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Abundance and Characteristics of Microplastics in Gastropods and Lotus Basin Water at Kasetsart University, Kamphaeng Saen Campus, Thailand

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Abstract

Microplastics (MPs) are widely distributed in nature due to the proliferation of plastic waste. However, their presence in lotus basins remains largely unknown compared to other aquatic bodies. This study investigates MP contamination in water and gastropods from 33 lotus basins at Kasetsart University, Kamphaeng Saen Campus, Thailand. Water and snail tissue samples were digested using 30% hydrogen peroxide. A total of 5,047 and 1,650 MP items were identified in water and snail tissue, respectively. The majority of MPs were smaller than 100 µm. The most common shapes and colors were fragments and violet. A two-way ANOVA revealed a significant difference ($p \leq 0.01$) in MP levels between basins and snail species. Fourier transform infrared spectroscopy identified 38 polymer types, with cellulose acetate and styrene acrylonitrile being the most prevalent. No correlation ($p > 0.05$) was found between snail tissue weight and MP abundance. These findings suggest that MPs in lotus basins may serve as a bioindicator of microplastic pollution in freshwater ecosystems, particularly surface water.

Introduction

The non-biodegradable nature of plastic, coupled with its mass production and improper disposal, has significant environmental consequences, leading to its accumulation in natural ecosystems (Schwarz et al., 2019; Yan et al., 2021). Over time, physical abrasion, water movement, photodegradation, and other environmental processes fragment this accumulated plastic, generating microplastics (MPs) (Barnes et al., 2009; Hale et al., 2020). Plastic particles of sizes between 0.1 µm and 5 mm are referred to as microplastics (Thompson et al., 2004). GESAMP (2016) states that MPs are classified as secondary when they are separated from larger plastic products and as primary when they are produced in small sizes. Environmental sampling from seawaters and rivers has revealed the presence of MPs, which are ingested by a wide range of species, including plankton and various vertebrates (De Sá et al., 2018; Borriello et al., 2022; de Carvalho et al., 2022). Aquatic species can ingest MPs through respiration, eating, or predation, leading to digestive system damage, malnutrition, oxidative stress, and other effects (Mkuye et al., 2022; Yücel & Kılıç, 2023). MPs are well documented to cause organ damage

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and oxidative stress in a variety of species (Kim et al., 2021; Qiao et al., 2019).

Bellasi et al. (2020) noted that a significant amount of microplastics in the digestive tracts of several freshwater species reduces fecundity, reproduction, survival rate, and food intake. Polystyrene microplastics caused neurotoxicity, digestive enzyme changes, and an oxidative state shift in the edible mollusks *Mactra veneriformis, Bullacta exarate,* and *Cyclina sinensis* (Wang et al., 2023). The freshwater gastropod snail *Pomacea paludosa* suffers significant harm to its digestive gland cells and impairment of its antioxidant system when it consumes polypropylene microplastics (Jeyavani et al., 2022). Microplastics (MPs) have been shown to exert harmful effects in previous studies; however, research on their impact on freshwater mollusks in Thailand remains limited (Khamboonruang et al., 2024).

Mollusks can accumulate MPs by ingesting them and serve as effective bioindicators (Su et al., 2018; Liu et al., 2021). *Indoplanorbis exustus* is a commonly found freshwater gastropod mollusk that feeds on algae and plankton in small ponds, pools, and lakes (Saijuntha et al., 2021). Sarkar et al. (2021) found that MPs were isolated from *I. exustus'* GI tract in freshwater wetland environments. This shows that snails ingest MPs from contaminated water sources. Four distinct snails from two rivers in West Africa have been found to contain microplastics in the form of films and fibers (Akindele et al., 2019). The majority of the MPs in *Bellamya aeruginosa* were found as polyethylene and polypropylene and ranged in size from 10 to 100 μm (An et al., 2021). MPs with a maximum feeding rate of 10 μm to 90 μm were consumed by *Physella acuta* (Scherer et al., 2017).

This study examines the abundance and types of MPs (morphotype, size, and color) in surface water and gastropod tissue collected from lotus basins at Kasetsart University, Kampkaeng Saen Campus, Nakhon Pathom, Thailand. We also investigate the polymer type and concentration of MPs in water and snail tissue. Urbanization and the unintended discharge of plastic garbage are increasingly damaging and degrading freshwater bodies. This study aims to enhance public awareness and advocate for governmental implementation of preventive measures and mitigation strategies to address microplastic pollution.

Materials and methods

1. Sampling and sample analysis

A field survey was conducted in July 2023 at Kasetsart University, Kaamphaeng Saen Campus, Nakhon Pathom Province, Central Thailand (N 14°01.280', E 099°59.427') (Fig.1), in 33 lotus basins that are separated from one another. The lotus basin was contaminated by pollutants from a variety of sources, including basin water fill, roadside operations, and tire wear particles. Each basin was sampled for approximately thirty similar-sized gastropod species that dwell on lotus leaves, basin walls, and bottoms. The samples were placed in glass bottles, transported to the laboratory, and frozen. To avoid collecting suspended particles from the bottoms of the collection locations, water samples were collected before gastropods were collected. The collection consisted of approximately 1.0 L of water directly from the basins using glass drinking containers.

In the laboratory, each individual sample was weighed (g), and then the entire soft tissue was extracted and weighed (ww). Each gastropod species' soft tissues were pooled to form a replica (three for each basin). Ten specimens of each snail species' soft tissues were combined to create a duplicate (3 for each basin).

2. Isolation of microplastics

Each water sample was placed in a 1000-mL beaker, then 20 mL of 30% hydrogen peroxide (H_2O_2) was added to the water and left for at least 24 h to break down biogenic material. After digestion, the residual water samples were filtered using a nylon membrane filter (Whatman, Kent, UK; pore size: 0.45 μm; 47 mm diameter) with a vacuum pump. Following filtering, the filter papers were dried at 50°C for 2 days and placed in glass Petri dishes for microscopic examination.

Microplastics were extracted from gastropods based on earlier studies by Su et al. (2016) and Li et al. (2015). Briefly, the soft tissue of the snail was weighed. The weight of soft tissue was used in the current investigation to determine the quantity of microplastics in gastropods. Three gastropod replicates were taken out and examined. To break down soft tissue, 50 mL of 30% hydrogen peroxide was added to a 100 mL flask containing the gastropods. The flask was then covered with aluminum foil and heated at 60°C in a shaken water bath at 150 rpm for 3 h or until all of the soft tissue was digested. After digestion, the liquid in the flask was filtered, and the filter was covered and stored in dry Petri dishes for further observation.

Fig. 1 The sampling site of the lotus basin is in Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom Province, Thailand (N 14°01.280', E 099°59.427'), covering both the environment and gastropods

3. Quality control of experiments

The containers and sample instruments were cleaned with filtered tap water (pore size 0.45 µm). To keep the instrument clean prior to use, they were packaged in an aluminum foil bag. To prevent contamination, the sample tools were prewashed in place with water. To correct and examine background contamination, blanks were run in the laboratory without any water or gastropod samples.

4. Observation and validation of microplastic

The suspect plastic particles on the filters were examined, and pictures were taken with a microscope (Leica EZ4E) at 35x magnification. We employed visual assessments to measure and identify suspected microplastics based on their properties (Yang et al. 2015). Su et al. (2016) identified four categories of microplastics in water and gastropod samples: fiber, rod, spherical, and fragments. The color and size of the microplastics were also measured and recorded using visual criteria.

Of the 5620 particles, 582 were selected for confirmation using a Hyperion 2000 FT-IR microscope equipped with a mercury-cadmium telluride detector (Bruker Daltonik, Billerica, MA, USA). The polymer composition was evaluated using an FT-IR with attenuated total reflection mode (Bruker). Data was collected at 4 cm^{-1} resolution with a scan length of 32 sec. The polymer type was determined by comparing all of the spectra to a Bruker database. Woodall et al. (2014) certified spectra with a quality value of >70. Finally, the number of microplastics reported was modified to remove verified non-plastic objects.

5. Data analysis

The size, color, and shape of microplastics discovered in water and gastropods in each replication were documented. Non-parametric statistics (Kruskal-Wallis H Test) were used to determine the differences in microplastic levels found in each type of gastropod tissue. A two-way ANOVA of the amount of microplastics discovered in each lotus basin and species of gastropod tissue was carried out. The statistical data were examined with the SPSS 22.0 (Statistical Package for the Social Sciences) software.

Results and discussion

1. Microplastic abundance profiles in surface water and gastropods in lotus basins

A total of 5,047 MP items were found in 33 lotus basins, with an average of 152.94 items/L. Surface water samples from all 33 lotus basins showed a diverse distribution of microplastic. The abundances ranged between 12.0±3.0 particles/L (19th lotus basin) and 173.3±78.1 particles/L (28th lotus basin) (Fig. 2). The amount of microplastics in the environment is influenced by weather, surrounding conditions, and human activity (Kim et al., 2015). Human activity near the study site lotus basins may have contributed to an increase in microplastic contamination. Microplastics in

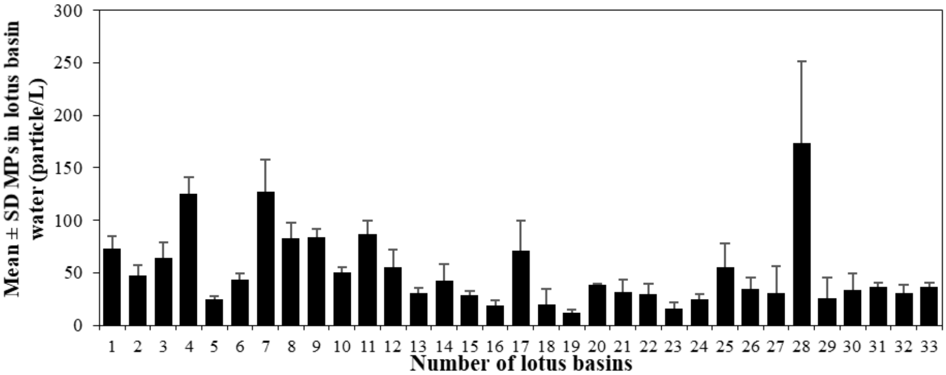

Fig. 2 The average number of microplastics discovered in water samples collected from 33 lotus basins

road dust include plastic litter, road particles, and tire wear particles. Wind, drainage, and human activity all contribute to the spread of microplastics along roadsides (Premarathna et al., 2025).

A total of 1,143 gastropods were investigated, including 287 *Indoplanorbis exustus,* 776 *Radix (Lymnaea) rubiginosa,* and 80 *Pomacea canaliculata* (Table 1). There were 1,650 MP items among the 1,143 gastropods (Table 1).

The average concentration of MPs (Fig. 3) in *Indoplanorbis exustus, Radix (Lymnaea) rubiginosa,* and *Pomacea canaliculata* were 4.43±3.96 items/individual, 2.19±2.87 items/individual, and 2.36±2.53 items/individual, respectively (Table 1).

The number of microplastics in each gastropod's wet weight tissue varied significantly (Chi-Square = 15.469, *df* = 2; Asymp. Sig. = 0.000), but the amount of microplastics per g individual did not differ significantly (Chi-Square = 4.541, *df* = 2; Asymp. Sig. = 0.103) (Table 1). Akindele et al. (2019) found that larger gastropod species (*L. varicus* and *M. tuberculata*) had significantly higher MP loads per individual than *T. fluviatilis*. The snails' body size and dietary (physiological) requirements may have altered the rate of MP uptake in each species.

Table 1 A summary of the microplastics identified in the wet weight tissues of 3 different snail species

Species	n	Total wet weight (g)	Total MPs (item)	MP items/ individual	MP items/ gram
Indoplanorbis exustus (IE)	287	16.06	573	4.43±3.96	106.70±146.33
Radix (Lymnaea) rubiginosa (RX)	776	27.77	1012	2.19±2.87	112.47±194.13
Pomacea canaliculata (PC)	80	7.10	65	2.36±2.53	34.80±35.82
Chi-Square				15.49	4.541
df				2	2
Asymp. Sig.				0.000	0.103

Several factors influence MP accumulation in gastropods, including the habitat (lotus basin) and species. The twoway analysis of variance (ANOVA) demonstrated that MP concentration in gastropods influenced both species and study sites (lotus basin) (p = 0.000) (Table 2).

Table 2 The results of the two-way ANOVA reveal the relationships between the lotus basins, gastropod species, and the number of MPs accumulated in the gastropods

Source of variation	Sum of squares	*df*	Mean square	F	Sig.
Lotus basins	4088.497	32	127.766	5.309	0.000
Species	88.606	2	44.303	1.841	0.165
Lotus basins * Species	1121.866	11	101.988	4.238	0.000
Error	2189.833	91	24.064		
Total	27576.000	137			

It is reasonable to believe that microplastics in water harm benthic filter feeders and deposit feeders. The *Indoplanorbis exustus, Pomacea canaliculata,* and *Radix* (*Lymnaea*) *rubiginosa* have distinct feeding behaviors from other freshwater snail species. They are invasive species that consumes both water and land (Gilioli et al., 2017; Sun et al., 2012). Depending on their environment, they can consume a wide range of aquatic plants, including seaweed, morning glory, water mimosa, *Azolla* spp., water lettuce, chufa, leaf blade, rice sprouts, and even rotting carcasses in the water. As far as we are aware, this is the first in situ study of microplastics in water and gastropods in a small freshwater area (lotus basin). Overall, we found that each

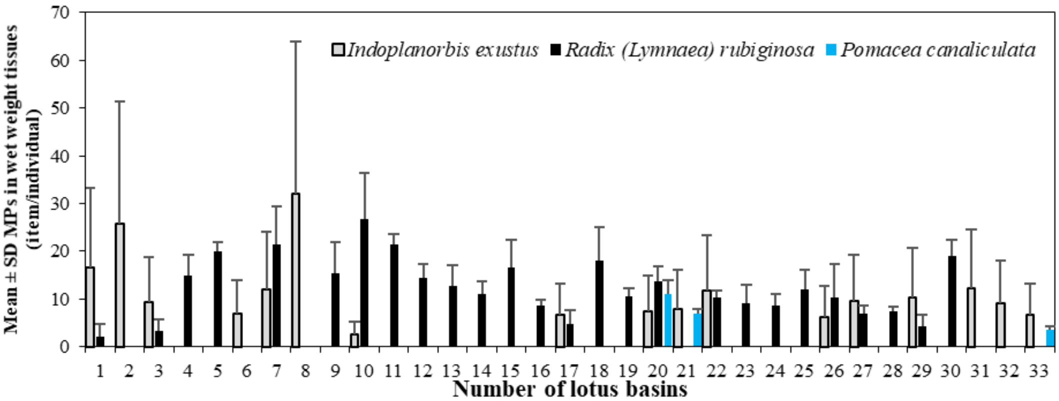

Fig. 3 The average number of microplastics discovered in the wet weight gastropod species collected from 33 lotus basins.

lotus basin's water samples and individual gastropods were contaminated with microplastic. The difference in the level of microplastic in water was reflected in the variation in gastropod microplastic concentrations. The snail is a good bioindicator of microplastic contaminants on a broad scale because it is easy to collect in a range of freshwater systems, and the fluctuation in its microplastic content corresponds to the surrounding environment. All gastropods had microplastics in their bodies. This level of microplastic presence (100%) is far higher than that reported for Asian clams from Taihu Lake, China (Su et al., 2018). The prevalence of microplastic contamination in gastropods is equivalent to that observed in Asian clams, allowing comparisons between freshwater habitats (Su et al., 2018). Furthermore, the significant level of microplastic contamination in gastropods suggests that freshwater systems are contaminated, and microplastics may infiltrate the food chain, especially since humans consume *P. canaliculata.* The levels of microplastic contamination discovered in water during this experiment were consistent with those reported in worldwide freshwater environments (Horton et al., 2017).

2. Microplastic size, shape, and color profiles in surface water and gastropods from the lotus basins

In the lotus basins, the sizes of the suspected microplastic items were classified into four categories: <100 µm, 100–500 µm, 500 µm–1 mm, and >1 mm. In the water, the majority of microplastics (59.26%) were less than 100 µm, followed by 100–500 µm (30.5%), 500 µm–1 mm (6.2%), and >1 mm (4.04%) (Fig. 4). This consistency with the majority of MPs in gastropod species was less than 100 µm (56.5%), followed by 100–500 µm (28.1%), 500 µm-1 mm (7.9%), and>1 mm (7.5%) (Fig. 5). The percentage of small-sized (<100 µm) microplastics in gastropods was similar in size to those in water. This finding may raise concern about the environmental dangers of smaller-sized plastic particles, which are more likely to be ingested by species and pose a greater risk (Besseling et al., 2014; Bergami et al., 2016).

Wang et al. (2021) and Barboza et al. (2018) support these findings, stating that smaller MPs are more frequent in aquatic environments due to their capacity to be suspended and transported by water flow. These particles,

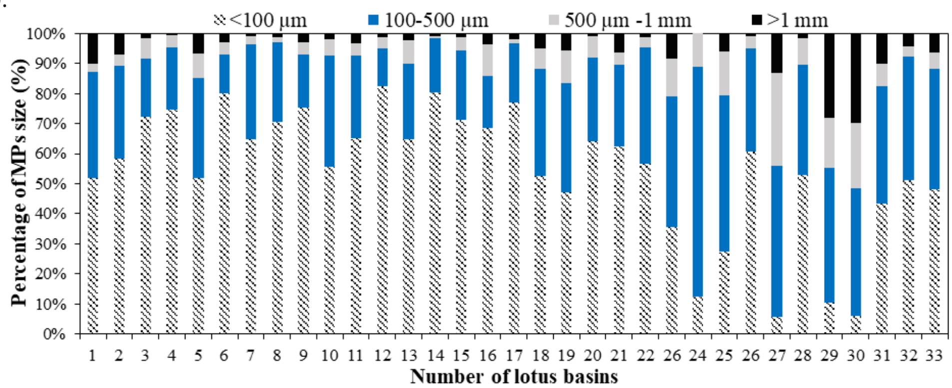

Fig. 4 MPs size in surface water in each lotus basin

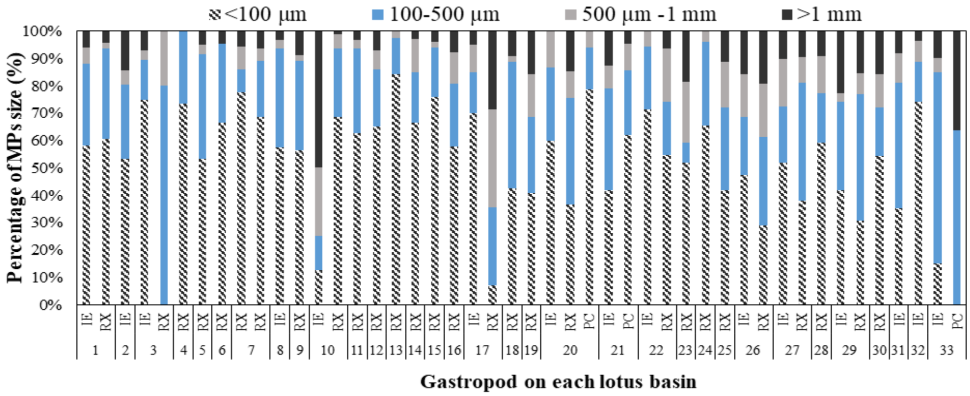

Fig. 5 MPs size in gastropod species in each lotus basin

which are generally smaller than 5 mm, have a high surface area-to-volume ratio and low density, allowing them to remain suspended for long periods of time (Guo et al., 2024). MPs' mobility and dispersion in aquatic environments are influenced by size, density, and environmental circumstances, resulting in their widespread prevalence in water bodies (Huang et al., 2024).

Fragments (70%), fibers (17.6%), lines (1.39%), spheres (1.13%), and films (0.1%) were among the MPs discovered (Figs. 6–7). Mechanical and chemical deterioration are the most typical sources of fragment generation. Fragments could appear from widely made plastic products such as containers, packing materials, and cleaning media (Zhang et al., 2015). The quantity of these plastics may appear based on irresponsible garbage disposal, which is a major issue in Thailand, particularly in densely populated areas. Fiber shedding from artificial textiles is a major source of microplastics in aquatic environments (Chen et al., 2023). We noticed that snails retain fibers more efficiently (Khamboonruang et al., 2024). These patterns could explain the widespread occurrence of fibers in all samples.

The samples showed ten distinct colors, with violet accounting for the highest percentage (76.4%). The other colors observed in the samples were blue (13.8%), transparent (3%), brown (2.7%), pink (2.5%), green (0.7%), orange (0.5%), black (0.3%), yellow (0.2%), and red (0.1%) (Figs. 8–9). In this study, the color patterns of microplastics in gastropods matched those found in the water. Violet, blue, and transparent microplastic items commonly found in gastropods have been reported in field studies of Asian clams (Su et al., 2018) and golden apple snails by Khamboonruang et al. (2024), who discovered that the highest prevalent items had colors of transparent and blue, and they predominated in all color classes in both sediment and water.

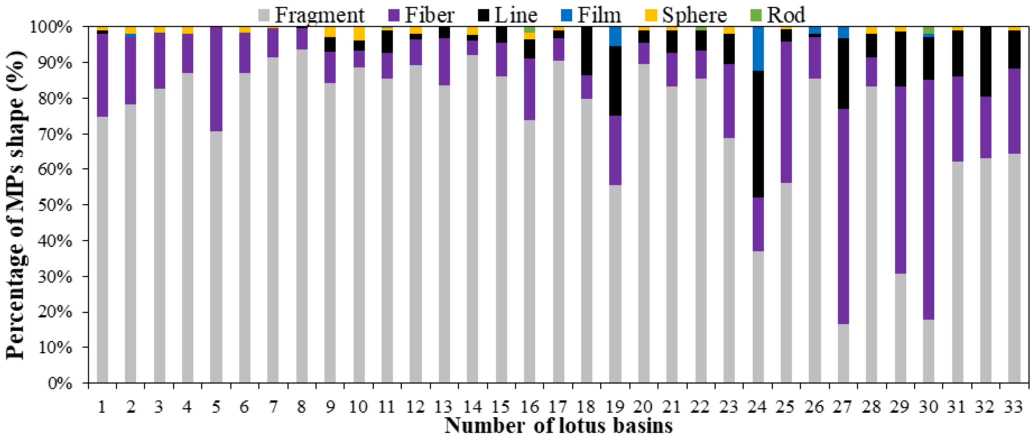

Fig. 6 MPs shape in surface water in each lotus basin

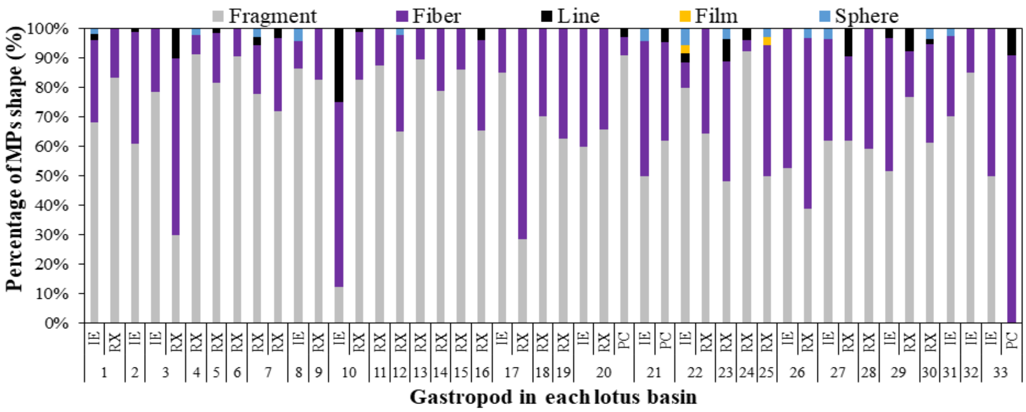

Fig. 7 MPs shape in gastropod species in each lotus basin

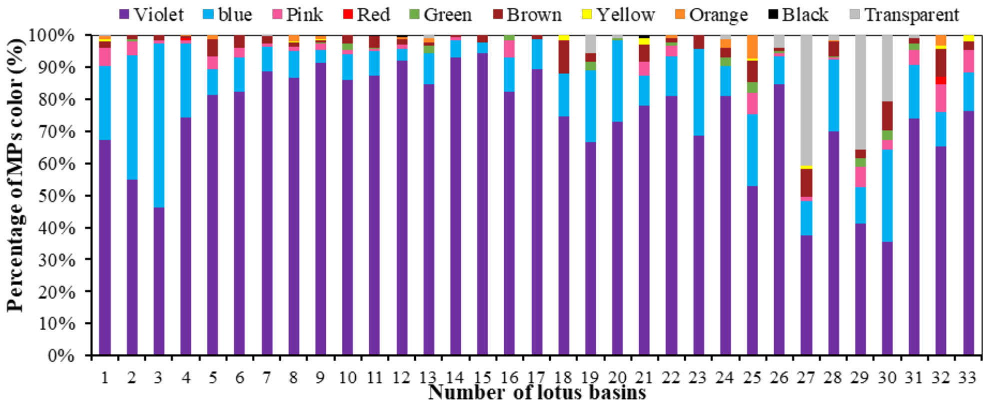

Fig. 8 MPs color in surface water in each lotus basin

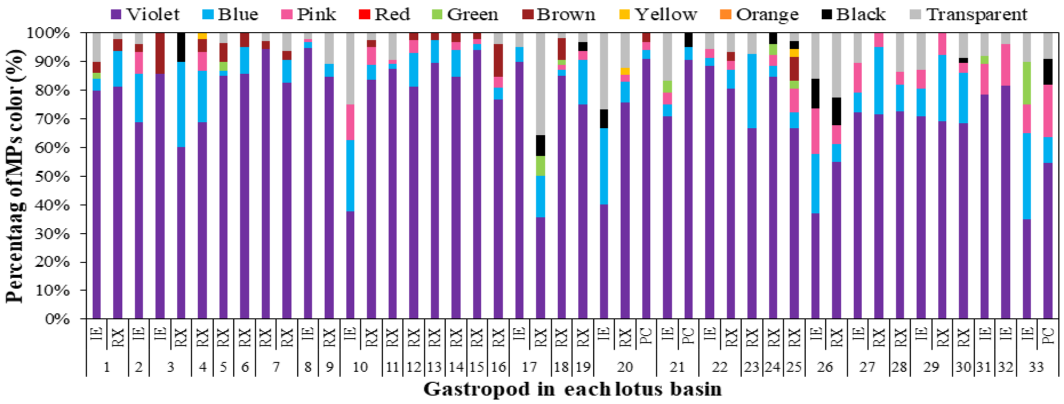

Fig. 9 MPs color in gastropod species in each lotus basin

Microplastics are mostly created through the gradual disintegration of larger plastic trash bits. The cube law idea is applicable to smaller particle sizes (Cózar et al., 2014). Large plastic pieces continue to degrade into smaller fragments. Over time, the number of smaller fragments increases. The number of microplastic fragments found in surroundings is related to their size. Microplastics are defined as particles measuring less than 5 mm in size. Eo et al. (2019) found that small microplastic objects (20-1000 µm) were over 30 times more abundant than large microplastic objects (1-5 mm) on South Korean sandy beaches. Enders et al. (2015) discovered that the number of microplastic particles bigger than 10 µm between the European Coast and the North Atlantic Subtropical Gyre decreased with time, leading to smaller particles and fibers. Previous studies (Cózar et al., 2014; Isobe et al., 2014) reported a considerable decrease in microplastic abundance on sea surfaces around 100 µm in size. Microplastic particles seep into sediment, increasing the rate of ingestion by benthic species. The presence of microscopic microplastic particles (<100 µm) in gastropods (56.5%) indicates that ingestion rates vary according to particle size. Gastropods' olfactory and taste senses let them distinguish between indigestible particles and appetizing food. However, gastropods are unable to filter the minute microplastic fragments that stick to the food in the aggregates they feed (Gutow et al., 2016). Small microplastic fragments may be digested by snails. They may degrade the larger microplastic fragments they feed into smaller ones. Microfragments were discovered in the stomachs and guts of common periwinkles (Littorina littorea) throughout feeding. They were not found in gastropods' midgut glands, the principal digestive organs (Gutow et al., 2016). As a result, marine snails break down microfragments even further. Dawson et al. (2018) discovered that Antarctic krill (Euphausia superba) can break microplastics 31.5 µm into particles <1 µm. Future study should focus on understanding the biological mechanisms underlying microplastic breakdown and disintegration in organisms.

3. Microplastic polymer profiles of surface water and gastropods from the lotus basins

Twenty-six polymers were found in the surface water samples (Table 3). Cellulose acetate (CA) predominated over the other polymers discovered in this investigation, followed by styrene acrylonitrile (SAN), styrene allyl alcohol (SAA), glycerol triacetate, styrene butadiene (SBR), poly (vinyl propionate), polystyrene (PS), N-butyl acetyl ricinoleate, and polyethylene (PE). Eight polymers were also identified in the gastropods, including cellulose acetate, cellulose propionate, glycerol triacetate, poly (1,4-Cyclohexanedimethyleneterephthalate), polyvinyl acetate, triethyl citrate, vinyl alcohol, and vinyl chloride. The majority of the polymers found in microplastic particles in this study were also found in an earlier study on *Pomecea canaliculata* tissue in a flood-prone lowland area (Khamboonruang et al., 2024). Runoff during the July rainy season increases the number of polymer types as well as the amount of microplastic. The presence of microplastics in gastropods indicates that microplastics have been found in the water (Vitheepradit et al., 2024).

The polymer type, cellulose acetate, which was more abundant in water and gastropod species than other polymers discovered in this study, was the cigarette filters, also known as "butts," one of the world's most prevalent litter items (Green et al., 2020). Cigarette filter microplastics are made of cellulose acetate, which cannot disintegrate biologically or photochemically. These microplastics are widely distributed and can be found in water, soil, and air. These fibers have a high absorption capacity, allowing them to collect and hold pollutants such as dangerous compounds (Soltani et al., 2025). A freshwater mesocosm experiment was conducted to assess the impact of leaching from smoked cellulose acetate and smoked cellulose filters, across a range of concentrations (0, 0.2, 1, and 5 butts/L), on the mortality and behavior of four freshwater invertebrate species (*Dreissena polymorpha, Polycelis nigra,*

Table 3 Polymer type distribution in water and gastropod species in the lotus basin

Polymer type/samples	Water	*Indoplanorbis exustus*	*Radix (Lymnaea) rubiginosa*	*Pomacea canaliculata*	Total
Cellulose acetate	339	49	72	3	470
Cellulose acetate butyrate	2				2
Cellulose propionate	7	1	3		11
Polyethylene	4				4
Ethylene propylene diene	1				1
Glycerol triacetate	18	2			20
Methyl acetyl ricinoleate	8				11
n-Butyl acetyl ricinoleate	7				7
N-Ethyl-p-toluenesulfonamide	2				2
Poly (1,4-butylene terephthalate)			1		1
Poly (1,4-Cyclohexane dimethyleneterephthalate)	3		1		4
Poly(4-methyl-1-pentene)				1	1
Poly(acrylamide)	2				2
Poly(dimethylamine-coepichlorohydrin)	3				3
Polyvinyl acetate	5	2	1	1	9
Polyvinyl alcohol	3				3
Poly (vinyl propionate)	7				7
Poly (vinyl pyrrolidone)	3				3
Polystyrene	6				6
Propylene glycol monostearate	1				1
Styrene/acrylonitrile copolymer	55				55
Styrene/allyl alcohol copolymer	31				31
Styrene/butadiene copolymer	10				10
Triethyl citrate	1			1	2
Vinyl alcohol	2	1	3		6
Vinyl chloride	1	1			2

Planorbis planorbis, and *Bithynia tentaculata*). Leachate from 5 butts/L of either type of filter resulted in 60-100% mortality for all species within 5 days. Adults exposed to 1 butt/L leachate of either type were less active compared to those exposed to no leachate or 0.2 butt/L. Cigarette butts, regardless of their perceived biodegradability, can cause mortality and reduced activity in critical freshwater invertebrates and should always be disposed of appropriately (Green et al., 2020).

In terms of polymer types, two low-density polymers were recognized in water but not in gastropod tissue: polyethylene (PE) and polystyrene (PS). The production and consumption of PE and PS in Thailand is reasonable. PS appears in foam items, plastic cups, protective packaging, bottles, and food containers. PE is a raw material used in bottles, homeware, pipes, and toys. As a result, these polymer types are widely distributed throughout the aquatic environment. Furthermore, our research discovered evidence of a non-polymer natural fabric. Under a stereo microscope, these non-polymer items can be difficult to distinguish from polymers and may be mistaken for microplastics. The FTIR spectra of representative microplastics from the water and gastropods, along with the shapes of selected microplastics under the FTIR microscope, are shown in Fig. 10. As a result, these polymer types are broadly distributed across the aquatic ecosystem. Furthermore, our research revealed evidence of a non-polymer natural fabric. Under a stereo microscope, these non-polymer objects can be difficult to distinguish from polymers and may be mistaken for microplastics. Fig.10 displays the FTIR spectra of representative microplastics from water and gastropods, as well as the shapes of selected microplastics seen through an FTIR microscope.

Multiple styrene copolymers have been identified, including styrene/acrylonitrile copolymers, styrene/allyl alcohol copolymers, and styrene/butadiene copolymers. Styrene-butadiene-isoprene copolymer is an elastomer often found in rubber tires, while poly (styreneallyl alcohol) is used in surface coatings and paint applications (Zhang et al., 2015). The presence of poly (styrene-co-allyl alcohol) in the particle may indicate that the microplastic's surface has been coated with another polymer.

More than 5000 synthetic polymers are produced globally (Wagner & Lambert, 2018). In theory, they should produce more than 5000 different forms of microplastic polymers that can be recognized in various conditions. In practice, however, it is practically impossible to distinguish between all polymer types of microplastics in complicated matrices. It is also nearly impossible to separate and evaluate every type of ambient microplastic. However, certain types of microplastic particles made of common polymers can

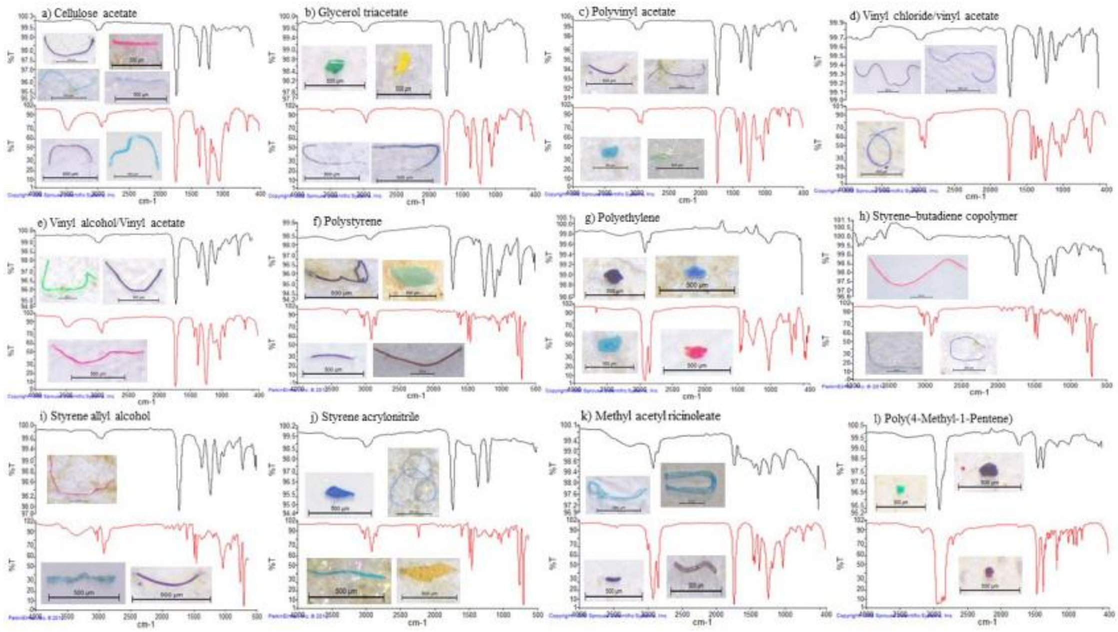

Fig. 10 FTIR spectra of microplastics from water and gastropod species in a lotus basin, as well as their morphology under an FTIR microscope

be monitored and identified. As a result, CA, CP, and polyvinyl acetate (PVA) may be indicated as promising prospective indicators of microplastic contamination in both abiotic and biotic environmental compartments, as they were discovered in both water and snails in the current investigation.

Conclusion

This study represents the first investigation of microplastic contamination in water and gastropod species from a lotus basin, a small reservoir where microplastics may be present. Microplastic particles smaller than 100 μm predominated in both biotic and abiotic samples. Within these environments, microplastic pollution in gastropods reflected the broader distribution of microplastics in water, with comparable abundance, size distribution, and color patterns. Based on these findings, we propose the use of gastropod species as bioindicators of microplastic pollution in freshwater ecosystems, particularly surface water.

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