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REVIEW ARTICLE

A Review of Cd, Cu and Zn in Cockle *Anadara Granosa* and Mussel *Perna Viridis*: Comparison of Food Safety Limits and Human Health Risk Assessment

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Abstract

The present study reviewed the concentrations of Cd, Cu, and Zn in the edible soft tissues (ST) of cockle *Anadara granosa* and mussel *Perna viridis* from the literature. Based on field collected samples, the Cd concentrations in the cockles were generally higher than those of mussels while Cu concentrations in the mussels were generally higher than those of cockles. However, Zn concentrations were comparable and not significantly ($P < 0.05$) different from each other. From the public health point of view, these metal concentrations were below the maximum permissible levels (MPL)'s set by different and agencies. For a more valid and reliable human health risk assessment, target hazard quotient (THQ) values were calculated based on the reviewed data of Cd, Cu and Zn in the cockles and mussels. The THQ values of Zn in the cockles and mussels were found to be all below 1.00, indicating no non-carcinogenic effects of Zn. Only the laboratory study of Cd exposure on the cockles and mussels exceeded a THQ value of 1.00. This indicated the Cd uptake can be highly accumulated in the ST of *A. granosa* and *P. viridis* without regulation of this non-essential metal. Therefore, there are always risks of Cd toxicity and non-carcinogenic effects of Cd to the consumers of this popular Asian seafood. All Cu THQ values in the cockles and mussels are found to be below 1.00 except for the Cu level in mussels collected from coastal waters of Hong Kong [1]. This strongly indicated that the ST of the field collected *P. viridis* has the capability of Cu accumulation that exceeding the Cu MPL and thus can pose a non-carcinogenic effect of Cu to the consumers. The recommended MPL for Zn should be revised especially since the Zn accumulation in the ST of both bivalves will not exceed the MPL for Zn due to partial regulation mechanism of the bivalves.

Keywords

Heavy metals, Mussels and cockles, Health risk assessment

Introduction

Food safety is a growing global concern, not only for its continuing importance to public health, but also because of its impact on international trade [2]. Recent trends in global food production, processing, distribution and preparation are creating an increasing demand for food safety research in order to ensure a safer global food supply.

Among the edible shellfish products, mussels and cockles are reported to be highly consumed by the Asians [3-5]. It is, therefore, most of the metal levels reviewed in this paper are collected from Asian countries. This is due to marine species provide cheap source of proteins for the human consumption since decades or centuries ago. Most of the coastal waters are highly populated and this has made the coastlines interesting from the ecotoxicological point of view. Questions like 'Are the seafood delicacies such as marine mussels and cockles contaminated by heavy metals?' and 'Are they safe for human consumption?' could be arisen from the public concerns.

Since heavy metals are inorganic chemicals that are nonbiodegradable, cannot be metabolized and will not break down into harmless forms [6], the measurement of levels of metals in the soft tissues of mussel *Perna viridis* is becoming more significant. They could simply accumulate through time, becoming more and more of a toxic threat as their concentrations increase. Levels of metals that were above the permissible limits would certainly give a notorious status on food safety from the public health point of view. Chronic exposure to heavy metals such as Cu and Zn was associated with Parkinson's disease and the metals might act alone or together over time to cause the disease [7]. Also, concerns on metal toxicity in humans are high judging from the many such related studies were reported in the literature.

From biological point of view, especially genetically, comparisons of metals in the different bivalve species are arguable. This is due to they are not of similar species and this makes the comparison of pollutant levels in a biomonitor of different geographical areas difficult and thus not reliable. In the biomonitoring study, a proposed biomonitor of a similar species should have low variability in its morphological features as well as genetic differentiation [8]. If a mussel is compared with cockles or other mollusks species, variability of their heavy metal concentrations is expected to be high since they have different sets of physiology, ecology and biology even though they are found in the same sampling site. Although these comparisons are seldom found in the literature if ecotoxicologists are monitoring different areas, our comparisons are able to answer several interesting questions like 'Do cockles accumulate higher levels of metals than those in mussels?' and 'Are the consumable molluscs safe for human consumption from the metal toxicological point of view?' Since most molluscs species are potential seafood delicacies, our comparison would provide a means of assessing toxicant risk to the public health when compared to the permissible limits (MPL)s.

Two latest papers on heavy metals were reported by Suprapto, et al. [9] and Qari, et al. [10] for *P. viridis* and Sudsandee, et al. [11] and Pradit, et al. [12] for cockle *Anadara granosa*. Suprapto, et al. [9] reported the concentration of Cd, in *P. viridis* at Semarang Bay while Qari, et al. [10] reported the heavy metals including Cd, Cu and Zn, in *P. viridis* collected from Paradise Point of Karachi coast. The above two papers did not include human health risk (HHR) based on target hazard quotient (THQ). Sudsandee, et al. [11] reported 8 heavy metals including Cd, Cu and Zn in *A. granosa* collected from three locations in the Upper Gulf of Thailand. They assessed the HHR based on THQ values. They found that THQ values were below 1 in all three sampling areas, indicating that adverse health effects were not likely to be caused by exposure to heavy metals in blood cockles over a human lifetime. Pradit, et al. [12] assessed levels of 5 trace metals including Cd, Cu and Zn in *A. granosa* collected from two coastal areas in Thailand (Pattani Bay) and Malaysia (the Setiu Wetlands). However, they did not estimate the HHR based on THQ.

The estimated daily intake (EDI) and THQ values were calculated to evaluate the non-carcinogenic health risk from individual heavy metal due to dietary intake [13]. THQ value proposed by USEPA [14] is an integrated risk index by comparing the ingestion amount of a pollutant with a standard reference dose and has been widely used in the risk assessment of heavy metals in contaminated foods [15].

The objectives of this review paper were to 1) Review the concentrations of Cd, Cu, and Zn in mussel *P. viridis* and cockle *A. granosa* in available sources in the literature (from 1983 to 2013), and 2) Assess the potential HHR from Cd, Cu and Zn via consumption of the two bivalves. This HHR was estimated through direct comparison of the metals with the established recommended guidelines for food safety limits, and calculations of EDI ad THQ of the three metals.

Methodology

The data of Cd, Cu, and Zn concentrations in the mussels and cockles were reviewed from available sources, from 1983 to 2013, in the literature especially based on papers by Yusof, et al. [5] and Yap, et al. [4] (Figure 1). These three-metal data were selected because the HHR based on THQ values have not been calculated.

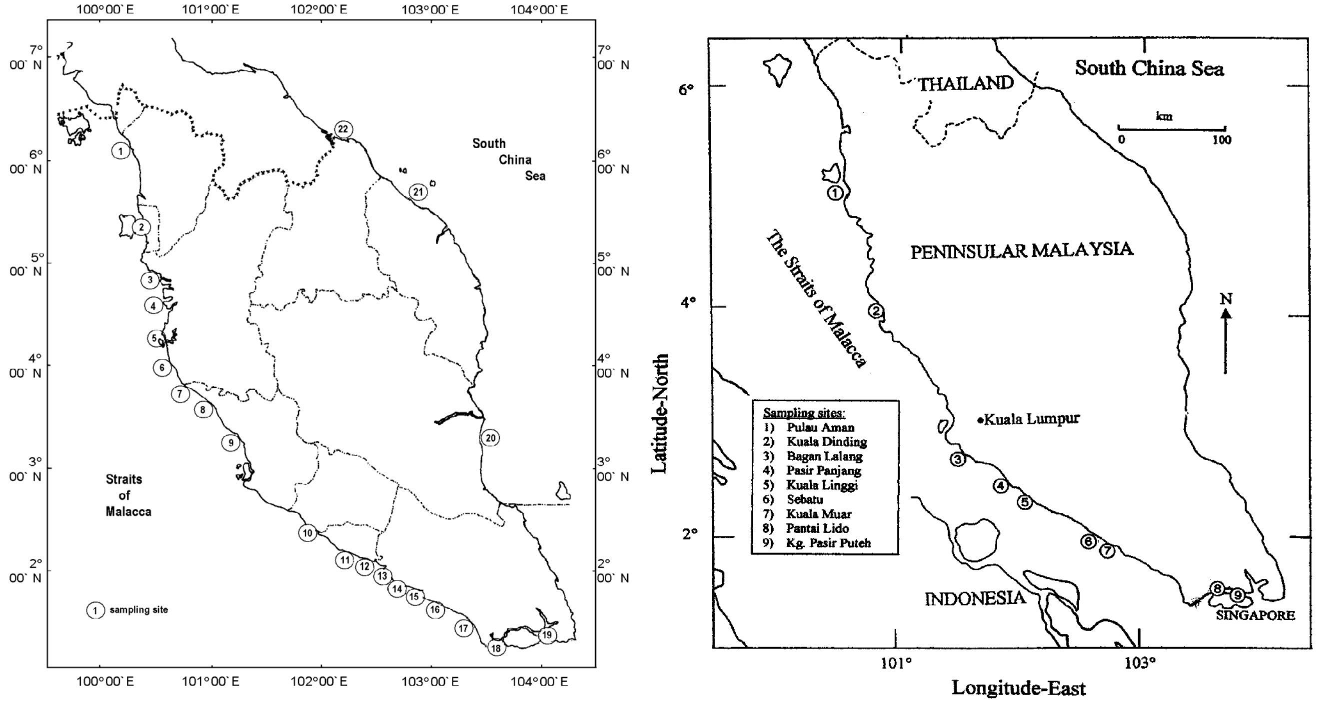

Figure 1: Sampling sites of *Perna viridis* (a and b) and *Anadara granosa* (a) in the coastal waters of Peninsular Malaysia (a: Yusof, et al. [5]; b: Yap, et al. [4]).

Data treatment

In order to evaluate a once-or long-term potential hazardous exposure to metals through consumption of snails [16] by the population of Peninsular Malaysia, the EDI and THQ values were calculated using the following formulas [14], respectively:

$$\text{EDI} = \frac{\text{Mc} \times \text{consumption rate}}{\text{body weight}}$$

Where Mc is the metal concentration (µg/g) in snail soft tissue was obtained on wet weight bases, body weight for adult was 60 kg and consumption rate as 17.86 and 35.7 g/day, for average and high-level mollusks consumers, respectively [17]. The oral reference dose (RfD) was used in this study to compare with the EDIs (µg/kg wet weight/day) of metals in bivalves.

In this study, a non-cancer risk assessment method is based on the use of THQ, a ratio between the estimated dose of contaminant and the reference dose below which there will not be any appreciable risk. The THQ determined with the formula described by USEPA [14]:

$$\text{THQ} = \text{EDI}/\text{RfD}$$

Where oral reference dose (RfD) (µg/kg wet weight/day) used in this study were Cd: 1.00; Cu: 40.0; and Zn: 300, provided by the EPA's Integrated Risk Information System online database [14,18,19].

Results and Discussion

Comparisons with food safety guidelines

All the reviewed concentrations of Cd, Cu, and Zn of *P. viridis* and *A. granosa* are mainly found from Asian coastal waters (Table 1 and Table 2) because both bivalve species are tropical indigenous species. All the data reviewed are samples collected from different environmental backgrounds, from pristine area to metal-polluted coastal waters such as Tolo Harbour of Hong Kong. From Table 1, all concentrations of Cd, Cu, and Zn in the soft tissues of *A. granosa* are within the different standards defined by different countries and below the maximum permissible limits (MPL)

Table 1: Concentrations (µg/g) of Cd, Cu, and Zn in *Anadara granosa* reviewed from regional studies and Malaysia.

No.	Locations	WB		Cd	Cu	Zn	Authors
Field collected samples							
1	Upper gulf of thailand	ww*	Min	0.36	0.27	15.0	Hungspreugs and Yuangthong [26]
		ww*	Max	0.53	0.42	17.5	
2	Some coastal areas in thailand and malaysia	ww*	Min	0.63	0.89	19.6	Everaarts and Swennen [27]
		ww*	Max	1.09	0.91	25.1	
3	Bombay harbour (1976-1980)	ww*	Min	0.53	1.54	13.3	Patel, et al. [28]
		ww*	Max	0.86	2.11	25.1	
4	Thailand coastal waters	ww*	Min	0.05	0.86	8.00	Phillips and Muttasasin [29]
		ww*	Max	0.15	1.40	11.0	
5	Northern part of peninsular malaysia	ww	Min	0.20	0.80	14.6	Devi [30]
		ww	Max	0.79	1.60	23.1	
6	Lekir, perak	ww*	Min	1.16	1.20	12.2	Mat, et al. [24]
7	Batu kawan and K. Selangor	ww*	Min	0.40	0.65	10.6	Mat and Maah [25]
		ww*	Max	1.31	0.86	12.2	
8	Retail outlets in kuala lumpur (4 sites)	ww	Min	1.23	0.64	12.9	Mat [31]
		ww	Max	1.42	0.80	14.7	
9	Coastal waters of peninsular malaysia (19 sites)	ww	Min	0.10	0.25	8.00	Liong [32]
		ww	Max	0.90	6.00	40.0	
10	Coastal waters of peninsular malaysia	ww	Avg	1.91	0.51	19.2	Jothy, et al. [33]
11	Kuala juru, penang, malaysia	ww*	Min	NA	NA	35.2	Ibrahim [34]
		ww*	Max	NA	NA	44.8	
12	Sg. Bahru	ww*	Avg	0.30	1.25	17.8	Yap and Lo [35]
13	Kuala juru	ww*	Avg	0.17	2.58	20.3	Yap and Lo [35]
14	Bayan lepas	ww*	Avg	0.24	1.27	24.9	Yap and Lo [35]
15	Batu melintang	ww*	Avg	0.22	3.82	36.1	Yap and Lo [35]
16	Sg. Ayam	ww*	Avg	0.23	1.22	18.8	Yap and Lo [35]
17	Minyak beku	ww*	Avg	0.33	0.51	12.0	Yap and Lo [35]
18	Kuala juru	ww*	Avg	0.25	1.40	38.7	Yap, et al. [36]
19	Kuala kurau	ww*	Avg	0.63	1.07	17.5	Yap, et al. [36]
20	Jeram	ww*	Avg	1.79	1.03	18.1	Yap, et al. [36]
21	KRG-1	ww*	Avg	0.13	0.38	10.9	Yusof, et al. [5]
22	KRG-2	ww*	Avg	0.65	0.62	18.8	Yusof, et al. [5]
23	KRG-3	ww*	Avg	0.49	0.32	10.7	Yusof, et al. [5]
24	KRG-4	ww*	Avg	0.47	0.60	14.7	Yusof, et al. [5]
25	KRG-5	ww*	Avg	0.41	0.46	14.0	Yusof, et al. [5]
26	KRG-6	ww*	Avg	0.28	0.49	13.3	Yusof, et al. [5]
27	KRG-7	ww*	Avg	0.51	0.58	25.8	Yusof, et al. [5]
28	KRG-8	ww*	Avg	0.84	0.41	30.0	Yusof, et al. [5]
29	KRG-9	ww*	Avg	0.51	0.56	18.9	Yusof, et al. [5]
30	KRG-12	ww*	Avg	0.12	1.73	18.8	Yusof, et al. [5]
31	KRG-14	ww*	Avg	0.16	0.37	10.1	Yusof, et al. [5]
32	KRG-16	ww*	Avg	0.28	0.51	12.5	Yusof, et al. [5]
33	KRG-20	ww*	Avg	0.12	0.93	19.8	Yusof, et al. [5]
34	KRG-21	ww*	Avg	0.07	0.42	12.3	Yusof, et al. [5]
35	KRG-22	ww*	Avg	0.03	0.35	7.94	Yusof, et al. [5]
Laboratory study							
1	Exposure of 0.25 mg Cd/L for 16 days	wet	Min	4.00	NA	NA	Chan, et al. [21]
		wet	Max	20.0	NA	NA	
2	Exposure of 0.28 mg Cu/L for 24 hours	ww*	Avg	NA	13.7	NA	Yap, et al. [37]
3	Six days of 0.100 mg Cu/L accumulation	ww*	Avg	NA	3.46	NA	Yap, et al. [38]
4	Four days of depuration after four days of 0.10 mg Cu/L exposure	ww*	Avg	NA	2.24	NA	Yap, et al. [38]
5	Six days of 1.0 mg Zn/L accumulation	ww*	Avg	NA	NA	50.4	Yap, et al. [38]
6	Four days of depuration after four days of 1.00 mg Zn/L exposure	ww*	Avg	NA	NA	35.5	Yap, et al. [38]

Note: BDL: Below Detection Limit; WB: Weight Basis; BDL: Below Detection Limit; *: indicated the wet weights are converted from dry weight by a multiplying factor of 0.19 (Yap, et al. [36]) for *Anadara granosa*; NA: Not Available; Avg: Average; min: Minimum; max: Maximum.

Table 2: Concentrations (μg/g) of Cd, Cu, and Zn in *Perna viridis* reviewed from regional studies and Malaysia.

No.	Location	WB		Cd	Cu	Zn	References
Field collected samples							
1	Upper Gulf of Thailand	ww*	Min	0.02	0.97	16.2	Hungspreugs and Yuangthong [26]
		ww*	Max	0.08	3.04	43.0	
2	Coastal waters of Hong Kong	ww*	Min	0.05	2.72	15.1	Phillips [1]
		ww*	Max	0.24	474	27.9	
3	Thailand coastal waters	ww*	Min	0.15	1.60	10.4	Phillips and Muttarasin [29]
		ww*	Max	1.17	2.65	12.8	
4	Some coastal areas in Thailand and Malaysia	ww*	Min	0.09	1.02	7.14	Everaarts and Swennen [27]
		ww*	Max	0.38	1.39	8.33	
5	The Gulf of Thailand	ww*	Min	0.00	0.26	4.37	Sukasem and Tabucanon [39]
		ww*	Max	3.25	1.92	13.4	
6	Putai coast of Taiwan	ww*	Min	NA	0.30	2.45	Han, et al. [40]
		ww*	Max	NA	0.92	4.37	
7	Southeast coast of India	ww*	Min	0.27	5.71	10.3	Senthilnathan, et al. [41]
		ww*	Max	0.75	8.36	16.0	
8	The Gulf of Thailand	ww*	Min	0.03	0.50	4.23	Ruangwises and Ruangwises [42]
		ww*	Max	0.55	2.55	36.2	
9	Tolo Harbour, Hong Kong	ww*	Min	0.08	1.02	15.3	Wong, et al. [43]
		ww*	Max	0.24	4.08	22.9	
10	Guangdong market, China	ww		0.38	2.05	9.90	Fang, et al. [44]
11	Fish cultured sites at Hong Kong waters	ww*	Min	0.05	3.23	16.4	Wong et al. [45]
		ww*	Max	0.15	3.42	34.2	
12	Coastal waters of Singapore (8 sites)	ww*	Min	0.00	3.91	31.5	Bayen, et al. [3]
		ww*	Max	0.03	5.95	75.8	
13	Penang, Malaysia	ww*	Avg	0.00	1.36	12.9	Sivalingam and Bhaskaran [46]
14	Ban Merbok, Perak	ww	Avg	0.05	1.93	13.8	Liong [32]
15	Lekir, Perak	ww	Avg	0.18	2.70	22.8	Devi [30]
16	Kg. Masai	ww*	Avg	0.27	2.07	12.3	Yap, et al. [47]
17	Kg. Sg. Melayu	ww*	Avg	0.14	0.94	10.8	Yap, et al. [47]
18	Pulau Aman	ww	Avg	0.15	1.84	18.6	Yap, et al. [47]
19	Kuala Dinding	ww	Avg	0.18	1.32	15.3	Yap, et al. [47]
20	Bagan Lalang	ww	Avg	0.19	1.39	16.4	Yap, et al. [47]
21	Pasir Panjang	ww	Avg	0.18	1.85	16.8	Yap, et al. [47]
22	Kuala Linggi	ww	Avg	0.22	1.55	17.2	Yap, et al. [47]
23	Sebatu	ww	Avg	0.18	1.90	12.8	Yap, et al. [47]
24	Muar Estuary	ww	Avg	0.14	1.35	13.4	Yap, et al. [47]
25	Pantai Lido	ww	Avg	0.12	1.60	19.9	Yap, et al. [47]
26	Kg. Pasir Puteh	ww	Avg	0.14	3.42	21.9	Yap, et al. [47]
27	KPG-1	ww*	Avg	0.29	0.60	12.3	Yusof, et al. [5]
28	KPG-10	ww*	Avg	0.03	0.36	8.90	Yusof, et al. [5]
29	KPG-11	ww*	Avg	0.02	0.81	13.2	Yusof, et al. [5]
30	KPG-12	ww*	Avg	0.18	0.67	10.5	Yusof, et al. [5]
31	KPG-13	ww*	Avg	0.06	0.66	11.4	Yusof, et al. [5]
32	KPG-14	ww*	Avg	0.22	1.06	12.9	Yusof, et al. [5]
33	KPG-15	ww*	Avg	0.05	0.89	11.7	Yusof, et al. [5]
34	KPG-17	ww*	Avg	0.04	1.35	15.3	Yusof, et al. [5]
35	KPG-18	ww*	Avg	0.20	1.45	14.9	Yusof, et al. [5]
36	KPG-19	ww*	Avg	0.49	0.96	16.2	Yusof, et al. [5]
Laboratory study							
1	Three days of 0.20 mg Cu/L accumulation	ww	Avg	NA	9.97	NA	Yap, et al. [48]
2	Two days of depuration after three days of 0.20 mg Cu/L accumulation	ww	Avg	NA	5.50	NA	Yap, et al. [48]
3	Four days of 1.21 mg Cd/L accumulation	ww	Min	20.0	NA	NA	Yap, et al. [23]
		ww	Max	25.0	NA	NA	
4	Four days of depuration after four days of 1.21 mg Cd/L accumulation	ww	Min	0.74	NA	NA	Yap, et al. [23]
		ww	Max	30.0	NA	NA	

5	Four days of 1.95 mg Zn/L accumulation	ww	Min	NA	NA	26.0	Yap, et al. [23]
		ww	Max	NA	NA	44.3	
6	Four days of depuration after four days of 1.95 mg Zn/L accumulation	ww	Min	NA	NA	20.8	Yap, et al. [23]
		ww	Max	NA	NA	27.0	

Note: WB: Weight Basis; BDL: Below Detection Limit: *: Indicated the wet weights are converted from dry weight by a multiplying factor of 0.17 [49] for *Perna viridis*; NA: Not Available; Avg: Average; min: Minimum; max: Maximum.

Table 3: Maximum permissible limits or guideline limits for Cd, Cu and Zn concentrations (µg/g) for fish/shellfish set by different countries and agencies.

Guideline limits	WB	Cd	Cu	Zn
Permissible limits set by Malaysian Food Regulation [50]	Wet	1.00	30.0	100
International Council for the Exploration of the Sea [51] for status: 'increased contamination'	Dry	1.80	NA	NA
Maximum permissible levels established by Brazilian Ministry of Health [52]	Dry	5.00	150	250
Permissible limit set by Ministry of Public Health, Thailand [53]	Dry	NA	133	667
Food and Drug Administration of the United States [54]	Dry	25.0	NA	NA
	Wet	3.70	NA	NA
Australian Legal Requirements [55]	Dry	10.0	350	750
Permissible limit set by the Hong Kong Environmental Protection Department [56]	Wet	2.00	NA	NA
FAO limits [20]	Wet	2.0	10-30	40-100
	Dry*	10.0	50-150	200-500

Note: WB: Weight Basis; *: Concentrations in wet weight have been converted into dry weight by multiplying by a factor of 5 [57]; NA: Not Available.

(Table 3). All reviewed data in Table 2 shows that the three metal levels are also well below the MPLs, except for Cu levels in *P. viridis* collected from coastal waters of Hong Kong reported by Phillips [1].

The metal levels in mussels and cockles are also compared with international standards for metals in mollusks/shellfish (Table 3) compiled by the Food and Agriculture Organization (FAO) of the United Nations [20]. Most of these limits were originally presented in standard units of fresh weight. A comparison with the concentrations reviewed in mussels and cockles are clearly below the converted FAO limits in dry weight basis.

It should be noted that the ability to accumulate high levels of metals in *P. viridis* and *A. granosa* should be an important factor in determining the levels of heavy metals in their soft tissues. It is difficult to judge or conclude the extent of metal accumulation in the bivalves if they are not found in highly polluted coastal waters. To answer this, data obtained from the laboratory studies are reviewed.

Based the laboratory study reviewed in Table 1, a study reported by Chan, et al. [21] found that the *A. granosa* accumulated 20 µg/g wet weight of Cd after exposure of 0.25 mg/L Cd for 16 days. Again, this Cd concentration is 10 times higher than the maximum permissible limit (2 µg/g wet weight) by FAO and a few times higher than those set by other countries and agencies (Table 3).

Based on Table 2, exposures of *P. viridis* to sublethal concentrations of Cd resulted in high levels of Cd accumulated in the soft tissues of *P. viridis*. These metal levels far exceeded the MPLs set for food safety by FAO [20] for Cd. After 4 days depuration, the levels were still 17 times higher than the maximum permissible limit for Cd. All the above laboratory studies indicated a possibility of elevated levels of Cd to be accumulated in the edible soft tissues of mussels, and these concentrations were far exceeding the MPLs as indicated in Table 3.

Under laboratory experimental condition, for Cu and Zn (Table 1 and Table 2), the levels accumulated in the ST of *A. granosa* and *P. viridis* were well below the MPLs for both metals. This indicated the incapability of cockles and mussels to accumulate to such elevated levels of Cu and Zn to exceed the MPLs. The Cu and Zn levels found in the ST and cockles and mussels were not exceeding the MPLs after sublethal level exposures for both metals and followed by depuration (Table 1 and Table 2). This could be due to partial regulation mechanism in the essential Cu and Zn. However, no such mechanism for the non-essential Cd since most Cd accumulated in the body

Table 4: Values of estimated daily intake (EDI) and target hazard quotient (THQ) of Cd, Cu and Zn in *Anadara granosa*. Numbers (No.) follows the names of sampling locations in Table 1.

No.		Cd	Cu	Zn	CdEDI	CuEDI	ZnEDI	CdTHQ	CuTHQ	ZnTHQ
	Field collected samples									
1	Min	0.36	0.27	15.01	0.11	0.08	4.47	0.106	0.002	0.015
	Max	0.53	0.42	17.48	0.16	0.12	5.20	0.157	0.003	0.017
2	Min	0.63	0.89	19.57	0.19	0.27	5.83	0.186	0.007	0.019
	Max	1.09	0.91	25.08	0.32	0.27	7.47	0.324	0.007	0.025
3	Min	0.53	1.54	13.30	0.16	0.46	3.96	0.158	0.011	0.013
	Max	0.86	2.11	25.08	0.25	0.63	7.47	0.255	0.016	0.025
4	Min	0.05	0.86	7.98	0.01	0.26	2.38	0.014	0.006	0.008
	Max	0.15	1.40	11.00	0.05	0.42	3.27	0.045	0.010	0.011
5	Min	0.20	0.80	14.60	0.06	0.24	4.35	0.060	0.006	0.014
	Max	0.79	1.60	23.10	0.24	0.48	6.88	0.235	0.012	0.023
6	Min	1.16	1.20	12.16	0.34	0.36	3.62	0.345	0.009	0.012
7	Min	0.40	0.65	10.64	0.12	0.19	3.17	0.119	0.005	0.011
	Max	1.31	0.86	12.16	0.39	0.25	3.62	0.390	0.006	0.012
8	Min	1.23	0.64	12.90	0.37	0.19	3.84	0.366	0.005	0.013
	Max	1.42	0.80	14.70	0.42	0.24	4.38	0.423	0.006	0.015
9	Min	0.10	0.25	8.00	0.03	0.07	2.38	0.030	0.002	0.008
	Max	0.90	6.00	40.00	0.27	1.79	11.91	0.268	0.045	0.040
10	Avg	1.91	0.51	19.20	0.57	0.15	5.72	0.569	0.004	0.019
11	Min	NA	NA	35.15	NA	NA	10.46	NA	NA	0.035
	Max	NA	NA	44.84	NA	NA	13.35	NA	NA	0.044
12	Avg	0.30	1.25	17.84	0.09	0.37	5.31	0.090	0.009	0.018
13	Avg	0.17	2.58	20.33	0.05	0.77	6.05	0.051	0.019	0.020
14	Avg	0.24	1.27	24.89	0.07	0.38	7.41	0.071	0.009	0.025
15	Avg	0.22	3.82	36.10	0.07	1.14	10.75	0.066	0.028	0.036
16	Avg	0.23	1.22	18.75	0.07	0.36	5.58	0.069	0.009	0.019
17	Avg	0.33	0.51	11.97	0.10	0.15	3.56	0.098	0.004	0.012
18	Avg	0.25	1.40	38.66	0.07	0.42	11.51	0.074	0.010	0.038
19	Avg	0.63	1.07	17.46	0.19	0.32	5.20	0.188	0.008	0.017
20	Avg	1.79	1.03	18.09	0.53	0.31	5.39	0.534	0.008	0.018
21	Avg	0.13	0.38	10.87	0.04	0.11	3.24	0.038	0.003	0.011
22	Avg	0.65	0.62	18.77	0.19	0.18	5.59	0.194	0.005	0.019
23	Avg	0.49	0.32	10.72	0.15	0.09	3.19	0.147	0.002	0.011
24	Avg	0.47	0.60	14.67	0.14	0.18	4.37	0.141	0.004	0.015
25	Avg	0.41	0.46	13.95	0.12	0.14	4.15	0.121	0.003	0.014
26	Avg	0.28	0.49	13.26	0.08	0.15	3.95	0.084	0.004	0.013
27	Avg	0.51	0.58	25.84	0.15	0.17	7.69	0.151	0.004	0.026
28	Avg	0.84	0.41	30.02	0.25	0.12	8.94	0.251	0.003	0.030
29	Avg	0.51	0.56	18.94	0.15	0.17	5.64	0.150	0.004	0.019
30	Avg	0.12	1.73	18.75	0.04	0.51	5.58	0.035	0.013	0.019
31	Avg	0.16	0.37	10.13	0.05	0.11	3.01	0.046	0.003	0.010
32	Avg	0.28	0.51	12.52	0.08	0.15	3.73	0.082	0.004	0.012
33	Avg	0.12	0.93	19.76	0.04	0.28	5.88	0.037	0.007	0.020
34	Avg	0.07	0.42	12.26	0.02	0.12	3.65	0.020	0.003	0.012
35	Avg	0.03	0.35	7.94	0.01	0.10	2.36	0.010	0.003	0.008
	Laboratory study									
1	Min	4.00	NA	NA	1.19	NA	NA	1.191	NA	NA
	Max	20.00	NA	NA	5.95	NA	NA	5.953	NA	NA
2	Avg	NA	13.68	NA	NA	4.07	NA	NA	0.102	NA
3	Avg	NA	3.46	NA	NA	1.03	NA	NA	0.026	NA
4	Avg	NA	2.24	NA	NA	0.67	NA	NA	0.017	NA
5	Avg	NA	NA	50.35	NA	NA	14.99	NA	NA	0.050
6	Avg	NA	NA	35.53	NA	NA	10.58	NA	NA	0.035

Note: NA: Not Available; Avg: Average; Min: Minimum; Max: Maximum.

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is detoxified in the forms of lysosomes and metallothionein [22,23].

A consumable *A. granosa* in general had a higher level of Cd [24,25] than those in *P. viridis* while *P. viridis* could accumulate higher levels of Cu than those in the cockles. The levels of Zn in the cockles were comparable to those of *P. viridis*. Although the comparison of metal concentrations in mussels with those in the cockles carried out in different coastal environments are difficult, we can at least be able to answer that cockles can accumulate higher levels of Cd and mussels had higher levels of Cu than those of *P. viridis*.

Although the present assessment of risk due to heavy metal contamination in mussels and cockles is based on the available food safety guidelines for fish products, the assessment and comparison can still be argued from several points of views. First, 'Why the metal accumulated in the biological samples were still lower than the safety limits although they were collected from known polluted sites? Are the recommended MPLs not suitable for the mussels and cockles and they should be revised? The revision of the safety guidelines is prompted by several reasons. First, if the mussels and cockles have low capability in the accumulation of certain metals to concentrations as high as 30 µg/g wet weight for Cu and 100 µg/g wet weight for Zn, why such high concentrations of metals are still set at those levels and applied to those organisms? This is clearly shown in Table 1 and Table 2 which included polluted site at Tolo Harbour. Second, the mussels and cockles possess different pathways of accumulation and regulation. The high difference in the metal levels accumulated in the ST of mussels and cockles indicated that the different species has different pattern of accumulation as a result of differences in their ecology and biology [22]. Not all the metals are accumulated in biological tissues. Some of them could be regulated. For example, *P. viridis* is known to have a partial regulatory mechanism for Zn in its soft tissues [1,23]. Therefore, 100 g/g wet weight Zn guideline limit set by most countries and agencies, which is meant for all fish products, should be revised for *P. viridis* and *A. granosa*. Also, revision for Cu safety limit could be suggested and advised.

Target hazard quotient

The present reviewed data of Cd, Cu and Zn are recalculated for EDI and THQ. We assume that this HHRA is more relevant and reliable when compared to just direct comparison to the MPLs. First, the amount of the edible ST of bivalves is taken into calculation. Second, the body weight of the consumer is also taken into consideration. The THQ values for cockles and mussels are presented in Table 4 and Table 5, respectively. Table 6 shows the overall statistical values of Table 4 and Table 5.

From Table 4, the THQ values of Cd, Cu and Zn in the cockles were found to be all below 1.00, indicating no non-carcinogenic effects of the three metals but the Cd THQ value based on laboratory study was higher than 1.00. This indicated the Cd accumulation capacity in cockles to a level that can pose human health risk of Cd toxicity.

From Table 5, the THQ values of Cd, Cu and Zn in the mussels were found to be all below 1.00 except for a study by Phillips [1] based on Cu level in mussels collected from coastal waters of Hong Kong which recorded 474 µg/g wet weight. Interestingly, even the data based on laboratory study of Cu exposure did not exceed a THQ value of 1.00. This strongly indicated that the soft tissues of *P. viridis* has the capability of Cu accumulation that exceeding the maximum permissible limit of Cu and thus can pose a non-carcinogenic effect of Cu to the consumers. In addition, only the laboratory study of Cd exposure on the mussels exceeded a THQ value of 1.00. This indicated the Cd uptake can be highly accumulated in the soft tissues of *P. viridis* without regulation of this non-essential metal. Therefore, there is always risks of Cu and Cd toxicity and non-carcinogenic effects of Cd and Cu to the consumers of this popular Asian seafood.

From Table 6, an interesting point to note is for the Zn levels in both cockles and mussels reviewed in this study. Apparently, the THQ values of Zn based on field

Table 5: Values of estimated daily intake (EDI) and target hazard quotient (THQ) of Cd, Cu and Zn in *Perna viridis*. Numbers follows the names of sampling locations in Table 2.

No.		Cd	Cu	Zn	CdEDI	CuEDI	ZnEDI	CdTHQ	CuTHQ	ZnTHQ
Field collected samples										
1	Min	0.02	0.97	16.15	0.01	0.29	4.81	0.005	0.007	0.016
	Max	0.08	3.04	43.01	0.02	0.91	12.80	0.023	0.023	0.043
2	Min	0.05	2.72	15.13	0.02	0.81	4.50	0.015	0.020	0.015
	Max	0.24	474.30	27.88	0.07	141	8.30	0.072	3.530	0.028
3	Min	0.15	1.60	10.37	0.05	0.48	3.09	0.045	0.012	0.010
	Max	1.17	2.65	12.75	0.35	0.79	3.80	0.347	0.020	0.013
4	Min	0.09	1.02	7.14	0.03	0.30	2.13	0.026	0.008	0.007
	Max	0.38	1.39	8.33	0.11	0.41	2.48	0.112	0.010	0.008
5	Min	0.00	0.26	4.37	0.00	0.08	1.30	0.001	0.002	0.004
	Max	3.25	1.92	13.43	0.97	0.57	4.00	0.967	0.014	0.013
6	Min	NA	0.30	2.45	NA	0.09	0.73	NA	0.002	0.002
	Max	NA	0.92	4.37	NA	0.27	1.30	NA	0.007	0.004
7	Min	0.27	5.71	10.27	0.08	1.70	3.06	0.080	0.043	0.010
	Max	0.75	8.36	16.00	0.22	2.49	4.76	0.223	0.062	0.016
8	Min	0.03	0.50	4.23	0.01	0.15	1.26	0.009	0.004	0.004
	Max	0.55	2.55	36.21	0.16	0.76	10.78	0.164	0.019	0.036
9	Min	0.08	1.02	15.30	0.02	0.30	4.55	0.023	0.008	0.015
	Max	0.24	4.08	22.95	0.07	1.21	6.83	0.073	0.030	0.023
10		0.38	2.05	9.90	0.11	0.61	2.95	0.113	0.015	0.010
11	Min	0.05	3.23	16.44	0.02	0.96	4.89	0.016	0.024	0.016
	Max	0.15	3.42	34.17	0.04	1.02	10.17	0.044	0.025	0.034
12	Min	0.00	3.91	31.45	0.00	1.16	9.36	0.000	0.029	0.031
	Max	0.03	5.95	75.82	0.01	1.77	22.57	0.010	0.044	0.075
13	Avg	0.00	1.36	12.92	0.00	0.40	3.85	0.000	0.010	0.013
14	Avg	0.05	1.93	13.80	0.02	0.57	4.11	0.015	0.014	0.014
15	Avg	0.18	2.70	22.80	0.05	0.80	6.79	0.054	0.020	0.023
16	Avg	0.27	2.07	12.29	0.08	0.62	3.66	0.080	0.015	0.012
17	Avg	0.14	0.94	10.78	0.04	0.28	3.21	0.043	0.007	0.011
18	Avg	0.15	1.84	18.60	0.05	0.55	5.54	0.045	0.014	0.018
19	Avg	0.18	1.32	15.30	0.05	0.39	4.55	0.054	0.010	0.015
20	Avg	0.19	1.39	16.40	0.06	0.41	4.88	0.057	0.010	0.016
21	Avg	0.18	1.85	16.80	0.05	0.55	5.00	0.054	0.014	0.017
22	Avg	0.22	1.55	17.20	0.07	0.46	5.12	0.065	0.012	0.017
23	Avg	0.18	1.90	12.80	0.05	0.57	3.81	0.054	0.014	0.013
24	Avg	0.14	1.35	13.40	0.04	0.40	3.99	0.042	0.010	0.013
25	Avg	0.12	1.60	19.90	0.04	0.48	5.92	0.036	0.012	0.020
26	Avg	0.14	3.42	21.90	0.04	1.02	6.52	0.042	0.025	0.022
27	Avg	0.29	0.60	12.33	0.09	0.18	3.67	0.085	0.004	0.012
28	Avg	0.03	0.36	8.86	0.01	0.11	2.64	0.010	0.003	0.009
29	Avg	0.02	0.81	13.21	0.01	0.24	3.93	0.005	0.006	0.013
30	Avg	0.18	0.67	10.51	0.06	0.20	3.13	0.055	0.005	0.010
31	Avg	0.06	0.66	11.36	0.02	0.20	3.38	0.017	0.005	0.011
32	Avg	0.22	1.06	12.89	0.07	0.31	3.84	0.066	0.008	0.013
33	Avg	0.05	0.89	11.65	0.01	0.26	3.47	0.014	0.007	0.012
34	Avg	0.04	1.35	15.33	0.01	0.40	4.56	0.012	0.010	0.015
35	Avg	0.20	1.45	14.94	0.06	0.43	4.45	0.061	0.011	0.015
36	Avg	0.49	0.96	16.22	0.15	0.28	4.83	0.146	0.007	0.016
Laboratory study										
1	Avg	NA	9.97	NA	NA	2.97	NA	NA	0.074	NA
2	Avg	NA	5.50	NA	NA	1.64	NA	NA	0.041	NA
3	Min	20.0	NA	NA	5.95	NA	NA	5.95	NA	NA
	Max	25.0	NA	NA	7.44	NA	NA	7.44	N	NA
4	Min	0.74	NA	NA	0.22	NA	NA	0.22	N	NA
	Max	30.0	NA	NA	8.93	NA	NA	8.93	NA	NA

5	Min	NA	NA	26.0	NA	NA	7.74	NA	NA	0.026
	Max	NA	NA	44.3	NA	NA	13.2	NA	NA	0.044
6	Min	NA	NA	20.8	NA	NA	6.19	NA	NA	0.021
	Max	NA	NA	27.0	NA	NA	8.04	NA	NA	0.027

Note: NA: Not Available; Avg: Average; Min: Minimum; Max: Maximum.

Table 6: Overall statistics of mean concentrations (µg/g wet weight) of Cd, Cu and Zn and their values of estimated daily intake (EDI) and target hazard quotient (THQ) in *Anadara granosa* (AG) and *Perna viridis*, based on field collected samples.

AG	**Cd**	**Cu**	**Zn**	**CdEDI**	**CuEDI**	**ZnEDI**	**CdTHQ**	**CuTHQ**	**ZnTHQ**
N	44	44	46	44	44	46	44	44	46
Minimum	0.03	0.25	7.94	0.010	0.070	2.360	0.010	0.002	0.008
Maximum	1.91	6.00	44.8	0.570	1.790	13.350	0.569	0.045	0.044
Mean	0.54	1.03	18.7	0.162	0.307	5.579	0.162	0.008	0.019
Standard error	0.07	0.15	1.30	0.021	0.046	0.388	0.021	0.001	0.001
Median	0.40	0.72	17.5	0.120	0.215	5.200	0.120	0.006	0.017
Standard deviation	0.46	1.03	8.85	0.136	0.307	2.635	0.137	0.008	0.009
PV	Cd	Cu	Zn	CdEDI	CuEDI	ZnEDI	CdTHQ	CuTHQ	ZnTHQ
N	45	47	47	45	47	47	45	47	47
Minimum	0.001	0.26	2.45	0.00	0.076	0.73	0.00	0.002	0.002
Maximum	3.25	474	75.8	0.967	141	22.6	0.967	3.530	0.075
Mean	0.26	12.0	16.8	0.077	3.58	5.01	0.077	0.090	0.017
Standard error	0.07	10.1	1.75	0.022	2.99	0.52	0.022	0.075	0.002
Median	0.15	1.55	13.8	0.045	0.46	4.11	0.045	0.012	0.014
Standard deviation	0.50	68.9	12.0	0.150	20.5	3.57	0.150	0.513	0.012

Note: N: Number of Samples.

collected samples and laboratory study samples, are all below 1.00. First, there is clearly no non-carcinogenic effects of Zn. Secondly, a question could be arisen is that 'Is the MPL level (100 pm) set for Zn is too high and unrealistic since the MPL is set for all fishes and shellfish? If we are looking at the bivalves such as cockles and mussels, can we revise the MPL level for Zn lower since these two bivalves will not exceed the MPL level and not to exceed the Zn THQ value of 1.00? These two questions should become an interesting point for future studies since we are now should look into the different types of molluscs with different capabilities of metal uptake, regulation, sequestration, assimilation and accumulation.

Conclusions

Present study revealed that the Cd concentrations in the cockles were generally higher than those of mussels but Cu concentrations in the mussels were generally higher than those of cockles. Zn concentrations were comparable and not significantly ($P < 0.05$) different from each other. From the public health point of view, these metal concentrations were below the MPLs set by different and agencies. The THQ values were calculated based on the reviewed data of Cd, Cu and Zn in the cockles and mussels. The THQ values of Zn in the cockles and mussels were found to be all below 1.00, indicating no non-carcinogenic effects of Zn. Only the laboratory study of Cd exposure on the cockles and mussels exceeded a THQ value of 1.00, indicating capability of Cd uptake in high concentrations in the ST of *A. granosa* and *P. viridis* without regulation of this non-essential metal. Hence, there is always a risk of Cd toxicity and non-carcinogenic effects of Cd to the consumers. All Cu THQ values in the cockles and mussels are found to be below 1.00 except for the Cu level in mussels collected from coastal waters of Hong Kong [1]. This strongly indicated that the St of the field collected *P. viridis* has the capability of Cu accumulation that exceeding the Cu MPL and thus can pose a non-carcinogenic effect of Cu to the consumers. The recommended guidelines for the food safety for Zn should be revised because Zn accumulation in the body of cockles and mussels would not reach the MPL of Zn.

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