Ecotoxicology and Environmental Safety 183 (2019) 109547

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Multiple evaluation of the potential toxic effects of sediments and biota collected from an oil-polluted area around Abu Ali Island, Saudi Arabia, Arabian Gulf

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ARTICLE INFO

Keywords:
AhR-mediated potency
Luminescence inhibition
Next generation sequencing
Microbial community
Effect-directed analysis
Sediments

ABSTRACT

After the Gulf War Oil Spill, there have been many investigations about distributions of oil-derived pollutants nearby areas, but lacking in ecotoxicological assessment. We evaluated the potential toxicity of asphalt mats, sediments, and biota (polychaetes, chitons, snapping shrimps, and crabs) by combining two bioassays (H4IIE-*luc* and *Vibrio fischeri*) and *in situ* microbial community (eDNA). Samples were collected from Abu Ali Island, and organic extracts were bioassayed and further fractionated according to the chemical polarity using silica gel column. Great aryl hydrocarbon receptor (AhR)-mediated potencies and inhibition of bioluminescence were mainly found in aromatics (F2) and saturates (F1) fractions of asphalt mat and sediments, respectively, while great toxicological responses in biota samples were found in resins and polar (F3) fraction. We also confirmed that potential toxicities of biota were species-specific; great AhR-mediated potencies were found in polychaetes and great bioluminescence inhibitions were found in crabs. In microbial communities, most genera (up to 90%) were associated with polycyclic aromatic hydrocarbons (PAHs)-degrading bacteria, supporting that PAHs are the primary stressors of the benthic community around Abu Ali Island. The present study provides useful information on the contamination status, risk assessment of environmental matrices and benthic organisms in Abu Ali Island.

1. Introduction

The northern shoreline of Abu Ali Island in the western Arabian Gulf was covered with a massive oil spill during the Gulf War of 1991 (Bejarano and Michel, 2010; Saeed et al., 2017). Although many endeavors have been attempted for restoration over the last three decades, stranded oils (asphalt mats) are still found in sediments, which serve as reservoirs for oil contamination around Abu Ali Island (Bejarano and Michel, 2010). Extensive monitoring and risk assessments have been carried out regarding of measuring petroleum-related hydrocarbons in seawater, sediments, and biota (Bejarano and Michel, 2010; de Mora et al., 2010; Yoon et al., 2019). In spite of the efforts in a variety of research fields, the chemical analysis has not been sufficiently performed since there are still many untargeted toxic chemicals and lack of information about bioavailability and bioactivity of compounds (Hong et al., 2012). Thus, toxicological methods such as *in vitro* bioassay are needed to complement existing chemical analyses. The advantages of *in vitro* bioassays are that they incorporate parameters that will react to the toxicity of unknown or unmeasured chemicals. Furthermore, unlike individual chemicals, toxicity is still detectable at very low levels, even if individual chemicals fall below the quantification limit (Escher et al., 2011). Despite these benefits, the results of bioassays are often criticized due to a shortage of a clear ecological relevance linked with their population- and community-level effects appearing limited (de Castro-Català et al., 2016). In the present study, reliable environmental risk assessments of exposure to a mixture of

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https://doi.org/10.1016/j.ecoenv.2019.109547
Received 3 April 2019; Received in revised form 30 July 2019; Accepted 5 August 2019

contaminants could be used to integrate several disciplines, including environmental chemistry, toxicology, and ecology.

For regional characterization regarding the oil impacts in Abu Ali Island, this region also contains many harbors and factories, which are continuously subjected to oil pollution that might lead to contamination with crude oil and products refined from oil (Al-Thukair et al., 2007; Barth, 2003). The oil residues in sediments might cause significant adverse effects on various marine organisms, especially to epi- and endo-benthic fauna as well as sediment microorganisms (Hong et al., 2012; Peterson et al., 2003). In fact, the stranded oil pollution is a well-known factor that may cause the changes and regulating dynamics in communities of micro-biotas which play an essential role in degrading spilled oil and/or residues (Lee et al., 2019a, b; Xie et al., 2018). Thus, the identification and enumeration of micro-biota communities in sediments that allows fine-scale analyses of ecosystems could be used as effectively and/or suitable strategies for assessing the effects of oil contamination. Furthermore, various biota affected through the food chain might lead to direct or indirect exposure in humans (Li et al., 2009). For this reason, continuous monitoring of impacted areas is needed, as well as identifying the toxic effects of residual oils in sediments and biota.

Crude oil is composed of several hundreds of chemicals containing polycyclic aromatic hydrocarbons (PAHs), heavy metals, and other hydrocarbons (Hong et al., 2015; Srogi, 2007). Many of these compounds, including PAHs and alkylated PAHs, are known to be toxic and persistent and are being major contributors to the toxicity of oil residues (Lee et al., 2011). Furthermore, the toxicity of PAHs changes under certain field conditions, identifying characteristics of toxic causative agents of stranded oil seems to be challenging due to their great complexity and mixture toxicity (Hong et al., 2012). It is very important to identify the major toxic substances and their characteristics in the monitoring and management of oil-contaminated sites. Effect-directed analysis (EDA) has made it easier to identify toxic causative chemicals in environmental mixtures such as crude oil. EDA is based on a combination of biotesting, fractionations, and chemical analysis, which separated fractions contain successively fewer groups or individual organic chemicals of similar functionality or polarity (Hong et al., 2015, 2016a; Vrabie et al., 2012). The greatest advantage of using EDA to identify contaminants in biotic versus abiotic components is that the bioavailability, bioaccumulation, and possible metabolization of compounds are also evaluated (Brack et al., 2009).

This study aimed to provide a comprehensive assessment of the potential toxic effects on oil-polluted areas, *in vitro* bioassays (H4IIE-*luc* and *Vibrio fischeri* assays) were conducted with environmental DNA (eDNA) metabarcoding to identify microbial responses as ecological relevance. The specific aims were to (1) evaluate aryl hydrocarbon receptor (AhR)-mediated potencies associated with organic extracts and fractions of sediments and biota samples collected from the Abu Ali Island, (2) identify the causative chemicals responsible for the observed biological activities in terms of potency balance by using both bioanalytical and instrumental analyses, (3) assess the inhibition of bioluminescence, and (4) characterize *in situ* response of indigenous bacterial communities to oil contamination in the Abu Ali Island.

2. Materials and methods

2.1. Sample collection and preparation

Asphalt mat, surface sediments, and biota (polychaetes, chitons, snapping shrimps, and crabs) were collected from an oil-contaminated area along the northern shoreline of the Abu Ali Island, Saudi Arabia, Arabian Gulf (otherwise known as the Persian Gulf), in December 2016. All samples were collected from three sites, except for the asphalt mat, which was only collected from site S2 (Fig. 1). Snapping shrimps and chitons were not available for analysis, because not enough specimens were collected from sites S1 and S3, respectively. All samples were collected using a stainless-steel spatula and hand capture and immediately transported to the laboratory and stored at −20 °C until analysis. The shells of the chitons were removed and whole somatic soft tissues were pooled and homogenized. Subsequently, all samples were freeze-dried.

2.2. Extraction and fractionation

Sample preparations for bioassays and chemical analyses have been performed following the methods described by Hong et al. (2012, 2015) with minor modification. In brief, 10 g of freeze-dried sediment sample or 1 g of biota sample was extracted by dissolving them in 350 mL dichloromethane (Burdick & Jackson, Muskegon, MI) in a Soxhlet extractor for 16 h. To remove elemental sulfur, the organic extracts were treated with activated copper powder (Sigma Aldrich, Saint Louis, MO) and concentrated to 2 mL. The organic extracts (Raw) were separated into two aliquots for use in the *in vitro* bioassays and the chemical analysis. The portion of the organic extract (1 mL) used in the bioassays was exchanged in dimethyl sulfoxide (DMSO, Sigma-Aldrich). For effect-directed analysis, 800 μL of the organic extract was passed through 8 g activated silica gel in a packed glass column for fractionation (Hong et al., 2015). The first fraction (F1), containing nonpolar compounds (e.g., saturates), was eluted with 40 mL hexane. The second fraction (F2), containing PAHs and organochlorine pesticides (e.g., aromatics), was collected by elution with 50 mL of 20% dichloromethane (DCM) in hexane (v/v). The third fraction (F3), containing polar compounds (e.g., resins), was eluted in 50 mL of 60% DCM in acetone.

2.3. H4IIE-*luc* bioassay and potency balance analysis

The H4IIE-*luc* bioassay was performed to detect AhR-mediated potencies in environmental samples, such as asphalt mat, sediments, and biota according to previously published methods (Hong et al., 2012). In brief, the trypsinized cells ($\sim 7.0 \times 10^4$ cells mL^{-1}) were seeded into 96 micro-well plates and incubated for 24 h. Then, dosed with the appropriate standards (benzo[*a*]pyrene (BaP) for 4 h; 0.1% dose), samples (raw and fractions; 0.1% dose), and solvent controls (0.1% DMSO). After 4 h of exposure, the results were expressed as relative luminescence units that were quantified using a Victor X3 multi label plate reader (PerkinElmer, Waltham, MA). The responses of the H4IIE-*luc* bioassay were converted to percentages of the maximum response according to 50 nM BaP ($= 100\%BaP_{max}$). AhR-mediated potencies were expressed as a BaP equivalent concentration (ng BaP-EQ g^{-1} dry mass (dm)) for direct comparison to instrumentally-derived BaP equivalent concentrations (BEQs). All the bioassays were conducted in triplicate.

A potency balance analysis between bioassay-derived concentrations (BaP-EQ) and instrument-derived concentrations (BEQs) in the samples was conducted to determine the contribution of each known chemical to total induced AhR-mediated potency. Instrument-derived BEQs were calculated as a BEQ sum by multiplying the concentration of seven individual AhR-active PAHs with previously established relative potency values (RePs) (Kim et al., 2019). Of note, the reported concentrations of PAHs measured in the same samples (Yoon et al., 2019) were utilized for the calculation of instrument-derived BEQs, and the raw data was also provided to aid understanding of PAHs contaminations cross samples in a comparative manner (Table S1 of the Supplementary Materials (S)).

2.4. *Vibrio fischeri* bioassay

The *Vibrio fischeri* bioassay was used to estimate the potential toxicity of the sediments and biota, which represent the bioavailability of sediment-bound pollutants (Adams et al., 2015). A bioluminescence test with *V. fischeri* (NRRL B-11177) was conducted using a luminescent bacteria toxicity measurement apparatus (N-TOX model 200; NeoEnBiz Inc., Bucheon, Korea) following the standard method specified by the

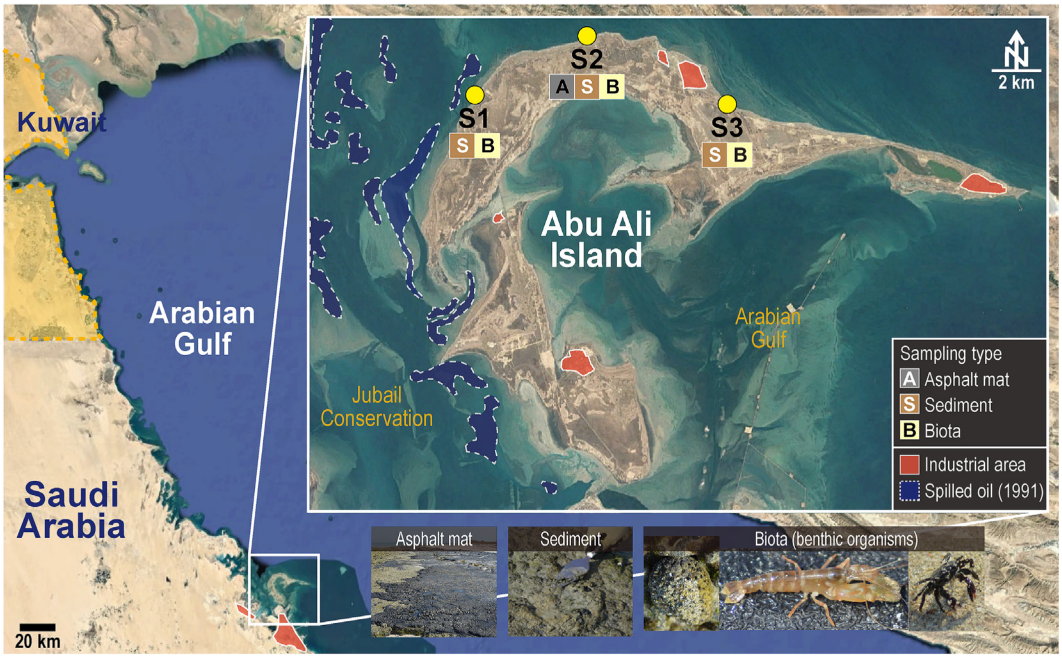

Fig. 1. Map showing the sampling sites from which asphalt mat, sediments, and biota were collected in Abu Ali Island, Saudi Arabia. The photographs show the sampled biota.

Ministry of Maritime Affairs and Fisheries of South Korea (MOMAF) (MOMAF, 2005). A detailed description of the bioassay has been published previously (Lee et al., 2019a, b). A zinc sulfate solution was used as a reference chemical. This standard was compared with every fresh vial of bacteria to ensure validity across all tests (The International Organization for Standardization (ISO), 1998). Extracts and fractionation samples of sediments and biota were used in the bioluminescence test. All of the bioluminescence inhibition measurements were performed in triplicate and were described as inhibition of luminescence (%) after 30 min (because 50% inhibitory effects were rarely observed) exposure to samples, which were calculated following standard methods (ISO, 1998).

2.5. Microbial metagenomic analysis

Microbial DNA in asphalt mat and sediments (0.5 g) were extracted using a Power Soil® DNA Isolation Kit (MoBio Laboratories, Solana Beach, CA) following the manufacturer's instructions. Bacterial 16s rRNA genes (V3 to V4 regions) were amplified using the Illumina overhang adaptor (Forward primer 5′- TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG-3'; reverse primer 5′-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTA TCT AATCC-3′). The genes were then purified and sequenced as described previously (Noh et al., 2018). Sequence data were quality trimmed using the Quantitative Insights into Microbial Ecology (QIIME) 1.9.1 v pipeline (Caporaso et al., 2010), and were clustered by operational taxonomic units (OTUs) at a sequence similarity cut off of 97% by using Vsearch, a versatile open-source tool for metagenomic bioinformatics (Rognes et al., 2016). The Shannon-Wiener index was used to calculate the species diversity of bacteria at the taxonomic level of class. To analyze metagenomes, read counts were transformed to relative abundance to normalize the number of valid reads, and were then square-root transformed. Bray – Curtis similarities (BCS) at the class level and genus level were analyzed by Primer software package 6.0.

3. Results and discussion

3.1. AhR-mediated potencies in asphalt mat, sediments, and biota

All organic extracts and fraction samples induced AhR activity of varying potencies between the matrices (asphalt mat, sediment, and biota), sampling sites, and types of samples (among biota) (Fig. 2A). These results indicated that general contamination by dioxin-like compounds on Abu Ali Island (Lee et al., 2017). Concentrations of BaP-EQs ranged from 8.0×10^2 to 1.2×10^6 ng BaP-EQ g^{-1} dm, 9.0×10 to 5.6×10^3 ng BaP-EQ g^{-1} dm, and 7.0 to 9.8×10^3 ng BaP-EQ g^{-1} dm in the asphalt mats, sediments, and biota samples, respectively. The overwhelming response of organic extracts and all fractions in the asphalt mat shows that it contains many chemicals that act as AhR agonists, in addition to (alkyl-) PAHs (Hong et al., 2012). The use of exhaustive extraction methods for abiotic compartments does not take into account the bioavailability of toxicants, and might overestimate actual exposure (Brack et al., 2009); however, some biota samples responded more than sediment samples as follows. In the biota samples, polychaetes had the greatest AhR-mediated potencies, while lesser responses were detected in chitons, snapping shrimps, and crabs (Fig. 2A). These results show that the bioaccumulation of AhR-active chemicals would vary across the target organisms, particularly between polychaetes and other target biota samples. The polychaetes collected from the study area are burrowing and known to be deposit feeders, which might have received greater or direct exposure to sedimentary residues of PAHs in the given region (Jørgensen et al., 2008; Joydas et al., 2017; Manokaran et al., 2013).

Greater concentrations of BaP-EQ were detected for sediments and biota extracts in S1 compared to S2 and S3 (Fig. 2A). In general, concentrations of BaP-EQs in sediments are related to oil pollution, as well as industrial and/or agricultural activities around the sampling locations or surrounding areas. S1 was located in an area near the Jubail City, which has undergone considerable development, including urbanization, industrialization (petrochemical company), port areas, and refineries (Al-Thukair et al., 2007; Barth, 2003). The geographical characteristics of the area (i.e., narrow inlet) also limit flushing, resulting in the sedimentation of localized AhR agonists at S1 (Lee et al.,

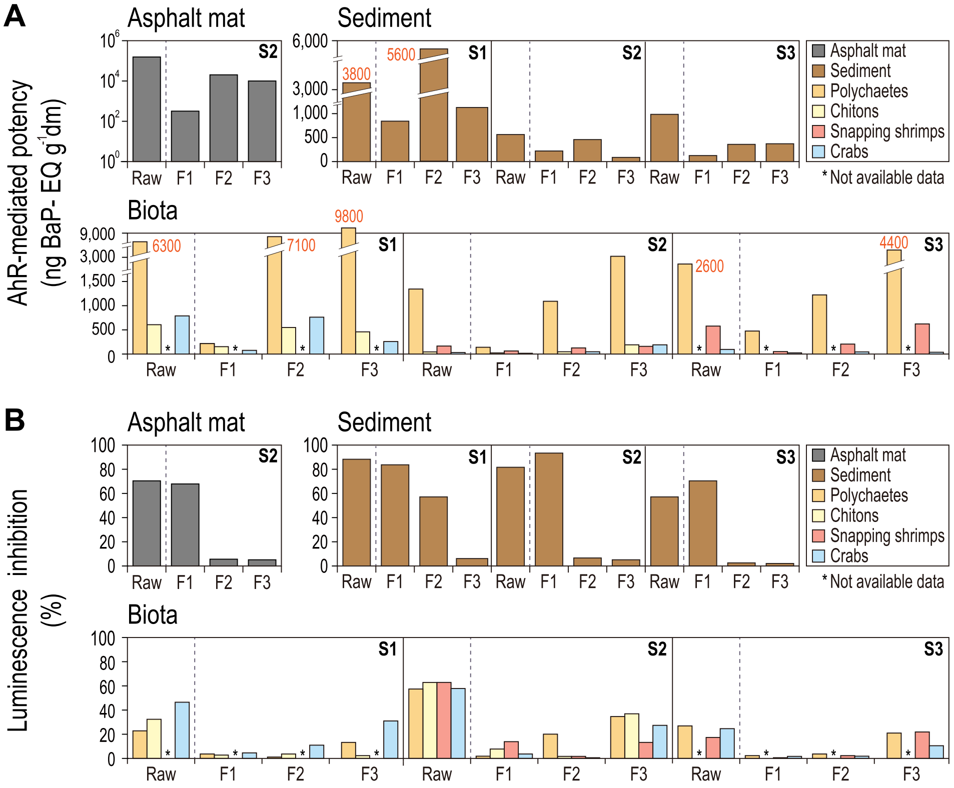

Fig. 2. (A) Distribution of BaP-EQ biological responses in asphalt mat, sediment, and biota. (B) Variation of bioluminescence inhibition (%) by the microbe *V. fischeri* to asphalt mat, sediment, and biota.

2018). In comparison, S2 and S3 are located in non-urbanized and open sea areas; consequently, both sites had lower concentrations of BaP-EQ in sediments and biota samples.

Among the three silica gel fractions, the F2 fractions generally induced greater AhR-mediated potencies (3.6×10^2–5.6×10^3 BaP-EQ ng g^{-1} dm) in the sediments, while the F3 fractions induced greater AhR-mediated potencies (2.6×10–9.8×10^3 BaP-EQ ng g^{-1} dm) in biota samples (Fig. 2A). In other words, the mean relative contribution of each fraction to the total (F1 + F2 + F3) values were 70% in the F2 of asphalt mat, 62% in the F2 of sediments, and 61% in the F3 of biota (Fig. 3A). These results indicated that the characteristics of AhR agonists differed among matrices of samples. In asphalt mat and sediments, the AhR-mediated potencies were mainly caused by the aromatic compounds associated with oil contamination due to their structural properties (Vrabie et al., 2012). In comparison, most AhR-mediated responses in biota appear to be caused by the bioavailable fractions of certain chemicals, such as untargeted PAHs (e.g., diclofenac, methyl-triclosan, keto-, dinitro-, hydroxyl-, and amino-PAHs and 2-hydroxyanthraquinone) in aquatic ecosystems (Regueiro et al., 2013; Xiao et al., 2016) (Table S2). AhR-mediated responses in biota might also be influenced by ingesting hydrocarbons, which are primarily dissolved and transformed to polar metabolites as dietary AhR ligands by biota (Jørgensen et al., 2008). The sampling of biota takes several factors into account that might influence the fate of environmental pollutants that accumulate in biota such as metabolism, depuration rates, excretion, stress, viability, and the condition of organisms (Vrana et al., 2005). It is difficult to quantify such effects when only sampling abiotic material. Thus, the monitoring and assessment of biotic samples are strongly recommended.

3.2. Potency balance analysis

The concentrations of PAHs, expressed as the sum of BaP equivalents (BEQs), ranged from below detection limit to 3.0×10^3 ng g^{-1} dm across all samples (Table 1). The greatest concentration was found in the asphalt mat at S2. Its concentration was almost hundred times higher than that in the corresponding sediment at S2. Similar to the AhR-mediated activities, the greatest concentration of BEQ (5.0×10 ng g^{-1} dm) was detected in the sediment at S1, out of all sediment samples. In contrast to sediment concentrations, biota at S2 had greater BEQs compared to other sites. The average concentrations of BEQ at S2 (1.1×10 ng g^{-1} dm) differed from the averages 3.7 ng g^{-1} dm and 2.3 ng g^{-1} dm at S1 and S3, respectively. This is because of the greatest relative contribution of dibenzo[*a,h*]anthracene (DBahA) in sediment at S2.

Comparison of the amount of toxic equivalents shown by mass balance indicated that only a small part of BaP-EQ (up to 26%) was accounted for by the seven AhR-active PAHs measured in the samples (Table 1). These results indicated that untargeted and unknown AhR agonists were probably present in the sediments and could show synergistic relationships among multiple pollutants. When the AhR-mediated potencies of untargeted AhR active agonists are considered, unidentified proportion of dioxin-like activities could be more explained, such as *o,p′*-DDD and *p,p′*-DDT, and alkyl-PAHs (Barron et al., 2004; Hong et al., 2016b). It has been reported that measurable concentrations of *o,p′*-DDD (0.04–0.30 mg kg^{-1}) and *p,p′*-DDT (0.01–0.05 mg kg^{-1}) were detected in sediments collected near the study area (Mohammed, 2013). More recently, relatively great concentrations of alkyl-PAHs such as C1-chrysene, C2-chrysene, C3-chrysene, and C4-phenanthrene were reported in the same samples of the present study (Yoon et al., 2019) (Table S1). However, the assay-specific ReP values of these compounds based on the 4 h exposure experiments are not available. Thus, accurate potency balance has been limited at present. Future study requires more elaborated work confirming and developing assay-specific RePs for new chemicals by determining time series dose-response relationship, combined with effect-directed analysis (Kim et al., 2019).

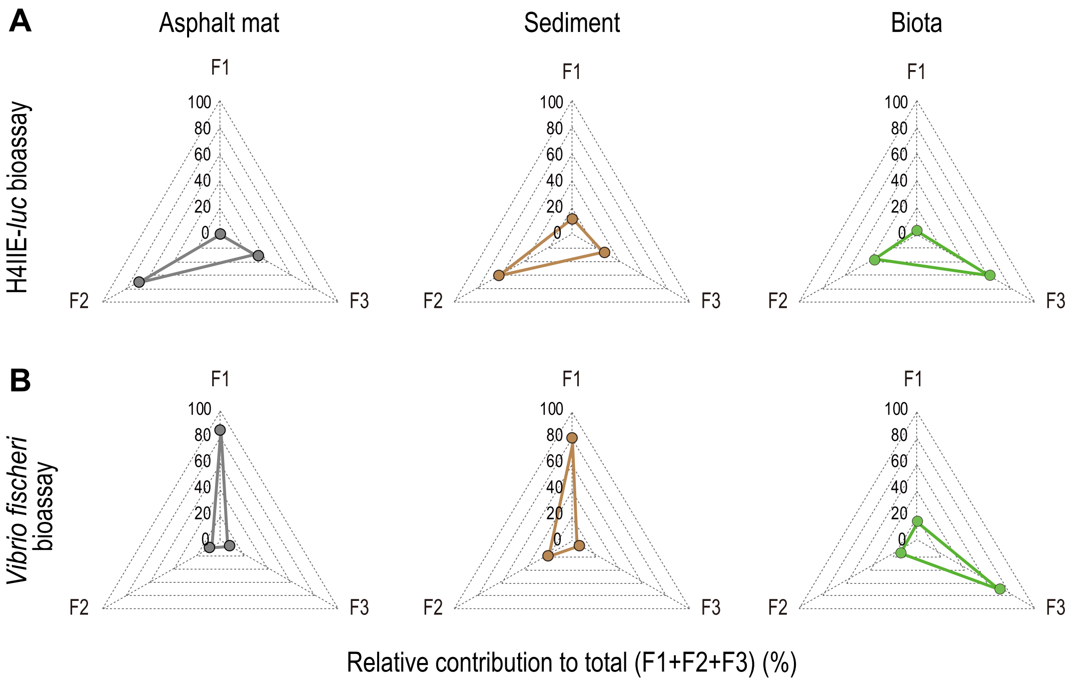

Fig. 3. The mean relative contributions of fractions to the organic extracts by all sampling sites from (A) H4IIE-*luc* bioassay and (B) *V. fischeri* bioassay.

Table 1
Relative potency values for the AhR-mediated activity of PAHs reported previously and overview of the results of instrument-derived equivalents and bioassay-derived equivalents (F2 fraction) of the samples (asphalt mat, sediment, and biota) collected from Abu Ali Island, Saudi Arabia.

Samples		RePs	Instrument-derived BEQ [a]								Bioassay-derived BaP-EQ [b]	Potency balance [c]
			BaA	Chr	BbF	BkF	BaP	IcdP	DbahA	ΣBEQs	(ng g^{-1})	(%)
			3.2 x 10^{-1}	8.5 x 10^{-1}	5.0 x 10^{-1}	4.8 x 10^{-1}	1.0	5.8 x 10^{-1}	6.6 x 10^{-1}	(ng g^{-1} dm)		
Asphalt mat		Site 2	160	770	280	160	360	380	840	3000	1.1×10^5	2.7
Sediment		Site 1	2.2	30	4.0	5.5	7.3	1.7	2.7	52	5.6×10^3	0.9
		Site 2	- [e]	0.9	–	–	0.7	1.0	1.2	3.8	4.6×10^2	0.8
		Site 3	–	–	–	–	–	–	–	–	3.6×10^2	–
Biota	Polychaetes	Site 1	0.5	4.5	1.1	–	1.0	1.1	2.0	10	7.1×10^3	0.1
	Chitons		–	–	–	–	–	0.2	0.3	0.5	5.4×10^2	0.1
	Crabs		–	–	–	–	–	–	0.3	0.3	7.6×10^2	–
	Polychaetes	Site 2	0.8	4.3	1.5	1.5	3.2	3.0	11	25	1.1×10^3	2.3
	Chitons		0.4	1.3	0.7	0.7	1.6	1.8	4.8	11	4.4×10	26
	S. shrimps [d]		0.5	1.7	0.9	0.5	2.2	2.3	6.0	14	1.3×10^2	11
	Crabs		–	0.4	–	–	–	0.6	1.5	2.5	1.7×10	15
	Polychaetes	Site 3	0.3	4.2	1.1	–	–	0.6	0.6	6.8	1.2×10^3	0.6
	S. shrimps		–	–	–	–	–	–	–	–	2.0×10^2	–
	Crabs		–	–	–	–	–	–	–	–	3.7×10	0.1

[a] BEQ values of PAHs were summed from the chemical concentrations of benzo[*a*]anthracene (BaA), chrysene (Chr), benzo[*b*]fluoranthene (BbF), benzo[*k*]flouranthene (BkF), benzo[*a*]pyrene (BaP), indeno[*1,2,3-c,d*]pyrene (IcdP) and dibenz[*a,h*]anthracene (DBahA) multiplied by the relative potency values (RePs) obtained in the previous study (Kim et al., 2019).

[b] Two significant digits were used in the table.

[c] Potency balance values were obtained from the percentage of instrument-derived values to the bioassay-derived values.

[d] S. shrimps: Snapping shrimps.

[e] -: Below limits of detection.

3.3. Bioluminescence inhibitions in asphalt mat, sediments, and biota

All organic extracts of asphalt mat, sediments, and biota are currently acutely toxic to the bacterium, *V. fischeri*. Mean inhibition rates were 71%, 76%, and 47% for the asphalt mat, sediment, and biota, respectively (Fig. 2B). Among the extracts of biota samples, crabs exhibited relatively greater inhibition, whereas the AhR-mediated potency induced by crab is low (Fig. 2A). Because crabs are carnivorous organisms and are not exposed to a great extent, to sediment particles, causative chemicals might differ from sediment-bound pollutants such as dioxin-like chemicals (Baumard et al., 1998).

Bioluminescence inhibition was caused by each of the fractions varied significantly in the matrix of samples. The F1 fraction of the asphalt mat and all sediment samples led to greater toxicity values compared to F2 and F3 fractions, presenting considerable differences polarity in causative compounds. The relative contribution of the F1 fraction to the total values (F1 + F2 + F3) samples of asphalt mat and sediment was 4–6 times greater than that of biota (Fig. 3B). The F1 fractions of the extracts allowed both non-polar organic pollutants and organo-metals affecting *V. fischeri* to be extracted (Roig et al., 2011).

Although F2 fractions from asphalt mat and sediments contained large quantities of AhR agonists, which induced greatest AhR activities, acute toxicity might have been reduced by the weathering process and the mechanisms of two bioassays were different (Ji et al., 2011). Thus, the acute ecotoxicological potential of the F2 fraction from sediments could be classified as low.

Greater induction of toxicity was observed in the F3 fraction in all biota samples, especially crabs, compared to those in F1 and F2 fractions, irrespective of sampling sites (Figs. 2B and 3B). These results might be attributed to their ability to biotransform organic substances into water-soluble components through metabolism after uptake (Baumard et al., 1998). In the marine food web, the trophic level of crabs is greater than that of others. Crabs had greater biotransformation capacities, and preferentially accumulated lower molecular weight PAHs over higher molecular weight PAHs (more hydrophobic) (Baumard et al., 1998). Thus, various parameters must be considered when selecting the sentinel organisms used in risk assessments. Overall, the hydrophobicity and hydrophilicity of compounds appears to drive the general toxicity of these building block chemicals to *Vibrio fischeri*.

3.4. Microbial responses to sediment contaminations

The relative abundance of the bacterial 16S rRNA gene sequences detected in samples was distinguished across the matrices and sampling sites (Fig. 4A). There were diverse bacterial communities, which were colonized by 60 bacterial phyla (> 0.1% across all samples). Proteobacteria was the most abundant and the largest occupied phylum in all samples, representing 84% of bacteria in asphalt mat, and 63%, 56%, and 41% of bacteria in S1, S2, and S3 sediments, respectively. But its classes showed different tendencies, within the Proteobacteria phylum, bacterial assemblages in asphalt mat and sediments from S2 were similar, while those of S1 and S3 differed. Gammaproteobacteria was predominantly detected in asphalt mat and S2 sediment while Alphaproteobacteria and Deltaproteobacteria were also primarily detected in S1 and S3 sediment (Fig. 4A). These results were consistent with the microbial diversity indices (Chao 1: OUT richness estimator; Shannon index: accounts for the number and evenness of species), which were lower in communities of asphalt mat and S2 sediment compared to S1 and S3 sediment (Fig. 4B). Although great concentrations of AhR agonists was shown in sediment from S1, microbial richness and evenness were greatest throughout the sites. The richness and evenness would have increased by organic matter and AhR agonists associated with oil pollution driven by a major source of carbon and electrons in an otherwise nutrient-starved marine environment (Kostka et al., 2011). In S3 sediment where relatively few AhR agonists were detected, various bacteria communities seem to have adapted and enriched due to fine conditions. These differences structure of microbial communities might be due to the variety of environmental factors such as nutrient, redox conditions, air-condition, pH, and temperature (Kostka et al., 2011; Xiong et al., 2015). Bacteroidetes and Actinobacteria were the most abundant groups, followed by Proteobacteria in sediment samples. Of note, the relative abundances of these two phyla in sediment samples were greater than those of in asphalt mat, which are well-known degraders of organic matter such as sulfides (Thomas et al., 2011).

The Bray-Curtis similarity index exceeded 50% between samples, regardless of the matrices at class level (Fig. 5A). Thus, the microbial composition was mainly associated with sampling sites, rather than difference in matrix types (asphalt mat and sediments). At the genus level, asphalt mat and sediment from S2 and sediments from S1 and S3 showed an apparent grouping despite of weak similarity. This pattern of grouping can be confirmed with dominant genera in each site. Despite the difference in the top five most abundant genera among the sites, the most abundant genera were shared between grouped sites (Table 2). *Pseudoalteromonas* and *Desulfococcus* were the dominant genera among grouped sites asphalt mat and sediment samples from S2, sediment samples from S1 and S3, respectively. Both two genera are known as PAH-degrading bacteria (Abed et al., 2011; Hedlund and Staley, 2006) (Table S3). Including both dominant genera, majority of the genera occupied up to 90% was related to PAH-degrading bacteria (Fig. 5B). Also, the dominant phyla classified to Alpha- and Gamma-proteobacteria were the potential key players in biodegradation of oil contaminants in the marine environment (Acosta-González and Marqués, 2016; Gao et al., 2015). These results indicated that Abu Ali Island is constantly affected by oil contamination or other sources of PAHs. Non-related oil degradation bacteria which contributes a great level of microbial diversity indices mostly exist in sediments from S1 and S3,

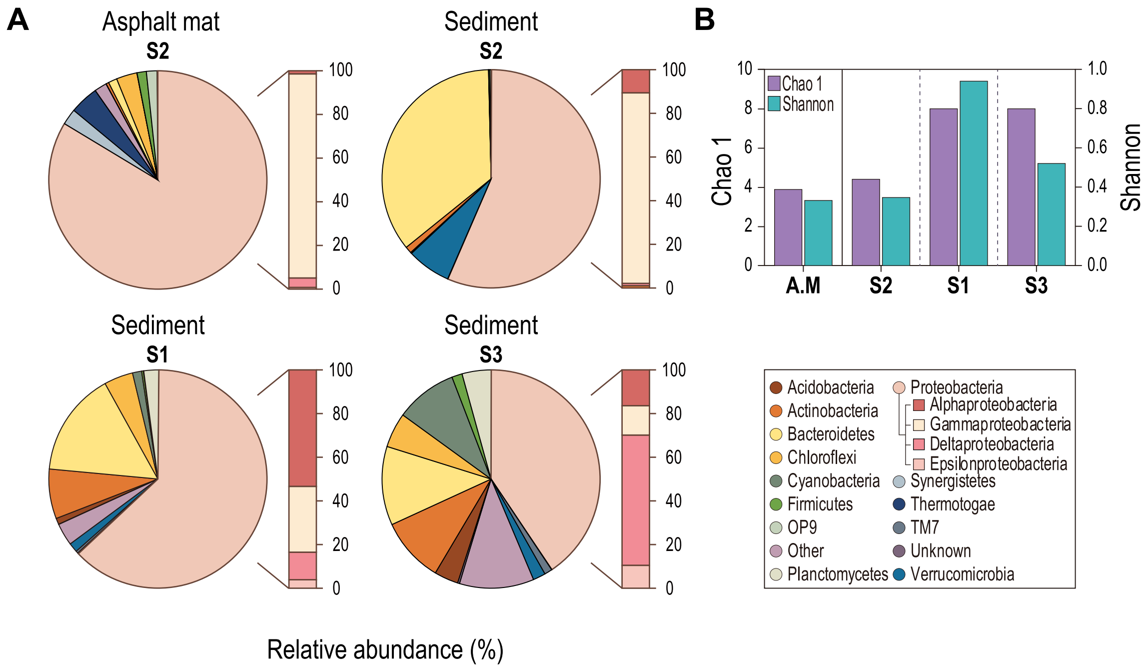

Fig. 4. (A) Relative abundance of the microbial community observed in asphalt mat and sediment based on different phyla and classes of Proteobacteria. Low abundance phyla (< 0.1%) were not presented. (B) Comparisons of microbial diversity indices (Chao 1 and Shannon index).

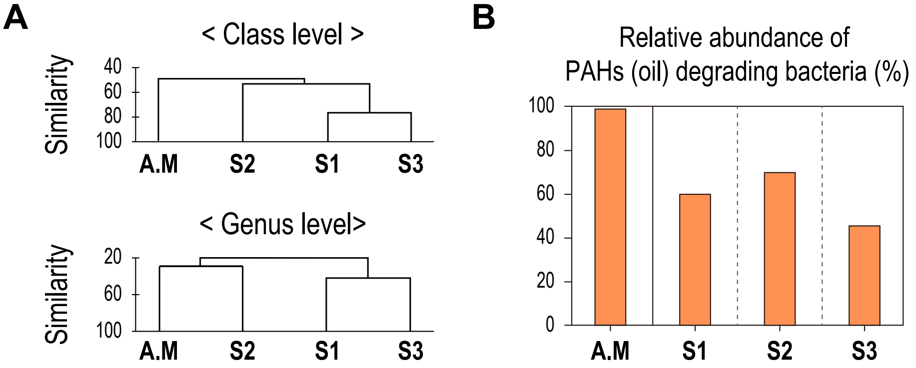

Fig. 5. (A) Dendrogram representing hierarchical clustering based on Bray – Curtis similarities (BCS) at the class level and genus level. (B) Relative abundance of related PAHs (oil) degrading bacteria to total genera in asphalt mat and sediments.

Table 2
Relative abundances of the top five genera (**bold**) from the microbial communities in the asphalt mat and sediment samples.

Microbial community contribution (%)					Asphalt mat	Sediments			PAHs (Oil)-degrading bacteria [a]
Phylum	Class	Order	Family	Genus		Site 1	Site 2	Site 3	
Actinobacteria	Acidimicrobiia	Acidimicrobiales	koll13	*mixed*	- [b]	0.80	–	**8.0**	
Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	*Mesonia*	–	–	**10**	0.03	
				Robiginitalea	–	**7.2**	0.07	**20**	
				Winogradskyella	–	0.28	**7.0**	–	✓
Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	*Marivita*	–	**11**	0.11	2.3	
				Oceanicola	–	**8.5**	0.06	2.5	✓
	Deltaproteobacteria	Desulfobacterales	Desulfobacteraceae	*Desulfococcus*	0.34	**16**	–	**31**	✓
		Syntrophobacterales	Syntrophobacteraceae	*Desulfoglaeba*	**5.4**	–	–	–	✓
	Gammaproteobacteria	Alteromonadales	Alteromonadaceae	*Marinobacter*	**26**	–	3.2	–	✓
		Chromatiales	Ectothiorhodospiraceae	*Halorhodospira*	0.43	**14**	–	–	
		Oceanospirillales	Halomonadaceae	*Halomonas*	**5.6**	–	2.2	–	✓
		Vibrionales	Pseudoalteromonadaceae	*Pseudoalteromonas*	**45**	–	**38**	–	✓
Spirochaetes	Spirochaetes	Spirochaetales	Spirochaetaceae	*Spirochaeta*	0.58	0.83	–	**3.8**	✓
Thermotogae	Thermotogae	Thermotogales	Thermotogaceae	*Petrotoga*	**5.3**	–	–	–	✓
Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales	Verrucomicrobiaceae	*Persicirhabdus*	–	–	**7.8**	–	
				Rubritalea	–	0.23	**5.6**	0.68	
				Verrucomicrobium	–	1.5	0.16	**4.0**	

[a] References and mini reviews of PAHs (oil) - degrading bacteria presented in Table S3.

[b] Not detected.

including *Halorhodospira*, *Marivita*, *Robiginitalea*, and *Verrucomicrobium*. These genera are related to sulfur-reducing bacteria (Čanković et al., 2017; Falcón et al., 2007). In the previous study, crude oil was degraded efficiently under sulfate-reducing and mixed electron-acceptor conditions (Boopathy et al., 2012). Overall, microbial communities from different matrices and sampling sites showed differences, which could not be fully explained by the limited factors determined in the present study. However, the present study provided that the microbial community affected by abiotic factors rather than types of matrix. Furthermore, our results generally supported that microbial communities were constantly influenced by oil-contamination.

To the best of our knowledge, this study provides the first approach to assess potential toxicity in multiple research area upon the regions in Abu Ali Island. The findings of this study advance our understanding of the persistence of oil spill-related damage through evaluating the presence of bioactive organic contaminants and major contributors to oil-related ecotoxicological impacts. The obtained results demonstrate that the potential toxicities of environmental residues of oils on Abu Ali Island continue to present significant risks. Ongoing monitoring of the area should use multiple lines of evidence to derive hazardous impacts with a holistic assessment method, including pollutant concentrations, ecotoxicological components, and hydrological factors.

Acknowledgments

This work was supported by the project entitled “Quantification of self-purification capacity of coastal wetlands through understanding of chemodynamics of land-driven coastal pollutants (NRF-2017R1E1A1A01075067)” funded by the Ministry of Science and ICT. This work was also supported by the projects entitled “Development of techniques for assessment and management of hazardous chemicals in the marine environment (2014-0342) funded by the Ministry of Oceans and Fisheries of Korea”. Authors also would like to thank Twasol Research Excellence Program (TRE Program) at King Saud University, Riyadh, Saudi Arabia, for support.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecoenv.2019.109547.

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<Supplementary Materials>

Multiple evaluation of the potential toxic effects of sediments and biota collected from an oil-polluted area around Abu Ali Island, Saudi Arabia, Arabian Gulf

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Table S1. Concentrations of polycyclic aromatic hydrocarbons (PAHs, ng g^{-1} dm) in asphalt mat, sediments, and biota samples from Abu Ali Island reported in the previous study (Yoon et al., 2019).

Matrix	**Asphalt mat**	**Sediment**			**Biota**									
Sites	**2**	**1**	**2**	**3**	**1**			**2**				**3**		
Compounds [b]					**Polychaetes**	**Chitons**	**Crabs**	**Polychaetes**	**Chitons**	**S. shrimps** [a]	**Crabs**	**Polychaetes**	**S. shrimps**	**Crabs**
Acl	4.3	-[c]	-	-	-	-	-	-	-	-	-	-	-	-
Ace	36.8	1.4	-	-	3.1	-	-	1.3	-	-	-	4.1	-	-
Flu	135.6	5.3	-	-	10.1	-	-	3.7	-	2.0	-	13.1	2.3	-
Dbthio	422.2	21.3	0.5	0.1	1.8	0.2	0.2	0.6	0.1	0.2	0.0	1.9	0.4	0.1
Phe	266.0	8.0	-	-	25.1	-	-	7.3	1.5	3.5	-	43.4	7.7	-
Ant	40.2	7.5	-	-	1.0	-	-	0.6	-	-	-	0.8	-	-
Fl	147.4	7.0	-	-	4.8	-	-	1.9	-	0.9	-	5.0	0.9	-
Py	428.5	17.6	-	-	13.3	-	-	3.4	-	1.4	-	8.6	1.7	-
BaA	509.6	6.9	1.3	-	1.5	-	-	2.7	1.2	1.4	-	1.0	-	-
Chr	908.9	33.9	1.2	-	5.4	0.0	0.0	5.1	1.5	2.0	0.5	4.9	-	-
BbF	556.1	8.1	0.7	-	2.2	-	-	3.0	1.4	1.9	-	2.1	-	-
BkF	332.5	11.5		-	-	-	-	3.2	1.5	1.1	-	-	-	-
BaP	358.0	7.3	0.9	-	1.0	-	-	3.2	1.6	2.2	-	-	-	-
Pery	338.5	6.4	0.6	-	0.5	-	-	0.6	-	-	-	-	-	-
IcdP	657.9	2.9	2.7	-	1.9	0.3	-	5.2	3.1	3.9	1.0	1.1	-	-
DbahA	1275.7	4.1	2.9	-	3.1	0.5	0.4	15.9	7.2	9.1	2.2	0.9	-	-
BghiP	925.7	12.9	1.9	0.9	2.5	-	-	5.7	2.9	3.5	0.9	1.6	-	-
C1-Na	521.4	1.2	0.2	0.2	88.0	0.2	0.3	68.3	10.2	27.3	5.5	97.5	22.0	0.3
C2-Na	4141.3	42.1	0.4	0.2	93.3	2.4	7.9	52.5	15.0	23.4	4.8	91.6	19.5	1.2
C3-Na	6738.4	404.6	0.5	0.6	67.1	2.0	2.6	20.8	3.7	8.7	1.6	63.2	13.3	0.4
C4-Na	20994.8	640.5	1.3	0.5	51.9	2.3	2.2	31.1	3.2	8.9	1.5	33.5	6.3	0.2
C1-Flu	1930.4	114.9	0.8	0.9	46.1	1.3	1.4	18.8	3.7	9.5	2.0	72.7	12.2	0.7
C2-Flu	6131.7	548.6	2.2	0.8	41.1	2.5	2.4	14.9	1.9	4.5	0.7	42.8	7.5	0.6
C3-Flu	14783.0	1073.1	4.8	1.1	72.5	4.2	4.3	24.7	3.4	7.9	1.3	58.8	11.5	1.1
C1-Dbthio	4539.8	214.0	1.9	0.4	11.3	1.1	1.1	5.7	0.3	0.7	0.2	4.8	1.0	0.2
C2-Dbthio	19375.4	1276.9	11.4	1.1	63.6	6.8	5.8	22.4	0.4	0.8	0.1	14.7	1.7	0.8
C3-Dbthio	53621.7	2602.6	38.3	2.1	179.3	10.1	8.7	26.3	0.0	0.8	0.1	24.1	3.5	1.7
C1-Phe	3099.0	203.6	2.2	0.6	33.2	1.6	1.7	12.5	1.5	3.6	0.7	49.4	9.3	0.4
C2-Phe	18119.8	1251.3	5.8	1.3	68.6	5.5	5.5	40.1	4.1	5.6	1.3	60.0	11.1	1.0
C3-Phe	21712.6	1774.7	18.8	2.5	161.8	9.1	8.5	25.7	2.0	3.8	0.8	59.4	10.0	1.5
C4-Phe	11724.0	758.6	5.6	4.6	127.0	6.5	8.3	7.4	0.6	1.0	0.2	33.5	6.8	1.6
C1-Chr	1434.4	365.4	1.3	4.9	27.2	2.1	1.8	0.9	0.1	0.2	0.0	17.8	2.8	0.8
C2-Chr	2470.4	438.9	2.5	9.8	40.2	2.5	3.0	0.6	0.1	0.2	0.0	18.2	4.1	1.4
C3-Chr	3282.6	473.3	3.4	11.4	31.8	1.7	3.1	1.0	0.2	0.3	0.1	12.5	3.8	1.4

[a] S. shrimps: Snapping shrimps.

[b] Abbreviations. Acenaphthylene (Acl), Acenaphthene (Ace), Fluorene (Flu), Dibenzothiophene (Dbthio), Phenanthrene (Phe), Anthracene (Ant), Fluoranthene (Fl), Pyrene (Py), Benzo[a]anthracene (BaA), Chrysene (Chr), Benzo[b]fluoranthene (BbF), Benzo[k]fluoranthene (BkF), Benzo[a]pyrene (BaP), Perylene (Pery), Indeno[1,2,3-cd]pyrene (IcdP),

Dibenz[a,h]anthracene (DbahA), Benzo[g,h,i]perylene (BghiP), C1-Naphthalene (C1-Na), C2-Naphthalene (C2-Na), C3-Naphthalene (C3-Na), C4-Naphthalene (C4-Na), C1-Fluorene (C1-Flu), C2-Fluorene (C2-Flu), C3-Fluorene (C3-Flu), C1- Dibenzothiophene (C1-Dbthio), C3-Dibenzothiophene (C3-Dbthio), C1-Phenanthrene (C1-Phe), C2-Phenanthrene (C2-Phe), C3-Phenanthrene (C3-Phe), C4-Phenanthrene (C4-Phe), C1-crysene (C1-Chr), C2-crysene (C2-Chr), and C3-crysene (C3-Chr).

[c] - : Below limits of detection.

Table S2. Profile of eluted compounds in fractions from different polarities of the solvent.

Elution solvent from other study	**Sample type**	**Fractions in this study**			**Eluted compounds**	**References**
		F1 Hexane (100%)	F2 Hexane: DCM (80:20, v/v)	F3 Acetone: DCM (40:60, v/v)		
Hexane	Sediments	○			PAHs with two aromatic rings (DDE, PCBs) & Bexachlorobenzene	Khim et al., 1999a
Hexane:DCM (80:20, v/v)			○		PAHs with two aromatic rings (Ace, Flu, Na) PAHs with four aromatic rings (BaA, Chr, Fl, Py) PAHs with five aromatic rings (BaP, BbF, BkF, DbahA) PAHs with six aromatic rings (BghiP)	
DCM:Methanol (50:50, v/v)				△	PAHs with two aromatic rings (Bisphenol A) & NP, OP	
Toluene	Sediments	△			PAHs with two aromatic rings (3,3',4,4',5,5'-Hexachlorobiphenyl 3,3',4,4',5-Pentachlorobiphenyl, PCN, 3,3',4,4'-Tetrachlorobiphenyl)	Khim et al., 1999b
Hexane:DCM (80:20, v/v)	Fish		○		PAHs with two aromatic rings (p,p -DDD, p,p -DDT) Chlordanes, HCHs	Kannan et al., 2000
Toluene	Sediments	△			PAHs with two aromatic rings (PCDFs)	Yamashita et al., 2000
Hexane	Sediments	○			PAHs with two aromatic rings (23478-PeCDF, 2,4,4'-Trichlorobiphenyl , 2,2',5,5'-Tetrachlorobiphenyl, 2,2',4,5,5'-Pentachlorobiphenyl, 2,3',4,4',5-Pentachlorobiphenyl, 2,2',3,4,4',5'-Hexachlorobiphenyl, 2,2',4,4',5,5'-Hexachlorobiphenyl, 2,2',3,4,4',5,5'-Heptachlorobiphenyl)	Hilscherova et al., 2001
Hexane:DCM (80:20, v/v)			○		OC pesticides	
DCM				△	Polar metabolites steroid compounds	
Hexane	Sediments	○			Non-polar aliphatic compounds	Brack et al., 2003
Hexane:DCM (95:5, v/v)			△		Non-polar aromatic compounds	
Hexane:DCM (90:10, v/v)	Sediments		△		PAHs	Grote et al., 2005

Table S2. (continued).

Elution solvent from other study	**Sample type**	**Fractions in this study**			**Eluted compounds**	**References**
		F1 Hexane (100%)	F2 Hexane: DCM (80:20, v/v)	F3 Acetone: DCM (40:60, v/v)		
Hexane:DCM (95:5, v/v)	Sediments		△		PAHs with two aromatic rings (Anthraquinone)	Varel et al., 2008
DCM				△	PAHs with three aromatic rings (Benzo[*b*]naphtho[2,1-*d*]thiophene, 4H-Cyclopenta[*def*]phenanthrene-4-one,2,2-Naphthalenylbenzothiophene, 9-Nitroanthracene) PAHs with four aromatic rings (4H-Cyclopenta[*cd*]pyrene-3[4*H*]-one, 1-Hydroxypyrene) PAHs with five aromatic rings (Dibenz[*a*,*j*]acridine)	
Acetonitrile				△	PAHs with two aromatic rings (2-Hydroxyanthraquinone)	
Hexane:DCM (50:50, v/v)	Sediments		△		BHT, Phthalate compounds	Kaisarevic et al., 2009
DCM	Sediments			△	Androgenic compounds	Weiss et al., 2009
Hexane		○			Aliphatic hydrocarbons	
Hexane	Soils	○			Non-polar aliphatic compounds	Wölz et al., 2010
Hexane:DCM (90:10, v/v)			△		Non-polar PAHs	
DCM				△	Polar compounds	
Hexane:DCM (90:10, v/v)	Sediments	△			PAHs with two aromatic rings (PBDEs)	Qu et al., 2011
Hexane:DCM (80:20, v/v)		△			PAHs with two aromatic rings (TBBPA) & HBCDs	
Hexane:DCM (50:50, v/v)	Sediments		△		PAHs, PCBs, and dioxins	Schmitt et al., 2011
DCM				△	Nitro-PAHs	
Hexane	Worm, sediments, crude oil	○			Saturate hydrocarbons	Vrabie et al., 2012
DCM				△	Aromatic compounds	

Table S2. (continued).

Elution solvent from other study	**Sample type**	**Fractions in this study**			**Eluted compounds**	**References**
		F1 Hexane (100%)	F2 Hexane: DCM (80:20, v/v)	F3 Acetone: DCM (40:60, v/v)		
Heptane	Sediments	△			PAHs with two aromatic rings (2,4′-DDT, TCDD)	Creusot et al., 2013
Heptane:DCM (50:50, v/v)			△		PAHs with two aromatic rings (BP3, Cypermethrin, Fenofibrate) PAHs with three aromatic rings (Clotrimazole, Fenvalerate, TPP)	
Ethyl acetate				△	PAHs with two aromatic rings (*n*-Benzylparaben) Dexamethasone, β-Estradiol, Isoproturon, Prednisolone, Spironolactone, and α-Zearalanol	
Hexane:Ace (97:3, v/v)	Sediments		△		PAHs with two aromatic rings (C6-Na, Diclofenac, Methyltriclosan, Triclosan, Benzophenone, Ketoprofen, Naproxen) PAHs with three aromatic rings (Carbamazepine) Butylparaben, Clofibric acid, Mecoprop, Methyl chlorophenoxy acetic acid, Methyl dihydrojasmonate, Propylparaben, Tertbuthylazine, Tris(2-chloroethyl) phosphate	Regueiro et al., 2013
Hexane:DCM (50:50, v/v)	Porewater		△		PAHs with two aromatic rings (Carbazole) PAHs with three aromatic rings (Retene)	Fang et al., 2014
Hexane	Crude oil	○			Aliphatic compounds	Radović et al., 2014
DCM				△	Aromatic compounds	
Methanol					Resins and polar compounds	
Hexane:DCM (80:20, v/v)	Crude oil		○		PAHs with two aromatic rings (Dbthio, C1-Dbthio, C2-Dbthio, C3-Dbthio, C1-Flu, C2-Flu, C1-Na, C2-Na, C3-Na, C4-Na) PAHs with three aromatic rings (C1-Phe, C2-Phe, C3-Phe, C4-Phe) PAHs with four aromatic rings (BeP, C1-Chr, C2-Chr, C3-Chr)	Hong et al., 2015
Hexane:DCM (80:20, v/v)	Sediments		○		PAHs with two aromatic rings (Styrene dimers) PAHs with three aromatic rings (Styrene trimers)	Hong et al., 2016

Table S2. (continued).

Elution solvent from other study	Sample type	Fractions in this study			Eluted compounds	References
		F1 Hexane (100%)	F2 Hexane: DCM (80:20, v/v)	F3 Acetone: DCM (40:60, v/v)		
Hexane	Sediments	○			PCBs, Co-planar PCBs without chlorination in ortho-position, PCNs with 3-6 Cl	Xiao et al., 2016
Hexane: DCM (95:5, v/v)			△		PAHs with two rings to seven aromatic rings Monoitro-PAHs	
DCM				△	(Hydroxy-)quinones, keto-, dinitro-, hydroxyl-PAHs and N-Heterocycles with rising polarity	
Acetonitrile				△	2-Hydroxyanthraquinone	
Hexane	Oiled sediments	○			Saturate hydrocarbons	Kim et al., 2017
Ace:DCM (40:60, v/v)				○	Resins and polar compounds	

Abbreviations. 23478-PeCDF (2,3,4,7,8-Pentachlorodibenzofuran), Ace (Acenaphthene), Acl (Acenaphthylene), Ant (Anthracene), BaA (Benzo[a]anthracene), BaP (Benzo[a]pyrene), BbF (Benzo[b]fluoranthene), BeP (Benzo[e]pyrene), BghiP (Benzo[g,h,i]perylene), BHT (2,6-Di-tert-butyl-4-methylphenol), BkF (Benzo[k]fluoranthene), BPA (Bisphenol A), C1-Chr (1-Methylchrysene and 3-Methylchrysene), C1-Dbthio (2-Methyldibenzothiophene), C1-Flu (1-Methylfluorene and 9-Methylfluorene), C1-Na (1-Methylnaphthalene and 2-Methylnaphthalene), C1-Phe (2-Methylphenanthrene and 3-Methylphenanthrene), C2-Chr (6-Ethylchrysene), C2-Dbthio (2,4-Dimethyldibenzothiophene), C2-Flu (1,7-Dimethylfluorene), C2-Na (2,6-Dimethylnaphthalene and 2,3-Dimethylnaphthalene), C2-Phe (1,2-Dimethylphenanthrene and 1,6-Dimethylphenanthrene), C3-Chr (1,3,6-Trimethylchrysene), C3-Dbthio (2,4,7-Trimethyldibenzothiophene), C3-Na (1,4,5-Trimethylnaphthalene and 2,3,5-Trimethylnaphthalene), C3-Phe (1,2,6-Trimethylphenanthrene and 1,2,9-Trimethylphenanthrene), C4-Na (1,2,5,6-Tetramethylnaphthalene), C4-Phe (1,2,6,9-Tetramethylphenanthrene), C6-Na (2,6-Diisopropylnaphthalene), CHLs (α and γ-chlordanes), Chr (Chrysene), DbahA (Dibenz[a,h]anthracene), Dbthio (Dibenzothiophene), DCM (Dichloromethane), DDE (L59p,p'-1,1-dichloro-2,2-bis(pchlorophenyl)ethylene), DDTs (p,p'-dichlorodiphenyldichloroethane and p,p' dichlorodiphenyldichloroethylene), Fl (Fluoranthene), Flu (Fluorene), HAHs (Halogenated aromatic hydrocarbons), HBCDs (Hexabromocyclododecanes), HCB (Hexachlorobenzene), HCHs (α-, β-, and γ-hexachlorocyclohexanes), IcdP (Indeno[1,2,3-cd]pyrene), Na (Naphthalene), NP (Nonylphenol), OH-PBDEs (Hydroxylated brominated diphenyl ethers), OH-PCBs (Hydroxylated polychlorinated biphenyls), OHPs (Halogenated phenols), OP (Octylphenol), PAHs (Polycyclic aromatic hydrocarbons), PBDEs (Polybrominated diphenyl ethers), PCBs (Polychlorinated biphenyls), PCDDs (Dibenzo-p-dioxins), PCDFs (Polychlorinated dibenzofurans), PCNs (Polychlorinated naphthalenes), PFASs (Perfluoroalkyl substances), Phe (Phenanthrene), Py (Pyrene), Styrene dimers (1,3-Diphenylproane, cis-1,2-Diphenylcyclobutane, 2,4-Diphenyl-1-butene, and trans-1,2-Diphenylcyclobutane), Styrene trimers (2,4,6-Triphenyl-1-hexene, 1e-Phenyl-4e-(1-phenylethyl)-tetralin, 1a-Phenyl-4e-(1-phenylethyl)-tetralin, 1a-Phenyl-4a-(1-phenylethyl)-tetralin, 1e-Phenyl-4a-(1-phenylethyl)-tetralin, and 1,3,5-Triphenylcyclohexane), TBBPA (Tetrabromobiphenol A), TFA (Trifluoroacetic acid), TPP (triphenyl-phosphate).

Table S3. Mini review of common bacterial species that degrade PAHs (Oil).

Phylum	Class	Genus	Species	Target Contaminant	References
Actinobacteria	Actinobacteria	Arthrobacter	gandavensis	Py	Isaac et al., 2015
		Micrococcus	luteus	MaCs, LMW-PAHs, medium chain alkanes, crude oil	LIori et al., 2000
		Mycobacterium	fluoranthenivorans	Fl	Hormisch et al., 2004
		Mycobacterium	frederiksbergense	MaCs, LMW-, HMW-PAHs, short and long chain alkanes	Willumsen et al., 2001
		Mycobacterium	hodleri	Ant, Fl, Flu, Phe, Py	Kleespies et al., 1996
		Mycobacterium	hydrocarbonoxydans	LMW-, HMW-PAHs, crude oil	Schippers et al., 2005
		Mycobacterium	sp. F27 sp. P18	HMW-PAHs	Isaac et al., 2015
		Mycobacterium	vanbaalenii	Ant,BaP, biphenyl, Fl, Na, Phe, Py, 1-nitopyrene, 3-methylcholanthene, 6-nitrochrysene,	Khan et al., 2002
		Mycobacterium	wratislaviensis	Py	Isaac et al., 2015
		Streptomyces	griseoflavus parvus plicatus	LMW-, HMW-PAHs	Barabás et al., 2001
Bacteroidetes	Cytophagia	Cytophaga		Crude oil	Khomiakova et al., 2003
	Flavobacteriia	Flavobacterium		HMW-PAHs, diesel, crude oil	Stucki & Alexander, 1987
	Flavobacteriia	Robiginitalea		Crude oil	Kostaka et al., 2011
	Flavobacteriia	Winogradskyella		Crude oil	Liu et al., 2017
	Sphingobacteriia	Pedobacter		MaCs, LMW-, HMW-PAHs, medium chain alkanes	Margesin et al., 2003
Firmicutes	Bacilli	Bacillus		MaCs, LMW-, HMW-PAHs, medium and long chain alkanes	Feitkenhauer et al., 2003
		Paenibacillus		LMW-PAHs	Meyer et al., 1999

Table S3. (continued).

Phylum	Class	Genus	Species	Target Contaminant	References
		Planomicrobium		Alkanes, diesel oil	Engelhardt et al., 2001
Proteobacteria	Alphaproteobacteria	Jannaschia		Alkanes, PAHs	Kwon & Kim, 2010
		Kordiimonas		LMW-, HMW-PAHs	Kwon et al., 2005
		Oceanicola		Crude oil	Gao et al., 2015
		Ochrobactrum		LMW, HMW-PAHs, crude oil	Peressutti et al., 2003
		Roseobacter		Crude oil	Kwon & Kim, 2010
		Silicibacter		Dimethylsulfonio propionate, crude oil	Kwon & Kim, 2010
		Sphingobium		MaCs, LMW-, HMW-PAHs	Kertesz & Kawasaki, 2010
		Sphingomonas		MaCs, LMW-, HMW-PAHs	Kertesz & Kawasaki, 2010
		Sphingomonas	formosensis	Na, Py, PCBs	Lin et al., 2012
		Sphiongopyxis		MaCs, LMW-, HMW-PAHs	Kertesz & Kawasaki, 2010
		Sulfitobacter		Crude oil	Liu et al., 2017
		Thalassospira		LMW-PAHs	Kodama et al., 2008
		Tranquillimonas		Medium, long chain alkanes	Harwati et al., 2008
		Tropicibacter		MaCs, LMW-PAHs	Harwati et al., 2009a
		Tropicimonas		Medium, long chain alkanes	Harwati et al., 2009b
	Betaproteobacteria	Acidovorax		LMW-PAHs	Meyer et al., 1999
		Alcaligenes		LMW-, HMW-PAHs, long chain alkanes	Toledo et al., 2008
		Alcaligenes	faecalis	Na,Phe, PCBs	John et al., 2012
		Alcaligenes	xylosoxidans	Polychlorobiphenyl	Murínová et al., 2014
		Burkholderia		LMW-, HMW-PAHs, medium and long chain alkanes	Parales et al., 2000
		Burkholderia	pseudomallei	HCHs	Manonmani et al., 2000
		Burkholderia	sartisoli	LMW-PAHs	Vanlaere et al., 2008
		Comamonas		LMW-PAHs	Meyer et al., 1999

Table S3. (continued).

Phylum	Class	Genus	Species	Target Contaminant	References
		Delftia		MaCs, LMW-PAHs	Parales, 2010
		Polaromonas		LMW-PAHs, Medium chain alkanes	Jeon et al., 2004
		Ralstonia		MaCs, LMW-PAHs	Parales et al., 2000
Proteobacteria	Deltaproteobacteria	Desulfatibacillum		Medium and long chain alkenes	Cravo-Laureau et al., 2004
		Desulfatiferula		Long chain alkenes	Cravo-Laureau et al., 2007
		Desulfobacterium		MaCs, LMW-PAHs, long chain alkanes	Harms et al., 1999
		Desulfococcus		Long chian alkanes	Abed et al., 2011
		Desulfoglaeba		Medium chain alkanes	Davidova et al., 2006
	Gammaproteobacteria	Acinetobacter	baumannii	Phe, crude oil	Kim et al., 2009
		Alcanivorax		Medium and long chain alkanes	Hassanshahian et al., 2012
		Alkanindiges		Short and long chain alkanes	Bogan et al., 2003
		Alteromonas		LMW-, HMW-PAHs	Jin et al., 2011
		Cycloclasticus		MaCs, LMW-, HMW-PAHs	Dyksterhouse et al., 1995
		Halomonas		MaCs, LMW-PAHs, long chain alkanes, crude oil	Wang et al., 2007
				Flu, Fl, Py,	Nanca et al., 2018
		Marinobacter		LMW-PAHs, Long chain alkanes	Hassanshahian et al., 2012
		Microbulbifer		MaCs, crude oil	Brito et al., 2006
		Neptunomonas		LMW-PAHs	Hedlund et al., 1999
		Oleibacter		Long chain alkanes	Liu et al., 2017
		Oleiphilus		Medium and long chain alkanes	Golyshin et al., 2002
		Oleispira		Alkanes	Yakimov et al., 2003
		Pseudoalteromonas		MaCs, LMW-PAHs	Liu et al., 2017
		Pseudomonas		MaCs, LMW-, HMW-PAHs, short and long chain alkanes	Zhang et al., 2011

Table S3. (continued).

Phylum	Class	Genus	Species	Target Contaminant	References
Proteobacteria	Gammaproteobacteria	Pseudomonas	fluorescens	Phe, HCHs	Abbasnezhad et al., 2011
		Pseudomonas	monteilii,	Na, Phe	Isaac et al., 2015
			aeruginosa	HCHs	Ilori et al., 2000
			diminuta	HCHs	Manonmani et al., 2000
			vesicularis	HCHs	Manonmani et al., 2000
		Shewanella		Crude oil	Gentile et al., 2003
		Stenotrophomonas	maltophilia	BaA, BaP, Chr, DbahA, Fl, Phe, coronene	Juhasz et al., 2000, Kim et al., 2009
		Vibrio		LMW-PAHs	Hedlund & Staley, 2001
Spirochaetes	Spirochaetaceae	Spirochaeta		Crude oil	Xiong et al., 2015
Thermotogae	Thermotogaceae	Petrotoga		Oil	Daryasafar et al., 2014

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