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Bioaccessibility of AhR-active PAHs in sediments contaminated by the *Hebei Spirit* oil spill: Application of Tenax extraction in effect-directed analysis

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HIGHLIGHTS

- Bioaccessibility of AhR-active PAHs in oil-contaminated sediments were assessed.
- EDA was performed using a battery of H4IIE-*luc in vitro* bioassay and GC/MSD analysis.
- Concentrations of PAHs in Tenax extracts were ~20-fold less than Soxhlet extracts.
- Major AhR-active PAHs were identified as C1- and C3-chrysene, and C4-phenanthrene.
- Bioaccessibility of PAHs appeared to depend on hydrophobicity and oil-weathering.

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ABSTRACT

Bioaccessibility of toxic substances in sedimentary residual oil is a crucial factor that needs to be considered for accurate risk assessments posed by oil spills. However, information on oil weathering processes and bioaccessibility of residual oil is often not sufficient and clear. In the present study, bioaccessibility of aryl hydrocarbon receptor (AhR)-active polycyclic aromatic hydrocarbons (PAHs) in coastal sediments near the site of the *Hebei Spirit* oil spill (Korea, 2007) was assessed by Tenax extraction in effect-directed analysis (EDA). Sediment samples collected 6 years after the oil spill were extracted using Soxhlet or Tenax, and EDA was performed using a battery of H4IIE-*luc* bioassay and GC/MSD analysis. Concentrations of PAHs and alkyl-PAHs in Soxhlet extracts ranged from 210 to 53,000 μg kg^{-1} dry mass. However, concentrations of PAHs and alkyl-PAHs in Tenax extracts were approximately 20-fold less compared to those in Soxhlet extracts. In Soxhlet and Tenax extracts, the major AhR-active PAHs were identified as C1-chrysene, C3-chrysene, and C4-phenanthrene. Concentrations of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) equivalents (TEQ_{PAHs}) explained 31% and 60% of the bioassay-derived TCDD-EQ concentrations in Soxhlet and Tenax extracts, respectively. Overall, bioaccessibility of PAHs and alkyl-PAHs in sedimentary residual oils depended on hydrophobicity (log K_{ow}) and degree of weathering of crude oil. The results of the present study provide further evidence in support of the biological and ecological recoveries of oil spill sites.

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1. Introduction

Oil spills in marine environments, particularly heavily populated coastal areas, remain one of the most serious threats to ecosystems and humans worldwide (Allan et al., 2012; Hong et al., 2014; Peterson et al., 2003). Crude oil consists of a complex mixture of hydrocarbons with varying properties that can cause adverse effects among all levels of biological organization (Jung et al., 2011; Peterson et al., 2003; Seo et al., 2014). Polycyclic aromatic hydrocarbons (PAHs) are one of the well-known classes of toxic compounds in crude oil (Allan et al., 2012). Once crude oil is spilled at sea, it undergoes natural processes of degradation and dissipation, referred to collectively as weathering (Hong et al., 2012; Yim et al., 2012). Weathering of petroleum hydrocarbons (PHs) in crude oil, involves biological, chemical, and physical changes, including volatilization, photolysis, microbial transformation, and, ultimately, mineralization. These changes occur over varying time periods depending on environmental conditions (Dutta and Harayama, 2000; Garrett et al., 1998; Peterson et al., 2003; Prince, 1993). Residual hydrocarbons are relatively persistent in sediments compared to air, water, and biota (Hong et al., 2014). Concentrations and relative compositions of residual PHs including PAHs and alkyl-PAHs change during natural weathering, and their potencies to cause adverse effects on benthic organisms can change due to degradation and bioavailability (Lee and Page, 1997; Peterson et al., 2003). Bioavailability of toxic substances in sedimentary residual hydrocarbons are crucial factor(s) that need to be considered before accurate assessments of risks of spills of PHs can be made (Neff et al., 2006). However, information on processes involved in weathering and bioavailability of PHs is often not available.

The *Hebei Spirit* oil spill (HSOS) was the worst oil spill that has ever occurred in South Korea. On December 7, 2007, approximately 10,900 tons of crude oil, including Iranian Heavy Crude, Kuwait Export Crude, and UAE Upper Zakum, were spilled along the Taean coast (west coast of South Korea) (Hong et al., 2012, 2014; Yim et al., 2012). Results of previous studies have indicated that concentrations of PAHs in water and biota along the Taean coasts had fully recovered five years after the HSOS (Hong et al., 2014). However, residual PHs in sediments could potentially be toxic to benthic communities, which have not yet fully recovered (Hong et al., 2014). PHs derived from spilled crude oil (e.g., PAHs and alkyl-PAHs) and potential toxicity (e.g., aryl hydrocarbon receptor (AhR)- and estrogen receptor (ER)-mediated activities) persist, particularly in semi-closed areas and mudflats (Hong et al., 2012, 2015; Ji et al., 2011). Major AhR agonists in residual oils contained in sediments were classified as three to four ring PAHs, in particular alkyl-PAHs, determined by use of effect-directed analysis (EDA) (Hong et al., 2015).

The EDA method has become a powerful tool for identification of key toxicant(s) in complex mixtures such as sediments, soils, and crude oil (Brack and Schirmer, 2003; Vrabie et al., 2012). EDA provides useful information on chemicals of concern and mechanisms of toxic effects in sediments contaminated with oil. However, since most EDA methods are based on sequential extraction with organic solvents, thus environmental realism could not be considered and organic pollutants in environmental samples often overestimate their environmental risk (Schwab et al., 2009). For example, the water solubility and volatility of PAHs decrease as molecular mass increases, and greater molecular mass chemicals are less soluble in the water column and persistent in sediments more (Peterson et al., 2003). PAHs in solution are the most bioavailable and toxic to marine organisms. Thus, PAHs of greater molecular mass containing four or more rings seem to cause fewer effects in living organisms due to their lesser solubility in water (Neff, 2002), even though concentrations are greater in residual PHs in sediment. Indeed, two years after the HSOS, macrofaunal communities exhibited signs of recovery despite relatively great concentrations of crude-derived hydrocarbons and great AhR-mediated potencies in sediments along the Taean coast (Hong et al., 2012). Matching chemical analyses of sediments with responses of benthic communities might be improved by considering bioaccessibility of chemicals in sedimentary residual oils.

Several experimental methods, including partial extractions (e.g., Tenax extraction) and passive samplers (e.g., semipermeable membrane devices, SPMDs), have been developed to assess bioavailability and/or bioaccessibility of hydrophobic organic contaminants (HOCs) in environmental samples (Cui et al., 2013). Extraction with Tenax has been widely used to estimate the rapidly desorbing fraction of HOCs in sediments and soils (Cornelissen et al., 2001; You et al., 2006), and applied in EDA and toxicity identification evaluation for identifying putative toxicants in sediments (Schwab and Brack, 2007; Yi et al., 2015). A single-point Tenax extraction (24 h) is the simplest and quickest method of determining bioaccessibility of HOCs in sediments, of which fraction is comparable to rapidly desorbing fractions determined by consecutive Tenax extraction (Cornelissen et al., 2001). Studies have shown that HOCs from 6 or 24 h Tenax extraction were well correlated with bioaccumulation in benthic animals (You et al., 2011). However, studies using Tenax extraction to determine bioaccessibility of AhR-active PAHs in oil-contaminated sediments are scarce.

In the present study, bioaccessibilities of AhR-active PAHs in coastal sediments were assessed using a single point Tenax extraction as part of the EDA. The aims of this work were to: i) assess the bioaccessibility of PAHs and alkyl-PAHs in sediments using Tenax extraction, ii) determine changes of bioaccessibility of PAHs and alkyl-PAHs in sediments according to the oil weathering, and iii) revisit the major AhR-agonists in sediments and their contributions using EDA six years after the HSOS.

2. Materials and methods

2.1. Preparation of oil-coated sand

Oil-coated sand was prepared to determine bioaccessibility of crude oil in sediments during initial stages of an oil spill. Clean sand was collected from a beach (Ganghwa Island, Incheon) located on the west coast of South Korea. It was passed through a 2 mm sieve, homogenized, and air-dried. Baseline PAH concentrations were measured prior to oil spiking (sum of 36 PAHs and alkyl-PAHs: < 10 $\mu g\ kg^{-1}$ dry mass (dm)). One kilogram of sand was placed in a 1 L brown glass bottle and spiked with 1 g of crude oil (28.8% mass loss of Iranian Heavy Crude) to obtain a nominal oil concentration of 1000 $mg\ kg^{-1}$ dm. The glass bottle with oil-spiked sand was placed on a rolling mixer for three days. Oil-coated sand was dried in a fume hood for 24 h, mixed for 24 h, then stored at 4 °C until use.

2.2. Sampling and sample preparation

Sediments contaminated with spilled crude oil that had weathered, were collected from the Taean coast approximately six years after the HSOS (September, 2013) (details in Fig. S1 of Supplemental Materials (S)). Three sites, Gureumpo, Shinduri, and Euihangri, were selected based on results of previous monitoring studies (Hong et al., 2012, 2014, 2015). Sediments were collected after visual confirmation of residual oil *in situ*. All samples were immediately transferred to the laboratory and stored at 4 °C until analysis. Organic carbon (OC) content of the sediments was analyzed using an Elementar Vario Micro cube (Hanau, Germany) after removing inorganic carbon by 1 M HCl. OC contents were

0.08%, 0.31%, 0.57%, and 0.32% for oil-coated sand, Gureumpo, Shinduri, and Euihangri sediments, respectively.

2.3. Soxhlet extraction and fractionation

Detailed preparation of Soxhlet extracts for EDA has been described previously (Brack and Schirmer, 2003; Hong et al., 2015). Briefly, 30 g of oil-coated sand or sediment was extracted in a Soxhlet extractor for 24 h with 350 mL dichloromethane (DCM, Burdick and Jackson, Muskegon, MI) (Mazeas and Budzinski, 2002). Extracts were concentrated and separated into three fractions (F1: saturates; F2: aromatics; F3: polar and resins) using an activated silica gel column chromatography (70–230 mesh, Merck, Darmstadt, Germany) with 40 mL of hexane (F1), 50 mL of 20% DCM in hexane (F2), and 50 mL of 60% DCM in acetone (F3), respectively. The aromatic fraction (F2) was fractionated into six sub-fractions based on the number of aromatic rings using a normal phase HPLC column (Nucleosil 100–5 NO_2, 250 mm length, 5 μm particle size, 4.6 mm i.d., Macherey–Nagel, Düren, Germany) (Brack and Schirmer, 2003; Hong et al., 2015). Separation and collection of fractions were performed using an Agilent 1260 HPLC System (Agilent technologies, Avondale, PA). The hexane:DCM (95:5, v/v) mobile phase was delivered isocratically at a flow rate of 0.7 mL min^{-1}. Detailed HPLC conditions and information of F2 sub-fractions are provided in Table S1.

2.4. Tenax extraction

Tenax extraction of oil-coated sand and field sediments was conducted using previously described methods with some modifications (Cornelissen et al., 1997; You et al., 2007). Tenax TA beads (60–80 mesh, Supelco, Bellefonte, PA) were rinsed with deionized water, acetone, and hexane containing 10 mL g^{-1} of Tenax (two rinses each) and dried overnight at 80 °C. For oil-contaminated sediments, 2 g of sediment, 20 mg of $HgCl_2$, 0.4 g of Cu powder (for removing sulfur), 35 mL of deionized water, and 0.5 g of Tenax beads were added to each test tube and placed on a horizontal shaker for 24 h. After extraction, Tenax beads were floated and separated by filtration (GF/F, Whatman Ltd., Kent, UK). All experiments were conducted in triplicate. Target PAHs and alkyl-PAHs were extracted from harvested Tenax beads with a mixture of hexane and acetone (50:50, v/v). After concentration with N_2 gas, extracts were fractionated using a silica gel column and HPLC, and instrumental analyses and bioassays were performed according to methods described for Soxhlet extracts above.

2.5. PAHs and alkyl-PAHs analyses

Concentrations of PAHs and alkyl-PAHs were identified and quantified by use of a previously described method (Hong et al., 2012). A total of 36 PAHs and alkyl-PAHs were quantified using an Agilent 7890 gas chromatograph (GC) coupled with a model 5975C mass-selective detector (MSD, Agilent Technologies). Target PAHs and alkyl-PAHs were detected primarily in the F2 fraction of the both Soxhlet and Tenax extracts of sediments, with these chemicals not being detected in the F1 and F3 fractions. Detailed instrumental conditions for PAHs and alkyl-PAHs analyses are provided in the Table S2. In a separate experiment, QA/QC for PAHs analysis was conducted using NIST standard reference material (1941b, n = 3), and recoveries were generally acceptable, ranging from 57 (for naphthalene) to 105 (for fluoranthene) (details in Table S3).

2.6. H4IIE-luc in vitro bioassay

The H4IIE-*luc* bioassay was performed according to previously reported methods (Hong et al., 2012). Viability of cells and cytotoxicity of all samples were determined using the MTT assay. Dilution factors of each sample were determined (% live cell > 80%). Trypsinized cells (~8.0×10^4 cells mL^{-1}) were seeded into the 60 interior wells of 96 micro-well plates at a volume of 250 μL per well. After 24 h, test and control wells were dosed with the appropriate standards (2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), 0.1% dose), sample extracts (all fractions of Soxhlet and Tenax extracts, 0.1% dose), and solvent controls (0.1% DMSO). Six concentrations of the calibration standard or extracts were tested and each exposure was in triplicate. After 72 h of exposure, luciferase was quantified by use of a ML3000 microplate reading luminometer (Tecan, Infinite 200, Mannedorf, Switzerland). Responses of the H4IIE-*luc* bioassay (expressed as average relative luminescence units, RLU) were converted to percentages of the maximum response (%-$TCDD_{max}$) observed for a 300 pM (= 100%-$TCDD_{max}$) of 2,3,7,8-TCDD. Finally, AhR-mediated potency was expressed as a TCDD standard equivalent concentration (TCDD-EQ). Duplicates of the same samples exhibit variations that are accepted in the error range (%RSD < 15).

2.7. Potency balance analyses

Potency balance analyses between bioassay-derived TCDD-EQs and instrument (GC/MS)-derived TEQs were conducted to determine the contribution of each known chemical to total induced AhR-mediated potency. Concentrations of TEQs were calculated as the sum of the products of concentrations of individual PAHs and alkyl-PAHs multiplied by their assay-specific relative potency values (RePs) reported previously (Villeneuve et al., 2002; Hong et al., 2015) (Table S4).

3. Results and discussion

3.1. PAHs and alkyl-PAHs in Soxhlet and Tenax extracts of sediments

Concentrations of PAHs and alkyl-PAHs in oil-coated sand were 3,420 and 19,100 μg kg^{-1} dm for Tenax and Soxhlet extracts, respectively (Fig. 1). The relative bioaccessibility of PAHs and alkyl-PAHs ($C_{Tenax}/C_{Soxhlet}$) were compound-specific, ranging from 2.7 (for C3-chrysene (Chr)) to 95% (for naphthalene), with a mean of 23% (details in Table S5). Bioaccessibility was significantly correlated with hydrophobicities of target chemicals, which decreased with increasing log K_{ow} values (Table S5 and Fig. S2). This result was in agreement with several previous studies (Cornelissen et al., 1997; Yang et al., 2010; You et al., 2007).

Concentrations of PAHs and alkyl-PAHs in Soxhlet and Tenax extracts six years after the HSOS were also determined (Table S6). Concentrations of 36 PAHs and alkyl-PAHs in Soxhlet extracts from the three test sites, Gureumpo, Shinduri, and Euihangri, were 53,200, 24,000, and 209 μg kg^{-1} dm, respectively. Compared to Soxhlet extracts, concentrations in Tenax extracts from the test sites (Gureumpo, Shinduri, and Euihangri) were 4–50 times less (6,520, 484, and 56.3 μg kg^{-1} dm, respectively). The $C_{Tenax}/C_{Soxhlet}$ values of sediments ranged from 3.4 to 63% (mean = 15%), from 0.1 to 40% (mean = 6.7%), and from 9.5 to 88% (mean = 41%) in Gureumpo, Shinduri, and Euihangri, respectively. Chemical compositions indicated that PAHs and alkyl-PAHs from Gureumpo and Shinduri were affected by the HSOS, while those in Euihangri did not originate from the HSOS (Fig. S3). Double ratios of C3-dibenzothiophene (Dbthio)/C3-phenenthrene (Phe) and C2-Dbthio/C2-Phe in Gureumpo and Shinduri sediments were associated with Iranian Heavy Crude and/or mixtures of the three crude oils that were spilled during the HSOS (Iranian Heavy Crude, Kuwait Export Crude, and UAE Upper Zakum) (Yim et al., 2011).

Overall, results indicated that six years after the HSOS, residues

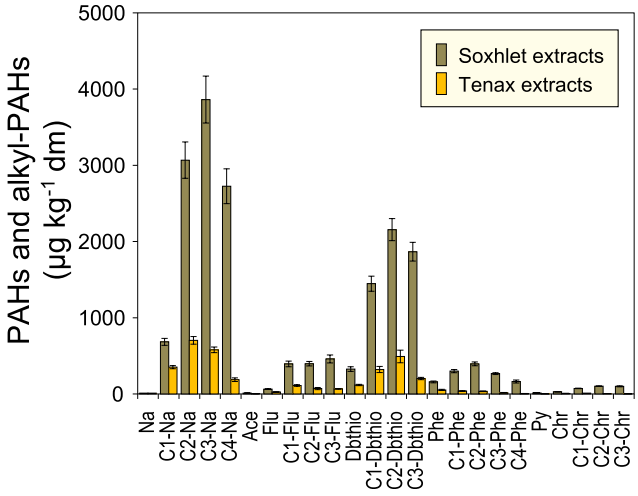

Fig. 1. Desorbed PAHs and alkyl-PAHs from oil-coated sand. Concentrations of PAHs and alkyl-PAHs in Soxhlet and Tenax extracts.

of crude oils still remain in some more affected areas of the Taean spill. Weathered PHs penetrated into sediments in the upper intertidal zone in the case of the Exxon Valdez oil spill (Neff et al., 2006). The buried oil has persisted in sediments over 16 years after the oil spill. This was attributed to lesser accessibility to tidal flushing and wave action (Short et al., 2007). Thus, potential biological effects of these residual oils might still be of concern.

3.2. Changes of bioaccessibility of PAHs and alkyl-PAHs during oil weathering

Degree of weathering of oil was estimated using alkyl-PAH double ratios, such as C2-Dbthio/C3-Dbthio and C2-Phe/C3-Phe (Hong et al., 2012, 2015) (Fig. 2). Results suggested that oil-coated sand was categorized into “not weathered” (similar to Iranian Heavy Crude), while a sediment from the Gureumpo region was found to be “slightly weathered” (Stage I), and sediment from the Shinduri region was identified as “severely weathered” (Stage III) based on results of previous studies (Hong et al., 2012; Yim et al., 2011). Sediment from the Euihangri region was excluded from this analysis, because it did not originate from the HSOS. Previous studies suggested that the degree of residual oil weathering in sediments could be affected by environmental parameters, including geographical, physical, and biological conditions (Douglas, 1996; Short and Heintz, 1997). In the present study, which was conducted approximately six years after the HSOS, relatively fresh crude oil (Stage I) was still found in sediments from some isolated sites, such as Gureumpo.

Bioaccessibility of individual PAHs and alkyl-PAHs in oil-coated sand and field sediments from Gureumpo and Shinduri were determined from the percentage of C_{Tenax} per $C_{Soxhlet}$ (Fig. 2). Values ($C_{Tenax}/C_{Soxhlet}$) of individual PAHs and alkyl-PAHs in sediments were inversely proportional to log K_{ow}. This trend was similar to those of the oil-coated sand. Also, bioaccessibility of individual PAHs and alkyl-PAHs in severely weathered sediments, such as those from the Shinduri region, were less than those from slightly weathered sediments from Gureumpo. In particular, bioaccessibility of PAHs of lesser molecular mass with the log $K_{ow} < 4.5$ apparently decreased with increasing oil weathering compared to those of greater molecular mass PAHs (Fig. 2 and Fig. S2). In addition, types of organic carbon might affect bioaccessibility of PAHs and alkyl-PAHs in sediments due to affecting sorption of PAHs. Relatively great organic carbon content was found in the Shinduri sediment, which also showed small bioaccessibility of PAHs presumably due to the relatively greater sorption in sediment. Meanwhile, the unresolved complex mixture (UCM) might act as a sedimentary super-sorbent, which can alter bioaccessibility of sedimentary PAHs by competition for sorption sites of sediments (Du et al., 2012; Jonker et al., 2003). Thus, bioaccessibility of PAHs and alkyl-PAHs in sedimentary residual oils appeared to depend on the i) hydrophobicity of chemicals, ii) organic carbon contents associated with grain size, and iii) the degree weathering of the UCM. Overall, these results suggested that bioaccessibility of AhR-active PAHs in residual oils in sediments appeared to decrease with increased oil weathering due to greater sorption of PAHs (Jonker et al., 2003).

Bioaccessibility results for sediments from Taean seemed to support data showing that organisms and macrofaunal community have recovered since the HSOS (Hong et al., 2014). Concentrations of PAHs in muscles of pelagic and benthic fishes collected from the Taean area decreased to background concentrations within 11 months of the HSOS (Jung et al., 2011), and total concentrations of PHs in seawater from the Taean coast decreased to match the Korean marine water quality standard of 10 μg L^{-1} within 10 months of the oil spill (Kim et al., 2010). These results are in contrast with reports of oil-impacted sediments approximately four years after the HSOS (Hong et al., 2014, 2015). It is important to note that despite concentrations of residual PHs in sediments, macrofaunal communities in intertidal areas were recovering two years after the oil spill (Hong et al., 2012). In addition, macrobenthic communities achieved a stable faunal composition 3 years after the HSOS, even though other aspects of the ecosystem had not yet fully recovered (Seo et al., 2014). Taken together, these data suggest that benthic community structures recover over time due to degradation of toxic chemicals and reduction of bioaccessibility of toxic compounds, regardless of whether sedimentary residual oils remain. Thus, ecological risk posed by chemical and/or toxicological assessment in oil-contaminated sediments could be overestimated if the bioavailability or bioaccessibility of toxic compounds and oil weathering are not assessed and considered.

3.3. Effect-directed analysis: Soxhlet and Tenax extracts of sediments

EDA was performed to identify key toxicant(s) in sediments from the three test sites (shown in Fig. S1). In particular, bioaccessibility of major toxicant(s) in sediments contaminated with PHs were determined. Results of the H4IIE-*luc* bioassays revealed that Soxhlet extract F2, which contained aromatics, had the greatest AhR-mediated potency (Fig. 3a). Fraction F1, which contained aliphatic PHs, and fraction F3, which contained polar residues and resins, contained fewer or no AhR agonists. This result is consistent with those of previous studies that have demonstrated that aromatic compounds, including PAHs and alkyl-PAHs, bind to the AhR (Hong et al., 2015; Radović et al., 2014).

Results of a previous study indicated that AhR-mediated potencies in fraction F3 were comparable to those of fraction of F2 crude oil (Iranian Heavy Crude), while extracts of Taean sediments showed a decreasing trend from 2007 to 2012 (Hong et al., 2015). Results of the present study were consistent with previous findings showing that AhR agonists in fraction F3 were degraded or transformed to less toxic chemicals during the oil weathering process. Another possible explanation of the lesser potency detected in F3 might be due to biotransformation during 72 h exposure. The F2 fraction was sub-fractionated by HPLC into fractions F2.1–F2.6. Analyses showed the greatest AhR-mediated potency was present in fractions F2.2 and F2.3 from Shinduri and Gureumpo sediments. Together, these results indicate that the major AhR agonists were

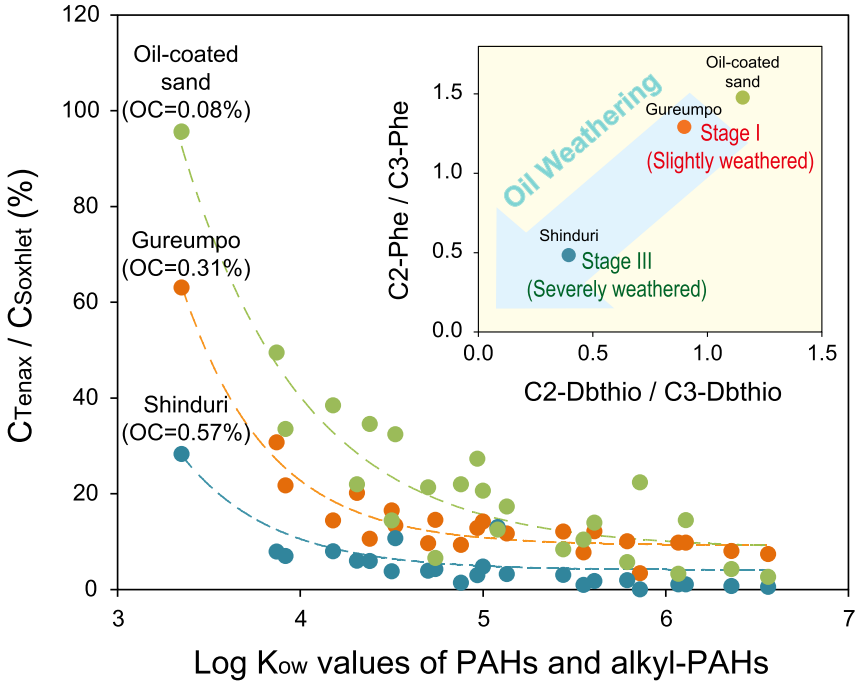

Fig. 2. Scatter plot for percentage of concentrations of PAHs and alkyl-PAHs of Tenax per Soxhlet ($C_{Tenax}/C_{Soxhlet}$) of oil-coated sand and oil-contaminated sediments (different degree of oil weathering estimated by alkyl-PAHs double ratios) vs. log K_{ow} values.

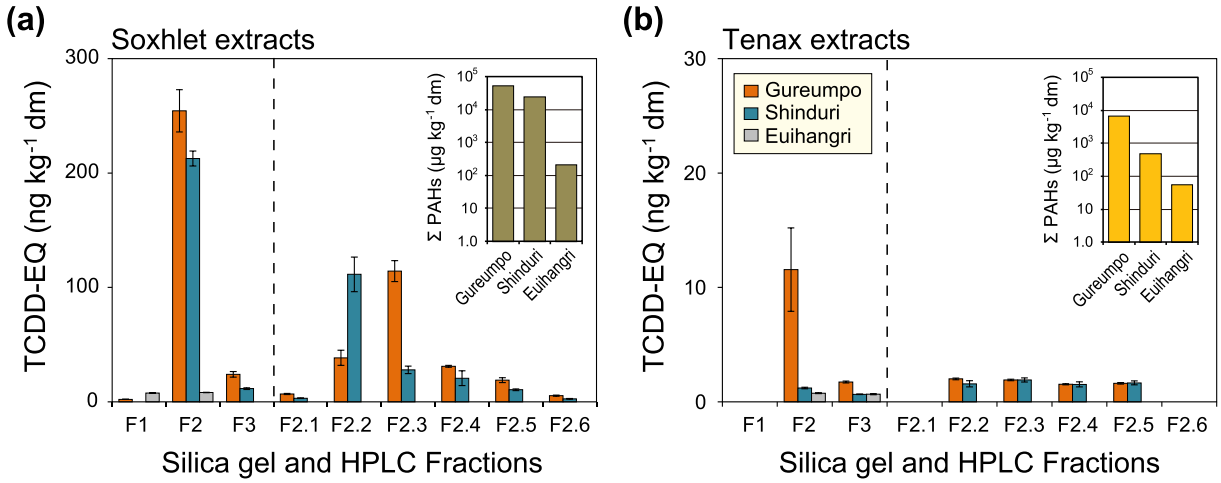

Fig. 3. Bioassay-derived TCDD-EQs in silica gel and HPLC fractions and PAHs concentrations of (a) Soxhlet extracts and (b) Tenax extracts of oil-contaminated field sediments (Error bar: mean ± SD; n = 3).

three to four ring aromatics (Fig. 3a and Table S1). AhR-mediated potency was also quantified in Tenax extracts. Greater AhR-mediated potency was found in fraction F2 of Gureumpo sediment (Fig. 3b). However, lesser AhR-mediated potency was observed in F2 sub-fractions from Tenax extracts (range: 1.5–2.0 ng TCDD-EQ kg^{-1} dm). AhR-mediated potencies in F2 Tenax extracts were approximately 20 times less than those detected in Soxhlet extracts. These results indicate AhR-active chemicals in sedimentary residual oil are less bioaccessible to organisms.

3.4. Potency balance analysis: contributions of PAHs and alkyl-PAHs

Contributions of PAHs and alkyl-PAHs to overall AhR-mediated potencies were determined by comparing bioassay-derived TCDD-EQs and instrument-derived TEQs (Table 1 and Fig. 4). Concentrations of TCDD-EQs of Soxhlet and Tenax F2 fractions were directly compared to concentrations of TEQs (sum of the 13 PAHs and alkyl-PAHs) (see Table S4) (Hong et al., 2015; Villeneuve et al., 2002). Percent contributions of AhR-active PAHs in Soxhlet extracts of sediments from Gureumpo, Shinduri, and Euihangri, were 32%, 56%, and 15%, respectively. The primary AhR-agonists were C1-Chr, C3-Chr, and C4-Phe, consistent with results from a previous study (Hong et al., 2015). However, relative contributions of the three major AhR-active PAHs in sediments were increased in this study. Unalkylated and analogous alkyl-PAHs in sediment extracts four years after the HSOS explained approximately 18% of the bioassay-derived TCDD-EQs (Hong et al., 2015). Compared to other unknown AhR agonists, these chemicals appeared to persist in coastal sediments despite natural oil weathering processes.

Table 1
Bioassay-derived TCDD-EQs and instrument-derived TEQs of Soxhlet and Tenax extracts of oil-contaminated sediments collected from the Taean coast six years after the *Hebei Spirit* oil spill.

Analysis		Unit	Fraction	Gureumpo		Shinduri		Euihangri	
				Soxhlet	Tenax	Soxhlet	Tenax	Soxhlet	Tenax
Bioassay-derived	TCDD-EQ	ng kg^{-1} dm	RE	7.7×10^2	1.3×10	3.0×10^2	1.0×10	6.1	0.72
			F1	2.2	–[e]	–	–	7.8	–
			F2	2.5×10^2	1.2×10	2.1×10^2	1.2	8.3	0.76
			F3	2.4×10	1.7	1.1×10	0.67	–	0.67
			F2.1	7.0	–	3.2	–	–	–
			F2.2	3.9×10	2.0	1.1×10^2	1.6	–	–
			F2.3	1.1×10^2	1.9	2.8×10	1.9	–	–
			F2.4	3.1×10	1.5	2.1×10	1.5	–	–
			F2.5	1.9×10	1.6	1.1×10	1.7	–	–
			F2.6	5.5	–	2.7	–	–	–
Instrument-derived	Parent-PAHs[a]	µg kg^{-1} dm	F2	1.6×10^3	1.8×10^2	6.0×10^2	2.1×10	7.5×10	1.5×10
	Alkyl-PAHs[b]		F2	5.2×10^4	6.3×10^3	2.3×10^4	4.6×10^2	1.3×10^2	4.2×10
	Total PAHs		F2	5.3×10^4	6.5×10^3	2.4×10^4	4.8×10^2	2.1×10^2	5.6×10
	TEQ_{PAHs}[c]	ng kg^{-1} dm	F2	8.2×10	7.1	1.2×10^2	1.0	1.3	0.28
Potency balance	%-Identified[d]	(%)	F2	32	61	56	84	15	37

[a] Parent PAHs: Sum of the concentrations of parent PAHs.
[b] Alkyl-PAHs: Sum of the concentrations of alkyl-PAHs.
[c] TEQ_{PAHs}: Sum of the TEQ values of all target PAHs calculated using concentrations and ReP values (Hong et al., 2015; Villeneuve et al., 2002).
[d] %-Identified: The percentage of total TEQ_{PAHs} to the bioassay-derived TCDD-EQs of F2 fraction.
[e] –: below detection limits.

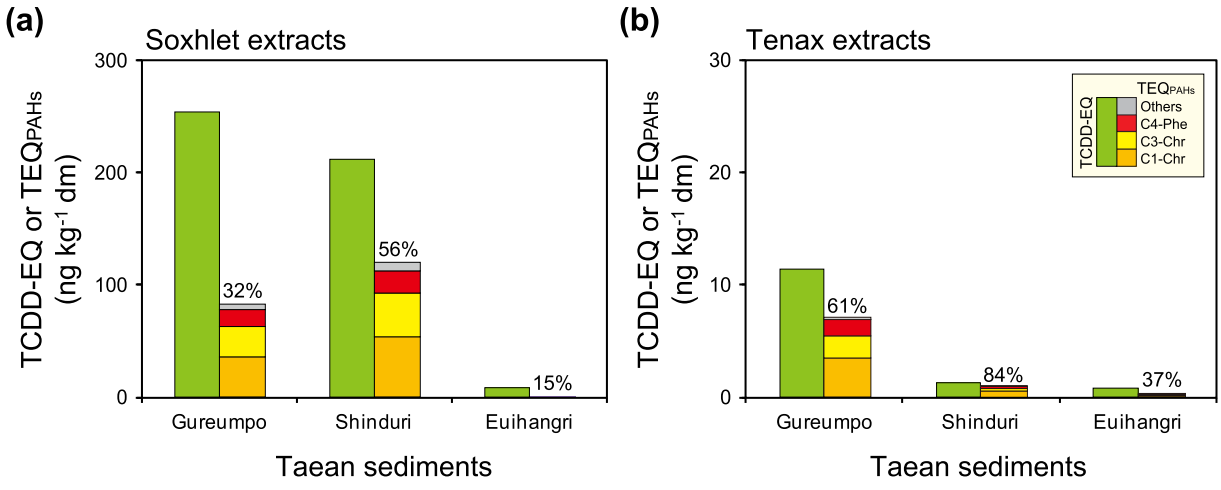

Fig. 4. Potency balance between bioassay-derived TCDD-EQs and instrument-derived TEQ_{PAHs} in (a) Soxhlet extracts and (b) Tenax extracts of oil-contaminated field sediments.

A greater proportion of compounds were identified in Tenax extracts compared to Soxhlet extracts (Fig. 4), and known AhR-active PAHs and alkyl-PAHs explained a greater proportion of the total AhR-mediated potency. Indeed, known PAHs and alkyl-PAHs explained 61%, 84%, and 37% of toxicity in Tenax extracts of Gureumpo, Shinduri, and Euihangri sediments, respectively. Among the target chemicals, three highly potent chemical groups including C1-Chr, C3-Chr, and C4-Phe were found to be the major bioaccessible PAHs. Results of a previous EDA study conducted on various oils and refined products found that the major AhR agonists were aromatic compounds with log K_{ow} values of 5–8 (Vrabie et al., 2012), which include C1-Chr (6.11), C3-Chr (6.56), and C4-Phe (6.07). In addition, major AhR-active compounds in North Sea crude and heavy fuel oil were alkyl-substituted three and four-ring aromatic chemicals as well as hetero-aromatic systems (containing S and N) (Radović et al., 2014). Those results were consistent with results of the present study, which indicates that the major AhR agonists in oil components are three to four ring aromatics. However, it should be noted that contributions to toxicity and bioaccessibility of PAHs and alkyl-PAHs in sedimentary residual oil could vary, depending on the region and duration of exposure.

Overall, the results of the study upon which we report here, indicated that concentrations of PAHs in sediments contaminated with crude oil, were generally well matched to potential toxic effects. However, ecological recovery from the oil spill was not clearly explained by concentrations or toxic potencies of target chemicals, such as PAHs and alkyl-PAHs. Difficulties in matching the hydrocarbon chemistry, crude oil toxicity, and benthic community recovery could be explained by several factors, including: i) bioavailability of residual oils, ii) site-specific oil weathering, and iii) unknown toxicants in crude oils. Moreover, the toxic effects of the mixtures could be largely missed and/or masked by each other. Thus, additional complementary studies on oil spill-affected environments would contribute toward integrated assessment in the future.

Acknowledgments

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Management of Hazardous Chemicals in the Marine Environment" funded by the Ministry of Oceans and Fisheries of Korea given to Prof. JSK. Prof. JPG was supported by the Canada Research Chair program, a Visiting Distinguished Professorship in the Department of Biology and Chemistry and State Key Laboratory in Marine Pollution, City University of Hong Kong, the 2012 "High Level Foreign Experts" (#GDW20123200120) program, funded by the State Administration of Foreign Experts Affairs, the P.R. China to Nanjing University and the Einstein Professor Program of the Chinese Academy of Sciences.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.chemosphere.2015.09.043.

References

Allan, S.E., Smith, B.W., Anderson, K.A., 2012. Impact of the deepwater horizon oil spill on bioavailable polycyclic aromatic hydrocarbons in gulf of Mexico coastal waters. Environ. Sci. Technol. 46, 2033–2039.

Brack, W., Schirmer, K., 2003. Effect-directed identification of oxygen and sulfur heterocycles as major polycyclic aromatic cytochrome P4501A-inducers in a contaminated sediment. Environ. Sci. Technol. 37, 3062–3070.

Cornelissen, G., Rigterink, H., Ten Hulscher, D.E.M., Vrind, B.A., Van Noort, P.C.M., 2001. A simple tenax® extraction method to determine the availability of sediment-sorbed organic compounds. Environ. Toxicol. Chem. 20, 706–711.

Cornelissen, G., Van Noort, P.C.M., Govers, H.A.J., 1997. Desorption kinetics of chlorobenzenes, polycyclic aromatic hydrocarbons, and polychlorinated biphenyls: sediment extraction with Tenax® and effects of contact time and solute hydrophobicity. Environ. Toxicol. Chem. 16, 1351–1357.

Cui, X., Mayer, P., Gan, J., 2013. Methods to assess bioavailability of hydrophobic organic contaminants: principles, operations, and limitations. Environ. Pollut. 172, 223–234.

Douglas, G.S., 1996. Environmental stability of selected petroleum hydrocarbon source and weathering ratios. Environ. Sci. Technol. 30, 2332–2339.

Dutta, T.K., Harayama, S., 2000. Fate of crude oil by the combination of photooxidation and biodegradation. Environ. Sci. Technol. 34, 1500–1505.

Du, J., Mehler, W.T., Lydy, M.J., You, J., 2012. Toxicity of sediment-associated unresolved complex mixture and its impact on bioavailability of polycyclic aromatic hydrocarbons. J. Hazard. Mat. 203, 169–175.

Garrett, R.M., Pickering, I.J., Haith, C.E., Prince, R.C., 1998. Photooxidation of crude oils. Environ. Sci. Technol. 32, 3719–3723.

Hong, S., Khim, J.S., Ryu, J., Kang, S.G., Shim, W.J., Yim, U.H., 2014. Environmental and ecological effects and recoveries after five years of the Hebei Spirit oil spill, Taean, Korea. Ocean. Coast. Manage 102, 522–532.

Hong, S., Khim, J.S., Ryu, J., Park, J., Song, S.J., Kwon, B.O., Choi, K., Ji, K., Seo, J., Lee, S., Park, J., Lee, W., Choi, Y., Lee, K.T., Kim, C.K., Shim, W.J., Naile, J.E., Giesy, J.P., 2012. Two years after the Hebei Spirit oil spill: residual crude-derived hydrocarbons and potential AhR-mediated activities in coastal sediments. Environ. Sci. Technol. 46, 1406–1414.

Hong, S., Lee, S., Choi, K., Kim, G.B., Ha, S.Y., Kwon, B.-O., Ryu, J., Yim, U.H., Shim, W.J., Jung, J., Giesy, J.P., Khim, J.S., 2015. Effect-directed analysis and mixture effects of AhR-active PAHs in crude oil and in coastal sediments contaminated by the *Hebei Spirit* oil spill. Environ. Pollut. 199, 110–118.

Ji, K., Seo, J., Liu, X., Lee, J., Lee, S., Lee, W., Park, J., Khim, J.S., Hong, S., Choi, Y., Shim, W.J., Takeda, S., Giesy, J.P., Choi, K., 2011. Genotoxicity and endocrine-disruption potentials of sediment near an oil spill site: two years after the Hebei Spirit oil spill. Environ. Sci. Technol. 45, 7481–7488.

Jonker, M.T.O., Sinke, A.J.C., Brils, J.M., Koelmans, A.A., 2003. Sorption of polycyclic aromatic hydrocarbons to oil contaminated sediment: unresolved complex? Environ. Sci. Technol. 37, 5197–5203.

Jung, J.H., Kim, M., Yim, U.H., Ha, S.Y., An, J.G., Won, J.H., Han, G.M., Kim, N.S., Addison, R.F., Shim, W.J., 2011. Biomarker responses in pelagic and benthic fish over 1 year following the Hebei Spirit oil spill (Taean, Korea). Mar. Pollut. Bull. 62, 1859–1866.

Kim, M., Yim, U.H., Hong, S.H., Jung, J.H., Choi, H.W., An, J., Won, J., Shim, W.J., 2010. Hebei Spirit oil spill monitored on site by fluorometric detection of residual oil in coastal waters off Taean, Korea. Mar. Pollut. Bull. 60, 383–389.

Lee, R.F., Page, D.S., 1997. Petroleum hydrocarbons and their effects in subtidal regions after major oil spills. Mar. Pollut. Bull. 34, 928–940.

Mazeas, L., Budzinski, H., 2002. Improved accuracy of GC-MS quantification of aliphatic and aromatic hydrocarbons in marine sediments and petroleums. Validation on reference matrices and application to the Erika Oil Spill. Intern. J. Environ. Anal. Chem. 82, 157–173.

Neff, J.M., 2002. Chapter 15-Polycyclic aromatic hydrocarbons in the ocean. In: Neff, J.M. (Ed.), Bioaccumulation in Marine Organisms. Elsevier, Oxford, pp. 241–318.

Neff, J.M., Bence, A.E., Parker, K.R., Page, D.S., Brown, J.S., Boehm, P.D., 2006. Bioavailability of polycyclic aromatic hydrocarbons from buried shoreline oil residues thirteen years after the Exxon Valdez oil spill: a multispecies assessment. Environ. Toxicol. Chem. 25, 947–961.

Peterson, C.H., Rice, S.D., Short, J.W., Esler, D., Bodkin, J.L., Ballachey, B.E., Irons, D.B., 2003. Long-term ecosystem response to the exxon Valdez oil spill. Science 302, 2082–2086.

Prince, R.C., 1993. Petroleum spill bioremediation in marine environments. Crit. Rev. Microbiol. 19, 217–240.

Radović, J.R., Thomas, K.V., Parastar, H., Díez, S., Tauler, R., Bayona, J.M., 2014. Chemometrics-assisted effect-directed analysis of crude and refined oil using comprehensive two-dimensional gas chromatography–time-of-flight mass spectrometry. Environ. Sci. Technol. 48, 3074–3083.

Schwab, K., Altenburger, R., Lübcke-Von Varel, U., Streck, G., Brack, W., 2009. Effect-directed analysis of sediment-associated algal toxicants at selected hot spots in the River Elbe basin with a special focus on bioaccessibility. Environ. Toxicol. Chem. 28, 1506–1517.

Schwab, K., Brack, W., 2007. Large volume TENAX extraction of the bioaccessible fraction of sediment-associated organic compounds for subsequent effect-directed analysis. J. Soils Sediments 7, 178–186.

Seo, J.Y., Kim, M., Lim, H.S., Choi, J.W., 2014. The macrofaunal communities in the shallow subtidal areas for the first 3years after the Hebei Spirit oil spill. Mar. Pollut. Bull. 82, 208–220.

Short, J.W., Heintz, R.A., 1997. Identification of Exxon Valdez oil in sediments and tissues from Prince Willam sound and the Northwestern Gulf of Alaska based on a PAH weathering model. Environ. Sci. Technol. 31, 2375–2384.

Short, J.W., Irvine, G.V., Mann, D.H., Maselko, J.M., Pella, J.J., Lindeberg, M.R., Payne, J.R., Driskell, W.B., Rice, S.D., 2007. Slightly weathered *Exxon Valdez* oil persists in Gulf of Alaska Beach sediments after 16 years. Environ. Sci. Technol. 41, 1245–1250.

Villeneuve, D.L., Khim, J.S., Kannan, K., Giesy, J.P., 2002. Relative potencies of individual polycyclic aromatic hydrocarbons to induce dioxinlike and estrogenic responses in three cell lines. Environ. Toxicol. 17, 128–137.

Vrabie, C.M., Sinnige, T.L., Murk, A.J., Jonker, M.T.O., 2012. Effect-directed assessment of the bioaccumulation potential and chemical nature of A h receptor agonists in crude and refined oils. Environ. Sci. Technol. 46, 1572–1580.

Yang, X., Wang, F., Gu, C., Jiang, X., 2010. Tenax TA extraction to assess the bioavailability of DDTs in cotton field soils. J. Hazard. Mater. 179, 676–683.

Yim, U.H., Ha, S.Y., An, J.G., Won, J.H., Han, G.M., Hong, S.H., Kim, M., Jung, J.-H., Shim, W.J., 2011. Fingerprint and weathering characteristics of stranded oils after the Hebei Spirit oil spill. J. Hazard. Mater. 197, 60–69.

Yim, U.H., Kim, M., Ha, S.Y., Kim, S., Shim, W.J., 2012. Oil spill environmental forensics: the Hebei Spirit oil spill case. Environ. Sci. Technol. 46, 6431–6437.

Yi, X., Li, H., Ma, P., You, J., 2015. Identifying the causes of sediment-associated toxicity in urban waterways in South China: Incorporating bioavailability-based measurements into whole-sediment toxicity identification evaluation. Environ. Toxicol. Chem. 34, 1744–1750.

You, J., Harwood, A.D., Li, H., Lydy, M.J., 2011. Chemical techniques for assessing bioavailability of sediment-associated contaminants: SPME versus Tenax extraction. J. Environ. Monit. 13, 792–800.

You, J., Landrum, P.F., Lydy, M.J., 2006. Comparison of chemical approaches for assessing bioavailability of sediment-associated contaminants. Environ. Sci. Technol. 40, 6348–6353.

You, J., Landrum, P.F., Trimble, T.A., Lydy, M.J., 2007. Availability of polychlorinated biphenyls in field-contaminated sediment. Environ. Toxicol. Chem. 26, 1940–1948.

<Supplemental Materials>

Bioaccessibility of AhR-active PAHs in sediments contaminated by the *Hebei Spirit* oil spill: Application of Tenax extraction in effect-directed analysis

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Table of Contents

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Table S1. HPLC instrumental conditions for the fractionation of aromatics (F2).

HPLC system		Agilent 1200 Series
Column		Nucleosil 100-5 NO_2 (25 cm, 5 μm, 4.6 mm), Normal phase column
Mobile phase		Hexane:DCM (95:5. v/v), Isocratic elution
Flow rate		0.7 mL/min
Delay time		0.3 min
Injection volume		10 μL
Fraction collect	0 – 4 min	discard
	4 – 6.6 min (F2.1)	Naphthalene (Na), C1-Na, C2-Na, C3-Na, and C4-Na
	6.6 – 9.2 min (F2.2)	Acenaphthylene (Acl), Acenaphthene (Ace), Fluorene (Flu), C1-Flu, C2-Flu, C3-Flu, Dibenzothiophene (Dbthio), C1-Dbthio, C2-Dbthio, C3-Dbthio, Phenanthrene (Phe), C1-Phe, C2-Phe, C3-Phe, C4-Phe, and Anthracene (Ant)
	9.2 – 11.8 min (F2.3)	Fluoranthene (Fl) and Pyrene (Py)
	11.8 – 14.4 min (F2.4)	Benzo[*a*]anthracene (BaA), Chrysene (Chr), C1-Chr, C2-Chr, and C3-Chr
	14.4 – 19.6 min (F2.5)	Benzo[*b*]fluoranthene (BbF), Benzo[k]fluoranthene (BkF), Benzo[e]pyrene (BeP), and Benzo[a]pyrene (BaP),
	19.6 – 25 min (F2.6)	Indeno[*1,2,3-cd*]pyrene (IcdP), Dibenz[*a,h*]anthracene (DbahA), and Benzo[*g,h,i*]perylene (BghiP)

Table S2. GC/MSD instrumental conditions for the determination of PAHs and alkyl-PAHs.

GC/MSD system	Agilent 7890A GC and 5975C MSD
Column	DB-5MS (30 m long, 0.25 mm i.d., 0.25 μm film thickness)
Gas flow	1 mL/min He
Injection mode	Split 1 mL/min (ratio 1:1)
Injection volume	2 μL
MS temperature	175 °C
Detector temperature	280 °C
Oven temperature	60 °C hold 2 min Increase 6 °C/min to 300 °C 300 °C hold 13 min
Target PAHs and alkyl-PAHs	Naphthalene (Na), C1-Na, C2-Na, C3-Na, C4-Na, Acenaphthylene (Acl), Acenaphthene (Ace), Fluorene (Flu), C1-Flu, C2-Flu, C3-Flu, Dibenzothiophene (Dbthio), C1-Dbthio, C2-Dbthio, C3-Dbthio, Phenanthrene (Phe), C1-Phe, C2-Phe, C3-Phe, C4-Phe, Anthracene (Ant), Fluoranthene (Fl), Pyrene (Py), Benzo[*a*]anthracene (BaA), Chrysene (Chr), C1-Chr, C2-Chr, and C3-Chr Benzo[*b*]fluoranthene (BbF), Benzo[*k*]fluoranthene (BkF), Benzo[*e*]pyrene (BeP), Benzo[*a*]pyrene (BaP), Indeno[*1,2,3-cd*]pyrene (IcdP), Dibenz[*a,h*]anthracene (DbahA), and Benzo[*g,h,i*]perylene (BghiP)

Table S3. QA/QC for PAHs analysis determined using standard reference material of marine sediment (1941b, NIST, n = 3).

Compounds	Certified Conc. (μg kg^{-1})		Measured conc. (μg kg^{-1})		%RSD	%REC
	Mean	SD	Mean	SD		
Naphthalene	848	95	483	114	24	57
2-Naphthalene	276	53	161	26	16	58
1-Naphthalene	127	14	76	12	15	60
Acenaphthylene	53.3	6.4	48	3	6	90
Phenanthrene	406	44	380	19	5	94
Anthracene	184	18	153	9	6	83
2- Anthracene	36	15	38	2	6	104
1- Phenanthrene	73.2	5.9	60	2	4	81
Fluoranthene	651	50	681	30	4	105
Pyrene	581	39	560	25	5	96
Benzo[*a*]anthracene	335	25	328	11	3	98
Chrysene	291	31	281	12	4	97
Benzo[*b*]fluoranthene	225	18	221	10	4	98
Benzo[*e*]pyrene	325	25	296	13	4	91
Benzo[*a*]pyrene	358	17	325	11	3	91
Perylene	397	45	353	14	4	89
Indeno[*1,2,3-cd*]pyrene	341	57	336	20	6	98
Benzo[*g,h,i*]perylene	307	45	304	23	7	99

Table S4. Relative potency values for AhR-mediated activity of PAHs and alkyl-PAHs reported previously.

Analytes	ReP values	References
2,3,7,8-TCDD	1.0	Hong et al., 2015
C4-Penanthrene	1.0×10^{-5}	
Dibenzothiophene	2.5×10^{-7}	
Chrysene	2.0×10^{-5}	
C1-Chrysene	1.0×10^{-4}	
C2-Chrysene	2.0×10^{-4}	
C3-Chrysene	3.5×10^{-7}	
C4-Chrysene	5.0×10^{-5}	
Benz[*a*]anthracene	1.9×10^{-6}	Villeneuve et al., 2002
Benzo[*b*]fluoranthene	5.1×10^{-6}	
Benzo[*k*]fluoranthene	1.4×10^{-4}	
Benzo[*a*]pyrene	1.6×10^{-6}	
Indeno[*1,2,3-c,d*]pyrene	1.5×10^{-5}	
Dibenz[*a,h*]anthracene	4.6×10^{-6}	

Table S5. Concentrations of PAHs and alkyl-PAHs in Soxhlet and Tenax extracts of oil-coated sand used in the present study (unit: μg kg^{-1} dry mass (dm)).

Analytes	Soxhlet extracts (n = 3)		Tenax extracts (n = 3)[a]		$C_{Tenax}/C_{Soxhlet}$ (%)
	Mean	SD	Mean	SD	
Naphthalene	11.0	0.62	10.5	0.47	95
C1-Naphthalene	683	45	352	21	52
C2-Naphthalene	3070	240	703	50	23
C3-Naphthalene	3860	310	580	37	15
C4-Naphthalene	2720	230	187	24	6.9
Acenaphthene	11.9	1.5	4.17	0.73	35
Fluorene	64.3	5.7	25.8	1.4	40
C1-Fluorene	393	37	112	11	28
C2-Fluorene	397	29	71.4	14	18
C3-Fluorene	460	52	66.7	4.1	15
Dibenzothiophene	327	30	118	9.2	36
C1-Dibenzothiophene	1450	99	321	41	22
C2-Dibenzothiophene	2160	140	492	82	23
C3-Dibenzothiophene	1870	120	202	15	11
Phenanthrene	158	13	53.3	7.6	34
C1-Phenanthrene	300	22	39.0	5.1	13
C2-Phenanthrene	395	26	34.4	4.2	8.7
C3-Phenanthrene	267	11	15.9	3.2	5.9
C4-Phenanthrene	164	20	5.59	0.99	3.4
Pyrene	13.3	0.59	2.85	0.66	21
Chrysene	29.3	2.1	6.80	1.7	23
C1-Chrysene	72.7	2.9	11.0	0.33	15
C2-Chrysene	103	4.8	4.63	0.86	4.5
C3-Chrysene	100	6.3	2.73	0.59	2.7
Sum of 24 PAHs	19100	1400	3420	280	18

[a] Extraction for 24h with 2 g of oil-coated sand, 0.5 g of Tenax, 20 mg of $HgCl_2$, 0.4 g of Cu, and 35 mL of DW.

Table S6. Concentrations of PAHs and alkyl-PAHs in Soxhlet and Tenax extracts of oil contaminated sediments (unit: μg kg^{-1} dm).

Analytes	Soxhlet extracts			Tenax extracts		
	Gureumpo	Shinduri	Euihangri	Gureumpo	Shinduri	Euihangri
Naphthalene	13.3	7.75	11.7	8.40	2.19	3.32
C1-Naphthalene	122	18.7	15.1	37.6	1.48	3.07
C2-Naphthalene	2980	82.3	19.9	600	4.92	4.51
C3-Naphthalene	8890	950	27.7	1450	36.4	5.07
C4-Naphthalene	7410	3230	18.2	1080	138	3.30
Acenaphthylene	1.73	2.77	0.42	0.19	0.16	0.19
Acenaphthene	16.5	89.1	0.98	3.58	6.23	0.43
Fluorene	121	10.9	3.15	17.5	0.87	1.19
C1-Fluorene	551	46.7	2.54	70.6	14.0	2.16
C2-Fluorene	1310	330	2.94	154	10.7	1.93
C3-Fluorene	1670	965	3.39	202	17.0	2.65
Dibenzothiophene	776	37.3	0.62	82.3	2.21	0.42
C1-Dibenzothiophene	3570	19.1	1.83	344	7.48	1.61
C2-Dibenzothiophene	6940	2910	2.93	644	41.8	1.36
C3-Dibenzothiophene	7710	7380	6.68	599	69.6	0.78
Phenanthrene	375	29.2	7.96	50.2	3.13	3.42
C1-Phenanthrene	1900	40.2	6.45	242	5.25	3.18
C2-Phenanthrene	3250	1150	3.68	393	34.8	2.85
C3-Phenanthrene	2550	2370	4.64	254	45.4	2.13
C4-Phenanthrene	1450	1910	6.62	141	21.0	2.75
Anthracene	29.9	2.08	0.30	-	-	0.21
Fluoranthene	8.08	21.8	9.80	1.26	1.35	1.26
Pyrene	40.2	57.5	11.6	5.70	2.75	1.10
Benz[*a*]anthracene	8.13	18.5	2.35	1.51	0.63	-
Chrysene	141	215	5.86	4.77	0.11	2.28
C1-Chrysene	353	529	4.19	34.6	5.56	1.19
C2-Chrysene	488	714	3.39	39.2	5.19	1.72
C3-Chrysene	529	764	3.88	39.1	4.41	1.26
Benzo[*b*]fluoranthene	7.88	25.5	5.47	0.79	0.55	0.53
Benzo[*k*]fluoranthene	1.17	3.85	2.24	-	0.13	-
Benzo[*e*]pyrene	17.5	38.9	3.34	3.52	0.72	0.42
Benzo[*a*]pyrene	5.05	9.72	2.25	1.09	0.14	-
Indeno[*1,2,3-cd*]pyrene	1.77	10.0	3.13	-	-	-
Dibenz[*a,h*]anthracene	1.37	4.04	0.52	-	-	-
Benzo[*ghi*]perylene	5.58	16.6	3.00	0.74	-	-
Sum of 36 PAHs	53200	24000	209	6520	484	56.3

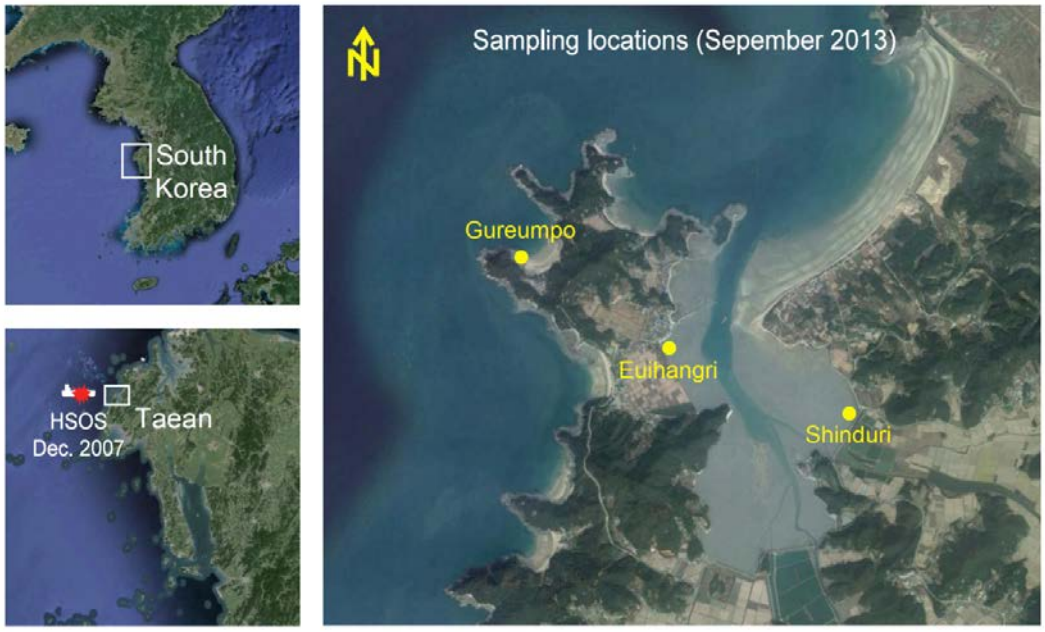

Fig. S1. Map showing the sampling sites of surface sediments from Taean study area (September 2013).

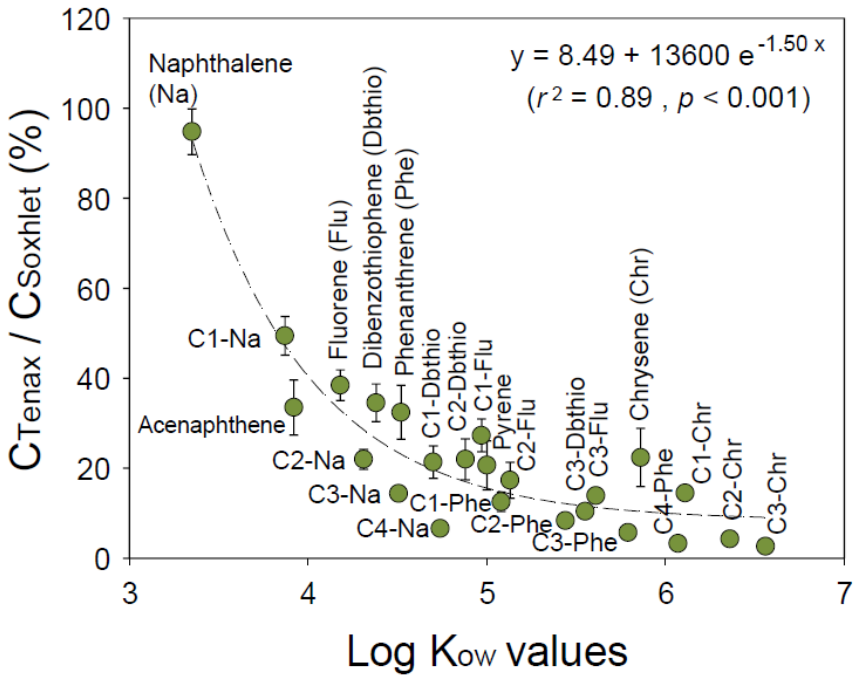

Fig. S2. Scatter plot for percentage of concentrations of PAHs and alkyl-PAHs of Tenax per Soxhlet (C_{Tenax} / $C_{Soxhlet}$) vs. log K_{ow} values (Error bar: mean ± SD; n = 3). The log K_{ow} values of PAHs and alkyl-PAHs were referred from Kang et al. (2014).

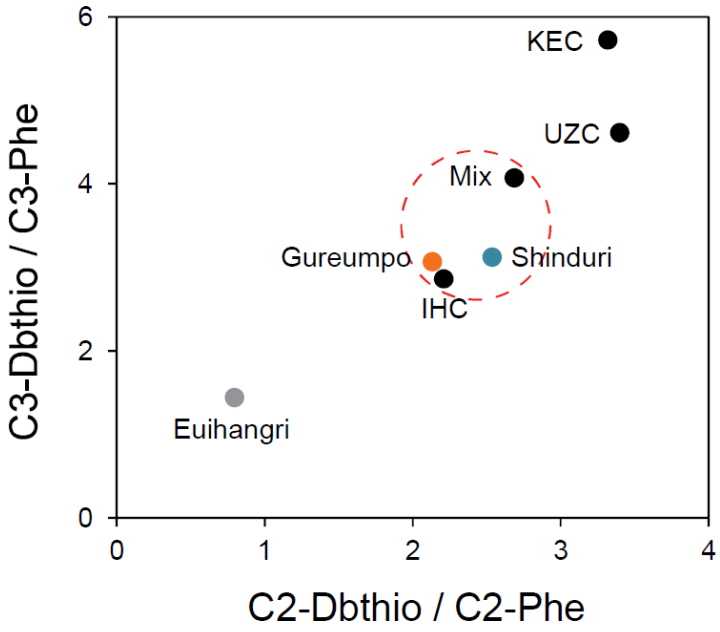

Fig. S3. Double ratio plots using alkylated phenanthrenes (C2- and C3-Phe) and dibenzothiophenes (C2- and C3-Dbthio) for source identifications of oil-contaminated sediments and crude oil (IHC: Iranian Heavy Crude, KEC: Kuwait Export Crude, UZC: UAE Upper Zakum, and Mix: mixture of IHC, KEC, and UZC) (refer from Yim et al. (2011)).

References

Hong, S., Lee, S., Choi, K., Kim, G.B., Ha, S.Y., Kwon, B.-O., Ryu, J., Yim, U.H., Shim, W.J., Jung, J., Giesy, J.P., Khim, J.S., 2015. Effect-directed analysis and mixture effects of AhR-active PAHs in crude oil and in coastal sediments contaminated by the *Hebei Spirit* oil spill. Environ. Pollut. 199, 110-118.

Kang, H.-J., Lee, S.Y., Roh, J.-Y., Yim, U.H., Shim, W.J., Kwon, J.-H., 2014. Prediction of ecotoxicity of heavy crude oil: Contribution of measured components. Environ. Sci. Technol. 48, 2962-2970.

Villeneuve, D.L., Khim, J.S., Kannan, K., Giesy, J.P., 2002. Relative potencies of individual polycyclic aromatic hydrocarbons to induce dioxinlike and estrogenic responses in three cell lines. Environ. Toxicol. 17, 128-137.

Yim, U.H., Ha, S.Y., An, J.G., Won, J.H., Han, G.M., Hong, S.H., Kim, M., Jung, J.-H., Shim, W.J., 2011. Fingerprint and weathering characteristics of stranded oils after the Hebei Spirit oil spill. J. Hazard. Mater. 197, 60-69.