TITLE: APPLICATION OF FECAL STEROLS AND STANOLS TO INVESTIGATE SEWAGE POLLUTION IN SEDIMENTS OF LANGAT RIVER

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Introduction

The Langat River basin has seen rapid developments in industrialization , urbanization and dramatic population increases during the past two decades (Alireza *et al.*, 2009). In urban areas, due to high rates of growth and population densities, the individual impact of poor sanitation and water related diseases as well as the overall impact on society is even greater.(Isobe, 2004).

Sewage pollution in Langat River is a severe health risk to people that live near rivers and waterways. Direct discharge of domestic waste, leaching from poorly maintained septic tanks and improper management of farm waste are suspected as the major sources of waterborne diseases.(Hunttly, 1990)

Coliform bacteria have been used as the indicator of sewage pollution for several decades. However, the methods suffers from some major constraints such as rapid biodegradation in marine environment and consequently fluctuations in their number (Mudge *et al*., 1999). In addition, fecal contamination from municipal sewage discharge cannot be unambiguously differentiated from those resulting from other animal feces only by coliform bacteria (Leeming *et al*.,1996).

One of the method that may have significant advantages is the use of chemical markers such as fecal sterols (Isobe et al.,2002). This organic molecular markers are organic compounds that maintain sufficient structural integrity for their sources to be recognized (Egenhouse, 1997).Coprostanol, mainly coming from human feces (comprising 40-60% of the total fecal sterols excreted in human waste) has been applied successfully due to its source specificity and environmental stability (Grimalt *et al*.,1990, Takada *et al*.,1994, Leeming *et al*.,1999, Maldonado et al.,2000, Isobe *et al*., 2002).

Coprostanol produced from hydrogenation of cholesterol by bacteria in the digestive system. Sterols in human feces show distribution profile considerably different from those in animals' excretion and plant sterols. Therefore, fingerprinting the fecal sterols together with other structurally related sterols can provide particularly useful information for source identification of fecal environmental pollution (Leeming *et al.*,1996, Isobe *et a*l.,2002).

When fecal matter is introduced to aquatic environment , lipophilic fecal sterols such as coprostanol are strongly bound to particulate matter (Hatcher and

McGillivary,1979). Although fecal sterols degrade under aerobic conditions in the water column in 1-2 weeks (Kirchmer ,1971) , the degradation of fecal sterols is very limited once they are incorporated into the sediments (Bartlett, 1987).

Problem statement

This study will investigate about sewage pollution in sediment. This investigation will be conducted in Langat river. The present study, will be concerned with the following research questions.

1. Does Langat river polluted with sewage pollution?
2. What are the sources that contribute to the sewage pollution in Langat river?
3. Does Langat river have a good treatment of sewage produces in this recent time?
4. Which part of Langat River that most polluted with fecal?

Significance of study

1. Observation about the water quality in Langat river in this recent times.
2. Determination and investigation of sterols distribution in Langat River will be an important information for the authority to take action on how to preserve and protect the river from been polluted of sewage.

Research objectives

1. To determine level of sewage pollution in Langat River
2. To investigate distribution of sterols and stanols in Langat River.

Literature review

1. Brown, R.C. and Wade, T.L. (1984) Sedimentary coprostanol and hydrocarbon distribution adjacent to a sewage outfall. *Water Research* **18**, 621-632
    - human feces contain a high concentration of coprostanol.
2. Barlett, P.D. (1987) Degradation of coprostanol in an experimental system. *Water Research* **18**, 27-29
    - fecal stanols are suitable molecular marker for sewage dispersion in coastal waters where the relatively shallow waters and slow current does not allow a long residual time for the sewage particles in the water column. Once incorporated into anoxic sediments, these stanols are expected to persist.
3. Mudge, S.M. and Norris, C.E. (1997) Lipid biomrkers in the Conwy Estuary (North Wales,UK):a comparison between fatty alcohols and sterols. *Marine Chemistry* **57**, 61-84
    - sterols are better preserved in sedimentary environment than many other biological products such as carbohydrates and amino acids.

4. Xianzhi, P. et al (2005) Tracing anthropogenic contamination in the Pearl River estuarine and marine environment of South China Sea using sterols and other organic molecular marker. *Marine Pollution Buletin* **50**, 856-865
    - fingerprinting the fecal sterols together with other structurally related sterols can provide particularly useful information for source identification of fecal environmental pollution.

Research methodology

Reflux

About 30-40g of sediment samples will be hydrolyzed using 50 ml of 6% potassium hydroxide (KOH) in methanol. The samples will be reflux for 4 hours. After reflux, the product will be centrifuged at 4000 rpm for 3 minutes to separate the sediment from liquid. The supernatant layer will be taken and transfer to the separating funnel for the liquid-liquid extraction process. While the bottom layer will be discarded.

Liquid-liquid extraction

The supernatant layer will be added with 20ml of hexane and 10 ml of distilled water. The mixture have to shake vigorously until two layers form which are consists of polar and non-polar layers. The non-polar layer will be taken and transfer to a flask. All of the procedure will be repeated to ensure that the extraction process are maximum. Sample will be concentrated using rotary evaporator at 40ºC. Sample will be diluted with 2-3ml of hexane and transfer to 14ml vial. Anhydrous sodium sulphate will be added to remove water that still left in the sample. While transferring to vial, the samples need to be filtered using filter paper. The vial have to blow down using nitrogen gas until dryness for the derivatization process.

Derivatization

Samples need to go through derivatization process to improve the chromatography of sterols. 2-3 drops of bis-(trimethylsilyl)triflouroasetamida (BSTFA) will be added to the sample and be heated on heater block for 10 minutes at 60ºC. The BSTFA will replaced hydrogen atom with trimethylsilyl that are more volatile and stable for analysis of GCMS. Sample will be blow down with nitrogen gas again until dryness before been diluted with 1ml of hexane and transfer to a 1.4ml vial. Sample then be stored in refrigerator at a temperature -20ºC before been analysed using GCMS.