



## Distribution of heavy metals (arsenic, cobalt, copper and zinc) composition and bacterial communities in sediments along tropical river east coast Malaysia

Noor Faizul Hadry Nordin<sup>a,\*</sup>, Mohd Huzaimi Mohd Amin<sup>b</sup>, Kamaruzzaman Yunus<sup>c</sup>, Ahmed Jalal Khan Chowdhury<sup>d,\*</sup>, Cristalina Jalil Marsal<sup>d</sup>, Mohd Hafiz Jamaludin<sup>d,e</sup>, Wanidawati Tamat<sup>f</sup>

<sup>a</sup>International Institute for Halal Research and Training, International Islamic University Malaysia, Jalan Gombak 53100, Kuala Lumpur Malaysia, email: faizul@iium.edu.my (N.F.H. Nordin)

<sup>b</sup>Department of Biotechnology, Kulliyah of Science, International Islamic University Malaysia, Jalan Sultan Ahmad Shah, Bandar Indera Mahkota, 25200, Kuantan, Malaysia

<sup>c</sup>Department of Marine Science, Kulliyah of Science, International Islamic University Malaysia, Jalan Sultan Ahmad Shah, Bandar Indera Mahkota, 25200, Kuantan, Malaysia

<sup>d</sup>Faculty of Agriculture, Universiti Islam Sultan Sharif Ali (UNISSA), Km 33, Tutong Kg. Sinaut Tutong TB1741, Brunei Darussalam, email: ahmed.chowdhury@unissa.edu.bn (A.J.K. Chowdhury)

<sup>e</sup>Faculty of Agrobased Industry, Universiti Malaysia Kelantan, Jeli Campus, 17600 Kelantan, Malaysia

<sup>f</sup>Department of Fisheries, Ministry of Primary Resources and Tourism, Brunei Muara, BT 1728 Brunei Darussalam

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### ABSTRACT

Sediment samples were collected from 19 sampling stations of Pahang River, east coast Malaysia to determine the concentration of arsenic (As), cobalt (Co), copper (Cu), zinc (Zn) and bacterial colony forming unit (CFU) distribution. Sampling was conducted during pre and post of North-East monsoon season for the years 2013 and 2022. Heavy metal concentrations were determined by using inductively coupled plasma mass spectrometry (ICP-MS). The concentration of heavy metals was compared with the world average concentration of shale values. The dry weight concentration of As ranged between  $4.387 \pm 0.586$  to  $15.922 \pm 8.738 \mu\text{g}\cdot\text{g}^{-1}$  during pre-monsoon. While it was  $3.989 \pm 0.758$  to  $11.336 \pm 6.748 \mu\text{g}\cdot\text{g}^{-1}$  during post-monsoon. The range of dry weight concentration for Co was found between  $1.730 \pm 0.318$  and  $4.569 \pm 0.586 \mu\text{g}\cdot\text{g}^{-1}$  during pre-monsoon while  $1.476 \pm 0.063$  and  $4.620 \pm 0.951 \mu\text{g}\cdot\text{g}^{-1}$  during post-monsoon. The dry weight concentration of Cu ranged between  $1.182 \pm 0.510$  to  $10.722 \pm 5.664 \mu\text{g}\cdot\text{g}^{-1}$  and  $0.900 \pm 0.222$  to  $6.514 \pm 3.749 \mu\text{g}\cdot\text{g}^{-1}$  during pre-monsoon and post-monsoon, respectively. The range dry weight concentration for Zn was found between  $7.964 \pm 4.857$  and  $26.289 \pm 2.636 \mu\text{g}\cdot\text{g}^{-1}$  during pre-monsoon while  $8.187 \pm 3.010$  and  $28.347 \pm 15.665 \mu\text{g}\cdot\text{g}^{-1}$  during post-monsoon. The bacterial community in sediments along Pahang River was determined using culture-based method. The bacterial CFU range was found between  $1526.67 \pm 64.68$  and  $16146.67 \pm 225.71$  CFU/g during pre-monsoon while  $1013.33 \pm 38.51$  and  $28826.67 \pm 418.47$  CFU/g during post-monsoon. This study revealed that the concentration of heavy metals does not influence the distribution of bacteria in the Pahang River sediments. Even though the concentrations of heavy metals studied are typically below reported toxic levels, ongoing monitoring of river ecosystems is an essential to safeguard this ecosystem and ensure that the pollution from heavy metals is under conducive condition throughout these increased industrialization and urbanization.

*Keywords:* Pollution; Heavy metals; Bacterial communities; Sediments; Pahang River

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\* Corresponding authors.

## 1. Introduction

### 1.1. River water pollution

Water is an essential element of human life and plays a significant function in ecological systems. Rivers are particularly prone to pollution since they are open areas of the environment. Because of ongoing anthropogenic disruptions and seasonal flooding conditions which have caused concentrations of heavy metals and microbial pollution in rivers to keep rising above the regional background value and have the potential to affect both human health and the ecological balance [1,2].

Heavy metals in watershed ecosystems are hard to remove and have a great tendency to conceal themselves, making them harmful for a long time. They displayed various risk levels, effects from point and non-point surface pollution, characteristics of bioaccumulation, and anthropogenic dominant sources [3].

Quality of river water is affected by human-induced or natural activities in the upstream watershed. As a result of the natural flow of the water, most pollutants are drained into a one-point collection site, such as reservoirs that can serve as a sink for different pollutants. Due to its potential and toxic environmental and public health effects and the ability to accumulate, heavy metal contamination of the aquatic ecosystems is becoming a potential global problem [4,5].

### 1.2. River sediment pollution

Sediment contamination with heavy metals could be either from natural geogenic sources or sourced from anthropogenic activities. The benthic environment of aquatic ecosystems receives and absorbs heavy metals from natural weathering, erosion, industrial wastes, and atmospheric deposition [6]. Anthropogenic activities, such as industrial and agricultural discharges, inappropriate disposal of industrial wastes, dumping of domestic and municipal wastes, faulty drainage systems are some of the causes for heavy metal contamination of aquatic ecosystems [7,8].

Several studies indicated that heavy metal concentration in stream sediments is relatively high due to significant anthropogenic metal loadings carried by tributary rivers. As a result, surficial sediments may serve as a metal puddle that can release metals to the overlying water that could potentially adversely affect the riverine ecosystems [9,10]. It is well-known that the mobility and availability of heavy metals in aquatic environments are primarily affected by physicochemical parameters of water, such as pH, dissolved oxygen, and organic matter content [11,12].

### 1.3. Sources of river pollution

The entry of contaminants into the environment due to human and natural activities is one of the most important issues facing today's communities. Due to the industrial and economic growth and the production of a variety of compounds and chemicals followed by increased consumption man makes some unwanted pollutants, many of which cause serious problems and risks for the environment and for man himself. The most important natural resources of environmental pollution are soil and rock

weathering and natural events such as earthquakes and floods [13].

The entry of municipal, industrial, and agricultural waste into the environment is another way of the environment pollution by human. Water resources are among the most critical resources. The importance of water resources, particularly surface waters (rivers), in meeting the water needs of humans, animals and industries indicates the essential need to protect them against contamination. As municipal, industrial, and agricultural waste enters the water, biological and chemical contaminants including heavy metals also enter water resources. Although some of these metals are essential as micronutrients, their high concentration in the food chain can cause toxicity and environmental impacts and endanger aquatic ecosystems and their users [3].

Although the concentrations of heavy metals studied are typically below reported toxic levels, ongoing monitoring of river ecosystems is required due to the level of increased industrialization and urbanization to protect this ecosystem and make sure that the pollution from heavy metals is under control. The main toolbar that controls the severity of disease outbreaks is the oscillation of changing water levels and environmental elements in connection to the biota and abiotic variables. Water serves as an intermediary medium in the fecal-oral route used to transfer these pathogenic pathogens [14].

### 1.4. River water quality and bacteriological contaminations

Seasonal changes in rainfall, surface runoff, inter-flows, groundwater flows, and pumped-in and -outflows during flood events show a considerable influence on river discharge and consequently on the concentration of contaminants in the river water [15].

These could build-up of domestic pollutants in river channels can all cause in a decline in the quality of the river's water. These associated organic and inorganic sediments were re-suspended and transported downstream during the disaster, where they were dispersed onto the flood plains implying negative effects on the ecological balance of the recipient environment and a variety of aquatic organisms [16,17].

The primary point sources for microbial contamination of natural aquatic resources include hospital and industrial emissions of pollutants, non-collective sewage systems, and water treatment plant discharges. The higher amounts of fecal indicator bacteria revealed that urban floodwater was fecally contaminated. The family Enterobacteriaceae includes numerous species of bacteria, including *Citrobacter*, *Enterobacter*, *Escherichia*, and *Klebsiella*, that make up the coliform bacteria group. This bacterial species primarily inhabits dirt, water, and the digestive tracts of both humans and animals. The main origins of microbial contamination of natural aquatic resources are related to the point sources such as the discharges of water treatment plants, non-collective sewage systems, or contaminants emissions from hospitals and industries [18].

Urban floodwater was found to be fecally contaminated which was demonstrated by the elevated concentrations of fecal indicator bacteria. The coliform bacteria group consists of several genera of bacteria belonging

to the family Enterobacteriaceae including *Citrobacter*, *Enterobacter*, *Escherichia* and *Klebsiella*. This group of bacteria mainly lives in soil, water and the digestive system of animals and human [19,20].

The World Health Organization (WHO) reports that more than 5 people have died from water-borne infections. According to the World Health Organization (WHO), the mortality of water-borne diseases has exceeded 5 million people/y and the most important bacterial diseases transmitted through the floodwater are listed in Table 1 [21].

### 1.5. Pahang River Malaysia

River surface water studies are among the preliminary topics in Malaysia, which provide an overview of the sedimentation problems on the specified river. Sedimentation is a problem that often occurs in the rivers in Malaysia, especially on the main river. Almost 60% of the rivers in Malaysia are regulated for domestic, farming and agricultural, industrial fields, residential, sewage disposal and urbanization the major pollution sources influencing the river equilibrium in Malaysia. Sedimentation is the process of bringing the material of erosion by water, wind or glaciers. Sediments are a solid material, which moved and deposited in a new location. Sediment consists of rocks and minerals as well as the remains of plants and animals, and all the materials were deposited and will become sediments [22].

The Pahang River is the principal river that traverses the Malaysian state of Pahang. The South China Sea is where the river empties into after starting at the junction of the Tembeling and Jelai rivers in the Titiwangsa Mountains. It is the longest river on the Peninsula of Malaysia. The length of the river is 440 km and it drains an area of 29,300 km<sup>2</sup>, of which 27,000 km<sup>2</sup> situated within Pahang (which is about 75% of the state) and 2,300 km<sup>2</sup> is located in Negeri Sembilan [17,23].

The monsoon climate-controlled hydrology of the Pahang River plays an important role in its sediment erosion, transportation, and deposition processes with a higher river discharge of 845.78–1,008.50 m<sup>3</sup>/s. The flow regime of Pahang River is characterized by low flows during the pre-monsoon season and extremely high flows during the

flooding and post-monsoon seasons. The scenario worsened during the annual flood tragedies since 2014 had devastated 18,000 km<sup>2</sup> of the lowland areas lying along Pahang River. The swift river flow has transported vast quantities of pollutant loads, including eroded sediments, debris and tremendous amount of toxic heavy metals and microbial pollution from the adjacent landmass into the river by inflowing water during the flood event. The Pahang River ecology has been negatively impacted towards benthic ecosystem and the flora-fauna by the extensive human activities such as farming and agricultural practices, mining, residential, urbanization, logging, industrial and seasonal flooding as well [24–27].

The soil biogeochemistry, temperature, organic content, pH, salinity, dissolved oxygen, turbidity, nutritional contents, and overall water quality index all interact intricately with the surface and subsurface microorganisms. After the monsoon flood events, higher quantities of microbial pathogens and fecal-indicator bacteria have been discovered in the floodwaters and sediments along the riverine environment depending on the location and sanitation situations. Based on the above perspectives, this study provides information on the likelihood and severity on the distribution of heavy metals and microbiological contamination in the Pahang River [28–30].

## 2. Methodology

### 2.1. Sample collection

Sediments samples were collected during pre and post North-East Monsoon. The samples were collected from 19 stations along the Pahang River with frequency of 20–30 km for each sampling station. The coordinate and site description of each station has shown in Table 2. The sediments were collected using Ponar grab sampler. The sediments samples were kept in ice during transportation to the laboratory.

### 2.2. Heavy metal analysis

The sediment samples were digested by following the published methodologies by [14,31]. Samples were digested

Table 1  
List of water-borne diseases related to river flooding [Source: 21]

No.	Water-borne bacteria	Diseases
1.	Water-borne diseases: Diseases spread through water which acts as a passive carrier for the infecting pathogens	Cholera, typhoid, bacillary dysentery, infectious hepatitis, leptospirosis, giardiasis, gastroenteritis
2.	Water-related diseases: Diseases spread by vectors and insects that live inside or close to water. Stagnant ponds of water provide the breeding place for the disease spreading vectors, such as mosquitoes, flies and insects	Yellow fever, dengue fever, encephalitis, malaria, filariasis (all by mosquitoes), sleeping sickness (Tsetse fly), onchocerciasis ( <i>Simulium</i> fly)
3.	Water-based diseases: Diseases caused by infecting agents driven by contact with or ingestion of water. Water supports an essential part of the life cycle of infecting agents	Schistosomiasis, dracunculosis, bilharziosis, phalarises, oncholersosis, treadworm and other helminthes
4.	Water-washed diseases: Diseases caused by the inadequate quantity of water for proper maintenance of personal hygiene	Scabies, trachoma (eye-infection), leprosy, conjunctivitis, salmonellosis, ascariasis, trichuriasis, hookworm, amoebic dysentery, paratyphoid fever

Table 2  
Coordinates and site description of each station along Pahang River

Stations	Coordinate	Site description
S1	N 04° 23' 1.0" E 102° 23' 59.0"	Kuala Tahan National Park, commercial centers, for example, 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
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

using Teflon bomb digestion method and heavy metal detection was carried out by using inductively coupled plasma mass spectrometry (ICP-MS). 0.05 g of dried sediment sample (<63 µm) was weighed. Subsequently, the sediment sample was transferred into Teflon bomb for digestion. 1.5 mL of mixed acid with the ratio of 3.0 hydrofluoric acid (HF): 3.5 nitric acid (HNO<sub>3</sub>): 3.5 hydrochloric acid (HCl) was added and heated at 150°C for 5–7 h. After heating, the Teflon bombs were cooled down at room temperature. 3.0 mL of boric acid (H<sub>3</sub>BO<sub>3</sub>) and EDTA were added and heated again for 5–7 h at 150°C. Subsequently, they were cooled down at room temperature before transferred into

15 mL Falcon tube. Lastly, Milli-Q water was added for dilution up to 10 mL. A laboratory standard sediments reference material (SRM 1646a) and a blank reagent were subjected to the same procedure in order to determine the precision of the analytical method.

### 2.3. Bacterial community distribution – culture of bacteria

In culture-based method, 5 g of sediment samples was weighted and transferred into sterile Falcon tube. 10 mL of sterile distilled water was added into each Falcon tube and vortexed for homogenization. 200 µl of supernatant was

pipetted and transferred into freshly prepared Luria Bertani agar plate. Subsequently, the mixture transferred was spread on individual plates for each sample. The plates were incubated at 37°C for 24 h. After incubation, all the colonies growth was calculated to obtain colony forming unit.

### 3. Result and discussion

The heavy metals composition studied at Pahang River were arsenic, cobalt, copper and zinc. Nine replicates were done at each station. The world average concentration of shale value for arsenic is 13  $\mu\text{g}\cdot\text{g}^{-1}$ , cobalt is 19  $\mu\text{g}\cdot\text{g}^{-1}$ , copper 45  $\mu\text{g}\cdot\text{g}^{-1}$  and zinc is 95  $\mu\text{g}\cdot\text{g}^{-1}$  [32].

#### 3.1. Arsenic (As) concentration in sediments

The mean concentration of arsenic at different sampling stations during pre and post-monsoon season. Analysis of Variance (ANOVA) showed significant differences of arsenic ( $p < 0.05$ ) between sampling stations (Fig. 1). During pre-monsoon season, the highest mean concentration of arsenic was recorded at Station 19 with  $15.922 \pm 8.738 \mu\text{g}\cdot\text{g}^{-1}$ . The lowest mean was recorded at the Station 16 with  $4.387 \pm 0.586 \mu\text{g}\cdot\text{g}^{-1}$ . Meanwhile, for the post-monsoon season, the highest mean concentration of arsenic was recorded at Station 10 with  $11.336 \pm 6.748 \mu\text{g}\cdot\text{g}^{-1}$ . The lowest mean was recorded at the Station 18 with  $3.989 \pm 0.758 \mu\text{g}\cdot\text{g}^{-1}$ . Analysis of variance (ANOVA) showed no significant differences of arsenic concentration ( $p > 0.05$ ) between sampling seasons. From the result, the concentration of arsenic at most station was lower compared with the average shale values except Station 19 which is estuary area during pre-monsoon season. This finding is similar with study conducted by other researchers which mention that arsenic concentration in sediment which exceeded the environmental guideline values is at locations close to the river mouth and in the mainstream [13]. The potential source of higher concentration of arsenic might be from industrial processes including waste recycling, burning of fossil fuels, paper production. In addition, other sources of arsenic are from terrestrial runoff, atmospheric deposition, pesticides, fertilizers from agriculture activities, ship waste, anticorrosive paints used on marine vessels and embarkation activities [33]. Field observation showed there are several activities that might lead in increasing arsenic

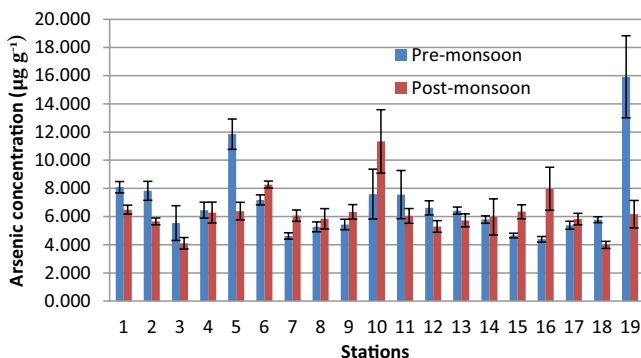


Fig. 1. Concentration of arsenic ( $\mu\text{g}\cdot\text{g}^{-1}$ ) at different monsoon season.

concentration at Pahang River including fishing activities especially at Station 19, sand and mineral mining along the river, palm oil plantations and urban or industrial effluent.

#### 3.2. Cobalt (Co) concentration in sediments

The mean concentration of cobalt at different sampling stations during pre- and post-monsoon season. Analysis of Variance (ANOVA) showed significant differences of cobalt concentration ( $p < 0.05$ ) between sampling stations (Fig. 2). During pre-monsoon season, the highest mean concentration of cobalt was recorded at Station 5 with  $4.569 \pm 0.586 \mu\text{g}\cdot\text{g}^{-1}$ . The lowest mean was recorded at the Station 16 with  $1.730 \pm 0.318 \mu\text{g}\cdot\text{g}^{-1}$ . Meanwhile, for the post-monsoon season, the highest mean concentration of cobalt was recorded at Station 6 with  $4.620 \pm 0.951 \mu\text{g}\cdot\text{g}^{-1}$ . The lowest mean was recorded at the Station 18 with  $1.476 \pm 0.063 \mu\text{g}\cdot\text{g}^{-1}$ . Analysis of Variance (ANOVA) showed significant differences of cobalt concentration ( $p < 0.05$ ) between sampling seasons. The concentration of cobalt at all station was lower compared with the average shale values. The industrial and agricultural activities are the potential source of copper. According to previous study, cobalt was claimed to enhance the health of aquaculture and grazing animals due to its function to trigger and develop some protein [34]. It is believed that a lot of aquaculture activities along Pahang River might be contributed to the level of cobalt concentration.

#### 3.3. Copper (Cu) concentration in sediments

Fig. 3 shows the mean concentration of copper at different sampling stations during pre- and post-monsoon

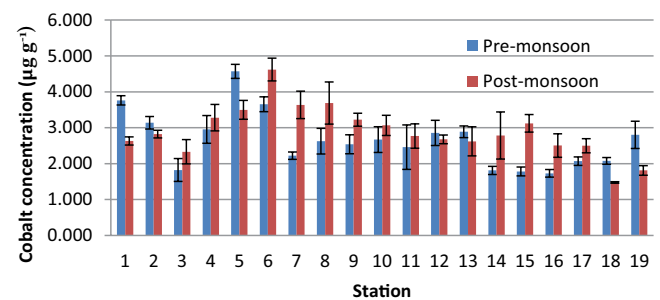


Fig. 2. Concentration of cobalt ( $\mu\text{g}\cdot\text{g}^{-1}$ ) at different monsoon season.

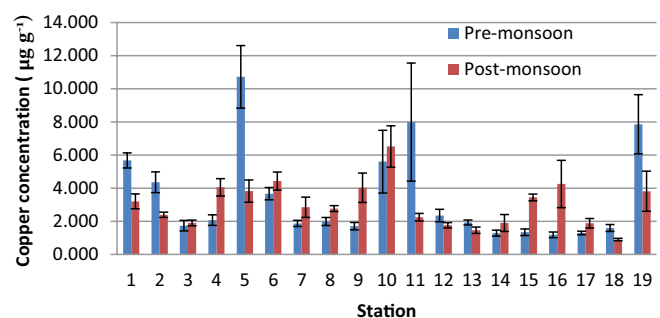


Fig. 3. Concentration of copper ( $\mu\text{g}\cdot\text{g}^{-1}$ ) at different monsoon season.

season. Analysis of Variance (ANOVA) showed significant differences of copper concentration ( $p < 0.05$ ) between sampling stations. During pre-monsoon season, the highest mean concentration of copper was recorded at Station 5 with  $10.722 \pm 5.664 \mu\text{g}\cdot\text{g}^{-1}$ . The lowest mean was recorded at Station 16 with  $1.182 \pm 0.510 \mu\text{g}\cdot\text{g}^{-1}$ . Meanwhile, for the post-monsoon season, the highest mean concentration of copper was recorded at Station 10 with  $6.514 \pm 3.749 \mu\text{g}\cdot\text{g}^{-1}$ . The lowest mean was recorded at Station 18 with  $0.900 \pm 0.222 \mu\text{g}\cdot\text{g}^{-1}$ . Analysis of Variance (ANOVA) showed no significant differences of copper concentration ( $p > 0.05$ ) between sampling seasons. The mean concentration of copper recorded at all station was much lower when compared with the world average shale value.

The higher concentrations of copper in this study might be from anthropogenic activities including domestic sewage and industrial effluent. The findings reveals that the higher concentration of copper is mostly at the urban and residential area. This is perhaps the sources from non-point city sewage and industrial effluents. Apart from that, application of herbicide and pesticide in agricultural area, boating activities and sand mining also a potential source of the higher concentration of copper at Pahang River estuary [35].

### 3.4. Zinc (Zn) concentration in sediments

Fig. 4 shows the mean concentration of zinc at different sampling stations during pre- and post-monsoon season. Analysis of Variance (ANOVA) showed significant differences of zinc concentration ( $p < 0.05$ ) between sampling stations. During pre-monsoon season, the highest mean concentration of zinc was recorded at Station 1 with  $26.289 \pm 2.636 \mu\text{g}\cdot\text{g}^{-1}$ . The lowest mean was recorded at Station 3 with  $7.964 \pm 4.857 \mu\text{g}\cdot\text{g}^{-1}$ . Meanwhile, for the post-monsoon season, the highest mean concentration of zinc was recorded at Station 10 with  $28.347 \pm 15.665 \mu\text{g}\cdot\text{g}^{-1}$ . The lowest mean was recorded at Station 18 with  $8.187 \pm 3.010 \mu\text{g}\cdot\text{g}^{-1}$ . Analysis of Variance (ANOVA) showed no significant differences of zinc concentration ( $p > 0.05$ ) between sampling seasons. The concentration of zinc at all station was lower compared with the average shale values. The potential source of zinc contamination in the study areas might be from industrial effluents, ports and transportation. In addition, many fishing boats as well as tourist boat might also influence the higher zinc concentration in the environment. Moreover, studies mentioned that painting

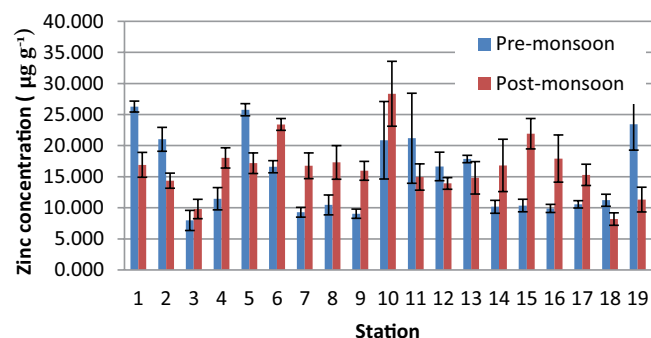


Fig. 4. Concentration of zinc ( $\mu\text{g}\cdot\text{g}^{-1}$ ) at different monsoon season.

and the used of antirust paint in shipping and fishing industries may affect the concentration of Zn in the sediments [36].

Natural loose sand, clay, and soil particles make up riverine sediment, which is a complex mixture of organic and inorganic elements, mostly silicates, carbonates, sulphur, and minerals. Via the processes of precipitation, ion exchange, adsorption, hydrolysis, and chelation, these substances are left behind at the bottom of the water body. Sediment is a well-known sensitive indicator of environmental and geochemical contaminations that can be used to detect secondary sources of heavy metal pollution in river water. The mineralogical compositions, organic matter contents, and textural properties of the sediment sequence may highlight the best natural archives of recent environmental changes as the major carrier and final storage site for dangerous chemical contaminants in the aquatic ecosystem [37].

### 3.5. Bacterial colony forming unit

Fig. 5 shows the mean colony forming unit (CFU) distribution at different sampling stations during pre- and post-monsoon season. Analysis of Variance (ANOVA) showed significant differences of CFU ( $p < 0.05$ ) between sampling stations. During pre-monsoon season, the highest mean of CFU was recorded at Station 19 with  $16146.67 \pm 225.71 \text{ CFU/g}$ . The lowest mean was recorded at Station 14 with  $1526.67 \pm 64.68 \text{ CFU/g}$ . Meanwhile, the highest mean of CFU was recorded for the post-monsoon season at Station 12 with  $28826.67 \pm 418.47 \text{ CFU/g}$ . The lowest mean was recorded at Station 2 with  $1013.33 \pm 38.51 \text{ CFU/g}$ . Analysis of Variance (ANOVA) showed significant differences of CFU ( $p < 0.05$ ) between sampling seasons. According [38], they mentioned that bacterial abundance generally increased in downstream direction with slow flow rates and have high level of nutrients in the water. Fig. 5 shows the number of bacteria increases in downstream direction especially at middle stations. Apart from that, in this study, the distribution of bacteria do not influence by the concentrations of heavy metals. It might be influence by other factors such as physicochemical parameters, sediments size and nutrient contents in the river.

### 3.6. Correlation between heavy metals and bacterial colony forming unit

The relationship between arsenic, cobalt, copper and zinc concentration with bacterial colony forming unit were

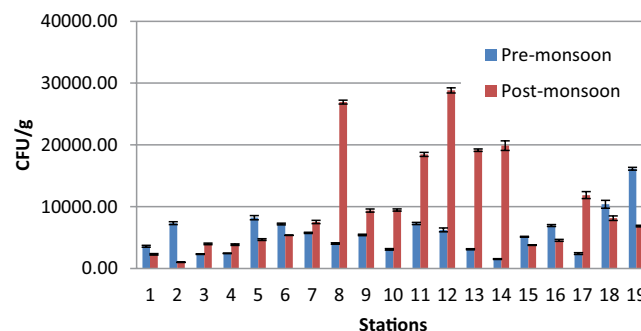


Fig. 5. Bacterial colony forming unit at different monsoon season.

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

Heavy metals	Bacterial colony forming unit
As	0.105
Co	0.023
Cu	0.043
Zn	0.063

analyzed by Pearson's correlation coefficient. Correlation results are presented in Table 3. Pearson correlation analysis revealed that there were no significant correlations found between arsenic, cobalt, copper and zinc with bacterial colony forming unit ( $p > 0.05$ ). This study is agreeing with the other researchers' findings. Ecological risk assessment of contaminants would gain accuracy from further research on the relative contribution of tolerance acquisition and co-tolerance processes on the functional response of microbial communities. They investigated that sediments contaminated with higher level of copper and zinc did not significantly affect the diversity of microbial communities [39,40].

#### 4. Conclusion

The current study evaluated the levels of heavy metal contamination, their ecological dangers, and their concentrations in sediments from the Pahang River. A significant level of possible ecological risk was present due to the moderate amounts of multi-heavy metal contamination that were observed due to its different environment fresh water and estuarine region as well. Investigations were performed into the combined effects of multi-heavy metal pollution on microbial community structure and ecological processes. In the conditions of an estuary environment, we discovered novel relationships between sedimentary microbial populations and several heavy metals.

It is anticipated that the distribution of microbial-metal resistance may be more strongly influenced by the characteristics of the microbial community and the sedimentary environment. In order to draw attention to the importance of carefully considering the dynamic influence and interacting mechanisms between various heavy metals and microbial populations in future studies and assessments, we pay emphasis to this in our conclusion. While trying to sort through the complexity of microbial ecotoxicology, this should be done in tandem with the main environmental elements. This will improve our fundamental understanding of the interactions between metals, biota, and environments and provide a stronger foundation for risk assessment and ecosystem management.

The concentration of arsenic (As), cobalt (Co), copper (Cu) and zinc (Zn) at most station along Pahang River showed lower concentration compared to the world average shale value. It can be said that the condition of Pahang River is still conducive, and the concentration of heavy metals do not influence the bacterial distribution. The concentrations of heavy metals studied are generally below reported

toxic levels. However, a continuous monitoring of river ecosystem is needed since industrialization and urbanization level increased to protect this ecosystem and to ensure the heavy metal pollution is controlled.

Nevertheless, the long-term research findings indicated that the ecosystem may suffer negative long-term biological effects. The newly arranged baseline data may prove to be a valuable resource for the precise assessment of heavy metal and bacterial contamination status under various environmental conditions, which is required for the preservation of aquatic systems and potential river basin restoration or rehabilitation.

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