HEAVY METAL CONCENTRATIONS (Cu, Pb, Ni and Zn) IN THE SURFACE SEDIMENTS FROM A SEMI-ENCLOSED INTERTIDAL WATER, THE JOHORE STRAITS: MONITORING DATA FOR FUTURE REFERENCE

¹*YAP, C. K., ¹EDWARD, F.B. AND ²TAN, S. G.

¹Department of Biology, Faculty of Science, ²Department of Cell and Molecular Biology, Faculty of Biotechnology and Biomolecular Science, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia. Tel: 603-89466616, Fax: 603-86567454.

*Corresponding author: yapckong@hotmail.com

Abstract: For decades, the 1909-built dam-like Johore Causeway has been of much environmental concerns. This is due to the rapid economic and industrial development in southern Johore of West Malaysia and Singapore which created a lot of anthropogenic pollutants into this semi-enclosed intertidal ecosystem via riverine inputs. In this study, surface sediments were collected from the western and eastern parts of the Johore Straits, in August 2004. The samples were analyzed for Cu, Zn, Pb and Ni. As a function of dry weight, the mean total concentrations of these metals were 28.6 μg/g (west) and 110 μg/g (east) for Cu; 137 μg/g (west) and 180 μg/g (east) for Zn; 33.7 μg/g (west) and 33.6 μg/g (east) for Pb and 22.6 μg/g (west) and 27.3 μg/g (east) for Ni. Geochemical studies revealed that the metal nonresistant fractions of the sediments were 52.3%, 67.3%, 29.2% and 64.9% for Cu, Zn, Pb and Ni, respectively. The non-resistant percentages indicate that the Johore Straits is receiving anthropogenic Zn, Ni, Cu and Pb. The present data indicate that some sites at the Straits are polluted with heavy metals to a certain degree based on the set Sediment Quality Guidelines/Criteria for similar metals. The data found in this study should provide useful reference if the dam-like Causeway were to be replaced by a proposed free-flow bridge in the future.

KEYWORDS: Johore Straits, heavy metal pollution, sediments, semi-enclosed strait, monitoring

Introduction

Aquatic sediments have always been studied for heavy metal pollution because of the advantages in employing the sediment samples as indicators of heavy metal pollution (Chandra Sekhar *et al.*, 2003; Ramessur, 2004). In particular, the semi-enclosed intertidal ecosystem is an interesting wetland for ecotoxicological studies. A good example is the Tolo Harbour of Hong Kong, a polluted semi-enclosed intertidal ecosystem in Asia. The Johore Straits, situated between the southern part of Peninsular Malaysia, Johore and the island nation of Singapore, needs pollution monitoring studies due to its semi-enclosed intertidal straits and it can also potentially accumulate pollutant loads from both countries.

Geographically, the Johore Straits is a relatively narrow and shallow waterway, mostly less than 6 km across and 25 m deep. The dam-like Johore Causeway has further limited the free flow of the intertidal waters. This, in turn, limits the natural flushing of riverine input, that potentially contain high levels of heavy metals (Abdullah *et al.*, 1999; Chua *et al.*, 2000), fulfilling the typical characteristics of a semi-enclosed ecosystem in this region. The Johore Straits are very important for the floating hatchery and net cage culture of prawns, fishes and mussels (Walford and Lam, 1987; Thomas, 1988).

In the early 1970s, Chung (1970) reported that parts of the Johore Straits near the Causeway were devoid of corals, due, presumably, to the decreased salinity, increased turbidity and

silting. These were triggered by the retarded flow of seawater and increased freshwater outflow on either shore along the Johore Straits. Increasing economic developmental activities and the ensuing pollution along the Straits may threaten preservation of biological resources in the adjacent seawater (MPP-EAS, 1999; Chua *et al.*, 2000). According to ASEAN/USCRPM (1991), the major pollution sources were domestic wastes from human settlements, agro-based wastes from palm oil mills and rubber processing factories, industrial effluents from estates and discharges from animal farms. There are 18 major tributaries which empty into the Johore Straits and might carry potentially toxic chemicals into this semi-enclosed Straits since sediments are sinks for such persistent pollutants as heavy metals. In addition, large shipyard repair and construction facilities, fossil fuel fired electrical power plants and shipping docks along the Johore Straits are located in the industrial area (Wood *et al.*, 1997). These pollution problems have adversely affected natural resources from the Straits such as fisheries, recreational activities as well as the general aesthetic quality of the coastal environment particularly the inner Johore Straits (Koh *et al.*, 1991). Our monitoring study conducted in August 2004 was therefore interested to know the possibility of heavy metal toxicity due to the anthropogenic inputs.

Sediment samples were focused upon in this paper because they received widespread attention in the literature regarding heavy metal pollution study. The advantages of using the sediment are widely discussed by many researchers and studies (Salomons *et al.*, 1987; Zwolsman *et al.*, 1996; Birch *et al.*, 2001). The pollution studies in the Johore Straits have been reported by a few researchers (Mat *et al.*, 1994; Wood *et al.*, 1997; Orli and Tang, 1999; Bayen *et al.*, 2004). For example, Wood *et al.*, (1997) reported the trace metal concentrations in the sediments collected from the Straits in 1993. Recently, Bayen *et al* (2004) reported heavy metal concentrations in the sediments collected near the Singapore side on the Johore Straits. However, since then, there has been no recent work reported regarding heavy metal pollution in the sediments collected from the Malaysian side along the Johore Straits.

On the other hand, the employment of the cluster analysis in the present was to get better information about the heavy metal contaminant status in the Johore Straits. According to Simeonov et al. (2003), multivariate analysis such as cluster analysis is useful and necessary to assess large and complex databases in order to get better information about the health of the environment, the design of sampling and the effective pollution control or management of the environment. Numerous studies have reported the application of multivariate analysis to better understand pollution problems. These include studies conducted by Simeonov et al. (2003) for surface water quality (Greece), Yap and Edward (2009) for mussels (Malaysia), Alkarkhi et al. (2009) for sediment (Malaysia) and Yap et al. (2010) for mollusks (Malaysia).

This paper, reports the concentrations of Cu, Ni, Pb and Zn in the surface sediments collected in August 2004 from the Johore Straits. To demonstrate better reliability, it estimates the anthropogenic input (nonresistant fractions) of the metals in the sediments by using a four-fractions sequential extraction technique (SET).

Materials and Methods

Sampling and Storage

The sampling sites cover the area of latitude-north between 01°26' and 01°29' and longitude-east between 103°55 and 103°40' from the western to the eastern parts of the Johore Straits (Table 1 and Figure 1). Descriptions for each of the sampling sites and their Global Position System (GPS) are given in Table 1. The samplings were conducted in August 2004. Ten sampling stations were

established along the Johore Straits (Figure 1). The samples from each station were collected by using an Ekmen grab and a stainless steel spatula. The top 3 to 5 cm of surface sediments were collected at each sampling site. Each sediment sample was placed in an acid-washed polyethylene bag and frozen (-10°C) prior to analysis.

Sample Preparation and Digestion

The sediment samples were dried at 60°C for at least 72 hours until a constant dry weight. Afterwards, the samples were sieved through a 63 mm stainless steel sieve and were shaken vigorously to produce homogeneity.

For the analyses of total concentrations of Cu, Ni, Pb and Zn in sediment samples, the direct aqua-regia method was used. About one g of each dried sample was weighed and digested in a combination of concentrated nitric acid (AnalaR grade, BDH 69%) and perchloric acid (AnalaR grade, BDH 60%) in the ratio of 4: 1. As described by Yap *et al.* (2002), this was first performed at low temperature (40°C) for 1 hour and then the temperature was increased to 140°C (and retained) for at least 3 hours.

The digested samples were then diluted to a certain volume (40 ml) with double distilled water (DDW). The sample was then filtered through Whatman No 1. filter-paper and the filtrate was stored in a refrigerator (< 15°C) until analysis.

Speciation of Cu, Ni, Pb and Zn in Sediment Samples

Geochemical fractions of Cu, Ni, Pb and Zn in the sediments were obtained by using the modified SET as described by Badri and Aston (1983) that was adopted by Yap *et al* (2002). The four fractions considered, the extraction solutions and the conditions employed were:-

- easily, freely, leacheable or exchangeable (EFLE): About 10 g of sample was continuously shaken for 3 hours with 50 ml 1.0 M ammonium acetate (NH₄CH₃COO), pH 7.0 at room temperature.
- 'Acid-reducible': The residue from (1) was continuously shaken for 3 hours with 50 ml 0.25 M hydroxylammonium chloride (NH₂OH.HCL) acidified to pH 2.0 with HCL, at room temperature.
- 3. 'Oxidisable-organic': The residue from (2) was first oxidized with 30% $\rm H_2O_2$ in a water bath at 90-95°C. After cooling, the metal released from the organic complexes was continuously shaken for 3 hours with 1.0 M ammonium acetate (NH₄CH₃COO) acidified to pH 2.0 with HCL, at room temperature.
- 4. 'Resistant': The residue from (3) was digested in a combination of concentrated nitric acid (AnalaR grade, BDH 69%) and perchloric acid (AnalaR grade, BDH 60%) as performed in the direct aqua-regia method.

The residue used for each fraction was weighed before the next fractionation was carried out. The residue was washed with 20 ml DDW. It was filtered through a Whatman No.1 (Filter speed: medium) filter paper in a funnel and the filtrate was stored. For each fraction of the sequential extraction procedure, a blank was employed using the same procedure to ensure that the samples and chemicals used were free of metal contaminants.

Determination of Cu, Ni, Pb and Zn

After filtration, the prepared samples were analyzed for heavy metals by using an air-acetylene flame atomic absorption spectrophotometer (AAS) Perkin-Elmer Model AAnalyst 800. The data are presented in mg/g dry weight. Multiple-level calibration standards were used to generate calibration curves against which sample concentrations were calculated. Standard solutions were prepared from 1000 mg/l stock solution of each metal (MERCK Titrisol).

Quality Control

A quality control sample (internal calibration) was routinely run through during the period of metal analysis. To avoid possible contamination, all glassware and equipment used were acid-washed. The percentages of recoveries were between 80-110%. The quality of the method used was checked with a Certified Reference Material (CRM) for Soil (International Atomic Energy Agency, Soil-5, Vienna, Austria). The agreement between the analytical results for the reference material and its certified values for each metal was satisfactory with the recoveries being 64-112% as shown in Table 2.

In order to check the accuracy of this method, the sum of all four-extraction steps of SET for each metal was compared with that found by using the direct digestion of aqua-regia method. Our method was acceptable since significant correlations of the four metals were found between the direct aqua-regia method and the summation of four geochemical fractions (R=0.69-0.99 at least P<0.05) (Table 3).

Statistical Analysis

Mean difference between the western and eastern parts of the Johore Straits were tested using Man-Whitney test, the analysis was carried out using SPSS 12. For cluster analysis, STATISTICA 99 edition was utilized.

Results and Discussion

Figure 2 and Table 4 show the total concentrations of Cu, Zn, Pb and Ni in the surface sediments collected from the Johore Straits. The mean total concentrations (μ g/g dry weight) of these metals in the surface sediments were 28.6 (west) and 110 (east) for Cu; 137 (west) and 180 (east) for Zn; 33.7 (west) and 33.6 (east) for Pb and 22.6 (west) and 27.3 (east) for Ni.

At the western part (Stations 1-7) of the Johore Straits (Figure 2), the highest concentrations of Cu in the surface sediments were recorded from Station (St) 3 (46.6 μ g/g dry weight) while the lowest Cu concentrations were found in St 5 (14.0 μ g/g dry weight) which is a remote rocky site with mangrove forest in the vicinity. The lowest concentrations of Zn, Pb and Ni were found in St 7, demonstrating the fact that Pantai Lido is characterized by sandy sediments, offering low affinity to bind heavy metals in the sediment samples. At the eastern part (St 8, 9 and 10) of the Johore Straits (Table 4 and Figure 2), the ranges of Cu, Zn, Pb and Ni were 85.4-122 μ g/g dry weight, 162-214 μ g/g dry weight, 29.3-37.4 μ g/g dry weight and 24.1-29.6 μ g/g dry weight, respectively.

As shown in Table 4, the total concentrations of heavy metals (Cu, Ni and Zn) at the eastern part of the Johore Straits were higher than the western part. The level of Zn was also higher in the eastern part than in the western part which in agreement with those found in the second and the third fractions. Although there were no significant value tested from Man-Whitney test for Pb and Ni, the levels of heavy metals were higher in the eastern part than in the western part of the Johore Straits.

The metal ranges were relatively narrow indicating that the anthropogenic inputs into the eastern part of the Straits. These elevated concentrations in the Johore Straits were further explained by the cluster analysis as presented in Figure 3. It was assumed that, similar clustering groups formed from the analysis could due to the common sources of contaminants shared by the differing sampling locations. For Cu (Figure 3), two distinct clustered groups were formed, namely the western part (St 1-St 7) and the eastern part (St 8-St 10). The higher level of metal concentrations recorded in the eastern part than in the western part of the Johore Straits were due to the coal-powered generator facility found in St 8 (Senibong), domestic wastes from St 10 (Kg. Masai) and the existence of shipping activities (Pasir Gudang Port) at St 9 (Teluk Jawa) and St 10 (Kg. Masai). As for Zn, it was found that St 3, where a mussel-processing factory is located, showed a significant different cluster in comparison to other sampling locations. On the other hand, the cluster analysis also revealed that the less-polluted site, St 7 was significantly clustered from the other locations for the metals of Pb and Ni. As previously addressed, low affinity of sediment from St 7 for heavy metals could be the major contributor to the clustering pattern.

The total metal concentrations (Figure 2) of the sediments showed that the ranges of Cu at the eastern part of the Straits were higher than most of the previous reported studies. As for Zn, Pb and Ni, the values were still within the ranges of the previous studies reported. In this regard, Mat *et al.* (1994) reported that the Johore Straits was relatively enriched with Cu, Ni, Pb and Zn. Generally, high concentrations of Cu were recorded at the eastern coast of the Johore Straits. The high levels of heavy metals found in the sediments in the eastern part of the Causeway were plausibly related to the discharge of effluents from the nearby domestic and industrial inputs. This Cu elevation was reported by Yap *et al.* (2003a) and Yap *et al.* (2002) in analysis of heavy metals in the mussel and the sediment samples collected in 2000 from Kg. Pasir Puteh which is located in the eastern part of Johore. The higher metal levels in the sediments collected from the eastern part than in the western part of the Johore Straits coincides with the findings of Yap *et al.* (2004; 2006) who found that the contaminated site at Kg. Pasir Puteh which is located in the eastern part of the Straits had higher metal bioavailability and contamination than those collected from the less contaminated site at Pantai Lido which is located in the western part of the Straits. Their samples were collected in 2000 (Yap *et al.*, 2004) and 2004 (Yap *et al.*, 2006).

High concentrations of Zn was found in St 3, where a mussel-processing factory is located. The factory is believed to throw the untreated wastes which contain high levels of Zn into the water. In the factory, tap water was used to boil the mussels before shucking them for packing. The water could contain high levels of dissolved Zn since Zn in the soft tissues of *Perna viridis* is partially regulated and not tightly bound to the binding sites of metallothionein (Yap *et al.*, 2003b). As Zn is easily mobilized in the soft tissues of *P. viridis*, high levels of Zn could be easily released during the boiling process (Yap *et al.*, 2007). Water containing high levels of Zn could then be released into the nearby coastal waters. Besides, Wood *et al.* (1997) also reported that, Zn pollution occurring in the Straits was likely attributed to wear from the vehicle tires, since Zn compounds are commonly used in catalysts in manufacturing tire rubber. In this study, high concentrations of Pb were found surprisingly in St 1 (Pantai Lido boat renting booth) where a famous recreational park and a floating restaurant are located. A potential cause of high concentrations of Pb at the Straits is the exhaust emissions from the large volume of vehicles passing daily over the Causeway (Wood *et al.*, 1997).

The total concentrations of Cd, Cu, Ni and Zn in Johore Straits sediments found in this study were compared with the established Sediment Quality Guidelines/Criteria as presented in Table 5. For the mean concentrations of Cu at the eastern part, the level was higher than 11 of the 14 established guidelines while the mean Cu level at the western part was only higher than 3 of all the comparative guidelines. For mean concentrations of Ni in the eastern and western parts were higher

than 3 and 2, respectively, of the 10 established guidelines. For the mean concentrations of Pb for the both parts of the Straits, the concentrations were higher than 6 of the 14 established guidelines. Meanwhile, for the mean concentrations of Zn in the eastern part, the level was higher than 8 of the 14 established guidelines while the mean Zn level in the western part, it was higher than 7 of all the guidelines. From the comparison above, it can be concluded that the Johore Straits (the western and eastern parts) are polluted by Ni, Pb and Zn while only the eastern part is polluted by Cu. Although the metal pollution is localized, the comparisons indicate that Cu, Pb, Ni and Zn concentrations in the surface sediments collected from some of the sampling sites are potentially and environmentally harmful to living biota in the Straits. This is attributed to increased solubility of all these elements during intense chemical weathering in the hot, humid tropical climate of the drainage basin.

From Figure 4, the geochemical studies reveal that the metal nonresistant fractions of the sediments are 52.3%, 67.3%, 29.2% and 64.9% for Cu, Zn, Pb and Ni, respectively. Geochemical study of the metals in the sediments (Figure 3) reveal that, the EFLE fraction contributes only a small portion of the total concentrations of Cu, Zn, Pb and Ni of the sediments collected from the Johore Straits. The low percentage of ELFE fraction indicates that the heavy metals in the sediments are not easily leached out by water. Even though this fraction is normally lower than the other fractions, this fraction is considered very important as it could be a model for what is 'bioavailable' to sediment-ingested animal (Jenne and Louma, 1977; Yap *et al.*, 2002).

In the 'acid-reducible' fraction, the percentage of Cu, Pb and Ni are lower than when the EFLE fraction but for Ni, the levels are higher (St 3, St 6, St 7, St 8, St 9 and St 10) than the EFLE fraction. The low percentages of heavy metal concentrations in this fraction showed that the affinities of this fraction in the sediments are not high. Calmano and Forstner (1983) reported that the reducible conditions are mainly caused by decomposition of organic matter that is mediated by microorganisms. The 'acid-reducible' fraction includes metals associated with manganese and iron dioxides and hydroxides and possibly also with carbonates (Badri and Aston, 1983; Yap *et al.*, 2002).

The 'oxidisable-organic' fraction show high levels of Cu, Zn, Pb and Ni in the sediments. It might be due to the high affinities of these metals to humic substances which are fractions of organic matters and chemically very active in complexing elements such as heavy metals (Forstner and Wittmann, 1981). The percentages of heavy metals in this fraction are mostly higher than the EFLE and the acid-reducible fractions could be due to the strong absorbent of organic matter such as living organisms, detritus or coating on mineral particles (Soares *et al.*, 1999; Tokalioglu *et al.*, 2000).

High levels of Pb in the 'resistant' fraction show that most of the heavy metals are due to natural origin. The 'resistant' fraction was probably trapped within silicate minerals (Badri and Aston, 1983). This natural fraction of sediment contained heavy metals strongly incorporated into the crystalline lattices of the minerals (Badri and Aston, 1983; Yap *et al.*, 2002). Therefore, the 'resistant' fraction is low in biological availability.

Conclusion

Based on aqua-regia method and sequential extraction conducted on the surface sediments, it is concluded that the Johore Straits could be polluted with Cu at the eastern part and by Zn, Ni and Pb at both parts of the Straits. This is in agreement with the cluster analysis where two distinct clustering groups were formed for Cu. Hence, it is suggested that proper management and control measures are implemented to protect the biological resources from the Straits. If the dam-like Causeway were to be removed in future, the findings of this study would serve as useful comparative metal concentrations for future references.

Acknowledgements

The authors wish to thank Mr. Reduan Bin Che Sallehhudin of Fisheries Department, Johore Bharu for assistance during the field trip in the Johore Straits. The financial support by 'Skim Lantikan Baru Vot: 53153' and Research University Grant Scheme (RUGS) from Universiti Putra Malaysia are highly appreciated.

References

- Abdullah, A.R., N.M. Tahir, T.S. Loong, F.M. Hoque, A.H. Sulaiman. (1999). The GEF/UNDP/IMO Malacca Straits demonstration project: Sources of pollution. *Mar. Pollut. Bull.* 39: 229–233.
- Alkarkhi, A.F.M., N. Ismail, A. Ahmed, A.M. Easa. (2009). Analysis of heavy metal concentrations in sediment of selected estuaries of Malaysia-a statistical assessment. *Environ. Monit. Assess.* 153: 179-185.
- ASEAN/USCRPM. (1991). The coastal Environmental Profile of South Johore, Malaysia. International Center for Living Aquatic Resources Management, Technical Report, 24, 65 pp. Manila, The Philippines, ISBN 971-1022-78-8.
- Badri, M.A., S.R. Aston. (1983). Observation on heavy metal geochemical associations in polluted and non-polluted estuarine sediments. *Environ. Pollut. Ser. B.* 6: 181-193.
- Bayen, S., G.O. Thomas, H.K. Lee, J.P. Obbard. (2004). Organochlorine pesticides and heavy metals in green mussel, *Perna viridis* in Singapore. *Wat. Air Soil Pollut*. 155: 103-116.
- Birch, G.F., S.E. Taylor, C. Matthai. (2001). Small-scale spatial and temporal variance in the concentration of heavy metals in aquatic sediments: a review and some new concepts. *Environ. Pollut.* 113: 357-72.
- Bowen, H.J.M. (1979). Environmental Chemistry of Elements. London: Academic Press 333pp.
- Cairney, T., D.M. Hobson. (1998). Contaminated land: problems and solutions, London. E and FN Spon.
- Calmano, W., U. Forstner. (1983). Chemical extraction of heavy metals in polluted river sediment in central Europe. *Sci. Tot. Environ.* 28: 77-90.
- CCME (Canadian Council of Ministers of the Environment). (1999). Canadian environmental quality guidelines. Canadian Council of Ministers of the Environment, Winnipeg, Man.
- Chandra Sekhara, K., N.S. Charya, C.T. Kamalaa, D.S. Suman Rajb, A. Sreenivasa Rao. (2003). Fractionation studies and bioaccumulation of sediment-bound heavy metals in Kolleru lake by edible fish. *Environ. Int.* 29: 1001-1008.
- Chapman, P.M., P.J. Allard, G.A. Vigers. (1999). Development of sediment quality values for Hong Kong special Administrative Region: a possible model for other jurisdictions. *Mar. Pollut. Bull.* 38: 161-169.
- Chua, T.E., I.R.L. Gorre, S.A. Ross, S.R. Bernad, B. Gervacio, M.C. Ebarvia. 2000. The Malacca Straits. *Mar. Pollut.* Bull. 41: 160-178.
- Chung, S.H. (1970). Animal life and nature in Singapore. Singapore University Press. 302pp.
- Forstner, U., G. Wittmann. (1981). Metal pollution in the aquatic environment. Berlin: Springer. 453pp.

Jenne, E.A., S.N. Luoma. (1977). Forms of trace metals. Forms in soils, sediments and associated waters: An overview of their determination and biological availability. In: Wildung R, Drucker H (eds.), Biological implications of metals in the environment, ERDA symposium series 42. Oak Ridge. Energy Res. Develop. Admin. 42: 110-143.

- Koh, H.L., P.E. Lim, Z. Midun. (1991). Management and control of pollution in Inner Johore Strait. *Environ. Monitor. Assess.* 19: 349–359.
- Lau Wong, M.M., R.C. Rootham. (1993). A strategy for the management of contaminated dredged sediment in Hong Kong. *J. Environ. Manage*. 38: 99-114.
- Long, E.R., D.D. MacDonald, S.L. Smith, F.D. Calder. (1995). Incidence of adverse biological effects within ranges of chemical concentrations in marine and estuarine sediments. Environ. Manage. 19: 81–97.
- Mason, B., C.B. Moore. (1982). *Principles of geochemistry*, 4th edn. New York: J. Wiley and Sons. 344pp.
- Mat, I., M.J. Maah, M.S. Yahya. (1994). The status of trace metal pollution in intertidal sediments from the Straits of Tebrau, Malaysia. *Jurnal Sains* 2: 221-228.
- MPP-EAS. (1999). Straits of Malacca environmental information system (CD-ROM). GEF/UNDP/IMO MPP-EAS. Philippines: Quezon City.
- Orli, C., S.M. Tang. (1999). Elemental depth profiles in marine sediments of Singapore coastal waters. Nucl. Instru. Methods Phys. Res. Sec B. 150: 291-297.
- Ramessur, R.T. (2004). Statistical comparison and correlation of zinc and lead in estuarine sediments along the western coast of Mauritius. *Environ. Int.* 30: 1039–1044.
- Salomons, W., N.M. de Rooij, H. Kerdijk, J. Bril. (1987). Sediments as a source for contaminants? *Hydrobiologia*. 149: 13-39.
- Simeonov, V., J.A. Stratis, C. Samara, G. Zachariadis, D. Voutsa, A. Anthemidis, M. Sofoniou, Th. Kouimtzis. (2003). Assessment of the surface water quality in Northern Greece. *Wat. Res.* 37: 4119-4124.
- Soares, H.M.V., R.A. Boaventura, A.S. Machado. (1999). Sediments as monitor of heavy metal contamination in the Ave river basin (Portugal): Multivariate analysis of data. *Environ. Pollut.* 105: 311-323.
- Tanner, P.A., L.S. Leong, S.M. Pan. (2000). Contamination of heavy metals in marine sediment cores from Victoria Harbour, Hong Kong. *Mar. Pollut. Bull.* 40: 769–779.
- Tokalioglu, S., S. Kartal, L. Elei. (2000). Determination of heavy metals and their speciation in lake sediments by flame atomic spectrometry after a four-stages sequential extraction procedure. *Anal. Chim. Act.* 413: 33-40.
- Turekian, K.K., K.H. Wedepohl. (1961). Distribution of elements in some major units of the earth's crust. *Bull. Geol. Soc. Am.* 72: 175-192.
- Walford, J., T.J. Lam. (1987). Floating hatchery and net cage culture of *Penaeus indicus* in the Straits of Johore, Singapore. Aqua. 62: 11-32.
- Wood, K., Z. Ahmad, N.A. Shazili, K. Yaakod, R. Carpenter. (1997). Geochemistry of sediments in Johor Strait between Malaysia and Singapore. *Continent. Shelf. Res.* 17: 1207-1228.
- Yap, C.K., A. Ismail, S.G. Tan, H. Omar. (2002). 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. (2003a). Background concentrations of Cd Cu, Pb and Zn in the green-lipped mussel *Perna viridis* (Linnaeus) from Peninsular Malaysia. *Mar. Pollut. Bull.* 46: 1035-1048.
- Yap, C. K., Ismail, A., Omar, H. and Tan, S. G. (2003b). 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., S.G. Tan, A. Ismail, H. Omar. (2004). Allozyme polymorphisms and heavy metal levels in the green-lipped mussel *Perna viridis* (Linnaeus) collected from contaminated and uncontaminated sites in Malaysia. *Environ. Int.* 30: 39-46.
- Yap, C.K., A. Ismail, Edward, F.B., S.G. Tan, S.S. Siraj. (2006). Use of different soft tissues of *Perna viridis* as biomonitors of bioavailability and contamination by heavy metals (Cd, Cu, Fe, Pb, Ni and Zn) in a semi-enclosed intertidal water, the Johore Straits. *Toxicol. Environ. Chem.* 88(4): 683 695.
- Yap, C. K., M. Rashiq, F.B. Edward. (2007). Is the mussel processing site a point source of Zn contamination? An Evidence of Zn Remobilization Based on 15-minutes boiled Mussel *Perna viridis*. Paper Presented at Third Colloquium Biology on the 12 December 2007 at the Marriott Hotel, Putrajaya.
- Yap, C.K., F.B. Edward. (2009). The different capability of metal uptake in the shell of *Perna viridis* compared to the different soft tissues: A statistical approach. *J. Sustain. Sci. Manage.* 4(1): 38-48.
- Yap, C.K., F.B. Edward, S.G. Tan. (2010). Similarities and differences of metal distributions in the tissues of molluses by using multivariate analyses. *Environ. Monit. Assess.* 165 (1-4): 39-53.
- Zheng, Q.H., Z.Q. Liang, Y.Q. He, W.Y. Wen. (1992). Geochemical behavior of the pollutants in surface sediments of Daya Bay. *Trop. Oceanol.* (in Chinese). 11: 65-71.
- Zwolsman, J.J.G., G.T.M. van Eck, G. Burger. (1996). Spatial and temporal distribution of trace metals in sediments from the Scheldt Estuary, Southwest Netherland. Estuar. Coast. *Shelf. Sci.* 43: 55-79.

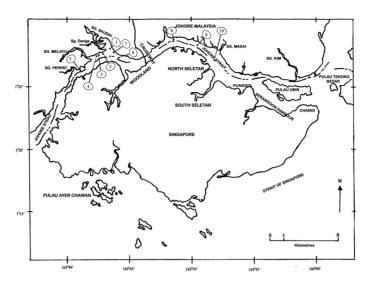


Figure 1: The sampling sites for surface sediments in the Straits of Johore. The numbers follow those presented in Table 1.

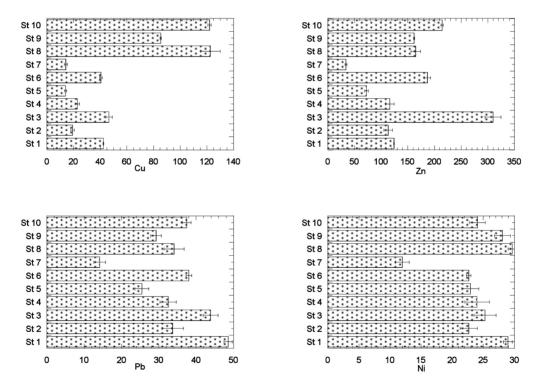


Figure 2: Total concentrations (mean $\mu g/g$ dry weight \pm standard error) of Cu, Zn, Pb and Ni in the surface sediments by using aqua-regia method collected in the Straits of Johore. The numbers follow those presented in Table 1.

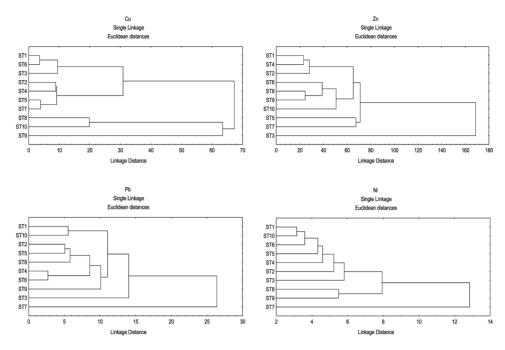


Figure 3: Hierarchical cluster analysis of the surface sediments collected from the Straits of Johore based on each metal concentrations [Log10(x+1)].

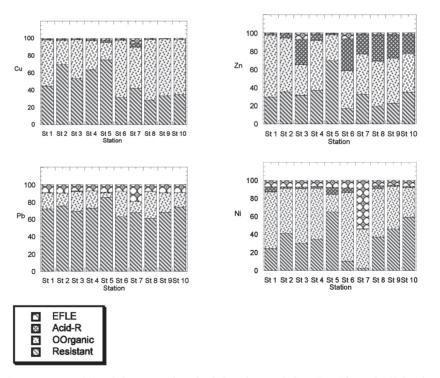


Figure 4: Percentages (%) of four geochemical fractions of Cu, Zn, Pb and Ni in the surface sediments collected in the Straits of Johore. The numbers follow those presented in Table 1.

St.	Sampling sites	GPS reading	Site description
	The western part	_	
1.	Pantai Lido-1	N 01° 28.130'	1. A small jetty
		E 103° 43.567'	2. A restaurant
			2. Boat repairing site
2.	Mussel farm-1	N 01° 27.595'	1. Fish culturing (floating cages)
		E 103° 42.808'	2. Mussels culturing (floating cages)
3.	Mussel	N 01° 27.626'	1. Housing area
	processing	E 103° 42.335'	2. Soft tissues are separated from the shells for
	house/center		market.
4.	Mussel farm- 2	N 01° 27.264'	1. Mussels culturing using buoyant
		E 103° 42.125'	
5.	Tebing Runtuh	N 01° 26.414'	1. Small forest
		E 103° 40.873'	2. Rocky beach area
			3. Erosion occurred at the seaside
6.	Pantai Lido-2	N 01° 27.460	1. Near the water gate of Hospital Sultanah
		E 103° 44.552'	Aminah
7.	Pantai Lido-3	N 02° 23.842'	1. Jetty
		E 101° 58.945'	2. By the roadside
			3. Recreation park
	The eastern part		
8.	Senibong	N 01° 28.993'	1. Mussels culturing (floating cages and buoyant)
	-	E 103° 48.748'	2. Power-generating facility
9	Teluk Jawa	N 01° 28.560'	Mussels culturing using buoyant
		E 103° 50.414'	2. Big seaport (Pasir Gudang)
10.	Kampung Masai	N 01° 27.910'	1. Mussels culturing using buoyant
		E 103° 51.772'	2. Big Seaport (Pasir Gudang)

Table 2: A comparison of heavy metal concentrations ($\mu g/g$ dry weight) between measured values and certified values in the Certified Reference Materials (CRM) for soil.

Metals	Certified value (C)	Measured value (M)	Percentage of recovery (M/C)
Cu	77.1 ± 4.7	72.9	94.6%
Ni	13	12.3 ± 3.0	94.6%
Zn	368 ± 8.2	238	64.7%
Pb	129.0 ± 26.0	144.7 ± 10.0	112.2%

Table 3: Correlation coefficient of heavy metals between direct aqua-regia method (AR) and sum of the four geochemical fractions (SET).

Metal	AR	SET	Correlation between AR and SET
Cu	53.02 ± 13.34	79.326 ± 11.596	R= 0.997; P< 0.01
Ni	24.014 ± 1.5761	22.9265 ± 2.6754	R = 0.887; $P < 0.01$
Pb	33.702 ± 3.0327	58.562 ± 9.2072	R = 0.692; $P < 0.05$
Zn	149.82 ± 24.484	146.923 ± 13.143	R= 0.989; P< 0.01

Table 4: Average (μ g/g dry weight) and minimum to maximum (in brackets) concentrations of Cu, Zn, Pb and Ni in the surface sediments between western and eastern parts of Johore Causeway. N= 3 for each parameter.

		The western part (7 sites)		The eastern part (3 sites)		
Metal	Fraction	Min-max	Mean ± SE (A)	Min- max	Mean ± SE (B)	Man whitney test between A and B
Cu	Total	14.0-46.6	28.6 ± 5.34	85.4-122	110 ± 12.3	P< 0.05
	F1	0.36-0.98	0.64 ± 0.10	0.88-1.80	1.49 ± 0.303	P> 0.05
	F2	0.34-0.77	0.45 ± 0.05	0.25-0.44	0.37 ± 0.06	P> 0.05
	F3	3.66-39.2	18.6 ± 5.37	94.8-132	115 ± 10.7	P < 0.05
	F4	7.18-35.7	20.1 ± 3.43	48.2-64.1	55.3 ± 4.66	P< 0.05
Ni	Total	12.0-28.9	22.6 ± 1.96	24.1-29.6	27.3 ± 1.66	P> 0.05
	F1	1.30-2.34	1.69 ± 0.13	1.72-1.96	1.84 ± 0.07	P> 0.05
	F2	0.01-1.40	0.75 ± 0.20	0.42-1.02	0.66 ± 0.18	P> 0.05
	F3	1.89-16.6	10.6 ± 2.21	10.1-16.6	14.3 ± 2.12	P> 0.05
	F4	0.117-11.0	6.08 ± 1.41	11.5-17.8	15.0 ± 1.86	P< 0.05
Pb	Total	14.2-48.6	33.7 ± 4.35	29.3-37.4	33.6 ± 2.37	P> 0.05
	F1	4.38-6.64	5.09 ± 0.284	4.97-6.17	5.66 ± 0.356	P > 0.05
	F2	0.01-0.59	0.15 ± 0.09	0.007-0.008	0.01 ± 0.00	P> 0.05
	F3	2.73-19.1	10.4 ± 2.29	10.9-20.4	15.3 ± 2.79	P> 0.05
	F4	23.0-50.5	40.2 ± 3.23	39.7-48.1	43.9 ± 2.42	P> 0.05
Zn	Total	34.2-310	137 ± 33.9	162-214	180 ± 17.0	P> 0.05
	F1	0.45-19.2	5.33 ± 2.85	3.81-4.44	4.10 ± 0.183	P > 0.05
	F2	0.91-78.7	24.6 ± 13.0	41.6-48.3	44.3 ± 2.04	P > 0.05
	F3	13.3-95.9	60.9 ± 12.4	83.0-85.9	84.7 ± 0.87	P > 0.05
	F4	9.75-88.3	42.1 ± 8.86	32.2-69.7	46.6 ± 11.6	P > 0.05

Note: Total: total metal concentration by using Aqua-regia method; F1: easily, freely, leacheable or exchangeable; F2: Acid-reducible; F3: Oxidisable-organic; F4: Resistant.

Table 5. Comparisons of heavy metal concentrations ($\mu g/g$ dry weight) in the intertidal sediments with the established.

No.	Sediment Quality Criteria/Guidelines	Cu	Ni	Pb	Zn
1.	Action Level according to Dutch Sediment	190	210	530	720
	Quality Criteria (Cairney and Hobson,				
	1998.)				
2.	Action Level according to Hong Kong	65	40	75	200
	Sediment Quality Criteria (Lau Wong and				
	Rootham, 1993).				
3.	Background values of Chinese coastal areas	30	NA	25	80
	(Zheng et al., 1992).				
4.	Background values of the marine sediments	15	25	50	94
_	in Hong Kong (Tanner et al., 2000).				
5.	Background values f the estuary sediments	10	15	29	70
	in Hong Kong (Tanner et al., 2000).				0
6.	Effect Range Low (Long et al., 1995)	34	20.9	46.7	150
7.	Effect Range Median (Long et al., 1995)	270	51.6	218	410
8.	Interim Marine Sediment Quality Guideline	18.7	NA	30.2	124
0	(CCME, 1999)	100	NT 4	110	271
9.	Probable Effect Levels (CCME, 1999)	108	NA	112	271
10.	Interim Sediment Quality Values (ISQV)-	65	40	75	200
	Low (Chapman et al., 1999).	270	NT 4	210	410
11.	Interim Sediment Quality Values (ISQV)-	270	NA	218	410
10	High (Chapman et al., 1999)	45.0	(0.0	20.0	05.0
12.	Average Shales (Turekian and Wedepohl,	45.0	68.0	20.0	95.0
12	1961) Maga Caustal Material (Magan and Magan	55.0	75.0	12.0	70.0
13.	Mean Crustal Material (Mason and Moore, 1982)	33.0	73.0	13.0	70.0
14.	Mean Crustal Material (Bowen, 1979)	50.0	80.0	14.0	75.0
14.	Eastern part of the Straits of Johore (3 sites)	110 ±	27.3 ±	33.6 ±	$\frac{73.0}{180 \pm}$
	Eastern part of the straits of Johore (3 sites)	110 ± 12.3	27.3 ± 1.66	33.0 ± 2.37	180 ± 17.0
	Western part of the Straits of Johore (7 sites)	$28.6 \pm$	22.6 ±	33.7 ±	17.0 137 ±
	western part of the straits of Johore (7 sites)	5.34	1.96	4.35	33.9
Note: SE	- standard error NA: Not available	3.37	1.50	7.33	33.9

Note: SE- standard error. NA: Not available.