THE FATE OF DISCHARGED POLYCYCLIC AROMATIC HYDROCARBONS (PAHs) IN WATER STREAMS

By

Law Ming Xian

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Bandar Seri Iskandar

31750 Tronoh

Perak Darul Ridzuan

CERTIFICATION OF APPROVAL

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Approved:

_____

Dr. Amirhossein Malakahmad

Project Supervisor

UNIVERSITI TEKNOLOGI PETRONAS

TRONOH, PERAK

MAY 2013

CERTIFICATION OF ORIGINALITY

This is to certify that I am responsible for the work submitted in this project, that the original work is my own except as specified in the references and acknowledgements, and that the original work contained herein have not been undertaken or done by unspecified sources or persons.

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Law Ming Xian

ABSTRACT

Polycyclic aromatic hydrocarbons (PAHs), also known as polynuclear aromatic hydrocarbons, are potent atmospheric pollutants consisting of fused aromatic rings and do not carry or contain heteroatoms/substituents. 16 of PAHs have been classified by Environmental Protection Agency (EPA) for possible carcinogenic properties. It is discovered that PAHs are found abundant in water stream. Most of the PAHs are discharged without getting treated properly at sewage treatment plant.

When exposed to the environment, the PAHs will be degraded either in microbial degradation or photo degradation pathway. The PAHs will be then transformed to various metabolites, creating 'daughters' and byproducts along its degradation. They may also be mutagenic or carcinogenic even if their parent compounds are not (International Association of Oil and Gas Producers, 2005).

A research study was conducted on former works on PAHs and its metabolites. This included the current study to identify various sources of PAHs in water stream in river of Sungai Perak near the area Teluk Kepayang. Samples were obtained from influent of water treatment plant (WTP) in Teluk Kepayang and were analysed with Gas Chromatography/Mass Spectrometry (GC/MS) to formulate conversion path of carcinogenic PAHs. Tests were performed as well on influent and effluent of Universiti Teknologi PETRONAS (UTP) sewage treatment plant (STP) for studies on sewage polluted urban streams. The samples were then used as test solution for guppy (*Poecilia reticulate*) as their living environment to establish toxicity intensity of carcinogenic PAHs parents and daughter products through 96hours acute toxicity test.

GC/MS analysis identified phthalic acid and benzoic acid as major derivatives found in all samples. The analysis showed the detection of Naphthalene and few other suspected derivatives in influent WTP Teluk Kepayang sample. Mortality rate of Poecilia reticulate against time exposure of 96 hours was 20% for influent Teluk Kepayang WTP and 10% for influent UTP STP whereas the rate is 0% for effluent UTP STP. It was observed that the amount of PAHs and its derivatives detected in both effluent and influent of UTP STP and influent of Teluk Kepayang WTP are not harmful to the environment.

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CHAPTER 1.0

INTRODUCTION

1.1 Project Background

Polycyclic Aromatic Hydrocarbon (PAHs) is one unique class of persistent organic hydrocarbon pollutant, semi-volatile organic compounds containing two or up to seven fused benzene aromatic rings of carbon and hydrogen atoms (Chen Y, Ho K F, Ho S S H, HoWK, Lee S C, Yu J Z et al, 2007).

PAHs originate mainly from anthropogenic processes, particularly from incomplete combustion of organic fuels. PAHs are distributed widely in the atmosphere. Natural processes, such as volcanic eruptions and forest fires, also contribute to an ambient existence of PAHs. PAHs can be present in both particulate and gaseous phases, depending upon their volatility. Light molecular weight PAHs (LMW PAHs) that have two or three aromatic rings are emitted in the gaseous phase, while high molecular weight PAHs (HMW PAHs), with five or more rings, are emitted in the particulate phase. In the atmosphere, PAHs can undergo photo-degradation and react with other pollutants, such as sulphur dioxide, nitrogen oxides, and ozone (Lee, 2010). PAHs have been thoroughly studied due to their toxicity, persistency and environmental prevalence. Such studies are often limited to 16 PAHs, designated as priority pollutants by the United States Environmental Protection Agency (US-EPA).

PAHs are nonpolar, hydrophobic compounds that do not ionize. Volatilization, photolysis, hydrolysis, microbial degradation, and adsorption and subsequent sedimentation determine the fate of PAHs in the environment (Southworth, G.R., 1979)

1.2 Problem Statement

Polycyclic aromatic hydrocarbons (PAHs) cause a public concern due to their potency as carcinogens and mutagens. PAHs are now found distributed in natural waters worldwide (Xu, S.F., Jiang, X., Wang, L.S., 2000). The distribution of PAHs in the aquatic environment is significantly affected by aquatic particulates, which act as aggregates of numerous complicated organic products (Tang, H.X., Qian, Y., Wen, X.H., 2000). Exposure of PAH compounds to light produces partially oxidized intermediates which are more susceptible to biodegradation than parent compounds (Lehto K.M., Vuorimaa E., Lemmetyinen H., 2000). Microbes are also known for their catabolic activity in bioremediation, but changes in microbial communities are still unpredictable and the microbial community is still termed as a 'black box' (M. Dua, A. Singh, N. Sethunathan, A.K. Johri, 2002). Furthermore, some environmental transformation products of PAHs may react directly with DNA, causing mutations and possibly cancer, without the need for metabolic activation (Moller M, Hagen I, Ramdahl T, 1985). Some of the short-lived metabolic products from enzymatic PAH degradation may be more toxic than the parent compounds. They may also be mutagenic or carcinogenic even if their parent compounds are not (International Association of Oil and Gas Producers, 2005). Hence, the daughters and conversion products produced by the discharged parent PAHs to water streams should be identified properly along with their respective possible toxicity/hazardous properties to the environment.

1.3 Objectives and Scope of Study

1.3.1 Objective:

a) To identify various source of PAHs in water stream.

b) To formulate conversion path of carcinogenic PAHs in natural water stream and sewage polluted urban streams.

c) To establish toxicity intensity of carcinogenic PAHs parents and daughter products.

1.3.2 Scope of Study:

It is begun by conducting a research study on PAHs. Details such as different point of sources, behaviour and physical structures of common carcinogenic PAHs, documented hazardous properties and chemical properties are properly sorted out. These studies will be useful for reference purposes in results and discussion session.

This research continues on investigating the parents-daughters of PAHs in the water stream upon getting discharged from sewage treatment plant (STP). Possible chemical/physical/biology reactions it will undergo are drafted out and Gas Chromatography/Mass Spectrometry will be performed to analysis the predicted degradation of this organic pollutant.

1.4 Significance of Project

This research will provide a clear view on what is happening to the treated polycyclic aromatic hydrocarbon after leaving STP to the mainstream by varying the possible reactions to the pollutants. Moreover, future investigations and methods can be carried out to ensure the identified hazardous degraded of treated polycyclic aromatic hydrocarbon to be properly handled before reaching environment.

1.4.1 Relevancy of Project

This research is important as it will play a major role in yet another milestone in wastewater engineering. Treating PAHs is considerably vital as it will reduce the exposure of PAHs to human and the environment, as production of PAHs is nearly unavoidable due to incomplete fuel combustion and various human activities (Sun P, Backus S, Blanchard P, Hites R A, 2006)

1.4.2 Feasibility of the Project

This project is feasible, given the ample of time to conduct research and adequate facilities such as laboratory. Samples can be easily obtained directly from the STP located inside place of study itself.

CHAPTER 2.0

LITERATURE REVIEW

2.1 Possible Source of PAHs in water stream

Studies have found that PAHs in the atmosphere are mostly (90% of most PAHs in Earth) in the gas phase (Lili Yan, Xiang Li, Jianmin Chen, Xinjun Wang, Jianfei Du, Lin Ma, 2012). The largest emissions of PAHs result from industrial processes and other human activities such vehicle exhausts, agricultural burning, residential wood burning, municipal and industrial waste incineration, and hazardous waste sites (WHO Denmark, 2000). It can be concluded that air is the primary carrier of PAHs.

Due to that, rainwater may be a primary non-point source of PAHs in the region because both gaseous and particulate PAHs can be easily scavenged by rainfall (Lili Yan, Xiang Li, Jianmin Chen, Xinjun Wang, Jianfei Du, Lin Ma, 2012). Through rainwater, PAHs in atmosphere are transferred as surface indirect runoff, resulting in contamination in water stream.

In our daily food, naphthalene, one of the common PAHs, was detected in six of eight samples of human milk (WHO Denmark, 2000). Cooking meat or other food at high temperatures, during grilling or charring, increases the amount of PAHs in the food. An experiment done with mice, where 80% of Benzo(a)pyrene was recovered from faeces after 7days, while a total of 42% was recovered from faeces and urine in rats (WHO Denmark, 2000), suggests that PAHs can be easily discharged into urban wastewater through human waste.

Another source of PAHs from domestic and commercial activities is the use of phenol and creosol in products such as wood preservatives. PAHs may be in groundwater near disposal sites where construction wastes or ashes are buried. Few sources of PAHs include liquid waste discharge of industrial activities, such as primary aluminium and coke production, petrochemical industries, rubber tire and cement manufacturing, bitumen and asphalt industries, wood preservation, commercial heat and power generation and waste incineration (Lee, 2010).

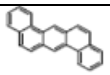
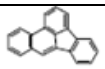
Identifying the source point of PAHs in water stream remains crucial and highly important, as source reduction is one clinical method to solve environmental issue.

2.2 Analysis of Documented Types of PAHs according to EPA and EU

2.2.1 Classification of Types of PAHs

Table 2.1 shows 16 EPA regulated PAHs and 7 of them have been classified as carcinogens by the International Agency for Research on Cancer (IARC), listed by the EPA as dominant groups present in the wastewater mixture from petrochemical effluents due to their toxic, mutagenic, and carcinogenic properties and can cause detrimental effect to flora and fauna. (Lerda, 2010)

Table 2.1: List of Polycyclic Aromatic Hydrocarbon (PAHs), its respective molecular structure according to Environment Protection Agency (EPA)

Common Name	Structure	Common Name	Structure
Acenaphthene		Chrysene	
Acenaphthylene		Dibenz[a,h]anthracene	
Anthracene		Indeno[1,2,2-cd]pyrene	
Benz[a]anthracene		Fluoranthene	
Benzo[b]flouranthene		Fluorene	
Benzo[k]flouranthene		Naphthalene	
Benzo[a]pyrene		Phenanthrene	
Benzo[ghi]perylene		Pyrene	

2.2.2 Identification of Carcinogenic/Toxic PAHs

USEPA formally adopted provisional guidance for estimating cancer risks associated with polycyclic aromatics hydrocarbons (Lerda, 2010). The procedure uses information from the scientific literature to estimate the carcinogenic potency of several PAHs relative to benzo[a]pyrene as benchmark and it is shown in Table 2.2.

Table 2.2: Compilation of USEPA listed PAHs and the carcinogenic potency value respectively

Compound PAHs	Relative Potency Factor
Acenaphthene	N/A
Acenaphthylene	N/A
Anthracene	N/A
Benz[a]anthracene	0.1
Benzo[b]flouranthene	0.1
Benzo[k]flouranthene	0.01
Benzo[a]pyrene	1
Benzo[ghi]perylene	N/A
Chrysene	0.01
Dibenz[a,h]anthracene	1
Indeno[1,2,2-cd]pyrene	0.1
Fluoranthene	N/A
Fluorene	N/A
Naphthalene	N/A
Phenanthrene	N/A
Pyrene	N/A

2.3 Organic Pollutants Detection Methods in Domestic Wastewater

2.3.1 Gas Chromatography Mass Spectrometry GC/MS

Gas Chromatography Mass Spectrometry (GCMS) will make an effective combination for chemical analysis as it involves technique to analysis and quantize organic volatile and semi volatile compounds (EAG-Evans Analytical Group: Materials Characterization, 2013).

Gas Chromatography (GC) is implemented in order to separate mixtures into individual components using a temperature controlled capillary column. Smaller molecules with lower boiling point travel much quicker than larger molecules with higher boiling point down the column.

Mass Spectrometry (MS) identifies various components from their mass spectra as each component has a unique mass spectrum that is comparable with their mass spectral databases and thus identified.

GCMS works on all phases of matters. For solids, analysis is carried out either by solvent extraction, out gassing or paralysis. For liquids, samples are directly injected into the GC whereas for gaseous, transfer of components are done by gas tight syringes into the GC.

Advantages of GC/MS are as such:

- Able to perform both qualitative and quantitative analysis
- Able to identify organic components by separating complex mixtures
- Able to determine trace-level organic contamination (Dynamic Headspace Analysis)

Disadvantages of GC/MS are as such:

- Presented samples must be volatile
- If it is a sample of headspace, pyrolysis, or direct probe, analysed material must be volatile.

2.4 Fate of discharged PAHs in water stream

2.4.1 Reactions of discharged PAHs released in natural environment

2.4.1.1 Photo degradation of discharged PAHs

Once the PAHs are discharged from STP into the environment, primary removal processes of low molecular weight PAHs are microbial degradation and evaporation. Higher molecular weight aromatics are less water soluble, which makes biodegradation difficult. Thus, these compounds are degraded by photochemical oxidation process (D. Dąbrowska, A. Kot-Wasik, J. Namieśnik, 2008). PAHs maybe degraded through either direct or sensitized photochemical reactions (Matthew P. Fasnacht and Neil V. Blough, 2002) Exposure of PAH compounds to light produces partially oxidized intermediates which are more susceptible to biodegradation than parent compounds (Lehto K.M., Vuorimaa E., Lemmetyinen H., 2000).

Many studies have been performed on the photolysis of individual PAHs in natural waters under irradiation. It has been found that the photolysis rates of selected PAHs in a natural water body were quite fast and the photolysis half-lives of PAHs ranged from several minutes to several hours. Half-lives for benzo(a)pyrene and benzo(a)anthracene in water under sunlight irradiation were 0.69 and 5.0h, respectively (D. Dąbrowska, A. Kot-Wasik, J. Namieśnik, 2008)

It is generally accepted that photo degradation of PAHs in solutions is an oxidative process which is highly accelerated by the presence of photo-initiators. In general, the more polar the solvent is, the faster the degradation process of PAH. The rate of the photo degradation process is also affected by the amount of dissolved oxygen, temperature and light intensity. (D. Dąbrowska, A. Kot-Wasik, J. Namieśnik, 2008)

2.4.1.2 Microbial Degradation of Discharged PAHs

PAHs-degrading bacteria generally use the PAHs as a carbon and energy source while fungi metabolize the PAHs to more water-soluble compounds, thereby promoting their subsequent excretion (Lundstedt, 2003).

As can be seen in Figure 2.1, fungi oxidize PAHs via the cytochrome P-450 enzyme system to form phenols and trans-dihydrodiols, which can be conjugated and excreted from the organism (Lundstedt, 2003).

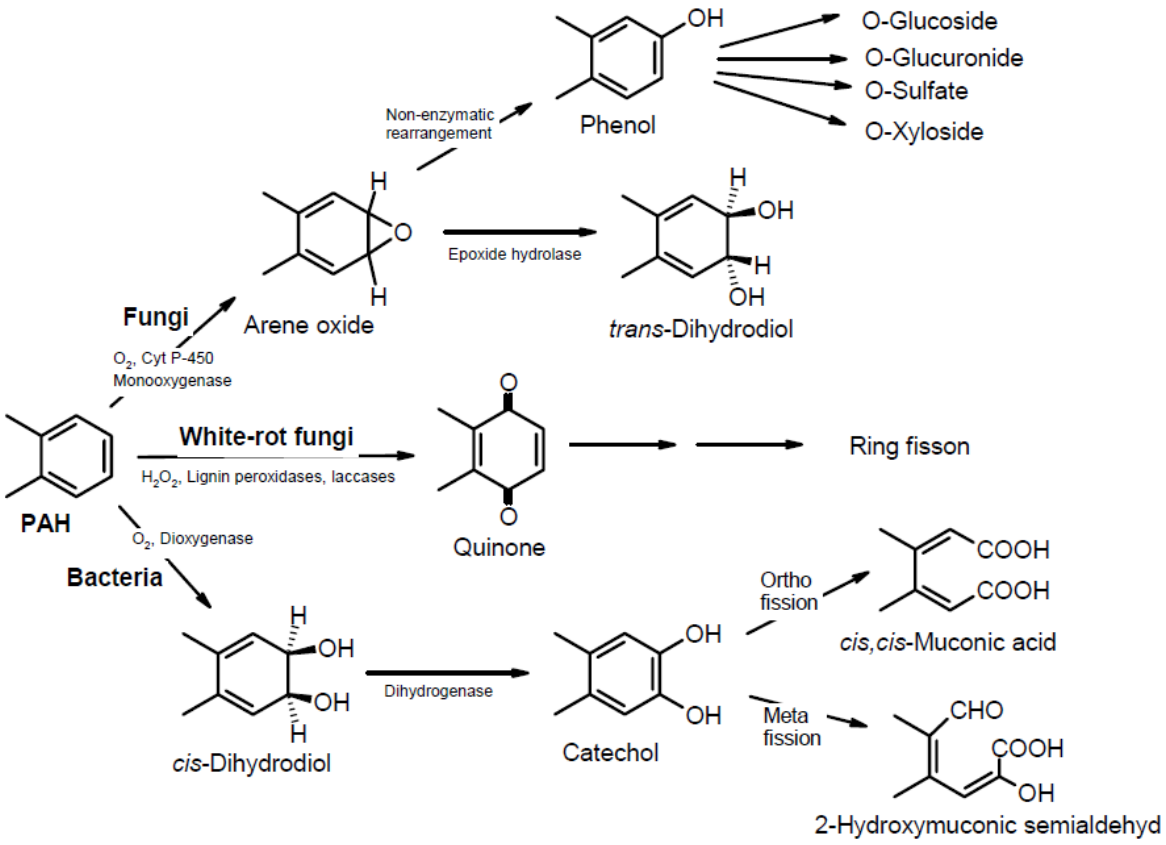


Figure 2.1: General pathways for the microbial degradation of PAHs, based on (Cerniglia, 1992)

From Figure 2.1, the bacterial degradation of PAHs generally begins with a dioxygenase attack on one of the aromatic rings to form a cis-dihydrodiol, which is subsequently dehydrated to catechol. Catechol is a key intermediate from which ring cleavage can occur (Cerniglia, 1992).

2.5 Identifying daughters-parents of discharged PAHs in water stream

2.5.1 Acenaphthene

Irradiations of acenaphthene in the presence of benzophenone and cupric pivalate under mercury and sodium lamps, and the analysis of products by GC-MS, showed that acenaphthene remains unreacted in a photolysis medium. Irradiations of acenaphthene in the presence of benzophenone and cupric pivalate yielded products only under concentrated sunlight. Acenaphthene dehydrogenation under concentrated sunlight resulted in the minor formation of acenaphthylene and the quantitative formation of acenaphthenone when the solution was aerated. Figure 2.2 shows the proposed pathway for photo hydrogenation of acenaphthene under sunlight (Nesibe Avcibasi, et al., 2003).

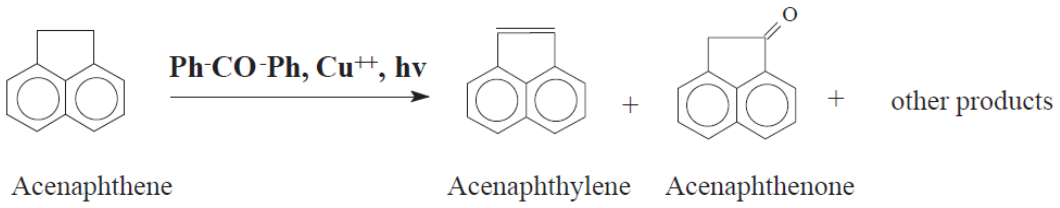


Figure 2.2: Proposed pathway for photo hydrogenation of acenaphthene

2.5.2 Acenaphthylene

Table 2.3 shows the compounds identified by GCMS on PAHs that has been exposed to UV radiation. Such radiation has been used to degrade acenaphthylene either as a single system or in combination with ozone (Francisco J. Rivas, et.al, 2000).

It is noticed that a similarity in the nature of intermediates formed regardless of the method of acenaphthylene degradation used. The formation of some compounds like furanes, biphenyls, pyranes, etc., suggests an increase in toxicity of the effluent if compared to the parent compound (Francisco J. Rivas, et.al, 2000).

Table 2.3: Intermediates identified in the degradation of acenaphthylene by UV/ UV + O^3 reactions

Compound Detected	**UV**	**UV/O^3**
2-methyl naphthalene	X	X
1-methyl naphthalene	X	

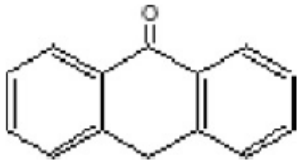
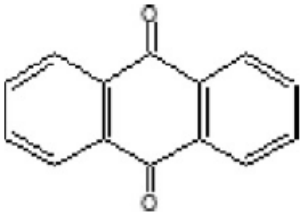
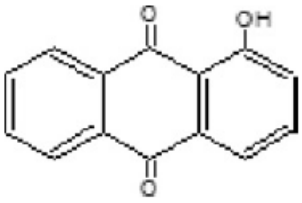
2-ethen-naphthalene	X	X
1,7-dimethyl naphthalene	X	X
2,7-dimethyl naphthalene	X	X
1-naphtalen carboxyl aldehyde	X	X
Dibenzofurane	X	X
1H,2H-1-oxaacenaphthylee-2-one	X	X
2-ethoxy-3-methoxyphenol	X	
1,1'-biphenyl 4-carboxyaldehyde	X	X
1,1'-biphenyl 2,2'diol	X	
1,2-acenaphthylenedione	X	
1H,3H-naphthen (1,8 cd) pyran 1-one	X	X
naphthalenecarboxylic acid	X	X
1,8-naphthalic anhydride	X	X
o-hydroxybiphenyl		X
1(3H)-isobenzofuranone		X
2-hydroxy-1-naphtalen carboxyaldehyde		X
Benzaldehyde		X
2-methyl-benzaldehyde		X

2.5.3 Anthracene

Three products, listed in Table 2.4, are anthrone, anthraquinone, and 1-hydroxy-anthraquinone, produced by photolysis of anthracene. Although no chemical oxidant or catalyst was used in this study to induce advanced oxidation processes, oxygenated products were produced. This observation was previously reported by other authors. Miller and Olejnik proposed possible photolysis reactions of the PAH molecules surrounded by water with dissolved oxygen (J.S. Miller, D. Olejnik, 2001).

Anthrone and anthraquinone have been previously reported to form as a consequence of anthracene degradation using lamps with an emission peak of 365 nm in the presence of titanium dioxide (O.T. Woo, W.K. Chung, K.H. Wong, A.T. Chow, P.K. Wong, 2009).

Table 2.4: Observed compounds and by-products identified after photolysis of Anthracene

Compounds	Structure	Molecular formula
Anthrone		$C_{14}H_{10}O$
Anthraquinone		$C_{14}H_8O_2$
1-Hydroxy-anthraquinone		$C_{14}H_8O_3$

2.5.4 Benz[a]anthracene

The degradation pathway of Benz[a]anthracene by photo catalytic oxidation (PCO) degradation (in 1% acetone solution) was investigated. Acetone, a water-miscible organic solvent, helps PAHs contact a photo-catalyst more readily to trigger PCO degradation in aqueous solution. Table 2.5 shows the intermediates detected in the test whereas Figure 2.3 shows the proposed degradation pathway of Benz[a]anthracene by photo catalytic oxidation (PCO) degradation (in 1% acetone solution) (O.T. Woo, W.K. Chung, K.H. Wong, A.T. Chow, P.K. Wong, 2009).

Table 2.5: Observed compounds and by-products identified by photo catalytic oxidation (PCO) degradation (in 1% acetone solution) of Benz[a]anthracene

No.	**Compounds Detected**	**Chemical Formulae**
26	1,2-Benzenedicarboxaldehyde	$C_8H_6O_2$
27	2H-1-Benzopyran-2-one	$C_9H_6O_2$
28	2-Hydroxy-1,4-naphthalenedione	$C_{10}H_6O_3$
30	Benzo[a]anthracene-7,12-dione	$C_{18}H_{10}O_2$

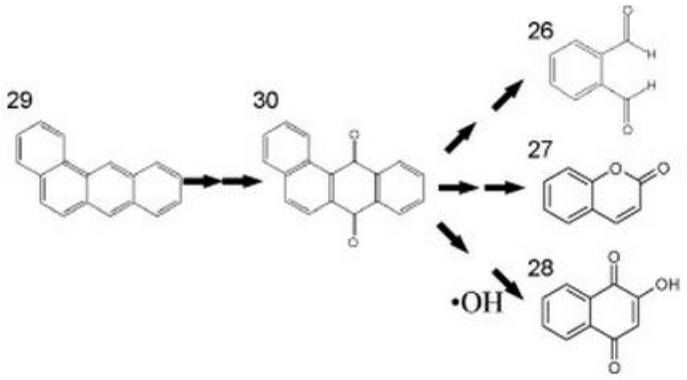
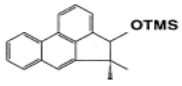
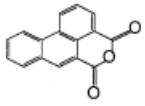
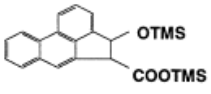


Figure 2.3: Proposed degradation pathway of Benz[a]anthracene based on the compounds details in Table 5

2.5.5 Benzo[b]flouranthene

Lack of growth on high molecular weight Benzo[b]flouranthene (B[b]F), as sole carbon sources may be due to low water solubility of this compound where growth is limited by a slow dissolution rate. In this study, B[b]F was provided as sole carbon sources incorporated into agar medium that contained Tween-80 to increase their concentration; this still did not support growth. Catabolic products of B[b]F was not detected with strain EPA505. This suggests that strainnEPA505 transforms B[b]F to a later non-detected intermediate(s). Hydroxyacephenanthroic acid and hydroxyacephenanthrene were detected at earlier stage, and phenanthrene anhydride and the open ring of B[b]F were detected at later stage. Table 2.6 shows the identification of hydroxyacephenanthrene and phenanthrene anhydride further support that removal of the carboxyl from the cyclopentane ring followed by a dioxygenase reaction are common mechanisms involved in further transformation of PAHs with a cyclopentane moiety by S. paucimobilis EPA505 (S. P. Story, et.al, 2004).

Table 2.6: Observed compounds identified by Sphingomonas paucimobilis Strain EPA505 degradation of Benzo[b]flouranthene (S. P. Story, et.al, 2004)

Compounds Observed	Chemical Formulae	Chemical Structure
hydroxyacephenanthrene	$C_{14}H_{10}O$	
phenanthrene anhydride	-	
Hydroxyacephenanthroic acid	-	

2.5.6 Benzo[k]flouranthene

In a static biodegradability test employing a domestic wastewater inoculum, 50-70% of benzo(k)fluoranthene was degraded in four successive weekly subcultures (Irwin, 1997). However, due to lack of resources, identifying the parents-daughters of Benzo[k]flouranthene, either through mircobial or photolysis reaction, is difficult and hence, omitted.

2.5.7 Benzo[a]pyrene

Warshawsky et al. found that Selenastrum capricornutum, a freshwater green alga metabolizes Benzo[a]pyrene (BaP) to cis-dihydrodiols using a dioxygenase enzyme system as found in heterotrophic prokaryotes. With increasing light energy from gold to white to UV-A in PAH-absorbing region, BaP quinone production increased. The study also concluded that only green algae almost completely metabolized BaP to dihydrodiols, whereas yellow algae and blue green algae failed in metabolizing the PAH. (A.K. Haritash, C.P. Kaushik, 2009).

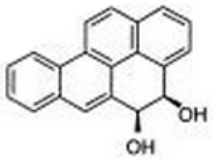
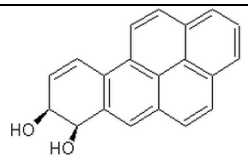
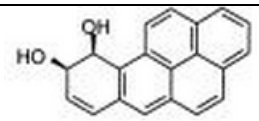
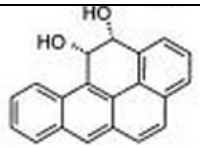
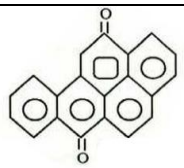
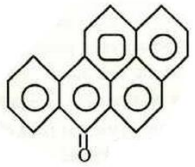
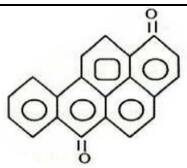
Lindquist and Warshawsky and Warshawsky et al. demonstrated that the oxidation of benzo[a]pyrene by the green alga Selenastrum capricornutum resulted in the formation of benzo[a]pyrene cis-4,5-, 7,8-, 9,10-, and 11,12-dihydrodiols (Joanna D. Moody, James P. Freeman, Peter P. Fu and Carl E. Cerniglia, 2004).

Many studies have been performed on photolysis of individual PAHs in natural waters under irradiation (D. Dąbrowska, A. Kot-Wasik, J. Namieśnik, 2008) found that the photolysis rates of selected PAHs in natural water body were quite fast and the photolysis half-lives of PAHs ranged from several minutes to several hours.

In addition, other isomers are probable to be formed, where both processes (oxidation and biodegradation) precede simultaneously, another compound was identified. This mass spectral fragmentation pattern suggests that this metabolite could be BaP-dione-like compound, for example, BaP-7, 10-dione, 2-hydroxy- BaP-1,6-dione, BaP-3,6-dione, or BaP-6,12-dione (A. Kot-Wasik, D. Dąbrowska, J.Namieśnik, 2004).

Above researches and its respective intermediates formed are listed and tabulated in the form of table shown in Table 2.7 and Table 2.8.

Table 2.7: Observed compounds and by-products identified after photolysis and microbial degradation of Benzo[a]pyrene.

Reaction	Compounds Observed	Chemical Structure
Microbial Degradation	benzo[a]pyrene cis-4,5-dihydrodiols	
	benzo[a]pyrene cis-7,8-dihydrodiols	
	benzo[a]pyrene cis-9,10-dihydrodiols	
	benzo[a]pyrene cis-11,12-dihydrodiols	
Photo Degradation	BaP-6,12-dione	
	BaP-3,6-dione	
	BaP-1,6-dione	

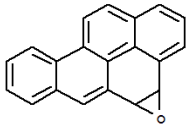
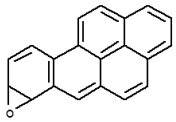
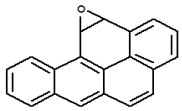
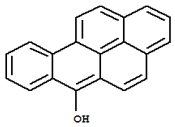
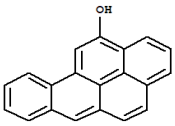
29 benzo(a)pyrene derivatives were tested for mutagenic activity without metabolic activation in SSalmonella typhimurium strains TA98, TA100, and TA1538 and in Chinese hamster V79 cells (Richard L. Chang, et al., 1976).

For oxides, Benzo(a)pyrene 4,5-oxide was the most mutagenic of the compounds tested in both the bacterial and mammalian systems. The other arene oxides benzo(a)pyrene 7,8-, 9,10-, and 11,12-oxides] were only weakly mutagenic in the S. typhimurium strains.

Among the phenols, 6-hydroxybenzo(a)pyrene and 12-hydroxybenzo(a)pyrene were moderately mutagenic in strain TA98 of S. typhimurium, and 6-hydroxybenzo(a)pyrene was moderately mutagenic in V79 cells.

The other 10 phenols, were either inactive or only weakly mutagenic. For Quinones, Benzo(a)pyrene 11,12-quinone was extremely cytotoxic to the V79 cells but had no observable toxicity in the bacterial strains (Richard L. Chang, et al., 1976).

Table 2.8: Observed compounds and by-products identified after photolysis and microbial degradation of Benzo[a]pyrene.

Compounds	Toxicity Level	Chemical Structure
Benzo(a)pyrene 4,5-oxide	High	
benzo(a)pyrene 7,8-oxides	Weak	
benzo(a)pyrene 11,12-oxides	Weak	
6-hydroxybenzo(a)pyrene	Moderate	
12-hydroxybenzo(a)pyrene	Moderate	

2.5.8 Benzo[ghi]perylene

Benzo(ghi)perylene is not expected to undergo hydrolysis in the environment due to the lack of hydrolysable functional groups (Lyman WJ, et.al., 1990). Within 7 days, an aerobic aqueous screening test inoculated with sewage showed a 60% loss of 1 ppm benzo(ghi)perylene. An anaerobic sludge digestion study found that a statistically significant amount benzo(ghi)pyrene was biodegraded over a 32 day incubation period (EG, 1981). However, due to lack of resources, identifying the parents-daughters of Benzo[ghi]perylene, either through mircobial or photolysis reaction, is difficult and hence, omitted.

2.5.9 Chrysene

Based on pathway in Figure 2.4, a study has been done to study heterogeneous photo degradation of chrysene is conducted in aqueous laponite suspensions, under conditions that simulate high light estuarine environment. Although PAH photolysis on dry particle surfaces has been reported to proceed through photoionization, the addition of water to the surface appeared to shift the reaction toward a pathway dominated by singlet oxygen. Chrysene photo degradation was accompanied by the formation of positively identified products, 1-4-chrysenequinone, 2-formyl benzoic acid and phthalic acid. Phthalic acid appeared to be the only stable product under experimental conditions and increased throughout the experiment. No degradation was observed in samples that were kept in the dark (Li Kong and John L Ferry, 2001).

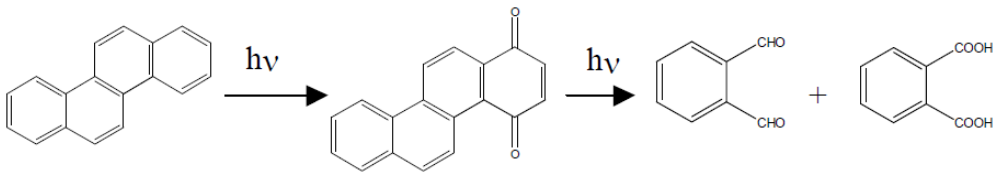


Figure 2.4: Photo degradation products of chrysene (coated on laponite) (Li Kong and John L Ferry, 2001)

2.5.10 Dibenz[a,h]anthracene

Dibenzo(a,h)pyrene is not expected to volatilize from water surfaces based on an estimated Henry's Law constant, developed using a fragment constant estimation method (Meylan WM, Howard PH, 1991). Polycyclic aromatic hydrocarbons with four or more rings, such as dibenzo(a,h)pyrene, are expected to be resistant to biodegradation (Boethling RS et al, 1994). However, due to lack of resources, identifying the parents-daughters of Dibenz[a,h]anthracene, either through mircobial or photolysis reaction, is difficult and hence, omitted.

2.5.11 Indeno[1,2,2-cd]pyrene

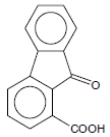
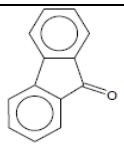
Indeno(1,2,3-cd)pyrene is not expected to volatilize from water surfaces based on a measured Henry's Law constant (Meylan WM, Howard PH, 1991). Indeno(1,2,3-cd)pyrene is not expected to undergo hydrolysis in the environment due to the lack of hydrolysable functional groups (Lyman WJ, et.al., 1990). 89% (mean) removal of

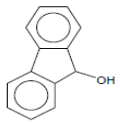
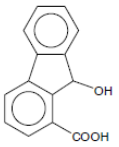
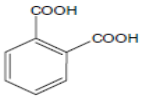
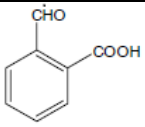
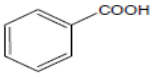
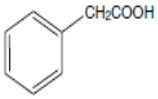
indeno(1,2,3-cd)pyrene was observed in six municipal waste water treatment plants (Van Luin AB, Van Starkenburg W, 1984). However, due to lack of resources, identifying the parents-daughters of Indeno [1,2,2-cd]pyrene, either through mircobial or photolysis reaction, is difficult and hence, omitted.

2.5.12 Fluoranthene

The toxicity of nine stable products of the biodegradation of fluoranthene with the pure common freshwater bacterial strain Pasteurella sp. IFA was studied and shown in Table 2.9. For their quantification, an improved analytical procedure with two-step liquid–liquid extraction, derivation and gas chromatographic-mass spectrometric detection was used. Growth inhibition and immobility tests for fluoranthene and its metabolites were carried out using algae (Scenedesmus subspicatus), bacteria (Pseudomonas putida) and crustaceans (Daphnia magna and Thamnocephalus platyurus). Tests using the alga S. subspicatus revealed that with the exception of 9-hydroxyfluorene, which was only four times less toxic than fluoranthene, all the other metabolites were 37 to 3000 times less toxic than the parent material. P. putida cells were resistant to fluoranthene and its primary metabolites, but were inhibited by low molecular weight intermediates, especially benzoic acid. Fluoranthene was not toxic to T. Platyurus, but was toxic to D. magna. Its primary metabolites (including 9-fluorenone and 9-hydroxyfluorene) were toxic to D. magna, and a low molecular weight metabolite (2 carboxybenzaldehyde) was highly toxic to T. platyurus (E. Sepic, M. Bricelj, H. Leskovsek, 2003).

Table 2.9: List of metabolites resulted from biodegradation of fluoranthene with the pure bacterial strain Pasteurella sp. IFA

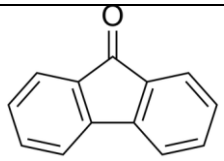
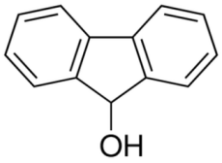
Metabolites identified	**Molecular mass**	**Structural Formula**
9-Fluorenone-1-carboxylic acid	224	
9-Fluorenone	180	

9-Hydroxyfluorene	182	
9-Hydroxy-1-fluorenecarboxylic Acid	226	
Adipic acid	146	
Phthalic acid	166	
2-Carboxybenzaldehyde	150	
Benzoic acid	122	
Phenylacetic acid	136	

2.5.13 Fluorene

Degradation of fluorene in selected organic solvents was monitored for more than 3 months. For the first 20 days, the concentration of fluorene remained constant in all examined solvents. After 80 days of exposure, only 40% loss was observed in dichloromethane solutions. On the basis of agreement of retention times and comparison of the UV absorption spectra obtained for samples of standard solutions of a reference and fluorene it was concluded that the conversions taking place in solutions of fluorene in dichloromethane and hexane result in the formation of 9-fluorenone. During attempted identification of trace amounts of compounds formed as a result of degradation of fluorene in dichloromethane and hexane, the presence of 9-hydroxyfluorene was established (D. Dąbrowska, A. Kot-Wasik, J. Namieśnik, 2008). All the compounds are shown in Table 2.10.

Table 2.10: Observed compounds of photo-degraded Fluorene with organic solvent (D. Dąbrowska, A. Kot-Wasik, J. Namieśnik, 2008)

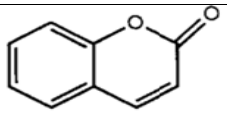
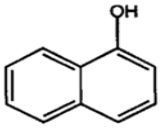
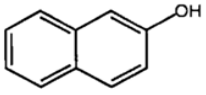
Compounds identified	Chemical Formulae	Structural Formulae
9-fluorenone	$C_{13}H_8O$	
9-hydroxyfluorene	$C_{13}H_{10}O$	

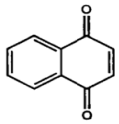
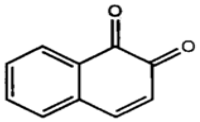
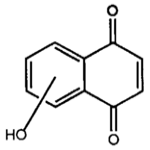
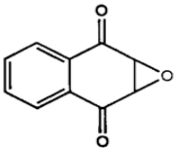
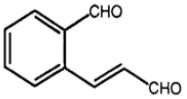
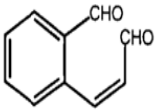
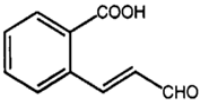
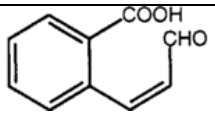
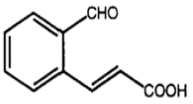
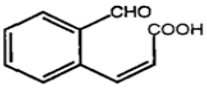
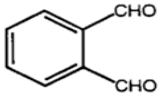
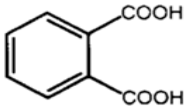
2.5.14 Naphthalene

Table 2.11 shows the identified intermediates of the naphthalene photo-degradation with TiO_2 as a photo-catalyst detected after 4 hours of irradiation. All intermediates were measured in the chloroform extract of the filtered suspension. Intermediates of relatively high concentrations were indicated as "main intermediates" while other by-products with relatively lower concentrations were indicated with "traces" or "small" amounts. (J. Theurich, D.W. Bahnemann, 1997).

The photo degradation of more stable intermediates, which tend to accumulate, such as phthalic acid, was studied separately under the same experimental conditions, using the respective component alone in water.

Table 2.11: Observed compounds and by-products identified after photo degradation with TiO_2 as a photo-catalyst of Naphthalene

Compounds	Concentrations	Structural formula
Coumarin	Main intermediate	
1-naphthol	Small amounts	
2-naphthol	Traces	

1,4-naphthalenedione	Main intermediate	
1,2-naphthalenedione	Traces	
x-hydroxy-1,4-naphthalenedione, x=2,5,6	Traces	
2,3-dihydro-2,3-epoxy-1,4-naphthalenedione	Main intermediate	
2-formyl-E-cinnamaldehyde	Traces	
2-formyl-Z-cinnamaldehyde	Main intermediate	
2-carboxyl-E-cinnamaldehyde	Traces	
2-carboxyl-Z-cinnamaldehyde	Traces	
2-formyl-E-cinnamic acid	Traces	
2-formyl-Z-cinnamic acid	Traces	
Phthalic acid (detected as anhydride)	Main intermediate	
1,2-benzenedicarboxaldehyde	Main intermediate	

In Figure 2.5, it shows the pathway of Oscillatoria sp., strain JCM, microbial commonly found in watering-troughs waters, grown photo-autotrophically in the presence of naphthalene oxidized the aromatic hydrocarbon to cis-l,2-dihydroxy- 1,2-dihydronaphthalene, 4-hydroxy- 1-tetralone and 1-naphthol. The major metabolite was 1-naphthol (Carl E. Cerniglia, L Chase Van Baalen and David T. Gibson, 1980) .

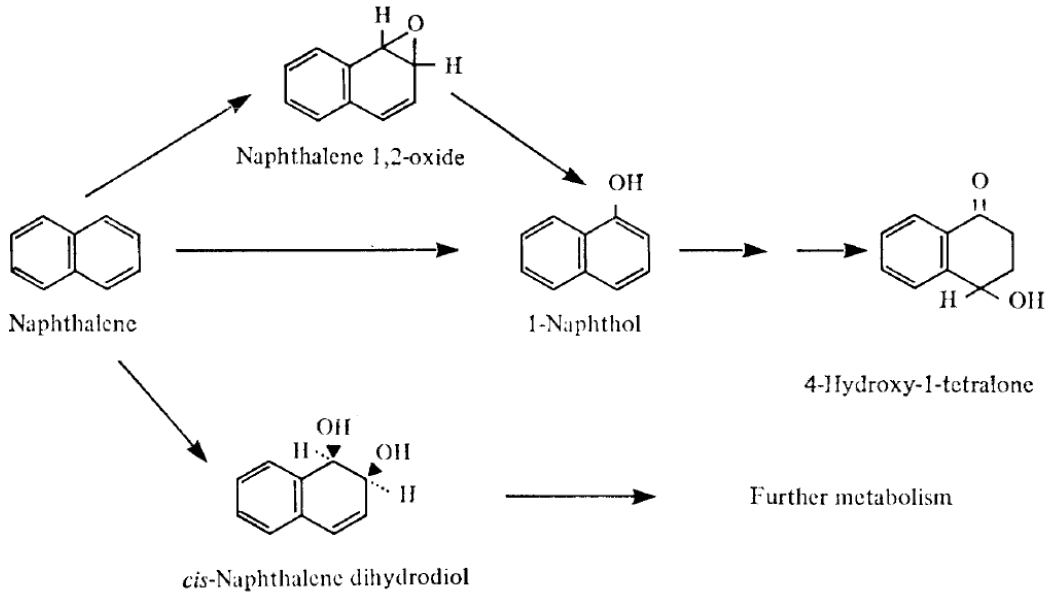


Figure 2.5: Proposed pathways for the metabolism of naphthalene by Oscillatoria sp., strain JCM

2.5.15 Phenanthrene

The degradation pathway of phenanthrene by photo catalytic oxidation (PCO) degradation (in 1% acetone solution) was investigated. Acetone, a water-miscible organic solvent, helps PAHs contact a photo-catalyst more readily to trigger PCO degradation in aqueous solution. Table 2.12 shows the intermediates detected in the test whereas Figure 2.6 shows the proposed degradation pathway of Phenanthrene by photo catalytic oxidation (PCO) degradation (in 1% acetone solution) (O.T. Woo, W.K. Chung, K.H. Wong, A.T. Chow, P.K. Wong, 2009).

Table 2.12: Observed compounds and by-products identified by photo catalytic oxidation (PCO) degradation (in 1% acetone solution) of Phenanthrene

No.	Compounds Detected	Chemical Formulae
14	(1,1′-Biphenyl)-2,2′-dicarboxaldehyde	$C_{14}H_{10}O_2$
15	Benzocoumarin	$C_{13}H_8O_2$
16	9-Phenanthrenol	$C_{14}H_{10}O$
17	9,10-Phenanthrenedione	$C_{14}H_8O_2$

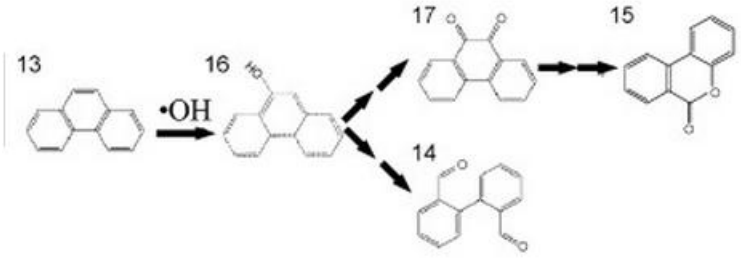


Figure 2.6: Proposed degradation pathway of Phenanthrene based on the compounds details in Table 2.12

2.5.16 Pyrene

Figure 2.7 shows the pathway of pyrene and its photolysis has been studied in water and in Brij 35 micellar media. Photolysis in both media lead to the formation of 1,6- and 1,8-pyrenequinones as stable products. The first step in the photochemical oxidation is proposed to involve an electron transfer from the excited singlet state of pyrene to molecular oxygen in a contact charge-transfer pair. 1-Hydroxypyrene is identified as a product of the initial photochemical oxidation and undergoes further photochemical oxidation to produce 1,6- and 1,8-pyrenequinones as illustrated in Figure 2.7 (Michael E. Sigman, et.al, 1998).

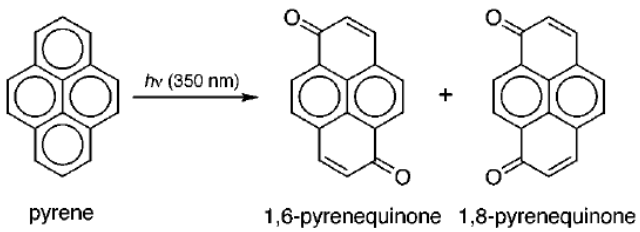


Figure 2.7: Proposed pathway for photolysis of Pyrene

2.6 Performing Toxicity Test on Organisms in PAHs environment

In an exposure experiment, the impact of B[a]P is examined on zooplankton community using conceivable concentrations in the environment (5 and 10µg/l) using typical zooplankton community in eutrophicated systems.

Despite the residence time of B[a]P in the water column was short as 4 days, it induced decrement of zooplankton abundance.

Consequently, B[a]P showed insecticide-like impacts, suppressing cladoceran populations and inducing the dominance of rotifers particularly under high concentration (10ug/l).

Results have suggested that, even such short duration of B[a]P in the water body can have impact on zooplankton abundance and community structure (Yoshinori Ikenaka, Masaki Sakamoto et.al., 2012).

Figure 2.8 shows the change of individual numbers of cladocerans in mesocosms. Arrows indicate the application of B[a]P on day 24. Control specimen has a healthy growth rate meanwhile the sample with low concentration of B[a]P suffers from growth inhibition. Sample with high concentration of B[a]P proves to be high in toxicity as the number of cladocerans decreases to zero at day 28, just 3 days after implementation of B[a]P (Yoshinori Ikenaka, Masaki Sakamoto et.al., 2012).

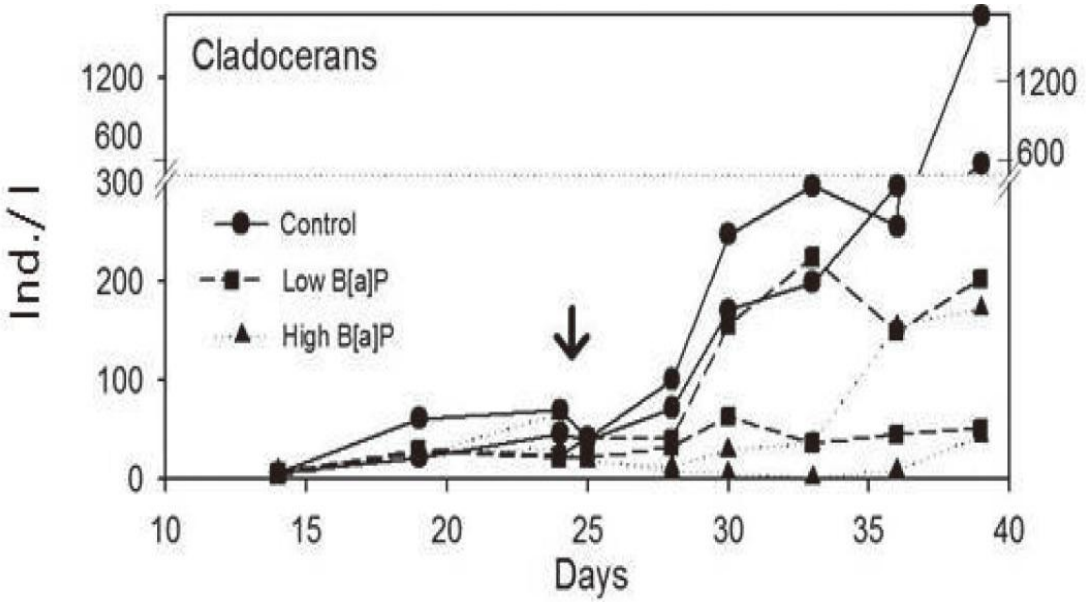


Figure 2.8: Change of individual numbers of cladocerans in mesocosms. Arrows indicate the application of B[a]P on day 24.

CHAPTER 3.0

METHODOLOGY

3.1 Sampling

This part was done in order to obtain data and information about PAHs content on particular sampling point and to which extent variations in PAHs content can be expected. Manual sampling method was used to collect raw river water (influent) sample from water treatment plant (WTP) of Teluk Kepayang as it was previously susceptible with PAHs-contaminants and hence suitable to be used as the place to perform case study on identification of parents-daughters of PAHs. Experiments were also performed on domestic urban wastewater. Hence, sewage treatment plant (STP) of Universiti Teknologi PETRONAS (UTP) was a preferred location as wastewater sampling point.

3.1.1 Sampling Procedure

Once the collection was decided and marked, a proper method/procedure was followed in sampling process. The site was approached from downstream, by standing facing upstream. The bottom sediment of the collection site was not disturbed.

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 with 20cm beneath the surface or midway between the surfaces and bottom if the water was shallow.

The bottle was later let to be filled by running current river water. Once full, the bottle was emptied downstream and it was repeated for three times for rinsing purposes. After the completion of rinsing, the bottle was filled again with river water, leaving one inch of air space to allow for shaking or mixing before analysis.

Sample bottles were labelled with bottle number, site identification, date and time, and packed in ice at the sampling location. The samples were stored in the cold room of Environmental Laboratory of Civil Engineering Department, UTP.

3.2 Liquid–Liquid Phase extraction

The liquid-liquid extraction was done using the Standard Method 6410 B. 1L of water sample was taken and by adding 100ml of dichloromethane, it allowed extraction of PAHs. The sample was shaken for 5-10 minutes to allow coagulation. If the emulsion cannot be broken, the extraction procedure was continued with an additional of 60 mL volumes of dichloromethane.

By using separate funnel, solvent was then separated from the water. Anhydrous Sodium Sulphate was applied to absorb any remained water in separated organic layer. The hydrated form of sodium sulphate was formed (clumped together) indicating the presence of water molecules in the extracted sample. The additional of salt was continued until no more clumped salt formed.

The solvent was later put into rotary evaporator at 40°C of water bath temperature with 120 rpm until the apparent volume of sample liquid reached 1 to 2 mL of dichloromethane.

Then, it was transferred into the 1.5 mL vial, ready for GCMS analysis. Samples were analysed within 40 days after the re-concentration.

3.3 GC/MS Analysis

GC/MS analysis was performed with a Car Erba GC8000 and mass spectrometer (Fisons MD800). 30M elite-5MS column (5% phenylmethy-silicone; 0.25mm I.D., 0.25mm film thickness) was installed with optimum temperature of 320 degree Celsius.

There were two methods of detection performed, namely qualitative and quantitative analysis. Qualitative method was based on standard library NIST05a.L, whereas quantitative method detection was based on the calibration of BPX5-SIM-PAH calib2013jun.M set by technician responsible for GCMS analysis. This research focused more on qualitative approach as formulation of conversion path of carcinogenic PAHs was performed through identification type of compounds. The result was later stored in the library of computer attached to GC/MS equipment in the result section.

3.4 Toxicity Test

This test was carried out in the Environmental Laboratory of Civil Engineering Department of UTP. The acute toxicity received from raw influent of WTP Teluk Kepayang, influent and effluent UTP STP to guppy, *Poecilia reticulate* species was estimated by using fully grown adults in 96 hours, constant renewal test. The effects included the synergistic, antagonistic, and additive effects of the all chemical, physical, and biological components which adversely affect the physiological and biochemical functions of the test organisms.

In this acute toxicity test, *Poecilia reticulate* was chosen as the test specimen for its wide availability. This species is commonly found in streams of freshwater in Malaysia and it is one of the fish species recommended for acute toxicity testing in OECD requirement (OECD, 2008).

3.4.1 Acclimation period

A number of 180 fully grown and healthy guppies that were breed in local fish farm (less than 4 weeks) were bought. Those fishes were quickly transferred immediately to laboratory within an hour in well aerated plastic bags.

Figure 3.1: The acclimation tank that holds all the fishes for 14 days

Those fishes were first acclimated for a total of 14 days with continuous aeration. The purpose of the acclimation was to allow the fishes to adapt to the new environment and it must be held in water of the quality to be used in the test. The acclimation process took place by placing all the fishes in a prepared 100 litre glass container containing filtered tap water. The tap water was tested for its pH, dissolved oxygen concentration and conductivity. The result was listed below:

Table 3.1: Condition of the treated tap water in acclimation tank

pH	6.8 – 7.2
Dissolved Oxygen Concentration	7.9 – 8.3 mg/l
Conductivity	80 µs

The tap water was treated to remove chlorine before it is channelled into the container. Sodium thiosulfate was used as the removing agent. 8 ml of sodium thiosulfate was required to remove chlorine from an amount of 30 litres of tap water. Hence, in this acclimation tank in which to be filled with 50 litres of tap water, 13ml sodium thiosulfate was required. The tank water was renewed every 48 hours with constant aeration.

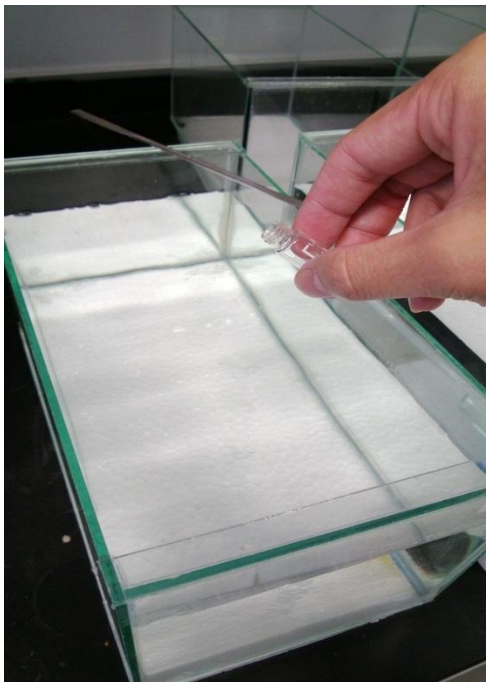


Figure 3.2: A prepared solution of sodium thiosulfate was poured to remove the dissolved chlorine from tap water

The condition of the lab was ensured to receive 12 hours of daylight and 12 hours of darkness daily. The temperature of the lab was optimized to about 24°C, as in the range of recommended test temperature for guppy which was between of 21-25°C. The feeding process was done with commercial fish food on daily basis and it was stopped 48 hours prior to beginning of test.

3.4.2 Test solutions

Test solutions were prepared by adapting the exact concentration of solution from samples. Fishes were exposed to raw sample of influent of WTP Teluk Kepayang, influent of UTP STP and effluent of UTP STP.

Figure 3.3: The collected 15L of raw influent WTP Teluk Kepayang samples as test solution

3.4.3 Test period and procedure

Acute toxicity test with *Poecilia reticulate* was performed according to OECD recommendations (OECD, 1993). Laboratory static tests were conducted to determine the mortality rate as static test was 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.

Figure 3.4: The transferring process of fishes to 5L test chamber

5L test chamber was prepared and filled with 5L raw sample of influent WTP Teluk Kepayang. The test chamber was stocked with fishes in a ratio of 1.0g/L water. As each fishes weigh 0.5g each, groups of 10 fishes of similar size were randomly sampled and transferred with the help of small hand net from the acclimation tank into the test chamber. Careful steps were taken to avoid any possibility of mechanical injury to the test fish. No dilution was required and the concentration of raw sample was preserved as originally obtained from sites.

A control group was produced with only presence of dilution water without any addition of samples and it should be maintained with zero mortality throughout the experiment.

The experiments were replicated three times in two other test chambers with similar raw source and concentration of sample used. The number of fishes and the environment was maintained as according to initial test. All the replicates run concurrently with the initial test to obtain consistent and more accurate data findings.

The static renewal method was used in which no flow of test solution occurs and remains unchanged throughout the duration of the test.

A flexible silicone tube was attached to a regulated air supply to provide sufficient amount of oxygen to avoid concentration of DO below 5mg/L. Physiochemical parameters (pH, conductivity, dissolved oxygen and temperature) were measured daily in each test chambers and the water temperature was maintained at 24±^{oc}. Feeding was discontinued during the 96 hours period.

Figure 3.5: An initial test of influent WTP Teluk Kepayang with two replicates and a control tank

Mortality and behavioural changes was observed and recorded for every interval of 12 hours throughout the test period with constant photographing as evidence purposes. During the experiment, dead fish were removed to prevent static bioassays that might deplete dissolved oxygen in the test chamber.

The mortality rate was then calculated based on number of death in each group.

The above mentioned procedures were repeated next by using influent and effluent of UTP STP as test solution.

3.5 Gantt Chart and Key Milestones

		FYP 1														FYP 2													
No.	Details/Week	1	2	3	4	5	6	7	8	9	10	11	12	13	14	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	Topic Selection																												
2	Proposal Submission																												
3	Proposal approval by research cluster																												
4	Preliminary research/literature review																												
5	Extended Proposal																												
6	Experiment/Research Familiarization																												
7	Proposal Defense Presentation Preparation																												
8	Research work																												
9	Interim Report Preparation (Draft)																												
10	Interim Report Submission																												
11	Preparation for FYP 2																												
12	Experiment Work																												
13	Initial Sub-Analysis Conclusion																												
14	Experiment Verification																												
15	Progress Report Submission																												
16	Intermediate Sub-Analysis Conclusion																												
17	Full Report Presentation Preparation																												
18	Pre-SEDEX preparation																												
19	Draft Report Submission																												
20	Dissertation Submission (Soft Bound)																												
21	Technical Paper Submission																												
22	Oral Presentation																												
23	Dissertation Submission (Hard Bound)																												

Key Milestone

CHAPTER 4.0

RESULT AND DISCUSSION

4.1 GC/MS Analysis

4.1.1 Influent WTF Teluk Kepayang

Using standard library of database\NITTO5a.L, GC/MS analysis was performed on prepared sample of vial from influent WTP Teluk Kepayang and the result of the possible compounds were identified based on given retention time on peak graphs in Figure 4.1. The data were tabulated in Table 4.1.

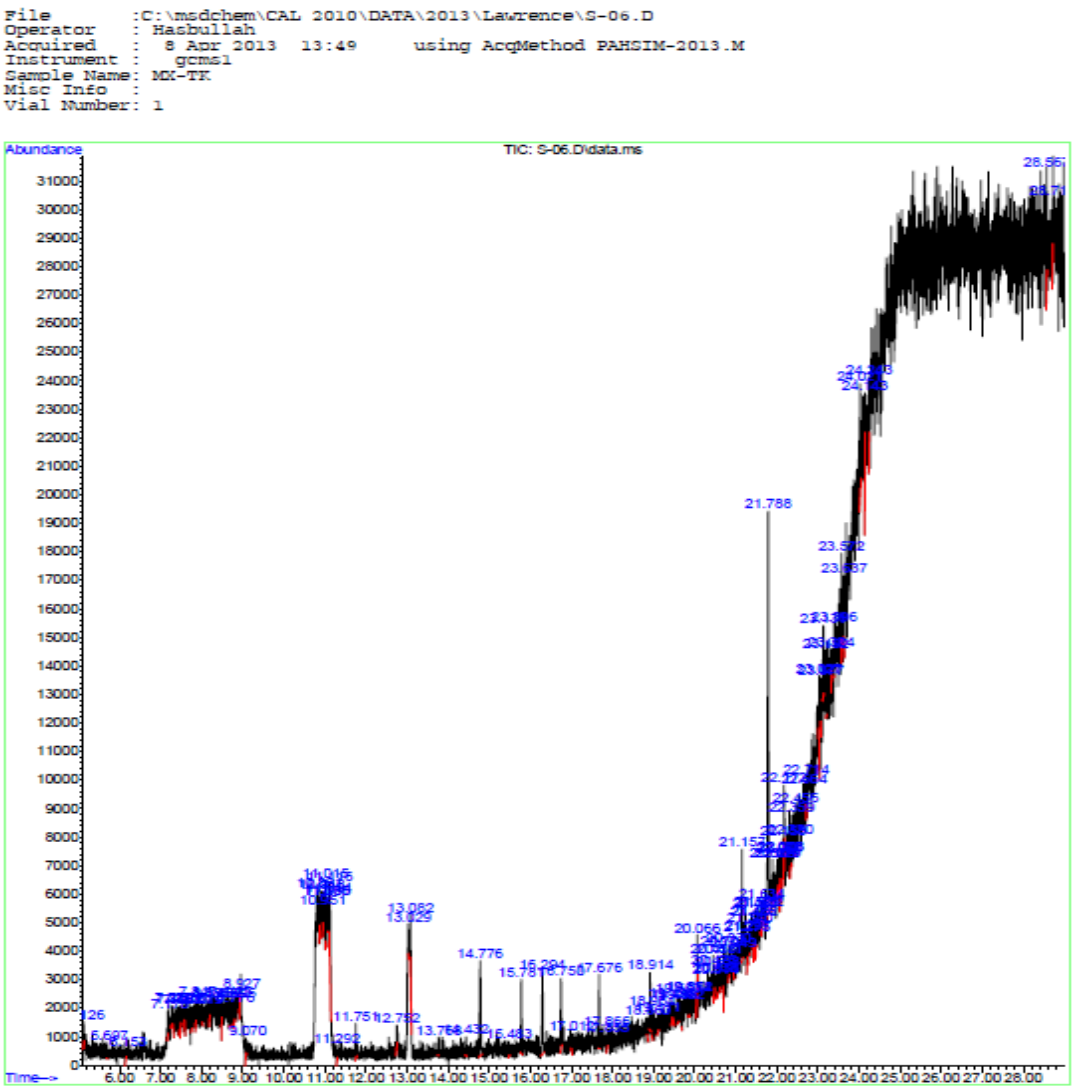


Figure 4.1: Graph of retention time against peak height in GC/MS analysis on influent Teluk Kepayang

Table 4.1: Tabulation of detected significant compounds of GC/MS analysis on influent Teluk Kepayang

Relation	Suspected PAHs/ Derivatives	Detected amount (% of total)	Classification	Maximum Threshold Limit	Remarks
Parent	Acenaphthylene	-	N/A	N/A	N/A
Daughters	1(3H)-isobenzofuranone	2.517	Not classified	Mammal: 2000 ppm (diet) Aquatic 96hr LD_{50} : 320 mg/l Algae 72hr LD_{50}: 1000 mg/l	- Irritant to eyes and inhalation.
	benzaldehyde	0.465	Not classified	Mammal LD_{50} : 1300 mg/kg (oral) Aquatic 96hr LD_{50} : 11.2 mg/l Algae 72hr LD_{50} : 340 mg/l	
Parent	Chrysene	-	N/A	N/A	N/A
Daughter	Phthalic acid	15.697	Not classified	Mammal LD_{50} : 7900 mg/kg (oral) Aquatic 96hr LD_{50} : >100 mg/l Algae 72hr LD_{50} : >100 mg/l	- The substance is toxic to mucous membranes.
Parent	Fluoranthene	-	N/A	N/A	N/A
Daughters	Benzoic acid	4.821	Slightly toxic	Mammal LD_{50} : >2100 mg/kg (oral) Aquatic 96hr LD_{50} : 180 mg/l Algae 72hr LD_{50} : 10 mg/l	- May cause urticaria, asthma, rhinitis and anaphylactic shock - Possible liver, kidney and brain toxicant
	Phenylacetic acid	1.358	Not classified	Mammal LD_{50} : 2250 mg/kg (oral) Aquatic 96hr LD_{50} : 127.37 mg/l	- The substance is toxic to lungs, mucous membranes
Parent	Naphthalene	0.564	Suspected Carcinogenic	Mammal LD_{50} : 490 mg/kg (oral) Aquatic 96hr LD_{50} : 305.2 mg/l Algae 72hr LD_{50} : 340 mg/l	- The substance is toxic to blood, kidneys, the nervous system, and multiple organ systems failure.
Daughters	1,4-naphthalenedione	0.364	Moderate toxic	Mammal LD_{50} : 190-202 mg/kg (oral) Aquatic 96hr LD_{50} : 3.5 mg/l Algae 72hr LD_{50} : 3.5 mg/	- Very toxic to aquatic organisms. - Very toxic by inhalation.
	Phthalic acid	Similar as mentioned above			

4.1.2 Influent and Effluent of UTP STP

Using standard library of database\NITTO5a.L, GC/MS analysis was performed on prepared sample of vials of influent and effluent of UTP STP. The result of the compounds were identified based on given retention time with peak graphs in Figure 4.2 and Figure 4.3 and was tabulated in Table 4.2.

```
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Instrument :   gcms1
Sample Name: Influent mx
Misc Info  :
Vial Number: 1
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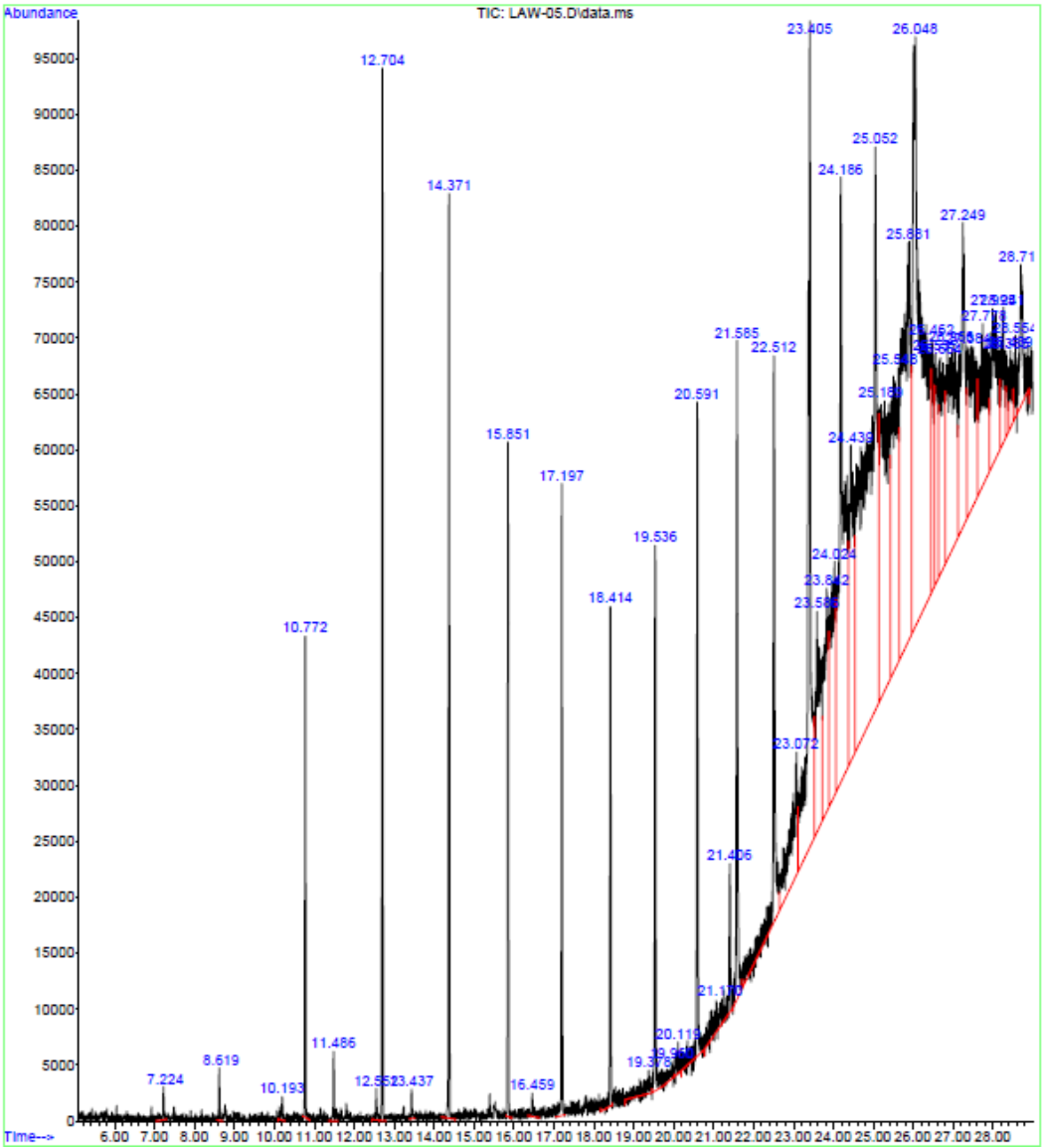

Figure 4.2: Graph of retention time against peak height in GC/MS analysis on influent UTP STP

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Acquired   : 29 Jul 2013  11:57     using AcqMethod BPX5-SIM-PAH200.M
Instrument :   gcms1
Sample Name: effluent mx
Misc Info  :
Vial Number: 1
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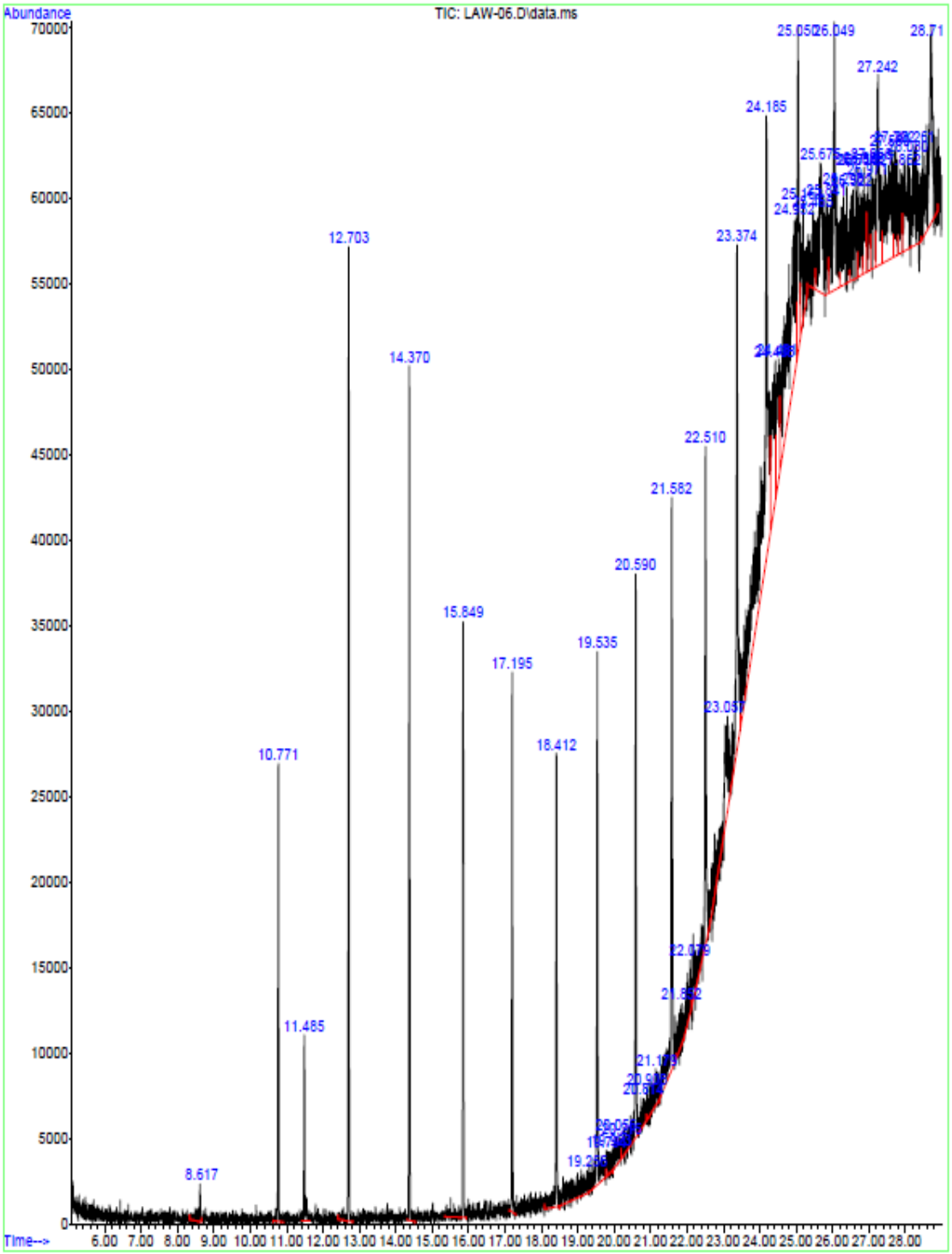

Figure 4.3: Graph of retention time against peak height in GC/MS analysis on effluent UTP STP

Table 4.2: Tabulation of detected significant compounds of GC/MS analysis on influent and effluent UTP STP

Relation	Suspected PAHs/ Derivatives	Detected amount (% of total)		Classification	Maximum Threshold Limit	Remarks
		Influent	Effluent			
Parent	Chrysene	-	-	N/A	N/A	N/A
Daughter	Phthalic acid	2.170	4.011	Not classified	Mammal LD_{50} : 7900 mg/kg (oral) Aquatic 96hr LD_{50} : >100 mg/l Algae 72hr LD_{50} : >100 mg/l	- The substance is toxic to mucous membranes.
Parent	Benz[a]anthracene	-	-	N/A	N/A	N/A
Daughter	2H-1-Benzopyran-2-one	-	3.020	Not classified	The toxicological properties have not been fully investigated	- Causes respiratory tract irritation. Irritant. Harmful if swallowed, inhaled, or absorbed through the skin. - Causes eye and skin irritation. May cause digestive tract irritation.
Parent	Fluoranthene	-	-	N/A	N/A	N/A
Daughters	Benzoic acid	3.944	4.515	Slightly toxic	Mammal LD_{50} : >2100 mg/kg (oral) Aquatic 96hr LD_{50} : 180 mg/l Algae 72hr LD_{50} : 10 mg/l	- May cause urticaria, asthma, rhinitis and anaphylactic shock - Possible liver, kidney and brain toxicant
	9-Hydroxyfluorene	-	0.921	Not classified	The toxicological properties have not been fully investigated	-
Parent	Naphthalene	-	-	N/A	N/A	N/A
Daughter	Phthalic acid	Similar as mentioned above				

4.2 Toxicity Test

Physico-chemical properties such as Dissolved Oxygen (DO), pH, temperature, turbidity and chemical oxygen demand (COD) were measured and recorded from the samples collected from the respective sampling points in Table 4.3.

Table 4.3: Recorded physico-chemical properties from the collected samples

Sampling Points	**Turbidity (NTU)**	**Dissolved Oxygen (mg/L)**	**pH**	**Chemical Oxygen Demand (mg/L)**
Teluk Kepayang WTP	17.4	8.1	6.72	12
Influent UTP STP	29.2	8.6	7.31	100
Effluent UTP STP	9.9	10.2	7.52	7.52

The number of mortalities of influent WTP Teluk Kepayang was observed and at 24hr, no fishes were observed dead in all three replicates. However, on 48hr, two mortalities were reported on the initial test chamber and a fish was found dead in 1st replicate test chamber. On 72hr, one fish is found dead on each 1st replicate and 2nd replicate test chamber respectively. The data has been tabulated in Table 4.4. For influent UTP STP, only one fish is found dead each on initial test chamber at 96hr and in 2nd replicate test chamber at 72hr respectively. No mortality was recorded at other intervals of influent UTP STP and the data has been tabulated in Table 4.5.

Table 4.4: Recorded mortalities for influent WTP Teluk Kepayang

	24 hours	48 hours	72 hours	96 hours
Initial	-	2	-	-
1st Replication	-	1	1	-
2nd Replication	-	-	1	-
Control	-	-	-	-

Table 4.5: Recorded mortalities for influent UTP STP

	24 hours	48 hours	72 hours	96 hours
Initial	-	-	-	1
1st Replication	-	-	-	-
2nd Replication	-	-	1	-
Control	-	-	-	-

Table 4.6: Recorded mortalities for effluent UTP STP

	24 hours	48 hours	72 hours	96 hours
Initial	-	-	-	-
1st Replication	-	-	-	-
2nd Replication	1	-	-	-
Control	-	-	-	-

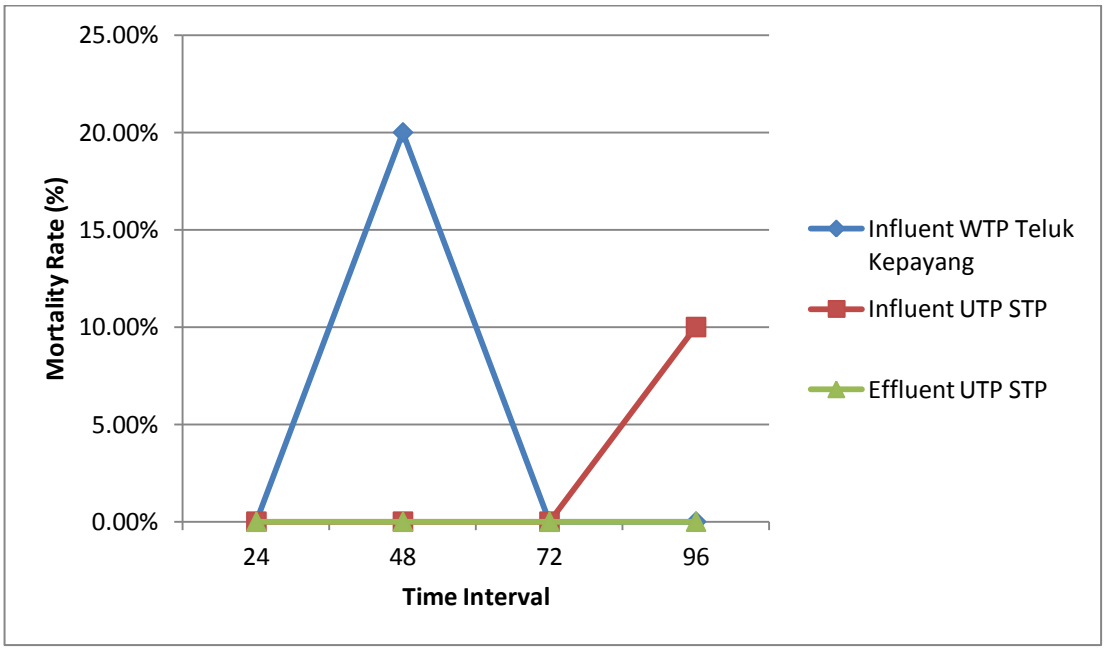

Figure 4.4: Graph of *Poecilia reticulate* mortality rate against time intervals of three respective sampling points

4.3 Discussion

4.3.1 Analysis of GC/MS Results

Based on each respective retention time in Figure 4.1 and 4.2, the types of detected compounds were tabled alongside with its percentage of abundance from GC/MS analysis respectively in Table 4.1 and 4.2. The overall result was attached as appendix A. It was observed that 1, 2-Benzenedicarboxylic acid (Phthalic acid) has highest peak in WTP Teluk Kepayang sample and high corresponding % max in sample of influent UTP STP. Hence, phthalic acid was categorized as dominant quantity among compounds identified in samples of influent WTP Teluk Kepayang and influent of UTP STP.

Phthalic acid is known to be one of the main intermediates of Naphthalene, Chrysene and Antharacene. In photocatalytic degradation of Naphthalene, phthalic acid and 9,10-anthraquinone have been found as relatively stable intermediates whereas phthalic acid are found as trace amounts in degradation of Anthracene (J. Theurich, D.W. Bahnemann, 1997). Phthalic acid can further degrade itself under photo degradation and its compound is to be further identified and compared based on other known studies. It is observed too in both Table 4.1 and 4.2, benzoic acid is identified and it is part of the common derivatives of chrysene and fluoranthene (E. Sepic, M. Bricelj, H. Leskovsek, 2003).

Both Table 4.1 and 4.2 included maximum threshold limit and its known hazardous properties for each compound. Phtahlic acid is known to be dangerous to aquatic life when it reaches above 100mg/l concentration in water stream. Naphthalene was identified in the sample of influent WTP Teluk Kepayang with lowest corresponding % max. 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. However, those compounds are known to be highly toxic to aquatic environments as well if it is 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.

4.3.2 Analysis of Toxicity Test

The turbidity, dissolved Oxygen, pH and chemical oxygen demand were measured accordingly from each of the sample before the test were conducted to ensure the physico-chemical environment of the test solution used is suitable and not harmful for the *Poecilia reticulate.*

For sample of influent WTP Teluk Kepayang, number of mortalities recorded in 96 hours test period was relatively low, with only an average on 20% mortality rate throughout the period tests.

In test of influent UTP STP, number of mortalities recorded was relatively low as well, with average mean of 10% mortality rate from the total of all three replicates throughout the period tests.

Number of mortalities recorded in sample of effluent UTP STP was zero, although in 2^{nd} replication there was a mortality recorded in 24^{th} hour interval, in which it was discovered the premature death was caused by human errors when transferring *Poecilia reticulate* from acclimation tank to test chambers.

There was no obvious recorded major change in the behaviour and swimming patterns of *Poecilia reticulate* for the whole test period of all samples. On the other hand, sudden jerks, mucosal secretion in the gills on the body surface and gathering at the surface of test chambers for breathing were noticed in few of the fishes gradually, which ultimately resulted in death as in the mentioned mortalities above.

No mortality rate was recorded and there was no change in the behaviour and the swimming patterns in all groups of control tank.

Both influents of two separate sampling locations were compiled and the mortality rate was compared against each other. The mortality rate was calculated based on ratio of number of mortalities and number of stock fishes in each test chamber at initial 0hr interval. The average rate were obtained and plotted with mortality rate (%) against time interval (h) in Figure 4.4.

With such low rate of mortality for samples of influent WTP Teluk Kepayang and influent UTP STP, and technically zero for effluent UTP STP, it was concluded the amount of PAHs and its derivatives present in the samples were not critical to be considered toxic and carcinogenic to the aquatic organisms in water streams.

CHAPTER 5.0

CONCLUSION

5.1 Relevancy to Objectives

This project was completed within given time frame to meet the relevant objectives. Literature review conducted has enabled a better understanding on polycyclic aromatic hydrocarbons (PAHs) and its possible linkage of derivatives identified in qualitative GC/MS analysis performed on influent WTP Teluk Kepayang, influent UTP STP and effluent UTP STP.

The conversion path of carcinogenic PAHs in both water stream and sewage polluted urban streams were analysed and tabulated according to its possible derivatives and toxicity. Phthalic acid and benzoic acid were found to be the major derivatives analysed and observed in GC/MS analysis of two different locations of influents. The toxicity intensity of carcinogenic PAHs parents and daughter products were established through 96 hours acute toxicity test and mortality rate of guppies against time exposure of 96 hours for influent WTP Teluk Kepayang and influent UTP STP is 20% and 10% respectively. It is observed that the amount of PAHs and its derivatives detected in both influents of UTP STP and Teluk Kepayang WTP are not harmful to the aquatic environment.

5.2 Recommendations

This project focuses on both analytical and biological approach. Hence, in the future, a comprehensive study should be done to understand more on sources and potential of PAHs and its derivatives to the environment. The project can also be expanded by including possible treatment and handling method on discharged PAHs. On the other hand, this research, in hope to be able to create breakthrough research on PAHs in Malaysia, is set to generate attention on discharged PAHs in water stream. The current law in Malaysia did not include the hazardous effects of such compounds. However, in future research on toxicity establishment, Finneys Probit Analysis Method of EPA should be practiced to find mean LC_{50} values by adopting synthetic compounds with different range of concentration instead of raw samples. Findings from such method can be compared with existing work to increase feasibility and consistency of such gathered data.

CHAPTER 6.0

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