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The first evidence of microplastic presence in the River Nile in Khartoum, Sudan: Using Nile Tilapia fish as a bio-indicator

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ABSTRACT

The extent of microplastics in African freshwater systems remains less investigated. In Sudan, there is no single study reporting microplastics in water bodies. This scoping study aimed to investigate the presence and characteristics of microplastics (MPs) in Nile Tilapia fish from the River Nile in Khartoum, Sudan. The digestive tracts of the fish were digested using 10% potassium hydroxide, and microplastic particles were extracted by density separation using sodium iodide. 567 particles of different sizes (0.04–4.94 mm), shapes (fibers, fragments, films, foams, and pellets), and colours (mostly green, black, blue, and grey) were identified as microplastics. The average abundance of microplastics was 72.02 ± 62.06 particles/kg, and the average intensity was 18.90 ± 9.17 MPs/fish. Small-sized (<1 mm), fibrous-shaped, and coloured microplastics were most abundant in all samples, representing 56%, 85%, and 84%, respectively. Surface examination by SEM showed signs of fragmentation such as cracks, pits, and pores. Two polymer types (high-density polyethylene and polypropylene) were identified by Raman spectroscopy. The predominance of fibers and fragments (94.5%) over pellets (0.35%) and the apparent signs of fragmentation may indicate that MPs are mostly secondary MPs. Wastewater effluent, domestic discharge, and recreational activities are the potential sources. This scoping investigation provided the first data on microplastic presence in the River Nile in Khartoum, and it could be used to guide future studies to fill research gaps in the region.

1. Introduction

Due to their exceptional properties such as durability, thermal and electric insulation, and corrosion resistance, plastics are widely used in countless applications [1–3]. This has led to an ongoing increase in plastic production and resulted in large volumes of plastic waste accumulating in the environment [4,5]. The persistence of plastic debris further affects the ecosystem with consequential effects on biodiversity, climate, human health, and global economies [6–8].

Over time, plastics break down into smaller pieces in a variety of shapes known as microplastics (plastic pieces < 5 mm) [9]. The contamination of global environments by MPs has gained increased attention, and more research has recently been directed at documenting MPs in different environmental compartments [10–12]. However, knowledge of MP contamination in the African context is lacking. Until recently, MP research on the continent was limited to marine and coastal areas [13], and this work is the first attempt to assess the presence of MPs in the River Nile in Khartoum, Sudan.

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The River Nile is one of the world's most famous rivers and is Africa's most important freshwater system. It supports significant populations as it flows through several densely populated cities and influences people living in 11 African countries. It covers about 10% of the African landmass and hosts 20% of the African population who depend on livestock and agriculture for their livelihoods [14–18].

Due to their correlation with aquatic compartments, several aquatic fauna are used as bio-indicators to monitor MPs in water systems [19–22].

Nile Tilapia (*Oreochromis niloticus*) is a popular African freshwater fish species and forms the basis of commercial fisheries in many African countries [23,24]. In Sudan, Tilapia is a dominant economic and ecological species, and it is widely consumed [25–27]. Thus, representing a suitable target organism to study the presence and level of contamination of MPs in the River Nile. The main objectives of this study were to investigate the distribution of MPs in the River Nile in Khartoum, Sudan (using Nile Tilapia as a bioindicator), to examine the physical and chemical characteristics of MPs, and to identify potential sources.

2. Methods

2.1. Study area and sample collection

The White Nile and the Blue Nile are the two main tributaries of the River Nile. The White Nile starts at Lake Victoria in Uganda, while the Blue Nile drains from Lake Tana in Ethiopia [28]. Once they merge at the capital city of Sudan, Khartoum (Fig. S1), the Nile continues north toward Egypt and drains into the Mediterranean Sea [16,18]. The confluence of the two Niles "Al-Mogran – in local Sudanese Arabic" is where the Nile begins. Khartoum, where the two Niles meet, is a densely populated city and urban centre, and is the largest industrial hub in Sudan. The city witnessed rapid population growth, from 250 thousand in the fifties to about eight million in 2018 [28–30].

The fish in this study were caught just after Al-Mogran. Thirty Nile Tilapia fish were bought (freshly caught) from a fisherman at Al-Mawrada fish market along the River Nile in Omdurman, Khartoum, in autumn (Aug 2021). Fish were frozen and transported to the microplastic laboratory at Witwatersrand University in South Africa and kept frozen at −20 °C until analysis.

2.2. Biometric characteristics

Fish were defrosted and rinsed with filtered deionized water. A routine physical examination was then done before dissecting the digestive tracts to record the biometric specifications of individual fish (length (cm), body mass (g), and sex). The total length was measured from the tip of the snout to the end of the caudal fin using a measuring tape with 0.1 cm precision.

External and internal reproductive features were used to identify the sex. Males were identified by the presence of two openings, the anus, which is situated immediately in front of the anal fin, and the urethral, which is located at the end of the genital papilla (an oval-shaped lobe directly beyond the anus), and is where urine and milt are discharged. Females, on the other hand, were identified by three body openings. In addition to the anus and urethra, females have a third opening, the oviduct (a crescent-shaped slit), from which eggs are released [31]. This was further confirmed by the presence and absence of testes and ovaries seen later during dissection.

Further, Using the recorded biometric data, namely, length and weight, the condition factor was calculated using equation (1) below:

$$\text{Condition Factor (K)} = 100\text{W}/\ \text{L3} \tag{1}$$

W is the weight in grams and L is the total length in cm.

The condition factor reflects the fish's growth, habitat conditions, and the general well-being and fitness of the aquatic environment, in this case, the River Nile in Khartoum, Sudan. Low K values (<1) reflect unfavourable environmental conditions and/or insufficient nutrition, whereas favourable conditions like habitat and prey availability are indicated by K values that are greater than one [32].

2.3. Sample pre-treatment and extraction of MPs

Fish were dissected to remove their gastrointestinal tracts; a longitudinal small incision was made from the anus to the lower jaw using forceps and a scalpel. The digestive tract of each individual was gently removed and placed in a 250 mL glass beaker, weighed, and covered (with aluminium foil). Alkaline digestion was accomplished by adding 10% KOH at a volume ratio of 1:3, and the glass beakers were covered and placed in a pre-heated incubator at 40 °C and rotated at 125 rpm for 24 h [33]. Following complete digestion, salt-based density separation was conducted using sodium iodide (supplied by Ace Chemicals South Africa ≥99.5%) to extract MPs. The density separation solution was added in a 1:2 vol ratio and left overnight [33]. 47 mm diameter with 1.6 μm pore size Whatman GF/A glass microfiber filters using a Nalgene vacuum filter were used to filter the samples.

To prevent cross-contamination, the filter funnel was thoroughly rinsed, and the sides were rinsed with filtered deionized water to ensure that all particles sticking to the sidewalls were washed into the filters. Filter papers were air-dried in a fume hood and stored in covered glass Petri dishes. All glassware and handling equipment were washed and rinsed with filtered deionized water before use to prevent contamination. All experiments were conducted in a fume hood. Blanks were prepared using filtered Milli-Q® Type 1

Ultrapure water and run concurrently with the samples to count for possible procedural contamination. No MPs were detected in the blanks.

2.4. Physical examination and chemical identification of MPs

Physical examination was performed using a stereomicroscope, by which all suspected particles were visually observed, measured, and photographed.

Visual criteria reported in the literature were used to identify MPs [13,33]. Potential MPs were classified into different shapes (e.g., fibers, pellets, films, foams, and fragments) and sorted to a maximum size of 5 mm. Scanning electron microscopy (TESCAN Vega SEM) was then used to examine the surface texture of selected particles of different shapes.

Polymer types were chemically identified using a Horiba Lab RAM HR Raman spectrometer. The software Lab Spec v5 was used to acquire and analyse spectra. The chemical compositions of polymers were then identified by comparing the acquired spectra with reference spectra in the polymer database of KnowItAll® Informatics system software (John Wiley & Sons, Inc., Hoboken, New Jersey, United States).

3. Results and discussion

3.1. Biometric measurements

Biometric data, including length, sex, weight, and condition factor of Nile Tilapia are presented in Table 1. The mass of the fish ranged from 0.11 to 1.39 Kg, and the length from 19.10 to 45.00 cm; of the thirty fish, twenty were identified as males, and ten were identified as females.

As the values in Table 1 show, all K values except for sample S1 were found to be greater than 1, indicating that fish were in good health and generally well-being. Furthermore, no significant differences in condition indices were found between groups, suggesting that they received equal nutrition.

3.2. MP prevalence, abundance, and intensity in the digestive tracts of Nile Tilapia

In this investigation, MPs were 100% prevalent in Nile Tilapia specimens. A number of 567 particles were identified as MPs, with an

Table 1
Biometric data of Nile Tilapia.

Sample Code	Sex	Body Mass (Kg)	Total Length (cm)	Width (cm)	Condition factor (K) (g/cm^3)
S_1	Male	0.120	26.0	07.0	0.68
S_2	Female	0.147	21.0	07.9	1.59
S_3	Male	0.118	20.8	07.7	1.31
S_4	Female	0.114	20.7	07.3	1.29
S_5	Male	0.132	22.6	07.4	1.14
S_6	Male	0.107	19.1	06.5	1.54
S_7	Male	0.116	19.5	06.8	1.56
S_8	Male	0.128	20.0	07.3	1.60
S_9	Female	0.160	21.5	07.4	1.61
S_{10}	Male	0.159	21.4	07.4	1.62
S_{11}	Male	0.307	27.6	08.6	1.46
S_{12}	Male	0.353	28.0	09.5	1.61
S_{13}	Male	0.310	30.1	09.4	1.14
S_{14}	Female	0.380	31.5	09.6	1.22
S_{15}	Female	0.305	28.1	09.4	1.37
S_{16}	Male	0.321	29.7	09.5	1.23
S_{17}	Female	0.304	28.0	08.8	1.38
S_{18}	Female	0.365	28.4	09.6	1.59
S_{19}	Female	0.325	27.6	09.7	1.55
S_{20}	Male	0.389	29.0	09.7	1.59
S_{21}	Male	0.738	37.5	12.3	1.40
S_{22}	Male	0.969	40.7	13.1	1.44
S_{23}	Female	1.132	42.1	15.3	1.52
S_{24}	Male	1.154	41.8	15.8	1.58
S_{25}	Male	1.388	45.0	15.8	1.52
S_{26}	Male	0.776	37.1	12.6	1.52
S_{27}	Male	0.720	37.2	12.6	1.40
S_{28}	Male	0.871	38.7	13.0	1.50
S_{29}	Male	0.946	40.2	13.2	1.46
S_{30}	Female	0.945	40.6	13.0	1.41

average intensity of 18.90 ± 9.17 particles/individual.

The intensity of MPs in fish samples varied with a maximum number of 47 particles in sample S11 and a minimum number of 5 particles in S24, as shown in Fig. 1.

The abundance of MPs ranged from 04.33 to 236.84 particles/Kg, with an average of 72.02 ± 62.06 particles/Kg. A relatively high MP abundance was observed in S1(216.67 particles/kg) and S4 (236.84 particles/kg), both were classified as small fish. On the other hand, minimum abundances were found in larger fish S23 (06.18 particles/kg) and S24 (04.33 particles/kg).

The relationship between fish size (body mass and length) and MP abundance was further examined statistically using the Spearman correlation test (Fig. 2). Low p-values of 0.001 (Fig.s. 2a) and 0.003 (Fig. 2b) implied a significant negative correlation between MP abundance and body mass and length, respectively, meaning the abundance of MPs increased as the fish size decreased.

The high prevalence of MPs in the fish, which may reflect the extent of plastic pollution in the River Nile, shows that the aquatic life in the Nile is at risk of ingesting MPs and associated contaminants. However, in the absence of any data on MP presence in the waters and sediment of the River Nile, any conclusion that relates the ingestion of MPs by the Tilapia solely to the actual abundance of MPs in the river would be inaccurate. In other words, biotic factors such as feeding habits may contribute to the high MP abundance and intensity in Nile Tilapia.

The ingestion of MPs by fish is increasingly reported [33–37]. Table 2 shows the findings of some studies reporting MP ingestion by Nile Tilapia.

3.3. *Physical characteristics of MPs recovered from Nile Tilapia*

3.3.1. *Size of MPs*

The size of MPs recovered from the digestive tracts of Nile Tilapia ranged from 0.04 to 4.94 mm. Size distribution is depicted in Fig. 3, which shows the dominance of smaller MPs as follows: 30.51% of MPs are <0.5 mm, 25.93% are between 0.5 and 1 mm, 27.69% in the range of 1–2 mm, and only 15.88% of the 567 particles are larger than 2 mm (2–5 mm).

The predominance of small-sized MPs is frequently reported in different aquatic fauna in several aquatic environments around the world, including the Great Lakes in the US, the Yangtze River in China, the Saigon River in Vietnam, Geoje Island in South Korea, and the Vaal River in South Africa [33,44–47]. This may indicate a higher abundance of small-sized MPs in the river due to frequent fragmentation by several environmental processes. Additionally, small-sized MPs have a larger surface area that facilitates bio-fouling and attachment of other contaminants, thus reducing the buoyancy and increasing their bioavailability [8,9,48].

3.3.2. *Shape of MPs*

Criteria reported by Jabeen and co-authors were used to classify the different shapes of MPs [49]. Particles with a slender and elongated appearance were categorized as fibers; particles of irregular shapes were categorized as fragments; very thin particles were categorized as films; particles with a sponge-like appearance were classified as foams; and round particles with spherical shapes were categorized as pellets. Based on these criteria, five shapes were identified, namely, fibers, fragments, films, foams, and pellets.

Fig. 4 displays the distribution of the detected shapes. Fibers were the most abundant, with 100% prevalence; of the 567 particles, 483 particles were fibers, representing 85% of the MPs recovered from the fish. Fragments and films were present in small percentages of 9.5% and 4.6%, respectively, while foam and pellets were detected in a minor percentage of 0.35%. This may indicate a higher

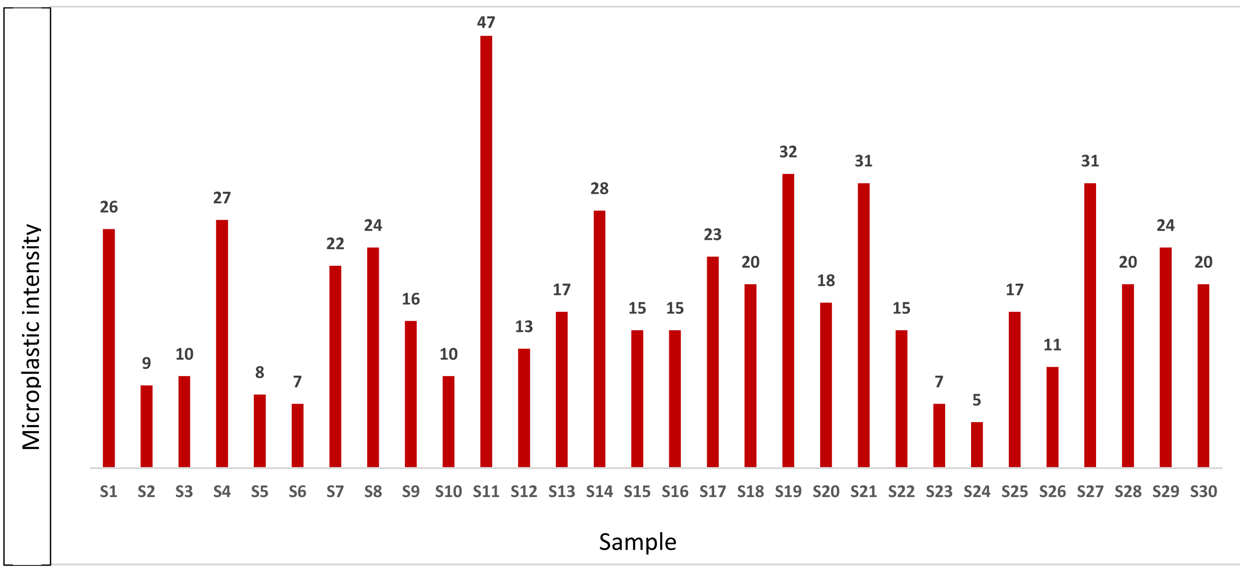

Fig. 1. Intensity of MPs in Nile Tilapia fish sampled from the River Nile (number of MPs per individual fish).

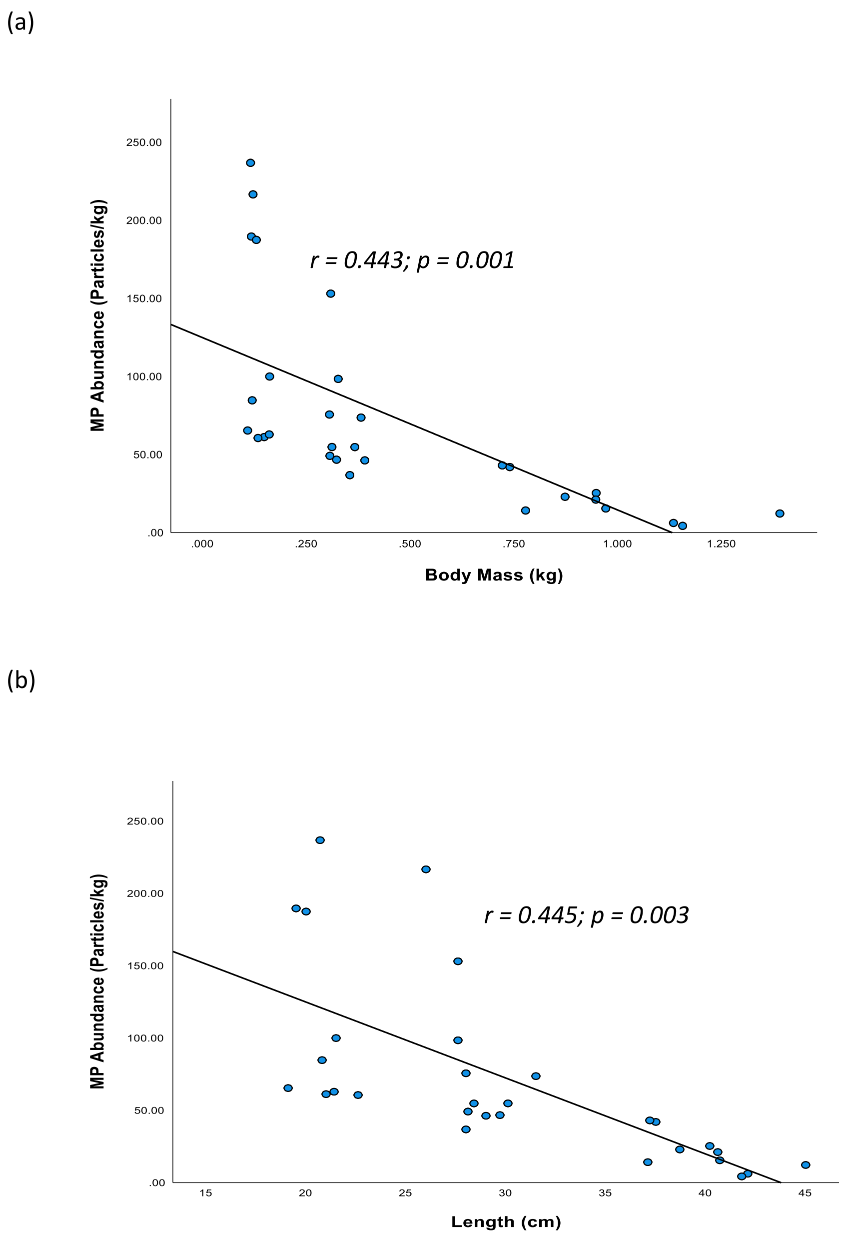

Fig. 2. Correlation between MP abundance and (a) body mass, (b) length.

Table 2
Some studies reporting MPs in Nile Tilapia.

Freshwater system	Region	Number of fish	Number of MP	prevalence %	Abundance particles/Kg	References
Lake Eleyele	Nigeria	43	1–4	34.0	–	[38]
Lake Victoria	Tanzania	07	–	35.0	–	[39]
Lake Hawassa	Ethiopia	30	147	36.67	4.03 ± 3.19	[40]
Atoyac River	Mexico	15	139	53.7	–	[41]
River Nile	Egypt	29	164	75.9	–	[42]
Lake Amatitlán	Guatemala	65	631	96.9	65.74 ± 57.55	[43]
River Nile	**Sudan**	**30**	**567**	**100**	**72.07 ± 63.10**	**Present study**

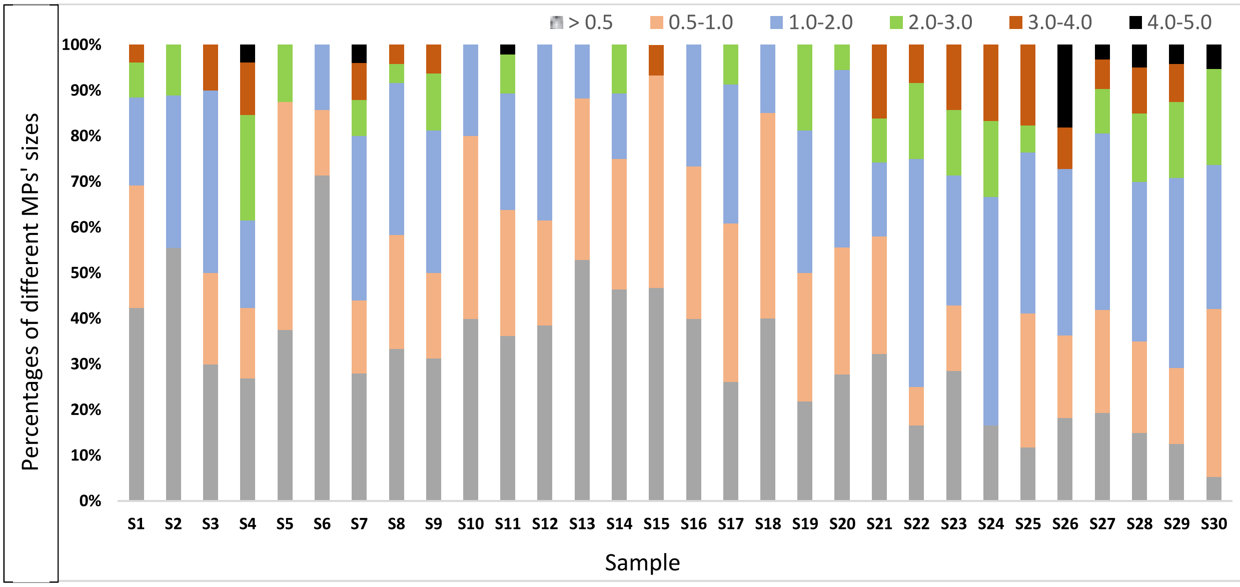

Fig. 3. Distribution of MPs' sizes per individual fish.

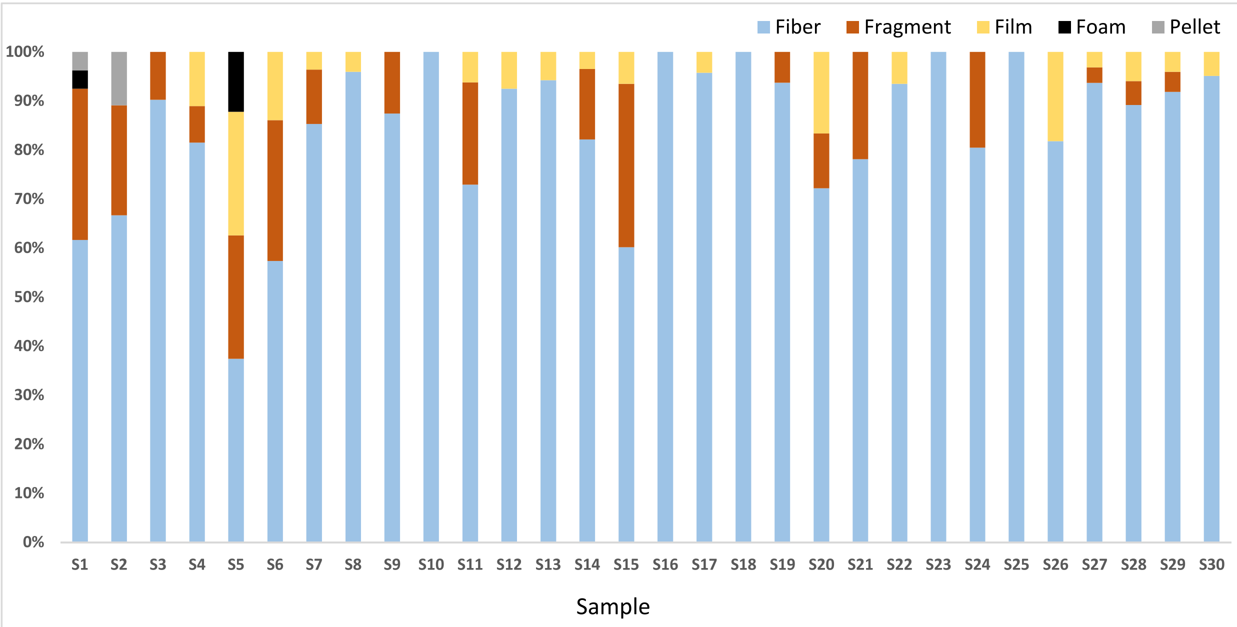

Fig. 4. Distribution of MPs shapes per individual fish.

abundance of fibers in the River Nile. However, it should be noted that the shape of MPs influences prey perception in visual foraging fish [50]. Thus, the abundance of a specific shape does not necessarily reflect its prevalence in a given water body. Consequently, the predominance of fibers in this study may suggest that Nile Tilapia fish prefer fiber-like particles. This is supported by the observation made by Yuan and co-authors, who reported that the proportion of fibers in goldfish from Poyang Lake, China, was higher than that found in the surrounding environment [51].

3.3.3. Colour of MPs

A great variety of colours were observed, of which, green was the most abundant (26.63%), followed by black (18.69%), blue (14.29%), red (8.82%), grey (7.23%), and white (3.53%). Other less frequently observed colours (purple, yellow, pink, and brown) were grouped under one category named others and represented (4.41%), altogether. Transparent MPs constituted 16.40% of the total

MPs (Fig. 5). However, it is essential to note that the colour of MPs may change/fade due to weathering processes [52].

Fig. 6a shows shape distribution per size range. Fibers were present across all size ranges, a large proportion of 4.34% was observed in the size range of 1.0–2.0 mm. Fragments were observed in three size ranges: <0.5 mm (6.53%), 0.5–1.0 mm (5.11%), and (1.41%) in 1.0–2.0 mm. Films were observed in two size ranges, 0.5–1.0 mm and 1.0–2.0 mm, with 3.35% and 0.53%, respectively. Pellets and foams were observed in only one size range of 0.5–1.0 mm (0.35%).

The distribution of colours per shape (Fig. 6b) revealed a higher abundance of coloured fibers (73.02%) compared to transparent fibers (11.82%). Similarly, coloured fragments were more abundant. The variety of colours indicates the diversification of MP pollution sources [53]. For instance, black fragments may come from car tires, whereas, translucent and white may result from packaging materials and plastic carry bags [54]. Transparent/white fibers are mostly from fishing robes, and coloured fibers come from clothes [55]. Wastewater and domestic effluent could be primary sources of these MPs [50].

3.3.4. Surface morphology of MPs

Fig. 7 shows SEM images of selected MPs of different shapes. The particles showed significant disintegration and/or fragmentation signs (cracks, pores, pits, and scratches). Which increases the surface area, thus facilitating the adsorption of chemicals and micro-organisms [56]. It is well known that the interaction of MPs with their surroundings, the development of biofilms, and the adsorption of other pollutants are greatly influenced by their surface texture [48].

In addition, deteriorated MPs are reported to emit dimethyl sulfide (DMS) odour [57]. Fish that feed on primary consumers like phytoplankton that produce DMS are shown to induce a foraging behavioural response to DMS and its chemical precursor dimethyl sulfoniopropionate. As such, Nile Tilapia may intentionally consume weathered MPs due to confusing the particles for the smell of their natural food [57,58].

3.4. Polymer types

Two polymers (polypropylene (PP) and high-density polyethylene (HDPE)) were identified. This may indicate both their ubiquitous usage and persistence in the environment. The Raman spectra for the two polymers are given in Fig. S2. The relatively low densities of PE and PP enable spatial dispersal in aquatic environments. As a result, these two polymers are commonly reported in all aquatic compartments [13]. According to Klein et al. (2015), PP and PE were reported to be predominantly abundant in the Rhine-main River in Germany [59]. The authors attributed this to their high demand and economic worth.

4. Biological parameters influencing MP uptake by Nile Tilapia

As discussed in previous sections, the physiochemical properties of MPs are of great importance and have significant impacts on their bio-availability for consumption by Nile Tilapia and other aquatic organisms. Some characteristics may increase the non-

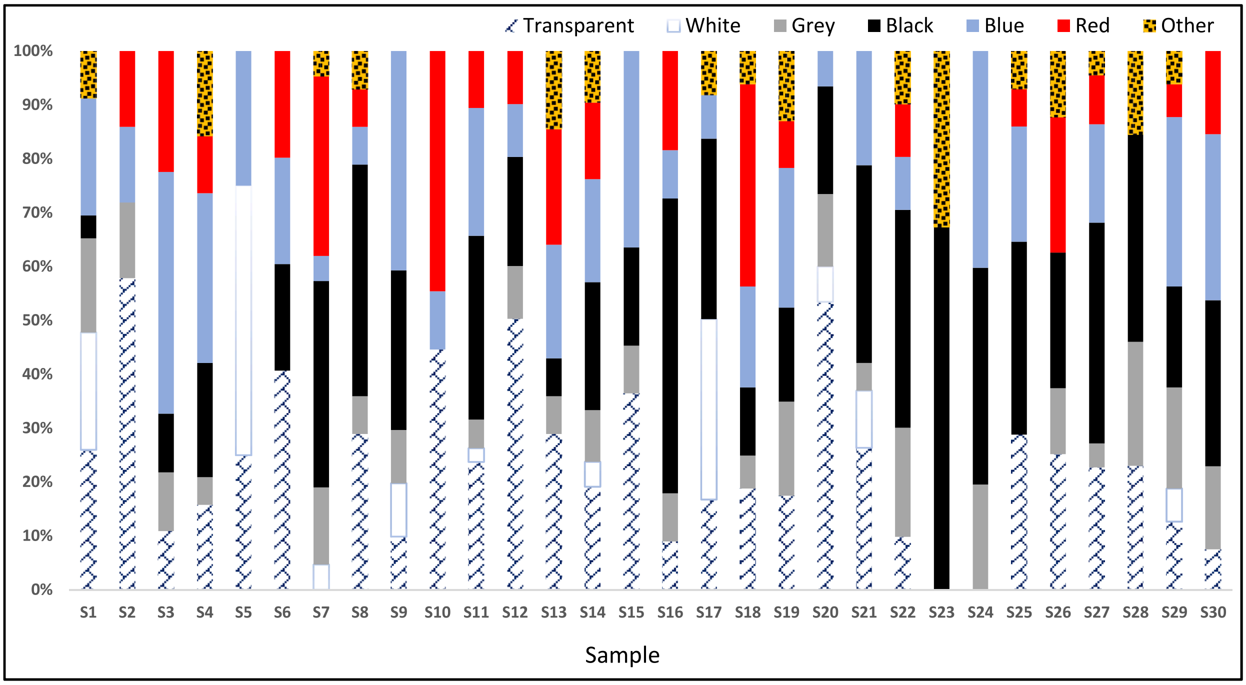

Fig. 5. Distribution of MPs colours per individual fish.

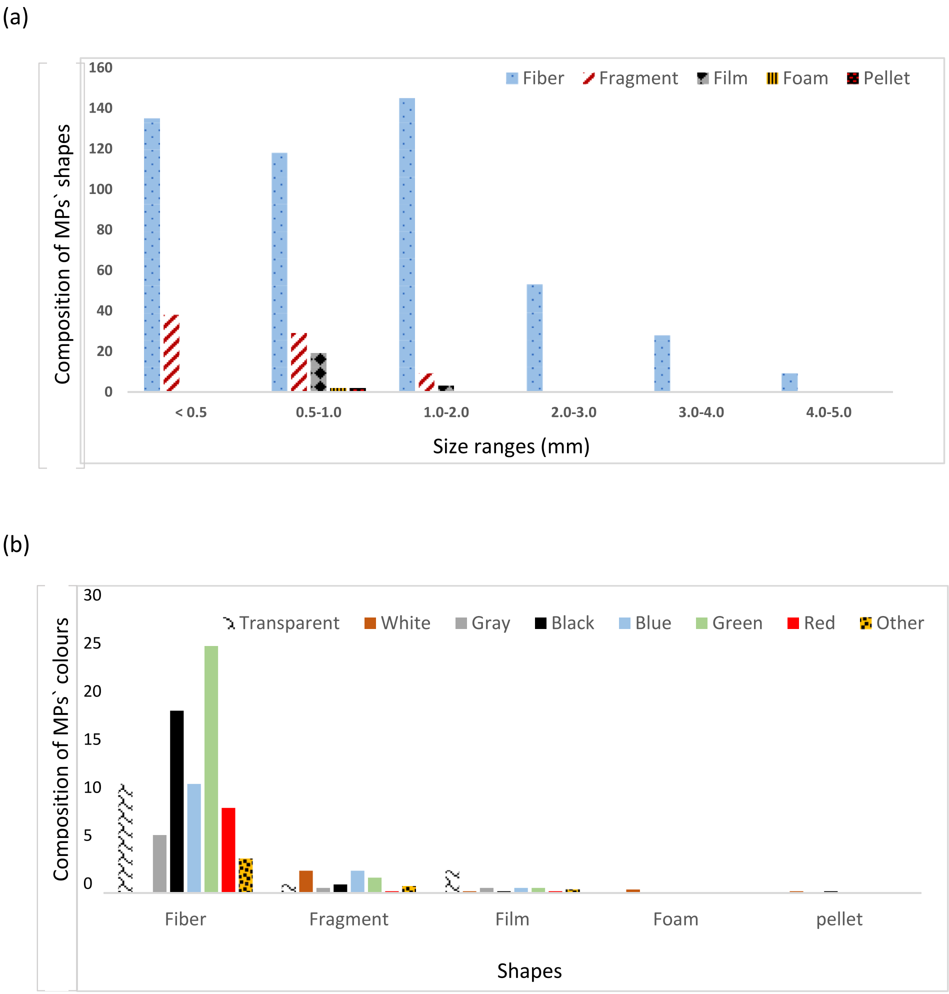

Fig. 6. Distribution of (a) shape per size and (b) colour per shape.

intentional uptake of MPs, for example, small-sized MPs are readily available for consumption by a wide range of species across all ontogenetic phases. Other characteristics may induce intentional uptake, such as confusing MPs of specific shapes and colours with natural food items [13,53,60].

Nonetheless, biotic factors such as trophic level, age, sex, and most importantly, feeding habits/foraging behaviour may also influence the uptake of MPs by the fish [61–63].

Feeding behaviour plays a key role in the ingestion of MPs by fish, while some fish ingest MPs accidently, others may intentionally consume MPs due to their similarity with their natural prey. This depends on the sensory capacities of the fish to locate food, their ability to catch and ingest food, and their biochemical and physiological capacities to digest the nutrients [33,49].

Nile Tilapia are versatile omnivorous foragers that consume a variety of organisms, including phytoplankton, zooplankton, periphyton, invertebrates, detritus, bacterial films, as well as smaller fish and fish eggs [23,24]. Thus, with such diverse feeding habits, Nile Tilapia are at higher risk of MPs' ingestion due to foraging across a wide range of food types and throughout the water column [60]. Further, planktivorous fish such as Nile Tilapia are known to swallow large volumes of water to filter sufficient food, and they may accidently ingest non-food items, such as MPs. They also have long and twisted intestines, which allow them to amass the MPs [64,65]. All this may explain the high prevalence of MPs in the Nile Tilapia specimens.

However, it is important to note that the composition of the Nile Tilapia diet may vary depending on seasonal and spatial conditions, fish size, and maturity. Some studies reported ontogenetic dietary shifts, indicating that small Nile Tilapia are mainly omnivorous to meet the high growth rate, which requires a higher protein diet, and, as they grow, they shift to herbivorous feeding behaviour [24,66]. In this study, it was noted that smaller fish (<0.25 kg) ingested a higher number of MPs compared to the larger fish (>0.5 kg). It is reported that omnivorous fish consume high amounts of MPs compared to herbivorous and carnivorous fishes, and depending on the trophic level, they may feed on, for example, macroinvertebrates that previously consumed MPs [67,68]. This agrees with the observation of the higher abundance of MPs in the smaller fish in this study.

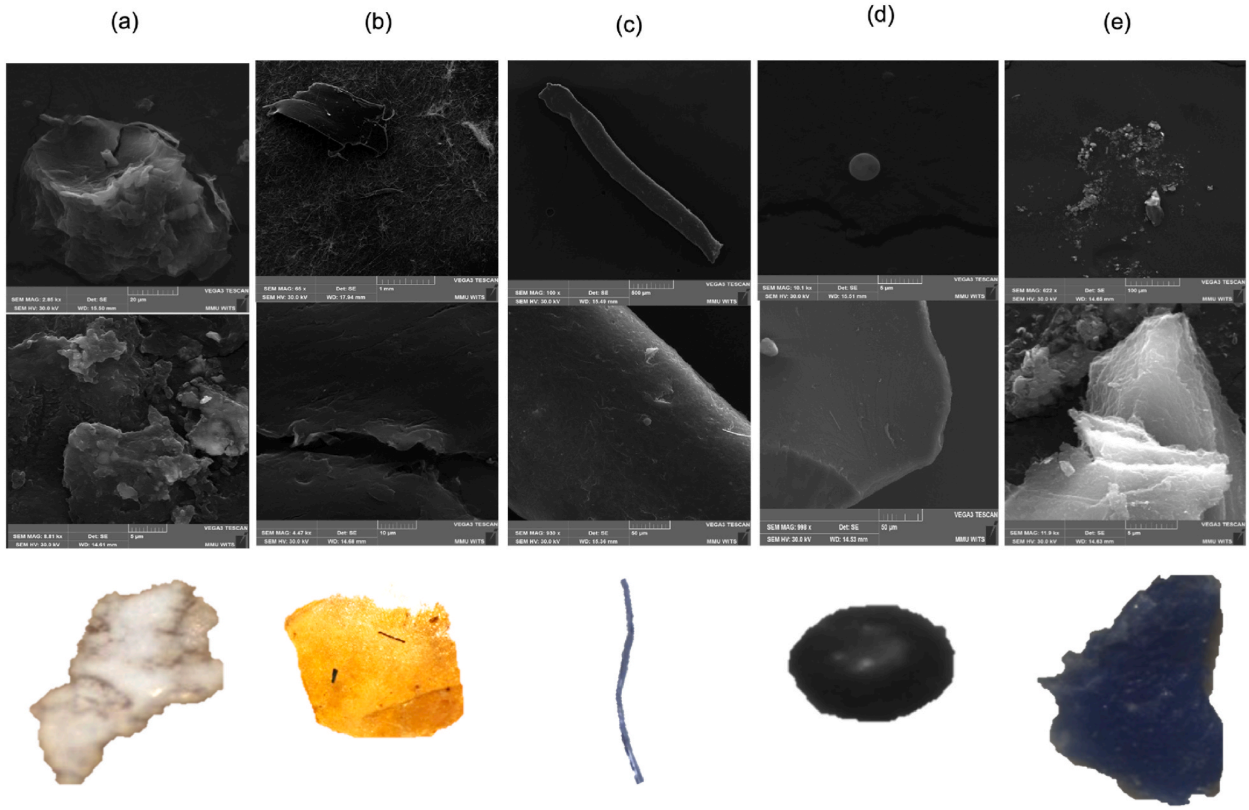

Fig. 7. Microscopic images of (a) foam, (b) film, (c) fiber, (d) pellet and (e) fragment.

5. Potential sources of MPs in the River Nile in Khartoum

Although no studies report MPs in water and sediments of the River Nile in Khartoum or elsewhere, the 100% prevalence of MPs in the Nile Tilapia is of great concern. It indicates their presence in the water and sediment of the river and reflects some level of MP contamination in the study region. Khartoum is confronted with poor waste management as a result of funds shortages, inadequate facilities for waste collection/disposal, and a lack of urban planning and environmental legislation. Thus, large quantities of solid waste, including plastics, end up in landfills and/or illegal dumping [69–71]. According to Abdel-Rahman et al. (2013), 65% of plastic waste in Khartoum is disposed of in open dumps, leading to contamination of the environment and water bodies [72]. This implies that the River Nile in Khartoum is exposed to massive plastic leakage.

The wastewater treatment system in Khartoum is inefficient, and the ongoing urban expansion has caused further deterioration. The three WWTPs in Khartoum state, namely, Karary, Wd-Daffiaa, and Soba, are outdated, do not meet local and international standards, and operate beyond the design capacity [29,73,74]. As such, the discharge of untreated effluent into the river from domestic, industrial, and agricultural activities could be a potential source of MP pollution.

The River Nile provides countless recreational sites in Khartoum [28,75]. The Nile Street is the most popular in the capital city, hosting several recreational activities along the river, such as water sports, restaurants, cafes, clubs, event venues, and hotels, as well as tea ladies (a common phenomenon in Khartoum where ladies serve hot beverages in makeshift mobile cafes along the river banks). Due to the absence of efficient waste collection and disposal practices in the city, these leisure activities contribute to large amounts of plastic litter leaking into the river stream and eventually degrading into MPs.

6. Conclusions

Thirty Nile Tilapia fish were used to bio-monitor the presence of MPs in the River Nile in Khartoum. Microplastics were 100% prevalent in Nile Tilapia specimens, with abundances ranging from 04.33 to 236.84 particles/Kg. The dominance of small-sized MPs (<2 mm = 84%) and fibers (85%), as well as the signs of fragmentation, indicate that they are predominantly secondary MPs. Anthropogenic activities, including agricultural, recreational, and industrial, are all potential sources of MP contamination. High-density polyethylene and polypropylene were identified by Raman spectroscopy.

This work presents the first evidence of MP presence in the River Nile in Khartoum. As such, it offers a basis to comprehend the fate of MPs in the River Nile. Further, such evidence is also essential for informed policy formulation and effective decision-making.

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Data availability statement

Data will be made available on request.

CRediT authorship contribution statement

Dalia Saad: Writing - review & editing, Writing - original draft, Supervision, Resources, Project administration, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Hadeel Alamin:** Writing - review & editing, Methodology, Formal analysis, Data curation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e23393.

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