



Mercury Determination in Biological Organism in the Estuary of Muar River, West Johor, Malaysia

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ABSTRACT

Determination of Mercury in Biological Sample in Muar River Estuary, West Johor, Malaysia. This study was carried out at the Estuary of Muar River, west Johor, Malaysia. Different types of biota species namely *Crassostrea irredalei*, *Perna viridis* and *Anadara granosa* were collected all through the torrential rainy season in November 2005. These biota species were dried and then cooked in an acid solution for Cold Vapour Atomic Absorption Spectrometric (CV-AAS) analysis. CV-AAS was used for decisive the mercury (Hg⁺⁺) concentration in the collected biota species. The average Hg⁺⁺ concentration in *C. irredalei*, *P. viridis* and *A. granosa* were $1.854 \pm 1.012 \mu\text{g g}^{-1}$, $0.336 \pm 1.125 \mu\text{g g}^{-1}$ and $1.178 \pm 1.182 \mu\text{g g}^{-1}$ respectively. These results directed that the biota especially *C. irredalei* and *A. granosa* are contaminated by Hg⁺⁺ and these are not safe to be consumed for publicly used due to the total Hg⁺⁺ concentration exceeding $0.5 \mu\text{g g}^{-1}$ according to WHO.

Keywords: biota, CV-AAS, mercury concentration.

INTRODUCTION

Mercury is considered as global pollutant element and many of its compounds are highly toxic and readily released into the environment because of their high volatility and mobility. Mercury is one of the hazardous, prevalent and toxic elements to

the environment ecosystem. This element can be present as diverse dissolved chemical species in natural water as well as rivers, lake and ocean. Inorganic and organic species of mercury are generated in different industrial activities, mainly pharmaceutical, paper, electrochemical (batteries) and plaguicide industries (Mason and Fitzgerald,

1990). According to Regional Water Quality Control (RWQC), the source of mercury come from residents (46%), water supply (22%), dentist (9%), storm water (3%), permitted industries (6%), unknown sources (11%) and employee-related human waste (3%). Although mercury is distributed throughout the environment, its toxic effects result mainly from ingestion of contaminated seafood. Unlike many other air pollutants, mercury residence times in the atmosphere are long and it may eventually be dispersed over large areas. As a result, adverse effects must be considered on both local and regional scales.

Trace metal bound to certain phases of the sediment are likely to be in thermodynamic equilibrium with the associated pore waters. These metals are likely to be those bound at surface adsorption sites in sulphide or organic phases, rather than those occluded within mineral phases (Fikriah *et al.* 2014). Adsorption occurs during sedimentation and re-suspension of particulates. Interaction of biota with natural sediment constituent and contaminants occurs at several trophic levels (Suhaimi *et al.* 2012).

In recent years, several author have reported on the usefulness of the common mussel as a biology indicator of heavy metal contamination in coastal waters, Mussels may accumulate these elements from ingested food material and sea water to concentration greatly excess of those found in their environment and to an extent which is related to the content of available metals in water. Since they are also worldwide distribution, Goldberg proposed the use of mussel as indicator organism for the global marine pollution monitoring programmed (Goldberg, 1978).

Muar river estuary is located at Johor, Malaysia lied in the proximity of industries area, primer forest and the town of Muar, Bandar Maharani. Muar river estuary is a famous place for seafood and even seafood source such as oysters, fish, and blood cockle. Thus there is a need to carry out a study to determine the mercury concentration in the water due to the development of the industrial areas at this river. Industries areas have a high tendency to send the pollutant to the Muar River Estuary and cause water contamination which lead to the aquatic

organism bioaccumulation via food chain. The aims of this study are to gain an insight on the level of total mercury that accumulated in biological samples from Muar River Estuary, Johor.

MATERIALS AND METHODS

Reagent Preparation

Bromine monochloride (BrCl) was prepared by adding 10.8 g of reagent grade Potassium bromide (KBr) into 1 liter volumetric flask containing 1 liter of concentrated hydrochloric acid (HCl) and stirred for an hour with stir bar. 15.2 g reagent grade Potassium Bromate (KBrO_3) was added slowly while stirring. This process will produce large free halogens and the color will change from yellow to red then yellow. Finally, the solution was allowed to stir for another addition hour. For Hydroammonium chloride ($\text{NH}_2\text{OH}\cdot\text{HCl}$) solution, it was prepared by adding 30g of $\text{NH}_2\text{OH}\cdot\text{HCl}$ into deionized water in 100ml volumetric flask and the solution was purged with mercury free nitrogen. Tin chloride solution was prepared by dissolving 25% Tin (II) chloride (SnCl_2) in 20% HCl by heating. For potassium bromide, it was prepared by diluting 10 g of potassium bromide into 100 ml deionized water and mixed well.

Sampling collection and preparation

The sampling stations located at Muar River Estuary, Johor is very unique due to the existence of industrial areas, primary forests and township of Muar around. The industrial and town area are located oppositely while mangrove is located near to sea. The types of industry laid at sampling area included semiconductor industries, steel mill industries, electrical industries, distillery industries, food industries, and oil stations. 10 sampling sites were sampled on November 2005 during high tide (Figure 1) within monsoon season. In situ water parameter was measured using Hydrolab before sample collection. Parameters that taken into account including temperature, conductivity, turbidity, salinity, dissolved oxygen, pH and depth. Biota sample was hand collected. All samples were placed in plastic bags, sealed, labelled and stored on ice for transportation to the laboratory.

Prior to sampling and laboratory analysis, all the glassware and equipment used were washed with tap water and distilled water before being immersed

in 10% nitric acid (HNO₃) solution for at least 24 hours. Next, the container was taken out and soaked in 1.0% hydrochloric acids solution to remove all trace of oxidizing compound that can destroy and stored double-bagged until used (Nguyen *et al.*, 2005)

Analytical Method

Biological samples were dried at 60°C until constant weights were reached. 0.5 g of each sample was weight in Teflon beaker and placed on the hot plate. 4 ml of nitric acid (HNO₃) was added followed with 2 ml sulfuric acid (H₂SO₄). The sample was heated constantly for 3 hours at 90°C temperature. 1 ml of 10% potassium dichromate (w/v) K₂Cr₂O₇ was added at the end of heating process. The samples were then brought to 50 ml volume with deionized water and 2 ml of Tin (II) chloride (SnCl₂) was added.

Analysis of total mercury was carried out using CV-AAS in its standard configuration. Results were quantified via an external calibration curve generated from the responses obtained from multiple dilutions of a single element calibration standard that was prepared from 1000 µg L⁻¹ mercury standard stock solution (Merck).

Concentration of mercury in biological samples was calculated by using the formula below:

$$\text{Metal conc. } (\mu\text{g g}^{-1} \text{ dry weight}) = \frac{\text{Concentration } (\mu\text{g mL}^{-1}) \times \text{dilution volume (50 ml)}}{\text{Weight of dry sample (g)}}$$

Detection of total mercury in biota samples that carried out using CV-AAS was blank corrected and expressed as µg g⁻¹ dry weight. Blank sample was used to investigate any effect of contaminant of mercury derived from instrument, environment or calculation error. Acid solution without actual biota sample was organized in a similar way for digestion process. The actual concentration of samples was produced by subtracting the values of the blank from the values obtained by the detection reading.

The recovery test was carried out and checked in a separate comparative study and undergo the same procedure of sample. Recovery test was checked using spike method. Recoveries obtained was 93.65%.

RESULTS AND DISCUSSION

The highest temperature was achieved at the mangrove and the lowest was found in industrial area. Generally, the result shows similar average temperature among all stations. There was significant different among all stations ($p < 0.05$).

There was significant difference observed in the conductivity among all stations ($p < 0.05$). The conductivity reading was ranged from 22.20 µs cm⁻¹ to 46.03 µs cm⁻¹ in which the highest conductivity was achieved by station 10 (mangrove area) and the lowest was station 2 (industrial area). From the result obtained, the conductivity increased from industrial area at upstream to the mangrove zone at

Table 1: Hydrology parameters from 10 sampling sites from Muar River Estuary, Johor

Station	Temperature (°C)	Conductivity (µs cm ⁻¹)	Turbidity (g L ⁻¹)	Salinity (ppt)	Dissolved oxygen (mg L ⁻¹)	pH	Depth (m)
1	29.12	24.01	14.46	13.32	5.00	6.71	1.71
2	29.03	22.20	13.92	12.78	5.28	6.77	1.59
3	29.22	35.26	21.18	21.23	5.97	7.05	2.09
4	28.70	29.95	18.05	19.96	6.03	7.07	2.07
5	29.09	36.75	22.13	21.09	5.93	7.17	1.72
6	29.10	34.38	21.32	21.79	6.23	7.29	1.84
7	28.97	41.17	25.46	24.81	6.34	7.44	1.91
8	29.83	45.42	27.12	26.64	6.83	7.75	1.97
9	29.62	45.92	27.42	29.96	6.36	7.93	1.90
10	29.63	46.03	27.49	27.04	6.47	8.03	2.10

downstream. Conductivity became higher when the station closer to open mouth of estuary to coastal, such as in mangrove zone. Salinity measurement increased constantly from station 1 to station 10. The highest salinity was recorded at mangrove and lowest salinity was found in industrial area, ranging from 12.78 ppt to 29.96 ppt. Mangrove exhibited relatively high salinity reading compared to other locations. Higher salinity in mangrove may due to its location nearby the coastal and influenced by

sea-level. There was significant different among all stations ($p < 0.05$).

As the sampling point getting closer to the mouth of estuary, the dissolved oxygen increased constantly from industrial area to mangrove zone. The lowest dissolved oxygen reading was showed by station 1 (5.0 mgL^{-1}) and the highest was found in station 8 (6.83 mgL^{-1}). Higher dissolved oxygen level in mangrove indicates an adequate supply

Table 2: Mean concentration of mercury in biological samples from Muar River Estuary

n	Sample	Mean concentration ($\mu\text{g g}^{-1}$)	
11	<i>Crassostrea iredalei</i>	1.854	1.012
9	<i>Perna Viridis</i>	0.366	1.125
5	<i>Anadara granosa</i>	1.178	1.182

Table 3: Ratio of mercury in water and biota (Fowler, 1988)

Range	Classification
1-10	Accumulation
11-50	Moderately accumulation
50-100	Severely accumulation
>100	DNA structure different

Table 4: Regression analysis total mercury and various parameters

	Temperature	Salinity	Dissolved oxygen R^2	pH (sediment)	Turbidity
Mercury	0.8701	0.7483	0.9777	0.1638	0.8312

and available of oxygen to support marine species growth and activity. Dissolved oxygen level is controlled by water mixing, temperature, salinity and decomposition of organic matter. pH values at Muar River Estuary was constantly increased from station 1 to station 10. The pH values were in between 6.71 to 8.03, which mangrove area has the highest pH value while industrial area has the lowest. The pH values for estuaries typically increase as salinity

Table 5: ANOVA test for biological samples

Samples	P-value
<i>C. iredalei</i>	0.000793
<i>P. viridis</i>	0.000115
<i>A. granosa</i>	0.083444

Table 6: Comparison of mercury concentration in this study with other studies

Region	Mercury concentration	Reference
<i>A. granosa</i>		
Penang	0.001-0.049 $\mu\text{g g}^{-1}$	(Devi, 1986)
Selangor	0.006-0.128 $\mu\text{g g}^{-1}$	
Perak	0.029 $\mu\text{g g}^{-1}$	
Penang	0.011-0.023 $\mu\text{g g}^{-1}$	(Jothy <i>et al.</i> , 1983)
Perak	0.007-0.065 $\mu\text{g g}^{-1}$	
Penang	0.038-0.072 $\mu\text{g g}^{-1}$	
Thailand	0.020 $\mu\text{g g}^{-1}$	(Phillips and Rainbow, 1989)
ADI (WHO)	0.500 $\mu\text{g g}^{-1}$	
<i>C. iredalei</i>	1.850 $\mu\text{g g}^{-1}$	This study
<i>P. viridis</i>	0.366 $\mu\text{g g}^{-1}$	
<i>A. granosa</i>	1.178 $\mu\text{g g}^{-1}$	

risers. This can be explained by go through the salinity measurement in this study area. Effect from seasonal also affects pH values. During monsoon season, the water flow in Muar River Estuary is fast, adds to the river natural flow and discharges pollutants into the water from surrounding. Runoff from domestic and industrial area may attribute to these pH levels recorded. No significant differences of dissolved oxygen and pH were found among stations ($p>0.05$).

The turbidity showed linear increasing from station 7 to station 10. Turbidity of industrial area and town area was not stable as somehow the water fluxes at upstream well mixed the water content more frequently than downstream. Hence, the water in industrial area and town area was clearer than mangrove, in which mangrove is nearly muddy

and consisted of many suspended solids. There was significant different among all stations.

Concentration of total mercury in biological samples

Mercury concentration in biota samples from Muar River estuary ranged between 0.158 - 3.481 $\mu\text{g g}^{-1}$ dry weight while the mean mercury concentration, 0.366 - 1.854 $\mu\text{g g}^{-1}$ dry weight (Table 2). *C. iredalei* and *P. viridis* was collected at station 2 while *A. granosa* was collected at station 8. It was found that *C. iredalei* has the highest mercury concentration in contrast with *P. viridis* which are from the same station and *A. granosa* from the sea. However, *A. granosa* found to have higher mercury concentration compare with *P. viridis*. There was significant differences found in total mercury concentration among samples for

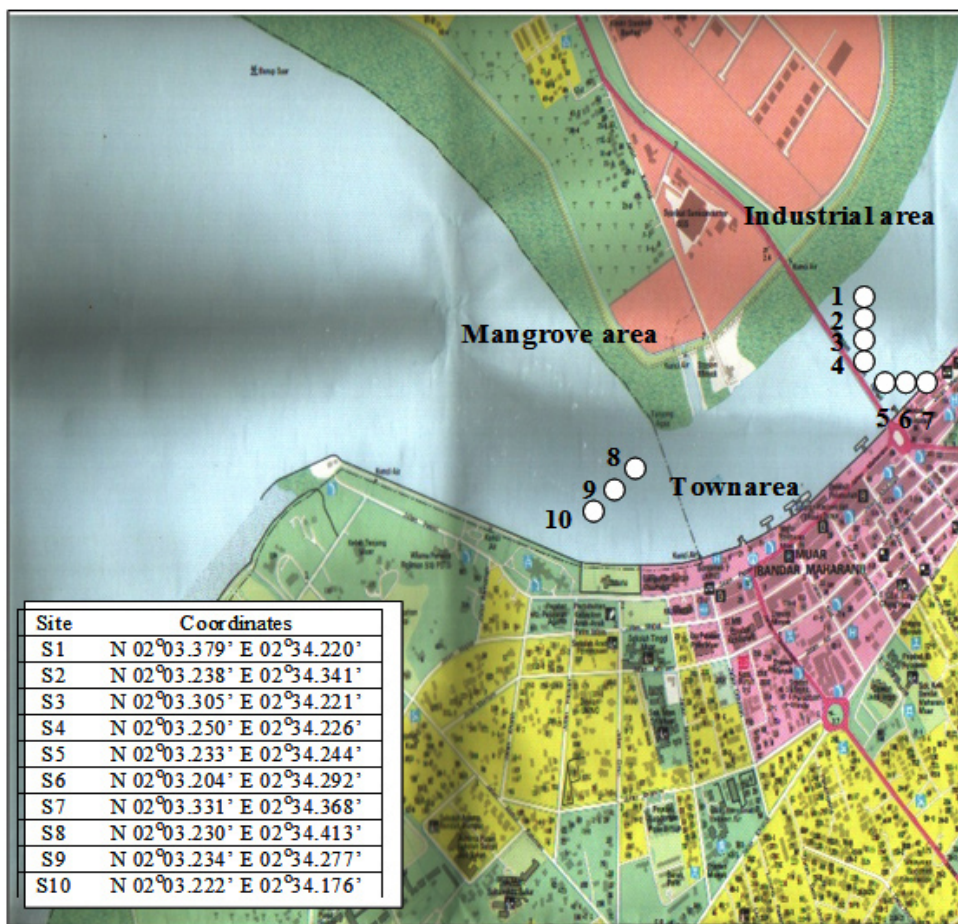


Fig. 1: 10 station locations with coordinates in Muar River Estuary, Malaysia

Site	Coordinates
S1	N 02°03.379' E 02°34.220'
S2	N 02°03.238' E 02°34.341'
S3	N 02°03.305' E 02°34.221'
S4	N 02°03.250' E 02°34.226'
S5	N 02°03.233' E 02°34.244'
S6	N 02°03.204' E 02°34.292'
S7	N 02°03.331' E 02°34.368'
S8	N 02°03.230' E 02°34.413'
S9	N 02°03.234' E 02°34.277'
S10	N 02°03.222' E 02°34.176'

C. iredalei and *P. viridis* ($p < 0.05$) while none was found for *A. granosa* ($p > 0.05$).

Analysis constructed to determine and classified the level of accumulation of the biota samples shows that *C. iredalei*, *P. viridis* and *A. granosa* have ratio of 5.80, 1.15 and 1.43. This means that this biota were in the earlier stage of bioaccumulation level referred to the Table 3.

The internal process of biological samples can strongly influence the organism metal concentration. Condition may influence by salinity and reproductive, tidal exposure and food supply. Size is the most important internal factor that may affect the accumulation rate of biological tissue. Smaller size of *A. granosa* proved to concentrate more mercury than the larger size (Fowler, 1988; Fowler *et al.*, 1993). It can be concluded that accumulation rates might produce different results even the biological samples have similar body mass.

Regression analysis

The concentration of total mercury in sediment was influenced by temperature, salinity, dissolved oxygen and turbidity with the R square value in the range 0.1638 to 0.9777 (Table 4). Dissolved oxygen has a greatest influence on total mercury concentration compare to temperature, salinity and turbidity. pH does not influenced the concentration of total mercury in sediment due to

the R square value lower than 0.5.

The P-value for the biological samples was in the range 0.000115 to 0.083444 (Table 5). The null hypothesis for *C. iredalei* and *P. viridis* was fail to reject due to the P-value was lowest than 0.05. These mean that the concentration of total mercury in the samples of *C. iredalei* and *P. viridis* was different. However, for *A. granosa*, the hypothesis was rejected. These mean that the concentration of total mercury in all samples was same.

Comparison with previous studies

Table 6 shows the concentration values of mercury from other studies. In comparing with biological samples from other areas in Malaysia, the mercury concentration in Muar River Estuary is very much higher. The concentration is also found to be higher than the acceptable daily intake standard of 0.500 $\mu\text{g g}^{-1}$ particularly for oysters and cockles. Concentration of mercury in mussel is the only one lowest than the ADI (WHO) standard which is 0.500 $\mu\text{g g}^{-1}$ (WHO, 1958).

CONCLUSION

The total mercury concentration in biota was between 0.366 $\mu\text{g g}^{-1}$ to 1.854 $\mu\text{g g}^{-1}$. From the data analysis, the dissolved mercury concentration in water was found to being influenced by the dissolved oxygen, pH, salinity, turbidity and the water temperature. The accumulation of mercury in tissues of *C. iredalei*, *P. viridis* and *A. granosa* were found in the early stage. *C. iredalei*, and *A. granosa* in Muar River Estuary was contaminated with the total mercury and the concentration was above the ADI (WHO) level, 0.5 $\mu\text{g g}^{-1}$ and considered not safe for consumption.

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