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Microplastics contamination in fish feeds: Characterization and potential exposure risk assessment for cultivated fish of Bangladesh

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ABSTRACT

Fish feed is becoming an increasingly vital source of nourishment for farmed fish, which are mainly coming from marine fish and agricultural sources. Anthropogenic particles, such as microplastics, are abundant in both marine fish and agricultural byproducts that are utilized to make fish feed. This study investigated whether fish feed could be a source of microplastic contamination, and revealed that a 20 weeks adult farmed tilapia fish might consume up to 268.45 ± 1.438 microplastic particles via fish feed where finisher type feeds were found to be mostly contributory in this number. The microplastics were initially observed with a stereomicroscope and FESEM-EDS. Polymeric composition of microplastics was determined to be polypropylene (PP), nylon-6 (NY-6), polyethylene terephthalate (PET), polystyrene (PS), polyvinyl alcohol (PVA), polyethylene (PE), high- and low-density polyethylene (HDPE, LDPE), ethylene vinyl acetate (EVA), polycarbonate (PC), poly vinyl acetate (PVAc), poly urethane (PU) and polyvinyl chloride (PVC) by FTIR. Results also revealed that the size of microplastic particles in all fish feed ranged from 14 μm to 4480 μm, with 550 ± 45.45 to 11,600 ± 56.1 microplastic particles/kg of fish feed. The FESEM-EDS data demonstrated to overlook the microplastic surface along with attachment of heavy metals onto that surface such as Pb, Ni, and Co in finisher type feed that could create additional health risks.

1. Introduction

Fish feed is regarded as one of the most significant factors influencing the outcome of aquaculture practices. There must be an adequate supply of nutritionally balanced fish feeds for traditional aquaculture systems. For aquaculture production to be successful, well-balanced and unwanted materials based commercial feeds must be utilized [1,2]. It is well-known that commercial feed increases the carrying capacity of culture systems, thereby multiplying the number of fish produced by several orders of magnitude [3]. In 2008, it was estimated that farmed fish around the world needed 29.3 million metric tons of specially made feed, and this number has grown at an exponential rate [4]. In 2019, 41 million metric tons of fish feed were used for aquaculture around the world [5]. Even though the demand for more and better fish feed in aquaculture of Bangladesh is growing, there could be some anthropogenic elements e.g.

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microplastic particles in commercial fish feed could create potential threats onto this sector. Contaminated feeds have a major impact on both the vulnerable consumers who rely on fish as a protein source and a staple diet, as well as the fish themselves. Diverse unwanted elements that can arise from the environment or the manufacturing process can contaminate fish feed and the materials utilized. These impurities are easily transferred from feed to farmed fish, and then to fish eaters [6]. One of the major contaminants is the microplastics that can concentrate into fish body through bioaccumulation [7–9]. Microplastics (≤5 mm) are usually defined as the breakdown byproducts of macroplastics or polymers [10]. Plastics produced as tiny plastic particles are known as primary microplastics, whereas plastics created as pieces of larger plastic goods are known as secondary microplastics [11,12]. Numerous scholars have conducted extensive investigations on the adverse effects of microplastics on various species, encompassing a wide range of detrimental outcomes such as disruption of biological processes and mortality [13]. The classification of microplastics poisoning is determined based on the characteristics of the microplastics following ingestion. There are three main consequences associated with the presence of microplastics in the gastrointestinal tract. Firstly, microplastics can accumulate and cause physical harm by obstructing and damaging the gastrointestinal tract. Secondly, the release of microplastics as pseudo feces can disrupt the energy transfer processes of organisms. Lastly, the transfer of microplastics within the body can expose inner organs and tissues to these particles [14]. These factors have the potential to result in tissue damage, oxidative stress, alterations in immune-related gene expression, and changes in the antioxidant status of fish. Fish may experience neurotoxicity, growth retardation, and behavioral abnormalities as a result of exposure to microplastics [15,16].

Fish feed is mainly composed of marine trash fish, mustard oilcake, rice bran, wheat lower, molasses, and salt. Marine trash fish are mainly used to add protein, fat, phosphorus, and calcium to the fish feed content, while rice brans, wheat flowers, mustard oil cakes, and molasses are used for mainly carbohydrate and fiber contents. Trash fish, usually used in fish feed, are caught in oceans and seas. Studies revealed that marine water had been immensely loaded with microplastics [17–19] and a significant amount of marine fish species had been found contaminated with microplastic particles [20,21]. The size of detected microplastics from sea food-based feed varied from 20 μm to 5 mm [22]. The average number of plastic particles detected in the examined feed (3.9 ± 1.4 PL/g) aligns closely with previously documented values in commercial diets for *Heteropneustes fossilis*, and catfish, which ranged from 2.0 to 5.7 plastic particles/g [23]. To the best of our understanding, the predominant focus of numerous studies has been on investigating the presence of microplastics (MPs) in feed ingredients, with particular emphasis on fishmeal. Fishmeal is a crucial component incorporated into the diets of carnivorous marine species. Its content of MP can exhibit a range of values, spanning from 0 to 17.3 PL/g. The specific value within this range is contingent upon the fishing grounds from which the fishmeal is sourced, as indicated by various studies [10, 24–26]. It is noteworthy to mention that the fishmeal obtained from fish captured in contaminated fishing regions exhibited the highest concentrations of MPs, thereby emphasizing the impact of marine pollution on the safety of feed constituents [24,26]. In addition, it is worth noting that plant-based meals, which are frequently employed as substitutes for fishmeal in the production of animal feed, demonstrated similar levels of microplastics (ranging from 0.8 to 1.7 particles per gram) when compared to specific sources of fishmeal [25]. In addition to considering the characteristics and composition of the ingredients, it is important to acknowledge the presence of MPs in fish feeds which have been used in different life stages of cultured fishes. However, it is crucial to note that the current body of research on this topic is emerging and thus necessitating further investigation to comprehensively explore this issue.

Therefore, we hypothesized that fish feed is associated with microplastics contamination. Again, since microplastics have been profoundly found in agricultural land-based crops [27–29] and terrestrial ecosystems [30,31], the transfer of microplastics from these spheres to fish feed cannot be neglected. In addition, microplastics could be added to fish feed during the feed processing, grinding, heating, cooking, and packaging processes. A good rule of thumb is that the pellet's size of fish feed should be 20–30% of the fish's mouth gape. Inefficient feeding occurs when a farmer feed too few pellets since it takes more effort to find and eat more pellets. On the other hand, overly large pellets will make it difficult to feed and may even result in choking. To avoid this problem, a feed pellet size guidance for various species and life stages is frequently offered by feed suppliers. Fish size influences feeding rates and frequency to some extent [32]. In consideration of this, five distinct varieties of fish feed have been made commercially under the labels Nursery, Pre-starter, Starter, Grower, and Finisher type fish feed. The primary distinction between these feed contents is the diameter of the feed pellets. Powdery in texture, nursery feed is generally used for fry staged fish. Pre-starter and starter type fish feed pellets with diameter of 0.8 mm and 1.5 mm are used to feed fingerlings in an aquaculture pond. Grower fish feed pellets with a 2 mm diameter are consumed by juvenile fish, whereas adult fish typically ingest both grower and finisher fish feed pellets (2.5 mm in diameter). Consequently, due to wide feed constituents, it is assumed that use of commercial fish feed poses a substantial danger of microplastic penetration in cultured fish. In this study, we calculated the potential microplastics exposure rate for different fish feed types that were calculated for fry, fingerlings, juveniles, and adult fish exposures. This research is one of the very insights to shed light on potential microplastic exposure through fish feed during various life stages of farmed fish. These findings will therefore serve as the focal point for subsequent research on the effects of microplastics on fish feed for cultured fish.

2. Materials and methods

2.1. Sample collection

Bangladesh is a country with a huge fish farming industry. In the nation, there are numerous types of fish feed. They are produced locally and commercially. Five different commercial fish feeds were used for this study from the local market: nursey (for feeding fry), pre-starter and starter (for feeding fingerlings), grower (for feeding juvenile fish), and finisher (for feeding adult fish). A total of 15 fish feed samples were collected (3 brands of each type of feed). For each feed type, similar feed compositions percentages were chosen

(Supplementary Table 1). Additionally, triplication was also performed for each feed type.

2.2. Microplastic extraction protocol

Fish feed is a complex organic matrix consisting of trash fish, mustard oilcake, soybean meal, sunflower oilcake, rice bran, maize, vegetable oil, wheat flower, molasses, salt, as well as vitamin supplement (Walkinshaw et al., 2022). Therefore, it was necessary to run a two-step optimized microplastic extraction procedure, including chemical digestion and density separation. With this focus, the extraction protocol was adopted from Karami et al., 2017a [33]. In brief, 20 gm of fish feed (n = 45) was taken into a 250-ml conical flask. After that, 100 ml of a 10% KOH solution (1:10 w/v) was added to it and sealed for 96 h (4 days) under a laminar hood to complete the digestion. This solution was then separated and poured into another conical flask. 40 ml of saturated NaCl solution were then added to the solution and kept for 24 h (1 day) for density separation. Bone and flesh materials including the microplastics particles of fish feeds could be compromised by acidic digestion. Therefore, KOH digestion protocol was applied that might reduce the spectroscopic difficulty in identifying the polymeric composition [34]. Again, fish feed is composed of complex biotic materials. Saline solution, e.g., NaCl, is denser than water and causes plastic materials to float to the surface [35,36] (. The supernatant was then vacuum filtered through 0.45 μm Whatman glass microfiber filter paper (GF/F Whatman TM, USA). These filter papers were then kept in Petri dishes, dried, and preserved for visual and polymeric inspection.

2.3. Visualization of microplastics by stereomicroscope

The conventional approach for identifying microplastics involves utilizing stereomicroscopy to visually detect them based on their size, shape, and color [37]. In this study, all the filter papers were visualized by Leica EZ4E stereomicroscope (Leica, Germany) with 16x, 20x, 30x and 35x zoom on the basis of needs. The characterization of microplastics utilizing this technology primarily relies on their morphological and physical attributes, contingent upon the specific research objectives. The size categories of polymer species exhibit variation, spanning a range of 1–5 mm [38]. This methodology does not furnish data regarding the specific identity of the polymer. Nevertheless, in order to assess the presence of potential plastic fragments, image processing software such as digital image J software was utilized to quantitatively determine the prevalence of microplastics [39,40]. Microscopy is a process that may be influenced by subjectivity, tedium, and reliance on the observer. However, the utilization of automation and signal processing through image J software has the potential to mitigate these limitations. Nevertheless, this analytical technique lacked the ability to effectively differentiate microplastic particles from other anthropogenic synthetic particles [41]. Therefore, in order to validate the existence of microplastic polymers, we further employed additional techniques such as SEM and FTIR spectroscopic analysis.

2.4. Visualization of microplastics by FESEM-EDS

The topography and elemental composition of a few selected microplastics were investigated and photographed using a Field Emission Scanning Electron Microscope (FESEM), model no. JSM-7610 F, JEOL, Japan, at a magnification range of 1000–10,000. A concentrated electron beam is utilized in FESEM to scan a sample's surface and create high-resolution pictures of it. The interaction between the beam's electrons and the sample results in a variety of signals that reveal details about the surface's topography, morphology, and composition. The elemental adherence to the microplastic surface was also determined using an energy-dispersive x-ray spectroscopy (EDS Oxford Instrument) [7].

2.5. Polymeric verification using FTIR

FTIR (Fourier Transform Infrared Spectrophotometer, Model no. IR Prestige-21, SHIMADZU, Japan) was utilized to validate the polymeric kinds of suspect microplastics. A representative number of samples were selected for characterization of microplastics by the FTIR. These microplastics were deemed to be indicative of the most often observed types of particles across all samples. Microplastics were evenly dispersed throughout a KBr crystal disc. Spectra were captured as the mean of 64 scans in the 4000-400 cm-1 spectral wave region at a resolution of 4cm-^{1} [42,43]. Each sample spectrum was verified by database from John Wiley & Sons, Inc.'s online spectral repository as well as by Jung et al., 2018 [44].

2.6. Microplastic ingestion through fish feed

Number of microplastic particles within fish feed samples ingested by tilapia fish **(*Oreochromis niloticus*)** were calculated by the following equations i and ii [25].

$$\boldsymbol{Fc = Average\ weight\ of\ Cultured\ Fish \times FCR} \tag{i}$$

$$\boldsymbol{Pe = Fc \times \%IFM \times MPFF} \tag{ii}$$

Several cultured species have been produced in Bangladesh around the year. In this study, we considered the tilapia fish **(*Oreochromis niloticus*)** which is one of the most widely cultured and consumed farmed fish species in Bangladesh as well as in many parts of the globe. Here, *Fc* represented the total amount of feed (kg) consumed by cultured (tilapia) fish. Since most of the fish including tilapia has to pass through the different life stages, therefore, we calculated the *Fc* value in the following segment: fry (0–4 weeks), fingerlings

(4–8 weeks), juveniles (8–12 weeks) adult (12–20 weeks). The average weight of tilapia fish in different life stages were considered according to the following manner-fry: 0.001 kg, fingerlings: 0.005 kg, juveniles: 0.125 kg and adult: 0.2 kg. *FCR* stood for food conversion ratio. *FCR* for fry, fingerlings, juvenile and adult tilapia fish were 2.86, 2, 1.69 and 1.4 respectively [32,45–47].

Again, *Pe* denoted the total number of microplastic particles ingested by tilapia in different life stages. *%IFM* indicated the inclusion of fish feed percentages by body weight. In this study, the *%IFM* for both tilapia fry and fingerlings were considered to be 3% while *% IFM* for juvenile and adult tilapia fish were considered to be 5% and 10% in order. *MPFF* stood for number of microplastic particles found in different fish feed (kg^{-1}) [32,45–47].

2.7. *Quality control*

To prevent potential contamination, extra precautions were taken throughout the entire procedure, including feed collection, preservation, alkali digestion, and microplastic analysis [42,48–51]. All equipment was rinsed with demineralized water three times to prevent any possible contamination. The filter papers were immediately placed in a Petri dish and covered to prevent cross-contamination. In the laboratory, non-plastic coats e.g. cotton lab coats, nitrile hand gloves, glassware, and metal wares were utilized. To avoid the possibility of error, blanks for the feed sample, KOH, and NaCl were performed concurrently [51]. By using this method, the instrument's and the environment's potential effects on the measurement of the sample's absorbance value will be reduced.

2.8. *Statistical and graphical analysis*

All the statistical analysis was done by using Microsoft Excel 2016. Each bar chart with errors, stacked columns, and FT-IR data plotting was generated using Origin 2022.

3. Results and discussion

3.1. *Microplastic abundance in fish feed*

Total of 15 fish feed samples (5 types with 3 different brands for each feed type) were examined. Interestingly all types of fish feeds (e.g. nursery, pre-starter, starter, grower and finisher) were found to be contaminated with microplastics. This finding confirmed the assumption of microplastic contamination in fish feed and posed potential threat of microplastic ingestion for cultured fish of Bangladesh. The number of calculated microplastic particles per kg in fish feed samples ranged from 550 ± 45.45 to 11,600 ± 56.1 with standard error while the total number of detected microplastic particles in each kg of all feed samples was 45,850 ± 548.95 with standard error. Average number of microplastics in different fish feed were displayed by Fig. 1.

Fig. 1 indicated that finisher feed type contained highest amount of microplastic particles in average (9150 ± 37.37 microplastic particles/kg with standard error) and nursery feed type contained least amount of microplastic particles in average (883.33 ± 39.71 microplastic particles/kg with standard error). Overall the microplastic findings in different fish feed showed the following descending order: finisher (microplastic abundance 59.87%) > starter (microplastic abundance 19.52%) > pre-starter (microplastic abundance 8.07%) > grower (microplastic abundance >6.76%) > nursery (microplastic abundance 5.78%). The one-way ANOVA test results for all types of fish feed indicated that they were statistically significant (0.000313). No significant study focusing on microplastic contamination in fish feed had been done yet. However, Rahman et al., 2022 [23] investigated the feed of cat fish (*Heteropneustes fossilis*) and found 45 microplastic particles. Limited research had also been done for fishmeal. There is a significance difference between fish meal and fish feed. Fish meal comes from raw marine fish, fish processing waste, or seafood processing industries. It is used

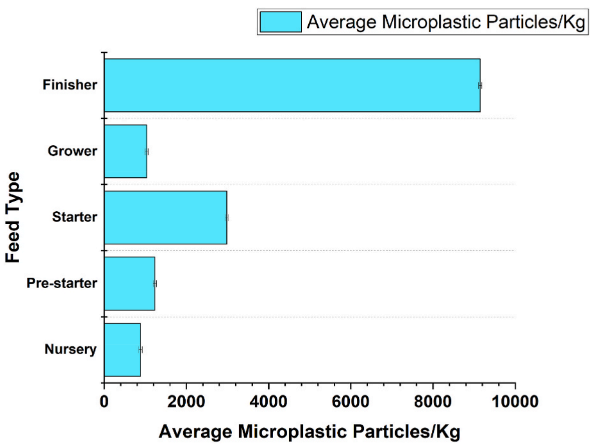

Fig. 1. Average number of microplastic particles/kg with standard error.

as fertilizer and animal feed, especially for livestock, poultry, farmed fish, and shrimp because it is high in essential amino acids, protein, and fatty acids [52]. 236 pieces of microplastic particles had been found in 20 gm of three different brands of fishmeal by Karbalaei et al., 2020 [53] and Hanachi et al., 2019 [10] isolated 226 microplastic particles from 10 gm of 4 types of fish meal. Walkinshaw et al., 2022 investigated both fishmeal and soybean meal and found relatively higher amount of microplastic particles (1070–2000 particles/kg).

3.2. Size, color and morphological variations of microplastics

Microplastic size varied greatly from feed to feed (Fig. 2). Longest microplastic particles found in nursery type fish feed (4480 μm) while shortest microplastic particle found in pre-starter feed (14 μm). Again the highest average size of microplastic was found in finisher type fish feed (574.98 ± 376.62 μm with standard error) and the lowest average size of microplastic was found in grower type fish feed (235.76 ± 135.71 μm with standard error). Since finisher type fish feed was assumed to be most potential contributory route of microplastic exposure (discussed in section 3.5), it was expected that cultured fish would mostly consume microplastic particles with the size of 574.98 ± 376.62 μm with standard error. The size variations in different feeds could be attributed to grain size of the feed types, packaging materials as well as the components of feed materials from various sources. Rahman et al., 2022 [23] found microplastics in cat fish (*Heteropneustes fossilis)* feed with average size range of 10.08–88.3 μm. Karbalaei et al., 2020 [53] analyzed fish meals and found the microplastic range of 180–7800 μm while Hanachi et al., 2019 [10] found 158–810 μm sized microplastics in fish meal.

Color variation is a typical indicator of rising demand and the usage of a wide range of plastic products in daily life, which produces significant amounts of plastic waste [54]. Total seven different types of color i.e. blue, brown, red, pink, transparent, grey and white had been found in all fish feed samples. The dominant color is white followed by transparent and blue (Fig. 3).

Color variations in nursery feed were found to be like blue (47.17%) > Transparent (37.74%)>white (9.43%). Brown, red and grey color showed equally (1.89%). For pre-starter feed, color variations were Transparent (43.24%) > blue (24.32%) > white (22.97%) > red (6.76%) >pink (2.70%). Again, starter feed showed the color variation order as white (64.25%) > Transparent (24.02%) > brown (5.59%) > blue (4.47%) > pink (1.12%) > red (0.56%). Microplastics in grower feed characterized by following composition-white (52.38%) > transparent (39.68%) > blue (1.58%) while brown and red showed the equal distribution (3.17%). Finally, finisher feed type also loaded with 6 colored of microplastics with the following descending order white (55.01%) > transparent (39.34%) > blue (2.73%) > red (1.27%) > brown (0.91%) > pink (0.73%). Yao et al., 2021 found 8 different colors including rarely found red colored microplastics in fish feed and shrimp feed while Rahman et al., 2022 found black, blue, translucent and brownish color in cat fish feed. Color variations could attract the fish especially during choosing the prey and the predator fish could mistakenly ingest these colored microplastics as thinking of their feed [10]. So identifying the color variation is important. There were also morphological variances in all fish meals. In varying proportions, filament, film, foam, fragment, and pellet were the observed morphotypes (Fig. 4).

Fig. 5 reveals that filament shape (49.06%) dominated the nursery feed, whereas film shape (43.24%) dominated the pre-starter feed. This kind of result was found to be similar with the Wang et al., 2022. The majority of starter (64.81%), grower (52.31%), and finisher (55.19%) feeds had a foam-like appearance. However, Rahman et al., 2022 discovered that filamentous shape predominated in catfish feed. Hanachi et al., 2019 [10] determined the morphological structure of fish meal to be fragment (67%), film (19%), pellet (8%) and fiber (6%). Karbalaei et al., 2020 [53] also found the results of a similar nature. The most prevalent morphological structure was fragment (78.2%), followed by filament (13.4%), and then film (8.2%). Surprisingly, they also found structures resembling pigment. The morphological variations in different fish feed had been displayed by Fig. 5(a–e).

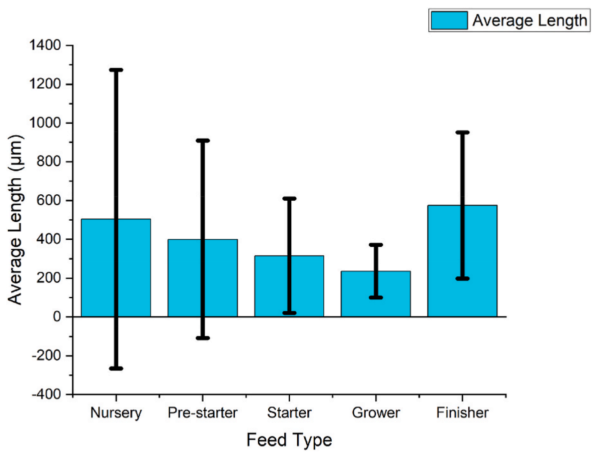

Fig. 2. Average length of microplastics in different fish feed with standard error.

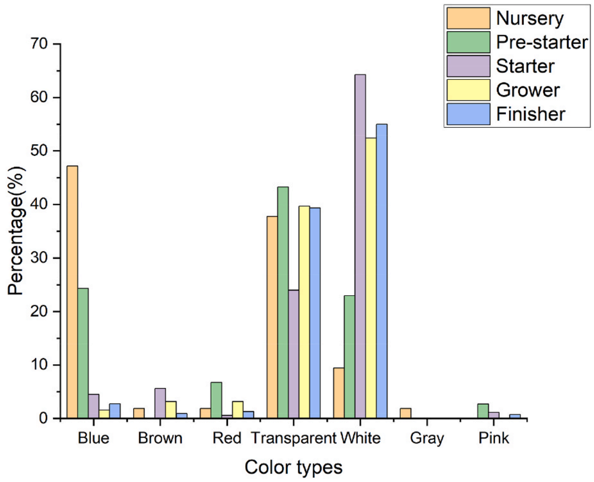

Fig. 3. Color variations in different fish feeds.

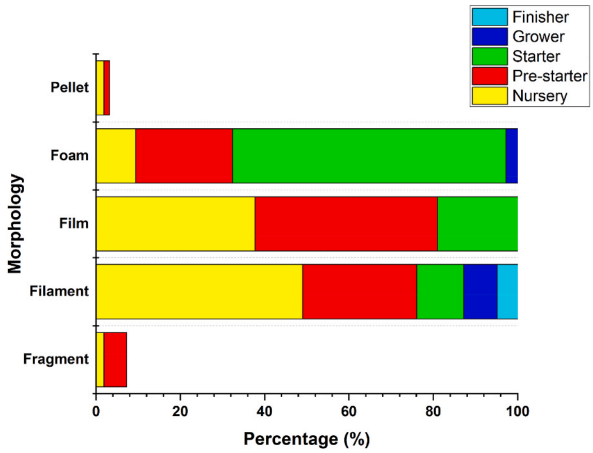

Fig. 4. Morphological variation in different fish feed.

3.3. Microplastic surface screening by FESEM-EDS

By creating a high-resolution image of the microplastic's surface, FESEM is frequently used to describe or identify MPs. Energy dispersive X-ray analysis is a further method that may be used to examine microplastics for specific elemental composition (EDS). EDS produces the chemical makeup of the surface of microplastics, whereas FESEM produces magnification, high-quality image of the surface of MPs (Haque et al., 2023). For the FESEM-EDS investigation, feed samples from a representative sample size were collected. Yet, it can be challenging to recognize the microplastics surface. The FESEM results showed that microplastic fibers contained flakes, cracks, fractures, and edges (Fig. 6). The fragments were composed of many layers of minute particles, each having a rough surface and multiple cracks along the edge. Once more, it has been reported that inorganic pollutants adsorbed by microplastics that were taken from the environment [55].

Since highest amount of microplastic particles were found in finisher type feed, we selected this feed type for EDS analysis. On the surface of microplastics, however, EDS examination on finisher feed revealed the presence of Na, Cl, Ni, Pb, Co, C, K and O (Fig. 7). Smooth plastic fragments have poor adhesive properties, but those with rough surfaces have more significant adhesion properties [56]. It might be originating from different anthropogenic or environmental sources. Inorganic chemicals may appear as contaminants in the environment and be linked to microplastics, or they may be additive compounds found inside the matrix of the microplastics polymer. Apart for C and O, these components were not innate to microplastics. Na, K and Cl could be coming from digestion and density separation procedure. Ni, Pb and Co could be coming from feed additives belonging to the functional groups of binders and anti-caking agents, fish oil, mineral mixtures of essential elements [57]. These heavy metals cause fatalities like allergies, poisoning, kidney damage, cardiovascular diseases and even cancer [58–60].

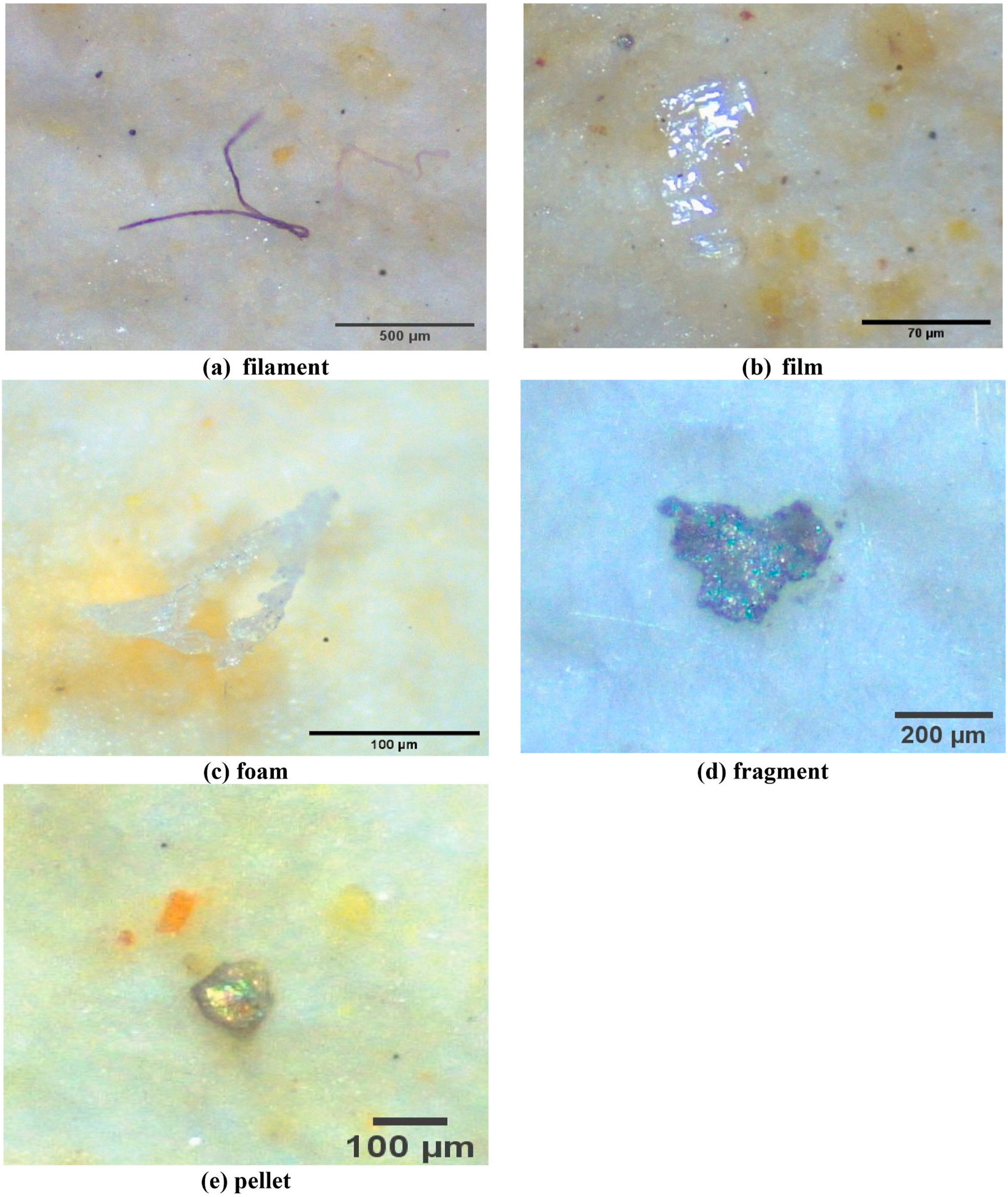

Fig. 5. (a–e): Morphological variations found in different fish feeds.

3.4. Polymeric composition of microplastics by FTIR

The polymer type of some selected samples from each morphotype for fish feed were identified using FTIR analysis. 13 different polymers were identified by FTIR in all types of fish feeds e.g. (a) polypropylene (PP), (b) nylon, 6, (c) polyethylene terephthalate (PET), (d) polystyrene (PS), (e) polyvinyl alcohol (PVA), (f) high density polyethylene (HDPE), (g) polyethylene (PE), (h) low density

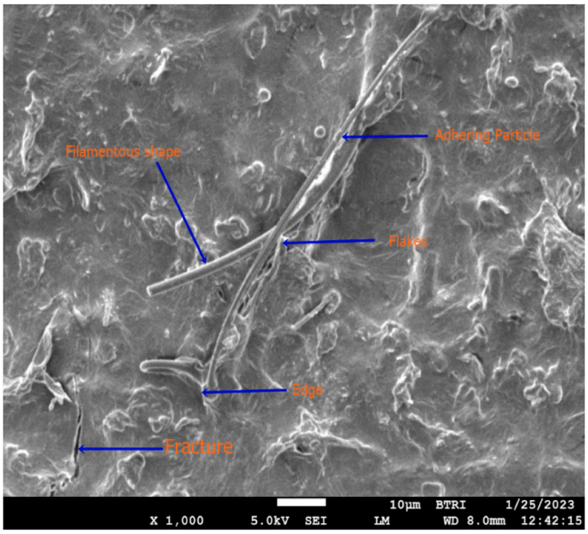

Fig. 6. SEM image of microplastics in fish feed sample × 1000 magnification.

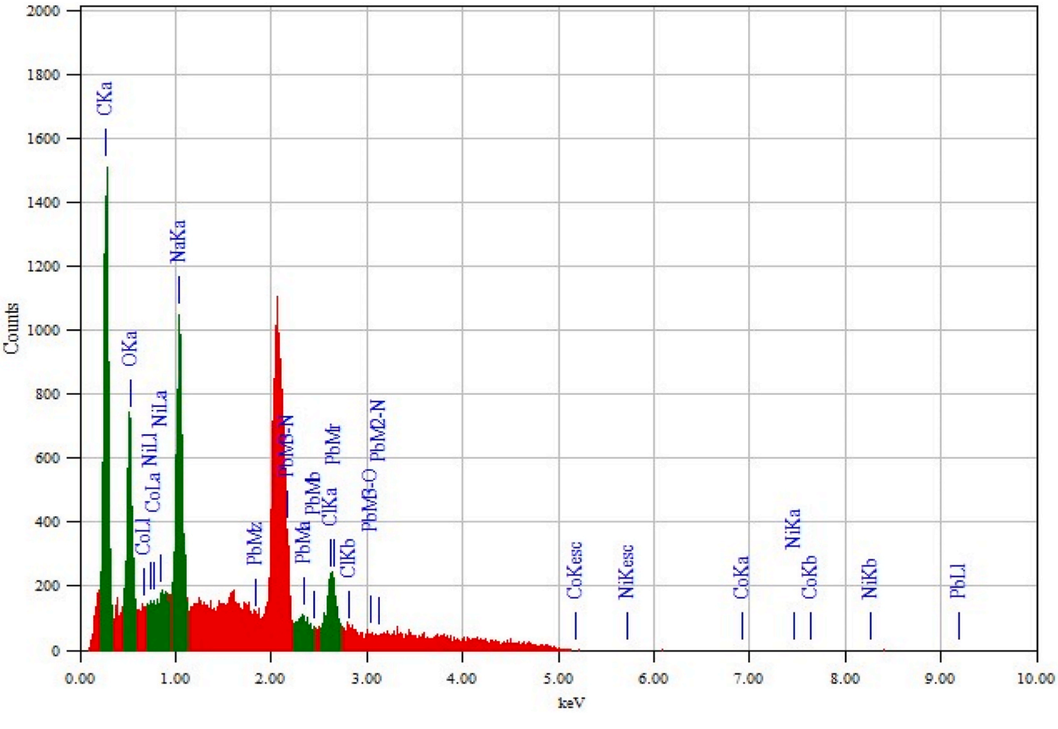

Fig. 7. EDS results for microplastics on fish feed sample.

polyethylene (LDPE), (i) ethylene vinyl acetate (EVA), (j) polycarbonate (PC), (k) polyvinyl acetate (PVAc), (l) poly urethane (PU), and (m) poly vinyl chloride (PVC). Representative polymeric spectrum of FTIR data were illustrated by Fig. 8.

Detected polymeric composition of micro plastic's functional groups were displayed by Fig. 9. Microplastics in nursery, pre-starter, grower and finisher feed dominated by PP in 20.83%, 33.33%, 33.33% and 35.71% respectively. Microplastic in starter type fish feed dominated by PET polymers. Significant number of particles (peaks in Fig. 9) also found to be unknown in all feed samples.

The absorption bands of each polymer were determined by examining previously published materials [7,8,44,61]. On the FTIR spectrum, some of the plastic polymers' typical peaks were missing, while others were present. The potential cause is that plastic waste may weather with time due to physical, chemical, and photo-degradation processes [62,63]. This weathering process could change MPs by adding or deleting other functional groups, such the hydroxyl group, through a slow hydrolysis process that could conceal some

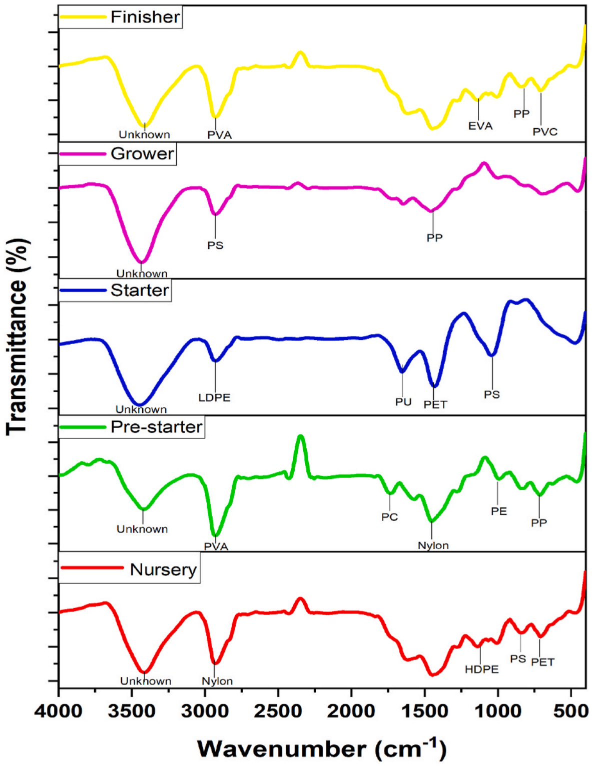

Fig. 8. Representative FTIR spectra of microplastics in different fish feed.

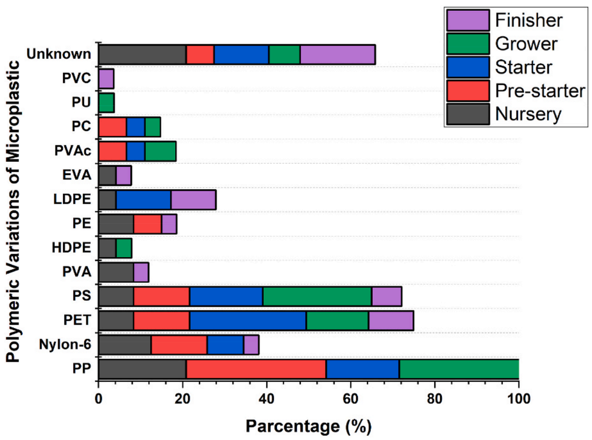

Fig. 9. Polymeric composition of microplastics in different fish feed.

other MPs-specific peaks [62]. In addition, many forms of functional groups, such as –OH stretching, C=O, and *C*–N, were reported to be responsible for absorbing or desorbing various types of heavy metals [64,65]. These fictitious groups' microplastics might easily serve as transporters for heavy metals [9]. Hanachi et al., 2019 [10] found polymeric composition as PP (45%) followed by PS (24%), PE (19%), PET (8%), and rayon (4%) in fish meal. Karbalaei et al., 2020 [53] found PP, PET and nylon, 6 in different fish meal.

3.5. Potential exposure of microplastics via fish feed among the lifecycle of cultured fish

Using our results, we calculated the potential microplastic exposure rate via different fish feeds for common cultured tilapia fish (Table 1). We divided the life span of a tilapia fish into four stages: fry (age: 0–4 weeks), fingerlings (age: 4–8 weeks), juveniles (age: 8–12 weeks), and adults (age: 12–20 weeks) [47]. Fry consume nursery-type fish feed, whereas fingerlings consume pre-starter and starter-type fish feed. Grower type fish feed used for juveniles and finisher types of fish feed are applied to adult fish. Since all types of

Table 1
Potential microplastic exposure rate at different life stages of cultured fish.

Potential Microplastic Consumption by Fry (Feed type: Nursery) (0–4 weeks)	Potential Microplastic Consumption by Fingerlings (Feed type: Pre-starter & Starter) (4–8 weeks)	Potential Microplastic Consumption by Juvenile (8–12 weeks) (Feed type: Grower)	Potential Microplastic Consumption by Adult (12–20 weeks) (Feed type: Finisher)	Potentially Ingested Total Microplastic Particles by cultured fish
0.075 ± 0.003	1.265 ± 0.021	10.911 ± 0.368	256.2 ± 1.046	268.45 ± 1.438

fish feed were found to be contaminated with microplastics, it was assumed that cultured tilapia fish could be exposed to high levels of microplastics throughout their lifecycles. Based on average data of microplastic particles presence in different fish feed, we calculated potential exposure levels for microplastics in 20 weeks of adult cultured tilapia fish. The hypothesized results revealed that 20 weeks of adult cultured tilapia fish could be exposed to 268.45 ± 1.438 pieces of microplastic particles. Table 1 also revealed that finisher type fish feed contained highest amount of microplastic particles that were supposed to be consumed by adult cultured tilapia fish. The potential exposure of microplastics via fish feed to different life stages of cultured tilapia fish followed the following descending order: finisher type feed (potential microplastic exposure by adult: 95.53%) > grower type feed (potential microplastic exposure by juvenile: 4.06%) > pre-starter + starter type feed (potential microplastic exposure by fingerlings: 0.471%) > nursery type feed (potential microplastic exposure by fry: 0.03%). The important part is that these calculated results only revealed the microplastic exposure from fish feed. Therefore, it is obvious that cultured fish could be exposed to more microplastic contamination via different natural weathering of plastic materials in the environment and anthropogenic sources. Walkinshaw et al., 2022 [25] found 706 and 116 particles from fish meal and between 1082 and 1353 particles from soybean meal.

It is likely that microplastic particles contained in the source material (i.e., fish GIT or, tissues, mustard oilcake, soybean meal, sunflower oilcake, rice bran, maize, vegetable oil, wheat flower, molasses, salt, as well as vitamin supplement) were broken down or eliminated during manufacture, either through mechanical abrasion or combustion, due to the utilization of elevated temperatures (up to 500 °C) during desiccation [66]. Detected polymers in the fish feed e.g. PP, nylon, 6, PET, PS, PVA, HDPE, LDPE, PE, EVA, PC, PVAc, PU, and PVC possessed the melting point temperature from 100 °C to 270 °C [25]. This high melting point characteristics indicated that feed processing stage would cause them to degrade. Future research may want to take into account the frequency of harmful by-products that are released by anthropogenic particles during combustion, such as poly aromatic hydrocarbons, free radicals, and toxic heavy metals [67,68]. It was assumed that the main source of microplastic particles in aquaculture feed are both pre and postharvest contamination. Different literatures suggested that farmed fish could be exposed to microplastic particles through their feed [69,70] which come from employees' clothing [71], aquaculture equipment, crushing, grinding and chopping of feed ingredients [72] as well as airborne deposition and packaging materials [73]. There is mounting evidence that microplastics could adversely affect growth and reproductive output in commercially exploited species, which could result in a longer time to market and decreased commercial and nutritional value [74,75]. The consumption of microplastic particles by finfish may have significant effects on farmed populations [25]. Upon ingestion, microplastics particles would by frequently transported through the digestive tract and eliminated through feces, where they sink into the water column [76–78]. This may result in hotspots of human-made particles in the benthos directly beneath aquaculture facilities in open-cage facilities, which could cause environmental disturbances for underlying benthic ecosystems [79]. There is currently debate over whether marine species raised in captivity or in the wild have higher levels of microplastics [80–83]. The data reported here shows that anthropogenic particle pollutants are found in aquaculture feeds, but we do not yet know what additional risk this poses to farmed creatures. More study is necessary to determine whether the quantity of man-made particles consumed by farm animals through their feed affects apical endpoints, which could be dangerous for food security. Considering the effects of contaminated feed on not only the health of farmed fish but also the nutritional value and human health, the sample of feed material and farmed fish from the same aquaculture locations may give insight on the potential exposure risk from unsafe aquaculture feeds.

Since the conventional fish feed is found to be contaminated with different anthropogenic contaminants including microplastics, the fish farmers should think of sustainable protein soured alternatives like insect protein, or plant protein. According to a recent study conducted by Magbanua and Ragaza, 2022 [84], regional sources of plant-based proteins, including soybean, corn, palm kernel, and pea meal, have been identified as highly promising substitutes for fishmeal. Plant-based proteins are readily accessible in the commercial sphere and exhibit a substantial quantity and commendable quality of protein content. Copra meal is a widely accessible raw material that is utilized on a global scale as an additive in animal feed, particularly in the context of poultry farming. In the field of aquaculture, copra meal has been identified as a viable option for supplementing protein in fish feed due to its ability to offer adequate apparent digestibility and promote improved growth rates [85].

In addition, insects are regarded as a highly promising and sustainable means of obtaining animal protein due to their notable nutritional value, composition of amino acids, and the ease with which they can be propagated [86]. Several insect species have shown promise as potential substitutes for fish meal and fish oils in animal feed. These species include the black soldier fly (*Hermetia illucens*), the yellow mealworm (*Tenebrio molitor*), and the common housefly (*Musca domestica*) [86,87]. Regardless of the various techniques employed for the propagation and production of diverse species, insects have demonstrated encouraging outcomes in terms of their potential utilization as a protein and oil resource in aqua feed [88].

Notwithstanding the encouraging outcomes documented regarding the incorporation of insects or plant as constituents in aqua feed, significant deficiencies persist with regard to their comprehensive exploitation in the field of aquaculture. Furthermore, there is a lack of clarity regarding the presence of anthropogenic pollutants including microplastics and their influences on developmental stages when it comes to incorporating insects into aqua feed, particularly in different culture systems [89]. In addition, it is also necessary to pay attention to the water environment used for aquaculture in order to clarify the pollution of microplastics in the future.

4. Conclusions

There were different amounts of microplastic particles in each of the 5 types of fish feed (3 brands × 5 of each type of feed = 15 fish feed) that were tested. PP, Nylon, 6, PET, PS, PVA, PE, LDPE, HDPE, EVA, PC, PVAc, PU, and PVC were found in the most common white, blue, and transparent colored foam, filament, and film type morphological structures. It was also assumed that an adult cultured tilapia fish could consume up to 268.45 ± 1.438 pieces of microplastic particles throughout its 20 weeks of lifespan whereas finisher type feed was found to be responsible in highest percentages (95.53%) for microplastic load. Regardless of the source of the feed, both fish- and plant-based feeds had substantial quantities of anthropogenic particles, therefore we assumed that the bulk of the particles and fibers could come from both pre and postharvest contamination. In addition, presence of Ni, Pb and Co heavy metal in finisher fish feed possess additional health risk to the consumers. It can be concluded that microplastic contamination could be reduced significantly if precautions are strictly followed in finisher fish feed preparation.

Author contribution statement

Md. Iftakharul Muhib: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper. Md. Mostafizur Rahman: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Data availability statement

Data will be made available on request.

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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e19789.

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