Booster biocides in sediments

DCOIT followed by diuron, dichlofluanid, Irgarol, and chlorothalonil were detected ($> \text{LOD}$) in 64%, 36%, 29%, 29% and 14% of the sediment samples, respectively (Table 1 and Figure 2C). Contamination levels ranged from <0.2 to 63.6 ng g^{-1} for DCOIT, <0.5 to 2.7 ng g^{-1} for diuron, <0.7 to 6.5 ng g^{-1} for dichlofluanid, <0.4 to 1.4 ng g^{-1} for Irgarol and <0.2 to 8.6 ng g^{-1} for chlorothalonil. Again, the lack of correlations between booster biocides with TOC ($p > 0.05$) and % of fines ($p > 0.05$) indicates the relevance of the sources (better discussed ahead).

3.3 APPs in sediments and corresponding booster biocides levels

Antifouling paint particles (APPs) were found in 13 out of 14 sites with levels ranging between <0.01 (ES11) and $5,969 \mu\text{g g}^{-1}$ (ES) (Figure 2D). Levels of booster biocides associated to APPs ranged from <17 to $1,670,000$ for diuron, <40 to 170 ng g^{-1} for Irgarol, <20 to $899,600 \text{ ng g}^{-1}$ for DCOIT and $<\text{LOD}$ for chlorothalonil and dichlofluanid (Figure 2D). No correlation was found between levels of APPs and booster biocides in the sediments.

3.4 Principal component analysis

The first principal component (PC1) explained 62.8 % of the variability associated with the variable APPs (loading value - 0.98) and, minorly, with Σ BTs (0.10), DCOIT (0.10) and COT (-0.10), while PC2 explained other 20.6 % of the variability associated with the variable DCOIT (loading value - 0.96) and, minorly, with Chlorothalonil (-0.20), Diuron (0.10) and -APPs (-0.10) (Figure 3).

4. Discussion

Although sediments provide an integration of contamination levels over the previous years, their characteristics (grain size, mineralogy, and organic carbon content) may influence the contaminant accumulation and bioavailability (Gray and Elliott, 2014). Since finer sediments provide more reactive surface area, correlations with organic matter are often expected (Artifon et al., 2019). In the present study, however, such parameters were not correlated due to the relatively high and homogenous levels

(most sediments have levels between 3 – 6 % TOC and 40 – 60 % fines), resultant of distinct input sources of suspended material and, consequently, distinct compositions of organic matter, as previously seen in this estuary (Costa et al., 2015). In fact, VES receives large volumes of untreated domestic and industrial effluents, as well as sediment particles and organic material derived from the mangroves located in the inner side of the system (Hadlich et al., 2018). In this sense, organic carbon contents (> 2 %) observed in VES indicate a relatively high capacity of adsorption and, consequently, preservation of BTs in the sediments. Nevertheless, TBT levels in the present study were relatively low and comparable to the lowest range found within Latin America (Batista-Andrade et al., 2018; Quintas et al., 2016) and worldwide (Chen et al., 2019; Wetzel et al., 2013). Although the highest TBT level was found in an area where the main activity is related to a commercial port (ES7 – 21.1 ng Sn g⁻¹), similar values (11.2 to 16.8 ng Sn g⁻¹) and the highest BTs levels were found in sediments of areas under the influence of fishing ports/boatyards used for fishing boat repairs (ES3 - 113 ng Sn g⁻¹; ES2 - 58 ng Sn g⁻¹ and ES13 – 48.1 ng Sn g⁻¹) and marinas, where maintenance of leisure boats may take place (ES14 - 54 ng Sn g⁻¹ and ES6 – 44.1 ng Sn g⁻¹) (Table 1). The constant dredging operations, required to increase or maintain the Vitória port operational depth (Baptista Neto et al., 2019), may explain such level of TBT in ES7 (21.1 ng Sn g⁻¹) and ES6 (14.4 ng Sn g⁻¹), since previously contaminated sediments can be exposed and/or contaminants trapped in anoxic sediments remobilized (Wang et al., 2019).

Disregarding sites ES9 and ES11 which are clearly away from any local source (levels <LOQ), the Butyltin Degradation Index (BDI) above 1 in most sites indicate the occurrence of chronic inputs of TBT (Table 1). Although BDI<1 in sites ES4, ES5, ES7 and ES10 indicate fresh inputs, the relatively low levels of TBT (6 – 21.1 ng Sn g⁻¹) suggest the occurrence of minor or punctual and less significant sources of TBT that may be associated to dredging events of mud depocenters along the Port channel and Vitória bay. Levels of BTs normalized to 1% TOC also indicate that, despite TOC content and fines were high within VES, lower levels of BTs might be circulating in these areas (Table S2). Even having the lowest levels of TOC, ES3 normalized to 1% TOC is still the most contaminated site.

In any case, levels of BTs have shown a drastic reduction in comparison to the contamination found a decade ago along the VES. Costa et al. (2014) observed high levels of TBT and Σ BTs, respectively, in sediments sampled in 2007 nearby sites ES13

(7,173 and 12,812 ng Sn g⁻¹), ES14 (448 and 1,625 ng Sn g⁻¹) and ES6 (509 and 1,442 ng Sn g⁻¹). Although examples otherwise have also been seen for Latin America (Abreu et al., 2020; Mattos et al., 2017), significant reductions were expected after the TBT banning by the IMO AFS Convention in 2008 and has been reported worldwide (Choi et al., 2010; Lahbib et al., 2011). In addition to the reduction in the use of TBT, this estuarine system, particularly the Port channel, has a low water residence time that favors the removal of the contamination load off this system (Neves et al., 2012).

Despite the trend of marinas, fishing ports and boatyards becoming more relevant current sources of TBT than commercial harbors (Abreu et al., 2020; Lagerström et al., 2017), it was not possible to track the specific origin of (even low) contamination among the multiple potential sources of TBT in VES. The clear reduction in the contamination levels, associated to the presence and proximity of multiple facilities of distinct maritime activities, as previously pointed out by Costa et al. (2017), makes it difficult to locate any residual source of TBT.

Regarding toxicity, all sites showed levels of TBT below the “no observed-effect concentration (NOEC)” (≤ 41 ng Sn g⁻¹) set by the Brazilian sediment quality guideline (Brasil, 2012), which has one of the least restrictive threshold limits worldwide. However, according to the classification proposed by OSPAR for TBT levels in sediments (OSPAR, 2011), the large majority of the concentrations fall into assessment classes A (ES9 - < LOD) and C (12 out of 14 sites - 2 to <50 ng g⁻¹), and would not be expected to affect the reproductive capability of gastropods. Only site ES7 is classified as class D (50 - <200 ng g⁻¹), indicating levels able to cause reproductive effects on the sensitive gastropod species. Based on the Australian Sediment Quality Guidelines (SQG), only site ES3 showed TBT levels that may induce deleterious effects (possible adverse effects) on benthic organisms, once they exceeded the threshold value (SQGV) of 9 ng Sn g⁻¹ (normalized to 1 % organic carbon) (Simpson et al., 2013) (Table S2).

Imposex incidence is well documented in VES. In a temporal and spatial study at Espírito Santo bay (2007-2014), Costa et al. (2017) assessed TBT pollution defining ecological status classes for the region using *Leucozonia nassa* as biomonitor. Although a decline of imposex parameters was observed from 2012, high levels of imposex were still detected in 2014. According to this study, sites located in the vicinity of ES13 and ES14 were classified in the *Good/Moderate* ecological status class (%I = 50 and 100%; VDSI = 2.75 and 1.55, respectively). Most recently, Podratz et al. (2020) showed that gastropods (*Leucozonia nassa*) collected in 2019 nearby ES14 (%I = 100%) and used to

feed pregnant female rats have caused reproductive abnormalities and offspring complications. Thus, BTs levels found in these gastropod tissues (TBT = 37.4 ± 14.4 , DBT = 95.6 ± 39.1 , and MBT = 39.0 ± 11.1 ng Sn g⁻¹, n = 4) have shown biological relevance at least to mammal models. In fact, even at its historical lower the current environmental levels in VES still pose a human health risks derived from the intake of OTs accumulated in seafood, especially to riparian populations (more 18,000 fishermen in VES).

Regarding to booster biocides, Irgarol, diuron, chlorothalonil, dichlofluanid and DCOIT were detected in sites under the influence of different maritime activities (port, marina, fishing ports, boatyards, and areas with traffic only). Therefore, this is the first record of environmental occurrences of booster biocides used in antifouling systems at VES. The lack of correlation between booster biocide levels, TOC% and %fine suggest the preferential partitioning into water phase. In fact, Voulvoulis et al. (2002) performed laboratory experiences observing that diuron remains mainly in the dissolved phase, whereas chlorothalonil and Irgarol will be present significantly in both adsorbed phase and particulate matter. Moreover, DCOIT and dichlofluanid exhibits stronger adsorption characteristics, partitioning into sediments (Voulvoulis, 2006).

Chlorothalonil was only detected at 2 boatyard sites located in the Port channel with predominance of leisure boats (ES4) and fishing boats (ES8) (Table 1 and Figure 2). Antifouling paints are important sources for this compound, as seen in marina areas from Greece (Albanis et al., 2002) and Korean bays (Lee et al., 2015). However, agricultural applications may also contribute to chlorothalonil inputs. In this sense, Zhen et al. (2019) found high levels of chlorothalonil in sediments of a Chinese river (max. of 2,500 ng g⁻¹) with higher loads associated to agricultural runoff. Chlorothalonil is registered for pesticide use in Brazil and, together with diuron, is among the top 15 active ingredients used as pesticides in Espírito Santo State (Ministério da Agricultura, 2019). However, fishing-related activities are more important than agriculture in VES. Considering the Environmental risk limits (ERL) of 50.6 ng g⁻¹ derived by Van Wezel and Van Vlaardingen (2004) using the equilibration partitioning method (EqP), the concentrations detected at VES (0.6 and 8.2 ng g⁻¹) do not pose a risk to the environment.

Dichlofluanid was detected at 4 out of 14 sites of VES mainly associated to fishing port/boatyards (ES8, ES13), marina/boatyard (ES12), and port (ES5) and with concentrations ranging from 1.1 (ES8) to 6.5 ng g⁻¹ (ES12) (Table1, Figure 2). The VES

has been considered an eutrophic and polluted environment due to anthropogenic activities such as boat traffic, discharge of domestic effluent and metallurgy (Costa et al., 2015). The relevant sedimentation rates ($> 6.3 \text{ mm y}^{-1}$) (Grilo et al., 2013) can contribute to the detection of dichlofluanid (Log K_{oc} 3.1) at Passagem channel (ES12 and ES13). The sites ES5 (3.6 ng g^{-1}) and ES8 ($<2.1 \text{ ng g}^{-1}$), located in the Port channel, are surrounded by marinas and repairing yards which may act as sources of dichlofluanid. Indeed, several studies have pointed the occurrence of dichlofluanid associated to antifouling particles (Hasan et al., 2014). Similar concentrations have been found in sediments from harbors of Gran Canarias Island (Spain) (<0.3 and 16.6 ng g^{-1}) (Sánchez-Rodríguez et al., 2011) and along the coastal area of Korea ($< \text{LOD} - 6.6 \text{ ng g}^{-1}$) (Lee and Lee, 2017). The concentrations detected at VES (<0.7 and 6.5 ng g^{-1}) are below the PNEC (predicted no-effect concentration) of $18 \mu\text{g Kg}^{-1}$ derived by Carvalho et al. (2015) using the EqP approach, indicating a low risk associate to dichlofluanid.

Irgarol was detected at sites under the influence of traffic only (ES9 – 1.3 ng g^{-1}) and fishing port (ES10 – 1.4 ng g^{-1}) at Vitoria bay, and fishing port/boatyard (ES13 – 1.3 ng g^{-1}) at Passagem channel (Table 1, Figure 2). Similar concentrations (<0.1 to 2.8 ng g^{-1}) were detected at coastal areas of Panama (Batista-Andrade et al., 2018). In fact, a worldwide reduction in Irgarol contamination is expected since its prohibition in antifouling paints by some European countries (Price and Readman, 2013). Irgarol is especially toxic to autotrophic aquatic species, causing the interruption of electron transport in chloroplasts during photosynthesis. Gallucci et al. (2015) also suggest that Irgarol (and diuron) threatens heterotrophic benthic communities by causing species loss and affecting the community structure. Based on the ERL of 1.4 ng g^{-1} proposed by Van Wezel and Van Vlaardingen (2004), only site ES10 presented concentrations capable of causing deleterious effects to benthic organisms of VES. However, according to the Norwegian sediment quality criteria, sites ES9, ES10, and ES13 (0.3 , 0.2 and 0.4 ng g^{-1} , normalized to 1% OC (Table S2)) can be classified as class III “moderate” ($>0.08 - \leq 0.5 \text{ ng g}^{-1}$, normalized to 1% OC), where toxic effects are likely seen following chronic exposure to Irgarol (Bakke et al., 2010). Additionally, Irgarol toxicity may be influenced by the organic carbon content in sediments (Ferraz et al., 2020), raising concern about its bioavailability, since VES has a high content of organic matter from anthropic and natural sources (Hadlich et al., 2018).

Diuron, in turn, was the second most frequent booster biocide, being detected at sites ES4, ES5, ES7 and ES13 ($<1.4 \text{ ng g}^{-1}$), and at site ES14 (2.7 ng g^{-1}), an area under

the influence of marina/boatyard. Similarly, Abreu et al. (2020) observed the highest concentration (9.9 ng g^{-1}) of diuron in areas under the influence of marinas at Santos and São Vicente Estuarine System. Diuron was also detected ($<0.06 - 144 \text{ ng g}^{-1}$) in sediments of the Korean coastal areas under intense maritime activity (ports and shipyards) (Lam et al., 2017). Based on the Norwegian sediment quality criteria, site ES14 (1.3 ng g^{-1} , normalized to 1% OC) (Table S2) can be classified as class III “moderate” ($>0.7 - \leq 6.4 \text{ ng g}^{-1}$, normalized to 1% OC) (Bakke et al., 2010). Thus, at this recreational marina/boatyard (ES14), chronic effects on autotrophic biota may happen since diuron is a photosynthetic inhibitor (Thomas and Brooks, 2010). Moreover, 3,4-Dichloroaniline (3,4-DCA), a degradation product of diuron, has been recognized as a Endocrine Disrupting Chemical (EDC) (Amara et al., 2018a).

DCOIT has recently been considered as a contaminant of emerging concern to coastal areas (Chen and Lam, 2017). Currently used in many antifouling paint formulations due to its high degradation rate and supposedly “environmentally friendly” behavior (Chen et al., 2015), DCOIT has been detected in many environmental matrices including seawater, sediments and biota (Chen and Lam, 2017). Due probably to its high partition coefficient ($\text{Log } K_{oc} - 4.2$) and an increasing frequency of use, DCOIT has been frequently detected in sediments (Lam et al., 2017). In VES, DCOIT was found in 9 (ES1, ES3, ES5, ES6, ES7, ES9, ES10, ES12 and ES14) out of 14 sites, including areas under the influence of marinas, port, boatyards (for fishing and pleasure boats) and traffic only. Measured concentrations ($<0.7 - 63.6 \text{ ng g}^{-1}$) were comparable to those reported in sediments of Panamá ($<0.4 - 82 \text{ ng g}^{-1}$) (Batista-Andrade et al., 2018), but lower than those found in Indonesia ($<0.04 - 150 \text{ ng g}^{-1}$) (Harino et al., 2012) and coastal areas of Korea ($30 - 281 \text{ ng g}^{-1}$) (Lee et al., 2015). In this sense, these higher levels may be related to a greater regional maritime trading in Asia (2018 - 260.8 million Twenty-foot Equivalent Unit (TEU)) (UNCTAD, 2020). In fact, areas under direct influence of the commercial fleet have shown the highest sedimentary concentrations of DCOIT (Tornero and Hanke, 2016), as seen for the Olympic Port (up 2600 ng g^{-1}) in Barcelona (Martínez et al., 2001) and Vitoria Port (ES7 - 63.6 ng g^{-1}) (present study). Also, the highest concentrations were seen in the VES sites ES12 (11.9 ng g^{-1}) and ES14 (40.3 ng g^{-1}) with marinas and boatyards for recreational boats, since DCOIT is present in modern paint formulations used by more expensive boats (Abreu et al., 2020). In fact, the PCA highlighted a distinction in sites characterized as Port (ES7 and ES5) and marinas with boatyards (ES12, ES14), which were grouped by the

indicative use of DCOIT in these areas (Figure 3). Furthermore, several studies have associated high levels of DCOIT in sediments to the occurrence of antifouling paint particles (Batista-Andrade et al., 2018; Soroldoni et al., 2018). DCOIT has shown to be more toxic (i.e., LC_{50} (48h) = 0.32 mg L⁻¹ for *Artemia* larvae) to animals than other antifouling biocides, such as chlorothalonil, zinc pyrithione, ziram, Irgarol, diuron, zineb and dichlofluanid (Jung et al., 2017). Considering a PNEC of 3.0 µg kg⁻¹ derived using EqP (Wang et al., 2014a) and a NOEC (no observed effect concentration) of 9.7 ng g⁻¹ (Onduka et al. (2013), not only sediments obtained in the sites ES7 (63.6 ng g⁻¹), ES14 (40.3 ng g⁻¹) and ES12 (11.9 ng g⁻¹), but also those of sites ES5 (3.6 ng g⁻¹), ES9 (3.5 ng g⁻¹) and ES10 (4.4 ng g⁻¹), are likely to cause toxicity to the biota.

Antifouling Paint Particles (APPs >500 µm) were found in all sediment samples, except at site ES11 located in the inner portion of the VES (Figure 2D). This site is the furthest away from any source of contamination, showing the lowest levels of antifouling residues (Figure 3), which may be the closest it can get from the actual background levels of this system. On contrary, the highest concentrations of APPs were associated to areas under the influence of boatyards used mostly by fishing boats (ES3 – 305 µg g⁻¹ and ES13 – 1,192 µg g⁻¹), leisure boats (ES12 – 154 µg g⁻¹) or a combination of both (ES1 – 5,969 µg g⁻¹), as confirmed in the PCA (Figure 3). Costa et al. (2017) have already indicated these areas as potential sources of APPs due to the presence of boatyards. In addition, based on information obtained through interviews with port authority employees, repairs and new paintings on vessel hulls take place along the VES without any specific control or supervision by the environmental agencies or maritime authority (Vianna, 2009). In fact, boat/ship maintenance activities seem to be associated to the incidence of APPs in sediments, as demonstrated by Soroldoni et al. (2018) for Patos Lagoon estuary (Southern Brazil) and Abreu et al. (2020) for Santos-São Vicente Estuarine System (Southeastern Brazil). However, since the APPs were assessed only on the > 500 µm fraction, the amount of paint particles, and consequently biocides, stored in the sediments may have been underestimated (Lagerström et al., 2017). APPs smaller than 500 µm are probably generated when sanding or dry/wet blasting instead of scraping off is used to remove the coatings.

Once associated to APPs, biocides increase their half-lives and, thus, persistence in the environment (Thomas et al., 2000). In this sense, APPs can severely impact aquatic environments since they can act as secondary sources of legacy and emerging antifouling biocides (Parks et al., 2010). However, APPs represent still an analytical

challenge given the compositional variation observed among the individual paint fragments, as illustrate in Figure 2A. Beyond of heterogeneous composition due to the presence of pigments, solvents, metals, and organic and organometallic biocides in antifouling coatings (Turner, 2010), the varied shapes and colors implies to heterogeneity from temporal (degrees of leaching or ages since deposition) and geographical variation (formulations applied and how the paint was removed) (Singh and Turner, 2009). This may explain the lack of correlation between levels of APPs and booster biocides in the sediments of VES. In fact, this complex mixture of chemicals constituting APPs can interact with the booster biocides and, consequently, increase even more their risk to the environment (Wang et al., 2011). In addition, APPs have recently been recognized as plastic particles due to their alkyd-resin base and their large quantity is contributing to the already abundant load of plastics present in the environment (Hartmann et al., 2019). They also represent a potential risk related to the transport of contaminants, since may present a relatively large surface area to volume ratio, releasing contaminants into interstitial waters and, by desorption or during sediment resuspension or dredging, to the overlying water column (Turner, 2010). APPs can also be ingested by a wide range of organisms inducing deleterious responses (Thompson et al., 2009). Deposit-feeding organisms, for instance, can feed on particles directly from sediments (Gallucci et al., 2015; Soroldoni et al., 2020)

The occurrence of Irgarol, diuron and DCOIT in APPs of VES confirmed their origin associated to antifouling paints (Figure 2D, Table S3). The highest biocide concentrations associated to APPs were observed in the vicinity of Vitoria Port, with simultaneous detection of diuron and DCOIT in ES7 (1,670,000 ng g⁻¹; 652,000 ng g⁻¹, respectively), ES8 (91,000 ng g⁻¹; 899,600 ng g⁻¹, respectively) and ES9 (1,200 ng g⁻¹; 1,950 ng g⁻¹, respectively). The presence of both biocides at the same particles indicates the use of modern paints with diuron and DCOIT in the formulation, such as Micron Premium (International®). However, APPs containing only DCOIT were found in sediments under the influence of marina/boatyard at Passagem channel (ES12 - 24,000 ng g⁻¹) and marina/fishing port/boatyard (ES1 - 1,900 ng g⁻¹) at Espírito Santo bay. Irgarol was detected (up to 170 ng g⁻¹) in APPs from four sites (ES6, ES9, ES10 and ES12) under different maritime activities (Figure 2D, Table S3). Zhou (2008) also found higher levels of Irgarol (up to 60 ng g⁻¹) in APPs than in sediments (<1.7–45 ng g⁻¹) of marinas from southern England. Chlorothalonil and dichlofluanid, however, were

not detected in any APPs, which may be related with their low (or no) use in antifouling paint formulations used in Brazil, as pointed out Paz-Villarraga (2019). Although Soroldoni et al. (2018) associated the occurrence of APPs near boat maintenance facilities with increasing levels of metal and booster biocides in sediments, the absence of correlations between biocides levels in particles and in sediment samples was probably related to the underestimation in the amount of APPs (the unmeasured <500 μm fraction) and/or to a long-distance input of dust or fine particles (Singh and Turner, 2009).

Considering the current situation, VES is threatened by antifouling biocide residues due to the multiple diffuse sources of contamination, which may be worsen considering the trend of expansion of navigation/maritime activities worldwide. In this regard, strategies combining public policies (i.e., regulating the use and disposal of antifouling residues) with monitoring programs that temporarily assess the effectiveness of any adopted measures are required. This is even more important considering that none of the studied booster biocides but diuron, which has permissible maximum value of $90 \mu\text{g L}^{-1}$ for drinking water (Brasil, 2011), is regulated in Brazil. Irgarol, at least, is under review by the Marine Environment Protection Committee (MEPC) of IMO to be included in Annex 1 to the AFS convention (IMO, 2020). Thus, the establishment of environmental quality standard (EQS) and sediment quality guidelines (SQG) covering such contaminants is also urgently needed. However, the lack of measured environmental concentrations and Environmental Risk Assessments for the Brazilian coastal areas are the perfect excuse for the stakeholders and decision-makers to do nothing to better protect the aquatic environments.

5. Conclusion

Even at its historical lower ($\Sigma\text{BTs} \leq 113 \text{ ng Sn g}^{-1}$), the current environmental levels of butyltins in areas with a predominance of boatyards still pose a risk to the local biota and human population. DCOIT was the most frequently detected booster biocide, especially in boatyards ($\leq 40 \text{ ng g}^{-1}$) and Vitoria Port (64 ng g^{-1}), while APPs were detected in 13 out of 14 sites mainly in sediments of boatyards ($\leq 5,969 \mu\text{g g}^{-1}$). Since levels of diuron and DCOIT in APPs were as high as 1,670,000 and 899,000 ng g^{-1} dry wt, respectively, they are acting as secondary sources of these antifouling biocides. Therefore, VES is threatened by antifouling biocide residues due to the multiple diffuse sources of contamination, which may be worsen considering the trend of expansion of

navigation/maritime activities worldwide. In fact, based on threshold values established by international sediment quality guidelines, TBT, DCOIT, diuron and Irgarol pose a risk to the environment of the VES. In this regard, strategies combining public policies (i.e., regulating the use and disposal of antifouling residues) with monitoring programs are needed actions. In addition, the establishment of environmental quality standard (EQS) and sediment quality guidelines (SQG) covering such contaminants are also urgently required.

6. Acknowledgment

The authors are thankful to Msc. Felipe Corteletti Pedruzzi and municipality of Vitoria for the support during field work and LACOM - *Laboratório de Análises de Compostos Orgânicos e Metais* (FURG) for LC-ESIMS/MS analyses. The present research was sponsored by FINEP – *Financiadora de Estudos e Projetos* (Proc. No 1111/13 – 01.14.0141.00) and CAPES - *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior* (CIMAR II No 1988/2014). F. E. L. Abreu (PhD grant) and R. M. Batista (PhD grant) were PhD fellows of CAPES, while I.B. Castro (PQ 302713/2018-2) and G. Fillmann (PQ 312341/2013-0 and 314202/2018-8) were research fellows of CNPq.

Table 1: Total organic carbon (%TOC), percentage of fines (%), levels of TBT, DBT, MBT and sum of BTs (Σ BTs) (ng Sn g⁻¹), butyltin degradation index (BDI), chlorothalonil (Chlor), dichlofluanid (Dichlo) and DCOIT (ng g⁻¹) in sediments of VES

Site Code	Site Location	Main Activity	TOC (%)	Fine (%)	(ng Sn g ⁻¹)					Diuron	Irgarol	Chlor	Dichlo	DCOIT
					TBT	DBT	MBT	Σ BTs *	BDI *					
ES1	Espírito Santo bay	Marina/Fishing port/Boatyard	2.3	56.2	≥1-<5	13.5	19.0	35.0	13.0	<0.5	<0.4	<0.1	<0.7	≥0.2-<0.7
ES2	Port channel	Fishing port/Boatyard	3.1	50.5	16.6	24.0	17.4	58.0	2.5	<0.5	<0.4	<0.1	<0.7	<0.2
ES3	Port channel	Fishing port/Boatyard	0.5	20.1	11.2	22.9	79.5	113.0	9.1	<0.5	<0.4	<0.1	<0.7	≥0.2-<0.7
ES4	Port channel	Marina/Boatyard	4.4	54.3	14.8	<1	≥1-<5	17.8	0.2	≥0.5-<1.4	<0.4	8.6	<0.7	<0.2
ES5	Port channel	Port	4.8	59.2	6.0	<1	≥1-<5	9.0	0.5	≥0.5-<1.4	<0.4	<0.1	3.6	3.6
ES6	Port channel	Marina/Boatyard	4.8	53.2	14.4	18.8	10.9	44.1	2.1	<0.5	≥0.4-<1.2	<0.1	<0.7	≥0.2-<0.7
ES7	Port channel	Port	4.3	48.3	21.1	12.3	≥1-<5	35.9	0.7	≥0.5-<1.4	<0.4	<0.1	<0.7	63.6
ES8	Port channel	Fishing port/Boatyard	3.5	58.3	5.6	≥1-<5	6.3	14.4	1.6	<0.5	<0.4	0.6	≥0.7-<2.1	<0.2
ES9	Vitoria bay	Traffic only	4.5	40.1	<1	<1	≥1-<5	<3.5	4.5	<0.5	1.3	<0.1	<0.7	3.5
ES10	Vitoria bay	Fishing port	6.3	66.5	6.6	≥1-<5	<1	9.6	0.5	<0.5	1.4	<0.1	<0.7	4.4
ES11	Vitoria bay	Traffic only	11.1	50.5	≥1-<5	≥1-<5	≥1-<5	<7.5	2.0	<0.5	<0.4	<0.1	<0.7	<0.2
ES12	Passagem channel	Marina/Boatyard	5.4	39.4	≥1-<5	8.4	6.6	17.5	6.0	<0.5	<0.4	<0.1	6.5	11.9
ES13	Passagem channel	Fishing port/Boatyard	3.1	47.9	16.8	16.9	14.4	48.1	1.9	≥0.5-<1.4	1.3	<0.1	5.7	<0.2
ES14	Espírito Santo bay	Marina/Boatyard	2.2	63.5	11.4	15.1	27.5	54.0	3.7	2.7	<0.4	<0.1	<0.7	40.3

* Whenever results were <LOD or < LOQ, one-half of the detection or quantification limit, respectively, was used for calculating Σ BTs and BDI.

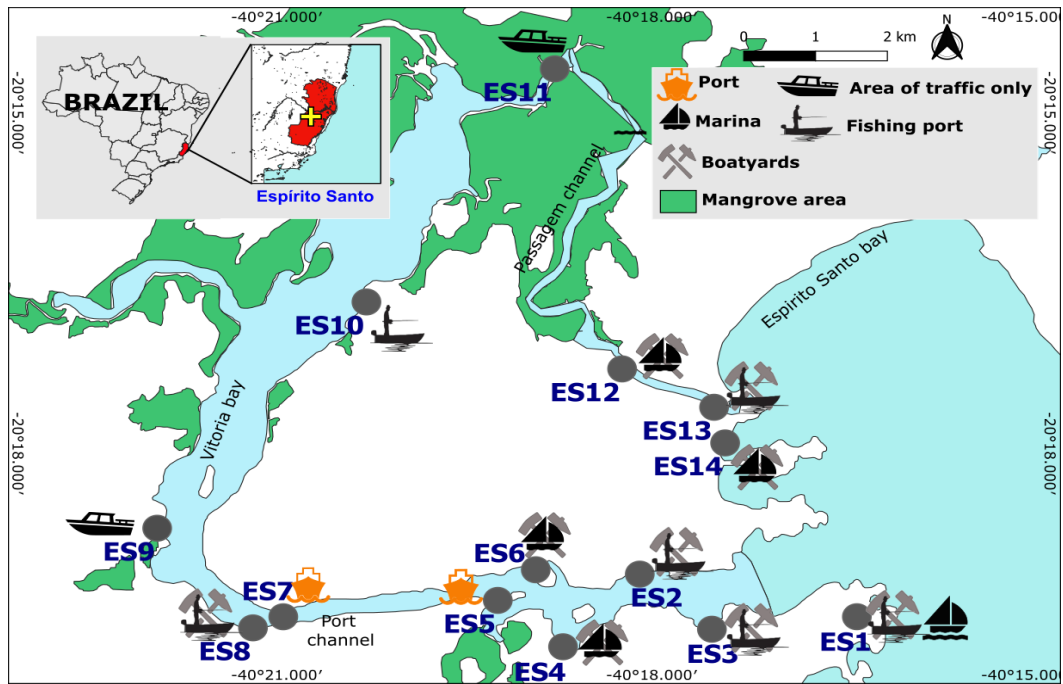


Figure 1: Sampling sites along the Vitoria Estuarine System (VES)

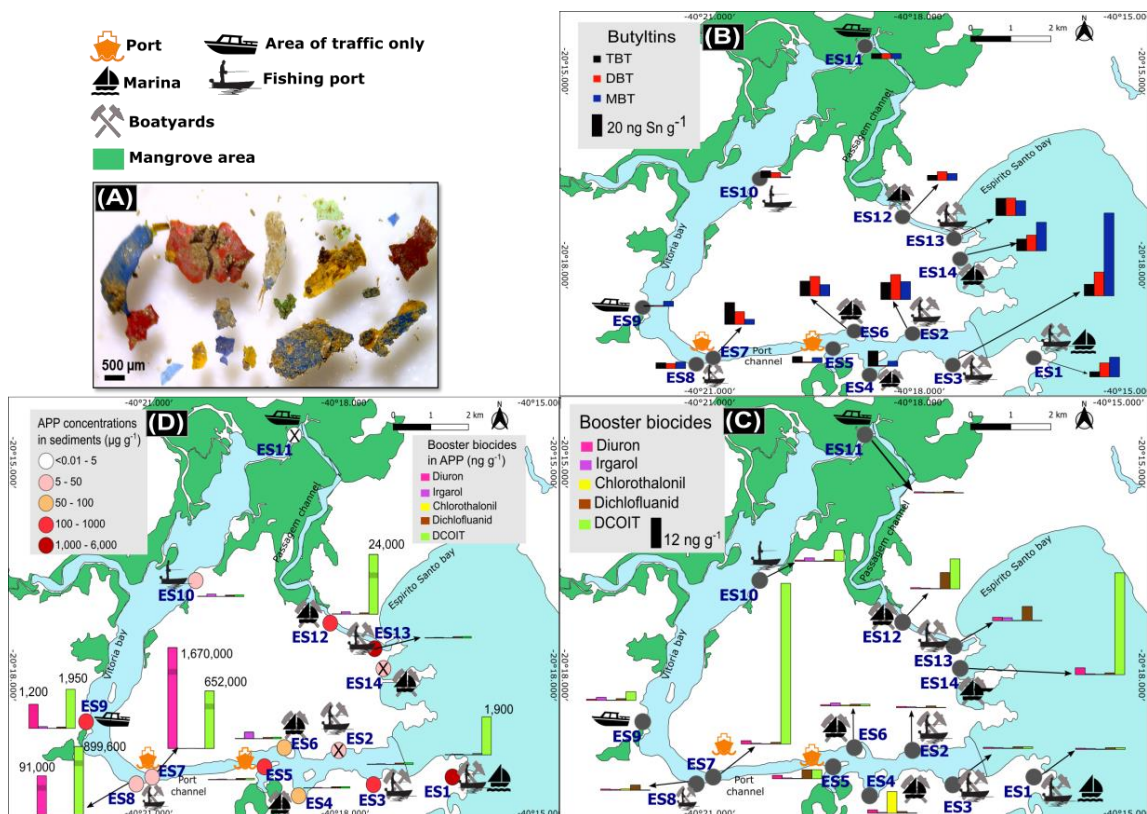


Figure 2: Distribution of antifouling biocides and antifouling paint particles (APPs) in VES. (A) APPs collected in sediments samples; (B) Concentrations of butyltins in sediments (ng Sn g^{-1}); (C) Concentrations of booster biocides in sediments (ng Sn g^{-1}) and; (D) Range of concentrations of APPs in sediments ($\mu\text{g g}^{-1}$) and their corresponding concentrations of booster biocides (ng g^{-1}). In figure 2D, circles with x indicate sites without analysis of booster biocides in APPs (run out of APPs)

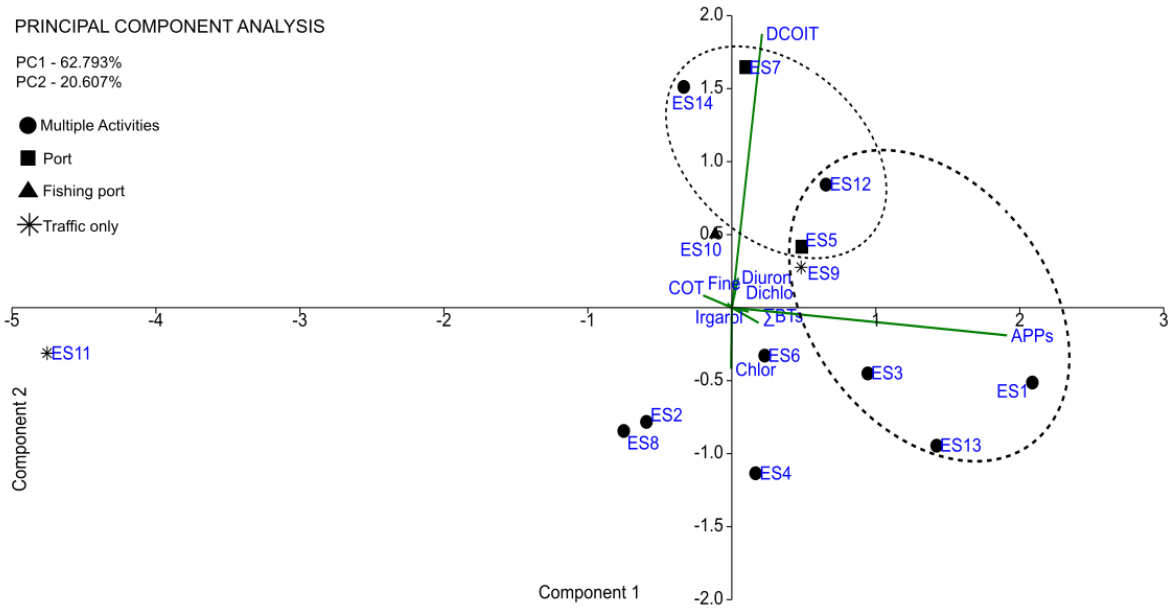


Figure 3: Biplot of PCA score and loading plots showing the PC1 (62.8 %) and PC2 (20.6 %). Loadings are indicated by green lines.

ARTIGO 3

Antifouling biocides in sediments along the Brazilian coast

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ABSTRACT

Contamination of antifouling biocides (TBT, DBT, MBT, diuron, Irgarol, chlorothalonil, dichlofluanid and DCOIT) and their residues (antifouling paint particles) were evaluated in sediments across the coast of Brazil. Effectiveness of the tributyltin ban was observed through low levels of butyltin (BTs) contamination on Brazilian ports (average of 26 ng Sn g⁻¹). Also, most of sites (95%) showed old TBT inputs based on BT degradation index. However, hotspot of contamination and recent inputs of BTs were also observed in boatyards due to presence of paint particles. Similarly, booster biocides (BB) were present mainly near marinas in lower concentrations (mean of \sum BB 13.7 ng g⁻¹) than butyltins (mean of \sum BTs 126.4 ng Sn g⁻¹). DCOIT was the most frequently booster biocide indicating its common use in modern antifouling paints. In addition, results indicated concerning about paint particles as it contains large and sporadic concentrations of antifouling biocides (DCOIT – 43,139 ng g⁻¹; TBT – 311,474 ng Sn g⁻¹). In conclusion, Brazilian coastal areas are impacted by antifouling biocides and their residues, including levels above safety environmental concentrations.

1. Introduction

Marine biofouling is a biological phenomenon characterized by settling and growing of organisms on surfaces exposed to natural waters (Almeida et al., 2007). Seeking to protect aquaculture and naval structures (oil platforms and vessels), antifouling systems have been widely used. Currently, antifouling system (AFS) usage is mandatory and includes different layers such as anticorrosive primer, pigments, preservatives and antifouling topcoat. The latter has changed over time in terms of the composition and characteristics of the binder and matrix type (Xie et al., 2019).

Organotins were initially used as co-toxicants in high-performance copper paints but gradually became the main active ingredient in AFS (Yebra et al., 2004), being the most used biocides in antifouling paints due to their durability and efficiency against biofouling (Yebra et al., 2004). However, their high toxicity, especially tributyltin (TBT), towards non-target organisms led the International Marine Organization (IMO) to propose a worldwide ban of TBT-based antifouling paints which came into force in 2008 (IMO, 2020). TBT is persistent in the environment (half-life up to 30 years in anoxic sediments) and its degradation products (dibutyltin – DBT and monobutyltin – MBT) are also deleterious (Fent, 1996). Therefore, even after the global ban, environmental concern rises due to the legacy of its residues and fresh inputs related to illegal and unregulated use (Abreu et al., 2020; Castro et al., 2018).

As an alternative to TBT, other biocides have been introduced as co-toxicants in copper-based paints (Thomas and Brooks, 2010). Nowadays, up to 30 compounds have been used as active biocides in antifouling formulations (Amara et al., 2018a; Konstantinou and Albanis, 2004; Thomas and Brooks, 2010). Among them, Irgarol, diuron, 4,5-dichloro-2-octylisothiazol-3(2H)-one (DCOIT), chlorothalonil, dichlofluanid, thiram, zinc pyrithione (ZnPT) and copper pyrithione (CuPT) are the most likely to occur in aquatic environments (Tornero and Hanke, 2016). Nevertheless, these compounds have distinct physicochemical properties. Irgarol and diuron are moderately persistent in sediment (half-life over 1 year) while chlorothalonil has shown a half-life of 8 days, and dichlofluanid and DCOIT can degrade in less than of one day (Konstantinou and Albanis, 2004). Moreover, Irgarol, diuron, chlorothalonil and dichlofluanid exhibit moderate $\log K_{oc}$ (3.3, 2.3, 2.9 and 3.1, respectively) while DCOIT is more likely to partition into the particulate phase ($\log K_{oc}$ 4.2) (Thomas and Brooks, 2010). These factors influenced the UK and Denmark to forbid Irgarol and

diuron use on ships less than 25 m long (Price and Readman, 2013) as well as support the authorization of DCOIT and dichlofluanid as an antifouling product by the Biocidal Products Regulation (ECHA, 2019, 2016).

More recently, antifouling paint particles (APPs) produced mainly during hull maintenance procedures (removal of old antifouling coatings) have been identified as an environmental issue due to its widespread occurrence and toxicity (Abreu et al., 2020; Soroldoni et al., 2020). Although such residues are progressively leached over time, they remain with huge concentrations of biocides which can cause deleterious effects to aquatic ecosystems over a long time and far from their primary sources (Singh and Turner, 2009b). In fact, the smaller the APP (such as dust and fine, weathered or eroded particles), the more prone to long-range transport (Parks et al., 2010). Considering sediments as the fate of APPs, benthic organisms are under threat (Soroldoni et al., 2020).

Antifouling biocides from shipping activity are the main source of organotins in marine and coastal waters (Sonak, 2009). In this sense, Brazil has a set of ports and navigation facilities with more than 1 billion tons of cargo handled (ANTAQ, 2019) and it is 23rd on worldwide ranking to production of fish from extractive fisheries which involve mainly small vessels artisanal fishing ports distributed along the coast (Alves et al., 2018). Regarding this antifouling contamination, recent assessments along the Brazilian coast have detected butyltins (BTs) (Artifon et al., 2016; Maciel et al., 2018; Moreira et al., 2017), booster biocides (Abreu et al., 2020; Soroldoni et al., 2018; Viana et al., 2020) and APPs (Abreu et al., 2020; Soroldoni et al., 2018) in the vicinity of commercial ports, marinas and shipyards. However, Brazil is a continental-size country with many other estuarine or coastal areas of ecological and economical relevance that remained unassessed regarding AF impacts. This study aimed to appraise spatial distribution of antifouling biocides (TBT, DBT, MBT, diuron, Irgarol, chlorothalonil, dichlofluanid and DCOIT) and APPs in representative systems across the coast of Brazil. Moreover, the present study aimed to build a robust baseline of this kind of contamination, which may be used to guide management strategies and threshold values to assessment impacts in coastal zones.

2. Materials and methods

2.1. Study area and sampling

Surface sediments (top 2 cm) were collected between September 2015 and April 2018 using an Ekman and Van Veen grabber in 8 coastal systems distributed along the Brazilian coast (Table 1; Figure 1). Samples were frozen (-20 °C) and stored for subsequent analysis. Sampling sites were representative of relevant nautical activities areas along the coast of Brazil: (1) Marajó and Guajará bay (Pará State – PA); (2) São Marcos Bay (Maranhão State - MA), (3) Fortaleza coast (Ceará State - CE), (4A) Suape and (4B) Capibaribe estuaries (Pernambuco State - PE), (5) Guanabara Bay (Rio de Janeiro State - RJ), (6) Itajaí-Açu River (Santa Catarina State - SC) and (7) Patos Lagoon estuary (Rio Grande do Sul State - RG) (Figure 1, Table 1). Each site was classified according to its main maritime activity (i.e. potential sources of antifouling biocides) into: traffic (T), a waterway for ship/boat navigation; boat/shipyard (B), ship and/or boat maintenance sites; marina (M), area for mooring fishing and/or recreational vessels with no evident maintenance site; port (P), area with berths for large commercial ships (Table 1).

2.2. Sediment and Antifouling Paint Particles characterization

Sediments were freeze-dried and homogenized in the laboratory. Grain size was determined according to Gray and Elliott (2014) and results were expressed as mud percent (< 0.0063 mm) in total dry sediment. Total organic carbon (TOC %) was analyzed according to Kristensen and Andersen (1987). Sediments were decarbonated with Hydrochloric Acid (HCl) (37%, w/w) and analyzed in a TOC-L SSM 5000 A (Shimadzu).

The presence of antifouling paint particles (APPs) was assessed according Abreu et al. (2021). Briefly, homogenized sediments (50 g) of São Marcos Bay (MA), Fortaleza coast (CE), Guanabara Bay (RJ) and Itajaí-Açu River (SC) were sieved and the fraction above 500 µm was searched for APPs under a stereo microscope (5x magnification). APPs in sediments of Marajó and Guajará bays (PA) as well as Capibaribe and Suape estuaries (PE) were not analyzed (NA) due to methodological issues. APP data from Patos Lagoon estuary were obtained from Soroldoni et al. (2018).

2.3. Chemical Analyses

2.3.1. Butyltins

Butyltins (TBT, DBT and MBT) were analysed according to Batista et al. (in preparation). Briefly, 2 g of freeze-dried sediment or 0.01 g of APPs (only particles from Patos lagoon estuary due to the availability) were spiked with surrogate standard

(100 ng of tripropyltin - TPrT) and left aside for equilibrium (30 min). Extraction was performed using tropolone 0.03% in methanol (w/v) (9 mL) and glacial acetic acid (1 mL). Samples were mixed on vortex for 1 min and sonicated (40 kHz, 132 W) for 30 min. Five milliliters of supernatant were collected and mixed with sodium acetate buffer (pH 4.5) (1 mL). One hundred microliters of sodium tetraethylborate (NaBEt₄) solution (10%, w/w) was used for derivatization. Clean-up was performed in a silica column (2.5 g) through elution with n-hexane (10 mL). One hundred microliters of tetrabutyltin solution (1000 ng Sn mL⁻¹) was added as the internal standard. Extracts were analyzed by gas chromatography (GC) using a Perkin Elmer Clarus 500MS coupled to a mass spectrometer (MS) and equipped with an Elite-5MS (5% diphenyldimethylpolysiloxane) capillary column (30 m × 0.25 mm × 0.25 μm).

Quality assurance (QA) and quality control (QC) were based on regular analyses of blanks, spiked matrices and certified reference material (CRM – PACS-3/National Research Council of Canada, Ottawa, Canada). Recovery of target analytes in the CRM was close to certified concentrations (88% for TBT; 85% for DBT and 114% for MBT). Recoveries of the surrogate ranged between 68 and 130%, while RSD (relative standard deviation) was below 20%. Limits of detection (LD) and quantification (LQ) were calculated by signal to noise (S/N) ratio (“peak to peak”) obtained by the Turbo Mass software (S/N = 3 for LD and S/N = 10 for LQ, respectively). For each butyltin, they were 1 and 5 ng Sn g⁻¹ in sediments and 250 and 500 ng Sn g⁻¹ in APPs, respectively. Analytical curves in solvent and in sediment extract were used for quantification of APPs and sediments, respectively. Results were reported as ng Sn g⁻¹ (dry weight).

Butyltin Degradation Index (BDI) was calculated based on the equation: $BDI = [DBT] + [MBT] / [TBT]$, where values of BDI < 1 indicate fresh inputs of TBT (Díez et al., 2002).

2.3.2. Booster Biocides (BB)

Booster biocides were extracted from sediments and APPs according to Abreu et al. (2020). Briefly, 1 g of freeze-dried sediment or 0.01 g of APPs were spiked with 20 ng L⁻¹ of Atrazine d₅ (surrogate used for LC-MS/MS analyses) and 20 ng L⁻¹ of PCB112 (surrogate used for GC-ECD analyses), mixed with acetonitrile (15 mL) and sonicated (40 kHz frequency – 50°C) for 30 min (twice). Supernatants were merged and concentrated down to 1 mL. The extract was left overnight in contact with activated copper. For clean-up, extracts were diluted in 50 mL of ultrapure water submitted to

solid-phase extraction (SPE) using C18 cartridges and cleaned up by SPE using C18 cartridges and eluted with 2 x 2 mL of ethyl acetate:hexane (1:1, v/v). Eluates were divided in two fractions: the first one was solvent exchanged to methanol (for LC-MS/MS) while the other was exchanged to hexane (for GC-ECD). The GC-ECD fraction was spiked with PCB30 (10 ng mL⁻¹) as internal standard. Irgarol, diuron, and DCOIT were analysed by LC-ESI-MS/MS (Alliance Separations, model 2695, Waters – Milford, MA, USA). Chlorothalonil and dichlofluanid were determined by GC-ECD (Perkin Elmer Clarus 500; Waltham, MA, USA) using two different capillary columns (30m x 25mm x 0.25µm): ZB-5MS (Phenomenex, Alcobendas, ES) for quantification and DB-1701 (Agilent, CA, USA) for confirmation.

QA/QC were based on regular analyses of blanks and spiked matrices. Recoveries of surrogate ranged between 59% and 140% while RSD was below 20%. For sediments, LOD and LOQ were 0.5 and 1.4 ng g⁻¹ for diuron, 0.4 and 1.2 ng g⁻¹ for Irgarol, 0.1 and 0.4 ng g⁻¹ for chlorothalonil, 0.7 and 2.1 ng g⁻¹ for dichlofluanid, and 0.2 and 0.7 ng g⁻¹ for DCOIT, respectively. For APPs, LD and LQ were 100 times higher than those reported for sediments. Results were reported as ng g⁻¹ (dry weight).

2.4. Statistical and data analysis

Normality and homogeneity of variances were verified using Shapiro-Wilk and Levene tests, respectively. Spearman rank order correlation analysis was used for investigating associations between concentrations of biocides and sediment properties, as well as APPs and their booster biocides levels. Correlations were used by pairwise comparisons of antifouling biocides in maritime activity (ports, marina, boatyard and traffic) using the Mann-Whitney analysis supported by Box plot graphs. Half of LD and LQ were used for statistical analysis, BDI calculations and summations of BTs and BB. All statistical analyses were performed using the software PAST for Windows (version 3.25) with a level of significance set at 0.05 (Hammer et al., 2001).

3. Results and Discussion

1.1 Characterization of sediment samples

Total organic carbon (TOC) in surface sediments collected in the study areas ranged from 0.01% (RJ6, RJ8 and MA8) to 5.1% (PE15) (Table 2). The lower TOC values were observed in the inner continental shelf at sites for disposal of dredging material or near beaches formed by coarse sediments. As expected, higher values

occurred in mangrove regions, but could also be related to domestic sewage (Grilo et al., 2013). Different sedimentation processes and sources of organic carbon are expected for distinct coastal systems distributed along the Brazilian coast (~7,000 km long) (Buruaem et al., 2012).

Muddy sediments (silt + clay) ranged between 1.4% (RJ5) and 89.6% (RJ4). The higher mud contents were found in sheltered areas with limited water circulation and close to zones of wave and current diffraction. A total of 30 sites were dominated by sandy/coarse sediments (mud fraction below 10%) due to either moderate to high hydrodynamics (RJ6-RJ8, CE3) or erosive margins on rivers (PA1, CE6). These results indicated the contrasted energy conditions observed in Brazilian estuarine systems (Araújo et al., 2020).

1.2 Levels of antifouling biocides in coastal areas of Brazil

1.2.1 Marajó and Guajará Bay, Pará

Butyltins (BTs) were detected in 6 out of 8 sediment samples from Marajó and Guajará bays, in Eastern Amazon (Brazil). TBT, DBT and MBT concentrations ranged from < 1.0 to $24.7 \text{ ng Sn g}^{-1}$, < 1.0 to 8.5 ng Sn g^{-1} and < 1.0 to $24.1 \text{ ng Sn g}^{-1}$, respectively (Table 2; Figure 1). The highest concentration of Σ BTs (55 ng Sn g^{-1} at PA6) were close to shipyard activities (repair and construction of ships and boats), even with presence of moderate content of fines and organic carbon (0.54% and 17.1%, respectively), confirming the relevant BTs input from repairs areas. Similar results was observed at Bragança shipyard (PA19, Σ BTs = $22.7 \text{ ng Sn g}^{-1}$). The second highest concentration of Σ BTs ($35.9 \text{ ng Sn g}^{-1}$) was registered in the largest open street market of Latin America (PA4). The intense navigation traffic in addition to the significant fraction of fines (51.8%), which are propitious to retain contaminants, could explain high concentrations. There is no clear evidence of fresh input of TBT since BDI values were above 1 at all sites. Conversely, Ribeiro-Brasil et al. (2021) calculated $\text{BDI} < 1$ at PA4, PA6 and PA19 in samples collected in the wet season (December to May 2016). These results might suggest a seasonality of fresh TBT inputs to the area. Due environment concern to TBT, some regulations defined Sediment Quality Guidelines Value (SQGV) for it. Although contamination of all sites is below the limit set to the Brazil legislative threshold for marine sediments effect level (Limit 1 $\geq 41 \text{ ng Sn g}^{-1}$), data normalization to 1% of organic carbon shows PA4 ($12.1 \text{ ng Sn g}^{-1}$) and PA6 ($45.8 \text{ ng Sn g}^{-1}$) has potential for TBT to induce deleterious effects according Australia Guidelines (Simpson

et al., 2013) (Table S1). These values are above SQGV of 9 ng Sn g⁻¹ (also normalized to 1% OC) (Simpson et al., 2013). In fact, our results showed that 5 out of 8 sites in Marajó and Guajará bays (PA4, PA6, PA7, PA9 and PA19) are classified as Class C according to OSPAR (2011). In such class, long term exposure may cause adverse effects to benthic organisms. Ribeiro-Brasil et al. (2021) also evaluated imposex (sexual alteration in gastropods induced by TBT exposure) in *Thaisella coronata* from intertidal marine sites between PA9 and PA19. Imposex incidence ranged from 0 to 100% in inner regions, but the imposex indices indicate low effects in individuals (Vas Deferens Sequence Index - VDSI up to 1).

All booster biocides were detected in Marajó and Guajará bays. DCOIT was detected in 4 out of 8 sites (< 0.7 to 6.5 ng g⁻¹). Dichlofluanid (< 2.1 to 3.8 ng g⁻¹), chlorothalonil (< 0.4 to 1.9 ng g⁻¹) and Irgarol (< 1.2 to 2.0 ng g⁻¹) were detected in 3 out of 8 sites, and diuron was detected in two sites: PA4 (< 1.4 ng g⁻¹) and PA19 (6.9 ng g⁻¹) (Table 2; Figure 1). The highest concentrations of diuron and DCOIT were found in a shipyard site (PA19) with many docked boats and moderate levels of TOC and muddy sediment (1.2% and 15.9%, respectively). Both biocides are commonly employed to modern antifouling paints (Abreu et al., 2020). Irgarol, chlorothalonil and dichlofluanid were found mainly in the Guajará Bay (PA1, PA3, PA4 and PA6) where there is prevalence of artisanal fishing. Nevertheless, the highest concentration of Irgarol and dichlofluanid were found in PA4 (2 ng g⁻¹ and 3.8 ng g⁻¹, respectively), and the highest level of chlorothalonil was close to boatyard on Marajó bay (PA9 – 1.9 ng g⁻¹). These concentrations are low and can be resultant of the high hydrodynamic in the whole system due to the tidal range, which varies from 4 to 6 meters of amplitude, and/or by freshwater output both due to the vast numbers of large rivers that make up the Amazonian basin and/or due to local seasonality, as the region exhibits an annual average precipitation of 2000 mm/year. Therefore, potential inputs from intense artisanal fishing activities, including boat maintenance and maritime transport, are constantly diluted and contaminants associated to suspended particulate matter are exported to open waters (Ribeiro-Brasil et al., 2021). Due to the influence of TOC on persistence and bioavailability of contaminants, the Norwegian Pollution Control Authorities developed a guideline for environmental health of marine sediments in which sites PA19 (normalized 1% OC) belong to class III for diuron (Bakke et al., 2010). This means that long-term exposure to diuron in those sites may cause effects on sufficient number of species to damage community structure and function. Regarding Irgarol, PA4 and PA6

are class V (Table S1). This class are associated to severe community effects from short-term exposure to Irgarol (Bakke et al., 2010). For DCOIT, in turn, only concentration at PA19 (6.5 ng g^{-1}) is above the predicted no effect concentration (PNEC) of 3 ng g^{-1} (Wang et al., 2014b) indicating a likely adverse effect to the local biota.

1.2.2 São Marcos Bay, Maranhão

TBT was detected ($< 5 \text{ ng Sn g}^{-1}$) in sediments of 2 out of 10 sites (MA3 and MA13) of São Marcos Bay, while DBT and MBT were only detected at site MA13 (6.7 and 7.1 ng Sn g^{-1} , respectively) (Table 2; Figure 1). MA3 and MA13 exhibited, respectively, high and moderate contents of organic carbon (2% and 0.4%) and fine sediments (61.9% and 31.5%). Both sites are under influence of marinas with intermediate level of activities of recreational and fishing boats (Viana et al., 2020). Although below the limit of quantification, TBT levels indicate that MA3 and MA13 are classified as class C according to the OSPAR (2011) guidelines. In fact, there are frequent observations of imposex in gastropods on Sao Marcos bay, especially, in site MA13 (personal comm.). Nevertheless, considering the low levels of TBT, its metabolites and no fresh inputs, São Marcos Bay does not seem to pose a risk to local biota.

On the other hand, all booster biocides were found in São Marcos Bay. Irgarol was detected in 5 out of the 10 sites (< 1.2 to 1.9 ng g^{-1}), while DCOIT was detected in 4 sites (4.7 to 9 ng g^{-1}). Diuron (< 1.4 to 1.7 ng g^{-1}), chlorothalonil (1.9 to 3.7 ng g^{-1}) and dichlofluanid (< 2.1 to 3.1 ng g^{-1}) were detected in 3 sites (Figure 1; Table 2). Chlorothalonil and dichlofluanid were detected in sampling points (MA3, MA4, MA5 and MA6) with constant mooring and repairing of artisanal fishing boats. These sites are located in a semi-enclosed region with low water circulation. Although dichlofluanid was not analyzed in sediments and porewaters by Viana et al. (2019), these authors found their metabolite (DMSA) in porewaters of São Marcos Bay. DMSA was detected in sites where our results show the presence of dichlofluanid (MA4 and MA8). These results are complementary since dichlofluanid is quickly degraded in the environment (Schouten et al., 2005). DCOIT was found in marinas with intense mooring of boats (MA2, MA5 and MA6) as well in a boatyard with frequent repair of boats and ships (MA1). This was also observed in other Brazilian estuaries such as Vitoria estuarine system (Abreu et al., 2021) and Patos Lagoon estuary (Soroldoni et al., 2018). DCOIT levels found in São Marcos Bay are above the PNEC of 3 ng g^{-1} (Wang

et al., 2014b) indicating likely adverse effects to biota. Regarding to Irgarol, it was found in marinas (MA1, MA4, MA6, MA13) and at a ferry boat terminal that transport people from São Luís to the continent (MA8). Although detected in low concentrations, levels of Irgarol pose potential toxic effects to biota, because according to Bakke et al. (2010), our results (1% OC) showed sites under classes IV (MA1 and MA6) and in the worst class V (MA13) which is likely to induce severe community effects on local biota. Similarly, Diuron was detected in sites MA1, MA2 but their levels (1% OC) not expected to pose risk on São Marcos bay according Bakke et al. (2010). The categorization of MA8 in class V and IV for Irgarol and diuron, respectively, is due the low content of organic carbon in this sample (0.2%) (Table S1). Also, in order to compare the results, Viana et al. (2019) reported high values to Irgarol (MA8) and diuron (MA2) (maximum of 45.6 and 55.2 ng g⁻¹, respectively) in sediments collect in the same period. However, the authors also reported high variation between replicates due to probable presence of antifouling paint particles (APP) could have affected the levels of those booster biocides in São Marcos bay. In fact, our findings confirmed the presence of APPs. Levels of APPs ranged from < 0.01 to 938 µg g⁻¹ (MA2), being detected in 9 out of 10 sites (Figure 2). Presence of booster biocides in APPs confirmed their origin associated to antifouling paints as indicated by Thomas et al. (2003). Among antifouling biocides, the levels on APP were detected only to chlorothalonil (669 ng g⁻¹) and the levels for dichlofluanid and DCOIT ranged from < 70 to 12,683 ng g⁻¹ and < 20 to 10,960 ng g⁻¹, respectively (Figure 2). The marina MA2 and shipyard MA3, region intense boats maintenance, showed high levels of chlorothalonil, dichlofluanid and DCOIT on APP. DCOIT also was the only biocide detected in APP from Itaquí Port (MA7) supporting their common use in commercial ships worldwide (Tornero and Hanke, 2016). These concentrations imply a long-term source of biocide contamination to São Marcos Bay as it has been observed in Santos-São Vicente Estuarine System (Abreu et al., 2020).

3.2.3. Fortaleza coast, Ceará

Concentrations of TBT, DBT and MBT ranged from < 1 to 254 ng Sn g⁻¹, < 1 to 83.7 ng Sn g⁻¹ and < 1 to 72 ng Sn g⁻¹, respectively, in sediments of Fortaleza coast. The highest levels of ΣBTs were observed in sites CE5 (409 ng Sn g⁻¹), an area under influence of a marina used by many small fishing and recreational boats, and CE4 (60.1 ng Sn g⁻¹), a site located in sheltered sites of Mucuripe port (Figure 1; Table 2). Due to the sheltering effect produced by jetties built in Mucuripe port (Maia, 1998), sites CE4

and CE5 presented relatively high percentage of mud (> 65%) and TOC (> 0.5%). In fact, Buruaem et al. (2020) observed levels of TBT ranging from 9.8 to 52.6 ng Sn g⁻¹ on commercial berths and the navigation channel of Mucuripe port. Moreover, artisanal fishing, an important economic activity in Ceará state (Vaisman et al., 2005), may also have contributed with fresh TBT input at CE5 and CE6, since BDI was less than 1 (Table 2). In case of CE5, TBT levels presented values between ‘limit 1’ and ‘limit 2’ of Brazilian guideline (Brasil, 2012) which is lower than threshold levels of legislations but it causes concern due to the possible biomagnification on TBT on seafood sold (fish and stingray). Also, according to more restrictive regulation from OSPAR (2011), CE5 is classified as class F where levels are able to impair reproduction on more sensitive gastropod species. Whereas CE1 is categorized as class C where long term exposure may cause adverse effects and sites CE4 and CE6 are classified as class D where effects on reproduction may happen. Considering potential bioavailability (1% OC), CE1 and CE4 exceeded the SQGV of 9 ng Sn g⁻¹ (1% OC) while CE5 and CE6 exceeded trigger value of 70 ng Sn g⁻¹ (Simpson et al., 2013), indicating a strong probability of adverse effects to biota (Table S1). Some sites with dredging events (CE3, CE4) and intense artisanal fishery (CE5) has been revealed 100% of imposex. Despite this, a general reduction on contamination and imposex (levels, VDSI and RPLI) has been observed in the Fortaleza coast from 2004 to 2018 (unpublished data).

Although Irgarol, chlorothalonil and dichlofluanid were not detected in any sediment samples, diuron (< 1.4 ng g⁻¹) and DCOIT (< 0.7 to 9.6 ng g⁻¹) were detected in 2 and 3 out of 6 analyzed sites, respectively. Santos et al. (2019) also did not detected chlorothalonil among various pesticides analysed in sediments from Timonha-Ubatuba estuarine system (western border of Ceara). However, an inventory of pesticides used in the Ceará backlands indicated wide use in agriculture of chlorothalonil and diuron (Gama et al., 2013). On the other hand, traffic of small boats (fishery and recreational boats) is the main source of Irgarol and DCOIT since they are associate exclusively with antifouling paints (Thomas and Brooks, 2010). In fact, DCOIT was found at CE1 and CE5 that are sites with fishing activities and mooring of small fishing boats take place. These contamination found is above Predicted No Effect Concentrations (PNEC) levels (3 ng g⁻¹) available in the literature (Wang et al., 2014b). Further, only DCOIT was detected in APP from the coast of Fortaleza at levels reaching 1,656 ng g⁻¹ at CE5 which contains also the highest APP occurrence in sediments from Fortaleza coast (CE5, 62.7 µg g⁻¹) (Figure 2). APPs also detected in sediments from CE1 – 26.1 µg g⁻¹

(marina), CE3 – 19.1 $\mu\text{g g}^{-1}$ (boat/shipyard) and CE4 – 26.1 $\mu\text{g g}^{-1}$ (Mucuripe Port). In fact, a recent study carried out at Mansa Beach (close to Mucuripe port) indicated that it is an accumulation area for debris related to navigation/fishing activities from the Cocó River, Mucuripe port and nearby beaches (Cavalcante et al., 2020).

1.1.4 Capibaribe and Suape estuaries, Pernambuco

Samples were collected in wet season (March to September 2018) and PE1, PE2 and PE3 had with high levels of TOC (2.8% - 3.8%) and moderate content of fine sediments (23% - 35.7%) (Table 2). On the other hand, PE4 and PE5 were predominantly sand (> 92%) and moderate TOC (< 0.6%). Although Suape Port shelters shipyards and it has high ship traffic and cargo-handling (Table 1), sedimentary BT were not detected in this area (PE1 – PE5) (Figure 1). The low contamination by BTs in Suape estuary can be explained due the potential to transport material and dilution process by output of residual flow from the port area (Zanardi-lamardo et al., 2018). In fact, Maciel et al. (2018) evaluated butyltins contamination in this estuary and observed differences of BTs concentrations depending of occurrence of dredging events. In those study, PE3 and PE4 (dredging area) showed higher concentration before being dredged (ΣBTs 8.1 and 51.3 ng Sn g^{-1} , respectively, wet season) with subsequent decrease to (4.3 and 6.4 ng Sn g^{-1} , respectively, dry season). In present study, no was observed dredging activity during this study sampling (Suape, 2018), even dredging is a constant practice in this area.

Capibaribe Estuary System (CES) (PE6 – PE15), which shelters Recife Port, had a different scenario: sediments had only degradations products of TBT (Table 1; Figure 1). DBT and MBT were detected in all samples from Capibaribe bay ranging from <5 to 12.7 ng Sn g^{-1} and 9.24 to 54.9 ng Sn g^{-1} , respectively. The high content of fine sediments (11.5% – 74.7%) and TOC (0.86% – 5.1%) in estuary also influence on deposition and retention of butyltins. The nautical activities in CES are more intense and with more potential source of antifouling biocides than at Suape estuary due several marinas, fishing harbors and the Port of Recife (Maciel et al., 2018). Besides that, Schettini et al. (2016) characterized CES and classified it as partially mixed estuary which suspend material is trapping efficiency. In addition, the $\text{BDI} > 1$ in all sites from Capibaribe bay indicates the TBT degradation is occurring (photodegradation and/or microbial activity) and the inputs are not recent. In comparison to previous data (Maciel et al., 2018) the reduction of BTs contamination also is clear. The highest levels (ΣBTs) reported by Maciel et al. (2018) were in port area PE7 (ΣBTs 281 ng Sn g^{-1})

and a boatyard in PE13 (Σ TBTs 542 ng Sn g⁻¹) for sediments collected in December 2011, while in the present study measured concentration (Σ TBTs) at the same places were 35.9 ng Sn g⁻¹ and 68.1 ng Sn g⁻¹, respectively. According to OSPAR (2011) which classified based in TBT concentrations, only sediments from PE11 and PE12 (TBT < 5 ng Sn g⁻¹) were classified as class C, that could cause adverse effects on long term exposure. However, Maciel et al. (2018) observed no imposex incidence in *Stramonita rustica* from Capibaribe bay and Suape Port. Thus, our results may indicate the effectiveness of global ban of TBT-based antifouling paints at least nearby commercial ports on Suape and Capibaribe bays (IMO, 2020).

Sediments from Suape area presented lower concentration of booster biocides than Capibaribe, except at PE1. Chlorothalonil was not detected, Irgarol varied from <1.2 to 1.8 ng g⁻¹, and diuron was below quantification limit (<1.4 ng g⁻¹) or not detected (Table 2). Irgarol (PE1 – 1.8 ng g⁻¹, PE2 – 1.5 ng g⁻¹ and PE3 - <1.2 ng g⁻¹) and dichlofluanid (PE5, 5.4 ng g⁻¹) were detected in the watershed of Suape estuary (Massangana and Tatuoca rivers) which are influenced by industrial effluent and dredging events from Suape Port (Pessoa et al., 2009). DCOIT was the highest biocide concentration observed in this area, with 227.5 ng g⁻¹ (PE1). Indeed, this region receives remobilized sediments from dredging area (Maciel et al., 2018) and contains residues associated to eventual releases by fishery or recreational vessels (Lemos et al., 2014) as APPs which are commonly related by other to elevated concentrations (Batista-Andrade et al., 2018). Unfortunately, due to the small amount of samples, it was not possible to analyze the APP in sediments from Suape and Capibaribe bay. Based in PNEC values available in literature (Carvalho et al., 2015), dichlofluanid did not pose risk to biota. Nevertheless, Irgarol may present toxic effects at PE1 and PE2 once their normalized concentration to carbon organic 1% (0.6 ng g⁻¹ and 0.4 ng g⁻¹, respectively) (Table S1) are classified as class IV and III (Bakke et al., 2010). Still, the punctual high concentration of DCOIT (PE1) was 75 times higher than the Predicted No Effect Concentration (PNEC) of 3 ng g⁻¹ indicated by Wang et al. (2014).

Contrary what was observed in Suape area, all booster biocides were detected at Capibaribe bay. Contamination levels of DCOIT, diuron, dichlofluanid, Irgarol and chlorothalonil, ranged from <0.2 to 25.1 ng g⁻¹, <0.5 to 2.4 ng g⁻¹, <0.7 to 17.9 ng g⁻¹, <0.4 to 1.5 ng g⁻¹ and <0.1 to 1.6 ng g⁻¹, respectively (Table 2). The highest concentration of Irgarol (PE8, PE14), chlorothalonil (PE14) and dichlofluanid (PE15) was found in the inner region of CES, the Pina Sound (Figure 1). In this region there are

many wharfs for fishing vessels and intense traffic of small boats and high retention capacity mainly due to the tide-dominated hydrodynamics favoring organic contaminants accumulation (Maciel et al., 2016). In addition, Irgarol levels (1% OC) on sites PE14 (class IV), and PE8 (class III) can cause adverse effects according Norwegian authorities (Bakke et al., 2010). The highest concentration of DCOIT was observed at PE7, located in front of the international Recife Port, suggesting the common use of DCOIT in antifouling paint worldwide (Paz-Villarraga et al., submitted). Also, the second highest concentration of DCOIT and the highest level of diuron were also found at PE11, an area under influence of marinas and Yacht Club point out in recent studies as concern zones of antifouling biocides contamination (Abreu et al., 2020; Wezenbeek et al., 2018). In this site, concentration of both compounds in Capibaribe bay are in a potentially toxic since according to Bakke et al. (2010), diuron was categorized in class III (Moderate) while DCOIT concentration was above the PNEC (3 ng g^{-1} , according to Wang et al. (2014)). Similarly, Recife Port (PE7) and inner region (PE14) also presented DCOIT levels above the PNEC value. However, the general lower levels of butyltins and booster biocides in Suape port region may indicate the predominant use of other antifouling paint as Zn and Cu pyrthione (not analysed in this study) as suggested by Tornero and Hanke (2016b).

1.1.5 Guanabara Bay, Rio de Janeiro

Levels of TBT, DBT and MBT ranged from < 1 to $25.8 \text{ ng Sn g}^{-1}$, < 1 to $12.6 \text{ ng Sn g}^{-1}$ and < 1 to $27.3 \text{ ng Sn g}^{-1}$, respectively. Except RJ5, butyltins were detected in all sites of inner Guanabara Bay. This area is characterized by intense navigation activities as well as the presence of shipyard and marinas which act as contamination sources (Borges et al., 2013). Only RJ3 (a large shipyard) presented BDI less than 1, indicating a likely fresh input of TBT from boat maintenance activities. The highest concentrations of Σ BTs were found in sediments close to an oil platform at RJ1 ($49.3 \text{ ng Sn g}^{-1}$) and a shipyard at RJ4 ($49.1 \text{ ng Sn g}^{-1}$). These sites also exhibited high contents of mud (47.9% and 89.6%, respectively) and organic carbon (2.6% and 3.6%, respectively). Considering 1% OC normalization, concentrations in these sites were above the limit of 9 ng Sn g^{-1} (adverse biological effects on living biota are expected to occur) set by Australia Authorities (Simpson et al., 2013). No butyltins were detected at sites RJ6, RJ7 and RJ8 of Guanabara Bay. Although these sites receive disposal of dredging material, intense currents facilitate dispersion and dilution of contaminants (Baptista Neto et al., 2019a). Assessment of butyltins contamination has been done in Guanabara

Bay since the 2000's (Fernandez et al., 2005). In sediments collected in 2000, Botafogo inlet (RJ5) and Marina da Gloria (RJ4) exhibited, respectively, 520 and 419 ng Sn g⁻¹ associated with high incidence of imposex (Fernandez et al., 2005). After the global TBT ban, Toste et al. (2013) observed a decrease in severity of butyltins pollution and recolonization of *Stramonita brasiliensis* at some sites. Currently, concentration of butyltins further decreased in Guanabara Bay, but they are still a threat to gastropods since RJ1 and RJ3 were classified as class D while RJ2 and RJ4 were categorized as Class C following the OSPAR regulation (OSPAR, 2011).

Similar to other Brazilian coastal areas, DCOIT was the most frequently detected booster biocide (5 out of 8 sites in Guanabara Bay) ranging from < 0.7 to 13.3 ng g⁻¹, while Diuron ranged from < 1.4 to 1.6 ng g⁻¹. Irgarol (RJ5 and RJ6) and dichlofluanid (RJ7) were detected below the limit of quantification, and chlorothalonil was not detected at any site. Diuron was detected in RJ7 (out of the bay) even with low diuron concentrations (1% OC) may be already involved in severe community effects from short-term exposure (class V according to Bakke et al., 2010) due to low total organic carbon (< 0.01%). DCOIT has been widely detected in distinct regions of maritime activities (Chen and Lam, 2017). At Guanabara Bay, DCOIT was found in the mooring area (RJ2), a marina (RJ5) and offshore sites (RJ6, RJ7 and RJ8). Only RJ8 presented levels below the LQ, all other samples exhibited concentrations above the PNEC of 3 ng g⁻¹ (Wang et al., 2014b). This indicates likely DCOIT adverse effects to local biota of those sites. The maritime facilities on Guanabara Bay also can generate APP with occurrence in sediments ranging from <0.01 µg g⁻¹ to 117.4 µg g⁻¹ (RJ4). Similarly, microplastics other than APPs, but coming from harbor activities, have been observed in high quantities on inner and out of Guanabara bay (Baptista Neto et al., 2019a). Such particles from this study presented diuron and DCOIT concentrations ranged from < 50 to 7,377 ng g⁻¹ and < 20 to 43,139 ng g⁻¹, respectively. Diuron and DCOIT have been observed (in some instances in the same formulation) in modern antifouling paints (Paz-Villarraga et al., submitted). Thus, APP derived from such paints may act as secondary sources of diuron and DCOIT. Thereby, in regard of Guanabara bay being recognized as an environmentally degraded area, our findings on antifouling biocides and APP may be corroborating to the high toxicity found in the sediments in that area (Soares-Gomes et al., 2016).

3.2.6 Itajaí-Açu estuary, Santa Catarina

BT residues were detected at two sites of Itajaí-Açu estuary. At SC2, concentrations of TBT, DBT and MBT were 118, 26.3 and 44.1 ng Sn g⁻¹, respectively, while site SC5 revealed levels of 6.4, 8.6 and 6.5 ng Sn g⁻¹. Itajaí-Açu estuary shelters Itajaí Port, fishery processing facilities, boatyards and the largest Brazil's fishing port which are potential sources of BTs to local sediments. Total BT levels found at SC2 (188 ng Sn g⁻¹) and SC5 (21.5 ng Sn g⁻¹) might be influenced by regular dredging that remobilizes contaminants deposited in sediment beds (Frena et al., 2017). In fact, BDI < 1 at SC2 indicates predominance of tributyltin released from sediment remobilization. According to Brazilian guidelines (Brasil, 2012a), SC2 is between 'limiar 1 and 2' could be required further ecotoxicological studies if necessary perform dredge. Furthermore, considering TOC (1% OC), site SC2 exceeded the trigger value (SQG-High) at which effects on the sediment biota are likely to be observed (Simpson et al., 2013). Everywhere else, no BTs were detected in Itajaí-Açu estuary. Although all samples were collected near boatyards (i.e. potential source of antifouling biocides), input of contaminants can vary according to frequency of vessel repairs and composition of antifouling paints used (Valero et al., 2020). Previous studies evaluated butyltins in the same estuary and reported predominance of TBT in docks used for ship/boat repairs (Oliveira et al., 2010). A decrease of butyltins was observed in this study when compared to Oliveira et al. (2010) which showed a BTs ranged from 87.5 (SC2) to 1,267 ng Sn g⁻¹ (SC4) in sediments from collect in July 2008. Despite this, current levels of TBT in Itajaí-Açu estuary still pose a risk to local biota since SC2 and SC5 were classified as classes E and C, respectively, according to OSPAR (2011).

All booster biocides were detected in sediments of Itajaí-Açu estuary. Levels of DCOIT, diuron, dichlofluanid, Irgarol and chlorothalonil ranged from < 0.7 to 62.2 ng g⁻¹, 1.7 to 2.1 ng g⁻¹, 2.4 to 2.8 ng g⁻¹, < 1.2 to 1.7 ng g⁻¹ and < 0.4 to 14.5 ng g⁻¹, respectively. Beside antifouling paints, sources of diuron, dichlofluanid and chlorothalonil include their use in crops as herbicide and fungicide (Thomas and Brooks, 2010). Chlorothalonil, for example, is the eighth best-selling active ingredient in Santa Catarina state (Ministério da Agricultura, 2019). In this case, Itajaí Mirim is the main tributary of Itajaí-Açu River and drains agricultural areas of Santa Catarina (Schettini, 2002). Regarding to diuron, it was detected in two boatyards areas (SC6 and SC8) in levels (both 1.1 ng g⁻¹ - 1% OC) categorized in class III according (Bakke et al., 2010). Conversely, Irgarol and DCOIT are exclusively associated to antifouling paints. In this study, Irgarol was found in boatyards SC2, SC4 (near to Itajaí Port) and SC8.

Among these areas, SC2 (1.5 ng g⁻¹ - 1% OC) and SC4 (1.2 ng g⁻¹ - 1% OC) shows levels in “class bad” where severe community effects are expected from short-term exposure (Bakke et al., 2010). DCOIT, in turn, was detected in 4 out of 9 of sediment samples with level above the PNEC value (3 ng g⁻¹) (Wang et al., 2014b) in SC2, SC4 and SC5. Itajaí-Açu estuary has one of the largest maritime facilities of Brazil occupying approximately 17 km² and counting around 40 companies for boat maintenance services (Aur and Catarina, 2019). APPs occurrence were also observed in sediment from Itajaí-Açu estuary with levels from <0.01 to 47.4 µg g⁻¹. Diuron (< 50 to 8,204 ng g⁻¹), Irgarol (< 40 to 583 ng g⁻¹), chlorothalonil (< 15 to 4,924 ng g⁻¹) and DCOIT (< 20 to 4,108 ng g⁻¹) were also detected in these APPs from Itajaí-Açu estuary. Among booster biocides, only DCOIT were correlated with APP concentration sediments (Spearman, $r_s = 0.71$, $p < 0.05$). However, levels of Irgarol, chlorothalonil and dichlofluanid in sediments were observed at SC4 which there are relevant APP occurrence (25.4 µg g⁻¹). These findings suggest that inputs of such antifouling biocides to Itajaí-Açu estuary may be derived from APPs. Navigation activities can cause significant impacts due to inputs of inorganic (Pereira-Filho et al., 2010) and organic contaminants (Frena et al., 2017). Valero et al. (2020) associated the enrichment of metals (Cu, Pb and Zn) in sediments of Guanabara Bay, Itajai-Açu estuary and Patos Lagoon estuary to inputs from APP.

3.2.7 Patos Lagoon estuary, Rio Grande do Sul

The highest concentrations of BTs in the Patos Lagoon estuary were found in boatyards and marinas where Σ BTs ranged from 3.5 to 5,311 ng Sn g⁻¹. Among public port areas of Brazil, Rio Grande Port ranks 4th in cargo handling and Twenty Foot Equivalent Unit (TEU) number (ANTAQ, 2020). However, detection of butyltins in the estuary was restricted to boatyard areas (RG1, RG6, RG8, RG11, RG12 and RG13) where contaminant inputs are directly released to the environment. The wide range of total BTs may be influenced by different boatyard working capacities (Valero et al., 2020). The Yacht Club of Rio Grande (RG8, 5,311 ng Sn g⁻¹) has 25 years of regular maintenance of boats, while RG6 (1,720 ng Sn g⁻¹) is the oldest local boatyard (100 years of activities). Although the latter had been decommissioned in 2008, residues still persist in local sediments. In contrast, Rio Grande shipyard (one the largest in Patos Lagoon) showed lower contamination (Σ BTs = 26.6 ng Sn g⁻¹) because it is limited to construct oil platforms that do not require antifouling coatings routinely (Soroldoni et al., 2018). Among sampling sites, boatyards exhibited high contents of mud (over 40%)

and TOC (over 0.8%), except RG8 and RG13. Data normalized to 1% TOC showed 203 ng Sn g⁻¹ (RG6) and 503 ng Sn g⁻¹ (RG8). Both levels exceed SQG-High and consequently may cause significant adverse effects on the local biota. Conversely, no BTs were observed close to the estuary mouth (RG4, RG5) and navigation channel (RG2, RG3, RG7, RG9 and RG10). These sites are influenced by seaward transport of sediments, high water renewal rate and intense hydrodynamics (Fernandes et al., 2002). All sites in the Patos Lagoon estuary, including those with high concentration of BTs, showed rapid degradation of TBT (BDI > 1). In fact, Castro et al. (2012) observed a significant reduction of imposex close to estuarine mouth and moderate levels of BTs in tissues (15.9 to 33.5 ng Sn g⁻¹) of *Stramonita braziliensis*. Boatyards in the Patos Lagoon estuary pose a risk to benthic organisms since particle-derived BT residues are a continuously released to the environment. Sediments from boatyard RG11 and RG12 were classified as class C (likely adverse effects by long exposure) according to OSPAR (2011). Furthermore, sites RG6 and RG8 are categorized in class F where populations of sensitive gastropods can be extinct. Given the extremely high concentration, RG6 is above 'limit 1' while RG8 is above 'limit 2' on the Brazilian regulation. It set a high concentration (> 410 ng Sn g⁻¹) as the threshold for high probability of causing adverse effects to the local biota.

Soroldoni et al. (2018) reported the presence of diuron, Irgarol and DCOIT in sediments from the Patos Lagoon estuary (Figure 1). According to the authors, antifouling biocide occurrence was associated to APP generated by shipyards and marinas. In this study, chlorothalonil and dichlofluanid were reported in the Patos Lagoon estuary. Concentrations ranged from < 0.1 to 7.5 ng g⁻¹ and < 0.7 to 5.8 ng g⁻¹, respectively. Contamination sources include intense traffic of fishing and recreational boats and spread use in agricultural practices. As pointed by Paz-Villarraga (2019), the highest values of DCOIT, Irgarol, diuron, chlorothalonil and dichlofluanid were found during summer and autumn due to higher traffic of recreational boats in the area. Diuron and dichlofluanid were found in gills of *Pseudobatos horkelii* at Cassino beach (next to the mouth of the Patos Lagoon estuary). Mean levels were 2.0 ng g⁻¹ and 19.3 ng g⁻¹, respectively (Martins et al., 2020). The authors suggested that contamination in gill is indicative of exposure through water. Environmental "hotspots" of metal and antifouling biocides contamination have been associated to the occurrence of APP (Turner et al., 2009). We observed high concentration of antifouling biocides in particles retrieved from sediments collected near boatyards, corroborating with the

above mentioned sources. Previous studies found Irgarol (3.2 ng g^{-1}), diuron (25.3 ng g^{-1}) and DCOIT (88.6 to $67,125 \text{ ng g}^{-1}$) in APPs collected from boat hulls in two boatyards (RG1 and RG8) (Soroldoni et al., 2018). In the present study, BT levels were analyzed in APP retrieved from boatyard sediments (RG1, RG6, RG8, RG11, RG12 and RG13). BTs levels in the estuary ranged from < 100 to $311,474 \text{ ng Sn g}^{-1}$ for TBT, < 100 to $4,300 \text{ ng Sn g}^{-1}$ for DBT and < 100 to $6,610 \text{ ng Sn g}^{-1}$ for MBT (Figure 2). Although high concentrations of antifouling biocides in APPs may be due to old paint coatings, these levels still indicate high persistence of contaminants in the environment (Turk et al., 2020).

1.1 Contamination overview

BTs were detected in all investigated estuarine systems. Among the 69 investigated sites, TBT, DBT and MBT were detected in 36% (< 5 to 549 ng Sn g^{-1}), 52% (< 5 to $1,252 \text{ ng Sn g}^{-1}$) and 49% (< 5 to $3,510 \text{ ng Sn g}^{-1}$), respectively. In addition, there was a strong correlation was found between TBT and its degradation products (for DBT $r^2 = 0.74$, and MBT $r^2 = 0.65$, $p < 0.05$). A strong correlation was also observed between DBT and MBT ($r^2 = 0.99$, $p < 0.05$). Such results indicate that DBT and MBT are probably originated from the degradation of TBT (Castro et al., 2018).

Overall, BDI showed an effective degradation of TBT in sediments of Brazilian coastal areas. BDI over 1 was calculated for 64 sites (93%), indicating prevalence of degradation rather than input. In addition to sediment properties for degradation occurs (anoxic sediments, darkness) (Araújo et al., 2020), the predominance of old TBT input indicates the effectiveness of its world ban of antifouling paints. Conversely, BDI below 1 was calculated for 5 remaining sites (MA3, CE5, CE6, RJ3 and SC2), indicating fresh input of TBT associated with ongoing illegal and/or unregulated use of TBT-based paints by fishing boats in marinas and boatyards. Lack of control on TBT use has been already observed in other Latin American countries such as Ecuador (Castro et al., 2012a) and Venezuela (Paz-Villarraga et al., 2015). Furthermore, TBT in paint particles ($> 20 \mu\text{g g}^{-1}$) were found at MA3, CE5 and SC2. This finding can also contribute with fresh input of TBT to the environment (Lagerström et al., 2017). Prevalence of old input and/or efficient degradation has been observed in other studies carried out on in estuarine systems of southern Brazil (Abreu et al., 2021, 2020). The authors observed in Vitoria estuarine system fresh inputs of TBT (72% of sites) in the port navigation channel due to regular dredging operations and numerous boat maintenance facilities. Whereas, Abreu et al. (2020), in the Santos-São Vicente estuarine system, reported a

combination of fresh and old TBT inputs in 40% of sites dominated by marinas and boatyards.

Total BTs measured along the Brazilian coast are close to those reported by other authors worldwide even after a decade has passed of the global TBT ban (Jokšas et al., 2019). Ten years after the ban, Filipkowska and Kowalewska (2019) observed total BTs in sediments of ports of the southern Baltic coastal zone in the range of 5.7 to 3,321 ng Sn g⁻¹. Therefore, the impact of BTs in Brazilian coastal areas cannot be neglected even considering the global ban. In this study, sediment sampling comprised 31 sites under direct influence of boat/shipyards, 15 influenced by marinas, 18 exposed to ship/boat traffic and 5 sites under direct influence of commercial ports (navigation channel). The highest concentrations of BTs in coastal areas of Brazil were observed close to boat/shipyards as observed in Patos Lagoon estuary, Itajaí-Açu river and Fortaleza coast. BTs contamination in sites influenced by boatyards was significantly higher than those collected in sites exposed to navigation (Mann-Witney, $p < 0.05$). No statistically significant differences were observed between the other groups (Figure 3). Although marinas can also offer repair services to boats (e.g. CE5 and PA6), difference of high concentrations of antifouling biocides in boat/shipyards can be related to absence of good management practices becoming these sites hotspots of contamination due paint particles presence (Kotrikla, 2009). In a recent study, Turk et al. (2020) reported BTs levels of 10,120 to 66,345 ng Sn g⁻¹ in the vicinity of service cranes and shipyards in the Croatian Adriatic Sea.

In general, booster biocides presented lower concentrations in sediments of the Brazilian coast. The sum of booster biocides ($\Sigma BB = \text{Irgarol} + \text{diuron} + \text{chlorothalonil} + \text{dichlofluanid} + \text{DCOIT}$) ranged from 0.8 to 284.8 ng g⁻¹ (Table 1). Among booster biocides, DCOIT was the most frequently detected (50.7%), exhibiting its highest concentrations at RG8 (273.7 ng g⁻¹) and PE1 (227.5 ng g⁻¹). The same pattern was also observed in other estuaries of Brazil such as Santos-São Vicente (Abreu et al., 2020) and Vitória (Abreu et al., 2021) where DCOIT was the most frequent booster biocide with highest concentration of 74.6 and 63.6 ng g⁻¹, respectively. Although, DCOIT is expected to degrade rapidly in environmental matrices, a recent review shows that its degradation potential dependent on environmental factors such as pH, temperature, sunlight and dissolved oxygen (Chen and Lam, 2017). In addition, DCOIT intensive usage around the world favors its high frequency of detection in aquatic environments (Tornero and Hanke, 2016). Irgarol, diuron, dichlofluanid and chlorothalonil were also

detected in Brazilian coastal areas with frequencies of 37.7%, 30.4%, 23.2% and 18.9% of samples, respectively. No significant differences were observed between booster biocides considering groups of activities (boat/shipyards, traffic zone, marinas and ports) (Figure 3). These results might be influenced from non-naval sources of booster biocides since detection of dichlofluanid and chlorothalonil have been commonly associated to their use as herbicide (Lee et al., 2015) while diuron is applied to rice crops in southern Brazil (Caldas et al., 2019). Further studies considering other regions under influence of agricultural areas can be addressed for evaluated the potential contribution of other biocides sources.

Higher contaminations tend to be observed in environments that also have antifouling paint particles (Turner, 2010). In the Panamá channel, the highest concentration of DCOIT (81.6 ng g^{-1}) was found in a site with high traffic of commercial ships and vessels under maintenance and repair (Batista-Andrade et al., 2018). The same authors observed high relative standard deviation in sediments contaminated by paint particles. Antifouling paint particles can be found in a variety of sizes and leaching states that depends on their method of generation during paint removal and subsequent weathering (Turner, 2010). Quantification of booster biocides in paint particles retrieved from sediments of MA, CE, RJ and SC confirmed these residues as secondary sources of biocides to the environment (Figure 2). Despite this, no significant correlations ($p > 0.05$) were observed between concentration of APP and biocides in sediments. The intrinsic heterogeneity of particles (Turner et al., 2010) and the mixture with extraneous particulates and paints of non-antifouling origin (Parks et al., 2010) may alter the release and distribution of antifouling paint particles.

There are few studies regarding toxicity of booster biocides to sediment-dwelling organisms. According to Bakke et al. (2010), there are thresholds values for diuron and Irgarol that can be used for comparison purposes with levels obtained in this study. Environmental risk limits for chlorothalonil can be only found in the study of Van Wezel and Van Vlaardingen (2004) (50 ng g^{-1}). However, this level was not observed in any of the sampling sites assessed in this study. Carvalho et al. (2015) derived a predicted non effect concentration (PNEC) of 18 ng g^{-1} for dichlofluanid in sediments. Although the highest concentration found at PE15 was 17.9 ng g^{-1} , it is still below the concentration for triggering adverse effects to biota. The assessment report for DCOIT in the European Union (ECHA, 2014a) suggests PNEC of 3 ng g^{-1} . It is necessary to refine this value for Brazil considering the wide occurrence this compound

and the higher sensitivity on marine organism as point out by Martins et al. (2018). Given the use and frequency of detection of booster biocides in Brazilian coastal areas, there is urgency in performing an ecological risk assessment (ERA) in order to compare their environmental occurrence with ecotoxicity data and to infer potential risks for Brazilian ecosystems.

4. Conclusion

This study provides a wide diagnostic of Brazilian coastal areas regarding antifouling biocide contamination. In general view, butyltins concentration were higher than booster biocides. Nevertheless, butyltins were mainly derived from old input except in sites under influence of paint particles or dredging operations. A clear difference between boatyard sites and traffic zones was observed for BTs contamination. Similar differences were not seen for booster biocides. The highest concentrations of BTs and booster biocides were associated with boats maintenance and/or the presence of paint particles (punctual secondary source). This diagnostic revealed that contamination of antifouling biocides in Brazil needs an urgent Environmental Risk Assessment to evaluated if Brazilian ecosystems are under threatening risk.

Acknowledgments

The authors would like to thank Pablo Teofilo, Eduarda Garcia, Msc. Marta Diniz and Dra Sanye Soroldoni for the great help in samples preparation and performing chemical analysis. Authors also thank LACOM - *Laboratório de Análises de Compostos Orgânicos e Metais* (FURG) for LC-ESI-MS/MS analyses. This research was sponsored by FINEP – *Financiadora de Estudos e Projetos* (Grant No 1111/13 – 01.14.0141.00) and CAPES - *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior* (CIMAR II - Grant No 1988/2014). F.E.L. Abreu was funded by CAPES (CIMAR II). J. L. M. Viana was sponsored by FAPEMA (Process BM-02411/17). R. M. Batista (PhD grant) was also funded by CAPES. I. B. Castro (PQ 302713/2018-2), G. T. Yogui (PQ 310554/2019-5), E. Zanardi-Lamardo (PQ 311771/2019-0) and G. Fillmann (PQ 312341/2013-0 and 314202/2018-8) are research fellows of CNPq.

Table 1: Description of the study area

State of Brazil	Area	Sampling date	N° sediment sampled	Port / Cargo-handling (million tons per year)	Area description
Pará (PA)	Marajó and Guajará bays	Jul. 2017	8	Belem port / 14	Situed in Eastern Amazon, the system of Pará river estuary comprehends the Marajó bay in the west side where gather Tocantins rivers and Guajara bay flows to Atlantic Ocean. The Guajará Estuary is located at the mouth of the Amazon delta and is part of the Marajó bay (Borba and Rollnic, 2016). The water circulation is highly dynamic with mesotidal regime and spring tidal variations of around 3.6 m. Deposition and dispersion of sediments are controlled by intense fluvial regime and tida currents. There are prevalence muddy sediments on deposits along the system (Gregório and Mendes, 2009).
Maranhão	São Marcos bay	Apr.2018	10	Itaqui port / 25	São Marcos bay is located in a transition region between Amazon region and the semi-arid northeast. The estuary zone is governed by semidiurnal tides with macrotidal variation amplitudes ranging between 4 and 7 meters (González-Gorbeña et al., 2015). The estuary also insert in the Macro-Tides Mangrove Coast of the Amazon, one of the largest in the Brazil (Menezes et al., 2008). The port region has a deep navigable waterway and harbor facilities including the Ponta da Madeira terminal, the largest private port in Brazil (exportation of 190 million of ton per year) (González-Gorbeña et al., 2015)
Ceará	Fortaleza coast	Jan.2018	6	Mucuripe port / 4	Ceará is located on Northeastern Brazil where the climate is largely influenced by the Intertropical Convergence Zone (ITCZ) as well El Niño - Southern Oscillation (ENSO)(Moreira et al., 2017). Ceará, Cocó and Pacoti rivers begins on a rural and suburban area and crosses one of the largest urbanized areas in Brazil (Fortaleza city) until reached the Atlantic Ocean (Maia, 1998). The grain size characteristics of Fortaleza coastline is conditioned by the influence of hydrodynamic conditions strongly dominated by coarse sediments (particle sizes >63 µm) (Marino et al., 2013). Coastal currents are usually flowing from east to west which created a circular current type inside of the harbor's area (Bezerra et al., 2007). The Mucuripe harbor infrastructure includes an access channel, anchorage areas, evolution basins, and a long jetty (1900m long).

Pernambuco	Suape Estuary	Mar.2018	5	Suape Port / 24	<p>Capibaribe and Suape estuaries are in Pernambuco State, northeastern coast of Brazil, a tropical area under influence of the intertropical convergence zone (ITCZ). Recife and Suape port are about 40 km apart (Maciel et al., 2018). Suape port was constructed in 1977 by interrupted flow of four rivers. The inundation of surrounding landscape, mangroves forests and high amount of suspend material requires constant dredging events (Neumann-Leitão and Matsumura-Tundisi, 1998). On the hand, Recife Port is insert in Capibaribe estuarine system where local circulation is determined by the tidal regime and freshwater outflow. This estuary includes the Pina Sound which receives the domestic and industrial sewage from Recife city but still preserves a narrow fringe of mangrove forest. The depths of Recife ports range from -12 to -8 m due to dredging (Schettini et al., 2016a).</p>
	Capibaribe Estuary	Apr.2018	10	Recife Port / > 1	
Rio de Janeiro	Guanabara bay	Ago.2017	8	Rio de Janeiro Port / 7	<p>Guanabara Bay is a eutrophic coastal bay located in the heart of Rio de Janeiro city. The bay is severely polluted due to industrial and domestic discharges derived from several municipalities. It has a mean depth of 5.7 m and it is driven mainly by mixed semi-diurnal tidal currents (tidal amplitude - 0.7 m) (Kjerve et al., 1997). Guanabara bay is partially stratified and presents anoxic sediments. Sediments have high contents of fine sand and mud (Quaresma et al., 2001). Guanabara bay holds Rio de Janeiro Port which shipping of automobiles, containers, and transport of tourists. Boatyards and navigation areas for water sports also are common in this region.</p>
Santa Catarina	Itajaí-Açu river	Dez.2017	9	Itajaí Port / 5	<p>The Itajaí-Açu estuarine system (central coast of Santa Catarina state, South Brazil) has a drainage basin of 15,500 km². It is a narrow salt-wedge system with a meandering river shape (C. A.F. Schettini, 2002). The harbor activities continue to develop with dredging events to deepen the main river channel for reached 14 meters and allowing the entrance of larger ships (Naval, 2020). The lower estuary (highly stratified) consists mainly of clay size sediments (> 70 %) and local tide is mixed semi-diurnal with a mean range of 0.8 m (C. A.F. Schettini, 2002).</p>

Rio Grande do Sul	Patos Lagoon Estuary	Sep.2015	13	Rio Grande Port / 26	<p>Patos Lagoon is the world largest choked lagoon with 250 km in extension (Niencheski and Windom, 1994) where estuary covers about 10% of the total surface area. The wind and rivers discharge are the main forces driving circulation (Fernandes et al., 2002). Northwest winds favor the discharge of fresh water from the lagoon, while the South and Southwest winds favor the entry of seawater into the estuary (Moller et al., 2001). The morphological characteristics of the lagoon shows a dominance of coarse sediments in shallow areas and fines in deeper ones (Calliari et al., 2009). Port activity is one the most important economic activity in Rio Grande with three ports (Super Porto, Porto Velho, and Porto Novo) on the main channel in Patos Lagoon estuary. Moreover, the maritime traffic is intense in this region, with large boatyards and boat maintenance facilities to fishing and leisure boats (Soroldoni et al., 2018)</p>
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Table 2: Maritime activity type, coordinates, total organic carbon (%TOC), percentage of fines, concentration of TBT, DBT, MBT and sum of BTs (Σ BTs) (ng Sn g⁻¹), butyltin degradation index (BDI), and concentration of diuron, Irgarol, chlorothalonil (Chlor), dichlofluanid (Dichlo), DCOIT, and sum of booster biocides (Σ BB) (ng g⁻¹) for sediment samples from Pará (PA), Maranhão (MA), Ceará (CE), Pernambuco (PE), Rio de Janeiro (RJ), Santa Catarina (SC), and Rio Grande do Sul (RG).

Site	Type	Coordinates (S; W)	TOC(%)	Fine (%)	ng Sn g ⁻¹				BDI	ng g ⁻¹					
					TBT	DBT	MBT	Σ BTs		Diuron	Irgarol	Chlor	Dichlo	DCOIT	Σ BB
PA1	Traffic	1°44'9.1";48°53'46.3"	1.8	12.0	<1	<1	<5	3.5	6.0	<0.5	<0.4	<0.1	2.8	2.2	5.5
PA3	Boat/Shipyard	1°29'24.85";48°27'39.86"	1.1	30.1	<1	<1	<1	1.5	2.0	<0.5	<0.4	<0.1	<2.1	<0.2	1.7
PA4	Traffic	1°27'7.93";48°30'11.66"	0.75	51.8	9.1	8.5	18.3	35.9	2.9	<1.4	2.0	<0.4	3.8	<0.2	6.8
PA6	Boat/Shipyard	1°13'24.28";48°31'31.29"	0.54	17.1	24.7	6.3	24.1	55.1	1.2	<0.5	1.6	0.6	<0.7	<0.2	2.9
PA7	Boat/Shipyard	0°45'29.06";48°30'54.11"	0.72	41.1	<5	<5	<5	10	3.0	<0.5	<1.2	<0.1	<0.7	<0.7	1.6
PA8	Boat/Shipyard	0°44'21.47";48°30'47.60"	1.3	26.4	<1	<5	<5	5.5	10.0	<0.5	<0.4	<0.1	<0.7	<0.2	1.0
PA9	Boat/Shipyard	0° 51' 30"; 48°9' 39"	2.6	21.5	<5	<5	<5	7.5	2.0	<0.5	<0.4	1.9	<0.7	2.3	5.1
PA19	Boat/Shipyard	1° 3'34.10";46°45'43.28"	1.2	15.9	7.6	<5	11.6	22.7	2.0	6.9	<0.4	<0.1	<0.7	6.5	14.0
MA1	Marina	2°32'53.09"; 44°18'22.66"	1.6	70.5	<1	<1	<1	<1.5	2.0	<1.4	1.4	<0.1	<0.7	5.3	7.8
MA2	Boat/Shipyard	2°32'28.00"; 44°18'42.00"	1.4	66.2	<1	<1	<1	<1.5	2.0	<1.4	<0.4	<0.1	<0.7	9.0	10.3
MA3	Marina	2°25'13.25"; 44° 5'38.29"	2.0	61.9	<5	<1	<1	3.5	0.4	<0.5	<0.4	1.9	<0.7	<0.2	2.8
MA4	Marina	2°25'15.04"; 44° 5'37.75"	1.3	73.9	<1	<1	<1	<1.5	2.0	<0.5	<1.2	<0.1	3.1	<0.2	4.1
MA5	Marina	2°25'16.70"; 44° 5'36.89"	1.1	67.2	<1	<1	<1	<1.5	2.0	<0.5	<0.4	3.7	<0.7	4.7	9.2
MA6	Marina	2°25'18.29"; 44° 5'36.20"	0.80	59.9	<1	<1	<1	<1.5	2.0	<0.5	1.9	3.7	<0.7	5.9	12.1
MA7	Port	2°35'5.80"; 44°22'5.60"	1.6	87.2	<1	<1	<1	<1.5	2.0	<0.5	<0.4	<0.1	<0.7	<0.2	1.0
MA8	Traffic	2°32'8.00"; 44°21'17.00"	0.01	4.4	<1	<1	<1	<1.5	2.0	1.7	1.7	<0.1	2.9	<0.2	6.5

MA9	Traffic	2°31'42.00"; 44°18'28.70"	0.70	59.4	<1	<1	<1	<1.5	2.0	<0.5	<0.4	<0.1	<2.1	<0.2	1.7
MA13	Marina	2°30'27.65"; 44°19'0.79"	0.40	31.5	<5	6.7	7.13	16.3	5.5	<0.5	1.4	<0.1	<0.7	<0.2	2.2
CE1	Marina	3°42'0.24"; 38°35'18.94"	0.32	16.1	6.3	7.0	6.8	20.1	2.2	<1.4	<0.4	<0.1	<0.7	9.6	10.9
CE2	Traffic	3°49'21.23"; 38°24'19.27"	0.44	14.3	<1	<1	<1	<1.5	2.0	<0.5	<0.4	<0.1	<0.7	<0.2	1.0
CE3	Boat/Shipyard	3°43'4.10"; 38°31'28.42"	0.31	2.2	<1	<1	<1	<1.5	2.0	<0.5	<0.4	<0.1	<0.7	<0.2	1.0
CE4	Port	3°42'20.76"; 38°29'4.07"	0.53	89.6	23.7	31.3	5.1	60.1	1.5	<1.4	<0.4	<0.1	<0.7	<0.2	1.4
CE5	Marina	3°43'4.91"; 38°28'36.11"	1.1	67.2	254	83.7	72	409	0.6	<0.5	<0.4	<0.1	<0.7	8.6	9.5
CE6	Traffic	3°46'25.94"; 38°26'14.76"	0.19	6.8	23.1	8.7	<5	34.3	0.5	<0.5	<0.4	<0.1	<0.7	<0.7	1.2
PE1	Traffic	8°21'33.87" ;34°58'22.44	3.1	35.7	<1	<1	<1	<1.5	2.0	<0.5	1.8	<0.1	<0.7	227	229
PE2	Boat/Shipyard	8°22'13.33" ;34°58'1.56"	3.8	23.7	<1	<1	<1	<1.5	2.0	<0.5	1.5	<0.1	<0.7	<0.2	2.3
PE3	Boat/Shipyard	8°22'57.14" ;34°58'46.92"	2.8	23.5	<1	<1	<1	<1.5	2.0	<1.4	<1.2	<0.1	<0.7	<0.2	1.8
PE4	Port	8°23'27.99" ;34°57'57.6"	0.54	8.2	<1	<1	<1	<1.5	2.0	<0.5	<0.4	<0.1	<0.7	<0.2	1.0
PE5	Traffic	8°21'37.8" ;34°57'48.96"	0.08	4.3	<1	<1	<1	<1.5	2.0	<0.5	<0.4	<0.1	5.4	<0.2	6.0
PE6	Marina	8°3'2.4";34°52'25"	3.4	17.8	<1	9.7	21.3	31.5	62.0	<0.5	<0.4	<0.1	4.8	<0.2	5.4
PE7	Port	8°3'14" ;34°52'3.7"	1.7	61.3	<1	<5	32.9	35.9	70.8	<0.5	<0.4	<0.1	<0.7	25.1	25.9
PE8	Boat/Shipyard	8°4'12.1" ;34°52'20.8"	3.0	20.7	<1	6.8	22.4	29.7	58.4	<0.5	1.5	<0.1	<0.7	<0.7	2.5
PE9	Marina	8°4'24.1";34°52'48.9"	2.9	20.1	<1	7.8	15.4	23.7	46.4	<0.5	<0.4	<0.1	<0.7	<0.2	1.0
PE10	Boat/Shipyard	8°4'44.8";34°52'38.9"	2.8	61.3	<1	6.5	20.7	27.7	54.4	<0.5	<1.2	0.8	<0.7	<0.2	2.1
PE11	Boat/Shipyard	8°4'44.1" ;34°53'25.2"	2.6	41.6	<5	7	28.4	37.9	14.2	2.4	<0.4	<0.1	<0.7	6.0	9.0
PE12	Boat/Shipyard	8°4'59.8" ;34°53'5.9"	3.4	14.2	<5	8.1	28.5	39.1	14.6	1.5	<0.4	<0.1	3.9	<0.2	5.7
PE13	Boat/Shipyard	8°5'2.3" ;34°53'20.6"	2.9	74.7	<1	12.7	54.9	68.1	135	1.5	<0.4	<0.1	<0.7	<0.7	2.5

PE14	Traffic	8°5'3.6" ;34°53'58.5"	0.86	11.5	<1	<5	14.5	17.5	34.0	<0.5	1.4	1.6	<0.7	3.9	7.5
PE15	Marina	8°5'21.1" ;34°53'41.7"	5.1	74.7	<1	6.5	9.2	16.2	31.5	<0.5	<0.4	<0.1	17.9	<0.2	18.5
RJ1	Marina	22°49'54.00" ;43°09'46.60"	2.6	47.9	25.1	11.8	12.4	49.3	1.0	<0.5	<0.4	<0.1	<0.7	<0.2	1.0
RJ2	Port	22°51'02.20" ;43°08'38.80"	1.5	19.7	13.2	9.5	8.0	30.7	1.3	<0.5	<0.4	<0.1	<0.7	6.3	7.2
RJ3	Boat/Shipyard	22°51'92.60" ;43°12'29.00"	2.6	55.9	25.8	12.6	10.7	49.1	0.9	<0.5	<0.4	<0.1	<0.7	<0.2	1.0
RJ4	Marina	22°54'97.20" ;43°10'07.50"	3.6	89.6	6.4	2.5	27.3	36.2	4.7	<0.5	<0.4	<0.1	<0.7	<0.2	1.0
RJ5	Marina	22°56'82.50" ;43°10'54.80"	0.06	1.4	<1	<1	<1	<1.5	2.0	<1.4	<1.2	<0.1	<0.7	13.3	15.0
RJ6	Traffic	23°02'284" ;43°06'527"	0.00	3.4	<1	<1	<1	<1.5	2.0	<0.5	<1.2	<0.1	<0.7	8.5	9.7
RJ7	Traffic	23°01'427" ;43°06'527"	0.03	1.5	<1	<1	<1	<1.5	2.0	1.6	<0.4	<0.1	<2.1	5.9	8.9
RJ8	Traffic	23°00'342" ;43°06'615"	0.01	3.7	<1	<1	<1	<1.5	2.0	<0.5	<0.4	<0.1	<0.7	<0.7	1.2
SC1	Boat/Shipyard	26°53'5.36"; 48°39'50.78"	1.3	64.6	<1	<1	<1	<1.5	2.0	<0.5	<0.4	<0.1	<0.7	<0.2	1.0
SC2	Boat/Shipyard	26°53'12.49";48°40'51.45"	1.1	76.7	118	26.3	44.1	188	0.6	<0.5	1.6	<0.1	<0.7	3.2	5.5
SC3	Boat/Shipyard	26°51'25.58";48°43'18.03"	1.9	45.4	<1	<1	<1	<1.5	2.0	<0.5	<0.4	<0.1	<0.7	<0.2	1.0
SC4	Boat/Shipyard	26°53'35.57"; 48°40'17.43"	1.4	87.9	<1	<1	<1	<1.5	2.0	<0.5	1.7	14.5	2.8	62.2	81.6
SC5	Boat/Shipyard	26°52'52.12";48°41'25.90"	1.4	61.9	6.4	8.6	6.5	21.5	2.4	<0.5	<0.4	<0.1	<0.7	3.5	4.3
SC6	Boat/Shipyard	26°51'52.16";48°41'40.72"	1.5	61.0	<1	<1	<1	<1.5	2.0	1.7	<0.4	<0.1	<0.7	<0.2	2.4
SC7	Boat/Shipyard	26°52'1.91;48°42'24.69"	0.93	46.0	<1	<1	<1	<1.5	2.0	<0.5	<0.4	<0.1	<0.7	<0.7	1.2
SC8	Boat/Shipyard	26°55'10.84";48°38'51.84"	1.9	47.4	<1	<1	<1	<1.5	2.0	2.1	<1.2	<0.4	2.4	2.8	8.1
SC9	Boat/Shipyard	26°54'43.40";48°39'10.18"	1.3	87.1	<1	<1	<1	<1.5	2.0	<0.5	<0.4	<0.1	<0.7	<0.2	1.0
RG1	Boat/Shipyard	32° 8'12.15"; 52° 6'14.33"	1.1	45.7	2.5	6.2	17.9	26.6	9.6	<0.1*	<0.1*	<0.1	<0.7	3.7*	4.2
RG2	Traffic	31°43'52.08";51°57'57.41"	1.6	27.2	<1	<5	<1	3.5	6.0	<0.5*	<0.5*	0.8	<0.7	2.9*	4.6

RG3	Traffic	31°44'5.46";52° 8'44.36"	1.6	45.4	<1	<5	<5	5.5	10	<0.1*	<0.1*	<0.1	2.5	<0.5*	2.9
RG4	Traffic	32°10'9.43";52° 5'17.11"	0.81	36.2	<1	<1	<1	<1.5	2.0	<0.1*	<0.1*	<0.1	<0.7	<0.5*	0.8
RG5	Marina	32° 8'13.33"; 52° 6'13.78"	1.2	16.1	<1	<1	<1	<1.5	2.0	<0.1*	<0.5*	<0.1	<0.7	<0.5*	1.0
RG6	Boat/Shipyard	32° 1'44.61";52° 4'53.52"	1.6	50.6	325	319	1076	1720	4.3	17.7*	7.8*	<0.1	<0.7	6.2*	32.1
RG7	Traffic	31°56'57.66";52° 4'27.03"	0.99	24.0	<1	<1	<1	<1.5	2.0	<0.5*	<0.1*	7.5	<0.7	<0.5*	8.4
RG8	Boat/Shipyard	32° 1'34.73";52° 6'32.50"	1.1	27.8	549	1252	3510	5311	8.7	5.0*	<0.5*	<0.1	5.8	273.7*	284
RG9	Traffic	31°42'26.01";51°57'25.00"	1.3	35.4	<1	<5	<1	3.5	6.0	3.7*	1.6*	<0.4	<0.7	<0.5*	6.0
RG10	Traffic	32° 2'28.57";52° 3'7.58"	0.81	24.8	<1	<1	<1	<1.5	2.0	<0.1*	<0.1*	<0.1	<0.7	<1.5*	1.3
RG11	Boat/Shipyard	32° 3'9.79"; 52° 2'44.16"	0.88	43.7	5.7	23.5	96.8	126	21.1	<0.1*	<0.1*	<0.1	<0.7	<1.5*	1.3
RG12	Boat/Shipyard	32° 8'8.84";52° 4'48.78"	1.6	53.1	5.8	21.4	42.9	70.1	11.1	<0.1*	<0.5*	<0.1	<0.7	<1.5*	1.5
RG13	Boat/Shipyard	32° 3'1.58";52° 2'42.55"	0.44	25.3	<5	8.3	12.8	23.6	8.4	<0.5*	<0.5*	<0.1	3.2	<1.5*	4.5

*Levels of Irgarol, diuron and DCOIT in sediments from Patos Lagoon estuary (RG1-RG13) are retrieved of Soroldoni et al. (2018)

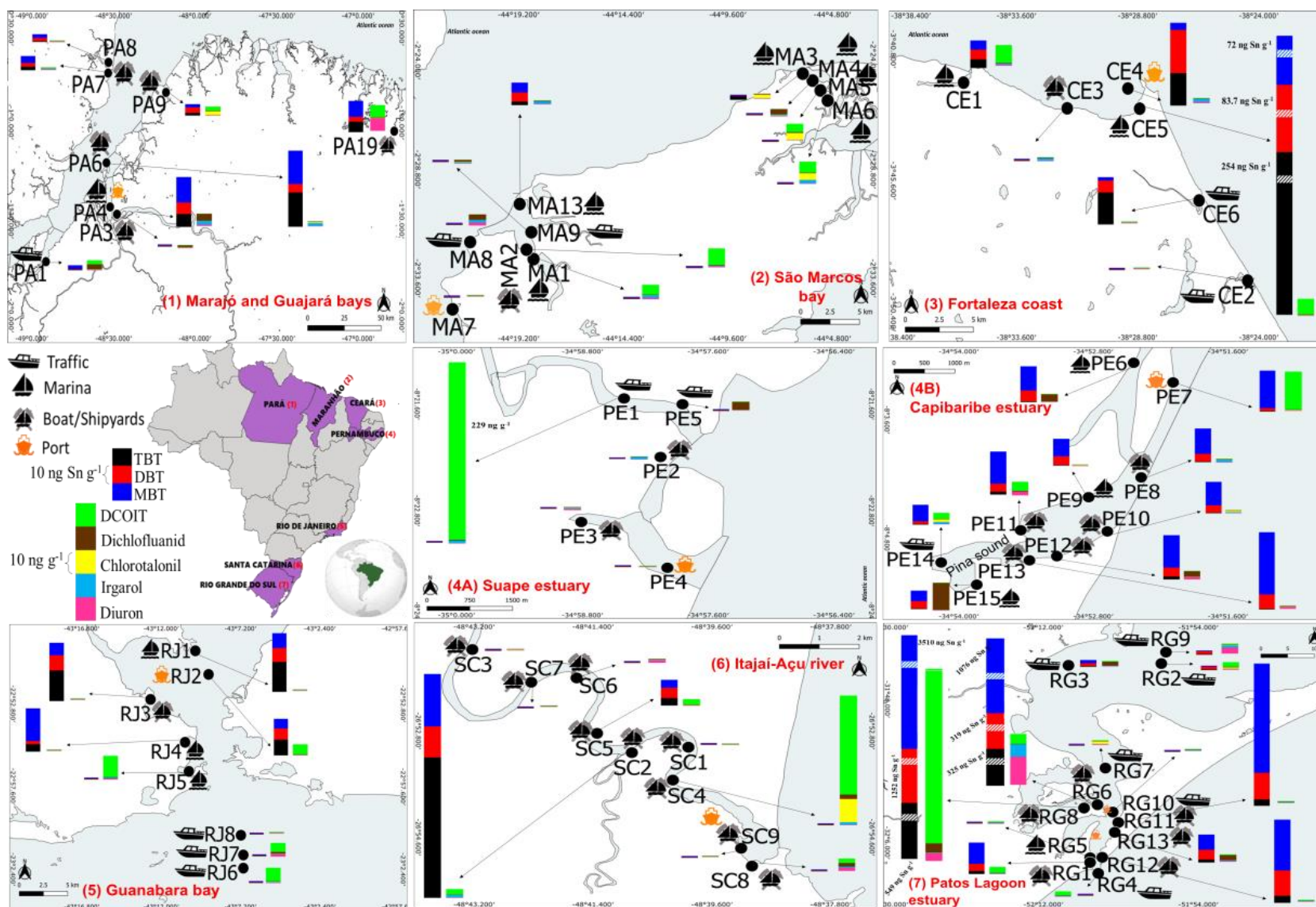


Figure 1: Concentrations of butyltins (ng Sn g⁻¹) and booster biocides (ng g⁻¹) in sediments along the Brazilian coast

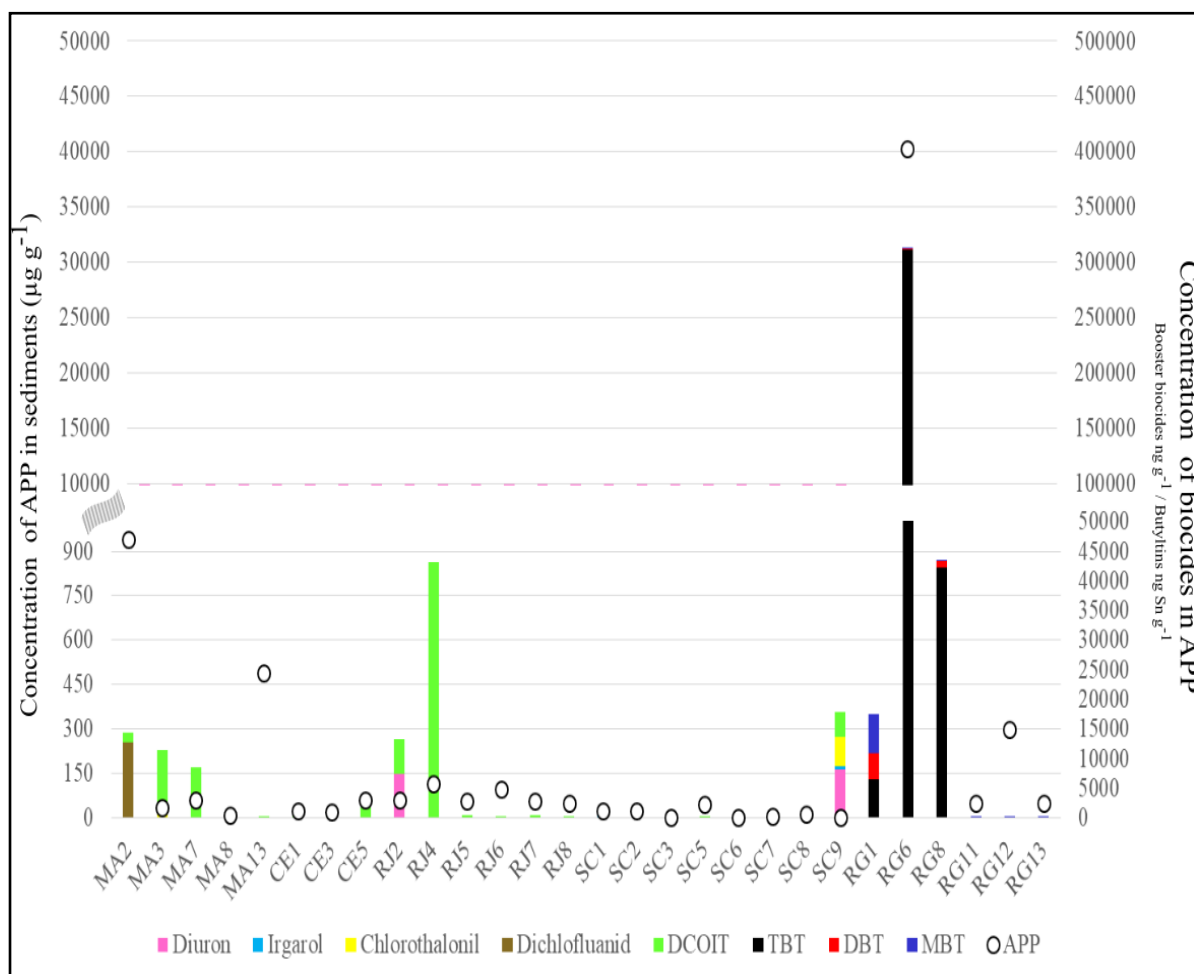


Figure 2. Concentration ($\mu\text{g g}^{-1}$) of APPs occurrence in sediments (white circles) and concentration of antifouling biocides in APPs (colored bars) from Maranhão (MA), Ceará (CE), Rio de Janeiro (RJ), Santa Catarina (SC) and Rio Grande do Sul (RG)

*Concentration of APP in sediments from Rio Grande do Sul (RG) was retrieved from Soroldoni et al. (2018)

** BTs contamination was analyzed only in APP from Rio Grande do Sul (RG). No was there enough paint particles for booster biocides analysis.

***No analysis of paint particles was evaluated in sediment from Para and Recife (no sample available).

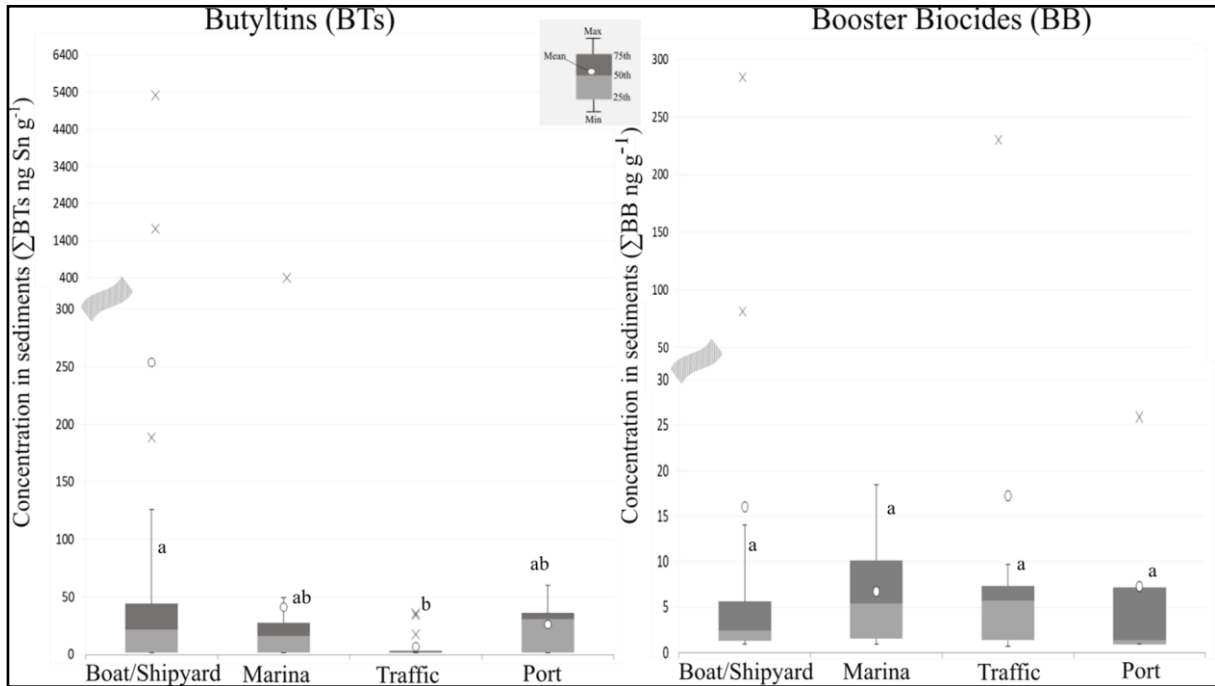


Figure 3. Boxplot of butyltins (sum of TBT, DBT and MBT) and booster biocides (sum of Irgarol, diuron, chlorothalonil, dichlofluanid and DCOIT) grouped according to the type of maritime activity (boatyard, marina, traffic and port). Symbol X represents the outliers. Different letters indicate significant differences ($p < 0.05$).

ARTIGO 4

Ecological Risk Assessment of booster biocides in sediments of the Brazilian Coastal Areas

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ABSTRACT

Although booster biocides (Irgarol, diuron, chlorothalonil, dichlofluanid, and DCOIT) have been detected in sediments along the Brazilian coastal areas, the risk associated to their occurrence and levels is still unknown. Thus, the ecological risk of booster biocides to sediment-dwelling organisms from the Brazilian coast was assessed using a risk characterization approach through the Risk Quotient (Measured environmental concentration (MEC) / Predicted no effect concentrations (PNECs)). Sedimentary PNECs for Irgarol, diuron, chlorothalonil and DCOIT were derived based on published ecotoxicological data from both freshwater and marine studies, while a NORMAN methodology was used to derived it for dichlofluanid. Results showed that DCOIT, diuron, Irgarol, chlorothalonil, and dichlofluanid can pose high risk on 47%, 35%, 15%, 1% and 1%, respectively, of the appraised 113 Brazilian sites. Considering the trend of expansion of navigation/maritime activities, DCOIT may worsen its impact over the coastal areas of Brazil, especially ports, but also ship/boatyards, marinas, and maritime traffic zones. In addition to bring this problem to the attention of stakeholders and policymakers, the environmental legislation may also incorporate these more robust threshold limits to improve the control over the use of antifouling products within Brazil.

1. INTRODUCTION

1.1 Contextualization

Chemical contaminants in the environment play an important role in the Anthropocene era (Hayes and Hansen, 2017). Transport to remote locations, bioaccumulation, biomagnification and persistence in the environment raise concerns associated to pesticides, biocides, pharmaceutical and personal care products, among others. Regarding environmental persistence, sediments are final repository of most contaminants that enter the aquatic environments (Simpson and Batley, 2019). The exposure to organic compounds in sediments are often associated to adverse effect on benthic biota (Halpern et al., 2019). In addition, climatic and hydrodynamic events (rainfall, tides or water circulation) can also remobilize these contaminants to the water column and groundwater (Simpson and Batley, 2019). For these reasons, regulatory attention must be given to the ecological risks that sediment contaminants might pose.

Regulation frameworks in the United States (EPA, 1992), Canada (FCSAP, 2012) and Europe (ECB, 2003) are based on ecological risk assessment (ERA), which evaluate how likely adverse ecological impacts may occur as a result of exposure to one or more environmental stressors (EPA, 1992). In such countries, this process is mandatory to approve the use and commercialization of products by the market. According to the Technical Guidance Document on Risk Assessment (TGD) (ECB, 2003) and the Guidance on the Biocidal Products Regulation (BPR) (ECB, 2017), both from the European Commission (one of the most strict environmental regulation), the risk assessment should proceed by phases of hazard identification (problem formulation), exposure assessment, dose-response assessment (ecological effects) and risk characterization (ECB, 2017, 2003).

The exposure assessment is performed through Measured Environmental Concentration (MEC) or Predicted Environmental Concentration (PEC), while ecological effects thresholds are determined by deriving Predicted No Effect Concentration (PNEC) for each environmental compartment (water, sediment, air) (ECB, 2017). Afterwards, a risk definition to environmental compartment follows, in general, a tiered approach to define a sequence of steps for risk assessments. In Tier-1, modelling and/or experimental data are used to assess ecotoxicity (PNEC) and characterize local or regional exposure scenarios (MEC) (ECB, 2017). Risk quotients ($RQ = MEC/PNEC$) are then calculated. In case of $RQ > 1$, indicating the substance is

“of concern”, further actions (Tier 2 and sequence) are requested. Examples of Tier-2 risk assessments include compound monitoring, improvements of analytical methods, PNEC refinement, which could be addressed for ensure the environment protection. Also, the applicant of ERA can choose for risk reduction measures or consider the risk acceptable (ECB, 2017). However, policymakers or stakeholders may ask for further actions (Tiers) until a final conclusion concerning environmental risk is drawn.

1.2 Problem formulation

The Brazilian coastal area stretches out for 8500 km throughout the tropical and subtropical zones, harboring many distinct ecosystems of high ecological and touristic relevance, such as coral reefs, mangroves, coastal lagoons, sandbanks, wetlands, beaches and dunes (Jablonski and Filet, 2008). Each environment has its unique richness, diversity and abundance, as pointed out by several studies (Amaral et al., 2016; Araújo et al., 2020). However, this biodiversity is being threatened by many anthropic activities, such as inputs of metals (Baeyens et al., 2019), oil spills (Magris and Giarrizzo, 2020), dredging events, and navigation (Moreira et al., 2017).

Antifouling biocides prevent biofouling in submersed structures (platforms or oil duct) and vessel hulls worldwide. Currently, antifouling paints formulations are based on zinc and copper compounds, which have their toxicity boosted by the addition of one or more organic or organometallic biocides (Amara et al., 2018). Biocides used in formulations of the contemporary antifouling paint aim to be less harmful to the environment compared to organotin biocides (Konstantinou and Albanis, 2004). Commercial booster biocides, such as Irgarol, 4,5-Dichloro-2-n-octil-4-isotiazolin-3-ona (DCOIT), diuron, chlorothalonil and dichlofluanid, are among the most commonly currently used (Thomas and Brooks, 2010; Tornero and Hanke, 2016). Therefore, these are the ones addressed in the present study.

Irgarol and diuron have half-lives of 300 and 100 days, respectively, in sediments (Thomas and Brooks, 2010). Due to the effects on autotrophic organisms, their use was restricted in European countries (Price and Readman, 2013) and Australia (Simpson and Batley, 2019). Chlorothalonil and dichlofluanid have low solubility and low stability in the environment (Thomas and Brooks, 2010). The use of chlorothalonil as active substance is not approved by the European commission (European Commission, 2019) and New Zealand, while dichlofluanid has approval for use as antifouling biocide in both regions (Soon et al., 2019). However, there are known effects of both compounds on animal functions, such as metabolism and reproduction

(Coimbra et al., 2020; Guerreiro et al., 2017). Although its affinity to the particular phase, DCOIT rapidly degrades in sediment or water (half-life < 24h), which granted it the EPA Green Challenge Award in 1996 (U.S. EPA, 1996). However, DCOIT causes adverse effects as impairment of the homeostasis of sex hormones in fish (Chen et al., 2014) and modulation of antioxidant defense system in polychaeta (Eom et al., 2019). In addition, due to its continuous inputs to the aquatic environment, DCOIT behaves as a pseudo-persistent contaminant (Chen and Lam, 2017; Soroldoni et al., 2018). Nevertheless, DCOIT use as antifouling biocide has been authorized in many countries, such as Japan, New Zealand and European Union (ECHA, 2019; Soon et al., 2019). Table 1 summarizes the main properties of diuron, Irgarol, dichlofluanid, chlorothalonil and DCOIT. The booster biocides bioavailability and toxicity are also influenced by drivers such as turbidity, temperature, salinity, potential redox and granulometry, especially when associated to processes such as bioturbation, dredging and remobilization by waves/tides (Thomas and Brooks, 2010). In addition, drivers such as light incidence and temperature directly affect the degradability of the compounds and, consequently, the fraction available to the biota that causes adverse effects (Voulvoulis, 2006). Hydrodynamic and boats/ships traffic are factors that rule the circulation of biocides in the environments and, consequently, can also affect their concentration (Abreu et al., 2020). Moreover, sediments are the main repository of organic compounds and their characteristic such as microbial communities and organic carbon content (binding sites for organic contaminants) influences the toxicity (Ferraz et al., 2020). On contrary, a high sedimentation rate could favor the storage of biocides in this compartment, reducing their bioavailability (and toxicity) in the environment. Figure 1 depicts a conceptual model by illustrating direct interactions and drivers, processes, factors, and effects of booster biocides on sediment-dwelling organisms (representation of problem formulation).

Despite the intense navigation and maritime activities, to the extent of our knowledge, only one work performed ecological risk assessment (ERA) of booster biocides to the pelagic community in port regions from Northeast Brazil (Viana et al., 2020), while no ERA of booster biocides were reported to the sediment-dwelling community along the Brazilian coastal. Therefore, determining risk quotients (Tier-1 risk assessment) of the selected booster biocides Irgarol, diuron, DCOIT, chlorothalonil and dichlofluanid, to sediments from areas under the influence of ship/boat maintenance

and traffic of fishing and recreational vessels, as well as commercial port zones, was the main goal of this study.

2. MATERIAL AND METHODS

2.1 Hazard assessment

2.1.1. Ecotoxicity data survey

The ecotoxicity data of each biocide (Irgarol, diuron, chlorothalonil, dichlofluanid and DCOIT) on sediments were obtained through a systematic review from two types of dataset: 1) database available online from networks groups and governmental environmental agencies (NITE, PubChem, EPA, LANUV, PPDB, NORMAN, ECHA, EnviroTox, Pan, ETOX, EFSA) and 2) from qualified peer-reviewed published papers available from Google Academic, Web of Science and PubMed (Adriaanse and Rensleigh, 2011). The keywords used for searching the ecotoxicity data for each biocide were “cas number” and a combination of “chemical name + sediment + toxicity OR ecotoxicity” and “synonymous of chemical name + sediment + toxicity OR ecotoxicity”.

From this search, the ecotoxicological studies with substantial information (organism test, test duration, endpoint and toxicity value) were evaluated for reliability using the SciRAP tool (<http://www.scirap.org/>). This tool follows the Criteria for Reporting and Evaluating Ecotoxicity Data (CRED) method, proposed by Moermond et al. (2016) for safeguard the reliability and relevance of ecotoxicity studies. Documents which reached reliability $\geq 70\%$ in the sum of “fulfilled” and “partially fulfilled” fields were selected for PNEC derivation. In addition, mainly results expressed as no observed effect concentration (NOEC) or no observed effect level (NOEL) were used due to the representativity for environment protection against antifoulants (long term exposure to low levels). Data from technical reports of environmental agencies were considered reliable and robust due to the recognized quality of the information. A spreadsheet was then prepared with the acquired and relevant information on ecotoxicity of biocides in sediments (Table 2).

2.1.3 Derivation of Predicted No Effect Concentration (PNEC)

On effect assessment, a protective value must be derived to ensure protection of the environment represented by a specific compartment. A Predicted No Effect Concentration (PNEC) is regarded as a concentration below which an unacceptable

effect will, most likely, not occur (ECB, 2017). However, for most chemicals the number of toxicity data for sediment organisms are limited and requires a PNEC derivation by the deterministic approach. The deterministic approach consists of dividing the lowest toxicity threshold value (i.e., for the most sensitive organism) by an assessment factor (AF).

For Irgarol, diuron, chlorothalonil and DCOIT, PNEC were derived following the European TGD on Risk Assessment for biocides (ECB, 2017). Due the lack of toxicity data for sediment, ecotoxicological tests in whole sediments with both freshwater and marine organisms were considered, according to TGD recommendations. Pathways through which test organisms are exposed to the chemical, their feeding regimen and the use of whole-sediment tests using benthic organisms were taken into account for Assessment Factor (AF) application. In addition, an appropriate AF (depending on the number of long-term tests and feeding habit of species tested) on the lowest toxicity value was applied according to the criteria established for the sediment compartment (ECB, 2017).

For dichlofluanid, no toxicity data for sediment organisms was found. In this case, the PNEC sediment ($PNEC_{sed}$) value was derived from the Lowest PNEC water ($PNEC_w$) value available in the NORMAN website (<https://www.norman-network.net/>). The $PNEC_{sed}$ then represents the concentration of a given contaminant in sediment, equivalent to its concentration in the water column when the system is at equilibrium (Dulio and Ohe, 2013). First, the lowest $PNEC_w$ is calculated using the equilibrium partitioning approach (EqP), applied on toxicity data predicted by QSAR models or obtained experimentally, available in the NORMAN database. NORMAN network also judges multiple criteria (relevance and reliability of the key study) for derivation of robust $PNEC_w$ (Dulio and Ohe, 2013). Thereafter, $PNEC_{sed}$ for dichlofluanid was derived through equation 1 (Dulio and Ohe, 2013). This equation is the result of assumptions of calculation from provisions of the TGD-EQS Guidance (ECB, 2011).

$$PNEC_{sed} = PNEC_w * 2.6 * (0.615 + 0.019 * K_{oc}) \quad \text{Eq. (1)}$$

Where $PNEC_{sed}$ is expressed in dry weight ($\mu\text{g Kg}^{-1}$); $PNEC_w$ is the lowest PNEC ($\mu\text{g L}^{-1}$) available in the NORMAN website, and K_{oc} is the partition coefficient between organic carbon and water (L Kg^{-1}). To compare with the deterministic method

applied in the present study, the EqP method was also applied for Irgarol, diuron, chlorothalonil and DCOIT.

2.1 Exposure characterization

Recent studies of our research group have detected concentrations of booster biocides (Irgarol, diuron, chlorothalonil, dichlofluanid and DCOIT) in sediments from 113 sites along the Brazilian coastal areas (Abreu et al., 2021, 2020; in preparation.; Soroldoni et al., 2018). These Measured Environmental Concentration (MEC) were used in the present study for performing the ERA. Sampling sites were chosen as representative of nine (9) relevant navigation areas along the Brazilian coast: (1) Marajó and Guajará bay (Pará – PA); (2) São Marcos bay (Maranhão – MA), (3) Fortaleza coast (Ceará – CE), (4A) Suape port and (4B) Capibaribe bay (Pernambuco – PE), (5) Vitoria Estuarine System (Espírito Santo – ES), (6) Guanabara bay (Rio de Janeiro, RJ), (7A) Santos São Vicente Estuarine System (SSES – Port area) (São Paulo – SP), (7B) Santos São Vicente Estuarine System (SSES – Marinas area) (São Paulo – SP), (8) Itajaí-Açu river (Santa Catarina – SC) and (9) Patos Lagoon estuary (Rio Grande do Sul – RS). In the above mentioned studies, the sediment samples were extracted by ultrasound-assisted extraction with acetonitrile and clean-up by solid phase extraction (C18 cartridge) (For detailed analytical methodology, see Abreu et al., 2020). The quantification of Irgarol, diuron and DCOIT was done by liquid chromatography (LC-MS/MS), while chlorothalonil and dichlofluanid were quantified by gas chromatography (GC-ECD). Limits of detection and quantification obtained in those previous studies are presented in supplementary material (Table S1).

2.2 Risk Characterization

The phase of risk characterization estimates the incidence and severity of the adverse effects that are likely to occur in the environmental compartments due to the measured (MEC – measured exposure concentration) or predicted (PEC – predicted exposure concentration) exposure concentration to a certain contaminant (ECB, 2003). Risk Quotient (RQ) is calculated through the ratio between the MEC and the respective PNEC values (for each biocide), i.e: $RQ = MEC/PNEC$.

For calculations, MEC below the limit of detection (LD) and limit of quantification (LQ) were considered ½ of respective value. The RQs were calculated for each sampling station and classified according to ECB (2003) with adaptations. The following classification was proposed for chlorothalonil, dichlofluanid and Irgarol: “low risk”/no likely adverse effects to biota ($RQ \leq 0.1$); “moderate risk” ($0.1 < RQ < 1$) or

“high risk”/potentially cause adverse effects to biota ($RQ \geq 1$). Due to the relatively high LDs/LQs obtained for DCOIT (Table S1), the category Low-Moderate risk (L-M) was added whenever the DCOIT measured concentrations were $< LQ$. Diuron and Irgarol, in turn, presented LDs/LQs above the PNEC values (Table S1). Therefore, whenever the measured concentrations were $< LD$ or $< LQ$, the risk could not be determined, while areas where they were measured $> LQ$ were categorized as “high risk” ($RQ \geq 1$). An exception was made for RQ to the Patos Lagoon estuary. Due to the lower LD/LQ values obtained for diuron by Soroldoni et al. (2018) for the Patos Lagoon estuary, it was possible to add the category L-M category whenever diuron was detected and $< LQ$.

3. RESULTS AND DISCUSSION

3.1 PNEC derivation

The literature review (including database from organizations and technical reports from environmental agencies) shows a limited number of results available from ecotoxicity tests in marine sediments. The number of ecotoxicity values from sediments tests found was 25, 5, 4, 7 for DCOIT, diuron, Irgarol and chlorothalonil, respectively. For dichlofluanid, no results were found for toxicity tests in sediments. Shortening the list for long-term tests results for PNEC derivation according to the Technical Guidance For Deriving Environmental Quality Standards (TGD) (ECB, 2017), the final data selection ended up restricted to 2 to 7 ecotoxicity values for each biocide (Table 2).

Seven ecotoxicity studies on sediments were selected for PNEC derivation of DCOIT, including ecotoxicity results for *Chironomus riparius* (deposit feeder), *Leptocheirus plumulosus* (filter feeder/deposit feeder), *Ampelisca abdita* (tube-dwelling feeder) and *Perinereis nuntia* (deposit feeder). Due to the availability of three long-term tests with animals from different feeding habits, an AF of 10 was applied to the 14d-NOEC of the most sensitive species (*P. nuntia*), resulting in a PNEC of $0.97 \mu\text{g Kg}^{-1} \text{ dw}$ (Table 2). For diuron, PNEC was based on two long-term tests for microalgae (*Chlorella vulgaris* and *Raphidoceles subcapitata*). In this case, due to the extrapolation from tests carried out with freshwater organisms and a limited number of trophic levels (only one), species (two) and taxonomic groups (only one), and variety of feeding strategies (only one), an AF of 1000 was applied on the lowest toxicity value (*R. subcapitata*) (ECB, 2003). The final PNEC for diuron was $0.15 \mu\text{g Kg}^{-1} \text{ dw}$. For chlorothalonil, only two ecotoxicity tests for sediments were also available and both

used deposit-feeding chironomids (*Chironomus riparius* and *Chironomus dilatus*). An AF of 100 was used due to the long-term test with *C. riparius* in sediments, resulting in a PNEC of 9.5 $\mu\text{g Kg}^{-1}$. Irgarol, in turn, presented ecotoxicity tests for three species (*Ampelisca abdita*, *Chironomus riparius* and *Hyaella azteca*) with different feeding habits (tube-dwelling feeder, deposit-feeding and omnivorous, respectively). So, an AF of 75 was applied to the chronic test with *C. riparius* since the results considered other long-term tests with distinct species (two amphipods), resulting in a PNEC of 16 $\mu\text{g Kg}^{-1}$ dw. Studies on the ecotoxicity of dichlofluanid to benthic organisms were not found, so that a PNEC of 16.6 $\mu\text{g Kg}^{-1}$ was derived from the lowest PNEC_w using the NORMAN methodology.

Although sediments are the main repository for many contaminants which may affect the benthic biota and, consequently, the whole food chain, the number of toxicity data for organic compounds, including booster biocides, using sediment organisms is extremely limited. Thus, the current PNEC values derived by the deterministic method, using assessment factors, were compared with previous values obtained by the Equilibrium partitioning method (EqP) (ECB, 2003) (Table 3). The deterministic method was previously applied in an Assessment Report (AR) of the European Commission for DCOIT (ECHA, 2014a) and Irgarol (ECHA, 2014b), resulting in PNECs of 3.4 $\mu\text{g Kg}^{-1}$ dw and 0.04 $\mu\text{g Kg}^{-1}$ dw, respectively. PNEC values to proceed with risk characterization in the present study were selected from Table 3, which includes results from the present study and previous results based on both the EqP (NORMAN) and deterministic method. Although the same methodology was used, the PNEC obtained for DCOIT in the present study was considered more reliable since it was based on a larger dataset (Table 2). In this sense, considering the most sensitive value observed by Onduka et al. (2013), a lower value of PNEC was derived for DCOIT (0.97 $\mu\text{g Kg}^{-1}$ dw) and chosen. For Irgarol, on the other hand, it made more sense to use the conservative value derived by ECHA (2014b), which was based on a short term exposure of *Monoporeia affinis* (Wiklund et al., 2009). This study was disregarded in the dataset (Table 2), which was focused on long-term exposure. However, *M. affinis* acutely exposed was much more sensitive than other marine species chronically exposed, compromising the reliability of the PNEC derived.

PNECs for diuron, chlorothalonil and dichlofluanid had only been previously derived based in Equilibrium partitioning (EqP). Based on adsorption-desorption distribution coefficient (K_d) (equilibrium distribution between water and sediment), the

Norwegian Environment Agency (NEA, 2018) and Sangchan et al. (2014) obtained less protective values for diuron and chlorothalonil ($0.71 \mu\text{g Kg}^{-1}$ and $11 \mu\text{g Kg}^{-1}$, respectively) than those obtained by the present study. Moreover, the EqP approach requires other several parameters (i.e. bulk density of wet suspended matter partition and concentration in sediment pore water), increasing the cost of the method and reducing the feasibility of results. A practical adaptation of EqP was proposed by the NORMAN Network using an equation for converting PNEC_w into PNEC_{sed} (Dulio and Ohe, 2013). NORMAN methodology (<https://www.norman-network.com/nds/ecotox/>) allows the derivation of PNEC by standards assumptions on density of the sediment, density of the solid phase and volume fraction of water in sediment. In addition, a conversion factor from sediment concentration in wet weight basis to dry weight basis is used in the equation (Dulio and Ohe, 2013). This methodology was used by Barbieri et al. (2019) on sediments from the Llobregat River basin (Catalonia, Spain) for five detected pesticides, including Irgarol. The lower derived value of PNEC ($0.005 \mu\text{g Kg}^{-1}$) may be due to the old values of PNEC_w available in the NORMAN website at the time (the database is constantly updated). In the case of dichlofluanid, the PNEC of $16.6 \mu\text{g Kg}^{-1}$ derived in the present study using only the NORMAN methodology is lower (more protective) than that obtained by Carvalho et al. (2015) ($18 \mu\text{g Kg}^{-1}$) using the traditional EqP approach.

Furthermore, PNECs derived for DCOIT and diuron by the deterministic method were more protective than those values derived using the NORMAN methodology and those available in the literature (Table 3). On the other hand, Irgarol and chlorothalonil presented less protective (higher) PNEC values using the deterministic method, except when chlorothalonil is compared with EqP approach from Sangchan et al. (2014). Hence, although the deterministic method may still overestimate the risk due to a small dataset consisting of only a few chronic tests in marine sediments, this method is preferred over EqP, as deterministic PNECs are at least based on experimental data representative of the sediment compartment. In this sense, EqP method is considered just a screening approach to compensate for the lack of toxicity data, since it only considers uptake via the water phase and overlook the uptake by ingestion or direct contact with sediment (ECB, 2003). On the other hand, the TGD (ECB, 2003) strongly recommends tests with benthic organisms using spiked sediment for a robust ecological risk assessment (ECB, 2003). Even with a limited number of toxicity data for sediment organisms, the use of AF considers uncertainties, and it

allows to extrapolate from single-species laboratory data to a multi-species ecosystem. Thus, the protection of the most sensitive species should protect the structure, and hence function on ecosystem (ECB, 2017). Therefore, PNEC_{sed} derived by determinist method from sediment ecotoxicological tests are preferable and more reliable for risk characterization than PNECs based on the EqP approach and/or the NORMAN methodology. Consequently, the PNECs derived in the present study using the deterministic method (Table 3) were chosen for risk characterization of DCOIT, diuron and chlorothalonil. For Irgarol, PNEC derived by ECHA (2014b) was selected, while PNEC for dichlofluanid was based on EqP methodology due to the absence of experimental data (Table 3).

3.2 Risk Characterization

In total, 113 sediment samples along the Brazilian coast were characterized for the ecological risk of Irgarol, diuron, chlorothalonil, dichlofluanid and DCOIT (Figure 2, Table S2). Although all biocides were detected in sediments along the study area (Abreu et al., 2021, 2020; Fiamma E.L. Abreu et al., n.d.; Soroldoni et al., 2018), the risk depends on a combination of exposure to the toxicant and the sensitivity of the sediment-dwelling community to the toxicant. As a result, all biocides showed high risk in at least one sampling site. DCOIT, diuron, Irgarol, chlorothalonil and dichlofluanid presented high risk in 47%, 35%, 15%, 1% and 1% of the sampling sites, respectively.

In fact, DCOIT presented high risk in, at least, 2 sites in each of the 9 regions sampled, including 18 sites under the influence of boatyards (from PA, MA, PE, ES, SP, SC, ELP), 17 of marinas (from MA, CE, ES, SP, RJ), 12 of boat traffic only (from PA, PE, ES, SP, RJ, ELP) and 6 of Ports (from ES, SP, PE, RJ) (Figure 2). Boatyards (and some marinas with boats maintenance facilities) are considered hot spots of contamination, since antifouling paint particles (APPs) can be generated during repair, cleaning and painting procedures of vessel hulls (Singh and Turner, 2009). The analysis of APPs retrieved from sediments and boat hulls also evidenced the use of DCOIT in Brazil (Abreu et al., 2020; Soroldoni et al., 2018). The high risk also detected at traffic zones suggests the release of DCOIT by water friction on vessel hulls. DCOIT also presented high risk in sheltered areas (i.e., SP24, RG6, RG8) and open sea areas (i.e., CE5, RJ6, RJ7). In addition, 6 out of 9 Port zones are likely to be highly impacted by DCOIT ($RQ > 1$), indicating the widespread use of DCOIT in antifouling paints. Thus, this ubiquitous detection and high risk of DCOIT in the Brazilian coastal areas can be

related to its presence in several paint formulations currently used, since DCOIT has a low half-life in sediments (<24 h) (Jacobson and Willingham, 2000). Indeed, Paz-Villarraga (2019) evaluated the current use of biocides in antifouling paint formulations from Brazil, Japan, UK, Australia, Malta and USA and found DCOIT in 9.3 % of the assessed paints.

Risk quotients for DCOIT could not be determined at 19 sites because environmental concentrations were < LQ (Table S1). In those cases, the category low/moderate risk was attributed. In this regard, a deeper investigation is needed in these areas under the influence of boatyards (PA7, ES1, ES3, ES6, PE13, PE8, SC7, RG1, RG12, RG13), marina (RG5) and boat traffic (RJ8, SP12, RG3, RG4, RG7, RG9, RG10) to improve the ERA for DCOIT. Thus, a further step (Tier-2) based on the reduction of the current LQ by the optimization of analytical methods and the use of more sensitive instruments, and by obtaining more refined exposure data is recommended. Nevertheless, almost half of the analyzed sites exceed the PNEC values (RQ > 1), suggesting DCOIT as a potential threat to the sediment-dwelling biota in the Brazilian ecosystems. DCOIT is a broad-spectrum action biocide (Voulvoulis, 2006) and its ecotoxicity has been reported for diverse species across different trophic levels (i.e., bacteria, phytoplankton, fish) (Martins et al., 2018). Chronic exposure showed endocrine disrupting effects on fish (Chen et al., 2017; Chen and Lam, 2017) and DCOIT has proven to be highly toxic among alternative antifouling booster biocides, showing toxicity similar to or higher than organotins compounds (Fernández-Alba et al., 2002; Jung et al., 2017).

The difficulty in assessing risk of DCOIT has been reported by other authors (Mochida et al., 2015; Wang et al., 2014b). Despite high frequency of detection in other areas of the world, the lack of effect assessments on sediment-dwelling organism hampers the derivation of PNECs using more robust methods, such as the probabilistic derivation of species sensitivity distributions (SSD), for example. In the present study, a toxicity database accounting for distinct endpoints (1 growth, 1 adult emergency, 3 survival and 1 not informed) was used, and the derived PNEC value of 0.97 $\mu\text{g Kg}^{-1}$ dw was based on the most sensitive marine species (the Polychaeta *P. nuntia*). The database also encompassed organisms from three different feeding strategies. Although this value is considered a reliable and protective PNEC, the continuity of studies on the toxicity of DCOIT to other representative sediment-dwelling species is encouraged.

As seen for DCOIT, diuron also presented high risk in at least one site of each sampled regions (Figure 2; Table S2). Diuron showed high risk in 18 sites under the influence of boatyards from PA, MA, PE, ES, SP, SC and RG; in 14 of marinas from MA, CE, ES, RJ, and SP; in 6 of boat traffic from PA, MA, RJ, SP and RG; and 2 of Ports from CE and ES. Diuron has been used as antifouling biocide since 1980's but, since included in the EU priority substance list, is prohibited for the EU market (Directive 2013/39/EC) (EU, 2013). Its high persistence in the environment (half-life > 250 days in sediments) can influence the continuous detection (and risk) in aquatic environment (Giacomazzi and Cochet, 2004). Interestingly, in some marinas (e.g., MA1, CE1, ES5, RJ1), both DCOIT and diuron showed high risk to the ecosystem. The Material Safety Data Sheet (MSDS) and laboratory analyses of some paint formulations have already indicated the simultaneous use of diuron and DCOIT (Abreu et al., 2020). In addition, it is worth mentioning the high number of marinas from Santos São Vicente Estuarine System (13 out of 18 sites) classified under high risk by diuron. The low water circulation, a likely wide use in antifouling paints on boats, and the input from APPs stored in local sediments (Abreu et al., 2020) are factors that may have contributed to this.

At the Patos Lagoon estuary (RG1 – RG13), the risk was categorized as low to moderate at sites RG1, RG3, RG4, RG5, RG10, RG11 and RG12, high at sites RG6, RG8, RG9, and could not be determined (<LQ) at sites RG2, RG7 and RG13. Sites at low-moderate risk of diuron were situated mainly in the main navigation channel (RG1, RG3, RG4, RG5, RG10), sites with under strong hydrodynamics (Moller et al., 2001), or under the influence of small boatyards (RG11, RG12) (Soroldoni et al., 2018). A high risk of diuron was observed in boatyards with intense current boat maintenance (RG6) and in the past (RG8), and upstream the mouth of São Gonçalo river (RG9). Additionally to navigation sources, the RG9 surroundings receive agricultural runoff from the coastal plain of Rio Grande do Sul state, which may contribute to the higher risk of diuron since this compound is also applied to crops, such as fruit, cotton, sugar cane and wheat (Caldas et al., 2019). However, RQ could not be calculated (the risk was not determined) in 66 areas distributed along PA, MA, CE, PE, ES, RJ, SP and SC regions because MEC was < LQ.

The ecological risk of diuron is associated to impairment of electron transport within algal chloroplasts and inhibition of photosynthesis (Menin et al., 2008). Although diuron has direct effects on primary productivity of coastal systems, the

degradation of diuron generates intermediate products (3,4-dichloroaniline and 1-(3,4-dichlorophenyl)-3-methylurea, for example) with higher toxicity than the parent compound to crustacean and fish (Tixier et al., 2000). Therefore, an effort in optimizing the analytical methods to determine diuron and its degradation products in sediments (application of Tier-2) is urgent to refine the risk assessment of diuron to the Brazilian ecosystem.

Irgarol presented high risk in 17 sites (PA4, PA6, MA1, MA6, MA8, MA13, PE1, PE2, PE8, PE14, ES9, ES10, ES13, SC2, SC4, RG6, RG9) associated with areas under the influence of marinas, boatyards, and boat traffic only (Figure 2; Table S1). As already mentioned, marinas and boatyards areas are considered hot spots of contamination due to the intense boat maintenance activities and APPs generation. In fact, the low concentrations found for Irgarol (max 7.8 ng g⁻¹) may be associate to the leaching of APPs acting as a secondary source, since its use is restricted in modern antifouling paints all over the world. Although identify in antifouling paints registered for use in Brazil (Paz-Villarraga, 2019), Irgarol use as a biocide in antifouling paints is already prohibited in the EU (Directives 2013/39/EC) (EU, 2013), Denmark and Australia (EPA, 2011; Wezenbeek et al., 2018), which lead to a worldwide environmental reduction of its levels (Batista-Andrade et al.,2018; Sapozhnikova et al. 2013). In this sense, it is comprehensive that low risk was found in Port regions of Fortaleza coast, Guanabara bay, and SSES. The risk for Irgarol could not be determined in 85% of sites, since MECs were <LQ. Therefore, the follow-on steps of ERA (Tier 2, Tier 3) through the optimization of analytical methods (to lower LQs) and continuous acquisition of environmental levels are strongly recommended to improve the risk assessment. Still, the risk characterization allowed the identification of priority areas for mitigatory actions, since several adverse ecological effects of Irgarol are related to primary production on coastal areas from photosynthesis inhibition of phytoplankton species to coral bleaching by inhibition of zooxanthellae photosynthesis (Buma et al., 2009; Jones, 2005).

High risk of chlorothalonil and dichlofluanid was observed only in one site, SC4 and PE15, respectively. These sites are surrounded by shipyards in sheltered areas with high water residence time (Schettini, 2002; Schettini et al., 2016). SC4 denotes a particular concern, since DCOIT and chlorothalonil pose high risk, and Irgarol and dichlofluanid present moderate risk. In addition, very high concentrations of other antifouling biocides (butyltins – 1,268 ng Sn g⁻¹) have been previously reported in SC4

(Oliveira et al., 2010). Chlorothalonil can act on biochemical and physiological processes of aquatic organisms, as shown for bivalves and fish (Haque et al., 2019), and accumulates in sediments being toxic to both zooplankton and fish (Martins et al., 2018). Thus, despite the high risk in a single site does not really denote a “red signal”, the widespread use of chlorothalonil in the agriculture (among the top 12 active ingredient marketed in Brazil) (Ministério da Agricultura, 2019) indicates that extra attention is required due to its likely runoff towards the aquatic ecosystem. Similarly, a high risk of dichlofluanid was found in one site only (PE15), which is a marina with mooring of fishing boats at the inner region of Capibaribe bay. The site shows high total organic carbon content (5.1%) and is in the maximum turbidity zone (Abreu et al., 2021; Schettini et al., 2016). Dichlofluanid is considered the least toxic compound among alternative antifouling booster biocides (Cima and Ballarin, 2020). Commonly, dichlofluanid presents toxicity threshold levels much higher than concentration found in the environment (Cima and Ballarin, 2020; Jung et al., 2017). According to the Watch List under the Environmental Quality Standards Directive (Carvalho et al., 2015), dichlofluanid does not show potential to cause long-term effects, since the highest concentration measured is below the derived PNEC (most measurements were < LQ) (Carvalho et al., 2015). Overall, the risk of dichlofluanid in Brazilian coastal areas is negligible, since dichlofluanid is very unstable in the environment (Hamwijk et al., 2005), and it is unlikely to occur at toxic levels (Martins et al., 2018). Although recent studies point out that dichlofluanid impairs the immune system defense in ascidian and mussel (Cima and Ballarin, 2020; Coimbra et al., 2020), information on the toxicity and mode of action of dichlofluanid is still scarce, as evidenced by the absence of toxicity studies to benthic species in sediment.

This is the first study concerning ecological risk assessment of antifouling booster biocides to sediment-dwelling community in Brazilian coastal areas. Overall, the Brazilian coastal ecosystem showed to be subject to low to moderate risk posed by booster biocides, with hotspots (mainly in marinas and boatyards) along the coast bringing risk to the sediment-dwelling communities. Antifoulants also pose high risk to the pelagic community in shipyard areas, as recently reported by Umbría-Salinas et al. (2021). These authors derived RQ of two fractions of copper, which is used as the main antifouling agent in most antifouling formulations, to representative aquatic species from the Brazilian coast. High risk of copper to the pelagic community was found in

shipyards from Itajai-Açu River, Guanabara bay and Patos Lagoon estuary, reinforcing the problems associated to antifoulants along the Brazilian coast.

The combination of intense maritime traffic of several types of vessels, their maintenance and the use of antifouling paints with up to 6 biocides in the same formulation on coastal waters (Paz-Villarraga, 2019; Tornero and Hanke, 2016) contribute significantly to the chemical cocktails found nowadays in the environment. As expected, more than one biocide was detected in several sites (Figure 2, Table S2). Complex mixtures in coastal systems may cause multiple interactions, resulting in different toxic effects on organisms (Nikinmaa, 2014). Synergistic effects on aquatic organisms exposed to the mixture of Irgarol, diuron, chlorothalonil, dichlofluanid and DCOIT (Fernández-Alba et al., 2002; Wang et al., 2011), as well as antagonistic effects caused by the mixtures of Irgarol and Chlorothalonil (Fernández-Alba et al., 2002), have already been reported. Thus, ecological effects may still occur even when single compounds pose a low risk. The ecotoxicology of mixtures is still poorly understood and a major knowledge gap for risk assessments, including the antifoulants mixture effects on sediments, call for more studies.

The decision sequence (Tier approach) is commonly proposed for helping decision-makers to create regulations, since Tiers show how to optimize the ERA and which further actions must be taken for the environment protection (ECB, 2003). Based on the Tier-1 risk assessment performed in the present study, the results showed that chlorothalonil and dichlofluanid (both $RQ < 1$ in 99% of sites) pose low risk to the benthic communities and do not need, at the moment, further information and/or testing, and no risk reduction measures are needed. Exceptions are one site of Itajaí-Açu river and one at Capibaribe bay, where a high risk of chlorothalonil and dichlofluanid, respectively, was found. Thus, more information about variations in local concentration should be provided for these sites. On the other hand, the results found for Irgarol ($RQ > 1$ in 15% of sites), diuron ($RQ > 1$ in 35% of sites) and DCOIT ($RQ > 1$ in 47% of sites) call for further Tier risk assessments, including an optimization of analytical methods (Tier 2) and subsequent long-term monitoring programs (Tier 3) to evaluate environmental concentrations, especially in SSES (Marinas area). Meanwhile, risk assessments to sediments from other aquatic ecosystems could be performed based on PNEC values derived and discussed in the present work.

The present study adds knowledge on the risk of several booster biocides to the sediment-dwelling community and brings to light that sediments at the Brazilian coast,

particularly near hotspot areas, are threatened by DCOIT, Irgarol and diuron. The outputs are useful for Brazilian regulatory bodies as a starting point to the development and implementation of an environmental policy to reduce the footprint from shipping-associated activities, such as the establishment of environmental quality standards for booster biocides, which are severely lacking in the country.

3.3 Conclusions

The present study presented a Tier-1 ERA of five biocides used in antifouling paints for sediments along the Brazilian coastal areas. More robust $PNEC_{sed}$ were derived for chlorothalonil, diuron, and DCOIT using ecotoxicological tests on sediments. However, the proposed PNEC values for the five biocides can be used as threshold limits for sediments all over the world.

Several sites showed that booster biocides pose high risk to the sediment-dwelling community. DCOIT, diuron, Irgarol, chlorothalonil and dichlofluanid presented high risk to at least one site. In this sense, further investigation in boatyards from Itajaí-Açu river (SC), marinas from Capibaribe bay (PE) and marinas of Santos-Sao Vicente Estuarine System (SP) are recommended. Diuron, DCOIT and Irgarol pose high risk to more than one third of the assessed environments, including all types of navigation activities (boatyards, marinas, boat traffic and Port zones). Particularly high risk of DCOIT is observed in most ports analyzed in the present study, rising the concern to the use of this antifouling biocide worldwide.

Some limitations were also detected and must be addressed for improving the current ERA. An optimization of analytical methods especially for quantification of diuron, Irgarol and DCOIT, as well a long-term monitoring to account for seasonal variations in the risk assessments are recommended. The present study is an important contribution to support advance on policy formulation concerning booster biocides in Brazil, particularly considering the lack of regulation on the use of antifouling biocides (except for tributyltin, which is already banned worldwide). The high risk of biocides to sediments from several aquatic environment in Brazil shows the urgency of regulatory measures.

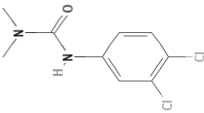
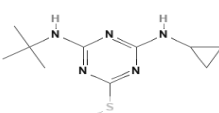
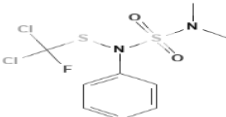
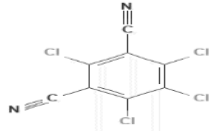
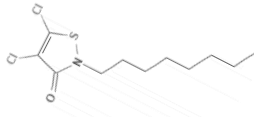
Predictive toxicity modelling has been evolving (e.g., QSAR and Bayesian network model) for water protection. However, despite being an important compartment that guarantees the recycling of nutrient and offers protection and food for several organisms, the risk assessment for sediment still lacks basic information, such as more

data on chronic ecotoxicity for different representative species. Therefore, the monitoring and regulation of chemical contaminants in this media deserves more attention to ensure adequate environmental protection.

Acknowledgments

The present research was sponsored by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – CAPES (CIMAR II N° 1988/2014) and FINEP – Financiadora de Estudos e Projetos (Proc. No 1111/13 – 01.14.0141.00). F. E. L. Abreu was sponsored by CAPES – finance code 001, while G. Fillmann (PQ 312341/2013-0 and 314202/2018-8) is a research fellow of CNPq. The authors also acknowledge the support of the Post-Graduate Program in Oceanology from CAPES.

Table 1: Physicochemical properties and mode of action of diuron, Irgarol, dichlofluanid, chlorothalonil and DCOIT

Parameter	Diuron	Irgarol	Dichlofluanid	Chlorothalonil	DCOIT
Cas number ¹	330-54-1	28159-98-0	1085-98-9	1897-45-6	64359-81-5
Chemical structure ²					
Chemical name ³	3-(3,4-Dichlorophenyl)-1,1-dimethylurea	2- <i>N</i> - <i>tert</i> -butyl-4- <i>N</i> -cyclopropyl-6-methylsulfanyl-1,3,5-triazine-2,4-diamine	N-[dichloro(fluoro)methyl]sulfanyl-N-(dimethylsulfamoyl)aniline	2,4,5,6 – Tetrachloroisophthalonitrile	4,5-dichloro-2-octyl-1,2-thiazol-3-one
Synonyms ⁴		Cybutryne			Kathon 930; Sea-Nine 211
Molecular mass (g mol ⁻¹) ⁵	233.09	253.37	333.2	265.9	282.2
Solubility in water (mg L ⁻¹) ⁶	35 – 36.4	7 - 33	0.006 - 1.3	0.6 - 0.8	4.7 - 6.5
Octanol–water partition coefficient (Log K _{ow}) ⁷	2.8	2.8 – 3.9	2.8 -3.7	2.6 – 4.3	2.8 – 6.4
Organic carbon partition coefficient (Log K _{oc}) ⁸	2.6	3.3	3.1	2.9	4.2
Degradation in sediment (half life - days) ⁹	14	100 - 200	7	> 1 - 8	> 1
Mode of action ¹⁰	It is inhibitor of PS II electron transport and affect planktonic and periphytic microalgae by reducing the chlorophyll a levels. This biocide also impacts the	It cause disturbs on electron transfer process within Photosystem-II and it is highly effective against freshwater and marine algae	It has a lower toxicity compared with other antifouling agents but causes physiological effects on adaptive response and antioxidant defense system	It is an inhibitor of mitochondrial electron transport. It can lead to genotoxicity and it is acutely toxic to fish. This	It shows acute toxicity to a wide range of aquatic organisms across different trophic levels. DCOIT presents similar or higher toxicities than other antifouling biocides (including organotin)

reproduction of green freshwater algae.

compound can accumulate in tissues and bioaccumulate.

Products of degradation¹¹

2-methylthio-4-tert-butylamino-6-amino-s-triazine (M1); N0-di-tert-butyl-6-methylthiol-s-triazine-2,4-diamine

1-(3-chlorophenyl)-3,1-dimethylurea CPDU, -(3,4-dichlorophenyl)-3-methylurea DCPMU, -(3,4-dichloro phenyl)urea CPU

N^o-Dimethyl-N-phenylsulphamide (DMSA)

4-hydroxy-2,5,6-trichlorisophtalonitrile; 1,3-dicarbamoyl-2,4,5,6-tetra chlorobenzene

N-octyl carbamic acid, N-octyl oxamic acid and

1, 2, 3, 4 and 5: Obtained from <http://pubchem.ncbi.nlm.nih.gov>

⁶: Cassi et al., 2008; Castro et al., 2011; Chen and Lam, 2017; Konstantinou and Albanis, 2004

⁷: Castro et al., 2011; Thomas and Brooks, 2010

^{8, 9}: Cassi et al., 2008; Chen and Lam, 2017; Konstantinou and Albanis, 2004; Thomas and Brooks, 2010

¹⁰: Amara et al., 2018; Chen and Lam, 2017; Fernández-Alba et al., 2002; Martins et al., 2018; Rola et al., 2017

¹¹: Chen and Lam, 2017; Sakkas et al., 2001; Thomas and Brooks, 2010

Table 2: Database for PNEC derivation of booster biocides from literature review. D: Days; h: hours; n.i: not informed; NA: not applicable

Compound	Ecological group	Species	Feeding habitat	Test duration	Parameter	Endpoint	Test media	Toxicity ($\mu\text{g Kg}^{-1}$)	Expression of results	Reliability	Reference
DCOIT	Freshwater midge	<i>Chironomus riparius</i>	Detritivore (select)	28 D	NOEL	n.i	Sediment	4,900	n.i.	NA	EPA (MRID 46816304)
DCOIT	Freshwater midge	<i>Chironomus riparius</i>	Detritivore (select)	10 D	NOEC	adult emergency	Sediment	9,700	n.i.	NA	EPA (MRID 46816304)
DCOIT	Freshwater midge	<i>Chironomus riparius</i>	Detritivore (select)	10 D	NOEC	survival	Sediment	3,100	n.i.	NA	(ECHA, 2014a)
DCOIT	Marine amphipod	<i>Leptocheirus plumulosus</i>	Suspension or Deposit feeding	28 D	NOEC	survival	Sediment	100	n.i.	NA	(ECHA, 2014)
DCOIT	Marine amphipod	<i>Ampelisca abdita</i>	Tube-dwelling feeder	10 D	NOEL	survival	Sediment	6,900	n.i.	NA	EPA (MRID 44755903)
DCOIT	Marine amphipod	<i>Leptocheirus plumulosus</i>	Suspension or deposit feeding	28 D	NOEC	n.i	Sediment	10,000	n.i.	NA	EPA (MRID 46780740)
DCOIT	Marine Polychaeta	<i>Perinereis nuntia</i>	Detritivore	14 D	NOEC	growth	Sediment	9.7	Per dry weight	90%	(Onduka et al., 2013)
Diuron	Marine microalgae	<i>Chlorella vulgaris</i>	Filter	72h	EC ₅₀	algae cell yields	Sediment	2,370	Per dry weight	95%	(Pei et al., 2020)
Diuron	Freshwater microalgae	<i>Raohidocetes subcapitata</i>	Filter	72h	NOEC	growth inhibition	Sediment river	150	Per dry weight	95%	(Zhang et al., 2012)
Chlorothalonil	Freshwater midge	<i>Chironomus riparius</i>	Detritivore (select)	28 D	NOEC	n.i	Sediment	950	n.i.	NA	(FAO, 2015)
Chlorothalonil	Freshwater midge	<i>Chironomus dilutus</i>	Detritivore (select)	10 D	NOEL	n.i.	Sediment	8,500	n.i.	NA	EPA (MRID 49524602)
Irgarol	Marine amphipod	<i>Ampelisca abdita</i>	Tube-dwelling feeder	10 D	NOEC	mortality	Sediment	44,000	Per dry weight	NA	(ECHA, 2014b)
Irgarol	Freshwater midge	<i>Chironomus riparius</i>	Detritivore (select)	28 D	NOEC	development	Sediment	1,200	Per dry weight	NA	(ECHA, 2014b)
Irgarol	Freshwater amphipod	<i>Hyalella azteca</i>	Omnivorous	10 D	NOEL	n.i.	Sediment	120,000	n.i.	NA	EPA (MRID 44998401)
Dichlofluanid						No sediment test available					

Table 3: PNEC results for booster biocides on sediment based in different methods

	PNECs							
	Deterministic method (present study)			NORMAN		Literature		
	Lowest toxicity value ($\mu\text{g Kg}^{-1}$)	AF	PNEC ($\mu\text{g kg}^{-1}$)	Lowest toxicity value ($\mu\text{g L}^{-1}$)	PNEC ($\mu\text{g kg}^{-1}$)*	PNEC ($\mu\text{g kg}^{-1}$)	Method	Reference
DCOIT	9.7	10	0.97	0.056	43.9	3.0	EqP	Wang et al., 2014
						3.4	Deterministic (AF 10)	ECHA, 2014a
Diuron	150	1000	0.15	0.2	4.3	0.71	EqP	NEA, 2018
Chlorothalonil	950	100	9.5	0.06	2.4	11	EqP	Sangchan et al., 2014 ¹
Irgarol	1,200	75	16	0.0035	0.4	0.005	NORMAN	Barbieri et al., 2019
						0.04	Deterministic (AF 1000)	ECHA, 2014b
Dichlofluanid	-	-	-	0.26	16.6	18	EqP	Carvalho et al., 2015

*Conversion by equation: $\text{PNEC}_{\text{sed}} = \text{Lowest PNEC}_{\text{fw}} * 2.6 * (0.615 + 0.019 * K_{\text{oc}})$

¹Database information from river sediment

AF: Assessment factor

EqP: Equilibrium partitioning method

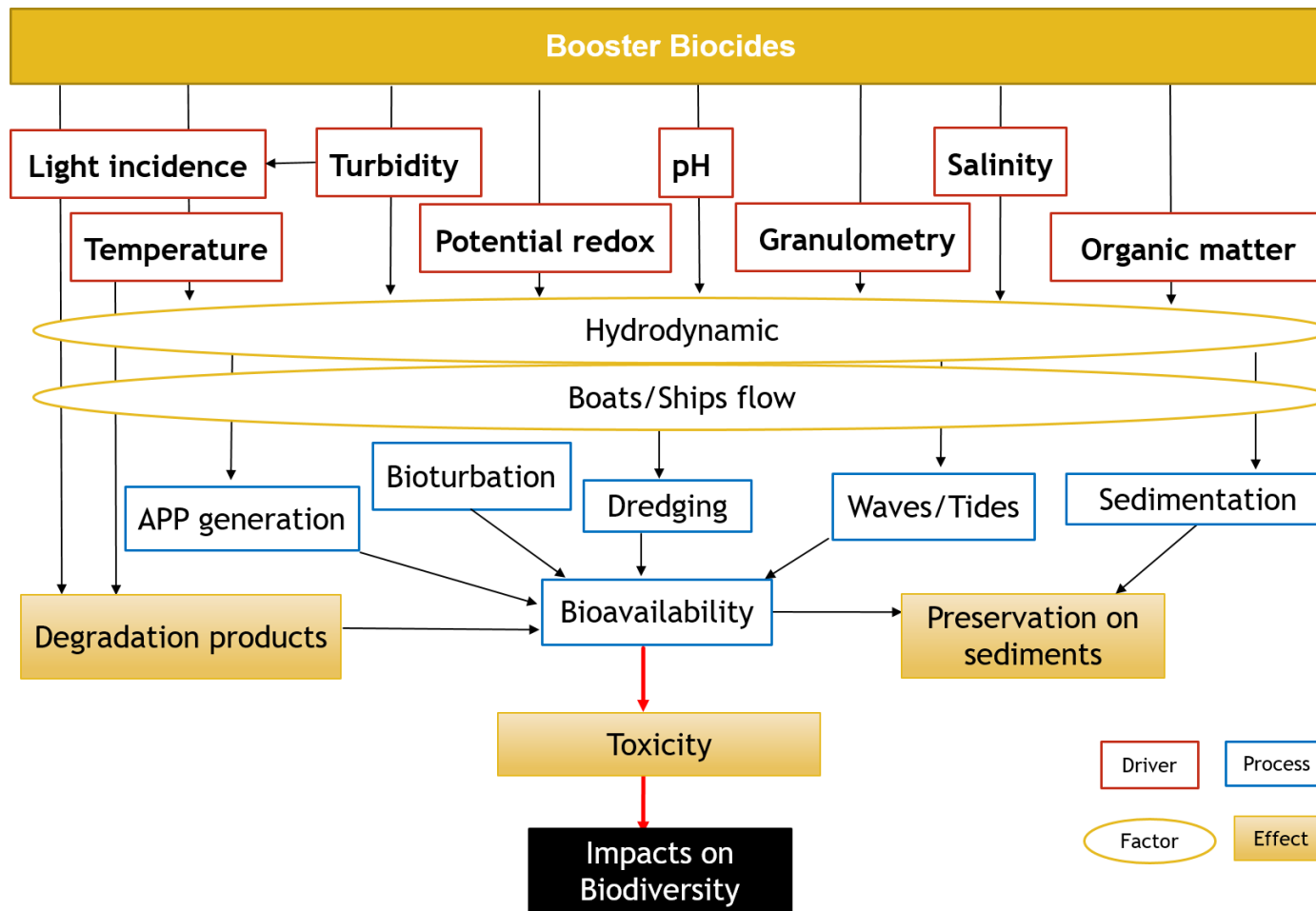


Figure 1: Conceptual model of the direct interactions and drivers, processes, factors, and effects of booster biocides on sediments of aquatic systems (representation of problem formulation).

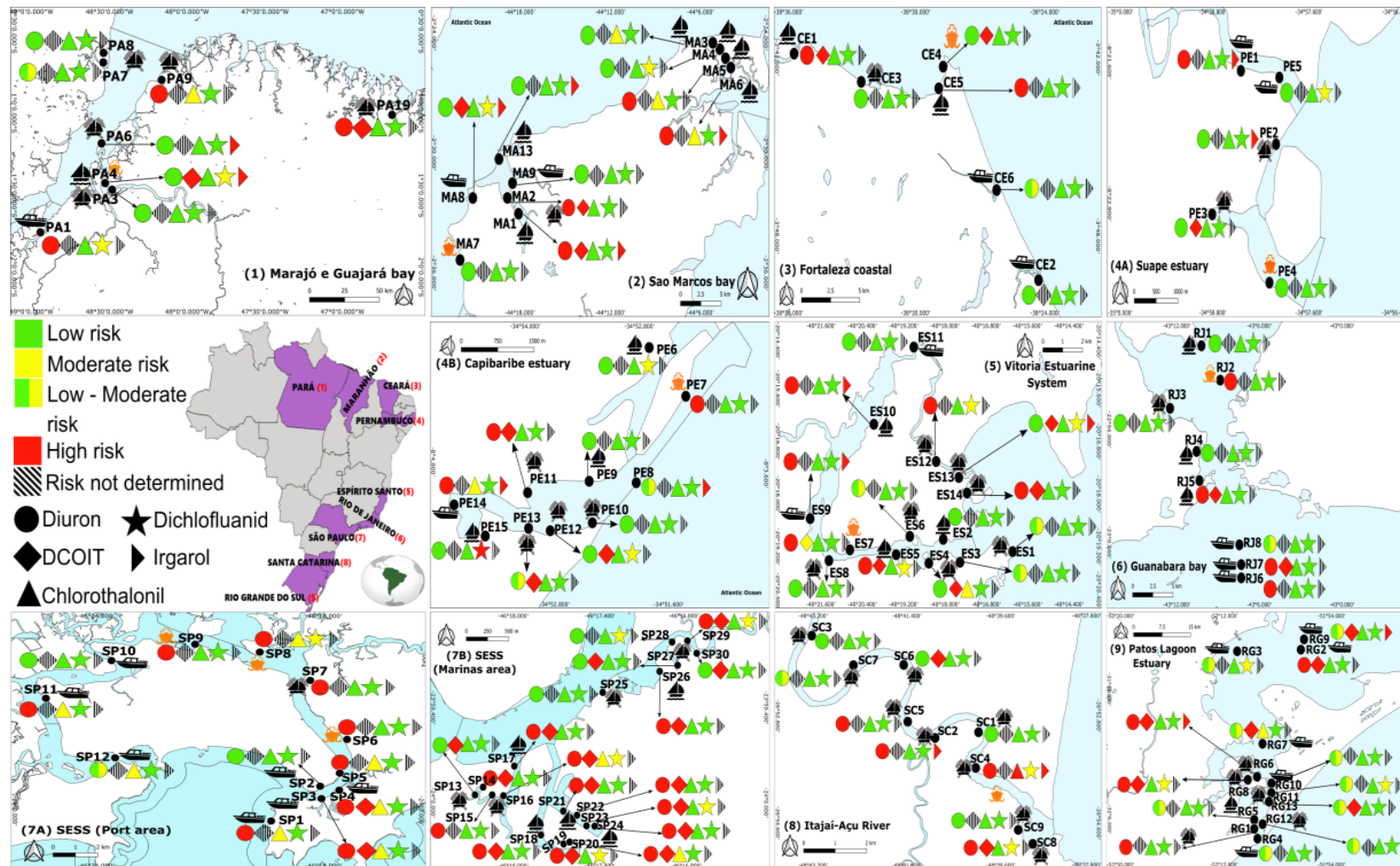


Figure 2: Risk characterization of DCOIT, diuron, chlorothalonil, dichlofluanid and Irgarol for sediments along the Brazilian coastal areas. More details about antifouling biocides contamination on Brazil coastal areas, see (Abreu et al., 2021, 2020; Abreu et al., in preparation; Soroldoni et al., 2018).

Capítulo VII: Síntese da Discussão e Conclusões

A presente Tese de Doutorado teve como objetivo geral avaliar a efetiva relevância ambiental dos principais biocidas e co-biocidas anti-incrustantes nas zonas costeiras do Brasil sob influência de atividades marítimas. As motivações para realização desse trabalho foram criadas a partir de uma base de dados construída ao longo de 20 anos pela Rede Nacional de Estudos em Anti-incrustantes (RNEA), onde observou-se a necessidade de atualização do status da contaminação de biocidas anti-incrustantes de 2ª geração e a ausência dos níveis de contaminação de biocidas anti-incrustantes de 3ª geração em sedimentos da costa do Brasil. No entanto, a fim de proporcionar produtos científicos mais aplicáveis para definição de medidas regulamentadoras, a Avaliação de Risco Ecológico para tais biocidas e co-biocidas também foi almejada na realização desse trabalho.

Devido ao volume de informações geradas, melhor discussão das mesmas e de acordo com a malha amostral, os resultados foram divididos em três artigos científicos quanto à ocorrência e distribuição dos butilestânicos (BTs), biocidas de reforço e partículas de tintas anti-incrustantes (PTAs). O primeiro artigo focado no Sistema Estuarino de Santos e São Vicente (São Paulo) foi realizado utilizando uma malha de 30 pontos na região estuariana do principal porto da América do Sul (Porto de Santos), bem como na sua área adjacente onde há grande influência de marinas, estaleiros e áreas com pequenos barcos de pesca. As análises mostraram que apesar do banimento mundial de TBT em tintas anti-incrustantes, ainda há a presença de relevantes níveis de butilestânicos ($> 300 \text{ ng Sn g}^{-1}$) em áreas sob influência de tráfego e estaleiros de barcos de pesca, enquanto os biocidas de reforço apresentaram as concentrações mais altas (até 74.6 ng g^{-1}) em regiões de marinas com barcos de recreação (iates e lanchas, por exemplo). Assim, apesar da presença do Porto de Santos no canal principal de navegação, o aporte de contaminação foi relacionado às demais atividades marítimas envolvendo barcos de pesca de pequeno e médio porte, estaleiros e marinas, bem como às condições oceanográficas das áreas adjacentes. Além disso, quantidades expressivas de PTAs ($> 200 \text{ } \mu\text{g g}^{-1}$) em sedimentos da região representam uma importante fonte secundária de contaminação por biocidas de reforço, chegando a níveis equivalentes a $200 \text{ } \mu\text{g}$ de DCOIT por m^2 de sedimento, por exemplo.

O estudo dos níveis ambientais e a possível influência do perfil das atividades marítimas como fonte de contaminação para os ambientes costeiros do Brasil continuou com o segundo trabalho realizado no Sistema Estuarino de Vitória (VES). Este sistema abrange um dos principais portos comerciais brasileiros, bem como estaleiros, marinas e portos pesqueiros. Apesar dos resultados terem demonstrado uma redução significativa nos níveis de butilestânicos quando comparados a estudos prévios na região, estes ainda são passíveis de causar efeitos adversos à biota local e, até mesmo, aos seres humanos. Além disso, embora altas concentrações dos biocidas de reforço (especialmente diuron e DCOIT) tenham sido detectados nas PTAs, as concentrações não foram refletidas em similar escala nos sedimentos. Ao contrário do observado no SESS, não foi possível estabelecer uma relação entre possíveis fontes e os perfis das atividades marítimas, uma vez que há diversas atividades atuando simultaneamente. No entanto, butilestânicos e DCOIT apresentaram as maiores concentrações no local próximo ao porto comercial (relacionado possivelmente às atividades de dragagens) e em sedimentos de áreas sob influência de portos de pesca/estaleiros usados para reparos de barcos de pesca e marinas onde ocorrem a manutenção de barcos de lazer.

O terceiro artigo agrupou os demais resultados provenientes da Baía de Marajó e Guajará (Pará), Baía de São Marcos (Maranhão), região costeira de Fortaleza (Ceará), Estuário de Suape e Capibaribe (Pernambuco), Baía de Guanabara (Rio de Janeiro), Estuário do rio Itajaí-Açu (Santa Catarina) e Estuário da Lagoa dos Patos (Rio Grande do Sul). Similar ao que foi encontrado no VES, os níveis de aportes de contaminação para butilestânicos foram menores do que encontrados em trabalhos anteriores e os biocidas de reforço não mostraram altos níveis de contaminação. Quando agrupamos os níveis ambientais em relação ao perfil predominante de atividades marítimas (porto, marinas, estaleiros e zonas de tráfego), diferenças significativas são encontradas nas concentrações de BTs dos sítios sob influência de estaleiros, indicando que estaleiros são prováveis fontes atuais de butilestânicos. No entanto, nenhuma diferença significativa foi observada para os biocidas de reforço. Isso possivelmente se justifica pelas baixas concentrações encontradas e às distintas fontes que estabelecem um padrão de aporte mais difuso. Considerando ainda os biocidas de reforço, o trabalho mostrou a predominância do DCOIT, sendo detectado tanto nos sedimentos quanto nas PTAs de todas as áreas costeiras estudadas. Esse trabalho confirma também o importante papel das PTAs como fonte secundária de biocidas anti-incrustantes devido à altas

concentrações na partícula e nos sedimentos circundantes (dependendo das condições hidrodinâmicas do local).

De modo geral, os artigos demonstram as principais tendências quanto à contaminação por biocidas anti-incrustantes ao longo da costa do Brasil. Exceto no Sistema Estuarino de Santos São Vicente e estaleiros do Estuário da Lagoa dos Patos, houve um predomínio de aportes antigos de TBT, demonstrando uma crescente eficácia das legislações nacionais e internacionais que baniram o seu uso nas tintas anti-incrustantes. Quanto aos biocidas de reforço, Diuron e DCOIT foram muitas vezes detectados simultaneamente, sugerindo o uso desses contaminantes em uma mesma formulação de tintas anti-incrustantes. Menos frequentemente detectados, os níveis de Irgarol, diclofluanida e clorotalonil não demonstraram uma clara relação com as atividades marítimas.

Em uma primeira avaliação do impacto por butiesltânicos em diversas regiões costeiras da América Latina, Castro (2011) sugeriu que as áreas até então impactadas por organoestânicos poderiam ser também atingidas pelos biocidas de reforço. Assim, o presente trabalho detectou níveis ambientais dos biocidas de reforço em algumas das áreas previamente estudadas. Nesse sentido, uma Avaliação de Risco Ecológico (quarto artigo) foi realizada para caracterizar o risco associado à ocorrência de Irgarol, diuron, clorotalonil, diclofluanida e DCOIT nos sedimentos. Baseando-se no reconhecido Documento de Orientação Técnica da Comissão Europeia (ECB, 2003), os valores protetivos para os organismos foram, pela primeira vez, derivados a partir de resultados provenientes de testes ecotoxicológicos em sedimento. Além do avanço metodológico, possibilitou a geração de valores de PNEC mais confiáveis para sedimento. Assim, com exceção da diclofluanida que seguiu a metodologia da rede NORMAN (Dulio and Ohe, 2013) devido à ausência de dados ecotoxicológicos, os PNECs foram derivados para os demais biocidas. A partir da relação entre os níveis ambientais e os respectivos PNECs foi possível caracterizar o risco dos biocidas Irgarol, diuron, clorotalonil, diclofluanida e DCOIT para cada local de estudo. Irgarol foi classificado como risco moderado para sedimentos em apenas seis locais e apresentou baixo risco nos demais pontos. Clorotalonil e diclofluanida apresentaram alto risco em pelo menos uma amostra analisada em cada estado, enquanto diuron e DCOIT representam alto risco para mais de um terço dos ambientes avaliados, especialmente nas áreas sob influência de portos e, no caso do SESS, as áreas adjacentes sob influência de marinas e estaleiros. O trabalho também indicou que áreas do estuário do rio Itajaí-Açu e estuário do

Capibaribe devem ser melhor investigadas considerando que apresentaram os únicos pontos com alto risco para clorotalonil e diclofluanida, respectivamente. Assim, apesar de limitações metodológicas pela falta de dados ecotoxicológicos, os resultados podem ser aplicados como valores protetivos, em relação aos biocidas de reforço, para organismos bentônicos por todo mundo além de dá o suporte para inclusão de limites seguros no Brasil em legislações vigentes como a CONAMA 454/2012 referente a sedimentos dragados.

Desse modo, apesar da ocorrência de aportes recentes de TBT ter sido detectada em áreas pontuais que apresentaram elevadas concentrações nos sedimentos (Artigo 1 e 3), os resultados confirmam parcialmente a hipótese da Tese quanto a tendência preferencial de redução dos níveis ambientais de TBT encontrados ao longo da costa do Brasil. Quanto aos biocidas de reforço, Irgarol, clorotalonil e diclofluanida, a hipótese foi confirmada por não apresentarem risco significativo (alto risco) na maioria dos locais estudados. No entanto, para DCOIT e diuron, a hipótese foi rejeitada por apresentarem alto risco de causar efeitos adversos em diversos ambientes costeiros brasileiros sob a influência de atividades marítimas (Artigo 4). Quanto às partículas de tintas anti-incrustante, não foi possível confirmar a hipótese devido à falta de evidências entre sua ocorrência no ambiente aquático e identificação de alto risco para a biota (Artigos 3 e 4).

Os dados apresentados na presente Tese representam uma parcela de uma ampla base de dados gerada pela Rede Nacional de Estudos em Anti-incrustantes (RNEA) para a costa do Brasil. Porém, as atividades da RNEA se estendem também a regiões costeiras de outros países da América Latina. Através da consolidação de toda essa informação, que vem sendo construída ao longo dos últimos 20 anos, é possível obter uma visão geral quanto ao status atual da contaminação por biocidas anti-incrustantes na América Latina. Assim, a partir do Doutorado Sanduíche realizado na Universidade de Aveiro (Portugal) sob a orientação do Prof. Dr. Carlos M. Miguez Barroso, está sendo realizado um diagnóstico ambiental ranqueando o impacto do TBT nas regiões costeiras ao longo de toda América Latina a partir dos índices do biomarcador *Imposex*. Seguindo critérios rigorosos da legislação europeia e utilizando a base de dados da RNEA, este será mais um trabalho para subsidiar à atualização de medidas regulamentatórias em países da América Latina.

Quanto aos biocidas de reforço, o presente trabalho apresenta um diagnóstico inicial sobre a sua distribuição espacial. Visando um melhor entendimento do comportamento desses contaminantes, é preciso realizar um monitoramento capaz de investigar eventuais variações temporais dos aportes, bem como ampliar a sua cobertura espacial a fim de identificar outras possíveis fontes de contaminação. Este estudo é premente principalmente para o DCOIT e diuron, que se mostraram amplamente distribuídos e podem causar efeitos adversos em diversas áreas costeiras do Brasil.

Além do contínuo monitoramento ambiental dos biocidas de reforço, a Avaliação de Risco Ambiental (ARE) que se baseia em valores de PNEC para sedimento deve ser refinada. Desta forma, considerando o baixo número de testes ecotoxicológicos utilizados no artigo 4 para derivação do PNEC, a realização de testes crônicos com organismos bentônicos expostos ao diuron, Irgarol, clorotalonil, diclofluanida e DCOIT em sedimento integral vem sendo demandada aos pesquisadores da RNEA.

- Abreu, F.E.L., Batista, R.M., Amado, L.L., Ribeiro-Brasil, D.R.G., Santos, T.C.R., Viana, J.L.M., Zanardi-Lamardo, E., Yogui, G.T., Fernandez, M.A. dos S., Castro, I.B. de, Fillmann, G., in preparation. Antifouling biocides in sediments along the Brazilian coast.
- Abreu, F.E.L., Batista, R.M., Castro, I.B. de, Gilberto, F., 2021. Legacy and emerging antifouling biocide residues in a tropical estuarine system (Vitória state, SE, Brazil). *Mar. Pollut. Bull.* 112255. <https://doi.org/https://doi.org/10.1016/j.marpolbul.2021.112255>
- Abreu, F.E.L., Juliane Natália Lima da Silva, Castro, I.B. de, Fillmann, G., 2020. Are antifouling residues a matter of concern in the largest South American port? *J. Hazard. Mater.* <https://doi.org/https://doi.org/10.1016/j.jhazmat.2020.122937>
- Adriaanse, L.S., Rensleigh, C., 2011. Comparing Web of Science, Scopus and Google Scholar from an Environmental Sciences perspective. *South African J. Libr. Inf. Sci.* 77. <https://doi.org/10.7553/77-2-58>
- Albanis, T.A., Lambropoulou, D.A., Sakkas, V.A., Konstantinou, I.K., 2002. Antifouling paint booster biocide contamination in greek marine sediments. *Chemosphere* 48, 475–485. [https://doi.org/10.1016/S0045-6535\(02\)00134-0](https://doi.org/10.1016/S0045-6535(02)00134-0)
- Almeida, E., Diamantino, T.C., de Sousa, O., 2007. Marine paints: The particular case of antifouling paints. *Prog. Org. Coatings* 59, 2–20. <https://doi.org/10.1016/j.porgcoat.2007.01.017>
- Amara, I., Miled, W., Ben, R., Ladhari, N., 2018a. Antifouling processes and toxicity effects of antifouling paints on marine environment . A review. *Environ. Toxicol. Pharmacol.* 57, 115–130. <https://doi.org/10.1016/j.etap.2017.12.001>
- Amara, I., Miled, W., Ben, R., Ladhari, N., Slama, R. Ben, Ladhari, N., Ben, R., Ladhari, N., 2018b. Antifouling processes and toxicity effects of antifouling paints on marine environment. A review. *Environ. Toxicol. Pharmacol.* 57, 115–130. <https://doi.org/10.1016/j.etap.2017.12.001>
- Amaral, A.C.Z., Corte, G.N., Denadai, M.R., Borzone, C., Omena, P., Zalmon, I.R., Rocha-barreira, C.D.A., Roberto, J., Souza, B. De, Cesar, T., Almeida, M. De, 2016. Brazilian sandy beaches: characteristics, ecosystem services, impacts, knowledge and priorities. *Brazilian J. Oceanogr.* 64, 5–16. <https://doi.org/http://dx.doi.org/10.1590/S1679-875920160933064sp2>

- Antizar-Ladislao, B., 2008. Environmental levels, toxicity and human exposure to tributyltin (TBT)-contaminated marine environment. A review. *Environ. Int.* 34, 292–308. <https://doi.org/10.1016/j.envint.2007.09.005>
- Araújo, M.P., Hamacher, C., de Oliveira Farias, C., Martinho, P., de Oliveira Chaves, F., Gomes Soares, M.L., 2020. Assessment of brazilian mangroves hydrocarbon contamination from a latitudinal perspective. *Mar. Pollut. Bull.* 150, 110673. <https://doi.org/10.1016/j.marpolbul.2019.110673>
- Artifon, V., Zanardi-Lamardo, E., Fillmann, G., 2019. Aquatic organic matter: Classification and interaction with organic microcontaminants. *Sci. Total Environ.* 649, 1620–1635. <https://doi.org/10.1016/j.scitotenv.2018.08.385>
- Baeyens, W., Mirlean, N., Bundschuh, J., Winter, N. De, Baisch, P., Manoel, F., Júnior, S., Gao, Y., 2019. Arsenic enrichment in sediments and beaches of Brazilian coastal waters : A review. *Sci. Total Environ.* 681, 143–154. <https://doi.org/10.1016/j.scitotenv.2019.05.126>
- Bakke, T., Källqvist, T., Ruus, A., 2010. Development of sediment quality criteria in Norway 172–178. <https://doi.org/10.1007/s11368-009-0173-y>
- Baptista Neto, J.A., Gaylarde, C., Beech, I., Bastos, A.C., da Silva Quaresma, V., de Carvalho, D.G., 2019. Microplastics and attached microorganisms in sediments of the Vitória bay estuarine system in SE Brazil. *Ocean Coast. Manag.* 169, 247–253. <https://doi.org/10.1016/j.ocecoaman.2018.12.030>
- Barbieri, M.V., Postigo, C., Monllor-Alcaraz, L.S., Barceló, D., López de Alda, M., 2019. A reliable LC-MS/MS-based method for trace level determination of 50 medium to highly polar pesticide residues in sediments and ecological risk assessment. *Anal. Bioanal. Chem.* 411, 7981–7996. <https://doi.org/10.1007/s00216-019-02188-0>
- Bastos, A.C., Vilela, C.G., Quaresma, V.S., Almeida, F.K., 2010. Mid- to late-Holocene estuarine infilling processes studied by radiocarbon dates, high resolution seismic and biofacies at Vitoria Bay, Espirito Santo, Southeastern Brazil. *An. Acad. Bras. Cienc.* 82, 761–770. <https://doi.org/10.1590/s0001-37652010000300022>
- Batista-Andrade, J.A., Caldas, S.S., Batista, R.M., Castro, I.B., Fillmann, G., Primel, E.G., 2018. From TBT to booster biocides: Levels and impacts of antifouling along coastal areas of Panama. *Environ. Pollut.* 234, 243–252. <https://doi.org/10.1016/j.envpol.2017.11.063>
- Batista, R.M., Soares, K.L., Fillmann, G., n.d. Analytical method for determination of organotin compounds in solid environmental matrices. *TrAC - Trends Anal. Chem.*
- Brasil, 2012. Resolução nº 454, de 1 de novembro de 2012. Estabelece as

- diretrizes gerais e os procedimentos referenciais para o gerenciamento do material a ser dragado em águas sob jurisdição nacional. URL <http://www2.mma.gov.br/port/conama/legiabre.cfm?codlegi=620> (accessed 1.January.20).
- Brasil, 2011. PORTARIA Nº 2.914, DE 12 DE DEZEMBRO DE 2011. Dispõe sobre os procedimentos de controle e de vigilância da qualidade da água para consumo humano e seu padrão de potabilidade.
- Buma, A.G.J., Sjollema, S.B., van de Poll, W.H., Klamer, H.J.C., Bakker, J.F., 2009. Impact of the antifouling agent Irgarol 1051 on marine phytoplankton species. *J. Sea Res.* 61, 133–139. <https://doi.org/10.1016/j.seares.2008.11.007>
- Buskens, P., Wouters, M., Rentrop, C., Vroon, Z., 2013. A brief review of environmentally benign antifouling and foul-release coatings for marine applications. *J. Coatings Technol. Res.* 10, 29–36. <https://doi.org/10.1007/s11998-012-9456-0>
- Caldas, S.S., Arias, J.L.O., Rombaldi, C., Mello, L.L., Cerqueira, M.B.R., Martins, A.F., Primel, E.G., 2019. Occurrence of Pesticides and PPCPs in Surface and Drinking Water in Southern Brazil: Data on 4-Year Monitoring 30, 71–80. <https://doi.org/http://dx.doi.org/10.21577/0103-5053.20180154> J.
- Carvalho, R.N., Ceriani, L., Ippolito, A., 2015. Development of the first Watch List under the Environmental Quality Standards Directive water policy. <https://doi.org/10.2788/101376>
- Cassi, R., Tolosa, I., Mora, S. d., 2008. A survey of antifoulants in sediments from Ports and Marinas along the French Mediterranean coast. *Mar. Pollut. Bull.* 56, 1943–1948. <https://doi.org/10.1016/j.marpolbul.2008.07.011>
- Castro, I.B., Arroyo, M.F., Costa, P.G., Fillmann, G., 2012. Butyltin Compounds and ImPOSEX Levels in Ecuador. *Arch. Environ. Contam. Toxicol.* 62, 68–77. <https://doi.org/10.1007/s00244-011-9670-2>
- Castro, Í.B., Westphal, E., Fillmann, G., 2011. Tintas anti-incrustantes de terceira geração: Novos biocidas no ambiente aquático. *Quim. Nov.* X, 1–11.
- Chen, C., Chen, L., Xue, R., Huang, Q., Wu, L., Ye, S., Zhang, W., 2019. Spatiotemporal variation and source apportionment of organotin compounds in sediments in the Yangtze Estuary. *Environ. Sci. Eur.* 31, 24. <https://doi.org/10.1186/s12302-019-0207-z>
- Chen, L., Au, D.W.T., Hu, C., Peterson, D.R., Zhou, B., Qian, P.Y., 2017. Identification of Molecular Targets for 4,5-Dichloro-2-n-octyl-4-isothiazolin-3-one (DCOIT) in Teleosts: New Insight into Mechanism of Toxicity. *Environ. Sci. Technol.* 51, 1840–1847. <https://doi.org/10.1021/acs.est.6b05523>
- Chen, L., Lam, J.C.W., 2017. SeaNine 211 as antifouling biocide: A

- coastal pollutant of emerging concern. *J. Environ. Sci.* 61, 68–79. <https://doi.org/10.1016/j.jes.2017.03.040>
- Chen, L., Xu, Y., Wang, W., Qian, P.Y., 2015. Degradation kinetics of a potent antifouling agent, butenolide, under various environmental conditions. *Chemosphere* 119, 1075–1083. <https://doi.org/10.1016/j.chemosphere.2014.09.056>
- Chen, L., Ye, R., Xu, Y., Gao, Z., Au, D.W.T., Qian, P.Y., 2014. Comparative safety of the antifouling compound butenolide and 4,5-dichloro-2-n-octyl-4-isothiazolin-3-one (DCOIT) to the marine medaka (*Oryzias melastigma*). *Aquat. Toxicol.* 149, 116–125. <https://doi.org/10.1016/j.aquatox.2014.01.023>
- Choi, M., Moon, H.B., Yu, J., Eom, J.Y., Choi, H.G., 2010. Temporal trend of butyltins in seawater, sediments, and mussels from Busan Harbor of Korea between 2002 and 2007: Tracking the effectiveness of tributyltin regulation. *Arch. Environ. Contam. Toxicol.* 58, 394–402. <https://doi.org/10.1007/s00244-009-9428-2>
- Cima, F., Ballarin, L., 2020. Immunotoxicity in ascidians: Antifouling compounds alternative to organotins - V. the case of dichlofluanid. *Comp. Biochem. Physiol. Part - C Toxicol. Pharmacol.* 169, 16–24. <https://doi.org/10.1016/j.cbpc.2014.12.007>
- Clarke, K.R., Gorley, R.N., Somerfield, P.J., Warwick, R.M., 2014. *Change in marine communities*, 3rd ed. Primer-e, Plymouth.
- CODESA, 2019. *Relatorio estatístico anula da movimentação de cargas 2018*.
- Coimbra, R., Silveira, A., Gabe, H., Alberto, M., Eduardo, C., Zomer, J., 2020. Antifouling biocide dichlofluanid modulates the antioxidant defense system of the brown mussel *Perna perna*. *Mar. Pollut. Bull.* 157, 111321. <https://doi.org/10.1016/j.marpolbul.2020.111321>
- Costa, E.S., Grilo, C.F., Wolff, G.A., Thompson, A., Figueira, R.C.L., Neto, R.R., 2015. Evaluation of metals and hydrocarbons in sediments from a tropical tidal flat estuary of Southern Brazil. *Mar. Pollut. Bull.* 92, 259–268. <https://doi.org/10.1016/j.marpolbul.2014.11.028>
- Costa, M.B., Zamprogno, G.C., Pedruzzi, F.C., de Moraes, L., Tognella, M.M.P., Godoi, A.F.L., Santos, D.M., de Marchi, M.R.R., Fernandez, M.A., 2014. Differential organotin sensitivity in two *Leucozonia* species from a ship traffic area in southeastern Brazil. *Mar. Biol. Res.* 10, 712–724. <https://doi.org/10.1080/17451000.2013.850512>
- Costa, M.B. da, Zamprogno, G.C., Otegui, M.B.P., de Moraes, L., Pedruzzi, F.C., Dalbem, G.B., da Silva, D.A., Resende, A.C.B., 2017. A temporal and spatial monitoring of organotin pollution in a harborside region of Brazil by imposex and ecological quality ratio using *Leucozonia nassa*. *Environ. Monit. Assess.* 189. <https://doi.org/10.1007/s10661-017-6367-7>

- Díez, S., Abalos, M., Bayona, J.M., 2002a. Organotin contamination in sediments from the Western Mediterranean enclosures following 10 years of TBT regulation. *Water Res.* 36, 905–918.
- Díez, S., Ábalos, M., Bayona, J.M., 2002b. Organotin contamination in sediments from the Western Mediterranean enclosures following 10 years of TBT regulation. *Water Res.* 36, 905–918. [https://doi.org/10.1016/S0043-1354\(01\)00305-0](https://doi.org/10.1016/S0043-1354(01)00305-0)
- Dulio, V., Ohe, P.C. Von Der, 2013. Working Group on Prioritisation of Emerging Substances NORMAN Prioritisation framework for emerging substances. Verneuil en Halatte.
- ECB, 2017. Guidance on the Biocidal Products Regulation: Volume II Part A. <https://doi.org/10.2823/31176>
- ECB, 2011. Guidance Document No . 27 Technical Guidance For Deriving Environmental Quality Standards. <https://doi.org/10.2779/43816>
- ECB, 2003. Technical Guidance Document on Risk Assessment, part II. European Chemical Bureau.
- ECHA, 2019. Information on biocides: DCOIT. URL <https://echa.europa.eu/pt/information-on-chemicals/biocidal-active-substances/-/disas/substance/100.058.930> (accessed 7.July.20).
- ECHA, 2014a. DCOIT (Sea - Nine 211) Regulation concerning the making available on the market and use of biocidal products Product type 21 (Antifouling products).
- ECHA, 2014b. Evaluation of active substance - Assessment Report - Cybutryne 21, 47.
- Eom, H., Haque, N., Nam, S., Lee, D., Rhee, J., 2019. Effects of sublethal concentrations of the antifouling biocide Sea-Nine on biochemical parameters of the marine polychaete *Perinereis aibuhitensis*. *Comp. Biochem. Physiol.* 222, 125–134. <https://doi.org/https://doi.org/10.1016/j.cbpc.2019.05.001>
- EPA, 2011. Reassessment of antifouling paints. New Zealand Environmental Protection Agency 1–19.
- EPA, 1992. Framework for ecological risk assessment. *Am. J. Obstet. Gynecol.* 23, 911. [https://doi.org/10.1897/1552-8618\(1992\)11\[1663:AFFERA\]2.0.CO;2](https://doi.org/10.1897/1552-8618(1992)11[1663:AFFERA]2.0.CO;2)
- EU, 2013. Directive 2013/39/EU of the European Parliament and of the Council of 12 August 2013 amending Directives 2000/60/EC and 2008/105/EC as regards priority substances in the field of water policy.
- European Commission, 2019. Final Renewal report for the active substance chlorothalonil. <https://doi.org/https://doi.org/10.2903/j.efsa.2018.5126>
- FAO, 2015. FAO Specifications and Evaluations for Agricultural Pesticides - Chlorothalonil. *Fao* 1–62.
- FCSAP, 2012. Ecological Risk Assessment Guidance. Federal Contaminated Sites Action Plan (FCSAP), Environment and Climate

- Change Canada.
- Fent, K., 1996. Ecotoxicology of Organotin Compounds. *Crit. Rev. Toxicol.* 26, 1–117.
<https://doi.org/10.3109/10408449609089891>
- Fernández-Alba, A.R., Piedra, L., Mezcua, M., Hernando, M.D., 2002. Toxicity of single and mixed contaminants in seawater measured with acute toxicity bioassays. *Anal. Chim. Acta* 303–312.
<https://doi.org/10.1100/tsw.2002.221>
- Ferraz, M.A., Choueri, R.B., Castro, Í.B., Simon da Silva, C., Gallucci, F., 2020. Influence of sediment organic carbon on toxicity depends on organism's trophic ecology. *Environ. Pollut.* 261.
<https://doi.org/10.1016/j.envpol.2020.114134>
- Fonseca, V.B., Guerreiro, A. da S., Vargas, M.A., Sandrini, J.Z., 2020. Effects of DCOIT (4,5-dichloro-2-octyl-4-isothiazolin-3-one) to the haemocytes of mussels *Perna perna*. *Comp. Biochem. Physiol. Part - C Toxicol. Pharmacol.* 232, 108737.
<https://doi.org/10.1016/j.cbpc.2020.108737>
- Gallucci, F., Castro, I.B., Perina, F.C., Abessa, D.M. de S., Paula Teixeira, A., 2015. Ecological effects of Irgarol 1051 and Diuron on a coastal meiobenthic community: A laboratory microcosm experiment. *Ecol. Indic.* 58, 21–31. <https://doi.org/10.1016/j.ecolind.2015.05.030>
- Giacomazzi, S., Cochet, N., 2004. Environmental impact of diuron transformation: A review. *Chemosphere* 56, 1021–1032.
<https://doi.org/10.1016/j.chemosphere.2004.04.061>
- Gray, J.S., Elliott, M., 2014. *Ecology of Marine Sediments*, Oxford University Press.
- Grilo, C.F., Neto, R.R., Vicente, M.A., Castro, E.V.R. De, Figueira, R.C.L., Carreira, R.S., 2013. Evaluation of the influence of urbanization processes using mangrove and fecal markers in recent organic matter in a tropical tidal flat estuary. *Appl. Geochemistry* 38, 82–91. <https://doi.org/10.1016/j.apgeochem.2013.08.009>
- Guerreiro, S., Coimbra, R., Tomazele, M., Rutz, S., Zomer, J., 2017. Antifouling biocides: Impairment of bivalve immune system by chlorothalonil. *Aquat. Toxicol.* 189, 194–199.
<https://doi.org/10.1016/j.aquatox.2017.06.012>
- Hadlich, H.L., Venturini, N., Martins, C.C., Hatje, V., Tinelli, P., Gomes, L.E. de O., Bernadino, A.F., 2018. Multiple biogeochemical indicators of environmental quality in tropical estuaries reveal contrasting conservation opportunities. *Ecol. Indic.* 95, 21–31.
<https://doi.org/10.1016/j.ecolind.2018.07.027>
- Halpern, B.S., Frazier, M., Afflerbach, J., Lowndes, J.S., Micheli, F., O'Hara, C., Scarborough, C., Selkoe, K.A., 2019. Recent pace of change in human impact on the world's ocean. *Sci. Rep.* 9, 1–8.

- <https://doi.org/10.1038/s41598-019-47201-9>
- Hamwijk, C., Schouten, A., Foekema, E.M., Ravensberg, J.C., Collombon, M.T., Schmidt, K., Kugler, M., 2005. Monitoring of the booster biocide dichlofluanid in water and marine sediment of Greek marinas. *Chemosphere* 60, 1316–1324. <https://doi.org/10.1016/j.chemosphere.2005.01.072>
- Haque, N., Eom, H., Nam, S., Shin, Y.K., 2019. Chlorothalonil induces oxidative stress and reduces enzymatic activities of Na⁺ / K⁺ - ATPase and acetylcholinesterase in gill tissues of marine bivalves. *PLoS One* 1–17. <https://doi.org/https://doi.org/10.1371/journal.pone.0214236>
- Harino, H.H., Guchi, S.E., Hji, M.O., 2012. Occurrence of antifouling biocides in Japan and Southeast Asia : The survey for 10 years. *Coast. Mar. Sci.* 35, 246–254.
- Hartmann, N.B., Hu, T., Thompson, R.C., Hassello, M., Verschoor, A., Daugaard, A.E., Rist, S., Karlsson, T., Brennholt, N., Cole, M., Herrling, M.P., Hess, M.C., Ivleva, N.P., Lusher, A.L., Wagner, M., 2019. Are We Speaking the Same Language ? Recommendations for a definition and categorization framework for plastic debris. *Environ. Sci. Technol.* 1039–1047. <https://doi.org/10.1021/acs.est.8b05297>
- Hasan, C.K., Turner, A., Readman, J., Frickers, T., 2014. Environmental Risks Associated with Booster Biocides Leaching from Spent Anti-Fouling Paint Particles in Coastal Environments. *Water Environ. Res.* 86, 2330–2337. <https://doi.org/10.2175/106143014X14062131178835>
- Hayes, T.B., Hansen, M., 2017. From silent spring to silent night: Agrochemicals and the anthropocene. *Elementa* 5. <https://doi.org/10.1525/elementa.246>
- IMO, 2020. Comprehensive information on the status of multilateral Conventions and instruments in respect of which the International Maritime Organization or its Secretary 557.
- IMO, 2008. Guidelines for survey and certification of anti-fouling systems on ships.
- Jablonski, S., Filet, M., 2008. Ocean & Coastal Management Coastal management in Brazil – A political riddle. *Ocean Coast. Manag.* 51, 536–543. <https://doi.org/10.1016/j.ocecoaman.2008.06.008>
- Jacobson, A.H., Willingham, G.L., 2000. Sea-nine antifoulant: An environmentally acceptable alternative to organotin antifoulants. *Sci. Total Environ.* 258, 103–110. [https://doi.org/10.1016/S0048-9697\(00\)00511-8](https://doi.org/10.1016/S0048-9697(00)00511-8)
- Jones, R., 2005. The ecotoxicological effects of Photosystem II herbicides on corals 51, 495–506. <https://doi.org/10.1016/j.marpolbul.2005.06.027>
- Jung, S.M., Bae, J.S., Kang, S.G., Son, J.S., Jeon, J.H., Lee, H.J., Jeon,

- J.Y., Sidharthan, M., Ryu, S.H., Shin, H.W., 2017. Acute toxicity of organic antifouling biocides to phytoplankton *Nitzschia pungens* and zooplankton *Artemia* larvae. *Mar. Pollut. Bull.* 124, 811–818. <https://doi.org/10.1016/j.marpolbul.2016.11.047>
- Kjerfve, B., Filippo, A., Dias, G.T. de M., Quaresma, V. da S., 1997. Oceanographic Characteristics of an Impacted Coastal Bay : Baía de Guanabara , Oceanographic characteristics of an impacted coastal bay : Baía de. *Cont. Shelf Res.* 4343. [https://doi.org/10.1016/S0278-4343\(97\)00028-9](https://doi.org/10.1016/S0278-4343(97)00028-9)
- Konstantinou, I.K., Albanis, T.A., 2004. Worldwide occurrence and effects of antifouling paint booster biocides in the aquatic environment: A review. *Environ. Int.* 30, 235–248. [https://doi.org/10.1016/S0160-4120\(03\)00176-4](https://doi.org/10.1016/S0160-4120(03)00176-4)
- Kristensen, E., Andersen, F., 1987. Determination of organic carbon in marine sediments: a comparison of two CHN-analyzer methods. *J. Exp. Mar. Bio. Ecol.* 109, 15–23. [https://doi.org/10.1016/0022-0981\(87\)90182-1](https://doi.org/10.1016/0022-0981(87)90182-1)
- Lagerström, M., Strand, J., Eklund, B., Ytreberg, E., 2017. Total tin and organotin speciation in historic layers of antifouling paint on leisure boat hulls. *Environ. Pollut.* 220, 1333–1341. <https://doi.org/10.1016/j.envpol.2016.11.001>
- Lahbib, Y., Abidli, S., González, P.R., Alonso, J.I.G., Trigui El Menif, N., 2011. Monitoring of organotin pollution in bizerta channel (Northern Tunisia): Temporal trend from 2002 to 2010. *Bull. Environ. Contam. Toxicol.* 86, 531–534. <https://doi.org/10.1007/s00128-011-0248-5>
- Lam, N.H., Jeong, H. ho, Kang, S. dong, Kim, D.J., Ju, M.J., Horiguchi, T., Cho, H.S., 2017. Organotins and new antifouling biocides in water and sediments from three Korean Special Management Sea Areas following ten years of tributyltin regulation: Contamination profiles and risk assessment. *Mar. Pollut. Bull.* 121, 302–312. <https://doi.org/10.1016/j.marpolbul.2017.06.026>
- Lee, M.R.N., Kim, U.J., Lee, I.S., Choi, M., Oh, J.E., 2015. Assessment of organotin and tin-free antifouling paints contamination in the Korean coastal area. *Mar. Pollut. Bull.* 99, 157–165. <https://doi.org/10.1016/j.marpolbul.2015.07.038>
- Lee, S., Lee, Y.W., 2017. Determination of five alternative antifouling agents in Korean marine sediments. *Environ. Earth Sci.* 76, 1–9. <https://doi.org/10.1007/s12665-017-6954-5>
- Lindholdt, A., Dam-Johansen, K., Olsen, S.M., Yebra, D.M., Kiil, S., 2015. Effects of biofouling development on drag forces of hull coatings for ocean-going ships: a review. *J. Coatings Technol. Res.* 12, 415–444. <https://doi.org/10.1007/s11998-014-9651-2>
- Lopes, F.C., Junior, A.S.V., Corcini, C.D., Sanchez, J.A.A., Pires, D.M.,

- Pereira, J.R., Primel, E.G., Fillmann, G., Martins, C.M.G., 2020. Impacts of the biocide chlorothalonil on biomarkers of oxidative stress, genotoxicity, and sperm quality in guppy *Poecilia vivipara*. *Ecotoxicol. Environ. Saf.* 109847. <https://doi.org/10.1016/j.ecoenv.2019.109847>
- Magris, R.A., Giarrizzo, T., 2020. Mysterious oil spill in the Atlantic Ocean threatens marine biodiversity and local people in Brazil. *Mar. Pollut. Bull.* 153, 2019–2021. <https://doi.org/https://doi.org/10.1016/j.marpolbul.2020.110961>
- Martínez, K., Ferrer, I., Hernando, M.D., Fernández-Alba, A.R., Marcé, R.M., Borrull, F., Barceló, D., 2001. Occurrence of antifouling biocides in the Spanish Mediterranean marine environment. *Environ. Technol. (United Kingdom)* 22, 543–552. <https://doi.org/10.1080/09593332208618258>
- Martins, S.E., Fillmann, G., Lillicrap, A., Thomas, K. V., 2018. Review: ecotoxicity of organic and organo-metallic antifouling co-biocides and implications for environmental hazard and risk assessments in aquatic ecosystems. *Biofouling* 34, 34–52. <https://doi.org/10.1080/08927014.2017.1404036>
- Mattos, Y., Stotz, W.B., Soledad, M., Bravo, M., Fillmann, G., Castro, Í.B., 2017. Butyltin contamination in Northern Chilean coast: Is there a potential risk for consumers? *Sci. Total Environ.* 595, 209–217. <https://doi.org/10.1016/j.scitotenv.2017.03.264>
- Menin, A., Ballarin, L., Bragadin, M., Cima, F., 2008. Immunotoxicity in ascidians: Antifouling compounds alternative to organotin - II. The case of Diuron and TCMS pyridine. *J. Environ. Sci. Heal. - Part B Pestic. Food Contam. Agric. Wastes* 43, 644–654. <https://doi.org/10.1080/03601230802352690>
- Ministério da Agricultura, P. e A., 2019. Agrofit: Consulta Aberta. URL http://agrofit.agricultura.gov.br/agrofit_cons/principal_agrofit_cons (accessed 10.October.19).
- Mochida, K., Hano, T., Onduka, T., Ichihashi, H., Amano, H., Ito, M., Ito, K., Tanaka, H., Fujii, K., 2015. Spatial analysis of 4,5-dichloro-2-n-octyl-4-isothiazolin-3-one (Sea-Nine 211) concentrations and probabilistic risk to marine organisms in Hiroshima Bay, Japan. *Environ. Pollut.* 204, 233–240. <https://doi.org/10.1016/j.envpol.2015.05.012>
- Moermond, C.T.A., Kase, R., Korkaric, M.K., Ågerstrand, M., 2016. CRED: Criteria for reporting and evaluating ecotoxicity data. *Environ. Toxicol. Chem.* 35, 1297–1309. <https://doi.org/10.1002/etc.3259>
- Moller, O.O., Castaing, P., Salomon, J.-C., Lazure, P., 2001. The Influence of Local and Non-Local Forcing Effects on the Subtidal Circulation of Patos Lagoon. *Estuaries* 24, 297. <https://doi.org/10.2307/1352953>

- Moreira, L.B., Castro, Í.B., Hortellani, M.A., Sasaki, S.T., Taniguchi, S., Petti, M.A.V. V, Fillmann, G., Sarkis, J.E.S.S., Bicego, M.C., Costa-Lotuf, L. V., Abessa, D.M.S.S., 2017. Effects of harbor activities on sediment quality in a semi-arid region in Brazil. *Ecotoxicol. Environ. Saf.* 135, 137–151. <https://doi.org/10.1016/j.ecoenv.2016.09.020>
- Muller-Karanassos, C., Turner, A., Arundel, W., Vance, T., Lindeque, P.K., Cole, M., 2019. Antifouling paint particles in intertidal estuarine sediments from southwest England and their ingestion by the harbour ragworm, *Hediste diversicolor*. *Environ. Pollut.* 249, 163–170. <https://doi.org/https://doi.org/10.1016/j.envpol.2019.03.009>
- NEA, 2018. Risk assessment of contaminated sediments Norway. Norwegian Environment Agency.
- Neves, R., Bastos, A.C., Neves, R.C., 2012. Transporte sedimentar em baías costeiras: estudo de caso nas Baías de Vitória e Espírito. *Rev. Bras. Geofísica* 30, 181–189.
- Nikinmaa, M., 2014. Modeling Toxicity, in: *An Introduction to Aquatic Toxicology*. Academic Press, Oxford, UK, pp. 207–219. <https://doi.org/10.1016/b978-0-12-411574-3.00018-9>
- Oliveira, C.R., Santos, D.M. dos, Madureira, L.A. dos S., Marchi, M.R.R. de, 2010. Speciation of butyltin derivatives in surface sediments of three southern Brazilian harbors. *J. Hazard. Mater.* 181, 851–856. <https://doi.org/10.1016/j.jhazmat.2010.05.091>
- Onduka, T., Ojima, D., Ito, M., Ito, K., Mochida, K., Fujii, K., 2013. Toxicity of the antifouling biocide Sea-Nine 211 to marine algae, crustacea, and a polychaete. *Fish. Sci.* 79, 999–1006. <https://doi.org/10.1007/s12562-013-0678-6>
- OSPAR, 2011. Background Document on Organic tin compounds, Hazards Substances Water.
- Parks, R., Donnier-Marechal, M., Frickers, P.E., Turner, A., Readman, J.W., 2010. Antifouling biocides in discarded marine paint particles. *Mar. Pollut. Bull.* 60, 1226–1230. <https://doi.org/10.1016/j.marpolbul.2010.03.022>
- Paz-Villarraga, C.A., 2019. Níveis ambientais e efeito de biocidas usados em sistemas anti-incrustantes da Lagoa dos Patos, Rio Grande, RS. Universidade Federal do Rio Grande.
- Pei, Z.T., Xu, R.R., Liu, H.Y., Wang, W.Q., Zhang, M., Zhang, L.L., Zhang, J., Wang, W.Q., Yu, R., Sun, L.W., 2020. Development and application of a novel whole sediment toxicity test using immobilized sediment and *Chlorella vulgaris*. *Ecotoxicol. Environ. Saf.* 189, 109979. <https://doi.org/10.1016/j.ecoenv.2019.109979>
- Podratz, P.L., Merlo, E., de Araújo, J.F.P., Ayub, J.G.M., Pereira, A.F.Z., Freitas-Lima, L.C., da Costa, M.B., Miranda-Alves, L., Cassa, S.G.S., Carneiro, M.T.W.D., Fillmann, G., Graceli, J.B., 2020. Disruption of

- fertility, placenta, pregnancy outcome, and multigenerational inheritance of hepatic steatosis by organotin exposure from contaminated seafood in rats. *Sci. Total Environ.* 723, 138000. <https://doi.org/10.1016/j.scitotenv.2020.138000>
- Price, R.G.A., Readman, W.J., 2013. Booster biocide antifoulants: is history repeating itself? Late lessons from early Warn. *Sci. precaution, Innov.* 265–278.
- Quintas, P.Y., Oliva, A.L., Arias, A., Domini, C.E., Alvarez, M.B., Garrido, M., Marcovecchio, J.E., 2016. Seasonal changes in organotin compounds in sediments from the Bahía Blanca Estuary. *Environ. Earth Sci.* 75. <https://doi.org/10.1007/s12665-016-5471-2>
- Ribeiro-Brasil, D.R.G., Castro, I.B. de, Petracco, M., Batista, R.M., Brasil, L.S., Ferreira, J.A., Borba, T.A. da C., Rollnic, M., Amado, L.L., Fillmann, G., 2021. Spatial distribution of butyltins and imposex in eastern Brazilian Amazon. *Mar. Pollut. Bull.* 165, 112155. <https://doi.org/https://doi.org/10.1016/j.marpolbul.2021.112155>
- Rola, R.C., Souza, M.M., Sandrini, J.Z., 2017. Hypoosmotic stress in the mussel *Perna perna* (Linnaeus, 1758): Is ecological history a determinant for organismal responses? *Estuar. Coast. Shelf Sci.* 189, 216–223. <https://doi.org/10.1016/j.ecss.2017.03.020>
- Sakkas, V.A., Konstantinou, I.K., Albanis, T.A., 2001. Photodegradation study of the antifouling booster biocide dichlofluanid in aqueous media by gas chromatographic techniques. *J. Chromatogr. A* 930, 135–144. [https://doi.org/10.1016/S0021-9673\(01\)01193-1](https://doi.org/10.1016/S0021-9673(01)01193-1)
- Sánchez-Rodríguez, Á., Sosa-Ferrera, Z., Santana-Rodríguez, J.J., 2011. Applicability of microwave-assisted extraction combined with LC-MS/MS in the evaluation of booster biocide levels in harbour sediments. *Chemosphere* 82, 96–102. <https://doi.org/10.1016/j.chemosphere.2010.09.064>
- Sangchan, W., Bannwarth, M., Ingwersen, J., Hugenschmidt, C., Schwadorf, K., Thavornyutikarn, P., Pansombat, K., Streck, T., 2014. Monitoring and risk assessment of pesticides in a tropical river of an agricultural watershed in northern Thailand. *Environ. Monit. Assess.* 186, 1083–1099. <https://doi.org/10.1007/s10661-013-3440-8>
- Sapozhnikova, Y., Wirth, E., Schiff, K., Fulton, M., 2013. Antifouling biocides in water and sediments from California marinas. *Mar. Pollut. Bull.* 69, 189–194. <https://doi.org/10.1016/j.marpolbul.2013.01.039>
- Schettini, C.A.F., 2002. Near bed sediment transport in the itajaí-açu river estuary, southern brazil., *Proceedings in Marine Science*. Elsevier B.V. [https://doi.org/10.1016/S1568-2692\(02\)80036-5](https://doi.org/10.1016/S1568-2692(02)80036-5)
- Schettini, C.A.F., Paiva, B.P., Batista, R. de A.L., de Oliveira Filho, J.C., Truccolo, E.C., 2016. Observation of an Estuarine turbidity maximum in the highly impacted capibaribe estuary, Brazil. *Brazilian J.*

- Oceanogr. 64, 185–190. <https://doi.org/10.1590/S1679-87592016115006402>
- Simpson, S., Batley, G., 2019. Introduction, 2^o. ed, Sediment Quality Assessment. CSIRO Publishing, Australia. <https://doi.org/10.1071/9781486303854>
- Simpson, S.L., Batley, G.E., Chariton, A.A., 2013. Revision of the ANZECC/ARMCANZ sediment quality guidelines. CSIRO L. Water Rep. 08/07 132. <https://doi.org/10.1016/j.proeng.2012.04.061>
- Singh, N., Turner, A., 2009. Trace metals in antifouling paint particles and their heterogeneous contamination of coastal sediments. Mar. Pollut. Bull. 58, 559–564. <https://doi.org/10.1016/j.marpolbul.2008.11.014>
- Soon, Z.Y., Jung, J., Jang, M., Kang, J., Jang, M., Lee, J., Kim, M., 2019. Zinc Pyrithione (ZnPT) as an Antifouling Biocide in the Marine Environment — a Literature Review of Its Toxicity , Environmental Fates , and Analytical Methods 1–18. <https://doi.org/https://doi.org/10.1007/s11270-019-4361-0>
- Soroldoni, S., Castro, Í.B., Abreu, F., Duarte, F.A., Choueri, R.B., Möller, O.O., Fillmann, G., Pinho, G.L.L., 2018. Antifouling paint particles: Sources, occurrence, composition and dynamics. Water Res. 137, 47–56. <https://doi.org/10.1016/j.watres.2018.02.064>
- Soroldoni, S., Silva, S.V. da, Castro, Í.B., Martins, C.M.G., Pinho, G.L., 2020. Antifouling paint particles cause toxicity to benthic organisms: Effects on two species with different feeding modes. Chemosphere 238, 124610. <https://doi.org/https://doi.org/10.1016/j.chemosphere.2019.124610>
- Thomas, K. V., Brooks, S., 2010. The environmental fate and effects of antifouling paint biocides. Biofouling 26, 73–88. <https://doi.org/10.1080/08927010903216564>
- Thomas, K. V, Blake, S.J., Waldock, M.J., 2000. Antifouling paint booster biocide contamination in UK marine sediments. Mar. Pollut. Bull. 40, 739–745. [https://doi.org/http://dx.doi.org/10.1016/S0025-326X\(00\)00010-2](https://doi.org/http://dx.doi.org/10.1016/S0025-326X(00)00010-2)
- Thompson, R.C., Moore, C.J., Saal, F.S.V., Swan, S.H., 2009. Plastics, the environment and human health: Current consensus and future trends. Philos. Trans. R. Soc. B Biol. Sci. 364, 2153–2166. <https://doi.org/10.1098/rstb.2009.0053>
- Tixier, C., Bogaerts, P., Sancelme, M., Bonnemoy, F., Twagilimana, L., Cuer, A., Bohatier, J., Veschambre, H., 2000. Fungal biodegradation of a phenylurea herbicide, diuron: Structure and toxicity of metabolites. Pest Manag. Sci. 56, 455–462. [https://doi.org/10.1002/\(SICI\)1526-4998\(200005\)56:5<455::AID-PS152>3.0.CO;2-Z](https://doi.org/10.1002/(SICI)1526-4998(200005)56:5<455::AID-PS152>3.0.CO;2-Z)
- Tornero, V., Hanke, G., 2016. Identification of marine chemical contaminants released from sea-based sources A review focusing on

- regulatory aspects. <https://doi.org/10.2788/258216>
- Turner, A., 2010. Marine pollution from antifouling paint particles. *Mar. Pollut. Bull.* 60, 159–171. <https://doi.org/10.1016/j.marpolbul.2009.12.004>
- U.S. EPA, 1996. Presidential Green Chemistry Challenge: 1996 Designing Greener Chemicals Award.
- Umbría-Salinas, K., Valero, A., Martins, S.E., Wallner-Kersanach, M., 2021. Copper ecological risk assessment using DGT technique and PNEC: A case study in the Brazilian coast. *J. Hazard. Mater.* 403. <https://doi.org/10.1016/j.jhazmat.2020.123918>
- UNCTAD, 2020. Review of maritime transport 2019.
- Vale, 2015. Port of Tubarão, in Vitoria: 49 years of history. URL <http://www.vale.com/en/aboutvale/news/pages/porto-tubarao-vitoria-completa-49-anos-historia.aspx> (accessed 4.April.20).
- Van Wezel, A.P., Van Vlaardingen, P., 2004. Environmental risk limits for antifouling substances. *Aquat. Toxicol.* 66, 427–444. <https://doi.org/10.1016/j.aquatox.2003.11.003>
- Viana, J.L.M., dos Santos Diniz, M., dos Santos, S.R.V., Verbinnen, R.T., Almeida, M.A.P., dos Santos Franco, T.C.R., 2020. Antifouling substances as a continuous threat to the aquatic environment: Sources, temporal trends and ecological risk assessment in an impacted region of Brazil. *Sci. Total Environ.* 730, 139026. <https://doi.org/10.1016/j.scitotenv.2020.139026>
- Vianna, C.B.E., 2009. Conflitos de usos na Baía de Vitória: Diagnóstico da gestão ambiental portuária e costeira 103.
- Voulvoulis, N., 2006. Antifouling paint booster biocides: Occurrence and partitioning in water and sediments. *Handb. Environ. Chem. Vol. 5 Water Pollut.* 5, 155–170. <https://doi.org/10.1007/698-5-053>
- Voulvoulis, N., Scrimshaw, M.D., Lester, J.N., 2002. Partitioning of selected antifouling biocides in the aquatic environment. *Mar. Environ. Res.* 53, 1–16. [https://doi.org/10.1016/S0141-1136\(01\)00102-7](https://doi.org/10.1016/S0141-1136(01)00102-7)
- Wang, H., Li, Y., Huang, H., Xu, X., Wang, Y., 2011. Toxicity evaluation of single and mixed antifouling biocides using the *Strongylocentrotus intermedius* sea urchin embryo test. *Environ. Toxicol. Chem.* 30, 692–703. <https://doi.org/10.1002/etc.440>
- Wang, J., Zhao, F., Yang, X., Han, W., Long, K., Zhou, Y., 2014a. Marine Environmental Risk Assessment Method for Active Substances Used in Antifouling Systems on Ships in China. *Adv. Mater. Res.* 864–867, 962–972. <https://doi.org/10.4028/www.scientific.net/AMR.864-867.962>
- Wang, J., Zhao, F., Yang, X., Han, W., Long, K., Zhou, Y., 2014b. Marine Environmental Risk Assessment Method for Active Substances Used in Antifouling Systems on Ships in China 867, 962–972.

- <https://doi.org/10.4028/www.scientific.net/AMR.864-867.962>
- Wang, X., Kong, L., Cheng, J., Zhao, D., Chen, H., Sun, R., 2019. Distribution of butyltins at dredged material dumping sites around the coast of China and the potential ecological risk. *Mar. Pollut. Bull.* 138, 491–500. <https://doi.org/10.1016/j.marpolbul.2018.11.043>
- Wetzel, M.A., Winterscheid, A., Wahrendorf, D.S., 2013. Baseline of the butyltin distribution in surface sediments (0-20cm) of the Elbe estuary (Germany, 2011). *Mar. Pollut. Bull.* 77, 418–423. <https://doi.org/10.1016/j.marpolbul.2013.09.021>
- Wezenbeek, J.M., Moermond, C.T.A., Smit, C.E., 2018. Antifouling systems for pleasure boats. RIVM Report 2018-0086.
- Wiklund, A.E., Vilhelmsson, S., Wiklund, S.J., Eklund, B., 2009. Contaminants and habitat choice in the Baltic Sea: Behavioural experiments with the native species, *Monoporeia affinis*, and the invasive genus, *Marenzelleria*. *Estuar. Coast. Shelf Sci.* 81, 238–246. <https://doi.org/10.1016/j.ecss.2008.10.023>
- Yebra, D.M., Kiil, S., Dam-Johansen, K., 2004. Antifouling technology - Past, present and future steps towards efficient and environmentally friendly antifouling coatings. *Prog. Org. Coatings* 50, 75–104. <https://doi.org/10.1016/j.porgcoat.2003.06.001>
- Zamprogno, G.C., 2016. The structural heterogeneity of an urbanised mangrove forest area in southeastern Brazil: Influence of environmental factors and anthropogenic stressors. *Brazilian J. Oceanogr.* 64, 157–172.
- Zhang, L.-J., Ying, G.-G., Chen, F., Zhao, J.-L., Wang, L., Fang, Y.-X., 2012. Development and application of whole-sediment toxicity test using immobilized freshwater microalgae *Pseudokirchneriella subcapitata*. *Environ. Toxicol. Chem.* 31, 377–386. <https://doi.org/10.1002/etc.734>
- Zhen, X., Liu, L., Wang, X., Zhong, G., Tang, J., 2019. Fates and ecological effects of current-use pesticides (CUPs) in a typical river-estuarine system of Laizhou Bay, North China. *Environ. Pollut.* 252, 573–579. <https://doi.org/10.1016/j.envpol.2019.05.141>
- Zhou, J.L., 2008. Occurrence and persistence of antifouling biocide Irgarol 1051 and its main metabolite in the coastal waters of Southern England. *Sci. Total Environ.* 406, 239–246. <https://doi.org/10.1016/j.scitotenv.2008.07.049>

ANEXOS

MATERIAL SUPLEMENTAR – ARTIGO 1

Are antifouling residues a matter of concern in the largest South American port?

Table S1: Description and geographic coordinates of sediment samples from Santos – São Vicente estuarine system

Site Code	Latitud	Longitud	Main Activity	Descriptions and vessel types
SP1	24°0'30.31"	46°19'25.40"	Ship traffic/ boat traffic	Santos Bay/Nearby MNC entrance (ships and small boats)
SP2	23°59' 30.75"	46° 18' 10,82"	Ship traffic/boat traffic	Entrance of MNC/Fishing boat mooring area (ships and small boats)
SP3	23°59'45.00"	46°17'56.98"	Ship traffic/boat traffic	Entrance of MNC/Entrance of Pouca Farinha river (ships)
SP4	23°59'47.65"	46°17'41.45"	Ship traffic/boat traffic	Entrance of Meio river (ships)
SP5	23° 59' 15.40"	46° 17' 40.27"	Ship traffic	MNC/Ferryboat jetty (ships)
SP6	23° 56' 55.88"	46°18' 22.85"	Ship traffic	MNC/Mooring area/ Port terminal (ships)
SP7	23° 55' 31.16"	46°19' 39.83"	Ship traffic / shipyard	MNC/Shipyard (ship and boats)
SP8	23° 55 '7.56"	46° 22' 9.11"	Ship traffic	MNC/Mooring area/ Port terminal 2 (ships)
SP9	23° 58' 34.55"	46° 17' 11.80"	Ship traffic	MNC/Mooring area/ Port terminal 3 (ships)
SP10	23°55' 39.15"	46° 23' 27.61"	Boat traffic	Craft and fishing boats
SP11	23° 57' 7.24"	46° 25' 30.36"	Boat traffic	Craft and fishing boats
SP12	23°58' 36.25"	46°23' 45.98"	Boat traffic	Marina with fishing and small boats (< 25m) (cheap boats)
SP13	23°59'55.32"	46°18'13.86"	Boat traffic/ boatyard	Maintenance of craft and fishing boats (< 25m) (cheap boats)
SP14	23°59'55.80"	46°18'13.32"	Boat traffic/ boatyard	Maintenance of craft and fishing boats (< 25m) (cheap boats)
SP15	23°59'58.50"	46°18'9.84"	Boat traffic/ boatyard	Maintenance of craft and fishing boats (< 25m) (cheap boats)
SP16	23°59'58.80"	46°18'4.80"	Boat traffic/ boatyard	Maintenance of craft and fishing boats (< 25m) (cheap boats)
SP17	23°59'50.94"	46°17'58.26"	Boat traffic/ marina	Marina with leisure boats / boat maintenance (expensive boats)
SP18	24° 0'14.70"	46°17'48.06"	Boat traffic/ marina	Marina with leisure boats / boat maintenance (expensive boats)

SP19	24° 0'18.36"	46°17'37.92"	Boat traffic/ marina	Marina with leisure boats / boat maintenance (expensive boats)
SP20	24°0'17.58"	46°17'35.28"	Boat traffic/ marina	Marina with leisure boats (expensive boats)
SP21	24° 0'5.04"	46°17'38.10"	Boat traffic/ marina	Marina with leisure boats / boat maintenance (expensive boats)
SP22	24° 0'10.98"	46°17'27.72"	Boat traffic/ marina	Marina with leisure boats / boat maintenance (expensive boats)
SP23	24° 0'6.72"	46°17'31.98"	Boat traffic/ marina	Marina with leisure boats / boat maintenance (expensive boats)
SP24	24°0'11.16"	46°17'24.06"	Boat traffic/ marina	Marina with leisure boats / boat maintenance (expensive boats)
SP25	23°59'18.84"	46°17'22.74"	Ship traffic/ shipyard	MNC/Maintenance of Ferryboat ships
SP26	23°59'17.04"	46°17'1.20"	Boat traffic/ marina	Marina with leisure boats / boat maintenance (expensive boats)
SP27	23°59'8.46"	46°16'50.76"	Boat traffic/ boatyard	Maintenance of vessels (mainly fishing boats)
SP28	23°59'0.78"	46°16'54.96"	Boat traffic/ boatyard	Maintenance of vessels (mainly fishing boats)
SP29	23°58'59.58"	46°16'46.86"	Boat traffic/ boatyard	Maintenance of vessels (mainly fishing boats)
SP30	23°59'4.38"	46°16'41.82"	Boat traffic/ boatyard	Maintenance of vessels (mainly fishing boats)

MNC – Main navigation channel

Table S2: Analytical curve equation prepared in solvent and sediment (matrix-matched), square of regression coefficient (R²) and matrix effect (% ME) for butyltins and booster biocides.

Compounds	Solvent calibration		Matrix-matched calibration		Matrix effect (EM%)
	Equation	R ²	Equation	R ²	
<i>Butyltins</i>					
TBT	$y = 0.00570193x - 0.00728584$	0.998	$y = 0.00454291x - 0.01832051$	0.998	20
DBT	$y = 0.00595851x - 0.00415379$	0.997	$y = 0.00514563x - 0.02247480$	0.996	13
MBT	$y = 0.00091140x + 0.00083390$	0.991	$y = 0.00140020x + 0.00417327$	0.991	-53
<i>Booster biocides</i>					
Diuron	$y = 97820x - 13995$	0.999	$y = 75043x + 8194.6$	0.998	23
Irgarol	$y = 3E+06x + 187361$	0.999	$y = 2E+06x + 131337$	0.999	44
Chlorothalonil	$y = 0.0807x - 0.0538$	0.995	$y = 0.0662x - 0.2037$	0.993	18
Dichlofluanid	$y = 0.0734x - 0.0161$	0.995	$y = 0.0585x - 0.0507$	0.990	20
DCOIT	$y = 79503x + 894.14$	0.999	$y = 26513x + 3241.9$	0.997	67

Table S3: Total organic carbon (%TOC) and concentrations of butyltins (TBTn, DBTn, MBTn, ng Sn g⁻¹) and diuron (ng Sn g⁻¹) normalized to 1% organic carbon in sediments collected along Santos – São Vicente estuarine system

Site Code	TOC (%)	Normalized to 1% organic carbon			
		TBTn	DBTn	MBTn	Diuron
SP1	1.5	<LD	<LD	<LD	<LD
SP2	0.9	1.3	<LD	2.6	<LD
SP3	3.5	2.3	3.1	4.3	1.7
SP4	3.2	7.4	5.7	11.2	0.4
SP5	1.3	<LD	3.2	2.3	<LD
SP6	3.0	<LD	0.6	<LD	<LD
SP7	2.7	0.9	3.3	24.0	<LD
SP8	2.8	6.0	2.9	1.0	<LD
SP9	3.4	6.9	1.3	1.0	<LD
SP10	3.5	<LD	2.5	1.5	<LD
SP11	1.1	1.3	<LD	<LD	<LD
SP12	1.0	7.9	4.0	<LD	<LD
SP13	5.4	127.5	56.2	56.7	<LQ
SP14	6.3	70.5	46.4	60.5	<LQ
SP15	6.7	57.1	33.5	120.8	<LD
SP16	3.7	28.1	16.2	44.3	<LQ
SP17	2.5	55.9	26.1	24.4	0.6
SP18	1.7	5.4	3.1	6.6	1.4
SP19	2.8	32.2	14.7	34.4	1.7
SP20	3.3	19.8	12.9	25.6	3.0
SP21	3.2	52.7	23.0	38.3	2.0
SP22	3.3	20.7	9.8	6.7	2.2
SP23	2.8	42.3	44.9	60.1	1.2
SP24	4.1	19.6	10.1	26.6	1.4
Site Code	TOC (%)	Normalized to 1% organic carbon			
		TBTn	DBTn	MBTn	Diuron
SP25	0.5	340.4	119.8	139.4	<LD
SP26	5.7	37.5	18.3	73.7	<LQ
SP27	5.3	87.8	38.1	137.1	<LQ
SP28	4.7	98.4	33.6	135.6	<LD
SP29	4.3	69.1	32.5	74.9	<LQ
SP30	3.3	105.0	39.5	80.5	<LQ

<LD – below limit of detection




<LQ – below limit of quantification

ANEXOS

MATERIAL SUPLEMENTAR – ARTIGO 2

Legacy and emerging antifouling biocide residues in a tropical estuarine system (Vitória state, SE, Brazil)

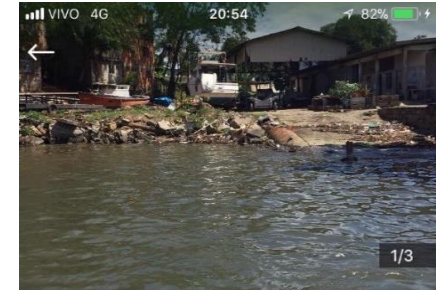
Table S1: Description and geographic coordinates of sediment samples from Vitoria Estuarine System (VES)

Site Code	Latitude (W)	Longitude (S)	Main Activity	Depth (m)	Descriptions and vessel types	Picture
ES1	40°16'18.30"	20°19'29.49"	Marina/Fishing port/Boatyard	0.5	Mooring of leisure and fishing boats / Boatyard (fishing boats)	
ES2	40°18'2.51"	20°19'6.99"	Fishing port/Boatyard	1.5	Mooring of fishing boats / Untreated sewage	
ES3	40°17'27.89"	20°19'36.08"	Fishing port/Boatyard	1	Mooring of fishing boats / Fishermen's Association / Untreated sewage	

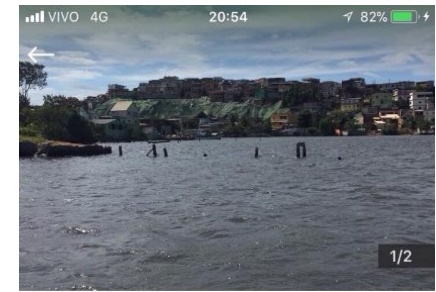
ES4	40°18'39.34"	20°19'45.36"	Boatyard	1.5	Boatyard (mainly fishing with some leisure boats) / Untreated sewage
ES5	40°19'15.69"	20°19'26.27"	Port	1.5	Roll-on-roll-off operations and supply boat
ES6	40°18'52.44"	20°19'4.69"	Marina/Boatyard	2	Leisure boats / Untreated sewage / Supply boats nearby
ES7	40°20'53.64"	20°19'29.52"	Port	7	Container port (Vitória port)



ES8 40°21'8.18" 20°19'35.37" Boatyard 6 Boatyard (fishing and commercial boats)



ES9 40°21'54.18" 20°18'42.75" Traffic 2 Traffic of fishing boats



ES10 40°20'13.72" 20°16'43.25" Fishing port 1.5 Mooring of fishing boats and some eventual leisure boats



ES11 40°18'43.32" 20°14'40.12" Traffic 2 Fishing boats and recreational boats traffic



ES12 40°17'55.93" 20°17'28.80" Marina/Boatyard 2 Small pier for leisure boats



ES13 40°17'26.84" 20°17'38.86" Fishing port/Boatyard 2 Fishermen's Association / Fishing boats / Untreated sewage



ES14 40°17'21.54" 20°17'57.69" Marina/Boatyard 3 Yacht Club / Boatyard (leisure boats)



Pictures source: Abreu, F. E. L, Pedruzzi, F. C, and Google®

Table S2: Total organic carbon (%TOC) and concentrations of butyltins (TBTn, DBTn, MBTn, ng Sn g⁻¹), diuron (ng Sn g⁻¹) and Irgarol (ng Sn g⁻¹) normalized to 1% organic carbon in sediments collected in Vitoria Estuarine System

Site Code	TOC (%)	Normalized to 1% organic carbon				
		TBTn	DBTn	MBTn	Diuron	Irgarol
ES1	2.3	<LD	5.8	8.2	<LD	<LD
ES2	3.1	5.3	7.7	5.6	<LD	<LD
ES3	0.5	22.9	46.7	162.2	<LD	<LD
ES4	4.4	3.3	<LD	<LD	<LQ	<LD
ES5	4.7	1.3	<LD	<LD	<LQ	<LD
ES6	4.8	3.0	3.9	2.3	<LD	<LQ
ES7	4.3	4.9	2.9	<LD	<LQ	<LD
ES8	3.5	1.6	<LD	1.8	<LD	<LD
ES9	4.5	<LD	<LD	<LD	<LD	0.3
ES10	6.3	1.0	<LD	<LD	<LD	0.2
ES11	11.1	<LD	<LD	<LD	<LD	<LD
ES12	5.4	<LD	1.6	1.2	<LD	<LD
ES13	3.1	5.4	5.4	4.6	<LQ	0.4
ES14	2.1	5.3	7.0	12.8	1.3	<LD

<LD – below limit of detection; <LQ – below limit of quantification

Table S3: Site, main activity, occurrence ($\mu\text{g g}^{-1}$) of antifouling paint particles (APPs) in sediments collected in the Vitoria Estuarine System and concentrations of diuron, Irgarol, chlorothalonil, dichlofluanid and DCOIT (ng g^{-1}) in the corresponding APPs.

Site	Site location	Main activity	APP	Diuron	Irgarol	Chlorothalonil	Dichlofluanid	DCOIT
ES1	Espírito Santo bay	Marina/Fishing port/ Boatyard	5,969	<50	<40	<10	<70	1,897
ES2	Port channel	Fishing port/ Boatyard	12	na	<40	na	na	na
ES3	Port channel	Fishing port/ Boatyard	305	<50	<40	<10	<70	<20
ES4	Port channel	Boatyard	85	<50	<40	<10	<70	<20
ES5	Port channel	Port	127	<50	<40	<10	<70	<20
ES6	Port channel	Marina/Boatyard	78	<140	170	<10	<70	<20
ES7	Port channel	Port	35	1,674,779	<40	<10	<70	65,1846
ES8	Port channel	Boatyard	19	90,997	<40	<10	<70	899,608
ES9	Vitoria bay	Traffic	144	1,210	<130	<10	<70	1,954
ES10	Vitoria bay	Fishing port	34	<50	<130	<10	<70	<20
ES11	Vitoria bay	Traffic	<0.01	na	na	na	na	na
ES12	Passagem channel	Marina/Boatyard	154	<50	<130	<10	<70	24,052
ES13	Passagem channel	Fishing port/Boatyard	1,192	<50	<40	<10	<70	<20
ES14	Espírito Santo bay	Marina/Boatyard	12	na	na	na	na	na

na: not analyzed (lack of APPs)

ANEXOS

MATERIAL SUPPLEMENTAR – ARTIGO 3

Antifouling biocides in sediments along the Brazilian coast

Table S2: Total organic carbon (%TOC) and concentrations of butyltins (TBTn, DBTn, MBTn, ng Sn g⁻¹), diuron (ng Sn g⁻¹) and Irgarol (ng Sn g⁻¹) normalized to 1% organic carbon in sediments collected in Vitoria Estuarine System. Table S2: Classification following Simpson and Bakker using data normalized for 1% considering the site above LQ

Site Code	TOC (%)	Normalized to 1% organic carbon					ANZEEC	Norway	
		TBTn	DBTn	MBTn	Diuron	Irgarol	TBT	Diuron	Irgarol
PA1	1.8	<1	<1	<1	<0.5	<0.4			
PA3	1.1	<1	<1	<1	<0.5	<0.4			
PA4	0.8	12.1	11.4	24.4	<1.4	2.7	SQGV		V
PA6	0.5	45.8	11.6	44.6	<0.5	3.0	SQGV		V
PA7	0.7	<5	<5	<5	<0.5	<1.2			
PA8	1.3	<1	<5	<5	<0.5	<0.4			
PA9	2.6	<5	<5	<5	<0.5	<0.4			
PA19	1.2	6.6	<5	10	5.9	<0.4		III	
MA1	1.6	<1	<1	<1	0.5	0.9			IV
MA2	1.4	<1	<1	<1	<1.4	<0.4			
MA3	2.0	<5	<1	<1	<0.5	<0.4			
MA4	1.3	<1	<1	<1	<0.5	<1.2			
MA5	1.1	<1	<1	<1	<0.5	<0.4			
MA6	0.8	<1	<1	<1	<0.5	2.4			IV
MA7	1.6	<1	<1	<1	<0.5	<0.4			
MA8	0.2	<1	<1	<1	8.5	8.5		IV	V
MA9	0.7	<1	<1	<1	<0.5	<0.4			
MA13	0.4	<5	16.8	17.8	<0.5	3.5			V
CE1	0.32	19.9	22.1	21.5	<1.4	<1.2	SQGV		
CE2	0.44	<1	<1	<1	<0.5	<0.4			
CE3	0.31	<1	<1	<1	<0.5	4.5			V
CE4	0.53	44.8	59.2	9.6	<1.4	3.0	SQGV		V
CE5	1.07	237.3	78.2	67.3	<0.5	<0.4	SQG-high value		
CE6	0.19	118.9	44.8	<5	<0.5	<0.4	SQG-high value		
PE1	3.14	<1	<1	<1	<0.5	0.6			IV
PE2	3.80	<1	<1	<1	<0.5	0.4			III
PE3	2.80	<1	<1	<1	<1.4	<1.2			
PE4	0.54	<1	<1	<1	<0.5	<0.4			
PE5	0.08	<1	<1	<1	<0.5	<0.4			
PE6	3.42	<1	2.8	6.2	<0.5	<0.4			
PE7	1.68	<1	<5	19.6	<0.5	<0.4			
PE8	3.03	<1	2.2	7.4	<0.5	0.5			III
PE9	2.93	<1	2.7	5.3	<0.5	<0.4			
PE10	2.83	<1	2.3	7.3	<0.5	<1.2			
PE11	2.57	<5	2.7	11.0	0.9	<0.4		III	
PE12	3.39	<5	2.4	8.4	0.4	<0.4			
PE13	2.88	<1	4.4	19.1	0.5	<0.4			
PE14	0.86	<1	<5	16.9	<0.5	1.6			IV
PE15	5.13	<1	1.3	1.8	<0.5	<0.4			
RJ1	2.60	9.6	4.5	4.8	<0.5	<0.4	SQG		
RJ2	1.48	8.9	6.4	5.4	<0.5	<0.4			
RJ3	2.60	9.9	4.9	4.1	<0.5	<0.4	SQG		
RJ4	3.57	1.8	0.7	7.6	<0.5	<0.4			
RJ5	0.06	<1	<1	<1	<1.4	<1.2			

RJ6	0.00	<1	<1	<1	<0.5	<1.2		
RJ7	0.03	<1	<1	<1	45.9	<0.4	V	
RJ8	0.01	<1	<1	<1	<0.5	<0.4		
SC1	1.25	<1	<1	<1	<0.5	<0.4		
SC2	1.05	112.1	25.0	41.9	<0.5	1.5	SQG-high value	IV
SC3	1.95	<1	<1	<1	<0.5	<0.4		
SC4	1.43	<1	<1	<1	<0.5	1.2		IV
SC5	1.43	4.5	6.0	4.6	<0.5	<0.4		
SC6	1.46	<1	<1	<1	1.2	<0.4	III	
SC7	0.93	<1	<1	<1	<0.5	<0.4		
SC8	1.93	<1	<1	<1	1.1	<1.2	III	
SC9	1.27	<1	<1	<1	<0.5	<0.4		
RG1	1.09	<5	5.7	16.4	<0.1	<0.1		
RG2	1.62	<1	<5	<1	<0.5	<0.5		
RG3	1.57	<1	<5	<5	<0.1	<0.1		
RG4	0.81	<1	<1	<1	<0.1	<0.1		
RG5	1.16	<1	<1	<1	<0.1	<0.5		
RG6	1.6	203.1	199	672	11.1	4.9	SQG-high value	IV V
RG7	0.99	<1	<1	<1	<0.5	<0.1		
RG8	1.09	503.7	1,148	3,220	4.5	<0.5	SQG-high value	III
RG9	1.25	<1	<5	<1	2.9	1.2	III	IV
RG10	0.81	<1	<1	<1	<0.1	<0.1		
RG11	0.88	6.5	26.7	110	<0.1	<0.1		
RG12	1.62	3.6	13.2	26.5	<0.1	<0.5		
RG13	0.44	<5	18.9	29.1	<0.5	<0.5		

SQGV: < 9 ng Sn g⁻¹ (normalized to 1% organic carbon) – It is induce deleterious effects

SQG-high: < 70 ng Sn g⁻¹ (normalized to 1% organic carbon) – It is high trigger value

Class III: Toxic effects following chronic exposure

Class IV: Toxic effects following short term exposure

Class V: Severe acute toxic effect

Table S2: Occurrence (ng g^{-1}) of antifouling paint particles (APPs) in sediments collected along the SSES and concentrations of diuron, Irgarol, DCOIT, dichlofluanid and BTs (ng g^{-1}) in the correspondent APPs.

Site Code	APPs ($\mu\text{g g}^{-1}$)	Diuron (ng g^{-1})	Irgarol (ng g^{-1})	Dichlofluanid (ng g^{-1})	DCOIT (ng g^{-1})	TBT (ng Sn g^{-1})	DBT (ng Sn g^{-1})	MBT (ng Sn g^{-1})
MA1	9.7	na	na	na	na	na	na	na
MA2	938	<50	<40	<15	12683	na	na	na
MA3	36.6	<50	<40	669	<20	na	na	na
MA4	3.2	n.a	n.a	n.a	n.a	na	na	na
MA5	0.01	n.a	n.a	n.a	n.a	na	na	na
MA6	9.3	n.a	n.a	n.a	n.a	na	na	na
MA7	59.4	<50	<40	<15	105	na	na	na
MA8	10.0	<50	<40	<15	<70	na	na	na
MA9	20.3	na	na	na	na	na	na	na
MA13	488	<50	<40	<15	<20	na	na	na
C1	26.1	<50	<40	<15	<20	na	na	na
C2	0.01	na	na	na	na	na	na	na
C3	19.1	<50	<40	<15	<20	na	na	na
C4	2.6	na	na	na	na	na	na	na
C5	62.7	<50	<40	<15	<20	na	na	na
C6	0.01	na	na	na	na	na	na	na
RJ1	4.0	na	na	na	na	na	na	na
RJ2	59.6	7,377	<40	<15	<20	na	na	na
RJ3	0.01	na	na	na	na	na	na	na
RJ4	117.4	<50	<40	<15	<20	na	na	na
RJ5	57.5	<50	<40	<15	<20	na	na	na
RJ6	98.3	<50	<40	<15	<20	na	na	na
RJ7	58.8	<50	<40	<15	<20	na	na	na
RJ8	48.4	<50	<40	<15	<20	na	na	na
SC1	24.8	<50	388	<15	105	na	na	na
SC2	23.2	<50	<40	<15	<20	na	na	na
SC3	4.3	<50	<40	<15	<20	na	na	na

SC4	25.4	na	na	na	na	na	na	na
SC5	47.4	<50	<120	<15	<20	na	na	na
SC6	4.1	<50	<40	<15	<20	na	na	na
SC7	6.7	na	na	na	na	na	na	na
SC8	14.2	<50	300	<15	<20	na	na	na
SC9	4.0	8,204	583	4,924	<20	na	na	na
RG1	840	na	na	na	na	6,600	4,300	6,610
RG2	<0.01	na	na	na	na	<250	<250	<250
RG3	<0.01	na	na	na	na	<250	<250	<250
RG4	<0.01	na	na	na	na	<250	<250	<250
RG5	<0.01	na	na	na	na	<250	<250	<250
RG6	44,300	na	na	na	na	311,474	1,526	735
RG7	<0.01	na	na	na	na	<250	<250	<250
RG8	1,176	na	na	na	na	42,308	1,138	<250
RG9	<0.01	na	na	na	na	<250	<250	<250
RG10	<0.01	na	na	na	na	<250	<250	<250
RG11	130	na	na	na	na	<250	<250	<250
RG12	350	na	na	na	na	<250	<250	<250
RG13	122	na	na	na	na	<250	<250	<250

n.a: not analysed due to limited availability of APPs.

ANEXOS

MATERIAL SUPLEMENTAR – ARTIGO 4

Table S1: Limits of detection (LD) and quantification (LQ) ($\mu\text{g Kg}^{-1}$) from previous studies (Abreu et al., 2020, 2021; Soroldoni et al., 2018; Abreu 2020)

Sediments from Biocide	Brazil coastal areas and Vitoria Estuarine System Abreu et al., 2021; Abreu 2020	Santos-São Vicente Estuarine System Abreu et al., 2020	Patos Lagoon Estuary Soroldoni et al., 2018
Irgarol	0.4 / 1.2	0.5 / 1.0	0.1 / 0.5
Diuron	0.5 / 1.4	0.5 / 1.0	0.1 / 0.5
Chlorothalonil	0.1 / 0.4	0.1 / 0.4	n.a
Dichlofluanid	0.7 / 2.1	0.7 / 2.1	n.a
DCOIT	0.2 / 0.7	0.2 / 0.7	0.5 / 1.5

n.a: not analysed

Table S2: Risk characterization for booster biocides in sediments along to Brazil coastal area.

L-M: Low to Moderate risk (<LQ)

ND: RQ not determined (environmental concentrations < LD/LQ)

*RQ have been calculated based on environmental concentrations below LQ (it is used ½ LQ)

Site	Risk Quotient (RQ)				
	DCOIT	Diuron	Chlorothalonil	Dichlofluanid	Irgarol
PA1	2.29	ND	0.01	0.16	ND
PA3	0.10	ND	0.01	0.06*	ND
PA4	0.10	4.67*	0.02*	0.22	50.00
PA6	0.10	ND	0.06	0.02	40.00
PA7	L-M*	ND	0.01	0.02	ND
PA8	0.10	ND	0.01	0.02	ND
PA9	2.39	ND	0.20	0.02	ND
PA19	6.75	46.00	0.01	0.02	ND
MA1	5.45	4.67*	0.01	0.02	35.00
MA2	9.32	4.67*	0.01	0.02	ND
MA3	0.10	ND*	0.20	0.02	ND
MA4	0.10	ND	0.01	0.17	ND
MA5	4.84	ND	0.39	0.02	ND
MA6	6.08	ND	0.39	0.02	47.50
MA7	0.10	ND	0.01	0.02	ND
MA8	0.10	11.33	0.01	0.16	42.50
MA9	0.10	ND	0.01	0.06*	ND
MA13	0.10	ND	0.01	0.02	35.00
CE1	9.90	4.67*	0.01	0.02	ND
CE2	0.10	ND	0.01	0.02	ND
CE3	0.10	ND	0.01	0.02	ND
CE4	0.10	4.67*	0.01	0.02	ND
CE5	8.87	ND	0.01	0.02	ND
CE6	L-M*	ND	0.01	0.02	ND
PE1	234.50	ND	0.01	0.02	45.00
PE2	0.10	ND	0.01	0.02	37.50
PE3	0.10	4.67*	0.01	0.02	ND
PE4	0.10	ND	0.01	0.02	ND
PE5	0.10	ND	0.01	0.31	ND
PE6	0.10	ND	0.01	0.27	ND
PE7	25.87	ND	0.01	0.02	ND
PE8	L-M*	ND	0.01	0.02	37.50
PE9	0.10	ND	0.01	0.02	ND
PE10	0.10	ND	0.09	0.02	ND
PE11	6.19	16.00	0.01	0.02	ND
PE12	0.10	10.00	0.01	0.22	ND

PE13	L-M*	10.00	0.01	0.02	ND
PE14	4.02	ND	0.17	0.02	35.00
PE15	0.10	ND	0.01	1.01	ND
ES1	L-M*	ND	0.01	0.02	ND
ES2	0.10	ND	0.01	0.02	ND
ES3	L-M*	ND	0.01	0.02	ND
ES4	0.10	4.67*	0.91	0.02	ND
ES5	3.71	4.67*	0.01	0.20	ND
ES6	65.57	4.67*	0.01	0.02	ND
ES7	3.61	ND	0.01	0.02	31.50
ES8	L-M*	ND	0.01	0.02	ND
ES9	4.54	ND	0.01	0.02	34.50
ES10	41.55	18.00	0.01	0.02	ND
ES11	0.10	4.67*	0.01	0.32	31.50
ES12	12.27	ND	0.01	0.37	ND
ES13	0.10	ND	0.06	0.06*	ND
ES14	0.10	ND	0.01	0.02	ND
RJ1	0.10	ND	0.01	0.02	ND
RJ2	6.54	ND	0.01	0.02	ND
RJ3	0.10	ND	0.01	0.02	ND
RJ4	0.10	ND	0.01	0.02	ND
RJ5	13.69	4.67*	0.01	0.02	ND
RJ6	8.74	ND	0.01	0.02	ND
RJ7	6.13	10.67	0.01	0.06*	ND
RJ8	L-M*	ND	0.01	0.02	ND
SP1	6.70	ND	0.51	0.02	ND
SP2	0.10	ND	0.01	0.02	ND
SP3	5.88	39.33	0.25	0.06*	ND
SP4	20.82	8.67	0.49	0.02	ND
SP5	12.58	ND	0.15	0.02	ND
SP6	11.65	ND	0.06	0.02	ND
SP7	5.57	ND	0.02*	0.06*	ND
SP8	65.67	ND	0.97	0.85	ND
SP9	8.76	ND	0.01	0.02	ND
SP10	0.10	ND	0.01	0.02	ND
SP11	7.94	ND	0.32	0.02	ND
SP12	L-M*	ND	0.21	0.02	ND
SP13	0.10	3.33*	0.06	0.06*	ND
SP14	8.76	3.33*	0.01	0.02	ND
SP15	1.03	ND	0.01	0.02	ND
SP16	1.24	3.33*	0.56	0.15	ND
SP17	9.69	10.67	0.02*	0.02	ND

SP18	3.30	15.33	0.01	0.02	ND
SP19	12.27	32.67	0.01	0.27	ND
SP20	17.84	66.00	0.22	0.02	ND
SP21	16.08	43.33	0.01	0.02	ND
SP22	76.91	48.67	0.01	0.02	ND
SP23	6.60	22.67	0.01	0.17	ND
SP24	28.25	38.67	0.01	0.02	ND
SP25	0.10	ND	0.01	0.02	ND
SP26	6.70	3.33*	0.02*	0.06*	ND
SP27	0.10	3.33*	0.02*	0.06*	ND
SP28	0.10	ND	0.01	0.06*	ND
SP29	2.78	3.33*	0.01	0.90	ND
SP30	0.10	3.33*	0.01	0.06*	ND
SC1	0.10	ND	0.01	0.02	ND
SC2	3.30	ND	0.01	0.02	40.70
SC3	0.10	ND	0.01	0.02	ND
SC4	64.16	ND	1.53	0.16	42.50
SC5	3.59	ND	0.01	0.02	ND
SC6	0.10	11.33	0.01	0.02	ND
SC7	L-M*	ND	0.01	0.02	ND
SC8	2.90	13.77	0.02*	0.13	ND
SC9	0.10	ND	0.01	0.02	ND
RG1	3.85	L-M	0.01	0.02	ND
RG2	3.01	ND	0.08	0.02	ND
RG3	L-M	L-M	0.01	0.14	ND
RG4	L-M	L-M	0.01	0.02	ND
RG5	L-M	L-M	0.01	0.02	ND
RG6	6.40	118.3	0.01	0.02	194.25
RG7	L-M	ND	0.79	0.02	ND
RG8	282.16	33.00	0.01	0.33	ND
RG9	L-M	24.53	0.02*	0.02	39.00
RG10	L-M*	L-M	0.01	0.02	ND
RG11	L-M*	L-M	0.01	0.02	ND
RG12	L-M*	L-M	0.01	0.02	ND
RG13	L-M*	ND	0.01	0.18	ND

