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Baseline

Presence of microplastics in two common dried marine fish species from Bangladesh

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ABSTRACT

We examined microplastics (MP) in two commercially important dried fish, Bombay duck (*Harpadon nehereus*) and ribbon fish (*Trichiurus lepturus*), collected from two sites on the Bay of Bengal (Cox's Bazar and Kuakata). The number of MP found in dried Bombay duck and ribbon fish from Kuakata was significantly higher (41.33 g^{-1} and 46.00 g^{-1}, respectively) than the MP present in samples collected from Cox's Bazar (28.54 g^{-1} and 34.17 g^{-1}, respectively). Fibers were the most common type of MP identified in all samples (41–64%), followed by fragments (22–34%), microbeads (9–16%), films (3–4%), foams (1–4%), and pellets (0–2%). ATR-FTIR analysis revealed three different types of MP polymer - polyethylene (35–45%), polystyrene (20–30%) and polyamide (30–45%) in the dried fish samples. The study confirms the presence of high MP loads in dried fish from the Bay of Bengal, with high potential of trophic transfer of MP to the human body.

Microplastics (MP) pollution of aquatic environments is a global concern. The ultimate destination of most plastic products is the marine environment (Barnes et al., 2009). In 2010, about 275 million tons of plastic products were manufactured throughout the world and significant portions of them ended up in marine environments (Jambeck et al., 2015). Evidence of marine MP pollution has been growing rapidly (Guzzetti et al., 2018; Savoca et al., 2019; Hossain et al., 2021). Worldwide, numerous studies have detected MP in fish (Ory et al., 2018; Hossain et al., 2019), crustaceans (Devriese et al., 2015), bivalves (Li et al., 2018; Cho et al., 2019), zooplankton (Botterell et al., 2019), corals (Hall et al., 2015) and seabirds (Zhao et al., 2016; Amelineau et al., 2016). Ingestion of MP in fish and bivalves has been studied in the field and under laboratory conditions (Hermsen et al., 2017; Wang and Wang, 2018; Hossain et al., 2019). Harmful effects of MP ingestion including physiological damage, inflammation, blockage of the digestive tract, and cellular toxicity have been observed in different organisms (Prokić et al., 2019; Savoca et al., 2019; Strungaru et al., 2019). MP in these food organisms may transfer to the human body through the food chain, posing a potential hazard for human health (Hossain et al., 2019; Crew et al., 2020).

MP enter the oceans from various sources including riverine output, atmospheric precipitation, sewage systems, and improper disposal (Cole et al., 2011; Sadri and ThoMPon, 2014). The Bay of Bengal is likely highly vulnerable to MP pollution as three major riverine systems (Padma, Meghna and Jamuna) composed of several hundreds of rivers transport MP to the Bay of Bengal from all over Bangladesh and from neighbouring countries upstream. Fishing gears made of monofilament line and nylon netting are regularly lost at sea, and create problems due to their intrinsic capacity to entangle marine organisms (Macfadyen et al., 2009). Ghost fishing nets are responsible for nearly 10% of all oceanic plastic. These mega- and macro-plastic compounds eventually disintegrate into smaller fragments – microplastics – due to intense cracking and gouging by sand, wind, and wave action (Chatterjee and Sharma, 2019; Allen et al., 2020). Wind-driven ocean circulation can drive MP accumulation in shallow coastal waters leading to high concentrations in these zones (Vianello et al., 2013). Moreover, each year about two million tourists in Bangladesh visit Cox's Bazar, the world's longest sandy beach, producing large quantities of plastic wastes that are

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ultimately deposited in the Bay of Bengal (Hossain et al., 2021). Similarly, tourists visiting Kuakata beach produce large amounts of plastic debris, much of which is deposited in coastal waters.

Dried fish is important in the diets and livelihoods of people across Asia and Africa. The dried fish sector has long played a significant role in the economy of Bangladesh (Hossain et al., 2015), where a large share of marine and freshwater capture fisheries landings are processed to produce dried, salted and fermented products (Belton et al., 2018). Marine fish drying has long been practiced along the entire coastal belt in Bangladesh, and it is estimated that nearly half of the total marine catch is dried, with most production occurring during the winter season (October to March). These dried products are in high demand in Bangladesh, and are also exported to overseas markets, particularly among the non-resident Bangladeshi diaspora. Belton et al. (2014a, 2014b) observed that dried fish were eaten more frequently than any other type of fish in several regions of the country. The contribution of dried products to fish consumption is disproportionately important for low-income consumer groups, making these products important for food and nutrition security.

Among all the aquatic organisms dried on Bangladesh's coast, the two most abundant and popular fish species are Bombay duck (*Harpadan nehereus*, or loitta in Bangla) and ribbon fish (*Trichiurus lepturus*, or churri) (Belton et al., 2014a, 2014b; Jahan et al., 2019). During 2018–19, *H. nehereus* (0.068 million MT) contributed 10.3% of the total marine fish catch (0.66 million MT) of Bangladesh (FRSS-DoF, 2020). Bombay duck and ribbon fish together account for approximately half the dried marine fish marketed in Bangladesh. In a survey of the largest dried fish wholesale market in Bangladesh (Asadgong in Chottogram), Faruque et al. (2012) found that Bombay duck and ribbon fish accounted for 25% and 22% of dried fish products for sale, respectively (a combined total of 47% of the products in the market). Potential health risks make it important to assess MP in food organisms consumed by humans (Savoca et al., 2019; Hossain et al., 2019). There are only a few studies on MP in fish (Hossain et al., 2019), shrimp (Hossain et al., 2020) and beach sediment (Hossain et al., 2021) in the Bay of Bengal. However, to date there has been no study of the occurrence of MP in dried fish products, which are commercially and nutritionally very important and consumed by millions of people. This study aims to address this important knowledge gap by presenting the first scientific record of the occurrence of MP in dried fish from the Bay of Bengal and South Asia.

Marine fish drying yards were selected for sampling in Nazirertek, Cox's Bazar (21.4630°N, 91.9541°E) and Kuakata, Patuakhali (21.8198°N, 90.1104°E) (Fig. 1). A total of 80 individual dried Bombay duck and ribbon fish (20 individuals per species from each site) were collected from the sampling sites between October and December 2020. During sampling, dried fish were labelled by site name and immediately wrapped in aluminum foil and transported to the Laboratory of Fish Ecophysiology, Bangladesh Agricultural University, Mymensingh, and stored in a dry place in the lab prior to analysis. The mean total length (cm) and weight (g) of sampled dried fish is presented in Table 1.

To isolate MP from the samples, we used a slight modification of combined KOH and H_2O_2 digestion methods (Rochman et al., 2015; Hurt et al., 2020). Dried fish were unpacked from the aluminum foil. The samples were then dried further in a hot air oven (Digisystem Laboratory Instruments Inc., Taiwan) at 55 °C–60 °C for 5 to 6 h to remove all residual moisture, following which fish were reweighed individually. The whole dried fish were then crushed using a high-performance blender (Miyako, Japan) and the weight (g) of the mash was recorded. For KOH digestion, 1 g of homogenized mash of dried fish was transferred to a cleaned 500 mL beaker and submerged by adding 60 mL of 1 M KOH and

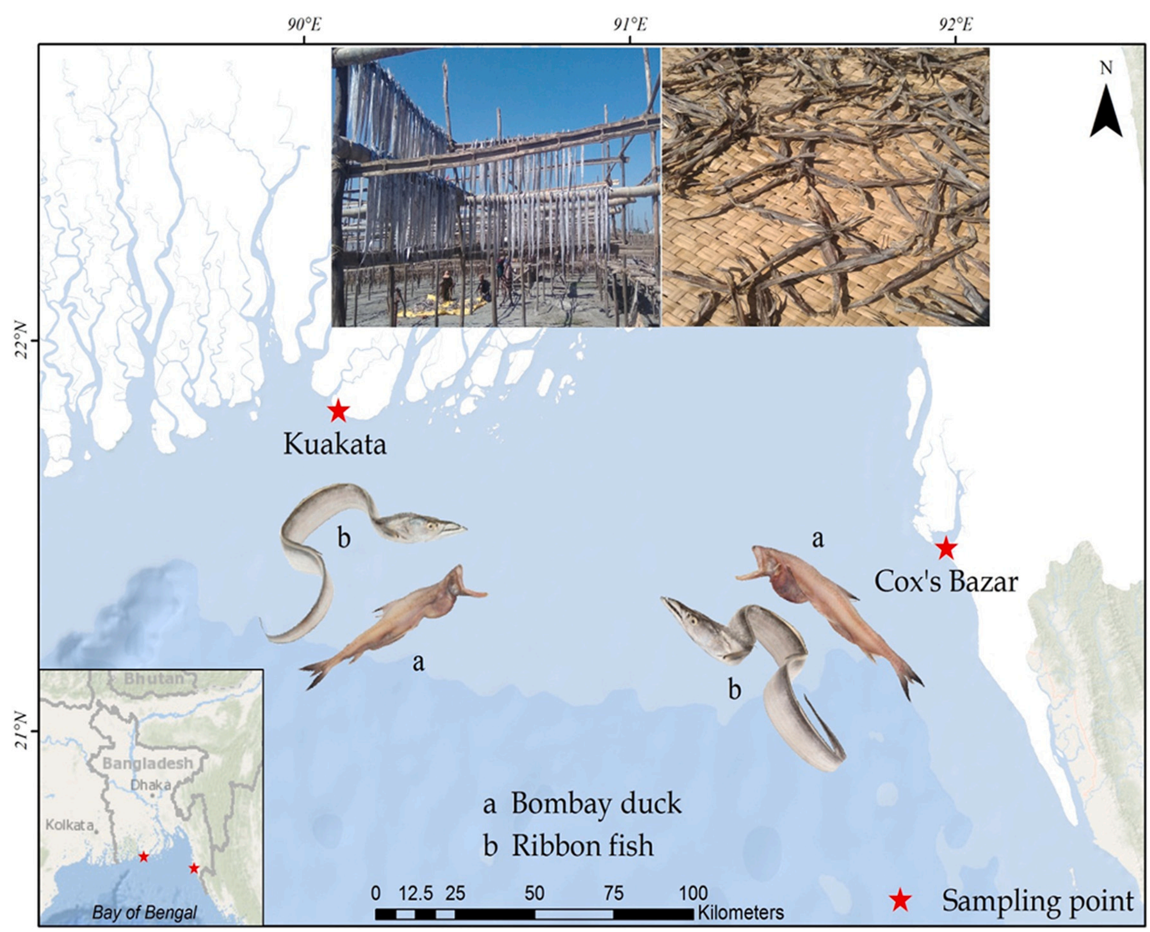

Fig. 1. Location of fishing grounds in the Bay of Bengal and drying yards of collected fish samples at Cox's Bazar and Kuakata in Bangladesh.

Table 1
Mean length (cm) and weight (g) of sampled dried fish from Cox's Bazar and Kuakata.

Sampling sites	Species	Mean length (cm)	Mean body weight (g)
Cox's bazar	Bombay duck (loitta), *Harpadon nehereus* (N = 20)	28.92 ± 1.42	32.21 ± 3.69
	Ribbon fish (churii), *Trichiurus lepturus* (N = 20)	64.75 ± 2.24	69.34 ± 5.38
Kuakata	Bombay duck (loitta), *H. nehereus* (N = 20)	29.30 ± 2.10	34.31 ± 3.95
	Ribbon fish (churi), *T. lepturus* (N = 20)	60.35 ± 4.91	65.28 ± 5.35

30 mL of Sodium lauryl sulphate ($C_{12}H_{25}NaSO_4$) (0.5% *w/v* [ca. 5 g L^{-1}]). The beaker was covered with aluminum foil and incubated in a water bath at 50 °C for 72 h. During incubation, the beaker was shaken smoothly several times. For wet peroxide oxidation after 72 h incubation, 6 g NaCl was fully mixed with 20 mL of 30% H_2O_2 and added to the beaker and left for 12 h at room temperature to allow digestion of the remaining organic materials. Additionally, the sample was centrifuged (Laboratory Centrifuge 800, China) at 2000 rpm at room temperature for 10 min to amalgamate the dilution properly. The sample was then filtered through a 0.2 μm cellulose nitrate filter (Whatman) by glass vacuum filtration unit (Duran, Germany) using a vacuum pump (Rocker 300, Taiwan). After filtration, the filter membrane was immediately covered with a petri dish to prevent contamination. For digestion and environmental control, one procedural blank was run in parallel with the samples without the dried fish mash. In brief, 60 mL of 1 M KOH and 30 mL of Sodium lauryl sulphate (0.5% *w/v* [Ca. 5 g L^{-1}]) was placed in a 500 mL beaker. Then a mixture of 6 g NaCl and 20 mL of 30% H_2O_2 was transferred to the beaker and the solution was filtered through a 0.2 μm cellulose nitrate filter. Final MP concentration was corrected by removing the corresponding number of blank sample MP from each fish sample.

Each filter membrane was examined under a Daffodil MCX100 microscope (Micros Austria) ranging between 40× and 1000× for visual identification and counting of MP. Filter membranes were carefully transferred to slides from petri dishes using clean tweezers and the slides were placed under the microscope. Additionally, for curved filters, 2–3 small drops of glue were applied directly to the filter edges. Filter membranes were slightly discoloured white, and gridded to ensure no double counting of MP. Particles were within the filtration perimeter and the light-coloured perimeters were easily distinguishable by naked eye or underneath the microscope. Each filter was examined from left to right, moved down one row, and read from right to left, and data from each section of the grid were recorded in turn. Particles were identified and categorized based on their morphological characteristics namely shape, size and colour. We categorized MP into six types: fibers, fragments, microbeads, films, foams and pellets. Each particle was photographed using an Amscope camera attached to the microscope and IScapture software. For 10% of particles, we used a hot needle test as an alternative identification method to distinguish between plastic pieces and organic matter to confirm positive identification. Other materials like algae, sand, and animal shells and parts observed on the filters were ignored throughout the identification period.

Fourier Transform Infrared (FTIR) (Nicolet iS5 FT-IR, Thermo Scientific Inc., USA) was used to identify the chemical composition of MP. A representative number of randomly selected visually scrutinized particles were individually placed on the surface of Attenuated Total Reflectance (ATR) (iD7, Thermo Scientific Inc., USA) crystal. Isopropanol was used to clean the crystal surface of ATR prior to placing each sample. After collecting the sample spectrum, the chemical composition of the sample was identified by comparing the sample spectrum with Omnic polymer reference Spectra library (Thermo Fisher Scientific Inc., USA).

All the recorded data was compiled in a Microsoft Excel spread sheet and analyzed using SPSS software version 22. Two-way Analysis of Variance (ANOVA) was employed to analyze variations in the abundance of microplastics between the dried fish samples by site followed by pairwise comparisons using Tukey's HSD test.

The number of MP per g was found to be 28.54 ± 4.21 and 41.33 ± 3.45 for Bombay duck and 34.17 ± 3.32 and 46.00 ± 4.62 for ribbon fish collected from Cox's Bazar and Kuakata, respectively (Fig. 2). The total number of MP per g of dried fish collected from Kuakata was significantly ($p < 0.05$) higher for both species than in samples taken from Cox's Bazar. MP contamination in aquatic organisms is an alarming worldwide trend, and numerous studies have been conducted on the assessment of MP in the wild and under in laboratory conditions. Most studies of wild aquatic organisms have assessed MP in the digestive tracts of freshly caught or preserved fresh fish. However, the potential for migration of MP from the digestive organs to other edible tissues has raised concerns about food safety (Browne et al., 2008; Avio et al., 2015; Lu et al., 2016). Moreover, aquatic organisms are often eaten whole, without discarding the digestive tract. This is especially common in the case of dried fish, which is a widely eaten food item around the globe.

All the dried fish samples in the present investigation were found to contain high numbers of MP, irrespective of species or sampling location, indicating the possibility of MP transfer to the human body for consumers of these products. The high abundance of MP in all the dried fish samples examined in the present study may not be directly comparable to other observational studies that mainly dealt with fresh fish. MP may reach higher concentrations in dried fish than in fresh fish, because 2–6 kg of fresh fish is needed to produce 1 kg of dried fish, depending on the species used. In a previous study in Bangladesh, Hossain et al. (2014) found that 5.97 kg of fresh Bombay duck and 2.99 kg of fresh ribbon fish are required to produce 1 kg of these respective dried fish products. Various other studies also report the presence of MP in samples of preserved products. Karami et al. (2017) isolated sixty one MP-like particles from the viscera and gills of four dried fish species in Malaysia; Greenback mullet (*Chelon* subviridis), Belanger's croaker (*Johnius belangerii*), Indian mackerel (*Rastrelliger kanagurta*) and Spotty-face anchovy (*Stolephorus waite*). MP were also isolated from canned sardines (Karami et al., 2018), and canned tuna and mackerel (Akhbarizadeh et al., 2020). Moreover, MP has been found in the edible seaweed nori *Pyropia* spp. (Li et al., 2020) and seaweed *Ulva prolifera* (Gao et al., 2020). The present study provides clear evidence that dried fish from the Bay of Bengal are contaminated with MP (Fig. 3). Hossain et al. (2019) identified MP in live Bombay duck from the Bay of Bengal,

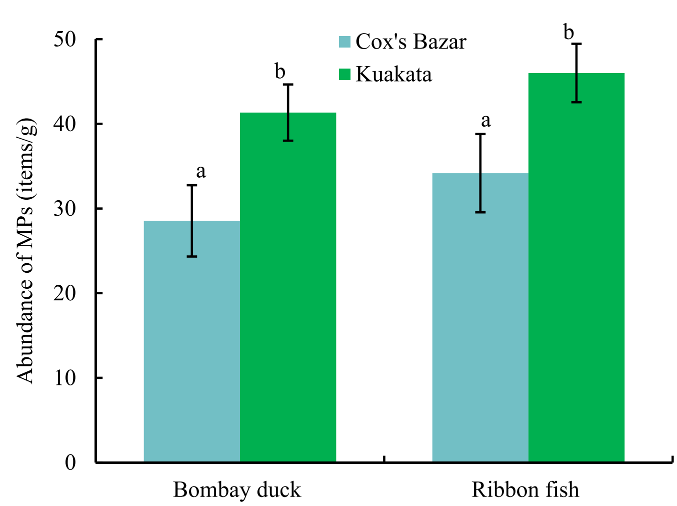

Fig. 2. Abundance (items g^{-1}) of microplastics in dried Bombay duck and ribbon fish from Cox's Bazar and Kuakata. Different superscripts indicate a significant difference ($p < 0.05$). All values are expressed as mean ± SD.

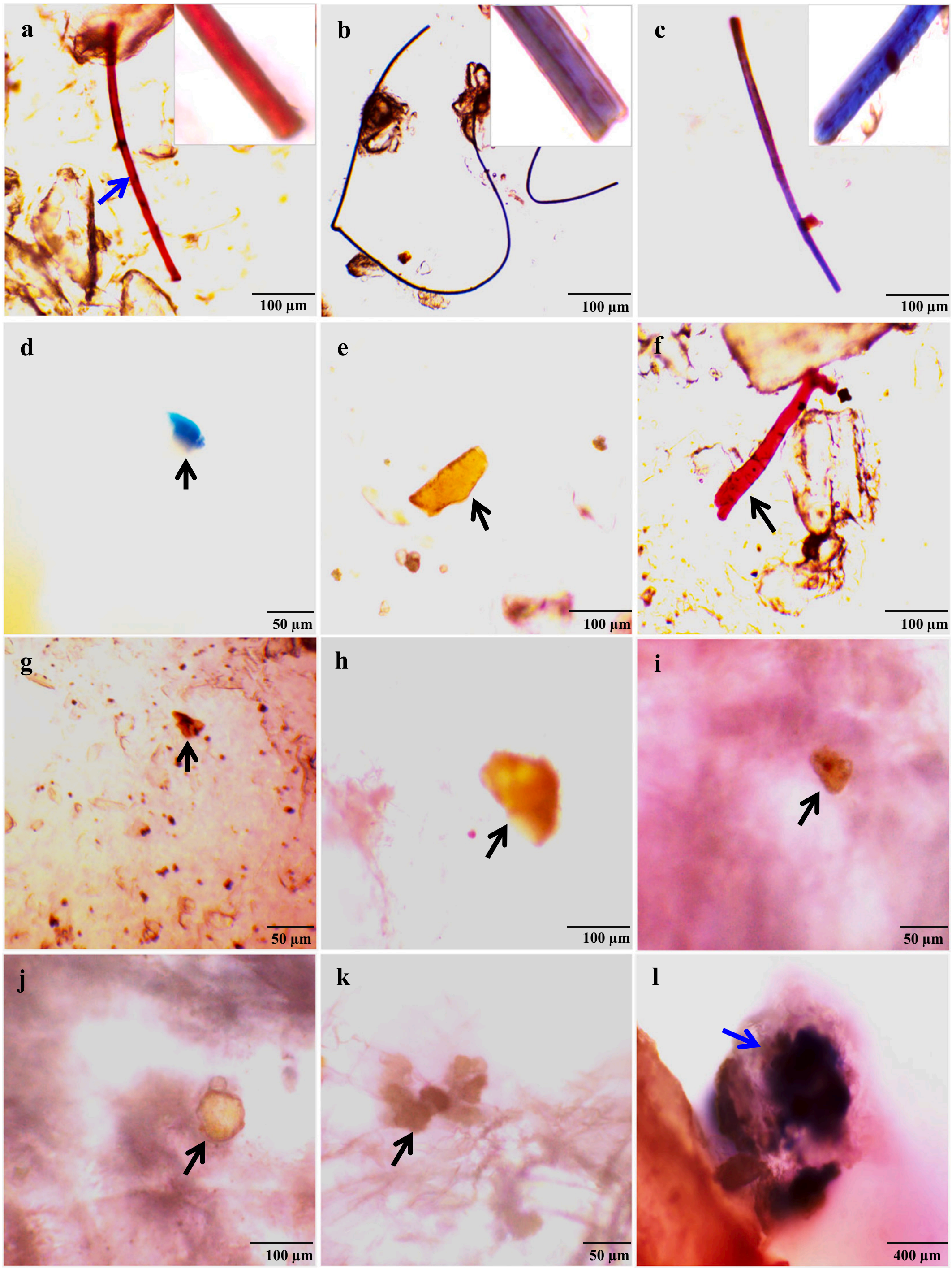

Fig. 3. Microscopic images of selected microplastics: a, b & c – fibers; d, e & f – fragments; g, h & i – films; j – microbeads; k – pellets; and l – foams.

which supports our findings. The presence of MP has also been reported in 35% of fish from the North Pacific Central Gyre (Boerger et al., 2010), 37% fish from the English Channel (Lusher et al., 2013), 20% fish off the Portuguese coast (Neves et al., 2015), 77% fish in Tokyo Bay, Japan (Tanaka and Takada, 2016), 68% fish around the Balearic Islands (Nadal et al., 2016), 18% fish from the Spanish coast (Bellas et al., 2016), 11% mesopelagic fish of the North Atlantic Ocean (Lusher et al., 2016), and 58% fish from the Mediterranean Sea (Güven et al., 2017).

Fiber dominated in all the samples (Fig. 4), accounting for 41–64% of MP particles, followed by fragments (22–34%), microbeads (9–16%), films (3–4%), foams (1–4%) and pellets (0–2%). A significantly ($p < 0.05$) higher proportion of fibers were found in both fish samples collected from Cox's Bazar compared to samples from Kuakata (Fig. 4). Similar to the results of other studies of fresh fish, fiber was the most prevalent among the types of MP detected in the dried fishes in the present study (Desforges et al., 2015; Steer et al., 2017; Sun et al., 2017). Many researchers in different regions report finding a variety of types of MP (Kolandhasamy et al., 2018; Teng et al., 2019; Frias and Nash, 2019). In the present study, fiber accounted for 41–64% of MP, which is lower than previous findings of 71–83% in fish from the Spanish coast (Bellas et al., 2016; Compa et al., 2018), 70% in fish from the Mediterranean Sea (Güven et al., 2017), 66% in fish taken from off the Portuguese coast (Neves et al., 2015), and 68% in fish from the English Channel (Lusher et al., 2013). Fishing nets, ropes, laundry, and urban

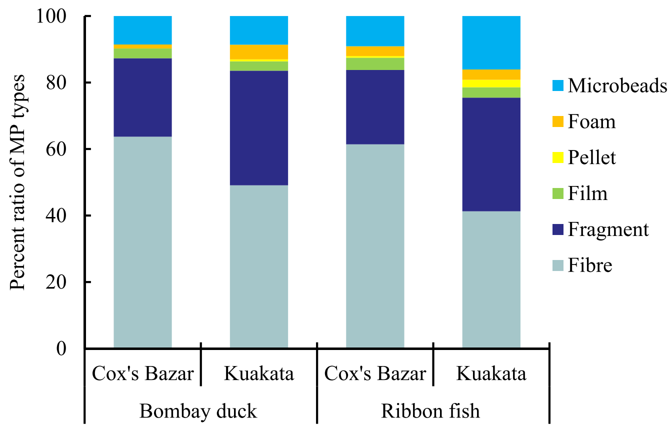

Fig. 4. Distribution of types of microplastics found in dried Bombay duck and ribbon fish from Cox's Bazar and Kuakata.

wastes may be possible sources of fibers in coastal waters (Browne et al., 2011; Claessens et al., 2011). Urban wastes might include significant quantities of microfibers from the garment industry (Bangladesh's number one export earning sector), and microfibers that are commonly used in health, beauty, and other industrial products, such as cleansers and toothpastes.

Three different types of MP polymer namely polyethylene (PE), polystyrene (PS) and polyamide (PA) of dried fish were identified using ATR-FTIR (Fig. 5). The most dominant polymer type was PE (40–45%) followed by PA (30%) and PS (25–30%) in Bombay duck. On the other hand, PA (40–45%) was the most abundant polymer in Ribbon fish followed by PE (35%), and PS (20–25%). The high quantity of polyethylene particles may be due to fishing and intensive tourism activities (plastic bags, bottles, cosmetics, fishing gear, rope, packaging materials and other products), and increased high traffic maritime routes and urbanized coastal areas, which are common adjacent to the sampling sites (IDCL, 2019). These factors are also reported by a number of other studies in different parts of the world (Baalkhuyur et al., 2018; Andrade et al., 2019). Disposal of damaged fishing nets, floats, ropes and fish baskets/bags in marine waters may increase the accumulation of polyamide among fishes - c.f. Thushari et al. (2017) and Hossain et al. (2019). On the other hand, fragments of PS polymer, which is the raw material used in plastic products such as nets, baskets, and packaging might become mixed in with fish during transport or storage, before, during or after processing (Wu et al., 2019).

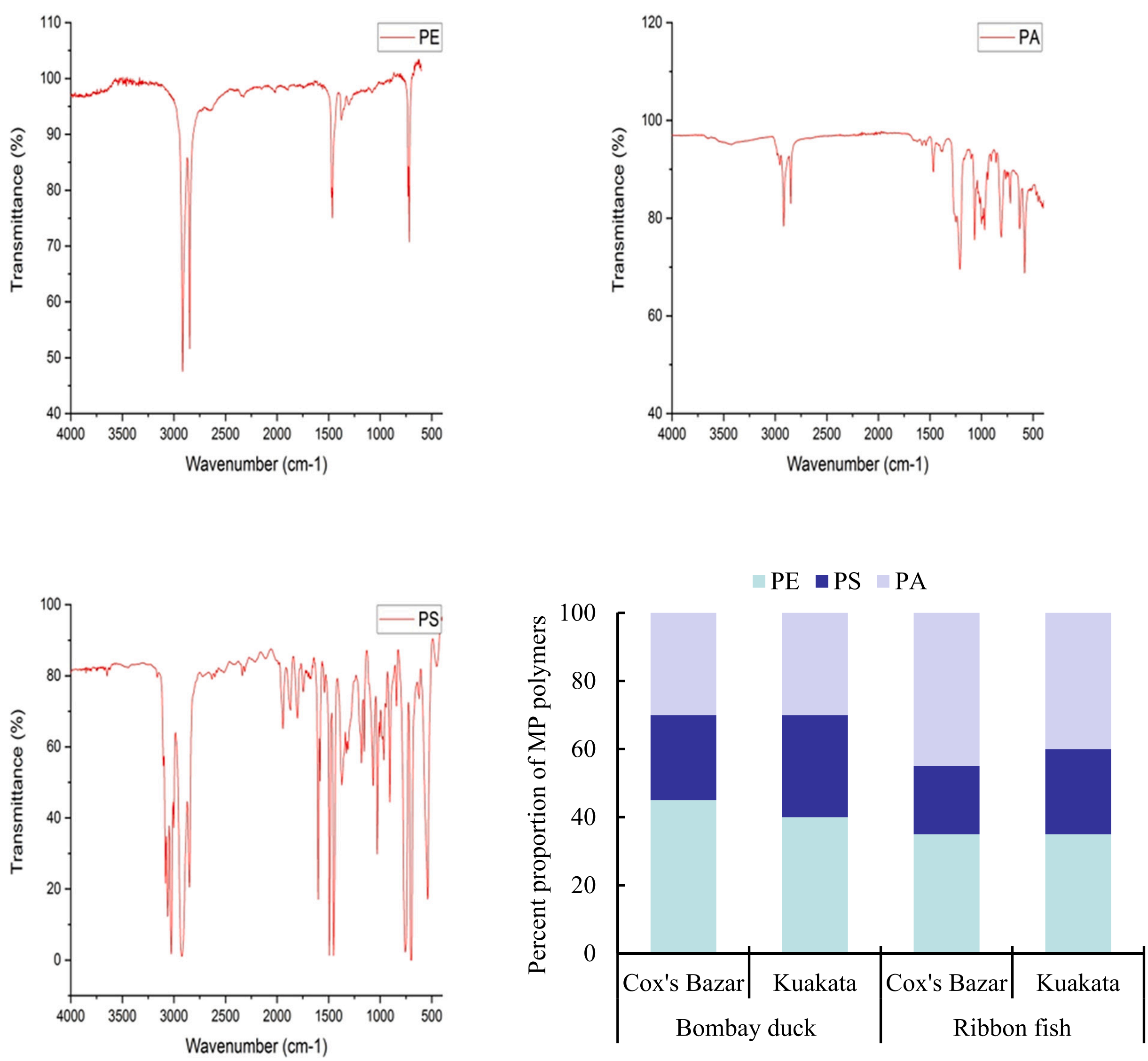

Fig. 5. Distribution of microplastics polymers found in dried Bombay duck and ribbon fish from Cox's Bazar and Kuakata.

MP identified in the present study were classified into three size groups - <0.5 mm, 0.5–1.0 mm, and 1.0–5.0 mm (Fig. 6a). Among the size groups, MP of 1.0–5.0 mm dominated in all the samples accounting for 50–67%, followed by 0.5–1.0 mm (19–35%) and <0.5 mm (12–16%). Larger sized MP were found to be more abundant in fish samples taken from Cox's Bazar, while fish from Kuakata were contaminated with smaller sized MP. The distribution of size ranges of MP reported in live Bombay duck (Hossain et al., 2019) and in sediments (Hossain et al., 2021) from the Bay of Bengal is similar to those in our findings. Limited feeding selectivity and ingestion of any food items of suitable size (Boerger et al., 2010; Cole et al., 2013) likely accounts for the significantly higher abundance of smaller MP in dried fish collected from Kuakata.

The most common colour of MP was blue (20–29%), followed by purple (17–25%), red (13–25%), pink (9–21%) and brown (8–14%). In addition, 6–17% of MP was categorized as 'other' colours (Fig. 6b). We detected blue, purple, red, pink and brown MP in the present study, which is similar to the distribution found in the sediments at the Cox's Bazar beach by Hossain et al. (2021). The colour of MP may vary due to their sources and manufacturing processes. Blue MP also dominated in all three commercially important fish species from North East Atlantic Ocean, as observed by Barboza et al. (2020). Coloured MP are considered hazardous to marine organisms as small aquatic animals are attracted by the appearance of MP that mimic their natural prey items (Cozar et al., 2014; Wang et al., 2016; Ory et al., 2017). Small fish and crustaceans that constitute normal prey items are often blue, purple, or red, meaning that microparticles of a similar colour may appear attractive and be preferentially consumed (Wieczorek et al., 2018). Through trophic transfer these MP reach the gastrointestinal tract in larger fish as they predate on small fish and other organisms.

Dried Bombay duck and ribbon fish contribute to the food and nutrition security to millions of people in Bangladesh, and production of these products supports the employment and livelihoods of large numbers of people. Both fishes are typically dried without degutting, scaling, or removal of fins, gills and viscera. Although dried fish are typically cut into smaller pieces or mashed during cooking, little matter is discarded so the fish is effectively eaten whole, including the digestive tract where the highest MP concentrations are likely to be found. Both fish species examined in this study are carnivorous. The benthopelagic Bombay duck is found on sandy-muddy bottoms in offshore waters and gathers in large shoals in river mouths. It is an aggressive predator (Frimodt, 1995) with a large mouth equipped with slender, arrow-shaped, depressible teeth of unequal size, and feeds actively on shrimp and small fishes (Balli et al., 2006). The elongated ribbon fish usually lives on muddy bottoms of shallow coastal waters. Ribbon fish often enter estuaries and feed aggressively on fishes, crustaceans and squid near the surface (Chiou et al., 2006). Thus, the ingestion of microplastics by both fishes may be influenced by their feeding habits, MP aggregations in water column and bottom sediments in their habitats and the characteristics of MP particles present in the marine environment.

The present study's findings are significant given that millions of people in Bangladesh regularly consume dried fish, and the country is producing increasing quantities of plastics. Bangladesh has more than 3000 small and large plastic manufacturing installations, and plastics were identified as twelfth among the top sources of foreign exchange earnings in 2017–18 (IDCL, 2019). The daily production of plastic of Bangladesh now stands at 3000 t, which is equivalent to 8% of the total waste produced by the country (Mahmudul, 2019). Plastic waste accumulation largely affects the marine ecosystems and biota of the Bay of Bengal. In a recent survey, 6705 pieces of debris were collected from Cox's Bazar beach, of which nearly two thirds were plastic (Mehedi, 2018). The high population density and increasing waste generation from urban and industrial sources make Bangladesh highly vulnerable to plastic pollution, and it is likely that the quantity of microplastics in aquatic ecosystems will increase in the coming years (Napper et al., 2021). In 2012–13, nearly 60,000 t dried fish (85% from marine fish) were produced in Bangladesh. Most dried fish are consumed locally, and millions of people in Bangladesh are fond of dried fish, which is eaten regularly. Some dried fish from Bangladesh is exported overseas for consumption by non-resident Bangladeshis living in Europe and North America, and migrant workers in the Middle East and East Asia (Hossain et al., 2015). The effects on many millions of consumers of regularly ingesting MP with dried fish are unknown. Consumers in Bangladesh are largely unaware of the possible dangers, and the government has not established a system for monitoring MP in food products. In light of the high levels of MP contamination identified in the present study we recommend that the relevant ministries and government departments work together to identify long-term solutions to the potentially grave risks posed by the increasing presence of MP in dried fish and other food products.

In conclusion, given that dried fish are usually consumed in their entirety, dried products may be responsible for the trophic transfer of a significant amount of MP to consumers' bodies. To date, scientific understanding of the sources, exposure to, bioaccumulation of, and resulting toxic effects of MP on the marine ecosystems and biota, and in humans, are limited. Yet, the sheer ubiquity of MP found in dried fish in Bangladesh, coupled with the potential harm that these contaminants may cause to health of consumers, should prompt action by the authorities to begin cleaning up aquatic habitats and monitoring way the dried fish is produced and consumed. Further scientific studies will play a key role in addressing these knowledge gaps to support the two-fold objectives of sustaining consumption of aquatic foods and safeguarding consumers from possible health risks posed by MP.

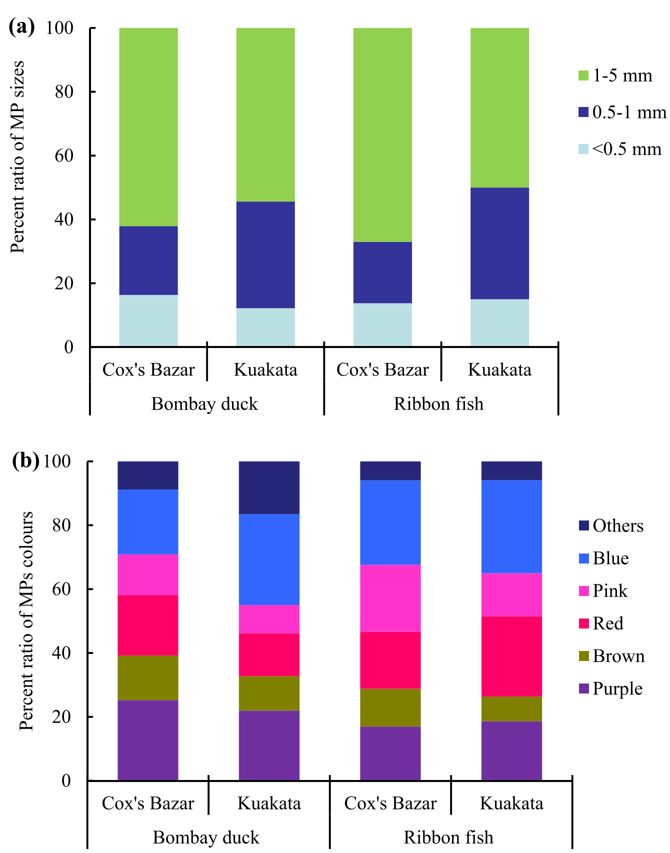

Fig. 6. Distribution of microplastics found in dried Bombay duck and ribbon fish from Cox's Bazar and Kuakata, by (a) particle size and (b) colour.

CRediT authorship contribution statement

Jabed Hasan and SM Majharul Islam: Sample collection and preparation, finalization of extraction protocol, extraction and analysis of MP, table and graph preparation. Md Samsul Alam and Ben Belton: Writing, interpretation, technical oversight to the manuscript, review & editing. Derek Johnson: Social science oversight of the manuscript, revision, editing, funding acquisition. Mostafa Ali Reza Hossain & Md Shahjahan: Conceptualization, visualization, supervision, data analysis, preparation of the first draft of the manuscript and final approval. All authors read the final version and approved the manuscript.

Declaration of competing interest

The authors do not have any known competing financial or personal interests that could influence the work reported in the current paper.

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