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SPATIAL AND TEMPORAL DISTRIBUTION OF BACTERIAL COMMUNITIES AND HEAVY METALS (CR, CD AND PB) COMPOSITION IN SEDIMENTS ALONG PAHANG RIVER, MALAYSIA

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Abstract

Bacterial communities show complex and sensitive response towards the environmental stimulation. Pahang River is one of the important inland aquatic biodiversity resources that provide food and excellent habitat for many organisms including microorganisms. Higher bacterial diversity is assumed to increase ecosystem capacity to resist and recover from perturbation. Hence, it is important to assess the impacts of heavy metals composition towards distribution of bacteria in sediments along Pahang River. A study on heavy metals composition such as chromium, cadmium and lead was carried out along the Pahang River using Teflon Bomb digestion processes and were analyzed using ICP-MS. Overall 19 sampling areas along Pahang River with frequency of 20-30 km for each site were chosen as our sampling stations. Sampling was conducted during pre and post of North-East monsoon season. The average dry weight concentration for chromium (Cr) was found between 3.250 and 21.950 µg/g, cadmium (Cd) ranged from 0.019 to 0.403 µg/g and lead (Pb) 8.024 to 20.660 µg/g. The bacterial community in sediments along Pahang River was determined using culture-based method. The bacterial colony-forming unit (CFU) range was found between 1013.33 CFU/g and 28826.67 CFU/g. This study demonstrated that the concentration of heavy metals and bacterial colony number different at each station during pre and post-monsoon. Apart from that, the heavy metals composition can influence the changes of bacterial colony number. However this changes also influence by other factors such as physicochemical parameters, sediments size, nutrient contents in the river and also sediments and water run-off. The condition of Pahang River is still conducive and activities that causing pollution should be stopped.

Keywords: Pahang river sediments, chromium, cadmium, lead, colony forming unit

1.0 INTRODUCTION

Pahang River is one of the important inland aquatic biodiversity resources in Peninsular Malaysia which stretched from Ulu Tembeling at the upstream to Kuala Pahang at river mouth to the South China Sea. However, there are some problems associated with the river ecosystem. Pollution of river mainly driven by wastewater from agricultural, municipal and industrial activities is widely focused as one of the major environmental problem.

Heavy metals give a significant effect on the ecosystem quality and are considered as a main contribution of pollution in the environment. Major contributors of heavy metals nowadays are anthropogenic activities including smelting, electroplating, mining and other metal processing industry [1, 2]. Other source of heavy metal contamination are urban runoff, industrial effluents and wastes, sewage treatment plants, boating activities, domestic garbage dumps and agricultural fungicide runoff [3]. Usually, industrial dumped the waste that contains the heavy metals into the rivers, stream to reduce the cost of disposal. Due to this, the pollution needs to be monitored and controlled.

Sediments of aquatic system are source of organic and inorganic material and known as place where the majority of decomposition process takes place [4]. Sediments have high physical-chemical stability. Their characteristics commonly represent average condition of system and often the representative of average water quality [2]. Moreover, sediments can act as scavenger agent for heavy metal and an adsorptive sink in aquatic environment. Therefore, sediment can be considered as appropriate indicator for pollution of heavy metal [5].

Bacterial communities may serve as an indicator for sediment environmental stress evaluation as sediment provides substrate for colonization and nutrients for the growth of bacteria [6, 7]. Thus, sediment is a complex habitats occupied by various groups of microorganisms, which play an important role in aquatic food webs, biogeochemical cycling, decomposition process and remobilization of heavy metals [8,9].

Apart from that, most of other study shown that microbial diversity can be affected by several environmental disturbance including heavy metals [10], herbicide and pollutant [11]. Sites contaminated by metal such as mercury and cadmium showing less microbial diversity, then it promote metal-resistant organism to grow [12]. However, this finding contradict with the study did by [13]. He investigated that sediments contaminated with higher level of cadmium, copper, lead and zinc did not significantly affect the diversity of microbial communities.

Moreover, microbes are known to facilitate changes in metal formation [14] through respiration [15] or detoxification reaction such as reduction [16] which may have indirect effect of increased toxicity levels. Currently, the comprehensive data on longitudinal dynamics and changes of the bacterial community and heavy metals composition in the Pahang River is not available. More studies are needed in order to understand the impacts of environmental pollution towards microbial diversity in freshwater ecosystem.

2.0 EXPERIMENTAL

2.1 Sampling

Sediments samples were collected from 19 stations along Pahang River with frequency of 20-30 km for each sampling station (Figure 1). The coordinate and site description of each station were shown in Table 1. The sediments were collected using Ponar grab sampler. Each sediments sample was put and sealed in plastic bags and stored in ice chest at 4-6°C immediately after collection. Sampling was conducted on 2013 and 2014during pre and post North-East Monsoon.

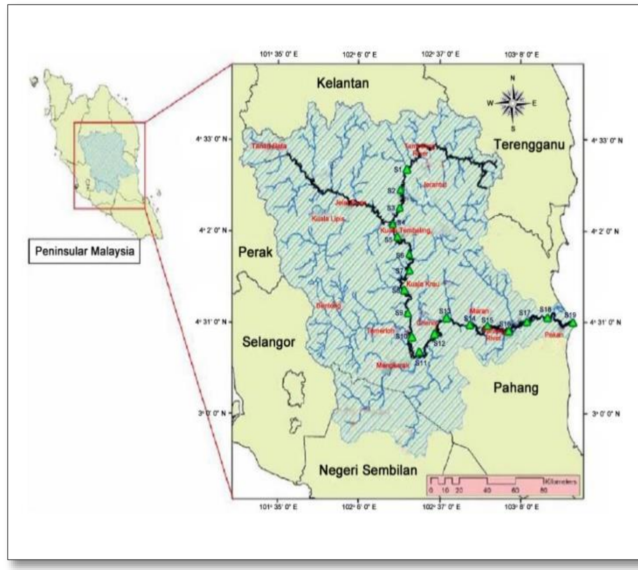

Figure 1 Sampling stations along Pahang River

Table 1 Coordinates and site description of each sttion along Pahang River

Station	Coordinate	Site Description
S1	N 04° 23’ 1.0” E 102° 23’ 59.0”	Kuala Tahan National Park, Commercial centers e.g. Chalet, Restaurants, Boating activities
S2	N 04° 15’ 54.2” E 102° 22’ 24.9”	Undisturbed area, Kuala Atok National Park
S3	N 04° 07’ 45.4” E 102° 20’ 31.2”	Oil palm and rubber tree plantations
S4	N 04° 04’ 14.4” E 102° 19’ 3.3”	Confluence of Tembeling and Jelai Rivers, Aquaculture, Residential areas, Boating activities
S5	N 03° 59’ 15.2” E 102° 20’ 30.7”	Oil palm plantation
S6	N 03° 54’ 14.4” E 102° 25’ 51.8”	Oil palm plantation, Aquaculture, School
S7	N 03° 47’ 58.3” E 102° 25’ 37.7”	Oil palm plantation
S8	N 03° 40’ 57.3” E 102° 23’ 14.1”	Agriculture, Aquaculture, Village
S9	N 03° 34’ 14.3” E 102° 24’ 14.5”	Aquaculture
S10	N 03° 25’ 23.5” E 102° 26’ 20.9”	Aquaculture, Village, Near Temerloh town
S11	N 03° 20’ 5.1” E 102° 28’ 48.5”	Village, School
S12	N 03° 25’ 49.0” E 102° 35’ 1.9”	Undisturbed area
S13	N 03° 31’ 39.5” E 102° 38’ 0.4”	Oil palm plantation, Aquaculture
S14	N 03° 29’ 43.1” E 102° 47’ 5.5”	Undisturbed area

Station	Coordinate	Site Description
S15	N 03° 29' 28.8" E 102° 53' 40.1"	Rubber tree plantation
S16	N 03° 27' 54.3" E 103° 03' 57.4"	Rubber tree plantation
S17	N 03° 33' 5.6" E 103° 12' 29.9"	Oil palm and rubber tree plantations
S18	N 03° 34' 1.6" E 103° 20' 42.4"	Oil palm, coconut and rubber tree plantations
S19	N 03° 31' 52.3" E 103° 27' 57.2"	Estuary, LKIM jetty, Residential area

2.2 Heavy Metals Determination

Each sediment sample was air-dried for approximately one week. Samples were oven-dried at 60 °C for approximately 3 days. Subsequently, the samples were crushed and sieved using electrical sieve with mesh size of 63 µm. The sediment samples were digested by following the published methodologies by [3]. Teflon bomb jackets were rubbed with sand paper in order to remove rust before they are used. The Teflon bomb containers were then be immersed in 69 % of concentrated nitric acid and heated for 3 hours. The acid being used considered as a wash solution. After cooling, the Teflon bomb beakers were rinsed using distilled water and put in the dryer. 0.05 g of dry sediment sample was weighed. The sediment sample was transferred into Teflon bomb for digestion. 1.5 mL of mixed acid with the ratio of 3.0 HF: 3.5 HNO_3: 3.5 HCl was added and heated at 150 °C for 5-7 hours. After heating, the Teflon bombs were cooled down at room temperature. 3.0 mL of boric acid and EDTA were added and heated again for 5-7 hours at 150 °C. They were cooled down at room temperature before transferred into 15 mL Falcon tube. Milli-Q water was added to mesh up to 10 mL. A laboratory standard sediments reference material and a blank reagent were subjected to the same procedure in order to determine the precision of the analytical method. Analysis of heavy metals was determined using ICP-MS (Perkin ELMER ELAN 9000 system).

2.3 Culture of Bacteria

Sediment sample (5g) was weighted and transferred into sterile Falcon tube. 10ml of sterile distilled water was added into each Falcon tube and vortexed for homogenization. A fixed amount of 200µl of supernatant was pipetted and transferred into freshly prepared Luria Bertani agar plate. The mixture transferred was spread on individual plates for each sample. The plates were incubated at 37°C for 24h. Colonies grew on the plate were counted.

3.0 RESULTS AND DISCUSSION

The heavy metals composition studied at all stations were chromium, cadmium and lead. Nine replicates were done at each station. The world average concentration of shale value for chromium is 90 µg g^{-1}, cadmium is 0.3 µg g^{-1} and lead is 20 µg g^{-1} [17].

3.1 Chromium (Cr) Concentration in Sediments

The average concentration trend of chromium at each station during pre and post-monsoon were illustrated in Figure 2. The chromium concentration ranged from 3.250 to 21.950 µg g^{-1}. The highest concentration of chromium was at Station 5 during pre-monsoon season while the lowest was at Station 14 in pre-monsoon season. The result showed that the chromium concentration fluctuated from upstream to the downstream of Pahang River during pre and post-monsoon season. The concentration of chromium at most station was lower compared with the average shale values. The higher concentrations of chromium in the river are might be from anthropogenic activities as mentioned in Table 1. This finding is similar with the study done by [18] which mentioned that the release of chromium is from various anthropogenic activities. Apart from that, applications of fertilizers in agriculture also a potential source of the higher concentration of chromium [19].

3.2 Cadmium (Cd) Concentration in Sediments

The average concentration trend of cadmium at each station during pre and post-monsoon were illustrated in Figure 3. The cadmium concentration ranged from 0.019 to 0.403 µg g^{-1}. The highest concentration of cadmium was at Station 1 during post-monsoon season while the lowest was at Station 16 in pre-monsoon season. The concentration of cadmium at most station was lower compared with the average shale values. The potential source of higher concentration of cadmium at Station 1 is might be from boating activities. Field observation at Station 1 showed there are a lot of boats that bring tourist and passengers pass through the river everyday which might influence the higher level of cadmium in the river. Cadmium is a component of diesel fuels, petrol and lubricating oil [2]. In addition, domestic sewage also might be contributes to the level of cadmium [18]. At that station there are a lot of restaurant and chalet nearby the river that release their sewage to the river.

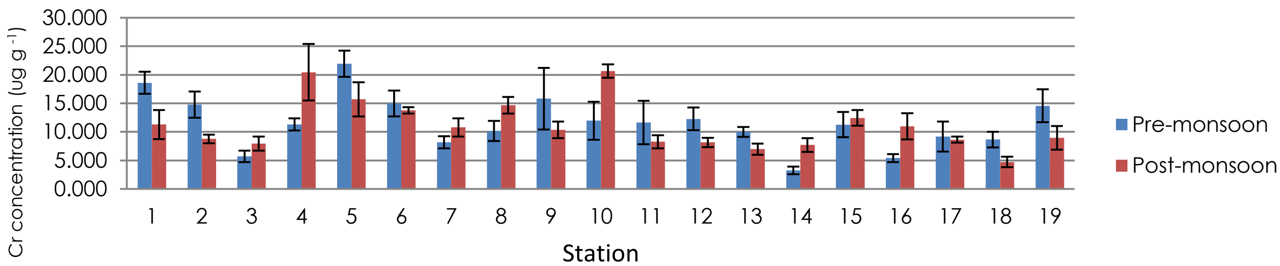

Figure 2 Concentration of chromium (µg g^{-1}) at different monsoon season

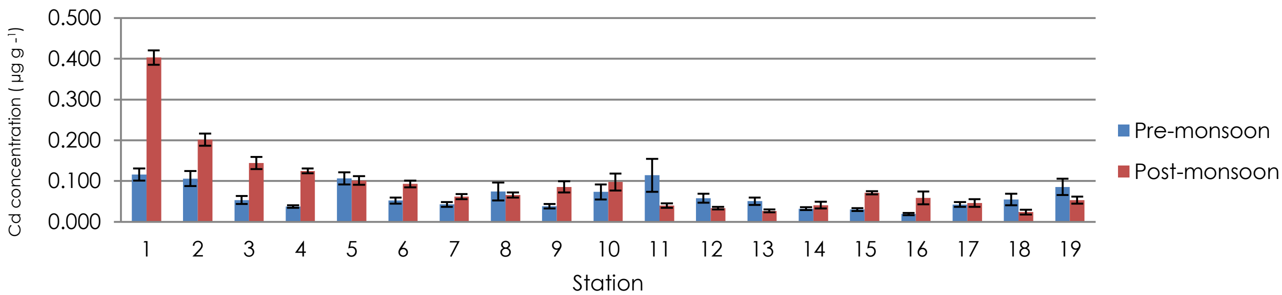

Figure 3 Concentration of cadmium (µg g^{-1}) at different monsoon season

3.3 Lead (Pb) Concentration in Sediments

The average concentration trend of lead at each station during pre and post-monsoon were illustrated in Figure 4. The lead concentration ranged from 8.024 to 20.660 µg g^{-1}. The highest concentration of lead was at Station 5 during pre-monsoon season while the lowest was at Station 3 in pre-monsoon season. The concentration of lead at most station was lower compared with the average shale values. The potential source of lead contamination is might be from leaded petrol, industrial effluents and residential sewage [5]. There are a lot of activities taking place along the Pahang River such as sand mining, aquaculture and agriculture that can lead to lead contamination.

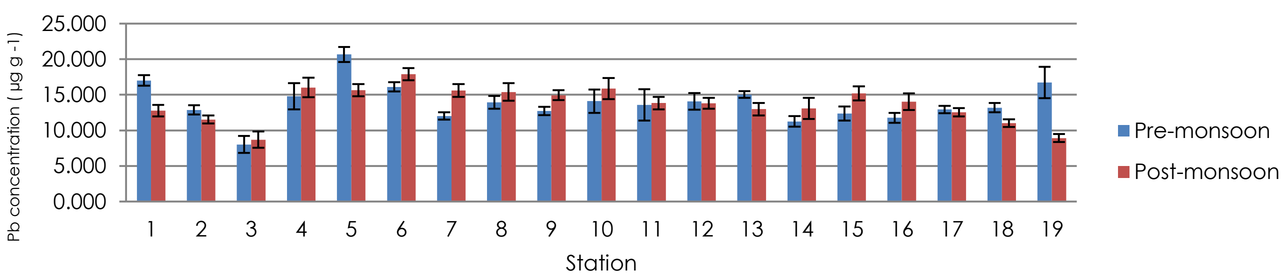

Figure 4 Concentration of lead (µg g^{-1}) at different monsoon season

3.4 Bacterial Colony Forming Unit

For the bacterial counting, nine replicates bacterial spreading on LB agar plates was done. The average CFU distribution trend at each station during pre and post-monsoon were illustrated in Figure 5. The CFU range was found between 1013.33 CFU/g and 28826.67 CFU/g. The highest bacterial colony number was at Station 12 during post-monsoon season while the lowest at Station 2 in pre-monsoon season. According to [20], abundant bacterial species make dominant populations where the location has the lowest flow and richest nutrition. This finding also similar to the research done by [21] mentioned that bacterial abundance generally increased in downstream direction with slow flow rates and have high level of nutrients in the water. As shown in Figure 5, the number of bacteria increases in downstream direction especially at middle stations.

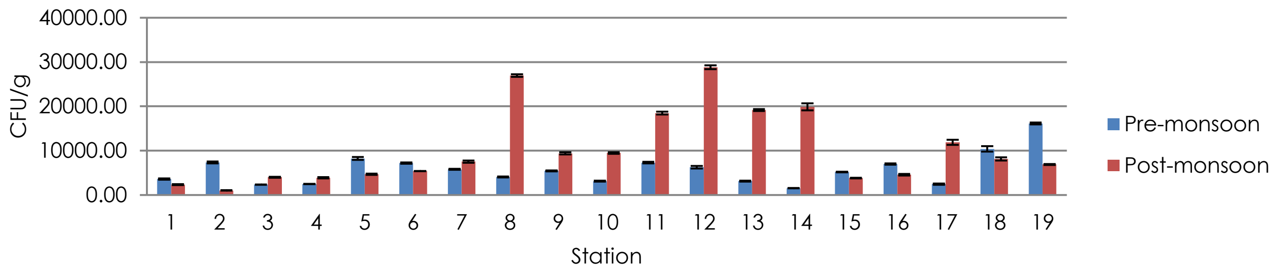

Figure 5 Bacterial colony forming unit at different monsoon season

3.5 Inter – Spatial and Temporal Distribution

The results of Analysis of Variance (ANOVA) showed that there were significant differences in chromium, cadmium and lead concentration and bacterial colony forming unit in each station along Pahang River ($p < 0.05$). For the temporal distribution, ANOVA showed that there were significant differences in cadmium concentration and bacterial colony forming unit ($p < 0.05$) while there were no significant differences for chromium and lead concentration during pre and post-monsoon season ($p > 0.05$) (Table 2). The variation of heavy metals concentration in each station depends on sources and rate of contamination [3]. Thus, it is important to study the contamination level at the study area.

Table 2 Analysis of Variance (ANOVA) inter – spatial and temporal distribution

	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Station	Chromium	4233.057	18	235.170	5.651	.000
	Cadmium	.904	18	.050	32.333	.000
	Lead	1362.762	18	75.709	7.314	.000
	CFU	5719721414.474	18	317762300.804	20.112	.000
Monsoon	Chromium	17.277	1	17.277	.415	.520
	Cadmium	.081	1	.081	52.185	.000
	Lead	2.663	1	2.663	.257	.612
	CFU	1809939079.605	1	1809939079.605	114.558	.000

3.6 Correlation Between Heavy Metals and Bacterial Colony Forming Unit

The relationship between chromium, cadmium and lead concentration with bacterial colony forming unit were analysed by Pearson's correlation coefficient. Correlation results are presented in Table 3. Pearson correlation analysis revealed negative and significant correlation between cadmium and bacterial colony forming unit ($p < 0.01$). This supports the finding studied by [12]. Sites contaminated by cadmium showing less microbial diversity. In contrast, there was no significant correlations found between chromium and lead with bacterial colony forming unit ($p > 0.05$). According to [13], he investigated that sediments contaminated with higher level of lead did not significantly affect the diversity of microbial communities.

Table 3 Correlation analysis of heavy metals with bacterial colony forming unit

	Bacterial colony forming unit
Cr	- .029
Cd	-.198**
Pb	.082

**. Correlation is significant at the 0.01 level (2-tailed)

4.0 CONCLUSION

The concentration of Chromium (Cr), Cadmium (Cd) and Lead (Pb) at most station along Pahang River showed lower concentration compared to the world average concentration of shale. It can be concluded that the condition of Pahang River is still conducive and no serious heavy metals contamination recorded. The bacterial community along the river also still abundance. The priority should be given for long term study of heavy metals composition and bacterial community distribution in order to get valid results. Moreover continuous study of Pahang River is needed to ensure the habitat is still conducive for sustainable development of aquatic resources.

Acknowledgement

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