Assessing the distribution, origins, and ecological risk of polycyclic aromatic hydrocarbons (PAHs) in the habitat of Medaka fish at Keramat Kebo River and Estuary, Tangerang, Banten

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Abstract. Indonesia has a variety of coastal systems such as coral reef, mangrove, seagrass, mudflat, and dune, each of which has high biodiversity of species. The primary concern in Indonesia is that rapid economic growth would endanger some essential natural ecosystems and resources, and cause deterioration of environmental condition. As a part of bioindicator development to recognize pollutants with small fish of the genus Oryzias, this study was conducted to assess baseline status of PAHs distribution and sources in seawater, sediments and Oryzias fish. The ecological risk of PAHs in sediments was also evaluated. Concentrations of fifteen USEPA PAH based on GCMS analysis in seawater, sediments, and Oryzias fish vary from 0.00 to 30,600 ng/l, 6.7 to 138.6 ng/g dry weight (dw) and 25.2 to 30.5 ng/g dw, respectively. Based on the diagnostic ratio of PAH compounds, the potential sources of PAHs originated mainly from pyrogenic sources. The status of sediment from this Oryzias fish habitat was considered to be low polluted with PAHs.

1. Introduction

PAHs are one of emerging organic pollutants that represent a severe worldwide concern for ecological and human health due to their characteristics such as persistent, bioaccumulate and also carcinogenic [1]. Toxicity of PAHs is increasing along with the increase of the number of compounds’ molecular weight. Also, the extreme environmental condition has a significant alteration on the environmental fate and behavior of chemical toxicants including PAHs to be more toxic to marine biota and human health [2,3]. The previous study also reported that PAH compounds also have the capability as endocrine disrupting chemicals which can disrupt endocrine development, reproduction activity, the immune and nervous system of organisms [4].

Different approaches were developed to determine PAHs sources such as the use of a unique compound as molecular markers and ratio diagnostic model by using differences in thermodynamic stability among PAH species. Those sources are petrogenic (crude oil and petroleum product), pyrogenic (incomplete combustion of organic matter, for example, wood, coal, and grass), and natural sources (oil seeps, biogenesis, and volcano) [5,6].

River and estuary, as the transitional area between land and sea, provide crucial habitat for the different type of biota [6,7]. The Medaka fish is one of the standard experimental fish used in the laboratory and also as a bioindicator for environmental monitoring [8]. However, in Indonesia, as this fish is economically unimportant, the information regarding its habitat, ecology, and taxonomy is scarce. Therefore, as part of an investigation to provide information for further assessment on
bioindicator selection, we made a baseline study to assess the health of the habitat of Medaka fish through determining PAHs contamination.

In detail, the objectives of this study are to determine PAHs compounds in seawater, sediments, and Medaka fish from Keramat Kebo river and estuary, and to identify PAH source and ecological risk assessment of PAHs in sediment samples.

2. Material and Methods

2.1. Sample Collection

Samples of seawater, sediment, and Medaka fish were taken from the river and estuary of Keramat Kebo, Tangerang, Banten known as the habitat of Medaka fish (figure 1). Stainless steel water sampler was used to collect surface water samples at a depth of less than 1.0 meter and the samples were transferred to 2.5 L amber glass bottle. Sediment samples were collected at each site with Van Veen Grab sampler. The surface layer of sediment was taken using an aluminum spoon previously cleaned with DCM and placed in a pre-cleaned glass bottle, covered with aluminum foil and stored under 4 °C until further analysis. The Medaka fish were taken using fish net and directly placed in the glass bottle similar with sediments.

Figure 1. Sampling location for seawater, sediments and Medaka fish in Keramat Kebo River and Estuary.

2.2. Sample preparation for GC/MS

Surface water samples were immediately filtered through 0.45 μm glass fiber filters (Whatman GF/C), and 2 L filtrate water was extracted with 60, 30 and 30 mL n-hexane using a separator funnel. The raw extracts were dried from water by passing through Na2SO4 and evaporated until 1 mL.

The sediment sample was divided into two parts. A subsample of 10gram of wet sediment was transferred to a pre-weighed porcelain cup and dried in an oven overnight (minimum 12hrs) at 105°C to analyze the dry weight (dw) of sediment. Another part, 40gram of wet sediment was dried in an oven overnight at 60 °C. After that, the dried sediment was added Na2SO4 and ground into fine powder. The extraction of PAHs was carried out according to the method reported by Holden and Marsden, 1969; Greve and Grevenstuk, 1975; Duinker and Hillebrand, 1978. Each sample was
subsequently placed to the Soxhlet extractor, added 120 mL of dichloromethane (DCM), and extracted for 8 hours. The extract was concentrated to 1 mL.

The resulting extract from sediment and water sample was loaded into alumina gel column and elution with 10 mL 4% diethyl ether in hexane for cleanup. The column was conditioned with DCM and hexane. The purified extracts were concentrated to a volume of 1 mL. After cleaned up with alumina, the purified extract was separated and fractionated using silica gel column. The column also was pre-conditioned with 10 mL DCM and hexane. Non-polar fraction (F1) was eluted with hexane for analysis of saturated hydrocarbon; and polar fraction (F2) by 10% diethyl ether in hexane for analysis of PAHs. Tissue samples of Medaka fish were prepared similarly to sediment samples except for the weight which is around 10g and it did not require drying in the oven. Na2SO4 is directly added to dry homogenize tissue.

2.3. GC/MS analysis

A qualitative and quantitative analysis of PAHs was carried out using Trace 1300 GC coupled to an ISQ LT– Single Quadrupole MSD and used under selected ion monitoring mode (Thermo Scientific, USA). External standards of QTM PAH Mix in the concentration of 2.5, 5 and 20 ng/µL were used for quantification. Fifteen EPA priority PAH compounds were determined and quantified based on retention time shifts. Primary and secondary ions of each compound include: naphthalene (Nap), acenaphthylene (Acethy), acenaphthen (Ace), fluorene (Fl), phenanthrene (Phe), anthracene (Ant), fluoranthene (Flu), pyrene (Pyr), benzo[a]anthracene (BaA), chrysene (Chr), benzo[b]fluoranthene (BbF), benzo[k]fluoranthene (BkF), benzo[a]pyrene (BaP), indeno[1,2,3-cd]pyrene (InP), dibenzo[a,h]anthracene (DBA), and benzo[g,h,i]perylene (BgP).

2.4. Quality control and quality assurance

All glassware was treated with different treatment to eliminate any remaining contaminants such as washing it with Teefol detergent, soaking it with hot water, and rinsing it with distilled water. Field blank was applied to control the quality of methodology, and no quantifiable PAH compounds were detected. If significant concentration in the blank sample is found, the areas of corresponding compounds will be subtracted. Quantitative analysis for each PAHs compound was based on Signal-to-Noise Ratios at minimal 3 and direct confirmation with a qualitative assessment. All calibration curve of each PAHs standard had satisfactory value for the coefficient of determination (R2>0.99).

2.5. Data Analysis

All data were calculated using Microsoft Office Excel 2007 for total PAHs concentration, individual composition, diagnostic ratio and analysis ecological risk assessment. Several ratios of individual PAHs were applied to distinguish the sources of PAH, including Flu/Pyr, Flu/ (Flu + Pyr), BaP/(BaP + Chr), and BaA/228. The higher value of Flu/Pyr (>1), Flu/(Flu+Pyr) (>0.5), and BaA/228 (>0.35) indicates the dominance of biomass combustion, for example, kerosene, grass, wood, coal combustions, and creosote, whereas corresponding values below 1, 0.4, and 0.2 represent petroleum sources. The last ratio is BaP/(BaP+Chr); the value more than 0.6 indicates petroleum sources, between 0.4 and 0.6 for a mixture between petroleum and combustion sources and less than 0.2 for combustion sources [6].

3. Result and Discussion

3.1. Concentration, composition, and distribution of PAHs

The ranges of total PAHs concentration in seawater, sediment and fish were from below MDL to 30,600 ng/l (Average: 11,823 ng/l), 6.7 to 138.6 ng/g dw (48.8 ng/g dw), and 25.2 to 30.5 ng/g dw (27.9 ng/g dw), respectively. The highest concentration of PAHs in sediment was obtained from station TNG8 (3083 ng/g ww), whereas the lowest concentration was measured at station TNG2 (113.7 ng/g dw) located in Keramat Kebo River, and it only detected five PAH compounds. While in seawater, the concentration of PAHs was high at station TNG2 (30,600 ng/l ) and varied across part of
the river from upstream to the estuary of Keramat Kebo River. The concentration of PAHs in Medaka fish was lower than in the sediment. Furthermore, different factors influenced the higher concentration of PAHs in sediments, including grain size of sediment, organic content, type of sediment, physical characteristics of water and also the distance to the sources of pollutants [9]. Compared to the national regulation for seawater quality guidelines for marine biota in Indonesia No. 51/2004, generally the total PAHs concentration in seawater in the present study is lower than 30,000 ng/L, except at station 8. This condition means that prolonged exposure to these contaminants will cause an adverse effect on organisms. However, high concentrations of PAHs in surface water depends on several factors such as fisherman vessels traffic and tidal current in the river. Therefore, the current finding cannot be generalized as a condition in Keramat Kebo River and estuary because Medaka fish as bioindicator species are still alive.

Furthermore, an assessment was conducted to determine the composition patterns of PAHs in sediment and surface water samples (figure 2). In the surface water, PAHs composition mainly consisted of 2-ring of benzene and Naphthalene which were predominant compounds, whereas in the sediment 4- and 5-ring showed marked predominance and benzo[a]pyrene as the highest. PAHs composition in Medaka fish showed the least variation of PAH compound; only three compounds of PAH were found. The results suggested that low molecular weight of PAHs were easy to dissolve in water and the heavier compounds of PAH with larger Kow would be adsorbed to organic matter and sink to sediment [10]. Furthermore, another study also found that LMW PAHs are less thermodynamically stable and able to degrade by a microorganism in sediment; therefore the composition of PAHs in sediment mainly consists of higher molecular weight PAHs with 4-6 numbers of rings. Due to their characters, persistent and potential carcinogenic, more studies suggested that HMW PAHs would be a major compound need to assess on ecological risk assessment of sediment [11].

Figure 2. Composition of PAHs in water, sediments and Medaka fish from Keramat Kebo river.

3.2. Sources and ecological risk assessment of PAHs in sediment
The results from diagnostic ratio analysis revealed that petrogenic sources from petroleum emission are indicated by ratio BaA/228 with a value below 0.2. Another ratio such as BaP/(BaP+Chr), Flu/Pyr and Flu/(Flu+Pyr) mainly confirms a mix between petroleum and biomass combustion or only biomass combustion with value ranged from 0.11 to 0.59, 0.92 to 16.68 and 0.48 to 0.94, respectively. Petroleum combustion is only represented by ratio Flu/(Flu+Pyr) in station TGN1 with value 0.48 (Table 1). The present calculation shows that in all stations, the dominance of PAHs sources in sediment is pyrogenic sources, and petrogenic sources are less detected. This estimation is consistent
with the abundance of high molecular weight of PAHs in sediments as a result of incomplete combustion of biomass.

The results from ecological risk assessment are shown in Table 2. Practically, the data of individual PAH compounds were compared to the values of effects range low (ERL) and effects range median (ERM) from the previous study. This assessment was to classify the station into three different ranges, below ERL, between ERL and ERM, and the last upper ERM which represents as rarely, occasionally, or frequently related to adverse effects [9] and [12]. The results revealed that individual PAHs in sediment from Keramat Kebo River were mainly below the ERL, only 1 or 2 stations were between ERL and ERM. No detected concentration of individual of PAHs was higher than ERM value. However, even though there was rarely adverse effect of each PAHs in the sediments, protection and future monitoring are needed to conduct in Keramat Kebo river as a candidate for Medaka fish habitat. Also, the river is vulnerable from contamination from rapid urbanization and industrial development in the upstream area.

| Station/ Diagnostic Ratio | Flu/Pyr | Flu/(Flu+Pyr) | BaP/(BaP+Chr) | BaA/228 |
|---------------------------|---------|-------------|---------------|---------|
| TGN1                      | 0.92    | 0.48        | 0.23          | 0.00    |
| TGN2                      | 1.4     | 0.60        | 0.11          | 0.00    |
| TGN3                      | 1.82    | 0.65        | 0.21          | 0.00    |
| TGN4                      | 4.63    | 0.82        | 0.43          | 0.00    |
| TGN5                      | 1.90    | 0.66        | 0.14          | 0.00    |
| TGN6                      | 1.57    | 0.61        | ND            | 0.00    |
| TGN7                      | ND      | ND          | 0.46          | 0.00    |
| TGN8                      | 16.68   | 0.94        | 0.59          | 0.00    |

| Compound                  | Concentration range | Mean values | Guideline values | Station |
|---------------------------|---------------------|-------------|------------------|---------|
|                           |                     |             | ERL               | ERM     | < ERL | ERL - ERM > ERM |
| Naphthalene               | ND                  | ND          | 160              | 2100    | all station | 3          |
| Acenaphthene              | ND                  | ND          | 44               | 640     | all station | 3          |
| Acenaphthylene            | ND                  | ND          | 16               | 500     | all station | 1          |
| Fluorene                  | 2-3                 | 3           | 19               | 540     | all station | 1          |
| Phenanthrene              | ND-243              | 81          | 240              | 1500    | 7     | 1          |
| Fluoranthene              | 12 – 744            | 242         | 600              | 5100    | 7     | 1          |
| Pyrene                    | 7 – 417             | 140         | 665              | 2600    | all station | 1          |
| Benzo (a) Anthracene      | 10 – 593            | 135         | 261              | 1600    | 7     | 1          |
| Chrysene                  | 32 – 622            | 253         | 384              | 2800    | 6     | 2          |
| Benzo (a) Pyrene          | ND – 478            | 132         | 430              | 1600    | 7     | 1          |
4. Conclusions
In this study, the baseline condition of Medaka fish's habitat had been studied. Total concentrations of PAHs from the highest to the lowest were found in the sediments, fish, and seawater, respectively. Variations of compound molecular weight were observed in all samples, whereas lower molecular weight was mainly found in surface water and high molecular weight in the sediments and fish. The potential sources of PAHs originated mainly from pyrogenic sources. Also, as compared to the values of effects range low (ERL) and effects range median (ERM), the status of sediments was considered to be low polluted with PAHs.

Acknowledgments
This study was supported by DIPA 2014 fiscal year in Research Center for Oceanography, Indonesian Institute of Sciences, Government of Indonesia. Furthermore, we would like to thank PI of this project, Suratno for his valuable support on this study and also to M. Reza Cordova for his technical assistance on ArcGIS software application. We also want to acknowledge Herman Rahayaan for his laboratory assistance. We acknowledge the reviewer and the editor of GCGE2017 for their constructive comments and suggestions.

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