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RESEARCH NOTES

HEAVY METAL POLLUTION IN THE JURU RIVER BASIN RECEIVING INDUSTRIAL EFFLUENTS: THE NEED FOR BIOCHEMICAL AND MOLECULAR STUDIES IN THE EDIBLE COCKLES *Anadara granosa*

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Kuala Juru is one of several thousand fishing villages on the coastline of Malaysia. Cockle farming is a major income-source for the fishermen in the Juru area since 1976 (ILO, 1986). This was due to the fishing activities in the Juru area being adversely affected by pollution from neighboring industries since Juru is an industrial area with food and electronic manufacturing industries (Lim and Kiu, 1995). As a result, fishing decreased dramatically since high mortality and low fecundity were found in the fish population. The villagers on their own initiative decided to turn to farming cockles, which are more resistant than fish to chemical pollution. They have been doing this on a collective basis since July 1976 (ILO, 1986). Besides thriving easily and needing little or no maintenance, cockles are also cheap and they can provide a rich source of nutrients (Lowe and Kendall, 1990). Today, Juru is one of the most productive mudflats for cockle farming in Peninsular Malaysia but the pollution inputs due to urban and industrial activities in the Juru area are of much ecotoxicological concern.

The results of a number of previous studies (Seng *et al.*, 1987; DOE-USM, 1992; Mat *et al.*, 1994; Lim and Kiu, 1995; DOE 2005) conducted at various periods indicate that the Juru River Basin is grossly polluted by domestic wastes and discharges from pig farms. Other than carrying highly polluting organic materials, these wastes are also contaminated with heavy metals. Recently, Abbas *et al.* (2007) still reported elevated concentrations of heavy metal concentrations in Kuala Juru based on the water samples collected in 2005. The poor water quality indicated continual sources of anthropogenic inputs of heavy metals into the Juru River Basin. However, extensive cockle rearing is found along the mudflats off the estuary of the Juru River, raising the question of the suitability of such an activity in view of the poor water quality in the area.

Therefore, the objectives of this study are to determine the total concentrations and geochemical (nonresistant and resistant) fractions of Cd, Cu, Fe, Ni, Pb and Zn in the surface sediments collected from three sites in the Juru River Basin receiving industrial effluents and in a firefly site at Kg. Kuantan, Kuala Selangor.

In this study, surface water and sediment were collected at three sites in the Juru River Basin receiving industrial effluents; the first site was at a drainage near an electronic factory at Juru [Long: 05º20.105' N; Lat: 100º26.011' E], the second site was at the downstream of the Juru River [Long: 05º19.772' N; Lat: 100º26.083' E] and the third site was at the estuary of the Juru River [Long: 05º20.410' N; Lat: 100º24.518' E] which was also a jetty (Table 1). For comparative purposes, samples from a reference site at Kg. Kuantan [Long: 03º21.745' N; Lat: 101º18.093' E], Kuala Selangor were also collected. This site was chosen because it is the habitat for fireflies and

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therefore an ecotourism site and it is considered to be a reference site only receiving domestic wastes (Table 1). To ascertain the extent of heavy metal pollution in the rivers, the total concentrations of Cd, Cu, Fe, Ni, Pb and Zn and their four geochemical fractions were obtained using the sequential extraction technique in sediment samples collected from three sites in the Juru River Basin. The geochemical study of heavy metals in the sediment samples estimated four operationally defined host fractions according to Badri and Aston (1981) and Yap *et al*. (2002, 2003, 2005). The four fractions considered, the extraction solutions and the conditions employed were: (1). "Easily, freely, leachable or exchangeable" (EFLE) -10g of the samples will mix with 50 ml of 1.0 M ammonium acetate (NH_4CH_3COO), pH 7.0, at room temperature and shaken continuously for about 3 hours. (2). "Acid-reducible"-The residue was continuously shaken in an orbital shaker for 3 hours with 50 ml of 0.25 M hydroxylammonium chloride ($NH_2OH.HCl$) acidified to pH 2 with HCl, at room temperature. (3). 'Oxidisable-organic' - About 90-95ºC of the temperature for water bath will be used. The residue will oxidized with 15 ml of 30% H_2O_2 inside the water bath. After cooling, the metal will released from the organic complexes was continuously shaken in an orbital shaker for 3 hours with 1.0M ammonium acetate (NH_4CH_3COO) acidified to pH 2.0 with HCl, at room temperature. (4). Resistant-The residue after that was digested in a combination of concentrated nitric acid (AnalaR grade, BDH 69%) and the perchloric acid (AnalaR grade, BDH 60%) same the procedure as in the direct aqua-regia method.

For the water samples, physico-chemical parameters including temperature, conductivity, TDS (total dissolved solid), salinity, DO (dissolve oxygen) and pH were measured '*in situ*' while the dissolved concentrations of heavy metals were analyzed based on collected water samples in the laboratory.

The physico-chemical parameters of the water samples recorded in the field are presented in Table 2. Interestingly, the highest conductivity was found in the Juru drainage but high levels of salinity and TDS were not recorded here. Therefore, conductivity in this site was mostly likely due to anthropogenic input. This conclusion was also supported by the finding of the lowest DO at this site. Conductivity of water samples has been suggested as a good indicator of water quality deterioration (Arimoro *et al*., 2007).

The dissolved concentrations of heavy metals in the water samples are presented in Table 3. The highest dissolved concentrations of Cd, Ni and Pb were found at Kuala Juru while elevated dissolved Cu concentrations were found in the Juru drainage and Kuala Juru (0.062-0.070 mg/L), followed by Kg. Kuantan (0.031 mg/L) and Sg.

Table 1. Site description, sampling dates and GPS of each site

No.	Site	Date of sampling	Color of sediment	Anthropogenic sources
1.	Sg. Juru, Penang	20 April 2005	Grey-black in color	An industrial area potentially receiving industrial effluents.
2.	IRE TEX (factory), a drainage at Juru Industrial Area, Penang	20 April 2005	Grey in color	An industrial area potentially receiving effluents from an electronic factory.
3.	A Jetty at Kuala Juru, Penang	20 April 2005	Grey in color	A fishing village and potentially receiving industrial effluents from Juru River.
4.	Kg. Kuantan, Selangor	18 April 2005	Brown in color	A firefly site potentially receiving domestic wastes.

Table 2. Mean values of physico-chemical parameters of the water samples at the sampling sites. N= 3.

No.	Location	Temp. (ºC)	Conductivity (µS/cm)	TDS (mg/L)	Salinity (ppt)	DO (mg/L)	pH
1.	Sg. Juru, Penang	35.3	2708	1.42	1.14	11.4	9.61
2.	IRE TEX (factory), a drainage at Juru Industrial Area, Penang	32.1	1436	0.822	0.62	0.42	8.54
3.	A Jetty at Kuala Juru, Penang	33.0	40324	22.73	21.8	8.59	9.87
4.	Kg. Kuantan, Selangor	29.3	87.0	0.052	0.04	2.17	7.63

Table 3. Dissolved concentrations (mean [mg/L] ± standard error) of heavy metals in the water samples. N= 3

No.	Location	Cd	Cu	Ni	Fe	Pb	Zn
1.	Sg. Juru, Penang	0.024 ± 0.004	0.005 ± 0.003	0.001 ± 0.001	2.520 ± 0.093	0.287 ± 0.075	0.240 ± 0.036
2.	IRE TEX (factory), a drainage at Juru Industrial Area, Penang	0.019 ± 0.013	0.070 ± 0.009	0.001 ± 0.001	35.7 ± 1.01	0.677 ± 0.215	0.609 ± 0.044
3.	A Jetty at Kuala Juru, Penang	0.194 ± 0.020	0.062 ± 0.003	0.269 ± 0.006	0.265 ± 0.180	0.998 ± 0.049	0.203 ± 0.094
4.	Kg. Kuantan, Selangor	0.015 ± 0.005	0.017 ± 0.007	0.001 ± 0.001	0.761 ± 0.060	0.255 ± 0.087	0.073 ± 0.018

Juru (0.005 mg/L). For Fe and Zn, the highest dissolved concentrations of both metals were found in the Juru drainage. The dissolved Fe concentration in the Juru drainage could reach as high as 35.7 mg/L.

According to the Interim National Water Quality Standards (INWQS) for Malaysia (DOE, 2005), the dissolved levels of Cd of the water samples found three sites at Juru River Basin and Kg. Kuantan were all categorized in Class V (worse than Class IV= suitable for irrigation only or the worst water quality classification according to Malaysian INWQS) while the dissolved levels of Cu in Kuala Juru and IRE TEX were categorized in Class IV. The dissolved levels of Fe were categorized in Class V for Sg. Juru and Class IV for IRE TEX. In water samples collected from Kuala Juru was categorized in Class V. The dissolved levels of Pb were categorized in Class IV for Sg. Juru, IRE TEX and Kuala Juru. Therefore, these water samples generally collected from Juru River Basin are classified as polluted, unhealthy and not suitable for water contact activities. For the dissolved levels of Cu, Fe and Pb of water samples collected from Kg. Kuantan, the water quality fell into the category as Class IIA (suitable for water supply and conventional treatment required) or IIB (suitable for recreational use with body contact). For Pb level at Kg. Kuantan, it was within Class III (suitable for water supply but extensive treatment required) and IV while for Zn, it was in the Class III. Therefore, based on this classification, the water quality at Kg. Kuantan was generally better than at least one of the sites at Juru River Basin.

The concentrations of heavy metals in the surface sediments are presented in Table 4. The highest concentrations of Cd, Cu, Ni, Pb and Zn were found in the drainage sediments collected from the Juru industrial area. These metal levels were also significantly ($P < 0.05$) higher in the Juru River and in the Juru Estuary sediment when compared to the Kg. Kuantan sediments. Lim and Kiu (1995) found that Zn was mainly associated with the reducible fractions of the Juru sediments. In this study, the Zn concentrations were mainly associated with the acid-reducible and oxidisable-organic fractions of Zn in the Juru sediments (Table 4). Concentrations of Zn and Fe found in the moderately reducible fraction were significantly correlated, indicating that iron oxide was the preferred host phase of Zn.

The reason why we chose Kg. Kuantan as a relatively unpolluted site for comparative purposes is due to the fact that the wide distribution and abundance of the firefly insect itself is a good bioindicator for clean ecosystem which is reported in the literature such as absence of firefly population due to toxicity of agricultural chemicals (Anazawa *et al.*, 2004). The hypothesis as a relatively unpolluted ecosystem at Kg. Kuantan is also verified by the better water quality and lower heavy metal concentrations in the surface sediments at Kg. Kuantan when compared to the three sites at Juru River Basin. Therefore, based on the above two reasons, Kg. Kuantan can be considered as a relatively unpolluted site although it is thought that it received urban effluents (but it is not significant).

By comparing with the established Sediment Quality Criteria in Table 5, the concentrations of Ni and Zn in the sediments collected from the Juru Industrial drainage were found to be higher than the Action Levels of these metals established by the Hong Kong Sediment Quality Criteria (Lau Wong and Rootham, 1993) and the Interim Sediment Quality Values-high (ISQVs-high) for Hong Kong (Chapman *et al.*, 1999). For the Kg. Kuantan's sediment data, except for Cd level the concentrations of Cu, Ni, Pb and Zn were found to be lower than the two mentioned guideline levels. All of the above comparisons indicated that these elevated metal concentrations were most likely contributed by nearby industrial activities and further studies on heavy metal pollution in the Juru Industrial area are suggested.

A previous study (DOE-USM, 1992) had estimated that domestic waste contributes about

Table 4. Concentrations (mean µg/g dry weight ± standard error [SE]) of heavy metals of the surface sediments collected from 3 sites at the Juru River Basin and a reference site at Kuala Selangor. N= 3

		Kg. Kuantan		Kuala Juru		Sg. Juru		Juru drainage	
Metal	Variable	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Cu	Total Aqua-regia	11.14	0.31	32.91	0.41	28.12	0.22	60.9	0.68
	Sum SET	12.04	0.29	45.54	0.07	34.88	0.12	84.7	0.38
	F1	0.14	0.02	0.52	0.00	0.04	0.01	0.15	0.01
	PerF1	1.21	0.22	1.15	0.00	0.12	0.02	0.17	0.01
	F2	0.09	0.00	0.14	0.01	0.14	0.03	0.05	0.01
	PerF2	0.74	0.00	0.31	0.01	0.41	0.10	0.06	0.01
	F3	3.16	0.14	18.60	0.06	11.35	0.16	49.9	0.56
	PerF3	26.22	0.52	40.85	0.08	32.53	0.35	58.9	0.40
	F4	8.65	0.17	26.27	0.01	23.35	0.08	34.6	0.20
	PerF4	71.83	0.31	57.69	0.06	66.94	0.47	40.9	0.41
Pb	Total Aqua-regia	26.74	1.02	30.20	1.48	40.83	0.81	65.3	1.82
	Sum SET	58.43	1.30	76.36	0.97	77.43	1.04	118	0.88
	F1	1.52	0.06	4.58	0.25	1.63	0.11	2.24	0.20
	PerF1	2.61	0.16	6.00	0.41	2.11	0.17	1.90	0.16
	F2	2.52	0.03	2.77	0.16	0.97	0.20	1.50	0.29
	PerF2	4.32	0.04	3.62	0.16	1.25	0.24	1.28	0.26
	F3	19.68	0.20	26.01	0.06	3.13	0.26	33.78	0.24
	PerF3	33.72	1.07	34.07	0.35	4.03	0.28	28.72	0.01
	F4	34.71	1.53	43.01	1.00	71.70	0.69	80.11	0.73
	PerF4	59.35	1.27	56.31	0.60	92.60	0.35	68.10	0.11
Cd	Total Aqua-regia	1.74	0.19	1.24	0.10	1.40	0.19	1.46	0.20
	Sum SET	1.85	0.04	3.68	0.01	4.68	0.26	2.58	0.18
	F1	0.19	0.02	0.23	0.02	0.03	0.01	0.09	0.02
	PerF1	10.41	1.19	6.28	0.59	0.58	0.12	3.44	0.62
	F2	0.26	0.01	0.11	0.01	0.07	0.00	0.13	0.01
	PerF2	13.92	0.01	3.07	0.15	1.53	0.20	5.09	0.80
	F3	0.21	0.03	0.50	0.08	0.67	0.06	0.56	0.04
	PerF3	11.50	1.21	13.67	2.12	14.36	0.51	21.8	0.05
	F4	1.18	0.02	2.83	0.11	3.91	0.20	1.80	0.13
	PerF4	64.16	0.03	76.98	2.86	83.53	0.43	69.7	0.12
Zn	Total Aqua-regia	119.72	2.09	317.39	2.48	461.33	1.86	484	3.01
	Sum SET	153.21	0.56	303.65	1.79	419.90	0.33	620	6.02
	F1	1.01	0.01	24.96	0.32	56.52	0.12	59.4	0.01
	PerF1	0.66	0.00	8.22	0.06	13.46	0.04	9.58	0.09
	F2	33.39	0.47	58.02	0.14	61.98	0.21	63.3	0.23
	PerF2	21.80	0.39	19.11	0.16	14.76	0.04	10.2	0.06
	F3	47.29	0.62	84.66	0.16	84.11	0.51	84.34	1.27
	PerF3	30.86	0.30	27.88	0.11	20.03	0.14	13.6	0.07
	F4	71.51	0.40	136.01	1.45	217.29	0.75	413.0	4.53
	PerF4	46.67	0.09	44.79	0.21	51.75	0.14	66.62	0.08
Ni	Total Aqua-regia	28.01	0.05	50.28	0.75	46.61	0.71	73.92	0.28
	Sum SET	15.14	0.34	56.48	1.97	46.38	0.10	75.36	0.04
	F1	0.31	0.01	0.37	0.03	1.67	0.18	1.54	0.16
	PerF1	2.03	0.00	0.67	0.08	3.59	0.39	2.04	0.22
	F2	0.37	0.08	1.23	0.07	1.98	0.07	2.46	0.05
	PerF2	2.42	0.45	2.19	0.20	4.27	0.17	3.27	0.07
	F3	3.45	0.12	30.92	1.32	24.42	0.06	42.35	0.27
	PerF3	22.75	0.27	54.72	0.41	52.65	0.02	56.20	0.33
	F4	11.02	0.14	23.96	0.76	18.31	0.07	29.01	0.02
	PerF4	72.80	0.72	42.43	0.13	39.48	0.24	38.50	0.04
Fe	Total Aqua-regia	27724	1204	27595	1795	40998	2683	40790	2994
	Sum SET	28017	128	32788	250	40761	55.4	51690	565
	F1	1.38	0.23	7.59	0.53	5.33	0.42	6.08	0.17
	PerF1	0.00	0.00	0.02	0.00	0.01	0.00	0.01	0.00
	F2	1861	85.31	529.9	9.83	1234	39.54	1167.56	41.37
	PerF2	6.64	0.27	1.62	0.04	3.03	0.10	2.26	0.10
	F3	2671	113.87	3691	73.24	17899	49.40	5723.73	28.13
	PerF3	9.53	0.36	11.25	0.14	4.39	0.12	11.08	0.17
	F4	23483	71.65	28559	186.47	37732	45.94	44792.89	635.05
	PerF4	83.8	0.64	87.1	0.10	92.6	0.01	86.65	0.28

Note: F1= EFLE, F2= acid-reducible, F3= oxidisable-organic, F4= resistant fractions and Sum= summation of the four geochemical fractions. Per= percentage of each geochemical fraction.

Table 5. Comparison of the heavy metal concentrations (µg/g dry weight) obtained in this study with some Sediment Quality Values of Cd, Cu, Pb, Ni and Zn established in this region

No.	Sources	Cd	Cu	Pb	Ni	Zn
1.	Action Level according to Hong Kong Sediment Quality Criteria (Lau Wong and Rootham, 1993).	1.5	65	75	40	200
2.	Interim ISQV- Low sediment quality values (Chapman, *et al*, 1999).	1.5	65	75	40	200
3.	Interim ISQV-High sediment quality values (Chapman, *et al*, 1999).	9.6	270	218	NA	410
4.	Juru River Basin (3 sites)	0.96–1.46	28.12–60.9	30.2–65.3	46.6–73.9	317–484
5.	Kg. Kuantan, Kuala Selangor (1 site)	1.74	11.14	26.74	28.01	119.72

Note: NA= not available.

80% of the BOD load in the Juru River. With the decline of pig-rearing activities because of the country's strict environmental regulations and the projected rapid increase in population in the river basin, the pollution load and the percentage in the river contributed by domestic waste will rise further. This could have a serious implication on the cockle-rearing activities found at the river estuary as bioaccumulation of heavy metals by bivalves is now well established.

The present finding of the heavy metal concentrations in the edible soft tissues of *A. granosa* indicates that 1) the elevated levels were due to the effluents of industries and urbanization along the Juru River Basin and 2) the heavy metal inputs due to the point source is still continuous. Therefore, control of the anthropogenic discharges in the form of special treatment should be enhanced. Finally, the authorities should strictly implement treatment regulations on industrial effluents into the rivers since they would find their way to the intertidal areas which support a high biodiversity of flora and fauna which are among the main components of biological resources.

Finally, from the genetic point of view, the long-term exposure of pollutants in the Juru estuary by cockles should pose a question 'What would happen to the population genetic structures of cockles inhabiting the Juru estuary which receives industrial and urban wastes ? From the genetic ecotoxicological point of view, this question is very interesting since the possibility of the development of high resistance to pollution could lead to population differentiation and eventually even speciation (Nevo *et al.*, 1986). Hence, studies using the biochemical (allozymes) and molecular (DNA) markers should be done on this cockle species since it is now a popular seafood delicacy among Malaysians.

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