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Ingestion of microplastics in market squid sold for human consumption

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Abstract The ingestion of microplastic in aqutic orgainism has become an emerging environmental issue, with implications for seafood safety. The microplastic presence in domestic squid (*Loligo chinensis*) sold in a fish market in Singhanakorn District, Songkhla Province was investigated. This species was chosen because it is widely consumed and commercially significant done, particularly in the southern Thailand region. The total microplastic concentration in the squid's samples were large *L. chinensis*, 1.22±0.03 n/g (digestive tract weight) and 3.21±0.01 n/individual, medium *L. chinensis*, 1.62±0.09 n/g (digestive tract weight) and 3.22±0.01 n/individual. Most of the microplastics found were fibres (75%) and and fragments (25%) for the large group of microplastics. The medium group of microplastics are found to be higher percentage of fibers (86%) and followed by fragments (24%). The findings suggested that microplastic contamination is occured in one of Thailand's commercial squid species.

Keywords: Microplastics, Squid, *Loligo chinensis*, Ingestion, Contamination

Introduction

Plastic garbage enters the water through unintentional release or indiscriminate disposal, and it is gradually divided into smaller particles through numerous natural processes. such as photodegradation (UV radiation), physical fragmentation, chemical deposition and biological degradation (Browne *et al.*, 2008; Andrady, 2017). Referred to as microplastics, plastic debris in the marine ecosystem can be of various sizes and, have different density, chemical compositions and morphological properties (colour and shape) (Hidalgo-Ruz *et al.*, 2012; Duis and Coors 2016). Microplastics (<5mm in diameter) (Cole *et al.*, 2015; GESAMP, 2015) consisting of microscopic flecks, fibres, fragments and granules and are ubiquitous and have high potential of being ingested by a wide range of marine organisms (Guzzetti *et al.*, 2018). Due to their persistency in the

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marine environment, microplastics have been reported as a threat which can cause detrimental impacts on ecosystems notably for marine organisms (CBD, 2016). In the marine environment, the polymers frequently reported as microplastics are polyethylene terepthalate (PET), polypropylene (PP), polystyrene (PS), polyethylene (PE) and polyvinylchloride (PVC) (Rocha-Santos and Duarte, 2015).

There have been numerous reported on microplastics ingestion in bivalves such as mussels (Browne *et al*., 2008;) and fishes (Bellas *et al*., 2016). Most previous research conducted in Thailand and other contries have studied marine debris, microplastics in sediment, and marine organisms (Pradit *et al*., 2020; Chinfak *et al*., 2021; Jiwarungrueangkul *et al*., 2021; Kalaiselvan *et al.,* 2022). Recently, a few studies have been publishedin Thailand, that describe microplastic ingestion and abundance in marine speciessuch as sessile invertebrates (oyster, striped barnacle, and periwinkle) collected along the eastern Thai coast (Thushari *et al*., 2017), fish samples caught in the southern Gulf of Thailand coastal zone (Azad *et al.*, 2018) and shrimp and fish samples from Songkhla Lagoon, southern Thailand (Pradit *et al.*, 2021). Yet to date, there is only one report (worldwide) on microplastics in cephalopods (Oliveira *et al*., 2020) particularly squid. Since squids possess a different feeding strategy to bivalves, crustaceans or fishes, it is imperative to investigate the ingestion of microplastics in squid through their predatory feeding approach (Roberts *et al*., 2012) as well as their demersal or semi-pelagic habitat (Jackson and David, 1990). From the consumer perspective, knowledge of microplastics in squid could be significant in ascertaining the role as a possible source of microplastic ingestion. Furthermore, squids (*Loligo chinensis*) are one of economically and highly sought after seafood in Thailand especially in southern coastal area. Its high rate of consumption could be a potential route of human exposure to microplastics or other pollutants absorbed by microplastics.

In a general sense, the human health effects depend on the microplastics' concentration exposure rate. Due to a data gap and poor evidence, there is currently no reliable estimate of the amount of microplastics consumed by humans through food.

The presence microplastics in marine organisms such as fish, bivalves, and crustaceans sold for human consumption is now well-known (Smith *et al*., 2018). As an example, the number of microplastics in *Mytilus edulis* and *Mytilus galloprovincialis* of five European countries has changed from 3 to 5 fibres per 10 g mussels (Nelms *et al*., 2016). Hence as proved by the capacity of synthetic particles smaller than 0.15 mm to traverse gastrointestinal epithelium in mammalian bodies, uptake of microplastics in humans is plausible via diet exposure. Scientists speculate that only a lower fraction of 0.3% of the particles that may able to trespass both organs and cellular membrans (Barboza *et al.*, 2018). The discovery of microplastics in human stools revealed that for every 10 g of stool, 20 plastic particles were discovered, primarily made of PE and PP and measuring between 5 and 500 mm in size (Schwabl *et al.*, 2019), confirmed the assumption of microplastics in human at a gastro-intestinal level. The human excretory system is expected to remove up to 90% of ingested plastic particles. (Smith *et al*., 2018).

A market-based survey is a suitable approach to assess microplastics in seafood. This study examined microplastic presence in Loligo chinensis sold at fishing market in Singhanakorn District. It is a popular seafood market in southern Thailand. The objective was to determine the quantity and morphological properties (number, shape, size, and colour) of microplastics in *L. chinensis* and to primarily study of the microplastic contamination in the coast of the southern Gulf of Thailand.

Materials and methods

Two hundred squid individuals were bought from Muang Ngam fishery market located in Singhanakorn District, Songhkla Province, southern Thailand. Based on the information acquired from the fishery market seller, the squids were caught from coastal areas of Singhanakorn and Sathing Phra Districts, as shown in Figure 1. The average length of adult *L. chinensis* range from 7.8 cm. to 37 cm. The samples purchased were then divided into two groups, a large size with length range of 18.6 cm to 37 cm and a medium size with length range of 7.8 cm. to 18.5 cm. Squids were shipped, frozen, and kept in storage at -20°C for further laboratory investigation.

After defrosting, each squid was dissected to remove the digestive system or tract that consists of radula, liver, stomach, mantle artery, and anus. During dissection, extraction, sorting, and visual identification, precautions were taken to avoid contaminating the material. All material and working surfaces were cleaned with alcohol distilled water and a cotton laboratory coat was worn at all times. All procedures were undertaken under a clean fume chamber in a standard laboratory, approved by Prince of Songkla University. After the dissection process, the wet weight of the digestive tract samples was recorded. Subsequently, the digestive tract samples were then digested using alkaline digestion method (Cole *et al.,* 2015) with modifications. Each sample placed in a 250 ml conical flask followed by the addition of 150 ml KOH (10%) solution before sealing the flask using aluminium foil. All samples were left for 12 hours at room temperature to ensure the process of assimilation. Afterwards the conical flasks were then heated on hotplate at 60°C for 12 hours with 2 minutes manual shaking during at 2-hour interval, to insure removal of organic matter. Without cooling down, the digested solutions were then immediately filtered over Whatman GF/F filter (0.7 µm pore size) using a vacuum pump. Before observation under a dissecting microscope, the filters were placed in clean petri dishes to be dried in a hot oven at 60ºC.

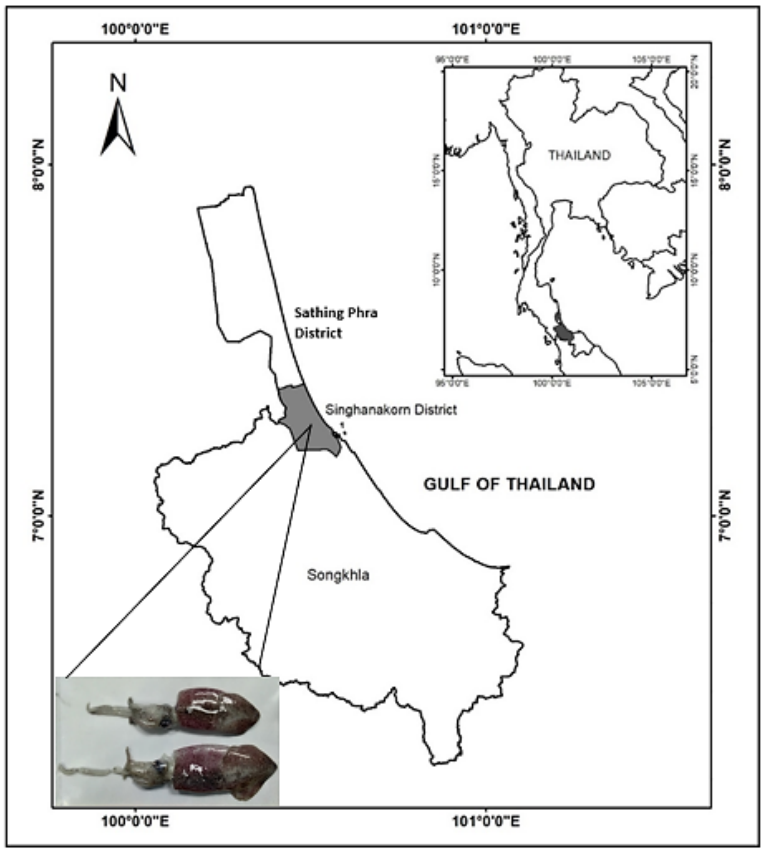

Figure 1. Map of study site in Songkhla Province, Thailand

Items that were suspected to be microplastics under microscope identification were photographed, and then the length was measured. The morphological features were records, including colour (black, blue, and red, white, transparent) and shape (fibre, film, sphere, or fragment). This identification method was done using Olympus SZ, lenses 110AL2X-2 microscope with Canon EOS 600D camera-Microplastics were recognized as products with synthetic polymer characteristics, such as mouldable items with constant thickness and color that did not break when pressed with forceps. Crossvalidation was applied using Fourier Transform Infrared Spectrophotometer (FT-IR).

Results

Microplastics were detected in squid (*Loligo chinensis*) samples sold at Singhanakorn fishery market. The samples were divided into two groups, large and medium squid groups based on their length measurements with 75% microplastic frequency for the large group and 86% for the medium group. The total microplastic concentration in the commercial squids were: large *L. chinensis*, 1.22±0.03 n/g (digestive tract weight) and 3.21±0.01 n/individual, medium *L. chinensis*, 1.62±0.09 n/g (digestive tract weight) and 3.22±0.01 n/individual (Table 1). The microplastics discovered were mainly composed of fibres (75%) followed by fragment (25%) for the large group. The microplastics found in the medium group of squid had higher percentage of fibres (84%) and followed by fragments (16%) (Table 1). A great variety of colours were found, with red (25.8%) the most common, followed by blue (23.9%), black (19.8%), and transparent (17.2%) (Figure 4).

Table 1. Total and concentration of microplastics in *L. chinensis* from Singhanakorn fishery market

Category	Large	Medium
No. of Individuals	100	100
Mean Weight (g)	27.7±1.42	7.45±1.18
Mean Length (cm)	28.9±1.6	8.01±0.05
Mean Weight of Digestive Tract (g)	1.97±1.29	1.71±1.14
Total no. of Microplastics	241 items	277 items
Microplastics Frequency (%)	75	86
Microplastics Shape (%):		
(i) Fibre	75	84
(ii) Fragment	25	16
Microplastics Concentration:		
(i) n/g wet weight	1.22±0.03	1.62±0.09
(ii) n/individual	3.21±0.01	3.22±0.01

The percentages of microplastics presented in squid samples were categorized into size classes and divided into fibres and fragment shape categories. The size of microplastics for fibres in large size squids ranged from 0.12-2.86 mm with the most common size class being 0.5-2 mm, and the least common size class was 2-2.5 mm (Figure 2). For fragments in the large size group, the microplastics found were in the smaller size class, with the most common size class of 0.1-0.5 mm (Figure 2). The size of microplastic particles for fibres in medium size squids ranged from 0.13 to 2.7 mm with the most common size class of 1-1.5 mm, and the least common class 2.5-3 mm (Figure 3). For fragments in the medium size group, the microplastics found were also in the smaller size class, with the most common size class of 0.1-0.5 mm (Figure 3). Since every object discorvered was smaller than 5 mm, it all qualified as microplastic.

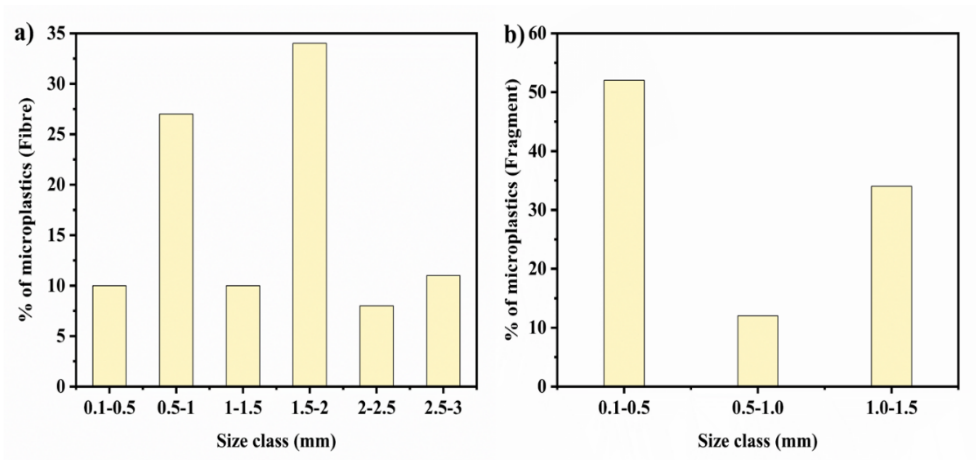

Figure 2. Percentage of microplastics (fibre and fragment) in *L. chinensis* (large size group) according to their size classes

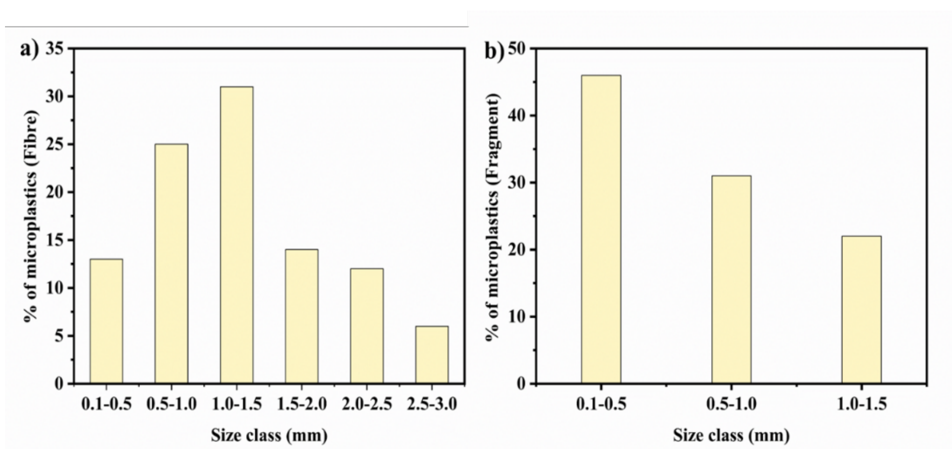

Figure 3. Percentage of microplastics (fibre and fragment) in *L. chinensis* (medium size group) according to their size classes

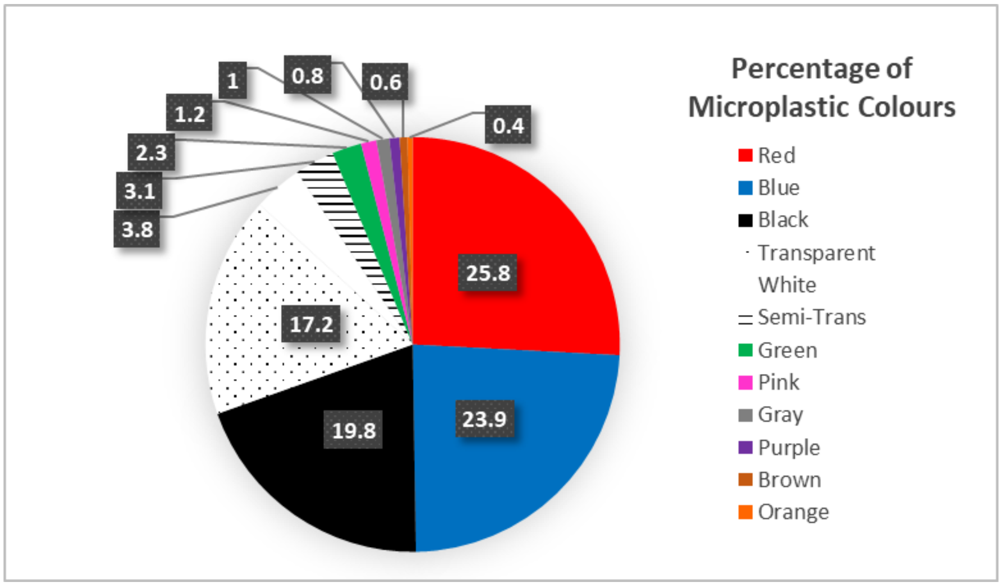

Figure 4. Pie chart showing the microplastic colours detected in *L. chinensis* and their relative frequency

Discussion

Even though the microplastics were not found in all squid sample, their digestive tracts contained two type of shapes and a diverse colour variation. *L. chinensis* are predatory feeders that consume other marine organisms such as fishes and bivalves (Islam *et al.*, 2018). The probability of this squid species indirectly ingesting microplastics through transfer from its source of food is high and they may directly ingest microplastics from the surrounding environment. Trophic transfer is considered as one of the main routes of exposure to microplastics for marine organisms and has been studied in several studies. For example, fluorescent PS (polystyrene) microspheres (0.5 µm) were examined for trophic transfer through a food chain experiment consisting of *C. maenas* and *M. edilus* (Farrell and Nelson, 2013). The *C. maenas* were found ingesting the PS microspheres in their stomach, hepatopancreas, ovary, and gills after they ate and ingested soft tissues of *M. edilus* that were exposed to PS microspheres. Another study used macroalgae (*Fucus vesiculosus*) and periwinkle (*Littorina littorea*) to investigate the trophic transfer of microbeads, microplastic fragments and fibres (Gutow *et al.*, 2015).

The squids sample from the present study had a relatively abundant number of microplastics inside their digestive tract. As mentioned, it is feasible that they ingested microplastics through indirect transfer from their prey or directly from their habitat. A recent study reported a comparison of microplastic presence in digestive gland/tract between wild-caught and cultured cuttlefish, Sepia officinalis (Oliveira *et al.*, 2020). The most predominant microplastics found were fibres (~90% total count) for both wild-caught and cultured cuttlefish. The fibre count/g digestive gland of wild-caught animals was twice that of the cultured cuttlefish; which emphasized on wild cuttlefishes have higher chance of ingesting microplastics from their source of food. Belonging to the same molluscan class cephalopods, in a sense cuttlefish and squid possesses similar digestive system activity. When compared to our research, *L. chinensis* also have more counts of fibres type with 75% for the large size group and 84% for the medium size group. Therefore, there is a potential health implication in the squid health, but this condition may change or be influenced by their active digestive system flow that eventually egests unwanted particles.

Another study focused on microplastics in fishes caught near the coastal area of Sathing Phra district (Azad *et al*., 2018) which was the same location as the caught squid samples in the present study. The fish samples in the previous studya were divided into three groups, demersal fish, pelagic fish, and reef associated fish with an overall mean average length of 9.5±0.1 to 23.5±4.3 cm. Since *L. chinensis* in general eat smaller prey species than their size, this comparison is suitable for large size group with a mean average length of 28.9±1.6 cm. From Azad *et al*., 2018 study, the most common microplastic types found ingested by the fish samples were fibres and fragments with the average size of microplastic ranging from 0.54 to 4.83 mm. In comparison with our study, *L. chinensis* in the large size group ingested microplastics with the size range of 0.12 to 2.86 mm, which is on the smaller side range. This is the probable evidence of indirect translocation of microplastics from their prey (smaller fishes). As for colours, microplastics found in the fishes comprised of various colours with the most predominant transparent (38.2%), black (24.5%) blue (16.5%), brown (16.5%) and red (6%). The microplastic colours found in *L. chinensis* in our study also had similar main colours with red (25.8%), blue (23.9%), black (19.8%) and transparent (17.2%). Similar colours of microplastics may not be the crucial evidence of trophic transfer but it may articulate the situation of both samples for these two studies which came from the same habitat or environment.

Our previous study (Goh *et al*., 2019) reported on microplastics ingestion in green mussels (*Perna viridis*) which were sampled from the same location as our squid samples. One hundred green mussels in the study recorded 1,273 items of microplastics, which were twice the number of microplastics found in two hundred squid samples from our current study with the total amount of 518 items. Microplastic concentrations in the green mussels were 21.10±0.15 n/g (wet weight) and 12.30±0.20 n/individual that were clearly higher than *L. chinensis*. This difference is primarily because green mussels are filter feeders, which is a non-selective feeding strategy that has the ability to consume and accumulate marine polluted particles with poor qualitative values, such as microplastics (Browne *et al*., 2008). This comparison may justify that feeding strategy factor influenced the concentration uptake of microplastics by a specific marine organism.

Through bioaccumulation and biomagnification processes, microplastics, either alone or in conjunction with harmful pollutants, can be transmitted down the food chain, contaminating biotic and abiotic marine products such as seafood and posing possible health concerns to humans (Van Cauwenberghe and Janssen, 2014). Microplastics in the marine ecosystem can absorb harmful chemicals, organic materials, nutrients, and living organisms. This can alter the bioavailability and toxicity of these substances (Galloway *et al*., 2017). Direct adverse effects towards marine organisms may vary due to microplastics ingestion and accumulation. These effects may include blockages of the gut tract that can cause pseudo-satiety sensation and physiological stress, internal and/or external injuries, alteration of feeding, growth retardation and reduction in fertility (Cole *et al*., 2015; Nelms *et al*., 2016).

When discussing these adverse effects, it may affect *L. chinensis* differently as this species has a distinct feeding strategy with an active digestion system (Islam *et al*., 2018). The source of food (fishes, bivalves, shrimps) consumed enters the stomach and digestive system through the mouth and is taken up by cells that line the digestive glands. In the stomach, the food is entirely digested and nutrients that are required are passed into the circulation. Undigested materials are compressed and discharged (egestion capability) through the anus into the mantel cavity (Jereb *et al*., 2010). This is the key point that distinguishes squid from other marine species, in which this particular squid is able to egest microplastics or other undigested particle from its body. Consequently, it becomes evident that foreign particles like microplastics may not permanently stay in the body (stomach/digestive tract) of squid. The liver of the squid produces digestive enzymes, which break down the food and allow the digestive process to take place (Roberts *et al*., 2012; Islam *et al*., 2018). In the context of microplastic contamination squids are most probably one of the safest seafood to consume because the whole digestive system, including the stomach are often cleaned or taken out before consumption by humans.

Surprisingly the microplastic types found were only polyethylene (PE). This may be due to the dilution factor, random sampling technique, and the sampling location. Polyethylene or PE is a common thermoplastic. It is extensively utilized in numerous applications, including food containers and packages containers (Li, 2013). From FTIR spectrum. The FTIR absorption peaks of PE were observed at 2850 and 2920 cm^{-1} assigned to CH_2 symmetric and CH_2 asymmetric vibrations respectively**.** A strong vibration in PE structure is shown at the wave number of 1470 cm^{-1}. The absorption peaks about 1500-1600 cm^{-1} might be a vibration of C=C bond. The strong absorption peak was observed at 1000-1100 cm^{-1} that could be come from contamination in the sample. PE is primarily utilized in the production of HDPE ropes and fishing nets. In the marine industry, this kind of rope is widely utilized for fishing and ship docking operations (Jang *et al*., 2014). Microplastic fibres contamination takes place when ropes are discarded when they are worn out and in time ends up polluting seawater, indirectly affecting marine organisms. The source of microplastic fibres in the squid samples may originate from the fragmentation of these ropes of fishing nets. This condition is evident since small-scale fishing has been the primary livelihood for local fishermen near Songkhla Lagoon and coastal areas of Songkhla Province for many generations (Hue *et al*., 2018).

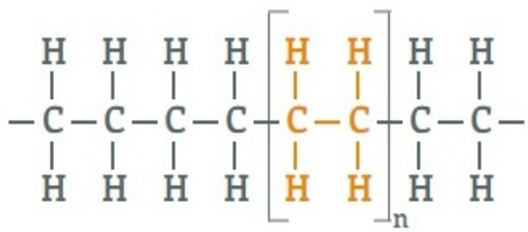

Figure 5. Structure of Polyethylene (PE)

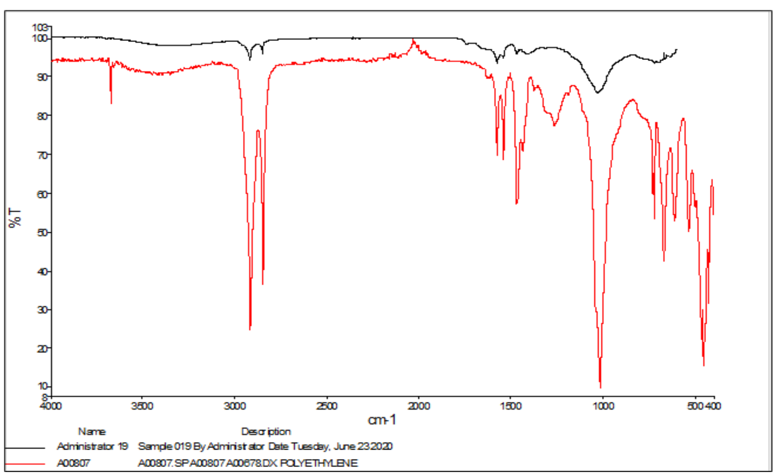

Figure 6. Microplastics polymer type polyethylene (PE) detected using FTIR

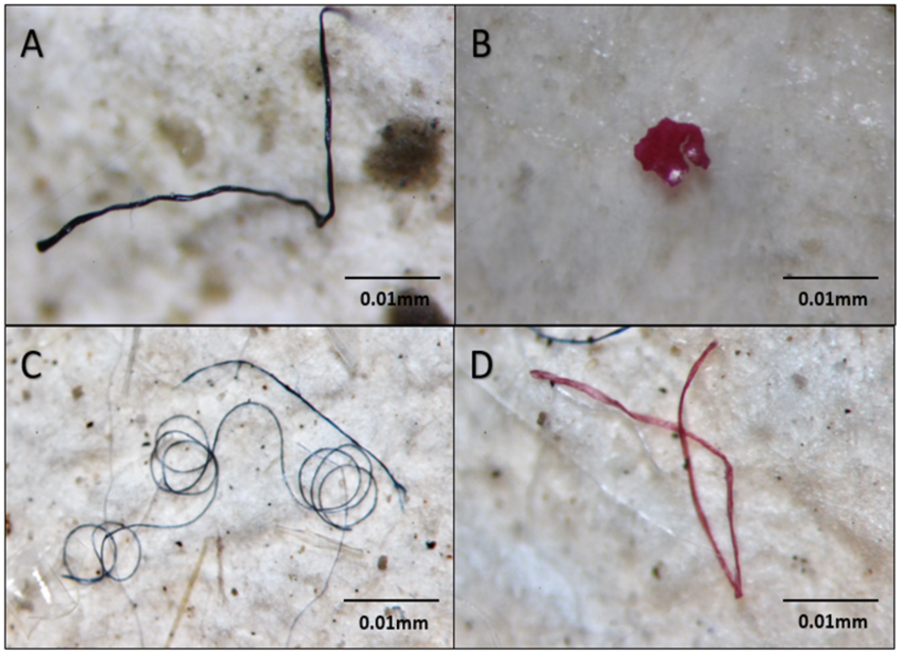

Figure 7. Examples of microplastics found in digestive system of *L. chinensis*. Common plastic fibres (A, C, D) and plastic fragment (B)

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