



**SPATIAL AND TEMPORAL DISTRIBUTION OF  
BACTERIAL COMMUNITY AND HEAVY METALS  
COMPOSITION OF SEDIMENTS ALONG PAHANG  
RIVER**

**BY**

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## ABSTRACT

Rapid expanding of industrialization and urbanization along the Pahang River requires continuous monitoring in order to know the current status and to protect this ecosystem. This study aimed to determine spatial and temporal distribution of bacterial community, physicochemical parameters, sediment grain size characteristics and heavy metals composition in Pahang River's sediments. The bacterial community in sediments along the Pahang River was determined using culture-based method. The bacterial colony forming unit (CFU) range was found between  $1527 \pm 65$  and  $16147 \pm 226$  CFU/g during pre-monsoon while  $1013 \pm 39$  and  $28827 \pm 418$  CFU/g during post-monsoon. The identified bacteria genera in the river sediments were *Bacillus*, *Enterobacter*, *Escherichia*, *Serratia*, *Aeromonas*, *Pseudomonas* and *Staphylococcus*. The physicochemical parameters were determined using HYDROLAB multiprobes DataSonde® 5. The temperature was found between  $24.671 \pm 0.516$  and  $31.171 \pm 0.314$  °C, pH ranged between  $6.639 \pm 0.040$  to  $9.042 \pm 0.028$ , salinity ranged between 0.000 to  $30.172 \pm 0.271$  ppt, dissolved oxygen (DO) ranged between  $1.698 \pm 0.035$  to  $9.383 \pm 0.508$  mg L<sup>-1</sup> and the depth ranged between  $0.352 \pm 0.156$  to  $973 \pm 3.009$  m. The sediment grain size consists of coarse or very coarse sand at all stations. The heavy metal concentrations were determined by using Inductively Coupled-Plasma Mass Spectrometry (ICP-MS). The dry weight concentration of arsenic ranged between  $4.387 \pm 0.586$  to  $15.922 \pm 8.738$  µg g<sup>-1</sup> during pre-monsoon while  $3.989 \pm 0.758$  to  $11.336 \pm 6.748$  µg g<sup>-1</sup> during post-monsoon, cobalt was found between  $1.730 \pm 0.318$  and  $4.569 \pm 0.586$  µg g<sup>-1</sup> during pre-monsoon while  $1.476 \pm 0.063$  and  $4.620 \pm 0.951$  µg g<sup>-1</sup> during post-monsoon, copper ranged between  $1.182 \pm 0.510$  to  $10.722 \pm 5.664$  µg g<sup>-1</sup> during pre-monsoon and  $0.900 \pm 0.222$  to  $6.514 \pm 3.749$  µg g<sup>-1</sup> during post-monsoon, zinc was found between  $7.964 \pm 4.857$  and  $26.289 \pm 2.636$  µg g<sup>-1</sup> during pre-monsoon while  $8.187 \pm 3.010$  and  $28.347 \pm 15.665$  µg g<sup>-1</sup> during post-monsoon, chromium from  $3.250 \pm 2.065$  to  $21.950 \pm 6.904$  µg g<sup>-1</sup> during pre-monsoon while  $4.719 \pm 2.811$  to  $20.664 \pm 3.548$  µg g<sup>-1</sup> during post-monsoon, cadmium was found between  $0.019 \pm 0.008$  and  $0.116 \pm 0.045$  µg g<sup>-1</sup> during pre-monsoon while  $0.024 \pm 0.017$  and  $0.403 \pm 0.053$  µg g<sup>-1</sup> during post-monsoon, lead ranged between  $8.024 \pm 3.542$  to  $20.660 \pm 3.126$  µg g<sup>-1</sup> during pre-monsoon and  $8.696 \pm 3.433$  to  $17.887 \pm 2.537$  µg g<sup>-1</sup> during post-monsoon, nickel was found between  $3.113 \pm 0.825$  and  $10.137 \pm 9.626$  µg g<sup>-1</sup> during pre-monsoon while  $3.284 \pm 1.221$  and  $8.073 \pm 4.519$  µg g<sup>-1</sup> during post-monsoon, aluminium ranged between  $42388.889 \pm 8940.610$  to  $93588.889 \pm 10347.938$  µg g<sup>-1</sup> during pre-monsoon and  $50846.667 \pm 13703.594$  to  $84091.111 \pm 34682.522$  µg g<sup>-1</sup> during post-monsoon. There were significant difference of bacterial CFU and all physicochemical parameters among sampling stations and seasons. For heavy metals composition, there were significant differences for all studied heavy metals between sampling stations while for sampling seasons, the differences were only found in arsenic, cadmium, cobalt and aluminium concentrations. The correlation results showed that the bacterial community distribution was influenced by the temperature, pH, DO, salinity and cadmium concentrations while the other parameters showed no significant correlation towards bacterial community distribution. As conclusion, the present study has provided the current condition of Pahang River to facilitate in the management and conservation of the river ecosystem in the future.

## خلاصة البحث

التوسع السريع في التصنيع والتنمية على طول ضفاف نهر باهانج يتطلب مراقبة مستمرة لمعرفة الوضع الحالي ولحماية هذا النظام البيئي. هدفت هذه الدراسة إلى تحديد التوزيع المكاني والزمني للأحياء الدقيقة البكتيرية، العوامل الفيزيائية وخصائص وحجم حبيبات الرواسب وأنواع المعادن الثقيلة في رواسب نهر باهانج. تم تحديد المستعمرات البكتيرية في الرواسب على طول نهر باهانج باستخدام الطريقة القائمة على التزريع. تم العثور على مجموعة وحدات تشكيل المستعمرات البكتيرية (CFU) بين  $65 \pm 1527$  و  $226 \pm 16147$  جرام/CFU خلال الفترة التي تسبق الرياح الموسمية في حين  $39 \pm 1013$  و  $418 \pm 28827$  جرام/CFU خلال مرحلة ما بعد الرياح الموسمية. وكانت أجناس البكتيريا التي تم تحديدها في رواسب النهر تتراوح من جنس العصوية، الأمعائية، الإشريكية، السراتية، الإيرومونات، الزائفة والمكورات العنقودية. تم تحديد الملزمات والعوامل الفيزيائية باستخدام multiprobes HYDROLAB DataSonde® 5. تم التعرف على درجة حرارة تتراوح بين  $0.516 \pm 24.671$  و  $0.314 \pm 31.171$  درجة مئوية، ودرجة الحموضة بين  $6.639 \pm 0.040$  إلى  $9.042 \pm 0.028$ ، وتراوحت نسبة الملوحة بين صفر (00.00) إلى  $0.271 \pm 30.172$  جزء في الألف، وتراوحت نسبة الأوكسجين المذاب (DO) بين  $1.698 \pm 0.035$  إلى  $9.383 \pm 0.508$  ملجم/لتر. وتراوح العمق بين  $0.352 \pm 0.156$  إلى  $973 \pm 3.009$  م. وجد أن حبيبات الرواسب تتكون من الرمل الخشن أو خشن جدًا في جميع المحطات. تم تحديد تراكيز المعادن الثقيلة باستخدام جهاز مطياف البلازما بالحث الكتلي (ICP-MS). تراوح تركيز الوزن الجاف للزرنخ بين  $4.387 \pm 0.586$  و  $8.738 \pm 15.922$  ميكروجرام/جرام خلال الفترة التي تسبق الرياح الموسمية في حين كانت ما بين  $3.989 \pm 0.758$  إلى  $11.336 \pm 6.748$  ميكروجرام/جرام خلال مرحلة ما بعد الرياح الموسمية. ووجد أن عنصر الكوبالت تراوحت نسبته بين  $1.730 \pm 0.318$  إلى  $4.569 \pm 0.586$  ميكروجرام/جرام خلال الفترة التي تسبق الرياح الموسمية في حين كانت بين  $1.476 \pm 0.063$  و  $4.620 \pm 0.951$  ميكروجرام/جرام خلال مرحلة ما بعد الرياح الموسمية. أما النحاس فتراوحت نسبة تركيزه بين  $1.182 \pm 0.510$  و  $10.722 \pm 5.664$  ميكروجرام/جرام خلال الفترة التي تسبق الرياح الموسمية و  $0.222 \pm 0.900$  إلى  $6.514 \pm 3.749$  ميكروجرام/جرام بعد الموسمية. أما الزنك فكان تركيزه بين  $7.964 \pm 4.857$  خلال مرحلة ما بعد الرياح الموسمية و  $26.289 \pm 2.636$  ميكروجرام/جرام خلال المرحلة تسبق الرياح الموسمية في حين  $3.010 \pm 8.187$  و  $15.665 \pm 28.347$  ميكروجرام/جرام خلال مرحلة ما بعد الرياح الموسمية. والكروم من  $3.250 \pm 2.065$  إلى  $21.950 \pm 6.904$  ميكروجرام/جرام خلال المدة التي تسبق الرياح الموسمية في حين وجد تركيزه ما بين  $4.719 \pm 2.811$  و  $20.664 \pm 3.548$  ميكروجرام/جرام. وايضا تم العثور على عنصر الكاديوم الذي وجد تركيزه خلال مرحلة ما قبل الرياح الموسمية بين  $0.019 \pm 0.008$  و  $0.116 \pm 0.045$  ميكروجرام/جرام في حين وجد أنه  $0.024 \pm 0.017$  و  $0.403 \pm 0.053$  ميكروجرام/جرام خلال مرحلة ما بعد الرياح الموسمية. وتراوحت نسبة تركيز الرصاص قبل الرياح الموسمية بين  $8.024 \pm 3.542$  إلى  $20.660 \pm 3.126$  ميكروجرام/جرام وكانت  $8.696 \pm 3.433$  إلى  $17.887 \pm 2.537$  ميكروجرام/جرام خلال مرحلة ما بعد الرياح الموسمية. تم العثور على النيكل بين  $3.113 \pm 0.825$  و  $10.137 \pm 9.626$  ميكروجرام/جرام خلال المرحلة التي تسبق الرياح الموسمية في حين  $3.284 \pm 1.221$  و  $8.073 \pm 4.519$  ميكروجرام/جرام خلال مرحلة ما بعد الموسمية، وتراوحت نسبة وجود الألومنيوم بين  $42388.889 \pm 8940.610$  و حتى  $93588.889 \pm 10347.938$  ميكروجرام/جرام خلال الفترة التي تسبق الرياح الموسمية و  $50846.667 \pm 13703.594$  حتى  $84091.111 \pm 34682.522$  ميكروجرام/جرام خلال مرحلة ما بعد الموسمية. كان هناك اختلاف كبير في (CFU) البكتيرية وجميع العوامل الفيزيائية بين محطات أخذ العينات والمواسم. وفيما يخص المعادن الثقيلة، كانت هناك فروق ذات دلالة إحصائية لجميع المعادن الثقيلة التي شملتها الدراسة بين محطات أخذ العينات. أما في مواسم أخذ العينات كانت الاختلافات موجودة فقط في تراكيز الزرنخ والكاديوم والألومنيوم. أظهرت دراسة النتائج أن توزيع المستعمرات البكتيرية مرتبط ويتأثر بدرجة الحرارة، ودرجة الحموضة، DO، والملوحة وتركيزات الكاديوم، في حين أظهرت غيرها من المعالم عدم وجود ارتباط كبير نحو توزيع المجتمعات البكتيرية. وفي الختام، يمكن القول أن هذه الدراسة قد قدمت صورة جلية للوضع الحالي لنهر باهانج وهذا يؤدي لتسهيل إدارة وحفظ النظام البيئي الطبيعي لهذا النهر في المستقبل.

## APPROVAL PAGE

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# CHAPTER ONE

## INTRODUCTION

### 1.1 BACKGROUND OF THE STUDY

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

Running waters act as natural integrators of surficial within their drainage basins processes, including many anthropogenic activities and transport large amount of nutrients and organic matter. Rivers are able to change the quantity and quality of the material transported to the ocean through transformation, consumption and production mediating these ecosystem processes as they catalyse the flux energy and matter. They are also important along the riverine continuum as a linkage of riverine metazoan food web (Pusch et al., 1998). The abundance and activity of bacterial communities, not only influenced by physicochemical properties, but also due to other environmental factors such as availability of organic matter, nutrients (Sinsabaugh and Foreman, 2001) and other xenobiotic compounds from terrestrial inputs.

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 (Güven and Akinci, 2011; Zahir et al., 2012). Other sources of heavy metal contamination are urban runoff,

industrial effluents and wastes, sewage treatment plants, boating activities, domestic garbage dumps and agricultural fungicide runoff (Kamaruzzaman et al., 2010a). Usually, industries dump the waste that contains the heavy metals into the rivers and stream to reduce the cost of disposal. Due to this, the pollution needs to be monitored and controlled.

Sediments of aquatic system are sources of organic and inorganic material and known as the place where the majority of decomposition process takes place (Liu et al., 2011). Sediments have high physical-chemical stability. Their characteristics commonly represent the average condition of the system and often the representative of average water quality (Zahir et al., 2012). Moreover, sediments can act as a scavenger agent for heavy metal and an adsorptive sink in the aquatic environment. Therefore, sediment can be considered as an appropriate indicator for pollution of heavy metal (Kamaruzzaman et al., 2011a).

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 (Kemp and Aller, 2004; Kostanjsek et al., 2005). Thus, sediment is a complex habitat occupied by various groups of microorganisms, which play an important role in aquatic food webs, biogeochemical cycling, decomposition process and remobilization of heavy metals (Ye et al., 2009; Haller et al., 2011).

Hydrolab system for determination of physicochemical conditions complemented by molecular biological analysis, such as Polymerase Chain Reaction (PCR) and deoxyribonucleic acid (DNA) sequencing can be powerful tools to study the bacterial distribution and the influence of environmental factors towards its changes.

Currently, the comprehensive data on the distribution of bacterial community, physicochemical parameters, sediment characteristics and heavy metal composition in



the Pahang River is not available. Therefore, the aim of this study was to provide information on the distribution of the bacterial community, physicochemical parameters, sediment characteristics and heavy metals composition along the Pahang River.

## **1.2 STATEMENT OF THE PROBLEM**

In recent years, the pollution of the river has become the main concern throughout the world. There are various ways pollutants enter into the river which, either from point or non-point sources including wastewater from agricultural, municipal and industrial activities. Moreover, climatic condition, especially heavy rainfall as well as human activities in the form of exploitation of natural resources and developments are always the external factors which affect and increase the river dynamic process which lead to river degradation (Jackson et al., 1995). Since sediments of aquatic system are the place where the majority of decomposition process takes place and source of organic and inorganic materials, they have been considered as a suitable site to study the effects of pollution on ecosystems. The changing of physicochemical parameters will also influence the ecosystem of the river. Thus, it is important to monitor the current status of physicochemical parameters in order to ensure the sustainability of the river ecosystem.

## **1.3 RESEARCH OBJECTIVES**

1. To determine the physicochemical parameters of Pahang River.
2. To determine the characteristics of sediment, including mean size, sorting and skewness in Pahang River.
3. To determine the heavy metals composition in Pahang River sediments.

4. To isolate and identify bacterial-strains from sediments and determine the bacterial distribution along the Pahang River.
5. To determine the correlation of physicochemical parameters, sediment size and heavy metals composition towards bacterial community distribution in sediment along the Pahang River.

#### **1.4 RESEARCH QUESTIONS**

1. How bacterial community distribution in sediment changes from upstream to downstream along the Pahang River?
2. What is the status of physicochemical parameters, sediments size and heavy metals compositions in Pahang River and how they influence the bacterial community distribution?

#### **1.5 RESEARCH HYPOTHESES**

1. The physicochemical parameters, sediment characteristics, heavy metals compositions and bacterial community distribution are different from upstream to downstream of Pahang River.
2. The physicochemical parameters, sediments size and heavy metals composition influence the distribution of bacterial community in sediments from upstream to downstream of Pahang River.

#### **1.6 SIGNIFICANCE OF THE STUDY**

Pahang River with 439 km in length is the longest river in Peninsular Malaysia and an important inland aquatic biodiversity resource in Peninsular Malaysia. However, the continuous threat from anthropological activities such as deforestation for the expansion of urbanization and industrialization degrades the quality of the water thus

influences its biodiversity richness. Therefore, this study was conducted to provide information on the distribution of the bacterial community, grain size characteristics, heavy metals composition and the physicochemical parameters along the Pahang River. This study also intended to determine the influence of physicochemical, grain size and heavy metals composition towards bacterial community distribution. At the end of the study, better understanding on how the physicochemical, grain size and heavy metals composition affect the distribution of the microbial community can be deduced for the best river management.

## **CHAPTER TWO**

### **LITERATURE REVIEW**

#### **2.1 PAHANG RIVER**

Pahang River basin is located in the eastern part of Peninsular Malaysia. The location of the river is between latitude N 2° 48' 45" and N 3° 40' 24" and between longitude E 101° 16' 31" and E 103° 29' 34". The Pahang River is the longest river in Peninsular Malaysia with the length about 439 km and drains an area of 29300 km<sup>2</sup>, which 75 % (27000 km<sup>2</sup>) lies within Pahang and others lies in Negeri Sembilan (Muhammad, 2007).

In addition, Pahang River originates at Kuala Tembeling as consequences of the confluence of Jelai and Tembeling rivers which are the main tributaries of Pahang River. The Jelai River originates from the Central Mountain Range while the Tembeling River originates from the Besar Mountain Range. The other tributaries of this river are Semantan River, Triang River, Bera River and Lepar River and the main reservoir are Chini Lake, Bera Lake and Sultan Abu Bakar Dam of Tenaga Nasional Berhad (TNB).

The Pahang River system begins to flow in the direction of southeast and south from the north, through or passing along a number of major towns such as Kuala Lipis, Jerantut and Temerloh, then finally turning eastward at Mengkarak in the central south flowing through Pekan town near the coast before being discharged into the South China Sea (Aminuddin et al., 2012).

The climate of the Pahang River Basin is mainly controlled by the North East and South West Monsoons. The North East Monsoon occurs from October to January while the South West Monsoon occurs between March and September. The North East Monsoon season is mainly responsible for the heavy rainfall, which can cause

flooding in Pahang state (Ashenafi, 2010). The transition period between these monsoons is known as the inter-monsoon period, which the basin experiences the low amount of rainfall. Annual rainfall of the Pahang River Basin is ranged from 1609.00 mm (Temerloh) to 2132.36 mm (Lubuk Paku) (Pan and Wang, 2011).

## **2.2 BACTERIAL COMMUNITY IN SEDIMENT OF RIVER**

Microbial communities represent the major biodiversity on earth and catalyze important process to sustain life on earth (Van der Gucht et al., 2007). According to Martiny et al. (2006), there are four hypotheses about the microbial composition and diversity in the ecosystem: (i) The composition and diversity of microbial is randomly distributed throughout the space, (ii) Differences in the microbial composition and diversity simply reflects the influence of variations of the existing environment, (iii) The differences in the microbial composition and diversity simply because of the lasting effects of the past ecological and evolution events and (iv) The differences in the microbial composition and diversity is similar to macroorganisms, reflecting the influences of both the past events and contemporary environmental variations. In contrast to our knowledge of freshwater microbiology, the ecological impact of attached and planktonic bacteria in the river ecosystems are poorly understood (Araya et al., 2003).

The function of microorganisms in aquatic ecosystem is well recognized. In the aquatic ecosystems, populations of bacteria play an important role in the demineralization and transformation of nutrients to maintain energy instability (Araya et al., 2003). Apart from that, microorganisms in river sediments are responsible in biogeochemical process such as carbon and nitrogen cycling in all water bodies. They are also major contributors to water quality control process and are critical to the process of removal and degradation of pollutants that are introduced into the

environment (Eggleton and Thomas, 2004; Greer, 2010; Huang et al., 2011). Moreover, microorganisms also considered as the main primary producer as well as the secondary producers and consumers in the river ecosystem (Al-Sayed et al., 2005).

In freshwater sediments, the dominant microbial community in that area are Betaproteobacteria and Gammaproteobacteria, in contrast to the enrichment of marine sediments that dominated by Alphaproteobacteria (Greer, 2010). In addition, Anderson-Glenna et al. (2008) also mentioned that some of the bacteria frequently encountered in the study of freshwater ecosystem, including Proteobacteria, Cyanobacteria, Bacterioidetes, Verrucomicrobia, Planctomycetes and Actinobacteria.

Apart from that, anthropogenic activities will influence the distribution of bacteria. A study on the sediment of the main rivers of the Scheldt drainage network, which are Lys River, Dendre River, Nethe River, Dyle River, Zenne River, Rupel River and Scheldt River found that there are abundant of faecal indicator bacteria which are *Escherichia coli*. The Scheldt watershed is characterized by a high population density, intense industrial activities and intensive agriculture and breeding (Ouattara et al., 2011).

Agbabiaka and Oyeyiola (2012) stated that there are numerous bacteria were isolated from the soil, sediment samples of Foma River, Nigeria, which is characterized by agricultural and other human activities at the nearby area. The Foma River is a good source of domestic water for some neighbouring villages and for recreational activities. The bacteria present include *Corynebacterium pseudotuberculosis*, *Corynebacterium renale*, *Corynebacterium kutscheri*, *Corynebacterium diphtheriae*, *Escherichia coli*, *Bacillus megaterium*, *Bacillus pasteurii*, *Bacillus subtilis*, *Bacillus licheniformis*, *Bacillus sphaericus*, *Bacillus cereus*, *Enterobacter aerogenes*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Micrococcus lutues*, *Micrococcus varians*, *Acinetobacter*

*calcoaceticus*, *Pseudomonas aeruginosa*, *Pseudomonas putida*, *Aeromonas hydrophila*, *Aeromonas punctata*, *Salmonella enteritidis*, *Streptococcus faecalis*, *Proteus vulgaris*, *Proteus mirabilis* and *Erwinia amylovora*. The presence of these potential bacterial pathogens in the sediments of the river was due to the anthropogenic activities.

In addition, according to Barua (2007), Coeur d'Alene River is also known as CDAR located in northern Idaho. It is one of the metal contaminated rivers in United State (US) due to mining since the late 1800's. The sediments of this river are enriched with various heavy metals such as arsenic, copper, zinc, antimony, manganese, mercury, iron, silver, lead and cadmium which are toxic and able to give hazardous effects to animals and humans. Since microorganisms living in the river sediment may remove the metals and detoxify the environment, he has done a study of this contaminated river in aiming to investigate the microbial communities existing in CDAR sediment. The major representative genera found were *Thiobacillus*, *Azoarcus*, *Acidobacterium*, *Burkholderia*, *Flavobacterium* and *Janthinobacterium*. There are thirteen different bacterial classes found in CDAR sediment which are *Proteobacteria* ( $\alpha$ -,  $\beta$ -,  $\delta$ - and  $\gamma$ -), *Acidobacteria*, *Actinobacteria*, *Flavobacteria*, *Bacteroidetes*, *Chloroflexi*, *Sphingobacteria*, *Chlorobia*, *Cyanobacteria* and *Clostridia*.

Another study that related to metal contamination is done by Hug et al. (2013) on the sediment core samples at the Rifle Integrated Field Research Challenge (IFRC, US) in July 2007, from within a 6m to 7m thick aquifer adjacent to the Colorado River. They showed that *Proteobacteria* (23 %) and *Chloroflexi* (14 %) represented a significant fraction of the most abundant bacterial phylum in the sediment. The Rifle IFRC is a uranium-contaminated aquifer with groundwater flow into the Colorado River. A study conducted by Miura et al. (2009) on sediment of Takagi River showed that *Bacteroides* spp. and fecal coliforms was detected. This might due to the presence

of the bay where oyster beds are placed at this river. Therefore, various anthropogenic activities will lead to the variation of bacterial community distribution in the river sediments. The soil sediments as well as the water of the river need to be treated in order to make it potable and safe for both domestic and recreational activities.

### **2.3 IDENTIFICATION OF BACTERIA USING MOLECULAR MICROBIAL TECHNIQUES**

In this study, we take the advantages of molecular approaches such as Polymerase Chain Reaction (PCR), Restriction Fragment Length Polymorphism (RFLP), bacterial deoxyribonucleic acid (DNA) extraction and sequencing to determine the diversity of bacterial communities along the Pahang River.

The introduction of molecular techniques, especially PCR has made it possible to obtain information on the composition of microbial communities. It is a molecular biology technique that allows a small amount of DNA to be amplified exponentially (Newton and Graham, 1997). An environmental sample can be inventoried for taxa with the direct isolation of nucleic acids, followed by specific marker gene amplification and sequence analysis of base pairs. The most commonly used marker gene in the identification of bacteria is *16S rRNA*.

In addition, RFLP is a method in which organisms will be differentiated based on the determination of patterns derived from the cleavage of their DNA. The distance of the cleavage site or known as recognition sequence is different between species. Therefore, the bands profile obtained will be different between organisms. The current application of the molecular techniques in a variety of habitats has created a large set of the sequences from this gene. The growing of the database has taught us that diversity of bacteria is larger and under estimate before the presence of molecular techniques (Zwart et al., 2002).