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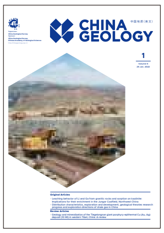

Ecological environment response of benthic foraminifera to heavy metals and human engineering: A case study from Jiaozhou Bay, China

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ABSTRACT

The estuary and coastal zone are the key areas for socio-economic development, and they are also the important channels for pollutants transported to the sea. The construction of the Jiaozhou Bay Bridge changed the hydrodynamic condition of the bay, which made the self-purification capacity of the bay weakened and the pollution in the estuary and adjacent coastal zone become more serious. In this study, 55 surface sediment samples were collected from the three seriously polluted estuaries and the adjacent coastal zone of Jiaozhou Bay to comprehensively study how the benthic foraminifera response to heavy metal pollution and human engineering, and to assess the ecological risks of the bay. A total of 80 species, belonging to 42 genera, were identified in this study. The results showed that Cu, Pb, Cr, Hg, Zn, and As had low to median ecological risks in the study area which would definitely affect the ecological system. The construction of the Jiaozhou Bay Bridge has resulted in pollutants accumulated at the river mouth of Loushan River, which has adverse effects on the survival and growth of benthic foraminifera. The lowest population density and diversity as well as the highest FAI (Foraminiferal Abnormality Index) and FMI (Foraminiferal Monitoring Index) occurred at Loushan River Estuary which indicated that the ecological environment of the northeastern part of Jiaozhou Bay (Loushan River Estuary) had been seriously damaged. Licun River and Haipo River estuaries and the adjacent coastal zone were slightly polluted and had low ecological risk. As a consequence, it suggested that the supervision of industrial and domestic waste discharge and the protection of the ecological environment in northeast Jiaozhou Bay should be paid more attention.

1. Introduction

As the connection between land and ocean, the estuary and coastal zones are the most active area of socioeconomic activities and the most sensitive area response to global change (Yin P et al., 2017). With the rapid development of the social economy, estuaries have become the important channels for rivers to carry pollutants into the sea. In recent years, environmental pollution is getting more and more serious especially in the estuary and coastal zone. Compared with other pollutants, heavy metals are most harmful to the ecological environment and human health (Liu R and Huang Y, 2019; Negar F et al., 2020). They are enriched in sediments, with the continued accumulation, toxic metals will be absorbed by benthic creatures. Furthermore, through the food chain, it will ultimately affect human health (Lawrence AL and Mason RP, 2001). Till now, the regional ecological risk assessment by using heavy metals is often inconsistent, according to the different evaluation indexes (Xiao CL et al., 2017). Therefore, it is necessary to combine the evaluation index with the bio-indicators to evaluate the pollution degree.

Benthic foraminifera, a kind of marine protozoa, most of which live in the ocean, while a few live in shallow water marginal environments (shoal, gulf lagoon, and estuary) (Jain S, 2017). Foraminifers are small individuals with a short life-

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cycle and are widely distributed with abundant quantity and high biodiversity (Murray JW, 1991). Their whole life processes are almost *in situ* (Kitazato H, 1988). When environmental factors (water depth, salinity, temperature, substrate type, food supply, sedimentation rate, and pollution, etc.) change, they will quickly respond (Boltovskoy E et al., 1991; Lo Giudice Cappelli E et al., 2019; Benito X, 2020). Foraminifera is much more sensitive and tolerant than other organisms (such as ostracods, crustaceans) which would generate malformation when encountering stressed environmental conditions (Le Cadre V and Debenay JP, 2006; Andreas AL and Bowser SS, 2021). Therefore, they are the most suitable organisms as bio-indicators of environmental status (Armynot du Châtelet E et al., 2004; Albani A et al., 2007). Among these influencing factors, heavy metals have the greatest impact on foraminifera. Recent studies have focused on the response of foraminiferal population density, diversity, assemblages, and morphological abnormalities to heavy metal and organic pollution (Alve E, 1995; Yanko V et al., 1994, 1998; Samir AM and El-Din AB, 2001; Geslin E et al., 2002; Ferraro L et al., 2006; Frontalini F et al., 2009; Mendes I et al., 2013; El Kateb A et al., 2020; Gildeeva O et al., 2021). A large number of studies have confirmed that heavy metals (Cr, Cu, Hg, Pb, and Zn) have significant effects on the distribution of foraminiferal assemblages in ports, lagoons, estuaries, and coastal areas (Coccioni R, 2000; Armynot du Châtelet E et al., 2004; Frontalini F et al., 2009; Nagendra R and Reddy AN, 2019). The population density and diversity of foraminifers often decrease in the polluted areas (Bergin F et al., 2006; Debenay JP and Fernandez JM, 2009; Li T et al., 2014, 2015), with the appearance of morphological abnormalities (Frontalini F and Coccioni R, 2008; Frontalini F et al., 2009). A few studies conducted on foraminiferal culture in the contaminated environment by a single trace element in the laboratory have also shown that there were various deformities under different concentrations of heavy metals (Le Cadre V and Debenay JP, 2006; Nigam R et al., 2009; Brouillette PE et al., 2019). Furthermore, a few studies have begun to explore the influence of heavy metals on foraminifera from the perspective of molecular structure, and the results show that Pb and Hg would lead to changes in the individual morphology and molecular structure (Frontalini F et al., 2015, 2017). However, the same genus of foraminifera may be sensitive in this region and resistant to contamination in another area, so the absence of a specific species cannot be used to access ecological quality (Alve E, 1995). Thus, more studies should be carried out in the worldwide area.

2. Geological and environment background

Jiaozhou Bay is located in the south of Shandong Peninsula and is a semi-closed bay (Fig. 1). Silt is the main type of sediment on the eastern coast which is mainly distributed in the shallow range of 5 m isobath, and the grain size becomes coarser near the Cangkou Waterway (Wang YP et al., 2000). The coast of Jiaozhou Bay is suffering from industrial and domestic pollution in recent years (Xu FJ et al., 2017; Liu S et al., 2020). The main ports and industries of Qingdao City are distributed along the eastern coast of Jiaozhou Bay, which is significantly affected by human activities. Especially, the Loushan River, Licun River, and Haipo River have become the main channels for industrial and domestic sewage to enter the bay (Qiao LL et al., 2019). Due to the reduced river runoff in recent years, the salinity of seawater in the estuary was close to the adjacent sea area (Dong H, 2012). Heavy metals were accumulated in surface sediments of the estuaries which were far higher than the background value (Li Y et al., 2006; Guo JH et al., 2012; Liu S et al., 2020). The reversing current of the Cangkou Waterway (Fig. 1) impedes the diffusion of pollutants from the east coast to the westward (Wang HJ et al., 2007), resulting in a high content of pollutants in the eastern Jiaozhou Bay (Li TT et al., 2016). Moreover, the topography of Jiaozhou Bay has been significantly changed by intensive anthropogenic activities, land reclamation, and the construction of the Jiaozhou Bridge over the last decades (Yuan Y et al., 2021). The cross-bay bridge has a relatively large impact on the hydrodynamic conditions which will definitely affect the ecological environment of Jiaozhou Bay (Li P et al., 2014; Chen YY et al., 2019). Therefore, Jiaozhou Bay is considered to be a natural laboratory to study how the benthic foraminifers respond to human activities including heavy metal pollution and coastal engineering.

In this study, the Loushan River, Licun River, and Haipo River estuaries and adjacent coastal zone were taken as the research object. The aims are: (1) Investigate the distribution of benthic foraminifera of eastern coast of Jiaozhou Bay and evaluate the ecological risks of the three estuaries by using ecological risks index; (2) try to explore how the distribution characteristics of benthic foraminifera (population density, diversity, morphological deformities) response to the environmental factors (including heavy metals) and Jiaozhou Bay Bridge based on the results of the investigation. The results will be of great significance for better understanding the effects of human activities on benthic communities and the complex ecosystem of Jiaozhou Bay.

3. Materials and methods

3.1. Study area and sampling sites

Four sewage outfalls including Loushan River Estuary, Qingdao Alkali Industry Co. LTD, Licun River Estuary, and Haipo River Estuary were found in the study area which was all seriously polluted (Fig. 2). Of these sewage outfalls, Licun River Estuary was mainly polluted by domestic sewage, while the other sewage outfalls were mainly discharged industrial sewage. The sewage outfalls of Qingdao Alkali Industry Co. LTD were seriously polluted (Fig. 2b). According to the preliminary survey, the mid-lower reaches of the Licun River were dry all year-round and the stench could be smelled during the investigation (Fig. 2d). A yellow-green liquid was

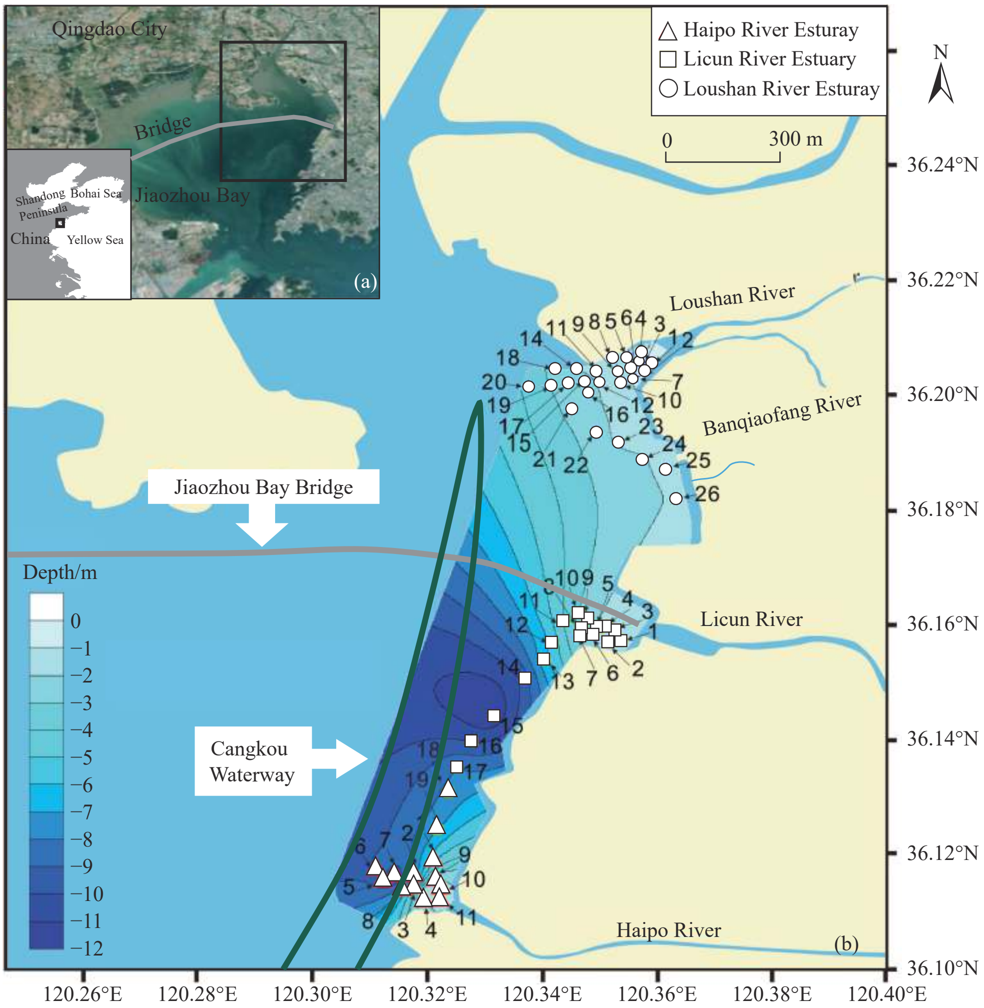

Fig. 1. (a) Map showing the location of the study area; (b) map showing the water depth of the study area with sampling stations. The locations of the Jiaozhou Bay Bridge and the Cangkou Waterway are also showed on this map.

observed and the pungent stench was smelled in the Haipo River Estuary (Fig. 2c). The mid-lower reaches of the Loushan River were dry and covered with weeds, and there was only a half-meter-wide channel in the middle which was filled with sewage (Fig. 2a).

A grab sampler was used to collect the upper 5 cm surface samples. Time and water depth were recorded at the same time. The surface sediments are mainly composed of silt and clayed silt. A total of 55 surface samples were obtained in this study (including 11 in the Haibo River Estuary, 19 in the Licun River Estuary, and 25 in the Loushan River Estuary) (Fig.1).

3.2. Foraminiferal analysis

The collected surface sediment samples were dried at 60℃ and soaked in the 10% oxyful solution. They were then gently washed with tap water through a diameter of 0.063 mm sieve to remove clay, silt, and any excess dye. The residual fraction was dried again and through flotation. The dry samples obtained were splitted up into eight equal parts. 1/8 samples were observed under a stereo-binocular microscope to identify the foraminifera. The data of living, dead and total individuals were counted respectively. The statistical parameters included: (1) Abundance, population density, that is, the total number of foraminifera in 50 g dry sample; (2) S, simple differentiation, that is, the number of foraminifera species in the sample; (3) H, Shannon-Weiner index, reflects the degree of species diversity and evenness of biological population. The calculation formula of the Shannon-Wiener index: $H=-\sum_{i=1}^{S} Pi\ln Pi$, where Pi is the ratio of the number of individuals to the total number of individuals in the group; (4) the percentage of major genera species (%), the ratio of major genera species to the total foraminiferal population quantity; (5) FAI (Foraminiferal Abnormality Index), the percentage of the population quantity of the deformities in the sample to the total number of the foraminiferal population; (6) FMI (Foraminiferal Monitoring Index), represents the percentage of the number of species of the deformities to the total number of species.

3.3. Total organic carbon, heavy metal analysis, and pH

The surface samples, as well as the water samples, were collected at the sewage outfalls in 2009 and the location was shown in Table 1. The water samples were only used for pH

Fig. 2. a –Pictures of the lower reaches of the Loushan River (cited from http://house.qingdaonews.com/news/2015-04/30/content_11035286_all.htm); b–sewage outfall of Qingdao Alkali Industry Co. LTD (cited from http://ocean.qingdao.gov.cn/n12479801/index.html); c–sewage outfall of Haipo River Estuary; d–Licun River Estuary.

Table 1. Heavy metal contents in eastern Jiaozhou Bay, China.

Stations	Longtitude/ (˚E)	Latitude/ (˚N)	As/ (mg/kg)	Cd/ (mg/kg)	Cr/ (mg/kg)	Cu/ (mg/kg)	Hg/ (mg/kg)	Pb/ (mg/kg)	Zn/ (mg/kg)
QB030-T-1	120.36	36.21	23.0	1.17	1160	151	0.88	190	531
QB031-T-1	120.36	36.20	6.13	0.46	46.8	59.6	0.76	58.6	78.2
QB033-T-1	120.35	36.16	6.66	0.28	37.8	48.4	0.35	66.2	306
QB035-T-1	120.34	36.29	4.42	0.11	53.5	46.8	0.029	18.9	79.0
QB036-T-1	120.28	36.18	11.9	0.69	59.6	24.2	0.078	32.3	86.2
QB037-T-1	120.28	36.18	5.88	0.29	17.4	18.2	0.40	92.4	90.8
QB038-T-1	120.24	36.20	7.30	0.12	33.6	49.4	0.037	23.6	64.3
Background level			3.6	0.13	31	13.2	0.04	31	69
effect range low (ERL) (Long ER et al., 1995)			8.2	1.2	81	34	0.15	46.7	150
Effect range median (ERM) (Long ER et al., 1995)			70	9.6	370	270	0.71	218	410

analyses and were measured by a pH meter. The surface samples were used for total organic carbon (TOC) and heavy metals (including Cu, Pb, Zn, Cd, Cr, As, Hg) analyses. Inductively Coupled Plasma Mass Spectrometry (ICP-MS) was used for Cu, Pb, Zn, Cd, Cr analysis. Atomic Fluorescence Spectroscopy (AFS) was used for As and Hg. The potassium bicarbonate oxidation-reduction volumetric method was used for the analysis of TOC. All samples were analyzed at the Experiment and Testing Center of Qingdao Institute of Marine Geology, China Geological Survey.

4. Results

4.1. Foraminiferal assemblages

A total of 80 species, belonging to 42 genera of benthic foraminifera were identified in 55 surface samples. All of the foraminifera found in the samples belongs to the benthic species, which is due to the estuarine and coastal environment.

The assemblages are mainly dominated by *Quinqueloculina bellatula* (25.86%), *Ammonia beccarii* (21.49%), *Elphidium magellanicum* (13.48%), and subordinately by *Trochammina inflata*, *Paratrochammina* sp., *Ammobaculites agglutinans* and *Arenoparella asiatica*, etc. *Q.bellatula* is the dominant species in Loushan River Estuary, with a relative abundance of 94.55% at station 5. *A. beccarii* and *E. magellanicum* have an advantage, with the highest relative abundance at station 20, reaching 60.47% in the offshore area in Loushan River Estuary. *A. beccarii*, with the highest relative abundance of 82.69% at station 3 in the inner area, is the dominant species in Licun River Estuary. *E. magellanicum* has the highest relative abundance in the coastal area, reaching 44.96% at station 10. The relative abundance of *A. beccarii* is very high in the inner-most area of Haipo River Estuary, which is highest at station 9, reaching 82.35%; *Atrochammina* sp. reaches 50% at the station 8 out of the estuary; *A. asiatica* enjoys an advantage near the shore, accounting for 37.72% at station 1.

The abundance and diversity of benthic foraminifera are lower than that of the normal shallow sea environment, and a trend of gradual increase from the estuary to the sea and from Loushan River Estuary to Haipo River Estuary (Figs. 3a–c). The abundance of foraminifera (Abundance) in the mouth of the Loushan River, Licun River, and Haipo River are in the range of 288 –3456, 56 –10272, and 136 –5328, with an average of 808, 1955, and 1804, respectively. The number of species (S) in the river mouth of Loushan River, Licun River, and Haipo River is in the range of 2–27, 2–43, 2–37, with an average value of 9.4, 19.6, 17.5, respectively. The Shannon-Wiener index (H) of the Loushan River Estuary, Licun River Estuary, and Haipo River Estuary is in the range of 0.25–2.35, 0.60–2.79, 0.47–2.62, with an average value of 1.11, 1.91, 1.82, respectively.

Other types of benthic organisms, such as Ostracoda, bivalve, and gastropod, are also found in this study, in which only one deformed test is found (Fig. 4), while foraminifera is identified as deformed of 28 species, accounting for 35% of the total species. It shows that foraminifera has a high sensitivity to pollution as a bio-indicator and is superior to other organisms. There are seven types of deformities showed in this study: (1) Abnormal additional chamber (s); (2) distorted chamber arrangement or changed in coiling; (3) reduced chamber size; (4) aberrant chamber shape and size; (5) distorted chamber arrangement or change in coiling and aberrant chamber shape and size; (6) spiroconvex; (7) complexity (Fig. 4).

FAI and FMI are concentrated in Loushan River Estuary, showing a decreasing trend from the estuary to the sea and from Loushan River Estuary to Haipo River Estuary (Figs. 3d, e). FAI (%) of the Loushan River Estuary, Licun River Estuary, Haipo River Estuary is in the range of 1.85–41.82, 0 –14.29, 0 –5.88, with an average of 10.95, 4.4, 2.62, respectively. FMI (%) of Loushan River Estuary, Licun River Estuary, and Haipo River Estuary is in the range of 0–100, 0 –25, 0 –50, with an average of 27.57, 9.87, 14.74, respectively.

4.2. Heavy metal and TOC concentrations

Heavy metals and TOC are obviously enriched in the estuaries (Fig. 5), indicating that rivers play important roles in carrying pollutants into the sea. Cu, Pb, Zn, Cr, Cd, Hg, As, and TOC are concentrated in Loushan River Estuary (Table 1). The values of pH varying from 7.5 to 8.4 in the study area (Fig. 5). The area between Loushan River Estuary and Licun River Estuary shows the highest pH value which may be related to the sewage outfall of Qingdao Alkali Industry Co. LTD.

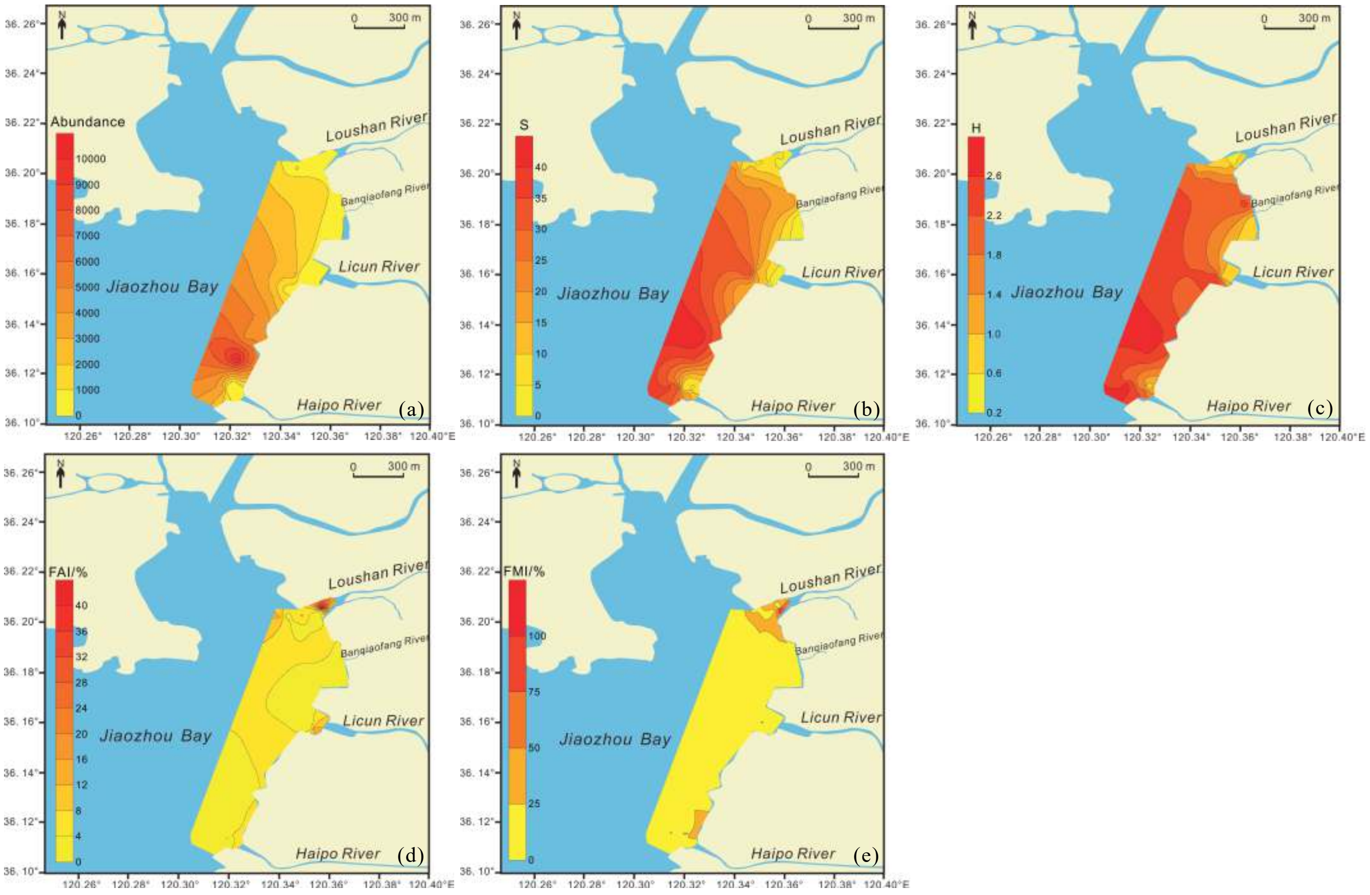

Fig. 3. Map showing the distribution of abundance (a), S (b), H (c), FAI (d), FMI (e) of foraminifera.

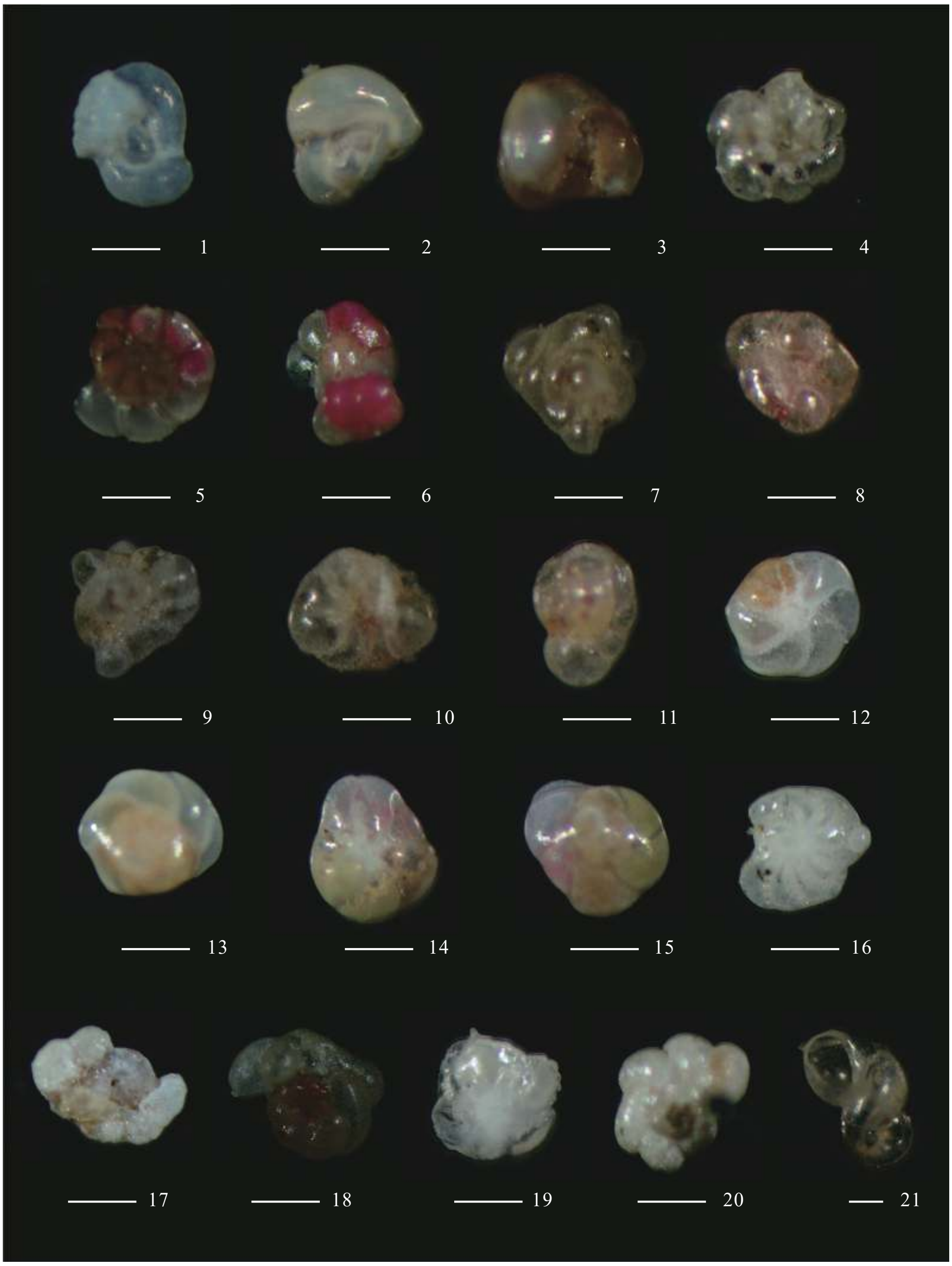

Fig. 4. SEM photomicrographs of benthic foraminifera bearing different morphological abnormalities: 1–3–*Quinqueloculina bellatula*; 4–6–*Ammonia beccarii*; 7–11–*Elphidium magellanicum*; 12–15–*Buccella frigida*; 16–*Elphidium advenum*; 17, 18–*Hanzawa* sp.; 19–*Ammonia compressiuscula*; 20–*Trochammina inflata* (all the scale bar=50 μm); 21–gastropod (as a contrast). 2, 3–distorted chamber arrangement or change in coiling; 5–reduced chamber size; 7–complexity; 1, 4, 6, 10, 12–15, 19–aberrant chamber shape and size; 8, 16–abnormally additional chamber; 9, 17, 18, 20–distorted chamber arrangement or change in coiling and aberrant chamber shape and size; 11–spiroconvex.

5. Discussion

5.1. Sediment transport after the construction of Jiaozhou Bay Bridge

According to the numerical results, the suspended sediment concentration is relatively low in Jiaozhou Bay which is consistent with the low runoff of rivers surrounding the bay (Fig. 6). It is higher in the west of the bay than that in the east. This is mainly related to the lower runoff of rivers (Loushan, Licun, and Haipo Rivers) in the Eastern Jiaozhou Bay. Dagu River on the western coast contributes a lot of fine sediments to the west of the bay. In addition, the suspended sediment concentration decreases rapidly from the bay head towards the bay mouth which indicated that the suspended sediments could hardly be transported out of the bay (Fig. 6). The construction of the Jiaozhou Bay Bridge seriously

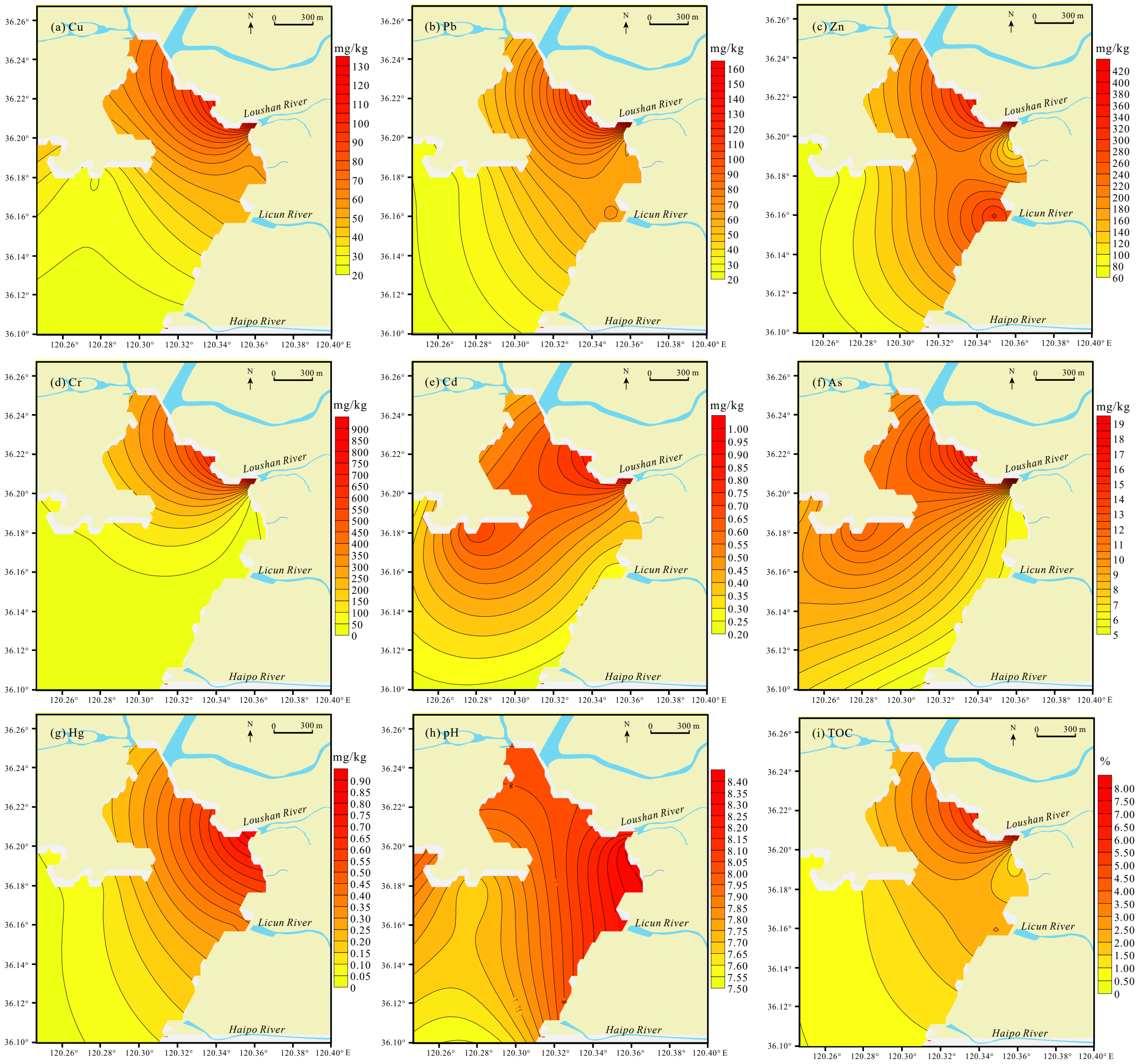

Fig. 5. Map showing the distribution of Cu (a), Pb (b), Zn (c), Cr (d), Cd (e), As (f), Hg (g), pH (h), and TOC (i) of the Jiaozhou Bay, China.

affected the hydrodynamic condition of the bay (Qiao LL et al., 2019). Residual current is supposed to be much more important in long-period mass transportation (Li P et al., 2014). The reduced number and strength of residual current circles indicate that water exchange capacity has been weakened due to the piers' blockage (Fig. 7; Li P et al., 2014; Qiao LL et al., 2019). Especially, the strength of residual current circles in the bay mouth has been greatly reduced which would suppress the capacity of sediments to be diffused into the ocean. As a result, the pollutants from the rivers would be deposited along with the sediment and are mainly trapped in the bay which would ultimately lead to the deterioration of the ecological environment. Above all, in the northeast of the bay, the loss of the residual current circles after the completion of the cross-bay bridge would reduce the capacity of pollutants exchange (Li P et al., 2014; Chen YY et al., 2019). Therefore, the ecological system of the northeast of the bay needs to be focused on in the future.

5.2. Assessing the pollution degree in Eastern Jiaozhou Bay

Compared with background concentrations of Cu, Pb, Zn, Cr, Cd, Hg, and As in soils of Qingdao City, the heavy metal concentration in each station has exceeded the background concentrations in Eastern Jiaozhou Bay which are mainly related to human impact. Especially, Cr and Hg contents of Loushan River Estuary exceed the background values by more than 10 times (Table 1). The heavy metals concentrations in the study area are compared with the

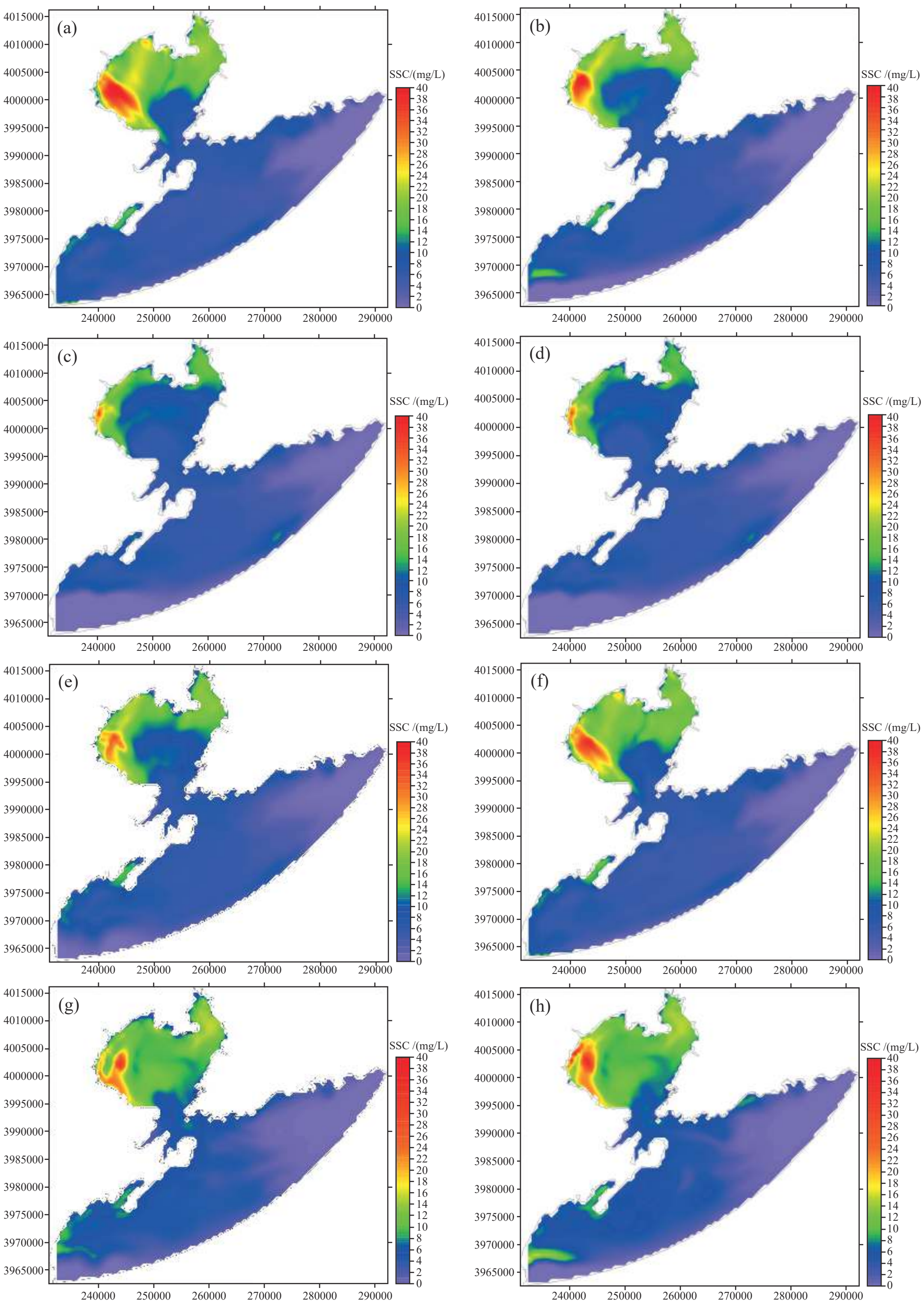

Fig. 6. Distribution of suspended sediment concentrations in Jiaozhou Bay during different periods. a–beginning of the flood tide for spring tides; b–maximum flood tide for spring tides; c–high tide for spring tides; d–beginning of the ebb tide for spring tides; e–maximum ebb tide for spring tides; f–low tide for spring tides; g–maximum flood tide for neap tides; h–maximum ebb tide for neap tides (cited from Zhang Y et al., 2019).

adverse biological effect values. The results show that the trace elements (except Cd) are exceeding the effect range low (ERL). Furthermore, Cr, Hg and Zn in Loushan River Estuary have exceeded effect range median (ERM) which indicates that the biological environment in Loushan River Estuary was bearing risks.

5.3. Foraminifera distribution responses to environmental factors

Q. bellatula, *A. beccarii,* and *E. magellanicum* are most abundant in this study. They are widely distributed in the worldwide intertidal zone (Woodroffe S et al., 2005). *A.*

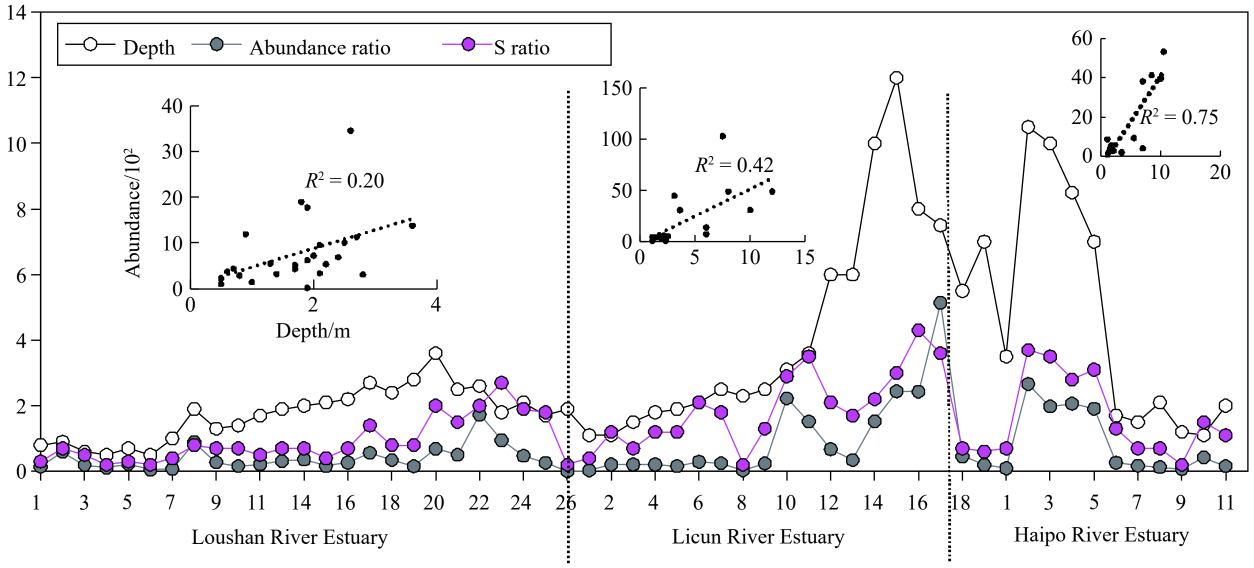

Fig. 7. Distribution of depth, abundance, and species of Loushan River Estuary, Licun River Estuary, and Haipo River Estuary. The three scatter plots above represent the correlation of depth and abundance of the three estuaries.

beccarri is considered to be the euryhaline shallow-water species in the world, which can survive in the salinity of 1 ‰ –40 ‰ (Debenay JP et al., 1998) and is common in estuaries, lagoons, and other land-continuation phases within the water depth of 20 m. *Quinqueloculina* and *Elphidium* usually live in an environment of salinity from 10‰ to 33‰ and pH larger than 7 (Bradshaw JS, 1957; Orabi OH et al., 2017). Due to the low runoff of the three rivers, the salinity of the study area varied little which was close to the normal marine environment in 2013 (Gong XB et al., 2015). According to the distribution of environmental factors (Fig. 5), the pH of eastern Jiaozhou Bay varied from 7.5 to 8.4. The salinity and pH in the study area are suitable for these species to survive. *Paratrochammina* sp., *Ammobaculites agglutinans,* and *Arenoparella asiatica* of the agglutinated species are mainly distributed in the near-shore area of Haipo River Estuary, which is supposed to be related to coarse-grained sediments (Wang PX et al., 1986).

Environmental factors such as salinity, dissolved oxygen, water depth will affect the foraminifera distribution (Boltovskoy E et al., 1991; Bergamin L et al., 2020). Salinity and water depth were considered to be the main factors affecting the foraminifera distribution through the castration analysis of the surface samples in the Pearl River Estuary (Wu J et al., 2013). Based on the above discussion, the effect of salinity can be basically eliminated. In this study, the authors analyzed the relationship between foraminiferal abundance (species) and water depth. The results show that from Loushan River Estuary to Haipo River Estuary, the influence of the water depth is gradually increased (Fig. 7). There is little correlation between the abundance and water depth in the Loushan River Estuary. As mentioned above, the study area is seriously polluted according to the contamination. As a consequence, heavy metal and organic pollution are deemed to be the main factor affecting the distribution of foraminifera in the Loushan River Estuary. The distribution of foraminifera is more influenced by the water depth than heavy metal or organic pollution in the coastal area near Haipo River Estuary. However, water depth is not an independent factor controlling the distribution of the organism but is the comprehensive manifestation of many factors. There is a residual current circle out of the Haipo River Estuary after the completion of the Jiaozhou Bay Bridge (Li P et al., 2014). The residual current circle will enhance the water exchange which is beneficial to foraminifera growth. Therefore, the abundance of benthic foraminifera is the highest out of the Haipo River Estuary.

5.4. Foraminifera distribution responses to heavy metal and organic pollution

The previous study has revealed that heavy metals do not support the survival of any species (Alve E and Olsgard F, 1999). The sensitive species would be reduced or even destroyed under the high concentrations of toxic metals, while the resistant species will rapidly multiply and take advantage which would result in low population density and diversity (Frontalini F and Coccioni R, 2008, 2011; Nagendra R and Reddy AN, 2019). Organic pollution may lead to oxygen depletion in the sediment and would further affect the benthic communities (Kaithwar A et al., 2020). This can be by which benthic foraminiferal assemblages were mainly affected by TOC, As, and Cd (El Kateb A et al., 2020). The hierarchically clustered heat map is a visualization method for analyzing the distribution of biological data and can be used to merge small clusters layer by layer from the bottom up (Fernandez NF et al., 2017). According to the heat map and hierarchical clustering of the relative abundance of foraminifera, the study area can be divided into two clusters (Cluster A and Cluster B) (Fig. 8). *Q.bellatula* is the dominant species in Cluster A (samples from stations 1 to 18 in Loushan River Estuary). *A.beccarii* and *E. magellanicum* are the dominant species in

Cluster B (samples from Licun River Estuary, Haipo River Estuary, and coastal area of eastern Jiaozhou Bay). Foraminiferal biotopes in the area of Loushan River Estuary (Cluster A) are obviously different from those of the other areas (Fig. 8). The foraminiferal abundance and diversity of Cluster A are much lower than those of Cluster B (Table 2). As mentioned above, the concentrations of heavy metals and TOC in Loushan River Estuary area were quite higher than those of the Licun River Estuary and Haipo River Estuary areas (Fig. 5). Therefore, the foraminiferal biotopes of Cluster A are deemed to be severely affected by heavy metals and TOC. As discussed above, the Jiaozhou Bay Bridge would reduce the capacity of water exchange in the northeastern part of the bay which would make the heavy metals and TOC contamination be concentrated in the northeastern part of the bay. Although samples from station 19 to 26 of Loushan River Estuary is located in the northeastern part of the bridge, the foraminiferal biotopes of these samples are close to those of the Haipo and Licun Rivers areas which may be related to the influence of the residual current circle (Li P et al., 2014). This can be used to interpret why the foraminiferal biotopes of Cluster A are different from those of the other areas. In consequence, the authors suggested that the change of the residual currents makes the difference between Cluster A and Cluster B more obvious.

In this study, foraminiferal biotopes and heavy metal distribution are possibly influenced by the changed hydrodynamic condition which is particularly affected by the residual current circles. The ecological environment of the estuary from the northeastern part of the bay has been seriously damaged due to both industrial and domestic waste discharge and the construction of the bridge. The previous study has shown that bacterial community structure is heavily affected by heavy metal contamination in the lower reaches of the Licun and Haipo Rivers (Yao X et al., 2016). This research indicates that samples from the outer part of the estuaries and coastal area are considered to be less influenced by heavy metal pollution due to the relatively open hydrodynamic environment after the completion of the bridge.

5.5. Relationship between deformities and heavy metals

Abnormal salinity value (Brasier MD,1973), low nutrient levels (Murray JW, 1963), rapidly changing environmental conditions (Boltovskoy E et al., 1991), and different types of pollution (Frontalini F and Coccioni R, 2011) would generate

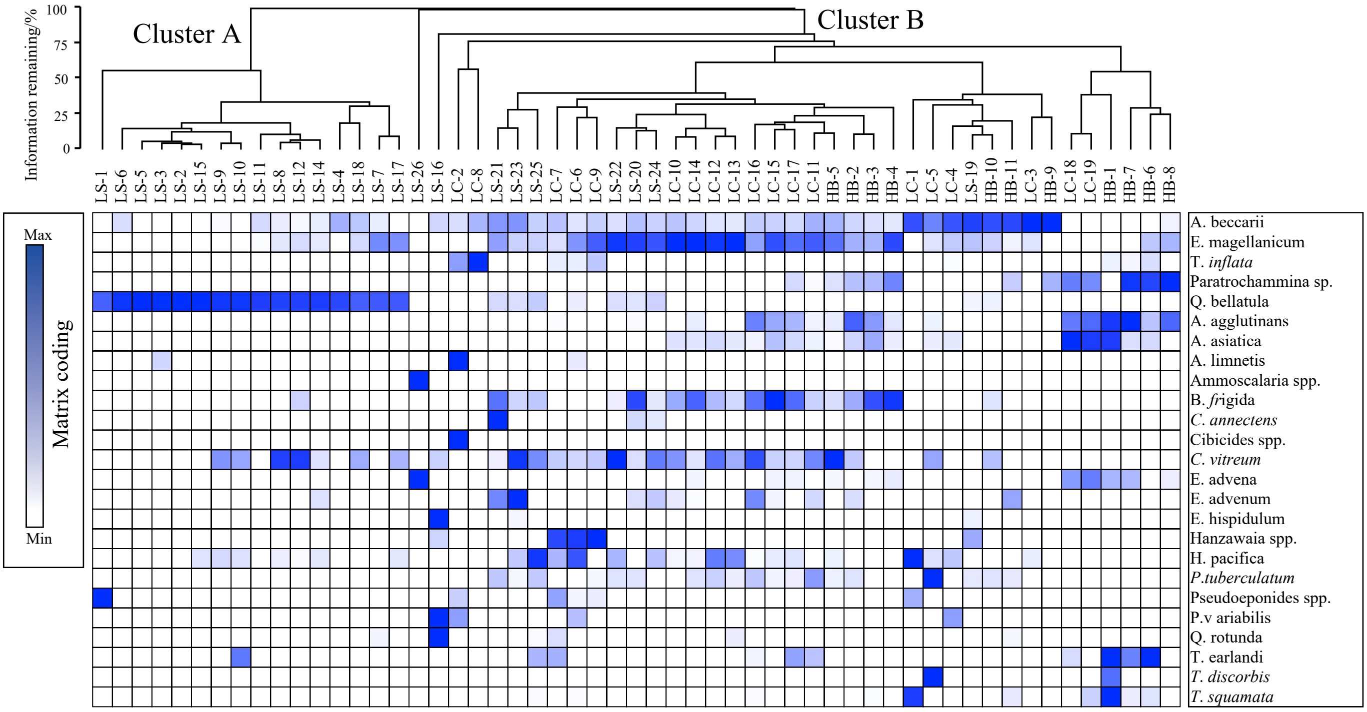

Fig. 8. Heat map of the relative abundance of each species (rows) in each sample (columns). Dendrogram classification of sampling sites produced by Hierarchical clustering method using Euclidean distance.

Table 2. Significant characteristics of the inner place of the three estuaries.

		Q. Bellatula /%	*A. Beccarii* /%	*E. Magellanicum* /%	Abundance	S	H	FMI/%	FAI/%
Cluster A	Min	58.33	0	0	104	2	0.25	14.29	4.65
	Max	94.55	29.63	22.22	1776	14	1.3	100	41.82
	Mean	72.05	10.40	5.24	580.71	5.88	0.79	33.5	12.81
Cluster B	Min	0	0	0	16	2	0.47	0	0
	Max	24.62	82.69	44.96	10272	43	2.79	50	14.29
	Mean	5.20	26.41	17.17	1750.53	17.61	1.86	12.38	3.63

deformed tests. The tests begin to dissolve when pH is below 7.8 (Alve E and Nagy J, 1990), and begin to regenerate when the pH returns to normal, which can also cause deformities (Le Cadre V et al., 2003). As discussed above, pH and salinity remained stable in the study area. Thus, the deformities are mainly related to the pollution in this study. The morphological deformities found in the estuaries and coastal zone mainly include pygmies, sinistral tests, the shape of chambers, distorted chamber arrangement, and changed in coiling which has been regarded to be caused by heavy metal pollution in previous studies (Yanko V et al., 1994; Samir AM and El-Din AB, 2001; Le Cadre V and Debenay JP, 2006; Linshy VN et al., 2013; El-Kahawy R et al., 2018). However, the mechanism of the effects of heavy metals on benthic foraminifera is still unclear. Currently, the main understanding is that the cytoskeleton determines the shape of the body, and heavy metals can penetrate the cytoskeleton together with food, which will lead to cell malformation (Samir AM and El-Din AB, 2001; Le Cadre V and Debenay JP, 2006). This understanding could be confirmed by the fact that the heavy metal contents in the deformed tests were much higher than those in the normal tests (El-Kahawy R et al., 2018). The previous studies suggested that Cu was better absorbed in foraminiferal tests than Cr, Zn, and Pb (Samir AM and El-Din AB, 2001; Frontalini F et al., 2009). Hg is a toxic metal and has shown a strong ecotoxicological effect on foraminifera according to Frontalini F et al. (2017). Loushan River Estuary was the most seriously polluted area as well as had the highest FMI and FAI. Since the concentrations of Cu, Pb, Zn, Cr, Hg, As exceed ERL and Pb, Cr, Hg exceed ERM in Loushan River Estuary, the excessive heavy metal concentrations are supposed to be the main factor influencing the morphology of benthic foraminifera. Although deformities were often caused by a series of contamination in the modern environment (Alve E, 1995), there were still a small number (1%–2%) of deformed tests in the non-polluted area (Stouff V et al., 1999; Morvan J et al., 2004). However, FAI of all the samples in Loushan River Estuary are nearly above 20%, which is often considered to be affected by pollutants (Yanko V et al., 1998; Coccioni R et al., 2009; Martins V et al., 2010).

The relative abundance of deformities also shows an obvious difference between Cluster A and Cluster B (Fig. 9). FAI and FMI in Cluster B are much lower than those of Cluster A which reveals the foraminifera can quickly and obviously respond to the contamination. The main species of deformities in Cluster A is *Q. bellatula*, while *A. beccarii* and *E. magellanicum* are the dominant species of deformities in Cluster B. *Q. bellatula* was most likely to generate deformities compared with the other species which could be regarded as the tolerant species. Therefore, *Q. bellatula* is supposed to have strong resistance to most trace elements and

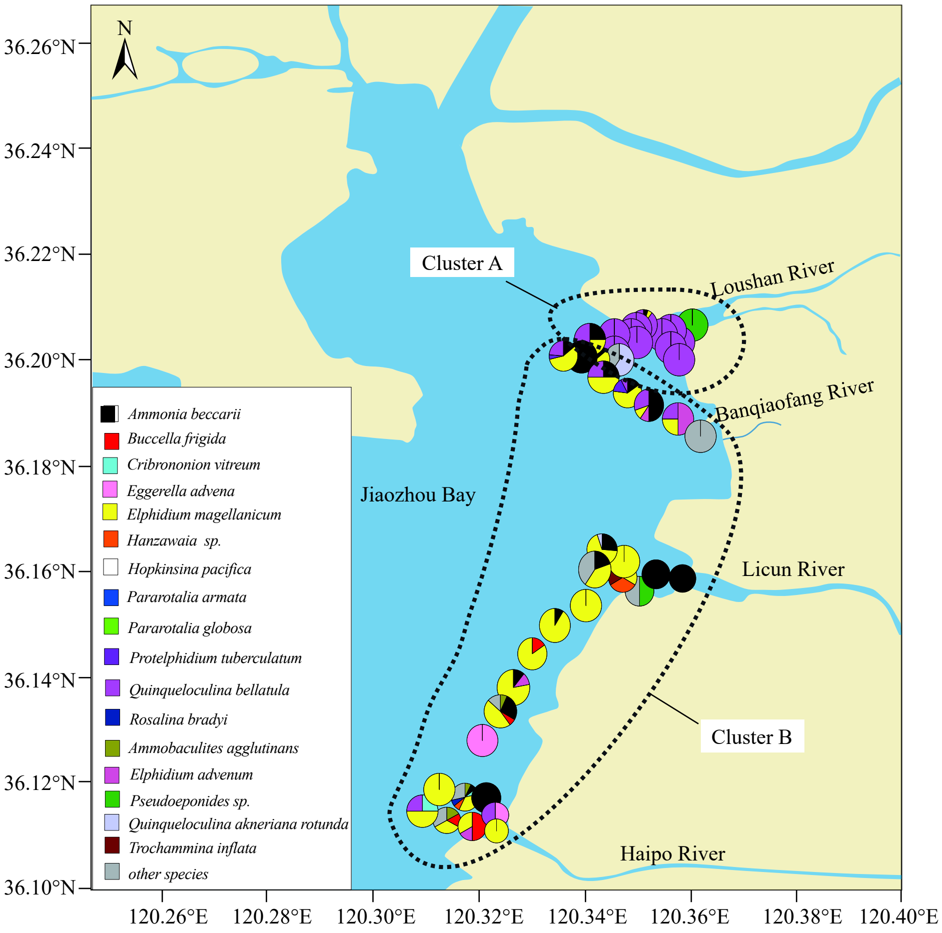

Fig. 9. Distribution of the relative abundance of the deformities.

organic matters in this study. *Ammonia* and *Elphidium* have been recognized as the tolerant species in previous studies (Zhu XD, 1994; Li T et al., 2014, 2015; Frontalini F et al., 2009, 2017). In this study, *A. beccarri* and *E. magellanicum* also show resistance to heavy metal contamination in Licun and Haipo River Estuaries and coastal areas of the eastern Jiaozhou Bay.

6. Conclusion

This study revealed that the three estuaries and the adjacent coastal zone of Jiaozhou Bay were seriously contaminated and faced with great ecological risks. Cu, Pb, Cr, Hg, Zn, As had low to median ecological risks in the study area and were mostly concentrated in Loushan River Estuary. The construction of the Jiaozhou Bay Bridge weakened the capacity of water exchange of the bay, resulting in pollutants concentrated in the northeast, which would further threaten the growth and reproduction of benthic foraminifera. Affected by the serious pollution, the abundance and species of the benthic foraminifera were lowest in Loushan River Estuary. Moreover, the distribution of total individuals and deformities in Loushan River Estuary were different from the other two estuaries and the adjacent coastal zone which was supposed to be influenced by the changing tidal current and residual current due to the bridge blockage. In this study, the authors suggested the construction of the Jiaozhou Bay Bridge aggravated the destruction of the ecological environment of the estuary in the northeastern bay. A series of suggestions are given according to this research: (1) The capacity of sewage treatment at the outlet of the northeastern part of the Jiaozhou Bay should be improved; (2) it should be paying attention to the ecological environment of estuaries in northeastern Jiaozhou Bay; (3) benthic foraminifers are sensitive to environmental pollution and quickly respond to environmental changs, hence need to be used in evaluating ecological quality in the future.

CRediT authorship contribution statement

Jing-yi Cong and Hai-yan Long conceived the presented idea and prepared the manuscript. Jing-yi Cong drew all the figures. Yong Zhang and Nan Wang supervised the findings of this work. All authors discussed the results and contributed to the final manuscript.

Declaration of competing interest

The authors declare no conflict of interest.

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