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RESEARCH PAPER

Separation and Identification of Microplastics in Digestive System of Bivalves

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Abstract: A pretreatment method was established for separating microplastics from digestive system of bivalve sample. Qualitative and quantitative analysis of microplastics was carried out by micro-Fourier transformed infrared (μ-FT-IR) spectroscope and Stereo microscope. The method was applied to analyze the microplastics in the digestive system in *Chlamys farreri* and *Mytilus galloprovincialis*. The results showed that the digestion system of using 10% KOH had high digestion efficiency. With this digestion system, the recoveries of polypropylene (PP), polyethylene (PE), polystyrene (PS) and polyvinyl chloride (PVC) ranged from 96.7% to 98.6%, with relative standard deviation (RSD, n = 3) of ≤ 3.19%. We collected *Chlamys farreri* from local markets (n = 50) and *Mytilus galloprovincialis* from both local markets (n = 50) and wild environments (n = 15) in Qingdao, China. The results showed that microplastics were found in over 80% of the individuals purchased from the market and 40% of the wild collected individuals. The average abundance of microplastics in *Chlamys farreri* purchased from different markets varied between 5.2 and 19.4 items/individual or between 3.2 and 7.1 items g^{-1} (wet weight of digestive system), while in *Mytilus galloprovincialis*, the numbers varied between 1.9 and 9.6 items/individual or between 2.0 and 12.8 items g^{-1}. Farmed mussels (*Mytilus galloprovincialis*) contained more microplastics (average 1.9 items per individual, 3.17 items g^{-1}) than wild mussels (average 0.53 items individual, 2.0 items g^{-1}). Three shapes of microplastics, including fibers, fragments and granules were separated from the samples above. Among which, fibrous microplastics, being the most dominant ones, took up 84.11% of total microplastics. The average size of fibrous microplastics ((0.66 ± 0.70) mm) was larger than that of the other two shapes of microplastics. The number of microplastics decreased with increasing microplastic sizes. Microplastics of less than 500 μm coming from different markets were in the range of 26% to 84%. And it was found that the most common polymer component in the samples was cellophane (CP), followed by polypropylene (PP). The method has some advantages such as simplicity, high efficiency, and low damage to the microplastics in the sample, and can be used to detect and analyze microplastics in seafood.

Key Words: Microplastic; Bivalves; Digestive system; Microplastic pollution; Infrared spectroscopy

1 Introduction

Plastics and their products are widely used by humans. While bringing convenience to human life and social production, a large amount of plastic wastes accumulate and pollute our environment. It is reported that more than 300 million tons of plastics are produced each year in the world, and about 10% of plastics will enter the ocean through the river or other ways[1]. Larger plastic debris is broken down into small pieces through collective chemical, physical forces and biological degradation[2]. When the small pieces of plastic have the longest dimension of less than 5 mm, they are called microplastics[3]. Plastics can stay in the oceans for hundreds or even thousands of years due to their relatively stable chemical

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properties[4]. Microplastics, with relative large surface area and hydrophobic properties, can also adsorb persistent organic pollutants (POPs)[5,6] and heavy metals[7]. Microplastics can float at the sea or sink and accumulate in the sediments[8]. When microplastics with adsorbed pollutants are ingested by zooplankton and benthic animals, they would have direct toxic effects on the organisms and be enriched and transferred through the food chain[9–12]. The result of laboratory experiments illustrated that desorption rate of pollutants in the microplastics under gut conditions could be up to 30 times greater than in seawater alone[13]. As a result, microplastics in the marine environment might eventually endanger human health.

Bivalves are often used as model organism in marine environmental assessment because they are dominant filter feeders. Microplastics in these bivalves ingested can easily enter the human body through the food chain. Although the biotoxicity of the microplastics ingested by these creatures has not been confirmed yet, studying the distribution of microplastics in the organisms is of great importance for evaluating their impacts. Therefore, it is of significant value to investigate and monitor the abundance of microplastics in those commercial bivalves to secure seafood safety. In the previous studies, the whole soft tissue of commercial bivalves are used to investigate the abundance of microplastics[14,15]. There are very few studies on using only the digestive systems of bivalves, and also the sample treatment methods still need to be improved. Since the digestive systems in bivalves are where microplastics accumulate, it is possible to detect microplastics in the digestive system of bivalves because it can reflect the abundance of microplastics in the animal.

The key step for separating microplastics from biological samples lies in the effective digestion of organic matter and does not affect the qualitative and quantitative analysis of microplastics. Currently, there are mainly three pretreatment methods for separating microplastics, including acid, alkaline, and enzymatic digestion[16]. Acid digestion may digest some types of microplastics while digesting biological samples[16]. For example, van Cauwenbergheetal *et al*[17] found only low abundance of microplastics (0.36 items g^{-1}) without any fibrous microplastics in mussels when using 69% HNO_3 as the treatment solution. In addition, nylon fibers were reported missing by Catarino *et al*[18] after overnight digestion with concentrated HNO_3 solution. Alkaline digestion can not only effectively remove organic matter, but it can ensure the qualitative and quantitative analysis of microplastics by controlling the alkaline solution concentration. For instance, Cole *et al*[19] reported that NaOH solution (1 M) has a high digestion rates (up to 90%) on plankton-rich seawater samples at room temperature. But the concentration of NaOH solution reached 10 M, it could damage some types of particles[19]. Enzymatic digestion is gentle to the microplastic, however, the high costs of enzymes greatly limit its application. The separated microplastics need to be further characterized for composition. There are multiple methods of microplastics identification, including pyrolysis gas chromatography/mass spectrometry (Pyr-GC/MS), Raman spectroscopy, and Fourier Transform Infrared (FT-IR) spectroscopy. Both Pyr-GC/MS and Raman spectroscopy are sensitive to the additives and other pollutants, which interfere with polymer type identification. Pyr-GC/MS would completely destroy the microplastics in the process of identifying polymers types, resulting in non-recyclable sample. FT-IR spectroscopy is a common and wildly used identification method. This method requires the samples to be dried before analysis[1].

In the present study, we collected *Chlamys farreri* and *Mytilus galloprovincialis* from five main local markets and natural wild *Mytilus galloprovincialis* from Huangdao district in Qingdao, China, and established a bivalve sample pretreatment method for separating the microplastics from their digestive systems. The abundance of microplastics in the digestive systems of *Chlamys farreri* and *Mytilus galloprovincialis* from local markets was investigated, and the abundance of microplastics between farmed and wild *Mytilus galloprovincialis* was compared. The research provided both methodological guidance and environmental assessment information to assess the microplastics pollution status and potential ecological risk in the coastal water of Qingdao, China.

2 Experimental

2.1 Instruments and reagents

Micro-Fourier Transformed Infrared Spectroscope (Thermo Nicolet iN 10MX, USA) and Nikon SMZ1270 Stereo microscope (Nanjing Hengqiao Instrument Co., Ltd., China) were used in this experiment for the qualitative and quantitative analysis of microplastics. Zhichu ZQLY-180S oscillation incubator (Shanghai Zhichu Instrument Co., Ltd., China) was used for the digestion. FSH-2A adjustable high-speed homogenizer (Jintan Chengxi Zhengrong Experimental Instrument Factory, China), KQ-400KDE high-power digital ultrasonic instrument (Kunshan Ultrasonic Instruments Co., Ltd., China), and SHB-III A circulating water multi-purpose vacuum pump (Zhengzhou Changcheng Science Industry & Trade Co., Ltd., China) were used for the sample treatment. Electric thermostat blast drying oven (Shanghai Jinghong Experimental Equipment Co., Ltd., China) was used for sample drying. Ultrapure water (18.2 MΩ cm) obtained through a Milli-Q system (Millipore Co. USA) was used throughout the experiment. Electronic balance ME104/02 (Mettler-Toledo Instruments Co., Ltd., Shanghai, China) was used for weighing samples.

Four different microplastics standards (PP, PE, PS and PVC, particle size: 50 μm) were obtained from Shanghai YangLi Electrical Technology Co., Ltd., China. Potassium hydroxide and 30% H_2O_2 purchased from Sinopharm Chemical Reagent

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