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Two Years after the *Hebei Spirit* Oil Spill: Residual Crude-Derived Hydrocarbons and Potential AhR-Mediated Activities in Coastal Sediments

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S *Supporting Information*

ABSTRACT: The *Hebei Spirit* oil spill occurred in December 2007 approximately 10 km off the coast of Taean, South Korea, on the Yellow Sea. However, the exposure and potential effects remain largely unknown. A total of 50 surface and subsurface sediment samples were collected from 22 sampling locations at the spill site in order to determine the concentration, distribution, composition of residual crudes, and to evaluate the potential ecological risk after two years of oil exposure. Samples were extracted and analyzed for 16 polycyclic aromatic hydrocarbons (PAHs), 20 alkyl-PAHs, 15 aliphatic hydrocarbons, and total petroleum hydrocarbons using GC-MSD. AhR-mediated activity associated with organic sediment extracts was screened using the H4IIE-*luc* cell bioassay. The response of the benthic invertebrate community was assessed by mapping the macrobenthic fauna. Elevated concentrations of residual crudes from the oil spill were primarily found in muddy bottoms, particularly in subsurface layers. In general, the bioassay results were consistent with the chemistry data in a dose-dependent manner, although the mass-balance was incomplete. More weathered samples containing greater fractions of alkylated PAHs exhibited greater AhR activity, due to the occurrence of recalcitrant AhR agonists present in residual oils. The macrobenthic population distribution exhibits signs of species-specific tolerances and/or recolonization of certain species such as *Batillaria* during weathering periods. Although the *Hebei Spirit* oil spill was a severe oil exposure, it appears the site is recovering two years later.

Two years after the *Hebei Spirit* oil spill, Taean, South Korea
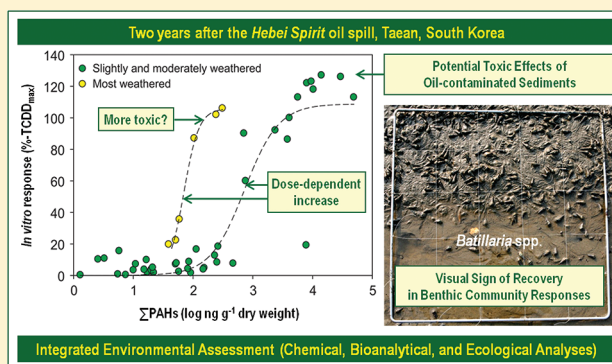
INTRODUCTION

In December 2007, the *M/V Hebei Spirit*, which was carrying 260 000 tons of crude oil, collided with a crane barge on the
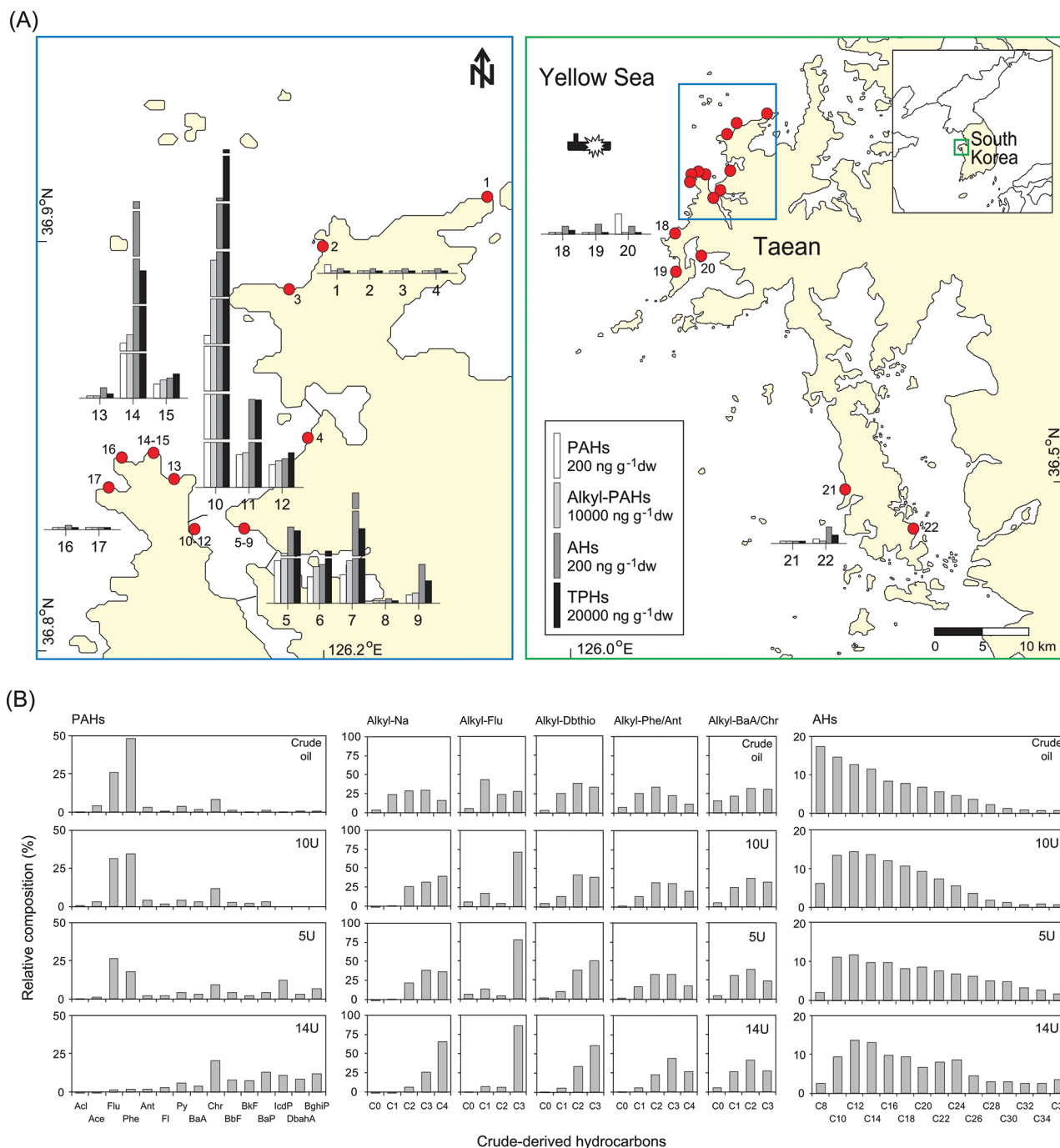
Figure 1. Results of chemical analysis. (A) Sampling sites and distribution of crude-derived hydrocarbons in surface sediment from Taean study area, Korea. (B) Relative composition (%) of PAHs, alkyl-PAHs, and AHs in selected sediments (surface sediments from sites 5, 10, and 14) and crude oil (Iranian heavy).

Yellow Sea approximately 10 km off the coast of Taean, South Korea, and spilled ~10 800 tons of oil.[1] The spill contained a mixture of UAE Upper Zakum, Kuwait export crude, and Iranian heavy crude and extended along >70 km of the Taean shoreline.[2] Most of the oil released in this accident was Iranian heavy crude primarily consisting of aliphatic/aromatic hydrocarbons and polar compounds as well as heavy metals and some volatile organic compounds.[3,4]

As crude oil is dispersed in the environment, it undergoes dissipation and degradation through a process known as "weathering." Weathering encompasses a variety of physical and biochemical changes such as evaporation, photo-oxidation,

solubilization, alkylation, and microbial degradation over periods of days, months, or many years.[5−7] Under environmental conditions, the concentration of spilled oil is gradually reduced by these processes. However, some higher molecular weight (HMW) compounds are more resistant to weathering and persist in sediments, which can cause long-term adverse effects on the aquatic environment.[8,9] The rate of crude oil weathering generally tends to increase with exposure time, although the rates of dissipation and transformation are site-specific[10] due to regional or temporal differences in wind, waves, tidal flushing, strength of currents, and microbial activities.[11,12] Sedimentary compositions of residual crude-

derived hydrocarbons such as polycyclic aromatic hydrocarbons (PAHs), alkyl-PAHs, and aliphatic hydrocarbons (AHs) change during weathering.[9,10,13]

Crude oils have been reported to cause short- and long-term toxic effects in marine organisms and humans.[8,14] PAHs are common constituents of crude oil and are one of the major contributors to the toxicity of crude oils, and can produce mutagenic, carcinogenic, and teratogenic effects.[15] HMW PAHs such as pyrene (Py), chrysene (Chr), and benzo[*a*]anthracene (BaA) are relatively strong agonists of the aryl hydrocarbon receptor (AhR).[16,17]

Because they integrate the potential toxic effects of complex mixtures, in vitro bioassays are used to assess the potential toxicity of chemicals present in environmental samples such as sediments. Bioanalytical screening tools are useful because they are simple, rapid, inexpensive, and provide a direct measurement in units of biological activity.[18−20] In particular, the rat hepatoma H4IIE-*luc* cell containing a luciferase reporter gene is used to measure the overall AhR-mediated activity induced by environmental dioxin-like chemicals such as polychlorinated dibenzo-*p*-dioxins, dibenzofurans (PCDD/Fs), polychlorinated biphenyls (PCBs), and PAHs in sediment extracts.[18−20]

We determined the concentration, distribution, and composition of residual hydrocarbons in sediments along the Taean coastal area two years after the *Hebei Spirit* oil spill. Weathering of residual crudes in sediment was investigated by examining patterns of concentration in alkyl-PAH homologues. The potential toxic effects of residual crudes were determined by use of the in vitro H4IIE-*luc* bioassay. Toxicity was further characterized through a combination of instrumental and bioanalytical measurements which included mass balance analysis. The macrobenthic communities of the intertidal areas were analyzed using the habitat mapping technique, which facilitates the understanding of community level responses to oil spills. This combined effort is sometimes referred to as the sediment triad approach to integrated environmental assessment, including chemical, bioanalytical, and ecological investigations.

■ MATERIALS AND METHODS

Sampling. A total of 50 surface and subsurface sediments from 22 sites along the coast of Taean were collected by use of acrylic core linears (30 cm long and 10 cm ID) in December 2009 (Figure 1A; for details see Table S1 of Supporting Information). The upper 10 cm of surface sediments were collected from all sites. At 14 locations, subsurface sediments were also collected to a depth of 30 cm and sectioned at 10 cm intervals (0−10 cm: upper (U); 10−20 cm: middle (M); 20−30 cm: lower (L)). All samples were immediately transferred to the laboratory and stored at −20 °C until analysis.

Sample Preparation. A detailed description of sample preparation procedures can be found in previous publications.[18,21,22] In brief, a 20-g sample of freeze-dried sediment was extracted with 400 mL of dichloromethane (DCM, Burdick and Jackson, Muskegon, MI, U.S.) in a Soxhlet extractor for 24 h. Elemental sulfur was removed by reaction with activated copper (Merck, Darmstadt, Germany) and the extracts were concentrated to 1 mL under a gentle stream of nitrogen on a heating block at 30 °C. The extract was divided into two equal portions for chemical analysis and bioassay. The solvent in one portion was replaced with dimethyl sulfoxide (DMSO, Burdick and Jackson) for bioassay and the other portion was passed through a 5 g silica gel (70−230 mesh, Merck) column and

eluted with 100 mL of hexane (Burdick and Jackson):DCM (80:20, v/v) for instrumental quantification. To compare the aliphatic and aromatic hydrocarbon composition in the sediment with the original crude oil, a 30 mg sample of Iranian heavy crude was prepared in the same manner.

Instrumental Analysis. Concentrations of PAHs, alkyl-PAHs, AHs, and total petroleum hydrocarbons (TPHs) were measured using an Agilent 7890 gas chromatograph (GC) coupled to a model 5975C mass-selective detector (MSD, Agilent technologies, Avondale, PA, U.S.). Detection limits for crude-derived hydrocarbons ranged from 0.1 to 0.5 ng g^{-1} dw for PAHs and alkyl-PAHs, 0.1 to 1.0 ng g^{-1} dw for AHs, and 100 ng g^{-1} dw for TPHs. Detailed information on target chemicals and instrumental conditions are presented in Table S2 and Figure S1 of the Supporting Information, SI.

In Vitro Bioassay. The H4IIE-*luc* cell bioassay was performed according to the modified method of Khim et al.[18] Trypsinized cells from a culture plate were diluted to a concentration of approximately 8.0×10^4 cells mL^{-1} and seeded into the 60 interior wells of 96 well micro plates at 250 μL per well. After overnight incubation, test and control wells were dosed with 2.5 μL per well (1% dose) of the appropriate standards, sample extracts, or solvent controls. For sample dose−response characterization, extracts were prepared at six concentrations using 3-fold serial dilution (100, 33.0, 11.0, 3.3, 1.1, and 0.3%). All samples were tested in triplicate wells in the same assay. Luciferase assays were conducted after 72 h of exposure using an ML3000 microplate reading luminometer (Dynatech Laboratories, Chantilly, U.S.). Cell viability and overall cytotoxicity of all samples were determined by use of the MTT assay described in detail elsewhere.[22]

Data Analysis. Bioassay responses expressed as mean relative luminescence units were converted to a percentage of the maximum response (%-TCDD$_{max}$) observed for a standard containing 40 nM (= 100%-TCDD$_{max}$) 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD, Wellington Laboratories inc., Guelph, OT, Canada). Significant responses (4.3%-TCDD$_{max}$) were defined as those resulting in a response three times as great as the standard deviation of the mean solvent control responses. Sample potencies expressed as TCDD standard equivalents (TCDD-EQs) were determined directly from sample dose−response relationships generated by testing samples at multiple (at least 3 points) of dilutions.[23] In order to account for uncertainty in the TCDD-EQ estimates caused by deviations of the sample dose−response curves from the TCDD standard curve (0.064, 0.32, 1.6, 8, and 40 nM TCDD), TCDD-EQs were calculated for a range of responses (TCDD-EQ$_{20−80}$) based on multiple-point estimates of relative potencies (REP$_{20−80}$). TCDD equivalents (TEQ) for dioxin-like PAHs (DL-PAHs) including BaA, Chr, BbF, BkF, BaP, IcdP, and DBahA were calculated using the toxic equivalency factor (TEF) described by Villeneuve et al.[24]

Macrobenthic Fauna Mapping. Abundances of macrobenthic species were determined by counting the number of observed individuals or burrows within five 50 × 50 cm areas at each sampling location. Individuals of some species (e.g., *Helice* spp., *Macrophthalmus japonicus*, *M. dilatatus*, and *Periserrula leucophryna*) were counted based on the number of active burrows with grazing and/or excavating prints near the burrow openings.[25] Sedimentary structures such as ripple marks and shell fragments were also noted to provide sedimentary and biological information to complement the ecological interpretation.

Table 1. Results for Instrumental Analysis of Crude-Derived Hydrocarbons in Sediments (Sites 5–15) and Their Potential Toxicity

		instrumental-based						magnitude-based[b]		potency-based[c]		
		PAHs	alkyl-PAHs	AHs	TPHs	DL-PAHs	TEQ_{PAHs}[a]	$TCDD_{max}$	$TCDD_{max}$ EQ	$TCDD$-EQ_{20}	$TCDD$-EQ_{50}	$TCDD$-EQ_{80}
site	depth	(ng g^{-1} dw)					(pg g^{-1} dw)	(%)	(pg g^{-1} dw)	(pg g^{-1} dw)		
5	U	1.9×10^{2}	1.0×10^{4}	3.3×10^{2}	3.1×10^{4}	7.3×10	6.8×10^{-1}	120	1.5×10^{3}	6.0×10^{2}	1.3×10^{3}	2.8×10^{3}
6	U	1.1×10^{2}	7.9×10^{3}	1.7×10^{2}	2.3×10^{4}	3.6×10	4.8×10^{-1}	120	1.9×10^{3}	1.6×10^{2}	3.4×10^{2}	7.3×10^{2}
7	U	1.3×10^{2}	9.4×10^{3}	4.7×10^{2}	3.2×10^{4}	4.7×10	5.0×10^{-1}	120	2.1×10^{3}	3.2×10^{2}	4.4×10^{2}	6.0×10^{2}
8	U	3.2	4.8×10	1.5×10	7.6×10^{2}	1.3	2.1×10^{-2}	7.2	2.3	$<10^{-2}$	$<10^{-2}$	$<10^{-2}$
	M	5.3	2.6×10^{2}	2.0×10	1.1×10^{3}	6.2×10^{-1}	2.8×10^{-2}	8.4	2.4	$<10^{-2}$	$<10^{-2}$	$<10^{-2}$
	L	8.1×10	3.7×10^{3}	1.5×10^{2}	1.2×10^{4}	5.4×10	6.4×10^{-1}	86	2.4×10^{2}	3.5×10	5.7×10	9.2×10
9	U	4.0×10	2.3×10^{3}	1.7×10^{2}	1.0×10^{4}	2.0×10	2.4×10^{-1}	92	3.3×10^{2}	3.2×10	5.5×10	9.6×10
10	U	6.5×10^{2}	4.8×10^{4}	1.2×10^{3}	1.4×10^{5}	1.4×10^{2}	1.7	110	1.1×10^{3}	3.4×10^{3}	5.2×10^{3}	8.1×10^{3}
11	U	1.5×10^{2}	7.7×10^{3}	3.8×10^{2}	3.1×10^{4}	9.6×10	1.3	19	4.6	$<10^{-2}$	$<10^{-2}$	$<10^{-2}$
	M	2.8×10^{2}	2.9×10^{4}	1.0×10^{3}	1.0×10^{5}	1.3×10^{2}	1.3	130	2.5×10^{3}	3.5×10^{2}	7.9×10^{2}	1.8×10^{3}
	L	1.5×10	9.9×10	3.6×10	1.3×10^{3}	8.8	1.6×10^{-1}	17	3.9	$<10^{-2}$	$<10^{-2}$	$<10^{-2}$
12	U	1.0×10^{2}	5.6×10^{3}	1.2×10^{2}	1.5×10^{4}	6.8×10	8.5×10^{-1}	110	1.1×10^{3}	4.6×10^{2}	8.3×10^{2}	1.5×10^{3}
13	U	4.5	1.0×10^{2}	4.4×10	2.0×10^{3}	2.1	3.4×10^{-2}	87	2.6×10^{2}	2.6×10	3.8×10	5.5×10
14	U	2.4×10^{2}	1.4×10^{4}	8.5×10^{2}	5.5×10^{4}	1.8×10^{2}	2.6	130	2.4×10^{3}	9.1×10^{2}	1.0×10^{3}	1.1×10^{3}
15	U	6.4×10	4.0×10^{3}	8.9×10	1.1×10^{4}	4.4×10	4.7×10^{-1}	100	5.3×10^{2}	6.8×10^{2}	1.5×10^{2}	3.4×10^{2}
	M	1.1×10^{2}	6.0×10^{2}	9.5×10	3.8×10^{3}	6.9×10	9.3×10^{-1}	90	2.9×10^{2}	2.0×10^{2}	4.18×10	8.8×10
	L	4.0×10	7.2×10^{2}	8.7×10	3.5×10^{3}	2.3×10	3.5×10^{-1}	60	5.0×10	1.9×10	2.3×10	2.8×10

[a] Instrumentally derived TCDD equivalents of PAHs associated with sediment samples. For TEQ_{PAHs} calculation, concentrations of BaA, Chr, BbF, BkF, BaP, IcdP, and DbahA were used (refer from Villeneuve et al.[24]). [b] Response magnitude presented as percentage of the maximum response observed for a 40 nM TCDD standard (set to 100%-$TCDD_{max}$) elicited by 100% sediment raw extracts. [c] Potency-based TCDD-EQs (TCDD-$EQ_{20-50-80}$) were obtained from sample dose−response relationships generated by testing samples at multiple levels of dilution. TCDD-$EQ_{20-50-80}$ refer to the TCDD-EQs generated from multiple point estimate for responses to 20, 50, and 80%- $TCDD_{max}$.

■ RESULTS AND DISCUSSION

Distribution and Concentration of Crude-Derived Hydrocarbons. Nearly all sediments from locations along the Taean coast contained detectable concentrations of residual crude-derived hydrocarbons, with the greatest concentrations occurring near Sinduri beach (sites 5–9), Gaemok harbor (sites 10–12), and Naetaebae (sites 14–15) (Figure 1A). Surface sediment from site 10 contained the greatest concentrations of PAHs (6.5×10^{2} ng g^{-1} dw), alkyl-PAHs (4.8×10^{4} ng g^{-1} dw), AHs (1.2×10^{3} ng g^{-1} dw), and TPHs (1.4×10^{5} ng g^{-1} dw) (sites 5–15 in Table 1, sites 1–4 and sites 16–22 in Table S3 of the SI). Lower concentrations of TPH were found in the northern (sites 1–3) and far southern portions of Taean (sites 21–22), which are situated further from the oil spill site (Figure 1A).

Concentrations of several target hydrocarbons in sediment were compared to existing corresponding sediment quality guidelines (ERL: effects range low; ERM: effects range median)[26,27] and presented in Figure S2 of the SI. Acenaphthene (Ace) exceeded the ERL value (16 ng g^{-1} dw) in surface samples from site 1 and subsurface sediments from site 10. Concentrations of fluoranthene (Flu) in sediments exceeded the ERL value (19 ng g^{-1} dw) at sites 5, 6, 7, 10, and 11. Among alkyl-PAHs, C2-naphthalene (Na), C1-dibenzothiophene (Dbthio), and C2-phenanthrene+anthracene (Phe+Ant) exceeded the ERL values (C2−Na: 150 ng g^{-1} dw; C1-Dbthio: 85 ng g^{-1} dw; C2-Phe+Ant: 200 ng g^{-1} dw) at Sinduri beach (sites 5–9), Gaemok harbor (sites 10–12), and Naetaebae (sites 13–15), and exceeded ERM values (C2−Na: 1450 ng g^{-1} dw; C1-Dbthio: 600 ng g^{-1} dw; C2-Phe+Ant: 2500 ng g^{-1} dw) in surface sediments from site 10. Total concentrations of PAHs in surface and subsurface sediments exceeded the ERL value (4022 ng g^{-1} dw) in 18% (9 samples) of all samples with the greatest concentration at site 10 (4.9×10^{4} ng g^{-1} dw). Although the mean concentrations of most hydrocarbon residues were less than the suggested sediment quality guidelines, their concentrations in some locations were near or exceeded the ERM, potentially causing toxic effects on benthic organisms.

The spatial distribution of hydrocarbon residues in the Taean sediments revealed greater concentrations in the interior of small bays and in muddy bottom locations such as Sinduri beach, Gaemok harbor, and Naetaebae (Table S1 of the SI). Sediments from locations in which the bottom was predominantly composed of muck (such as sites 8, 11, 18, and 20) accumulated greater concentrations of crude-derived hydrocarbons in the subsurface sediments relative to surface sediments (Figure S3 of the SI). The relatively great concentrations of TPH in samples from semiclosed areas and the significantly greater concentrations of PAH in subsurface sediments could be explained by the lack of flushing of oil residues under these low-energy conditions. Concentrations of hydrocarbons measured at some locations were consistent with penetration to deeper layers, mobility in sediment layers, and accessibility of remaining subsurface oil.[8,9]

Concentrations of PAHs, alkyl-PAHs, and AHs detected in Taean sediments accounted for 2–40% of TPHs by weight. Unidentified toxic substances, such as unverified PAHs, alkylated PAHs, alkylated phenols, and organic sulfur compounds are suspected to occur in crude oils.[28,29] Certain of these unidentified chemicals can be toxic to benthic organisms and humans following long-term exposure. In addition, deeply buried oil appeared to be resistant to weathering and could cause long-term biological effects.[30]

Weathering Characteristics of Residual Crudes. The compositions of crude-derived hydrocarbons including PAHs, alkyl-PAHs, and AHs in coastal sediments and crude oil
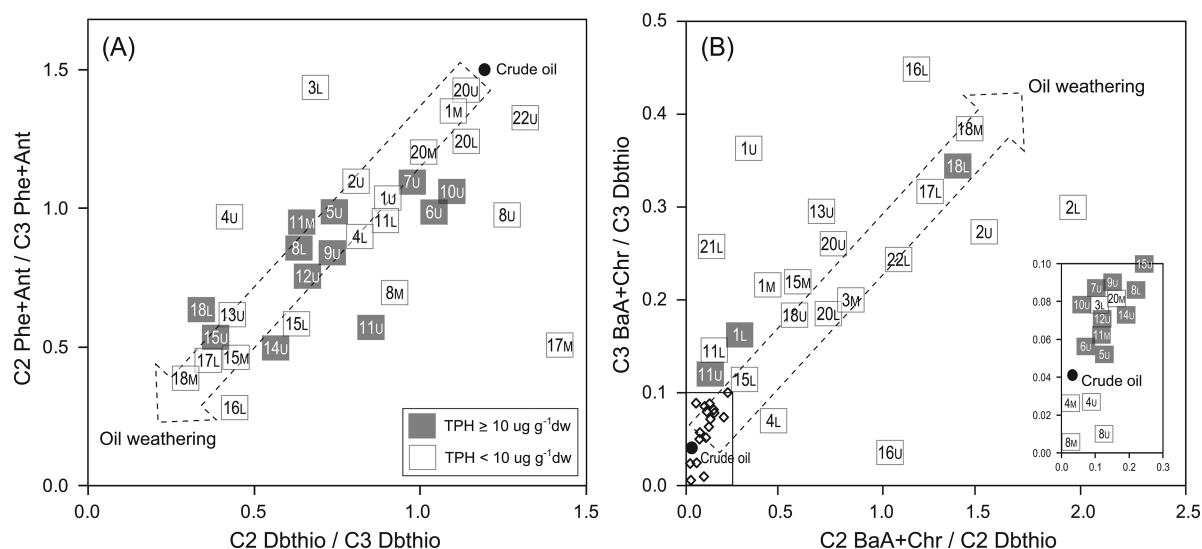
Figure 2. Weathering characteristics of residual crudes. (A) C2-Dbthio/C3-Dbthio and C2-Phe+Ant/C3-Phe+Ant double ratios, and (B) BaA+Chr/C2-Dbthio and C3-BaA+Chr/C3-Dbthio double ratios of all sediment samples (modified from Sauer et al.[10] and Michel and Hayes[13]).

samples (Iranian heavy crude) were compared (Figure 1B). Surface sediments from sites 5, 10, and 14 were selected for comparison because they contained the greatest concentrations of hydrocarbons. Na was excluded from composition analysis because it accounted for ~62% of the total of 16 PAHs in crude oil but represented <1% in sediment samples due to its volatility.[6] Low molecular weight (LMW) PAHs containing ≤3 benzene rings comprised ~80% of the crude oil. However, LMW-PAHs represented a smaller fraction of samples from coastal sediments, including 70% at site 10, 48% at site 5, and 5.6% at site14. PAHs at site 14 were predominantly high molecular weight species (4−6 rings), which suggests greater weathering occurred at site 14 (outer region) than sites 5 or 10 (inner region). These results are consistent with previous reports which indicated that the composition of PAHs shifts during weathering processes.[10]

The distribution of alkyl homologues depends on the degree of alkylation during weathering, and might be used to assess weathering of oil residues in sediments.[10] The alkyl-Na content in crude oil exhibited a bell-shaped distribution (C3=C2 > C1 > C4 > C0−Na), and the fraction of C3 and C4 Na species gradually increased from the inner (C3 > C4 > C2 > C1 > C0−Na) to the outer regions (C4 > C3 > C2 > C1 > C0−Na). The trends were similar for alkylated Flu, Dbthio, and Phe+Ant compounds, whereas alkylated BaA+Chr displayed a somewhat different distribution. Alkylation of PAHs during weathering was greater for two to three ring PAHs such as Na, Flu, Dbthio, and Phe+Ant than four ring PAHs such as BaA+Chr. The relative concentrations of individual AHs (C8−C36, even number of carbons) in Iranian heavy crude exhibited a general pattern of abundance of *n*-alkanes being inversely proportional to the total number of carbon atoms. The fractions of C8 and C10 compounds at sites 5, 10, and 14 were less than in crude oil, possibly due to weathering processes such as degradation, volatilization, and dispersion. In particular, C22 to C26 hydrocarbons at site14 were more abundant among AHs. This could result from their greater resistance to weathering and/or biodegradation. Therefore, the composition of PAHs, alkyl-PAHs, and AHs in coastal sediments could be used to identify the degree of weathering, with each site exhibiting a specific pattern two years after the oil spill.

The degree of weathering of residual crude oil surface and subsurface sediments was estimated from C2-Dbthio/C3-Dbthio and C2-Phe/C3-Phe double ratios and C2-BaA+Chr/C2-Dbthio and C3-BaA+Chr/C3-Dbthio double ratios by use of a procedure adapted from Sauer et al.[10] and Michel and Hayes[13] (Figure 2). Overall, the two methods for determining the degree of oil weathering in sediments yielded similar results, although some less contaminated samples including 1M (middle layer), 4M, 20U (upper layer), and 20L (lower layer) displayed differences in the degree of weathering between methods. This could be due to masking effects from background noise.[13] Samples 5U, 6U, 7U, 10U, and 11U from Sinduri beach and Gaemok harbor were slightly weathered, 14U, 15U, 15M, and 15L from Naetaebae were moderately weathered, and 18M, 17L, 16L, 13U, and 18L were most weathered. Understanding the degree of weathering is important in assessing persistency and toxicity of crude oil residues during long-term monitoring and environmental management of oil spill-affected areas.

AhR-Mediated Activity in Sediment Extracts. In vitro H4IIE-*luc* bioassays were conducted to assess the potential AhR-mediated potency of residual crude oils in sediments (sites 5−15 in Table 1, sites 1−4 and 16−22 in Table S3 of the SI). No cytotoxic effects were observed in H4IIE cells during exposure to sediment extracts in all samples checked with MTT bioassay (>80% cell survival). The responses varied between locations, with values ranging from <4.3 to 130%-TCDD$_{max}$. Half (sites 5−15 and 18) of the 22 locations exhibited greater responses (>80%-TCDD$_{max}$) to organic sediment extracts. In those higher response samples, the responses appeared to be due to the greater concentrations of known target chemicals. Subsurface sediments at sites 8, 11, and 18 displayed greater bioassay responses than did surface sediments, which was consistent with chemical analyses which showed greater oil concentrations in the deeper sediments.

To fully evaluate the potency of AhR-active compounds present in the sediment extracts, bioassay-derived TCDD-EQs were estimated directly from the dose−response curves for the samples and for the TCDD standard (Table 1, Table S3 of the SI). The greatest concentrations of TCDD-EQ$_{50}$ were located in sediments from site 10 (5.2×10^3 pg g^{-1} dw, TCDD-EQ$_{20−80}$:
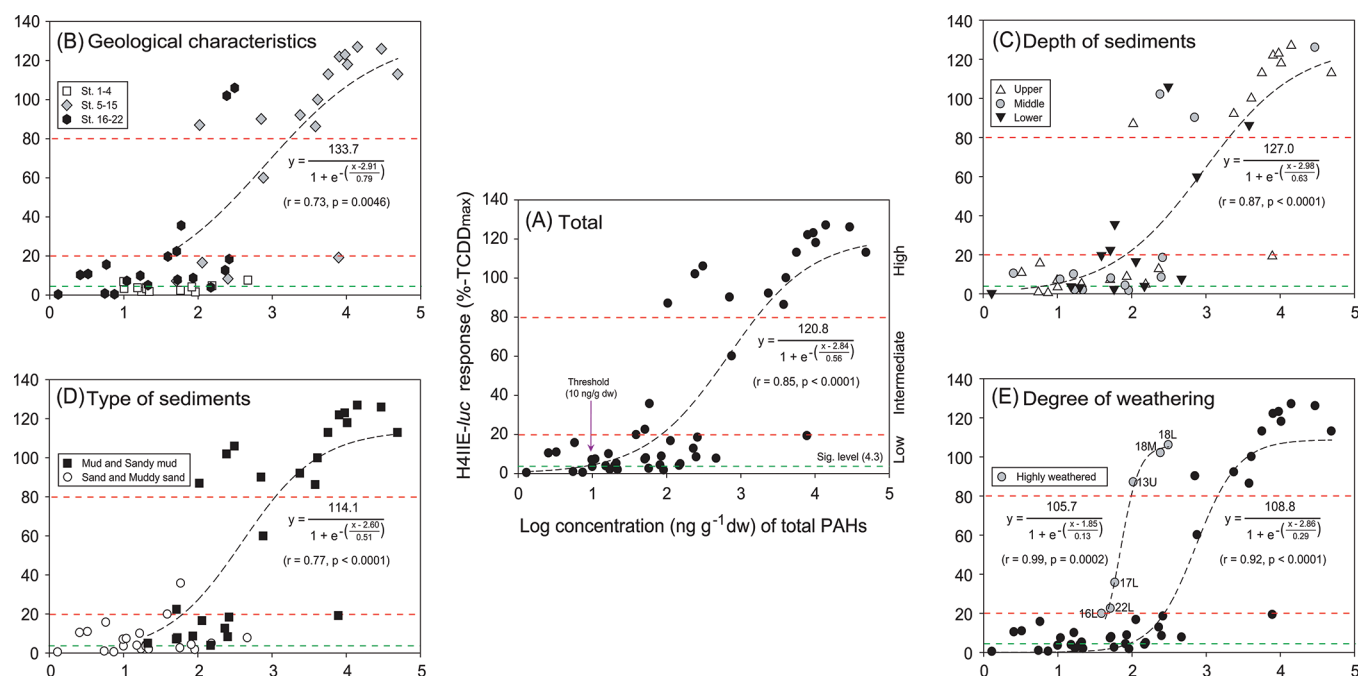
Figure 3. Scatter plots indicating dose−response relationships between concentration of total PAHs (log concentration) and H4IIE-*luc* response (%-TCDD$_{max}$) for all sediment samples. (A) total, (B) geological characteristics, (C) depth of sediment, (D) type of sediments, and (E) degree of weathering.

3.4×10³−8.1×10³ pg g⁻¹ dw), followed by site 5 (1.3×10³ pg g⁻¹ dw, TCDD-EQ$_{20-80}$: 6.0×10²−2.8×10³ pg g⁻¹ dw) and site 14 (1010 pg g⁻¹ dw, TCDD-EQ$_{20-80}$: 9.1×10²−1.1×10³ pg g⁻¹ dw). However, when a mass balance was conducted, the TCDD-EQs contributed by the DL-PAHs accounted for between 0 and 34% of the TCDD-EQ value, indicating the presence of additional unidentified AhR-active compounds present in the sediments.

The bioassay results suggest that sediments from the oil spill area contain large amounts of dioxin-like compounds, in addition to endocrine-disrupting and genotoxic compounds as recently reported.[31,32] There is a need for long-term follow-up studies of the potential toxicity of residual crude oil in sediments in order to protect wildlife and human health. The present study was one of the first few efforts that applied a combined use of both instrumental and bioanalytical assessment to evaluate the potential toxic effects of oil-contaminated sediments.

AhR-Mediated Activity Characterization. In order to characterize the agents responsible for the observed TCDD-EQs determined using the H4IIE-*luc* assay, the mathematical relationship between concentrations and TCDD-EQ were investigated by use of the sigmoid model equation (SigmaPlot 2001 for Windows Version 7.0, SPSS inc., Chicago, IL, U.S.). The model for response (R) and concentration was calculated using eqs 1 and 2:

$$R = \frac{a}{1 + e^{-\left(\frac{c - c_0}{b}\right)}} \quad (1)$$

$$c = \log_{10} C \quad (2)$$

in which c_0, a, and b are nonlinear regression parameters for eq 1 and c is the logarithm of the total PAH concentration (C, eq 2). Total PAH concentrations were significantly correlated with responses of the H4IIE-*luc* bioassay (r^2 = 0.85, p <0.0001)

(Figure 3A). This relationship is consistent with the hypothesis that PAHs accounted for most of the observed TCDD-EQ. The relationships between total concentrations of PAH and %-TCDD$_{max}$ were classified according to geographical characteristics (Figure 3B), depth profile (Figure 3C), sediment facies (Figure 3D), and weathering stage (Figure 3E). Most of the H4IIE-*luc* responses (>80-% TCDD$_{max}$) were associated with total PAH concentrations in inner region samples, surface sediments, and muddy bottom sediments.

Meanwhile, several samples exhibited greater dioxin-like responses at relatively lesser PAHs concentrations (<500 ng g⁻¹ dw), of which data points showed notable deviations from the predicted concentration−response relationship (Figure 3A). By extracting those data points (18L, 18M, 13U, 17L, 22L, and 16L), which were all classified as highly weathered, another dose−effect relationship could be found (r^2 = 0.99, p < 0.0002) (Figure 3E). This finding was in contrast to earlier studies reporting that oil weathering generally tended to decrease toxicity.[7,14,33−35] However, in most previous studies, toxicities were evaluated by exposing various aquatic organisms to water-soluble fractions of crude oil such as WAF (water accommodated fraction), weathered oil, and/or oil residues in sediment. This test method has the advantage of providing information about the toxicity of bioavailable fractions.[36] However, few compounds with large K_{ow} values are extracted in the WAF technique, and concentrations of LMW chemicals were effectively reduced during weathering which could affect the results of the toxicity analysis.[35] In vitro toxicity assessments performed using organic sediment extracts (as was done in this study) can assess the overall toxic potency of the mixture, including both water-extractable compounds and particle-adsorbed AhR agonists in often sensitive and specific manner. Differences in toxicity between more and less weathered crude oils could be explained by the modified composition of chemicals with varying potency of AhR activity present in the

Table 2. Macrobenthic Fauna and Sedimentary Structures Visually Observed at Sampling Sites[a]

	macrobenthic fauna (ind./50 × 50 × 5 cm)									sedimentary structures		
sites	Helice spp[b]	Ilyoplax spp[b]	Macrophthalmus japonicus[b]	Macrophthalmus dilatatus[b]	Umbonium thomasi	Batillaria spp.	Lunatia fortunei	Asterina pectinifera	Periserrula leucophryna	total burrows	ripple[c]	shell[c]
1				2						5	4/5	5/5
2		2									1/5	3/5
3		2			1						3/5	2/5
4		1			46			2		2	0/5	3/5
5−9			1			205				94	0/5	2/5
10−12	1	2	13			77	1		1	68	0/5	0/5
14−15			5		5	308				32	0/5	3/5
16											0/5	0/5
17										3	0/5	0/5
18										13	0/5	3/5
19											0/5	0/5
20			7							144	0/5	1/5
21											5/5	1/5
22			2							22	0/5	3/5

[a]T13 data are not available. [b]Number of individuals was estimated from counting burrows identified as active (refer from Pandya and Vachhrajani[25]). [c]Number of corresponding cases among five photographs.

corresponding samples, seemingly due to chemical and/or biological transformation.[35]

Overall, the use of the H4IIE-*luc* cells has several advantages in assessment of potential toxicity of residual crudes in sediment. First, the H4IIE-*luc* assay is more sensitive to residual crudes. For example, the threshold value of H4IIE-*luc* assay was found to be 10 ng g^{-1} of total PAHs in this study, while that of amphipod mortality was 2600 ng g^{-1} of total PAHs in sediments following the *Exxon Valdez* oil spill in Prince William Sound, Alaska, U.S.[14] Also, in a parallel study,[31] a microbial (Microtox®); and an acute waterflea toxicity test were used, and only one sample each was determined to be toxic. Second, the use of H4IIE-*luc* assay provides much greater details of the mechanistic specificity of the contaminants under study, compared to the in vivo assessment. Third, the H4IIE-*luc* test characterizes the potential toxic effect for known or unknown, and bioavailable organic fractions in residual crudes. Finally, we reported bioassay data with multiple ranges of responses (viz. 20−50−80%-TCDD-EQs), of which values and range are reported as an estimate of the uncertainty in expressing sample potency. All together, the H4IIE-*luc* bioassay utilized in the present study was rapid, sensitive, inexpensive, and integrative,[24,37] thus a valuable tool for assessing the environmental status of oil spill-affected areas.

Macrobenthic Fauna Community. While macrobenthic populations were completely eradicated immediately after the *Hebei Spirit* oil spill, the organisms had almost completely recovered two years later.[38] This includes populations of crabs (*Helice* spp., *Ilyoplax* spp., *M. japonicus*, and *M. dilatatus*), gastropods (*Umbonium thomasi*, *Batillaria* spp., and *Lunatia fortunei*), sea stars (*Asterina pectinifera*), and polychaetes (*Periserrula leucophryna*). Despite the results of chemical and bioanalytical assessments, the macrofauna community exhibited relatively large diversity, abundance, and burrow activity at Sinduri beach, Gaemok harbor, and Naetaebae (Table 2). It is unlikely that the relatively great concentrations of residual crude oils in the sediments at these locations have affected the benthic community. For example, the deposit feeding gastropods *Batillaria* were present in high density in areas with elevated PAH concentrations but were not present in other locations. These species are widely distributed along the west

coast of Korea including this region and are known as deposit feeders, grazing the mudflat biofilm composed of microalgae, bacteria, and detritus.[39−41] It is likely that *Batillaria* recolonized the sites through immigration by tidal current and/or direct development from benthic egg capsules at the site following the oil spill.[42] It is unknown why *Batillaria* were dominant in the three most polluted sites, whether the mudflat regions are suitable for inhabitation, or if residual crude oil serves as an additional nutrient source based on which benthic microalgae flourish. It is evident that two years after the *Hebei Spirit* oil spill the macrobenthic communities have recovered.

Overall, our integrated environmental assessment (chemical, bioanalytical, and ecological) was successful in identifying high-risk regions, determining the relationship between toxicity and weathering, and quantifying community responses, and will provide useful information during long-term monitoring and management efforts in future oil spills.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information
Additional tables and figures as noted in the text. This material is available free of charge via the Internet at http://pubs.acs.org.

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