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Biomonitoring of Heavy Metals in the West Coastal Waters of Peninsular Malaysia Using the Green-lipped Mussel *Perna viridis*: Present Status and What Next?

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ABSTRAK

Kertas kerja ini bertujuan untuk mengulas kembali kertas-kertas kerja yang telah diterbitkan menggunakan kupang Perna viridis *sebagai agen pemantauan bagi pencemaran logam berat di rantau ini dan khasnya dari Malaysia dan membincangkan pengetahuan yang sedia ada. Juga dicadangkan bahawa penyelidikan lanjutan yang berpotensi pada masa hadapan yang akan menambahkan pemahaman kita dan pengetahuan dalam ekotoksikologi logam berat di Malaysia dengan menggunakan kupang* P. viridis. *Kajian menggunakan kupang ini boleh dikatakan sebagai sebahagian daripada program 'Mussel Watch' di rantau ini. Kerja-kerja dalam membuktikan* P. viridis *sebagai agen pemantauan, yang berdasarkan kriteria yang telah dicadangkan bagi sesuatu agen pemantauan yang baik, boleh diterima pakai untuk spesies moluska yang lain.*

ABSTRACT

This paper aims to review the papers published using Perna viridis *as a biomonitoring agent of heavy metals in this region and particularly from Malaysia and to discuss the existing knowledge. Further research which will enhance our understanding and increase our knowledge on heavy metal ecotoxicology in Malaysia by using* P. viridis *is also suggested. This can be part of Mussel Watch monitoring program in this region. The work in establishing* P. viridis *for the biomonitoring of heavy metals, which is fundamentally based on the recommended criteria for a good biomonitoring agent, can be applied in other mollusks species.*

INTRODUCTION

Since the Mussel Watch monitoring approach was initially proposed by Goldberg (1975), the use of the green-lipped mussel *Perna viridis* as a biomonitoring agent for heavy metal pollution studies in the Asia-Pacific coastal waters has been reported in the literature for over a quarter of a century. In the Southeast Asian region, perhaps the earliest reported study was conducted in Thailand's coastal waters (Menasveta and Cheevaparanpiwat 1981). In Malaysia, the monitoring of contaminant levels in the coastal waters of Penang using Mussel Watch approach was reported in the 1980s (Sivalingam and Bhaskaran 1980; Sivalingam *et al.* 1982; Sivalingam 1985). However, there has been a long absence of reported data using this mussel species until Ismail (1993a) reported the general levels of heavy metals in *P. viridis* from the west coast of Peninsular Malaysia and Din and Jamaliah (1994) reported those from Penang Island. This paper aims to review the papers published in this region and particularly from Malaysia and to discuss the existing knowledge and potential research area for future studies. In this paper, most reported work on *P. viridis* particularly for biomonitoring of contaminants is reviewed from other regions and discussion is focused upon from those reported from Malaysia.

Overview of Biomonitoring Studies Using Perna viridis *from this Region*

The use of *P. viridis* for the biomonitoring studies in the coastal waters has been implemented in

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Asia Pacific coastal waters since 1980s (Tanabe 2000; Tanabe *et al.* 2000; Nicholson and Lam 2005) such as in China (Klumpp *et al.* 2002), Singapore (Bayen *et al.* 2004), Hong Kong (Nicholson and Szefer 2003), Malaysia (Ismail *et al.* 2003; Yap *et al.* 2003a) and Thailand, Philippines and India (Tanabe *et al.* 2000). The mussel species has been shown to be a good biomonitoring agent for contaminants such as organochlorines (Klumpp *et al.* 2002; Monirith *et al.* 2003; Bayen *et al.* 2004), buytltin compounds (Kan-atireklap *et al.* 1997; Fung *et al.* 2004; Sudaryanto *et al.* 2004), heavy metals (Wong *et al.* 2000; Nicholson and Szefer 2003; Yap *et al.* 2003a; Wang *et al.* 2004) and polychlorinated biphenyls (Tanabe *et al.* 1987). Other ecotoxicological studies using *P. viridis* include the uptake and assimilation of metals (Pan and Wang 2004; Wang *et al.* 2004), feeding behaviors (Wong and Cheung 1999; 2001), DNA strand breakage (Siu *et al.* 2003), metal exposure studies (Yap *et al.* 2004a; Shi and Wang 2004) and other physiological studies on its cytological, lysosomal, ecocytological and cardiac responses to metals (Nicholson 1999a, 1999b, 2001, 2003) and biomarkers (Lau and Wong 2003). All the mentioned work implied the importance of biomonitoring studies and *P. viridis* will continue to be used and focused upon for biomonitoring purposes in future.

WHY *PERNA VIRIDIS* ?

The state of metal pollution on the west coast of Peninsular Malaysia has been assessed by measuring metal levels in 1) sediments (Ismail 1993b; Ismail *et al.* 1993; Yap *et al.* 2002a, 2003b, 2003c) and 2) mollusks as biomonitoring agents. Based on our work done since 1998, we tested the suitability of *P. viridis* as a biomonitoring agent of heavy metals for Peninsular Malaysia based on the recommended criteria for a good biomonitoring agent (Phillips and Rainbow 1993; Tanabe 2000; Monirith *et al.* 2003). The eight criteria recommended are given below and the studies done so far to test each criterion are reviewed.

Criterion 1: Wide Geographical Distribution in the Coastal Waters

Mussels can easily be found in the west coast of Peninsular Malaysia during our field trip from 1998 to 2004. Background levels of Cd, Cu, Hg, Pb and Zn in the soft tissues of *P. viridis* were found and reported in the literature (Ismail *et al.* 2000, Yap *et al.* 2003a, 2003d) and this baseline is very important for future reference.

In April 2004, a survey and sampling was conducted from Tumpat to Mersing off the east coast of Peninsular Malaysia and the green-lipped mussels were found in very small numbers from Tumpat (Kelantan) and Nenasi (Pahang) and relatively high abundance of natural mussel population at Kuala Pontian (Pahang) (Yap *et al.* 2004b). This confirmed the fact that the abundance of *P. viridis* along the east coast is not as high as that in the west coast of the peninsula. Thus, the geographical distribution of mussel populations in the coastal waters of Peninsular Malaysia is mainly in the west coast, including the Straits of Johore. Since the natural habitats of mussels are found at Kuala Pontian, this information is important for mussel transplantation purpose because the east coast is regarded less contaminated than in the west coast.

Criterion 2: Sedentary Lifestyle after the Pelagic Lifespan

The biology of *P. viridis* is interesting since they experience a short (2-3 weeks) period of mobile pelagic larvae in the beginning of their life stage and become non-mobile after their attachment to substrata.

Criterion 3: Easy Sampling since They Can be Found in Abundance

Since most mussels can be collected under buoys, nibong poles or hanging ropes by raft (for mariculture sites), they are relatively easy for sampling.

Criteria 2 and 3 can be easily justified based on our field samplings and from the literature.

Criterion 4: Simple Correlations between the Metal Levels in the Mussels and Those in Their Environments

For this criterion, Yap *et al.* (2002b) found that significant ($P< 0.05$) correlations were observed between Cd in *P. viridis* and Cd in the sediment (EFLE fraction and total Cd), Cu in *P. viridis* and Cu in the sediment (EFLE and 'acid-reducible' fractions and total Cu) and Pb in *P. viridis* and Pb in the sediment ('oxidisable-organic' fraction and total Pb). No significant correlation ($P> 0.05$) was found between Zn in *P. viridis* and all the sediment geochemical fractions of Zn and total Zn in the sediment.

This indicates that Zn is possibly 'partially regulated' by the soft tissue of *P. viridis*. The results support the use of *P. viridis* as a suitable biomonitoring agent for Cd, Cu and Pb.

Criterion 5: Easy Identification of the Species due to Low Variability

We identified *P. viridis* by using morphological characters such as the green colour found specifically on the periphery of the shell and the total absence of the anterior adductor muscle (Siddall 1980). However, the question is 'Are they genetically similar so that the various geographical populations of the mussels can be used as a biomonitoring agent?' By using the isozyme approach, Yap *et al.* (2002c) studied the genetic structures of *P. viridis* collected from the west coast of Peninsular Malaysia and found an interesting phenomenon. From the eight geographical populations studied, fourteen polymorphic loci were observed. The observed mean heterozygosity ranged from 0.108 to 0.334, while the expected mean heterozygosity ranged from 0.133 to 0.301. The populations studied could be divided into two groups by the UPGMA dendrogram based on Nei's (1978) genetic similarities. The groupings seemed to indicate differentiation into local populations. These results suggested that *P. viridis* had a tendency to split into a number of geographical populations regardless of larval dispersal as a potential agent of gene flow. The mean *Fst* value of 0.149 indicated that the mussel populations showed a moderate degree of genetic differentiation. However, the mean genetic distance from the study (0.048 ± 0.004) fell within the range of genetic distances between conspecific populations of mussels (0.0-0.14). Therefore, the study supported the use of the local mussel *P. viridis* as a suitable biomonitoring agent for heavy metals (Yap *et al.* 2002d). The range of genetic distance values (0.004-0.091) that were obtained would also serve as baseline data with which results of similar studies in the future can be compared to determine whether genetic divergence of mussel populations from the west coast of Peninsular Malaysia is taking place. At the molecular level, Chua *et al.* (2003) reported that the population genetic structure of the green-lipped mussel *P. viridis* (L.) as indicated by Randomly Amplified Polymorphic (RAPD) molecular markers, was associated with the transplantation of mussels from Johore to Langkawi for mariculture purpose.

Criterion 6: Capacity to Accumulate Pollutants in the Tissues of the Mussels

The bioaccumulative properties of Cd, Cu, Pb and Zn were investigated. Although marine mussels are generally used as biomonitoring agents of heavy metals, regulative mechanisms of essential metals in the soft tissues of mussels have been reported (Phillips and Rainbow 1993). Earlier, Phillips (1985) and Chan (1988, 1989) studied the potential of *P. viridis* collected from Hong Kong coastal waters as a biomonitor of Cd, Cu, Pb and Zn. In Malaysia, Yap *et al.* (2002a, 2003e, 2003f) used similar mussel species for the same purpose but those mussels were collected from the coastal waters of Peninsular Malaysia and they used different methodology to find evidences to prove the suitability of *P. viridis* as a biomonitoring agent of heavy metals.

Yap *et al.* (2003e, 2003f) did ecotoxicological tests on *P. viridis* under laboratory conditions. Different rates of accumulation and depuration of Cd, Cu, Pb and Zn in the soft tissues were found and this might be due to different mechanisms for metal binding and regulation. At the end of depuration, Cd levels in the soft tissues of *P. viridis* were 10-30 times higher than those before exposure, while Zn levels in the soft tissues were almost similar to the levels before exposure (Yap *et al.* 2003e). These results indicate that *P. viridis* is a good biomonitoring organism for Cd but Zn levels may be actively regulated. It remains uncertain whether or not *P. viridis* is a good biomonitoring organism of environmental Zn contamination (Yap *et al.* 2003e). The high ratio of maximum to minimum Pb and Cu levels and the similar patterns (although at different rates) of accumulation and depuration in the different soft tissues for Pb and Cu indicate that *P. viridis* is a good biomonitoring organism for Cu and Pb (Yap *et al.* 2003f, 2004a). The conclusion on Cu and Pb was also supported by analysis of field samples collected from contaminated and uncontaminated sites (Yap *et al.* 2003f; Yap *et al.* 2004a). Recently, Yap *et al.* (2005) suggested that crystalline style and metal redistribution in the different soft tissues of *P. viridis* can be used as indicators of Cu and Pb bioavailabilities and contamination in coastal waters.

Criterion 7: They are Tolerant but Relatively Sensitive to Chemical Pollutants

To prove that they are tolerant but sensitive to heavy metal stress, studies on toxicities and tolerances of Cd, Cu, Pb and Zn in *P. viridis* were conducted by short-term bioassays using endpoint mortality (Yap *et al.* 2004c). The LC_{50} values for the mussels were 1.53 mg/L for Cd, 0.25 mg/L for Cu, 4.12 mg/L for Pb and 3.20 mg/L for Zn. These LC_{50} values were within the concentration ranges that were reported by other authors who used *P. viridis* as the test organism. Based on these LC_{50} values, the mussel seems to be most sensitive to Cu, followed by Cd, Zn and Pb.

In addition to endpoint mortality, toxicity tests of the effects of Cd, Cu, Pb and Zn based on the endpoint filtration rate (FR) of *P. viridis* were also conducted (Yap *et al.* 2003g). Probit analysis was used to calculate the metal concentrations that inhibited 50% of the FR (EC_{50}) of the experimental samples when compared to the control samples. For mussels with size ranging from 6-8 cm shell length (at salinity: 26 ppt, temperature: 28°C, pH: 7.87 and dissolved oxygen: 7.10 mg/L), the EC_{50} values were: 0.43 mg/L for Cd, 0.31 mg/L for Cu, 2.81 mg/L for Pb and 1.67 mg/L for Zn. The results suggest that the mussels are most sensitive to Cu, followed by Cd, Zn and Pb. This result agrees well with those that were obtained from the experiment which used mortality as an endpoint as has been mentioned earlier. Yap *et al.* (2004d) found that the smaller size group *P. viridis* is more sensitive, based on lower LC_{50} values, than those of the larger size group mussel.

To enhance our understanding of the sensitivity of *P. viridis* toward heavy metals (Cd and Pb), the relationships between condition index (CI) and accumulated concentrations of Cd and Pb were determined in field samples of *P. viridis* and those under experimental conditions. The field samples included those from uncontaminated and contaminated sites (Yap *et al.* 2002e). In the field samples, significant ($P < 0.05$) negative relationships between CI and metal concentrations were found. However, the results could also have been due to variations in nutritional state and reproductive status of the mussels. To clarify these uncertainties, an experimental study was conducted and the results agreed with that found in the field samples. Therefore, the results indicated that if the heavy metal levels of the field collected mussels were significantly ($P < 0.001$) different, the influences caused by the nutritional and reproductive states would be masked by the ecotoxicological effects of heavy metal contamination, provided that mussels with similar size range were selected for the CI determination. The CI can be used to show that *P. viridis* is a sensitive organism to Cd and Pb. This work also showed that the sensitivity of *P. viridis* to Cd and Pb when CI was used as a physiological index. Recently, Al-Barwani *et al.* (2004) found that CI can be used as a physiological index for the reproductive status of *P. viridis*.

Criterion 8: Public Concern from the Health Point of View Since They are a Commercially Important Protein Source

This criterion is important from the public health standpoint. The mussels collected between 1999 and 2001 from the wild and from aquacultural sites, off the west coast of Peninsular Malaysia were analysed for Cd, Cu, Hg, Pb and Zn concentrations and these values were compared to permissible limits from established guidelines for food safety in US, UK, Hong Kong, Australia, China and maximum permissible limits established by Malaysian Food Regulations 1985 (Yap *et al.* 2004e). The concentrations of these heavy metals should result in no acute toxicities of the metals since they were lower than the permissible limits for human consumption. In addition, these metal concentrations were also considered to be low when compared with regional data based on *P. viridis* as a biomonitoring agent. However, the risk would depend on the amount of mussels being consumed daily and weekly.

Other Studies on Perna viridis

Apart from the above criteria, related work that can enhance our understanding of the use of *P. viridis* as a biomonitoring agent includes the potential use of the occurrence of shell deformities (Yap *et al.* 2002f) as an indicator, and byssus (Yap *et al.* 2003h) and shell (Yap *et al.* 2003i, 2004f) as better biomonitoring materials of heavy metal contamination in coastal waters. Yap *et al.* (2003h) found that when compared to the soft tissues, the byssus was a more sensitive biomonitoring organ for Zn while it could be a complementary organ for Cd and Pb in the total soft tissues. The potential of the byssus of *P.*

viridis to monitor the metal pollution in the coastal waters was shown by Sivalingam *et al.* (1983). Since the total soft tissue of *P. viridis* had been reported to have a regulatory mechanism for Zn, the byssus can be used as a biomonitoring organ for the identification of coastal areas exposed to Zn pollution. Our suggestion is supported by the data reported by Nicholson and Szefer (2003) in which the byssus of *P. viridis* collected from a contaminated site at Kennedy Town of Hong Kong coastal waters accumulated about 3 times higher Zn concentration than that of an uncontaminated site at Kat O.

Apart from the soft tissues and byssus, the hard tissue, the shell of *P. viridis*, is also shown to be potential as a biomonitoring material of heavy metals (Yap *et al.* 2003i; Yap *et al.* 2004f). Yap *et al.* (2003i) found that the depuration of heavy metals in the shell was not affected by the physiological condition of the mussels. Although Zn could be regulated by the soft tissues, the incorporated Cd, Pb and Zn remained in the shell matrices. The present results supported the use of the total shell of *P. viridis* as a potential biomonitoring material for long-term contamination by Cd, Pb and Zn. By using correlation analysis between Zn levels in the soft tissue of *P. viridis* and those in the geochemical fractions of sediments, Yap *et al.* (2004f) found a higher correlation coefficient of Zn between shells-sediment than those of soft tissues-sediments. Therefore, the mussel shells can be used to monitor Zn pollution in the coastal waters more effectively than the soft tissue of *P. viridis*. Yap *et al.* (2004g) also suggested that allozyme polymorphism in *P. viridis* was a potential biomonitoring tool for heavy metal contamination.

In agreement with Boyden's formula (1977), Yap *et al.* (2003j) showed that the plotting of metal content against dry body flesh weight on a double logarithmic basis gave good positive straight lines. It was found that the smaller mussels (lower total soft tissue dry weight) had higher concentrations of Cd, Pb and Zn than the larger ones. Since shell thickness could be considered to be an estimate of the age of the mussel (Yap *et al.* 2003k), it was also found that the younger mussels accumulated more Cd, Pb and Zn than the older ones. This indicated that *P. viridis* has a different metabolic strategy for each of the studied metals which may be related to age. Nonetheless, the physiological conditions of the mussels, as indicated by the CI, was found not to be a significant factor affecting the accumulations of Cd, Cu, Pb and Zn in the mussel.

What are the Next Endeavors?

All the previous studies have contributed towards the use of *P. viridis* as a suitable biomonitoring agent of trace toxic contaminant levels in the west coast of Peninsular Malaysia. However, there are still a lot of unknowns to be looked into in the future. A lot of questions arise. We will again look into the previously mentioned criteria one after another and discuss what is still lacking in our knowledge of *P. viridis*.

For criterion 1, although *P. viridis* is widely found and distributed in the west coast of Peninsular Malaysia, the decline in spatfalls in certain areas has prompted massive transplantations from high spatfall coastal areas to low spatfall sites. This phenomenon needs further attention. Another question that arises is a) Why are there hardly (although they can still be found) any mussels found naturally in the east coast of Peninsular Malaysia although there have been reports in the literature that they are found in the Gulf of Thailand (Hungspreugs *et al.* 1984; Sukasem and Tabucanon 1993; Ruangwises and Ruangwises 1998) which is located just above the east coast of the peninsula.

Generally, we think that the topography, water quality, attachment substrates and the big waves of the east coast are factors that limit the growth of *P. viridis* on the peninsula's east coast. Therefore, detailed studies to find out the exact reasons for the rarity there should be undertaken.

For criterion 2, its sedentary adulthood is the determinant for the successful survival of *P. viridis* in coastal waters. This is much related to the attachment properties of the mussel byssus. a) What are the chemicals responsible for the detachment of byssus that would ultimately affect the abundance of mussels in coastal waters? b) What attachment substrates in spat collectors are more suitable to collect spats in the high spatfall waters? c) What is the composition in the byssus responsible for the attachment on hard substrates and d) Can the attachment plaque properties of the mussel byssus be applied for the benefit of humans?

For criterion 3, the occurrence in abundance of mussels in the west coastal waters of Peninsular

Malaysia is localized although they are widely found along this coast. This abundance is much related to the spatfalls in certain areas during transplantation. Even in the west coast, a) Is the spatfall density related to the attachment substrates? b) What are the reasons for the low spatfall in certain areas? c) What are the peak seasons of reproductive cycles in the west coast of Peninsular Malaysia (the Straits of Malacca)? d) How can we maintain the spatfalls of the high reproduction sites? e) How can we enhance high spat collection at a less productive spat area? and f) Could the low spatfall area be a result of pollution?

The success of mussel farming depends heavily on spatfalls in the natural environment. The origin of the spats is unknown and the supply is highly variable. Apart from the problem of insufficient supply of spats for seeding, genetic problems can arise from the use of wild stocks of unknown origins. Therefore, selection and improvement of cultured mussels may be hindered by the limited amount of genetic information available (Yap *et al.* 2002c, 2002d). Based on this aspect, information about the population genetic structure of *P. viridis* collected along the west coast of Peninsular Malaysia is undoubtedly very important. This particular aspect is now being looked into, using single-locus codominant microsatellite markers.

For criterion 4, simple correlations between the biomonitoring agent and its environment could only be found for certain metals such as Cd, Cu and Pb but not for Zn (Yap *et al.* 2002b, 2003h). This was due to the fact that not all metals are effectively accumulated in the soft tissues of *P. viridis.* 'Partial regulation' of Zn in the soft tissues of *P. viridis* was reported (Phillips 1985, Yap *et al.* 2003h) and this could affect the accuracy of the estimation of Zn pollution levels in coastal waters. Still, a lot of information on Zn levels in mussel soft tissues had been obtained from Hong Kong, Thailand, India and recently Malaysia. Perhaps, the question should be 'At what degree of metal regulation should the mussel be rejected as a biomonitoring agent of a particular metal?'

For criterion 5, low variability in morphological features and genetic variations need further validation. So far, only studies using isozyme markers have been reported to investigate levels of genetic variations of this species collected along the west coast of Peninsular Malaysia (Yap *et al.* 2002c). However, isozyme based studies are limited by low polymorphisms and the neutrality of isozymes is still doubtful (Jarne and Lagoda 1996). Therefore, hypervariable markers should be obtained at the DNA level. Polymorphisms detected at the DNA level are higher and give more information. Several questions could arise here; a) What are the DNA patterns of the local populations or indigenous species of *P. viridis*? b) What are the genetic variations of *P. viridis* collected along the west coast of Peninsular Malaysia, based on DNA markers (microsatellites)? c) How can the genetic information be used in maintaining the brookstocks of *P. viridis*? d) What are the useful genetic markers to distinguish between male and female mussels? e) What are the DNA level diagnostic markers to distinguish between contaminated and uncontaminated mussel populations?

For criterion 6, we found *P. viridis* is accumulative of Cd, Cu, Hg, Pb and Zn but we are lacking the knowledge and understanding on the chemical interactions among these metals in affecting the accumulation of metals in the mussel tissues. Also, other anthropogenic hazardous trace metals like As, Co, Ni, Fe and Se in the soft and hard tissues of *P. viridis* collected from Malaysia have not been studied yet to our knowledge.

For criterion 7, to further test the tolerances and sensitivities of *P. viridis* to heavy metals, other physiological indices should be tried and used. Also, the effects of single and multiple metals in affecting the tolerances and toxicities of metals that could cause 'synergistic' or 'additive' phenomenon are interesting research topics for future studies when this criterion is being focused upon.

For criterion 8, our risk assessment of heavy metal consumption through shellfish in Malaysia by comparison with established guidelines for food products from other countries should be improved. This could be done by establishing Malaysian guidelines based on our data spanning at least 10 years. To achieve this, the heavy metal concentrations in the soft tissues of *P. viridis* along the west coast of the peninsula should be studied yearly. 'What are the Malaysian hazardous indices/guidelines for shellfish consumption (rather than fish product in general)?' is also a question that should prompt further studies.

CONCLUSION

To establish an organism to be a good biomonitoring agent needs a lot of scientific research. Although workers from other regions have studied similar mussel species for the biomonitoring purpose, relatively similar studies should be conducted to test the local species and the existing information should be considered for comparison rather than extrapolation of the same phenomenon and results if the local similar species is used for biomonitoring studies. Our experience using the mussel *P. viridis* is interesting and by reviewing all the work, there is still a lot of interesting research areas to be investigated. Therefore, we suggest some of the potential work to be carried out in future studies but that would be greatly dependent on the numbers of researchers and the research funding available to study them. The work in establishing *P. viridis* for the biomonitoring of heavy metals, which is fundamentally based on the recommended criteria for a good biomonitoring agent, can be applied in other mollusks species such as the popular cockles and fish tilapia.

REFERENCES

AL-BARWANI, S., A. ARSHAD, B. JAPAR SIDIK, S. SITI SHAPOR and C. K. YAP. 2004. Comparison of condition index of the green-lipped mussel *Perna viridis* between Sebatu and Pasir Panjang. Presented at Second Biology Colloquium, Department of Biology, UPM. Biology: Unity and diversity. 22 December 2004, Marriot Putrajaya.

BAYEN, S., G.O. THOMAS, H.K. LEE and J.P. OBBARD. 2004. Organochlorine pesticides and heavy metals in green mussel, *Perna viridis* in Singapore. *Wat. Air Soil Poll.* **155:** 103-116.

BOYDEN, C.R. 1977. Effects of size upon metal contents of shellfish. *J. Mar. Biol. Assoc. U. K.* **57:** 675-714.

CHAN, H.M. 1988. Accumulation and tolerance to cadmium, copper, lead and zinc by the green-lipped mussel *Perna viridis. Mar. Ecol. Prog. Ser.* **48:** 295-303.

CHAN, H.M. 1989. Temporal and spatial fluctuations in trace metal concentrations in transplanted mussels in Hong Kong. *Mar. Poll. Bull.* **20:** 82-86.

CHUA, B. H., S. H. YONG, C. H. TEH, S. G. TAN, C. K. YAP and A. ISMAIL. 2003. Population genetic structure of the green-lipped mussel *Perna viridis* (L.) as indicated by Randomly Amplified Polymorphic (RAPD) molecular markers. In *Aquatic Resource and Environmental Studies of the Straits of Malacca: Managing the Straits Through Science and Technology*, ed. B. Japar Sidik, A. Arshad, H. H. Zakaria and A. Kawamura, p. 291-301. Serdang: MASDEC and JICA, UPM Malaysia.

DIN, Z.B. and M.R.S.N. JAMALIAH. 1994. Trace metal pollution in the coastal areas of Penang Island, Malaysia. In *ASEAN criteria and monitoring: Advances in marine environmental management and human health protection*, eds. D. Watson, S. O. Koh and G. Viger, p. 240-245. ASEAN-Canada Co-operative Programme on Marine Science.

FUNG, C.N., J.C. W. LAM, G.J. ZHENG, D.W. CONNELL, I. MONIRITH, S. TANABE, B.J. RICHARDSON and P.K.S. LAM. 2004. Mussel-based monitoring of trace metal and organic contaminants along the east coast of China using *Perna viridis* and *Mytilus edulis. Environ. Poll.* **127:** 203-216.

GOLDBERG, E. D. 1975. The Mussel Watch - A first step in global marine monitoring. *Mar. Poll. Bull.* **6:** 111.

HUNGSPREUGS, M., S. SILPIPAT, C. TONAPONG, R. F. LEE, H. L. WINDOM and K. R. TENORE. 1984. Heavy metals and polycyclic hydrocarbon compounds in benthic organisms of the Upper Gulf of Thailand. *Mar. Poll. Bull.* **15:** 213-218.

ISMAIL, A. 1993a. Heavy metals Cd, Cu, Pb and Zn in green mussel (*Perna viridis* L.) from the west coast of Peninsular Malaysia. 9th – 12th December. *IRPA/UKM Workshop and Seminar*, Allson Kelana, Seremban Malaysia.

ISMAIL, A. 1993b. Heavy metal concentration in sediments off Bintulu, Malaysia. *Mar. Poll. Bull.* **26:** 706-707.

ISMAIL, A., C. K. YAP, M.P. ZAKARIA, S. TANABE, H. TAKADA and A. RAHIM ISMAIL. 2000. Green-lipped mussel *Perna viridis* (L.) as a biomonitoring agent for heavy metals in the west coast of Peninsular Malaysia. In *Towards Sustainable Management of the Straits of Malacca,* *Technical and Financial Options*, ed. M. Shariff, F. M. Yusoff, N. Gopinath, H. M. Ibrahim and A. Nik Mustapha, p. 553-559. MASDEC, Serdang, Malaysia: Universiti Putra Malaysia.

ISMAIL, A., M.A. BADRI and M. N. RAMLI. 1993. The background levels of heavy metal concentration in sediments of the west coast of Peninsular Malaysia. *Sci. Tot. Environ.* **Suppl. 1993:** 315-323.

ISMAIL, A., S. TANABE and C.K. YAP. 2003. Mussel Watch: Heavy metals and tributyltin of the Straits of Malacca. Paper presented at *UPM-JICA Project Workshop: Managing the Straits Through Science and Technology*, 14-16 January. Renaissance Palm Garden Hotel Putrajaya.

JARNE, P. and P.J.L. LAGODA. 1996. Microsatellites, from molecules to populations and back. *TREE* **11:** 424-429.

KAN-ATIREKLAP, S., S. TANABE, J. SANGUANSIN, M.S. TABUCANON and M. HUNGSREUGS. 1997. Contamination by butyltin compounds and organochlorine residues in green mussel (*Perna viridis*, L.) from Thailand coastal waters. *Environ. Poll.* **97:** 79-89.

KLUMPP, D.W., H. HUASHENG, C. HUMPHREY, W. XINHONG and S. CODI. 2002. Toxic contaminants and their biological effects in coastal waters of Xiamen, China. I. Organic pollutants in mussel and fish tissues. *Mar. Poll. Bull.* **44:** 752-760.

LAU, P.S. and H.L. WONG. 2003. Effect of size, tissue parts and location on six biochemical markers in the green-lipped mussel, *Perna viridis*. *Mar. Poll. Bull.* **46:** 1563-1572.

MENASVETA, P. and V. CHEEVAPARANAPIWAT. 1981. Heavy metals, organochlorines, pesticides and PCBs in green mussels, mullets and sediments of river mouth in Thailand. *Mar. Poll. Bull.* **12:** 19-25.

MONIRITH, I., D. UENO, S. TAKAHASHI, H. NAKATA, A. SUDARYANTO, A. SUBRAMANIAN, S. KARUPPIAH, A. ISMAIL, M. MUCHTAR, J.S. ZHENG, B.J. RICHARDSON, M. PRUDENTE, N.D. HUE, T.S. TANA, A.V. TKALIN and S. TANABE. 2003. Asia-Pacific mussel watch: monitoring contamination of persistent organochlorine compounds in coastal waters of Asian countries. *Mar. Poll. Bull.* **46:** 281-300.

NEI, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* **89:** 583-590.

NICHOLSON, S. 1999a. Cytological and physiological biomarker responses from green mussels, *Perna viridis* (L.) transplanted to contaminated sites in Hong Kong coastal waters. *Mar. Poll. Bull.* **39:** 261-268.

NICHOLSON, S. 1999b. Cardiac and lysosomal responses to periodic copper in the mussel *Perna viridis* (Bivalvia: Mytiloidae). *Mar. Poll. Bull.* **38:** 1157-1162.

NICHOLSON, S. 2001. Ecocytological and toxicological responses to copper in *Perna viridis* (L.) (Bivalve: Mytilidae) haemocyte lysosomal membranes. *Chemosphere* **45:** 399-407.

NICHOLSON, S. 2003. Cardiac and branchial physiology associated with copper accumulation and detoxication in the mytilid mussel *Perna viridis* (L.). *Journal of Experimental Marine Biology and Ecology* **295:** 157-171.

NICHOLSON, S. and P.K.S. LAM. 2005. Pollution monitoring in Southeast Asia using biomarkers in the mytilid mussel *Perna viridis* (Mytilidae: Bivalvia). *Environ. Int.* **31:** 121-132.

NICHOLSON, S. and P. SZEFER. 2003. Accumulation of metals in the soft tissues, byssus and shell of the mytilid mussel *Perna viridis* (Bivalvia: Mytilidae) from polluted and uncontaminated locations in Hong Kong coastal waters. *Mar. Poll. Bul.* **46:** 1040-1043.

PAN, J-F. and W-X. WANG. 2004. Influences of dissolved and colloidal organic carbon on the uptake of Ag, Cd, and Cr by the marine mussel *Perna viridis*. *Environ. Poll.* **129:** 467-477.

PHILLIPS, D.J.H. 1985. Organochlorines and trace metals in green-lipped mussels *Perna viridis* from Hong Kong waters: A test of indicator ability. *Mar. Ecol. Prog. Ser.* **21:** 251-258.

PHILLIPS, D.J.H. and P.S. RAINBOW. 1993. *Biomonitoring of Trace Aquatic Contaminants*. 371 pp. London: Elsevier Science.

RUANGWISES, N. and S. RUANGWISES. 1998. Heavy metals in green mussels *(Perna viridis)* from the Gulf of Thailand. *J. Food Protection* **61:** 94-97.

SHI, D. and W-X. WANG. 2004. Modification of trace metal accumulation in the green mussel *Perna viridis* by exposure to Ag, Cu, and Zn. *Environ. Poll.* **132:** 265-277

SIDDALL, S.E. 1980. A clarification of the genus *Perna* (Mytilidae). *Bull. Mar. Sci.* **30:** 858-870.

SIU, W.H.L., C.L.H. HUNG, H.L. WONG, B.J. RICHARDSON and P.K.S. LAM. 2003. Exposure and time dependent DNA strand breakage in hepatopancreas of green-lipped mussels (*Perna viridis*) exposed to Aroclor 1254, and mixtures of B[*a*]P and Aroclor 1254. *Mar. Poll. Bul.* **46:** 1285-1293.

SIVALINGAM, P.M. 1985. An overview of the Mussel Watch monitoring programme in conjunction with the Westpac programme. *J. Asian Environ.* **6:** 39-50.

SIVALINGAM, P.M. and B. BHASKARAN. 1980. Experimental insight of trace metal environmental pollution problems in mussel farming. *Aquaculture* **20:** 291-303.

SIVALINGAM, P.M., H. SCHWENKE and J. KNOTH. 1983. Mussel byssus as biomonitor of heavy metals. *J. Singapore Nat. Acad. Sci.* **10-12:** 34-37.

SIVALINGAM, P.M., I. ALLAPITCHAY, H. KOJIMA and T. YOSHIDA. 1982. Mussel watch of PCBs and persistent pesticides residues in *Perna viridis* Linnaeus from Malaysian and Singapore waters. *Appl. Geography* **2:** 231-237.

SUDARYANTO, A., S. TAKAHASHI, H. IWATA, S. TANABE and A. ISMAIL. 2004. Contamination of butyltin compounds in Malaysian marine environments. *Environ. Poll.* **130:** 347-358

SUKASEM, P. and M. TABUCANON. 1993. Monitoring heavy metals in the Gulf of Thailand using Mussel Watch approach. *Sci. Tot. Environ.* **139/140:** 297-305.

TANABE, S. 2000. Asia-Pacific Mussel Watch progress report. *Mar. Poll. Bull.* **40:** 651.

TANABE, S., M.S.S. PRUDENTE, KAN-ATIREKLAP and A. SUBRAMANIAN. 2000. Mussel watch: marine pollution monitoring of butyltin and organochlorines in coastal waters of Thailand, Phillips and India. *Ocean Coast. Manage.* **43:** 819-939.

TANABE, S., R. TATSUKAWA and D.J.H. PHILLIPS. 1987. Mussels as bioindicators of PCB pollution: A case study on uptake and release of PCB isomers and congeners in green-lipped mussel (*Perna viridis*) in Hong Kong waters. *Environ. Poll.* **47:** 41-62.

WANG, W-X., R.S.K. WONG, J. WANG and Y-F. YEN. 2004. Influences of different selenium species on the uptake and assimilation of Hg(II) and methylmercury by diatoms and green mussels. *Aquat. Toxicol.* **68:** 39-50.

WONG, C.K., R.Y.H. CHEUNG and M.H. WONG. 2000. Heavy metal concentrations in green-lipped mussels collected from Tolo Harbour and markets in Hong Kong and Shenzhen. *Environ. Poll.* **109:** 165-171.

WONG, W.H. and S.G. CHEUNG. 1999. Feeding behaviour of the green mussel, *Perna viridis* (L.): Responses to variation in seston quantity and quality. *J. Exp. Mar. Biol. Ecol.* **236:** 191-207.

WONG, W.H. and S.G. CHEUNG. 2001. Feeding rates and scope for growth of green mussels, *Perna viridis* (L.) and their relationship with food availability in Kat O, Hong Kong. *Aquaculture* **193:** 123-137.

YAP, C.K., A. ISMAIL, S.G. TAN and H. OMAR. 2002a. Concentrations of Cu and Pb in the offshore and intertidal sediments of the west coast of Peninsular Malaysia. *Environ. Int.* **28:** 467-479.

YAP, C.K., A. ISMAIL, S.G. TAN and H. OMAR. 2002b. Correlations between speciation of Cd, Cu, Pb and Zn in sediment and their concentrations in total soft tissue of green-lipped mussel *Perna viridis* from the west coast of Peninsular Malaysia. *Environ. Int.* **28:** 117-126.

YAP, C.K., S.G. TAN, A. ISMAIL and H. OMAR. 2002c. Genetic variation of the green-lipped mussel *Perna viridis* (L.) (Mytilidae: Mytiloida: Mytilicae) from the west coast of Peninsular Malaysia. *Zool. Stud.* **41:** 376-387.

YAP, C.K., A. ISMAIL, S.G. TAN and H. OMAR. 2002d. Genetic structures of populations of the green-lipped mussel *Perna viridis* (Linnaeus): A biomonitoring agent for the

Straits of Malacca. In *Tropical Marine Environment: Charting Strategies for the Millennium*, ed. F.M. Fatimah, M. Shariff, H.M. Ibrahim, S.G. Tan and S.Y. Tan, p. 641-650. Serdang, Malaysia: MASDEC, Universiti Putra Malaysia.

YAP, C.K., A. ISMAIL and S.G. TAN. 2002e. Condition index of green-lipped mussel *Perna viridis* (Linnaeus) as a potential physiological indicator of ecotoxicological effects of heavy metals (Cd and Pb). *Malaysian Appl. Biol.* **31:** 37-45.

YAP, C.K., A. ISMAIL and S.G. TAN. 2002f. The occurrence of shell deformities in green-lipped mussel *Perna viridis* (Linnaeus) collected from Malaysian coastal waters. *Bull. Environ. Contam. Toxicol.* **69:** 877-884.

YAP, C.K., A. ISMAIL and S.G. TAN. 2003a. Background concentrations of Cd, Cu, Pb and Zn in the green-lipped mussel *Perna viridis* (Linnaeus) from Peninsular Malaysia. *Mar. Poll. Bull.* **46:** 1035-1048.

YAP, C.K., A. ISMAIL and S.G. TAN. 2003b. Cd and Zn in the straits of Malacca and intertidal sediments of the west coast of Peninsular Malaysia. *Mar. Poll. Bull.* **46:** 1348-1353.

YAP, C.K., A. ISMAIL and S.G. TAN. 2003c. Mercury levels of the surface sediments of the intertidal area along the west coast of Peninsular Malaysia. *Toxicol. Environ. Chem.* **85:** 13-21.

YAP, C.K., A. ISMAIL and S.G. TAN. 2003d. Mercury levels in the green-lipped mussel *Perna viridis* (Linnaeus) from the west coast of Peninsular Malaysia. *Bull. Environ. Contam. Toxicol.* **71:** 570-576.

YAP, C.K., A. ISMAIL, H. OMAR and S.G. TAN. 2003e. Accumulation, depuration and distribution of cadmium and zinc in the green-lipped mussel *Perna viridis* (Linnaeus) under laboratory conditions. *Hydrobiologia* **498:** 151-160.

YAP, C.K., A. ISMAIL and S.G. TAN. 2003f. Different soft tissues of the green-lipped mussel *Perna viridis* (Linnaeus) as biomonitoring agents of copper: field and laboratory studies. *Malay. Appl. Biol.* **32:** 9-18.

YAP, C.K., A. ISMAIL, S.G. TAN and H. OMAR. 2003g. Effects of heavy metals (Cd, Cu, Pb and Zn) on the filtration rate of the green-lipped mussel *Perna viridis* (Linnaeus). *Malaysian Appl. Biol.* **32:** 7-13.

YAP, C.K., A. ISMAIL, S.G. TAN and H. OMAR. 2003h. Can the byssus of green-lipped mussel *Perna viridis* (Linnaeus) from the west coast of Peninsular Malaysia be a biomonitoring organ for Cd, Pb and Zn? Field and laboratory studies. *Environ. Int.* **29:** 521-528.

YAP, C.K., A. ISMAIL, S.G. TAN and I. ABDUL RAHIM. 2003i. Can the shell of the green-lipped mussel *Perna viridis* (Linnaeus) from the west coast of Peninsular Malaysia be a potential biomonitoring material for Cd, Pb and Zn ? *Estuar. Coast. Shelf Sci.* **57:** 623-630.

YAP, C.K., A. ISMAIL and S.G. TAN. 2003j. Effects of body size (total soft tissue) and shell thickness on the accumulation of heavy metals (Cd, Cu, Pb and Zn) in the green-lipped mussel *Perna viridis* (Linnaeus). *Russian J. Mar. Biol.* **29:** 323-327.

YAP, C.K., A. ISMAIL and S.G. TAN. 2003k. Water content, conversion factor, shell thickness and condition index of the green-lipped mussel *Perna viridis* (Linnaeus) from Malaysian coastal waters. *Malay. Nature J.* **56(4):** 387-396.

YAP, C.K, A. ISMAIL and S.G. TAN. 2004a. Assessment of different soft tissues of the green-lipped mussel *Perna viridis* (Linnaeus) as biomonitoring agents of Pb: Field and laboratory studies. *Wat. Air Soil Poll.* **153:** 253-268.

YAP, C.K., W. H. CHENG, A. ISMAIL and S.G. TAN. 2004b. Tissue distribution of heavy metals (Cd, Cu, Pb and Zn) in the green-lipped mussel *Perna viridis* from Nenasi and Kuala Pontian, east coast of Peninsular Malaysia. Presented at *Second Biology Colloquium*, Department of Biology, UPM. 22 December. Biology: Unity and diversity. Marriot Putrajaya.

YAP, C.K., A. ISMAIL, H. OMAR and S.G. TAN. 2004c. Toxicities and tolerances of Cd, Cu, Pb and Zn in a primary producer (*Isochrysis galbana*) and in a primary consumer (*Perna viridis*). *Environ. Int.* **29:** 1097-1104.