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Baseline

Sterols as biomarkers in the surface microlayer of the estuarine areas

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ABSTRACT

This study aims to determine the concentration of sterols used as biomarkers in the surface microlayer (SML) in estuarine areas of the Selangor River, Malaysia. Samples were collected during different seasons through the use of a rotation drum. The analysis of sterols was performed using gas chromatography equipped with a flame ionisation detector (GC–FID). The results showed that the concentrations of total sterols in the SML ranged from 107.06 to 505.55 ng L^{-1}. The total sterol concentration was found to be higher in the wet season. Cholesterol was found to be the most abundant sterols component in the SML. The diagnostic ratios of sterols show the influence of natural sources and waste on the contribution of sterols in the SML. Further analysis, using principal component analysis (PCA), showed distinct inputs of sterols derived from human activity (40.58%), terrigenous and plant inputs (22.59%) as well as phytoplankton and marine inputs (17.35%).

Sterols are organic compounds, known as steroid alcohols, which are essential components of eukaryotic cells (ranging from microorganisms to macroalgae) and vascular plants. They are predominantly found in the organisms' fat storage areas (Puglisi et al., 2003). As a hydrophobic compound, sterols easily transfer from aqueous to organic phases in aquatic systems (Mudge and Duce, 2005; Harwood, 2014). As they have a longer residence time in the environment, sterols have been successfully applied as organic molecular markers to evaluate and distinguish various sources of sewage contamination (Eganhouse, 1997; Bull et al., 2002; Shah et al., 2007; Tyagi et al., 2009). Based on specific structures, sterols can provide useful information on source identification of faecal matter in the environment (Isobe et al., 2002; Mudge and Duce, 2005; Piah et al., 2008). Previous studies have reported that sterol compounds, such as coprostanol and epicoprostanol, can indicate the sources of anthropogenic activities (Venkatesan and Kaplan, 1990; Jeng and Han, 1994). Cholesterol and cholestanol are predominantly found to be the main sterols in marine systems due to their ubiquitous distribution in zooplankton and phytoplankton (Volkman, 2005; Loh et al., 2008). In addition, sterols such as β-sitosterol, stigmasterol and campesterol are commonly known as markers for algae, dinoflagellate, diatom, bacteria and vascular plants' input into surface water (Volkman et al., 1998; Peng et al., 2005).

Identification of pollution sources and their contribution to environment system is of great importance to obtain a clear overview of the pollutant profile. Principal component analysis (PCA) was recommended as an exploratory tool which would reveal unknown patterns in a large and complex dimension dataset (Wenning and Erickson, 1994; Smith, 2002). Based on the characteristics of both magnitude and variations in the sterol compounds, biogenic or anthropogenic sources can be identified (Mudge, 2007; Shlens, 2009). This technique has been applied by other researchers, such as by Saim et al. (2009), Adnan et al. (2012), Osman et al. (2012), Yao et al. (2013), Rushdi et al. (2014) and Zubir et al. (2014) for similar purposes.

The estuarine area is the place where pollution can occur rapidly due to the movement of pollutants from river system and sea water intrusion to the river during low and high tide respectively. The surface microlayer (SML) constitutes the uppermost tens to hundreds of micrometres (μm) of the river's/ocean's surface and it is this part which is in direct contact with the atmosphere. The amount of organic substances, such as sterols in the SML within an estuarine area can contribute to the quantity of organic substances in marine ecosystem. The objectives of this study are to

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determine the concentration and sources of sterols in the SML using diagnostic ratios and principal component analysis (PCA).

The Selangor River is located on the west coast of the Malaysian Peninsula and is one of the main sources of public water supplies in Malaysia. Industrial and agricultural activities, such as oil palm and rubber plantations can be found along the river banks. The estuarine area of the Selangor River has several remaining patches of upland forest, swamp forest and mangroves. Urbanisation processes and residential activity, as well as small industries, are expected to impact the water quality of the Selangor River (Ali et al., 2009; Alsalahi et al., 2014). For this study, samples of the SML were collected from 13 stations within the estuarine area of the Selangor River (Fig. 1). The stations represent different backgrounds, such that S1–S5 are located along the river and S6–S13 are located in the coastal areas facing mangrove areas.

The collection of the SML samples was conducted using a sampling method suggested by Harvey (1966). Samples were collected during both the wet (October, 2010) and dry (March, 2011) seasons around estuarine areas of the Selangor River. A thickness of approximately 50 μm of the SML was collected using a wiping device rotation drum (Stolle et al., 2010). The samples were then stored in an ice box prior to being transported to the laboratory. In the laboratory, the samples were filtered within 48 h using a glass microfibre GF/C filter paper with a 0.45 μm pore size and 47 mm diameter (Whatman, UK) and then stored in the fridge at −4 °C until further analysis.

The procedure of the sterol analysis in the SML was undertaken based on Peng et al. (2005), with some modifications for enhancement. Sterols from the SML samples were extracted using a 20 mL dichloromethane (DCM) with three replications using a Teflon separate funnel. These extracted samples were then combined and concentrated to a small volume. Pre-baked anhydrous sodium sulphate (Na_2SO_4) was used to remove excess water in the extracts. The Na_2SO_4 from the extract was removed by filtration using 1.6 μm glass-fibre filter paper (Whatman, UK). The extract was then concentrated to 1 mL using a rotary evaporator. About 5 mL n-hexane was added as a solvent to enhance the fractionation step of sterols in the chromatography column.

For the purification and fractionation of the organic extracts, a liquid chromatography column was used. This step was performed before the quantification of sterols. The short column (1 cm i.d., 25 cm height) was isolated from the extract in a glass column and filled with silica gel-alumina (5% water deactivated). First, the aliphatic hydrocarbon fraction was eluted using a 20 mL mixture of n-hexane and DCM (7:3). Next the sterol compounds were eluted with 20 mL of ethyl acetate (EtOAC). The sterol fraction was then concentrated to 2 mL using a rotary evaporator which was then followed by a gentle flux of purified N_2. The fraction comprising the sterols was treated with N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) (50 μL, 80 °C, 1 h) for the purpose of converting the sterols into their corresponding trimethylsilyl ethers. Finally, the samples were evaporated and kept in a desiccator at room temperature overnight before the determination of the sterols' composition.

In this study, a gas chromatography with flame ionisation detector (GC–FID) (Agilent Technology, 6890 N, USA) was used to quantify the sterols in all the samples. Fused silica capillary columns with different non-polarity were used: HT-5 (25 m × 0.22 mm i.d., film thickness 0.1 μm) and helium was used as a carrier gas at 200 kPa. This procedure was undertaken in accordance with gas chromatography (GC) operating conditions: maintaining the injection port at 250 °C, a detector temperature of 360 °C and injecting the 1 μL of the sample in the split/splitless mode followed by a 1 min purge after the injection. The column temperature was held at 80 °C for 1 min and then it was programmed at 15 °C/min to 300 °C, 5 °C/min to 350 °C and held for 15 min.

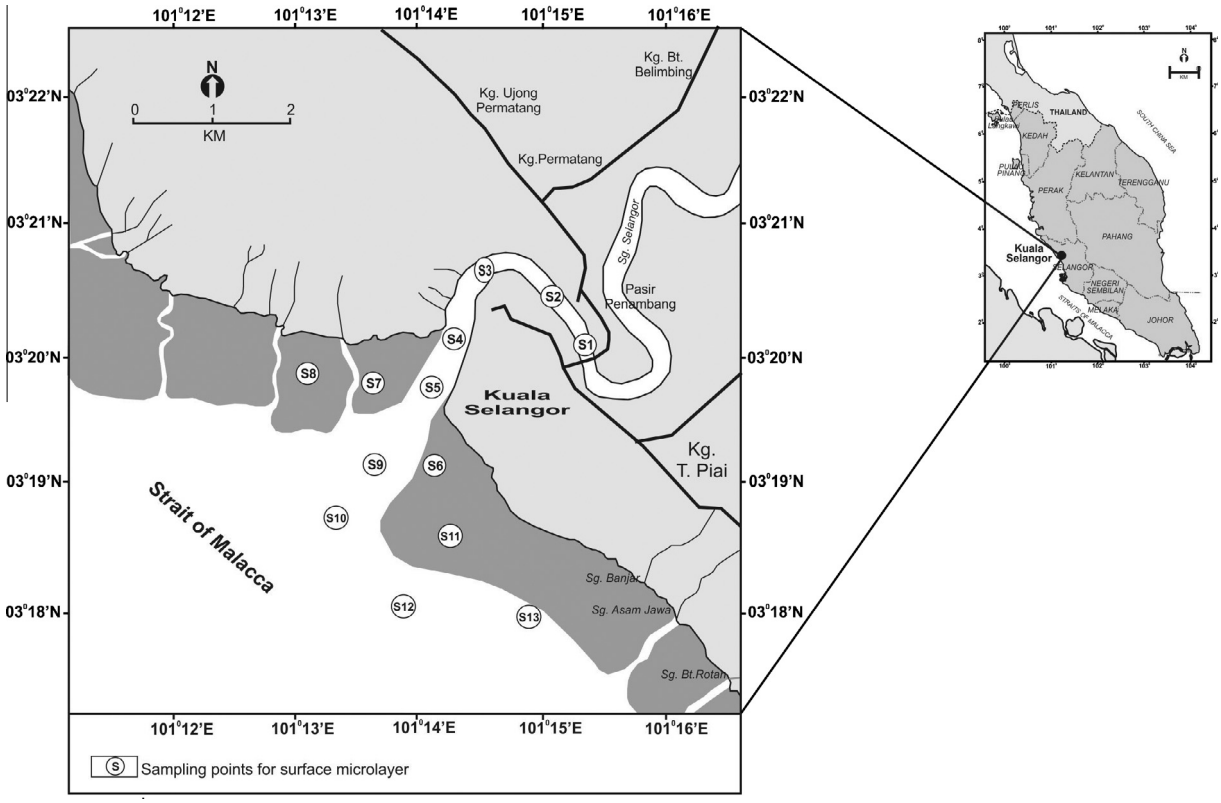

Fig. 1. Coordinates of 13 sampling stations for the surface microlayer in Kuala Selangor, Malaysia.