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Relating metals with major cations in oyster *Crassostrea hongkongensis*: A novel approach to calibrate metals against salinity

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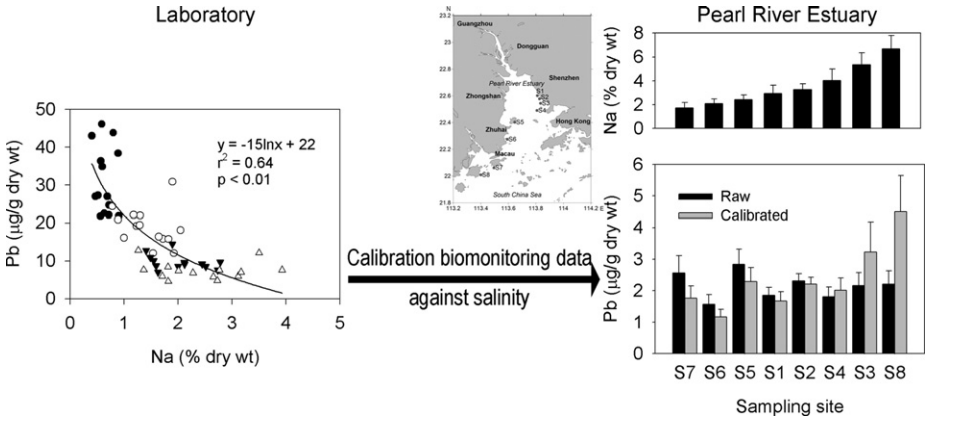
HIGHLIGHTS

- A novel method was developed to calibrate the biomonitoring data against salinity.
- Negative or positive correlations between tissue concentrations of trace elements and Na were observed.
- In contaminated estuary, salinity influenced the bioaccumulation of trace elements.
- Quantitative relationships between salinity and tissue metals could facilitate the interpretation of biomonitoring data.

GRAPHICAL ABSTRACT



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ABSTRACT

Despite salinity has been well documented for its significant effects on the bioaccumulation of many trace elements in biomonitors, no calibration method has been proposed to reduce such influences. For the first time, the present study established a novel method to calibrate biomonitoring data against salinity. Relationships between trace element concentration in oyster *Crassostrea hongkongensis* and the biological proxy for salinity were quantified based on laboratory exposure experiments. The method was then verified by the biomonitoring data of Pearl River Estuary (PRE). Tissue concentrations of trace elements (Cu, Zn, Ag, Cd, Pb, Cr, As, Se, and Ni) and major cations (Na, Mg, K, and Ca) in oysters exposed at 4 salinities (5, 12, 20, and 28 psu) and low concentrations for 6 weeks were measured to establish such quantitative relationships. Tissue Na, Mg, and K could be the proxy for salinity, while Na was the best one. Negative correlations between tissue concentrations of trace elements and Na after exposure were observed for metal cations such as Cu, Zn, Ag, Cd, and Pb, while tissue As, Se, and Ni were positively correlated with Na. In PRE, salinity significantly influenced the bioaccumulation of trace elements even under the multifactor-affected field conditions. The calibration method applied to the biomonitoring of PRE was verified to be feasible, and effectively reduced the influences of salinity. Therefore, calibration against salinity could facilitate the interpretation, comparability, and analysis of biomonitoring data.

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1. Introduction

Rapid economic growth has caused increasing pressure of metal contamination in aquatic ecosystems, especially the estuarine and coastal areas, which usually receive direct discharges from various sources (Kumar et al., 2015; Pan and Wang, 2012; Wang et al., 2014). Due to the difficulties of accurately measuring trace metal concentration in seawater, biomonitoring is much more preferable to integratively reflect the water contamination level and its comprehensive effects on organisms (Phillips and Rainbow, 1993). Bivalves, such as oysters, mussels, and clams, are often employed in biomonitoring of aquatic environments because of their wide geographical distribution, abundance, sedentary, and ability of strong tolerating and accumulating various environmental contaminants (Zhou et al., 2008). The biomonitoring data, usually the tissue concentrations of pollutants, however, are influenced by many factors, such as salinity, which vary significantly from site to site. Therefore, data from different sampling sites or even different times may not be comparable and need to be corrected.

Oyster *Crassostrea hongkongensis* is one of the common biomonitors and normally found in estuaries in Southern China, where it suffers a wide range of salinity change (from almost freshwater to 100% seawater). Salinity can greatly affect the bioaccumulation of trace elements by changing the water chemistry or organism physiology. On the one hand, more chloride ions at higher salinity can affect the speciation of most divalent metals due to increasing complexation, thus reducing free metal ions, which are believed to be the most bioavailable form (Wright, 1995). Meanwhile, more cations (mainly Na, Mg, Ca, and K) lead to enhanced competition with metals for biological uptake sites, because some cations and metal ions share the same uptake pathways, such as Ca and Cd (Hollis et al., 2000; Qiu et al., 2005). On the other hand, salinity can cause physiological changes of animals, such as apparent water permeability, in which cases a totally reverse relationship between bioaccumulation of trace elements and salinity has been observed (Rainbow and Black, 2005, 2002). Little is known, however, about the combined effects of water chemistry and animal physiology on bioaccumulation of trace elements, as well as the quantitative relationships between metal burden in animals and external salinity. In addition, few literatures available attempted to calibrate aquatic biomonitoring data by salinity for better interpretation, although the significant influences of salinity on metal bioaccumulation have long been well known.

Bivalves, which are frequently employed as biomonitors, can form microenvironments different from the ambient waters when they close shells (Gosling, 2015). For example, bivalves deploy the strategy of shell closure to protect themselves from the drastic changes of salinity in estuaries (Gosling, 2015; Shumway, 1977), and may be exposed to the air at low tides. Under such circumstances, the measured salinity of the ambient waters cannot represent the microenvironments that bivalves actually face. In addition, salinity is often not collected simultaneously when sampling organisms. Therefore, a biological parameter that can link the variation of salinity and change of bivalve physiology is required to substitute for salinity. This is especially important for estuarine bivalves facing great variations of salinity over tidal cycles. The major impact of salinity change on aquatic organisms involved the disturbance of osmotic pressure balance between internal and external environments, causing osmoregulation of living bodies. Considering that all bivalves are osmoconformers, osmolytes in organisms, such as inorganic cations (Na^+, Mg^{2+}, K^+, and Ca^{2+}) and anions (Cl^- and SO_4^{2-}), free amino acids (alanine, arginine, and taurine), and other small organic molecules (betaine and homarine), vary with salinity (Gosling, 2015; Pierce, 1970, 1971; Potts, 1954; Shumway, 1977). Therefore, the tissue concentration of major cations in oyster may be a good proxy for salinity. Liu and Wang (2015) quantified the effects of salinity on trace element bioaccumulation by analyzing correlations between trace elements and major cations in mussel tissues based on global field data. They concluded that 12% to 84% of trace element variations were associated with major cations. Besides salinity, however, results of field study are usually interfered by many other factors like metal contamination level, needing laboratory study to provide quantifiable relationships for biomonitoring calibration.

The objectives of this study were therefore to quantify the relationships between tissue concentrations of trace elements and major cations in oyster *C. hongkongensis*, and establish a method of calibrating biomonitoring data against salinity. We further verified the feasibility and effectiveness of the method using biomonitoring data from the Pearl River Estuary (PRE). Tissue concentrations of trace elements (Cu, Zn, Ag, Cd, Pb, Cr, As, Se, and Ni) and major cations (Na, Mg, K, and Ca) in oysters exposed at 4 salinities (5, 12, 20, and 28 psu) and low concentration of trace elements for 6 weeks were measured to establish the quantitative relationships. The established relationships were used to calibrate the effects of salinity on trace element bioaccumulation in the oysters collected from field, to enhance the interpretation, comparability, and analysis of biomonitoring data.

2. Materials and methods

2.1. Oyster collection and acclimation

Oysters *C. hongkongensis* (0.29 ± 0.09 g dry weight) for laboratory exposure were collected from Dafeng Estuary (Guanjing Pier, Beihai, Guangxi, China) in November 2015, where salinity was around 20 psu. Oysters were acclimated under laboratory conditions for 15 days, and then sequentially acclimated to 4 salinities (5, 12, 20, and 28 psu) by 4 psu every 2 days to minimize the stress of an abrupt salinity change. During acclimation and following experiments, seawater was continuously aerated, and oysters were fed with algal powder (ORI-GO, Skretting) daily.

Oysters *C. hongkongensis* of similar sizes were sampled from 8 stations in PRE (Guangdong, China, Fig. 1) in December 2015. Trace

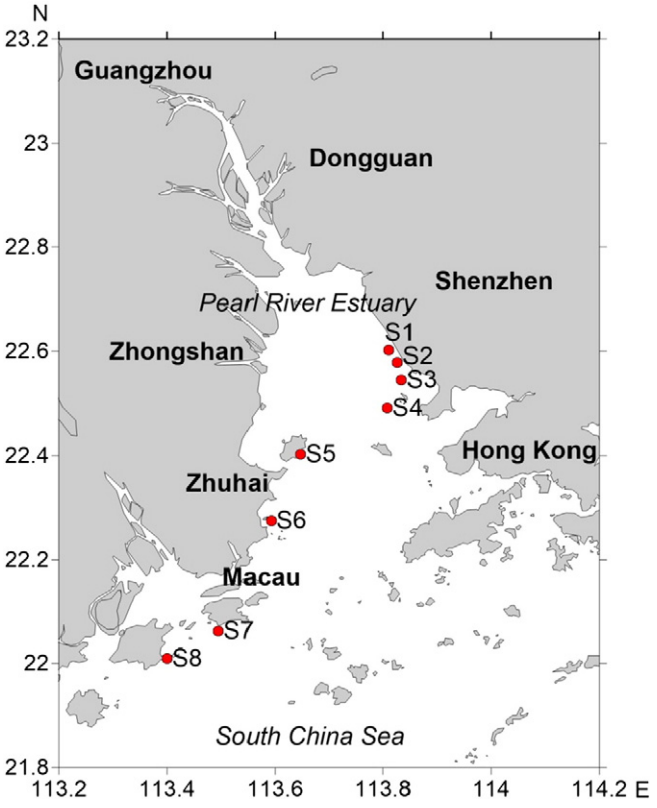

Fig. 1. Sampling stations in Pearl River Estuary.