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MICROPLASTIC ASSESSMENT IN LIVER, KIDNEY, BRAIN, AND MUSCLE TISSUE OF THE FISH SPECIES *CYPHOCHARAX VOGA*

AVALIAÇÃO DE MICROPLÁSTICOS EM FÍGADO, RIM, CÉREBRO E TECIDO MUSCULAR DA ESPÉCIE DE PEIXE *CYPHOCHARAX VOGA*

EVALUACIÓN DE MICROPLÁSTICOS EN HÍGADO, RIÑÓN, CEREBRO Y MÚSCULO DEL PEZ *CYPHOCHARAX VOGA*

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ABSTRACT
Microplastic (MP) pollution represents an emerging threat to aquatic ecosystem health. This study investigated MP concentrations in liver, brain, kidney, and muscle tissue of *Cyphocharax voga* individuals collected from the Sinos River, a water resource located in an urbanized region of southern Brazil. Using Nile Red staining and fluorescence microscopy, MP concentrations per gram were determined and MPs were identified by form and size. The highest mean MP concentration per gram was observed in brain tissue (192 ± 124 MP/g), followed by liver (65 ± 37 MP/g), kidney (47 ± 25 MP/g), and muscle tissue (39 ± 24 MP/g). Analysis revealed significant differences in MP concentration between fish sexes in liver and brain tissues. A statistically significant correlation was observed between kidney and liver (r = -0.520, p = 0.019), as well as between kidney and brain (r = -0.424, p = 0.039). Regarding MP morphology, fragments were the predominant form across all organs and muscle tissue, significantly different from all other forms (foams/films, spheres, and fibers). These results demonstrate MP presence in all sampled tissues and highlight the brain as a sensitive organ for biomonitoring purposes. This work provides critical insights into MP occurrence in freshwater fish that may inform future studies on the hazards these pollutants pose to aquatic environments, as they may serve as models for other vertebrates and potentially humans.

Keywords: Microplastics. *Cyphocharax voga*. Nile Red. Bioaccumulatio. Freshwater Contamination.

RESUMO
A poluição por microplásticos (MPs) representa uma ameaça emergente à saúde dos ecossistemas aquáticos. Este estudo investigou as concentrações de MPs nos tecidos do fígado, cérebro, rim e músculo de indivíduos da espécie *Cyphocharax voga* coletados no Rio

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dos Sinos, um recurso hídrico localizado em uma região urbanizada do sul do Brasil. Utilizando coloração com Nile Red e microscopia de fluorescência, foram determinadas as concentrações de MPs por grama de tecido, e as partículas foram identificadas quanto à forma e ao tamanho. A maior concentração média de MPs por grama foi observada no tecido cerebral (192 ± 124 MP/g), seguida pelo fígado (65 ± 37 MP/g), rim (47 ± 25 MP/g) e tecido muscular (39 ± 24 MP/g). A análise revelou diferenças significativas na concentração de MPs entre os sexos dos peixes nos tecidos hepático e cerebral. Também foi observada correlação estatisticamente significativa entre rim e fígado (r = -0,520; p = 0,019), bem como entre rim e cérebro (r = -0,424; p = 0,039). Em relação à morfologia dos MPs, os fragmentos foram a forma predominante em todos os órgãos e no tecido muscular, diferindo significativamente das demais formas (espumas/filmes, esferas e fibras). Esses resultados demonstram a presença de MPs em todos os tecidos analisados e destacam o cérebro como um órgão sensível para fins de biomonitoramento. Este trabalho fornece importantes informações sobre a ocorrência de MPs em peixes de água doce, podendo subsidiar estudos futuros sobre os riscos que esses poluentes representam para os ambientes aquáticos, uma vez que esses organismos podem servir como modelos para outros vertebrados e potencialmente para os seres humanos.

Palavras-chave: Microplásticos. *Cyphocharax voga*. Nile Red. Bioacumulação. Contaminação em Água Doce.

RESUMEN
La contaminación por microplásticos (MPs) representa una amenaza emergente para la salud de los ecosistemas acuáticos. Este estudio investigó las concentraciones de MPs en los tejidos de hígado, cerebro, riñón y músculo de individuos de la especie *Cyphocharax voga* recolectados en el Río dos Sinos, un recurso hídrico ubicado en una región urbanizada del sur de Brasil. Utilizando la tinción con Nile Red y microscopía de fluorescencia, se determinaron las concentraciones de MPs por gramo de tejido y se identificaron según su forma y tamaño. La mayor concentración media de MPs por gramo se observó en el tejido cerebral (192 ± 124 MP/g), seguida por el hígado (65 ± 37 MP/g), el riñón (47 ± 25 MP/g) y el tejido muscular (39 ± 24 MP/g). El análisis reveló diferencias significativas en la concentración de MPs entre los sexos de los peces en los tejidos hepático y cerebral. También se observó una correlación estadísticamente significativa entre riñón e hígado (r = -0,520; p = 0,019), así como entre riñón y cerebro (r = -0,424; p = 0,039). En cuanto a la morfología de los MPs, los fragmentos fueron la forma predominante en todos los órganos y en el tejido muscular, presentando diferencias significativas en comparación con las demás formas (espumas/films, esferas y fibras). Estos resultados demuestran la presencia de MPs en todos los tejidos analizados y destacan el cerebro como un órgano sensible para estudios de biomonitoreo. Este trabajo aporta información relevante sobre la ocurrencia de MPs en peces de agua dulce y puede contribuir a futuros estudios sobre los riesgos que estos contaminantes representan para los ambientes acuáticos, ya que estos organismos pueden servir como modelos para otros vertebrados y potencialmente para los seres humanos.

Palabras clave: Microplásticos. *Cyphocharax voga*. Nile Red. Bioacumulación. Contaminación de Agua Dulce.

1 INTRODUCTION

Microplastics (MPs) are small plastic fragments smaller than 5 mm, frequently derived from the fragmentation of larger plastics or industrial products directly released into the environment (LAMBERT, WAGNER, 2018, RIBEIRO *et al.* 2024). They can have primary or secondary origins and are currently considered an emerging and ubiquitous contaminant in ecosystems (MONTAGNER *et al.* 2021).

The first evidence of MP pollution in water dates to the 1970s, when studies such as Carpenter, Smith (1972) reported the presence of small plastic spheres along the New England coast. Since the 1970s, numerous studies have documented the presence of these fragments in ecosystems worldwide, including aquatic ecosystems (CRAWFORD, QUINN, 2017, GAMBOA *et al.* 2025). According to Bauer *et al.* (2022), MP concentrations in rivers are strongly correlated with factors such as population density and urbanization.

In Brazil, the Sinos River, located in Rio Grande do Sul state, exemplifies an aquatic ecosystem impacted by intense anthropogenic activity, receiving large quantities of plastic waste from urban and industrial areas, making it a critical source of MP pollution (SHI *et al.*, 2024, ROSSATTO *et al.*, 2024). Studies conducted in this same water resource by Ferraz *et al.* (2020) and Bauer *et al.* (2022) identified the presence of approximately 330.2 particles per liter of water and MPs in 38% of fish gastrointestinal tracts (GIT) from the river headwaters, respectively.

According to IO-USP (2025), in polluted environments, chemical and toxic substances are absorbed by organisms directly or indirectly, and when accumulated in organs and tissues, can reflect the quantity of elements ingested (IO-USP, 2025). Recent experimental studies demonstrate that MPs can accumulate in organs such as liver, brain, kidney and muscle tissue of fish, causing adverse effects including oxidative stress, cellular damage and inflammation (KELLY *et al.*, 2024).

Beyond their widespread presence in ecosystems, MPs can accumulate in the digestive tract, gills and skin of fish (HUANG *et al.*, 2020). Once absorbed, they can accumulate in tissues and organs, causing metabolic problems and potentiating the absorption of other pollutants, thereby amplifying ecotoxicological risk (AHMADI *et al.*, 2022). This causes damage such as inflammation and oxidative stress, immunological dysfunctions, reproductive damage and behavioral alterations, significantly compromising fish health and aquatic ecosystem sustainability (HUANG *et al.*, 2020, PARKER *et al.*, 2024). Complementarily, studies with nanoparticles, such as Mattsson *et al.* (2014), observed behavioral alterations in fish, including reduced activity, increased feeding time and more rapid activity decline, even with available food. To analyze the impacts of such factors on fish

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health, the condition factor (Kn) is used, as it relates weight to total length of individuals and reflects their nutritional status and well-being. This index is employed in ecological studies, allowing assessment of pollution effects and food availability on populations (ROCHA *et al.*, 2005).

Beyond environmental factors, physiological differences related to reproduction can result in distinct patterns between sexes regarding pollutant accumulation and physiological responses (ADEOGUN *et al.*, 2020, GUILLANTE *et al.*, 2023). Studies addressing this dynamic with heavy metals, such as Bastos *et al.* (2016), El-Ghazaly *et al.* (2017) and Al-Yousuf *et al.* (2000), found higher concentrations in internal organs of female fish compared to males. However, according to Guillante *et al.* (2023), there is no single pattern of xenobiotic concentration between sexes. This variation may depend on species, pollutant and environment, with one possible explanation being that females eliminate contaminants through transfer to oocytes, embryos and, in mammals, to breast milk (BURGER, 2007). Additionally, in fish, these variations may also be associated with sexual dimorphism (BURGER, 2007, POLVERINO *et al*. 2023).

Regarding MPs, studies addressing this analysis remain scarce, but there is evidence of sex influence. Studies by Bermúdez-Guzmán *et al.* (2020) and Sbrana *et al.* (2020), for example, reported differences in mean MP ingestion between fish sexes. Thus, MP accumulation likely occurs differently in liver, brain, kidney and muscle tissue, as already noted in the literature for other xenobiotics (BASTOS *et al.*, 2016, EL-GHAZALY *et al.*, 2017, AL-YOUSUF *et al.* (2000), GUZMAN *et al.*, 2022, SBRANA *et al.*, 2020, HARIKRISHNAM *et al.*, 2023).

Cyphocharax voga (Hensel, 1869) is a native species of the Sinos River, classified as detritivorous and iliophagous, with feeding based on ingesting detritus present on the river bottom, making it particularly susceptible to MP ingestion in polluted environments (HARTS, BARBIERI, 1993, SCHIFINO *et al.*, 1998, CORRÊA, PIEDRAS, 2008, MAHIDEV *et al.* 2024, SULTANA *et al.* 2024). Detritivorous fish like *C. voga* exhibit substrate-associated feeding habits (SILVA *et al.* 2021) and consequently are adapted to consume, besides detritus, periphyton, filamentous algae, cladocerans, sediments and microorganisms (CORRÊA, PIEDRAS, 2008), which potentiates ingestion of MPs deposited on aquatic environment substrates (ISLAM *et al.*, 2024, AHMADI *et al.*, 2022).

The feeding characteristics of *Cyphocharax voga*, combined with the fact that fish, living their entire lives in water, are particularly effective as biological indicators (MASSON *et al.*, 2021), make this species especially susceptible to ingesting pollutants deposited in the benthic environment (SILVA *et al.*, 2021, FERNANDES *et al.*, 2022).

MP particle internalization can occur through two main routes: through dietary ingestion, following the path ingestion → intestine → blood → tissues, or through direct water absorption via gills, following the path water → gills → blood → tissues (KIM *et al.*, 2021). Considering that the liver is identified as the central organ in MP detoxification and metabolism (YIN *et al.*, 2022), we expect to find higher MP concentrations in this organ.

The objectives of this study were: (i) to analyze MPs in liver, kidney, brain and muscle tissue of *C. voga*, (ii) to compare MP concentrations identified in females and males, (iii) to relate MP concentrations in organs and muscle tissue with the Condition Factor of these individuals, (iv) to verify the characteristics of MP forms found in the studied fish species.

2 MATERIALS AND METHODS

2.1 STUDY AREA

The study was conducted in the Sinos River, the main watercourse of the Sinos River Hydrographic Basin (RSBH), located in the eastern region of Rio Grande do Sul State, Brazil, with Serra Geral to the north, where it borders the upper course of the Caí River. The basin covers approximately 3,800 km² and 32 municipalities, representing a prominent industrial region in the country. The river presents three distinct sections according to bed slope: the upper section, with 25 km length between 600 and 60 meters altitude, the middle section, with 125 km between 60 and 5 meters, and the lower section, with 50 km and almost zero slope (COMITESINOS, 2014).

The analyzed fish originated from commercial capture and were acquired from a professional fisherman from Prainha locality, located in the lower section of Sinos River, in Novo Hamburgo municipality, RS (RIO GRANDE DO SUL, 2023), at coordinates 29°44'14.95"S and 51°05'26.39"W. The location selection along the river considered the degree of area urbanization. The sampling period (between April and May) was selected to avoid the reproductive season and potential temporal variation in samples.

2.2 FISH CHARACTERISTICS AND COLLECTION DETAILS

After capture, fish were immediately stored on ice at approximately -20°C to preserve their biological conditions until laboratory arrival. In the laboratory, specimens underwent screening, were separated by sex, and physical determinations of total length, standard length and mass were performed using a 50 cm millimeter aluminum ichthyometer and scale with 0.1 g precision.

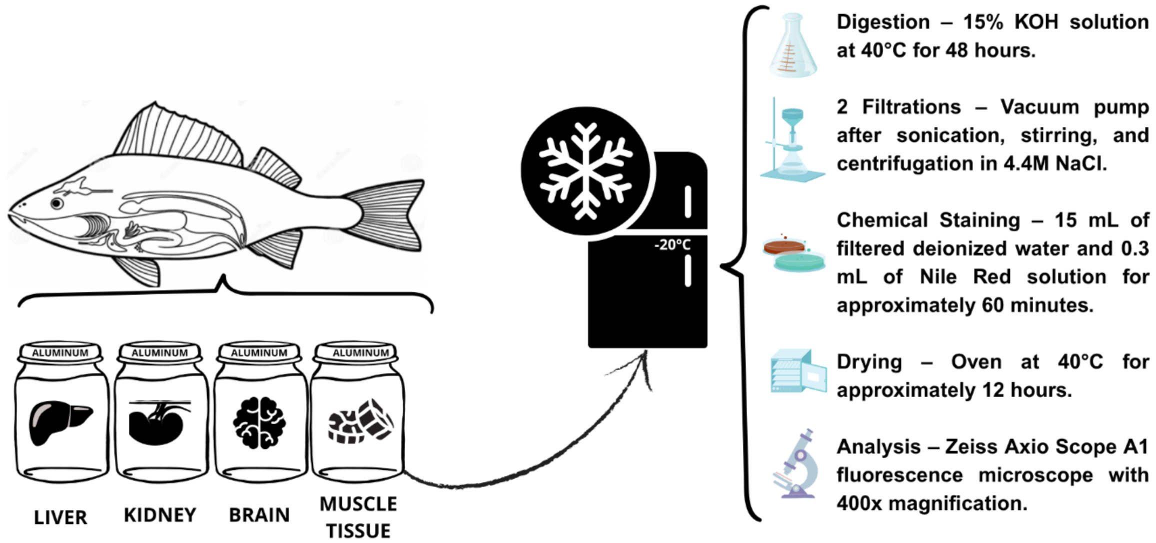
Fish were dissected using surgical scissors and scalpels, through straight incisions from the anal portion to the mouth region, exposing visceral content, which was removed to allow access and kidney extraction. Subsequently, muscle tissue samples were removed from the distal abdominal region, and brains were extracted from the skull after opening with surgical scissors and forceps. To avoid cross-contamination, after removing each organ and muscle tissue, instruments (scissors and forceps) were sanitized with alcohol and rinsed with ultrapure water. Organs and muscle tissue were stored in glass containers and preserved in the freezer at -20°C until processing.

2.3 MICROPLASTIC EXTRACTION AND IDENTIFICATION

Fish organ and muscle tissue samples were thawed and weighed at room temperature. For extraction and characterization of possible MPs, methods previously described by Silveira *et al.* (2024) were used, based on references from Karami *et al.* (2017) and Ferraz *et al.* (2020). Figure 1 presents the methodology applied in the study, outlining key steps adopted for data collection and subsequent analysis.

Figure 1

Infographic of the methodology applied in the study



The Nile Red (NR) dye (9-diethylamino-5H-benzo(α)phenoxazinone-5-one) has proven efficient in microplastic analysis due to its affinity for various polymer types, rapid incubation time, high adsorption capacity, fluorescent intensity and low cost (Guzman *et al.*, 2022). Its application was described by Elsey *et al.* (2007) and Maes *et al.* (2017) and has been widely used in aquatic environment studies (Ferraz *et al.*, 2020, Salla *et al.*, 2024).

To identify, quantify and measure possible MPs in samples, filters were examined with a Zeiss Axio Scope A1 fluorescence microscope, using Carl Zeiss Axio Vision Rel. 4.8 software. A 40x objective and 10x ocular were used. Analysis occurred under Rhodamine light, with excitation wavelength between 575-640 nm and emission of 590-620 nm, as indicated by Elsey *et al.* (2007) and Salla *et al.* (2024).

MPs found were classified as particles, films, spheres and fibers (HENDRICKSON *et al.*, 2018). MP particles were measured based on their largest dimension, a characteristic parameter for assessing particle size (LI *et al.*, 2021).

2.4 CONTAMINATION PREVENTION

To avoid MP contamination during procedures, contamination control measures were adopted. This included preferential use of laboratory materials such as glassware and utensils made of glass and metal, washing equipment with detergent and alcohol, rinsing twice with ultrapure water and drying in an oven before use. All solutions were prepared with ultrapure water and filtered again after preparation. During procedures, room access was restricted, and 100% cotton lab coats and masks were used, as well as nitrile gloves. Procedural blanks were performed for contamination control. The total number of microplastic particles from each sample was composed of counts of all fragments, spheres, films/pellets and fibers. These counts were corrected by subtracting counts in control filters.

2.5 DATA ANALYSIS

MP concentrations were expressed as plastic particles per gram of organ or tissue (MPs/g). Normality tests (Kolmogorov-Smirnov) were performed in SPSS. Results were not significant ($p = 0.000$) for all samples and evaluated groups (MP/g for all organs and muscle tissue), suggesting data follow a normal distribution.

With normal distribution confirmed, single-factor ANOVA evaluation proceeded, with 95% confidence interval to assess differences in MP concentrations between organs and tissues and sex-specific organs and tissues.

The equation $M=a.C^b$, where M is mass, C is total length, a and b are coefficients determined from observed data, describes how fish mass varies as a function of length. From this equation, the condition factor K_n was calculated for individuals, where K_n values close to 1 indicate fish are in good condition, while divergent values may suggest environmental or health problems in the population. Pearson correlation analysis was conducted to examine potential associations between MP/g concentrations across different tissues and their relationship with the fish condition factor. In this analysis, correlation values close to 1 indicate strong association between variables, while values close to 0 indicate absence or weak correlation.

For analysis of MP particle forms and sizes in different organs and muscle tissue, distribution comparison of forms between tissues was performed. ANOVA test was applied to verify significant differences in form distribution between groups. When significant differences were identified, Tukey post hoc test was used to identify between which groups these differences occurred.

3 RESULTS AND DISCUSSION

3.1 MICROPLASTICS IN LIVER, KIDNEY, BRAIN AND MUSCLE TISSUE OF *C. VOGA*

Figure 2 shows the highest MPs/g accumulation in brain, followed by liver, kidney and muscle tissue, and the percentage of contaminated samples relative to the number of analyzed samples (n=54).

Figure 2

Distribution of MPs/g concentrations in different analyzed tissues

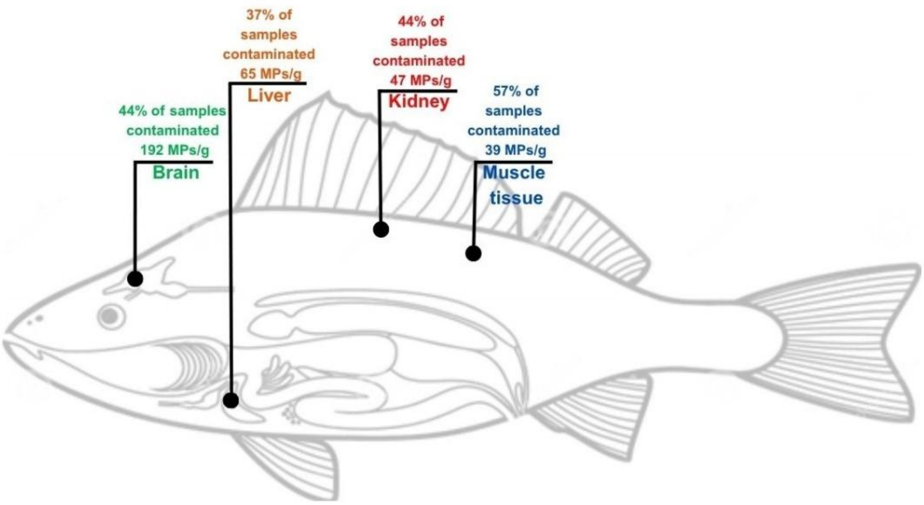
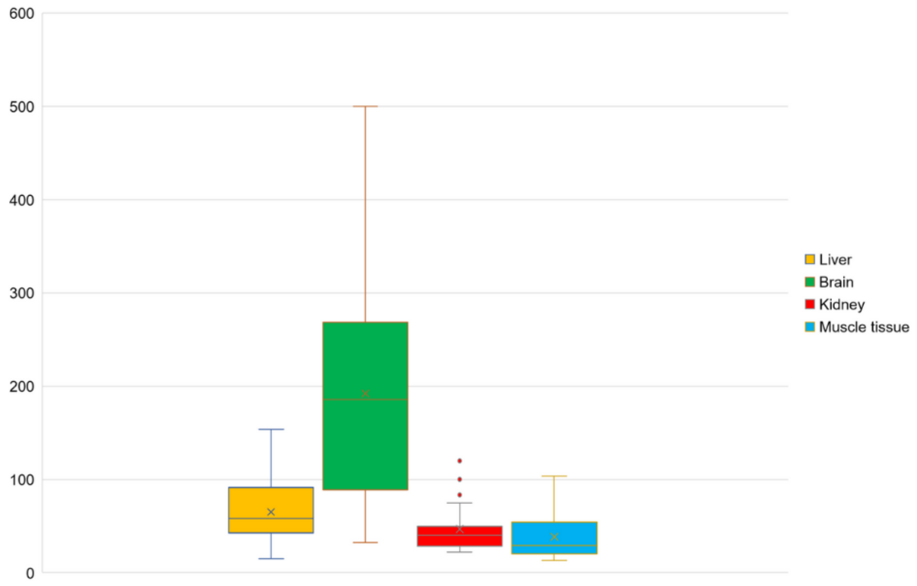


Figure 3 presents the distribution of MPs/g concentration in different analyzed organs and tissues (liver, brain, kidney and muscle tissue), expressed through boxplot graph.

Figure 3

Distribution of MPs/g concentration in different organs and muscle tissue of analyzed fish



The brain was the organ with highest mean MPs/g concentration compared to kidneys, livers and muscle tissue of studied fish, reaching 262 MPs/g in males and 157 MPs/g in females. The MPs/g values found in the present study greatly exceed those observed by Barboza *et al.* (2023) and Blonç *et al.* (2023), who found, respectively, 7 ± 10 MPs/g in *Dicentrarchus labrax* and up to 30 MPs/g in *Oreochromis niloticus*. Although methodological differences may influence these variations, the data clearly reinforce MPs' capacity to reach fish brains and strengthen the hypothesis of blood-brain barrier transposition, as discussed by Atamanalp *et al.* (2021) and Nihart *et al.* (2025).

The liver, in turn, also stood out as a relevant organ for MP accumulation, with females presenting higher means than males (78 and 42 MPs/g, respectively). As the central organ in xenobiotic detoxification and metabolism, the liver is highly vascularized and frequently cited in the literature as the primary target of systemic plastic particle retention (WANG *et al.*, 2022, RAHMAN *et al.*, 2024).

The higher MP concentration observed in brain and liver corroborates findings by Nihart *et al.* (2025), who identified these same organs as sites with tendency for higher MP concentration accumulation, including in studies conducted with human brains.

In kidney, males presented higher mean concentrations than females (55 vs. 38 MPs/g). Although the kidney did not exhibit the highest absolute values, its excretory function and high blood irrigation make it a relevant compartment for systemic MP retention, especially after gastrointestinal translocation. The data corroborate studies by Rahman *et al.* (2024), who identified the kidney as an important accumulation site in fish exposed to plastic pollution.

Muscle tissue, despite presenting the lowest mean concentration (40 MPs/g in females and 58 MPs/g in males), indicated the highest frequency of contaminated individuals (79%). This result suggests MPs may be absorbed through routes alternative to the gastrointestinal tract, such as transcutaneous exposure — a hypothesis supported by evidence on fish epidermis permeability (OLIVEIRA *et al.*, 2022) and studies with nanoplastics in humans (YEE *et al.*, 2021).

The MP distribution among different organs and muscle tissue of C. voga revealed an accumulation route consistent with classic contaminant toxicokinetic models. Using studies such as Olson, 2014 and Pompêo *et al.*, 2022 on xenobiotic absorption and redistribution routes — such as heavy metals, pharmaceuticals and pesticides — described by ecotoxicology, it is possible to trace a plausible path for MPs in fish: entry through ingestion (gastrointestinal tract), absorption through branchial and/or cutaneous epithelia, passage to bloodstream and subsequent distribution to highly vascularized and metabolically active organs such as liver, brain and kidneys.

In our study, the mean MPs/g concentration in different organs (Table 1) followed the descending contamination order brain > liver > kidney > muscle tissue.

Table 1

Contamination ratio compared to muscle tissue (lowest value)

Organ	Mean concentration (MPs/g)	Ratio relative to muscle concentration
Brain	192	5 times higher
Liver	65	1.7 times higher
Kidney	47	1.2 times higher
Muscle tissue	39	— (reference)

The brain, as one of the most lipid-rich organs, heavily depends on transport and distribution of these molecules to maintain homeostasis and neuronal function (RAULIN *et al.*, 2022), which may favor retention of hydrophobic compounds like MPs. This hypothesis is reinforced by results from Dar *et al.* (2025), who, investigating nanoparticle (NP) behavior in *Daphnia* intestine, observed these compounds interact with lipid granules, which may facilitate their retention in lipid-rich tissues. This is because, according to Nihart *et al.* (2025), lipid uptake favors selective transfer to the brain, which may explain its greater susceptibility to accumulation of these contaminants.

Habumugisha *et al.* (2023) and Blonç *et al.* (2023) also analyzed MP accumulation in multiple fish tissues experimentally, comparing between organs. The study by Habumugisha *et al.* (2023), with Danio rerio under laboratory conditions, identified the contamination order liver > muscle tissue > brain. However, exposure time in experimental studies may be a variable influencing translocation in controlled environments. In the work by Blonç *et al.* (2023), with tilapia in a recirculating aquaculture system (RAS), evaluating MP presence, the sequence muscle tissue > brain > liver was found, with brain being one of the organs with highest MP presence, as in the present study with *C. voga*. It should be noted that our evaluation considered MP concentration in different organs and not just MP count. The differences observed between the present study and Habumugisha *et al.* (2023) and Blonç *et al.* (2023) regarding descending contamination order may be attributed to factors such as type and size of polymers used in experimental studies, evaluated species, exposure environment (laboratory, cultivation or nature) and, especially, employed methodologies.

It is important to highlight that gills are highly vascularized organs and act as an efficient entry route for suspended contaminants in water from highly polluted natural environments (Bhagat *et al.*, 2021), unlike controlled environments, which may partially justify the high MP concentration in brain observed in our study. Another possible explanation for our results is based on the fact that micro or nano-scale particles, once in systemic circulation, have potential to reach distant tissues like the brain, transposing selective barriers such as the blood-brain barrier, as demonstrated in studies with fish and mammals (Mattsson *et al.* (2017) and Shan *et al.* (2022). This entry route may be particularly relevant in natural environments, where organisms are continuously exposed to suspended MPs of varied compositions, sizes and origins.

This research suggests that particulate pollutant (MPs and NPs) toxicokinetics follows principles analogous to classic xenobiotics and persistent organic pollutants (POPs), where tissue distribution and retention depend on properties such as size, form, persistence and solubility (OLIVEIRA, BALDAN, 2022), with priority accumulation in nervous and hepatic tissues (POMPÊO *et al.*, 2022). Furthermore, MP detection in muscle tissue, even at lower concentration per gram of tissue, reinforces the hypothesis of direct environmental absorption, possibly via cutaneous route, considering teleosts present highly water- and solute-permeable epidermis (POMPÊO *et al.*, 2022, OLIVEIRA, BALDAN, 2022).

Pearson correlation analysis revealed significant associations between some organs regarding MPs/g concentration. A statistically significant correlation was observed between kidney and liver ($r = -0.520$, $p = 0.019$), as well as between kidney and brain ($r = -0.424$, $p = 0.039$). (Figures 4 e 5). These results indicate that, in analyzed individuals, higher MPs/g concentrations in kidney are associated with lower concentrations in liver and brain. Although mechanisms are not yet fully elucidated, our results, like those of Deng *et al.* (2017), support the hypothesis that reduced particle size is associated with accumulation in internal organs.

Figure 4

Correlation between MP concentrations found in kidney and liver

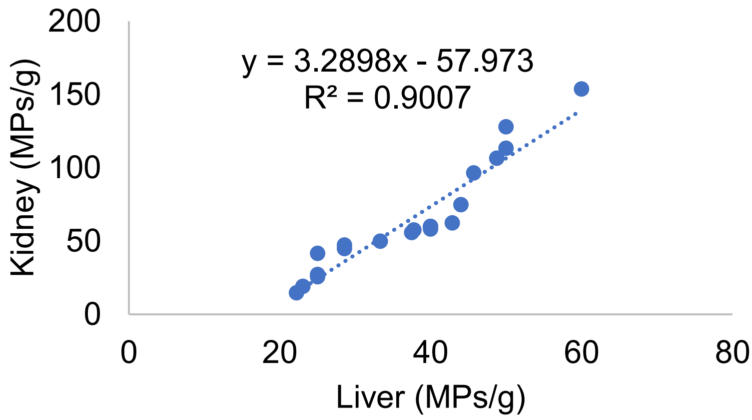
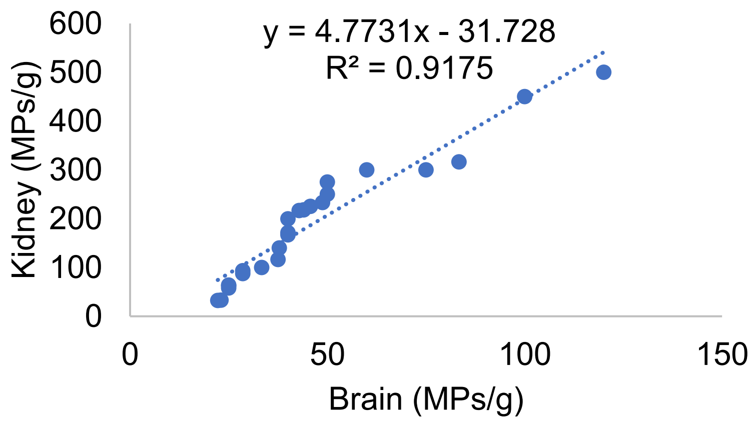


Figure 5

Correlation between MP concentrations found in kidney and brain



3.2 CONCENTRATION OF MPS IDENTIFIED IN FEMALES AND MALES OF *C. VOGA*

Results from evaluation of MPs/g presence and concentration in samples (46% contaminated by MPs) demonstrated that MPs can be absorbed and reach all analyzed organs. Additionally, single-factor ANOVA test demonstrated that MPs/g concentration varies significantly between biological compartments both in general analysis and when individuals were separated by sex (Table 2).

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Table 2

ANOVA results for MPs/g concentration in liver, brain, kidney and muscle tissue of *C. voga*, considering general analysis, females and males

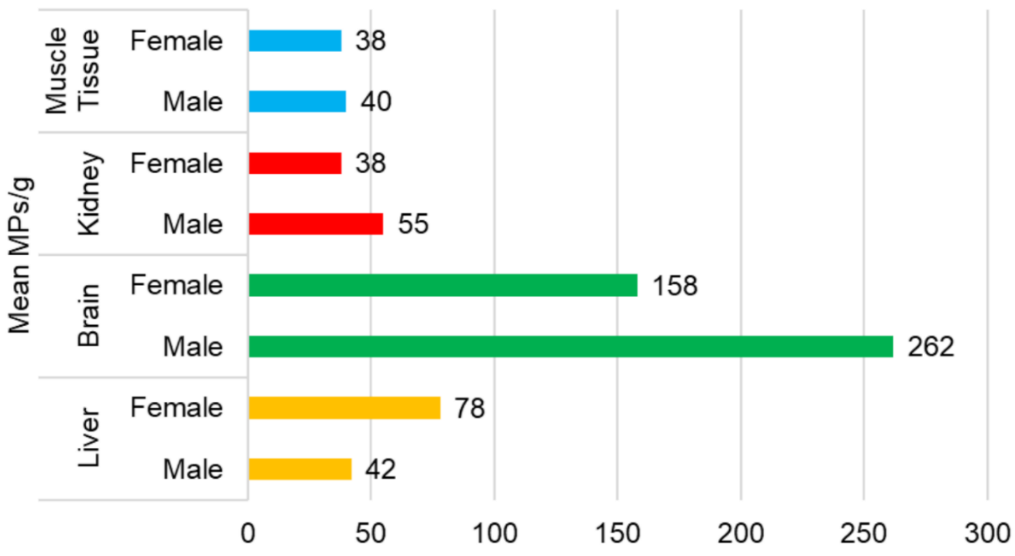
Source of variation	SS	df	MS	F	p-value	Critical F
Evaluation among all 54 fish (MPs/g in each organ and muscle tissue)	384842.29	3	128280.76	29.517188	1.426E-13	2.7004091
Evaluation among all 29 females (MPs/g in each organ and muscle tissue)	145269.44	3	48423.147	15.372754	2.559E-07	2.7791143
Evaluation among all 25 males (MPs/g in each organ and muscle tissue)	305237.54	3	101745.85	21.583177	2.502E-08	2.8517413

Significant differences exist (p=1.42E-13, p=2.559E-07, p=2.502E-08, respectively for general, female and male evaluations) when relating MP concentrations found in studied organs and muscle tissue. Tukey HSD multiple comparison test indicated that brain (both in males and females) is the organ with results significantly different from other organs and muscle tissue. Verification was performed pairwise between MPs/g results in each of the four organs (kidney, brain, liver and muscle tissue), evaluated separately in female and male fish.

Males presented higher concentrations in brain and kidney, while the opposite was verified in liver, with higher results in females. In muscle tissue, mean values were similar between sexes (Figure 6).

Figure 6

Comparison between mean concentrations (MPs/g) found in different organs and muscle tissue of female and male C. voga



To assess statistical significance of these differences, variance analysis (single-factor ANOVA) was performed for each organ in relation to sex. Results indicated that, in liver, there was significant difference between males and females ($p = 0.0345$, $f = 5.23 >$ critical $f = 4.41$), showing that MP accumulation in this organ is associated with sex. The same occurred in brain, where significant difference was verified between males and females ($p = 0.0475$, $f = 4.40 >$ critical $f = 4.30$). In contrast, in kidney ($p = 0.1022$, $f = 2.90 <$ critical $f = 4.30$) and muscle tissue ($p = 0.7919$, $f = 0.07 <$ critical $f = 4.18$), observed differences were not statistically significant.

Literature analysis reveals that sex influence on bioaccumulation and pollutant effects in fish has been addressed mainly for traditional xenobiotics, such as heavy metals and persistent organic compounds, but still in initial form for MPs, an emerging contaminant. Studies such as Gewurtz *et al.* (2011), El-Ghazaly *et al.* (2017), Adeogun *et al.* (2020) and Al-Yousuf *et al.* (2000), identified significant differences between males and females in heavy metal accumulation. However, although Collard *et al.* (2017), Atamanalp *et al.* (2021), Blonç *et al.* (2023) and Rahman *et al.* (2024) analyzed MP presence in brain, liver, gastrointestinal tract, gills and muscle tissue of different fish species, none of these authors performed analyses on MP concentration differences between males and females.

Our results regarding differential accumulation between females and males exposed to MPs compared to traditional xenobiotics align with findings by Adeogun *et al.* (2020) who identified variations in hepatic tissues between males and females, despite absence of differences in muscle tissue, behavior also observed in our analysis. El-Ghazaly *et al.* (2017) and Al-Yousuf *et al.* (2000) reported higher accumulation in females, particularly in liver, a result also verified in our study. On the other hand, Gewurtz *et al.* (2011) observed higher mercury and PCB accumulation in male walleye, which resembles the pattern observed in some organs analyzed in the present study.

When specifically addressing MPs, studies remain scarce and focused mainly on GIT accumulation, but there is evidence of sex influence. Bermúdez-Guzmán *et al.* (2020) reported higher mean MP ingestion by *Opisthonema sp*. females, although without statistical significance, while Sbrana *et al.* (2020) observed significantly higher ingestion by *Boops boops* males, suggesting behavioral aspects may modulate this difference.

In controlled environments, Harikrishnam *et al.* (2023) showed that *Danio albolineatus* males presented more intense physiological responses to MPs, such as greater immunosuppression and oxidative stress, even without direct accumulation evaluation. Thus, data from the present study strengthen evidence that sex is a relevant variable in MP exposure and response dynamics, both in terms of bioaccumulation and physiological effects, reiterating the need for future ecotoxicological studies to systematically consider this variable.

Currently, few studies investigating MP presence in freshwater fish have also analyzed the existence of statistical differences between males and females regarding accumulation of these pollutants in different organs and tissues. Our study is among the pioneers to present, besides mean MPs/g concentrations in liver, kidney, brain and muscle tissue, an analysis of variations between sexes and among analyzed organs.

3.3 RELATIONSHIP BETWEEN MP CONCENTRATION IN ORGANS AND MUSCLE TISSUE OF *C. VOGA* WITH CONDITION FACTOR K_N

Evaluation results between different MP concentrations in organs and muscle tissue and condition factor indicated no significant relationship between them (K_n and Liver: r = –0.086, p = 0.718, K_n and Brain: r = –0.373, p = 0.072, K_n and Kidney: r = –0.152, p = 0.478, K_n and Muscle tissue: r = –0.218, p = 0.246).

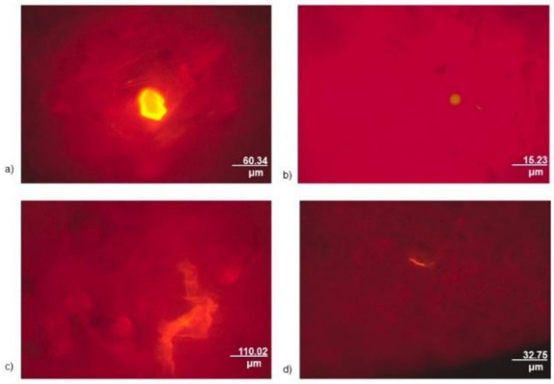
These results align with James *et al.* (2020) and Blankson *et al.* (2022) who previously sought to relate MP presence in liver and GIT of marine fish and Mancuso *et al.* (2022) and Gomez *et al.* (2023) in eyes, liver and GIT of aquatic fish without finding any significant influence of MP concentration on this variable.

3.4 CHARACTERIZATION OF FORM AND SIZE OF MP PARTICLES FOUND IN DIFFERENT ORGANS AND MUSCLE TISSUE OF *C. VOGA*

Four MP forms were found in analyzed samples: fragments, spheres, films/pellets and fibers. Figure 7 presents representative examples of MP morphological forms stained with NR under fluorescence microscopy.

Figure 7

Examples of plastic particles stained with NR under fluorescence: (a) fragment, (b) sphere, (c) film and pellets, (d) fiber



Tukey test was performed separately for each studied organ and tissue, verifying in all situations that Fragment-type particles differed significantly from all others.

Figures 8 to 11 present the distribution of MP forms found in each analyzed sample, morphologically categorized. On average, 72% of MPs found in fish are Fragments, 26% are Spheres, 1.2% are Films/pellets and 1.2% are Fibers.

Figure 8

Distribution of different MP forms found in liver samples (n=20)

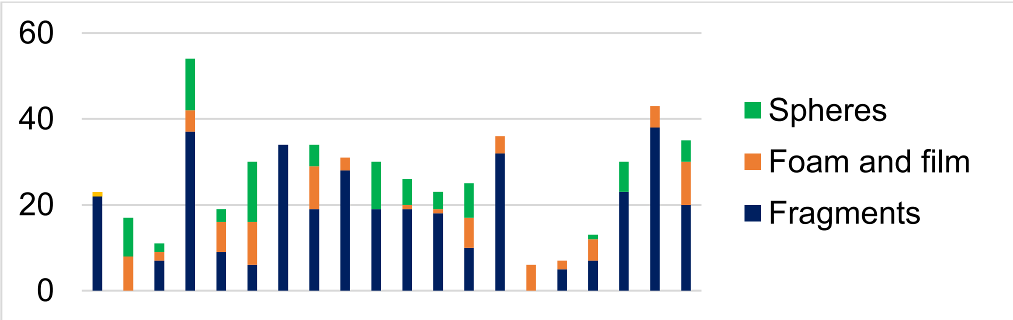


Figure 9

Distribution of different MP forms found in brain samples (n=24)

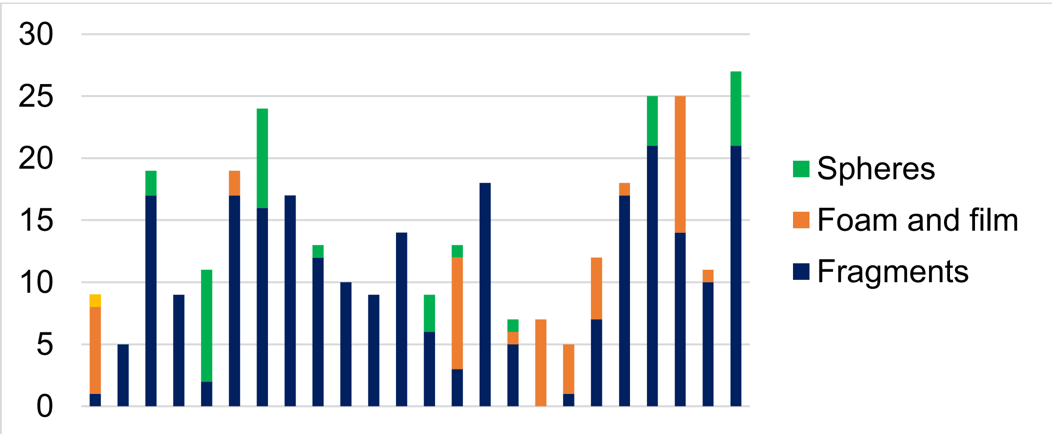


Figure 10

Distribution of different MP forms found in kidney samples (n=24)

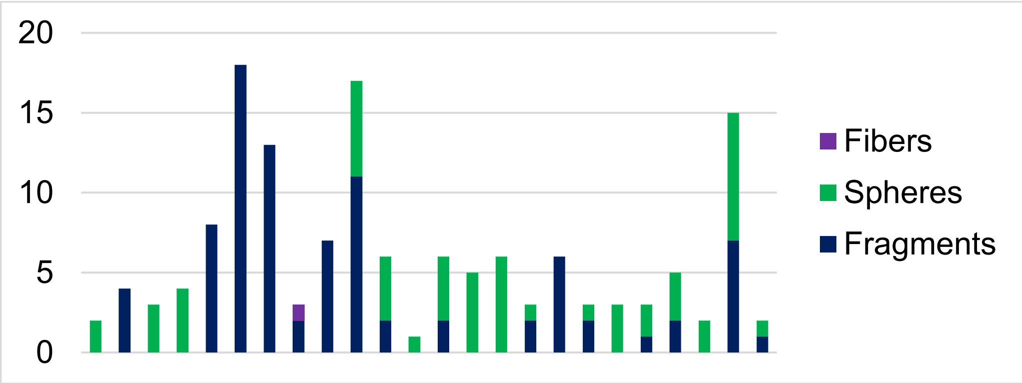


Figure 11

Distribution of different MP forms found in muscle tissue samples (n=31)

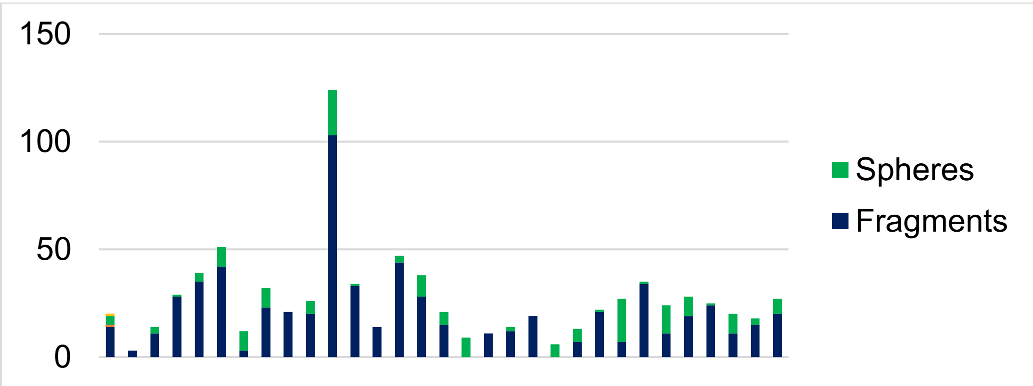


Table 3 presents the summary of each form number identified per analyzed sample, as well as mean sizes, standard deviation and minimum and maximum sizes of each MP particle form found in each compartment of analyzed fish.

Table 3

Distribution of MP forms found in analyzed organs and muscle tissue

Sample	Shape	Nº of particules found	Average size ± Standard deviation (µm)	Minimum size (µm)	Maximum size (µm)
Liver	Fragments	142	52.0±49.0	7.3	372.5
	Sphares	50	12.2±11.2	3.2	71.0
	Foam and films	5	45.5±28.5	22.0	88.0
	Fibres	0	NC	NC	NC
Brain	Fragments	134	38.4 ± 43.0	3.0	237.1
	Sphares	60	14.3 ± 23.0	2.5	162.0
	Foam and films	3	35.4 ± 17.0	16.0	47.0
	Fibres	0	NC	NC	NC
Kidney	Fragments	59	32.0 ± 27.0	3.0	146.1

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	Spheres	38	14.2 ± 11.0	2.3	55.2
	Foam and films	0	NC	NC	NC
	Fibres	2	88.2 ± 114.5	7.2	169.1
Muscle tissue	Fragments	199	47.9 ± 42.0	5.0	279.0
	Spheres	41	17.0 ± 18.1	5.0	105.0
	Foam and films	0	NC	NC	NC
	Fibres	0	NC	NC	NC

NC: MP form was not present in samples

Results corroborate previous studies indicating fragments as the main MP form ingested or internalized by freshwater fish, especially in environments impacted by urban activities and domestic effluents (COLLARD *et al.*, 2017, RAHMAN *et al.*, 2024). Fragment prevalence may be associated with greater secondary plastic fragmentation degree in contaminated water bodies, as well as ease of ingestion of these particles by detritivorous/iliophagous species like *C. voga*.

The presence of microspheres, especially in the kidney, may point to specific sources of contamination, such as effluents from cosmetics and cleaning products (AVIO *et al.*, 2015), emphasizing the kidney's role in filtering smaller particles. The lower occurrence of fibers and films/pellets may be associated with their lower retention rate or greater degradation in fish digestive systems.

This information reinforces the importance of morphological characterization as complement to quantification, providing clues about origin, entry route and possible MP accumulation mechanisms in aquatic organisms.

Regarding size, particles presented overall mean size of 36 ± 39.5μm, compatible with the interval reported in previous studies with marine and freshwater fish (ATAMANALP *et al.*, 2021, RAHMAN *et al.*, 2024, BARBOZA *et al.*, 2020). Generally, fragments (43.6 ± 42.2), films and pellets (52.9 ± 38.1), as well as fibers (88.2± 114.5), due to their irregular form and tendency to secondary fragmentation, present larger mean size than spheres (14± 16.2), which tend to be smaller and more uniform.

Particle size distribution by organs and tissue revealed that kidney concentrated the smallest particles (26.4 ± 27.1 μm), followed by brain (31 ± 39.1 μm), liver (41.4 ± 45.4) and muscle tissue (42.8 ± 40.6μm). This trend is corroborated by Rahman *et al.* (2024), who identified preferential accumulation of smaller MPs in kidneys of market and experimental fish.

The presence of particles in brain tissues, even with mean dimensions below 35 μm, raises concerns about systemic translocation and biological barrier crossing. Previous studies demonstrate that particles <20 μm can cross the blood-brain barrier (BBB)

(ALEXANDER *et al.*, 2016, YEE *et al.*, 2021), potentially interacting with microglial cells and triggering neurotoxicity (ATAMANALP *et al.*, 2021, BARBOZA *et al.*, 2020).

The observed variability (sizes from 2.3 to 372.7μm) also indicates many particles may have undergone fragmentation processes in the environment, increasing their bioavailability (BOUCHER *et al.*, 2019, YEE *et al.*, 2021). Fragment dominance may be associated with environmental wear and secondary ingestion, while sphere presence may indicate industrial or cosmetic sources (RAHMAN *et al.*, 2024).

4 CONCLUSION

The present study represents the first record of MP presence and characterization in liver, brain, kidney and muscle tissue of *Cyphocharax voga* in the Sinos River Hydrographic Basin. The species demonstrated systemic MP bioaccumulation, with emphasis on the brain, which presented the highest mean MPs/g concentration among analyzed organs, followed by liver, kidney and muscle tissue.

High frequency of contaminated individuals was observed, mainly in muscle tissue (79%), which, despite presenting the lowest mean MPs/g concentration, suggests systemic and prolonged species exposure to contaminants. Statistical analysis indicated significant differences in MP concentration between organs, with brain presenting values significantly higher than other biological compartments in both sexes.

Additionally, statistically relevant differences were identified between males and females in MPs/g accumulation in brain and liver, indicating that, as occurs with other xenobiotics, there may be possible sex influence on accumulation patterns, reinforcing the importance of considering this variable in ecotoxicological evaluations.

Significant correlations between MP concentrations in kidney with liver and brain show possible physiological interactions between these organs in particle retention and translocation. Regarding identified forms, fragments predominated (72%), followed by spheres, foams/films and fibers, with kidney concentrating particles of smallest mean size, possibly due to its physiological role in filtration.

Results did not indicate significant relationship between MPs/g concentration in organs and individual K_n condition factor, suggesting that MP physiological effects may not immediately reflect in body metrics such as mass and length, but does not exclude possible long-term cumulative impacts.

Given the exponentially increasing environmental presence of this pollutant, our findings underscore the need to broaden the scope of studies on plastic pollution in freshwater environments. This includes the evaluation of non-traditional organs in ecotoxicological analyses and the investigation of potential alternative pathways for MP entry into organisms, in order to support future actions in environmental monitoring and plastic waste management.

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- **Authors' Contributions** - Daniela da Cunha Silveira, Uwe Horst Schulz and Luciana Paulo Gomes contributed to the study conception and design. The first draft of the manuscript was written by Daniela da Cunha Silveira and Luciana Paulo Gomes and all authors (Daniela da Cunha Silveira, Kelly Correia de Lima, Uwe Horst Schulz, Amanda Gonçalves Kieling, Marcelo Oliveira Caetano and Luciana Paulo Gomes) commented on previous versions of the manuscript. All authors (Daniela da Cunha Silveira, Kelly Correia de Lima, Uwe Horst Schulz, Amanda Gonçalves Kieling, Marcelo Oliveira Caetano and Luciana Paulo Gomes) read and approved the final manuscript."

- **Ethical Approval** - The authors are aware of the rules stated in and confirm that they comply with all ethical issues mentioned.

- **Consent to Participate** - Does not apply.

- **Consent to Publish** - All authors agree to the publication of the work.

- **Competing Interests** - The authors have no relevant financial or non-financial interests to disclose.

- **Data Availability Statement** - The data involved in the research will be made available upon justified request.

In Brazil, research involving animals generally requires approval from an Animal Use Ethics Committee (CEUA). However, the need for approval may depend on the type of study. If the research involves only the analysis of commercially caught fish, without experimental manipulation, there may be exceptions. Brazilian legislation, such as Law No. 11,794/2008 (Arouca Law), regulates the scientific use of animals. Commercially purchased fish do not require approval. The thesis and/or publication must state that the samples were acquired commercially.

This is the paragraph that mentions the purchase of fish: "The analyzed fish originated from commercial capture and were acquired from a professional fisherman from Prainha locality, located in the lower section of Sinos River, in Novo Hamburgo municipality, RS (RIO GRANDE DO SUL, 2023), at coordinates 29°44'14.95"S and 51°05'26.39"W. The location selection along the river considered the degree of area urbanization. The sampling period (between April and May) was selected to avoid the reproductive season and potential temporal variation in samples".

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