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Integrated toxicity evaluation of metals in sediments of Jiaozhou Bay (China): Based on biomarkers responses in clam *Ruditapes philippinarum* exposed to sediment extracts

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ABSTRACT

To evaluate the integrated toxicity of metals in sediments of Jiaozhou Bay, we exposed clam (*Ruditapes philippinarum*) to sediments extracts obtained using of sediment extraction with deionised water adjusted to pH 4 which simulated the weak acidity in the digestive juice of clams and tested the selected biomarkers responses in clams for exposure over 15 days. At the same time, the contents of metals in sediments were assessed with method of the mean sediment quality guideline quotient (SQG-Q). The integrated biomarker response version 2 (IBR_{v2}) was used to assess the integrated toxicity induced by metals in sediment extracts based on biomarkers response in clams: the results demonstrated that site S7 located in the mouth of Nanxin'an River show higher IBR_{v2} values compared to the other sites. The IBR_{v2} values exhibited the good consistency with SQG-Q values.

1. Introduction

Significant quantities of metals from anthropogenic activities (river effluence, port development, shipbuilding, and mariculture, etc.) have been discharged into the marine environment in recent years, and are mainly deposited in sediments through the adsorption of suspended particulate matter (Yuan et al., 2012; Chaudhary et al., 2013). Metals accumulated in sediments can pose a threat to marine organism health and the equilibrium of a marine ecosystem once their contents exceed the corresponding threshold (Kalantzi et al., 2013). A variety of studies have demonstrated that sediments are a more effective reservoir of metals, as well as other pollutions, compared with the water column as they can record long-term changes in the environment, and thus metal contents in sediments are often used to evaluate the impact of anthropogenic activities on the estuarine and offshore environment (Long et al., 2006; Viguri et al., 2007; Pazi, 2011). However, the total amount of metals cannot characterise the toxicological and environmental hazards of sediments, because only those bioavailable metals have potential bio-toxicity (Luoma, 1989; Di Toro et al., 1990; Yu et al., 2012). In addition, the toxic effects of metals in the form of combined pollution on organisms cannot be simply obtained by chemical analysis (Tsangaris et al., 2011a, 2011b). Therefore, the assessment of environmental quality based on biological effects caused by pollution stress has attracted increasing research attention (Lehtonen et al., 2006; Lam, 2009).

A biomarker is signal indicator of abnormal changes at molecular and cellular levels when organisms suffer from external environmental stress (Depledge et al., 1995). A biomarker is sensitive to pollutants and also provided with an "early warning" function, and thus it has been considered as an effective tool for monitoring and assessing biological effects of pollutants (Nigro et al., 2006; Hylland et al., 2016; Bouhallaoui et al., 2017). Furthermore, the biomarker assay was conducted as an important indicator of metals pollution in marine ecological health assessment by the International Council for the Exploration of the Sea (ICES) in 2007 (Galloway et al., 2007). According to the coexistence of various pollutants in the marine environment, the integrated biomarker response version 2 (IBR_{v2}) was proposed by Sanchez et al. (2013) in the view of integrated biomarker response (IBR) proposed by Beliaeff and Burgeot (2002). This approach aims mainly to reflect the extent and severity of marine pollution from the simultaneous responses of multiple biomarkers to spot bioindicators, and meets the need for a comprehensive assessment of environmental

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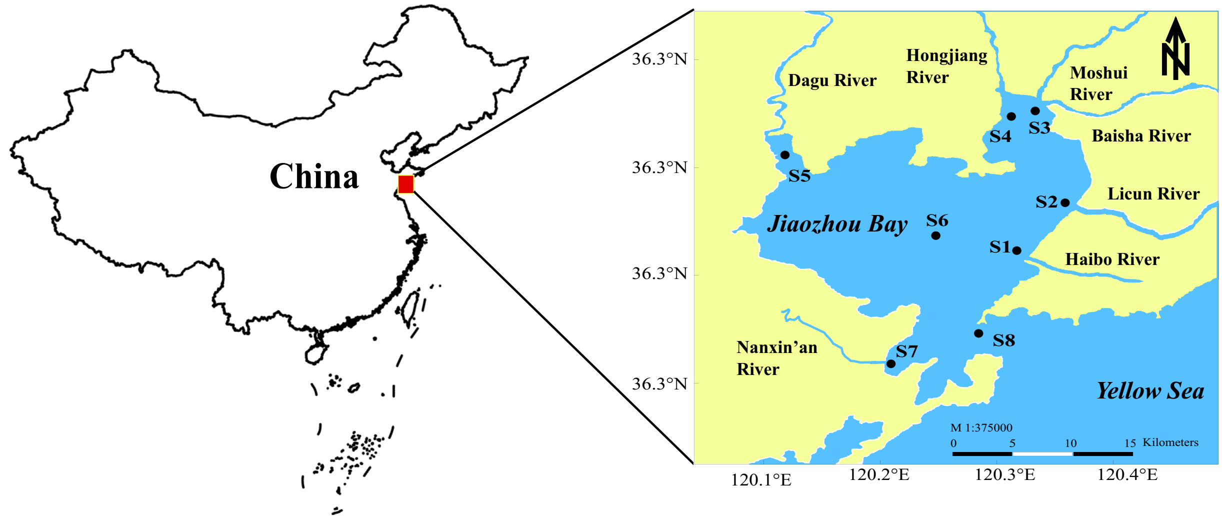

Fig. 1. Map of the sediment sampling sites in Jiaozhou Bay.

quality in compound polluted maritime space. In nearly a decade, the IBR_{v2} index has been applied to evaluate the combined pollution stress in marine environment, and the differences in extent of pollution between sites (Sanchez et al., 2013; Marques et al., 2016; Raphael et al., 2016). In particular, large size, wide distribution, stable life, and strong enrichment ability, as well as other characteristics of bivalves make them have been widely used as bioindicators in various marine organisms (Viarengo and Canesi, 1991; Moschino et al., 2012). *Ruditapes philippinarum*, an important commercially mollusc, is widely distributed in coastal waters of China and is also the main bioindicator used in the Mussel Watch Programme (China), and thus it is suitable to be used as a sentinel organism in this study (Meng et al., 2011).

Nevertheless, there is a crucial limiting factor that the availability of bioindicators for studying area pollution assessment using biomarkers (Bolognesi et al., 2004; Chariton et al., 2010). The target bioindicators cannot always be collected in some sites because the density of marine organisms in different areas varies, which cannot meet the requirements for biomarker determination. An effective method of solving the aforementioned problem is to expose the previously prepared bioindicators with the same physiological condition to sediments collected from study area under laboratory controlled condition. At the same time, this method can also eliminate the differences in non-pollution factors (pH, salinity, temperature, etc.) between diverse sites (Luedeking and Koehler, 2004; Holmstrup et al., 2010; Dafforn et al., 2012). Currently, the exposure culture way in most of previous studies is that place bioindicators into a tank containing contaminated sediment and clean seawater (De Domenico et al., 2011; Kerambrun et al., 2012; De Domenico et al., 2013; Edge et al., 2014). A promising exposure method is to expose the bioindicators to sediment extracts (Amiard et al., 2007; Rigaud et al., 2012). The sediment extracts were prepared using a chemical extractant which simulated the digestive absorption of particulate substances and was adjusted to physiological pH. Moreover, metals in sediments can be more easily absorbed by bioindicators compared with the original exposure method because they are transferred into the liquid phase after treatment by the appropriate extractant: this is helpful when enhancing the response of biomarkers to different pollution sites (Rigaud et al., 2012). Amiard et al. (2007) reported that metals accumulated in organisms were significantly correlated with the desorbed metals from sediments treated with sediment extractant with buffers adjusted to pH 4 for *Mytilus edulis* or pH 6 for *Crassostrea gigas*.

Jiaozhou Bay, located in the southern Shandong Peninsula and encircled by Qingdao City (China), is a typical semi-enclosed shallow bay with a surface area of about 390 km^2, and forms a diversified complex ecosystem in combination with the surrounding terrestrial environmental (Fu et al., 2007; Liu et al., 2008). Jiaozhou Bay is also an important economic zone for mariculture, the salt-production industry, and tourism in the sea of northern China (Gao et al., 2003). Nevertheless, with booming urbanisation and industrialisation around the coast of Jiaozhou Bay, a large number of pollutants such as metals were discharged into bay via several seasonal rivers (Shi et al., 2011). According to the statistics of the official agency, there were > 22.6 tons of metals and metalloid discharged into Jiaozhou Bay from streams in 2013 (Oceanic and Fishery Administration of Qingdao (OFAQ), 2015). As for distribution, sources, and ecological risk assessment of metals in sediments, Jiaozhou Bay has been investigated by dozens of researchers considering its important economic status and severely polluted status in the last 30 years (Dai et al., 2007a, 2007b; Deng et al., 2010; Zhao et al., 2015; Lin et al., 2016; Xu et al., 2016). However, the study on the integrated toxicity of metals in sediments based on multi-biomarker responses has not yet been reported. In the current study, the clams *R. philippinarum* were exposed to metals extracts obtained from sediments of Jiaozhou Bay for 15 days, and the response characteristics of selected biomarkers in clam digestive glands were also investigated, including metallothioneins (MTs), glutathione *S*-transferase (GST), 7-ethoxyresorufin *O*-deethylase (EROD), and malondialdehyde (MDA), as well as lysosomal membrane stability (LMS) and micronucleus frequency (MNF) in haemocytes. Eventually, the integrated toxicity of metals in the sediments of Jiaozhou Bay was evaluated using of calculated IBR_{v2} index based on biomarker responses.

2. Material and methods

2.1. Study area and sample collection

Jiaozhou Bay lies 35° 58′ to 36° 18′ N and 120° 23′ to 120° 23′ E and is part of the Yellow Sea. The coast of Jiaozhou Bay is dominated by silt and rocky coasts. There are various types of sediments in the study area, and the sedimentary environment exhibits strong reducibility (Dai et al., 2007a, 2007b). The tidal current in Jiaozhou Bay shows the characteristics of reciprocating flow and standing waves; the tide is a regular semidiurnal tide and its Significant Wave Height (SWH) is < 5 m on the whole (Chen et al., 2012). Several small seasonal rivers with various water, and sediment, loads discharge into the Jiaozhou Bay, namely, Haibo, Licun, Baisha, Moshui, Hongjiang, Dagu, and Nanxin'an Rivers (Fig. 1).

In the current study, site selection was mainly based on a previous study of metals contamination and feature of major anthropogenic