v. 3, n. 33, 2023

Journal of
Engineering
Research

FIRST EVIDENCE OF MICROPLASTIC IN THE ROOTS OF *EICHHORNIA CRASSIPES* (MART.) SOLMS (1883) AT THE DELMIRO GOUVEIA PAULO AFONSO RESERVOIR – BA - SUBMEDIO SÃO FRANCISCO

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Abstract: The work aimed to analyze the presence of microplastics (MPs) in Eichhornia crassipes roots and in water samples, in order to understand the dynamics, adherence and influence on tilapiculture. Root and water collections were carried out in Oct and Dec/2021 and February, April, July and October/2022 at the Delmiro Gouveia Reservoir, close to a fish farm in Paulo Afonso – BA. A total of 228 MPs were identified in the water samples including the control point and 211 MPs in the root samples. In the months of April, February, July and October/2022 there was no collection due to the opening of the hydroelectric dam gates in the region. The frequency of occurrence varied with 41.89% items identified in the water samples, 45.88% in the control point water samples and 12.21% in the root samples and presented higher averages in the months of October and December/2021 and February/2022 with a significant difference between the months and points studied. The filament category was predominant in all samples. The work identified for the first time the presence of MP's in the roots of *E. crassipes* in the sub-middle São Francisco and contributed to elucidating the adhesion of suspended solids in them.

Keywords: Environmental impacts; Macrophytes, Plastic; Pollution.

INTRODUCTION

Latin America represents 5% of global plastic production, with Brazil responsible for almost half of this production in 2019 and the fourth largest producer of plastic waste by millions of tons per year (ATLAS DO PLÁSTICO, 2020). Used in various types of materials by human beings in their daily lives, plastic is used in packaging, automotive, construction, textiles, electronics, agriculture, domestic applications, health and safety equipment industries (TERRA LUCIO, et al 2019). Due to characteristics such as high resistance, low production cost and lightness, the advancement of technology has made it possible to explore the various functionalities of the plastic material, revolutionizing the way of life. However, with production growing exponentially, plastic has become a global environmental problem (BERTOLDI, 2022).

Microplastics (MP's) are particles that have a size on the scale < 5 mm in diameter (MASURA et al. 2015), their origin can be primary, produced on a small and large scale for industrial use and for domestic use, and secondary, arising from the fragmentation of macroplastics, through UV radiation, temperature and friction, which can become even smaller and more toxic (TERRA LUCIO, et al 2019).

In this sense, in the last decade, several studies on MP's have been carried out with the aim of providing information about their characteristics, their distribution in environments (continental and marine) and the impacts caused to ecosystems (BHATTACHARYA, 2016). In Brazil, there are more studies on PMs in marine environments than in fresh water.

Several studies have demonstrated the impacts of microplastic on biota, mainly via ingestion (VON MOOS et al., 2012; BROWNE et al., 2013; DE SÁ et al., 2014), therefore it is a potential problem for living organisms, since ingesting plastic can cause intestinal blockage or stomach ulcers, reducing the absorption of nutrients, in addition to causing a false feeling of satiety and hormonal changes that are harmful to the growth and reproduction of animals.

According to Rehse et al, (2016), significant effects of microplastics were found in marine organisms in species from the zooplâncton (*Trigiopus japonicus*), in bivalves (*Mytilus edulis*) and in fish species (*Pomatochistus microps*). Of the few studies carried out in freshwater, effects were found on *Dafnia magna* and fish species: *Clarias gariepinus, Oryzis latipes and Perca fluviatilis* (LAMBERT e WAGNER, 2016).

Based on this principle, the Embrapa Research and Development Bulletin (2020) shows that in Brazil, aquaculture has transformed significantly in recent years, moving from small-scale production to a business sector with high technological intensity. Thus, tilapiculture has been the main driver of this transformation, practiced in new spaces, such as lakes, dams and dams (TENÓRIO et al, 2017). According to Bueno et al. (2011), being one of the fastest growing activities in Brazil, mainly in reservoirs in the intensive system using net tanks and cages.

Furthermore, reservoirs and lakes are extremely important for supplying water for various purposes, including agriculture. However, inadequate use of soil causes the loading of nutrients into the bed of water bodies, in addition to urban and industrial waste, these being the main factors responsible for the existing imbalance, accelerating the exaggerated growth of undesirable aquatic vegetation (MARTIN, D et al, 2009).

Otherwise, aquatic macrophytes constitute one of the main communities of limnic ecosystems as they contribute to biological diversity and have high biomass and high productivity, playing an important role in the nutrient cycle and energy flow (WETZEL, 1993; ESTEVES, 1998). These also act as nutrient stores, influencing the physical-chemical characteristics of water bodies (PAGIORO & THOMAZ, 1999).

Anthropogenic actions that contribute to the high production of aquatic macrophytes have caused economic impacts, mainly on the Bahian bank of the São Francisco River, with the closure of fish farms, decreased tourism, interference with navigability and water supply (SOUZA, ANDREZA. et al, 2019).

The appearance of aquatic plants, popularly called baroneses, in the micro-region of Paulo Afonso (BA) is seen as a consequence of the inadequate dumping of organic materials on the banks of lakes and rivers, and, even more severely, the amount of untreated sewage dumped into the river, which no longer experiences periods of flooding, further problematizing the situation (CAVALCANTI, 2018). Being able to retain on their surface pollutants present in the aquatic environment, such as heavy metals, among others, it is assumed that macrophytes can be potential sinks for PM's (POMPÊO; MOSCHINI-CARLOS, 2003, KALČÍKOVÁ, 2020a).

The present work aimed to analyze the presence of MP's in the roots of Eichhornia crassipes near a fish farm in the Delmiro Gouveia Hydroelectric Reservoir, in the municipality of Paulo Afonso – Bahia, seeking to understand the adsorption dynamics of this species, namely, of MP's to in order to satisfy the growing demand for animal protein through plastic pollution-free tilapiculture.

MATERIAL AND METHODS

The Moxotó hydroelectric reservoir has an approximate surface area of 98 km², with a storage capacity of around 1.2 billion cubic meters of water. No relevant industrial activities are observed in the region, although urban occupations located on the banks of the reservoir are observed, as well as collections for public supply in Jatobá - PE, Glória - BA, Canafistula - PE and Paulo Afonso - BA (AURELIANO, et al 2007).

According to the City Hall Portal, Paulo Afonso has a population of 117,014 inhabitants (IBGE, 2021). Its region is highlighted in the development of fish farming, mainly in the creation of tilapia, which attracts industries for the manufacture of feed for fish farming and other purposes. The location of the Delmiro Gouveia hydroelectric reservoir area, the georeferenced collection point (09°22'15.9" S, 038°12'57.5" W) and the Paulo Afonso Hydroelectric Complex are shown in Figure 1.

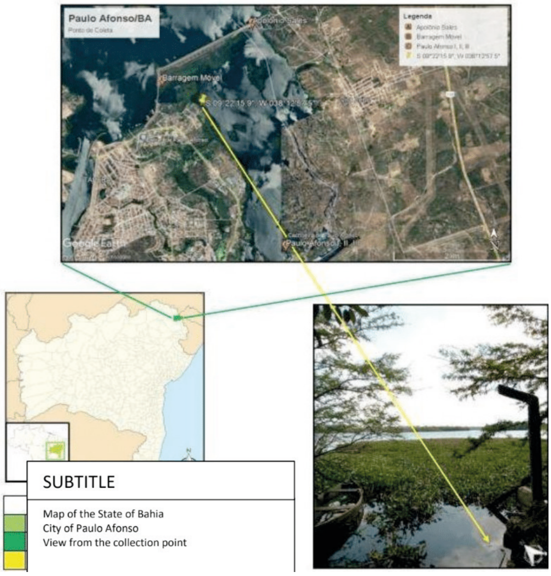


Figure 1: Georeferencing of the collection point for baroness roots and water samples at the Delmiro Gouveia Hydroelectric Reservoir on the São Francisco River, Paulo Afonsina bank; Floodgates of the Paulo Afonso I, II, III, Apolônio Sales Plants and Port of the Mobile Dam.

Source: Google Earth adapted by Alves (2022).

Formed by the damming of the São Francisco River, the Delmiro Gouveia and Moxotó hydroelectric reservoirs supply the Paulo Afonso I, Paulo Afonso II, Paulo Afonso III plants, the pilot plant, located three kilometers upstream of the group of Paulo Afonso plants (Figure 2), supply the Apolônio Sales Hydroelectric Plant, boosting the waters of the lake of the same name. The Paulo Afonso IV reservoir is supplied by a channel excavated from the right bank of the Moxotó Reservoir (LIMA REIS, 2007).

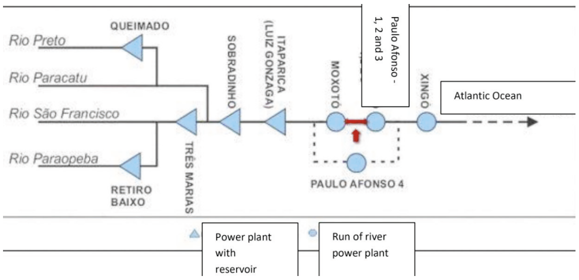


Figure 2: Informative flowchart of the characterization of the study site, focusing on the collection point marked in red.

Source National Water and Supply Agency – (ANA, 2022).

Lakes enriched with effluents of anthropogenic origin are prone to the proliferation of aquatic macrophytes. For example, Eichhornia crassipes has been gaining prominence in increasingly frequent research, as it causes great economic damage, with high production of biovegetable mass, causing greater visual impact on the São Francisco hydroelectric reservoirs (SOUZA, 2019). In recent years, these have been increasingly common in resorts in the municipality of Paulo Afonso/BA, and being floating, the stands invade tourist attractions and affect the local economy (Figure 3).

Figure 3: Impacts caused by Eichhornia crassipes in spas in the municipality of Paulo Afonso – BA.

Collections were carried out during the period of October and December/2021, February, April, July and October/2022 in locations close to a fish farm located in the municipality of Paulo Afonso, Delmiro Gouveia hydroelectric reservoir, sub-middle São Francisco. This reservoir was chosen due to the increasingly frequent occurrence of aquatic macrophytes and its proximity to a fish farm (Figure 4).

Figure 4: Collection site for aquatic macrophytes and water samples in the Delmiro Gouveia hydroelectric reservoir.

Source: Alves (2022).

Aquatic macrophytes were selected and collected for visual uniformity in root size, at three points distant from the waterline at 1.0 m, 3.0 m and 5.0 m. Next, the roots were pruned and weighed in the amount of 1 kg and placed in duly labeled plastic bags. They were then placed in an oven at 100° C for a period of 24 hours for drying and sorting. This methodology was implemented because there is no other work with microplastics in E. Crassipes roots.

Water samples were collected in the amount of 1 liter at points similar to the location of the macrophytes; placed in previously sanitized plastic bottles that were submerged approximately 30 centimeters from the surface with their opening favoring the movement of water. After collection, the bottles, duly identified, were left at room temperature (25°C) for analysis in the laboratory.

In the laboratory, some measures were taken to guarantee the quality of sample processing, including: I. The entire team worked wearing cotton lab coats and nitrile gloves; II. The analyzes were carried out in a room with a maximum of three people; III. The bench and the material used were previously disinfected with 70% alcohol for each sample analyzed; IV. All material analyzed was closed and opened only when used, with the intention of avoiding atmospheric contamination.

Despite the importance of care to ensure no cross-contamination of samples, some studies consider that atmospheric contamination becomes insignificant, observing that the number of PM's found in control samples was lower than those found in real samples (BIRNSTIEL et al, 2019; PAZOS et al, 2020).

Root and water samples were taken to the laboratory for screening. MP's were quantified and categorized according to the morphological types of the material: filaments, soft plastics and hard plastics (LIMA et al., 2014). Regarding classification by color (colored, white, aged and colorless) according to Endo et al., 2005; Sobral et al., 2011.

After sorting, the items visually identified in each type of category were taken for image capture using an optical magnifying glass in order to characterize the categories found in the different samples. Subsequently, the samples were separated in Petri dishes, then they were subjected to the nitric acid (HNO^3) 65% test to remove organic matter and better observe plastic debris, as this test is used effectively in the rapid dissolution of biogenic material, so that if any particle is mistaken for biological material, it will be destroyed.

The digestion of biogenic organic matter is generally carried out through the application of different chemical agents or enzymes, such as: oxidizing agents, acids, bases and specific enzymes (MATTHIAS TAMMINGA, 2021). Subsequently, all material was reviewed under an optical magnifying glass to definitively confirm the non-organic material. Statistical analyzes were carried out using Analysis of Variance (ANOVA) and the means were compared using the Tukey Test at 5% probability, using the Statistical Software for Analysis and Teaching of Statistics (SISVAR 5.7).

RESULTS AND DISCUSSION

The microplastics (MPs) identified using an optical magnifying glass were categorized according to morphological types and colors, respectively (Figure 5).

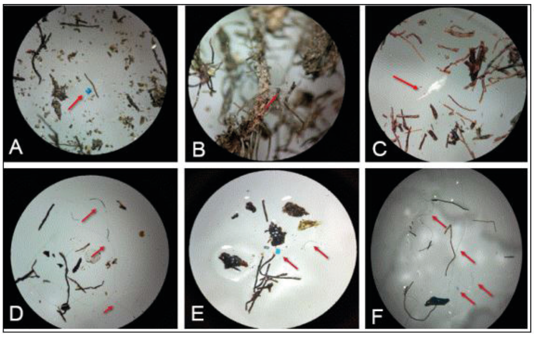


Figure 5: Microplastics categorized by morphological type and colors, respectively: Blue hard plastic (A); Colorless filaments (B); White soft plastic (C); Blue, pink and colorless filaments (D); Soft plastic and blue filaments (E); Blue and colorless filament (F).

Source: Alves (2022).

After acid digestion of organic matter, a total of 228 MPs were found in all water samples (including the control point - PC), 217 of which were for the filament category. Figure 6 shows the morphological types identified in the screening stage using an optical magnifying glass after confirmation with the nitric acid (HNO^3) 65% test.

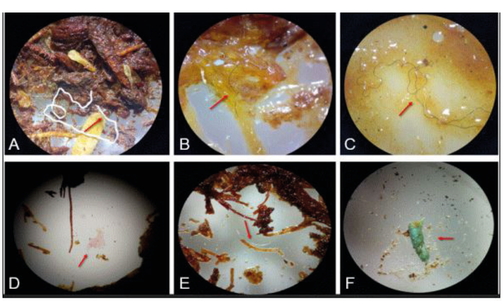


Figure 6: Baroness root samples after nitric acid (NHO^3) 65% test. White hard plastic (A); Black filament (B and C); Pink soft plastic (D); Colorless thread type fiber (E); Blue soft plastic (F).

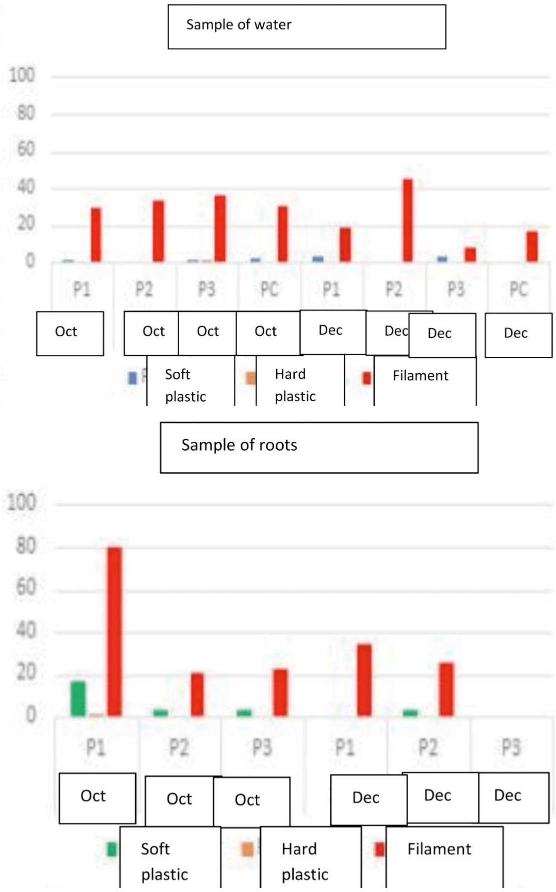
Source: Alves (2022).

In the roots of *E. crassipes*, this category (filament) also stood out, presenting 184 of the 211 identified items in the filament category. The same happened in recent studies in the waters of Lake Paranoá - DF, according to Oliveira Alves (2021), where filaments were mostly identified in blue, green and black.

To assess the presence of MP's, observation through the optical magnifying glass was based on capturing images for greater details focusing on particles that presented characteristics similar to Olivatto (2017).

The distribution of microplastics in water resources is influenced by several factors, the most determining ones being the material composed of polymers, the exposure time of the material and the hydrological dynamics of the environment (COLE, M. et al., 2011). In order to compare the distribution of MPs in water and root samples, the quantitative results at all points in the months of October and December 2021 are shown in graph 1.

The PM particles identified in the plastic (soft and hard) and filament categories showed a different spatial distribution in the water samples, 24 where the filament category exhibited quantitative similarity in the month of October, at all points, including the control point. In the month of December, there was a considerable increase in point 2 and an abrupt drop in point 3. It was observed that the night before the collection carried out in December/2021 it had rained. According to Xia et al. (2020), studies on the influence of rain and climate change demonstrated a significant influence on the concentration of PM's in water bodies.



Graph 1: Quantitative representation of the total distribution of microplastics found in water samples and root samples in the months of October and December/2021.

It is worth mentioning that in the month of October/2021, the reservoir flow was 795.00 m^3/s and in the month of December/2021 820.00 m^3/s and that at point 1 and point 3 there was greater friction of the plants, in function of the current and wind, while at point 2, the macrophytes possibly suffered less friction due to the density between them, presenting a higher quantity of MP's (Figure 7). Santos et.al, 2020 observed that the number of PMs in the water column decreased considerably during the period of invasion and proliferation of baroneses in a study carried out in the Moxotó reservoir - sub-middle São Francisco.

Figure 7: Collection points marked in yellow highlighting the distances between them (1 m, 3 m and 5 m).

Source: Alves (2022).

For the root samples, it was possible to observe that the majority of MPs found consisted of the colorless, blue, pink and black filament fiber category. The same happened in a study carried out with macrophytes from coastal areas, where a total of 97.3% of microfibers were identified in their sampling (ESIUKOVA. et al, 2021). The distribution of MP's found in the month of October showed a quantitative difference for point 1 and in the month of December for point 3, which in turn was not present in the sampling. This fact may have possibly occurred due to the higher flow rate than in October, in addition to the occurrence of rain the night before the collection took place (Table 1). Another factor that may have led to the absence of PMs at point 3 was the friction between the macrophytes in a location with greater volume and current that simulates a "washing" of the plants.

Months of collection	Flow (m³/s)	Temperature of water	Weather conditions
October/2021	795,00	27°C	Cloudy
December/2021	820,00	29°C	Previous rain
February/2022	1.910,00	28°C	Cloudy
April/2022	1.195,00	27°C	Previous rain
July/2022	1.703,00		
October/2022	1.017,00	28°C	Partially cloudy

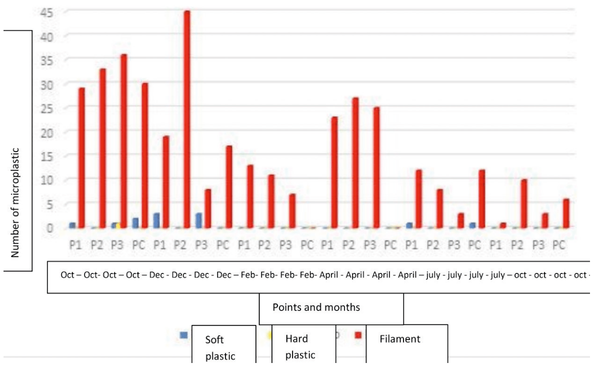
Table 1: Informative data on the characteristics of the location on the day of collections.

Source: Reservoir Monitoring System – SAR, adapted by Alves (2022).

According to the 2019 Aquatic Macrophyte Monitoring Report, the presence of baronesses varies according to the weather, characteristics of different areas and depends on the interaction between the action of winds, the variation in water level in the reservoirs, the increase in flow and the degree of phenological development of the stands, especially of the most abundant species. Therefore, due to the large volume of water resulting from the opening of the floodgates (Jan/2022), it was impossible for the presence of macrophytes that were carried away by the force of the water and for this reason it was not possible to collect baronesses in the Delmiro Gouveia Reservoir in the months of February, April, July and October/2022.

The hydrological dynamics of the studied reservoir fluctuates according to the flow controlled by the São Francisco Hydroelectric Company (CHESF). Due to the increase in the storage volume of the reservoirs at the Sobradinho (BA) and Luiz Gonzaga (PE) plants, resulting from the incidence of rain, CHESF resumed the pouring procedure at Cachoeira de Paulo Afonso in January 2022 after 12 years, to control reservoir levels, lasting through the months of February, March and April of the current year (CHESF PORTAL, 2022).

It was possible to observe that the MPs identified in the water samples in the months of October and December/2021 and in the months of February, April, July and October/2022 showed a quantitative difference (Graph 2). With a flow between 795.00 and 820.00 m³/s, the months of October and December/2021 presented a higher quantity than the others. The characteristics of the MP's found in this research corroborate Mendoza et al, 2018, who observed that fibers are the items of greatest quantitative relevance, arising from the washing of fabric, which can emit approximately 1900 fibers per washed item.



Graph 2: Distribution of MP's found in all water samples in the months of October, December/2021 and February, April, July and October/2022.

The high number of PMs in the initial months of the present study was possibly due to the long period of drought and stability of the reservoir flow. However, the month of April/2022 presented a higher number compared to the other months of the year mentioned, except for the control (Table 1). In the soft plastic and hard plastic categories in water samples, there was a significant difference for the control point (October/2021) and at points 1 and 3 for December/2021. For the filament category, there was a significant difference in relation to point 3 for the month of October/2021 and for point 2 and December/2021 (Table 1).

Months of collection	Water - Category: soft plastic Distances between points			
	Point 1 (1m)	Point 2 (3m)	Point 3 (5m)	Point of control
Oct/21	1bB	0aC	1bB	2aA
Dec/21	3aA	0aB	3aA	0bB

Months of collection	Water - Category: hard plastic Distances between points			
	Point 1 (1m)	Point 2 (3m)	Point 3 (5m)	Point of control
Oct/21	1bB	0aC	1bB	2aA
Dec/21	3aA	0aB	3aA	0bB

Months of collection	Water - Category: filament Distances between points			
	Point 1 (1m)	Point 2 (3m)	Point 3 (5m)	Point of control
Oct/21	29aD	33bB	36aA	30aC
Dec/21	19cB	45aA	8cD	17bC

Table 1: Variance of microplastics found in water samples, at different distances, close to a fish farm in Paulo Afonso/BA, in the months of October and December/2021, between the points represented by a lowercase letter and between the months, represented by a letter uppercase.

**Averages followed by the same lowercase letter in the column and capital letter in the row do not differ from each other using the Tukey Test at 5% probability.

Months of collection	Roots - Category: soft plastic Distances between points		
	Point 1 (1m)	Point 2 (3m)	Point 3 (5m)
Oct/21	17aA	3aB	3aB
Dec/21	2bA	0bB	3aC

Months of collection	Roots - Category: hard plastic Distances between points		
	Point 1 (1m)	Point 2 (3m)	Point 3 (5m)
Oct/21	2aA	0aB	0aB
Dec/21	1bA	0aB	0aB

Months of collection	Roots - Category: filament Distances between points		
	Point 1 (1m)	Point 2 (3m)	Point 3 (5m)
Oct/21	80aA	21bC	23bB
Dec/21	21bC	34aA	26aB

Table 2: Variance of microplastics found in root samples, at different distances, close to a fish farm in Paulo Afonso/BA, in the months of October and December/2021, between the points represented by a lowercase letter and between the months, represented by a letter uppercase.

**Averages followed by the same lowercase letter in the column and capital letter in the row do not differ from each other using the Tukey Test at 5% probability.

In the results of the root samples, it was possible to observe that there was significance for point 1 in the month of October/2021 in the soft plastic and hard plastic categories. For the filament category, there was significance at point 1 in October/2021 and at point 2 in December/2021 (Table 2). In these two moments, rain occurred the night before (the day before collections), which probably corroborated the aforementioned fact, being consistent with studies that present an average difference in the presence of suspended solids during rainy periods, according to Oliveira, 2020.

In January/2022, the floodgates were opened and, consequently, flows were higher than the collection months in 2021 (Table 1) by the company CHESF, due to the large volume caused by rain in Alto São Francisco, according to circular letter SSO – 011/2022 (ANNEX 1). Therefore, in the months of February, April, July and October/2022 there was no root collection (Figure 8). According to Souza, in 2020 the speed and volume of water influence the presence of plants and retention of floating solids such as PET bottles, plastic bags, among others. However, it possibly facilitates the adsorption of filamentous MPs in the roots. In this sense, the data presented in Table 3 only includes PM sampling in water samples.

Journal of Engineering Research ISSN 2764-1317 DOI 10.22533/at.ed.3173332325097

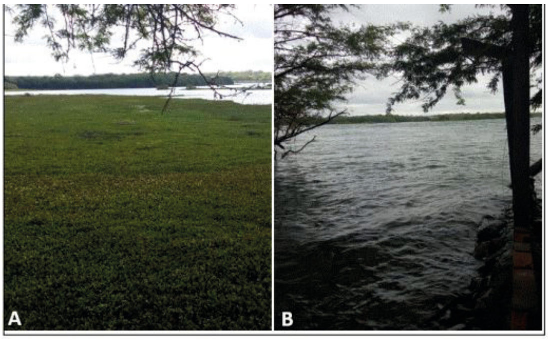


Figure 8: Collection point with low flow before the opening of the gates at the Apolônio Sales Plant (A - Oct and Dec/2021); Collection point with high flow, after opening the floodgates (B – Feb/2022).

The soft plastic category showed a significant difference at point 1 and the control point in July/2022 in relation to the other months. However, there was no significant difference between them. MPs categorized as hard plastic were not identified in water samples in the months of February, April, July and October/2022. The filament category (predominant) showed a significant difference at point 2 in April/2022 and at the control point in July/2022.

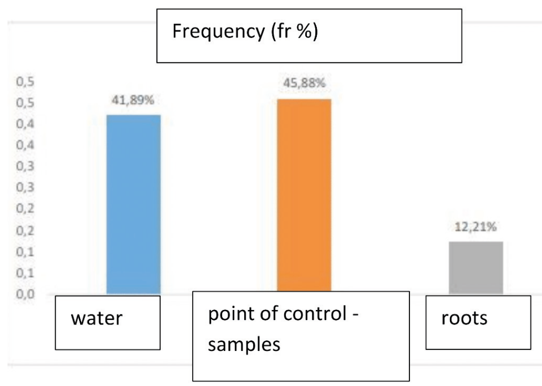
Months of collection	Roots - Category: soft plastic Distances between points			
	Point 1 (1m)	Point 2 (3m)	Point 3 (5m)	Point of control
Feb/2022	0aA	0aA	0aA	0aB
April/2022	0aB	0aA	0aA	0aB
July/2022	1aA	0bA	0bA	1aA
Oct/2002	0aB	0aA	0aA	0aB

Months of collection	Roots - Category: hard plastic Distances between points			
	Point 1 (1m)	Point 2 (3m)	Point 3 (5m)	Point of control
Feb/2022	0aA	0aA	0aA	0aA
April/2022	0aA	0aA	0aA	0aA
July/2022	0aA	0aA	0aA	0aA
Oct/2002	0aA	0aA	0aA	0aA

Months of collection	Roots - Category: filament Distances between points			
	Point 1 (1m)	Point 2 (3m)	Point 3 (5m)	Point of control
Feb/2022	13aB	11bB	8cB	0dC
April/2022	23cA	27aA	25bA	0dC
July/2022	12aC	8bD	3cC	12aA
Oct/2002	1Dd	10aC	3cC	6bB

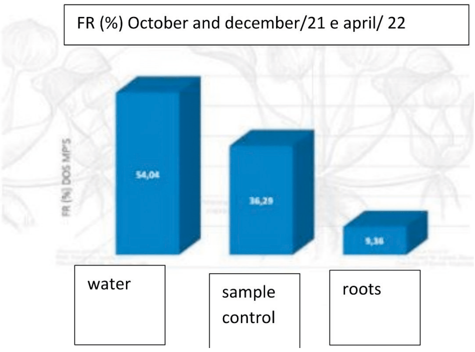
Table 3: Variance of microplastics found in water samples, at different distances, close to a fish farm in Paulo Afonso/BA, in the months of Feb, April, Jul and Oct/ 2022, between the points represented by the lowercase letter and between the points represented by capital letters.

**Averages followed by the same lowercase letter in the column and capital letter in the row do not differ from each other using the Tukey Test at 5% probability.



Graph 3: Relative frequency (Fr%) of microplastics in all samples from the months of October and December/21.

The entry of MPs into the Delmiro Gouveia Reservoir may differ for each point, as the sampled locations have different characteristics such as: depth, water speed and distance from the banks. Approaching fish farms can lead to the release of plastic waste, such as improperly discarded fishing gear. The frequency of PM's in water samples in all months of the present study are described in graph 4, in order to generally visualize their occurrence.



Graph 4: Frequency of occurrence (%) of water and root samples in the months of October and December/2021 and February, April, July and October/2022.

According to Kalčíková (2020a), MPs that enter reservoirs and lakes float on the water surface until they interact with floating plants. Their high interaction capacity can cause adverse ecological effects, since aquatic macrophytes serve as habitat, shelter and food. for several species, implying trophic transfer of MP's in the system.

Regarding contamination by PM's, some intrinsic characteristics of the study site can be highlighted. For example, the location of the area is in a small reservoir close to the region's Power Plant Complex and holds turbine waters from a larger reservoir (Moxotó Reservoir) that is part of the São Francisco River Basin. According to the São Francisco River Basin Committee (CBHSF), only the municipality of ``Lagoa da Prata`` – MG, of the 507 municipalities bathed by the river, has 100% basic sanitation.

CONCLUSIONS

The present work identified for the first time the presence of MP's in the roots of E. crassipes in a reservoir located in the sub-middle São Francisco and contributed to elucidating the adhesion of suspended solids in them.

MP's were present in water samples and roots of the aquatic macrophyte studied, demonstrating anthropogenic impacts on water bodies and their distribution and transport are influenced by several factors, being a vehicle for plastic pollution. However, E. crassipes can be a storage barrier for plastic pollution, acting as potential PM sinks.

The information collected also indicated filament as the predominant form of microplastic in the samples and suggests a secondary source of plastic waste in the water body, possibly arising from the fragmentation of plastics used in aquaculture and mainly from the dumping of domestic sewage, factors that could be monitored, minimizing impacts.

A more in-depth study on the absorption potential of the studied species on MP's is necessary, with a broad and, above all, inter and multidisciplinary approach to better understand the dynamics of this species as a filter feeder, in order to understand the damage to the production of fish and their contribution to plastic pollution-free tilapiculture.

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