Petroleum Hydrocarbon in Mud Clam (*Polymesoda erosa*) and its Ambient Medium in the Coastal Area of Putatan and Tuaran, Sabah

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Abstract
Total petroleum hydrocarbon (TPH), total organic carbon (TOC) content and particle size were investigated in mud clam (*Polymedosa erosa*) and sediment in the coastal area of Putatan and Tuaran, Sabah. Mud clams and sediments were collected by hand-picking and trowel in five stations respectively for both coastal area. Mud clams and sediment samples were extracted for TPH using standard method APHA 5520 and analyzed using UV/VIS spectrophotometer. The determination of TOC content was based on United Nations Environment Programme (UNEP)/ MAP Athens (2006). The overall range of TPH concentrations in mud clam and sediments in both study areas were recorded at 0.72 – 2.16 mg/kg dw and 0.15 – 0.62 mg/kg dw Miri crude oil equivalents, respectively. The overall mean and range of TOC content from coastal area of Putatan and Tuaran were 9.30% (5.37 – 15.82%) and 7.98% (4.21 – 12.93%) respectively and sandy particles are majority in these two sites. In the meantime, the statistical analysis by Pearson correlation showed a moderately positive correlation with coefficient for TPH concentration in mud clam and sediment (r=0.6340 for Putatan; r=0.6978 for Tuaran), meanwhile showed a strong positive correlation for TPH concentration and TOC content in sediment (r=0.9637 for Putatan; r=0.9340 for Tuaran). Therefore, TOC can be used as the screening for TPH in sediment for both areas.

Introduction

Petroleum hydrocarbon contamination of the marine and coastal environment is a common issue, but the impact of the contamination is quite difficult to assess due to the fact that the survey data are scarce and fragmentary, especially across Southeast Asian countries (UNEP, 1988). The research about petroleum hydrocarbons has been improving since then. Crude oil and its refined products are subject to physical, chemical and biological processes in the environment, which can alter the composition and environmental impact. These important processes affect the behavior of hydrocarbon compounds in the environment and as an aid to predict the exposure to human being (USEPA, 2014). In general, hydrocarbon distributions can be varied qualitatively with the particle size within given sediment (Thompson & Eglinton, 1977). Finer particles are generally richer in adsorbed organic matter, for example hydrocarbons, due to the comparable weight basis as the surface area is larger (Boehm & Quinn, 1977; Kruitwagen *et al.*, 2008).

Polymedosa erosa also known as mud clam, is a class under Mollusca, where they are highly adaptive in diverse habitat (Leal, 2013). Mud clam, which is a filter feeder, depends on the organic particles and microorganisms like phytoplankton as their food source (Eyre & Ferguson, 2006; Maulana *et al*., 2010). Sediment size variation and selective uptake by the clam cannot explain the observed hydrocarbons of bivalves and sediment. This is because the strong affinity of hydrocarbons for solid surfaces, ingestion of whole sediment particles may not result in any transfer of hydrocarbons from the sediment to benthic organisms. But as suggested by Farrington & Quinn (1973), the selective uptake process by the clams could account for the observed hydrocarbon patterns with sediments (Boehm & Quinn, 1977).

Sediment and sand is a good indicator for petroleum hydrocarbons contamination (USEPA, 2000). The organic matter in the sediment often related with the accumulation of hydrocarbons. Hence, total organic carbon (TOC), which is a gross measure of all forms of organic carbon, including petroleum hydrocarbons and natural organic matter tested will support the reading and value of TPH more accurately (Schreier *et al*., 1999). Sediment particle size is tested on the surface sediment of the coastal area as the quantity of the pollutants and organic substances are often related to the texture of the sediment, as in high clay content are thought to have high TOC and TPH as well. This helps to improve and support the TPH reading as the general indicator of petroleum hydrocarbon contamination. Marine sediment also more stable than the overlying water, hence it is commonly used to monitor the environmental quality of the sea (Zhou *et al*., 2014).

This study is for the screening of the TPH concentrations in study sites and act as data collection to provide more data for future study as the data and research of petroleum hydrocarbon in Sabah region is scarce. It can provide, as well as improving and update the data for the research regarding petroleum hydrocarbons contamination that could be happening in Sabah, by taking sediment and Mollusca as the bio-accumulator of hydrocarbons. Since there are too many chemicals exist in crude oil and also other petroleum products, it is literally not practical and troublesome to measure each and every one separately. Therefore, by measuring the total amount of TPH at the site helps to provide an overview of the contaminants on the site (ATSDR, 1999). Mollusca have been well known for its ability to accumulate hydrocarbons as well as other pollutants. Therefore, many countries in the world have recognized Mollusca as the bio-indicator of petroleum hydrocarbons contamination.

This study focused on Putatan and Tuaran coastal area due to the interest to know whether the mud clam and its ambient are polluted or not with petroleum hydrocarbon due to the location is not far from the city center. Besides that, many points of discharge such as road runoff, urban and villages, industrial area, development activities and entrance of around several rivers are located at the study area. Those potential areas will discharge effluents, sewage, wastes and pollutants directly to coastal or from the river to coastal which will increase the existing petroleum concentration (Siti Aishah *et al.*, 2015). The information on the distribution of petroleum hydrocarbons in the coastal environment is necessary to determine the extent of oil pollution input into the oceans (Venkatachalapathy *et al.*, 2010).

Therefore, the aim of this study is to determine the total hydrocarbon concentration (TPH) concentration, total organic carbon (TOC) percentage and particle size classification in mud clam and its ambient from coastal area of Putatan and Tuaran, Sabah. Correlations with TPH concentration in mud clam and sediment and between TPH concentration with TOC content in sediment from sampling stations are also been discussed.

Methodology

Sample collection

This study was conducted in January 2015. Mud clam with preferably size around 60 mm are collected by hand-picking and trowel randomly within the 25 m^2 frame (5m x 5m), together with sediment samples with 3 replication in 5 stations for each areas as shown in Figure 1. The coordinate of sampling locations were recorded by Global Positioning System (GPS) device (Table 1). The mud clam and sediment samples were wrapped in aluminum foil and placed on ice before transported to the laboratory. The mud clam tissues are removed from its shell using pre-cleaned knife, wrapped in aluminium foil and sealed in thick polythene bag. Both mud clam tissues and sediments were kept and preserved in the refrigerator at -18 ˚C before analysis (USEPA, 1997).

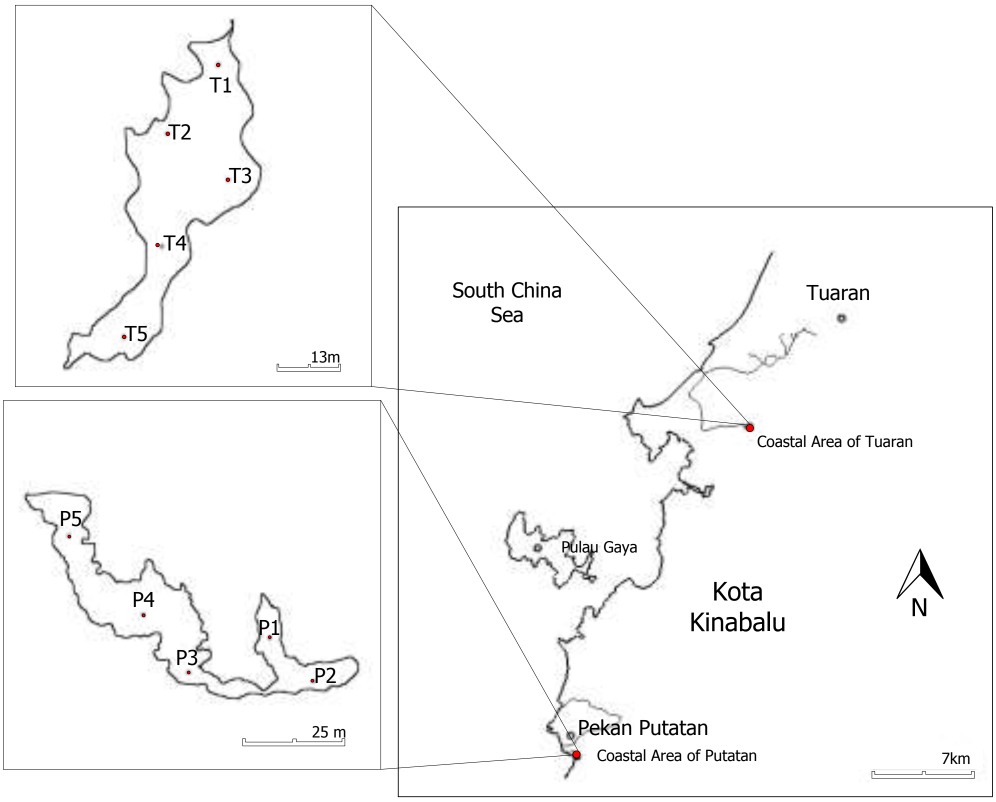

Figure 1. Study area and sampling stations.

Table 1. The coordinate of sampling stations.

Stations (Putatan)	Latitude / Longitude	Remarks	Stations (Tuaran)	Latitude / Longitude	Remarks
P1	5° 52’ 41.43” N 116°3’ 12.04” E	Residential area, facing school and road	T1	6° 6’ 4.38” N 116° 10’ 11.87” E	Near to domestic and highway
P2	5° 52’ 40.87” N 116°3’ 12.59” E	Commercial area, presence of workshop	T2	6° 6’ 3.72” N 116° 10’ 11.49” E	Undisturbed area
P3	5° 52’ 40.98” N 116°3’ 10.99” E	Centre of mangrove	T3	6° 6’ 3.64” N 116° 10’ 11.93” E	Route for local people to collect mud clam
P4	5° 52’ 41.72” N 116°3’ 10.41” E	Domestic area; village	T4	6° 6’ 3.20” N 116° 10’ 11.50” E	Area of collecting mud clam
P5	5° 52’ 42.74” N 116°3’ 9.45” E	Near to a big empty land	T5	6° 6’ 2.62” N 116° 10’ 11.25” E	Nearest to industrial area; highways

Petroleum hydrocarbon analysis

The standard method for the petroleum hydrocarbon content analysis was based on the APHA 5520. Mud clam tissue samples were thawed and then undergo extraction process, according to the method used by El Nemr *et al*. (2003). 10 g of wet tissues is weighed and added with 30 g of sodium anhydrous sulfate. After blended the mixture, the samples was extracted with 200 ml methanol for 8 hour in Soxhlet extractor. 20 ml of 0.7M potassium hydroxide and 30 ml of distilled water is added into the flask for another 2 hour. The content in the extraction flask is then extracted using separator funnel with 80 ml of hexane and dried with anhydrous sodium sulfate. The extracts were saturated using rotary evaporator and cleaned-up by silica gel columns. For sediment analysis, 10 g of sediments sample was dried in the oven at 105°C for 2 hours to determine the moisture content analysis. About 10 g of sediment was extracted with saturated hydrochloric acid (HCl) and 15 g magnesium sulphate ($MgSO_4$). After mashing the whole sediment sample with pestle, the sample was extracted with Soxhlet for 4 hours with 250 ml petroleum ether. The extracts were saturated using rotary evaporator and cleaned-up by silica gel columns. The eluate from the columns was concentrated with pure nitrogen. TPH concentration for mud clam tissues and sediment were analysed by using UV/VIS spectrophotometer Lambda 25 Perkin Elmer (excitation was at 239 nm and emission at 259 nm). A calibration curve was generated using Miri crude oil as arbitrary standard (concentration range between 0 -120 ppm).

Particle size analysis

For the relative proportions of sand, silt and clay in the study site’s sediments, the sediment grain size analysis is carried out using dry sieving (62.5 µm) and the pipette method for silt and clay by Folk (1980) as the main reference.

Total organic carbon analysis

The TOC percentage of the sediment was measured based on United Nations Environment Programme (UNEP)/MAP Athens (2006) method, where the sediment is put into oven and heated for 24 hour at 60 ˚C. Then approximately 1g of dry sediment was then placed into a furnace at 450˚C for 3 hour for ignition. TOC percentage was calculated using formula 1:

$$TOC\ \% = \frac{(D_0 - D_t)\ g / 1.724}{D_0} \times 100 \qquad (1)$$

where; D_0 = dry sediment mass before ignition and Dt = dry sediment mass after ignition (in gram).

Result and discussion

Table 2 shows the overall mean TPH (mg/kg), TOC (%) and particle size classification for each station in mud clam and its ambient at coastal area of Putatan and Tuaran, Sabah. The overall mean and range of TPH concentrations in mud clam and sediments in both study areas were recorded at 1.57 (0.72 – 2.16 mg/kg) dw and 0.36 (0.15 – 0.62 mg/kg) dw Miri crude oil equivalents, respectively. According to Marchand *et al*. (1996), sediments containing hydrocarbon levels less than 100 mg/kg dry weight can be classified as not anthropogenically contaminated. Based on the result, it can be stated that the levels of TPH obtained from both coastal areas are still below the alarming pollution level.

Table 2. Overall mean TPH (mg/kg), TOC (%) and particle size classification for each station in mud clam and its ambient at coastal area of Putatan and Tuaran, Sabah.

Area	Station	TPH in mud clam (mg/kg)	TPH in sediment (mg/kg)	TOC (%)	Particle size classification
Putatan	P1	1.73	0.35	5.87	Sandy loam
	P2	1.73	0.49	14.00	Sandy loam
	P3	1.21	0.28	5.40	Sandy loam
	P4	1.57	0.62	15.82	Sandy loam
	P5	0.72	0.26	5.37	Sandy loam
Tuaran	T1	2.01	0.33	7.74	Clay loam
	T2	0.89	0.15	4.21	Sandy loam
	T3	1.67	0.45	12.93	Sandy clay loam
	T4	2.01	0.32	7.09	Loamy silt
	T5	2.16	0.35	7.92	Sandy loam
Mean		**1.57**	**0.36**	**8.64**	

The result shows that the highest TPH concentrations in mud clam tissues are found at station T5 (2.16 mg/kg) which is in Tuaran area, where as the lowest concentrations are found at station P5 (0.72 mg/kg) which is Putatan area. Meanwhile for TPH concentrations in sediment, the highest value are found at P4 (0.62 mg/kg) and the lowest concentrations are found at station T2 (0.15 mg/kg). In the meantime, the statistical analysis by Pearson correlation showed a moderately positive correlation with coefficient for TPH concentration in mud clam and sediment for Putatan (r=0.6340) and Tuaran (r=0.6978). This suggests that the petroleum hydrocarbon in the sediments affects the concentration of TPH in the mud clam moderately in both areas.

The highest TPH concentration for mud clam and sediment was observed in different station which was T5 and P4 respectively. The TPH concentration for mud clam was observed highest in T5, which is the station nearest to industrial area and was surrounded by two main highways. They may absorb the hydrocarbons from the air which comes from the incomplete combustion of vehicles' exhaust. The possible source of hydrocarbons in the air is mainly due to the anthropogenic input which includes pyrogenic (combusted) such as vehicle exhaust and petrogenic (non-combusted) which are some unburned crude oil and the derivatives such as diesel fuel or lubricating oil which may be the source of TPH in this area (Keshavarzifard *et al*., 2014). It was also suggested that mud clam are not exposed directly to the contamination absorbed onto sediment grains, but could be from the water column (de Mora *et al*., 2010). Besides the location factor, Connell (1997) stated that bioavailability and organic physiology also can affect the contaminant body burden. The physiological variables include lipid levels, the rates of uptake and the elimination rate of the contaminants by the biota which made the concentration of TPH detected subjectively.

P4 which was the highest concentration of TPH for sediment was the station nearest to the village and domestic area as per observation. The possible source of TPH could be the improper handling and dumping of municipal wastes which includes mostly plastics. Plastics are known to eventually break down in to small pieces and the core ingredients to make plastics are hydrocarbons. The sediment's TPH contamination was mainly due to the land based sources such as the improper municipal wastes and the atmospheric fallout (Vicent *et al*., 2013). The petroleum hydrocarbons in the water and the air could have entered the sediment by precipitation, runoff and then adsorption and deposition. Pyrogenic inputs are commonly higher in aquatic ecosystems due to the increasing number of vehicles, due to increased population and affluence (Wang *et al*., 2001; Stout *et al*., 2004 ; Boitsov *et al*., 2013). The vehicles exhaust gases and the soot particles from motor vehicles are the common mobile sources for pyrogenic petroleum hydrocarbons. Research by van Metre *et al*. (2000) stated that the petroleum hydrocarbons in water shed are significantly related with the combustion derivative particulate matter, such as the vehicles' exhausts, gasoline fuel.

The lowest TPH concentration in mud clam and its ambient were seen in P5 and T2 respectively, most probably due to the location where it is the empty undisturbed land and there is no presence of industrial or people having activities over the sampling station, based on the observations and local people's contribution. P5 does not encounter much anthropogenic sources of petroleum hydrocarbons, and the possible source of the petroleum hydrocarbons was predicted to be from naturally occurring substances such as the biological process of diagenesis in the phytoplankton, algae and plants (Wang & Fingas, 2003; Suratman *et al*., 2012).

Total organic carbon is an important variable that affects the accumulation and the release of various micro pollutants (Massoud *et al*., 1996). The overall mean and range of TOC content in Putatan and Tuaran were 9.30% (5.37 – 15.82%) and 7.98% (4.21 – 12.93%) respectively. Generally, the sediment in Putatan has the higher percentage of TOC compared to Tuaran sediment. The highest level of TOC was recorded at station P4 (15.82%), meanwhile the lowest level was recorded at station

T2 (4.21%). All stations in the area of Putatan has the same soil texture identified, which are sandy loam, whereas Tuaran has a few mixture of soil texture, mostly consists of loam. The average percentages of clay, silt and sand were 12.6%, 16.6% and 70.8% for Putatan and 21%, 14% and 65% for Tuaran. Sandy sediments contain lower organic content due to the fewer surfaces as compared with fine particles such as silt (Kruitwagen *et al*., 2008). Therefore, the accumulation of TPH concentration and total organic carbon in sediment in the two study sites is considered low.

The correlation between TPH concentration and TOC content in sediment for Putatan and Tuaran coastal area had been tabulated and showed a strong positive correlation with r = 0.9637 and r = 0.9340 respectively. The result clearly shows that the TPH concentrations significantly influence on TOC accumulations in the sediment for both areas. TOC is effective as one way to screen for petroleum hydrocarbons contamination in the sediment, as TOC encompasses all of the weight fractions of TPH (Schreier *et al*. 1999).

The petroleum hydrocarbon concentrations results are compared with previous studies that have been reported for coastal areas of Sabah as shown in Table 3. The results reveal that the range concentrations of the petroleum hydrocarbon in this study is comparatively low while TOC% showed apparently higher percentage from previous studies. Increase in organic content of sediment maybe accompanied by increases in chemical contaminants that are co-varying in relation to a common environmental factor such as increasing proportions of finer-grained sediment particles (Thompson & Lowe, 2004)

Table 3. Comparison of petroleum hydrocarbon (mg/kg) and TOC % in sediment of coastal regions of Sabah.

Location	TPH (mg/kg dw)	TOC (%)	References
Papar and Putatan, Sabah	0.26 - 4.59	0.35 – 2.32	Siti Aishah *et al.* (2013)
Papar to Tuaran, Sabah	0.24 – 20.65	0.03 – 4.02	Siti Aishah *et al.* (2015)
Putatan and Tuaran, Sabah	0.72 – 2.16	4.21 – 15.82	Present study

Conclusion

Petroleum hydrocarbons concentration, total organic carbon percentage and particle size classification in sediment and mud clam from Putatan and Tuaran coastal area of Sabah were quantified and compared. The overall mean TPH in mud clam and its ambient for both study areas are recorded with 1.57 mg/kg dw and 0.36 mg/kg dw Miri crude oil equivalents respectively. The mud clam and sediment samples in study areas can be concluded as non-polluted by petroleum hydrocarbon and statistical analysis using Pearson correlation clearly shows that the TPH concentrations significantly influence on the TOC accumulations in the sediment for both areas.

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