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SPECIALTY SECTION

This article was submitted to Marine Pollution, a section of the journal Frontiers in Marine Science

RECEIVED 09 June 2022 ACCEPTED 13 January 2023 PUBLISHED 01 February 2023

CITATION

Zhang Q, Xie J, Ma S, Chen Y, Lin F and Diao X (2023) Occurrenceand characteristics of microplastics in benthic species from mangrove wetlands of Hainan, South China. *Front. Mar. Sci.* 10:965059. doi: 10.3389/fmars.2023.965059

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Occurrenceand characteristics of microplastics in benthic species from mangrove wetlands of Hainan, South China

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Microplastics (MPs) are emerging contaminants that pose a global threat to the environment. Mangrove ecosystems, which contribute to biogeochemical cycles, are vulnerable to various anthropogenic disturbances and chemical pollutants. In this study, the abundance and the characteristics of MPs were investigated in 10 species of benthic organisms, including crabs, bivalves, and snails, from seven typical mangrove distribution areas, with a total of 15 sampling sites in Hainan, South China. The abundance of MPs in each sampling site ranged between 0.83 + 1.32 and 12.00 + 0.00 items/individual, with an average of 3.90 + 3.31 items/ individual, while the abundance of MPs varied between 0.17 and 2.00 items/individual for the different species. Fibers (80.13%) were the most abundant MPs, most of the MPs were brown (37.18%) or blue (26.64%), and more than 80% were small-sized plastic (<2 mm). Raman analysis showed that polypropylene (94.44%) was the most dominant type of polymer. In addition, crabs (with an average abundance of 1.10 ± 0.59 items/ individual) showed a higher potential for accumulating MPs than the other species investigated in the present study. This study shows that MPs are widely distributed throughout benthic species in the mangrove wetlands of Hainan.

KEYWORDS

microplastics, mangrove wetland, invertebrates, contaminates, Hainan Island

1 Introduction

Microplastic (MP; particles <5 mm) pollution is one of the emerging threats to both aquatic and terrestrial ecosystems, even in polar regions, due to their long-distance migration in the environment (Wright et al., 2013; de Souza Machado et al., 2018). Their widespread presence in different environmental media, including atmospheric (Cai et al., 2017; Liu et al., 2019), terrestrial (He et al., 2018; Zhou et al., 2018; Corradini et al., 2019; Chia et al., 2021), freshwater (Wang et al., 2018; Caradini et al., 2019; Chia et al., 2021), freshwater (Wang et al., 2018; Caradini et al., 2019; Chia et al., 2021), freshwater (Wang et al., 2018; Caradini et al., 2019; Chia et al., 2021), freshwater (Wang et al., 2018; Caradini et al., 2019; Chia et al., 2021), freshwater (Wang et al., 2018; Caradini et al., 2019; Chia et al., 2021), freshwater (Wang et al., 2018; Caradini et al., 2019; Chia et al., 2021), freshwater (Wang et al., 2018; Caradini et al., 2019; Chia et al., 2021), freshwater (Wang et al., 2018; Caradini et al., 2019; Chia et al., 2021), freshwater (Wang et al., 2018; Caradini et al., 2019; Chia et al., 2021), freshwater (Wang et al., 2018; Caradini et al., 2019; Chia et al., 2021), freshwater (Wang et al., 2018; Caradini et al., 2019; Chia et al., 2021), freshwater (Wang et al., 2018; Caradini et al., 2018;

2017; Fu and Wang, 2019; Mintenig et al., 2019; Meng et al., 2020; Yang et al., 2021), and marine (Cincinelli et al., 2017; Wang et al., 2019; Zhang et al., 2020; Gao et al., 2022; Jiang et al., 2022) ecosystems, has been increasingly reported. MPs are ingested directly and indirectly by fish, bivalves, crustaceans, and other animals (Chan et al., 2019; Teng et al., 2019; Carlin et al., 2020; Savoca et al., 2020; Sequeira et al., 2020; Pequeno et al., 2021; Yin et al., 2022). MPs may transfer from lower to higher trophic levels along the food chain and may cause potential threats to human health (Santillo et al., 2017).

Mangrove forests are saline and tidal habitats and are considered one of the most carbon-dense ecosystems on Earth (Bai et al., 2021). Mangrove wetlands provide numerous ecological services and functions, including water purification, coastal protection, and marine animal habitats (Lovelock and Duarte, 2019). Several studies have reported the distribution of MPs in global mangrove forests. Li et al. (2018) found that the abundance of MPs ranged from 15 to 12,852 items/kg in sandy beaches and mangrove wetlands. The average abundance of MPs in mangrove sediments in Singapore was 9.2 ± 5.9 particles/250 g (Mohamed Nor and Obbard, 2014) and varied from 0.6 to 8.0 items/ individual in fish species collected from the mangrove wetland of Zhanjiang (Huang et al., 2020). In addition, Zhou et al. (2020) investigated the distribution of MPs along the coast of China and found that the abundance of MPs in mangrove sediments was 8.5 times higher than that in mangrove-free sediments. Nevertheless, in the Muara Angke Wildlife Reserve of Indonesia, the concentrations of MPs in sediments were higher outside than inside mangrove areas (Cordova et al., 2021). However, few studies have focused on MP pollution in benthic species, especially invertebrates, in mangrove wetlands. Hainan Island represents nearly 20% of the mangrove forest areas in China, and these areas are distributed in Dongfang, Danzhou, Lingao, Chengmai, Dongzhaigang, Wenchang, and Sanya. Among them, the Dongzhaigang National Mangrove Reserve, which was established in 1986, was designated as one of the most important wetlands in the world in 1992 (Qiu et al., 2011; Wu et al., 2013).

In this study, wild benthic species were collected from 15 sites along seven typical mangrove wetlands in Hainan Island, and their abundance, morphotype, size, color, and polymer composition were investigated. The main objectives were to quantify and characterize the MPs in benthic organisms from mangrove areas. This study provides basic data on the contamination level of MPs in benthic organisms in the mangrove wetlands of Hainan.

2 Materials and methods

2.1 Study area

Seven typical mangrove distribution areas around Hainan Island, including Danzhou, Lingao, Chengmai, Dongzhaigang, Wenchang, Sanya, and Dongfang, were chosen as the sampling sites for mangrove benthos. A total of 15 sites were included: Huachong, Caiqiao, Fuli mangrove bay, Beijie, Kunshan, Wuli, He Harbor, Changwan, Nanjie, Dongye, Pai Harbor, Xiachang, Sanya River, Qingmei Harbor, and Dongfang. The layout of the stations in each area is shown in Figure 1.

2.2 Sample collection

A total of 10 species of mangrove benthos were collected from the Hainan mangrove area in April 2019. All the benthos were sieved from sediments collected with a Van Veen grab at 30 cm depth from a



~10 m × 10 m area in each sampling site, using a five-point sampling method according to previous studies, with slight modifications (Ryan, 2004). A total of 135 individuals were identified down to the species level (Table 1) by referring to the *Atlas of molluscs in Dongzhai bay*, *Hainan*, and the *Atlas of marine animals in mangrove wetland in Beibu Gulf, Guang Xi.* There were six species of crabs (n = 80), three species of bivalves (n = 49), and one species of snails (n = 6). To avoid contamination of MPs during transportation, the collected samples were packed in aluminum foil, transported to the laboratory under $-4^{\circ}CC$, and stored at $-20^{\circ}CC$.

2.3 Microplastic extraction

The MPs were extracted as described by Munno et al. (2018), with some modifications. Briefly, the soft tissues of animals (crabs, bivalves, and snails) were collected and weighed and then placed in 500-ml glass beakers individually. Subsequently, 180 ml of 10% (m/v) KOH and 20 ml of 30% H₂O₂ were added for the digestion process. Each beaker was covered with aluminum foil and placed in an oven at 60°CC for at least 48 h. To ensure complete digestion, the beaker was shaken every 6 h. The digestate was then cooled and vacuum filtered through a GF/F glass microfiber filter (0.7 mm pore size, 47 mm diameter; Whatman plc, Maidstone, UK). Afterward, the filters were placed in clean Petri dishes and dried at room temperature (25°CC) until analysis.

2.4 Microplastic identification

Suspected plastic particles were observed using a stereoscopic microscope (GL6545T; Guilin, China) equipped with a high-resolution digital camera. The MPs were classified and counted according to shape (classified into fibers, granules, fragments, pellets, and films), size (classified into <1, 1–2, 2–3, 3–4, and 4–5 mm) (Cui et al., 2022), and color (Nie et al., 2019). In addition, a laser confocal microscope and a Raman spectrometer (DXR2; Thermo Fisher Scientific, Waltham, MA, USA) were used to analyze the suspected plastic particles according to the methods of Di and Wang (2018). To identify the chemical composition, a spectral database based on OMNIC software (Thermo Fisher Scientific) was used to compare the spectra of the samples, and the level of certainty was set to 60% (Woodall et al., 2014; European Commission, 2013).

2.5 Quality assurance and quality control

All of the experimental equipment used in this study were made of non-plastic materials and were rinsed carefully with filtered distilled water several times to avoid potential contamination from other sources. All solutions, including the distilled water, KOH, and H_2O_2 , were filtered through a 0.45 µm filter paper under vacuum before use. During all experimental processes, all containers were covered with aluminum foil, and polymer-free gloves and cotton lab coats were worn. In addition, the blank samples were corrected for potential procedural contamination.

2.6 Statistical analysis

A location map of the sampling areas was drawn using ArcGIS 10.2. All data were analyzed using Microsoft Excel and are shown as the mean \pm standard deviation (SD). The abundance of MPs in benthic species at each site was expressed as items per individual (items/individual). In addition, the characteristics of the MPs were plotted using GraphPad Prism software, and SPSS 16.0 was used for statistical analysis. Differences in the abundance of MPs were determined using one-way ANOVA with Dunnett's test, and significance was set at p < 0.05.

3 Results and discussion

MP pollution in the benthos of Hainan's mangrove wetlands was studied for the first time. A total of 135 benthic organisms, including six species of crab, three species of bivalves, and one species of snail from 15 sampling sites in seven typical mangrove wetlands of Hainan, were analyzed to determine the abundance and characteristics of MP contamination. The different sizes, shapes, colors, and chemical compositions of the MPs were examined in the benthos samples from different mangrove wetland areas.

3.1 MP abundance in benthos from different mangrove wetland areas

The abundance of MPs in the benthos from different mangrove areas is shown in Table 2. The abundance of MPs ranged between 0.83 \pm 1.32 and 12 \pm 0.00 items/individual (average, 3.90 \pm 3.31 items/ individual), with the highest abundance in Changwan, Dongzhaigang $(12.00 \pm 0.00 \text{ items/individual})$, while the lowest abundance $(0.83 \pm 1.32 \text{ items/individual})$ was found in Fuli mangrove bay, Chengmai. This result was similar to the number of MPs observed in organisms collected from the mangrove region of Zhanjiang (0.6-8.0 items/individual) (Huang et al., 2021). Our results also demonstrated that there were differences in the MP abundance between each sampling site (Figure 2A). The abundance of MPs in the benthos collected from Changwan was significantly higher than that from other sites in the Hainan wetlands (p < 0.05), which was largely due to the input of plastic debris from the tourism industry and the semi-closed bay with weak hydrodynamic conditions. It has been reported that Dongzhaigang is a mangrove wetland nature reserve in China and is the biggest bay in Hainan Island (Li et al., 2020). Furthermore, the abundance of MPs in Pai Harbor, He Harbor, and Sanya River was relatively high, with values of 9.00 ± 8.08, 7.50 \pm 0.58, and 6.33 \pm 6.66, respectively, which may due to port transportation and the urban communities around these areas.

TABLE 1 Abundance of microplastics in different benthic species.

Family	Species	Benthos samples (n)	Microplastics (items)	Abundance (items/individual)
Crab	Uca vocans	9	2	0.22
	Uca arcuata	19	22	1.16
	Perisesarma bidens	15	16	1.07
	Uca dussumieri	6	12	2.00
	Helicana wuana	9	7	0.78
	Sesarma plicata	22	30	1.36
Bivalve	Vignadula atrata	23	5	0.22
	Geloina erosa	13	3	0.23
	Saccostrea echinata	13	24	1.85
Snail	Littoraria melanostoma	6	1	0.17

TABLE 2 Abundance of microplastics in benthos samples from different sample sites.

Mangrove areas	Sampling sites	East longitude North latitude	Abundance (items/individual)
Danzhou	Huachong (HC)	109°C15′21" 19°C46′3"	4.40 ± 4.45
Lingao	Caiqiao (CQ)	109°C34'0" 19°C51′23"	2.40 ± 1.82
Chengmai	Fuli mangrove bay (FL)	109°C59 '13" 19°C54′32"	0.83 ± 1.32
	Beijie (BJ)	110°C35'24" 19°C55′47"	1.00 ± 0.00
	Kunshan (KS)	110°C37 '3" 19°C55′35"	1.50 ± 0.58
Dereshtiene	Wuli (WL)	110°C36'53" 19°C55′27"	3.00 ± 0.00
Dougznaigang	He Harbor (HH)	110°C33'9" 19°C55′27"	7.50 ± 0.58
	Changwan (CW)	110°C37'53" 19°C55′43"	12.00 ± 0.00^{a}
	Nanjie (NJ)	110°C35'40" 19°C55′10"	3.00 ± 1.15
	Dongye (DY)	110°C50'21" 19°C33′32"	1.00 ± 1.46
Wenchang	Pai Harbor (PH)	110°C50'18" 19°C37′37"	9.00 ± 8.08
	Xiachang (XC)	110°C47'47" 19°C36'29"	3.50 ± 3.20
Secure	Sanya River (SR)	109°C30'17" 18°C15′33"	6.33 ± 6.66
Sanya	Qingmei Harbor (QH)	109°C36'60" 18°C13′21"	2.00 ± 2.68
Dongfang	Dongfang (DF)	109°C38'54" 19°C5′50"	1.00 ± 1.22

^aSignificant differences in microplastic abundance in the benthos between sampling sites.



FIGURE 2

Microplastic abundance in the different sample sites (A) and in each benthic species (B) from the mangrove areas of Hainan. Lowercase letter "a" indicate significant differences between sampling sites.

In addition, we calculated the abundance of MPs in the different benthic species (Table 1 and Figure 2B) and found that it varied from 0.17 to 2.00 items/individual in each species, with the highest MP abundance found in crabs (including *Uca vocans*, *Uca arcuata*, *Perisesarma bidens*, *Uca dussumieri*, *Helicana wuana*, and *Sesarma plicata*), which showed an average abundance of 1.10 ± 0.59 items/ individual; among them, *S. plicata* had the highest abundance (1.36 items/individual), followed by *U. arcuata* and *P. bidens* (1.16 items/individual for both). The average abundance of MPs in bivalves (including *Vignadula atrata*, *Geloina erosa*, and *Saccostrea echinata*) was 0.77 \pm 0.94 items/individual, with the highest found in *S. echinata* (1.85 items/individual), followed by *G. erosa* (0.23 items/ individual) and *V. atrata* (0.22 items/individual); the snails *Littoraria melanostoma* had the lowest MP abundance (average, 0.17 items/ individual). We compared our results with those of previous studies that focused on the abundance of MPs in benthos from different areas (see Table 3). The MP abundance values found in our study were consistent with those detected in crabs from the English Channel and the Atlantic Ocean (Welden et al., 2018), but the concentrations of MPs in crabs and bivalves from the Arctic and sub-Arctic regions (Fang et al., 2018) were slightly lower than those in the present study. Moreover, the bivalve species from Qingdao (Ding et al., 2021),

TABLE 3 Comparison of microplastic abundance in benthic species with previous studies.

Species and sources	MP concentrations (Items/individual)	Dominant types and polymers of microplastics	Reference
Crabs, bivalves, snails Hainan mangrove wetlands, South China	0.17–2.00	Fibers (80.13%) PP (94.44%), PS (5.56%)	This study
Spider crab (<i>Maja squinado</i>) English Channel and Atlantic Ocean	(1.39) ^a	_b _	Welden et al., 2018
Crab (Chionoecetes opilio), bivalves (Astarte crenata and Macoma tokyoensis) Arctic and sub-Arctic regions	$0.17 \pm 0.12 - 0.83 \pm 0.43$	Fibers (87%) PA, PE	Fang et al., 2018
Four locally cultured bivalve species Qingdao, China	0.5–3.3	Microfiber PVC and rayon	Ding et al., 2021
Nine bivalve species Shanghai's biggest fishery market, China	4.3-57.2	Fibers -	Li et al., 2015
Four oyster species Coastline of China	(2.93) ^a	Fibers CP, PE, and PET	Teng et al., 2019
Predatory snail (<i>Thais mutabilis</i>) Persian Gulf	3.70-17.70	Fibers PE, PET, and PA	Naji et al., 2018

PP, polypropylene; PA, polyamide; PE, polyethylene; PVC, polyvinyl chloride; CP, cellophane; PET, polyethylene terephthalate.

^aAverage abundance.

^bData not shown in the article.

Shanghai's biggest fishery market (Li et al., 2015), and the coastal areas of China (Teng et al., 2019) have been reported to have considerably higher MP abundance compared to this study. The average abundance of MPs in snails from the Hainan mangrove areas was lower than that in predatory snails from the Persian Gulf (Naji et al., 2018). Our results suggest that the levels of MPs in biota species from Hainan's mangrove wetlands were low to moderate compared to those reported in previous studies.

3.2 Morphological properties of the MPs in benthos from different mangrove areas

Five different morphotypes of MPs—fibers, granules, fragments, pellets, and films—were observed in the sampled benthic organisms from these mangrove areas. The most common type of MP in all collected benthic species was fiber (80.13%) (Figure 3A), which was consistent with those detected in benthic organisms from other areas (Table 3) and in fish species from the mangrove wetland of Zhanjiang (70%) (Huang et al., 2020), as well as in mussels from 25 sites along the coastal waters of China (Qu et al., 2018). In addition, the site proportion of fibrous MPs in Sanya River, Dongye, Wuli, Beijie, Fuli mangrove bay, and Caiqiao accounted for 100% (Figure 3D), all of which are close to urban communities and fishing areas. It was speculated that the high levels of fibrous MPs may be associated with human activities such as the disposal of municipal wastewater and the fishery.

The sizes of the MPs in the present study were classified into five ranges, i.e., <1, 1–2, 2–3, 3–4, and 4–5 mm, which accounted for 48.08%, 32.69%, 10.90%, 5.13%, and 3.21% of the MPs, respectively (Figure 3B). The proportion of MP size in benthos from the Beijie site accounted for 100%, while Changwan contained more smaller-sized MPs (<1 mm, 86.61%), which also had the highest abundance of MP in benthic species (Figure 3E). The main size ranges were <1 mm (48.08%) and <2 mm (>80%), which were similar to the proportions of small MPs found in benthic species from other mangrove and coastal areas of China (Courtene-Jones et al., 2017; Wang et al., 2019; Filgueiras et al., 2020). The size range of the MPs can be explained by the feeding habits of benthic species (Bour et al., 2018). It has been reported that mussels are more likely to ingest smaller MPs (Qu et al., 2018).

In addition, the color can affect the ingestion of MPs by aquatic species (Filgueiras et al., 2020). MPs of five different colors—brown (37.18%), blue (25.64%), white (13.46%), red (1.28%), transparent (1.28%), and other artificial colors (21.15%)—were observed (Figure 3C). Among the examined species, brown and blue MPs were the predominant ingested items, similar to other studies on mussels (Digka et al., 2018) and sea snails (Courtene-Jones et al., 2017). The differences in the colors of the MPs in each site are shown in Figure 3F, with multicolor or brown and blue MPs being the most prevalent. Moreover, in the present study, crabs were the dominant species with the highest proportion of MPs (72.95%), followed by bivalves (22.95%) and snails (4.10%) (Figure 4A). The higher intake of small and colorful particles may be explained by their feeding habits, as crabs are visual predators and may confound plastic particles with their natural food (Nanninga et al., 2020).



3.3 Chemical properties of the MPs in the benthos from different mangrove areas

A laser confocal microscope and a Raman spectrometer were used to identify the polymer types of the MPs ingested by the benthic species in Hainan's mangrove wetland areas. As shown in Figures 4B, C, two types of MPs, polypropylene (PP) and polystyrene (PS), were identified. PP accounted for 94.44% of the MPs in benthic species, which was inconsistent with previous studies reporting that polyethylene (PE), polyamide (PA), and/or polyethylene terephthalate (PET) were the major polymer types of MPs in benthic organisms collected from other areas (Fang et al., 2018; Naji et al., 2018). On the contrary, other studies reported that PP fibers were the most abundant in sediments from the Beibu Gulf Sea (Xue et al., 2020). PP is commonly used in packaging, containers, pipes, textiles, and fishing equipment (Park et al., 2004; Cai et al., 2018). Around the mangrove region, there are a number of fish ports and mariculture sites in the harbor; concurrently, the mangrove wetlands are tourist areas. The present study suggests that artificial disturbance, including urban wastewater treatment, mariculture, and port transportation, might be the sources of MP contamination in the Hainan mangrove wetlands.

4 Conclusion

Mangrove ecosystems are important coastal resources that create unique ecological environments hosting various species. This study is the first to quantify MP pollution in the benthic species from Hainan's mangrove wetlands. In this study, the MPs were extensively characterized in 10 benthic species collected from 15 sampling sites within seven typical mangrove wetlands in Hainan Island. The average abundance of MPs ranged between 0.83 \pm 1.32 and 12.00 \pm 0.00 items/individual in each sampling site. According to Raman analysis, most detected MPs were PP, with an abundance rate of 94.44%, mainly in the form of fibers (80.13%). Ingesting MPs and associated contaminants in other organisms through the food chain is a great risk to human health. Our results are indicative of the



bioavailability of MPs to benthic marine organisms. Future studies on the abundance and distribution of MPs in various organisms from different geographical locations are needed to assess the risk of MPs to public health and ecosystems.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

QZ: Conceptualization, data curation, formal analysis, and writing—original draft. JX: Conceptualization, data curation, formal analysis, and writing—reviewing and editing. SM: Investigation, methodology, and data curation. YC and FL: Investigation. XD: Conceptualization, supervision, and funding acquisition. All authors contributed to the article and approved the submitted version.

Funding

This project was funded by the Ministry of Science and Technology of the People's Republic of China (no. 2017FY100703); the Key Research and Development Project of Hainan Province (nos. ZDYF2018122 and ZDYF2020178); the Key Science and Technology Program of Haikou City (no. 2017052); and the Initial Fund from Hainan University for R&D [KYQD(ZR)1870].

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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