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CONTAMINAÇÃO POR PLÁSTICOS EM AMBIENTE DE MARISMA E SUA INTERAÇÃO COM O PROCESSO DE BIOINCRUSTAÇÃO

LARA MESQUITA PINHEIRO

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

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por

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Resumo

A contaminação por resíduos sólidos é um problema global principalmente devido ao uso de itens plásticos, atualmente categorizados de acordo com seu tamanho em macroplásticos (MAP), mesoplásticos (MEP) e microplásticos (MIP). Seu exacerbado consumo e persistência ambiental vêm causando problemas nos mais diversos ecossistemas, incluindo estuários. Outra problemática associada aos resíduos sólidos é que estes vêm sendo utilizados como substratos para o processo de bioincrustação. Apesar de se saber bem como esse processo ocorrem em superfícies naturais, ainda pouco se sabe dessa relação com resíduos sólidos como os plásticos. A comunidade bioincrustante em plásticos vem sendo chamada de Plastisfera, sendo que estudos nessa temática são escassos em ambientes estuarinos como marismas. Assim, o presente estudo teve como objetivo caracterizar a contaminação por plásticos em uma marisma e avaliar a sua interação com o processo de bioincrustação. Para tal, foi escolhida a Marisma do Molhe Oeste, no Estuário da Lagoa dos Patos (ELP), que apresenta zonação bem definida pela vegetação e taxa de alagamento. As hipóteses testadas foram: (*i*) a Marisma do Molhe Oeste está contaminada por resíduos sólidos, predominantemente plásticos, com variação negativa de distribuição em relação à taxa de alagamento, e (*ii*) a bioincrustação na marisma é afetada por diferentes características dos resíduos sólidos e pela zonação. Os níveis de contaminação por resíduos sólidos atingiram 5,35 ± 6,02 itens m^{-2} (maioria plásticos), 8,89 ± 8,75 MIPs L^{-1} em água, 279,63 ± 410,12 MEPs e MIPs kg^{-1} de sedimento seco superficial, e 366,92 ± 975,18 MEPs e MIPs kg^{-1} de sedimento seco ao longo da coluna sedimentar. Quantidades significativamente maiores de resíduos foram vistas nas zonas mais secas da marisma do que em relação às zonas mais alagadas para MAPs, MEPs e MIPs, o que confirma a primeira hipótese. Isso pode ser explicado por uma combinação de fatores como anta entrada de material (principalmente de origem doméstica, de pesca ou portuária), o papel de aprisionamento da vegetação nessa zona, e a hidrodinâmica do ELP. Foram encontrados 13 grupos de macro-organismos associados aos resíduos sólidos, além de micro-organismos (bactérias, fungos e microalgas) em MEPs e MIPs. Os organismos demonstraram ocorrência variadas nas zonas da marisma e características do substrato, o que pode estar associado aos diferentes níveis de resistência a variações de disponibilidade de água e luz e à comunidade previamente estabelecida, sendo então a segunda hipótese confirmada. Futuros estudos temporais de monitoramento dessa contaminação e de investigação mais profunda da comunidade epiplástica (i.e. Plastisfera) são necessários para entender suas consequências para os ecossistemas de marismas.

Palavras-Chave: Lagoa dos Patos, Plastisfera, Sedimento, Zonação.

Abstract

Contamination by solid waste is a global problem mainly due to the use of plastic items, currently categorized according to their size into macroplastics (MAP), mesoplastics (MEP) and microplastics (MIP). Its exacerbated consumption and environmental persistence have been causing problems in the most diverse ecosystems, including estuaries. Another problem associated with solid waste is that it has been used as substrates for the biofouling process. Although it is well known how this process occurs on natural surfaces, less is known about this relationship with solid waste such as plastics. The biofouling community in plastics has been called Plastisphere, and studies on this subject are scarce in estuarine environments such as salt marshes. Thus, the present study aimed to characterize the contamination by plastics in a salt marsh and evaluate its interaction with the biofouling process. The Molhe Oeste salt marsh, located at the Patos Lagoon Estuary (PLE), was chosen due to its well-defined zonation according to vegetation and flooding rate. The hypotheses tested were: (*i*) the Marisma do Molhe Oeste is contaminated by solid waste, predominantly plastic, with a negative variation in distribution in relation to the rate of flooding, and (*ii*) biofouling in the marsh is affected by different characteristics of solid waste and by zoning. The levels of contamination by solid waste reached 5.35 ± 6.02 items m^{-2} (mostly plastic), 8.89 ± 8.75 MIPs L^{-1} in water, 279.63 ± 410.12 MEPs and MIPs kg^{-1} of dry surface sediment, and 366.92 ± 975.18 MEPs and MIPs kg^{-1} of dry sediment along the sedimentary column. Significantly greater amounts of residues were seen in the drier areas of the marsh than in the more wetted areas for MAPs, MEPs, and MIPs, which confirms the first hypothesis. This can be explained by a combination of factors such as high material input (mainly of domestic, fishing or port origin), the role of vegetation entrapment in this zone, and the hydrodynamics of the PLE. Thirteen groups of macro-organisms associated with solid waste were found, in addition to microorganisms (bacteria, fungi and microalgae) in MEPs and MIPs. The organisms showed varied occurrence in the salt marsh areas and substrate characteristics, which may be associated with the different levels of resistance to variations in water and light availability and to the previously established community, so the second hypothesis was confirmed. Fture temporal studies of monitoring this contamination and deeper investigation of the epiplastic community (i.e. Plastisphere) are necessary to understand its consequences for the marsh ecosystems.

Keywords: Patos Lagoon, Plastisphere, Sediment, Zonation.

Capítulo I: Introdução

A presença humana vem causando inúmeras perturbações na Terra ao longo do tempo. As atividades de origem antropogênica estão intrinsecamente ligadas à geração de impactos no ambiente, principalmente desde o final do século XIX após a Revolução Industrial [Sayadi *et al.* 2009]. Um exemplo amplamente difundido de impacto antropogênico é a emissão exacerbada de dióxido de carbono (CO_2) para a atmosfera terrestre que, por sua vez, vem desencadeando efeitos a longo prazo como alterações climáticas em níveis globais [Naidu *et al.* 2021]. Entretanto, a degradação ambiental também pode estar diretamente associada à quantidade exorbitante de produtos químicos nocivos que são produzidos e utilizados em atividades humanas diariamente. Mais de 140 milhões de substâncias e misturas químicas tem registro na Associação Americana de Química (ACS), e uma análise recente de inventários nacionais e regionais de substâncias químicas feita por Wang *et al.* [2020] mostrou que mais de 350.000 substâncias já foram registradas para produção e uso no mundo. Ao considerar as taxas de produção e diversificação das substâncias químicas sintéticas, esse tipo de impacto já se estabeleceu como um agente de mudança global [Bernhardt *et al*. 2017].

Apesar dos inegáveis benefícios das substâncias químicas para a vida humana, o uso e descarte indiscriminado desses compostos ocasiona a sua atualmente inevitável liberação para o ambiente. Atividades como mineração, agricultura, construção civil e produção de energia descarregam anualmente trilhões de materiais quimicamente ativos no ambiente [Cribb 2021]. Visto que todo material presente no planeta acabar por fazer parte dos ciclos biogeoquímicos, essas substâncias químicas têm sido identificadas nos registros geológicos e têm sido interpretadas como marcos da presença humana na Terra [Waters *et al*. 2016]. Assim, existe a proposta de criação de uma nova época geológica, o Antropoceno, na qual a humanidade estaria inserida e teria inegáveis impactos na estrutura do planeta [Crutzen 2016, Zalasiewicz *et al.* 2016].

Alguns dos grupos de substâncias químicas que despertam preocupação ambiental devido à sua ubiquidade e efeitos deletérios são os metais e metaloides [Nag *et al.* 2022], hidrocarbonetos [Fernandes *et al*. 2022], pesticidas e retardantes de chama [Michałowicz *et al*. 2022], e resíduos sólidos. Resíduos sólidos são considerados um dos principais grupos de contaminantes de ambientes naturais, [Akhtar *et al*. 2021]. Segundo a Legislação Brasileira, resíduos sólidos são definidos como quaisquer "*material, substância, objeto ou bem descartado resultante de atividades humanas em sociedade, a cuja destinação final se procede, se propõe proceder ou se está obrigado a proceder, nos estados sólido ou semissólido, bem como gases contidos em recipientes e líquidos cujas particularidades tornem inviável o seu lançamento na rede pública de esgotos ou em corpos d'água, ou exijam para isso soluções técnica ou economicamente inviáveis em face da melhor tecnologia disponível*" [Brasil, 2010]. Esses materiais podem ser provenientes das mais diversas atividades como domésticas, hospitalares, industriais, pesqueiras, agrícolas, urbanas, dentre outras. Resíduos sólidos são considerados atualmente um dos maiores problemas na humanidade, sendo que seus impactos vêm sendo amplamente estudados em ambiente aquáticos.

Dada a diversidade de materiais que são utilizados na sociedade para diversas finalidades, consegue-se encontrar resíduos sólidos feitos de plástico, vidro, metal, papel/papelão, resíduos orgânicos, dentre outros. Os resíduos plásticos são os encontrados em maior quantidade nos ambientes aquáticos, podendo corresponder a até mais de 95% da composição dos resíduos em algumas regiões do mundo [Galgani *et al.* 2015; Lechthaler *et al.* 2020].

Plásticos são materiais sintéticos feitos principalmente a partir de petróleo ou gás natural e que apresentam estrutura química de uma cadeia polimérica de hidrocarbonetos, que se diferenciam de acordo com o tipo de polímero (Figura 1). É um material amplamente versátil devido à sua ampla gama de propriedades interessantes como maleabilidade, baixa densidade, baixa condutividade elétrica e térmica, resistência à temperatura e à corrosão, durabilidade, baixo custo de produção, dentre outras, a depender dos tipos de polímeros e dos aditivos que podem ser utilizados durante a sua produção [Andrady *et al.* 2011, Lechthaler *et al.* 2020].

Nome do polímero	Fórmula química	Exemplos de usos	Código de reciclagem
Polietileno tereftalato (PET)	$(C_{10}H_8O_4)_n$	Garrafas, fibras têxteis, lâminas	1
Polietileno de alta densidade (PEAD)	$(C_2H_4)_n$	Uso industrial, brinquedos, utensílios domésticos, armazenamento	2
Policloreto de vinila (PVC)	$(C_2H_3Cl)_n$	Uso na construção civil, fraldas, mangueiras, revestimento de fios elétricos	3
Polietileno de baixa densidade (PEBD)	$(C_2H_4)_n$	Sacolas e embalagens plásticas flexíveis	4
Polipropileno (PP)	$(C_3H_6)_n$	Sacos para grãos e cereais, cadeiras, brinquedos, copos	5
Poliestireno (PS)	$(C_8H_8)_n$	Cosméticos, copos, escovas de dentes, uso na construção civil	6
Outros: poliuretano (PU), poliamida (PA), policarbonato (PC), ...	várias	Esponjas, coberturas, isolantes térmicos e acústicos, cordas e redes	7

Figura 1. Principais tipos de produtos plásticos utilizados atualmente, com suas respectivas fórmulas químicas, usos comuns e numeração de código de reciclagem.

A utilização de itens plásticos iniciou com a criação do primeiro polímero sintético em 1907, o Bakelite. Entretanto, a popularização e produção em massa dos polímeros sintéticos se deu apenas a partir dos anos 1950, sendo que a produção anual aumentou cerca de 200 vezes de 1950 até 2015, quando chegou à marca de 381 milhões de toneladas produzidas em um ano [Geyer *et al*. 2017]. Dados mais recentes mostraram que em 2020 a produção mundial foi de 367 milhões de toneladas, sem considerar a produção de plásticos reciclados [PlasticsEurope, 2021]. A Figura 1 traz os principais tipo de plásticos que são produzidos atualmente no mundo, com suas respectivas estruturas químicas e usos comuns na sociedade.

Ao longo dos anos, a visão dos plásticos como um material revolucionário e benéfico foi dando lugar às problemáticas associadas à produção e utilização exacerbada desse material. Primeiramente, sabe-se que a grande maioria dos itens plásticos produzidos no mundo são itens de uso único, i.e., tem vida útil extremamente curta por serem feitos para ser usados uma vez só [UNEP 2022]. Dos 8.300 milhões de toneladas de plástico já produzidos no mundo até 2015, 6.300 milhões de toneladas se tornaram resíduos sólidos. Destes, apenas 9% foram reciclados, 12% foram incinerados, e os 79% restantes permanecem acumulando em aterros ou no ambiente [Geyer *et al.* 2017]. Alguns autores já apresentaram estimativas referentes às quantidades de resíduos plásticos que entram nos oceanos a cada ano. Jambeck *et al.* [2015] estimaram valores entre 4,8 e 12,7 milhões de toneladas por ano entrando no oceano através de áreas costeiras, já Lebreton *et al.* [2017] mostraram valores médios de 1,15 a 2,41 milhões de toneladas por ano vindos apenas de rios, enquanto Ryberg *et al.* [2019] falam em 9,2 milhões de toneladas de resíduos plásticos por ano sendo perdidos para o ambiente em geral. Outros números mundiais mostram que cerca de um milhão de garrafas plásticas são compradas a cada minuto enquanto 5 trilhões de sacolas plásticas são usadas a cada ano [UNEP 2022]. De qualquer forma, tem-se uma enorme pressão ambiental relacionada à contaminação plástica em ambientes naturais, o que ressalta a importância de estudos nessa temática.

Lechthaler *et al.* [2020] indicou que a produção de plásticos mundial se dá principalmente para seu uso em embalagens (39,9%), em construções (19,8%), na indústria automotiva (9,9%), em eletrônicos (6,2%), em itens de uso doméstico e de lazer (4,1%), na agricultura (3,4%), dentre outros. Essa variedade de usos implicam que, além de diferentes tipos de polímeros sendo produzidos, os plásticos podem se apresentar em diferentes formatos, cores, texturas, tamanhos, dentre outras características. Para fins de monitoramento ambiental, os estudiosos do assunto vêm utilizando nomenclaturas baseadas em diferentes faixas de tamanho que estão descritas na Tabela 1. Essas diferentes categorias vêm sendo cada vez mais investigadas de forma separada, sendo que microplásticos têm ganhado bastante atenção principalmente a partir de 2004, quando esse termo foi usado pela primeira vez por Thompson *et al.* [2004]. Plásticos de todos os tamanhos já vêm sendo considerados "*poluentes de importância e um agente de mudanças globais*" [Bernhardt *et al.* 2017, Souza Machado *et al*. 2018, Rillig *et al.* 2021], visto que as quantidades encontradas na natureza se encontram em tendência crescente e esse cenário deve se manter no futuro [Brandon *et al.* 2019, Isobe *et al.* 2019]. Além disso, esses contaminantes são conhecidos por sua persistência ambiental e consequências para diversos ecossistemas aquáticos, biota e até para a saúde humana [Karbalaei *et al.* 2018].

Tabela 1. Categorias de tamanhos de plástico para fins de monitoramento ambiental propostas na literatura. NI = não informado.

Nomenclatura	**Macroplástico (MAP)**	**Mesoplástico (MEP)**	**Microplástico (MIP)**	**Nanoplástico (NP)**	**Ref.***
Variação de tamanho	> 20 cm	20 – 5 cm	5 mm – 1 µm	< 1000 nm	1
	NI	NI	5 mm – 1 µm	1000 nm – 1 nm	2, 3
	NI	5 – 10 mm	5 – 0,2 mm	NI	4
	1 m – 2,5 cm	2,5 cm – 5 mm	5 – 0,1 mm	NI	5
	> 200 mm	4,76 – 200 mm	0,33 – 4,75 mm	NI	6

***Referências:** **1** Hanvey *et al.* [2017]; **2** Frias & Nash [2019]; **3** Gigault *et al.* [2018]; **4** Collignon *et al.* [2014]; **5** Young & Elliot [2016]; **6** Eriksen *et al.* [2014].

De acordo com o *Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection* (GESAMP), microplásticos podem ser de fonte primária, i.e., já são fabricados nessa faixa de tamanho para desempenhar uma função específica, como *pellets* usados como matéria prima de itens plásticos maiores e microesferas abrasivas presentes em produtos de higiene pessoal [GESAMP 2019]. Já outros microplásticos podem ser de origem secundária, originando-se da quebra ou desgaste de objetos plásticos maiores devido ao seu uso ou por intempéries no ambiente, como por exemplo fibras de tecidos sintéticos, partículas de tinta, fragmentos, fibras de pneus, dentre outros [GESAMP 2019].

Diversos estudos verificaram a interação de organismos marinhos com plásticos de todos os tamanhos como mamíferos [ex.: Moore *et al.* 2009, Plaza & Lambertucci 2017], aves [ex.: Kühn *et al.* 2015], tartarugas [ex.: Tomás *et al.* 2002, Nelms *et al.* 2016], peixes ósseos [ex.: Ferreira *et al.* 2019, Neto *et al.* 2020] e cartilaginosos [ex.: Cliff *et al.* 2002, Parton *et al.* 2020], e diversos grupos de invertebrados: ouriços-do-mar [ex.: Nobre *et al.* 2015], caranguejos [ex.: Watts *et al.* 2015, 2016], cefalópodes [ex.: Matsuoka *et al.* 2005, Freitas *et al.* 2022], mexilhões [ex.: Li *et al.* 2019], cracas [ex.: Goldstein & Goodwin 2013], dentre outros [ver Pinheiro *et al.* 2020].

Essas interações frequentemente se dão na forma de emaranhamento, quando animais ficam presos em resíduos disponíveis na água, ou através da ingestão ou inalação de itens plásticos. Ambas as situações têm consequências diretas para o desenvolvimento e até a sobrevivência desses organismos, sendo que diversos estudos verificaram diversos efeitos biológicos para esses organismos, a maioria utilizando experimentos controlados de laboratório [de Sá *et al.* 2018]. Alguns efeitos reportados relacionados à captação de plásticos foram reduções nas taxas de alimentação e no metabolismo [Wright *et al.* 2013, Green *et al.* 2016], respostas imunológicas [Avio *et al.* 2015], e estresse oxidativo [Canesi *et al.* 2015], além de efeitos físicos como aprisionamento, obstrução do trato digestivo e/ou respiratório, fecalomas e machucados [Kühn *et al.* 2015, Rizzi *et al.* 2019]. Além disso, substâncias adicionadas à estrutura polimérica durante a produção dos plásticos ou que são adsorvidas à sua superfície no ambiente também podem causar efeitos tóxicos para organismos aquáticos [Anbumani & Kakkar 2018, Celis-Hernandez *et al.* 2021]. Alguns exemplos de substâncias associadas aos plásticos que já tiveram toxicidade identificada são fármacos e produtos de cuidados pessoais [Wu *et al.* 2016, Guo & Wang 2019, Zhou *et al.* 2019], compostos orgânicos [Bakir *et al.* 2014], e metais [Holmes *et al.* 2014, Turner *et al.* 2015, Turner 2016]. Além desses efeitos diretos para organismos, a contaminação por plásticos também acarreta problemas de saúde pública e perdas na biodiversidade, além de reduzir valores estéticos associados aos ambientes aquáticos naturais, causar danos à navegação e consequentemente causar perdas econômicas [Horton *et al.* 2017]. Ademais, a poluição ambiental por plásticos tem ligação direta com as mudanças climáticas já que estas podem aumentar a propagação desse tipo de poluição [Ford *et al.* 2022]. Ainda, o ciclo de vida desses produtos contribui para a emissão de gases de efeito estufa, além das espécies aquáticas estarem concomitantemente suscetíveis a esses dois grandes problemas ambientais [Ford *et al.* 2022].

Outra situação de interação dos plásticos no ambiente aquático se dá por meio da incrustação das superfícies desses itens por organismos chamados de bioincrustantes, formando uma comunidade biológica epiplástica que atualmente é chamada de Plastisfera. Esse termo foi usado pioneiramente por Zettler *et al.* [2013] para designar a comunidade de micro-organismos que se estabelecem em plásticos em ambientes marinhos. No presente trabalho, é proposta a utilização do termo de forma mais ampla, incluindo organismos de todos os tamanhos (macro- e micro-organismos) e em todos os tipos de ambientes aquáticos.

A bioincrustação pode ser definida como a associação direta ou indireta de organismos a substratos consolidados uma vez que estes são expostos ao ambiente aquático [Agostini *et al.* 2018, 2019]. Assim, qualquer resíduo sólido além do plástico está sujeito à bioincrustação. As etapas do processo foram descritas por Kerr e Cowling [2003], e se iniciam logo quando a superfície a ser colonizada entra em contato com a água. Em um primeiro momento, ocorre a sorção (adsorção e absorção) de moléculas, íons e nutrientes na superfície do substrato, formando um filme condicionante. Esse filme se torna atrativo tanto para micro-organismos que usam o substrato em si como fonte de carbono quanto para os que apenas utilizam os nutrientes sorvidos. Após essa fase inicial, a colonização bacteriana se estabelece de forma mais intensa até que as células bacterianas iniciam a produção da matriz de substâncias poliméricas extracelulares (MSPE), que envolve as células e as colônias em formação, dando origem a uma estrutura em multicamadas chamada de biofilme bacteriano. De acordo com Flemming *et al.* [2016], cada biofilme possui características individuais que lhes conferem proteção contra predação, agentes químicos externos (ex.: antibióticos), agentes físicos externos (ex.: dessecação), além de conferir alta capacidade passiva de sorção de nutrientes e gases através da MSPE, o que aumenta a disponibilidade dessas substâncias exógenas para as células do biofilme.

Após o estabelecimento da colonização primária, o biofilme bacteriano atrai outros organismos chamados de colonizadores secundários como vírus, microalgas (ex.: cianobactérias, diatomáceas), protozoários (ex.: ciliados, flagelados) e fungos

[Quero & Luna 2017]. Em estágios mais avançados, colonizadores terciários como larvas de invertebrados (ex.: cracas, esponjas) e urocordados (ex.: ascídias) podem se assentar no substrato [Astudillo *et al.* 2009], além de organismos vágeis que também poderão utilizar o substrato como nicho ecológico como anfípodes, poliquetas, isópodes, ácaros, caranguejos e insetos [Agostini *et al.* 2018].

Já se têm evidências de que diversos fatores têm influência no processo de bioincrustação, que podem estar relacionados às condições ambientais, às características do substrato, ou às interações ecológicas acontecendo na superfície em colonização. Por exemplo, viu-se que a colonização bacteriana é diferenciada a depender de condições ambientais de salinidade e concentração de nutrientes [Oberbeckmann *et al.* 2018]. Outras evidências mostram a formação de comunidades bacterianas distintas em plásticos em diferentes estações do ano, o que pode estar relacionado a diferenças sazonais de temperatura e concentração de oxigênio na água [Oberbeckmann *et al.* 2014]. Um estudo realizado em plásticos coletados na costa sul do Brasil observou diferenças significantes na composição da comunidade epiplástica coletada em duas áreas diferentes, o que foi atribuído possivelmente a variações de temperatura, pluviosidade e produtividade entre as regiões [Lacerda *et al.* 2021].

Já se tem evidências de que o tipo de material do qual o substrato é feito também altera a colonização, apesar de diferentes trabalhos mostrarem resultados contrastantes. Por exemplo, Ogonowski *et al.* [2018] verificaram que as comunidades epiplásticas em polietileno, polipropileno e poliestireno se diferenciavam de outros substratos como celulose e vidro, o que foi atribuído aos diferentes graus de hidrofobicidade dos materiais. Outro fator já investigado foi a textura, que parece ser importante em estágios mais iniciais da colonização [Bravo *et al.* 2011]. Substratos com diferentes cores também podem ter colonização bacteriana inicial afetada, como mostrado por Dobretsov *et al.* [2013]. Todos esses estudos foram mais focados em condições marinhas, sendo que ambientes de água doce ou de transição permanecem pouco investigados.

Um exemplo importante de investigação nessa área foi realizado na região sul do Brasil por Sousa [2022], buscando analisar diferenças na composição da Plastisfera em diferentes tipos de polímeros (PE e PP) e estações do ano no Estuário da Lagoa dos Patos (Rio Grande, RS). Os plásticos expostos por um ano ao ambiente estuarino apresentaram diferenças significativas de riqueza de eucariotos entre tipos de polímeros, e de composição das comunidades epiplásticas tanto para eucariotos quanto para procariotos entre estações do ano. Além disso, foram encontrados organismos potencialmente patogênicos e degradadores de hidrocarbonetos, o que ressalta a importância da investigação dessas comunidades nos mais variados ambientes para entender e até prever potenciais consequências ecológicas da utilização desses substratos principalmente considerando a diversidade de resíduos plásticos disponíveis para colonização e a sua ubíqua presença ambiental.

Tratando ainda da ubiquidade ambiental da contaminação plástica, existem relatos em regiões costeiras como praias arenosas [ex.: Pinheiro *et al.* 2019, de Ramos *et al.* 2021] e costões rochosos [ex.: McWilliams *et al.* 2018, Weideman *et al.* 2020], em plataformas continentais [ex.: Martin *et al.* 2017, Gerigny *et al.* 2019], no fundo oceânico [ex.: Perkins 2014, Barrett *et al.* 2020], em águas superficiais [ex.: Lima *et al.* 2021, Lacerda *et al.* 2022], e em ilhas oceânicas [ex.: Andrades *et al*. 2018, Monteiro *et al.* 2020]. Apesar da constante atenção para o oceano, há também disponível na literatura artigos científicos que demonstraram contaminação por plásticos em outros ambientes como o terrestre [ex.: Fuller & Gautam 2016, Huerta Lwanga *et al.* 2016], o ar [ex.: Dris *et al.* 2016, Beaurepaire *et al.* 2021], os polos [ex.: Obbard *et al.* 2014, Kelly *et al.* 2020], os rios [ex.: Mai *et al.* 2020], as lagoas [ex.: Chico-Ortiz *et al.* 2020], e os estuários [ex.: Lorenzi *et al.* 2020].

Estuários são zonas costeiras de transição entre o ambiente terrestre e o marinho, que apresentam características únicas devido ao fluxo bidirecional de água doce vinda do continente e de água salgada vinda do oceano. Esse encontro de ‘águas com propriedades físico-químicas diferentes criam uma espécie de barreira para diversos tipos de contaminantes, como por exemplo os plásticos, que podem acumular na região interna do estuário em situações de maior fluxo vindo do oceano ou serem exportados para fora do estuário quando o fluxo de água doce consegue quebrar essa barreira [Lebreton *et al.* 2017, Lima *et al.* 2014]. Assim, pode-se dizer que estuários atuam como vias de exportação de plásticos oriundos do ambiente terrestre [Lima *et al.* 2020], e que resíduos plásticos são, portanto, de natureza transfronteiriça em ambientes aquáticos [Krelling *et al.* 2017]. Mai *et al.* [2020] argumentam que de 57 a 265 mil toneladas entraram nos oceanos através de rios no ano de 2018, sendo que essas quantidades podem representar 90% do input de resíduos plásticos do continente para o oceano [Lima *et al.* 2020, Santos 2021].

Dentro dos estuários, destacam-se as marismas. Marismas são definidas como ambientes entremarés que ocorrem em regiões de médias e altas latitudes e apresentam cobertura por vegetação halófita, i.e., que são tolerantes a grandes variações de salinidade [Seeliger 1992, NOAA 2018]. Devido a essa ampla variação de salinidade, taxas de alagamento e consequentemente de composição da vegetação, esses ambientes podem formar uma estrutura em zonas distintas dentro de uma mesma marisma [Costa *et al.* 2013]. As espécies vegetais presentes podem formar uma extensa e densa cobertura [Nieva *et al.* 2001], que pode servir de armadilha para aprisionamento de sedimento e outros materiais em suspensão quando consideradas também as variações no nível da água [Kakeh *et al.* 2016, Stead *et al.* 2020]. Além disso, marismas são ambientes de importância ecológica elevada pelo seu papel desenvolvido na proteção costeira contra erosão [Shepard *et al.* 2011], na produtividade primária [Jinks *et al.* 2020], na ciclagem de nutrientes [Tagliani *et al.* 2003], e no sequestro e armazenamento de carbono antropogênico [McLeod *et al.* 2011], sendo atualmente consideradas ambientes de carbono azul [Duarte *et al.* 2013].

Os impactos antropogênicos em ambientes de marismas vêm sendo relatados há algum tempo [Fraser *et al.* 2020], porém apenas alguns estudos investigaram a contaminação ambiental por resíduos sólidos nesses locais, sendo todos localizados no hemisfério norte. Três estudos foram realizados nos Estados Unidos [Gilligan *et al.* 1992, Uhrin & Schellinger 2011, Viehman *et al.* 2011], e um na Espanha [Masarraza *et al.* 2019]. Nenhum desses estudos consideraram a presença da Plastisfera nesses ambientes ou as possíveis consequências ecológicas da formação dessa comunidade, o que representa então uma grande lacuna científica dentro dessa temática.

Capítulo II: Hipóteses

O presente trabalho de Tese é norteado por duas hipóteses principais:

(i) A Marisma do Molhe Oeste, Estuário da Lagoa dos Patos, RS, Brasil está contaminada por resíduos sólidos, predominantemente por plásticos, com variação negativa de distribuição em relação à taxa de alagamento da Marisma.

(ii) A bioincrustação na Marisma do Molhe Oeste é afetada quali- e quantitativamente por diferentes características dos resíduos sólidos como tamanho, cor e tipo de polímero, e pela zonação da marisma.

Capítulo III: Objetivos

Este trabalho tem como objetivo geral caracterizar a contaminação por plásticos em uma marisma do Estuário da Lagoa dos Patos e avaliar a sua interação com o processo de bioincrustação. Como objetivos específicos, tem-se:

(i) Investigar, a partir de um estudo de revisão, o status da contaminação/poluição por plásticos em ambientes estuarinos e sua relação com a bioincrustação, outros contaminantes e a sua toxicidade;

(ii) Caracterizar a distribuição de resíduos sólidos em um ambiente de marisma e a sua relação com o processo de bioincrustação, bem como a influência das taxas de alagamento e circulação estuarina nesses processos;

(iii) Caracterizar a distribuição de meso- e microplásticos em sedimento (superfície e coluna sedimentar) e em água de um ambiente de marisma e sua relação com a bioincrustação;

(iv) Investigar a interação entre plásticos e a bioincrustação em ambiente de marisma, avaliando a influência de diferentes características dos polímeros.

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Capítulo IV: Área de estudo

A Lagoa dos Patos (LP) está inserida na planície costeira do estado do Rio Grande do Sul, na região sul do Brasil, entre 30º e 32º S. É a maior laguna estrangulada do mundo e a maior laguna costeira da América do Sul [Kjerfve 1986], cobrindo uma área de pouco mais de 10.000 km^2 [Fernandes *et al.* 2002]. A bacia de drenagem na qual a laguna está inserida se estende por uma área de aproximadamente 200.000 km^2, que abrange aproximadamente 260 municípios, incluindo a capital do estado do Rio Grande do Sul, Porto Alegre, que também é a mais populosa do estado com mais de 1,4 milhões de habitantes [IBGE, 2010].

A laguna recebe contribuição hídrica de diversos corpos d'água, sendo os principais o Rio Guaíba, o Rio Camaquã e a Lagoa Mirim através do Canal de São Gonçalo, que contribuem com médias de descarga de 1.500, 300 e 700 $m^3\ s^{-1}$ mensais respectivamente [Vaz *et al.* 2006]. A Lagoa dos Patos tem uma média anual de volume de descarga de água doce de 2.000 $m^3\ s^{-1}$, podendo chegar até 12.000 $m^3\ s^{-1}$ durante eventos relacionados ao El Niño - Oscilação Sul (ENSO) [Fernandes *et al.* 2002, Marques *et al.* 2014]. O clima na região da laguna abrange regiões subtropicais e temperadas quentes, com temperatura anual média de 18 ºC [Abreu *et al.* 2017]. O regime de precipitação pluviométrica na região é de grandes volumes anuais, com média anual variando de 1.250 a 2.000 mm, com forte influências de eventos ENSO e anomalias de precipitação, que ocasionam picos de vazão fluvial [Grimm *et al.* 1998]. Além disso, a região apresenta um complexo padrão de fluxos de tributários da bacia de drenagem, o que forma um sistema altamente dinâmico e variável [Asmus 1998]. A Lagoa dos Patos apresenta na sua porção mais ao sul uma região estuarina estrangulada que se conecta ao Oceano Atlântico por meio de um canal de < 1 km de largura e se estende laguna adentro até a Ponta da Feitoria (Figura 2). O Estuário da Lagoa dos Patos (ELP) representa cerca de 10% da área total da laguna, e abrange as cidades de Rio Grande (212.881 habitantes) e Pelotas (343.826 habitantes) na sua margem oeste, e de São José do Norte (27.866 habitantes) na sua margem leste.

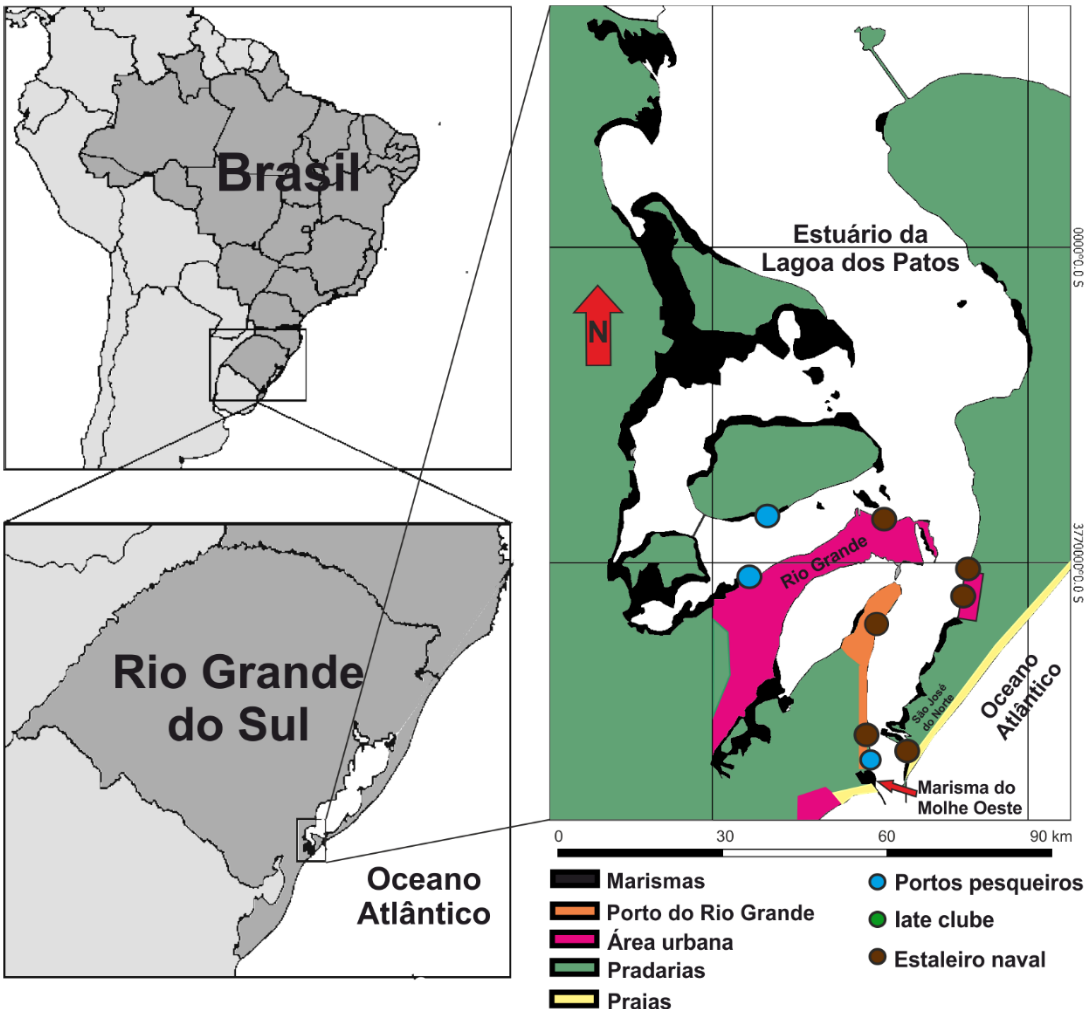

Figura 2. Mapa da localização do Estuário da Lagoa dos Patos, com indicação da localização da Marisma do Molhe Oeste (32° 09' 09,3" S 52° 06' 03,1" O).

A hidrodinâmica do estuário relacionada sobretudo ao balanço entre entrada e saída de água salina do oceano para o estuário é governado por forças locais e não locais associadas principalmente com o regime de ventos, força da descarga fluvial e equilíbrio precipitação/evaporação [Möller *et al.* 2001, Castelão & Möller 2003]. Em ocasiões de vento nordeste, que ocorre principalmente no final do inverno e na primavera, o regime de vazante é favorecido no estuário devido à alta descarga de água doce. Em situações de vento de quadrante sul a intrusão de água salina se intensifica e a entrada de água doce no estuário diminui, favorecendo o regime de enchente, ocorrendo durante as estações do outono e inverno [Möller *et al*. 2001]. Assim, a salinidade no ELP varia consideravelmente (de 0 a 35) ao longo do ano, com os menores valores na primavera e os maiores no verão [Vaz *et al.* 2006], sendo que os três tipos de estruturas verticais de salinidade propostos por Cameron & Pritchard [1963] podem ocorrer no ELP (cunha salina, parcialmente estratificado, bem misturado) a depender da descarga fluvial e da ação dos ventos [Möller & Castaing 1999].

Diversas atividades antropogênicas foram se desenvolvendo ao longo dos anos nas margens do ELP como a expansão de centros urbanos, instalação de indústrias, atividades agropecuárias e a construção e expansão do segundo maior porto do Brasil, o Porto de Rio Grande, localizado na cidade de Rio Grande. Essas atividades vêm causando impactos diretos nos ecossistemas presentes no estuário como as marismas. Um estudo recente estimou, a partir de dados socioeconômicos, que a produção de plástico na bacia hidrográfica da Lagoa dos Patos é principalmente ligada a produtos como garrafas, sacolas e embalagens plásticas, que foi de 4,54 milhões de toneladas de 2010 a 2017, sendo que a entrada de resíduos plásticos para a laguna foi estimada entre 0,5 e 3,2 gramas por pessoa por dia [Santos 2021].

O ELP apresenta 24 unidades de marismas em seu território que juntas representam uma área de 69,84 km^2, dentre marismas de margem e de ilha [Costa *et al.* 1997, Marangoni & Costa 2009]. Esses ambientes vêm sofrendo variações em sua extensão devido a erosão causada pela ação de ventos NO-SO na margem leste (São José do Norte) e ventos NE-NE na margem oeste (Rio Grande), mas a área total se manteve quase inalterada ao longo de meio século [Costa *et al.* 1997, Marangoni & Costa 2009]. Ainda, as marismas realizam trocas de contaminantes, nutrientes, matéria orgânica dissolvida e particulada com o estuário, associadas principalmente à produção primária que varia de 669 a 4.700 g peso seco m^{-2} ano^{-1} [Silva *et al.* 1993, Cunha 1994, Gaona *et al.* 1996, Seeliger *et al.* 1997].

As marismas podem apresentar uma zonação paralela à costa que pode ser definida pela vegetação e pelas taxas de alagamento que sofrem ao longo da sua extensão. A zona mais frequentemente alagada (quase 100% do tempo) e que não apresenta vegetação é chamada de Plano Lamoso (PL); a zona dominada por *Spartina alterniflora* e que apresenta taxa de alagamento anual de 64% é chamada de Marisma Inferior (MI); a zona dominada por *Spartina densiflora* e que tem taxa de alagamento entre 37,4% e 20,1% é chamada de Marisma Médio (MM); e a zona mais seca, com taxa de alagamento de 20,1% – 3,1% e com ocorrência de *Scirpus maritimus*, *Scirpus olneyi*, *Juncus* effusus, *Myrsine parvifolia*, dentre outras espécies juntamente com *S. densiflora* é chamada de Marisma Superior (MS) [Seeliger *et al.* 1998, Marangoni & Costa, 2009]. Esses valores de taxas de alagamento e vegetação podem variar de acordo com a marisma, sendo que as espécies vegetais também podem ocorrer ou em manchas ao longo da marisma ao invés de faixas paralelas à costa devido a competição interespecífica [Seeliger *et al.* 1998]. As marismas do ELP se diferenciam em diversos aspectos como extensão, localização, biodiversidade e impactos antrópicos que vêm sofrendo [Marangoni & Costa, 2010]. Esses impactos foram identificados e esquematizados em 2009 por Marangoni e Costa e incluem pastejo, corte, fogo, presença de resíduos sólidos, aterro, canais de drenagem, descontinuidade vegetal e erosão [Marangoni & Costa, 2009]. Dentre as 24 marismas do ELP, a Marisma do Molhe Oeste tem destaque em relação à sua localização e a presença de zonação marcada, facilmente identificável. Ela apresenta área de 0,16 km^2 [Marangoni & Costa 2009], e está localizada em 32° 09' 09,3" S e 52° 06' 03,1" O, na desembocadura da Lagoa dos Patos para o Oceano Atlântico (Figura 2). Apesar de ter sofrido com variações na extensão de cobertura vegetal entre o período de 1947 e os anos 2000, essa marisma sofreu aumento total de área superior a 0,05 km^2 nesse período e serve de hábitat para pelo menos 47 espécies de plantas e 32 espécies de aves [Silva *et al.* 1993, Eichenberger 1999, Marangoni 2003], além de beneficiar espécies aquáticas estuarinas, incluindo as de interesse comercial [Costa *et al.* 1997].

Ainda, devido à sua localização, a Marisma do Molhe Oeste pode sofrer influência do aporte de material tanto do oceano quanto de toda a bacia hidrográfica da laguna a depender do balanço de enchente/vazante do estuário. Além disso, evidências iniciais mostram que essa marisma apresenta perturbações como fogo e pastejo nas zonas MM e MS, aterros, e que resíduos sólidos podem ser identificados ao longo de toda sua extensão mesmo em níveis que os autores consideraram baixos [Costa *et al.* 1997, Marangoni & Costa, 2009].

Capítulo V: Material e Métodos

As metodologias utilizadas no presente trabalho estão descritas abaixo, sendo separadas de acordo com cada artigo científico proveniente da tese de doutorado.

5.1. Busca da literatura sobre contaminação plástica em ambientes estuarinos (Artigo 1)

A busca na literatura foi realizada no Periódicos Capes – Brasil, que é uma plataforma de dados de publicações científicas financiada pelo Governo Brasileiro e que inclui bases de dados mundialmente utilizadas como a *Scopus*, a *Web of Science*, e o *Science Direct*. Para a busca, foram utilizadas as palavras-chave *estuary* e *plastic/polymer* em combinação com *salt marshes*, *mangrove*, *biofilm/biofouling*, *contaminant interaction* e *toxicity*. Após as buscas, os resultados obtidos foram triados e artigos foram selecionados baseados nos seguintes critérios obrigatórios:

i. Estar publicado em um periódico revisado por pares;

ii. Reportar contaminação/poluição por plásticos;

iii. Reportar quantificação em campo e/ou ter realizado experimento de campo ou de laboratório envolvendo o ambiente estuarino.

Outra busca adicional foi realizada na mesma plataforma utilizando as palavras-chave *estuary* e *plastic/polymer* em combinação com *correlative models* e *particle tracking*. O objetivo dessa busca foi investigar fatores que são considerados importantes na distribuição de plásticos no ambiente e para encontrar modelos computacionais de transporte de plástico em estuários. Todos os artigos que se enquadravam nos critérios pré-estabelecidos e que que estavam disponíveis até Setembro de 2021 foram incluídos na revisão. Documentos como teses, dissertações, resumos apresentados em congressos e relatórios técnicos não foram incluídos na revisão.

5.2. Coleta e análise de resíduos sólidos e seus organismos bioincrustantes na Marisma do Molhe Oeste (Artigo 2)

5.2.1. Coleta e análise dos resíduos sólidos

Para a análise da contaminação por resíduos sólidos foi selecionada uma área de aproximadamente 2.300 m^2 dentro da Marisma do Molhe Oeste (Figura 3), que compreendia as quatro zonas da marisma: Marisma Superior (MS), Marisma Médio (MM), Marisma Inferior (MI) e Plano Lamoso (PL). Transectos de 10 x 2 m foram delimitados a partir da linha do deixa presente na zona MS e abrangendo as áreas de transição entre as quatro zonas, como esquematizado na Figura 3. Sete transectos foram demarcados com pelo menos 2 m de distância entre si em cada uma das áreas, que daqui em diante serão referidas como área seca (entre MS e MM), área intermediária (entre MM e MI), e área alagada (entre MI e PL), totalizando 21 transectos (representados em tons de vermelho na Figura 3). Nove dos 21 transectos foram coletados em Outubro de 2017, e os outros 12 transectos em Agosto e Setembro de 2018. Todos os itens de resíduos sólidos maiores que > 5 mm foram coletados e acondicionados manualmente em sacos plásticos identificados e armazenados até triagem em laboratório. Itens muito grandes que não puderam ser carregados foram fotografados e contabilizados, porém foram deixados em campo.

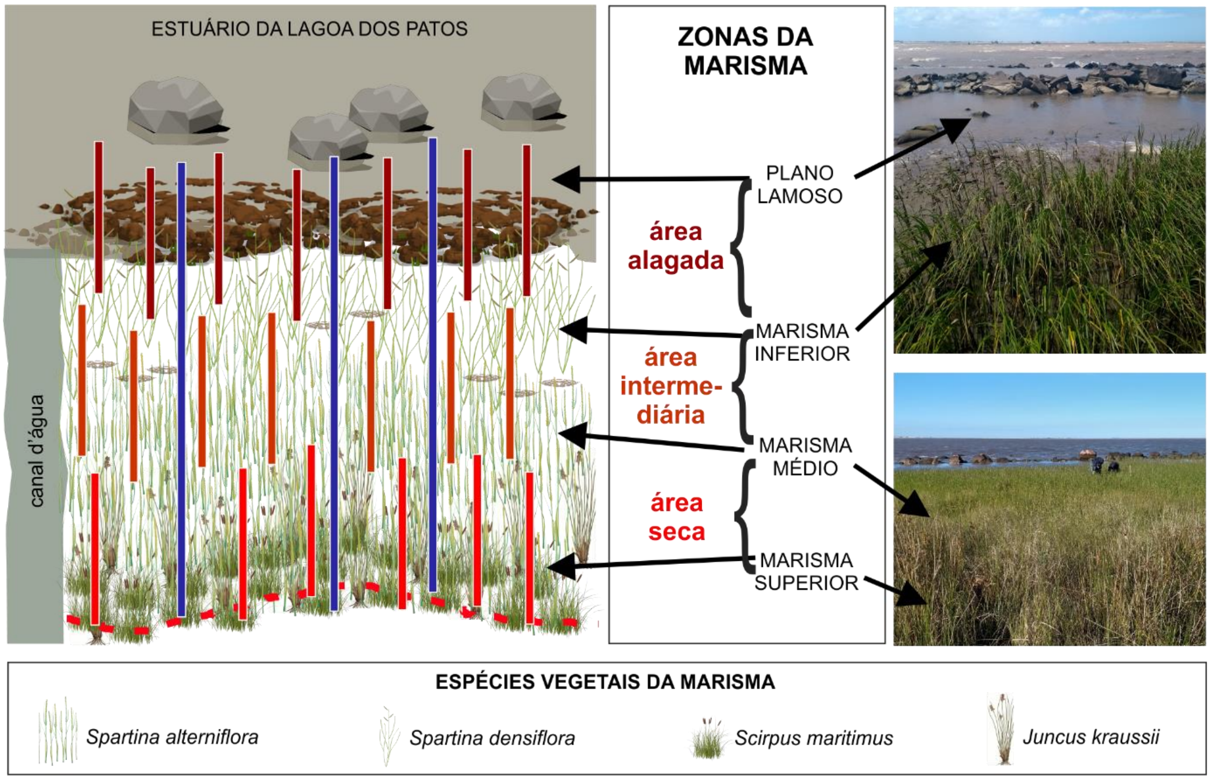

Figura 3. Esquema da zonação presente na Marisma do Molhe Oeste e dos transectos (barras) traçados para as coletas de resíduos sólidos (em tons de vermelho) e análise de bioincrustação (em azul). A linha tracejada vermelha indica a linha do deixa, presente na Marisma Superior.

A triagem do material coletado em campo foi realizada nas dependências do Laboratório de Microcontaminantes Orgânicos e Ecotoxicologia Aquática (CONECO - FURG). Cada item encontrado foi analisado e classificado como descrito na Tabela 2. As classificações utilizadas foram baseadas na publicação do GESAMP [2019], com modificações.

Tabela 2. Descrição das características dos resíduos sólidos analisados na triagem do material coletado na Marisma do Molhe Oeste. NI = não identificado.

Característica analisada	**Classificações utilizadas**
Composição	plástico, metal, madeira, cera, papel/papelão, vidro, espuma/esponja, orgânico/ Tetra Pak, borracha, tecido, mistura, NI
Tipo de plástico (se identificável)	polietileno tereftalato (PET), Nylon, poliestireno (PS), polietileno (PP), polietileno de alta densidade (PEAD), polietileno de baixa densidade (PEBD), cloreto de polivinila (PVC), látex, TNT, NI
Fragmentação	fragmentado, não fragmentado
Textura	duro, mole
Cor	amarelo, bege, cinza, dourado, marrom, preto, roxo, verde, azul, branco, colorido, laranja, metálico, rosa, transparente, vermelho
Uso prévio (atividade na qual o item poderia ter sido utilizado durante sua vida útil, podendo ser mais de uma para o mesmo item)	alimentício, cosmético, uso doméstico, pesca, resíduo hospitalar, uso em embarcação, transporte, uso pessoal, vestuário, NI
Tamanho	em centímetros, medido no maior lado

Outra classificação utilizada foi em relação ao grau de degradação dos itens, como descrito e ilustrado em Siqueira *et al.* [2017]. Esses autores desenvolveram uma escala visual, e, portanto, subjetiva, para estimar o estágio de decomposição de resíduos sólidos. Itens que ainda possuíam sua cor original, rótulo e/ou código de barras claros e legíveis e que ainda não tinham começado a se fragmentar foram classificados como *recentes*; itens que já apresentavam sinais de degradação como alterações de cor e bioincrustação, mas que ainda eram possíveis de serem identificados em relação ao uso prévio, foram classificados como *intermediários*;

itens em estágio avançado de degradação, frágeis, quebradiços, com muita bioincrustação e que não podiam ter seu uso prévio identificado foram classificados como *antigos*. Para fins de análise dos dados, a nomenclatura usada para essa escala foi de deg1, deg2 e deg3 para itens recentes, intermediários e antigos, respectivamente. Ao final da análise dos itens, todos foram separados em recicláveis e não recicláveis e destinados corretamente ao serviço de coleta seletiva disponível nas dependências da FURG.

5.2.2. Análise da bioincrustação em resíduos sólidos

Para a análise dos organismos bioincrustantes em resíduos sólidos da Marisma do Molhe Oeste, foram coletados resíduos ao longo de três transectos de 30 x 2 m de comprimento de forma a compreender as quatro zonas da marisma representados em azul na Figura 3). As coletas foram realizadas em Maio, Agosto e Setembro de 2018, e todos os itens encontrados ao longo dos transectos foram analisados para as mesmas características descritas na Tabela 2. Adicionalmente, os itens também foram classificados em relação ao seu grau de degradação baseado em Siqueira *et al*. [2017] como descrito anteriormente, além de características relacionadas à bioincrustação descritas na Tabela 3.

Tabela 3. Descrição das características relacionadas à bioincrustação analisadas na triagem dos resíduos sólidos coletada na Marisma do Molhe Oeste. NI = não identificado.

Característica analisada	Classificações utilizadas
Presença de bioincrustação	presença, ausência
Grupo de organismo incrustante (identificado visualmente)	ácaro, alga, anfípode, mexilhão, caranguejo, casulo, craca, fungo, gastrópode, hidrozoário, inseto, isópode, poliqueta
Percentual de cobertura do item (calculado através de estimativa visual)	cover1 (< 10% da superfície do item), cover 2 (11 – 25%), cover3 (26 – 50%), cover 4 (51 – 75%), cover5 (76 – 100%)

5.2.3. Análise de dados

Os dados de quantidade de resíduos sólidos foram reportados em número de itens por área amostrada (nº de itens m^{-2}), e as variáveis qualitativas (composição, tipo de plástico, cor, textura, fragmentação) foram reportadas e analisadas como percentuais do número total de itens de cada transecto. O tamanho médio dos itens foi calculado pela média aritmética dos itens em cada transecto. A bioincrustação foi reportada como a frequência (%) de ocorrência da presença de bioincrustação em cada transecto. O nível de degradação foi reportado como a média ponderada do grau de degradação atribuído para os itens de cada transecto, como descrito na Equação 1:

$$x^1 = \left((x^1 \times 1) + (x^2 \times 2) + (x^3 \times 3)\right)/x^1 + x^2 + x^3 \qquad (1)$$

Testes individuais de Shapiro-Wilk foram usados para testar a normalidade dos dados de quantidade de resíduos sólidos, textura, fragmentação, grau de degradação e tamanho médio. Todos os dados apresentaram distribuição normal (p

< 0,01), e os resíduos dos modelos também foram analisados para homoscedasticidade e normalidade. Testes individuais de análises de variância (ANOVA) com teste a *post-hoc* de Tukey foram realizados para avaliar se as zonas da marisma (seca, intermediária, alagada) mostraram diferenças significativas em relação a quantidade de itens, textura, fragmentação, grau de degradação, tamanho médio e ocorrência de bioincrustação. Além disso, uma análise de Percentual de Similaridade (SIMPER) foi feita para estimar a contribuição de cada grupo de organismos incrustantes e a sua preferência em relação às diferentes zonas da marisma, tipos de material e cores dos resíduos sólidos. Todos os testes previamente citados foram realizados no software Past versão 3.21 [Hammer *et al*. 2001].

O Modelo Aditivo Generalizado (GAM) [Hastie & Tibshirani 1990] foi utilizado para investigar diferenças significativas entre a quantidade de itens, as zonas da marisma e os meses de coleta. O GAM é uma extensão do Modelo Linear Generalizado (GLM) com um preditor linear envolvendo a soma de funções suavizadoras das covariáveis [McCullagh & Nelder 1989, Wood 2017]. O grau de suavidade dos termos do modelo foi estimado como parte de um ajuste usando *splines* de regressão cúbica penalizados. Para a análise GAM, foi utilizado um modelo de erro Gaussiano com uma função *link identify*. Foi considerado para a construção do modelo que as médias e variâncias das três zonas da marisma são dependentes, já que são áreas adjacentes e com continuidade espacial. Assim, para a análise GAM as categorias “alagado”, “intermediária” e “seca” foram categorizadas em “1”, “2” e “3”, respectivamente. Outras análises GAM foram feitas para investigar relações entre a ocorrência de bioincrustação (total, por algas e por anfípodes) e as mesmas covariáveis. Apenas a ocorrência de bioincrustação foi considerada, assim um modelo de erro binomial com uma função *logit link* foi utilizado. Devido às limitações do modelo, apenas os grupos com ocorrência maior de 10% puderam ser modelados (algas e anfípodes). A seleção do modelo foi baseada no Critério de informação de Akaike (AIC), em que menores valores são considerados melhor ajustados ao modelo. Todas as análises GAM foram feitas com o pacote *mgcv* no software R versão 1.7-5 [Wood 2011].

5.3. Coleta e análise de meso- e microplásticos e seus organismos bioincrustantes na Marisma do Molhe Oeste (Artigo 3)

Para análise dos níveis de contaminação por meso- e microplásticos na Marisma do Molhe Oeste e da comunidade biológica associada a eles (i.e., a Plastisfera), foram coletadas amostras de sedimento superficial, testemunhos sedimentares e de água como esquematizado na Figura 4 e de acordo com os métodos descritos a seguir.

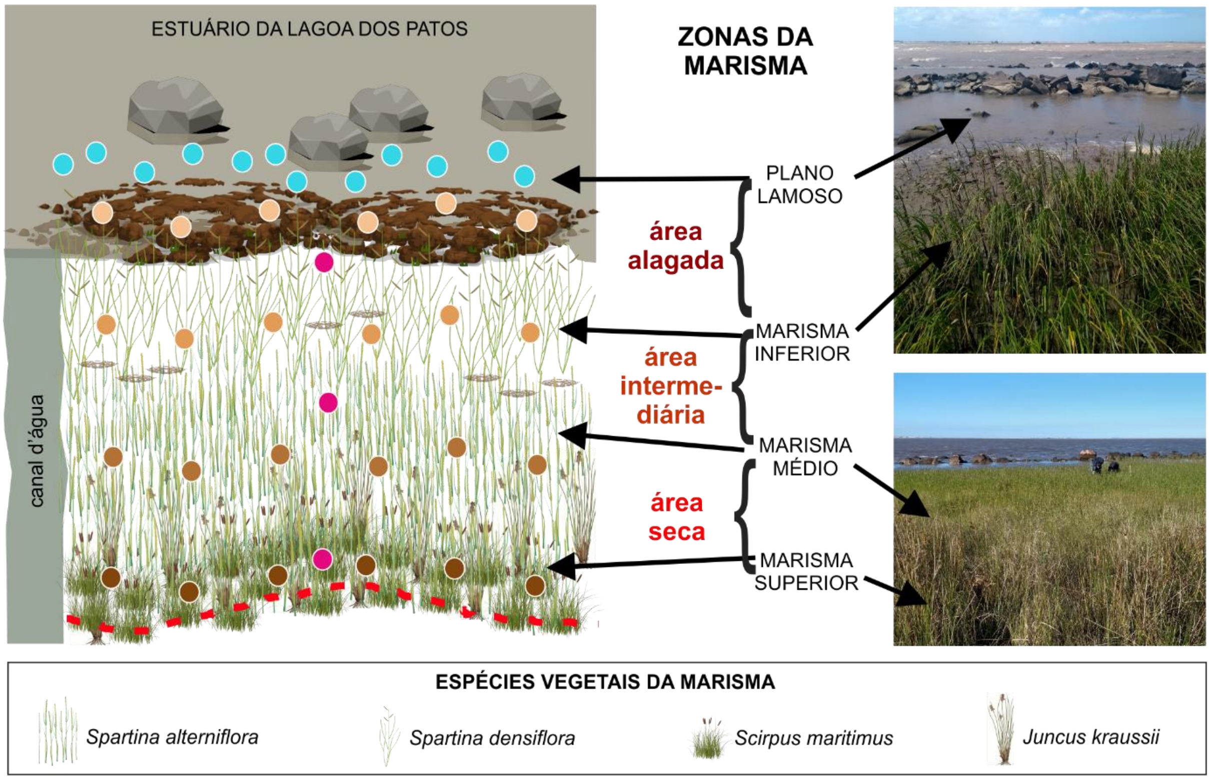

Figura 4. Esquema da zonação presente na Marisma do Molhe Oeste e dos pontos de coleta de sedimento superficial (em tons de marrom), de água (em azul), e dos testemunhos sedimentares (em rosa). A linha tracejada vermelha indica a linha do deixa, presente na Marisma Superior.

5.3.1. Coleta de sedimento superficial, testemunhos e água

Para sedimento superficial, foram coletadas em Julho de 2018 amostras dos primeiros 5 cm de sedimento em seis pontos localizados em cada uma das quatro zonas da marisma (MS, MM, MI e PL), em um total de 24 amostras (em tons de marrom na Figura 4). Para a coleta foi utilizado um amostrador do tipo *corer* de metal e com 50 cm de comprimento e 47 mm de diâmetro. As amostras foram acondicionadas em bandejas metálicas identificadas, fechadas com tampa de papelão e armazenadas até análise em laboratório.

Para os testemunhos, foi coletado em Maio de 2018 um testemunho em um ponto localizado na zona MS, um na região entre as zonas MM e MI, e um na região entre as zonas MI e PL, num total de 3 testemunhos sedimentares (em azul na Figura 4). Os amostradores foram inseridos no sedimento até a maior profundidade alcançada utilizando amostradores do tipo *corer* de PVC de 1,5 m de comprimento e 47 mm de diâmetro. Após retirada do testemunhador, suas extremidades foram seladas e o testemunhos foram identificados e transportados para o laboratório. Lá, cada testemunho foi fatiado a cada 2 cm, e cada fatia (subamostra) foi acondicionada em marmitas metálicas identificadas, fechadas com tampa de papelão e armazenadas até posterior análise. Além disso, a camada de serrapilheira eventualmente presente acima do sedimento nos testemunhos também foi separada e tratada como subamostra nas análises subsequentes.

Para as amostras de água, um total de doze amostras (em azul na Figura 4) de 150 mL de água cada foram coletadas em Julho de 2018 na zona PL da marisma utilizando uma proveta. Ainda em campo, as amostras foram filtradas em uma seringa de polipropileno acoplada a filtro GF/F de 45 mm de diâmetro e poro 0,7 µm. Os filtros resultantes foram então armazenados em envelopes metálicos e identificados e transportados para laboratório para posterior análise.

5.3.2. Isolamento e identificação de meso- e microplásticos

Todas as amostras de sedimento (superfície e testemunho) foram secas em estufa a 40 ºC até peso constante. Após esse período, as amostras foram pesadas individualmente em balança analítica e seu peso seco foi anotado. Para isolamento dos meso- e microplásticos, foi utilizado um método de flutuação salina adaptado de Pinheiro *et al*. [2019]. O princípio do método é baseado na diferença de densidade entre o sedimento e as partículas plásticas presentes na amostra. Cada amostra de sedimento seco foi colocada em um béquer de 1 L contendo um volume de solução salina supersaturada de NaCl (densidade 1,2 g cm^{-3}) na proporção de 1:5 (peso seco do sedimento em g : volume da solução em mL). Essa mistura foi agitada em agitador magnético por 30 minutos, seguidos de mais 30 minutos sem agitação para que o material mais denso se assentasse. Espera-se que materiais poliméricos menos densos que a solução salina como produtos à base de polipropileno (0,85 – 0,92 g cm^{-3}), polietileno (0,89 – 0,98 g cm^{-3}) e poliestireno (0,01 – 1,06 g cm^{-3}) permanecerão boiando no sobrenadante, enquanto produtos à base de polímeros mais densos como o poliuretano (1,20 – 1,26 g cm^{-3}), polietileno tereftalato (1,38 – 1,41 g cm^{-3}) e cloreto de polivinila (1,38 – 1,41g cm^{-3}) irão afundar [Frias 2018]. Após o tempo de descanso, o sobrenadante foi filtrado em sistema de filtração à vácuo com filtro de celulose de 90 mm de diâmetro e poro < 12 µm. Para cada amostra, esse procedimento foi repetido três vezes para assegurar a recuperação total das partículas plásticas.

Os filtros resultantes das filtrações das amostras de água e de sedimento foram acondicionados individualmente em placas de Petri de vidro tampadas e então secas a 40 ºC em estufa por pelo menos 12h. Após esse período, cada filtro foi analisado individualmente para visualização de potenciais partículas plásticas sob um estereomicroscópio (OPTSZ Opticam) acoplado à uma câmera e ao software Opticam Microscopia OPTHD versão 3.7.11443.20180326. As potenciais partículas plásticas foram então contabilizadas e descritas em relação às características apresentadas na Tabela 4.

Tabela 4. Descrição das características utilizadas para classificação de meso- e microplásticos coletados em sedimento e água na Marisma do Molhe Oeste. NI = não identificado.

Característica analisada	**Classificações utilizadas**
Cor	branco, transparente, azul, preto, verde, vermelho, amarelo, marrom, laranja, cinza, bege, rosa, colorido
Formato	fragmento, fibra, pellet, filme, esfera
Tamanho	em centímetro, medindo o maior lado
Classe de tamanho	mesoplástico (5 – 25 mm), microplástico (< 5 mm) [GESAMP 2019]

Para confirmação da natureza polimérica das potenciais partículas plásticas, foi realizada caracterização química em pelo menos 40% das partículas encontradas para identificação do tipo de polímero por microscopia de Infravermelho Transformada de Fourier (FTIR). As partículas encontradas em sedimento superficial e água foram analisadas no Laboratório Greenpeace, na Universidade de Exeter (Reino Unido), utilizando um µ-FTIR usando um sistema de imagem Perkin Elmer Spotlight 400 acoplado a um módulo de reflectância total atenuada (ATR), equipado com software Perkin-Elmers Spectrum^{TM} 10 (versão 10.5.4.738) que continham 8 livrarias de polímeros comercialmente disponíveis (*adhes.dlb*, *Atrpolym.dlb*, *ATRSPE~1.DLB*, *fibres.dlb*, *IntPoly.spl*, *poly1.dlb*, *polyadd1.dlb* e *POLYMER.DLB*). As partículas encontradas nos testemunhos sedimentares foram analisadas no Centro Universitario Regional del Este (CURE), na Universidad de la Republica (Uruguai), usando um Perkin Elmer model Frontier acoplado com um acessório U-ATR equipado com uma base de dados de espectros poliméricos de Primpke *et al*. [2018]. Uma média de 60 escaneamentos foi feita por amostra, e os espectros foram feitos na região entre 4.000 e 450 cm^{-1}.

5.3.3. Análise da Plastisfera em meso- e microplásticos

Dentre partículas plásticas encontradas em sedimento superficial e água na Marisma do Molhe Oeste, um total de 35 partículas foram selecionadas para análise da Plastisfera utilizando um método adaptado de Agostini *et al*. [2021]. As partículas foram fixadas em glutaraldeído 1% por pelo menos 24h, e depois foram retiradas da solução e secas em estufa a 40 ºC por pelo menos 12h. Depois, as partículas foram colocadas em placas de alumínio e cobertas com pó de ouro antes de serem analisadas em Microscópio Eletrônico de Varredura (MEV) modelo EOL JSM-6060, no Centro de Microscopia Eletrônica do Sul da FURG (CEME-SUL - FURG). Os micro-organismos observados nas fotografias do MEV foram então contados e classificados em bactérias, microalgas ou fungos.

5.3.4. Controle de qualidade das amostras ambientais de meso- e microplásticos

Diversos métodos são comumente utilizados para evitar a contaminação de amostras ambientais para análise de plásticos principalmente em tamanhos pequenos como meso- e microplásticos. No presente trabalho, jalecos de algodão e luvas de nitrila foram utilizadas em todos os momentos ao manusear amostras no laboratório. As amostras foram sempre cobertas com papel alumínio ou tampa de vidro, além de somente serem manuseadas dentro de uma estrutura fechada de acrílico que foi desenvolvida no laboratório CONECO para minimizar a contaminação proveniente do ar. Todas as soluções (água destilada e solução salina de NaCl) foram filtradas em filtro de celulose com poro < 12 µm antes de entrar em contato com amostra ou vidrarias a serem utilizadas, que por sua vez também foram lavadas com água destilada filtrada antes do uso. Um filtro de celulose úmido foi utilizado como “branco” do método, sendo sempre deixado em uma placa de Petri destampada ao lado da amostra correspondente durante a análise. Esses filtros foram analisados juntamente com os filtros das amostras em estereomicroscópio e FTIR como descritos anteriormente. A quantidade de partículas encontradas em filtro um do "branco" foi então subtraída da quantidade encontrada na sua respectiva amostra ambiental, como sugerido por Wright *et al*. [2021]. Contaminação pelos amostradores do tipo *corer* não foi identificada, já que nenhuma partícula identificada como PVC possuía a mesma cor do amostrador.

5.3.5. Análise dos dados

O nível de contaminação da Marisma do Molhe Oeste por meso- e microplásticos foi reportado como abundância de plásticos, sendo em nº de itens kg^{-1} de sedimento seco para amostras de sedimento superficial e testemunhos, e em nº de itens L^{-1} para amostras de água. Os dados das amostras de água não foram analisados estatisticamente e não puderam ser comparados às quantidades reportadas no sedimento devido à diferença entre as unidades reportadas. A normalidade dos dados das amostras de sedimento superficial foi testada usando um teste de Shapiro-Wilk, e os resíduos dos modelos foram também analisados para homoscedasticidade e normalidade. As diferenças significativas entre abundância de plásticos em sedimento superficial entre as zonas da marisma (MS, MM, MI, PL) foram avaliadas usando um teste de Kruskall-Wallis, seguido de um teste de Mann-Whitney *pairwise* para localizar as diferenças entre os grupos. As diferenças entre a abundância de plásticos no sedimento superficial entre categorias de tamanho de plásticos (mesoplásticos e microplásticos) e entre camadas do testemunho sedimentar (0 - 10 cm, 10 – 30 cm, abaixo de 30 cm) foram testadas usando uma análise de variância (ANOVA). Todos os testes estatísticos foram realizados no programa Past versão 3.21 [Hammer *et al*. 2001]. As quantidades de organismos observados no MEV foram reportadas como nº de células para bactérias e microalgas, ou como área (μm^2) para fungos.

5.4. Influência das características dos plásticos e da zonação da Marisma do Molhe Oeste na bioincrustação

5.4.1. Desenho experimental

Foram realizados três experimentos de campo com duração de 21 dias cada a fim de investigar a influência de três diferentes características dos resíduos plásticos no processo de bioincrustação ao longo de três áreas da Marisma do Molhe Oeste. As características testadas foram:

i. Experimento 1 - Tamanho dos plásticos: 6 x 2 mm, 30 x 10 mm, 60 x 20 mm;

ii. Experimento 2 - Cores dos plásticos: branco, vermelho, preto;

iii. Experimento 3 - Tipo de polímero dos plásticos: etileno acetato de vinila (EVA), polipropileno (PP), poliestireno (PS).

Os diferentes tamanhos, cores, e tipos de polímeros foram escolhidos baseados em sua importância e presença ambiental e na disponibilidade de materiais comercialmente acessíveis. Cada experimento contava com três níveis de cada fator "*características dos plásticos*", além do fator "*zonação da marisma*" com três níveis: área seca (entre zonas MS - MM), área intermediária (entre zonas MM - MI) e área alagada (entre zonas MI - PL). Assim, cada experimento tinha um desenho experimental do tipo 3 x 3.

O experimento 1 foi realizado entre Junho e Julho de 2021, em que corpos de prova nos tamanhos mencionados acima foram confeccionados manualmente a partir de copos descartáveis comerciais de PS na cor branca. O experimento 2 foi realizado entre Outubro e Novembro de 2021, em que corpos de prova foram confeccionados no tamanho 30 x 10 mm a partir de folhas de EVA nas cores mencionadas acima. O experimento 3 foi realizado entre Fevereiro e Março de 2022, em que corpos de prova foram confeccionados no tamanho 30 x 10 mm a partir de folhas de EVA e de copos descartáveis comerciais de PS e de PP, todos na cor branca.

Em cada experimento, os corpos de prova foram acondicionados dentro de estruturas metálicas mostradas na Figura 5. Para o experimento 1, os plásticos de tamanho 6 x 2 mm foram colocados dentro de tubos feitos com malha metálica de porosidade ~ 500 µm presos à uma grade metálica. Para os outros tamanhos de plástico em todos os experimentos, os plásticos foram colocados diretamente na grade metálica que foi recoberta com a mesma malha metálica de forma a conter os plásticos. Além disso, a estrutura foi montada com boias nas extremidades confeccionadas a partir de flutuadores de piscina. Essa estrutura foi construída de forma a (*i*) minimizar, dentro do possível, o uso de outros polímeros sintéticos que pudessem servir de substrato alternativo para colonização biológica; (*ii*) impedir que os corpos de prova escapassem da estrutura , (*iii*) permitir a entrada de íons, moléculas e organismos planctônicos que fazem parte do processo de colonização primária como bactérias, fungos e microalgas; e (*iv*) permitir que se mantivesse acompanhando as flutuações naturais no nível da água ao longo das três áreas da marisma onde foram colocadas. Ao final de cada experimento, as estruturas metálicas contendo os corpos de prova foram levadas às dependências do laboratório CONECO, onde os plásticos foram destinados em réplicas para cada uma das análises descritas a seguir.

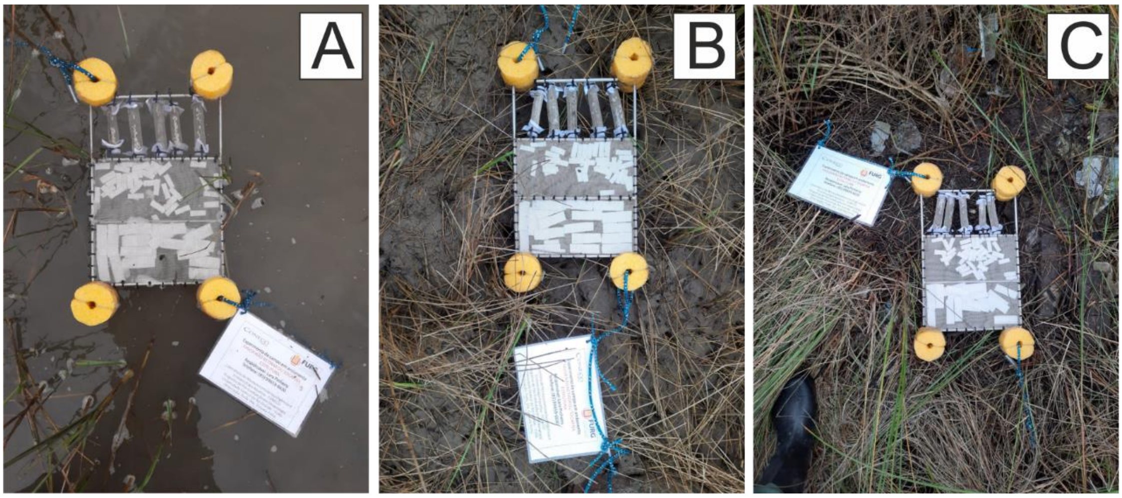

Figura 5. Exemplo de fotografias dos experimentos de campo nas diferentes áreas da Marisma do Molhe Oeste. As fotos acima foram tiradas durante o experimento 1 (tamanho x zona), por isso podem ser observados os tubos onde foram acondicionados os corpor de prova de tamanho 6 x 2 mm. A: área alagada; B: área intermediária, C: área seca.

5.4.2. Microscopia Eletrônica de Varredura (MEV)

Para visualização da estrutura da comunidade microbiana incrustante em MEV, foi utilizada metodologia adaptada de Agostini *et al*. [2021]. Cinco plásticos de cada tratamento foram selecionados, lavados três vezes com solução salina estéril 0,9% para retirada de material solto ou organismos planctônicos, e fixados em solução de glutaraldeído 1% por pelo menos 24h. Depois, os plásticos foram retirados da solução e secos em estufa a 40 ºC por pelo menos 12h. Em seguida, foi retirada uma subamostra de cada plástico utilizando um bisturi ou tesoura, evitando as bordas dos plásticos. Essa etapa se fez necessária para os plásticos de tamanho 30 x 10 mm e 60 x 20 mm, pois a estrutura do MEV não comportava o plástico inteiro nesses tamanhos. As subamostras foram então colocadas em placas de alumínio e cobertas com pó de ouro antes de serem analisadas em Microscópio

Eletrônico de Varredura (MEV) modelo EOL JSM-6060, no Centro de Microscopia Eletrônica do Sul da FURG (CEME-SUL - FURG). Os micro-organismos observados nas fotografias do MEV foram então contados e classificados em bactérias, microalgas ou fungos.

5.4.3. Microscopia de Epifluorescência (MEF)

Para determinação da densidade bacteriana em células mm^{-2}, foi utilizada uma metodologia de microscopia de epifluorescência (MEF) adaptada de Agostini *et al*. [2021]. Para essa análise, foram analisadas 5 réplicas de cada tratamento em cada experimento, representadas por 1 plástico cada réplica, exceto nos plásticos de tamanho 6 x 2 mm que foram agrupados de 10 em 10 plásticos para compor uma réplica.

Inicialmente, os substratos foram lavados 3x com solução salina estéril 0,9% para retirada de material solto ou organismos planctônicos. Depois, cada substrato foi raspado manualmente utilizando uma lamínula de vidro, e todo o material raspado juntamente com o substrato foram colocados em tubos individuais contendo 5 mL de solução salina estéril 0,9%. Os tubos foram então agitados em vórtex por 30 segundos, depois colocados em ultrassom (Ultronique, 40 KHz) por 2 minutos, e então agitados novamente em vórtex por 30 segundos. Esse procedimento garante que o material biológico (Plastisfera) seja desprendido dos substratos e fique em suspensão. Após esse procedimento, 2 mL de amostra foram fixados com volume de formaldeído a obter concentração final de 4% e armazenados a 8 ºC até posterior análise.

Uma alíquota de 0,5 mL de amostra foi então diluída de 1:1 usando 0,5 mL de água destilada antes de ser filtrada em filtros de policarbonato Whatman (25 mm de diâmetro, poro 0,2 µm) escurecidos com Irgalan Black. Essa diluição se fez necessária para melhorar a posterior visualização no MEF, sendo que um teste foi realizado previamente com as diluições de 1:0, 1:1 e 1:10 para cada experimento. Após a filtração, os filtros foram corados com laranja de acridina 1%, lavados com água destilada filtrada (GF/F), e colocados em lâminas para posterior visualização em MEF (Zeiss Axioplan) em aumento de 1000x. Cinco fotografias foram tiradas para cada lâmina/réplica, e a média aritmética da contagem de células das cinco fotografias foi calculada. A densidade bacteriana (DB, células mm^{-2}) foi calculada utilizando a Equação 2:

$$DB = \frac{C_M \times A_F \times V_S}{A_C \times V_F \times A_S} \qquad (2)$$

Onde C_M = média de contagem de células por lâmina; A_F = área do filtro; V_S = volume de amostra em solução; A_C = área do campo visual; V_F = volume de amostra filtrada; A_S = área do substrato.

5.4.4. Pesagem da Plastisfera

Para determinar a massa do material biológico depositado na superfície dos substratos, réplicas dos corpos de prova foram inicialmente lavados 3x com solução salina estéril 0,9% para retirada de material solto ou organismos planctônicos, depois secos em estufa a 40 ºC até peso constante. Após esse tempo, os substratos foram pesados individualmente em balança analítica de precisão. Depois de ter sua massa inicial anotada, o material incrustado foi desprendido dos substratos através de raspagem seguido de vórtex e ultrassom como descrito na seção *5.4.3*. Após esse procedimento, a solução resultante foi descartada e os substratos foram novamente secos a 40 ºC em estufa até peso constante, quando então a massa final foi aferida em balança analítica de precisão (precisão 0,001g). A massa da Plastisfera foi então calculada através da diferença entre a massa final e a massa inicial dos substratos.

5.4.5. Cromatografia Líquida de Alta Performance (HPLC)

Para quantificação dos principais pigmentos de microalgas presentes nos substratos, foi aplicada a metodologia de cromatografia líquida de alta performance (HPLC) adaptada de Mendes *et al.* [2007]. Para os plásticos nos tamanhos 30 x 10 mm e 20 x 60 mm, um substrato de cada tratamento foi selecionado e cortado em pedaços menores para otimizar a extração. Para os plásticos no tamanho 6 x 2 mm, dez substratos foram utilizados para compor uma amostra a ser extraída. Os substratos foram inicialmente lavados 3x com solução salina estéril 0,9% para retirada de material solto ou organismos planctônicos. Os pigmentos foram extraídos a frio, no escuro, com 4 mL de solução de metanol 95% tamponado com 2 % de acetato de amônia, contendo 0,05 mg L^{-1} de um padrão interno (trans-β-apo-8'-carotenal), durante 60 minutos a -20 ºC, sendo que as amostras foram sonicadas por 5 minutos no início do processo de extração. Os extratos foram então centrifugados a 1.100 g por 5 minutos a 4 ºC, e em seguida filtrados em membrana Fluoropore PTFE de poro 0,2 µm para eliminação de qualquer detrito ainda em suspensão. Antes de inserir no auto injetor do HPLC, houve a mistura de 1000 µL desta solução filtrada com 400 µL de água Milli-Q em um frasco de vidro de 2.0 mL a fim de ajustar as polaridades do extrato com a metodologia de HPLC usada [Zapata *et al.* 2000]. O equipamento HPLC Shimadzu incluí um módulo de distribuição de solventes (LC-20AD) com um desgasificador acoplado (DGU-20A5), um controlador de sistema (CBM-20A), um auto injetor refrigerado (SIL-20AC), um forno de coluna (CTO-20AC), um detector de fotodiodos (SPD-M20A) e um detector de fluorescência (RF-10AXL). A separação cromatográfica dos pigmentos foi realizada utilizando uma coluna monomérica OS C8 (Waters® SunFire; 15 cm de comprimento, 4.6 mm de diâmetro, e 3.5 µm de tamanho da partícula), de acordo com método de Zapata *et al.* [2000] e adaptado por Mendes *et al.* [2007], usando um fluxo de 1 mL min^{-1}, um volume de injeção de 100 µL e duração de corrida de 40 minutos.

A identificação e quantificação dos picos referentes aos pigmentos fotossintéticos foram realizadas usando como referência padrões comerciais da DHI (Institute for Water and Environment, Denmark). A concentração foi calculada a partir do sinal obtido pelo detector de fotodiodos e/ou pelo detector de fluorescência, para o caso dos pigmentos clorofilianos. Os picos foram integrados usando o software LC-Solution, sendo checados manualmente e corrigidos quando necessário. Para correção das perdas e/ou alterações de volume, as concentrações dos pigmentos foram normalizadas pelo padrão interno.

5.4.7. Análise dos dados

Os dados de MEV foram reportados em número de células por área do substrato (mm^2) para bactérias, fungos unicelulares e microalgas; e em percentual de área dos filamentos em relação à área do substrato (mm^2) para fungos filamentosos. Os dados de MEF foram reportados como densidade bacteriana (células mm^{-2}). Os dados de pesagem são apresentados como massa por área do substrato (mg mm^{-2}). A normalidade dos dados quantitativos foram testados através de um teste de Shapiro-Wilk, e os resíduos dos modelos também foram testados para normalidade e homoscedasticidade usando teste de Shapiro-Wilk e de Levene, respectivamente. Para dados que não apresentaram distribuição normal, as diferenças significativas entre as variáveis em diferentes áreas da marisma e características dos plásticos foram testadas através de teste de Kruskall-Wallis seguindo de teste de Dunn *posthoc* para localizar as diferenças entre os grupos. Para dados que apresentem distribuição normal, serão usadas análises de variância (ANOVA) seguido de teste de Tukey *posthoc*. Todas as análises estatísticas foram realizadas no programa *Past* versão 4.10 [Hammer et al. 2001].

Capítulo VI: Síntese dos resultados

Os resultados apresentados neste capítulo são referentes às metodologias da coleta de resíduos sólidos e seus organismos bioincrustantes (Artigo 2, Capítulo VII), da coleta de meso- e microplásticos e seus organismos bioincrustantes (Artigo 3, Capítulo VIII), e dos experimentos de campo investigando a influência das características dos plásticos e da zonação na bioincrustação.

6.1. Contaminação da Marisma do Molhe Oeste por resíduos sólidos e sua relação com organismos bioincrustantes (Artigo 1)

Um total de 2247 itens de resíduos sólidos foram coletados e caracterizados na Marisma do Molhe Oeste. A média de quantidade de resíduos foi 5,45 ± 6,02 itens m^{-2}, variando de 0 a 22,15 itens m^{-2}. As Figuras 3 e 4 do Artigo 2 (Capítulo VIII: Artigo 2) mostram os vários tipos de material encontrados, incluindo detalhes em relação ao tipo de polímeros, cores e uso prévio. Materiais feitos de plástico foram os mais comumente encontrados na Marisma, atingindo 92,4%, seguido de madeira (3%) e papel/papelão (1,2%). Outros materiais como metal, tecido, orgânicos, esponja/espuma, misturas, vidro e borracha representaram juntos aproximadamente 3%. Dentre os plásticos, foi possível identificar polímeros como PET (4,8%) e Nylon (4,5%), os mais comumente encontrados, seguidos de PS (3,1%), PP (1,6%) e outros (< 1,5%). As cores mais encontradas foram transparente (28%), branco (20,8%), e colorido (15,9%). Outras cores como azul (12%), verde (5,6%) e marrom (4,1%) também foram encontradas, dentre outras (< 12,5%). Um total de 27,5% dos itens puderam ser associados com algum tipo de uso prévio, sendo que destes a maioria eram embalagens alimentícias (12,4%), relacionados à atividades pesqueiras e portuárias (5,2%), uso pessoal (5,1%), e outros (< 3%). Itens presentes na zona alagada eram principalmente ligados à fonte alimentícia, uso pessoal e doméstico, enquanto itens ligados à pesca e ao porto foram mais encontrados na zona mais seca (Figura 5, Capítulo VIII: Artigo 2).

As análises de ANOVA e GAM mostraram que a contaminação por resíduos sólidos na zona mais seca era significativamente maior do que nas zonas intermediária e alagada (Figura 6A, Capítulo VIII: Artigo 2; Figura S1 e Tabela S1, ANEXO II). Foi encontrada também uma maior ocorrência de itens de textura dura nas zonas intermediária e seca quando comparadas à alagada (Figura S2, ANEXO II). Não houve diferença significativa entre zonas em relação à fragmentação dos itens, grau de degradação e tamanho médio (Figura S2A, S2C e S2D, ANEXO II). A maioria das cores e tipos de material estavam mais associados às zonas intermediária e seca, enquanto itens na zona alagada eram menos diversos em relação a essas características (Figura 6B e 6C, Capítulo VIII: Artigo 2).

Um total de 13 grupos de organismos bioincrustantes foram encontrados em associação com os resíduos sólidos coletados na Marisma do Molhe Oeste, como exemplificados na Figura 3 do Capítulo VIII: Artigo 2. A ocorrência de bioincrustação se mostrou decrescente da zona alagada para a mais seca, embora essa tendência não tenha se mostrado significativa (ANOVA $p>0,05$; Figura 7A, Capítulo VIII: Artigo 2). Entretanto, foi encontrado algum nível de explicação na análise GAM (15,1%) em relação ao número de itens por zona ($p>0,05$), período de coleta ($p<0,01$), tamanho do item ($p<0,03$) e nível de degradação ($p>0,05$) (Figura S3 e Tabela S2, ANEXO II).

O grupo bioincrustante mais encontrado em associação com os resíduos sólidos foi algas (53,3%), que também ocorreu em alta classe de cobertura (Figura S4, Anexo III), seguido de anfípodes (18,94%) e gastrópodes (5,73%). Os demais grupos representaram juntos 22,03%. A presença desses grupos vários em relação à zona da marisma, tipo de material e cores, como mostrado na Tabela 1 e na Figura 7A (Capítulo VIII: Artigo 2). Algas estavam mais presentes na zona seca, e em itens plásticos e transparentes (GAM $p<0,05$) e brancos (GAM $p<0,03$) (Figura S5 e Tabela S3, ANEXO II). Anfípodes também estavam mais presentes na zona seca (GAM $p<0,01$) (Figura S6A, ANEXO II), preferencialmente em itens marrons e feitos de madeira (Tabela 1, Capítulo VIII). Essa preferência de ocorrência na zona seca também foi observada para fungos e isópodes (Tabela 1, Capítulo VIII: Artigo 2), no entanto a maioria dos grupos (61,5%) estava mais presente na zona intermediária. De forma geral, a maioria dos grupos (38,5%) ocorreram preferencialmente em itens plásticos, e em itens de cor marrom.

6.2. Contaminação da Marisma do Molhe Oeste por meso- e microplásticos e sua relação com organismos bioincrustantes (Artigo 3)

6.2.1. Sedimento superficial e água

Um total de 777 e 83 potenciais partículas plásticas foram encontradas em amostras ambientais e em brancos do procedimento, respectivamente, representando uma contaminação de procedimento média de 16,69%. As variâncias do número de partículas em amostras ambientais e dos brancos foram semelhantes ($p=0,42$), porém as amostras ambientais apresentaram significativamente mais partículas do que as do branco ($p<0,01$) (Tab. S5, ANEXO III). A abundância média de plásticos em amostras de sedimento superficial foi de 279,63 ± 410,12 itens kg^{-1}

de sedimento seco e 8,89 ± 8,75 itens L^{-1} em água. Considerando as quantidades de potenciais plásticos (amostras ambientais menos brancos do procedimento), houve um decréscimo na abundância de plásticos da zonas mais secas em direção às mais alagadas (Kruskal-Wallis p=0,003) (Figura 2A, Capítulo IX: Artigo 3). A maioria das partículas em sedimento superficial se encaixavam na categoria de microplásticos (89,5% das partículas medidas). A abundância de microplásticos (132,54 ± 252,26 itens kg^{-1} sedimento seco) foi maior do que a de mesoplásticos (15,91 ± 40,33 itens kg^{-1} sedimento seco), porém essa diferença não se mostrou significativa (ANOVA $F_{(1,38)}$=3,89; p=0,053). Todas as partículas encontradas em água eram microplásticos.

Os plásticos encontrados em sedimento superficial e água tinham tamanho médio de 9,05 ± 3,19 mm e 1,73 ± 1,15 mm para meso- e microplásticos, respectivamente. Itens eram principalmente brancos (38,55%), transparentes (25,36%) e azuis (21,35%). O padrão de cores variou bastante entre zonas da marisma (Figura 4, Capítulo IX: Artigo 3), sendo partículas azuis mais associadas com a Marisma Superior, enquanto itens brancos e transparentes foram menos associados à Marisma Inferior e Plano Lamoso (PCA 99,89% de explicação, Figura S2A, ANEXO III). Plásticos tinham principalmente o formato de fragmentos na Marisma Superior (84,75%) e Médio (80,45%), enquanto na Marisma Inferior, Plano Lamoso e água eram principalmente fibras (55,81%, 74% e 93,75%, respectivamente) (PCA 99,99% de explicação, Figura S2B, ANEXO III). A maioria das partículas foi identificada como polímero sintético (84,61% das partículas analisadas no FTIR). Os principais tipos de polímero encontrados foram PEAD (34,78%), PE (25,92%) e PP (23,15%), enquanto outros polímeros representavam juntos 16,15% (PVC, PES, Nylon, PA, PS e 13 outros) (Figura 4C, Capítulo IX:

Artigo 3). PEAD estava mais associado à Marisma Superior, enquanto PP e PE estavam mais relacionados à Marisma Médio e PES à Marisma Inferior e Plano Lamoso (PCA 99,70% de explicação, Figura S2C, ANEXO III).

6.2.2. Coluna sedimentar

Um total de 1628 e 573 potenciais partículas plásticas foram encontradas em amostras ambientais de testemunhos sedimentares e em brancos do procedimento, respectivamente, representando uma contaminação de procedimento média de 10,45%. As variâncias do número de partículas em amostras ambientais e dos brancos foram diferentes ($p<0,01$), porém as amostras ambientais apresentaram significativamente mais partículas do que as do branco ($p<0,01$) (Tab. S5, ANEXO III). A abundância média de plásticos ao longo das colunas sedimentares foi de 366,92 ± 975,18 itens kg^{-1} de sedimento seco. A maior abundância de plásticos foi encontrada no testemunho coletado na Marisma Superior (827,59 ± 1473,98 itens kg^{-1}), seguido da Marisma-Médio - Marisma Inferior (92,40 ± 95,97itens kg^{-1}), e da Marisma Inferior - Plano Lamoso (85,21 ± 87,05 itens kg^{-1}) (Fig, 5A, Capítulo IX: Artigo 3). Em todas as zonas, os plásticos foram mais abundantes nos 10 cm mais superficiais do que no restante do testemunho, mesmo não considerando a camada de serrapilheira, mas essa diferença só foi estatisticamente significativa no testemunho coletado na Marisma Superior (ANOVA $p<0,001$). (Figura 5B, Capítulo IX: Artigo 3).

As principais cores das partículas encontradas foram azul (46,80%), branco (23,34%), e preto (14,16%), e os principais formatos encontrados foram fibras (54,32%) e fragmentos (18,80%). Os principais tipos de polímeros encontrados foram PEAD (30,99%), PE (30,51%), PET (11,73%) e PP (11,26%). Os padrões dessas características foram variáveis ao longo dos três testemunhos (Figura 6, Capítulo IX: Artigo 3).

6.2.3. Bioincrustação em meso- e microplásticos de sedimento superficial e água

Uma área de aproximadamente 693720,84 μm^2 da superfície dos plásticos foi analisada, o que correspondeu a uma média de 0,5% da área de cada plástico. Todos os plásticos analisados (n=35) tinham organismos bioincrustantes em sua superfície, em todas as zonas da marisma. Foram contabilizados no total 1683 bactérias, 318 células de microalgas (inteiras ou fragmentadas), e uma área de 20049,93 μm^2 de fungos filamentosos. A cobertura por bioincrustação nos plásticos coletados na Marisma Superior era frequentemente vista em manchas, enquanto em plásticos coletados em zonas mais alagadas como a Marisma Inferior e o Plano Lamoso a superfície dos plásticos era completamente coberta por material biológico. Bactérias e microalgas foram observadas em todos os formatos e zonas, enquanto fungos apenas foram vistos em fragmentos e pellets mas não em fibras e filmes, e apenas nas zonas de Marisma Médio e Superior.

6.3. Evidências da influência da zonação e características dos plásticos na bioincrustação – resultados preliminares

Apesar da limitação na identificação morfológica que é consequência do método de secagem utilizado para o MEV no presente estudo, uma grande variedade de microrganismos puderam ser observados compondo a Plastisfera nos três experimentos realizados na Marisma do Molhe Oeste incluindo bactérias isoladas e em colônia, em formato cocoide e bacilos; fungos unicelulares (leveduras) e filamentosos; microalgas arredondadas, penadas, e fragmentadas (Figura 6).

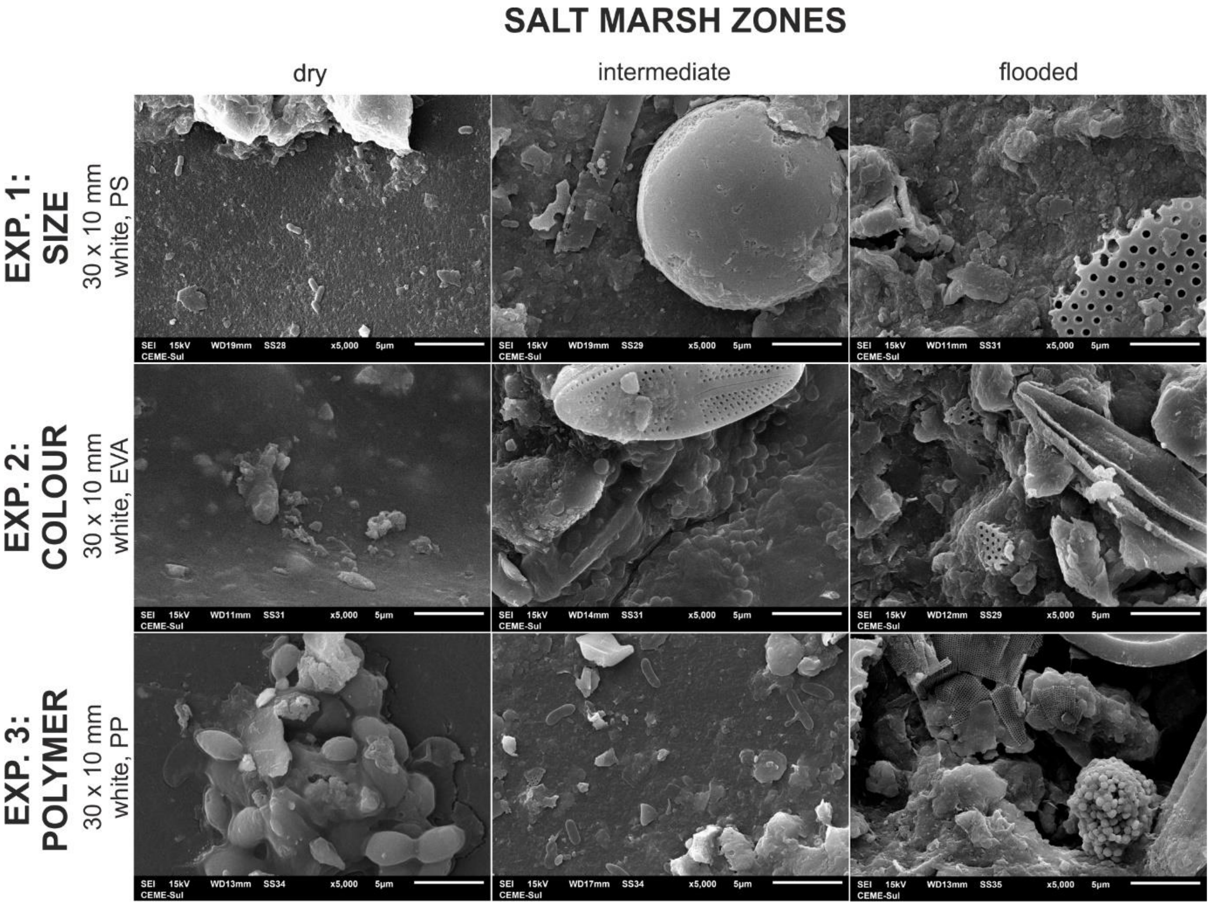

Figura 6. Fotomicrografias de microscopia eletrônica de varredura (MEV) exemplificando a comunidade epiplástica, i.e., Plastisfera, em alguns dos diferentes substratos expostos à diferentes zonas da Marisma do Molhe Oeste.

Para a massa da Plastisfera, observou-se no experimento 1 um valor médio total de 0.008 ± 0.006 mg mm^{-2}, sendo que na zona seca não houve diferença de massa da Plastisfera entre os três tamanhos de substrato (Figura 7A). Na zona intermediária, os substratos de tamanho 6 x 2 mm tiveram significativamente mais massa na sua superfície do que outros tamanhos. Para a zona alagada, o cenário foi bem diferente: os substratos com tamanho 60 x 20 mm tiveram significativamente mais material depositado na sua superfície do que os de tamanho 6 x 2 e 30 x 10 mm. No experimento 2, não houve diferença significativa de massa em relação a nenhuma zona da marisma ou cor de substrato (Figura 7B). No experimento 3, não foi observada diferença entre as massas da Plastisfera entre tipos de polímero na zona seca nem na intermediária (Figura 7C). Contudo, na zona alagada houve um decréscimo significativo na massa seguindo o padrão EVA > PP > PS). Além disso, os valores de massa encontrados na zona intermediária foram significativamente maiores do que os encontrados na zona alagada, sendo a massa encontrada no EVA na zona intermediária também significativamente maior que todos os polímeros da zona seca.

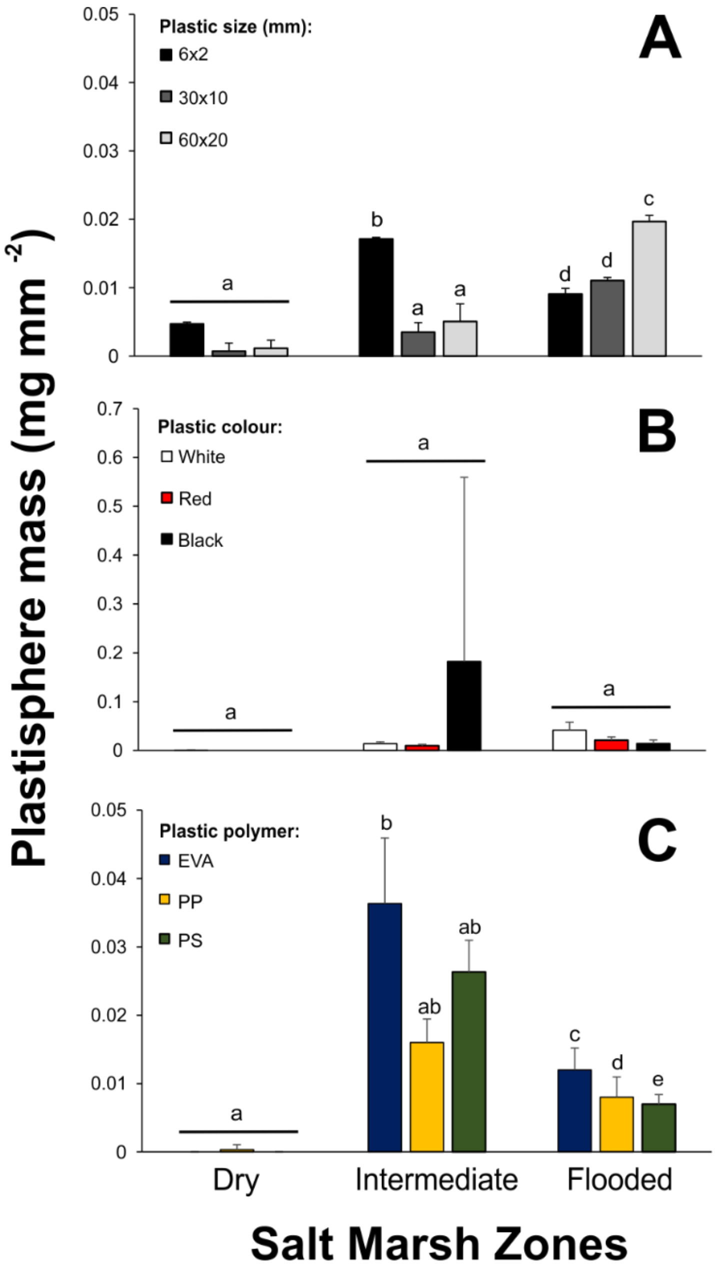

Figura 7. Massa da Plastisfera (mg mm^{-2}) encontrada nos três experimentos de campo envolvendo diferentes características dos substratos e diferentes zonas da marisma (seca, intermediária, alagada). A: Experimento 1 – Tamanho; B: Experimento 2 – Cor; C: Experimento 3 – Tipo de polímero.

Em relação à densidade bacteriana (DB, Figura 8A), observou-se no experimento 1 um padrão geral ao longo das três zonas da marisma de DB decrescente dos plásticos de tamanho 6 x 2 mm, para os de 30 x 10 mm e os de 60 x 20 mm. No experimento 2, não houve diferença significativa entre cores dos substratos em nenhuma zona da marisma, mas a DB foi significativamente maior na zona alagada em comparação com a zona seca (Figura 8B). Similarmente, no experimento 3 não houve diferença significativa entre os tipos de polímeros em nenhuma zona da marisma, mas a DB foi significativamente maior na zona alagada em comparação com as zonas intermediária e seca (Figura 8C).

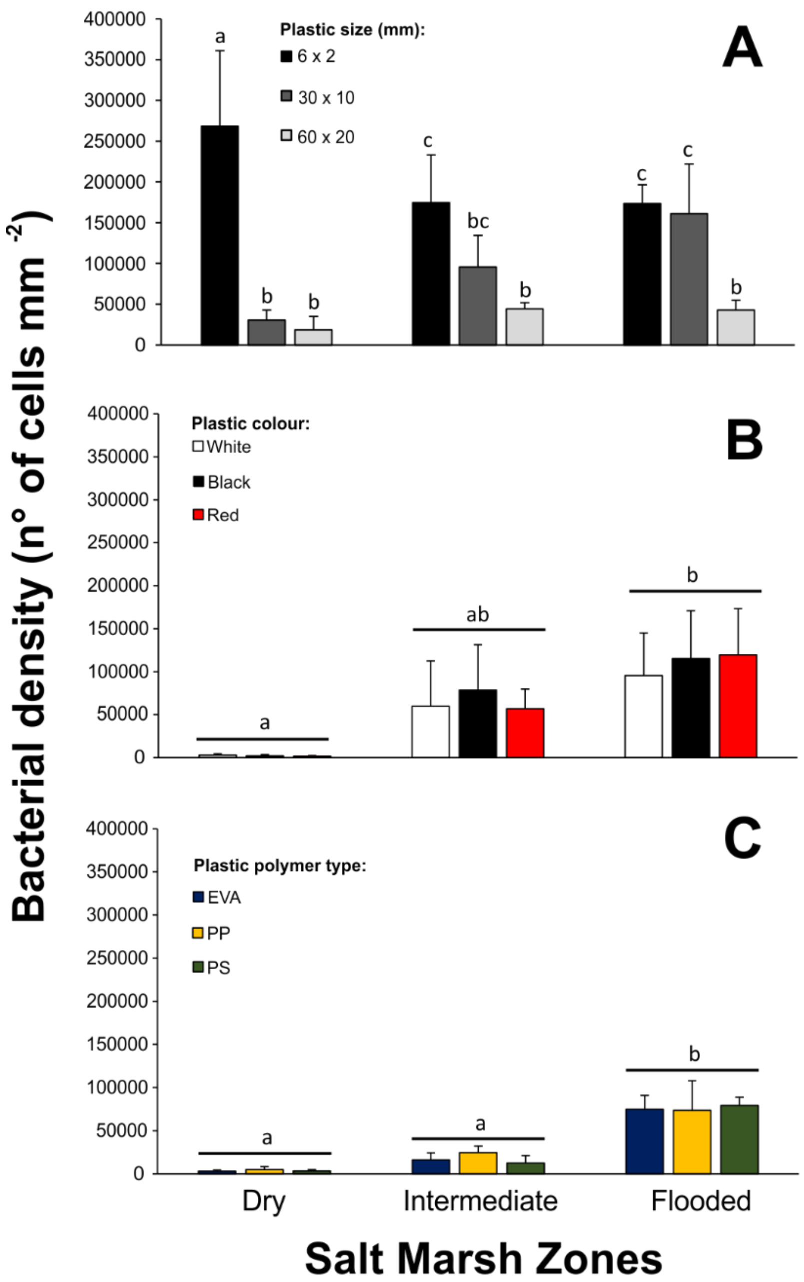

Figura 8. Densidade bacteriana (DB, células mm^{-2}) encontrada nos três experimentos de campo envolvendo diferentes características dos substratos e diferentes zonas da marisma (seca, intermediária, alagada). A: Experimento 1 – Tamanho; B: Experimento 2 – Cor; C: Experimento 3 – Tipo de Polímero.

Em relação às microalgas, a concentração de clorofila *a*, que por estar presente em todas as microalgas pode ser lida como biomassa total, foi muito pouco representativa nas zonas seca e intermediária em todos os experimentos (Figura 9). Na zona alagada, existe uma tendência geral de aumento da biomassa de microalgas e da maioria dos pigmentos fotossintéticos secundários (clorofila *b* e fucoxantina) com o aumento do tamanho do substrato (6 x 2 < 30 x 10 < 60 x 20 mm) (Figura 9A). Comparando as cores do substratos, os pigmentos também apresentaram uma tendência de aumento na concentração seguindo o padrão dos substratos brancos < pretos < vermelhos (Figura 9B), mas com maior expressão apenas das clorofila *a* e *b*. Para os tipos de polímero, houve uma tendência de aumento da biomassa total de microalgas (clorofila *a*) seguindo o padrão EVA < PP < PS, enquanto para a fucoxantina esse padrão foi EVA < PS < PP (Figura 9C).

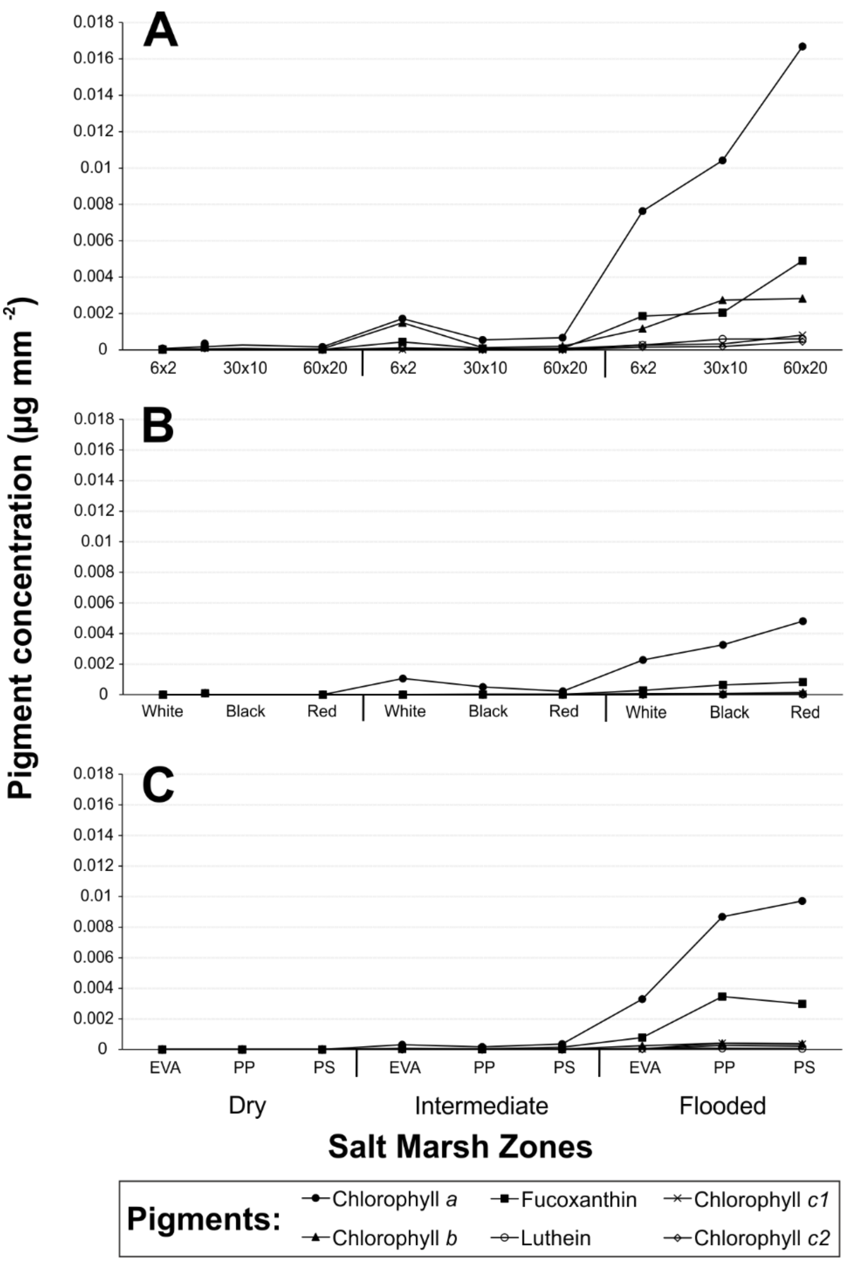

Figura 9. Concentração de pigmentos de microalgas (µg mm^{-2}) encontrada nos três experimentos de campo envolvendo diferentes características dos substratos e diferentes zonas da marisma (seca, intermediária, alagada). A: Experimento 1 – Tamanho; B: Experimento 2: Cor; C: Experimento 3 – Tipo de Polímero.

Capítulo VII: Artigo 1

O primeiro artigo científico proveniente desta Tese de Doutorado é apresentado nesse capítulo. O manuscrito é de autoria de Lara Mesquita Pinheiro, Vanessa Ochi Agostini, André Ricardo de Araújo Lima, Raymond Ward e Grasiela Lopes Leães Pinho, é intitulado "***The fate of plastic litter within estuarine compartments: An overview of current knowledge for the transboundary issue to guide future assessments***" e foi publicado no periódico *"**Environmental Pollution**"* e está disponível no link https://doi.org/10.1016/j.envpol.2021.116908 (0269-7491 / © 2021 Elsevier Ltd. All rights reserved). O material suplementar do artigo se encontra no ANEXO I desta Tese.

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Review

The fate of plastic litter within estuarine compartments: An overview of current knowledge for the transboundary issue to guide future assessments☆

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ABSTRACT

Plastics can enter biogeochemical cycles and thus be found in most ecosystems. Most studies emphasize plastic pollution in oceanic ecosystems even though rivers and estuaries are acknowledged as the main sources of plastics to the oceans. This review detected few studies approaching the transboundary issue, as well as patterns of estuarine gradients in predicting plastic distribution and accumulation in water, sediments, and organisms. Quantities of plastics in estuaries reach up to 45,500 items m^{-3} in water, 567,000 items m^{-3} in sediment, and 131 items per individual in the biota. The role of rivers and estuaries in the transport of plastics to the ocean is far from fully understood due to small sample sizes, short-term approaches, sampling techniques that underestimate small plastics, and the use of site-specific sampling rather than covering environmental gradients. Microfibres are the most commonly found plastic type in all environmental matrices but efforts to re-calculate pathways using novel sampling techniques and estimates are incipient. Microplastic availability to estuarine organisms and rising/sinking is determined by polymer characteristics and spatio-temporal fluctuations in physicochemical, biological, and mineralogical factors. Key processes governing plastic contamination along estuarine trophic webs remain unclear, as most studies used "species" as an ecological unit rather than trophic/functional guilds and ontogenetic shifts in feeding behaviour to understand communities and intraspecific relationships, respectively. Efforts to understand contamination at the tissue level and the contribution of biofouling organisms as vectors of contaminants onto plastic surfaces are increasing. In conclusion, rivers and estuaries still require attention with regards to accurate sampling and conclusions. Multivariate analysis and robust models are necessary to predict the fate of micro- and macroplastics in estuarine environments; and the inclusion of the socio-economic aspects in modelling techniques seems to be relevant regarding management approaches.

1. Introduction

Plastic waste is one of the world's most pressing environmental problems driven by international mismanagement; accounting for 100 million tonnes found in the oceans (Anderson et al., 2018; Lebreton et al., 2017; Ockelford et al., 2020). Nearly 90% of this waste enters the ocean from land-based sources as estuaries are the main pathway exporting plastics from the land to the sea (Lima et al., 2020). The bi-directional freshwater-seawater flow creates heterogeneous boundaries with potential to accumulate plastics into these systems. The relative abundance of plastics increases upstream when tidal influx is the main factor structuring the estuarine gradient, and then increases seawards whenever river

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flows break this gradient, as observed for other pollutants worldwide (Lebreton et al., 2017; Lima et al., 2014). This highlights that plastic pollution has a transboundary nature, with complex spatiotemporal patterns that are not fully understood (Krelling and Turra, 2019; Lima et al., 2020). Assessments of riverine systems are rare, which leads to knowledge gaps and estimations of plastic emissions to the oceans (Li et al., 2018a,b). In addition, controversial concepts regarding buoyancy vs. settling hampers accurate predictions concerning the fate of plastics within biogeochemical cycles (Zalasiewicz et al., 2016).

Plastics are ubiquitous and commonly recognized as stratigraphic markers, and they have been used to support the proposal of a new geological epoch called the Anthropocene (Zalasiewicz et al., 2016). Due to their low density and portability, plastic polymers such as polyester (PES), polyethylene (PE), polypropylene (PP), polyvinyl chloride (PVC), polystyrene (PS) are readily found in every aquatic environment (Wang et al., 2018). Once in the aquatic environment, plastics typically break down into smaller fragments, known as microplastics (<5 mm). Microplastics' size can be compared to plankton (<0.2 μm to >20 cm) or even sediment grains (fine gravel to clay – 0.98 μm to 8 mm), which influences their capacity to cause harm, to become bioavailable and to be transferred along the trophic web (Crooks et al., 2019; Farrell and Nelson, 2013; Murray and Cowie, 2011; Ferreira et al., 2019a).

This work provides a critical review of knowledge gaps regarding plastic contamination in estuarine ecosystems, especially concerning methodological efforts, composition, toxicity and interaction with biota and other contaminants through estuarine compartments. A total of 133 selected publications in 46 journals (Table S1) from studies in 26 countries were evaluated (Fig. 1). These ranged from 1972 until our pre-established time limit of September 2020 (Figure S1). Details of the search methods can be found in the supplementary material.

2. Plastic contamination from rivers to estuaries

It is estimated that 57,000–265,000 MT of plastic entered the oceans from riverine systems in 2018, according to a recent model considering the Human Development Index (HDI) (Mai et al., 2020). These estimates are much lower than those reported by Lebreton et al. (2017) (1.15–2.41 million MT year^{-1}), which are based on annual production and the concept of Mismanaged Plastic Waste (MPW). However, the strong correlation between model estimates and field measurements ($r^2 = 0.71$) suggests that HDI models are better indicators to estimate global riverine plastic outflows. Asian rivers accounted for ~69% of the total global input, suggesting discharges of up to 173,000 MT year^{-1}. The remainder comes from South America (13%), North (7.1%) and Central America (5.5%), Europe (5%), and Africa and Oceania (0.5%) (Mai et al., 2020).

Estimates concerning microplastic inputs are still doubtful (Bellasi et al., 2020; Li et al., 2018a,b; Strungaru et al., 2019). The abundance of microplastics reported by studies using pumping or grab are at least three orders of magnitude higher than those collected with plankton net tows, as small sized plastics and flexible fibres are not efficiently collected by nets even though they represent >50% – 90% of the microplastic present in the aquatic ecosystems (Lima et al., 2021). In the Austrian Danube (Austria) and Grand Paris, microplastics had an average abundance of 0.317 and 30 items m^{-3} when collected with plankton nets with mesh sizes of 80 μm and 500 μm, respectively (Dris et al., 2015; Lechner et al., 2014), but this increased up to 2516.7 items m^{-3} in the Yangtze river (China) (Zhao et al., 2014) and up to 10^5 items m^{-3} in the Dutch River Delta and Amsterdam Canals (Leslie et al., 2017), when samples collected by water pumping were passed through a 32 μm sieve and filtered over a 0.7 μm glass filter, respectively. Although comparisons between methods must be performed at least in the same region and between similar size ranges, it is likely that microplastic emissions have been underestimated due to the high divergence in abundances estimated by different methods,

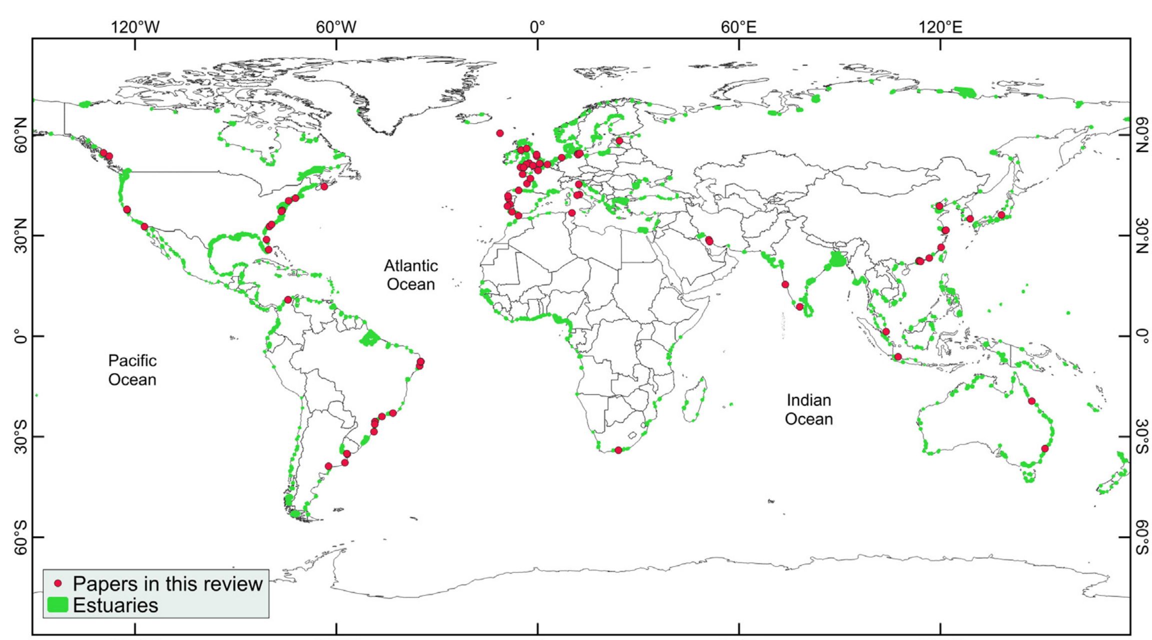

Fig. 1. World distribution of publications (red dots) on plastic contamination in estuarine environments up to September 2020 found in this literature review (search: *estuary* and *plastic/polymer* in combination with *salt marshes*, *mangrove*, *biofilm/biofouling*, *contaminant interaction*, *toxicity*). The global distribution of estuaries (in green) was retrieved from the Global Estuary Database available at https://data.unep-wcmc.org/datasets/23 (Alder, 2003; Watson et al., 2004). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

regardless of the riverine system (Li et al., 2018a,b). Therefore, efforts to understand smaller-size plastic emissions are still necessary (Blettler et al., 2018).

Little is known about plastic pollution in riverine sediments in comparison to water. In the Thames River (UK), for example, microplastic concentration ranged between 18.5 ± 4.2 and 66 ± 7.7 items per 100 g of dry sediment (1000 to 4000 μm) (Horton et al., 2017). In the Rhine and Main rivers (Germany), microplastic concentration varied from 1,784 to 30,106 items m^{-2} (63–5000 μm) (Klein et al., 2015). Although methodological comparison is not possible, it is interesting to note that fibres and fragments were the most common types in both studies, and sources were related to domestic effluents and local breakdown, respectively.

Fragments and fibres are commonly found in freshwater systems. Tyre wear particles (TWP, < 2.5 μm) account for 5–10% of all microplastics originated in land and ending up in the oceans (Bellasi et al., 2020). In Germany, 11,000 tons $year^{-1}$ of TWP reach surface waters through rainwater runoff (Bellasi et al., 2020). Another important source of microplastics are Wastewater Treatment Plants (WWTPs), especially in highly populated areas in developing countries, where microbeads and microfibres are the main contaminants. Microplastic emissions through WWTPs have been estimated around 209.7 trillion $year^{-1}$ in mainland China (Cheung and Fok, 2017); 50,000 - 15 million microplastics day^{-1} in the United States (Mason et al., 2016), and ~30 billion microplastics $year^{-1}$ in Vancouver (Canada) (Gies et al., 2018). Washing machines also discharge large amounts of synthetic fibres into wastewater that eventually reach aquatic systems (Dris et al., 2015). Synthetic fibres represent 60% of the 9 million tons of fibres produced worldwide, and approximately 2.5 million tons $year^{-1}$ of polyester fibres enter the oceans via river input (Carr, 2017).

3. The occurrence of plastics in estuaries

3.1. Modelling the distribution and accumulation of plastics in estuaries

Few studies have implemented modelling techniques to investigate plastic distribution and accumulation in estuaries according to spatial and temporal factors (Waldschläger et al., 2020). Hydrodynamic models are, for instance, useful tools to predict particle tracking, including Lagrangian and Eulerian approaches; and these have been widely used to simulate the pathways of plastics according to estuarine physical properties (Cohen et al., 2019; Krelling et al., 2017). However, processes such as the influence of rainfall, tidal waves and flow rates are still not well discussed because correlative modelling is missing (Lourenço et al., 2017; Naidoo et al., 2015).

Large plastics are easy to track, and most studies investigating macroplastic pollution have focussed on understanding how urbanization, industrialization, and proximity to Wastewater Treatment Plants influence accumulation patterns (Nelms et al., 2020; Viehman et al., 2011; Willis et al., 2017a). In the lower Paranaguá estuary (Brazil), for example, large plastic fragments dominate marine debris (74.8%) (Krelling and Turra, 2019). For this estuary, debris are exported seaward after a residence period of 5 days, as revealed by a simplified hydrodynamic model of dispersion (Krelling et al., 2017). Once in the outer estuary, no movement upstream was observed and, thus, the ocean is suggested to act as a sink. The study highlights that transboundary approaches must be implemented to manage marine debris across the land-river-sea continuum (Krelling et al., 2017).

Tracking microplastic distribution is far more difficult as a result of the high number of types and sources, fragmentation routes, and the complex relationship between abundance and physico-chemical factors within aquatic systems. Tyre and road wear particles (TRWP) degrade to smaller particles with estimates of 1.8 kg $inhabitant^{-1}$ yr^{-1} in the Seine watershed (France), as revealed by a geospatially- and temporally-resolved mass balance model (Unice et al., 2019). The model considers terrestrial transport to soil, air and roadways, and freshwater transport processes. These estimates indicate that 49% of TRWP produced on the road are transported to freshwater systems, 19% is transported through rivers, and ~2% is eventually exported to estuaries.

In Delaware Bay (USA), 3D hydrodynamic simulations applying a regional ocean modelling system within the Coupled-Ocean-Atmospheric-Wave-Sediment Transport Modelling System were performed to determine the transport and distribution of positively buoyant microplastics (Cohen et al., 2019). The model suggested that microplastics quickly organize into hotspots with high spatial and temporal variability influenced by currents, winds and tides; and the upper bay was found to have the highest microplastic densities. However, physical process such as river runoff influences were not identified due to limited and short-term sampling design (Cohen et al., 2019).

Both sediment and water samples were evaluated in five estuaries in Durban (South Africa) to understand patterns of microplastic accumulation under a simple spatial approach (Naidoo et al., 2015). Significant differences were observed among different estuarine reaches, but the Durban Bay estuary presented the highest abundance of microplastics (up to 7.4 ± 1.3 × 10^{-6} particles m^{-3}). Microplastics had a positive relationship with the large number of stormwater outfalls and rivers that drain into the Durban harbour. In the Tejo Estuary (Portugal), the distribution of microfibres was investigated in intertidal sediments using a General Linear Model (Lourenço et al., 2017). The spatial distribution of microfibres was positively influenced by the percentage of fine sediments (characterizing areas of slow current velocity), and by human settlement in adjacent areas. This suggests that hydrodynamics, local domestic sewage, and textile washing were the main factors influencing the distribution of microfibres.

Given the assumptions reported using modelling approaches, it is not surprising that more studies on plastic dynamics are still necessary to fully address their fate in estuaries (Ockelford et al., 2020). Although it was expected that sediments may be the final sink for microplastics (Van Cauwenberghe et al., 2013), more recent studies suggest that this might not be true for all polymers especially buoyant polymers (Erni-cassola et al., 2019). Therefore, driving forces influencing polymer distribution need to be evaluated as a whole considering as many steps of biogeochemical cycles as possible.

3.2. Methodologies to estimate plastic contamination in estuarine environments

3.2.1. Size categories

There appears to be a consensus about the upper limits of micro and nanoplastics but not meso and macroplastics (Table S2). A division between macroplastics (>5 mm), microplastics (1 μm–5 mm) and nanoplastics (1 nm– 1000 nm) was therefore used in this review.

Fewer papers on macroplastics (25.5%, considering plastics and debris in general) in estuaries were retrieved compared to microplastics (82.7%). This reflects the interest in microplastics in recent years (2014 onwards, Figure S1), which is justified by the interest in ingestion as microplastics are harmful to smaller estuarine species as planktonic organisms that can ingest particles even in the nano-size range (Rist et al., 2017). However, no papers discussed nanoplastics in estuarine environments, which is likely due to methodological limitations to analyse such particles in environmental samples (Koelmans et al., 2015; Mattsson et al., 2018). Attention appears to be directed towards lower trophic levels (see "*The presence of plastics in estuarine biota*"), since they represent a base link to the trophic web and microplastics are potentially more harmful to these organisms. However, 9% of studies did not specify the size of the debris analysed, leading to inconclusive results regarding effects to biota (Ye and Andrady, 1991; Turner et al., 2015).

3.2.2. Water sampling in estuaries

Water quality in estuaries is often defined by local climates, sediment cycles, fluctuation of physicochemical parameters, and human changes (Lima et al., 2020; Seeliger et al., 1998; Ward et al., 2014, 2016). The salinity gradient, which also influences parameters including pH and suspended material (through flocculation), is also acknowledged to induce particle movement in the water column (Niencheski and Windom, 1994). Indeed, it has been shown that litter accumulates in saline fronts, probably related to the low circulation and high sedimentation rates normally found in these areas during drought periods (Acha et al., 2003).

Saline fronts, also named as estuarine turbidity maximum (ETM) zones, can vary in their position dependent on the river/ocean flow balance (Day et al., 2013). In macrotidal estuaries, the volume of water exchanged between river and ocean during tidal cycles is much higher than in microtidal estuaries, where other factors such as rain and wind patterns will determine the flow balance and consequently the salinity gradient (Ward et al., 2016). The presence of an ETM zone and associated factors and temporal conditions should therefore be considered in investigations concerning suspended contaminants, especially because small-sized plastics seems to have a positive correlation with the amount of fine sediments (Lourenço et al., 2017).

Plastic contamination in estuaries can be assessed directly using water samples. In this review, four papers quantified macroplastics in estuarine water. Sadri and Thompson (2014) used a manta (300 μm) net to collect plastic debris from surface water of the Tamar estuary (UK). Although they found mostly microplastics (82%), particles > 5 mm were also sampled and quantified. Morrit et al. (2014) used modified fyke nets, used for fishing, to trap macro-litter items for almost three months in the River Thames (UK). All litter collected in that survey was submerged (the net was deployed at a depth of 40 cm), and not on the river surface.

Plastic items can have their density altered by degradation and/or biofouling, while water density variations in estuaries are dominated by salinity (Maccready et al., 2018), which is influenced by freshwater/seawater inflows. The differences between plastic density and water density will determine their buoyancy, and therefore need to be considered in such studies. Yet, contamination studies should also consider different depths inside the water matrix i.e. the feeding zones of burrowing organisms where plastic particles can accumulate due to bioturbation activity (Näkki et al., 2017; Gebhardt and Forster, 2018).

Microplastics are usually isolated from water samples using filtration/sieving methods. In this review, the most common sampling devices were nets with 300–333 μm mesh size. This is recommended by the Guidance on Monitoring of Marine Litter in European Seas for microplastic sampling in seawater (Galgani et al., 2013) in order to increase comparability. However, most fibres can pass through this mesh due to flexibility and small size; therefore, quantities of microfibers estimated using these methods are probably highly underestimated (Lima et al., 2021). Posterior filtration using sieves or paper filters with mesh sizes varying from 0.02 μm to 3 mm were also used (see Table S3).

The studies from two papers used water pumps to collect microplastic samples from estuarine water (Zhao et al., 2014, 2015). This method has the advantage of a precise volume filtered through the pump, so reported results are more reliable for fibres. Also, Setälä et al. (2016) have compared this method with manta trawl sampling and stated that pumps allow method control, use of different filter sizes and sampling at different depths. However, water collection through pumping must be performed under a continuous sampling intake to allow the coverage of a larger area, as plankton tows do, rather than the collection of point samples. Therefore, coupling plankton tows and pumping methods for water samples is a good step to guarantee accurate quantifications of the diversity of microplastics found in aquatic systems.

Initially microplastics were assessed as a sub-product of plankton surveys. Now they are being targeted at their own right, which explains why most studies used sampling with nets (Table S3). Consequently, a great amount of organic material in the same size class is collected together with microplastics. Therefore, methods are required to clean up samples to allow for effective polymer characterization. Three papers used digestion with hydrogen peroxide (H_2O_2) to minimise biological interference in water samples for microplastic analysis (Stolte et al., 2015). This is a common method for removing organic material in sediment analysis (Jensen et al., 2017), and therefore it is especially encouraged in highly productive environments, such as estuaries (Day et al., 2013) as it can help sample characterization and further microplastic identification, increasing the reliability of results.

3.2.3. Sediment sampling in estuaries

Sediments in estuaries are derived from river input, erosion, primary production, the sea and the atmosphere, although mudflats can be important lateral sediment sources when present in estuaries (Schubel, 1982). Estuaries can entrap sediments during low river flow, where they accumulate before entering the oceans when runoff increases seaward (Ward and Lacerda, 2021). This process has been used to explain patterns of dispersal of suspended solids and contaminants such as heavy metals (Teuchies et al., 2013; Celis-Hernandez et al., 2020a; Lacerda et al., in press), and can therefore be extended to plastics.

To perform sampling of macroplastics in sediment, collection using transects appeared to be the most common procedure in estuaries (e.g. Araújo and Costa, 2007; Ivar do Sul and Costa, 2013). This method allows a quick visual identification and sampling of plastic items in the environment, which can be analysed in the field or taken to a research facility for posterior analysis. Parameters such as number of items per unit area, item size, degradation stage and possible source are commonly used to describe environmental macroplastics and therefore to report a contamination scenario of the area. For microplastics, the great majority of works dealt with superficial layers of sediment (up to 5 cm deep) (e.g. Vianello et al., 2013; Talley et al., 2020), which are expected to comprise recent deposition of contaminants (Zalasiewicz et al., 2016). Usually sampling is performed in quadrats, with the sediment collected using grabbers or simple instruments like shovels, so the results are usually reported in number of microplastics per unit of area (e.g. Fok and Cheung, 2015; Fok et al., 2017; Cheung et al., 2016).

Both transects and quadrats represent simple methods for plastic sampling providing comparability among studies. However, estuarine regions have many different scenarios of tidal regimes, flooding rates and vegetation, and these must be considered in order to select an adequate sampling strategy. For example, sediment in estuaries can be found covered by a significant plant litter layer in salt marsh environments (Adam, 1993). In areas where this occurs, these different compartments (sediment/plant litter) should be considered individually when analysing plastic contamination, as the deposition times and dynamics are likely to differ among them (Ward et al., 2014; Ward, 2020).

In order to isolate microplastic particles from sediment, it is very common to use saline flotation techniques followed by filtration, using high density solutions that allow lighter plastic particles to float. Fok and Cheung (2015) isolated microplastics from sediment using seawater from their sampling site. This likely allowed the isolation of both lighter plastic items and items whose original polymer density was higher than seawater density but were weathered and became lighter. Thus, this methodology is appropriate for lower parts of the estuary, where seawater has a stronger influence and therefore a greater amount of plastics are likely to float. However, in the upper parts of the estuary, where seawater intrusion is low or non-existent, this method may not be as efficient due to a lower freshwater density (~1.0 g cm^{-3}).

For other saline solutions, preparation can require various salts such as NaCl (1.0–1.2 g cm^{-3}), $Na_2WO_4 \cdot 2H_2O$ (1.40 g cm^{-3}), NaBr (1.37–1.40 g cm^{-3}), $3Na_2WO_4 \cdot 9WO_3$ H_2O (1.40 g cm^{-3}), $Li_6(H_2W_{12}O_{40})$ (1.6 g cm^{-3}), $ZnCl_2$ (1.6–1.8 g cm^{-3}), $ZnBr_2$ (1.7 g cm^{-3}), and NaI (1.80 g cm^{-3}) (Frias, 2018). Interestingly, most papers (9 of 17 using saline flotation) in this review used NaCl, with three papers using NaI, two using $ZnCl_2$, and the other three papers using other solutions (Table S3). The NaCl solution may still be largely in use due to its low cost and efficiency, this method has been found to be highly efficient at isolating microplastics, although $ZnCl_2$ is more efficient for denser polymers (Coppock et al., 2017). Plastic particles in estuaries are likely to undergo high degradation levels due to physical forces such as periodical sunlight exposure and abrasion and thus their density may be lower than in other environments, so a lower density solution may be suitable to catch these items (Erni-cassola et al., 2019).

For sediment samples, digestion procedures can also be used to remove biological material and enhance plastic identification, including H_2O_2 (Jensen et al., 2017). However, only four papers in this review used such technique for this type of matrix (Table S3). According to the authors, this prevented large amounts of organic matter interfering with plastic isolation during the density separation process and subsequent counting. In addition, treatment with H_2O_2 can help remove natural coating such as biofilms on plastic surface (Christensen et al., 1990), which can make them resemble natural particles and be missed during visual identification (Isobe et al., 2019).

The sedimentation process is highly influenced by the action of waves, tides, atmospheric pressure and currents (Teasdale et al., 2011; Ward et al., 2014, 2016; Lima et al., 2020). As estuaries are very dynamic, sediment deposition is highly influenced by those forces and can vary greatly within the sediment column (Willis et al., 2017b). Sediments deposited on the surface can be translocated to deeper layers through sediment mixing or bioturbation processes (Martinetto et al., 2016; Ward, 2020), taking plastic particles with it. Extreme events such as storms and typhoons can cause stratigraphic mixing, even if the area is protected by vegetation such as salt marshes (Feagin et al., 2009; Li et al., 2020a). Also, sediment permeability can differ among sediment types and depths, which has also been suggested to influence microplastic dynamics (Misic et al., 2019). Therefore, although it may be very difficult to correlate plastics and sediment deposition rate, it is quite important to analyse deeper fractions of sediment in order to fully understand microplastic dynamics in estuaries, as has been undertaken for other contaminants (Cundy and Croudace, 1996; Celis-Hernandez et al., 2020a,b).

3.2.4. Laboratory and field experiments under estuarine conditions

Laboratory experiments were described in seventeen papers retrieved in this review. One paper used estuarine sediment to investigate bacterial colonization on microplastic particles in a microcosm system (Harrison et al., 2014), while two others investigated sorption aspects of the interaction between heavy metals (Holmes et al., 2014) and organic compounds (Bakir et al., 2014) with microplastics. We could only identify two works that have performed laboratory feeding trials using estuarine species (Table S3), although a recent study has assessed the trophic build-up of microplastics from *Mytilus edulis* to *Necora puber* through predation (Crooks et al., 2019). These approaches are important to answer specific questions by isolating factors of interest, and they will be discussed in further sections in this review.

Experimental procedures performed in the field were described in four papers (see Table S3 for details). They involved implantation of plastic items in the environment for different purposes. Two papers investigated the biofouling process in macroplastic items (Lobelle and Cunliffe, 2011; Ye and Andrady, 1991). One interesting work observed the formation of microplastics from implanted macroplastics in a salt marsh environment (Weinstein et al., 2016), but they did not quantify these particles in the environment.

One paper used a different approach in their field experiment, performing a recovery experiment by releasing tagged macroplastic items in a mangrove unit in Northeast Brazil and then recollecting them six days (Ivar do Sul et al., 2014). Their strategy was defined to understand the retention and exportation capacity of that specific environment, and therefore they did not quantify actual amounts of plastic litter in the environment, water or sediment. However, they showed how plastic contamination was influenced by hydrodynamics and vegetation, with more items being trapped in higher elevation areas, with weaker currents and denser vegetation. This recognized the role of vegetation in trapping debris on estuarine areas, as also observed by Araújo and Costa (2007) and similar to the processes influencing sediment (Ward et al., 2014), showing that vegetation is a key factor influencing plastic dynamics in estuaries.

Most of the aforementioned works were the result of field investigations (58.7%), with a few others conducting laboratory experiments (12.8%). Although these are very informative, some uncertainties remain about how plastic contamination can alter and affect estuarine environments and associated organisms. Experiments performed in natural environments under semi-controlled conditions can be considered a very useful strategy to answer questions that cannot be fully assessed with other approaches alone because it portrays the multi-faceted processes that plastic items suffer in estuarine environments.

3.3. Factors influencing plastic quantities in estuaries

In our literature review, 41 and 31 studies out of 100 papers quantified plastic in sediment and water, respectively. The abundance of macro and microplastics found in the studied estuarine matrices are shown in Tables S4 and S3, respectively, reaching up to 567,000 items m^{-3} in sediment and 45,500 items m^{-3} in water. Morritt et al. (2014) quantified macro litter in the upper portion of the Thames river estuary (UK) where a total of 8,490 items were collected inside the river catchment in three months using a pyke net with a non-specified mesh size. In sediments, abundance of macroplastics (manually collected) ranged from < 0.1 items m^{-2} in an isolated Brazilian beach (Araújo and Costa, 2007) up to 163 ± 154 items m^{-2} in the at the Pearl River Estuary (China) (Fok et al., 2017), where plastics from 0.315 to 10 mm were visually sorted in the top 4 cm of sediment (Table S4). Regarding microplastics (Table S3), the Mtamvuna River estuary (South Africa) had the highest contamination per volume of sediment (567,000 items m^{-3}), collected to a depth of 5 cm, sieved through a 1 mm mesh sieve and isolated using a NaCl solution (De Villiers, 2019). The Mosquito Lagoon in the northern Indian River Lagoon system, Florida (US), had the highest microplastic abundance per volume in water (up to 45,500 items m^{-3}), which was collected with bottles and filtered through a 0.45 μm mesh (Waite et al., 2018). The least contaminated areas were the Bay of Brest (France), with 0–8.74 items kg^{-1} of dry sediment collected with a Van Veen grab, from which microplastics were isolated using NaCl and Na_2WO_4 solutions followed by filtration with a 1.6 μm mesh size (Frère et al., 2017), and the Citarum River (Indonesia) with 0.000666 ± 0.000577 items m^{-3} of water, collected with a manta trawl (125 μm mesh) and a grab sampling method, from which microplastics were isolated visually (Sembiring et al., 2020).

It is inconclusive to compare microplastic abundance between sediments and water samples due to divergences in sampling methods, sampling sizes and sampling designs. Physical properties of an estuary such as tidal movements, currents, river discharge and winds have a strong influence on water flow, in a way that sampling in water only portrays a snapshot of the contamination. Also, microplastics can overlap in size and settling rates with sediment particles (Vermeiren et al., 2016), so forces acting on the sediment particles can also act on microplastics.

The transfer of energy, material and organisms between the water column and the benthic environment, i.e. the benthic-pelagic coupling, is an important process that occurs in estuaries (Griffiths et al., 2017). Plastic items in estuarine water or sediment can therefore be influenced by several processes included in the benthic-pelagic coupling such as sinking (Kaiser et al., 2017), (re) suspension (Critchell and Lambrechts, 2016), bioturbation (Näkki et al., 2017), among others (Wolanski and Elliot, 2015). Studies analysing surface sediments should be concerned about the exchange of plastics with water and the influence of estuarine organisms on these processes.

Similar forces may have implications on both sediment and microplastic deposition through the sediment column (Chubarenko et al., 2018; Willis et al., 2017b). Few studies looked at the sediment column below the surface in sandy beaches (e.g. Turra et al., 2014). Two papers investigated plastic in deeper sediment layers in estuaries, with quantities varying from 4.8 to 15.9 items m^{-3} up to 20 cm depth in a mangrove (Costa et al., 2011) and more than 100 g of plastics accumulated to a depth of 50 cm in a mudflat (Iribarne et al., 2000). Researchers should also consider local hydrodynamics before associating plastic deposition with time, as sedimentation rates in estuaries can vary greatly as a result of river flow variability (Butzeck et al., 2014; Ward, 2020), and mixing might occur due to dredging and fishing devices, such as bottom trawls (Bardos et al., 2020). Bioturbation activity of sediment-dwelling species can bury synthetic particles (Gebhardt and Forster, 2018), e.g. the burrowing activity of the intertidal crab *Neohelice granulata* can trap debris inside the sediment of salt marshes (Iribarne et al., 2000), and therefore needs to be considered. Solid materials can also be retained by estuarine vegetation (Ivar do Sul et al., 2014), and it is reasonable to relate these interactions with particle size according to species and distance from open water (Ward et al., 2014). Therefore, it is suggested that research in this subject should increase in quality in order to couple all information into single predictions instead of simple quantifications as observed until recently.

4. The presence of plastics in estuarine biota

In this review, both macro and microplastics contaminated organisms that inhabit estuaries (e.g. Dantas et al., 2019; Kazour et al., 2020). The most common approaches were analysis of the stomach content (e.g. Kartar et al., 1976; Possatto et al., 2011) and digestion with H_2O_2 of the whole organism (e.g. Pazos et al., 2017; Waite et al., 2018). A combination of digestion with saline flotation followed by filtration also seems to be a suitable option to analyse microplastics in tissues (Mathalon and Hill, 2014). In addition, a few studies have analysed excrement for plastic presence (Bravo Rebolledo et al., 2013; Mathalon and Hill, 2014) and even brain tissue (Crooks et al., 2019).

Given that plastics can be found in animals' stomach, tissues or excrements, plastic contamination is likely to negatively affect aquatic organisms. The toxic effects of plastic contamination can include lower feeding activity and loss of energy budget (Wright et al., 2013a), immune responses and oxidative stress (Avio et al., 2015; Canesi et al., 2015), and changes in metabolic rates (Green et al., 2016).

These effects have been shown for fully marine species but the information available for estuarine biota is limited to 34 studies looking at presence inside the organism, and only seven which have looked at the impact. For example, 17 works showed plastic ingestion by estuarine fish (e.g. Possatto et al., 2011; Ramos et al., 2012), but only 2 investigated effects. Dantas et al. (2019) looked at alterations in the condition factor (CF), which is a measure of health considering the weight and length for the Guri sea catfish *Genidens genidens* as proposed by Richardson et al. (2011). They found lower CF values related to plastic ingestion, while Miranda et al. (2019) showed a reduction in post exposure predatory performance and acetylcholinesterase activity (an enzyme used in neurotransmission) in the common goby *Pomatoschistus microps*. For other groups, microplastic exposure caused oxidative stress for the peppery furrow shell clam *Scrobicularia plana* (O'Donovan et al., 2018) and decrease in coelomocytes viability in the polychaete *Hediste diversicolor* (Revel et al., 2020).

Ingestion can result in direct physical harm to the animal's gastrointestinal tract such as obstruction or internal abrasions, and can ultimately result in death (Wright et al., 2013b). Besides that, during production of polymeric materials many additives such as plasticizers, stabilizers, antioxidants and biocides are commonly used (Hahladakis et al., 2018). These chemicals can be released to the environment due to plastic degradation and can also be toxic to the estuarine biota (Anbumani and Kakkar, 2018; Celis-Hernandez et al., 2020b).

Whilst it is important to understand the impact of microplastic ingestion by estuarine species, there are some limitations to field studies. There is an ethical issue about animal handling, as the procedures for plastic analysis are invasive and mainly lethal. Sampling faeces and other residues may be a good alternative but does not provide a full perspective as animals can retain plastic particles (Gebhardt and Forster, 2018). The effects of ingestion and trophic transfer of plastic items amongst estuarine organisms in the field have not been directly assessed.

Ingestion of plastic particles by both freshwater (e.g. Andrade et al., 2019) and marine organisms (e.g. Hall et al., 2015) have been previously reported and reviewed (Wagner et al., 2014; Wesch et al., 2016). We identified two papers analysing the ingestion of macroplastics. Guebert-Bartholo et al. (2011) investigated the stomach content of the green turtle (*Chelonia mydas*) in a Brazilian estuary, finding plastics in their gut. Although the green turtle is considered a marine species, the individual studied was part of a group that performs their foraging activities in this estuary. Bravo Rebolledo et al. (2013) investigated stomach, intestine, scats from the harbour seal (*Phoca vitulina*), finding plastics in 11% of individuals' stomachs, 1% for intestines, and 0% for scats. The investigation of plastic ingestion in marine/freshwater species that visit estuaries is important as they can be part of the plastic cycling in these environments, either removing plastics through ingestion or depositing them through excretion.

We identified 25 papers reporting microplastic ingestion by estuarine species, distributed in many animal taxa with different feeding habits (Table S5). Although microplastic abundance in organisms is commonly reported in percentage of individuals containing plastics (see Table S5), this type of representation is not comparable among species as they vary in size and therefore have different uptake capacities. However, a comparison can be made by analysing the percentage of organisms of different feeding guilds or trophic levels, for example, to have an idea of what group is more affected by this type of contamination.

The abundance of microplastics varied from 0 to 131 items per individual in predators (fishes and seals) and from 1.4 to 36 items per individual in deposit and filter feeders (bivalves and polychaetes) (Table S5). These differences point to an expected scenario where organisms in higher trophic levels (predators) tend to ingest more contaminants than animals in lower trophic levels (filter/deposit feeders). However, one should not compare these directly as plastics were analysed in different body parts: digestive tract for predators, whole organisms for filter feeders and excrement for deposit feeders. Yet, the trophic transfer of microplastics have been demonstrated in the laboratory (Crooks et al., 2019; Farrell and Nelson, 2013; Santana et al., 2017), but deeper investigation of consequences for estuarine species are still lacking.

In the Goiana Estuary (Brazil), at least eleven fish species were evaluated in order to understand how seasonal patterns of estuarine use by different phases of their life cycle affects rates of microfiber ingestion (Dantas et al., 2012; Ferreira et al., 2016, 2019a, 2019b; Possatto et al., 2011; Ramos et al., 2012; Silva et al., 2018). Within this system, contamination is enhanced during the late rainy season in the middle estuary, lower estuariy and coastal zone, coinciding with the highest availability of microplastics, when river runoff increases and flushes plastics seaward (Lima et al., 2014). Ingestion averaged 2.3 items individual^{-1} in lower trophic level fishes and up to 13 items individual^{-1} in higher trophic level fishes (Lima et al., 2020). Although every ontogenetic phase was contaminated, a positive relationship was observed between the number of microfibres and fishes ingested by adult piscivorous fishes. In such case, piscivorous fishes seem to be more susceptible to contamination through trophic transfer, especially because ~50% of the fishes ingested were also contaminated (Ferreira et al., 2016; 2019a; 2019b). Therefore, despite fishes exhibiting complex spatial ranges, those depending on estuaries often spend a whole season using estuarine resources during one life phase, which may coincide with peaks in microplastic availability.

Other works mainly analysed species widely used for human consumption (fish, oysters and mussels). Researchers have warned that in Iran humans may consume up to 555 microplastic items per 300 g of fish week^{-1} (Akhbarizadeh et al., 2018). However, studies regarding the effects of plastic consumption in humans are still lacking. Some species that were used to indicate plastic pollution are not directly consumed by humans but can be preyed upon by larger fish of economic importance (Dantas et al., 2012). Such species may have ecological roles that can be crucial to an estuary and therefore should also be surveyed. Most of these studies highlighted that better assessments of aquatic animals are further necessary to improve planning regarding environmental contamination with plastics.

5. Plastic toxicity in estuaries

In this review, three papers investigated plastic toxicity alone in estuarine biota. Li et al. (2020b) could not see any effects on retention time, gene expression related to metal-related stress, antioxidant defence or metabolic impact in the estuarine mussel *Mytilus edulis* after exposure to PVC particles alone or combined with cadmium. Meanwhile, exposure to microplastics caused oxidative stress in the European seabass *Dicenthrarchus labrax* (Barboza et al., 2018) and neurotoxicity in the common goby *Pomatochistus microps* (Miranda et al., 2019). Barboza et al. (2018) also reported that mercury was more bioconcentrated in the gills and bioaccumulated in the liver when European seabass were exposed to both metal and microplastics. On the contrary, Miranda et al. (2019) reported that the effects of simultaneous exposure of the common goby to chromium and microplastics caused an antagonistic effect (i.e. decreased neurotoxicity). Similarly, there is some evidence that the presence of microplastics decreased mortality associated with chromium toxicity in the common goby (Luís et al., 2015). These results are controversial, which indicates that different metals might interact differently with plastic particles resulting in changes in toxicity. Moreover, no paper emphasized the implications of estuarine conditions on those effects, which highlights the importance of investigating these interactions and with other types of contaminants in estuaries.

Estuaries often receive high levels of urban effluent, which carry contaminants from human activities such as pharmaceuticals, pesticides, antibiotics, personal care products and other contaminants of emerging concern (Loos et al., 2013; Pintado-Herrera et al., 2017; Celis-Hernandez et al., 2020b). Also, port and fishing activities often release chemicals to estuaries such as heavy metals from antifouling paints (AFPs) (e.g. Turner, 2010). These contaminants are toxic and some have the potential to bioaccumulate and biomagnify through the food chain (Farrell and Nelson, 2013; Celis-Hernandez et al., 2020a), and to interact with plastic items that can serve as a carrier for these compounds in estuaries (see "*Plastics and other contaminants in estuaries*").

The first paper reporting plastics in estuaries also reported polychlorinated biphenyl (PCBs) on the surface of plastic pellets (Carpenter and Smith, 1972). The authors suggested that this interaction was due to the presence of PCBs in water, as they were not used as plastic additives. PCBs are highly toxic for a number of species including humans and have been prohibited in many countries for many years (Penteado and Vaz, 2001).

Estuarine conditions can affect contaminant bioavailability. Differences in salinity/chlorinity, pH, temperature and suspended organic material directly influence chemical speciation of metals and organic contaminants and therefore their biological affinity (Salomons and Förstner, 2012; Xu et al., 2018). If estuarine species are subjected to such contaminants, it is important to understand how this combined exposure affects them. Estuarine species are threatened in three ways: (i) by plastics and leaching of their additives; (ii) by contaminants released from wastewater treatment plants, and (iii) by local activities such as port and fishing.

6. Biofouling on plastic in estuaries

Once in contact with water, any hard substrate such as plastics becomes rapidly covered by particles such as ions that form a conditioning film. Following this, microorganisms such as bacteria begin a process called biofouling, that can be defined as the direct or indirect biological association to either natural or synthetic hard substrates. An initial biofilm formed by bacteria and its extracellular polymeric substances (EPS) allows other organisms such as viruses, fungi, algae, protozoans and invertebrates to colonize a surface, which can support the development of a macroscopic community called the Plastisphere (Fig. 2) (Agostini et al., 2018; Galloway et al., 2017; Zardus et al., 2008; Zettler et al., 2013).

An important implication of the plastisphere is that the fouling community can change the probability of plastic particles being ingested. This has been shown for zooplankton, where Vroom et al. (2017) identified the preference of *Calanus finmarchicus* for biofouled microplastics over pristine ones. They associated this behaviour with the excretion of chemical attractors by biofilms that led zooplankton to mistake plastics for food. In contrast, Kaposi

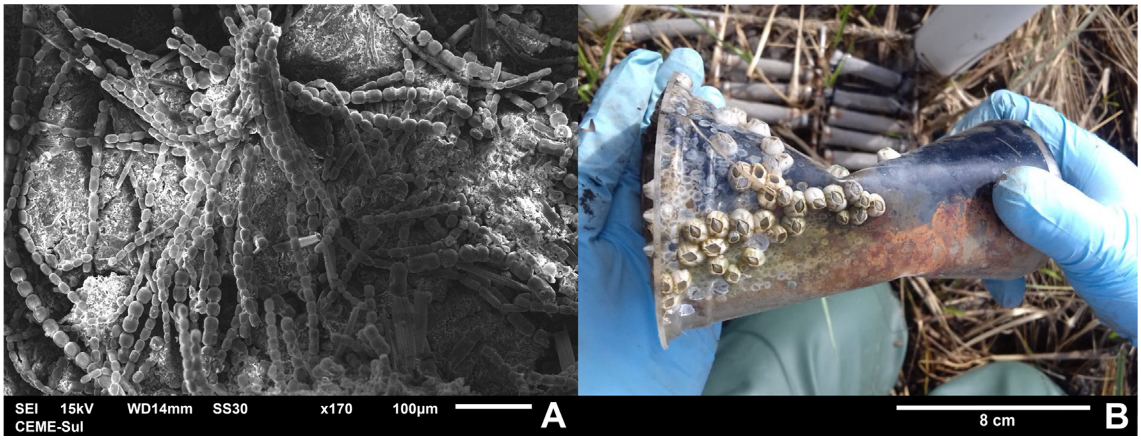

Fig. 2. A: Scanning electron microscopy of the surface of a microplastic particle (covered by gold), showing its rich microscopic biofouling community, mainly filamentous cyanobacteria. B: Macroplastic item (disposable cup) covered by algae and Cirripedia. Both plastic items were collected in the Patos Lagoon Estuary (Brazil). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

et al. (2014) showed that the percentage of *Tripneustes gratilla* larvae with microplastics in their stomach was higher when they were fed with non-fouled microplastics in comparison with fouled particles. They associated this with the behaviour of particle selection by larvae based on size, which was larger on fouled particles.

It has been proposed that some physical characteristics of the substrate and of the environment can interfere in biofouling (Agostini et al., 2017, 2018). Hence, this may be valid for plastic characteristics such as polymer type, size, colour and texture. In fact, Kettner et al. (2019) observed that the microbial community growing on polyethylene and polystyrene microplastics surface was different from that on wood or in the surrounding water. Environmental parameters such as pH, salinity, temperature, nutrient availability and light can also be determining factors in microbial associations in aquatic systems (Harrison et al., 2018; Oberbeckmann et al., 2018; Rummel et al., 2017). As Oberbeckmann et al. (2018) have shown, some bacterial assemblages colonize only polyethylene and polystyrene microplastics but not wood, especially under a high salinity, low nutrient level scenario. These characteristics can vary greatly in estuaries, which affects the response of associated microbial communities.

Substrates can face alternated inundation periods caused by tides in estuaries. The fouling process can therefore be interrupted as the substrate is exposed to air, therefore the survival of the plastisphere will depend on the protection provided by its own structure (EPS) and the time it remains exposed to drought. This has been suggested to have a potential effect on the plastisphere community, but has not yet been investigated (Harrison et al., 2018).

Other factors such as plastic age (weathering) can vary in estuaries. Rivers, streams and WWTPs are often considered plastic sources (Jambeck et al., 2015), so plastics from those sources can be relatively new. In contrast, plastic residence time in the ocean is normally higher (Gewert et al., 2015), which could enhance weathering. Either way, plastic weathering will influence the biofouling process, e.g. by favouring the adherence of microorganisms to plastic (Rummel et al., 2017). Therefore, plastisphere formation and composition are likely to differ between upper and lower areas of estuaries, although this remains under investigated.

To date there are no studies investigating the effects of plastic pollution on primary producers (e.g., cyanobacteria and diatoms), but it has been shown that biofouling organisms can alter the physical properties of plastics. For example, biofouling can make plastics denser, making them sink faster (Fazey and Ryan, 2016; Long et al., 2015). Ye and Andrady (1991) exposed macroplastic items to natural conditions at the Biscayne Bay (USA) for about seven months. They observed all stages of the microfouling process and that defouling (dispersion) can occur due to chemical changes in the water as the item submerges due to increasing density, and this may be followed by a new fouling cycle.

Microorganisms that degrade polymers can also increase their buoyancy, favouring an upward movement of plastics (Rummel et al., 2017). However, biofouling can lower plastic exposure to sunlight (UV radiation) and oxygen, which slows chemical degradation processes (Kershaw et al., 2011). Either way, plastic dynamics are potentially altered by associated organisms. Most evidence shows that biofouling leads to sinking, e.g. Kaiser et al. (2017) established that the sinking velocity of biofouled PS, a negatively buoyant polymer, increased by 16% in estuarine water and 81% in marine water after 6 weeks. However, a recent study by Nguyen et al. (2020) provides different scenarios, showing that negatively buoyant polymers (PVC, polyurethane, and polyethylene terephthalate) have their sinking velocity increased when high density biofilm is attached to them, but they tend to become neutrally buoyant or even rise when aggregated with low-density biofilm. They also showed that positively buoyant polymers (PP and high-density PE) had their rising velocity enhanced when fouled by low-density biofilm but started to settle when high-density biofilm is attached due to the effect of the increasing size of the aggregate (Nguyen et al., 2020). Therefore, they suggested that the formation of biofouling on microplastic surfaces depend on factors such as the plastic density, size, and shape but also strongly on the biofilm density (Nguyen et al., 2020). Kooi et al. (2017) developed a model for the effects of biofouling on this vertical movement of microplastics but environmental validation is still lacking.

Plastics and their plastisphere can be transported for long distances. In an estuary, this means that species living in the upper part, where freshwater predominates, might be transported to lower parts where salinity is higher. If the salinity range is high and the species is stenohaline, it might not survive the osmotic change. In contrast, euryhaline species may be able to survive a wide salinity change. Also, species transport due to biofouling of plastic items may result in the establishment of exotic species in non-native environments. Therefore, these invasions can have a range of outcomes for the environment and for the invading species itself (Grosholz and Ruiz, 2003; Thiel and Gutow, 2005).

7. Plastics and other contaminants in estuaries

Plastics can act as vectors for contaminants in the aquatic environment due to their high capacity to adsorb such components on their surface. These interactions are directly influenced by physicochemical properties of the surrounding matrix such as dissolved and particulate organic matter, pH and chlorinity (Salomons and Förstner, 2012; Xu et al., 2018). These properties can vary greatly in estuaries due to freshwater-saltwater mixing, dependent on local hydrodynamic conditions. Organic matter concentrations tend to be higher in the upper estuary than in the lower section as the input of organic material tends to be greater in this area (Middelburg and Herman, 2007). The chlorinity/salinity gradient is naturally accentuated in estuaries, and together with differences in biological activity they can also influence pH variation along these gradients (Howland et al., 2000).

The sorption capacity of polymers also depends on their properties (polymer type, colour, degradation level) and environmental properties (salinity, pH, organic matter, presence of other sorbents) (Wang et al., 2018). In general, plastics are excellent transport agents for hydrophobic and metallic chemicals dissolved in the water, e.g. plastic polymers might have a greater sorption capacity for some persistent organic pollutants (POPs) than minerogenic sediments (Teuten et al., 2009). It can therefore be complicated to understand sorption dynamics in estuarine plastics.

Estuarine environments are often close to contamination sources such as ports, marinas and harbours, where antifouling paints (AFPs) are widely used in order to protect boats and ships from biofouling organisms (Thomas and Brooks, 2010), but their properties also act on non-target species in the environment (Soroldoni et al., 2017). A study by Onduka et al. (2013) showed toxic effects of commercial DCOIT (4,5-Dichloro-2-octyl-4-isothiazolin-3-one) in four species of algae, two crustaceans and one polychaeta, all coastal species. Therefore, AFPs act as contamination sources by releasing toxic chemicals that can potentially achieve significant concentrations in the environment and even interact with other particles such as plastics.

Another contamination source in estuaries are Wastewater Treatment Plants (WWTP), which are also a major source of microplastics (Conley et al., 2019; Xu et al., 2019) and other contaminants such as perfluoroalkyl substances (PFASs) and pharmaceutical and personal care products (PPCPs) to estuaries (Zhou et al., 2019). Some of these compounds interact with plastic particles (Wu et al., 2016), but no papers investigating this in estuaries were found in this review.

We identified seven research papers regarding plastic interaction with heavy metals and five papers with organic compounds in estuaries. Turner (2016) and Turner et al. (2015) found high concentrations of Cu, Pb, Zn and Sn, which is an important indicator of organotin compounds banned years ago. Holmes et al. (2014) performed a field experiment by exposing beached and virgin microplastics to estuarine conditions and found that pH and salinity changes through the estuary alter adsorption rates of metals, and that adsorption was much higher in beached (degraded) pellets. One paper investigated the adsorption of dichloro-diphenyl-trichloroethane (DDT) and phenanthrene using realistic salinity levels in order to simulate riverine, estuarine and marine environments in laboratory experiment (Bakir et al., 2014). Although concentrations of DDT and phenanthrene were slightly higher under estuarine than riverine and marine conditions, the effect of salinity on sorption kinetics was not significant. Other properties such as contaminant concentration, proximity to contamination source and plastic transport may have a stronger influence on this interaction.

Regarding biota, the capacity of microbial biofilms to absorb or even metabolize contaminants in the surrounding environment has been documented for heavy metals (Ancion et al., 2010) and organic compounds (Writer et al., 2011). If these organisms can occupy plastic surfaces, it can be expected that they will affect plastic sorption for other contaminants, but it remains unclear whether biofilms would increase or decrease plastic sorption capacity. Indeed, concern over the role of the plastisphere is increasing as it has been recently proposed that understanding how biofilms in microplastics interfere with primary production processes and interactions between organisms is largely understudied (Harrison et al., 2018).

8. Conclusions and remarks

Estuaries are key systems acknowledged to be systematically contaminated by plastics in both biotic and abiotic compartments. This review adds information in the so called “source-to-sea” approach in order to support future research on the estimates of plastics in rivers and estuaries. Many estuaries around the globe have not yet been investigated for plastic contamination (Fig. 1), and even worse is the case of riverine systems. Within this review, just under 100 of the more than 1200 estuaries in the world were discussed, with large gaps in the knowledge particularly concerning Africa, eastern Europe, Oceania, Central America, and western South America, that are absent from study. Despite a range of sampling methods deployed, current efforts should focus on standardizing procedures to avoid underestimations and to increase comparability in different environmental settings. Sampling designs must consider links among biological, sedimentary, and physicochemical factors to assess and predict contamination accounting for spatial, temporal, and seasonal fluctuation of environmental gradients, such as those observed in estuaries. Plastic quantities appear to be higher in river and estuarine sediments than in the water column, as expected for contaminants as a result of the mixing of water masses, accretion of bottom sediments and high sedimentary input from terrestrial sources. Thus, the water matrix is more relevant to understand episodic variation in contamination, while sediments might be more suitable for long term investigations. This review has also highlighted that semi-controlled field experiments are a valuable approach to achieve reliable results in realistically relevant scenarios and thus should be encouraged.

Plastic dynamics in estuaries are not fully understood and future studies are recommended to use the following spatial-temporal approaches: (i) water sampling at different depths and estuarine reaches to assess differences between freshwater and seawater according to the vertical stratification throughout the estuary; (ii) sediment sampling at deeper depths to account for stratigraphic variation (e.g. to 0.5 m), considering sedimentation rate, sediment permeability relative to particle size, vegetation, bioturbation, human action (e.g. dredging) and extreme weather events.

Plastics interact with both lower trophic level organisms and top predators, showing a generalized exposure within estuaries but key processes remain unclear. Some questions should be addressed in future surveys: (i) how plastics bioaccumulate and are transferred between trophic levels along the river-estuary-sea continuum, and (ii) how patterns of use of estuaries during different life phases influence contamination and interaction rates. Investigation should focus on trophic/functional guilds and ontogenetic shifts in feeding behaviour, in order to consider community structures and intra-specific relationships, respectively, rather than use “species” as an ecological unit, in order to provide insights for management based on monitoring of economically and ecologically important species.

Estuaries are often associated with highly urbanized centres, which is associated with the release of environmental contaminants such as persistent organic and metallic compounds. Both plastics and biofouling organisms can interact with these chemicals, and efforts are increasing to understand the contribution of biofouling organisms as possible vectors of contaminants onto plastic surfaces, but it remains uncertain whether these interactions increase the bioavailability of chemical contaminants, and, consequently their toxicity to organisms.

The discussion of plastic pollution mitigation and toxicity has to include synthetic fabrics, as fibres from these sources are abundant. Accordingly, fibres are commonly ingested by aquatic organisms and, thus, financial support to quickly understand how hazardous fibres are is another step to couple this information with those available for other contaminants. This is needed by organizations such as the European Environment Agency (EEA), U.S. Environmental Protection Agency (EPA), Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) to establish safe levels of microplastics in aquatic organisms for human consumption. Robust sampling is needed to predict how, where and when plastic ingestion, absorption by animal tissues and toxicity peak in the natural environment. Once these are elucidated beyond simple ingestion, efforts can be made to evaluate links with human health.

In summary, economic activities surrounding river basins, estuaries and adjacent coastal waters have been neglected concerning the risk assessments for plastic contamination, even though these are necessary to guarantee the ecological functions of these systems. Models are necessary to predict the fate of micro- and macroplastics in aquatic environments; and the inclusion of the above socio-economic aspects in modelling techniques is relevant regarding management approaches.

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Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2021.116908.

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Capítulo VIII: Artigo 2

O segundo artigo científico proveniente desta Tese de Doutorado é apresentado nesse capítulo. O manuscrito, de autoria de Lara Mesquita Pinheiro, Isadora Vieira Carvalho, Vanessa Ochi Agostini, Gustavo Martinez-Souza, Tamara Susan Galloway e Grasiela Lopes Leães Pinho, é intitulado "***Litter contamination at a salt marsh: An ecological niche for biofouling in South Brazil***" e foi publicado no periódico *"**Environmental Pollution**"* e se encontra disponível no link https://doi.org/10.1016/j.envpol.2021.117647 (0269-7491 / © 2021 Elsevier Ltd. All rights reserved). O material suplementar do artigo se encontra no ANEXO II desta Tese.

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Litter contamination at a salt marsh: An ecological niche for biofouling in South Brazil☆

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ABSTRACT

The presence of solid litter and its consequences for coastal ecosystems is now being investigated around the world. Different types of material can be discarded in areas such as salt marshes, and various fouling organisms can associate with such items forming the Plastisphere. This study investigated the distribution of solid litter along zones (dry, middle, flooded) of a salt marsh environment in the Patos Lagoon Estuary (South Brazil) and the association of biofouling organisms with these items. Solid litter quantities were significantly higher in the dry zone when compared to the middle and flooded zones, showing an accumulation area where the water rarely reaches. Most items were made of plastic, as shown for many other coastal areas, and originated from food packaging, fishery and shipping activities and personal use. Although not statistically significant, there was a tendency of increased biofouling towards the flooded zone. Thirteen groups were found in association with solid litter items, mainly algae, amphipods, and gastropods. The preference for salt marsh zones, types of material and items' colour was highly variable among groups of organisms, which can be related to their varied physiological requirements. In summary, significant plastic contamination of salt marshes of the Patos Lagoon was associated with a heterogeneous distribution of fouling communities.

1. Introduction

Anthropogenic litter is now a major problem around the world (Galgani et al., 2015). Several studies have reported tons of litter entering the world each year (Jambeck et al., 2015). It is known that several types of material can be found in marine and coastal environments, characterizing situations of environmental contamination and/or pollution (Bergmann et al., 2015). Plastic is usually the main component of marine litter, but also metal, glass, paper, and wood are commonly found in marine and coastal ecosystems (Tekman et al., 2019) and can interact with biota in many ways such as ingestion, entanglement, fouling and toxicity caused by the interactions with other contaminants (GESAMP, 2019).

Among the different interactions between solid waste and biota, biofouling is one of the least studied issues. This type of interaction among organisms and litter in the environment has received some attention in recent years, mainly concerning microfouling (Zettler et al., 2013). This association begins with a process that occurs naturally in aquatic environments - mainly in estuarine and marine environments - when biological deposits (fouling, sedentary and vagile organisms) are associated directly or indirectly on hard surfaces (Agostini et al., 2018). This process consists of (*i*) contact of the material with water, (*ii*) adhesion of ions and other compounds on surface, (*iii*) colonization from microorganisms to macroorganisms (Agostini et al., 2018).

There is evidence in the literature for what types of material are more attractive to biofouling organisms. In wastewater treatment structures,

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Noble et al. (2016) showed that the fouling microbial community differed among ethylene–propylene-diene monomer (EPDM), polyurethane, and silicone substrates, which at that point was associated with differences in chemical and physical characteristics of the material such as the availability of organic compounds (e.g., plasticizers). In the marine environment, invertebrate larvae have preference for plastic surfaces over wood or concrete (Pinochet et al., 2020), and evidence for colonization preference for more hydrophilic and textured surfaces was shown for mussels (Carl et al., 2012). Microtopography is important for some coral larvae settlement (Whalan et al., 2015). Surface colour also influenced the macrofouling community composition, as shown by Dobretsov et al. (2013), but further implications of preferences of the fouling community and subsequent differences in its structure in solid litter is still understudied in aquatic systems.

The presence of organisms attached to solid litter surfaces can have consequences for the items and/or the organisms. Some items are positively buoyant in water due to their material density or the presence of air but can sink if organisms colonise the surface. This was previously considered as one of the main explanations for the missing amounts of plastic items in the sea surface (Cózar et al., 2014; Ryan, 2015). Also, organisms can alter plastic degradation as they might act as a protection for abiotic forces that degrade polymers such as UV radiation (Weinstein et al., 2016). On the other hand, some microorganisms have the ability to degrade synthetic polymers and therefore enhance plastic degradation (Shah et al., 2008).

Organisms associated with solid litter items can be carried to different sites, where they can encounter adverse environmental conditions. On the other hand, if organisms find favourable conditions, it can result in bioinvasion of other habitats (Therriault et al., 2018). Another consequence is the potential toxicity of some materials such as

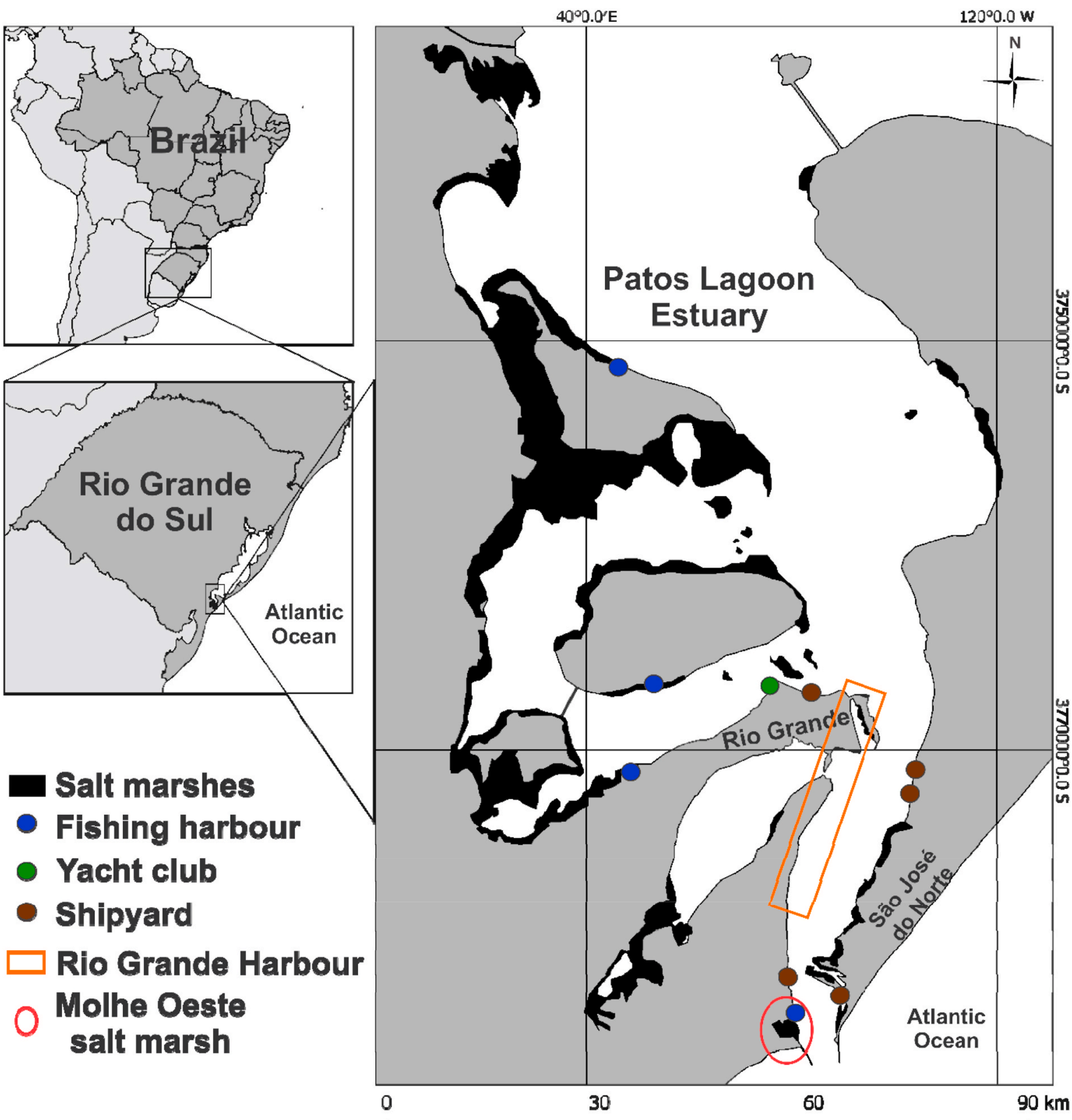

Fig. 1. Location of Molhe Oeste salt marsh (red circle) at the Patos Lagoon estuary, in the Rio Grande do Sul state, southern Brazil. The Patos Lagoon estuary has 24 salt mash units (in black), described by Marangoni and Costa (2009). Main cities (Rio Grande and São José do Norte) and activities in the estuary are indicated in the figure. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

plastics associated with additives added during production (Hahladakis et al., 2018). In this sense, fouling organisms are then susceptible to the effects caused by such chemicals.

Although the biofouling process is well studied when associated to man-made aquatic structures (Flemming, 2002), there is a gap regarding association of organisms on solid waste items of anthropogenic origin such as macroscopy litter and organisms in estuarine ecosystems (Pinheiro et al., 2021). Estuaries are highly productive ecosystems as they serve as a niche for freshwater, estuarine and marine species. Estuaries are submitted to both riverine and oceanic inputs of organisms, water and, consequently, contamination (European Commission, 2011), resulting in a complex hydrodynamics, which can lead to temporary storage of suspended material, including contaminants (Hartmann and Schettini, 1991). Habitats such as mangroves, seagrasses, mud flats and salt marshes can be present in such regions (Zhang, 2017).

Salt marshes are intertidal coastal environments that occur in regions of middle and high latitudes around the world (NOAA, 2018). This type of environment is strongly influenced by tide and therefore constantly flooded with water, with different flooding rates among zones. Lower zones in the marshes are flooded more often than higher zones, and different plant species can be found according to the flooding rate (Costa et al., 2003). These species can intensively occupy the area forming a dense canopy (Nieva et al., 2001), and can act as a trap for debris in different intensities according to species composition or distribution in the zones.

Studies focusing on solid waste contamination in salt marsh environments are scarce. A few studies have looked specifically into this environment in the northern hemisphere - United States (Gilligan et al., 1992; Uhrin and Schellinger, 2011; Viehman et al., 2011) and in Spain (Mazarrasa et al., 2019) - but do not cover the extensive salt marsh distribution around the globe (Mcowen et al., 2017).

The Patos Lagoon, located in south of Brazil, is the world's largest choked lagoon and the largest coastal lagoon in South America (Kjerfve, 1986) (Fig. 1). Its margins host various urban centres including large cities, agricultural areas and one of the biggest harbours in Brazil, the Rio Grande harbour. Human activities have impact on natural ecosystems of the lagoon such as the salt marshes located in the estuary, causing degradation on the salt marshes over the last decades (Marangoni and Costa, 2009a).

Salt marshes in the Patos Lagoon Estuary suffer from solid litter contamination (Marangoni and Costa, 2009b). The origin of litter has been associated with direct or indirect deposition, and the type of deposition and the accumulation can differ among higher and lower zones of the marsh (Marangoni and Costa, 2009b), but this was not deeply investigated. Also, there is no previous report of the association of this type of contamination with biofouling organisms and the possible consequences for this ecosystem.

The aim of this work was to investigate litter contamination in a salt marsh (Patos Lagoon Estuary - PLE, Brazil) and its characteristics and distribution in different zones, evaluating how macroorganisms can be colonizing items with different characteristics. The authors hypothesize that (i) litter quantities are higher in higher areas, with lower flooding rates and denser vegetation, (ii) biofouling occurrence is higher lower, more flooded areas, and (iii) the attached organisms vary along salt marsh zones and between types of solid litter.

2. Materials and methods

2.1. Study area

The Patos Lagoon is a choked coastal lagoon located between 30 and 32° S (Möller and Castaing, 1999). The lagoon covers an area of >10, 000 km^2 with approximately 7 million habitants on its margins and drains an area of nearly 200,000 km^2 (Moller et al., 2001; Tavora et al., 2019). Its estuary is under the influence of a microtidal regime, and the main factor affecting water level is the southwest-northeast wind regime (Moller et al., 2001). Its 24 salt marsh units were defined by Marangoni and Costa (2010), which vary in size, location, vegetation, and type of impact suffered. These intertidal environments receive a large amount of waste due to human use, its location and the surrounding hydrodynamics, which can influence the type and amount of waste (Siqueira et al., 2017).

The Molhe Oeste marsh (at 32° 09′ 09.3″S and 52° 06′ 03.1″W) is located at the mouth of the PLE (Fig. 1). It is influenced by both Rio Grande and São José do Norte cities and also by the Atlantic Ocean. It has four zones well defined by vegetation (Fig. 2) and flooding rate: mud flat – MF (flood index 100%), low marsh – LM (flood index 64%), middle marsh – MM (flood index 20.1%) and high marsh – HM (flood index 3.1%) (Perillo et al., 1999).

2.2. Litter sampling

To assess litter contamination in the Molhe Oeste salt marsh, litter items >5 mm were sampled above the sediment in twenty-one 10 × 2 m transects delimited within an area of approximately 2,300 m^2 inside the marsh where all four zones were present. Nine of the 21 transects were sampled in a first survey in October of 2017 and the remaining twelve in August and September 2018. This second survey was performed to increase our sample size. The transects were delimited in three areas of transition between the four zones of the marsh. There was a total of seven transects per transition area, hereafter referred as flooded (MF – LM, flood index 64–100%), middle (LM – MM, flood index 20.1–64%) and dry (MM – HM, flood index 20.1–3.1%) zones. The flooded zone is partially not vegetated and partially vegetated by *Spartina alterniflora* specimens, while the species *Spartina densiflora* appears as the flooding rate increases in the intermediate zones. Species other than *Spartina* are present in the dry zone, such as *Scirpus maritimus*, *Juncus kraussii, Hydrocotyle bonariensis,* and *Myrsine parvifolia* (Fig. 2).

Items that were too large to be carried (>1 m) were photographed and left at the sampling site. In the laboratory, collected items were screened considering their composition (e.g.: plastic, metal, wood, wax, paper/cardboard, glass, foam/sponge, organic, Tetra Pak®, rubber, fabric, mixture, and not identified – NI), plastic type (if identifiable), size, fragmentation, texture (hard or soft) and colour. Another variable implemented to classify the items was their degradation level, which is described by Siqueira et al. (2017). These authors used a visual scale based on physical properties to estimate the decomposition stage of solid litter. Items that had their original colours present, clear label and/or bar code, and that were not yet fragmenting can be classified as *recent*; items that are starting to weather, present some colour alterations, possible biofouling, but it is still possible to identify their use can be considered *intermediate*; items that are highly weathered and fragmented, brittle, present biofouling, or cannot be identified can be considered *old* (Siqueira et al., 2017). In this work, we used the nomenclature deg1, deg2 and deg3 for recent, intermediate, and old items, respectively. After analysis, litter items were disposed appropriately according to the recycling policy at the laboratory.

For biofouling analysis, litter surveys were performed in May, August, and September 2018 in three 30 m-long transects delimited in the marsh, comprehending all four zones. All observed solid waste items found 1 m to the right and 1 m to the left of the transect were analysed. The following variables were analysed visually: composition of the item, size, degradation level (as described above, based on Siqueira et al., 2017), fragmentation, texture (hard or soft), colour, and presence of biofouling. For items that presented biofouling, we also analysed the groups of associated animals (e.g.: algae, hydrozoans, isopods), and percentage of coverage by fouling (cover1: <10%, cover2: 11–25%, cover3: 26–50%, cover4: 51–75%, cover5: 76–100%), which were both determined by a single qualified observer for consistency. These percentages were estimated by visually comparing the extension of coverage (for algae and fungi) or body sizes (for identifiable individuals) of each group of organisms to the item's size, as at this point it was not

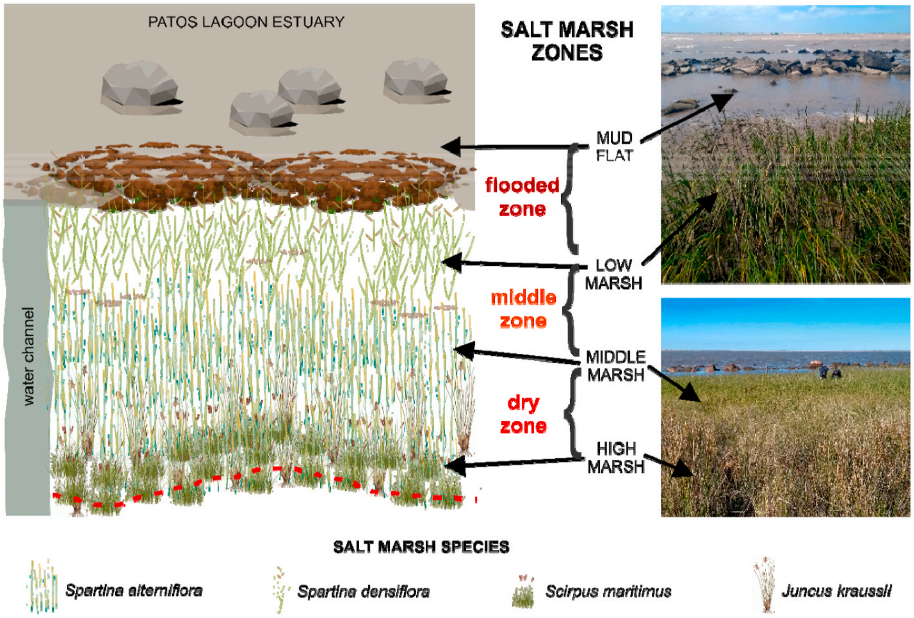

Fig. 2. Representation of the Molhe Oeste salt marsh at the Patos Lagoon estuary, in the Rio Grande do Sul state, southern Brazil, including common plant species and photographs of the zones. Other species such as *Hydrocotyle bonariensis* and *Myrsine parvifolia* were also present at the High Marsh but are not represented in this figure. The dashed red line indicates the upper limit of the sampling (i.e., the strandline). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

possible to actively measure the coverage as vagile organisms would rapidly dissipate. Examples of these estimations for biofouling groups can be seen on Fig. 3.

2.3. Data analysis

Litter quantities were reported in number of items per area sampled (nº of items m^{-2}). Qualitative variables of composition, colour, texture, and fragmentation were reported and analysed as percentages of the total number of items in each transect. Average size was reported by calculating the average size (cm) of the items in each transect, and average degree of degradation was reported as the weighted arithmetic mean of degradation level, $x'=((x_1*1)+(x_2*2)+ (x_3*3))/x_1+x_2+x_3)$. Data normality for litter quantities (nº of items m^{-2}), texture (% of hard items), fragmentation (% of fragmented items), average degree of degradation, and average size (cm) was tested using individual Shapiro-Wilk normality tests. All data fit normal distribution ($p < 0.01$), and the residues from the models were also analysed for homoscedasticity and normality. Other variables such as composition, plastic type, and colour were also reported as percentages of total number of items in each transect. Biofouling was reported as the frequency of biofouling occurrence (% of fouled items in each transect). To evaluate whether the areas inside the marsh (*i.e.,* flooded, middle and dry) showed differences in the item's quantity, texture, fragmentation, degree of degradation, average size, and biofouling, ANOVA tests were performed for each variable, with a *posthoc* Tukey test. All aforementioned tests were performed in the Past software version 3.21 (Hammer et al., 2001).

To investigate differences in the number of items between salt marsh

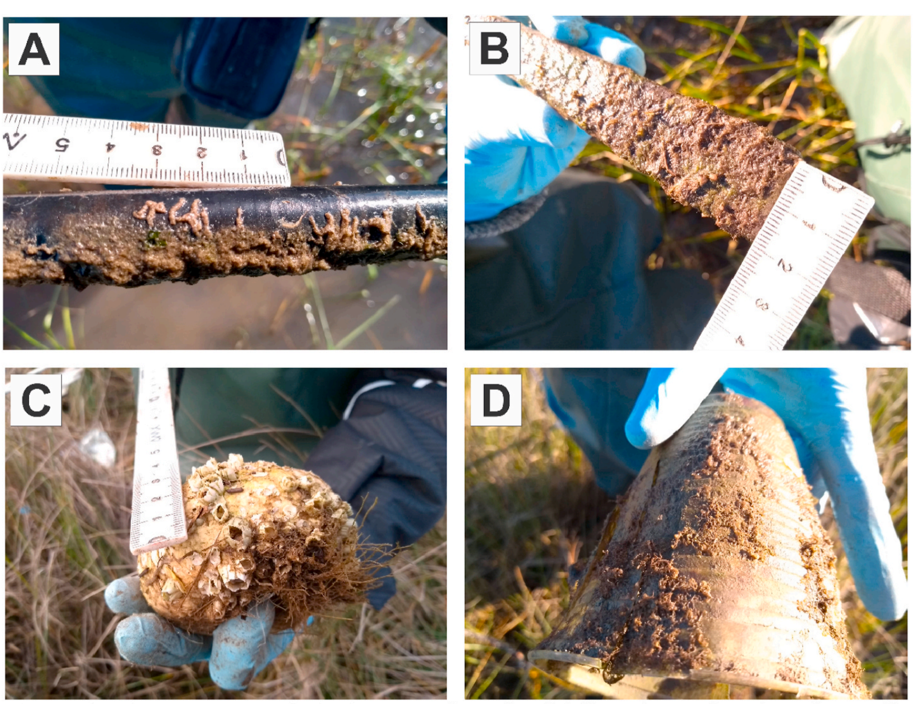

Fig. 3. Solid litter items found at the Molhe Oeste salt marsh at the Patos Lagoon estuary, in the Rio Grande do Sul state, southern Brazil. The biofouling coverage (cover1: <10%, cover2: 11–25%, cover3: 26–50%, cover4: 51–75%, cover5: 76–100%) for each group was estimated visually as described in the materials and methods section, and it is indicated inside parenthesis. A: metal stick with polychaete marks (cover1) and algae (cover3); B: wooden stick covered in algae (cover5); C: buoy with barnacles (cover3), algae (cover5), and hydrozoans (cover4); D: disposable cup with algae (cover5), fungi (cover2), among other groups.

zones and sampling months (October 2017, August 2018, September 2018), a Generalized Additive Modelling (GAM) analysis was employed (Hastie & Tibshirani, 1990). The GAM is an extension of the generalized linear model (GLM) with a linear predictor involving a sum of smooth functions of covariates (McCullagh & Nelder, 1989; Wood, 2006). The degree of smoothness of model terms was estimated as part of a fitting using penalized cubic regression splines. A Gaussian error model was used in the GAM analysis, with a link identity function. It is proposed that the means and variances in three salt marsh zones are dependent as they are adjacent areas, so for GAM analysis the categories "flooded", "middle" and "dry" were transformed in "1", "2" and "3", respectively (Fig. S1). Additional GAM analyses were also used to investigate the relationship between the presence of biofouling (by all species, algae, and amphipods) and the same candidate covariates (number of items, fragmentation, hardness, degradation level, size, colour, % biofouling and coverage classes, salt marsh zones). Only the presence of biofouling was considered, and therefore a Binomial error model was used in the GAM analysis, with a logit link function. Only biofouling groups with occurrence higher than 10% (algae and amphipod) could be modelled. Model selection was guided by Akaike's Information Criterion (AIC), where lower values as better-fitted models. All GAMs were implemented using the *mgcv* library of R (package version 1.7–5) (Wood, 2011). Finally, to assess the predominance of biofouling groups in different zones, types of material and colours of litter, the contribution index (CI) of each group was calculated by the Similarity Percentage test (SIMPER).

3. Results

3.1. Litter composition in the salt marsh

A total of 2,247 items were collected in the Molhe Oeste salt marsh during samplings. A Generalized Additive Model showed that the sampling period did not influence the number of items in each zone (Fig. S1 and Table S1), so this factor was not further considered for data explanation. The average litter quantity was 5.35 ± 6.02 items m^{-2}, with a range from 0 to 22.15 items m^{-2}. Figs. 3 and 4 shows the variety of materials found, including details of plastic polymer types, their colours and previous use. Plastic was the main type of material found in all zones in the marsh (92.4%), followed by wood (3%) and paper/cardboard (1.2%) (Fig. 4A). The other materials represented together approximately 3%. Among plastic items, it was possible to identify some polymer types, although the majority of the items (79%) had no indication of its polymer type. PET (4.8%) and Nylon (4.5%) were among the most common plastic types, followed by PS (3.1%), PP (1.6%) and others (< 1.5%) (Fig. 4A). Regarding to colours, items were mainly transparent (28%), white (20.8%) and colourful (15.9%), but other colours such as blue (12%), green (5.6%) and brown (4.1%) were also present, among other colours (< 12.5%) (Fig. 4B). Items that could have their previous use identified (25.7%) were mainly from food packaging (12.4%), fishery and shipping (5.2%), personal use (5.1%), and others (< 3%) (Fig. 4C). Items in the flooded zone were mainly from food and from personal and domestic use, while fishery and shipping material were mostly found in the higher zones (Fig. 5).

Litter from seven transects in each of the three salt marsh zones were sampled and analysed in order to verify differences among solid litter distribution and among some characteristics of the items. Both ANOVA (Fig. 6A) and GAM (60.8% of explanation, Fig. S1 and Table S1) analyses showed a significant increase in litter quantity (i.e., number of items per m^{-2}) in the dry zone, the zone with lower flooding rate, when compared to flooded and middle zones. Also, there was a higher occurrence of hard materials then soft materials in zones middle and dry, when compared to flooded (Fig. S2B). There was no significant difference regarding to fragmentation of the items, their degree of degradation, and average size

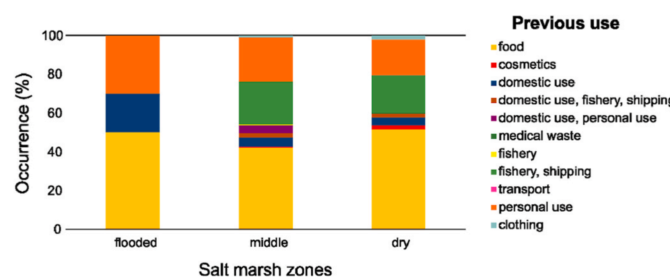

Fig. 5. Previous use of solid litter found in each zone of the Molhe Oeste Salt marsh, without not identified items (n = 576).

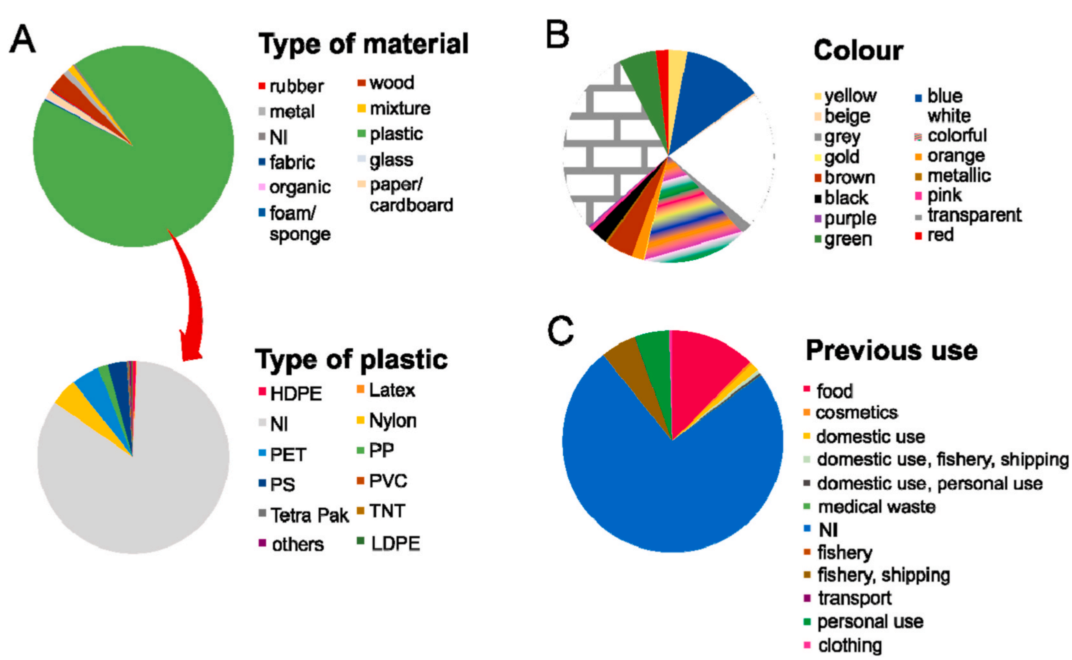

Fig. 4. Types of material of solid litter (n = 2,247) found in the Molhe Oeste Salt marsh with details of A: plastic types (n = 2,112); B: solid litter colours (n = 2,247); and C: previous use (n = 2,247). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

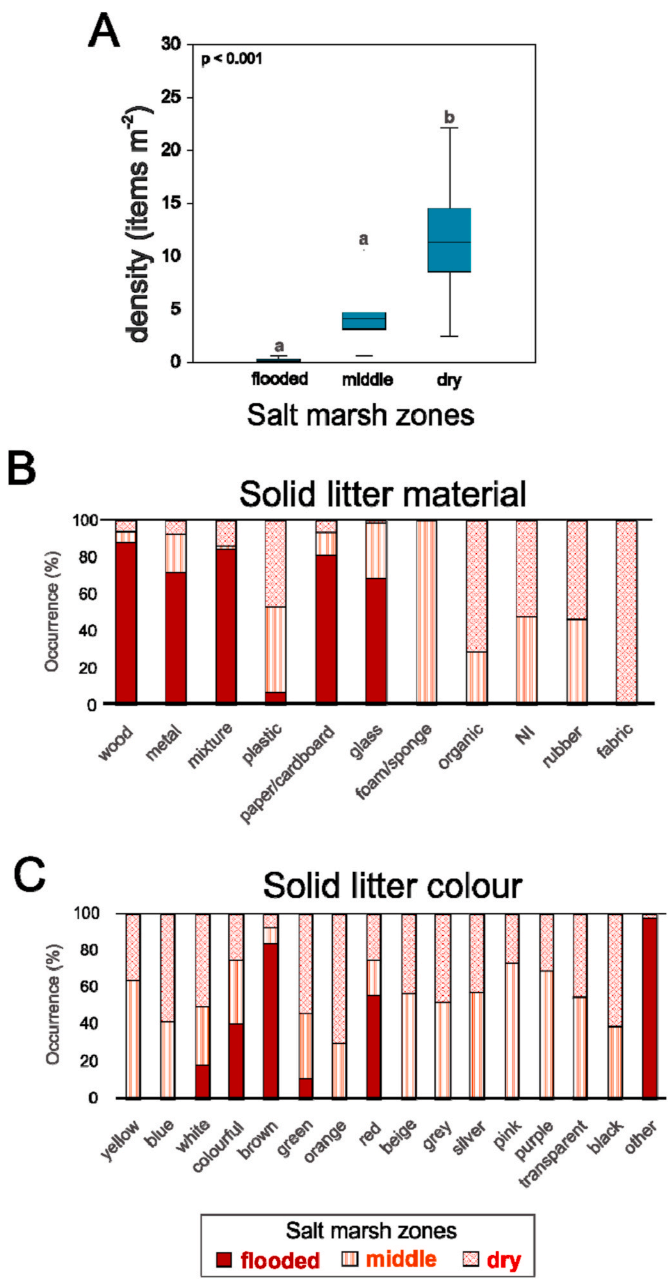

Fig. 6. Solid litter physical characteristics by salt mash zones (n = 2,247). A: Solid litter quantity (items m^{-2}), where similar lowercase letters indicate similarities (ANOVA, $p > 0.05$), and different lowercase letters indicate statistical differences ($p < 0.05$) among the salt marsh zones; B: Distribution of solid litter material among salt marsh zones (n = 430); C: Distribution of solid litter colours among salt marsh zones (n = 430). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

among zones (Figs. S2A, S2C and S2D).

Fig. 6B and C shows the distribution of the solid litter materials and colour regarding to salt marsh zones, respectively. It appears that the majority of material types and colours were more associated with the middle and dry zones, while items found in the flooded were less diverse, with items made mainly by wood, glass, and metal and with colours brown, red, and colourful.

3.2. Biofouling of litter in the salt marsh

The association of fouling organisms with solid litter items and its characteristics was evaluated among the salt marsh zones (Fig. 7). Although the biofouling occurrence on the plastic tended to increase from dry to flooded, this was not statistically significant when considered only total number of items per transect (ANOVA $p > 0.05$, Fig. 7A). However, GAM analyses showed some level of explanation (15.1%) of the number of items by salt marsh zone ($p > 0.05$), sampling period ($p < 0.01$), items size ($p < 0.03$), and degradation level ($p > 0.05$) (Fig. S3 and Table S2).

A total of 13 groups of organisms were identified: mite, algae, amphipod, mussel, crab, coccum (insect), barnacle, fungi, gastropod, hydrozoan, insect, isopod and polychaeta. The biofouling groups presented different biofouling coverages, as exemplified in Fig. S4. In general, the group that was most found in association with the litter items was algae (53.3%), which also commonly occurred in high coverage classes (Fig. S4). The other most common fouling groups were amphipods (18.94%) and gastropod (5.73%). The other groups represented together 22.03% of the total found in all items.

It was possible to identify the preference of each group according to the salt marsh zone, the solid litter material, and its colour (Fig. 7B and C, and Table 1). Algae were preferentially present in the dry zone, mainly associated with transparent plastic items. This significant association of algae with transparent items ($p < 0.05$) was also seen on the GAM analysis, as well as for white items ($p < 0.03$), considering the different salt marsh zones, sampling periods and items size (Figure S5 and Table S3). Amphipods were more present in the dry zone, preferably in brown, wooden material (Table 1). This strong relationship with the dry zone was also seen in the GAM analysis ($p < 0.01$) (Figure S6A), when the model also considered the variables sampling period, items size and degradation level. This preference for the dry zone and wooden material was also observed for fungi and isopods (Table 1). However, the majority of the groups (61.5%) were preferentially present in items found in the middle zone. Among the ten different types of material found in the salt marsh, plastic items were preferred by most of the groups (38.5%), like algae, crabs, insects, and mite (Table 1). Brown items were also preferred over other colours, as found for mussels, fungi, gastropods, and isopods (Table 1).

4. Discussion

The results shown here are the first to investigate solid litter contamination in a salt marsh environment in South America, and they represent a snapshot from an important coastal ecosystem in a temperate region. Salt marsh environments are considered areas of permanent preservation in Brazil (Brazilian Federal Law no. 4771/1965), but anthropogenic impacts at the Patos Lagoon estuary still occur, including solid litter contamination (Marangoni and Costa, 2009a). This might relate to the fact that in the city of Rio Grande, where this study was performed, the waste collection system is highly inefficient regarding recycling, management, and destination (de Ramos et al., 2021). Debris can cause serious damage to salt marsh vegetation such as broken stems and leaves, and grasses might not be able to recover from damage caused by large items such as tyres even after 56 weeks of the item's removal (Uhrin and Schellinger, 2011). Implications for this can include impairment of normal salt marsh ecological roles such as protection against erosion and waves, nutrient cycling, species habitat, and participation in the hydrological cycle (Shepard et al., 2011; Sousa et al., 2010). These might be aggravated considering that macroplastics can persist in estuarine environments for long periods of time in a cycle of transport, deposition, and remobilisation (Tramoy et al., 2020).

Litter densities (items m^{-2}) in the Molhe Oeste salt marsh were much higher than those observed by Viehman et al. (2011), that found less than one item per square metre in North Carolina's (US) salt marshes. These authors showed that litter was mostly concentrated in higher zones in the salt marsh where they could identify wrack lines or patches. They argued that litter accumulation can be attributed to flooding

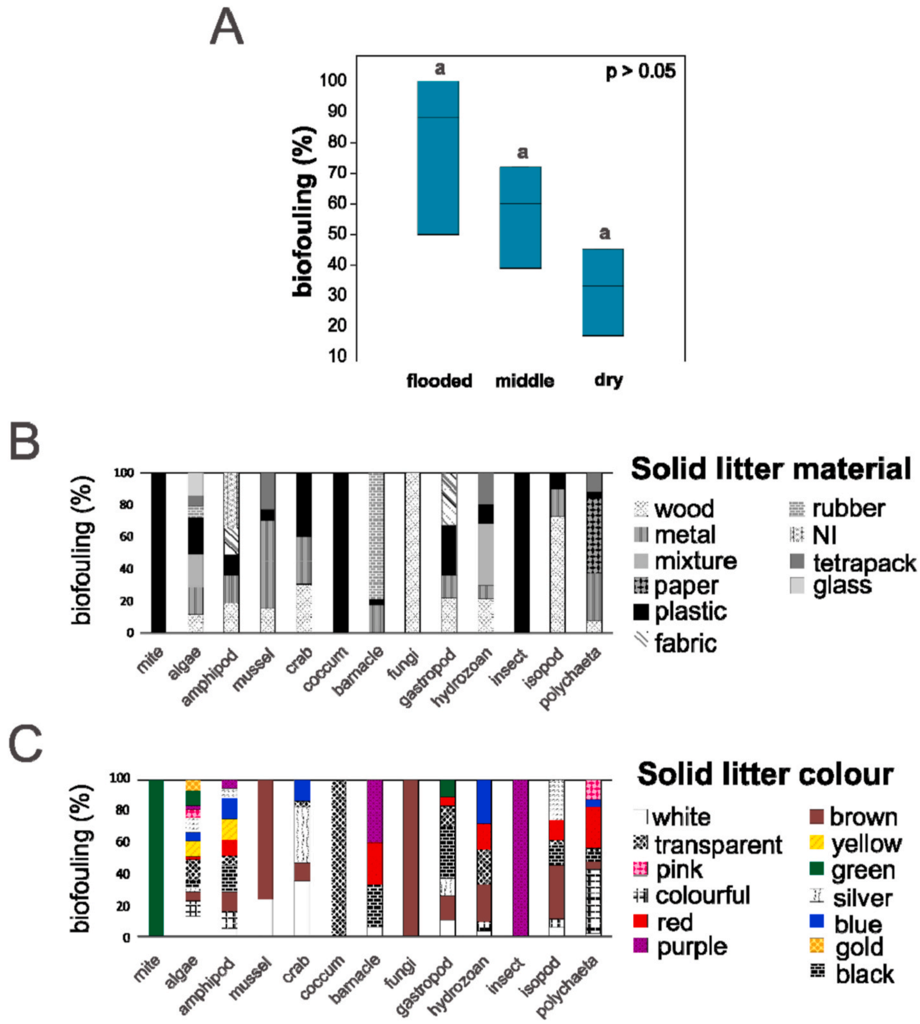

Fig. 7. Frequency of biofouling occurrence (%) in the salt marsh zones (A) (n = 419), where similar lowercase letters in indicate similarities (ANOVA, $p > 0.05$), and different lowercase letters indicate statistical differences (ANOVA, $p < 0.05$) among zones; and the biofouling (%) recorded on the different solid litter material (B) and colour (C) (n = 419). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

frequency that normally occurs in intertidal areas such as salt marshes, but less frequent events such as storms can move the items further up in the salt marsh. These explanations apply to the Patos Lagoon Estuary, as even though it is under a microtidal regime it can suffer from occasional storms and cyclones (Parise et al., 2009). Indeed, it was also possible to notice a litter concentration zone in a line at the High Marsh zone, parallel to the coastline, which might correspond to the highest water level, i.e. the strandline, during such extreme events.

Higher litter quantities in higher zones in the salt marsh was also found in three estuaries in Spain (Mazarrasa et al., 2019), although the average quantity (1.31 ± 0.14 items m^{-2}) was lower than at the Molhe Oeste salt marsh. Still, the quantities found in the present study are likely to be underestimated as items under the thick plant litter layer were not collected. It is not possible to compare these results with the reported by Gilligan et al. (1992), as these authors only presented the quantities of litter per length (m) of the samples area. At that point, they found 0.73 items m^{-1} in a salt marsh area in Chatham County, Georgia (US), which cannot be transformed in unit of area with the information given in the paper. Nevertheless, they state that litter was collected from the water line until the highest tide line, which is likely to be larger than the transect areas collected in this investigation. Therefore, quantities would still be higher at Molhe Oeste salt marsh.

In a recent study in Brazil, de Ramos et al. (2021) monitored the contamination by solid litter items (> 2.5 cm) in a touristic beach located at the mouth of the Patos Lagoon estuary, the Cassino beach. They found concentrations of up to 0.42 items m^{-2} in the summer, 0.19 items m^{-2} in the fall, 0.09 items m^{-2} in the winter, and 0.16 items m^{-2} in the spring (de Ramos et al., 2021). The solid litter concentrations found by these authors are at least one order of magnitude lower than the concentrations found at the Molhe Oeste salt marsh, which might indicate that the inner part of the estuary is being more affected by that type of contamination that its adjacent coastal areas.

Investigation of contamination sources to aquatic environments are crucial to protect these areas. At the Cassino Beach (Brazil), solid litter items were mainly originated from beach users (37.1%) or from fishery (10%) (de Ramos et al., 2021). Meanwhile, most of the items from the Molhe Oeste salt marsh that could have their previous use identified were mainly related to food packaging (12.4%), fishery and shipping activities (5.75%), and personal use (5.08%) (Figs. 4C and 5). This corroborates with the fact that the packaging industry is the main producer of plastics in the world (Lechthaler et al., 2020). Such disposable products are commonly found in the environment, mainly due to this high level of production and its short service life (Lechthaler et al., 2020).

The litter sources might vary between Cassino beach and the Molhe Oeste salt marsh due to differences in the environment use, but it is clear that both areas are affected by intense fishery activities and human presence in the region. However, it is important to notice that the

Table 1
SIMPER results indicating the contribution index (CI) of the main biofouling groups by factors evaluated (salt marsh zones, solid litter material, and solid litter colour) (n = 419).

Taxa	Av. dissim	Contrib. %	Cumulative %	Preference
Among salt marsh zones				
Algae	19.57	28.8	28.8	dry zone
amphipod	11.85	17.44	46.24	middle zone
polychaeta	9.493	13.97	60.21	flooded zone
gastropod	7.443	10.95	71.16	flooded zone
hydrozoan	5.861	8.62	79.79	dry zone
mussel	4.567	6.72	86.51	middle zone
isopod	3.496	5.14	91.65	dry zone
barnacle	3.216	4.73	96.38	middle zone
crab	1.686	2.48	98.87	flooded zone
fungi	0.6098	0.89	99.76	dry zone
coccum	0.05369	0.08	99.84	middle zone
insect	0.05369	0.08	99.92	middle zone
mite	0.05369	0.08	100.00	middle zone
Among solid litter materials				
Taxa	**Av. dissim**	**Contrib. %**	**Cumulative %**	**Preference**
algae	22.28	31.18	31.18	plastic
amphipod	12.78	17.89	49.06	wood
polychaeta	10.96	15.35	64.41	metal
gastropod	6.67	9.34	73.75	tissue
hydrozoan	5.37	7.52	81.28	mixture
mussel	4.56	6.38	87.66	metal
barnacle	4.26	5.96	93.62	rubber
isopod	2.67	3.74	97.36	wood
crab	1.34	1.88	99.24	plastic
fungi	0.40	0.56	99.81	wood
coccum	0.04	0.06	99.87	plastic
insect	0.04	0.06	99.94	plastic
mite	0.04	0.06	100.00	plastic
Among solid litter colours				
Taxa	**Av. dissim**	**Contrib. %**	**Cumulative %**	**Preference**
algae	16.39	30.79	30.79	transparent
amphipod	8.63	16.22	47.01	black
polychaeta	7.04	13.22	60.24	colourful
gastropod	6.97	13.09	73.33	brown
isopoda	3.29	6.18	79.51	brown
crab	2.94	5.53	85.05	white/silver
hydrozoan	2.63	4.95	90.00	blue
barnacle	2.08	3.92	93.92	purple
mite	1.22	2.29	96.21	green
insect	1.21	2.28	98.49	purple
mussel	0.46	0.87	99.36	brown
fungi	0.24	0.46	99.82	brown
coccum	0.09	0.17	100.00	transparent

contamination of the salt marsh, where touristic activities normally do not take place at the time sampled, was much higher than the highest concentrations found nearby at the Cassino beach in the summer, when beach use is intensified due to a five-fold increase in the coastal population (Prefeitura Municipal do Rio Grande, 2020). This leads to a hypothesis that inner parts of the Patos Lagoon estuary are more contaminated by solid litter than near coastal areas, but monitoring efforts are needed to confirm this.

Marangoni & Costa (2009a) have argued that items related to direct deposition (food packaging, personal use) might predominate in higher areas of the marsh i.e., the dry zone, while items that arrive from the water and are therefore indirectly deposited in the salt marsh might predominate in areas with higher water influence such as the intermediate and flooded zones. This pattern was not observed in the Molhe Oeste salt marsh, as items originating from fishing or shipping activities were present in the higher zones (Fig. 5). Therefore, deposition of litter in this area seems to vary regarding sources along the salt marsh and with time. Furthermore, even though closer sources of litter such as the human occupation and port/fishery activities near the Molhe Oeste salt marsh are present, it is worth mentioning that litter might also be originating farther from the ocean and being transported down the Patos Lagoon, especially because increased water movement originated from extreme events can also remobilise macroplastics from river bottoms and transport them further (Hurley et al., 2018).

Other items' characteristics such as % fragmentation and degradation level did not differ among zones (Figure S2). This might indicate that solid litter items are suffering from degradation forces throughout the salt marsh at similar levels, which can include sunlight exposure, the air/water exposure balance, and interaction with the vegetation and the biota. In fact, there was no significant difference between the % biofouling among zones (Fig. 7A), which indicates that organisms are colonizing the items regardless of the zone. However, the % biofouling tended to be higher at the more flooded zones, which was expected due to the requirement of water for the biofouling process to occur, and thus this correlation remains to be more deeply investigated.

It is important to notice that even though the average size of the items did not differ between zones, there was a wide variation in items' size at the HM-MM zone (Figure S2D). This was due to the presence of very large items (> 1 m) like anthropogenic wood in that zone, which were not found in lower zones. This might again be related to extreme events that are able to carry these large, heavy items further up the salt marsh where they get trapped in the vegetation, while smaller pieces are more easily transported between zones by more frequent water level oscillations. This trapping effect related to debris size was described in a work by Cozzolino et al. (2020), where these authors found that the saltmarsh vegetation was able to trap significantly more macroplastics than the seagrass vegetation and an adjacent unvegetated area, while there was no difference between those types of vegetation for trapping microplastics (< 5 mm).

Salt marshes such as the Molhe Oeste have unconsolidated substrate such as mud, and natural solid surfaces such as rocks and shells thus provide an important substrate for colonization by biofouling organisms in these environments. This colonization has been referred to as the Plastisphere for microorganisms attached to plastic marine debris (Amaral-Zettler et al., 2015; Zettler et al., 2013). Here, we extend that definition to macroorganisms attached to any solid litter and to any aquatic environment such as the salt marshes, as coastal systems are now widely contaminated. This association has led to many consequences such as the colonization of engineered structures and transport of exotic species (Campbell et al., 2017). Therefore, it has become essential to understand the factors influencing this association in order to prevent unwanted outcomes.

The implications for the presence of solid litter and its associated fouling organisms can include transport of exotic (Rech et al., 2018) or pathogenic (Zettler et al., 2013) species, changes in organic matter cycling (Arias-Andres et al., 2018a), increases in gene exchange (Arias-Andres et al., 2018b), among others. Coastal species in the Plastisphere can travel for longer distances as plastics are often buoyant in seawater. This can increase their colonization potential and ultimately alter species composition in affected areas (Pinochet et al., 2020). All these can be applied to a salt marsh environment where freshwater and seawater mix, even though at this point we did not investigate whether the biota associated with solid litter at the Molhe Oeste salt marsh was native or not.

In general, the organisms' preference for different zones in the salt marsh varied among groups (Table 1). Secondary colonizers such as algae were more present in the dryer zone, where they can be protected against desiccation by the plant cover and against photoinhibition that could impair their development (Dodds et al., 1999).

Larger sessile organisms such barnacles were more present in the middle zone. Factors related to substrata influencing the settlement of barnacle cyprid larvae has been previously studied (e.g. Mendez et al., 2013). Recently, Joesting et al. (2020) have shown that the barnacle *Chthamalus fragilis* prefer parts of the cordgrass *Spartina alterniflora* that can keep them protected against thermal and desiccation stress and predation at the same time. Parallelly, the dry zone in the Molhe Oeste salt marsh represent an area with lower water availability and thus higher susceptibility to high temperatures, while the flooded zone can represent higher risk of predation. Therefore, the middle zone could be offering a similar balance to barnacles in the Molhe Oeste salt marsh.

Biofouling organisms can show preferences for some substrates characteristics available in aquatic systems (Carl et al., 2012; Noble et al., 2016). In this study, it was possible to notice a preference for plastics in the majority of the biofouling groups (5 of 13) (Table 1). This corroborates with the findings from Pinochet et al. (2020), that showed the preference of a marine invertebrate larvae for plastics over concrete and wood surfaces, and from Li et al. (2016), that showed higher bryozoan settlement in plastics than in glass surfaces. However, this last study also showed that for barnacles the settlement was higher in glass surfaces, which could be associated with chemical leaching from plastic items.

The preference for different surfaces was not the same for all groups in this study (Table 1), which might reflect their individual settling mechanisms involving sensory capabilities, resistance to chemical leachates, or the pre-existent fouling community. For example, algae preferred transparent plastic items (Table 1), which were represents mainly by disposable cups. These cups were widely available in the higher zone, where algae were most present, and they might serve as an optimum niche for algae colonization as it allows the passage sunlight which is required for photosynthesis.

Barnacles and other groups such as algae, bryozoans, copepods, and insect larvae show higher abundance in substrates without gastropod grazers (Anderson and Underwood, 1997). This corroborates with the results found in the SIMPER analysis in this study (Table 1), where barnacles, algae and insects did not have the same preferences for zone, material, or colour as gastropods, which indicates that these groups occurred in different items. Barnacles are able to discriminate between surfaces prior to settlement following physical and biological cues (Aldred and Clare, 2008; Siddik and Satheesh, 2019), which can be associated with the presence of certain bacterial taxa in biofilms in the surface (Aldred and Nelson, 2019). These preferences for surfaces with previously attached organisms need further investigation in this salt marsh environment.

5. Conclusions

This work presents an assessment of solid litter contamination in South American salt marshes and contributes to the understanding of the interaction of these items with biofouling organisms in these coastal areas. Quantities reported here can now serve as a baseline for further monitoring studies at the Patos Lagoon Estuary and as a comparative for future studies investigating litter distribution along salt marshes zones according to plant species composition and flooding systems. Future time-related investigations should consider local hydrodynamics and the various deposition sources in the estuary. Higher salt marsh zones seem to be as accumulation spots, and future studies should investigate accumulation dynamics at this estuary.

Biofouling preference for lower, wetter zones was not significant at this point but future studies are encouraged to investigate this tendency. The heterogeneous distribution of the fouling community in salt marshes can be related to their physiological requirements regarding water and light availability. Also, preferences for item's characteristics such as material types and colour were different for each of the 13 groups of organisms, which is likely to be related to previously attached organisms. These preferences are especially interesting for the anti-fouling industry, which might benefit from ecologically friendly strategies to reduce fouling in man-made structures. Also, it was not possible to identify species associated with solid litter from the Molhe Oeste salt marsh at this point, so implications for the salt marsh community remains to be further investigated, especially related to the identification of exotic species.

Credit author statement

Lara M. Pinheiro: Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft, Visualization, Project administration; Isadora V. Carvalho: Investigation, Visualization; Vanessa O. Agostini: Conceptualization, Methodology, Formal analysis, Investigation, Writing – review & editing; Gustavo Martinez-Souza: Formal analysis, Writing – review & editing; Tamara S. Galloway: Writing – review & editing, Supervision; Funding acquisition; Grasiela L.L. Pinho: Investigation, Resources, Writing – review & editing, Supervision, Funding acquisition.

Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2021.117647.

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Capítulo IX: Artigo 3

O terceiro artigo científico proveniente desta Tese de Doutorado é apresentado nesse capítulo. O manuscrito, de autoria de Lara Mesquita Pinheiro, Larisa Midori Konishi Britz, Vanessa Ochi Agostini, Andrés Pérez-Parada, Felipe Garcia-Rodriguez, Tamara Susan Galloway e Grasiela Lopes Leães Pinho, é intitulado "***Salt Marshes as the Final Watershed Fate for Meso- and Microplastic Contamination: A Case Study from Southern Brazil***" e foi publicado no periódico "***Science of the Total Environment***" e se encontra disponível no link http://dx.doi.org/10.1016/j.scitotenv.2022.156077 (0048-9697/ © 2022 Elsevier B.V. All rights reserved). O material suplementar do artigo se encontra no ANEXO III desta Tese.

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Salt marshes as the final watershed fate for meso- and microplastic contamination: A case study from Southern Brazil

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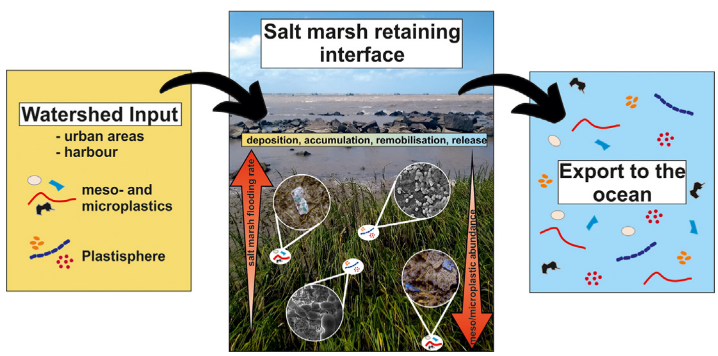
HIGHLIGHTS

- Salt marshes are transitional biotopes heavily contaminated by meso/microplastics.
- Microplastic abundance reached 1123.16 items kg^{-1} in sediment surface.
- Sediment mixing processes promote microplastic contamination within deep layers.
- Plastic sequestration was modulated by vegetation, flooding rate and marsh zonation.
- A diverse Plastisphere in salt marsh was composed by bacteria, microalgae and fungi.

GRAPHICAL ABSTRACT



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ABSTRACT

Plastics pose a major threat to aquatic ecosystems especially in smaller size fractions. Salt marshes play a crucial role in maintaining the coastal zone and aquatic food web, yet their contamination, including by plastic materials, is still poorly investigated. This work investigated meso- (MEP, 5–25 mm) and microplastic (MIP, 1 μm–5 mm) contamination of a salt marsh, which reached average levels of 279.63 ± 410.12 items kg^{-1}, 366.92 ± 975.18 items kg^{-1}, and 8.89 ± 8.75 items L^{-1} in surface sediment, sediment cores and water, respectively. Photomicrographs revealed a complex fouling community on plastics surface for both different salt marsh zones and plastic formats. Abundance of plastics in sediment was higher in the dryer, vegetated zones compared to flooded, unvegetated zones. This is consistent with the role of vegetation as a trap for solid litter and final fate of plastic deposition, but also with local hydrodynamics influencing deposition pattern. Plastics were detected up to 66 cm-depth, presenting higher levels at surface sediments. It was also possible to identify the main groups of microorganisms (1638 bacterial cells, 318 microalgae cells, and 20049.93 μm^2 of filamentous fungi) composing the Plastisphere communities on all plastic items recorded in the different zones. These results are a pioneer contribution, highlighting that regional salt marshes participate in sequestration and longstanding accumulation of plastic particles in estuarine environments, before exportation to the ocean.

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1. Introduction

The continental export of terrigenous material to the marine environment consists of a source-to-sink process, which includes both erosion and resuspension mechanisms within the basins, fluvial transport and deposition on the coastal zone and the inner shelf (Liu et al., 2016). Such a process is subject to geological, geomorphological, sedimentological, and oceanographic processes, but its original magnitude is modulated by rainfall, wind, and geological attributes such as watershed slope and sedimentology (Milliman et al., 2008; Vinzon et al., 2009; Tyaquiçã et al., 2017; Jung et al., 2020; Perez et al., 2021; Bortolin et al., 2022). Particularly in humid regions, such as the east coast of South America, which holds the world's largest coastal lagoon, salt marshes act as a retaining interface of continental material before it is finally exported to the ocean (Reed et al., 2009; Vinzon et al., 2009). Therefore, coastal marshes are subject to long-distance pollution (Pinheiro et al., 2021b).

Plastic contamination and pollution have been a hot topic in environmental science since the beginning of this century, and awareness of the harmful effects of this pollutant in aquatic environments is still being unravelled. Deterioration of aquatic environments is mainly related to ingestion by biota and includes concentration-dependent changes in filtration/feeding/clearance rate (e.g., Browne et al., 2013; Rist et al., 2016), changes in energy budget (e.g., Watts et al., 2015; Wright et al., 2013), changes in metabolic rate (e.g., Green et al., 2016), oxidative stress responses (e.g., Canesi et al., 2015; O'Donovan et al., 2018), and even cellular and DNA damage (e.g., Avio et al., 2015; Revel et al., 2020). Such effects are aggravated by the large amounts of plastic waste entering environmental compartments, for which transport occurs mainly through rivers and was estimated to be 57000–265000 MT year^{-1} in 2018 but is expected to attain 62400–290000 MT year^{-1} in 2028 (Mai et al., 2020). Given the global extent and ubiquity of such contamination, the microplastic size fraction (MIP) is also being proposed as a stratigraphic marker for the Anthropocene onset, as a new geological epoch flagged by anthropogenic modification of biogeochemical processes on Earth (Zalasiewicz et al., 2016).

As for MIPs, estimated amounts within the ocean approach 24.4 trillion items (8.2×10^4–57.8×10^4 tons, Isobe et al., 2021). MIPs represent the category of plastic items defined according to a size class ranging between 1 μm and 5 mm (Frias and Nash, 2019; GESAMP, 2019). Other categories such as megaplastics (>1 m), macroplastics (MAP, 25–1000 mm), mesoplastics (MEP, 5 to 25 mm), and nanoplastics (NP, <1 μm), are recognized by the scientific community although their size definition has not been completely standardized yet (GESAMP, 2019).

Once MIPs are deposited in sedimentary systems, items of any size represent a source for biological colonization for macro/microorganisms, forming a community collectively known as the Plastisphere (Pinheiro et al., 2021b; Zettler et al., 2013). Such a fouling community can compromise complex webs including viruses, bacteria, algae, fungi, invertebrates and even urochordates (Amaral-Zettler et al., 2020; Astudillo et al., 2009; Barnes and Fraser, 2003). The fouling biological material on plastic surfaces exert diagenetic effects on plastic dynamics such as changes in density/buoyancy (Amaral-Zettler et al., 2021), not only for invasive species transport (Rech et al., 2016), but also for the transport of other contaminants (Richard et al., 2019). All of these emerging issues and implications are still under study, and hence, the community composition of the different compartments of the Plastisphere needs further scientific attention.

Current investigations have detected overwhelming plastic contamination in soil (e.g., Ng et al., 2018), atmosphere (Brahney et al., 2021), oceans (e.g., Fischer et al., 2015), cryosphere (e.g., Peeken et al., 2018), lakes (e.g., Ballent et al., 2016), islands (e.g., Monteiro et al., 2018), rivers (Blettler et al., 2017), and estuaries (e.g., Tramoy et al., 2020). Estuaries represent important transitional environments of high ecological connectivity value, through the source-to-sink process observed along the freshwater-seawater continuum (Liu et al., 2016). These areas are frequently the final fate of MIPs, and accordingly are highly contaminated by plastic waste of all size categories, but most estuaries around the world still remain uninvestigated (Pinheiro et al., 2021a).

Among estuarine environments, salt marshes are transitional biotopes of special concern due to their diverse ecological roles in temperate systems as protective buffers for coastlines under erosion processes (Shepard et al., 2011), food web productivity source (Jinks et al., 2020), nutrient cycling (Sousa et al., 2010), contaminant removal (Teuchies et al., 2013), nursing and fisheries activities (Tagliani et al., 2003), carbon sequestration and storage (Mcleod et al., 2011). Anthropogenic activities have long affected the ecology of salt marsh environments (Fraser et al., 2020), but the extent of plastic litter contamination still remains understudied, even though such systems are considered as blue carbon environments sensitive to sequestration and accumulation of plastic particles within the sediment (Duarte et al., 2013).

Studies of MIPs contamination in salt marshes have been only recently reported (Yao et al., 2019). Weinstein et al. (2016) investigated MIPs formation, although they did not quantify particle total abundance. For other systems such as the open ocean a lot more attention has been paid for such contamination (Thompson et al., 2004). To our knowledge there are less than ten publications aiming to quantify this type of contamination in different salt marsh compartments and only one addressed the Plastisphere (Seeley et al., 2020). In addition, none of them was undertaken in South America or in Atlantic Ocean estuaries. Therefore, as an attempt to generate knowledge on contamination for Southeast South America, this work aimed to set reference baseline contamination levels for MEPs and MIPs in a salt marsh area contained within the largest coastal lagoonal system of the world. The study encompassed zones of different flooding rates and an associated vegetation cover gradient. We hypothesized that this salt marsh environment is highly contaminated with MEPs and MIPs as substrates for proliferation of a variety of microorganisms, and that plastic abundance is directly proportional to vegetation cover and inversely proportional to the flooding rate.

2. Materials and methods

2.1. Study area

Patos Lagoon is a coastal freshwater body flowing into the ocean thus conforming to an estuarine system. The Molhe Oeste salt marsh is located at 32° 09′ 09.3″ S and 52° 06′ 03.1″ W (Fig. 1), at the mouth of the Patos Lagoon (South Brazil), which covers >10,000 km^2 and drains an area of approximately 200000 km^2 (Moller et al., 2001; Tavora et al., 2019). The lagoon exports a mean annual freshwater discharge of 2000 m^3 s^{-1} (Fernandes et al., 2002). Local hydrodynamics are mostly governed by local and non-local forces such as wind regime and the evaporation/precipitation balance, while tide has a minor influence (Moller et al., 2001; Castelão and Moller, 2003). The fluvial discharge also plays an important role in controlling estuarine hydrodynamics, especially at longer time scales (Barros et al., 2014; Tavora et al., 2019). The estuarine region holds 24 previously described salt marsh units, which differ in size, location, biodiversity, and human influence (Marangoni and Costa, 2010). The Molhe Oeste salt marsh is influenced by two surrounding cities, Rio Grande and São José do Norte, with ~210000 and ~25000 inhabitants, respectively (IBGE, 2010). It is also located adjacent to the Atlantic Ocean and to the second largest harbour in Brazil, the Rio Grande harbour, with an area of 55.6 km^2, where transport operations account for up to 50 million tons per year (Governo do Estado do Rio Grande do Sul, 2021). Therefore, this intertidal environment is subject to large amounts of urban waste (Pinheiro et al., 2021b). This salt marsh consists of four well-defined zones that differ in vegetation and flooding rate: Mud Flat – MF (flood index 100%), Low Marsh – LM (flood index 64%), Middle Marsh – MM (flood index 20.1%) and High Marsh – HM (flood index 3.1%) (Perillo et al., 1999).

The surface sediment composition of Patos Lagoon is overall strongly related to the hydrodynamics and consists of littoral zones dominated by sediment resuspension processes, where the sandy fraction is the most abundant (i.e., Mz values ranging between 1 and 3, Calliari et al., 2009, Bortolin et al., 2020), and current velocity is mostly higher than 0.2 m s^{-1} and depth is

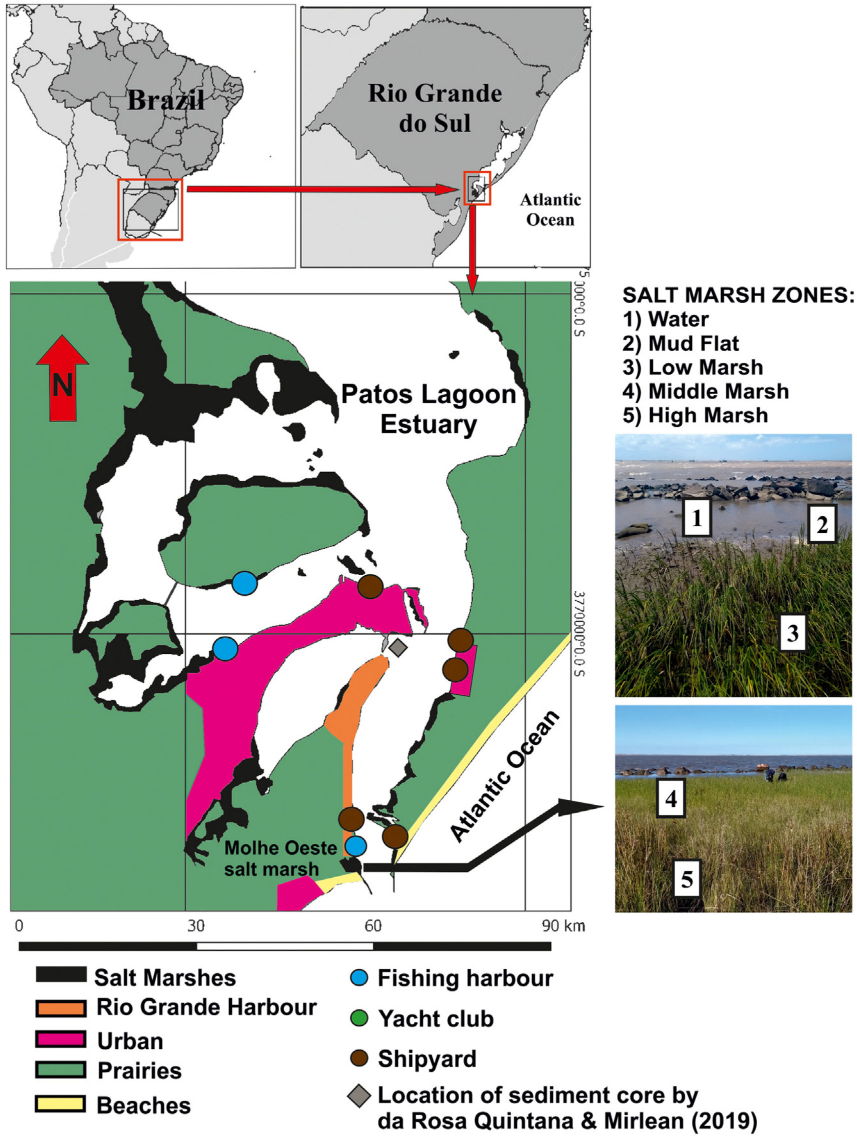

Fig. 1. Location and main anthropogenic activities and land use within the study site, i.e., Molhe Oeste salt marsh, Brazil (32° 09′ 09.3″ S, 52° 06′ 03.1″ W). Photographs of the salt marsh zones where water and sediment samples were collected from are shown to the right.

<4–5 m. On the other hand, central zones are dominated by the silt sediment fraction (i.e., Mz values ranging between 4 and 8), and current velocity is close to 0.1 m s^{-1} and depth was >5 m.

2.2. Surface sediment, coring, and water sampling

Surface sediment and water sampling was performed in 07/05/2018, and sediment cores were retrieved in 05/17/2018. The top 5 cm of sediment was collected using a 47 mm diameter PVC core sampler in each zone of the Molhe Oeste salt marsh, attaining a total of 24 samples (6 samples per zone). Twelve water samples were taken by filtering 150 mL of water above the Mud Flat zone using a 45 mm diameter GF/F filter attached to a 50 mL polypropylene syringe. The filters were stored in metallic envelopes until laboratory analysis. Three sediment cores were collected using the same PVC corer for surface samples in three spots at the Molhe Oeste salt marsh: one at the High Marsh zone (66 cm long), one between the middle and the Low Marsh zone (52 cm long), and one between the Low Marsh and the Mud Flat zone (56 cm long). The cores were immediately sealed with PVC lids and transported to the laboratory. The upper plant litter layer above the core was separated and treated as a subsample. Each core was then sliced every 2 cm, and each subsample was analysed individually. Subsamples were identified and stored in metallic trays with lids until analysis.

2.3. Sample processing and meso- and microplastic isolation

All sediment samples were dried to a constant weight in an oven at 40 °C. Then, sediment was weighed, and plastic particles were isolated using a density separation method adapted from Pinheiro et al. (2019). The dried sediment was placed in a 1 L beaker and a volume of a supersaturated saline solution (NaCl, 1.2 g cm^{-3}) was added to the beaker in a proportion of 1:5 (mass/volume). The mixture was shaken for 30 min using a magnetic shaker, and then left to settle for another 30 min in order to let denser particles sink. It is then expected that less dense polymers such as polypropylene (0.85–0.92 g cm^{-3}), polyethylene (0.89–0.98 g cm^{-3}) and polystyrene (0.01–1.06 g cm^{-3}) will remain within the supernatant, while denser polymers such as polyurethane (1.2–1.26 g cm^{-3}), polyethylene terephthalate (1.38–1.41 g cm^{-3}) and polyvinyl chloride (1.38–1.41 g cm^{-3}) will settle down. The supernatant was filtered in a vacuum filtration system using a 90 mm diameter cellulose filter (<12 μm mesh size). This procedure was performed three times for each sediment sample to ensure total plastic particles recovery.

Filters from water and sediment samples were dried overnight in an oven at 40 °C and then visually analysed (lower detection limit of 0.1 mm) for the presence of plastic particles under a stereomicroscope (OPTSZ Opticam) coupled with a camera and the Opticam Microscopia OPTHD software version 3.7.11443.20180326. The particles on the filters were considered to be potential plastic particles when they presented visual characteristics adapted from Hidalgo-Ruz et al. (2012), i.e., clear, homogeneous color, no cellular or other organic structure, fibers were equally thick throughout their length, and did not shatter when softly pressed with a needle. Other potential particles generating doubts on their synthetic nature (e.g.: brown color and/or smaller size than 1 mm) were also selected for further chemical confirmation. The potential plastic particles were counted and assorted by their size (mm), color (white, clear, blue, black, green, red, yellow, brown, orange, grey, beige, pink and colorful), and format (fragment, fiber, pellet, film, sphere). The items were classified according to their size class (MEPs, 5–25 mm, MIPs, <5 mm), as determined by the GESAMP Guidelines for the Monitoring and Assessment of Plastic Litter in the Ocean (GESAMP, 2019). Particles larger than 25 mm were not considered for this study. Particles from sediment cores were not measured due to equipment limitation. In addition, a chemical characterization was performed for at least 40% of potential plastic particles (Table S1) to confirm their synthetic nature and thus identify corresponding polymer type. This subsample set was selected to encompass different combinations of both plastic format and color, i.e., a small number of particles were picked from a group of several visually similar particles. Surface sediment and water samples were analysed by micro-Fourier-transform infrared spectroscopy (μ-FTIR) using a Perkin Elmer Spotlight 400 imaging system with attenuated total reflectance (ATR). For sediment core samples, identification of polymers was performed using a Perkin-Elmer model Frontier FTIR spectrophotometer with a U-ATR accessory equipped with Perkin Elmer ATR database of polymers spectra and microplastics FTIR database from Primpke et al. (2018). A total of 60 scans were averaged per sample, and the spectra were collected in the range of 4000–450 cm^{-1}. A spectral match was considered when the search score between sample and library spectra was equal to or higher than 0.7 (at least 70% similarity).

2.4. Plastisphere analysis on surface sediment and water

A total of 35 plastic particles were selected from the salt marsh surface sediment and water for Plastisphere analysis. The presence of microorganisms on the plastic surface i.e., the Plastisphere, was analysed following a methodology adapted from Agostini (2018). Particles were fixed in 1% glutaraldehyde and dried in the laboratory at 40 °C overnight. Then, particles were placed in aluminium plates and covered with Au powder and analysed under a scanning electron microscope (SEM) (EOL JSM-6060). Microorganisms from the Plastisphere were counted in each SEM image and classified as bacteria, microalgae, or fungi. These were visually identified to the most accurate possible taxonomical level using morphological features as shown in previous SEM studies on the structure of the Plastisphere community (Ramsperger et al., 2020; Gkoutselis et al., 2021).

2.5. QC/QA

Several procedures were adopted to avoid post-sampling contamination. Cotton lab coats and nitrile gloves were worn at all times. Samples were kept covered with aluminium foil or a glass lid at all times, and all sample handling were performed inside a closed acrylic structure in order to prevent airborne contamination. The solutions (distilled water and NaCl solution) were previously filtered (<12 μm pore size), and all glassware was cleaned with filtered distilled water before use. No contamination from the core sampler was identified as the plastic particles identified as PVC in the FTIR analysis did not have the same color as the core sampler. A clean, damp filter (procedural blank filter) was left uncovered next to each sample in order to catch airborne particles that would eventually fall on the filter. The blank filters were visually analysed under a stereomicroscope and particles were chemically characterized as described above (FTIR) for environmental samples. To mitigate contamination, the quantities of particles found in each blank filter were subtracted from the quantities detected in the corresponding environmental sample, as suggested by Wright et al. (2021).

2.6. Data analysis

The level of plastic contamination in the Molhe Oeste salt marsh was reported as plastic abundance (number of items per kg of dry sediment or per L of water). Data from water samples were simply reported and not analysed statistically as they could not be compared to sediment samples due to their different units. Data variances from blank and environmental samples were compared in surface and core sediment data using an F-test, and differences among blank and environmental samples were tested using a one-sided *t*-test for equal variances and a Welch F-test for unequal variances. Data normality was tested using a Shapiro-Wilk test, and the residues from the models were also analysed for normality using a Shapiro-Wilk test and homoscedasticity using a Levene's test. Differences in plastic abundance in the sediment surface between zones (HM, MM, LM, MF) were tested using a Kruskal-Wallis followed by a Dunn's *posthoc* test to identify the differences between groups. In addition, a Principal Component Analysis (PCA) was performed to visualize the distribution of plastic colors, formats, and polymer types among salt marsh zones. For this, data for colors and formats was used as abundance (number of items per kg of dry sediment), while polymer type data was transformed by dividing the number of items by their standard deviation. Differences in surface plastic abundance between plastic size categories (MEP and MIP) and in the sediment core layers (0 to 10 cm, 10 to 30 cm, below 30 cm) were tested using two-way ANOVA followed by a Tukey's *posthoc* test. All statistical tests

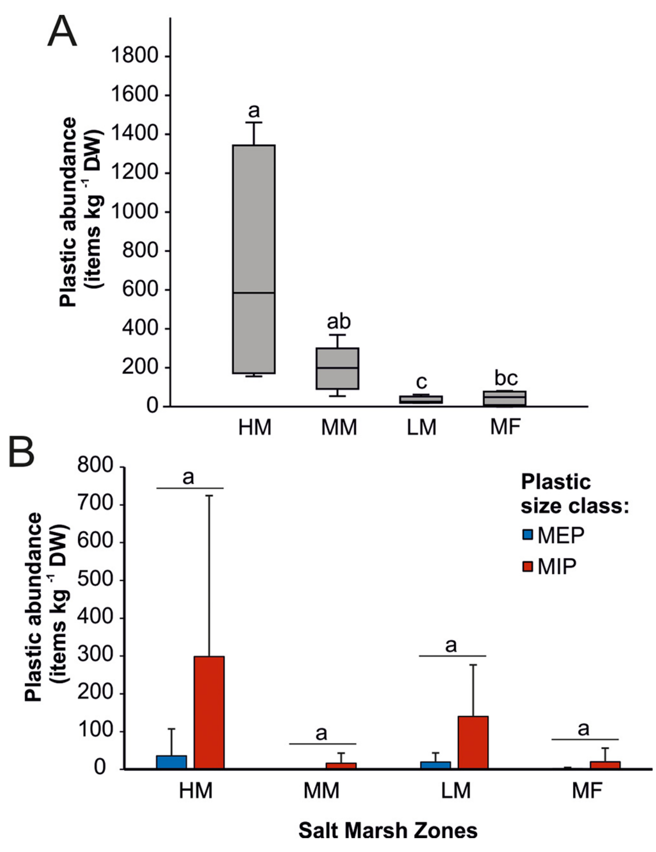

Fig. 2. Plastic abundance (items kg^{-1} DW) in surface sediment samples collected. A: Plastic average abundance in each salt marsh zone (Kruskal Wallis + Dunn's post hoc, $p = 0.003$). B: Plastic average abundance in each size class (MEP: >5 mm, MIP: 1 μm–5 mm) and each salt marsh zone (*two-way* ANOVA: $F_{(1,3)} = 1.14$; $p = 0.034$). Different lowercase letters indicate statistical difference between groups ($\alpha = 0.05$). DW: dry weight; HM: High Marsh; MM: Middle Marsh; LM: Low Marsh; MF: Mud Flat, MEP: mesoplastics, MIP: microplastics.

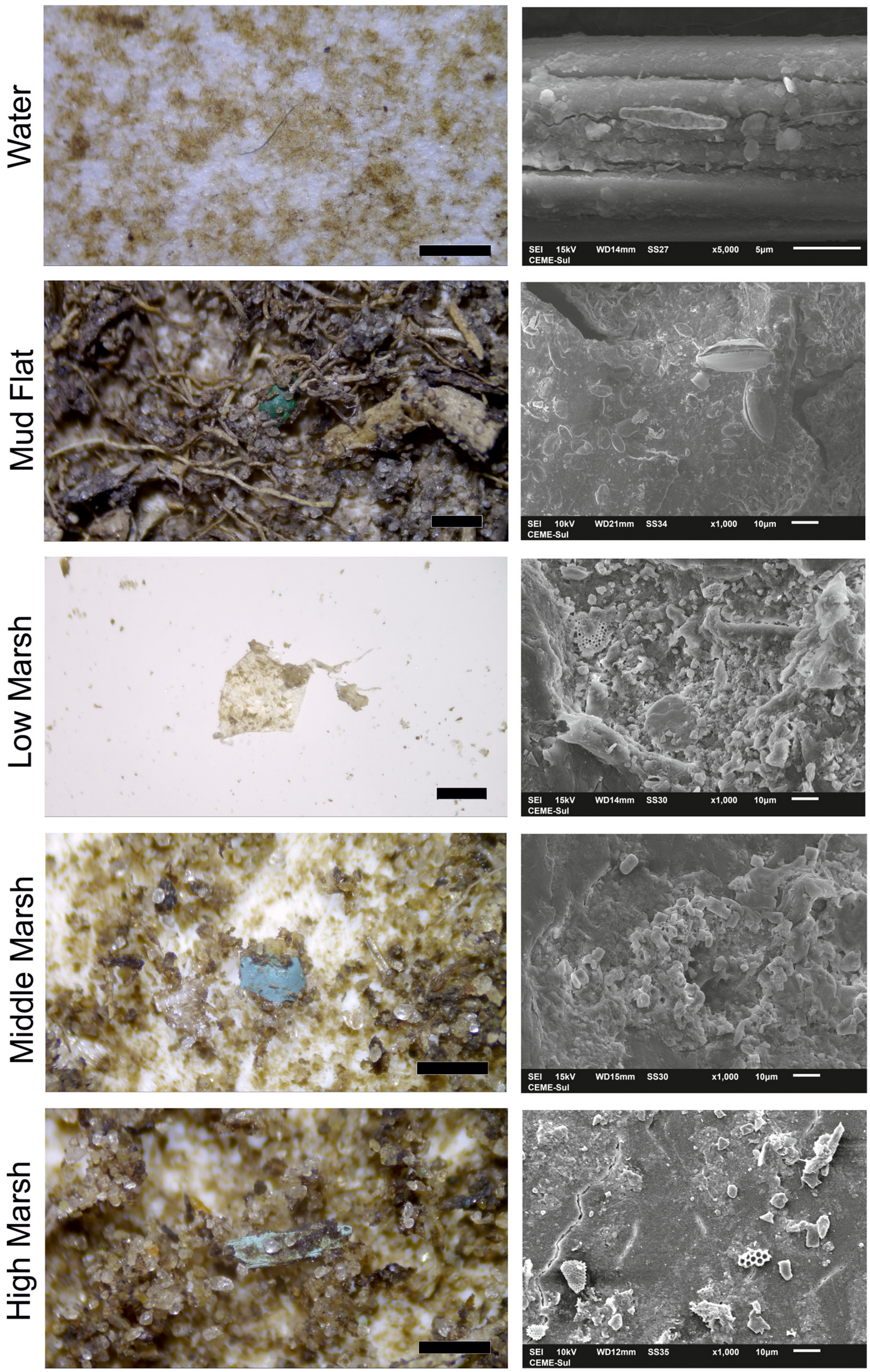

Fig. 3. Examples of plastic particles found in sediment and water samples collected at different salt marsh zones. The photos on the right column were taken with a stereomicroscope (OPTSZ Opticam) coupled with a camera and the Opticam Microscopia OPTHD software version 3.7.11443.20180326, and the photos on the left column were taken with an electron microscope (EOL JSM-6060). Black bars = 1 mm.

were performed considering a significance level of $\alpha = 0.05$ using the Past software version 4.10 (Hammer et al., 2001). The quantity of organisms observed in SEM images was reported as number of cells of bacteria and microalgae. As fungi could only be identified as filaments instead of individual cells, they were reported as unit of area (μm^2).

3. Results

3.1. Meso- and microplastic contamination in the sediment surface and water

A total of 773 and 83 potential plastic particles was found in the environmental samples and in the blank filters, respectively. The average of procedural contamination was 16.69% in environmental samples. Variances among the number of potential plastic particles in blank and environmental samples were similar ($p = 0.42$), but environmental samples showed significantly higher amounts of particles than those blank samples ($p < 0.01$) (Table S5).

The average plastic abundance in sediment surface samples was 279.63 ± 410.12 items kg^{-1} dry weight and 8.89 ± 8.75 items L^{-1} in the water at the Molhe Oeste salt marsh. Considering the corrected quantities of potential plastics (i.e., environmental samples minus blank filters), MIP abundance in the sediment was found to significantly decrease from higher to lower zones (Fig. 2A, Kruskal-Wallis: $p = 0.003$). Details on the amounts and characteristics of potential plastics found in procedural blanks can be found in Table S6.

Particles in the sediment were mostly dominated by MIPs rather than MEPs when considering all particles (89.5% of 394 measured particles) but also throughout all salt marsh zones (Fig. 2B and Table S2). The total abundance of MIPs (132.54 ± 252.26 items kg^{-1} sediment DW) was usually higher than the total abundance of MEPs (15.91 ± 40.33 items kg^{-1} sediment DW), although not significantly (ANOVA: $F_{(1,38)} = 3.89$; $p = 0.053$). All particles recorded in the water samples were <5 mm and were therefore classified as MIPs.

Fig. 3 shows some examples of the plastic items documented at the Molhe Oeste salt marsh, which had an average size of 9.05 ± 3.19 mm and 1.73 ± 1.15 mm for MEPs and MIPs, respectively. Considering all salt marsh zones, items in both sediment surface and water were mostly white (38.55%), clear (25.36%), and blue (21.35%), while other colors together represented 14.75%. However, this pattern was rather variable among zones (Fig. 4A). Blue particles were associated more strongly with the High Marsh zone, while white and clear particles were less strongly associated with the Low Marsh and Mud Flat zones (99.89% of explanation, Fig. S2A). Plastics were mostly fragments in the High Marsh (84.75%) and Middle Marsh (80.45%) zones, while in the Low Marsh, Mud Flat, and in the water, they were mostly fibers, with 55.81%, 74% and 93.75% of total plastics in each zone, respectively (Fig. 4B). This pattern was also observed in the PCA (99.99% of explanation; Fig. S2B). Only two pellets and one sphere were found, located in the High Marsh and in the Middle Marsh, respectively.

Most particles were confirmed to be synthetic polymers in the environmental samples (84.61% of particles analysed in the FTIR). The most common recorded polymers were High-Density Polyethylene (HDPE, 34.72%), Polyethylene (PE, 25.92%), Polypropylene (PP, 23.15%). Other polymers identified included Polyvinyl Chloride (PVC, 6.01%), Polyester (PES, 1.38%), Nylon (0.92%), Polyamide (0.46%), Polystyrene (0.46%), and 13 other polymers (6.98%) (Figs. 4C and S1). Two out of 11 different polymer types could be identified in each salt marsh zone, as the pattern of polymer composition varied among them (Figs. 4C, S2C). HDPE was more associated with the High Marsh zone, while PP, PE, and PVC were more closely related to the Middle Marsh zone, and PES to the Low Marsh and the Mud Flat zones (99.70% of explanation, Fig. S2C).

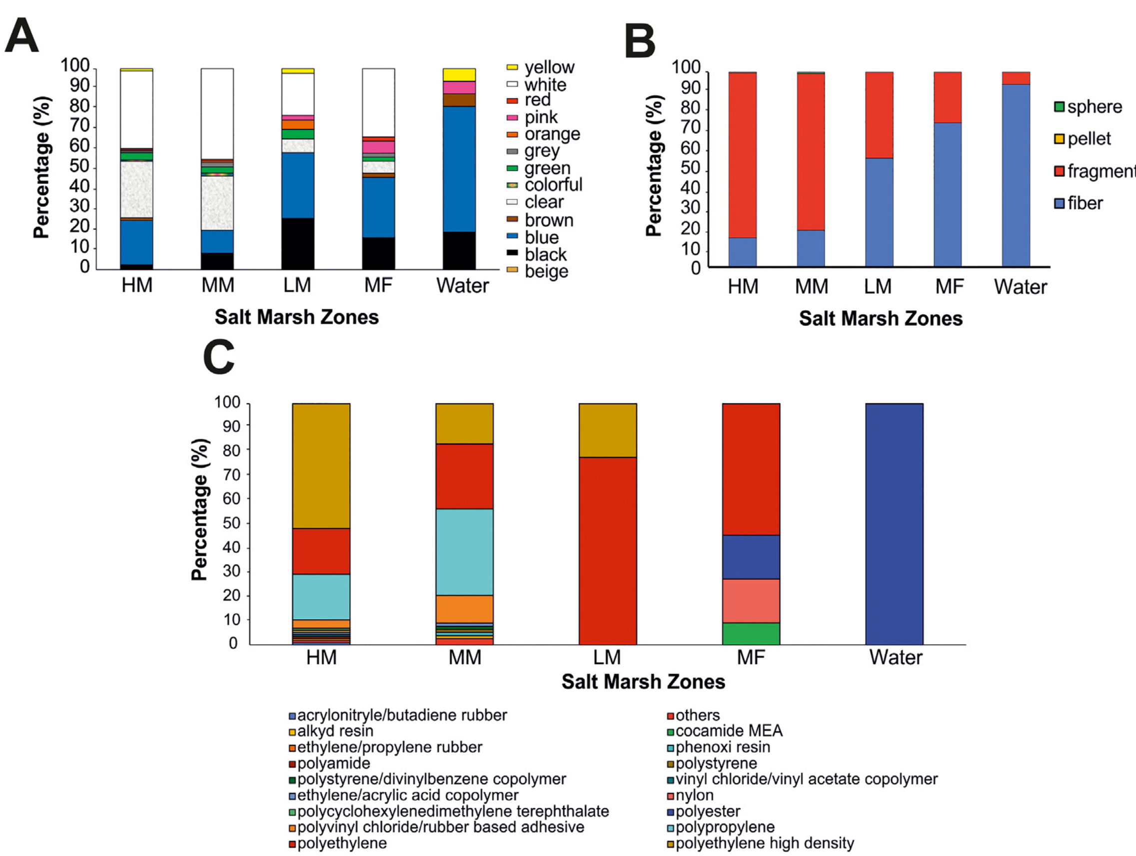

Fig. 4. Characteristics of the plastics recorded in sediment and water samples collected at different salt marsh zones. A: colors, B: formats, C: polymer types. HM: High Marsh; MM: Middle Marsh; LM: Low Marsh; MF: Mud Flat.

3.2. Meso- and microplastic contamination in the sediment cores

A total of 1628 and 573 potential plastic particles was found in the environmental samples and in the blank filters, respectively, with an average of 10.45% of contamination. Variances among the number of potential plastic particles in blank and environmental samples were different ($p < 0.01$), but a Welch test for unequal variances still showed that environmental samples exhibited significantly higher amounts of particles than blank samples ($p < 0.01$) (Table S5).

The total average plastic abundance in sediment core samples was 366.92 ± 975.18 items kg^{-1} dry weight at the Molhe Oeste salt marsh. Most plastics were present in the High Marsh core (827.59 ± 1473.98 items kg^{-1}), followed by the Low-Marsh - Mud Flat (92.40 ± 95.97 items kg^{-1}), and the Middle Marsh – Low Marsh (85.21 ± 87.05 items kg^{-1}) (Fig. 5A). In all zones the plastics were more abundant within the top 10 cm, even without considering the plant litter layer, but this was only significant in the High Marsh core (ANOVA, $p < 0.001$) (Fig. 5B).

Particles were mostly blue (46.80% of total), white (23.34% of total), and black (14.16% of total) in all salt marsh zones, even though this pattern was rather variable throughout each core (Fig. 6A). The most common format was fiber (54.32% of total), followed by fragments (18.80% of total), pellets (0.18% of total), and sphere (0.09% of total). The distribution of format predominance was present in all sediment cores, although it varied with depth (Fig. 6B). The main polymer type found in the cores was high density polyethylene (HDPE, 30.99% of the total), polyethylene (PE, 30.51% of the total), polyethylene terephthalate (PET, 11.73% of the total), and polypropylene (PP, 11.26% of the total) (Figs. 6C, S1). The other types accounted together for 15.49%. The composition of polymer types also varied with depth and among salt marsh zones (Fig. 6C). Even though HDPE was the most common recorded polymer, it was only identified at the High Marsh zone. Polymers such as poly(1-butene) and polyacrylamide were only found at the High Marsh. Polyethylene, on the other hand, was found nearly throughout all cores.

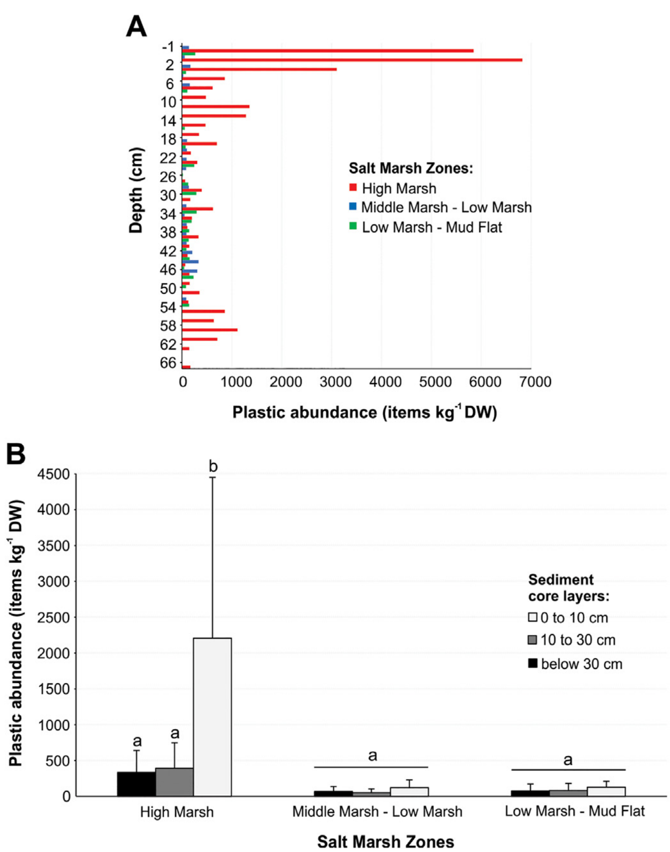

Fig. 5. Plastic abundance (items kg^{-1} DW) in sediment core samples. A: Depth profile of plastic abundance in cores collected at the three salt marsh zones. The uppermost negative value at the y axis represent the plant litter layer present above the sediment surface at the High Marsh and the Middle Marsh - Low Marsh zones. B: Plastic abundance in different sediment layers (0 to 10, 10 to 30, and below 30 cm deep; plant litter layer not included) from all three salt marsh zones (ANOVA: $F_{(1,8)} = 6.88$; $p < 0.001$). Different lowercase letters indicate statistical difference between groups ($\alpha = 0.05$). DW: dry weight.

3.3. Biofouling on meso- and microplastics from surface sediment and water

A total plastic surface area of 693720.84 μm^2 was photographed and analysed, corresponding to an average of 0.5% of each plastic surface. All plastics analysed for biofouling, belonging to all four salt marsh zones, exhibited at least one group of colonizing organisms on their surface (n = 35). A total of 1683 bacteria, 318 microalgae (either entire or fragmented), and 20049.93 μm^2 of filamentous fungi were counted. Biofouling in the High Marsh was often observed in patches when considering the entire plastic surface. On the other hand, in wetter zones such as the Low Marsh and the Mud Flat, the plastics were entirely covered by biological material (Fig. 3). Microorganisms such as bacteria and microalgae were recorded in all plastic formats and salt marsh zones, while fungi were only detected in fragments and pellets but not fibers and films, and only at the Middle Marsh and the High Marsh zones.

4. Discussion

4.1. Water column and surface sediment

Plastic contamination in estuarine waters has been described for several places around the globe as reviewed by Pinheiro et al. (2021a). In this regard, Lima et al. (2014) reported a total MIPs abundance of 0.26 items m^{-3} considering both surface and bottom waters in the Goiana Estuary (Brazil), collected with a conical plankton net (300 μm). Zhao et al. (2015) measured 100 to 4100 MIPs m^{-3} in subsurface waters (30 cm depth) from three urban estuaries in China, but they used a different sampling technique with filtration step with 1.2 μm mesh size, allowing broader large size recovery. Castro et al. (2016) reported 16.4 MIPs m^{-3} at the Jurujuba Cove (Brazil) using a sampling cylindro-conical plankton net (150 μm). All these abundances are much higher than reported here at the Molhe Oeste salt marsh water (less than 0.01 MIPs m^{-3}). This is because our sampling effort was reduced in extent compared to Lima et al. (2014), Zhao et al. (2015) and Castro et al. (2016), but provides unprecedented baseline information for salt marshes from the largest lagoon of the world.

Since papers on MEPs size are difficult to find [5–25 mm according to GESAMP (2019)], the comparison of contamination levels is therefore limited. For example, Liu and Fang (2020) reported 0 up to 65 items kg^{-1} in sediments from an artificial lake in China. However, these values represent both MEPs and MIPs together, so they cannot be genuinely compared to the values recorded in the present work. Young and Elliott (2016) reported 1208 and 1828 plastic items at the Kamilo and the Kahuku beach respectively, both in Hawaii (USA). However, their results reported the total amount (not relativized by either area or mass) and for plastics in size categories from 2–4 and 4–8 cm. Bancin et al. (2019) used the same size definition as the present work, but they only reported the total number of plastic items (399) as a proxy for MEPs alone, thus the quantities per area (items m^{-2}) were reported for all size categories together (Bancin et al., 2019). Sarkar et al. (2019) reported quantities of MEPs from 4.37 to 215.43 items kg^{-1} in sediments from the Ganga River, in India. However, the size fraction of MEPs was constrained to >5 to <10 mm. Isobe et al. (2014) reported MEPs (>5 mm) contamination levels at several locations in the western part of the Seto Inland Sea, in Japan, but only in coastal waters, so sediment comparisons to our work are not possible.

The MEPs contamination values found in surface sediments from the Molhe Oeste salt marsh, show the same pattern as for MIPs, being more abundant than MEPs as also reported by Young and Elliott (2016), Bancin

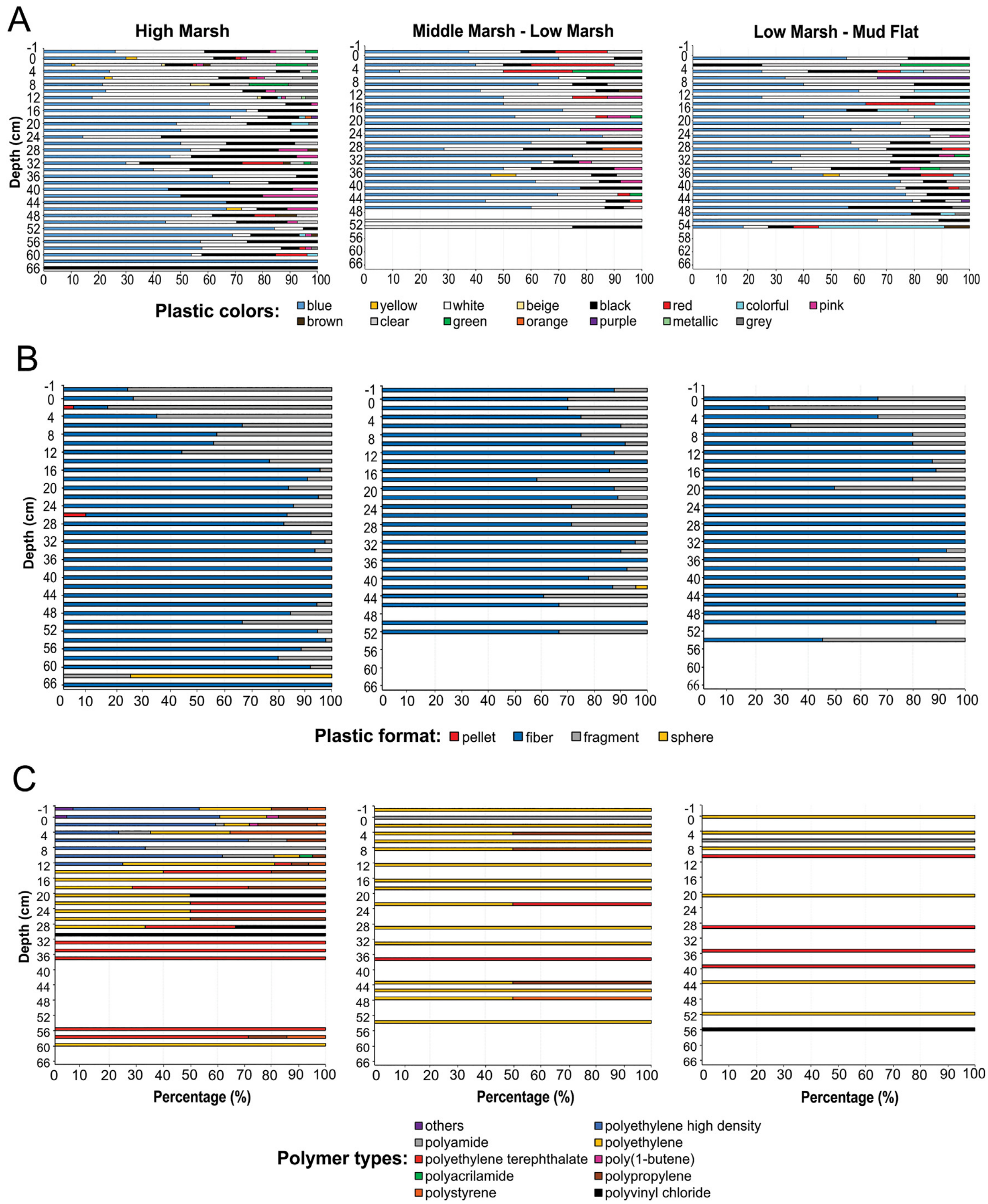

Fig. 6. Characteristics of the plastics recorded in sediment core samples. A: colors, B: formats, C: polymer types.

et al. (2019), Sarkar et al. (2019), Liu and Fang (2020). This highlights the overwhelming significance of MIPs contamination in aquatic environments over other larger size classes. However, it is important to mention that MEPs can alone represent a more dangerous threat to specific faunal groups that might preferably uptake more plastic items within such size category, as it has been reported that an animal's size alone can explain 42% of the variation in the size of plastics ingested (Jâms et al., 2020).

Considering only MIPs, comparison with other studies of the surface sediment of salt marsh environments, shows that our results are similar to those found at the Yangtze Estuary (Wu et al., 2020) and the Angdong salt marsh (Fraser et al., 2020), both in China, and at least one order of magnitude higher than those of Ria Formosa Lagoon, in Portugal (Cozzolino et al., 2020) (Table S3).

The Yangtze River (China) is the world's third largest river, with a catchment area of over 1.8 million km^2 (Zhang et al., 2010), which is about nine times larger than the Patos Lagoon basin of nearly 200000 km^2 (Moller et al., 2001). On the other hand, the Qiantang River (China), where the Angdong salt marsh is located, holds a much smaller catchment area than the Patos Lagoon, with 55058 km^2 (Ministry of Water Resources, 2009). The Ria Formosa Lagoon drains an even smaller area of 741 km^2 (Stigter et al., 2013). Even though the above-mentioned basins are very different in size, MIPs contamination levels were similar in all four studies, which indicates that river basin size itself would not explain MIPs input in estuarine environments. The average MIPs contamination at the sediment surface found in this work, was slightly lower than that recorded at salt marshes in Hong Kong (Lo et al., 2018), and at least one order of magnitude lower than at the Rainbow Haven Beach back lagoon, in Canada (Mathalon and Hill, 2014), and the Spiekeroog Island, in Germany (Liebezeit and Dubaish, 2012) (Table S3). The most polluted areas of Lo et al. (2018) receive freshwater input from the Pearl River of 10600 $m^3 s^{-1}$ and about 2 million tonnes of waste per year (Lam and Lam, 2004; Pearl River Water Resources Committee, 1991). It was therefore expected that areas around the Pearl River would be more polluted than in the Patos Lagoon, as its average discharge is much higher than the average in the present study site (2000 $m^3 s^{-1}$), even though this sampling was performed in the winter when freshwater input reaches its maximum annual peak (Moller et al., 2001). However, the Spiekeroog Island presented much higher MIP abundance despite the low riverine influence, as the nearest river (River Ems) has an average discharge of only 100 $m^3 s^{-1}$ (Van Leussen, 1994).

Multiple environmental variables are clearly needed to explain plastic contamination in estuarine areas. Small scale hydrological variables such as water depth and flow, and submergence time were correlated to MIPs abundance (Wu et al., 2020), and modelling studies have argued that larger scale variables such as river runoff, currents, winds, waves, and tides also exert influences on MIPs deposition in estuarine environments (Cohen et al., 2019). Anthropogenic activities occurring at the catchment area of rivers/lagoons have also been suggested to help understand plastic contamination levels in estuaries (Pinheiro et al., 2021a). In the specific case of Patos Lagoon, the city of Rio Grande has exerted strong historical industrial and harbour impacts leading to environmental contamination since the early colonial period by 1770s intensifying from the 1920s onwards (da Rosa Quintana and Mirlean, 2019).

The pattern of decreasing MIPs abundance from High Marsh to Low Marsh and Mud Flat shown for macroplastics at the Molhe Oeste salt marsh (Pinheiro et al., 2021b) was also detected for both MEPs and MIPs densities in surface sediment (Figs. 2A and 5). Other papers investigating the influence of vegetation on macroplastic accumulation also registered significantly higher contamination levels at vegetated areas when compared to unvegetated areas at North Carolina salt marshes (Viehman et al., 2011), the Cantabria region in Spain (Mazarrasa et al., 2019), and the Ria Formosa Lagoon in Portugal (Cozzolino et al., 2020).

For MIPs, this pattern was also observed during neap tide at the Nanhui tidal flat in China (Wu et al., 2020). Yao et al. (2019) also found the vegetated sites of the Linkun Island, at the mouth of the Ou River in China, to be more contaminated with both MAPs and MIPs than the lower, unvegetated bareflat site. However, they claim that even higher, vegetated sites at the interior of the salt marsh had lower MIPs abundance than the bareflat sites. They argued that the edge area, where the vegetation attains the adjacent water, has higher potential to retain MIPs, and therefore impeded their transport into higher zones in the interior of the salt marsh (Yao et al., 2019). For the Molhe Oeste salt marsh, the potential for retaining both MAPs and MIPs seems to be modulated by both vegetation and flooding degree, as the highest abundances are observed for the zone with denser vegetation and lower water influence (HM).

Accumulation of MIPs in areas protected by solid structures (e.g., beachrocks) has been suggested for sandy beach environments (Pinheiro et al., 2019), and this could be extended to salt marsh environments such as the Molhe Oeste due to the presence of large rocks at the end of the Mud Flat zone (Fig. 1). These rocks have been introduced in the area in the 1960s and the 1970s to prevent erosion and allowed initial plant colonization and subsequent full plant cover of the Low, Middle and High Marsh Zones by the 1980s (Marangoni and Costa, 2009) but also accumulation of plastic items.

In general, the predominant colors of MEPs and MIPs (white and clear, Fig. 4A) in sediment were the same as a previous work at the same study site (Pinheiro et al., 2021b). This incites two different, but not exclusionary, hypotheses: (1) larger deposited items are degrading and thus originating smaller particles, or (2) both large and small particles come from similar sources outside the Molhe Oeste salt marsh and are being deposited in this area. Given the high degradation levels of macroplastics reported in all salt marsh zones by Pinheiro et al. (2021b), the first hypothesis seems suitable for supratidal, drier zones of the salt marsh where macroplastic items might get trapped within the denser vegetation and degrade as they accumulate, because the removal by rising water level is rare. For lower, more frequently flooded zones, the MEPs and MIPs contamination levels were significantly lower, as highly degraded macroplastics still originate MIPs, but they are less likely to settle and remain in such a zone due to high hydrodynamics.

4.2. Sediment cores

The MEP and MIP record in sediment cores collected at the Molhe Oeste salt marsh, come from a dynamic littoral environment, where sediment resuspension and remobilisation are high. In this regard, Bortolin et al. (2020) inferred the hydrodynamic conditions evolved from predominant winds and morphometry, leading to the occurrence of littoral zones of sediment resuspension dominated by sand, where current velocity is normally higher than 0.2 $m s^{-1}$ and depth is very shallow, i.e., less than 1 m. Because of the resuspension processes, the long-term sedimentary record is highly disturbed and the occurrence of plastics in deep layers is a mixing artifact rather than a chronological deposition. Therefore, the presence of plastics in deep layers cannot be used as an Anthropocene marker in the studied area. It is not possible at this point to make historical assumptions of plastic contamination throughout the sediment column at the Molhe Oeste salt marsh. Nevertheless, the present results allow comparisons with similar areas around the globe and contribute to the understanding of plastic contamination levels at estuarine environments.

The average abundance of MIP items observed in the sediment cores was much higher than the maximum values reported for dredged sediments from the Aa River in France (Constant et al., 2021) and from two rivers in Wenzhou in China (Ji et al., 2021), for sediments from the Qinhuai River (Niu et al., 2021) and the Qiantang River (Fraser et al., 2020), both in China (Table S4). Comparison with riverine environments must be carefully interpreted as estuarine areas subject to strong hydrodynamic processes. Works with plastic accumulation along the sediment column are scarce in more similar environments, but there are examples of mangrove (Martin et al., 2020), coastal lagoon (Chico-Ortiz et al., 2020), and estuaries (Willis et al., 2017; Wu et al., 2020). MEP and MIP abundance values in the Molhe Oeste salt marsh still appear to be higher, although comparisons with values reported in different units can be difficult (Table S4).

The depth profile of MEP and MIP contamination found at the Molhe Oeste salt marsh could also be found in other regions of the world. The decrease in plastic abundance with increasing depth was also reported by Fraser et al. (2020) in a highly populated area at the Qiantang River, China, by Chico-Ortiz et al. (2020) at a coastal lagoon in Ghana, and by Martin et al. (2020) in mangroves from the Red Sea and the Arabian Gulf. However, the former was only observed below 11-15 cm depth, with decreased abundances within the top sediment layers (Chico-Ortiz et al., 2020). On the other hand, several works have reported higher MIP abundance in deep when compared to surface layers (e.g. Ji et al., 2021; Niu et al., 2021; Willis et al., 2017). Hence, it is clear that different deposition patterns might occur in different environments, which has been related to sediment remobilisation by dredging (Constant et al., 2021) bioturbation (Xue et al., 2020), procedural contamination (Brandon et al., 2019; Turner et al., 2019), pore water transport (Courtene-Jones et al., 2020), and downward transport or surficial movement at the sediment-water layer (Martin et al., 2022).

Higher amounts of plastic were recorded at the sediment surface than at deeper layers in all salt marsh zones, even by excluding the plant litter layer (roughly 5 cm thick) above the sediment (Fig. 5B). This plant litter layer is more likely to hold reworked content due to its natural dynamics, but it could also interfere with plastic deposition from the sediment immediately below. Strong wind and water action in the study area might cause plant material to be deposited, transported, or even removed from the sediment surface, which definitely influences the plastic load in this layer. Indeed, this pattern was also found by Yao et al. (2019), who reported plastic abundance increasing from sediment subsurface, to litter layer, and then the surface at salt marsh environment in Linkun Island, China. Similarly, it was previously suggested that the water-sediment interface is a highly dynamic layer which content is constantly removed and deposited, which prevents the final accumulation of plastic particles (Frère et al., 2017; Turra et al., 2014; Xue et al., 2020).

Fibers were the predominant format in surface, sediment cores and water (Figs. 4B and 6B), which agrees with global production and with a number of survey studies assessing their abundance in different environments and organisms around the globe (Constant et al., 2021; Lima et al., 2021; Rebelein et al., 2021). However, there are findings showing prevalence of plastic fragments over other formats (e.g., Cozzolino et al., 2020; Ji et al., 2021). Willis et al. (2017) have suggested that the current prevalence of fibers, particularly in remote locations, can be explained by contamination, which highlights the importance of well-stablished QC/QA procedures to avoid overestimation of the environmental loads of plastics.

Similar to the findings for sediment surface, the main polymers observed throughout the sediment column correspond to the main commodity plastics produced worldwide in the last years, such as polyethylene and polypropylene (Geyer et al., 2017). These polymers exhibit density slightly lower than water (Frias, 2018), and therefore are likely to float in aquatic systems. However, degradation processes that normally occur in the environment can even cause their buoyancy to increase (Kowalski et al., 2016; Rummel et al., 2017). This fact has been evidenced by Kaiser et al. (2017) and Karkanorachaki et al. (2021). Hence, MEP and MIP items can sink to sediment and be buried along normal sedimentation processes, as also observed here at 60 cm depth (Fig. 6C). On the other hand, denser polymers such as PVC could only be found below 20 cm at the High Marsh and only at 54 cm deep at the Low Marsh – Mud Flat zone (Fig. 6C). This could indicate that even though sediment can be remobilised throughout the column, denser plastics can remain at higher depths due to its higher density. Thus, the hypothesis that the same factors act on both MIPs and sediment dynamics, which is discussed by Vianello et al. (2013) for example, needs to be further reviewed.

4.3. Biofouling

All MEP and MIP items analysed for biofouling were covered with organisms such as bacteria, fungi, and microalgae, thus reinforcing the significant strong association of the Plastisphere with plastic contamination. It has been shown that factors such as initial surface conditioning by adhered macromolecules (Lorite et al., 2011), surface hydrophobicity (Ogonowski et al., 2018), and polymer types differing in surface roughness (Cai et al., 2019) modulate bacterial adhesion and subsequent biofouling succession. Also, environmental conditions such as nutrient concentration, salinity, temperature, and oxygen in the water column have been reported as causes for differences in bacterial communities forming the Plastisphere (Oberbeckmann et al., 2018, 2014). Differences in the structure of the biological community among marsh zones and plastic types was not investigated at this point, but it can be suggested that organisms producing a protective extracellular matrix such as bacteria would be more resistant to desiccation in higher, dryer zones than microalgae and fungi, who do not produce such a matrix. It has been suggested for macroorganisms inhabiting macroplastics at the Molhe Oeste salt marsh that biofouling occurrence is higher at the wetter, lower zones (Pinheiro et al., 2021b). Given the variety of plastics size, format, color, or even polymer type found at this site, these characteristics could also be affecting colonization but further investigation on the causes or differences among surface characteristics are needed.

The consequences of this association to the Molhe Oeste salt marsh or the Patos Lagoon estuary still remain unclear, as at this point the species composition, preference to plastic types, or alterations to the plastics dynamics were not investigated. However, it can be assumed that the Plastisphere exerts an effect on plastic dynamics as it has been demonstrated that biofouling can increase the sinking behavior (Fazey and Ryan, 2016; Kaiser et al., 2017) or the buoyancy (Rummel et al., 2017) of such particles, which in turn might depend on both plastic and biofilm densities (Nguyen et al., 2020). Future analysis of larger plastic quantities and areas and/or using controlled experiments to investigate the salt marsh Plastisphere at species/metagenomic level will allow further interpretations on these subjects.

4.4. Study limitations

The Patos Lagoon Estuary hosts most of the area covered by this type of environment in the Rio Grande do Sul, Brazil (Marangoni and Costa, 2009). Both the present study and a previous study (Pinheiro et al., 2021b) were performed in a single salt marsh located at the mouth of the lagoon that closely represents the adjacent marine environment. There are 23 further salt marsh estuarine units distributed across a salinity gradient (Fig. 1) still uninvestigated for MAP, MEP, and MIP contamination. Additional sampling efforts distributed in a defined time scale are needed to confirm the plastic accumulation in these areas and to allow comparison with other areas worldwide.

The visual identification of MEPs and MIPs and subsequent chemical identification by Fourier-Transformed Infrared spectroscopy limited the identification of several plastic particles, mainly in the sediment cores, probably due to extensive biofouling coverage that was present in some particles. A digestion step to remove this material from the plastic surface could be further used to improve polymer identification in this type of sample.

Another limitation regarded the Plastisphere analysis, which could not be performed in a more detailed way. Visual identification of the main biofouling groups allowed an initial perception of community diversity, but more appropriate methods such as molecular analysis are needed to identify microorganisms at a lower taxonomic level and to determine diversity indexes. Specific methods to quantify the colonization such as pigment analysis (for algae) of cell density determination (for bacteria) are also indicated. These are required to identify significant differences in the Plastisphere among salt marsh zones and different plastic types. Field experiments testing different plastic types in different zones inside salt marshes could help elucidate such gaps.

5. Conclusions

MIP is a category of contamination that is being found in alarming quantities in several locations, MEP, continue to be a poorly described size category. Comparison of contamination levels for this category is limited, and standardization of size limits for this category is therefore imperative.

The Molhe Oeste salt marsh was previously found to be heavily contaminated with macroplastics, a fact further confirmed here for smaller categories as MEPs and MIPs. Similarly, macroplastics were shown to provide suitable habitat for macroorganisms, and our evidence shows extensive biofouling by microorganisms on smaller plastics.

The vegetation present in the Molhe Oeste can act as a trap for incoming MEPs and MIPs, as found for other vegetated coastal habitats. Studies on the role of vegetation and local hydrodynamics in influencing the quantities of plastics in these areas are crucial to understand plastic dynamics in estuarine areas. Even though the construction of a long-term temporal record of MEPs and MIPs at the Molhe Oeste salt marsh was not possible at this moment, these results contribute to the understanding of plastic contamination and its dynamics in disturbed sediments.

CRediT authorship contribution statement

Lara M. Pinheiro: Conceptualization, Methodology, Formal analysis, Investigation, Writing - original draft, Visualization, Project administration, Funding acquisition; **Larissa M. K. Britz**: Investigation, Visualization; **Vanessa O. Agostini**: Conceptualization, Methodology, Formal analysis, Investigation, Writing - review & editing; **Andrés Pérez-Parada**: Investigation, Resources, Writing - review & editing; **Felipe García-Rodríguez**: Writing - review & editing; **Tamara S. Galloway**: Resources, Writing - review & editing, Supervision; Funding acquisition; **Grasiela L. L. Pinho**:

Conceptualization, Investigation, Resources, Writing - review & editing, Supervision, Funding acquisition.

Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2022.156077.

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Capítulo X: Síntese da Discussão e Conclusões

Nesse capítulo são apresentados os principais pontos das discussões e das conclusões destacados nos artigos científicos apresentados nos Capítulos VI, VII e VIII. Após a discussão, é apresentada a conclusão geral da Tese através da verificação da Hipótese proposta. Por fim, são apresentadas as considerações finais e perspectivas futuras para avanços do presente trabalho.

10.1. Síntese da discussão da Tese

10.1.1. Visão geral da contaminação plástica em estuários e direcionamentos para estudos futuros

A revisão de literatura científica a respeito da contaminação por plásticos em ambientes estuarinos revelou que as estimativas de quantidade deste material de fontes terrestres que entram nos oceanos através dos rios chega à ordem de trilhões de partículas ou milhões de toneladas por ano [Cheung & Fok 2017, Mai *et al.* 2020]. Entretanto, existem razões para acreditar que esses valores são subestimados, devido principalmente à grande divergência nas estimativas feitas com diferentes metodologias, além da limitação de informações em categorias de plástico menores como microplásticos e principalmente no formato de fibras. Estudos que investigaram a distribuição e acúmulo de plásticos em ambientes estuarinos comumente se utilizam de técnicas de modelagem para considerar fatores temporais e espaciais na explicação dessas variáveis. Entretanto, processos como a influência do regime de chuvas, das ondas e da descarga dos corpos d'água ainda não são bem discutidos nesse tipo de análise [Lourenço *et al.* 2017, Naidoo *et al.* 2015]. De forma geral, itens maiores de plástico (macroplásticos) são mais fáceis de rastrear e modelar, o que se reflete numa maior quantidade de estudos com essa classe de tamanho [ex.: Krelling *et al.* 2017]. Trabalhos com modelagem de microplásticos são mais difíceis devido à sua ampla variedade de fontes e rotas de fragmentação, além da complexa relação entre a abundância dessas partículas e as variáveis físico-químicas do ambiente onde estão inseridos. Para ambientes estuarinos, essa complexidade de variáveis parece de fato ser limitante para o desenvolvimento de estudos nessa temática. Isso foi evidenciado pela grande lacuna espacial de publicações com contaminação plástica em estuários do mundo (Figura 1, Capítulo VII: Artigo 1).

As matrizes ambientais presentes em estuários (água, sedimento e biota) apresentam características distintas que são comumente consideradas para entender a contaminação por plásticos nesses ambientes. A densidade dos plásticos é um dos fatores que influencia o transporte desses contaminantes em água, sendo que a diferença entre a densidade da água e a das partículas é que vai determinar o seu comportamento de flutuar ou afundar na coluna d'água, o que por sua vez é influenciado pela degradação e bioincrustação atuando simultaneamente nos plásticos. Trabalhos quantificando plásticos em águas estuarinas também consideraram a presença de matéria orgânica na superfície dos plásticos como um problema a ser resolvido a fim de aumentar a confiabilidade das análises para identificação dos polímeros, já que ambientes estuarinos são altamente produtivos e portanto apresentam alta concentração de matéria orgânica [Day et al. 2013].

Assim, técnicas de digestão para remoção desse material são comumente usadas para investigações nesses ambientes [Stolte *et al.* 2015, Jensen *et al.* 2017]. A existência de um gradiente de salinidade causado pela intrusão de água salina em água doce é outro fator que tem influência direta na movimentação de partículas plásticas em suspensão na água, como já observado por Acha *et al.* [2003] em um trabalho no estuário do Rio da Prata que demonstrou o papel da frente salina como uma barreira para itens plásticos, que acumulavam na parte interna do estuário. Considerando essa diversidade de fatores e das propriedades físico-químicas das água estuarinas, coletas realizadas nessa matriz representam portanto um registro momentâneo da contaminação ambiental por plásticos.

A composição da matriz sedimentar em ambientes estuarinos normalmente leva em consideração a contribuição dos rios, dos processos erosivos, da produção primária, do oceano, da atmosfera e até de planos lamosos, quando presentes em estuários [Schubel 1982]. Assim, se considera que o material plástico presente em sedimentos também deriva dessas contribuições, além de ser influenciado por processos similares aos sedimentos naturais como balanço na entrada de água salina e doce, ondas, maré, pressão atmosférica, correntes, bioturbação, permeabilidade do sedimento e presença de vegetação [Teasdale *et al.* 2011, Ward *et al.* 2014, 2016, Misic *et al.* 2019, Martinetto *et al.* 2016, Ward 2020, Ward & Lacerda 2021]. Percebe-se também que a correlação direta entre a deposição de plásticos e as taxas de sedimentação pode ser desafiadora em estuários, já que estes são altamente dinâmicos e isso pode causar mistura estratigráfica mesmo em regiões onde há proteção por vegetação devido à ocorrência de eventos extremos [Feagin *et al.* 2009, Li et al. 2020] e principalmente nas camadas mais superficiais de sedimento [Critchell & Lambrechts 2016, Willis *et al.* 2017]. Assim, interpretações temporais da contaminação plástica na matriz sedimentar são válidas desde que realizadas em frações mais profundas de sedimento onde se acredita ter menor perturbação por variações no fluxo d'água [Butzeck *et al.* 2014, Ward 2020], bioturbação [Gebhardt & Forster 2018], ou por ações humanas como pesca ou dragagem [Bardos et al. 2020], como já é feito para outros grupos de contaminantes [Cundy & Croudace 1996, Celis-Hernandez *et al.* 2020, 2021].

A contaminação plástica presente na matriz biótica (i.e., nos organismos estuarinos) foi confirmada através de análises de conteúdos estomacais, de excrementos, ou da digestão dos organismos inteiros. Na revisão de literatura realizada, até setembro de 2020 o número de estudos com organismos estuarinos era limitado a 34 artigos publicados, sendo apenas sete investigando os efeitos da presença de plásticos nos organismos. Além dos riscos de obstrução e abrasão do trato gastrointestinal ou das vias aéreas, os organismos também estão sujeitos aos efeitos tóxicos das substâncias que são adicionadas aos plásticos durante sua fabricação, e que são liberadas ao longo da sua vida útil, ou que são adsorvidas à superfície dos plásticos uma vez que eles estão no ambiente [Anbumani & Kakkar 2018, Celis-Hernandez *et al.* 2020]. Essas substâncias adsorvidas incluem metais, fármacos, praguicidas, antibióticos, produtos de cuidados pessoais e outros contaminantes de preocupação emergente [Loos *et al.* 2013, Pintado-Herrera *et al.* 2017, Celis-Hernandez *et al.* 2020, Li *et al.* 2020], que por sua vez já são reportadas em grandes quantidades em efluentes de grandes centros urbanos, onde estuários estão comumente localizados [Conley *et al.* 2019, Xu *et al.* 2019, Zhou *et al.* 2019].

A transferência trófica de plásticos entre organismos estuarinos e suas possíveis consequências ainda não foram investigadas em campo. Além disso, os esforços para investigar presença e efeitos dos plásticos na biota estão mais concentrados em espécies de valor comercial, que são utilizadas para consumo humano como peixes, ostras e mexilhões [ex.: Ferreira *et al.* 2016, 2019a, 2019b]. Estratégias de monitoramento ambiental devem incluir espécies e/ou guildas tróficas com diferentes papéis ecológicos facilitando o planejamento para mitigação da contaminação em ambientes estuarinos.

Outra importante interação entre plásticos e biota em ambientes aquáticos se dá pela bioincrustação, formando a Plastisfera [Agostini *et al.* 2018, Zettler *et al.* 2013]. Para estuários, essa interação se mantém pouco estudada mas algumas inferências podem ser feitas considerando a literatura disponível. Sabe-se que as características do substrato podem interferir no processo de bioincrustação [Agostini *et al.* 2017, 2018], e que parâmetros ambientais como pH, salinidade, temperatura, disponibilidade de nutrientes e luz são também determinantes para a formação de comunidade microbianas [Harrison *et al.* 2018, Oberbeckmann *et al.* 2018, Rummel *et al.* 2017]. Em ambientes estuarinos, a variedade de condições ambientais e de substratos em termos de combinações de cores, formatos, texturas, tamanhos e tipos de polímero é bastante significante. Além disso, as peculiaridades em termos das diferentes atividades que são desenvolvidas ao longo do estuário também contribuem para a variabilidade de fontes de contaminação e, consequentemente, de substratos e propriedades físico-químicas do ambiente ao longo do estuário. Assim, tem-se a possibilidade de formação de inúmeros cenários a partir das combinações de condições ambientais e de substratos, sendo então de fundamental importância entender as consequências da bioincrustação de plásticos nesses cenários.

10.1.2. Contaminação por resíduos sólidos na Marisma do Molhe Oeste e sua atuação como nicho para a bioincrustação

O presente trabalho de Tese investigou de maneira inédita a contaminação ambiental por resíduos sólidos em uma marisma do Estuário da Lagoa dos Patos (RS). Os plásticos representaram 92,4% dos materiais encontrados, mas itens de madeira, papel/papelão, vidro metal e outros materiais também foram encontrados. As quantidades de resíduos encontradas na Marisma do Molhe Oeste foram maiores do que as encontradas em marismas na Carolina do Norte (EUA) por Viehman *et al.* [2011], sendo que esses autores também encontraram um padrão de maior deposição de resíduos nas zonas mais secas da marisma, assim como visto na Marisma do Molhe Oeste. Esse padrão também foi observado em marismas de três estuários da Espanha [Mazarrasa *et al.* 2019], apesar da quantidade média ter sido menor do que a encontrada no presente estudo. A explicação dada para o maior acúmulo de resíduos nas zonas mais secas pode ser atribuída à menor taxa de alagamento dessas zonas, o que indica menor frequência de remoção dos resíduos, que por sua vez são depositados nessas zonas em ocasiões de eventos extremos como tempestades. De fato, a região do Estuário da Lagoa dos Patos sofre com ocasionais tempestades e ciclones [Parise *et al.* 2009]. Além disso, a presença de vegetação mais densa na zona mais elevada e seca da marisma causa um efeito de aprisionamento de itens de resíduos sólidos, como descrito por Cozzolino *et al.* [2020]. Esses autores viram que a vegetação de marismas pode prender significativamente mais macroplásticos do que uma área não vegetada adjacente, apesar de não terem observado esse efeito de aprisionamento para microplásticos.

Um trabalho de monitoramento de resíduos sólidos realizado em uma área de praia arenosa adjacente ao Estuário da Lagoa dos Patos, a praia do Cassino, foi realizado por de Ramos *et al.* [2021]. Nesse trabalho, as quantidades de resíduos encontradas foram até uma ordem de magnitude menor do que as encontradas na Marisma do Molhe Oeste. Essa comparação representa um indício de que a região do interior do estuário está sendo mais afetada pela contaminação por resíduos sólidos do que a área costeira adjacente. Além disso, as fontes dos resíduos identificadas na praia do Cassino indicam origens predominantes de usuários de praia ou de atividades pesqueiras, enquanto na marisma a maioria dos itens eram embalagens alimentícias, itens de pesca ou relacionados a embarcações e itens de uso pessoal. Apesar dessas diferenças, é possível perceber a grande contribuição das atividades pesqueiras para a contaminação ambiental na região costeira da cidade de Rio Grande.

Ambientes de marismas normalmente apresentam substratos não consolidados como lama, sendo que estruturas sólidas como conchas e rochas são então utilizadas por organismos bioincrustantes nessas regiões. O presente estudo mostrou que os substratos antropogênicos na forma de resíduos sólidos estão servindo como substrato adicional para esses organismos. Essa associação tende aumentar de frequência da zona mais seca em direção à zona mais alagada (Figura 6A , Capítulo VIII: Artigo 2), sendo que os grupos de organismos ocorrendo nas zonas variaram provavelmente de acordo com seus mecanismos individuais de seleção de substrato, sua resistência a lixiviados químicos ou a comunidade incrustante pré-existente. Colonizadores secundários como algas estavam mais presentes na zona seca (Tabela 1, Capítulo VIII: Artigo 2), onde esses organismos podem encontrar maior cobertura vegetal protegendo-os conta dessecação e fotoinibição que impactaria seu desenvolvimento [Dodds *et al.* 1999]. Colonizadores terciários como cracas estavam mais presentes na zona intermediária (Tabela 1, Capítulo VIII: Artigo 2), onde podem encontrar um ambiente que balanceia estresse térmico, dessecação e predação de forma vantajosa para esse grupo.

As análises de preferência de ocorrência dos grupos de organismos por diferentes substratos também mostrou, de forma geral, que a maioria dos grupos de organismos ocorre mais em plásticos do que em outros tipos de materiais como madeira ou metal. Esse resultado já foi encontrado também por outros estudos como o de Pinochet *et al.* [2020] e Li *et al.* [2016], que mostraram maior ocorrência de grupos bioincrustantes em plásticos do que em concreto, madeira e vidro. Considerando a crescente disponibilidade de substratos plásticos nos ambientes aquáticos, essa preferência pode favorecer a ocorrência de espécies e potencialmente modificar as relações ecológicas em uma determinada região, trazendo impactos ecológicos de magnitude ainda incerta.

Alguns grupos de organismos bioincrustantes como cracas, algas, briozoários, insetos e copépodos já foram vistos com maior ocorrência em substratos sem gastrópodes raspadores [Anderson & Underwood 1997]. Indícios desse padrão também foram encontrados no presente estudo, visto que cracas, algas e insetos não tiveram a mesma preferência de ocorrência de gastrópodes para zona, material ou cor. Em relação às cores, a preferência de ocorrência dos grupos variou bastante. Por exemplo, as algas ocorreram mais em itens transparentes como copos descartáveis, que por sua vez ocorreram bastante na zona seca onde algas também foram mais frequentes, o que pode ser explicado pela providência de passagem de luz solar necessária para a atividade fotossintética desse grupo. Assim, a ocorrência dos grupos incrustantes na Marisma do Molhe

Oeste em diferentes tipos de substratos antropogênicos ainda precisam de investigações adicionais a fim de elucidar preferências por cores, materiais ou outras características dos resíduos sólidos.

10.1.3. Contaminação por meso- e microplásticos na Marisma do Molhe Oeste e sua atuação como nicho para a bioincrustação

O presente trabalho também quantificou de forma inédita a contaminação por mesoplásticos (MEP, 5 - 25 mm) e microplásticos (MIP, < 5 mm) em amostras de água, sedimento superficial e coluna sedimentar na Marisma do Molhe Oeste. Os valores de abundância de plástico encontrados em águas foram menores do que os reportados em outras regiões estuarinas do mundo como no Rio Goiana (Brasil) [Lima *et al.* 2014], na Baía de Jurujuba (Brasil) [Castro *et al.* 2016], e em três estuários urbanos da China [Zhao *et al.* 2015]. Porém, é importante ressaltar que o esforço amostral realizado na Marisma do Molhe Oeste foi muito pequeno quando comparado a esses outros estudos, e representam apenas um registro momentâneo da contaminação desse ambiente.

A comparação entre as quantidade de MEPs encontradas na marisma e outros estudos publicados foi limitada devido à falta categorização padronizada dos tamanhos de plásticos. Por exemplo, estudos como o de Liu & Fang [2020] trazem quantidades de MEP e MIP agrupados, enquanto outros estudos como o de Young & Elliott [2016] reportam a quantidade total de plásticos sem relativizar por massa ou volume, o que torna a comparação direta inviável. Assim, estudos que tragam categorias de tamanhos mais definidas e padronizadas devem ser encorajados, principalmente considerando que diferentes grupos de organismos poderão absorver essas partículas em diferentes tamanhos, já que essas duas variáveis são diretamente relacionadas [Jâms *et al.* 2020].

Ao observar apenas MIPs, as quantidades encontradas no sedimento superficial da Marisma do Molhe Oeste foram similares às encontradas em dois estuários da China [Fraser *et al.* 2020, Wu *et al.* 2020], porém muito maiores do que as encontradas por Cozzolino *et al.* [2020] em Portugal. Essas regiões apresentam bacias de drenagem de tamanhos muito diferentes do que a do Estuário da Lagoa dos Patos, o que indica que esse fator sozinho não explica a entrada de MIPs em regiões estuarinas. O mesmo acontece para os volumes de descarga dos corpos d'água em estuários: a contaminação na Marisma do Molhe Oeste foi apenas um pouco menor do que a encontrada em marismas de Hong Kong [Lo *et al.* 2018], mesmo considerando que a entrada média de água doce da Lagoa dos Patos é mais de cinco vezes menor do que o na região do estudo supracitado.

Assim, pode-se afirmar que são necessários múltiplos fatores para explicar a contaminação ambiental em ambientes estuarinos. De fato, variáveis de pequena escala como profundidade da coluna d'água, fluxo e tempo de submersão já foram correlacionados com abundância de MIP [Wu *et al.* 2020], assim como variáveis de larga escala como correntes, ventos, ondas e marés [Cohen *et al.* 2019]. Atividades antropogênicas ocorrendo na região também ajudam a entender esse tipo de contaminação, como visto no segundo artigo desta Tese [Pinheiro *et al.* 2021]. Para a região da Lagoa dos Patos, as atividades industriais e portuárias vêm exercendo pressão ambiental no estuário desde o início do período colonial nos anos 1770, mas foi intensificado após 1920 [da Rosa Quintana & Mirlean 2019].

O padrão de maior contaminação nas zona mais seca da marisma que diminuía em direção à zona mais alagada foi encontrando também para MEPs e

MIPs (Figura 2A, Capítulo IX: Artigo 3), assim como para resíduos sólidos maiores (Figura 6A, Capítulo IX: Artigo 3). Outros estudos observaram essa distribuição de MIPs em regiões de marismas na China [Yao *et al.* 2019, Wu *et al.* 2020], o que é frequentemente relacionado à presença mais densa de vegetação nessas regiões. Assim, variação na vegetação e na taxa de alagamento parecem modular a abundância de MAPs, MEPs e MIPs na Marisma do Molhe Oeste. Além disso, a presença de enrocamento paralelo à linha de margem também pode ser um fator que favorece o acúmulo de plásticos na região, como já demonstrado para estruturas sólidas similares (*beachrocks*) em ambientes de praias arenosas [Pinheiro *et al.* 2019].

Os formatos e cores dos MEPs e MIPs predominantes na Marisma do Molhe Oeste foram similares aos dos itens plásticos maiores descritos no artigo 2 desta Tese, o que leva à formulação de duas hipóteses não-excludentes: (*i*) macroplásticos depositados na marisma estão degradando e originando microplásticos secundários; (*ii*) macro-, meso- e microplásticos estão vindo de fontes similares externas à marisma e sendo depositadas na região. A primeira hipótese parece plausível para a zonas mais secas, considerando que foi observado alto grau de degradação dos itens ao longo de todas as zonas da marisma (Figura S2C, ANEXO II) e que a remoção de resíduos pela subida do nível da água não ocorre com frequência. Para as zonas mais alagadas, as partículas têm menos probabilidade de assentar e acumular devido à constante variação hidrodinâmica.

A contaminação por MEP e MIP na Marisma do Molhe Oeste alcançou até 66 cm de profundidade, indicando que as partículas plásticas depositadas na superfície são transportadas para camadas mais profundas na coluna sedimentar, onde podem permanecer por tempo indeterminado. Inferências temporais de deposição dos plásticos ao longo da coluna sedimentar não puderam ser realizadas no presente estudo, pois a região da marisma está sujeita a mistura estratigráfica por ressuspensão e remobilização do sedimento devido às condições hidrodinâmicas [Bortolin *et al.* 2020]. Assim, apesar de não poder ser utilizado para marcador do Antropoceno, o presente estudo contribui para o entendimento da contaminação em estuários e permite comparação com estudos em área similarmente dinâmicas.

As quantidades de MIPs nos testemunhos coletados na Marisma do Molhe Oeste foram maiores do que os encontrados em diversas regiões estuarinas ao redor do mundo como França [Constant *et al.* 2021], Arábia Saudita [Martin *et al.* 2020], Gana [Chico-Ortiz *et al.* 2020], e China [Fraser *et al.* 2020, Ji *et al.* 2021, Niu *et al.* 2021]. Alguns destes trabalhos mostraram maior abundância de plásticos nas camadas mais superficiais dos testemunhos coletados [ex.: Fraser *et al*. 2020, Martin *et al.* 2020], enquanto outros viram a abundância aumentar em camadas mais profundas [ex.: Ji *et al.* 2021, Niu *et al.* 2021]. No presente estudo, o tipo de distribuição observado foi o primeiro, com quantidades de plástico significativamente mais altas nos primeiros 10 cm de sedimento (Figura 5B, Capítulo IX: Artigo 3). Portanto, diferentes padrões de deposição de MEP e MIP podem ocorrer em diferentes regiões estuarinas, a depender de fatores ligados à remobilização sedimentar como dragagem [Constant *et al.* 2021], bioturbação [Xue *et al.* 2020], transporte de água intersticial [Courtene-Jones *et al.* 2020], e transporte para camadas profundas ou movimentação na interface água-sedimento [Martin *et al.* 2022], além de contaminação durante os métodos de análise dessas partículas [Brandon *et al.* 2019, Turner *et al.* 2019]. Entretanto, apesar da discussão de que os mesmos fatores atuam na dinâmica dos plásticos e do sedimento, apresentada por Vianello *et al.* [2013] por exemplo, a presença de plásticos de alta densidade como o PVC apenas em camadas mais profundas pode indicar que apesar da remobilização sedimentar plásticos mais densos podem permanecer em camadas mais profundas devido à sua maior densidade.

Todos os MEPs e MIPs apresentaram organismos como bactérias, fungos e microalgas colonizando sua superfície, o que reforça a intrínseca associação da Plastisfera com a contaminação plástica em ambientes de marisma. Apesar da relação entre a presença desses grupos e as diferentes zonas da marisma ou características dos plásticos não ter sido profundamente investigada nesse estudo, pode-se sugerir que organismos que produzem matriz polimérica extracelular como bactérias sejam mais resistentes à dessecação nas zonas mais secas, enquanto fungos e microalgas que não possuem tal proteção devem ser menos resistentes nessas áreas. As consequências ecológicas dessa associação para a região da Marisma do Molhe Oeste, do Estuário da Lagoa dos Patos e as regiões costeira e marinha adjacentes permanecem incertas, a depender de estudos que investiguem a composição de espécies, a preferência por tipos diferentes ou as alterações na dinâmica de plásticos utilizando experimentos controlados e em maior escala amostral.

10.2. Conclusão Geral da Tese

Estuários são sistemas complexos que estão sendo sistematicamente contaminados por resíduos sólidos, principalmente por plásticos. Com o presente trabalho, o Estuário da Lagoa dos Patos passa a ser melhor consolidado dentro dessa premissa, uma vez que essa contaminação foi identificada e caracterizada em um importante ambiente de marisma localizado dentro dessa região, a Marisma do Molhe Oeste. As quantidades e composição de resíduos sólidos em todas as categorias de tamanho reportadas nesta Tese de doutorado trazem uma linha de base para futuros esforços de monitoramento e de comparação da distribuição dos resíduos sólidos, além da influência da composição vegetal e dos regimes de alagamento em áreas de marisma. O papel de aprisionamento feito pela vegetação mais abundante em zonas mais secas da marisma foi observado, o que sugere que futuros estudos buscando medidas preventivas ou mitigatórias devam focar nessas regiões. Mais investigações são necessárias para estabelecer essa relação na Marisma do Molhe Oeste, principalmente para partículas plásticas menores como MEPs e MIPs.

O estabelecimento de uma comunidade bioincrustante chamada de Plastisfera nas superfícies de MEPs e MIPs pôde ser observada na Marisma do Molhe Oeste, e a distribuição heterogênea dessa comunidade nas diferentes zonas da marisma e nos diferentes tipos de substrato disponíveis pôde ser confirmada. Os fatores que explicam essa distribuição parecem estar relacionados às necessidades individuais de cada grupo incrustante como a resistência a variações de disponibilidade de água e luz e à comunidade previamente estabelecida, mas também pode-se considerar outros fatores como o tempo de permanência desses itens no ambiente.

10.3. Considerações finais e perspectivas futuras

Ao retomar os objetivos geral e específicos desta Tese de Doutorado (Capítulo III), temos que o primeiro objetivo, i.e., "(i) *investigar, a partir de um estudo de revisão, o status da contaminação/poluição por plásticos em ambientes estuarinos e sua relação com a bioincrustação, outros contaminantes e a sua toxicidade*", foi alcançado a partir da publicação do primeiro artigo da Tese (Capítulo

VII: Artigo 1), que compilou com sucesso informações científicas disponível na literatura a respeito da grande temática deste trabalho, identificando lacunas e estabelecendo guias para novas investigações. O segundo objetivo específico, i.e., "c*aracterizar a distribuição de resíduos sólidos em um ambiente de marisma e a sua relação com o processo de bioincrustação, bem como a influência das taxas de alagamento e circulação estuarina nesses processos"*, também foi alcançado a partir da publicação do segundo artigo desta Tese (Capítulo VIII: Artigo 2), onde foram reportadas as quantidades de resíduos sólidos na Marisma do Molhe Oeste, os organismos associados a esses itens e como eles se distribuíram nas zonas e tipos de substratos disponíveis. O terceiro objetivo específico, i.e., "c*aracterizar a distribuição de meso- e microplásticos em sedimento (superfície e coluna sedimentar) e em água de um ambiente de marisma e sua relação com a bioincrustação"*, também foi alcançado através da metodologia e dos resultados apresentados no terceiro artigo da Tese (Capítulo IX: Artigo 3), onde foram reportadas as quantidades de MEPs e MIPs e dos micro-organismos da Plastisfera em diferentes matrizes abióticas da Marisma do Molhe Oeste. Por fim, os esforços para atingir o quarto objetivo específico, i.e., "*investigar a interação entre plásticos e a bioincrustação em ambiente de marisma, avaliando a influência de diferentes características dos polímeros"*, foram iniciados e continuam em andamento, sendo que resultados preliminares já dão indícios de que essa investigação será bem-sucedida através de uma quarta publicação futura resultante do trabalho desenvolvido nesta Tese.

A primeira hipótese formulada e testada neste trabalho de Tese de Doutorado, i.e., "*A Marisma do Molhe Oeste, Estuário da Lagoa dos Patos, RS, Brasil está contaminada por resíduos sólidos, predominantemente por plásticos,* *com variação negativa de distribuição em relação à taxa de alagamento da Marisma*", foi totalmente aceita, visto que esse tipo de contaminação foi confirmada em abundâncias médias de 5,35 ± 6,02 itens m^{-2} para resíduos sólidos grandes (a maioria plásticos), 8,89 ± 8,75 MIPs L^{-1} em água, 279,63 ± 410,12 MEPs e MIPs kg^{-1} de sedimento seco superficial, e 366,92 ± 975,18 12 MEPs e MIPs kg^{-1} de sedimento seco ao longo da coluna sedimentar. As abundâncias de MAPs, MEPs e MIPs em sedimento acompanharam uma tendência do tipo Marisma Superior > Marisma Médio > Marisma Inferior > Plano Lamoso, i.e., decrescente com o aumento da taxa de alagamento. Futuros estudos temporais de acúmulo de resíduos plásticos nessas regiões devem considerar a hidrodinâmica local e as mais variadas fontes de contaminação que estão presentes ao longo de toda a bacia hidrográfica. As zonas mais secas e com vegetação mais densa presentes nas marismas no Estuário da Lagoa dos Patos podem estar atuando como *hotspots* de acumulação de plásticos, o que deve ainda ser confirmado a partir de estudos mais abrangentes.

A segunda hipótese formulada e testada neste trabalho de Tese de Doutorado, i.e., "*A bioincrustação na Marisma do Molhe Oeste é afetada quali- e quantitativamente por diferentes características dos resíduos sólidos como tamanho, cor e tipo de polímero, e pela zonação da marisma.*", foi aceita visto que tendências de maior ocorrência de grupos incrustantes puderam ser percebidos para diferentes zonas da marisma, tipos de material do qual o substrato era feito e suas cores. Os resultados apresentados na subseção 6.3 indicam que a zonação e as cores do substrato são um fator decisivo para a colonização inicial, porém mais variáveis como a composição da comunidade precisam ser analisadas para fortalecer a segunda hipótese deste Tese.

Como perspectivas futuras, tem-se o aprofundamento dos estudos a respeito as implicações da ocorrência da Plastisfera em ambientes de marisma para a ecologia local, principalmente em relação ao estabelecimento de espécies exóticas e as potenciais consequências para a ecologia loca. Em termos de preservação ambiental, é de fundamental importância que estudos de maior abrangência temporal e espacial sejam realizados no Estuário da Lagoa dos Patos a fim de suportar medidas de mitigação e planejamento do manejo de resíduos sólidos nessa importante região da América do Sul.

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ANEXO I

The fate of plastic litter within estuarine compartments: an overview of current knowledge for the transboundary issue to guide future assessments

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Supplementary material

Literature searching methods

An extensive literature review was performed using Periódicos Capes - Brazil, a database comprising collections including Scopus, Web of Science and ScienceDirect Journals. The keywords *estuary* and *plastic/polymer* were used in combination with *salt marshes*, *mangrove*, *biofilm*/*biofouling*, *contaminant interaction*, and *toxicity*. Retrieved papers were screened and selected according to these mandatory criteria: (*i*) published in an indexed peer reviewed journal); (*ii*) reporting contamination and/or pollution by plastics); (*iii*) reporting field observations and/or performing field or laboratory experiments involving estuarine environment. Grey literature (theses and dissertations, conference abstracts and technical reports) were not considered in this review.

An additional search was performed in the same database using the keywords *estuary* and *plastic/polymer* in combination with *correlative models* and *particle tracking* in order to investigate factors considered to influence plastic distribution and to find computational models of plastic transport in estuaries. Ten papers published from 2015 to 2020 were retrieved regarding estuaries. Five papers concerning freshwater systems were also retrieved and used to discuss plastic sources to estuaries.

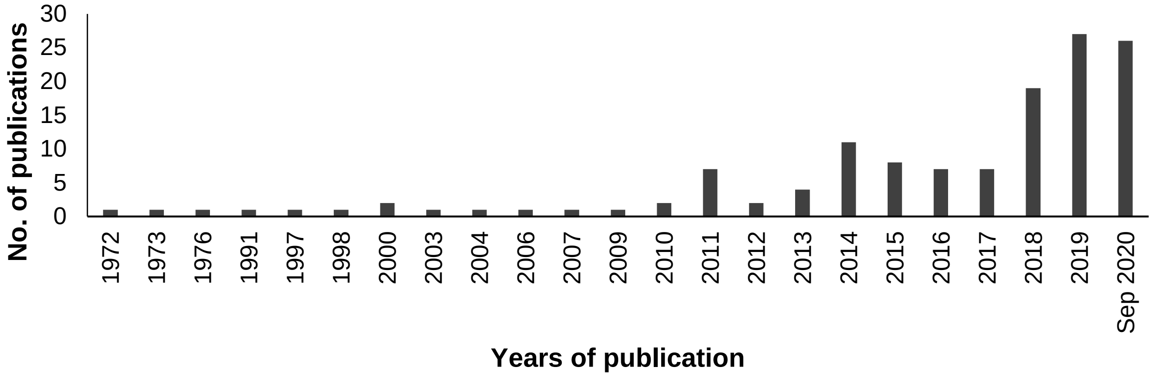

Figure S1. Publications on plastic contamination in estuarine environments between 1972 and September 2020. The papers were retrieved from the Periódicos Capes – Brazil database.

Table S1. Journals where the papers analysed in this review were published.

Journal name	N° of publications
Marine Pollution Bulletin	33
Environmental Science and Pollution Research	14
Environmental Pollution	8
Nature	6
PLoS ONE	6
Estuarine Coastal and Shelf Science	4
Frontiers in Marine Science	4
International Journal of Environmental Research and Public Health	4
Water Air Soil Pollution	4
Bulletin of Environmental Contamination and Toxicology	3
Frontiers in Microbiology	3
Journal of Coastal Research	3
Environmental Science and Technology	3
Environmental Science: Processes & Impacts	2
Environmental Toxicology and Chemistry	2
Food Additives & Contaminants: Part A	2
Science	2
Science of the Total Environment	2
Annual Review of Marine Science	1
Antonie van Leeuwenhoek	1
Applied Microbiology and Biotechnology	1
Aquatic Biology	1

BMC Microbiology	1
Chemistry and Ecology	1
Chemosphere	1
Critical Reviews in Environmental Science and Technology	1
Ecotoxicology	1
Ecotoxicology and Environmental Safety	1
Endangered Species Research	1
Environmental Chemistry	1
Environmental Earth Sciences	1
Journal of Food Science and Technology	1
Management of Environmental Quality	1
Marine Chemistry	1
Marine Environmental Research	1
Marine Policy	1
Nuclear Inst. and Methods in Physics Research B	1
Particle and Fibre Toxicology	1
PeerJ	1
Philosophical Transactions of the Royal Society B	1
Proceedings of the Estonian Academy of Sciences	1
South African Journal of Science	1
Talanta	1
Water	1
Water AS	1
Water Research	1

Table S2. Size categories proposed to classify plastics in the environment. NI: not informed.

Nomenclature	Macroplastic	Mesoplastic	Microplastic	Nanoplastic	Ref.*
Size scales	> 20 cm	20 – 5 cm	5 mm – 1 µm	< 1000 nm	1
	NI	NI	5 mm – 1 µm	1000 nm – 1 nm	2, 3
	NI	5 – 10 mm	5 – 0.2 mm	NI	4
	1 m – 2.5 cm	2.5 cm – 5 mm	5 – 0.1 mm	NI	5
	> 200 mm	4.76 – 200 mm	0.33 – 4.75 mm	NI	6

***References: 1** Hanvey et al. (2017); **2** Frias and Nash (2019); **3** Gigault et al. (2018); **4** Collignon et al. (2014); **5** Young and Elliot (2016); **6** Eriksen et al. (2014).

Table S3. Compilation of data from the papers analysed in this review, focusing on methodologies and concentrations of microplastics found in the field. It was not possible to fit the size classification of Lee et al. (2013) (ref. nº 42 in the table) to our size classification so their nomenclature was kept in this table. Browne et al. (2010) (ref. nº 40 in this table) reported microplastics as particles > 1 mm. References 46 to 49 in this table did not discriminate sizes when reporting concentrations, but they were included in this table as they found mostly microplastics. S: sediment; W: water; FS: field sampling; FE: field experiment; LE: laboratory experiment.

Country	Matrix analysed	Type of work	Methodology used	Microplastic concentration in the field	Ref.*
United Kingdom	S	FS	S: sampling from the strandline with a trowel and from the subtidal using an Eckman grab, saline flotation with NaCl, filtration (1.6 µm)	S: 0 to 120,000 items m^{-3};	1
Singapore	S	FS	sampling of top 3-4 cm sediment in three separate 1.5 m x 1.5 m quadrats using a spatula; saline flotation with NaCl; filtration (1.6 µm)	12.0 ± 8.0 - 62.7 ± 27.2 items kg^{-1} DW; average 36.8 ± 23.6 items kg^{-1} DW	2
Tunisia	S	FS	sampling of top 2-3 cm sediment in 0.25 m x 0.25 m quadrats with a spatula; saline flotation with NaCl; filtration (~1 µm)	141.2 ± 25.9 to 461.2 ± 29.7 items kg^{-1} DW, average 316.0 ± 123.7 items kg^{-1} DW	3

China	S	FS	sampling with a box corer; saline flotation with NaCl; filtration (1 µm)	20 to 340 items kg^{-1} DW; average 121 ± 9 items kg^{-1} DW	4
Belgium	S	FS	sampling with a Van Veen grab; saline flotation with NaCl; filtration (38 µm)	66.9 ± 7.9 to 390.7 ± 32.6 items kg^{-1} DW; average of 166.7 ± 92.1 items kg^{-1} DW; 0.8 ± 0.1 to 7.2 ± 0.8 mg kg^{-1} DW	5
Italy	S	FS	sampling with a box-corer (0-5 cm)	672 to 2175 items kg^{-1} DW	6
China	S	FS	sampling of the top 4 cm in 50 X 50 cm quadrats using a shovel; saline flotation with seawater; filtration (315 µm)	94 ± 44 to 2098 ± 1705 items m^{-2}, average of 520 ± 688 items m^{-2}; 0.1 ± 0.1 - 3.1 ± 3.8 g m^{-2}, average of 0.5 ± 0.7 g m^{-2}	7
United Kingdom	S	LE	sediment microcosm spiked with LDPE microplastics	Not applicable	8
Canada	S	FS	sampling in 225 cm^2 quadrats, 3 to 4 cm deep, using a trowel; digestion with H_2O_2, saline flotation with NaCl (1.2 g cm^{-3}), filtration (0.8 µm)	2,000 to 8,000 items kg^{-1} DW	9
United States	W	FS	sampling with a surface trawl (330 µm) at a depth of 15 cm; filtration (50 to 300 µm)	< 1.0 x 10^{-6} g m^{-2} to 245.7 ± 271.7 x 10^{-6} g m^{-2}; 0 to 0.3 ± 0.2 items m^{-2}	10

Brazil	W	FS	sampling with a conical plankton net (300 µm)	0 - 0.1 items m^{-3}; total of 0.3 items m^{-3}	11
United Kingdom	W	FS	sampling with a plankton net (300 µm), filtration (1.6 µm)	3 to 937 items; total of 2759 items	12
China	W	FS	sampling with Teflon pump: 20 L subsurface water at a 30 cm depth; filtration (1.2 µm)	100 to 4,100 items m^{-3}	13
Brazil	W	FS	sampling with cylindro-conical plankton net (150 µm); filtration (0.02 µm)	16.4 items m^{-3}	14
China	W	FS	sampling with Teflon pump: depth of 1 m, filtration (32 µm steel sieve)	500 to 10,200 items m^{-3}, average of 4137.3 ± 2461.5 items m^{-3}	15
United Kingdom	W	LE	Adsorption experiment	Not applicable	16
United Kingdom	W	LE	Adsorption experiment	Not applicable	17
China	W	FS	30 L of bulk surface water at the top 20 cm, filtration (20 µm)	not informed, but correlation with biota (R^2=0.778, p =0.000)	18
United States	W	FS	sampling with oblique plankton tows by using the National Academy of Sciences (NAS) reference net (333 µm)	0 - 14.1 items m^{-3}; Average of 1 items m^{-3}	19
United States	W	FS	W: sampling with 1 L plastic bottles in 0.5-m depth water, filtration (0.45 µm)	average of 15,600 ± 8,400 to 33,900 ± 11,600 items m^{-3}	20

France	W, S	FS	W: sampling of the top 20 cm water with a standard manta trawl with a 335 mm mesh net, tweezers, filtration (1.6 mm); S: top 5 cm with a Van Veen grab, saline flotation with NaCl (1.2 g cm^{-3}) and sodium tungstate (1.56 g cm^{-3}); filtration (1.6 mm)	S: 0 to 8.7 items kg^{-1} DW; 0 to 6,700 items m^{-3}; average of 1 ± 2.1 items kg^{-1} DW; W: 0 to 1.4 items m^{-3}; 0 to 0.2 items m^{-2}; average of 0.2 ± 0.3 items m^{-3}	21
Germany	W, S	FS	S: sampling of top 1–2 cm sediment, saline flotation with $CaCl_2$ (1.30–1.35 g cm^{-3}), filtration (55 µm); W: top layer at a depth of 2–4 cm was allowed to flow freely into 5–10 L canisters; digestion with H_2O_2, filtration (55 µm)	S: 14 to 532 items kg^{-1} DW; W: 0 to 5,000 items m^{-3}	22
India	W, S	FS	S: sampling using tweezers; W: cylindro-conical WP2 net (100 µm)	3000 pellets in six beaches	23
United States	W	FE	Incubation of PLA and PE MIPs	Not applicable	24
China	S	FS	top 5 cm in 50 cm × 50 cm quadrants, flotation (distilled H_2O), visual inspection	Not informed	25
China	S	FS	top 4 cm of the seabed surface, sieving (5 and 0.3 mm), flotation ($ZnCl_2$ 1.6 mg cm^{-3}), digestion (H_2O_2)	95 to 298 items kg^{-1}, average of 194.5 ± 49.9 items kg^{-1}	26
Italy	W	FS	net sampling (333 µm), visual inspection (> 300 µm)	Po: 0.641± 0.231 items m^{-3}; Tevere: 0.568± 0.156 items m^{-3}	27

Iran	S	FS	grab sampler, sieving (63 µm), digestion with H_2O_2, filtration (2 µm), flotation (NaI 1.6 mg cm^{-3}), filtration (< 2 mm)	47.25 ± 43.7 items per sampling location	28
South Africa	S	FS	sampling of top 5 cm; sieving (1 mm), flotation (NaCl 1.2 g cm^{-3}), sieving (40 µm)	0 to 567,000 items m^{-3}	29
Australia	W	FS	water: sampling with plankton net (355 µm mesh), filtration (37 µm), visual inspection	547 items, median 0.12 to 0.25 items m^{-3}, range 0.04 – 0.47 items m^{-3}	30
China	W, S		W: sampling with water pump, sieving (48 µm), digestion with H_2O_2, filtration (1 µm); S: top 5 cm of 1m x 1 m quadrats, digestion with H_2O_2, flotation (NaI 1.6 g cm^{-3}), sieving (48 µm), filtration (1.0 µm)	W: 788.0 ± 464.2 to 1333.3 ± 782.1 items m^{-3}; S: 85.0 ± 35.1 items to 234.7 ± 89.6 items kg^{-1}	31
United Kingdom	S	FS	sampling with sediment cores, filtration (≤ 1.6 µm), visual inspection	0	32
Indonesia	W, S	FS	W and S: net sampling (manta trawl) and Eckman grab sampling, filtration (125 µm), digestion by Fenton oxidation, flotation (ZnCl 1.5 g cm^{-3}); biota: digestion by Fenton oxidation, filtration (1.2 µm)	W: 0.000666 ± 0.000577 items m^{-3}; S: 116.65 ± 5.77 items kg^{-1}	33

United Kingdom	W	FS	sampling of the sea surface microlayer using the glass plate method	average of 7.5 items m^{-2}	34
United States	S	FS	10-cm diameter x 5-cm depth cores, visual inspection	average of 9,544 ± 1,413 items m^{-2}	35
Germany	W	FE	incubation of PS and PE MIPs	Not applicable	36
United States	W	FS, FE	net sampling (80µm); incubation of PE, PP, PC and PS MIPs	0.07 items m^{-3}	37
United States	S	FE	incubation of PE, PU, PVC and PLA MIPs	Not applicable	38
China	S	FS	sampling of the top 4 cm of S using a metal shovel from a 0.25-m^2 quadrat; saline flotation with seawater; filtration (315 µm)	6675 ± 7021 items m^{-2}; 4.6 ± 6.2 g m^{-2}	39
United Kingdom	S	FS, LE	sampling of underlying 3 cm of sediment (500 mL), saline flotation with NaCl; Filtration (1 µm)	0 to 180,000 items m^{-3}	40
China	S	FS	sampling of 4-cm deep surface sediments from a 50 x 50 cm quadrat in a 30-m-long transect using a metal shovel; saline flotation with seawater; filtration (315 µm)	87%of all plastics: overall 3,242 ± 1,991 items m^{-2}; 3.2 ± 1.8 g m^{-2}; in dry season 889 ± 350 items m^{-2}; 0.8 ± 0.2 g m^{-2}, in wet season 5,595 ± 3,950 items m^{-2}, 5.6 ± 3.5 g m^{-2}	41

South Korea	S	FS	sampling in 0.5 X 0.5 m quadrats, sieving (5000 and 1000 µm Tyler sieves)	Microplastics: 343.2 to 1,228.8 items m^{-2}; Mesoplastics: 16 to 150.4 items m^{-2}	42
United States	S	FE	exposure of plastic strips (high-density polyethylene, polypropylene, extruded polystyrene), 8 cm above the surface of a salt marsh, for 4-wk, 8-wk, 16-wk, and 32-wk.	not applicable	43
Japan	W	FS	net sampling (335 µm), filtering (100 µm), digestion with H_2O_2, filtering (100 µm), visual inspection	0.03 to 63.89 items m^{-3}; average of 4.34 items m^{-3}; 80 to 1,6150,000 kg m^{-3}, average of 790,000 kg m^{-3}	44
Colombia	S	FS	surface sediment sampling, flotation ($(NaPO_3)_6$ 2.5 g L^{-1}), sieving (1–5 mm), filtration (8 µm)	31 to 2863 items kg^{-1} DW	45
Portugal	S	FS	sampling of sediment in top 2–3 cm from 50×50 cm quadrats	plastics (99%of items): average 666 ± 2,642 items m^{-2}; items (68% of plastics): average 454 ± 1,908 items m^{-2}	46
United Kingdom	W	FS	sampling with a manta net (300 µm mesh); filtration (3000, 1000 and 270 µm)	average < 0.1 items m^{-3}	47

Canada	S	FS	sampling of top 5 cm in 0.5 m x 0.5 m quadrats, flotation (NaCl and sea salt 1.35 g cm^{-3}), sieving (5 mm), filtration (1.6 µm)	100 to 25000 kg^{-1} DW	48
Brazil	W	FS	net sampling (300 µm), filtration (500 µm), visual inspection	0.0022 ± 0.0025 up to 0.0245 ± 0.0294 items m^{-3}	49

***References: 1** Thompson et al. (2004); **2** Mohamed Nor and Obbard (2014); **3** Abidli et al. (2018); **4** Peng et al. (2018); **5** Claessens et al. (2011); **6** Vianello et al. (2013); **7** Fok and Cheung (2015); **8** Harrison et al. (2014); **9** Mathalon and Hill (2014); **10** Yonkos et al. (2014); **11** Lima et al. (2014); **12** Gallagher et al. (2016); **13** Zhao et al. (2015); **14** Castro et al. (2016); **15** Zhao et al. (2014); **16** Bakir et al. (2014); **17** Holmes et al. (2014); **18** Li et al. (2018); **19** Carpenter and Smith (1972); **20** Waite et al. (2018); **21** Frère et al. (2017); **22** Stolte et al. (2015); **23** Veerasingam et al. (2016); **24** Schönlau et al. (2019); **25** Shi et al. (2020); **26** Cheang et al. (2018); **27** De Lucia et al. 2018); **28** Abbasi et al. (2019); **29** De Villiers (2019); **30** Jensen et al. (2019); **31** Wu et al. (2019); **32** Knight et al. (2020); **33** Sembiring et al. (2020); **34** Stead et al. (2020); **35** Talley et al. (2020); **36** Kallscheuer et al. (2019); **37** Laverty et al. (2020); **38** Seeley et al. (2020); **39** Fok et al. (2017); **40** Browne et al. (2010); **41** Cheung et al. (2016); **42** Lee et al. (2013); **43** Weinstein et al. (2016); **44** Nihei et al. (2020); **45** Garcés-Ordóñez et al. (2019); **46** Antunes et al. (2018); **47** Sadri and Thompson (2014); **48** Kazmiruk et al. (2019); **49** Lorenzi et al. (2020).

Table S4. Compilation of data from the papers analysed in this review, focusing on methodologies and concentrations of macroplastic (> 5 mm) found in the field. The paper from Costa et al. (2011) (reference no. 16 in this table) found plastics > 1mm up to 160 cm^2; and the paper from Turner et al. (2015) (reference no. 17 in this table) analysed paints directly from boats or from particles with no specified size, so information from both papers was included in this table. S: sediment; W: water; FS: field sampling; FE: field experiment.

Country	Matrix analysed	Type of work	Methodology used	Macroplastic concentration in the field	Ref.*
Brazil	S	FS	manual sampling of debris in a 50 m wide transect	11.4 to 19.7 items m^{-2}	1
Brazil	S	FS	manual sampling in 20 m wide transects	Average of 0.11 ± 0.01 items m^{-2}	2
United Kingdom	S	FS	manual sampling in transects (10 - 200 m)	77 to 124 items per beach	3
United Kingdom	W	FS	sampling with four types of fyke net, a standard eel net and three modified nets	8490 items	4
United Kingdom	W	FE	exposure of polyethylene plastic food bags 2 m below the water's surface for 3 weeks	Not applicable	5
Brazil	W, S	FE	release and track of debris in mangrove environment; counted at 24, 48, 72, 96, 120 and 144 h	Not applicable	6
Portugal	W	FE	incubation of PET and PE in the environment	Not applicable	7

China	S	FS	sampling of the top 4 cm of sediment using a metal shovel from a 0.25-m^2 quadrat; saline flotation with seawater; filtration (315 μm)	163 ± 154 items m^{-2}; 1.9 ± 2.3 g m^{-2}	8
United Kingdom	S	FS, LE	manual sampling on the strandline	0 to 100,000 items m^{-3}	9
China	S	FS	sampling of 4-cm deep surface sediments from a 50 x 50 cm quadrat in a 30-m-long transect using a metal shovel; saline flotation with seawater; filtration (315 μm)	13% of total plastics: 0 to 20,000 items m^{-2}; 0 to 75 g m^{-2}	10
South Korea	S	FS	manual sampling in 10 X 10 m quadrats	0.4 to 2.1 items m^{-2}	11
United States	S	FE	exposure of plastic strips (high-density polyethylene, polypropylene, extruded polystyrene), 8 cm above the surface of a salt marsh, for 4-wk, 8-wk, 16-wk, and 32-wk.	not applicable	12
Colombia	S	FS	sampling in 50 x 2 m transects	0.0015 to 0.0728 items m^{-2}, with 73 to 96% plastic	13
Spain	S	FS	all litter at the beach was collected	1.26 to 4.57 items m^{-2}	14

United States	W	FE	exposure of plastic (PVC) pipes and styrofoam floats to marine environment for 11 weeks, then for 7 months at 3-4.5 m depth with a monthly basis sampling	not applicable	15
Brazil	S	FS	sampling with a cylindrical corer (20 cm in diameter X 20 cm height); sieving (1 mm)	4.8 to 15.9 items m^{-3}; total of 59 items m^{-3}	16
Various countries in the European Union	paint particles	FS	sampling of paint particles from boatyards, shipyards, slipways and from boats abandoned on foreshores or archived samples	not applicable	17

***References: 1** Araújo and Costa (2007); **2** Ivar do Sul and Costa (2013); **3** Turner (2016); **4** Morritt et al. (2014); **5** Lobelle and Cunliffe (2011); **6** Ivar do Sul et al. (2014); **7** Tuccori et al. (2019); **8** Fok et al. (2017); **9** Browne et al. (2010); **10** Cheung et al. (2016); **11** Lee et al. (2013); **12** Weinstein et al. (2016); **13** Garcés-Ordóñez et al. (2019); **14** Ibabe et al. (2020); **15** Ye and Andrady (1991); **16** Costa et al. (2011); **17** Turner et al. (2015).

Table S5. Data concerning microplastic (MIP) contamination in estuarine-associated organisms reported in the papers analysed in this review. FS: field sampling; FE: field experiment; LE: laboratory experiment.

Type of work	Organism	Feeding behaviour	Methodology used	Abundance of microplastics	Ref.*
FS	fish: 3 species	predator	sampling with otter-trawl net; stomach content	0 to 18 MIP individual^{-1}	1
FS	fish: 4 species	carnivore, predator	sampling not specified; stomach content	0 to 25% of organisms had MIP	2
FS	fish: 1 species	carnivore, predator	sampling not specified; stomach content	up to 30 MIP individual^{-1}	3
FS	fish: 2 species	carnivore, predator	sampling with fyke nets; stomach content	0.2 ± 0.42 to 0.85 ± 1.17 MIP individual^{-1}	4
FS	fish: 12 species	detritivore, planktivore, omnivore and ichthyophagous	sampling with fyke nets; digestion with H_2O_2; stomach content	1 - 89 MIP individual^{-1}; 1 - 8 'others' individual^{-1}; average of 18.5 ± 18.9 MIP and 0.7 ± 1.7 for 'others' individual^{-1}	5
FS	mussel: 1 species; polychaetes: 2 species	filter feeder, deposit feeder	polychaete worm fecal cast samples, live mussels from beach, mussels from grocery store; digestion with H_2O_2, saline flotation with NaCl (1.2 g cm^{-3}), filtration (0.8 µm)	Average of 34 MIP per wild mussel and 75 per farmed mussel; 4 to 6 MP g^{-1} of polychaetes casts	6
FS	fish: 2 species	carnivore, predator	sampling from fishers; stomach content	(60%) 12 out of 40 organisms had MIP	7

FS	fish: 2 species	predators	sampling with otter-trawl net; stomach content	7.9% of individuals (6.9% and 9.2%)	8
FS	oyster: 1 species	filter feeder	sampling at site, analysis of soft tissue, saline flotation with NaCl (1.2 g cm^{-3});	1.4 to 7.0 MIP $individual^{-1}$; 1.5 to 7.2 MIP g^{-1} WW	9
FS	fish: 14 species; chaetognath: 1 species	carnivore, predator	sampling with oblique plankton tows by using the National Academy of Sciences (NAS) reference net (333 µm)	57% (n=14) of fish species examined and a chaetognath. 2.1 - 33% of fish had plastic. *S. elegans* had 1 MIP in its intestine	10
FS	crab: 1 species; oyster: 1 species	carnivore, filter feeder	sampling from reef, digestion with H_2O_2, filtration (0.45 µm)	Average of 4.2 MIP per individual in crabs, 16.5 MIP $individual^{-1}$ in oyster	11
FS	seal: 1 species	carnivore, predator	scat; stomach and intestine contents	11% (n=107) of stomachs had plastic: average of 0.26 items and 0.0244 g $individual^{-1}$ (maximum 8 items and 1.4228 g per stomach), 1% (n=100) of intestines had plastic: 7 items and 0.0436 g $individual^{-1}$, no scats had plastics (n=125)	12

FS	fish: 3 species	predators, epibenthophagous	sampling with otter-trawl net; stomach content	18% to 33% of individuals had plastic	13
FS, LE	lobster: 1 species	omnivorous	sampling with beam trawl; feeding trial	83% of organisms (n=120)	14
LE	amphipod, barnacle, lugworm	detritivore, filter feeder, deposit feeder	feeding trial	not informed	15
FS	fish: 1 spp.	carnivore, predator	stomach dissection, visual inspection	up to 0.46 individuals; up to 0.002 g	16
LE	Bivalve: 1 spp.	deposit and filter feeder	feeding trial	Not applicable	17
LE	fish: 1 spp.	carnivore	feeding trial	Not applicable	18
LE	Bivalve: 1 spp.	filter feeder	feeding trial	Not applicable	19
LE	fish: 1 spp.	carnivore, predator	feeding trial	Not applicable	20
LE	fish: 2 spp. (1 estuarine and 1 freshwater)	carnivore, detritivore, herbivore	feeding trial	Not applicable	21
FS	fish: 1 spp.	carnivore, predator	gastrointestinal tract dissection, digestion with KOH, filtration (1.6 µm)	1.67 ± 1.43 to 2.04 ± 1.93 MIP individual^{-1}l	22
FS	fish: 1 spp.	carnivore, predator	gastrointestinal tract digested with H_2O_2, filtration (8 µm)	3 to 28 MIP individual^{-1}, average of 12.1 ± 6.2 MIP individual^{-1}	23
FS	fish: 4 spp.	detritivore, carnivore	gastrointestinal tract digested with NaOH, filtration (100 µm)	0-2 MIP individual^{-1}; 5 to 21.7% of occurrence in each species	24

FS	fish: 3 spp.	carnivore, predator	gastrointestinal tract dissection, visual inspection	1.51 ± 0.13, 1.43 ± 0.11 and 1.21 ± 0.18 MIP individual^{-1}	25
FS, LE	fish: 1 spp. (feeding trial) + 4 spp. (fish meals)	omnivorous	fish meals: digestion with KOH, filtration (149 µm), flotation (NaI 1.5 g mL^{-1}), filtration (8 µm); feeding trial	Not informed	26
FS	fish: 1 spp.	carnivore	water: sampling with plankton net (355 µm mesh), filtration (37 µm), visual inspection; fish: gastrointestinal tract dissection, visual inspection	445 MIP (n=60); medians of 4.0 (inshore) and 4.5 (offshore) individual^{-1}; 0 to 131 individual^{-1}	27
FS	bivalves: 2 spp.	filter and deposit feeders	digestion with KOH, dying with Rit Dye More, filtration (11µm), digestion with H_2O_2	0 to 2.92 ± 1.00 MIP individual^{-1}; 0 to 46% of individuals with MIP	28
FS	polychaete: 1 spp.	deposit feeder	sampling with sediment cores, filtration (≤ 1.6 µm), visual inspection; biota: casts visual inspection	260,000 ± 66,000 particles m^{-3}	29
FS	polychaete: 1 spp.	filter feeder	hand sampling	average of 20,800 ± 3,700 particles kg^{-1} in polychaetes tubes	30
LE	polychaete: 1 spp.	deposit and filter feeder	feeding trial	Not applicable	31

FS	fish: 1 spp.	carnivore	digestion by Fenton oxidation, filtration (1.2 µm)	guts and gills: 1.166 ± 0.983 MIP $individual^{-1}$; tissues: 1.111 ± 0.838 MIP $individual^{-1}$	32
FS	fish: 3 spp.	carnivore, herbivore, predator	sediment: 10-cm diameter x 5-cm depth cores, visual inspection; biota: gastrointestinal content dissection, visual inspection	2.86 ± 1.37 MIP $individual^{-1}$	33
FS, LE	crab: 1 spp.	omnivorous, predator	feeding trial	FS: 5% of individuals had MIP; LE: up to 45% of individuals had MIP	34

***References:** **1** Ramos et al. (2012); **2** Kartar et al. (1976); **3** Kartar et al. (1973); **4** McGoran et al. (2017); **5** Pazos et al. (2017); **6** Mathalon and Hill (2014); **7** Kumar et al. (2018); **8** Dantas et al. (2012); **9** Li et al. (2018); **10** Carpenter and Smith (1972); **11** Waite et al. (2018); **12** Bravo Rebolledo et al. (2013); **13** Possatto et al. (2011); **14** Murray and Cowie (2011); **15** Thompson et al. (2004); **16** Dantas et al. (2019); **17** O'Donovan et al. (2018); **18** Miranda et al. (2019); **19** Li et al. (2020b); **20** Barboza et al. (2018); **21** LaPlaca and Hurk (2020); **22** Kazour et al. (2020); **23** Arias et al. (2019); **24** Calderon et al. (2019); **25** Ferreira et al. (2019); **26** Hanachi et al. (2019); **27** Jensen et al. (2019); **28** Bendell et al. (2020); **29** Knight et al. 2020); **30** Piazzolla et al. (2020); **31** Revel et al. (2020); **32** Sembiring et al. (2020); **33** Talley et al. (2020); **34** Torn (2020).

ANEXO II

LITTER CONTAMINATION AT A SALT MARSH: AN ECOLOGICAL NICHE FOR BIOFOULING IN SOUTH BRAZIL

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Supplementary Material

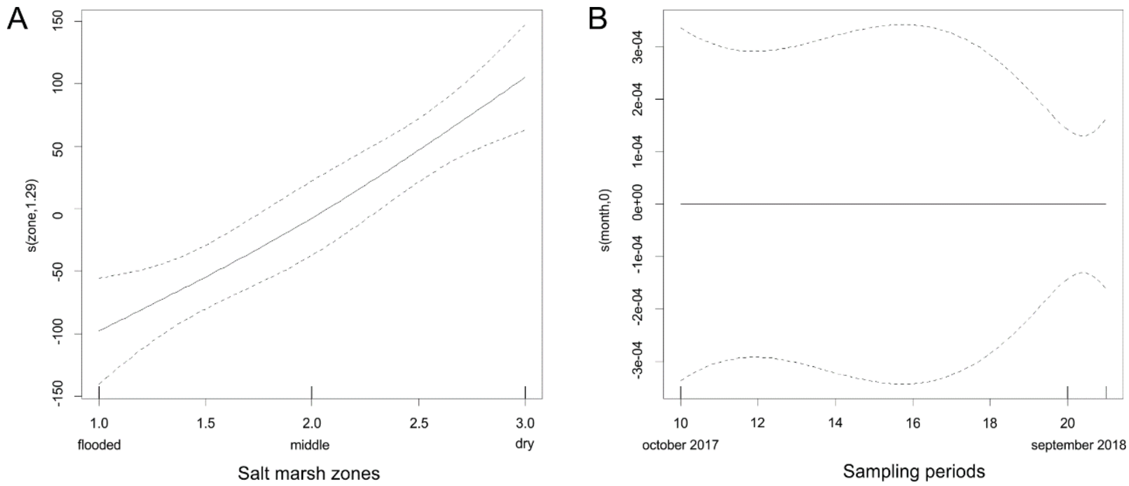

Figure S1. Generalized Additive Model for the quantities of litter items describing the response of the number of items (n=2,247) per transect (21) according to salt marsh zones (*zones*) and sampling periods (*months*). The y-axis is a relative scale, with a positive y-value on the plots, indicating a positive effect of that explanatory variable (zones or sampling months) on the dependent variable (no. of litter items), and a negative y-value indicates a negative effect of that variable. As the range of the smoothed function indicates the relative importance of each predictor, all y-axes have been adjusted to approximately the same range to aid comparison of the predictors.

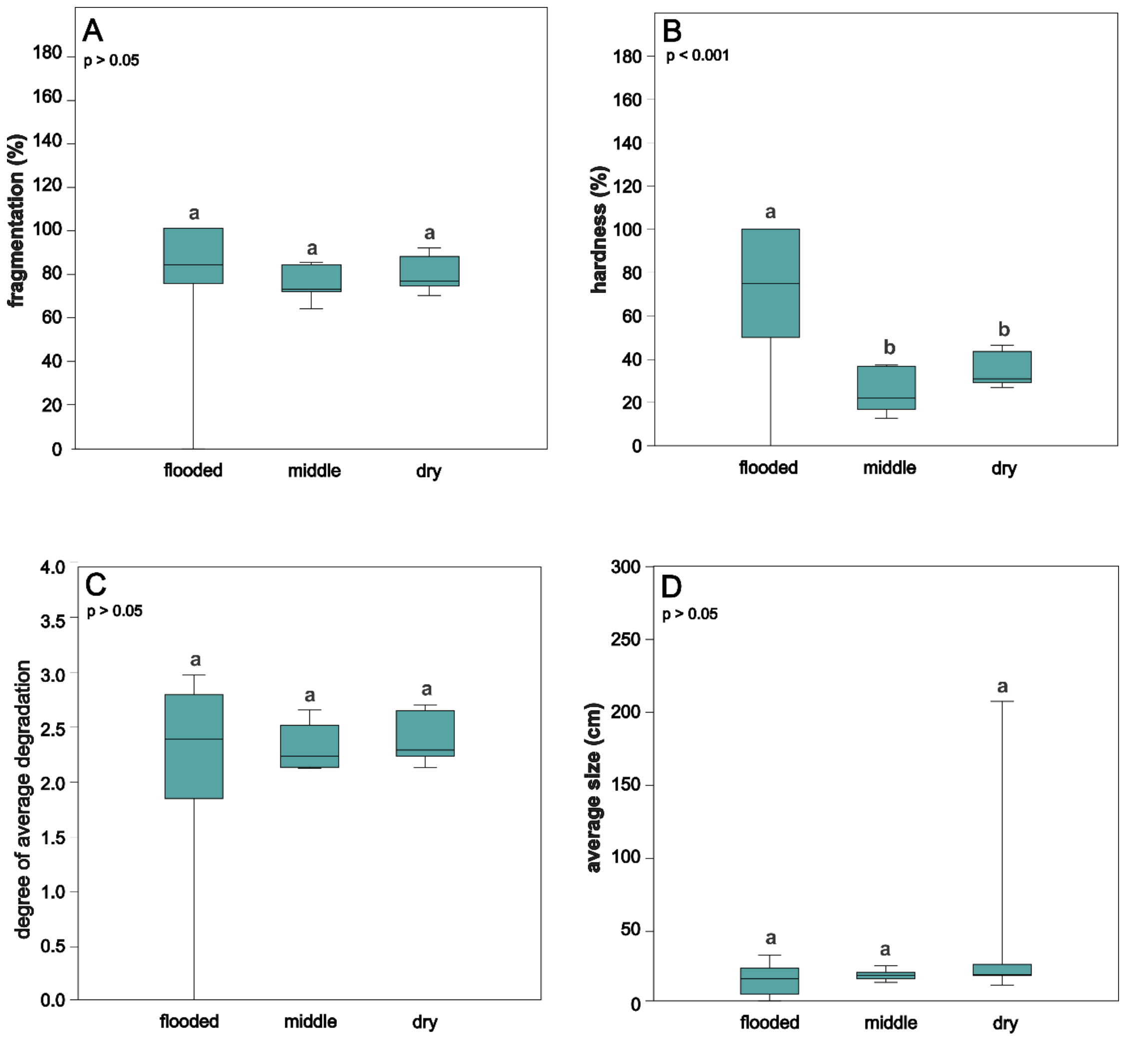

Figure S2. Solid litter physical variables by salt mash zones (n=2,247). Similar lowercase letters indicate similarities (ANOVA, $p > 0.05$), and different lowercase letters indicate statistical differences (ANOVA, $p < 0.05$) among the salt marsh zones.

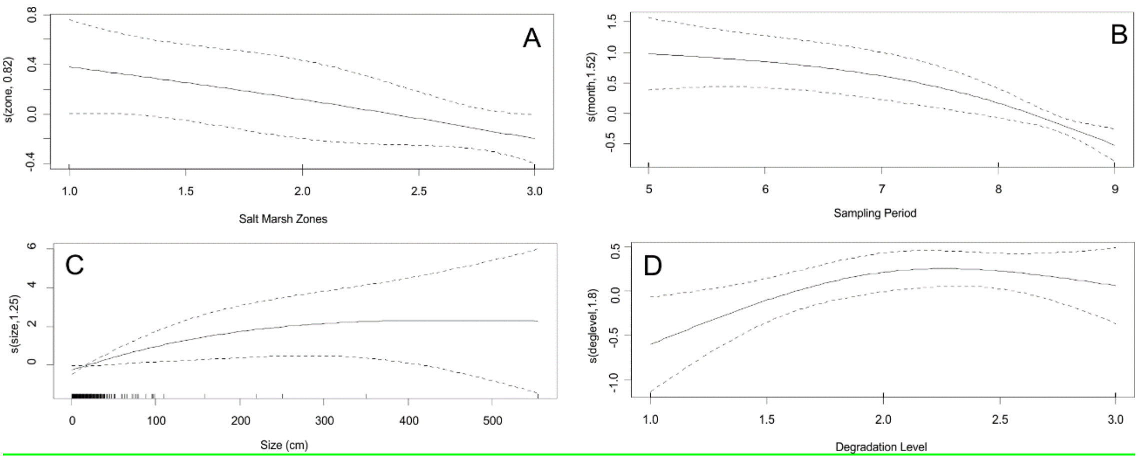

Figure S3. Generalized Additive Model (biofouling ~ s(*zone*) + s(*month*) + s(*size*) + s(*deglevel*)) describing the response of biofouling occurrence in litter items (n=419) according to salt marsh zones (A), sampling periods (B), items' size (C), and degradation level (D). The y-axis is a relative scale, with a positive y-value on the plots, indicating a positive effect of that explanatory variable (zones, sampling months, size, and degradation level) on the dependent variable (occurrence of biofouling), and a negative y-value indicates a negative effect of that variable. As the range of the smoothed function indicates the relative importance of each predictor, all y-axes have been adjusted to approximately the same range to aid comparison of the predictors.

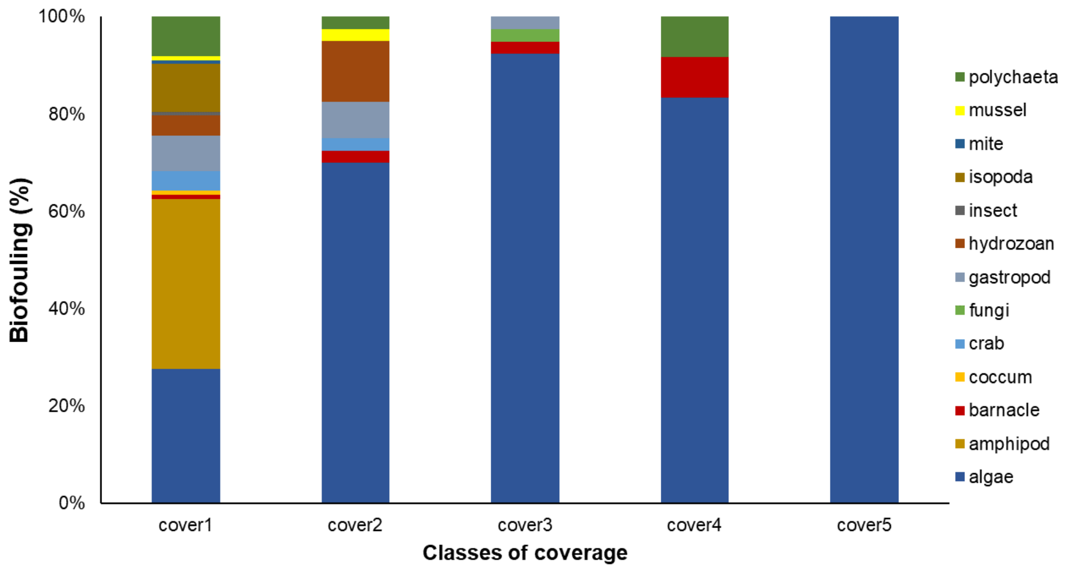

Figure S4. Percentage of coverage of the surface by biofouling groups in items collected at the Molhe Oeste salt marsh at the Patos Lagoon estuary, in the Rio Grande do Sul state, southern Brazil. Cover1: <10% surface coverage, Cover2: 11-25% surface coverage, Cover3: 26-50% surface coverage, Cover4: 51-75% surface coverage, Cover5: 76-100% surface coverage.

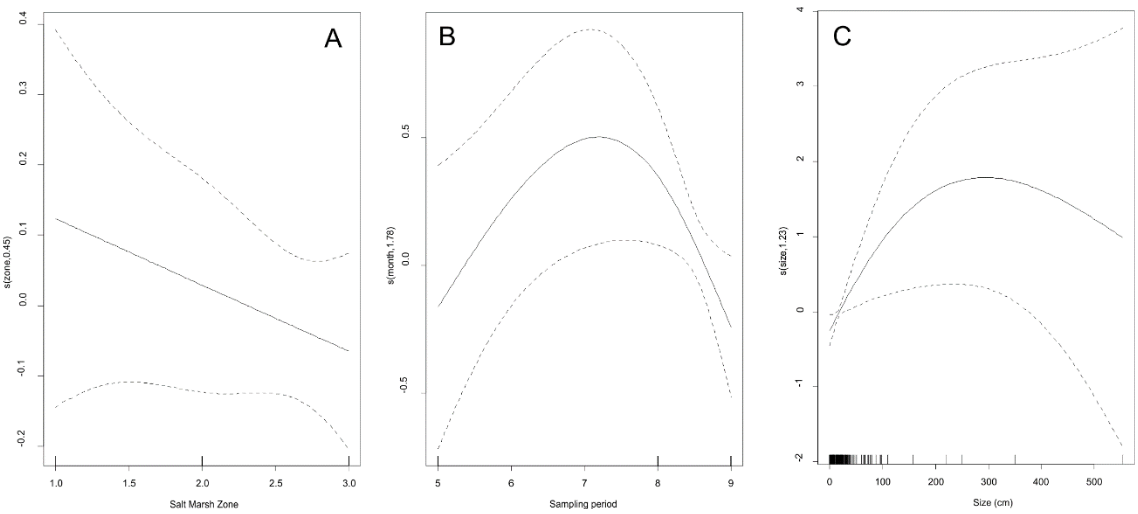

Figure S5. Generalized Additive Model (algae ~ s(zone) + s(month) + s(deglevel)) describing the response of occurrence of biofouling by algae in litter items according to salt marsh zones (A), sampling periods (B), and items' size (C). The y-axis is a relative scale, with a positive y-value on the plots, indicating a positive effect of that explanatory variable (zones, sampling months, and size) on the dependent variable (occurrence of algae), and a negative y-value indicates a negative effect of that variable. As the range of the smoothed function indicates the relative importance of each predictor, all y-axes have been adjusted to approximately the same range to aid comparison of the predictors.

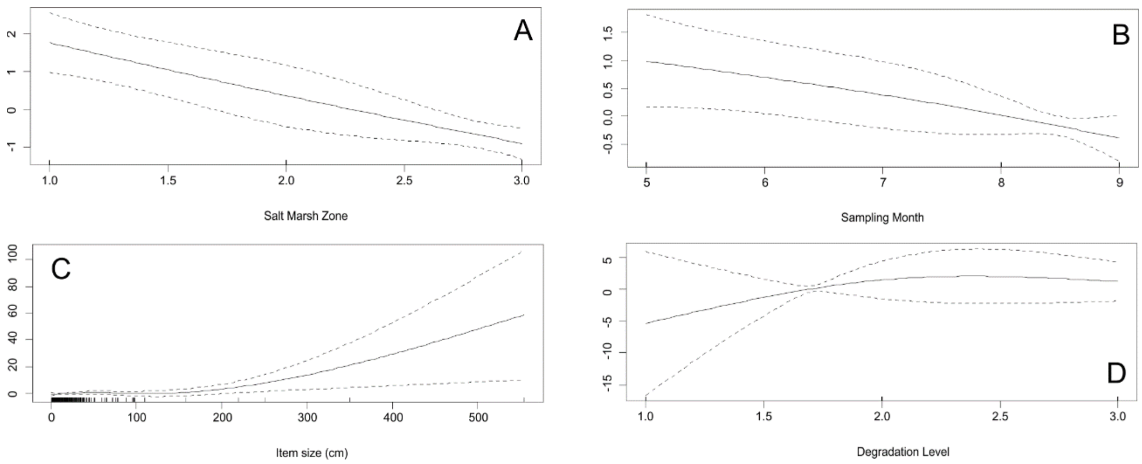

Figure S6. Generalized Additive Model (amphipoda ~ s(*zone*) + s(*month*) + s(*size*) + s(*deglevel*) + not_fragmented) describing the response of occurrence of biofouling by amphipods in litter items according to salt marsh zones (A), sampling periods (B), items' size (C), and degradation level (D). The y-axis is a relative scale, with a positive y-value on the plots, indicating a positive effect of that explanatory variable (zones, sampling months, size, and degradation level) on the dependent variable (occurrence of amphipods), and a negative y-value indicates a negative effect of that variable. As the range of the smoothed function indicates the relative importance of each predictor, all y-axes have been adjusted to approximately the same range to aid comparison of the predictors.

Table S1. Generalized Additive Model (GAM) describing the response of the number of items per transect (n=21) according to salt marsh zones (*zones*) and sampling periods (*months*). E.d.f.: degrees of freedom estimated for the model; F: statistical test to evaluate the significance of smoothed terms. Stars (*) indicate significance.

Model quantity ~ s(*zone*) + s(*month*)

	coefficient (std. error)	**t (F)-value**	**P-value**	**R-square (adj)**	**E.d.f.**
Intercept	107 (17)	6.291	5.23e-06*	0.581	-
s(*zone*)	-	15.248	0.000238*	-	1.290e+00
s(*month*)	-	0.118	0.999998	-	3.333e-11

Deviance explained: **60.8%**

Table S2. Generalized Additive Model (GAM) describing the response of biofouling occurrence in litter items (n=419) according to salt marsh zones (*zones*), sampling periods, items' size (*size*), and degradation level (*deglevel*). E.d.f.: degrees of freedom estimated for the model. Stars (*) indicate significance.

Model biofouling ~ s(*zone*) + s(*month*) + s(*size*) + s(*deglevel*)

	coefficient (std. error)	**z value**	**P-value**	**R-square (adj)**	**E.d.f.**
Intercept	-0.4948 (0.1130)	-4.381	1.18e-05*	0.174	
		Chi.sq			
s(*zone*)		6.171	0.042241*		1.701
s(*month*)		13.890	0.000939*		1.539
s(*size*)		9.185	0.025029*		2.314
s(*deglevel*)		6.002	0.047316*		1.739

Deviance explained: **15.1%**

Table S3. Generalized Additive Models (GAM) describing the response of occurrence of biofouling by algae (*algae*) and amphipods (*amphipods*) in litter items (n=419) according to salt marsh zones (*zones*), sampling periods (*months*), items' size (*size*), items' colours (*colour*), and degradation level (*deglevel*). E.d.f.: degrees of freedom estimated for the model; F: statistical test to evaluate the significance of smoothed terms. Stars (*) indicate significance.

Model algae ~ s(*zone*) + s(*month*) + s(*deglevel*)

	coefficient (std. error)	**z value**	**P-value**	**R-square (adj)**	**E.d.f.**
Intercept	-0.9411 (0.1107)	-8.502	< 2e-16*	0.0267	
		Chi.sq			
s(*zone*)		2.126	0.1504		0.7918
s(*month*)		8.009	0.0179*		1.8373
s(*deglevel*)		1.140	0.4954		1.4381
					Deviance explained: **3.15%**

Model	amphipoda ~ s(*zone*) + s(*month*) + s(*size*) + s(*deglevel*) + not_fragmented				
	coefficient (std. error)	**z value**	**P-value**	**R-square (adj)**	**E.d.f.**
Intercept	-2.9004 (0.3269)	-8.874	<2e-16*	0.34	
not_fragmented	-0.8546 (0.5383)	-1.587	0.122		

	Chi.sq		
s(*zone*)	18.706	4.41e-05*	1.0206
s(*month*)	4.134	0.0563	0.8623
s(*size*)	11.504	0.0165*	3.0146
s(*deglevel*)	8.847	0.0119*	1.8631
			Deviance explained: **40.3%**

Model	algae ~ s(*zone*) + s(*month*) + s(*size*) + *colour*				
	coefficient (std. error)	**z value**	**P-value**	**R-square (adj)**	**E.d.f.**
Intercept	-2.766e+00 (1.056e+00)	-2.620	0.00878*	0.0963	
colourblue	8.062e-01 (1.148e+00)	0.702	0.48259		
colourbrown	1.484e+00 (1.137e+00)	1.305	0.19195		
colourtransparent	2.163e+00 (1.074e+00)	2.015	0.04390*		
colourcolourful	1.500e+00 (1.125e+00)	1.333	0.18247		
colourgreen	1.297e+00 (1.196e+00)	1.085	0.27801		
colourgrey	1.791e+00 (1.452e+00	1.233	0.21741		
colourmetal	2.884e+00	1.960	0.05002		

lic	(1.472e+00)			
colourNI	2.572e+00 (1.777e+00)	1.447	0.14790	
colourorange	-1.339e+02 (1.292e+07)	0.000	0.99999	
colourpink	2.615e+00 (1.405e+00)	1.861	0.06280	
colourpurple	1.400e+02 (6.711e+07)	0.000	1.00000	
colourred	9.906e-01 (1.504e+00)	0.659	0.51016	
colourwhite	2.504e+00 (1.081e+00)	2.315	0.02061*	
colouryellow	1.172e+00 (1.203e+00)	0.974	0.32993	
		Chi.sq		
s(*zone*)		0.857	0.3545	0.4528
s(*month*)		6.889	0.0309*	1.7834
s(*size*)		6.885	0.0244*	1.2289

Deviance explained: **12.7%**

ANEXO III

Supplementary Files to:

SALT MARSHES AS THE FINAL WATERSHED FATE FOR MESO- AND MICROPLASTIC CONTAMINATION: A CASE STUDY FROM SOUTHERN BRAZIL

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Table S1. Percentage of potential plastics analysed in the FT-IR spectrometer in each salt marsh zone from the Molhe Oeste salt marsh, Brazil. HM: High Marsh; MM: Middle Marsh; LM: Low Marsh; MF: Mud Flat.

	% of blank samples	**% of environmental sample**
HM	28.12	22.68
MM	41.66	69.92
LM	40.91	44.18
MF	47.06	52.00
water	00.00	62.50

Table S2. Quantities of measured MEPs and MIPs in environmental samples in each salt marsh zone from the Molhe Oeste salt marsh, Brazil. HM: High Marsh; MM: Middle Marsh; LM: Low Marsh; MF: Mud Flat, MEP: mesoplastic, MIP: microplastic.

	nº of MEPs	**nº of MIPs**	**total**	**%MEP**	**%MIP**
HM	26	215	241	10.78838174	89.21161826
MM	12	85	97	12.37113402	87.62886598
LM	0	11	11	0	100
MF	2	23	25	8	92
Water	1	15	16	6.25	93.75

Table S3. Comparison of microplastic abundances in sediment surface reported in publications on salt marsh/tidal flat/mud flat environments. MIP: microplastic.

Location	MIP average abundance	Maximum MIP abundance	Minimum MIP abundance	Unit reported	Reference
Yangtze estuary, China	143 ± 30	not informed	not informed	items kg^{-1}	Wu et al. (2020)
Andong salt marsh, China	150 ± 30	270 ± 50	40 ± 20	items kg^{-1}	Fraser et al. (2020)
Hong Kong	268	2,116	0.99	items kg^{-1}	Lo et al. 2018)
Ria Formosa lagoon, Portugal	22.7 ± 17.6	12	0	items kg^{-1}	Cozzolino et al. (2020)
Rainbow Haven Beach back lagoon, Canada	3,567	6,000	2,200	items kg^{-1}	Mathalon & Hill (2014)
Spiekeroog Island, Germany	3,800	5,800	1,500	items kg^{-1}	Liebezeit & Dubaish (2012)
Molhe Oeste salt marsh, Brazil	**132.54 ± 252.26**	**1,123.16**	**0**	**items kg^{-1}**	**This work**

Table S4. Examples of microplastic abundances found in sediment cores reported in publications in different environments. NI: not informed, MIP: microplastic.

Location	**Environment/samples type**	**Maximum sampling depth (cm)**	**Maximum depth with MIPs (cm)**	**MIP average abundance**	**Maximum MIP abundance**	**Minimum MIP abundance**	**Unit reported**	**Ref.**
Aa River, France	riverine/dredged sediment	140	140	NI	0.78	2,800	items kg^{-1}	Constant et al. (2021)
Qinhuai River, China	riverine	50	50	NI	46.92 ± 13.34	20.42 ± 9.38	items kg^{-1}	Niu et al. (2021)
Wenzhou, Zhejiang Province, China	riverine/dredged sediment	30	30	24,784 ± 6,953; 29,031 ± 5,869	37,780	13,710	items kg^{-1}	Ji et al. (2021)
Red Sea and Arabian Gulf	mangrove	30	30	14 ± 3	NI	NI	items per core	Martin et al. (2020)
Ghana	coastal lagoon	30	30	NI	25.94 ± 3.13	11.22 ± 2.69	items m^{-3}	Chico-Ortiz et al. (2020)

Qiantang River, China	riverine	15	15	NI	400 ± 150	50 ± 1	items kg^{-1}	Fraser et al. (2020)
Yangtze Estuary, China	estuarine	10	10	143 ± 30	153 ± 23	131 ± 28	items kg^{-1}	Wu et al. 2020
Derwent Estuary, Australia	estuarine	104	104	NI	4,200	2,450	items kg^{-1}	Willis et al. 2017
Belgian coast	beach	NI (equivalent to years 1993-2008)	NI (equivalent to years 1993-2008)	NI	156.2 ± 6.3	54.7 ± 8.7	items kg^{-1}	Claessens et al. 2011
Lake Ontario, Canada	lacustrine, nearshore sediment	15	15	2,130	NI	NI	items kg^{-1}	Ballent et al. 2016
Lake Ontario, Canada	lacustrine, offshore sediment	30	8	NI	0.03	0.09	% (w/w)	Corcoran et al. 2015

Molhe Oeste salt marsh, Brazil	**estuarine**	**66**	**66**	**366.92 ± 975.18**	**6,822.32**	**0**	**items kg^{-1}**	**This work**

Table S5. Test parameters for equal variances and equal means for meso- and microplastic found in procedural blank and environmental samples from sediment surface and sediment core collected at the Molhe Oeste salt marsh, Brazil.

	Sediment surface samples		**Sediment core samples**	
F-test for equal variances				
	log(BLANK plastics)	**log(ENVIRONMENTALplastics)**	**log(BLANK plastics)**	**log(ENVIRONMENTALplastics)**
N:	15	20	78	78
Variance:	0.16979	0.25899	0.082984	0.1748
Calculated F value: 1.5253			**Calculated F value:** 2.1064	
Critical F value (p=0.05): 2.8607			**Critical F value (p=0.05):** 1.5684	
p(same var.): 0.42499			**p(same var.):** 0.001275	
t-test for equal means			**Welch F- test for unequal variances**	
N:	15	20	78	78
Mean:	0.57683	1.2916	0.91773	1.0733
95% conf.:	(0.34865 0.80502)	(1.0534 1.5298)	**F =** 7.324	
Difference between means: 0.71479			**df =** 136.7	
t = 4.4501			**p =** 0.00767	
p(same mean): 0.000092304				
Critical t value (p=0.05): 2.0345				

Table S6. Data on quantities of meso- and microplastic quantities and their characteristics (size, colour, and format) found in procedural blank samples. The symbol (-) indicates data that was not assessed. HM: High Marsh; MM: Middle Marsh; LM: Low Marsh; MF: Mud Flat.

	Sediment surface					Sediment core		
Salt marsh Zones	**HM**	**MM**	**LM**	**MF**	**Water**	**HM**	**MM-LM**	**LM-MF**
nº of MEP	0	0	0	0	0	-	-	-
nº of MIP	25	5	1	1	15	-	-	-
Colour								
beige	0	0	0	0	0	1	0	0
black	6	7	8	8	3	46	17	42
blue	20	3	11	3	11	130	151	120
brown	0	0	0	0	0	1	0	1
clear	1	0	1	2	0	0	5	7
colourful	0	0	0	0	0	0	0	2
green	1	0	0	0	0	1	0	5
grey	0	1	0	0	0	0	0	2
orange	1	0	0	0	0	0	0	0
pink	2	0	1	1	0	5	4	3
purple	0	0	0	0	0	2	0	2

red	0	0	0	0	0	4	4	5
white	1	1	0	3	1	5	6	14
Format								
fibre	32	12	20	17	15	194	185	194
fragment	0	0	1	0	0	1	2	0
Avg. size (mm)	1.304	1.354	2.08	1.04	1.37	-	-	-

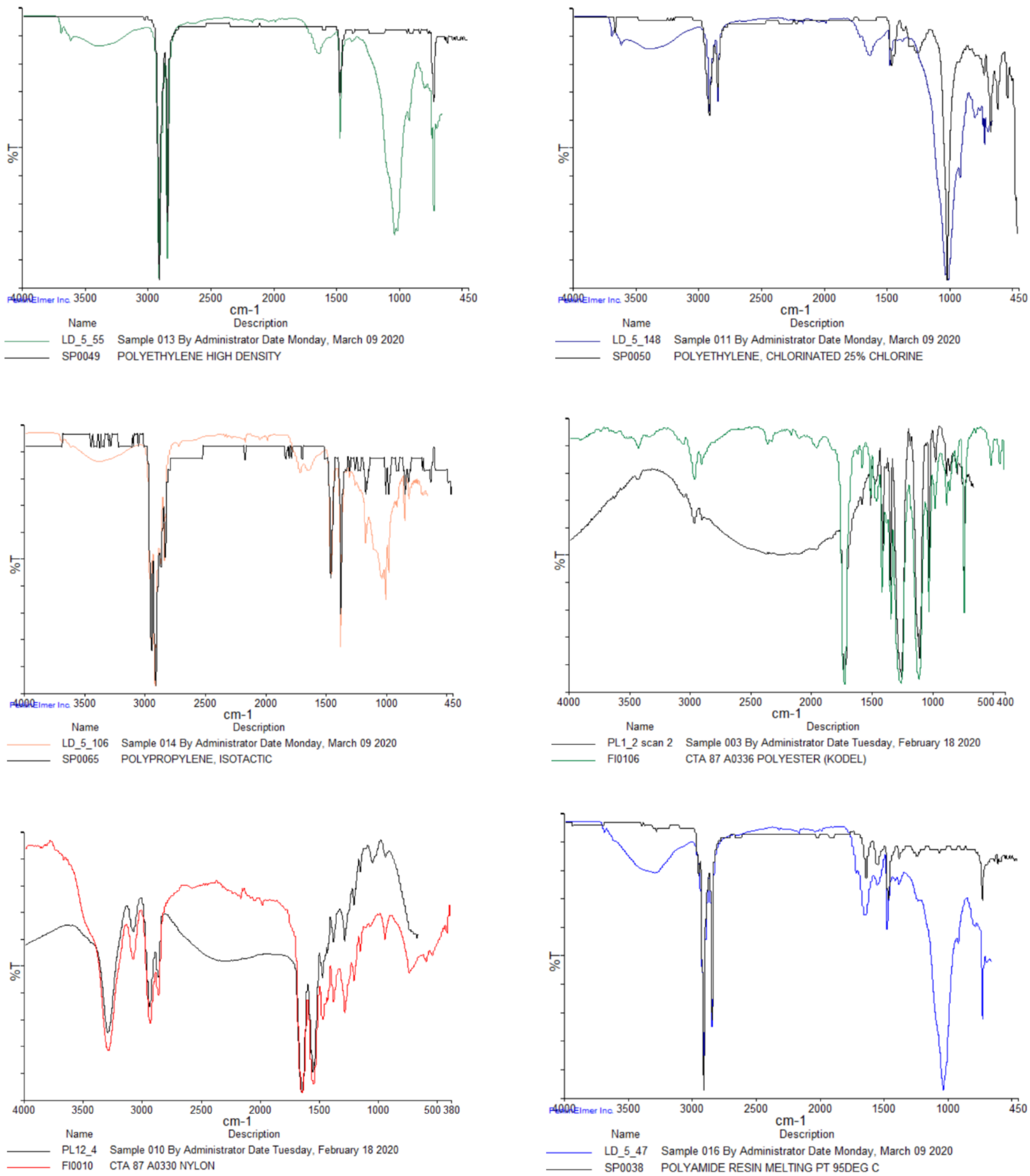

Figure S1. Examples of FT-IR spectra from the main polymer types identified in plastic particles found at the Molhe Oeste salt marsh, Brazil. Spectra were obtained with a Perkin Elmer Spotlight 400 Microscope (μ-FTIR) with a Frontier FT-IR Spectrometer (ATR).

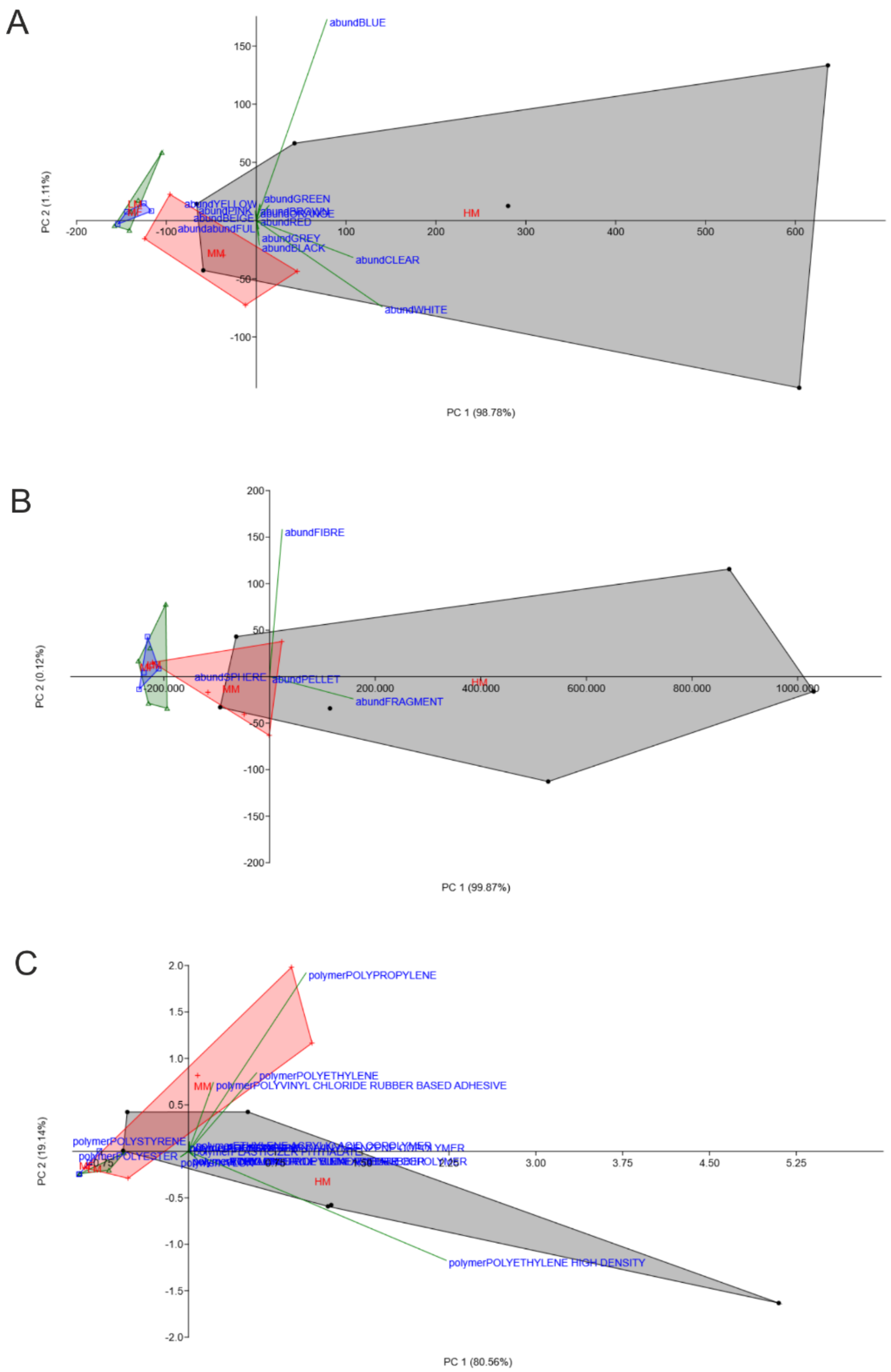

Figure S2. Principal Component Analysis showing the distribution of plastic characteristics (A: colours. B: formats; C: polymer types) in surface sediment among salt marsh zones (HM, MM, LM, MF). Variance explanation scores are indicated in the axis titles. HM: High Marsh; MM: Middle Marsh; LM: Low Marsh; MF: Mud Flat.