UNIVERSIDADE FEDERAL DE LAVRAS

MARINA FERREIRA MOREIRA

SPATIO-TEMPORAL DYNAMICS OF MICROPLASTIC CONTAMINATION IN TROPICAL FRESHWATER FISH

LAVRAS – MG
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Orientador
Prof. Dr. Paulo dos Santos Pompeu

Coorientadora
Profa. Dra. Cecília Gontijo Leal

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MARINA FERREIRA MOREIRA

SPATIO-TEMPORAL DYNAMICS OF MICROPLASTIC CONTAMINATION IN TROPICAL FRESHWATER FISH

DINÂMICA ESPAÇO-TEMPORAL DA CONTAMINAÇÃO POR MICROPLÁSTICOS EM PEIXES DE ÁGUA DOCE TROPICAIS

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“Como sei pouco, e sou pouco,
faço o pouco que me cabe
me doando por inteiro.”
(Thiago de Mello)

RESUMO

A produção e o consumo de plástico têm aumentado de forma contínua nas últimas décadas, contribuindo para o acúmulo de resíduos no ambiente, entre os quais os microplásticos são atualmente amplamente detectados. Embora as pesquisas sobre a contaminação por microplásticos tenham se expandido rapidamente, os ecossistemas de água doce, especialmente em regiões tropicais, permanecem pouco estudados, e abordagens de longo prazo ainda são raras. Essa tese avança na compreensão da contaminação por microplásticos em ambientes de água doce tropicais ao abordar dimensões espaciais e temporais. Primeiramente, uma revisão da literatura sintetiza o estado atual do conhecimento sobre microplásticos em águas doces tropicais, identificando os principais avanços e lacunas entre regiões, ecossistemas e grupos de organismos. Em seguida, a ingestão de microplásticos por peixes é avaliada em diferentes ambientes de água doce dentro de uma bacia hidrográfica, incluindo ecossistemas lóticos e lênticos, destacando o papel do contexto hidrológico e a complexidade dos padrões de contaminação. Por fim, tendências de longo prazo na ingestão de microplásticos por peixes são avaliadas ao longo de um período de 24 anos em regiões com contextos contrastantes de uso do solo, representando a primeira análise desse tipo para peixes de água doce tropicais. De modo geral, os resultados mostram que ambientes lênticos podem atuar como importantes zonas de acúmulo, expondo os organismos a níveis mais elevados de contaminação, e que as tendências temporais da contaminação em peixes não são uniformes, mas dependem do contexto ambiental regional. Além disso, a turbidez da água influencia a ingestão de microplásticos em espécies forrageadoras visuais. Essa tese também destaca a importância do monitoramento ecológico de longo prazo e de arquivos biológicos como ferramentas essenciais para reconstruir a contaminação passada e avançar o conhecimento sobre a poluição por plásticos.

Palavras-chave: contaminante emergente; peixes de água doce; poluição plástica.

ABSTRACT

Plastic production and consumption have increased steadily over recent decades, contributing to the accumulation of debris in the environment, among which microplastics are now widely detected. Although research on microplastic contamination has expanded rapidly, freshwater ecosystems, particularly in tropical regions, remain comparatively understudied, and long-term perspectives are still rare. This thesis advances the understanding of microplastic contamination in tropical freshwater environments by addressing spatial and temporal dimensions. First, a literature review synthesizes the current state of research on microplastics in tropical freshwaters, identifying major advances and gaps across regions, ecosystems, and organism groups. Second, microplastic ingestion by fish is evaluated across different freshwater environments within a river basin, including lotic and lentic ecosystems, highlighting the role of hydrological context and the complexity of contamination patterns. Finally, long-term trends in microplastic ingestion by fish are assessed over a 24-year period across regions with contrasting land-use contexts, representing the first analysis of this kind for tropical freshwater fish. Overall, the results show that lentic environments can act as important accumulation zones, exposing organisms to higher contamination levels, and that temporal trends in fish contamination are not uniform but depend on regional environmental context. In addition, water turbidity influences microplastic ingestion in visual foragers. This thesis also highlights the importance of long-term ecological monitoring and biological archives as essential tools for reconstructing past contamination and advancing knowledge on plastic pollution.

Keywords: emerging contaminant; freshwater fish; plastic pollution.

INDICADORES DE IMPACTO

Essa tese gera impactos científicos, ambientais, sociais e institucionais ao ampliar o entendimento sobre a contaminação por microplásticos em ecossistemas de água doce tropicais, um tema ainda pouco explorado no contexto global. Ao fornecer uma abordagem temporal inédita para peixes de água doce, os resultados contribuem para reduzir lacunas de conhecimento sobre a dinâmica da contaminação por microplásticos fora de ambientes marinhos e de regiões temperadas. Os resultados demonstram que ambientes lênticos podem atuar como importantes áreas de acúmulo de partículas, o que tem implicações diretas para a conservação da biodiversidade aquática e para a avaliação de riscos ambientais. Além disso, a tese evidencia que a variação temporal da ingestão de microplásticos por peixes não ocorre de forma semelhante em toda a bacia, sendo dependente do contexto ambiental e do uso do solo da região. A diferença na contaminação entre espécies similares reforça a necessidade de cautela na extrapolação de resultados baseados em poucas espécies para a avaliação da contaminação de um determinado local. Do ponto de vista social e ambiental, o estudo contribui para o entendimento de pressões antrópicas em sistemas aquáticos amplamente utilizados pela população humana, seja para abastecimento, recreação ou alimentação, podendo apoiar futuras ações de gestão ambiental e políticas públicas voltadas à qualidade da água. Em termos institucionais, a pesquisa reforça a importância de programas de monitoramento ecológico de longo prazo e da manutenção de acervos científicos, como coleções biológicas e depósitos de material histórico, demonstrando seu valor não apenas para o avanço do conhecimento atual, mas também para a reinterpretação de condições ambientais passadas. A tese apresenta alinhamento com a área temática de Meio Ambiente da Política Nacional de Extensão e os Objetivos de Desenvolvimento Sustentável da ONU, especialmente os ODS 6 (Água Potável e Saneamento), 12 (Consumo e Produção Responsáveis) e 14 (Vida na Água).

IMPACT INDICATORS

This thesis generates scientific, environmental, social, and institutional impacts by expanding the understanding of microplastic contamination in tropical freshwater ecosystems, a topic that remains poorly explored in the global context. By providing a novel temporal approach for freshwater fish, the results help reduce knowledge gaps regarding the dynamics of microplastic contamination outside marine environments and temperate regions. The findings demonstrate that lentic environments can act as important accumulation areas for particles, with direct implications for the conservation of aquatic biodiversity and for environmental risk assessment. In addition, the thesis shows that temporal variation in microplastic ingestion by fish does not occur uniformly across the entire basin, being strongly dependent on the environmental context and regional land use. Differences in contamination among similar species reinforce the need for caution when extrapolating results based on a limited number of species to assess contamination at a given site. From a social and environmental perspective, the study contributes to the understanding of anthropogenic pressures on aquatic systems widely used by the human population, whether for water supply, recreation, or food, and may support future environmental management actions and public policies aimed at water quality. Institutionally, the research highlights the importance of long-term ecological monitoring programs and the maintenance of scientific collections, such as biological collections and historical material repositories, demonstrating their value not only for advancing current knowledge but also for reinterpreting past environmental conditions. The thesis is aligned with the Environmental thematic area of the National Extension Policy and with the United Nations Sustainable Development Goals, particularly SDGs 6 (Clean Water and Sanitation), 12 (Responsible Consumption and Production), and 14 (Life Below Water).

SUMÁRIO

PRIMEIRA PARTE

INTRODUÇÃO GERAL

1 INTRODUCTION

Indispensable in the current world, plastic has been suggested as a geological indicator of the Anthropocene due to its widespread distribution in ecosystems (ZALASIEWICZ et al., 2016). Most plastic polymers (e.g., nylon, polystyrene, polyvinyl chloride) started to be produced between the 1930s and 1940s (ZALASIEWICZ et al., 2016). Since then, they have become essential. They have enabled important advances in food hygiene, health, and the construction sector. Because of this, since the 1950s, the global annual plastic production has increased 150 times (ZALASIEWICZ et al., 2016). Although recycling has been indicated as an important solution to plastic pollution, only 9% of the plastic ever produced has been recycled. Meanwhile, most plastic (60%) has been discarded and is now accumulating in the environment (GEYER; JAMBECK; LAW, 2017), reaching freshwater and marine ecosystems.

Once in the environment, plastic can fragment through physical, chemical and biotic mechanisms, reaching even nano and millimetric sizes (ZHANG et al., 2021). When smaller than 5 mm, these particles are known as microplastics, and now have evidence of negative effects across different levels of biological organization (THOMPSON et al., 2024). The risks of microplastics are also attributed to the adsorption of other contaminants by the particles. Heavy metals, persistent organic pollutants, pathogens, among other contaminants present in the environment, can adhere to microplastics and increase their harmful effects (HAMID et al., 2018). Although research on microplastics is still recent, especially in freshwater environments, these particles are ubiquitous worldwide, with detections even in remote areas.

The first records of microplastics occurred in the 1970s in coastal waters (e.g., BUCHANAN, 1971; CARPENTER; SMITH, 1972). Nevertheless, research on the subject only started to grow substantially more than four decades after the first studies (ZHANG et al., 2020). Before 2015, less than 100 papers were published (ZHANG et al., 2020). Although since then, marine ecosystems have been the most studied environments (YANG et al., 2025), the recognition of the significance of land-based sources for ocean microplastic pollution and the role of rivers as the main pathway could have brought attention to the contamination in freshwater environments (JAMBECK et al., 2015; LEBRETON et al., 2017).

Although freshwater environments have been the subject of an increasing number of studies in recent years (YANG et al., 2025), there are still important knowledge gaps.

Most studies are concentrated in temperate regions and are restricted to samples collected over a few months or years, resulting in a lack of historical understanding of freshwater contamination. In this context, the aim of this thesis is to advance the knowledge on microplastic contamination in tropical freshwater environments by assessing the current research status and evaluating spatial and temporal aspects. To achieve this, the thesis is divided into three manuscripts, which analyze: (1) the state of microplastic research in tropical freshwater environments through a literature review, (2) microplastic ingestion by fish from different freshwater environments across a river basin, and (3) temporal trends in microplastic ingestion by fish over 24 years across different regions of a river basin.

SEGUNDA PARTE

ARTIGOS

Artigo 1 - Redigido conforme norma do periódico científico - Versão publicada	
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ARTIGO 1

Trends and gaps in microplastics research in Tropical freshwater ecosystems

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Abstract

Although the contamination of microplastics is a very recent topic, knowledge increased rapidly, especially in the last decade. Despite this, freshwater ecosystems have received less attention than marine ones, particularly in the tropics. Considering the particularities of Tropical freshwater ecosystems that can be important in the microplastics dynamic, such as the high rate of untreated water, the flooding dynamics and high number of reservoirs, our review aims to present the state of the research of microplastic contamination in Tropical freshwater ecosystems, emphasizing the knowledge advances and existing gaps. We compared studies in different countries, ecosystems and type of samples, and identified gaps and needs for futures research. Although research and the complexity of studies have increased over the years, Tropical freshwater ecosystems are still understudied. We emphasized the need for more studies in African countries and Tropical Australia, as well as in reservoirs across all Tropical regions. It is also crucial to evaluate contamination related to flooding dynamics and in aquatic invertebrates. Advancements in research on this topic are essential to provide a clear picture of the real problem in the tropics, leading to the possibility of better mitigation and conservation actions in the future.

Key-words

Aquatic environment, bibliometric review, plastic pollution, Tropical ecosystems

Introduction

The contamination of microplastics in different environmental matrices is a major concern worldwide. These particles are defined as synthetic polymers ranging in size from 1 µm to 5 mm (Frias & Nash 2019, Thompson et al. 2024). In the ocean, only 20% of the microplastic found is the result of direct anthropic activities in this environment, the remaining 80% reaches the marine ecosystem from terrestrial sources, with rivers being the main pathway (Lebreton et al. 2017, Rochman 2018, Osman et al. 2023). Microplastics have been documented in several different organisms, including in humans (de Sá et al. 2018, Osman et al. 2023). Although the knowledge of their effects is still limited and mostly focused on model organisms (Bhuyan 2022), it is known that the consequences of exposure to microplastics are not only related to the particle itself, but also to several contaminants that can be adsorbed by the particles (Li et al. 2018, Bhuyan 2022, Osman et al. 2023).

The amount of microplastic particles found in aquatic environments is strongly related to anthropic activities. Both sewage and solid waste are recognized as sources of microplastic contamination. Landfills can produce microplastics due to plastic litter degradation (Kabir et al. 2023), these particles can reach water bodies and groundwater through runoff and the leachate (Ferronato & Torretta 2019; He et al. 2019). Although wastewater treatment can remove more than 90% of microplastics, this rate can vary greatly since its efficiency depends on microplastic characteristics and treatment conditions (Ahmed et al. 2024). Regardless, the wastewater effluent is still a major source of contamination in freshwater environments (Kay et al. 2018). This is a particularly critical problem in the Tropical region, where only seven countries are classified as developed (high-income) (Fantom & Serajuddin 2016). Most developing countries face extensive problems related to the treatment and disposal of waste and lack of policies and infrastructure to deal with solid waste and water treatment (WWAP 2017, Ferronato & Torretta 2019). The rate of untreated wastewater in those countries, which can be released into water courses, can reach up to 92% (WWAP 2017).

Microplastics can also enter aquatic environments from terrestrial sources through flood pulses. The flooding of terrestrial areas during the rainy season is a key aspect of tropical river dynamics (Welcomme 1985). This process is an important source of trophic resources, influencing the entire river basin and its trophic web (Junk et al. 1989). However, the microplastics retained inland can enter the river during the flooding events (Song et al. 2020). In addition to these factors, the presence of large reservoirs in tropical rivers could also be significant in microplastic dynamics, playing a key role in freshwater ecosystems, as they can act as sinks for these particles (Zhang et al. 2015, Watkins et al. 2019, Liu et al. 2022).

Although it is a very recent topic, the knowledge on microplastic contamination increased rapidly, with a gradual rise in the last decade (Liu et al. 2023). Despite this, freshwater ecosystems have been receiving less attention than marine ecosystems. By 2018, only 4% of the publications focused on freshwater environments (Wagner & Lambert 2018). Nevertheless, microplastics were reported in different freshwater ecosystems across the world (Eerkes-Medrano et al. 2015) and some freshwater bodies can present higher concentrations of particles than estuarine and coastal areas (Luo et al. 2019). Considering the particularities of Tropical freshwater ecosystems that can be important in the microplastics dynamic, such as the high rate of untreated water, the flooding dynamics and high number of reservoirs, our review aims to present the state of research on microplastic contamination in Tropical freshwater ecosystems. We compared studies in different countries, ecosystems and types of samples, in addition to presenting gaps and needs for future research.

Material and Methods

Tropical region

We considered as Tropical countries those with at least half of their territory in the tropics (23.5º N and 23.5º S). Although we found some studies in the Himalayan and Andean regions that were within the tropics, they were not considered tropical due to the high altitude, which results in significantly different climatic conditions. Nearly half of Australia's territory lies within the Tropics, but only a small portion of the country has tropical conditions. Because of this, when screening publications from Australia, each study was assessed for whether it was conducted in tropical Australia; if not, it was excluded.

Search strategy

We used Web of Science (all databases) and Scopus to search for peer review studies on microplastics in the Tropics and followed the PRISMA guidelines for systematic reviews (Page et al. 2021). We combined terms in English that described the three aspects of the studies we were interested in: microplastic, freshwater, and tropical region (Table I). We selected papers that were published up to the date of the search (July 8, 2024).

Inclusion and exclusion criteria

We included papers that were not exclusively focused on tropical regions, freshwater environments, or microplastic contamination, as long as part of the study also addressed these aspects. We did not include (i) gray literature, (ii) experimental studies, since most of what we found was performed with model organisms which are not in the scope of our review, (iii) studies in brackish water and estuarine environments, and (iv) global review papers because they typically provide little or no information on tropical freshwater environments (Table I).

Table I. Methods used for the identification and exclusion of studies on microplastic contamination in Tropical freshwater ecosystems, along with the data extracted from these studies.

Sections	**Web of Science:** topic **Scopus:** title-abs-key
Identification	**Topic:** microplastic* **Environment:** freshwater* OR river* OR stream* OR lake* OR lagoon* OR reservoir* OR groundwater OR "retention pond" OR peatland OR borehole **Region:** tropic* OR neotropic* OR "Central America" OR "South America" OR Caribbean OR "South Asia" OR "Southeast Asia" OR "Middle East" OR Africa OR Australia OR "Papua New Guine" OR Mexico
Exclusion criteria	Marine, estuary or brackish water studies Gray literature (i.e., thesis, conference abstracts, reports, papers in pre-print format, notes) Experimental studies Global review
Data extraction	Year Country Environment (e.g., river, lake, reservoir) Type of sample (e.g., water, biota, sediment) Aim

Data extraction

We extracted five variables from each paper: the year of publication, the country where the study was conducted, the studied environment, the type of sample studied, and the aim of the publication (Table I). We divided the aim of the studies into five categories: (1) descriptive, (2) analytical, (3) modelling, (4) systematic review and, (5) meta-analysis. We considered a paper descriptive when it only describes the microplastic contamination in a specific environment or organism (i.e., quantification and types of particles found). As for the analytical aim, we considered studies that not only described the contamination, but also focused on the causes, consequences and/or variables influencing the contamination; temporal and/or spatial patterns; and/or risk assessments indices based on microplastic contamination. For the review papers, we were interested in only the year of publication and studied country to avoid overestimating and duplication of the results. Many studies involved multiple environments, countries, or sample types. In these cases, each was counted individually.

Initially, our search returned a total of 1,165 publications. After two rounds of screening, we found 190 papers that met all of our criteria (Figure 1).

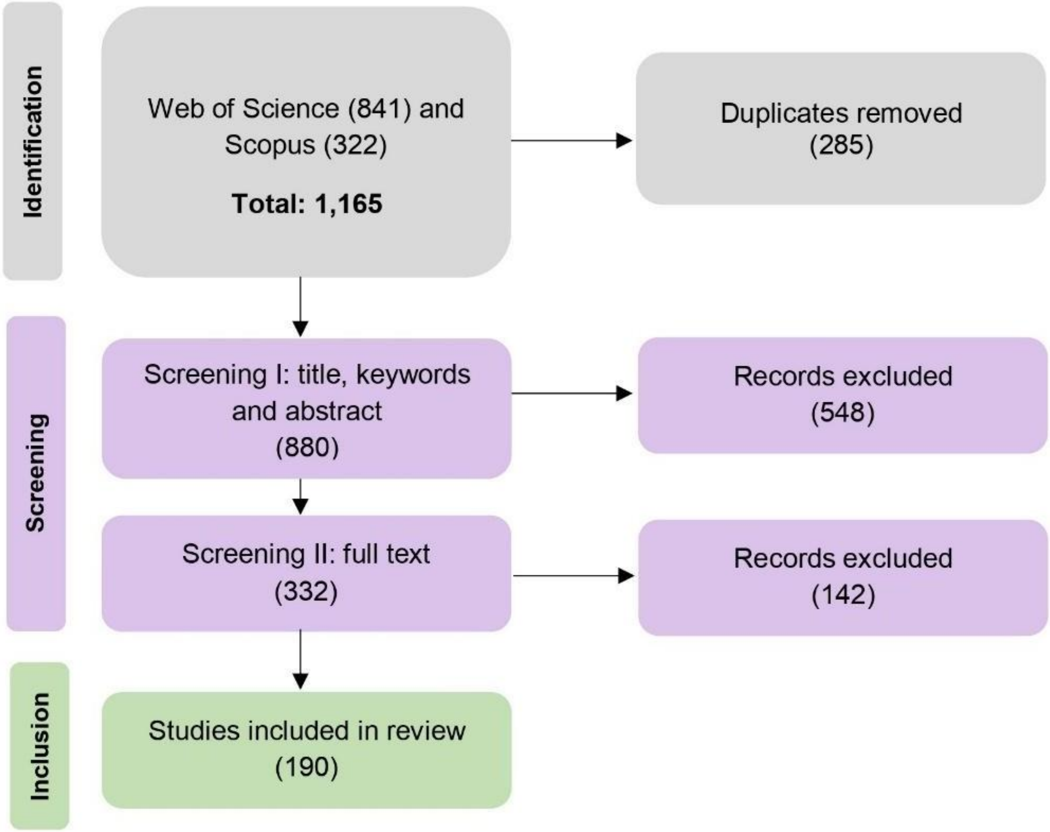

Figure 1. Preferred reporting items for systematic reviews and meta-analysis (PRISMA) flowchart adapted from Page et al., 2021 describing the search protocol.

Results

Out of the 190 papers included, the first study was published in 2015, which was the only publication from that year. Before 2019, only five studies had been published. After this, there was a significant increase, with 61 studies published in 2023 alone (Figure 2). The years of 2021 and 2022 showed the smallest increase in the number of publications, compared to the previous year, with four and eight studies published, respectively. This marked a notable difference compared to others years after 2019, which had an increase around 20 papers per year.

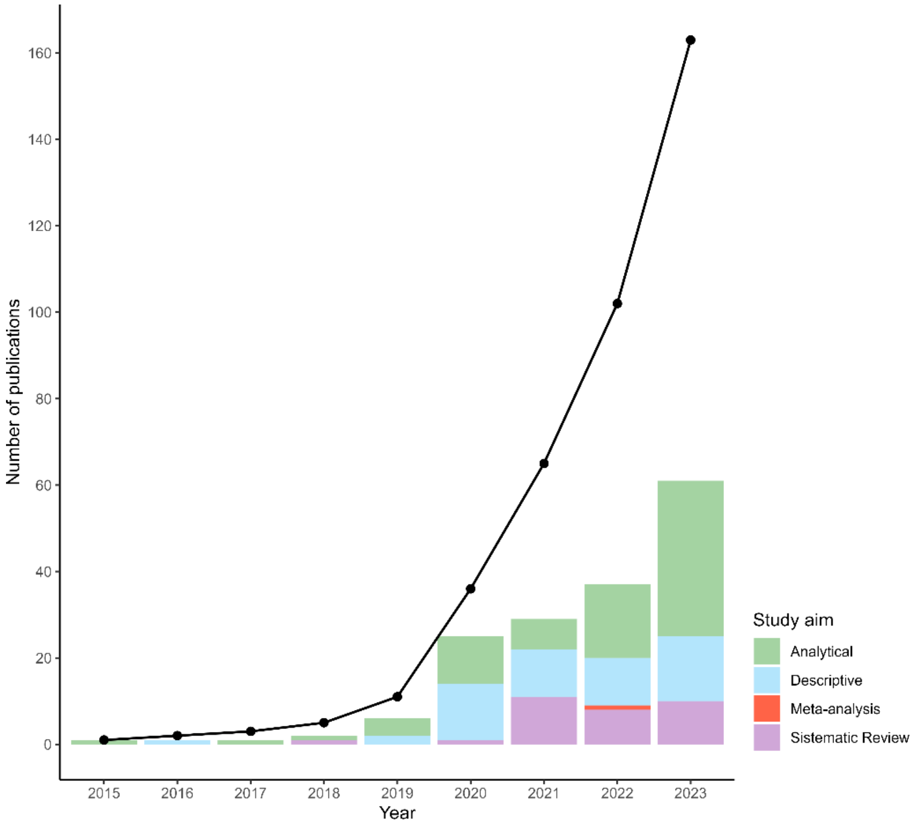

Figure 2. Number of publications on microplastics contamination in freshwater ecosystems of the Tropical region along with the aims of the studies for each year. The black line represents the cumulative number of publications.

We found papers covering all the aims that we previously categorized but this varied by year and region (Figure 3). Most studies were analytical and descriptive (46% and 33%, respectively). We found only one paper using modeling, which was published in 2024, in addition to only two meta-analyses, in 2022 and 2024. The year of 2021 showed differences in the patterns of study type. In general, after 2019, there was a constant number of descriptive papers along the years, while analytical studies increased. However, 2021 presented more descriptive studies, in addition to a high number of review papers, making it the year with the most published reviews.

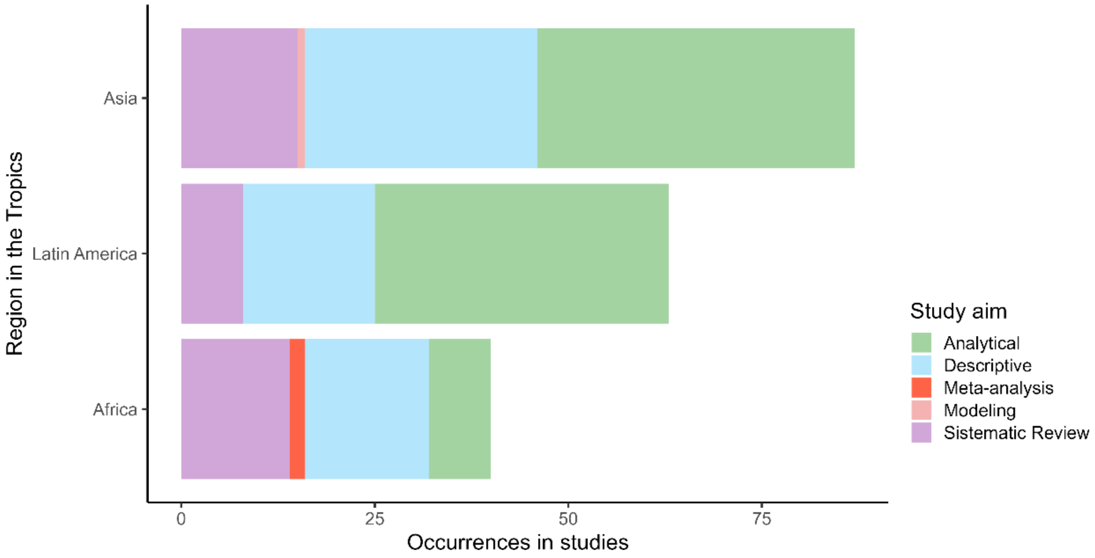

Figure 3. Number of publications in each region of the Tropics, along with the aims of the studies for each region.

India and Brazil were the most studied countries, representing, together, 48% of the total number of papers in the tropical region. Asia was the most studied region, followed by Latin America and Africa (Figure 4). While we found studies for most countries in Asia and Latin America, in Africa, only eight countries were studied. Additionally, we didn’t find papers for the tropical region of Australia.

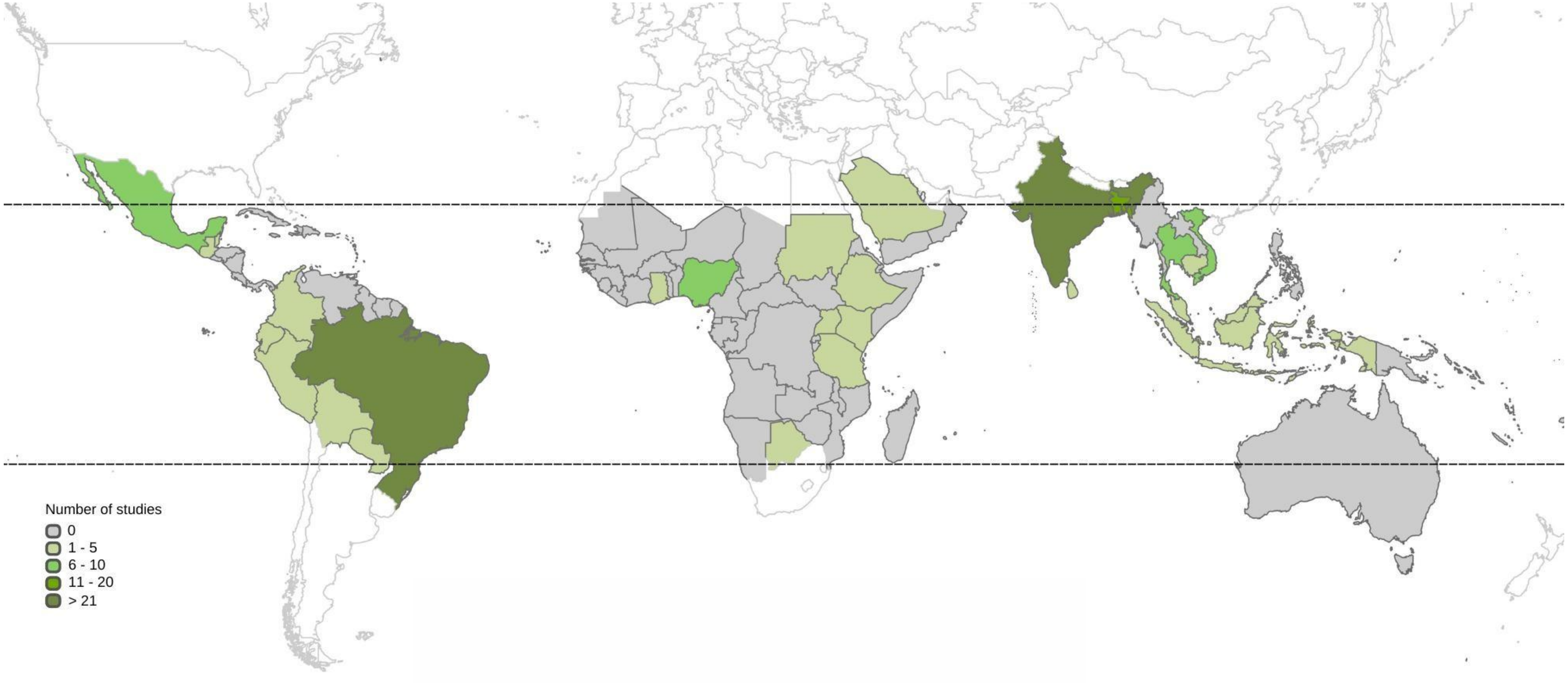

Figure 4. Map showing the countries in the Tropical region, along with the number of publications per country. The dotted line indicates the Tropics, while the countries shown in white were excluded from the tropical classification.

Eight different Tropical freshwater ecosystems were studied for microplastic contamination (Figure 5). Rivers were the most studied (57%), followed by lakes (21%) and reservoirs (7%). Our search identified multiple artificial water bodies in urban environments, such as channels, lakes and stormwater retention reservoirs. All of these environments were grouped into the category urban water bodies, representing 4.85% of the total. In all the environments, the contamination of one or more of the following was studied: water (40%), biota (32%) and sediment (27%). Among the biota, fish and fish eggs, crustacean, mollusk, insect, plant and stingray were studied (Figure 5). There was a predominance of studies with fish, regardless of the region, which were studied at least ten times more than other organisms. In addition, nine of the studies with fish used fish bought from markets to analyze the microplastic contamination. The others organisms were represented by only one to five occurrences in the publications.

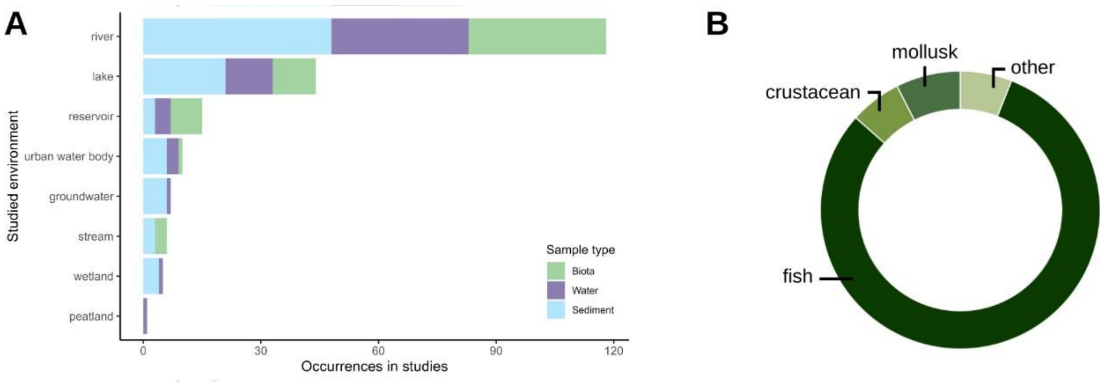

Figure 5. Number of publications in freshwater ecosystems in the Tropical region along with the sample type analyzed in each one (a) and, the freshwater organisms that were studied within these ecosystems (b).

Discussion

Microplastic contamination is a recent research topic, especially for freshwater environments. Our results show that studies in the Tropical region began less than ten years ago. In the following four years, only ten studies were published. Publications over the years for the Tropical region followed the pattern found for freshwater environments for the whole globe, which had its first studies in 2013 and then showed a slow increase until 2019, followed by a rapid development after 2019 (Wang et al. 2023).

Some factors could be responsible for the rapid development of microplastic research after 2019. The recognition of terrestrial environments as the main source of microplastics and rivers as the primary pathway for these particles to reach the ocean (Jambeck et al. 2015; Lebreton et al. 2017; Rochman 2018) may have drawn attention to the significance of studying these environments. Public awareness regarding this topic also grew, likely due to the identification of microplastic particles in drinking water (e.g. Koelmans et al. 2019; World Health Organization 2019), coupled with a better understanding of their impacts on human health (e.g. Wright & Kelly 2017, Cox et al. 2019, World Health Organization 2019). Additionally, the adoption of regulations by some countries, such as the banning of microbeads in cosmetics (Thompson et al., 2024), may have further contributed to the growing public concern. There was also a rise in international collaboration between researchers (Wang et al., 2023). Furthermore, although there is still no consensus on the methods for microplastic extraction and identification, in recent years there were advances in their detection (Thompson et al., 2024) coupled with an increase in the number of publications evaluating the existing methods and indicating best practices (e.g. Munno et al. 2018, Wang & Wang 2018, Lusher et al. 2020), which could have encouraged research groups started studying microplastics contamination. Although Tropical freshwater environments have been gaining interest and a fast increase in publications in the last years, by 2022 it still accounted for only 14% of the studies worldwide (Wang et al. 2023).

With the exception of 2021, there has been a continuous increase in the number of analytical studies over the years. In addition to this, the studies from the last three years have increased in complexity. In 2022, a meta-analysis was published, followed by another in 2024, as well as a modeling study in the same year (Masiá et al. 2022, Brooks et al. 2024, Nyaga et al. 2024). Although both meta-analyses encompass the entire African continent, including countries outside the tropics, both are, to date, the only studies of their kind that include freshwater environments in the tropical region. From plastic fibers sampled in the Mekong River, Brooks et al. (2024) developed a model to predict the settling velocity of fibers. In addition to predicting the behavior of these particles, they observed that the amount of settling particles is underestimated by conventional methods, representing an advancement and a valuable contribution toward more complex studies in the Tropical region. The increase in the number of publications and more complex studies shows significant progress in the knowledge of microplastic contamination in the tropics and in the freshwater environments, a trend that is likely to continue. As of mid-2024, there are 25 publications encompassing all five types of study: descriptive, analytical, modeling, meta-analysis, and review.

The reviews have presented important syntheses and overviews of the knowledge available for some regions of the tropics, representing an advance in this field. They focused on different aspects, such as the contamination of water, sediment, fish and others (e.g. Chen et al. 2021; Enyoh et al. 2023; Malli et al. 2023; Morais et al. 2024). However, they are often focused on a specific river basin, country or continent.

The small increase in the number of publications between 2021 and 2022, together with the high number of descriptive and review papers instead of analytical, especially in 2021, is a clear reflection of the consequences of the COVID-19 pandemic on science. Although there was growth in productivity, grants offered, and publications in research related to COVID-19, other areas were significantly affected (Omary et al. 2020). The social distancing and increase in remote work have led to a decrease in the productivity of scientists of different areas for multiple reasons, such as increased responsibilities of parents due to the daycares centers being closed, poor mental health, the absence of an adequate work environment at home and difficulty in finding new funding for research (Heo et al. 2022). Moreover, besides these consequences, the research areas that need laboratory facilities and field work, which is the case for microplastic contamination studies, were severely affected (Omary et al. 2020, Heo et al. 2022). The restricted access to laboratories and canceled field trips slowed and decreased the productivity in these areas. Even in the beginning of the COVID-19 pandemic, some journals in aquatic sciences presented low submission rates (Hobday et al. 2020).

India and Brazil represent significant advances in the knowledge of microplastic contamination in Tropical regions. Both are among the largest producers of plastic waste in the world (Law et al. 2020). Although the total number of publications in these countries is still small, especially in relation to their size, most of their studies are more complex, including, for example, spatial and temporal assessments and the association of microplastic particles with other contaminants, and the evaluation of contamination in different samples, such as water, sediment, and various organisms (e.g. Amrutha & Warrier 2020, Costa et al. 2023, Pandey et al. 2023, Trindade et al. 2023, Pavithra et al. 2024; Sarkar et al. 2021; Souza et al. 2023). In addition to these, the studies in India and Brazil include descriptive analyses (e.g. Lima et al. 2021, Maheswaran et al. 2022,), which are essential for developing a strong base of knowledge, as well as reviews that consolidate the existing knowledge (e.g. Rani-Borges et al. 2021, Vaid et al. 2021).

Africa was the region with the fewest studied countries, Nigeria is the only country with more than six papers. Most of the tropical countries in Africa are classified as low or lower-middle income (Fantom & Serajuddin 2016). Along with inadequate funding for the research (Hydén 2017), these countries struggle both to access high-quality scientific journals and to publish in them. This is a problem caused by the high fees charged to publish in the Open Access system, where low and lower-income countries lack funding to cover these expenses and cannot personally pay with an ecologist’s salary (Mekonnen et al. 2022, Tomillo et al. 2022). Unlike other countries in Africa, Nigeria has a fund to pay for publications fees, it is still a highly competitive process, but this explains the higher number in studies of that country (Mekonnen et al. 2022).

Rivers accounted for more than half of the total studied environments and were studied across all Tropical regions. In addition to their high representation, compared to other environments, rivers also exhibited a balanced number of studies in all the sample types: water, sediment and biota. Although less studied than marine environments (Wagner & Lambert 2018), rivers are the main pathway for microplastic from terrestrial to estuarine and marine ecosystems (Lebreton et al. 2017, Lambert & Wagner 2018, Rochman 2018), and their contamination can also be similar to or higher than that of marine environments (Eerkes-Medrano et al. 2015, Luo et al. 2019).

Lentic ecosystems, especially reservoirs, play a key role in microplastic dynamics. They can act as sinks, accumulating these particles, particularly in sediments (Watkins et al. 2019). The lentic ecosystems represent 32.6% of the studies in the tropics. Research on reservoirs in the tropics began only in 2019 (Shruti et al. 2019) and, although reservoirs of all regions were studied, there are only nine publications on this ecosystem. Across the Northern Hemisphere, microplastics have been well documented in lakes and reservoirs within a wide range of population densities and sizes, with concentrations higher than some very affected locations in the ocean (Nava et al. 2023). Although tropical countries have a high number of reservoirs (Lehner et al. 2011, 2024), this is still an understudied environment that needs further research, especially because, compared to temperate reservoirs, they generally have large areas and longer residence time (Lehner et al. 2024).

The interaction of microplastic with several aquatic organisms was documented around the world, with ingestion rates and effects varying among different species (Tang et al. 2021). The studied organisms in Tropical freshwaters follow a global trend – with fish, crustaceans, and mollusks being the most studied from both fresh and seawater (de Sá et al. 2018). Globally these three organisms are targeted by a high number of publications (de Sá et al. 2018), however, in the tropical region, the numbers were uneven, with fish being studied at least ten times more than all the others. This emphasizes the significant knowledge advances on tropical fish, with both descriptive and more complex studies on fish throughout the tropics, in different lentic and lotic ecosystems (e.g. Biginagwa et al. 2016, Kasamesiri & Thaimuangphol 2020, Urbanski et al. 2020, Yofukuji et al. 2024). These fish were mainly sampled in the wild but there are also studies investigating the contamination in fish that are sold in markets for human consumption (e.g. Kutralam-Muniasamy et al. 2023). Additionally, one study evaluated the eggs of commercially exploited fish (Mukhopadhyay & Valsalan 2024).

Insects, stingrays, and plants only appeared once in our search. While the first two were part of descriptive studies (Akindele et al. 2020, Trindade et al. 2023), the study on plants is more complex, analyzing the efficiency of microplastic removal by two different species (Cabañas-Mendoza et al. 2024). Although crustaceans were the focus of only four papers, these include all Tropical regions and both lentic and lotic environments (Guimarães et al. 2023, Boateng et al. 2024, Chesnokova et al. 2024, Haque et al., 2023). As for mollusks, the studies are mainly analytical and were conducted in Africa and Asia (e.g. Ditlhakanyane et al. 2023, Haque et al. 2023).

Research gaps and future directions

We presented an overview of the current research on microplastic contamination in Tropical freshwater environments, emphasizing the knowledge advances and existing gaps. Although research and the complexity of studies have increased over the years, the Tropical region and its freshwater environments are still understudied. Furthermore, we highlight the need for future research in African countries and tropical Australia, which are still understudied compared to other tropical regions. Regarding the environments, reservoirs deserve more attention, since recent research has shown high levels of contamination of microplastics in these environments (Watkins et al. 2019), which can be acting as sinks for these particles. Whether the trapping of particles in reservoirs represents a beneficial process for the downstream areas and, subsequently, the oceans, or poses a significant risk to aquatic organisms of the reservoir, remains unclear and needs further investigation.

Tropical river basins often flood over large terrestrial areas through flood pulses in the rainy season (Welcomme 1985). The flooding process could be acting both as a source of microplastic for aquatic environments, transporting the particles from terrestrial areas, and as a sink for terrestrial particles. This dynamic has not been thoroughly explored in the tropics and deserves more attention. Moreover, less than 5% of the studied environments in the Tropical region are urban water bodies. We emphasize the need for future research in these environments, since they can provide valuable insights of the contamination in urban centers. Additionally, some of these environments can be discharged in natural waterbodies, being a source of microplastics in these ecosystems.

The ingestion and effects of microplastic particles on aquatic invertebrates also need to be addressed in future studies, in addition to the consequences for these organisms. They could also be impacting the food web and, some of them, such as crustaceans and mollusks, are consumed by humans, exposing them to health issues (Alberghini et al. 2022, Bhuyan 2022).

Freshwater environments provide several ecosystem services for humans, with their quality being essential to human use (Sterner et al. 2020, Petsch et al. 2023). Some of these services can be a source of microplastics in these environments, potentially affecting human health (Alberghini et al. 2022, Bhuyan 2022). This is a major challenge in developing countries of the tropical region, which are still facing problems with water quality and waste treatment (WWAP, 2017). Advancements in research on this topic are essential to provide a clear picture of the real problem in the tropics, leading to the possibility of better mitigation and conservation actions in the future.

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ARTIGO 2

Microplastic contamination of freshwater fish in lotic and lentic environments in a tropical river basin

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Abstract

Freshwater ecosystems can act as major reservoirs of microplastic particles. Lotic and lentic environments differ in hydrology and sediment transport capacity, shaping microplastic behavior and leading to distinct contamination patterns and exposure levels for fish. In this context, our study aimed to investigate whether microplastic contamination differs among fish species from floodplain lagoons, streams, the main river stem, and tributaries, and to evaluate the influence of land use and seasonality on contamination. We analyzed microplastics contamination in the gastrointestinal tracts of three Acestrorhamphidae species at 16 sampling sites along the Velhas River Basin, Brazil. The tracts were digested, filtered, and analyzed under a microscope and with Raman spectroscopy. Fish from lagoons showed higher ingestion levels. Ingestion also differed among species, and we did not find a significant relationship between the number of microplastics ingested by the fish and either land use or season. We highlighted the importance of studying the floodplains to understand the microplastic dynamics in the tropics. We also found that even closely related species may respond differently to contamination within the same environment, and showed that the influence of external factors may be challenging to identify. Altogether, these findings emphasize the complexity of contamination dynamics in tropical freshwater ecosystems and the importance of advancing our understanding to support conservation and management efforts.

Keywords

Aquatic ecosystem, emerging contaminant, floodplain, São Francisco Basin, spatial distribution, plastic pollution.

Introduction

Microplastics are ubiquitous in freshwater ecosystems worldwide (Eerkes-Medrano et al. 2015). Nevertheless, our current understanding of the magnitude and consequences of microplastic pollution for freshwater ecosystems and biodiversity remains limited (Eerkes-Medrano et al. 2015). Microplastics have been recorded in the world's major river basins, in urbanized and more remote watercourses, natural lakes, artificial reservoirs, and even in groundwater (Dikareva and Simon 2019; Rico et al. 2023; Nava et al. 2023; Lee et al. 2024). Rivers are considered the major pathway of plastic pollution to the oceans (Strokal et al. 2023). However, less than 3% of plastic waste reaches the sea, indicating that freshwater environments can act as major reservoirs for plastic pollution (van Emmerik et al. 2022). Particles can be trapped in plants, riverbed sediments, in infrastructure and lentic water bodies such as floodplains, lakes, and reservoirs (van Emmerik et al. 2022).

Most microplastics entering freshwater ecosystems are the result of land-based activities, such as littering, landfills, wastewater effluents, and surface runoff from roads, construction sites, and agricultural areas (Waldschläger et al. 2020). Flood events can also be a key aspect of macro and microplastic dynamics in aquatic systems. Flooding can remobilize plastic that is retained in rivers as well as being a source of new particles for floodplains and lotic environments due to the transport from terrestrial areas (Windsor et al. 2019; Waldschläger et al. 2020; van Emmerik et al. 2022). Once in the water, the fate of microplastics depends on hydrological aspects (e.g., river flow), on particle characteristics (e.g., density), and on the formation of biofilm on the particle surface (Eerkes-Medrano et al. 2015; Waldschläger et al. 2020; van Emmerik et al. 2022).

The accumulation of microplastic particles has direct impacts on freshwater biota (de Sá et al., 2018). Microplastics have been detected in multiple organs of fish species from different habitats and feeding guilds (Wootton et al. 2021). The main form of contamination is through ingestion, which can occur intentionally when a particle is mistaken for prey, or accidentally through the consumption of prey that is already contaminated (Jovanović 2017). Additionally, microplastics in the water column can also adhere to fish gills (Zhang et al., 2021). A broad range of consequences from microplastics uptake has been documented and can be attributed not only to the particles themselves but also to additives used during manufacturing and to contaminants adsorbed onto the particles in the environment (Bhuyan 2022). Examples of these consequences are gastrointestinal blockage, DNA and tissue damage, growth reduction, behavioral changes, and mortality (de Sá et al. 2018; Bhuyan 2022). Some effects can extend to organisms that consume fish, including humans (Bhuyan 2022). Microplastics have been found in fish sold in markets (Karami et al. 2017; Wootton et al. 2021) and since smaller particles can be transported into tissues, the removal of viscera prior human consumption is not enough to guarantee the absence of particles (Karami et al. 2017).

Microplastic pollution can also vary across different freshwater environments within a river basin. Rivers, streams, lakes, and floodplains vary in hydrological characteristics, including size, flow, and water residence time, as well as in their capacity to transport matter and sediment (Vercruysse et al. 2017) . These can influence microplastic behavior, resulting in distinct contamination patterns and varying levels of exposure for fish species (Rodríguez-Seijo and Pereira 2017). However, microplastic contamination studies integrating lotic environments of varying sizes with floodplain systems are still scarce. Studying them together across an entire river basin allows for a comprehensive view of microplastic distribution and exposure, rather than focusing on individual habitats. This integrated approach is particularly important in tropical freshwater systems, where information is still limited and the role of floodplain lagoons in accumulating microplastics is not yet fully understood (Moreira et al. 2025).

This study seeks to obtain a comprehensive understanding of fish contamination throughout a tropical river basin. Our aim is to describe and test whether microplastic contamination differs among fish species from floodplain lagoons, streams, the main river stem, and tributaries, as well as to assess whether land use and seasonality influence fish contamination. We hypothesize that microplastic contamination in fish differs among freshwater environments. Specifically, we expect higher contamination in fish from lagoons and the most downstream site of the main river. Additionally, we predict higher contamination at more urbanized sites and during the wet season, and we expect no differences in contamination among the three studied species.

Methods

Study area

The study was conducted in the Velhas River basin, a tributary of the São Francisco River, Southeastern Brazil. The Velhas basin covers 27,820 km² and drains 51 municipalities (CBH Rio das Velhas 2010), with a total population of over 4 million inhabitants (IBGE 2022). The Velhas River is 806 km long and is the largest tributary of the São Francisco River basin (Alves and Pompeu 2010). In the lower part of the Velhas Basin, there are several lagoons that typically flood during the rainy season. The upper part of the Velhas basin encompasses the third largest metropolitan region in Brazil, Belo Horizonte. This region is the major source of pollution for the basin due to the discharge of domestic and industrial sewage, and inadequate solid waste disposal (Pompeu et al. 2025). The Velhas Basin is also affected by mining activities, deforestation, and excessive water extraction (Pompeu et al. 2025).

We selected 16 sampling sites, four from each different environment of the Velhas River basin: main river stem, tributaries, streams, and lagoons (Fig. 1). For each site, we calculated land use within a 500 m buffer using the Mapbiomas database and Google Earth Engine (Gorelick et al. 2017; Souza et al. 2020).

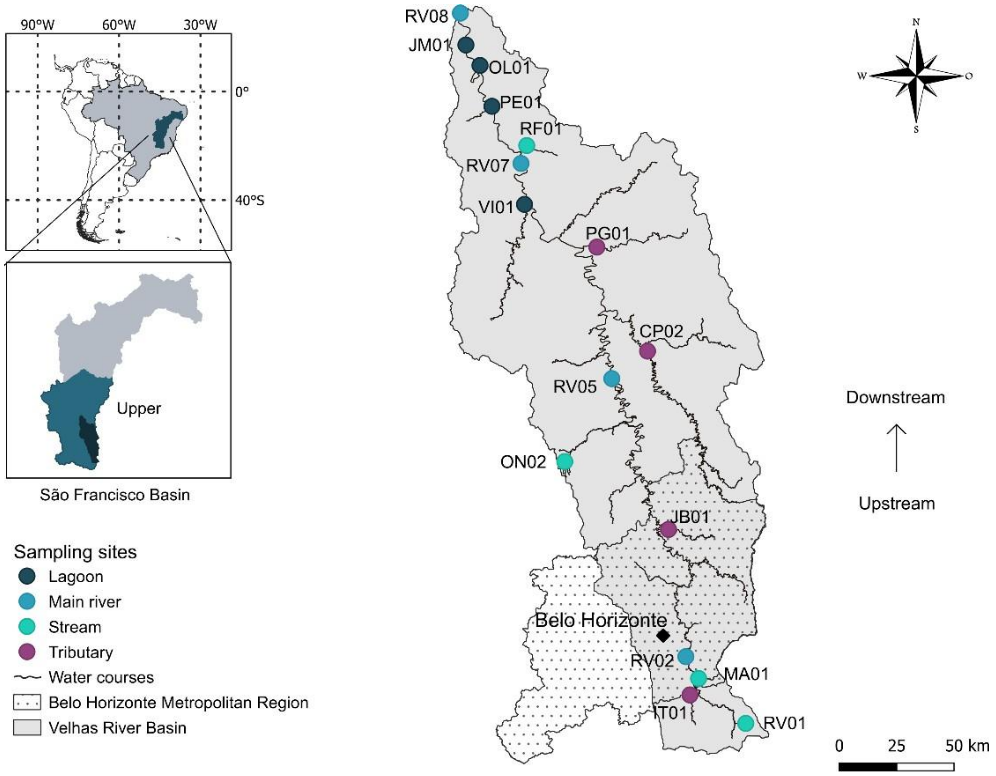

Fig. 1 Map of the Velhas River basin, indicating the sampling site locations

Sampling

Fish were sampled in 2020, 2021, 2022, and 2023, depending on the site. Fish from the main river stem, tributaries and streams were sampled during dry (March to September) and rainy seasons (October to February), while the lagoons sampling took place only in the dry season due to the lack of access in the rainy season. We used gillnets (3-16 cm between adjacent nodes, 10 m long, 1.5-1.75 m high), trawl nets (0.3 mm between adjacent nodes, 5 m long, 1.5 m high) and semicircular hand nets (1 mm mesh size, 80 cm in diameter). Fish were euthanized with eugenol solution, preserved in 10% formaldehyde, and later transferred to 70% alcohol. In the laboratory, species were identified, measured, and weighed. Fish samplings were in accordance with the Ethical Committee for Animal Use in Experiments of the Federal University of Lavras (CEUA number 024/2020) and SISBIO Sampling License number 73184.

Species

We selected 235 individuals from three species of the Acestrorhamphidae family: *Astyanax lacustris* (Lütken 1875), *Psalidodon fasciatus* (Cuvier 1819) and *Psalidodon rivularis* (Lütken 1875). These species are opportunistic, diurnal, omnivorous with a frequent preference for insects and vegetal matter, water-column swimmers, and show multiple spawning events throughout the year (Bastian et al., 2021; Casatti and Castro, 1998; Pires et al., 2024; Souza et al., 2015). For streams, tributaries, and the main river stem we analyzed individuals from two or three of the species. In lagoons, only *A. lacustris* was analyzed, as it was the only species found.

Sample processing

To analyze the ingestion of microplastic particles, we extracted the gastrointestinal tract of each specimen and stored them in clean glass bottles. Gastrointestinal tracts were submitted to a digestion process to remove organic matter without damaging microplastic particles. This consisted of placing the samples in a 10% potassium hydroxide solution in a proportion of three times the gastrointestinal volume, in an oven at 60ºC for 24 hours, which accelerates the dissolution process (Munno et al. 2018). Then, samples were filtered using a filtration system coupled to a vacuum pump and a Mixed Cellulose Ester membrane filter (47 mm diameter, 0.22 µm porosity). The membranes were stored in Petri dishes covered with aluminum foil and dried at room temperature before the microscopic analysis.

We used a microscope equipped with a camera to detect microplastic particles. After identifying the particles in the sample, their color and morphology were recorded. For microplastic morphology, we considered three groups: beads, fibers, and fragments (Lusher et al. 2020). We selected a subset of the samples to perform a chemical identification using Raman spectroscopy (RS) with a commercial micro-Raman spectrometer (WITec Alpha 300 RA). Measurements were performed with a 532 nm excitation laser (0.64 mW), a Nikon CF Plan ELWD 50×/0.55 objective, and a 600 g/mm grating (BLZ = 500 nm). Spectra were acquired over a Raman shift range of 0–3600 cm^{-1}, centered at 2100 cm^{-1}, with 10 accumulations of 5 s integration time each.

Quality control

Several measures were adopted to minimize contamination during sample processing, as suggested by Hermsen et al. (2018). The room where the samples were processed had no windows and the door was kept closed at all times. No more than two people were working in the room. Gloves and a cotton lab coat were used during processing. The work surface was carefully wiped before processing. The distilled water used was filtered using a filtration system with the same membrane filter used for the samples. This water was used to prepare the KOH solution and rinse the utensils and glassware thoroughly before and between sample manipulations. We also carried out visual inspections of the membranes before use. After the gastrointestinal tract extraction and during the dissolution process, glass bottles were covered with an aluminum-foil lid and processed quickly to prevent airborne contamination.

To check for possible airborne contamination, we placed three Petri dishes with a membrane filter on the work surface for each sample batch during all three steps of processing: dissection, filtration, and microscopic analysis. As we found a large number of transparent fibers in both blank and fish samples, we excluded this type of particle from our results. Afterwards, we estimated the number of microplastic particles that could have fallen into each sample by dividing the total number of microplastics found in the blank batch by the number of samples processed with that same batch. Across all steps, the potential contamination ranged from 0 to 1 particle per sample, with most values being below one. Therefore, we chose not to apply blank correction.

Data analysis

To understand the factors influencing the ingestion of microplastic particles in fish, we performed a Generalized Linear Model (GLM). We tested the influence of five independent variables: environment type (lagoon, main river stem, stream, and tributary), species, season, percentage of urbanization, and percentage of agricultural land use. We used the mean number of particles ingested per species at each sampling site in each season as the dependent variable and the five variables mentioned above as independents. Because multiple samples were collected at the same site, we first built a Generalized Linear Mixed Model (GLMM) using the sampling site as a random factor. In this first model, there was no variation attributed to the random effect (variance = 0.00 and standard deviation = 0.00). For this reason, we opted to build a GLM with Gaussian family.

The analyses were conducted using RStudio (R Core Team 2021). We used lme4 package for the GLMM (Bates et al. 2015). To check the fitting of our GLM, we tested the visual residuals, multicollinearity, and overdispersion. For this, we used the packages DHARMa (Hartig 2022), car (Fox and Weisberg 2019) and performance (Lüdecke et al. 2021), respectively. We tested the significance of the model using an Anova with the car package (Fox and Weisberg 2019).

Results

We found a total of 246 microplastic particles in 131 (55.7%) fish specimens. Fish from all sampling sites were contaminated, and the prevalence of contaminated individuals per site ranged from 31.8% to 77.7% (Table 1). The number of microplastic particles ingested differed between environments (p = 0.01; Fig. 2). Fish from lagoons showed higher ingestion levels, with values consistently high across sites. There was also a consistently higher proportion of contaminated individuals in all four lagoons (66%–77%). In contrast, fish from all lotic environments exhibited similar ingestion but showed greater variation across sites. Contrary to our hypothesis, the most downstream site in the main river stem (RV-08) showed the lowest fish contamination, both in mean number of ingested particles and in the frequency of contaminated individuals. The ingestion also differed between species (p = 0.03), with *A. lacustris* ingesting more particles than *P. fasciatus* and *P. rivularis* (Fig. 3).

Table 1 Number of analyzed fish, percentage of contaminated individuals, and mean number of ingested particles per sampling site. Data from lagoons in the rainy season are not available because these sites were not sampled during that period.

		Dry		**Rainy**	
Site	**Analyzed fish**	**% contaminated**	**Mean particle**	**% contaminated**	**Mean particle**
Lagoon					
JM01	9	77.7	1.77	-	-
OL01	11	72.72	1.81	-	-

PE01	12	66.66	2.25	-	-
VI01	6	66.66	1.83	-	-
Main stem					
RV02	11	-		45.45	1.00
RV05	10	50	1.33	50	0.75
RV07	18	55.55	0.88	77.77	1.00
RV08	22	36.36	0.45	27.27	0.36
Stream					
MA01	16	60	0.80	33.33	0.50
ON02	21	70	0.80	36.36	0.54
RF01	14	50	0.60	100	2.00
RV01	13	70	2.30	33.33	0.33
Tributary					
CP02	17	50	0.70	28.57	0.28
IT01	21	54.54	0.63	60	1.20
JB01	14	50	0.75	60	1.00
PG01	20	60	0.90	60	1.00
Total	235	59.71	1.19	50	0.82

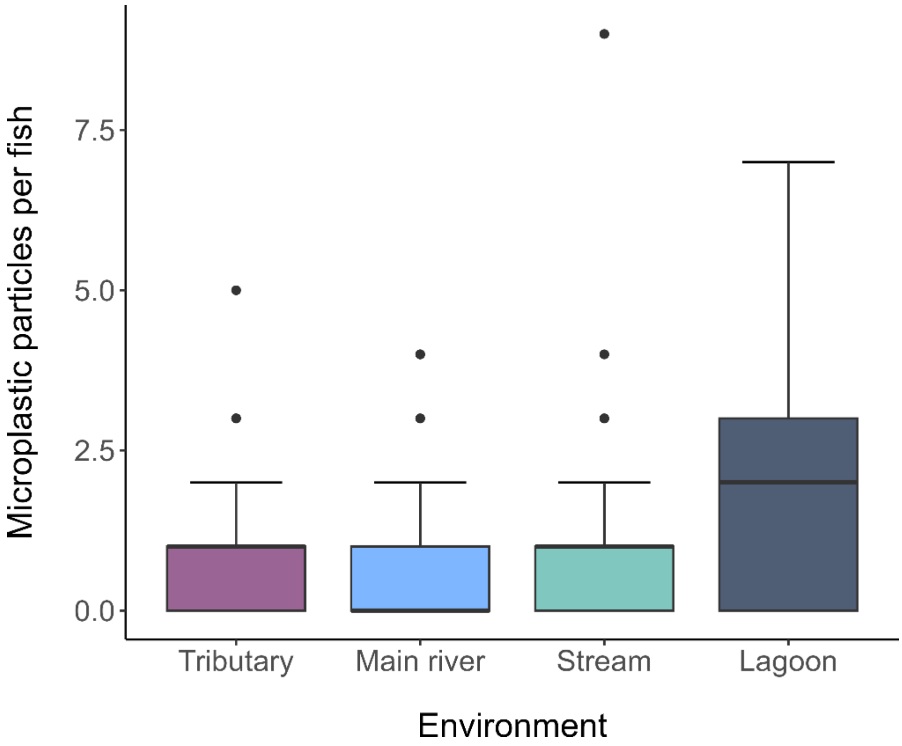

Fig. 2 Number of particles found per individual in each environment. The box represents the interquartile range (25%–75%), the line inside the box indicates the median, the whiskers show the data variation, points outside the whiskers are outliers

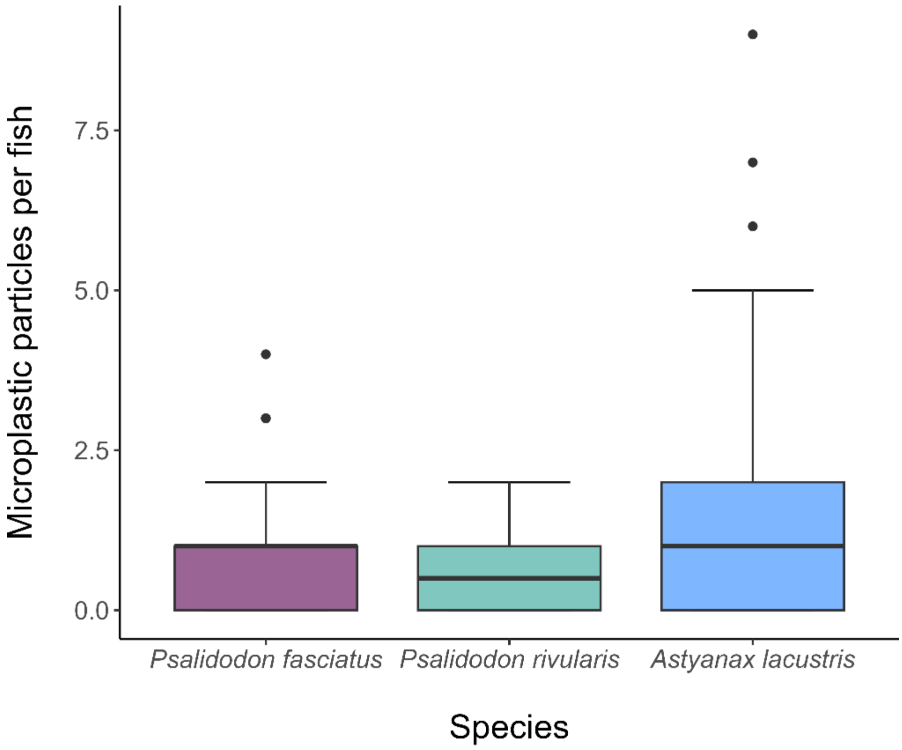

Fig. 3 Number of particles found per individual for each species. The box represents the interquartile range (25%–75%), the line inside the box indicates the median, the whiskers show the data variation, points outside the whiskers are outliers

Land use among sites varied from 14% to 87% agricultural and from 0% to 45% urban (Table 2). Nevertheless, urban coverage showed little variation, with 11 of the 16 sites showing 0% of this land use. We did not find a significant relationship between the number of microplastics ingested by fish and land use or season.

Table 2 Land use and river flow of the sampling sites. The land use was calculated within a 500-meter buffer.

Site	Main land use	Native (%)	Urban (%)	Agricultural (%)	Flow
Lagoon					
JM01	Native	66.3	0	33.7	-
OL01	Native	84.3	0	15.7	-
PE01	Native	85.3	0	14.7	-
VI01	Agricultural	38.4	0	61.5	-
Main stem					
RV02	Urban	33	45	22	23.4
RV05	Agricultural	12	0.7	87.3	120.5

RV07	Native	62.5	0	37.5	295
RV08	Agricultural	28.7	8.6	62.7	326.6
Stream					
MA01	Native	66.1	0.7	33.2	0.3
ON02	Agricultural	29.3	0	70.7	0.3
RF01	Agricultural	31.4	0	68.6	1.2
RV01	Native	54.9	0	45.1	1.1
Tributary					
CP02	Agricultural	22.7	0	77.3	26.8
IT01	Native	82.8	0.2	17	7.6
JB01	Agricultural	20.4	0	79.6	7.1
PG01	Agricultural	16.2	0	83.8	21.9

We found all three morphologies of particles: beads, fragments, and fibers (Fig. 4). Fragments were the most abundant in all four environments, followed by fibers. Beads were rare, with only five particles recorded, occurring at three sampling sites of the main river stem, one tributary, and one lagoon (Fig. 5a). Nine microplastic colors were identified: black, blue, gray, green, pink, red, transparent, white, and yellow. Blue and black were the most abundant (63.8% and 23.5%, respectively), and blue was present in all sampling sites. The other seven colors represented only a small portion of the ingested particles (Fig. 5b). Streams and lagoons showed lower color diversity, with four and five colors, respectively. In contrast, tributaries and the main river stem had seven and eight colors, with green and red being exclusive to these environments. Through Raman spectroscopy, we identified the polymers: polypropylene (PP, 14.8%), polyethylene terephthalate (PET, 18.5%), polyurethane (PU, 7.4%), and polystyrene (PS, 7.4%). Dyes such as copper phthalocyanine (33.3%) and diarylide yellow (3.7%) were also detected. Inorganic carbon was identified in 11% of the particles (Fig. 5c).

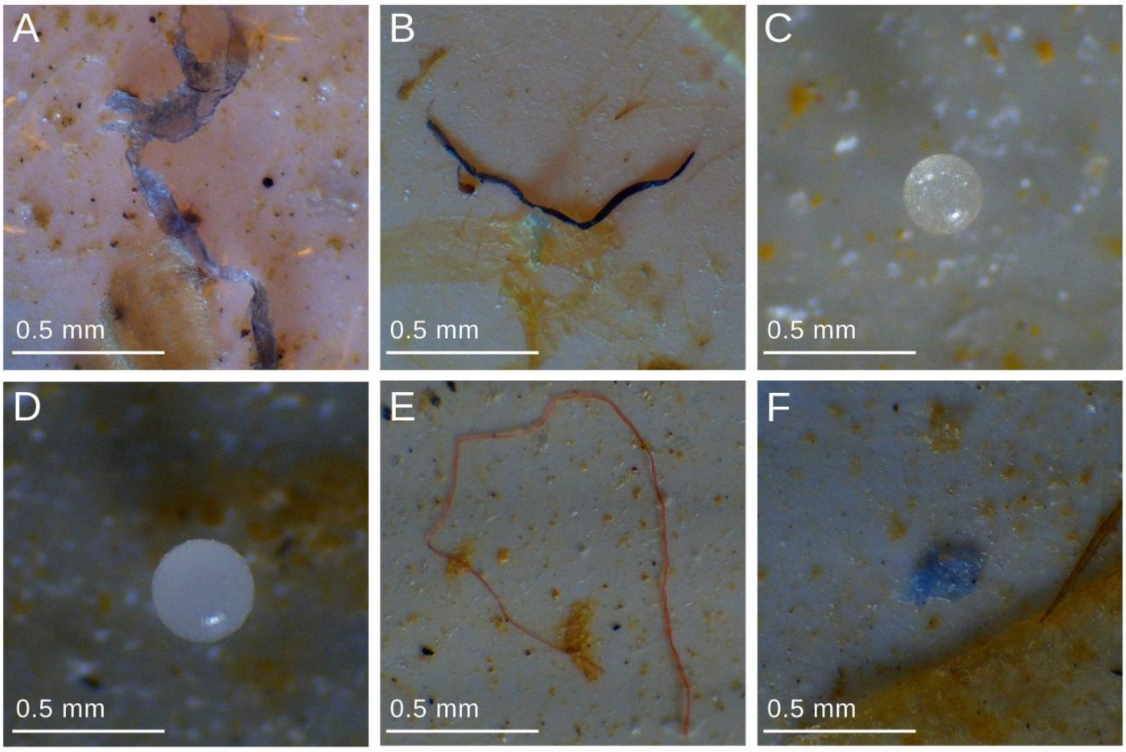

Fig. 4 Microplastic particles found in the studied fish: fragments (A, F), fibers (B, E), and beads (C, D)

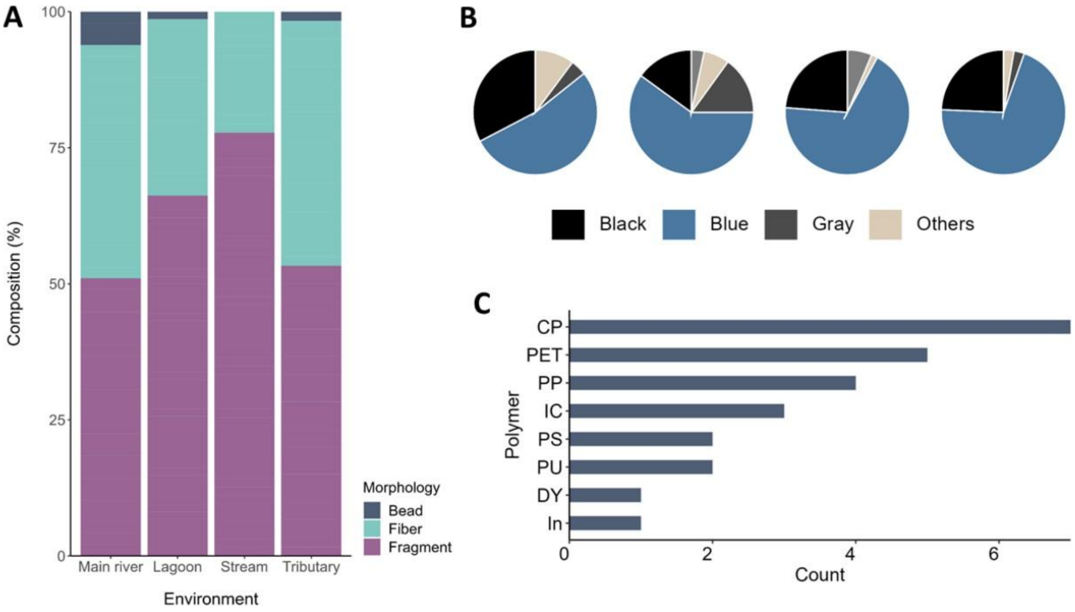

Fig. 5 Overview of microplastic composition in fish from the studied environments, showing particle morphologies (A) and colors (B) by environment, and polymers in the total sample (C). CP, copper phthalocyanine; PET, polyethylene terephthalate; PP, polypropylene; IC, inorganic carbon; PS, polystyrene; PU, polyurethane; DY, yellow of diarylide; In, inconclusive

Discussion

Our study presents new insights into microplastic contamination in tropical freshwater ecosystems. The higher ingestion by fish from lagoons highlights the role of lentic environments as sinks for microplastic particles. Our results also demonstrate that even congeneric, morphologically similar species in the same environment can ingest microplastics in different amounts. These findings, combined with the similarity of contamination between streams and large rivers, as well as the absence of a relationship between contamination and commonly related factors such as land use and seasonality, highlight the complexity of contamination dynamics in freshwater environments.

Floodplains can act as sinks for microplastic particles (Lechthaler et al. 2021; van Emmerik et al. 2022). Although most studies conducted in floodplains focused on their terrestrial portion (e.g., Lechthaler et al., 2021; Weber and Opp, 2020), the higher microplastic ingestion by fish from the lagoons in our study suggests that floodplain lagoons are also areas of microplastic accumulation. Since lentic water bodies are static, with low water velocity and usually isolated during most of the year, they can retain particles (Windsor et al. 2019; Waldschläger et al. 2020; Lechthaler et al. 2021). Furthermore, the high residence time in lentic environments can lead to the fragmentation of macroplastics into microplastics, making their concentration higher than in lotic ecosystems, such as the main river stem (van Emmerik and Schwarz 2020; Christensen et al. 2020; Lechthaler et al. 2021). The high contamination in this environment can pose serious risks to the biota, including important commercial fish species that rely on the floodplain during their early development (Welcomme, 1985).

Although our lotic sampling sites have different levels of conservation, the contamination of fish from the main river stem, tributaries and streams was similar. The fact that contamination in headwater streams was comparable to larger watercourses is particularly concerning and highlights the widespread presence of microplastics throughout the Velhas River basin. Considering the sampling sites individually, we expected fish from the most downstream site of the river stem to show high contamination due to particle transport from upstream areas, especially from the metropolitan region. Contrary to this expectation, it was one of the least contaminated sites. This result suggests that most particles from upstream are either retained along the river (van Emmerik et al. 2022) or, if they reach downstream areas, are transported to the lagoons during flooding, supporting the idea that floodplain areas act as sinks for particles from the entire basin. However, a lower direct input of plastic and microplastic particles in the downstream area could also contribute to this pattern. Additionally, the inflow of tributaries in better conservation conditions along the main river may contribute to dispersing the microplastic concentration originating from the upstream metropolitan area, as previously observed for water quality (Pompeu et al. 2025).

Our study showed significant differences in microplastic ingestion among three species, with *A. lacustris* consistently showing higher ingestion regardless of the environment. Differences in species foraging could lead to higher ingestion of microplastics. The coexistence of morphologically similar and even congeneric species can be attributed to resource partitioning, as evidenced by low diet overlap among *Astyanax* species (Comte et al. 2016; Bonato et al. 2018; Neves et al. 2018). Since microplastics can accumulate in some regions, such as areas with reduced flow or in flooded and riverbank vegetation, *A. lacustris* could be exploring areas and resources with greater microplastic load (Waldschläger et al. 2020; van Emmerik et al. 2022). Additionally, in the adult stage, *A. lacustris* shows an herbivorous tendency, whereas the other species are predominantly invertivorous (Pompeu and Godinho, 2003). Since the digestion of plant material is slower, it may result in longer retention of microplastics in the gastrointestinal tract, leading to higher contamination in this species. This is also relevant regarding the observed differences in contamination between environments. The higher contamination in the lagoons may stem not only from floodplain dynamics but also from differences in species foraging strategies.

Microplastic contamination can be affected by several factors and is often closely related to anthropogenic activities, such as land use (Talbot and Chang 2022). Nonetheless, this relationship is not always easily detected (Talbot and Chang 2022). The effects of urbanization and agricultural use could be overshadowed by the downstream transport and atmospheric deposition throughout the study area (Talbot and Chang 2022). More recently, atmospheric transportation is increasingly recognized as an important contribution to microplastic pollution worldwide, in both urbanized and remote areas, given the dominance of fibers in the samples (Can-Güven 2021). In our study, fibers were abundant throughout the basin, even in more isolated sampling sites.

We did not find a relationship between fish contamination and seasonality. Freshwater environments usually exhibit higher levels of contamination during the wet season, as a result of the resuspension of sediments and the runoff from terrestrial environments (Talbot and Chang 2022). However, in the dry season, the higher water transparency and lower food availability could be leading to high microplastic ingestion by these species, which are visual feeders, resulting in similar rates of ingestion throughout the seasons. Still, seasonality is probably a key factor in the microplastic contamination of lagoons that are part of a floodplain system. The flooding of the lagoons in the wet season, along with their connection to the terrestrial environment and the main river, is likely to contribute to the incorporation of a significant number of particles into the lagoons (Roebroek et al. 2021; Rolf et al. 2022). Unfortunately, evaluating seasonal variation in the lagoons was not possible in our study due to the lack of data from the wet season.

Fibers and fragments accounted for the majority of the particles ingested by fish in our study. Secondary microplastics are the most common particles found in aquatic ecosystems and can be formed and released into the environment through fragmentation of large plastic items and during the use of some products (Li et al. 2020; Thompson et al. 2024). In contrast, primary microplastics are already produced in this size, usually as beads and can occur as small particles (e.g., glitter), be added to products, or as pre-production materials (Cole et al. 2011; Wagner et al. 2014). Natural degradation can alter the shape of beads, making them harder to identify (Rodríguez-Seijo and Pereira, 2017), which may also help explain the low number of these particles in our study.

Microplastic can be mistaken for food and be actively consumed by organisms, especially by visual consumers, leading to a higher ingestion of specific colors (Wright et al. 2013; Ríos et al. 2022). In freshwater ecosystems, blue and black particles are commonly the most abundant colors and are also the most frequently ingested by fish (Costa et al., 2023; Nava et al., 2023). An experimental approach with a *Psalidodon* species showed color preferences, with a higher ingestion of yellow and blue particles, in contrast with a lower intake of white particles (Ríos et al. 2022). Although we also found a high ingestion of blue particles and a low ingestion of white ones for both *Psalidodon* and *Astyanax* species in all environments, yellow microplastics were rarely found in individuals from the three lotic environments, and they were not detected in fish from lagoons. This difference demonstrates that, even for the same species group, color preference may vary across environments. Two of the identified polymers in our study (PP and PET) are frequently found in freshwater environments, whereas PU is less commonly detected and is primarily associated with building and construction sources (Geyer et al. 2017; Sun et al. 2022). The ingestion of such plastic polymers by freshwater organisms has been associated with negative effects such as stress, growth inhibition, reduced body length, and mortality (Anbumani and Kakkar 2018).

When microplastic contamination is only analyzed in water, sediment, or the biota, the influence of external factors, such as spatial and temporal variables, can be difficult to detect. Because plastic polymers have different densities, they concentrate in distinct areas in aquatic ecosystems, leading to differences in microplastic abundance and diversity even in the same environment (Rodríguez-Seijo and Pereira 2017). The absence of a relationship between land use and seasonality in our study suggests that fish contamination may not fully reflect the contamination of the environment. This may result from fish preference for specific particle morphologies and from the type of polymers that tend to accumulate where these species forage. Future research on contamination in environmental compartments and its comparison to fish ingestion could help determine whether fish show selective uptake of particles under natural conditions. Moreover, it would allow investigation into whether their contamination reflects the quantity and types of particles present in the environment, and if fish are a reliable proxy for the contamination of a specific site or environment.

We presented a novel comparison of microplastic contamination in fish from different freshwater environments, highlighting the importance of studying floodplains to understand the microplastic dynamics in the tropics. We also showed that even closely related species may respond differently to contamination within the same environment and that the influence of external factors may be challenging to identify. Altogether, these findings emphasize the complexity of contamination dynamics in tropical freshwater ecosystems and the importance of advancing our understanding to support conservation and management efforts in the future.

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Twenty-four years of microplastic contamination in fish in a tropical river basin

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Running title

Two decades of microplastics in tropical fish

Abstract

Microplastic research in tropical freshwaters is relatively recent, and long-term contamination patterns in these environments remain poorly understood. In this context, we investigated microplastic contamination in tropical freshwater fish over a 24-year period across regions with contrasting environmental contexts within a large river basin, and evaluated the influence of turbidity on microplastic ingestion. The gastrointestinal tracts of small-sized characids from the Velhas River Basin, Brazil, were used for this assessment. The tracts were digested, filtered, and analyzed under a microscope. We found significant changes in fish contamination over the last two decades, however, temporal trends differed among regions with contrasting land uses. Fish ingestion was also related to water turbidity, with higher ingestion occurring in clearer waters. Our study provides the first long-term assessment of microplastic contamination in tropical freshwater fish. The results indicate that, although plastic production and disposal have increased over the last decades, biological responses are not straightforward and depend on the environmental context.

Key-words

Emerging pollutant, freshwater fish, land use, long-term monitoring.

Introduction

Plastic production and consumption have increased steadily over the past decades, with about 60% of its total being discarded in the environment (Geyer, Jambeck and Law, 2017). Because of their resistance, plastics can persist in the environment for hundreds to thousands of years, during which they break down into microplastic particles (Zhang *et al.*, 2021). Microplastics are widely defined as particles smaller than 5 mm and are present in most environments worldwide (Thompson *et al.*, 2024). The first studies on plastic pollution focused on marine ecosystems and considered freshwater systems merely as pathways through which particles reach the oceans. However, more recently, research on freshwater has expanded, and watercourses are increasingly recognized as reservoirs of microplastic particles, highlighting their importance for understanding contamination and its relationship to human health (Wang *et al.*, 2023; Moreira, Leal and Pompeu, 2025).

Microplastic contamination has been associated with land-use changes surrounding freshwater ecosystems (Soltani *et al.*, 2022). Urbanization is a major source of microplastics through multiple pathways, including the fragmentation of plastic resulted from littering, wastewater treatment plant (WWTP) effluents, untreated sewage discharges, and road runoff (Horton *et al.*, 2017). However, agricultural activities can also be important sources of microplastics. Crop and animal production rely on several plastic products that can fragment (Horton *et al.*, 2017). In protected areas and regions characterized by extensive native vegetation cover, lower levels of microplastic contamination are generally expected, reflecting the absence or reduced intensity of direct anthropogenic activities. However, microplastics have been documented in freshwater ecosystems within protected areas, although at lower concentrations than in less preserved sites (Granados-Sánchez, Sedeño-Díaz and López-López, 2024). This highlights the role of transport processes in microplastic dynamics, such as atmospheric deposition, and contamination from upstream sources. In this context, terrestrial vegetation can act as temporary sinks for microplastics, which can later be transported again to water bodies through wind and during floods (Liu *et al.*, 2020).

Freshwater ecosystems permeate all landscapes and are linked to all land uses. The plastic contamination of watercourses is not only a result of the input from the surrounding terrestrial systems, but also of water quality parameters that determine particle degradation, changes in properties, and their adsorption of pollutants (Li *et al.*, 2023; Sodré *et al.*, 2023). For instance, turbidity, conductivity, and temperature can influence the abundance of microplastics in rivers (Buwono, Risjani and Soegianto, 2021; Rivera-Gutiérrez *et al.*, 2025). However, the relationship between water quality and microplastic ingestion by freshwater biota has been understudied. The uptake of microplastics by fish can occur either directly, through the consumption of particles that are accidentally ingested or mistaken for food, or indirectly, through the ingestion of contaminated prey (Jovanović, 2017). Foraging strategy is an important factor influencing microplastic detection and ingestion (Roch, Friedrich and Brinker, 2020). In freshwater fish, visually foraging species ingest more particles, as microplastics can resemble natural food items and are less easily discriminated than by chemosensory species (Roch, Friedrich and Brinker, 2020). For the former, microplastic ingestion is likely influenced by turbidity that affect particle detectability.

Although nanoplastic particles may pass through the gastrointestinal tract wall of fish, microplastics do not appear to accumulate in the gastrointestinal tract (Jovanović, 2017; Roch, Friedrich and Brinker, 2020). Therefore, their presence reflects recent ingestion, enabling accurate inference of temporal patterns by comparing fish collected in different years. Despite this advantage, long-term studies on microplastic contamination in fish remain scarce, possibly reflecting the limited availability of historical biological material and continuous monitoring programs, together with the novelty of microplastic research. Existing long-term assessments have relied on biological archives, such as museum collections and historical samples. However, they are restricted to temperate marine ecosystems (Beer *et al.*, 2018; Courtene-Jones *et al.*, 2019; Stefanelli-Silva *et al.*, 2024).

Our aim is to investigate microplastic contamination in tropical fish over the last 24 years across regions with different environmental contexts within a large river basin, and to assess whether turbidity influences microplastic ingestion by fish. We hypothesize that microplastic ingestion by fish differs in both abundance and diversity among different regions, with higher ingestion and a greater diversity of particles in the urban portion of the basin compared to agriculture and reference regions. We also hypothesize that there has been an increase in microplastic ingestion over the last 24 years, mainly in the urban region. Finally, we expect lower microplastic ingestion in regions with higher water turbidity. We focus on the Velhas River basin, Southeast Brazil, a system that has been monitored since 1999, providing a unique opportunity for a temporal approach. The basin includes a range of both anthropogenic and natural landscapes, draining a major city while also including protected areas, making it a representative example of many tropical river contexts.

Methods

Study area

The study was conducted in Velhas River basin, Minas Gerais state, Brazil, a tributary of the São Francisco River. This basin encompasses regions with contrasting environmental contexts, subject to different types and intensities of anthropic pressures, providing a valuable framework to understand long-term microplastic contamination in freshwater fish across a gradient of impact. We selected three regions within the basin, classified according to their dominant land use characteristics: urban, agriculture, and reference regions, the latter characterized by a high proportion of native vegetation (Figure 1, 2). The urbanized region has over 4 million inhabitants and is located in the upper portion of the basin. Belo Horizonte is the major urban center, accounting alone for 49% of the total population of the entire basin (CBHVelhas, 2015). The main sources of impact in this region are the discharge of untreated domestic and industrial sewage, as well as solid waste and effluents from illegal mining activities (CBHVelhas, 2015; Pompeu *et al.*, 2025).

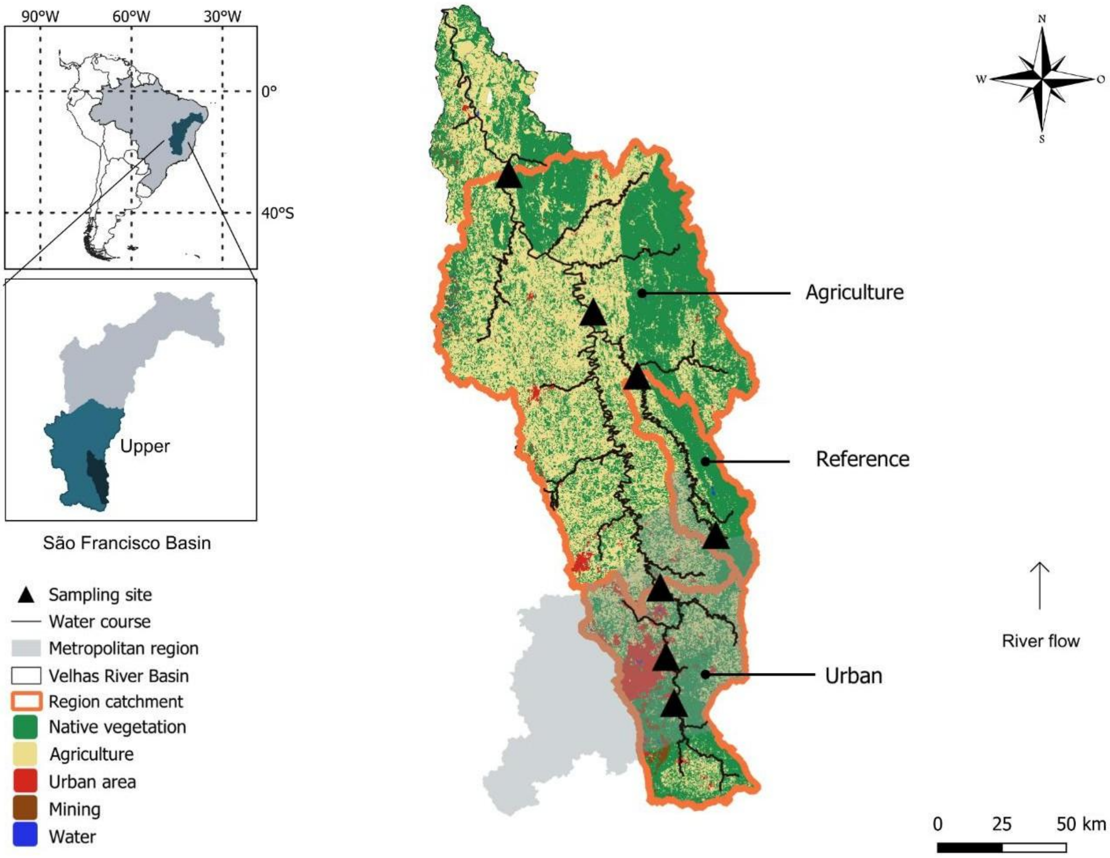

Figure 1. Map of the Velhas River basin, indicating the sampling regions and site locations.

The agriculture region has a lower population density, but because of its lower position, it receives inputs from the entire basin. This portion also includes a floodplain with several lagoons that connect to the main channel during the rainy season. The reference region corresponds to the Cipó River basin. The Cipó River is 252 km long, a fifth-order tributary of the Velhas River, and the most preserved of the basin (Pompeu *et al.*, 2025). It rises in the Serra do Cipó National Park, which was established in 1984 and covers an area of 31,639 ha, harboring several endangered species (ICMBio, 2009). Because of its ecological importance, the Cipó River and its tributaries were designated a ‘Permanent Preservation River’ status, which prohibits several activities that could degrade the river and its margins, including alterations to the riverbed and banks, mineral extraction activities, and other actions that could endanger aquatic fauna (Estado de Minas Gerais, 2004). There are only four municipalities within the Cipó River basin, and agriculture activities are low.

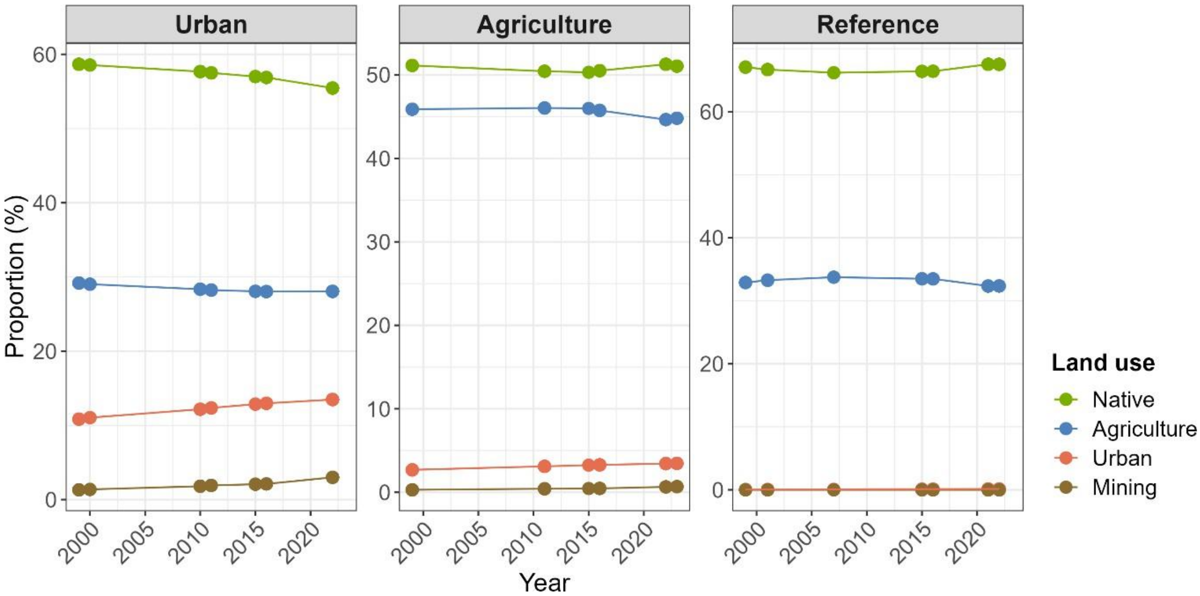

Figure 2. Temporal variation in land use proportion across the three study regions. Panel titles indicate the study regions, while colors represent the contribution of different land use classes. Land use data were extracted from the MapBiomas database (Souza *et al.*, 2020).

Sampling

Fish were sampled during the dry (March to September) and rainy (October to February) seasons at three sites within the urban region, two agriculture sites, and two reference sites over a 24-year period (1999–2023). All three regions were sampled repeatedly throughout the study period, although the specific sites sampled varied among years (Table S1). We used gillnets (10 m x 1.5 m in height, with mesh sizes ranging from 3 to 16 cm between opposite knots), trawl nets (5 m x 1.5 m high, and 0.3 cm mesh), and semicircular hand nets (80 cm in diameter, 1 mm mesh). Specimens were euthanized with a eugenol solution, fixed in 10% formalin, and transferred to 70% alcohol for preservation. In the laboratory, individuals were identified to species level, measured, and weighed. All sampling was authorized by the SISBIO collection license no. 73184 and procedures complied with the Ethical Committee for Animal Use in Experiments of the Universidade Federal de Lavras (CEUA 024/2020).

Species

We chose four species for this approach: *Astyanax lacustris* (Lütken 1875), *Psalidodon* cf. *bockmanni* (Vari & Castro 2007), *Psalidodon fasciatus* (Cuvier 1819), and *Psalidodon rivularis* (Lütken 1875), totaling 287 individuals. Small-sized characids of the family Acestrorhamphidae, they are widespread in South American basins and characterized by diurnal, opportunistic, and omnivorous habits, predominantly occupying the water column and reproducing throughout the year (Casatti and Castro, 1998; Souza *et al.*, 2015; Pires, Souza and Silva, 2024). In addition to being visual foragers, which allows testing the hypothesis that ingestion is related to turbidity, these species are highly abundant throughout the basin and occurred in all sampling years.

Sample processing

Microplastic particles were extracted from the gastrointestinal tracts of the specimens. A 10% potassium hydroxide (KOH) solution was used to dissolve the organic matter, with samples placed in an oven at 60 °C for 24 hours to accelerate dissolution (Munno *et al.*, 2018). The resulting liquid was filtered using a vacuum filtration system with a Mixed Cellulose Ester membrane filter (47 mm diameter, 0.22 μm porosity). The membranes were stored in glass Petri dishes, dried at room temperature, and analyzed under a microscope equipped with camera to identify microplastic particles. The microplastics detected were categorized according to their morphology (fiber, fragment, or bead) and color (Lusher *et al.*, 2020).

Quality control

We adopted a series of measures to ensure minimal contamination of the samples (Hermsen *et al.*, 2018). Processing was performed in a room without windows, with the door kept closed at all times and no more than two people allowed. A cotton lab coat was used, and all equipment and glassware were thoroughly rinsed with filtered distilled water, including between samples, to avoid cross contamination. The KOH solution was also filtered before use. To evaluate possible contamination during sample processing, blanks samples were included at all steps. For each batch of samples, three blanks were used during dissection and filtration, and one during microscope analysis. Transparent fibers were found in large amounts in both blanks and samples, therefore, to avoid overcounting, these were excluded. Based on the analysis of the blanks, we calculated the mean number of particles that could have fallen into our samples. As this number were consistent across batches and always lower than one particle per sample, we decided to not apply blank correction.

Data analysis

Turbidity data were obtained from fluviometric stations of the Instituto Mineiro de Gestão de Água (IGAM-MG) located near our sampling sites, through the Portal HidroWeb (https://www.snirh.gov.br/hidroweb/serieshistoricas). Because turbidity data were not available for all sampling sites and years, its relationship with microplastic ingestion could be tested only for one third of the data. For this analysis, we used a Generalized Linear Mixed Model (GLMM) with a negative binomial distribution. We evaluated site, species, and season as potential random effects and found that they explained little variance and presented similar AIC values. Site was then used as a random effect to account for the non-independency of the data. Using the complete data, we tested the effects of region, time (sampling year), and particle morphology on microplastic abundance using a GLMM. We also evaluated different random effects, and site was found to best explain the variance. The final model was a GLMM with a negative binomial distribution, with site as a random effect, that we tested the significance using ANOVA from the car package (Fox and Weisberg, 2019). Model fit was assessed through visual inspection of residuals, multicollinearity, and overdispersion using DHARMa, car, and performance packages (Fox and Weisberg, 2019; Lüdecke *et al.*, 2021; Hartig, 2022). All statistical analysis was performed in RStudio (R Core Team, 2021).

Results

Overall, 74.5% of fish were contaminated with a total of 590 microplastic particles. We found a significant effect of water turbidity on microplastic ingestion ($p<0.05$, Table 2, S2) across all three regions combined, indicating reduced ingestion under higher turbidity (Figure 3, 4). Fish from the urban region had the highest ingestion and proportion of contaminated individuals, followed by agriculture and reference region (Table 1; Figure 5). Microplastic abundance changed in the last 24 years, but with different patterns across regions ($p<0.05$; Table S2). Microplastic abundance decreased in the reference region over the years, increased in the agriculture region, and remained stable in the urban (Figure 6).

Table 1. Microplastic contamination in fish from the three studied regions.

Area	Analyzed fish	Contaminated fish (%)	Number of particles	Mean particle per fish
Urban	97	84.5	248	3.02
Agriculture	94	68.0	156	2.43
Reference	96	70.8	186	2.73
Total	287	74.5	590	2.75

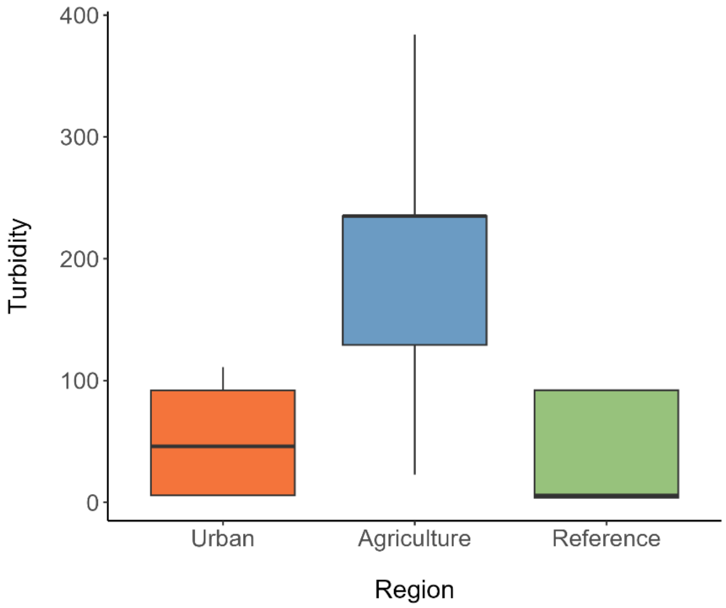

Figure 3. Turbidity levels from the three studied regions. The box represents the interquartile range (25%–75%), the line inside the box indicates the median, the whiskers show the data variation within the interquartile range, points outside the whiskers are outliers.

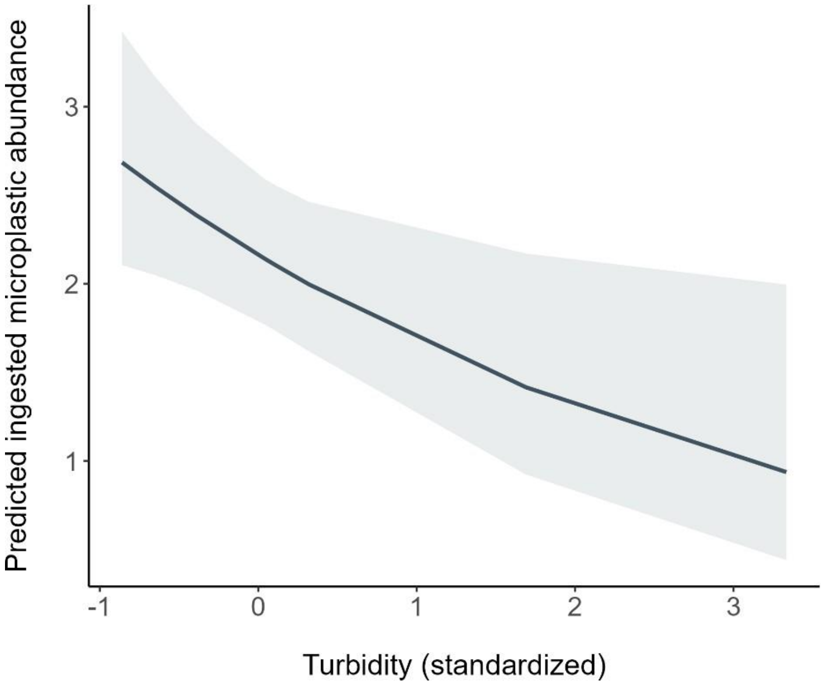

Figure 4. Predicted relationship between standardized turbidity and ingested microplastic abundance. The solid line represents the model prediction, and the shaded area indicates the confidence interval.

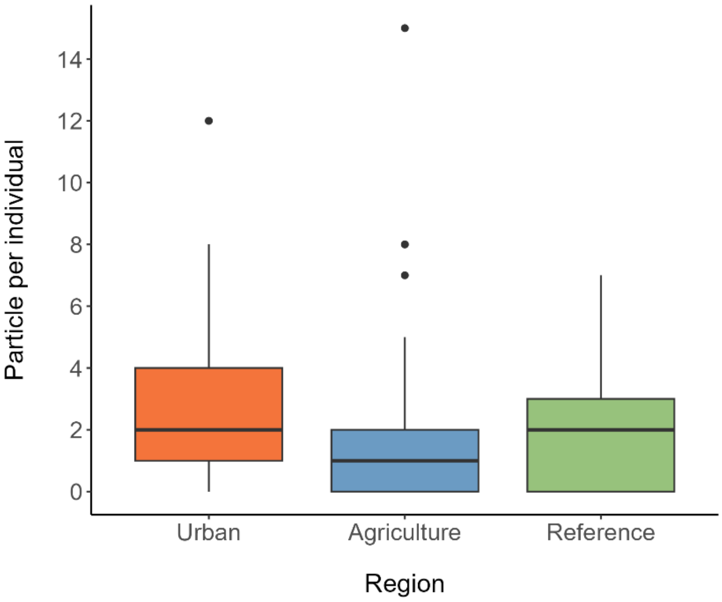

Figure 5. Microplastic contamination in fish from the three studied areas. The box represents the interquartile range (25%–75%), the line inside the box indicates the median, the whiskers show the data variation within the interquartile range, points outside the whiskers are outliers.

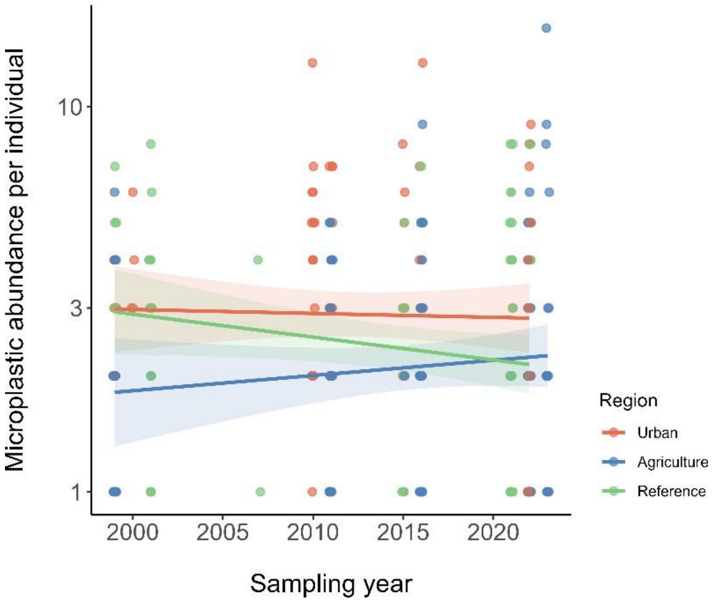

Figure 6. Microplastic contamination in the last 24 years in fish from the three studied areas.

We found particles of the three morphologies (fibers, beads, and fragments) and a variation over the years and among regions ($p < 0.05$). The urban and reference regions exhibited a similar pattern, where fragments were more abundant, while beads and fibers presented similar proportions. Conversely, the agriculture showed a higher proportion of fibers (Figure 7). Over time, the abundance of bead decreased in the urban and reference region, while the other two remained constant. At the agriculture region, fibers increased over the years, with no changes in bead and fragments. In total, nine different colors of particles were found: black, blue, gray, green, orange, pink, red, white, and yellow. Black and blue were the most abundant throughout the studied period and the three regions (Figure 7).

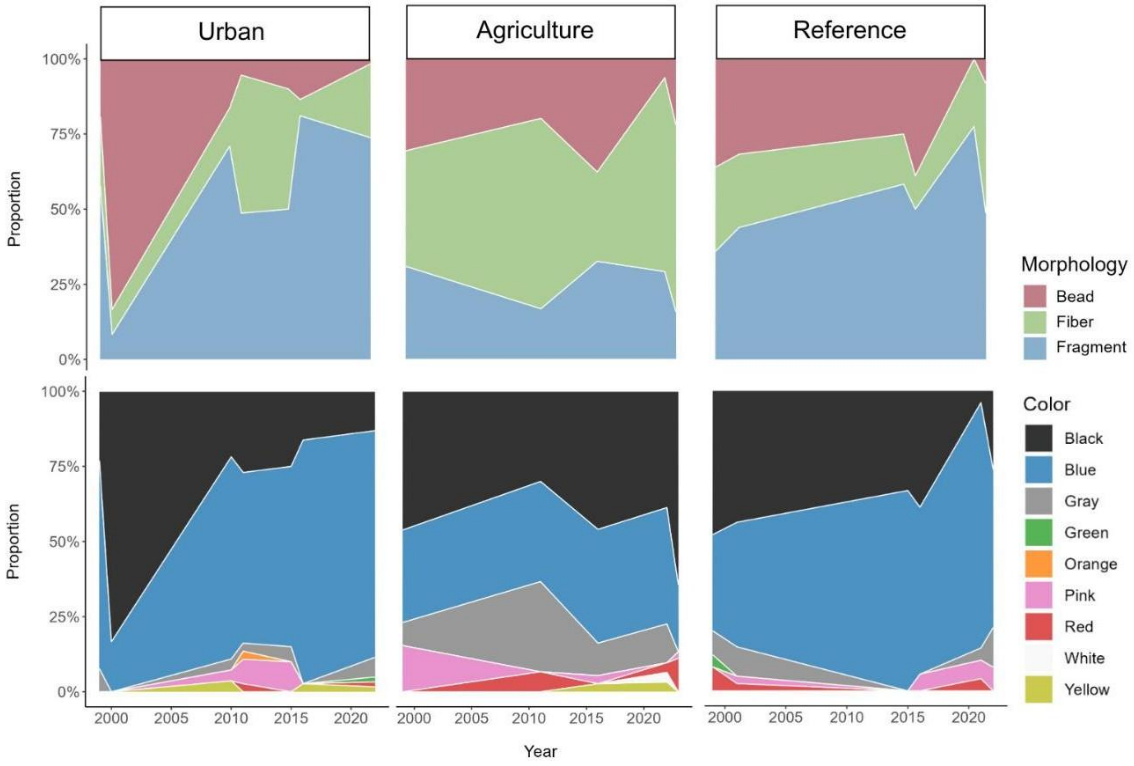

Figure 7. Temporal variation in microplastic morphologies and colors across the studied regions.

Table 2. Generalized Linear Mixed Model (GLMM) results showing the effects of morphology, year, region, and their interactions on microplastic abundance in fish. All models were fitted with a Negative Binomial distribution and site as a random effect (variance = 0.058). Significant p-values are indicated in bold. The adjusted pseudo-R² for the model is 11.7%.

Fixed effect	**p-value**
Morphology	**2.636×10^{-12}**
Year	**0.001**
Region	0.491
Morphology*Year	**0.001**
Morphology*Region	**3.428×10^{-07}**
Year* Region	**0.004**
Morphology*Year* Region	0.394
Turbidity	**0.020**

Discussion

This is the first study to conduct a long-term assessment of microplastic contamination in tropical freshwater fish across an entire basin. Our results showed that microplastic ingestion by tropical fish changed over the last 24 years in the Velhas River basin. However, the temporal trends differed among regions with contrasting land use. This suggests that the environmental context, shaped by different patterns of human use, influences temporal patterns of contamination in fish. In addition to this, our findings highlight that water parameters like turbidity can be a key to understanding microplastic ingestion by visual feeders.

As hypothesized, we found that microplastic ingestion decreased with an increase in water turbidity. The studied species are visual feeders, exhibiting preferences for specific particle colors, thus, higher turbidity may reduce their ability to detect and ingest microplastic particles (Roch, Friedrich and Brinker, 2020; Ríos, Tesitore and de Mello, 2022). This indicates that anthropic activities can also affect biota ingestion of microplastics indirectly, by changing water characteristics that may affect the probability of an individual finding particles. Water quality parameters, such as turbidity, are determined by natural geological drivers but are also affected by land use. In urbanized areas, the occupation of river margins, urban runoff, and the discharge of effluents can alter turbidity. Similarly, farming and livestock activities may accelerate soil erosion and runoff, leading to a higher input of sediments into waterbodies (Nelson and Booth, 2002; Allan, 2004). Therefore, although intensified human influence is expected to increase microplastic contamination in the environment, the associated increase in water turbidity may mask this effect when contamination is assessed through fish ingestion, especially in visual feeders.

The differences in microplastic contamination among the three regions reflect the contrasting influence of anthropogenic pressures. As we predicted, the urban area showed the highest contamination. This region concentrates several cities and industrial activity established along the Velhas River channel, which can result in river contamination from both treated and untreated industrial and domestic effluents. Fragmentation of plastic debris directly dumped into the river, together with land and road runoff may also represent an important source of contamination (Luo *et al.*, 2021; Zhang *et al.*, 2021). The agriculture region captures the cumulative influence of the entire basin, receiving transported particles from upstream while also being subject to local input of microplastic from agriculture. This was the region with the lower contamination but turbidity levels up to four times higher than those of the other regions. Therefore, this result should be interpreted with caution, since increased turbidity may reduce microplastic detectability for these species. Moreover, this portion of the basin includes a floodplain with several lagoons, that act as sinks for microplastics, thereby reducing the number of particles in the main channel (Moreira *et al.*, 2025, manuscript 1). The reference region, in turn, showed intermediate contamination**.** Although it is a preserved area, a portion of its catchment is occupied by agriculture, which is a source of microplastics (Lwanga *et al.*, 2022). Moreover, this river exhibits the clearest waters among the three regions and fish may have a higher probability of detecting and ingesting the particles.

Fish ingestion remained constant in the urban region despite the intensification of urbanization and the growing production and disposal of plastics in recent decades (Browning, Beymer-Farris and Seay, 2021), however, this increase was not reflected in the fish contamination. The transport of particles along the Velhas River, supported by the rise in agriculture in the region’s contamination over time, together with a likely increase in turbidity in this region, could explain this pattern. Although we did not test whether there were changes in turbidity over time, the expansion of urban activities and even mining in the upstream portion of the basin (Figure 2) probably led to higher turbidity in the urban region, restricting fish from detecting the particles. Consistent with this scenario, microplastic contamination in the agriculture region increased over the past 24 years, likely as a consequence of the growing particle load transported along the basin, particularly those originating from the urban region. Tributaries transporting both runoff from surrounding areas and wastewater treatment plant (WWTP) effluents from the whole basin may also intensify contamination in the agriculture region. In the reference region, fish showed a decrease in microplastic ingestion, reaching the lowest levels in the basin by 2020, highlighting the importance of protected areas also in aspects related to emerging pollutants. The reference river, the Cipó River, has restrictions on human activities along its channel and margins since 2004, and its upstream portion has been protected by a national park since 1984 (Estado de Minas Gerais, 2004). These combined actions may help maintain low contamination levels by preserving riparian vegetation, reducing land disturbance, and limiting pollutant inputs at the river source.

The regions also differed in the relative contribution of microplastic morphologies and in how these proportions changed over time, reflecting differences in sources and the transport along the basin. The higher proportion of fragments in the urban region indicates that, despite the multiple sources of all plastic morphologies, mismanaged plastic waste represents a key contributor of contamination. This is likely intensified by the close proximity of urban centers to the river channel. Field observations from 2011 revealed a high presence of plastic fragments in this region, with one sampling net completely filled with debris (Figure S1, C. B. M. Alves, personal communication). In addition, part of these fragments may originate from the degradation of tire-wear particles, which are transported to the river through road runoff (Luo *et al.*, 2021). In the reference region, the predominance of fragments likely reflects the gradual degradation of plastic items that entered the system before the protection of the river and its source area and also the inputs from agriculture (Lwanga *et al.*, 2022; Pal *et al.*, 2024). Additionally, although the presence of a national park ensures protection for the area, recreational activities associated with tourism in the park, may still contribute to the introduction of plastic waste, which can be transported downstream (Pásková *et al.*, 2024). In contrast, the high proportion of fibers in the agriculture region, is probably a reflection of the transport of these particles, especially due to WWTP effluents along the main channel and tributaries. Although the efficiency of WWPTs in removing microplastics varies widely, the high volume of effluents discharged into the environment still makes them a key source of this contaminant (Ziajahromi, Neale and Leusch, 2016; Acharya *et al.*, 2021). Combined to that, the low density of fibers, allows a long-distance transport of these particles (Acharya *et al.*, 2021).

The agriculture region also presented a distinct trend in the temporal changes of microplastic morphology. The increase in fibers over time in the agriculture region supports the hypothesis that inputs associated with WWTP effluents have become increasingly relevant with population growth in the basin. This pattern may also be reinforced by an increase in per capita textile consumption over time (Acharya *et al.*, 2021). In contrast, there was a decrease in beads in the urban and reference regions. Microplastic beads are commonly associated with WWTP effluents (Ziajahromi, Neale and Leusch, 2016), and changes in population dynamics, consumption patterns, or wastewater management practices may influence their availability over time. Additionally, in recent years, beads have been banned from products in several countries (Thompson *et al.*, 2024). Although until now there is no specific regulation addressing their use in Brazil, changes in product formulations adopted globally may also be reflected in other countries.

The similarity in ingestion of particle colors among the three regions, as well as the consistency of this pattern across the studied years, suggests that contamination in the studied species may be driven both by selectivity and environmental availability. Although color preference can be species-specific (Horie *et al.*, 2024), blue and black microplastics are commonly reported as the most ingested colors by tropical freshwater fish (Andrade *et al.*, 2019; Miranda *et al.*, 2025). For *Psalidodon eigenmanniorum*, a congeneric species from the Uruguay River basin, experimental studies have shown black to be among the preferred colors (Ríos, Tesitore and de Mello, 2022).

Long-term assessments of microplastic contamination remain scarce. Existing research is restricted to marine environments from temperate regions. Despite covering several decades (up to 40 years), most of these studies did not test for long-term temporal trends or did not detect consistent changes in microplastic contamination over time (Goldstein, Rosenberg and Cheng, 2012; Beer *et al.*, 2018; Courtene-Jones *et al.*, 2019; Stefanelli-Silva *et al.*, 2024), with only one study suggesting a temporal increase (Claessens *et al.*, 2011). Our study provides the first long-term assessment of microplastic contamination in tropical freshwater fish. Our findings show that temporal changes in microplastic ingestion over the last 24 years were not homogeneous within the same river basin. Beyond contamination levels, we demonstrated that water parameters play a key role in shaping microplastic ingestion patterns for visual feeders. To our knowledge, this study provides the first evidence linking water turbidity to microplastic ingestion in freshwater fish. These results indicate that, although plastic production and disposal have increased over the last decades, responses in biota are not straightforward and depend on the environmental context. Future studies incorporating local assessments to identify point sources may allow a more integrated understanding of the factors influencing microplastic ingestion by fish across different contexts. We also reinforce how valuable biological archives and collections are for the development of new knowledge and encourage others with access to these materials to use them to advance the understanding of microplastic contamination in freshwater environments and its historical patterns.

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Supplementary Material

Twenty-four years of microplastic contamination in fish in a tropical river basin

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Table S1. Sampling sites, regions, and years of fish collection in the Velhas River Basin. Sampling points labeled RV are located in the Velhas River, and those labeled CP are located in the Cipó River.

Sampling site	Region	Sampled Year
RV02	Urban	2000, 2010, 2011, 2015, 2016, 2022
RV03	Urban	1999, 2022
RV04	Urban	1999, 2011, 2022
RV06	Agriculture	1999, 2011, 2016, 2023
RV07	Agriculture	1999, 2011, 2015, 2016, 2022, 2023
CP01	Reference	2001, 2015, 2016, 2021,2022
CP02	Reference	1999, 2001, 2007, 2021, 2022

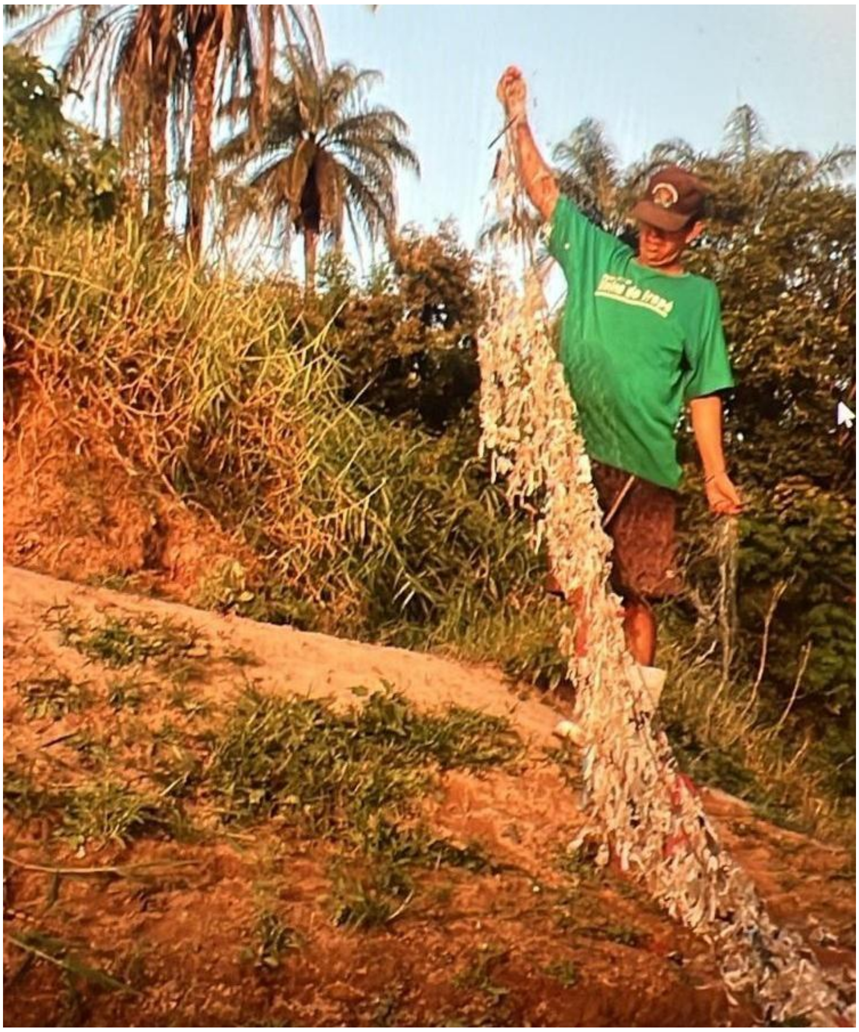
Table S2. Variable and parameters for the first Generalized Linear Mixed Model. Microplastic abundance is the depend variable and site was used as random effect.

Fixed effect	Estimate	Std. Error	z value	p-value
(Intercept)	0.77198	0.09649	8.001	1.23×10^{-15}
Turbidity	-0.25108	0.10802	-2.324	0.0201

Table S3. Variables and parameters for the second Generalized Linear Mixed Model. Microplastic abundance is the depend variable and site was used as random effect.

Fixed effect	**Estimate**	**Std. Error**	**z value**	**p-value**
(Intercept)	-1.4664	0.3037	-4.828	< 0.001
Morphology (fiber)	0.9043	0.3101	2.917	0.0035
Morphology (fragment)	1.9407	0.2904	6.683	< 0.001
Year (scaled)	-0.7305	0.2345	-3.116	0.0018
Region (DOWN)	0.4744	0.4038	1.175	0.240
Region (REF)	0.1950	0.4211	0.463	0.643
Morphology (fiber) × Year	0.8326	0.2994	2.781	0.0054
Morphology (fragment) × Year	0.9760	0.2760	3.537	< 0.001
Morphology (fiber) × Region (DOWN)	-0.1524	0.4075	-0.374	0.708
Morphology (fragment) × Region (DOWN)	-1.9344	0.4103	-4.714	< 0.001
Morphology (fiber) × Region (REF)	-0.3498	0.4292	-0.815	0.415
Morphology (fragment) × Region (REF)	-0.6945	0.4020	-1.728	0.084
Year × Region (DOWN)	0.8220	0.3290	2.499	0.012
Year × Region (REF)	-0.1616	0.3087	-0.524	0.601
Morphology (fiber) × Year × Region (DOWN)	-0.4448	0.4208	-1.057	0.291
Morphology (fragment) × Year × Region (DOWN)	-0.8291	0.4260	-1.946	0.052
Morphology (fiber) × Year × Region (REF)	-0.1278	0.3875	-0.330	0.742
Morphology (fragment) × Year × Region (REF)	-0.1791	0.3597	-0.498	0.619

Figure S1. Field observation from 2011 showing a high accumulation of plastic fragments in a sampling net at the RV04 site in the Velhas River.



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3 CONCLUSION

Despite the rapid advances in the understanding of microplastic contamination in tropical freshwater systems, important gaps remain, with several ecosystems, organism groups, and regions still poorly studied. This thesis contributes to filling part of this gap by showing that lentic environments, although little explored in the literature, are key to the contamination dynamics in freshwater ecosystems. Lagoons accumulate microplastic particles, exposing organisms to higher contamination levels. This work also brings, for the first time, long-term data on contamination in freshwater, showing that temporal variation in microplastic ingestion by fish is not as straightforward, differing according to the anthropogenic context of each region of the basin. Moreover, even among species with similar ecological traits, particle ingestion can be distinct. This finding highlights the need for caution when using one or a few species as proxies for environmental contamination. Finally, this work reinforces the value of long-term ecological monitoring and the maintenance of biological archives, such as collections and stored biological material. These data can become especially valuable for future discoveries, including topics that were not recognized at the time of sampling but have since emerged as major environmental concerns.

The findings of this thesis provide a starting point for discussions on prevention and mitigation of microplastic contamination in Velhas River basin. A broader understanding of the problem in the basin is still needed and should include the investigation of different environmental matrices, such as water and sediments, as well as migratory fish species that are frequently consumed by the local population. It is also important to evaluate the efficiency of wastewater treatment plants in retaining microplastic particles and to discuss the feasibility of implementing technologies capable of reducing the emissions into rivers. The identification of direct sources of contamination, including areas used for inadequate waste disposal, should also be prioritized. Although many questions remain, some actions can already be implemented to improve monitoring and reduce emissions. Existing hydrological monitoring stations along the basin, managed by the Instituto Mineiro de Gestão das Águas (IGAM), which continuously measure several water parameters, could incorporate microplastic quantification as an additional variable, enabling long-term and spatially distributed monitoring and supporting the identification of priority areas for intervention. Additionally, measures such as restricting the use of beads in products, as well as regulating products composed of microplastics, such as glitter, could contribute to reducing primary microplastic inputs. Finally, public awareness is essential, as increasing societal understanding of the environmental and potential human health implications of microplastics can strengthen support for policies aimed at regulating plastic and microplastic production, use, disposal, and management.

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