Journal of Oceanology and Limnology
Vol. 41 No. 4, P. 1519-1536, 2023
https://doi.org/10.1007/s00343-022-1387-8

Assessment of heavy metal pollution in Laizhou Bay (China) using the ecological risk index and the integrated biomarker response of the goby *Acanthogobius ommaturus**

Maosheng LIU[1, #], Hua XU[2, #], Qiang ZOU[3], Fei FANG[1], Shan SUN[4], Yuting ZHAO[4], Xin HE[4], Yonghui BO[1], Lei YAO[1], Yan FANG[1, **]

[1] *School of Agriculture, Ludong University, Yantai 264025, China*
[2] *Yantai Ecological Environment Monitoring Center, Yantai 264010, China*
[3] *Yantai Branch of Shandong Technology Transfer Center, Chinese Academy of Sciences, Yantai 264003, China*
[4] *Shandong Provincial Key Laboratory of Restoration for Marine Ecology, Shandong Marine Resource and Environment Research Institute, Yantai 264006, China*

Received Nov. 17, 2021; accepted in principle Mar. 31, 2022; accepted for publication May 23, 2022

Abstract We used the Integrated Biological Responses version 2 (IBRv2) method to evaluate the biological effects of heavy metals in the sediments in Laizhou Bay, China on the benthic goby *Acanthogobius ommaturus*. In December 2018, gobies and sediments were collected from 15 stations. We measured the activities of defense enzymes and the contents of malondialdehyde (MDA) and metallothionein (MT) in the goby liver as well as the levels of heavy metals in the sediments and goby muscle tissue. Most of the heavy metal concentrations in sediment at each station were below the Class I criteria set by Chinese Standards for Marine Sediment Quality, and the Håkanson ecological risk index suggested low risk for the heavy metals. We found that *A. ommaturus* could effectively accumulate mercury, cadmium, arsenic, and zinc and that the contents of MT and MDA and the activities of glutathione peroxidase and glutathione reductase were suitable biomarkers of heavy metal pollution in this species. The IBRv2 method integrated these four biomarkers and discriminated stations according to heavy metal pollution. Higher IBRv2 values suggested more adverse effects in gobies, corroborating more serious heavy metal contamination. The stations with high IBRv2 values and high contents of heavy metals were mainly distributed in the west and northeast parts of the bay. These results show that the IBRv2 approach is a feasible strategy for assessing heavy metal pollution through biological response and biological status and that it can be implemented for environmental monitoring in Laizhou Bay.

Keyword: integrated biomarker responses; heavy metal; assessment; goby *Acanthogobius ommaturus*; Laizhou Bay

1 INTRODUCTION

Bays are located at the intersection of the lithosphere, hydrosphere, atmosphere, and biosphere. They are the carriers of environmental resources; thus, they have high ecological value and an important strategic position for humans to develop and utilize the ocean. Many pollutants generated by industrial and agricultural production and human activities are discharged directly or indirectly into bays via surface runoff, atmospheric deposition, or direct coastal discharge, resulting in degradation of the habitat and resources (Duarte et al., 2017).

Heavy metals are one of the most prevalent persistent pollutants in the marine environment. They

* Supported by the National Key Research and Development Program of China (No. 2019YFD0900704), the Yantai Key Research and Development Program (No. 2019XDHZ097), the National Natural Science Foundation of China (No. 42076137), the Natural Science Foundation of Shandong Province (No. ZR2020QD003), and the Shandong Key Laboratory of Coastal Environmental Processes, YICCAS (No. 2019SDHADKFJJ16)
** Corresponding author: fly12689@163.com
# Maosheng LIU and Hua XU contributed equally to this work and should be regarded as co-first authors.

are toxic to the immune, nervous, and reproductive systems of marine animals, and they endanger living beings through food chain transfer (La Colla et al., 2018). Heavy metals have been detected by chemical monitoring in many bays of the world (De Jesus et al., 2021; Ota et al., 2021), including in China (Liu et al., 2019, 2021). In China, about 7.13 billion tons of sewage was discharged directly into the sea from 442 pollution sources in 2020, including 17.2 tons of chromium (Cr), lead (Pb), mercury (Hg), and cadmium (Cd) (Ministry of Ecology and Environment of the People's Republic of China, 2021). Through chemical analysis, we can determine what kinds of heavy metals exist in the environment and their concentrations. However, this does not reflect the toxic effects of heavy metals on organisms.

The effects of pollutants on organisms begin at the molecular level and gradually appear at the levels of cells, tissues, organs, individuals, populations, and communities. Biomarkers are biological measures of the response of organisms to environmental changes at molecular, biochemical, or cellular levels (WHO, 1993). They can provide an early warning about problems that could seriously damage organisms, thus they are a powerful tool for monitoring the biological effects of pollutants.

A single biomarker often cannot fully explain the response of organisms to a complex and changeable environment, but the use of multiple biomarkers can help solve this problem. The Integrated Biomarker Response (IBR) method integrates the values of multiple biomarkers into an index of IBR, which allows for the holistic assessment of the effects of environmental pollution from the perspective of biological response (Beliaeff and Burgeot, 2002). The IBR was updated to Integrated Biological Responses version 2 (IBRv2) to avoid weakness of this integrative tool (Sanchez et al., 2013). The combination of biomarker monitoring and chemical monitoring provides an ecotoxicological diagnosis that can be used for marine environmental management (Vieira et al., 2019).

Laizhou Bay, located in the southern part of the Bohai Sea in China, is the main spawning and nursery ground, habitat, and traditional fishing ground of fishery species in the Yellow Sea and Bohai Sea. Industry (e.g., marine chemical engineering, oil and gas exploitation, and salt production), agriculture (e.g., aquaculture and fishing), and urbanization are rapidly developing around this area. These activities together with the many rivers that empty into the bay mean that a large quantity of heavy metals is discharged into Laizhou Bay, and they pose a threat to the marine environment and fishery species (Liu et al., 2019). Chemical monitoring of heavy metals in this area has been reported (Li et al., 2019), but biomarker monitoring has rarely been conducted.

The first step of biomarker monitoring is to select suitable bioindicators. Gobies (family Gobiidae) are resident benthic fish species in Laizhou Bay. They are the food of almost all of the large bottom fishes (such as members of the Trichiuridae, Sciaenidae, and Rajiformes as well as *Paralichthys olivaceus*, *Lateolabrax japonicus*, and *Liparis tanakae*) in the Bohai Sea, with the exception of members of the Pleuronectidae (Liu et al., 1997). Because gobies are an intermediate link between the benthos and benthic fishes, they play an important ecological role, and they have been widely used in toxicology and environmental monitoring. Biomarkers in gobies have been applied successfully as indicators of sediment pollution (Pauletto et al., 2019; Salgado et al., 2021). In recent years, goby resources have increased, and the sedentary and large-sized *Acanthogobius ommaturus* has become the dominant species in the fish community of Laizhou Bay (Zhang et al., 2019). Therefore, *A*. *ommaturus* is a potential bioindicator for pollution monitoring in Laizhou Bay.

The next step of biomarker monitoring is to select appropriate biomarkers. Esterase acetylcholinesterase (AChE, EC 3.1.1.7) is widely used in biomonitoring programs due its sensitivity to pollutants (Barhoumi et al., 2014). The components of the animal's defense system, such as antioxidant enzymes and metallothionein (MT), protect organisms against stress. Heavy metals cause oxidative stress in organisms. Malondialdehyde (MDA) is the product of lipid peroxidation, and its level can reflect the degree of oxidative stress. The activities of antioxidant enzymes and AChE as well as contents of MT and MDA in gobies respond sensitively to heavy metals (McDonald et al., 2021; Salgado et al., 2021), thus they are candidates for biomarker monitoring in *A*. *ommaturus*.

To describe the effects of pollution on organisms and provide a better understanding of environmental risk in Laizhou Bay, multiple biomarkers in *A*. *ommaturus* were analyzed. The levels of heavy metals in *A*. *ommaturus* and the sediment were also measured. The correlation between the biomarkers in *A*. *ommaturus* and the heavy metal levels in the sediment were analyzed to verify the feasibility of

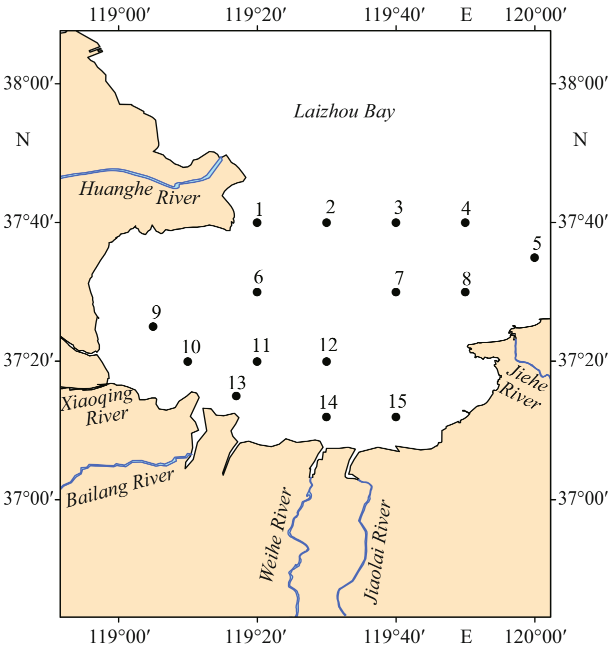

Fig.1 Map of the study area and sampling stations in Laizhou Bay

The dots indicate sampling stations.

biomarker monitoring. Consequently, the heavy metal contamination in Laizhou Bay was assessed using the combination of biomarker and chemical monitoring.

2 MATERIAL AND METHOD

The field survey was conducted according to the Specification for Marine Investigation (General Administration of Quality Supervision, Inspection and Quarantine of the People's Republic of China, China National Standardization Administration, 2008a) and the Specification for Marine Monitoring (General Administration of Quality Supervision, Inspection and Quarantine of the People's Republic of China, China National Standardization Administration, 2008b) in Laizhou Bay in December 2018. The goby and surface sediment samples were collected at 15 stations (Fig.1). The surface water temperature, pH, dissolved oxygen content, and salinity were measured using a multi parameter water quality detector (556MPS, YSI, Yellow Springs, OH, USA).

2.1 Sampling of sediments and fishes

Surface sediments were collected using a grab sampler (van Veen 437332, HYDRO-BIOS, Altenholz, Germany), and three sediment samples were obtained at each station. Sediments were preserved in glass jars for Hg analysis and in polyethylene bags for copper (Cu), Pb, zinc (Zn), Cd, Cr, and arsenic (As) analysis. Sediment samples were transported in low temperature to the Shandong Marine Environment Monitoring Center who has passed the qualification certification for inspection and testing institutions for the above heavy metal monitoring projects.

Acanthogobius ommaturus were collected at each site using a fish net. Ten fishes from each site with average body length of 89.02±19 mm and body weight of 57.96±17 g were used for biomarker analysis. The fishes were anaesthetized with 20 mg/L of tricaine methane sulfonate (MS222, Sigma-Aldrich, St. Louis, MO, USA). The fish were washed with ultrapure water, and then 10–20 g of muscle tissues were dissected from the dorsal side of the fish without skin and bone. The muscle tissues were collected into plastic bags and transported to the Shandong Marine Environment Monitoring Center in an icebox for heavy metal analysis. The livers were dissected and stored in liquid nitrogen for biomarker analysis. Livers or muscles from three or four fishes were pooled as one replicate, with three replicates for each station.

2.2 Heavy metal analysis

The muscle samples were freeze-dried, homogenized, and sieved trough a 154-μm nylon mesh. The analysis of heavy metals in fish muscle tissues and sediment samples was performed according to the Specification for Marine Monitoring (General Administration of Quality Supervision, Inspection and Quarantine of the People's Republic of China, China National Standardization Administration, 2008b). Hg and As contents were measured using the atomic fluorescence spectrometry method in an atomic fluorescence spectrophotometer (PF72, Persee, Beijing, China). Cu, Pb, Cd, and Cr levels were measured using flameless atomic absorption spectrometry in an atomic absorption spectrophotometer (AA-7000, Shimadzu, Kyoto, Japan). Zn content was measured by flame atomic absorption spectrometry using the atomic absorption spectrophotometer. The detection limits for Hg, Cu, Pb, Zn, Cd, Cr, and As in the sediment were 0.002, 0.5, 1.0, 6.0, 0.04, 2.0, and 0.06 mg/kg, respectively. The detection limits for Hg, Cu, Pb, Zn, Cd, Cr, and As in the biological samples were 0.002, 0.4, 0.04, 0.4, 0.005, 0.04, and 0.2 mg/kg, respectively. Organic carbon and sulfide contents in the sediment were measured using the potassium dichromate oxidation reduction volumetric method and the methylene blue spectrophotometry method under the guidance of the Specification for Marine Monitoring (General Administration of Quality Supervision, Inspection and Quarantine of the People's Republic of China, China National Standardization Administration, 2008b).

2.3 Quality assurance and quality control (QA & QC)

Quality assurance and quality control measures included one reagent blank sample, 10% parallel samples, one matrix spiked sample, and one sediment certified standard sample (National Standard Sediments GBW07333) or one certified reference material (National Standard Croaker Material GBW08573) for each sample batch. The relative deviation of the heavy metal content for the parallel samples was less than 8%. The recovery rate of matrix spiked with standard varied between 94% and 106%. The measured value of the heavy metal content for the certified standard sample was in the range of the guaranteed value.

2.4 Assessment of heavy metals in sediments

2.4.1 Geo-accumulation index

The heavy metal pollution was assessed according to the heavy metal content and geochemical background value as follows (Müller, 1960; Supplementary Table S1):

$$I_{geo}=\log_2(C_n/1.5B_n), \tag{1}$$

where I_{geo} is the geo-accumulation index, C_n is the measured concentration of the heavy metal n, and B_n is the geochemical background level of the heavy metal n. The geochemical background levels of Hg, Cu, Pb, Zn, Cd, Cr, and As in Laizhou Bay are 0.15, 24.68, 17.50, 79.82, 0.156, 61.0, and 15.0 mg/kg, respectively (Li et al., 1994; Liu et al., 2019). The constant 1.5 is a matrix correction factor that eliminates the influence of background value variation caused by sediment diagenesis.

2.4.2 Håkanson ecological risk index

The Håkanson ecological risk index assesses the degree of potential ecological hazard of heavy metals considering their biological, toxicological, and sedimentological characteristics (Håkanson, 1980). It can comprehensively reflect the potential impact of heavy metals on the environment based on the following equations:

$$C_f^i=C_s^i/C_n^i, \tag{2}$$

$$E_r^i=T_r^i\times C_f^i, \tag{3}$$

$$\mathrm{RI}=\sum_{i=1}^{7}E_r^i, \tag{4}$$

where C_f^i is the pollution index of heavy metal i; C_s^i is the content of the heavy metal i; C_n^i is the background content of the heavy metal i in the studied area; and n is the number of measured heavy metal species. T_r^i is the toxic response factor for heavy metal i, and T_r^i values of Hg, Cu, Pb, Zn, Cd, Cr, and As are 40, 5, 5, 1, 30, 2, and 10, respectively. E_r^i is the potential risk of heavy metal i, and RI is the sum of the potential risks of measured heavy metals. Because the number of pollutants selected in this study differ from that of Håkanson (1980), modification of the risk scale was made (Lv et al., 2018), as shown in Supplementary Table S2.

2.5 Calculation of the bioaccumulation factor

The bioaccumulation factor (BAF) was used to evaluate the ability of *A. ommaturus* to accumulate metals from sediment. The BAF was calculated as reported for benthic fishes *Zosterisessor ophiocephalus* (Barhoumi et al., 2009) and *Cottus gobio* (Pastorino et al., 2020):

$$\mathrm{BAF}=C_B/C_s, \tag{5}$$

where C_B is the metal content in goby tissues and C_s is the metal content in the sediment, and BAF >1 indicates effective accumulation of heavy metals by organisms.

2.6 Biomarker analysis

Half of the livers were homogenized at 4 °C in 0.25-mol/L sucrose with a weight:volume ratio of 1:9. The homogenate was centrifuged (20 000×*g*) at 4 °C for 40 min (Fang et al., 2012). The supernatants were collected for the determination of MT content using the Ag saturation method (Scheuhammer and Cherian, 1986) with some modifications. MT content was calculated according to the content of Ag^+ bound by MT.

The other half of the livers were homogenized at 4 °C in 0.1-mol/L Tris-HCl buffer (pH 7.4, containing 1-mmol/L EDTA, Sinopharm Chemical Reagent Co., Shanghai, China) with a weight:volume ratio of 1:9. The homogenate was centrifuged (13 000×*g*) at 4 °C for 30 min (Zhu et al., 2019). The contents of MDA and protein as well as activities of antioxidant enzymes and AChE in the supernatant were analyzed by spectrophotometry (WFZ UV-2800AH, Unico, Dayton, NJ, USA) according to the instructions for each kit (Nanjing Jiancheng Bioengineering

Institute, Nanjing, China).

Superoxide dismutase (SOD, EC 1.15.1.1), catalase (CAT, EC 1.11.1.6), glutathione peroxidase (GPX, EC 1.11.1.9), glutathione reductase (GR, EC 1.6.4.2), and glutathione S-transferase (GST, EC 2.5.1.18) are important components of the antioxidant defense system. SOD inhibits the production of superoxide anion radical ($O_2^-\cdot$) generated by xanthine and the xanthine oxidase reaction system, then reduces the amount of colored compound generated in the reaction system. SOD activity was measured at 505 nm, and one unit was defined as the amount of enzyme inhibiting 50% of the color production per milligram of tissue protein. Ammonium molybdate stops CAT from decomposing H_2O_2 and combines with H_2O_2 to form a yellow complex. The yellow complex can be detected at 405 nm. One unit of CAT activity was expressed as the enzyme amount that decomposes 1 μmol of H_2O_2 per second per milligram of tissue protein. GPX catalyzes oxidation of reduced glutathione (GSH) by H_2O_2. The compound formed by the combination of GSH and 5,5′-Dithiobis-(2-Nitrobenzoic Acid) has an absorption peak at 412 nm. At this wavelength, the decrease of absorbance can reflect GPX activity. One unit of GPX activity was defined as the enzyme amount required to oxidize 1 mol of GSH per minute per milligram of tissue protein. GR catalyzes the reduction of oxidized glutathione (GSSG) by receiving the hydrogen supply of nicotinamide adenine dinucleotide phosphate in the reduced form (NADPH). GR activity was calculated by detecting the amount of NADPH at 340 nm, and one unit was expressed as the amount of enzyme required to change the concentration of NADPH by 1 mmol/L per minute per milligram of tissue protein. The reaction products of GSH and 1-chloro-2,4-dinitrobenzene catalyzed by GST can be detected at 412 nm. A unit of GST activity was defined as the amount of enzyme required to reduce 1-μmol/L GSH per minute per milligram of tissue protein.

Malondialdehyde is the product of lipid peroxidation, and its level can reflect the degree of oxidative stress. MDA combines with thibabituric acid to form a red product detected at 532 nm. MDA content was expressed as the mole number of MDA per milligram of protein in the tissue. AChE hydrolyzes acetylcholine to choline, which reacts with a sulfhydryl chromogenic agent to produce sym-trinitrobenzene, which is detected at 412 nm. One unit of AChE activity was defined as the amount of enzyme required to hydrolyze 1 μmol of acetylcholine over a 6-min period per milligram of tissue protein. Protein content was measured based on the Bradford (1976).

2.7 IBRv2

Biomarkers significantly related to the content of heavy metals in sediments were screened using Pearson correlation analysis. The screened biomarkers were integrated by IBRv2 (Sanchez et al., 2013). The multi-biomarker responses in *A. ommaturus* were assessed based on the IBRv2 scores and star plots. Data processing of IBRv2 calculations were as follows:

A certain biomarker value (X_i) of the polluted station was compared to a reference value (X_0) of the reference station. The station with low pollution investigated at the same time could be used as the reference station (Sanchez et al., 2013). A log transformation was processed to reduce variance as follows:

$$Y_i=\log(X_i/X_0). \quad (6)$$

The general mean (μ) and standard deviation (σ) were calculated to standardize Y_i:

$$Z_i=(Y_i-\mu)/\sigma. \quad (7)$$

The biomarker deviation index (A) was calculated using the mean of the standardized biomarker response (Z_i) and reference biomarker data:

$$A=Z_i-Z_0. \quad (8)$$

The absolute value of A for each biomarker at every polluted station was summed as

$$\text{IBRv2}=\sum|A|. \quad (9)$$

The biomarker star plots for all stations were generated using Sigmaplot (version 13.5; Systat Software, San Jose, CA, USA). For a certain station, the A parameters were shown in a star plot to represent the reference deviation of every analyzed biomarker. The value of A parameters >0 indicates biomarker induction, and the value of A parameters <0 indicates biomarker inhibition.

2.8 Statistical analysis

The data were expressed as mean±standard deviation (SD). Three repeated measurements were performed for every biomarker of each station. One-way analysis of variance was applied to analyze the differences of the biomarker among the stations using SPSS (version13.0; IBM, Armonk, NY, USA). Tukey's test was used with the significance level set at P<0.05. Correlations between heavy metal content, biomarker level, RI, and IBRv2 value were analyzed

Table 1 Geo-accumulation index (I_{geo}) of heavy metals in Laizhou Bay

Station	Hg	Cu	Pb	Zn	Cd	Cr	As
1	-2.860	-0.504	-0.560	-0.924	-0.513	-0.998	-0.895
2	-3.170	-1.210	-1.364	-1.515	-1.212	-1.283	-0.956
3	-4.354	-1.477	-1.336	-1.600	-1.585	-1.334	-1.006
4	-3.059	-1.098	-1.482	-1.515	-1.661	-1.395	-1.404
5	-5.006	-0.903	-1.489	-1.564	-1.461	-1.223	-1.304
6	-5.006	-0.613	-1.404	-1.349	-1.170	-1.187	-1.086
7	-4.229	-0.527	-2.287	-1.968	-1.940	-1.804	-1.558
8	-3.907	0.363	-0.732	-0.842	-0.522	-0.721	-0.981
9	-4.229	0.259	-0.520	-0.656	-0.212	-0.685	-1.086
10	-3.644	-0.853	-0.723	-0.613	-0.057	-1.106	-0.644
11	-4.492	-0.607	-1.560	-1.457	-1.700	-1.480	-1.072
12	-4.006	-0.275	-1.229	-1.377	-1.037	-1.155	-1.006
13	-4.113	-0.978	-1.378	-1.575	-0.241	-1.548	-1.032
14	-4.354	-0.650	-2.061	-1.820	-1.411	-1.734	-1.458
15	-4.006	-0.561	-1.548	-1.571	-1.461	-1.562	-1.454

Table 2 Ecological risk index for heavy metals in Laizhou Bay

Station	E_r^i							RI
	Hg	Cu	Pb	Zn	Cd	Cr	As	
1	8.267	5.288	5.086	0.791	31.538	1.502	8.067	60.537
2	6.667	3.241	2.914	0.525	19.423	1.233	7.733	41.737
3	2.933	2.694	2.971	0.495	15.000	1.190	7.467	32.751
4	7.200	3.505	2.686	0.525	14.231	1.141	5.667	34.954
5	1.867	4.011	2.671	0.507	16.346	1.285	6.073	32.762
6	1.867	4.903	2.834	0.589	20.000	1.318	7.067	38.577
7	3.200	5.207	1.537	0.383	11.731	0.859	5.093	28.010
8	4.000	9.643	4.514	0.837	31.346	1.820	7.600	59.760
9	3.200	8.975	5.229	0.952	38.846	1.866	7.067	66.134
10	4.800	4.153	4.543	0.981	43.269	1.393	9.600	68.740
11	2.667	4.923	2.543	0.546	13.846	1.075	7.133	32.734
12	3.733	6.199	3.200	0.578	21.923	1.348	7.467	44.448
13	3.467	3.809	2.886	0.504	38.077	1.026	7.333	57.101
14	2.933	4.781	1.797	0.425	16.923	0.902	5.460	33.221
15	3.733	5.085	2.566	0.505	16.346	1.016	5.473	34.725

by Pearson correlation analysis followed by a two-tailed test, with the significance level set at $P<0.05$. Graphics were depicted using ArcGIS (version 10.0; Environmental systems research institute, Redlands, CA, USA) and Sigmaplot (version 13.5; Systat Software).

3 RESULT

3.1 Physicochemical parameters of water and sediment

The physicochemical parameters of water were: pH 8.24–8.35, temperature 3.70–5.10 °C, salinity 25.74–27.52, and dissolved oxygen content 9.70–11.40 mg/L (Supplementary Table S3). The content of organic carbon and sulfide in the sediment met the Class I criteria set by Chinese Standards for Marine Sediment Quality (General Administration of Quality Supervision, Inspection and Quarantine of the People's Republic of China, 2004).

3.2 Heavy metal levels in sediments

The Class I criteria set by Chinese Standards for Marine Sediment Quality (General Administration of Quality Supervision, Inspection and Quarantine of the People's Republic of China, 2004) state that levels of Cu, Pb, Zn, Cd, Cr, As, and Hg should be below 35, 60, 150, 0.50, 80, 20, and 0.20 mg/kg, respectively. Most of the heavy metal levels at all stations were below these values (Supplementary Table S4). However, Cu concentrations at station 8 (northeast bay) and station 9 (southwest bay) were 47.6 and 44.3 mg/kg, respectively, which exceeded the Class I criteria. The concentrations of heavy metals were ranked as: Zn > Cr > Cu > Pb > As > Cd > Hg. The stations with high contents of Pb, Zn, Cd, and Cr were mainly distributed in the west and northeast parts of the bay (Fig.2a–d). The stations with high contents of Hg and As were mainly distributed in the northwest part of the bay and in the Xiaoqing River estuary (Fig.2e–f). The low Cu concentration areas distributed in the middle of the bay mouth, but its concentration was high in other areas of the bay (Fig.2g).

Most I_{geo} values for heavy metals were <0, which indicated that the surface sediments in Laizhou Bay were mostly unpolluted (Table 1). However, the I_{geo} values of Cu at stations 8 and 9 were between 0 and 1, which indicated that these areas were unpolluted to moderately polluted. The I_{geo} values of the heavy metals were arranged in the following order Cu > Cd > As > Pb > Cr > Zn > Hg.

The E_r^i value of Cd at station 10 was between 40 and 80, indicating that Cd posed a moderate ecological risk in this area (Table 2). The remaining E_r^i values were <40, indicating low ecological risk in most areas of Laizhou Bay. The sequence of ecological risk of heavy metals was Cd > As > Cu > Hg > Pb > Cr > Zn. The RI at each station suggested that levels of all of

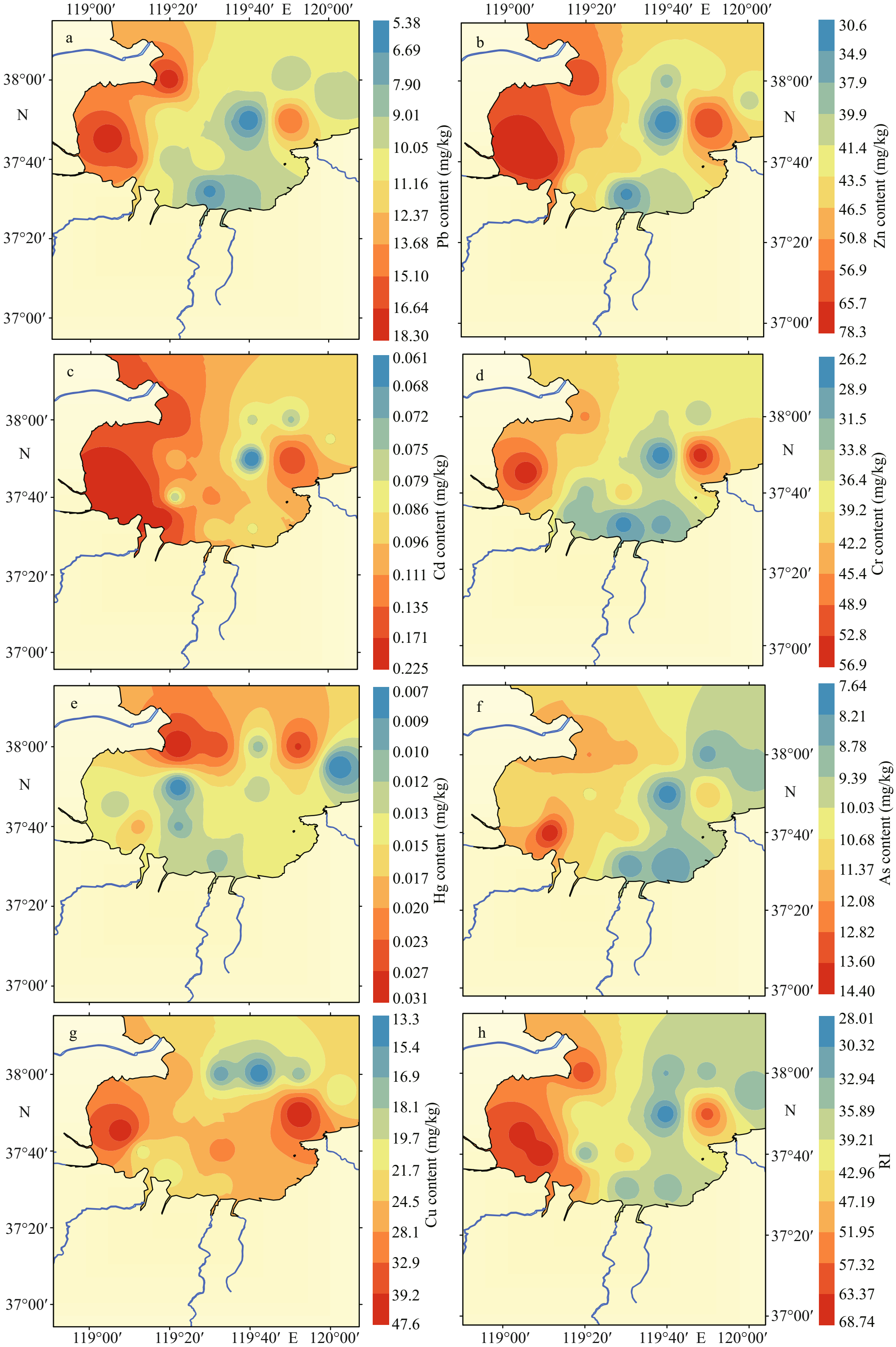

Fig.2 Distribution of heavy metals and RI in Laizhou Bay

a. Pb; b. Zn; c. Cd; d. Cr; e. Hg; f. As; g. Cu; h. RI.

Table 3 Heavy metal content in muscles of *A. ommaturus* (mg/kg, dry weight)

Station	Hg	Cu	Pb	Zn	Cd	Cr	As
1	0.082±0.008	1.03±0.26	0.19±0.03	52.23±7.31	0.231±0.055	2.30±0.32	5.10±1.65
2	0.062±0.005	1.32±0.37	0.06±0.01	47.21±6.51	0.055±0.008	3.33±0.96	23.28±6.21
3	0.042±0.004	1.94±0.21	0.10±0.02	30.99±7.27	0.056±0.007	5.48±0.87	18.16±1.91
4	0.072±0.019	0.82±0.15	0.12±0.02	32.64±3.60	0.013±0.004	0.51±0.13	16.74±1.67
5	0.038±0.007	1.81±0.26	0.04±0.01	42.68±4.00	0.134±0.045	0.59±0.07	8.40±1.52
6	0.076±0.011	0.90±0.11	0.11±0.02	36.44±5.85	0.032±0.006	0.53±0.09	15.75±2.68
7	0.051±0.013	1.35±0.33	0.07±0.01	27.70±6.54	0.066±0.007	0.73±0.07	6.37±1.93
8	0.053±0.008	3.17±0.63	0.19±0.02	41.59±3.31	0.174±0.023	13.04±2.96	4.02±0.64
9	0.046±0.005	2.15±0.20	0.20±0.06	68.57±6.87	0.067±0.009	4.43±1.45	3.45±0.78
10	0.055±0.011	2.60±0.46	0.32±0.05	62.54±8.47	0.564±0.062	1.64±0.17	18.21±2.22
11	0.059±0.005	1.56±0.46	0.08±0.01	35.68±7.31	0.136±0.033	7.80±0.95	15.40±2.78
12	0.069±0.014	1.90±0.55	0.13±0.03	50.11±7.86	0.071±0.013	4.74±0.76	13.99±2.04
13	0.055±0.007	2.04±0.23	0.16±0.06	44.40±11.27	0.215±0.063	1.17±0.11	11.77±2.73
14	0.041±0.010	1.36±0.14	0.13±0.02	30.53±7.45	0.312±0.046	3.48±0.65	4.43±0.61
15	0.047±0.007	1.20±0.13	0.05±0.01	30.49±8.53	0.194±0.031	0.78±0.14	3.37±0.33

the studied heavy metals were of low ecological risk.

3.3 Heavy metal level and BAF in *A. ommaturus*

The average contents of heavy metals analyzed in goby muscle tissue followed the order: Zn > As > Cr > Cu > Cd > Pb > Hg (Table 3). According to the maximum allowable levels of Cd, Cr, and Pb in foods set by the National Food Safety Standard Maximum Levels of Contaminants in Foods (National Health Commission of the People's Republic of China, State Administration for Market Regulation, 2017), the contents of Cd, Cr, and Pb in *A. ommaturus* were below these maximums except for Cd content at station 10 and Cr content at station 8. The BAF values followed a descending order of Hg > Cd > As > Zn > Cu > Pb > Cr (Table 4). These results indicate that *A. ommaturus* can efficiently bioaccumulate Hg, Cd, As, and Zn.

Table 4 The BAF values of heavy metals in *A. ommaturus*

Station	BAF						
	Hg	Cu	Pb	Zn	Cd	Cr	As
1	2.642	0.040	0.011	0.828	1.406	0.050	0.422
2	2.496	0.083	0.006	1.127	0.543	0.089	2.007
3	3.819	0.146	0.010	0.785	0.722	0.151	1.622
4	2.685	0.047	0.012	0.779	0.180	0.015	1.969
5	5.389	0.092	0.004	1.054	1.571	0.015	0.922
6	10.895	0.037	0.011	0.775	0.307	0.013	1.486
7	4.264	0.052	0.014	0.905	1.084	0.028	0.834
8	3.518	0.067	0.012	0.623	1.068	0.235	0.352
9	3.795	0.049	0.011	0.902	0.331	0.078	0.325
10	3.053	0.127	0.020	0.799	2.508	0.039	1.264
11	5.876	0.064	0.008	0.818	1.888	0.238	1.439
12	4.902	0.062	0.011	1.087	0.626	0.115	1.249
13	4.198	0.108	0.016	1.104	1.085	0.038	1.070
14	3.698	0.058	0.020	0.900	3.542	0.126	0.541
15	3.389	0.048	0.005	0.757	2.277	0.025	0.410

3.4 Biomarker level

The biomarker levels differed significantly among stations (Fig.3). The minimum (1.84±0.63 nmol Ag^+/g wet weight (ww)) and the maximum (28.19±3.39 nmol Ag^+/g ww) of MT content occurred at stations 3 and 10, respectively (Fig.3a). Station 11 exhibited the highest AChE activity (0.138±0.019 U/mg prot.), and station 7 exhibited the lowest AChE activity (0.036±0.014 U/mg prot.; Fig.3b). The highest SOD activity (325.62±43.23 U/mg prot.) was found at station 12, and it was significantly higher than that at the other stations (Fig.3c). The lowest SOD activity (70.40±13.63 U/mg prot.) were found at station 7. CAT activity was lowest at station 6 (1.57±0.45 U/mg prot.) and highest at station 5 (8.18±1.61 U/mg prot.; Fig.3d). The lowest and highest values of GPX activity were 27.95±1.48 U/mg prot. at station 4 and 103.30±12.75 U/mg prot. at station 13, respectively (Fig.3e). GR activity was highest at station 10 (35.63±6.51 U/mg prot.), which was significantly higher than that at the other stations, and

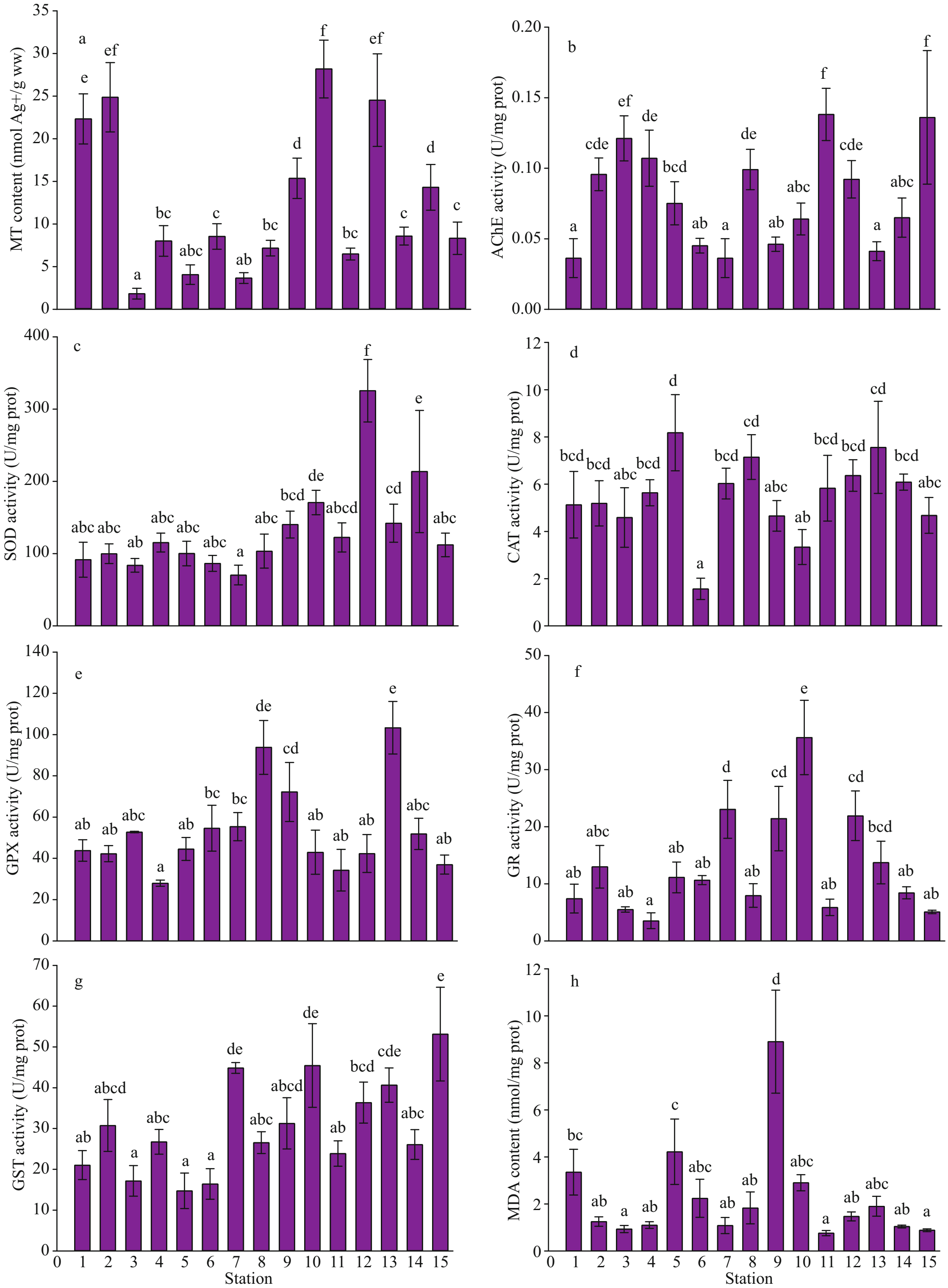

Fig.3 Biomarkers in the liver of *A. ommaturus* collected in Laizhou Bay

a. MT; b. AChE; c. SOD; d. CAT; e. GPX; f. GR; g. GST; h. MDA. The values are presented as mean±SD. Different lowercase letters indicate significant differences among stations (P<0.05).

lowest at station 4 (3.53±1.38 U/mg prot.; Fig.3f). The lowest GST activity was at station 5 (14.73±4.35 U/mg prot.), and the highest value was at station 15 (53.15±11.49 U/mg prot.; Fig.3g). MDA content at station 9 was 8.90±2.19 nmol/mg prot., which was significantly higher than that at the other stations

Table 5 Correlation coefficient between biomarker values and heavy metal contents in the sediment

Biomarker	Hg	Cu	Pb	Zn	Cd	Cr	As	RI
MT	0.527*	0.049	0.481	0.502	0.514*	0.299	0.615*	0.567*
AChE	-0.028	-0.149	-0.227	-0.228	-0.473	-0.152	-0.101	-0.411
SOD	-0.085	0.155	0.021	0.068	0.178	0.015	0.144	0.158
CAT	-0.044	0.106	-0.190	-0.292	-0.092	-0.111	-0.295	-0.107
GPX	-0.268	0.459	0.258	0.214	0.548*	0.321	0.152	0.480
GR	-0.084	0.148	0.271	0.451	0.536*	0.187	0.430	0.478
GST	0.086	0.075	-0.076	0.042	0.241	-0.254	-0.056	0.186
MDA	-0.076	0.497	0.655**	0.644**	0.568*	0.688**	0.196	0.575*
IBRv2	0.223	0.254	0.765**	0.770**	0.846**	0.648*	0.707**	0.848**

The asterisk indicates significant correlation (* $P<0.05$, ** $P<0.01$).

(Fig.3h). The minimum MDA content was 0.76±0.11 nmol/mg prot. at station 11.

3.5 Correlations among biomarkers and contents of heavy metals in the sediment

Metallothionein content was significantly correlated with As, Cd, and Hg contents in the sediment and RI (Table 5). GPX and GR activities were significantly correlated with Cd content. MDA content was significantly correlated with Cr, Zn, Cd, and Pb contents. Thus, the contents of MT and MDA as well as the activities of GPX and GR were integrated by IBRv2 to assess the heavy metal contamination in Laizhou Bay.

3.6 IBRv2

Considering the pollution degree of heavy metals in sediments and the response of biomarkers in the goby, station 3 was selected as the reference station. The IBRv2 value ranged from 3.12 to 8.93, with an average of 5.27 (Fig.4). The scores of GPX activity at some stations such as station 1 and station 2 were <0, which indicated that pollution inhibited the biomarker level. The scores of other biomarkers were >0, which indicated that pollution induced the biomarker level. IBRv2 values were significantly correlated with RI and heavy metal content in sediments (Table 5).

4 DISCUSSION

4.1 Heavy metal levels in surface sediment samples

Anthropogenic activities have a great impact on the content of heavy metals in Laizhou Bay (Liu et al., 2019). More than 10 rivers transport large amounts of pollutants from industrial and agricultural wastewater and domestic sewage into Laizhou Bay, and heavy metals are concentrated in areas where the pollutants are discharged from the rivers. The areas with the highest content of heavy metals were located in the northwest and southwest regions of the bay, in which Huanghe (Yellow) River and Xiaoqing River meet in the bay, respectively. These two rivers discharged as much as 316 tons of heavy metals into Laizhou Bay in 2016 (Li et al., 2019).

Comparing survey results from Laizhou Bay, the content of heavy metals in sediments fluctuated slightly in the period from 2010 to 2018 (Table 6). The contents of Pb, Zn, Cd, Cr, and Hg in our study were in the range of the previous survey results. The highest contents of Cu and As in our survey were slightly higher than those in other surveys (Table 6). It is generally accepted that Cu mainly originates from natural sources, such as coastal erosion, rock weathering, and loess matter transportation by the Huanghe River (Li et al., 2019). The sources of Cu may also be related to anthropogenic organic pollutants (Lü et al., 2015) and acid volatile sulfide (Di Toro et al., 1990). In addition to the western regions of the bay, Cu was concentrated in the northeast regions, where Longkou Port and many chemical and gold mining enterprises are located. A large amount of industrial sewage is discharged into the northeastern waters of Laizhou Bay, and rivers and sewage pipes are sources of Cu from both anthropogenic activities and natural contributions (Lü et al., 2015; Zhang et al., 2017). Therefore, we speculate that natural and human activities have caused the increased levels of Cu content. Largely, As contamination is also due to anthropogenic activities (Xu et al., 2015). Discharge from the smelting industry, fuel burning and industrial plants contribute to As contamination (Zhao et al., 2014). We previously mentioned the discharge capacity of the Xiaoqing River. A large

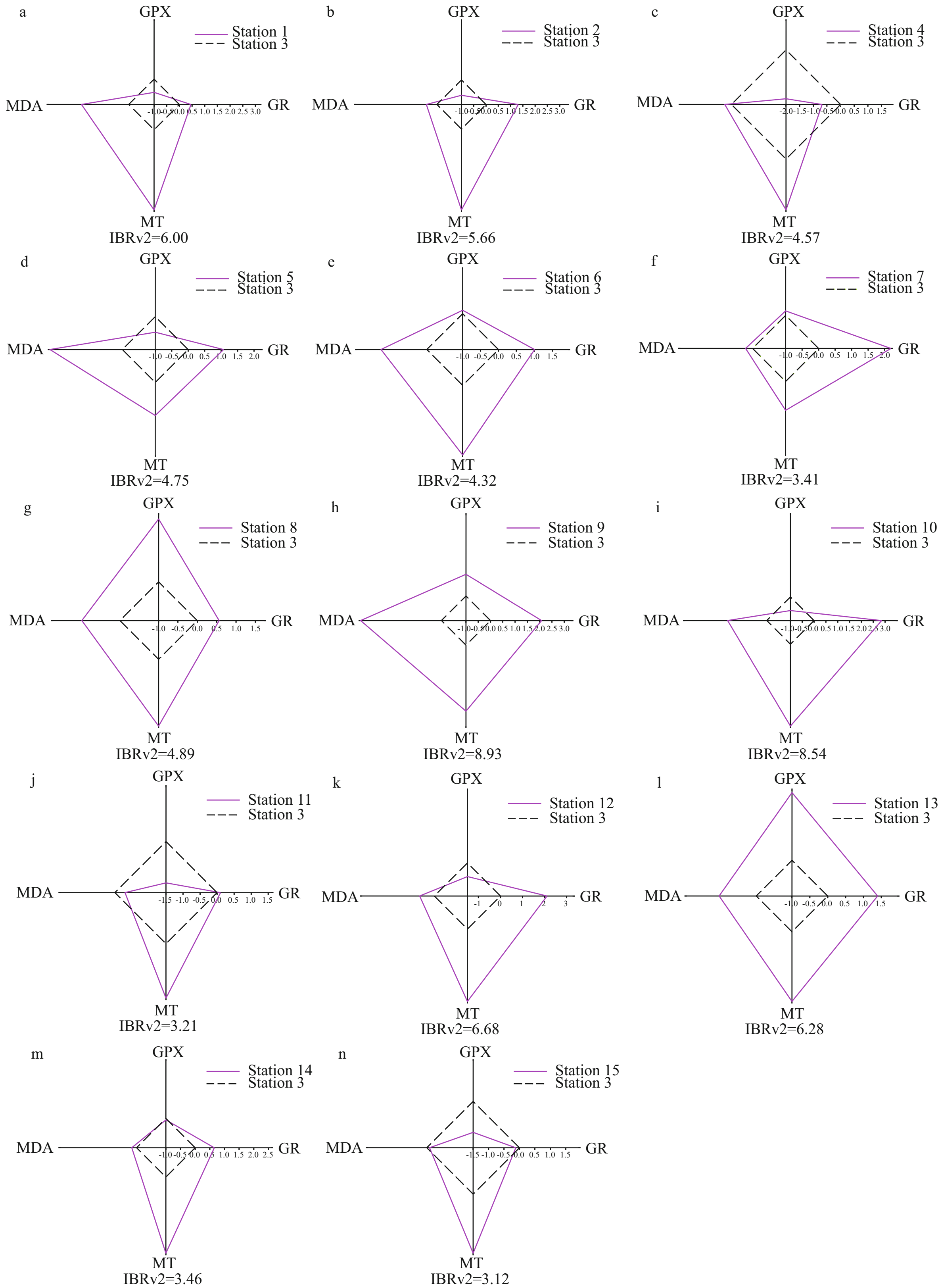

Fig.4 IBRv2 star plots at each station in Laizhou Bay

a. station 1; b. station 2; c. station 4; d. station 5; e. station 6; f. station 7; g. station 8; h. station 9; i. station 10; j. station 11; k. station 12; l. station 13; m. station 14; n. station 15. The values of biomarker deviation index at each station were used as the coordinates of radar axis to depict the star plot. The four coordinates representing four values of biomarker deviation index at the reference station were connected with black dashed lines. The four coordinates representing four values of biomarker deviation index at any other stations were connected with purple solid lines.

Table 6 Concentration ranges (mg/kg) of heavy metals in the surface sediments in Laizhou Bay and other coastal waters

Area	Date	Hg	Cu	Pb	Zn	Cd	Cr	As	Reference
Laizhou Bay, China	December, 2018	0.007–0.031	13.30–47.60	5.38–18.30	30.60–78.30	0.061–0.225	26.20–56.90	7.64–14.40	This study
Laizhou Bay, China	September–October, 2012	ND	7.18–34.77	7.77–39.89	29.13–98.53	0.180–0.840	29.23–115.03	ND	Li et al., 2021
Laizhou Bay, China	July–August, 2011	0.018–0.163	9.17–31.73	ND	ND	0.070–0.290	40.96–71.16	ND	Liu et al., 2019
Laizhou Bay, China	July–August, 2010	0.003–0.212	4.51–30.50	8.94–32.20	ND	0.180–0.670	ND	1.13–2.37	Lü et al., 2015
Western Laizhou Bay, China	September, 2016	0.022–0.035	17.60–20.50	13.40–15.80	34.40–43.50	0.160–0.180	31.50–37.0	9.20–11.90	Li et al., 2019
Haizhou Bay, China	May, 2016	0.003–0.212	3.09–29.10	9.32–32.30	6.45–93.40	0.043–0.144	12.58–79.10	2.50–13.70	Liu et al., 2021
Northern Liaodong Bay, China	April, August, November, and December, 2009	0.010–0.590	0.70–6.20	0.60–17.20	1.20–82.80	0.100–1.400	ND	1.92–10.10	Zhang et al., 2017
Yellow Sea of China	2018	0.003–0.430	4.83–83.00	13.70–60.50	26.30–380.00	0.058–0.920	27.00–93.00	2.25–65.90	Tian et al., 2020
Yellow Sea of the Republic of Korea	2018	0.001–0.080	4.80–33.20	15.40–51.20	15.00–124.00	0.013–0.200	9.20–83.60	3.30–12.10	Tian et al., 2020
Coastal area of Suva, Fiji	–	ND	78.43–490.18	116.96–233.92	18.55–68.78	5.490–9.160	ND	ND	Arikibe and Prasad, 2020
Red Sea coast, Saudi Arabia	–	0.160–0.580	20.80–54.40	4.40–10.80	38.00–118.00	0.140–0.550	16.80–42.20	2.80–12.40	El-Sorogy et al., 2021
Coastal area, Palau	June 2013	0.002–0.070	1.10–66.40	0.10–2.70	0.70–52.5	0.001–0.052	4.90–451.00	0.90–43.50	Jeong et al., 2021
Egypt Bay, USA	–	ND	1.30–18.40	1.90–25.70	6.10–84.40	0.240–0.340	ND	ND	Osher et al., 2006

– and ND: no data or not detected.

amount of wastewater from petrochemical industries was discharged into the Guangli River, where the Dongying Port Industrial Zone and Shengli Oilfield are located. Discharges from the Xiaoqing River and Guangli River have become a considerable source of high contents of As in these estuarine areas. In addition, a large amount of As is present in phosphate fertilizers, which was used intensively for agriculture (Xia et al., 2011). Due to agricultural development along the upstream of the Huanghe River, extensive usage of fertilizers and pesticides contributes greatly to the increased contents of heavy metals in the Huanghe River Delta. This is the main source of As pollution in the Huanghe River estuary reported in this study. Heavy metal levels in surface sediment samples collected from Laizhou Bay were comparable to those of other global offshore systems (Table 6).

The I_{geo} assessment suggested that the degree of heavy metal pollution in the sediments at the 15 stations in our study was low (i.e., unpolluted), except for two stations where the Cu level was ranked as unpolluted to moderately polluted. The Håkanson ecological risk index indicated low ecological risk for the heavy metals evaluated, except for a moderate ecological risk for Cd at one station. These evaluations were consistent with those reported in other studies (Li et al., 2019; Liu et al., 2019). The results of the two assessment methods were similar for the degree of pollution of heavy metals but differed for the order of pollution degree. Heavy metals with high pollution risk were Cu, Cd, and As in Laizhou Bay. The I_{geo} assessment showed that Cu was the most serious pollutant, whereas the Håkanson ecological risk assessment showed that Cd was the most serious pollutant. According to the order of degree of pollution from heavy to light, Hg ranked seventh and fourth among the seven heavy metals in the I_{geo} assessment and Håkanson ecological risk assessment, respectively. In the I_{geo} assessment, only the measured values and geochemical background values of heavy metals were considered, whereas the toxicity of heavy metals to organisms was also considered in the Håkanson ecological risk evaluation. The toxic response factors for Cd, Cu, and Hg were 30, 5, and 40, respectively. Furthermore, Cd was reported to be more toxic to aquatic organisms than Cu, and Hg was reported to be the most toxic heavy metal in toxicological research (Rebolledo et al., 2021). In our study, Cd content was correlated with biomarkers in *A. ommaturus*. Accordingly, we recommend using the Håkanson ecological risk evaluation because it considers the ecological risk of heavy metals to organisms and the contents of heavy metals. In addition, control of Cd pollution should be a focus of management strategies.

4.2 Biomarker response

As a cysteine-rich, low-molecular-weight protein, MT plays an important role in the metabolism of essential metals and the detoxification of toxic metals. MT is the first detectable biomarker of metal exposure at the cellular level (Filipović and Raspor, 2003). The presence of heavy metals resulted in the elevation of MT content in the trahira fish *Hoplias intermedius* and armoured catfish *Hypostomus affinis* (Weber et al., 2020). MT was induced in the goby *Pseudogobius* sp. and *Gobius niger* under Cd and Zn exposure (Migliarini et al., 2005; McDonald et al., 2021). Similar to these findings, higher MT content in *A. ommaturus* was observed at the stations with higher content of heavy metals. The significant correlation between MT content and heavy metal levels in the sediments and the fishes indicated that heavy metals in the habitat were the main contributors to MT induction. MT scavenges the excess metals via the metal-thiolate bond, which relieves the fish of the metal body load (Swaleh et al., 2019). Based on our results, we suggest that MT is a preferred biomarker of heavy metals in *A. ommaturus*.

Acetylcholinesterase is the neurotransmitter at cholinergic synapses and the primary target of some pollutants. Thus, it is an indicator of neurotoxic effects. In the Bizerte Lagoon in Tunisia, AChE activity of the grass goby *Z. ophiocephalus* was negatively correlated with the body burden of hexachlorobenzene and dichlorodiphenyltrichloroethane (Barhoumi et al., 2014). The inhibitory effect of heavy metals on AChE activity has also been reported in zebrafish *Danio rerio* (Richetti et al., 2011), the neotropical fish *Prochilodus lineatus* (Simonato et al., 2016), and the benthic fish *Hoplosternum littorale* (De Araújo et al., 2018). However, no significant correlations were found between AChE activity and the contents of heavy metals in our study, thus AChE activity in *A. ommaturus* is not a suitable biomarker of heavy metal contamination.

Heavy metals induce the generation of reactive oxygen species (ROS) in aquatic organisms. Antioxidant enzymes can protect organisms from oxidative damage, and they have been applied as key nonspecific biochemical biomarkers for ecological monitoring (Lin et al., 2018; De Jesus et al., 2021). SOD converts superoxide radicals into hydrogen peroxide (H_2O_2). CAT catalyzes the decomposition of H_2O_2 into oxygen and water. GPX can reduce toxic peroxide to the non-toxic hydroxyl compound and promote the decomposition of H_2O_2. GR can reduce GSSG to GSH, which can scavenge free radicals and some organic peroxides. GST can inhibit lipid peroxidation and catalyze the combination of GSH with toxic substances to detoxify them. These enzymes act sequentially to resist oxidative stress.

In heavy metal polluted waters, the activities of SOD, CAT, GPX, GR, and GST increased in the milk fish *Chanos chanos* (Rajeshkumar et al., 2013), mullet fish *Liza parsia* (Moniruzzaman et al., 2020), and *H. intermedius* (Weber et al., 2020). The activities of SOD, GPX, GR, and GST in *A. ommaturus* differed significantly among stations and were positively correlated with RI and the content of most heavy metals in sediments. The results of this study are consistent with those of previous studies. Fishes absorbed pollutants in dissolved form from their habitat. BAF values indicated that *A. ommaturus* could effectively accumulate Hg, Cd, Zn, and As. The bioaccumulated pollutants caused oxidative stress, and antioxidant enzymes were activated to combat the oxidative stress (Javed et al., 2020).

However, there was no significant correlation between the content of heavy metals and the activities of SOD and GST in our study. These biomarkers responded to the pollutants in species- and tissue-specific manners. In other studies, the liver SOD activity of the large-sized *H. intermedius* with a piscivorous feeding habit in an area contaminated with mining waste was higher than that in the control area, while the liver SOD activity of the medium-sized fish *H. affinis* with a benthic periphyton feeding habit in the mining waste contaminated area was the same as that in the control area (Weber et al., 2020). Compared with the reference site, the SOD activity in the gill, kidney, and brain of *Channa striatus* collected in a heavy metal contaminated site was lower and the SOD activity in the liver was higher (Fatima et al., 2015). In summary, the niche, feeding habit, and excretion rate of the fish changed the biomarker response by affecting the bioaccumulation of pollutants.

Other types of pollutants can also affect biomarker response. Polycyclic aromatic hydrocarbons (PAHs) and pharmaceuticals and personal care products (PPCPs) present in sediments were found to contribute to the pronounced changes of antioxidant activities in the violet goby *Gobioides broussonnetii* (Salgado et al., 2021). PPCPs and PAH are reportedly distributed in Laizhou Bay (Han et al., 2020; Qi et al., 2021). The insignificant correlation between the content of heavy metals and the activities of SOD and GST may also be due to the complexity of the pollutants present in the study area. The variations of these biomarkers were comprehensive responses to the environment, and pollutants other than heavy metals may have a greater impact on the activities of SOD and GST.

When the formation of ROS exceeds the organism's defense ability, they will cause oxidative damage through lipid peroxidation (Grbin et al., 2019), and then the MDA content will increase. In the present study, MDA level rose with the increase of the content of most heavy metals. This result suggested that higher content of heavy metals posed risks to cellular lipids and increased oxidative damage. Thus, the antioxidant defense system was impaired and the antioxidant status in the organisms was challenged by heavy metals.

Overall, our results show that the contents of MT and MDA as well as the activities of GPX and GR in *A. ommaturus* are suitable biomarkers of heavy metal contamination. In view of the different responses of each biomarker to pollution, combined use of a series of biomarkers is recommended to evaluate biological impacts of contaminants (Tagliaferro et al., 2018). Our results illustrate the importance of using an approach that integrates multiple biomarkers.

4.3 Application of IBRv2 in the assessment of coastal pollution

The Integrated Biomarker Response approach is a powerful tool for integrating multiple biomarkers to assess environmental pollution through biological response. IBR was applied previously to studies of the goby *G. broussonnetii* and the goby *Z. ophiocephalus* to describe marine sediment contamination (Pauletto et al., 2019; Salgado et al., 2021). As reported by the founders of the IBR method, it has two shortcomings: the influence of biomarker arrangement on the IBR value and consideration only of up-regulation or down-regulation of a certain biomarker. IBRv2, which is based on reference deviation, was developed to avoid these shortcomings (Sanchez et al., 2013). They suggested using the station with low pollution investigated at the same time as the reference station. Sanchez et al. (2013) chose the upstream station with low environmental pressures as the reference to obtain IBRv2 values for downstream stations, and they found that IBRv2 values could discriminate the response at stations with different degrees of pollution. In our study, selection of a reference station was crucial to the IBRv2 assessment. Stations with a low RI value, such as stations 3, 7, and 11, were potential reference stations. We then considered biomarker responses to select the reference station. MT content at station 3 was significantly lower than that at station 11 and was non-significantly lower than that at station 7. There was no significant difference in GPX activity between station 3 and station 7 and between station 3 and station 11. GR activities at station 3 and station 11 were not significantly different, and they were significantly lower than that at station 7. There was no significant difference in MDA content among stations 3, 7, and 11. Therefore, the levels of four biomarkers indicated a good physiological condition of *A. ommaturus* at station 3. In view of heavy metal pollution and biomarker response, we selected station 3 as the reference station.

Biomarker responses with different intensities resulted in varied IBRv2 values. IBRv2 value was high at the stations with high levels of heavy metals in the sediments. The IBRv2 results for the goby *A. ommaturus* for heavy metal pollution in Laizhou Bay were consistent with those of chemical monitoring. Similar results were reported for grass goby *Z. ophiocephalus* (Barhoumi et al., 2014), three-spined stickleback *Gasterosteus aculeatus* L. (Raphael et al., 2016), and clam *Ruditapes philippinarum* (Lin et al., 2018). High IBRv2 values suggested a strong biological response and poor health status of the organisms as well as serious environmental pollution (Baudou et al., 2019).

Marine organisms are exposed to many kinds of pollutants that are ubiquitously present in the environment, and changes in biological indexes are the result of the comprehensive action of all pollutants. Chemical monitoring is performed to identify the main pollutants affecting biological indexes. In addition, biomarker monitoring is carried out to describe biological responses caused by the corresponding pollutants. The IBRv2 approach would provide a feasible strategy for evaluation of contaminants based on the biological responses and health status of affected organisms.

5 CONCLUSION

In this study, the IBRv2 approach was used to assess the heavy metal pollution in Laizhou Bay in view of the health status of the goby *A. ommaturus*. The activities of GR and GPX as well as the contents of MT and MDA in the goby were responsive to the presence of heavy metals in the sediments. IBRv2 could efficiently integrate these effective biomarkers. IBRv2 values could discriminate the response at stations with different degrees of pollution, and IBRv2 assessment matched well with chemical assessment. The results showed that IBRv2 method is a feasible tool for evaluating biological responses to heavy metals in Laizhou Bay. The combination of chemical monitoring and the IBRv2 approach improves the comprehensiveness of environmental monitoring and provides valuable data for environmental management.

6 DATA AVAILABILITY STATEMENT

The data that support the findings of the current study are available from the corresponding author upon reasonable request.

References

Arikibe J E, Prasad S. 2020. Determination and comparison of selected heavy metal concentrations in seawater and sediment samples in the coastal area of Suva, Fiji. *Marine Pollution Bulletin*, **157**: 111157, https://doi.org/10.1016/j.marpolbul.2020.111157.

Barhoumi B, Clérandeau C, Gourves P Y et al. 2014. Pollution biomonitoring in the Bizerte lagoon (Tunisia), using combined chemical and biomarker analyses in grass goby, *Zosterisessor ophiocephalus* (Teleostei, Gobiidae). *Marine Environmental Research*, **101**: 184-195, https://doi.org/10.1016/j.marenvres.2014.07.002.

Barhoumi S, Messaoudi I, Deli T et al. 2009. Cadmium bioaccumulation in three benthic fish species, *Salaria basilisca*, *Zosterisessor ophiocephalus* and *Solea vulgaris* collected from the Gulf of Gabes in Tunisia. *Journal of Environmental Sciences*, **21**(7): 980-984, https://doi.org/10.1016/S1001-0742(08)62371-2.

Baudou F G, Ossana N A, Castañé P M et al. 2019. Use of integrated biomarker indexes for assessing the impact of receiving waters on a native neotropical teleost fish. *Science of the Total Environment*, **650**: 1779-1786, https://doi.org/10.1016/j.scitotenv.2018.09.342.

Beliaeff B, Burgeot T. 2002. Integrated biomarker response: a useful tool for ecological risk assessment. *Environmental Toxicology and Chemistry*, **21**(6): 1316-1322, https://doi.org/10.1002/etc.5620210629.

Bradford M M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, **72**(1-2): 248-254, https://doi.org/10.1016/0003-2697(76)90527-3.

De Araújo M C, De Assis C R D, Silva K C C et al. 2018. Characterization of brain acetylcholinesterase of bentonic fish *Hoplosternum littorale*: perspectives of application in pesticides and metal ions biomonitoring. *Aquatic Toxicology*, **205**: 213-226, https://doi.org/10.1016/j.aquatox.2018.10.017.

De Jesus W B, Andrade T D S D O M, Soares S H et al. 2021. Biomarkers and occurrences of heavy metals in sediment and the bioaccumulation of metals in crabs (Ucides cordatus) in impacted mangroves on the Amazon coast, Brazil. *Chemosphere*, **271**: 129444, https://doi.org/10.1016/j.chemosphere.2020.129444.

Devin S, Burgeot T, Giambérini L et al. 2014. The integrated biomarker response revisited: optimization to avoid misuse. *Environmental Science and Pollution Research*, **21**(4): 2448-2454, https://doi.org/10.1007/s11356-013-2169-9.

Di Toro D M, Mahony J D, Hansen D J et al. 1990. Toxicity of cadmium in sediments: The role of acid volatile sulfide. *Environmental Toxicology and Chemistry*, **9**(12): 1487-1502, https://doi.org/10.1002/etc.5620091208.

Duarte I A, Reis-Santos P, França S et al. 2017. Biomarker responses to environmental contamination in estuaries: a comparative multi-taxa approach. *Aquatic Toxicology*, **189**: 31-41, https://doi.org/10.1016/j.aquatox.2017.05.010.

El-Sorogy A S, Youssef M, Al-Kahtany K. 2021. Evaluation of coastal sediments for heavy metal contamination, Yanbu area, Red Sea coast, Saudi Arabia. *Marine Pollution Bulletin*, **163**: 111966, https://doi.org/10.1016/j.marpolbul.2020.111966.

Fang Y, Yang H S, Liu B Z. 2012. Tissue-specific response of metallothionein and superoxide dismutase in the clam *Mactra veneriformis* under sublethal mercury exposure. *Ecotoxicology*, **21**(6): 1593-1602, https://doi.org/10.1007/s0646-012-0938-8.

Fatima M, Usmani N, Firdaus F et al. 2015. *In vivo* induction of antioxidant response and oxidative stress associated with genotoxicity and histopathological alteration in two commercial fish species due to heavy metals exposure in northern India (Kali) river. *Comparative Biochemistry and Physiology Part C*: *Toxicology & Pharmacology*, **176-177**: 17-30, https://doi.org/10.1016/j.cbpc.2015.07.004.

Filipović V, Raspor B. 2003. Metallothionein and metal levels in cytosol of liver, kidney and brain in relation to growth parameters of *Mullus surmuletus* and *Liza aurata* from the Eastern Adriatic Sea. *Water Research*, **37**(13): 3253-3262, https://doi.org/10.1016/S0043-1354(03)00162-3.

General Administration of Quality Supervision, Inspection and Quarantine of the People's Republic of China. 2004. GB 18668-2002 Marine Sediment Quality. Standards Press of

China, Beijing. (in Chinese)

General Administration of Quality Supervision, Inspection and Quarantine of the People's Republic of China, China National Standardization Administration. 2008a. GB/T 12763.1-2007 Specifications for Oceanographic Survey—Part 1: General. Standards Press of China, Beijing. (in Chinese)

General Administration of Quality Supervision, Inspection and Quarantine of the People's Republic of China, China National Standardization Administration. 2008b. GB 17378.1-2007 The Specification for Marine Monitoring—Part 1: General Rules. Standards Press of China, Beijing. (in Chinese)

Grbin D, Sabolić I, Klobučar G et al. 2019. Biomarker response of Mediterranean mussels *Mytilus galloprovincialis* regarding environmental conditions, pollution impact and seasonal effects. *Science of the Total Environment*, **694**: 133470, https://doi.org/10.1016/j.scitotenv.2019.07.276.

Håkanson L. 1980. An ecological risk index for aquatic pollution control. A sedimentological approach. *Water Research*, **14**(8): 975-1001, https://doi.org/10.1016/0043-1354(80)90143-8.

Han B, Liu A, Wang S et al. 2020. Concentration level, distribution model, source analysis, and ecological risk assessment of polycyclic aromatic hydrocarbons in sediments from Laizhou Bay, China. *Marine Pollution Bulletin*, **150**: 110690, https://doi.org/10.1016/j.marpolbul.2019.110690.

Javed M, Abbas K, Ahmed T et al. 2020. Metal pollutants induced peroxidase activity in different body tissues of freshwater fish, *Labeo rohita*. *Environmental Chemistry and Ecotoxicology*, **2**: 162-167, https://doi.org/10.1016/j.enceco.2020.09.001.

Jeong H, Choi J Y, Choi D H et al. 2021. Heavy metal pollution assessment in coastal sediments and bioaccumulation on seagrass (*Enhalus acoroides*) of Palau. *Marine Pollution Bulletin*, **163**: 111912, https://doi.org/10.1016/j.marpolbul.2020.111912.

La Colla N S, Botté S E, Marcovecchio J E. 2018. Metals in coastal zones impacted with urban and industrial wastes: insights on the metal accumulation pattern in fish species. *Journal of Marine Systems*, **181**: 53-62, https://doi.org/10.1016/j.jmarsys.2018.01.012.

Li M K, Zhu S Y, Ouyang T P et al. 2021. Magnetic properties of the surface sediments in the Yellow River Estuary and Laizhou Bay, Bohai Sea, China: implications for monitoring heavy metals. *Journal of Hazardous Materials*, **410**: 124579, https://doi.org/10.1016/j.jhazmat.2020.124579.

Li S Y, Liu G X, Miao F M. 1994. The distribution and environmental background values of the heavy metals in sediment of the Bohai sea. *China Environmental Science*, **14**(5): 370-376. (in Chinese with English abstract)

Li X, Chi W Q, Tian H et al. 2019. Probabilistic ecological risk assessment of heavy metals in western Laizhou Bay, Shandong Province, China. *PLoS One*, **14**(3): e0213011, https://doi.org/10.1371/journal.pone.0213011.

Lin Y F, Liu Q Q, Meng F P et al. 2018. Integrated toxicity evaluation of metals in sediments of Jiaozhou Bay (China): based on biomarkers responses in clam *Ruditapes philippinarum* exposed to sediment extracts. *Marine Pollution Bulletin*, **131**: 180-190, https://doi.org/10.1016/j.marpolbul.2018.04.024.

Liu J H, Cao L, Dou S Z. 2019. Trophic transfer, biomagnification and risk assessments of four common heavy metals in the food web of Laizhou Bay, the Bohai Sea. *Science of the Total Environment*, **670**: 508-522, https://doi.org/10.1016/j.scitotenv.2019.03.140.

Liu Q, Su Z M, Zeng X Q et al. 1997. Studies on ichthyofauna and ecology of goby fishes in the Bohai Sea. *Journal of Ocean University of Qingdao*, **27**(2): 29-37, https://doi.org/10.16441/j.cnki.hdxb.1997.02.004. (in Chinese with English abstract)

Liu R, Jiang W W, Li F J et al. 2021. Occurrence, partition, and risk of seven heavy metals in sediments, seawater, and organisms from the eastern sea area of Shandong Peninsula, Yellow Sea, China. *Journal of Environmental Management*, **279**: 111771, https://doi.org/10.1016/j.jenvman.2020.111771.

Lü D W, Zheng B, Fang Y et al. 2015. Distribution and pollution assessment of trace metals in seawater and sediment in Laizhou Bay. *Chinese Journal of Oceanology and Limnology*, **33**(4): 1053-1061, https://doi.org/10.1007/s00343-015-4226-3.

Lv J S, Xia Q, Yan T et al. 2018. Identifying the sources, spatial distributions, and pollution status of heavy metals in soils from the southern coast of Laizhou Bay, eastern China. *Human and Ecological Risk Assessment*: *An International Journal*, **25**(8): 1953-1967, https://doi.org/10.1080/10807039.2018.1478275.

McDonald S, Hassell K, Cresswell T. 2021. Effect of short-term dietary exposure on metal assimilation and metallothionein induction in the estuarine fish *Pseudogobius* sp. *Science of the Total Environment*, **772**: 145042, https://doi.org/10.1016/j.scitotenv.2021.145042.

Migliarini B, Campisi A M, Maradonna F et al. 2005. Effects of cadmium exposure on testis apoptosis in the marine teleost *Gobius niger*. *General and Comparative Endocrinology*, **142**(1-2): 241-247, https://doi.org/10.1016/j.ygcen.2004.12.012.

Ministry of Ecology and Environment of the People's Republic of China. 2021. Bulletin of Marine Ecological Environment in China in 2020, https://www.mee.gov.cn/hjzl/sthjzk/jagb/. (in Chinese)

Moniruzzaman M, Kumar S, Das D et al. 2020. Enzymatic, non enzymatic antioxidants and glucose metabolism enzymes response differently against metal stress in muscles of three fish species depending on different feeding niche. *Ecotoxicology and Environmental Safety*, **202**: 110954, https://doi.org/10.1016/j.ecoenv.2020.110954.

Müller G. 1969. Index of geoaccumulation in sediments of the Rhine River. *GeoJournal*, **2**(3): 108-118.

National Health Commission of the People's Republic of

China, State Administration for Market Regulation. 2017. GB 2762-2017 National Food Safety Standard Maximum Levels of Contaminants in Foods. Standards Press of China, Beijing. (in Chinese)

Osher L J, Leclerc L, Wiersma G B et al. 2006. Heavy metal contamination from historic mining in upland soil and estuarine sediments of Egypt Bay, Maine, USA. *Estuarine*, *Coastal and Shelf Science*, **70**(1-2): 169-179, https://doi.org/10.1016/j.ecss.2006.06.009.

Ota Y, Suzuki A, Yamaoka K et al. 2021. Geochemical distribution of heavy metal elements and potential ecological risk assessment of Matsushima Bay sediments during 2012-2016. *Science of the Total Environment*, **751**: 141825, https://doi.org/10.1016/j.scitotenv.2020.141825.

Pastorino P, Prearo M, Bertoli M et al. 2020. Accumulation of As, Cd, Pb, and Zn in sediment, chironomids and fish from a high-mountain lake: First insights from the Carnic Alps. *Science of the Total Environment*, **729**: 139007, https://doi.org/10.1016/j.scitotenv.2020.139007.

Pauletto M, Lopparelli R M, Pegolo S et al. 2019. Significance of the goby *Zosterisessor ophiocephalus* as a sentinel species for Venice Lagoon contamination: Combining biomarker responses and bioaccumulation. *Science of the Total Environment*, **660**: 959-973, https://doi.org/10.1016/j.scitotenv.2019.01.033.

Qi Y J, He Z S, Yuan J J et al. 2021. Comprehensive evaluation of organophosphate ester contamination in surface water and sediment of the Bohai Sea, China. *Marine Pollution Bulletin*, **163**: 112013, https://doi.org/10.1016/j.marpolbul.2021.112013.

Rajeshkumar S, Mini J, Munuswamy N. 2013. Effects of heavy metals on antioxidants and expression of HSP70 in different tissues of Milk fish (*Chanos chanos*) of Kaattuppalli Island, Chennai, India. *Ecotoxicology and Environmental Safety*, **98**: 8-18, https://doi.org/10.1016/j.ecoenv.2013.07.029.

Raphael S, Aude J, Olivier P et al. 2016. Characterization of a genotoxicity biomarker in three-spined stickleback (*G asterosteus aculeatus* L.): biotic variability and integration in a battery of biomarkers for environmental monitoring. *Environmental Toxicology*, **31**(4): 415-426, https://doi.org/10.1002/tox.22055.

Rebolledo U A, Páez-Osuna F, Fernández R. 2021. Single and mixture toxicity of As, Cd, Cr, Cu, Fe, Hg, Ni, Pb, and Zn to the rotifer *Proales similis* under different salinities. *Environmental Pollution*, **271**: 116357, https://doi.org/10.1016/J.ENVPOL.2020.116357.

Richetti S K, Rosemberg D B, Ventura-Lima J et al. 2011. Acetylcholinesterase activity and antioxidant capacity of zebrafish brain is altered by heavy metal exposure. *NeuroToxicology*, **32**(1): 116-122, https://doi.org/10.1016/j.neuro.2010.11.001.

Salgado L D, Marques A E M L, Kramer R D et al. 2021. Sediment contamination and toxic effects on Violet Goby fish (*Gobioides broussonnetii*-Gobiidae) from a marine protected area in South Atlantic. *Environmental Research*, **195**: 110308, https://doi.org/10.1016/j.envres.2020.110308.

Sanchez W, Burgeot T, Porcher J M. 2013. A novel "Integrated Biomarker Response" calculation based on reference deviation concept. *Environmental Science and Pollution Research*, **20**(5): 2721-2725, https://doi.org/10.1007/s11356-012-1359-1.

Scheuhammer A M, Cherian M G. 1986. Quantification of metallothioneins by a silver-saturation method. *Toxicology and Applied Pharmacology*, **82**(3): 417-425, https://doi.org/10.1016/0041-008X(86)90277-2.

Simonato J D, Mela M, Doria H B et al. 2016. Biomarkers of waterborne copper exposure in the Neotropical fish *Prochilodus lineatus*. *Aquatic Toxicology*, **170**: 31-41, https://doi.org/10.1016/j.aquatox.2015.11.012.

State Environmental Protection Administration of the People's Republic of China. 2004. GB 3097-1997 Marine Water Quality Standard. China Environmental Press, Beijing. (in Chinese)

Swaleh S B, Banday U Z, Usmani N. 2019. Comparative study of biochemical, histological and molecular biomarkers of heavy metal contamination in *Cyprinus carpio* collected from warm-monomictic lake and government culture pond. *Chemosphere*, **236**: 124182, https://doi.org/10.1016/j.chemosphere.2019.06.152.

Tagliaferro M, Gonçalves A M M, Bergman M et al. 2018. Assessment of metal exposure (uranium and copper) by the response of a set of integrated biomarkers in a stream shredder. *Ecological Indicators*, **95**: 991-1000, https://doi.org/10.1016/j.ecolind.2017.10.065.

Tian K, Wu Q M, Liu P et al. 2020. Ecological risk assessment of heavy metals in sediments and water from the coastal areas of the Bohai Sea and the Yellow Sea. *Environment International*, **136**: 105512, https://doi.org/10.1016/j.envint.2020.105512.

Vieira C E D, Costa P G, Caldas S S et al. 2019. An integrated approach in subtropical agro-ecosystems: Active biomonitoring, environmental contaminants, bioaccumulation, and multiple biomarkers in fish. *Science of the Total Environment*, **666**: 508-524, https://doi.org/10.1016/j.scitotenv.2019.02.209.

Weber A A, Sales C F, Faria F D S et al. 2020. Effects of metal contamination on liver in two fish species from a highly impacted neotropical river: A case study of the Fundão dam, Brazil. *Ecotoxicology and Environmental Safety*, **190**: 110165, https://doi.org/10.1016/j.ecoenv.2020.110165.

WHO. 1993. Biomarkers and risk assessment: concepts and principles. *In*: Safety I P O C ed. Environmental Health Criteria. United Nations Environment Programme, International Labour Organisation, World Health Organization, Geneva.

Xia P, Meng X W, Yin P et al. 2011. Eighty-year sedimentary record of heavy metal inputs in the intertidal sediments from the Nanliu River estuary, Beibu Gulf of South China Sea. *Environmental Pollution*, **159**: 92-99, https://doi.org/10.1016/j.envpol.2010.09.014.

Xu G, Liu J, Pei S F et al. 2015. Sediment properties and trace metal pollution assessment in surface sediments of the Laizhou Bay, China. *Environmental Science and Pollution Research*, **22**(15): 11634-11647, https://doi.org/10.1007/s11356-015-4393-y.

Zhang L C, Li F, Lv Z B et al. 2019. Stock distribution and community structure of members in Gobioidei in Laizhou Bay. *Journal of Dalian Ocean University*, **34**(4): 588-594, https://doi.org/10.16535/j.cnki.dlhyxb.2019.04.019. (in Chinese with English abstract)

Zhang P, Hu R J, Zhu L H et al. 2017. Distributions and contamination assessment of heavy metals in the surface sediments of western Laizhou Bay: Implications for the sources and influencing factors. *Marine Pollution Bulletin*, **119**(1): 429-438, https://doi.org/10.1016/j.marpolbul.2017.03.046.

Zhao L, Xu Y F, Hou H et al. 2014. Source identification and health risk assessment of metals in urban soils around the Tanggu chemical industrial district, Tianjin, China. *Science of the Total Environment*, **468-469**: 654-662, https://doi.org/10.1016/j.scitotenv.2013.08.094.

Zhu N, Yang Y Y, Xu H et al. 2019. Bioaccumulation of decabromodiphenyl ether affects the antioxidant system in the clam *Mactra veneriformis*. *Environmental Toxicology and Pharmacology*, **68**: 19-26, https://doi.org/10.1016/j.etap.2019.03.004.

Electronic supplementary material

Supplementary material (Supplementary Tables S1–S4) is available in the online version of this article at https://doi.org/10.1007/s00343-022-1387-8.