RESEARCH ARTICLE

Occurrence and Source Apportionment of Polycyclic Aromatic Hydrocarbons (PAHs) in Green Mussel (*Perna viridis*) from Cilincing Waters of Jakarta Bay, Indonesia

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Abstract

Green mussels (*Perna viridis*) as sedentary organisms have been adopted as bioindicators in the marine environment for many decades. They have been potentially affected by anthropogenic activity and organic contaminants such as polycyclic aromatic hydrocarbons (PAHs) in an aquatic environment. This study aims to determine concentration, distribution and potential sources of PAHs in green mussel biomass obtained from Cilincing waters, Jakarta Bay. Fresh green mussels were collected from aquaculture facilities at Cilincing waters of Jakarta Bay with various size categories of shell length and were extracted with solvent n-hexane-dichloromethane mixture using Soxhlet method. The concentrate of sample extract was purified using silica gel/sodium sulfate anhydrous with n-pentene-DCM effluent in fractionation. Finally, the purified sample was injected into the GC-MS instrument. The result showed that the highest concentration of total PAH (TPAH) was 126.47 ng.g^{-1} wet weight (ww), founded in the guts from the biggest group of green mussel (length of 6.00 to 7.99 cm), and Σ3-aromatic rings were predominantly detected in all group sizes of green mussel. The result of the ΣLMW/ΣHMW and diagnostic binary ratio indicated that PAH contamination is caused by a mixed source of petrogenic and pyrogenic processes. It may be due to the presence of oil spilled and petroleum waste from shipyard and cargo activities and atmospheric deposition. Based on excess cancer risk (ECR) value and the concentration level of PAH carcinogenic according to National Agency of Drug and Food Control (BPOM) regulation, PAH level in green mussels are acceptable and minor negative impact due to typical human consumption. Nevertheless, it needs to be circumspect to exploiting green mussels as seafood resources for daily consumption due to the carcinogenic content.

Keywords: Polycyclic Aromatic Hydrocarbons (PAHs), green mussel, Cilincing, petrogenic, pyrogenic

Introduction

Within few decades, there has been an awareness of potential organic contaminants such as polycyclic aromatic hydrocarbons (PAHs). This compound consists of more than hundreds of different chemical pollutants containing two or more fused aromatic rings in linear, angular or group arrangements (Ghosal, Ghosh, Dutta, & Ahn, 2016). This pollutant belongs to the persistent organic pollutants (POPs), PAHs are ubiquitous in the environment (Wang et al., 2016) and have potentially adverse effects on organisms and public health due to their toxic, genotoxic, mutagenic, and carcinogenic properties (Croxton, Wikfors, & Schulterbrandt-Gragg, 2012; EFSA, 2008; Gu, Li, & Lu, 2017). They are generally formed by incomplete combustion or pyrolysis of organic matter like coal, tar, oil and gas (Li et al., 2015), while those of natural forms are from forest burn, volcanic eruptions and bacterial reactions (Abdel-Shafy & Mansour, 2016; Tobiszewski & Namieœnik, 2012). PAHs enter a marine ecosystem through the atmosphere, municipal run-off,

waste of industry and household as well as oil spills (Huang, Wang, Yan, & Bay, 2012) and it persists in the environment for extended periods (Dsikowitzky et al., 2011). In an estuary, PAHs may create an aggregate with particulates and dissolved organic matters due to their low aqueous solubility and high hydrophobicity (Countway, Dickhut, & Canuel, 2003) and they may be deposited on sediments (Katayama et al., 2010). The dissolved and suspended PAHs can be recycled in the surface sediment due to its bioavailability (Song et al., 2016). Marine invertebrate such as mussel which is living in PAH-contaminated environments are able to accumulate these compounds in many pathways (e.g. through adsorption of their body surface and gills, ingestion of contaminated sediment or particles), which then transfer them through the aquatic food web (Patrolecco, Ademollo, Capri, Pagnotta, & Polesello, 2010).

Mussels, as marine macroinvertebrates, have been routinely utilized for environmental monitoring and assessment purposes (Schøyen et al., 2017). They are a filter-feeding biota and are extensively distributed in the aquatic environment, especially in estuarine; hence they are feasible for collecting (Beyer et al., 2017). They perform as a bioaccumulator of organic contaminants and tolerantly live in a wide range of contaminant habitats (Farrington et al., 2016). In addition, they have many remarkable capabilities, including adaptable to variation of environmental parameters such as temperature, salinity, dissolved oxygen, and food availability hence particularly appropriate for verifying the biological impact of pollutants (Martínez-Gómez et al., 2017). As a bioindicator organism, this biota can provide much information about the various pollutants, including organic contaminants present from the ocean to the neritic waters environment (Kasiotis et al., 2015; Schøyen et al., 2017). This organism's use as a bioindicator has been validated over the Asian countries (Richardson, Tse, Luca-Abbott, Martin, & Lam, 2005), including Indonesia (Dwiyitno et al., 2016). Furthermore, marine mussel such as green mussel has been considerably used to reflect one of the most helpful biological indicators of organic contaminants; PAH is one of them (Isobe et al., 2007).

Cilincing waters as part of Jakarta Bay is a foreshore area bordered with a densely populated area with more than 400,000 inhabitants (BPS-Jakarta, 2020). In addition, there are hundreds of manufacturing plant, agriculture, and service industries (PTSP, 2015), as well the Tanjung Priok international port which is neighboring the Cilincing coastal (BPS-Jakarta, 2020). Consequently, Cilincing water receives a lot of the household and industrial waste effluents, municipal run-off, discharge of shipyard and cargo and oil spills with cause a severe pollution potential (Rinawati et al., 2012). Therefore, chemical pollutants such as PAHs in this water area have increased in the last two decades (Dwiyitno et al., 2016), along with increasing anthropogenic activities (Dsikowitzky et al., 2018; Rositasari, Puspitasari, Nurhati, Purbonegoro, & Yogaswara, 2017). Regarding this situation, the marine mussel, including cultured mussel in Jakarta Bay, is the appropriate choice as a sentinel and essential biota to reveal ecosystem health data.

Research on PAHs in green mussel in Jakarta Bay has been begun since 2007 (Augustine, 2008). In the previous period in the Jakarta Bay area, the research focuses on PAHs level in whole green mussel tissue (Dwiyitno et al., 2016; Falahudin & Khozanah, 2012), without specific analysis of PAHs in a particular organ of green mussels, such as flesh and guts. As lipophilic substances, PAHs accumulate more in guts than in flesh because guts contain more lipid than flesh (Sun et al., 2016). Furthermore, there is still limited data on PAHs level on particular mussel organs such as flesh and guts. Therefore, this gap needs to be answered through this study. Accordingly, this study investigated PAH concentrations, compositions and sources of PAHs in green mussels (*Perna viridis*) at an aquaculture facility site in the Cilincing water of Jakarta Bay. This site area has suffered from anthropogenic activities and previously has not been long yet investigated in recent years. This study will provide better understanding than previous studies on the distribution and fate of PAH compounds in the green mussels. Another advantage of this study is assessing risk analysis of level consumption of green mussels, based on the regulation of the National Agency of Drug and Food Control (BPOM).

Material and Methods

Sample Collection

The green mussel samples were collected at the aquaculture facilities belonging to local fishermen in April 2019 in Cilincing water of Jakarta Bay at 6.072510°S; 106.94109°E (Figure 1). The fresh green mussels were divided into four groups of shell length size as follows: the smallest (< 2.00 cm), small (2.00-3.99 cm), medium (4.00-5.99), and the biggest (6.00-7.99 cm) (Figure 2). This grouping of sizes proposed comparing PAH level with previous studies that had measured PAH levels in green mussels in Jakarta Bay. Each group of shell length size has one replicate and consisted of 10-20 individuals of green mussels. The samples were stored in an icebox at 4 °C by adding several ice packs to maintain the temperature during transportation to the laboratory for further analysis.

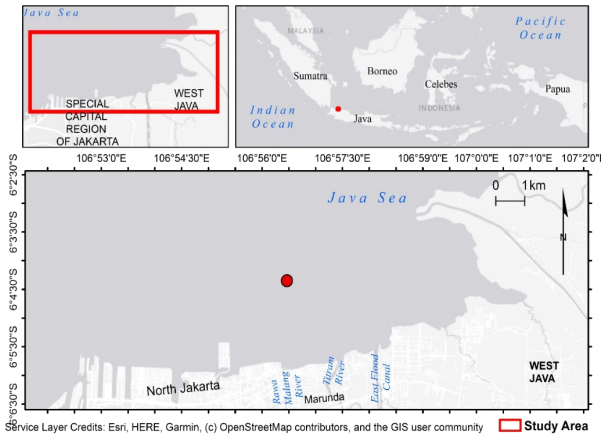


Figure 1. Location of green mussels aquaculture facility (red dot) in Cilincing waters, Jakarta Bay.

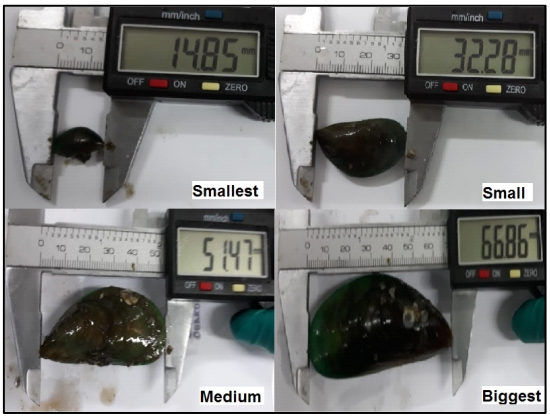


Figure 2. Photo of green mussels in various sizes consisting of the smallest (< 2.00 cm), small (2.00-3.99 cm), medium (4.00-5.99 cm), and the biggest sizes (6.00-7.99).

Sample Preparation

Preparation of flesh and gut samples were only carried out for medium and the biggest sizes, but not for the smallest and small sizes, due to their separation difficulty. Analysis of the samples used a method developed and applied by Vorkamp et al. (2010). Briefly, 5 g of all various green mussel sizes (including flesh and guts for medium and the biggest sizes only) were extracted with n-hexane-dichloromethane mixture (60:60 mL) using a Soxhlet extraction at 50 °C. The extracts were then concentrated to 1 mL by rotary evaporation at 70-80 rpm with pressure at 8-10 psi and temperature 40 °C for solvent reduction. Activated copper was used to purify the obtained concentrate from sulfur contaminant. Activated silica gel 60 (0.063-0.2 mm) (3 g)/sodium sulfate anhydrous (2 g) column fractionation was applied for clean up with 15 mL of n-pentane-dichloromethane mixture (40:60) as an eluent prior to analysis using GCMS instrument (Khozanah et al., 2019). All solvents used in this experiment were of pro analysis grade and purchased from Merck, Germany.

Analysis of PAHs Source

The best methods to identify and estimate PAHs sources is by determining ratios of low molecular weight (LMW) and high molecular weight (HMW) as well as by determining the binary ratios of anthracene-phenanthrene, fluoranthene-pyrene, and benzo(a) anthracene-chrysene (Table 1) (Yunker et al., 2002; Tobiszewski & Namieœnik, 2012; Huang et al., 2012).

Human Health Risk Assessment

US-EPA (2000) recommends using potency equivalent concentration (PEC) to express carcinogenic compounds such as benzo(a)pyrene of total PAHs to assess bivalve consumption risk to human health was estimated following Zhao, Zhang, Cai, & Chen (2014) methods. PEC was represented by the benzo(a)pyrene equivalent (BaPeq) concentration, was calculated according to Equation 1.

$$PEC = \sum_{i=1}^{n} TEF_i \times C_i \quad (1)$$

PEC is a toxic equivalent factor for each PAH compound and C for each PAH concentration observed in target sample i. The toxicity equivalency factors (TEF) value consist of 0.001 (for naphthalene (Nap), acenaphthene (Ace), fluorene (Flu), fluoranthene (Flt), phenanthrene (Phe), and pyrene (Pyr)); 0.01 (for anthracene (Ant), chrysene (Chr) and benzo[ghi]-perylene (BgP)); 0.1 (for benzo[a]anthracene (BaA), benzo[b]fluoranthene (BbF), benzo[k]fluoranthene (BkF), and indeno[1,2,3-cd]pyrene (InP)); and 1(for benzo[a]pyrene (BaP) and dibenzo[a,h]anthracene

Table 1. Estimated PAH sources based on PAH ratio compounds (Yunker et al., 2002).

Ratio	Source
LMW/HMW > 1	Petrogenic
LMW/HMW < 1	Pyrogenic
An/(An+Phe) < 0.1	Petroleum
An/(An+Phe) > 0.1	Grass, wood & coal combustion
Fluo/(Fluo+Pyr) < 0.4	Petroleum
0.4 ≤ Fluo/(Fluo+Pyr) < 0.5	Petroleum combustion
Fluo/(Fluo+Pyr) ≥ 0.5	Grass, wood & coal combustion
B(a)A/(B(a)A+Chry) < 0.2	Petroleum
0.2 ≤ B(a)A/(B(a)A+Chry) < 0.35	Petroleum combustion
B(a)A/(B(a)A+Chry) ≥ 0.35	Grass, wood & coal combustion

(dBA)) (Barhoumi et al., 2016). Furthermore, the lifetime cancer risk level of dietary exposure was estimated using the excess cancer risk (ECR) according to Equation 2 (Ke et al., 2017).

$$ECR = \frac{PEC \times ED \times CR \times CSF}{AT \times BW} \times 10^{-6} \quad (2)$$

Where ED is exposure duration (30 years for adults), CR is the consumption rate of seafood for Asian countries in urban areas (7 g.person^{-1}.day^{-1}; (Gu et al., 2017), CSF is cancer slope factor for oral of BaP of 7.3 mg.kg^{-1}.day^{-1} (Sun et al., 2016), AT is the average exposure time (assumed to be 70 years), and BW is the average body weight of Asian adults (60 kg) (Shi et al., 2016).

Quality Control

All sample procedures were processed with each sample batch identically to the samples, including a standard series mix of 16 PAH congeners and a spike of internal standard (i.e. naphthalene-d8, acenaphthene-d10, phenanthrene-d10, chrysene-d12, and perylene-d12) and blank samples. Identification of PAH compound was verified by comparing the gas chromatographic retention times and mass spectra with those of the reference compounds in library tools. Quantitative data of PAHs was performed by gas chromatography-mass spectrometry (GC/MS) to obtain the integration of the selected ion chromatograms extracted from the total ion current and selected mass molecule of standard series. The obtained data must have a satisfactory value based on the determination coefficient (R^2 > 0.90). All concentration results in this study were reported in ng.g^{-1} ww. The method detection limit (MDL) was reported as < 0.1 ng.g^{-1} for total PAHs, while the recovery for spiked blanks was 60-65%.

The PAH analysis was conducted using GCMS system (ISQ-LT 1310 Thermo Scientific, USA) was equipped with a 30 m TG5-SilMS fused silica capillary column with a specification of 0.25 mm ID and coated with a 0.25 µm thick 5% diphenyl-95% dimethylpolysiloxane film. Helium (99.99% purity of GCMS grade) was used as a carrier gas. The GC temperature program used the initial oven temperature of 50 °C (held for 0.5 min). It was increased to 160 °C (held for 15 min), and was further increased to 290 °C at the rate of 13 °C/min (held for 13 min), and was finally increased to 300 °C at the rate of 1.5 °C/min (held for 4 min). Helium was used as carrier gas at a constant rate of 1.2 mL/min with a split flow rate was 10 mL/min. The injection was made in splitless at half a min. The mass spectrometer was operated in a full-scan mode in EI$^+$ (70 eV), ion source temperature 250 °C, scanning from 50 to 650 amu with a rate inter-scan time of 0.3/s (Khozanah et al., 2019).

Statistical Analysis

All concentration data were presented on a wet weight (ww) basis. Principal component analysis (PCA) has been utilized to further understand the relationship between types of PAH congeners with various mussel organism sizes (Glad, Bihari, Jaksic, & Fafandel, 2017). Statistical analysis was performed with Primer 7 software version 7.0.17 (Quest Research Limited, New Zealand). One way analysis of variance (ANOVA) was conducted to determine the level of significance at p< 0.05 to compare PAH level in green mussel sizes, e.g., the smallest, small, medium, and the biggest. To obtain a normal distribution, the logarithm function was applied in data analysis of PAH level.

Results and Discussion

Concentration and Characteristic of PAHs

The total concentrations from 16 parents of PAHs in all various green mussels size were ranged from 15.45 to 126.47 ng.g^{-1} ww, and the means +/- SD for the smallest, small, medium, and the biggest sizes were 1.29 ± 0.142; 1.65 ± 0.187; 1.85 ± 0.155; and 1.97 ± 0.190 g, respectively (Figure 3a). The highest average concentration of total PAHs was detected in the biggest size. The average of total PAH concentration in flesh and guts parts in medium and biggest size of green mussels were ranged from 14.04 to 88.49 ng.g^{-1} ww. The means +/- SD for flesh (medium), guts (medium), flesh (biggest), and guts (biggest) parts were 1.43 ± 0.394; 1.62 ± 0.002; 1.55 ± 0.035; and 1.74 ± 0.292 g, respectively (Figure 3b). The highest average concentration of total PAHs was detected in the guts of the biggest size. The smallest size mussel contained the lowest PAH exposure among other sizes. The PAH level in the smallest size was almost 5 folds, the small size was 2.1 folds, and the medium size was 1.3 folds lower than those in the biggest size of green mussels. The ANOVA test was used to evaluate PAH levels' interspecific differences in various green mussel sizes and flesh and guts. The result did not show statistical significance p > 0.05. In flesh and guts, the ratio of PAH level percentage was 37-44% (flesh) and 56-63% (guts) identified in the medium and biggest sizes.

The increasing size of green mussel may accumulate the PAH more than the smaller one, due to the capability of the biggest size to absorb more PAH compounds than the other sizes. In addition, PAHs are more prone to be accumulated in guts than in flesh of the mussel because this organ plays an important role in digesting and processing the ingested food (which probably also

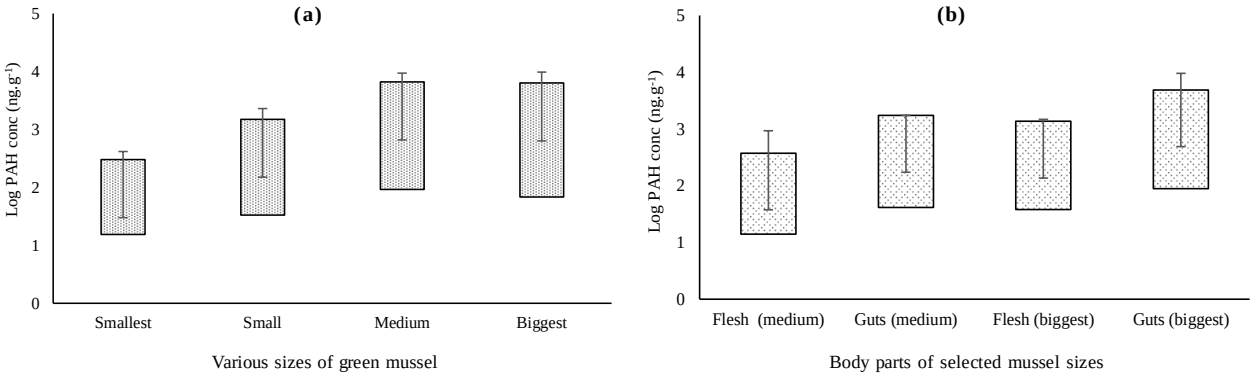


Figure 3. The average of PAH concentration in green mussels; (a) whole and (b) flesh and guts.

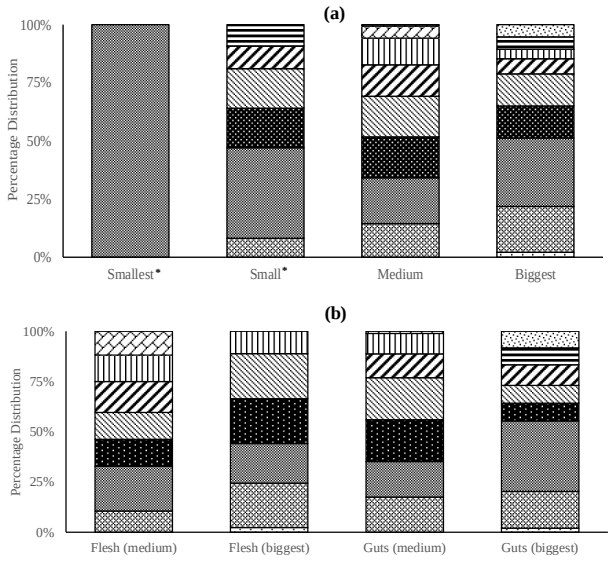
contains other pollutants PAHs). The digestion system in bivalve organisms was reported to be the predominant storage of organic contaminants (Wang et al., 2020). Furthermore, PAH concentrations in green mussel in this study showed to have similar results from those observed in a previous study (i.e. 20-390 ng.g^{-1}), even though the previous study did not reflect Cilincing waters specifically (Dwiyitno et al., 2016; Falahudin & Khozanah, 2012). This condition could occur due to PAH pollutants entering Jakarta Bay annually (Rinawati et al., 2012). The existence of green mussels as a filter feeder biota can accumulate pollutants and can adapt to a polluted environment (Richardson et al., 2005). The PAH level in the present study has nearly similar to those of previous studies that took place in India, Malaysia, Thailand, and Philippine coastal areas (Isobe et al., 2007), which is massive with anthropogenic activities and industries (OECD, 2019).

One of the environmental quality parameters of Jakarta Bay was represented by the PAH level in seawater and sediment. In the previous studies, PAH level in seawater was ranged from 30-982 ng.L^{-1} (Edward, 2017), and PAH level in sediment was ranged from 30 to 2,600 ng.g^{-1} (Dwiyitno et al., 2016). The occurrence of PAH in seawater and sediment will certainly impact the living biota around the Cilincing water ecosystem. In the water column, green mussel absorbed PAH contaminants present in seawater and accumulated them in its body's compartment. Hence, those PAH contaminants source in green mussel were absorbed from seawater, and it could reflect actual PAH level in the aquatic environment.

A similar study of PAH level in oyster (*Crassostrea* sp.) was conducted in Can Gio coastal wetland Vietnam. The result showed that PAH levels in oysters ranged from 3.26 to 64.45 ng.g^{-1} ww with Σ3-aromatic rings as the predominant in PAH compounds (Pham et al., 2020). It has a similar result with the present study. Another study of PAH level in green mussel showed similar results such as those in Straits of Johore in Singapore (36–244 ng.g^{-1}) (Yap, Shahbazi, & Zakaria, 2012), Penang Bridge of Penang in Malaysia (265-273 ng.g^{-1}), Yaring of Pattani in Thailand (85 ng.g^{-1}), and Sapian Bay of Capiz in Philippines (75 ng.g^{-1}) (Isobe et al., 2007).

The Σ3-aromatic rings, such as acenaphthene, fluorene, anthracene and phenanthrene (> 70%), were predominantly detected in the biggest size mussels (Figure 4a). The flesh and guts contained massive exposure of PAHs over the medium size (Figure 4b). The ring domination of PAH compounds decreased from high to low as the following order: 3-rings (71.83%) > 2-rings (15.00%) > 4-rings (9.26%) > 5-rings (3.72%) > 6-rings (0.19%).

These LMW of PAHs were in accordance with those previous studies in similar sites (Dwiyitno et al., 2016) and identical distribution patterns of 2 – 3 rings PAHs were in accordance to the previous studies (Olenycz et al., 2015; Wang et al., 2020). Eleven types of total PAH congeners were identified in the green mussel. The PAH congeners were widely distributed in the big sizes (such as in the biggest and medium sizes of mussel) compared to those in the smallest size. For example, the PAH types in the guts and flesh of the biggest and medium sizes contained six to nine types of PAH, where as only one PAH type was observed from the smallest size (Fig. 4). Acenaphthene compound was predominantly more than 34% of TPAHs. The dominance of acenaphthene as LMW group was found regularly with the PAH congeners found in mussels from other marine environment systems (Kucuksezgin, Gonul, Pazi, Ubay, & Guclusoy, 2020; Roldán-Wong, Kidd, Ceballos-Vázquez, Rivera-Camacho, & Arellano-Martínez, 2020; Wang et al., 2020). In addition, indeno[123-cd]pyrene was detected as the lowest PAHs composition (<1%), followed by benzo[a]pyrene and benzo[k]fluoranthene (>3%) (Figure 4b). These results indicated that mussels can accumulate PAHs compounds and have the ability to uptake particle-associated contaminants in the water column both as particulate organic matter or



*Flesh and guts in mussel's size of smallest and small were not analyzed due to their separation difficulty.

Figure 4. Characteristics, composition and distribution of PAH congeners in four different green mussels groups: (a) the smallest, small, medium, and the biggest sizes; (b) flesh and guts parts of medium and the biggest sizes group only

resuspended cycle of particulate on surface sediment (Farrington et al., 2016).

Principal component analysis (PCA) has been utilized to further understand the relationship between PAH congeners' relationship with various mussel organism size (Glad et al., 2017). All the statistical data in PCA analysis were performed with Primer 7. Two principal components (PC) accounted for 88.9% of the total variance size of green mussels. The first principal component (PC1) explains 51.1% of the total variance. It is dominated by naphthalene, acenaphthene, chrysene, and benzo[k]fluoranthene (Figure 5a). As a result, these PAH congeners likely originated combinate from LMW and HMW group. The second component (PC2) accounting 37.8% of the total variance. It is characterized by 2-bromonaphthalene, anthracene, phenanthrene, pyrene, benzo[a]pyrene, and indeno[1,2,3-cd]pyrene (Figure 5a). A combination of PAH congeners from LMW and HWM indicated incomplete combustion from engine vehicle activities and emission from industrial activities (Ghanavati, Nazarpour, & Watts, 2019). In this study, PCA analysis was divided into three cluster groups; they were smallest and biggest size as the first cluster, medium size as the second cluster, and small size as the third cluster. The PCA results statistically showed that the PAH contaminant distribution was smaller along with the smaller green mussels' size (Figure 5a).

Furthermore, two (PCA) accounted for 84.2% of total variances in flesh and guts samples. The first principal component (PC1) explains 49.7% of the total variance. It is dominated by acenaphthene, chrysene, benzo[k]fluoranthene, 2-bromonaphthalene, and naphthalene. The second component (PC2) accounts for 34.5% of the total variance. It is characterized by benzo(a)pyrene and phenanthrene (Figure 5b). The PCA analysis in flesh and guts showed a minor significant correlation between flesh and guts both in the medium and the biggest size. Each part has a different type of contaminant and is not related to each other.

Previously, several PAH exposure studies have been conducted for various species of mussels and locations worldwide, including Indonesia. The average exposure of PAH in green mussel (*P. viridis*) in the present study (20-97.29 ng.g^{-1}) was lower by 93 folds, 29 folds and 12 folds than those reported in the mussel of *Mytilus galloprovincialis* in the Marmara Sea of Turkey (664-9083 ng.g^{-1}), *Mytilus edulis* in coastal of Algerian (68.1-2892.1 ng.g^{-1}), and *Mytella guyanensis* in Gulf of Paria in Trinidad (201.80-1200.08 ng.g^{-1}), respectively (Balcýoðlu, 2016; Benali et al., 2017; Balgobin & Singh, 2018). Baumard et al. (1998) proposed a category of PAH concentration in mussels which divided into three classifications as follows: low (0-100 ng.g^{-1}), moderate (100-1,000 ng.g^{-1}), and high (1,000-10,000 ng.g^{-1}). The present study was classified into a low category of exposure of PAHs in mussels. Meanwhile, the exposure of PAH in several types of mussels in Europe and East Asia was less than 500 ng.g^{-1} (low to moderate category), such as in Harbour of Norway, Mediterranean Sea of France-Italy, Rijeka Bay of Croatia, and Pacific Coast of Japan (Table 2) (Andral et al., 2011; Glad et al., 2017; Onozato, Nishigaki, & Okoshi, 2016; Schøyen et al., 2017). The Mediterranian sea and its surrounding (Europe) and the Pacific coast of Japan (East Asia) have low to moderate category due to high flushing rate, as a consequence, the dilution effect is greater than that of organic pollutants (Andral et al., 2011; Onozato et al., 2016).

Source Apportionment of PAHs

The LMW PAHs commonly come from pyrogenic processes which are the incomplete combustion of

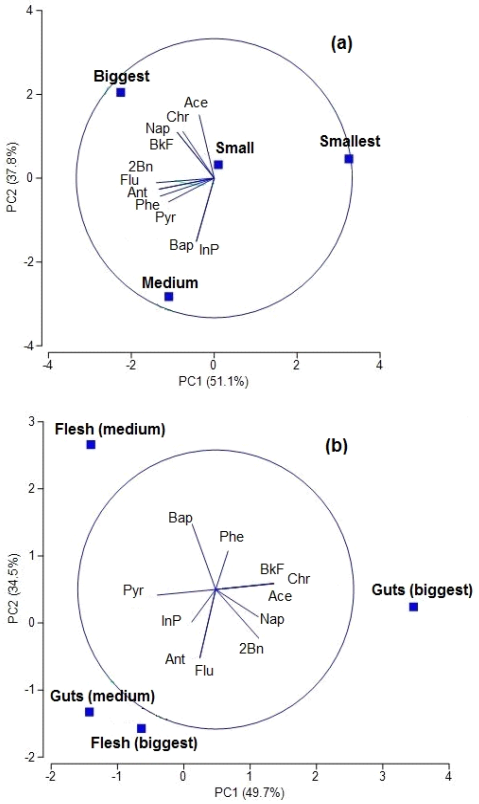


Figure 5. PCA analysis to identify the correlation between PAH congeners in green mussel: (a) whole part (the smallest, small, medium, and the biggest) and (b) flesh and gut parts (medium and the biggest only).

fossil fuels and biomass (> 300 °C), or from an oil spill (Tobiszewski & Namieœnik, 2012) and slow maturation of organic matter (Olenycz et al., 2015). The data relating to the ratio of ΣLMW/ΣHMW showed that the main source of PAHs pollutants was predominantly affected by the petrogenic process (> 1) in green mussel (Figure 6) (Tobiszewski & Namieœnik, 2012). These petrogenic source uptake in green mussel might be related to the extensive shipyard and cargo activities in Tanjung Priok International Port, geographically located adjacent to aquaculture facilities in Cilincing waters. A report from Rinawati et al. (2012) mentioned that the source of PAH compounds in Cilincing water (as habitant of green mussel) was a petrogenic signature with a relatively abundant functional group of alkyl-hydrocarbons.

The parent PAH diagnostic binary ratios have been widely used to detect the source of PAH and its derivate (Yunker et al., 2002). The PAH diagnostic binary ratios show the substantial variability and substantial similarity source (Galarneau, 2008). In this study, anthracene/(anthracene+phenanthrene) (Ant/(Ant+Phe)) and anthracene/178 (Ant/178) were used to distinguish between petrogenic and pyrolytic potential sources (Huang et al., 2012). Ant/(Ant+Phe)'s diagnostic binary ratio can be applied to identify PAHs' potential sources (Figure 7a). On the other hand, the source of PAH pollutants can be determined using characteristics and the diagnostic ratio of Ant/178 (Figure 7b). Suppose the ratio of (An/178) is < 0.1. In that case, this indicates of petrogenic contamination, whilst ratio 0.1, indicates the incomplete combustion produced from vehicle or biomass (Yunker et al., 2002).

Based on the diagnostic binary ratio of Ant/(Ant+Phe) and Ant/178,the present study result showed that PAHs probably came from the mixed sources of petrogenic and pyrogenic processes. It was deducted with a presumption that primarily originated from oil

Table 2. Comparison of PAH distribution at global scale

Location/country	Species/genus	Concentration (ng.g^{-1})	Reference
Harbour of Norway	*Mytilus edulis*	77-265	Schøyen et al. (2017)
Coastal of Algerian	*Mytilus galloprovincialis*	68.1-2,892.1	Benali et al. (2017)
Jakarta Bay, Indonesia	*Perna viridis*	20-390	Dwiyitno et al. (2016)
Rijeka Bay, Croatia	*Mytilus galloprovincialis*	108-486	Glad et al. (2017)
Gulf of California, USA	*Modiolus capax*	147.01-271.09	Roldán-Wong et al., (2020)
Marmara Sea, Turkey	*Mytilus galloprovincialis*	664-9,083	Balcıoğlu (2016)
Mediterranean sea, France-Italy	*Mytilus galloprovincialis*	38.40-150.50	Andral et al. (2011)
Birch Harbor, Maine, USA	*Mytilus edulis*	105-180	Apeti et al. (2010)
Gulf of Paria, Trinidad	*Mytella guyanensis*	201.80-1,200.08	Balgobin & Singh (2018)
Port and coastal, France	*Mimachlamys varia*	177.12-212.56	Breitwieser et al. (2016)
Pacific coast of Japan	*Mytilus galloprovincialis*	268-351	Onozato et al. (2016)
Cilincing water, Jakarta Bay	*Perna viridis*	20-61.35	This study

Note: Low (0-100 ng.g^{-1}), Moderate (100-1,000 ng.g^{-1}), and High (1,000-10,000 ng.g^{-1})

spills, shipyard and cargo activities, and leakage tanker shipping (Isobe et al., 2007). Petrogenic process predominantly affected in the small, medium and the biggest size of Green mussels. This finding results reported by Isobe et al. (2007), who mentioned that mussels in South and Southeast Asia are heavily affected by the petrogenic process. While pyrogenic process predominantly affected the smallest size of green mussel, it was possibly sourced from the waste of industry and household, land run-off, and vehicle combustion from the mainland.

Distribution and source of PAH pollutants in Cilincing waters as the habitat of green mussel were affected by many factors. Physical hydrodynamic processes influences temporal and spatial variations of tides, wind and riverine inputs particularly in Jakarta Bay. Northwest monsoon (NWM) and southeast monsoon (SEW) as monsoon regimes predominantly control winds over the Jakarta Bay Sea, including over Indonesian seas (Koropitan, Ikeda, Damar, & Yamanaka, 2009), as well as affect precipitation, river discharges and water circulation (Aldrian & Susanto, 2003).

Human Health Risk Assessment

The carcinogenicity and endocrine activities disrupted the documentation of PAHs. Variances individual PAHs have different toxic levels, both carcinogenicity in LMW and HMW of PAH compounds. Toxic potency was characterized by using toxic equivalent concentration (TEQ_{BaP}) value based on the toxic equivalent factor (TEF) (Qu et al., 2020). PECwas used to estimate carcinogenic contaminants and evaluated biota consumption risk to human health. PEC values in all various sizes of the smallest, small, medium, and the biggest were 0.020; 0.086; 3.954; and 0.624 $ng.g^{-1}$, respectively. The contributions of different PAHs in various sizes of green mussel to the total PEC were decreased as follows: medium > biggest > small > smallest. These results were lower than those of other studies of PAH levels in bivalve organisms in coastal areas (Barhoumi et al., 2016; Pham et al., 2020). In this study, the BaP concentration was 3.84 $ng.g^{-1}$ which was lower than the maximum level of 6 $ng.g^{-1}$ set by the National Agency of Drug and Food Control (BPOM) for smoked mussels (BPOM, 2018).

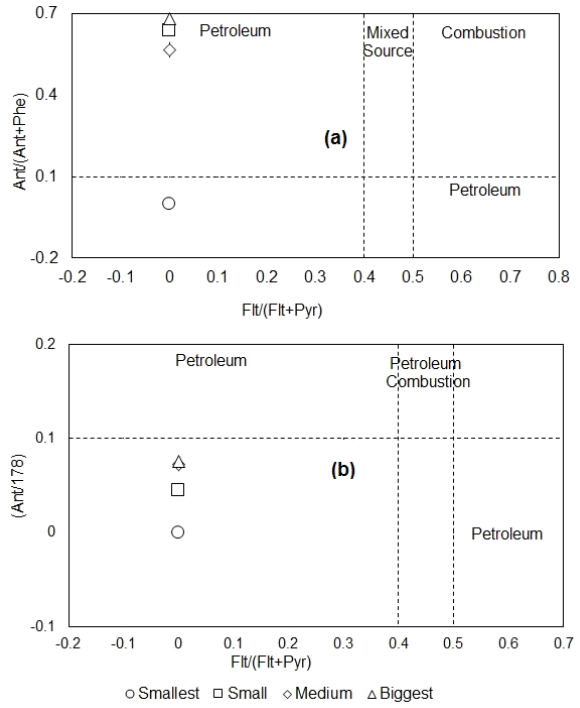


Figure 7. The diagnostic binary ratio of Ant/(Ant+Phe) (a) and Ant/178 (b) of PAH compounds according to Yunker et al., (2002).

The medium size of blue mussel can absorb enormous nutrients and other organic substances higher than the biggest size (Tagliarolo, Clavier, Chauvaud, Koken, & Grall, 2012). Therefore, it can be presumed that the medium size of green mussel can absorb higher PAH contaminants, including carcinogenic compounds, than the biggest size. In addition, the metabolic system of the biggest mussel is more stable so that PAH levels will also decrease indirectly, while in the medium mussel, the metabolic system is growing up continuously toward the adult phase (Rajagopal, Van Der Gaag, Van Der Velde, & Jenner, 2005). The biggest size mussel has less mortality due to higher resistance to PAH contaminant than that of the small one (Rajagopal et al., 2005). Metabolism of PAH compound in bivalve is closely associated with high protein content in its body. Several proteins function as enzyme biomarkers that metabolize PAH such as acetylcholinesterase (AChE), benzo[a]pyrene hydroxylase (BPH), multixenobiotic resistance (MXR), glutathione S-transferase (GST), catalase (CAT) and malondialdehyde (Kamel et al., 2014). In addition, several environmental factors affected the fluctuation of PAH levels in mussel, such as the level concentration of PAH in the water column, the kinetics of PAH compounds, and mussel's ability to absorb and metabolize PAH contaminants (Galgani et al., 2011). However, it should be considered that every green mussel (small, medium, or big mussels) that contains carcinogenic compounds has the potential to cause adverse health effects.

The exces cancer values (ECR) values in green mussels ranged from 0.007 x 10^{-6} to 1.443 x 10^{-6} with an average of 0.429 x 10^{-6}. These results are less than those reported in the previous study for seafood such as in mussel (*Mytilus galloprovincialis*) in Bizerte Lagoon - Tunisia (2.1 x 10^{-8} and 1.0 x 10^{-6}) (Barhoumi et al., 2016), oyster (*Crassostrea rivularis*) in

Figure 8. Comparison of PAH carcinogenic (PAH_4) on the smallest, small, medium, and the biggest sizes of green mussel in Cilincing waters, Jakarta Bay

Guangdong Coastal - China (5.1×10^{-6} and 6.8×10^{-6}), and fish (*Drapane africana*) in Tema Port - Ghana (7×10^{-7} and 4×10^{-4}). Conversely, the result in this study of ECR value is higher than that of oyster (*Crassostrea sp*.) in Can Gio Coastal Wetland - Vietnam (0.37×10^{-8} and 4.2×10^{-8}) (Pham et al., 2020). Shi et al. (2016) proposed two categories of ECR over the lifespan (70 years): (1) 1 in 1,000,000 (10^{-6}) is considered as "acceptable"; (2) 1 in 10,000 (10^{-4}) is considered as "serious risk". In our study, the observed ECR values of Green Mussel from Cilincing water (Jakarta Bay) were categorised as acceptable concentration based on study of Shi et al (2016), hence its consumption by human might caused a minor negative impact of carcinogenic risk.

Furthermore, the impact of PAH compound in mussels could be identified through the level of carcinogenic PAH such as benzo[a]pyrene, benzo[a]anthracene, benzo[b]fluoranthene, and chrysene. They are known as appropriate PAHs indicators in mussel consumption (BPOM, 2018; EU-Commission, 2011). The results of carcinogenic PAH (PAH_4) level for the smallest, small, medium, and the biggest sizes were undetected; 4.35; 3.84; and 5.21 $ng.g^{-1}$, respectively. These values were still lower than the standard value of the National Agency of Drug and Food Control (BPOM) regulation for bivalve biota, which is 35 $ng.g^{-1}$ (Figure 8). PAH_2 compounds (benzo [a]pyrene and chrysene) and PAH_8 (benzo[a]pyrene, benzo[a]anthracene, benzo[b]fluoranthene, benzo[k] fluoranthene, benzo[ghi]perylene, chrysene, dibenzo [a,h]anthracene, and indeno[1,2,3-cd] pyrene) are common indicators used for food, but the PAH_4 is more acceptable (EFSA, 2008). Based on the food safety standards, green mussels in Cilincing waters are acceptable for consumption. However, green mussels needs to be consumed cautiously because of its carcinogenic content.

It needs to be considered that this data probability showed a description of the potential health risk of consuming green mussel contaminated by PAH carcinogenic. Many other factors affect the risk assessment, such as duration of contaminant exposure, misinterpretation of consumption data, or level concentration of carcinogenic contaminants. These factors might cause under or overestimation of dietary exposure of carcinogenic compounds intake to humans. Therefore, further study is important to carry out to fill the gap of the data and to consider other effects of other organic contaminants.

Conclusion

The study of PAH in green mussel only assesses the level concentration and the potential risk that might be occurred based on theoretical estimates, with PAH compounds as a single parameter. The 16 major parents of PAH were analyzed, and the highest concentration of TPAHs was detected in guts of the biggest size of the green mussel. Based on the obtained value of diagnostic binary ratios (ΣLMW/ ΣHMW, Ant/ (Ant+Phe), and Ant/178), the PAHs originated from mixed sources of petrogenic and pyrogenic process, with a presumed that mainly originated from oil spills and petroleum waste from shipyards and cargo activities. Oil spills is predominated with Σ3-rings aromatic of PAH congeners, such as acenaphthene, fluorene, anthracene, and phenanthrene. Based on ECR value, the PAH levels in the green mussels in this study are considered as an acceptable concentration. Following the threshold level of food safety standards set by BPOM, the green mussels from the Cilincing water are acceptable for human consumption due to low risk of carcinogen. However, in the presence of carcinogenic compound content in green mussel, a precaution must be taken for longterm consumption.

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