ASSESSMENT OF SELECTED HEAVY METALS IN *Perna viridis* MUSSEL AND SURFACE SEAWATER IN THE COASTAL AREA OF PASIR GUDANG, MALAYSIA

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This is especially dedicated to my beloved parents (Mak and Abah), siblings, close family members, dearest one and friends…

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ABSTRACT

Owing to its nutritional values, consumption of cultured marine bivalve such as *P. viridis* has gained popularity in many countries including Malaysia. Besides, the use of *P. viridis* as biomonitoring agent for heavy metal pollutions in coastal waters has also been suggested. The previous study on levels of heavy metal contaminants in *P. viridis* collected from Kampung Pasir Putih, Pasir Gudang, Johor was reported in 2013. Because temporal changes might have occurred at this important harvesting site, this present research acquires public health consideration. Concentrations of lead (Pb), cadmium (Cd) and copper (Cu) as well as mercury (Hg) in the soft tissue of *P. viridis* and surface seawater sampled from the site during January to March 2015 were investigated using Flame Atomic Absorption Spectrometry (FAAS) and Flow Injection Mercury System (FIMS), respectively. Results revealed significantly higher concentrations of these heavy metals in *P. viridis* ($p < 0.05$) than that of surrounding surface seawater samples. Alarmingly, the ranges of concentrations for Pb (4.27-6.55 µg/g) and Cd (1.55-2.21 µg/g) in *P. viridis* mussel (wet weight) exceeded the maximum permitted proportion prescribed by the Malaysian Food Act. Furthermore, the concentrations of Pb (2.62-3.62 mg/L), Cd (0.72-0.78 mg/L), Cu (0.27-0.38 mg/L) and Hg (0.21-1.49 µg/L) in surface seawater samples exceeded the Malaysia Marine Water Quality Criteria and Standards (MMWQCS). While no significant correlation ($p > 0.05$) was found between Hg ($r = -0.110$) in *P. viridis* mussel and the surrounding surface seawater, significant correlations ($p < 0.05$) were observed for Pb ($r = 0.787$), Cd ($r = -0.620$) and Cu ($r = -0.794$). Considering the high concentrations of heavy metals found in both *P. viridis* and surface seawater at the study site, the negative impacts on human health following consumption of this seafood product could not be ruled out. Therefore, continuous assessment on this aspect proves to be relevant.

ABSTRAK

Pengambilan hasil ternakan dwicengkerang laut seperti *P. viridis* telah meraih populariti di banyak negara termasuk Malaysia disebabkan oleh nilai pemakanannya. Selain itu, penggunaan *P. viridis* sebagai agen biomonitor bagi pencemaran logam berat di perairan pantai juga telah disyorkan. Kajian terdahulu ke atas bahan cemar logam berat dalam *P. viridis* yang telah diambil dari Kampung Pasir Putih, Pasir Gudang, Johor telah dilaporkan pada tahun 2013. Oleh sebab perubahan temporal yang mungkin berlaku di tapak penternakan yang penting ini, kajian ini adalah penting terhadap kesihatan awam. Kepekatan plumbum (Pb), kadmium (Cd) dan kuprum (Cu) dan juga merkuri (Hg) dalam tisu lembut *P. viridis* dan air laut permukaan yang disampel dari tapak tersebut dari Januari hingga March 2015 telah dianalisis menggunakan Spektrometri Penyerapan Atom Nyala Api (FAAS) dan Sistem Merkuri Suntikan Aliran (FIMS). Hasil kajian mendapati logam-logam berat ini mempunyai kepekatan tinggi yang signifikan dalam *P. viridis* ($p < 0.05$) berbanding sampel air laut permukaan. Julat kepekatan Pb (4.27-6.55 μg/g) dan Cd (1.55-2.21 μg/g) dalam kupang *P. viridis* (berat basah) melebihi kadar maksimum yang dibenarkan oleh Akta Makanan Malaysia. Tambahan pula, kepekatan Pb (2.62-3.62 mg/L), Cd (0.72-0.78 mg/L), Cu (0.27-0.38 mg/L) dan Hg (0.21-1.49 μg/L) dalam sampel air laut permukaan melebihi Standard dan Kriteria Kualiti Air Marin Malaysia (MMWQCS). Tiada korelasi yang signifikan ($p > 0.05$) didapati antara Hg ($r = -0.110$) dalam kupang *P. viridis* dan air laut permukaan di sekitarnya, manakala korelasi yang signifikan ($p < 0.05$) dapat dilihat bagi Pb ($r = 0.787$), Cd ($r = -0.620$) dan Cu ($r = -0.794$). Mengambilkira kepekatan tinggi logam berat yang terdapat dalam kedua-dua *P. viridis* dan air laut permukaan di kawasan kajian, kesan negatif ke atas kesihatan manusia berikutan pengambilan produk makanan laut ini tidak harus dipandang remeh. Oleh itu , penilaian yang berterusan ke atas aspek ini adalah wajar.

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LIST OF SYMBOLS AND ABBREVIATIONS

°C	–	Degree Celsius
%	–	Percent
Cd	–	Cadmium
cm	–	Centimetre
Cu	–	Copper
DOE	–	Department of Environment
DOF	–	Department of Fisheries
FAAS	–	Flame Atomic Absorption Spectrometry
Fe	–	Iron
FIMS	–	Flow Injection Mercury System
g	–	Gram
Hg	–	Mercury
LOD	–	Limit of detection
LOQ	–	Limit of quantification
m	–	Metre
min	–	Minute
µg/g	–	Microgram per gram
µg/L	–	Microgram per litre
µL	–	Microlitre
mg/L	–	Milligram per litre
mL	–	Millilitre
mm	–	Millimetre
MWQI	–	Marine Water Quality Index
MMWQCS	–	Malaysia Marine Water Quality Criteria and Standards
MΩ·cm	–	Megohm-centimetre
ng/g	–	Nanogram per gram

P. viridis	–	*Perna viridis*
Pb	–	Lead
ppb	–	Parts per billion
ppm	–	Parts per million
ppt	–	Parts per trillion
U.S. EPA	–	United States Environmental Protection Agency
U.S. FDA	–	United States Food and Drug Administration
Zn	–	Zinc

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

The Asian green mussel, *Perna viridis* is an economically important coastal bivalve mollusc belonging to the family Mytilidae; known as the Philippine green mussel or green-lipped mussel in certain parts of the world (National Introduced Marine Pest Information System, NIMPIS, 2016). The mussels are commonplace along the coastal marine waters of the Indo-Pacific region (Gosling, 2003) and at several portions of coastal areas of Peninsular Malaysia (Ismail *et al*., 2000). The use of *P. viridis* as a biomonitoring agent for heavy metal pollutions within the coastal environment has been reported (Nicholson & Szefer, 2003; Hadibarata *et al*., 2012; Vasanthi *et al*., 2012), attributable to its cost effectiveness as well as reliability (Yap *et al*., 2006a). Furthermore, as a biomonitoring agent, *P. viridis* mussels have been indicated as sedentary organisms, long living, easily identifiable, reasonably abundant and available throughout the year, tolerant to environmental changes and pollution (Wagner & Boman, 2004), as well as having good net accumulation capacities (Yap *et al*., 2004a).

Considering that *P. viridis* mussels are made up by about 60% of protein for every 100 g of its dry weight (Choo & Ng, 1990), having substantial amounts of vitamins and trace elements (Gopalakrishnan & Vijayavel, 2009), while abundantly found within the coastal region of Peninsular Malaysia; they become an important source of nutrients for human consumption (Yap *et al*., 2004a). Interestingly, while

Johor has been the largest producer of *P. viridis* mussels in Malaysia (Department of Fisheries Malaysia, DOF, 2013), its major harvesting area (Kampung Pasir Putih at the Straits of Tebrau) for this aquaculture product is located around the Pasir Gudang Seaport and industrial areas (Yap *et al*., 2004b). It has been indicated that Kampung Pasir Putih has been subjected to various industrial and socioeconomic activities, mainly petrochemical and its related industries as well as shipping, land reclamation and urbanization (Yap *et al*., 2004b). Hence, the possibility of contamination by heavy metals from the seaport and industrial areas into the surrounding seawater where the mussels are reared could not be ruled out. Recognising the possibilities of contamination by heavy metals in the mussels harvested in Kampung Pasir Putih, a number of studies conducted between 2002 to 2013, have been reported (Yap *et al*, 2002, 2003, 2004a, 2004b, 2004c, 2005a, 2006a, 2006b; Eugene Ng *et al*., 2013). Due to the possible temporal changes in socioeconomic and environmental factors that may have occurred at the harvesting area and since review of the literature reveals no specific research on this aspect beyond year 2013, the current level of safety for consuming such product could not be ascertained.

It is pertinent to indicate here that acute and/or chronic exposure towards heavy metals (e.g. lead, Pb; cadmium, Cd; copper, Cu; mercury, Hg) would lead to detrimental health implications among humans (Cope *et al*., 2004; Flanagan *et al*., 2008). While acute exposure to high levels of heavy metals may result in brain damage, paralysis, anaemia and disruption of gastrointestinal system, chronic exposure of such contaminants has been attributable to damages of the kidneys, reproductive, immune, nervous, respiratory and cardiovascular systems (Cope *et al.*, 2004; Flanagan *et al*., 2008). Alarmingly, even a low level of exposure may possibly cause disturbance in the intellectual development among children. In addition, miscarriage, stillbirth, premature birth and low birth weight, as well as malformations of the foetus and/or infants among pregnant women those were exposed to heavy metals have been reported (WHO, 2015). Therefore, continuous evaluation of these contaminant levels in such aquaculture product as *P. viridis* mussels, in view of its toxicological risks towards public health, merits serious consideration.

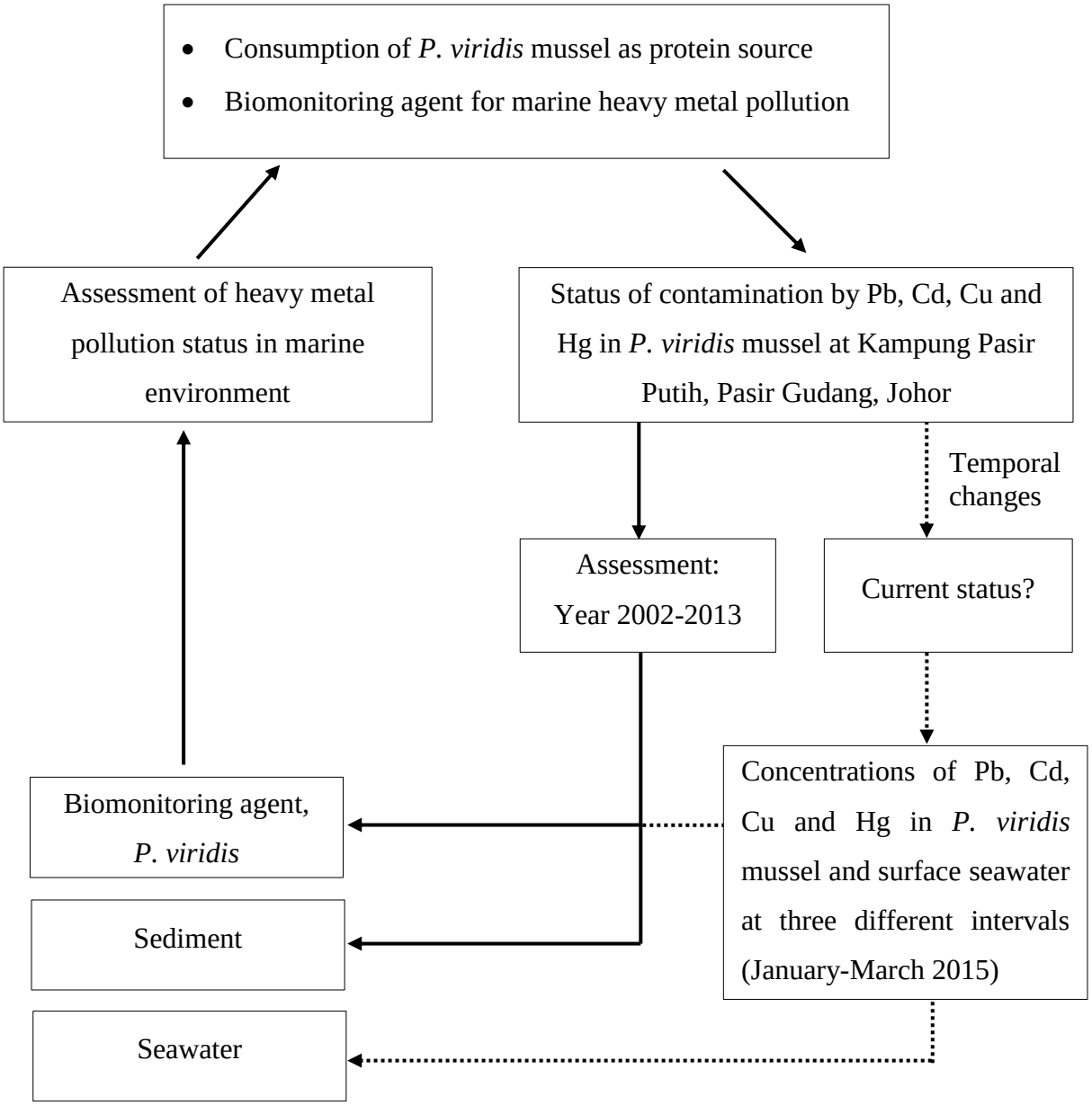
1.2 Problem Statement

It is pertinent to indicate here that the last ecotoxicological assessment covering this area of aquaculture of interest was reported in 2013 (Eugene Ng *et al*., 2013). Considering the (a) role of *P. viridis* mussels as a cheap source of nutrients, (b) its good net accumulation capacities of pollutants, (c) toxicities of heavy metals towards humans and (d) temporal changes that may have occurred, continuous assessments on the concentrations of heavy metals as contaminants, both in water and in the mussels are paramount. Because high concentrations of Pb, Cd and Cu had been reportedly found in mussels from the different sites in Johor (Yap *et al.*, 2003, 2004b, 2005a, 2006b, 2007) and since awareness on toxicities of Hg exposure associated with fish and seafood consumption has been increasing (Hajeb *et al.*, 2012), the choice of these four contaminants appears justifiable. Therefore, this present research designed to assess the concentrations of Pb, Cd, Cu and Hg in the harvested *P. viridis* mussels from the different sampling sites within Kampung Pasir Putih harvesting area as well as its surrounding seawater samples at three different intervals (January-March 2015) acquires public health consideration. The conceptual framework of this present research is presented in Figure 1.1.

1.3 Objectives and Hypotheses

Taking into account all the relevant information discussed above, this present research was set to achieve the following objectives:

i. Determine the concentrations of Pb, Cd, Cu and Hg in the sampled *P. viridis* as well as surrounding seawater samples during January to March 2015.
ii. Compare and correlate the concentrations of Pb, Cd, Cu and Hg in the sampled *P. viridis* with that of surrounding seawater samples.
iii. Compare the differences in the concentrations of Pb, Cd, Cu and Hg in the sampled *P. viridis* as well as the surrounding seawater samples during the different sampling intervals (January-March 2015).



Note: Dotted lines indicate the contextual importance of the present research for assessing contamination of Pb, Cd, Cu and Hg in *P. viridis* mussel and surface seawater.

Figure 1.1: Conceptual framework of the research

In view of all the objectives listed above, it was hypothesised that:

a) The concentrations of Pb, Cd, Cu and Hg in the sampled *P. viridis* would be significantly different ($p < 0.05$) and highly correlated with that of surrounding seawater samples.
b) The concentrations of Pb, Cd, Cu and Hg in the sampled *P. viridis* and the surrounding seawater samples would be significantly different among the three sampling intervals (January-March 2015).

1.4 Scope of Study

This present research involved samples of *P. viridis* mussels as well as the surrounding surface seawater from different sampling sites within the Kampung Pasir Putih harvesting area of Johor (1°25'−26'N 103°55'−57'E); those were collected at three different sampling intervals (January-March 2015). Following the analytical methods described by previous researchers (U.S. Environmental Protection Agency, EPA, 1992; Rahman *et al*., 2012; Chadid *et al*., 2014), samples collected were analysed for Pb, Cd, Cu and Hg. Analysis of Pb, Cd and Cu using a Flame Atomic Absorption Spectrometry (FAAS) (Perkin Elmer PinAAcle 900T). In addition, analysis of Hg was done using the Flow Injection Mercury System (FIMS) (Perkin Elmer FIMS 100). The data were presented in microgram per gram (µg/g) of sample dry weight (dw). Partial validation (linearity, limit of detection (LOD), limit of quantitation (LOQ) and percentage recovery) of the analytical methods used was also attempted.

1.5 Significance of Study

The findings reported here would be able to elucidate the levels of contaminations by Pb, Cd, Cu and Hg in the surrounding surface seawater as well as *P. viridis* mussels harvested in one of the major harvesting areas in Johor i.e. Kampung Pasir Putih. In this context, determining if such contaminants in the *P. viridis* mussels and surrounding surface seawater samples were within the maximum permitted proportions of metal contaminants prescribed in the Fourteenth Schedule of the Food Act 1983 (Act 281) & Regulations (2013) for assessing its potential threat to public health was found pertinent. The findings may be of applied values for the environmental and health authorities for formulating suitable intervention programmes for managing this significant issue of public interest.

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