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Accumulation of Trace Metals in *Anadara granosa* and *Anadara inaequivalvis* from Pattani Bay and the Setiu Wetlands

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Abstract This study was undertaken to assess the levels of trace metals (As, Cd, Cu, Pb, and Zn) in two common species of cockles (*Anadara granosa* and *Anadara inaequivalvis*) from two coastal areas in Thailand (Pattani Bay) and Malaysia (the Setiu Wetlands). A total of 350 cockles were collected in February and September 2014. Trace metals were determined by Inductively Coupled Plasma Mass Spectrometry. We observed that cockles in both areas had a higher accumulation of metals in September. Notably, the biota-sediment accumulation (BSAF) of Cd was highest in both areas. A strong positive correlation of Cd with the length of the cockles at Pattani Bay ($r^2 = 0.597$) and the Setiu Wetlands ($r^2 = 0.675$) was noted. It was suggested that As could be a limiting element (BSAF < 1) of cockles obtained from Pattani Bay. In comparison with the permissible limits set by the Thailand Ministry of Public Health and the Malaysia Food Regulations, mean values of As, Cd, Cu, Pb, and Zn were within acceptable limits, but the maximum values of Cd and Pb exceeded the limits for both areas. Regular monitoring of trace metals in cockles from both areas is suggested for more definitive contamination determination.

Keywords ICPMS · Sediment · Cockle · BSAF

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Metal accumulation in aquatic organisms, especially filter feeders like bivalves, is a serious concern, due to their capacity for bioaccumulation. The abundance of metals may jeopardize human health, due to the consumption of contaminated bivalves (Lias et al. 2013). For example, Cd may be a carcinogen, and Pb can damage blood circulation (Rahman et al. 2012; Arnich et al. 2012). Bivalve mollusks, including mussels, oysters, and clams, have been widely analyzed for toxic metal levels to ensure shellfish food safety. They are also regularly used as biomonitors to assess the environmental health of coastal ecosystems with respect to the bioavailability of toxic metals (Boening 1999; Wang 2002; Bustamante and Miramand 2005; Hamed and Emera 2006; Luoma and Rainbow 2008).

The blood clam (cockle) is a favorite seafood consumed by locals in Thailand and Malaysia. *Anadara granosa* is common in Pattani Bay, while *Anadara inaequivalvis* is common in the Setiu Wetlands. These bivalves are a source of cheap protein in tropical areas, especially in the Indo-Pacific region (Bardach et al. 1972). In Thailand, the national production of *A. granosa* is high, with an average yield of 80 billon kilograms per year (Suwanjarat et al. 2009). Since the cockle is a filter-feeding organism, contamination of the highly productive mudflats with metals leads to accumulation in their whole body tissue (Hossen et al. 2014). Due to concerns over this accumulation and their toxic effects to humans consuming these organisms, monitoring programs for metals in environmental (biotic and abiotic) samples have been widely established and implemented (Sasikumar et al. 2006).

Pattani Bay is located in southeastern Thailand. The coastal zone of the bay is characterized by muddy and sandy sediments with abundant natural invertebrates and widespread clam farming and local fisheries (Swennen et al. 2001). Industries and homes are situated along the shore of Pattani Bay, where their wastes, some of which contain metal contaminants, drain directly into the sea (Suwanjarat et al. 2009). The Setiu Wetlands are also characterized by mudflats and sandy sediments. The two coastal areas face the South China Sea. Few studies have focused on trace metals in cockles (*A. granosa* and *A. inaequivalvis*) in both areas.

The aim of this study was to determine accumulation of trace metals (As, Cd, Cu, Pb, and Zn) in the soft tissue of cockles (*A. granosa* and *A. inaequivalvis*). This soft tissue can directly influence human health through cockle consumption. Study results will be used as a baseline of data for the present state of metal in cockles in the region.

Materials and Methods

Two coastal areas were selected for this study, Pattani Bay (5 stations) and the Setiu Wetlands (5 stations) (Fig. 1). Pattani Bay ($\sim$74 km^2) is located in the southern part of Thailand. The bay is a semi-enclosed estuary, protected on the northeast side by a 12 km long sand spit. It is an important water body in southern Thailand, as it is a major producer and nursery ground for local fisheries (Ruangchuay et al. 2007). Two major rivers, the Pattani and Yamu, drain into the bay. The bay currently faces issues associated with industrial zone expansion, coastal aquaculture, and seaport development (Sowana et al. 2011).

The Setiu Wetlands, part of the Setiu River Basin and the larger Setiu-Chalok-Bari-Merang Basin wetland complex, lies in Terengganu on peninsular Malaysia's east coast. The Setiu Wetlands are a unique habitat in Malaysia consisting of nine interconnected ecosystems: the sea, beach, mudflat, lagoon, estuary, river, islands, coastal forest, and mangrove forest. This study covered about 13 km^2 of the Setiu Wetlands lagoon area.

Cockles were sampled in February and again in September 2014. In total, 200 individual cockles (*A. granosa*) were collected from Pattani Bay (120 individuals in February and 80 individuals in September). Cockles were purchased from the local fishermen, who gathered them from five stations located from the middle to the inner area of the bay. All the fishing stations of cockle are muddy, small area and quite shallow (<1 m) therefore all the samples were collected in the aforementioned areas (information obtained from interviewing the local fishermen by this study). A total of 140 individual samples (*A. inaequivalvis*) were collected from the Setiu Wetlands (90 individuals in February and 60 individuals in September) which were handpicked at low tide from five stations. Cockles were cleaned externally before being placed in a plastic ziplock bag and stored on ice for transportation. At the laboratory, samples were kept in seawater for 24 h to purify the digestive tract. After that, samples were cleaned with Milli-Q water (18 Ω). The total length, weight, and fresh tissue for each cockle were measured before the soft tissue was freeze-dried.

Freeze-dried samples were ground to a fine powder with a mortar and pestle. A constant weight of about 0.05 g dry weight was transferred to a Teflon vessel for the acid digestion process using 1.5 ml HNO_3 (Merck, 65 %, suprapur). The decomposition vessel was sealed tightly and heated in a drying oven at 120°C for 7 h. After cooling to room temperature, the contents were diluted to 10 ml with deionized water. Metal concentrations were determined by Inductive Couple Plasma Mass Spectrometry (ICP-MS) using a Perkin Elmer ELAN 900. Results were expressed in μg/g dry weight. Blanks and standards were digested with each sample set for quality control. Method accuracy was verified with mussel tissue (BCR-668) as the standard reference material.

All calculations and statistics were carried out using MS Excel 2007. All cockles from Pattani Bay were composited for analysis, whereas cockles from the Setiu Wetlands were analyzed by station. The extent of chemical bioaccumulation can be expressed using a bioaccumulation factor (BAF). In sediment-dwelling organisms, the biota-sediment accumulation factor (BSAF) is used for the relative concentration of a substance in the tissues of an organism compared to the concentration of the same substance in the sediment (Baumard et al. 1999; Cortazar et al. 2008; Gobas and Morrison 2000; USEPA and USACE 1998) according to the formula

$$BSAF = C_t/C_s,$$

where C_t is the concentration in benthic organisms and C_s is the concentration in the sediment. BSAF $>$ 1 indicates the organism contains more metals than in sediment, whereas BSAF $<$ 1 is a sign of the limited ability of selected metals to accumulate from the sediment.

In a related paper (unpublished), mean concentrations of metal in the sediment at Pattani Bay in February/September for Cu, Zn, As, Cd, and Pb were 3.3/2.0, 9.3/5.0, 3.4/6.5, 0.003/0.007, and 9.6/7.7 μg/g dry weight, respectively. Mean concentrations of metal in the sediment at the Setiu Wetlands in February/September for Cu, Zn, As, Cd, and Pb were 0.4/0.8, 2.2/1.3, 0.7/2.7, 0.001/0.001, and 1.6/1.0 μg/g dry weight, respectively.

Differences in metal concentrations of cockle tissue and cockle length were evaluated. Correlations between metal concentrations and cockle length were determined using Excel 2007.

To compare with the permissible limits, the concentrations were converted into wet weight. The conversion factor from dry weight to wet weight was divided by 5 (the water content is about 80 %).

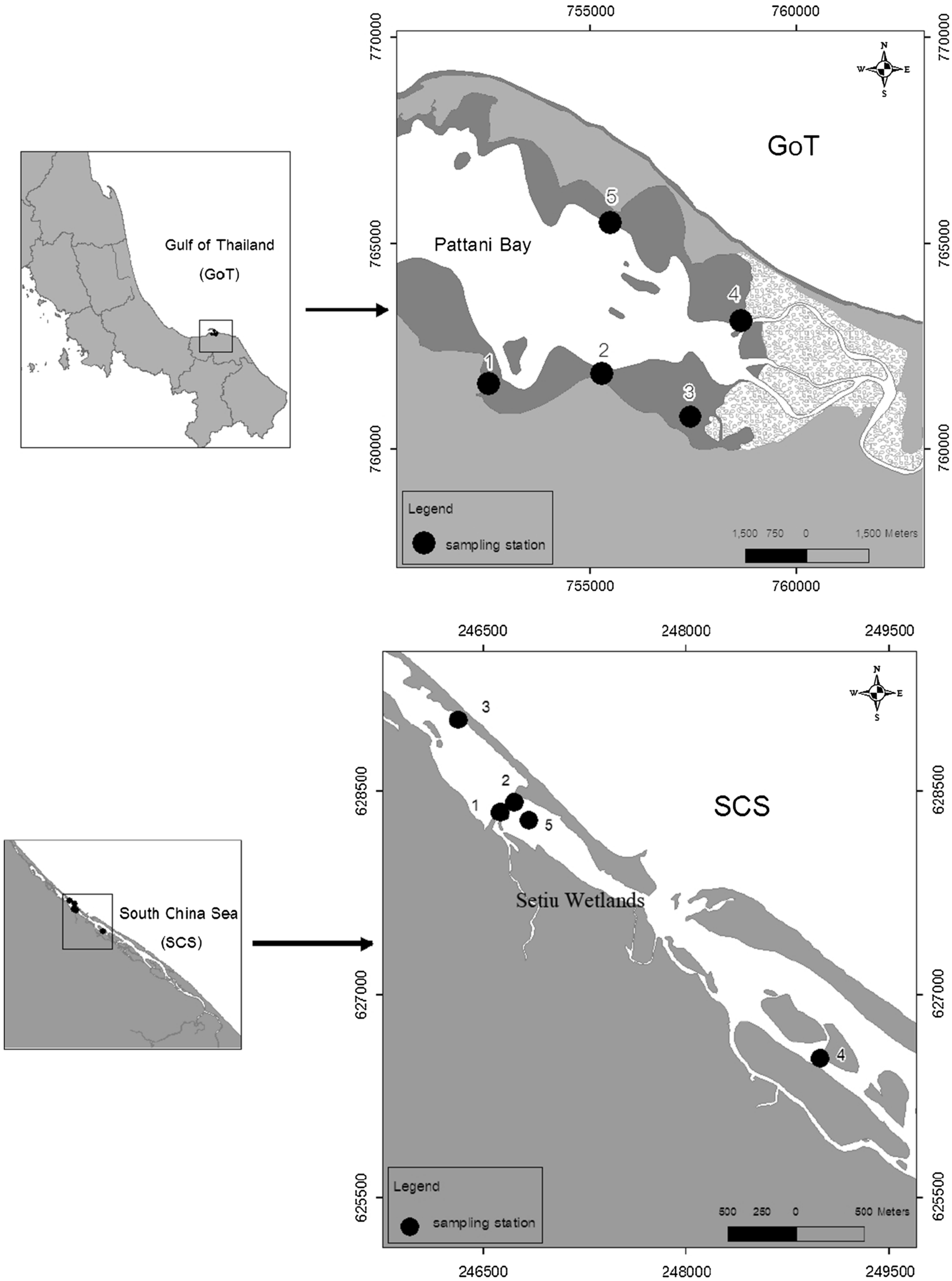

Fig. 1 Locations of sampling area from the mudflats of Pattani Bay, Thailand and the Setiu Wetlands, Malaysia

Results and Discussion

At Pattani Bay, concentrations of Cd (2.83 μg/g dry weight) and Cu (4.21 μg/g dry weight) were highest in February, while Zn (76.70 μg/g dry weight) was highest in September (Table 1). At the Setiu Wetlands, concentrations of As (5.68 μg/g dry weight) and Pb (2.85 μg/g dry weight) were highest in September. Cockles from both areas had a higher accumulation of As and Pb in September. Suwanjarat et al. (2009) reported that, in August (at Pattani Bay), the reproductive activity of *A. granosa* took place mostly in the developing and maturing stages. During these stages, clams required considerable energy consumption, and this led to metal intake and accumulation in tissue (Mathieu and Lubet 1993). Additionally, February is characterized by light rain, but the rain is heavy in September which may bring metal waste from point sources by runoff.

In this study, the BSAF > 1, except for As and Pb in September at Pattani Bay and for Pb in February at the Setiu Wetlands (Table 2). The BSAF value was higher in September for Cu, Pb, and Zn at Pattani Bay and for Cd, Pb, and Zn at the Setiu Wetlands. The BSAF of As was high in February for both areas. The BSAF value of all of the elements at the Setiu Wetlands was higher than that at Pattani Bay. The BSAF was highest for Cd in both areas and highest at the Setiu Wetlands in September, but the concentration of Cd in the sediment of the two areas was very low. A possible explanation may be that Cd accumulated in the cockles for a long time, thus, the source of Cd may come from water and suspended particles. Since the BSAF of As is <1 at Pattani Bay, As could be a limiting element for accumulation from the environment. Since As is a toxic element, it could be harmful to the organism if it is obtained at concentrations beyond those necessary. To deal with this essentiality/toxicity duality, biological systems have developed the ability to recognize a metal and deliver it to the target without allowing the metal to participate in toxic reactions (Luk et al. 2003).

A strong positive correlation of Cd with length was found in September at Pattani Bay (composited data) ($r^2 = 0.597$) and at the Setiu Wetlands ($r^2 = 0.678$). Zn had both a positive correlation ($r^2 = 0.501$ at station 1) and a negative correlation ($r^2 = 0.515$ at station 3) at the Setiu Wetlands in February. Cu and As at Station 1 showed a strong negative correlation, of $r^2 = 0.636$ and 0.500, respectively, at the Setiu Wetlands. For bivalves, it is known that the strength of the net accumulation patterns of metals varies, both between metals and between one metal and a species (Rainbow 2002; Luoma and Rainbow 2008). Differentiation between size and growth greatly affects the distribution of metals in mollusks. Normally, the accumulated metal of bivalves increases with length, which is similar to our result of Cd (both areas) and Zn (station 1).

Table 1 Cockle length, weight and trace metal concentrations at Pattani Bay and the Setiu Wetlands

Location	Length (cm)	Weight (g)	Dry weight (μg/g)				
			As	Cd	Cu	Pb	Zn
February							
Pattani Bay							
Mean ± SD	3.37 ± 0.21	14.03 ± 2.51	2.31 ± 1.75	2.83 ± 0.92	4.21 ± 5.17	1.63 ± 0.65	54.66 ± 12.44
Min	2.56	8.16	0.06	0.02	0.13	0.15	1.01
Max	3.90	21.86	9.65	4.75	46.25	3.20	84.70
Setiu Wetlands							
Mean ± SD	2.88 ± 0.73	9.50 ± 7.08	4.762 ± 3.23	2.07 ± 2.04	4.16 ± 5.08	0.60 ± 1.04	57.99 ± 48.54
Min	1.80	1.70	0.28	0.25	1.17	0.00	15.91
Max	4.60	28.70	15.20	16.19	37.92	4.99	341.50
September							
Pattani Bay							
Mean ± SD	3.20 ± 0.49	17.59 ± 8.67	2.84 ± 1.38	1.82 ± 2.04	4.62 ± 1.16	2.37 ± 2.55	76.70 ± 16.15
Min	2.10	8.19	0.88	0.05	2.18	0.08	50.22
Max	4.31	44.11	7.10	7.58	8.73	11.09	117.29
Setiu Wetlands							
Mean ± SD	3.48 ± 0.71	16.79 ± 9.85	5.68 ± 2.91	2.84 ± 2.21	2.28 ± 0.82	2.85 ± 3.65	56.05 ± 23.18
Min	2.10	3.14	0.61	0.74	1.00	0.01	6.09
Max	5.00	48.37	16.81	12.48	4.69	14.97	131.10

Table 2 Biota-sediment accumulation factor (BSAF) of blood cockles from Pattani Bay and the Setiu Wetlands

Location	BSAF (February)					BSAF (September)				
	As	Cd	Cu	Pb	Zn	As	Cd	Cu	Pb	Zn
Pattani Bay										
Mean ± SD	0.68 ± 0.52	943.82 ± 306.46	1.28 ± 1.57	0.17 ± 0.07	5.88 ± 1.34	0.44 ± 0.21	259.49 ± 291.62	2.31 ± 0.58	0.31 ± 0.33	15.34 ± 3.23
Min	0.02	7.37	0.04	0.02	0.11	0.13	7.38	1.09	0.01	10.04
Max	2.84	1584.33	14.02	0.33	9.11	1.09	1083.27	4.37	1.44	23.46
Setiu Wetlands										
Mean ± SD	6.80 ± 4.61	2070.47 ± 2041.93	10.41 ± 12.69	0.38 ± 0.65	26.36 ± 22.07	2.10 ± 1.08	2842.76 ± 2207.02	2.85 ± 1.03	2.85 ± 3.65	43.11 ± 17.83
Min	0.40	252.00	2.91	0.00	7.23	0.22	739.59	1.25	0.01	4.68
Max	21.71	16,190.00	94.80	3.12	155.23	6.23	12,482.00	5.86	14.97	100.85

Metal concentrations of Cu and Zn of subtidal *Arctica islandica* decreased with increasing shell length (Swaileh and Adelung 1994). This result is similar to concentrations of As, Cu, and Zn (station 3) in this study, which decreased with cockle length. The greater metabolic rate of small organisms may partially account for the higher concentrations of the essential elements Cu and Zn (Williamson 1980). Body size and weight also play an important role in the bioaccumulation of metals.

The pattern of mean metal concentrations in cockles was Zn > Cu > Cd > As > Pb for Pattani Bay in February and Zn > As > Cu > Cd > Pb for the Setiu Wetlands in February. Mean metal content in cockles was Zn > Cu > As > Pb > Cd for Pattani Bay in September and Zn > As > Pb > Cd > Cu for the Setiu Wetlands in September. Zn was the highest in both areas, whereas Pb, Cd, and Cu were lowest in Pattani Bay and the Setiu Wetlands.

Permissible limits of metals have been set by the Ministry of Public Health in Thailand (MPHT) (1986) and the Malaysia Food Regulations (MFR) (1985) (Table 3). Mean values of Cd, Cu, Pb, and Zn were within limits, but exceedances of Cd and Pb occurred in both areas. At Pattani Bay in September, the concentration of Cd exceeded MFR and MPHT by 8 %, and Pb exceeded MPHT by 15 % and MFR by 1 %. At the Setiu Wetlands in February, Cd exceeded the MFR and MPHT by 4 %, and Pb exceeded MPHT by 1 %. At the Setiu Wetlands in September, Cd exceeded MFR and MPHT by 10 %, and Pb exceeded MPHT and MFR by 23 % and 7 %, respectively. Two potential sources of Pb at Pattani Bay were lead as galena (PbS) and lead oxide (Pb_3O_4) in boat repairing at the end of the Pattani River (Simachaya et al. 2003). At the Setiu Wetlands, potential sources of Pb would likely be boating and sand mining (Kamaruzzaman et al. 2002). River discharge resulted from a combination

Table 3 Maximum permission limits on heavy metals (in µg/g wet weight) for food safety set by Thailand and Malaysia

	Cd	Cu	Pb	Zn
Thailand[a]	2	20	1	100
Malaysian[b]	1	30	2	100
This study				
Pattani Bay (February)	1	9	1	17
Pattani Bay (September)	1.5[d]	2	2.2[c,d]	23
The Setiu Wetlands (February)	3[c,d]	8	1	68
The Setiu Wetlands (September)	2.5[c,d]	1	3[c,d]	26

[a] Ministry of Public Health, Thailand (MPHT 1986)

[b] Malaysian Food Regulation (MFR 1985)

[c] Exceeded the MPHT

[d] Exceeded the MFR

of factors, including industrial discharges to the river, inputs from weathering, and the effects of local activities nearby, which were likely contributors to Cd and Pb. It is possible that sources of Pb contamination include the atmospheric input of Pb from the use of leaded petrol by motor vehicles, as well as by fishing boats that frequent ports.

Regular monitoring of trace metal levels in cockles and other benthic species should be conducted. Most metal pollutants could easily move and disperse through aquatic ecosystems. This can translate into effects on the food chain and eventually reach humans.

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