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Status of Heavy Metals Concentrations in Oysters (*Crassostrea* sp.) from Setiu Wetlands, Terengganu, Malaysia

(Status Kepekatan Logam Berat dalam Tiram (*Crassostrea* sp.) dari Tanah Bencah Setiu, Terengganu, Malaysia)

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ABSTRACT

This study aimed at documenting the potentially toxic metal levels in oysters from the aquaculture area of the Peninsular Malaysia east coast. Concentrations of essential (Zn and Cu) and non-essential (Pb and Cd) heavy metals were analyzed in total soft tissue, different sex, selected organs and shells of Crassostrea *sp. from cultured and wild area of Setiu Wetlands. The heavy metal contents among the sampling sites are statistically not significant (*$p>0.05$*) indicating no systematic and site-specific trend between cultured and wild environment. Zinc was the highest metal detected in total soft tissue, sex and shells with the mean concentration of 28.55±6.76, 30.55±3.89 and 8.22±2.98 µg/g, respectively. The analysed metals were highly accumulated in gills than other organs with the mean value of 74.11±13.03 µg/g of Zn, 4.82±0.82 µg/g of Cu, 0.61±0.06 µg/g of Pb and 0.45±0.1 µg/g of Cd. The varying metals content in the different soft tissues might be due to the different affinity of metals that bind with metallothioneins. The metal levels measured in oysters did not exceed the maximum permissible limits for Zn, Cu, Pb and Cd. The measured metal levels may represent baseline values reflecting background conditions that contain a certain degree of human impact.*

Keywords: Aquatic; Crassostrea *sp.; heavy metals; Setiu wetlands*

ABSTRAK

Kajian ini bertujuan untuk mendokumentasi tahap logam yang berpotensi toksik di dalam tiram dari kawasan akuakultur di pantai timur Semenanjung Malaysia. Kepekatan logam berat perlu (Zn dan Cu) dan tidak perlu (Pb dan Cd) telah dianalisis dalam keseluruhan tisu lembut, kelainan jantina, organ terpilih dan cengkerang Crassostrea *sp. dari kawasan liar dan kultur di tanah bencah Setiu. Kandungan logam berat antara kawasan persampelan secara statistik adalah tidak signifikan (*$p>0.05$*) menunjukkan tiada corak yang sistematik dan khusus antara persekitaran liar dan kultur. Zink adalah logam yang paling tinggi dikesan di dalam keseluruhan tisu lembut, kelainan jantina dan cengkerang dengan kepekatan minimum 28.55±6.76, 30.55±3.89 dan 8.22±2.98 µg/g. Logam yang dianalisis terkumpul di dalam insang berbanding organ lain dengan nilai minimum 74.11±13.03 µg/g bagi Zn, 4.82±0.82 µg/g bagi Cu, 0.61±0.06 µg/g bagi Pb dan 0.45±0.1 µg/g bagi Cd. Perbezaan kandungan logam dalam tisu lembut mungkin disebabkan oleh pertalian berbeza logam yang terikat dengan metalotionein. Tahap logam di dalam tiram tidak melebihi had maksimum yang dibenarkan bagi Zn, Cu, Pb dan Cd. Tahap logam yang telah diukur mungkin mewakili nilai asas yang mencerminkan latar belakang yang mengandungi tahap tertentu kesan manusia.*

Kata kunci: Akuatik; Crassostrea *sp.; logam berat; tanah bencah Setiu*

INTRODUCTION

In recent years, people are concerned about the increasing heavy metal pollutants in the aquatic environment. Heavy metals can be originated either from natural or anthropogenic sources but it is the latter that result in elevated levels of potentially toxic metals in aquatic food systems. These chemical substances can be categorized as essential or non-essential. Some of them become toxic to living organisms when subject to high concentrations (i.e. Zn, Cu, Co and Fe), but others are toxic even at low concentrations (i.e. Pb, Cd, As and Hg).

Bivalve mollusks are the organisms that are highly affected by the heavy metal pollutants due to their sessile character and feeding mechanism. These organisms conduct filter feeding on suspended particles from surrounding waters, thus becoming a source of heavy metal uptake in their body (Wong et al. 2000). They tend to accumulate contaminants at much higher level than the natural background concentration in the environment (Rainbow 2002). Thus, they are the most suitable bio-indicator to monitor the heavy metal contamination in the aquatic environment (Fang et al. 2003). Oysters *Crassostrea* sp. has been used as bio-indicator in several environmental studies for a long time (Beliaeff et al. 1998; Paéz-Osuna et al. 1995). This organism exhibits high filtration rates of suspended particles and capable to bio-accumulate heavy metals (Wallner-Kersanach et al. 2000). Therefore, the concentration of metals in the tissues of *Crassostrea* sp. is reliable to reflect the magnitude of environmental contamination.

Studies on metal contents of oysters in tropical environments, including Malaysia, are limited, especially investigation on metals in various organs of oysters. Therefore, in this study we investigated the concentration of essential metals (Zn and Cu) and non-essential metals (Pb and Cd) in total soft tissue, different sex, selected organs and shells of *Crassostrea* sp. in the aquaculture area of Setiu Wetlands, Terengganu, Malaysia. The obtained results were compared with those of other studies as well as some permissible guidelines.

METHODS

STUDY AREA

The Setiu Wetlands is an important tropical aquaculture area involving a large production of seafood-based delicacies and is, therefore, a suitable place for heavy metal concentrations study in oysters *Crassostrea* sp. due to large oyster farming. The Setiu Wetlands is located in Terengganu on Peninsular Malaysia's east coast (Figure 1). This wetland complex encompasses several distinct ecosystems including mangrove, riparian forest, peat swamp, wetland, lagoon and estuary. There are two main sources of freshwater input to this wetland, Setiu River and Lake Berambak. Brackish water aquaculture activities such as cage culture, pen culture and oyster farming are the major economic activities in Setiu Wetlands (Suratman et al. 2014).

SAMPLE COLLECTION

A total of 60 individuals of *Crassostrea* sp. were sampled from wild (5° 40' 52" N, 102° 42' 39" E), rack culture (5° 40' 59" N, 102° 42' 37" E) and string culture (5° 40' 53" N, 102° 42' 42" E) in July 2008. The specimens were packed in clean vinyl plastic bags and kept cold in the ice-chest containers. The flesh tissues of *Crassostrea* sp. was detached from the shell and separated to several subsamples; total soft tissue, sex, adductor muscle, stomach, flesh and gills. The samples were wet weighed and then freeze-dried at -20°C for 24 h. The dried samples were finely ground and homogenized by ceramic mortar and pestle and stored in the clean plastic vials for further chemical analysis.

LABORATORY ANALYSIS

Five shell pairs of adult oysters were selected from each site. The shells were washed and scrubbed in deionized water with a cleaned toothbrush to remove the attached organic and inorganic substrate. The clean shells thus obtained were dried in oven at 50°C for at least 48 h. The individual shells were finely ground and homogenized by ceramic mortar and pestle. The powdered samples were stored in the clean plastic vials for the chemical analysis. Soft tissue digestion was carried out by the following established procedures of US EPA Method 200.3 (1991) with some modifications of quantity of reagents used which are given as follows. Approximately 1 g of homogenized sample was digested in the glass test tube with 10 mL

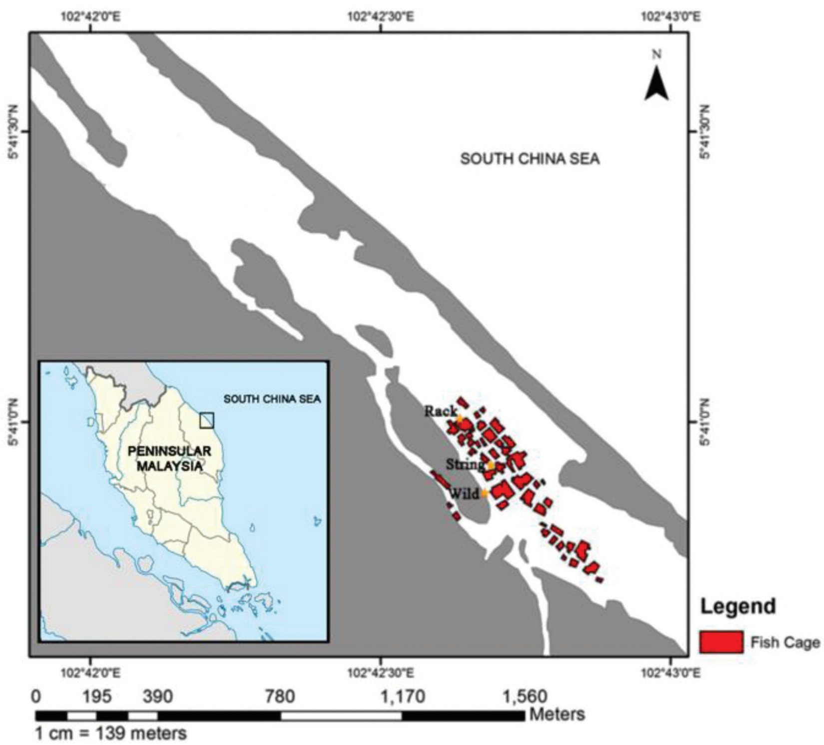

FIGURE 1. Map showing location of Setiu Wetlands where cultured (rack and string) and wild oysters were collected. The point (filled star) indicates the approximate position of the sampling sites

HNO_3 at 95°C and left to reflux for 15 min. The similar procedure was repeated twice with 5 mL HNO_3 at 95°C and left to reflux for 30 min. After evaporation to dryness, the residue was dissolved in 10 mL of H_2O_2 and diluted with Milli-Q ultrapure water. At this stage, a clear solution was obtained from complete digestion process. The digested sample was brought to 50 mL with 5% HNO_3 as a diluent. Analysis of the Zn, Cu, Pb and Cd contents was performed using inductively coupled plasma mass spectrometry (ICP-MS). The method accuracy was verified by the certified reference material of dogfish liver (DOLT-3). The recovery of the DOLT-3 ranged from 93.33 to 108% (Table 1).

The shell sample digestion was carried out following the established method described by Cravo et al (2007). Approximately 0.1 g homogenized shell sample was totally digested in a mixture of concentrated HNO_3 and 50% w/v H_2O_2. After evaporation to dryness, the residue was dissolved in 2 mL of HCl and fumed to dryness. Then, the final residue was re-dissolved in 10 mL of 0.05 M HCl. Analysis of metals was performed using ICP-MS. The samples were collected and analyzed using standard quality assurance/quality control (QA/QC) protocol, in accordance with standard practices. All reagents used were of analytical reagent grade or better. Metal concentrations are reported as µg/g dry weight.

STATISTICAL ANALYSIS

The analytical variance (ANOVA) was applied to understand the variations in measured metal concentrations in *Crassostrea* sp. and sampling sites.

RESULTS AND DISCUSSION

Concentrations of measured metals in total soft tissue, sex and shells are shown in Table 2. The mean concentration of heavy metals in the body (soft tissue and shell) from all sampling sites follow a descending order of Zn > Cu > Pb > Cd. The ANOVA analysis showed no significant difference (p>0.05) of metals between sampling sites. The highest concentration of Zn was observed in total soft tissue of 34.23 µg/g for string, 21.34 µg/g for rack and 29.57 µg/g for wild. The concentration of Cu was 2.44 µg/g for string, 1.54 µg/g for rack and 1.96 µg/g for wild. Green-Ruiz and Paez-Osuna (2003) found that oysters in the lagoon system are the strong accumulators of both metals, especially of Zn. As essential metal zinc is usually accumulated at higher concentration than other studied metals because the presence of this metal is needed as ionic cofactor (Zn^{2+}) for enzymes of metabolic activities in the tissue (Frausto Da Silva & Williams 1991). Copper is also an essential metal to metabolic processes of oysters (Launestein et al. 2002). However, the measured values of Cu in the present study are considerably low compared to that of Zn. According to Pan and Wang (2009) the uptake rate of Cu by bivalves are correlated with the surrounding copper concentration, the uptake rate decrease when the concentration is low. The low concentration of Cu may reflect the low Cu content of sediment and water in the lagoon system of Setiu Wetlands. The source of Cu cannot be verified due to the lack of availability of baseline values of Cu in the study area. Meanwhile, the concentration of both Cd and Pb were relatively low in the whole body from string, rack and wild of 0.23 and 0.18 µg/g, 0.22 and 0.14 µg/g and 0.31 and 0.38 µg/g, respectively. Kargin (1998) found that the concentrations of non-essential metals in the aquatic organisms depend predominantly on the environmental levels. Hence, we suppose that the oysters in this ecosystem accumulated lower Cd and Pb is due to the exiguous content of both metals in the surrounding water column.

The average metal concentration found in both male and female follow the decending order of Zn > Cu > Cd > Pb. The highest metal in male and female was Zn with the average value of 33.30±13.05 and 27.80±7.3 µg/g, respectively. The mean concentration of Cu in male and female were 1.84±0.33 and 1.81±0.15 µg/g, respectively. Meanwhile, both male and female showed comparable mean concentrations of Cd with the value of 0.24±0.03 µg/g. The lowest concentration of metal in both male and female was Pb of 0.14±0.02 and 0.16±0.04 µg/g. The ANOVA analysis shows no significant difference (p>0.05) of analyzed metals between sampling sites and different sex. However, the concentration of Zn was slightly higher in male than female *Crassostrea* sp. This may be due to the bio-concentration of zinc during their gonadal stage (Latouche & Mix 1982). Guo et al. (1998) reported that juvenile oysters usually growth up as male and change to be a female at the later life cycle. Thus, we suppose that *Crassostrea* sp. in the study area may accumulated some higher Zn during early stages of life cycles as male individual.

The results indicated the similar pattern of accumulated metals in the shells and total soft tissue of oysters. There were no statistically significant differences (p>0.05) between the sampling sites for concentrations of all metals in shell. Zinc was the highest metal detected in shell samples similar to the total soft tissue with mean value

TABLE 1. Recovery test using DOLT-3 standard

Metals	Certified value (µg/g)	Results (µg/g)	Recovery (%)
Cd	1.94±0.06	1.84±0.05	94.8
Cu	3.12±0.1	3.39±0.21	108
Pb	0.03±0.005	0.028±0.005	93.33
Zn	8.66±0.24	8.52±2.7	98.4

TABLE 2. The concentration Cd, Cu, Zn and Pb (μg/g) in total soft tissue, sex and shell

	Total soft tissue				ANOVA
Samples	Cd	Cu	Zn	Pb	
String	0.23	2.44	34.74	0.18	$p<0.05$
Rack	0.22	1.54	21.34	0.14	$p<0.05$
Wild	0.31	1.96	29.57	0.38	$p<0.05$
Average	0.25	1.98	28.55	0.23	$p<0.05$
Std.	0.05	0.45	6.76	0.13	
			Sex		
Samples	Cd	Cu	Zn	Pb	
String (M)	0.26	2.19	48.30	0.14	$p<0.05$
Rack (M)	0.21	1.80	24.54	0.12	$p<0.05$
Wild (M)	0.24	1.54	27.06	0.15	$p<0.05$
Average	0.24	1.84	33.30	0.14	$p<0.05$
Std.	0.03	0.33	13.05	0.02	
String (F)	0.18	1.93	36.22	0.12	$p<0.05$
Rack (F)	0.29	1.85	23.26	0.16	$p<0.05$
Wild (F)	0.25	1.64	23.91	0.19	$p<0.05$
Average	0.24	1.81	27.80	0.16	$p<0.05$
Std.	0.06	0.15	7.30	0.04	
			Shell		
Samples	Cd	Cu	Zn	Pb	
String	0.03	0.62	8.85	0.54	$p<0.05$
Rack	0.04	0.65	10.83	0.68	$p<0.05$
Wild	0.01	0.54	4.98	0.57	$p<0.05$
Average	0.03	0.60	8.22	0.60	$p<0.05$
Std.	0.01	0.06	2.98	0.07	

* M-male, F-female
** $p<0.05$ no significant difference between sampling sites

of 8.22±2.98 μg/g. Meanwhile, the concentration of Cu, Pb and Cd were 0.6±0.07, 0.6±0.01 and 0.03±0.01 μg/g, respectively. Rebelo et al. (2003) reported that oysters can concentrate up to 50 times more Zn than found in the bottom sediments using the biota-sediment accumulation factor technique. This much higher Zn suggests significant accumulation which may be due to slow or inefficient depuration mechanisms in oysters. According to Huanxin et al. (2000), heavy metal accumulation in oyster tissue and shell is influenced by oyster metabolism and availability of the different metals from environment. A few metals in shells are derived from tissues because the mantle of oysters secrete a viscid organic film at the edge of the valves where mineralization process occurs (Carriker et al. 1980). Then, the metal in the form of inorganic detritus incorporates into the shell at the mantle edge during shell formation (Huanxin et al. 2000). Foster and Chacko (1995) found that some incorporated metals in the mollusks shell occurs via the substitution of the Ca^{2+} in the crystalline phase or association with the organic matrix of the shell.

Table 3 shows the concentration of heavy metal in different organs for all sampling sites. The statistical ANOVA shows a significant difference ($p<0.05$) between oyster organs. The average concentrations of heavy metal is in the descending order of Zn > Cu > Pb > Cd. The highest concentrations of metals were measured in gills for all sampling sites with the mean values of 74.11±13.03 μg/g for Zn, 4.82±0.82 μg/g for Cu, 0.61±0.06 μg/g for Pb and 0.45±0.10 μg/g for Cd. According to Deb and Fukushima (1999), the organs that serve as the place for uptake and storage (e.g. digestive gland, intestine and gill) tend to bio-accumulate metals, thus, may exhibit relatively high concentration of metals than other organs. This is due to the greatest capacity of metallothionein induction in these tissues (Deb & Fukushima 1999). Some of previous reports showed that the presence of high metallothioneins enzyme in gills of mollusks (Carpene & George 1981; Nolan & Duke 1983). Metallothionein is a family of low molecular weight proteins localized to the membrane of the Golgi apparatus and have the capacity to bind heavy (Sigel et al. 2009). This suggest that the dissimilarity of metals attraction to the binding sites of metallothioneins in different soft tissues (Viarengo et al. 1985) could affect the variable concentration of metals in the oyster's organ of the study area. Furthermore, higher metal accumulation in gills than other organs might be caused by direct contact of this organ with seawater (Yap et al. 2003) and the large surface area of gills should increase metal uptake through facilitated diffusion (Franklin et al. 2008). Meanwhile, the presence of heavy metal in the flesh may due to the increasing adsorption of metal on the body surface and uptake of organic ligand complex metals through the surface of soft

TABLE 3. Heavy metal concentrations (μg/g) in different part of selected soft tissues

Samples	Cd	Cu	Zn	Pb	ANOVA
			String		
Abductor	0.07	1.08	22.48	0.43	$p<0.05$
Stomach	0.24	1.89	26.81	0.52	$p<0.05$
Flesh	0.15	1.94	32.80	0.60	$p<0.05$
Gills	0.34	4.10	59.14	0.66	$p<0.05$
Average	0.20	2.25	35.31	0.55	
Std.	0.12	1.3	16.44	0.10	
			Rack		
Samples	Cd	Cu	Zn	Pb	
Abductor	0.23	1.09	23.35	0.55	$p<0.05$
Stomach	0.56	2.98	43.39	0.55	$p<0.05$
Flesh	0.21	3.33	36.98	0.55	$p<0.05$
Gills	0.53	5.71	82.92	0.62	$p<0.05$
Average	0.39	3.28	46.66	0.57	
Std.	0.19	1.90	25.57	0.03	
			Wild		
Samples	Cd	Cu	Zn	Pb	
Abductor	0.17	1.69	26.90	0.54	$p<0.05$
Stomach	0.59	3.02	36.36	0.60	$p<0.05$
Flesh	0.31	4.07	51.12	0.50	$p<0.05$
Gills	0.47	4.65	80.28	0.55	$p<0.05$
Average	0.39	3.36	48.67	0.55	
Std.	0.18	1.30	23.31	0.04	

*$p<0.05$ significant difference between selected organs

part of this bivalve (Martincic et al. 1986). The lowest concentration of heavy metal in the present study is observed in the abductor muscle. The low metal content in adductor muscle was due to the absence of absorption or secretion functions (Senthilnathan & Balasubramanian 1998). Thus, we suppose that the metallothioneins protein and principal function of the organs may affect the metals accumulation in the different organ of this species.

Wild and cultured oysters did not indicate a trend of metal enrichment and/or depletion due to the environment. Average metal levels of Zn, Cu, Pb and Cd in wild oysters registered similar values to the cultured oysters (Table 3). Both essential metals, Zn and Cu, showed similar distribution pattern in different organs and followed the descending order as gills > flesh > stomach > abductor muscle. We assumed that the high concentrations of Zn and Cu could be due to the uneaten food pellets and fish feces from the cage-culture. These products transform into suspended particles in the water column and attached to the mucus layer covering the gills and may result in greater and faster accumulation of both metals. The concentration of Cd and Pb in stomach was 0.59±0.2 and 0.27±0.02 μg/g, respectively. The pattern of Cd accumulation in the tissue samples of *Crassostrea* sp. followed the sequence of stomach ≥ gill > flesh > abductor muscle. There were no significant differences of mean Cd concentrations between the stomach and gill. Lead accumulation in the tissues of *Crassostrea* sp. in descending order of gill > stomach > flesh > abductor muscle. The high content of both metals in stomach of *Crassostrea* sp. could be due to the fact that the bioaccumulation rate exceeds the depuration rate.

The comparison of heavy metals in the present study and other studies are shown in Table 4. The studied metal concentrations were found to be lower than those from Pulau Aman (Yap et al. 2004), Likas and Kota Belud estuary (Abdullah et al. 2007), Muar estuary (Kamaruzzaman et al. 2008), Tok Bali (Abdullah et al. 2009) and Pekan (Kamaruzzaman et al. 2011) and some of the world studies (Rojas de Astudillo et al. 2002; Usero et al. 2005). The measured values of metals in this study were also significantly lower than in the bivalves from location that have been considered as contaminated area with heavy metals (Biswas et al. 2013; Heidari et al. 2013; Osuna-Martınez et al. 2011). This observation indicates that there was no abnormality of heavy metal in the oysters from the study area and reflects the low level of heavy metal in the ecosystem.

In order to assess the possible risk to human health, the analyzed metals of total soft tissues were compared with the guideline of permissible limit proposed by local and international authorities (Table 5). The metals level in total soft tissue of *Crassostrea* sp. from Setiu Wetlands was lower than those stated in the Malaysian Food and Regulation (MFR 1985), which sets the maximum value of 1 μg/g for Cd, 30 μg/g for Cu, 100 μg/g for Zn and 2 μg/g for Pb. The measured value of Cu, Zn and Cd in the total

TABLE 4. Comparison of metals in total soft tissue with local and world studies. All value in µg/g

Location	Species	Cd	Cu	Zn	Pb	Reference
*Pekan	*Perna viridis*	0.3	19.05	45.54	0.47	Kamaruzzaman et al. 2011
*Likas estuary	*Meretrix meretrix*	3.27	6.62	106.7	1.72	Abdullah et al. 2007
*Kota Belud estuary	*Meretrix meretrix*	1.68	5.78	83.1	1.09	Abdullah et al. 2007
*Tok Bali	*Soletellina* sp.	-	4.7	80.0	1.6	Abdullah et al. 2009
*Pulau Aman	*Perna viridis*	0.87	10.80	110.0	4.76	Yap et al. 2004
*Muar Estuary	*Perna viridis*	0.58	8.96	86.73	2.28	Kamaruzzaman et al. 2008
Gulf of Paria, Trinidad	*Crassostrea rizophorae*	0.31	29.8	346	-	Rojas de Astudillo et al. 2002
Southern Spanish Coast	*Crassostrea gallina*	0.33	38	72	1.3	Usero et al. 2005
**West of Bengal, India	*Saccostrea cucullata*	37.01	294	573	23.53	Biswas et al. 2013
**Lengeh Port coast, Iran	*Saccostrea cucullata*	11.13	323.90	748.00	41.19	Heidari et al. 2013
**SE Gulf of California Mexico	*Crassostrea gigas*	13.9	58	478	2.1	Osuna-Martínez et al. 2011
Setiu Wetland	*Crassostrea* sp.	*0.25*	*1.98*	*28.55*	*0.23*	This Study

*study in Malaysia
**polluted area

TABLE 5. Comparison of metals in total soft tissue with the various established permissible guidelines

Guideline	Cd	Cu	Zn	Pb	Reference
World	1	10	150	-	FAO/WHO (1984)
Australia & New Zealand	2	3	130	2	FSANZ (2005)
Thailand	-	26.6	133	1.33	MPHT (1983)
Malaysia	1	30	100	2	MFR (1985)
Setiu Wetlands	0.25	1.98	28.55	0.23	This Study

• The metals value in µg/g

soft tissue was also lower than the permissible standards in Thailand (MPHT 1986). This study also showed that Cd, Cu and Zn levels did not exceed the permissible concentration level of 1, 10 and 150 µg/g, respectively, set by the FAO/WHO (1984), as well as the maximum levels of Cd and Pb (2 µg/g) that was declared by FSANZ (2005) for bivalve mollusks.

CONCLUSSION

Cultured and wild oysters metal concentrations were similar and did not register a systematic trend between sampling sites. The metal levels based on sex did not show a systematic trend with exception of Zn which was found to be slightly higher in male than the female. Metal level in gills of oyster *Crassostrea* sp. was found to be higher among all organs with an order of decreasing as stomach ≥ gill > flesh > abductor muscle. Overall, heavy metal levels (Zn, Cu, Pb and Cd) in oysters were found to be well below the permissible concentration level for human consumption and probably represent prevailing background metal levels. In this respect, it was presumed that wild and cultured oysters from Setiu Wetlands were likely not to be toxic to public consumption.

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