Bulletin of Environmental Contamination and Toxicology
https://doi.org/10.1007/s00128-018-2270-3

Concentrations of Cadmium, Copper, and Zinc in *Macrobrachium rosenbergii* (Giant Freshwater Prawn) from Natural Environment

Farah Akmal Idrus[1] · Masania Mohd Basri[1] · Khairul Adha A. Rahim[1] · Nur Syazwani Abd Rahim[1] · Melissa Dennis Chong[1]

Received: 20 April 2017 / Accepted: 5 January 2018

Abstract

This study analyzed the levels of cadmium (Cd), copper (Cu), and zinc (Zn) by the flame atomic absorption spectrophotometer (FAAS), in the muscle tissues, exoskeletons, and gills from freshwater prawn (*Macrobrachium rosenbergii*) (n = 20) harvested from natural habitat in Kerang River, Malaysia on 25th November 2015. Significant increase of the metals level in muscle tissue and gill ($r > 0.70$, $p < 0.05$) were observed with increase in length except for Cu in gills. No relationship was found between metals level in exoskeleton and length. The concentrations of Cd, Cu and Zn were significantly higher ($p < 0.05$) in males (muscle tissues and exoskeleton) except for Cd in exoskeleton. In gills, only Cu was significantly higher ($p < 0.05$) in female than male. All samples contained metals below the permissible limit for human consumption (i.e., Cd < 2.00 mg/kg; Cu < 30.00 mg/kg; Zn < 150 mg/kg). Annual metals monitoring in prawn and environmental samples is recommended to evaluate changes of metals bioaccumulation and cycling in the system, which is useful for resources management.

Keywords Freshwater prawn · Metals concentration · Sex and body length · Kerang River

Metals contamination in the aquatic ecosystems has become one of the greatest concerns towards the aquatic environmental bio-monitoring worldwide. Metals such as Cd and Zn had been known to occur in the phosphate fertilizers that used to enhance crop production especially in the nutrient-deficient land (Lambert et al. 2007). However, the cultivated land with the usage of phosphate fertilizers is susceptible to Cd, Cu and Zn contamination because these metals are naturally present in the phosphate rocks mainly used in the production of these fertilizers (Lambert et al. 2007). Moreover, the Cd, Cu and Zn absorbed by the cultivated soil can enter the river ecosystem by the leaching process and later accumulated through the food chain.

Kerang River floodplain is located in the Balai Ringin, Sarawak. Balai Ringin area was dense with mixed dipterocarp forest (Kendawang et al. 2004) and some part of oil palm plantation and cultivated area (Mohizah 2003). The basis of water quality of this river can be grouped into two different types which are brown water and black water (Rahim et al. 2009). The black water of this river is contributed from its tributaries which flow into the brown water river. Black water or commonly known as dark 'tea' colour water column in certain river or floodplain can happen naturally in the lowland river ecosystem and usually associated with high dissolved organic matter and low dissolved oxygen level (Howitt et al. 2007). Shuhaimi-Othman et al. (2009) emphasized that in black water surrounding, metals content in the sediments were generally higher than in the underlying water column due to the conversion of dissolved to the particulate metal and indirectly shows the pollution over long time. Kerang River is generally known to have abundant of *M. rosenbergii* in their water body that provide for livelihood for people resides around Kerang River. This may indirectly cause the metals accumulation in prawn body in this river to be higher than other river.

Macrobrachium rosenbergii is a nocturnal feeders and remains half-buried in sediments during daytime to hide from direct sunlight (Murthy et al. 2012) thus being susceptible to metals accumulation. Furthermore,they serves as regional and local fisheries importance and economic value since they meet a high demand as a source of protein and international export activities (Banu and Christianus 2016;

✉ Masania Mohd Basri
masania.mohdbasri@yahoo.com

[1] Faculty of Resource Science and Technology, Universiti Malaysia Sarawak, 94300 Kota Samarahan, Sarawak, Malaysia

Hameed and Bonami 2012; Islam et al. 2017). Hence, the assessments of the metals level in *M. rosenbergii* are crucial to determine the safety level for human consumption. There were several previous studies that indicate the significant correlation of metals and length of prawn and the significant difference between male and female prawn.

Therefore, the aims of this study were to determine the correlation between total length of *M. rosenbergii* and Cd, Cu and Zn concentrations in muscle tissues, exoskeleton, gills and eggs (if any) and to determine the significant difference of Cd, Cu and Zn concentrations in muscle tissue, exoskeleton and gills in male and female *M. rosenbergii*.

Materials and Methods

Wild-captured *M. rosenbergii* were caught using the one-layer fishing net in the Kerang River (Fig. 1). The net was employed for 4 h in the morning from 6:00 am to 10:00 am during the low tide. The sampling was done on 25th November 2015 during the *M. rosenbergii* season.

The chosen site for prawn sampling is the regular site (01°04.82′N, 110°45.97′E) for *M. rosenbergii* capture during the prawn season by the fishermen for trading or consumption purposes. Prawns were then stored in the zipper polyethylene bags, labelled properly and placed in the cooler box with ice during the transportation to the laboratory. The total wet weight and total body length (Table 1) of the individual prawn were recorded prior to dissection. The mature male and female *M. rosenbergii* can be identified by observing their second pleopod. In male *M. rosenbergii*, the mature male prawn develops the appendix masculina in their second pleopod which characterized especially for copulation (Karplus and Sagi 2010).

Table 1 Characteristics of *M. rosenbergii*

Sex	n	Reproductive state	Total length (cm) (Mean ± SD)	Total weight (g) (Mean ± SD)
Female	12	Mature	13.44 ± 1.29^{a}	22.11 ± 7.11^{a}
Male	8	Mature	14.34 ± 3.34^{b}	34.54 ± 23.09^{b}

Different letters (a, b) in the same column indicate the significant difference ($p < 0.05$)

Mature female generally have smaller proportion of head and claw than male prawn. They also have a brood chamber specify to carry their eggs by the first, second and third abdominal pleurae (Karplus and Sagi 2010). The dissected muscle tissues, exoskeleton and gills were placed in the clean acid-washed petri dish and were oven dried at 60°C until achieve a constant dried weight. The dried samples were ground to powder form using the mortar and pestle. Digestion process was done by heating the 0.2 g of powdered samples in the 10 mL of 65% v/v HNO_3 (Tu et al. 2008) on the hot plate until the solution turns clear.

The multi-elements preconcentration on the commercially available chelating resin (Toyopearl AF Chelate-650M) as describe by Arslan and Paulson (2002) were done for prawn samples. As the preconcentration step by using this chelating resin is pH dependent, all samples pH were adjusted to 5.5 ± 0.05 by adding the ammonium acetate buffer. The 2 M HCl solution were prepared to flush out all possible contaminations in the preconcentration system following by deionised water prior to the sample preconcentration process. The samples were analysed by using Flame Atomic Absorption Spectrophotometer (Thermo Scientific iCE 3500) (FAAS). The calibration of

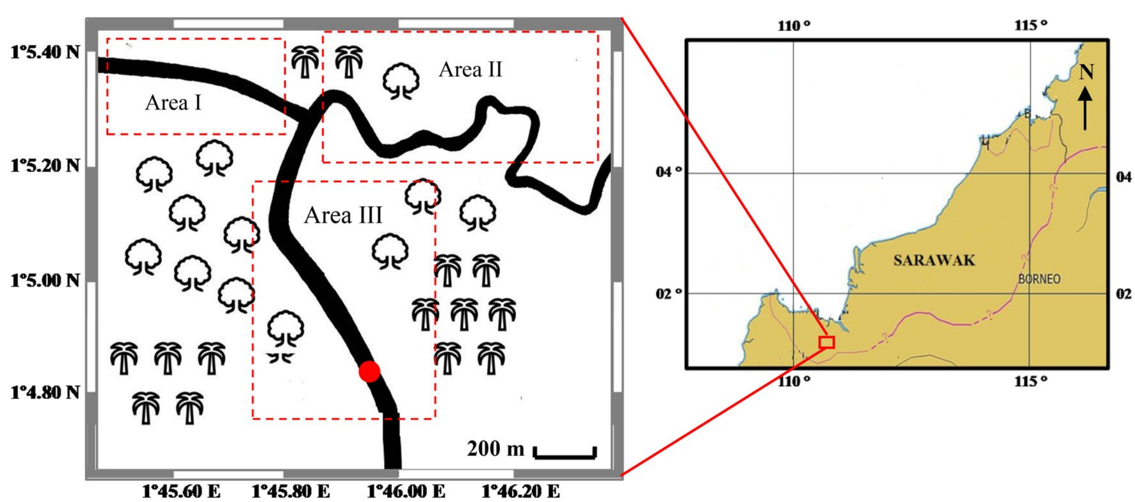

Fig. 1 Location of sampling area in Kerang River. Area I: Mixing of black and brown water, Area II: Barai River (blackwater tributaries), Area III: Brown water river. Red dot represents the sampling site of *M. rosenbergii*

Table 2 The metals standard concentration of Cd, Cu and Zn (mg/L) determined by FAAS

Metal	Standard 1	Standard 2	Standard 3	Correlation coefficient (r)
Cd (mg/L)	0.5	1.0	1.5	0.9983
Cu (mg/L)	1.0	3.0	5.0	0.9982
Zn (mg/L)	0.3	0.6	1.0	0.9996

standard was performed as the following concentrations shown in Table 2.

The certified reference material used was LUTS-1 non-defatted lobster hepatopancreas (National Research Council of Canada). All collected metals concentration data were expressed in flesh weight (FW). The collected data were analyzed by using Pearson's correlation coefficient to test the relationship of metals concentration and total length, while non-parametric test were done to test the mean difference between males and females, using the Minitab 17 Statistical Software, with the level of significance at $p<0.05$. All data are expressed as the mean ± standard deviation. Finally, the metals concentrations in edible muscle tissues were compared to the safety limits provided by Joint FAO/WHO Expert Committee on Food Additives (JECFA 2012).

Results and Discussion

The obtained values and metals recovery of Cd, Cu and Zn were 91 ± 4%, 108 ± 7% and 104 ± 5% respectively as stated in Table 3, which suggest the efficiency of the chelation methods used.

A positive correlation ($r^2=0.820$) was found between the total length and the total weight of *M. rosenbergii* collected from Kerang River (Fig. 2). Therefore, in order to study the metals concentrations in muscle tissues, exoskeleton and gills of *M. rosenbergii*, only the total length of prawn were used.

Cd, Cu and Zn concentrations in muscle tissues and gills were positively correlated ($r^2>0.70$) to the total length of *M. rosenbergii* (Fig. 3). Pattern of metals accumulation in the organisms body were normally associated with their size in which often explained in term of their exposure period to the contamination. Bigger size samples were

Table 3 The metals recovery of Cd, Cu and Zn (mg/kg) in LUTS-1

LUTS-1	Cd (mg/kg)	Cu (mg/kg)	Zn (mg/kg)
Certified values	14.2 ± 0.9	107 ± 8	82.9 ± 5.4
Obtained values	12.9 ± 0.6	116 ± 11	86.4 ± 3.5
Recovery (%)	91 ± 4	108 ± 7	104 ± 5

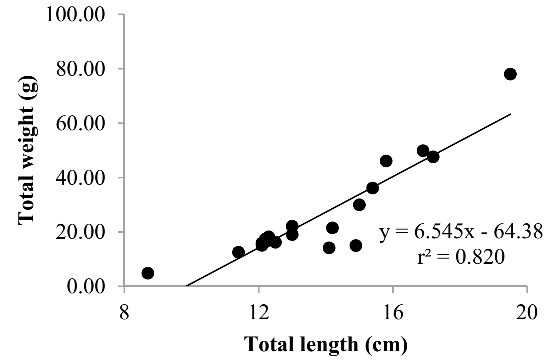

Fig. 2 Relationship between total length (cm) and total weight (cm) of *M. rosenbergii*

generally older thus more susceptible to their surrounding especially on the contaminated sediments because they preferred to live on river bed (benthic) rather than pelagic like other smaller prawn (Elahi et al. 2012; Lavalli and Spanier 2007; Wickins and Lee 2008). The results obtained were similar to the study done by Elahi et al. (2012) on the three species of crustaceans from Persian Gulf, Iran. Metals content in exoskeleton may be reduced in the process of moulting which involves the shedding of the chitinous exoskeleton layer and replaced by a new layer during the active grow of crustaceans (Pourang et al. 2004; Weis 2013; Stanek et al. 2014).

In this study, *M. rosenbergii* accumulates higher Cu and Zn concentrations than Cd. *M. rosenbergii* from Kerang River accumulated more Cu in gills than exoskeleton while muscle tissue possessed the lowest Cu. This result was in agreement with the study done by Canli and Furness (1993) on the Norway lobster *N. norvegicus*. Although same pattern of Cu accumulation was found, this study recorded lower Cu concentration (highest was 52.25 ± 9.37 mg/kg FW in gills) than that of *N. norvegicus* (250 ± 105 mg/kg). Cu is a component of respiratory system of crustaceans called hemocyanin (Rainbow 2002; Kouba et al. 2010; Stanek et al. 2014) hence cause the Cu concentration to be high in gills. Zn level detected in this study was highest in gills (14.69 ± 3.76 mg/kg FW). Metals in water have a high binding tendency to gills of aquatic organisms as they passed through the gills as the starting point (Playle 1998). Because Zn is a constituent of more than 200 metalloenzymes and other metabolic compounds, concentration of Zn in body of organism can be exceptionally high until it reaches the threshold level (Kouba et al. 2010). Zn concentrations in muscle tissue of *M. rosenbergii* in this study were 1.20 ± 0.41 mg/kg FW, which is considerably lower compared to Zn detected in muscle tissue of *P. inflatus* (Paez-Osuna et al. 1995) and *P. japonicus* (Canli et al. 2001). Cd in muscle tissue of *M. rosenbergii* in this study was lower than Cu and Zn because they may

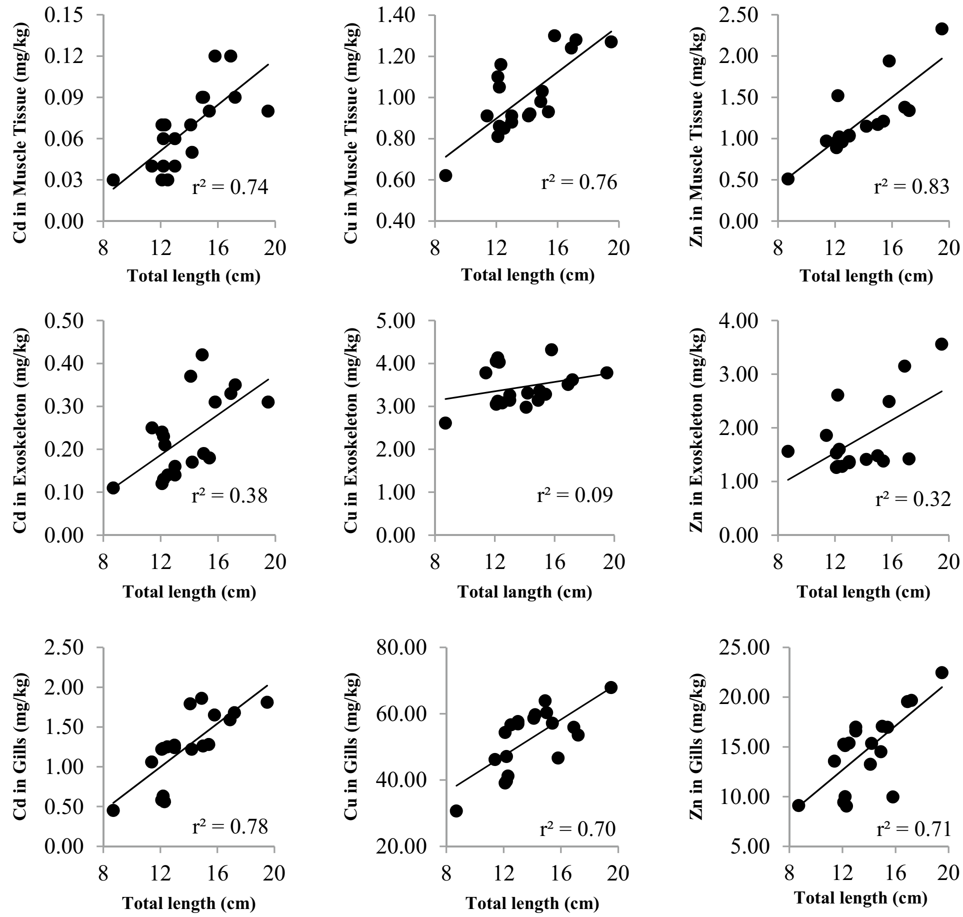

Fig. 3 Relationship between total length (cm) and Cd, Cu and Zn concentrations (mg/kg FW) in muscle tissue, exoskeleton and gills of *M. rosenbergii*. All metals in muscle tissue and gills were showing the strong positive correlation ($r^2 > 0.70$)

develop the mechanism in order to remove or excrete the harmful contaminants from their body (Burger 2007).

Several studies have documented the difference in metals accumulations in different sex of invertebrates and crustaceans, where several factors were influenced the difference such as diet (Beckvar et al. 1996; Elahi et al. 2012), changes in metabolic rate, hormonal and reproductive status, as well as size variations (Burger et al. 2007). In this study, Cd, Cu and Zn were found to be significantly higher in muscle tissue of male than in female ($p < 0.05$) similar to the Cu and Zn in exoskeleton (Fig. 4). However, Cd in exoskeleton and gills of male and female does not differ significantly ($p > 0.05$). Similar pattern was shown in the Zn concentrations in gills of male and female sample. Only Cu in gills accumulated significantly higher ($p < 0.05$) in female than in male (Fig. 4).

These results were in agreement with the several studies done on the *Palaemonetes varians* (Frenet and Alliot 1985) and *Panaeus californiensis* (Paez-Osuna and Tron-Mayen 1995). Both studies reported that Cd, Cu and Zn concentrations in some tissues, including muscle tissues of *P. varians* and *P. califoniensis* were higher in males than in females. Pourang et al. (2004) emphasize that the female crustaceans can be expected to possess a lower metals accumulation in their body that may be due to their higher growth rate. Energy consumption of females will be high to facilitate their gonadal development (Karplus and Sagi 2010; Ra'Anan et al. 1991) thus may cause the metals to be consumed rapidly through metabolic process.

Overall, the levels of Cd, Cu and Zn in muscle tissue of *M. rosenbergii* collected from Kerang River ranged from 0.03 to 0.12 mg/kg with an average of 0.07 ± 0.03 mg/kg for Cd, 0.62 to 1.28 (1.00 ± 0.18 mg/kg FW) for Cu and 0.51–2.33 (1.20 ± 0.41 mg/kg FW) for Zn, and all of the selected metals were below the safety limit set by JECFA (2012) of 2 mg/kg (Cd), 30 mg/kg (Cu) and 150 mg/kg (Zn).

In conclusion, *M. rosenbergii* collected from Kerang River were safe to be consumed in a considerable amount on a regular basis. Because the Cd, Cu and Zn in muscle tissues and gills of *M. rosenbergii* were significantly correlated to their total body length, it is expected that the bigger male could possess the higher concentration of these metals in their body especially in the muscle tissue.

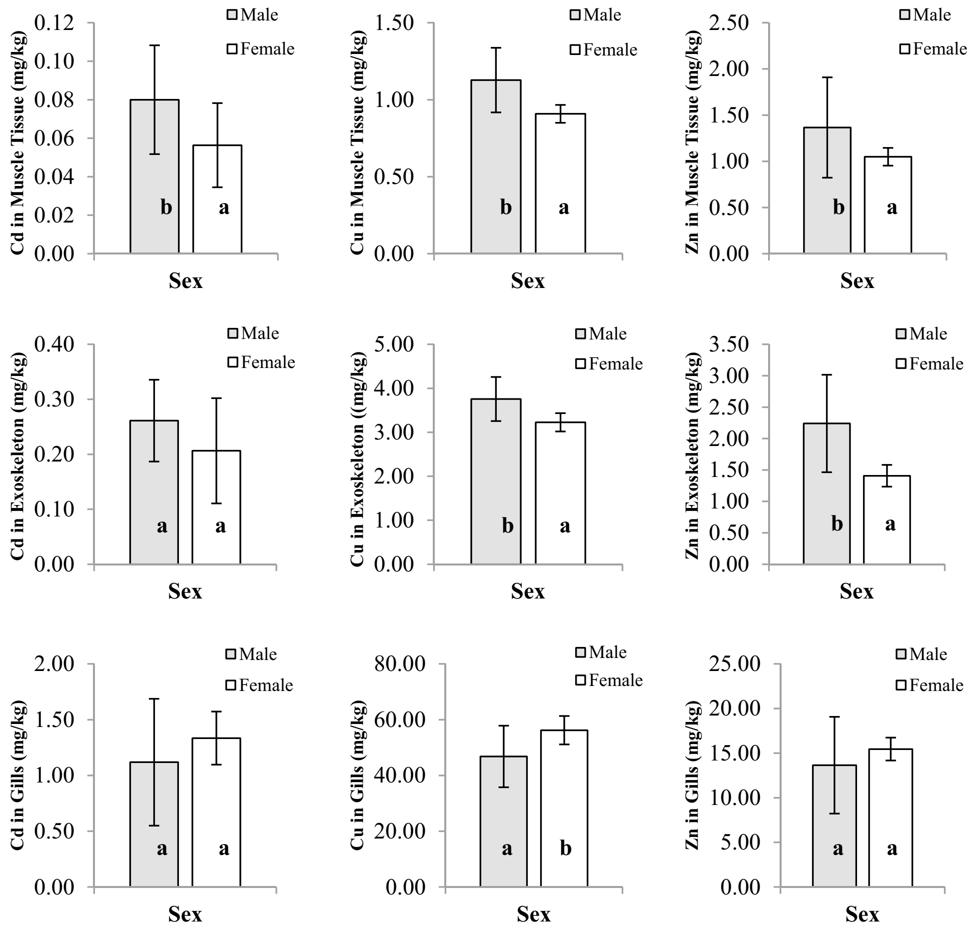

Fig. 4 Comparison of Cd, Cu and Zn (mg/kg FW) between males and females of *M. rosenbergii*. Different letters (a, b) in the same axis graph indicate the significant difference

Acknowledgements The authors would like to express our gratitude towards the provider of Grant RAGS/STWN01(01)/1178/2014(01), UNIMAS for facilities support and staffs of Faculty of Resource Science and Technology who involved during the sample collection and laboratory works.

References

Arslan Z, Paulson AJ (2002) Analysis of biogenic carbonates by inductively coupled plasma-mass spectrometry (ICP-MS). Flow injection on-line solid-phase preconcentration for trace element determination in fish otoliths. Anal Bioanal Chem 372:776–785

Banu R, Christianus A (2016) Giant freshwater Prawn *Macrobrachium rosenbergii* Farming: a review on its current status and prospective in Malaysia. J Aquac Res Dev 7:2

Beckvar N, Field J, Salazar S, Hoff R (1996) Contaminants in aquatic habitats at hazardous waste sites: mercury. NOAA, Seattle

Burger J (2007) A framework and method for incorporating gender-related issues in wildlife risk assessment: Gender- related differences in metal levels and other contaminants as a case study. Environ Res 104:153–162

Burger J, Fossi C, McClellan-Green P, Orlando EF (2007) Methodologies, bioindicators, and biomarkers for assessing gender related differences in wildlife exposed to environmental chemicals. Environ Res 104:135–152

Canli M, Furness RW (1993) Heavy metals in tissues of the Norway lobster Nephrops norvegicus: effects of sex, size and season. Chem Ecol 8:19–32

Canli M, Kalay M, Ay Ö (2001) Metal (Cd, Pb, Cu, Zn, Fe, Cr, Ni) concentrations in tissues of a *fish Sardina pilchardus* and a prawn *Peaenus japonicus* from three stations on the Mediterranean Sea. Bull Environ Contam Toxicol 67:75–82

Elahi M, Esmaili-Sari A, Bahramifar N (2012) Total mercury levels in selected tissues of some marine crustaceans from Persian Gulf, Iran: variations related to length, weight and sex. Bull Environ Contam Toxicol 88:60–64

Frenet M, Alliot A (1985) Comparative bioaccumulation of metals in *Palaemonetes varians* in polluted and non-polluted environments. Mar Environ Res 17:19–44

Hameed ASS, Bonami JR (2012) White tail disease of freshwater prawn, *Macrobrachium rosenbergii*. Indian J Virol 23:134–140

Howitt JA, Baldwin DS, Rees GN, Williams JL (2007) Modelling blackwater: Predicting water quality during flooding of lowland river forests. Ecol Model 203:229–242

Islam GR, Habib MR, Waid JL, Rahman MS, Kabir J, Akter S, Jolly Y (2017) Heavy metal contamination of freshwater prawn (*Macrobrachium rosenbergii*) and prawn feed in Bangladesh: a market-based study to highlight probable health risks. Chemosphere 170:282–289

JECFA (2012) Joint FAO/WHO food standards programme Codex committee on contaminants in food. Sixth Session

Karplus I, Sagi A (2010) The biology and management of size variation. In: New MB, Valenti WC, Tidwell JH, D'Abramo LR, Kutty

MN (eds) Freshwater prawns biology and farming. Wiley-Blackwell, New Jersey, pp 316–347

Kendawang JJ, Tanaka S, Ishihara J, Shibata K, Sabang J, Ninomiya I, Ishizuka S, Sakurai K (2004) Effects of shifting cultivation on soil ecosystems in Sarawak, Malaysia: I. Slash and burning at Balai ringin and Sabal experimental sites and effect on soil organic matter. J Soil Sci Plant Nutr 50:677–687

Kouba A, Buřič M, Kozák P (2010) Bioaccumulation and effects of heavy metals in crayfish: a review. Water Air Soil Pollut 211:5–16

Lambert R, Grant C, Sauvé S (2007) Cadmium and zinc in soil solution extracts following the application of phosphate fertilizers. Sci Total Environ 378:293–305

Lavalli KL, Spanier E (2007) The biology and fisheries of the slipper lobster. CRC press, New York

Mohizah M (2003) Floristic and structural variation in early successional tropical rain forest communities following shifting cultivation in Sarawak, Malaysia. Universiti Malaysia Sarawak (UNIMAS), Malaysia

Murthy SH, Kumarswamy R, Palaksha J, Sujatha H, Shankar R (2012) Effect of different types of shelters on survival and growth of giant freshwater prawn, *Macrobrachium rosenbergii*. J Mar Sci Tech 20:153–157

Paez-Osuna F, Tron-Mayen L (1995) Distribution of heavy metals in tissues of the shrimp *Penaeus californiensis* from the northwest coast of Mexico. Bull Environ Contam Toxicol 55:209–215

Paez-Osuna F, Perez-Gonzalez R, Izaguirre-Fierro G, Zazueta-Padilla H, Flores-Campana L (1995) Trace metal concentrations and their distribution in the lobster *Panulirus inflatus* (Bouvier, 1895) from the Mexican pacific coast. Environ Pollut 90:163–170

Playle RC (1998) Modelling metal interactions at fish gills. Sci Total Environ 219:147–163

Pourang N, Dennis JH, Ghourchian H (2004) Tissue distribution and redistribution of trace elements in shrimp species with the emphasis on the roles of metallothionein. Ecotoxicology 13:519–533

Ra'Anan Z, Sagi A, Wax Y, Karplus I, Hulata G, Kuris A (1991) Growth, size rank, and maturation of the freshwater prawn, *Macrobrachium rosenbergii*: analysis of marked prawns in an experimental population. Biol Bull 181:379–386

Rahim KAA, Daud SK, Siraj SS, Arshad A, Esa Y, Ibrahim ER (2009) Freshwater fish diversity in Batang Kerang floodplain, Balai Ringin, Sarawak, Pertanika. J Trop Agric Sci 32:7–16

Rainbow PS (2002) Trace metal concentrations in aquatic invertebrates: why and so what? Environ Pollut 120:497–507

Shuhaimi-Othman M, Ahmad A, Lim E (2009) Metals concentration in water and sediment of Bebar peat swampy forest river, Malaysia. J Biol Sci 9:730–737

Stanek M, Dabrowski J, Dlugosz J, Janicki B (2014) Impact of the anthropogenisation on the metals bioaccumulation and distribution in the Spiny-Cheek Crayfish (*Orconectes limosus* Raf.) from Lake Gaplo, Poland. Int J Environ Res 8:1315–1322

Tu NPC, Ha NN, Ikemoto T, Tuyen BC, Tanabe S, Takeuchi I (2008) Bioaccumulation and distribution of trace elements in tissues of giant river prawn *Macrobrachium rosenbergii* (Decapoda: Palaemonidae) from South Vietnam. Fish Sci 74:109–119

Weis JS (2013) Physiological, developmental and behavioral effects of marine pollution. Springer, Dordrecht

Wickins JF, Lee DOC (2008) Crustacean farming: ranching and culture. Wiley, Hoboken