UNIVERSITI PUTRA MALAYSIA

ACCUMULATION AND DISTRIBUTION OF HEAVY METALS IN GREEN-LIPPED MUSSEL *PERNA VIRIDIS* (LINNAEUS) FROM THE WEST COAST OF PENINSULAR MALAYSIA

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By

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Thesis Submitted in Fulfilment of the Requirements for the Degree of Master of Science in the Faculty of Science and Environmental Studies Universiti Putra Malaysia

August 1999

ACKNOWLEDGEMENTS

I am most indebted and grateful to my supervisor, Associate Professor Dr. Ahmad Ismail, who has guided me along the way and given me a lot of constructive comments, advice and ideas no matter how busy he is. He is certainly the person responsible for the successful completion for my project. I certainly cannot make it this far without his consistent 'inputs'. Sincerely, thank you.

I would also like to extend my sincere thanks to my co-supervisors Dr. Abdul Rahim Ismail for commenting and advising on my statistical analysis, and Mr. Mohamad Pauzi Zakaria for his comments on my project and help during the field-trip. Thank you. Special thanks are also given to Miss Rosniza Ramli, Mr. Mansor Rashid, Mr. Navies Maisin, Mr. Hidir Hashim, Mr. Zahar Jaafar, Mr. Ali Mashinchian and all the staff in the Biology department. Also thanks to my friends whom I know Kak Leha, Hena, Latif, Nik, Katty, Sem and my housemates.

Not forgetting my family and my girl friend, Miss Choh Mew Seong, who have become my constant source of inspiration and motivation. I could not have made it without you all. Thanks and I love you all.

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LIST OF ABBREVIATIONS

$\mu g.g^{-1}$	microgram per gram
ANOVA	analysis of variance
CI	condition index
CV	coefficient of variation
DDW	double distilled water
$g.cm^{-2}$	gram per centimeter square
$g.cm^{-3}$	gram per centimeter cubic
H_2SO_4	acid sulphuric
HCl	asid hydrochloric
$HCLO_4$	acid perchloric
HNO_3	acid nitric
LH	shell length : shell height
$mg.l^{-1}$	microgram per litre
ml	mililitre
STI	soft tissue or flesh
SNK	Student-Newman-Keuls
SPM	suspended particulate materials
ST	shell thickness
WH	shell width : shell height
WL	shell width : shell length

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Master of Science.

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August 1999

Chairman : Associate Professor Ahmad Ismail, Ph.D.

Faculty : Science and Environmental Studies

The main aim of this study is to establish mussel *Perna viridis* as a biomonitoring agent along the west coast of Peninsular Malaysia. In this study, the levels of heavy metals had been studied in the mussel *P. viridis* collected from 13 stations along the coastline of Peninsular Malaysia and 2 stations in Sabah. The results showed that, in general, the levels of heavy metals in total soft tissue of mussel *P. viridis* were within or lower than the levels found elsewhere. The ranges varied widely from 0.19 to 1.56 $\mu g.g^{-1}$ dry weight for Cd, 5.78 to 15.14 $\mu g.g^{-1}$ dry weight for Cu, 1.17 to 8.27 $\mu g.g^{-1}$ dry weight for Pb and 46.78 to 145.95 $\mu g.g^{-1}$ dry weight for Zn.

Mussels *P. viridis* collected from one population were dissected into eight parts (byssus, mantle and gills, posterior adductor muscle, retractor byssal muscle, foot, crystalline style, gonad and remainder visceral mass) and the concentrations of heavy metals were measured. The highest concentration of Cu was obtained in the crystalline style while elevated Cu, Pb and Zn concentrations were noted in the byssus. Female's gonad generally accumulated higher metals levels than male's gonad. In the study of

different parts in the shell of mussel *P. viridis*, Cu and Zn levels were found elevated in the periostracum layer while Cd and Pb levels were highly elevated in the inner nacreous shell layer.

The relationships between the concentrations ($\mu g.g^{-1}$) and contents ($\mu g.individual^{-1}$) of heavy metals and body size (total STI dry weight, shell thickness and shell length) were also investigated. The results indicated that smaller mussels have higher metals accumulation especially for Cd, Zn and Pb. The metal accumulations in total STI of *P. viridis* were also affected by metal concentrations in sediment and suspended particulate materials. Besides, physico-chemical parameters, condition index, population structure and sex also played a role in affecting heavy metal accumulation in mussel *P. viridis*.

Apart from STI, mussel shell and byssus also can be considered as good bioindicators for Cu, Zn, Cd and Cu. Pearson's correlation coefficients in these metals in the organs showed positive correlations and suggested the organ to be a goosd bioindicator. The study on coefficient of variation indicated that if analysis of individuals are used, a very limited size range of samples from all stations should be selected to increase the ability of biomonitoring programmes.

In general, this study showed that Cu, Pb, Zn and Cd accumulated in the STI of *P. viridis* and showed some agreements that *P. viridis* is a good bioindicator for Cu, Pb, Zn and Cd. Tissue distribution of Cu, Pb, Zn and Cd varied in the organs and this phenomenon probably depends on physiology, ecology and biology of the mussel *P. viridis*.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi syarat untuk ijazah Master Sains.

AKUMULASI DAN TABURAN LOGAM BERAT DI DALAM KUPANG *PERNA VIRIDIS* (LINNAEUS) DARI PERAIRAN PANTAI BARAT SEMENANJUNG MALAYSIA

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Tujuan utama kajian ini adalah mencadangkan dan menggunakan kupang *Perna viridis* sebagai agen penunjuk biologi di sepanjang perairan pantai barat Semenanjung Malaysia. Dalam kajian ini, tahap logam berat telah dikaji dalam kupang *P. viridis* yang disampel dari 13 stesen di sepanjang perairan pantai Semenanjung Malaysia dan 2 stesen di Sabah. Keputusan menunjukkan bahawa secara amnya, tahap logam berat dalam seluruh isi lembut kupang *P. viridis* adalah dalam lingkungan atau lebih rendah daripada kajian di tempat-tempat lain. Lingkungannya berubah daripada 0.19 ke 1.56 $\mu g.g^{-1}$ berat kering untuk Cd, 5.78 ke 15.14 $\mu g.g^{-1}$ berat kering untuk Cu, 1.17 ke 8.27 $\mu g.g^{-1}$ berat kering untuk Pb dan 46.78 ke 145.95 $\mu g.g^{-1}$ berat kering untuk Zn.

Kupang *P. viridis* yang disampel dari satu populasi telah dibedah kepada 8 bahagian (bisus, mantel dan insang, otot aduktor posterior, otot retraktor bisus, kaki, stil berhablur, gonad dan tinggalan jisim viseral) dan kepekatan logam berat telah ditentukan. Kepekatan tertinggi bagi Cu telah diperolehi di dalam stil berhablur manakala kepekatan yang tinggi bagi logam Cu, Pb dan Zn telah diperhatikan di dalam

bisus. Gonad betina secara amnya menimbun logam lebih tinggi daripada gonad jantan. Dalam kajian mengenai bahagian berlainan dalam cangkerang kupang *P. viridis*, tahap Cd dan Zn adalah lebih tinggi dalam lapisan periostrakum manakala tahap Cd dan Pb adalah sangat tinggi dalam lapisan dalaman nakreous cangkerang.

Perkaitan di antara kepekatan ($\mu g.g^{-1}$) dan kandungan ($\mu g.individu^{-1}$) bagi logam berat dan saiz badan (jumlah isi lembut berat kering, ketebalan cangkerang dan kepanjangan cangkerang) telah dikaji. Keputusan menunjukkan kupang bersaiz kecil lebih banyak menimbum logam berat terutamanya bagi Cd, Zn dan Pb. Kandungan logam dalam seluruh isi lembut *P. viridis* adalah dipengaruhi oleh kepekatan logam dalam sedimen dan partikel terampai. Selain itu, parameter fizikokimia, indeks 'condition', struktur populasi dan jantina juga memainkan peranan dalam mempengaruhi kandungan logam berat di dalam kupang *P. viridis*.

Selain tisu lembut, cangkerang and bisus juga boleh dianggap sebagai penunjuk biologi kepada Cu, Zn, Cd and Cu. Korelasi koefisien Pearson bagi logam-logam ini dalam organ-organ tersebut menunjukkan keputusan positif yang mencadangkan organ-organ ini menjadi penunjuk biologi yang baik bagi logam-logam tersebut. Keputusan bagi koefisien variabiliti menunjukkan bahawa sekiranya analisis sampel secara individu digunakan, sampel dalam julat saiz yang terhad harus dipilih bagi meningkatkan keupayaan program pengawasan bioawas.

Secara umumnya, kajian ini menunjukkan Cu, Pb, Zn and Cd menimbun di *P. viridis* dan mempersetujui *P. viridis* sebagai penunjuk biologi yang baik kepada logam-logam tersebut. Taburan Cu, Pb, Zn and Cd berubah-ubah di dalam organ dan keadaan ini mungkin bergantung kepada fisiologi, ekologi dan biologi bagi kupang *P. viridis*.

CHAPTER I

INTRODUCTION

For the last fifty to a hundred years in particular, marine organisms have been faced with an increasing input of a wide variety of potentially toxic chemicals (Livingstone, 1992). Many of these chemicals are bioaccumulated within the tissues of biota to concentrations significantly above ambient levels in the environment (Phillips *et al.*, 1982; Rainbow, 1993) and can in some cases be up to 100 or 1000 times higher than in sea water (Farrington *et al.*, 1983). The rapid increase in inputs of chemicals, either mobilized or synthesized by man into the estuarine and coastal environment was due to growth in industrial activities during the twentieth century (Krishnakumar *et al.*, 1987). With the steady growth of industrial activities in South East Asia over the decades, there has been increasing concern over the environmental degradation of coastal waters and the diverse and unique environments which they support (Brown, 1985). Malaysia, as a fast-developing country, should be made aware of the seriousness that may arise if toxic chemicals were continually discharged into the coastal environments due to rapid industrialization. In this study, coastal areas were focused because these areas are hotspots of human activities (Ismail *et al.*, 1993) and have been the most productive areas for all sort of aquaculture activities (Thomas, 1988).

Aquatic contamination by heavy metals is extremely harmful since these elements are not degradable in the marine environments (Jordao *et al.*, 1997) and keep accumulating in the shellfish. Heavy metals, notably, Cu, Zn, Cd and Pb, have been common by-products of anthropogenic activities since the earliest civilized times. Furthermore, these heavy metals are potentially harmful to most organisms at a level of exposure and absorption above a minimum threshold and thus induce adverse biological effects. In addition, it is well-known that the consumption of fish and shellfish containing excessive amount of heavy metals can be harmful to man. For instance, the Minamata Disease outbreak in Japan in 1950s was caused by the consumption of fish and shellfish contaminated by methylmercury (Forstner and Whittmann, 1981).

Studies on heavy metals' pollution in Malaysian coastal waters have been done (Law and Singh, 1988; Din, 1992; Ismail, 1993a; Ismail *et al.*, 1993; Ismail and Ramli, 1997). Some of these studies have shown elevated concentrations of heavy metals in these areas. This information has certainly inferred the need for regular monitoring of heavy metals along the Malaysian coastlines. The west coast of Peninsular Malaysia has been targetted because more than 60% of the Malaysian population is concentrated here and most of the areas are being developed heavily (Ismail *et al.*, 1993). Beck (1995) suggested that the spreading and accumulation of persistent chemicals, and the destruction of oceans, are two of the most pressing environmental problems by the year 2020. This is exactly the year by which Malaysia has been expected to reach the status of a developed country. Therefore, intensive and regular monitoring of heavy metals' levels in Malaysia's coastal waters should be done in order to provide comprehensive baseline information for future reference.

A study of mussels is of interests to the public (Cheung and Wong, 1992) because these animals are important sources of food (Vakily, 1992). Elevated levels of heavy metals in mussels could mean contamination of this marine food delicacy. Mussels are among the hardiest and most easily gathered organisms and the mussel culture is the most productive form of saltwater aquaculture (Bardach *et al.*, 1972).

Marine mussels are recognized as a good bioindicator of heavy metals' pollution (Goldberg, 1975; Viarengo *et al.*, 1995). Mussels have been used for the 'Mussel Watch' monitoring programmes in which the mussels' species such as *M. edulis* and *M. galloprovincialis* had been studied since the late 1960s (Morita, 1988). Until today, there are reports on using mussels as bioindicators of heavy metals' levels (Boonchalermkit *et al.*, 1998; Senthilnathan *et al.*, 1998). An outstanding feature of using mussels as bioindicator, when compared to other environmental components, is that it is capable of assessing the biological availability of the pollutants measured (Kristensen and Tyle, 1990). Mussels being inhabitants of the coastal waters are more prone to be affected by pollutants derived from many sources (Prabhudeva and Menon, 1987).

Are there any mussels abundantly found along the coastlines of Malaysia? If mussels are found, are they as effective as a good bioindicator as described in the system developed by 'Mussel Watch'? These questions can be answered if a survey is conducted and the samples are analysed in order to compare with other environmental variables. In Malaysia, reports on heavy metals' levels in the tissues of mussel *P. viridis* are scant (Sivalingam and Bhaskaran, 1980; Ismail, 1993b).

This study also examined the bioavailability of shell and byssus of mussel *P. viridis* as better bioindicators, if possible, of heavy metals' levels on the basis of heavy metals' concentration in the shell and total STI, and byssus and total STI. These two studies were prompted by the questions arising in the usual assessment of the total STI results in blue mussels *M. edulis*. Do all the tissues in the mussels accumulate the same amount of heavy metals? Do the shells or perhaps the byssus has different accumulation patterns as compared to heavy metals' levels in the total STI of the mussels? The first question could be answered through the investigation and comparisons of heavy metals' levels in the byssus and the different soft tissues in the mussel *P. viridis*.

This study was conducted to :-

1. assess the current status of heavy metals' (Cu, Cd, Pb and Zn) levels and contamination in the west coast of Peninsular Malaysia,

2. investigate the distribution of heavy metals in different tissues of STI in mussel *P. viridis* as well as different layers and sections in the mussel's shell,

3. examine the uses of byssus and shell as biomarkers and biomonitoring materials for heavy metals,

4. find out if sediment, suspended particulate materials, physico-chemical parameters, size, CI, population structure and sex, could be the factors affecting the accumulation of heavy metals in the total STI of mussel *P. viridis*, and

5. to investigate the concentrations of heavy metals' variability among-individuals, referred to here as inter-station and intra-station's variability, so as to increase the understanding of this aspect.