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Proximate Composition and Microplastic Content of Fish Feed Available in UAE (United Arab Emirates) Markets

Maryam Aghaei, Meera Aljneibi, Salama Al Qadawi, Vijo Poulose, Thies Thiemann*

Department of Chemistry, College of Science, United Arab Emirates University, Al Ain, United Arab Emirates
Email: 201721360@uaeu.ac.ae, 201808650@uaeu.ac.ae, 201735024@uaeu.ac.ae, vijo.p@uaeu.ac.ae, *thies@uaeu.ac.ae, *thiesthiemann@yahoo.de

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Abstract

21 fish feed samples were acquired commercially in UAE markets. Their ash and moisture contents were found to be 1.96% - 12.49% and 3.22% - 9.59%, respectively. The mineral content of the ash of 7 products was determined by WD-XRF. 13 products were analyzed for MP contamination and showed an MP count of 0.7 - 9 particles per gram. The MP concentration in the fish feed products showed no correlation with the price of the products.

Keywords

Fish Feed, Microplastic, Mineral Content

1. Introduction

The quality of fish feed used in aquaculture is determined by numerous factors, including the content and balance of nutrients [1], protein digestibility [2] as well as the absence of contaminants in the feed such as microplastics (MPs). Microplastics (MPs) are plastic particles of less than 5 mm in size [3]. In the last two decades, MPs have been identified as ubiquitous contaminants [4]. MPs have been found to enter the human food web through MP contaminated produce [5]. This includes meat [6], fish [7], vegetables and fruit [8]. While some MPs enter the produce during food processing [9] [10], much of it is taken up before the food is "harvested". In the case of fruit and vegetables, MPs are taken up through irrigation water [11], fertilizer [12] or mulch [13] or through Aeolian deposition [14]. In the case of meat producing farm animals and fish, MPs are often taken up with the feed [15] [16] especially with fish meal [17]-[19]. Significant uptake of MPs by fish and other sea organisms causes marked physiological and behavioral changes, impacting the health of the animals [20]. Thus, the growth of the fish can be affected by MPs [21] [22]. MPs can also alter movement patterns of fish [23]. Health implications include improper gill function [24], immuno-suppression [25], and compromised reproduction, including decreased fecundity and increased abnormal offspring [26]. With this, MPs have detrimental effects on aquaculture [27]-[29]. Additionally, there is the worry of the cross-over of MPs into the human food-web with the consumption of seafood [5]. Equally it has been reported that the presence of MPs affects the health of farmed ruminants [30] [31] and other livestock [32]. Yang *et al.* have shown that polystyrene MPs disturb muscle angiogenesis in piglets and affect the quality of pork meat [32]. Again, the possibility of entry of MPs into the human food chain through contaminated meat and dairy products in general is worrisome [30] [31].

While it is understood that animals are generally exposed to MPs, little work has been done on the exposure of pets to MPs through their food intake. J. Zhang *et al.* who looked at cat and dog foods available in the United States, found polycarbonates and polyethylene terephthalate, the latter at concentrations of 4.6 - 12 μg/g [33].

Looking at necessary nutrients, it has been noted that in fish farms feed is the main input route of N, P, K, and Zn, with Ca, Mg, S, Fe, Cu being partially supplied by the water used [1]. It has also been noted that a site-specific optimization is always necessary [1]. In the case of aquarium fish, the feed is the major source of nutrients as in this case the water itself offers few nutrients. Mineral deficiency signs in fish include reduced bone mineralization, anorexia, lens cataracts (Zn), skeletal deformities (P, Mg, and Zn), fin erosion (Cu and Zn), nephrocalcinosis (Mg), thyroid hyperplasia (I), muscular dystrophy (Se) and hypochromic microcytic anemia (Fe) [34].

Here, the authors have looked at the MP content of 13 fish feed products bought in UAE markets. Also, 7 of the fish feed products were selected for the analysis of the elemental composition of the ash acquired by combustion of the feeds at 600˚C to determine the original nutrient levels of the feeds. While the majority of the analyzed feeds targeted ornamental pet fish, the makers of these feeds advertise some of these also as supplemental feed for aquacultural fish farming. The choice of fish feed products for MP content analysis was carried in such a way that all brands, all countries of origin and all price ranges of the products were covered.

2. Materials and Methods

2.1. Analysis of the Feeds

2.1.1. Water Content

A pre-weighed feed sample (3 - 4 g) was heated in a crucible (79C-00, Waldenwanger, Berlin) in a Carbolite electric oven ELE 11/6 at 100˚C (21 samples) or 120˚C (2 samples) for 24 h. The cooled feed was re-weighed. The moisture content of the feed is given in percent.

2.1.2. Digestion of the Feeds

A mixture of 1 g of feed in aq. KOH (2.0 g, 0.036 mol KOH in 20 mL distilled water) was stirred at 70˚C (Stuart CB 162 magnetic stirrer) for 24 h. Thereafter, the mixture was filtered (filter paper: Fioroni and Schleicher Schuell 589[2], thereafter Whatman 1 (Cytiva), pore size 0.5 µm), and the filter cake was washed with distilled water (3 × 15 mL). The filter with the filter cake was dried in an Ecocell drying cabinet (MMM Medcenter Einrichtungen GmbH) at 37˚C for 12 h. The digestion was carried out in triplicate for each fish feed.

2.1.3. Analysis of the MP Content of the Fish Feeds

The filter paper with the residual filter cake was optically scrutinized directly for any adhering microplastics using an Amscope 7× - 45× Zoom Trinocular stereomicroscope (at 4× magnification). A 5.1 MP Amscope microscope digital camera was mounted on the stereoscope and the Amscope ×64, 3.7.1443.2018036 software was used for processing the microphotos. The MPs were recorded as to abundance, color, shape (type) and size. The lower size limit of MPs to be clearlv discerned was seen as 30 µm. In this study, the MPs were not identified as to their polymer type by spectroscopic means. However, suspected MP particles were subjected to a hot needle test [35].

The laboratory work was carried out within a clean, designated laboratory space. At all points in time, researchers were wearing laboratory coats and gloves to minimize contamination of the work-place. Filter papers were scrutinized for contaminants under the microscope before use to ascertain the absence of MPs before experimentation. In addition, blanks were run that used aq. KOH (2.0 g KOH in 20 mL distilled water). Again, no MP presence could be detected on the filter papers after filtration.

2.2. Ash Analysis of the Feeds

2.2.1. Combustion and FT-IR Analysis of the Ash Content

500 - 900 mg samples of fish food were heated in a crucible (79C-00, Waldenwanger, Berlin) at 600˚C for 3 h (Carbolite electric oven ELE 11/6), during which all adhering organic components combusted. The thermolysis experiments were carried out in triplicate for the solid of each fish food brand analyzed. After cooling, the ash content was weighed and subjected to FT-IR spectroscopic analysis as KBr (Sigma Aldrich) pellets on a Perkin Elmer Spectrum Two and a Thermo Nicolet Nexus 670 FT-IR spectrophotometer, where the transmittance of the KBr sample pellet was recorded in the range 4000 - 500 cm^{-1} and processed after 32 scans. Thereafter, to understand their elemental composition, the ash contents of selected fish foods were examined with X-ray fluorescence spectroscopy (WD-XRF). Furthermore, many of the fish samples were subjected to IR spectroscopic analysis before and after ashing.

2.2.2. Wavelength Dispersive XRF (WD XRF) Analysis of the Ash

1) Sample preparation

The ash sample obtained after thermolysis was ground to a fine powder. The resulting fine powder was pressed in a 13 mm bore steel die in a manually operated hydraulic press (Specac). Pressure was applied until the reading was stable at 10 tons and left for 40 s. This produced mechanically stable round pellets of 13 mm diameter. The pellets were generally analyzed within an hour and great care was taken that the two flat surfaces intended for XRF analysis were not touched. The weight and exact diameter of the pellet was measured and used for the semi-quantitative X-ray analysis.

2) XRF analysis

The XRF analysis was done on a wavelength dispersive (WD) XRF spectrometer (Rigaku ZSX Primus IV) equipped with a Rh X-ray tube. The instrument is controlled by ZSX Guidance software intended for analysis of approximately 70 elements from F to U. The resulting pellet was placed in a sample holder cup with the aid of 10 μm polypropylene film which had a high X-ray transmission rate and low level of impurities. All samples were arranged on sequential basis controlled by an automated autosampler system. The spectra were processed with a semi quantitative SQX software package, capable to automatically correct all matrix effects, including line overlaps. SQX also corrected for secondary excitation effect by photoelectrons (light and ultra-light elements), varying atmospheres, impurities, and different sample sizes. Finally, the spectra of each sample were matched with a library and Perfect Scan Analysis Programs [36].

3. Results and Discussion

21 fish feeds (**Figure 1**) were bought in commercial markets in Al Ain, Abu Dhabi, UAE: The fish feeds cost 0.05 - 1.31 AED/g (or mL) [0.014 - 0.36 USD/g (or mL) product].

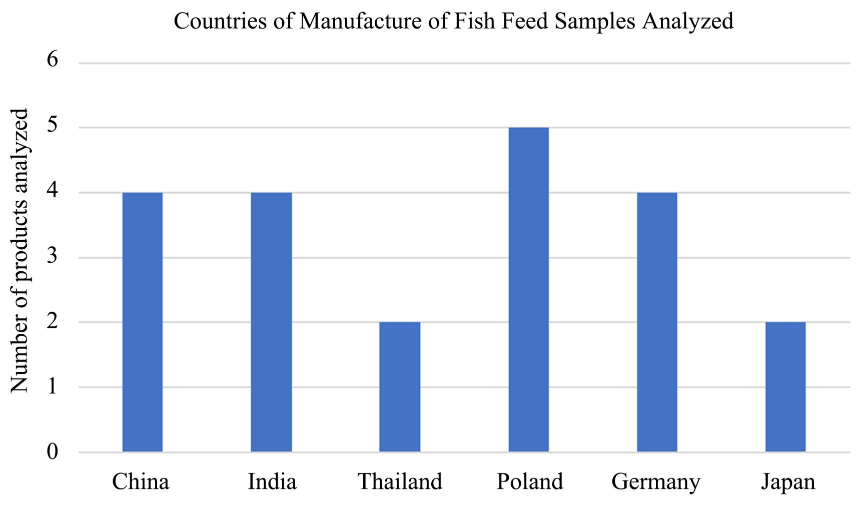

Figure 1. Origin of the 21 fish feeds that were analyzed.

Initially, fish feeds were subjected to thermal gravimetry (TGA) to gauge the mass loss of the products as a function of temperature. Thus, product No. 5 lost 7.58 w% of its weight when heated up to 124˚C. This loss was associated with moisture content of the fish. Between 127˚C - 226˚C, the sample lost another 4.55 w% of its weight. This is due to the emission of low weight volatile constituents of the products. By TGA, it was also determined that heating the samples to 550˚C was not sufficient for total combustion of the organic constituents. This is why the combustion of the feed samples to obtain their ash content was carried out at 600˚C.

The moisture and ash contents of the respective feed products are shown in Table 1. The highest percentage of water loss was 9.59% (for sample 1) and the lowest percentage of water loss was 3.22% (for sample 17). The highest percentage of ash content was 12.49% (for sample 6) and the lowest percentage of ash content was 1.96% (for sample 10), where sample 9 (ash content: 21.77 ± 0.89 w%) was excluded from this statement. Sample 9 is composed of amphipod crustaceans of the family Gammaridae. Exoskeletons of shrimps are known to contain proteins, chitin and calcium carbonate and usually give high ask contents. With 21.77 ± 0.89 w% the ash content of sample 9 was lower than that found for the Northern shrimp (*Pandalus borealis*) with 34 ± 2 w% [37] and on the lower side of contents reported for shrimps in general (20 - 40 w%) [38].

Table 1. Water and ash content of 21 fish feed bought in the UAE.

Product	Water content			Ash content	
No.	Dry w% (avg.)	Water w% (avg.)	STD	Ash w% (avg.)	STD
1	90.41	9.59	0.08	5.43	0.06
2	91.95	8.05	0.72	10.98	0.06
3	94.87	5.13	0.25	10.21	0.09
4	95.83	4.17	0.49	11.55	0.07
5	92.68	7.32	0.46	9.54	0.14
6	94.98	5.02	0.11	12.49	0.68
7	96.70	3.30	0.34	9.23	0.12
8	96.54	3.46	0.59	6.74	0.24
9	99.42	0.58	0.03	21.77	0.89
10	94.43	5.57	0.47	1.96	0.03
11	92.18	7.82	0.13	8.11	0.21
12	95.29	4.71	0.44	6.87	0.34
13	95.31	4.69	0.05	9.05	0.06
14	93.70	6.30	0.07	10.29	0.14
15	95.85	4.15	0.93	6.14	0.43
16	96.31	3.69	0.14	10.34	0.37
17	96.78	3.22	0.37	9.78	0.03
18	94.07	5.93	0.06	5.09	0.05
19	95.05	4.95	0.74	9.75	0.22
20	93.47	6.53	0.48	5.21	0.01
21	95.00	5.00	0.28	5.24	0.19

The average percentage of K in the 7 feed ash samples analyzed was noted to be 21.02% ± 6.16% (Table 2). The samples can be divided into 3 groups according to their K content: 1) 15.8% - 16.7%, 2) 19% - 20.5% and 3) 27.4% - 31.7.%. The average percentage of Ca in the 7 feed ash samples was found to be 31.6% ± 7.6% (Table 2). The samples can also be divided into 3 groups according to their Ca content: 1) 16.6%, 2) 28% - 34.5% and 3) 37.6% - 39.5.%. Ca can be added to the feeds as monobasic calcium phosphate ($CaHPO_4$). From the feed product labels, it was evident that Ca can also be added as calcium iodate [$Ca(IO_3)_2$], calcium-L-ascorbyl-2-monophosphate as a vitamin C of improved chemical stability and bioavailability [39], and calcium pantothenate. In the fish, iodate in [$Ca(IO_3)_2$] is reduced to iodide and absorbed almost completely from the gastrointestinal tract [40]. According to product labels, Zn, found in 5 of 7 samples (Table 3), is added to the feeds as zinc oxide and zinc sulfate. Mn and Fe can also be brought in with the fish feed as manganese sulfate ($MnSO_4$) and ferrous sulfate ($FeSO_4$), respectively.

Table 2. Composition (in %) of the ash of 7 fish feed products.

No.	Na	K	Ca	Mg	Al	Mn
10	19.8	20.5	16.6	NA	NA	NA
11	9.05	19	37.6	2.72	0.112	NA
12	NA	31.7	28	8.75	NA	NA
16	7.23	15.8	39.5	3.43	0.780	NA
17	9.50	16.7	33.8	3.88	0.56	0.194
18	7.98	16.1	34.3	3.58	0.865	0.213
19	19.8	27.4	31.2	6.39	NA	NA

Table 3. Composition (in %) of ash of 7 fish feed products (continued).

No	Fe	Zn	Sr	P	Si	S	Cl	Br
10	NA	NA	NA	22.9	NA	3.3	16.0	NA
11	0.385	0.151	0.215	16.0	1.04	1.82	11.8	0.106
12	NA	NA	NA	29.5	NA	NA	NA	NA
16	NA	0.288	0.112	17.3	3.54	3.17	7.58	NA
17	1.19	0.226	0.111	18.4	2.96	3.23	7.58	NA
18	1.89	0.155	0.037	18.6	4.30	0.964	10.9	0.037
19	NA	NA	NA	24.5	4.17	1.15	5.24	NA

13 fish feeds were analyzed for MP content. In all 13 fish feeds, MP content was found with in a range between 0.7 ± 0.6 MP/g and 9.0 ± 2.6 MP/g. MP concentration did not correlate with the price of the feed (Table 4). The breakdown of the

MPs found in the fish feed by color is shown in **Figure 2**. Most common were blue MPs, followed by black, white/transparent, and red MPs (**Figure 2**), Fibers (69%) were the most common MP type, followed by fragments (22%), rectangular shaped MPs (6%), and films (3%) (**Figure 3**). Photos of typical MPs found in the fish feed are shown in **Figure 4**. MP content in fish feed can derive from manufacturing and packaging processes. However, it must be noted that fish meal itself is a considerable ingredient of fish feed, and thus MP content in fish feed can originate from the MP uptake of fish from which fish meal has been prepared to be included in the fish feed [41]-[48]. This, in turn, creates a self-sustaining cycle. Altogether, the authors found more MPs in these animal feeds of repute available in the UAE than they did in packaged products available in the UAE that come into contact with the human oral tract such as toothpastes [49].

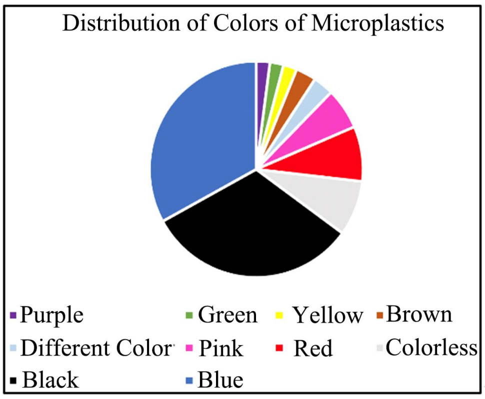

Figure 2. Breakdown of the MPs found in fish feed by color.

Table 4. Concentration of MPs in 13 fish feed products analyzed.

Sample	MPs/per/g	Price (AED)/1g or 1ml
1	4.0 ± 0.6	0.2
2	2.3 ± 2.1	0.23
3	3.0 ± 0.6	0.13
4	0.7 ± 0.6	0.53
5	3.0 ± 1.7	0.6
6	2.3 ± 1.2	0.12/ml
8	2.3 ± 2.1	1.31
9	9.0 ± 2.6	0.61
10	2.0 ± 1.0	0.23
13	3.0 ± 0.6	0.25
16	1.7 ± 2.1	0.05/ml
18	2.0 ± 1.0	0.74
19	2.7 ± 0.6	0.26

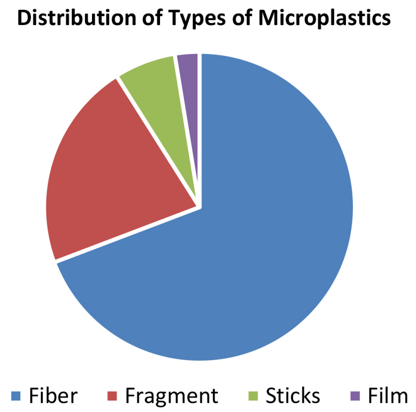

Figure 3. Breakdown of MPs by particle type.

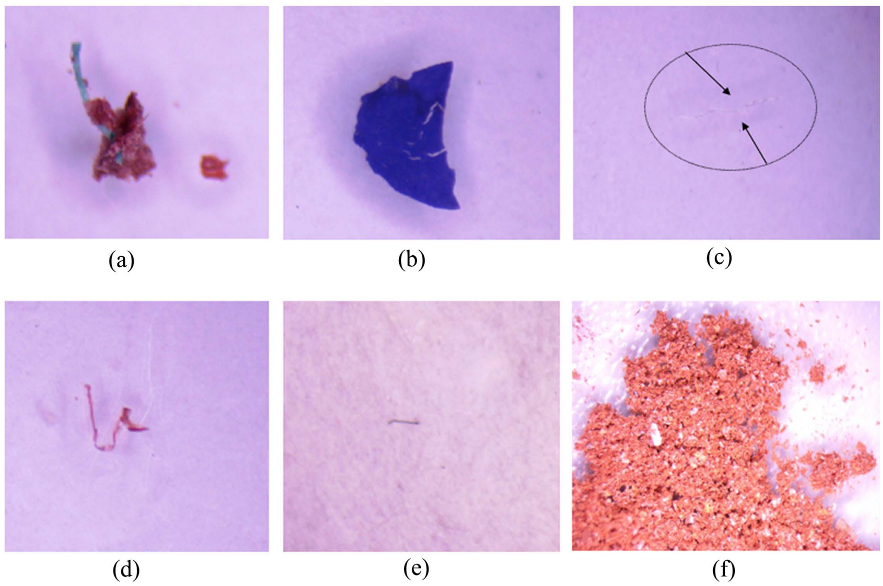

Figure 4. Microphotos of isolated MPs from fish feed ((a)-(e)). Microphoto (f) shows fish feed ash after combustion at 600˚C.

4. Conclusions

XRF analysis of the ash of 7 fish feed produced by well-known brands commercially available in the UAE showed a Na content of 7.3% - 19.8% Na, 15.8% - 31.7% K, 16.6% - 39.5% Ca, 2.7% - 8.8% Mg, 16.0% - 29.5% P, and 5.3% - 16.0% Cl. Of the 13 fish feeds investigated, all of them showed MP content, where polymeric fibers were the main type of MP.

For future work, it would be interesting to investigate the polymeric nature of the MPs detected in fish food to better understand their origin and their point of entry.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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