



Toxic metals in *Perna viridis* mussel and surface seawater in Pasir Gudang coastal area, Malaysia, and its health implications

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Abstract

Contamination of toxic metals in *P. viridis* mussels has been prevalently reported; hence, health risk assessment for consuming this aquaculture product as well as the surrounding surface seawater at its harvesting sites appears relevant. Since Kampung Pasir Puteh, Pasir Gudang is the major harvesting site in Malaysia, and because the last heavy metal assessment was done in 2009, its current status remains unclear. Herein, flame atomic absorption spectrometry and flow injection mercury/hydride system were used to determine the concentrations of Pb, Cd, Cu and total Hg in *P. viridis* mussels and surface seawater (January–March 2015), respectively. Significantly higher concentrations of these metals were found in *P. viridis* mussels ($p < 0.05$) than that of surface seawater samples. The concentrations for Pb (4.27–6.55 $\mu\text{g/g}$) and Cd (1.55–2.21 $\mu\text{g/g}$) in *P. viridis* mussels exceeded the maximum permitted proportion prescribed by the Malaysian law. The concentrations of all metals in surface seawater also violated the Malaysia Marine Water Quality Criteria and Standards. Significant ($p < 0.05$) and high strength of association ($r = 0.787$) observed between Pb concentration in *P. viridis* mussel with the surface seawater indicates its possible application for inferring Pb concentrations in the mussel. Since both the calculated target hazard quotient and hazard index for Pb and Cd exceeded 1, the possible detrimental health impacts on human for consuming *P. viridis* mussels from this rearing site cannot be ignored. Hence, promoting continuous monitoring programmes and developing efficient toxic metal removal techniques prior to entering the market are required.

Keywords *P. viridis* mussel · Toxic metals · Risk assessment · Surface seawater · Flame atomic absorption spectrometry · Flow injection mercury/hydride system

Introduction

Perna viridis (family: Mytilidae) is an economically important coastal bivalve mollusc, known as the Asian green mussel, Philippine green mussel or green-lipped mussel in certain parts of the world. The mussels are commonplace along the coastal marine waters of the Indo-Pacific region (Gosling 2003), as well as at several portions of coastal areas of Peninsular Malaysia (Ismail et al. 2000). Since *P. viridis* mussels contain about

60% of protein for every 100 g of its dry weight (Choo and Ng 1990) with substantial amounts of vitamins and trace elements (Gopalakrishnan and Vijayavel 2009), they become an important source of nutrients for human (Yap et al. 2004a). Alarming, toxic metals from natural and anthropogenic sources that continuously enter aquatic ecosystem would increase the possibility of posing serious threat towards aquatic organisms due to their toxicity, long-term persistence, bioaccumulation and biomagnification in the food chain (Papagiannis et al. 2004; Chua et al. 2014; Edbeib et al. 2016, 2017). Bivalves like *P. viridis* have the ability to accumulate toxic metals in their soft tissues to relatively higher levels than that of their environment without exhibiting any detrimental effects (Wagner and Boman 2004). Therefore, the use of *P. viridis* as a biomonitoring agent for heavy metal pollutions within the coastal environment has also been suggested (Nicholson and Szefer 2003; Arockia et al. 2012; Hadibarata et al. 2012), attributable to its cost effectiveness, as well as reliability (Yap et al. 2006).

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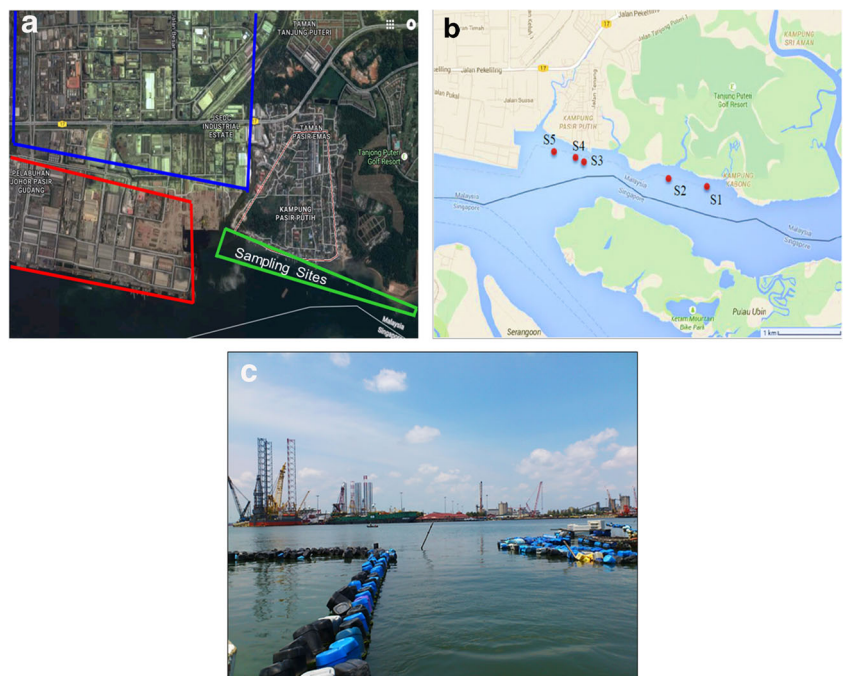
Interestingly, while Johor has been the largest producer for *P. viridis* mussels in Malaysia (Department of Fisheries 2013), its major harvesting area (i.e. Kampung Pasir Puteh) is located adjacent to the Pasir Gudang Seaport and industrial zones (Yap et al. 2004b). Kampung Pasir Puteh has been exposed to petrochemical and its related industries, as well as shipping, land reclamation and urbanisation (Yap et al. 2004b). Pertinently, the cumulative rearing area for this important aquaculture species at Kampung Pasir Puteh has soared tremendously from 133,501 m² (in 2009) to 287,248 m² (in 2015), a twofold increase over the span of 6 years (Department of Fisheries Malaysia 2015). In addition, the million freight weight tonnes (FWT) managed by the Pasir Gudang Seaport has increased from 23.9 million FWT in 2009 to 32.9 million FWT for 2015, a staggering increment of 38% of commodity load over 6 years (MMC Corporation Berhad 2015). Since the strait that borders Malaysia and Singapore is narrow and because the rearing site is limited within the Malaysian border alone, the ever-increasing rearing area has grown closer to the bustling Pasir Gudang Seaport and JSEDC Industrial Estate (Fig. 1a–c). Hence, the possibility of increasing contamination by toxic metals from the seaport and industrial activities into the surrounding seawater where the mussels are reared could not be ruled out.

Since ecotoxicological studies performed on *P. viridis* mussel at Kampung Pasir Puteh between 1998 to 2009 (Yap et al. 2002, 2003, 2004a, b, 2005, 2006; Ng et al. 2013) reported substantial amounts of lead (Pb) and cadmium (Cd), and because similar analysis on such mussels sampled beyond 2009 remains unavailable, this present study that evaluated such aspect acquires considerable pertinence. Considering the

adjacent increasing industrial activities, especially those related to mercury (Hg), as well as public awareness on toxicities of Hg associated with seafood consumption (Hajeb et al. 2012; Dong et al. 2015), its presence in *P. viridis* mussel was also assessed here. Similarly, Cu contamination may originate from industrial effluents, municipal waste outfalls and sediment dredging as well as from leaching of antifouling paints of ships, entering marine and estuary waters. Although Cu is considered as an essential element for aquatic environment, its toxicity can occur when the concentration exceeds certain ranges (Baltas et al. 2016). To date, only the Indian jurisdiction (Food Safety and Standards Authority of India, FSSAI 2011) has provided the maximum permitted proportion for Cu in mussels. Interestingly, while investigation on the physiological response *P. viridis* when exposed to Cu (in view of its potential use as cytological marker of pollution) has been reported (Nicholson 2001), the health risk assessment of Cu in the mussel for inferring potential human toxicity following its ingestion remains unreported.

Acute exposure to high levels of toxic metals may result in brain damage, paralysis, anaemia and disruption of gastrointestinal system. On the other hand, chronic exposure of such contaminants has been associated with damages of the kidneys, as well as reproductive, immune, nervous, respiratory and cardiovascular systems (Cope et al. 2004; Flanagan et al. 2008). In addition, miscarriage, stillbirth, premature birth and low birth weight, as well as malformations of foetus among pregnant women exposed to toxic metals, have been reported (World Health Organisation, WHO 2016). In view of such toxicological risks towards public health, continuous assessment of toxic metals in seafood such as *P. viridis* mussel

Fig. 1 a Aerial view of the sampling sites of *P. viridis* mussels at Kampung Pasir Puteh, Pasir Gudang, Johor, b harvesting sites (S1–S5), and c its proximity to the Pasir Gudang Seaport



merits serious consideration. In this context, determining if the concentrations of such contaminants in *P. viridis* mussels and surrounding seawater samples are within the maximum permitted proportions of metal contaminants prescribed by the Malaysian and international laws proves relevance.

Hence, this cross-sectional research that assessed the concentrations of Pb, Cd, Cu and total Hg in the harvested *P. viridis* mussels, as well as its surrounding seawater samples, from different sampling sites within Kampung Pasir Puteh harvesting area during January–March 2015 acquires significance. The findings may be beneficial for the environmental and health authorities for formulating suitable intervention programmes for managing this significant issue of public interest for benefiting the community at large.

Materials and methods

Standards and reagents

The reagents used in this study were analytical-grade stannous chloride dihydrate and potassium permanganate purchased from Merck (Darmstadt, Germany) and used without further purification. Standards for Pb, Cd and Cu in 2% HNO₃ and total Hg in 10% HNO₃ were both purchased from Merck (Germany) (1000 mg/L for each standard). All standards and sample solutions were prepared using deionised water produced by Sartorius arium® pro ultrapure water system (Gottingen, Germany). Concentrated acids, i.e. HNO₃ (65%), H₂SO₄ (95–97%) and HCl (37%), as well as H₂O₂ (35%), for sample digestion were acquired from QR&C (Malaysia).

Sampling and sample preparation

In three replicates, *P. viridis* mussels and surrounding surface seawater samples were collected from the five different harvesting sites (labelled as S1–S5) of Kampung Pasir Puteh, Pasir Gudang, Johor, Malaysia (1°25′–26′N, 103°55′–57′E) (Fig. 1a–c) during January, February and March 2015. Each *P. viridis* sample (a pooled of 15 mature mussels) was collected from each harvesting site, placed in a cold box that contained ice cubes, transported to the laboratory and kept at –20 °C prior to analysis. Using the same cold box, the surface seawater samples (in the different acid-washed, 10% HNO₃, polyethylene bottles) collected from all the harvesting sites were acidified using concentrated HNO₃ (67%, 5 mL). The samples were then transported to the laboratory and kept at 4 °C before analysis.

To prevent contamination from sand and dirt from the external surface of mussel, the shell surface was cleaned using running tap water and a brush. Then, the mussel was thawed at room temperature (about 26 °C) on a clean tissue paper with

the posterior part facing downwards for draining the water content in the shell. Upon completion, the flesh of each mussel was removed and dried at 60 °C for 72 h to a constant dry weight. All the 15 *P. viridis* mussels that formed a sample were then homogenised by grinding with a porcelain mortar and pestle to a fine powdery form and stored in an airtight plastic container at room temperature, prior to analysis. Considering better homogeneity of samples as well as the availability of suitable guidelines for inferring toxicity, dry weight of *P. viridis* mussel was used in this research. All *P. viridis* mussels and surface seawater samples collected during the three different sampling intervals (January, February and March 2015) were analysed quantitatively for Pb, Cd, Cu and total Hg.

For analysing Pb, Cd and Cu using flame atomic absorption spectrometry (FAAS), the *P. viridis* sample was acid-digested following the method described by previous researchers (Rahman et al. 2012) with several modifications. The modifications introduced here included the use of hotplate instead of water bath, initial heating temperature at 40 °C for 1 h, as well as longer duration of digestion (from 20 min to 2 h). A sample (0.5 g dry weight) of powdered mussel was placed in a conical flask through a filter funnel, followed by the addition of concentrated H₂SO₄ (2 mL) and HNO₃ (4 mL). After the initial vigorous reaction subsided, the mixture was heated on a hot plate for 1 h at 40 °C. Upon completion, H₂O₂ was added drop wise into the mixture until a clear solution was obtained. For total Hg analysis using flow injection mercury/hydride system (FI-MHS), the *P. viridis* sample was acid digested following the method described by Chahid et al. (2014). A sample (0.5 g dry weight) was digested using a mixture that consisted of concentrated HNO₃ (4 mL), H₂SO₄ (2 mL) and HCl (1 mL). The mixture was heated at 80 °C until it was fully digested. Upon cooling to room temperature, both mixtures were filtered using Whatman No. 1 filter papers into two different 50-mL volumetric flasks, subsequently diluted with deionised water and subjected to analysis with FAAS and FI-MHS, respectively.

Surface seawater sample was digested according to the standard method (U.S. EPA 3005A) prescribed by the US Environmental Protection Agency (US EPA 1992). Fifty millilitres of well-mixed sample was transferred into a 100-mL beaker, followed by the addition of concentrated HNO₃ (1 mL) and HCl (2.5 mL). The sample was heated on a hot plate at about 90 °C until the volume was reduced to approximately 10 mL. The sample was allowed to cool to room temperature, filtered into a 50-mL volumetric flask and made up to the mark with deionised water. For total Hg determination, the surface seawater sample was filtered using a Whatman No. 1 filter paper and analysed directly without any pre-treatment.

Analysis of toxic metals with FAAS and FI-MHS

Air-acetylene FAAS (Perkin Elmer PinAAcle 900 T, USA) was used for quantifying the concentrations of Pb, Cd and Cu in all *P. viridis* and surface seawater samples, while total Hg was determined using FI-MHS (Perkin Elmer FIAS 100, U.S.A.). The conditions for FAAS were air flow 10 L/min, acetylene flow 4 L/min and wavelengths at 283.3, 228.8 and 324.8 nm for Pb, Cd and Cu, respectively, with a slit width of 0.7 nm. The conditions for FI-MHS were argon flow 90 mL/min, 20 s integration time, wavelength at 253.7 nm and a slit width of 0.7 nm.

The working standards for Pb (0–5 mg/L), Cd (0–1 mg/L), Cu (0–1 mg/L) and total Hg (0–10 µg/L) were prepared by diluting the concentrated stock solutions (1000 mg/L) with suitable amounts of deionised water. As for total Hg standard, 3% HCl solution was used. For total Hg analysis, a reducing agent (1.1% SnCl₂) was prepared by placing 11 g of SnCl₂·2H₂O into a 50-mL beaker. Then, 10 mL of deionised water and 3 mL of concentrated HCl were added into the beaker, and the solution was left to stand for 10 min for complete dissolution. The solution was transferred into a 1000-mL volumetric flask, diluted up to the mark using deionised water and then inverted and shaken well. A carrier solution (3% HCl) for the FI-MHS analysis was prepared by transferring 30 mL of concentrated HCl into a 1000-mL volumetric flask. The solution was diluted by adding deionised water. A KMnO₄ solution (5%) was prepared by placing 1.25 g of KMnO₄ into a 25-mL volumetric flask. The compound was then diluted using deionised water. The calibration standard solutions of total Hg and blank were stabilised by adding four drops of a 5% KMnO₄ solution into 100 mL of solution. The concentrations of toxic metals in *P. viridis* mussels were expressed in µg/g dry and wet weights, while mg/L was used for the surface seawater samples. For enabling appropriate comparison with the concentrations of toxic metals in molluscs as prescribed by different international standards, the dry weight was converted to wet weight using a conversion factor of 0.17 recommended by previous researchers (Yap et al. 2004a).

Analytical method validation

This research utilised the analytical methods for analysing Pb, Cd and Cu (Rahman et al. 2012) as well as total Hg (Chahid et al. 2014) in seafood described by previous researchers, and the methods were partially validated once again here. Calibration curves for Pb (0, 1, 2, 3, 4 and 5 mg/L), Cd (0, 0.2, 0.4, 0.6, 0.8 and 1.0 mg/L), Cu (0, 0.2, 0.4, 0.6, 0.8 and 1.0 mg/L) and total Hg (0, 2, 4, 6, 8 and 10 µg/L) were prepared by spiking the samples with known amount of analyte via serial dilution. Using the coefficient of determination (R^2), the calibration curves were constructed using the absorbance values versus the concentrations of analyte added. The obtained calibration curves were accepted only when (1) the

coefficient of determination (R^2) was > 0.995 and (2) the relative standard deviation (RSD) was ≤ 20% (US EPA 2010). The limit of detection (LOD) and limit of quantitation (LOQ) for all the heavy metals were mathematically determined from the standard calibration curves using the equations (Sanagi et al. 2009) described below:

$$LOD = \frac{3 \times \text{standard deviation}}{\text{slope}} \quad (1)$$

$$LOQ = \frac{10 \times \text{standard deviation}}{\text{slope}} \quad (2)$$

Using six replicates, the recovery of Pb, Cd, Cu and total Hg in *P. viridis* mussel and surface seawater was investigated. Standard solutions containing Pb, Cd, Cu and total Hg were individually used to spike samples of *P. viridis* mussel (0.5 g) and surface seawater (50 mL). After acid digestion, the spiked sample in a calibrated volumetric flask was further added with deionised water to the mark, prior to analysis. The percentage recovery of each of the four heavy metals analysed was calculated using Eq. 3 (Vipulanandan 2004) described below:

$$\text{Percentage recovery} = \frac{A_T - A_i}{A_{\text{added}}} \times 100 \quad (3)$$

where A_T refers to the total amount measured in the spike sample, A_i represents the amount measured in the unspiked sample and A_{added} is the amount of known analyte (standard) added to the sample.

Estimation of daily intake of toxic metals and health risk assessment

The adult estimated daily intake (EDI) for Pb, Cd, Cu and total Hg through ingestion of *P. viridis* mussel was calculated following the equation suggested by Song et al. (2009) (Eq. 4), and compared with the provisional tolerable daily intakes (PTDI) suggested by the Joint FAO/WHO Expert Committee on Food Additives (JECFA 1999, 2003) and the US EPA (2011):

$$EDI = \frac{C_{\text{metal}} \times W_{\text{mussels}}}{B_w} \quad (4)$$

EDI refers to an adult daily intake of a heavy metal via consumption of *P. viridis* mussels (in µg/g/day), C_{metal} is the concentration of a heavy metal in such mussels (in µg/g, wet weight), W_{mussels} being the daily consumption of mussels (in g/day), and B_w represents the body weight of an adult (in kg).

Because specific data on daily consumption of mussels remains unavailable for Malaysia, W_{mussels} was estimated from the data reported by the US EPA (2000), i.e. 17.5 g. In addition, the Malaysian Adult Nutrition Survey reported 63 kg as the average B_w for Malaysians (Azmi et al. 2009). For

assessing the risk of Pb, Cd, Cu and total Hg through consumption of *P. viridis* mussels, the target hazard quotient (THQ) was also calculated based on the US EPA Region III risk-based concentration table (US EPA 2000) (Eq. 5):

$$THQ_i = \frac{EF_r \times ED \times W_{mussel} \times C_i^{metal}}{RfD \times Bw \times AT_n} \times 10^{-3} \quad (5)$$

While EF_r represents the exposure frequency (350 days/year), ED , $W_{mussels}$ and C_i^{metal} are the exposure duration (70 years), daily consumption of *P. viridis* mussels (in g/day) and the individual concentration of heavy metal in *P. viridis* mussels (in $\mu\text{g/g}$, wet weight), respectively. In addition, RfD refers to the oral reference dose (in $\mu\text{g/g/day}$) provided by the US EPA (2011), B_w is the average body weight of an adult (63 kg) and AT_n being the average exposure time for non-carcinogens ($ED \times 365$ days/year).

Since the RfD value for Pb remains unreported and there is no evidence of a threshold below which a non-harmful intake could be allowed for Pb (USEPA 2004), the THQ for Pb was calculated using the equation suggested by Jović and Stanković (2014) detailed below:

$$THQ = C/MRL \quad (6)$$

where C represents Pb concentration in mussels (mg/kg w/w) and MRL (maximum residue limit) set by the Regulation (EC) No 1881/2006 (European Communities 2006).

Considering the exposure of multiple toxic metals within the *P. viridis* mussels to human, the total hazard index (HI) was computed by summing all the calculated THQ values of toxic metals as suggested by Li et al. (2013) (Eq. 7):

$$HI = \sum_{i=1}^n THQ_i \quad (7)$$

while THQ_i refers to the target hazard quotient of an individual heavy metal, HI is the total hazard index for all the four toxic metals analysed here viz. Pb, Cd, Cu and total Hg with the n value of 4.

Statistical analysis

All data were analysed using the IBM SPSS version 20.0. Kolmogorov–Smirnov and Shapiro–Wilk tests were used to determine the normality of the distribution of data. Because the data were all normally distributed, the one-way ANOVA with Tukey–Kramer post hoc test was used to determine the significant differences among groups (January, February and March 2015). Independent samples t test was used for comparing the concentrations of Pb, Cd, Cu and total Hg between *P. viridis* mussel with that of surrounding surface seawater. For studying the association between concentrations of Pb, Cd, Cu and total Hg in *P. viridis*

mussel with that of surface seawater samples, Pearson correlation coefficient analysis was utilised. For defining the strength of correlation coefficient, the categorical definition (very high 0.90–1.00; high 0.70–0.89; moderate 0.50–0.69; low 0.26–0.49; little 0.00–0.25) suggested by Munro (2005) was used. Level of significance of 0.05 ($\alpha < 0.05$) was utilised for determining the significant differences among groups (Munro 2005).

Results and discussion

Analytical method validation

The calibration parameters, LOD and LOQ, as well as percentage recovery, are presented in Table 1. It has been indicated that to accept a calibration curve, the R^2 must be greater than 0.995 with RSD of less than or equal to 20% (Man et al. 2006). Therefore, considering that all the calibration curves included in this present research had fulfilled such prescribed criteria, it can be construed that the responses obtained were linear and hence determination of those analytes in the *P. viridis* mussel and surface seawater samples proved to be appropriate. LOD is the lowest concentration of an analyte determined with signal-to-noise ratio of at least 3:1, while LOQ refers to the lowest concentration of an analyte in a calibration curve with signal to noise ratio of at least 10:1 (Sanagi et al. 2009). Using the data from calibration curves, the LODs for Pb, Cd, Cu and Hg were calculated as 0.140, 0.026, 0.022 and 0.360 $\mu\text{g/L}$, respectively (Table 1). In addition, the LOQs were 0.460, 0.087, 0.075 and 1.200 $\mu\text{g/L}$, correspondingly (Table 1). Results also revealed that the percentage recoveries for Pb, Cd, Cu and Hg in *P. viridis* mussel ranged between 94.0 and 95.9%, 101 and 113%, 97.5 and 102% and 80.0 and 83.0%, respectively (Table 1). While the percentage recovery for Hg in surface seawater was not determined because the sample was analysed directly without any digestion, the percentage recoveries for Pb, Cd and Cu in this matrix ranged between 97.3 and 98.0%, 94.2 and 102% and 99.3 and 102%, respectively (Table 1). Considering that the percentage recoveries for all the analytes analysed here fell well within the acceptable range (75.0–120%) prescribed by previous researchers (AOAC 2002; Ryu et al. 2016), the analytical method used in this present research appears reliable.

Toxic metals in *P. viridis* mussel and surface seawater at Kampung Pasir Puteh, Pasir Gudang, Johor

Concentrations of four toxic metals (Pb, Cd, Cu and total Hg) that are commonly associated with various adverse effects in human as well as environment in *P. viridis* mussels were investigated. While high concentrations of Pb, Cd and Cu in the soft tissues of *P. viridis* mussels collected from Kampung Pasir Puteh, Johor had been reported between 2002 and

Table 1 Analytical figures of merit (calibration curves, LOD and LOQ) as well as percentage recovery (%) of heavy metals in *P. viridis* mussel and surface seawater samples

		Heavy metals					
		Pb			Cd		
Calibration curves	Range	0–5 mg/L			0–1 mg/L		
	R ²	0.9996			0.9996		
Equation		$y = 0.01550x + 0.000020$			$y = 0.2285x + 0.0013$		
LOD; LOQ		0.14 mg/L; 0.46 mg/L			0.026 mg/L; 0.087 mg/L		
		Pb			Cd		
Spiked conc. (µg/g)		1	2	4	0.2	0.5	0.7
<i>P. viridis</i> mussel	Recovered concentration (µg/g)	0.959 ± 0.0259	1.88 ± 0.0714	3.78 ± 0.0982	0.202 ± 0.0105	0.566 ± 0.0181	0.759 ± 0.0433
	Percentage recovery (%)	95.9	94.0	94.5	101	113	108
	%RSD	2.7	3.8	2.6	5.2	3.2	5.7
Surface seawater	Recovered concentration (µg/g)	0.975 ± 0.0186	1.96 ± 0.0216	3.89 ± 0.0661	0.203 ± 0.010	0.471 ± 0.0132	0.668 ± 0.0214
	Percentage recovery (%)	97.5	98.0	97.3	102	94.2	95.4
	%RSD	1.9	1.1	1.7	4.9	2.8	3.2

		Heavy metals					
		Cu			Hg		
Calibration curves	0–1 mg/L				0–10 µg/L		
	0.9997				0.9992		
Equation	$y = 0.1485x + 0.0012$				$y = 0.02500x + 0.00090$		
LOD; LOQ	0.022 mg/L; 0.075 mg/L				0.36 µg/L; 1.2 µg/L		
		Cu			Hg		
Spiked conc. (µg/g)	0.2	0.5	0.7	0.002	0.005	0.007	
<i>P. viridis</i> mussel	0.195 ± 0.0132	0.492 ± 0.0301	0.711 ± 0.0405	0.00166 ± 0.000126	0.00240 ± 0.000213	0.00401 ± 0.000289	
	97.5	98.4	102	83.0	80.0	80.2	
	6.8	6.1	5.7	7.6	8.9	7.2	
Surface seawater	0.201 ± 0.0182	0.508 ± 0.0117	0.695 ± 0.0167	–	–	–	
	101	102	99.3	–	–	–	
	0.9	2.3	2.4	–	–	–	

The percentage recovery for all the analytes in *P. viridis* mussel and surface seawater was evaluated using three different concentrations within calibration curves. Six replicates were utilised for each concentration. Since the concentration of Hg in surface seawater samples was analysed without any digestion; the percentage recovery for this analyte for such matrix was not calculated

2013 (Yap et al. 2002, 2003, 2004a, b, 2005, 2006; Ng et al. 2013), status of contamination beyond 2013 remains unclear. Yap et al. (2016) reanalysed Cd, Cu, Fe, Ni, Pb and Zn concentrations in *P. viridis* mussels reported by the previous studies conducted in Peninsular Malaysia (between year 2002 and 2009), including those from Kampung Pasir Puteh (10 June 2006, 10 May 2007, 13 May 2008 and 28 August 2009). The authors (Yap et al. 2016) concluded that because the Pb THQ values were high, ‘the Pb levels in some mussel populations could create a health risk problem’ and therefore, ‘the consumption amounts of mussels should be limited for minimizing potential health risks of heavy metals to the HLM consumers’. Because a recent sampling conducted at a nearby *P. viridis* harvesting site (Kong Kong Laut, about 60 km up north) revealed a moderate level of contamination of Pb and

Cu in catfish and *P. viridis* mussel (Mohamat-Yusuff et al. 2015), investigating the current status at the Kampung Pasir Puteh harvesting site becomes pertinent. This aspect is further exacerbated by the fact that the harvesting sites at Kampung Pasir Puteh are located close to a bustling seaport and industrial areas, possible sources of contaminations (Fig. 1a, c).

In Malaysia, the maximum permitted proportions for Pb, Cd and total Hg for mussels (wet weight) as prescribed by law (Food Act 1983 (Act 281) and Regulations 2013) are 1.5, 2.0 and 0.5 µg/g, respectively. As for the general marine water, the maximum levels for Pb, Cu and total Hg are 8.5, 2.9 and 0.16 µg/L, respectively (Department of Environment Malaysia, DOE 2015). Interestingly, while the maximum permitted proportion for Pb, Cd and Hg in bivalve molluscs have been gazetted by the Malaysian law, the same remains lacking

for Cu. The lack of maximum permitted proportion for Cu also prevails in the European Community (EC 2006), Food Standards Australia New Zealand Act (FSANZ 2015) and the US Food and Drug Administration (US FDA 2007). The only available standard, i.e. the Indian jurisdiction, prescribes the maximum limit for Cu in mollusc as 30 µg/g (FSSAI 2011). Taking into account that excessive amount of Cu can still lead to undesired human toxicity (Baltas et al. 2016), and because mussels like *P. viridis* are consumed routinely, establishing its specific maximum permitted proportion in the Malaysian Food Act appears necessary.

The concentrations of Pb, Cd, Cu and total Hg in *P. viridis* mussel as well as the surrounding surface seawater samples during January–March 2015 are presented in Table 2. The means concentrations of Pb, Cd, Cu and total Hg (Table 2) in *P. viridis* mussel (dry weight) during the 3 months of sampling period ranged between 25.10 and 38.60 µg/g, 9.10 and 13.00 µg/g, 11.20 and 13.80 µg/g and 30.10 and 38.90 ng/g, respectively. These concentrations were equivalent to 4.27–6.55 µg/g, 1.55–2.21 µg/g, 1.90–2.35 µg/g and 5.12–6.60 ng/g of Pb, Cd, Cu and total Hg in wet weight of *P. viridis* mussels, correspondingly. It was observed that the concentration of Pb in *P. viridis* mussel differed significantly ($p < 0.05$) among the 3 months of sampling period. While the highest concentrations of Pb in the *P. viridis* mussel (dry weight) (38.60 ± 2.22 µg/g) was recorded during January, its lowest concentration (25.10 ± 2.11 µg/g) was observed during March (Table 2, $p < 0.05$). Like Pb, the highest mean concentrations for Cd (13.00 ± 0.20 µg/g), Cu (13.80 ± 2.10 µg/g) and total Hg (38.90 ± 4.20 ng/g) in *P. viridis* mussel were observed during January (Table 2, $p < 0.05$). Interestingly, while the lowest mean concentration of Pb in *P. viridis* mussel was observed during March, the lowest mean concentrations of Cd, Cu and total Hg were all found during February. Notably, the means concentrations of Pb and Cd in *P. viridis* mussel reported here were greater than the previous studies conducted at the same harvesting site (Yap et al. 2004a, 2005). Such observations indicate the possibility of temporal changes that might have taken place at this important mussel harvesting site, possibly due to more extensive industrial and seaport activities that resulted in severe contamination. Alarmingly, because the ranges of mean concentration of Pb and Cd in *P. viridis* mussel reported here exceeded the maximum permitted proportions (µg/g wet weight) prescribed by the Malaysian Food Act 1983 (Act 281) and Regulations (2013), its negative impacts on human health could not be ruled out.

In addition, significantly lower mean concentrations of Pb (2.62–3.62 mg/L), Cd (0.72–0.78 mg/L), Cu (0.27–0.38 mg/L) and total Hg (0.21–1.49 µg/L) were observed in the surrounding surface seawater samples when compared with that of *P. viridis* mussels (Table 2, $p < 0.05$). Such finding appears consistent with those reported by previous researchers (Hadibarata et al. 2012; Putri et al. 2012; Tewari et al. 2001), attributable to higher bioaccumulation ability of the bivalves (Hadibarata et al. 2012;

Table 2 Concentrations of Pb, Cd, Cu and total Hg in *P. viridis* mussel and surface seawater samples collected from Kampung Pasir Putih, Pasir Gudang, Johor (January–March 2015)

	Comparison of heavy metals												
	Months of sampling						Surface seawater						
	January (2015)		February (2015)		March (2015)		Jan vs Feb		Feb vs Mar		Jan vs Mar		
Pb	<i>P. viridis</i>	38.60 ± 2.22	33.90 ± 1.02	25.10 ± 2.11	S	S	S	S	S	S	S	S	S
	Seawater	3.62 ± 0.21	2.86 ± 0.24	2.62 ± 0.26	S	S	S	S	S	S	S	S	S
Cd	<i>P. viridis</i>	13.00 ± 0.20	9.10 ± 0.20	11.40 ± 0.80	S	S	S	S	S	S	S	S	S
	Seawater	0.72 ± 0.014	0.78 ± 0.018	0.75 ± 0.012	S	S	S	NS	NS	NS	NS	NS	NS
Cu	<i>P. viridis</i>	13.80 ± 2.10	11.20 ± 0.60	12.60 ± 1.70	S	S	S	S	S	S	S	S	S
	Seawater	0.27 ± 0.03	0.38 ± 0.04	0.35 ± 0.04	S	S	S	S	S	S	S	S	S
Hg	<i>P. viridis</i>	38.90 ± 4.20	30.10 ± 3.01	30.30 ± 6.01	S	S	S	NS	NS	NS	NS	NS	NS
	Seawater	0.89 ± 0.11*	1.49 ± 0.27+	0.21 ± 0.08	S	S	S	S	S	S	S	S	S

The data are presented as mean ± standard deviation. The concentrations for Pb, Cd and Cu in *P. viridis* mussel and surface seawater are expressed as µg/g and mg/L, respectively. As for total Hg in *P. viridis* mussel and surface seawater, the units are ng/g and µg/L, respectively. ANOVA with Tukey-Kramer post hoc test with the significant level of 0.05 was used for determining the significant differences among groups (January, February and March 2015). Independent samples *t* test was used for comparing the concentrations of Cd between *P. viridis* mussel with that of surrounding surface seawater. S statistically significant with $p < 0.05$, NS statistically insignificant with $p > 0.05$

Thorp 2010). Furthermore, the high bioaccumulation ability of *P. viridis* mussel towards marine contaminants has resulted in its utilisation as a biomonitoring agent for pollution (Yap et al. 2002; Nicholson and Szefer 2003; Arockia et al. 2012).

As for the surrounding surface seawater sample, the mean concentrations of Pb, Cd and Cu as well as total Hg (Table 2) ranged between 2.62 and 3.62 mg/L, 0.72 and 0.78 mg/L, 0.27 and 0.38 mg/L and 0.21 and 1.49 µg/L, correspondingly. In view of regulating the level of contaminations in marine water areas where seafood for human consumption is applicable, the DOE (2015) via its Malaysia Marine Water Quality Criteria and Standards (MMWQCS) has prescribed that the levels of Pb, Cd, Cu and total Hg shall not exceed 8.5, 3.0, 2.9 and 0.04 µg/L, respectively. Since the levels of Pb, Cd, Cu and total Hg in the surface seawater samples exceeded the maximum levels allowable by the MMWQCS (DOE 2015), the possibility of such water being a source of contamination for seafood (especially *P. viridis* mussels) for human consumption proves alarming. This observation appears to be consistent with the moderate Marine Water Quality Index (MWQI) for the coastal water around Pasir Gudang seaport categorised in the Malaysia Environmental Quality Report (DOE 2015).

While the highest concentration of Pb in surface seawater samples was recorded during January (3.62 ± 0.21 mg/L), the lowest concentration of such analyte was observed during March (2.62 ± 0.26 mg/L) (Table 2, $p < 0.05$). Moreover, the highest mean concentrations of Cd and Cu and total Hg were all observed in the surrounding surface seawater during February (Table 2, $p < 0.05$). Although the lowest mean concentrations of Cd and Cu in the surrounding surface seawater was observed during January (Table 2, $p < 0.05$), the lowest mean concentration of total Hg in the same matrix was evident during March (Table 2, $p < 0.05$). In this context, it is pertinent to indicate that higher temperature (Lee and Lee 2005; Mubiana and Blust 2007) as well as lower salinity (Belabed et al. 2013) and pH (Pascal et al. 2010) in the surrounding seawater would increase the proportion of ionic metals, leading to better bioaccumulation of toxic metals in molluscs such as *P. viridis*. The significant temporal variations observed may further be explained by differences in amount of pollutants entering the surrounding marine ecosystem, in addition to the possible run-off effect of rainfall and water current. Hence, further studies incorporating the temporal physicochemical characterisation (temperature, salinity and pH) of surrounding surface seawater and its possible influence on bioaccumulation of toxic metals by *P. viridis* mussel at Kampung Pasir Puteh, Pasir Gudang, Johor merit consideration.

Estimation of daily intake of toxic metals and health risk assessment

Presence of toxic metals in coastal areas, especially from anthropogenic discharges, would lead to their unwanted bioaccumulations in aquaculture products (e.g. *P. viridis*

mussels), causing detrimental health implications towards human, particularly over long-term consumption (Li et al. 2013; Chua et al. 2014; Edbeib et al. 2017). Therefore, continuous assessments of health risk indices such as THQ and HI, following the consumption of contaminated *P. viridis* mussels harvested at Kampung Pasir Puteh, Pasir Gudang, Johor may prove necessary. Being a ratio of the consumed dose of toxic metals through oral ingestion, THQ has been commonly used for evaluating such toxic metal intake through contaminated foods (Chary et al. 2008). While it is likely that the exposed population with $THQ > 1$ may ‘experience obvious deleterious effects’, higher THQ has been associated with the probability of having greater hazard risk (Li et al. 2013). In addition, because multiple toxic metals can occur in aquaculture products such as *P. viridis* mussels, estimating the HI values for explaining the risk of having non-cancer human health impacts deserves public health consideration. In this context, occurrence of non-cancer human health impact is the least expected whenever the $HI < 1$, and vice versa (Griffin 2009).

To enable calculations of THQ and HI values, the EDI values for Pb, Cd and Cu and total Hg for an adult via consumption of mussels were calculated. The calculated EDI values, as well as the relevant PTDI data suggested by the JECFA (1999, 2003), are provided in Table 3. Results revealed high EDI values for Pb (7.12–10.94 µg/kg/day) and Cd (2.58–3.69 µg/kg/day) in *P. viridis* mussels examined here when compared with that reported by previous researchers (Franco et al. 2002; Yap et al. 2004a; Li et al. 2013) and the PTDI values (JECFA 1999, 2003). While the EDI values for Pb and Cd observed here were (1219–1873% and 2504–3582%, respectively) substantially higher than that reported by a previous study in Malaysia (Yap et al. 2004b), the values were also 199–306% and 258–369% higher than the PTDI reported by JECFA (1999, 2003). Although the EDI value for total mercury in *P. viridis* mussels (0.01 µg/kg/day) was observably higher than the EDI values (0.005–0.054 µg/kg/day) reported by previous researchers (Franco et al. 2002; Li et al. 2013), the value remains lower than the reported PTDI for Hg (0.228 µg/kg/day) (JECFA 1999, 2003). The fact that the EDI and PTDI values for Cu in mussels remain unavailable, suitable comparison with the findings reported in this present research could not be made.

Considering that specific study focusing on the risk assessment of ingesting toxic metals viz. Pb, Cd, Cu and total Hg in *P. viridis* mussels in Malaysia and its implication on public health remains lacking, this present study that utilised THQ and HI indices for assessing the health risk of consuming such mussels deserves consideration. The THQ and HI values of Pb, Cd, Cu and total Hg by consuming *P. viridis* mussels for a healthy adult are presented in Table 4. Like the finding reported from countries surrounding the Adriatic Sea (Jović and Stanković 2014), the THQ values for Cu (0.089–0.194) and total Hg (0.030–0.081) observed here were also largely lower

Table 3 Estimated dietary intakes (EDI) of toxic metals by consuming mussels collected from Kampung Pasir Putih, Pasir Gudang, Johor, Malaysia from January–March 2015

Heavy metals	Month of sampling	Concentrations ($\mu\text{g/g}$ wet weight)	EDI ^a ($\mu\text{g/kg/day}$)	EDI ^b ($\mu\text{g/kg/day}$)	EDI ^c ($\mu\text{g/kg/day}$)	EDI ^d ($\mu\text{g/kg/day}$)	PTDI ^e ($\mu\text{g/kg/day}$)
Pb	Jan	38.60	10.94	0.130	0.383	0.584	3.570
	Feb	33.90	9.61				
	Mac	25.10	7.12				
Cd	Jan	13.00	3.69	0.206	0.127	0.103	1
	Feb	9.10	2.58				
	Mac	11.40	3.23				
Cu	Jan	13.80	3.91	NA	NA	NA	NA
	Feb	11.20	3.18				
	Mac	12.60	3.57				
Hg	Jan	0.04	0.01	0.005	0.054	NA	0.228
	Feb	0.03	0.01				
	Mac	0.03	0.01				

NA unavailable data

^a EDI values of heavy metal by consuming mussels from this present research

^b EDI values of heavy metal by consuming oyster from China (Li et al. 2013)

^c EDI values of heavy metal by consuming mollusks from Spain (Franco et al. 2002)

^d EDI values of heavy metal by consuming mollusks from Malaysia (Yap et al. 2004a)

^e PTDI values (in micrograms per kilogram per day) of Hg, Pb and Cd were based on the data suggested by the Joint FAO/WHO Expert Committee on Food Additives (JECFA 1999, 2003). The PTDI value of methyl mercury was adopted as that of total Hg

than 1, indicating that the amount of Cu and total Hg in *P. viridis* mussels may not likely cause negative health effect on human. However, because results also highlighted on the

Table 4 Target hazard quotients (THQs) of toxic metals by consuming mussels. Average consumer (AC); high consumer (HC)

Month sampling	Target hazard quotients (THQs)				HI	
	Cu	Cd	Pb	Hg		
January ^a	AC	0.097	3.714	25.712	0.040	33.41
	HC	0.194	7.426	25.712	0.081	
February ^a	AC	0.079	2.600	22.581	0.030	28.00
	HC	0.158	5.199	22.581	0.061	
March ^a	AC	0.089	3.257	16.719	0.030	23.47
	HC	0.177	6.512	16.719	0.061	
Albania ^b	AC	0.015	0.024	0.393	0.049	0.57
	HC	0.030	0.048	0.393	0.099	
Montenegro ^b	AC	0.011	0.095	0.667	0.072	1.02
	HC	0.022	0.190	0.667	0.144	
Slovenia ^b	AC	0.015	0.042	0.227	0.023	0.38
	HC	0.029	0.083	0.227	0.045	
Croatia ^b	AC	0.033	0.182	0.540	0.161	1.29
	HC	0.066	0.363	0.540	0.321	
Italy ^b	AC	0.018	0.101	0.673	0.048	1.01
	HC	0.036	0.202	0.673	0.096	

^a Findings of this present research

^b Reported by Jovic and Stankovic (2014)

alarmingly high THQ values for Pb (16.719–25.712) and Cd (2.600–7.426) by consuming *P. viridis* mussels harvested at Kampung Pasir Putih, Johor, Malaysia, their roles in causing detrimental health effects on humans cannot be completely ruled out. Such a proposition is further supported by the extremely high values of HI reported in this present research (23.47–33.41) when compared to those reported by Jović and Stanković (2014). In fact, chronic exposures to Pb and Cd have been associated with numerous health problems that include toxicity of haematopoietic, nervous, reproductive, respiratory and urinary systems, as well as impairment of cognitive ability and tumours (Cope et al. 2004; Flanagan et al. 2008). Therefore, having the ability to screen the concentrations of these toxic metals in *P. viridis* mussels, probably by associating their concentrations in the surface seawater surrounding its harvesting sites, attempted in this present research too, may prove useful.

Association between the concentrations of Pb, Cd, Cu and total Hg in *P. viridis* mussel with that of surface seawater

While moderate to high strengths of associations between concentrations of Pb, Cd and Cu in *P. viridis* mussels and surface sediments in the west coast of Peninsular Malaysia have been reported (Yap et al. 2002), specific studies that explore the association between those elements in *P. viridis* mussel and surface seawater in Malaysia remain lacking. The

fact that ‘suspended filter feeder for *P. viridis* is not in actual contact with the sediments’ (Yap et al. 2002), and because the sampling of surface seawater is relatively easier than that of sediments, exploring such association for inferential purposes merits consideration.

Table 5 represents the correlation coefficients and regression parameters observed between the concentrations of Pb, Cd, Cu and total Hg in *P. viridis* mussels and the surrounding surface seawater at Kampung Pasir Puteh harvesting site. It was found that the concentration of Pb in the *P. viridis* mussel was highly and positively correlated ($r = 0.787$; $p < 0.05$) with that of the surrounding surface seawater. Such significant and high strength of association observed between the concentrations of Pb, in *P. viridis* mussel, with that of surface seawater indicates the possible application of surface seawater for inferring the concentrations of Pb, in *P. viridis* mussel. In contrast, associations between the concentrations of Cd ($r = -0.620$; $p < 0.05$) and Cu ($r = -0.794$; $p < 0.05$) in *P. viridis* mussels versus that of the surrounding surface seawater were negatively moderate and negatively high, respectively. Such negative associations observed for Cd and Cu although statistically significant ($p < 0.05$) may not provide meaningful insights for inferring their concentrations in the *P. viridis* mussels from those found in the surface seawater samples. Similar conclusion can also be made for total Hg, considering the insignificant ($p > 0.05$) as well as negative and little ($r = -0.110$) strength of association was observed in the *P. viridis* mussels with that of surface seawater samples.

Review of literature also reveals no specific study, focusing on the association between Hg concentration in *P. viridis* mussel with that of sediments and/or surface seawater in Malaysia. Therefore, further studies for exploring the associations between concentrations of various toxic metals that are prevalently found in the coastal waters with that of economically important marine commodities such as *P. viridis* mussel over a longer duration appear useful. This is due to relatively easier analysis of toxic metals in water than that of animal tissues that would enable more frequent periodic assessments to be carried out for ensuring safer human consumption of this important aquaculture product.

Table 5 Association between the concentrations of toxic metals in *P. viridis* mussel versus that of surface seawater in Kampung Pasir Putih, Pasir Gudang, Johor, Malaysia during January–March 2015

Heavy metals	Pearson correlation coefficient (r)		Regression (r^2)	
	r values	p values	r^2 values	Regression equations
Pb	0.787	$p < 0.05$	0.619	$y = 3.729 + 9.508x$
Cd	-0.620	$p < 0.05$	0.384	$y = 31.068 - 26.540x$
Cu	-0.794	$p < 0.05$	0.630	$y = 22.415 - 29.006x$
Hg	-0.110	$p > 0.05$	0.012	$y = 0.036 - 0.708x$

Conclusions

This present research assessed the concentrations of Pb, Cd, Cu and total Hg in *P. viridis* mussel and surface seawater samples collected at Kampung Pasir Puteh, Pasir Gudang, Johor, Malaysia during January to March 2015. It was found that the concentrations (dry weight) of Pb, Cd, Cu and total Hg in *P. viridis* mussel during the 3 months of sampling period ranged between 25.10 and 38.60 $\mu\text{g/g}$, 9.10 and 13.00 $\mu\text{g/g}$, 11.20 and 13.80 $\mu\text{g/g}$ and 30.10 and 38.90 ng/g , respectively. Since enormous values of THQ for Pb and Cd as well as HI were found, the possibility that prolonged consumption of such mussels would lead to unwanted detrimental impact on public health appears disturbing. Therefore, continuous monitoring on the level of toxic metals in such mussels and developing an efficient toxic metal removal technique prove pertinent. The fact that significant and moderate correlation was observed between the concentration of Pb, in *P. viridis* mussels and the surrounding surface seawater, its application for monitoring activities deserves to be further explored.

Limitations

Because *P. viridis* is a suspension feeder, and since the biogeochemical cycle of trace metals depends on pH and salinity, analyses of trace metals in suspended particulate matter (SPM) along with the data on seawater pH and salinity would provide more accurate interpretation relating to the temporal changes. Considering that in this present research the sampling was done and completed in year 2015, analysing new samples from the site now may no longer be representative to the actual conditions that prevailed during that time. Therefore, further studies focusing on these aspects at the same *P. viridis* harvesting site merit consideration.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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