Heavy metals in sediment, microplastic and sea cucumber *Apostichopus japonicus* from farms in China

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**ABSTRACT**

The concentrations of eight heavy metals (As, Cd, Cr, Cu, Mn, Ni, Pb and Zn) were measured in the sediment, the isolated microplastics from the sediment and the body wall of sea cucumbers from farms in China. Accordingly, the heavy metal concentrations in the sediment were below the class I upper limit of Chinese sediment quality guidelines. Among heavy metals, the median concentrations of Cd and As were higher in the body wall than in the corresponding sediment. Additionally, the median concentrations of Cd, Pb, and Zn were higher on the microplastics than in the corresponding sediment. Furthermore, there was no significant correlation among heavy metals in sediment, sea cucumber and microplastics. This study contributes to the understanding of the heavy metal accumulation in the sediment, the microplastics and the body wall of the sea cucumber.

1. Introduction

Heavy metals are natural components that exist in the ecosystem. Some of these components are essential nutrients, such as iron, zinc, copper and manganese, while others are non-essential and highly toxic at high quantities, such as cadmium, mercury, and lead (reviewed by Tchounwou et al., 2012). In the marine environment, sources of heavy metals often include contributions from human activities such as industrial and municipal wastes, agricultural discharge, sediment release, atmospheric sedimentation (Gao et al., 2014) and microplastics as carriage (Brennecke et al., 2016).

Heavy metals are reportedly associated with microplastics (MPs), which are small plastic particles that measure < 5 mm in diameter (Arthur et al., 2009); heavy metals were reported from the isolated MPs from water such as copper (Cu) and zinc (Zn) (Brennecke et al., 2016) or from the isolated MPs from sediment such as cadmium (Cd), lead (Pb) and bromine (Br) (Massos and Turner, 2017). In addition, aquatic sediment has been known as a sink for heavy metals (de Groot, 1995). Both MPs and sediment could be vectors that transport heavy metals when ingested by bottom feeders (Fan et al., 2014; Hodson et al., 2017).

In laboratory experiments, the release of Zn in synthetic earthworm gut was higher in association with MPs (40–60%) compared with sediment (2–15%); hence, MPs might increase the bioavailability of metals to the bottom feeders (Hodson et al., 2017).

Sea cucumber *Apostichopus japonicus*, the most valuable species among sea foods in China (Yang et al., 2015), is a deposit feeder that ingests the sedimentary detritus as its main food source (Gao and Yang, 2015). Moreover, our previous work has shown that sea cucumber (*A. japonicus*) ingests MPs in addition to sediment (Mohsen et al., 2019). Therefore, the quantification of heavy metals in association with both MPs and sediment is key to assessing the hazardous effects of heavy metals on the sea cucumber. Although the heavy metal adsorption to the sediment is well documented in China (reviewed by Fan and Wang, 2012; Wang et al., 2013; Gao et al., 2014), few studies have examined the concentration of heavy metals in the adults of sea cucumber (Wen and Hu, 2016; Wang et al., 2012) or in the MPs (Wang et al., 2017) in China.

The aims of the current study were as follows: 1) assess the...
concentrations of heavy metals in the sediment and on the MPs in the field; 2) determine the heavy metals content in the body wall of farmed sea cucumbers; 3) examine the sediment quality of sea cucumber farms, and 4) investigate the correlation between the concentrations of heavy metals in the body wall of sea cucumber, the sediment, and the MPs.

2. Materials and methods

2.1. Samples collection and preparation

Samples of sea cucumber and sediment were collected during the year 2017–2018 from eight farming sites of the sea cucumber *A. japonicus* in China (Fig. 1) (Table S1). Six of these sites were culture ponds, and two sites were open areas for culturing the sea cucumber. In each site, the sea cucumbers (n = 3) were collected by divers, while the sediment samples (n = 5) were collected via Van Veen grab. The sea cucumbers from each site were dissected, and the internal viscera was removed. The wet weight of the average body wall ranged from 27 to 51.5 g (Table S2).

The body wall and the sediment were transferred in foil bags to the laboratory and kept frozen until analysis. The sediment and the body wall were dried at 60 °C for 72 h to obtain a dry weight. The dried sea cucumbers were ground in a Tissue Lyser-24 (Shanghai Jingxin Industrial Development Co., Ltd., China) at 70 Hz for 30 s. To achieve a reasonable weight of the MPs for heavy metal analysis, 50 g of dried sediment was exposed to the glass beaker to degrade the organic matter and calcium compounds. A total of 5 mL H2O2 (30%) was added in the 250 mL glass beaker. The homogenized powder and were then sieved and stored until heavy metals analysis.

2.2. Microplastics isolation

To achieve a reasonable weight of the MPs for heavy metal analysis, 50 g of dried sediment was exposed to the flotation test, with minor modifications (Thompson et al., 2004). The sediments were mixed with 200 mL of Na-Cl solution (ρ = 1.20 g/mL) for one minute and then held to settle for 5 min. Most of the supernatant was carefully transferred to a 250 mL glass beaker. The flotation test was repeated three times to increase recovery. A total of 5 mL H2O2 (30%) was added in the 250 mL glass beaker to degrade the organic matter and calcium floats and then held to settle for 24 h. The supernatant was filtered through an 8-μm glass microfiber filter paper (Shanghai Xingya Purification Material Factory, China) with vacuum filtration, and then, it was transferred to a new petri dish for later inspection by a dissecting microscope (SMZ-161-BLED, Motic, China). The MPs were identified depending on colour homogeneity, resistance to the stress of tweezers, and clearance of any tissue structure (Hidalgo-Ruz et al., 2012). MPs were isolated under a dissecting microscope without adhesion of the sediment and then transferred to a clean filter paper. The structure of the MPs was confirmed by Fourier transform infrared micro spectroscopy (FT-IR) (Nicolet™ iN10, Thermo Fisher Scientific, USA) (Directive, 2013). The FT-IR was supplied with an ultra-fast motorized phase and mercury cadmium telluride (MCT) sensor. During the process of MP identification, the MCT sensor was cooled using liquid nitrogen. The spectrum of the particle was within a range of 650–4000 cm⁻¹ by co-adding 128 scans at a resolution of 8 cm⁻¹. The aperture was adjusted to 150 × 150 μm, using knife-edges. Afterward, the obtained spectra were compared with OMNIC polymer spectra library; only matches above 70% were accepted.

To prevent contamination, the procedures were performed under a clean hood with air flow. Polymer free gloves and coats made of cotton were worn during all the experimental procedures. Every glass beaker was washed three times, including two times by filtered water and the last time by ultrapure water. Additionally, three blanket replicates were set up, with the floatation test for background correction.

2.3. Metals analysis

2.3.1. Sediment

The digestion of the dry sediment (0.1 g) was performed (AOAC, 1995) by using a mix of 5 mL hydrofluoric acid (HF), 5 mL nitric acid (HNO₃) and 1 mL perchloric acid (HClO₄) at a temperature of 140–220 °C. The residual was mixed with 1 mL HNO₃ (1:1, V/V) and diluted to 15 mL with Millipore water. The solution was examined for the occurrence of Cd by inductively coupled plasma mass spectrophotometer (ICP-MS, Thermo Fisher Icap-Qc) and for the occurrence of Cu, Zn, Cr and Pb by inductively coupled plasma-optical emission spectrometry (ICP-OES; Perkin-Elmer 7300 DV).

For As examination, the dried sediment (0.1 g) was digested at 100 °C for 1 h using 10 mL aqua regia (HNO₃:HCl:water = 1:3:4, V/V/V). Millipore water was used to dilute the residual to 25 mL and held overnight. Then, the supernatant (2 mL) was mixed with 10 mL diluted HCl (HCl:water = 1:1, V/V) and thiourea-ascorbic acid reducing agent (5 g thiourea and 5 g ascorbic acid dissolved in 100 mL water). The residual was then diluted to 100 mL with Millipore water and held overnight. Then, atomic fluorescence spectroscopy (Jitian AFS-8w) was performed to detect As. All the detection limits were based on a 98% confidence level with three standard deviations.
Table 1
Comparison of the average heavy metals content from the current study and nearby regions in China or other regions (mg/kg).

|  | As     | Cd     | Cr      | Cu     | Mn     | Ni     | Pb      | Zn     | Sampling date | Reference |
|---|--------|--------|---------|--------|--------|--------|---------|--------|---------------|-----------|
| **Bohai Sea** |        |        |         |        |        |        |         |        |               |           |
| Panshan (S1) | 6.37 ± 0.31 | 0.17 ± 0.02 | 47.75 ± 2.18 | 27.09 ± 1.92 | 18.23 ± 6.02 | 20.78 ± 1.38 | 11.86 ± 0.83 | 34.01 ± 0.37 | 2018 | Current study |
| Lvshunkou (S2) | 9.03 ± 0.17 | 0.15 ± 0.01 | 53.01 ± 2.63 | 30.85 ± 0.76 | 40.17 ± 8.10 | 32.49 ± 0.66 | 11.81 ± 0.99 | 44.44 ± 0.61 | 2018 | Current study |
| Tangshan (S7) | 5.31 ± 0.18 | 0.09 ± 0.001 | 35.61 ± 0.99 | 20.67 ± 0.31 | 24.26 ± 7.37 | 22.48 ± 0.13 | 10.17 ± 0.79 | 27.45 ± 0.41 | 2018 | Current study |
| Laizhou (S8) | 3.01 ± 0.14 | 0.04 ± 0.0002 | 11.14 ± 0.37 | 4.75 ± 0.37 | 4.25 ± 0.47 | 6.46 ± 0.15 | 10.62 ± 1.19 | 12.16 ± 0.04 | 2018 | Current study |
| Liaodong Bay, China | 8.30 | NA | 46.40 | 19.40 | NA | 22.50 | 31.80 | 71.70 | 2009 | (Hu et al., 2013) |
| Central Bohai Sea (Summer-winter) | NA | 1.65–0.199 | 16.8–18.2 | 503–535 | 18.5–17.4 | 11.4–12.0 | 41.9–39.7 | 2011 | (Liu et al., 2016) |
| Shuangtai Estuary, China | 3.53 | 0.15 | 26.80 | 9.77 | NA | NA | 11.90 | 16.90 | 2011 | (Li et al., 2018) |
| Jinzhou Bay, China | 19.90 | 2.48 | NA | 32.70 | NA | NA | 32.50 | 257.70 | 2015 | (Li et al., 2018) |
| Laizhou Bay, China | 3.07 | 0.16 | 40.40 | 19.20 | NA | NA | 17.35 | 45.20 | 2015 | (Li et al., 2018) |
| Laizhou Bay, China | NA | 0.22 | 56.70 | 12.00 | NA | 25.90 | 19.40 | 41.5 | 2011 | (Zhang and Gao, 2014) |
| **Yellow Sea** |        |        |         |        |        |        |         |        |               |           |
| Rongcheng, Weihai (3) | 3.71 ± 0.28 | 0.06 ± 0.01 | 19.98 ± 0.42 | 10.29 ± 0.47 | 257.75 ± 5.76 | 8.70 ± 0.24 | 10.62 ± 1.40 | 10.33 ± 0.19 | 2018 | Current study |
| Haiyang, Yantai (4) | 7.23 ± 0.15 | 0.16 ± 0.004 | 41.70 ± 0.66 | 28.60 ± 0.52 | 479.53 ± 2.76 | 23.86 ± 0.59 | 15.88 ± 0.47 | 58.80 ± 0.78 | 2015 | Current study |
| Chengyang, Qingdao (5) | 12.42 ± 0.32 | 0.13 ± 0.001 | 76.51 ± 1.27 | 52.66 ± 1.16 | 1854.67 ± 24.17 | 44.71 ± 0.95 | 25.72 ± 0.46 | 82.72 ± 1.31 | 2013 | Current study |
| Pingdao Island, Rizhao (6) | 5.02 ± 0.17 | 0.13 ± 0.001 | 37.65 ± 1.48 | 26.65 ± 0.55 | 981.23 ± 53.64 | 26.60 ± 0.20 | 15.35 ± 0.62 | 50.13 ± 1.08 | 2018 | Current study |
| Jiaozhou Bay, China | NA | 0.30 | 86.17 | 27.31 | NA | 32.35 | 38.54 | 76.00 | 2015 | (Li et al., 2018) |
| Jiaozhou Bay, China | 9.10–20.77 | 0.07–0.37 | 83.3–140.6 | 12.8–124.5 | 42.0–93.1 | 66.8–243.4 |
| Yellow Sea (summer-winter) | NA | 0.51–0.115 | 15.1–15.9 | 410–373 | 18.6–18.8 | 12.3–11.3 | 47.3–46.2 | 2011 | (Jiang et al., 2014) |
| Weihai coast, China | 9.0 | 0.14 | 23.9 | 11.6 | NA | NA | 20.0 | 40.0 | 2009–2013 | (Li et al., 2017) |
| Swan Lake, Rongcheng, China | 5.95 | 0.35 | 72.35 | 24.98 | NA | NA | 46.82 | 68.86 | 2015 | (Wang et al., 2016a) |
| Mariculture zone, Hailing Bay, China | 16.43 | 0.187 | 50.8 | 34.54 | NA | 25.2 | 46.6 | 137 | 2010 | Zhang et al., 2012 |
| Sea cucumber habitat, Malaysia | NA | 1.01 | 7.75 | 0.62 | 0.52 | 0.21 | 2.73 | 3.38 | NA | (Husimi et al., 2002) |
| Sea cucumber habitat, Pakistan | NA | 1.88–2.42 | 4.92–5.52 | 132–137 | NA | 33–37 | 14–39 | 2014 | (Ahmed et al., 2017) |
| Sea cucumber habitat, Iran | NA | 1.18–3.07 | 5.99–9.04 | 134–143 | NA | 47–56 | 15–22 | 2014 | (Ahmed et al., 2017) |
| Sea cucumber habitat, Iran | NA | 0.6–1.15 | 118.31–51.89 | 11.18–28.79 | 28.26–55.78 | 2014 | (Mohammadzadeh et al., 2015) |
| Class 1 upper limit | 20.00 | 0.50 | 80.00 | 35.00 | NA | NA | 60.00 | 150.00 | SEPA (2002) |
| Class 2 upper limit | 56.00 | 1.50 | 150.00 | 100.00 | NA | NA | 130.00 | 350.00 | SEPA (2002) |
| Class 3 upper limit | 93.00 | 5.00 | 270.00 | 200.00 | NA | NA | 250.00 | 600.00 | SEPA (2002) |
| TEL | 7.30 | 0.68 | 52.30 | 18.70 | NA | 15.90 | 30.20 | 124.00 | TEL |
| PEL | 41.60 | 4.20 | 160.00 | 108.00 | NA | 42.80 | 112.00 | 271.00 | PEL |

NA = Not available.
2.3.2. Sea cucumber and microplastics

Dry samples of the body wall of sea cucumber (0.1 g) or MPs (1 mg) were exposed to the aqua regia digestion (Holmes et al., 2012), and then, inductively coupled plasma mass spectrometry (ICP-MS) was used to analyse the chosen heavy metals.

2.4. Quality assessment guides

2.4.1. Sediment quality assessment

The concentrations of the heavy metals in the sediment were compared to the Chinese Sediment Quality Guidelines (SEPA, 2002) as follows: Class I criteria (suitable for nature reserves, mariculture, endangered species reserves, seawater bathing, etc.), Class II criteria (suitable for general industrial use and coastal tourism) and Class III criteria (suitable for harbour activities). Additionally, the heavy metal concentrations of the sediments were compared with the guidelines for the threshold effects level (TEL) and probable effects level (PEL) (MacDonald et al., 1996; Long et al., 1998). The TEL and PEL are sediment quality evaluation guidelines that were developed based on toxicity tests of benthic community animals. The TEL has been used to identify uncontaminated sediment with a limited effect range, while the PEL has been used to identify sediment with elevated chemical concentrations which warrant further assessment. Generally, a value below the TEL indicates that heavy metals abundance is not associated with adverse biological effects, while a value between the TEL and PEL (≥TEL and < PEL) indicates that heavy metal concentration may occasionally cause adverse biological effects. A value above the PEL indicates that heavy metals concentrations are frequently associated with adverse biological effects.

2.4.2. Biota-sediment accumulation factor (BSAF)

BSAF is a parameter that has been used to evaluate the bioaccumulation of sediment-associated metals or organic contaminants in the tissues of an organism (Burkhard, 2009). Biota-sediment accumulation factor (BSAF) was calculated using the formula: (BSAF) = CA/CS, where CA is the concentration of heavy metals in the animals (dry weight), and CS is the concentration of heavy metals in the sediment (dry weight). According to this factor, a value above 2 indicates the organism is a macro-concentrator, a value between 1 and 2 indicates the organism is a micro-concentrator, and a value below 1 indicates the organism is a de-concentrator.

2.5. Statistical analysis

SPSS Statistics 20.0 statistical software (SPSS Inc., Chicago, IL) was used to conduct all statistical analyses. The Shapiro–Wilk normality test with 95% confidence level was used to examine the normality of the data. The data were not normally distributed and included outliers. Outliers were identified by box plot for the values > 1.5 times the interquartile range. Since the data were not normally distributed, non-parametric statistical analyses were used subsequently. Spearman’s rank correlation was used because it is more appropriate for outliers than Pearson’s correlation coefficient (Chok, 2010). Additionally, Kruskal-Wallis H followed by Mann-Whitney test were used to detect the significant differences in the heavy metal concentrations among sites (SI).

3. Results and discussion

3.1. Heavy metals in the sediment

In the current study, heavy metal concentrations in the sediments at all the sites were less than the upper limits of Chinese sediment quality Class I guidelines. The range of the average concentrations of As, Cd, Cr, Cu, Mn, Ni, Pb and Zn were 3.01–12.42 mg/kg, 0.04–0.17 mg/kg, 11.14–76.51 mg/kg, 4.75–52.66 mg/kg, 24.25–1854.76 mg/kg, 6.48–44.71 mg/kg, 10.17–25.72 mg/kg, 10.33–82.72 mg/kg, respectively. These concentrations significantly differed among sites (Table S3).

In the Bohai Sea, the values of As, Cr at S2 and Cu at S1, S2 and S7 were ≥TEL and < PEL, which indicate that adverse biological effects may occasionally occur at these sites. The heavy metal concentrations were the lowest in the sediment of sea cucumber pond at Laizhou Bay (S8), although several studies highlighted the pollution of the heavy metals in the Laizhou Bay (Table 1). Hence, this site might be considered as a typical farming site. The main source of heavy metals pollution in the Bohai Sea includes industrial waste, river discharge, sediment release and atmospheric sedimentation, which cause higher concentrations of the heavy metals, especially in the coastal water of the Bohai Sea (Gao et al., 2014). Furthermore, the excess concentration of Cu in the Bohai Sea may be due to the use of copper sulfate in aquaculture, or because of other anthropogenic activities (Zhang et al., 2017). Moreover, the high Mn abundance in the sediment may be due to the deposition in coarse sediments with high oxygen content or may be linked to the abundance of the biogenic CaCO3 (Yuan et al., 2012).

In the Yellow sea, the concentrations of As, Cr, Cu at S5, Jiaozhou Bay, Qingdao indicate that adverse biological effects may rarely occur (≥TEL and < PEL). Also, Ni was higher than PEL guidelines, indicating frequent adverse biological effects at site S5. In addition, Cu and Ni were ≥TEL and < PEL at S4 and S6, indicating that adverse biological effects may rarely occur. The heavy metals pollution in the Jiaozhou Bay is mainly from rivers discharges (Xu et al., 2017), municipal and harbour activities (Lin et al., 2016), industrial and agricultural development (Liang et al., 2018). Moreover, Cu, Cr, Ni pollution is mostly from industrial resources in the Jiaozhou Bay (Liang et al., 2018).

In the current study, the sediment of the A. japonicus habitats had lower concentrations of Zn and Pb than those detected in the mariculture zone (Hailing Bay, south China). In addition, the farms of A. japonicus had higher concentrations of Mn when compared with the sea cucumber habitats at different countries (Table 1). This might be attributed to different geological backgrounds or different pollution sources.

The correlation of heavy metal concentrations in the sediment at different sites mostly showed a significant high value except for Cd (Table S4), indicating similar adhesion process or similar sediment characteristics. The sediment characteristics such as organic matter content and grain size. The organic matter content could increase the heavy metals adsorption on the sediment (Liang et al., 2018). Additionally, smaller grain sizes indicate higher metal concentrations of Cu, Cd, Cr, Ni, Pb and Zn (Zhao et al., 2010). Furthermore, concentrations of heavy metals on the sediment showed no significant correlation with the concentration of the heavy metals on MPs (Table 2).

Table 2

| Heavy metal | As   | Cd   | Cr   | Cu   | Mn   | Ni   | Pb   | Zn   |
|------------|------|------|------|------|------|------|------|------|
| Sediment & MP | −0.381 | 0.429 | 0.31 | 0.524 | 0.452 | 0.548 | 0.405 | 0.405 |
| Sediment & animal | 0.095 | 0.5  | −0.33 | −0.69 | 0.214 | −0.143 | −0.024 | −0.024 |
| MP & animal    | 0.405 | −0.167 | −0.357 | 0.119 | −0.048 | −0.0476 | −0.119 | −0.119 |
### Table 3
Comparison of the average heavy metals content between the sea cucumber *Apostichopus japonicus* and some commercial species of sea cucumber (mg/kg).

| Study place | Scientific name | As  | Cd  | Cr  | Cu  | Mn  | Ni  | Pb  | Zn  | References  |
|-------------|----------------|-----|-----|-----|-----|-----|-----|-----|-----|-------------|
| S1          | *A. japonicus*  | 10.47 ± 0.28 | 0.85 ± 0.02 | 3.64 ± 0.27 | 2.78 ± 0.49 | 39.24 ± 1.02 | 1.77 ± 0.10 | 2.18 ± 0.36 | 20.30 ± 1.02 | Current study |
| S2          | *A. japonicus*  | 10.88 ± 0.29 | 0.38 ± 0.03 | 2.43 ± 0.17 | 3.20 ± 0.12 | 28.34 ± 0.70 | 1.55 ± 0.10 | 2.59 ± 0.21 | 24.24 ± 0.94 |          |
| S3          | *A. japonicus*  | 5.99 ± 0.11 | 0.36 ± 0.04 | 4.61 ± 0.18 | 4.00 ± 0.13 | 30.43 ± 2.30 | 1.65 ± 0.05 | 3.05 ± 0.13 | 26.17 ± 0.41 |          |
| S4          | *A. japonicus*  | 5.38 ± 0.83 | 0.82 ± 0.03 | 2.88 ± 0.55 | 2.99 ± 0.08 | 29.82 ± 2.25 | 1.21 ± 0.04 | 1.76 ± 0.07 | 23.72 ± 0.75 |          |
| S5          | *A. japonicus*  | 5.25 ± 0.42 | 0.57 ± 0.05 | 2.29 ± 0.23 | 1.55 ± 0.16 | 58.91 ± 2.83 | 1.29 ± 0.07 | 2.72 ± 0.08 | 24.35 ± 0.53 |          |
| S6          | *A. japonicus*  | 12.39 ± 0.25 | 0.31 ± 0.03 | 2.77 ± 0.17 | 7.79 ± 0.26 | 17.25 ± 0.45 | 1.18 ± 0.11 | 1.56 ± 0.25 | 29.98 ± 0.52 |          |
| S7          | *A. japonicus*  | 9.86 ± 0.11 | 0.54 ± 0.02 | 3.55 ± 0.33 | 8.21 ± 0.19 | 52.67 ± 1.10 | 1.61 ± 0.08 | 1.94 ± 0.15 | 36.21 ± 0.15 |          |
| S8          | *A. japonicus*  | 4.26 ± 0.87 | 0.50 ± 0.01 | 2.37 ± 0.15 | 3.55 ± 0.55 | 16.37 ± 0.42 | 1.36 ± 0.18 | 4.25 ± 0.23 | 23.10 ± 0.63 |          |

Italy, Marseille and France.

| Scientific name | As  | Cd  | Cr  | Cu  | Mn  | Ni  | Pb  | Zn  | References  |
|-----------------|-----|-----|-----|-----|-----|-----|-----|-----|-------------|
| Holothuria tubulosa | NA  | 0.38–2.84 | NA  | 0.76–5.78 | NA  | NA  | 1.23–18 | 8.87–26 | Warnau et al., 2006 |
| Holothuria scabra | NA  | 0.15 | NA  | 57.85 | NA  | NA  | 1.92 | 23.29 | Mohammadizadeh et al., 2015 |
| Holothuria aranciaca | NA  | 0.12–1.42 | NA  | 0.43–2.23 | 2.45–5.32 | NA  | 0.92–2.33 | 11–28 | (Ahmed et al., 2017) |
| Holothuria pardeis | NA  | 0.21–0.99 | NA  | 1.13–3.34 | 1.23–3.91 | NA  | 0.76–1.56 | 17–25 |          |
| Holothuria verrucosa | NA  | 0.76–1.76 | NA  | 1.98–3.76 | 0.76–2.47 | NA  | 0.54–1.03 | 12–30 |          |
| Holothuria atrata | NA  | 0.52–1.11 | NA  | 2.03–3.89 | 1.09–2.49 | NA  | 0.69–1.23 | 18–24 |          |
| Holothuria cineraescens | NA  | 2.67 | NA  | 8.93 | 4.64 | NA  | 2.12 | 37 |          |
| Holothuria leucospilota | NA  | 1.02 | NA  | 8.64 | 7.12 | NA  | 2.19 | 46 |          |
| SriLanka | Holothuria edulis | NA  | 0.114 | 0.003 | 1.84 | NA  | 0.0337 | 20.95 |          |
|              | Thelenota anax | NA  | 0.08415 | 0.0002 | 2.92 | NA  | 0.29757 | 22.81 |          |
|              | Bohadschia marmorata | NA  | 0.137 | 0.00046 | 2.81 | NA  | 0.22702 | 16.06 |          |
|              | Stichopus chloronotus | NA  | 0.0851 | 0.00099 | 7.25 | NA  | 0.68336 | 16.20 |          |
|              | Bohadschia sp. | NA  | 0.12893 | 0.00117 | 4.30 | NA  | 0.49164 | 12.68 |          |
|              | Holothuria spinosa | NA  | 0.04823 | 0.00131 | 4.42 | NA  | 0.20456 | 8.77 |          |
|              | Actinopyga miliaris | NA  | 0.05266 | 0.00385 | 9.18 | NA  | 2.28705 | 12.11 | (Jinadasa et al., 2014) |
|              | Bohadschia similis | NA  | 0.05497 | 0.0047 | 5.70 | NA  | 0.45053 | 16.22 |          |

Supermarket in Guangzhou, China.

| Scientific name | As  | Cd  | Cr  | Cu  | Mn  | Ni  | Pb  | Zn  | References  |
|-----------------|-----|-----|-----|-----|-----|-----|-----|-----|-------------|
| Stichopus hermanni | NA  | 1.6 | 3.0 | 9.1 | 0.3 | NA  | 33 | (Wen and Hu, 2010) |
| Stichopus chloronotus | NA  | 1.5 | 3.0 | 2.2 | 0.3 | NA  | 16 |          |
| Thelenota ananas | NA  | 2.7 | 4.4 | 16 | 2.5 | NA  | 46 |          |
| Holothuria furcogilva | NA  | 1.3 | 57 | 9.4 | 1.5 | NA  | 11 |          |
| Holothuria furcunculata | NA  | 5.5 | 74 | 12 | 3.0 | NA  | 25 |          |
| Holothuria mexicana | NA  | 2.2 | 30 | 1.6 | 1.1 | NA  | 16 |          |
| Actinopyga mauritiana | NA  | 9.6 | 14 | 9.2 | 4.2 | NA  | 57 |          |
| Actinopyga caerulea | NA  | 1.3 | 4.0 | 1.1 | 0.5 | NA  | 20 |          |
| Bohadschia argus | NA  | 4.9 | 18 | 3.7 | 5.1 | NA  | 100 |          |

NA = not available.
The biota-sediment accumulation factor showed that macro-concentrator for Cd (BSAF > 2) at all the sites (Table 4). Cd accumulation was observed in the worms A. japonicus (Table 4). This finding might be due to the tendency of sea cucumber to accumulate Zn in the body wall. Zn concentration in the body wall of the sea cucumber was at higher rate than of Cu in sea cucumber to accumulate Zn in the body wall. Zn concentration in the body wall at di-47
day of skin epithelium cells (Warnau et al., 2006). Furthermore, the correlation between Cu and Zn in the body wall at different sites was high (Table S6), which might indicate a similar biochemical pathway in the body wall of A. japonicus. The biochemical pathway is the chemical reactions that take place in the normal operation of living systems. Furthermore, there was no significant correlation between heavy metals concentrations in the sea cucumber body wall and in the sediment or in the MPs (Table 2).

### 3.2. Heavy metals concentration in the body wall of sea cucumber

The range of the average concentrations of As, Cd, Cr, Cu, Mn, Ni, Pb and Zn in the body wall of the sea cucumbers were 4.26–12.39 mg/kg, 0.31–0.85 mg/kg, 2.29–4.61 mg/kg, 1.55–8.21 mg/kg, 16.37–58.91 mg/kg, 1.18–1.77 mg/kg, 1.05–4.25 mg/kg, 20.30–36.21 mg/kg, respectively (Table 3) (Table S5). The analysis of the biota-sediment accumulation factor showed that A. japonicus was a macro-concentrator for Cd (BSAF > 2) at all the sites (Table 4). Cd accumulation was observed in the worms Neanthes japonica (BSAF > 1) at a higher rate than the other metals at Jinzhou Bay, China (Fan et al., 2014). In the sea cucumber A. japonicus, Cd accumulation was observed in laboratory experiments in the body wall from a dietary intake of 500 mg/kg (Wang et al., 2016b). Furthermore, cadmium accumulation is reported for many sea cucumber species such as Thelenota ananas, Holothuria tubulosa, Holothuria arenicola, Holothuria verrucosa, Holothuria atra, and Holothuria cinerascens. Additionally, some holothurians are suggested to be micro-concentrators for Cd in Pakistan (Ahmed et al., 2017) (Table 3). Hence, our study suggests that A. japonicus could be considered as a bio-monitor for Cd. Similarly, Ziyaadini et al. (2017) reported the possibility of using the mollusc Chiton lamyi as a bio-indicator for Cd, depending on whether they are macro-concentrators. Additionally, some sea cucumber species, such as H. tubulosa, are useful bioindicators for heavy metal concentrations in sediment (Warnau et al., 2006). Furthermore, the biota-sediment accumulation factor showed that A. japonicus is a macro-concentrator for Zn at S3 (Table 4). This finding might be due to the tendency of sea cucumber to accumulate Zn in the body wall. Zn concentration in the body wall of the sea cucumber was at higher rate than of Cu in sea cucumber H. leucospliota (Xing and Chia, 1997). Moreover, A. japonicus was mostly a micro-concentrator for As and a de-concentrator for Cr, Cu, Mn, Ni, Pb. This finding might indicate that sea cucumber might has a strategy for accumulating some heavy metals in the body wall. For instance, Pb accumulation in the body wall of sea cucumber might be due to its high affinity to the calcium-rich skeleton (Warnau et al., 2006). Also, Zn concentrations in the body wall of the sea cucumber H. leucospliota decreased when the sea cucumber left without sediment for forty days (Xing and Chia, 1997).

The correlation between Cu and Zn in the body wall at different sites was high (Table S6), which might indicate a similar biochemical pathway in the body wall of A. japonicus. The biochemical pathway is the chemical reactions that take place in the normal operation of living systems. Furthermore, there was no significant correlation between heavy metals concentrations in the sea cucumber body wall and in the sediment or in the MPs (Table 2).

### 3.3. Heavy metals association with MPs

The composition of the MPs isolated was mainly fibres and was reported in our previous study from the same sites (Mohsen et al., 2019). In addition, no MPs were detected in the blank replicates. FT-IR analysis indicated that the polymer types were mainly cellophane and polyester with smaller amounts of polypropylene and polyethylene terephthalate (Table S7) (Fig. S1).

The eight heavy metals examined in this study were detected in association with isolated MPs from all the sites, with variation among sites (Fig. 2). The range of the average concentrations of As, Cd, Cr, Cu, Mn, Ni, Pb and Zn in association with the MPs were as follows: 0.35–2.89 mg/kg, 0.058–0.99 mg/kg, 4.43–37.47 mg/kg, 1.37–21.67 mg/kg, 7.57–98.35 mg/kg, 1.31–43.2 mg/kg, 2.56–40.8 mg/kg, 16.44–1190 mg/kg, respectively (Table S8). These concentrations are lower than the heavy metals concentrations of Cu, Zn, Pb, Cd, Mn, Ni, Pb and Zn in association with the MPs were higher than those associated with the corresponding sediment at all the sites. Additionally, Pb accumulated in higher concentrations than the corresponding sediment at some sites (Fig. 2). This is likely due to an increase over time of heavy metals adsorption on the plastics particles (Rochman et al., 2014). Accumulation of Cd and Pb was reported with MPs in beach sediments that exceeded 10^3 mg/kg (Massos and Turner, 2017). Additionally, Zn accumulation was observed on the suspended sediments or incubation times.

![Fig. 2. Average heavy metal concentrations in the sediment, MP, the body wall of sea cucumber at different sites (mg/kg).](image-url)
MPs in the sea water from antifouling paint (Brennecke et al., 2016). The affinity of the MPs to a certain metal might be affected by the polymer type. For instance, polystyrene polymer adsorbs lower amounts of Cu and higher amount of Zn than polyvinylchloride (Brennecke et al., 2016). Additionally, high-density polyethylene polymer adsorbs lower amounts of Cd, Ni, Pb and Zn than of polyethylene terephthalate, polypropylene, and low-density polyethylene (Rochman et al., 2014). Other heavy metals showed lower concentrations in association with the MPs than those of the corresponding sediments at all the sites. The MPs isolated from site S6 showed the highest accumulation of heavy metals, while the lowest accumulation was observed at the MPs isolated from site S1. Total heavy metal concentrations on the MPs were sequenced as follows: Zn > Mn > Pb > Cr > Ni > Cu > As > Cd.

The correlation between heavy metal concentrations associated with the MPs were mostly significant except for As (Table S9), suggesting similar adhesion process or similar pollution sources. In addition, there was no significant correlation between the heavy metal concentration associated with the MPs and in the sediment, or between the heavy metal concentrations associated with the MPs and in the body wall of sea cucumber. These findings might indicate that heavy metal concentration associated with the MPs is not an indicator for the heavy metal concentrations in the sea cucumber or in the sediment. This is likely due to the following reasons: 1) concentrations of the heavy metal that associated with the MPs may vary among sites (Ashton et al., 2010; Vedolin et al., 2018), which is similar with the current study; 2) heavy metal accumulate on the MPs over time (Rochman et al., 2014); 3) metal adsorption is higher on the aged MPs than of the more recent one (Holmes et al., 2014).

CRediT authorship contribution statement

Mohamed Mohsen: Conceptualization, Investigation, Formal analysis, Writing - original draft. Qing Wang: Data curation, Writing - review & editing. Linbin Zhang: Funding acquisition, Visualization, Writing - review & editing. Lina Sun: Resources, Writing - review & editing. Chengang Lin: Investigation, Writing - review & editing. Hongsheng Yang: Funding acquisition, Project administration, Supervision, Validation.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version, atdoi https://doi.org/10.1016/j.marplanbul.2019.04.025. These data include the Google map of the most important areas described in this article.

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