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# Microplastic accumulation in oysters: Insights from aquaculture and laboratory conditions

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## ABSTRACT

Microplastics, emerging pollutants, are present in seafood and pose a potential health risk to consumers. The objective of this study was to assess microplastic concentrations in the surface water, sediments, and oysters of the Cha Va River, an aquaculture production area in southern Vietnam. Laboratory experiments were also conducted to examine rates of ingestion by and bioconcentration of microplastics in oysters. Microparticles including microplastics were measured in all samples collected from the field, and presented 9–27 items  $m^{-3}$  in surface water, 3300–8000 items  $kg^{-1}$  dry weight in sediments, and 0.67–8.33 items  $ind^{-1}$  or 0.02–0.33 items  $g^{-1}$  wet weight in oysters. Fibre shapes and blue colour were the most common while polypropylene, polyester, polyethylene, and polyethylene terephthalate acrylate, alkyd were identified. The presence of microparticles in surface water and sediment was influenced by the complex vertical motion characteristic of tidal environments whereas the microparticles in natural oysters seemed to be closer related to those in sediment than in the surface water. Laboratory experiments showed that oysters incubated in 10 and 1000 microplastics  $L^{-1}$  tanks accumulated  $4.1 \pm 2.0$  and  $19.0 \pm 11.0$  microplastics per individual, respectively. However, bioconcentration of microplastics in oysters was higher ( $12.37 \pm 6.95$ ) in tanks with lower microplastic concentrations than in tanks with higher microplastic concentrations ( $0.62 \pm 0.35$ ). It is a high potential human health risk to the local oyster-consumers because of the annually microparticle ingestion from 657 to 3942 items per person. We suggested further studies on the severity of this potential health risk and policies for minimising microplastic emissions into the environment in Vietnam.

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## 1. Introduction

Plastic pollution causes serious environmental and ecological issues, with potential threats to human health worldwide ([Chae and An, 2017](#); [Kurniawan et al., 2021](#)). Plastic use has increased dramatically over the past several decades getting over 400 tons per year, but most plastic (~9%) is not recycled and accumulates in the environment over time ([Anderson et al., 2016](#); [UNEP, 2022](#)). Microplastics (MiPs, size range: 1  $\mu m$ –5 mm) occur in different forms (e.g., fibre, fragment, foam, pellet, film) and colours (e.g., blue, red, black, white, yellow). Typically, they derive from the breakdown of plastic materials produced for industrial

or domestic use, or are secondarily formed through the fragmentation of macroplastics in the environment ([Arthur et al., 2009](#); [Auta et al., 2017](#); [GESAMP, 2019](#)). MiPs are of increasingly significant global environmental concern ([Phuong et al., 2022](#); [Eerkes-Medrano et al., 2015](#)

Anthropogenic sources of microplastic (MiP) release into the environment are diverse, ranging from wastewater treatment, industrial manufacturing and domestic sewage, personal care products, fishery industries and aquaculture, agriculture, daily activities of residents and tourists, transportation, surface runoff, and dumping of waste; they may even be spread by precipitation and atmospheric currents ([Kang et al., 2018](#); [Yukioka et al., 2020](#); [Phuong et al., 2022](#)). MiPs accumulate close

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to cities and urban areas but are also found in remote regions such as the north and south poles (Anderson et al., 2016; Waller et al., 2017; Lahens et al., 2018). MiPs are widely distributed in both marine and freshwater systems (Jambeck et al., 2015; Anderson et al., 2016; Phuong et al., 2022). Rivers and coastal areas are the two main pathways of MiP transport from inland to the oceans (Phuong et al., 2022). In aquatic systems, MiPs are found in sediments throughout the water column and in numerous organisms (Anderson et al., 2016; Auta et al., 2017; Waddell et al., 2020). MiP presence in aquatic animals can result from filter feeding, suspension feeding, consumption of MiP-exposed prey, or direct ingestion (Anderson et al., 2016). Some species (e.g., bivalves) are capable of excretion or egestion of MiPs, whereas others retain, accumulate, and immobilise MiPs into their circulation (e.g., Anderson et al., 2016; Auta et al., 2017; Nelms et al., 2018). Oysters, for instance, a common bivalve group that are consumed worldwide, have recently been found to ingest and accumulate MiPs in their tissues (Phuong et al., 2018; Abidli et al., 2019; Zhu et al., 2019; Martinelli et al., 2020; Ding et al., 2021).

Asia has the highest rates of plastic waste production of any region in the world, and Vietnam is the fourth largest source of plastic waste that enters the ocean (Jambeck et al., 2015). Investigations of MiPs originating from Vietnam over the past several years have focused on their occurrence and distribution in riverine, lake, and coastal waters (e.g., Lahens et al., 2018; Strady et al., 2021; Strady et al., 2020), sediments (Strady et al., 2021; Duong et al., 2022; Le et al., 2022; Tran-Nguyen et al., 2020), atmospheric fallout (Truong et al., 2021), and bivalves (Phuong et al., 2019; Kieu-Le et al., 2022). However, little is known about the occurrence and distribution of MiPs in the surface waters, bottom sediments, and biota (e.g., fish, bivalves) of fisheries and aquaculture areas in Southeast Asia, including Vietnam (Thushari et al., 2017; Fathoniah and Patria, 2021; Ta et al., 2022). Here, we examined MiP abundance and distribution in the Cha Va River, which supports numerous aquaculture operations, in Ba Ria, Vung Tau Province, southern Vietnam. The objectives of this study were to (i) assess MiP concentrations in the surface waters, sediments, and oysters of

aquaculture facilities under field conditions, and (ii) evaluate MiP accumulation in oysters (*Crassostrea angulata*) under both field and laboratory conditions. To do so, we performed both in situ assessments and laboratory experiments.

## 2. Materials and methods

### 1. Study area

The Long Son District (21,000 inhabitants, density of 400 inhabitants per square km; DARDVT, 2017), Vung Tau City, Ba Ria Vung Tau Province (southern Vietnam), consists of an island 51 km<sup>2</sup> in size. The local economy is based on fisheries, aquaculture, and saltmarsh activities. Aquaculture was first established in 2007 and consists of fish and oyster cultivation, with oyster production predominant, involving 130 households, covering a water surface area of 0.127 km<sup>2</sup>, and producing 288 tons of oysters per year. The substrates used for oyster cultivation are composed of fibrocement materials and old tyres, whereas nets are used for fish cultivation (DARDVT, 2017). The area is subjected to a monsoon tropical climate, with an average annual temperature of 27 °C and a rainy season lasting from May to November, with an average annual rainfall of 1600 mm (DARDVT, 2017). The aquaculture zone is located in the Cha Va River estuarine system, an area extending 10 km in length and 200–600 m in width (DARDVT, 2017) and subjected to semi-diurnal tides, as the estuarine opens to the Ganh Rai Gulf (Costa-Boddeker et al., 2017). Six sites were chosen along the Cha Va River from which surface water and sediment samples were collected (Fig. 1). Sites 1, 2, 3, and 4 were located in the midst of the oyster and fish rearing zones; site 5 was located at the edge of the zone (i.e., few cages present); and site 6 was located 1 km from any aquaculture cages.

### 2. Sample collection in the field

#### 1. Surface water sample collection

Surface water samples were collected following the method



**Fig. 1.** Map of the sampling area and the six sampling sites (1–6) in the Cha Va River. The dash line with arrow indicates the water flow in the Cha Va River from site 6 to site 1 during low tide. Coordinates for the sites are: (1) 10.40.09 N, 107.11.80 E; (2) 10.44.26 N, 107.11.99 E; (3) 10.43.44 N, 107.11.02 E; (4) 10.43.13 N, 107.10.65 E; (5) 10.46.65 N, 107.11.73 E; and (6) 10.46.91 N, 107.11.52 E.

described by [Strady et al. \(2021\)](#). Water was collected from a boat using an 80  $\mu\text{m}$  mesh and 50 cm diameter plankton net coupled to a flowmeter (General Oceanics®) to determine the volume of sampled water (range of 20 – 285  $\text{m}^3$ ). After 10 min of exposure, the net was rinsed from the outside to the inside, and all particles attached to the inside were recovered in a 500 mL glass container. Sample bottles were stored at 4 °C in the laboratory until further use.

## 2. Bottom sediment sample collection

Bottom estuarine sediments were collected using a Petersen grab sampler ([APHA, 2012](#)) at the depth from 9 to 17 m. Sediment collected at each site was transferred into a metal container, with ~0.5 kg of wet sediment placed in a plastic bag and kept in cool conditions until further use.

## 3. Natural oyster collection

Fifteen oysters (*Crassostrea angulata*) of similar size (length: 12.1  $\pm$  1.4 cm, width: 8.6  $\pm$  3.0 cm, height: 4.1  $\pm$  0.8 cm) were purchased from a local fisherman collected near sampling site 1 ([Fig. 1](#)) and kept under cool conditions during transport to the laboratory. The natural oysters, caught by the local fisherman, commonly live on concrete or wood near the bottom of the river.

### 2.3. Laboratory experiment: microplastic ingestion by oysters

Natural oysters (*C. angulata*) originating from Ba Ria Vung Tau Province were kept in the laboratory in seawater (pH 7.8; salinity 30‰; aeration system) previously filtered through a screen mesh size of 50  $\mu\text{m}$  to prevent MiP contamination. The oysters were not fed for 2 days prior to the experiment to ensure the same hunger level ([Xu et al., 2017](#)) and MiP depuration from their soft tissues. Standardised plastic fragments (CRT332, polystyrene, size 150 – 250  $\mu\text{m}$ , median size 200  $\mu\text{m}$ , various colours; CARAT GmbH) were used in the experiment. The fragments were mostly in square shape. The number of fragments in 1 mg of plastic fragment was evaluated at 217.2  $\pm$  12.5 fragments ( $n = 10$  counts). We used the MiP fragment for present experiment because (i) fragment is one of the most common shapes in natural oysters ([Thushari et al., 2017](#); [Phuong et al., 2018](#); [Teng et al., 2019](#); [Zhu et al., 2019](#); [Jang et al., 2020](#); [Martinelli et al., 2020](#); [Liao et al., 2021](#)), (ii) the natural oysters in our study could already have fibres in their body which make us difficulty to distinguish the fibres from nature (background fibres) or from the addition if we used fibres for bioaccumulation study, and (iii) the available commercial fragment product that we had in our laboratory.

Thirty oysters (length: 10.7  $\pm$  0.9 cm, width: 8.0  $\pm$  0.8 cm, height: 3.2  $\pm$  0.5 cm, weight: 191  $\pm$  27 g) were used in the experiment and separated into three treatments consisting of: (i) Control: no MiP addition; (ii) MiP = 10: addition of MiPs at a concentration of 10 items  $\text{L}^{-1}$ ; and (iii) MiP = 1000: addition of MiPs at a concentration of 1000 items  $\text{L}^{-1}$ . In each condition, 10 oysters were raised in a 10 L tank and fed diatoms (*Thalassiosira* sp.) at a concentration of  $2 \times 10^8$  cells  $\text{L}^{-1} \text{d}^{-1}$ . Tanks were thoroughly cleaned and rinsed with filtered seawater prior to use. Seawater in the tanks was well mixed via an aeration system to ensure full suspension of diatoms (i.e., food) and MiPs (excluding Control tanks). Seawater, food, and MiPs (in exposure treatment tanks) were renewed daily ([Xu et al., 2017](#)). The experiment was run at a temperature of 28  $\pm$  1 °C, with a light intensity of 3000 Lux, and under a 12/12 h light/dark cycle. At the end of 5 d, each oyster was gently removed, rinsed with filtered seawater, and dissected to remove soft tissue. The tissues were individually weighed, placed in a flask, and subjected to the MiP analysis and observation protocols described in [Sections 2.4.3 and 2.5](#).

## 4. Microparticle and MiP analysis

### 1. Surface water

Microparticles and MiPs in the surface water samples were analysed

following the procedures described by [Strady et al. \(2021\)](#). First, field water samples were filtered through a 1 mm sieve to remove larger particles (algae, mineral, organic,) and keep plastic particles in a separated glass vial until the end of the analysis. The water passing through the sieve was collected in glass bottles and treated for a volume sample equivalent to 350 mL with (i) sodium dodecyl sulfate (Merck®) at a dose of 1 g at 40 °C for 24 h, (ii) bioenzymes SE (protease and amylase, Spinnrad®; 1 mL) and F (lipase, Spinnrad®; 1 mL) at 50 °C for 48 h, and (iii) hydrogen peroxide (30%, Merck®; 15 mL) at 50 °C for 48 h. The digested water sample was filtered through a 250  $\mu\text{m}$  sieve and all retrieved materials were kept for use in density separation; this was performed by overflowing, with a sodium chloride solution

(Merck®, 1.18  $\pm$  0.02  $\text{g cm}^{-3}$ ) used to recover the supernatant, including microparticles and MiPs. Consecutive overflows were performed until no further MiPs were observed. Finally, the supernatant was passed through a glass fibre filter (Whatman®, 1.6  $\mu\text{m}$  pore size) and kept in sterile petri dishes until further observation.

### 2.4.2. Sediments

Microparticles and MiPs in the sediment samples were analysed following the procedures described by [Strady et al. \(2021\)](#). Approximately 10 g of dry homogenised sediment was used in the analysis. Sediments were filtered through a 1 mm mesh sieve to remove large particles and microparticles and MiPs larger than 1 mm, then digested with hydrogen peroxide (30%, Merck®) at 40 °C in a glass beaker and covered with aluminium foil. Digested samples were filtered through a 250  $\mu\text{m}$  mesh sieve using a sodium chloride solution (density: 1.18  $\text{g mL}^{-1}$ ). Density separation and filtration procedures were similar to those used for the surface water samples as mentioned in 2.4.1.

### 2.4.3. Oysters

Microparticles and MiPs were analysed in oyster soft tissues in both field and laboratory experiments. First, the size and weight of each individual were measured before digestion, following which all soft tissues were removed from the shells, weighed, and stored in 250 mL Erlenmeyer flasks. For oysters collected to study MiP accumulation in the natural environment, a pool of three individuals constituted a sample; therefore, five samples were analysed. For oysters used in laboratory experiments, a single oyster constituted a sample. Oyster samples were digested according to a protocol adapted from [Dehaut et al. \(2016\)](#). Each sample was placed in a flask and digested with 150 mL of KOH 10% at 40 °C for 72 h on a hot plate ([Fig. 2](#)). After digestion, the sample was rinsed through a 75  $\mu\text{m}$  mesh sieve, with the portion captured by the sieve placed in a glass beaker that was then filled with a sodium chloride solution (density: 1.18  $\text{g mL}^{-1}$ ). Density separation and filtration procedures were the same as those described above for the surface water and sediment samples, and from [Strady et al. \(2021\)](#).

## 2.5. MiP observation

Microparticles and MiPs on the GF/A filters were observed using a Leica stereoscope S6D coupled with LAS software®. Based on the GESAMP recommendation when visual observation is performed without systematic analysis of the polymers, we set up the minimum length at 300  $\mu\text{m}$  (e.g. maximum 5,000  $\mu\text{m}$ ) and minimum area size at 45,000  $\mu\text{m}^2$  (e.g. maximum at 25000,000  $\mu\text{m}^2$ ) ([GESAMP, 2019](#)). The minimum observation size was set at 300  $\mu\text{m}$  for all samples, whereas the maximum size was 5 mm; all items smaller than 300  $\mu\text{m}$  were excluded from the analysis. Microparticles were counted and classified according to their forms (e.g., fibre, fragment, pellet), and their length and area were measured. Microparticle fibres were defined as being equally thick throughout their entire length and lacking cellular or organic structures, or tapered towards the ends and featuring three-dimensional bending; Microparticle fragments were defined as irregularly shaped hard particles with the appearance of once having been part of a larger piece of litter, flat flexible particles with smooth or



Fig. 2. Protocol for analysis of microplastic (MiP) particles in oyster tissues.

angular edges, or near-spherical or granular particles that deform readily under pressure and can be partly elastic; and microparticle pellets were defined as round and regular, with a constant diameter and no internal structure. Only microparticles exhibiting a clear and homogeneous colour throughout were included; however, straight, transparent, or whitish fibres were excluded to avoid contamination by material of biological or organic origin. Microparticle colours were determined during observation.

#### 6. Chemical characterization of MiPs

The nature of MiPs was determined on 110 random items from field samples using Fourier Transform Infrared Spectrometry (FTIR-ATR iS50 Thermo Scientific) coupled with the microscope (Nicolet Continuum FTIR microscope). Each sample was measured on the microscope FTIR combined to a MCT/A detector, a KBr beam splitter. The FTIR spectra were obtained with reflectance mode, 64 scans, resolution 4 (data spacing  $0.48\text{ cm}^{-1}$ ), automatic atmospheric suppression. The spectrum width was in the range of  $4000\text{--}650\text{ cm}^{-1}$ . Each spectrum was compared with the standard polymers database, and matching superior to 70% of reference spectra were accepted for the polymers characterization. Six types of polymers were identified including acrylate (9.1%), alkyl (18.2%), polypropylene (21.8%), polyester (2.7%), polyethylene (16.4%), and polyethylene terephthalate (6.4%). Besides, 25.5% were non plastic materials (e.g., silicate, cargill, cellulose, chitin, calcium carbonate, and rayon; supplementary 1).

#### 7. Quality control

Quality control procedures based on recommendations presented in Dehaut et al. (2016) were followed to prevent contamination from external MiPs and to ensure data integrity. Laboratory benches were wiped with wet cotton prior to sample digestion or stereoscopic observations, and cotton clothes and gloves were worn; additionally, air conditioning was used instead of fans or ventilation. All glassware was rinsed with filtered tap water (Whatman® GF/A,  $1.6\text{ }\mu\text{m}$  pore size) and covered with aluminium foil. Three control samples were prepared and checked during digestion and observation of the MiP samples. The sieving atmospheric control (SAC) was established by placing a GF/A filter in an open Petri dish during sample digestion and filtration. SAC aids in the avoidance of airborne MiP contamination of samples. An observation atmospheric control (OAC) was prepared by leaving an uncovered GF/A filter during stereomicroscope observation to check for

airborne MiP contamination, and a positive extract control (PEC) was performed by adding 10 yellow PE (CRT 171, CARAT GmbH) to the original sample before digestion to confirm recovery efficiency. Observation of MiPs in controls indicated that both SAC and OAC had values of 0 items ( $n = 10$ ), whereas PEC had a mean value of 96% and a range of 80–100% (supplementary 2). The results showed that almost no MiPs were present on the blank filters, suggesting that we did not exclude any MiPs from the samples relevant to contamination.

#### 2.8. Data treatment

Microparticle fibres and fragments on the filters were counted, and the total fibre and fragment concentrations were expressed differently for each type of sample: items  $\text{m}^{-3}$  for water samples, items  $\text{kg}^{-1}$  of dry sediment for sediment samples, and items  $\text{g}^{-1}$  of oyster wet weight or items  $\text{individual}^{-1}$  for oyster samples.

MiP bioconcentration in experimental oysters was calculated in accordance with the procedures described by Walker et al. (2006) as the ratio of MiP concentration in oysters to that in the ambient medium. We assumed that the density of seawater used in laboratory experiments involving oysters was approximately  $1\text{ kg L}^{-1}$ . Bioconcentration factors are presented as mean values  $\pm$  one standard deviation (SD).

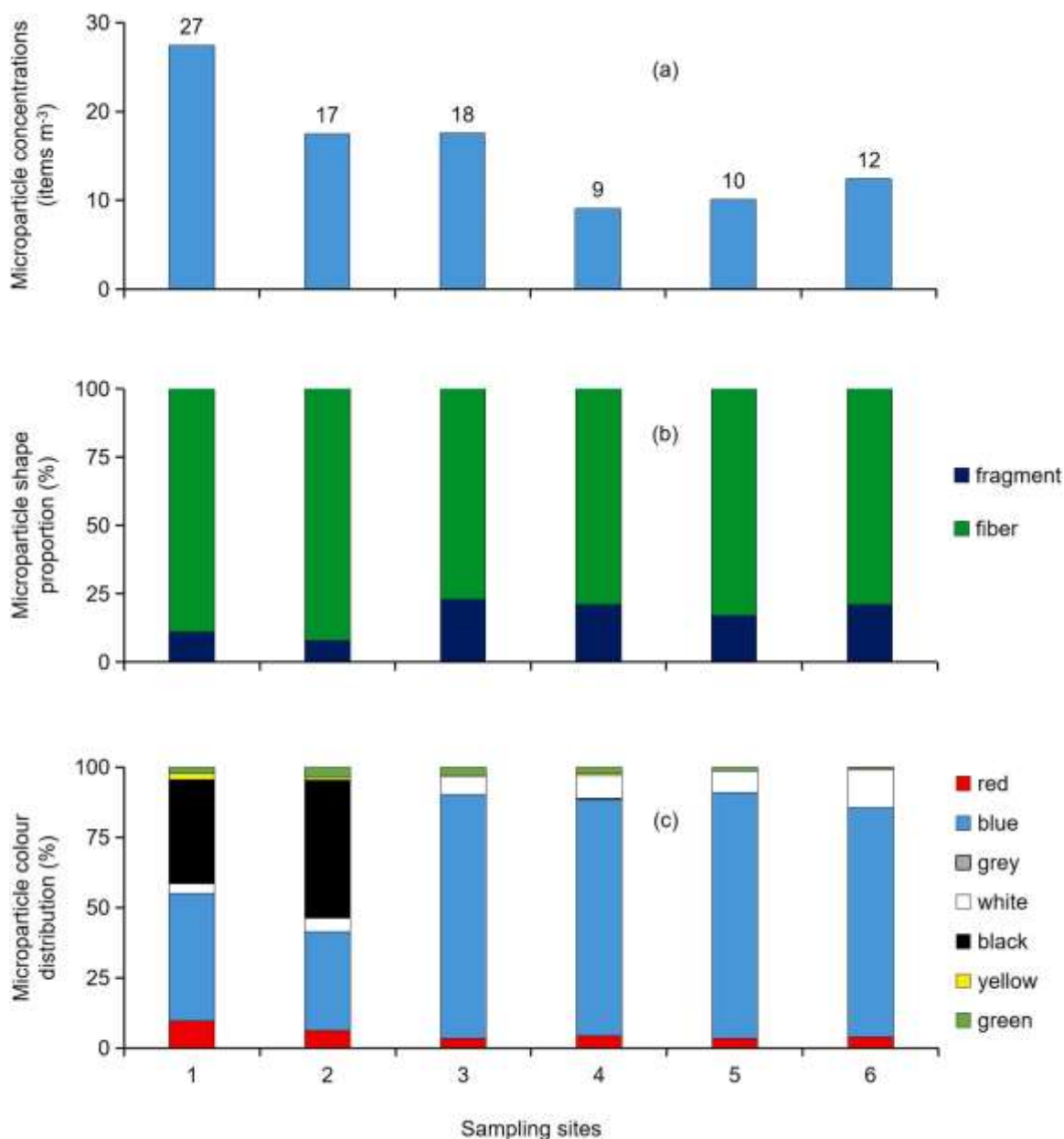
A Kruskal - Wallis test (Sigma Plot, version 12.0) was used to evaluate significant differences ( $p < 0.05$ ) in fibre length and diameter between the surface water, sediment, and oyster samples, as well as to detect differences in MiP concentrations and bioconcentration factor between the two MiP treatments in the laboratory experiments.

### 3. Results

#### 1. Microparticles and MiP characteristics in surface water and bottom sediments

Microparticle concentrations in surface waters ranged from 9 items  $\text{m}^{-3}$  (site 4) to 27 items  $\text{m}^{-3}$  at (site 1) (Fig. 3a) and did not exhibit clear decreasing or increasing pattern from site 1 to site 6. Microparticles in water were primarily fibres (77–92% of total MiPs) (Fig. 3b) and fragments (8–23% of total MiPs) (Fig. 3b), with no pellets observed. Seven colours of MiPs were identified in the water samples (red, blue, grey, white, black, yellow, and green), with blue the most common at sites 3, 4, 5, and 6, whereas blue and black were the most common at sites 1 and 2 (Fig. 3c). Fibre length in the surface water samples ranged from  $321\text{ }\mu\text{m}$  to  $4837\text{ }\mu\text{m}$ , with median values at each site varying from





**Fig. 3.** a) Microparticle concentration (items  $m^{-3}$ ), b) relative proportion of different microparticle forms (%), and c) colour distribution in surface waters collected from the six sampling sites.

1186  $\mu m$  to 1461  $\mu m$  (Table 1) and the first and third quartile values of fibre length across all sites ranging from 835  $\mu m$  to 1110  $\mu m$  and 1737  $\mu m$  to 2420  $\mu m$ , respectively. Fibre diameter in all samples varied from 6  $\mu m$  to 77  $\mu m$ , with the median at each site varying from 14  $\mu m$  to 18  $\mu m$  and the first and third quartile values of the fibre diameter across all sites ranging from 12  $\mu m$  to 15  $\mu m$ , and from 17  $\mu m$  to 22  $\mu m$ , respectively (Table 1). Fragments observed in the water samples varied greatly, from 45,802  $\mu m^2$  to 5936,710  $\mu m^2$ , with median values at each sampling site varying from 96,865  $\mu m^2$  to 221,256  $\mu m^2$  and the first and third quartile values of fragments across all sites ranging from 77,893  $\mu m^2$  to 116,613  $\mu m^2$  and 142,362  $\mu m^2$  to 623,668  $\mu m^2$ , respectively (Table 1).

Microparticle concentrations in sediments varied from 3300 items  $kg^{-1}$  (site 5) to 8000 items  $kg^{-1}$  (site 1) (Fig. 4a), with the highest concentrations found at sites 1 and 6, and the lowest at sites 4 and 5. As with surface water samples, only fibres and fragments were detected in sediments, with fibres predominant, composing 97–100% of total

microparticle concentrations (Fig. 4b). Although microparticles of four different colours were observed in the sediment (green, white, blue, and red), 80–90% of all sediment microparticles were blue in colour (Fig. 4c). Fibre length in all sediment samples varied from 308  $\mu m$  to 4815  $\mu m$ , with median values at each site varying from 770 to 1340  $\mu m$  and the first and third quartile fibre length values ranging from 536  $\mu m$  to 844  $\mu m$  and from 1281  $\mu m$  to 2045  $\mu m$ , respectively (Table 1). Fibre diameter in all sediment samples varied from 5  $\mu m$  to 60  $\mu m$ , with median diameters at each site varying from 12  $\mu m$  to 16  $\mu m$  and the first and third quartile values ranging from 10  $\mu m$  to 14  $\mu m$  and from 15  $\mu m$  to 21  $\mu m$ , respectively (Table 1). The number of microparticle fragments in sediment samples was very limited, with only 0–1 fragment found per sample, ranging in size from 62,674  $\mu m^2$  to 4858,332  $\mu m^2$ .

Analysis (Kruskal–Wallis test) of fibres collected from surface waters ( $n = 1855$ ) and sediments ( $n = 305$ ) revealed that fibres in sediments were significantly shorter in length and smaller in diameter than those in surface waters ( $P < 0.001$ ).

**Table 1**

Fibre length and diameter, and fragment area (median, mean  $\pm$  one standard deviation [SD], minimum, maximum, first quartile [Q25], and third quartile [Q75]) of microparticles including MiPs collected from surface water and sediment samples.

Samples	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6
Surface water						
Fiber length ( $\mu\text{m}$ )						
Median	1407	1443	1218	1186	1680	1461
Mean ( $\pm$ SD)	1595 ( $\pm$ 859)	1623 ( $\pm$ 862)	1450 ( $\pm$ 759)	1339 ( $\pm$ 677)	1881 ( $\pm$ 979)	1525 ( $\pm$ 707)
Min - max	329 -4746	321 -4502	437 -4661	321 -3760	423 -4837	327 -4319
Q25	961	962	991	835	1110	974
Q75	2074	2071	1769	1737	2420	1790
Fiber diameter ( $\mu\text{m}$ )						
Median	18	17	15	15	17	14
Mean ( $\pm$ SD)	19 ( $\pm$ 7)	18 ( $\pm$ 6)	16 ( $\pm$ 7)	16 ( $\pm$ 6)	19 ( $\pm$ 8)	15 ( $\pm$ 6)
Min - max	6 -77	6 -60	6 -73	7 -50	9 -76	8 -60
Q25	15	15	12	13	15	12
Q75	22	20	17	17	20	17
Fragment area ( $\mu\text{m}^2$ )						
Median	221256	159477	159400	160270	96865	104061
Mean ( $\pm$ SD)	537155 ( $\pm$ 756336)	449864 ( $\pm$ 956969)	628794 ( $\pm$ 1191382)	374332 ( $\pm$ 542260)	387249 ( $\pm$ 927536)	159311 ( $\pm$ 201436)
Min - max	52744 -3620791	52369 -5376190	45802 -5635607	51234 -3415053	47927 -5936710	45816 -866091
Q25	116613	103023	77893	85683	78205	79456
Q75	623668	517849	387386	404466	211069	142362
Sediment						
Fiber length ( $\mu\text{m}$ )						
Median	770	1009	1008	1340	855	842
Mean ( $\pm$ SD)	1079 ( $\pm$ 811)	1433 ( $\pm$ 1104)	1269 ( $\pm$ 888)	1523 ( $\pm$ 904)	987 ( $\pm$ 510)	1027 ( $\pm$ 679)
Min - max	310 -4474	313 -4078	346 -4213	411 -4815	367 -2198	308 -4014
Q25	552	607	655	844	627	536
Q75	1358	1873	1545	2045	1281	1305
Fiber diameter ( $\mu\text{m}$ )						
Median	15	16	16	12	14	14
Mean ( $\pm$ SD)	16 ( $\pm$ 5)	16 ( $\pm$ 8)	17 ( $\pm$ 7)	13 ( $\pm$ 4)	15 ( $\pm$ 4)	14 ( $\pm$ 5)
Min - max	7 -30	6 -60	6 -55	6 -28	9 -23	5 -27
Q25	13	12	14	10	10	11
Q75	21	18	20	15	17	17
Fragment area ( $\mu\text{m}^2$ )*	113029	62674	N/A	4858332	1128978	N/A

\* only a single fragment was found in sediment samples; N/A, not available.

### 3.2. Microparticle and MiP characteristics in natural oysters

Microparticles and MiPs were observed in all natural oysters collected from the Cha Va River, with concentrations ranging from 0.67 items individual<sup>-1</sup> to 8.33 items individual<sup>-1</sup> (Fig. 5a) and with a mean value of  $4.07 \pm 3.09$  items individual<sup>-1</sup>. In oyster tissues (expressed in wet weight, ww), microparticle concentrations varied from 0.02 items g<sup>-1</sup> ww to 0.33 items g<sup>-1</sup> ww (Fig. 5b), with a mean concentration of  $0.15 \pm 0.13$  items g<sup>-1</sup> ww. Only fibres were observed in natural oysters, and only two colours were recorded (red and blue), in which blue was the most common (83 - 100% of observed fibres per oyster sample) (Fig. 5c). Fibre length of MiPs in oysters varied from 311  $\mu\text{m}$  to 2989  $\mu\text{m}$ , with a mean value of  $927 \pm 504$   $\mu\text{m}$  (Table 2). Median fibre length per natural oyster sample varied from 627  $\mu\text{m}$  to 906  $\mu\text{m}$  and median fibre diameter ranged from 4.1  $\mu\text{m}$  to 25.9  $\mu\text{m}$ , with a mean of  $12.7 \pm 4.3$   $\mu\text{m}$  (Table 2).

Analysis (Kruskal - Wallis test) of fibres in surface waters (n = 1855), sediments (n = 305), and oysters (n = 61) revealed that fibre length was significantly shorter in oysters than in surface waters ( $P < 0.001$ ) but not in sediment ( $P = 0.073$ ), and that mean fibre diameter was significantly smaller in oysters than in the surrounding water and sediment ( $P < 0.001$ ).

### 3.3. Bioconcentration of MiPs in oysters from laboratory experiments

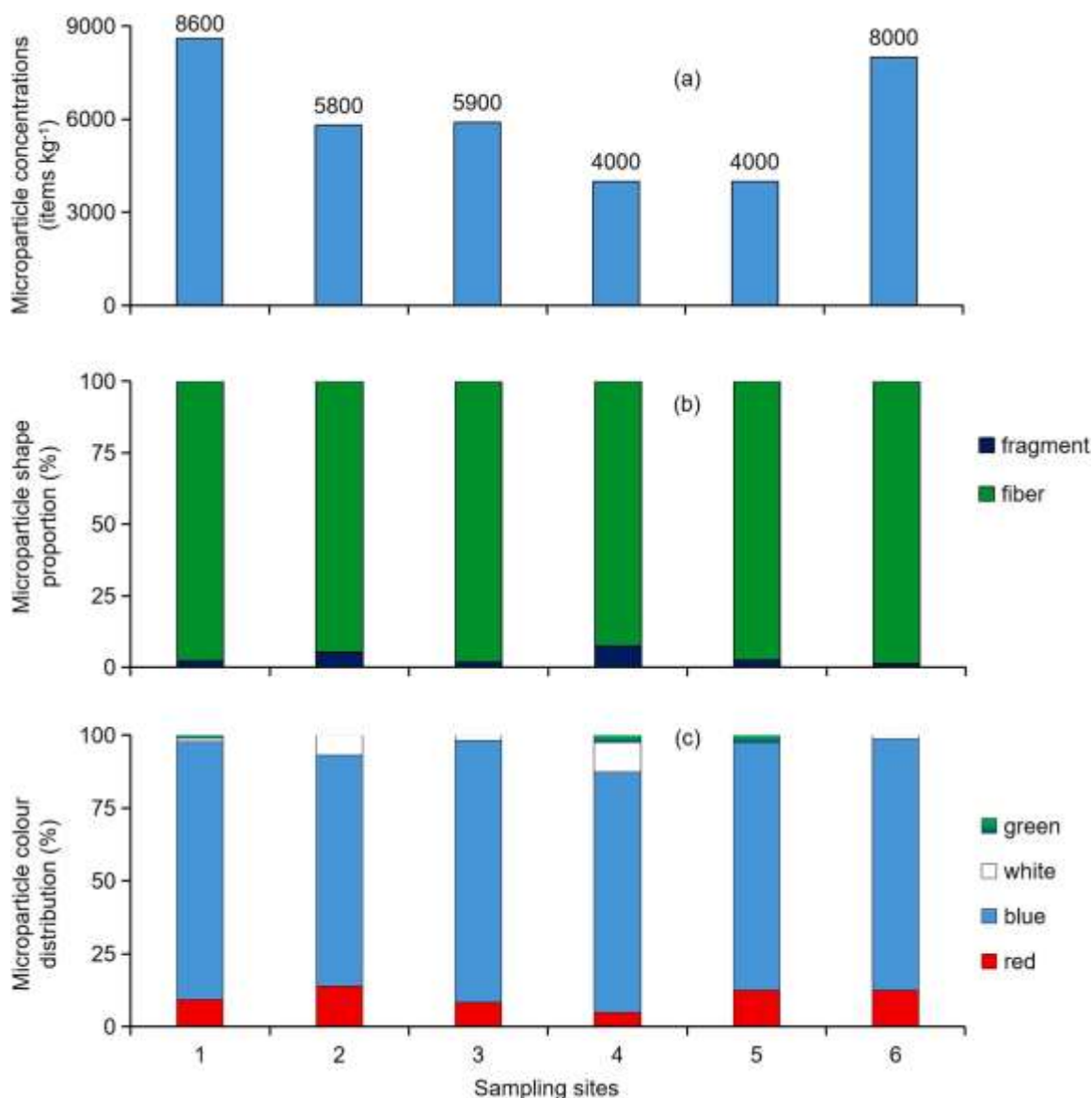
Following 5 d of incubation, we measured MiP accumulation in oysters at both exposure levels (10 and 1000 MiPs L<sup>-1</sup>). MiP concentrations in oysters in tanks containing 10 MiPs L<sup>-1</sup> was  $4.1 \pm 2.0$  items individual<sup>-1</sup> ( $0.12 \pm 0.06$  items g<sup>-1</sup> ww), while concentrations in oysters in tanks containing 1000 MiPs L<sup>-1</sup> was  $19.0 \pm 11.0$  items individual<sup>-1</sup> ( $0.62 \pm 0.35$  items g<sup>-1</sup> ww) (Table 3), suggesting that MiP

concentrations in oysters displayed a positive relationship with MiP exposure level. However, MiP bioconcentration factors in oysters in the 10 MiPs L<sup>-1</sup> tanks was  $12.37 \pm 6.95$ , whereas that of oysters in the 1000 MiPs L<sup>-1</sup> tanks was considerably lower, at  $0.62 \pm 0.35$  (Table 3).

## 4. Discussion

### 4.1. Comparison to global aquaculture and potential sources of microparticles and MiPs in surface waters and sediments

Understanding of MiP abundance, distribution, and transport in aquaculture zones in Asia is limited (e.g., Chen et al., 2018; Zhu et al., 2019; Liao et al., 2021), and especially so in Southeast Asia. This study provides the first assessment of microparticle and MiP levels in aquaculture areas in Vietnam and contributes to global assessments of the threat posed by MiP pollution to aquaculture. It is important to note that in previous studies, the term MiPs (from field samples) was used for their results even though not all particles were MiPs. Therefore, in present paper we prefer to use the term microparticles to illustrate the items in water, sediment and biological samples. The term MiPs is used whenever the plastic polymeric of items was identified. Microparticle concentrations (in which MiP concentrations gained 74.5%) measured in surface waters of the Cha Va River (mean:  $15.7 \pm 6.8$  items m<sup>-3</sup>) were within the same range as reported for mariculture operations in Xiangshan Bay, China ( $4.6 \pm 0.5 - 20.1 \pm 0.2$  MiPs m<sup>-3</sup>; Chen et al., 2018), and lower than in both an intensive aquaculture zone in China's Maowei Sea ( $1200$  to  $10,100$  items m<sup>-3</sup>; Zhu et al., 2019) and oyster farms in Taiwan ( $7000 \pm 3460$  items m<sup>-3</sup>; Liao et al., 2021). Microparticle concentrations in the bottom sediments of the Cha Va River ( $3300 - 8000$  items kg<sup>-1</sup>) were higher than those measured in a previous study in nearby recreational sandy beaches at Vung Tau, Vietnam ( $1542 - 2024$  items kg<sup>-1</sup>; Strady



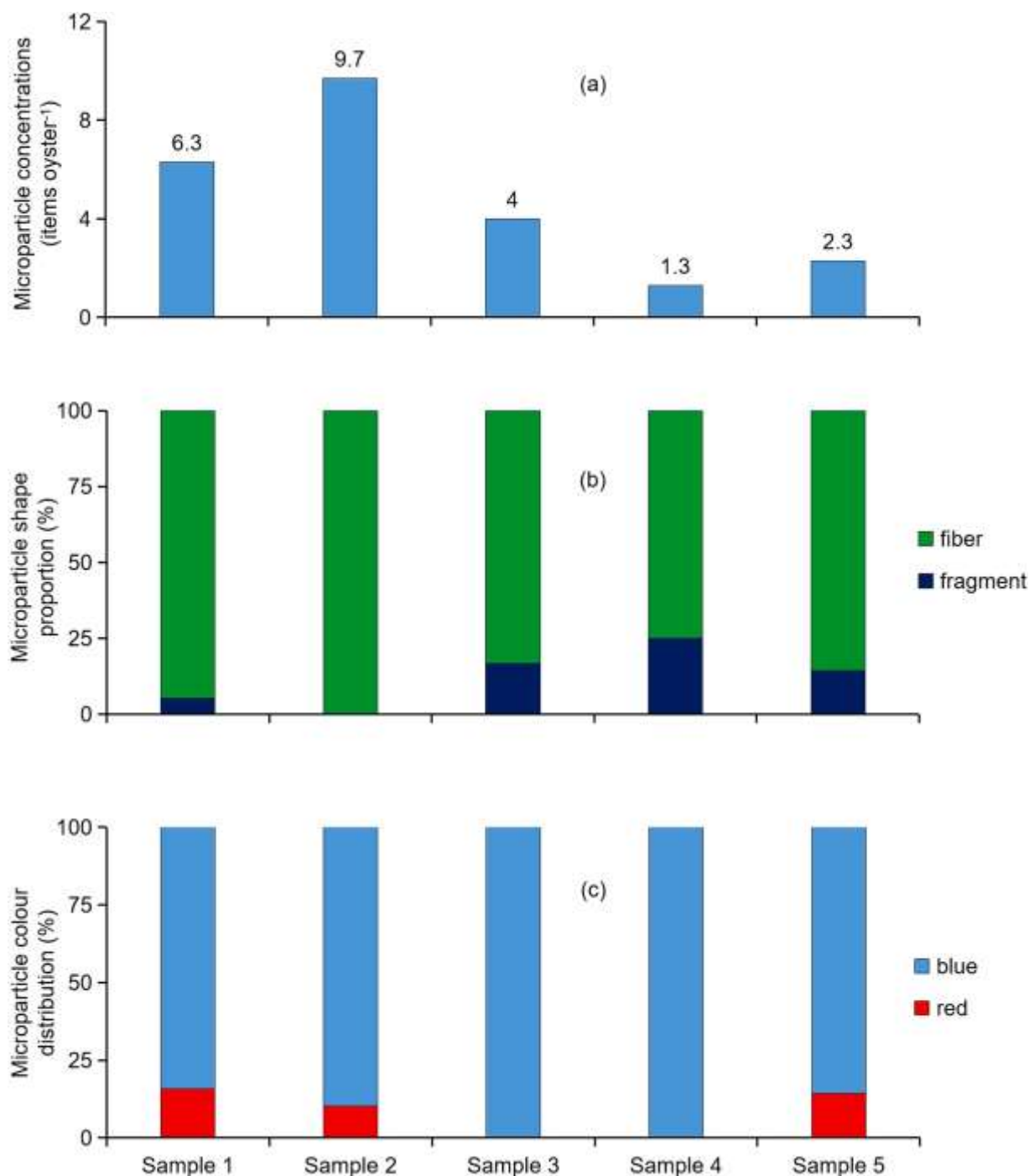
**Fig. 4.** a) Microparticle concentration (items kg<sup>-1</sup> dry weight [dw]), b) relative proportion of different microparticle forms (%), and c) colour distribution in dw sediments collected from the six sampling sites.

et al., 2021) but higher than those in the Xiangshan Bay mariculture zone ( $417 \pm 71 - 985 \pm 120$  items kg<sup>-1</sup> sediment, with the exception of one sampling site, for which concentrations of  $5570 \pm 296$  items kg<sup>-1</sup> were found in the sediment; Chen et al., 2018). It is important to note that there were a little difference in the sizes of the microparticles in our study (300 - 5000  $\mu$ m) and previous ones (from some  $\mu$ m to > 9 mm) which should closely related to the different microparticle concentrations among the studied areas. For example, Chen et al. (2018) took into the account of microparticle concentrations with the length of < 100  $\mu$ m to > 9 mm for water and sediment samples. The microparticles of < 250  $\mu$ m gained > 15% of total microparticle concentration in water in the study of Zhu et al. (2019). Similarly, around 53% of total microparticle concentration in aquacultural water along the Taiwanese coastal line had the length of less than 100  $\mu$ m (Liao et al., 2021). On the other hand, because of lacking a standardised method for MiP study, different pretreatment processes of samples might result in variation of study outcomes (Li et al., 2023; Metz et al., 2020)

Fisheries and aquaculture operations along the Cha Va River provide livelihoods and sustenance to approximately 200 local households. Although these households are not connected to wastewater treatment

systems, MiPs released from domestic sources in this region can be considered to be low, as almost no commercial products commonly available in Vietnam contain microbeads. Additionally, most of these household do not own washing machines; as such, given that the release of MiPs from synthetic clothes is mainly caused by the stresses that fabrics are subjected to during machine washing (De Falco et al., 2019), few synthetic fibres are released into the environment via laundry-related mechanical appliances. Plastic materials used in aquaculture and fisheries mainly consist of netting and old rubber tyres used as supports for oyster beds (DARDVT, 2017), and this equipment is generally used until failure. Therefore, seawater coupled with tropical climate characteristics — intense UV radiation, extreme dry and rainy seasons due to monsoons, and high ambient temperatures — exacerbates the wear and tear of plastics through greater rates of photo-degradation and oxidation, leading to fragmentation and MiP release, especially fibres, into surrounding environments. The predominance of fibres over fragments we observed in the surface waters and sediments of the Cha Va River (Fig. 3b; Fig. 4b) could be related to the degradation of fisheries and aquaculture gear (e.g., netting, rubber tyres); moreover, this pattern has also been observed in the aquaculture areas of both Xiangshan Bay





**Fig. 5.** a) Microparticle concentration expressed as items individual<sup>-1</sup> and b) items g<sup>-1</sup> wet weight (ww), and c) colour distribution in natural oysters from the Cha Va River. A sample consists of a pool of three natural oysters; all five oyster samples (15 individuals in total) were obtained near site 1.

(Chen et al., 2018) and the Maowei Sea (Zhu et al., 2019). As such, we conclude that fishery and aquaculture operations are likely the main sources of microparticle and MiP contamination in this section of the Cha Va River. Besides, regarding the colour and polymer types of the microparticles in present study, the six polymers of acrylate, alkyd, polypropylene, polyester, polyethylene and polyethylene terephthalate were determined in the blue MiPs. The polyethylene was found in all six MiP colours from the Cha Va River. The found polymers are primarily materials to produce packaging, storage containers, rope, gear, strapping, textile, floats and boat products (GESAMP, 2019) which could be used for multi-purposes including domestic use and aquaculture. Hence the, MiPs in the Cha Va River could be partly from the solid domestic

wastes of the local inhabitants.

#### 4.2. Transfer of microparticles and MiPs between surface water and sediment

The Cha Va River estuary is a semi-diurnal tidal environment, and as such the hydrodynamics of the zone are complex and characterised by periodic fluctuations in flow direction and velocity (Costa-Boddeker et al., 2017). In both surface waters and sediments, microparticle fibres were far more common than fragments, representing 77–92% and 97–100% of all microparticles collected from surface waters and sediments, respectively (Fig. 3b, Fig. 4b). The relative infrequency

**Table 2**

Microparticle concentration, form, size (mean, SD; range of min-max), and colour in natural oysters.

Scientific names	Concentrations	Shapes	Size (µm)	Colors	Countries	References
<i>Crassostrea angulata</i>	4.07 ± 3.09 items individual <sup>-1</sup> 0.15 ± 0.13 items g <sup>-1</sup> ww	Fiber <sup>a</sup>	Length Mean = 927 (SD, ± 504) Min -max, 311 -2989 Diameter Mean = 12.7 (SD, ± 4.3) Min -max, 4.1 -25.9 µm	Blue <sup>b</sup> , red	Vietnam	Present study
<i>Crassostrea gigas</i>	3.75 -6.21 particles g <sup>-1</sup> dw	Fiber <sup>a</sup> , other particles	Min -max, 1 -5000 (size of 1 -300, 75 -80%; size of 300 -5000, 20 -25% of total concentration)	N/A	The Netherlands	<a href="#">Leslie et al. (2013)</a>
<i>Crassostrea gigas</i>	0.47 ± 0.16 items g <sup>-1</sup> dw	Particle <sup>a</sup>	Min -max, 5 -> 25 (size of > 25 was dominant, >40% of total concentration)	Blue, red	France	<a href="#">Van Cauwenberghe and Janssen (2014)</a>
<i>Crassostrea forskalii</i>	0.35 -0.57 particles g <sup>-1</sup> ww	Fiber <sup>a</sup> fragment,	N/A	Red, brown, blue, white, transparent	Thailand	<a href="#">Thushari et al. (2017)</a>
<i>Crassostrea gigas</i>	2.1 ± 1.71 items individual <sup>-1</sup> 0.18 ± 0.16 items g <sup>-1</sup> ww	Fragment <sup>a</sup> , fiber	Size of 20 -100, 47% of total concentration Size of > 100, 53% of total concentration	Grey <sup>b</sup> , black, green/yellow, red, white, blue	France	<a href="#">Phuong et al. (2018)</a>
<i>Saccostrea cucullata</i>	4-7.0 items individual <sup>-1</sup> 5-7.2 items g <sup>-1</sup> ww	Fiber <sup>a</sup> , fragment, pellet, sheet	Min -max, 20 -5000 (size of 20 -100 gaining ~84% of total concentration)	Transparent, white, green, yellow, blue, brown, black, red	China	<a href="#">Li et al. (2018)</a>
<i>Crassostrea</i> spp.	2.93 items individual <sup>-1</sup> 0.62 items g <sup>-1</sup> ww	Fiber <sup>a</sup> , fragment, film, pellet	Mean = 902 (SD, ± 782) Min -max, 20 -4807 (size of 50 -1500 gaining >80% of total concentration)	N/A	China	<a href="#">Teng et al. (2019)</a>
<i>Crassostrea hongkongensis</i>	4.7 ± 0.3 particles individual <sup>-1</sup> 0.8 ± 0.2 particles g <sup>-1</sup> ww	Fiber <sup>a</sup> , flake, fragment, foam	Min -max, < 250 -5000 (size of < 250 gaining 31% of total concentration)	White <sup>b</sup> , blue, yellow, black	China	<a href="#">Zhu et al. (2019)</a>
<i>Crassostrea gigas</i>	1.5 items g <sup>-1</sup> ww	Fiber <sup>a</sup> , fragment, film,	Min -max, 50 -> 1000 (size of 100 -1000 gaining ~70% of total concentration)	Black <sup>b</sup> , blue, red, yellow, white	Tunisia	<a href="#">Abidli et al. (2019)</a>
<i>Crassostrea gigas</i>	Median, 1.13 ± 0.84 particles g <sup>-1</sup> ww	Fragment <sup>a</sup> , fiber,	N/A	Colorless <sup>b</sup>	Korea	<a href="#">Jang et al. (2020)</a>
<i>Crassostrea virginica</i>	0.72 ± 0.33 particles individual <sup>-1</sup> 0.08 ± 0.17 particles g <sup>-1</sup> ww	Fiber ~ fragment, bead	N/A	N/A	Korea	<a href="#">Kleisling et al. (2020)</a>
<i>Crassostrea gigas</i>	10.95 ± 0.77 items individual <sup>-1</sup> 0.35 ± 0.04 items g <sup>-1</sup> ww	Fiber <sup>a</sup> , fragment, bead, film, foam, unknown shape	Mean = 1340 Min -max, 100 -8720	Colorless <sup>b</sup> , blue, grey, black	USA	<a href="#">Baechler et al. (2020)</a>
<i>Crassostrea gigas</i>	0.69 -3 particles individual <sup>-1</sup> 0.02 -0.3 particles g <sup>-1</sup> ww	Fiber <sup>a</sup> , flake, fragment	Mean = 621 Min -max, 102 -2885	Dark (blue and black), light (yellow, white, silver), red, purple, green	USA	<a href="#">Martinelli et al. (2020)</a>
<i>Crassostrea angulata</i>	7.41 ± 10.45 items g <sup>-1</sup> ww	Fragment <sup>a</sup> , fiber, flake, sphere	Min -max, 9 -1921 (size of < 100 gaining 53% of total concentration; size of > 500 gaining 8% of total concentration)	Transparent <sup>b</sup> , black, yellow, orange	Taiwan	<a href="#">Liao et al. (2021)</a>
<i>Saccostrea</i> sp.	7.75 ± 12.34 items g <sup>-1</sup> ww					
<i>Crassostrea gigas</i>	1.2 -3.3 items individual <sup>-1</sup> 0.3 -3.0 items g <sup>-1</sup> ww	Film <sup>a</sup> , fiber, granule, fragment	Mean = 1145 Min -max, 7 -5000	Transparent <sup>b</sup> , blue, black, white, grey, red, blue	China	<a href="#">Ding et al. (2021)</a>

ww, wet weight; dw, dry weight; a, most common form; b, most common colour; N/A, not available.

**Table 3**

Microplastic concentration (mean values ± SD, n = 10) and bioconcentration factor in oysters under laboratory conditions.

	Treatments		
	Control	10 MiPs L <sup>-1</sup>	1000 MiPs L <sup>-1</sup>
Concentrations of MiPs (items individual <sup>-1</sup> )	0	4.1 ± 2.0	19.0 ± 11.0 *
Concentrations of MiPs (items g <sup>-1</sup> ww)	0	0.12 ± 0.06	0.62 ± 0.35 *
Bioconcentration factor of MiPs in oysters	0	12.37 ± 6.95	0.62 ± 0.35 *

ww, wet weight of oyster tissues. \*indicates significant differences in MiP concentrations between the two MiP treatments (P &lt; 0.001; Kruskal - Wallis test).

fragments in sediments could be due to the fact that many fragments can float or be suspended in the water column, and thus may be transported downstream of the sampling area. Most items constructed of plastics used in aquaculture are floating equipment (e.g., buoys, barrels), which are made of polyethylene or expanded polystyrene, which can continue to float even after fragmentation into MiPs (assuming low levels of biofouling and that constant particle density is maintained). In fact, in this study, the proportion of low density items such as polypropylene, polyethylene and polyethylene terephthalate ([Anderson et al., 2016](#)) was higher (44.6%) than high density items such as polyester and acrylate (11.8%; [Section 2.6](#)). We propose that the relative paucity of microparticle fragments in this section of the Cha Va River is due to downstream transport outside of the system.

Analysis of fibre size revealed that fibres in sediments were significantly shorter in length and smaller in diameter than fibres in surface waters (Table 1;  $P < 0.001$ ), suggesting that larger fibres have lower potential to move downward through the water column than smaller ones. In Xiangshan Bay, larger fibres were observed in surface waters

(mean:  $1540 \pm 1530 \mu\text{m}$ ) than in sediments (mean:  $1330 \pm 1690 \mu\text{m}$ ) (Chen et al., 2018). Laboratory experiments have shown that the spatial orientation of fibres is an important parameter that controls the rate at which fibres sink through the water column (Bagaev et al., 2017), and that fibre movement becomes unsteady and three-dimensional, resulting in decreased total downward flux because the particles are retained within the convective mixing layers of the water column (Khatmullina and Chubarenko, 2021). The authors of the latter study concluded that the vertical flux of MiPs and thin synthetic fibres is highly dependent on the vertical motion of water and the degree of water turbulence. Based on these findings, we postulate that the complex vertical motion characteristic of tidal environments influences the vertical distribution (i.e., between surface waters and sediments) of fibres of different sizes, and recommend that greater focus be placed on how fibres of lengths spanning the range that occurs in the environment (i.e., from a few  $\mu\text{m}$  to  $5000 \mu\text{m}$ ) respond to vertical motion.

#### 4.3. Microparticles and MiP accumulation in natural oysters

Numerous studies on the abundance, form, size, and colour of MiPs in oysters have been conducted over the past decade in Europe (Leslie et al., 2013; Van Cauwenberghe and Janssen, 2014; Phuong et al., 2018), North America (Baechler et al., 2020; Martinelli et al., 2020), Africa (Abidli et al., 2019), and Asia (Thushari et al., 2017; Li et al., 2018; Teng et al., 2019; Zhu et al., 2019; Jang et al., 2020; Kleisling et al., 2020; Liao et al., 2021; Ding et al., 2021). By comparison, average microparticle concentrations in oysters in the Cha Va River were lower than in oysters in the Netherlands, Thailand, Tunisia, South Korea, the United States, and Taiwan, and within the range of concentrations found in oysters in France and China (Table 2). As the matter of fact, the recorded microparticle size in our study ( $300 - 5000 \mu\text{m}$ ) and that in previous studies ( $1 - 5000 \mu\text{m}$ ) were not in the same/ similar range. For example, Leslie et al. (2013) noted that MiP of  $1 - 300 \mu\text{m}$  gained  $75 - 80\%$ , whereas MiP of  $300 - 5000 \mu\text{m}$  gained  $20 - 25\%$  of total MiP concentration in the oyster from The Netherlands. The mean MiP (all size) concentration in oyster from China was  $4.7 \pm 0.3$  particles individual<sup>-1</sup> (Zhu et al., 2019), however, the concentration of MiP with size from  $250$  to  $5000 \mu\text{m}$  was around  $70\%$  of total or  $3.29 \pm 0.2$  particles individual<sup>-1</sup>. The size of MiPs in the oysters from Thailand and Korea was not available (Table 2) which diminished the MiP concentration comparison among the studies. The microparticles that accumulated in oysters from the Cha Va River consisted solely of fibres, and no fragments were observed (Table 2). This finding is consistent with the low proportion of microparticle fragments recovered from the surface water and sediment samples ( $89\%$  and  $99\%$ , respectively, Figs. 3b, 4b) collected at site 1, where the oyster samples were collected, further reinforcing our assumption that fragments did not settle but rather were carried downstream.

In terms of colour, only blue and red microparticles were observed in oysters from the Cha Va River, a finding similar to that reported by Van Cauwenberghe and Janssen (2014). Blue fibres were the most common in both surface water and sediment samples at site 1 (Figs. 3c, 4c); likewise, dominance of a single MiP colour has also been observed in both water and oyster samples in other areas (e.g., Zhu et al., 2019, white; Jang et al., 2020, colourless/transparent; and Liao et al., 2021, transparent). The length of microparticle fibres collected from oysters ranged from  $311 \mu\text{m}$  to  $2989 \mu\text{m}$  (mean length:  $927 \pm 504 \mu\text{m}$ ; mean diameter:  $13 \pm 4 \mu\text{m}$ ; Table 2), with the maximum fibre length in oysters within the range of fibre lengths occurring in both surface waters ( $329 - 4746 \mu\text{m}$ ) and sediments ( $310 - 4474 \mu\text{m}$ ) (Table 1). Ward et al. (2019) demonstrated that oysters rejected and ingested equal numbers

of fibres regardless of size ( $75 \mu\text{m}$ ,  $587 \mu\text{m}$ , and  $1075 \mu\text{m}$ ), and that rejection of fibres in pseudofaeces was variable and not correlated with size. On the other hand, we found that the the median and mean length of the blue items (the dominant colour; Fig. 5c) in the oyster was  $848 \mu\text{m}$  and  $974 \mu\text{m}$ , respectively, which was much shorter than that in the surrounding environments, median length of  $1363 \mu\text{m}$  and mean length of  $1553 \mu\text{m}$ . As such, the role that oyster gills and labial palps play in determining particle size ingestion remains undetermined.

#### 4.4. Potential health risks of oyster MiP bioconcentration

A number of studies focusing on MiP uptake by marine bivalves have been undertaken in recent years, including mussels (Qu et al., 2018; Fernandez and Albentosa, 2019; Ward et al., 2019), clams (Xu et al., 2017; Kieu-Le et al., 2022), and oysters (Graham et al., 2019; Ward et al., 2019). Graham et al. (2019), for instance, in an examination of the

uptake ratio of microbeads (size range:  $100 - 500 \mu\text{m}$ ) by *Magallana gigas* at a level of  $60$  items L<sup>-1</sup>, found that MiP uptake varied from  $12.9 \pm 2\%$  to  $19.4 \pm 2\%$  after  $24$  h of incubation, equivalent to the ingestion and retention of  $3.9 \pm 0.6 - 5.8 \pm 0.6$  beads per oyster (Graham et al., 2019). Similarly, when testing uptake rates at levels of  $49$ ,  $495$ , and  $1050$  items L<sup>-1</sup>, Ward et al. (2019) reported that, in the eastern oyster (*Crassostrea virginica*), ingestion of spheres (diameter of  $113 - 510 \mu\text{m}$ ) resulted in  $2.6 \pm 6.3 - 315.5 \pm 151.8$  items individual<sup>-1</sup> and ingestion of fibres (length of  $75 - 1075 \mu\text{m}$ ) resulted in  $26.1 \pm 26.6 - 241.3 \pm 193.6$  items individual<sup>-1</sup> after  $2$  h of incubation. Graham et al. (2019) and Ward et al. (2019) noted that larger MiP items (beads, spheres, and fibres) were consumed at lower rates by oysters than were smaller items. Although MiP abundances in our study ( $4.1 \pm 2.0$  items individual<sup>-1</sup> for oysters in the  $10$  MiP items L<sup>-1</sup> treatment and  $19.0 \pm 11.0$  items individual<sup>-1</sup> for oysters in the  $1000$  MiP items L<sup>-1</sup> treatment) were comparable to those reported by both Graham et al. (2019) and Ward et al. (2019), differences in experimental design prevent comparisons of MiP uptake or ingestion status between the studies for several reasons: (i) MiP form influences ingestion rates (Ward et al., 2019) and different forms of MiPs were used in the studies (beads in Graham [et al., 2019], spheres and fibres in Ward [et al., 2019], fragments in the present study); (ii) various exposure periods were used, ranging from  $5$  d (this study), to  $24$  h (Graham et al., 2019), to  $2$  h (Ward et al., 2019); and (iii) the studies focused on different oyster species that likely have different ingestion rates (*C. angulata* in this study, *Magallana gigas* in Graham [et al., 2019], and *C. virginica* in Ward [et al., 2019]). In addition, research has shown that oysters can egest MiP beads ( $500 \mu\text{m}$  in diameter), spheres ( $510 \mu\text{m}$  in diameter), and fibres ( $1075 \mu\text{m}$  in length) (Graham et al., 2019; Ward et al., 2019); in some cases, more than  $90\%$  of anthropogenic particles were depurated by oysters within  $2$  d post-treatment (Ward et al., 2019). Thus, examination of MiP accumulation in bivalves must be conducted on a species-specific basis, and under standardised conditions of MiP concentration, form, exposure levels, and duration.

Different trends were observed for fragment ingestion by oysters in the Cha Va River, in which no fragments corresponding to the observation size range ( $300 - 5000 \mu\text{m}$ ) were detected, and oysters used in laboratory experiments, in which fragments with a mean length of  $200 \mu\text{m}$  were found. This discrepancy may be due to the size bias induced by the observation size range at site 1, with minimal and median areas of  $52,744 \mu\text{m}^2$  and  $221,256 \mu\text{m}^2$ , respectively (Table 1), and by the size of added fragments with a mean length of  $200 \mu\text{m}$ , corresponding roughly to  $40,000 \mu\text{m}^2$  maximum, which is approximately five times smaller than the median area of fragments in situ. As such, small fragments may accumulate in oyster tissues, but not large fragments. In addition, MiPs can be both ingested and egested by oysters (Craig et al., 2022), as demonstrated by the fact that oysters collected in the field did not contain MiP fragments of the same size as those recorded in Cha Va River surface waters.

Bioconcentration of MiPs was higher among oysters exposed to less

concentrated treatments (Table 3). Accumulation of MiP particles in oysters is a consequence of particle selection, which is closely related to particle size, form, nutritive value, and surface chemicals (Arapov et al., 2010). Even particles that enter the mantle cavity and reach the pallial organs of the oyster may be either ingested (Arapov et al., 2010) or egested as faeces and/or pseudofaeces (Ward et al., 2019; Craig et al., 2022; Graham et al., 2019). At present, no experimental evaluations of MiP accumulation in oysters has involved exposure to different concentrations of MiP fragments. In our study, the fragment abundances in the two treatments (i.e., 10 items L<sup>-1</sup> and 1000 items L<sup>-1</sup> in 10 L tanks) were 100 and 10,000 MiPs, respectively. We found that the amount of MiPs that one oyster ingested and accumulated was higher in tanks

containing 1000 items L<sup>-1</sup> (19.0 ± 11.0 items individual<sup>-1</sup>) than in tanks containing 10 items L<sup>-1</sup> (4.1 ± 2.0 items individual<sup>-1</sup>; Table 3). However, because of the very large difference in MiP abundances (100 and 10,000) between the two treatments, the bioconcentration factor in the low MiP (10 items L<sup>-1</sup>) treatment was much higher than that in the high MiP (1000 items L<sup>-1</sup>) treatment (Table 3). This suggests that ingestion of MiP particles by oysters is proportional to particle abundance (e.g., algae as food) in the surrounding environment, until a threshold is reached that limits ingestion due to the filtering rate, at which point MiP concentrations in oysters would stabilise (Tenore and Dunstan, 1973).

In Vietnam, oysters are widely consumed by both local and non-local residents. In the absence of data on oyster consumption rates in the country, we assumed a daily oyster consumption rate similar to that in Europe, or approximately 12–72 g day<sup>-1</sup> (Cauwenberghhe and Janssen, 2014), and a mean microparticle concentration of 0.15 items g<sup>-1</sup> ww (Table 2). Based on these assumptions, the potential annual microparticle intake by Vietnamese consumers ranges from 657 to 3942 items per person. Recently, Kieu-Le et al. (2022) estimated the intake of MiP fibres from consumption of clams (*Meretrix lyrata*) to be 324 fibre items per person per year. Exposure of humans to MiPs through consumption and ingestion of MiP-contaminated bivalves may cause or contribute to a diversity of disorders, including oxidative stress, cytotoxicity, neurotoxicity, immune system disruption, and transfer of MiPs to other tissues (Bhuyan, 2022). MiPs have also been shown to inhibit lipid digestion (Tan et al., 2020). It must therefore be concluded that consumption of oysters harvested from the Cha Va River poses a health risk to local populations, and further research on the severity of this potential hazard is urgently needed.

## 5. Conclusions

We provide here the first assessment of microparticle and MiP concentrations in the environment and natural oysters of a prominent aquaculture area in Vietnam, and evaluated oyster bioconcentration of MiP fragments under laboratory conditions. The proportion of fibre microparticles in surface water, sediment, and oyster samples was very high, comprising 77–99%, 97–100%, and 100% of total microparticle concentrations, respectively. Bioconcentration factor in oysters was higher when exposed to lower levels of MiPs. Microparticles in this part of the Cha Va River derive primarily from the activities of local residents, and aquaculture and fishery operations. The presence of MiPs in oysters poses a potential health risk to residents, and policies aimed at minimising MiP emissions into the environment, particularly in areas with extensive aquaculture operations, have been implemented throughout Vietnam. However, further research on the effectiveness of these strategies in reducing MiPs in oysters is needed to ensure future food safety.

## CRediT authorship contribution statement

Thanh-Son Dao: Conceptualization, Investigation, Writing – original draft, Writing – review & editing. Thuy-Chung Kieu-Le: Conceptualization, Investigation, Writing – original draft. Xuan-Thao La:

Investigation, Writing – original draft. Tran-Nguyen-Sang Truong: Investigation, Writing – review & editing. Van-Tai Nguyen: Investigation, Writing – review & editing. Emilie Strady: Conceptualization, Writing – original draft, Writing – review & editing, Supervision.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

Data will be made available on request.

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## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at

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