UNIVERSITI PUTRA MALAYSIA

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By

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master Science

STEROL BIOMARKERS AND CHEMOMETRIC TECHNIQUES FOR TESTING SEWAGE CONTAMINATION IN SEDIMENT OF LANGAT RIVER, MALAYSIA

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July 2012

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Sewage contamination that origin from multiple sources such as human, animals and domestic sources has been recognised as one of the major cause of deterioration in water quality especially in Selangor state. For many years, the Langat River has been experiencing anthropogenic inputs that possibly originates from more than three million inhabitants surrounding in the river basin. Sterols, one of the chemical biomarkers has been successfully applied as a sewage biomarker as it remains in the environment longer than microbial indicators and can be used to discriminate different sources of faecal pollution in sediments.

In this study, we analysed the distribution of sterols in surface sediments of Langat River and evaluate the level of sewage pollution by using diagnostic ratios (biomarkers) and chemometric techniques. Sediment samples were collected from 22 stations along Langat River then extracted and analysed for sterols by using GC-MS instrument. Six different sterols were identified and quantified with total sterol concentrations ranging between 11.50 to 618.25 ng g^{-1} dry weight. The highest total sterol concentration was found at Sg. Balak (SL02) at 618.25 ng g^{-1} dry weight). This is a tributary of Langat River that is densely populated with residential and industrialised areas to identify the sources of sewage. Three types of diagnostic ratios were used; coprostanol/cholesterol, coprostanol/(coprostanol+ cholestanol) and epicoprostanol/coprostanol. From the results of the majority of the diagnostic ratios, it was found that sewage contamination was occurring in some of the sampling sites, the other sampling sites had low to moderate sewage pollution levels. The chemometric techniques led to a better understanding of the data in term of source of pollution. With the use of statistical analysis, the sterols compounds which act as different biomarkers were able to be separated and thus, different sources of sewage origin could be revealed. This is the first report on sewage pollution that is based on the combination of biomarkers and chemometric techniques that can established a new approach for sewage detection using faecal sterol and stanol.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PENANDA BIOLOGI STEROL DAN TEKNIK KIMOMETRIK SEBAGAI PENGESAN PENCEMARAN KUMBAHAN DALAM SEDIMEN DI SUNGAI LANGAT, MALAYSIA

Oleh

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Pencemaran kumbahan yang berasal dari pelbagai sumber seperti manusia, haiwan dan sumber dalam negeri telah dikenalpasti sebagai salah satu punca utama kemerosotan kualiti air yang dialami terutamanya di negeri Selangor. Selama bertahun-tahun, Sungai Langat telah mengalami input antropogenik yang mungkin berasal dari lebih daripada tiga juta penduduk sekitar di Lembah Sungai Langat. Sterol, salah satu penanda bio kimia telah berjaya digunakan sebagai penanda bio kumbahan yang kekal dalam persekitaran lebih lama daripada penunjuk mikrobiologi. Sterol boleh digunakan sebagai bahan untuk membezakan dan mendiskriminasi sumber pencemaran najis yang berbeza di dalam sedimen.

Dalam kajian ini, taburan sterol dalam sedimen permukaan Sungai Langat telah dianalisa dan mengaitkannya dengan tahap pencemaran kumbahan dengan menggunakan nisbah diagnostic (penanda biologi) dan teknik kimometrik agar mendapat pemahaman yang lebih baik daripada data yang diperolehi. Sampel sedimen yang dikumpul daripada 22 stesen yang dipilih di sepanjang Sungai Langat dikumpul, diekstrak dan dianalisa dengan menggunakan instrumen GC-MS. Terdapat enam sterol yang berbeza yang telah dikenal pasti dan dikira dengan kepekatan antara 11.49 hingga 446.48 ng g^{-1} berat kering. Kepekatan sterol tertinggi ditemui dalam SL02 (618.29 ng g^{-1} berat kering) yang dikesan di Sg. Balak, anak sungai Sungai Langat yang padat dengan penduduk dan kawasan perindustrian yang mengelilingi lokasi pensampelan. Tiga jenis nisbah diagnostik digunakan sepanjang kajian ini; i) koprostanol / kolesterol, ii) koprostanol / (koprostanol + kolestanol), iii) epikoprostanol / koprostanol untuk mengenal pasti sumber-sumber kumbahan dalam sampel yang diambil. Daripada majoriti keputusan nisbah diagnostik, ia jelas menunjukkan bahawa pencemaran kumbahan berlaku di beberapa lokasi pensampelan, manakala kebanyakan titik persampelan yang lain telah mengalami tahap pencemaran kumbahan rendah ke sederhana. Analisis menggunakan teknik kimometrik telah membawa kepada pemahaman yang lebih baik terhadap data yang diperolehi kerana ia dikemukakan dengan menggunakan dua rajah dimensi. Dengan gabungan menggunakan analisis statistik, sebatian sterol yang bertindak sebagai penanda bio yang berbeza dapat diasingkan dan dengan itu, sumber-sumber berbeza yang berasal dari kumbahan boleh diketahui. Ini merupakan laporan pertama mengenai pencemaran kumbahan yang berdasarkan kombinasi penanda biologi dan teknik kimometrik yang akan mewujudkan

satu pendekatan baru untuk mengesan pencemaran kumbahan menggunakan sterol kumbahan.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Master Science Environmental Quality and Conservations. The members of the Supervisory Committee were as follows:

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DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any degree at Universiti Putra Malaysia or at any other institution.

NUR HAZIRAH ADNAN

Date: 30 July 2012

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CHAPTER ONE

INTRODUCTION

1.1 General Introduction

Environmental problems that are associated with deterioration of water quality will become the world's major problem in the coming years (Pratt, 2005). Deterioration of water quality is not only considered for drinking water supply, but also relates to recreational and aquaculture activities. Sewage discharge into the aquatic system is one of the major contributors to the deterioration of water bodies. This is due to the organic or nutrient content (nitrogen and phosphorus) of the sewage itself as well as specific contaminants that are associated with the discharge (Mudge and Duce, 2005).

Water pollution mainly comprised of sources from domestic sewage, manufacturing industries, domestic effluent and animal farms (Department of Environment, 2009). The total numbers of polluted river basin in Malaysia increases from 7 river basins in 2007 to 13 river basins in 2010 including Langat River and the problem was strongly related with human activities such as industrial, agricultural and construction activities (Conpendium of Environment Statistics, 2011).

Due to the increasing population which Malaysia is experiencing, the local sewage treatment plants (STPs) have come under enormous pressure and more often than not the primary sewage is released into the aquatic environment (Poon *et al*., 2000; Eganhouse and Sherblom, 2001; Seguel *et al*., 2001). Serious environmental problems that occur such as sewage contamination will result in the spread of most of the waterborne diseases that are wide-ranging in these regions (Isobe *et al*., 2002). Water supply and sanitation-related diseases comprise of hepatitis, diarrheal, cholera and typhoid. The waterborne diseases in Malaysia that were reported in 2009 comprised of cases such as 40 hepatitis, 276 cases of cholera and 303 cases of typhoid fever that are associated with poor drinking water quality and inadequate sanitation (Conpendium of Environment Statistics 2011)

Domestic sewage (with or without treatment) that is discharged indiscriminately into the aquatic environment will affect water quality and the geochemical composition of the sediments (Sandro *et al*., 2010). Raw sewage has high levels of bacteria and viruses that come from human pathogens. Nutrients and pathogens will accumulate in sediments up to certain concentrations as they are not easily dispersed, and in the end they will affect marine communities (Coelho *et al*., 1999). Sediments can act as a sink for pollutants as well as contaminant source which can be detrimental to aquatic organisms living in or using bottom substrates (Gagne *et al*., 2001; Bachtiar *et al*., 1996).

Microbiological markers such as faecal coliform bacteria have been widely used as indicators of sewage contamination especially in surface water. These bacteria are very common to both human and other animals. Differentiating the sources of bacteria that

exist in surface waters is important because the potential to transmit human disease is greater if the source of the bacteria is human sanitary waste. Traditional microbiological assay in monitoring sewage contamination experiences serious downfall and shortcomings that it has resulted in the urge for researchers to seek another more specific indicator of sewage pollution. One of the promising methods that has more reliability in tracing sewage inputs that has been suggested by some researchers (Hatcher and McGillivary, 1979; Venkatesan and Mirsadeghi, 1992; Writer *et al*., 1995) is the use of faecal sterols which are the metabolites of cholesterol that are very useful for this purpose.

In sewage impact studies, the most cited sterols are coprostanol and epicoprostanol which are normally known as faecal sterols. They are not naturally exists in aquatic sediments. Coprostanol is the major human sewage biomarker that is produced in the digestive tract of higher mammals through the process of microbial reduction of cholesterol (Isobe *et al*., 2002). Together with structurally closely related sterols, a number of studies have shown that sterol compounds have the ability to discriminate between sources of faecal contamination from humans, agriculture, animals and so on (Leeming *et al*., 1996). Sterols have also been shown to have the ability to discriminate between algal and terrestrial sources which is important in the distribution and mixing of marine versus freshwater environments (Jaffe *et al*., 2001). Due to its characteristics that are unaffected by high temperature and various treatments such as chlorination, sterols present a large number of other advantages that make them suitable for sewage detection within water or sediment samples.

Many of the faecal sterol study done by researchers have focused within the area with temperate and cool environment such as the United Kingdom and United States (Hatcher and McGillivary, 1979; McCalley *et al*., 1981). However, there are a few studies that have concentrated on faecal study within the tropical climate. Therefore, in this study, we have examined the approach of sterols as biomarker in sediments from tropical climate, which is Malaysia, more specifically in Langat River, to detect the sewage impact in this area. Although some studies have assessed and described the environmental quality of Langat River (Azrina *et al*., 2006; Juahir *et al*., 2009), data on sterols are scarce. Thus, to elucidate the recent status of sewage contamination, an effort has been made in this study to focus on faecal sterols in sediments from selected areas of Langat River.

1.2 Significance of study

Within Peninsular Malaysia, especially in the state of Selangor, the problems of river pollution which are related to the changes of land use throughout the years have become a major concern. The principal and prominent river in Selangor which is Langat River is heavily populated with human population and industrial areas and it has become an important water supply to half of the population in Selangor in spite of it being used as a source of hydropower and control of flood discharges (Juahir, 2009). Rosnani (2001) said that Malaysian rivers are polluted with sewage disposal that is discharged from small to large scale industrial activities due to the inefficiency of treatment facilities which are without proper effluent treatment system. Furthermore, the existing wastewater treatment facilities are unable to process the increasing number of domestic

sewage throughout the year. Due to the concern of environmental problems that occur in Malaysia, especially Selangor, this study was conducted in the selected areas along Langat River to provide information on the sanitary condition for this current status which can be further investigate to improve the river water quality in Malaysia.

1.3 Research aim and objectives

The aim of this research is basically to provide the current status of our sanitary condition in Langat River. The study will investigate about sewage sources that contribute to the conditions of Langat River by using two approaches with are diagnostic ratios and chemometric techniques. This research has three main objectives which are:

1. To investigate the distribution of faecal sterols and stanols in sediments along Langat River, Selangor.
2. To determine the level of sewage pollution along Langat River based on selected sewage diagnostic index.
3. To determine the sources of sewage pollution in Langat River using combination of chemometric techniques and diagnostic ratios.

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