STATE UNIVERSITY OF PONTA GROSSA

PRO-RECTORY OF RESEARCH AND POSTGRADUATE

MASTER DEGREE PROGRAM IN ENVIRONMENTAL AND SANITARY ENGINEERING

VALÉRIA DE ALMEIDA

DISTRIBUTION AND CHARACTERIZATION OF MICROPLASTICS IN SEDIMENT OF THE ALAGADOS WATER SUPPLY RESERVOIR

DISTRIBUIÇÃO E CARACTERIZAÇÃO DE MICROPLÁSTICOS NO SEDIMENTO DA REPRESA DE ABASTECIMENTO DE ÁGUA ALAGADOS

PONTA GROSSA

2024

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Dissertation submitted to the Master Degree Program in Environmental and Sanitary Engineering of the State University of Ponta Grossa as a requirement for obtaining the title of Master.

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PONTA GROSSA

2024

Almeida, Valéria de
A447 Distribution and characterization of microplastics in sediment of the Alagados water supply reservoir / Valéria de Almeida. Ponta Grossa, 2024.
96 f.

Dissertação (Mestrado em Engenharia Sanitária e Ambiental - Área de Concentração: Saneamento Ambiental e Recursos Hídricos), Universidade Estadual de Ponta Grossa.

Orientadora: Profa. Dra. Giovana Katie Wiecheteck.
Coorientadora: Profa. Dra. Susete Wambier Christo.

1. Microplastics. 2. Water supply reservoir. 3. Sediment. I. Wiecheteck, Giovana Katie. II. Christo, Susete Wambier. III. Universidade Estadual de Ponta Grossa. Saneamento Ambiental e Recursos Hídricos. IV.T.

CDD: 628.4

Ficha catalográfica elaborada por Maria Luzia Fernandes Bertholino dos Santos- CRB9/986

CERTIFICADO - 2403547/2024 - PPGESA

PROGRAMA DE PÓS-GRADUAÇÃO EM ENGENHARIA SANITÁRIA E AMBIENTAL
Certificado nº 2403547/2024 PPGESA

CERTIFICADO DE APROVAÇÃO - DISSERTAÇÃO DE MESTRADO

Título da Dissertação: **"DISTRIBUTION AND CHARACTERIZATION OF MICROPLASTICS IN SEDIMENT OF THE ALAGADOS WATER SUPPLY RESERVOIR"**

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Ponta Grossa, 16 de dezembro de 2024

Documento assinado eletronicamente por **Giovana Katie Wiecheteck**, **Professor(a)**, em 11/02/2025, às 14:40, conforme Resolução UEPG CA 114/2018 e art. 1º, III, "b", da Lei 11.419/2006.

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A autenticidade do documento pode ser conferida no site https://sei.uepg.br/autenticidade informando o código verificador **2403547** e o código CRC **E6F9927A**.

24.000079028-3 2403547v2

ACKNOWLEDGEMENTS

I would like to thank my parents Helena and Janes for all the support during my research and my life, and for always reminding me of the importance of knowledge and perseverance. In addition, my boyfriend João, for all the help and kindness on my journey.

To Dr. Giovana Kátie Wiecheteck for preparing, guiding, and inspiring me since my undergraduate and throughout my study, and for making this research possible. I would also like to thank Dr. Susete Wambier Christo for accepting me as part of the team, and for the knowledge and help.

To the Master Degree Program in Environmental and Sanitary Engineering - PPGESA, professors, and the State University of Ponta Grossa - UEPG, for the opportunity to improve my career and future.

To Water and Sanitation Company of Paraná State (SANEPAR) for the support on Alagados expeditions.

To Augusto and Gabriel, thank you for your patience, help, and assistance.

To Felipe and Kali, for all the help and support. I will always be waiting for you.

To Muki, for bringing a new ray of sunshine.

To Billy, Jimmy and Pelúcio, for being so supportive.

ABSTRACT

The contamination assessment and management in water supply reservoirs is essential under a sanitary aspect, once their presence is associated with environmental impacts and can also compromise human health. In this scenario, microplastics perform a major role, since the microscopic dimensions ease their spread and turn their detection, quantification, and removal into a difficult task. In this manner, microplastic characterization in freshwater environments constitutes a necessity, by retrieving data and diagnosing an outlook of this modern pollution. It also contributes to highlighting the problem, calling attention to the scientific community to the development of an intertwined effort toward effective solutions. This research presents the results of the distribution and characterization of microplastic contamination in the Alagados Reservoir, in Ponta Grossa, south of Brazil, which is responsible for a part of the city's water supply. This is extremely important for involving not only the protection of the environment but also the public health. It evaluates the temporal and spatial variation of the particles and their correlation to environmental parameters, such as water transparency, depth, and temperature. The sampling was conducted over twelve months, from June 2023 to April 2024, with samples collected bi-monthly from six different sites. Microplastics were found in each of the 108 samples, totaling 3,459 particles. The concentration ranged from 40 to 810 particles.kg^{-1}, a mean of 320.28 ± 162.83 particles.kg^{-1} of wet sediment. The predominant shapes were filaments (43.25%) and fibers (34.58%). 18.65% of microplastics were colored, 645 in total. It was performed a two-way variance analysis (ANOVA), Tukey's honestly significant difference (HSD) test, and the Person test. This way, it was possible to investigate variations of the microplastic concentrations associated with the month and site of each sample, as well as relate the microplastic concentrations to the environmental parameters. Therefore, microplastic particles were identified in all collected samples, exhibiting a diverse range of characteristics. This indicates a widespread presence of microplastics, which poses an environmental problem for the ecosystem. Filaments and fibers were the primary shapes and most of the microplastics observed were uncolored. When microplastics were found with fading color, it could indicate pigment release into the environment, water, and sediment. There was a statistical correlation between the concentration of microplastics in sediment and the time, water clarity, and water temperature; however none between microplastic concentration and water depth. This research also presents a deep learning model for the microplastic and algae automated classification, developed in Python using the Google Colab environment. 1140 images were used, 80% for training and 20% for validation. The model had an 80% accuracy, reaching a recall of 93% and 92% for algae and microplastic filaments, respectively, despite still improvement in other classes and aspects. Overall, the accuracy of the model is encouraging considering the dataset size and all the challenges that involve the automatic identification of microplastics, with all their shape variations and nuances, but the results are promising. This research underscores the importance of enhanced data collection and analysis of microplastics in freshwater and riverbed sediments, especially within water supply reservoirs. Although microplastic contamination in the Alagados Reservoir is relatively low compared to similar locations, further studies and consistent monitoring remain crucial.

Keywords: Microplastics, water supply reservoir, sediment.

RESUMO

A poluição de corpos hídricos por microplásticos é um problema sanitário e ambiental relevante, com potenciais impactos na saúde pública. Nesse contexto, microplásticos configuram um papel importante, uma vez que suas dimensões microscópicas facilitam sua dispersão e tornam sua detecção, quantificação e remoção em um desafio. Nesse sentido, a avaliação de microplásticos em ambientes dulcícolas constitui uma necessidade, por retornar dados e diagnosticar um panorama dessa poluição moderna. Além disso, também contribui para evidenciar o problema, chamando a atenção da comunidade científica tratar de soluções efetivas. A presente pesquisa apresenta uma avaliação da contaminação por microplásticos na Represa de Alagados, responsável por parte do abastecimento hídrico da cidade de Ponta Grossa, analisando a variação espacial e temporal das partículas e a correlação a parâmetros ambientais, como a transparência, profundidade e temperatura da água. Isso é de extrema importância por envolver não apenas o cuidado com o meio ambiente, como também uma questão de saúde pública. A amostragem ocorreu no período de doze meses, de junho de 2023 a abril de 2024, em coletas bimensais em seis pontos diferentes. Microplásticos foram encontrados em cada uma das 108 amostras, um total de 3 459 partículas. A concentração variou de 40 a 810 partículas.kg^{-1}, com média de 320.28 ± 162.83 partículas.kg^{-1} de sedimento úmido. As formas predominantes foram filamentos (43.25%) e fibras (34.58%). 18.65% dos microplásticos eram coloridos, 645 no total. Realizou-se uma análise de variâncias (ANAVA), teste de Tukey de diferença honestamente significativa e teste de Pearson. Com isso foi possível investigar mudanças na concentração relacionadas ao mês e ao ponto da coleta, bem como relacionar as concentrações com os parâmetros ambientais. Portanto, microplásticos foram identificados em todas as amostras coletadas, apresentando uma gama de características. Isso indica uma onipresença, logo um problema para o ecossistema. Filamentos e fibras foram os principais formatos encontrados e a maior parte dos microplásticos observados era incolor ou com desbotamento, podendo indicar uma liberação de pigmentos para o meio, água e sedimento. Houve correlação estatística entre a concentração de microplásticos no sedimento e o decorrer do tempo, transparência e temperatura da água, mas não entre concentração de microplásticos e profundidade da coluna d'água. A presente pesquisa apresenta adicionalmente um modelo de aprendizado profundo para a classificação automatizada de microplásticos e algas, desenvolvido em Python por meio do ambiente do Google Colab. 1140 imagens foram utilizadas, 80% para treino e 20% para validação. O modelo teve uma acurácia de 80%, atingindo um recall de 93% e 92% para alga e filamentos de microplásticos, respectivamente, apesar de ainda serem necessários aprimoramentos em outras classes e aspectos. De modo geral, a acurácia do modelo é encorajadora considerando o tamanho do conjunto de dados e todos os desafios que envolvem a classificação automática de microplásticos, que possuem tamanha diversidade em sua forma, mas os resultados são promissores. Quanto aos resultados encontrados nesta pesquisa, ressalta-se a importância do aumento de dados e análises de microplásticos em água doce e sedimentos de leito de rios, especialmente aqueles relacionados de algum modo ao consumo humano. A contaminação de microplásticos na Represa de Alagados pode ser considerada relativamente baixa comparada a outros locais similares, contudo são necessários estudos adicionais e monitoramento consistente.

Palavras-chave: Microplásticos, reservatório de abastecimento de água, sedimento.

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LIST OF ABBREVIATIONS

ANOVA	Analysis Of Variance
ATR	Attenuated Total Reflectance
CNN	Convolutional Neural Network
DW	Dry Weight
EDS	Energy Dispersive Spectroscopy
FTIR	Fourier Transform Infrared
HDPE	High Density Polyethylene
HSD	Honestly Significant Difference
LDPE	Low-Density Polyethylene
MWD	Mean Weight Diameter
NOAA	National Oceanic And Atmospheric Administration
PC	Polycarbonate
PCB	Polychlorinated Biphenyl
PCR	Polymerase Chain Reaction
PET	Polyethylene Terephthalate
PNRS	National Solid Waste Policy
POP	Persistent Organic Pollutants
PP	Polypropylene
PS	Polystyrene
PU	Polyurethanes
PVC	Polyvinyl Chloride
ROV	Remotely Operated Vehicles
SEM	Scanning Electron Microscopy

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1 INTRODUCTION

The demand for plastics due to modern human activities presents a significant global challenge (Pilapitiya et al., 2024; Ahamad et al., 2022). This issue arises from the massive production of plastic, while current waste management solutions are insufficient to effectively mitigate environmental pollution and its sanitary impacts (Alliance to end plastic waste, 2023; Masud et al., 2023). Microplastics, defined as synthetic polymer particles smaller than 5 millimeters (Fath et al., 2022; Defu, et al., 2020), play a significant role in environmental issues. Their small size allows them to spread easily, making detection, quantification, and removal challenging (Stanton et al., 2020). Consequently, microplastics represent a silent yet urgent concern that is widespread across the globe.

This research assumes that a significant amount of microplastics may enter the Alagados Reservoir in Ponta Grossa, Parana, Brazil, as a result of both urban and rural activities. These microplastics can be introduced through various factors, including weather conditions, surface runoff, river transport, and improper wastewater discharge, which may ultimately lead to their accumulation in the sediment. This investigation focuses on the presence of microplastics, examining their quantity, characteristics, properties, and their correlations with the surrounding environment and seasonal variations. Evaluating this contamination is crucial for environmental and human health, as the resuspension and chemical release from microplastics can significantly affect water quality (Li et al., 2024; Xia et al., 2021; Crawford et al., 2017). This issue is particularly concerning given the purpose of the Alagados Reservoir and the associated risk of microplastic accumulation (Kasamesiri et al., 2020).

This work aims to provide a clearer perspective on global waste generation, specifically focusing on plastic and microplastic pollution in freshwater environments. It will also explore the current state of microplastic pollution in Alagados Reservoir, providing a local evaluation. This analysis may lead to future solutions for addressing the issue, ultimately benefiting both the ecosystem and human well-being. Studies on microplastics in freshwater environments are currently a significant topic of interest, reflecting global scientific concerns (Ribeiro, 2022; Kumar et al., 2024, Sorensen et al., 2021). This issue is widely discussed but still lacks comprehensive understanding (Guo et al., 2024; Mihai et al., 2023).

2 OBJECTIVES

2.1 GENERAL OBJECTIVE

Evaluate the spatial and temporal distribution and characteristics of microplastic in the sediment collected at different sites of the Alagados Reservoir; propose and evaluate a deep learning model to automate the classification of microplastics and algae through a Convolutional Neural Network (CNN).

2.2 SPECIFIC OBJECTIVES

Analyze the variation in microplastic concentrations in sediment samples collected from six different sites in Alagados Reservoir.

Correlate the microplastic concentrations with factors such as water depth and transparency, temperature, and the seasonality of the sample collections.

Assess the characteristics of the microplastics in terms of abundance, shape, color, size, and composition, and identify any potential hazardous elements.

Create and optimize an architecture for feature mapping of images.

Train a deep learning model for microplastics and algae differentiation.

Optimize and evaluate the multiclass classification of microplastics and algae predicted via Transfer learning.

3 LITERATURE REVIEW

3.1 POPULATIONAL GROWTH AND POLLUTION

The world's population is growing and is expected to continue throughout the century (Gu et al., 2021). It took a long way for humankind to reach its first billion people on Earth, around 1804 (Population Connection, 2024) but since then a lot has changed. The industrial revolution of the 19th century caused significant rural flight, resulting in uncontrolled urban growth, widespread diseases, and significant deprivation (Varma et al., 2021). Furthermore, modern medicine evolved and had crucial advances like antibiotics and vaccines (Zikiryayevna et al., 2024) which, allied to sanitation improvement over time (Budge, et al., 2022), contributed to decreasing death rates (widespread globally after 1920), fertility and life expectancy to rise and humans to thrive (Davenport et al., 2021).

The population has tripled since the 20th century and increased by 1 billion individuals since 2010 (UN, 2024). Such a rapid and substantial increase places harmful pressure on the biosphere (Barnett et al., 2023), with humankind using Earth's resources 1.7 times faster than its biocapacity can regenerate (Global Footprint Network, 2024). This scenario implies vast consumption under the natural expanse and a favorable outlook for abundant product offerings and waste generation. In 2021, it was estimated that 2.1 billion tons per year of municipal solid waste was generated worldwide (UNEP, 2024).

There are currently more than 8 billion people on Earth (UNFPA, 2024) in a complex network of activities and interactions with each other and the environment. To meet the pressure of such demand, natural resource exploitation by industries is intense and related to many environmental issues, such as global warming, water pollution, and deforestation (Danish et al., 2023; Ukpong et al., 2021). Consumption has become the driving force for the global economy (Featherstone et al., 2018), leading to profit-based approaches by sellers, resulting in superfluous accumulation, excessive discard, and programmed obsolescence of consumer goods (Penny et al., 2024). This implies a growing waste generation, potentially harmful to the environment and society depending on its management (Masud et al., 2023), requiring global

coordination in a rigid application of the concepts of environmental education and sustainable production and consumption (Glavič et al., 2021).

Sustainable Solid Waste Management (SSWM) encompasses the principle of 3R: reducing, reusing, and recycling, which aims to lower consumption and increase the use of products, thereby reducing the speed and quantity of waste generation (Das et al., 2019). However, responsibility does not summarize only the final consumer, despite its major role (Broz et al., 2023). The industries are responsible for the adoption of production processes that aim to minimize waste and the emission of pollutants and contaminants, incorporating practices like Cleaner Production (P+L) (Silva et al., 2021) and reverse logistics (Cricelli et al., 2021), also generating products with optimized efficiency and durability. Media outlets must also participate by highlighting the importance of responsible consumption and sustainable waste management, and spreading awareness about sanitary health-related issues (Hossain et al., 2022). This matter is fundamentally political, socioeconomic, and institutional (Fadugba et al., 2022).

A variety of factors can affect waste management, such as the volume generated, limited destination space, inadequate urban infrastructure, insufficient public policies and interventions, investment, supervision, and control laws (Chandrappa et al., 2024). These factors tend to harm the waste's correct destination, so instead of being recycled, properly incinerated, or stored in sanitary landfills (Nanda et al., 2020; Rubio-Jimenez et al., 2023), it ends up going astray and coming into potentially harmful contact with nature. This can occur due to open waste burning, waste being discarded directly in improper locations like vegetated areas, rivers, and soil. Consequently, open dumps, and extensive unregulated garbage deposits that lack management and structure, are prone to cause severe environmental damage and pose risks to public health (Ramadan et al., 2022, Yadav et al., 2019).

Although harmful to the environment, open dumps still pose a concrete problem, serving as a final destination for roughly 40% of the world's waste (UNEP, 2024). In Brazil, Law 12.305, of August 2nd, 2010, institutes the National Solid Waste Policy (PNRS), which deals with principles and guidelines related to the integrated management of solid waste (Brasil, 2010). Despite being a strong tool for correction, change of perspective, and introduction of solutions to the destination of waste, even imposing goals for the elimination and recovery of open dumps in national territory, the

deadlines set end with unsatisfactory progress in multiple cities (Sant'anna et al., 2024).

The situation of waste pollution is also concerning in rivers and lakes around the world, where there is an accumulation of trash and contaminants from various sources, the majority of which are land-derived and mainly found in urban centers (Hamilton et al., 2023). In addition, there is the recurrent problem of irregular discharge of domestic and industrial sewage (Deshpande et al., 2022) and the contaminants coming from surface runoff and infiltration, a problem even more pronounced in regions close to large waste deposits with leachates threatening groundwater quality (Alao et al., 2023).

Neglected waste poses a significant threat to the environment. In addition to its detrimental presence, certain materials like metal, glass, and plastic have long decomposition periods (Adekomaya et al., 2021; Dey et al., 2024). This prolonged existence leads to negative impacts on ecosystems (Osuji et al., 2023) and can contribute to the spread of potentially toxic elements such as mercury, lead, and cadmium (Siddiqua et al., 2022). Moreover, improper disposal of these materials can harm living organisms through physical interactions causing entanglement, strangulation, or ingestion (Isangedighi et al., 2020).

3.2 PLASTIC WASTE

Synthetic polymers have a wide variety of compositions, properties, and applications (Ray et al., 2024). They can be classified based into two main categories based on their mechanical properties: thermoplastics and thermosetting polymers. Thermoplastics can be molded when heated and this process is reversible, although it has its limits. In contrast, thermosetting polymers lose their structural integrity when heated beyond their melting temperature after undergoing a curing process (Bîrcă et al., 2019). Examples of thermoplastics include polyethylene terephthalate (PET), which is used for bottles and clothing fibers (Nisticò et al., 2020; Mather et al., 2023), low-density polyethylene (LDPE) for food packaging (Mooninta et al., 2020), high-density polyethylene (HDPE) for tubes and connectors (Omar et al., 2023), polyvinyl chloride (PVC) for ceiling panels and footwear soles (Egbeyale et al., 2024; Adamus-Włodarczyk et al., 2022), polypropylene (PP) for automotive components (Norrrahim

et al., 2021), polycarbonate (PC) for electronics and glazing (Sushmita et al., 2021; Mohammed et al., 2023), and polystyrene (PS) used in cups and packaging (Sembiring et al., 2023). It is estimated that these thermoplastics account for approximately 80% of all plastic consumption worldwide (Iuvshin, et al., 2020). Thermosetting polymer examples include polyurethanes (PU), commonly used in insulating foams (Ates et al., 2022), and Bakelite, which is often found in cookware handles (Phuakphewvong et al., 2020).

The versatility of plastic, combined with its low cost and ease of production, has made it a widely used material around the globe (Ahamad et al., 2022). Plastic is found not only in packaging and everyday items but also in specialized equipment serving important roles in technological advancements in fields such as biomedicine, aerospace, and naval engineering (Dhandapani, et. al, 2024). Microchips made of polymeric substrates, such as PMMA, PC, and PS, are a rapid, efficient, and low-cost solution applied in nucleic acid manipulation, and used also in PCR tests for COVID-19 detection (Li, et. al, 2023). In submarines and other remotely operated vehicles (ROV), PC offers resistance to pressure at great depths without detriment in visibility while used in portholes and domes (Smith et al., 2023). In aircraft, plastic materials have the advantage of being light, increasing fuel efficiency, and offering design flexibility in complex shaped components.

Despite its positive aspects, the widespread use of plastic results in substantial waste generation, exacerbated by the ease of low-cost production (Pilapitiya et al., 2024). According to the “Plastics – the Fast Facts 2023” bulletin published by Plastics Europe, global plastic production in 2022 reached 400,3 million tons (Mt), with 90,2% derived from fossil fuels and only 8,9% mechanically recycled. This indicates a significant volume of plastic that has not been reused. It is estimated that 360 Mt of plastic waste is produced annually worldwide. In 2021, about 50% of generated plastic waste ended up in landfills, 20% ended up in open dumpsites or was burned, 20% was incinerated, and 10% was recycled (Alliance to end plastic waste, 2023).

3.3 MICROPLASTICS

Microplastics are a significant component of plastic pollution, defined as tiny particles measuring less than 5 millimeters (Fath et al., 2022). They can be generated

as primary microplastics, which are found in various personal care and cosmetic products due to their abrasion properties (Guerranti et al., 2019). Alternatively, microplastics can originate from the breakdown of larger plastic items caused by usage and environmental wear (Laskar et al., 2019). In 2019, it was estimated that microplastics accounted for 12% of the total weight of plastic waste that ended up in nature (OECD, 2022).

Recent research on microplastic pollution has demonstrated a widespread contamination scenario (Khatoon et al., 2024). However, there is currently no established limit for microplastics in either drinking water or wastewater discharge (Zhao et al., 2024). This raises concerns about the potential microplastic ingestion through drinking water (Danopoulos et al., 2020), including both tap and bottled water (WHO, 2019). Additionally, a significant amount of microplastics is released into rivers through treated wastewater. Despite high removal efficiencies of up to 99% (Parashar et al., 2023), wastewater treatment plants are considered the primary source of microplastic contamination in water bodies, contributing between 5.0×10^5 to 1.4×10^{10} particles per day (Komorowska-Kaufman et al., 2024). Fibers are often the most abundant shape, relating to laundering of synthetic textile products (Acharya, et al., 2021; Weis, et al., 2022).

Sources of diffused microplastics include urban activities, transportation, tourism, and agricultural land use (Hamidian et al., 2023). These particles are dispersed into the environment through surface runoff, infiltration, and airstreams, which serve as pathways for microplastics to eventually enter water bodies (Kumar et al., 2023). This dispersion has significant environmental impacts (Osman et al., 2023) because microplastics can accumulate harmful substances (Li et al., 2024) that are chemically attracted to them, such as heavy metals and persistent organic pollutants, including polychlorinated biphenyls (PCBs) a well-known carcinogen (Crawford et al., 2017). Furthermore, microplastics can be mistakenly ingested by aquatic animals such as fish (Sacco et al., 2024), crustaceans (Bos et al., 2023), and gastropods (Curren et al., 2024), and they are found in 94% of oysters globally (Liu et al., 2023). This bioaccumulation of microplastics poses a risk to the food web, potentially affecting higher trophic levels, including humans (Sinitsyna et al., 2023).

Microplastic pollution has recently become a major global issue (Kumar et al., 2024). Numerous studies have examined various environmental media, including water, sediment, snow, plants, and animals (Nirmala et al., 2023; Li et al., 2023;

Parolini et al., 2021; Qiu et al., 2023; Lim et al., 2023). While much of the focus has been on microplastic accumulation in marine environments, freshwater sources are increasingly receiving attention (Sorensen et al., 2021). This is critically important for human and animal health, environmental integrity, and the safety of drinking water. Furthermore, rivers serve as a major pathway for plastics entering the oceans, with an estimated export of about 0.5 million tons of plastics to the seas each year (Strokal et al., 2023).

3.3.1 Previous studies of microplastics in freshwater

In order to enlighten the current scenario of microplastic research worldwide, a review was conducted comparing 31 studies published in 21 articles. They were chosen among recent studies published in peer-reviewed journals over the past five years. In addiction of being recent, these studies examined microplastic pollution in freshwater environments across all continents (see Figure 1 and Table 1) and had different approaches. The studies in this review encompass various sources, including rivers, lakes, lagoons, streams, reservoirs, catchments, and wetlands, as well as analyses of water samples (both superficial and column), sediment, and living organisms. The studies revealed significant variations in methodologies, highlighting the ongoing issue of a lack of standardization in the field of microplastic research, particularly in sample collection, processing, and analysis (Pérez-Guevara et al., 2022). This review serves as a source for better understanding the current dynamics of microplastics research, presenting a perception of relevant protocols and results worldwide.

Figure 1 – Location of microplastic pollution studies in freshwater environments around the world.

Source: The author, 2024 (adapted from Google Maps).

A variety of equipment for water sampling has been used, including trawl nets and buckets. Neuston, manta, drifting, and plankton nets came in mesh sizes ranging from 20 to 500 µm and were often paired with a flowmeter. Processing typically involves several steps, including sieving, organic matter digestion (usually with H_2O_2 30%), density separation (using NaCl or $ZnCl_2$), and filtration with a vacuum pump. Microplastic concentrations in water have been measured in terms of the number of particles per liter ($n.L^{-1}$), per cubic meter ($n.m^{-3}$, the most common measurement), and per 1000 cubic meter (n. 1000 m^{-3}). The equipment which has been used for collecting sediment samples includes a hand trowel, Van Veen and Petit Ponar samplers, push and kayak corers, and shovels. The processing of these samples has been similar to that of water samples, but it includes additional drying stages.

Table 1 - Summary of data and methodologies used in the analysis of microplastics in freshwater environments. (To be continued)

Location	Medium	Sample collection	Sample processing	MP concentration	MP size	Validation	Reference
Rio de Janeiro, Brazil	River, water column	Neuston net (300 µm), from 10 - 15 minutes.	According to Masura et al., (2015).	3.6 - 51166.5 (3651.5) $n.m^{-3}$	300 - 5000 µm	Stereo microscope, ATR-FTIR	Drabinski et al., 2023
Santa Catarina, Brazil	Lagoon, water	Bucket, plankton net (20 µm, 7000 ± 120 L) and flowmeter.	Density separation (NaCl), vacuum filtration, digestion (H_2O_2 30%) and filtration.	0.00 - 0.38 (0.06) $n.L^{-1}$	Length of 20 - 3000 µm, width 20 - 500 µm	Stereo microscope, ATR-FTIR	Castro et al., 2024
	Urban river, water			0.06 - 0.59 (0.32) $n.L^{-1}$			
	Non-urban river, water			0.06 - 0.47 (0.32) $n.L^{-1}$			
Brazil (div)	River water	Plankton net (55 µm).	Filtration, sieving, digestion (H_2O_2 40%), density separation (NaI 1.8 g/ml), filtration.	8 – 39 $n.m^{-3}$; 5 - 152 $n.m^{-3}$; 50 – 126 $n.m^{-3.}$	300–1000 µm (47%), 100–300 µm (32%), 1000–5000 µm (18%), 55–100 µm (3%).	Stereo microscope, µ-FTIR	Rico et al, 2023
	Stream water			178 - 74550 $n.m^{-3}$; 167 – 3095 $n.m^{-3}$; 59 - 248 $n.m^{-3}$.			
Amazônia, Brazil	River, sediment	Van Veen sampler.	Drying, sieving, digestion (H_2O_2 30%), density separation ($ZnCl_2$), and vacuum filtration.	417 - 8178 $n.kg^{-1}$ dw*; 0 - 5725 $n.kg^{-1}$ dw	63 – 5000 µm	Stereo microscope	Gerolin et al., 2020
Vietnam (div.)	River, surface water	80 µm mesh size plankton net (diameter 50 cm) coupled to a flowmeter for up to 10 minutes (0.05 to 143 m3).	Sieving, digestion by Sodium Dodecyl Sulfate, protease, amylase, lipase, and H_2O_2 30%, sieving, density separation, and filtration.	2.3 - 2522 $n.m^{-3}$	761 - 1121 µm 76 487 -125 935 $µm^2$	Stereo microscope, FTIR-ATR	Strady et al., 2021
	Reservoir, surface water			1.5 $n.m^{-3}$	760 µm, 846 µm 125 607, 157 463 $µm^2$		
	Urban lake, surface water			70 - 611 $n.m^{-3}$	908 - 1894 µm; 67,806 to 138,020 $µm^2$		
Angers, France	River, water column	Directly sampled.	Filtering without digestion.	>100 $n.kg^{-1}$ dw	100 - 500 µm (36.4 – 70.6%)	Stereo microscope, µ-FTIR	Vidal et al., 2023

Table 1 - Summary of data and methodologies used in the analysis of microplastics in freshwater environments. (To be continued)

Lijiang, China	Lake, sediment	Stainless steel grabs.	Sieving, digestion by H_2O_2 30%, flotation with NaCl, and vacuum filtration.	10 - 115 n.kg^{-1}	<100 µm	Stereo microscope, SEM-EDS	Duan et al., 2024
	Lake, water	20 L through stainless-steel mesh sieves (<5 mm) and plankton mesh (<0.112 mm).		90 - 770 n.m^{-3}	<100 - 3000 µm		
Puerto Misahualli, Ecuador	Riverine depositional sediment	500 g of top sediment (10 cm deep) collected with hand trowel.	According to Gimiliani et al. (2020)	0 - 4200 n.kg^{-1} dw	<500 - 5000 µm	Stereo microscope	Lucas - Solis et al., 2021.
Dhaka, Bangladesh	Lake and river, sediment	Handmade push corer.	According to Gerolin et al., (2020).	13607 ± 13733 n.kg^{-1} dw	<100 - 5000 µm	Stereo microscope, FTIR, SEM-EDS	Parvin et al., 2022.
	Lake and river, surface water	Directly sampled.	Digestion (H_2O_2 30%), vacuum filtration and drying.	36000 ± 32 n.m^{-3}			
Thailand	Reservoir, fish	Gill nets.	Digestion (H_2O_2 30%), density separation (NaCl 300 g/l), and filtration.	2.92 ± 1.30 n/fish	<100 - 5000 µm	Stereo microscope	Kasamesiri et al., 2021
United Kingdom	River, catchment	The metal bucket on a telescopic pole and sieving (63 µm).	Digestion (H_2O_2 30%) and filtration.	max 0.4 n.L^{-1}	<100 - 3500 µm	Stereo microscope, ATR-FTIR	Stanton et al., 2020
Bursa, Turkey	River, sediment	Van Veen Grab sampler and Kajak Corer.	According to Masura et al. (2015).	276 n.m^{-2},	49 – 4951 (1380 ± 900) µm	Stereo microscope, ATR-FTIR	Almas et al., 2022
Kermanshah, Iran	Lake, sediment	Metal shovel (30 × 30 cm²) and stainless steel spoon (0–5 cm).	Drying, sieving, digestion (H_2O_2 30%), density separation (NaI, 1.60 g/cm3), centrifugation, vacuum filtration, and drying.	1733.33 - 4400 (2483.59 ± 805.30) n.kg^{-1} dw	25 – 1000 µm (62%); 1000 - 2000 µm (38%)	Stereo microscope, FTIR, SEM-EDS	Nayeri et al., 2023
Łomianki, Żyrardów, Poland	Lake and reservoir, water	Plankton nets (20, 200, and 500 µm; 14.4, 57.6, and 100.8 dm³).	Drying, digestion (69% HNO_3, 5 cm³ of H_2O_2 30%), vacuum filtration and drying.	174 – 3125 n.m^{-3}; 10 – 1250 n.m^{-3}	0.1 - 5000 µm	Digital microscope	Karaban et al., 2023
Guimarães, Portugal	River, sediment	Directly sampled.	According to Masura et al. (2015); Prata et al. (2020) and Prata et al. (2022).	2130.3 ± 377.6 - 3018.2 ± 416.8 n.kg^{-1} dw	< 5000 µm	Optical microscope, fluorescence under 470 nm blue light on orange filter	Ribeiro et al., 2022
	River, benthic macroinvertebrates	Hand net (500 µm).		558.6 ± 100.7 MPs·g^{-1} of fresh weight	10 to 70 µm; 20 to 90 µm		

Table 1 - Summary of data and methodologies used in the analysis of microplastics in freshwater environments (Conclusion)

North Carolina, USA	River, water	Neuston net (>335 µm) from 3 to 15 minutes (9 to 134 m3) and sieve (64 µm).	Digestion (H_2O_2 29-32%, $FeSO_4$), density separation (NaCl), sieving, sonication, and desiccation.	0.02 - 221 (0.44) $n.m^{-3}$; 20 - 130 $n.m^{-3}$	65 - 3587 µm; 64–335 µm	Stereo microscope, µFTIR-ATR	Kurki-Fox et al., 2023
	River, sediment	Directly sampled.	Sediment-Microplastic Isolation (SMI) units and $ZnCl_2$, rinsing, digestion (H_2O_2 30%, Fe^{2+}), vacuum filtration, UV light inspection, and drying.	0 - 808 (147) $n.kg^{-1}$ dw	65 - 1457 µm	Fluorescence microscope, Raman	
Ontario, Canada	River, sediment	Petite ponar grab sampler.	Drying, sieving, density separation (sodium polytungstate), filtration, and drying.	6 - 2444 (612) $n.kg^{-1}$ dw	63 - 5000 µm	Stereo microscope, ATR-FTIR	Corcoran et al., 2019
Vereeniging, South Africa	River, surface water	Plankton net (55µm), flowmeter.	Digestion (H_2O_2 30% and $FeSO_4$) and density separation (NaCl, NaI).	0.68 ± 0.64 $n.m^{-3}$	55 - 5000 µm	Stereo microscope, Raman	Saad et al., 2024
Uganda	Lake, surface water	Manta trawl (300 µm).	Sieving, drying, (H_2O_2 30% and $FeSO_4$), density separation (NaCl), filtering, and drying.	0.02–2.19 $n.m^{-3}$	300 - 5000 µm	Stereo microscope, ATR-FTIR	Egessa et al., 2020
Livingston Island, Antarctic	Stream, water	Drifting net (333 µm and 100 µm, 21.9 ± 4.4 m3/h).	Digestion (33% H_2O_2), filtration, and density separation (NaCl).	0.47–1.43 (0.95) n.1000 m^{-3}	400–3546 (1118) µm; 10–1026 (199) µm.	Stereo microscope, µFTIR	González-Pleiter et al., 2020
Victoria, Australia	Stream, wetland and river, surface water	Directly sampled.	Vacuum filtration.	0.40 ± 0.27 $n.L^{-1}$	36 - 4668 (942 ± 835) µm	Stereo microscope, µFTIR-ATR	Nan et al., 2020
	Freshwater shrimp	Dip net.	Rinsing, dissolving (NaOH), and vacuum filtration.	0.52 ± 0.55 $n.ind^{-1}$	190 - 4214 (764 ± 575) µm	Stereo microscope, µFTIR-ATR	

* Dw: dry weight.
Source: Indicated, edited by the author.

The NOAA Marine Debris Program protocol, as outlined by Masura et al. (2015) in the Laboratory Methods for the Analysis of Microplastics in the Marine Environment, was frequently used for analyzing both water and sediment samples. The measurements were reported in two main units: particles per kilogram of dry sediment ($n.kg^{-1}$ dw, which was the most common) and particles per square meter ($n.m^{-2}$). The identification of microplastics varied, but it often involved visual inspection using a stereomicroscope, along with validation techniques such as Scanning Electron Microscopy coupled with Energy Dispersive Spectroscopy (SEM-EDS), Attenuated Total Reflectance-Fourier Transform Infrared (ATR-FTIR) and Raman spectroscopy.

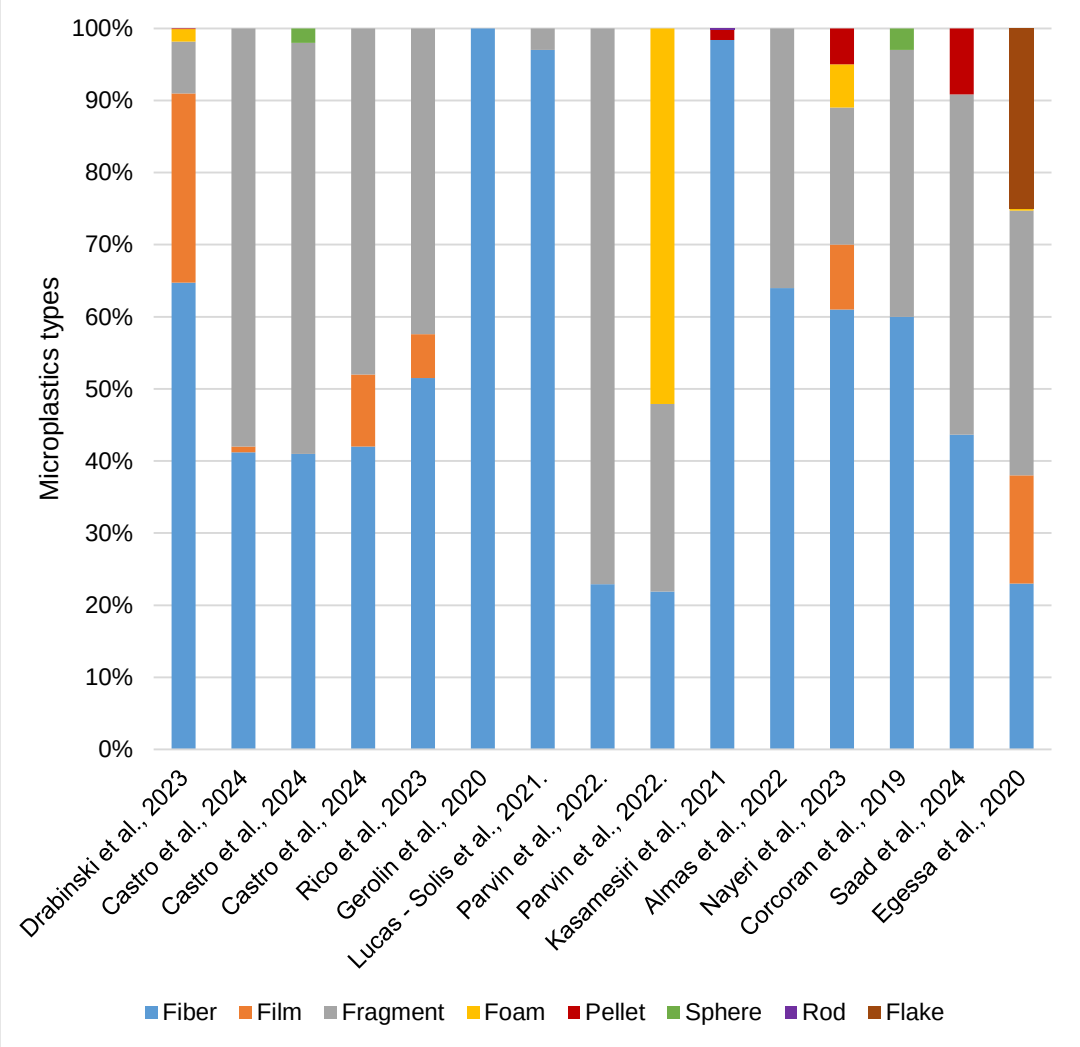
All of the studies identified microplastics in various environments, including rural, preserved areas, and even the Antarctic, highlighting the widespread presence of this pollutant. Ribeiro et al. (2022) found that locations with higher ecological status do not necessarily exhibit lower microplastic concentrations. The contamination in remote areas underscores the ease with which microplastics spread and the significant risks they pose globally. Proximity to urban centers and industries has contributed to higher concentrations in many findings, reflecting the human impact on the dispersion of microplastic pollution. Additionally, the studies emphasized the influence of river flow and morphology on the transport and accumulation of microplastics in sediments.

The highest concentration of microplastics found in water samples from referred studies was 74550 $n.m^{-3}$, observed in a Brazilian urban stream in Manaus, a tributary of the Amazon River, as reported by Rico et al. (2023). Additionally, a significant concentration of microplastics was detected in sediment from the Amazon River, measuring 8178 $n.kg^{-1}$ dry weight (dw), according to Gerolin et al. (2020). This situation is concerning, especially considering that the Amazon Basin boasts the highest concentration of biodiversity globally (Guayasamin et al., 2024). Furthermore, the Amazon River contributes the largest river discharge in the world, exceeding 5.10^{12} m^3 per year into the Atlantic Ocean, which accounts for 17% of the freshwater entering the ocean globally (Tosetto, et al., 2022; Rico et al, 2023).

The studies reporting microplastic types and their percentages are presented in Figure 2. The majority of shapes identified were fibers and fragments; however, other shapes such as films, foams, pellets, spheres, rods, and flakes were also noted. Eleven assessments mentioned the types of microplastic without their percentages. The variety of shapes found in each study suggests that there are likely multiple sources are contributing to the presence of microplastics. Overall, fibers were the predominant

shape, appearing in all but one of the studies. The high prevalence of fibers in freshwater ecosystems is concerning, as they are the most commonly ingested type by animals, which can be harmful to zooplankton and other aquatic organisms (Rebelein et al., 2021). Additionally, blue fibers are the most abundant type of microplastic found in the Amazon Basin (Silva, 2024; Lucas-Solis et al, 2021).

Figure 2 – Percentages of types of microplastic found in different studies.



Source: Indicated, edited by the author.

Since the complete removal of microplastic pollution is virtually impossible due to their microscopic dimensions, it is essential to understand their sources and how they enter the environment to mitigate the problem. Drabinski et al. (2023) highlighted the failure or lack of proper sanitation and sewage treatment, which contributes to higher concentrations of microplastics in freshwater systems. Additionally, Lucas–Solis et al. (2021) recommended investigating the microplastic removal capacity of

wastewater treatment plants, alongside a global effort to improve sewage management practices.

Castro et al. (2024) emphasized the importance of considering meteorological factors, such as wind and precipitation, when analyzing the transportation of microplastics. Duan et al. (2024) highlighted the necessity of managing the watersheds to reduce the input of microplastics into freshwater systems. Additionally, considering interesting factors in microplastics dynamics, Almas et al. (2022) utilized a paleolimnological approach, specifically focusing on plastic chronostratigraphy, which serves as a valuable tool for retrieving crucial data in microplastic analysis. This particular study identified microplastics in sediment cores that date back to the 1960s in Turkey.

Stanter et al. (2020) highlighted that temporal variability is a significant characteristic of microplastic pollution, and ignoring it could result in misrepresentation and substantial errors in the findings. Karaban et al. (2023) identified another potential source of errors: clogging in sampling nets, particularly in the finest meshes, due to organic matter such as phytoplankton. They also suggested obtaining larger water samples instead of smaller ones. Additionally, Kurki–Fox (2023) pointed out that some microplastics, especially fibers, might be lost during sample processing.

Gerolin et al. (2020) discovered that microplastic concentrations are higher in the sediments of shallow river waters with lower velocities compared to deeper ones with higher velocities. This finding confirms that hydrodynamics play a role in the transportation, settling and sedimentation of these particles, considering flow velocity, water level, tidal current, flooding events and overall discharge of the rivers (Kumar et al., 2021). As for the microplastics, factors such as the shape, size and density are linked to the settling velocity (Zhang et al., 2022).

Castro et al. (2024) observed higher concentrations of microplastics in the surface water of tributaries than in the lagoon to which they flow. This is consistent with he findings of Rico et al. (2023), which reported significantly higher microplastic concentrations in tributaries than in the mainstem river. Corcoran et al. (2019) noted that higher microplastic concentrations were associated with the finest grain sizes of freshwater sediment that contained more organic debris.

Strady et al. (2021) highlighted the disparities in facilities, as well as financial and human resources, between countries, which contribute to the challenges of standardizing methodologies and analyses for microplastic protocols worldwide. Given

this context, adaptations are necessary to reflect local circumstances, particularly in developing countries where assessments are essential for addressing and monitoring microplastic pollution.

Parvin et al. (2022) identified sites where microplastics pose a significant risk to freshwater biota, correlating with the research of Vidal et al. (2023), which found evidence of bioaccumulation in all sampled organisms, including bivalves, worms, and eels. They emphasized a relationship between concentrations of microplastics in bivalves and sediment. Additionally, Kasamesiri et al. (2020) reported high levels of microplastic ingestion by fish found in the middle to bottom layers of a reservoir's water column, predominantly blue fibers. Ribeiro et al. (2022) discovered a higher concentration of microplastics in low-weight organisms, potentially linking this to malnutrition and digestive disorders.

The generation of waste is a significant concern today, with plastic being a major contributor to the problem. The world is still seeking effective solutions for proper plastic waste management, and addressing the issue of microplastics is a crucial and long-overdue improvement. In recent years, extensive research has been conducted on microplastics in freshwater environments. The findings from these studies provide essential insights to the scientific community and lay the groundwork for future research. Notably, all studies referenced in this review detected microplastics in various locations across all continents, highlighting the widespread presence of this contaminant. This underscores the need for a global effort to monitor microplastics effectively, assess their sources and transportation pathways, and understand their fate, ultimately aiming to reduce their spread and accumulation.

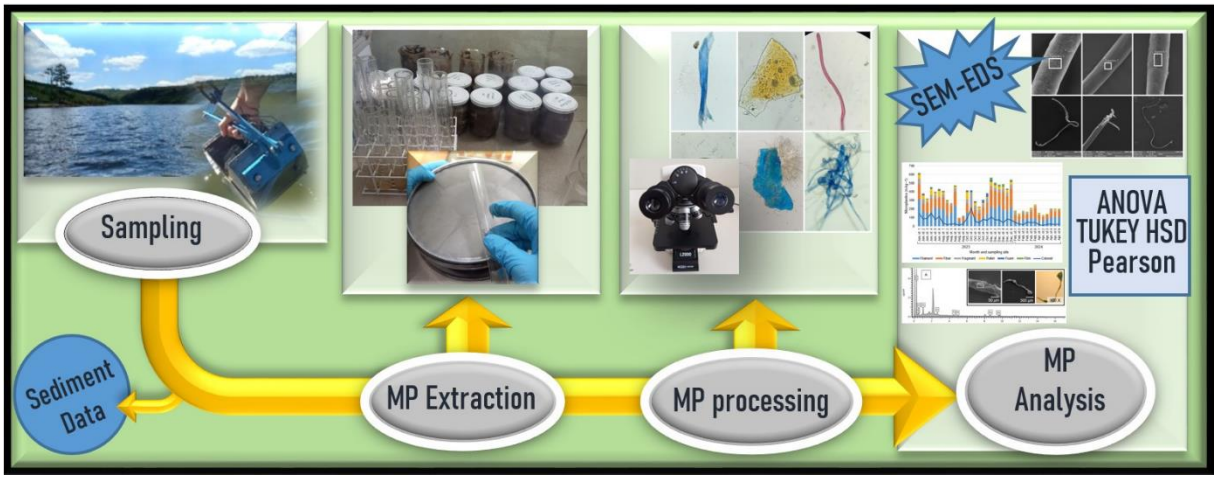
4 ARTICLE 1 - DISTRIBUTION AND CHARACTERIZATION OF MICROPLASTICS IN SEDIMENT IN A WATER SUPPLY RESERVOIR IN SOUTHERN BRAZIL

Abstract

Microplastics, which are synthetic particles smaller than five millimeters, constitute a significant environmental problem due to their long decomposition period and their ability to disperse easily in various environments. These particles can also retain chemicals and heavy metals, accumulating in the ecosystem or being ingested and entering the food web. Conducting studies to analyze this issue is essential, particularly through the collection, identification, and processing of data obtained in the field, especially near water catchments, as this could directly impact human health. This paper presents a temporal and spatial distribution and characterization of microplastics in the sediment of Alagados Reservoir, located in Parana, Brazil. The data were obtained over one year through bimonthly sampling at six different sites. In all 108 samples, a total of 3,459 microplastic particles were found. The main concentration was 320.28 ± 162.83 particles per kilogram of wet sediment. The most common shapes were filaments (43.25%) and fibers (34.58%). 18.65% of microplastics were colored, totaling 645, an average of 5.97 ± 5.98 per sample of sediment, of which 36.9% were blue and 21.5% were yellow. Statistical analysis was conducted using RStudio Software to perform a two-way ANOVA, Tukey's honestly significant difference (HSD) test, and the Person test. The seasonality of water collection, turbidity, and water temperature statistically affected microplastic concentrations. Analysis using SEM-EDS revealed the presence of zinc chloride, used during microplastics isolation, in 83.33% of the samples tested, despite being thoroughly rinsed with distilled water. This highlights the tendency of microplastics to chemically absorb substances and indicate the particles came from the samples instead of contamination. SEM-EDS images of the microplastics also displayed deterioration in different ways, suggesting the potential entry of even smaller plastics into the environment (nanoplastics).

Keywords: Microplastics; sediment; freshwater.

Graphical abstract.



Source: The author.

4.1 INTRODUCTION

Plastic is an industrially consolidated and widely used material (Lin et al., 2022) due to its production being faster and less expensive than other materials such as metal and wood. It is also moldable, light, versatile, and mainly resistant and durable (Costa et al., 2017). Plastic resistance is a desirable property but leads to long decomposition (Ghaffar et al., 2022), causing accumulation in landfills and the environment. This creates a complex problem due to its range of compositions (Erdle et al., 2023).

The amount of plastic waste continues to increase despite the availability of alternatives such as biodegradable plastics, recycling, and incineration (Thew et al., 2023). It is estimated that only 30% of all plastic ever produced is currently in use (Geyer et al., 2017). Each year, around 400 million tons of plastic are produced, with approximately 21 million ending up in aquatic ecosystems (UNEP, 2021).

For a long time, there has been concern about environmental pollution caused by easily detectable plastics, known as macroplastics, such as PET bottles and packaging (Ortiz et al., 2023). However, the recent focus has been on smaller plastics called microplastics, particularly over the last decade (Ramkumar et al., 2020). This is a less visible problem, but equally worrying (Simon et al., 2022).

Microplastics are small polymeric particles, each less than five millimeters in size (Defu, et al., 2020). They can originate as primary particles, already produced at a millimeter scale, or as secondary particles, resulting from the fragmentation of larger

plastics (Costa et al., 2022). Primary microplastics are vastly used in cosmetics and hygiene products, as pellets and fibers (Mathew et al., 2024). Secondary microplastics from textile products are the major polluter of the environment, mostly composed of fibers (Mušič, 2023). Microplastics pose a significant environmental threat (Ahmed et al., 2022) as they represent a form of contamination that is challenging to control and remove.

The analysis of microplastic presence in a given environment involves several steps. These include choosing the medium to be analyzed, the procedure for collecting and preparing samples (aimed at isolating microplastics), and the method for identifying and analyzing them (Park et al., 2021). There are various methods for determining the qualitative and quantitative characteristics of microplastic contamination in a sample (Kaile et al., 2020), such as quantity, shape, dimensions, and composition. However, there is currently no standardized method or unit of measurement used universally by researchers. This leads to methodological differences that make it difficult to compare the results obtained in characterization studies (Kutralam-Muniasamy et al., 2021).

When defining the environment to be analyzed for the presence of microplastics, it is important to consider how easily the particles can spread. Microplastics can be found in various environments due to factors such as particle size and density. They can be transported through atmospheric precipitation, percolation, outflow, and effluent release, as well as through direct discharge of wastewater into rivers by both the general population and industries. Microplastics can be found in aquatic and terrestrial environments, suspended in the atmosphere, in rainwater, in rural areas, and even at the poles (Sun et al., 2022; Sajjad et al., 2022; Truong et al., 2021; Koutnik et al., 2022; Chen, 2021; Materić et al., 2022).

The significant negative impact of microplastics on organisms and the food web is noteworthy (Bostan et al., 2023). In aquatic ecosystems, microplastics can be easily consumed, accumulating in an animal’s digestive system and persisting for long periods. This poses a high risk of toxic effects due to both direct and indirect ingestion (Li et al., 2023). In fishes, the microplastic ingestion is linked to behavioural abnormalities, reproductive impairment, decreased growth, compromised immunity, inflammatory responses, cellular damage and even death (Hasan et al., 2024; Hossain et al., 2022; Chen et al., 2023). There is also the potential for microplastics to transfer to humans, through the consumption of these affected animals (Benson et al., 2022).

Additionally, microplastics can adsorb and carry compounds such as pesticides, drugs, and metals (Tang et al., 2020), thereby increasing their potential for ecological harm (Martinho et al., 2022). Research indicates the presence of microplastics even in the bloodstream (Kutralam-Muniasamy et al., 2022) and human breast milk (Ragusa et al., 2022), and exposure to these particles may be linked to risks for the digestive system (including cancer) and reproductive system (CalSPEC, 2023).

The collection of microplastics can take place in the most varied ways, resulting in samples of water, sewage, mud, sand, and even animals like fish and oysters (Costa et al., 2023; Parashar et al., 2022; Yuan et al., 2022; Ben-Haddad et al., 2022; Park et al., 2022; Do et al., 2022). There are different methods for sample preparation (Fang et al., 2023), such as sieving, separation by density, and organic matter degradation to isolate microplastics (Masura et al., 2015). After the preparation, there is the identification and quantification of particles, including determining their morphological aspects, chemical composition, and concentration in the environment.

There are various methods for analyzing microplastics after they have been isolated, and several new techniques have been developed in recent years (Huang et al., 2023). Visual identification of microplastics, whether through naked-eye observation or microscopy, is a simple and commonly used method (Pico et al., 2019). However, it is laborious and prone to errors (Lv et al., 2021); particularly as the size of the particles decreases (Kotar et al., 2022). Electron microscopy is a powerful form of microscopy, involving the emission of an electron beam directed at a point on the sample, which interacts with it to obtain a two-dimensional image without causing damage. This method allows for the inference of properties of the analyzed materials, such as dimensions and shape (Gondikas et al., 2023), and includes transmission electron microscopy (TEM) and scanning electron microscopy (SEM).

Visual identification methods rely on the interpretation of the analyst, so it is recommended to use them along with analytical methods (Cutroneo et al., 2020) to determine the chemical composition of particles (Song et al., 2021). For SEM, an Energy Dispersive X-ray Spectroscopy (SEM-EDS) component can be added (Vasudeva et al., 2024) to provide not only high-resolution microscopic images but also detailed information about the elemental composition of the particle (Adelugba et al., 2024). The images and data obtained enable the analysis of the particle’s shape and composition but do not provide information about their color. This method can be used

to analyze microplastics if the particles are conductive and a specific preparation, such as gold coating the sample, is used.

Numerous studies are being conducted worldwide to investigate the presence of microplastics in aquatic environments (Leistenschneider et al., 2024; Samandra et al., 2023; Ditlhakanyane et al., 2022; Zhou et al., 2023; Jiménez-Contreras et al., 2024). However, there is still a limited understanding of the extent of microplastic pollution in riverbeds (Daele et al., 2024). The presence of microplastics in freshwater sediment is a cause for concern, as indicated by various studies (Gerolin et al., 2020; Kurzweg et al., 2024; Patidar et al., 2023; Forrest et al., 2023; Salikova et al., 2024; Cofano et al., 2023). This type of pollution can vary significantly due to factors such as flow hydrodynamics, channel characteristics, discharges, and the proximity of populated areas and water catchment facilities (Boos et al., 2024; Matjašič et al, 2023).

Some types of plastics, like polyvinyl chloride (PVC) and polyethylene terephthalate (PET) (Pakbar et al, 2023), are heavier than water and sink, while others, like polyethylene (PE) and propylene (PP), can float. However, even lighter plastics can get trapped on the riverbed due to turbulence and biofouling (Molazadeh et al., 2024; Melkebeke et al., 2020). Various factors, such as shape, surface roughness, and rainfall (Costa et al., 2023), can affect how microplastics settle. Microplastics are more likely to accumulate in sediment than in surface waters, and sediment can serve as a source for releasing settled plastic particles back into the water (Xia et al., 2021).

This paper addresses the issue of microplastic pollution in a rural reservoir in the South of Brazil. The reservoir is a source of water for the population of the city of Ponta Grossa, state of Parana, which means that the microplastics could impact not only the animals living in the reservoir but the population of the city as well. This way, it is necessary to address the microplastic pollution in order to analyze and the situation and investigate solutions in order of environmental protection and public health maintenance. Therefore, the main objective of this study is to evaluate the spatial and temporal distribution and characteristics of microplastic in the sediment collected in different sites of the Alagados Reservoir. The specific objectives are to assess the variation in microplastic concentration in the sediment of the reservoir over time and space; to characterize the morphological aspects of the microplastics; and to investigate the composition of the microplastic particles and their potential impact on human health and the environment.

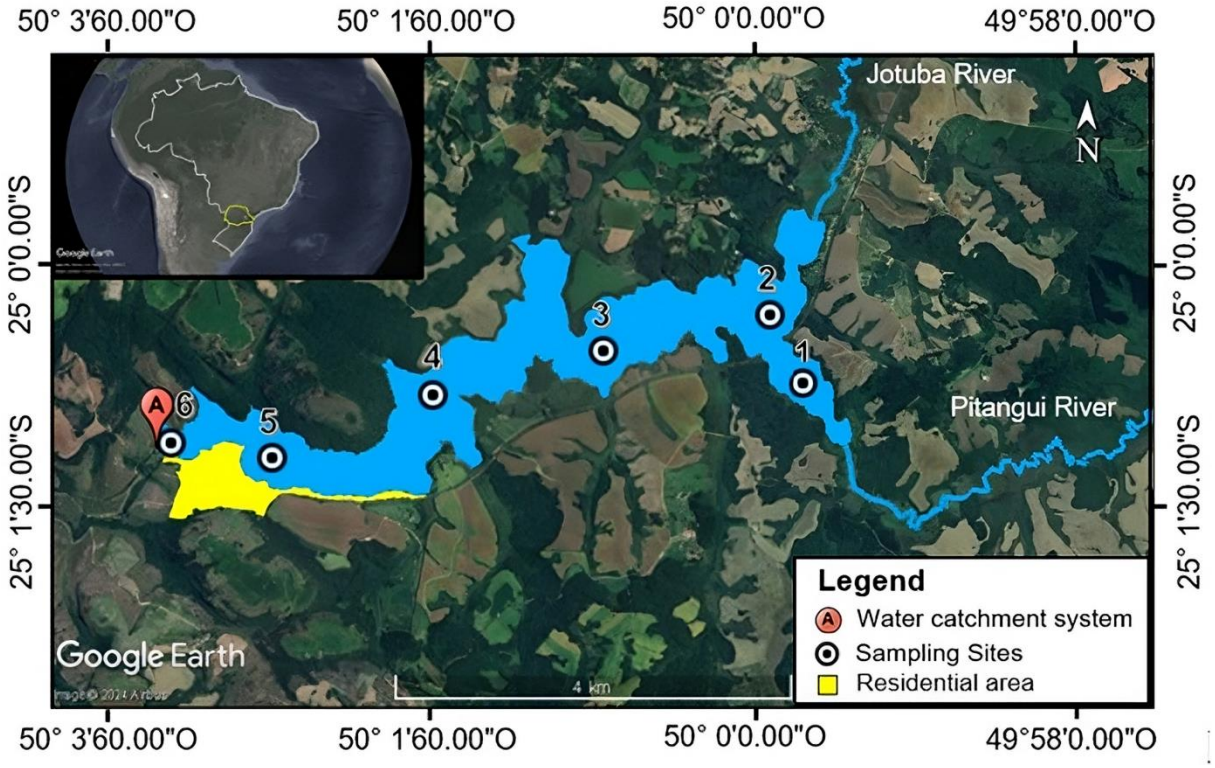
4.2 METHODOLOGY

4.2.1 Study area

Alagados is an artificial reservoir located in a rural area in southern Brazil, 20 km from Ponta Grossa, Parana (Rocha et al., 2011). Ponta Grossa has an estimated population of 372.562 inhabitants (IBGE, 2024). The reservoir area is also part of the municipalities of Carambeí and Castro. It is situated between the coordinates 24°59’ to 25º01' S and 49°58’ to 50º03' W (Lemos et al., 2014). The flooded area covers 7, 31 km² with an approximate length of 10,7 km (IAP, 2017). Alagados was created in 1929 by damming the Pitangui River for hydroelectric purposes (Paraná Government, 2024). Since 1969, it has been a source of water supply and currently provides 30% of the water demand for Ponta Grossa’s population (ARAS, 2017; Sanepar, 2024). The reservoir also receives water from the Jotuba River. The surrounding area is highly influenced by agricultural activity and livestock breeding (Beló, 2018). Additionally, a yacht club has been active near the water catchment of the Water and Sanitation Company of Parana (Sanepar) since 1970 (IPEA, 2024).

Six sampling sites were chosen (Figure 3) and strategically distributed along the reservoir to represent specific characteristics. Sites 1 and 2 are located at the Pitangui and Jotuba rivers mouths, in areas with some vegetation and relatively less human intervention. Site 3 and 4 are intermediary to sites 5 and 6, which are close to the most populated area in Alagados and the water catchment facility, respectively.

Figure 3 – Map of the study area (Alagados Reservoir), indicating the sampling sites.



Source: Alagados Reservoir and the location of sampling sites (adapted from Google Earth).

4.2.2 Sample collections

A previous analysis of the reservoir sediment was conducted to assess microplastic pollution and determine the required amount of sediment to be collected. Microplastics were detected in all 10 previous samples analyzed, which were collected in April and May of 2023 from sites 1, 2, 4, and 6. After this previous evaluation, triplicate samples were collected continuously bimonthly from June 2023 to April 2024, totaling 18 samples each time and 108 in total. In addition to the sediment samples, water column depth and transparency were measured on-site during sampling using respectively, a handheld echo sounder and a Secchi disk.

The sampling sites were accessed using a metal self-rowing motor boat to transport personnel and equipment, which could cause resuspension, so the collection of the sediment was the last activity performed in each site, after approximately 15 minutes. Each sampling activity took place in a single day, following the order of sites 1 to 6. A Petit Ponar grab sampler was used to collect sediment from each site and rinsed thoroughly throughout the process to avoid cross-contamination. The sediment was divided into three full 330 mL paper containers. These containers were then

labeled, covered with aluminum foil to prevent plastic contact, and transported to the laboratory for analysis.

4.2.3 Sample processing

The samples were weighed and stored in glass canning jars with metallic lids, each containing 100 grams. The remaining amount was set aside to assess the organic matter, water content, and granulometric data. The analysis of microplastics was adapted from Laboratory Methods for the Analysis of Microplastics in the Marine Environment, by NOAA Marine Debris Program (National Oceanic and Atmospheric Administration) (Masura et al., 2015), a method used by many other authors (Chen et al., 2021; Lenaker et al., 2019; Olivatto et al., 2019; David et al., 2023; Polanco et al., 2020).

In each wet sediment sample, a zinc chloride solution was added until it reached 1 cm above the solids. The mixture was stirred until fully homogeneous and then left to settle, to perform the density separation of the microplastics. In recent studies (Rodrigues et al., 2018; Dronjak et al., 2022; Konechnaya et al., 2020), it was found that using a zinc chloride solution for sedimentation instead of lithium metatungstate improved effectiveness and efficiency. A concentration of 700 g/L (1.6–1.8 g/cm^3) was achieved by diluting the salt in distilled water (Rodrigues et al., 2020). The sedimentation process was faster for medium sand sediment, taking only one hour to settle, while the majority of medium and coarse clay sediment took much longer, about 12 hours. The lids were kept on the jars during this process to prevent microplastic contamination from the air.

After the sedimentation time had been completed, 4 mL of the surface liquid was collected using a 10 mL glass syringe and placed into labeled glass test tubes. Hydrogen peroxide 35% was then added to the samples. The test tubes were placed in a drying oven at 50 °C for 24 hours. The liquid portion was sieved using a 75 µm stainless still mesh and rinsed with distilled water to remove the salt. This mesh was chosen due to equipment availability. The microplastics were washed into a petri dish and then onto a labeled microscopic slide, which was also placed in a drying oven at 50 °C until dry. Throughout the process, the temperature was monitored and kept at

50 °C; respecting the 60 °C limit to preserve the plastic particles from heat exposure, which could cause damage or volume loss (Munno et al., 2018).

The particles were visually identified, examined, and classified under an optical biological microscope (Labor L2000) (Labor, 2024) at magnifications ranging from 40X to 400X. The microplastics were categorized based on their size, type, and color. This process provided information on the quantity of plastic particles per unit of sediment ($n.kg^{-1}$ dry weight). To validate the findings, randomly selected particles were analyzed using SEM-EDS (Tescan Mira) (Tescan, 2024).

Nitrile gloves were used to handle the samples to minimize contamination. The samples were also kept covered or confined for most of the process. Microscopic slides were used to assess ambient contamination from air, particularly during exposed periods, being left uncovered and then examined for microplastics with the microscope. The zinc chloride and distilled water were also verified for contamination, with the collection of the supernatant and examination. All laboratory glassware and instruments that came into direct contact with the samples were cleansed with distilled water.

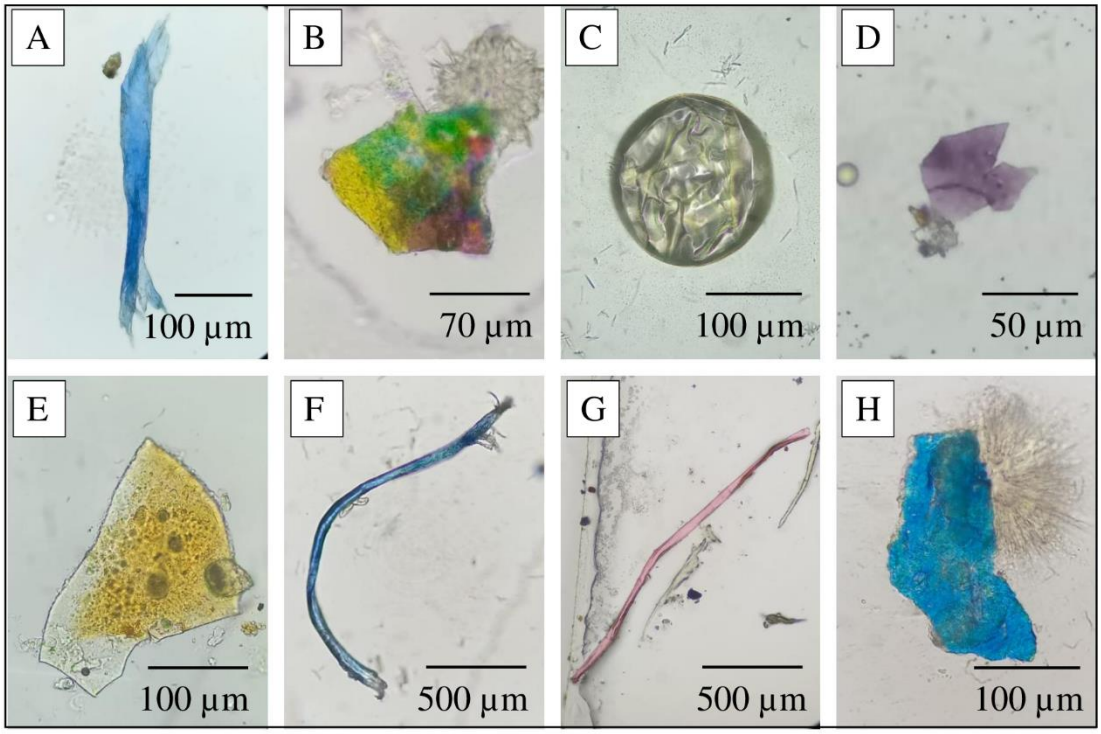
The depth and transparency of the water were measured on-site during sampling using a handheld echo sounder and a Secchi disk, respectively. The separated sediment reserved for further analysis was weighted and placed in a drying oven at 100 °C for 48 hours. Once fully dried, the samples were weighed again and disaggregated. The water content was calculated (Ishibashi et al., 2015). Sediment granulometric data was obtained using a Laser Diffraction Particle Size Analyzer (Bettersizer 2600) and for organic matter determination, the samples were dried in a muffle furnace at 540 °C for 3 hours and 30 minutes in porcelain crucibles.

4.3 RESULTS AND DISCUSSION

The study cataloged microplastics ranging from 5 mm to 75 µm. The visual identification follows the criteria of Horton et al., 2017 and Castro et al., 2024, meaning particles had to present an absence of organic and cellular structures, no segmentation, vivid color, bright or vitreous appearance, and constant thickness throughout the length, if fiber, to be considered as microplastic. In addition, a pin needle was used to verify the particles when necessary. Microplastics were found in all 108

samples taken from different sites on the Alagados Reservoir, including less populated areas near the river mouths and the water catchment area. A total of 3,459 particles were found, with 43.25% being filaments, 34.58% fibers, 10.38% fragments, 7.31% pellets, 2.49% foams, and 1.99% films (Figure 4). Throughout the year, the main concentration of microplastics in the sediment was 320.28 ± 162.83 n/kg of wet sediment.

Figure 4 – Microplastics found in the sediment from the Alagados Reservoir samples: Fibers (A, F, G), fragments (B, D, E, H), and pellet (C).



Source: The author.

The contamination was constantly monitored and accounted and was already subtracted from the presented values. During every sample preparing and examination under a microscope, which could take from 30 to 90 minutes, a clean microscopic slide was positioned nearby for monitoring the contamination from air. The slides were examined under a microscope after those periods and the microplastics were accounted and classified. In total, 604 particles were found, 53.64% filaments, 33.44% fibers, 7.28% fragments and 5.63% pellets. Foams and films were not found.

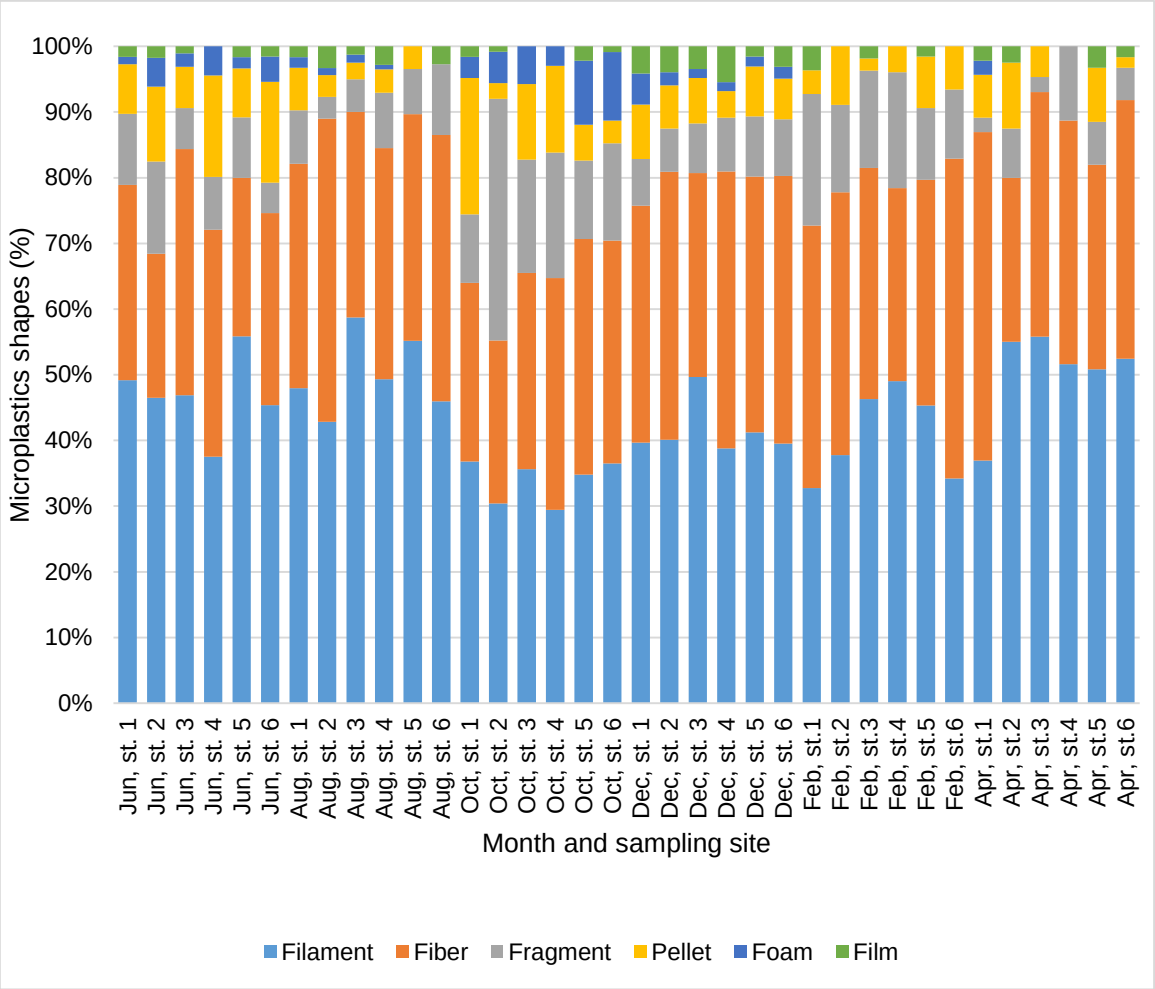
Microplastics concentrations were relatively low compared to similar studies conducted in Brazil. Queiroz *et al*. found a mean concentration of 6690 ± 1674.67 n/kg dw, in a study of a freshwater reservoir in a metropolitan area of São Paulo city, the largest city in Latin America (Queiroz et al., 2024; BBC, 2024). Guerolin *et al.* found concentrations ranging from 417 to 8178 n/kg dw in Amazon rivers, with the highest

values coming from shallow sites near the city of Manaus, an important industrial center (Gerolin et al, 2020). Those studies indicate a strong effect of the population density in the microplastics concentrations. This highlight the importance of monitoring and interventions regarding to waste generation and pollution along the development of small cities, in order to secure the control over the environmental scenario considering the urbanization process. The majority of microplastic studies in Brazil focus on marine environments, with beach sand being the main source of microplastic assessment in sediment. Therefore, there is a lack of data regarding freshwater environments (Castro et al., 2018).

Regarding global studies on freshwater sediment, the concentrations found in this research were higher than those found by Duan et al. (2024), on lake sediment samples in Lijiang, China, ranging from 10 to 115 n/kg. However, it was lower than those found by Lucas-Solis et al. (2021), on riverine depositional sediment in Puerto Misahualli, Ecuador, reaching up to 4200 n/kg dw. The presented concentration was also lower than that found by Corcoran et al. (2019), on river sediment of Ontario, Canada, ranging from 6 to 2444 n/kg dw, a mean of 612 n/kg dw. Parvin et al. (2022) found even higher concentrations, a mean of 13607 ± 13733 n/kg dw in lakes and rivers sediment of Dhaka, Bangladesh.

For the microplastic classification, filaments were considered as elongated stiff cylindrical forms; fibers were similar to filaments, but more thread-like and bendy. Fragments were considered as rigid particles with wider and plane surface areas. Pellets were particles with a regular spherical shape and clean edges, and foams were irregular shapes, with volume and irregular edges. Films were thin and wide pliable particles. Regarding the samples, 43.25% of the microplastics identified were filaments, 34.58% were fibers, 10.38% were fragments, 7.31% were pellets, 2.49% were foams and 1.99% were films (Figure 5). Those particles may have come from common daily activities of the community nearby the Alagados Reservoir, such as laundering and showering, releasing filaments, fibers and pellets of synthetic clothing and hygiene products into the environment (Luo et al., 2022). Other possible sources are tourism activities such as swimming, jet-skiing and hiking, agricultural interventions as plastic mulching and amateur and professional fishing (Gül, 2023; Khalid et al., 2023; Sharma et al., 2024). Additionally, microplastics could also come to the Alagados Reservoir transported by currents from upstream rivers (He et al., 2021).

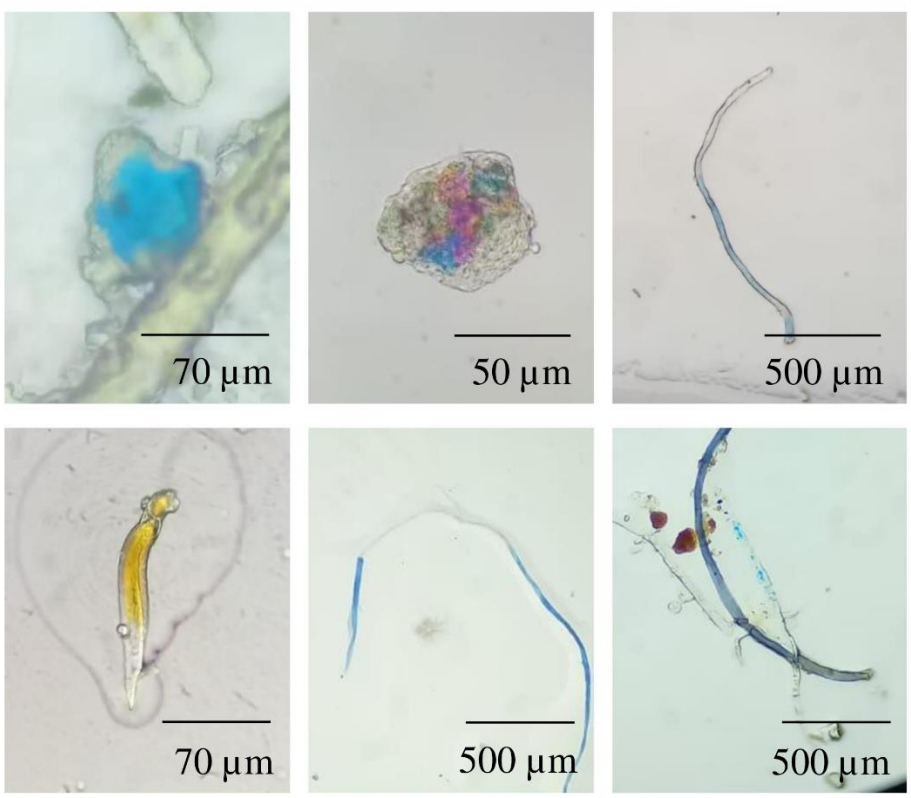
Figure 5 – Percentages of microplastic shapes in the Alagados Reservoir sediment sampling sites on each analyzed month from June 2023 to April 2024.



Source: The author.

4.73% of the microplastics measured between 5 and 2 mm, 45.40% measured between 2mm and 500µm, and 49.86% were smaller than 500 µm. 18.65% of the microplastics were colored, totaling 645, with an average of 5.97 ± 5.98. Among the colored microplastics, 36.9% were blue, 11.6% were pink, 21.5% were yellow, 9.3% were red, 3.9% were orange, 10.4% were brown, and 6.3% were green. The remaining microplastics were either white, transparent, or black. Some particles showed signs of color degradation (Figure 6), indicating the release of chemicals into the water and environment (Chen et al., 2022).

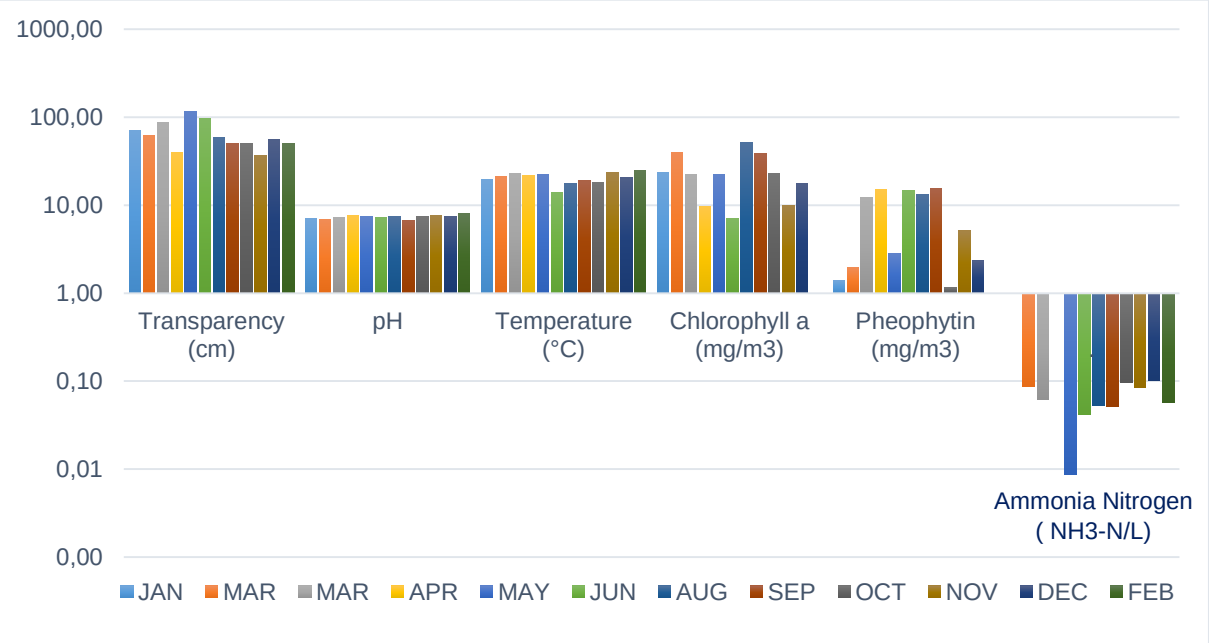
Figure 6 – Microplastics with fading color found in the sediment of Alagados Reservoir.



Source: The author.

The physical-chemical parameters of the water of the Reservoir are represented in Figure 7. The transparency had a mean of 64.28 ± 24.15 cm, measured with the Secchi disk. The pH was overall neutral and had a mean of 7.37 ± 0.34, measured with a portable pH meter. The temperature had a mean of 20.36 ± 3.03 ºC measured with a mercury thermometer. The chlorophyll a had a mean of 24.11 ± 13.92 mg/m³, the pheophytin had a mean of 7.76 ± 6.23 mg/m³ and the ammonia nitrogen had a mean of 0,06 ± 0.03 NH_3-N/L (CETESB, 2014).

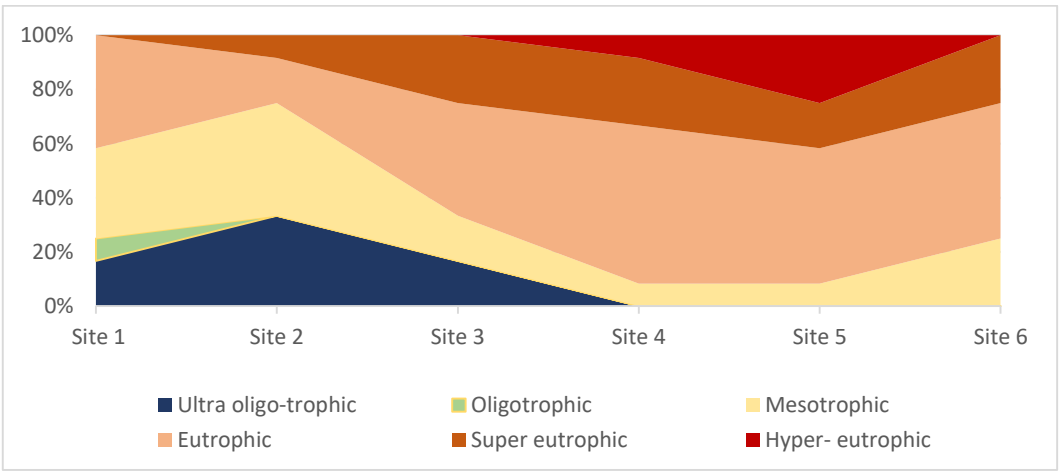
Figure 7 - Physical-chemical parameters of the water of the Reservoir



Source: The author.

The Trophic State Index (TSI) of the sites is shown in Figure 8, classified according to Lamparelli (2004) as ultra-oligotrophic, oligotrophic, mesotrophic, eutrophic, super-eutrophic and hyper-eutrophic. The graphic illustrates the classifications based on chlorophyll-a values. It is evident that the sites nearer to the river mouths exhibited lower concentrations of phytoplankton biomass compared to those closer to urbanized areas, particularly site 5. This trend may suggest a higher nutrient presence in the reservoir's water associated with human activities. Overall, the reservoir can be categorized as predominantly eutrophic.

Figure 8 - Trophic State Index (TSI) of the sites.



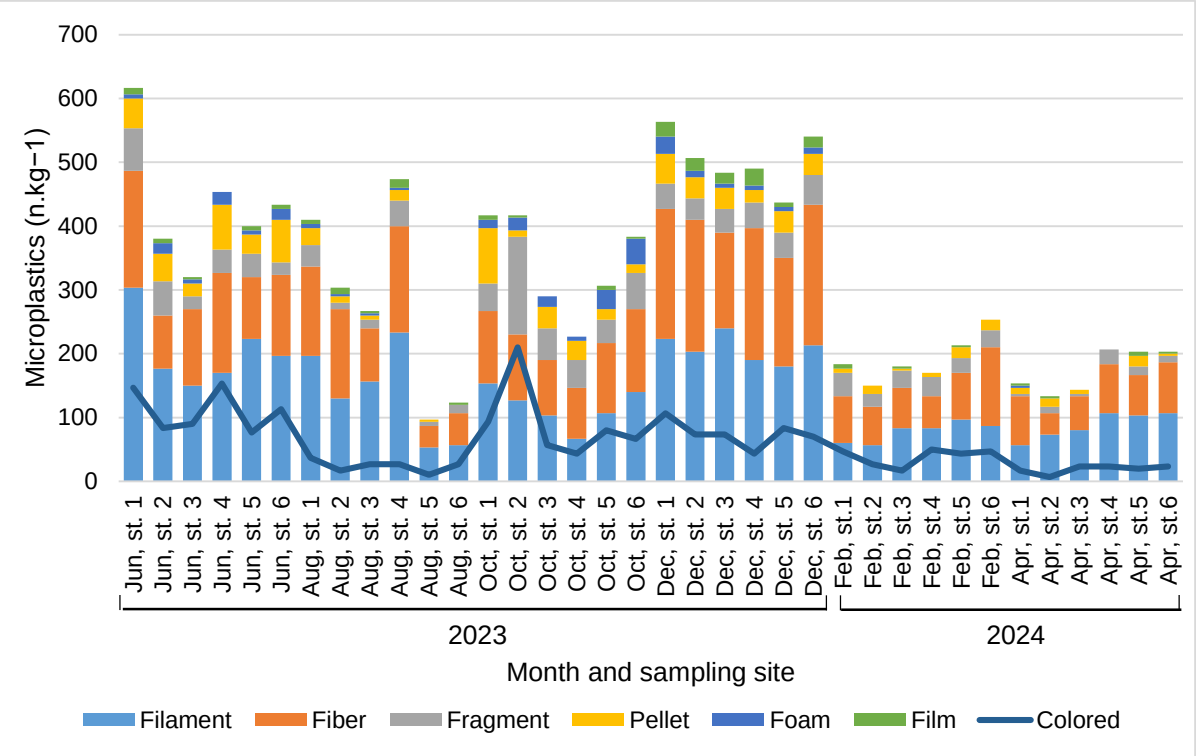
Source: The author.

The data on microplastic concentrations did not meet the normal distribution requirement for ANOVA, as indicated by the Shapiro-Wilk test (p-value of $1.53e^{-05}$). To meet this requirement, a logarithmic transformation was applied, after which the data met the normality requirement (p-value = 0.111). Subsequently, a two-way ANOVA and a Tukey's honestly significant difference (HSD) test were performed.

Based on the variance analysis chart and the Tukey's Test, it is evident that both the month and the sampling site impacted the microplastic concentrations. For instance, at site 1, the highest concentrations were in June, December, October, and August of 2023. At site 2, the concentrations were similar, with the samples from August 2023 and February 2024 showing intermediate values. Furthermore, at site 3, the highest concentration was observed in December 2023, while the lowest was in April 2024. At site 4, December, August, and June 2023 had the highest concentrations, whereas October 2023, February, and April 2024 had the lowest. Lastly, at sites 5 and 6, December 2023 still exhibited high concentrations, with the lowest concentrations observed in August 2023. Although there were variations in concentrations between months at each sampling site, the F-test revealed no significant statistical differences in concentrations among the sites for each month. The only exception was in August 2023, when the concentrations at sites 5 and 6 were lower than those at the other sites.

Statistical analysis of the samples from 2023 revealed higher concentrations compared to those from 2024. Notably, the peak values were recorded in June and December of 2023. This increase may be attributed to the Brazilian summer vacation, which leads to favorable conditions for fishing and aquatic activities, resulting in a higher occupancy at the yacht club and nearby residences close to the reservoir. The influx of visitors could contribute to the release of microplastics into the water and sediment due to various daily activities such as laundering, swimming, fishing, canoeing, and jet-skiing, among others.

Figure 9 – Characterization of microplastics found in the sediment, considering the average of all collection sites, from June 2023 to April 2024.



Source: The author.

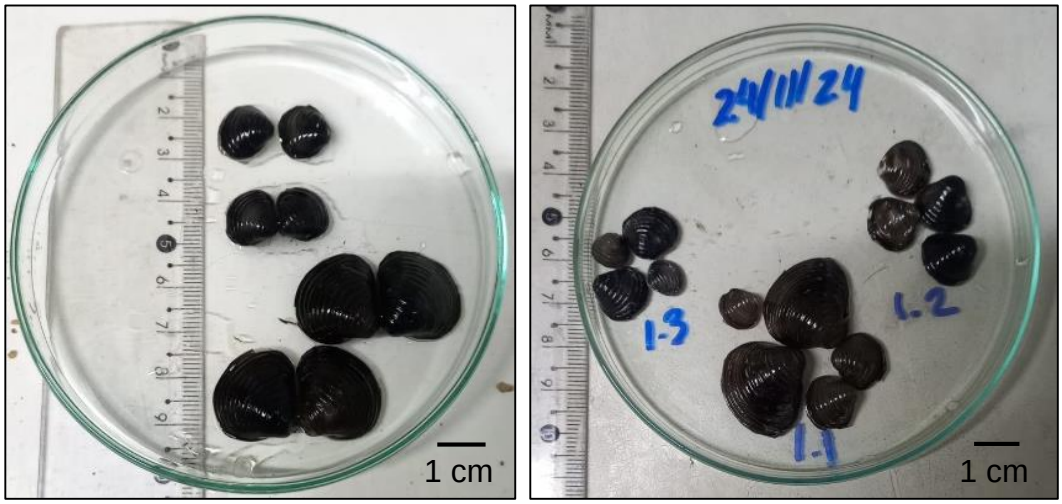
Based on the Pearson Test, it was found that there is a positive correlation between microplastic concentration in sediment and water transparency ($r = 0.37$, $p = 0.026$). It means that higher values of transparency are linked to higher concentrations of microplastics in the sediment, indicating that the sedimentation of the suspended solids implies their deposition on the bottom of the riverbed. It could be a consequence of previous turbidity currents with higher concentrations of suspended solids, which contributed to the deposit of microplastics, after their settling (Pohl et al., 2020; Zhang et al., 2024).

Additionally, there is a negative correlation between microplastic concentration and water temperature ($r = -0.41$, $p = 0,01$) which had a mean of 19.8ºC ± 3.5. This could be a sign that the cold temperature of the surface water favors the deposition of the microplastics, given that the cold water has a higher density than the warm water. This difference of the densities based on temperature would lead the microplastics to float in warm temperatures and settle in it is cold. There was no significant correlation between microplastic concentration and water depth ($r = 0.32$, $p = 0.061$).

The sediment had an average humidity of 70.15% with a standard deviation of 14.93 and organic matter of 2.71% with a standard deviation of 0.82. During sample processing, bivalve shells (*Corbicula fluminea*) were found in the sediment (Figure 10) (Araujo et al., 1993), mainly at sites 1 and 2. The shells were mostly still hanging in pairs at the processing of the sample, indicating the organisms were likely captured alive or recently deceased.

The presence of microplastics in the sediment suggests a potential impact on benthic organisms in Alagados Reservoir. Bivalves living in the sediment can ingest these particles, leading to behavioral impacts (Urban-Malinga et al., 2021) and potentially passing microplastics to their predators, including fish and even humans (Zhang et al., 2019). Vidal et al. (2023) found a mean concentration of 8.8 ± 10.6 microplastics per individual of *Corbicula fluminea* in a study of the Loire River, in Paris, highlighting a correlation between the clams and sediment microplastic concentration. Regarding filtrating organisms, according to Wootton et al. (2022), globally microplastics are present in 94.4% of oysters, with an average of 1.41 ± 0.33 particles per individual.

Figure 10 – Bivalve Shells found in the Alagados Reservoir sediment.



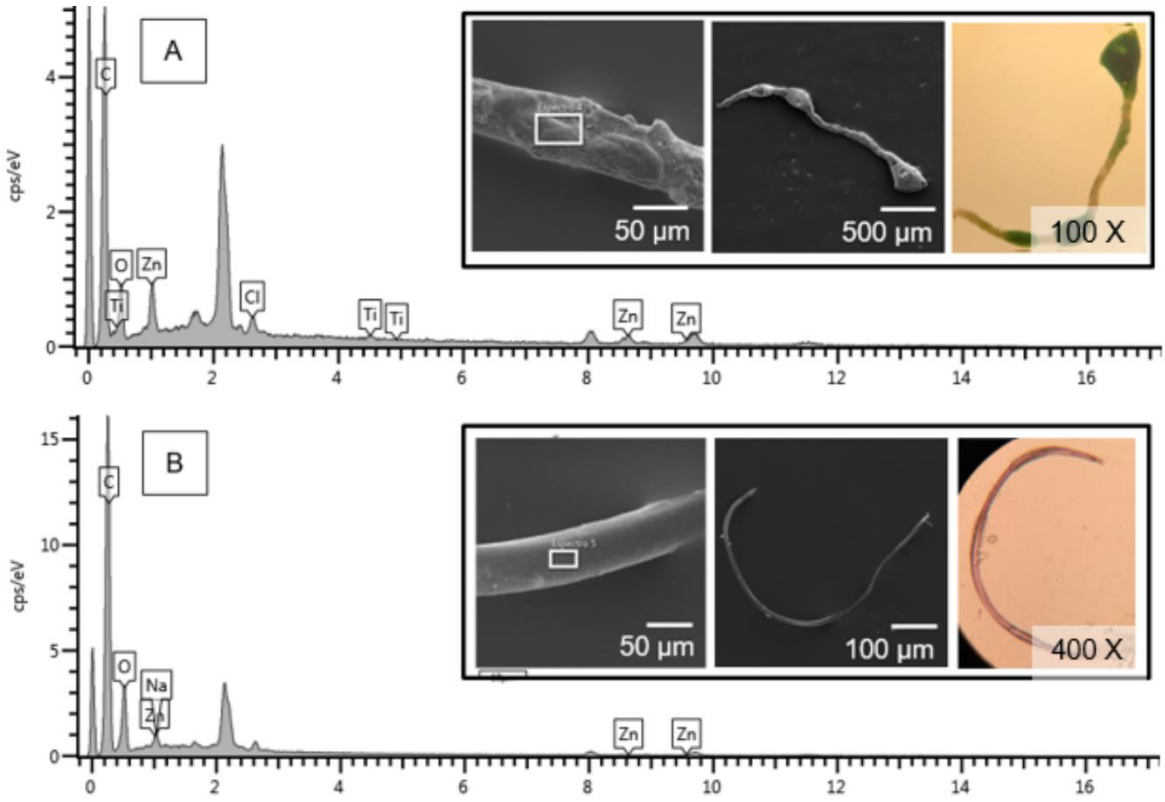
Source: The author.

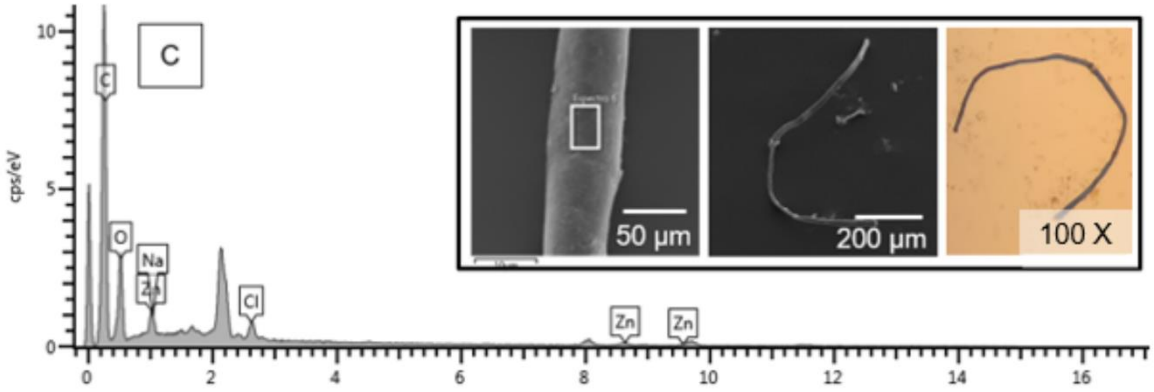
The samples were classified into Udden grades (1914) (Uden et al., 1914, apud Valentine et al., 2019), based on their Mean Weight Diameter (MWD). The samples from sites 1 and 2 were classified as medium clay, while those from sites 3, 4, and 5 were classified as coarse clay. The samples from site 6 had both coarse clay and medium sand. It was observed that the samples often had two distinct granulometries depending on the specific location they were collected from.

Additionally, in some cases, the petite ponar grab sampler had to be thrown more than once to collect sediment, suggesting the presence of a rock bed under the river close to the water catchment area.

Analysis using SEM-EDS revealed the presence of zinc chloride in 83.33% of the 18 samples tested (Figure 11). The concentration ranged from 1.64% to 11.60%, with an average of 5.92% (± 3.24). This was despite the samples being thoroughly rinsed with distilled water. This finding supports the idea that microplastics have a tendency to chemically absorb substances, including pharmaceuticals, toxic metals, and other harmful materials, posing a risk to the environment and human health (Fred-Ahmadu et al., 2020). Furthermore, Persistent Organic Pollutants (POPs), which can be absorbed by microplastics, have recently been discovered in the roe, viscera, and muscle of fish in Alagados Reservoir (Stremel et al, 2023). The SEM-EDS analysis also detected traces of titanium (Figure 11 A), similar to Nayeri et al. (2023), which is commonly used in pigments such as titanium dioxide (Turner et al., 2023).

Figure 11 – SEM-EDS results of fiber with green beads (A), pink rigid fiber (B), and blue soft fiber (C).

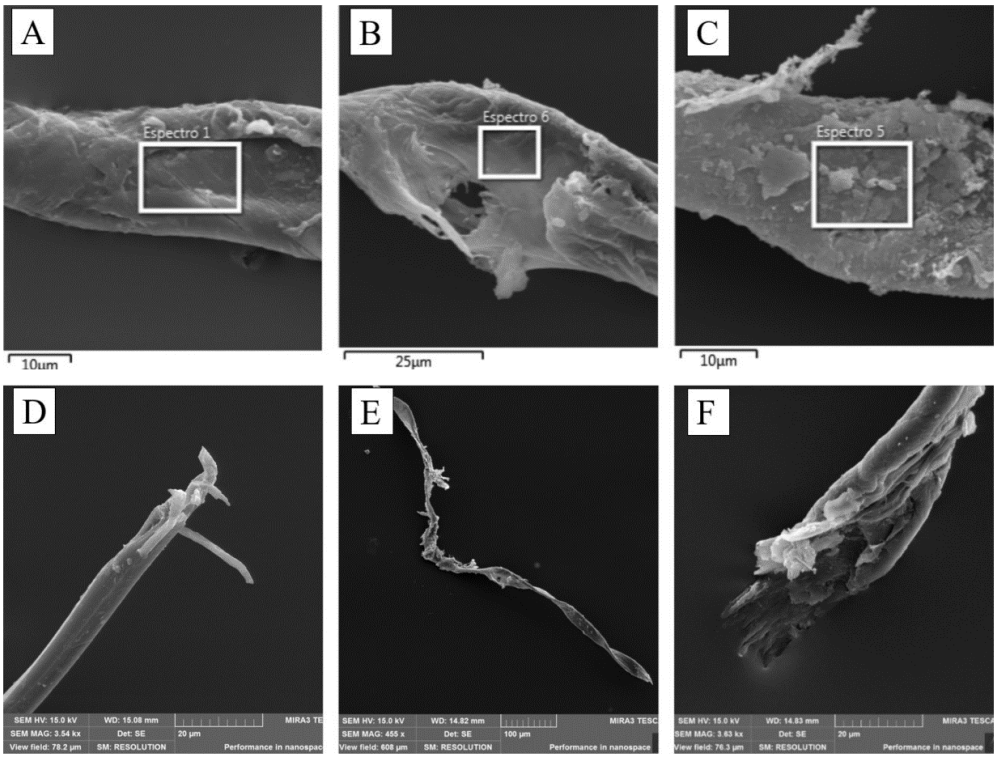




Source: The author.

In the analysis of 18 samples, SEM-EDS revealed the presence of mainly carbon, oxygen, chlorine, and silicon, supporting their identification as microplastics (Ratnam et al., 2022). There were also traces of sodium, iron, sulfur, aluminum, titanium, and calcium, which could be from pigments or a result of the microplastics retaining elements on the sediment. Certain particles in the images (Figure 12) showed signs of deterioration, suggesting the potential entry of even smaller plastics into the environment (nanoplastics). These may have different shapes and properties, resembling natural colloids with negatively charged surfaces

Figure 12 – Microplastics details. Superficial fissures (A), deterioration (B), flaking (C), and fraying (D, E, F).



Source: The author.

Microplastics were found to be widespread in the sediment of the Alagados Reservoir, even in its most preserved areas. This assessment offers valuable insights into microplastic pollution, which can inform future interventions and help trace the sources of the problem, ultimately aiding in mitigating its impact on the environment and public health. Recommended control and mitigation measures include investigating and eliminating improper sewage discharges in and around the Alagados Reservoir, supervising fishing activities, and imposing penalties for violations such as littering and the abandonment of fishing nets along the shores. Although the concentrations of microplastics were relatively low compared to larger cities, the situation in the Alagados Reservoir could deteriorate with population growth and the increased expansion of human activities, underscoring the need for further studies and proactive measures regarding microplastics.

4.4 CONCLUSION

In this study was found microplastic particles in all samples, in various shapes and sizes, throughout the year. This indicates a widespread presence and an environmental issue for the aquatic ecosystem. The most common shapes observed were filaments and fibers, and the majority of microplastics were uncolored. Some microplastics were also found with faded color, suggesting pigment released into the water and sediment. There was a statistical correlation between microplastic concentrations in the sediment and elapsed time, transparency of the water, and water temperature, but not with water depth. Based on these results, it is important to gather more data and analyze microplastics in freshwater and riverbed sediments, especially those related to human consumption. While the microplastic contamination in the Alagados Reservoir is relatively low compared to similar locations, further studies and consistent monitoring are necessary.

4.5 INTEREST CONFLICT DECLARATION

The development of this article did not involve any conflicts of interest.

ACKNOWLEDGEMENTS

The authors would like to thank the Water and Sanitation Company of Parana (Sanepar), the State University of Ponta Grossa, and the Multi-User Laboratory Complex (C – LABMU) for all the assistance received during the processing of the analysis. This study was financed in part by the *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES)* – Finance Code 001 and the *Fundação Araucária* (agreement 211/2022).

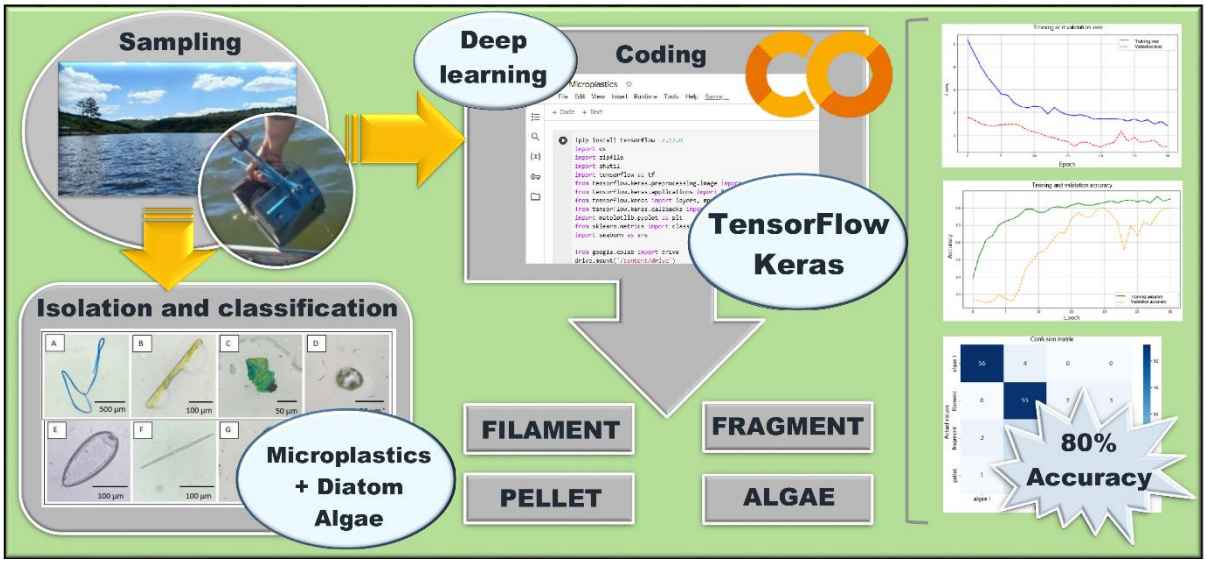
5 ARTICLE 2 – APPLICATION OF A CONVOLUTIONAL NEURAL NETWORK FOR AUTOMATED MULTICLASS IDENTIFICATION OF MICROPLASTICS FROM OPTICAL MICROSCOPY IMAGES

Abstract

Microplastics are present all around the globe, and they are a major threat to the environment because of the challenges they represent. Their sampling, isolation, and analysis are laborious and difficult due to their size, shape, and spreading dynamics. Furthermore, the lack of standardized protocols in microplastic research makes it difficult to compare results and unify the progress of the field. In this context, this work proposes and evaluates a model architecture based on deep learning to classify microplastic images using a dataset of real microplastics sampled from a freshwater reservoir, with Convolutional Neural Network (CNN) and Transfer Learning. Moreover, the model also identifies diatom algae frustules, which can persist the hydrogen peroxide degradation in the process of microplastic isolation due to their biosilica composition. The model was developed in Python using the Google Colab environment. 1140 images were used, 80% for training and 20% for validation. The model had an 80% accuracy, reaching a recall of 93% and 92% for algae and microplastic filaments, respectively, despite still improvement in other classes and aspects. Overall, the accuracy of the model is encouraging considering the dataset size and all the challenges that involve the automatic identification of microplastics, with all their shape variations and nuances, but the results are promising. To our knowledge, this is the first work addressing diatom presence after one of the most common microplastic isolation techniques and their automated classification among microplastics as well.

Keywords: Deep learning; diatoms; freshwater; microplastics.

The article's graphical abstract summarizes the research methodology.



Source: The author.

5.1 INTRODUCTION

Microplastics are defined as polymeric particles up to 5 millimeters. They can be classified as primary, which are produced at microscopic dimensions, such as those found in hygiene products for exfoliation, or secondary, which are fragments of larger plastics that have deteriorated due to environmental factors. Assessing microplastics presents significant challenges due to their characteristics, sources, distribution dynamics, and persistence in the environment (BLL, Waterhouse, 2023). Microplastics are ubiquitous, and analyzing them is complicated by the lack of standardized methodologies and access to affordable, proper equipment. Additionally, the analysis of microplastics tends to be labor-intensive and time-consuming. Many studies rely on human visual inspection using stereomicroscopy to examine particles, with only a small fraction undergoing further validation through techniques like FEG-EDS, Raman or FTIR (Drabinski et al., 2023; Castro et al., 2024; Gerolin et al., 2020; Strady et al., 2021; Vidal et al., 2023; Duan et al., 2024; Lucas - Solis et al., 2021; Parvin et al., 2022; Nayeri et al., 2023; Kurki-Fox et al., 2023; Corcoran et al., 2019; Saad et al., 2024; Egessa et al., 2020; Nan et al., 2020).

Those studies usually classify microplastics according to their shape. This is relevant for understanding microplastic dynamics, how these particles are present in different environments, as well as their behavior, sources, properties, interactions, and

implications within the environment. This could lead to future specific solutions applied to the reduction and removal of microplastic, enhancing its efficiency. However, the classification process, as it is, implies subjectivity and bias from various researchers around the world. To solve this problem, this work aims to present and evaluate the application of deep learning in the multiclass classification of microplastics through convolutional neural networks (CNNs).

In addition, it presents the simultaneous identification of algae, specifically diatom, since these were a common encounter in this work while handling microplastics, thus, the differentiation of one another was required. Diatoms are unicellular algae that have cell walls made of amorphous silica dioxide in two overlapping frustules, capable of remaining after organic matter degradation with hydrogen peroxide, which is often used in microplastic isolation protocols (Barsanti, Gualtieri, 2023; Sun et al., 2024). Therefore, diatoms can remain after the microplastic isolation process and be found among them.

Deep learning consists of an evolved machine learning technique in which the algorithm can learn rules, decision boundaries, or a mathematical function to process the input, in this case, microplastic images, and output the desired information, namely the microplastic class. The ResNet50 is a previously trained model using the ImageNet database, capable of classifying images by learning feature maps (MathWorks, 2024; Annable, 2024).

Applying deep learning for the classification of microplastics presents significant challenges. First, a dataset of microplastic images is necessary, but currently, there is no available database. To accurately depict the true characteristics of microplastics as a pollution agent, it is essential to collect samples directly from the environment instead of generating artificial particles and images. The process involves collecting microplastics through media sampling, isolating and preparing the samples, and conducting thorough analyses. Afterward, images must be captured under suitable conditions and at an ideal resolution. These images must be properly sorted, treated, and of sufficient quantity to provide substantial input for the model.

The most challenging aspect is the heterogeneity of real microplastic shapes, even under the same class, when compared to artificially generated images or even microplastics obtained under controlled conditions (Giardino et al., 2023). For example, fibers could be large, thin, big, small, tangled, straight, bright colored, opaque, or transparent, similar to other classes, with many other singularities. While the human

brain naturally recognizes complex visual patterns, achieving this through an automated algorithm presents a challenging task. (Nielsen, 2024).

Many authors have addressed the automated processing of microplastics in recent years, but the solutions often require expensive, specific equipment, products, or software. Giardino et al. (2023) proposed a semi-automatic image processing method for quantifying and measuring stained fluorescent microplastics using Nile Red dye. Lee et al. (2023) investigated the automatic identification of microplastics from their Raman spectra, similar to Shi et al. (2022), through scanning electron micrographs, but with also classification.

Lorenzo-Navarro (2021) developed an architecture to count and classify microplastics in images taken on a digital camera or a mobile phone with a resolution of at least 16 million pixels, without the need for microscopes, but only considering microplastics in the size range of 1 to 5 millimeters.

Since locations with significant pollution and sanitation problems are linked to higher microplastic concentrations and consequently environmental vulnerability (Strady et al., 2021), it is necessary to develop easy access, affordable, and effective solutions to facilitate the study of microplastics, especially in developing and underdeveloped countries.

5.2 METHODOLOGY

5.2.1 Microplastic isolation

The microplastics used to train the model were isolated from sediment samples of Alagados Reservoir, which is responsible in part for the city of Ponta Grossa's water supply. It is located in South Brazil, between the coordinates 24°59’ to 25º01' S and 49°58’ to 50º03' W (Lemos et al., 2014). The samples were collected with a ponar grab six different times over 12 months. The sample processing was adapted from Laboratory Methods for the Analysis of Microplastics in the Marine Environment, by NOAA Marine Debris Program (National Oceanic and Atmospheric Administration) (Masura et al., 2015), and consisted of density separation, sieving, organic matter digestion and drying.

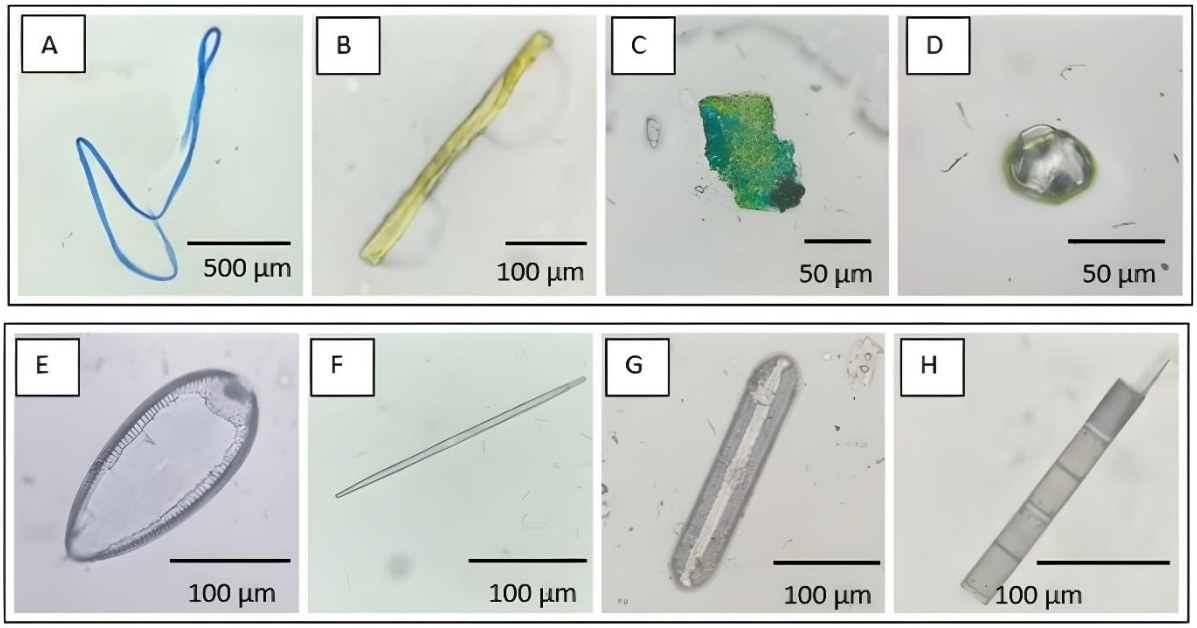
The microplastics were visually identified with an optic microscope at magnifications ranging from 40X to 400X, retrieving particles in the range of 0.075 to 5 millimeters, which were classified and photographed. The photos were taken with a smartphone Galaxy A22, in 2250 per 4000 pixels. A parcel of the microplastics was submitted to SEM-EDS (Tescan Mira) for further investigation and validation (Tescan, 2024).

5.2.2 Input images

A total of 3,459 particles were identified through visual inspection. Of these, 43.25% were classified as filaments, 34.58% as fibers, 10.38% as fragments, 7.31% as pellets, 2.49% as foams, and 1.99% as films. Although all particles were photographed, not all images were used as input for training the model due to issues with image quality or cluttered backgrounds. Additionally, the classes for fragments, foams, and films lacked sufficient images to enable further classification. For pretreatment, the images were cropped to sizes ranging from 600 x 600 to 1700 x 1700 pixels. These images were then organized into folders, compressed into zip files, and uploaded to Google Drive.

A total of 953 fibers, 938 filaments, 300 fragments, 240 pellets, and 300 algae from the genus *Surirella* were manually selected and treated in this analysis. Other diatom genera, such as *Synedra, Pinnularia,* and *Aulacoseira* were also observed during the microplastic analysis, however, since they had 60 or fewer photographs each, they were excluded from the analysis for insufficient input to train the model. To ensure that the number of images was equal across each class for the training process, we standardized the dataset to include 300 algae, 300 filaments, 300 fragments, and 240 pellets. This resulted in a total of 1,140 images, with 912 allocated for training and 228 for validation. An ideal scenario would involve increasing the dataset size to further enhance the model’s training and precision. The microplastics and algae are illustrated in Figure 13.

Figure 13 – Microplastics and Algae observed in the samples. (A): Fiber, (B): filament, (C): fragment, (D): pellet, (E): *Surirella sp.*, (F): *Synedra sp.*, (G): *Pinnularia sp.*, (H): *Aulacoseira sp.*.



Source: The author.

5.2.3 Model

The algorithm was coded in Python version 3.10.12 via Google Colab, using TensorFlow version 2.17.0 with Keras API. The architecture was based on the books by Ansari (2024), Chollet (2018), Zhang et al. (2023), and Gosh and Math (2023) with the structure and debugging assistance of artificial intelligence tools. The code was structured to use TensorFlow's deep learning to train the identification of microplastics in classes with a dataset of classified images of real microplastics, using Resnet50 architecture of convolutional neural network (CNN) and transfer learning to classify new images. The code was executed in a Dell Inspiron 15 5000, core i5 with a RAM of 4 gigabytes.

The process of coding optimization involves multiple iterations of architecture and input modifications. The final model architecture begins with the installation of TensorFlow and the importation of necessary libraries. It then proceeds to extract the microplastic images stored in a zip file on Google Drive, creating a directory for the extracted folder. The microplastic shapes can vary in color, primarily being transparent or displaying faded hues. After testing and consideration, the next step in the code was to convert the images to grayscale. This transformation ensures that the classification

focuses on the shape of the particles rather than associating specific colors with particular shapes.

Data augmentation was employed to expand the input dataset. The images were resized to 256 by 256 pixels, which is a standard size for CNN analysis. This size strikes a balance between higher detail compared to smaller sizes and better computational efficiency than larger sizes. Larger dimensions, such as 526 or 1024 pixels would offer more detail, but the available equipment could not support them. The dataset was split into 80% for training and 20% for validation, with class weights set inversely proportional to the number of images in each class.

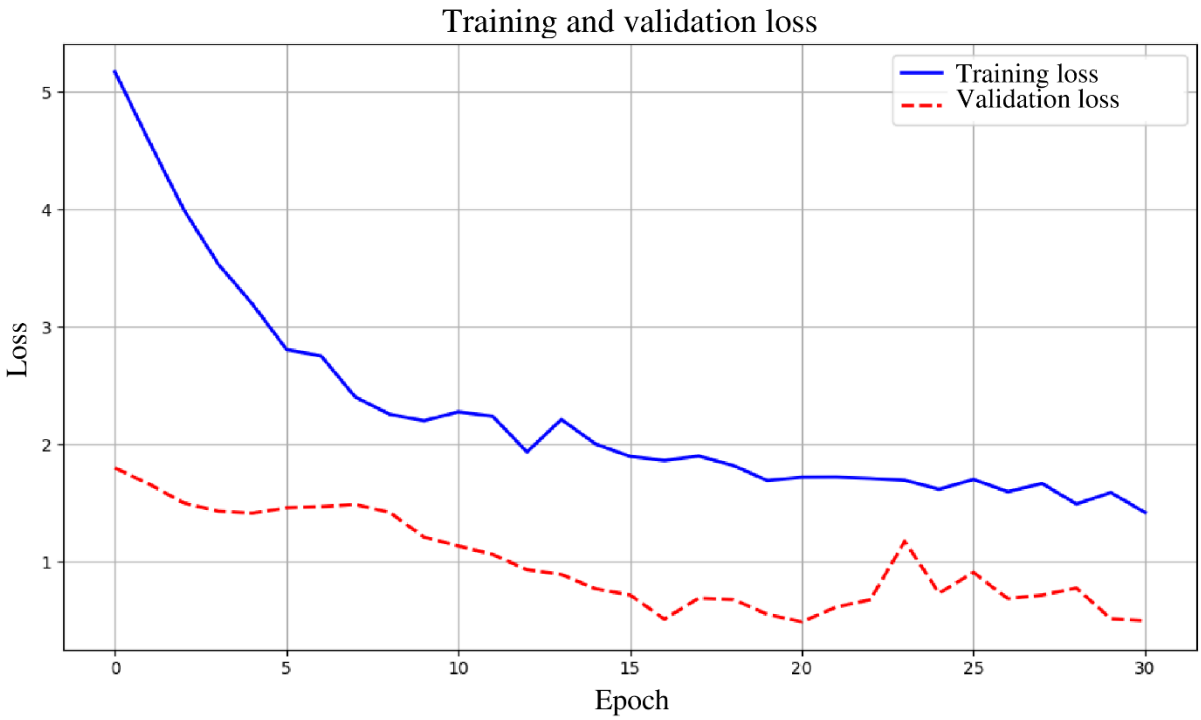
Since ResNet50 is a pre-trained model, fine-tuning was achieved by unfreezing the first 100 layers for training. The output layer corresponds to the classes of the microplastics and algae. The model was then compiled using the Adam optimizer to adjust the weights during deep learning. To prevent overfitting - where the model performs well on the training dataset but fails to generalize to new data - an early stopping function was implemented with a patience of 10 epochs. The accuracy and loss for each epoch were printed during training for monitoring purposes. After the conclusion of the training, it was shown the details about the process, precision, recall, F1-score, and confusion matrix.

5.3 RESULTS AND DISCUSSION

The execution of the model resulted in 32 batches of 29 images per epoch, taking an average of 21 seconds per step, and approximately 594 to 669 seconds per epoch. The training was halted at epoch 31 of 50 due to early stopping, which was triggered by a patience of 10. This means that after epoch 31, the validation accuracy stopped improving and the model was not generalizing any better, despite the increase in training accuracy.

The loss metrics during training and validation displayed in Figure 14 shows a decline in both cases over the epochs, indicating that the model is learning and that the predictions are increasingly close to the ground truth. The training loss decreased significantly until epoch 10, while the validation loss exhibited a more gradual decline throughout all epochs. Notably, epoch 24 marked a peak invalidation loss.

Figure 14 – Graphical representation of the training and validation loss.



Source: The author.

The training and validation accuracy are illustrated in Figure 15. The training accuracy showed an increase throughout the process, rising at a higher rate during the first 9 epochs, as expected. In the first epochs, the model is most likely adjusting the weights while learning basic features. As the training progresses, it begins to learn more refined and complex features, which slows down the pace of improvement after the initial epochs. It began at 35.80% in the first epoch and reached 86.14% by epoch 31.

The validation accuracy started at 26.32% and was steady at the first epochs until greatly improved, reaching 79.20% at epoch 21 after consistently increasing since epoch 8. It was also expected, as the model was beginning to adjust its parameters and had not yet developed sufficient learning to generalize well to the validation set. However, it experienced a fluctuation, dropping to 55,70% in epoch 24, before finishing at 79.82% in epoch 31.

Figure 15 – Graphical representation of the training and validation accuracy.

Source: The author.

The model report, presented in Table 2, outlines the prediction results derived from the validation dataset. Among the various types of microplastics assessed, fragments were identified with the highest precision at 86%, followed by pellets at 76% and filaments at 70%. Notably, the model achieved an impressive precision rate of 93% in identifying the algae class, likely due to the consistent shape of algae compared to the diverse shapes observed in microplastics, even within the same classification. This variability underscores the necessity for larger datasets to enhance the model's training. By expanding the dataset, we can improve pattern recognition and produce more accurate predictions.

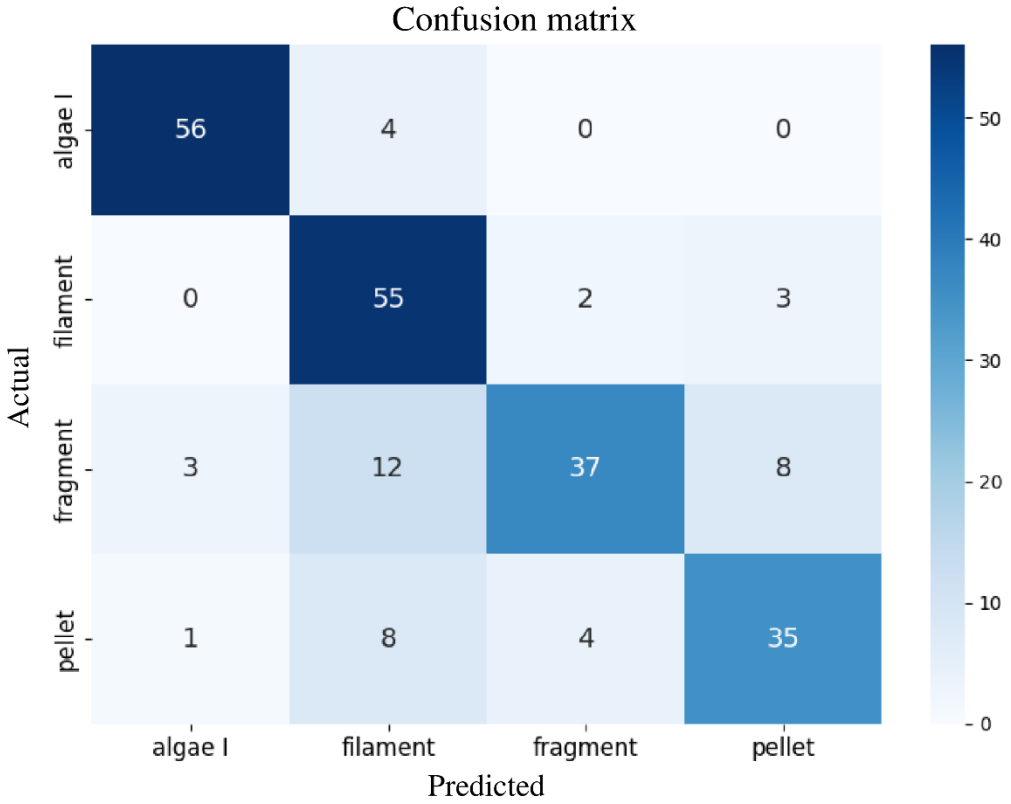
Table 2 – Graphical representation of the training and validation accuracy.

	Precision	Recall	F-1 score	Support
filament	0.70	0.92	0.79	60
fragment	0.86	0.62	0.72	60
pellet	0.76	0.73	0.74	48
Algae	0.93	0.93	0.93	60
Macro avg	0.81	0.80	0.80	228
Weighted avg	0.82	0.80	0.80	228
Accuracy			0.80	228

Source: The author.

In contrast, filaments showed the highest recall among the microplastics with a rate of 92%. This was followed by pellets at 73% and fragments at 62%. Algae also had a high recall value of 93%. The difference between the precision and recall for the filament category indicates that the model is generating more false positives and fewer false negatives, accurately predicting real positives at a rate of 92%. Conversely, for the fragment class, the model tends to generate more false negatives than false positives. This discrepancy is reflected in the F1 score, which is the harmonic mean of precision and recall. The support refers to the size of the validation sample, while accuracy is the mean of the F1 score. An accuracy value of 80% is satisfactory given the dataset size; however, there is still a need for improvement with a larger training input. The confusion matrix (Figure 16) illustrates the actual values, false negatives, and false positives of the predictions.

Figure 16 – Confusion matrix of the validation dataset, with actual values (ground truth), false negatives, and false positives of the predictions.



Source: The author.

The algae class had the highest number of true positive predictions, followed by filaments, fragments, and pellets. The most significant errors involved misidentifying

actual fragments as filaments (12 instances), actual pellets as filaments (8 instances), and actual fragments as pellets (8 instances). This suggests that the model has a tendency to over-predict filaments and struggles with accurately classifying pellets, resulting in numerous false positives and false negatives for this category. These issues also impacted the precision and recall for the fragments.

After conducting training and validation, the model was prepared to predict new images, displaying their filenames and predicted classes. The architecture of the model was designed to classify each image from a specific folder, returning results in the format: "Prediction for the image ({image_path}): {predicted_class}". Each prediction was completed in less than a second. The model achieved an overall accuracy of 80% when tested with various folders.

5.4 CONCLUSION

This study is the first to address the presence of diatom frustules following the most common microplastic isolation protocol, which involves degrading organic matter using hydrogen peroxide. Additionally, it introduces an automatic classification system that categorizes microplastics into four distinct groups. The model’s overall accuracy is 80%, and its precision in recognizing algae is 93. These results are promising, especially considering the size of the dataset and the challenges associated with the automatic identification of microplastics, which come in various shapes. Future research should expand the dataset to include over 1,000 images per class and incorporate additional diatom species in the classification, as these can resemble microplastic filaments. Accurate identification of these algae is essential.

5.5 INTEREST CONFLICT DECLARATION

The development of this article did not involve any conflicts of interest.

ACKNOWLEDGEMENTS

The authors would like to thank the Water and Sanitation Company of Parana (SANEPAR) and the State University of Ponta Grossa for all the assistance received

during the processing of the analysis. This study was financed in part by the *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES)* – Finance Code 001 and the *Fundação Araucária* (agreement 211/2022).

6 FINAL CONSIDERATIONS

The global population's growth has significantly increased waste generation over the last few centuries. Plastics, while being useful for a wide range of applications, also present a complex and urgent challenge, particularly in the form of microplastics. These tiny particles can easily spread unnoticed, contaminating various environments, including air, water, sediment, snow, and living organisms like plants, animals, zooplankton, worms, fishes, and even humans. The pressing concern is no longer whether microplastics are present in our surroundings, but rather how much of them exist.

The assessment of microplastics in the Alagados Reservoir highlights a significant environmental issue. All sediment samples collected during the analyzed period contained microplastics, indicating their persistent presence in the reservoir. This situation exposes aquatic organisms, particularly benthic species, to these tiny particles and the potentially hazardous chemicals they may retain or release. Consequently, there is a risk of microplastics entering the food web; however, further studies are necessary to confirm this.

Certain conditions can lead to the resuspension of microplastics from sediment back into the water column. Given that Alagados serves as a water supply reservoir, it is essential to monitor water quality. By tracking and removing microplastics from water sources, we can prevent their accumulation in water treatment plants and ensure better drinking water quality. Microplastic concentrations and their physical-chemical characteristics should be included as measured parameters in drinking water assessments. While microplastics have been found in the human body, research on their long-term effects on human health is ongoing, making it crucial to take precautions to minimize any potential risks.

Although the level of microplastic contamination in the Alagados Reservoir is significantly lower compared to other similar studies, it remains a source of concern that require further investigation and the implementation of preventive strategies. These strategies are necessary to reduce microplastic contamination and prevent worsening conditions that could harm both the ecosystem and human health. Numerous studies have shown the negative effects of improper wastewater discharge and waste disposal near water bodies. Therefore, it is vital to ensure proper

infrastructure, sanitation, and vigilance policies in areas surrounding the reservoir, which have experienced increased residential development in recent decades.

Future research should focus on the pathways through which microplastics enter the reservoir, their composition, and their correlation with concentrations in water and aquatic organisms. Additionally, risk assessments and the dynamics of sedimentation and resuspension warrant attention. Continuous and enhanced data collection is vital for a thorough assessment of microplastics in the Alagados Reservoir.

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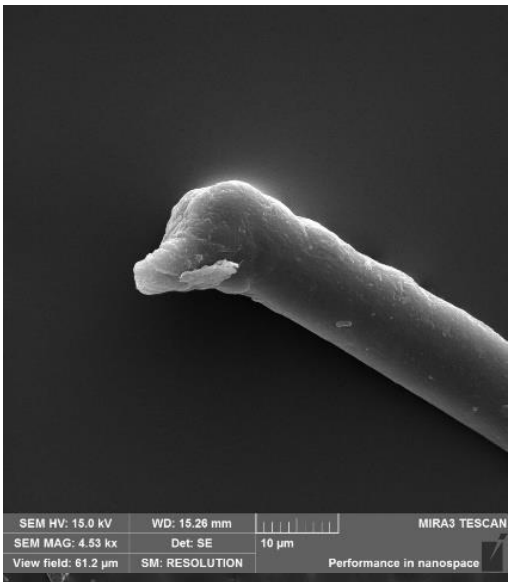
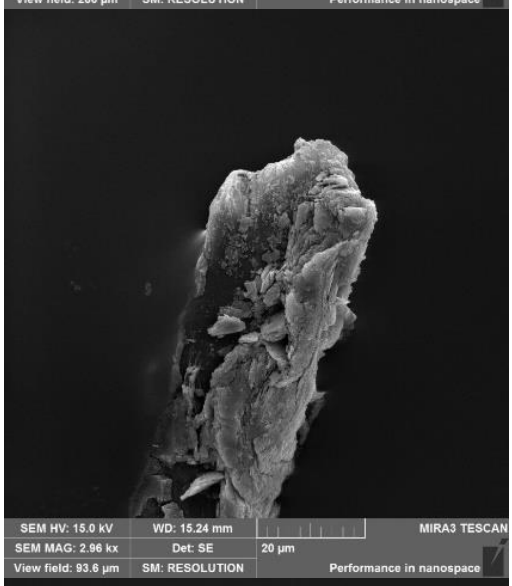
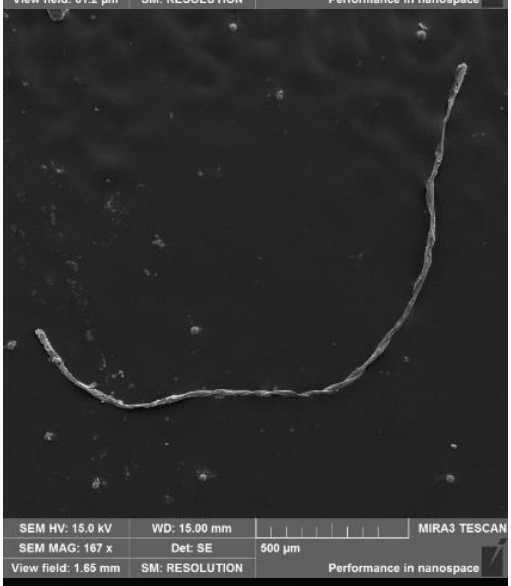
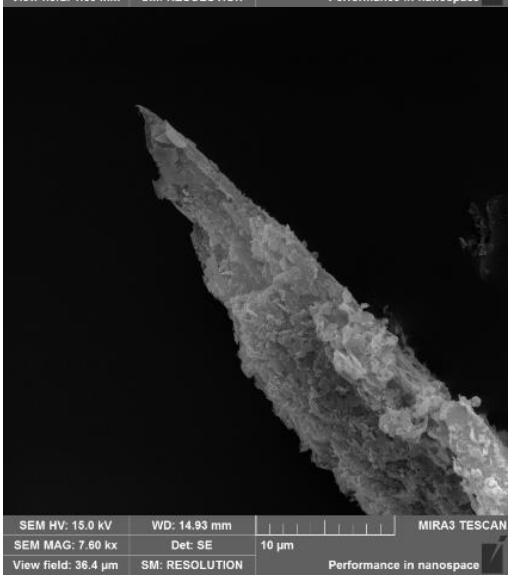
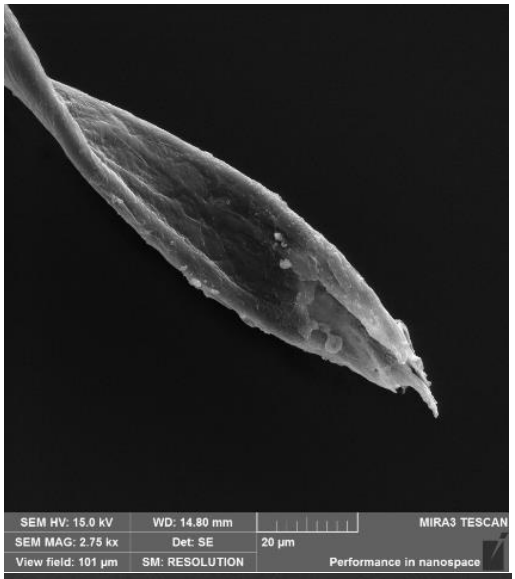
ANNEX A – IMAGES OF MICROPLASTICS FROM SEM



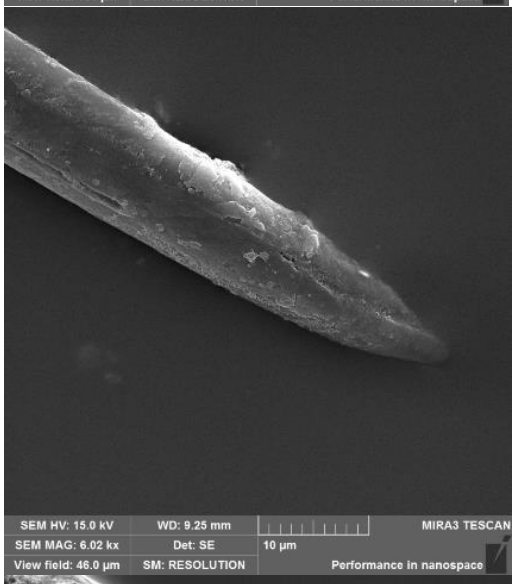







SEM HV: 15.0 kV
WD: 14.80 mm
MIRA3 TESCAN
SEM MAG: 2.75 kx
Det: SE
20 µm
View field: 101 µm
SM: RESOLUTION
Performance in nanospace

SEM HV: 15.0 kV
WD: 14.82 mm
MIRA3 TESCAN
SEM MAG: 455 x
Det: SE
100 µm
View field: 608 µm
SM: RESOLUTION
Performance in nanospace


SEM HV: 15.0 kV
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Det: SE
10 µm
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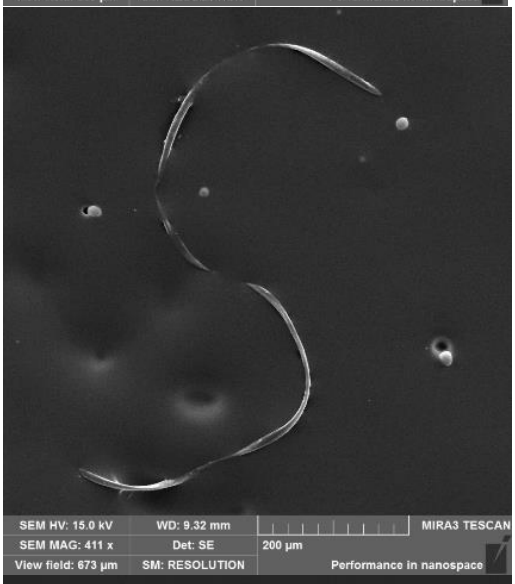
SM: RESOLUTION
Performance in nanospace


SEM HV: 15.0 kV
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MIRA3 TESCAN
SEM MAG: 411 x
Det: SE
200 µm
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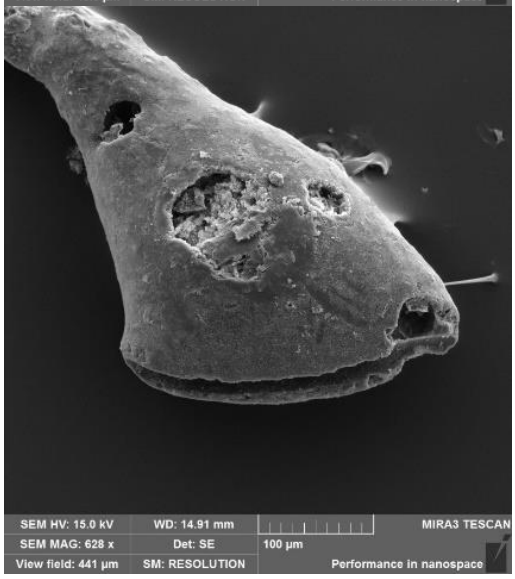
SM: RESOLUTION
Performance in nanospace


SEM HV: 15.0 kV
WD: 14.91 mm
MIRA3 TESCAN
SEM MAG: 628 x
Det: SE
100 µm
View field: 441 µm
SM: RESOLUTION
Performance in nanospace

SEM HV: 15.0 kV
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MIRA3 TESCAN
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Det: SE
20 µm
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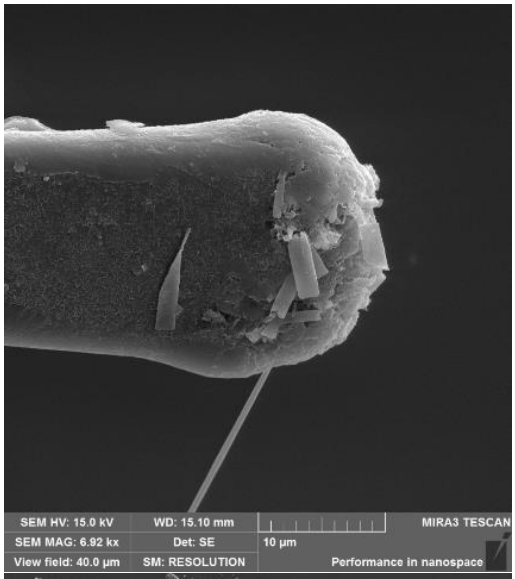
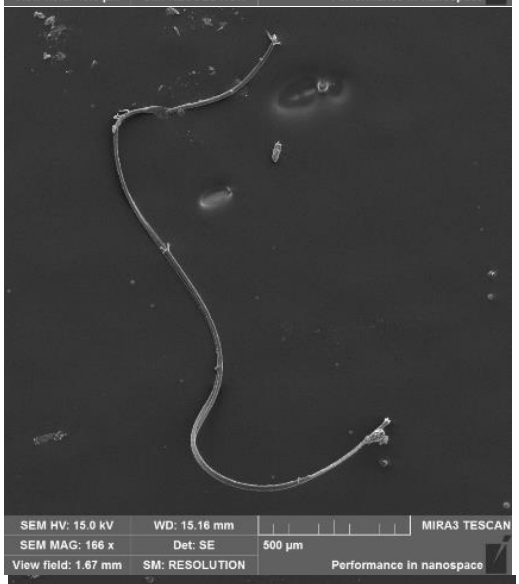
SM: RESOLUTION
Performance in nanospace

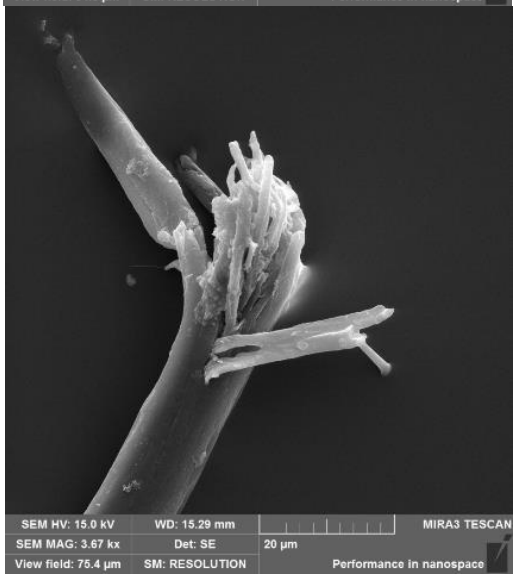

SEM HV: 15.0 kV
WD: 15.10 mm
MIRA3 TESCAN
SEM MAG: 6.92 kx
Det: SE
10 µm
View field: 40.0 µm
SM: RESOLUTION
Performance in nanospace

SEM HV: 15.0 kV
WD: 15.13 mm
MIRA3 TESCAN
SEM MAG: 412 x
Det: SE
200 µm
View field: 672 µm
SM: RESOLUTION
Performance in nanospace


SEM HV: 15.0 kV
WD: 15.16 mm
MIRA3 TESCAN
SEM MAG: 166 x
Det: SE
500 µm
View field: 1.67 mm
SM: RESOLUTION
Performance in nanospace

SEM HV: 15.0 kV
WD: 14.71 mm
MIRA3 TESCAN
SEM MAG: 2.92 kx
Det: SE
20 µm
View field: 94.8 µm
SM: RESOLUTION
Performance in nanospace

SEM HV: 15.0 kV
WD: 15.10 mm
MIRA3 TESCAN
SEM MAG: 267 x
Det: SE
200 µm
View field: 1.04 mm
SM: RESOLUTION
Performance in nanospace

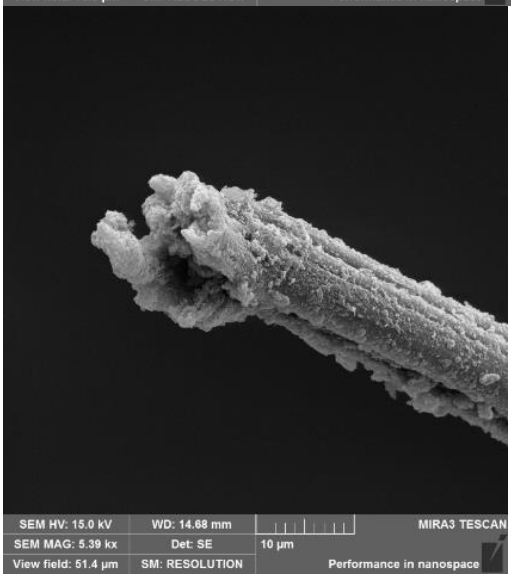

SEM HV: 15.0 kV
WD: 15.29 mm
MIRA3 TESCAN
SEM MAG: 3.67 kx
Det: SE
20 µm
View field: 75.4 µm
SM: RESOLUTION
Performance in nanospace

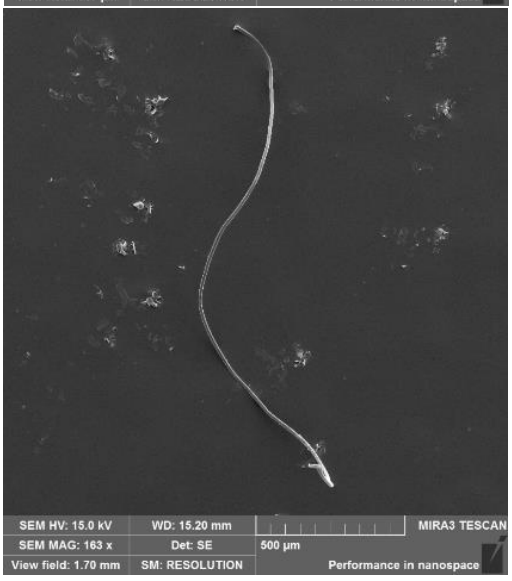
SEM HV: 15.0 kV
WD: 15.05 mm
MIRA3 TESCAN
SEM MAG: 537 x
Det: SE
100 µm
View field: 515 µm
SM: RESOLUTION
Performance in nanospace

SEM HV: 15.0 kV
WD: 15.00 mm
MIRA3 TESCAN
SEM MAG: 132 x
Det: SE
500 µm
View field: 2.10 mm
SM: RESOLUTION
Performance in nanospace

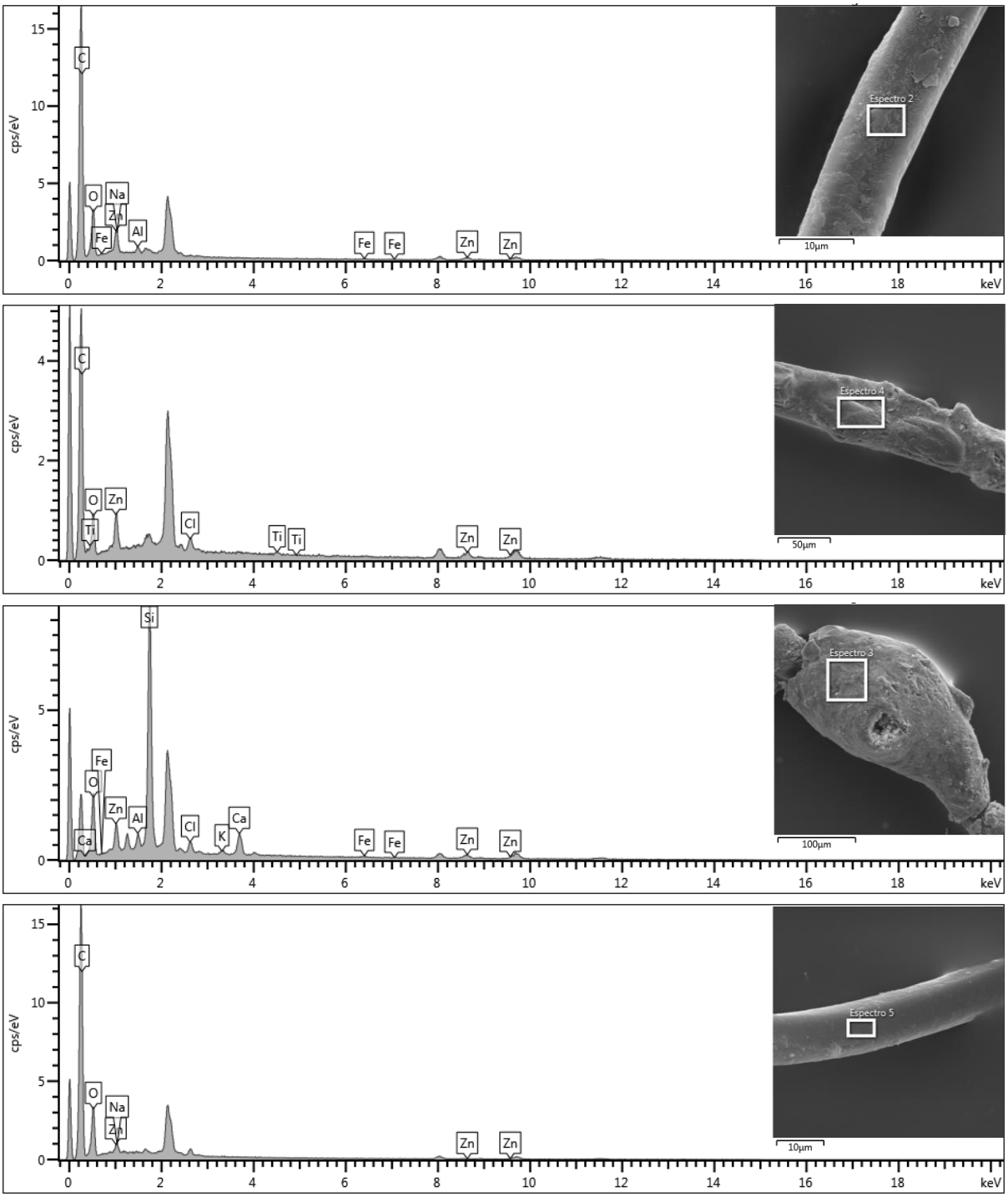
SEM HV: 15.0 kV
WD: 14.83 mm
MIRA3 TESCAN
SEM MAG: 3.63 kx
Det: SE
20 µm
View field: 76.3 µm
SM: RESOLUTION
Performance in nanospace

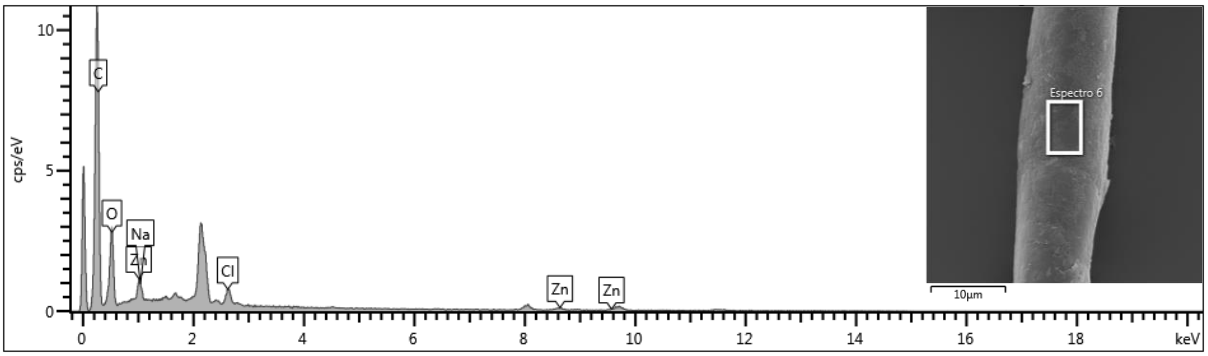
SEM HV: 15.0 kV
WD: 14.86 mm
MIRA3 TESCAN
SEM MAG: 715 x
Det: SE
100 µm
View field: 387 µm
SM: RESOLUTION
Performance in nanospace

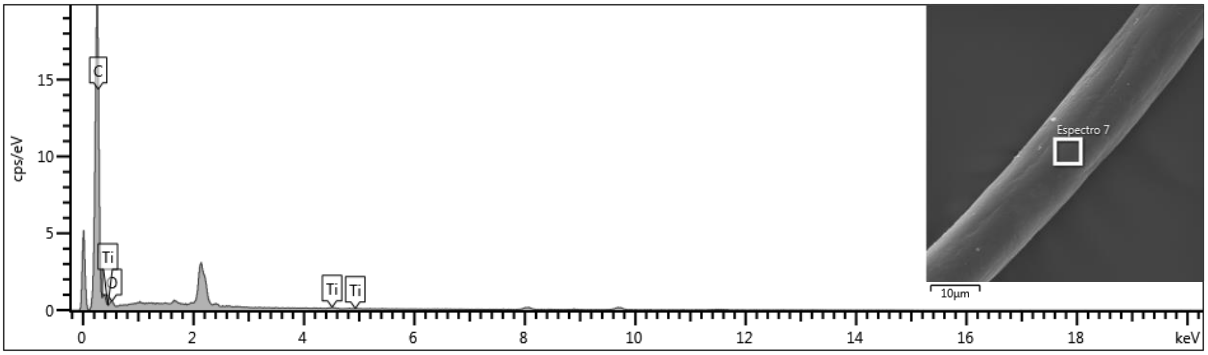

SEM HV: 15.0 kV
WD: 14.68 mm
MIRA3 TESCAN
SEM MAG: 5.39 kx
Det: SE
10 µm
View field: 51.4 µm
SM: RESOLUTION
Performance in nanospace

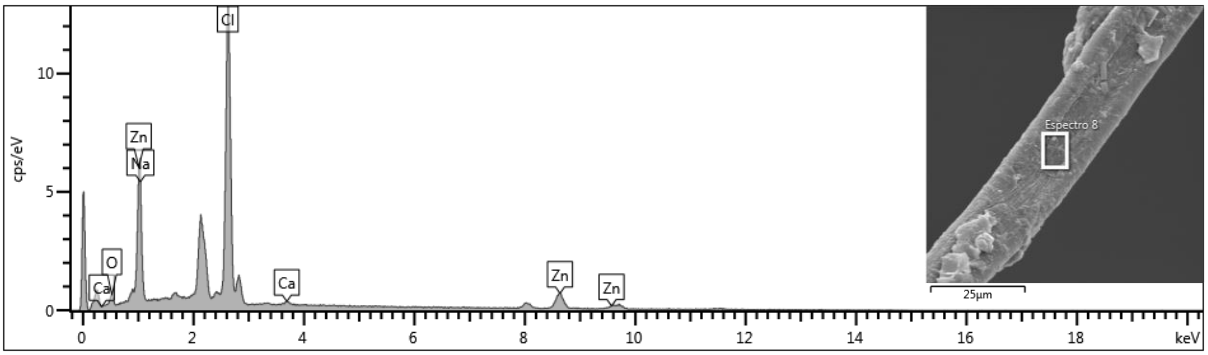

SEM HV: 15.0 kV
WD: 15.20 mm
MIRA3 TESCAN
SEM MAG: 163 x
Det: SE
500 µm
View field: 1.70 mm
SM: RESOLUTION
Performance in nanospace

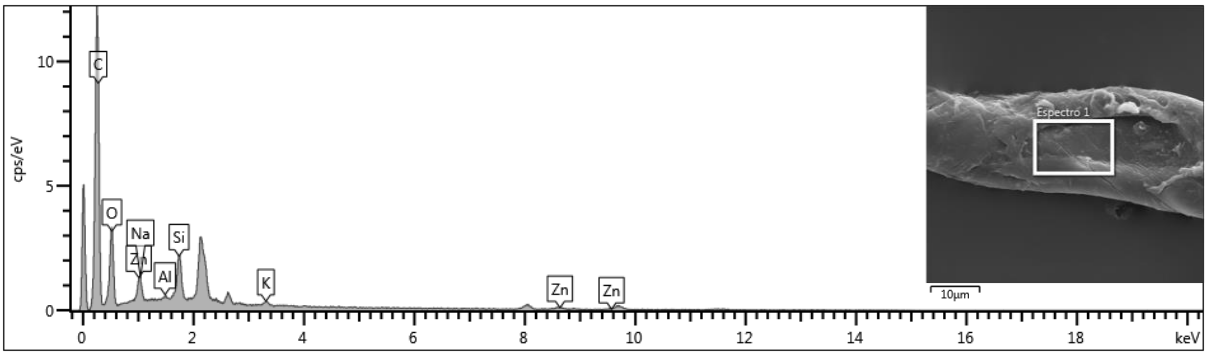
ANNEX B – SEM-EDS ELEMENTAL IDENTIFICATION OF MICROPLASTICS

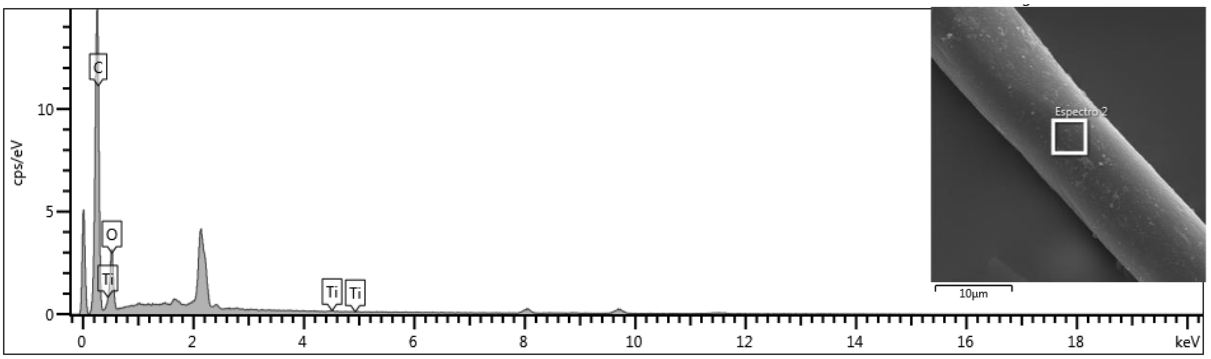


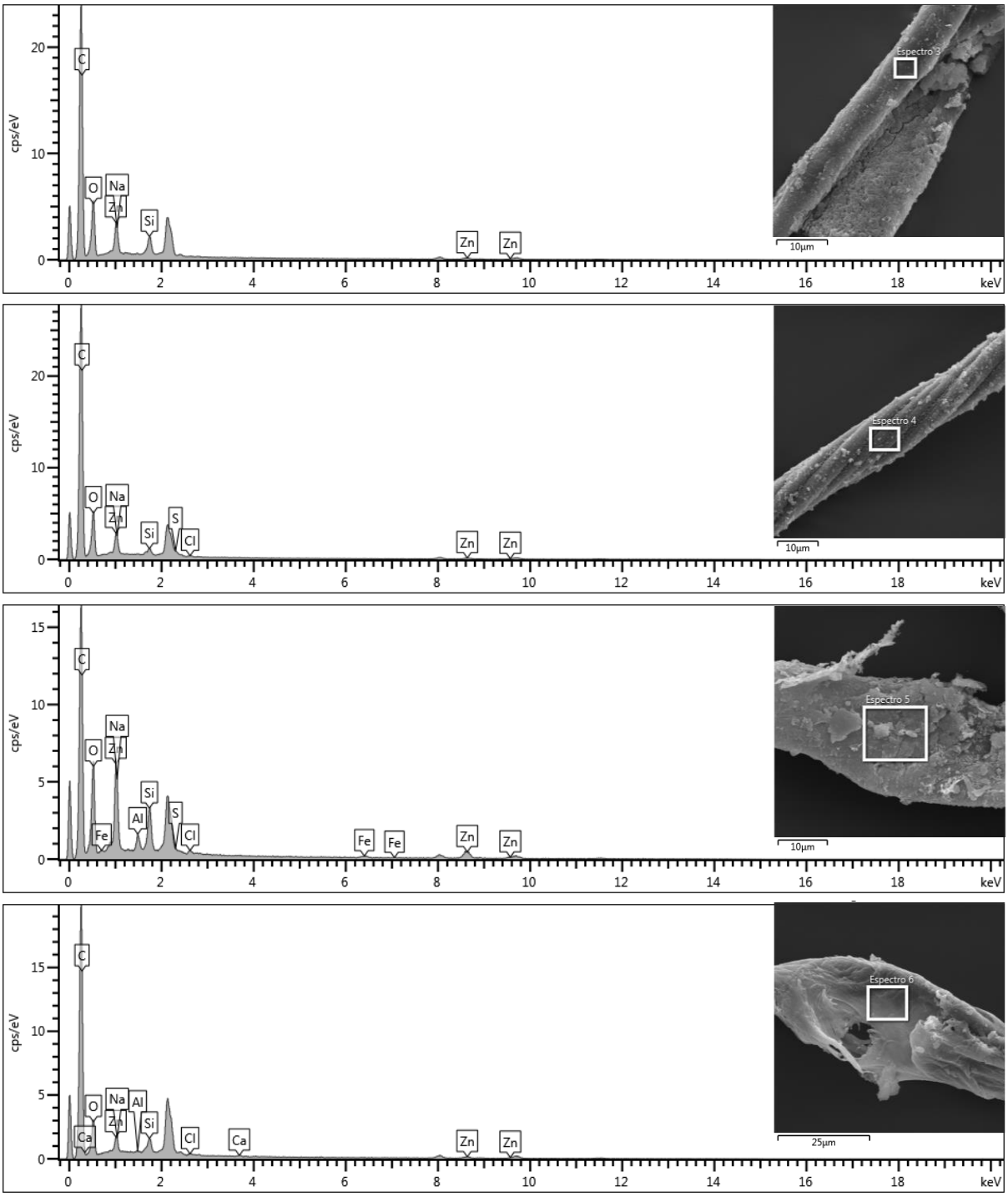

cps/eV
C
O
Na
Zn
Cl
Zn
Zn
keV
Espectro 6
10µm


cps/eV
C
Ti
O
Ti
Ti
keV
Espectro 7
10µm


cps/eV
Cl
Zn
Na
O
Ca
Ca
Zn
Zn
keV
Espectro 8
25µm


cps/eV
C
O
Na
Zn
Al
Si
K
Zn
Zn
keV
Espectro 1
10µm


cps/eV
C
O
Ti
Ti
Ti
keV
Espectro 2
10µm


cps/eV
keV
C
O
Na
Zn
Si
Zn
Zn
Espectro 3
10µm
C
O
Na
Zn
Si
S
Cl
Zn
Zn
Espectro 4
10µm
C
O
Na
Zn
Si
Al
S
Cl
Fe
Fe
Fe
Zn
Zn
Espectro 5
10µm
C
O
Na
Zn
Al
Si
Ca
Cl
Ca
Zn
Zn
Espectro 6
25µm

ANNEX C – MODEL ARCHITECTURE

```
!pip install tensorflow==2.17.0

import os
import zipfile
import shutil
import tensorflow as tf
from tensorflow.keras.preprocessing.image import ImageDataGenerator
from tensorflow.keras.applications import ResNet50
from tensorflow.keras import layers, models
from tensorflow.keras.callbacks import EarlyStopping, ModelCheckpoint
import matplotlib.pyplot as plt
from sklearn.metrics import classification_report, confusion_matrix
import seaborn as sns
from google.colab import drive
from tensorflow.keras.models import load_model


drive.mount('/content/drive')
zip_file_path = '/content/drive/My Drive/cod2/testing.zip'
extracted_folder_path = '/content/testing_extracao/'


with zipfile.ZipFile(zip_file_path, 'r') as zip_ref:
    zip_ref.extractall(extracted_folder_path)


print("Conteúdo das pastas descompactadas:", os.listdir(extracted_folder_path))
data_dir = extracted_folder_path


def preprocess_gray_image(img_path, target_size=(256, 256)):

    img = tf.keras.preprocessing.image.load_img(img_path, target_size=target_size)
    img_array = tf.keras.preprocessing.image.img_to_array(img)
    gray_img = tf.image.rgb_to_grayscale(img_array)
    gray_img_3_channels = tf.image.grayscale_to_rgb(gray_img)
    return gray_img_3_channels / 255.0


datagen = ImageDataGenerator(

    rescale=1./255,
    validation_split=0.2,
    rotation_range=40,
```

```
    width_shift_range=0.2,
    height_shift_range=0.2,
    shear_range=0.2,
    zoom_range=0.2,
    horizontal_flip=True,
    fill_mode='nearest'
)


train_gen = datagen.flow_from_directory(

    data_dir,
    target_size=(256,256),
    batch_size=32,
    class_mode='categorical',
    subset='training',
    shuffle=True,
    color_mode='rgb'
)


val_gen = datagen.flow_from_directory(

    data_dir,
    target_size=(256, 256),
    batch_size=32,
    class_mode='categorical',
    subset='validation',
    shuffle=False,
    color_mode='rgb'
)

class_weights = {0: 3.8,
                 1: 3.8,
                 2: 3.8,
                 3: 4.75}

print(f"Pesos das classes: {class_weights}")


base_model = ResNet50(weights='imagenet', include_top=False,
input_shape=(256,256, 3))


base_model.trainable = True
fine_tune_at = 100
for layer in base_model.layers[:fine_tune_at]:
```

```
    layer.trainable = False


model = models.Sequential([
    base_model,
    layers.GlobalAveragePooling2D(),
    layers.Dense(256, activation='relu'),
    layers.Dense(4, activation='softmax')
])


model.compile(optimizer=tf.keras.optimizers.Adam(learning_rate=1e-5),
loss='categorical_crossentropy', metrics=['accuracy'])


checkpoint_path = '/content/drive/My Drive/cod2/modelo_best_testing.keras'
model_checkpoint = ModelCheckpoint(checkpoint_path, save_best_only=True,
save_weights_only=False)


early_stopping = EarlyStopping(monitor='val_loss', patience=10,
restore_best_weights=True)


initial_epoch = 0


history = model.fit(
    train_gen,
    epochs=50,
    validation_data=val_gen,
    class_weight=class_weights,
    callbacks=[early_stopping, model_checkpoint],
    initial_epoch=initial_epoch
)


loss, accuracy = model.evaluate(val_gen)
print(f"Loss: {loss}, Accuracy: {accuracy}")


plt.figure(figsize=(10, 6))
plt.plot(history.history['loss'], label='Perda de treinamento', color='blue', linestyle='-',
linewidth=2)
plt.plot(history.history['val_loss'], label='Perda de validação', color='red', linestyle='--',
linewidth=2)
plt.xlabel('Épocas', fontsize=14)
```

```
plt.ylabel('Perda', fontsize=14)
plt.title('Perda de Treinamento vs Validação', fontsize=16)
plt.legend(loc='upper right', fontsize=12)
plt.grid(True)
plt.tight_layout()
plt.show()


plt.figure(figsize=(10, 6))
plt.plot(history.history['accuracy'], label='Acurácia de treinamento', color='green',
linestyle='-', linewidth=2)
plt.plot(history.history['val_accuracy'], label='Acurácia de validação', color='orange',
linestyle='--', linewidth=2)
plt.xlabel('Épocas', fontsize=14)
plt.ylabel('Acurácia', fontsize=14)
plt.title('Acurácia de Treinamento vs Validação', fontsize=16)
plt.legend(loc='lower right', fontsize=12)
plt.grid(True)
plt.tight_layout()
plt.show()


y_true = val_gen.classes
y_pred = model.predict(val_gen)
y_pred_classes = tf.argmax(y_pred, axis=-1).numpy()


print("Relatório de Classificação:\n", classification_report(y_true, y_pred_classes))


cm = confusion_matrix(y_true, y_pred_classes)
plt.figure(figsize=(8, 6))
sns.heatmap(cm, annot=True, fmt='d', cmap='Blues', xticklabels=['algae I', 'filament',
'fragment', 'pellet'], yticklabels=['algae I', 'filament', 'fragment', 'pellet'],
annot_kws={"size": 14})

plt.title('Matriz de Confusão', fontsize=16)
plt.xlabel('Predições', fontsize=14)
plt.ylabel('Valores Reais', fontsize=14)
plt.xticks(fontsize=12)
plt.yticks(fontsize=12)
plt.tight_layout()
plt.show()


def predict_image(image_path, model):
```

```
    img = preprocess_gray_image(image_path)
    img_array = tf.expand_dims(img, axis=0)


    prediction = model.predict(img_array)
    class_idx = tf.argmax(prediction, axis=-1).numpy()[0]

    class_labels = {0: 'algae I', 1: 'filament', 2: 'fragment', 3: 'pellet'}
    predicted_class = class_labels[class_idx]
    print(f"Prediction for the image ({image_path}): {predicted_class}")
    return predicted_class


def predict_all_images_in_folder(folder_path, model):

    for filename in os.listdir(folder_path):
        if filename.lower().endswith(('.png', '.jpg', '.jpeg')):
            image_path = os.path.join(folder_path, filename)
            predict_image(image_path, model)


folder_path = '/content/drive/My Drive/teste/test'
predict_all_images_in_folder(folder_path, model)
```