Faculty of Resource Science and Technology

DISTRIBUTION OF HEAVY METALS, HYDROCARBONS AND HYDROCARBON-DEGRADING BACTERIA IN FRESHWATER SEDIMENTS

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by

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DECLARATION

I hereby declare that no portion of this work referred to in dissertation has been submitted in support of an application for another degree or qualification to this university or any other institution of higher learning.

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LIST OF ABBREVIATIONS

µm	Micrometer
mL	Milliliter
mm	Millimeter
cm	Centimeter
sp.	Species
GC	gas chromatography
AAS	atomic absorption spectrophotometer
PAHs	polycyclic aromatic hydrocarbons
DCM	Dichloromethane
TEL	total extractable lipids
TAHs	total aliphatic hydrocarbons
SBM	saline basal medium
NA	nutrient agar
EDTA	ethylenediaminetetraacetic acid

ABSTRACT

Freshwater sediments from the Batang Ai hydroelectric Dam at Lubok Antu, Sri Aman were analyzed for heavy metals, hydrocarbons and hydrocarbon-degrading bacteria content. A total of 7 sampling sites within vicinity of Batang Ai Hydroelectric Reservoir were selected for investigation. The heavy metals content in sediment was analysed using atomic absorption spectrophotometer (AAS), while the aliphatic and aromatic hydrocarbons in sediments were analyzed by gas chromatography-flame ionization detector (GC-FID). Furthermore, distribution of hydrocarbon-degrading bacteria in sediments was analyzed using molecular biological technique. Heavy metal content in sediments show varying concentrations of nickel (Ni), copper (Cu), manganese (Mn), zinc (Zn), chromium (Cr), argentum (Ag), stannum (Sn) and bismuth (Bi). The highest concentration of Ni, Cu, Mn, Zn and Cr were detected at St2, while the highest concentration of Ag and Sn wrere detected at St1 and St4, respectively. Bismuth (Bi) was only detected at St5 and St6 with concentration 4.79 and 5.28 μg/g, respectively. Lead (Pb), cadmium (Cd) and arsenic (As) was not detected in all sampling station. The aliphatic hydrocarbons were ranged from 2 - 421145 ng/g dry weights. St1 and St2 indicate the higher content of aliphatic hydrocarbons, while St3 showed the lowest *n*-alkanes content. The total PAHs concentration of sediments in Batang Ai Dam ranged 2 - 5958 ng/g dry weights. The PAH composition pattern in sediments of Batang Ai suggest dominance by medium to high molecular weight compounds, and the ratio of certain related PAHs indicate anthropogenic sources and natural processes. The highest number of bacterial colonies was detected at St7 with 9.9×10^5 CFU/ml. The number of bacterial colonies in sediment is higher near the jetty than other sites of Batang Ai Hydroelectric Dam. Other sediments show moderate bacterial number with the lowest bacteria count was at St3 (1.4×10^1 CFU/ml).

Keywords: heavy metals, aliphatic hydrocarbons, aromatic hydrocarbons, hydrocarbon-degrading bacteria, sediments

ABSTRAK

Sedimen air tawar dari empangan hidroelektrik Batang Ai di Lubok Antu, Sri Aman telah dianalisis untuk logam berat, hidrokarbon dan kandungan bakteria pengurai hidrokarbon. Sebanyak 7 lokasi pensampelan di lingkungan empangan hidroelektrik Batang Ai telah dipilih untuk kajian. Kandungan logam berat dalam sedimen telah dianalisis dengan menggunakan spektrofotometer serapan atom (SSA), sementara hidrokarbon alifatik dan aromatik dalam sedimen yang diperolehi dianalisis dengan kromatografi gas pengesan pengionan nyala (KG-PPN). Selain itu, taburan bakterai pengurai hidrokarbon dalam sedimen telah dianalisis menggunakan teknik biologi molekul. Kandungan logam berat dalam sedimen menunjukkan pelbagai kepekatan nikel (Ni), tembaga (Cu), mangan (Mn), zink (Zn), kromium (Cr), perak (Ag), timah (Sn) dan bismut (Bi). Kepekatan tertinggi Ni, Cu, Mn, Zn dan Cr didapati daripada St2, manakala kepekatan tertinggi Ag dan Sn telah dikesan pada St1 dan st4, masing-masingnya. Bi hanya boleh dikesan di St5 dan St6 dengan kepekatan 4.79 dan 5.28 μg/g, masing-masingnya. Plumbum (Pb), kadmium (Cd) dan arsenik (As) tidak dijumpai di semua lokasi pensampelan. Kepekatan hidrokarbon alifatik adalah dalam julat 2 - 421145 ng/g berat kering. St1 dan St2 menunjukkan jumlah hidrokarbon alifatik tinggi, manakala St3 menunjukkan jumlah hidrokarbon alifatik yang terendah. Kepekatan hidrokarbon aromatic polisiklik (HAP) dalam sedimen adalah dalam julat 2 - 5958 ng/g berat kering. Corak komposisi HAP dalam sedimen Batang Ai didominasi oleh sebatian berat molekul yang tinggi dan sederhana, dan nisbah HAP tertentu menunjukkan sumber antropogenik dan proses semulajadi. Bilangan tertinggi koloni bakteria dikesan di St7 dengan 9.9×10^5 CFU/ml. Bilangan koloni bakteria berhampiran jeti lebih tinggi berbanding lokasi lain di empangan hidroelektrik Batang Ai. Sedimen lain menunjukkan bilangan bakteria sederhana dengan hitungan bakteria terendah di St3 (1.4×10^1 CFU/ml).

Kata kunci: logam berat, hidrokarbon alifatik, hidrokarbon aromatic polisiklik (HAP), bakteria pengurai hidrokarbon, sedimen

CHAPTER 1

INTRODUCTION

1.1 General Introduction

Sediments refer to the depositional site of mineral and organic particles that are transported from the catchment area, as well as particles that forms and settles from within the water body (Lopez and Lluch, 2000). Sediments efficiently sequester hydrophobic chemical pollutants that inflowing water bodies such as lakes (Harikumar *et al*., 2009). During the formation and absorption process of sediments in freshwater lakes, it accumulates various compounds such as heavy metals, hydrocarbons and hydrocarbon-degrading bacteria. Therefore, sediments can be good indicators to monitor level of contaminants and pollution in aquatic environment (Harikumar *et al*., 2009).

Heavy metals and organic pollutants normally accumulate in the sediments linked with organic particles, clay surfaces, sulphides and iron manganese hydroxides (Leivuori *et al.*, 2000). Heavy metals refer to metals of relatively high density, or of high relative atomic weight. Examples of heavy metals are cadmium, copper, iron, manganese, lead, zinc, and nickel. Moreover, some of heavy metals are known to be dangerous to health and to the environment.

Hydrocarbons refer to compounds that consists only the elements carbon and hydrogen. Hydrocarbons are divided into two types: aliphatic hydrocarbons and aromatic hydrocarbons (Loudon, 2002). Due to the tendency of aliphatic and polycyclic aromatic hydrocarbons to accumulate in sediments, they are considered to be ubiquitous sedimentary contamination. The

aliphatic hydrocarbons can be divided into three hydrocarbon families: alkanes, alkenes and alkynes (Loudon, 2002). Aromatic hydrocarbons that consist of two or more fused rings are known as polycyclic aromatic hydrocarbons (Loudon, 2002). Polycyclic aromatic hydrocarbons (PAHs) known to be highly mutagenic and carcinogenic. PAHs are derived from incomplete combustion of organic material and known as environmental pollutants (Landvik *et al.*, 2006). Example of PAHs is naphthalene which is the simplest of the polycyclic aromatic hydrocarbon (Loudon, 2002). The pathway of aliphatic hydrocarbon and polycyclic aromatic hydrocarbon in the freshwater environment is usually linked to anthropogenic sources.

The ability to catalyze the degradation of hydrocarbons is exhibited by wide variety of bacterial and fungal genera (Leahy and Colwell, 1990). This microorganism is known as hydrocarbon-degrading bacteria. Hydrocarbon-degrading bacteria are widely distributed in freshwater lake and marine environments and normally degrade numerous contaminating petroleum hydrocarbons and cleansing the ocean of oil pollutants (Sutiknowati, 2007). Thus, hydrocarbon-degrading bacteria plays a significant role in accelerate the pollution degradation in aquatic ecosystem.

In the study, the surface sediments were collected from freshwater lake (Batang Ai hydroelectric dam). Batang Ai hydroelectric dam is located at Lubuk Antu, of Sri Aman Division and built to generate hydroelectric power for Sarawak.

1.2 Objectives of the Project

The objectives of this project are:

a. to determine the distribution of heavy metals and hydrocarbons (aliphatic and aromatic) in freshwater sediments from Batang Ai Hydroelectric Dam,
b. to evaluate the distribution of the hydrocarbon-degrading bacteria in freshwater sediments,
c. to assess environmental status of Batang Ai Lake based on concentration of heavy metals, hydrocarbons and hydrocarbon-degrading bacteria in sediments.

CHAPTER 2

LITERATURE REVIEW

2.1 Heavy metals in sediments

Heavy metals are usually accumulated and incorporated in the sediments, hence added to a body of natural water (Harikumar *et al.*, 2009). Heavy metals are one of natural components of the environment which are mainly occurring in the bedrock and soil. Heavy metals enter pristine lakes from various sources, especially by the weathering of bedrock in catchments and consequently transported to the lakes as one of the important components of the accumulating material as dissolved or particulated form through runoff, and as wind-blown soil dust particles (Lepane *et al.*, 2007). Heavy metals is distinguishable from other toxic pollutants with its main properties which is they are not biodegradable. The concentration of heavy metal in water column can be relatively low compare to the concentrations of heavy metal in the core sediment which may be elevated (Kabarssi *et al.*, 2005). The occurrence of elevated levels of heavy metals mainly originate in the sediments can be a sensitive indicator for monitoring contaminants and man induced pollution and high levels of heavy metals can frequently be attributed to anthropogenic sources, rather than natural enrichment of sediment by geological weathering (Kabarssi *et al.*, 2005).

2.2 Hydrocarbons in sediments

One of significant parts of the land-derived organic inputs towards coastal regions is the hydrocarbons (Wu *et al*., 2001). Hydrocarbons which are known as sedimentary aliphatic hydrocarbons (AHCs) have both natural (including biogenic and petrogenic) and anthropogenic sources (Peng X. *et al.*, 2008). n-Alkanes are originate mainly from a large range of organisms. The assemblage of n-alkanes in both aquatic biota and in the surface waxes of higher plants is differentiated (Wu *et al*., 2001). Polycyclic aromatic hydrocarbons (PAHs) have carcinogenic properties and normally known as group of environmental pollutants derived mainly from all sorts of incomplete combustion and consequently may be considered to be ubiquitous (Jacob, 1996). This is especially true for many urban coastal areas where there are high anthropogenic sources and various environmental pollutants sources are presence (Wang *et al*., 2006).

2.3 Hydrocarbon-degrading bacteria in sediments

Hydrocarbons in the environment are mainly biodegraded by the bacteria and fungi (Leahy and Colwell, 1990). These microorganisms rely on nutrients for survival and these nutrients will be used to synthesize enzymes for the degradation of hydrocarbons (Hamzah *et al.*, 2010). Bacteria are normally considered to symbolize the predominant hydrocarbon degrading element of microbial community especially in the marine environment (Leahy and Colwell, 1990). In some environments, bacterial attack can be crucial process and specific strains have been investigated and identified to determine their abilities to degrade particular hydrocarbons (Cormack and Fraile, 1997). Hydrocarbon-degrading bacteria that are capable of utilizing n-

alkanes of chain length C_{10}-C_{40} as a sole source of carbon is *Acinetobacter sp.* (Throne-Holst *et al*., 2007).

2.4 Importance of sediments studies

Sediments provide useful information about the events that occurred in the past period in the lakes and its catchments area. The history of sediment reflects the contamination history of an area (Harikumar *et al*., 2009). The study of the chemical composition of sediments from lake helps researchers to understand many processes occurring within the total lake system including its groundwater drainage basin (Lopez and Lluch, 2000). Sediments can be good indicators for monitoring contaminants in aquatic environments (Harikumar *et al.*, 2009). Many researchers have used sediments to study the contamination caused by hydrocarbon (Wang *et al.*, 2006; Peng *et al*., 2008). There were also researchers that used sediment to study the pollution history of aquatic ecosystem (Karbassi *et al.*, 2005; Lopez and Lluch, 2000; Mohamed, 2005).

CHAPTER 3

MATERIALS AND METHODS

3.1 Study Area and Sample Collection

The study area is Batang Ai Hydroelectric Dam at Lubok Antu District of Sri Aman Division, Sarawak. This area is a man-made freshwater lake. A total of seven **(**7) sampling sites were selected for sediments collection as shown in Figure 3.1. The description of each sampling sites is presented in Table 3.1

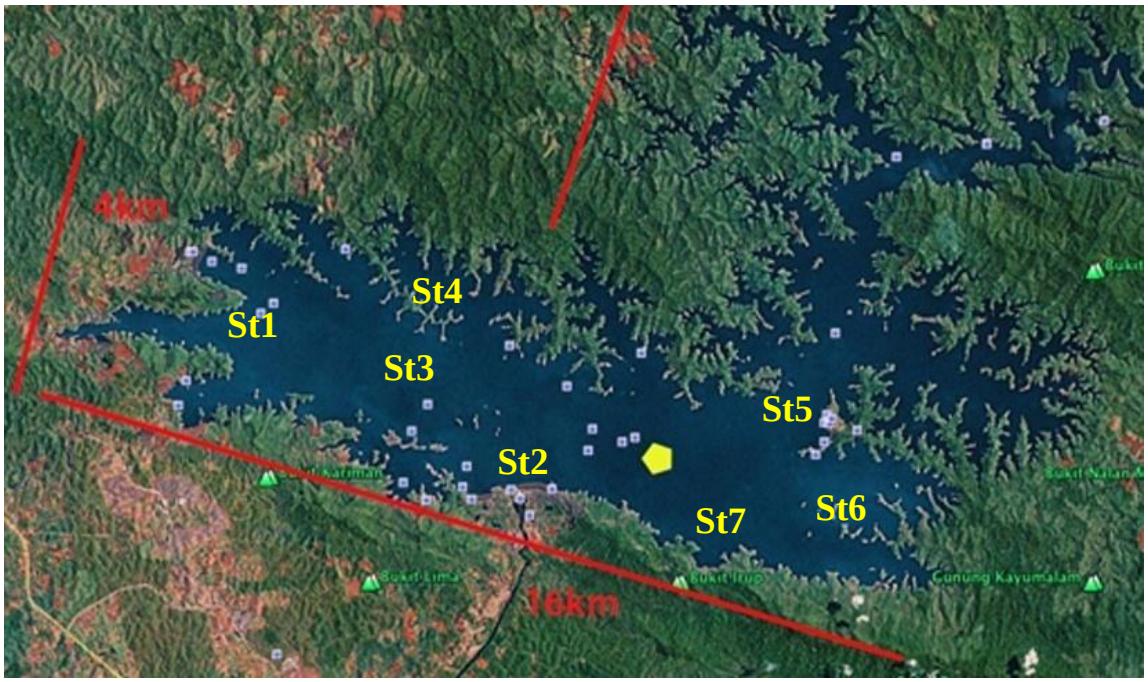

Figure 3.1: Location of sampling sites at Batang Ai Hydroelectric Dam at Lubok Antu District

Table 3.1: Description of each sampling sites

Sampling sites	Description
ST1	Adjacent to natural forest
ST2	Near the overflow of the Dam
ST3	Middle of the Lake
ST4	Jetty of Hilton Resort
ST5	Near the flow of river
ST6	Near the agricultural areas (rubber tree plantation)
ST7	Near the main jetty of the lake

The sediment samples were collected using a stainless steel grab sampler. The surface sediments for heavy metals analysis were placed into plastic bag. The samples were brought to the laboratory using plastic bag and placed in the cooler box for transportation. Whereas, the surface sediment samples for hydrocarbon analysis were wrapped with aluminum foil and placed in the cooler box for transportation. Upon arriving UNIMAS, the samples were stored frozen (-20 ^{o}C) until analysis in the laboratory. Sediment samples were dried and the dried sediments were sieve (125 µm) to remove large particles and debris before extraction (Wang *et al.*, 2006). For microbiological analysis, to avoid any possible contamination, stringent measures against contamination were maintained throughout the sample collection and microbial isolation. As soon as the sediment was brought on board, the samples were placed into a sterile plastic bag, stored frozen (-4^{o}C) and transported to the laboratory for analysis.

3.2 Heavy Metal Analysis

The sediment sample preparation was carried out according to procedure described by Binning and Baird (2001). The sediments were dried in petri-dishes at room temperature and then ground into a powder. Approximately 0.5 g of each sample was weighed and placed into the volumetric flask. Then, 20 ml Aqua Regia (1:3 $cHNO_{3:}$ cHCl) was added into the sample. The mixture was placed on hot plate and heated to near dryness for two hours. The volumetric flask was covered by beaker to avoid the evaporation. Then, the mixture was allowed to cool before 5 ml HNO_3 solution and 20 ml of deionized water were added. The mixture was again placed on hot plate and heated to near dryness for one hour. The samples were cooled and then were filtered through filter paper. The filtrates were transferred to a 100 ml volumetric flask and made up to the mark with deionized water.

The metal determinations of the solution were performed on AAS by using the calibration curve method. Concentrations of the following metals were determined: Cu, Mn, Ni, Cd, Pb, Zn, Cr, Ag, As, Bi, Sn and Ba. Model of AAS used was Thermo SCIENTIFIC iCE 3000 SERIES.

The concentration of respective heavy metals standard used during calibration analysis is shown in Table 3.2.