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BIOACUMULAÇÃO DE METAIS PESADOS E SUAS INTERAÇÕES AMBIENTAIS COM MICROPLÁSTICOS E PARÂMETROS FÍSICO-QUÍMICOS EM MOLUSCOS BIVALVES.

Orientador: Estefan Monteiro da Fonseca.

Niterói – RJ
2021

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Tese apresentada ao curso de Pós-Graduação em Dinâmica dos Oceanos e da Terra da Universidade Federal Fluminense como requisito à avaliação para obtenção do grau de Doutor.

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Aos meus pais, minha esposa e, especialmente, ao meu filho, Davi!

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A vida é assim: esquenta e esfria, aperta e daí afrouxa, sossega e depois desinquieta.
O que ela quer da gente é coragem.
(Guimarães Rosa)

APRESENTAÇÃO

Tese apresentada ao curso de Pós-Graduação em Dinâmica dos Oceanos e da Terra da Universidade Federal Fluminense como requisito à avaliação para a obtenção do grau de Doutor. Teve como objetivo o entendimento da bioacumulação de metais pesados e suas interações ambientais com microplásticos e parâmetros físicos e químicos em moluscos bivalves. Sua aplicação traz alternativas ao monitoramento de ambientes aquáticos, inclusive com tecnologias inovadoras na interação entre poluentes.

Para tanto, inicialmente foi realizado referencial teórico e introdutório sobre os temas circundantes ao tema, tais como: metais pesados nos ambientes aquáticos, parâmetros de qualidade de água (salinidade, temperatura, pH, OD e SST), microplásticos, moluscos bivalves como indicadores de poluição ambiental, bem como a caracterização dos locais avaliados. Foi descrito ainda o fluxograma de realização da Tese e a metodologia geral das avaliações realizadas. Os resultados foram concentrados em formas de artigos científicos, resultando em três manuscritos.

Os estudos concentraram-se em dois locais ao longo da costa brasileira, Vitória/ES e Paranaguá/PR, ambos municípios com atividade portuária relevante e com o monitoramento aquático como condicionante para o licenciamento ambiental. Na Baía de Vitória foram realizados dois trabalhos. O primeiro, intitulado “Avaliação da biodisponibilidade de metais traço e arsênio por meio de bioindicadores em um sistema estuarino urbanizado no sudeste do Brasil”, foi aceito e publicado em janeiro de 2021 na revista *Environmental Monitoring and Assessment*. Ainda com dados provenientes de Vitória foi gerado o segundo artigo, “Avaliação do risco à saúde humana associado ao consumo de mexilhões (*Perna perna*) e ostras (*Crassostrea rhizophorae*) usados como biomonitores no canal estuarino de baía da vitória, sudeste do brasil”. Esse manuscrito foi submetido à revista *Marine Pollution Bulletin*, em maio de 2021 e aprovado com revisões em junho de 2021.

No Complexo Estuarino de Paranaguá foi realizado o estudo “Ocorrência de microplásticos e acúmulo de metais pesados em ostras nativas *Crassostrea gasar* no Sistema Estuarino de Paranaguá, Brasil”. Esse trabalho foi publicado em março de 2021 na revista *Marine Pollution Bulletin*. Por fim, as conclusões gerais do trabalho foram destacadas.

ABSTRACT

Marine pollution is a growing problem in the world today. The oceans are full of problems caused by pollutants, which can be summarized in chemicals and garbage. Whatever the types of contamination in marine environments, there is an immense possibility of negative consequences for health, the environment and economic activities. It is also clear that many sources of these pollutants may be present, and that environmental variations and various human activities may also affect the environment and the availability of contaminants. It is important to understand not only each of these variables separately, but their interactions. Thus, this work aimed to evaluate the bioaccumulation of heavy metals (Al, Ba, Cd, Zn, Pb, Mn, Cu, Ni, Hg, Cr, Fe, As) and their interactions with microplastics and physical and chemical parameters in bivalve molluscs, culminating in the development of three scientific articles on the initially proposed theme and some of its variations. The studies concentrated on two locations along the Brazilian coast. In the Bay of Vitória (State of Espírito Santo), the work of "**Evaluation of bioavailability of trace metals and arsenic through bioindicators in a urbanized estuarine system in southeast Brazil**" and "**Human health risk assessment associated with the consumption of mussels (*Perna perna*) and oysters (C*rassostrea rhizophorae*) used as biomonitors in the estuarine channel of vitória bay, southeast brazil**". In the Estuarine Complex of Paranaguá (State of Paraná) the study "**Occurrence of microplastics and heavy metals accumulation in native oysters** *Crassostrea gasar* **in the paranaguá estuarine system, Brazil**" was carried out. Among the results, there was an influence of ocean waters on parameters as salinity and turbidity, which may also have influenced the concentration of some metals, both in mussels (Al, Zn, Mn, Cu, Ni, Cr, Fe), as well as in water (Cd, Zn and Pb). The bioavailability of all the metals tested was observed at some point, and variations in the accumulation of these metals between oysters and mussels, possibly related to the environmental and physiological conditions of each species worked. In the same way, Microplastics were found in all sampled locations (CEP), demonstrating the spread of this pollutant in the marine environment, its bioaccumulation capacity in oysters and the possible adsorption of some metals present in the marine environment, evaluated from MEV/EDS.

Keywords: Bioaccumulation, Heavy metals, Environmental parameters, Microplastics, Bivalve molluscs.

RESUMO

A poluição marinha é um problema crescente no mundo atual. Os oceanos estão repletos de problemáticas trazidas especialmente por poluentes, os quais podem ser resumidos em químicos e lixo. Quaisquer que sejam os tipos de contaminação nos ambientes marinhos, existe a imensa possibilidade de consequências negativas à saúde, ao ambiente e às atividades econômicas. Percebe-se também que muitas podem ser as fontes desses poluentes, sendo que as variações ambientais e as diversas atividades antrópicas podem ainda afetar o ambiente e a disponibilidade de contaminantes. É importante entender não só cada uma dessas variáveis separadamente, mas suas interações. Desse modo, esse trabalho teve o objetivo de avaliar a bioacumulção de metais pesados (Al, Ba, Cd, Zn, Pb, Mn, Cu, Ni, Hg, Cr, Fe, As) e suas interações ambientais com microplásticos e parâmetros físico-químicos em moluscos bivalves, culminando com o desenvolvimento de três artigos científicos acerca do tema inicialmente proposto e algumas de suas variações. Os estudos concentraram-se em dois locais ao longo da costa brasileira. Na Baía de Vitória (Estado do Espírito Santo) foram realizados os trabalhos de "**Avaliação da biodisponibilidade de metais traço e arsênio por meio de bioindicadores em um sistema estuarino urbanizado no sudeste do Brasil**" e "**Avaliação do risco à saúde humana associado ao consumo de mexilhões (***Perna perna***) e ostras (***Crassostrea rhizophorae***) usados como biomonitores no canal estuarino de baía da vitória, sudeste do brasil**". No Complexo Estuarino de Paranaguá (Estado do Paraná) foi realizado o estudo "**Ocorrência de acúmulo de microplásticos e metais pesados em ostras nativas** *Crassostrea gasar* **no Sistema Estuarino de Paranaguá, Brasil**". Dentre alguns dos resultados, percebeu-se influência de águas oceânicas em parâmetros como salinidade e turbidez, o que pode ter também influenciado a concentração de certos metais, tanto em mexilhões (Al, Zn, Mn, Cu, Ni, Cr, Fe), bem como na água (Cd, Zn e Pb). Observou-se a biodisponibilidade de todos os elementos testados em algum momento, e variações para no acúmulo desses metais entre ostras e mexilhões, possivelmente relacionados às condições ambientais e fisiológicas de cada espécie trabalhada. Da mesma maneira, encontrou-se Microplásticos em todos os locais amostrados (CEP), demonstrando a disseminação desse poluente no ambiente marinho, sua capacidade de bioacumulação nas ostras e a possível adsorção de alguns metais presentes no ambiente marinho, avaliados a partir do MEV/EDS.

Palavras-chave: Bioacumulação, Metais pesados, Parâmetros ambientais, Microplásticos, Moluscos bivalves.

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INTRODUÇÃO

A poluição marinha é um problema crescente no mundo atual. Os oceanos estão repletos de problemáticas trazidas especialmente por poluentes, os quais podem ser resumidos em químicos e lixo.

Quaisquer que sejam os tipos de contaminação nos ambientes marinhos, existe a imensa possibilidade de consequências negativas à saúde, ao ambiente e às atividades econômicas. Percebe-se que muitas podem ser as fontes desses poluentes. As variações ambientais e as diversas atividades antrópicas podem também afetar o ambiente e a disponibilidade de contaminantes.

É importante entender não só cada uma dessas variáveis separadamente, mas suas interações. Considerando os metais pesados como principal alvo desse estudo e sua biodisponibilidade para animais aquáticos, segundo Moore e Ramamoorthy (1984), diversos fatores interferem na disponibilidade e consequente bioacumulação dos metais, tais como: características e componentes presentes na água.

Assim, variações como parâmetros físicos e químicos da água, presença de microplásticos como potenciais superfícies de adsorção e fisiologia das espécies testadas podem alterar o acúmulo de metais em determinados indivíduos.

Metais pesados

Os metais pesados são geralmente definidos como metais com altas densidades, altos pesos atômicos ou altos números atômicos, podendo variar de acordo com o autor e o contexto (Pourret, 2018). Segundo Pereira et al. (2002), esses contaminantes são substâncias químicas consideradas como mais reincidentes, em termos de contaminação. O mesmo autor ainda relata que os metais nos ecossistemas aquáticos podem ocorrer de modo natural, devido à geoquímica da região e, por ação humana, como reflexo das atividades domésticas e/ou industriais, corroborando com Ramesh e Damodhram (2013). No segundo caso, a contaminação por metais pode ocorrer a partir de fontes difusas, como através da atmosfera, deposição de sólidos e lixiviação do solo, principalmente em áreas agrícolas, ou fontes pontuais caracterizadas principalmente devido a emissões de efluentes industriais, porões de esgoto, coleta de lixo e despejos de mineração (Campos, 2002).

Assim, entende-se que aportes elevados de metais pesados em ecossistemas podem causar contaminação ambiental, danos à saúde humana e de animais aquáticos. São considerados altamente tóxicos ou prejudiciais, podendo variar de acordo com sua forma e quantidade (Duffus, 2002).

Alguns elementos como cromo, arsênio, cádmio, mercúrio e chumbo têm o maior potencial de causar danos devido ao seu uso extensivo, à toxicidade de algumas de suas formas combinadas ou elementares e à sua ampla distribuição no ambiente (Baird & Cann, 2012). Esses cinco elementos, por exemplo, possuem alta afinidade com o enxofre, possibilitando alteração no controle de velocidade das reações metabólicas e causando sérios danos (Rusyniak et al, 2010). Ainda é importante citar o cromo e o arsênio como cancerígenos; o cádmio causando doença óssea degenerativa; e mercúrio e chumbo atingindo o sistema nervoso central (Rusyniak et al, 2010).

Outros metais pesados conhecidos por sua natureza potencialmente perigosa, geralmente como poluentes ambientais tóxicos, incluem manganês (dano ao sistema nervoso central) (Emsley, 2011); cobalto e níquel (carcinógenos) (Wiberg, 2001); cobre (Tokar et al, 2013), zinco (Eisler, 1993) e selênio (Lemly, 1997) (rompimento endócrino, distúrbios congênitos ou efeitos tóxicos gerais em peixes, plantas, pássaros ou outros organismos aquáticos); e estanho (dano ao sistema nervoso central) (Scott, 1989).

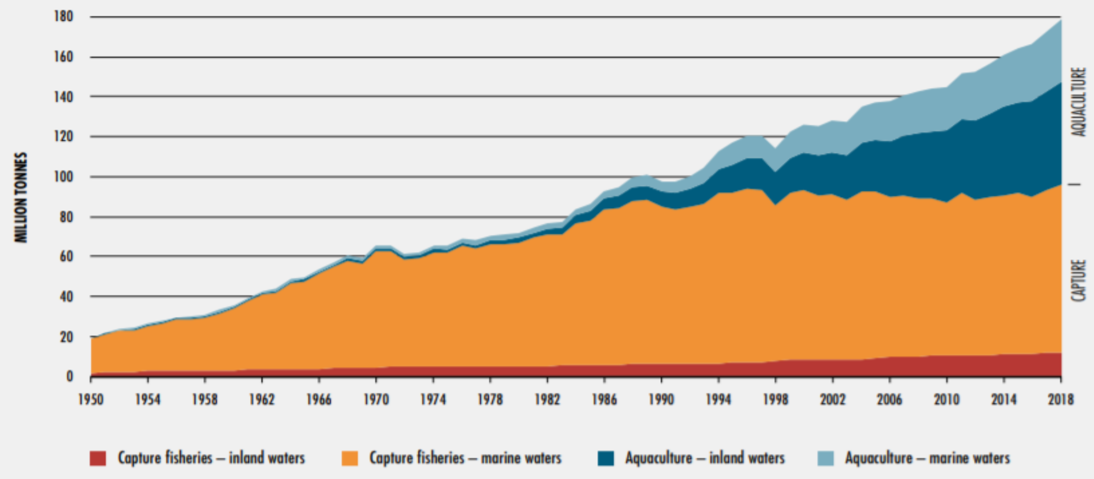
Desse modo, o monitoramento de contaminantes nos ecossistemas pode utilizar vários métodos diferentes, dependendo do objeto de uma pesquisa. A avaliação dos níveis de poluentes inorgânicos (por exemplo, metais pesados) no ambiente pode ser alcançada por análise química direta de amostras de água e sedimentos (por exemplo, Superville et al., 2014). No entanto, essa abordagem resulta em pouca informação sobre a biodisponibilidade de poluentes, uma vez que nem todos os metais são igualmente reativos, tóxicos e disponíveis para a biota (Hare, 1992).

As toxinas biodisponíveis são a fração poluente mais crítica do ponto de vista ecotoxicológico (Kim et al., 2015), na medida em que resultam em um risco potencial de equilíbrio para a cadeia da vida (Schöne & Krause Jr., 2016). Por outro lado, o consumo de frutos do mar contaminados por poluentes persistentes pode resultar em potenciais riscos humanos (AbdElGhany, 2017) já listados anteriormente. A contaminação por poluentes nos tecidos dos organismos e sua relação com o risco humano foram profundamente estudadas (El Nemr et al., 2016; El Nemr et al., 2012a; El Nemr et al., 2012; Jia et al., 2010).

Moluscos bivalves como indicador de poluentes marinhos

O consumo de pescados no Brasil está em franco crescimento, porém ainda é considerado baixo quando comparado à média mundial. Alguns fatores influenciam diretamente neste incremento progressivo, tais como aumento populacional, urbanização, crescente preocupação com hábitos alimentares saudáveis e ascensão do poder de compra nos países em desenvolvimento.

No entanto, com a visível estagnação da atividade pesqueira e a crescente demanda por pescado nos cenários brasileiro e mundial, o cultivo de organismos aquáticos, também conhecido como aquicultura, torna-se ferramenta potencial para atender o mercado consumidor. Atualmente, a produção de organismos aquáticos oriundos da aquicultura já é cerca de 50% dos pescados produzidos mundialmente (Fig. 1) (FAO, 2020).



NOTE: Excludes aquatic mammals, crocodiles, alligators and caimans, seaweeds and other aquatic plants.
SOURCE: FAO.

Fig. 1 – Produção mundial da pesca e da aquicultura – Fonte: FAO, 2020.

Neste contexto, além da tradicional atividade pesqueira, o Brasil se insere no panorama internacional como um dos países com maior potencial para o desenvolvimento da aquicultura por apresentar condições climáticas e hidrológicas que favorecem a atividade, além de seu extenso litoral e a disponibilidade de aproximadamente 8 bilhões de m³ de águas interiores.

Destaca-se também que a maior parte da demanda por frutos do mar ainda é por peixes. Outrossim, a produção de moluscos, especialmente os bivalves, é também importante no atendimento desta demanda crescente. Embora a colheita destes bivalves em estoques naturais continuará a acontecer, muitas populações selvagens provavelmente já estão perto de seus limites máximos sustentáveis, sendo a aquicultura a alternativa para a produção destas populações (Helm & Bourne, 2004).

Em relação aos moluscos, são animais predominantemente marinhos, embora existam espécies de água doce e terrestre. Os principais moluscos cultivados no Brasil são: o mexilhão (*Perna perna*), a ostra do pacífico (*Crassostrea gigas*) e as ostras nativas (*Crassostrea gasar e Crassostrea rhizophorae*), as vieiras (*Nodipecten nodosus*), além de outras espécies cujas metodologias de cultivo se encontram em fase experimental, como o berbigão (*Anomalocardia brasiliana*) e o sururu (*Mytella* spp.) (Silveira, não publicado).

O cultivo de moluscos é uma atividade que pode ter grande alcance social e econômico, beneficiando diversos setores da sociedade (produtores, consumidores, indústrias). Tal atividade gera empregos, ajuda a fixar populações nativas litorâneas em seu ambiente tradicional e torna-se um complemento de renda para pescadores artesanais (Rosa, 1997).

No entanto, além de sua importância comercial, para fins de monitoramento ambiental, as espécies de moluscos bivalves têm se mostrado excelentes ferramentas no ecossistema costeiro e estuarino (Chandurvelan et al., 2013), devido a suas particularidades fisiológicas como natureza séssil, abundância e modo de alimentação (filtração) (Diop et al., 2016). Além disso, sua capacidade de incorporar poluentes metálicos pode resultar em efeitos biológicos negativos (Chandurvelan et al., 2012, Chandurvelan et al., 2013). Finalmente, devido à sua abundância e ampla distribuição, os bivalves também têm sido usados como sentinelas há décadas para monitorar a poluição de metais costeiros (Wang e Lu, 2017).

A razão de incorporação de metais vestigiais nos bivalves depende principalmente de fatores biológicos e geoquímicos. Os fatores biológicos incluem idade, tamanho, sexo, genótipo, fenótipo, atividade alimentar, estado reprodutivo e condições fisiológicas dos animais (Saavedra et al., 2004; Mubiana et al. 2006). É importante ressaltar que é preciso reconhecer a diversidade dos padrões de bioacumulação de metais, tanto nos próprioes elementos metálicos quanto nas espécies bivalves (Wang e Lu, 2017).

Adicionalmente, também considerando seu modo alimentar, os bivalves podem ser utilizados para a identificação de microplásticos no ambiente marinho, uma vez que esse poluente pode ser facilmente confundido com plâncton (Egbeocha et al., 2018) e já encontrados, inclusive, no trato digestivo de algumas espécies de moluscos (Li et al, 2015; Teng et al., 2018).

Parâmetros Oceanográficos e Físico-Químicos

Salinidade

A salinidade é um importante fator ecológico que influencia a comunidade biológica que habita um corpo d'água. Juntamente a outros fatores, a salinidade afeta os processos fisiológicos de moluscos bivalves e pode determinar suas possibilidades para alimentação, a duração de vida planctônica e a habilidade para selecionar locais para fixação (Albuquerque et al., 2012).

Dentre todos os fatores abióticos, a salinidade pode ser considerada um parâmetro ambiental importante, especialmente em regiões estuarinas e outros ambientes costeiros, por apresentar altas e constantes variações, as quais afetam a fisiologia e a ecologia dos organismos (Albuquerque et al., 2012). Algumas espécies de moluscos, tais como aquelas encontradas em manguezais ou em estuários tropicais, são adaptadas a viver em condições de salinidade variável em ciclos diários e/ou sazonais (Glenn et al., 1999), assim chamadas de eurihalinas.

Assim, é válido destacar a importância do conhecimento dos limites de tolerância de salinidade por parte dos moluscos, uma vez que, esta informação pode ser útil em alguns processos fisiológicos (Maia et al., 2017). O mesmo autor também ressalta a importância de estudos que abordam os impactos da salinidade sobre a população de moluscos, sobretudo em áreas com variação do aporte de águas doces e salgadas.

A variação da salinidade ainda pode afetar o balanço osmótico dos moluscos, acarretando em gasto energético para reajustar sua concentração osmótica (Cheng et al., 2002), influenciando diretamente na bioacumulação de poluentes. Segundo Boening (1999), a biodisponibilidade de poluentes, como metais pesados, também pode ser dependente de fatores geoquímicos e biológicos. Os estuários, ambientes onde é comum a mistura de água doce e salgada, mostram grande peculiaridade na dinâmica de remobilização de metais (Noegrohati 2005).

Temperatura

Conforme amplamente difundido, o conceito de temperatura a define como uma grandeza física escalar do grau de agitação de moléculas que compõem um corpo. Quanto maior tal agitação molecular, maior será a temperatura do corpo e mais quente ele estará e vice-versa.

Em estuários, caso dos estudos em tela, parâmetros como a temperatura são influenciados especialmente por fatores climáticos (chuvas e ventos) que, por sua vez, podem regular a intensidade de ação das marés (IGIA, 2010). O mesmo trabalho destaca que, de modo geral, a temperatura apresenta um padrão de distribuição espacial semelhante à salinidade e as variações verticais e horizontais encontradas nestes ambientes estuarinos são de pequena magnitude.

Nesses locais a temperatura pode influenciar numerosos fatores físicos e químicos, com alterações na especiação química de metais entre os mais importantes (Byrne et al. 1988). A especiação química indica que o aumento da temperatura geralmente resulta em aumento nas concentrações e atividades de formas metálicas biodisponíveis, portanto, melhora a absorção (Mubiana e Blust 2007).

A temperatura ainda pode atuar na velocidade dos processos metabólicos em algumas espécies aquáticas, influenciando a sobrevivência, desenvolvimento e comportamento dos organismos (Newell e Branch, 1980). Como a maioria dos organismos aquáticos, ostras e mexilhões não podem regular sua temperatura corporal de acordo com o ambiente ao redor, como resultado, a maioria dos processos fisiológicos e bioquímicos depende da temperatura (Dame, 1996).

Assim, processos essenciais e indicadores da saúde destes moluscos, as taxas de filtração, respiração, excreção e absorção de alimentos, por exemplo, tendem a aumentar com o aumento da temperatura para ostras e mexilhões (Resgalla Jr. et al. 2007), cada um dentro de seus limites ótimos de cada espécie. Ainda é preciso destacar que tais limites de tolerância podem variar de acordo com a fase de vida destes animais aquáticos.

Adicionalmente, destaca-se que a temperatura pode influenciar na velocidade de adsorção dos metais em algumas superfícies (Jimenez et al. 2004), tais como os microplásticos.

Potencial Hidrogeniônico (pH)

O Potencial Hidrogeniônico (pH) é uma escala numérica adimensional utilizada para especificar a acidez ou basicidade de uma solução aquosa, definido como o cologaritmo da atividade de íons hidrônio (IUPAC, 1997). As soluções com valores de pH 7 são consideradas neutras, valores menores que 7 são ácidas pois apresentam quantidade maior de íons hidrônio e soluções com valores maiores do que 7 são básicas pois apresentam quantidade menor de íons hidrônio em relação aos íons hidróxido (Lim, 2006). O mesmo autor ainda enfatiza que, embora não seja habitual, a escala pode assumir valores abaixo de zero e acima de quatorze em casos de ácidos ou bases muito fortes, respectivamente.

O pH é uma propriedade de importância crucial para a maioria das soluções aquosas (Rasouli et al. 2020). As medições de pH podem ser realizadas em aplicações muito diferentes, tais como monitoramentos ambientais, industrias e ciências biomédicas, por exemplo (Wencel et al. 2013). No ambiente, o pH é um fator importante para processos químicos e biológicos na água do mar, água doce e solos, podendo afetar o equilíbrio de muitas espécies, solubilidade de metais, disponibilidade de nutrientes e atividade de microrganismos (Bargrizan et al. 2019), fatores intimamente ligados à bioacumulação de poluentes em animais aquáticos.

O pH do ambiente é um fator importante para a maioria dos metais pesados, uma vez que a disponibilidade dos mesmos pode ser relativamente baixa em valores de pH próximos à neutralidade, em torno de 6,5 a 7 (Kabata-Pendias e Pendias 1987). Este fator vai ao encontro da maioria das águas salinas onde são encontrados os moluscos de valor econômicos no Brasil, que segundo a Resolução CONAMA 357/2005, para águas salinas de classe I, o pH deve estar entre 6,5 - 8,5, valores próximos aos necessários para o desenvolvimento dessas espécies (Ramos et al. 2010).

Oxigênio Dissolvido (OD)

Oxigênio dissolvido é a medida da quantidade de oxigênio em um determinado fluido ou por ele transportado. O oxigênio dissolvido é elemento determinante em ambientes aquáticos, especialmente para os organismos vivos que ali habitam.

A poluição marinha, especialmente matéria orgânica em grandes quantidades, possivelmente proveniente de ações antrópicas, pode afetar drasticamente os níves de OD do ambiente, levando-os à hipóxia, como resultado do consumo desse carbono orgânico e fazendo com que bactérias aeróbicas aumentem seu consumo de oxigênio.

Em casos de hipóxia ou, principalmente, anóxia, conforme descrito acima, pode haver influência sobre elementos presentes no ambiente, possibilitando novas funções aos metais, sendo que outros receptores de elétrons (nitrato, nitrito, ferro, óxidos de manganês, sulfato e dióxido de carbono) passam a atuar na mediação da decomposição anaeróbica da matéria acumulada (Boyd et al., 2002).

O valor mínimo de OD para a preservação da vida aquática, estabelecido pela Resolução CONAMA 357/2005 é de 5,0 mg/L, mas existe uma variação na tolerância de espécie para espécie. Geralmente, os valores de oxigênio dissolvido menores que 2 mg/L podem apresentar risco para as espécies presentes naquele habitat. Os moluscos, por exemplo, estão presentes em ambientes próximos a superfície da água, onde há a maior produtividade primária (alimento) e, consequentemente, oxigênio.

Fatores que influenciam fortemente na quantidade de OD são temperatura, profundidade e pressão atmosférica, sempre ligados a outros processos físicos, químicos e biológicos desses corpos d’água. Em ambientes aquáticos, observar fatores que contribuem para as perdas de OD também são importantes, especialmente quando tratamos de cultivo ou extração de animais aquáticos. Poli e Arana (2004) destacam que nesses ambientes as perdas de oxigênio são ocasionadas pela respiração dos organismos, perdas para a atmosfera em casos de supersaturação da água, oxidação da matéria orgânica e oxidação de ions metálicos, como Ferro e Manganês, por exemplo.

Ainda, tratando especificamente de regiões estuarinas, estas podem apresentar diferentes níveis de OD, chegando a valores baixos devido à deficiência em renovação de água proveniente dos oceanos e ricas em oxigênio.

Turbidez e Sólidos Suspensos Totais (SST)

A turbidez, considerada uma medida subjetiva, pode ser definida como propriedade física dos fluidos que se traduz na redução da sua transparência devido à presença de materiais em suspensão que interferem a passagem da luz através daquele corpo d’água (Sousa, 2001). O parâmetro é considerado de grande relevância em temas ligados à oceanografia, uma vez que pode determinar a espessura da zona eufótica (Moreira et al. 2001), onde concentram-se muitos organismos vivos.

A turbidez está intimamente ligada à presença de Sólidos Suspensos Totais, os quais são definidos como toda a matéria, com excepção da água contida naquele fluido, ou, mais especificamente, matéria que permanece como resíduo após evaporação de 103 a 105 ºC, podendo ser divido em sólidos em suspensão e sólidos filtráveis (Sousa, 2001).

O mesmo autor ainda sugere que uma variedade desses materiais em suspensão podem causar a turbidez, sejam eles de origem orgânica ou inorgânica, as quais variam desde partículas coloidais até sólidos de diversas dimensões. Elevados valores para turbidez também podem ser associados a processos erosivos relacionados à ações antrópicas (Santos & Cunha, 2015), comuns em zonas estuarinas. Estas zonas, por sua vez, ainda podem ter problemas com a presença de SST ou altos níveis de turbidez por geralmente possuírem rios desaguando e afetando-as através da remoção de materiais adensados no fundo que são trazidos de volta para a coluna d’água.

Desse modo, os elevados índices de sólidos suspensos e turbidez podem prejudicar processos metabólicos dos corpos d’água e causar desequilíbrio na cadeia alimentar, prejudicando a procura por alimento de peixes e crustáceos, por exemplo, e reduzindo a fotossíntese, o que afeta diretamente o desenvolvimento de moluscos (Santos et al. 2014). Boyd e Tucker (1998) ainda definem que, em geral, para o melhor crescimento e sobrevivência de espécies aquáticas é ideal que a turbidez seja considerada moderada, equilibrando ídices de fitoplancton e oxigênio. Ions metálicos naturais, tais como Ferro e Manganês, também podem afetar esses índices e causar desequilíbrios no sistema e nesses parâmetros (Vaz et al. 2010).

Microplásticos (MPs)

Os microplásticos são considerados pequenos pedaços de plástico disponíveis no meio ambiente. Esses poluentes são também definidos como fragmentos de plástico com tamanho menor que 5 mm, podendo ser primários, quando adicionados intencionalmente a determinados produtos, ou secundários, quando da degradação física, química e/ou biológica proveniente de resíduos plásticos maiores em processos ambientais naturais (Wang et al, 2018). Pode-se citar uma variedade de fontes para esses MPs, tais como cosméticos, roupas, lixo plástico em geral e diversos processos industriais.

Mais especificamente, os microplásticos primários são quaisquer fragmentos de plástico naturalmente com dimensões inferiores a 5 mm, mesmo de entrarem no ambiente, podendo citar as microfibras de roupas, microesferas e esferas de plástico resultantes da fabricação de peças plásticas maiores (Boucher & Friot 2017). Os mesmos autores ainda descrevem os microplásticos secundários como aqueles criados a partir da degradação de produtos plásticos maiores quando entram no ambiente. Em geral, sua deteriorização ocorre por meio do desgaste por ação do tempo e dos diversos processos existentes no meio (temperatura e luz solar, por exemplo), bem como as características do próprio plástico (tamanho, densidade, composição) (Auta et al. 2017), exemplificando como garrafas PET, redes de pesca e sacos plásticos, muito comuns em ambientes marinhos e estuarinos.

São muitas as formas de chegada dos microplásticos em ambientes marinhos (Auta et al. 2017) (Fig. 2), tais como tratamentos de águas residuais, tempestades, esgotos, vento e correntes (Murphy et al., 2016) ou até mesmo o escoamento superficial urbano (Cole et al., 2011). No ambiente marinho, as naturezas flutuantes e persistentes dos microplásticos permitem que eles se dispersem fácil e amplamente através de hidrodinâmica processos e correntes oceânicas (Carvalho e Baptista Neto, 2016). Para exemplificar, Gouin et al. (2011) relataram que a população dos EUA emite cerca de 263 toneladas de microplásticos de polietileno por ano, principalmente do uso de produtos de cuidado pessoal. Eles estimaram o consumo per capita de microplásticos para 2,4 mg/pessoa.dia^{-1}, o que compõe 25% de plásticos no giro subtropical do Atlântico Norte (Auta et al. 2017).

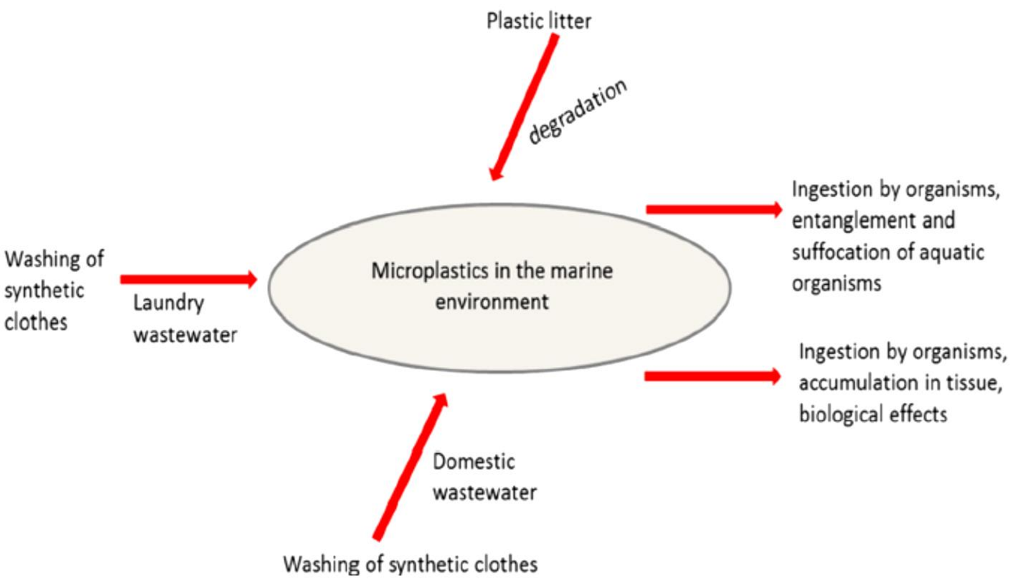


Fig. 2 – Causas e consequências de MPs em ambientes aquáticos – Fonte: Auta et al., 2017.

Nesse contexto, fica claro que os MPs já estão disseminados pelos mais diversos ambientes contíguos aos mares e oceanos, tais como praias, águas superficiais dos sedimentos do fundo do mar, bem como em uma grande diversidade de organismos marinhos, como aves marinhas, peixes, moluscos bivalves, mamíferos e crustáceos (DeWitte et al., 2014; Gauquie et al., 2015).

Com sua degradação lenta, ao longo de centenas, senão milhares de anos, é possibilitada sua ingestão e acúmulo nos corpos e tecidos de muitos organismos, tais como organismos marinhos citados, possivelmente confundidos com plâncton (Sleight et al., 2017; Egbeocha et al., 2018) e já encontrados, inclusive, no trato digestivo de algumas espécies de moluscos em trabalhos específicos (Li et al, 2015; de Sá et al., 2018; Teng et al., 2018).

Por sua característica alimentar de filtração, os moluscos são extremamente susceptíveis aos MPs. Eles podem filtrar grandes volumes de água, com taxas de até 300 mL.min^{-1}, aumentando sua suscetibilidade a substâncias transmitidas pela água (Widdows, 1973). Assim, diversos estudos sugerem que os microplásticos podem representar riscos potenciais à saúde humana quando os humanos consomem essas ostras contaminadas (Cole et al., 2011; Li et al., 2015; Auta et al., 2017; Teng et al., 2018; Li et al., 2018).

Adicionalmente, sob exposição simultânea a microplásticos e outros contaminantes ambientais, interações toxicológicas podem ocorrer e modificar o tipo e/ou a magnitude dos efeitos tóxicos (Chen et al., 2017; Guilhermino et al., 2018; Pacheco et al., 2018; Rainieri et al., 2018).

Produtos químicos que possuam metais pesados podem ser adicionados a polímeros virgens durante fabricação para melhorar as propriedades específicas do material (Cherif Lahimer et al., 2017) ou contaminação por metais pode ocorrer durante diferentes processos de fabricação (Whitt et al., 2013). O ambiente natural marinho também pode ser a fonte desses metais, uma vez que estudos vêm sendo desenvolvidos e observando que detritos de plástico também absorvem metais do ambiente circundante (Rochman et al., 2014; Turner e Holmes, 2015).

Os MPs podem, então, servir como transportadores e/ou facilitadores para produtos químicos tóxicos, como metais pesados e poluentes orgânicos no meio ambiente sejam eles marinhos ou dulcícolas, mar aberto ou estuários (Koelmans et al, 2016; Gao et al, 2019; Wu et al, 2019). Isso é possível através de mecanismos de sorção, especialmente adsorção de poluentes na superfície dos microplásticos (Gaylard, Neto and Fonseca, 2020). Esse fenômeno ocorre principalmente devido à grande área de superfície de microplásticos (tão alto quanto 4,37 m^2/g; medido usando o Método Brunauer – Emmett – Teller (BET) (Teuten et al., 2009). Além disso, a carga eletrostática induzida no plástico altamente resistivo causado pela fabricação de alta velocidade equipamento durante a produção de plástico pode aumentar a captação de poluentes no meio ambiente, além de sua composição (Verla et al., 2019). Entretanto, os mecanismos de adsorção e dessorção de substâncias tóxicas produtos químicos para plásticos são variados e complexos e ainda permanecem relativamente inexplorado por pesquisadores (Lusher et al., 2017).

ÁREA DE ESTUDOS

Os estudos concentraram-se em dois locais ao longo da costa brasileira.

Na Baía de Vitória (Estado do Espírito Santo) foram realizados os trabalhos de "**Avaliação da biodisponibilidade de metais traço e arsênio por meio de bioindicadores em um sistema estuarino urbanizado no sudeste do Brasil**" e "**Avaliação do risco à saúde humana associado ao consumo de mexilhões (*Perna perna*) e ostras (*Crassostrea rhizophorae*) usados como biomonitores no canal estuarino de baía da vitória, sudeste do brasil**". No Complexo Estuarino de Paranaguá (Estado do Paraná) foi realizado o estudo "**Ocorrência de microplásticos e acúmulo de**

metais pesados em ostras nativas *Crassostrea gasar* **no Sistema Estuarino de Paranaguá, Brasil**".

Baía de Vitória-ES

A Ilha de Vitória, sede da capital do estado do Espírito Santo, Vitória, está compreendida pelo Canal da Passagem, a Baía de Vitória e a Baía do Espírito Santo. A Baía de Vitória, alvo deste estudo, estende-se desde a desembocadura do Rio Santa Maria da Vitória até a Baía do Espírito Santo, contendo o Porto de Vitória e seu canal de acesso. A largura varia desde 1,60 km na região da Ilha das Caieiras, onde a profundidade é menor (cercade 4 m na parte mais profunda) até 160 m emfrente ao Morro do Penedo na região do Porto de Vitória, onde a profundidade alcança 24 m (Rigo, 2004) (Fig. 3).

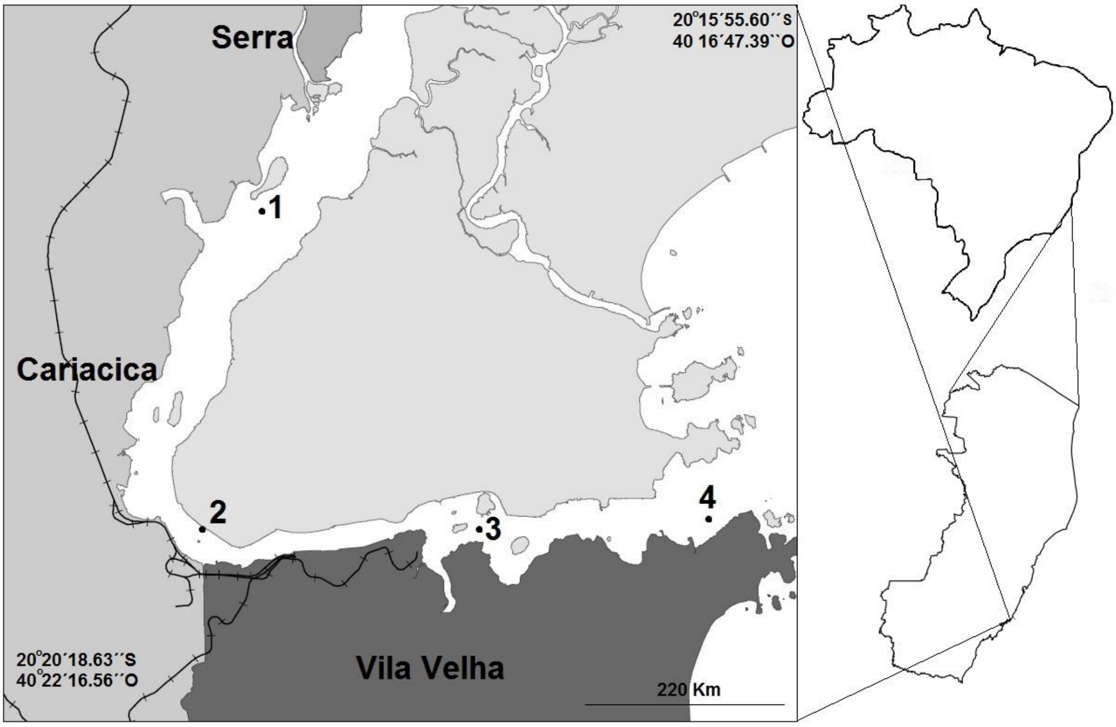


Fig. 3 – Quatro estações de amostragem na ilha de Vitória: BMP1 (S 20 ° 14'56,4 "; W 40 ° 19'35,7 "), BMP2 (S 20 ° 19'32,1 "; W 40 ° 21'03,2"), BMP3 (S 20 ° 19'22,3 "; W 40 ° 19'48,6 ") e BMP4 (S 20 ° 19'17,4 "; W 40 ° 16'56,8").

Toda essa região estuarina sofre a influência de água doce proveniente de rios de pequeno e médio porte que juntamente com a influência de águas oceânicas formam a paisagem principal do entorno da capital capixada, os manguezais (Jesus et al., 2004).

O principal rio formador da Baía de Vitória, o traz algumas de suas características para esse corpo d'água. A bacia do rio Santa Maria da Vitória apresenta razoável contraste térmico, com a temperatura na região próxima ao baixo curso do rio tende a se mostrar mais amena, devido à proximidade do mar, próximo aos 20ºC (EIA/RIMA, 2018). O mesmo Estudo de Impácto Ambiental dessa Bacia Hidrográfica descreve a cidade de Vitória como circundada de morros, o que faz a região ser uma das mais chuvosas no litoral espírito-santense, como a precipitação média anual está em torno de 1.275,7 mm, enquanto que a evaporação é de apenas 931,6 mm. Além disso, quanto à predominância de ventos, verifica-se que na primavera-verão, os ventos são geralmente de nordeste, seguidos de sudoeste e, no período de outono-inverno, o predomínio passa a ser do vento sudoeste, seguido pelo nordeste (fonte: Companhia Siderúrgica de Tubarão - CST).

Entretanto, além de algumas características naturais do meio, com o crescimento e urbanização de diversas cidades, o sistema estuarino da Ilha de Vitória tem sofrido ao longo de várias décadas uma forte degradação ambiental, por ocupação populacional de seu entorno, aterros, implantação de indústrias, atividades portuárias e, principalmente, devido ao lançamento de esgotos, a maioria deles (em torno de 70%) in natura (HABTEC, 1997). Além do município de Vitória, a Baía recebe também contribuições antrópicas de outros três municípios circunvizinhos (Vila Velha, Cariacica e Serra) (Jesus et al., 2004).

A fauna associada ao manguezal da Baía de Vitória possui a predominância de crustáceos, moluscos, peixes, pequenos mamíferos, entre outros (Carmo, 1987), destaca-se que muitas destas espécies são utilizadas pela população local como alimentação, tais como caranguejos, peixes, ostras do mangue e sururu (Sá, 1995).

Contudo, novamente o estudo de Jesus et al. (2004) já mostravam que a poluição dos ambientes no entorno da Ilha de Vitória necessitava de maiores informações e monitoramento. Os autores identificaram que as concentrações médias dos metais, por exemplo, encontradas para sedimentos do sistema estuarino da Baía de Vitória refletiram a contribuição de origem natural, associada à geologia local, bem como a contribuição antrópica pelo descarte de efluentes domésticos e industriais.

Nas regiões do Canal da Passagem e canal do Porto de Vitória (basicamente os extremos da baía), em geral, foram encontradas as maiores concentrações de metais, confirmando a influência antrópica nestas regiões, principalmente pelo lançamento de esgotos. Ainda de acordo com o estudo de Jesus et al. (2004), demonstrou-se que os sedimentos de alguns pontos desta região localizados próximos à Ilha de Vitória podem ser considerados antropizados com relação aos metais Cu, Pb, Zn, Mn e Hg, sendo necessários estudos adicionais para verificação da extensão da contaminação. Tais dados demonstram os riscos de consumo de alguns organimos aquáticos dessas regiões, conforme citado por Sá (1995).

Ainda assim, os municípios locais dependem do desenvolmento dessa região, possibilitando o confronto com a qualidade ambiental, uma vez que a economia de Vitória é voltada especialmente para as atividades portuárias, contando com dois dos Portos mais importantes do país (Porto de Vitória e o Porto de Tubarão) (Fig. 4).

Fig. 4 – Imagem aérea do Porto de Vitória – Fonte: www.skyscrapercity.com.

Complexo Estuarino de Paranaguá (CEP)-PR

Com similaridade ao cenário descrito para a Baía de Vitória, aqui novamente destaca-se que os estuários são importantes ecossistemas fornecedores alimento e habitat de uma grande quantidade de organismos aquáticos e pertencentes à cadeia alimentar marinha, além de promover serviços biogeoquímicos para outros ambientes devido às suas conexões entre bacias hidrográficas e águas costeiras (Barletta et al., 2010; Costa e Barletta, 2016). Entretanto, é visível o aumento da interferência antropogênica nesses habitats, muitas vezes causados pela atividade agrícola, desenvolvimento industrial e uso múltiplo do abastecimento de água, levando ao descarte inadequado de resíduos, descargas de esgoto e controle de fluxo, especialmente em países sul-americanos em crescimento (Barletta et al., 2019).

O panorama descrito é similar a algumas condições apresentadas no litoral do Estado do Paraná, Brasil, o qual possui cerca de 100 km de costa oceânica e 1300 km de costa estuarina (IGIA, 2010), com grande destaque para o Complexo Estuarino de Paranaguá – CEP (Fig. 5).

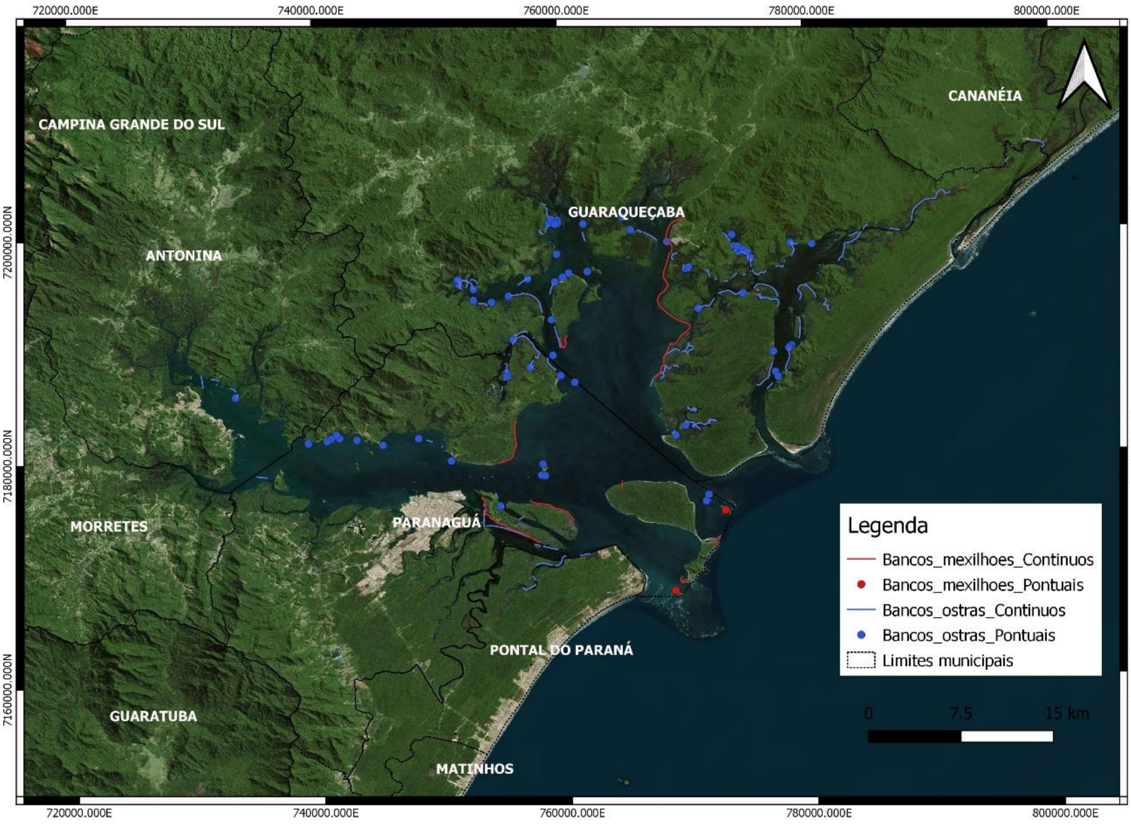


Fig. 5 – Representação do Complexo Estuarino de Paranaguá.

Para melhor entendimento da região, de acordo com Andriguetto Filho & Marchioro (2002), o litoral do Paraná apresenta oito ecossistemas estuarinos e marinhos, dentre os quais serão caracterizados abaixo aqueles utilizados como área de estudo para o presente trabalho e que foram descritos nos Planos Locais para Desenvolvimento da Maricultura do Paraná (IGIA, 2010):

1. *Baía de Antonina: região genuinamente estuarina, sendo considerada homogênea no inverno e estratificada no verão. O sedimento de fundo é predominantemente síltico-argiloso com manchas de areia, muito mal classificado, e com alto teor de matéria orgânica. A concentração de oxigênio dissolvido junto ao fundo, em geral, é mais baixa. O pH geralmente é inferior a 8 e a salinidade oscila entre 9 a 21, sendo que a média de salinidade é de 13. Dentre as baías do Complexo Estuarino de Paranaguá, ela é um das regiões mais ricas*

em nutrientes com alta produtividade primária, configurando-se como fonte ou zona de regeneração de nutrientes. Sua profundidade média é de até 2 metros.

2. *Setor mediano da Baía de Paranaguá (da Ilha do Teixeira até a Ilha da Cotinga): classificado como estuário homogêneo no inverno e estratificado no verão. Seu ambiente é de baixa energia com profundidade média de 4,3 m e fundo de areia síltico-argiloso. O pH é superior à 8 e a salinidade oscila entre 22 e 29, com média de 26. Neste local ocorre regeneração de fósforo e grande consumo de nitrogênio, devido à alta produtividade.*
3. *Zona nerítica da Baía de Paranaguá (da Ilha da Cotinga até a barra): esse ambiente é caracterizado pela sua alta energia, com profundidade média de 7 m e máximo de 33. Essa zona caracteriza-se como uma área de grande dinâmica e de mistura das águas das Baías de Paranaguá e da Baía de Laranjeiras na maré vazante. O sedimento de fundo é de areia fina bem selecionada. O pH é superior a 8 e a salinidade é alta, com média de 31 e valores entre 25 e 32.*
4. *Baía de Laranjeiras: região menos conhecida em suas características físicas. Ambiente estuarino de média a alta energia, com fundo de areia e manchas de silte e argila. Sua profundidade média é de 2,5 m. A salinidade fica em torno de 19 e 23, com valores médios de 26 a 28.*
5. *Baía de Guaraqueçaba: zona estuarina de média energia, com profundidade média de 3 metros. Predomínio dos sedimentos de areia síltica e areia argilosa. O pH é inferior a 8 e a salinidade varia entre 10 e 23.*
6. Os demais ecossistemas presentes no litoral do Parana, mas que não fizeram parte da área de atuação desse estudo, são: Baía dos Pinheiros, Baía de Guaratuba e Plataforma continental interna (região de mar aberto).

Ainda como caracterização do local, ressalta-se que a temperatura e salinidade de regiões estuarinas são principalmente controladas por fatores climáticos, os mesmos que podem também afetar a intensidade de ação das marés (regime de chuvas e ventos). A variação de temperatura (Fig. 6), de modo geral, apresenta um padrão de distribuição espacial semelhante ao da salinidade, descritas por regiões nos tópicos acima. Novamente de acordo com o estudo realizado pelo Instituto GIA (2010), no inverno (temperaturas mínimas) observa-se que a baía das Laranjeiras, eixo Norte-Sul, apresenta temperaturas entre 18 e 19° C na sua porção mais aberta, pois esta sofre uma maior influência da desembocadura do CEP. Nas áreas mais internas da baía percebe-se uma maior influência dos rios, sendo que na região de Antonina podemos perceber essa influência até a região do porto de Paranaguá. As temperaturas máximas (verão) observadas no CEP apresentam um padrão semelhante ao inverno, porém as mínimas temperaturas encontradas foram de 25°C.

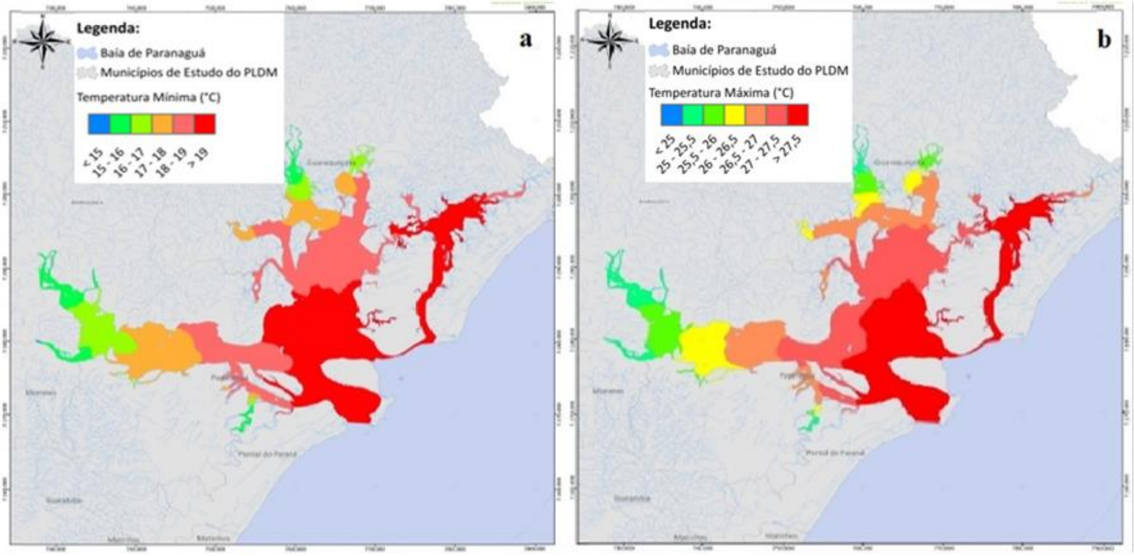


Fig. 6 – Temperaturas mínimas (a) e máximas (b) distribuídas ao longo do Complexo Estuarino de Paranaguá – Fonte: IGIA, 2010 – Adaptado pelo autor.

A pluviosidade também exerce importante função nas características ambientais da região. O litoral do Paraná apresenta os mais elevados índices pluviométricos do Estado (IGIA, 2010), conforme detalhado na Fig. 7.

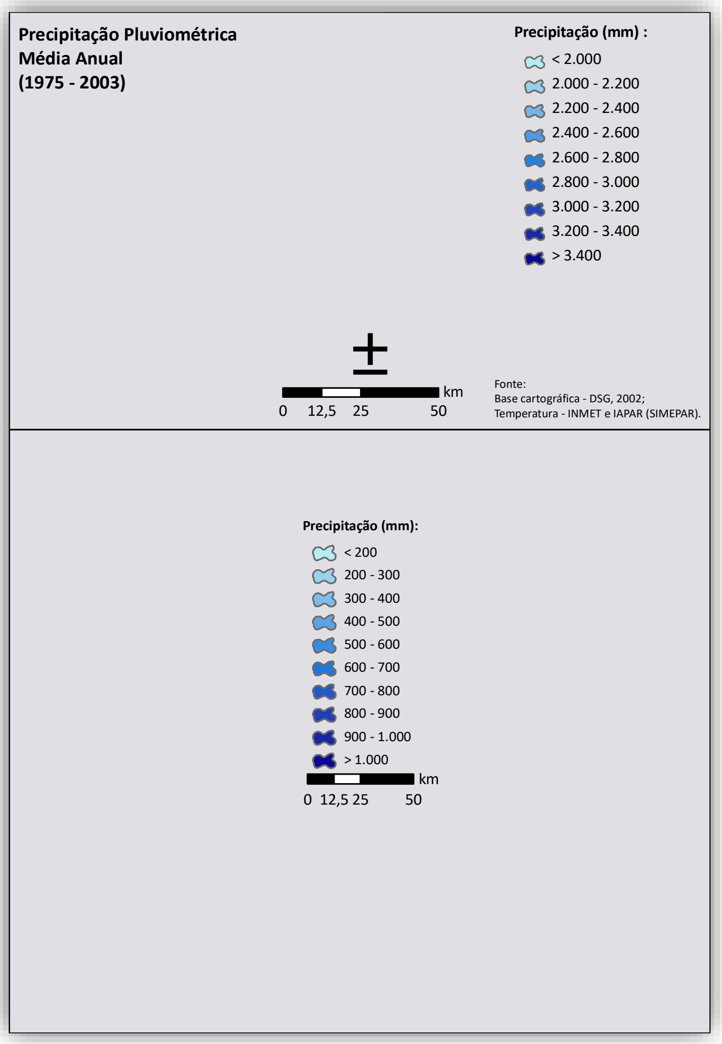


Fig. 7 – Pluviosidade média anual e sazonal do Litoral do Paraná – Fonte: IMMET e IAPAR, Retirado de IGIA, 2010.

Historicamente, essa região possui a pesca e a aquicultura como vocação e atividades potenciais para desenvolvimento social e econômico. No entanto, o aumento da urbanização e atividades industriais em regiões costeiras possibilitou o incremento de poluentes em ecossistemas aquáticos (Pereira et al., 2006), podendo causar contaminação ambiental, danos à saúde humana e à fauna local.

As principais fontes poluidoras no litoral paranaense, que podem influenciar na qualidade da água, tanto subterrânea como estuarina e marinha são as atividades industriais, portuárias e o despejo do esgoto diretamente nos ambientes aquáticos. Outro grave problema é a falta de aterros sanitários em nosso litoral. O lixo doméstico é coletado e depositado em lixões, sem nenhum tipo de prevenção, como por exemplo, o lixão do Embocuí em Paranaguá (SEMA, 2006).

A Baía de Paranaguá, principalmente na região próxima ao porto, historicamente concentra diversas fábricas de adubos e fertilizantes, sendo, portanto, considerada uma área de risco ambiental (Egler, 1996). O município de Paranaguá é o que possui um maior número de indústrias localizadas em regiões próximas de rios e a baía, setores estes que apresentam o lençol freático próximo a superfície e onde a drenagem continental ocorre via restinga e manguezal, o que pode ocasionar a destruição da cobertura vegetal e também a contaminação das águas (Caneparo, 2001).

Diante desse contexto, alguns dos principais poluentes disponíveis nos ambientes marinhos e estuarinos, gerados pelas mais distintas atividades, podem disponibilizar metais pesados e, mais recentemente, microplásticos. Esses elementos são diretamente absorvidos pela fauna aquática local, muitas vezes comercializada para alimentação humana e composta por uma enorme variedade de moluscos (Lana, 1986) (destaque para ostras e mexilhões), crustáceos (Melo et al., 1989), peixes (Haluch et al., 2004), entre outros.

METODOLOGIA

Com o objetivo de avaliar a bioacumulção de metais pesados e suas interações ambientais com microplásticos e parâmetros físico e químicos em moluscos bivalves, foram realizadas as seguintes etapas para a consolidação da Tese, disposto na Fig. 8, a qual desenvolveu três artigos científicos acerca do tema inicialmente proposto e algumas de suas variações.

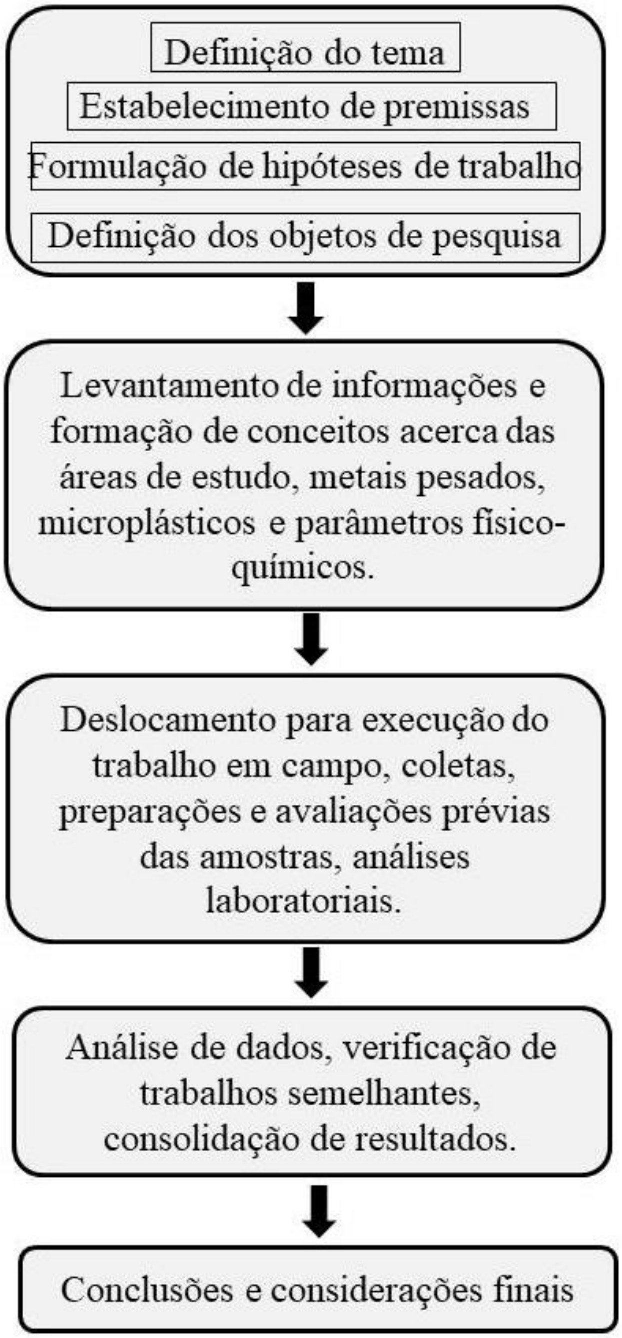


Fig. 8 – Fluxograma de realização da Tese.

Coleta

Baía de Vitória-ES

Para dois experimentos realizados na Baía de Vitória, visando o Monitoramento da Bioacumulação de Metais Pesados em mexilhões e ostras, foram utilizadas quatro estações, nomeadas como BMP1, BMP2, BMP3 e BMP4 e com suas localizações expressas por meio de coordenadas geográficas na Tabela 1.

Tabela 1 – Coordenadas e nomenclaturas das Estações Amostrais do Programa de Monitoramento de Bioacumulação dos Metais na Baía de Vitória – ES.

PONTOS DE COLETA		**COORDENADAS**	
		X	**Y**
Bioacumulação dos Metais	BMP1	S 20°14'56.4"	W 40°19'35.7"
	BMP2	S 20°19'32.1"	W 40°21'03.2"
	BMP3	S 20°19'22.3"	W 40°19'48.6"
	BMP4	S 20°19'17.4"	W 40°16'56.8"

Para a consecução do biomonitoramento e experimentos, um local de referência foi selecionado em uma fazenda marinha localizada no município de Anchieta, ES, Brasil (20º 48 '21 "S; 40º 38' 44" W), distante de qualquer complexo industrial. Visando confirmar baixos níveis de contaminação das amostras coletadas (Fig. 9a), um lote de organismos foi destinado para análises preliminares de quantificação dos metais presentes nos tecidos dos animais.

Após a confirmação de ausência de níveis elevados de metais nos animais deste local, três sacos contendo mexilhões adultos foram distribuídos em cada uma das estações de amostragem na Baía de Vitória (Fig. 9b), por 60 dias. Após esse período, as amostras foram coletadas e armazenadas sob refrigeração até o momento da análise.

O procedimento acima mencionado foi realizado três vezes por um ano, em triplicata para cada estação de amostragem. Os espécimes de mexilhões tiveram seus tecidos moles extraídos até a soma de 200g por estação de monitoramento (Fig. 9c). Essas amostras foram secas em estufa a 60ºC, moídas até um pó fino com um pilão de porcelana.

Para as coletas de ostras *Crassostrea rhizophorae* utilizadas no experimento 2, os animais foram amostrados na mesma campanha onde objetivou-se sua comparação com a bioacumulação de metais nos mexilhões. Entretanto, destaca-se a dificuldade de cultivos dessa espécie na região, o que direcionou os esforços às coletas em bancos naturais próximos às estações de cultivo e coleta.

Ressalta-se que com o intuito de garantir a exposição dos mexilhões e preservar a integridade das estruturas de cultivo, vistorias foram realizadas, em intervalos de 20 dias, onde se reforçou as fixações e realizou a limpeza de organismos incrustantes (epifauna) que por ventura se assentaram nas carapaças e nas redes das estruturas de cultivo. Devido à grande vazão e ataque de predadores em alguns pontos, algumas estruturas foram perdidas (Fig. 9d), mas como já se previa este problema e um número a mais de estruturas foram armazenadas, esta perda não prejudicou o resultado dos trabalhos.

Fig. 9 – (a) Mexilhões levados para a análise prévia de contaminação; (b) Estruturas de cultivo (sacos de germinação) de mexilhões *Perna perna*; (c) Tecido mole extraído de mexilhões *Perna perna*; (d) Estrutura de cultivo de mexilhões danificada, sem os organismos, resgatada em uma vistoria.

Ao mesmo tempo, amostras de água das camadas superficial e inferior foram coletadas na maré vazante, nas mesmas posições em que os organismos de monitoramento foram localizados. As amostras de água foram colhidas com uma garrafa Niskin. Os parâmetros da coluna de água (temperatura, salinidade, oxigênio dissolvido, pH e turbidez) foram analisados com uma sonda Horiba U10 multiparâmetros. Paralelamente, metais pesados também foram analisados em amostras de água.

Complexo Estuarino de Paranaguá-PR

As ostras foram coletadas em 10 locais ao longo do Complexo Estuarino de Paranaguá/PR, em comunidades dos municípios de Guaraqueçaba, Pontal do Paraná, Paranaguá e Antonina (Tabela 2). A coleta concentrou-se em locais importantes de cultivo, extração e comercialização das ostras, bem como os possíveis efeitos de poluentes em tais sítios.

Tabela 2 – Coordenadas e nomenclaturas das Estações Amostrais do biomonitoramento de Metais e Microplásticos no Complexo Estuarino de Paranaguá – PR.

PONTOS COLETA – LOCALIDADE		COORDENADAS	
		X	**Y**
K1	Guaraqueçaba Centro	25°17'57.51"S	48°19'53.38"O
K2	Guaraqueçaba – Ilha Rasa	25°20'10.22"S	48°23'10.23"O
K3	Laranjeiras	25°21'38.02"S	48°19'40.09"O
K4	Comunidade de Mariana	25°21'10.54"S	48°25'34.06"O
K5	Comunidade de Medeiros	25°22'32.66"S	48°27'12.85"O
K6	Ilha do Mel – Ponta Oeste	25°30'18.79"S	48°22'57.68"O
K7	Paranaguá – Comunidade de Europinha	25°27'38.18"S	48°36'28.34"O
K8	Antonina – Porto	25°27'31.10"S	48°40'23.75"O
K9	Paranaguá – Ilha do Teixeira	25°29'0.41"S	48°38'42.05"O
K10	Paranaguá – Ilha de Eufrasina	25°27'49.90"S	48°33'47.76"O

Destaca-se que na maioria dos locais foi realizada a coleta a partir de bancos naturais (Fig. 10a) e em outros as ostras foram provenientes de cultivos estabelecidos na região (K3, K5 e K6) (Fig. 10b). Em cada local foram coletadas ao menos 15 ostras com tamanho médio de 8 cm de altura (Fig. 10c e d), embaladas (Fig. 10e) e levadas à refrigeração.

Fig. 10 – (a) Coleta em bancos naturais no CEP; (b) Fazenda de cultivo de ostras nativas; (c e d) Ostras Crassostrea gasar coletadas em Paranaguá; (e) Ostras embaladas e prontas para serem colocadas na refrigeração.

Análises

As análises variaram de acordo com os experimentos e monitoramento previstos. Da mesma maneira, as análises estatísticas foram implementadas a partir da necessidade de relações e/ou comparações entre cada tipo de dado gerado e especificadas nos artigos científicos.

Metais na água, ostras e mexilhões – Baía de Vitória-ES

As amostras dos tecidos moles extraídos dos organismos foram analisadas pelos métodos USEPA 3051 e SMEWW 3120B (APHA 1992). Foi possível quantificar a concentração de metais (Al, Ba, Cd, Zn, Pb, Mn, Cu, Ni, Hg, Cr, Fe, As) presentes e acumulados nos tecidos dos organismos avaliados. Os limites de detecção para Al, Ba, Cd, Cu, Mn, Ni, Pb Zn Hg, Cr, Fe e As foram: 0,03, 0,005, 0,01,0,01, 0,01, 0,01, 0,03, 0,31,0,08 x10- 3, 0,002, 0,03, 0,005 mg/kg, respectivamente, e a recuperação variou de 98,45 a 104,5%. O material certificado também foi analisado (CRM NIST 2782).

Os mesmos metais avaliados na biomassa do organismo também foram analisados em amostras de água. Após agitação, as amostras foram transferidas para tubos de polipropileno de 50 mL para centrifugação a 3.000 RPM durante 5 min. Após filtração e acidificação com três gotas de HCl (preservação) e refrigeração, os metais pesados foram determinados usando um espectrômetro de emissão óptica de plasma acoplado indutivamente (ICP-OES, marca Jobin Yvon Horiba).

Metais e microplásticos em ostras no Complexo Estuarino de Paranaguá-PR

A preparação prévia dos moluscos consistiu na utilização de 10 indivíduos por cada um dos 10 locais amostrados (K1-K10). Em duplicata, visando atender a quantificação de metais e visualizar a presença de microplásticos, as ostras foram abertas, retirados 150 mg do hepatopâncreas, mesclados e homogeneizados.

Metais

Para a quantificação dos elementos metálicos (Al, Ba, Cd, Zn, Pb, Mn, Cu, Ni, Hg, Cr, Fe, As), foi realizado o procedimento de digestão das amostras em forno micro-ondas com a adição de 2,0; 1,0 e 4,0 mL de HNO3, H2O2 e H2O, respectivamente. O gás argônio com uma pureza de 99,996% foi utilizado como o gás principal, auxiliar e gás nebulizador (Air Liquide) no Espectrômetro de Massa com Fonte de Plasma (ICP-MS).

Microplásticos

Para a avaliação da presença dos microplásticos, também foi realizado o processo de digestão dos tecidos das ostras. Semelhante a Rochman et al. (2015), as amostras foram tratadas com KOH (10%, 3 × volume de tecido), incubados com agitação a 60 ° C por 48 horas (Fig. 11a). Em seguida, as amostras digeridas foram colocadas em provetas de 100 ml, adicionado NaCl até o limite das provetas e mantidas em repouso por 15 minutos (Fig. 11b) (aguardando os compostos orgânicos decantarem e os MPs acumularem-se na superfície por conta da diferença de densidade dessas substâncias) e submetido 50 ml da superfície ao processo de filtração em membranas de nitrato de celulose 8 µm (Fig. 11c). Por fim, as amostras foram levadas à estufa por 30 minutos para secagem e posterior visualização e identificação de MPs na Lupa (Fig. 11d).

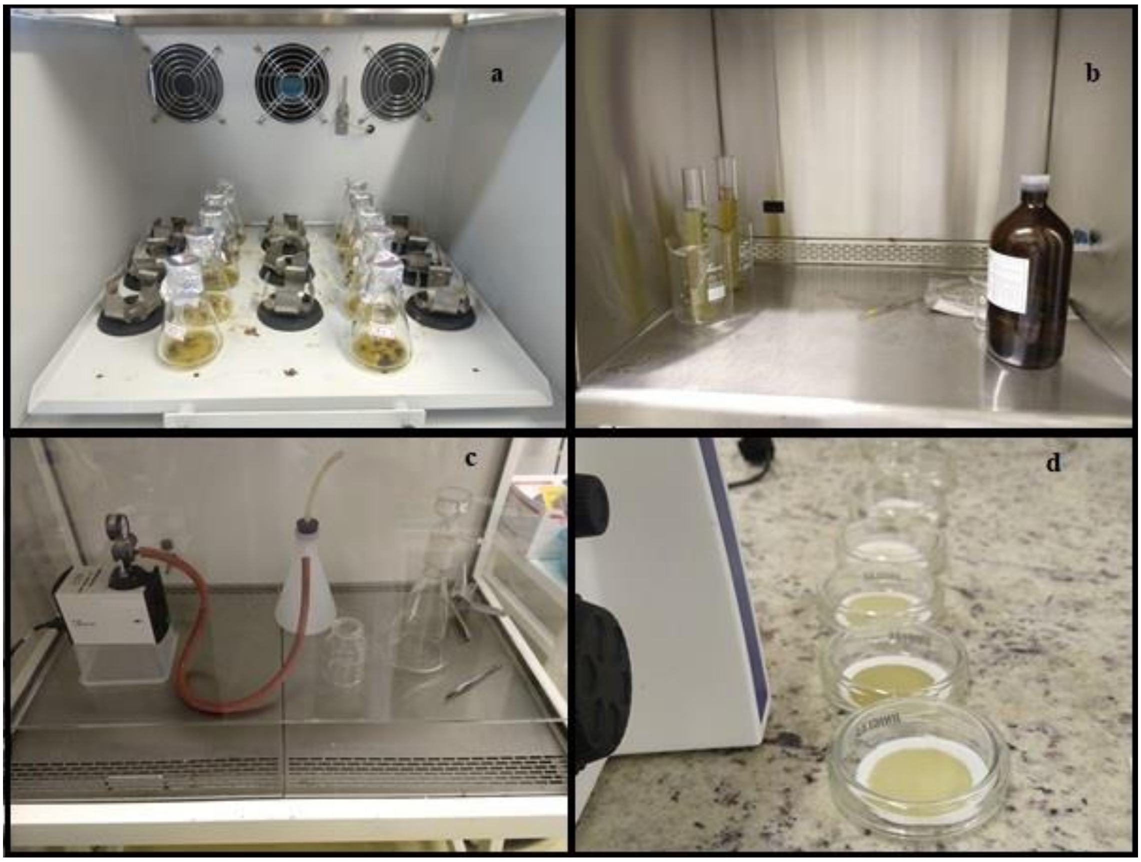


Fig. 11 – (a) Incubação das amostras para processo de digestão; (b) Adição de NaCl prévio à filtração; (c) Montagem sistema de filtração; (d) Amostras filtradas e prontas para serem observadas no Microscópio.

A identificação visual dos MPs foi baseada em estudos prévios, de acordo com o tamanho, formato e coloração (Shim et al., 2017; Abbasi et al., 2018; Naji et al., 2018; Neto et al., 2019;). As partículas com atributos compatíveis aos MPs foram retiradas individualmente das membranas de filtração por meio de pipetas (Fig. 12) e dispostas em stubs (Fig. 13) e levadas ao Microscópio Eletrônico de Varredura com o Energy Dispersion Spectroscopy acoplado (MEV/EDS), para identificação e quantificação dos elementos químicos presentes, confirmando a estrutura dos MPs e os possíveis metais adsorvidos.

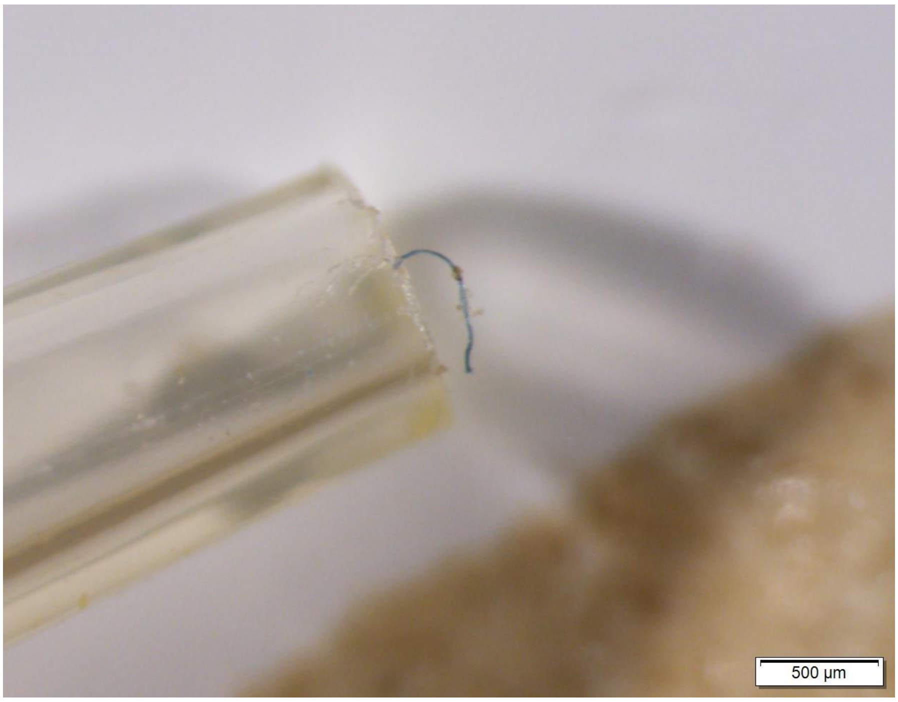


Fig. 12 – Fibra de microplástico sendo retirada da membrana de filtração para transferência aos stubs.

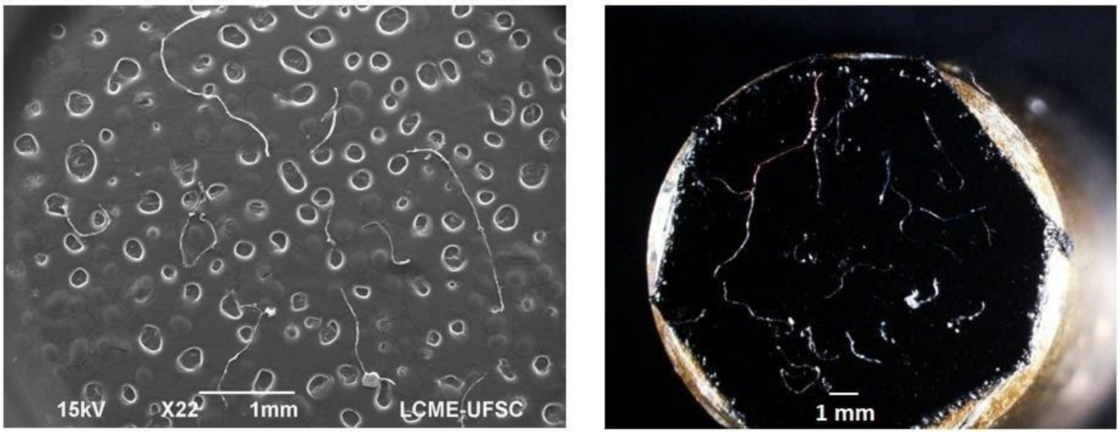


Fig. 13 – Visualização de microplásticos colocados em stubs a partir de MEV (esq.) e lupa (dir.).

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ARTIGO 1 – EVALUATION OF BIOAVAILABILITY OF TRACE METALS AND ARSENIC THROUGH BIOINDICATORS IN A URBANIZED ESTUARINE SYSTEM IN SOUTHEAST BRAZIL.

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PUBLICADO EM JANEIRO DE 2021 NA REVISTA *ENVIRONMENTAL MONITORING AND ASSESSMENT.*

Resumo

O mexilhão *Perna perna* é um dos bioindicadores mais utilizados em zonas costeiras e a espécie mais explorada economicamente no Brasil através da maricultura. No presente estudo, *Perna perna* foi utilizado para investigar a poluição por metais na área estuarina da Baía de Vitória. Quatro pontos de amostragem foram localizados ao longo de um braço estuarino da Baía de Vitória e as estações foram amostradas durante três campanhas. Foram avaliados metais traços nos tecidos de *P. perna*, metais traços dissolvidos e outras variáveis auxiliares na coluna d'água. As concentrações de Cd, Pb, Cu, Ni e Fe dissolvidos superaram os limites de tolerância estabelecidos pela legislação em todas as campanhas de amostragem. *Perna perna* apresentou concentrações em desacordo com a legislação brasileira para Cr e As. Uma tendência geral de maiores concentrações nas estações externas foi observada para a maioria dos metais, o que sugeriu a ocorrência de processo de floculação no estuário inferior, reduzindo as concentrações dos elementos dissolvidos e aumentando sua biodisponibilidade para a biota através da forma particulada. O Cd se destacou com elevadas concentrações na fração dissolvida, mas não detectado em *P. perna*, provavelmente devido à formação do complexo de cloro sob influência de águas mais salinas. Al, Ba, Mn, Fe, Cu, Zn, Ni, Cr, Pb e As foram considerados biodisponíveis, uma vez que se acumularam nos tecidos dos mexilhões. O Índice de Perigo (HI) e o Risco Alvo de Câncer (TCR) mostraram que os mexilhões oferecem riscos à saúde quando consumidos, sendo o ferro e o arsênio os principais contribuintes para os altos índices.

Palavras-chave: metais traço, bioindicadores, *Perna perna*, estuários, Baía de Vitória, poluição.

Abstract

The mussel *Perna perna* is one of the most used bioindicators of coastal areas and the most economically exploited species in Brazil through mariculture. In the present study, *Perna perna* was used to investigate metal pollution in the estuarine area of Vitória Bay. Four sampling sites were located along an estuarine branch of Vitória Bay and stations were sampled during three campaigns. Trace metals in the tissues of *Perna perna* were evaluated as well as dissolved trace metals and other ancillary variables in the water column. Dissolved Cd, Pb, Cu, Ni and Fe concentrations surpassed the tolerance limits stablished by legislation in all the sampling campaigns. *Perna perna* exhibited concentrations in disagreement with the Brazilian legislation for Cr and As. A general trend of higher concentrations in outer stations was observed for most metals, what suggested the occurrence of flocculation process in the lower estuary, reducing the concentrations of dissolved elements and increasing their bioavailability for the biota through the particulate form. Cd was highlighted with elevated concentrations in dissolved fraction but not detected in *Perna perna*, probably due to chlor-complex formation under influence of more saline waters. Al, Ba, Mn, Fe, Cu, Zn, Ni, Cr, Pb and As were considered bioavailable, once they were accumulated in the mussels' tissues. Hazard Index (HI) and Target Cancer Risk (TCR) showed that the mussels offer health risk issues when consumed, being iron and arsenic the main contributors for the high indexes.

Keywords: trace metal, bioindicators, *Perna perna*, estuaries, Vitória Bay, pollution.

Introduction

Brazil is among the 15 largest aquaculture producers in the world, with a production of approximately 562 thousand tons per year (FAO, 2018). Bivalve production was estimated in 20,800 tons/yr, generating approximately US$ 25 million. The main species produced in Brazil are: brown mussel (*Perna perna*), pacific oyster (*Crassostrea gigas*) and scallop (*Nodipecten nodosus*) (Pereira and Rocha 2015).

In addition to their commercial importance, many of the commercially exploited aquatic organisms can be used to demonstrate the effects of exposure to environmental contaminants as well as their toxicity (Pereira et al. 2006). Bivalve animals, such as mussels, are worldwide abundant, sessile and easily collected (Phillips, 1991). Mussels are also filter-feeding organisms, which make them ideal to evaluate the uptake of contaminants, since they filter a large amount of water and suspended particulate matter and can bioaccumulate several types of contaminants, including trace metals (Waykar and Deshmukh, 2012; Birch and Apostolatos, 2013).

Trace metals and metalloids reach aquatic ecosystems naturally, through geochemical weathering, or through anthropogenic domestic and industrial activities (Ramesh and Damodhram 2016). In the case of anthropogenic activities, the contamination by metals may originate from diffuse sources such as atmospheric diffusion, solid deposition and soil leaching, particularly in agricultural areas, or point sources characterized mainly by industrial effluent emissions, sewage household, garbage disposal and mining residues (Campos, 2002). Trace elements such as cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), mercury (Hg), nickel (Ni), and zinc (Zn) and metalloids like arsenic (As) can accumulate in marine organisms (Borak and Hosgood 2007; Mok et al. 2010).

Trace metals and metalloids bioaccumulation can be used in order to elucidate the aquatic behavior of environmental contaminants, once such elements from sea water and marine sediment can be accumulated by *Perna perna*, through their filter-feeding habits (Diop et al. 2016).

The aim of the present study was the evaluation of bioaccumulation of trace metals in the tissues of mussel *Perna perna* at sites located in the estuarine system of Vitória Bay, in order to investigate the potential contamination of waters and sediment from this coastal urbanized area of strong economic importance, where mariculture zones play a key role in the local economy.

Materials and Methods

Sampling

For the biomonitoring scope, a reference site was selected at a marine farm located in the city of Anchieta, ES, Brazil (20º 48' 21'' S; 40º 38' 44'' W), which is distant from the influence of any industrial complexes (Fig. 14). To confirm the lack of contamination by metals and metalloids in the collected specimens, a batch of organisms was submitted to a preliminary analysis to investigate if these elements were present in the animals' tissues.

After the confirmation that the mussels were not contaminated by trace metals and metalloids, the organisms were distributed in four sampling stations along the estuarine system of Vitoria Bay (Fig. 14), for 60 days. After that time, the samples were collected and stored under refrigeration until the arrival at the laboratory. This procedure was repeated three times, in October/2015 (Campaign 1); June/2016 (Campaign 2) and May/2017 (Campaign 3) for each sampling station.

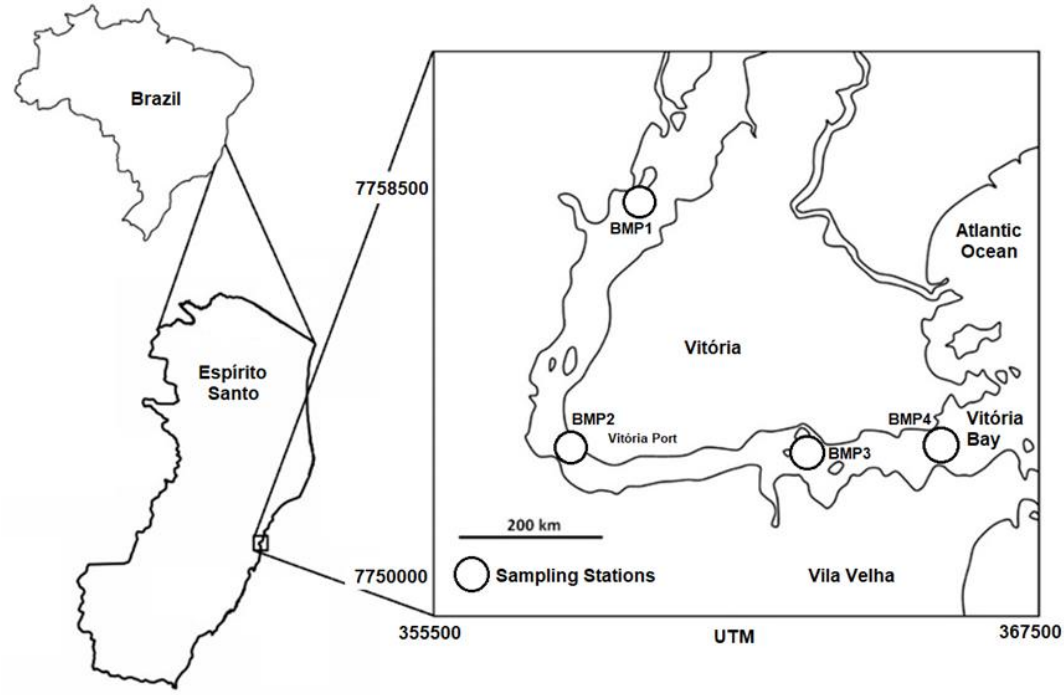


Fig. 14 – Study area and sampling stations: BMP1 (S 20°14'56.4"; W 40°19'35.7"), BMP2 (S 20°19'32.1"; W 40°21'03.2"), BMP3 (S 20°19'22.3"; W 40°19'48.6") and BMP4 (S 20°19'17.4"; W 40°16'56.8").

Water samples from surface and bottom layers were collected during ebb tide, in the same stations where the monitoring organisms were positioned, with a total depth of approximately 2 m. Water samples were obtained with a Niskin bottle. Aliquots for the determination of trace metals were stored in 50 ml polypropylene bottles previously decontaminated with HNO_3 10% (v/v) and rinsed with Milli-Q water, and samples were acidified with concentrated HNO_3 for preservation until the moment of analysis. The water column variables, temperature, salinity, dissolved oxygen (DO), pH, total suspended solids (TSS) and turbidity, were measured *in situ* with a multiparameter Horiba U10 probe.

Laboratory Analysis

In the laboratory, water samples for the determination of trace metals were vacuum filtered with 0.45 µm cellulose membranes. Trace metals were determined in the filtrate by inductively coupled plasma optical emission spectrometer (ICP-OES, brand Jobin Yvon Horiba) according to SMEWW 3120B (APHA 1992) method.

Mussels specimens had their soft tissue extracted up to the sum of 200 g per monitoring station. The samples were oven-dried at 40°C, grounded to a fine powder with a porcelain pestle and mortar. Trace metals in the soft tissues extracted from the organisms were analyzed using the method proposed by USEPA 3051a. All glassware was washed with neutral Extran® and HNO_3 10% (v/v) and rinsed with Milli-Q water. Wet digestion was made through the addition of 4.5 ml of HNO_3 and 1.5 ml of HCl to approximately 0.250g of sample and extraction proceed in a Microwave 3000, Anton Paar®. After the extraction in the microwave, samples were filtered in 0.45 µm cellulose membranes and the filtrate was completed to 35 ml with Milli-Q water. The determined metals in the tissues of the selected organisms were Al, Ba, Cd, Zn, Pb, Mn, Cu, Ni, Hg, Cr, Fe, As. CRM NIST 2782 was used as certified material for metals and arsenic analysis in each batch of samples and the recovery ranged from 98.45 to 104.5%. The detection limits for Al, Ba, Cd, Cu, Mn, Ni, Pb Zn Hg, Cr, Fe and As were: 0.03, 0.005, 0.01,0.01, 0.01, 0.01, 0.03, 0.31,$0.08x10^{-3}$, 0.002, 0.03, 0.005 mg/kg respectively.

Data treatment

For the evaluation of dissolved metals and metalloids, the Brazilian guideline CONAMA 357/2005 was used. Trace metal values in the organisms' tissues were evaluated based on the Maximum Tolerance Limit established in the Brazilian legislation (Decree nº 55.871/1965) for human consumption purposes. However, it is important to highlight that the refereed decree does not include all the metals determined in the present study. For the evaluation of limits of tolerance that were not included in the Brazilian legislation Decree 55.871/65, the standards of the USEPA (1997) World Health Organization (WHO 1993) were also used.

Human health risk assessment was used the following indexes: Estimated Daily Intake (EDI); Target Hazard Quotient (THQ); Hazard Index (HI) and Target Cancer Risk (TCR). All the indexes were calculated concerning average level (ALM) and high level (HLM) mussel consumers. The estimated values for ALM and HLM consumers used were the ones established by USEPA (2004), respectively 17.86g/day and 35.71 g/day, considering 60 kg body weight for an adult. Concentrations of metals and arsenic used in the calculation were the ones obtained from wet weight of the mussels (ww).

Estimated Daily Intake was calculated through the following formula:

EDI= (MC x *Consumption rate)/body weight*

The evaluation of EDI in mussels was made using the oral reference dose (ORD) in µg/day/kg body weight proposed by USEPA (2015) for Al (1000); Ba (200); Zn (300); Mn (140); Cu (40); Ni (20); Cr (3); Fe (700) and As (0.3). No ORD was established for lead, since USEPA did not find a threshold value below which no adverse effects were observed.

Target Hazard Quotient was obtained based on the calculation described by USEPA (2000), as follows:

$$THQ = \frac{EFxEDxCRxMC}{ORFxABWxAET}\ x\ 10^{-3}$$

where:

EF = exposure frequency (365 days/year)

ED = exposure duration (70 yrs)

CR = consumption rate (ALM = 17.86 g/day; HLM = 35.71 g/day)

MC = metal concentrations in mussels (ww)

ORF = oral reference dose for each element

ABW = average body weight (and adult of 60 kg)

AET = average exposure time (365 days x 70 yrs)

Hazard Index considers that the consumption of a particular food type corresponds to the simultaneous exposure to several potentially toxic elements. Therefore, even if THQ is <1, the sum of the individual THQ could cause a combined effect that could lead to adverse health effects. HI consists in the sum of the individual target hazard quotient for each element analyzed:

$$HI = \sum_{i=1}^{n} THQi$$

Target Risk Cancer ascertains the probability of excess cancer risk along one's lifetime of exposition to a potentially toxic element. An oral slope factor established by USEPA is used. In the case of arsenic the oral slope factor is 1.5 mg/kg/day.

$$TCR = \frac{EF\ x\ \ ED\ x\ CR\ x\ MC\ x\ CPSo}{ABW\ x\ AETc}\ x\ 10^{-3}$$

where:

EF = exposure frequency (365 days/year)

ED = exposure duration (70 yrs)

CR = consumption rate (ALM = 17.86 g/day; HLM = 35.71 g/day)

MC = metal concentrations in mussels (ww)

CPSo = oral slope factor

ABW = average body weight (and adult of 60 kg)

AET = average exposure time to carcinogen (365 days x 70 yrs)

The software Statistica 7.0® was used to perform the non-parametric Kruskal-Wallis test with 95%confidence level ($p<0.05$) in order to evaluate significant differences among the stations and campaigns concerning trace metal and metalloids concentrations as well as ancillary variables.

Results and Discussion

Table 3 presents the mean values of the physico-chemical variables registered in the water column during the three sampling periods.

Table 3 – Mean values (N=16) and standard error of temperature, pH, salinity, dissolved oxygen, turbidity and total suspended solids.

Sample area	Temperature (ºC)	pH	Salinity	DO (mg/L)	Turbidity (NTU)*	TSS (mg/L)
BMP_1	23.10±0.57	6.77±0.11	25.10±0.74	6.03±0.13	21.25±2.03	11.58±2.22
BMP_2	22.70±0.24	6.50±0.18	27.10±0.79	6.06±0.12	24.43±4.15	10.33±0.94
BMP_3	22.93±0.24	6.43±0.16	29.69±0.31	6.43±0.16	18.19±0.96	15.75±2.96
BMP_4	22.82±0.24	6.65±0.11	30.84±0.46	6.23±0.04	14.94±0.74	11.25±1.21

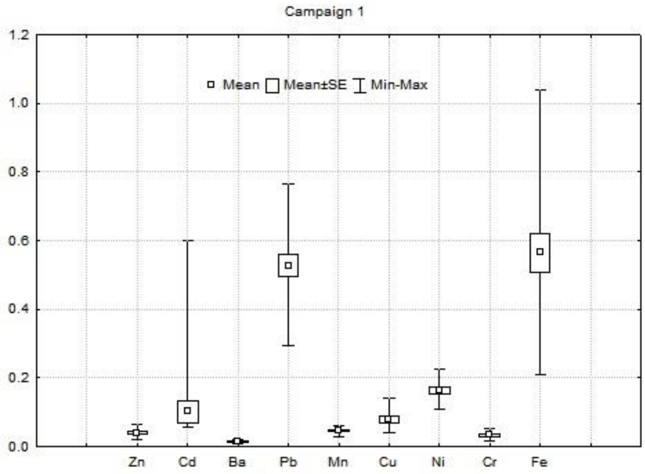
According to Boening (1999), the bioavailability of pollutants, like trace metals, depends on geochemical and biological factors. Estuaries, environments where the mixing of fresh and salt water occurs, show great peculiarity in the dynamics of remobilization of metals (Noegrohati 2005). According to Noegrohati (2005), high salinity environments favor the mass increase of total suspended solids composed of clay and organic matter, and these particles suffer decantation. In the present study, salinity varied between 25.1 and 30.8 with significant statistical difference among the sampling stations ($p <0.05$). Stations closer to the sea (BMP_3 and BMP_4) had higher salinity values, suggesting the influence of oceanic waters.

Temperature varied between 22.7 and 23.1C° showing no significant difference among the sampling stations, even with an indication of lower values in the station BMP_4. In seawater, temperature also influences numerous physiochemical factors, with changes in the chemical speciation of metals (Byrne et al. 1988). Mubiana and Blust (2007) showed that in cases of temperature variation, the accumulation of some metals by mussels showed a positive relationship between temperature and metal uptake. Temperature can also influence the variation of pH (Byrne et al. 1988). The pH of Vitoria bay presented small variation, between 6.43 and 6.77 (Table 3), corroborating the data obtained by Jesus et al. (2004) in the estuarine system of Vitória Bay.

Water was considered well oxygenated at all the monitored stations (Table 3) with little variations and no significant differences among the stations. Turbidity values presented significant differences among the stations (p< 0.05) and decreased towards Vitória Bay, pointing to the influence of marine waters at BMP$_4$ where the lowest value was registered. Total suspended solids showed no significant differences (p>0.05) among the stations. It should be emphasized that suspended solids are considered a potential source of trace metals for the marine environment (Pfeiffer 1980; Gregori et al. 1996), usually presenting significant statistical correlation with the growth of mollusks (Santos et al. 2008).

Fig. 15 presents the results of dissolved metal concentrations and arsenic for the three campaigns consolidating the four sampled stations. Aluminum, arsenic and mercury presented values below the detection limit. Zinc, manganese and barium also presented values below detection limits in campaign 3.

Significant differences were identified for trace metals concentrations among the three campaigns for Cd ($p<0.05$) and Pb ($p<0.05$). For Zn, Ba and Mn, which were only detected in the first two campaigns, no significant differences were observed between the samplings.



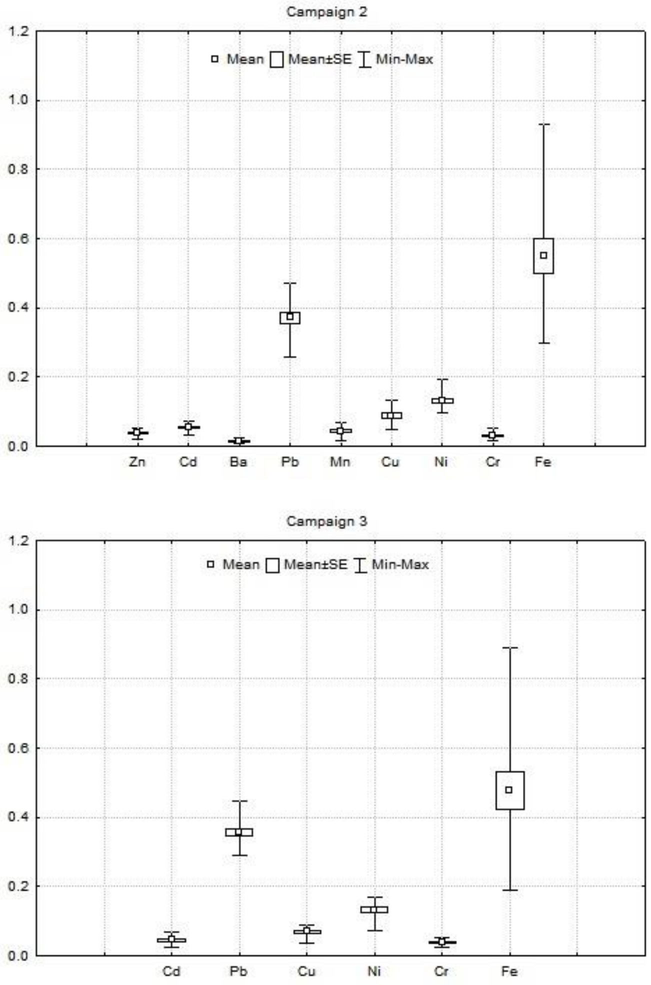


Fig. 15 – Basic statistics (N=16) for dissolved concentrations (mg/l) of zinc (Zn), cadmium (Cd), barium (Ba), lead (Pb), manganese (Mn), copper (Cu), nickel (Ni), chromium (Cr) and iron (Fe) in the water column of the estuarine system of Vitória Bay.

Cadmium, Pb, Cu, Ni and Fe concentrations in water column surpassed the limits stablished by CONAMA 357/2005 (Table 4), in the three campaigns in every station, probably a result of a continuous anthropogenic input in Vitória Bay's estuarine waters. Some anthropogenic sources of trace metals include agriculture, inputs of domestic and industrial sewage, accidents containing chemical residues that suffer runoff from continents to the aquatic systems (Velusamy et al., 2014).

The concentration of Fe in water ranged between 0.19 and 1.04 mg/l varying significantly among the stations ($p<0.05$) and presenting higher averages at stations BMP2 and BMP3. Concentrations of Fe in the bottom layers, were significantly higher than the ones on the surface ($p<0.05$) what can be associated remobilization of bottom sediments, especially in BMP$_3$, the port area (Fig. 14). Iron and Pb presented the most elevated concentrations in every campaign, and Cd also presented very elevated concentrations in the first campaign (Fig. 15). The high values registered for dissolved metals are related to the presence of mining and steel industries that are located in areas near the bay.

Table 4 – Maximum concentrations of trace metals (mg/L) in water established by CONAMA 357/2005.

Zn	Cd	Ba	Pb	Mn	Cu	Ni	Cr	Fe
0.09	0.005	1.0	0.01	0.1	0.005	0.025	0.05	0.3

Trace metals in the water column can be absorbed by filter-feeding organisms through the soluble fraction, but these animals also remove particles from the water column (Rainbow, 2002, Birch and Apostolatos, 2013). Metals in the dissolved fraction can be absorbed by primary producers which feed a variety of herbivorous, such as mussels. Besides that, after deceased these organisms can be consumed by detritivores and incorporated into the trophic chain (Mishra et al., 2008). Usually filter-feeding organisms such as mussels and oysters are considered complex matrices and have a limited metabolic activity, and the accumulation of pollutants in their tissues make them ideal pollution bio-indicators (Kasiotis and Emmanouil, 2015).

Fig. 16 shows the concentrations of trace metals in the dry tissues of *Perna perna* in the estuarine system of Vitória Bay. No significant differences for metal concentrations were found among the campaigns, except for Cu ($p<0.05$). Among the stations the differences in trace metal concentrations were not considered significant ($p>0.05$).

The Brazilian legislation Decree Decree 55.871/65 was used as guideline for the evaluation of trace metals and arsenic found in *Perna perna*. Overall, it was noticeable that the organisms collected from stations closer to Vitória Bay (MPB_3 and MPB_4) presented the higher concentrations of metals. This is probably due to flocculation effects caused by the mixing of marine and fresh waters, increasing the concentration of metals in the particulate form and uptake of these particles by the primary consumers. Cadmium and Hg were below the limit of detection (1 and 0.5 mg/kg, respectively).

Regarding Al in the water column, which was not detected, Kadar et al. (2002) described that this metal is commonly found in rivers and lakes, especially in the particulate form, what could corroborate the fact that dissolved aluminum was not detected in the present study. Nevertheless, the concentrations of aluminium in *Perna perna* (Fig. 16) were detected (4.72 and 193.87 mg/kg) and were probably incorporated through Al adsorbed onto particulate material. No significant differences were observed among the campaigns ($p>0.05$). Specific sources of Al in marine areas are usually related to urban runoff and acid sulfate soils, mining activities throughout the drainage basin, among others. The Al results presented here are smaller than the ones described by Campolim et al. (2017), who also found high concentrations of this metal in *Perna perna* specimens in Santos Bay, a very polluted estuarine area in southeast Brazil (Table 5).

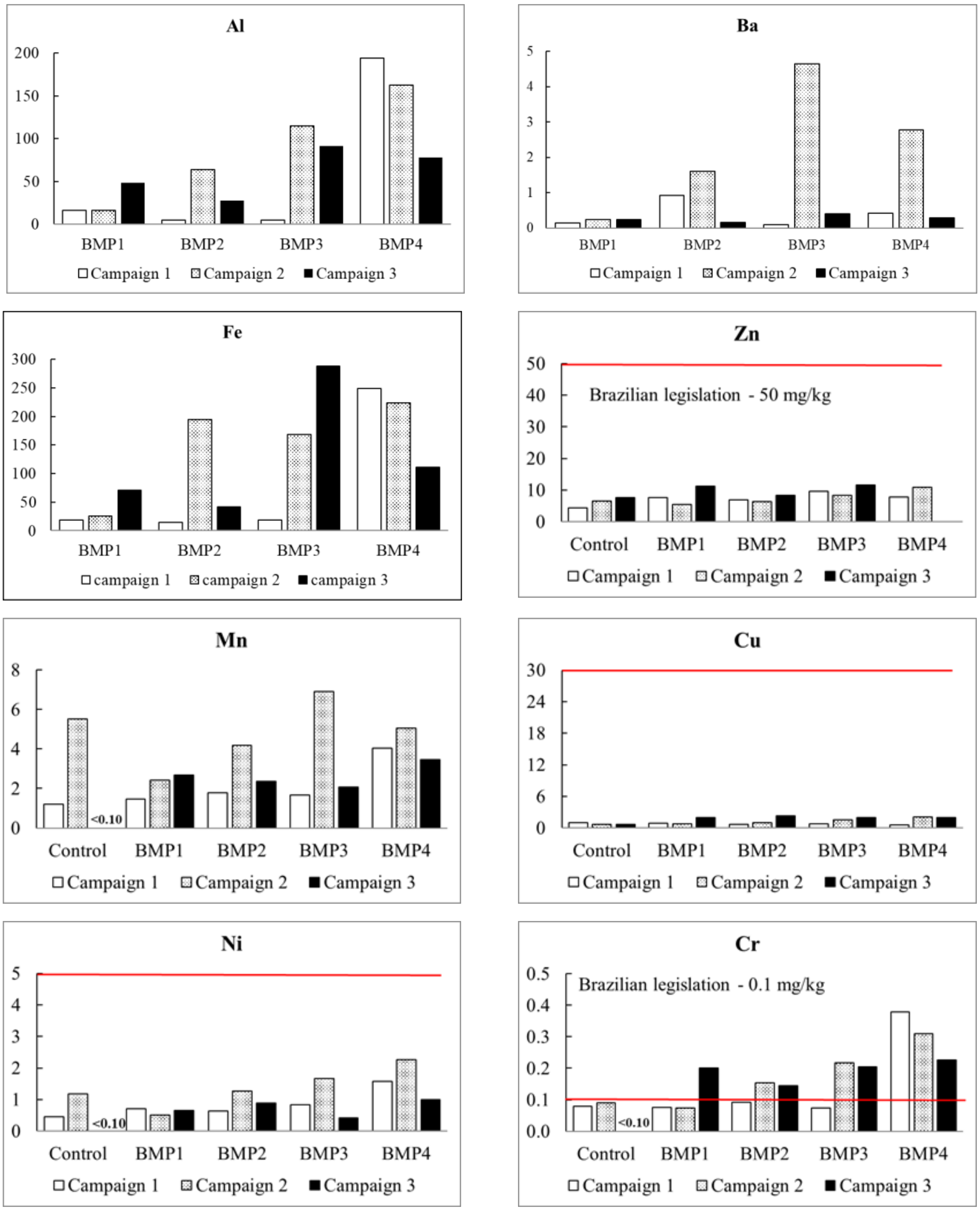
Barium concentrations in the dry tissue of *Perna perna* varied from 0.10 to 4.65 mg/kg (Fig. 16). Studies that monitor Ba through marine species are scarce, however higher concentrations of Ba in *Perna perna* were also registered in the most external stations (BMP_3 and BMP_4), especially in the second campaign (Fig. 16). Bioavailable Ba for incorporation into bivalve shells typically comes from two environmental pools: food and water (Stecher et al. 1996). Lira et al. (2011) mentioned that the association of Ba with sea salts demonstrates a high solubility and low bioavailability, which was not confirmed with the data obtained in the present study, since barium uptake was higher in the stations with more saline waters (Fig. 16), however this could be associated with the ingestion of Ba in the particulate form.

Iron varied from 14.14 to 248.49 mg/kg and no significant differences were observed among the three campaigns ($p>0.05$). Brazilian legislation stablishes no tolerance limit for Fe. The World Health Organization (WHO, 1993) sets a daily limit value for human consumption of 0.8 mg/kg, a value much lower than the ones found in the mussels from the present study (14.14-248.49 mg/kg) suggesting a potential risk of intoxication by Fe. Concentrations of Fe in mussels above the value recommended by WHO (1993) for daily consumption have been reported in several studies from southeast Brazil (Table 5), the most industrialized and developed part of the country, indicating the anthropogenic influence on the seafood products from this region. Kumar et al. (2015) found Fe concentrations extremely elevated (150.77-1,964.34 mg/kg) in *Perna perna* mussels from Vitória Bay corroborating the elevated values found in the present study and revealing the strong anthropogenic influence on the study area. According to Turner (1998), all the dissolved Fe in rivers occurs in the colloidal form and a significant part of dissolved iron in estuaries is through colloidal aggregation, what could justify higher concentrations of Fe in *Perna perna* mussels collected from the most saline stations.

According to Wuana and Okieimen (2011), the main issue of natural zinc is by erosion and among their anthropogenic sources are fertilizers, pesticides and biosolids. Zinc concentrations found in mussels were below the limit established by the Brazilian legislation (50 mg/Kg), reaching minimum and maximum values of 5.47 and 11.56 mg/kg, respectively (Fig. 16). Zinc concentrations in mussels can be very high as the one found in a *Perna perna* (175.1 mg/kg) by Sokolowski et al. (2004) (Table 5). Zn concentrations in *Perna perna* from Vitória Bay corroborated the low values of this element found in the water (Fig. 15) without significant differences among the campaigns ($p>0.05$).

For copper the maximum concentration allowed in aquatic organisms for human consumption is 30 mg/kg according to the Brazilian legislation Despite the high concentrations of dissolved Cu, during the three campaigns (Fig. 15), the concentrations of this metal in the dry tissue of mussels were considered low (0.60-2.29 mg/kg). Except for BMP_4, campaign 3 presented the highest Cu concentration (Fig. 16). High concentrations of Cu in *Perna perna* mussels were registered in the coastal area of southeast Brazil, as shown by Lino et al. (2016) in Table 5.

As observed for dissolved concentrations, significant differences in Ni contents in the mussels were not observed among the stations ($p>0.05$). It should be emphasized though that, dissolved nickel has a strong tendency to adsorb to suspended solids (Turner et al., 1998), and this should be one of the main sources of this element for filter-feeding organisms like *Perna perna*. The fact that higher Ni concentrations were registered in the most external stations sustain the hypothesis of flocculation and subsequent ingestion of particulate Ni by filter-feeding organisms. Hassani et al. (2017) has described a good removal of Ni through flocculation process in estuarine conditions, compared to other metals, highlighting that dissolved oxygen and redox conditions play a key role in this process.


Al
200
150
100
50
0
BMP1
BMP2
BMP3
BMP4
□Campaign 1 ▨Campaign 2 ■Campaign 3
Ba
5
4
3
2
1
0
BMP1
BMP2
BMP3
BMP4
□Campaign 1 ▨Campaign 2 ■Campaign 3
Fe
300
250
200
150
100
50
0
BMP1
BMP2
BMP3
BMP4
□campaign 1 ▨campaign 2 ■campaign 3
Zn
50
40
30
20
10
0
Brazilian legislation - 50 mg/kg
Control
BMP1
BMP2
BMP3
BMP4
□Campaign 1 ▨Campaign 2 ■Campaign 3
Mn
8
6
4
2
0
<0.10
Control
BMP1
BMP2
BMP3
BMP4
□Campaign 1 ▨Campaign 2 ■Campaign 3
Cu
30
24
18
12
6
0
Control
BMP1
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BMP4
□Campaign 1 ▨Campaign 2 ■Campaign 3
Ni
5
4
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2
1
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Control
BMP1
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BMP3
BMP4
□Campaign 1 ▨Campaign 2 ■Campaign 3
Cr
0.5
0.4
0.3
0.2
0.1
0.0
Brazilian legislation - 0.1 mg/kg
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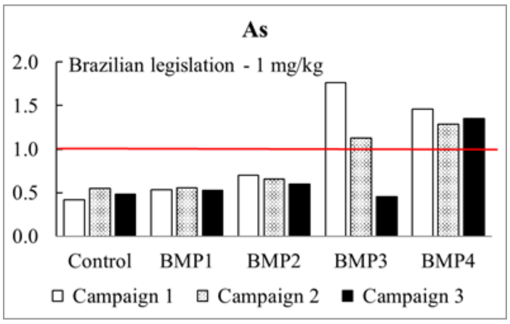

Fig. 16 – Concentrations of Al, Ba, Fe, Zn, Mn, Cu, Ni, Cr and As in *Perna perna* (mg/kg, dry weight) in the estuarine system of Vitória Bay and reference values from Brazilian legislation (Decree 55.871/65) (red lines indicates legislation limits).

Manganese concentrations in mussels varied from 1.47 to 6.92 mg/kg (Fig. 16), however, the Brazilian legislation establishes no limit for Mn in food. A study conducted by USEPA (2003) described concentrations of Mn in fruits and vegetables around 40-50 mg/kg, and values between 0.16 to 0.31 mg/kg in tuna fish. Considering that Mn is a nutrient present in the human diet, the values obtained for *Perna perna* in the present study should not pose toxic effects. Joksimovic et al. (2011) identified a wide variation in the concentration of Mn in Southeastern Adriatic Sea with the mussel *Mytilus galloprovincialis*, with similar values to the ones presented in this study on the Brazilian coast with *Perna perna*. Lino et al (2016) and Campolim et al. (2017) also found similar concentrations of Mn in *Perna perna* in the coasts of Rio de Janeiro and Santos, respectively (Table 5). For most stations concentrations of Mn were higher during campaign 2, and highest values were registered in the most external stations (BMP_3 and BMP_4).

Chromium concentrations varied between 0.07 and 0.38 mg/kg surpassing the established limit by the Brazilian legislation in the three campaigns (Fig. 16). The highest concentrations were registered in the most external stations and significant differences were observed among the stations ($p<0.05$), with the highest concentrations registered in BMP_4. Corroborating the results of the present study, Christophe Joyeux et al. (2004) identified Cr contamination in estuarine fishes from Vitória Bay. The contamination of mussels with Cr appears frequently in studies from the coastal area of Brazil as show by Table 5. Chromium is not found in a free form in nature, but mostly in the composition of oxides, sulfates, chromates, dichromate, basic salts and, in elemental form, overlaying metal and plastic parts in the processes of surface treatment (Pfeiffer 1980), what may be a significant anthropogenic source in port areas and justify the contamination of marine organisms from coastal areas.

Arsenic concentrations varied between 0.52 and 1.76 mg/kg, surpassing the guideline from the Brazilian legislation in the most external stations (Fig. 16). The difference in As concentrations among the stations was considered significant ($p<0.05$), with the highest values registered in BMP_4, except for campaign 1 when As was highest at BMP_3. In the coast of São Paulo state – Brazil, As concentrations above the tolerance limit established by the Brazilian legislation were also observed in *Perna perna* (Catharino et al. 2008). Arsenic tends to accumulate in bottom sediments and can be mobilized depending on physico-chemical processes and reach the aquatic biota, such as mussels (Magalhães et al. 2001). This explains why marine organisms tend to accumulate more arsenic than those living in freshwater or terrestrial environments (WHO 1993).

Lead in *Perna perna* was detected only in campaign 1 at BMP_4 with a concentration of 0.76 mg/kg and in campaign 2 in BMP_2, BMP_3 and BMP_4 with the respective values of 0.95; 0.85 and 0.91 mg/kg. All results of Pb in *Perna perna* were in accordance with the Brazilian legislation (2.0 mg/Kg). Studies conducted in the coastal area of southeast Brazil also presented very low concentrations of Pb, or it was not detected at all (Table 5), except for Campolin et al. (2017) who found higher concentrations of this metal in *Perna perna* (Table 5). Results of Pb in *Perna perna* are low in spite of the high concentrations of this metal registered in the dissolved fraction. Hasani et al. (2017) described the low effectiveness of Pb removal through flocculation in estuaries and a conservative behavior of this element along a salinity gradient. This behavior could justify the elevated concentration of Pb in dissolved fraction opposed to low concentrations in *Perna perna* from Vitória Bay.

Table 5 – Average concentrations of heavy metals (mg/kg dry weight) determined in the soft tissue of bivalve molluscs, collected from different coastal areas of the world.

Reference	**Specie**	**Location**	**Al**	**Ba**	**Cd**	**Zn**	**Pb**	**Mn**	**Cu**	**Ni**	**Hg**	**Cr**	**Fe**	**As**
Rezende and Lacerda (1986)	*Perna perna*	Guanabara Bay/RJ, Brazil	-	-	-	-	-	-	14.5	-	-	-	171.5	-
Carvalho et al. (1998)	*Perna perna*	Macaé/RJ, Brazil –	-	-	0.3	-	-	-	5.1	-	-	1.3	675	-
Ferreira et al. (2004)	*Perna perna*	Rio de Janeiro, Brazil	-	-	0.03 – 0.4	-	-	-	0.86 - 6	-	-	0.24 – 1.7	170 - 1190	-
Sokolowski et al. (2004)	*Perna perna*	Yemen (Gulf of Aden)	-	-	25.0	175.1	0.33	14.5	103.1	-	-	-	113,2	-
Catharino et al.(2008)	*Perna perna*	Cocanha/SP, Brazil	-	-	0.1	15.9	-	-	-	-	0.02	0.2	48	2,74
Catharino et al. (2008)	*Perna perna*	Ilhabela/SP, Brazil	-	-	0.11	15.4	-	-	-	-	0.01	0.09	22	1.88
Mol and Alakavuk (2011)	*Mytilus galloprovincialis*	Marmara Sea, Turkey	-	-	0.3 – 0.7	55 - 97	0.2 – 1.2	-	0.9 – 3.5	-	-	-	-	-
Mok et al.(2014)	*Mytilus galloprovincialis*	Changseon, Korea	-	-	0.2	15.9	0.17	-	1.5	0.37	0.007	0.23	-	-
Liu and Wang(2015)	*Perna viridis*	Port shelter, Hongkong			1.2	165	11		8	28				
Lino et al. (2016)	*Perna perna*	Rio de Janeiro, Brazil	-	-	<0.03 – 0.7	33 - 65	<0.6	3 - 40	3 - 3200	<1.4 – 4.0	-	1.0 – 7.5	104 - 477	-
Campolim et al. (2017)	*Perna perna*	Santos Bay, Brazil	1000 - 1750	-	0.24 - 0.87	59 - 170	0.3 - 2.6	5.0 - 9.6	4 - 8	4.5 - 11.5	-	0.5 - 2.5	430 - 770	-

In high energy estuaries with high to moderate turbidity sorption is a very efficient particle-water exchange (Turner et al., 1998). The flocculation of trace metals is a common feature in estuaries that causes the decrease of colloidal forms (<0.10µm) during the mixing of fresh and sea waters. During flocculation colloidal particles become micro flocks and the load of dissolved material decreases (Hassini et al., 2017). When submitted to favorable physico-chemical and hydrodynamic conditions these micro flocks can aggregate to form macro flocks and this influences their settling velocity (Mikes et al., 2004). The particulate material formed could then settle, be transported further from where it was originated or be incorporated by biota. Suspended particulate material is known to be one of the main controlling factors on metal behavior and bioavailability in estuaries, and its composition can comprise organic detritus, clays, metal oxides, among others (Zhang et al., 2008). In the present study a general trend of higher metal concentrations in *Perna perna* sample from the outer stations (BMP_3 and BMP_4) was highlighted. The outer stations receive stronger influence from marine waters what could enhance the flocculation of dissolved elements and, consequently, increase the contents of these elements in local biota. The pH of the environment is an important factor concerning the bioavailability of trace metals that tends to be relatively low in pH values between 6.5 to 7 (Kabata-Pendias and Pendias 1987). Even so, there was evidence in the present study that contradicts the assertion of Kabata-Pendias and Pendias (1987), since the increase of pH along the salinity gradient towards the bay was not observed, nevertheless, metal bioavailability for the mussels increased. Zhang et al. (2008), on the other hand, observed a sharply increase in metal adsorption onto suspended particulate metal for a pH difference of less than 2 units for several metals, varying from 6-7 to Cu up to 6-8 pH units for Zn, among other elements.

A highlight should be given to Cd, which presented very elevated concentrations in the dissolved phase, however, it was not detected in the dry tissue of *Perna perna*. Dai et al. (1995) describes the low affinity of Cd for colloidal matter, therefore the removal of this element by adsorption in suspended particulate matter less effective. The geochemistry of Cd in estuaries is highly dependent on salinity and redox conditions. Cadmium can be removed from dissolved phase in estuaries by adsorption onto Mn oxides and this is reverted when salinity increased in the lower estuary by the strong affinity between Cd and chloride forming a complex and increasing the dissolved phase again (Jiann and Ho, 2014).

Estimated Daily Intakes (EDI)

Mean EDI values were higher for Campaign 2 for most elements, except for Zn and Cu which were higher for Campaign 3 (Table 6). EDI for metals were all below the ORD in the three campaigns both for ALM and HLM (Table 6) for Al, Ba, Zn, Cu, Mn, Ni and Cr, which means that the average or high consumption of mussels would not offer significant health risks regarding these elements. For Fe, EDI values overcame ORD (700 µg/day/kg) for HLM in the three campaigns, reaching the maximum value in Campaign 3, 1,167.57 µg/day/kg, and revealing potential toxic effects for high level mussel consumers. Iron is an essential micro nutrient in human diet assisting the transport of oxygen through blood, therefore, it should not pose a health threat, unless a very large dose could be ingested, which seems to be the case for HLM consumers. The highest concern was posed by arsenic, which surpassed ORD (0.3 µg/day/kg) in the three campaigns for ALM and HLM consumers, even in the stations where the metalloid did not surpass the limits established by the Brazilian legislation.

Target hazard Quotient (THQ) and Hazard Index (HI)

For Al, Ba, Zn, Mn, Cu, Cr, Ni, mean values of THQ were <1 for both ALM and HLM consumers (Table 7). Maximum values of THQ for these elements presented maximum values much lower than 1, suggesting that the ingestion of mussels was not supposed to cause any adverse effects. Iron presented mean values of THQ also <1 for ALM and HLM consumers, however, maximum concentrations of THQ were >1 for HLM consumers in the three campaigns. In campaign 1 THQ was 1.48 for BMP4; in campaign 2 THQ was 1.15 and 1.33, respectively for BMP2 and BMP4, and in campaign 3, THQ was 1.71 in BMP3. These results reveal a higher contribution of iron contents in the most external stations, closer to Vitória Bay, suggesting the anthropogenic influence of this coastal area. Arsenic presented very elevated values of THQ, much higher than 1 both for ALM and HLM consumers. The highest mean and maximum values were registered during campaign 1. Maximum THQ for arsenic was registered at BMP 3 during campaign 1 and at BMP4 during campaigns 2 and 3, both closer to Vitória Bay, that could be a source of the metalloid bioavailable for biota, especially for filter-feeding organisms such as mussels. Health risks associated to *Perna perna* ingestions are considered elevated regarding arsenic concentrations.

Table 6 – Estimated daily intakes (EDI) (µg/day/kg body weigh) for average (ALM) and high (HLM) level mussel consumers for each element.

	EDI (ALM)	Al	Ba	Zn	Mn	Cu	Ni	Cr	Fe	As
Campaign 1	mean±SE	111.07±93.93	0.81±0.38	16.15±1.20	4.53±1.21	1.45±0.10	1.9±0.44	0.37±0.20	151.98±117.02	2.26±0.60
	min	9.56	0.19	13.87	2.97	1.21	1.30	0.15	28.62	1.08
	max	392.41	1.87	19.54	8.15	1.71	3.20	0.77	502.97	3.58
campaign 2	mean±SE	181.26±64.07	4.7±1.89	15.69±2.41	9.4±1.89	2.75±0.59	2.89±0.74	0.46±0.09	310.19±88.83	1.83±0.36
	min	32.96	0.50	11.07	4.91	1.60	1.04	0.31	52.55	1.12
	max	329.61	9.41	21.89	14.00	4.19	4.58	0.63	453.11	2.61
campaign 3	mean±SE	124.66±29.02	0.56±0.10	19.61±2.07	5.36±0.60	4.17±0.16	1.49±0.26	0.39±0.04	258.95±112.12	1.48±0.42
	min	56.59	0.34	15.32	4.19	3.91	0.83	0.29	84.29	0.91
	max	185.40	0.83	23.41	7.00	4.63	2.00	0.46	584.11	2.74
	EDI (HLM)									
Campaign 1	mean±SE	222.02±187.76	1.65±0.79	32.28±2.41	9.06±2.43	2.89±0.21	3.81±0.88	0.74±0.40	303.78±233.91	4.52±1.20
	min	19.11	0.40	27.72	5.94	2.42	2.59	0.30	57.21	2.16
	max	784.39	3.84	39.05	16.30	3.42	6.40	1.54	1,005.37	7.16
Campaign 2	mean±SE	362.32±128.07	9.66±3.88	31.36±4.81	18.8±3.78	5.5±1.17	5.77±1.48	0.92±0.18	620.03±177.57	3.67±0.72
	min	65.88	1.03	22.13	9.82	3.19	2.08	0.62	105.04	2.24
	max	658.84	19.34	43.75	27.98	8.37	9.16	1.26	905.71	5.21
Campaign 3	mean±SE	249.19±58.00	1.15±0.21	39.19±4.13	10.72±1.21	8.33±0.32	2.97±0.52	0.79±0.07	517.61±224.11	2.96±0.85
	min	113.12	0.69	30.62	8.38	7.81	1.66	0.58	168.49	1.82
	max	370.60	1.70	46.79	14.00	9.25	4.00	0.92	1,167.57	5.47

The Hazard Index, that considers the cumulative effects of the ingestion of potentially toxic elements was much higher than unit in all campaigns both for ALM and HLM consumers. Arsenic and Fe contributed to the elevated HI for ALM and HLM, since both elements presented high THQ. For ALM consumers the highest values of HI occurred at stations BMP3 and BMP 4 during campaign 1, respectively 12.22 and 11.50. During campaign 2, the highest HI values were registered at the same stations, 8.94 for BMP3 and 10.39 for BMP4. In campaign 3, the highest HI was found at BMP3, with the value of 10.08. For HLM consumers HI was higher at the same stations. During campaign 1, BMP3 and BMP4, respectively presented HI of 24.42 and 23.05. During campaign 2 BMP4 HI value was 20.80, and for campaign 3, the value of HI for BMP4, was 20.16. Hazard Index results suggested risk of health issues through the consumption of mussels, in every station, however, the outer stations, closer to Vitória Bay, presented higher risks, what could be due to higher bioavailability of trace metals and arsenic resulting from flocculation effects.

The only element for which Target Cancer Risk (TRC) was calculated was arsenic, since USEPA (2010) has an established cancer oral slope. The method established by USEPA considers cancer risk negligible when TCR<10^{-6}; and TCR values between 10^{-6} and 10^{-4} are considered acceptable. The threshold value for TCR then, concerning arsenic, is 10^{-4} (Shaheen et al., 2016; Antoine et al., 2017).

Risk Cancer for As varied between 0.001 and 0.005 for ALM consumers and from 0.003 and 0.011 for HLM consumers (Fig. 17), all above the threshold value of 10^{-4}. Results of TCR leave no doubt that consumers of mussels originated from the study area are exposed to the metalloid pollution with carcinogenic risks as the main consequence. It is important to highlight though, that the calculation of TCR was made with total arsenic concentrations, and calculations made with inorganic arsenic only, which is the form that produces toxic effects, results could be lower.

Table 7 – Target Hazard Quotient (THQ) and Total Hazard Index (HI) for average (ALM) and high (HLM) level mussel consumers for each element.

	THQ (ALM)	Al	Ba	Zn	Mn	Cu	Ni	Cr	Fe	As	**HI (ALM)**
Campaign 1	mean±SE	0.11±0.19	0.004±0.004	0.05±0.01	0.03±0.02	0.04±0.01	0.1±0.04	0.12±0.12	0.22±0.33	7.53±4.00	8.17±4.29
	min	0.01	0.001	0.05	0.02	0.03	0.06	0.05	0.04	3.60	3.93
	max	0.39	0.009	0.07	0.06	0.04	0.16	0.26	0.72	11.94	12.21
Campaign 2	mean±SE	0.18±0.13	0.02±0.02	0.05±0.02	0.07±0.03	0.07±0.03	0.14±0.07	0.15±0.05	0.44±0.25	6.12±2.41	7.21±2.96
	min	0.03	0.003	0.04	0.04	0.04	0.05	0.10	0.08	3.74	4.01
	max	0.33	0.047	0.07	0.10	0.10	0.23	0.21	0.65	8.69	10.39
Campaign 3	mean±SE	0.12±0.06	0.003±0.001	0.07±0.01	0.04±0.01	0.1±0.01	0.07±0.03	0.13±0.02	0.37±0.32	4.93±2.82	5.84±2.83
	min	0.06	0.00	0.05	0.03	0.10	0.04	0.10	0.12	3.04	4.23
	max	0.19	0.00	0.08	0.05	0.12	0.10	0.15	0.83	9.12	10.08
	THQ (HLM)										
Campaign 1	mean±SE	0.22±0.19	0.01±0.004	0.11±0.01	0.06±0.02	0.07±0.01	0.19±0.04	0.25±0.13	0.45±0.34	15.05±4.00	16.35±4.30
	min	0.02	0.002	0.09	0.04	0.06	0.13	0.10	0.08	7.20	7.85
	max	0.78	0.02	0.13	0.12	0.09	0.32	0.51	1.48	23.86	24.42
Campaign 2	mean±SE	0.36±0.13	0.05±0.02	0.1±0.02	0.13±0.03	0.14±0.03	0.29±0.07	0.31±0.06	0.9±0.26	12.22±2.41	14.44±2.96
	min	0.07	0.01	0.07	0.07	0.08	0.10	0.21	0.15	7.47	8.02
	max	0.66	0.09	0.15	0.20	0.21	0.46	0.42	1.33	17.38	20.80
Campaign 3	mean±SE	0.25±0.06	0.01±0.001	0.13±0.01	0.08±0.01	0.21±0.01	0.15±0.03	0.26±0.02	0.76±0.33	9.85±2.82	11.69±2.83
	min	0.11	0.003	0.10	0.06	0.20	0.08	0.19	0.25	6.08	8.47
	max	0.37	0.01	0.16	0.10	0.23	0.20	0.31	1.71	18.23	20.16

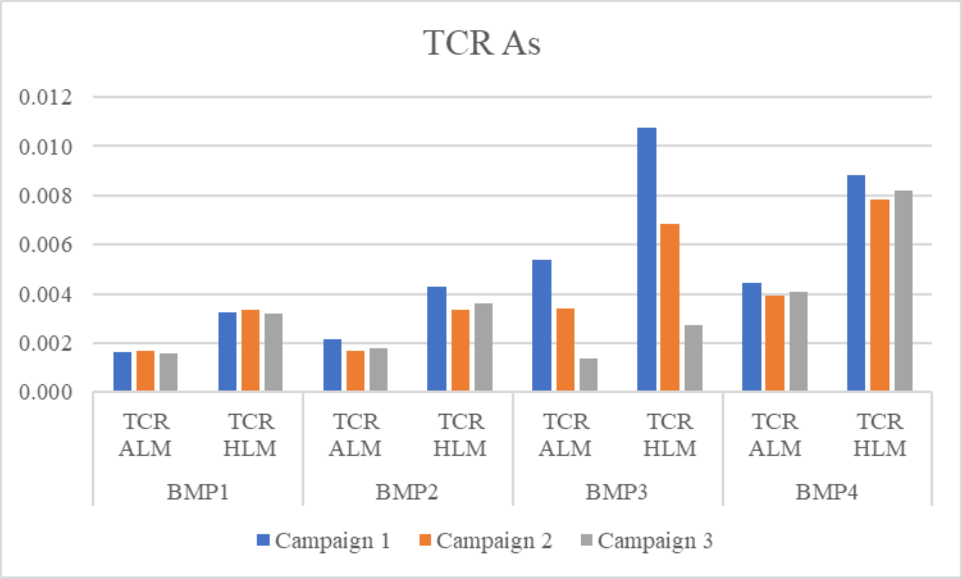

Fig. 17 – Target cancer risk (TCR) for arsenic in the estuarine system of Vitória Bay.

Conclusion

Most of the studied metals, when compared with their concentrations in water, could be considered bioavailable, once the mussels were able to accumulate these in their tissues. Pb had low concentrations in mussels after presenting high levels in water, and Cd, despite presenting high concentrations in the dissolved fraction was not detected in the mussels, suggesting reduced bioavailability of these metals in the studied area. A general trend of higher concentrations in the outer stations was observed for most metals, suggesting the influence of flocculation process increasing the particulate form and becoming available for the local biota due to the proximity with more saline waters. Port activities as well as the anthropogenic ones in the surroundings of the outer stations can also be held accountable for the higher metal concentrations in these locations. Cr and As in *Perna perna* surpassed the tolerance limit established by the Brazilian legislation suggesting potential of intoxication through consumption. The same was observed for Fe, which presented concentrations in *Perna perna* above the limit recommended by the international guidelines. Health risk issues were confirmed through the Target Hazard Quotient and Target Cancer Risk for arsenic. Therefore, even if individually, most of the metals accumulated in the mussel’s tissues do not offer health risks, the cumulative effect makes the ingestion of theses organisms dangerous for human health, being iron and arsenic the main contributors of this danger.

Considering that the fishery resources have great importance for the local economy, public actions should be taken to implement monitoring and preventive measures to guarantee the conservation of resources, human health and the preservation of local coastal area.

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ARTIGO 2 – HUMAN HEALTH RISK ASSESSMENT ASSOCIATED WITH THE CONSUMPTION OF MUSSELS (*Perna perna*) AND OYSTERS (*Crassostrea rhizophorae*) USED AS BIOMONITORS IN THE ESTUARINE CHANNEL OF VITÓRIA BAY (ES), SOUTHEAST BRAZIL.

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APROVADO EM JUNHO DE 2021 NA REVISTA *MARINE POLLUTION BULLETIN*.

Resumo

O canal estuarino da Baía de Vitória (ES), no sudeste do Brasil, foi avaliado quanto à bioacumulação de metais traço (Al, Ba, Mn, Fe, Zn, Cu, Cr) e As em moluscos bivalves, *Perna perna* e *Crassostrea rhizophorae*. Mexilhões foram retirados de uma fazenda marinha e transplantados para o canal estuarino em quatro estações, enquanto ostras foram coletadas nas mesmas estações. Concentrações de metais e arsênio foram determinadas no tecido seco dos bivalves após 60 dias do transplante dos mexilhões. A coluna d'água das quatro estações foi monitorada quanto à temperatura, salinidade, pH, oxigênio dissolvido e turbidez. A absorção de metais pelos mexilhões *Perna perna* foi maior no estuário externo, com valores de salinidade mais elevados. As concentrações de Al, Mn, Fe, Cr e As foram maiores em *Perna perna*, enquanto *Crassostrea rhizophorae* bioacumulou mais Ba, Zn e Cu. Em relação à legislação brasileira, as concentrações de Zn nas ostras estavam acima do limite de tolerância e as concentrações de Cr nos mexilhões e ostras estavam acima do limite estabelecido. A concentração em *Perna perna* também ultrapassou o limite da legislação brasileira na foz externa. A salinidade pareceu influenciar a absorção de metal em ambos os organismos, com mexilhões absorvendo mais metal em salinidades mais altas e ostras em áreas de salinidade mais baixa. Risco Alvo de Câncer para As revelou ameaça à saúde humana associada ao consumo de mexilhões e ostras da área de estudo.

Palavras-chave: Baía de Vitória, metais traço, arsênio, Perna perna, Crassostrea rhizophorae, risco à saúde.

Abstract

The estuarine channel of Vitória Bay (ES) in southeast Brazil, was evaluated regarding bioaccumulation of trace metals (Al, Ba, Mn, Fe, Zn, Cu, Cr) and As in bivalve mollusks, *Perna perna* and *Crassostrea rhizophorae*. Mussels were taken from an aquaculture farm and transplanted to the estuarine channel in four stations, whereas oysters were collected at the same stations. Concentrations of metals and arsenic were determined in the dry tissue of the bivalves after 60 days of mussels transplantation. The water column of the four stations was monitored for temperature, salinity, pH, dissolved oxygen and turbidity. Metal uptake for Perna perna mussels was higher in the outer estuary, with more elevated salinity values. Concentrations of Al, Mn, Fe, Cr and As were higher in *Perna perna*, whereas *Crassostrea rhizophorae* bioaccumulated more Ba, Zn and Cu. Regarding the Brazilian legislation, Zn concentrations in oysters were above the tolerance limit and Cr concentrations in mussels and oysters were higher than the established limit. As concentration in *Perna perna* also surpassed the limit of the Brazilian legislation in the outer estuary. Salinity seemed to influence metal uptake in both organisms, with mussels absorbing more metal at higher salinities and oysters in areas of lower salinity. Target Cancer Risk for As revealed threat for human health associated with the consumption of mussels and oysters from the study area.

Keywords: Vitória Bay, trace metals, arsenic, *Perna perna*, *Crassostrea rhizophorae*, health risk.

Introduction

Trace metals are among the most common and persistent pollutants found in aquatic environment. While some of them are considered essential for the biochemical functioning of several organisms, the excess concentrations may cause deleterious ecological impacts on biota and during the last decades, trace metal and metalloids pollution has got the attention of the scientific community due to the potential negative impact in the food chain (Achary et al., 2017).

The monitoring of contaminants in marine and estuarine ecosystems can follow a number of different methods, depending on the object of the study. Evaluating the levels of inorganic pollutants in the environment can be achieved by direct chemical analysis of water and sediment samples (Superville et al., 2014), however, this approach results in little information about the real pollutant bioavailability.

Bioavailable toxins are the most critical pollutants fraction from an ecotoxicological perspective (Kim et al., 2015), in that they result in a potential equilibrium risk to the trophic chain (Schöne and Krause., 2016). On the other hand, the consumption of seafood contaminated by persistent pollutants can cause human health issues (AbdelGhany, 2017). Pollutant contamination in the tissues of organisms and its relation to human health risk have been deeply studied (El Nemr et al., 201 6; El Nemr et al., 2012a; El Nemr et al., 2012; Jia et al., 2010). Regarding contamination by trace metals, the accumulation of these elements in organisms' tissues can cause toxicity affecting ecological performance concerning physiology, metabolism and ion-regulation (Walker et al., 2012; Kumar et al., 2015).

All species have the ability to reflect to negative environmental conditions, such as anomalous metal pollution levels, by stimulating a physiological stress behavior that changes the metabolism and redistributes the anabolic energy in repairing the stress which induces molecular damage (Pytharopoulou et al., 2013).

For the purpose of environmental monitoring, bivalve mollusks species have proven to be excellent tools in coastal and estuarine ecosystem (Chandurvelan et al., 2013), due to their physiological particularities like their sessile nature and filter-feeding ability (Diop et al., 2016). Additionally their capacity to incorporate metal pollutants, can result in negative biological effects (Chandurvelan et al., 2012,Chandurvelan et al., 2013). Finally, because of their abundances and widespread distribution, bivalve mollusks have also been used as sentinels for decades to monitor coastal areas regarding trace metal pollution (Wang and Lu, 2017).

Some of the biological factors that influence the uptake of trace metals are age, size, sex, genotype, phenotype, feeding activity, reproductive state and physiological conditions of the animals (Saavedra et al., 2004; Mubiana et al. 2006). Additionally, it is important to recognize the diversity of metal bioaccumulation patterns across different bivalve species (Wang and Lu, 2017). Studies about the contamination dynamic of species in potential polluted environments are of great importance, because they contribute significantly to the understanding of their biology, uptake mechanisms for different pollutants and the risks that these species can offer through human consumption.

The present study aimed to perform biomonitoring for trace metals and arsenic in the estuarine channel of Vitória, ES, southeast Brazil, through the evaluation of metal contents in the tissue of transplanted mussels and local oysters, both largely consumed by local population and, of great economic importance along Brazilian coastline.

Materials and Methods

Sampling

The estuarine system of Vitória Bay is located in the state of Espírito Santo in southeast Brazil (Fig. 18) the most urbanized area in the country, comprising more than 400 thousand industries. The estuary of Vitória bay has two main channels, Porto and Passagem, and both receive mixed waters from several tributaries that mix with oceanic waters. The area has been suffering environmental degradation along several decades due to urbanization of its surroundings, with over one million inhabitants, industries installations, port activities and input of raw sewage, since less than 30% of it is treated before reaching the system (Jesus et al., 2004, Joyeux et al., 2004). Local climate is typically tropical, characterized by dry winter and wet summer which directly influence physicochemical changes in water column (Joyeux et al., 2004).

The sampling occurred in 2017, during the dry season, along the estuarine channel of Vitória Bay, in the state of Espírito Santo, Brazil. Four sampling points were distributed from the inner estuary towards the mouth (Fig. 18). Some basic physico-chemical data was obtained *in situ* with the aid of a multi probe Horiba U10 during ebb tide, and measures of temperature, salinity, pH, dissolved oxygen and turbidity were taken approximately 0.5m from the bottom. The monitoring of physicochemical variables in the water column occurred twice within 60 days.

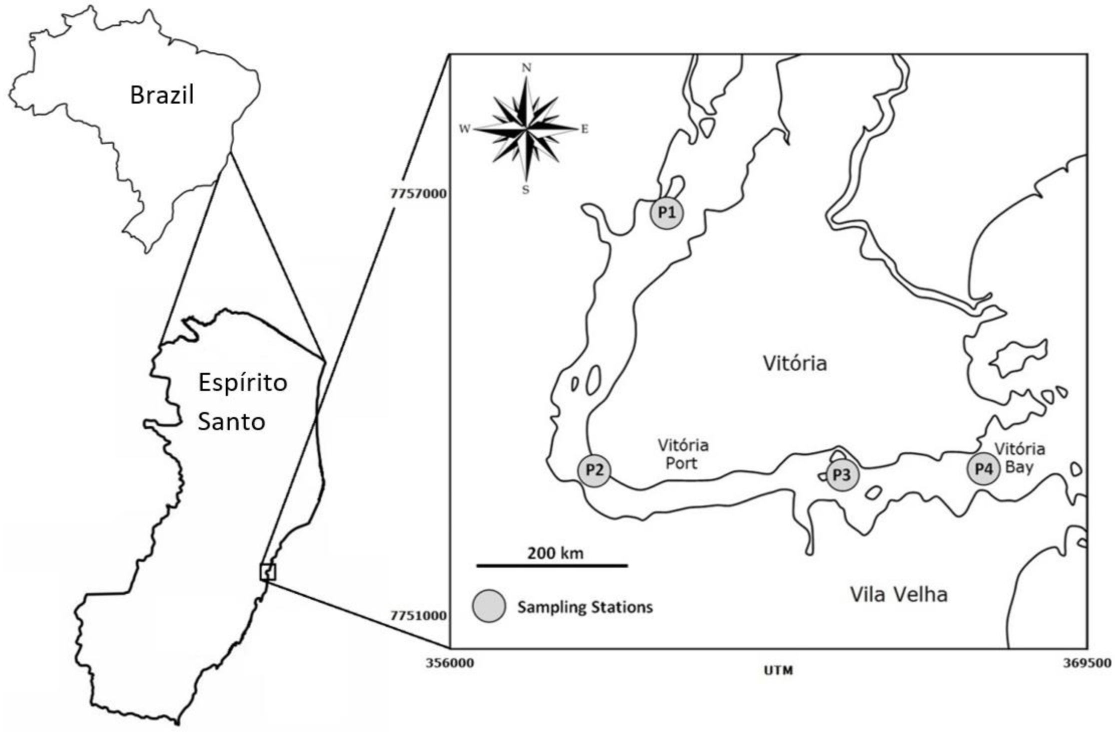

Fig. 18 – Study area and sampling stations along the estuarine channel of Vitória Bay, ES, Brazil.

Mussels *Perna perna* were originated from a mussel farm in the city of Anchieta in the state of Espírito Santo and subsequently transplanted to the four sampling points and left there for 60 days, before being collected. *Crassostrea rhizophorae* oysters, were collected, at the same time of the mussels from locally available consolidated substrates in the four sampling points. The animals were transported to the laboratory in pre cleansed polyethylene flasks right after the sampling. Mussels from the farm were collected in two different periods for the determination of trace metals and arsenic before transplantation into the stations along the estuarine system of Vitória Bay.

Laboratory Analysis

In the laboratory mussels and oysters were left separately in seawater tanks for approximately 48h to clear their gut content. The soft tissue of the organisms from each sampling station was extracted separately up to the sum of 200g per station. The samples were oven dried at 80ºC and subsequently ground to a fine powder with mortar and pestle. Metal extraction (Al, Ba, Zn, Cr, Cu, Pb, As, Mn, Fe) was made with the addition of 5 ml HNO_3 and 3 mL of $H_2O_2$to approximately 0.500 g of dried sample, followed by heating a Microwave 3000 Anton Paar®. The digested samples were then filtered on 0.45 µm cellulose acetate membranes and the filtrated was diluted with Milli-Q to 10 ml. Samples were then read in an ICP-MS. Blanks and standard reference material were digested with the same method, and the recovery of metals was within 10% deviation from certificate values (SRM 1566b-oyster tissue). All reagents were of analytical grade and all the glassware was previously cleaned with nitric acid and rinsed with Milli-Q water.

Risk Assessment Calculations

The evaluation of trace metal content in oysters and mussels was evaluated based on the Brazilian legislation (Decree nº 55.871/1965) that establishes the tolerance limit for metals in food and contemplates some of the elements determined in the present study except for Al, Ba, Fe and Mn. For Mg the study conducted by USEPA (2003) was used as reference for tolerance limit. For Fe, the tolerance limit was the one proposed by WHO (1993).

Regarding risk assessment through human consumption of mussels and oysters, the approach was based on the following indexes: Estimated daily intake (EDI), Target Cancer risk (TCR), Target Hazard Quotient (THQ) and Hazard Index (HI). All these indexes were calculated following some guidelines proposed by USEPA (2004), which establishes differences between Average Level Consumers (ALM) and High Level Consumers (HLM), respectively, 17.86 g/day and 35.71 g/day of mussels/oysters in the case of the present study. All the indexes considered a body weigh of 60 kg for an adult. All the indexes were calculated with concentrations of metals and arsenic obtained from wet weight of the mussels of mussels and oysters (ww)(Yap et al. 2003, 2016).

Estimated Daily Intake (EDI): This index considers the oral reference dose (ORD) in µg/day/kg body weigh as described in USEPA (2015). The ORD for the different metals are the ones in Table 8, and the index is calculated through the formula:

EDI = (MC x Consumption rate)/body weigh

MC = metal concentration

Table 8 – Oral Reference Dose (ORD) proposed by USEPA (2015)

Metal	Al	Ba	Fe	Zn	Cu	Cr	Mn	As
ORD	1000	200	700	300	40	3	140	0.3

Target Hazard Quotient (THQ): The calculation of this index is described in USEPA (2000), and THQ>1 suggests potential risk to human health:

$$THQ = \frac{EFxEDxCRxMC}{ORFxABWxAET}\, x\, 10^{-3}$$

EF = exposure frequency (365 days/year); *ED* = exposure duration (70 yrs); *CR* = consumption rate (ALM = 17.86 g/day; HLM = 35.71 g/day); *MC* = metal concentrations in mussels (ww); *ORF*= oral reference dose for each element; *ABW* = average body weight (and adult of 60 kg); *AET* = average exposure time (365 days x 70 yrs).

Hazar Index (HI): The calculation of HI is based on the THQ, since it considers that the risk of food ingestion is associated to the simultaneous ingestion of several toxic elements, which means that a THQ< 1 does not necessarily suggests absence of risk for food ingestion, for the combined effect of the elements can result in a HI>1.

$$HI = \sum_{i=1}^{n} THQi$$

Target Risk Cancer (TRC): This index evaluates the probability of an excessive cancer risk due to a lifetime exposure to a potentially toxic element. In the present study TCR was calculated for arsenic based on the slope factor established by USEPA, 1.5 mg/kg/day.

$$TCR = \frac{EF\ x\ \ ED\ x\ CR\ x\ MC\ x\ CPSo}{ABW\ x\ AETc}\, x\, 10^{-3}$$

EF = exposure frequency (365 days/year); *ED* = exposure duration (70 yrs); *CR* = consumption rate (ALM = 17.86 g/day; HLM = 35.71 g/day); *MC* = metal concentrations in mussels (ww);

CPSo = oral slope factor; *ABW* = average body weight (and adult of 60 kg); *AET* = average exposure time to carcinogen (365 days x 70 yrs)

Statistical Analysis

The analysis of data set was evaluated through non-parametric statistical tests, due to small sample size and non- normality. For physicochemical variables the Kruskal-Wallis test ($p<0.050$) was applied to check significant differences among the four sampling stations. Mann-Whitney test ($p<0.050$) was performed to test significant differences between metal concentrations in mussels before and after transplantation.

Results and Discussion

As filter feeding or/and deposit feeders bivalve mollusks have the ability to concentrate trace metals and metalloids from the aquatic environment (Krishnakumar et al., 2018). The pathways of incorporation of trace elements in bivalve mollusks depend on biological and physico-chemical factors (Boening, 1999). Examples of physico-chemical factors include salinity, temperature, pH, sediment grain size, dissolved oxygen, water hardness and hydrological features of the system (Elder and Collins, 1991). The capacity of an aquatic species to survive in the environment is controlled by its tolerance limits towards abiotic factors like salinity, temperature, dissolved oxygen and pH. It is noteworthy that oysters and mussels are considered resistant animals, supporting a wide variation of physico-chemical changes. In this sense, temperature which is crucial for the filtration process, presented values closer to the optimum conditions (Fig. 19) for mussel growth (Ferreira and Magalhães, 2004) when compared to oysters (Frias and Rodrigues, 1991), with no significant differences among the sampling stations ($p>0.050$).

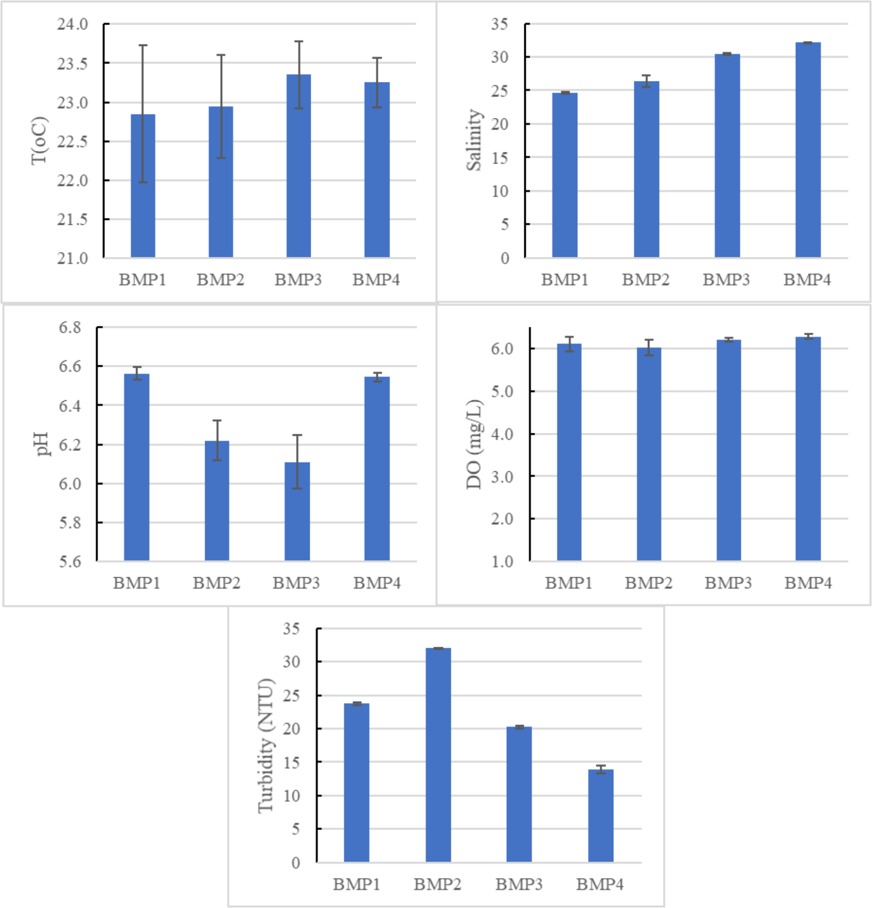

Fig. 19 – Mean and standard error for temperature, salinity, pH, dissolved oxygen and turbidity along the estuarine channel of Vitória Bay.

Salinity is among the main abiotic factors influencing distribution, abundance and survival of marine invertebrates. During larval phases of mollusks, salinity drives physiological processes, the duration of the planktonic phase, feeding habits and ability to select settlement sites (Horodeski et al., 2019). Salinity presented significant differences among the four stations ($p<0.050$) and a slight increase towards the estuary mouth was observed, with values above 30 in stations BMP3 and BMP4, revealing the influence of marine waters. Salinity variation favored the development of both groups of mollusks, since mussels prefer more saline environments (Ferreira and Magalhães, 2004; Resgalla Jr. et al., 2007) and C. rhizophorae oysters are favored by values around 27(Guimarães et al.,2008).

In shallow estuarine water, the physico-chemical fluctuations can be large; dissolved oxygen can vary from levels that characterize anoxia to supersaturation, and pH can fluctuate by a full pH unit or more, as a result of the equilibrium between photosynthesis and respiration (Wallace et al., 2014). In the present study, higher values of dissolved oxygen were registered in the most outer stations towards Vitoria Bay (Fig. 19), with variations between 5.68 and

6.42 mg/L and no significant differences among the stations (p>0.050). Variations of pH characterized a slightly acidic environment, with values between 6.00 and 6.59, typical of estuarine environments and corroborated the data obtained by Jesus et al. (2004) in the same area, with no significant differences were observed among the stations (p>0.050). Mussels (Mytilus galloprovincialis) subjected to long-term low pH, like 7.3, presents lower metabolic, growth and calcification rates (Michaelidis et al., 2005). Kroeker et al. (2010) suggests that the organisms tend compensate the greater solubility of the carbonate ion by increasing calcification rate, increasing their energy demand, limiting growth rates. In the present study all pH values obtained were below 7.0. (Table 8), suggesting a potential impact over the organism's metabolism and further metal accumulation.

According to Kumar et al. (2015) it is important to distinguish two routes for metal uptake from sediments, especially for burrowing infauna. The first route would be the one associated with sediment ingest by deposit feeders, and the second one refers to the interstitial pore water that contains metals released from sediments. For filter-feeders, however, metals associated with suspended particulate material are more important than the aforementioned routes. The higher the suspended particulate metal concentrations in the estuary the higher the turbidity values, which could also influence particulate metal concentrations available for filter-feeders organisms. In the Vitória estuarine system, turbidity values tended to decrease towards the estuary mouth (Fig. 19), probably an effect caused by flocculation and settlement due to increase of salinity. Water column turbidity can negatively impact organisms by reducing the quantity and quality of light available for photosynthesis (Petrou et al., 2013; Chartrand et al., 2016). Trace metal bioavailability in the water, on the other hand, can also be impacted by increase of turbidity. When the flocculation process takes place in an estuary, a considerable number of dissolved elements is decanted and leave the dissolved phase (Hassani et al., 2017).

In addition to physicochemical factors and availability of contaminants in the environment, the physiological characteristics of each species (e.g. species, age, sex, soft-body weight, gametogenesis and physiological status) and the characteristics of each metal also directly affect their biological uptake (Fernandez-Tajes et al., 2011). In general, the bioavailability of metals with biological functions is influenced by biotic and abiotic factors. Non-essential metals, on the other hand, may be influenced only due to external factors (Bordin et al., 1992), such as anthropogenic inputs.

Concerning salinity differences along the estuarine channel of Vitória, it is possible to observe that metal uptake was higher where salinity values were lower, for oysters (Fig. 20).

This was clear for oysters in the case of zinc, copper, aluminum, manganese, iron, chromium and arsenic. On the other hand, for mussels, maximum concentrations of metals were registered at the stations with higher salinity, such as zinc, manganese, iron, chromium, aluminum and arsenic. Overall, metal concentrations were higher in mussels, which presented higher mean concentrations for Al, Mn, Fe, Cr and As, whereas oysters presented higher mean values for Zn and Cu, elements for which they are known as hyperaccumulators, and Ba. Differences in metal bioaccumulation can be caused by several differences, including different geochemical behavior for the diverse elements. The increase of salinity in the outer estuary can lead to flocculation of metals, which means there would be less free ions available. Some studies have already demonstrated the increase of metal uptake by invertebrates with salinity decrease (Mo and Neilson, 1993; Wang and Dei, 1999, Kumar et al., 2015). Metal incorporation by invertebrates can also be affected by interactions among the different elements, since the exposure to one metal can drive changes in the process of uptake of other metal presenting similar physicochemical characteristics (Wang et al., 2011).

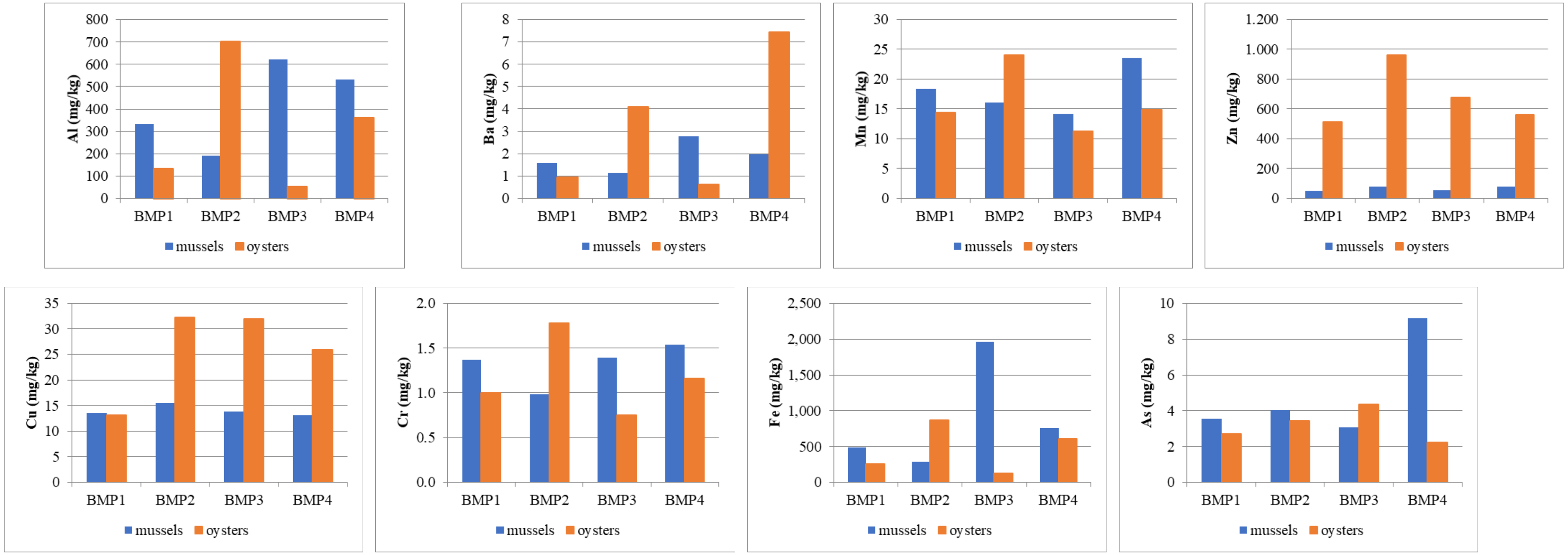

Fig. 20 – Concentrations of Al, Ba. Mn. Fe, Zn, Cu, Cr and As in mussels (*Perna perna*) and oysters (*Crassostrea rhizophorae*) along the estuarine channel of Vitória Bay.

Table 9 presents trace metals and arsenic concentrations in *P. perna* mussels from the mariculture farm before (BT) and after transplantation (AT) to the estuary. Mann-Whitney test (*p<0.050*) revealed no significant differences in trace metals and arsenic concentrations between BT and AT for *P. perna* mussels.

Table 9 – Mean±SE concentrations (mg/kg) of Al, Ba. Mn. Fe, Zn, Cu, Cr and As in mussels (*Perna perna*) from the farm before (BT) and after (AT) transplantation into the Vitória Estuarine system.

	Al	Ba	Zn	Mn	Cu	Cr	Fe	As
BT	745±248	2.12±1.62	59.97±15.43	19.00±18.50	4.74±0.16	0.89±0.39	1,120±169	9.54±0.68
AT	419±97	1.87±0.35	65.87±6.94	18.01±2.03	14.00±0.53	1.32±0.12	870±377	4.97±1.42

Until the last years, aluminum was classified as a harmless metal for the human ingestion as it is quickly excreted through urine. The studies conducted at that time were developed under unreal environmental conditions (pH of 6 or lower and in waters with low dissolved organic carbon and hardness concentrations). Additionally, there was little concern about variables affecting Al bioavailability. However, chemical species of Al vary significantly across the pH range of 6 to 8 found in most natural surface aquatic systems (Adams et al., 2018). Toxicologic studies carried out in recent years suggested Al as a cause of many diseases in humans, animals and plants (Barabasz et al., 2001). Increasing acidification of the environment water chemistry promotes the increase of aluminum bioavailability in the water resulting in detrimental impacts on all aquatic organisms (Bjerknes et al., 2003; Griffitt et al., 2010). Despite all that, aluminum has no reference value in Brazilian legislation. In the present study, concentrations of Al found in oysters were between 54.42 and 703.33 mg/kg, with the maximum value a little higher than the one found in mussels. Concentrations of Al in *P. perna* varied between 331.63 and 662.85 mg/kg, much higher than the ones found in other Brazilian coastal areas, however lower the results found by Campolim et al. (2017) in the same species in the heavily polluted estuarine system of Santos, also in southeast Brazil (Table 10). Mean Al concentrations in *P. perna* was a little higher before the transplantation (Table 9), but ranges of concentrations were very similar before and after transplantation.

Barium also has no reference in the Brazilian legislation, and very low concentrations were registered in mussels and oysters (Fig. 20) with no significant differences in concentrations between mussels and oysters ($p>0.050$). The insolubility of Ba in sea salts and its consequent low bioavailability has been demonstrated in other studies (Lira et al., 2011). According to Golding et al. (2018), sulfate levels highly determine the solubility of Ba in aquatic systems, as insoluble barium sulfate, considered less bioavailable and toxic than dissolved barium. In *P. perna* mussels mean Ba concentrations before and after transplantation were very similar (Table 9).

Manganese presented higher concentrations for oysters in less saline stations, whereas for mussels highest concentration was registered in BMP4 which presented the highest salinity value (Fig. 20). Similar mean Mn concentrations were observed in *P. perna* before and after transplantation.

Zinc and copper are considered essential elements, and oysters, among marine filter-feeders animals, are considered hyperaccumulators of these metals (Zarezadeh et al. 2017). Differences in zinc concentrations among mussels and oysters were very expressive reflecting oysters'hability to hyperaccumulate this metal (Ke and Wang 2001; Wang et al. 2011). Concentrations of Zn in oysters varied from 511.94 to 962.44 mg/kg and were above the limit established by the Brazilian legislation of 290 mg/kg. The values of Zn in mussels were under Brazilian guideline and varied between 51.46 and 78.64 mg/kg. Mena zinc concentrations before and after transplantation were similar for *P. perna* (Table 9). Both mollusks presented zinc concentrations within the range of values for mussels and oysters in Brazilian coast found in previous studies (Table 10).

Copper concentrations for mussels and oysters varied between 13.12-15.55 mg/kg and 13.17-32.24 mg/kg, respectively, exceeding the limit established by Brazilian legislation (30 mg/kg) in C. *rhizophorae*. These results were also comparable to the ones registered in other parts of the Brazilian coast (Table 10) except for the ones found by Lino et al. (2011) in the southeast coast of Brazil, which reached up to 3,200 mg/kg of Cu in *P. perna*. Copper values found in the present study were close to the ones found in some other coasts around the world, such as China and Venezuela (Table 10). These data corroborate the study of Rojas de Astudillo et al. (2005), who tested oysters and mussels to obtain similar patterns for these metals, reaching up to ten times higher concentrations of Zn and Cu in oysters. This result can be explained by the ability of oysters to tolerate high concentrations of Zn and Cu without affecting their normal development (Soto-Jimenez et al., 2001). Among the studied elements Cu concentration in *P.*

perna was the only one who showed an expressive increase after transplantation to the estuary, suggesting higher levels of copper in the estuarine channel, and corroborating the results obtained by Kumar et al. (2015) at the same location, who found Cu concentrations between 6.24 and 13.55 mg/kg in *P. perna* mussels. Kumar et al. (2015) found a direct and significant correlation between Cu concentrations in the sediments and *P. perna*' tissue in the estuarine system of Vitória Bay, suggesting the influence of sedimentary metal concentrations in bioaccumulation. A significant source of sedimentary Cu in the study area could be originated from antifouling paintings due to intense port activities in the study area.

Cr presented values high above the Brazilian legislation (0.58 mg/kg) for both mussels and oysters in every station (Fig. 20). The solubility of Cr is driven by pH of the water and under neutral to alkaline conditions Cr precipitates whereas under acidic pH it tends to solubilize (Oliveira, 2012). Values of pH found in the present study, between 5.83 and 6.64, corroborate the hypothesis of soluble Cr in the water column, which would facilitate the uptake by filter feeding organisms, such as mussels and oysters. Chromium concentrations were within the range of values of this element found in mollusks from other coastal areas of Brazil (Table 10). Mean Cr concentrations in *P.perna* before and after transplantation presented little difference.

Iron concentrations in oysters were much lower than in mussels, except for BMP2, where the highest value of Fe was registered, 868.96 mg/kg in an area of lower salinity. Mussels presented higher Fe concentration in the station with higher salinity (>30) (Fig. 20). and concentrations varied from 283.18 to 1,962.3 mg/kg. Mena iron concentrations in *P. perna* before and after transplantation presented a little decrease (Table 9), however values were still within the range of values found by Kumar et al. (2015) in the same species of mussel in the estuarine system of Vitória in the higher salinity area (150.77-1964.34 mg/kg).

Arsenic concentratins in mussels and oysters exceeded the limit allowed by Brazilian legislation (1 mg/kg) in every station (Fig. 20). Concentrations of As were higher in mussels, except for BMP3, where the maximum arsenic concentration was registered for oysters, 4.36 mg/kg (Fig. 20). Concentrations of As in *P. perna* varied between 3.07 and 9.19 mg/kg, reaching the maximum value at the BMP4 with the highest salinity value (>30). For *C. rhizophorae*, arsenic concentrations varied from 2.22 to 4.36 mg/kg, with increasing values towards the estuary mouth, except for BMP4, where As concentration lowered again, what could be an effect of flocculation due to higher salinity (Fig. 20). Mean arsenic concentration in *P. perna* was much higher before transplantation (Table 9). The lowering of As concentrations after transplantation into the estuarine channel suggests the depuration of mussels. Okay et al. (2016) also registered depuration of trace elements for mussels transplanted from farm to coastal areas.

Arsenic concentrations in the present study are within the range of values found in mollusks along other Brazilian coastal areas (Table 10). The results of the present study corroborate the ones found by Martins et al. (2020), who registered high As values in sediments in the study area, and, although mollusks do not feed directly from substrate, elevated concentrations of arsenic in the study area suggest anthropogenic input, and might as well reflect the pollutant concentrations in seafood. Among the several variables that involve the accumulation of trace elements by mollusks, the filtering and selection capacity of each of the evaluated species should be highlighted. According to Tang and Riisgard (2017), each mussel can filter from five to ten liters of seawater per hour and from 15 mg to 150 mg of organic and inorganic particles per hour, thus reducing turbidity and removing nutrients from the water. Oysters, in turn, can also filter up to ten liters of water per hour, but are considered more selective in adverse conditions such as polluted environments (Rodrigues and Carvalho Filho, 2011).

Elevated arsenic body burdens can be a result of increased As in sediments and water column, and is directly related to the organim's feeding mechanisms, also reflecting the proximity with the source (Taylor et al. 2017). In seawater As concentrations are usually low with little variability, ranging around 1-5 µg/L, and As(V) is the predominant form. In freshwater systems, arsenic is also predominately in inorganic form, however, concentrations are generally higher if compared to the ones in seawater. In spite of low concentrations of As in seawater, much higher values may be found in seafood, what can be due to transformation of inorganic arsenic into its organic forms at the base of the marine food web. The arsenic compounds in sea food are divided into classes considering their structures and properties and, among these substances, arsenobetaine (AB) is the major As species in most shellfishes. For bivalve mollusks AB corresponds to a significant fraction of the soluble form of As. Arsenobetaine is a highly stable compound to breakdown or metabolize, and its source and formation in the foodweb is still unclear (Taylor et al., 2017). Concentrations of arsenic in the tissue of marine invertebrates may vary between 1 and 100 mg/kg (dry weigh) mostly under the form of arsenobetaine and usually inorganic As composes up to 22% of total arsenic (Klarić et al. 2004).

Usually with the decrease of salinity it is expected an increase in trace element uptake by marine organisms, although there are exceptions. Kumar et al. (2015) found maximum concentrations of trace metals in mussels in lower salinities areas in the estuarine system of

Vitória bay. The mussel *P. perna*, however, is a high salinity water inhabitant and the referred author registered highest concentrations of Fe, Mn, Zn and Cu in this species at higher salinity (>30) areas, corroborating the results of the present study, which also presented maximum concentrations in the stations with salinity above 30. Oysters, on the other hand, presented maximum values of metals and As (except Ba) in stations with lower salinity values, corroborating the hypothesis that bioaccumulation of metals by mollusks are favored by lower salinities.

Table 10 – Concentrations of heavy metals (mg.kg-1 dry weight) determined in the soft tissue of bivalve molluscs, collected from different coastal areas of the world.

Reference	**Specie**	**Location**	**Al**	**Ba**	**Zn**	**Mn**	**Cu**	**Cr**	**Fe**	**As**
Brazilian legislation	**-**	**-**	**-**	**-**	**290**	**-**	**174**	**0.58**	**-**	**5.8**
Shulkin et al. (2003)	*Crassostre gigas*	Russia	-	-	5284	2.5	3946	-	-	-
Shulkin et al. (2003)	*Crenomytilusgrayanus*	Russia	-	-	616	1.3	108	-	-	-
Fung et al. (2004)	*Pernaviridis* and *Mytilus edulis*	China	-	-	76.89 – 141.3	-	1.83 – 22.58	2.18 – 10.93	120.6 – 681.5	12.93 – 24.55
Rojas de Astudillo (2005)	*Crassostrea rhizophorae*	Venezuela	-	-	488 ± 22	-	14.6 ± 0.5	0.23 ± 0.02	-	-
Rojas de Astudillo (2005)	*Pernaviridis*	Venezuela	-	-	18.8 ± 0.7	-	1.8 ± 0.1	0.22 ± 0.01	-	-
Orescanin et al. (2006)	*Mytilus galloprovincialis*	Europe	-	-	105 – 127.9	4.5 – 7.0	5.3 – 6.9	1.8 – 1.9	85.8 – 261.3	13.3 – 15.6
Tureck et al. (2006)	*Crassostrea gigas*	Santa Catarina, Brazil	-		53.1 – 184.6	-	1.27 – 53.62	-	-	0.17 – 2.58
Catharino et al. (2008)	*Pernaperna*	Ilhabela/SP, Brazil	-	-	15.4	-	-	0.09	22	1.88
De Souza et al. (2011)	*Crassostrea rhizophorae*	Bahia, Brazil	202 ± 3.26	-	1273 ± 13.5	18.9 ± 0.31	28.4 ± 0.30	0.15 ± 0.00	346 ± 8.97	5.55 ± 0.37
Nice and Fisher (2011)	*Mytilus edulis planulatus*	Australia	12 - 61	-	6 – 9.6	3.3 - 28	**1**.7 – 2.2	0.05 – 0.17	-	0.46 – 0.75
Kumar et al. (2015)	*P. perna and Mytela*sp.	Vitória, Brazil.	-	-	80 - 120	8 - 55	5 - 40	-	100 - 2000	-
Lino et al. (2016)	*Pernaperna*	Rio de Janeiro, Brazil	-	-	33 - 65	3 - 40	3 – 3,200	1.0 – 7.5	104 - 477	-
Campolimet al. (2017)	*Pernaperna*	Santos Bay, Brazil	1,000 – 1,750	-	59 - 170	5.0 - 9.6	4 - 8	0.5 - 2.5	430 - 770	-

Human Health Risk Assessment (HHRA)

Human exposure to trace metals and metalloids for non-occupationally exposed population is achieved through diet and the relationship between these elements and increasing trend of incidence rate of cancers among human population is object of several studies (Jarup, 2003, Yap, 2011, Koki et al., 2015, Antoine et al., 2016). Human health risk assessment characterizes potential adverse health effects on human population exposed to toxic elements, among them, organic pollutants, trace metals and metalloids (Koki et al., 2015). According to Koki et al. (2015), the steps involved in the characterization of HHRA involve hazard identification, exposure assessment, dose-response assessment and, finally, risk characterization. Pollutants can be classified regarding their carcinogenicity and elements considered non-carcinogenic are supposed to have a threshold value, below which no adverse health effects will be observed. Carcinogens, on the other hand, are supposed to have no effective threshold value, which means there is the risk of developing cancer even under low doses exposure. Human health risk assessment regarding exposure to trace metal and metalloids are, therefore, considered of primordial importance (Yap et al., 2011). Despite being considered a significant source of protein for human consumption, shellfish, crustacean and fish are inexorably affected by industrial pollution (Ahmed et al., 2015), and, therefore, ingestion of seafood is one of the main sources of trace metal and metalloids among other pollutants for humans.

Estimated daily intake revealed no potential risk for Al, Ba, Mn, Zn, Cu, Cr and Fe, for average level (ALM) and high level consumers (HLM), since all these elements presented EDI values (Fig. 21 and Fig. 22) under their respective oral reference dose (ORD) (Table 8). For the essential elements Zn and Cu, EDI values were higher in oysters for both ALM and HLM, and significant differences ($p<0.050$) were observed between mussels and oysters for Zn EDI values.

For arsenic, the ORD was surpassed for ALM in mussels at BMP4 (Fig. 21), coinciding with higher salinity values, where Asconcentration was also highest for *Perna perna*. Regarding high level consumers, EDI-HLM for As overcame the ORD at every station for mussels, and at stations BMP2 and BMP3 for oysters (Fig. 22), suggesting health risk through ingestion of these organisms. Ingestion of arsenic-contaminated foods and water is a primary route of exposure to this element (Keil et al., 2011). Approximately 95% of an ingested content of trivalent arsenic compounds is absorbed from the gastrointestinal tract (Rossman, 2007).

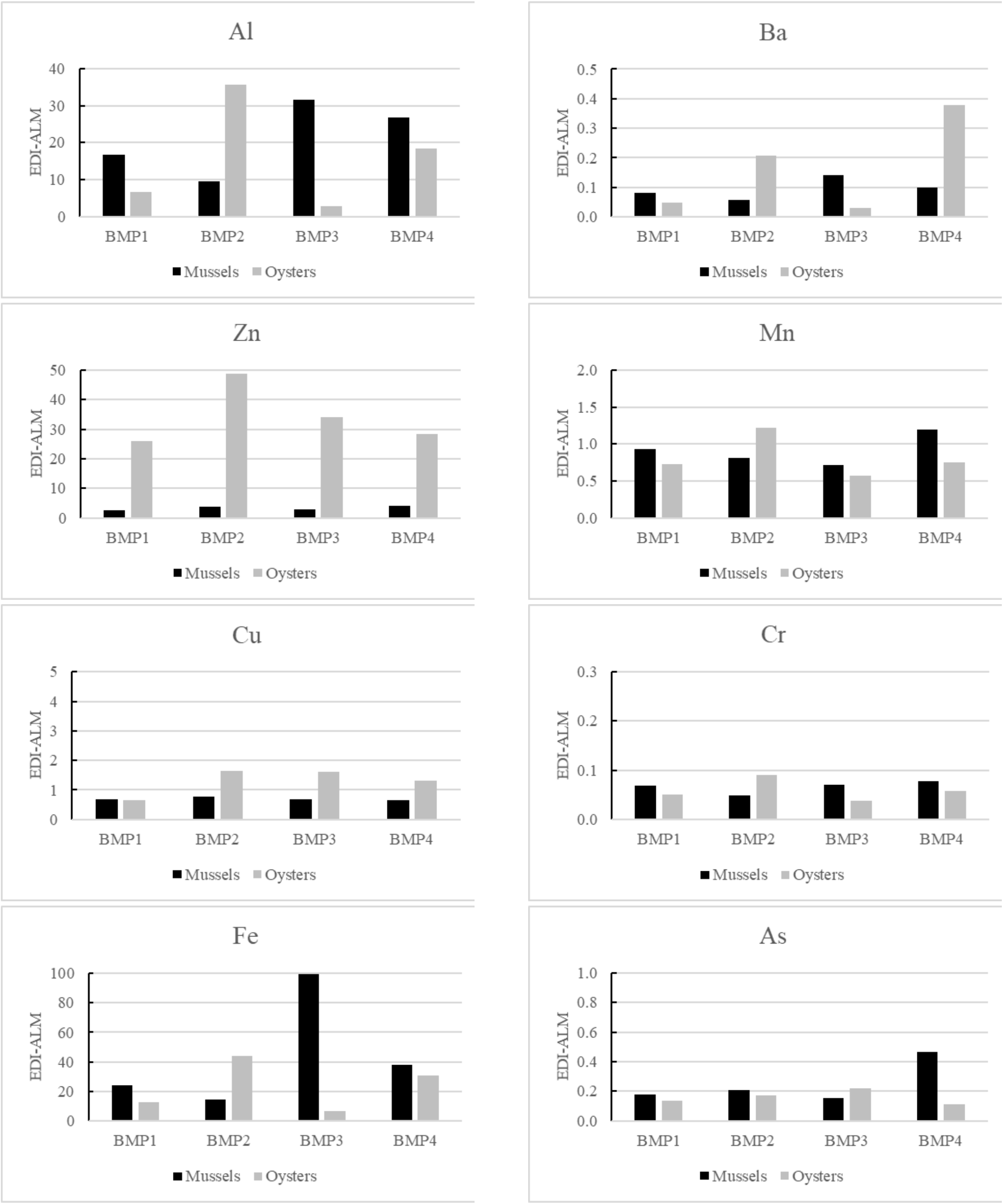

Fig. 21 – Estimated Daily Intake (µg/day/kg body weigh) for average level consumers (ALM) regarding Al, Ba, Zn, Mn, Cu, Cr, Fe and As for mussels and oysters from the estuarine system of Vitória Bay.

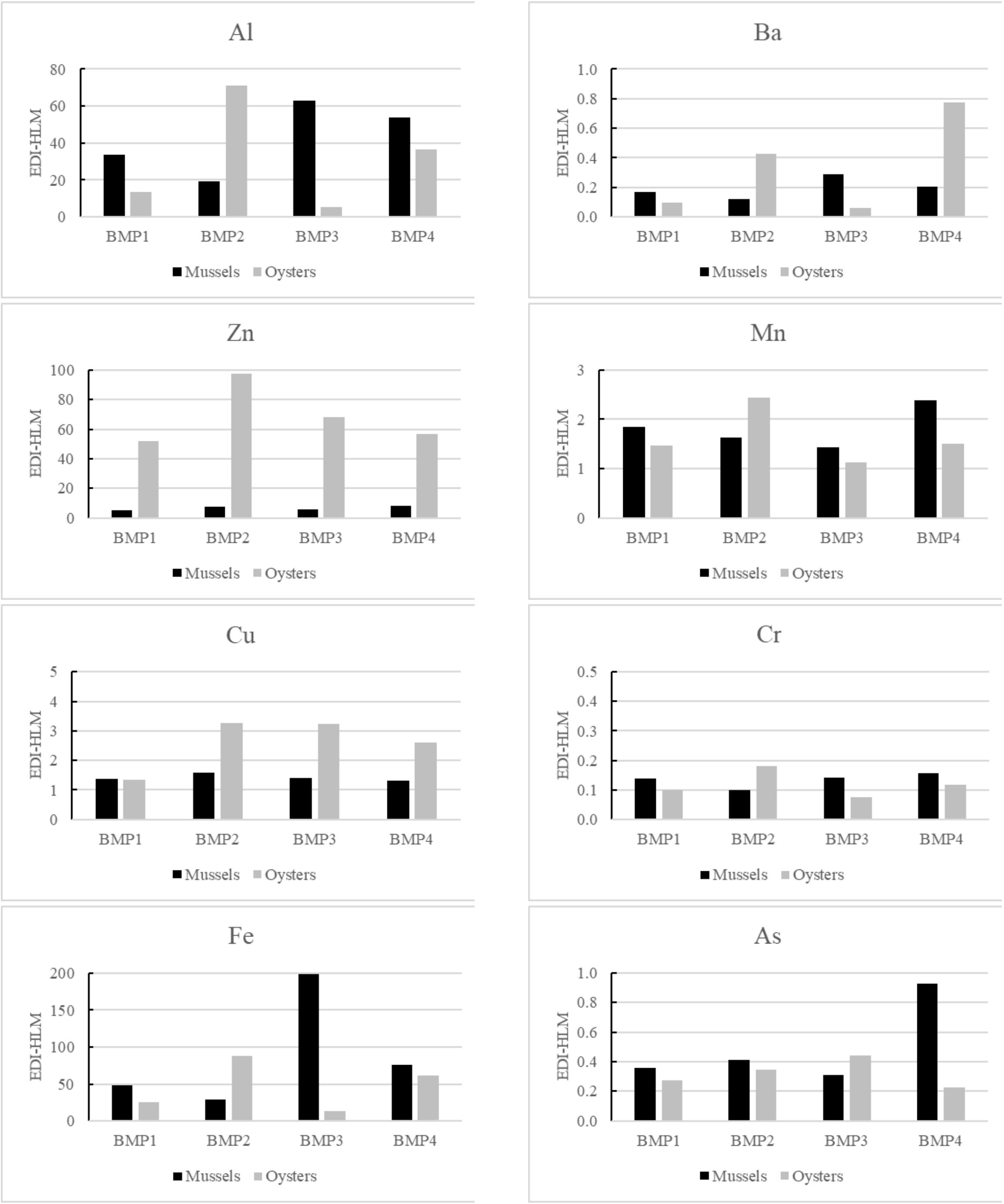

Fig. 22 – Estimated Daily Intake (µg/day/kg body weigh) for high level consumers (HLM) regarding Al, Ba, Zn, Mn, Cu, Cr, Fe and As for mussels and oysters from the estuarine system of Vitória Bay.

A Target Hazard Quotient (THQ) >1 means that the level of exposure to the element surpassed ORD, and therefore, the daily exposure at such level is supposed to cause deleterious human health issues along a lifetime consuming these contaminated organisms.

In the present study, THQ for ALM was <1 for all trace elements, except for arsenic (Fig. 23). Target hazard quotient values for ALM regarding Zn were significantly higher in oysters ($p<0.050$) in every station. For Cu, THQ-ALM were also higher in oysters in every station, except for BMP1, however, no significant differences were observed in relation to mussels. Values of THQ-ALM for Ba were also higher in oysters, but with no significant differences were observed between the two species of mollusks ($p>0.050$).

For As THQ-ALM was 1.55 at BMP4 for mussels (Fig. 23), suggesting health risk issues regarding consumption of mussels.

Results of THQ-HLM followed the trend for average level consumers and presented values <1 for all elements, except As (Fig. 24). Values of THQ-HLM for Zn, Cu and Ba were much higher in *Crassostrea rhizophorae* than in *Perna perna*, however, only for zinc these differences were considered significant ($p<0.050$). Arsenic results for THQ-HLM were concerning since all values were >1 for mussels, and also>1 in BMP2 and BMP3 for oysters (Fig. 24).

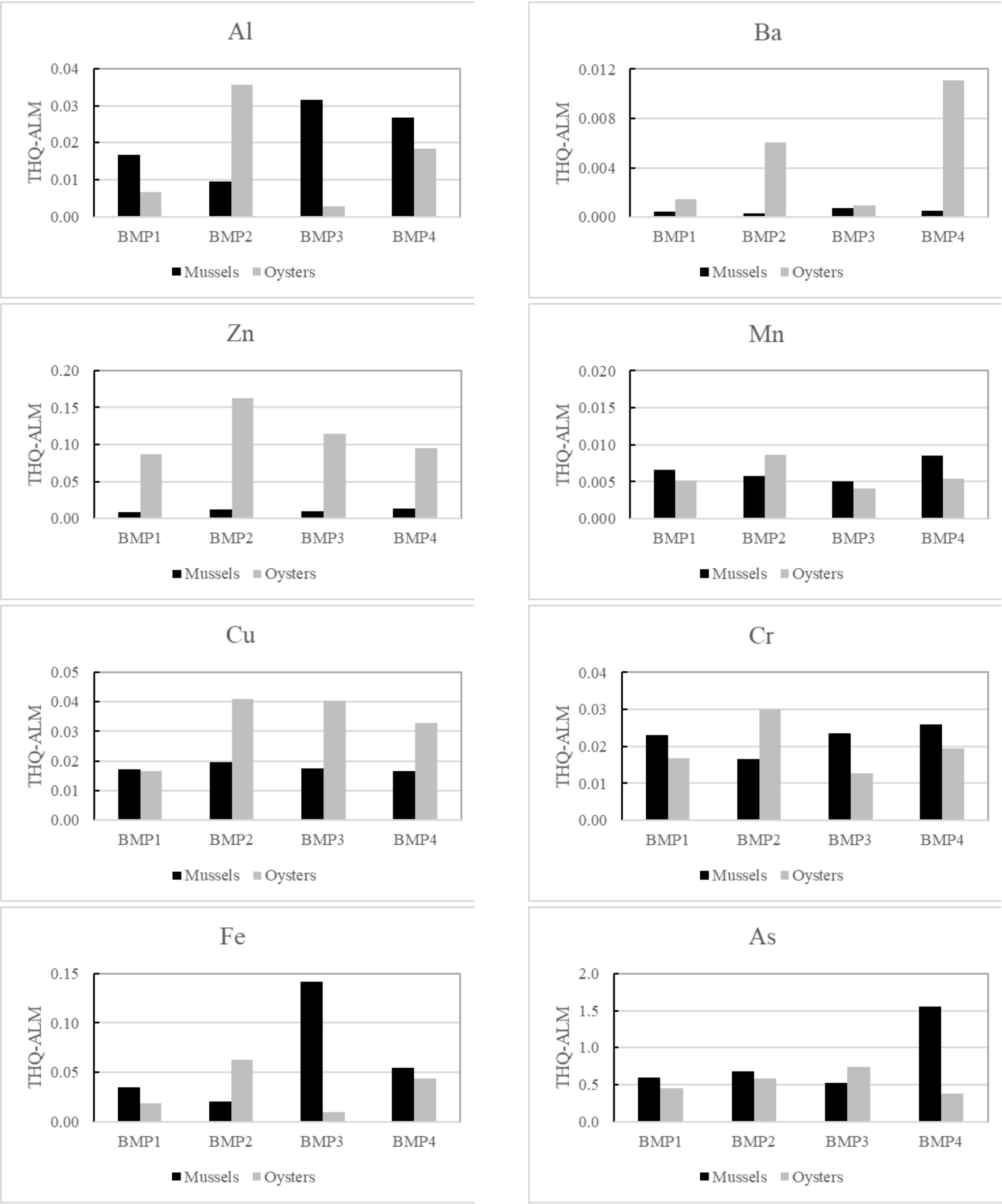

Fig. 23 – Target Hazard Quotient for average level consumers (ALM) regarding Al, Ba, Zn, Mn, Cu, Cr, Fe and As for mussels and oysters from the estuarine system of Vitória Bay.

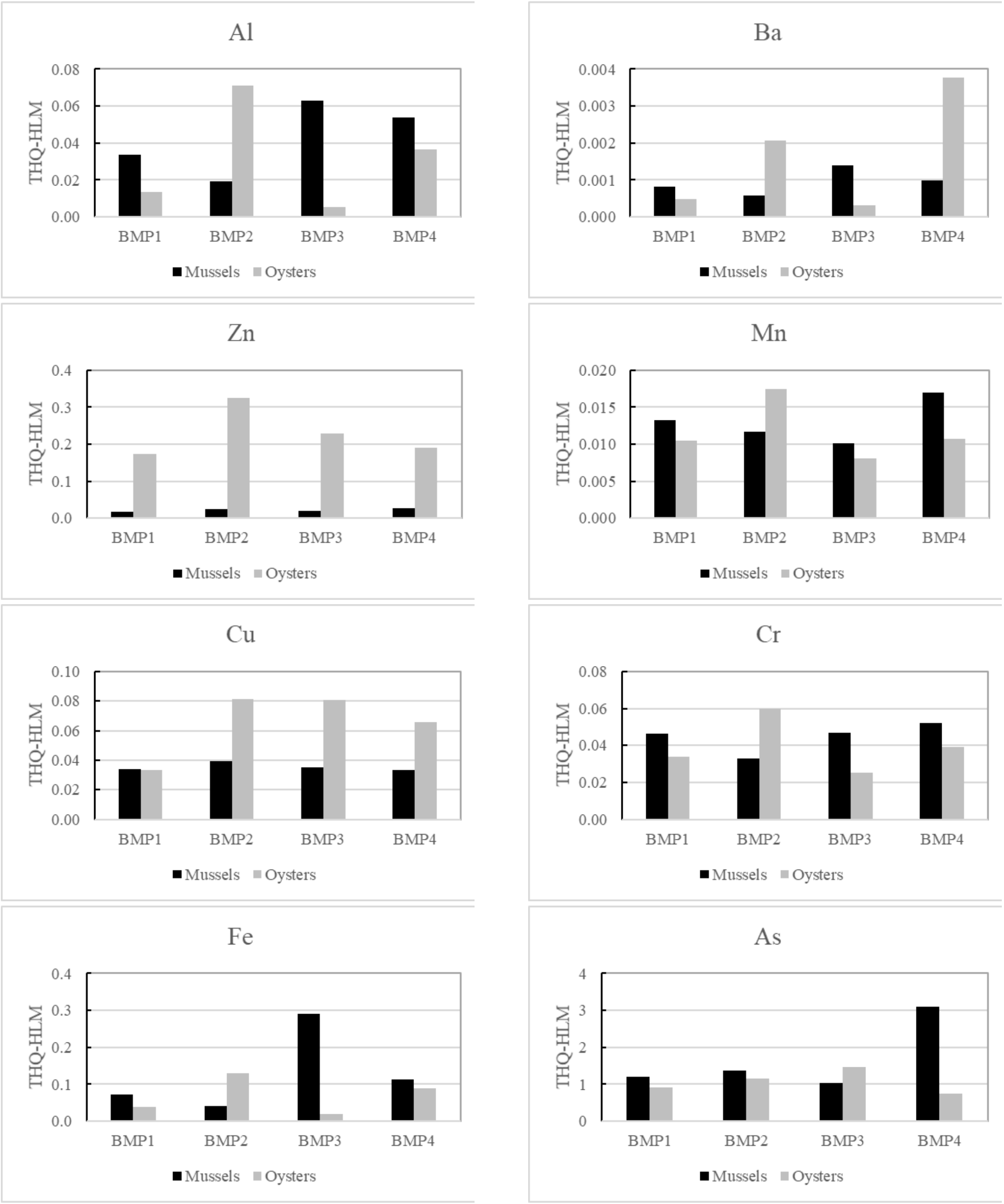

Fig. 24 – Target Hazard Quotient for average level consumers (HLM) regarding Al, Ba, Zn, Mn, Cu, Cr, Fe and As for mussels and oysters from the estuarine system of Vitória Bay.

The Hazard Index (HI) considers the cumulative effects regarding the ingestion of potentially toxic elements, and HI>1 represents human health risk. For average level consumers (ALM), HI was <1 for oysters in every station. For mussels, HI>1 was registered at BMP4, with a value of 1.71 (Fig. 25), suggesting human health risk for mussel consumption at this point. High level consumers presented HI>1 for oysters and mussels in every station, and no significant differences were observed between the two mollusks species ($p>0.050$).

Arsenic is considered carcinogenic and among the elements studied it presented THQ values that suggested risk for human health, and was also the main responsible for HI>1 in oysters and mussels for high level consumers. The Target Cancer Risk (TCR) was calculated for As based on the oral slope established by USEPA (2010). This index considers cancer risk as negligible if TCR$<10^{-6}$ and $10^{-6}<$TCR$<10^{-4}$ values are considered acceptable. Therefore, the value of 10^{-4} for TCR is considered to be the threshold for cancer risk through ingestion of contaminated food by arsenic.

Target Cancer Risk for As was $>10^{-4}$ for average and high level consumers, for both oysters and mussels, with no significant differences between the species ($p>0.050$). Apart from results at BMP3, TCR values were higher for mussels (Fig. 25). Results corroborate the higher uptake of As by mussels in the region of the outer estuary. Results of TCR revealed the possibility of occurrence of health risk issues associated to the consumption of *Perna perna* and *Crassostrea rhizophorae* from the estuarine system of Vitória.

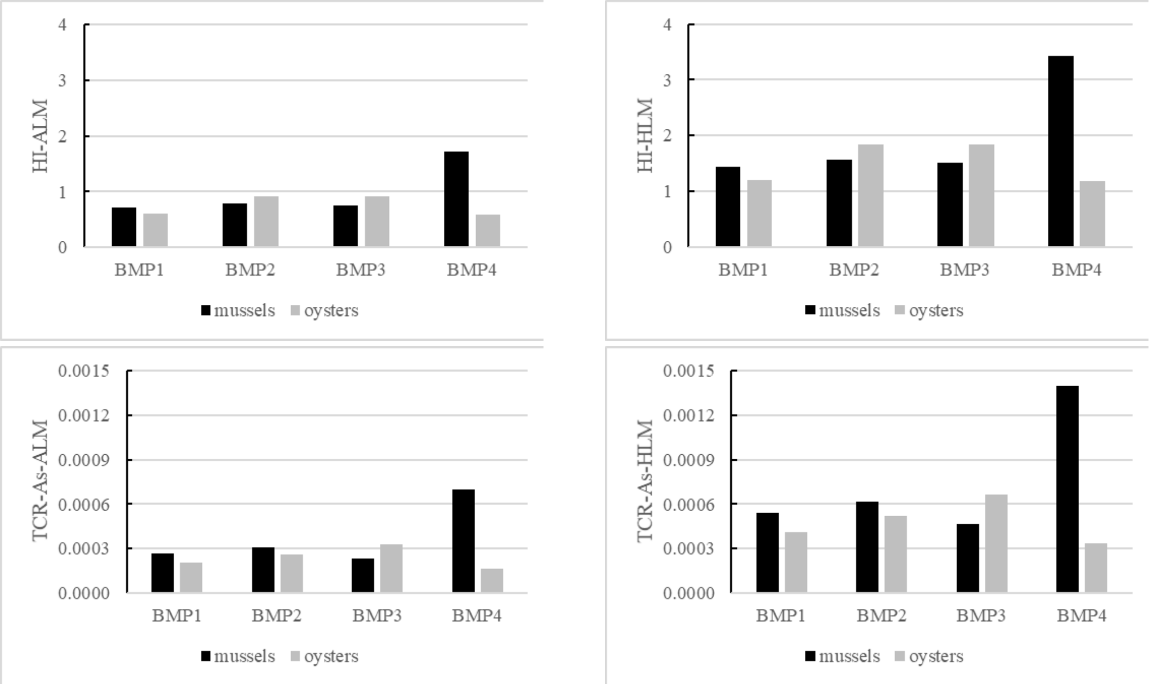

Fig. 25 – Hazard Index regarding Al, Ba, Zn, Mn, Cu, Cr, Fe and As and Target Cancer Risk for As in mussels and oysters for average level consumers (ALM) and high level consumers (HLM) from the estuarine system of Vitória Bay.

Conclusion

In the estuarine channel of Vitória Bay, the transplanted mussels *Perna perna* presented higher concentrations of arsenic and trace metals than local oysters *Crassostrea rhizophorae*. The exception were the essential elements, zinc and copper, which were higher in *Crassostrea rhizophorae* reflecting oysters' ability to hyperaccumulate these metals. Salinity seemed to influence metal and arsenic uptake for both organisms, with *Perna perna* mussels absorbing more arsenic than oysters in higher salinities. For *Crassostrea rhizophorae* oysters, trace metal uptake was higher in the areas of lower salinity in the estuarine channel for most of the studied elements. Considering the Brazilian legislation for metal and arsenic concentrations in food, oysters presented Zn and Cr concentrations above the tolerance limits in every station and higher uptakes in the lower salinity portion of the estuary. Mussels also presented Cr concentrations above the tolerance limit in every station and higher absorption in the higher salinity zone. Arsenic concentration above the tolerance limit allowed by Brazilian legislation also occurred for *P. perna* and *C. rhizophorae* all along the estuarine system. Hazard Index presented health risk for consumption of mussels and oysters from every station regarding high level consumers. Arsenic posed threat to human health as suggested by Edible Daily Limit for *Perna perna* and *Crassostrea rhizophorae* considering high level consumers, which also influenced the results of Hazard Index for the same class of consumers. Target Cancer Risk suggested probable human health deleterious issues associated with the consumption of *Perna perna* and *Crassostrea rhizophorae* from the estuarine system of Vitória Bay.

Results of the present study represent a preliminary evaluation of the estuarine system of Vitória Bay concerning human health risk through the ingestion of mollusks. A more detailed monitoring of the system including evaluation of water and sediments, besides biomonitoring organisms is needed to understand the local dynamics aiming at the potential risks of seafood consumption since mariculture is a relevant economic activity in the Brazilian coastline.

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ARTIGO 3 – OCCURRENCE OF MICROPLASTICS AND HEAVY METALS ACCUMULATION IN NATIVE OYSTERS Crassostrea gasar IN THE PARANAGUÁ ESTUARINE SYSTEM, BRAZIL

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PUBLICADO EM MARÇO DE 2021 NA REVISTA *MARINE POLLUTION BULLETIN*.

RESUMO

A presença onipresente de contaminantes no ambiente marinho é considerada uma ameaça global para os organismos marinhos. Metais pesados e microplásticos são duas classes distintas de poluentes, mas há interações entre esses dois estressores que ainda são pouco compreendidas. Nós examinamos a relação potencial entre metais pesados (Al, Cr, Mn, Fe, Ni, Cu, Zn, As, Cd, Ba, Hg, Pb) e partículas microplásticas em ostras amostradas ao longo do Sistema Estuarino de Paranaguá. Os resultados sugeriram altos níveis de As e Zn nos bivalves, que são destinados ao consumo humano. Partículas microplásticas foram encontradas nas ostras de todos os locais amostrados, demonstrando a disseminação desse poluente no meio marinho e sua capacidade de bioacumulação nas ostras. No entanto, nossos dados não demonstraram uma relação direta entre microplásticos e metais pesados, sugerindo que essas partículas não são a principal rota de contaminação por metais pesados de ostras no Sistema Estuarino de Paranaguá.

Palavras-chave: metais pesados, microplásticos, adsorção, ostras, poluição.

ABSTRACT

The ubiquitous presence of contaminants in the marine environment is considered a global threat to marine organisms. Heavy metals and microplastics are two distinct classes of pollutants but there are interactions between these two stressors that are still poorly understood. We examined the potential relationship between heavy metals (Al, Cr, Mn, Fe, Ni, Cu, Zn, As, Cd, Ba, Hg, Pb) and microplastic particles in oysters sampled along the Paranaguá Estuarine System. The results suggested high levels of As and Zn in the bivalves, which are destined for human consumption. Microplastic particles were found in oysters from all sampled locations, demonstrating the spread of this pollutant in the marine environment and its ability to bioaccumulate in oysters. However, our data did not demonstrate a direct relation between microplastics and heavy metals, suggesting that these particles are not the main route to heavy metal contamination of oysters in the Paranaguá Estuarine System.

Key-words: heavy metal, microplastics, adsortion, oyster, pollution.

INTRODUCTION

Estuaries are important ecosystems that provide food and habitat for a large number of aquatic organisms, in addition to promoting biogeochemical processes for other environments through their connection between watersheds and coastal waters (Barletta et al., 2010; Costa and Barletta, 2016). However, there is an increasing anthropogenic interference in these environments, often caused by activities such as agriculture, industrial development and multiple uses of water, leading to inadequate waste disposal, sewage discharges and flow control (Barletta et al., 2019). Estuarine environments are thus considered important pools of heavy metals and other contaminants (Ip et al. 2004) and have attracted much attention from researchers (Hu et al. 2013; Venkatramanan et al. 2015; Wang et al. 2015; Wang et al. 2014; Xu et al. 2014; Yang et al. 2015; Zhang et al. 2015a; Zhang et al. 2015b).

Heavy metals are widespread contaminants in aquatic ecosystems, presenting particular features like high toxicity, multiple origins, refractory properties, and accumulative behavior that make them an environmental concern (Zhao et al., 2017). Heavy metal pollution can give rise to bioconcentration and bioaccumulation of toxic metals in aquatic species, resulting in long-term negative impacts for human and ecosystem health (Ip et al. 2007).

In the same way, microplastics (MPs) can cause significant environmental impacts, being ingested by marine organisms, especially filter feeders (Sleight et al., 2017; Egbeocha et al., 2018). MPs are plastic fragments smaller than 5 mm; they can be primary, manufactured for addition to certain products, or secondary, formed by physical, chemical and/or biological degradation from larger plastic residues in natural environmental processes (Wang et al, 2018).It is estimated that there are >250,000 tons of plastic in the global oceans (Eriksen et al., 2014). MPs can negatively impact animals through physical mechanisms (Browne et al., 2008), or by their physico-chemical properties (material, size, shape etc.) that can favor its toxicity, varying between several possible situations and interactions (Lithner et al., 2011), as well as via any externally adsorbed materials (Teuten et al., 2009).

Marine species can incorporate pollutants from bottom sediments, suspended particulate material, from the water column or food sources (Laffon et al. 2006). Thus biomonitoring can be an effective approach for evaluating the contamination. Bivalves are good sentinel organisms frequently used to evaluate marine environmental quality (Solé et al. 1994; Porte et al. 2000; Serafim and Bebianno 2001). In addition, they stand out for their wide distribution in Brazil (native species) and in the world, vital ecological niches, susceptibility to pollutant absorption and close connection with marine predators and human health, as described by Li et al. 2019.

At the same time, exposure of aquatic organisms, such as bivalves to toxic contaminants could be a risk to human health if the contaminants are incorporated in the food chain (Lemiere et al. 2005). The aim of the study was to quantify heavy metals and microplastic particles in oysters (*Crassostrea gasar*) from sites distributed along the Paranaguá Estuarine System (PES) and and its possible adsorption ratio.

Study Site

The Paranagua Estuarine System (PES) is located on the north coast of the state of Paraná (SE Brazil) and consists of several regions and environments: to the west, the Bays of Paranaguá and Antonina, and to the north, the bays of Laranjeiras, Guaraqueçaba and Pinheiros and the inlets of Itaqui and Benito. The estuarine shore is occupied by extensive mangroves and hydrographical basins. In addition to the marine influence, the estuary receives considerable freshwater input from several rivers, primarily in the rainy season (summer). Two important harbors are installed in this area, Antonina and Paranaguá; the latter is one of the largest harbor for grain exportation in Latin America, reaching more than 8 million tons of shiploads in 2009 (Martins et al., 2010), posing risks to this bay. In addition, an artisanal fishery and aquaculture are two main economic and social activities in this region. However, the increase in urbanization and industrial activities in coastal regions have increased the level of pollutants in aquatic ecosystems (Pereira et al., 2006). This can lead to environmental contamination and cause health problems to humans and local fauna. Among some of the main pollutants in marine and estuarine environments, heavy metals play a prominent role and, more recently, microplastics have been shown to be important contaminants in aquatic environments (Barletta et al., 2019).

METHODOLOGY

Oysters were collected from 10 sampling stations along the PES (Fig. 26). These sites are representative of the estuary, being close to oyster producing communities, natural banks used for extractivism and containing large human populations, with potential for high environmental pollution.

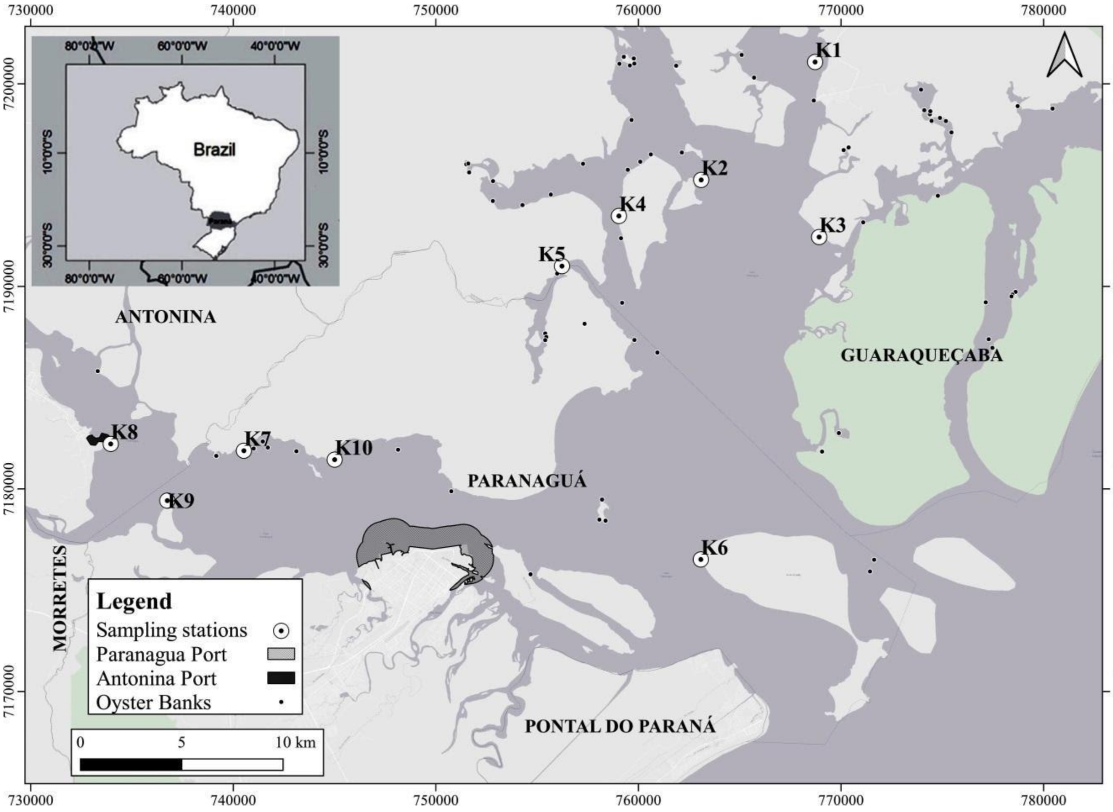

Fig. 26 – Study Area and sampling stations (K1-K10).

Ten oysters (n = 10) were collected at each location, which were called K1 to K10, totaling 100 organisms. In the laboratory, after collection, all oysters from each site were dissected and 30 mg of hepatopancreas from each oyster were extracted, resulting in 300 mg of total tissue for each site. The total tissue was homogenized and divided into equal parts in Falcon tubes, 150 mg of tissue for analysis of metals and 150 mg for analysis of microplastics. In this way, a duplicate of the total tissue of the hepatopancreas was generated for each monitored site.

Metal concentrations (Al, Cr, Mn, Fe, Ni, Cu, Zn, As, Cd, Ba, Hg, Pb) were analyzed in the oyster tissue using an inductively coupled plasma mass spectrometer (ICP-MS). The equipment used was a microwave (Milestone, model MLS 1200, Sorisole, Italy) and an ICP-MS (Perkin Elmer, model Nexion 300 D, Shelton, USA). For each sample, approximately 150mg was weighed in duplicate and then digested in a microwave oven with 2.0, 1.0 and 4.0 mL of HNO3, H2O2 and H2O, respectively. For the determination of the elements in the ICP-MS, argon (99.996% pure. Air Liquide, Palhoça, Brazil) was used as the main, auxiliary and nebulizer gas. The detection and quantification limits for each metal can be seen in Table 11.

Table 11 – Detection limits (DL) and quantification limits (QL) for each metal.

Element	DL mg.kg^{-1}	QL mg kg^{-1}
Al	$4.1E^{-05}$	$1.4E^{-04}$
Cr	$3.9E^{-05}$	$1.3E^{-04}$
Mn	$1.5E^{-05}$	$5.1E^{-05}$
Fe	$2.7E^{-05}$	$9.1E^{-05}$
Ni	$9.0E^{-06}$	$3.0E^{-05}$
Cu	$1.2E^{-04}$	$4.0E^{-04}$
Zn	$8.7E^{-05}$	$2.9E^{-04}$
As	$2.1E^{-05}$	$7.0E^{-05}$
Cd	$4.6E^{-06}$	$1.5E^{-05}$
Ba	$1.3E^{-06}$	$4.4E^{-06}$
Hg	$2.5E^{-04}$	$8.4E^{-04}$
Pb	$2.2E^{-05}$	$7.4E^{-05}$

For the evaluation of microplastics (MPs), oyster tissue was digested using the method of Rochman et al. (2015), with modifications. In brief, the samples were treated with potassium hydroxide (KOH 10%) in a volume (ml) proportional to 3 times the mass of the tissue, incubated with constant shaking at 40 ° C for 48 hours. The digested samples were then placed in 100 ml beakers, NaCl was added up to the beaker limit and left to stand for 15 minutes to allow MPs to float to the surface. Subsequently, 50ml was removed from the surface and was subjected to filtration through 8 µm cellulose nitrate membranes. Finally, the samples were oven dried for 30 minutes prior to visualization and identification of MPs in the dissecting microscope.

The visual identification of MPs was based on previous studies, according to size (<5 mm), varied coloring and morphology: spheres (beads, pellets, and granules - primary MPs), fibers (filaments and lines), foams, films and fragments (fragmentation of larger plastic debris - secondary MPs) (Karami, 2017; Shim et al., 2017; Abbasi et al., 2018; Naji et al., 2018; Neto et al., 2019). The particles with attributes compatible with MPs were observed with a stereoscopic microscope (Olympus BX41). They were categorized according to their color and morphology, counted and measured using the scales present in the microscope lenses. They were then placed on stubs (Fig. 27) and examined by scanning electron microscopy (SEM) with Energy Dispersive X-ray Spectroscopy (EDS) (JEOL; JSM 6390 LV, 15 kV). SEM/EDS it is not commonly applied to determine the type of polymer of the suspected MPs particles, but it allows to determine the elemental composition of the suspect particles. It is noteworthy that all the MPs selected in the aforementioned steps had carbon spikes and morphology compatible with other works, being considered as plastic (Li et al., 2016; Abbasi et al., 2018; Naji et al., 2018; Baptista Neto et al., 2019) and following the evaluations.

To prevent sample contamination with airborne microplastic, all material and equipment used was cleaned with deionized water prior to use and the procedures were performed in a fume hood. The lab coat worn during the assay was 100% cotton and flasks and other apparatus, whenever possible, were made of glass. Despite conducting standardized contamination prevention measures, no blanks were carried out during the extraction and analysis of the samples. Thus, the MP concentrations reported may be slightly overestimated.

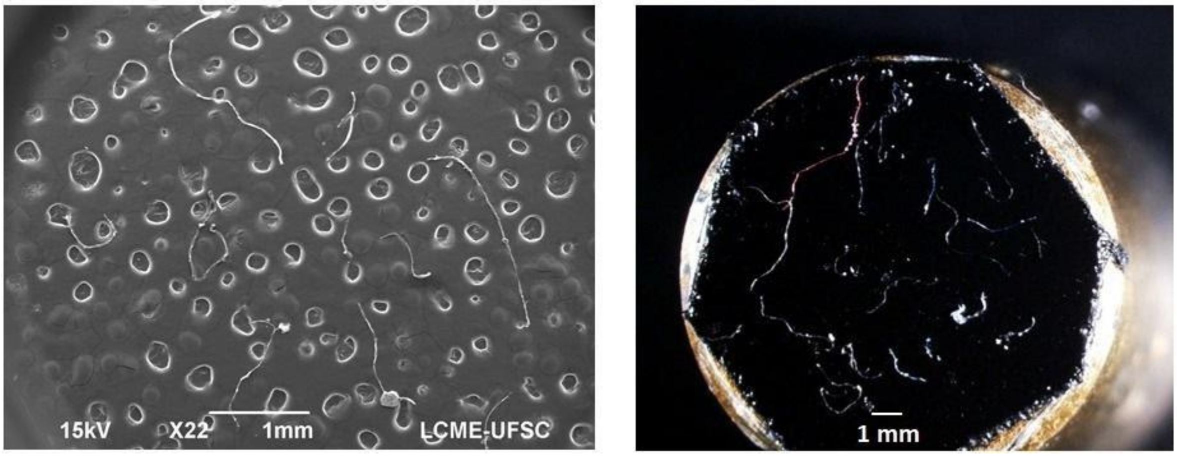

Fig. 27 – Microplastics visualized on stubs: SEM (left) and dissecting microscope (right).

Statistical tests were performed on the software Statistica 7.0®. The normality of the data was assessed using the Shapiro Wilk test. Then, assuming the normality of the data (p < 0,05), in order to evaluate the potential relationship between MP particles and the assimilation of metals by oysters, the Pearson correlation (r) between the values of metals and MPs concentrations was calculated. Values above 0.7 ($p < 0.05$) were considered significant, in agreement with Dancey and Reidy (2006), who consider that r = 0.10 to 0.30 shows weak correlation; r = 0.40 moderate and r = 0.70 to 1 strong correlation. In addition, considering the metal data pattern and that <QL and <DL were values close to zero, it was assumed to be zero for statistical tests, when used.

RESULTS AND DISCUSSION

Some metals are considered essential elements since they participate in important physiological processes (Hogstrand and Haux, 2001). Others, however, are strong toxins causing dysfunctions in a variety of living organisms (Damek-Proprawa and Sawicka-Kapusta, 2003). Unfortunately, only Cr, Ni, Cu, Zn, As, Cd, Hg and Pb have maximum levels in tissues for human consumption established by Brazilian legislation (MS Ordinance nº 685/98 and Decree nº 55.871/1965). Metal levels determined in the present study were compared with the legal tolerance limit (TL) (Table 11). The results (Table 12) were below the tolerance limit for Cr (<detection limit, DL - 05 mg Kg^{-1}), Ni (0.1 - 1.1 mg Kg^{-1}), Cu (<DL - 11.54 mg Kg^{-1}), Cd (0.04 - 0.38 mg Kg^{-1}), Hg (<DL - 04 mg Kg^{-1}) and Pb (<DL - 05 mg Kg^{-1}).

Table 12 – Concentrations of heavy metals (mg.Kg^{-1} wet weight) in oysters collected along the Paranaguá Estuarine System.

Heavy Metal Concentrations (mg Kg^{-1})											
Metal	**TL**	**Sampling Stations**									
		K1	**K2**	**K3**	**K4**	**K5**	**K6**	**K7**	**K8**	**K9**	**K10**
Al	-	7.4 ± 0.4	28.1 ± 5.1	31.0 ± 9.1	<LD	4.9 ± 0.2	11.3 ± 0.5	22.4 ± 0.8	77.3 ± 0.6	12.4 ± 0.6	8.0 ± 0.3
Cr	0.1	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
Mn	-	1.0 ± 0.1	3.4 ± 0.1	2.19 ± 0.03	2.25 ± 0.05	0.9 ± 0.1	1.12 ± 0.06	3.02 ± 0.05	2.31 ± 0,02	1.2 ± 0.1	1.0 ± 0.1
Fe	-	<DL	<DL	<DL	60.80 ± 3.94	2.37 ± 1.49	4.8 ± 1.2	<DL	<DL	<DL	<DL
Ni	5	0.10 ± 0.05	0.4 ± 0.1	1.1 ± 0.1	0.17 ± 0.06	0.15 ± 0.03	0.2 ± 0.1	0.3 ± 0.1	0.8 ± 0.1	0.10 ± 0.04	<DL
Cu	30	<DL	<DL	11.54 ± 0.94	<DL	<DL	<DL	3.5 ± 0.1	6.6 ± 0.5	10.7 ± 1.1	5.7 ± 0.3
Zn	50	245.2 ± 24.4	205.6 ± 7.0	136.2 ± 7.6	177.2 ± 6.2	206.7 ± 4.9	153.7 ± 5.6	303.7 ± 7.5	516.2 ± 9.8	326.5 ± 2.4	232.7 ± 3.1
As	1	0.7 ± 0.1	1.56 ± 0.03	1.0 ± 0.1	0.7 ± 0.1	0.5 ± 0.1	1.2 ± 0.1	1.1 ± 0.1	0.8 ± 0.1	0.9 ± 0.1	1.1 ± 0.1
Cd	1	0.15 ± 0.04	0.38 ± 0.03	0.06 ± 0.02	0.04 ± 0.02	<DL	0.25 ± 0.02	0.14 ± 0.02	0.17 ± 0.04	0.17 ± 0.05	0.20 ± 0.04
Ba	-	<DL	1.1 ± 0.1	1.22 ± 0.05	<DL	0.02 ± 0.01	0.40 ± 0.05	0.33 ± 0.06	3.1 ± 0.1	<DL	<DL

Hg	0.5	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
Pb	2	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL

*TL – Tolerance Limit / DL – Detection Limit

Arsenic levels were also above the legal limit in 5 sampling stations, varying between 0.5 and 1.56 mg Kg-1, which, even so, were less than those found by Hsiung and Huang (2004), McSheehy et al. (2001) and Castello (2010). According to Borba et al. (2009), the toxicity of As compounds is higher in its inorganic form, that is, not available for molluscs. Thus, the observed levels are possibly related to organic As. Even so, this metal must be monitored, since it can cause problems to human health, even resulting in death (Pereira et al., 2009).

Al, Mn and Fe have no legal limits in Brazilian legislation. In addition, there are few studies assessing their concentrations and toxicity (Carvalho et al., 2001; Joksimovic et al., 2011). In the present study, the concentrations can be considered low when compared to some studies of bioaccumulation of these elements in Brazil and around the world (Table 13) (Sokolowski et al., 2004; De Souza et al., 2011; Lino et al., 2016; Campolim et al., 2017). Ba levels were also low (between <LD and 1.22 mg Kg-1), agreeing with Wang et al. (2017) and suggesting the insolubility of this metal in saline waters and consequent low bioavailability in these environments (Lira et al., 2011).

Zn, on the other hand, was present at high concentrations (between 136.2 and 516.2 mg Kg-1) in oysters from all sites, echoing the results of Frías-Espericueta et al. (2005) in Mexico, Amado-Filho et al. (2008) in Bahia and, especially, Castello (2010), who worked on 8 sites along the coast of Paraná. It is important to emphasize that Zn is one of the components found naturally at high concentration in oysters (Sandstrom, 1997).

Table 13 – Average concentrations of heavy metals (mg.kg-1 wet weight) in the soft tissue of bivalve molluscs, collected from different coastal areas of the world.

Reference	**Species**	**Location**	**Al**	**Ba**	**Cd**	**Zn**	**Pb**	**Mn**	**Cu**	**Ni**	**Hg**	**Cr**	**Fe**	**As**
Brazilian legislation	**-**	**-**	**-**	**-**	**1**	**50**	**2**	**-**	**30**	**5**	**0.5**	**0.1**	**-**	**1**
Present study	*Crassostrea gasar*	PES/PR, Brazil.	20.22	061	0.16	250.3	-	1.84	3.80	0.33	-	-	6.80	0.96
Sokolowski et al. (2004)*	*Perna perna*	Gulf of Aden, Yemen	-	-	4.31	30.2	0.06	2.5	17.76	-	-	-	19.51	-
Rojas de Astudillo (2005)	*Crassostrea rhizophorae*	Venezuela	-	-	0.43 ± 0.02	488 ± 22	-	-	14.6 ± 0.5	0.17 ± 0.01	0.04 ± 0.00	0.23 ± 0.02	-	-
Tureck et al. (2006)	*Crassostrea gigas*	Santa Catarina, Brazil	-	-	<1 – 3.08	53.1 – 184.6	-	-	1.27 – 53.62	<1 – 7.59	-	-	-	0.17 – 2.58
Castello (2010)*	*Crassostrea rhizophorae*	PES/PR, Brazil	-	-	0.05	474.3	-	-	57.06	0.55	-	-	-	1.35
De Souza et al. (2011)*	*Crassostrea rhizophorae*	Bahia, Brazil	34.8 ± 0.56	-	-	220 ± 2.33	1.1± 0.0	3.26 ± 0.5	4.90 ± 0.05	-	0.02 ± 0.0	0.03 ± 0.0	59.65 ± 1.55	0.96 ± 0.06
Lino et al. (2016)*	*Perna perna*	Rio de Janeiro, Brazil	-	-	<0.005 – 0.12	5.68 – 11.2	<0.10	0.51 – 6.90	0.51 – 551.7	<0.24 – 0.69	-	0.17 – 1.29	17.9 – 82.2	-
Campolim et al. (2017)*	*Perna perna*	Santos Bay, Brazil	172 - 301	-	0.04 – 0.15	10.1 – 29.3	0.05 – 0.44	0.86 – 1.65	0.69 – 1.38	0.78 – 1.98	-	0.09 – 0.43	74.1 – 132.7	-
Wang et al. (2017)*	*Bathymodiolus platifrons*	China	137.9	-	1793	463.8	2793	2000	579.3	-	-	344.8	-	986.2
Suami et al. (2019)	*Egeria Congica*	Congo	-	-	0.14	112.6	0.34	17.46	16.28	0.49	-	1.06	457.3	-

* Articles with concentrations converted to wet weight according to Ricciardi and Bourget (1998).

Microplastics were found at all 10 sites, with an average of 9.6 items per 150 mg of hepatopancreas (Fig. 28). This result is above most of the studies at national or global level (Li et al., 2016; Teng et al., 2019; Martinelli et al., 2020). However, it should be remembered that our work used a pool of oysters and extracted only hepatopancreas, unlike the others studies that, in general, use only one animal and/or the whole body. It is important to note that hepatopancreas in molluscs is constantly studied and used to indicate the bioaccumulation of substances (Livingstone and Farrar, 1984; Le Pennec and Le Pennec, 2001). Still, comparatively, we consider a high concentration of MPs in Paranagua Estuarine System.

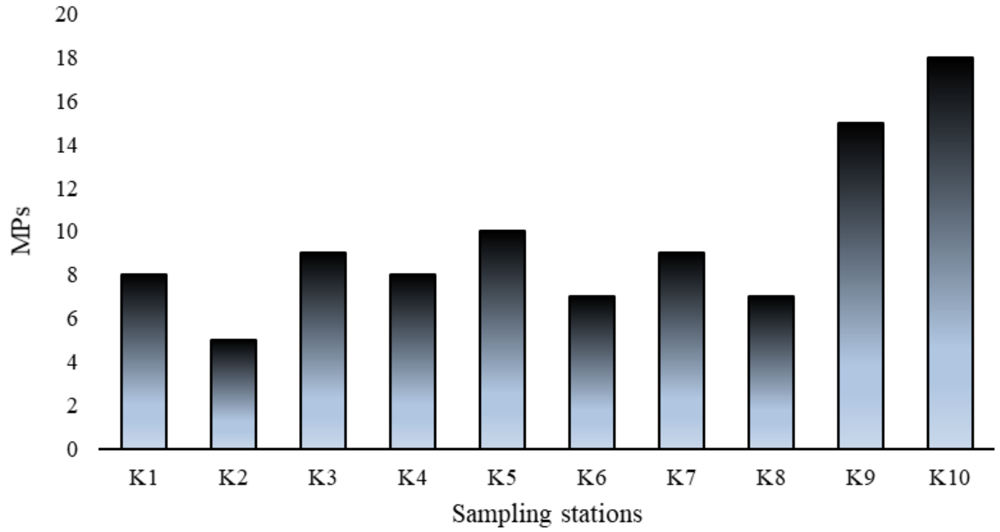

Fig. 28 – Abundance of microplastics in oysters along the Paranagua Estuarine System – items/150mg of hepatopancreas.

The greatest amount was observed at sampling stations K9 and K10, located in front of the Antonina and Paranaguá harbors, respectively. This confirms that aquatic organisms, especially filter feeders, can bioaccumulate MPs and contribute to their magnification through the trophic chain (Li et al., 2019).

Contamination by MPs was, indeed, expected, since these contaminants have already been observed throughout the Paranaguá Estuarine System (Possatto et al., 2015), as well as in Estuaries in South America (Fok and Cheung, 2015; Silva-Cavalcanti et al., 2017; Pazos et al., 2018) and around the world (Wessel et al., 2016; Abbasi et al., 2018; Wang et al., 2018; Conley et al., 2019). We have confirmed that they are taken up by oysters in the Paranaguá estuary.

Microplastics could be divided as to type only into fibers and fragments, with both being found in all locations, although fibers made up at least 60% in all samples, with an average of 78%

considering all sampled locations (Fig. 29). The predominance of fibres in environmental MPs has been observed in several studies around the world (Li et al., 2016; Davidson et al., 2016; McGoran et al., 2017; Li et al., 2018; Sun et al. 2018; Li et al., 2019; Zhu et al., 2020). There are many sources of fibers, as well as other MP fragments; these include the textile industry, wastewater, domestic and industrial sewage, atmospheric deposition and fishing activities (Zubris and Richards, 2005; Hartline et al., 2016). The fact that the samples in the current project were all taken from near ports and fishing locations like the municipality of Guaraqueçaba may have contributed to the low variation in MP levels detected in the oysters of the Paranaguá Estuarine System.

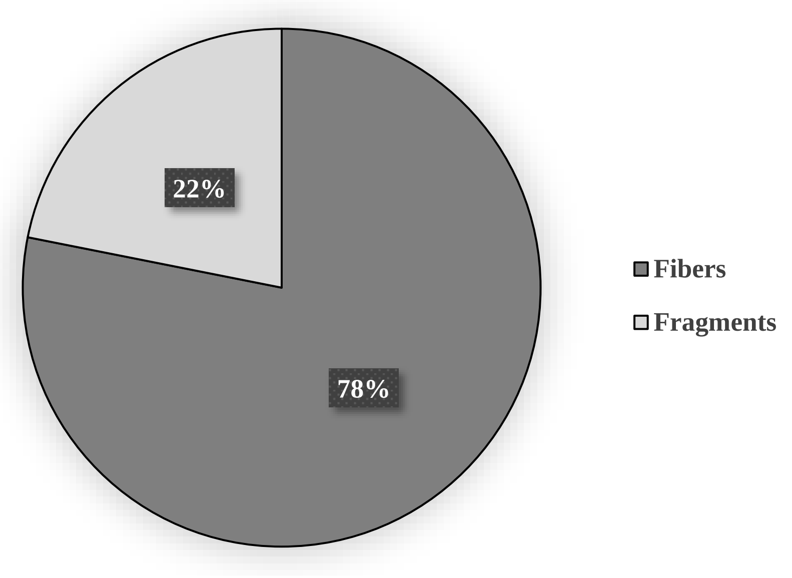

Fig. 29 – Proportion of fibers and microplastic fragments found at all sites.

There is a high variation in the characteristics of microplastics around the world, whether by type, color or size, often justified by activities adjacent to fishing environments and methods of analysis (Auta et al., 2017). The polymers analyzed in the dissecting microscope and by SEM-EDS (Fig. 30 and Fig. 31showed various colors, with the following predominance: blue> black> red> transparent> yellow> purple. The predominance of blue fibers is in agreement with other studies in Brazil and China, for example those of Neto et al. (2019) and Zhu et al. (2020).

MP sizes varied between 0.03 mm (mainly fragments) and 5.0 mm (fibers), which was within the range of filters used. This is similar to that defined for the first time by Thompson et al. (2004) as microplastic, with a variation from nanometers to 5.0 mm. Lusher et al. (2017)

compiled laboratory studies on the ingestion of MPs by several animals; the range of particle size ingested by oysters varied between 0.0001 mm and 1 mm. Some of the fibers found in the present study are outside this range (Fig. 30 and Fig. 31), suggesting that tests with longer MPs should be performed to determine the true limits.

Fig. 30 – MP fibers and fragments under the dissecting microscope.

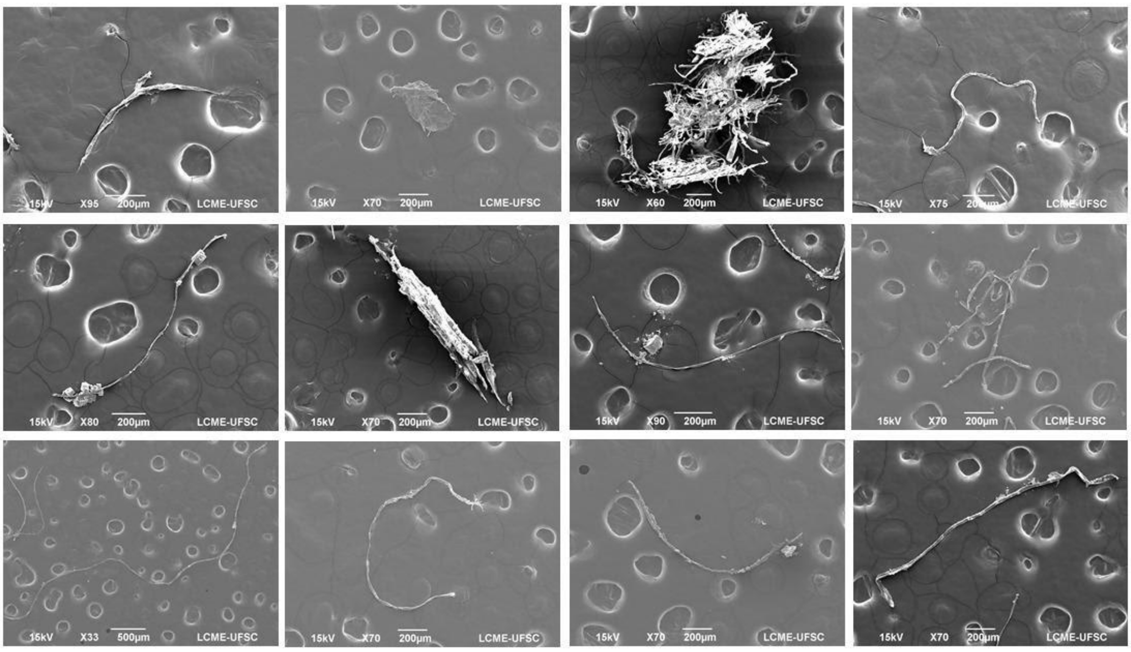

Fig. 31 – Fibers and fragments in the SEM.

The analyses carried out by SEM-EDS detected the presence of the metals Hg, Al, Ni, Cr, Pd, Fe and As on the surface of the MPs, in addition to other elements such as Si, B, Fl and components of the salts used (Na, Cl, Mg and K) (Fig. 32). In agreement with other authors

(Holmes et al., 2012; Brennecke et al., 2016; Auta et al., 2017; Foshtomi et al., 2019), it was possible to identify some interaction between MPs and metals and the potential of filter feeders to accumulate and carry metals by their uptake of MPs.

Thus, it is assumed that microplastics can adsorb heavy metals (vector), especially as observed in other studies (Auta et al., 2017; Foshtomi et al., 2019; Guo and Wang, 2019) and according to the first view in the SEM-EDS. Even so, the statistical test did not show a direct relationship between the concentrations of metals and MP particles; the correlation was weak for all metals (bellow 0,3), according to the standards described by Dancey and Reidy (2006). Despite the high concentrations of some metals, the existence of plastic particles does not seem to have a significant impact on the bioavailability of these trace elements in the case under analysis. This relationship should be better tested in the laboratory or with specific methodologies for adsorption tests among pollutants, as listed by Verla et al. (2019).

Some possible reasons why no correlation was found are:

1)The adsorption of metal ions is usually carried out through the mechanism of electrostatic interactions and there are many factors that affect this interaction, such as the conditions of the medium (water salinity, pH, ionic strength) and the MPs (mainly, the polymer type, their functional groups and polarity) (Gaylard, Neto and Fonseca, 2020). For example, Pb has a greater affinity with PVC MPs, because PVC has the chlorine functional group, which is polar and generates complexations with the Pb ion, however, PVC is not a common polymer in the form of PMs, unlike PP, PE, PS, for example (Gao et al., 2019).

2) Although the adsorption studies in the laboratory assess the ideal conditions (controlled concentrations, 1 single contaminant in pure medium, 1 type of MPs, etc.), in fact, it is much more complex. The study by Wang et al. (2018b) determined that the combined adsorption of heavy metals and the antibiotic levofloxacin can be beneficial or negative (in terms of adsorption) depending on the type of heavy metal present. This is due to the complexations that occur between some heavy metals and some polymers. Generally, pollutants "compete" for spaces to be adsorbed on microplastics or, in the specific case of ions, they generally compete for spaces with other substances (Torres et al., 2020). The salinity of the water can also affect. For example, Qiu et al. (2019) mentions that the adsorption of polyhalogenated carbazoles (PHCs) in PVC MPs decreased with increasing salinity of the medium, due to the fact that the ions displaced the PHCs from some locations on the PVC surface. So, the metals may be being displaced by other contaminants that are not evaluated and have a greater presence.

The main elemental components of hydrocarbons (carbon, oxygen and nitrogen) were also detected (Fig. 32), mainly with carbon spikes, confirming the samples as MPs, as defined by Shim et al (2017), who state that confirmatory analysis of MPs involves physical characterization (such as microscopy) followed by chemical characterization (for example, spectroscopy), also used by Liu et al. (2019).

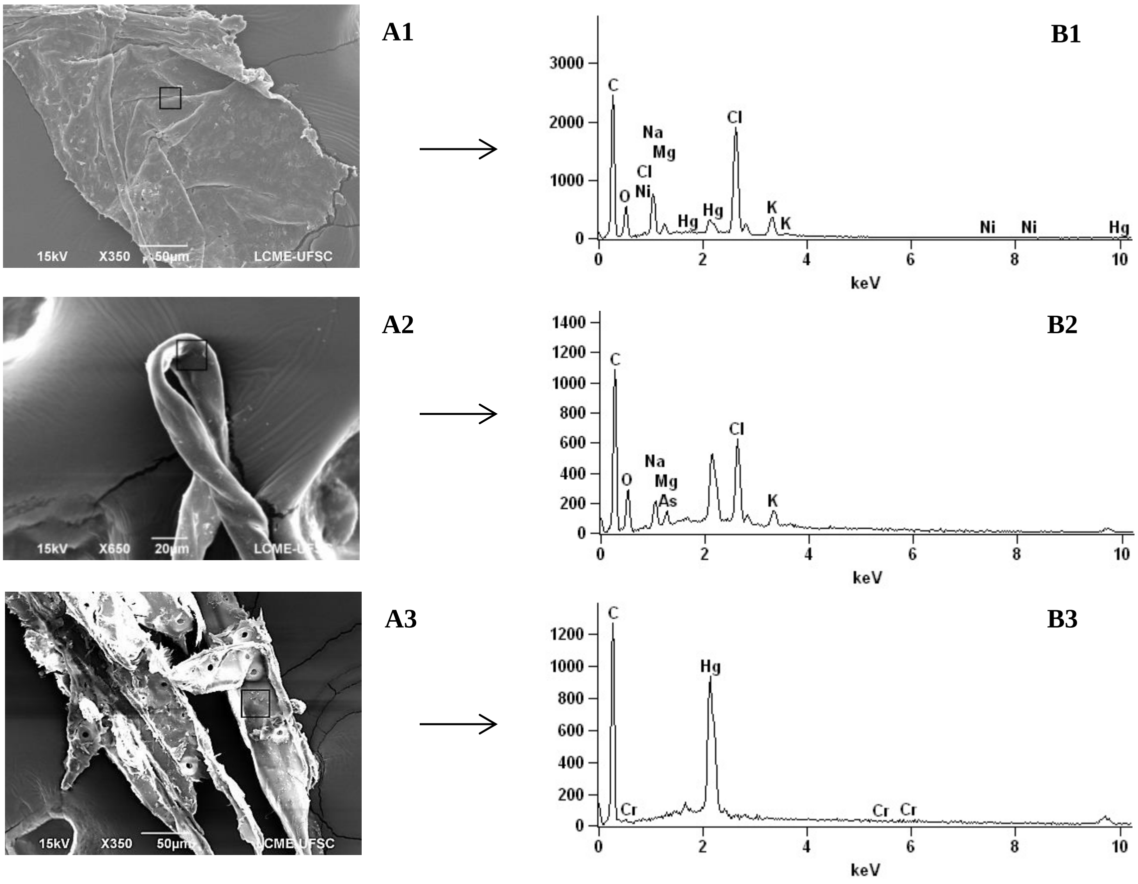

Fig. 32 – SEM of MPs, fragment (A1), fiber with regular characteristics (A2), and agglomerate of fibers with irregularities (A3), together with their respective EDS analyses (B1, B2 and B3).

The images from the SEM revealed different surface characteristics of microplastics, varying in their morphology, roughness, porosity and conservation. In line with the observations of Liu et al. (2019), even with a variation in physical characteristics in the middle of the fragments and/or fibers, all showed wear marks at least at the ends. Such variations may suggest different sources, materials and type of transport of the MPs, which could also influence the adsorption of metals on their surfaces. Holmes et al. (2014) tested the adsorption of several metals by plastics under different conditions and observed that polymers, specifically pellets in their case, with a higher degree of exposure to the environment were more susceptible to metal adsorption than virgin pellets. In the present study, however, it was not possible to observe relevant qualitative and quantitative differences between the metals adsorbed on more, or less, degraded MPs, but only the variable uptake overall (Fig. 32).

The type of polymer may also affect adsorption capacity for different metals (Mao et al., 2020). Li et al. (2019) observed that polystyrene, for example, has a negative charge and can directly adsorb positively charged metal ions, and this process may be related to the physical and/or chemical properties of the metal ions and MPs themselves. There is a need to identify the affinity between the various metallic elements and the composition of the plastic, as suggested by Gao et al. (2019). The aquatic environments and their physicochemical parameters must also be considered. For instance, authors working on estuaries have studied the influence on sorption of environmental factors such as salinity, pH and Dissolved Organic Matter (Holmes et al., 2014; Zhang et al., 2020).

Mercury stood out in our results, being present in over 70% of the MPs. However, Hg values were low in mollusk tissue (below the detection limit) and hence it is not possible to say that this adsorbed Hg directly influenced bioaccumulation. The Hg may have been in its non-bioavailable state. This metal is found abundantly in bottom sediments, where it is generally deposited in the insoluble form as HgS (Micaroni et al., 2000). Nevertheless, Hg has been found to show increased toxicity when mixed with plastics in experiments with Dobalent Sea bass labrax, where it caused oxidative stress in juveniles of the species (Barboza et al., 2018).

Arsenic was found adsorbed in one of the samples at site K10, where tissue samples also yielded high values of this metal. Like Hg, As tends to accumulate in the bottom sediment, being mobilized by various processes and affecting aquatic biota, such as oysters in crops or natural banks (Magalhães et al., 2001). The availability of As for molluscs or adsorption to MPs may be linked to its uses as a leather and wood preservative, an additive in lead and brass alloys, and in pesticides (Eisler, 1994).

It is important to highlight that some heavy metals may be incorporated into plastics during their manufacture, generally acting as stabilizers, anti-oxidants and dyes (Murphy, 2001). Metals like Cu, Zn, Cd, Hg and As, for example, can also be released from anti-fouling paints that are a source of heavy metal contamination in ports and marinas through paint deterioration (Almeida et al., 2007), serving as an exogenous source of the pollutant in the aquatic environment.

The statistical test did not show a direct relationship between the concentrations of metals and MP particles; the correlation was weak, according to the standards described by Dancey and

Reidy (2006). This relationship should be tested in the laboratory or specific methodologies for adsorption tests among pollutants, as listed by Verla et al. (2019). Despite the high concentrations of some metals, the existence of plastic particles does not seem to have a significant impact on the bioavailability of these trace elements.

CONCLUSION

Oysters were confirmed as effective bioindicators of environmental quality. The results identified a potential risk to human health from oysters produced for consumption from PES, taking into consideration the high levels of heavy metals such as As and Zn. Likewise, MPs were found at all sampled locations, confirming the spread of this pollutant in the marine environment; its ability to bioaccumulate in oysters was also shown. However, it was not possible to establish a direct relationship between the pollutant concentrations in oysters and MPs.

The results suggest that bivalves should be quality controlled by an increased number of control stations, which should identify metals and microplastics in the Paranaguá Estuarine System. This is an environmentally important location on the coast of the state of Paraná, which has great gastronomic potential for seafood, as well as being the location of port activities that can influence the marine and estuarine ecosystem.

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CONSIDERAÇÕES FINAIS

As ostras e os mexilhões analisados ao longo dos locais amostrados mostraram-se eficientes no monitoramento e identificação de poluentes em ambientes aquáticos, mais especificamente na Baía de Vitória e Complexo Estuarino de Paranaguá. Ao mesmo tempo que os resultados demonstram a capacidade de monitoramento das espécies, confirma-se o perigo de consumo sem controle desses moluscos em determinados locais. Além disso, as particularidades fisiológicas de cada espécie devem sempre ser considerada para análise mais acurada, demonstrado nesse estudo com diferentes absorções de metais por mexilhões e ostras, quando comparados.

Os altos níveis encontrados, destacando As, Cr e Zn, precisam ser constantemente monitorados. Especialmente porque observou-se que os metais estavam, de fato, bioacumulando, nos moluscos.

Houve ainda uma tendência geral de maiores concentrações nas estações externas (caso possível de se observar em Vitória) para a maioria dos metais, sugerindo a influência do processo de floculação aumentando a forma particulada e ficando disponível para a biota local devido à proximidade com águas mais salinas e, então, confirmando a interação da bioacumulação dos metais com a variação dos parâmetros ambientais. As atividades portuárias e antropogênicas no entorno das estações externas também podem ter influência por maiores concentrações de metais nesses locais.

Os microplásticos foram encontrados em todos os locais avaliados no Complexo Estuarino de Paranaguá, confirmando a disseminação desse poluente no meio marinho e sua capacidade de bioacumulação em moluscos. Os resultados obtidos com MPs em Paranaguá demonstram que a mesma avaliação deve ser realizada ao longo da costa brasileira, especialmente por seu potencial de adsorção de outros poluentes. Mesmo que não comprovado diretamente por esse trabalho, percebemos indícios dessa hipótese através da visualização de diversos metais na superfície dos MPs avaliados. Destaca-se a técnica de utilização do MEV/EDS para a consecução do trabalho e abertura de novas possibilidades para trabalhos com esse poluente.

Por fim, considerando que os recursos pesqueiros têm grande importância para a economia local, ratifica-se que ações públicas devem ser tomadas para implementar medidas de monitoramento e prevenção que garantam a conservação dos recursos, da saúde humana e da preservação da zona costeira local.