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Jaemin Lee , Byeongwook Choi , Haein Yang , Kyoungjin Lee , Taeyeong Heo , Eunhea Jho , Deokjong Song , Sungjong Lee *

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A Study on the Calculation of TEQ and MEQ of 16 PAHs in Kuwait's Crude Oil-Contaminated Soil and Ecotoxicity Assessment of Bioluminescent Bacteria

Jaemin Lee [1], Byeongwook Choi [1], Haein Yang [2], kyoungjin Lee [3], Tae- Young Heo [4], Eun Hea Jho [5], Deokjong Song [6] and Sungjong Lee [7,*]

[1] Department of Environmental Science, Hankuk University of Foreign Studies, Yongin 17035, Republic of Korea
[2] CENTENNIAL TECHNOLOGY Co., Ansan 15588, Republic of Korea
[3] DONGMOONENT Co. Ltd., Seoul 08377, Republic of Korea
[4] Department of Statistics, Chungbuk National University, Cheongju 28644, Republic of Korea
[5] Department of Agricultural and Biological Chemistry, Chonnam National University, Gwangju 61186, Republic of Korea
[6] Korea Environment Industry & Technology Institute (KEITI), Republic of Korea
* Correspondence: Sungjong Lee; Tel.: +82-31-330-4496, gopzzangno1@naver.com; Tel.: +82-31-330-4778

Abstract: The oil spilled from the Gulf war caused land pollution and various petroleum compounds. Among petroleum compounds, polycyclic aromatic hydrocarbons (PAHs) are known for toxicity, and have been designated as carcinogenic, mutagenic, and endocrine disrupting substances by the United States Environmental Protection Agency (USEPA). According to the USEPA, toxic equivalents quotient (TEQ), and mutagenic equivalents quotient (MEQ) can be calculated by multiplying the toxic equivalency factor (TEF) and mutagenic equivalency factor (MEF) by the concentration of PAHs. The USEPA calculates the relative TEF for other PAHs by setting the TEF value of benzo(a)pyrene, which is highly toxic, to 1. Relative MEF is also calculated in the same way. In this study, the TPH concentrations of crude oil-contaminated soils collected from the Burgan in Kuwait were measured to be 5, 8, and 20% and TEQ and MEQ of the soils were calculated by multiplying the 16 PAHs concentrations measured for each sample by TEF and MEF, respectively. When the TPH concentration was increased by 4 times from 5% to 20%, the TEQ was increased by about 9 times and the MEQ was increased by about 10 times. Therefore, it was concluded that as the TPH concentration increases, the carcinogenicity and mutation rate increase greatly. In addition, ecotoxicity assessment was performed using luminescent bacteria to compare the relationship between the calculated TEQ and MEQ and actual ecotoxicity, and benzo(a)pyrene among 16 PAHs showed a coefficient of determination of 0.95. In this study, ecotoxicity in PAHs-contaminated environment can be estimated by analyzing benzo(a)pyrene as a representative substance.

Keywords: crude oil-contaminated soil; PAHs; bioluminescent bacteria; ecotoxicity assessment

1. Introduction

The Gulf War oil spill was one of the most catastrophic spills in history. During the course of the war between August 1990 and February 1991, approximately 240 million gallons of crude oil were released into the Persian Gulf and Kuwaiti desert causing serious pollution. As a result, more than 700 km of coastlines in Kuwait and Saudi Arabia and 49 kms^2 of Kuwaiti desert were contaminated with crude oil (Ali et al. 2020) [1]. The Iraqi forces that had invaded Kuwait destroyed and burned down hundreds of oil wells as they retreated after losing the war. In this process, oil wells in Kuwait were abandoned as hundreds of oil lakes with an average depth of 2 m. Since then, the released crude oil has been exposed to the natural environment such as the sunlight (UV rays) and winds, and the

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physical and chemical properties of the crude oil were changed that it became extremely challenging to treat the spilled oil with conventional technologies (Mukhopadhyay et al. 2008) [2]. Until today, about 700 of these oil lakes remain neglected, incessantly spreading the land pollution.

Among various petroleum compounds in crude oil, polycyclic aromatic hydrocarbons (PAHs), in particular, are known for their high toxicity, so the United States Environmental Protection Agency (US EPA) has designated these substances as carcinogenic, mutagenic, and endocrine disrupting substances(Gearhart-Serna et al. 2018; Chibwe et al. 2019) [3,4]. PAHs in nature have very low solubility in water, and therefore, are strongly bound to soil (Olayinka et al. 2018) [5]. Also, as the number of benzene rings increases to 4 to 5, environmental persistence and genotoxicity increase. PAHs released can be bioaccumulated by organisms residing in the environment and may ultimately cause fatal damage to human health (Girardin et al. 2020) [6]. When crude oil is released into the environment, substances such as benzene and naphthalene are relatively easily removed due to their high volatility, while substances with many benzene rings and low solubility such as pyrene and benzo(a)pyrene are detoxified through weathering and biodegradation over a long period of time(Hassanshahian et al. 2015) [7]. In particular, high-molecular weight PAHs exhibiting greater mutagenicity and carcinogenicity(Misaki et al. 2016) [8], such as benzo(a)pyrene, remain in water or sediment for a long time. Many pollutants in the environment are degraded by microorganisms in nature, but the process is very slow(Sui et al. 2021) [9]. The presence of microorganisms capable of oxidizing hydrocarbons in natural environment has been the subject of major interests in oil-contaminated areas because of their potential for biodegradation (Rosenberg and Ron 2009) [10]. Studies have been conducted on the metabolic processes of microorganisms for each of aliphatic hydrocarbons, aromatic hydrocarbons, and gaseous hydrocarbons, the genetics of hydrocarbon-utilizing microorganisms, the biological remediation technologies of petroleum hydrocarbons in soil and aquatic environment, and applicability of petroleum hydrocarbon degrading microorganisms for remediation (Adeniji et al. 2019) [11].

When the toxicity mechanism of individual compounds in a group of compounds are the same as in PAHs, the toxic effect of the individual compound can be determined relative to the most toxic or representative compound in the group of compounds. When exposed to a mixture of compounds with the same toxic mechanism, the exposure assessment of these mixture compounds can be conducted after setting toxic equivalency factors (TEF) of each compound based on the toxicity of the substance that can represent each of these compounds(Jung et al. 2010) [12]. The US EPA sets the TEF value of benzo(a)pyrene, which is highly toxic, to 1 and determines the relative TEF for other PAHs. Similarly, mutagenic equivalency factor relative to benzo(a)pyrene (MEF), carcinogenic equivalent quotient (TEQ), and mutagenic equivalent quotient (MEQ) can be determined.

In this study, the concentrations of 16 PAHs in crude oil-contaminated soil collected from the Burgan area in Kuwait at the TPH concentration levels of 5, 8, and 20% were analyzed, and then the 16 PAHs concentrations were multiplied by the TEF and MEF to calculate the TEQ and MEQ, respectively. In order to compare the calculated toxic equivalent values and the actual degree of ecotoxicity, ecotoxicity assessment was performed using bioluminescent bacteria.

2. Materials and Methods

2.1. Sample Collection and Preparation

The crude oil-contaminated soils used in the study was directly collected in Kuwait with the help of the Kuwait Oil Company from the Burgan oil field. The crude oil-contaminated soils with the TPH concentrations of 5, 8, and 20% were collected. The TPH concentration was measured by the *n*-Hexane Extractable Material (HEM) method [13]. [13]. Depending on the depth of the soil collected from the oil lake, the TPH concentration distribution was different. The TPH concentration increased up to 20% with increasing depth. Before the analysis of TPH, the contaminated soil samples were washed with hexane, sieved through a 2 mm sieve, and air-dried. The soil texture of the contaminated soil was sandy loam consisting of 81% sand and 19% silt.

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2.2. PAHs Analysis

The extraction and purification of PAHs from the soil samples were performed according to the US EPA method (1999). PAHs were extracted with *n*-hexane (95%, J.T. Baker) using a Soxhelt apparatus for 16 h. For PAHs analysis, the PAHs internal standard 5 mix (naphthalene-D8, acenaphtene-D10, phenanthrene-D10, chrysene-D12, perylene-D12) and dibenzothiophene-D8 were used as surrogates, and *p*-terphenyl-D14 was used as an internal standard. All internal standards and surrogates were purchased from Accustandard. The concentrations of PAHs were analyzed using a gas chromatography-mass spectrometer (GC-MS; Agilent 6890/HP 5973, Agilent, DE, USA). The column used for the analysis was HP-5MS Ultra Inert (30 m × 0.25 mm i.d. × 0.25 um film thickness), and the spilt mode (1:10) injection method was used. The injector and detector temperatures were maintained at 260°C and 300°C, respectively. The flow rate of He was 1 mL min^{-1}. The GC-MS conditions are summarized in Table 1.

Table 1. Operation conditions of GC-MS.

Parameters	GC-MS Conditions
Injector temperature	260°C
Detector temperature	300°C
Flow gas	He
Flow rate	1mL min^{-1}
Injection volume	1 uL
Column	Silica capillary column HP-5MS Ultra Inert (30m x 0.25mm id x 0.25um)

2.3. Benzo(a)pyrene Equivalent Estimation

Toxic equivalency factors (TEFs) were developed for a number of individual PAHs to express their potency relative to benzo(a)pyrene, which has a TEF of 1. The concentration of the individual PAH compound was multiplied by its respective proposed TEF (Nisbet and LaGoy 1992) [14] (Table 2), and then, all values were summed up to yield benzo(a)pyrene equivalent concentrations, TEQ_{BaP} (Eq. (1)) [15] (Eq. (1)) [15]. The mutagenicity of individual PAH relative to benzo(a)pyrene was also computed using the MEF proposed by Durant et al. (1996, 1999) as shown in Table 2. The concentration of each PAH compound was multiplied by the corresponding MEF and then summed up to give the MEQ_{BaP} (Eq. (2)).

$$\boldsymbol{TEQ_{BaP}} = \sum(\boldsymbol{TEF_i} \times \boldsymbol{C_i}) \quad (1)$$

$$\boldsymbol{MEQ_{BaP}} = \sum(\boldsymbol{MEF_i} \times \boldsymbol{C_i}) \quad (2)$$

where C_i is the measured individual PAH concentration for the "i^{th}" compound with the assigned TEF_i or MEF_i.

Table 2. Proposed benzo(a)pyrene equivalent factors for carcinogenic (TEF) and mutagenic toxicity (MEF).

PAH compound (Abbreviation)	TEF (Nisbet and LaGoy 1992) [14]	MEF (Durant et al. 1996, 1999) [16,17]
Naphthalene(NaP)	0.001	-
Acenaphthylene(Acy)	0.001	-
Acenaphthene(Ace)	0.001	-
Fluorene(Flu)	0.001	-
Phenanthrene(Phe)	0.001	-
Anthracene(Ant)	0.01	-
Fluoranthene(Flr)	0.001	-

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Pyrene(Pyr)	0.001	-
Benzo(a)anthracene(BaA)	0.1	0.082
Chrysene(Chr)	0.001	0.017
Benzo(b)Fluoranthene(BbF)	0.1	0.25
Benzo(k)fluoranthene(BkF)	0.01	0.11
Benzo(a)pyrene(BaP)	1.0	1.0
Indeno(1,2,3-cd)pyrene(InP)	0.1	0.31
Dibenzo(a,h)anthracene(DahA)	1.0	0.29
Benzo(g,h,i)perylene(BghiP)	0.01	

2.4. Ecotoxicity Assessment of Bioluminescent Bacteria

The crude oil-contaminated soil samples extracted using hexane for the PAHs analysis were further treated to use in the assessment of the ecotoxicity with bioluminescent bacteria. The PAHs in the hexane extracted samples were substituted by dimethyl sulfoxide (DMSO), and the concentration of substituted DMSO did not exceed a maximum of 1% (v/v). DMSO was tested for luminescence inhibition as a negative control, and no toxicity was expressed at 1%.

The toxicity test was performed using bioluminescent bacteria(*Allivibrio fischeri*), and the test was carried out following the ISO 11348-3:2007 method. The change in luminescence before and after sample exposure was determined using the Microtox model 500 analyzer (Modern Water, United States). The acute toxicity after 5 min exposure was determined using the 81.9% method. The samples were serially diluted nine times to measure the bioluminescence. The half maximal effective concentration (EC50) of the samples was estimated using the Microtox omni software (Modern Water, United States). The luminescence inhibition rate is calculated as follows.

$$\textbf{inhibition rate}(\%) = (\mathbf{1} - \frac{\boldsymbol{L_t}}{\boldsymbol{I_o R_t}}) \quad (3)$$

where R_t is the correction factor obtained when the bioluminescence intensity of the control after time t is divided by the initial intensity of the control, I_t is the bioluminescence intensity of the samples after time t, and I_0 is the initial bioluminescence intensity of the samples.

3. Results & Discussion

3.1. PAHs in the Crude oil-Contaminated Soils

The TPH concentrations of the crude oil-contaminated soils at different depth analyzed by the HEM method were 5, 8, and 20%. Total petroleum hydrocarbon is a generic term for all hydrocarbon compounds derived from crude oil. Hydrocarbon compounds with C1 to C40, such as hexane, benzene, xylene, toluene, etc. are included in TPH. Polycyclic aromatic hydrocarbons are hydrocarbons with two or more fused benzene rings, which are generated either by pyrolysis of organic substances composed of carbon and hydrogen at high temperatures or by incomplete combustion (United Nations Environment Programme International Labour Organisation, Inter-Organization Programme for the Sound Management of Chemicals (IOMC) 1998; IARC Working Group on the Evaluation of Carcinogenic Risks to Humans (1988 : Lyon 2014) [18,19]. They are easily accumulated in the environment, and some are known to be carcinogenic and mutagenic (United Nations Environment Programme International Labour Organisation, Inter-Organization Programme for the Sound Management of Chemicals (IOMC) 1998; IARC Working Group on the Evaluation of Carcinogenic Risks to Humans (1988 : Lyon 2014) [18,19]. In this study, 16 PAHs suggested by the US EPA were analyzed, and the results are shown in Table 3.

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Table 3. Concentrations of 16 PAHs in the crude oil-contaminated soils with different TPH concentrations.

PAH Compounds	Mean±SD, (ug g^{-1})		
	Soil A (TPH 5%)	Soil B (TPH 8%)	Soil C (TPH 20%)
Naphthalene	0.0305±0.0047	0.2156±0.0251	0.8948±0.1544
Acenaphthylene	0.0840±0.0014	0.1077±0.0067	0.1018±0.0214
Acenaphthene	0.0497±0.0036	0.1152±0.0213	0.3942±0.0058
Fluorene	0.2565±0.0179	0.6579±0.0136	2.3564±0.1300
Phenanthrene	2.6463±0.0514	5.5680±0.4078	14.5326±0.8767
Anthracene	3.0338±0.0589	7.5540±0.4675	16.6606±1.0051
Fluoranthene	0.1394±0.0033	0.1587±0.0030	0.7432±0.0708
Pyrene	0.3829±0.0557	0.4252±0.0700	2.1732±0.1266
Benzo(a)anthracene	0.0757±0.0112	0.1057±0.0060	0.3839±0.0156
Chrysene	0.5561±0.0017	0.8569±0.0302	2.9539±0.1031
Benzo(b)Fluoranthene	0.0546±0.0020	0.1539±0.0186	0.0089±0.0015
Benzo(k)fluoranthene	0.0112±0.0007	0.0627±0.0017	0.4498±0.0213
Benzo(a)pyrene	0.0218±0.0031	0.2973±0.0362	0.4105±0.0317
Indeno(1,2,3-cd)pyrene	0.0030±0.0005	0.0064±0.0005	0.0220±0.0023
Dibenzo(a,h)anthracene	0.0065±0.0004	0.0189±0.0024	0.0376±0.0052
Benzo(g,h,i)perylene	0.0136±0.0010	0.0665±0.0072	0.2013±0.0340
∑16PAHs	7.3659	16.3708	42.3246

As the TPH concentration increased from 5% to 20%, the ∑16PAHs also increased (from 7.3659 to 42.3246 ug g^{-1}), but the ratio of the ∑16PAHs compared to TPH did not significantly increase. The ∑16PAHs ratios of the Soil A, B, and C were analyzed to be about 0.015, 0.020, and 0.021% of the TPH concentration, respectively. This can be attributed to about 30 year long period of weathering processes during which the PAHs in crude oil have either naturally reduced or transformed to alkylated PAHs.

3.2. Estimation of Toxic Equivalence Coefficient and Mutation Equivalence Coefficient of PAHs

Each PAH compound has a different toxicity, so it is not suitable to evaluate the degree of contamination by the sum of each PAH concentration. Therefore, many researchers have tried to indicate the equivalent toxicity of PAHs, and a number of institutions including the US EPA have published the equivalent coefficient for each compound to indicate the equivalent toxicity (Nisbet and LaGoy 1992; USEPA 1993; van den Berg et al. 1998) [14,20,21].

In this study, the equivalence coefficients (TEF, MEF) from Nisbet and LaGoy (1992) and Durant et al. (1996, 1999) were used to calculate the TEQ and MEQ (Table 2). The TEQ and MEQ corresponding to the Soil A, B, and C are shown in Table 4. The total TEQ of the 16 PAHs was calculated to be 0.07642~0.68684. When the TPH concentration increased by about four times (from 5% to 20%), the total TEQ value increased by about nine times, and the total MEQ value increased by about 10 times. This means that as the TPH concentration in the crude oil-contaminated soils increases, the degree of carcinogenesis and mutation rate may appear more rapidly.

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Table 4. TEQ_{BaP} and MEQ_{BaP} determined for 16 PAHs for the crude oil-contaminated soil samples with different TPH concentrations.

PAHs compounds	TEQ			MEQ		
	Soil A (TPH 5%)	Soil B (TPH 8%)	Soil C (TPH 20%)	Soil A (TPH 5%)	Soil B (TPH 8%)	Soil C (TPH 20%)
Naphthalene	0.00003	0.00022	0.00089	-	-	-
Acenaphthylene	0.00008	0.00011	0.00010	-	-	-
Acenaphthene	0.00005	0.00012	0.00039	-	-	-
Fluorene	0.00026	0.00066	0.00236	-	-	-
Phenanthrene	0.00265	0.00557	0.01453	-	-	-
Anthracene	0.03034	0.07554	0.16661	-	-	-
Fluoranthene	0.00014	0.00016	0.00074	-	-	-
Pyrene	0.00038	0.00043	0.00217	-	-	-
Benzo(a)anthracene	0.00757	0.01057	0.03839	0.00621	0.00867	0.03148
Chrysene	0.00056	0.00086	0.00295	0.00945	0.01457	0.05022
Benzo(b)Fluoranthene	0.00546	0.01539	0.00089	0.01365	0.03849	0.00222
Benzo(k)fluoranthene	0.00011	0.00063	0.00450	0.00123	0.00690	0.04948
Benzo(a)pyrene	0.02182	0.29733	0.41051	0.02182	0.29733	0.41051
Indeno(1,2,3-cd)pyrene	0.00030	0.00064	0.00220	0.00093	0.00200	0.00682
Dibenzo(a,h)anthracene	0.00654	0.01890	0.03759	0.00190	0.00548	0.01090
Benzo(g,h,i)perylene	0.00014	0.00067	0.00201	-	-	-
Total	0.07642	0.42777	0.68684	0.05519	0.37342	0.56162

3.3. Results of Acute Toxicity Assessment Using Bioluminescent Bacteria

The acute toxicity of the soil samples were assessed using bioluminescent bacteria following the Microtox 81.9% basic test method, and the results are shown in Table 5. Bioluminescent bacteria was exposed to each sample for 5 min to calculate the EC50. For quality control of bioluminescent bacteria, $K_2Cr_2O_7$ was used as suggested in the ISO 11348-3:2007, and the average EC50 was evaluated to be 50.47 mg L^{-1}.

Table 5. Acute toxicity the crude oil-contaminated soils determined using bioluminescent bacteria.

PAHs compounds	DMSO 1%	Soil A (TPH 5%)	Soil B (TPH 8%)	Soil C (TPH 20%)
EC50(%)	>100	25	2.5	0.75
∑16PAHs(ug/g)	-	7.3659	16.3708	42.3246

The EC50 of the crude oil-contaminated soils increased as the value of the ∑16PAHs increased. The coefficient of determination between EC50 and ∑16PAHs was to 0.55. Toxic unit (TU) values can be calculated by dividing 100 by EC50, so the lower the EC50 value, the stronger the toxicity. The coefficient of determination between each PAH compound and ecotoxicity ranged from 0.02 to 0.95, and benzo(a)pyrene, among the 16 PAHs compounds, was calculated to have the highest value of 0.95. It indicates that among 16 PAHs benzo(a)pyrene has the highest correlation with bioluminescent bacterial ecotoxicity and determines the overall toxicity of PAHs. For each of the 16 PAHs, he physicochemical properties and the magnitude of toxicity determined using the bioluminescent bacteria are shown in Table 6.

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Table 6. Physicochemical properties and toxicity of individual PAH compound.

Compound	Cas No.	MW (g mol^{-1})	Water Solubility (mg L^{-1} (25℃))	Log Kow	EC50 (mg L^{-1})
Naphthalene	91-20-3	128.1	31.6	3.4	0.55
Acenaphthylene	208-96-8	125.1	16.0	4.0	0.24
Acenaphthene	83-32-9	154.2	4.5	3.9	0.45
Fluorene	86-73-7	166.2	1.8	4.2	0.32
Phenanthrene	85-01-8	178.2	1.3	4.6	0.15
Anthracene	120-12-7	178.2	0.07	4.5	0.52
Fluoranthene	206-44-0	202.3	0.24	5.2	0.34
Pyrene	129-00-0	202.3	0.14	5.2	0.27
Benzo(a)anthracene	56-55-3	228.3	0.01	5.9	0.13
Chrysene	218-01-9	228.3	0.003	5.7	0.07
Benzo(b)Fluoranthene	205-99-2	252.3	< 0.001	5.8	0.08
Benzo(k)fluoranthene	207-08-9	252.3	< 0.001	6.0	0.08
Benzo(a)pyrene	50-32-8	252.3	< 0.001	6.0	0.05
Indeno(1,2,3-cd)pyrene	193-39-5	276.3	< 0.001	7.7	0.13
Dibenzo(a,h)anthracene	53-70-3	278.4	< 0.001	6.8	0.05
Benzo(g,h,i)perylene	191-24-2	276.3	< 0.001	6.5	0.08

The 16 individual PAH compounds do not cause the same toxic effects (Rengarajan et al. 2015; Abdel-Shafy and Mansour 2016) [22,23]. Many PAHs are mutagenic, carcinogenic, teratogenic, and immunotoxic to living organisms including microorganisms, animals, and humans (Characteristics 2005; Rengarajan et al. 2015; Bolden et al. 2017) [22,24,25] They are also known to have ecotoxic effects on aquatic organisms (Abdel-Shafy and Mansour 2016) [23]. Benzo(a)pyrene is considered as one of the most carcinogenic PAHs and generally used as an exposure marker for risk assessment (Guo et al. 2011) [26]. Significant accumulation and bioavailability of PAHs in the internal organs, which are rich in adipose tissue, after exposure are due to the high lipophilicity (Guo et al. 2011; Abdel-Shafy and Mansour 2016) [23,26]. Some of the organs most susceptible to tumor formation due to long-term exposure to PAHs include lung, skin, esophagus, colon, pancreas, bladder, and women's breast (Yu 2002; Rajpara et al. 2017) [27,28].

The exploration of the ecotoxicity of Polycyclic Aromatic Hydrocarbons (PAHs) encompasses a critical dimension within environmental research. This complex endeavor involves the assessment of the adverse effects these organic compounds exert on aquatic organisms, serving as valuable indicators of environmental health. Among the array of aquatic organisms available for such assessments, bioluminescent bacteria and daphnia emerge as prominent candidates due to their sensitivity and significance in ecological systems.

Daphnia, often referred to as water fleas, stands as a vital representative of aquatic invertebrates commonly employed in ecotoxicity testing. Daphnia possess inherent sensitivity to various pollutants and environmental stressors, making them an excellent model organism for evaluating the potential impacts of PAHs. In this context, the ecotoxicity assessment carried out using daphnia in the context of the presented study provides crucial insights into the hazardous nature of specific PAHs.

As elucidated by Table 7, the compounds fluoranthene and pyrene, both belonging to the PAH family, exhibited the highest levels of ecotoxicity when assessed using daphnia. This indicates the significant adverse effects these PAHs can impose on aquatic invertebrates, potentially disrupting their life cycles and ecological roles. The choice of daphnia as the experimental organism underscores its ecological importance and its capacity to illuminate the potential ecological ramifications of PAH contamination.

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Table 7. Results of PAHs ecotoxicity assessment of daphnia and bioluminescent bacteria among aquatic organisms.

PAH Compounds	*Daphnia magna* LC50 (mg L^{-1})	*Aliivibrio fischeri* (This study) EC50 (mg L^{-1})	Reference
Acenaphthene	41 (48 h)	0.45	(LeBlanc 1980) [29]
Phenanthrene	0.843 (48 h)	0.15	(Eastmond et al. 1984) [30]
Anthracene	0.02 (1 h)	0.52	(Kagan et al. 1985) [31]
Flouranthene	0.004 (1 h)	0.34	(Kagan et al. 1985) [31]
Pyrene	0.004 (1 h)	0.27	(Kagan et al. 1985) [31]
Benzo[a]pyrene	0.215 (48 h))	0.05	(Atienzar et al. 1999) [32]

Moreover, the study reinforces the idea that the degree of ecotoxicity elicited by PAH substances can significantly vary based on the chosen aquatic organism for assessment. Bioluminescent bacteria, employed alongside daphnia in this research, exemplify the intricate interplay between different organisms and their responses to PAH-induced stress. The divergent outcomes gleaned from the assessments employing these two distinct organisms underscore the complex nature of ecotoxicity within aquatic ecosystems.

Considering the trophic level and the broader food chain dynamics, the selection of appropriate organisms for ecotoxicity assessments becomes paramount. Different aquatic organisms occupy varying positions within these chains, and their sensitivities to contaminants can provide valuable information about potential impacts on entire ecosystems. The insights gained from assessments involving diverse organisms, such as bioluminescent bacteria, microalgae, daphnia, and fish, contribute to a more comprehensive understanding of how PAH contamination can propagate through aquatic environments.

Crude oil, a primary source of PAHs, presents a multidimensional challenge in environmental contamination. While the soil studied here originated from oil wells destroyed during conflict, real-world scenarios more commonly involve the transportation of crude oil through sea and land routes. The diverse composition of oil introduces a spectrum of PAHs, each with varying degrees of toxicity and environmental persistence. The ubiquity of PAHs in oil-contaminated areas underscores the need for meticulous ecotoxicity assessments to gauge potential risks to aquatic life.

Importantly, this study underscores the pivotal role of benzo[a]pyrene, a specific PAH, as a reliable indicator of ecotoxicity within PAH-contaminated environments. With a Toxic Equivalency Factor (TEF) and Mutagenic Equivalency Factor (MEF) of 1.0, benzo[a]pyrene stands out for its high carcinogenic and mutagenic potential. The demonstrated correlation between benzo[a]pyrene and the ecotoxicity observed in bioluminescent bacteria lends weight to its potential as a representative marker for estimating the magnitude of ecological risk in PAH-contaminated ecosystems.

In conclusion, the comprehensive assessment of PAH-induced ecotoxicity in aquatic environments is an intricate process that involves the meticulous selection of organisms, accounting for trophic interactions and considering the broader ecological context. The results of ecotoxicity assessments, as exemplified by the utilization of daphnia and bioluminescent bacteria in this study, underscore the diversity of responses organisms exhibit toward PAHs. These findings contribute to a deeper understanding of the potential ecological consequences of PAH contamination, enhancing our ability to manage and mitigate the impacts of such pollutants on aquatic ecosystems.

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