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MINI REVIEW

Occurrence and pathways of microplastics, quantification protocol and adverse effects of microplastics towards freshwater and seawater biota

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Abstract

Plastic has become one of the major contributors to the world's pollution. As it may degrade into smaller particles known as microplastics (<5 mm), which has become a new threat towards marine ecosystems. Due to their size, microplastics can easily enter the food chain due to seafood consumption, entering the digestive system. Moreover, microplastics also bring negative impact towards freshwater biota and sea life. Despite the extensive studies on microplastic contamination in the marine environment, research on microplastic in seafood and freshwater environments, specifically regarding ingestion and effect of microplastics in aquatic organisms, freshwater biota as well as human health along the supply chain are still scarce. Microplastic ingestion in aquatic animals causes health hazards such as leaking gut, transferring pathogenic bacteria into the blood and increasing toxic levels in the body. This scenario could potentially introduce similar hazards to human health if consumed. This paper reviewed the sources, detection method, allocation and adverse effects of microplastics contamination in seafood and freshwater ecosystems. The focus of this review is on biota which includes samples of aquatic animals as well as seawater samples from various sources including riverine (river), lacustrine (lake) and wastewater treatment plants (WWTPs) throughout the world. Since microplastics are related to synthetic substances from waste, there is an alarming concern regarding their physical and chemical compounds that might be present in the aquatic life and food supply chain. Therefore, recommendations and improvements on future studies of microplastic contamination in marine ecosystems regarding identification and quantification protocol along with the study on microplastics towards the freshwater organism also are addressed in this paper.

1. Introduction

Plastics are used worldwide and processed with various chemical additives to fit the intended purpose (Piringer, 2007). The production of plastic nanocomposites had been developed due to the advancement of new applications of elements based on nanoscales. The problem occurs when macroplastics from plastic waste start to break down in the environment, thus degrading into smaller pieces, ultimately becoming microplastics (Chae *et al*., 2019). Basically, macroplastics are defined as plastics with a size greater than 5 mm, meanwhile, mesoplastics are less than or equal to 5 to greater than 1 mm, microplastics are less than or equal to 1 mm to greater than 1.0 µm and nano plastics are less than or equal to 0.1 µm (Lambert *et al*., 2014).

Sources of degraded plastics may come from the land or industrial area as the plastics break down into microplastic dust and enter the drains, thus ending up in the seawater and sinking to the ocean floor (Caruso, 2020). Generally, plastics are introduced into the ocean through various ways such as land littering, personal care products and littering which threaten marine ecosystems as microplastics are sometimes mistaken as food by the sea or aquatic creatures. These microplastics could indirectly harm human health if they enter the food web (Smith *et al*., 2018; Waring *et al*., 2018). Plastics can leach high concentrations of polyethylene (PE) and polystyrene (PS) even in small quantities which can affect freshwater ecosystems (Phuong *et al*., 2016). As a result, bioaccumulation may occur with long-term exposure, and this can be an indicator to determine the

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degree of this toxic substance to be transported inside the food networks. Bioaccumulation is characterized as the net take-up of impurities such as microplastics from the surrounding by every conceivable course from any source (Binetti *et al*., 2008). Long-time exposure to high-density polyethylene may bring harmful towards human health (Okunola *et al*., 2019)

It is estimated that 55% of plastic waste is discarded into the environment and the rest is incinerated or recycled (Our World in Data, 2018). Plastics accumulate toxic compounds from the water and shuttle them, along with any chemicals that originated from the plastics, entering marine organisms, concentrating up the food chain and eventually ending up on human plates. Due to plastic sustainability and undecomposed properties, now it has become one of the threats to nature (Thompson *et al*., 2009). Other than marine life being affected by microplastic contamination, microplastic also can be found in every part of freshwater throughout the world including riverine, estuarine and lacustrine. In accordance with the environmental issue, much research has been developed focusing on the identification and detection of microplastic in biota for all trophic levels mostly from the marine organism but leaving the issues of seafood consumption's effects towards humans. For the freshwater ecosystem, research is still scarce and the protocol for identification is not fully standardized. Therefore, the objective of this paper includes reviewing the source and pathways of this microplastic contamination in seafood and freshwater biota, as well as identifying the research gaps in the microplastic study itself for a better understanding.

2. Overview of microplastics

2.1 Characteristics and morphological properties of microplastics

Ecological plastics are commonly described under multiple descriptors. Microplastic particles have infinite shapes (Claessens *et al*., 2013). Microplastics presumably come in varieties of shapes such as granules (beads), filaments (fibres), fragments (round, angular) and pellets (cylinders, discs, and spherules) (European Commission, 2013). Working Group on Environmental Status (WG-GES) has outlined specific size division for plastic litter: macroplastics (>5 mm), mesoplastics (1 to 5 mm), large microplastics (1 μm to 1 mm) and small microplastics (<0.1 μm). Other than that, plastic size that falls below 20 μm will therefore be classified as nanoplastics (European Commission, 2013). In addition, microplastics have a maximum size of 5 mm and the minimum size of about 335 microns which the minimum is the mesh size of the plankton that is used to collect them (Peters and Bratton, 2016). Microplastics are separated into two categories: primary and secondary. They can be divided based on the type of resin where the list of resin materials classified for microplastics are polyethyleneterephthalate (PET), polystyrene (PS), polyether (PT), polypropylene (PP), polyamide (PA), polyester (PES), polyurethane (PU), polyvinylchloride (PVC), cellophane (CP), acrylic polymers (AC) and not specified (NS) (De Sá *et al*., 2018). Other than that, there are also sub-polymers such as ethylene-vinyl cetate copolymer (EVA/PEVA), polyvinyl acetate (PVA), poly (methyl methacrylate) (PMMA) and polyacrylonitrile or known polyvinyl cyanide (PAN) (De Sá *et al*., 2018). However, the most plentiful manufactured plastic polymers found in seawater were polypropylene, polyethylene and some forms of polystyrene (Erni-Cassola *et al*., 2019). Polyethylene, polypropylene and polystyrene are the common plastics used abundantly in the packaging industry (food and plastics bottles), textiles industry (Claessens *et al*., 2011; Di and Wang, 2018) and cosmetic products (Cole *et al*., 2011).

2.2 Origin and source of microplastics

Primary microplastics are produced as microbeads, capsules, pellets or fibres (Peñalver *et al*., 2020). The sources of primary microplastics may comprise microbeads from cosmetic goods and personal care, chemical scrubber applied for polishing, microfibers from garments and natural resin pellets in plastic manufacturing (Duis and Coors, 2016). Other than that, secondary microplastics are produced from the breakdown of those large materials including fragments from a plastic bottle, films from a plastic bag, fibers from netting, rope and even from synthetic clothing (Karapanagioti and Klontza, 2008). On the other hand, secondary microplastics can also be defined as the outcome of larger plastic pieces which shatter down into smaller particles when plastic waste is exposed to sunlight and the environment (Bergmann *et al*., 2015). As it is continuously being exposed to the sun's radiation and ocean waves, the plastics will undergo deformation and disintegration producing secondary microplastics (Andrady *et al*., 2017).

Microplastics are also able to accumulate in food webs (Smith *et al*., 2018). It is reported that 80% of microplastics came from terrestrial sources with rapid development, industrialization and urbanization occurring along with high population density (Li *et al*., 2015). This includes waste from land littering such as plastics and polystyrene from food packaging and plastic shopping bags including personal care products (Fauziah *et al*., 2015). This intact debris may also be associated with the abundance of offshore activities such as commercial fishery, waste or sewage disposal, navigation activities and shellfish culture site that contribute to the accumulation of plastic debris which end up in the tissue of filter feeders (Thushari and Senevirathna, 2020). Plastics are broken down when they are consistently exposed to harsh solar radiation and consistent abrasion of wind and waves, creating microplastics. Seafood such as shrimp, fish and shellfish have been highly associated with microplastics presence (Devriese *et al*., 2015). This explains the magnification that refers to the exponential increase in a microplastic contaminant as it goes up through levels in a food web. Wastewater outflow from sewer systems and deterioration of large quantities of microplastics in water including the use of sludge have indeed been proposed as the major sources of freshwater ecological microplastics as more wastewater is being contributed from the population growth (Carr *et al*. 2016). However, through consistent disposal and accumulation of wastewater, these will cause a large quantity of microplastics entering the sludge thus transferring microplastic to freshwater biota such as rivers and lakes (Wong *et al*., 2020).

3. Microplastic contamination in marine life and freshwater biota

3.1 The occurrence of microplastics in marine life

Plastics are associated with a whole complex array of chemical waste from the manufacturing industry that might absorb the particles when they are in the environment (McDermid *et al*., 2004). The legacy contaminants such as Polychlorinated biphenyls (PCB) and dichloro-diphenyl-trichloroethane (DDT) are highly toxic industrial compounds. Many of these chemicals are known to be potentially harmful to organisms where the chemicals will transfer into the animal tissue and bring harm (Hosomi *et al*., 2012). Given statistics of fish utilization globally, it is unavoidable that individuals are exposed to microplastics at some point in the supply chain as there is a study which indicates that microplastics with unequivocal qualities can displace beyond cells in mammalian structures (Wright and Kelly, 2017). Studies have shown that microplastics are present in different species of marine life. Plastic particles have been reported to be found in the digestive tract and the gills except in the abdominal muscle tissue of a whole shrimp (*Crangon crangon)* from coastal waters of the Southern North Sea and Channel area (Devriese *et al*., 2015). In concurrence with the findings, microplastic is also found in the gastrointestinal tract of both species of pelagic and demersal fish species which are famous for human consumption (Hantoro *et al*., 2019). In addition, microplastics are also commonly found in shellfish due to their filter-feeding properties. Most investigations were focused on the blue mussel (*Mytilus edulis*) and shellfish (*Crassostrea gigas*). There were also a few additional research involving microplastics in shellfish (*Venerupis philippinarum*), rock clams (*Saccostrea forskalii*) and ocean snails (*Littorina littorea*) (Hantoro *et al*., 2019).

The effect of microplastic on marine biota is an issue as it prompts the entrapment and ingestion in marine life. The microplastic pieces presence in marine life are essentially from earth-bound sources and in this manner, waterfront biological systems which include coral reefs are in incredible threat due to microplastic contamination (Monterey Bay Aquarium, 2017). Corals develop energy by benefiting from microscopic fishes (plankton) to secure significant supplements which are basic for their development, improvement, and reproduction. The negative impact of microplastics on corals includes the preservation of plastic parts in mesenterial tissue which causes bringing down in energy reserves (Nuelle *et al*., 2014). The entrance of microplastics along the cell mass of phytoplankton creates a decrease in chlorophyll absorption (Mao *et al*., 2018). The presence of microplastics in different seafood species such as brine shrimp and fish are depicted in Figures 1 and 2.

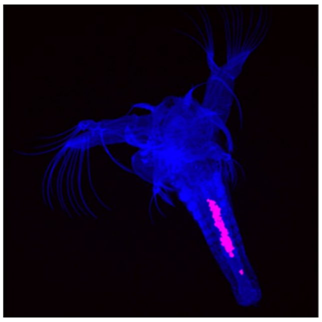

Figure 1. Microplastics in brine shrimp (Hale *et al*., 2020).

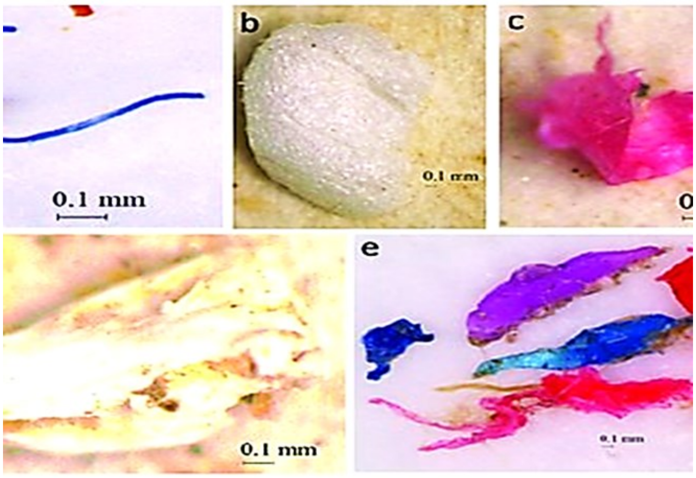

Figure 2. Microplastics obtained from the GI tract of different fish species at Skudai River, (a) fibre, (b) foam, (c) film, (d) fragment, (e) colourful film and fragment (Sarijan *et al*., 2019)

3.2 Occurrence of microplastics in freshwater biota

Previously, extensive research has been done to identify microplastic contamination in marine animals, but less study focuses on freshwater areas. Microplastic contamination study in freshwater is estimated to be five-fold less than in the marine animal study (O'Connor *et al*, 2019). However, recently, attention has been paid towards the detection and identification of microplastics contamination in the biota elements of the freshwater ecosystem. To date, microplastics studies on freshwater organisms have indeed been focused on the top levels of taxa, in specific fish, with minimal findings evaluating lower taxa (O'Connor *et al*., 2019). All studies portrayed in Table 1 show evidence of ingestion of microplastics in the studied species. Examples of images of microplastics under microscopic view are also shown in Figure 2, Figure 3 and Figure 4 which show microplastics' existence, incident, and type of microplastic which are prone to higher taxa levels, specifically the freshwater fish.

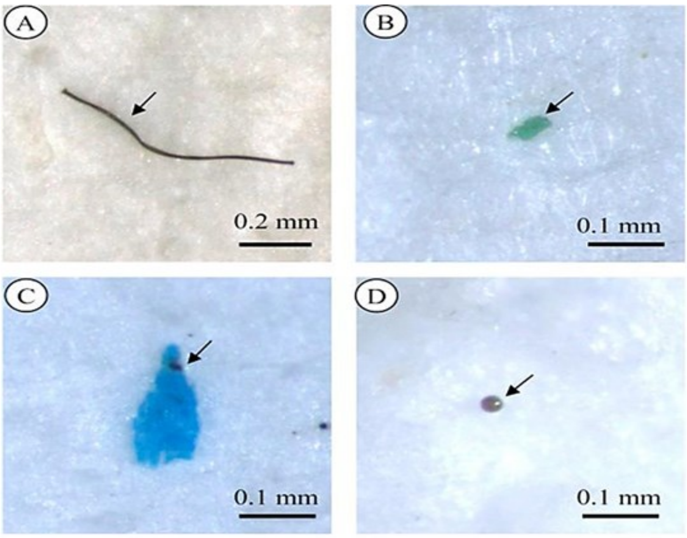

Figure 3. Photographs of microplastics isolated from the gut of various fish species from Taihu Lake, China. The morphotype included were (a) fibre, (b, c) fragment, (d) pellet (Jabeen *et al*., 2017).

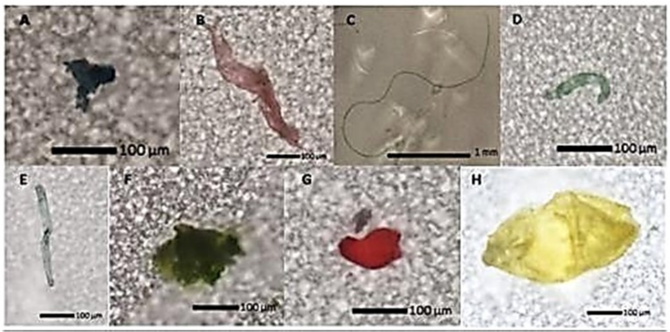

Figure 4. Identified microplastics from the gastrointestinal tract of Gobio gobio which is one type of small fish from Flemish River where (a, f) foam, (b, d) film, (c, e) fibre, (g) fragment and (h) pellet (Slootmaekers *et al*., 2019).

3.3 Trophic transfer of microplastics

The consumption of microplastics may take place either by direct transfer or secondary ingestion although there is no specific determination on the pathways. In addition, there are laboratory studies of nano plastics (fluorescent nano-sized polystyrene plastics) trophic transfer in a chain of four freshwater species (Chae *et al*., 2019). In this study, the test species used was *Chlamydomonas reinhardtill* as the primary producer, followed by primary consumer *Daphnia magna* (small planktonic crustacean), then *Oryzias senensis* (the Chinese rice fish) as secondary consumer and *Zacco temmenckii* (Japanese dark chub fish a species of cyprinid) as top predator. From the study, the analysis shows that the nano-sized polystyrene can penetrate and attach to the *Chlamydomonas reinhardtii* and enter the gut of the consumer species through the consumption of its food (prey). As a consequence, nano plastics also cause histological damage in the *Daphnia magna.* This finding can be an indicator that microplastics could potentially be transferred to higher trophic levels based on prey-predator systems and unintentionally enter our supply chain risking the health of seafood consumers when the hazardous compounds transfer into the organisms in the food web (Karami *et al*., 2018; Zaki *et al*., 2021)

4. Detection, identification, and quantification protocol of microplastics contaminants

The determination of microplastic numbers found in biota is sample-dependent and related to sampling sites including the method of investigation. The critical step is the sample collection at the sampling site followed by the separation process. Extraction of microplastic from samples takes place through digestion using acid or alkaline digestion, especially in biota and soft tissues (Claessens et *al*., 2013). The final steps for microplastic determination are identification and quantification by physical and chemical analysis (Hidalgo-Ruz *et al*., 2012). Factors such as measurement in mass and significant volume of the sample, the pore dispersity or mesh size of the filters, density segregation and digestion solutions are crucial to obtain reliable data on microplastics validity and verifiability in freshwater systems (Stock *et al*., 2019). Nonetheless, novel methods such as advancement in visual analysis with the use of dyeing chemical and chemical interpretation are also able to improve in giving relevant results in the analysis. The analytical and basic process of analyzing microplastics in biota is shown in Figure 5.

4.1 Extraction and purification process

Tissue digestion and removal of organic contaminants can be conducted either by using acids, bases, oxidative agents, or new advancements in using enzymes (Claessens *et al.,* 2013). However, these solutions used for digesting the tissue sample give an adverse effect on the microplastic numbers since it could cause undesirable disruption of microplastics plastics

Table 1. Microplastic contamination research on different freshwater species at different places between 2016 and 2020.

Species	Area	Treatment	Prevalence	Polymer	Shape	Colour	Size	References
Dorosoma cepedianum and *Micropterus salmoides*	Evergreen Lake and Bloomington Lake	Digestion: Potassium hydroxide (KOH) and 30% Hydrogen Peroxide (H_2O_2) for 24 hrs at 50°C in the water bath	100% 1-49 particles per fish	Visualization	Fibre, fragment	Coloured		Hurt *et al*. (2020)
Mystus bocourti, Puntioplites proctozysron, Henicorhynchus siamensis, Laides longibarbis, Labiobarbus siamensis, Cyclocheilichthys repasson, Hemibagrus spilopterus, Labeo chrysophekadion	Chi River, Thailand	30% hydrogen peroxide (H_2O_2) at 65°C for 24 hrs	72.9%	Visualization	Fibre, rod-shaped, pellet, fragment	Blue, red, black, white, transparent and brown.	0.03-3.84 mm	Kasamesiri and Thaimuangpho (2020)
Three trophic guilds (16 species): -Carnivorous (Piranha) -Omnivore -Herbivore	Xingu Basin, Brazil		81.3%	PE, PVC, PA, PP, PET, PMMA and rayon	Fragment, filament	Black, blue, red, white, translucent	1.0-4.9 mm	Andrade *et al*. (2019)
Oreochromis mossambicus, Cyclocheilichthys apogon, Clarias gariepinus, Anabas testudineus, Pangasius hypophthalmus, Oxyeleotris marmorata	Skudai river, Malaysia	10% potassium hydroxide (KOH)	40%	Visualization	Film, fragment, fibre, foam	Blue, white, red, black, yellow	≤0.01-5 mm	Sarijan *et al*. (2019)
Gobio gobio	Flemish rivers, Belgium	Separation: Sodium iodide (NaI) and Digestion: 15% H_2O_2 at 60°C	9%	EVA, PP, PET, PVC, CP, PVA and PA	Fibre, fragment, film, foam, pellet	Green, red, blue, yellow	≤ 2 mm	Slootmaekers *et al*. (2019)
Anadonta anatina	Swedish River, Sweden	65% nitric acid (HNO_3) for 24–72 hrs	100%	Visualization	Fibre	Transparent, black, red, green, grey, blue, and purple		Berglund *et al*. (2019)
Carassius auratus	Poyang Lake, China	10% KOH for 48 hrs in rocking bed at 40°C of speed 100 rpm. Then, 30% H_2O_2 overnight in the room temperature	25.7%	Visualization	Fibre, film, fragment, pellet	White, black, transparent, and coloured	≥0.5 mm	Yuan *et al*. (2019)
Dorosoma cepedianum, Catostomus commersonii, Pimephales promela, Carpoides cyprinus, Notropis stramineus, Notropis hudsonius, Fundulus diaphanous, Micropterus sp., Notropis atherinoides, Neogobius melanostomus, Cyprinella spiloptera	Lake Michigan, USA	30% H_2O_2 and 0.05M Fe (II) solution	85% with 13 particles per fish	Visualization	Fibre, fragment	White, blue, grey, red, black		McNeish *et al*. (2018)

Table 1 (Cont.). Microplastic contamination research on different freshwater species at different places between 2016 and 2020.

Species	Area	Treatment	Prevalence	Polymer	Shape	Colour	Size	References
Squalius cephalus	Marne and Seine River, France	14 g/l NaClO solution overnight	40%	PET, PP, PAN, PEVA, PE, and PS	Fibre, fragment		390 µm to 7.38 mm	Collard *et al.* (2018)
Gymnocypris przewalskii	Qinghai Lake, China	10% KOH		PE, PS, PP, and nylon	Fibre			Xiong *et al*. (2018)
Rutilus rutilus	River Thames, United Kingdom		32.8%	PE, PP, and PES	Fibre, fragment, pellets			Horton *et al.* (2018)
Chironomus spp. larvae	Bloukrans River, South Africa	55% HNO_3 for 6 hrs	98%	Visualization				Nel *et al*. (2018)
Involved wide and various sample of species (46 species)	Amazon river estuary, Brazil	Visual	30.4%	PE, PA, and rayon	Pellets, sheets, fragment, threads	Blue, yellow, transparent	0.38-4.16 mm	Pegado *et al.* (2018)
Cyprinus carpio, Carassius auratus, Hypophthalmichthys molitrix, Pseudorasbora parva, Megalobrama amblycephala, Hemiculter bleekeri	Taihu Lake, China	30% H_2O_2 at 65°C in an oscillation incubator of speed 80 rpm for 24-72 hrs	95.7%	Visualization	Fibres, fragments, pellets	Transparent, black, coloured	0.04-5mm	Jabeen *et al.* (2017)
Esox lucius, Catostomus commersoni, Notropis atherinoides, Pimephales promelas, Eucalia inconstans	Waskana creek, Canada	10% NaClO	73.5%	Visualization	Fibre, fragment, bead			Campbell *et al.* (2017)
Hoplosternum littorale	Pajeú river, Brazil	Visual	83%	Visualization				Silva-Cavalcanti *et al.* (2017)
Luciopimelodus pati, Pseudoplatystoma corruscans, Oligosarcus oligolepis, Parapimelodus valenciennes, Odontesthes bonariensis, Astyanax rutilus, Cyprinus carpio, Pimelodus maculatus, Prochilodus lineatus, Hypostomus commersoni, Cyphocharax voga	Freshwater zone of Río de la Plata estuary, Argentina	30% hydrogen peroxide at 60°C	100%	Visualization	Fibre	Red, green, yellow, white, black, and blue	0.06 to 4.7 mm	Pazos *et al.* (2017)
Involved wide and various sample of species (69 species)	Paraiba and Mamanguape River basins, Brazil	Visual	9% 1-4 particles per fish	Visualization	Fibre, film, fragment		0.02 mm and 0.04 mm based on figure	Vendel *et al.* (2017)

Table 1 (Cont.). Microplastic contamination research on different freshwater species at different places between 2016 and 2020.

Species	Area	Treatment	Prevalence	Polymer	Shape	Colour	Size	References
Corbiculafluminea	Taihu Lake, China	30% H_2O_2		CP, PET, PS, PP and terephthalic acid	Fibre, fragment, film, and pellet	Transparent, black, white, blue, yellow, red, and green	0.05-5 mm	Su *et al*. (2016)
Lepomis macrochirus and *Lepomis megalotis*	Brazos River Basin, USA	Visual	45%	Visualization				Peters and Bratton (2016)
Lates niloticus and *Oreochromis niloticus*	Lake Victoria, Tanzania	Digestion: 10 M NaOH at 60°C for 24 hrs	20%	PE, PU, PES, PP copolymer and silicone rubber			1.0-5 mm	Biginagwa *et al*. (2016)

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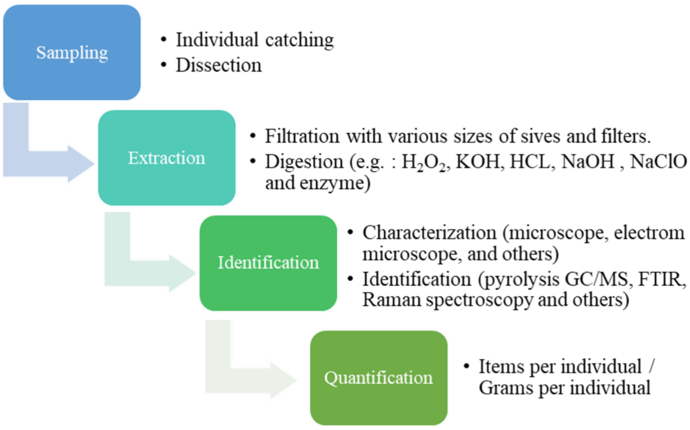

Figure 3. Photographs of microplastics isolated from the gut of various fish species from Taihu Lake, China. The morphotype included were (a) fibre, (b, c) fragment, (d) pellet (Jabeen *et al*., 2017).

(Ibrahim *et al*., 2018). The most important aspect in choosing the solution for complete tissue digestion is by ensuring that the solution is able to provide a high digestion rate without damaging the microplastics particles. Acid-like nitric acid (HNO_3) and base-like sodium hydroxide (NaOH) used in the digestion usually may cause dissolution and microplastic degradation which may cause inaccuracy of the results obtained (Enders *et al.,* 2017). The usage of hydrochloric acid (HCl) is not recommended to be used for tissue digestion as it is not efficient and unable to achieve optimum digestion of the organic material (Cole *et al*., 2014). Other chemical solutions for tissue digestion include potassium hydroxide (KOH) which can yield better results in conjunction with the resistance of the polymer (Kühn *et al*., 2017). In addition, another chemical agent used for the tissue digestion process is hydrogen peroxide (H_2O_2) which is an oxidizer and is also involved in the wet peroxide oxidation process. Despite its effectiveness in the digestion of organic contaminants, the use of H_2O_2 may cause foam formation and result in materials loss (Wang *et al*., 2018). Another upsurge method to remove the organic material is by using enzymatic degradation known as Basic Enzymatic Purification Protocol (BEPP) (Löder *et al*., 2017). Enzymes used are specifically designed to hydrolyze the protein only and break down the tissue of organic material. In contrast with chemical digestion, the enzymes do not cause any degradation, destruction or structural changes to the microplastics. The use of enzymes is more promising in obtaining more accurate results. However, enzyme usage is more time-consuming and costly which makes it difficult to implement into the analysis for massive scale (Klein *et al*., 2018). Standards used on the type of solution for microplastic detection and identification need to be established to produce more reliable results, giving a better comparison for each study.

4.2 Spectroscopic and chromatographic confirmation

Most of the studies only focus on the identification of microplastic ingestion in marine ecosystems by visual method or through physical characterization by using microscopic visualization (Claessens *et al*., 2013). Less effort has been made to confirm the identity of the microplastic which can be conducted by using chemical analysis such as spectroscopic and chromatographic methods (Kühn *et al*., 2017). Consequently, not all microplastics found are known for their polymer. Spectroscopic confirmation can be done by using Fourier -Transform Infrared (FTIR) spectroscopy, RAMAN spectroscopy, and Scanning Electron Spectroscopy (Liu *et al*., 2018). Meanwhile, for chromatographic methods, thermo-analytical methods such as pyrolysis gas chromatography-mass spectrophotometer (GC/MS) and high-performance liquid chromatography (HPLC) are used (Liu *et al*., 2018). Examples of analysis by the spectroscopic and chromatographic instruments are shown in Figure 6 using conventional Raman spectra, Figure 7 as a chromatographic method using IR spectra. Meanwhile, Figure 8 shows the comparison between GC/MS and FTIR spectra on microplastic analysis (Primpke *et al*., 2020). The analysis of spectroscopic and chromatography is able to present a quick and high detection rate rather than visual identification. Table 2 shows studies that applied spectroscopic to confirm microplastic polymers in different aquatic species. More studies on microplastic polymer type identification and characterization need to be addressed since most microplastic studies involve the interaction of different types of polymers (Liu *et al*., 2018).

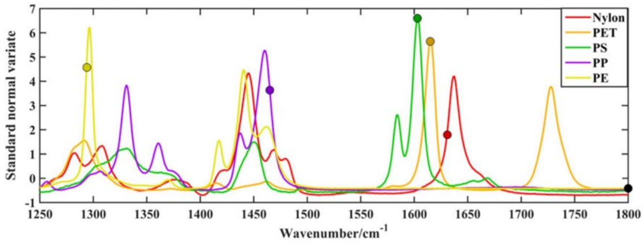

Figure 6. Conventional Raman spectra on five different polymers of microplastics (Zada *et al.,* 2018).

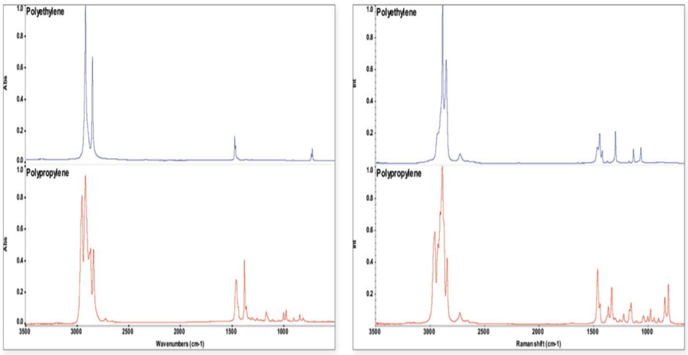

Figure 7. Left side: IR spectra of polyethylene and polypropylene, Right side: Raman spectra of polyethylene and polypropylene (ThermoFisher Scientific, 2018).

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Table 2. Spectroscopic confirmation of microplastic extracted from different aquatic species.

Species	Instrument	Purpose of use	Polymer detected	References
Three trophic guilds (16species): - Carnivorous (Piranhas) - Omnivore - Herbivore	ATR-FTIR spectroscopy	Reliable method for identification of microplastics' decomposition	PE, PVC, PA, PP, PET, PMMA, rayon	Andrade *et al*. (2019)
Gobio gobio	µ-FTIR and Raman spectroscopy	Distinguishing the synthetic background	Ethylene-vinyl acetate copolymer (EVA), PP, PET, PVC, CP, polyvinyl acetate (PVA) and PA	Slootmaekers *et al*. (2019)
Squalius cephalus	Raman spectroscopy	Verification of suspected microplastics	PET, PP, PAN, PEVA, PE, PS	Collard *et al*. (2018)
Gymnocypris przewalskii	Raman spectroscopy	Verification of suspected microplastics	PE, PS, PP, nylon	Xiong *et al*. (2018)
Rutilus rutilus	Raman spectroscopy	Verification of suspected microplastics	PE, PP, PES	Horton *et al*. (2018)
Involved wide and various samples of species (46 species)	ATR-FTIR spectroscopy	Reliable method for identification of microplastics' decomposition	PE, PA, rayon	Pegado *et al*. (2018)
Corbicula fluminea	µ-FTIR and SEM/ EDSspectroscopy	Verification of suspected microplastics	CP, PET, PS, PP and terephthalic acid	Su *et al*. (2016)
Lates niloticus and *Oreochromis niloticus*	ATR-FTIR spectroscopy	Identification of chemical composition of suspected microplastic	PE, PU, PES, PP copolymerand silicone rubber	Biginagwa *et al*. (2016)

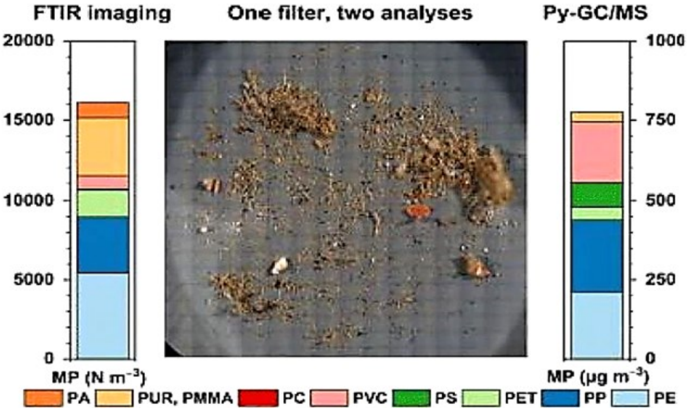

Figure 8. Comparison between GC/MS and FTIR spectroscopy on microplastics analysis (Primpke *et al*., 2020).

5. Adverse location of microplastics contamination

Microplastic contamination has become a worldwide issue. Malaysia has been one of the countries that face similar issues. East bank of Peninsular Malaysia such as Batu Burok Beach and Seberang Takir Beach (Terengganu Beach) have high levels of plastic contamination which is possibly caused by wave flows and tides from the South China Sea pushing the plastic to the shores (Fauziah *et al*., 2015). On the other hand, Asian countries including Indonesia found 20,000 items flowing into the ocean from the Ciliwung River per hour. Items include plastic bags, food wrappings and household waste, spilling into the ocean. The study also discovered that the plastic waste from all streams in Jakarta was 2.1 million kg (Emmerik, 2020). As in Singapore, polyamides (PA), polyethylene (PE), polypropylene (PP) and polyvinyl chloride (PVC) were detected in Singapore coastal mangroves (Hazimah *et al*., 2014). Meanwhile, at China Beibu Gulf coastline, polyethylene (PE), high-density polyethylene (HDPE), low-density polyethylene (LDPE), polyethylene terephthalate (PET), polyethylene vinyl acetate (PEVA), polypropylene (PP) and polystyrene (PS) were detected (Yu *et al*., 2016). Meanwhile, in Taiwan along the Northern Coast, acrylonitrile butadiene styrene (ABS), polyethylene (PE), polypropylene (PP) and polystyrene (PS) were detected (Kunz *et al*., 2016). Furthermore, at South Korea Soya Island beach, microplastics contaminants detected were polyethylene (PE), polypropylene (PP), polystyrene (PS), expanded polystyrene and polyurethane (PU) (Kim *et al*., 2015). Other than Asian countries, in African Great Lakes, microplastic contamination has been reported where a variety of polymers, including polyethylene, polypropylene and silicone rubber were recovered from the gastrointestinal tracts of Nile perch (*Lates niloticus*) and Nile tilapia (*Oreochromis niloticus*) fish from Lake Victoria (Biginagwa *et al*., 2016). In Nigeria, a study has been done which analyzed surface silt from four seashores in Lagos to explore microplastic levels in the Nigerian seaside condition and they found microplastics in all silt tests gathered from the seashores (Ilechukwu, 2019). This was trailed by pellets and afterwards fibres (Ilechukwu, 2019).

6. Adverse effects of microplastic contaminants towards freshwater organisms and human health

6.1 Hazard analysis from chemical and physical properties

The adverse effects of microplastic contamination are related to the chemical that may be incorporated during the transmission of the microplastic into the food chain as the microplastics exert physical and chemical properties associated with them (Wright and Kelly, 2017). Physical contamination is viewed based on the size and shape, including the concentration of microplastics in the freshwater environments which may cause entanglement and ingestion of organisms (Wardrop *et al*., 2016). Despite the report on the entanglement of microplastics in marine species, however, in freshwater species, there is no incidence of this present (Gall and Thompson, 2015). Basically, a plastic polymer is reported as non-toxic because it is not reactive and due to its size, it does not easily pass through the biological membrane (Smith *et al*., 2018). However, non-polymeric compounds such as toxic contaminants, harmful pollutants and the residue of monomers may nevertheless be detrimental to human health and the environment while leaching out from the plastic polymer matrices (Lusher *et al*., 2017). Taking into that account, microplastics also potentially contain hazardous chemicals which are divided into two categories. First, chemicals that originate from microplastics itself such as additives and polymeric raw materials. Plastics could continuously break into smaller particles causing an increase of the surface area to volume ratio and hazardous chemicals are presumably to leach (Teuten *et al*., 2009). These pollutants are feasible to accumulate naturally in seawater ecosystems (Teuten *et al*., 2009). The following category which is more potent where the chemicals which have been absorbed or carried by the surrounding as synthetic contaminants which can be correlated with plastic waste. Furthermore, microplastics are known with the ability to absorb persistent organic pollutants from the water such as polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs) and organochlorine pesticides such as dichloro-diphenyl-trichloroethane (DDT) or 10-hexachlorobenzene (HCB) (Mato *et al*., 2001; Rochman *et al*., 2013).

6.2 Ecotoxicological impacts of microplastics on seawater organisms.

The intensity of the negative effects of microplastics is based on the chemical toxicity exposure, organism vulnerability, the course of action of the subject being exposed and the hazards control (Wang *et al*., 2020). Therefore, the ecotoxicological harm of microplastics on seawater organisms has been modelled by a few studies. Early studies on the adverse effects of the combination of microplastics with organic pollutants have been done using Japanese rice fish or also known as medaka fish, *Oryzias latipes*. Results showed signs of stress and liver damage in the organism (Rochman *et al*., 2013). Other than that, the ingestion of polyethylene in medaka fish has been associated with chemical pollutants which affect the endocrine hormone activity as it changes the expression of genes in the fish (Rochman *et al*., 2014). Moreover, the ingestion of low-density polyethylene (LDPE) microplastics associated with phenanthrene in freshwater juvenile African catfish (*Clarias gariepinus*) had altered the degree of tissue change in the liver, reduced blood levels of high-density lipoprotein (HDL) and affect the synthesis of protein (Karami *et al*., 2016).

The effect of microplastic on fish health has been conducted by Lei *et al*. (2018). The study used zebrafish (*Danio rerio*) as experiment's subject where freshwater fish are exposed to five types of microplastics polymers including polyethylene (PE), polyamides (PA), polypropylene (PP), polystyrene (PS) and polyvinyl chloride (PVC). Results showed that the surviving and dead fishes have different morphologies (Lei *et al*., 2018). Based on the observation, the abdomen of the dead fish became swollen while the surviving fish showed the same overall body morphology as the control. However, after 10 days of exposure to various microplastics such as PA, PE, PVC and PE in pelagic water along with consistent feeding of commercial foods twice a day, the surviving fish have intestinal damage with villi cracking and enterocytes splitting for those exposed to those microplastics. As for surviving fish exposed to PS, it showed no significant difference with control (Lei *et al*., 2018). This indicates accumulation of microplastics may cause intestinal damage based on the polymer of the microplastics (Wright and Kelly, 2017).

A similar finding was reported in another study where the digestive feature of the amphipod crustacean, *H. Azteca* is disrupted due to exposure to two different types of microplastics which are polypropylene fibre and fluorescent polyethylene particles (Au *et al*., 2015). The authors also speculate that the toxicity of polypropylene fibre is higher than the polyethene particles and associated with the prolonged timeframe of the finer in the digestive tract (Au *et al*., 2015). Following that, the egestion rate of polypropylene microplastics in fibre shape is longer compared to polyethylene particles in rounded shape (Karami *et al*., 2016). This study concludes that the sphere or rounded shape of microplastics is easily transported throughout the digestive system compared to the fibrous one and suggests that the arrangement of microplastics during the digestion period probably influences the capability of the organisms to egest the microplastics creating a significant retention time in the gastrointestinal tract (Au *et al*., 2015).

6.3 Adverse impacts of microplastics on human health

Plastics can affect humans both physically and chemically. Plastic-derived contaminants such as polychlorinated biphenyls (PCB) and dichloro-diphenyl-trichloroethane (DDT) are highly toxic industrial compounds which potentially bring harmful to organisms where the chemicals may transfer into animal tissue causing tissue lacerations (Hosomi *et al*., 2012). Regarding the physical effects of microplastics towards human health, long exposure to microplastics could result in biological responses including inflammation, oxidative stress, genotoxicity, necrosis, and apoptosis. If these conditions are sustained, they could cause health issues including tissue damage, carcinogenesis, and fibrosis (Wright and Kelly, 2017). The chemical additives and monomers can pose risks to human health such as reproductive toxicity, carcinogenicity, and mutagenicity as well as phthalates which are one of the most harmful chemical additives which can bind with molecular targets in the body and disrupt hormones (Wright and Kelly, 2017). In contrast, the human body's excretory system discharges microplastics, likely eliminating more than 90 % of ingested micro- and nano-plastics via faeces (Halden, 2010). Factors affecting retention and clearance rates are based on size, shape, additive chemicals, and polymer types of microplastics consumed by humans. The ability of microplastics to accumulate persistent organic pollutants (POPs) inclines concern that microplastics could transfer hazardous POPs to marine organisms and subsequently humans. Nevertheless, further work is required to estimate the dose of chemicals exposed to humans from the ingestion of microplastics in seafood and the related effects, including studies of the supply chain of seafood intake, chemical characterization, and kinetic studies.

7. Suggestions and recommendations for future research

Many studies have already proved the presence of microplastics in seawater organisms. Studies are done mostly on the ingestion of microplastic in the biota focusing on the detection in the gastrointestinal tract (Collard *et al*., 2018). Therefore, it is recommended to carry out research on the microplastic accumulation in the body and organs of the animals as microplastics can also translocate to other parts of the body and other organs as well. In addition, other than using fish as research samples, it is crucial to widen and broaden the type of organisms for future research to expand the knowledge on microplastic consumption at every trophic level. Furthermore, further study is preferred to explore reducing the likelihood of microplastics being transferred through trophic levels until it reaches human consumption to reduce physical and chemical hazards. Moreover, the pathways and behaviour of microplastic being ingested, stored, and eliminated in the organism including the egestion rate of each species involved with plastic contamination also need to be explored for every trophic level. Besides, studies on types, polymers, shapes, and sizes of microplastics that could penetrate the gut and translocate to other tissues and the possible effects on humans should also be conducted. It is speculated that sphere-shaped microplastics may be easier and faster to transmit across, while sharp-edged fragments are more likely to damage the tissues and fibre-like structures are likely to create tangles and block the gut and other systems. The information generated from these studies will be useful in ensuring safe food consumption.

Conflict of interest

The authors declare no conflict of interest.

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