The Fate and Toxicity assessment of Polycyclic Aromatic Hydrocarbons (PAHs) in Water Streams of Malaysia

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Abstract

Polycyclic aromatic hydrocarbons (PAHs) are pollutants released to the environment through natural and anthropogenic activities. PAHs if not being treated properly, are able to be transformed to various derivatives (daughters). There are possibilities that both PAHs and their daughter products to be toxic to environment and human. In this study, samples from influent of a water treatment plant (WTP) and effluent of sewage treatment plant (STP) were obtained to investigate presence of PAHs and their derivatives. Analysis of samples were conducted using Gas Chromatography Mass Spectrometry (GC/MS) and conversion formulation of the identified PAHs were found out. Furthermore, toxicity study carried out using guppy (\textit{Poecilia reticulate}) to establish toxicity intensity of PAHs parents and daughters. The experiments were performed with three replicates using a total of 100 guppies for all definite tests through steady state method of acute toxicity test and mortality rates were investigated in 24, 48, 72 and 96 hours. Results indicated that phthalic acid and benzoic acid are significant compounds in both sampling points. Phthalic acid was found to be derived from Chrysene and Naphthalene whereas benzoic acid resulted from degradation of Fluoranthene. Mortality rates were 20\% and 10\%, in WTP influent and STP effluent, respectively. No mortality was observed in control tank which indicated accuracy of toxicity test. Although, the pollution levels in both samples did not produce 50\% mortality, the findings could indicate the conditions affecting fish populations in prolonged duration.

1. Introduction

Polycyclic Aromatic Hydrocarbons (PAHs) are unique class of persistent and semi-volatile organic pollutant containing two to six fused benzene aromatic rings of carbon and hydrogen atoms [1]. PAHs originate mainly from anthropogenic processes, particularly from incomplete combustion of organic fuels and get distributed widely in the environment. Natural processes, such as volcanic eruptions and forest fires, also contribute to ambient existence of PAHs in the ecosystem. PAHs are divided into two groups based on their molecular weights. Light molecular weight (LMW) PAHs are those with two to three aromatic rings and high molecular weight (HMW) PAHs have four to six rings in their structures. LMW and HMW PAHs demonstrate different behaviors when they being released to water. LMW PAHs such as Naphthalene and Fluorine usually get emitted to the atmosphere while HMW PAHs like Benzo [a] pyrene and Benzo [ghi] perylene will remain in the water or settles in sediment at the bottom of water courses. PAHs (parent) can undergo degradation and react with other pollutants, such as sulphur dioxide,
nitrogen oxides, and ozone and produce other derivatives (daughter) [2]. Other than that, PAHs may also get degraded biologically (biodegradation). Biodegradation happens by various types of microorganisms or enzymes that break PAHs chemical bonds [3]. PAHs have been studied due to their toxicity, persistence and environmental prevalence. Distributions of PAHs in aquatic environment is significantly affected by aquatic particulates, which act as combinations of numerous complicated organic products [4]. Exposure of PAHs to light produces partially oxidized intermediates which are more susceptible to biodegradation than parent compounds [5]. Some of the short-lived metabolic products from enzymatic PAHs degradation may be also more toxic than the parent compounds. They may also be mutagenic or carcinogenic even if their parent compounds are not [6]. Fluoranthene is a PAH compound with no acute toxicity and carcinogenicity reported, whereas benzoic acid as one of the fluoranthene’s daughters is slightly toxic [7-8].

Aquatic animals such as zebra fish (Danio rerio) [9], gold fish (Carassius auratus) [10] and guppy fish (Poecilia reticulate) [11] have been used widely in bioassays to monitor surface water and effluents quality [12]. In acute conditions, PAHs have been found to induce adverse effects on fish growth, maturity, reproduction and survival. Furthermore, after biotransformation, these compounds may originate reactive products that bind DNA and may cause mutations or other alterations on the genetic material [13]. Organization for Economic Cooperation and Development (OECD) [14] has provided list of recommended aquatic animals for toxicity studies. In toxicity studies, the chosen aquatic animal is subjected to sample of water with certain concentration level. The behaviors of aquatic organisms is being studied in different time interval and mortality rates are investigated. The recommended aquatic animal is subject of research for evaluating the impact of toxic effect of chemicals on fishes. PAHs toxicity studies have been conducted on numerous fish species. Anyhow, investigations of PAHs and their daughter’s toxicity levels on Poecilia reticulate (guppy fish) are not abundant. Poecilia reticulate is widespread and cultured all over Asia, parts of Europe and America continents. This species is listed as one of the recommended test species for acute toxicity test according EPA [15] and OCED [14]. Therefore, in this study identification of various types of PAHs and their conversion paths to the daughter compounds in two different water streams in Malaysia was conducted. In addition, mortality rates of Poecilia reticulate exposed to the sampled sources were investigated to establish toxicity intensity of PAHs parents and daughter products.

2. Materials and Methods

2.1. Sampling points

Two locations were identified and selected in this research. River water intake of a local water treatment plant (Teluk Kepayang WTP) situated adjacent to Perak River was chosen to investigate its toxicity levels. The water intake for the influent of WTP Teluk Kepayang is directly from Perak River, the main river of Perak district which is the key water source for most residential, agricultural and industrial areas. Activities in those areas may directly and/or indirectly contribute to amount of PAHs in that water stream. The water at this source after treatment get distributed to the nearby districts as source of potable water. Second sampling point was effluent of sewage treatment plant (STP) of Universiti Teknologi PETRONAS (UTP). The point was selected to investigate the PAHs level and their derivatives as well as toxicity of treated wastewater which get discharged to a nearby natural lake which is habitation of various flora and fauna.

2.2. Collection and Preparation of Samples

The sites were approached from downstream, by standing facing upstream. The bottom sediment of the collection site was not disturbed during collection of water samples. The cap was removed from bottle just before sampling. Any of the parts of the bottle (outer and inner) was avoided having contact with anything. The bottle was held near its base and plunged (with the opening downward) below the water surface. The bottle was filled by running current stream water. Once full, the bottle was emptied downstream and the procedure was repeated three times for rinsing purposes. After the completion of rinsing, the bottle was filled again with the water sample, leaving around one inch of air space to allow for shaking or mixing before analysis. Sample bottles were labeled with bottle number, site identification, date and time, and packed in ice at the sampling location. They were stored at laboratory cold room at 4 °C for later usage.

2.3. Analytical Methods

The PAHs identification of the samples was conducted using Gas Chromatography Mass Spectrometry (GC/MS) 5975C model by Agilent with Agilent 7890A GC system, direct insertion probe and pyrollzer coupled to detector- Triple Axis inert XL EI/CI MSD and mass spectrometer- Quadrupole mass analyzer. A 30 m × 250 μm × 0.25 μm film thickness HP-5MS cross-linked 5% phenylmethyl-silicone column was used with the following temperature program: 60 °C for 2 minutes, ramp at 10 °C/min to 300 °C in 1 min and hold at 300 °C until 29 min run time. The injector port was 360 °C and the carrier gas was...
helium. The physico-chemical properties including pH, dissolved oxygen (DO), turbidity, chemical oxygen demand (COD) and temperature were analyzed in each sample in accordance to standard methods for the examination of water and wastewater [16].

2.4. Test Species

Aquatic animals especially fishes have vital role for the flow of energy in aquatic ecosystems as they are exposed continuously to contaminants in the natural habitat [17]. *Poecilia reticulate* (guppy fish) species were obtained from a local breeder and transported immediately within 30 minutes to the laboratory in properly aerated plastic bags. In the laboratory, a total of 100 fishes were kept in 100 L glass container. The fishes were first acclimated for a total of 14 days with continuous aeration to allow them being adapted to the new environment. The container was filled with tap water (pH = 7±0.2, DO = 8.1±0.2 mg/L and conductivity = 80±1 µs). The tap water was initially treated with sodium thiosulphate (Na2S2O3·5H2O) (8L Na2S2O3·5H2O / 30L of tap water) to remove chlorine before it is channeled into the container. The tank water was changed every 48 hours with constant aeration. The environmental conditions in the laboratory were controlled to receive 12 hours of daylight and 12 hours of darkness daily and temperature was optimized at recommended range of 23 ± 1 °C [14]. The feeding process was done with commercial fish food on daily basis and it was stopped 48 hours prior to beginning of the test.

2.5. Acute Toxicity Test

Acute toxicity test with *Poecilia reticulate* was performed according to Organization for Economic Cooperation and Development (OECD) [14]. Laboratory static tests were conducted to determine the mortality rate of fishes. Static test is more suitable for short range of 96 hours toxicity test in order to preserve the quality of the water sample and minimizing changes in test environment to the utmost condition. Test chamber was prepared and filled with 5L raw samples of influent from WTP Teluk Kepayang (NTU = 17.4±0.2, pH = 6.7±0.1, DO = 8.1±0.3 mg/L, COD = 12±1 mg/L) and effluent from UTP STP (NTU = 9.9±0.3, pH = 7.5±0.1, DO = 10.2±0.2 mg/L, COD = 10±2 mg/L). The test chamber was stocked with fishes in a ratio of 1.0 g/L water. As each fishes weigh approximately 0.5g, 10 fishes of similar size were randomly sampled and transferred with a small hand net from the acclimation tank into the test chamber. Careful steps were taken to avoid any possibility of injuries to the fishes. No dilution was done and concentration of raw samples were maintained as originally obtained from sites. A control group was made with only usage of filtered and un-chlorinated tap water without any addition of samples. The test chamber was inserted with flexible silicone tube, attaching the chamber with constant air supply for adequate dissolved oxygen throughout the test. The physico-chemical parameters were measured daily to ensure that the test solution used is not harmful for the fishes. Mortality and behavioral changes of fishes were observed at interval of 24 hours. During the experiment, dead fish were removed to prevent depletion of dissolved oxygen, hence affecting the test chamber. The mortality rate was calculated based on the number of death in each group.

3. Results and Discussion

3.1. Polycyclic Aromatic Hydrocarbons (PAHs) and their fates

The GC/MS analysis of samples collected at WTP Teluk Kepayang influent and UTP STP effluent are shown in Tables 1 and 2. It was observed that 1, 2-Benzenedicarboxylic acid (Phthalic acid) has highest concentration in WTP Teluk Kepayang influent samples and high chemical mass distribution in sample from STP effluent.

| Compound name   | Type    | Chemical mass distribution % | Classification      |
|-----------------|---------|------------------------------|---------------------|
| Acenaphthylene  | Parent  | -                            | N/A                 |
| 1(3H)-isobenzofuranone | Daughters | 2.517                       | Not classified      |
| benzaldehyde    | Daughters | 0.465                       | Not classified      |
| Chrysene        | Parent  | -                            | N/A                 |
| Anthracene      | Parent  | -                            | N/A                 |
| Phthalic acid   | Daughter | 15.697                      | Not classified      |
| Fluoranthene    | Parent  | -                            | N/A                 |
| Benzoic acid    | Daughters | 4.821                      | Slightly toxic      |
| Phenylacetic acid | Daughters | 1.358                      | Slightly toxic      |
Phthalic acid is known to be one of the main intermediates of Naphthalene, Chrysene and Anthracene. The chemical process of Naphthalene had gone through 1,2-dihydroxynaphthalene ortho-type cleavage (intradiol) or 2,3-dihydroxynaphthalene meta-type cleavage to form 2-carboxycinnamic acid. To form Phthalic Acid, 2-carboxycinnamic acid, may undergo further degradation [118]. In photocatalytic degradation of Naphthalene, phthalic acid and 9,10-anthraquinone have been found as relatively stable intermediates whereas phthalic acid were found as trace amounts in degradation of anthracene [119]. The chemical process of Anthracene to Phthalic Acid is more complex than Naphthalene. There are 3 steps to achieve Phthalic Acid from Anthracene. The first step is to undergo multicomponent dioxygenases to produce cis-1,2-dihydrodiol. Enzymatic attack will eventually form 1,2-dihydroxyanthracene. The first product will need to go through meta-ring cleavage and 2-hydroxy-3-naphthoic acid will be the second product. Lastly, Mycobacterium sp. will change the second product to phthalic acid [20]. Phthalic acid is known to be dangerous to aquatic life when it reaches above 100 mg/L concentration in water stream. The high concentrations of phthalic acid at Teluk Kepayang is due to agriculture activities, existence of two highways and also resident area near Teluk Kepayang. Discharges from these kinds of anthropogenic activities cause presence of high concentrations of PAHs in the river. Meanwhile, the increase in concentrations of naphthalene, chrysene and anthracene in Teluk Kepayang water treatment plant influent cause higher concentration of phthalic acid (Table 1). Benzoic acid which is part of the common derivatives of chrysene and Fluoranthene [21] was identified in both samples. Naphthalene was identified in the sample of influent WTP Teluk Kepayang with low corresponding % max (threshold limits of naphthalene in aquatic system is 2 µg/l [22]). Other derivatives such as 1(3H)-isobenzofuranone, benzaldehyde, phenylacetic acid and 1,4-naphthalenedione were detected in the analysis with separate retention time in influent WTP Teluk Kepayang but it was insignificantly low in corresponding % max. The identified compounds are known to be highly toxic to aquatic environments only if they are above the threshold limit. No other compounds of PAHs were detected in both analyses, prompting the possibility of complete degradation of all compounds of PAHs in the water source, their release to the atmosphere or their sedimentation at the bottom of water course.

3.2. Toxicity Test

The fish were exposed to the samples for a period of 96 hours. Mortalities were recorded at 24, 48, 72 and 96 hours. Results achieved in control tank showed no mortality and no changes on the behavior and the swimming patterns of Poecilia reticulate. Healthy fishes can be known based on their swimming style [23]. Zero per cent mortality in the control tank indicated the validity of test. For the test to be valid the mortality should not exceed 10 per cent in control at the end of the test [14]. The observed mortality rates in control tank agrees well with the others in literature [24-26]. Unlike control tank, sudden jerks, swimming abnormalities, mucosal secretion in gills and on body surface were observed in tanks of samples from WTP Teluk Kepayang influent and UTP STP effluent. It was also observed that some of the fishes gathered at the surface of test chambers for breathing. Those symptoms ultimately contributed to the recorded mortalities of Poecilia reticulate in both samples from WTP Teluk Kepayang influent and UTP STP effluent.

### Table 2: Detected compounds in effluent from the university sewage treatment plant

| Compound Name | Type       | Chemical Mass Distribution % | Classification   |
|---------------|------------|-----------------------------|------------------|
| Chrysene      | Parent     | -                           | N/A              |
| Phthalic Acid | Daughter   | 2.170                       | Not classified   |
| Fluoranthene  | Parent     | -                           | N/A              |
| Benzoic Acid  | Daughter   | 3.944                       | Slightly toxic   |
| Naphthalene   | Parent     | -                           | N/A              |
| 1,4-naphthalenedione | Daughters | 0.364                       | Moderate toxic  |
| Naphthalene   | Parent     | -                           | N/A              |

Phthalic acid is known to be one of the main intermediates of Naphthalene, Chrysene and Anthracene. The chemical process of Naphthalene had gone through 1,2-dihydroxynaphthalene ortho-type cleavage (intradiol) or 2,3-dihydroxynaphthalene meta-type cleavage to form 2-carboxycinnamic acid. To form Phthalic Acid, 2-carboxycinnamic acid, may undergo further degradation [118]. In photocatalytic degradation of Naphthalene, phthalic acid and 9,10-anthraquinone have been found as relatively stable intermediates whereas phthalic acid were found as trace amounts in degradation of anthracene [119]. The chemical process of Anthracene to Phthalic Acid is more complex than Naphthalene. There are 3 steps to achieve Phthalic Acid from Anthracene. The first step is to undergo multicomponent dioxygenases to produce cis-1,2-dihydrodiol. Enzymatic attack will eventually form 1,2-dihydroxyanthracene. The first product will need to go through meta-ring cleavage and 2-hydroxy-3-naphthoic acid will be the second product. Lastly, Mycobacterium sp. will change the second product to phthalic acid [20]. Phthalic acid is known to be dangerous to aquatic life when it reaches above 100 mg/L concentration in water stream. The high concentrations of phthalic acid at Teluk Kepayang is due to agriculture activities, existence of two highways and also resident area near Teluk Kepayang. Discharges from these kinds of anthropogenic activities cause presence of high concentrations of PAHs in the river. Meanwhile, the increase in concentrations of naphthalene, chrysene and anthracene in Teluk Kepayang water treatment plant influent cause higher concentration of phthalic acid (Table 1). Benzoic acid which is part of the common derivatives of chrysene and Fluoranthene [21] was identified in both samples. Naphthalene was identified in the sample of influent WTP Teluk Kepayang with low corresponding % max (threshold limits of naphthalene in aquatic system is 2 µg/l [22]). Other derivatives such as 1(3H)-isobenzofuranone, benzaldehyde, phenylacetic acid and 1,4-naphthalenedione were detected in the analysis with separate retention time in influent WTP Teluk Kepayang but it was insignificantly low in corresponding % max. The identified compounds are known to be highly toxic to aquatic environments only if they are above the threshold limit. No other compounds of PAHs were detected in both analyses, prompting the possibility of complete degradation of all compounds of PAHs in the water source, their release to the atmosphere or their sedimentation at the bottom of water course.

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![Mortality Rate (%) vs Time (hr)](image-url)
Maximum mortality rates of 20 and 10 per cent was found at WTP Teluk Kepayang influent and UTP STP effluent, respectively (Figs. 1 and 2). In influent from WTP Teluk Kepayang no mortality was observed in first 24 hours. Though, one and two mortalities were detected on the second day in replicates 2 and 1, respectively. On the third day, one fish was found dead in each replicates 2 and 3. No further mortality was recorded on the fourth day in tanks contain influent WTP Teluk Kepayang. Samples of the UTP STP have shown lower mortality rates compared to influent WTP Teluk Kepayang. In effluent from UTP STP, only one fish was found dead after 24 hours in replicate 3. The concentrations of PAHs and their daughters in the samples did not produce acute symptoms or serious mortality in fish groups within the experimental duration. In fact, the pollution levels in collected samples did not produce a 50% mortality until 96 hours exposure. However, these results could indicate the conditions letting lengthy survival still affect fish populations [24].

4. Conclusion

The study was conducted to investigate the presence of Polycyclic Aromatic Hydrocarbons (PAHs) and their daughters in two sampling points (a water treatment plant influent and a sewage treatment plant effluent). The toxicity levels of samples subjected to Organization for Economic Cooperation and Development (OECD) acute toxicity test using guppy fish. The conversion paths of PAHs in both sampling points show that Phthalic and benzoic acids were the major derivatives (daughters) of their PAHs parents, Chrysene, Naphthalene and Fluoranthene. The concentrations of PAHs and their daughters in the samples produced up to 20% mortality in fish groups within the experimental duration. Although the achieved results in this study are applicable in details to specific experimental conditions used, cumulative or continuing toxic effects of certain pollutants at low concentrations are possibly a wide-ranging phenomenon. The water quality conditions required for mortality study of fish are complex and the occurrence of cumulative or continuing toxicity also makes the purpose of safe pollution levels for fish challenging. The findings of this work is significance to further investigate the sub-lethal effects of PAHs and their derivatives in longer duration. The expansion of sampling locations and investigation on sediment samples are also recommended in the area of industrial and agricultural activities where proven to be latent for PAHs releases to watercourses.

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