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Analysis of Microplastics and Heavy Metals in Most Consumed Molluscs cultured in Can Gio Biosphere Reserve of Vietnam

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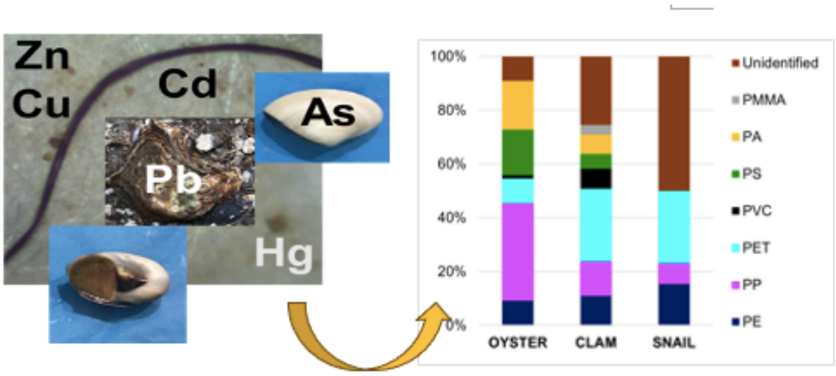
GRAPHICAL ABSTRACT

Microplastics and heavy metals were accumulated in oysters, snails and clams cultured in Can Gio Biosphere Reserve of Vietnam, with lower contents than other regions



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ABSTRACT

Can Gio, designated in 2000, is the first Biosphere Reserve of Vietnam. In recent years, local agriculture, tourism, households, nearby industrial zones, and maritime transport have released substances into rivers flowing through Can Gio Mangrove to the East Sea and transported into creatures. This study aims to investigate microplastics and heavy metals in the famous seafood of Can Gio. A Raman microscope was used to identify microplastics from the tissue of oysters, grease snails, and clams after enzymatic treatment with trypsin. Heavy metals were quantified with ICP-MS after acidic treatment. As a result, microplastics were found in almost all samples, even up to 10 in a random individual of bivalve. Fibers dominated, especially from 30 to 150 µm long. The metal with the highest content was zinc, followed by copper, especially in oysters. Non-essential metals were also present in the soft tissue in the order As > Pb > Cd. The accumulation of metals in Can Gio's molluscs was generally lower than in other regions and still in the allowed range for consumption of Vietnamese Standard. Although there have not been any Vietnamese regulations on the permissible level of microplastics for consumption, regular seafood intake will increase the risk of chronic poisoning and unknown disorders caused by the accumulation of microplastics and heavy metals.

1. INTRODUCTION

The pollution of marine microplastics has increasingly threatened the sustainable viability of aquatic ecosystems such as lakes [1], rivers [2], estuaries [3], and oceans [4]. Microplastics (MPs) are synthetic polymers in different forms smaller than 5 mm. Primary microplastics include products derived from households, cosmetic preparations, and other industrial production. They are broken into smaller fragments under environmental (physical and chemical) and microbiological factors [5-6]. Thus, they have diverse shapes and colors depending on their original products [7] and environmental behaviors. As plastic debris comes from various sources, it is observed in numerous appearances, which makes the standardization of its analytical procedure challenging. Microplastic analysis usually includes a set of techniques, such as physical and visual characterization or chemical analysis, to identify the composition of suspected microplastics. Infrared spectroscopy has become a conventional technique for detecting microplastics in environmental samples. Recently, micro-Raman has been used to determine microplastics in different samples such as sediment [8], freshwater, seawater, and salt in Can Gio [9-11], and shellfish [12] due to the fast and straightforward analytical procedure and insignificant effects of moisture or water remaining on the filter paper once spectra recording.

Heavy metals have become a major environmental pollutant, causing many potential and persistent risks. They are divided into two groups: (1) essential metals – Fe, Mn, Mg, Co, Zn, Cu are elements essential for the growth of organisms, (2) non-essential and toxic metals – Cd, Pb, Hg, As have no significant biological functions in metabolisms, but cause acute and chronic toxicity even at low concentrations [13]. The metal concentration in the surface water could be affected by season, position, and oceanic processes such as tide due to the highly dynamic hydrological regime of the sea. The season can influence the concentration of metals in tidal-river water through various pathways/mechanisms, including transport and dilution of the metals. Urban rivers and canals are supposed to be the primary sources of metals. Their type and content depend on the characteristics of catchments.

Bivalves are the significant bottom creatures that play a crucial role in nutrient recycling, regulating the habitat, and affecting food webs in marine ecosystems [14]. They are filter feeders that can be used to indicate pollution in the water ecosystems where they live [15]. The size of microplastics falls in the range of phytoplankton's size (< 20 µm for nanoplankton); therefore, they could mistake anthropogenic fibers or fragments for food. Metal uptake includes ingesting metal-enriched sediments, suspended particles, and water uptake [16]. Indeed, plastic debris was seen in the digestive system of some fish species like goby and freshwater carp [17], mollusc species such as mussels [18-19], and clams [15, 20-21]. There are adverse health effects from plastic resin, particularly oyster reproduction caused by PS 2-6 µm [22], but also hazardous substances such as heavy metals and persistent organic pollutants adsorbed on the surface of plastic debris [23]. Metals such as Zn, Cu, Cd, Cr, and Pb were present in high levels in the water, sediment [24], *Crassostrea belcheri* oyster [25], and *Meretrix lyrata* clam [26] in Can Gio. Moreover, the accumulation of chemicals in their tissue will cause the risk for higher organisms in the food chain.

Can Gio district is the deltaic confluence of Sai Gon, Dong Nai, and Vam Co rivers, which stretch out the most populated Ho-Chi-Minh City and Dong Nai province with heavy industrial zones. The aquaculture activities occur along the coastal zones, intensively in sea estuaries in Can Thanh, along with improperly managed tourism. The presence of contaminants in seafood will have significant economic and social influences on the community in this biosphere reserve and consumers throughout the country. To date, publications on the chemical contaminants in organisms in this reserve still need to be expanded, especially since there are no studies on the accumulation of microplastics and heavy metals in grease snails. Our study is the first to use enzymatic digestion to extract and determine microplastics with micro-Raman spectroscopy in the bivalves of Can Gio. In addition, heavy metals were also investigated in Pacific oysters (*Crassostrea gigas*), white clams (*Meretrix lyrata*), and grease snails. They are the local community and tourists' favorite and famous seafood. Finally, the paper summarizes the potential harmful impacts of bio-accumulated plastic debris and metals. This report contributes to the knowledge about the contamination of microplastics and heavy metals in biota in Vietnam and in a UNESCO Biosphere Reserve in particular.

2. INFORMATION COLLECTION AND EXPERIMENTAL METHODS

2.1. Interviews and Sampling

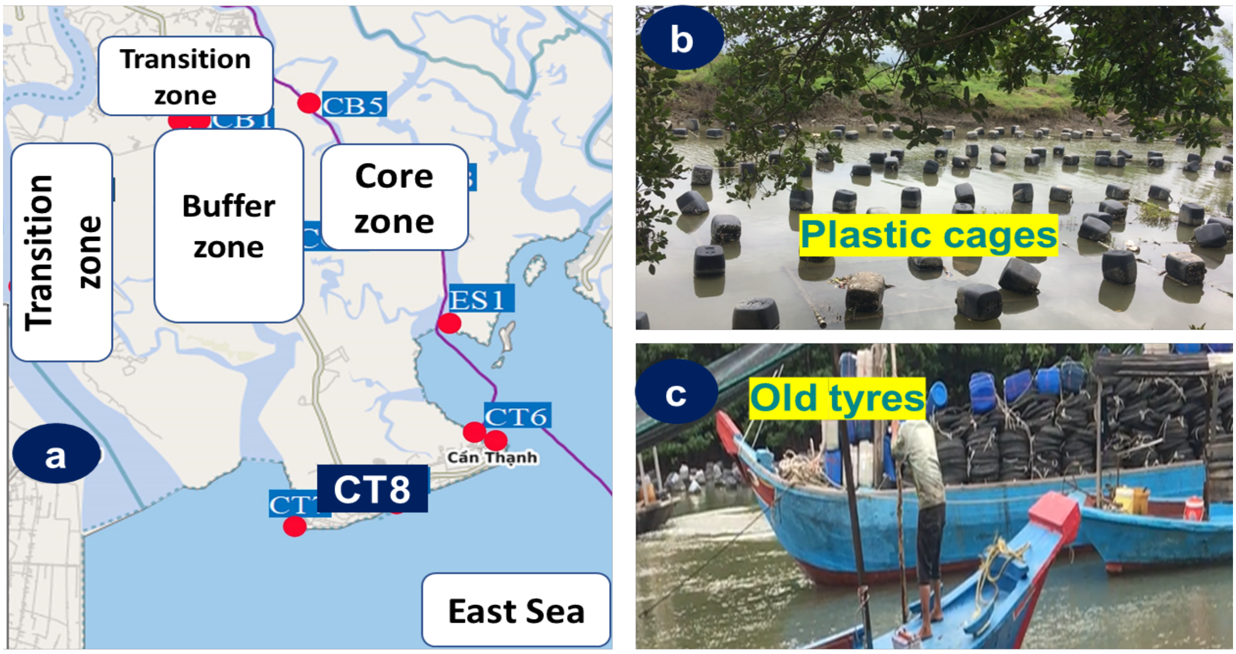

Figure 1. Sampling map in Can Gio (CT8) and Oyster cultivation (source: the authors)
(1) The sampling position in the map (CT8); (2) & (3) oyster cultivation in Rach Lo.

Oysters are more quickly caught in nature than clams and snails. Hence, they are raised in natural conditions at the sea estuaries. Oyster cultivation is very famous in Can Thanh town, Long Hoa, and Thanh An communes. In 2020, the estuary clean oyster farming project was first piloted in Can Gio, where the tires replaced the plastic category 1 b, c). Oysters were collected from the Rach Lo canal (10.394366, 106.935078) (CT8 in the map, Figure 1a). The adult oysters had an average shell length of 10 – 12 cm, and a dry weight of 160 ± 15 g. White clams and grease snails were supplied by local people during the rainy season. Individuals of similar shell sizes were selected for the study. Oysters, grease snails, and clams (Figure S1) were dissected to remove the shells. Soft tissues were washed thoroughly with filtered water, placed in separate clean glass Petri dishes, dried, and weighed (dry weight).

2.2. Procedure to identify microplastics

Four protocols with oxidizing agents (H_2O_2 33 %), acid (HNO_3 65 %, [27]), base (NaOH 5.0 M, 2.5 M and 1.0 M [28]), and enzyme, respectively were tested at the same condition (70°C in 3 hours before to 24-hour incubation at room temperature) on pure plastics of polyethylenes (LDPE, HDPE), polypropylene (PP), polystyrene (PS), polyvinyl chloride (PVC), polyethylene terephthalate (PET), polyamide (PA), poly(methyl methacrylate) (PMMA) to find out the best option for tissue removal without degrading effects on the plastic.

Enzymes are known not to cause effects on the chemical structure of the plastic resin; thus, this research employed a protease to digest the tissue of molluscs. 250 mg Trypsin was dissolved in 250 mL of 1 mM HCl to obtain Trypsin 1 mg/mL. The pH of the enzyme solution was recorded and adjusted to 3.0, which protects the enzyme's activity during storage at 2–8 °C for two weeks. In the last protocol (4), after adding Trypsin 1 mg/mL in HCl 1 mM, the solution was heated to 60 °C and kept for 3 hours, followed by 24-hour incubation at room temperature [29]. Additionally, peroxide hydrogen was utilized after enzymatic treatment. Each individual was immersed in the acidic enzyme solution with the following volumes: 10 mL for each clam and snail and 20 mL for each oyster. The bottles were covered with aluminum foil, placed on a heated magnetic stirrer, and stirred gently at 60 °C for 24 hours. The mixture was finally cooled gradually to room temperature until complete digestion.

All the digested solutions were filtered, and all the filter papers were observed directly under the ZEISS Stemi 508 Greenough Stereo Microscope with 8:1 Zoom. Every single suspicious item was then determined with the Raman microscope XploRA One 532 nm, as described in Khuyen, 2022 [11]. Each had the measured shape, color, and spectrum data, which were exported directly from the instrument.

Therefore, microplastics were sorted manually based on observing the data. The data on color and shape provide information on standard shapes and colors observed in each plastic type.

Distilled water used for rinsing bivalves, glassware, and preparing chemicals was filtered through glass fiber filters (Whatman ® GF/A, 1 6 µm pore size, 47 mm diameter). Samples were covered with aluminum foil or glass lid during the incubation to avoid airborne contamination. Quality atmospheric controls were performed during the two steps: sample treatment control (STC) and observation control (in a dark room) (OC). The controls presented very little/no airborne contamination: 1 to 5 short fibers (n = 3) in STC and 0 to 1 fiber in OC.

2.3. Procedure to identify heavy metals

The treatment of soft tissues was carried out based on guideline EPA Method 200.7 (EPA-821-R-01-010) [30]. Briefly, the dried tissue of each bivalve was incubated overnight in 30 ml of concentrated nitric acid. The solution was heated to 200 ºC until the solution was discolored and had the final volume of 2 ml. The solution was diluted and filtered through a GF filter paper 0.22 µm pore size. An autosampler injected the final solution into ICP-MS PerkinElmer NexION 2000 (USA). The metals were quantified by standard calibration method and reported as mg/kg dry weight for each type of metal in every individual mollusc.

3. RESULTS AND DISCUSSIONS

3.1. Accumulation of Microplastics

3.1.1. Sample treatment protocol selection

Strong acids [27] and bases [28] were known to give very high efficiencies in dissolving biotic samples containing proteins, carbohydrates, and lipids [31-32]. In the first protocol, the soft tissue of small bivalves - snails and clams was digested almost wholly, and oyster tissue was dissolved to a large extent in concentrated nitric acid. Despite the fast digestion, treatment with this acid resulted in color leaching and yellowing of after-treatment solutions (Figure S2. a1, e) due to the formation of reddish-brown nitrogen oxides at high temperatures [33]. Yellowed filter papers (Figure S2. a2) received after filtration will be observed as inaccurate in microplastics' color. The spectra measurement on pure plastics treated with nitric acid showed shifts of peaks of PA and intensity change of the peaks of PVC and PMMA but no effects on PE and PP. Thus, nitric acid was not selected. In the second protocol with NaOH, the treated mixture was viscous, and its color became green (Figure S2. d, e) and black (Figure S2. d) when the concentrations of NaOH increased. Furthermore, in the spiked samples of PA, PVC, and PE, the appearance of opaque white patches made the supernatant turbid, interfering with the observation of complete or incomplete tissue digestion. Moreover, alkali hydroxides were proved to be less effective for the biogenic and organic matter, for instance, wood debris, leaf, and algae that comprise cellulose or hemicellulose, chitin, lignin, tannin, and humic substances [34-35]. This hydroxide could also degrade PET [33, 36]. Therefore, sodium hydroxide was not chosen either.

Concentrated hydrogen peroxide (30 to 35 % H_2O_2) is the most common and best oxidizing agent regarding high treatment yields and the most minor effects on the chemical properties of investigated plastic types. In the third procedure, the peroxide could completely digest the tissue of small bivalves such as clams and snails (Figure S2. b1). However, it was not highly effective for more extensive seafood such as oysters (Figure S2. e) since the remaining tissue was too much in the solution to be filtered. Hence, combining this oxidizing agent with another agent is essential to speed digestion and increase its yield. Recently, enzymatic digestion with industrial proteases such as proteinase K, proteases, trypsin, and lipases [29] has been employed to dissolve biogenic matter in biotic samples with little to no effects on polymers [37] and high digestion efficiency [28]. To optimize the yields, the enzyme is used with an oxidizing agent, such as proteinase K with H_2O_2, to dissolve biofilms simultaneously with organic removal [38]. We observed that the digesting condition with trypsin did not affect the Raman spectra of pure plastics. However, the tissue dissolution was the slowest among the four protocols, especially for oysters. More importantly, the solution treated with only trypsin had a milky color, interfering with the color observation under the microscope. Microplastics were analyzed using vibrational spectroscopy methods, and the surface of microplastics strongly influences the spectra. Thus, the pure enzyme with subsequent oxidation could sufficiently purify our seafood samples.

Temperature is a vital factor in the treatment. The rise in temperature could accelerate the speed of digestion. Still, it would cause the degradation of plastics, especially when immersed in concentrated agents, such as PET digested with bases or PA digested with acids [39]. Treatment with H_2O_2 from room temperature to 70 °C was reported to have minor influences on the size, weight, discoloration, and spectra of surveyed polymers [11, 39], except for the case of weight loss of PA when being heated at high temperature [35-36]. We combined hydrogen peroxide with trypsin, and the temperature was controlled at 60 °C to maintain the enzyme activity [28] and ensure no effects on the properties of plastic types. After many of our trials, the mixture was heated with H_2O_2 33% for 12 hours for oysters and 6 hours for grease snails and clams to oxidize the matter after enzymatic digestion. At the end of the heating, the solution was let cool gradually to room temperature to limit thermally induced damage to microplastics during the exothermic reaction.

3.1.2. Microplastics extracted from the bivalves

Tiny objects were called microplastics based on shape, color, and plastic-type. They do not have biological structures such as cellular images. Their colors must be seen and homogenous. Special attention is essential for whitish and transparent particles to avoid misrecognition of the white color of filter papers [11-12]. Figure 2 describes microplastics extracted from commercial grease snails, clams, and oysters after being treated with hydrogen peroxide and trypsin. They were encountered in the forms of fibers (41.3 %), fragments (30.4 %), round beads (25.7 %), rectangle films and other shapes. Interestingly, plastic debris found in all investigated individuals had a similar appearance in terms of the color of each shape. The popular colors of fibers were purple, blue, pink, and white, and the color of non-fibers (fragments, particles, films) was white. Microplastics were all smaller than 200 µm. Colored fibers such as blue-purple with a petite length of 10 µm could also be observed. However, they were too thin to give Raman signals enough to determine their plastic-type; they were categorized into an "unidentified group."

As shown in Figure 3, polyethylene (PE) was found in any experimented molluscs. This reveals that almost all plastic debris in the sea estuary is in the status of breakdown and might be deliberately ingested by filter feeders. In clam and snail samples, PET-polyester was the most predominant (regardless of the unidentified group), whereas polypropylene dominated in oysters. PET and PA were usually encountered as fibers, while PE and PP were fragments and particles. Some PA fibers looked similar to PET fibers in shape and color, but their different spectra indicated their type. Other plastic types appeared in random shapes, such as films, tiny rounds, bars, or fragments.

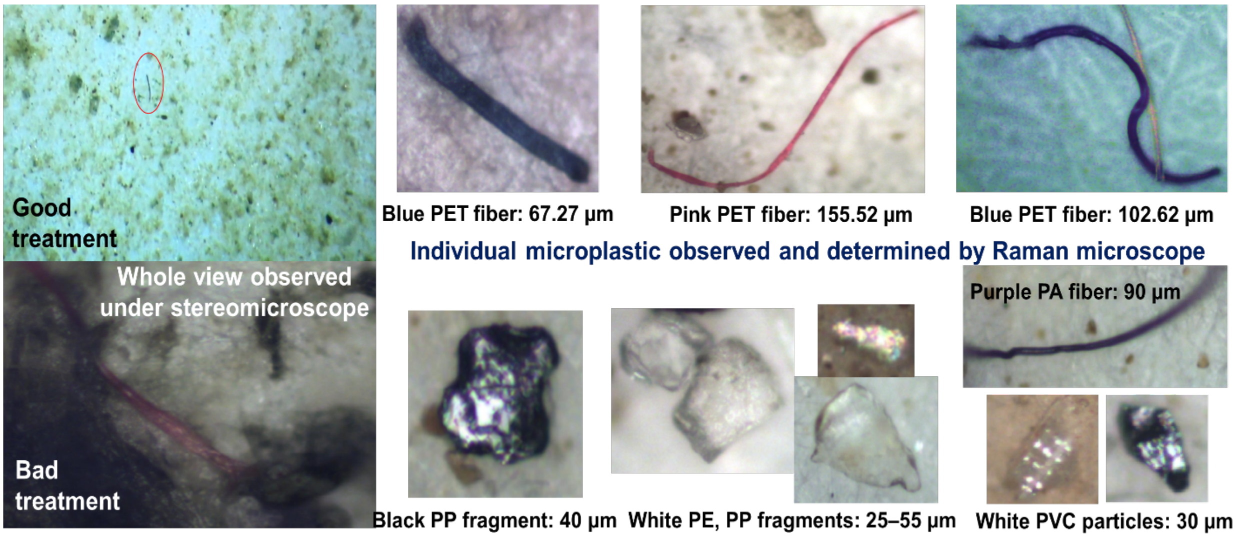

Figure 2. Common microplastics detected in soft tissue of bivalves in Can Gio.

Some other studies calculated the amount of microplastics in a particular bulk of seafood. Nevertheless, in our research, plastic items were calculated per individual piece of seafood to determine the range of plastics that can be accumulated in the seafood. The fact shows that some clams, snails, or oysters individuals did not contain a particle, while others could accumulate up to 11 particles. This is extremely dangerous since we cannot know which individuals we have eaten. The average results show that the number of microplastics in clams (average dry weight 1.3988 ± 0.1445 g) was 3.25 ± 1.35 per individual, higher than the data of previous years (only 1.33 MPs) [40]. Despite the large size and higher weight of tissue (3.0273 ± 0.2361 g), microplastics were present in oysters with a smaller amount, only 1.32 ± 0.46 MPs/individual, which was similar to the findings (1.11 MPs) of Pham et al. (2022) [40]. This value is lower than the number of oysters from French Atlantic coasts (2.1 ± 1.7 MP per individual). Almost all microplastics ranged from 50 µm to 100 µm [41], similar to our findings. The abundance of microplastics in the grease snails was the same as in clams, which was 3.27 ± 1.28 MPs in each individual of 2.4884 ± 0.8253 g. There were several studies on microplastic accumulation in shellfish, 0.36 ± 0.07 MPs/g of *Mytilus edulis* mussels [27], 0.61 ± 0.56 MPs per individual of green mussels (*Mytilus edulis*) in French Atlantic coast [41], 3 to 5 fibers per 10 g of *Mytilus edulis* and *Mytilus galloprovincialis* mussels from five European countries [42], and 2.60 ± 1.14 microplastics in the individual of Asian green mussels (*Perna viridis*) in Tinh Gia, Thanh Hoa province of Vietnam [43].

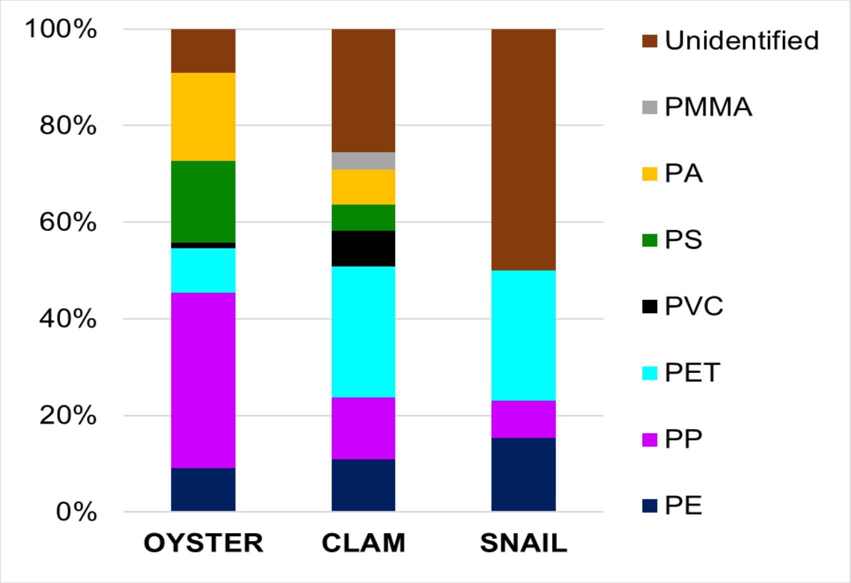

Figure 3. Types of plastic debris extracted from bivalves in Can Gio.

Microplastics are easily uptaken in aquatic organisms, especially fish and benthos [27] since they usually mistake plastic debris for food. EC50 values observe the toxicity of microplastics and are dependent on the properties of microplastics (size, abundance, plastic type). Microplastics are believed to be linked to physical disorders like behavior changes, reduced food intake, bowel obstruction [44], and serious diseases such as inflammation, hepatic stress, and reduced reproductive output [45]. The mechanisms of toxicity of microplastics on human health have not been well understood. As mentioned in a previous article, the microplastics found in our experiments were smaller than 150 µm, which could translocate across living cells to the circulatory systems and gastrointestinal lymphatics in the human body [46]. Only 0.1 % of particles larger than ten µm can go to cellular membranes and pass through the blood-brain barrier and placenta [47]. Most of the severe effects of microplastics are induced by compounds adsorbed on their surfaces, hefty metals, persistent organic pollutants, or plastic additives. In Vietnam, waste pickers or scavengers sorting rubbish on the streets, dumps, and landfills have a high risk of exposure to health hazards from plastics and other complex waste substances.

3.2. Accumulation of Heavy metals

Bivalves are an essential human nutrition source, producing more than 15 million tons annually, accounting for 14% of global marine production [48]. Due to their filter-feeding habits, bivalves, particularly oysters, can accumulate toxic substances, including heavy metals. Because of the small size, people always eat the whole body of an individual, so heavy metals will be transferred from their tissue to the human body and have the potential to cause adverse health effects. As shown in Table I, the content of heavy metals in mollusc species in Can Gio was low and within the allowable limit range of Vietnamese National Technical Regulations, except for Zn. According to ICP-MS measurement, the contents of metals were consistently highest in oyster samples, except for arsenic, in the decreasing order Zn > Cu > As > Pb > Cd > Hg, which is similar to the findings in 2017 published in Phu et al. (2021) on *Crassostrea belcheri* oyster collected from the western estuaries of Ganh Rai bay which is connected to Can Gio sea estuary [25]. Finally, mercury is a lethal metal; fortunately, no trace of mercury was found in all of our samples.

Filter-feeding molluscs prefer to accumulate copper and zinc, particularly oyster [49]. They are terrestrial elements that accumulate to extraordinary concentrations in eastern oysters, especially Zn, up to 228.228 mg/kg in the rainy season. They are exclusively sequestered in oyster amebocytes and so retained in the oyster longer than other metals, in spite of an available elimination mechanism [49-50]. Zinc (Zn) plays an important role in the activities of our body. It is a cofactor and a component of over 300 enzymes essential for catalytic activity and structural stabilization in the human and animals' body [51]. However, too high accumulation of Zinc will cause diseases such as anemia, dysfunction of the central nervous system, ulcers of the digestive system, pneumonia, and bronchitis. Copper is both essential and toxic to organisms. Firstly, it is an essential metal because it is a cofactor for enzymes which participate in making red blood cells, metabolism, energy production, supporting the immune system, protection of damage to proteins, nucleic acids, neovascularization and neuroendocrine functions. The results of metal content analysis showed that the Cu content in oysters was the highest among the molluscs surveyed, 6.834 ± 2.183 doubling than in clams. Population exposure to copper is mainly through drinking water, and the acute toxicity due to copper excess is usually considered as relatively rare conditions of exposure. The chronic toxicity of copper is involved in Wilson disease – an autosomal recessive genetic disorder, Indian childhood cirrhosis and Tyrolean infantile cirrhosis [52].

TABLE I. Concentration (mg/kg) of heavy metals in the bivalve tissues in Can Gio.

Metals	Oyster	Clam	Snail	Permissible limits[1]
Zn	228.228 ± 18.943	12.192 ± 1.012	22.193 ± 1.842	100
Cu	6.834 ± 2.183	3.761 ± 1.251	0.312 ± 0.135	-
Cd	0.330 ± 0.023	0.249 ± 0.017	0.189 ± 0.013	2
As	0.931 ± 0.059	1.714 ± 0.110	0.739 ± 0.037	1
Pb	0.437 ± 0.026	0.256 ± 0.015	0.144 ± 0.008	1.5
Hg	ND	ND[2]	ND	0.5

[1] QCVN 8-2:2011/BYT, 46/2007/QĐ-BYT; [2] ND: Not detected (below MDL)

Cadmium (Cd) and zinc and phosphorus compounds are present in nature, but unlike these nutrients, cadmium is not considered essential for life. Its contamination from drinking water is usually negligible. Tobacco plants have been documented as the dominant source of cadmium due to their ready accumulation in the soil. Food is considered the primary source of cadmium contamination for non-smokers. Shellfish, especially oysters and mussels, are among the most significant dietary sources [53]. When entering the mammal body, it is commonly found in the kidneys and liver and can be retained in the kidneys over a relatively long period, up to 10 or even 35 years. Eating foods containing high amounts of cadmium will cause digestive disorders such as nausea, vomiting, abdominal pain, and diarrhea. Long-term poisoning will damage kidney function and create kidney stones. Cadmium poisoning also causes calcium metabolism disorders, leading to bone diseases such as bone weakness, bone deformation, bone tissue destruction, osteoporosis, and bone pain [54]. Cadmium also causes damage to the respiratory tract with symptoms of rhinitis, decreased sense of smell, and loss of smell. Owing to the similar chemical properties of Zn and Cd, both elements compete to accumulate in the organism. Different from clams and oysters, the amount of Cd is not much lower than that of Cu in grease snails. Fortunately, their contents are still within the safe limit for consumption in Vietnam – QCVN 8-2:2011/BYT [55], 46/2007/QĐ-BYT [56].

Bioaccumulation occurs when an organism absorbs or excretes a substance faster than its catabolism and excretion. Thus, the longer biological half-life of a toxic substance increases the risk of chronic toxicity, even if its content is not very high at the actual time in the environment. Bioaccumulation of heavy metals by organisms may be passive or selective, which differs depending on their absorption and digestion [57]. Lead (Pb) is non-essential and has no metabolic role in ecological systems, and its poisoning can be acute or chronic. Despite its low geochemical mobility, lead is categorized as the most widespread and most hazardous heavy metal. After entering our body, it disrupts red blood cell formation, causes anemia, and damages the kidneys, bones, muscles, and nervous system. Fish and seafood are the main contributors of arsenic (As) in the diet [58]. Arsenic at low concentrations has a role in DNA methylation and metabolism of sulfur amino acids. The dominating arsenic is the organoarsenical arsenobetaine (AB). Blue mussels and other molluscs contain less AB but more arsenosugars and inorganic arsenic (iAs), including sulfur-containing arsenic compounds. If the concentration is high, it will cause poisoning, liver dysfunction, kidney failure, and motor neuron weakness. Toxic and carcinogenic inorganic arsenic is metabolized in the human body and excreted in the urine as the carcinogens dimethylarginine (DMA) and methyl arsenate (MA), producing reactive intermediates during processing [58].

4. CONCLUSIONS

This work detected plastic debris and heavy metals in popularly consumed bivalves caught and cultured naturally in the sea estuary in Can Gio. Trypsin combined with hydrogen peroxide is recommended to dissolve the soft tissue of biota samples. Our findings show the prevalence of different plastic polymer types in the tissue of oysters, grease snails, and clams, noticeably PE, PP, and PET. Most were short fibers and tiny fragments; the smallest size was 10 µm. Thus, they were considered secondary microplastics, which have spread out in the coastal ecosystems. Fish and shellfish are much more susceptible to heavy metal contamination, especially mercury and arsenic. The heavy metal contents in the molluscs cultivated and caught in Can Gio were generally lower than the acceptable limit given by Vietnamese national technique regulations issued by the Ministry of Health. Concentrations of essential metals were higher than non-essential metals in mollusc tissue, in the order Zn > Cu > As > Pb > Cd > Hg. Concerning food safety, at the investigation time, cadmium, arsenic, lead, and mercury contents were lower than the permissible limits; therefore, these bivalves are considered edible. Oyster is the best zinc source, containing the highest content of Zn, even much higher than the legal limit. This preliminary research reveals an accumulation of plastic debris and heavy metals in bivalves cultivated in Can Gio. This alerts the potential toxicity of microplastics and heavy metals if humans cannot control their seafood consumption habits. Further research on the toxicology of these pollutants on different species will be designed to have a comprehensive profile of heavy metals and microplastics in marine benthos and fishes. Pollution management is of the utmost importance to prevent and minimize the harmful impacts of plastic debris and hazardous metals on the ecosystem and human health.

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Conflicts of Interest

The authors declare no conflict of interest. The funders had no roles or decisions in the study as well as publishing the results.

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SUPPLEMENTARY INFORMATION

Figure S1. Images of oysters, clams and grease snails in Can Gio (source: the authors)

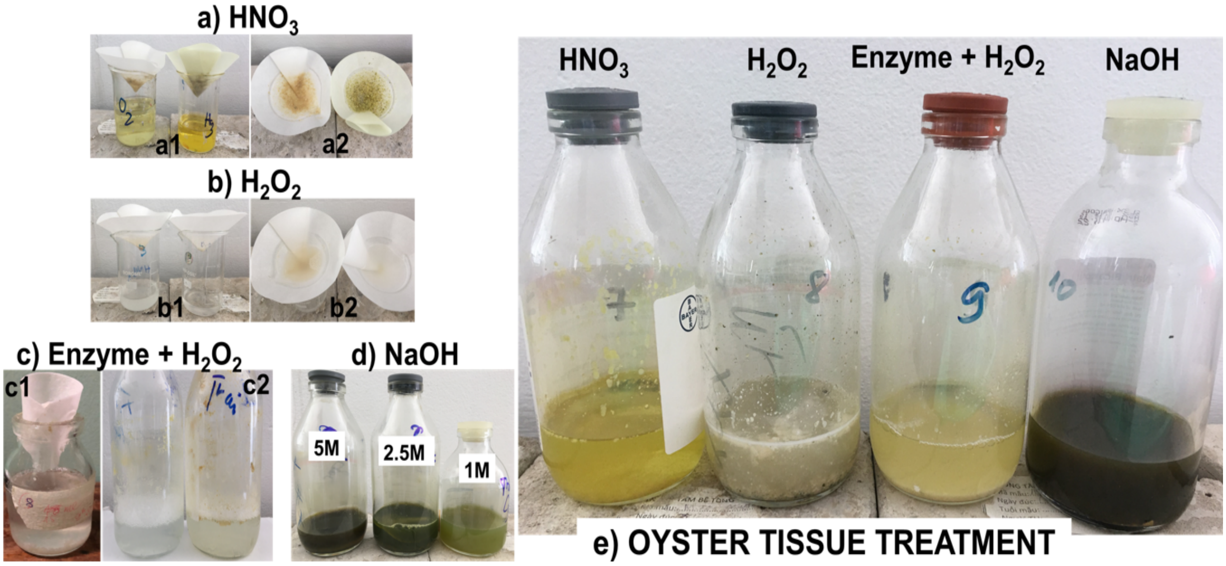

Figure S2. After digestion of bivalve's soft tissues

a) Digestion with HNO_3: a1) Dissolved snail (left) and oyster (right), a2) Undissolved remainings;
b) Digestion with H_2O_2: b1) Dissolved snail (left) and oyster (right), b2) Undissolved remainings;
c) Digestion with Trypsin: c1) After treated clam, c2) After treated oyster (left), snail (right);
d) Oyster after treated with NaOH – 5.0 M, 2.5 M, and 1.0 M;
e) After digestion of an oyster with different agents (HNO_3; H_2O_2; Trypsin - H_2O_2; NaOH).