Determination of 1-Hydroxypyrene and 2-Napthol in intertidal rocky shore macrobenthos following oil spill at Ao Prao, Samed Island

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Abstract
Biological monitoring of exposure to Polycyclic Aromatic Hydrocarbons (PAHs) by determination of PAHs metabolite products (1-hydroxypyrene (1-HOP) and naphthalene metabolite 2-napthol (2-NAP)) in three species of intertidal rocky shore molluscs; coiled snail (*Planaxis sulcatus*), whelk (*Morula sp*.) and rock oyster (*Saccosstrea cucullata*) by Gas Chromatography Mass Spectrophotometry were carried out. Specimens were collected in October, December 2013 and March 2014 at Ao Phrao beach, Samed Island where a PTTGC heavy oil spill accident was seriously impact to the beach in July 2013 compared with specimens from control site (Lam yaa beach). 2-NAP was detected in all molluscs species with the mean range from 0.048-0.972 µg kg^{-1}wet weight. However, 1-HOP was not detected in the present study. The concentration of 2-NAP in grazer, coiled snail, was highest and increased through time. Concentration of 2-NAP in whelk (*Morula sp*.) which is one of predator in rocky shore was lower possibly due to they got 2-NAP from their prey whereas the concentration was decreased in rock oyster. This finding suggests that 2-NAP might be an important biomarker in intertidal rocky shore macrobenthos contaminated with crude oil.

Keywords: Biomarker, Gas Chromatography Mass Spectrophotometry, 1-hydroxypyrene, Intertidal rocky shore, Macrobenthos, Oil spill, Polycyclic aromatic hydrocarbons, 2-napthol
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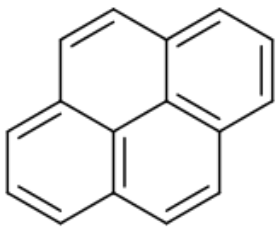
Introduction

In 28 July 2013, 50,000 litres of the crude oil leaked from a pipeline owned by PTTGC Plc, and spread to rocky shore and beach on the western coast of Samed Island in the following day resulted in serious impact to the shore. In general, a large quantity of crude oil spill cause acute and long-term damage to marine ecosystems. The biological impacts after an oil spill, intertidal rocky shore macrobenthos living in crude oils contaminated environments absorb these compounds through their body surface and

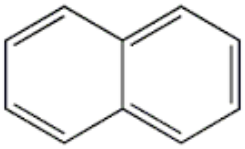
respiratory organ or by ingesting contaminated sediment or food. There are several reports about effects of oil spill to intertidal rocky shore organism both mobile and sessile species such as snail, limpet, whelk and oyster.

Crude oil is a complex compound consisted of aliphatic, aromatic and polar compounds. When the crude oil spilled to the sea, it spread on the sea surface and process involved in the weathering of crude oil including evaporation, dispersion, dissolution, photo-oxidation reaction and biodegradation (Kim *et al*., 2013). Once the oil spread on the sea surface, it form a thin film call an oil slick (Maki *et al*., 2001) All crude oil contains volatile organic compounds (VOCs), which readily evaporate into the air and by polycyclic aromatic hydrocarbons (PAHs). The polycyclic aromatic hydrocarbons are the major pollutants related to shipping activities and oil exploitation, it can persist in the environment for long years and form to chocolate mousse or tarball. Harmful Vocs typically are acutely toxic but that have a high vapor pressure in room temperature while PAHs are high molecular weight and low vapor pressure, they can stay in the environment for long periods of time. The toxicity of PAHs is lower than Vocs (Lim and Shin, 2013).

Polycyclic aromatic hydrocarbons (PAHs) are widespread contaminants in the marine environment. The major sources of PAHs in the marine environment are atmospheric fallout, spillage of petroleum and oil products. Generally, environmental exposure of organisms to PAHs is assessed by monitoring their environment, and monitoring of the levels of contaminants involves determining the PAHs in sediment and animal tissue samples (Zhanna *et al*., 2009). PAHs are metabolized to hydroxy PAHs, because pyrene and naphthalene is a major constituent of PAHs contained in crude oil , 1-hydroxypyrene (1-HOP) and 2-naphthol (2-NAP) must be considered as biomarker of PAHs exposure (Fillmann *et al*., 2004).



Pyrene ($C_{16}H_{10}$)



Naphthalene ($C_{10}H_8$)

Fig. 1 Formula of Pyrene ($C_{16}H_{10}$) Naphthalene ($C_{10}H_8$) (Marcia, 2001)

Biomarker studies using 1-hydroxypyrene (1-HOP) and 2-napthol (2-NAP), especially 1-HOP have previously dealt with body fluids (blood, bile and urine) and tissues such as liver, hepatopancreas, kidney, muscle and gills have been to determine the concentrations and types of PAHs that accumulation in organisms (Lim and Shin, 2013). Usually, Gas chromatography with mass spectrometric or GC-MS methods using for identification and quantification of PAHs metabolite products (Jana *et al*., 2010). This technique has been widely used for the routine monitoring or characterization of oils, these methods involve electron impact ionization-mass spectrometry (GC–EI-MS) and use high resolution mass spectrometry (GC–HRMS) (Lim and Shin, 2011).

The objective of this study was to determine of Polycyclic aromatic hydrocarbons (PAHs) metabolite products (1-Hydroxypyrene (1-HOP) and naphthalene metabolite 2-napthol (2-NAP)) in three species of intertidal rocky shore molluscs; coil snail (*Planaxia sulcatus*), whelk (*Morula sp.*) and rock oyster (*Saccosstrea cucullata*) were impacted by the oil spill (Ao Prao) by Gas Chromatography Mass Spectrometry (GC-MS).

Materials and Methods

Study area

The study was carried out in Ao Prao (impact site) comparing with rocky shore in Lam Yaa where was not impacted by the oil spill accident (non impact site) (Fig.2).

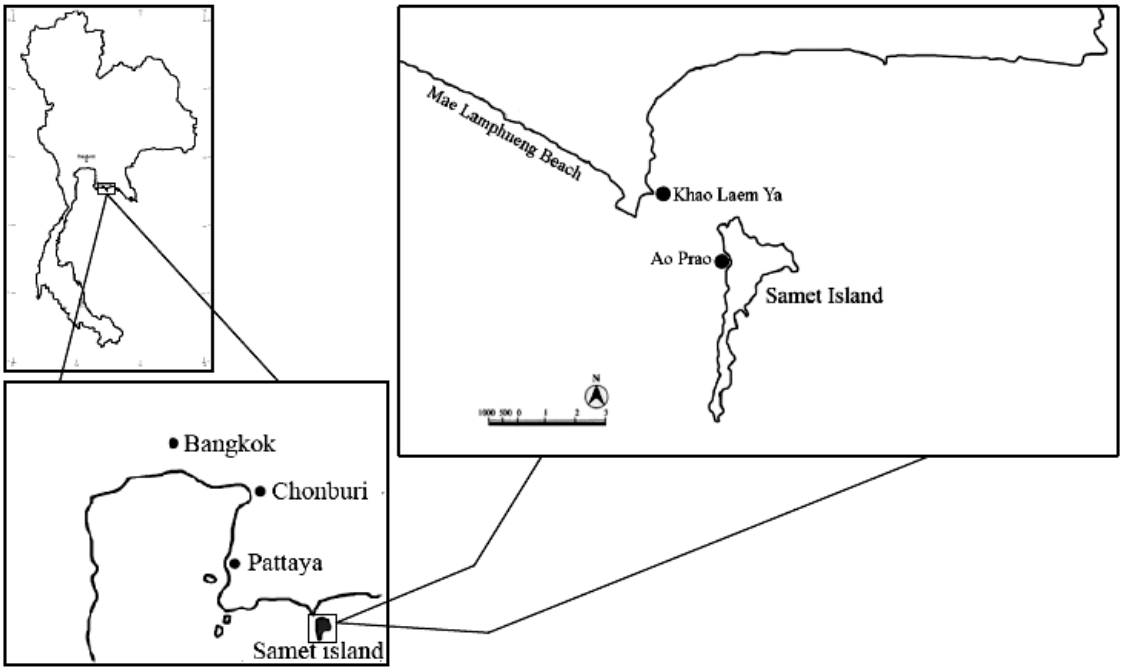


Fig. 2 Map of studied sites (Ao Prao, Samed Island and Lam Yaa rocky shore)

Sampling

The species of rocky shore mollusks namely *Planaxis sulcatus, Saccosstrea cucullata* and *Morula sp.* were collected in October 2013, December 2013 and March 2014 (3, 5 and 8 month following the oil spill accident) from Ao Prao and Lam Yaa. The samples were frozen at -20^{o}c.

Chemical analysis

1. Spiking

We used rocky oyster (*Saccosstrea cucullata)* for spiked samples. The spiked samples were prepared with 20-200 µl of standard solution ay concentrations of 0.001-0.1 mg/l and with 20 µl of the internal standard solution (1-HOP d-9) at a concentration of 0.1 mg/l.

2. Calibration Standards

Calibration curve for 1-hydroxypyrene (1-HOP) and 2-napthol (2-NAP) were established by extraction after adding 0.1, 0.5 and 1 ng of standards and 2 ng of internal standard in 2 g of rocky oyster (*Saccosstrea cucullata)* tissue. 1-HOP-d 9 was used as the internal standard.

3. Sample Preparation

Add 5.0 ml volume of 2 M KOH and internal standard (1-HOP d-9) in 2.0 g of shellfish tissue. The sample homogenized with homogenizer until the shellfish tissue and reagent mixed after sonicated with an ultrasonic bath for 10 minutes. The solution was hydrolyzed in oven at 90^{o}c for 90 min. The solution was extracted two times with 10 ml of n-pentane, and collected the inorganic layer. Added 5 ml solution of 2 M HCl to the solution and controlled to pH 2-3. The solution was extracted two times with 10 ml n-pentane. Then added of 50 µl of MTBDMSTFA to the total extract and shake with shaker for 10 minutes, the extract was dried with a nitrogen stream. The dry sample was dissolved with 50 µl of MTBDMSTFA solution and heated with oven for 30 min at 80^{o} c. The sample was injected into the gas chromatography mass spectrometry (GC-MS)

4. Gas chromatography–mass spectrometry

The gas chromatographic mass spectrometric conditions of 1-HOP and 2-NAP were performed according to our previous research result (Lim and Shin, 2011). The gas chromatograph used was an Agilent 7890 A with a split/splitless injector (Agilent Technologies, Santa Clara, CA, USA). Oven temperature program began at 100^{o}C, held for 1 min, raised to 320^{o} C at 20^{o}C/min and held for5 min. All mass spectra were obtained with an Agilent 5975 B instrument. The ion source was operated in the electron ionization mode (EI; 70 eV, 230^{o}C). Full-scan mass spectra (m/z 50–800) were recorded for the identification of analyses at a high concentration. Confirmation of trace chemicals was completed by three MS characteristic ions, and the ratio of the three MS characteristic ions and the GC-retention time matched the known standard compound. The ions selected by SIM were m/z 185, 201, 258 for TBDMS-2-NAP, m/z 259, 275, 332 for TBDMS-1-HOP, and m/z 267, 284, 341 for TBDMS-1-HOP-d9.

Results

Intertidal rocky shore mollusks were collected in October 2013, December 2013 and March 2014 at Ao Prao beach, Samed Island where a PTTGC heavy oil spill accident and Lam yaa beach (control site) using the sample preparation and GC-MS described (Fig. 2, 3 and 4). The concentration of 2-NAP was detected in all molluscs species with the mean range from 0.048-0.972 µg kg^{-1}wet weight. However, 1-HOP was not detected in the present study (Table 1).

At Ao Phrao beach, where a PTTGC heavy oil spill accident was seriously impact to the beach. 2-NAP was detected in a highest concentration range (0.972±0.312 µg kg^{-1}wet weight) in coiled snail (*Planaxis sulcatus*) in

December 2013 and increased through time (Fig 2; Table 1). The concentration of 2-NAP was decreased in rock oyster (*Saccostrea cucullata*), it was decreased from 0.811 µg kg^{-1}wet weight in October 2013 to 0.557 µg kg^{-1}wet weight in March 2014 (Fig 3; Table 1). The lowest of 2-NAP concentration range (0.048±0.014 µg kg^{-1}wet weight) was detected in whelk (*Morula sp*.) in October 2013 and the concentration of 2-NAP was increased from 0.048 µg· kg^{-1}wet weight in October 2013 to 0.120 µg kg^{-1}wet weight in March 2014 (Fig 4; Table 1).

The specimens from control site (Lam yaa beach), we found low concentration of 2-NAP, the concentration was detected in some molluscs species with the mean range from 0.285±0.041 and 0.052±0.021 µg kg^{-1}wet weight in coiled snail (*Planaxis sulcatus*) and rock oyster (*Saccostrea cucullata*) respectively while 2-NAP concentration not detected in whelk (*Morula sp*.) samples (Table 1).

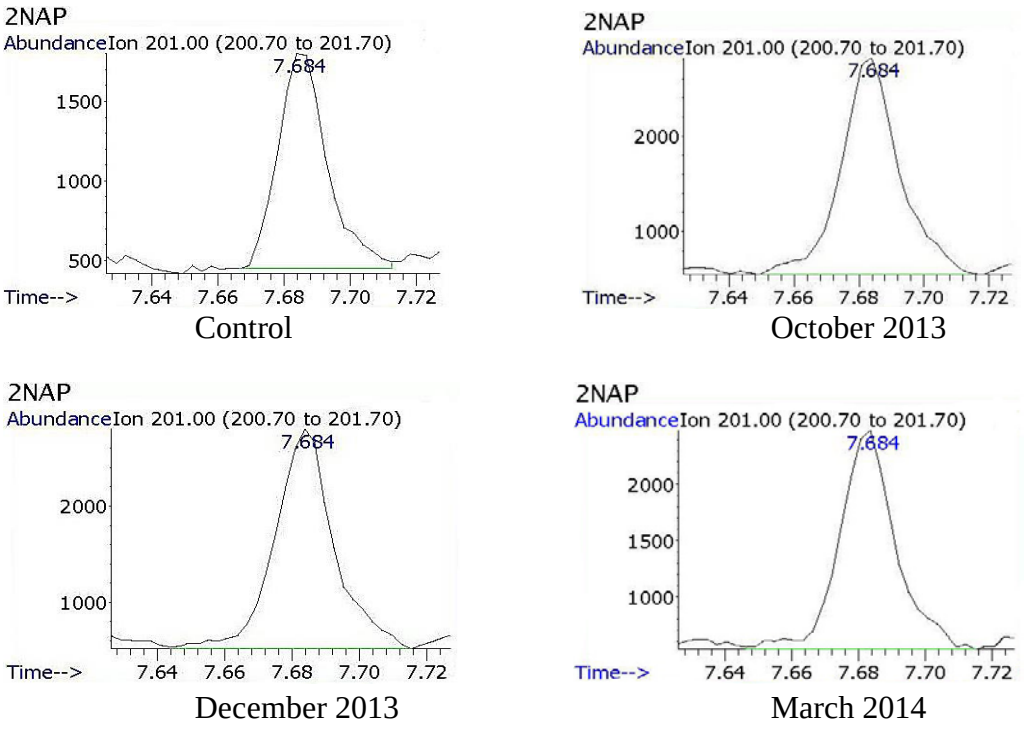


Fig. 2 Chromatograms of coiled snail (*Planaxis sulcatus*) samples in Ao Prao and Lam Yaa.

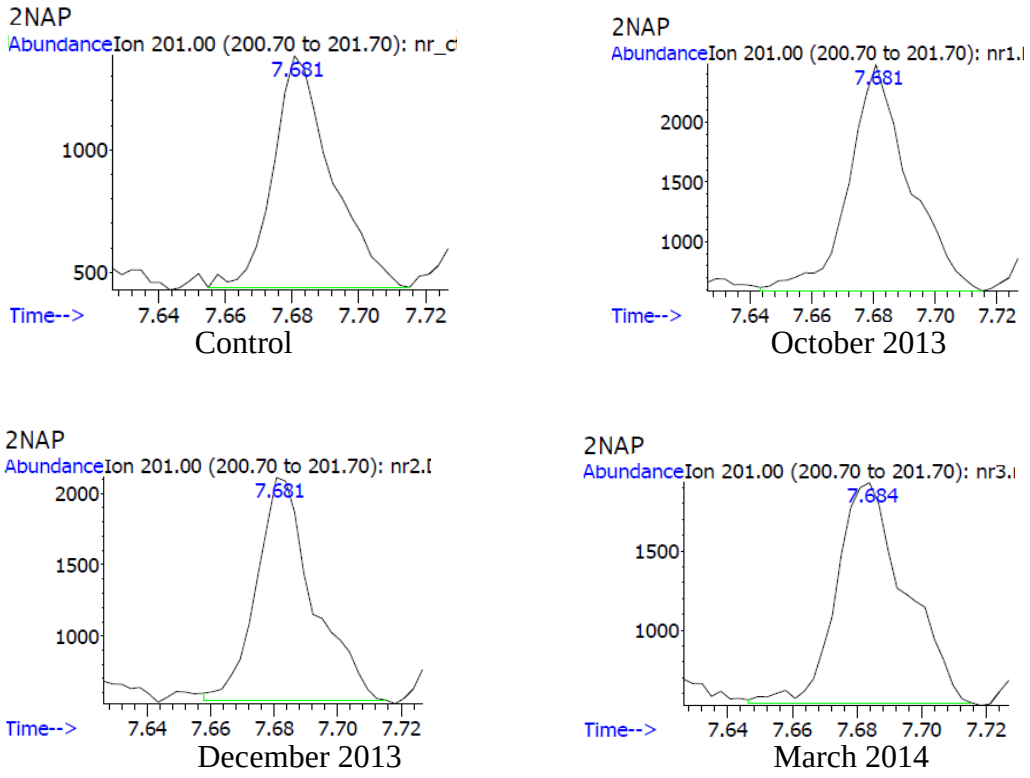
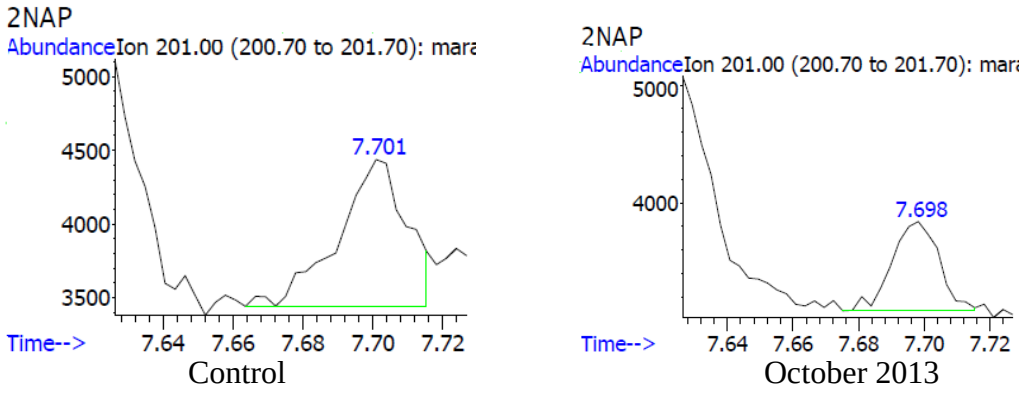


Fig. 3 Chromatograms of rock oyster (*Saccostrea cucullata*) samples in Ao Prao and Lam Yaa.



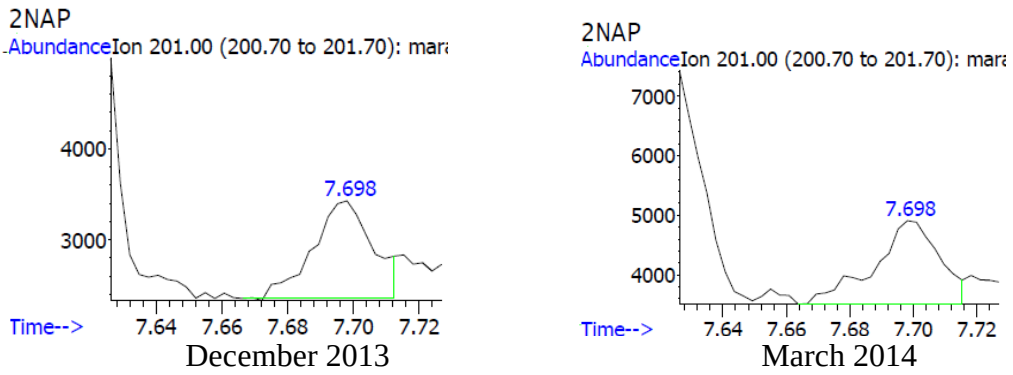


Fig. 4 Chromatograms of whelk (*Morula sp*.) samples in Ao Prao and Lam Yaa.

Table 1. The concentration of 2-NAP (µg kg^{-1} wet weight) detected in molluscs samples taken in impact site (Ao Prao) and non-impact site (Lam Yaa).

Samples	The concentration of 2-NAP (µg kg^{-1} wet weight)		
	Planaxis sulcatus	*Saccostrea cucullata*	*Morula* sp.
Control	0.285±0.041	0.052±0.021	0.00±0.00
October 2013	0.884±0.226	0.811±0.374	0.048±0.014
December 2013	0.972±0.312	0.625±0.215	0.066±0.022
March 2014	0.969±0.287	0.557±0.154	0.120±0.083

Discussions

We found the concentration of 2-NAP was detected in grazer, coiled snail (*Planaxis sulcatus*) was highest and increased through time. Concentration of 2-NAP in whelk (*Morula sp*.) which is one of predator in rocky shore was lower while the 2-NAP concentration in rock oyster (*Saccostrea cucullata*) was decreased because they got 2-NAP from their prey. This research illustrates, the intertidal rocky shore macrobenthos were impacted by the oil spill, the pollution is associated with the food chain. There are several studies indicated crude oil spill accident impact to marine organisms (phytoplankton, zooplankton, small fish, large fish and benthic invertebrates) in food chain (Karina *et al*., 2001). Charles (2001) reported the "Exxon Valdez" oil spill in Alaska, impacted on the ecosystem. Oil spill effect to algae, are eaten by grazing limpets, periwinkles, mussels and barnacles.

A gas chromatography–mass spectrometric (GC–MS) method is commonly used for PAHs analysis (Dianne *et al*., 2006). 1-HOP is the

predominant biotransformation product of pyrene and 2-NAP is the naphthalene metabolite. Generally 1-HOP has been used as a suitable indicator for PAHs exposure in organism such as human, fish and marine polychaetes. However, naphthalene is a major constituent of PAHs containing in crude oil. 2-naphthol must be considered as important biomarker in case of organism contaminated with crude oil. The results showed that 2-NAP as biomarker in intertidal rocky shore macrobenthos contaminated with crude oil and it is recommended as biomarker of environmental PAHs exposure to organisms.

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