



Bioaccumulation of microplastics and its *in vivo* interactions with trace metals in edible oysters



Xiaotong Zhu^a, Liyuan Qiang^a, Huahong Shi^a, Jinping Cheng^{a,b,*}

^a State Key Laboratory of Estuarine and Coastal Research, East China Normal University, Shanghai, 200062, China

^b State Key Laboratory of Marine Pollution & Department of Ocean Science, School of Science, Hong Kong University of Science and Technology, Clear Water Bay, Kowloon, Hong Kong, China

ARTICLE INFO

Keywords:

Microplastics
Oyster
Bioaccumulation
Translocation
Trace metals
In vivo interactions

ABSTRACT

Microplastics have been an emerging threat to marine environments and marine life. Oysters as seafood are popular worldwide, yet also a high-risk group to accumulate pollutants due to their filter-feeding nature. In this study, edible oysters were collected from Yantai, a coastal city in China, to study the uptake, accumulation and translocation of microplastics in marine life, as well as their *in vivo* interactions with other persistent pollutants. Microplastics were found in all of the studied oyster tissue samples with an average concentration around 4.53 items/g wet weight (24.49 items/g dry weight). Microplastics were mainly translocated and distributed in the gills and mantle of the studied oysters in the form of fibers. The detected microplastics in the oysters were mostly cellophane and polyester as identified by the micro-Fourier transform infrared spectroscope. Cellophane tends to accumulate in gills, mantle and muscle while polyester tends to accumulate in digestive glands of the studied oysters. Excessive concentrations of trace metals (30.484, 4.415, 0.395 and 181.044 $\mu\text{g/g}$ dry weight of Cr, Cd, Pb and Cu, respectively) were detected in the studied oysters using inductively coupled plasma mass spectrometry. Trace metals of Cr, Mn, Ni, Cu, Zn, Cd and Pb were found to be adsorbed on the surface of microplastics isolated from the oysters. There was a correlation between the *in vivo* concentration of microplastics and the *in vivo* concentration of four trace metals: Cd, Cr, Cu and Pb, which suggests potential *in vivo* interactions between microplastics and trace metals. This study can help to understand the impacts of microplastics and their joint toxicity with other pollutants on marine life, especially on aquaculture seafood. This study will be an important reference for the assessment of health risks associated with consumption of edible coastal oysters in China.

1. Introduction

Microplastics are plastic particles with a diameter of < 5 mm (Andrady, 2011). Some of the microplastics in the environment originate from personal care products such as cosmetics (Fendall and Sewell, 2009), and man-made plastic fibers like polyester and additives (Browne et al., 2011). Others are derived from larger plastic pieces or particles (Andrady, 2011). Microplastics can hardly degrade under natural conditions and cannot be removed with the current sewage treatment processes. This is because microplastics are small in size and have stable physicochemical properties (Cózar et al., 2014). Microplastics have been widely detected in the marine environment (Corcoran et al., 2015; Klein et al., 2015), even in the Arctic polar area (Lusher et al., 2015b).

There is an increasing health risk concern regarding the potential

ingestion of microplastics by aquaculture products and subsequent bioaccumulation and biomagnification along food chains. Microplastics have been increasingly detected in a wide range of marine organisms, including marine aquaculture species such as oysters and mussels (Table 1). In particular, microplastics with a size smaller than $20 \mu\text{m}$ are more difficult for marine life to eliminate. It has been reported that microplastics cannot be effectively depurated in *Potamocorbula* or *M. edulis* (Cauwenbergh and Janssen, 2014). The accumulation of microplastics in planktons (Desforges et al., 2015; Kang et al., 2015) can be further amplified along the food chain (Moore et al., 2001; Mallory et al., 2006). Histological evidence has shown that microplastics can be spread to the intestines, lumina of primary and secondary ducts, and the tubules of digestive glands of marine organisms (Moos et al., 2012; Sussarellu et al., 2016). The waterborne exposure to microplastics can enhance microplastic accumulation in filter-feeding marine organisms

* Corresponding author at: State Key Laboratory of Marine Pollution & Department of Ocean Science, School of Science, Hong Kong University of Science and Technology, Clear Water Bay, Kowloon, Hong Kong, China.

E-mail address: jincheng@ust.hk (J. Cheng).

<https://doi.org/10.1016/j.marpolbul.2020.111079>

Received 28 September 2019; Received in revised form 15 March 2020; Accepted 16 March 2020

Available online 29 March 2020

0025-326X/© 2020 Elsevier Ltd. All rights reserved.

Table 1
Reported detection of microplastics in marine life in different regions.

Country	Target	Reported concentration	Reference
China	Oyster <i>C. gigas</i>	35.6 ± 11.3 items/g, d.w.	This study
	Bivalves (<i>Sc. subcrenata</i> , <i>T. granosa</i> , etc.)	2.1–10.5 items/g	(Li et al., 2015)
	Mussel <i>M. edulis</i>	2.7 items/g wild groups and 1.6 items/g farmed groups	(Li et al., 2016)
Canada	Copepod <i>N. cristatus</i>	1/34 item/organism	(Desforges et al., 2015)
	Zooplankton <i>Euphausiids</i>	1/17 item/organism	
France	Mussel <i>M. edulis</i>	0.2(0.3) items/g	(Cauwenberghe et al., 2015)
German	Oyster <i>C. gigas</i>	0.47 ± 0.16 items/g	(Cauwenberghe and Janssen, 2014)
	Mussel <i>M. edulis</i>	0.36 ± 0.07 items/g	
France	Atlantic herring <i>C. harengus</i>	11 particles or 1.89 mg/ stomach	(Collard et al., 2015)
	Sardine <i>S. pilchardus</i>		
	European anchovy <i>E. encrasicolus</i>		
South Africa	Mullet <i>M. cephalus</i>	3.8 items/individual	(Naidoo et al., 2016)

(Moos et al., 2012). Previous studies also employed lugworms, sea cucumbers and mussels as objects to study the bioaccumulation of microplastics. Results have shown that exposure to microplastics induced inflammation, accumulation of microplastics in the circulatory system, as well as reduction of ingestion activity (Browne et al., 2008; Graham and Thompson, 2009; Moos et al., 2012; Besseling et al., 2013). In addition, exposure to high-density polyethylene (PE) microplastics reduced the stability of lysosomal membranes in oysters (Moos et al., 2012). Microplastic exposure also imposed inheritable negative impacts on the reproductive system of *Xenopus laevis* (Tussellino et al., 2015).

New evidence suggested a potential pollutant carrier role of microplastics. Microplastics was shown to adsorb and accumulate pollutants from seawater (Engler, 2012; Avio et al., 2015). Currently, most of the studies on the carrier role of microplastics have focused on organic pollutants. Polychlorinated biphenyls (Engler, 2012), as one of the persistent, bioaccumulative and toxic chemicals, were reportedly present in freshwater along with micro-polyethylene, in a linear relationship with Freundlich exponents (n) close to 1 (Velzeboer et al., 2014). Trace metals can also be adsorbed by microplastics in the sediment and further exposed to marine life in a combined manner (Ashton et al., 2010; Holmes et al., 2012). It has been reported that the increased adsorption of trace metals, such as cadmium (Cd), cobalt (Co), lead (Pb) and nickel (Ni), in microplastics can be affected by pH and salinity of water (Holmes et al., 2014).

Oysters are saltwater bivalve mollusks which live in estuarine or brackish habitats. As filter feeders, oysters ingest food through filtering large volumes of seawater. During the filtering process, oysters can uptake pollutants in marine water (Abdou et al., 2018). Therefore, oysters are often used as the model organism to monitor pollution levels in marine environments (Tan et al., 2017). Oysters are commonly cultured and consumed by human because of their high productivity and nutritional value. Oyster production in China has surpassed 4.83 million tons in 2016 with a 5% annual growth. In particular, Shandong province in China is known for its abundance of seafood, and oyster production in Shandong occupied 18.1% of the total in China (MAFB, 2017). Study on pollutants associated with the cultured oysters will be a beneficial reference for aquaculture farms and future risk assessments.

This study employed edible oysters from an aquaculture farm to study the bioaccumulation and *in vivo* distribution of microplastics in marine life, especially in aquaculture products. Concentration, form and composition of microplastics in different tissues of oysters were investigated to understand their entry pathways. The trace metal contents in oysters were also examined, and the *in vivo* interactions between trace metals and microplastics were further studied. This study can help to understand microplastic and metal contamination in a typical seafood oyster in China.

2. Materials & methods

2.1. Sample collection

Pacific oysters (*C. gigas*) of 11–13 cm shell length were randomly collected from a coastal commercial oyster farm in Haiyang, Yantai, Shandong, China. The oyster samples were cultured on sediments at 2–5 m deep for 3–4 years before collection in November 2015. A total of 40 oyster samples were collected, packed in boxes with ice bags and transported to the laboratory within three days. The oyster samples were kept in the laboratory at 4 °C for further experimental use. The whole study was completed in three months.

2.2. Isolation of microplastics from oysters

The soft tissues of oysters were extracted using a scalpel (#10, Feather, Japan) and sealed using aluminum foil and kept at –20 °C. Microplastics present in dissected soft tissues were isolated through chemical digestion using hydrogen peroxide (H₂O₂). Extracted soft tissues were added to conical flasks with 30% H₂O₂ (Analytical grade, Chinese Medicine Group Pharmaceutical Limited by Share Ltd., Shanghai, China), which were filtered over glass fiber filter membranes (GF/B, 1.0 μm pore size and 47 mm diameter, Whatman, U.K.). After 24 h in room temperature, the decomposing liquid was diluted five times with saturated NaCl solution (Analytical grade, Chinese Medicine Group Pharmaceutical Limited by Share Ltd., Shanghai, China), which was filtered over glass fiber filter membranes (GF/B, 1.0 μm pore size and 47 mm diameter, Whatman, U.K.). After resting in room temperature for 12 h, the solution was filtered over a 5.0 μm pore size, 47 mm diameter mixed cellulose ester membrane (SMWP04700, Millipore, Massachusetts, U.S.A.) using a vacuum system (JINTENG, Tianjin, China). The filter membrane containing the isolated microplastics was then placed in a clean petri dish for further observation and analysis. A total of 20 oysters ($n = 20$) were used for the isolation of microplastics, of which 11 oysters ($n = 11$) were used for the component analysis of microplastics using micro-Fourier transform infrared spectroscopy.

2.3. Abundance and distribution of microplastics in oyster tissues

Soft tissues of 20 oysters ($n = 20$) were extracted and divided into three parts: digestive glands, gills and mantle, and muscles. Microplastics in each part of every oyster were isolated as described above. The filter membranes containing isolated microplastics were observed under a Carl Zeiss Discovery V8 Stereo microscope (MicroImaging GmbH, Göttingen, Germany) at 80 × magnification. Images of each microplastic piece were recorded with an AxioCam digital camera (Carl Zeiss AG, Germany). The total number of microplastics in each soft tissue part was counted to determine the concentration and *in vivo* distribution of microplastics in different organs of

oysters ($n = 20$). Forms of microplastics were divided into fibers and fragments through differences in physical morphology.

2.4. Component analysis of microplastics using micro-Fourier transform infrared spectroscopy

Microplastics on filter membranes of each tissue part (in total of three parts: digestive glands, gills and mantle, and muscles) from 11 oysters ($n = 11$) were picked out using a dissecting needle under an Olympus DP80 microscope (Olympus Corporation, Japan). Isolated microplastics of similar color, shape and size were divided into groups based on a detailed examination under the microscope. Microplastic sample from each group was randomly selected for further component analysis. The component analysis of microplastics was performed via micro-Fourier transform infrared spectroscopy (μ -FTIR) (Nicolet iN10 MX, Thermo Fisher Scientific). The spectrum range was set to the mid-infrared region of $4000\text{--}500\text{ cm}^{-1}$ with 128 co-scans and with a spectral resolution of 4 cm^{-1} . The transmittance mode was set for transparent and semi-transparent particles and an attenuated total reflection mode was set for colored particles. All the collected spectra were then compared with the library (Hummel Polymer and Additives, Polymer Laminate Films) to verify the polymer type. A matching rate of over 60% was accepted.

2.5. Quantitative analysis of trace metals using inductively coupled plasma mass spectrometry

The whole soft tissues of seven oysters ($n = 7$) were dissected and freeze-dried using the freeze dryer (Sigma-Aldrich, St. Louis, U.S.A.). Freeze-dried soft tissues were crushed in a ceramic mortar to obtain homogenous powder samples. For each sample, 1.0 g of the dry powder were taken for quantitative detection of microplastics. Microplastic extraction was performed as described in 'Isolation of microplastics from oysters'; microplastic quantification was conducted as described in 'Abundance and distribution of microplastics in oyster tissues'. Then, for each sample, another 0.2 g of the dry powder were used to examine the concentration of metals in the oysters. The digestion process was performed in a fume cupboard; the 0.2 g of dry powder were added to a Teflon digestive bottle with 2.0 mL of 67% HNO_3 (Guaranteed grade, Chinese Medicine Group Pharmaceutical Limited by Share Ltd., Shanghai, China). The Teflon bottles were placed in an oscillating incubator for 2 h at $180\text{ }^\circ\text{C}$ until the solutions were clear, indicating that the samples were completely digested. Each solution was added with 1.0 mL of 30% H_2O_2 (Analytical grade, Chinese Medicine Group Pharmaceutical Limited by Share Ltd., Shanghai, China) and then heated at $120\text{ }^\circ\text{C}$ until only 0.2 mL remained. Solutions were then transferred to a 5.0 mL centrifuge tube. Finally, 4.8 mL of Milli-Q water (Millipore, $18.2\text{ M}\Omega\cdot\text{cm}$) was added to the solution with a final HNO_3 concentration of 2% for further analysis. The quantitative content analysis of trace metals in the solution was performed via inductively coupled plasma mass spectrometry (ICP-MS) (Neptune, Thermo Fisher Scientific). Four trace metal elements were selected: Cd, Pb, chromium (Cr) and copper (Cu).

2.6. Surface elements analysis by scanning electron microscope and energy dispersive X-ray spectroscopy

To understand the surface characteristics of microplastics isolated from oysters, a total of nine representative particles were examined using the scanning electron microscope (SEM) ($n = 9$). Isolated microplastic particles of both fiber and fragment forms were picked out with a dissecting needle under a Carl Zeiss Discovery V8 Stereo microscope (Microlmaging GmbH, Göttingen, Germany) at $80\times$ magnification. The samples were then taped on an aluminum sample holder with conductive carbon tape and plated with platinum (Pt). Morphological and chemical characterizations of microplastic samples

were performed using the field emission SEM (S-4800, Hitachi) equipped with an energy dispersive X-ray spectroscopy system (EDX) (Oxford INCA, Oxford, U.K.). By operating at 20 keV under backscatter mode, qualitative elemental composition of the surface particles was detected. Several trace metals were detected by SEM/EDX analysis including Cd, Cr, Cu, Ni, Pb, manganese (Mn) and zinc (Zn).

2.7. Statistical analysis

All of the results were expressed as mean \pm standard deviation (SD). The original data were analyzed using SPSS 17.0 (SPSS Inc., Chicago, U.S.A.). Difference in microplastic content in oyster tissues was tested using two-tailed t -test. Spearman's rank correlation was used to assess the degree of association between two variables. The diagrams were generated using GraphPad Prism 5.0 (GraphPad Software Inc., San Diego, U.S.A.) and Origin 8.5 (OriginLab Inc., Northampton, U.S.A.).

3. Results

3.1. Microplastics detected in the studied oysters

In this study, the average detected tissue microplastic content in the studied oysters was calculated through a weighted mean. Results reported a value of 4.53 items/g w.w. (24.49 items/g d.w.), and 35.6 items/g d.w. (7.12 items/g w.w) for another set of data using freeze-dried tissues. Two forms of microplastics, fibers and fragments, were found in the studied oyster samples (Fig. 1. A & B). Most fibers were dark blue or hyaline, whereas fragments had various colors such as red, yellow, blue and white. Fragments were usually $< 0.2\text{ mm}$ in size, much shorter than thread-like fibers. There is a higher concentration of fibers than fragments in the studied oysters (Fig. 1. C), although the difference is statistically insignificant.

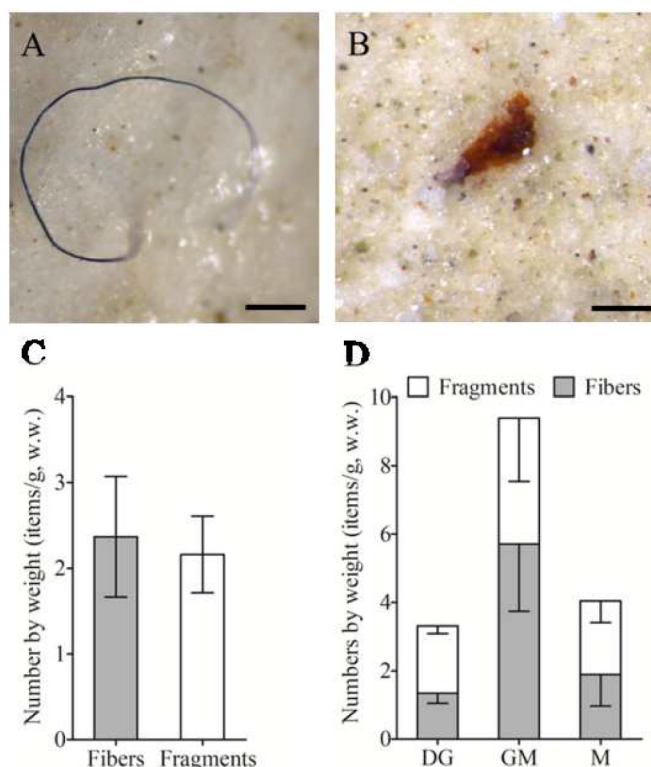


Fig. 1. Presence of microplastics (A & B) in the studied oysters. Concentrations of microplastics in the form of fibers and fragments (C), and the *in vivo* distribution of microplastics in major tissues of Pacific oysters (D). Results were expressed as mean \pm SD. Scale bars = $200\text{ }\mu\text{m}$. DG = Digestive glands; GM = Gills and mantle; M = Muscles. $n = 20$.

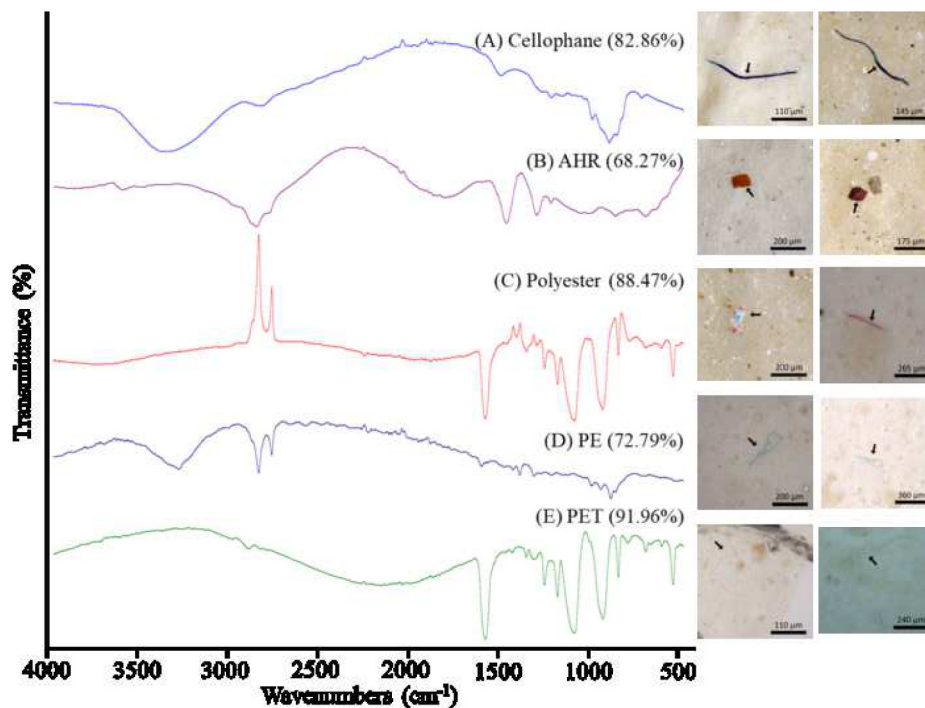


Fig. 2. Component analysis of microplastics using μ -FTIR. The spectra on the left indicates the matching values of representative microplastic samples. AHR: aromatic hydrocarbon resin; PE: polyethylene; PET: polyethylene terephthalate. The arrows on the pictures on the right indicate the microplastic samples and its form. $n = 11$.

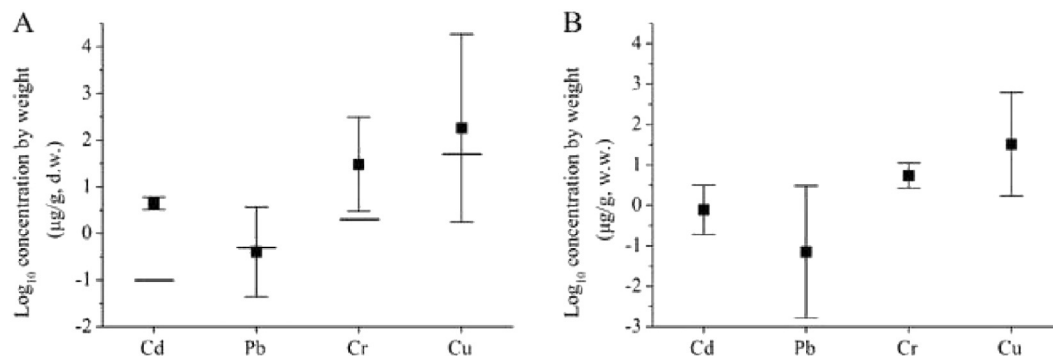


Fig. 3. Detected trace metal concentrations in Pacific oysters by dry weight (A) and wet weight (B). Short horizontal lines represent the metal concentration limit standardized by GB18406.4 - 2001. Results were expressed as mean \pm SD. $n = 7$.

As shown in Fig. 1. D, over half (56.06%) of the microplastics were found in the gills and mantle of oysters. On the other hand, the remaining amounts of microplastics in digestive glands (3.31 items/g, w.w.) and muscles (4.05 items/g, w.w.) were similar. Microplastics detected in gills and mantle were mainly in the form of fibers ($p < 0.05$, two-tailed t -test) while microplastics detected in digestive glands were mainly in the form of fragments ($p < 0.001$, two-tailed t -test). In the muscles of the studied oyster samples, fibers (46.65%) and fragments (53.35%) had similar detection frequencies. The recorded differences suggest that microplastics in the form of fibers and fragments utilize different pathways to enter the oysters.

3.2. Identification of microplastics in studied oysters

The results of μ -FTIR test showed that five main types of microplastics were detected in oysters: polyester, aromatic hydrocarbon resin (AHR), PE, polyethylene terephthalate (PET) and cellophane (Fig. 2). In oysters, polyester, cellophane and AHR accounted for nearly 80% of all microplastics. PE and PET accounted for just below 20% of microplastics in oysters.

Microplastic particles which had similar color, shape and size were picked out for μ -FTIR analysis, and the results demonstrated that microplastic particles with similar morphological features were of the same components (Fig. 2). In this study, cellophane was the most widely distributed and abundant microplastic in the studied edible oysters. The accumulation of cellophane and polyester, which were mostly fibers, were more likely to occur in gills and mantle, matching previous observations. PE and PET were the least common types of microplastics in the studied oysters. And in terms of *in vivo* distribution, PE and AHR were found to be equally distributed among the three studied soft tissue parts of oysters.

3.3. Tissue metal content and *in vivo* interaction between microplastics and trace metals in oyster tissues

For the following results, the average concentration of microplastics in the sample oysters was reported to be 35.6 items/g d.w. (7.12 items/g w.w.). The concentrations of Cd, Cr, Cu and Pb in studied oysters were detected by ICP-MS (Fig. 3A & B). In this study, tissue trace metal content analysis results showed that Cu had the highest concentration

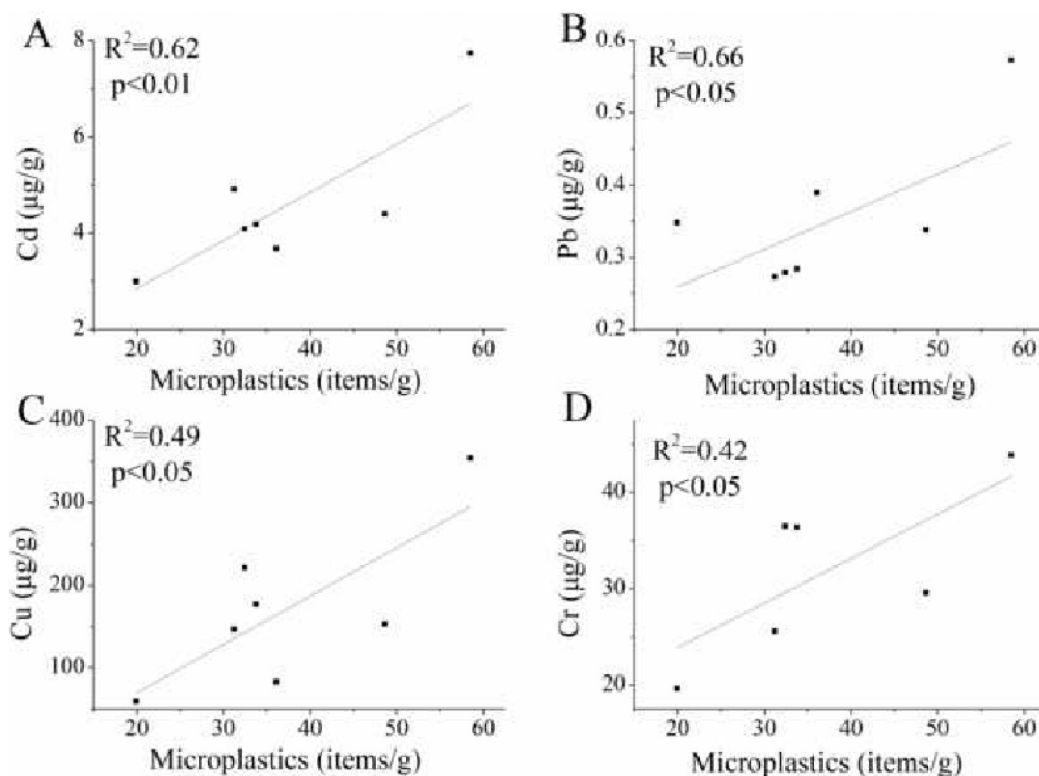


Fig. 4. Correlation between *in vivo* microplastic concentration and concentration of trace metals Cd (A), Pb (B), Cr (C) and Cu (D) in Pacific oysters. Straight lines represent linear regression analysis. The coefficients of determination (R^2) and p -values are indicated. $n = 7$.

of 181 μg/g, d.w. while Pb had the lowest concentration of 0.395 μg/g, d.w.. The mean concentrations of Cd and Cr were 4.06 and 29.51 μg/g, d.w., respectively. As shown in Fig. 3A, concentrations of Cd, Cr and Cu in oysters exceeded Chinese standards for aquatic products (GB18406.4-2001; GAQSIQ, 2001). To put it in perspective, Cd concentration exceeded by 40 times the standard (0.1 μg/g, d.w., GB18406.4-2001), Cr concentration exceeded by 15 times (2.0 μg/g, d.w., GB18406.4-2001) and Cu concentration exceeded by three times (50 μg/g, d.w., GB18406.4-2001). Only Pb concentration was below Chinese standards for aquatic products (0.5 μg/g, d.w., GB18406.4-2001).

The relationships between microplastic concentrations in oyster and precipitation of detected trace metals Cd, Pb, Cu and Cr on microplastics were shown in Fig. 4. Linear regression was used to model the relationship. The results of the regressions are as follows:

$$C_{Cd} = (0.10 \pm 0.03) \times C_{\text{microplastics}} + (0.86 \pm 1.18) \quad (R^2 = 0.62);$$

$$C_{Pb} = (0.0059 \pm 0.0027) \times C_{\text{microplastics}} + (0.13 \pm 0.10) \quad (R^2 = 0.66);$$

$$C_{Cu} = (5.86 \pm 2.25) \times C_{\text{microplastics}} - (47.56 \pm 87.84) \quad (R^2 = 0.49).$$

$$C_{Cr} = (0.46 \pm 0.21) \times C_{\text{microplastics}} + (14.66 \pm 8.50) \quad (R^2 = 0.42);$$

Significant correlations were found ($p < 0.05$) as all concentrations of Cd, Pb, Cu and Cr in oysters increased following the rise of microplastic concentration. These results suggested that microplastics may be responsible for *in vivo* adsorption and precipitation of trace metals such as Cd, Pb, Cu and Cr in the studied oyster.

3.4. Surface element analysis of microplastics in oyster tissues

The SEM images of microplastics in oysters were presented in Fig. 5A& B. As shown in Fig. 5C, D& E, the EDX analysis data demonstrated the presence of Cr, Mn, Ni, Cu, Zn, Cd and Pb on the surface of microplastics isolated from studied oysters. Metal Pb was shown to

precipitate and adsorb on every detected plastic particle with high detection frequency. Furthermore, Pb also had the highest weight compared to the other three metal elements, which also means that it had the highest *in vivo* concentration. Results showed that microplastics can adsorb and enrich heavy metal elements on their surface as four elements Cd, Cr, Cu and Pb were quantitatively detected on the surface of the microplastics. This effect has broader applications as potential *in vivo* adsorption of up to seven metal elements were also recorded on the surface of microplastics inside the oyster.

4. Discussion

The present study aimed to investigate the increasing human health risk concerns arising from microplastics invading food chains involving commercial aquaculture species. Bioaccumulation of microplastics within Pacific oysters is a fundamental variable to be monitored and studied. In this study, microplastic concentration in oysters is in line with a previous study on microplastics in commercial bivalves in China (Li et al., 2015), meaning a similar concentration of microplastics has been accumulated on both occasions over time. Bioconcentration factor (BCF) is a metric used to assess such accumulation of pollutants from environment to organism. The BCF for microplastics in oysters in this study is defined as follows:

$$BCF = \frac{C_{\text{biota}}}{C_w}$$

where C_{biota} is the microplastic concentration in oysters in this study (items/g, d.w.) and C_w is the microplastic concentration in surface water of the sampling area (items/g). According to Wang et al. (2018), microplastics in surface seawater of the Yellow Sea region had a concentration of 0.324×10^{-6} items/g. Therefore, the calculated BCF for microplastics in oysters can reach 1.09×10^8 . Currently, the production of oysters have reached over six million tons in the whole world and China contributed 80% of the total productivity (MAFB 2017). If all

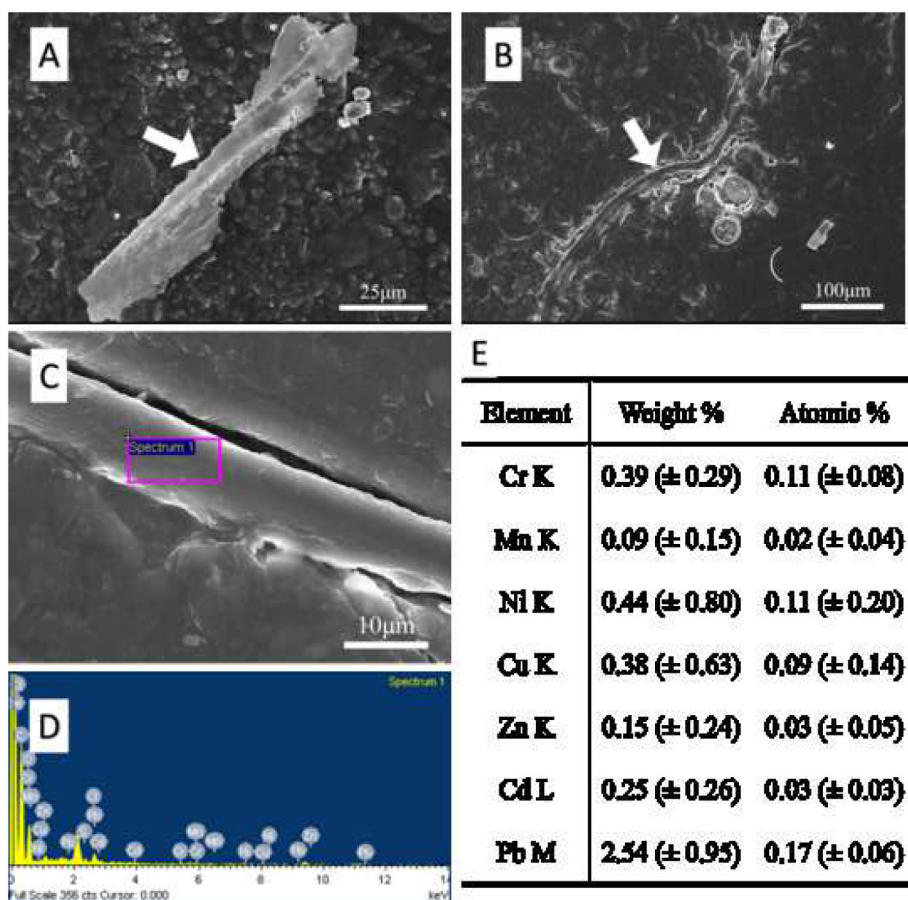


Fig. 5. Surface element analysis of microplastics detected in Pacific oysters using scanning electron microscopy (SEM). Microplastic fragment (A) and fiber (B) on filter membrane, with intact morphological features, are indicated by the arrows. Agglomerates adsorbed on the microplastic surface (C) were analyzed using the energy dispersive X-ray spectroscopy (EDX) (D) (E).

the cultured oysters were consumed by human, using the calculated BCF, an estimate of 1.7×10^{12} microplastic particles would be transferred into human body through oysters; each human consumer on average would take up 16,320 microplastic particles solely through oysters. Although the estimated number may not seem overwhelming, it justifies a need for constant monitoring.

Half of the microplastics in the studied edible oysters were found in the gills and mantle. In oysters, the gills are responsible for filtering marine water and then passing materials and organic matters to other tissues. The current result indicated that the filter-feeding through the gills is indeed a major pathway for microplastics to enter oysters. This is also in conformity with previous study reporting accumulation is more likely to occur in gills of marine life (Brennecke et al., 2015). Gills in oysters can be a major protective organ against the accumulation of allogeic materials (Azevedo et al., 2015). Gills are also important for metabolic processes of marine life. According to the study by Trevisan et al. (2016), after CNDB (1-chloro-2, 4-dinitrobenzene) exposure, the consumption and upregulated synthesis of glutathione (GSH) occurred in gills of oysters. Gills act as a GSH-dependent metabolic barrier in oysters, protecting oysters from oxidative attack, so it is possible that microplastics inside of oysters can have side effects on gills as a source of disturbance. As previously shown, most microplastic accumulation tended to occur in gills and mantle, of which approximately two thirds of the microplastics were in the more common form of fibers. Microplastics have two main ways to translocate *in vivo* after entering marine organisms. Some microplastics are transferred to digestive glands following the ingestion behavior of oysters (Hall et al., 2015). Others tend to be adsorbed on the surface of gills and mantle then endocytosed (Moos et al., 2012). Therefore, from the present results, microplastics in the form of fibers may tend to enter the oysters' body through endocytosis in gills. In contrast, microplastics in the form of fragments may have easier access to the oysters' body through the digestive

system. Previous studies have suggested that microplastics in the form of fibers are more likely to be ingested by marine life (Desforges et al., 2015; Rochman et al., 2015; Lusher et al., 2015a; Hamer et al., 2014). However, this is not supported by the current results as most of the fiber contents have accumulated in gills and mantle instead.

The detected microplastics of different components have an uneven distribution pattern in the studied edible oysters, as observed during the μ -FTIR examination. For instance, the most common cellophane tends to accumulate in gills, mantle and muscle while polyester tends to accumulate in digestive gland. This finding on components and distribution may be region-specific. The types of microplastics in oyster tissues from the Yellow Sea in the present study had very different components when compared with other countries and inland China. This difference might have been caused by the differences in industries and lifestyle between regions. In the Three Gorges area, the types of microplastics most frequently detected are polypropylene and PE. These were used in cables or building materials (Zhang et al., 2015) which may have originated from the construction wastes of the Three Gorges Dam project. In residential areas, the most common types of microplastics are polystyrene and PE arising from urban wastes and civil engineering, as reported by studies at Rhine River shore in Germany (Klein et al., 2015) and Lake Ontario in Canada (Corcoran et al., 2015). In this study, the most frequently recorded types of microplastics are polyester, cellophane and AHR. These microplastics are commonly used for fishing nets, packaging and painting. This is not surprising for Haiyang in Yantai, the sampling site in this study, as it is reliant on its fishing industry and transportation sector. Jinhae Bay of Korea is a comparable study area but with a different regional background. As a result, the types of microplastics detected were not very similar between each other. In Jinhae Bay, the most frequently detected microplastics are nitrocellulose, followed by polypropylene and PE (Chae et al., 2015). The different infrastructures of the two regions may be a potential

factor, as electronics and high-tech industries are more influential and well-developed in Korea. Future studies on microplastic uptake and accumulation should put emphasis on the sources of microplastics to aid data interpretation and monitoring work. For instance, microplastics of lower density such as PE may be of higher availability at surface water and sediments, which can be more impactful due to potential overlap and interference with feeding patterns of organisms reliant on planktons. Long term impacts on the ecosystem and risks arising from processes such as selected bioaccumulation and biomagnification are expected to be site-specific.

Trace metals are also posing a serious risk on human health by entering and biomagnifying through food webs (Svecevicus et al., 2014). The concentrations of Pb, Cr, Cd, and Cu in surface sediments of Yellow Sea were reported to be 11.36, 57.8, 0.115 and 15.9 µg/g, respectively, in 2013 and 2014 (Li et al., 2013; Jiang et al., 2014). In the present study, the concentrations of Cd and Cu detected in the studied oysters from Yellow Sea were 4.415 and 181.044 µg/g. d.w., respectively. The high *in vivo* Cd and Cu content detected in the studied oysters suggests significant bioaccumulation and biomagnification. Compared to another mollusk *M. edulis*, oysters showed a significantly higher ability to bioaccumulate trace metals from the marine environment (Zhang et al., 2012). Previous study by Lu et al. (2017) has shown that the average Pb and Cr concentrations in the oysters of Yellow Sea were around 0.5 and 5 µg/g d.w., respectively. In comparison, the present study reported the mean concentrations of Pb and Cr in Pacific oysters to be 0.395 and 29.51 µg/g, d.w., respectively. Enhanced bioaccumulation of toxic metals in edible oysters is a consistent observation in the Yellow Sea region, and should be considered a warning signal for increased health risks associated with seafood consumption, both locally and on a global scale.

The bioaccumulation of metals in edible oysters can be further magnified by *in vivo* microplastics. Previous study has shown that plastic pellets adsorbed trace metals like Cd, Co, Cr, Cu, Ni and Pb in estuarine freshwater (Holmes et al., 2014). In addition, Al and Zn were also shown to be adsorbed on marine plastic particles (Ashton et al., 2010; Holmes et al., 2012). Microplastics were also reported to contain some trace metals as additives (Imhof et al., 2016). Furthermore, microplastics have been reported to exhibit higher capability to adsorb pollutants when aged in the environment because of their more heterogeneous and reactive surfaces (Holmes et al., 2012). All of these point towards the possibility of an accelerated bioaccumulation of metals from the environment resulting from microplastic stress. In this study, the SEM/EDX results demonstrated the presence of metal element precipitation on microplastics' surface in oysters. In particular, the *in vivo* concentration of Cd, Cr, Pb and Cu increased with the *in vivo* abundance of microplastics. From the SEM results, the present study also found that microplastic fibers were more likely to enrich metal elements especially Ni, Mn, Cu and Cd on its surface than microplastic fragments, which is likely a result of thread-like fibers having smaller average size, hence a larger surface area to volume ratio. Aside from the form, the type of microplastic also makes a difference. For instance, cellophane fibers, which is likely the most common type of microplastics in oysters, contains chlorine. Chlorine is known to attract Cd which can form organometallic compounds (Nasr et al., 2015). A linkage between high detection frequency of cellophane microplastics and high level of Cd in oysters may be established.

5. Conclusion

Edible oysters, as filter-feeding organisms, collected from aquaculture farm in the Yellow Sea were detected with high levels of microplastic content and metal. Microplastics in edible oysters were mainly detected in the gills and mantle. Exposure to edible oysters consumed as a whole pose human health concerns. The most common types of microplastics in studied Pacific oysters are cellophane and polyester. The study also revealed the abundance of several toxic trace

metals such as Cr, Cd and Pb in the studied oysters. Precipitation and adsorption of several trace metals on microplastics inside the studied oysters were also observed. Furthermore, significant correlations were found between concentrations of the detected trace metals and microplastic content in studied oysters. The bioaccumulation of microplastics occurring in these oysters suggest a widespread of microplastics in the coastal environment and marine life. The potential widespread of microplastics in marine life, such as edible oysters, may become a threat to human health not only because of its presence but also its potential adsorption of many other contaminants. More exposure and toxicity data are needed to protect consumers from negative health effects resulting from microplastics entering the marine environment.

CRedit authorship contribution statement

Xiaotong Zhu:Data curation, Methodology, Investigation, Formal analysis, Writing - original draft.**Liyuan Qiang:**Formal analysis, Writing - original draft, Writing - review & editing.**Huahong Shi:**Methodology, Resources, Writing - review & editing.**Jinping Cheng:**Conceptualization, Resources, Supervision, Funding acquisition, Data curation, Formal analysis, Writing - original draft, Writing - review & editing.

Acknowledgements

This work was supported by State Key Laboratory of Estuarine and Coastal Research at East China Normal University, and the Hong Kong Branch of Southern Marine Science and Engineering Guangdong Laboratory (Guangzhou) (Project: SMSEGL20SC01). The work was also partially supported by the State Key Laboratory of Marine Pollution (City University of Hong Kong). We thank Mr. Linus Lo for his kind help in the revision of this manuscript.

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.

References

- Abdou, M., Dutruich, L., Schäfer, J., Zaldibar, B., Medrano, R., Izagirre, U., Gil-Díaz, Bossy C., Catrouillet, C., Hu, R., Coyne, A., Lerat, A., Cobelo-García, A., Blanc, G., Soto, M., 2018. Tracing platinum accumulation kinetics in oyster *Crassostrea gigas*, a sentinel species in coastal marine environments. *Sci. Total Environ.* 615, 652–663.
- Andrady, A.L., 2011. Microplastics in the marine environment. *Mar. Pollut. Bull.* 62, 1596–1605.
- Ashton, K., Holmes, L., Turner, A., 2010. Association of metals with plastic production pellets in the marine environment. *Mar. Pollut. Bull.* 60, 2050–2055.
- Avio, C.G., Gorbi, S., Milan, M., Benedetti, M., Fattorini, D., d'Errico, G., Paoletto, M., Bargelloni, L., Regoli, F., 2015. Pollutants bioavailability and toxicological risk from microplastics to marine mussels. *Environ. Pollut.* 198, 211–222.
- Azevedo, C.C., Guzmán-Guillén, R., Martins, J.C., Osório, H., Vasconcelos, V., da Fonseca, R.R., Campos, A., 2015. Proteomic profiling of gill GSTs in *Mytilus galloprovincialis* from the north of Portugal and Galicia evidences variations at protein isoform level with a possible relation with water quality. *Mar. Environ. Res.* 110, 152–161.
- Besseling, E., Wegner, A., Foekema EM, van den Heuvel-Greve MJ, Koelmans AA. 2013. Effects of microplastic on fitness and PCB bioaccumulation by the lugworm *Arenicola marina* (L.). *Environ Sci Technol* 47: 593–600.
- Brennecke, D., Ferreira, E.C., Costa, T.M., Appel, D., da Gama, B.A., Lenz, M., 2015. Ingested microplastics (> 100 µm) are translocated to organs of the tropical fiddler crab *Uca rapax*. *Mar. Pollut. Bull.* 96, 491–495.
- Browne, M.A., Dissanayake, A., Galloway, T.S., Lowe, D.M., Thompson, R.C., 2008. Ingested microscopic plastic translocates to the circulatory system of the mussel, *Mytilus edulis* (L.). *Environ Sci Technol* 42, 5026–5031.
- Browne, M.A., Crump, P., Niven, S.J., Teuten, E., Tonkin, A., Galloway, T., Thompson, R., 2011. Accumulation of microplastic on shorelines worldwide: sources and sinks. *Environ Sci Technol* 45, 9175–9179.
- Cauwenberghe, V.L., Janssen, C.R., 2014. Microplastics in bivalves cultured for human consumption. *Environ. Pollut.* 193, 65–70.
- Cauwenberghe, V.L., Claessens, M., Vandegheuchte, M.B., Janssen, C.R., 2015. Microplastics are taken up by mussels (*Mytilus edulis*) and lugworms (*Arenicola marina*) living in natural habitats. *Environ. Pollut.* 199, 10–17.

- Chae, D.H., Kim, I.S., Kim, S.K., Song, Y.K., Shim, W.J., 2015. Abundance and distribution characteristics of microplastics in surface seawaters of the Incheon/Kyeonggi coastal region. *Arch Environ Con Tox* 69, 269–278.
- Collard, F., Gilbert, B., Eppe, G., Parmentier, E., Das, K., 2015. Detection of anthropogenic particles in fish stomachs: an isolation method adapted to identification by Raman spectroscopy. *Arch Environ Con Tox* 69, 331–339.
- Corcoran, P.L., Norris, T., Ceccanese, T., Walzak, M.J., Helm, P.A., Marvin, C.H., 2015. Hidden plastics of Lake Ontario, Canada and their potential preservation in the sediment record. *Environ. Pollut.* 204, 17–25.
- Cózar, A., Echevarría, F., Gonzálezgordillo, J.I., Irigoien, X., Ubeda, B., Hernández, L.S., Palma, A.T., Navarro, S., García-de-Lomas, J., Ruiz, A., Fernández-de-Puelles, M.L., Duarte, C.M., 2014. Plastic debris in the open ocean. *Proc. Natl. Acad. Sci. U. S. A.* 111, 10239–10244.
- Desforges, J.P.W., Galbraith, M., Ross, P.S., 2015. Ingestion of microplastics by zooplankton in the northeast Pacific Ocean. *Arch Environ Con Tox* 69, 320–330.
- Engler, R.E., 2012. The complex interaction between marine debris and toxic chemicals in the ocean. *Environ Sci Technol* 46, 12302–12315.
- Fendall, L.S., Sewell, M.A., 2009. Contributing to marine pollution by washing your face: microplastics in facial cleansers. *Mar. Pollut. Bull.* 58, 1225–1228.
- Graham, E.R., Thompson, J.T., 2009. Deposit- and suspension-feeding sea cucumbers (*Echinodermata*) ingest plastic fragments. *J. Exp. Mar. Biol. Ecol.* 368, 22–29.
- Hall, N.M., Berry, K.L.E., Rintoul, L., Hoogenboom, M.O., 2015. Microplastic ingestion by scleractinian corals. *Mar. Biol.* 162, 725–732.
- Hamer, J., Gutow, L., Kohler, A., Saborowski, R., 2014. Fate of microplastics in the marine isopod *Idotea emarginata*. *Environ Sci Technol* 48, 13451–13458.
- Holmes, L.A., Turner, A., Thompson, R.C., 2012. Adsorption of trace metals to plastic resin pellets in the marine environment. *Environ. Pollut.* 160, 42–48.
- Holmes, L.A., Turner, A., Thompson, R.C., 2014. Interactions between trace metals and plastic production pellets under estuarine conditions. *Mar. Chem.* 167, 25–32.
- Imhof, H.K., Laforsch, C., Wiesheu, A.C., Schmid, J., Anger, P.M., Niessner, R., Ivleva, N.P., 2016. Pigments and plastic in limnetic ecosystems: a qualitative and quantitative study on microparticles of different size classes. *Water Res.* 98, 64–74.
- Jiang, X., Teng, A., Xu, W., Liu, X., 2014. Distribution and pollution assessment of heavy metals in surface sediments in the Yellow Sea. *Mar. Pollut. Bull.* 83, 366–375.
- Kang, J.H., Kwon, O.Y., Shim, W.J., 2015. Potential threat of microplastics to Zooplanktivores in the surface waters of the Southern Sea of Korea. *Arch Environ Con Tox* 69, 340–351.
- Klein, S., Worch, E., Knepper, T.P., 2015. Occurrence and spatial distribution of microplastics in river shore sediments of the Rhine-Main area in Germany. *Environ Sci Technol* 49, 6070–6076.
- Li, G.G., Hu, B.Q., Bi, J.Q., Leng, Q.N., Xiao, C.Q., Yang, Z.C., 2013. Heavy metals distribution and contamination in surface sediments of the coastal Shandong peninsula (Yellow Sea). *Mar. Pollut. Bull.* 76, 420–426.
- Li, J.N., Yang, D.Q., Li, L., Jabeen, K., Shi, H.H., 2015. Microplastics in commercial bivalves from China. *Environ. Pollut.* 207, 190–195.
- Li, J.N., Qu, X.Y., Su, L., Zhang, W.W., Yang, D.Q., Kolandhasamy, P., Li, D.J., Shi, H.H., 2016. Microplastics in mussels along the coastal waters of China. *Environ. Pollut.* 214, 177–184.
- Lu, G., Ke, C., Zhu, A., Wang, W., 2017. Oyster-based national mapping of trace metals pollution in the Chinese coastal waters. *Environ. Pollut.* 224, 658–669.
- Lusher, A.L., Hernandez-Milian, G., O'Brien, J., Berrow, S., O'Connor, I., Officer, R., 2015a. Microplastic and macroplastic ingestion by a deep diving, oceanic cetacean: the True's beaked whale *Mesoplodon mirus*. *Environ. Pollut.* 199, 185–191.
- Lusher, A.L., Tirelli, V., O'Connor, I., Officer, R., 2015b. Microplastics in Arctic polar waters: the first reported values of particles in surface and sub-surface samples. *Sci. Rep.* 5, 14947.
- Mallory, M.L., Roberston, J., Moenting, A., 2006. Marine plastic debris in northern furmors from Davis Strait, Nunavut, Canada. *Mar. Pollut. Bull.* 52, 813–815.
- Moore, C.J., Moore, S.L., Leecaster, M.K., Weisberg, S.B., 2001. A comparison of plastic and plankton in the North Pacific central gyre. *Mar. Pollut. Bull.* 42, 1297–1300.
- Moos, V.N., Burkhardt-Holm, P., Kohler, A., 2012. Uptake and effects of microplastics on cells and tissue of the blue mussel *Mytilus edulis* L. after an experimental exposure. *Environ Sci Technol* 46, 11327–11335.
- Naidoo, T., Smit, A.J., Glassom, D., 2016. Plastic ingestion by estuarine mullet *Mugil cephalus* (*Mugilidae*) in an urban harbour, KwaZulu-Natal, South Africa. *Afr. J. Mar. Sci.* 38, 145–149.
- Nasr, B.M., Aubert, E., Espinosa, E., Lefebvre, F., Nasr, B.C., 2015. Synthesis and physico-chemical studies of a novel organo-metallic compound CdCl₂(C₆H₄FNH₂)₂. *J. Mol. Struct.* 1082, 162–169.
- Rochman, C.M., Tahir, A., Williams, S.L., Baxa, D.V., Lam, R., Miller, J.T., Teh, F.C., Werorilangi, S., Teh, S.J., 2015. Anthropogenic debris in seafood: plastic debris and fibers from textiles in fish and bivalves sold for human consumption. *Sci. Rep.* 5, 14340.
- Sussarellu, R., Suquet, M., Thomas, Y., Lambert, C., Fabioux, C., Pernet, M.E.J., Goic, N.L., Quillien, V., Mingant, C., Epelboin, Y., Corporeau, C., Guyomarch, J., Robbens, J., Paul-Pont, I., Soudant, P., Huvet, A., 2016. From the cover: oyster reproduction is affected by exposure to polystyrene microplastics. *P Natl Acad Sci USA* 113 (9), 2430–2435.
- Svecevičius, G., Sauliute, G., Idzelis, R.L., Grigeleviciute, J., 2014. Accumulation of heavy metals in different body tissues of Atlantic salmon, *Salmo salar* L., exposed to a model mixture (Cu, Zn, Ni, Cr, Pb, Cd) and singly to nickel, chromium, and lead. *Bull. Environ. Contam. Toxicol.* 92, 440–445.
- Tan, Q.G., Zhou, W., Wang, W.X., 2017. Modeling the toxicokinetics of multiple metals in oyster *Crassostrea hongkongensis* in dynamic estuarine environment. *Environ Sci Technol* 484–492.
- The People's Republic of China Ministry of Agriculture, Fisheries Bureau (MAFB), 2017. China Fishery Statistical Yearbook, 2017. China Agriculture Press, pp. 39–55.
- Trevisan, R., Mello, D.F., Delapiedra, G., Silva, D.G., Arl, M., Danielli, N.M., Metian, M., Almeida, E.A., Dafre, A.L., 2016. Gills as a glutathione-dependent metabolic barrier in Pacific oysters *Crassostrea gigas*: absorption, metabolism and excretion of a model electrophile. *Aquat. Toxicol.* 173, 105–119.
- Tussellino, M., Ronca, R., Formigini, F., Marco, N.D., Fusco, S., Netti, P.A., Carotenuto, R., 2015. Polystyrene nanoparticles affect *Xenopus laevis* development. *J. Nanopart. Res.* 17, 1–17.
- Velzeboer, I., Kwadijk, C.J., Koelmans, A.A., 2014. Strong sorption of PCBs to nano-plastics, microplastics, carbon nanotubes, and fullerenes. *Environ Sci Technol* 48, 4869–4876.
- Wang, T., Zou, X., Li, B., Yao, Y., Li, J., Hui, H., Yu, W., Wang, C., 2018. Microplastics in a wind farm area: a case study at the Rudong offshore wind farm, Yellow Sea, China. *Mar. Pollut. Bull.* 128, 466–474.
- Zhang, G.S., Liu, D.Y., Wu, H.F., Chen, L.L., Han, Q.X., 2012. Heavy metal contamination in the marine organisms in Yantai coast, northern Yellow Sea of China. *Ecotoxicology* 21, 1726–1733.
- Zhang, K., Gong, W., Lv, J., Xiong, X., Wu, C., 2015. Accumulation of floating microplastics behind the three gorges dam. *Environ. Pollut.* 204, 117–123.