frontiers | Frontiers in Environmental Science

TYPE Original Research
PUBLISHED 01 April 2025
DOI 10.3389/fenvs.2025.1566507

OPEN ACCESS

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RECEIVED 24 January 2025
ACCEPTED 11 March 2025
PUBLISHED 01 April 2025

CITATION
Eid Z, Mahmoud UM and Sayed AE-D (2025) "The paper cups Nile": microplastics and other hazardous substances leached from paper cups: paper cups aquatic environmental bane in the River Nile, Egypt.
Front. Environ. Sci. 13:1566507.
doi: 10.3389/fenvs.2025.1566507

"The paper cups Nile": microplastics and other hazardous substances leached from paper cups: paper cups aquatic environmental bane in the River Nile, Egypt

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Recent studies suggest that paper cups may also contribute to environmental pollution, particularly through the release of microplastics (MPs). The Nile River, one of the world's most vital water sources, faces alarming contamination levels, raising concerns about its ecological health. This study investigated whether paper cups release MPs, ions, and heavy metals into water and assessed the potential impact of MPs on fish. In order to completely comprehend the nature and scope of the issue, 1 L of water was collected from the Nile River in Assiut, Egypt and the paper cups were ripped into tiny pieces. Paper cups were similarly soaked in similar volumes of distilled and tap water. Four months later, the leachate from each trail (three replicates for each) was analyzed to determine and compare the distribution of specific ions, heavy metals and microplastics. In order to clarify the availability of MPs in freshwater fish, the intestines of two common fish species (*Oreochromis niloticus* and *Bagrus bajad*) were collected from the River Nile in Assiut and examined. Polyethylene, polystyrene and polypropylene were the three main forms of microplastics identified in water samples from the Nile. Also, paper cups soaked in tap water leached the same three groups of MPs, but in lower amounts. Some microplastics may take longer to biodegrade in water, as evidenced by the absence of other forms of microplastics like rayon and polyvinyl chloride in any of the water samples under investigation. The present findings also indicate a noteworthy accumulation of MPs in the intestines of *O. niloticus* and *B. bajad*. In conclusion, these results indicated release of some ions, heavy metals, and microplastics from paper cups into water and the River Nile water is polluted with paper cups which have a negative effect on aquatic organisms. This study brings us one step closer to investigating and fully understanding the nature and extent of the problem posed by paper cups and their effects on the River Nile and freshwater fish, which will ultimately be reflected in human health risks.

KEYWORDS

paper cups, leachates, microplastics, River Nile, Assiut city, fish

1 Introduction

The Nile River, Mediterranean Sea, and the Red Sea have become significantly polluted with plastics and microplastics, even though they are among the most vital resources for human survival (Chaudhry and Sachdeva, 2021). MPs pollution has recently spread to freshwater environments such as lakes, rivers, wetlands, estuaries and even ground water (Du et al., 2021; Wang et al., 2020). Rivers are essential for the lives of countless people and have been demonstrated to be the primary routes by which large volumes of MPs and plastic debris are transported from land-based sources into the oceans (Lebreton et al., 2017; Schmidt et al., 2017). Consequently, microplastics have recently been detected in the water, organisms, and sediment of several major river systems worldwide, including the Thames in the United Kingdom (Horton et al., 2017), the Seine in France (Dris et al., 2015), the Rhine in Germany (Mani et al., 2015), and the Danube in Austria (Lechner and Ramler, 2015); the Amazon in South America (Andrade et al., 2019; Schmidt et al., 2017); the Yangzte in Asia China (Zhang et al., 2015); and the St. Lawrence in North America (Castañeda et al., 2014). Despite being one of the most well-known river in the world, the Nile River is left off this list. Consequently, this study tried to fill in the gap in identifying microplastics as a component of paper cups in the River Nile and freshwater fish. Li et al. (2020) found that microplastic pollution of freshwater ecosystems is increasing at an unprecedented and concerning rate, despite the fact that the concentration of microplastics in freshwater ecosystems is lower than in marine habitats. Even though the majority of MP research has focused on the marine environment, there is growing interest in identifying MPs in river systems. Thus, this study directed attention toward the examination of microplastics in some freshwater fish from the River Nile in Assiut City, due to the global production of plastic continues to increase and eventually release into the environment.

Given that plastic waste is believed to pose serious threats to wildlife, human health, and climate change, it has been reported in every region of the natural environment worldwide (Simantiris, 2024). Consequently, there has been a swift global shift in recent decades toward alternative solutions for plastic waste, prompting governments and industries to replace single-use plastics with paper products. However, several studies have highlighted the negative impacts of both single-use plastics and paper products, concluding that paper is not a sufficient solution due to various harmful effects on the environment and human health (Fidan and Ayar, 2023; Joseph et al., 2023; Ranjan et al., 2021).

It is recognized that one of the most polluting industries in the world is the paper sector (Kumar et al., 2020; Singh et al., 2022), primarily due to the use of chlorine compounds in paper processing, which have detrimental environmental effects (Kumar et al., 2012). Paper cups are often discarded in landfills or improperly disposed of, contributing to (micro) plastic waste and potentially polluting the world's oceans (Fidan and Ayar, 2023; Foteinis, 2020). Paper and paperboards account for 31% of the global packaging market and they are mostly utilized in food packaging for the purposes of containing and safeguarding food products, making them convenient to store or consume and informing customers about pertinent information, including marketing aspects (Jones and Comfort, 2017). Paper is the preferred material for the food companies due to its good standing for being environmentally friendly (Oloyede and Lignou, 2021). It is widely used in primary applications, such as those in which food products come into direct contact with it, as well as secondary applications, such as those in which primary packaging needs to be moved and stored (Khwaldia et al., 2010).

Additionally, paper and paperboard are the primary materials used in the production of beverage cups, ice cream cups, microwave popcorn bags, baking paper, milk cartons, and fast food packaging, such as pizza boxes and other similar items (Chen et al., 2023; Deshwal et al., 2019). In spite of, the paper cups are perceived as ecofriendly alternatives or biodegradable frequently due to greenwashing efforts (Viera et al., 2020). However, a thin layer of polylactic acid added to the inner face of many paper cups to create a waterproof layer between the beverage and the paper. Nevertheless, along with pesticides and other environmental pollutants, the vast and complex set of chemicals emitted from packaging material is considered a food hazardous agent (Grob et al., 2006). This could seriously endanger people's health, depending on how much of specific paper chemicals and components find their way into food products and are consumed (Joseph et al., 2023; Poças et al., 2010). According to Biedermann-Brem et al. (2016) and Joseph et al. (2023), the issue of food migrants has grown more complicated because to their wide variety and differing degrees of toxicity. Paper straws may include a variety of hazardous compounds (such as heavy metals, formaldehyde, fluorescent materials, ink, etc.) that can be harmful to people and/or promote microbial contamination, according to a study by Qiu et al. (2022). Research on the environmental toxicity of single-use items and materials that come into contact with take-out meals is necessary to fill in these knowledge gaps. A few previous studies have investigated the toxicity of leachates from particular kinds of plastic food packaging materials. According to research by Thaysen et al. (2018), leachate from expanded polystyrene cups was toxic to aquatic invertebrates. Almroth et al. (2023) indicated that, single-use take-away beverage cups of different materials can all induce toxic effects in midges and mismanaged waste has a detrimental effect on aquatic biota.

Despite the fact that paper has an environmentally safe component, there are significant problems associated with its production and recyclable nature (Deshwal et al., 2019). Less than 1% of paper coffee cups are recycled worldwide, despite major efforts and gradual improvements in wealthier economies to increase recycling rates, this estimate was determined for three reasons, according to Triantafillopoulos and Koukoulas (2020): (a) the belief that the paper and plastic combination used to make paper coffee cups is difficult to recycle; (b) the fact that many recycling facilities are unable to commit to processing waste streams contaminated with food; and (c) the fact that recycling programs within communities and venues are inconsistent and ineffective. Disposable paper cups are composed of 90%–95% paper and 5%–10% hydrophobic plastic film by weight (Arumugam et al., 2018; Constant, 2016). Thus, unfortunately, recycling these paper cups would be extremely difficult as paper cups are coated with polyethylene terephthalate (PET), which prevents them from being recycled or decomposed (Biswal et al., 2013; Foteinis, 2020).

In our daily lives, poor disposal of single use plastics (SUP) items like paper cups causes to plastic pollution in aquatic habitats as they become more prevalent (Foteinis, 2020). According to Foteinis (2020), there are two main paths for paper cup disposal, namely landfilling and recycling, assuming that each route accounts for

FIGURE 1
(A) Map of Egypt showing the catch site of *Oreochromis niloticus* and *Bagrus bajad* from the River Nile at Assiut, Egypt. **(B, C)** are two sites of plastic piles observed on the Nile shore at Assiut, from which the fish and water samples were obtained.

100% of the waste stream. In recent years, post-consumer garbage has accounted for around 35% of municipal solid trash by weight and has become a significant component in many landfill sites (Kapukotuwa et al., 2022). In recent surveys, Miarc (2020) estimated that the yearly market value of single-use OOH hot paper coffee cups is 118 billion, and by 2025, it is expected to grow at a 1.8% CAGR to reach 294 billion units. Reusing and recycling waste materials in an economical, safe, efficient and environmentally friendly way is one of the best ways to cut down on the amount of trash produced (Arumugam et al., 2018). Owing to these issues, there was a push to fully understand the nature and extent of problem of paper cups by some analysis trials to contribute to close this information gap. Further studies need to be carried out to get a better understanding of the environmental pollution by paper cups.

There are various solutions that have been suggested for limiting plastic discharges into the environment (Suzuki et al., 2024; Thaysen et al., 2018). In all over the world many countries, states, townships, and cities are making a great attention towards banning plastics from OOH items (Triantafillopoulos and Koukoulas, 2020). In India the Kerala state government has gone a step further in banning production, sale, and use of single-use plastics including paper coffee cups (Triantafillopoulos and Koukoulas, 2020). In a recent life cycle study, Foteinis (2020) showed that current methods for disposing paper cups has the global environmental footprint of 1.5 million Europeans and that recycling could decrease this impact by 40 percent. Collaborations on a national and international level should be established to carry out circular economy initiatives (such as implementing a system of fees and levies for single-use products), policy development, public and academic education (including information on the problems with paper alternatives), industrial company disposal fees and regulations, and sustainable solutions to keep the market system from collapsing (Simantiris, 2024).

2 Material and methods

2.1 Study area

The study was conducted along the River Nile in the Assiut Governorate, with two primary sampling sites selected based on

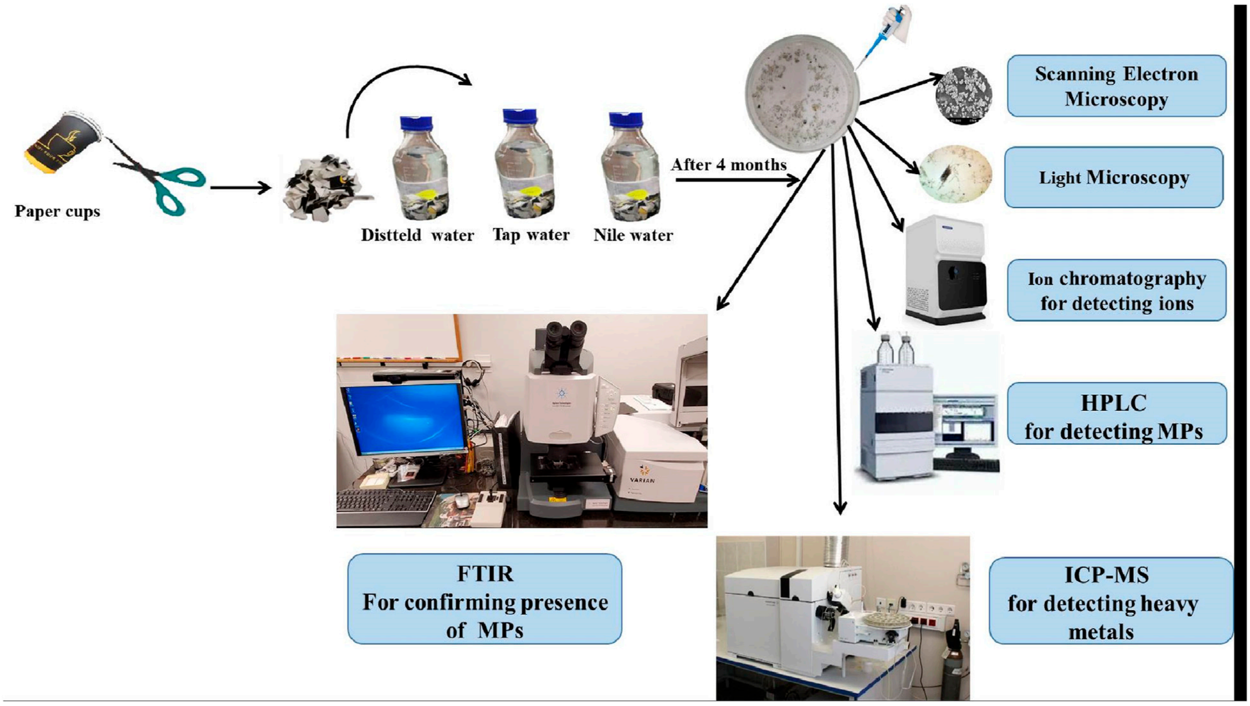

FIGURE 2
Summarized technique for the analysis of paper cups leachate following 4 months of soaking.

observed plastic and paper cups accumulation along the riverbank. These sites were chosen to obtain the fish and water samples (Figure 1). The first sampling site was located near a densely populated urban region within Assiut City (Figure 1B). This area is characterized by high human activity, including residential neighborhoods, and markets. The riverbank here showed visible signs of anthropogenic pressure, with significant plastic and waste piles consisting of disposable cups, plastic bags, and other debris. The proximity of this site to informal waste disposal points and active fishing areas raises concerns about direct pollution pathways into the River Nile. The surrounding area includes agricultural fields irrigated by river water, which may further spread contaminants to the soil and crops.

The second sampling site was situated in a more rural setting, downstream from Assiut City (Figure 1C). This site is relatively less populated but still exhibited noticeable plastic waste, particularly from agricultural runoff and domestic activities in nearby villages. The surrounding environment consists of small farms and natural vegetation, with some local fishing and livestock watering activities observed along the riverbank. Although human activity is less concentrated here, the accumulation of waste from upstream sources is evident, as plastic debris and paper cups are carried downstream by the river's flow. Both sites provide distinct settings for examining the impact of plastic and paper cups pollution in different environmental and socio-economic contexts.

2.2 Paper cups analysis trial

A number of paper cups were purchased from the commercial market. Three cups, each weighing 3.95 g (with a total weight of 11.87 g), were cut into small pieces using scissors and placed into 1 L of water collected from the River Nile in Assiut City. The same number of paper cups were also placed in an equal volume of tap and distilled water. After 4 months, the leachate from each trial (three replicates for each) and the River Nile water before adding paper cups fragments were analyzed to determine and compare the distribution of specific ions, heavy metals, and microplastics (Figure 2).

2.3 Analysis of the paper cups leachate and water of the River Nile

2.3.1 Ion chromatography

The ions were detected in samples of water from the River Nile, leachate of tap water contained paper cups and tap water without paper cups using an Ion Chromatography system according to Michalski (2006) and Ranjan et al. (2021). Water samples were prepared before analysis to ensure compatibility with the ion chromatography system. Standard solutions with known ion concentrations were also prepared, and a specific volume (10–50 µL) of the water sample was introduced into the ion chromatography system. As the sample passes through the column, the ions interact with the charged stationary phase and are separated according to their charge, size, and affinity for the column. The detector captures the ions as they exit the column, generating a chromatogram. Each peak in the chromatogram represents a specific ion, with the peak area indicating its concentration. Finally, the sample's chromatogram is compared to the calibration curve to quantify the ions.

2.3.2 High-performance liquid chromatography (HPLC) analysis for microplastics detection

The samples of water from the River Nile and leachate of tap water contained paper cups were stored in glass bottles to avoid contamination. The samples were filtered using polycarbonate membranes with a pore size of 0.45 µm to isolate microplastics. Filters were rinsed with distilled water to remove residual salts and particulate matter. The filtered samples were mixed with a saturated NaCl solution and left to settle for 24 h. The floating fraction, containing microplastics, was collected for further analysis. To remove organic contaminants, 30% hydrogen peroxide (H_2O_2) was added to the collected samples, and the solution was heated at 60°C for 24 h. The resulting microplastic residue was dried at 40°C to avoid polymer degradation. Adsorbed chemicals or monomers were extracted using methanol as an organic solvent, with ultrasonication applied for 20 min to enhance the extraction process. Finally, the solvent extract was filtered through a 0.22 µm syringe filter to prepare the sample for HPLC analysis. A high-temperature gradient HPLC system, PL XT-220 (Polymer Laboratories, Church Stretton, England), was utilized for the analysis according to Heinz and Pasch (2005). A high-temperature gradient HPLC system (PL XT-220) with a Nucleosil 500 stationary phase (25 × 0.46 cm, 5 µm particle size) was used for analysis. An ELSD detector (PL-ELS 1000) operated at 160°C nebulization, 270°C evaporation, and 1.5 L/min air velocity. The eluent flow rate was 1 mL/min. A robotic system (PL-XTR) automated sample preparation and injection, maintaining temperatures of 140°C for the column, 150°C for the injection port and transfer line, and 160°C for the sample block and robotic arm tip. Data processing was performed using "WinGPC-Software".

2.3.3 Inductively coupled plasma mass spectrophotometer (ICP-MS) for heavy metals detection

The samples of water from the River Nile, leachate of tap water contained paper cups and tap were filtered using 0.45 µm or 1.2 µm membrane filters to remove particulates, debris, and large organic matter. The filtered samples were then diluted with Milli-Q water (ultrapure water) at a ratio that ensured the analyte concentrations fell within the linear range of the ICP-MS. To the diluted samples, concentrated nitric acid (HNO_3) (trace metal grade) was added to ensure the complete dissolution of metal ions and to prevent precipitation. The concentration of heavy metals was measured using ICP-MS and compared to the calibration curve following the methodology described by Ranjan et al. (2021).

2.3.4 Post-analysis for microplastics

Following the ICP-MS analysis for heavy metals, any particulate matter remaining in the digestion residue was collected and isolated. Fourier Transform Infrared Spectroscopy (FTIR) was then utilized to confirm the presence of microplastics and determine their composition in the water samples according to Albrecht et al. (2007).

2.3.5 Scanning electron microscopy (SEM)

SEM was used for viewing and clearing up what were found in the River Nile water before putting paper cups and in the leachates of the same water after containing paper cups for 4 months at scanning electron microscopy unit, Assiut University (JEOL JEM-1200 EX II). The films were viewed under different magnifications ranging from 1,000 to 1,5000X. 5 µL of the tested water were airdried and viewed under the SEM.

2.3.6 Light microscopy

It was also used for examination the three tested water (River Nile, tap and distilled water) before and after containing paper cups under a ×10 objective with a ×1 eyepiece using Omax microscope with 14 MP USB Digital Camera (CS-M837ZFLR-C140U) (A35140U3; China).

2.4 Fish collection

The Nile Tilapia *Oreochromis niloticus* and *Bagrus bajad* (12 fish/species) were obtained from the River Nile at Assiut and transported to the fish Pollution Laboratory, Assiut University, Egypt (Figure 1).

2.5 Microplastics (MPs) quantification

Intestine (the digestive tract) samples were collected and digested in 10 mL of hydrogen peroxide (30%, v: v) at 70°C for 2 h, and 100 µL of the resultant solution was then examined under the light microscope for microplastic detection according to Deng et al. (2017) and photographed under a ×40 objective with a ×10 eyepiece using Omax microscope with 14 MP USB Digital Camera (CS-M837ZFLR-C140U) (A35140U3; China).

3 Results and discussion

3.1 Detection of ions and heavy metals in River Nile water, tap water and tap water contained in paper cups

Indeed, during the COVID-19 epidemic, there was a surge in the usage of food packaging materials and the corresponding creation of solid waste (de Oliveira et al., 2021). Many previous studies have investigated that harmful chemicals and substances can leach from paper and cardboard-based food packaging (Vandermarken et al., 2019). Owing to these issues, this study had a shed on the problem of paper cups by detection of microplastics, heavy metals and ions in samples of water from the River Nile, leachate of tap water contained paper cups and tap water only. The results of this research indicated that, the highest levels of some ions like fluoride, chloride, nitrite, sulfate and nitrate were detected in the water of the River Nile then leachate of tap water contained paper cups but only chloride ion was detected in the tap water without paper cups (Figures 3c, d). Similarly, other study has shown that, ions such as sulfate, nitrate, chloride and fluoride might be found in paper cups, all may originated from the chemical treatment of paperboards (Ranjan et al., 2021). This interpretation is also consistent with Ozaki et al. (2004), who reported that a number of chlorine-containing chemicals were used to bleach the paper pulp in order to remove lignin and give the final product a brighter white colour.

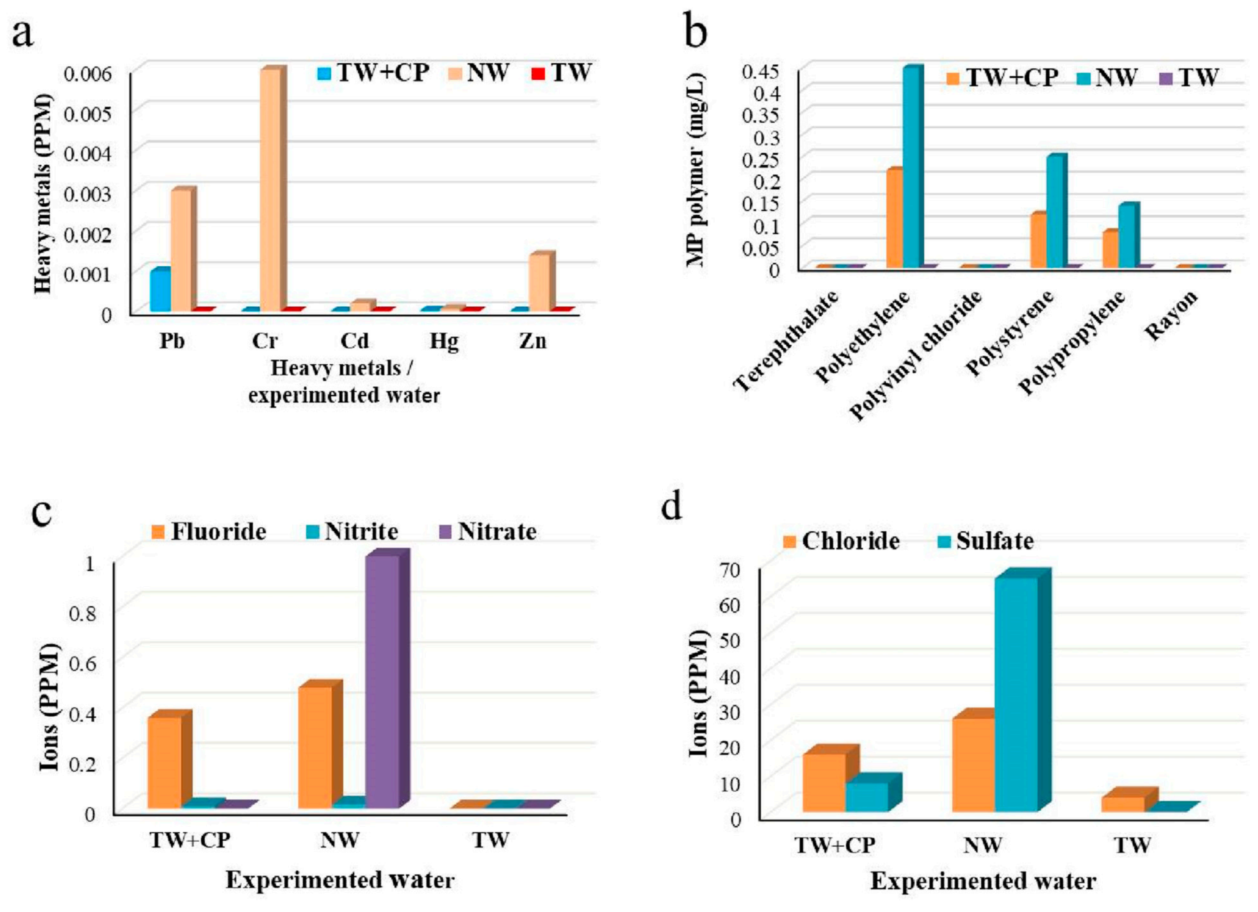

FIGURE 3
Showing heavy metals **(a)**, microplastics **(b)** and ions **(c, d)** detected in the water of the River Nile at Assiut city (NW), tap water (TW) and the leachate of tap water contained paper cups (TW + CP) (by Inductively Coupled Plasma Mass Spectrophotometer (ICP-MS), High-Performance Liquid Chromatography (HPLC) and Ion Chromatography respectively).

Additionally, paper boards used in food packaging are treated with per- and polyfluoroalkyl substances (PFASs) and other fluorinated chemicals; these compounds have the lipophobic and hydrophobic qualities that make paper containers waterproof and stain-resistant (Cantoral et al., 2019; Trier et al., 2018). Joseph et al. (2023) estimated that the average daily intake of fluoride from hot beverages in paper cups was 7.04 ± 8.8 μg/kg body weight. Rezvani Ghalhari et al. (2021) and Karami et al. (2019) had analysed fluoride and nitrate in tea and water. Health risk related with fluoride intake through tea and coffee was analysed by Satou et al. (2021).

This study revealed that the highest levels of certain heavy metals, including Pb, Cr, Cd, Hg, and Zn, were detected in the water of the River Nile. In the leachate of tap water contained paper cups, only Pb and Hg were detected, while no heavy metals were detected in tap water without paper cups (Figure 3a). Likewise, these heavy metals are well-known additives used in the manufacturing process to provide paper and paperboards specific properties (Ranjan et al., 2021). These heavy metals are not degradable, hazardous heavy and toxic even at low concentrations (Ranjan et al., 2021). According to Zeng et al. (2023), disposable food containers such paper cups, plastic cups, plastic bags, and plastic bowls offer new ways for metals and other elements to leak into drinking water, beverages and fast food in hot environments. In addition, lab experiments were conducted by Almroth et al. (2023) to examine the toxicity of paper and plastic cups and lids on aquatic midge larvae. According to the results of their investigation, paper products have a tendency to dissolve quickly in fluids, ultimately resulting in release chemicals and breaking down into tiny pieces that may impact the environment by damaging sediments, aquatic life, and the water column.

The presence of heavy metals (Pb, Cr, Cd, Hg, and Zn) in the water of the River Nile aligns with findings from other global river systems, where heavy metal pollution is often associated with microplastic (MP) contamination (Ta and Babel, 2023). For instance, studies on the Chao Phraya River in Thailand recorded high abundance of MPs in both water and sediments, with Pb and Cu adsorbed onto their surfaces (Ta and Babel, 2020). Similarly, Purwiyanto et al. (2020) observed significant adsorption of Pb and Cu on various polymer types, including PP, PES, OVC, PE, and nylon, in the Musi River, Sumatera, Indonesia. This adsorption phenomenon is particularly concerning because MPs can act as carriers for heavy metals, enhancing their persistence in aquatic environments and increasing their bioavailability to aquatic organisms (Cao et al., 2021). The toxic effects of heavy metals and MPs on biota have been widely reported (Foley et al., 2018;

Khalid et al., 2021b; Walkinshaw et al., 2020). Comparing our results with those from Poland, where studies have detected variations in Cr, Ni, Cu, Zn, Cd, and Pb concentrations in the Nida River and higher heavy metal concentrations in other rivers such as the Warta River (Bhat and Janaszek, 2024; Jaskuła et al., 2021; Sojka and Jaskuła, 2022), highlights the global scale of this issue. Whether in Africa, Asia, or Europe, rivers are becoming repositories for hazardous pollutants, which can have long-term ecological and health consequences (Gwenzi and Chaukura, 2018; Mushtaq et al., 2020; Singh et al., 2024).

3.2 Detection of microplastics in River Nile water, tap water and tap water contained in paper cups

This study revealed the presence of polyethylene, polystyrene, and polypropylene MPs in the River Nile, with a greater abundance than in tap water contained in paper cups (Figure 3b). This suggests that river systems act as major reservoirs for MP pollution from various sources, a trend that has been extensively documented in other freshwater systems. For example, studies in China have confirmed the presence of MPs in nearly all investigated water bodies, with concentrations reaching millions of particles per cubic meter (Gupta et al., 2023). The long residence time of MPs in rivers, influenced by water volume and movement, contributes to their widespread distribution (Khalid et al., 2021a). Furthermore, flood events can cause MPs and their associated heavy metals to be redistributed across different aquatic systems, including lakes and reservoirs (Harrison et al., 2018). Notably, in this study no MPs were detected in tap water without paper cups (Figure 3b), indicating that drinking water sources may initially be free from MPs but could become contaminated through contact with paper cups or plastic-based materials. In Thailand, the Chao Phraya River an essential source of drinking water and aquaculture has been found to contain MPs across various regions, from agricultural to urban and estuarine zones (Ta and Babel, 2023). Such contamination poses a direct threat to food security and public health.

According to He et al. (2022) microplastics can be categorized into six groups: polyethylene, polystyrene, polypropylene, polyurethane, polyvinyl chloride, and polyethylene terephthalate based on their molecular composition. Ranjan et al. (2021) and Joseph et al. (2023) examined how many microplastics an average person would consume while drinking hot liquids in a paper cup, such as tea or coffee. Other forms of microplastics such as Polyvinyl chloride and Rayon were not detected in any type of tested water, these indicated that microplastics need more time for biodegradation in the water. Nevertheless, there are worries that paper products may be exposed to all the drawbacks of plastic products in addition to the unfavorable effects of their chemical makeup because the majority of single-use paper products are said to include polymer coatings to keep the paper pulp from combining with the food or beverage (Ranjan et al., 2021; Simantiris, 2024). The investigation in this study indicated the release of certain ions, heavy metals, and microplastics from paper cups into water. Additionally, the River Nile is polluted with paper cups, which have a negative effects on aquatic organisms.

The FTIR analysis of water samples collected from the Nile River and the leachate of tap water containing paper cups respectively, revealed the presence of distinct peaks that can be attributed to various polymers, indicating potential contamination with microplastics (Figures 4A,B). In both spectra, the broad peak around 3,400 cm^{-1} corresponds to O-H stretching vibrations, which are likely due to water absorption but could also suggest the presence of cellulose-based polymers such as rayon (Coates, 2000; Premraj and Doble, 2005). The prominent peaks around 2,900 cm^{-1} are characteristic of C-H stretching vibrations, which are commonly observed in hydrocarbon-based polymers like polyethylene (PE), polypropylene (PP), and polystyrene (PS) (Pavia et al., 2015; Shah et al., 2008). Specifically, these peaks suggest the presence of aliphatic chains, which are a defining feature of these polymers. The appearance of peaks near 1700 cm^{-1} in the spectrum may be attributed to C=O stretching vibrations, which are a signature of ester groups present in polyethylene terephthalate (PET) (El-Azazy et al., 2022). Additionally, peaks around 1,400–1,500 cm^{-1} are indicative of CH2 and CH3 bending vibrations, further supporting the presence of polypropylene or polyethylene (Stuart, 2004). The peaks near 700 cm^{-1} are significant, as they may correspond to aromatic C-H bending, which is characteristic of polystyrene and PET, or C-Cl stretching, which would point to polyvinyl chloride (PVC) (Coates, 2000). The spectrum also displays peaks in the region of 1,000–1,300 cm^{-1}, which could be associated with C-O stretching in PET or C-Cl bonds in PVC.

Comparing the two spectra, the differences in peak intensities and sharpness suggest varying levels of polymer contamination in tap water containing paper cups and Nile water, with Nile water showing more pronounced peaks, indicative of a higher microplastic concentration. Overall, the analysis strongly indicated the presence of multiple polymers, including PET, PE, PP, PS, and potentially PVC and rayon, reflecting diverse sources of microplastic pollution in the River Nile, with paper cups playing a significant role in this contamination. These findings highlight the widespread issue of plastic and paper cup pollution in water sources and emphasize the need for further research into the environmental and health effects of microplastics and paper cups.

3.3 Microscopic analysis of microparticles in water samples before and after soaking with paper cups

In the current study, the films of the river water, both the leachate soaked with paper cups after 4 months and non-soaked water under the SEM showed particles and microparticles with different size and distribution that increased after putting the paper cups (Figure 5). Light microscopy photomicrographs of the three tested water (River, distilled and tap water) before and after soaking with paper cups showed that River Nile water was not clear and contained impurities but tap and distilled water seemed clear. After soaking with paper cups there were impurities which take different shapes such as rodlet, fibrous and spherical fragments and increased in River Nile water (Figure 6). Along with this study, various irregular shapes of the released MP particles from paper cups and the water contained in the disposable cups were investigated

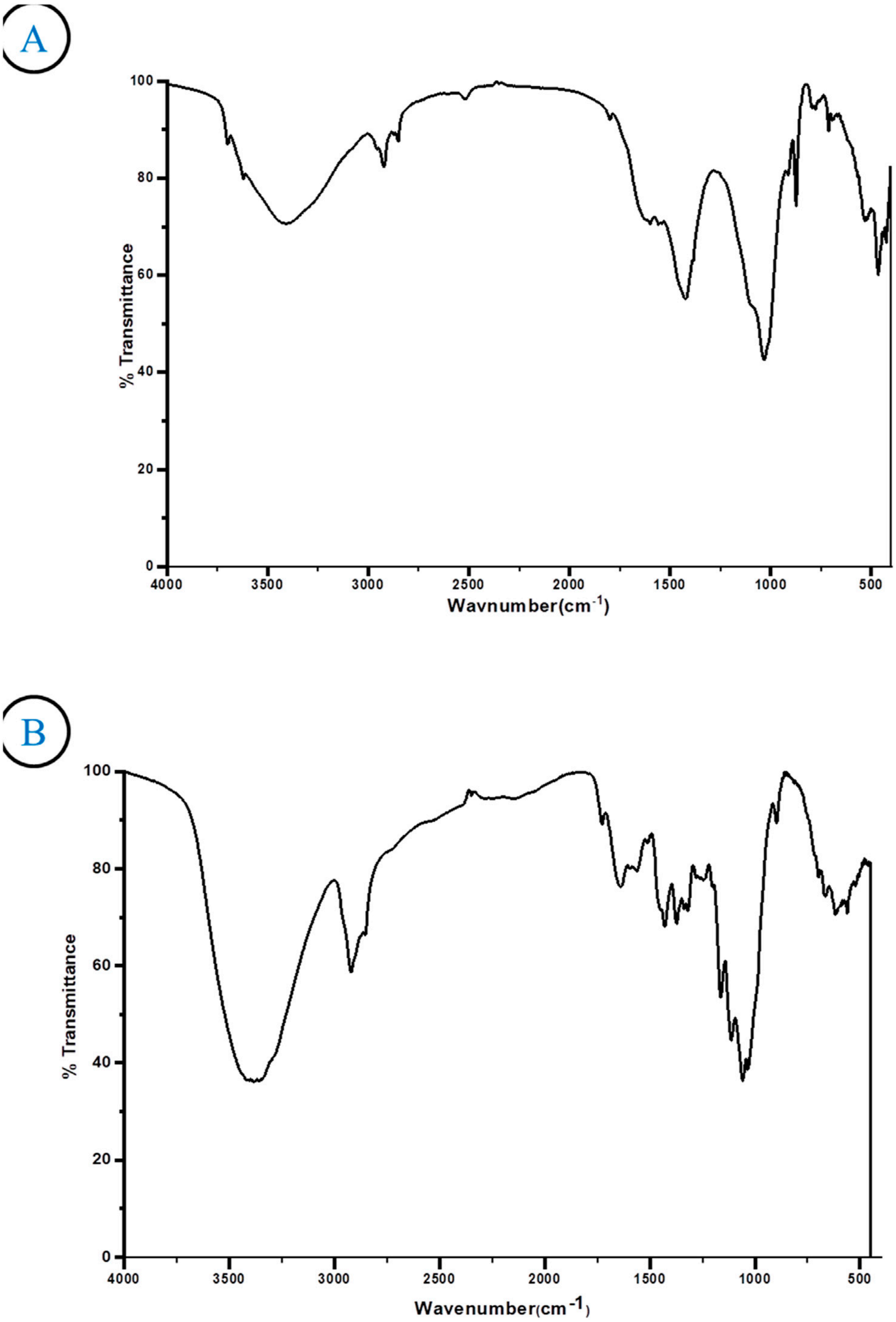

FIGURE 4
FTIR spectra of **(A)** River Nile water and **(B)** leachate of tap water containing paper cups.

in studies by Ranjan et al. (2021) and Chen et al. (2023) respectively, that could be taken up by humans and therefore pose health risks. Plastic fibers and microparticles were previously detected in tap waters (Pivokonsky et al., 2018), mineral water bottles in various countries and mineral water bottles (Koelmans et al., 2019; Schymanski et al., 2018).

According to Ranjan et al. (2021) the amount of particles that can be consumed when hot liquid is consumed in a 100 mL paper cup is (102.3 + 21.1) × 106 particles/mL. According to Batel et al. (2016), microplastics can be separated into five basic types based on their physical characteristics: fragments, fibers, films, foams, and pellets. Plastic, both whole and fragmented, has been found on beaches (Browne et al., 2011), floating on the surfaces of seas and lakes (Biginagwa et al., 2016), in the deep sea (Woodall et al., 2014) and in a wide range of species (Gall, 2015). The size and structure of microplastics are assumed to be markers of their fate and dissolution

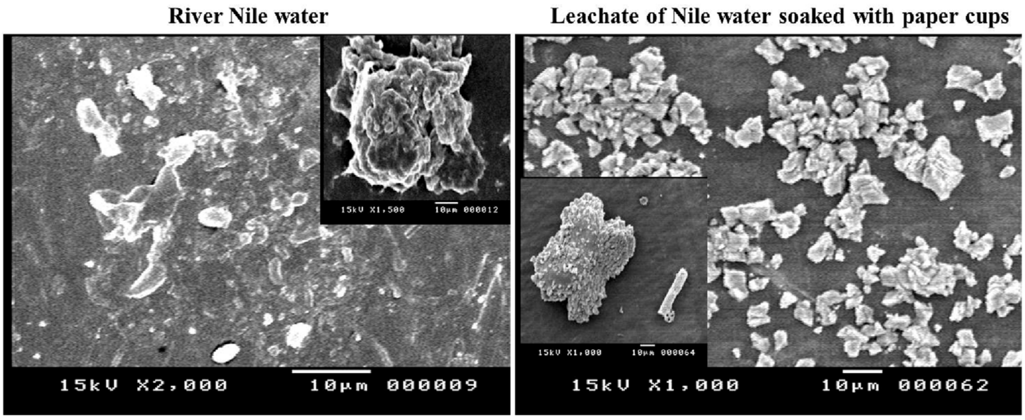

FIGURE 5
Photomicrographs of Scanning Electron Microscopy (SEM) comparing the appearance of river water, both the leachate soaked with paper cups after 4 months and non-soaked water.

in the environment (Biedermann-Brem et al., 2016). Significant volumes of microplastics infiltrate the sediment and water as the climate continues to warm and the use of plastic goods increases (Bioplastics, 2017).

3.4 Microplastic contamination in *O. niloticus* and *B. bajad*: an indicator of River Nile pollution

The results of this study revealed that, most *O. niloticus and B. bajad* that were analyzed for detection of microplastics as an indicator to microplastics pollution had contained irregular-shaped microplastics particles as showing in (Figures 7A, B) respectively. Accordingly, these findings suggested that microplastic contamination may be endangering fish in the River Nile, if environmental authorities are not drive a concerned actions toward its control. The presence of MPs in these fish could provide further information that could help in understanding the fate of MPs in freshwater bodies. Evidence of microplastic accumulation in fish, both demersal and pelagic, was revealed by the studies (Boerger et al., 2010; Lusher et al., 2013). Also, Neves et al. (2015) reported that up to one-third of the fish under study had ingested microplastics, ranging from 0.2 to 1.9 particles/fish. Polycyclic aromatic hydrocarbons (PAHs), herbicides and heavy metals are among the various contaminants that MPs can carry and absorb. As a result, they are more easily absorbed by organisms, transported throughout different fish organs and ultimately become more toxic (Bollainpastor and Agullo, 2019; Vedolin et al., 2018). Tissue-accumulated MPs influence the ability of organism to breathe, alter cell osmotic pressure, which can ultimately cause organ malfunctions and death (Ma et al., 2018). Feeding and skin absorption are among the main routes of MPs entry into organism tissues (Andrade et al., 2019). Microplastics impede an organism's digestive system, prevent animals from eating, or cause false satisfaction once they enter its body (Arumugam et al., 2018). When *Mytilus edulis* fed polyethylene microspheres (less than 80 μm) and *Carcinus maenas* fed polystyrene microspheres (10 μm) via gill respiration, intestinal damage was observed in the two species (Baker, 2013). *Daphnia magna* exposed to leachates from teabags containing microplastics showed anatomical anomalies (Hernandez et al., 2019).

Furthermore, microplastics could interfere with food, mechanically injure living things, and cause fish to lose their ability to allocate food and forage (Batel et al., 2016). Fishing productivity may drop as a result of these pollutants, according to reports that seriously endanger the growth and health of fish in Lake Amatitlán (Oliva-Hernández et al., 2021). Secondary and tertiary treatment could effectively eliminate the majority of MPs (Lares et al., 2018; Murphy et al., 2016). Whoever, Murphy et al. (2016) indicated that this technique couldn't stop the MPs effluents into aquatic systems. Some major factors, including water quality, human activity, urbanization, and wastewater treatment technology, limit the amount of microplastic pollution in freshwater systems (Zhang et al., 2022). The effects of exposure to microplastics can differ depending on whether it was received directly or indirectly (Enyoh et al., 2020). Therefore, MP pollutants have harmful effects on aquatic life, such as impairing their immune systems and digestive systems, which could cause fish, oysters, mussels and sea turtles to become extinct (Caron et al., 2018; Hipfner et al., 2018; Matsuguma et al., 2017). According to Du et al. (2020), direct exposure occurs when contaminants come into direct contact with an organism, usually leading to acute toxicity over a short period of time. Chronic organ toxicity is caused by indirect exposure, which is the incorporation of contaminants and microplastics into the food chain (Siddiqui et al., 2023). Pollutants including microplastics are incorporated into the food chain through indirect exposure, which results in long-term organ damage (Alijagic et al., 2024). The ingestion of microplastics has been associated with several hazards in a variety of aquatic organisms including oxidative stress, growth suppression, changed behaviour, cytotoxicity, reproductive

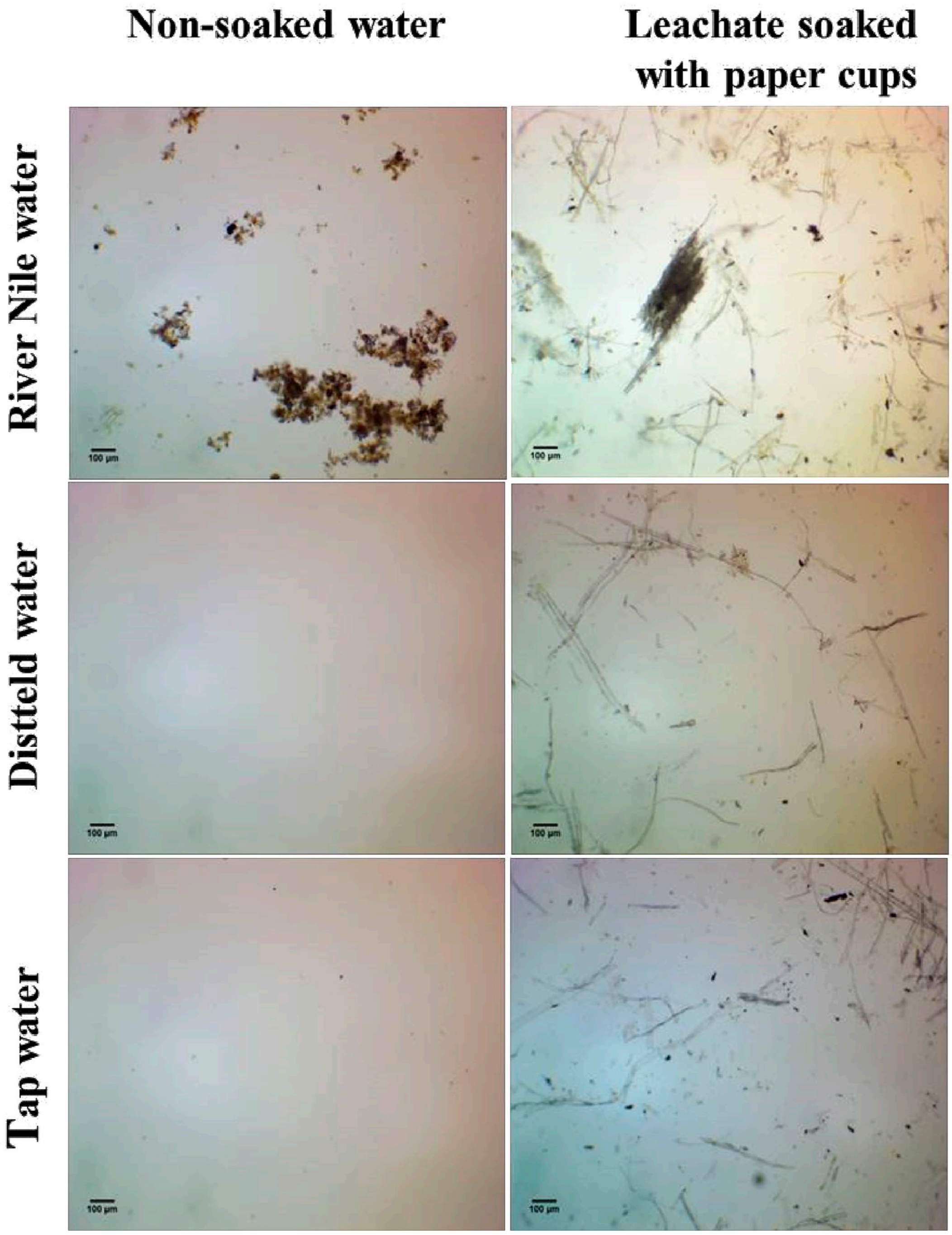

FIGURE 6
Light microscopy photomicrographs indicating the appearance of the three tested water (River, distilled and tap water) before and after soaking with paper cups.

failure, and differential gene expression (Amin et al., 2020; Hamed et al., 2019; Kedzierski et al., 2020; Meaza et al., 2021; Osman et al., 2023; Ugwu et al., 2021).

Overall, the presence of microplastics in fish from the River Nile indicates ongoing contamination, which may have an impact on the trophic network and fish consumers. Therefore, environmental and health authorities must pay close attention to limit microplastic pollution in the River Nile, particularly from widely used paper cups that often end up in the environment and persist in freshwater ecosystems.

4 Conclusion

The current findings concluded that paper cups released certain ions, heavy metals, and microplastics into the water. While some

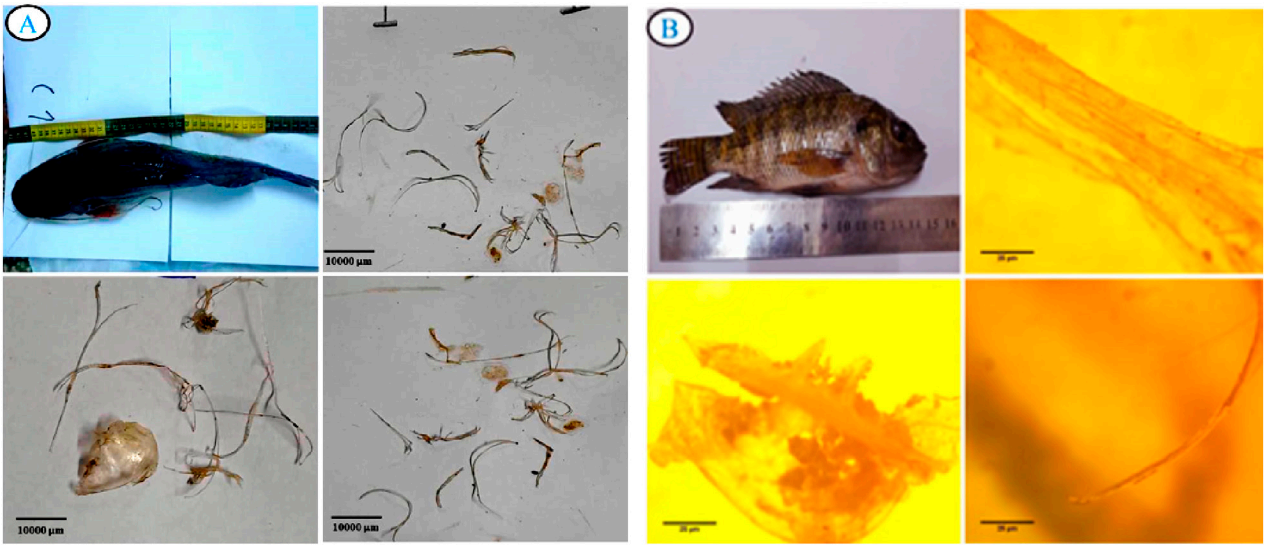

FIGURE 7
Photographs of the two investigated fish species from the River Nile, Assiut with their intestinal loads of microplastics, *Bagrus bajad* **(A)** and *Oreochromis niloticus* **(B)**.

microplastics, such as PVC, PET, and rayon, were not detected, their absence suggests they require more time to biodegrade. Our study identified microplastics in specific fish species from the River Nile at Assiut, highlighting potential threats from microplastic pollution and paper cup waste. The contamination caused by paper cups affected water quality, harming aquatic organisms and potentially endangering human health through fish consumption. While single-use paper products have harmful environmental impacts, banning them outright without providing viable alternatives is not a practical solution. Thus, this study recommends that future research should focus on developing strategies to minimize paper product pollution at its source. They should also assess the benefits of consumer education campaigns and other initiatives aimed at encouraging eco-friendly behavior, as well as the immediate and long-term environmental effects of these actions. Overall, the most promising solution to mitigate pollution from paper cups is to combine the environmental consciousness of consumers with economic measures for purchasing single-use products to reduce their usage.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding authors.

Ethics statement

The animal studies were approved by Assiut university committee, MBRSI-Research Ethics Committee. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent was obtained from the owners for the participation of their animals in this study.

Author contributions

ZE: Conceptualization, Investigation, Methodology, Resources, Writing–original draft, Writing–review and editing. UM: Conceptualization, Supervision, Writing–original draft, Writing–review and editing. A-DS: Conceptualization, Investigation, Methodology, Resources, Supervision, Writing–original draft, Writing–review and editing.

Funding

The author(s) declare that no financial support was received for the research and/or publication of this article.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

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