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Integrated assessment of trace elements in a marine ranching area based on multi-species and multi-level biomarkers: a case study in China's national-level marine ranching demonstration area

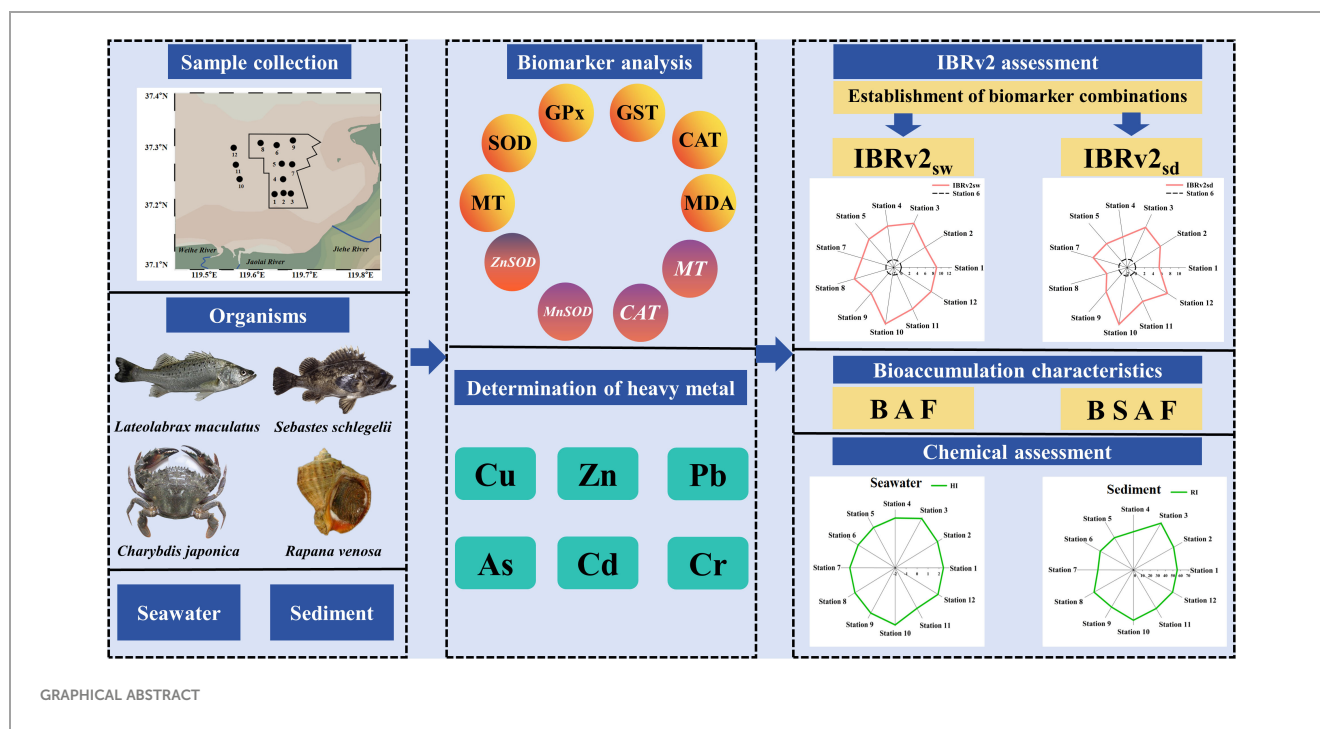
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The goal of this study was to evaluate the trace element pollution in a marine ranching area in China based on molecular responses (expression of metallothionein and antioxidant enzyme genes), and biochemical biomarkers (metallothionein content, antioxidant enzyme activities, and malonaldehyde level) in four indicator species. We collected samples of two fish (*Lateolabrax maculatus* and *Sebastes schlegelii*), one crustacean (*Charybdis japonica*), and one gastropod (*Rapana venosa*) from the western Furong Island marine ranching area and from an adjacent area in March 2022 and measured the trace element content in these indicator species as well as in the seawater and sediment. We found that the bioaccumulation characteristics of trace elements and the response patterns of biomarkers were species specific. Moreover, not every biomarker was significantly correlated with environmental trace element content. We then established two biomarker combinations indicative of trace element pollution in seawater and sediment, respectively, based on the correlation between biomarkers and trace element contents. The selected biomarkers were integrated using integrated biomarker response version 2 (IBRV2). IBRV2 values in the studied marine ranching area were lower than those in the adjacent area. Additionally, these values were consistent with the bioaccumulation of trace elements in the indicator species, the integrated trace element pollution index for seawater, and the potential risk index for sediment. These results show that this multi-biomarker and multi-species IBRV2 approach provided a comprehensive diagnosis of trace element pollution in the marine ranching area. Therefore, its application may be beneficial for marine environmental monitoring and management in view of the ecotoxicological impact of pollutants on organisms.

KEYWORDS

biomarker, trace element, fish, crustacean, gastropod, IBRV2



Highlights

- Biological responses to trace elements in four indicator species were analyzed in a marine ranching area in China.
- The bioaccumulation characteristic of trace elements and biomarker responses exhibited species specificity.
- Two biomarker combinations indicative of trace element pollution in seawater and sediment were established.
- The multi-biomarker and multi-species IBRv2 approach successfully assessed trace element pollution in the study area.

1 Introduction

The growing human population, increasing industrial development, and domestic activities have resulted in the discharge of huge quantities of chemicals into the marine environment. Trace elements are among these ubiquitous contaminants, and they are characterized by persistence, non-degradability in the environment, and accumulation in organisms (Lebrun and Gismondi, 2020). Even when trace element levels are generally low, long-term exposure can result in irreversible damage to aquatic organisms (Yang et al., 2023). Hence, national and local authorities oversee environmental trace element monitoring.

Although the degree of trace element pollution in the oceans has become a global concern and numerous studies have focused on measuring pollution levels (Zhao et al., 2021; Lazár et al., 2024), chemical monitoring of trace element content does not reflect the toxic effects of these pollutants on aquatic organisms. To improve

the reliability of monitoring schemes, the biomarker approach based on biological changes has evolved considerably in recent decades (Catteau et al., 2022; Yamamoto et al., 2023; Bouzahouane et al., 2024). This approach is designed to identify the effect of pollutants on organisms, explain how organisms respond to pollutants, and supplement biological and ecological dimensions of chemical monitoring.

The integrated approach of measuring multiple biomarkers to assess the quality of aquatic environments has become popular because it does not depend on a single biomarker, for which values can vary greatly (Duarte et al., 2017; Lin et al., 2018; Yang et al., 2023). This approach also means that molecular and biochemical biomarkers can be monitored and act as early warning signs of exposure to and effects of pollutants before the environment is damaged. Metallothionein (MT) is a family of ubiquitous low molecular weight proteins that are rich in cysteine and metal content, and they play a vital role in the homeostasis of essential trace metals, detoxification of toxic metals, and quenching of oxidative stress (Beg et al., 2015; Mijošek et al., 2019). One of the main mechanisms of trace element toxicity in aquatic organisms is induction of oxidative stress via increased amounts of reactive oxygen species (ROS). Antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione S-transferase (GST) interact as a sophisticated network to neutralize ROS and protect organism from oxidative damage, which can be measured as malonaldehyde (MDA) content. MT and antioxidant enzyme levels in aquatic organisms have been widely used as early warning signs of metal exposure in the evaluation of environmental metal contamination (Lin et al., 2018; Astani et al., 2023; Stroglyoudi et al., 2023).

The responses of these biomarkers in organisms result from systemic exposure to multiple contaminants in the aquatic

environment. The selection of appropriate biomarkers for certain contaminants is critically important for a precise assessment of their effects on organisms and the environment. Therefore, it is crucial to understand the ability of each biomarker to discriminate the status of trace element pollution and the relationship between biomarkers and trace element levels.

The biomarker response is also influenced by species sensitivity, living habits, feeding behaviors, and exposure pathways (Catteau et al., 2022), yet the vast majority of previous monitoring studies have measured biomarker levels in only one indicator species (Lin et al., 2018; Ji et al., 2019; Chalhmi et al., 2020; Astani et al., 2023; Benito et al., 2023). Therefore, assessment of multiple species is needed for holistic assessment of the effects of environmental pollution. To date, this approach has been applied in only a few monitoring studies (Duarte et al., 2017; Desforges et al., 2021; Yamamoto et al., 2023), and more multi-species studies are urgently needed to improve assessment strategies and to provide information for regional marine environmental management.

The Chinese government attaches great importance to the development of marine ranching, which is characterized by the restoration of marine environments, the maintenance of marine biodiversity, and the benefits of ecological importance (Qin and Sun, 2021). Shandong Province has constructed numerous national-level marine ranching demonstration areas in places with superior coastline and geographical location to provide scientific experience for the development and management of marine ranching worldwide. The marine ranching environment in offshore areas is highly vulnerable to the effects of human activities, riverine inputs, and aquaculture practices. The western Furong Island marine ranching initiatives in Laizhou Bay along the Shandong coast were initiated in 2011, and in 2015, it was designated as national-level marine ranching demonstration area. The bay is flanked by Longkou Port and numerous gold mining enterprises, leading to substantial pollution carried into the bay by rivers (Xu et al., 2015; Li et al., 2019; Liu et al., 2023). The primary activities within this marine ranching area include the construction of artificial reefs (ARs) to mitigate the degradation of marine habitats and living resources, the cultivation of aquaculture species to produce regular yields, and the development of offshore wind farms (OWFs) to synergize marine ranching with OWFs. ARs have been shown to influence the ecological environment not only in their immediate vicinity but also in adjacent non-ARs areas, altering trace element contents in seawater and surface sediments (Yang et al., 2019; Chen and Chen, 2020; Wilking et al., 2023). Additionally, galvanic anodes, used as a corrosion protection system for OWFs, result in the continuous release of trace elements into the marine environment (Kirchgeorg et al., 2018). Consequently, the western Furong Island marine ranching area functions both as a receptor for pollutants from human activities and riverine inputs and as a source of pollutants discharged by OWFs.

Understanding the impact of marine ranching construction on the marine environment is essential for the sustainable development of marine ranching. Environmental monitoring, especially in view of the effect of pollution on organisms, is crucial for the management of marine ranching areas. However, the biomarker approach had not yet been applied to environmental assessment of marine ranching prior to our study.

In this context, we developed a multi-level and multi-species biomarker approach to evaluate trace element pollution in the western Furong Island marine ranching area. We selected two fish, one crustacean, and one gastropod species from different niches as indicator species due to their common use for the assessment of trace element pollution (Duarte et al., 2017; Astani et al., 2023; Yamamoto et al., 2023). Spotted sea bass (*Lateolabrax maculatus*) is a middle-upper water dweller that is broadly distributed from the Bohai Sea to the Indo-China Peninsula, and black rockfish (*Sebastes schlegelii*) is a demersal reef-associated species that is distributed along the coasts of northern China, Korea, and Japan. The benthic-dwelling Asian paddle crab (*Charybdis japonica*) is widely distributed in China, Korea, Japan, and Malaysia, and the rapa whelk (*Rapana venosa*) originated in Asia and invaded the Black Sea. These four species are of high economic and ecological importance and are potent indicators of marine trace element pollution (Mao et al., 2012; Kim and Kang, 2017; Huang et al., 2022; Ryabushko et al., 2022).

We measured the accumulation of trace elements and the responses of MT and antioxidant enzymes at molecular and biochemical levels in these four indicator species collected from the marine ranch and an adjacent area. Based on the ability of the various biomarkers to distinguish trace element pollution and on their correlation with environmental trace element levels, we used combinations of six biomarkers in the four indicator species to assess trace element pollution in seawater, and then we repeated this process for the sediment. We used integrated biomarker response version 2 (IBRv2) to comprehensively evaluate trace element pollution in view of the impact of contaminants on organisms (Sanchez et al., 2013; Marques et al., 2016; Lin et al., 2018; Catteau et al., 2022). This monitoring strategy based on multi-level and multi-species biomarkers can be used to facilitate marine environmental management.

2 Materials and methods

2.1 Field survey and sample collection

The western Furong Island marine ranching covers an area of 11333 ha, including an OWF area of 4800 ha and an AR area of 3666 ha. Positioned 12 km to the coastline and south of the ARs, the OWF features fish farming cages tethered to the wind turbine foundations. This innovative integration of underwater aquaculture with overwater power generation optimizes space and resources. Within this marine ranching, stock enhancement has been implemented, resulting in the release of 1.23 billion fish, shellfish, crustaceans, and sea cucumbers into the marine ecosystem. These organisms are cultivated within the ranching area and subject to annual scientific harvesting.

To assess the environmental impact of these activities, the marine ranching has been segmented into three distinct areas: the OWF area with stations 1, 2, and 3; the AR area with stations 4, 5, and 6; and the AR surrounding area with stations 7, 8, and 9 (Figure 1). Additionally, three reference stations (10, 11, and 12) have been established in the adjacent area of the marine ranching. By analyzing the trace elements content and biomarkers across

these areas, we can discern the effects of various ranching activities on the marine environment.

We conducted the field survey in March 2022 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, 2007a). Bottom seawater, surface sediment, and *L. maculatus*, *S. schlegelii*, *C. japonica*, and *R. venosa* were sampled at all 12 stations.

Three bottom water samples were collected at each station using a water sampler (Slimline436 976, HYDRO-BIOS, Altenholz, Germany). The bottom water temperature, dissolved oxygen content, depth, conductivity, salinity, and turbidity were measured *in situ* using a multi parameter water quality detector (556MPS, YSI, Yellow Springs, OH, USA). Three surface sediment samples were collected at each station using a grab sampler (van Veen 437332, HYDRO-BIOS). The seawater and sediment samples were transported to the lab for measurement of copper (Cu), zinc (Zn), lead (Pb), arsenic (As), cadmium (Cd), and chromium (Cr) contents.

The indicator species *L. maculatus*, *S. schlegelii*, and *C. japonica* were collected using gillnets, and *R. venosa* was collected by divers. The collected organisms were placed on ice and transported immediately to the lab. The livers of *L. maculatus* and *S. schlegelii* and the digestive glands of *C. japonica* and *R. venosa* were removed and stored at -80°C until used for trace element and biomarker analyses. The body weight and body length of each specimen are listed in Supplementary Table S1.

2.2 Trace element analysis

Trace element contents in the samples were measured following the Specification for Marine Monitoring (General Administration of

Quality Supervision, Inspection and Quarantine of the People's Republic of China, China National Standardization Administration, 2007b). Cu, Pb, Cd, and Cr levels were measured using flameless atomic absorption spectrometry, and Zn level was measured by flame atomic absorption spectrometry in an atomic absorption spectrophotometer (AA-7000, Shimadzu, Kyoto, Japan). As content was measured using an atomic fluorescence spectrophotometer (PF72, Persee, Beijing, China).

The detection limits for trace elements in seawater samples were established at $0.2\ \mu\text{g/L}$ for Cu, $0.03\ \mu\text{g/L}$ for Pb, $3.1\ \mu\text{g/L}$ for Zn, $0.01\ \mu\text{g/L}$ for Cd, $0.4\ \mu\text{g/L}$ for Cr, and $0.5\ \mu\text{g/L}$ for As. In sediment samples, the detection limits were quantified at $0.5\ \text{mg/kg}$ for Cu, $1.0\ \text{mg/kg}$ for Pb, $6.0\ \text{mg/kg}$ for Zn, $0.04\ \text{mg/kg}$ for Cd, $2.0\ \text{mg/kg}$ for Cr, and $0.06\ \text{mg/kg}$ for As. For biological specimens, the detection limits for these elements were determined to be $0.4\ \text{mg/kg}$ for Cu, $0.04\ \text{mg/kg}$ for Pb, $0.4\ \text{mg/kg}$ for Zn, $0.005\ \text{mg/kg}$ for Cd, $0.04\ \text{mg/kg}$ for Cr, and $0.2\ \text{mg/kg}$ for As.

Quality assurance and quality control protocols encompassed a reagent blank sample, 10% proportion of parallel samples, a matrix-spiked sample, and a certified standard sample from either the National Standard Seawaters (Cu, Pb, Zn, Cd, and Cr from GBW(E) 080040) or As from GBW(E)080230), the National Standard Sediments GBW07333, or the National Standard Croaker Material GBW08573, for each batch of samples. The relative deviation in trace elements content among the parallel samples was maintained below 8%. The recovery rate for matrix-spiked samples with standard solutions ranged from 94% to 106%.

2.3 Assessment of trace element pollution

Trace element pollution in seawater was assessed using the single-factor index method (Equations 1, 2).

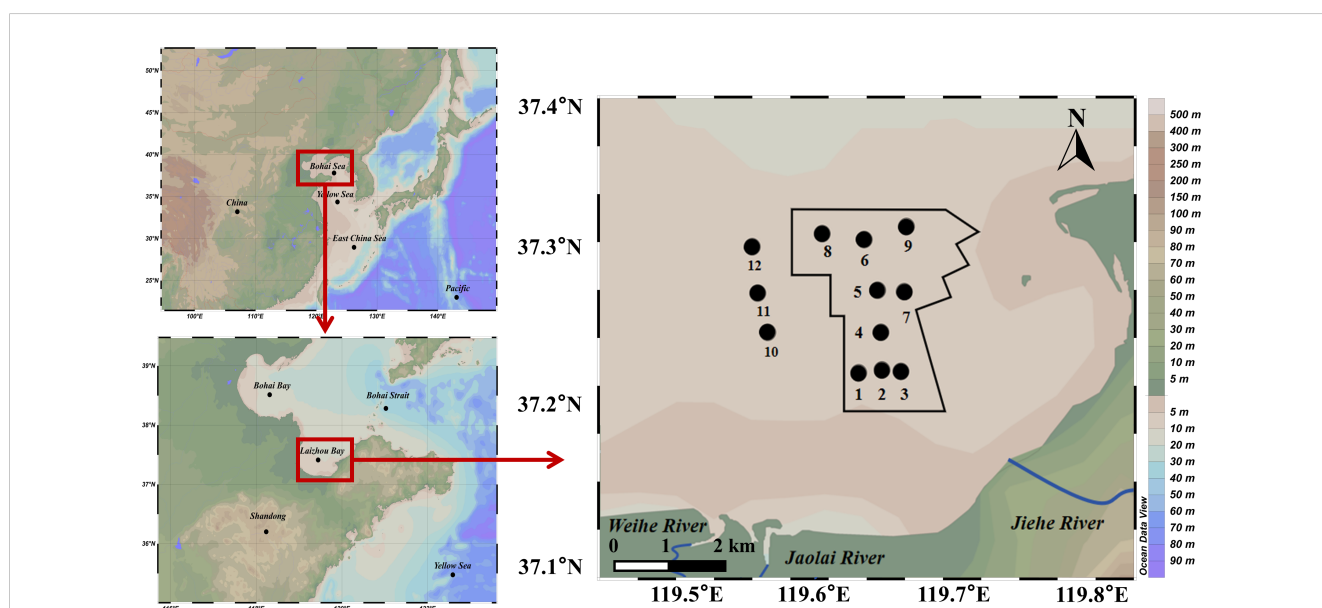


FIGURE 1

Survey stations in the western Furong Island marine ranching area, which is located within the black solid line. Stations 1, 2, and 3 were in the offshore wind farm area; stations 4, 5, and 6 were in the artificial reef area; stations 7, 8, and 9 were in the area surrounding the artificial reefs; and stations 10, 11, and 12 were in the area adjacent to the marine ranching.

$$P_i = C_i/S_i \tag{1}$$

$$HI = \sum_{i=1}^6 P_i \tag{2}$$

where P_i is the trace element pollution index; C_i is the measured concentration of a certain trace element; and S_i is the class I criterion of China Seawater Quality Standard (State Environmental Protection Administration of the People’s Republic of China, 1997). Class I criteria for Cu, Pb, Zn, Cd, Cr, and As are 5, 1, 20, 1, 50, and 20 $\mu\text{g/L}$, respectively. HI is the integrated trace element pollution index, and n is the number of measured trace elements. A larger HI value indicates a higher degree of trace element pollution.

The ecological risks of trace elements in the sediment were assessed using the ecological risk assessment method (Håkanson, 1980; Equations 3–5; Table 1):

$$C_f^i = C_s^i/C_n^i \tag{3}$$

$$E_r^i = T_r^i \cdot C_f^i \tag{4}$$

$$RI = \sum_{i=1}^7 E_r^i \tag{5}$$

where C_s^i is the measured concentration of a given trace element; C_n^i is the background concentration of a given trace element in the studied area; E_r^i is the potential risk of a given trace element; T_r^i is the toxic response factor for a given trace element; and T_r^i values of Cu, Pb, Zn, Cd, Cr, and As are 5, 5, 1, 30, 2, and 10, respectively (Håkanson, 1980). RI is the potential risk index. Table 2 shows categorization of E_r^i and RI .

The biota-seawater accumulation factor (BAF) and biota-sediment accumulation factor (BSAF) are indicators of the ability of organisms to accumulate trace elements in the seawater and sediment (Szefer et al., 1999; Usero et al., 2005). They were calculated as follows (Equations 6, 7):

$$BAF = M_{tissue}/M_{seawater} \tag{6}$$

$$BSAF = M_{tissue}/M_{sediment} \tag{7}$$

where M_{tissue} is the trace element content in the tissue; $M_{seawater}$ is the trace element concentration in the seawater; $M_{sediment}$ is the trace element concentration in the sediment.

TABLE 1 Categorization of potential risk assessed using Håkanson ecological risk assessment.

E_r^i	Potential ecological risk level	RI	Potential ecological risk level
$E_r^i < 40$	Low	$RI < 60$	Low
$40 \leq E_r^i < 80$	Moderate	$60 \leq RI < 120$	Moderate
$80 \leq E_r^i < 160$	Considerable	$120 \leq RI < 240$	High
$160 \leq E_r^i < 320$	High	$240 \leq RI$	Very high
$320 \leq E_r^i$	Very high		

2.4 Biomarker analysis

2.4.1 Analysis of biochemical biomarkers

The tissue samples used for MT content analysis were homogenized in 0.25 mol/L sucrose with a weight volume ratio of 1:9 at 4°C. After centrifugation (20,000 × g) at 4°C for 40 min, the supernatants were collected for MT content measurement using the Ag saturation method (Scheuhammer and Cherian, 1986). MT content was expressed as the amount of Ag⁺ bound by MT.

The samples used for measure antioxidant enzyme including SOD, CAT, GPx, and GST activities and MDA content were homogenized in 0.1 mol/L Tris-HCl buffer containing 1 mmol/L EDTA (pH 7.4, Sinopharm Chemical Reagent Co., Shanghai, China) at 4°C with a weight volume ratio of 1:9. After centrifugation (13,000 × g) for 30 min at 4°C, the supernatants were collected for biomarker analysis using spectrophotometry according to the instructions provided with the commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). SOD effectively suppresses the formation of the superoxide anion radical produced by the interaction of xanthine with the xanthine oxidase reaction system, thereby diminishing the concentration of colored compounds within the reaction medium. The activity of SOD was quantified spectrophotometrically at a wavelength of 505 nm, with one unit of the enzyme being characterized as the quantity required to inhibit 50% of the color development per milligram of tissue protein. Ammonium molybdate serves to inhibit CAT from decomposing H₂O₂ and instead reacts with H₂O₂ to form a yellow complex. This yellow complex can be spectrophotometrically detected at a wavelength of 405 nm. One unit is defined as the enzyme amount capable of decomposing 1 μmol of H₂O₂ per second per milligram of tissue protein. GPx catalyzes the oxidation of reduced glutathione (GSH) through the utilization of H₂O₂. The reaction product resulting from the interaction of GSH with 5,5'-Dithiobis-(2-Nitrobenzoic Acid) exhibits an absorption peak at 412 nm. At this specific wavelength, the reduction in absorbance serves as an indicator of GPx activity. One unit of GPx activity is defined as the enzyme quantity necessary to oxidize 1 mol of GSH per minute per milligram of tissue protein. The reaction products formed by the catalysis of GST on the interaction between GSH and 1-chloro-2,4-dinitrobenzene can be spectrophotometrically detected at a wavelength of 412 nm. One unit of GST activity is defined as the enzyme amount necessary to reduce 1 μM of GSH per minute per milligram of tissue protein. MDA reacts with thiobarbituric acid to yield a red product, which can be spectrophotometrically detected at a wavelength of 532 nm. The MDA content is quantified by expressing the molar amount of MDA per milligram of protein present in the tissue.

2.4.2 Measurement of gene expression

Only gene sequences of *ZnSOD*, *MnSOD*, and *CAT* in *S. schlegelii* and *MT* in *C. japonica* were available from the National Center for Biotechnology Information. Database. Herein, we used the SYBR Green RT-PCR assay to measure the expression of these genes, and β -actin was used as the internal reference. The specific

TABLE 2 Sorted rotated factor loadings of the original variables on principal factors of the studied marine ranching area.

Variable %Variable		F1 33.31	F2 15.36	F3 12.73	F4 9.76
Seawater	Cu	0.614	-	0.584	-
	Pb	-	0.744	0.478	-
	Zn	0.439	0.514	0.586	-
	Cd	0.454	-	-	-
	Cr	0.725	-	0.445	-
	As	0.73	-	-	-
Sediment	Cu	-	0.858	-	-
	Pb	0.878	-	-	-
	Zn	0.673	-	-	-
	Cd	0.669	0.546	-	-
	Cr	-	0.633	-	-
	As	-	-	-	0.864
<i>L. maculatus</i>	MT content	-	0.631	-	-
	SOD activity	-	-	-	-
	GPX activity	0.476	-	0.468	-
	GST activity	-	0.843	-	-
	CAT activity	-	-	0.451	0.633
	MDA content	0.736	-	-	-
<i>S. schlegelii</i>	MT content	0.529	0.414	-	-
	SOD activity	-	-	0.856	-
	GPX activity	-	0.827	-	-
	GST activity	0.425	-	-	-
	CAT activity	-	-	0.675	-
	MDA content	-	-	-	-
	ZnSOD expressions	0.717	-	-	-
	MnSOD expressions	-	-	0.841	-
CAT expressions	-	-	0.727	-	

(Continued)

TABLE 2 Continued

Variable %Variable		F1 33.31	F2 15.36	F3 12.73	F4 9.76
<i>C. japonica</i>	MT content	0.777	0.494	-	-
	SOD activity	-	0.828	-	-
	GPX activity	-	0.541	0.405	-
	GST activity	0.592	0.573	-	-
	MDA content	-	-	-	0.867
	MT expressions	0.669	-	-	-
<i>R. venosa</i>	MT content	0.486	-	0.526	-
	SOD activity	-	-	0.698	-
	GPX activity	-	-	-	0.53
	GST activity	-	-	0.717	-
	CAT activity	0.408	-	-	0.482
MDA content	0.879	-	-	-	

primers were designed using Primer Premier 5.0 (Premier Biosoft International, Palo Alto, CA, USA) and are listed in [Supplementary Table S2](#). Total RNA was extracted using a Trizol kit (Invitrogen, Carlsbad, CA, USA), digested by DNase (Promega, Madison, WI, USA), and reverse transcribed into single strand cDNA with reverse transcriptase (Promega). The PCR amplification volume contained 1 μL of cDNA, 0.4 μL each of forward and reverse primers, 10 μL of SYBR Green Master Mix (Vazyme, Nanjing, China), and 8.2 μL of diethylpyrocarbonate-treated water (Vazyme). The SYBR Green RT-PCR was performed on a CFX Connect™ Real-Time PCR System (Bio-Rad, Hercules, CA, USA), and the profile was 95°C for 5 min followed by 40 cycles of 95°C for 15 s and 60°C for 30 s. See [Fang et al. \(2010\)](#) for details about this process.

2.5 Integration of lines-of-evidence

For each investigated station, the relationships among variables were assessed by factor analysis (FA) and principal component analysis (PCA) as extraction procedure previously employed in studies by [Choueri et al. \(2009\)](#) and [Choueri et al. \(2010\)](#). These analyses were based on the trace elements content in seawater and

sediment and the biomarkers in the four studies indicators. The variables were autoscaled to ensure equal importance in the analysis. Our focus was on the cause-effect relationships stemming from environmental trace elements and their biological impacts. Notably, the data used for multivariate analysis excluded bioaccumulations, as these are phenomena rather than effects, as per Chapman (1990). The analyses were conducted using SPSS software (version 13.0; IBM, Armonk, NY, USA).

2.6 IBRv2 calculation

We conducted Pearson correlation analysis to assess the relationship between biomarkers and trace element contents in seawater or sediment. We established two biomarker combinations indicative of trace element pollution in seawater and sediment, respectively, based on the correlation between biomarkers and trace element contents. The selected biomarkers were integrated using IBRv2 (Sanchez et al., 2013). We used the station with the lowest pollution as the reference station. To reduce variance, a log transformation of each biomarker was conducted as follows (Equation 8):

$$Y_i = \log(X_i/X_0) \quad (8)$$

where Y_i represents transformation of the biomarker; X_i represents the biomarker value at the polluted station; and X_0 represents the biomarker value at the reference station.

The general mean (μ) and standard deviation (σ) of Y_i were used to obtain the standardized biomarker response (Z_i , Equation 9):

$$Z_i = (Y_i - \mu)/\sigma \quad (9)$$

The biomarker deviation index (A) was calculated using Z_i at the polluted station and Z_0 at the reference station (Equation 10):

$$A = Z_i - Z_0 \quad (10)$$

The IBR was obtained by calculating the sum of the absolute value of A for each biomarker at the polluted station (Equation 11):

$$IBR = \sum |A| \quad (11)$$

2.7 Statistical analysis

The data were expressed as mean \pm standard deviation (SD). Three repeated measurements were performed for every index for each station. The differences in the biomarker values among stations were analyzed using one-way analysis of variance followed by Duncan's test using SPSS. $P < 0.05$ was considered to be statistically significant. Correlations among measured indexes were assessed using Pearson correlation analysis followed by Duncan's test at a significance level of $P < 0.05$. Prior to conducting Pearson correlation analysis, the Shapiro-Wilk test was employed to ascertain the normality of the data distribution. The RNA expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001). Graphics were depicted using

ArcGIS (version 10.0; Environmental Systems Research Institute, Redlands, CA, USA) and SigmaPlot (version 13.5; Systat Software, San Jose, CA, USA).

3 Results

3.1 Trace element contents in seawater, sediment, and indicators

Supplementary Table S3 shows the physicochemical parameters of the seawater samples. The contents of Cu, Pb, Zn, Cd, Cr, and As in seawater were within Class I criteria, except for the Zn content at stations 1 and 3 in the offshore wind farm and station 10 in the area adjacent to the marine ranch (Supplementary Table S4). Zn contents at these three stations were within Class II criteria (50 $\mu\text{g/L}$, Figure 2).

The trace element contents in sediment at all stations were within Class I criteria of China Sediment Quality Standards (General Administration of Quality Supervision, Inspection and Quarantine of the People's Republic of China, 2002). There values were as follows: Cu, 35 mg/kg; Pb, 60 mg/kg; Zn, 150 mg/kg; Cd, 0.50 mg/kg; Cr, 80 mg/kg; and As, 20 mg/kg (Supplementary Table S5).

Overall, the trace element contents in seawater and sediment in the AR area and the area surrounding AR were generally lower than those in the offshore wind farm area and the area adjacent to the marine ranch. According to the HI of seawater, the ranking of trace element pollution is as follows: station 6 < station 5 < station 11 < station 7 < station 4 < station 8 < station 1 < station 9 < station 2 < station 12 < station 3 < station 10. The E_r^i values were ranked as: Cd > Pb > As > Cr > Cu > Zn at all stations (Figure 2). Every trace element at each station was of low ecological risk, except for Cd at stations 2, 3, 8, 10, 11, and 12, where it posed moderate ecological risk. Moreover, the ecological risk of the sum of all studied trace elements at every station was low, with the exception of moderate risk at stations 3 and 10. According to the RI of sediment, the ranking of trace element pollution is as follows: station 7 < station 5 < station 6 < station 4 < station 9 < station 1 < station 12 < station 8 < station 11 < station 2 < station 10 < station 3.

Cu, Zn, Pb, As, Cd, and Cr were detected in all four indicator species (Supplementary Table S6). The maximum content of these trace elements was 19.41 mg/kg Cu in *C. japonica* at station 11; 65.50 mg/kg Zn in *R. venosa* at station 1; 1.70 mg/kg Pb in *R. venosa* at station 12; 11.75 mg/kg As in *C. japonica* at station 11; 0.098 mg/g Cd in *L. maculatus* at station 12; and 1.98 mg/kg Cr in *C. japonica* at station 1. Trace element contents in *C. japonica* and *R. venosa* were relatively higher than those in *L. maculatus* and *S. schlegelii*.

The bioaccumulation characteristics of trace elements in the four indicators exhibited species specificity (Figure 3). The values of BAF and BASF for *C. japonica* and *R. venosa* were higher than those for *L. maculatus* and *S. schlegelii*. These values showed that *L. maculatus* could efficiently accumulate Cu and Zn from seawater and Cu from sediment and that *S. schlegelii* could efficiently accumulate Cu and As from seawater and Cu from

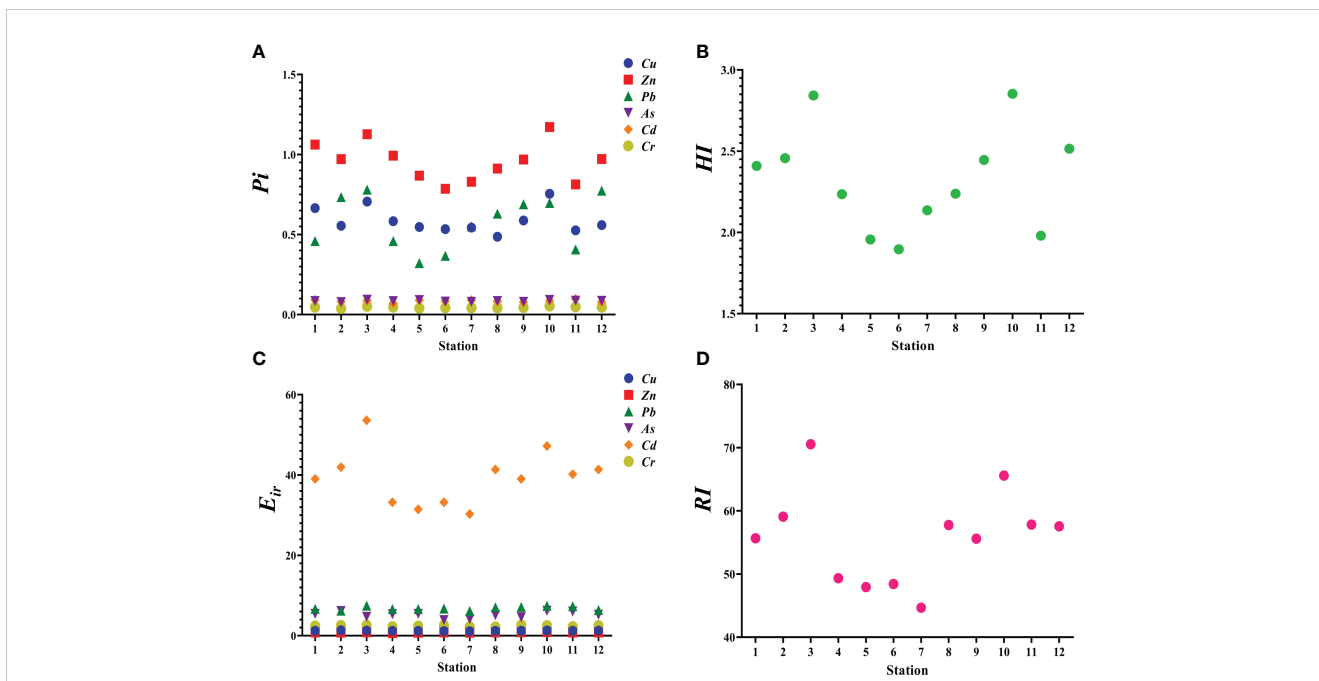


FIGURE 2 Assessment of trace element contents in seawater and sediment collected from the studied marine ranching area. (A) trace element pollution index (P_i) in seawater assessed using the single-factor index method. (B) integrated trace element pollution index (HI) in seawater. (C) potential risk of a given trace element (E_{ir}) in sediment assessed using the Håkanson ecological risk assessment method. (D) sum of E_{ir} (RI).

sediment. *C. japonica* and *R. venosa* could efficiently accumulate Cu, Zn, Pb, and As from seawater and Cu, Zn, and As from sediment.

3.2 Biomarker analysis

The studied biomarker response showed significant differences among stations, indicating spatial variation of biomarkers (Figures 4–

7). The biomarker values in the AR area and the area surrounding AR were generally lower than those in the offshore wind farm area and the area adjacent to the marine ranch, which suggested good health status of the indicator species. Furthermore, differences in biomarker responses were observed among species, which suggests that they have distinct mechanisms for adjusting to trace element exposure.

S. schlegelii and *L. maculatus* had higher SOD activity compared to *C. japonica* and *R. venosa*. The maximum MDA content (6.79 nmol/L) was found in *S. schlegelii* at station 8. *C. japonica* had the

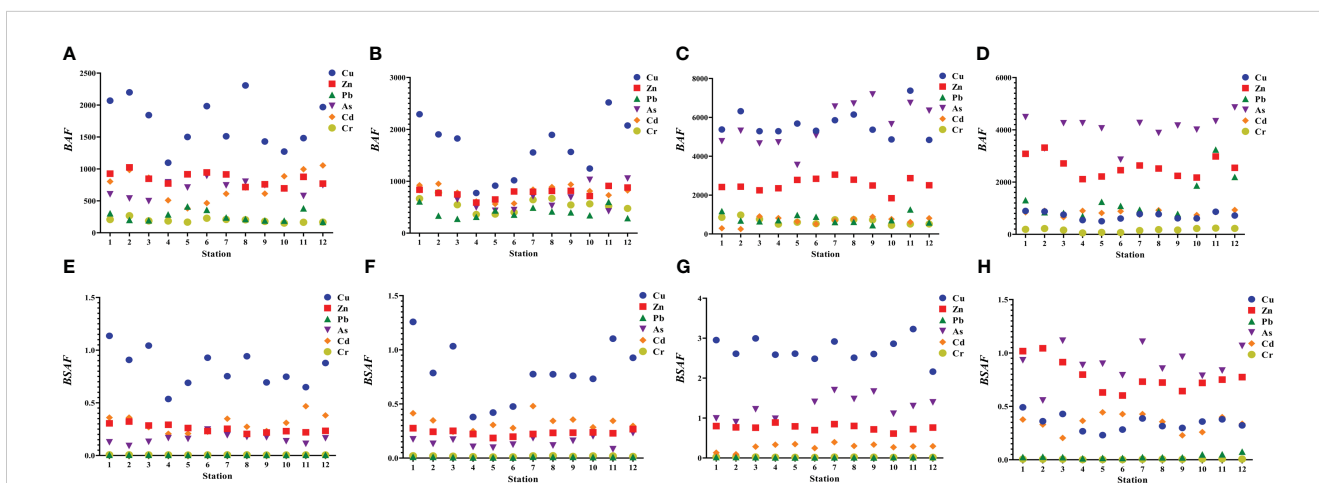


FIGURE 3 Biota-seawater accumulation factor (BAF) and biota-sediment accumulation factor (BSAF) in the four indicator species. (A) BAF in *L. maculatus*. (B) BAF in *S. schlegelii*. (C) BAF in *C. japonica*. (D) BAF in *R. venosa*. (E) BSAF in *L. maculatus*. (F) BSAF in *S. schlegelii*. (G) BSAF in *C. japonica*. (H) BSAF in *R. venosa*.

highest GPx activity and *R. venosa* had the lowest GST activity among the four indicator species. CAT activity was not detected in *C. japonica* and was highest in *L. maculatus*.

Biomarker values exhibited different response characteristics in the offshore wind farm area and the area adjacent to the marine ranch. The values of some biomarkers were higher in the offshore wind farm area compared to the adjacent area (e.g., MT content in *R. venosa* and GPx activity in *S. schlegelii*), whereas the opposite was true for other biomarkers (e.g., CAT activity in *S. schlegelii* and GST activity in *R. venosa*). As referred to the relationship between molecular and biochemical levels of a certain biomarker, only SOD activities were significantly correlated with *MnSOD* expressions in *S. schlegelii*.

3.3 Multivariate analysis

The factor analysis streamlined the original dataset into four principal factors, collectively accounting for 71.16% of the total variance present in the original data (Table 2).

The predominant factor (F1) accounted for 33.31% of the variance and related contents of 5 specific trace elements (Cu, Zn, As, Cd, and Cr) in seawater and 3 specific trace elements (Pb, Zn, and Cd) in sediment and 2 biomarkers in *L. maculatus* (GPx activity and MDA content), 3 biomarkers in *S. schlegelii* (MT content, GST activity, and *ZnSOD* expressions), 3 biomarkers in *C. japonica* (MT content, GST activity, and MT expressions), and 3 biomarkers in *R. venosa* (MT content, CAT activity, and MDA content).

The predominant factor (F2) accounted for 15.36% of the variance and related contents of 2 specific trace elements (Pb and Zn) in seawater and 3 specific trace elements (Cu, Cd, and Cr) in

sediment and 2 biomarkers in *L. maculatus* (MDA content and GST activity), 2 biomarkers in *S. schlegelii* (MT content, and GPx activity), and 4 biomarkers in *C. japonica* (MT content, SOD activity, GPx activity, and GST activity).

The predominant factor (F3) accounted for 12.73% of the variance and related contents 4 specific trace elements (Cu, Pb, Zn, and Cr) in sediment and 2 biomarkers in *L. maculatus* (GPx activity and CAT activity), 3 biomarkers in *S. schlegelii* (SOD activity, CAT activity, *MnSOD* expressions, and CAT expressions), GPx activity in *C. japonica*, and 3 biomarkers in *R. venosa* (MT content, SOD activity, and GST activity).

The predominant factor (F4) accounted for 9.76% of the variance and related contents of As content in sediment and CAT activity in *L. maculatus*, MDA content in *C. japonica*, and 2 biomarkers in *R. venosa* (GPx activity and CAT activity).

All stations exhibited positive scores across all four factors, with the exception of station 6, which registered a negative score for F4 (Table 3).

3.4 IBRv2 analysis

The data of trace element contents in environmental samples and biomarker levels conform to a normal distribution. According to Pearson correlation analysis, biomarkers significantly related to trace element contents in seawater or sediment were considered for IBRv2 calculation (Supplementary Figures S1, S2). The precise calculation of IBRv2 requires at least 5–6 biomarkers. Therefore, we used two sets of biomarkers from these four indicator species as an indicator of trace element pollution in seawater and sediment, respectively. *ZnSOD* expression and MT content in *S. schlegelii*

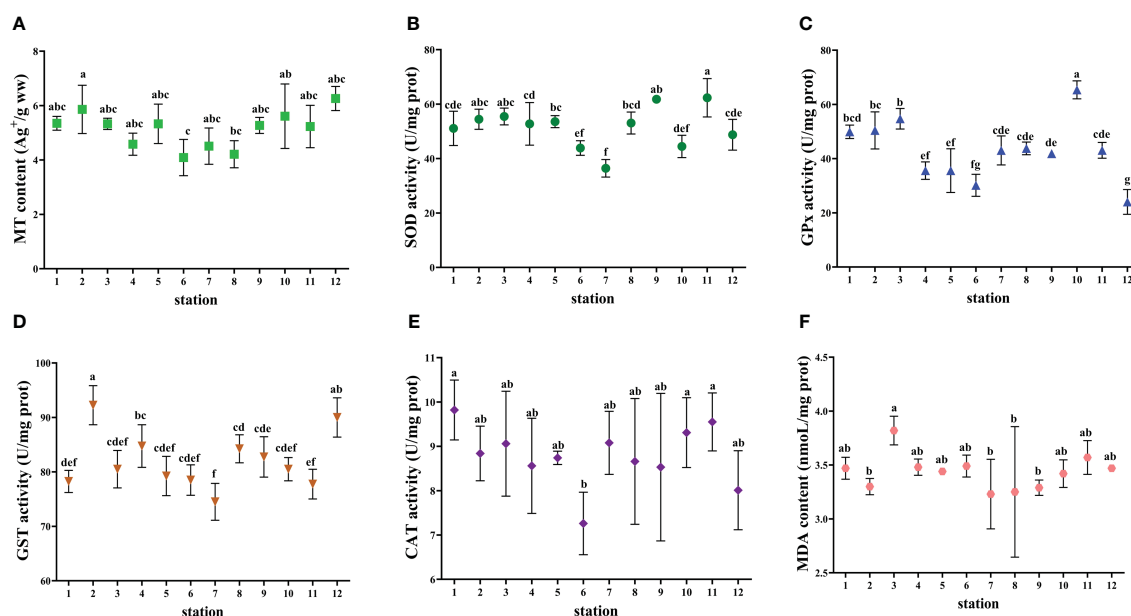
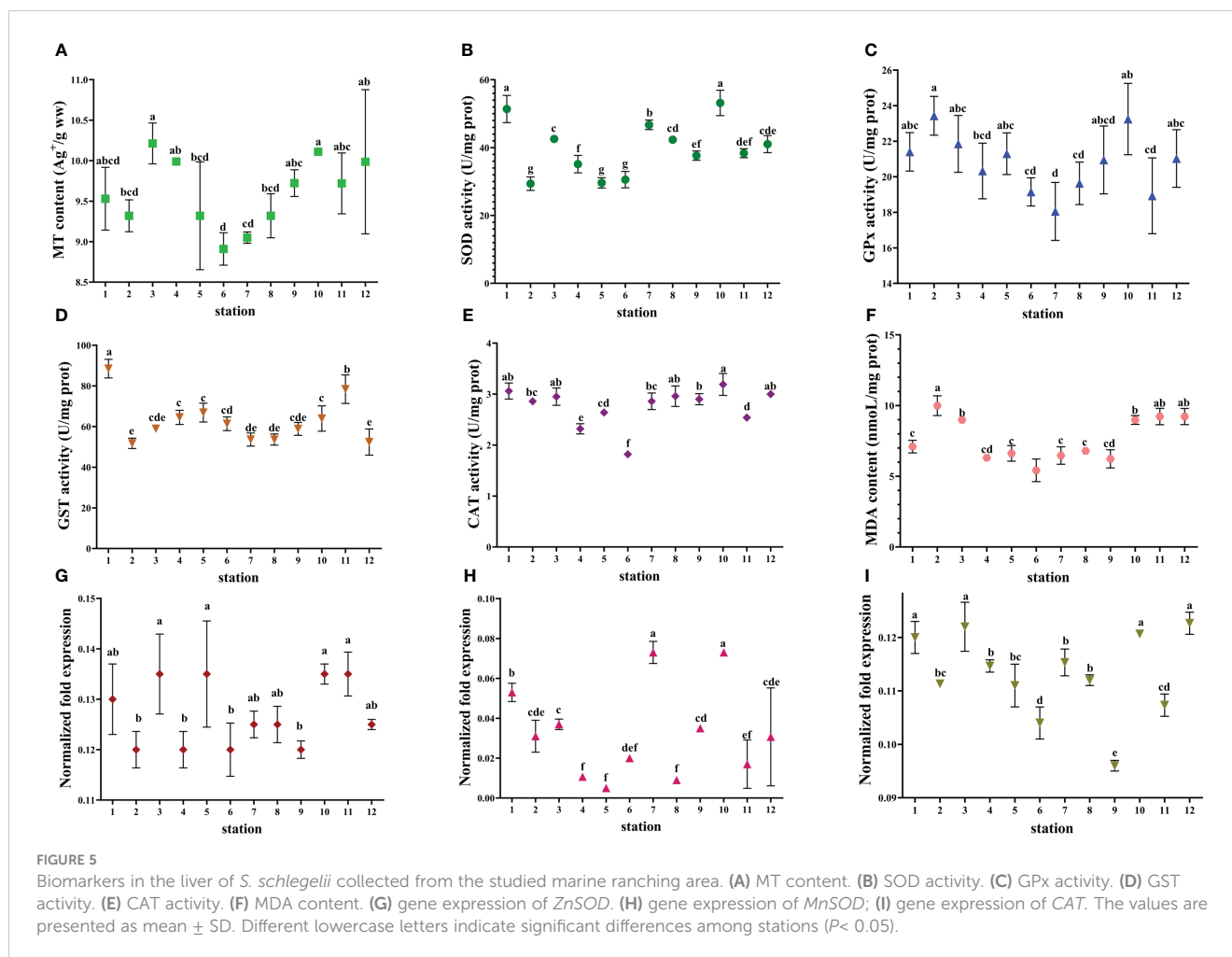


FIGURE 4

Biomarkers in the liver of *L. maculatus* collected from the studied marine ranching area. (A) MT content. (B) SOD activity. (C) GPx activity. (D) GST activity. (E) CAT activity. (F) MDA content. The values are presented as mean \pm SD. Different lowercase letters indicate significant differences among stations ($P < 0.05$).



(*SsZnSOD*, *SsMT*), GPx activity and GST activity in *L. maculatus* (*LmGPx*, *LmGST*), GST activity in *R. venosa* (*RvGST*), and GPx activity in *C. japonica* (*CjGPx*) were selected for the IBRv2 calculation for seawater. MDA content and CAT activity in *R. venosa* (*RvMDA*, *RvCAT*), GST activity and MT content in *C. japonica* (*CjGST*, *CjMT*), MT content in *L. maculatus* (*LmMT*), and GPx activity in *S. schlegelii* (*SsGPx*) were selected for the IBRv2 calculation for sediment. Based on the pollution status of trace elements and the health status of organisms, we selected station 6 as the reference station for IBRv2 analysis.

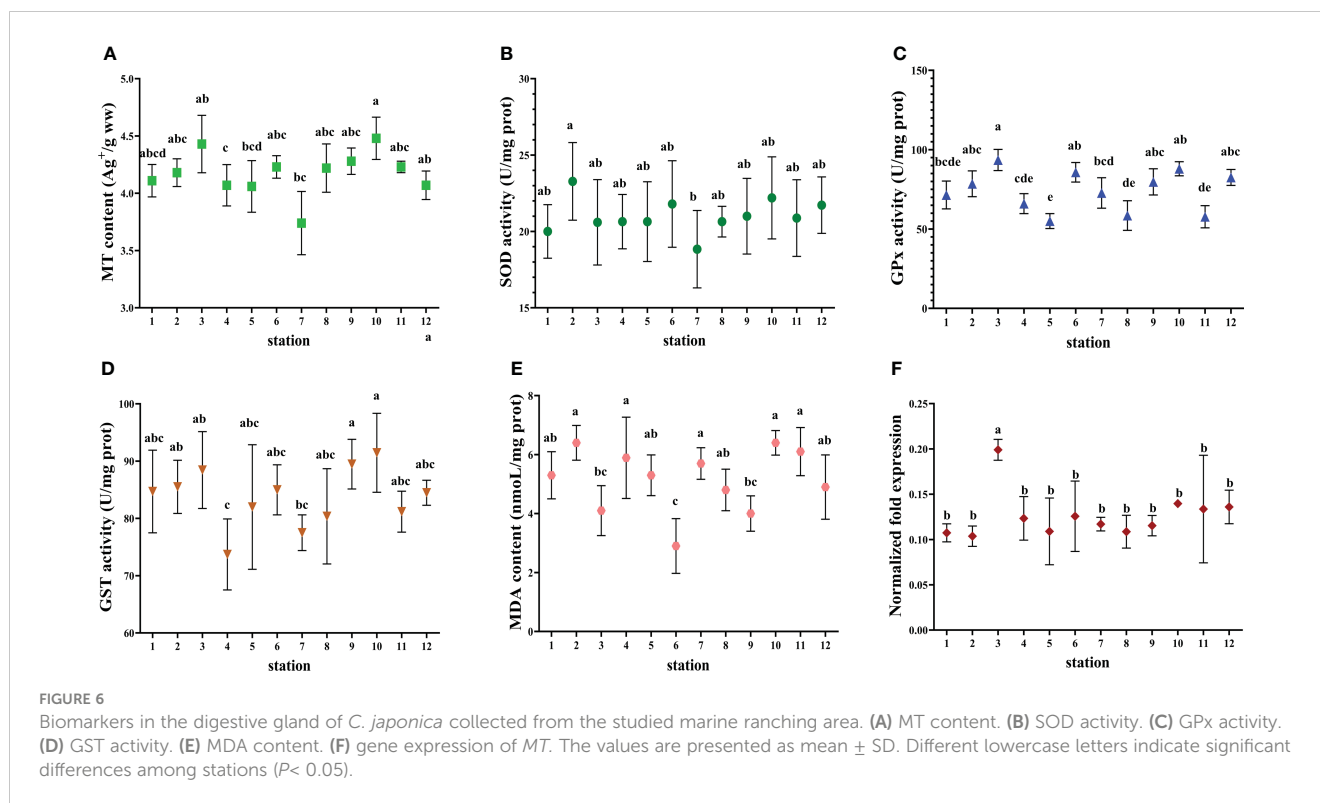
The calculated IBRv2 values can distinguish the low and high impact stations. Values were generally higher for the AR area and the area surrounding AR compared to the offshore wind farm area and the area adjacent to the marine ranch. The IBRv2 results indicated that trace elements in seawater and sediment at station 10 ($\text{IBRv2}_{\text{sw}} = 12.45$, $\text{IBRv2}_{\text{sd}} = 11.32$, Figures 8, 9) had the strongest biological impacts. According to the IBRv2_{sw} , the ranking of trace element pollution is as follows: station 7 < station 9 < station 5 < station 2 < station 8 < station 4 < station 1 < station 12 < station 11 < station 3 < station 10. According to the IBRv2_{sd} , the ranking of trace element pollution is as follows: station 8 < station 4 < station 5 < station 1 < station 9 < station 7 < station 11 < station 2 < station 3 < station 12 < station 10. The star plots of the studied stations show varied deviation of biomarkers from the baseline, which suggests

different response patterns to the pollution profiles of the trace elements. The content of certain trace elements in seawater or sediment or the four indicator species was significantly correlated with IBRv2 values (Supplementary Figures S3-S5). Moreover, significant correlations between IBRv2_{sw} values and *HI* and IBRv2_{sd} values and *RI* were found. These results indicated that the contamination pressure in seawater and sediment caused by trace elements was in agreement with the biomarker responses in these indicator species.

4 Discussion

4.1 Trace elements in the marine environment and organisms

Trace element contents in the surface seawater and sediment of the western Furong Island marine ranching area and adjacent areas were on the same order of magnitude as those along coasts around the world (Supplementary Tables S7, S8). The high accumulations of trace elements in Laizhou Bay contributed to the higher contents of some trace elements (e.g., Zn, Pb, and Cr) there compared to those reported for other coast (Lv et al., 2018). Trace element contents in the AR area and the area surrounding AR were lower



than those in adjacent areas and in a 2018 investigation (Yuan et al., 2022), which indicated that AR construction improved the quality of the marine environment. A similar scenario was reported by Chen and Chen (2020).

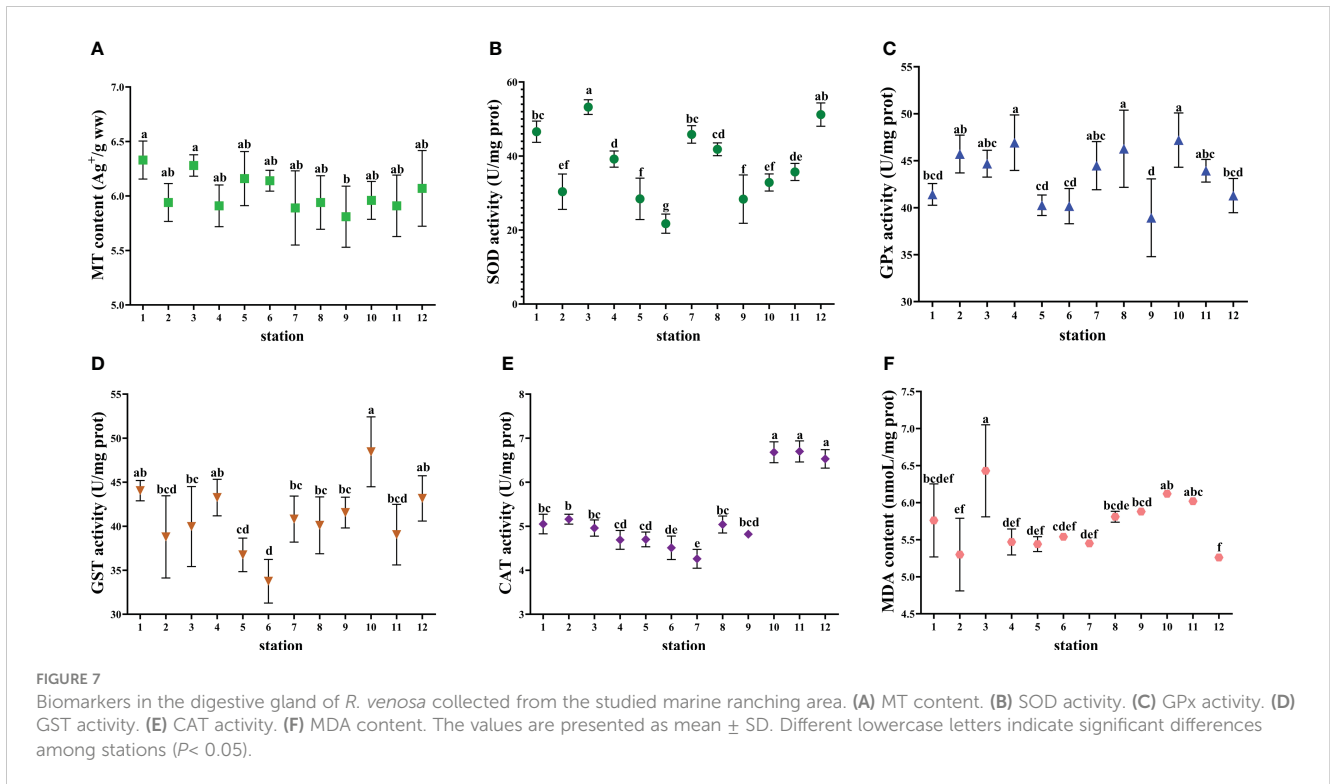
The contents of Zn, Cu, As, Pb, and Cd in the sediment and Zn in the seawater in the offshore wind farm area were higher than those in the other areas tested in the western Furong Island marine ranch. The galvanic anodes used for corrosion protection in offshore wind farms can leak 26 metal elements, with mass fractions of Zn, Cd, and Pb, and they are considered to be one of the highest sources of metal elements emitted into the marine environment (Reese et al., 2020). Thus, we propose that the construction and operation of the offshore wind farm led to the trace element pollution of the seawater and sediment detected in our study, which is in agreement with Zaborska et al. (2017) and Kirchengorg et al. (2018). Nevertheless, the impact of these trace element emissions is limited and weak (Wang et al., 2023). In addition, the offshore wind farm area is located relatively close to the south coast of Laizhou Bay, into which the Jiaolai, Jie, and Wei rivers flow. Coastal erosion and weathering products carried by the rivers also contributed to the high level of Cu and Zn in this area (Li et al., 2019). Xu et al. (2015) reported that high concentrations of As, Pb, and Cd were associated with human activities such as mining, smelting, and domestic sewage discharge.

Although the Cd level was relatively low in the sediment, the E_r^i value of Cd was highest among the tested trace elements due to its high response factor. Therefore, prevention and control of Cd is crucial in the environment management of western the Furong Island marine ranching area.

The BAF and BASF values suggested that the indicator species could accumulate trace elements from seawater and sediment and that they had strong enrichment capacity for Cu, Zn, As, and Pb. The high BAF and BASF values of Cu, Zn, As, and Pb were predominately associated with high contents of these trace elements in seawater and sediment. Moreover, the active absorption of essential trace elements during metabolism by the organisms also contributed to the high accumulation capability of Cu and Zn, which is consistent with results of previous studies (Liu et al., 2019; Gao et al., 2021).

Living habits are thought to affect trace element enrichment in species. *C. japonica* and *R. venosa* showed stronger ability to accumulate trace elements compared to *S. schlegelii* and *L. maculatus*. *C. japonica* and *R. venosa* usually bury themselves in the sediments, where they are subjected to relatively high levels of trace elements. Similar results were found in previous studies (Zhao et al., 2012; Gao et al., 2021).

A discrepancy was observed between the environmental concentrations and tissue levels of trace elements. The situation at station 10, characterized by almost the highest environmental concentrations of trace elements yet relatively low BAF and BASF values, is particularly noteworthy. This discrepancy can be explained by considering several intrinsic and extrinsic factors that significantly influence BAF and BASF, including dietary habits, ecological needs, trace element concentrations in water and sediments, seasonal variations, physicochemical properties of water and sediments, and the tolerance and adaptability of organisms to pollutants (Dadar et al., 2016; Marques et al., 2016; Shakouri and Gheytasi, 2018; Abkenar et al., 2021).



While chemical concentrations in tissues typically correlate with environmental exposure levels (Monroy et al., 2014), organisms possess mechanisms to counteract the toxic effects through various biochemical and physiological adaptations (Hansen, 2003). Once trace elements begin to bioaccumulate in tissues, organisms often initiate multiple detoxification responses. These elements dissolve in the cytosol, predominantly forming complexes with metal-binding proteins such as MTs, or are incorporated into metal-rich granules and other insoluble forms. Notably, the MT content was also relatively high in the four

indicator organisms at station 10 compared to other stations (Figures 4–7). Additionally, trace elements can be excreted from cells via adenosine triphosphate-binding cassette transporters (Hu et al., 2019). Consequently, the bioaccumulation in tissues does not always linearly correlate with environmental exposure levels, particularly at higher environmental concentrations (Cano-Rocabayera et al., 2022).

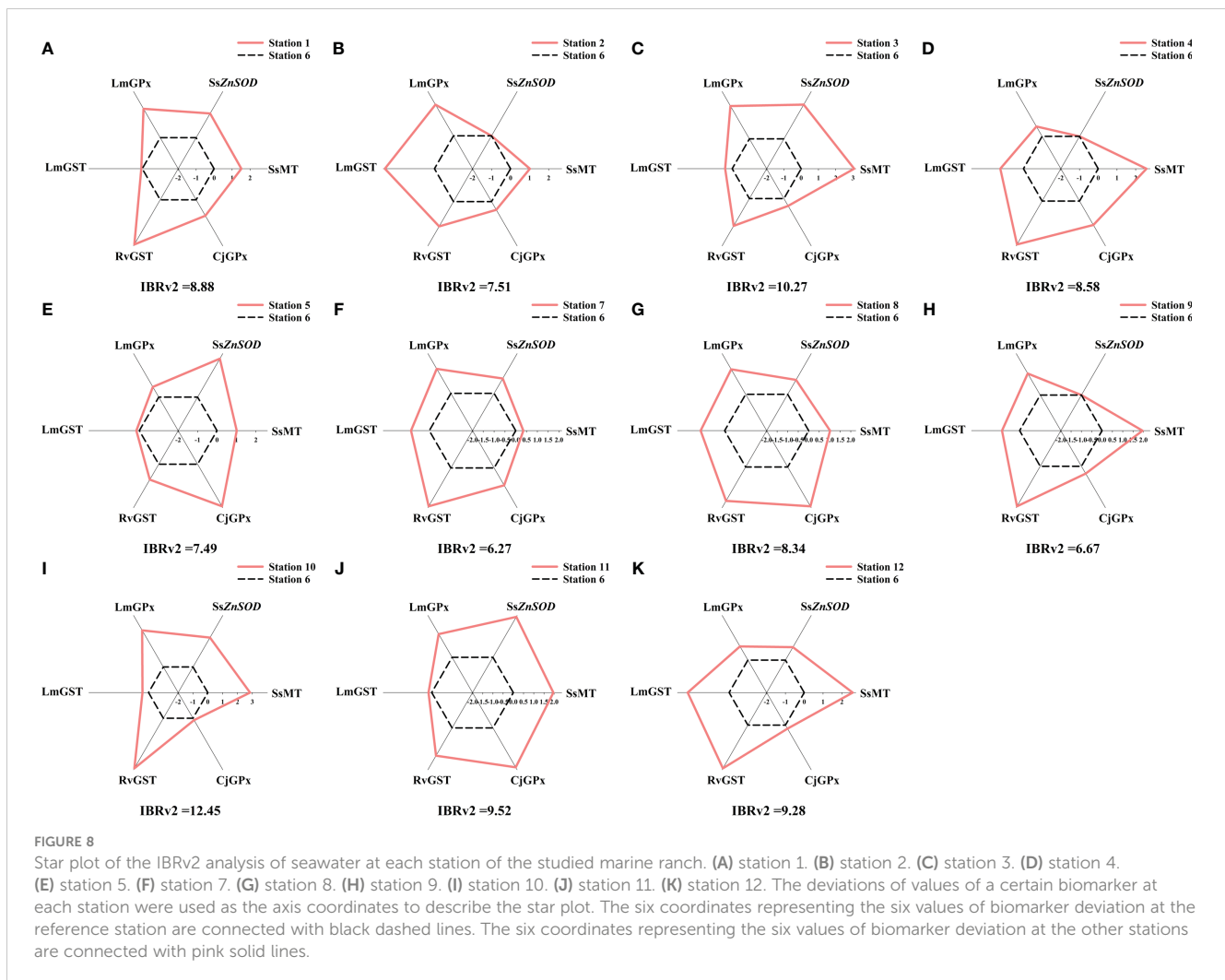
Therefore, in addition to trace element concentrations in water and sediments, the physicochemical properties of these mediums, trace element concentrations in prey, and the detoxification processes within organisms also impact bioaccumulation in tissues. This interplay results in the observed mismatch between environmental concentrations and tissue levels of trace elements in the present study. Similar scenarios were also reported in other studies (Hao et al., 2019; Cano-Rocabayera et al., 2022; Bettoso et al., 2023). A more detailed examination of the bioaccumulation process is warranted for further understanding.

TABLE 3 Estimated factor scores for the sampling stations in the studied marine ranching area.

Station	F1	F2	F3	F4
1	0.77	0.24	0.41	0.20
2	0.39	0.50	0.22	0.21
3	1.12	0.44	0.45	0.16
4	0.36	0.24	0.21	0.21
5	0.47	0.19	0.13	0.19
6	0.39	0.18	0.06	-0.02
7	0.25	0.08	0.31	0.13
8	0.46	0.24	0.23	0.20
9	0.59	0.38	0.20	0.09
10	1.07	0.51	0.52	0.27
11	0.76	0.19	0.22	0.27
12	0.56	0.45	0.33	0.17

4.2 Biomarker responses

MTs participate in the handling of intracellular metal levels and metal detoxification. They have been proposed as the biomarker of metal exposure, as they are the first detectable cellular sign of metal challenge (Filipović and Raspur, 2003). In our study, MT content and gene expression in the marine ranch area was generally lower than that in the adjacent area. MT content and gene expression in the four indicator species were positively correlated with levels of some trace elements in seawater and sediment. These results suggested a relationship between MT response and trace element level. MT in the four indicators could be induced by trace element



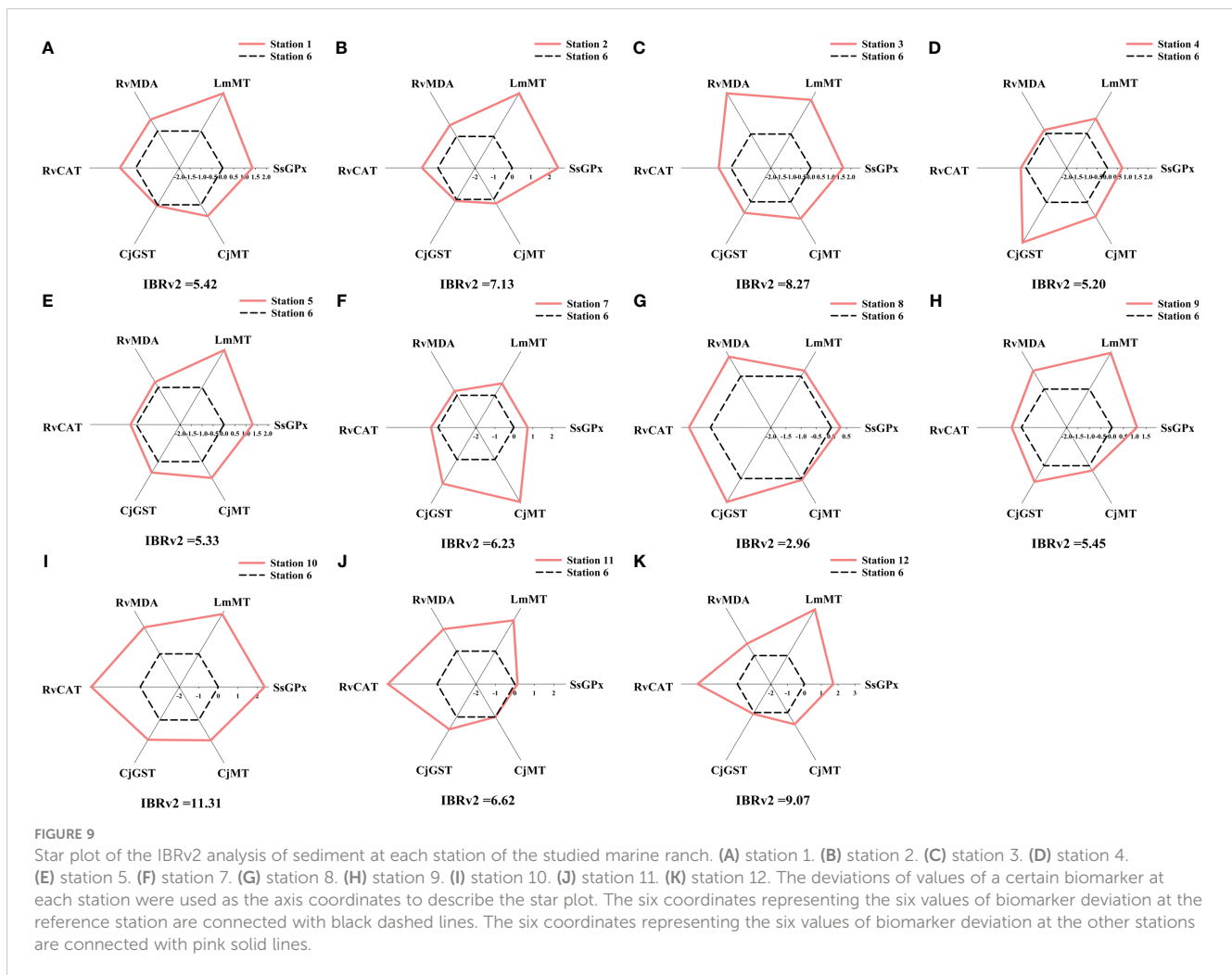
exposure and confirmed its involvement in trace element homeostasis and detoxification. This finding supports previous reports of the MT response and trace element concentrations in aquatic environments conducted in fish, gammarids, and mesozooplankton as indicators (Abril et al., 2018; Mijošek et al., 2019; Stroglyoudi et al., 2023).

MTs can also remove ROS via oxidation of the thiol group in cysteine and thereby protect organisms from oxidative damage induced by environmental stress. Trace elements commonly induce ROS generation and cause oxidative stress in organisms (Beg et al., 2015). Therefore, the observed MT response in the indicator species probably participated in quenching the effects of ROS resulting from trace element pollution.

MDA contents are generally considered to be the pivotal indicator of lipid peroxidation caused by oxidative stress. In our study, high MDA contents corresponded to high environmental levels of trace elements, further confirming the occurrence of oxidative stress caused by trace elements. Antioxidant enzymes also combat oxidant stress and maintain the cellular redox state. SOD dismutates superoxide radicals into hydrogen peroxide (H_2O_2). The neutralization of H_2O_2 is catalyzed by CAT, GPx, and GST. These enzymes act sequentially to resist oxidative stress.

The responses of antioxidant enzymes have been reported in various aquatic organisms, such as fish, bivalves, and crustaceans (Weber et al., 2020; Yu et al., 2020; Telahigue et al., 2022) that inhabit trace element-contaminated aquatic areas. Similarly, we found that the activities and transcripts of antioxidant enzymes in the four indicator species varied among stations and were related to trace element contents. These results suggest collaboration among the enzymes to counter trace element-induced oxidative stress. Moreover, the species-specific responses of these antioxidant enzymes suggest that multiple taxa must be considered as indicators in environmental biomonitoring.

The discrepancy between contents and transcripts of MT in *C. japonica*, between SOD activities and *MnSOD* transcripts, and between activities and transcripts of CAT in *S. schlegeli* are noteworthy. This scenario can be explained as follows. On the one hand, there are many members of the MT family and many sub-types of SOD. We measured the gene expression of only one MT member or one SOD sub-type, whereas we measured total MT protein content and total SOD activity. On the other hand, there is a time delay between transcription and protein synthesis or induction of enzyme activity. Post-transcriptional modification, translation, and post-translational modification can all regulate



protein content and enzyme activity (Desclaux-Marchand et al., 2007).

4.3 Multivariate analysis

The findings from multivariate analysis revealed that the MDA content in *L. maculatus* and *R. venosa*, the ZnSOD expressions in *S. schlegelii*, and the MT expressions in *C. japonica* were influenced by the presence of Cd and As in seawater, as well as Pb, Zn, and Cd in sediment. The MT content and GST activity in *L. maculatus*, the GPx activity in *S. schlegelii*, and the SOD activity in *C. japonica* were impacted by Cu in seawater and Cr in sediment. The MDA content in *C. japonica* and the GPx activity in *R. venosa* were affected by As in sediment, with the exception of station 6, which exhibited a negative score for F4, indicating superior sediment conditions relative to other stations.

Contrastingly, the SOD activity in *L. maculatus* and the MDA content in *S. schlegelii* did not exhibit any correlation with the principal components, suggesting that these two biomarkers were not significantly influenced by environmental trace elements. Therefore, they were not included in the IBR selection process.

The stations generally showed positive scores across all four factors, indicating clear cause-effect relationships between environmental trace elements and biomarkers in the four indicators, with the exception of the SOD activity in *L. maculatus* and the MDA content in *S. schlegelii*.

4.4 Integration of multi-species and multi-level biomarkers

Biomarkers reflect the pollutants' bioavailability and also organisms' responses to them and their ability to cope with pollutants. A single biomarker is not capable of comprehensively indicating the individual's fitness, whereas a battery of biomarkers ranging from the molecular to the individual level can allow integrated evaluation of the toxic effects of pollutants (Catteau et al., 2022). Different biomarkers have distinct sensitivity to pollutants. Because oxidative stress occurs along with trace element accumulation in indicator species, researchers have used the combination of MTs as proteins that scavenge trace elements and antioxidant enzymes that quench oxidative stress to comprehensively assess the effects of trace elements in aquatic environments (Beg et al., 2015; Pan et al., 2022). In our study, we

detected spatial differences in MT content and antioxidant enzyme activities in the four indicator species, which we attributed to variations in trace element pollution among the stations. Furthermore, as described above, biomarkers at the transcriptional level may not necessarily be more sensitive to pollutants than biomarkers at the protein level, even though transcription occurs earlier than protein synthesis. Accordingly, we recommend that researchers consider both the sensitivity and early warning functions when selecting biomarkers for evaluating the biological effects of pollutants.

The multi-species approach, which involves integrating responses and broadening the evaluation of aquatic pollutants, overcomes the shortcomings of the single-species approach, as it considers the influence of species' habits, lifestyles, and feeding behaviors on biomarker responses. The multi-species approach has been applied in a few comprehensive assessments of water pollution or sediment pollution (Marques et al., 2016; Catteau et al., 2022; Yamamoto et al., 2023).

In our study, we chose two fish species, one crustacean species, and one gastropod species to cover various habitats, niches, feeding behaviors, and commercial importance. These four indicator species exhibited distinctive accumulation and response patterns resulting from their different foraging behaviors, pollution pathways, and detoxification abilities. Moreover, we also found that certain biomarkers in every indicator species were correlated with trace element contents in seawater and sediment. These results suggested that certain biomarkers in each indicator species were able to indicate trace element pollutions in seawater and sediment. All four of the indicator species directly contact seawater and sediment, and the seawater and sediment interact with each other. Trace elements can accumulate in sediments from seawater through various physicochemical and biological processes. On the other hand, sediment resuspension due to hydraulic disturbances may cause the release of trace elements from the sediments into the overlying seawater (Pourabadehei and Mulligan, 2016). Accordingly, trace elements in seawater and sediments affected the middle-upper water-dweller *L. maculatus*, the demersal fish *S. schlegelii*, and benthic organisms *C. japonica* and *R. venosa*. However, not every biomarker was associated with environmental trace element contents. Therefore, we used only associated biomarkers for our IBRv2 analysis to allow accurate interpretation of the results (Li et al., 2020).

The selection of biomarkers is crucial for IBRv2 assessment, and a minimum of 5–6 biomarkers should be used (Novais et al., 2014). Drawing inspiration from Broeg and Lehtonen (2006), we used only the biomarkers that were correlated with the different types of trace elements for our IBRv2 calculation to avoid overemphasizing the presence or absence of a specific trace element in the overall assessment of trace elements. In contrast to previous multi-species studies in which IBRv2 was calculated for every studied species separately (Catteau et al., 2022; Yamamoto et al., 2023), biomarkers from the four indicator species were integrated for IBRv2 calculations to achieve improved and comprehensive evaluations. We also used two sets

of six biomarkers from the four indicators to assess water and sediment pollution, respectively.

We used station 6 in the AR area as the reference station because it had minor pollution levels and biomarker levels. The IBRv2 values clearly discriminated trace element pollution among stations. The different shapes of the star plots revealed the dissimilar pattern of trace element pollution. The highest IBRv2 value occurred in the area adjacent to the marine ranch, which suggested that the trace elements in that area had the strongest biological impacts. These results are consistent with the chemical assessment.

Undoubtedly, the integration of species with distinctive functional traits and biomarkers with different biological levels benefited the environmental pollution assessment. Together, our results illustrate that the multi-level and multi-species IBRv2 approach is a useful and reliable tool for assessing the trace element pollution status and biological responses in aquatic environments. Despite the different rankings of trace elements pollution at the survey stations using HI, RI, and IBR, a comprehensive analysis of these rankings revealed that the trace elements pollution in AR area and AR surrounding area was better than that in OWF area and marine ranching adjacent area. Therefore, we concluded that the construction of ARs was beneficial for improving marine trace elements pollution. The trace elements pollution in OWF area and marine ranching adjacent area were comparable. Therefore, further research and long-term environmental monitoring are necessary to properly assess the impact of OWF on the marine environment.

5 Conclusion

In this study, the four indicator species exhibited distinctive patterns of trace element accumulation and biological responses, which may be related to their habitats, niches, and feeding behaviors. The four species complemented each other in the assessment of the toxic impacts of trace elements on the whole ecosystem of the western Furong Island marine ranching area. Certain biomarkers from these indicator species were correlated with trace element levels in the seawater and sediment. The IBRv2 approach described herein integrated multi-level biomarkers from multiple species to provide a holistic description of the status of trace element pollution and the ecotoxicological effect of trace elements in the study area. In agreement with the chemical evaluation results, the IBRv2 results showed that trace elements had the strongest biological impacts in the area adjacent to the marine ranch. In contrast, trace elements in the AR area and the area surrounding the AR exhibited weak biological impacts of trace element pollution.

Our multi-biomarker and multi-species IBRv2 approach could become a common strategy for marine environmental assessment, as it is convenient for managers to use to make environmental decisions and for trace element control. The selection of target indicators and appropriate biomarkers is crucial for environmental monitoring in regional marine areas.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding authors.

Ethics statement

The animal study was approved by Science and Technology Ethics Review Committee of Ludong University. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

MZL: Conceptualization, Investigation, Methodology, Writing – original draft. ZC: Formal analysis, Resources, Writing – original draft. ML: Investigation, Resources, Writing – original draft. JZ: Data curation, Methodology, Writing – original draft. BX: Data curation, Methodology, Writing – original draft. YY: Formal analysis, Visualization, Writing – original draft. XL: Investigation, Methodology, Writing – original draft. LF: Investigation, Methodology, Writing – original draft. BL: Conceptualization, Data curation, Writing – review & editing. TZ: Conceptualization, Data curation, Writing – review & editing. YF: Funding acquisition, Supervision, Writing – review & editing, Writing – original draft. FL: Investigation, Supervision, Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmars.2024.1413847/full#supplementary-material>

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