Contents lists available at ScienceDirect

Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul

Aliphatic hydrocarbons and triterpane biomarkers in mangrove oyster (*Crassostrea belcheri*) from the west coast of Peninsular Malaysia

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ARTICLE INFO

Keywords:
n-Alkane
Hopane
Petroleum
Pollution
Oyster
Malaysia

ABSTRACT

The Straits of Malacca is one of the world's busiest shipping routes where frequent oil spills occur. Rapid development in the west coast of Peninsular Malaysia is the other major source of petroleum pollution in this narrow waterway. In order to identify occurrence and origin of hydrocarbons in the Straits, mangrove oysters (*Crassostrea belcheri*) were collected from five sampling locations and analysed for *n*-alkanes and biomarkers. Soxhlet apparatus and two step column chromatography were used for extraction, purification and fractionation of the oysters. Petroleum origin *n*-alkanes were detected in majority of the sampling locations which is indicative of anthropogenic activities in this region. Using source and maturity diagnostic ratios for hopanes revealed used crankcase oil as the main source of petroleum hydrocarbons in oysters from all sampling locations except for the Pulau Merambong where signature of South East Asia crude oil (SEACO) was detected.

1. Introduction

Petroleum pollution has been a prominent problem since commercial drilling of oil in the late 19th century. Pollutants derived from petroleum and its products seem to be increasing in the future. In industrialized and urbanized areas with intense anthropogenic activities, petroleum pollution is more significant. Peninsular Malaysia's intense industrialization and urbanization in recent decades has led to high consumption of petroleum and its products. The western coast of Peninsular Malaysia has experienced higher levels of development than that of the eastern seaboard of the country. Moreover, the west coast lies directly to the Straits of Malacca which is a narrow waterway with intense oil tanker traffic. The Straits of Malacca is the shortest shipping route for transportation of oil tankers from Middle East and North Europe to Northeast Asian countries such as Japan, Korea and China. Furthermore, a huge number of merchant ships pass through the Straits turning it into one of the busiest shipping routes in the world. As a result, large and small accidental oil spills as well as spillage from ballast water and tanker washing discharges occur frequently (Thia-Eng et al., 2000). Consequently, the west coast of Peninsular Malaysia receives pollutants including those from petroleum and its products from both land and sea.

n-Alkanes are saturated hydrocarbons with C_nH_{2n+2} chemical formula consisting of hydrogen and carbon with the utmost number of bonds. *n*-Alkanes mainly have petroleum origin in the environment, however, natural sources also contribute to the occurrence of *n*-alkanes. The cuticle of land plants is covered by epicuticular wax which contains high amounts of high molecular weight (HMW) *n*-alkanes predominated by odd carbon atoms. However, short chain *n*-alkanes predominated by odd carbon atoms, especially C_{17} have cyanobacteria and algea origins (Eglinton and Eglinton, 2008). Moreover, odd numbered mid-chain *n*-alkanes including C_{21}, C_{23} and C_{25} are abundant in submerged and emersed vegetation (Ficken et al., 2000). Predominance of even carbon atom *n*-alkanes in the environment can signify petrogenic inputs from different anthropogenic activities (Sakari et al., 2008). Terrigenous/aquatic ratio (TAR) which is the ratio of ($C_{27} + C_{29} + C_{31}$) over ($C_{17} + C_{19} + C_{21}$) can demonstrate alternation in marine based and land based natural sources of *n*-alkanes. Average chain length (ACL) is indicative of the average number of carbon atoms in *n*-alkanes originating from land plant in a specific geographical area (Jeng, 2006). Different kinds of plants have different chain length of carbon atoms. Generally, chain length of carbon atoms generated by warm climate plants is longer (Poynter et al., 1989). The ACL values might decrease in the presence of petrogenic inputs. Carbon preference index (CPI) is used as a tool to describe the predominance of long chain *n*-alkanes with odd number carbon atoms over even number carbon atoms in a definite range of carbon chain. CPI draws a comparison between biogenic and petrogenic inputs. CPI of 5 to 10 is indicative of hydrocarbons coming from land plants which contain high loads of *n*-alkanes with 27, 29, and 31 carbon atoms (Rielley et al., 1991) while CPI of about 1 is a sign of petroleum as

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http://dx.doi.org/10.1016/j.marpolbul.2017.07.008
Received 27 March 2017; Received in revised form 11 May 2017; Accepted 3 July 2017

well as recycling organic material and/or marine organism origins of *n*-alkanes (Pendoley, 1992; Tanner et al., 2010). Isoprenoids (pristane and phytane) can be used to identify source of *n*-alkanes. Due to higher resistance of pristane and phytane to degradation, the ratios of C_{17}/pristane and C_{18}/phytane below one might indicate weathered oil inputs to the environment. The ratio of pristane/phytane below one can be indicative of petrogenic origins of *n*-alkanes since phytane is more predominant in petroleum origins, while biogenic sources have higher concentrations of pristane than phytane.

Hopanes are among pentacyclic triterpanes originating from the cell membrane of prokaryotes in marine ecosystem. Various types of hopanes function in bacterial cell membrane (Ourisson et al., 1987; Prince et al., 1994). Hopanes can be found in petroleum and its products such as lubricating oil, tires and asphalts. Hopanes are present in high boiling point products of petroleum and cannot be found in gasoline and diesel fuels (Lough et al., 2006). Different types of hopanes have various numbers of carbon, ring configurations and side chains. The 17α(H),21β(H)-, 17β(H),21β(H)- and 17β(H),21α(H)-hopanes are the major sterioisomeric forms of hopanes. Hopanes with 27 to 35 carbon numbers and αβ configuration are molecular markers of petroleum due to their higher thermodynamic stability. The ββ and βα configurations convert to αβ configuration during the high temperature process of diagenesis and catagenesis (Boitsov et al., 2011). However, ββ-isomers are commonly found in living organisms (Wang et al., 2006). Being produced naturally, hopanes are found in large quantities on earth. Hopanes are among the last products of biohopanoids in the process of diagenesis (Hedges and Prahl, 1993; Saito and Suzuki, 2011). Nevertheless, hopanes are not looked upon as pollutants in the environment. Hopanes can be applied as molecular markers for petroleum pollution as they are resistant to degradation, semivolatile and present in different quantities in petroleum of different origins and maturity (Huang et al., 2014). Hopanes were used as biomarkers to identify weathered oil origin 10 years after spillage (Wang et al., 1994). It was suggested that the interval between release and biodegradation of 17α,21β(H) C_{30} hopane (C_{30} 17α) in the environment outweighs the periods of time needed for environmental recovery from spillage of petroleum. As a result, C_{30} 17α can be referred to as an indication of contamination spread after an oil spill (Farias et al., 2008).

Domestic crude oil from Southeast Asian oil fields i.e., South East Asia crude oil (SEACO), has waxy nature and is abundant in pentacyclic triterpanes with higher plant origin. Previously, source diagnostic ratios besides maturity ratio of hopanes were applied to distinguish SEACO from Middle East crude oil (MECO) in Peninsular Malaysia (Zakaria et al., 2000; Zakaria et al., 2001). Both 17α,21β (H) C_{29} hopane (C_{29} 17α), C_{30} 17α and common 17α,21β(H), 22S and 22R homohopanes from C_{31} to C_{35} abound in MECO and SEACO, however, the ratios of C_{29}/C_{30} and C_{31}–C_{35}/C_{30} are lower in SEACO compared to MECO. This can be due to nonmarine (i.e., lacustrine and deltaic) shale source rock origin of SEACO, whereas MECO derives from marine carbonate source rocks (Murray et al., 1994). As a result, the ratios of C_{29}/C_{30} and ΣC_{31}–C_{35}/C_{30} are reliable tools in recognition SEACO and MECO origins of hydrocarbons (Volkman et al., 1997). In other words, MECO is recognized by abundance of C_{29} 17α over C_{30} 17α, while SEACO is signified by depletion of C_{31}–C_{35} homohopanes (Zakaria et al., 2000). Therefore, the aforementioned ratios which are both normalized by C_{30} 17α are fruitful tools for identification of petroleum origin. Various geological procedures the two crude oils go through can explain the differences in the ratios of C_{29}/C_{30} and ΣC_{31}–C_{35}/C_{30}. Ts (18α(H)-22,29,30-trisnorneohopane) and Tm (17α(H)-22,29,30-trisnorhopane) and their ratio is a sensitive tool used to investigate thermal maturity of various samples with the same origin (Wang et al., 2006).

Shellfish such as oysters are filter feeders and can absorb petroleum hydrocarbons either dissolved or sorbed to particulate matter from water column through partitioning while passing through the gills or inside digestive tract (Neff, 2002). In order to be absorbed by organisms, petroleum hydrocarbons need to desorb from the organic matter and partition to porewater and subsequently pass through organism membrane. Oysters are among benthos species with long larval stage, living in saltwater regimes (Avise, 1994). These organisms are widely distributed in tropical and subtropical regions between 64°N and 44°S. Mangrove oyster (*Crassostrea belcheri*) habitat is mangrove ecosystems located in coastal areas, therefore, petroleum pollutants entering in coastal areas can be available to the oysters. Once hydrocarbons enter aquatic environment, aquatic organisms are exposed to these compounds, therefore, they can be absorbed by the aquatic organisms. Suspended matters and re-suspended sediment can be swallowed by filter feeders such as oysters leading to bioaccumulation of hydrocarbons in their fat tissue.

Considering the strategic location of the Straits of Malacca and huge number of oil tankers passing through the Straits, identification of petroleum pollution origins is necessary. However, the data on the origin of petroleum pollution is scarce, especially in the narrower south part of the Straits. This research aims to apply aliphatic hydrocarbons and hopanes for identification of petroleum pollution occurrences and origins across the Straits of Malacca using mangrove oyster (*Crassostrea belcheri*) as a sentinel species.

2. Materials and methods

2.1. Sampling

Twenty five individual oysters were collected from five sampling locations along the west coast of Peninsular Malaysia in January–May 2013. Approximately, similar sizes of oysters were selected in the sampling locations including the Merbok, Prai, Klang, Muar Estuaries and Pulau Merambong (Fig. 1). Oysters were placed on ice and later stored at − 20 °C. For preparation of the oysters, they were first defrosted at room temperature overnight. Later, a NISSEI AM-7, (0 − 200) rpm × 100 homogenizer (Japan) was used for homogenization of the soft tissues of the oyster at 8000–10000 rpm and for 12 min. A freeze-drier was used to remove the moisture from the samples.

2.2. Analytical procedure

The digestion processes were conducted on oysters based on a method described by Perugini et al. (2007). Briefly, 10 ml of 1 M KOH ethanolic solution was added to the freeze-dried oyster samples and the samples were transferred to a reflux system at 80 °C for 3 h. Later the liquid phase was separated and extracted using 10 ml of cyclohexane in a separation funnel and under 30 min of severe shaking. The liquid layer was transferred to a separation funnel and rinsed with 10 ml of cyclohexane for another 2 times. The extract was successively passed through an anhydrous sodium sulphate column and a florisil column and a rotary evaporator was used to concentrate the extract after passing through each column. Purification and fractionation of the samples were performed based upon a method described previously (Vaezzadeh et al., 2015a; Zakaria et al., 2002). In brief, samples were soxhlet extracted using dichloromethane (DCM) as solvent. Later, the extracts were concentrated with the help of a rotary-evaporator and added into a glass column chromatography (1 cm i.d. × 9 cm) packed with 5% H_2O deactivated silica gel. A total of 20 ml hexane/DCM (3:1, v/v) was used as elution solvent to get the hydrocarbon fractions. The fractions were concentrated to near dryness via a rotary-evaporator and subsequently fractionated using a column chromatography (0.47 cm i.d. × 18 cm) packed with fully activated silica gel. About 4 ml of high purity hexane was added to elute aliphatic hydrocarbon and hopane