UNIVERSIDADE FEDERAL DO PARÁ
INSTITUTO DE CIÊNCIAS BIOLÓGICAS
PROGRAMA DE PÓS-GRADUAÇÃO EM FARMACOLOGIA E BIOQUÍMICA

ERIKA MONTEIRO DOS SANTOS

MICROPOLUENTES ORGÂNICOS EMERGENTES NA REGIÃO AMAZÔNICA: EFEITOS DE CONCENTRAÇÕES AMBIENTALMENTE REALISTAS DE FTALATO EM *Hyphessobrycon heterorhabdus* (Teleostei: Characidae)

BELÉM-PA

2024

UNIVERSIDADE FEDERAL DO PARÁ
INSTITUTO DE CIÊNCIAS BIOLÓGICAS
PROGRAMA DE PÓS-GRADUAÇÃO EM FARMACOLOGIA E BIOQUÍMICA

MICROPOLUENTES ORGÂNICOS EMERGENTES NA REGIÃO AMAZÔNICA: EFEITOS DE CONCENTRAÇÕES AMBIENTALMENTE REALISTAS DE FTALATO EM *Hyphessobrycon heterorhabdus* (Teleostei: Characidae)

Autor(a): Erika Monteiro dos Santos

Orientador(a): Prof.ª Dr.ª Lílian Lund Amado

Coorientador(a): Prof.ª Dr.ª Daniele Salgado de Sousa

Documento de Dissertação apresentado ao Programa de Pós-graduação em Farmacologia e Bioquímica do Instituto de Ciências Biológicas da Universidade Federal do Pará como requisito para a obtenção do título de Mestre em Farmacologia e Bioquímica.

BELÉM-PA

2024

Dados Internacionais de Catalogação na Publicação (CIP) de acordo com ISBD
Sistema de Bibliotecas da Universidade Federal do Pará
Gerada automaticamente pelo módulo Ficat, mediante os dados fornecidos pelo(a) autor(a)

M772m Monteiro dos Santos, Erika.
Micropoluentes orgânicos emergentes na região amazônica: efeitos de concentrações ambientalmente realistas de ftalato em Hyphessobrycon heterorhabdus (Teleostei: Characidae) / Erika Monteiro dos Santos. — 2024.
VII, 63 f. : il. color.

Orientador(a): Profª. Dra. Lílian Lund Amado
Coorientação: Profª. Dra. Daniele Salgado de Sousa
Dissertação (Mestrado) - Universidade Federal do Pará, Instituto de Ciências Biológicas, Programa de Pós-graduação em Farmacologia e Bioquímica, Belém, 2024.

1. Micropoluentes na Amazônia. 2. DBP. 3. Ecotoxicologia. 4. Estresse oxidativo. I. Título.

CDD 612.015

AGRADECIMENTOS

À minha família, meus pais Lucimar e Valentim, minha avó Maria de Lourdes, minha irmã Jéssica e meus sobrinhos André e Ícaro, que sempre me apoiaram nessa trajetória, me incentivando sempre com amor e muito carinho em cada passo dado. Um muito obrigada aos meus pais e minha avó que com certeza foram os meus maiores incentivadores e apoiadores desde a infância, sempre me mostrando que a educação era o caminho certo e fazendo o impossível pra que eu pudesse ter a melhor educação possível. Eu amo muito vocês!

À minha orientadora Profa. Dra. Lílian Lund Amado, que é um ser humano além da curva que foi colocado em minha vida acadêmica, e a melhor orientadora que eu poderia ter. Obrigada por toda compreensão e ensinamentos ao longo desses anos. À minha coorientadora Profa. Dra. Daniele Salgado de Sousa por todos ensinamentos de vida e pelos conselhos profissionais durante esse período.

Ao meu grupo de pesquisa, o BioPaq, por todo suporte durante esse período, em especial aos meus parceiros de laboratório: Irina Carvalho, Yure Nascimento, Danilo Moutinho e Amanda Julião. Agradeço imensamente por todo companheirismo, incentivo, disponibilidade, ajuda nos experimentos e nas dosagens, nas rotinas laboratoriais e por cada momento de descontração dentro e fora do laboratório. Muito obrigada pela amizade e parceria além dos caminhos científicos! Serei eternamente grata a todos vocês.

Aos meus amigos dentro e fora da rotina acadêmica: Douglas Silva, Rogério Corrêa, Danielson Amaral, Rafaela Lima, Thaynara Oliveira, Izabele Chagas, e a todos os meus outros amigos próximos. Obrigada por cada conversa, incentivos e ensinamentos que vocês me deram, em especial ao Danielson Amaral, que foi a primeira pessoa que me ensinou com maestria os primeiros passos na ciência ainda em 2017. Agradeço vocês imensamente!

À Giulia Dal Castel, a melhor companheira de vida que eu poderia ter. Você foi peça fundamental pra conquista desse título. Obrigada, meu amor, por todo incentivo, preocupação, carinho, amor e paciência nesse período. Sem o teu apoio incondicional e parceria inestimável nada disso teria sido possível. Eu te amo infinitamente!

Por fim, o meu muito obrigada à todas as pessoas aqui citadas. Tenham certeza que vocês tiveram grande importância na minha trajetória até aqui. Amo cada um de vocês e serei eternamente grata!

RESUMO

Micropoluentes emergentes (MEs) são qualquer produto químico que não faça parte dos programas de monitorização ambiental e que esteja diretamente associado a atividades antropogênicas. Um exemplo são os ftalatos, produtos sintéticos amplamente utilizados na indústria de plastificantes. Podemos citar o di-butil ftalato (DBP), um dos mais encontrados no meio ambiente, que pode causar efeitos como genotoxicidade, apoptose, neurotoxicidade, hepatoxicidade, etc. Estudos recentes em todo o mundo têm identificado e quantificado DBP no meio ambiente, investigando seus efeitos em organismos aquáticos. No entanto, no Brasil, mais especificamente na região amazônica, ainda não existem estudos nesse sentido, deixando uma lacuna sobre a presença de MEs no ambiente aquático e seus efeitos sobre as espécies nativas. Assim, o objetivo geral desta dissertação foi identificar e quantificar MEs em rios urbanos da cidade de Belém, PA, Brasil, e avaliar as respostas de estresse oxidativo da espécie *Hyphessobrycon heterorhabdus* (Tetra Bandeira) à exposição a maior concentração de DBP encontrada na região. A dissertação está estruturada em dois capítulos: I) um estudo de campo, no qual foram identificados e quantificados MEs em rios urbanos de Belém, uma das cidades mais urbanizadas da Amazônia Oriental e; II) um estudo experimental, no qual foram avaliadas as alterações bioquímicas em *H. heterorhabdus* expostos a três concentrações de DBP. O estudo de campo coletou amostras de dois rios urbanos, os canais Tamandaré e Tucunduba. As amostras de água foram coletadas em garrafas âmbar, identificadas e enviadas ao laboratório para quantificação dos MEs por cromatografia líquida. Os peixes foram obtidos no Parque Ecológico do Gunma e aclimatados em laboratório para o experimento. Os animais foram expostos a três concentrações de DBP: (i) controle acetona (CA); grupo 1 (G1): 10 µg/L; grupo 2 (G2): 100 µg/L e grupo 3 (G3): 1000 µg/L durante sete dias. Amostras de água foram coletadas de todos os grupos em momentos específicos para a quantificação de DBP no meio experimental. Os animais foram dissecados nas porções anterior, média e posterior para análises bioquímicas, como a capacidade antioxidante total (ACAP), atividade da glutationa-S-transferase (GST) e lipoperoxidação (LPO). Foram identificados três grupos de MEs: organoclorados, hidrocarbonetos aromáticos policíclicos (HPAs) e ftalatos. Entre os ftalatos, o DBP e o di(2-etilhexil) ftalato (DEHP) foram os MEs com as concentrações mais elevadas, com 10.428 µg/L e 7.547 µg/L, respectivamente. No estudo experimental, a ACAP não variou entre os grupos nas porções anterior e posterior, enquanto na porção média observamos um efeito concentração-dependente. A GST não variou na porção posterior, mas na porção anterior, houve um aumento da GST no grupo G2 e uma diminuição no G1 na porção média. O LPO mostrou efeitos concentração-dependente e hormético nas porções anterior e posterior, respectivamente. Na porção média, a LPO não variou entre os grupos. Em geral, concluímos que organoclorados, HPAs e ftalatos estão presentes nos rios estudados e que a exposição ao DBP resulta em estresse para os organismos testados. Além disso, nossos resultados são de grande relevância para a região amazônica, pois este é um estudo pioneiro.

Palavras-chave: Micropoluentes na Amazônia; DBP; ecotoxicologia; estresse oxidativo.

ABSTRACT

Emerging micropollutants (EMs) are any chemical products that are not part of environmental monitoring programs and are directly associated with anthropogenic activities. One example is phthalates, synthetic products widely used in the plasticizer industry. We can mention di-butyl phthalate (DBP), one of the most commonly found in the environment, which can cause effects such as genotoxicity, apoptosis, neurotoxicity, hepatoxicity, etc. Recent studies around the world have identified and quantified DBPs in the environment, investigating their impact on aquatic organisms. However, in Brazil, more specifically in the Amazon region, there are still no studies in this regard, leaving a gap in the presence of EMs in the aquatic environment and their effects on native species. Thus, the general objective of this dissertation was to identify and quantify EMs in urban rivers in the city of Belém, PA, Brazil, and to evaluate the oxidative stress responses of the species *Hyphessobrycon heterorhabdu*s (Tetra Bandeira) to exposure to the highest concentration of DBP found in the region. The dissertation is structured into two chapters: I) a field study, in which EMs were identified and quantified in urban rivers in Belém, one of the most urbanized cities in the Eastern Amazon, and II) an experimental study, in which the biochemical changes in *H. heterorhabdus* exposed to three concentrations of DBP were evaluated. The field study collected samples from two urban rivers, the Tamandaré and Tucunduba canals. Water samples were collected in amber bottles, identified, and sent to the laboratory for quantification of EMs by liquid chromatography. The fish were obtained from the Parque Ecológico do Gunma and acclimatized in the laboratory for the experiment. The animals were exposed to three concentrations of DBP: (i) acetone control (CA); group 1 (G1): 10 µg/L; group 2 (G2): 100 µg/L and group 3 (G3): 1000 µg/L for seven days. Water samples were collected from all groups at specific times for the quantification of DBP in the experimental medium. The animals were dissected into anterior, middle, and posterior portions for biochemical analysis, such as total antioxidant capacity (ACAP), glutathione-S-transferase (GST) activity, and lipoperoxidation (LPO). Three groups of EMs were identified: organochlorines, polycyclic aromatic hydrocarbons (PAHs), and phthalates. Among the phthalates, DBP and di(2-ethylhexyl) phthalate (DEHP) were the EMs with the highest concentrations, at 10.428 µg/L and 7.547 µg/L, respectively. In the experimental study, ACAP did not vary among the groups in the anterior and posterior sections, while in the middle section, we observed a concentration-dependent effect. GST did not vary in the posterior section, but in the anterior section, there was an increase in GST in the G2 group and a decrease in G1 in the middle section. The LPO showed a concentration-dependent and hormetic effects in the anterior and posterior sections, respectively. In the middle section, the LPO did not vary among the groups. In general, we conclude that organochlorines, PAHs, and phthalates are present in the rivers studied and that exposure to DBP results in stress to the organisms tested. Furthermore, our results are of great relevance to the Amazon region, as this is a pioneering study.

Keywords: Micropollutants in the Amazon; DBP; ecotoxicology; biomarkers.

LISTA DE SIGLAS E SÍMBOLOS

ACAP	Capacidade antioxidante total contra radicais peroxil
DBP	Dibutil ftalato
DEHP	Di(2-etilhexil) ftalato
ETEs	Estações de tratamento de esgoto
GST	Glutationa-S-transferase
HPAs	Hidrocarbonetos policíclicos aromáticos
LPO	Lipoperoxidação
MEs	Micropoluentes emergentes
PCBs	Bifenilas policloradas

SUMÁRIO

1. VISÃO INTEGRADORA DO PROBLEMA 1

2. ARTIGO 1: A INFLUÊNCIA DE MARÉ NA DISTRIBUIÇÃO DE MICROPOLUENTES ORGÂNICOS EMERGENTES EM RIOS URBANOS NA REGIÃO AMAZÔNICA 5

3. ARTIGO 2: EFEITOS DE CONCENTRAÇÕES AMBIENTALMENTE REALISTAS DE DIBUTIL FTALATO NA REGIÃO AMAZÔNICA EM *Hyphessobrycon heterorhabdus* **(Teleostei: Characidae) 38**

4. CONCLUSÕES INTEGRADORAS 55

5. REFERÊNCIAS 56

6. COMPROVANTE DE SUBMISSÃO/ACEITE DE ARTIGO CIENTÍFICO 62

1. VISÃO INTEGRADORA DO PROBLEMA

Os plásticos são uma importante matéria-prima para a indústria em geral, porém desde o seu surgimento no século XX, este produto vem trazendo diversos impactos negativos para o meio ambiente devido seu descarte inapropriado (Conceição et al., 2019). Segundo o *World Wide Fund for Nature Inc.* (2016), a China é o segundo maior produtor de plástico do mundo, produzindo cerca de 54,7 milhões de toneladas de plástico por ano, ficando atrás apenas dos Estados Unidos com uma produção anual de cerca de 70,7 milhões de toneladas. Embora a China seja responsável por uma alta produção de plástico, o país recicla aproximadamente 22% de todo o plástico gerado. Segundo a *World Wide Fund for Nature Inc.* (2019), o Brasil se encontra em quarto lugar no ranking dos maiores produtores de plástico, produzindo aproximadamente 11,3 milhões de toneladas de plástico por ano e reciclando menos de 1,3% desse total, onde 2,4 e 7,7 milhões de toneladas são descartadas de formas irregulares e ficam em aterros sanitários, respectivamente.

O plástico é hoje um dos insumos mais utilizados na indústria, principalmente na indústria alimentícia constituindo suas embalagens, devido às suas características particulares como, resistência, maleabilidade e baixo custo (Kirwan e Strawbridge, 2003; Thompson et al., 2009), contudo para obter essas características, muitos aditivos prejudiciais para o meio ambiente, conhecidos como micropoluentes emergentes, são inseridos em seu processo de produção (Staples et al., 1997; Schecter et al., 2013; Malveda et al., 2015).

Micropoluentes emergentes podem ser entendidos como qualquer produto químico que ainda não faça parte de programas de monitoramento ambiental (Ternes et al., 2006; Reemtsma et al., 2008). Estes são considerados emergentes por só poderem ser detectados e quantificados mais recentemente com o desenvolvimento de técnicas analíticas mais sensíveis, uma vez que essas substâncias são detectadas no ambiente em concentrações baixíssimas (Luo et al., 2014). Esses contaminantes já foram detectados em sistemas hídricos em faixas de concentração variando entre ng/L à µg/L (Luo et al., 2014), uma vez que é improvável que essas substâncias sejam removidas pelas estações de tratamento de efluentes tradicionais (ETEs) dada a sua atual ineficiência (Bhatt, Bhandari e Bilal, 2022), o que pode ser descrita como prejudicial à saúde humana e ao ecossistema em geral (Guerra-Rodríguez et al., 2021).

Um dos grupos de micropoluentes emergentes mais estudados atualmente são os ftalatos, que são produtos sintéticos amplamente difundidos em diversas aplicações industriais (Staples et al., 1997; Shin et al., 2020; Radke et al., 2019), constituindo mais de 70% dos plastificantes usados no mundo (Staples et al., 1997; Schecter et al., 2013; Malveda et al., 2015). Apesar de ser empregado largamente na indústria de plastificantes, os ftalatos são muito utilizados na indústria como um todo, sendo assim, são encontrados em diversos tipos de produtos como tintas, artigos infantis, adesivos, perfumes, materiais de construção, cosméticos, shampoos, detergentes, sabonetes, esmaltes, pisos, *sprays* para cabelo, dispositivos médicos, entre outros (Latini, 2005; Wormuth et al., 2006; Benjamin et al., 2015; Brčić Karačonji et al., 2017).

O dibutil ftalato (DBP) é um dos ftalatos mais frequentes encontrados no meio ambiente, devido ser utilizado na fabricação de diversos produtos do dia-a-dia, tais como tintas em geral, medicamentos, cosméticos, embalagem de alimentos e uma variedade de produtos de higiene pessoal (Wittassek et al., 2011; Benjamin et al., 2015), expondo os seres humanos continuamente a este contaminante. A contínua exposição ao DBP fez com que vários estudiosos se questionassem quanto à sua toxicidade (Barbaud e Lafforgue, 2021; Heudorf, Mersch-Sundermann e Angerer, 2007), uma vez que este pode entrar no organismo humano por via digestiva, inalatória e dérmica (Wittassek et al., 2011).

Em humanos, além de estar diretamente relacionado com a má qualidade de espermatozoides produzidos (Pant et al., 2008; Jurewicz et al., 2013), o DBP também é apontado, segundo o Centro Nacional de Toxicologia para Avaliação de Riscos para Reprodução Humana, como causador de potenciais danos no desenvolvimento humano devido ao DBP estar inserido em diversos produtos de uso humano contínuo (NTP, 2003). Já em organismos aquáticos como peixes, estudos recentes relatam que o DBP causa imunotoxicidade (Xu et al., 2015) toxicidade no desenvolvimento (Sun e Li, 2019), hepatoxicidade (Song et al., 2021), neurotoxicidade (Paquette et al., 2023), genotoxicidade e estresse oxidativo no cérebro (Jiang et al., 2022) e apoptose (Hou et al., 2024).

Desse modo, tendo em vista o aumento da poluição de ambientes naturais, é válido ressaltar a importância do monitoramento ambiental de áreas afetadas para que sejam implementadas ações corretivas. Porém, essas áreas não podem ser somente avaliadas em nível de análises químicas, uma vez que estas análises não são capazes de indicar se a biota presente no local está sofrendo efeitos deletérios da poluição decorrente dos contaminantes presentes (Baršiené et al., 2006; Cajaraville et al., 2000). Sendo assim, é necessário verificar e analisar os efeitos que tais contaminantes possuem sobre os organismos vivos do local por meio dos marcadores biológicos ou como são comumente conhecidos: os biomarcadores (Wells et al., 2001; Fuentes-Rios et al., 2005).

Os biomarcadores correspondem a uma resposta biológica a algum xenobiótico, podendo essas respostas se apresentarem como alterações a nível bioquímico, celular, comportamental, histológico e fisiológico (Depledge, 1992; Lam e Gray, 2003). Estes podem ser divididos em: (i) biomarcadores de exposição, que são capazes de nos informar o nível de exposição a uma fonte estressora, como a capacidade antioxidante total contra radicais peroxil (ACAP) e a glutationa-S-transferase e; (ii) biomarcadores de efeito, os quais podem nos informar sobre danos ou efeitos deletérios, como a lipoperoxidação (LPO) (Sogorb et al., 2014; Amado et al., 2009; Zamek-Gliszczynski et al., 2006; Javed et al., 2016).

Além dos biomarcadores, os organismos biomonitores também consistem em uma importante ferramenta para estudos ecotoxicológicos, uma vez que são indicativos biológicos de uma determinada condição ambiental (Le et al., 2016). O *Hyphessobrycon heterorhabdus,* comumente conhecido como Tetra Bandeira é um peixe amazônico que se distribui por toda coluna d'água dos corpos hídricos amazônicos, alimentando-se de pequenos insetos aquáticos e terrestres e de matéria orgânica (Moreira et al., 2002; García-Alzate et al., 2008; Lima et al., 2014). No que diz respeito à abundância de peixes do gênero, a espécie *H. heterorhabdus* é uma das mais abundantes em igarapés da região (Cruz, 2006). Algumas espécies do gênero *Hyphessobrycon* já foram utilizados em estudos ambientais recentes visando analisar a toxicidade de certos contaminantes encontrados no meio ambiente, tendo ele apresentado boas características como organismo experimental, como por exemplo: boa adaptabilidade às condições laboratoriais, fácil disponibilidade, fácil manutenção, boa resistência e boa sensibilidade e suscetibilidade à exposição à contaminantes (Sotero-Santos, Rocha and Povinelli, 2007; Scalon et al., 2010; Carraschi, 2011; Damato and Barbieri, 2012), fazendo do *H. heterorhabdus* um excelente organismo biomonitor para testes ecotoxicológicos.

Deste modo, esse estudo teve por objetivo identificar e quantificar micropoluentes orgânicos emergentes em rios urbanos da cidade de Belém e também investigar quais efeitos o DBP (o poluente de maior concentração encontrado) em uma espécie de peixe amazônica, o *H. heterorhabdus*, através de biomarcadores de estresse oxidativo. Sendo assim, esta dissertação é composta por dois capítulos: (i) o primeiro capítulo que objetivou identificar e quantificar cinco grupos de micropoluentes, sendo eles os organoclorados, os hidrocarbonetos policíclicos aromáticos (HPAs), as bifenilas policloradas (PCBs), hormônios esteróides e os ftalatos em rios urbanos da região Metropolitana de Belém do Pará, verificando também como a influência de maré impactava em suas distribuições, o qual foi submetido a *Journal of Hazardous Materials*; (ii) já o segundo capítulo se tratou de uma extensão do capítulo 1, onde foram realizados ensaios em laboratório de exposição crônica à diferentes concentrações de DBP, semelhantes às encontradas nos rios urbanos, em *H. heterorhabdus*, de modo a avaliar os efeitos deste micropoluente através de biomarcadores de estresse oxidativo, o qual também será submetido a *Journal of Hazardous Materials*.

2. ARTIGO 1: A INFLUÊNCIA DE MARÉ NA DISTRIBUIÇÃO DE MICROPOLUENTES ORGÂNICOS EMERGENTES EM RIOS URBANOS NA REGIÃO AMAZÔNICA

The tidal influence on the distribution of emerging organic micropollutants in urban rivers in the Amazon region

Erika Monteiro[ac], Yure Jefferson da Cruz do Nascimento[ac], Caroline da Silva Montes[bc], Danilo Serrão Moutinho[c], Ilene Crestani[d], Cassia Katrin Reinke[d], Jocinei Dognini[d], Leandro Machado de Carvalho[e], Daniele Salgado[bc], Lílian Lund Amado[abc*]

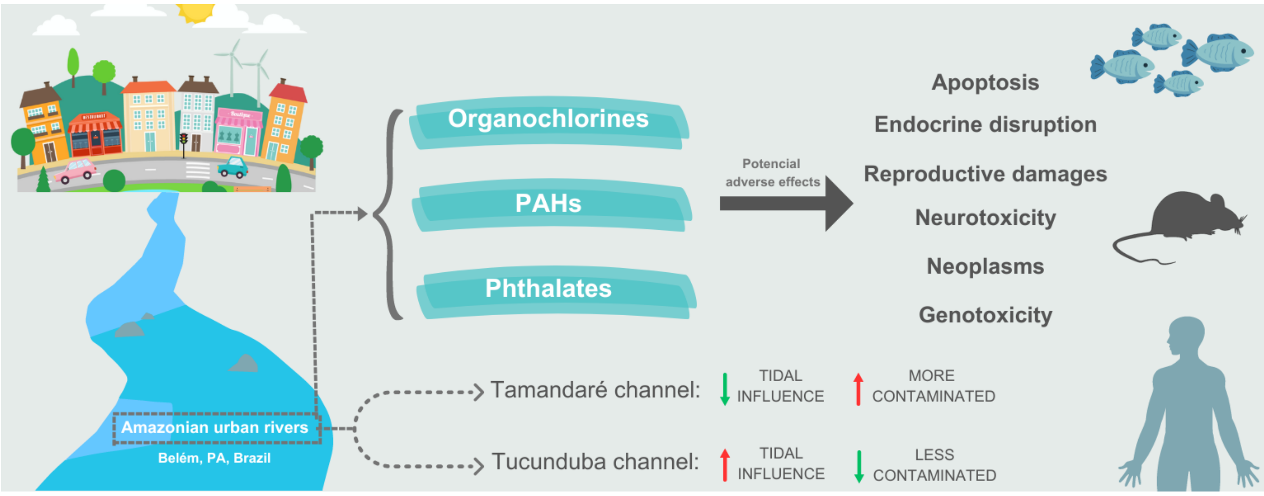
[a]Programa de Pós-graduação em Farmacologia e Bioquímica, Instituto de Ciências Biológicas, Universidade Federal do Pará, Belém, PA, Brazil.
[b]Programa de Pós-graduação em Oceanografia, Instituto de Geociências, Universidade Federal do Pará, Belém, PA, Brazil.
[c]Laboratório de Ecotoxicologia (LABECOTOX) e Laboratório de Pesquisas em Monitoramento Ambiental Marinho (LAPMAR), Universidade Federal do Pará, Belém, PA, Brazil.
[d]Instituto SENAI de Tecnologia Ambiental, Blumenau, SC, Brazil.
[e]Departamento de Química, Universidade Federal de Santa Maria, Santa Maria, RS, Brazil.

* Corresponding author: Laboratório de Ecotoxicologia, Instituto de Ciências Biológicas, Universidade Federal do Pará, Avenida Augusto Corrêa, 01, Guamá, 66075-110, Belém, Pará, Brazil.
E-mail address: lla@ufpa.br, lilian.amado@gmail.com (L.L. Amado).

Abstract:
Emerging micropollutants refer to chemical substances found in extremely low concentrations in the environment. These have earned the label "emerging" due to the recent advancement of susceptible analytical techniques, capable of detecting substances even at very low concentrations (from ng/L to μg/L). Some micropollutants are priorities due to their continuous input in the environment, such

as organochlorines, polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), hormones, phthalates and others. We identified and quantified different groups of micropollutants in urban rivers in the city of Belém, PA, the most urbanized cities in the Eastern Amazon. Water samples were collected in the rainy season along two urban rivers of Belém, the Tamandaré and Tucunduba channels. Tamandaré channel proved to be more contaminated due to a lower tidal influence, making it more susceptible to the accumulation of these contaminants. Our analyses demonstrated the presence of organochlorines, PAHs and phthalates in the rivers studied. These findings underscore the necessity for ongoing monitoring of Amazonian water bodies. The importance of this approach is due to its harmful effects, such as neurotoxicity, apoptosis, genotoxicity, damage to the reproductive system, neoplasms, endocrine disruption and developmental toxicity. These impacts extend to both aquatic organisms and human health.

Graphical abstract:



Keywords: Micropollutants in the Amazon; emerging contaminants; urban ecotoxicology; environmental pollution; ODS-14.

Highlights:

- Organochlorines, PAHs, and phthalates stood out as the most prevalent as micropollutants in Amazonian urban rivers.
- An inversely proportional relationship exists between tidal influence and the concentration of pollutants detected in water samples across these urban rivers.
- The imperative to monitor emerging organic micropollutants in Amazonian urban rivers arises from their potential to induce harmful effects.

1. Introduction

Emerging micropollutants encompass chemicals not yet integrated into environmental monitoring programs (Ternes and Joss, 2007; Reemtsma et al., 2008). These substances are intricately tied to human activities, including domestic, industrial, agricultural, and hospital-related sources (Komolafe et al., 2021). Examples of emerging pollutants include pharmacologically active wastes, plasticizers, personal care products, steroid hormones, and other toxic chemicals (Ternes and Joss, 2007; Reemtsma et al., 2008; Santos et al, 2022), that have already been detected in water systems in concentration ranges ranging from ng/L to µg/L (Luo et al., 2014), being harmful to human health and the ecosystem in general even at low concentrations (Lou et al., 2014; Guerra-Rodríguez et al., 2021).

The organochlorines, for example, constitute a collection of compounds characterized by having at least one chlorine atom directly bonded to a carbon chain. They possess low molecular mass, rendering them insoluble in water while exhibiting solubility in organic compounds (Hermes, 2004). These substances are highly resistant in the environment, and may persist for many years in the ecosystem (Andréa, 1998). This group of chemicals are considered toxic and easily absorbed by cutaneous, respiratory, and digestive routes (Cavalcanti et al., 2016).

Another example of emerging pollutants found in the environment are polycyclic aromatic hydrocarbons (PAHs), which are of special environmental relevance because they are harmful both to the health of the environment in general and to human health (Boonyatumanond et al, 2006; Chen et al., 2007; IARC, 2010), as they exhibit mutagenic, carcinogenic, teratogenic characteristics to living organisms, including microorganisms, animals and humans even at minimal concentrations

(Simpson et al., 2005; Rengarajan et al., 2015; Bolden et al., 2017). In general, these compounds have ecotoxic effects on aquatic life (Abdel-Shafy & Mansour, 2016).

Phthalates are a group of contaminants of great environmental concern, as they are present in food, air, water and soil. Their occurrences lead to significant human exposure to these kinds of contaminants (Ge et al., 2007; U.S. National Library of Medicine, 2009). Some studies over the years have linked exposure to phthalates to several human health consequences, such as: damage to the reproductive system, as well as malformations or even infertility (European Chemicals Agency, 2017), urogenital neoplasms in men and women, breast and endometrial neoplasms (Singh and Li, 2012), among others. In animals such as fish, phthalates can cause hormonal dysregulation, oxidative stress, metabolic disorders, developmental toxicity, apoptosis, and even genotoxicity (Zhang et al., 2021).

Congeners of polychlorinated biphenyls (PCBs) and steroid hormones are also emerging micropollutants commonly found in the environment (Gioia et al., 2014; Ojoghoro et al., 2021). PCBs are an important class of persistent, bioaccumulative pollutants with potential environmental and human health damage (Meeker and Hauser, 2010). Steroid hormones, on the other hand, are substances considered endocrine disruptors, because they directly affect the endocrine system, since these substances can mimic the body's natural hormone, altering the ideal functioning of the endocrine system of an organism, thus causing several effects on human health and other organisms (Lintelmann et al., 2003; Zhang et al., 2011).

Several studies on the identification and quantification of micropollutants in urban areas have been carried out around the world and in Brazil (Wong et al., 2015; Xie et al., 2019; Rodrigues et al., 2020; Gemusse et al., 2021; Song et al., 2022; Bertrand et al., 2023), but no study has yet been carried out in Belém, the largest city in the eastern Amazon (IBGE, 2010), thus, this study highlights its importance given its pioneering role in the Amazon region.

The Amazon Rainforest occupies almost 40% of Brazil's total area, with around 6.9 million km^2 of river basin, housing the largest river drainage system on the planet and the largest continuous area of tropical rainforest (Macedo and Castello, 2015). It is the largest biological reservoir in the world, containing diverse species of plants, vertebrates and invertebrates, many of which have not yet been identified (Macedo and Castello, 2015).

Thus, it is of great environmental relevance to know which micropollutants are present in the rivers of an important biome, such as Amazonia, thus inferring the level of hazard that they present to human health and what kind of damage they can cause to the environment. Thus, the objective of this study is to identify and quantify different groups of emerging micropollutants in urban rivers in the city of Belém, PA.

2. Material and methods

2.1 Sampling sites

The city of Belém (1º27'21''S 48º30'14''W), located in the northern region of Brazil, covers a total area of 1,059.458 km^2 and is a densely populated municipality, with a population density of 1,315.26 inhabitants/km^2 (IBGE, 2010). Belém is considered the eleventh most populous city in Brazil, and is also the second largest city in the Legal Amazon and the largest city in the Eastern Amazon, with an approximate population of almost 1.5 million inhabitants (IBGE, 2010).

To verify the environmental concentrations of emerging organic micropollutants in the Amazon region, eighteen water samples were collected in amber glass flasks along two urban channels of Belém (Figure 1), the Tamandaré (1º27'24''S 48º29'45''W) and Tucunduba (1º28'34''S 48º27'14''W) channels. both of which are natural water bodies derived from rivers in the Amazon region (Targa et al., 2012; Mácola et al., 2017). The samples were collected in the rainy season (ebb tide), which is the period when there is an increase in the leaching process and, consequently, the increase of pollutants in the waters (Wijngaard et al., 2017). These samples were collected in triplicate at three different points in each of the channels (nine samples for each sampling site) to verify a concentration gradient, considering that there is a strong anthropic influence along the two studied channels.

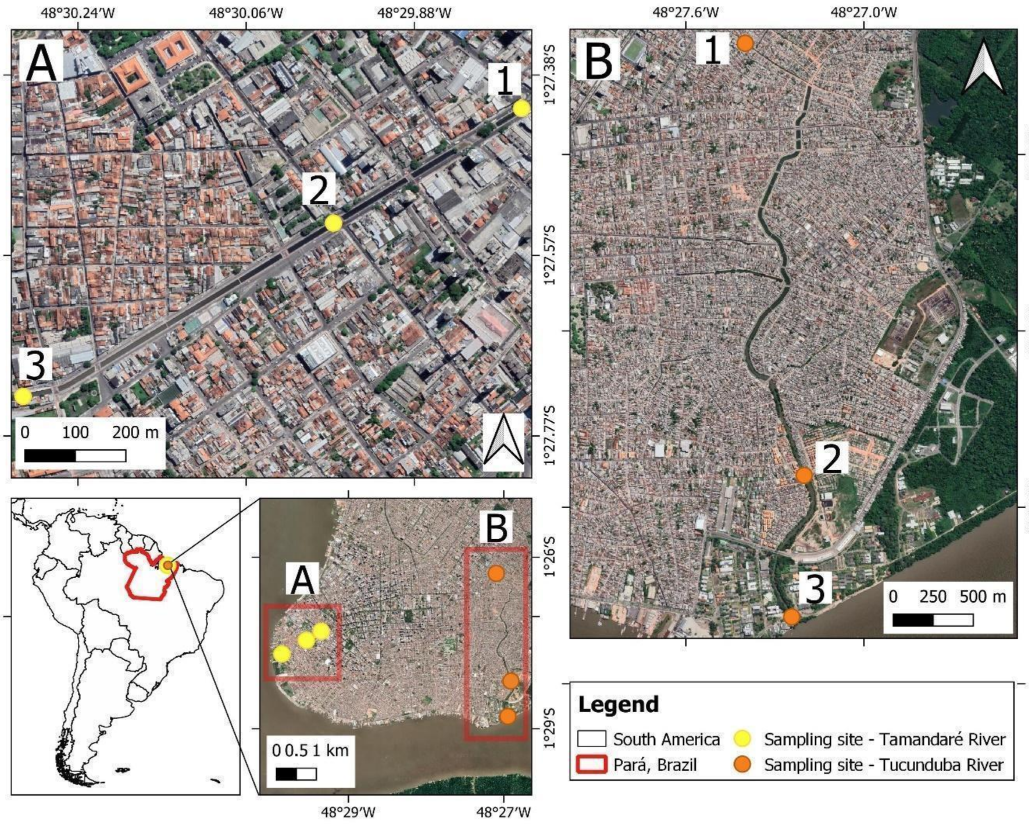


Fig. 1. Tamandaré and Tucunduba channels, Belém, PA, showing the sample collection points. (A) The points in yellow show the three sampling sites in the Tamandaré canal; (B) The points in orange show the three sampling sites in the Tucunduba canal.

The Tamandaré channel consists of a natural water arm that comes from the Guajará Bay, while the Tucunduba channel is a natural water arm that comes from the Guamá River and both channels are located in the urban area of Belém with a high level of anthropic influence (França et al., 2013; Silva et al., 2020). The Tucunduba channel is the second largest basin in Belém, having greater tidal influence than the Tamandaré channel (França et al., 2013; Bletter et al., 2018). Point 1, in both channels, is the most internally sampled area (inside the urban zone) and the most anthropized, while point 3 is the most external point and the closest to the river mouth (Figure 1).

2.2 Chemicals and standards

The chemicals sodium sulphate**,** ammonium formiate, dichloromethane, and acetonitrile, which were used for extraction procedures and chromatographic analysis, were purchased from Sigma Aldrich (St. Louis, USA).

The analytical standards of the organochlorines were acquired from AccuStandard, Inc. (New Haven, Connecticut, USA): Heptachlor Epoxide, Metribuzin, Pendimethalin, trans-Permethrin.

The analytical standards of polycyclic aromatic hydrocarbons (PAHs) were acquired from Sigma Aldrich (St. Louis, USA): Fluorene, Phenanthrene, Anthracene, Pyrene, Benz(a)anthracene, Chrysene, Dibenz(a,h)anthracene, Naphthalene, 2-methylnaphthalene, Acenaphthylene, Acenaphthene, Fluoranthene, Benzo(b)fluoranthene, Benzo(k)fluoranthene, Benzo(a)pyrene, Indeno(1,2,3-cd)pyrene, Benzo(g,h,i)pyrene.

The analytical standards of polychlorinated biphenyls (PCBs) were acquired from Sigma Aldrich (St. Louis, USA): 2,2,5-trichlorobiphenyl, 2,4,5-trichlorobiphenyl, 2,2,5,5-tetrachlorobiphenyl, 2,2,3,5-tetrachlorobiphenyl, 2,3,4,4-tetrachlorobiphenyl, 2,2,4,5,5-pentachlorobiphenyl, 2,2,3,4,5-pentachlorobiphenyl, 2,3,3,4,6-pentachlorobiphenyl, 2,2,3,5,5,6-hexachlorobiphenyl, 2,2,4,4,5,5-hexachlorobiphenyl, 2,2,3,4,4,5-hexachlorobiphenyl, 2,2,3,4,5,5-hexachlorobiphenyl, 2,2,3,4,5,5,6-heptachlorobiphenyl, 2,2,3,4,4,5,6-heptachlorobiphenyl, 2,2,3,4,4,5,5-heptachlorobiphenyl, 2,2,3,3,4,4,5-heptachlorobiphenyl, 2,2,3,3,4,4,5,5,6-nonachlorobiphenyl.

The analytical standards of Phthalates were acquired from Sigma Aldrich (St. Louis, USA): Dimethyl phthalate, Diethyl phthalate, Di-n-butyl phthalate, Di(2-ethylhexyl)phthalate.

The analytical standards of Hormones were acquired from Sigma Aldrich (St. Louis, USA): hydrocortisone, prednisone, progesterone, 17-ethynylestradiol, estriol, estrone 3 Meo, estrone, estradiol, hexestrol.

2.3 Analytical procedures

2.3.1 Sample treatment

The samples were prepared using the liquid-liquid extraction method, where 50 mL of sample, without pre-concentration, was extracted with dichloromethane and concentrated to 0.5 mL. Three extractions were performed with 15 mL of dichloromethane each, stirring for 2 minutes, letting stand for 10 minutes, and filtered with sodium sulphate into a concentrator tube, where it was concentrated with nitrogen to a volume of 0.5 mL. The vial with the concentrated sample was measured by the validated gas chromatographic methods (GC-MS) and liquid chromatographic methods (LC-MS/MS) for the following classes of compounds: (i) organochlorines; (ii) polycyclic aromatic hydrocarbons (PAHs); (iii) polychlorinated biphenyl species (PCBs), (iv) phthalates and (v) hormones.

2.3.2 Chromatographic conditions of analysis

The determination of semi-volatile organic compounds (SVOCs) from the organochlorine, PAH, PCB, phthalate compound classes was performed on a GC-MS systems (Agilent, model GC-7890B/MS-5977A) with an autosampler (model G6501B). The components were separated on a HP-5MS fused silica capillary column with a length of 20 m and an internal diameter of 0.18 mm, as well as a film thickness of 0.18 µm, consisting of 5% phenyl, 95% dimethylsiloxane stationary phase. The conditions for the chromatographic system were: injector temperature at 280°C; injection volume at 2 µL; splitless injection mode pulsed at 50 psi up to 0.5 min; 50 mL/min purge in 0.5 min; septum purge switched to 3 mL/min flow mode; oven with initial temperature of 40°C (temperature maintained at 2.5 min), with heating rate of 25°C/min to 125°C, where from this temperature, the heating rate becomes 16°C/min to 300°C (this temperature is maintained for 4.162 minutes); helium carrier gas with constant flow of 0.7 mL/min; total run time of 21 minutes. The mass spectrometer conditions were: transferline temperature of 300°C; ionization source temperature of 300°C and 150°C for the quadrupole; electron impact ionization source (70 eV) in SIM mode.

2.3.3 Method for the quantification of hormones by liquid chromatography (LC-MS/MS)

For the analytical method for identification and quantification of hormones by LC-MS/MS, ammonium formiate and acetonitrile solvent were purchased from Sigma Aldrich (St. Louis, USA). Reverse phase liquid chromatography analysis for hormone determination was performed using a LC-MS/MS system (Agilent, model GC-7890B/MS-5977A), solvent pump (model G1311B 1260 QuatPump), sampler (model G1329B 1260 ALS), column oven (model G1316A 1260 TCC), and a mass spectrometer (model G6430A). In the analysis of the compounds, the elution gradient was using 80% Ammonium Formate 0.1% in ultrapure water and 20% Acetonitrile, being: 0 min 20% B and 80 A %, 5 min 90 % Acetonitrile and 10 % Ammonium Formate until 12 minutes, rebalancing the column with 80% Ammonium Formate 0.1% in ultrapure water 20% B until the end of the run in 17.2 minutes. The column used was a reversed phase column (Poroshell SB C18 model) of size 300x100 mm, particle size of 2.7 µm (Agilent), injection volume of 50 µL, flow rate of 0.700 mL/min and the column temperature used was 45°C.

The measurements were performed using an ESI source with gas temperature of 350°C, gas flow of 8 L/min, nebulization of 36 psi and capillary voltage of 4.000V. The identification mode in the mass spectrometer was performed using multiple reaction monitoring (MRM) with 300 positive electromultiplier delta and 300 negative electromultiplier delta.

The accuracy of the measurements were evaluated by determining the concentration of each analyte in the following certified samples: DRH-006S for PAHs; M-8060 for phthalates; M-8082A for PCBs; M-8081-SC, M-508.1-X2, and AE-00023 for organochlorines (all from AccuStandard Inc., New Haven, USA). The accuracies were determined as in range 91-108% for PAHs, 92-107% for phthalates, 96-107% for PCBs, and 80-111% for organochlorines.

2.4 Statistical analysis

We conducted data analyses by examining concentrations of emerging organic micropollutants (ECs) that exceeded the quantification limit. This allowed us to establish profiles for different ECs along the channels, including their respective contributions expressed as percentages (%). We explored potential relationships among the occurrence of ECs by conducting correlations analyses using Spearman's rank test (significance level of 0.05), visualized as a chord diagram. Following data normalization, exploratory data analysis, Principal Component Analysis (PCA) and a clustered heatmap, was executed. The PCA Bi-Plot was constructed for aggregating ECs data from different points as descriptors, aiding in constructing classification trees that highlight major contributing variables for grouping. The primary axis of PCA captures the highest variation within the dataset, while subsequent axes are introduced with the condition of orthogonality to previously derived ones. All statistical analyses were performed using the R statistical software interface R-Studio (R version 3.6.3) using “FMSB”, “ggplot2”, “chordiagram” and “heatmaply” packages.

3. Results and discussion

Anthropogenic activities contribute to the release of harmful compounds into the aquatic environment. Studies identifying and characterizing these substances are extremely pertinent to mitigating and remedying their damage to human health and the ecosystem. In this context, we investigated the occurrence of emerging organic micropollutants in two urban rivers situated in Belém, the most populous city in eastern Amazonia, Brazil, and revealed their potential risk to the environment and the population. The groups of contaminants investigated in this study were: (i) organochlorines; (ii) polycyclic aromatic hydrocarbons (PAHs); (iii) polychlorinated biphenyl species (PCBs), (iv) phthalates and (v) hormones.

3.1 Presence of organochlorines, polycyclic aromatic hydrocarbons (PAHs); polychlorinated biphenyl species (PCBs), phthalates and hormones in the river waters

Our analysis revealed the presence of three of the five groups of micropollutants investigated: organochlorines, PAHs and phthalates. The micropollutants detected are shown below (Table 1).

Table 1. Comparison of the concentrations of micropollutants found in the two canals studied.

Group of micropollutants	Micropollutants	Tucunduba Channel (n=9)		Tamandaré Channel (n=9)	
		Lower range (µg/L)	Highest range (µg/L)	Lower range (µg/L)	Highest range (µg/L)
Phthalates	DBP	5370	7.720	7.217	10.428
Phthalates	DEHP	3.896	7.547	3.644	6.850
Phthalates	DEP	1.850	3.805	2.765	4.703
Phthalates	DMP	0.466	0.790	0.632	0.810
PAHs	Anthracene	ND	ND	0.038	0.319
Organochlorines	Pendimethalin	0.151	0.198	0.183	0.277
PAHs	Pyrene	ND	ND	0.051	0.259
Organochlorines	Metribuzin	ND	ND	0.177	0.252
PAHs	Phenanthrene	0.005	0.041	0.030	0.217
PAHs	Dibenz(a,h)anthracene	ND	ND	0.121	0.207
PAHs	Chrysene	ND	ND	0.015	0.041
Organochlorines	*trans*-permethrin	0.013	0.041	0.015	0.032
PAHs	Fluorene	0.004	0.011	ND	ND
PAHs	Benz(a)anthracene	0.007	0.007	0.008	0.008
Organochlorines	Heptachlor epoxide	ND	ND	0.001	0.003

ND: Not Detected

The environmental concentrations of the organochlorines found can be understood by two characteristics: liposolubility and slow metabolism. These properties favor the persistence of these compounds in the environment, which accumulate in the food chain and in fat tissue (Jayaraj et al., 2016). Numerous studies conducted over the years have showcased the detrimental impacts of organochlorines on the well-being of diverse organisms. (Singh et al., 2016). Damage such as gill hypertrophy, pyknotic core, hydropic degeneration in the liver, and damage to the reproductive system in males have already been demonstrated in fish (Da Cuna et al., 2011). In rats, damage such as dopaminergic neurotoxicity, dysfunction of mitochondrial activity (Sharma et al., 2010), apoptosis, biochemical changes, genotoxicity (Pelletier et al., 2009) and a high production of reactive oxygen species (Yu and Yang, 2007). In humans, besides organochlorines being associated with different types of cancer (Enan and Matsumura, 1998; Diel et al., 2002; Ndebele et al., 2010; Wong et al., 2015), they are also capable of causing damage to the male reproductive system from changes in sperm count in spermatogonia to morphological changes in spermatozoa (Cable and Doherty, 1999).

Among the 27 analyzed organochlorines, only 4 of them were identified in at least one of the sampling points. The identified organochlorines include heptachlor epoxide, metribuzin, pendimethalin, and *trans*-permethrin. Among the detected organochlorines, pendimethalin showed the highest concentration (0.277 ± 0.037 µg/L), while the lowest concentration was found for heptachlor epoxide (0.001 ± 0.0001 µg/L). These compounds are toxic and their presence in water, even at low concentrations, can be a significant source of pollution. Furthermore, pendimethalin has been found to be a moderately to extremely toxic compound for fish and aquatic organisms (Ikpesu et al., 2017; Qiao et al., 2021). Heptachlor Epoxide was quantified only in the samples from the Tamandaré channel in all sampling points. This substance was the organochlorine that presented the lowest concentrations, ranging from 0.001 to 0.003 µg/L. Metribuzin was also found only in the Tamandaré channel, but was quantified only in one of the sampling points in its three replicates, presenting a concentration ranging from 0.177 to 0.252 µg/L (0.226 ± 0.043 µg/L).

Considering the pollutant pendimethalin, it was found in almost all sampling points in the Tamandaré channel, presenting a concentration ranging from 0.183 to 0.277 µg/L (0.227 ± 0.031 µg/L). This substance was also found in its three replicates from a single sampling point in the Tucunduba channel, unlike heptachlor epoxide and metribuzin, which were not found in any concentration at this site. In the Tucunduba channel, pendimethalin showed concentrations ranging from 0.151 to 0.198 µg/L (0.174 ± 0.024 µg/L). In addition, *trans*-permethrin, like pendimethalin, was detected at both sampling sites. However, *trans*-permethrin was found at all sampling points in the Tamandaré channel and almost at all points in the Tucunduba channel. In the Tamandaré channel, the highest concentration found of *trans*-permethrin was 0.032 µg/L, while the lowest concentration found at this site was 0.013 µg/L (0.023 ± 0.024 µg/L). In the Tucunduba channel, the lowest and highest concentrations found, respectively, were 0.013 and 0.041 µg/L (0.024 ± 0.011 µg/L).

Studies have shown that pendimethalin can bioaccumulate in different fish tissues, such as *Oncorhynchus mykiss*, in bile and muscle due to its high bioconcentration factor. Pendimethalin can bioaccumulate in specific tissues and can disrupt different physiological parameters (Danion et al., 2012; 2014). Chronic exposure to metribuzin has had deleterious effects on fish development, being associated with increased mortality in embryos and larvae in *Cyprinus carpio* (Štěpánová et al., 2012).

Comparatively, the Tamandaré channel proved to be more contaminated than the Tucunduba channel with respect to organochlorines. This may be the result of the greater tidal influence in the Tucunduba channel, making it less susceptible to the accumulation of these contaminants (Al-Zawaidah et al., 2021). However, no discrepant difference was observed along the sampling points for each channel. The environmental importance of the detected organochlorines concentrations can be explained by taking into account the octanol-water partition coefficient (log Kow), because this ratio provides information about the bioaccumulation of these contaminants in the environment (Howard and Muir, 2010, 2011). Log Kow values higher than 3 indicate that the compound in question is considered very hydrophobic, which contributes to its bioaccumulation in the environment (Cumming and Rücker, 2017; Moldoveanu and David, 2021). Thus, heptachlor epoxide, pendimethalin, and *trans*-permethrin have higher bioaccumulation potential than metribuzin (log Kow = 1.7), since their log Kow range from 5.1 to 6.5.

3.2 Presence of PAHs in the river waters

The introduction of PAHs into the environment occurs by different routes, but the main ones are oil and natural gas production, chemical industries, gas stations, among others (Hussain et al., 2018). These substances are considered potentially toxic to the ecosystem, since they are able to bioaccumulate and cause various damages to aquatic life, for example (Sverdrup et al., 2002; Hylland, 2006). Several studies over the years have already demonstrated harmful effects of PAHs to fish and shellfish, such as metabolic dysfunction, reduced immunity, tumor development, and problems in the reproductive system (Almeda et al., 2013; Abdel-Shafy and Mansour, 2016).

The properties of PAHs, such as stability, bioaccumulation capacity, and long-range transport, allow them to disperse to different parts of the environment, such as soil, air, water, and biological tissues (Habibullah-Al-Mamun et al., 2019). Due to their potential to bioaccumulate in the environment, PAHs are able to travel through the entire food chain and can even harm human health due to the consumption of contaminated foods such as fish and seafood (Maletić et al., 2019; Ameur et al., 2023). All 7 contaminants detected in our samples are part of the list of 16 HPAs considered as priority HPAs due to their high environmental importance according to the US Environmental Protection Agency (USEPA, 2003). According to the International Agency for Research on Cancer, species such as chrysene, benz(a)anthracene, dibenz(a,h)anthracene found in our samples are potentially carcinogenic to humans (IARC, 1983, 2001).

The PAHs found at both sampling sites were phenanthrene and benz(a)anthracene, but phenanthrene was the only one found in all the replicates. In addition, benzo(a)anthracene was detected only at one sampling point at both sites. For the Tamandaré channel, phenanthrene concentrations ranged from 0.030 to 0.217 µg/L (0.094 ± 0.069 µg/L). For the Tucunduba channel, concentrations ranged from 0.005 to 0.041 µg/L (0.014 ± 0.011 µg/L). For benz(a)anthracene, the concentrations quantified at both sites were very low, being 0.007 µg/L (0.005±0.004 µg/L) for the Tamandaré channel and 0.008 µg/L for the Tucunduba channel (0.008 ± 0.0001 µg/L). In addition, anthracene, pyrene, chrysene and dibenzo(a,h)anthracene were detected only in the Tamandaré channel in at least one of the sampling points. Anthracene was detected at only one sampling point in its three replicates, with concentrations ranging from 0.038 to 0.319 µg/L (0.135 ± 0.159 µg/L). Pyrene was detected at the same point as anthracene in the same three replicates, with concentrations ranging from 0.051 to 0.250 µg/L (0.124 ± 0.117 µg/L). Dibenz(a)anthracene was detected in the three sampling points of the Tamandaré channel, presenting concentrations ranging from 0.121 to 0.207 µg/L (0.160 ± 0.025 µg/L). Chrysene was detected in two of the three sampling points in the Tamandaré channel, with the lowest and highest concentrations being 0.015 and 0.041 µg/L (0.020 ± 0.014 µg/L), respectively. The lowest concentration of PAH found was for to fluorene (0.004 µg/L) while the highest concentration found for this substance was 0.011 µg/L (0.008 ± 0.004 µg/L).

The study carried out by Khursigara et al. (2022) showed that acute exposure to pyrene caused a significant reduction in column length, standard length, total length and brain size in the estuarine fish *Sciaenops ocellatus*. The concentration of 2.2 µg/L caused total mortality of *S. ocellatus* larvae, so the concentration used in this study was 0.36 µg/L, while the highest concentration found in our analysis was 0.259 µg/L, i.e. the concentration of pyrene in urban Amazonian rivers is seven times higher than that already reported in the literature, causing harmful effects in fish.

In *D. rerio* larvae, exposure to phenanthrene resulted in morphological changes, such as spinal curvature, pericardial edema and body shortening (Xu et al., 2022). In adult *D. rerio*, exposure to phenanthrene causes changes in both oxidative stress and the immune system (Xu et al., 2021). The joint exposure of phenanthrene with microplastics causes an increase in oxidized damage in zebrafish, thus stimulating immune function and altering the composition of the intestinal microbiota, which can lead to damage to the animal's health (Xu et al., 2021).

In *Cyprinus carpio*, studies demonstrated that anthracene is capable of increasing the activity of aminotransferase enzymes in the liver after exposure to 7.5 mg/L anthracene (Lateef et al., 2021), while the same species showed DNA damage after exposure to 25 µg/L and 47 µg/L Dibenzo(a,h)anthracene (Kim and Kim, 2016). Even in plants, PAH exposure tests have shown negative results on plant physiology. A medicinal plant, *Amaranthus cruentus*, showed PAH accumulation associated with a reduced production of secondary metabolites (pigments and bioactive compounds) and an imbalance in redox status after being exposed to a mixture of PAHs (a solution that contains substances such as anthracene, dibenzo(a,h)anthracene, pyrene, and phenanthrene) (Tandey et al., 2021). These analyses demonstrate the isolated and combined action of PAHs on several biological systems, proving the harmful effects of these substances in the biota and their potential risk to human populations.

As observed for organochlorines, the Tamandaré channel was found to be more contaminated than the Tucunduba channel with PAHs. Again, this may be a result of the tidal influence being stronger in the Tucunduba channel. (Al-Zawaidah et al., 2021) and also there was no discrepant difference in the concentrations of contaminants in relation to the sampling points in each channel. The environmental importance of detected concentrations of PAHs can also be explained by the Log Kow, which ranged from 4.18 to 6.75, indicating that all the detected PAHs have a high hydrophobic level. Thus, it contributes to the ability of these contaminants to bioaccumulate, as found for the organochlorines.

3.3 Presence of phthalates in the river waters

Phthalates, unlike some substances belonging to organochlorines and PAHs, were detected at both sampling sites and in all replicates at each point, and their lowest concentration (0.466 µg/L) was almost twice as high as the highest concentration found in the other analyzed groups. Taking into account the high consumption of phthalates by industrial processes, this substance is considered ubiquitous in the environment, being found in air, soil, water and food (Naveen et al, 2022). Thus, the China Environmental Monitoring Center and the United States Environmental Protection Agency have listed phthalates as a group of priority pollutants of great environmental relevance (Das et al., 2021), and among the pollutants on this list are DMP, DEP, DBP, and DEHP, all of which were detected in our environmental samples.

The most abundant and commonly detected phthalates are DBP and DEHP (Schecter et al., 2013; Net et al., 2015), corroborating our results, where these presented the highest concentrations found in the environmental samples analyzed. Several harmful effects caused by DBP and DEHP have already been documented in animal tests, both known to cause toxicity in the reproductive system and development in animals for example (Kavlock et al., 2002; Mariana et al., 2016). Other damages caused by these substances are related to changes in the cardiovascular system (Gillum et al., 2009; Lee et al., 2016; Sun and Li, 2019), changes in liver function (Rowdhwal and Chen, 2018; Radha and Basha, 2020), among others.

Recent studies have demonstrated adverse effects of DBP and DEHP in zebrafish (*Danio rerio*), such as: alteration of gill calcium homeostasis (Rodrigues et al., 2020), oxidative stress, genotoxicity and lipid peroxidation in liver cells (Song et al, 2022), genotoxicity and oxidative stress in adult zebrafish brain (Jiang et al., 2022), oxidative stress, apoptosis and toxicity during early development in zebrafish larvae (Huang et al., 2022). According to some studies, the micropollutants detected in our analyses come from human activities (Liu et al., 2021). Therefore, it is believed that the origin of the three groups of micropollutants found comes from activities such as domestic and industrial discharges, dumping of untreated sewage waste, among others, activities which are largely present in the study sites due to the high degree of urbanization.

All the phthalates analyzed (DMP, DEP, DBP and DEHP) were quantified in all sampling points, differing only in their concentration. The lowest concentration found was for DMP (0.466 μg/L) and the highest concentration found was DBP (10.428 μg/L). The contaminant that presented the highest concentrations was DBP, ranging from 5.370 to 10.428 μg/L (7.149 ± 1.259 μg/L). The second was DEHP, ranging from 3.646 to 7,547 μg/L (4.837 ± 1.180 μg/L). The third was DEP, with concentrations ranging from 1.850 to 4.703 μg/L (3.013 ± 0.864 μg/L). DMP had the lowest concentrations found, ranging from 0.466 to 0.810 μg/L (0.683 ± 0.098 μg/L).

For phthalates, there was not a great difference with regard to the level of contamination of both studied channels, since the analyzed phthalates were detected at all sampling points in both channels. As seen for organochlorines and PCBs, for phthalates there was no discrepant difference in the concentrations found at the different sampling points for both channels. Regarding the log Kow of the phthalates, two of the detected phthalates have values below to 3, while the other two above 3, suggesting that DMP and DEP have a lower bioaccumulation potential compared to DBP and DEHP, which have a log Kow of 4.50 and 7.50 respectively. Lastly, the high concentrations of some phthalates found in 50mL of sample from each point can be explained by their physicochemical characteristics, since they have low solubility in water and good stability to light and heat (Gomez-Hens and Aguiar-Caballos, 2003).

3.1.3 Absence of PCBs and hormones in the river waters

PCBs are synthetic substances formed from the gradual chlorination of biphenyl in the presence of a catalyst, in other words, PCBs are chemically chlorinated hydrocarbons (Erickson and Kaley, 2011). These compounds are considered substances of environmental relevance and have been listed as persistent organic pollutants (POPs) by the Stockholm Convention (Zhu et al., 2022), due to presenting themselves as a threat to ecosystems and humans, as they have optimal stability in the environment and are able to bioaccumulate along the food chain (Merhaby et al., 2019).

Even though PCBs are not detected in our samples, many studies report various harmful health effects of PCBs in humans, mammals, and fish, and can cause adverse harm such as neurotoxicity, hepatoxicity, reproductive toxicity, among others (Pessah et al., 2019; Xie et al., 2019; Berghuis and Roze, 2019). These are also classified by the International Agency for Research on Cancer and the United States Environmental Protection Agency as compounds that are potentially carcinogenic to humans (IARC, 1990; EPA, 2015), and taking this into consideration, PCBs have their use totally banned by several countries, yet the population still remains exposed to these substances due to their great ability to bioaccumulate in the environment (Meeker and Hauser, 2010), so it was theoretically expected to find no trace concentrations of PCBs. It's possible that PCBs are present in very low concentrations and could be detected if the samples were pre-concentrated before analysis.

Among the hormones analyzed, none of the substances were detected in any of the sampling points. The hormones analyzed were: Hydrocortisone, Prednisone, Progesterone, 17-Ethynylestradiol, Estriol, Estrone 3 Meo, Estrone, Estradiol, Hexestrol. The non-detection of hormones in Amazonian urban rivers can be explained by the sample volume used sample volume used during the analysis and the concentration range commonly found for hormones, these being found in the environment in the range of ng/L (Almazrouei et al., 2023), that is, the volume of 50 mL used during the analyses may not have been enough to detect hormones, since these can be in extremely small concentrations in the environment, however, analyses with a larger sample volume may indicate the presence of hormones in Amazonian urban rivers.

Regarding hormones, these are considered endocrine disruptors and, even though they are not detected in our samples, they are one of the most studied emerging micropollutants today due to their environmental and human health importance (Bila and Dezotti, 2007). Endocrine disruptors are of great importance because they are able to impair the functioning of the endocrine system, since these substances are able to prevent the action of the natural hormone produced in the body, resulting in several consequences for the body's endocrine system, since this deregulation can affect from the synthesis to the excretion of a hormone produced by the body (Lintelmann et al., 2003).

3.2 Distribution of emerging organic micropollutants in urban rivers

The contribution of each pesticide class to the total pesticide levels in the investigated samples is showed in figure 2. As can be seen, the profile of the contaminants in this study area in the Amazon region is overall characterized by

PAHs and phtalates, and in general very similar in all points. Furthermore, during a detailed analysis of each compound and its contribution across various locations, a discernible pattern of contribution based on compound type and location emerged as can be seen in figure 3. In this figure, it is evident that the Tamandaré channel exhibits the presence of nearly all compounds, each making significant contributions, with the exception of fluorene. This stands in strong contrast to the situation observed in Tucunduba channel.

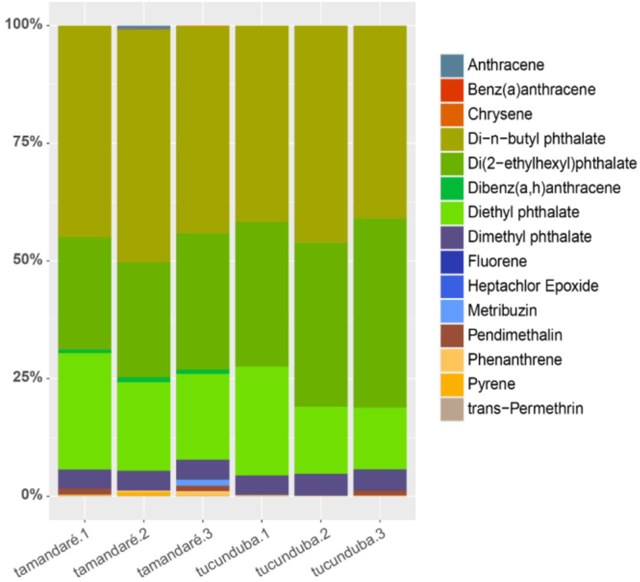


Fig. 2. Relative contribution of different compounds to total concentration of contaminants found in all studied samples.

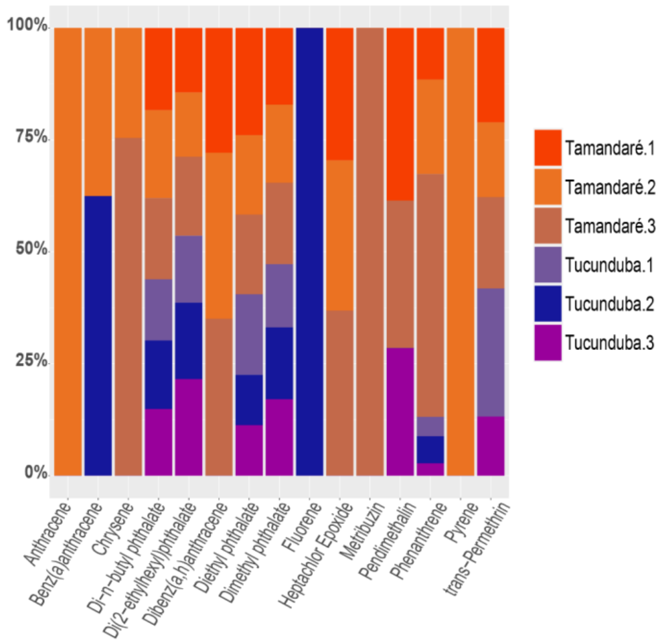


Fig. 3. Relative distribution pattern of the contaminants for each sampled point.

3.3 Multivariate analysis

The multivariate analysis facilitates the exploration of multidimensional data by encompassing multiple variables. It enables a more precise and comprehensive exploration of intricate chemical phenomena and data patterns compared to the limitations of univariate or bivariate approaches. Moreover, the insights garnered through the application of multivariate analysis techniques would have remained inaccessible had solely univariate or bivariate methods been employed. Therefore, the insights and deductions drawn from this study gain augmented validation through the incorporation of multivariate models in the analysis.

Figure 4 illustrates a clustered heatmap designed to depict the prevalence of contaminants traced in water across distinct sampling points in this study. Here, a notable disparity in the presence of contaminants at various points becomes evident, as evidenced by a color range.

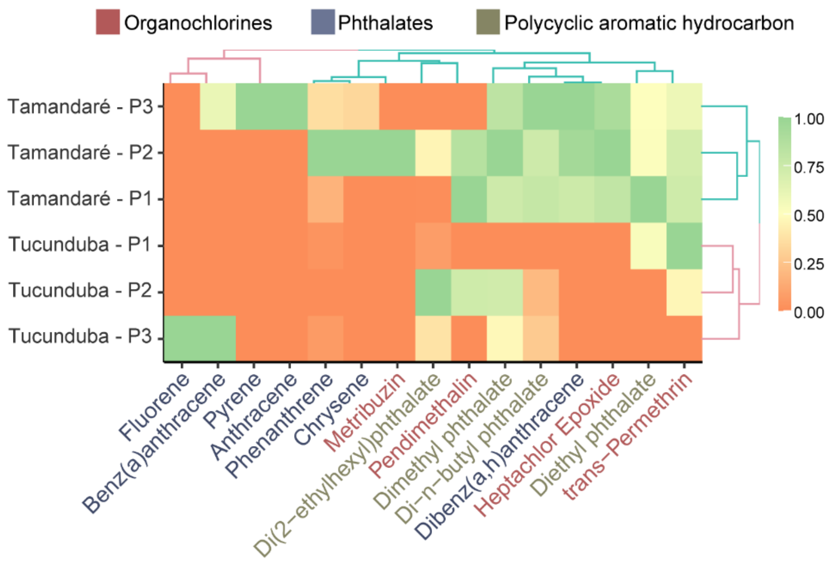


Fig. 4. Heatmap of the different classes of contaminants data from the selected points in a Brazilian urban stream (Tamandaré and Tucunduba channel). Rows are clustered using correlation distance and average linkage. The Profile of the variables is displayed considering the abundance represented through the color range (red to green).

The Biplot - PCA (Figure 5) was constructed to discern the relative significance of variables (contaminants) through group demarcations and their contributions within varying clusters. Our investigation highlights the prominent influence of the area factor. The data highlights that in the Tucunduba area, the compounds Benz(a)anthracene and Fluorene exhibited higher prevalence. Conversely, in the Tamandaré area, Dibenz(a,h)anthracene and Heptachlor epoxide emerged as the most prominent compounds.

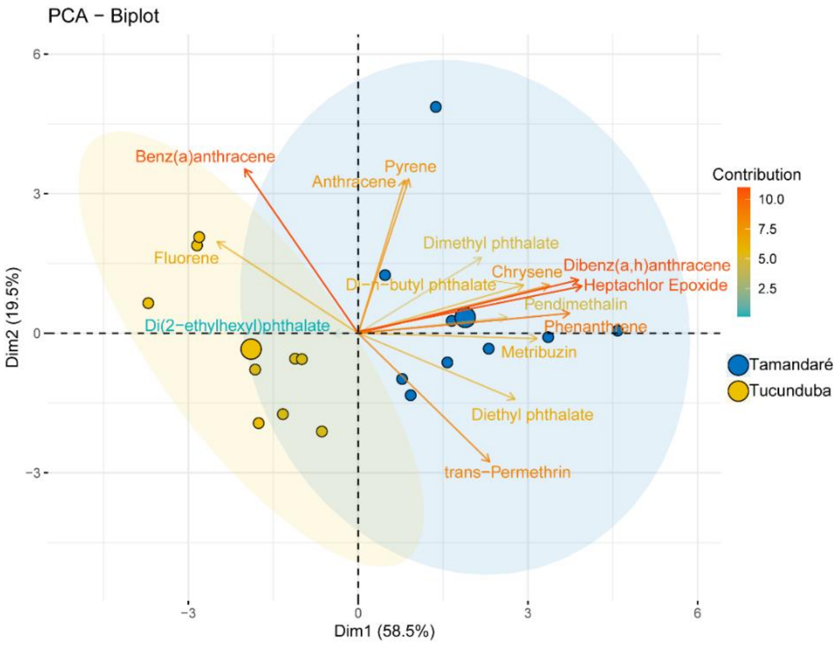


Fig. 5. PCA - Biplot of 15 contaminants collected different points in two different channels (Tamandaré and Tucunduba) in a Brazilian Urban stream from Amazon. The PCA was plot using the first and second principal components showing the correlation of each variable (arrows) and the respective contributions (Color range). The length of the arrows approximates the variance of the variables, whereas the angles between them approximate their correlations.

The chord diagram (Figure 6) displays the inter-relationships between data in a matrix that contains only significant correlations (over 0.5). Besides, it is possible to observe the strength of a variable over correlated variables. In this diagram, the size of each chord for every compound signifies its degree of correlation with the points-specifically, a larger chord indicates a stronger association or higher presence. Hence, from this depiction, it becomes immediately apparent that the compounds most prevalently represented are Di-(2-ethylhexyl) phthalate, Di-n-butyl phthalate, and Diethyl phthalate.

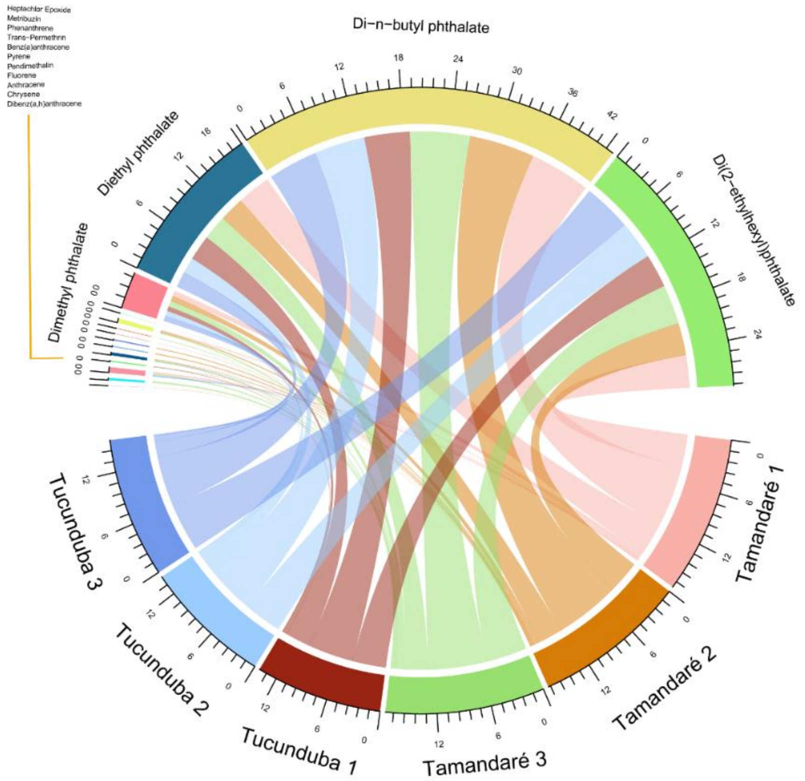


Fig. 6. The Chord diagram is designed with the contaminants data collected from different points in two channels (Tamandaré and Tucunduba) located in a Brazilian urban stream. The size of the arc is proportional to the importance of the flow and the nodes were plot considering only the significantly spearman correlations, ($p < 0.05$).

4. Conclusions

Considering the propensity for micropollutants like organochlorines, PAHs, and phthalates to undergo bioaccumulation, their existence within Amazonian urban rivers becomes a matter of heightened concern, since they are capable of bioaccumulating along the food chain and can easily reach humans through food, causing them various health problems.

Our data unveil greater contamination within the Tamandaré channel compared to the Tucunduba channel. This discrepancy can be attributed to the Tucunduba channel's heightened tidal influence, rendering it less susceptible to the buildup of these contaminants. Furthermore, no noteworthy disparities were observed among the various sampling points along both channels, as these points exhibited remarkably similar concentrations.

In our analyses, micropollutants with significant environmental impact were detected, such as DBP and DEHP, which were found in the highest concentrations and are among the most abundant and dangerous phthalates due to their chemical properties that contribute to their bioaccumulation. In the environment, it can cause serious damage to the human reproductive system and that of other organisms. The other micropollutants found, such as organochlorines and PAHs, even if in lower concentrations, also show up as worrying data since they also cause harmful effects to human and environmental health, considering that water is an essential resource for the survival of various organisms.

Thus, studies focused on monitoring this kind of pollutants are of great environmental relevance because, even at low concentrations, emerging contaminants can cause adverse health effects ranging from endocrine disruption to potential carcinogenic effects both for humans and for the aquatic biota of the Amazon region.

5. Acknowledgments

Erika Monteiro and Yure Jefferson da Cruz do Nascimento are post-graduation students fellowship from Brazilian agency CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior), processes N° 88887.688665/2022-00 and 88887.688681/2022-00, respectively. Lílian Lund Amado and Leandro Machado de Carvalho are researchers productivity fellowship from the Brazilian agency CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico), processes N° 309628/2022-9 and 315969/2021-0, respectively.

6. References

Abdel-Shafy, H. I., Mansour, M. S. M., 2016. A review on polycyclic aromatic hydrocarbons: source, environmental impact, effect on human health and remediation. Egyptian journal of petroleum, 25, 107-123. https://doi.org/10.1016/j.ejpe.2015.03.011.

Almazrouei, B. et al., 2023. Steroid hormones in wastewater: Sources, treatments, environmental risks, and regulations. Emerging Contaminants, 9, 100210. https://doi.org/10.1016/j.emcon.2023.100210.

Almeda, R. et al., 2013. Interactions between zooplankton and crude oil: toxic effects and bioaccumulation of polycyclic aromatic hydrocarbons. PloS one, 8, e67212. https://doi.org/10.1371/journal.pone.0067212.

Al-Zawaidah, H., Ravazzolo, D., Friedrich, H. 2021. Macroplastics in rivers: present knowledge, issues and challenges. Environmental Science: Processes & Impacts, 23, 535-552. https://doi.org/10.1039/D0EM00517G.

Ameur, W. B. et al., 2023. Polycyclic aromatic hydrocarbons in mullet (Chelon auratus) from two lagoons of great ecological and economic importance in Tunisia: Levels, sources and human health risk implications. Journal of Sea Research, 192, 102325. https://doi.org/10.1016/j.seares.2022.102325.

Andréa, M. M., 1998. Contaminação do solo por pesticidas. O Biológico, 60, 63-65. http://repositorio.ipen.br/bitstream/handle/123456789/6351/07834.pdf?sequence=1 (accessed 21 March 2023).

Berghuis, S. A., Roze, E., 2019. Prenatal exposure to PCBs and neurological and sexual/pubertal development from birth to adolescence. Current problems in pediatric and adolescent health care, 49, 133-159. https://doi.org/10.1016/j.cppeds.2019.04.006.

Bertrand, L. et al., 2023. Risk evaluation and prioritization of contaminants of emerging concern and other organic micropollutants in two river basins of central Argentina. Science of The Total Environment, 878, 163029. https://doi.org/10.1016/j.scitotenv.2023.163029.

Bila, D. M., Dezotti, M., 2007. Desreguladores endócrinos no meio ambiente: efeitos e consequências. Química nova, 30, 651-666. https://doi.org/10.1590/S0100-40422007000300027.

Blettler, M. C. M., Abrial, E., Khan, F. R., Sivri, N., Espinola, L. A., 2018. Freshwater plastic pollution: recognizing research biases and identifying knowledge gaps. Water Res. 143, 416-424. https://doi.org/10.1016/j.watres.2018.06.015.

Bolden, A. L. et al., 2017. Polycyclic aromatic hydrocarbons and female reproductive health: a scoping review. Reproductive toxicology, 73, 61-74. https://doi.org/10.1016/j.reprotox.2017.07.012.

Boonyatumanond, R. et al., 2006. Distribution and origins of polycyclic aromatic hydrocarbons (PAHs) in riverine, estuarine, and marine sediments in

Thailand. Marine pollution bulletin, 52, 942-956. https://doi.org/10.1016/j.marpolbul.2005.12.015.

Cable, G. G., Doherty, S., 1999. Acute carbamate and organochlorine toxicity causing convulsions in an agricultural pilot: a case report. Aviation, space, and environmental medicine, 70, 68-72. 9895024.

Cavalcanti, L. P. A. N. et al., 2016. Intoxicação por organofosforados: tratamento e metodologias analíticas empregadas na avaliação da reativação e inibição da acetilcolinesterase. Revista Virtual de Química, 8, 739-766. 10.5935/1984-6835.20160056.

Chen, S. J. et al., 2007. Emissions of polycyclic aromatic hydrocarbons (PAHs) from the pyrolysis of scrap tires. Atmospheric Environment, 41, 1209-1220. https://doi.org/10.1016/j.atmosenv.2006.09.041.

Cumming, H., Rücker, C. 2017. Octanol–water partition coefficient measurement by a simple 1H NMR method. ACS omega, 2, 6244-6249. https://doi.org/10.1021/acsomega.7b01102.

Da Cuna, R. H. et al., 2011. Assessment of the acute toxicity of the organochlorine pesticide endosulfan in Cichlasoma dimerus (Teleostei, Perciformes). Ecotoxicology and Environmental Safety, 74, 1065-1073. https://doi.org/10.1016/j.ecoenv.2011.02.002.

Danion, M., Le Floch, S., Kanan, R., Lamour, F., & Quentel, C., 2012. Effects of in vivo chronic exposure to pendimethalin/Prowl 400® on sanitary status and the immune system in rainbow trout (*Oncorhynchus mykiss*). Science of the total environment, 424, 143-152.

Danion, M., Le Floch, S., Lamour, F., & Quentel, C., 2014. Effects of in vivo chronic exposure to pendimethalin on EROD activity and antioxidant defenses in rainbow trout (*Oncorhynchus mykiss*). Ecotoxicology and environmental safety, 99, 21-27.

Das, M. T. et al., 2021. Remediation strategies for mitigation of phthalate pollution: Challenges and future perspectives. Journal of Hazardous Materials, 409, 124496. https://doi.org/10.1016/j.jhazmat.2020.124496.

Diel, P. et al., 2002. Effects of the environmental estrogens bisphenol A, o, p′-DDT, p-tert-octylphenol and coumestrol on apoptosis induction, cell proliferation and the expression of estrogen sensitive molecular parameters in the human breast cancer cell line MCF-7. The Journal of steroid biochemistry and molecular biology, 80, 61-70. https://doi.org/10.1016/S0960-0760(01)00173-X.

Enan, E., Matsumura, F., 1998. Activation of c-Neu tyrosine kinase by o, p′-DDT and β-HCH in cell-free and intact cell preparations from MCF-7 human breast cancer cells. Journal of Biochemical and Molecular Toxicology, 12, 83-92. https://doi.org/10.1002/(SICI)1099-0461(1998)12:2<83::AID-JBT3>3.0.CO;2-K.

EPA – Environmental Protection Agency, 2015. Learn About Polychlorinated Biphenyls (PCBs). https://www.epa.ie/our-services/monitoring--assessment/waste/chemicals/pcbs (accessed 12 March 2023).

Erickson, M. D., Kaley, R. G., 2011. Applications of polychlorinated biphenyls. Environmental Science and Pollution Research, 18, 135-151. 10.1007/s11356-010-0392-1.

European Chemicals Agency, 2017. Bis(2-ethylhexyl) phthalate. https://echa.europa.eu/brief-profile/-/briefprofile/100.003.829#collapseSeven (accessed 15 March 2023).

França, C. F. 2013. Degradação ambiental de planícies de inundação e qualidade de vida na cidade de Belém, Pará, Brasil, in: Prost, M. T., Mendes, A. C. (Eds.), Ecossistemas costeiros: impactos e gestão ambiental. Museu Paraense Emílio Goeldi, Belém, pp. 91-101.

Ge, R. et al., 2007. Phthalate ester toxicity in Leydig cells: developmental timing and dosage considerations. Reproductive Toxicology, 23, 366-373. https://doi.org/10.1016/j.reprotox.2006.12.006.

Gemusse, S. L. et al., 2021. Micropollutants impair the survival of Oreochromis niloticus and threat local species from Iguaçu River, Southern of Brazil. Environmental Toxicology and Pharmacology, 83, 103596. https://doi.org/10.1016/j.etap.2021.103596.

Gillum, N. et al, 2009. Clinically relevant concentrations of di (2-ethylhexyl) phthalate (DEHP) uncouple cardiac syncytium. Toxicology and Applied Pharmacology, 236, 25-38. https://doi.org/10.1016/j.taap.2008.12.027.

Gioia, R., et al., 2014. Polychlorinated biphenyls (PCBs) in Africa: a review of environmental levels. Environmental Science and Pollution Research, 21, 6278-6289. https://doi.org/10.1007/s11356-013-1739-1.

Gomez-Hens, A., Aguilar-Caballos, M. P., 2003. Social and economic interest in the control of phthalic acid esters. TrAC Trends in Analytical Chemistry, 22, 847-857. https://doi.org/10.1016/S0165-9936(03)01201-9.

Guerra-Rodríguez, S. et al., 2021. UV-A activation of peroxymonosulfate for the removal of micropollutants from secondary treated wastewater. Science of The Total Environment, 770, 145299. https://doi.org/10.1016/j.scitotenv.2021.145299.

Habibullah-Al-Mamun, M. et al., 2019. Distribution of polycyclic aromatic hydrocarbons (PAHs) in commonly consumed seafood from coastal areas of Bangladesh and associated human health implications. Environmental geochemistry and health, 41,1105-1121. https://doi.org/ 10.1007/s10653-018-0202-0.

Hermes, L. C., Silva, A. S., 2004. Avaliação da qualidade das águas: manual prático. Embrapa Informação Tecnológica. Brasília, DF.

Howard, P., Muir, D. C. G. 2010. Identifying new persistent and bioaccumulative organics among chemicals in commerce. Environmental Science & Technology, 44, 2277-2285. https://doi.org/10.1021/es903383a.

Howard, P., Muir, D. C. G. 2011. Identifying new persistent and bioaccumulative organics among chemicals in commerce II: pharmaceuticals. Environmental Science & Technology, 45, 6938-6946. https://doi.org/10.1021/es201196x.

Huang, W. et al., 2022. Effects of di-(2-ethylhexyl) phthalate (DEHP) on behavior and dopamine signaling in zebrafish (*Danio rerio*). Environmental Toxicology and Pharmacology, 93, 103885. https://doi.org/10.1016/j.etap.2022.103885.

Hussain, K. et al., 2018. Monitoring and risk analysis of PAHs in the environment (Eds). Handbook of environmental materials management, 1-35. https://doi.org/10.1007/978-3-319-58538-3_29-2.

Hylland, K., 2006. Polycyclic aromatic hydrocarbon (PAH) ecotoxicology in marine ecosystems. Journal of Toxicology and Environmental Health, Part A, 69, 109-123. 10.1080/15287390500259327.

IARC – International Agency for Research on Cancer, 1983. Summaries & Evaluations. v. 32. p. 135-299. https://www.iarc.who.int/ (accessed 15 March 2023).

IARC – International Agency for Research on Cancer, 1990. Summaries and Evaluations, Chlorinated Paraffins (Group 2B), p. 55. https://www.iarc.who.int/ (accessed 15 March 2023).

IARC – International Agency for Research on Cancer, 2001. Cumulative Cross Index to IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. https://www.iarc.who.int/ (accessed 15 March 2023).

IARC – International Agency for Research on Cancer, 2010. Some non-heterocyclic polycyclic aromatic hydrocarbons and some related exposures. IARC Monographs on the evaluation of carcinogenic risks to humans, 92, 1-853. Lyon, France.

IBGE – Instituto Brasileiro de Geografia e Estatística, 2010. Atlas do Censo Demográfico 2010. https://censo2010.ibge.gov.br/resultados.html (accessed 15 March 2023).

Ikpesu, T. O. et al., 2017. Anthropogenic Activities and the Quality of Aquatic Habitat with Reference to Heptachlor in Ethiope River Basin. Journal of Environmental Science and Public Health, 1, 75-85. 10.26502/JESPH.008.

Jayaraj, R. et al., 2016. Organochlorine pesticides, their toxic effects on living organisms and their fate in the environment. Interdisciplinary toxicology, 9, 90-100. https://doi.org/10.1515/intox-2016-0012.

Jiang, N. et al., 2022. Dibutyl phthalate induced oxidative stress and genotoxicity on adult zebrafish (*Danio rerio*) brain. Journal of Hazardous Materials, 424, 127749. https://doi.org/10.1016/j.jhazmat.2021.127749.

Kavlock, R. et al., 2002. NTP center for the evaluation of risks to human reproduction: phthalates expert panel report on the reproductive and developmental toxicity of di (2-ethylhexyl) phthalate. Reproductive toxicology (Elmsford, NY), 16, 529-653. 10.1016/s0890-6238(02)00032-1.

Khursigara, A. J., Ackerly, K. L., & Esbaugh, A. J., 2022. Pyrene drives reduced brain size during early life exposure in an estuarine fish, the red drum (*Sciaenops ocellatus*). Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology, 259, 109397.

Kim, JH. & Kim, WK. Use of the Integrated Biomarker Response to Measure the Effect of Short-term Exposure to Dibenz[a,h]anthracene in Common carp (*Cyprinus carpio*). Bull Environ Contam Toxicol 96, 496–501 (2016). https://doi.org/10.1007/s00128-015-1726-y

Komolafe, O. et al., 2021. Occurrence and removal of micropollutants in full-scale aerobic, anaerobic and facultative wastewater treatment plants in Brazil. Journal of Environmental Management, 287, 112286. https://doi.org/10.1016/j.jenvman.2021.112286.

Lateef, N. A. et al., 2021. Toxicity of Anthracene on the Function of the Liver and Kidney of the Common Carp *Cyprinus carpio*. Egyptian Journal of Aquatic Biology and Fisheries, 25(3), 831-840. https://dx.doi.org/10.21608/ejabf.2021.182302

Lee, K. et al., 2016. Maternal exposure to di-(2-ethylhexyl) phthalate exposure deregulates blood pressure, adiposity, cholesterol metabolism and social interaction in mouse offspring. Archives of toxicology, 90, 1211-1224. 10.1007/s00204-015-1539-0.

Lintelmann, J. et al., 2003. Endocrine disruptors in the environment (IUPAC Technical Report). Pure and Applied Chemistry, 75, 631-681. https://doi.org/10.1351/pac200375050631.

Liu, L. et al., 2021. Electrochemical adsorption of perfluorooctanoic acid on a novel reduced graphene oxide aerogel loaded with Cu nanoparticles and fluorine. Journal of Hazardous Materials, 416, 125866. https://doi.org/10.1016/j.jhazmat.2021.125866.

Luo, Y. et al., 2014. A review on the occurrence of micropollutants in the aquatic environment and their fate and removal during wastewater treatment. Science of the total environment, 473, 619-641. https://doi.org/10.1016/j.scitotenv.2013.12.065.

Macedo, M., and L. Castello. 2015. State of the Amazon:freshwater Connectivity and Ecosystem Health. (D. Oliveira,C. C. Maretti, S. Charity, eds). (Pp. 136). WWF LivingAmazon Initiative, Brasılia, Brazil.

Mácola, R. J. D. S. L., et al., 2017. I-076-Levantamento das condições de saneamento da microbacia da bacia da Tamandaré do município de Belém/PA. https://tratamentodeagua.com.br/wp-content/uploads/2018/05/I-076.pdf (accessed 15 March 2023).

Maletić, S. P. et al., 2019. State of the art and future challenges for polycyclic aromatic hydrocarbons is sediments: sources, fate, bioavailability and remediation techniques. Journal of hazardous materials, 365, 467-482. https://doi.org/10.1016/j.jhazmat.2018.11.020.

Mariana, M. et al., 2016. The effects of phthalates in the cardiovascular and reproductive systems: A review. Environment international, 94, 758-776. https://doi.org/10.1016/j.envint.2016.07.004.

Meeker, J. D., Hauser, R., 2010. Exposure to polychlorinated biphenyls (PCBs) and male reproduction. Systems biology in reproductive medicine, 56, 122-131. https://doi.org/10.3109/19396360903443658.

Merhaby, D. et al., 2019. Overview of sediments pollution by PAHs and PCBs in mediterranean basin: Transport, fate, occurrence, and distribution. Marine Pollution Bulletin, 149, 110646. https://doi.org/10.1016/j.marpolbul.2019.110646.

Moldoveanu, S., David, V. 2021. Modern Sample Preparation for Chromatography, second ed. Elsevier, Amsterdam.

Naveen, K. V. et al., 2022. Impact of environmental phthalate on human health and their bioremediation strategies using fungal cell factory-A review. Environmental Research, 214, 113781. https://doi.org/10.1016/j.envres.2022.113781.

Ndebele, K. et al., 2010. Estrogenic activity of coumestrol, DDT, and TCDD in human cervical cancer cells. International Journal of Environmental Research and Public Health, 7, 2045-2056. https://doi.org/10.3390/ijerph7052045.

Net, S. et al., 2015. Occurrence, fate, behavior and ecotoxicological state of phthalates in different environmental matrices. Environmental Science & Technology, 49, 4019-4035. https://doi.org/10.1021/es505233b.

Ojoghoro, J. et al., 2021. Steroid hormones in the aquatic environment. Science of The Total Environment, 792, 148306. https://doi.org/10.1016/j.scitotenv.2021.148306.

Pelletier, G. et al., 2009. Contribution of methylmercury, polychlorinated biphenyls and organochlorine pesticides to the toxicity of a contaminant mixture based on Canadian Arctic population blood profiles. Toxicology letters, 184, 176-185. https://doi.org/10.1016/j.toxlet.2008.11.004.

Pessah, I. N. et al., 2019. Neurotoxicity of polychlorinated biphenyls and related organohalogens. Acta neuropathologica, 138, 363-387. https://doi.org/10.1007/s00401-019-01978-1.

Qiao, L. et al., 2021. Determination of pendimethalin dynamic residual distribution in crucian carp tissues and associated risk assessment. International Journal of Analytical Chemistry, 2021, 9. https://doi.org/10.1155/2021/9984230.

Radha, D. M. J., Basha, P. M., 2020. Hepatotoxic evaluation of Di-n-butyl phthalate in Wistar rats upon sub-chronic exposure: A multigenerational assessment. Toxicology Reports, 7, 772-778. https://doi.org/10.1016/j.toxrep.2020.06.008.

Reemtsma, T. et al., 2008. Organophosphorus flame retardants and plasticizers in water in air: I. Occurrence and fate. Trends in Analytical Chemistry, 27, 727-737. https://doi.org/10.1016/j.trac.2008.07.002.

Rengarajan, T. et al., 2015. Exposure to polycyclic aromatic hydrocarbons with special focus on cancer. Asian Pacific Journal of Tropical Biomedicine, 5, 182-189. https://doi.org/10.1016/S2221-1691(15)30003-4.

Rodrigues, K. et al., 2020. Dibutyl phthalate rapidly alters calcium homeostasis in the gills of *Danio rerio.* Chemosphere, 258, 127408. https://doi.org/10.1016/j.chemosphere.2020.127408.

Rowdhwal, S. S. S., Chen, J., 2018. Toxic effects of di-2-ethylhexyl phthalate: an overview. BioMed research international, 2018. https://doi.org/10.1155/2018/1750368.

Santos, A. D. O. et al., 2022. Pharmaceuticals, natural and synthetic hormones and phenols in sediments from an eutrophic estuary, Jurujuba Sound, Guanabara Bay, Brazil. Marine Pollution Bulletin, 184, 114176. https://doi.org/10.1016/j.marpolbul.2022.114176.

Schecter, A. et al., 2013. Phthalate concentrations and dietary exposure from food purchased in New York State. Environmental health perspectives, 121, 473-479. https://doi.org/10.1289/ehp.1206367.

Sharma, H. et al., 2010. Organochlorine pesticides dieldrin and lindane induce cooperative toxicity in dopaminergic neurons: role of oxidative stress. Neurotoxicology, 31, 215-222. https://doi.org/10.1016/j.neuro.2009.12.007.

Silva, B. D. A., Nylander, J. D. A., Pereira, J. A. R. 2020. Análise do Lançamento de Esgoto Sanitário no Canal de Drenagem da Bacia Hidrográfica da Tamandaré/Belém-Pará. Journal Gestão & Sustentabilidade Ambiental, 9, 92-109.

Simpson, M. J. et al., 2005. Comparison of polycyclic aromatic hydrocarbon distributions and sedimentary organic matter characteristics in contaminated, coastal sediments from Pensacola Bay, Florida. Marine Environmental Research, 59, 139-163. https://doi.org/10.1016/j.marenvres.2003.09.003.

Singh, S., Li, S. S., 2012. Bisphenol A and phthalates exhibit similar toxicogenomics and health effects. Gene, 494, 85-91. https://doi.org/10.1016/j.gene.2011.11.035.

Singh, Z. et al., 2016. Toxic effects of organochlorine pesticides: a review. Am. J. BioSci, 4, 2016040301-13. 10.11648/j.ajbio.s.2016040301.13.

Song, P. et al., 2022. Ecotoxicological evaluation of zebrafish liver (*Danio rerio*) induced by dibutyl phthalate. Journal of Hazardous Materials, 425, 128027. https://doi.org/10.1016/j.jhazmat.2021.128027.

Štěpánová, Stanislava, et al., 2012. The effects of metribuzin on early life stages of common carp (*Cyprinus carpio*). Pesticide biochemistry and physiology, 103(2), 152-158. https://doi.org/10.1016/j.pestbp.2012.04.013.

Sun, G.; Li, Y., 2019. Exposure to DBP induces the toxicity in early development and adverse effects on cardiac development in zebrafish (Danio rerio). Chemosphere, 218, 76-82. https://doi.org/10.1016/j.chemosphere.2018.11.095.

Sverdrup, L. et al., 2002. Soil ecotoxicity of polycyclic aromatic hydrocarbons in relation to soil sorption, lipophilicity, and water solubility. Environmental science & technology, 36, 2429-2435. https://doi.org/10.1021/es010180s.

Tandey, R., Chouhan, K.B.S., Sen, K.K. et al. Physiological and biochemical responses of Amaranthus cruentus to polycyclic aromatic hydrocarbon pollution caused by thermal power units. Environ Sci Pollut Res 27, 14790–14806 (2020). https://doi.org/10.1007/s11356-020-07971-6

Targa, M. D. S. et al., 2012. Urbanização e escoamento superficial na bacia hidrográfica do Igarapé Tucunduba, Belém, PA, Brasil. Revista Ambiente & Água, 7, 120-142. https://doi.org/10.4136/ambi-agua.905.

Ternes, T., Joss, A., 2007. Human pharmaceuticals, hormones and fragrances: The challenge of micropollutants in urban water management, first ed. IWA publishing, London.

U.S. Environmental Protection Agency, 2009. National Primary Drinking Water Regulations: Minor Revisions to Public Notification Rule and Consumer Confidence Report Rule. https://www.federalregister.gov/ (accessed 15 March 2023).

USEPA – U. S. Environmental Protection Agency, 2003. Procedures for the derivation of equilibrium partitioning sediment benchmarks (ESBs) for the protection of benthic organisms: PAHs mixtures. Washington, DC.

Wijngaard, R. R. et al., 2017. The impact of climate change on metal transport in a lowland catchment. Water, Air, & Soil Pollution, 228, 1-20. 10.1007/s11270-017-3261-4.

Wong, L. I. L. et al., 2015. p, p′-Dichlorodiphenyltrichloroethane (p, p′-DDT) and p, p′-dichlorodiphenyldichloroethylene (p, p′-DDE) repress prostate specific antigen levels in human prostate cancer cell lines. Chemico-biological interactions, 230, 40-49. https://doi.org/10.1016/j.cbi.2015.02.002.

Xie, X. et al., 2019. PCB52 induces hepatotoxicity in male offspring through aggravating loss of clearance capacity and activating the apoptosis: sex-biased effects on rats. Chemosphere, 227, 389-400. https://doi.org/10.1016/j.chemosphere.2019.04.077.

Xu, K., Ai, W., Wang, Q., Tian, L., Liu, D., Zhuang, Z., & Wang, J., 2022. Toxicological effects of nanoplastics and phenanthrene to zebrafish (*Danio rerio*). Gondwana Research, 108, 127-132. https://doi.org/10.1016/j.gr.2021.05.012.

Xu, K., Zhang, Y., Huang, Y., & Wang, J., 2021. Toxicological effects of microplastics and phenanthrene to zebrafish (*Danio rerio*). Science of The Total Environment, 757, 143730. https://doi.org/10.1016/j.scitotenv.2020.143730.

Yu, H., Yang, K., 2007. Mechanisms of mitogen-activated protein kinase pathway in male reproductive toxicity induced organochlorine pesticides. Wei Sheng yan jiu = Journal of Hygiene Research, 36, 756-758, 2007. 18303644.

Zhang, X. et al., 2011. Estrogenic compounds and estrogenicity in surface water, sediments, and organisms from Yundang Lagoon in Xiamen, China. Archives of environmental contamination and toxicology, 61, 93-100. 10.1007/s00244-010-9588-0.

Zhang, Y. et al., 2021. Hazards of phthalates (PAEs) exposure: A review of aquatic animal toxicology studies. Science of the Total Environment, 771, 145418. https://doi.org/10.1016/j.scitotenv.2021.145418.

Zhu, M. et al., 2022. Environmental contamination and human exposure of polychlorinated biphenyls (PCBs) in China: A review. Science of the Total Environment, 805, 150270. https://doi.org/10.1016/j.scitotenv.2021.150270.

3. ARTIGO 2: EFEITOS DE CONCENTRAÇÕES AMBIENTALMENTE REALISTAS DE DIBUTIL FTALATO NA REGIÃO AMAZÔNICA EM *Hyphessobrycon heterorhabdus* (Teleostei: Characidae)

Effects of environmentally realistic concentrations of Dibutyl phthalate in the Amazon region in *Hyphessobrycon heterorhabdus* (Teleostei: Characidae)

Erika Monteiro[ac], Irina Sofia Cardoso de Carvalho[bc], Danilo Serrão Moutinho[c], Yure Jefferson da Cruz do Nascimento[ac], Amanda Julião da Silva[c], Ilene Crestani[d], Cassia Katrin Reinke[d], Jocinei Dognini[d], Leandro Machado de Carvalho[e], Daniele Salgado[c], Lílian Lund Amado[abc*]

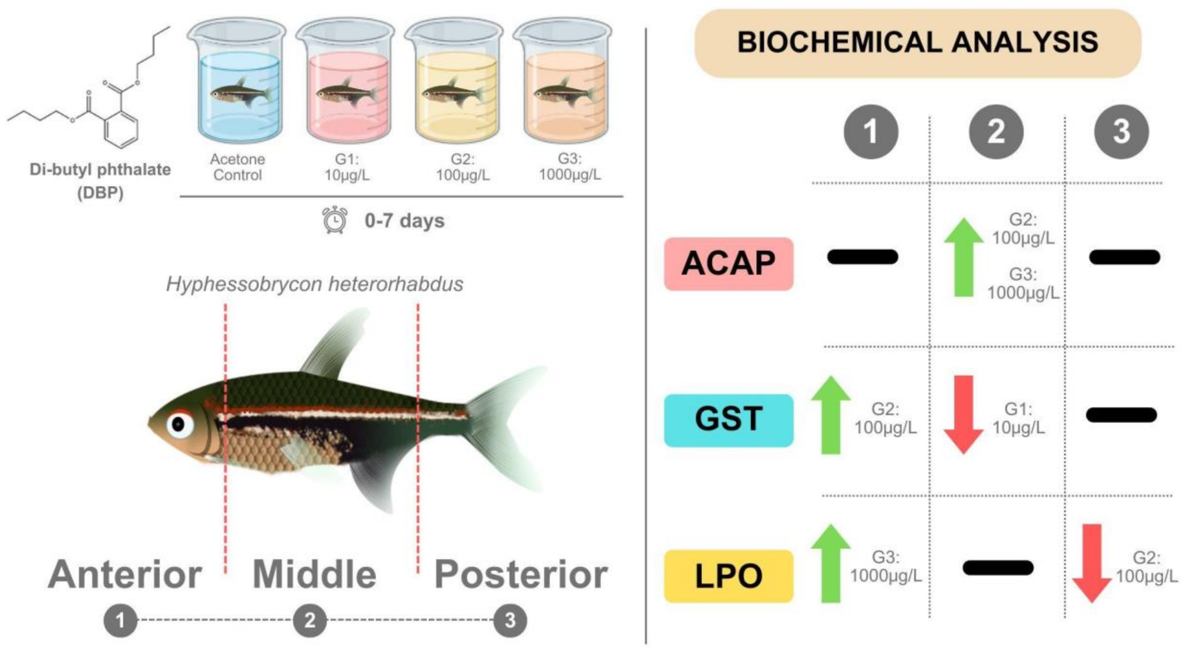
[a]Programa de Pós-graduação em Farmacologia e Bioquímica, Instituto de Ciências Biológicas, Universidade Federal do Pará, Belém, PA, Brazil.
[b]Programa de Pós-graduação em Ecologia Aquática e Pesca, Núcleo de Ecologia Aquática e Pesca da Amazônia, Universidade Federal do Pará, Belém, PA, Brazil.
[c]Laboratório de Ecotoxicologia (LABECOTOX) e Laboratório de Pesquisas em Monitoramento Ambiental Marinho (LAPMAR), Universidade Federal do Pará, Belém, PA, Brazil.
[d]Instituto SENAI de Tecnologia Ambiental, Blumenau, SC, Brazil.
[e]Departamento de Química, Universidade Federal de Santa Maria, Santa Maria, RS, Brazil.

* Corresponding author: Laboratório de Ecotoxicologia, Instituto de Ciências Biológicas, Universidade Federal do Pará, Avenida Augusto Corrêa, 01, Guamá, 66075-110, Belém, Pará, Brazil.
E-mail address: lla@ufpa.br, lilian.amado@gmail.com (L.L. Amado).

Abstract:
Previously our group have shown that urban amazonian rivers in Belém, Pará, Brazil displayed high levels of emerging pollutants, including Dibutyl phthalate (DBP). In this sense, this study evaluated the biochemical changes in *Hyphessobrycon heterorhabdus* exposed to three different concentrations of DBP. The experimental

groups were: solvent control (acetone), G1: 10 µg/L (urban river Amazon environmental concentration), G2: 100 µg/L, and G3: 1000 µg/L. The anterior, middle, and posterior sections of *H. heterorhabdus* were established for the analysis of biomarkers such as total antioxidant capacity (ACAP), glutathione S-transferase (GST), and lipoperoxidation (LPO). ACAP did not vary among the groups in the anterior and posterior sections, showing a concentration-dependent effect in the middle section. GST increased in G2 in the anterior section, preventing cell damage, and there was also a decrease in GST in G1 in the middle section, no variations in the posterior section. For LPO, we observed a concentration-dependent and hormetic effects in the anterior and posterior sections, respectively, but no variations in the middle section. In conclusion, our results showed concentration-dependent and hormetic effects of biomarkers when exposed to different concentrations of DBP, suggesting that the progressive increase of DBP in the environment is related to progressive cell damage and essential interference in the antioxidant system. We also highlight *H. heterorhabdus* as a potential biomonitor organism for the Amazon region since it has shown promising responses to exposure to contaminants.

Graphical abstract:



Keywords: Micropollutants in the Amazon; DBP; emerging micropollutants; environmental pollution; biomarkers.

Highlights:

- DBP causes a concentration-dependent effect on ACAP and LPO in the middle and anterior sections, respectively;
- The environmental concentration of DBP for the Amazon region causes a decrease in GST in the middle section;
- Low DBP concentrations result in a hormetic effect on LPO in the posterior section.

1. Introduction

Phthalates are synthetic products that are widespread in various industrial applications, mainly in plastic-based consumer products (Staples et al., 1997; Shin et al., 2020; Radke et al., 2019) which are widely used in industry as a whole, and are also found in other products such as paints, toys, perfumes, building materials, cosmetics, detergents, soaps, medical devices, among other products (Latini, 2005; Wormuth et al., 2006; Benjamin et al., 2015; Karačonji et al., 2017). Phthalates have an unstable characteristic because they are not covalently attached to the plastic matrix (Bošnir, 2003), facilitating their disaggregation from polymers (Kashyap and Agarwal, 2018; Zhang et al., 2021), and are carried into the environment through evaporation, leaching, and abrasion of plastic materials (Benjamin et al., 2015; Zhang et al., 2021).

Studies in various regions have already detected phthalates in the environment, such as in China (Wang et al., 2023), the Maldives (Saliu et al., 2019), Iran (Gholaminejad et al., 2024), and southeastern Brazil (Neves et al., 2023). However, in the Amazon, there are not enough studies to detect these pollutants in environmental compartments, with only one recent analysis in urban rivers in the northeastern part of the Amazon (Monteiro et al., 2024) detecting not only phthalates but other groups of emerging contaminants, with the compound present in the highest concentration in this region being dibutyl phthalate (DBP).

DBP is one of the most commonly found phthalates in personal care products, leading humans and other organisms to be directly and continuously exposed to this compound (Zhang et al., 2021; Barbaud and Lafforgue, 2021; Heudorf, Mersch-Sundermann and Angerer, 2007), with the ability to enter animals via three routes: digestive, inhalation and dermal (Wittassek et al., 2011). In addition, epidemiological studies and tests on animals and cell lines have shown that exposure to DBP causes potential damage to the reproductive axis (Alam et al., 2010; Bao et al., 2011; Chen, Wang, and Liang., 2024), neurotoxicity (Chen, Wang and Liang, 2024) and promotes oxidative damage (Aly et al., 2016; Hou et al., 2024). However, given the presence of DPB in Amazonian aquatic environments, it is worth emphasizing the importance of biomonitoring this region using native organisms as a biological model in order to obtain a realistic understanding of the effects of these contaminants on the environment studied (Krull and Barros, 2012).

In this context, the analysis of oxidative stress biomarkers is an excellent tool for assessing the toxicity of contaminants, such as DBP and other compounds, at a biological level (Gabriel et al., 2020). This definition is because biomarkers are capable of providing an immediate response to the effects of contaminants on organisms, indicating the level of exposure to a stressful source resulting from changes in the activity of antioxidant enzymes and oxidative damage suffered by biological molecules (Hampel et al., 2016; Gonçalves et al., 2021). In this way, we investigated the effects of environmentally relevant concentrations of DBP for the Eastern Amazon on the native fish *Hyphessobrycon heterorhabdus*, an animal that is a potential model organism for ecotoxicological tests in this region.

2. Material and methods

2.1 Fish collection and acclimation

The adult specimens of *H. heterorhabdus* (n = 20) were collected from a stream in the Parque Ecológico do Gunma (Pará, Brazil) with no direct influence from anthropogenic activity. The federal government agency SISBIO – ICMBIO (63470-2) authorized the collection of the animals, and the experimental procedures were approved by the Ethics Committee for the Use of Animals in Experiments of the Federal University of Pará (7369290224). The animals were acclimatized in the laboratory in glass aquariums (dimensions 35 cm x 30 cm x 35 cm) containing reconstituted reverse osmosis water for seven days, with a 14C/10E photoperiod, biological filtration, a constant aeration system, a temperature of 25ºC and partial water changes or as needed. The animals were maintained by feeding them TetraMin commercial feed with 37% protein twice a day, with feeding being interrupted 24 hours before the experiment.

2.2 Chemical substance and experimental design

A 99% purity DBP was purchased from Sigma-Aldrich (CAS 524980). Briefly, the DBP was diluted in Acetone P.A. to a nominal stock concentration of 20.79 g/L, after which part of this solution was diluted in distilled water to a nominal concentration of 100 mg/L, which was used for the subsequent dilutions of the experiments. From this stock solution, the solutions for the exposure media were prepared. The treatment concentration of 10 µg/L was chosen based on previous studies on the quantification of micropollutants in the Amazon (unpublished results).

The specimens were divided into four experimental groups with five replicates each: 1) the acetone group (CA) was defined using only the same volume of the diluent (acetone); 2) group 1 (G1) received the highest concentration found in the study carried out previously (10 µg/L); 3) groups 2 (G2) and 3 (G3) received, respectively, a concentration ten times and one hundred times higher than the highest concentration found (100 µg/L and 1000 µg/L), in order to assess the possible effects of increasing environmental concentrations of DBP for seven days. Partial changes were made every 24 hours.

After the experimentation period, the animals were euthanized following the precepts of the National Council for the Use of Animals in Experiments (CONCEA - RN nº13/2013) and then dissected to obtain the anterior, middle, and posterior sections for biochemical analysis. The anterior section was defined from the beginning of the head to the beginning of the vertebral column, covering the entire opercular area of the animal; the middle section was delimited from the vertebral column to the anus of the animal, with the posterior section being from the anus to the end of the caudal fin.

2.3 Biochemical analysis

The samples (anterior, middle, and posterior) were homogenized in a buffer solution (Tris-HCl 100 mM, EDTA 2 mM, MgCl2.6H2O 5 mM, pH 7.5) in a ratio of 1:4 (weight: volume) using a Potter-type tissue homogenizer according to the method of Bainy et al. (1996). They were then centrifuged at 10,000 x g for 20 minutes at 4ºC. The supernatant was removed, aliquoted, and placed in a freezer at -80ºC for further analysis. A commercial kit (Labtest) based on the Biuret test for proteins was used to analyze total proteins, using bovine albumin as a standard. Readings were taken using a multimodal microplate reader (PerkinElmer VICTOR X3) at 550 nm, and the results obtained were expressed in milligrams of protein/mL.

ACAP analysis followed the method described by Amado et al. (2009), which determines the antioxidant capacity of samples with and without exposure to a radical generator, peroxyl 2'2'-azobis-2-methylpropiamidine dihydrochloride (ABAP, 4nM), using 2′,7′-dichlorofluorescein diacetate (H2DCF-DA) as a substrate. Peroxyl radicals are produced by thermal decomposition (37°C) of ABAP. In the presence of peroxyl radicals, H2DCF-DA intercepts the radicals' electrons and generates a fluorochrome, which is detected using wavelengths of 488 and 525 nm for excitation and emission, respectively. The principle of this method is that samples with a higher antioxidant capacity will have less peroxyl radical formation. The results were expressed as the difference in the area of UF/min in the same sample with and without ABAP and relativized by the area without ABAP. The inverse of the relative area difference with and without ABAP is considered a measure of antioxidant capacity.

The analysis of GST was based on the work of Habig and Jakoby (1981), which evaluated the conjugation of 1 mM GSH with 1 mM 1-chloro-2,4 dinitrobenzene, a process that used GST as a catalyst and a reaction medium consisting of 0.1 M phosphate, pH 7.0. The readings were taken using a spectrofluorimeter with a microplate reader, and the results were expressed as UGST/mg of protein.

Lipoperoxidation was determined using the thiobarbituric acid reactive substances (TBARS) fluorimetry method, as described by Oakes and Kraak (2003). This method quantifies a by-product of lipid peroxidation, malondialdehyde (MDA). In the assay, MDA reacts with thiobarbituric acid (TBA, 0.8%) in an acidic medium (20% acetic acid) at a temperature of 95ºC, forming the pink-colored MDA-TBA2 complex, which is detected by the fluorimeter using wavelengths of 515 and 553 nm for emission and excitation, respectively. Butylated hydroxytoluene (BHT) was used as an antioxidant for the samples, and 1,1,3,3-tetramethoxypropane (TMP) was used as a standard. Sodium dodecyl sulfate (SDS, 8.1%) was used as a surfactant, and n-Butanol was used to separate the organic from the inorganic phase. The results were expressed as nmol MDA/g wet tissue.

2.4 Statistical analysis

The assumptions of normality (Shapiro-Wilks test) and homoscedasticity (Levene test) were tested on the biomarker data for subsequent application of analysis of variance (one way ANOVA) with Tukey's post-hoc test. The results were expressed as mean ± standard error. When these assumptions were not met, the non-parametric Kruskall-Wallis test was applied to compare biomarkers between DBP concentrations, with results expressed as median ± first quartile. The significance level adopted was 5% in all cases ($p<0.05$).

3. Results

The total antioxidant capacity (ACAP) showed no significant difference between the groups in the anterior and posterior sections (p>0.05) (Fig. 1A, 1C), while in the middle section, there was a significant decrease in antioxidant capacity among the diluent control group (2.980 ± 0.216) and treatment groups 10 µg/L (4.077 ± 0.790), 100 µg/L (5.856 ± 1.569), and 1000 µg/L (7.962 ± 0.533). (Fig. 1B).

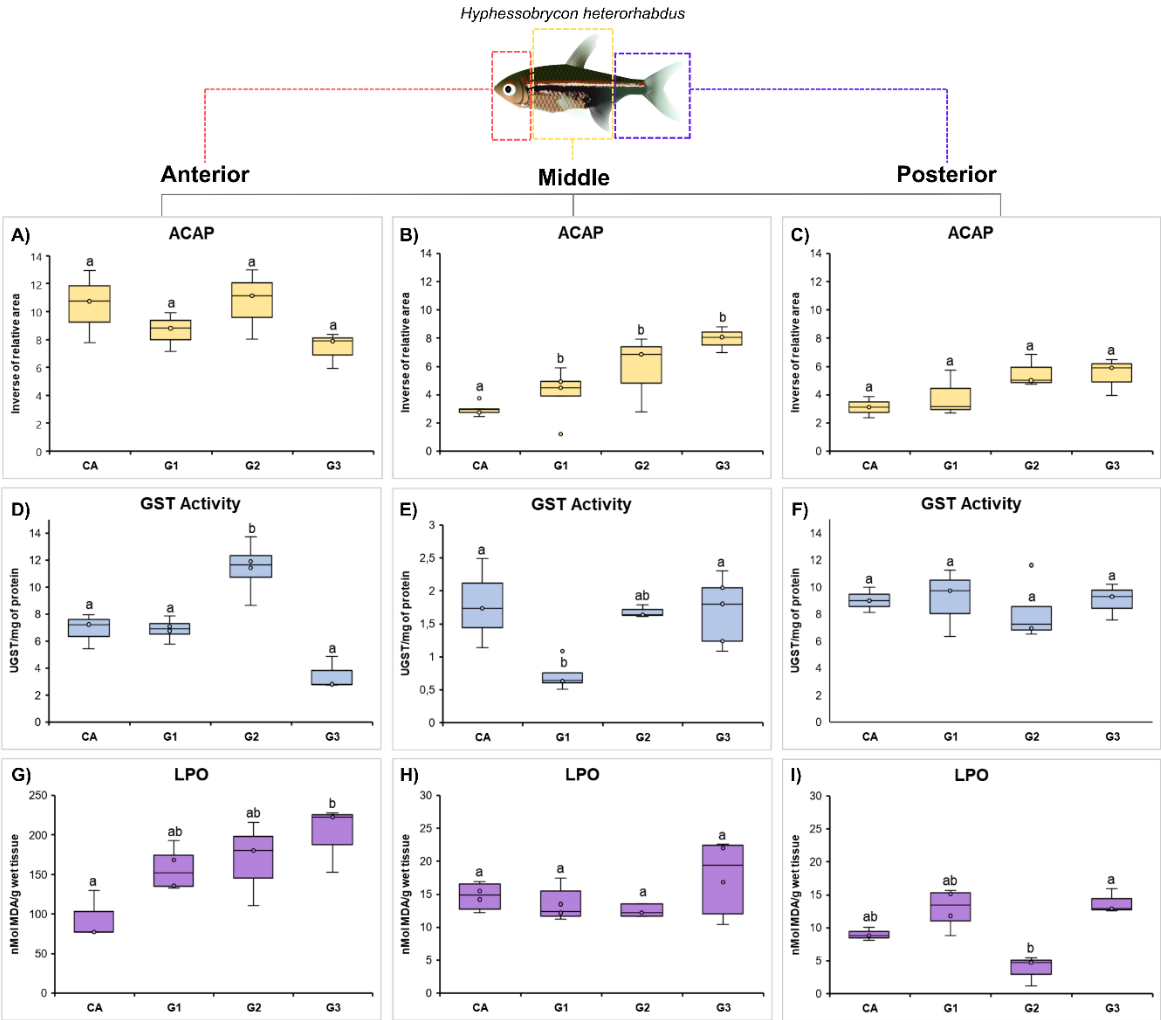


Fig. 1. Biomarkers of oxidative stress in the anterior, middle, and posterior portions of *H. heterorhabdus* at three concentrations of dibutyl phthalate. (A; B; C): total antioxidant capacity (mean ± standard error); (D; E; F): glutathione-S-transferase activity (mean ± standard error); (G; H; I): lipoperoxidation levels (median ± 1^{st} quartile). CA: acetone control; G1: 10 µg/L; G2: 100 µg/L; G3: 1000 µg/L. Note the different scales on the graphs. Different letters represent a significant difference between the groups (p<0.05).

Glutathione-S-transferase (GST) activity was higher in 100 µg/L (11.430 ± 1.045) than in the other groups in the anterior section (Fig. 1D). In the middle section, there was a decrease in GST of 10 µg/L (0.720 ± 0.127) compared to the control group (1.791 ± 0.392) and 1000 µg/L (1.700 ± 0.234). The other groups did not differ statistically ($p>0.05$) (Fig. 1E). In the posterior section, GST activity did not vary among the groups studied ($p>0.05$) (Fig. 1F).

Lipoperoxidation (LPO) for the anterior section only differed among groups control (94.656 ± 17.556) and 1000 µg/L (201.001 ± 24.240), where there was an increase in LPO in 1000 µg/L (Fig. 1G). CA group, G1 and G2, did not differ in terms of MDA in the anterior section of the ($p>0.05$) (Fig. 1G). In the middle section, there was no difference among the groups ($p>0.05$) (Fig. 1H). In the posterior section, there was only a difference among 100 µg/L (4.742 ± 2.957) and 1000 µg/L (12.890 ± 12.715), where there was an increase in MDA in 1000 µg/L (Fig. 1I). The other groups showed no difference in MDA ($p>0.05$) (Fig. 1I).

4. Discussion

Over the years, several studies have been carried out identifying and quantifying micropollutants in urban areas around the world, specifically in Brazil (Wong et al., 2015; Xie et al., 2019; Rodrigues et al., 2020; Song et al., 2022). However, in the Eastern Amazon there is still a lack of studies on the quantification of micropollutants, especially on their effects on native species. Thus, in this study, we evaluated the effects of increasing concentrations of dibutyl phthalate, using as a reference the concentration of this contaminant found in rivers in the Amazon region, on the native fish *H. heterorhabdus* by analyzing biomarkers of oxidative stress in the anterior, middle, and posterior portions of the animals.

When the organism is exposed to a contaminant, radicals are formed inside the cell, and ACAP is a biochemical biomarker that measures the cell's ability to neutralize prooxidants (Amado et al., 2009). In the anterior portion, where organs such as the gills and brain are located (Kardong, 2022), there were no differences in ACAP levels, probably due to global response of enzymatic (catalase, reduced glutathione, glutathione peroxidase, among others) and non-enzymatic (glutathione, β-carotene, etc.) factors (Amado et al., 2009) accessed by this method. However, when we analyzed the activity of the GST, another essential biomarker of the antioxidant system, which participates in the elimination of xenobiotics (Prysyazhnyuk et al., 2021) we observed an increase in this enzyme in 100 µg/L. This increase may have been an attempt by the organism to minimize cell damage, as evidenced by the levels of MDA, an effect biomarker that analyzes whether or not the organism has suffered oxidative damage from exposure to a contaminant (Regoli and Giuliani, 2014; Javed et al., 2016; Sies, Berndt and Jones, 2017).

A study carried out in China by Jiang et al. (2022) on *Danio rerio* showed an increase in MDA on the 28th day with DBP concentrations starting at 0.08 mg/L, where they observed a concentration-dependent effect, increasing MDA levels in the brain as the exposure concentration increased. Although at lower concentrations, our results were similar to those found by Jiang et al. (2022), where we observed an increase in MDA levels in G3 in anterior section. So it is possible to infer that the higher exposure concentration caused an increase in the formation of ROS, causing the antioxidant system to be unable to remove the excess of generated prooxidants. This situation caused an increase in the level of lipoperoxidation in *H. heterorhabdus*. This finding is cause for concern due to the damage caused even at low concentrations since recent studies show that DBP is a neurotoxic contaminant (Paquette et al., 2022; Chen, Wang, and Liang, 2024).

In the middle section, a concentration-dependent effect of ACAP was observed, with the levels of this biomarker of antioxidant competence capacity increasing with increasing concentration of DBP and preventing lipid peroxidation in the exposed groups. To prevent the increase in cell damage the Amazonian fish *H. heterorhabdus*, may have developed physiological adaptations against pro-oxidant attack in which basal levels of antioxidant enzymes, such as GST, are sufficient to protect the organism from cell damage (Cantanhêde et al., 2022). Despite the decrease in GST in 10 µg/L, there was no increase in MDA in this group, suggesting that other defense mechanisms of the antioxidant system may have been activated, as we observed a concentration-dependent effect of ACAP.

Although the decrease in GST did not prevent the increase in MDA in the middle section, the environmental concentration of 10 µg/L, found in urban rivers in the Amazon region, was sufficient to decrease GST activity, an essential enzyme in the antioxidant defense system. Another factor contributing to these results is the tissue heterogeneity in the middle section since it contains organs such as the heart, intestine, liver, and muscle tissue (Kardong, 2014), which are present in different proportions and may have diluted other information. Enzyme levels can also be explained by biological factors such as the absorption rate and distribution of DBP to different tissues (Whyte et al., 2000).

In the posterior section, where the muscle is primarily present, there were no changes in ACAP or GST activity, which may have contributed to the increase in MDA levels in 10 µg/L, a decrease in 100 µg/L, and a further increase in 1000 µg/L, resulting in a phenomenon known as hormesis (Agathokleous et al., 2021). Hormesis can be understood as a biphasic response characterized by two premises. The first is to inhibit biological functions when exposed to large amounts of stress, and the second is to stimulate biological processes when exposed to low amounts of stress (Calabrese and Baldwin, 2003). In other words, hormesis infers that organisms such as animals, plants, and microorganisms, among others, exhibit a stimulation of health in response to low amounts of stress (Agathokleous and Calabrese, 2020; Carvalho, Castro and Azevedo., 2020; Shahid et al., 2020). Recent studies show that leached chemical traces of microplastics, metals, and phthalates can induce hormesis in various organisms (Chae, Kim and An, 2019; Schiavo et al., 2021).

Relating the hormetic concept to the results found for LPO in the posterior section, we found that the lowest concentration of 10 µg/L of DBP used, although not significant to the acetone group, increased MDA levels. In comparison, the intermediary concentration of 100 µg/L of DBP resulted in a decrease in MDA levels, indicating that this concentration would be the necessary concentration to stimulate the antioxidant system, thus preventing cell damage (Agathokleous et al., 2021). However, MDA levels increased again in 1000 µg/L, as seen in 10 µg/L, which may be explained by the fact that the higher concentration of exposure ends up making the antioxidant system's defenses ineffective (Agathokleous et al., 2021), causing an increase in lipid peroxidation levels.

Thus, our analyses indicate that the possible increase in environmental concentrations can cause cell damage, as evidenced by the increased LPO in the anterior section, where in this region, the brain, an essential organ of the nervous system, and the gills, the organ responsible for gas exchange, are present, in addition to the concentration-dependent effect, evidencing the hormetic phenomenon. GST proved to be an essential biomarker of the antioxidant defense system, but more is needed to fully understand the ecotoxicological effects of DBP on *H. heterorhabdus*. It is necessary to investigate the activity of other biomarkers of the antioxidant system, such as glutathione peroxidase (GPx), glutathione reductase (GR), and acetylcholinesterase (AChE), thus highlighting the importance of investigating other biomarkers for a better understanding of the effects of DBP on native aquatic organisms.

In addition, *H. heterorhabdus* proved to be an excellent biomonitor organism for the Amazon region, as it shows good responses to exposure to contaminants and is a resistant species that is easy to adapt to and maintain in the laboratory.

5. Acknowledgments

We thank the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Brazilian CAPES) for the scholarship provided to E. Monteiro (Process Number 88887.688665/2022-00). L. L. Amado is researcher productivity fellowship from the Brazilian agency CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico), process N^{o} 309628/2022-9)

6. References

Agathokleous, E., Calabrese, E. J. (2020). A global environmental health perspective and optimisation of stress. Science of the Total Environment, 704, 135263.

https://doi.org/10.1016/j.scitotenv.2019.135263.

Agathokleous, E., Iavicoli, I., Barceló, D., Calabrese, E. J. (2021). Micro/nanoplastics effects on organisms: a review focusing on 'dose'. Journal of hazardous materials, 417, 126084. https://doi.org/10.1016/j.jhazmat.2021.126084.

Alam, M. S., Ohsako, S., Matsuwaki, T., Zhu, X. B., Tsunekawa, N., Kanai, Y., …, Kurohmaru, M. (2010). Induction of spermatogenic cell apoptosis in prepubertal rat testes irrespective of testicular steroidogenesis: a possible estrogenic effect of di (n-butyl) phthalate. Reproduction, 139(2), 427. https://doi.org/10.1530/rep-09-0226.

Aly, H. A., Hassan, M. H., El-Beshbishy, H. A., Alahdal, A. M., Osman, A. M. M. (2016). Dibutyl phthalate induces oxidative stress and impairs spermatogenesis in adult rats. Toxicology and industrial health, 32(8), 1467-1477. https://doi.org/10.1177/07482337145668.

Amado, L. L., Garcia, M. L., Ramos, P. B., Freitas, R. F., Zafalon, B., Ferreira, J. L. R., …, Monserrat, J. M. (2009). A method to measure total antioxidant capacity against peroxyl radicals in aquatic organisms: application to evaluate microcystins toxicity. Science of the total Environment, 407(6), 2115-2123. https://doi.org/10.1016/j.scitotenv.2008.11.038.

Bainy, A. C., Saito, E., Carvalho, P. S., Junqueira, V. B. (1996). Oxidative stress in gill, erythrocytes, liver and kidney of Nile tilapia (*Oreochromis niloticus*) from a polluted site. Aquatic Toxicology, 34(2), 151-162. https://doi.org/10.1016/0166-445X(95)00036-4.

Bao, A. M., Man, X. M., Guo, X. J., Dong, H. B., Wang, F. Q., Sun, H., …, Sha, J. H. (2011). Effects of di-n-butyl phthalate on male rat reproduction following pubertal exposure. Asian journal of andrology, 13(5), 702. https://doi.org/10.1038/aja.2011.76.

Barbaud, A., Lafforgue, C. (2021). Risks associated with cosmetic ingredients. In Annales de Dermatologie et de Vénéréologie (Vol. 148, No. 2, pp. 77-93). Elsevier Masson. https://doi.org/10.1016/j.annder.2020.04.027.

Benjamin, S., Pradeep, S., Josh, M. S., Kumar, S., Masai, E. (2015). A monograph on the remediation of hazardous phthalates. Journal of Hazardous Materials, 298, 58-72. https://doi.org/10.1016/j.jhazmat.2015.05.004.

Bošnir, J., Puntarić, D., Škes, I., Klarić, M., Šimić, S., Zorić, I. (2003). Migration of phthalates from plastic products to model solutions. Collegium antropologicum, 27(1), 23-30. https://pubmed.ncbi.nlm.nih.gov/12955888/.

Calabrese, E. J., Baldwin, L. A. (2003). Toxicology rethinks its central belief. Nature, 421(6924), 691-692. https://www.nature.com/articles/421691a.

Cantanhêde, S. M., de Carvalho, I. S. C., Hamoy, M., Corrêa, J. A. M., de Carvalho, L. M., Barbas, L. A. L., …, Amado, L. L. (2022). Evaluation of cardiotoxicity in Amazonian fish *Bryconops caudomaculatus* by acute exposure to aluminium in an acidic environment. Aquatic Toxicology, 242, 106044. https://doi.org/10.1016/j.aquatox.2021.106044.

Carvalho, M. E., Castro, P. R., Azevedo, R. A. (2020). Hormesis in plants under Cd exposure: from toxic to beneficial element?. Journal of Hazardous Materials, 384, 121434. https://doi.org/10.1016/j.jhazmat.2019.121434.

Chae, Y., Kim, D., An, Y. J. (2019). Effects of micro-sized polyethylene spheres on the marine microalga *Dunaliella salina*: focusing on the algal cell to plastic particle size ratio. Aquatic Toxicology, 216, 105296. https://doi.org/10.1016/j.aquatox.2019.105296.

Chen, H., Wang, Y., Liang, H. (2024). The combined neurotoxicity of DBP and nano-TiO2 in embryonic zebrafish (*Danio rerio*) revealed by oxidative activity, neuro-development genes expression and metabolomics changes. Aquatic Toxicology, 106881. https://doi.org/10.1016/j.aquatox.2024.106881.

Gabriel, F. Â., Hauser-Davis, R. A., Soares, L., Mazzuco, A. C. A., Rocha, R. C. C., Saint Pierre, T. D., …, Bernardino, A. F. (2020). Contamination and oxidative stress biomarkers in estuarine fish following a mine tailing disaster. PeerJ, 8, e10266. https://doi.org/10.7717/peerj.10266.

Gholaminejad, A., Mehdizadeh, G., Dolatimehr, A., Arfaeinia, H., Farjadfard, S., Dobaradaran, S., …, Ramavandi, B. (2024). Phthalate esters pollution in the leachate, soil, and water around a landfill near the sea, Iran. Environmental Research, 248, 118234. https://doi.org/10.1016/j.envres.2024.118234.

Gonçalves, A. M., Rocha, C. P., Marques, J. C., Gonçalves, F. J. (2021). Enzymes as useful biomarkers to assess the response of freshwater communities to pesticide exposure – A review. Ecological Indicators, 122, 107303. https://doi.org/10.1016/j.ecolind.2020.107303.

Habig, W.H., Jakoby, W.B. (1981). Assays for Differentiation of Glutathione S-Transferases. Methods in Enzymology. 77, 398-405. https://doi.org/10.1016/S0076-6879(81)77053-8.

Hampel, M., Blasco, J., Díaz, M. M. (2016). Biomarkers and effects. In Marine Ecotoxicology (pp. 121-165). Academic Press. https://doi.org/10.1016/B978-0-12-803371-5.00005-9.

Heudorf, U., Mersch-Sundermann, V., Angerer, J. (2007). Phthalates: toxicology and exposure. International journal of hygiene and environmental health, 210(5), 623-634. https://doi.org/10.1016/j.ijheh.2007.07.011.

Hou, T., Fan, X., Zhang, Q., Zhang, H., Zhang, D., Tao, L., Wang, Z. (2024). Dibutyl phthalate exposure induced mitochondria-dependent ferroptosis by enhancing

VDAC2 in zebrafish ZF4 cells. Environmental Pollution, 123846. https://doi.org/10.1016/j.envpol.2024.123846.

Javed, M., Ahmad, I., Usmani, N., Ahmad, M. (2016). Bioaccumulation, oxidative stress and genotoxicity in fish (*Channa punctatus*) exposed to a thermal power plant effluent. Ecotoxicology and Environmental Safety, 127, 163-169. https://doi.org/10.1016/j.ecoenv.2016.01.007.

Jiang, N., Song, P., Li, X., Zhu, L., Wang, J., Yin, X., Wang, J. (2022). Dibutyl phthalate induced oxidative stress and genotoxicity on adult zebrafish (*Danio rerio*) brain. Journal of hazardous materials, 424, 127749. https://doi.org/10.1016/j.jhazmat.2021.127749.

Karačonji, I. B., Jurica, S. A., Lasić, D., Jurica, K. (2017). Facts about phthalate toxicity in humans and their occurrence in alcoholic beverages. Archives of Industrial Hygiene and toxicology, 68(2), 81-92. https://doi.org/10.1515/aiht-2017-68-2951.

Kardong, K. V. (2014). Vertebrados: anatomia comparada, função e evolução. Roca.

Kashyap, D., Agarwal, T. (2018). Concentration and factors affecting the distribution of phthalates in the air and dust: a global scenario. Science of the total environment, 635, 817-827. https://doi.org/10.1016/j.scitotenv.2018.04.158.

Krull, M., Barros, F. (2012). Key issues in aquatic ecotoxicology in Brazil: a critical review. Ecotoxicology and Environmental Contamination, 7(2). https://doi.org/10.1016/j.scitotenv.2018.04.158.

Latini, G. (2005). Monitoring phthalate exposure in humans. Clinica chimica acta, 361(1-2), 20-29. https://doi.org/10.1016/j.cccn.2005.05.003.

Neves, R. A., Miralha, A., Guimarães, T. B., Sorrentino, R., Calderari, M. R. M., Santos, L. N. (2023). Phthalates contamination in the coastal and marine sediments of Rio de Janeiro, Brazil. Marine Pollution Bulletin, 190, 114819. https://doi.org/10.1016/j.marpolbul.2023.114819.

Oakes, K. D., Van Der Kraak, G. J. (2003). Utility of the TBARS assay in detecting oxidative stress in white sucker (*Catostomus commersoni*) populations exposed to pulp mill effluent. Aquatic Toxicology, 63(4), 447-463. https://doi.org/10.1016/S0166-445X(02)00204-7.

Paquette, E., Mumper, N., Rodrigues, A., Voulo, M., Rich, S., Roy, N. M. (2022). Hindbrain defects induced by Di-butyl phthalate (DBP) in developing zebrafish embryos. Neurotoxicology and Teratology, 92, 107093. https://doi.org/10.1016/j.ntt.2022.107093.

Prysyazhnyuk, V., Sydorchuk, L., Sydorchuk, R., Prysiazhniuk, I., Bobkovych, K., Buzdugan, I., …, Prysyazhnyuk, P. (2021). Glutathione-S-transferases genes-promising predictors of hepatic dysfunction. World journal of hepatology, 13(6), 620. https://doi.org/10.4254/wjh.v13.i6.620.

Radke, E. G., Galizia, A., Thayer, K. A., Cooper, G. S. (2019). Phthalate exposure and metabolic effects: a systematic review of the human epidemiological evidence. Environment international, 132, 104768. https://doi.org/10.1016/j.envint.2019.04.040.

Regoli, F., Giuliani, M. E. (2014). Oxidative pathways of chemical toxicity and oxidative stress biomarkers in marine organisms. Marine environmental research, 93, 106-117. https://doi.org/10.1016/j.marenvres.2013.07.006.

Rodrigues, K., Batista-Silva, H., de Moura, K. R. S., Van Der Kraak, G., Silva, F. R. M. B. (2020). Dibutyl phthalate rapidly alters calcium homeostasis in the gills of *Danio rerio*. Chemosphere, 258, 127408. https://doi.org/10.1016/j.chemosphere.2020.127408.

Saliu, F., Montano, S., Leoni, B., Lasagni, M., Galli, P. (2019). Microplastics as a threat to coral reef environments: detection of phthalate esters in neuston and scleractinian corals from the Faafu Atoll, Maldives. Marine pollution bulletin, 142, 234-241. https://doi.org/10.1016/j.marpolbul.2019.03.043.

Schiavo, S., Oliviero, M., Chiavarini, S., Dumontet, S., Manzo, S. (2021). Polyethylene, Polystyrene, and Polypropylene leachate impact upon marine microalgae *Dunaliella tertiolecta*. Journal of Toxicology and Environmental Health, Part A, 84(6), 249-260. https://doi.org/10.1080/15287394.2020.1860173.

Shahid, M., Niazi, N. K., Rinklebe, J., Bundschuh, J., Dumat, C., Pinelli, E. (2020). Trace elements-induced phytohormesis: A critical review and mechanistic interpretation. Critical Reviews in Environmental Science and Technology, 50(19), 1984-2015. https://doi.org/10.1080/10643389.2019.1689061.

Shin, H. M., Dhar, U., Calafat, A. M., Nguyen, V., Schmidt, R. J., Hertz-Picciotto, I. (2020). Temporal trends of exposure to phthalates and phthalate alternatives in California pregnant women during 2007–2013: comparison with other populations. Environmental science & technology, 54(20), 13157-13166. https://doi.org/10.1021/acs.est.0c03857.

Sies, H.; Berndt, C.; Jones, D. P. (2017). Oxidative stress. Annual review of biochemistry, v. 86, p. 715-748. https://doi.org/10.1146/annurev-biochem-061516-045037.

Song, P., Jiang, N., Zhang, K., Li, X., Li, N., Zhang, Y., …, Wang, J. (2022). Ecotoxicological evaluation of zebrafish liver (*Danio rerio*) induced by dibutyl phthalate. Journal of Hazardous Materials, 425, 128027. https://doi.org/10.1016/j.jhazmat.2021.128027.

Staples, C. A., Peterson, D. R., Parkerton, T. F., Adams, W. J. (1997). The environmental fate of phthalate esters: a literature review. Chemosphere, 35(4), 667-749. https://doi.org/10.1016/S0045-6535(97)00195-1.

Wang, D., Jiang, S. Y., Fan, C., Fu, L., Ruan, H. D. (2023). Occurrence and correlation of microplastics and dibutyl phthalate in rivers from Pearl River Delta, China. Marine Pollution Bulletin, 197, 115759. https://doi.org/10.1016/j.marpolbul.2023.115759.

Whyte, J. J., Jung, R. E., Schmitt, C. J., Tillitt, D. E. (2000). Ethoxyresorufin-O-deethylase (EROD) activity in fish as a biomarker of chemical exposure. Critical reviews in toxicology, 30(4), 347-570. https://doi.org/10.1080/10408440091159239.

Wittassek, M., Koch, H. M., Angerer, J., Brüning, T. (2011). Assessing exposure to phthalates – the human biomonitoring approach. Molecular nutrition & food research, 55(1), 7-31. https://doi.org/10.1002/mnfr.201000121.

Wong, L. I., Labrecque, M. P., Ibuki, N., Cox, M. E., Elliott, J. E., Beischlag, T. V. (2015). p, p′-Dichlorodiphenyltrichloroethane (p, p′-DDT) and p, p′-dichlorodiphenyldichloroethylene (p, p′-DDE) repress prostate specific antigen levels in human prostate cancer cell lines. Chemico-biological interactions, 230, 40-49. https://doi.org/10.1016/j.cbi.2015.02.002.

Wormuth, M., Scheringer, M., Vollenweider, M., Hungerbühler, K. (2006). What are the sources of exposure to eight frequently used phthalic acid esters in Europeans?. Risk Analysis, 26(3), 803-824. https://doi.org/10.1111/j.1539-6924.2006.00770.x.

Xie, X. L., Zhou, W. T., Zhang, K. K., Yuan, Y., Qiu, E. M., Shen, Y. W., Wang, Q. (2019). PCB52 induces hepatotoxicity in male offspring through aggravating loss of clearance capacity and activating the apoptosis: sex-biased effects on rats. Chemosphere, 227, 389-400. https://doi.org/10.1016/j.chemosphere.2019.04.077.

Zhang, Y., Jiao, Y., Li, Z., Tao, Y., Yang, Y. (2021). Hazards of phthalates (PAEs) exposure: A review of aquatic animal toxicology studies. Science of the Total Environment, 771, 145418. https://doi.org/10.1016/j.scitotenv.2021.145418.

5. CONCLUSÕES INTEGRADORAS

Considerando a periculosidade dos micropoluentes detectados em nossas análises como os organoclorados, HPAs e ftalatos, e o seus potenciais de bioacumulação, a sua presença em rios urbanos na região amazônica torna-se um fator preocupante, uma vez que esses são capazes de adentrar a cadeia alimentar podendo chegar até mesmo aos seres humanos, causando diversos problemas de saúde.

Nossos dados evidenciaram uma maior contaminação do canal da Tamandaré em relação ao canal do Tucunduba, o que pode ser explicado pela influência de maré, que é menor em relação ao canal do Tucunduba, tornando-o mais suscetível ao acúmulo desses contaminantes. Além disso, nossas análises revelaram a presença de micropoluentes relevantes ambientalmente como o DBP e o DEHP, que foram encontrados em maiores concentrações e estão entre os ftalatos mais comumente encontrados no meio ambiente devido ao seu potencial de bioacumulação. Os demais micropoluentes encontrados como os HPAs e organoclorados, mesmo que em concentrações menores, também se mostram como dados preocupantes, pois assim como o DBP e o DEHP, causam efeitos nocivos tanto para a saúde humana quanto para a biota aquática.

Verificamos também que a exposição ao DBP causa efeitos adversos a nível bioquímico em *H. heterorhabdus*, espécie nativa da região amazônica, tais como efeitos concentração-dependente e hormético. A concentração ambiental de 10µg/L para a região amazônica causa interferências significativas no sistema de defesa antioxidante, evidenciados por exemplo pela diminuição da GST na porção média. O aumento progressivo do DBP nos ambientes aquáticos amazônicos pode resultar futuramente em sérias complicações celulares, uma vez que nossos dados mostram que o aumento progressivo das concentrações do DBP resultou em um efeito concentração-dependente, ocasionando peroxidação lipídica.

Sendo assim, estudos de monitoramento ambiental como este são de suma importância para uma melhor compreensão sobre a presença e efeitos de micropoluentes em espécies nativas como *H. heterorhabdus*, o qual se mostrou como um potencial organismo biomonitor para a região. Ademais, ressaltamos também a relevância desse estudo para a região amazônica, uma vez que este consiste em um estudo pioneiro para a região, abrindo portas para estudos posteriores sobre micropoluentes emergentes na Amazônia.

6. REFERÊNCIAS

Amado, L. L., Garcia, M. L., Ramos, P. B., Freitas, R. F., Zafalon, B., Ferreira, J. L. R., …, Monserrat, J. M. (2009). A method to measure total antioxidant capacity against peroxyl radicals in aquatic organisms: application to evaluate microcystins toxicity. Science of the total Environment, 407(6), 2115-2123.

Barbaud, A., Lafforgue, C. (2021). Risks associated with cosmetic ingredients. In Annales de Dermatologie et de Vénéréologie (Vol. 148, No. 2, pp. 77-93). Elsevier Masson.

Baršienė, J., Lehtonen, K. K., Koehler, A., Broeg, K., Vuorinen, P. J., Lang, T., ..., Kopecka, J. (2006). Biomarker responses in flounder (*Platichthys flesus*) and mussel (*Mytilus edulis*) in the Klaipėda-Būtingė area (Baltic Sea). Marine Pollution Bulletin, 53(8-9), 422-436.

Benjamin, S., Pradeep, S., Josh, M. S., Kumar, S., Masai, E. (2015). A monograph on the remediation of hazardous phthalates. Journal of Hazardous Materials, 298, 58-72.

Bhatt, P., Bhandari, G., Bilal, M. (2022). Occurrence, toxicity impacts and mitigation of emerging micropollutants in the aquatic environments: Recent tendencies and perspectives. Journal of Environmental chemical engineering, 10(3), 107598.

Cajaraville, M. P., Bebianno, M. J., Blasco, J., Porte, C., Sarasquete, C., Viarengo, A. (2000). The use of biomarkers to assess the impact of pollution in coastal environments of the Iberian Peninsula: a practical approach. Science of the total environment, 247(2-3), 295-311.

Carraschi, S. P., Cubo, P., Schiavetti, B. L., Shiogiri, N. S., da Cruz, C., Pitelli, R. A. (2011). Efeitos tóxicos de surfactantes fitossanitários para o peixe mato grosso (*Hyphessobrycon eques*). Acta Scientiarum: Biological Sciences, 191-196.

Conceição, M. M. et al. (2019). O plástico como vilão do meio ambiente. Revista Geociências-UNG-Ser, v. 18, n. 1, p. 50-53.

Cruz, R. M.; Goch, Y. G. F. (2006). A comunidade de peixes como bioindicadora da qualidade da água de igarapés da bacia do Maicá, próximos ao aterro sanitário do município de Santarém – Pará. Monografia – Graduação em Ciências Biológicas da Universidade Federal do Pará. Santarém.

Damato, M., Barbieri, E. (2012). Estudo da Toxicidade aguda e alterações metabólicas provocadas pela exposição do Cádmio sobre o peixe *Hyphessobrycon callistus* utilizado como indicador de saúde ambiental. O Mundo da Saúde, 36(4), 574-580.

Depledge, M. H. (1992). Conceptual paradigms in marine ecotoxicology. In Proceedings of the 12 TH Baltic Marine Biologists Symposium; Bjornstad, E., Hagerman, L., Jensen, K., Eds (pp. 47-52).

Fuentes-Rios, D., Orrego, R., Rudolph, A., Mendoza, G., Gavilán, J. F., Barra, R. (2005). EROD activity and biliary fluorescence in *Schroederichthys chilensis* (Guichenot 1848): Biomarkers of PAH exposure in coastal environments of the South Pacific Ocean. Chemosphere, 61(2), 192-199.

García-Alzate, C. A., Román-Valencia, C., Taphorn, D. (2010). Two new species of *Hyphessobrycon* (Pisces: Characiformes: Characidae) from Putumayo River, with keys to the Colombian *Hyphessobrycon heterorhabdus*-group species. Brenesia, 70, 33-46.

Guerra-Rodríguez, S., Ribeiro, A. R. L., Ribeiro, R. S., Rodríguez, E., Silva, A. M., Rodríguez-Chueca, J. (2021). UV-A activation of peroxymonosulfate for the removal of micropollutants from secondary treated wastewater. Science of The Total Environment, 770, 145299.

Heudorf, U., Mersch-Sundermann, V., Angerer, J. (2007). Phthalates: toxicology and exposure. International journal of hygiene and environmental health, 210(5), 623-634.

Hou, T., Fan, X., Zhang, Q., Zhang, H., Zhang, D., Tao, L., Wang, Z. (2024). Dibutyl phthalate exposure induced mitochondria-dependent ferroptosis by enhancing VDAC2 in zebrafish ZF4 cells. Environmental Pollution, 123846

Jiang, N., Song, P., Li, X., Zhu, L., Wang, J., Yin, X., Wang, J. (2022). Dibutyl phthalate induced oxidative stress and genotoxicity on adult zebrafish (*Danio rerio*) brain. Journal of hazardous materials, 424, 127749.

Jurewicz, J., Radwan, M., Sobala, W., Ligocka, D., Radwan, P., Bochenek, M., ..., Hanke, W. (2013). Human urinary phthalate metabolites level and main semen parameters, sperm chromatin structure, sperm aneuploidy and reproductive hormones. Reproductive toxicology, 42, 232-241.

Karačonji, I. B., Jurica, S. A., Lasić, D., Jurica, K. (2017). Facts about phthalate toxicity in humans and their occurrence in alcoholic beverages. Archives of Industrial Hygiene and toxicology, 68(2), 81-92.

Kirwan, M. J., Strawbridge, J. W. (2003). Plastics in food packaging. Food packaging technology, 1, 174-240.

Lam, P. K., Gray, J. S. (2003). The use of biomarkers in environmental monitoring programmes. Marine pollution bulletin, 46(2), 182-186.

Latini, G. (2005). Monitoring phthalate exposure in humans. Clinica chimica acta, 361(1-2), 20-29.

Le, Q. A. V., Sekhon, S. S., Lee, L., Ko, J. H., Min, J. (2016). *Daphnia* in water quality biomonitoring-“omic” approaches. Toxicology and Environmental Health Sciences, 8, 1-6.

Luo, Y., Guo, W., Ngo, H. H., Nghiem, L. D., Hai, F. I., Zhang, J., ..., Wang, X. C. (2014). A review on the occurrence of micropollutants in the aquatic environment and their fate and removal during wastewater treatment. Science of the total environment, 473, 619-641.

Malveda, M. et al. (2015). Plasticizers. In: Chemical economics handbook. New York: HIS Inc.

Moreira, C. R., Landim, M. I., Costa, W. J. E. M. (2002). *Hyphessobrycon heliacus*: a new characid fish (Ostariophysi: Characiformes) from the upper rio Tapajós basin, Central Brazil. Copeia, 2002(2), 428-432.

National Toxicology Program (NTP). (2003). NTP-CERHR monograph on the potential human reproductive and developmental effects of di-nbutyl phthalate (DBP). NTP CERHR MON, v. 4, p. i–III90, 2003.

Pant, N., Shukla, M., Patel, D. K., Shukla, Y., Mathur, N., Gupta, Y. K., Saxena, D. K. (2008). Correlation of phthalate exposures with semen quality. Toxicology and applied pharmacology, 231(1), 112-116.

Paquette, E., Rodrigues, A., Fumo, M., Giacalone, J. P., Roy, N. M. (2023). Di-butyl phthalate disrupts muscle, motor and sensory neuron development in embryonic zebrafish. Neurotoxicology and Teratology, 97, 107179.

Radke, E. G., Galizia, A., Thayer, K. A., Cooper, G. S. (2019). Phthalate exposure and metabolic effects: a systematic review of the human epidemiological evidence. Environment international, 132, 104768.

Reemtsma, T., Quintana, J. B., Rodil, R., Garcı, M., Rodrı, I. (2008). Organophosphorus flame retardants and plasticizers in water and air I. Occurrence and fate. TrAC Trends in Analytical Chemistry, 27(9), 727-737.

Scalon, M. C. S., Rechenmacher, C., Siebel, A. M., Kayser, M. L., Rodrigues, M. T., Maluf, S. W., ..., Silva, L. B. D. (2010). Evaluation of Sinos River water genotoxicity using the comet assay in fish. Brazilian Journal of Biology, 70, 1217-1222.

Schecter, A., Lorber, M., Guo, Y., Wu, Q., Yun, S. H., Kannan, K., ..., Birnbaum, L. S. (2013). Phthalate concentrations and dietary exposure from food purchased in New York State. Environmental health perspectives, 121(4), 473-479.

Shin, H. M., Dhar, U., Calafat, A. M., Nguyen, V., Schmidt, R. J., Hertz-Picciotto, I. (2020). Temporal trends of exposure to phthalates and phthalate alternatives in California pregnant women during 2007–2013: comparison with other populations. Environmental science & technology, 54(20), 13157-13166.

Sogorb, M. A., Estévez, J., Vilanova, E. (2014). Biomarkers in biomonitoring of xenobiotics. In Biomarkers in Toxicology (pp. 965-973). Academic Press.

Song, P., Jiang, N., Zhang, K., Li, X., Li, N., Zhang, Y., …, Wang, J. (2022). Ecotoxicological evaluation of zebrafish liver (*Danio rerio*) induced by dibutyl phthalate. Journal of Hazardous Materials, 425, 128027.

Sotero-Santos, R. B., Rocha, O., Povinelli, J. (2007). Toxicity of ferric chloride sludge to aquatic organisms. Chemosphere, 68(4), 628-636.

Staples, C. A., Peterson, D. R., Parkerton, T. F., Adams, W. J. (1997). The environmental fate of phthalate esters: a literature review. Chemosphere, 35(4), 667-749.

Sun, G., Li, Y. (2019). Exposure to DBP induces the toxicity in early development and adverse effects on cardiac development in zebrafish (*Danio rerio*). Chemosphere, 218, 76-82.

Ternes, T. A., Joss, A. (2006). Introduction. In: Ternes, T. A., Joss, A. Human Pharmaceuticals, Hormones and Fragrances: The challenge of micropollutants in urban water management. London: IWA Publishing.

Thompson, R. C., Swan, S. H., Moore, C. J., Vom Saal, F. S. (2009). Our plastic age. Philosophical Transactions of the Royal Society B: Biological Sciences, 364(1526), 1973-1976.

Wells, P. G., Depledge, M. H., Butler, J. N., Manock, J. J., Knap, A. H. (2001). Rapid toxicity assessment and biomonitoring of marine contaminants – exploiting the potential of rapid biomarker assays and microscale toxicity tests. Marine Pollution Bulletin, 42(10), 799-804.

Wittassek, M., Koch, H. M., Angerer, J., Brüning, T. (2011). Assessing exposure to phthalates – the human biomonitoring approach. Molecular nutrition & food research, 55(1), 7-31.

World Wide Fund for Nature Inc (WWF). (2019) Brasil é o 4º país do mundo que mais gera lixo plástico. Disponível em: https://www.wwf.org.br/?70222/Brasil-e-o-4-pais-do-mundo-que-mais-gera-lixo-plastico. Acesso em: 10 de março de 2023.

Wormuth, M., Scheringer, M., Vollenweider, M., Hungerbühler, K. (2006). What are the sources of exposure to eight frequently used phthalic acid esters in Europeans?. Risk Analysis, 26(3), 803-824.

Xu, D. D., Liu, D. S. (2014). The experiment study of the pollution of phthalate compounds and heavy metals to rice in the film. Advanced Materials Research, 864, 303-306.

Xu, H., Dong, X., Zhang, Z., Yang, M., Wu, X., Liu, H., ..., Li, C. (2015). Assessment of immunotoxicity of dibutyl phthalate using live zebrafish embryos. Fish & Shellfish Immunology, 45(2), 286-292.

Zamek-Gliszczynski, M. J., Hoffmaster, K. A., Nezasa, K. I., Tallman, M. N., Brouwer, K. L. (2006). Integration of hepatic drug transporters and phase II metabolizing enzymes: mechanisms of hepatic excretion of sulfate, glucuronide, and glutathione metabolites. European journal of pharmaceutical sciences, 27(5), 447-486.

7. COMPROVANTE DE SUBMISSÃO/ACEITE DE ARTIGO CIENTÍFICO

7.1 COMPROVANTE DE SUBMISSÃO DO ARTIGO 1: The tidal influence on the distribution of emerging organic micropollutants in urban rivers in the Amazon region

Journal of Hazardous Materials

The tidal influence on the distribution of emerging organic micropollutants in urban rivers in the Amazon region

--Manuscript Draft--

Manuscript Number:	HAZMAT-D-24-04037
Article Type:	Research Paper
Keywords:	Micropollutants in the Amazon; emerging contaminants; urban ecotoxicology; environmental pollution; ODS-14.
Corresponding Author:	Lilian Lund Amado Universidade Federal do Pará BRAZIL
First Author:	Erika Monteiro
Order of Authors:	Erika Monteiro
	YURE JEFFERSON DA CRUZ DO NASCIMENTO
	CAROLINE DA SILVA MONTES
	DANILO SERRÃO MOUTINHO
	ILENE CRESTANI
	CASSIA KATRIN REINKE
	JOCINEI DOGNINI
	LEANDRO MACHADO
	DANIELE SALGADO
	Lilian Lund Amado
Abstract:	Emerging micropollutants refer to chemical substances found in extremely low concentrations in the environment. These have earned the label "emerging" due to the recent advancement of susceptible analytical techniques, capable of detecting substances even at very low concentrations (from ng/L to µg/L). Some micropollutants are priorities due to their continuous input in the environment, such as organochlorines, polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), hormones, phthalates and others. We identified and quantified different groups of micropollutants in urban rivers in the city of Belém, PA, the most urbanized city in the Eastern Amazon. Water samples were collected in the rainy season along two urban rivers of Belém, the Tamandaré and Tucunduba channels. Tamandaré channel proved to be more contaminated due to a lower tidal influence, making it more susceptible to the accumulation of these contaminants. Our analyses demonstrated the presence of organochlorines, PAHs and phthalates in the rivers studied. These findings underscore the necessity for ongoing monitoring of Amazonian water bodies. The importance of this approach is due to its harmful effects, such as neurotoxicity, apoptosis, genotoxicity, damage to the reproductive system, neoplasms, endocrine disruption and developmental toxicity. These impacts extend to both aquatic organisms and human health.
Suggested Reviewers:	Damià Barceló dbcqam@idaea.csic.es
	Shoji Nakayama fabre@nies.go.jp
	Kyungho Choi kyungho@snu.ac.kr
	Nicolas Roth nicolas.roth@scaht.org
	Yu Ait Bamai

	u-aitbamai@med.hokudai.ac.jp
	Linda Birnbaum birnbaumls@niehs.nih.gov