



Assessment of potential biological activities and distributions of endocrine-disrupting chemicals in sediments of the west coast of South Korea



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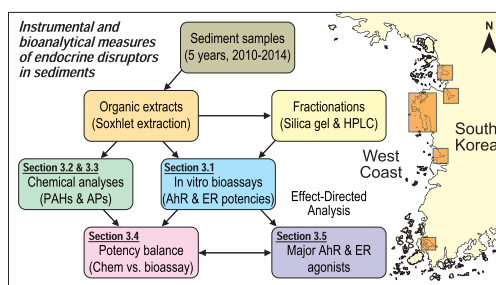
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HIGHLIGHTS

- AhR and ER potencies in sediments from the west coast of Korea were characterized.
- Bioassay results generally showed a low-to-moderate contamination of sediments.
- Distributions of PAHs and APs were generally affected by human activities.
- Five- and six-ring PAHs were the major AhR-active compounds in sediments.
- Known and unknown aromatics with log K_{ow} 5–8 are likely major AhR agonists.

GRAPHICAL ABSTRACT



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ABSTRACT

The west coast of Korea has experienced environmental deterioration for more than half a century. In the present study, we specifically aimed to: i) evaluate potential toxicities of contaminants in sediments that cause effects mediated through the aryl hydrocarbon receptor (AhR) and estrogen receptor (ER); ii) determine spatio-temporal distributions of polycyclic aromatic hydrocarbons (PAHs) and alkylphenols (APs); and iii) identify causes of greater potencies of samples. From 2010 to 2014, sediments were collected from 12 major estuarine and coastal regions along the west coast of South Korea. *In vitro* cell bioassays were performed to determine AhR- and ER-mediated potencies using H4IIE-*luc* and MVLN cells, respectively. Fifteen PAHs and six APs in sediments were identified by GC/MSD. Results of bioassays generally showed a low-to-moderate degree of contamination, however, greater AhR- and ER-mediated potencies were measured at some locations. Concentrations of PAHs and APs varied among locations, which indicated that sources were independently affected by the surrounding environment (e.g.,

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industrial complex and cities). Results of bioassays were generally well correlated with concentrations of putative causative chemicals. Benzo[*k*]fluoranthene, dibenz[*a,h*]anthracene, and benzo[*b*]fluoranthene were the major AhR agonists, explaining approximately 30% of the bioassay-derived benzo[*a*]pyrene equivalent concentration (BaP-EQ). Unknown AhR and ER agonists and potential mixture effects remain in question. Overall, the present study provides baseline information on chemical contaminations and potential toxicity of sediments in a fairly wide geographical region of the west coast of South Korea.

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1. Introduction

The Yellow Sea, together with nearby coastal and riverine areas, is a major commercial artery of East Asia that has been significantly urbanized and industrialized (Naile et al., 2011). Rapid social and economic development in the surrounding countries has brought economic development, however, it has also contributed to local contamination with persistent toxic substances (Hong et al., 2012a; Naile et al., 2011). Several major rivers, including the Han, Geum, and Yeongsan of South Korea, discharge directly into the Yellow Sea. Drainage basin of these rivers are used for both agricultural and chemical production, which can release inorganic and organic contaminants from both point- and nonpoint-sources (Hong et al., 2012a; Naile et al., 2011). Because the Yellow Sea is a semi-enclosed system, exchange of water with the Pacific Ocean is relatively slow. As a result, organic and inorganic contaminants tend to accumulate in coastal sediments (Hong et al., 2012a).

Polycyclic aromatic hydrocarbons (PAHs) and alkylphenols (APs) have previously been reported to be widely distributed in sediments and have been identified as prominent contaminants along the west coast of South Korea (Hong et al., 2012a; Khim and Hong, 2014; Naile et al., 2011). PAHs are composed of two or more fused aromatic rings and are listed as priority pollutants by the US Environmental Protection Agency. PAHs are emitted into the atmosphere mainly from anthropogenic sources such as vehicles (Jo and Lee, 2009; Marr et al., 1999) and industrial facilities (Yang et al., 2005). Because of their mutagenicity, carcinogenicity, and toxic effects that are similar to those of dioxins, their concentrations have been evaluated in a variety of environmental matrices including sediments (Hong et al., 2012a, 2016a; Khim and Hong, 2014). Alkylphenol ethoxylates (APEOs) are widely used nonionic surfactants, which are the reaction products of APs with ethoxylates. Nonylphenol ethoxylates (NPEOs) and octylphenol ethoxylates (OPEOs) are two of the most common nonionic surfactants (Ying et al., 2002). Nonylphenols (NPs) and octylphenols (OPs) belong to a category of compounds that are capable of mimicking hormones of organisms and thus disrupt their hormonal balance (Konieczko et al., 2014). They influence the synthesis, transportation, binding, action, and excretion of hormones that occur naturally in the system and are responsible for maintaining homeostasis as well as the reproduction and the behavior of living organisms (US EPA, 2010).

Instrumental analysis is widely used to evaluate concentrations of organic compounds in environmental samples. However, instrumental analysis can provide little information regarding the integrated biological potency of mixtures in environmental matrices. Furthermore, instrumental analyses cannot identify unknown toxic chemicals, which may act independently or interact with known agonists to influence the biological potency of the mixture of organic contaminants present in the environment. Also, *in vitro* cell-based bioassays have been used to assess pollution of sediments because they are simple, rapid, sensitive, cost-effective, and require a small amount of sample (Khim et al., 2001). H4IIE-

luc and MVLN bioassays are widely used to assess aryl hydrocarbon receptor (AhR)- and estrogen receptor (ER)-mediated potencies in environmental samples such as sediments (Giesy and Kannan, 1998; Hilscherová et al., 2000; Hong et al., 2012a, 2012b, 2016a, 2016b; Khim et al., 1999a, 1999b, 1999c; Palermo et al., 2005).

AhR-mediated potencies can be caused not only by known dioxin-like compounds, such as polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), coplanar-polychlorinated biphenyls (Co-PCBs), and some PAHs, but also can be caused by unknown compounds present in environmental samples (Giesy and Kannan, 1998; Hilscherová et al., 2000; Palermo et al., 2005). In addition, ER-mediated potencies can be caused by well-known estrogenic compounds such as APs and bisphenol A (BPA) (Krishnan et al., 1993; Nimrod and Benson, 1996; Steinmetz et al., 1997; White et al., 1994). Thus, *in vitro* bioassays using recombinant cells with a luciferase reporter gene can be used to examine the overall toxic potency of environmental samples.

In this study, potency balance analysis and effect-directed analysis were utilized to identify agents responsible for *in vitro* bioassay responses observed. Results of bioassays were compared to responses predicted from measured concentrations of targeted compounds by use of published relative potencies for the target compounds. The use of the bioassay-based potency balance approach was important because extracts of sediments can contain many bioactive compounds that cannot be analyzed by instrumental methods (Khim et al., 1999a, 1999b, 1999c, 2000). Some of these assays have been applied to estimate the potency of individual compounds as well as of complex mixtures that elicit biological responses mediated through specific nuclear receptors, such as AhR or ER (Macíková et al., 2014). Thus, the combination of the *in vitro* bioassay with instrumental analysis is a powerful tool to detect dioxin-like and estrogenic compounds in sediments (Khim et al., 2001; Kinani et al., 2010; Macíková et al., 2014; Wang et al., 2012).

There has been little long-term monitoring of persistent toxic substances in coastal sediments from the west coast of Korea. An ongoing study from 2010 to 2014 (5 years) was conducted to determine distributions of PAHs and APs as well as their potential biological effects using recombinant cell lines. This study employed two mechanism-specific, *in vitro* bioassays to support and complement instrumental characterization of coastal sediments. Specific aims were to: i) screen the potential effects of sedimentary contaminants mediated through the mechanisms of AhR and ER; ii) determine the spatio-temporal distributions of sedimentary PAHs and APs; iii) assess contributions of PAHs and APs to overall induced AhR- and ER-mediated potencies, respectively, by use of potency balance analysis; and iv) identify the primary fraction responsible for the biological response by use of effect-directed analysis. The present study provided relatively long-term changes (five years) of target chemicals and biological responses in fairly wide geographical regions in the west coast of Korea, which certainly aids the current information on environmental database following future directions on their management and control.

2. Materials and methods

2.1. Sampling and sample preparations

From 2010 to 2014, surface sediments were collected from estuarine and coastal areas along the west coast of South Korea (Fig. 1). All samples were transported on ice at 4 °C to the laboratory and frozen at –20 °C until analysis. Samples were freeze-dried and ground with a mortar and pestle. Approximately 20 g of sediment was extracted on a Soxhlet extractor with 350 mL of dichloromethane (DCM, Burdick and Jackson, Muskegon, MI) for 16 h. The organic extracts were treated with activated copper powder (Merck, Darmstadt, Germany) to remove elemental sulfur and concentrated to 2 mL.

Extracts were divided into two aliquots for use in the *in vitro* bioassay or for instrumental analysis. The portion of the extract (1 mL) used in the bioassay was exchanged into dimethyl sulfoxide (DMSO, Sigma-Aldrich, Saint Louis, MO). For chemical analysis and effect-directed analysis, 1 mL of the organic extract was passed through 8 g of activated silica gel (70–230 mesh, Merck) in a packed glass column for fractionation (Hong et al., 2016b; Khim et al., 1999a, 1999c). The first fraction (F1), containing nonpolar compounds, was eluted with 40 mL of hexane. The aromatic fraction (F2) was collected by elution with 50 mL of 20% DCM in hexane (v/v). The third fraction (F3) containing polar compounds, was eluted in 50 mL of 60% DCM in acetone. The fourth fraction (F4) containing column residues, was eluted with 50 mL of acetone. All eluents were concentrated for instrumental analysis and further fractionation using a rotary evaporator and a flow of N₂ gas. F2 and F3 were used for the analyses of PAHs and APs, respectively.

2.2. *In vitro* bioassay

The AhR-mediated potencies in the organic extracts of the sediments were measured by the H4IIE-*luc* bioassay (Khim et al., 1999b, 1999c). The cell viability and cytotoxicity of all samples

were determined using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (Yoo et al., 2006). Dilution factors for samples were determined from the results of the MTT assay, namely, samples with the percentage of live cells >80% were used for the bioassay. Trypsinized cells from a culture plate were diluted to a concentration of approximately 7×10^4 cells mL⁻¹ and seeded into the 60 interior wells of a 96-well plate with 250 μL of medium per well. After incubation for 24 h, the cells were exposed to test chemicals or sample extracts dosed at 0.25 μL (0.1% dose) with two different durations of exposure (4 h or 72 h), in order to identify metabolically labile compounds or stable AhR-binding compounds (Louiz et al., 2008). After 4 h or 72 h of exposure, the luminescence of luciferase was quantified by use of a Victor X3 multi label plate reader (Perkin-Elmer, Waltham, MA).

ER-mediated potencies in the organic extracts of the sediments were determined by the MVLN bioassay (Khim et al., 1999b, 1999c). Trypsinized cells from a culture plate were diluted to a concentration of approximately 1.25×10^5 cells mL⁻¹ and seeded into the 60 interior wells of a 96-well plate, with 250 μL of medium per well. After incubation for 24 h, the cells were exposed to test chemicals or sample extracts dosed at 0.25 μL for a 72-h incubation period. Luciferase assays were conducted after 72 h of exposure using a microplate luminometer.

2.3. Bioassay data analysis

Significant responses were defined as those that were three times as great as the standard deviation of the mean of solvent controls. Responses of the H4IIE-*luc* assay were converted to percentages of the maximum response (%BaP_{max} or TCDD_{max}) observed after exposure to 50 nM benzo[*a*]pyrene (BaP) for 4 h or 300 pM 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) for 72 h. The AhR-mediated potency was expressed as a BaP equivalent concentration (ng BaP-EQ g⁻¹ dry mass (dm)), which was determined directly from sample dose-response relationships generated by testing samples at multiple dilutions. The responses of the MVLN

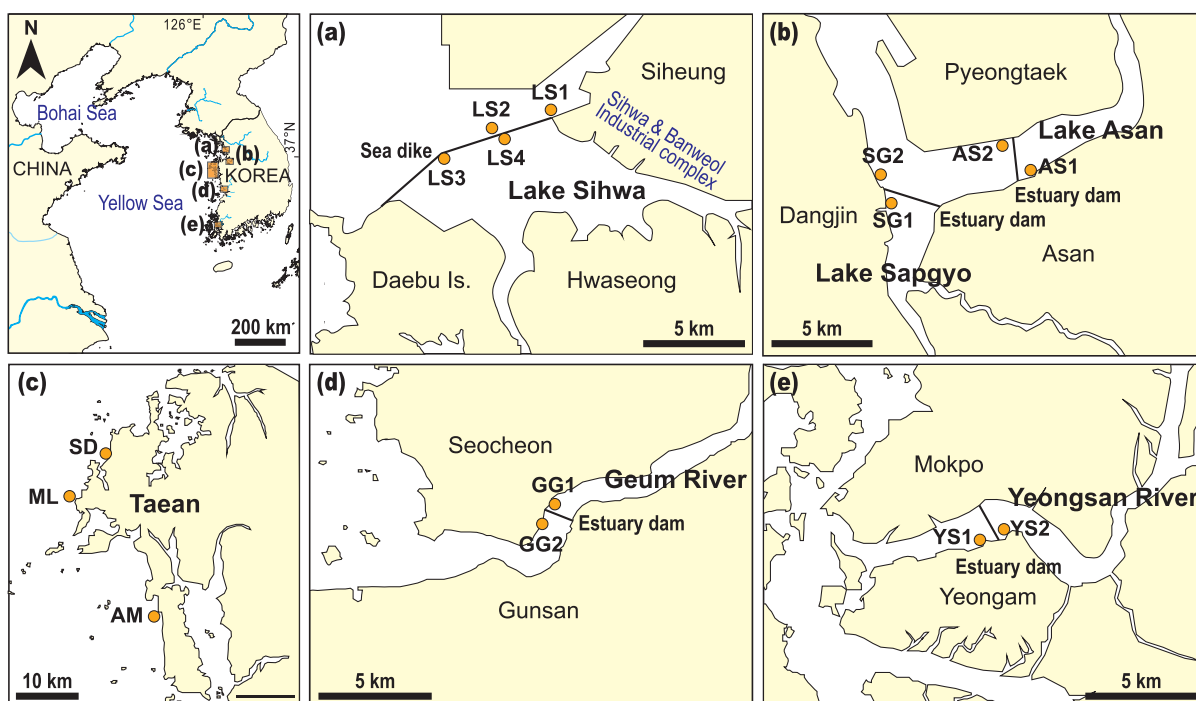


Fig. 1. Map showing the locations of the study areas. (a) Lake Sihwa, (b) Lakes Asan and Sapggyo, (c) Taean coast, (d) Geum River Estuary, and (e) Yeongsan River Estuary.

bioassay were converted to percentages of the maximum response (%E_{2max}) observed for 1235 nM 17 β -estradiol (E₂). Meanwhile, the E₂ standard equivalent concentration (E₂-EQ) could not be calculated due to the small inductions of ER-mediated potencies.

2.4. Chemical analysis

PAHs and APs in the sediment extracts were quantified by an Agilent 7890 gas chromatograph (GC) coupled with a 5975C mass-selective detector (MSD, Agilent Technologies, Avondale, PA) in selected ion monitoring mode (details in Fig. S1 of Supplemental Materials (S)). A total of 15 PAHs, including acenaphthylene (Acl), acenaphthene (Ace), fluorene (Flu), phenanthrene (Phe), anthracene (Ant), fluoranthene (Fl), pyrene (Py), benzo[a]anthracene (BaA), chrysene (Chr), benzo[b]fluoranthene (BbF), benzo[k]fluoranthene (BkF), BaP, indeno[1,2,3-c,d]pyrene (IcdP), dibenz[a,h]anthracene (DBaA), and benzo[g,h,i]perylene (BghiP), were quantified. APs, including NPs (isomeric mixture), nonylphenol monoethoxylate (NP1EOs), nonylphenol diethoxylate (NP2EOs), 4-*tert*-octylphenol (4-*tert*-OP), 4-*tert*-octylphenol monoethoxylate (OP1EO), and 4-*tert*-octylphenol diethoxylate (OP2EO), were quantified after silyl derivatization. For quality control, five surrogate standards (acenaphthene-d10, phenanthrene-d10, chrysene-d12, and perylene-d12 for PAHs; bisphenol A-d16 for APs) were used to assess the recoveries of PAHs and APs. The recoveries of surrogate standards were generally acceptable (75–108% for PAHs; 72% for APs). In addition, accuracy of determination of PAHs was assessed by use of certified reference material (CRM) 1941b (marine sediment, Gaithersburg, MD), previously, recoveries ranged from 74 to 112% (average 93%, n = 3). Method detection limits for the PAHs and APs ranged from 0.2 to 1.3 ng g⁻¹ dm and from 0.2 to 1.0 ng g⁻¹ dm, respectively.

2.5. Potency balance analysis

Potency balance analysis between bioassay-derived BaP-EQs and instrument-derived BaP equivalent concentrations (BEQs) were conducted to identify the relative contribution of each known chemical to the total induced AhR-mediated potency. The BEQ values were calculated as the sum of the BEQs by multiplying the concentration of individual PAHs by the relative potency values (RePs) (Equation (1)) obtained from previous studies (Louiz et al., 2008; Villeneuve et al., 2000) (Table S1).

$$BEQs = \sum_{i=1}^n (C_i \times ReP_i) \quad (1)$$

where: C_i is concentrations of individual PAHs and ReP_i is the ReP value of individual PAHs.

2.6. HPLC fractionation

The F2 and F3 fractions, containing PAHs and APs, respectively, were further fractionated into 10 subfractions based on the log K_{ow} values by use of a reverse phase (RP)-HPLC column (PrepHT XBD-C18, 21.2 × 250 mm, 7 mm, Agilent Technologies) (Hong et al., 2016a, 2016b). Separation and fraction collection were performed in an Agilent 1260 HPLC System with UV detection at 254 and 280 nm. The water:MeOH mobile phase (40:60, v/v) was delivered isocratically at a flow rate of 10 mL min⁻¹. Separation conditions of RP-HPLC were optimized through several tests with GC/MSD confirmation using 34 polychlorinated biphenyls (PCBs), 16 PAHs, 7 alkylphenols, and 5 phthalates (with known log K_{ow} values) (Hong et al., 2016a). Acceptable elution efficiency with all compounds was

achieved previously (>85%). Standards of the PAHs and APs were successfully fractionated and identified according to log K_{ow} values. The time windows chosen for collecting the ten consecutive subfractions corresponded to the following log K_{ow} ranges: < 1, 1–2, 2–3, 3–4, 4–5, 5–6, 6–7, 7–8, 8–9, and >9 (Table S2).

3. Results and discussion

3.1. Screening of AhR- and ER-mediated potencies in sediments

Significant AhR-mediated potencies were found in sediment extracts, indicating a general contamination by dioxin-like compounds on the west coast of South Korea. Responses of H4IIE-*luc* bioassay varied among extracts from various locations. The magnitude of AhR induction ranged from 15 to 120%BaP_{max} after exposure for 4 h (Fig. 2a), and ranged from not significant (n.s.) to 8.1%TCDD_{max} after exposure for 72 h (Fig. 2b). The great AhR-mediated potencies were found mainly in samples of sediments after exposure for 4 h, while lesser responses were detected after exposure for 72 h. This result indicates that transformation of compounds by H4IIE-*luc* cells occurred during the longer duration of exposure (Hong et al., 2016b). Thus, in general, AhR-active compounds in sediments along the west coast of South Korea were more labile compounds, like PAHs (Kinani et al., 2010; Louiz et al., 2008). Meanwhile, AS2 showed only relatively greater %TCDD_{max} values, indicating the presence of stable AhR-binding compounds, such as PCDDs, PCDFs, and Co-PCBs (Larsson et al., 2014; Lee et al., 2013; Louiz et al., 2008).

Dose-response curves were fairly parallel to the dose-response curves for the reference chemical (BaP) only after 4 h of exposure. This allowed us to use EC₅₀ values to calculate the biological BaP-EQ concentrations (Table 1). Concentrations of BaP-EQs ranged from less than the limit of detection (<DL) to 57 ng BaP g⁻¹ dm in the sediments. The greatest concentration of BaP-EQ was detected in LS2. Generally, concentrations of BaP-EQs in sediments were related to industrial and/or agricultural activities around the sampling locations or areas (Hong et al., 2012a). Meanwhile, ER-mediated potencies were small in sediment extracts, indicating a lesser distribution of estrogenic compounds on the west coast of South Korea (Fig. 2c). Relative ER-mediated potencies varied among sampling sites, ranging from 3.0 to 22%E_{2max}. The greatest magnitudes of %E_{2max} were detected in the Geum River Estuary (GG2).

Annual variations of AhR-mediated potencies (%BaP_{max}) did not show significant differences from 2010 to 2014 (Fig. 3a). It has been suggested that contaminations of major AhR-active compounds have not decreased during the last 5 years. Meanwhile, the ER-mediated potencies varied: relatively greater %E_{2max} values were present in 2012 and 2014, compared to those of 2011 and 2013. These findings suggested that ER-active compounds were heterogeneously distributed in coastal sediments and/or released irregularly along the west coast of South Korea.

3.2. Spatio-temporal distributions of PAHs in sediments

PAHs were detected in sediments at all locations (Fig. 2d and Table S3). Concentrations of PAHs in sediments from the west coast of South Korea ranged from 1.1 to 93 ng g⁻¹ dm (mean: 21 ng g⁻¹ dm). The greatest concentration of PAHs was found at GG2 (63 ng g⁻¹ dm), followed by LS2 (47 ng g⁻¹ dm) and GG1 (43 ng g⁻¹ dm). Measured concentrations of PAHs were comparable to those reported in our previous study conducted in 2008 (30–210 ng g⁻¹ dm) (Hong et al., 2012a). Concentrations of PAHs were less than those reported at other sites of the South Korean coasts (Khim and Hong, 2014). Many studies have reported that PAH contaminations are related to industrialization and



Fig. 2. AhR-mediated potencies after (a) a 4-h exposure and (b) a 72-h exposure using the H4IIE-*luc* bioassay; (c) ER-mediated potency using the MVLN assay, and distributions of (d) PAHs and (e) APs in sediments along the west coast of South Korea from 2010 to 2014.

Table 1

Comparisons of bioassay-derived BaP equivalents (BaP-EQ) and instrument-derived BEQs in sediments of the west coast of South Korea.

Sampling			AhR-mediated potency				
			Bioassay-derived (4 h exposure)		Instrument-derived	BEQ ^b /BaP-EQ (%)	
Location	Sampling year	n	%BaP _{max} (%)	BaP-EQ (ng BaP g ⁻¹ dm)	BEQ ^a (ng BaP g ⁻¹ dm)		
Lake Sihwa	LS1	2010–2014	5	120	32	11	36
	LS2	2011	1	110	57	33	59
	LS3	2014	1	87	21	1.0	4.8
	LS4	2010, 12, 14	3	40	19	3.2	17
Lake Asan	AS1	2010–2014	5	49	36	6.4	18
	AS2	2010–2014	5	110	33	9.0	28
Lake Sappgyo	SG1	2010–2014	5	73	24	2.9	12
	SG2	2010–2014	5	120	35	16	44
Taeon coast	SD	2010–2014	5	19		0.58	
	ML	2010–2014	5	18		0.70	
	AM	2010–2014	5	15		0.76	
Geum River Estuary	GG1	2010–2014	5	100	34	18	53
	GG2	2010–2014	5	120	35	29	84
Yeongsan River Estuary	YS1	2010–2014	5	72	26	4.3	16
	YS2	2010–2014	5	68	30	5.4	18

^a BEQ values of PAHs were summed by use of the chemical concentrations of Acl, Flu, Py, BaA, Chr, BbF, BkF, BaP, IcdP, and Dbah multiplied by the ReP values obtained in a previous study (Louiz et al., 2008).

^b BEQ/BaP-EQ (%): The percentage of instrument-derived BEQs to the bioassay-derived BaP-EQs.

urbanization, however, relatively greater concentrations of PAHs in sediments were found in low industrial regions, such as near the Geum River and Lake Sappgyo. These findings might be explained by the atmospheric inputs of the PAHs released from a variety of combustion sources and manufacturing processes (Choi et al., 2011).

High-molecular-weight PAHs with four to six rings, such as BkF and Dbah were predominant in the sediment samples (Hong et al., 2012a; Koh et al., 2002, 2004, 2005). The diagnostic ratios between individual PAHs in sediments were applied to assess the contribution of petroleum or combustion sources of PAHs (Fig. S2).

Double ratios of Ant/(Ant + Phe) and Fl/(Fl + Py), and BaA/(BaA + Chr) and IcdP/(IcdP + BghiP) in sediments indicated that the PAHs originated mainly from the combustion of petroleum, grass, wood, and coal (Yunker et al., 2002). The sources of PAHs in sediments along the west coast of Korea were not significantly different between the sampling sites (Hong et al., 2012a; Jiao et al., 2012). Although specific sources of PAHs could not be fully attributed by the use of the reported diagnostic ratios of PAHs, the sources of the PAHs were found to be slightly different among the sampling sites. Relatively greater concentrations of PAHs were detected in outer regions (LS1, LS2, AS2, SG2, GG2, and YS1) than in inner regions.

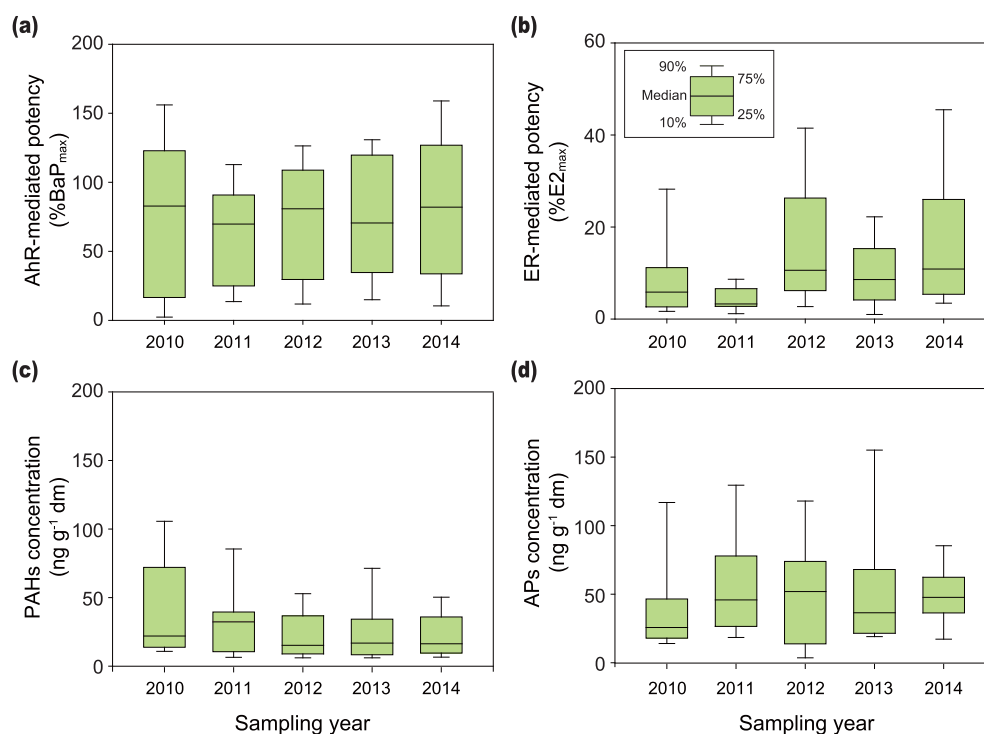


Fig. 3. Annual variations of (a) AhR-mediated potency, (b) ER-mediated potency, and concentrations of (c) PAHs and (d) APs in sediments along the west coast of South Korea from 2010 to 2014.

PAHs seemed to be introduced into the estuarine and coastal areas from inner regions of sea dikes or estuary dams, and they could be adsorbed onto suspended particles and/or sediments in coastal areas.

The PAH concentrations in sediments of the west coast of South Korea were compared to existing corresponding sediment quality guidelines, such as the effects range low and median (ERL and ERM) (Hahladakis et al., 2013). The concentrations of 15 PAHs did not exceed the ERL for any of the sampling sites. Thus, the results of the sedimentary PAHs implicated that there is little or no toxic effect on benthic organisms by PAH contaminants. Meanwhile, although the limited sample number in the present study and heterogeneous distributions, annual variations of the PAH concentrations from 2010 to 2014 showed a slightly decreasing trend (Fig. 3c). Recent regulations on flue gases from waste incinerators might be curbing PAHs contamination on the west coast of South Korea.

3.3. Spatio-temporal distributions of APs in sediments

APs were detected in sediments at all locations (Fig. 2e and Table S4). The greatest concentration of APs was found at LS2 (130 ng g⁻¹ dm), followed by GG1 (110 ng g⁻¹ dm) and LS1 (95 ng g⁻¹ dm). The Lake Sihwa region is a well-known hot spot area contaminated by APs, which affect the surrounding anthropogenic activities (Choi et al., 2009; Hong et al., 2010; Khim et al., 1999c; Koh et al., 2005). Concentrations of APs observed during this study were less than those observed during previous studies, which is indicative of success of chemical controls and regulations. The Korean government has been trying to reduce contamination of NPs by designating them as priority chemicals, prohibiting their use as components of household cleaners in 2001, for all domestic applications in 2007, and for some industrial applications such as paints and inks in 2010 (Choi et al., 2011).

NPs and OPs are degradation products of NPEOs and OPEOs,

respectively, and greater concentrations of the parent compounds indicated the fresh input of compounds (Li et al., 2008). At all sites, the concentrations of OP1EO and OP2EO were greater than OP concentrations, while the NP concentrations were greater than the concentrations of NP1EOs and NP2EOs. These findings can be attributed to the prohibition of use of NPs by the government (Choi et al., 2011); however, some sampling sites (LS1 collected from 2010 to 2011, SG2 collected from 2012, and GG2 collected from 2012) still demonstrated fresh inputs. Concentrations of NPs detected in sediments did not exceed sediment quality guidelines promulgated in Canada (CCME, 2001).

Meanwhile, annual variations in concentrations of APs between 2010 and 2014, obtained during this study did not show significant differences (Fig. 3d). These results revealed that APs from the surrounding areas consistently contaminate sampling sites. At all sites, concentrations of NPs and NP1EOs were relatively greater than those of NP2EOs, OP, OP1EO, and OP2EO. These findings seemed to be correlated to the production amounts of NPEOs and OPEOs and the influence of the carbon chain length on the hydrophobicity of APEOs (Ying et al., 2002).

3.4. Bioassays vs. instrumental analysis: potency balance

Results of quantification of PAHs and the H4IIE-*luc* bioassay in sediments were compared. The AhR-mediated potencies (%BaP_{max}) were generally well correlated with concentrations of PAHs in sediments, and a dose-response relationship was present ($r^2 = 0.70$, $p < 0.01$) (Fig. 4a). It is indicated that the total induced AhR-mediated potencies in sediments were partly explained by concentrations of PAHs in sediments along the west coast of South Korea. The correlation between ER-mediated potencies (%E2_{max}) and concentrations of APs was weak for the same set of sediment samples ($r^2 = 0.11$, $p < 0.05$) (Fig. 4b). ER-mediated potencies in sediments could not be fully explained solely by the APs suggesting

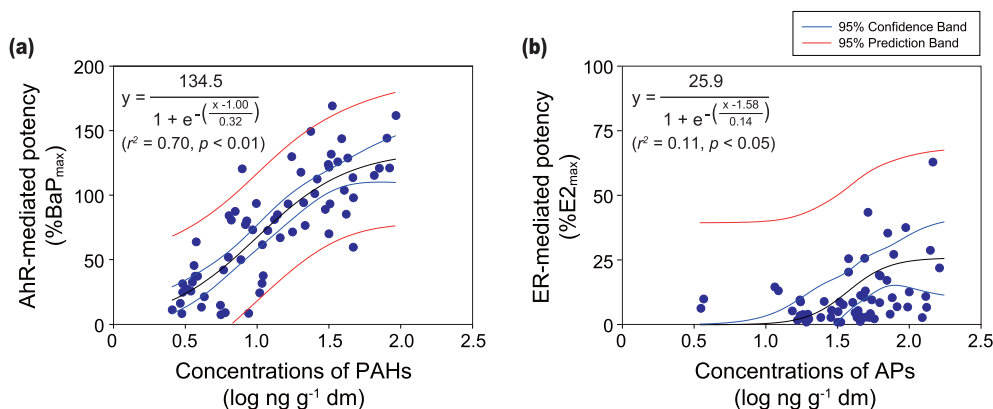


Fig. 4. Relationships between bioassays and chemical analysis results. (a) Concentrations of PAHs vs. AhR-mediated potency and (b) concentrations of APs vs. ER-mediated potency in sediments along the west coast of South Korea from 2010 to 2014.

that other untargeted ER-active compounds are present in sediments.

Comparison between results of bioassays and instrumental analysis to identify the contribution of each known chemical in sediments to the overall induced toxic potencies can be performed by potency balance analysis (Giesy et al., 2002; Hong et al., 2012a, 2014, 2016b; Khim et al., 2001; Koh et al., 2004). A potency balance analysis between H4IIE-*luc*-derived BaP-EQs and PAHs-derived BEQs was conducted to determine contributions of known AhR-active PAHs. Contributions of known AhR agonists in sediments were variable due to the sample-specific chemical compositions. Overall trends of major AhR agonists in sediments along the west coast of Korea were determined using mean concentrations of instrument-derived BEQs and bioassay-derived BaP-EQs of all sediments.

The proportion of BaP-EQ accounted for approximately 30% of the BEQ, based on the concentrations of PAHs in sediments (Fig. S3a). BkF was the predominant contributor to the BEQs, followed by DbahA and BbF. The proportions of BaP-EQ explained by measured chemicals varied among sediments and years (Fig. S3). Results of potency balances were comparable to previous studies: high-molecular-weight PAHs such as BkF and IcdP were the major AhR agonists in sediments (Hong et al., 2012a, 2016b; Macíková et al., 2014). Overall, these findings indicated that known AhR-active compounds such as PAHs represented only a small portion of total AhR-mediated potencies in sediments. Thus, unknown AhR-active compounds were commonly distributed along the west coast of South Korea.

3.5. Effect-directed analysis

In order to investigate the major AhR and ER agonists in sediments, further fractionation by use of RP-HPLC was conducted. Two sediment samples, GG2 in 2011 and in 2014, were selected to conduct the effect-directed analysis, because those samples elicited the greatest %BaP_{max} and %E2_{max}, respectively. In the H4IIE-*luc* bioassay, among four silica gel fractions, F2 of the GG2 sediment extract, containing aromatic compounds, elicited the greatest AhR-mediated potencies (%BaP_{max} and %TCDD_{max}) (Fig. 5a). Among 10 subfractions of F2, greatest AhR-mediated potency was found in fraction F2.7, followed by F2.8 and F2.6. Magnitudes of AhR-mediated potency were less than the level deemed significant in all of the RP-HPLC subfractions at 72 h after exposure in the H4IIE-*luc* assay. This result indicated that major AhR agonists in extracts of sediments might be aromatic compounds, with log *K*_{ow} values between 5 and 8, and metabolically labile compounds, such as PAHs

(Fig. 5a).

Fraction F2.7 contained five- and six-ring PAHs, such as BbF, BkF, BaP, IcdP, and DbahA. In fraction F2.6, four- and five-ring PAHs were present, including Fl, Py, BaA, and Chr. Although there were no target PAHs in fraction F2.8, it elicited a relatively great AhR-mediated potency. Target PAHs partially contributed to total concentrations of BEQs in F2.6 (2.5%) and F2.7 (31%). This pattern is similar to results of a previous study conducted in sediments of inland creeks of the Lake Sihwa region (Hong et al., 2016b). Results of the present study will provide useful information on unknown AhR agonists in sediments, such as polarity and log *K*_{ow} values. For example, untargeted four to five ring PAHs and their derivatives in sediments could also activate the AhR, as reported previously (Larsson et al., 2014). The 16 EPA PAHs were recognized as representatives for all PAHs during the last 40 years due to the limitations of analytical standards, environmental occurrences, and available toxicity data in 1976 (Andersson and Achten, 2015). Thus, environmental occurrences and ecotoxicity of other non-targeted PAHs (e.g., oxy-, nitro-, sulfur-, alkyl-, cyano-, and amino-PAHs) are still largely unknown. In the future, integrated environmental risk assessments on polycyclic aromatic compounds (PACs) are strongly recommended.

In the MVLN assay, fraction F3, containing polar compounds induced the greatest ER-mediated potency (%E2_{max}) (Fig. 5b). Among subfractions of F3, a significant response was detected in F3.2, indicating that the major ER agonists were polar compounds with log *K*_{ow} values between 1 and 2. In the present study, targeted ER agonists, such as APs, had log *K*_{ow} values between 4 and 6 (Ahel and Giger, 1993). OPs and NPs existed in F3.4 and F3.5 confirmed previously (Hong et al., 2016a), however, those fractions showed no significant induction of ER-mediated potency. Meanwhile, the fraction F3.2 showed the greatest ER-mediated potency induction, however, there were no targeted ER agonists observed in this study. Methoxychlor, 2,2-bis(*p*-hydroxyphenyl)-1,1,1-trichloroethane, tamoxifen, 4-OH-tamoxifen, diethylstilbestrol, and BPA are known to be ER-active chemicals in the environments (Barkhem et al., 1998; Muroso and Derk, 2005; Peterson and Barnes, 1991; Wilson et al., 2004). Therefore, more studies concerning unknown ER agonists in sediments are needed in the future.

Overall, unknown toxic compounds might contribute to the total induced toxicity in coastal sediments and are neglected in the monitoring programs (Brack, 2003). Thus, the identification of compounds responsible for potential toxicity in environmental samples is of great interest in order to update the priority lists and for further regulation (Regueiro et al., 2013). Additional investigations focusing on unknown highly potent compounds in

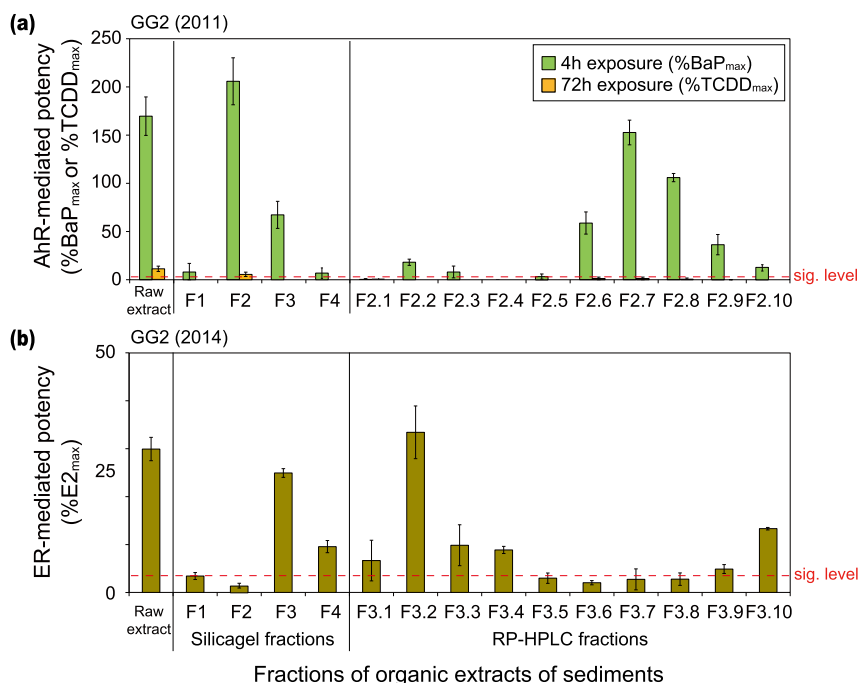


Fig. 5. Effect-directed analysis using silica gel column chromatography (step 1) and RP-HPLC (step 2) for (a) AhR-mediated potency and (b) ER-mediated potency in organic extracts of sediments collected from site GG2 in 2011 and 2014, respectively (sig. level: significant level).

sediments as well as assessment of potential interactions such as synergism or antagonism among compounds are needed to obtain further insight on the environmental identification of toxic active compounds.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.chemosphere.2016.10.089>.

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Assessment of potential biological activities and distributions of endocrine-disrupting chemicals in sediments of the west coast of South Korea

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Table S1. Relative potency values of PAHs for the AhR-mediated activities used in this study.

Target compound	ReP value	
	4h ^a	72h ^b
Acenaphthylene	5.6 x 10 ⁻³	-
Acenaphthene	-	-
Fluorene	1.4 x 10 ⁻²	-
Phenanthrene	-	-
Anthracene	-	-
Fluoranthene	-	-
Pyrene	3.6 x 10 ⁻³	-
Benzo[a]anthracene	2.6 x 10 ⁻¹	1.9 x 10 ⁻⁶
Chrysene	2.9 x 10 ⁻¹	2.3 x 10 ⁻⁶
Benzo[b]fluoranthene	6.9 x 10 ⁻¹	5.1 x 10 ⁻⁶
Benzo[k]fluoranthene	2.94	1.4 x 10 ⁻⁴
Benzo[a]pyrene	1	1.6 x 10 ⁻⁶
Indeno[1,2,3-c,d]pyrene	8.4 x 10 ⁻¹	1.5 x 10 ⁻⁵
Dibenz[a,h]anthracene	3.66	4.6 x 10 ⁻⁶
Benzo[g,h,i]perylene	-	-

^a Louiz et al., 2008.

^b Villeneuve et al., 2002.

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Table S2. Instrumental conditions of reverse phase-HPLC for fine fractionations.

RP-HPLC system	Agilent 1260 HPLC system
Column	C18 (25 cm, 7 μ m, 21.2 mm), Reverse phase column
Mobile phase	Water:MeOH (40:60, v/v), Isocratic elution
Flow rate	10 mL/min
Injection volume	1 mL
Test standard	16 PAHs mixture
Fractions collected	0 – 5.06 min: discard 5.06 – 8.30 min: F1.1 ($\log K_{ow} < 1$) 8.30 – 14.78 min: F1.2 ($\log K_{ow}$ 1-2) 14.78 – 21.27 min: F1.3 ($\log K_{ow}$ 2-3) 21.27 – 27.75 min: F1.4 ($\log K_{ow}$ 3-4) 27.75 – 34.24 min: F1.5 ($\log K_{ow}$ 4-5) 34.24 – 40.73 min: F1.6 ($\log K_{ow}$ 5-6) 40.73 – 47.21 min: F1.7 ($\log K_{ow}$ 6-7) 47.21 – 53.70 min: F1.8 ($\log K_{ow}$ 7-8) 53.70 – 60.18 min: F1.9 ($\log K_{ow}$ 8-9) 60.18 – 66.67 min: F1.10 ($\log K_{ow} > 9$) 66.67 – 70 min: discard

Table S3. Concentrations of PAHs in sediments collected from the west coast of South Korea.

Sampling		PAHs concentration (ng g ⁻¹ dw)															
Location	Year	Acl	Ace	Flu	Phe	Ant	Fl	Py	BaA	Chr	BbF	BkF	BaP	IcdP	DbahA	BghiP	ΣPAHs
LS1	2010	<DL ^a	0.4	1.4	4.5	0.5	7.8	4.4	0.9	2.4	3.6	2.9	1.0	2.6	1.2	2.9	37
	2011	<DL	<DL	2.7	3.9	0.4	6.7	2.4	0.7	1.4	2.9	1.6	0.6	1.2	<DL	1.6	26
	2012	<DL	<DL	1.7	1.3	0.2	1.4	0.5	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	5.1
	2013	<DL	<DL	2.5	3.6	0.5	7.4	3.3	1.5	1.2	3.1	2.0	0.9	1.9	1.8	2.9	33
	2014	<DL	<DL	2.4	2.6	0.3	6.7	2.8	1.3	0.7	2.5	1.5	0.5	0.6	<DL	1.4	23
LS2	2011	<DL	0.5	3.7	4.5	0.4	8.8	4.2	1.3	1.4	5.0	2.5	1.9	3.6	4.6	4.1	47
LS3	2014	<DL	0.7	2.1	2.5	<DL	<DL	0.4	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	5.7
LS4	2010	<DL	<DL	1.0	2.8	0.3	3.5	1.4	<DL	1.1	2.0	1.3	0.5	1.3	<DL	1.4	17
	2012	<DL	<DL	1.2	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	1.2
	2014	<DL	<DL	1.8	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	1.8
AS1	2010	<DL	<DL	2.6	1.2	0.3	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	4.1
	2011	<DL	<DL	3.2	2.9	0.5	4.9	2.5	1.1	1.5	3.5	1.7	0.9	2.3	5.0	2.1	32
	2012	<DL	<DL	1.7	0.9	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	2.6
	2013	<DL	<DL	1.5	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	1.5
	2014	<DL	<DL	1.7	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	1.7
AS2	2010	<DL	<DL	1.1	1.7	0.4	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	3.3
	2011	<DL	0.45	3.3	4.6	0.7	9.9	3.8	1.2	1.9	4.7	3.0	1.6	3.1	<DL	3.3	42
	2012	<DL	<DL	1.7	1.0	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	0.6	<DL	1.1	4.3
	2013	<DL	<DL	2.2	2.9	0.3	5.8	2.0	<DL	<DL	2.1	<DL	0.5	0.9	<DL	1.4	18
	2014	<DL	<DL	2.4	4.0	0.3	8.2	3.6	0.6	2.2	3.5	2.8	0.8	1.8	<DL	2.8	33
SG1	2010	<DL	<DL	0.7	2.2	0.2	2.3	0.8	<DL	0.7	0.9	<DL	<DL	<DL	<DL	0.5	8.2
	2011	<DL	<DL	3.1	1.8	0.2	2.3	1.1	<DL	<DL	0.6	<DL	<DL	<DL	<DL	0.5	9.5
	2012	<DL	<DL	2.8	2.4	0.2	2.6	0.9	<DL	<DL	1.0	<DL	<DL	0.6	<DL	0.9	12
	2013	<DL	<DL	1.8	1.4	0.2	1.4	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	4.7
	2014	<DL	<DL	1.9	1.5	<DL	1.8	0.6	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	5.8
SG2	2010	<DL	1.7	2.6	14.6	1.3	19.1	11	3.2	2.3	7.5	5.2	2.7	4.1	0.6	4.4	81
	2011	<DL	0.6	2.9	4.4	0.7	8.0	3.3	0.8	1.3	3.1	1.4	0.7	1.1	<DL	1.6	30
	2012	<DL	0.9	1.8	6.7	0.5	11	5.2	1.1	1.7	3.5	2.9	1.6	2.5	<DL	3.0	43
	2013	<DL	0.4	1.7	2.5	0.2	5.3	2.3	<DL	0.5	1.5	<DL	0.3	<DL	<DL	0.9	16
	2014	<DL	0.7	3.1	3.9	0.4	11	5.1	1.0	1.1	4.9	2.9	1.4	1.8	<DL	2.8	40
SD	2010	<DL	<DL	1.0	1.5	0.3	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	2.8
	2011	<DL	<DL	2.8	1.2	0.5	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	4.5
	2012	<DL	<DL	1.8	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	1.8
	2013	<DL	<DL	1.7	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	1.7
	2014	<DL	<DL	1.8	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	1.8
ML	2010	<DL	<DL	2.5	1.3	0.3	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	4.0

	2011	<DL	<DL	2.1	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	2.1	
	2012	<DL	<DL	1.7	0.9	0.2	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	2.8	
	2013	<DL	<DL	2.4	<DL	0.3	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	2.7	
	2014	<DL	<DL	2.3	0.9	<DL	<DL	1.1	<DL	<DL	<DL	<DL	<DL	<DL	3.4	<DL	7.7	
AM	2010	<DL	<DL	3.1	1.8	0.5	<DL	1.6	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	7.0	
	2011	<DL	<DL	1.6	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	1.6	
	2012	<DL	<DL	1.8	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	1.8	
	2013	<DL	<DL	1.3	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	1.3	
	2014	<DL	<DL	1.8	<DL	<DL	<DL	0.5	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	2.3	
GG1	2010	<DL	0.51	3.9	7.1	1.2	13	6.5	2.2	5.0	8.8	5.6	2.0	4.7	0.6	4.6	66	
	2011	<DL	<DL	2.0	4.0	0.4	7.9	3.3	1.7	1.1	4.2	2.7	0.9	0.6	<DL	2.6	31	
	2012	<DL	<DL	2.1	4.1	0.4	8.3	3.2	0.8	2.2	4.1	1.5	0.6	1.5	<DL	2.1	31	
	2013	<DL	0.4	3.7	5.8	0.5	10	6.2	1.6	1.3	6.4	2.9	1.4	3.3	<DL	3.4	47	
	2014	<DL	<DL	3.4	4.3	0.5	8.3	4.8	2.5	2.6	4.0	2.6	1.0	1.8	<DL	2.6	38	
GG2	2010	0.4	0.7	4.7	8.1	1.0	14.9	8.3	12.4	6.0	12	5.9	3.6	7.7	0.8	7.5	93	
	2011	<DL	<DL	1.9	3.6	0.3	6.2	2.8	<DL	1.0	1.4	1.9	0.3	<DL	<DL	0.9	20	
	2012	<DL	0.5	2.5	5.3	0.5	10	5.0	1.1	3.4	5.8	4.1	1.3	3.0	<DL	4.0	47	
	2013	0.4	<DL	3.8	7.5	0.7	14	7.6	4.7	4.9	8.2	5.1	2.3	4.8	0.6	6.0	71	
	2014	0.4	1.1	3.8	7.8	0.7	16	11	2.4	5.8	10	5.6	2.3	7.1	1.6	8.9	84	
YS1	2010	<DL	<DL	3.3	2.1	0.3	2.4	0.8	<DL	<DL	0.9	<DL	<DL	<DL	<DL	<DL	0.5	10
	2011	<DL	<DL	1.5	1.5	0.2	3.2	1.8	<DL	0.6	0.6	<DL	<DL	<DL	<DL	<DL	0.4	9.8
	2012	<DL	<DL	1.7	1.3	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	2.9
	2013	<DL	<DL	1.9	1.6	<DL	2.0	0.8	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	6.3
	2014	<DL	0.6	3.4	3.7	0.3	4.3	4.3	1.1	3.0	3.1	1.6	1.2	2.1	<DL	2.8	32	
YS2	2010	<DL	<DL	2.8	2.0	0.4	6.9	4.4	2.0	0.8	2.0	<DL	1.0	0.8	<DL	1.0	24	
	2011	<DL	<DL	1.1	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	1.1
	2012	<DL	0.4	2.3	3.5	0.3	2.9	1.1	<DL	<DL	0.9	<DL	0.3	<DL	<DL	<DL	0.6	12
	2013	<DL	<DL	2.7	2.1	0.2	1.6	0.8	<DL	<DL	0.9	<DL	<DL	<DL	<DL	<DL	0.8	9.0
	2014	<DL	<DL	2.8	1.1	<DL	1.8	1.4	<DL	<DL	1.3	<DL	<DL	<DL	2.4	1.4	12	

^a< DL: below detection limit.

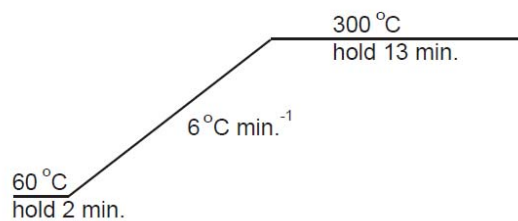
Table S4. Concentrations of APs in sediments collected from the west coast of South Korea.

Sampling		APs concentration (ng g ⁻¹ dw)						
Location	Year	NPs	NP1EOs	NP2EOs	OP	OP1EO	OP2EO	ΣAPs
LS1	2010	35	57	35	0.8	1.7	2.7	130
	2011	30	42	22	0.8	1.2	2.4	98
	2012	49	22	9.0	0.7	0.7	1.2	83
	2013	55	28	11	1.3	1.2	4.5	100
	2014	25	22	9.2	1.4	1.5	3.8	63
LS2	2011	62	33	26	1.4	0.8	9.7	130
LS3	2014	8.1	5.5	1.0	0.4	<DL ^a	<DL	15
LS4	2010	16	19	7.1	<DL	0.5	1.2	44
	2012	2.1	0.8	<DL	<DL	<DL	<DL	2.9
	2014	9.5	7.4	0.9	<DL	<DL	<DL	18
AS1	2010	9.4	6.4	1.1	<DL	0.2	<DL	17
	2011	35	15	5.4	<DL	0.5	1.6	57
	2012	36	9.1	1.6	<DL	<DL	<DL	47
	2013	9.7	7.4	1.3	<DL	<DL	<DL	18
	2014	48	9.7	3.2	0.4	0.3	<DL	62
AS2	2010	12	6.3	1.0	<DL	0.3	<DL	20
	2011	14	11	2.8	<DL	0.5	<DL	28
	2012	12	2.5	1.4	<DL	<DL	<DL	16
	2013	23	11	2.7	0.5	0.3	<DL	37
	2014	20	13	2.8	0.5	0.4	<DL	37
SG1	2010	13	5.8	1.0	<DL	0.3	<DL	20
	2011	18	13	2.2	<DL	0.4	<DL	33
	2012	44	18	5.3	0.7	1.2	2.7	71
	2013	25	11	2.3	1.0	0.5	<DL	40
	2014	9.3	6.3	1.1	<DL	<DL	<DL	17
SG2	2010	22	18	5.6	0.5	0.7	2.4	49
	2011	25	18	4.2	0.3	0.5	2.2	50
	2012	21	25	14	0.4	1.2	9.4	70
	2013	27	4.3	2.6	<DL	0.4	1.1	35
	2014	30	15	3.3	0.4	0.4	<DL	48
SD	2010	9.0	6.2	0.9	<DL	<DL	<DL	16
	2011	13	9.1	1.8	<DL	0.2	<DL	24
	2012	17	8.4	1.4	<DL	0.2	2.1	29
	2013	10	7.2	1.5	<DL	0.2	<DL	19

ML	2014	30	9.9	1.3	0.3	0.4	2.1	44
	2010	7.2	4.3	<DL	<DL	<DL	<DL	12
	2011	27	9.9	4.7	<DL	0.3	3.7	46
	2012	36	12	2.3	0.3	0.3	<DL	51
	2013	12	6.5	1.6	<DL	0.3	<DL	20
AM	2014	30	17	4.2	0.5	0.6	2.8	55
	2010	11	6.0	<DL	<DL	<DL	<DL	17
	2011	9.7	6.4	1.0	<DL	<DL	<DL	17
	2012	1.8	1.0	<DL	<DL	<DL	<DL	2.8
	2013	13	8.4	2.0	<DL	<DL	<DL	24
GG1	2014	26	12	2.5	0.4	0.9	4.0	46
	2010	14	13	3.8	<DL	0.3	1.0	32
	2011	69	38	13	2.1	0.7	1.6	120
	2012	21	21	8.8	0.5	0.6	1.4	53
	2013	120	43	6.4	3.9	0.7	<DL	180
GG2	2014	110	35	2.8	1.8	0.5	<DL	150
	2010	34	34	16	0.4	0.8	9.9	95
	2011	25	20	5.9	0.3	0.6	1.6	54
	2012	40	48	40	0.7	1.2	11	140
	2013	21	21	5.2	0.6	0.5	1.7	50
YS1	2014	42	27	5.8	1.5	0.6	1.9	79
	2010	18	11	2.6	<DL	0.3	<DL	32
	2011	27	12	1.7	<DL	0.3	<DL	41
	2012	66	7.5	2.5	<DL	<DL	<DL	76
	2013	17	11	3.0	0.3	0.4	<DL	31
YS2	2014	32	23	5.2	0.4	0.4	<DL	61
	2010	16	7.6	1.7	<DL	<DL	<DL	25
	2011	11	7.1	1.5	<DL	<DL	<DL	19
	2012	6.8	3.7	<DL	<DL	<DL	<DL	10
	2013	59	12	2.5	0.5	0.4	<DL	74
	2014	29	12	3.6	0.3	0.2	<DL	45

^a< DL: below detection limit.

GC/MSD conditions



Agilent 7890A GC and 5975C MSD

Column: DB-5MS
(30 m x 0.25 mm i.d. x 0.25 µm film)

Carrier gas: He

Injection temperature: 280 °C

Injection mode: splitless

Column flow: 1.0 ml min⁻¹

Ionization: EI mode (70 eV)

Acquisition mode: Scan (50-550 m/z) for non-targeted analysis
SIM for PAHs and Alkylphenols

Fig. S1. Instrumental conditions of GC/MSD.

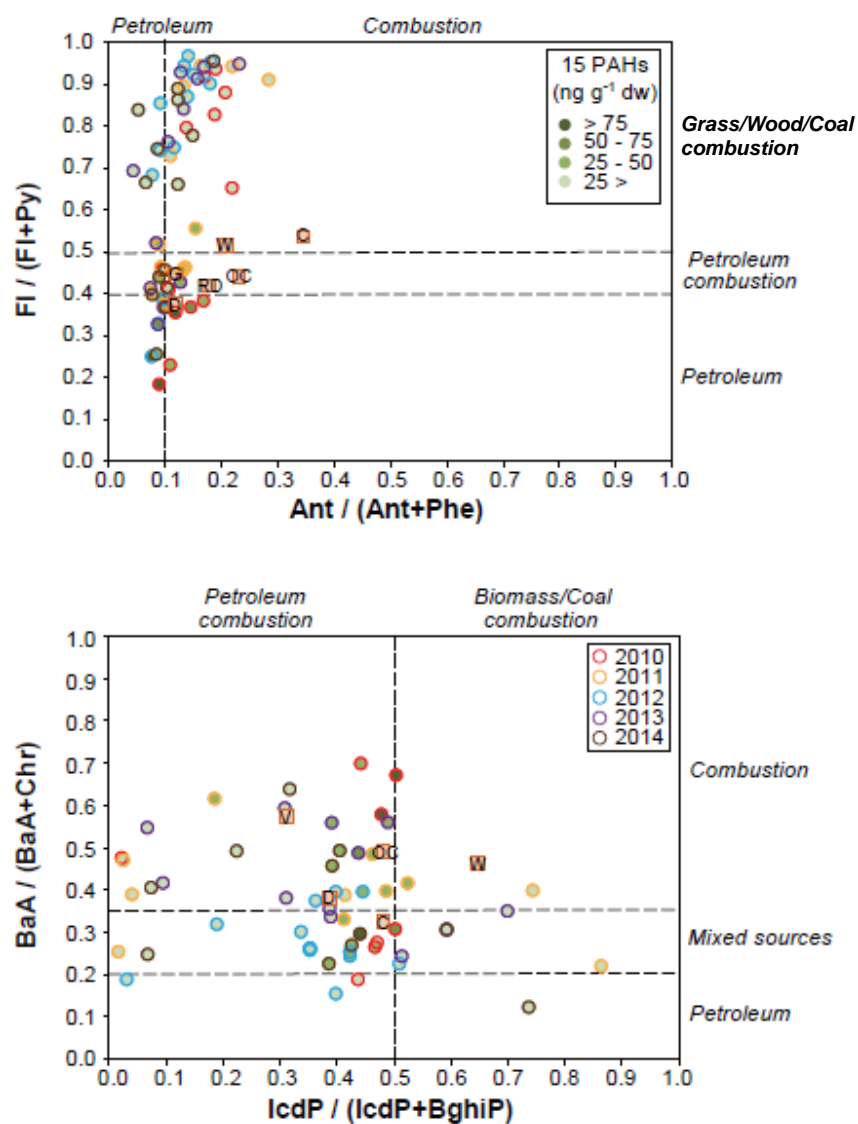


Fig. S2. Cross plots for the ratios between $FI / (FI + Py)$ and $Ant / (Ant + Phe)$ and the ratios between $BaA / (BaA + Chr)$ with $IcdP / (IcdP + BghiP)$ for source identifications of PAHs.

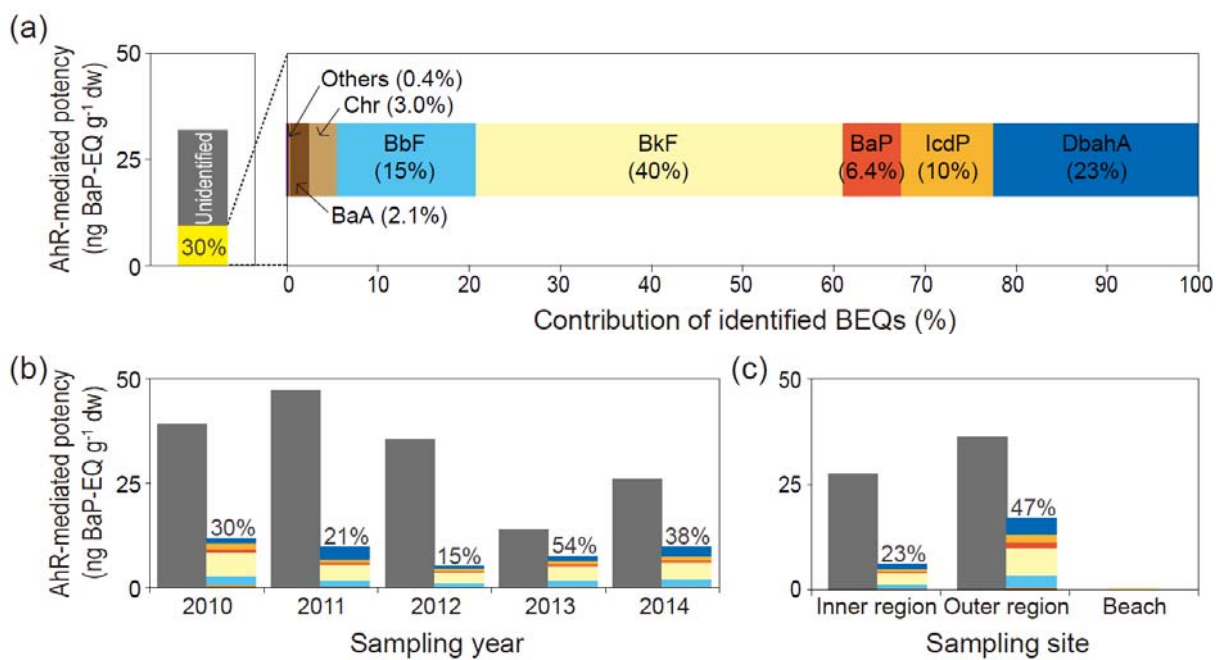


Fig. S3. (a) Contribution of identified BEQs by known PAH compounds to the overall induced BaP-EQs calculated by the average values of all sediment samples, (b) annual variations of BaP-EQs and BEQs in sediments, and (c) BaP-EQs and BEQs in sediments according to the area types.