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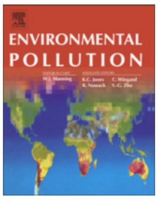

AhR-mediated potency of sediments and soils in estuarine and coastal areas of the Yellow Sea region: A comparison between Korea and China

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ABSTRACT

Extracts of sediments ($n = 45$) and soils ($n = 37$) collected from the coast of the Yellow Sea, in Korea and China, were screened for their ability to induce dioxin-like gene expression *in vitro* using the H4IIE-*luc*, transactivation bioassay. Significant dioxin-like potency was observed except for a few soils from Korea. Concentrations of TCDD-EQ (2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents) in sediments were comparable between Korea and China, but concentrations of TCDD-EQ in soil were 2-fold greater from Korea. Mass balance analysis indicated that concentrations of TCDD-EQ were to some degree chemical- and/or matrix-dependent, but were much more site-specific. For example, the proportion of the TCDD-EQ that could be identified varied among locations, which suggests different sources. Unidentified AhR-active compounds represented a greater proportion of the TCDD-EQ in samples from Korea, which suggests that sources in Korea were more complex than those in China. Potential sources of TCDD-EQ were investigated by considering land-uses and local activities.

1. Introduction

The aryl hydrocarbon receptor (AhR) is a ligand-dependent transcription factor which can be activated by numerous chemicals that have structures similar to that of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). Chemicals that have, or can attain, a planar configuration of approximately 3 × 10 å can bind to the AhR and result in expression of AhR-mediated responses (Giesy et al., 2002). These “dioxin-like” chemicals, among others, include polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), polychlorinated biphenyls (PCBs), and polycyclic aromatic hydrocarbons (PAHs) (Giesy and Kannan, 1998; Hilscherova et al., 2000; Palermo et al., 2005). It has been reported that the affinity with which chemicals bind to the AhR is directly proportional to the toxicity, including enhanced gene transcription and enzyme activities (Behnisch et al., 2001).

AhR agonists are of natural and human origin and include products and/or unwanted byproducts of combustion and due to their characteristics are widely distributed in environmental media and can be bioaccumulated and biomagnified (Tillitt et al., 1995; Van den Berg et al., 2006; Bittner et al., 2009). Risk assessments for dioxin-like compounds in sediments and soils are difficult due to the presence of complex mixtures of AhR agonists of differing persistence and accumulation and metabolic susceptibility (Giesy et al., 2002; Dévier et al., 2011).

There are several ways to characterize the overall potency of complex mixtures of AhR agonists of differential potency. The first is to measure all of the AhR agonists and then using relative potency factors to determine the overall potency of the mixture (Mousa et al., 1998). In this approach adopted by the World Health Organization (WHO) 2,3,7,8-TCDD equivalency factors are multiplied by the concentration of each AhR-active chemical in a mixture and reported as 2,3,7,8-TCDD equivalents (TEQ) (Van den Berg et al., 1998). Concentrations of TEQs in an extract are calculated as the sum of the product of the congener-specific toxic equivalency factor (TEF) and the concentration of the respective congener (Van den

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Berg et al., 1998; Villeneuve et al., 2000). Values for TEFs are consensus values to be used in risk assessments and are based on a range of endpoints. While TEFs correct for the relative potency of AhR agonists at the receptor, they do not correct for differential solubility, sorption, bioaccumulation, and biotransformation (Sanderson et al., 1996; Sanderson and Giesy, 1998). The TEFs used by the WHO were developed for use in risk assessments and are meant to be protective and not predictive (Van den Berg et al., 1998). The greatest limitation of the TEQ approach is the fact that all of the AhR agonists in a mixture need to be identified and quantified (Newsted et al., 1995). This can be highly challenging without comprehensive *a priori* knowledge concerning what AhR agonists might be present and/or without established analytical methods and standards for measuring them (Giesy et al., 2002). An additional limitation is the fact that TEFs have not been developed for many of the potential AhR agonists.

Because of the inherent limitations of the TEQ approach, a bioassay-based approach has been developed to measure the entire AhR-mediated potency of mixtures (Denison et al., 1993; Garrison et al., 1996). The H4IIE-*luc* transactivation bioassay measures the total potency of responses mediated by the AhR (Giesy et al., 1994a, 1994b). The H4IIE-*luc* assay is based on stably transfected cells in which the luciferase reporter gene has been inserted into the genome (El-Fouly et al., 1994). This transactivation assay uses the endogenous AhR of cells and the amount of light produced by the luciferase enzyme is directly proportional to binding of AhR agonists to the AhR. Results of the assay are expressed as 2,3,7,8-TCDD equivalents (TCDD-EQ). The emission of light, measured by use of a luminometer, is dependent on time, dose, persistence, and the potency of the AhR-active chemicals found in the sample/extract (Murk et al., 1996). The H4IIE-*luc* assay has been widely used as a screening tool for AhR-mediated potency of various complex mixtures in contaminated sediments, soils, and biological samples, such as human serum and plasma, and breast milk (Murk et al., 1996; Brown et al., 2000; Schroijen et al., 2006; Hasegawa et al., 2007; Hui et al., 2007; Kaisarevic et al., 2011). The results of the H4IIE-*luc* bioassay have been shown to be correlated with adverse outcomes caused by dioxin-like chemicals (Tillitt et al., 1991). Such a bioanalytical screening tool is a suitable and powerful alternative, because it is a relatively simple, rapid, integrative, and inexpensive, and reads out directly in total AhR-mediated potency expressed as concentrations of TCDD-EQ. Thus, the H4IIE-*luc* bioassay has been successfully applied in ecological risk assessment during the last few decades (Khim et al., 1999a; Hilscherova et al., 2001; Behnisch et al., 2003; Song et al., 2006).

The Yellow Sea, together with nearby coastal and riverine areas, is a major commercial artery of East Asia and has been significantly urbanized and industrialized (Luo et al., 2010; Naile et al., 2010). Rapid social and economic development in surrounding countries has brought economic development, but has also contributed to local contamination with persistent organic pollutants, metals and metalloids (Kim et al., 2007; Hu et al., 2010; Luo et al., 2010). Several major rivers including the Yellow, Liaohe, Haihe, Luanhe, and Dalinhe from China and Han, Geum, and Yeongsan from South Korea discharge directly into the Yellow Sea. The drainage areas of these rivers in both countries are used for both agricultural and chemical production, which can release inorganic and organic contaminants from both point- and nonpoint-sources. Because the Yellow Sea is a semi-enclosed system, water exchange with the Pacific Ocean is relatively slow; and as a result, such persistent pollutants tend to sediment and accumulate.

There have been studies of well-known AhR agonists such as PCDD/Fs, PCBs, and PAHs in sediments and soils of estuarine and riverine areas of Korea and China (Khim et al., 1999a; Lee et al., 2001; Naile et al., 2011). However, few studies have reported total concentrations of AhR-mediated potency by these compounds and/or other compounds, including unidentified natural and synthetic chemicals in sediments and soils from north coastal and riverine regions of the Yellow Sea. In particular, this information was not previously available for the Bohai Sea area of China. Therefore, baseline information on AhR-mediated potency of sediments and soils was needed to assess current environmental conditions and associated risk in aquatic and coastal environments of the region. In addition, in support of management decisions, it was necessary to know the nature of the compounds, including novel chemicals, that comprise the TCDD-EQ and if possible to identify their sources.

The present study was designated primarily for the purpose of investigating 1) the AhR-mediated activities of sediments and soils by use of H4IIE-*luc* bioassay, 2) the concentrations and sources of AhR-agonists using chemical analysis, and 3) the contributions of each AhR agonists to the total induced AhR-mediated potency (viz., mass balance analysis). Locations were chosen to detect possible point sources which were major freshwaters along the coasts of the West Sea of Korea and North Bohai Sea of China discharging directly into the Yellow Sea system. The results of this study will provide baseline information on AhR-mediated potency of sediments and soils in Yellow Sea regions and useful for regulation of chemicals of concern and predictive ecological risk assessment.

2. Materials and methods

2.1. Sampling

Sediments and soils were collected from 47 and 41 locations in Korea and China, respectively. Samples were collected from areas with different land uses, along the estuarine and coastal areas of the Yellow Sea during April and May, 2008 (Fig. 1 and Table S1 of Supplemental Materials). Surface sediments (0–10 cm) were collected from 12 sites in Korea and 35 sites in China. Soils were collected from 11 sites in Korea and 30 sites in China. Samples of individual soils consisted of approximately 15 cm of top soil from a central point and 15 cm of top soil from each of four additional points located 10–20 m in the four primary directions (N, E, S, and W) from the central point. Composite soil samples were then prepared after thoroughly mixing the sub-samples. All samples were immediately transferred to the laboratory and stored at −20 °C until analyses.

2.2. Sample preparation

Detailed descriptions of sample preparation have been published previously (Khim et al., 1999a; Koh et al., 2006). In brief, a 10 g sample of freeze-dried sediment or soil was extracted for 24 h by use of 400 mL of dichloromethane (DCM, Burdick and Jackson, Muskegon, MI, USA) in a Soxhlet extractor. Elemental sulfur was removed by reaction with activated copper (Merck, Darmstadt, Germany) and the extracts were concentrated to 1 mL. The extract was divided into two aliquots for use in the bioassay or identification and quantification of individual compounds. The portion of the extract to be used in the bioassay was transferred into dimethyl sulfoxide (DMSO, Burdick and Jackson) by use of differential volatilization.

2.3. In vitro bioassay

The H4IIE-*luc*, transactivation, reporter bioassay was performed by use of a slight modification of the methods of Khim et al. (1999b). 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 by adding 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 by 5-fold serial dilution (100, 20, 4.0, 0.8, 0.16, or 0.03%). All samples were tested in triplicate wells in the same assay. Emitted light was measured after 72 h of exposure by use of a 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 as described in detail elsewhere (Yoo et al., 2006). No cytotoxic effects were observed in H4IIE-*luc* cells during exposure to sediment or soil extracts of any samples.

2.4. Determination of TCDD-EQ

Responses of the bioassay, expressed as mean relative luminescence units were converted to a percentage of the maximum response ($\%2,3,7,8\text{-TCDD}_{max}$) for a standard containing 75 nM (=100 $\%TCDD_{max}$) 2,3,7,8-TCDD (Wellington

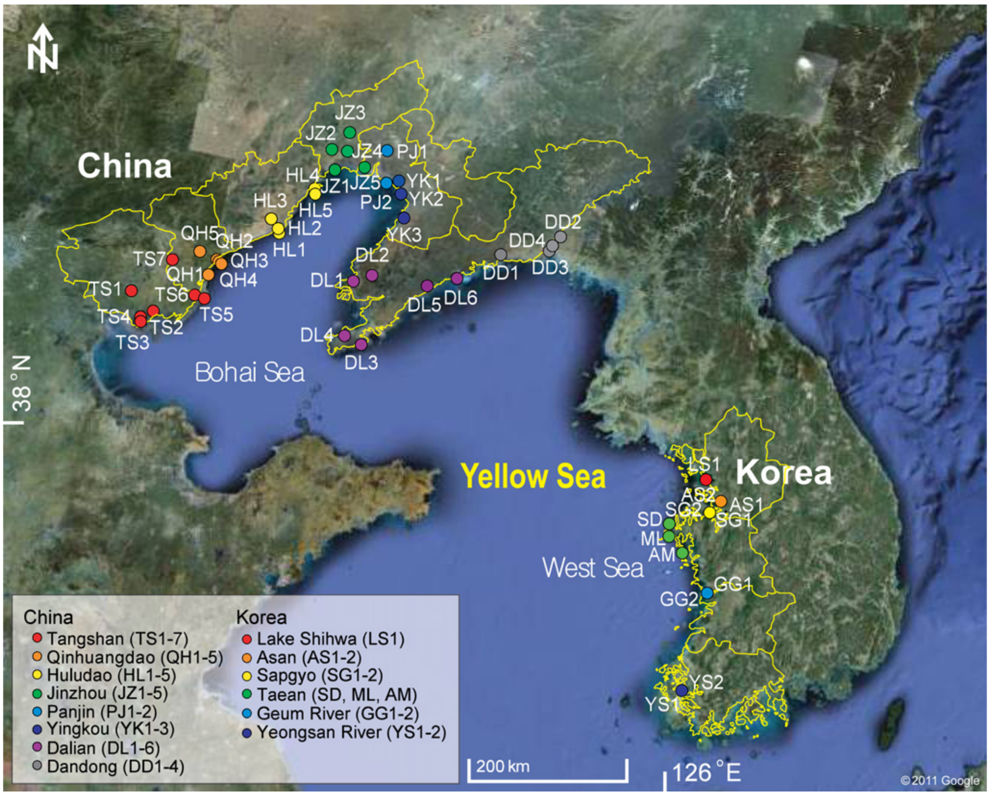

Fig. 1. Location map of sediment and soil samples from estuarine and coastal areas of the Yellow Sea.

Laboratories Inc., Guelph, OT, Canada). Significant responses (=3.7 %$TCDD_{max}$) 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 2,3,7,8-TCDD equivalents (TCDD-EQs) were determined directly from sample dose–response relationships generated by testing samples at multiple (at least 3 points) of dilution (0.030, 0.15, 0.75, 3.8, 18.8, or 75 nM TCDD) (Villeneuve et al., 2000).

2.5. Identification and quantification of individual chemicals

PAHs were quantified by use of previously published methods (Jiao et al., 2009, 2012). The extract was fractionated, and interferences were removed by use of a silica gel column. PAHs in sediments and soils were identified and quantified by use of an Agilent model 6890 gas chromatograph (GC) equipped with a model 5973 mass selective detector (MSD) in selective ion monitoring mode. A HP-5 fused silica capillary column (60 m × 0.25 mm i.d. × 0.25 μm film thickness) was used with helium as a carrier gas at a constant flow rate of 1 mL min^{-1}. Sixteen US EPA priority PAHs including naphthalene (Na), acenaphthylene (Acl), acenaphthene (Ace), fluorine (Flu), phenanthrene (Phe), anthracene (Ant), fluoranthene (Fl), pyrene (Py), benzo[*a*]anthracene (BaA), chrysene (Chr), benzo[*b*]fluoranthene (BbF), benzo[*k*]fluoranthene (BkF), benzo[*a*]pyrene (BaP), indeno[*1,2,3-c,d*]pyrene (IcdP), dibenz[*a,h*]anthracene (DBahA), and benzo[*g,h,i*]perylene (BghiP) were quantified. Detection limits for PAHs ranged from 1.7 to 4.9 ng g^{-1} dw.

2.6. Calculation of TEQ

Total concentrations of TEQs based on chemical analysis of AhR agonists were calculated as the sum of TEQs by multiplying the concentration of each AhR-active chemical by appropriate relative potency (ReP) values. In addition to total concentrations of TEQ (TEQ_{Total}) related to two classes of chemicals were also calculated, including PCDD/Fs ($TEQ_{PCDD/Fs}$) and PAHs (TEQ_{PAH}) (Jiao et al., 2012; Naile et al., 2011). Relative potencies for AhR-active compounds in the H4IIE-*luc* assay were used for $TEQ_{PCDD/Fs}$ according to Behnisch et al. (2003). Seven dioxin-like PAHs (DL-PAHs) including BaA, Chr, BbF, BkF, BaP, IcdP, and DBahA were used to calculate concentrations of TEQ_{PAH} for AhR-active PAHs by use of ReP as described by Villeneuve et al. (2002).

2.7. Statistics

SPSS 12.0 and SigmaPlot 2001 (for Windows, SPSS Inc., Chicago, IL, USA) were employed for statistical analyses. Correlation-based principal component analyses (PCA) were conducted by use of relative contributions (%) of AhR agonists (chemical analysis-derived) to total concentrations of TCDD-EQ activity (bioassay-derived) in sediments and soils of Korea and China. The PCA was used to convert the contribution of variables into two or three principal components (PCs) which were used to identify the dominant AhR agonists in sediments and soils. Results are shown in Fig. S2 of Supplemental Materials.

3. Results and discussion

3.1. TCDD-EQ in sediments and soils

Ten out of 12 sediments from Korea and 25 out of 33 sediments from China exhibited detectable concentrations of TCDD-EQ in organic extracts (Table 1, Fig. 2). Concentrations of TCDD-EQ in sediments ranged from <3.4 to 11 pg g^{-1} dw (max. at location GG1, mean: 4.6 pg g^{-1} dw) and from <3.4 to 28 pg g^{-1} dw (max. at location JZ4, mean: 4.9 pg g^{-1} dw) in samples from Korea and China, respectively. In soils, 9 of 11 samples in Korea and 22 of 26 samples in China were significantly induced (Table 1, Fig. 2). Concentrations of TCDD-EQ ranged from <3.4 to 23 pg g^{-1} dw (max. at location AS2, mean: 9.2 pg g^{-1} dw) and from <3.4 to 9.7 pg g^{-1} dw (max. at location HL4, mean: 3.8 pg g^{-1} dw) in samples from Korea and China, respectively. In general, concentrations of TCDD-EQ did not exceed sediment (<30 pg g^{-1}, possible-effect level) and soil (<50 pg g^{-1}, screening level) quality guidelines of the United States for dioxin-like compounds (De Rosa et al., 1999; Zhang et al., 2009). However, some sites both in Korea and China exceeded the Canadian sediment and soil quality guidelines (<0.85 pg g^{-1} for sediment; <4 pg g^{-1} for soil) (CCME, 2001, 2002).

The West coast of Korea is highly industrialized and urbanized and water and sediment have been frequently reported to be contaminated with various classes of organic and inorganic chemicals, especially in areas near industrial complexes and major cities (Khim et al., 1999a; Lee et al., 2001; Hong et al., 2010; Naile

Table 1

Overview of results for *in vitro* bioassay and chemical analysis of sediments and soils collected from Korea and China.

Analysis		Unit	Range	Sediment		Soil	
				Korea ($n = 12$)	China ($n = 34$)	Korea ($n = 11$)	China ($n = 31$)
In vitro bioassay	H4IIE-*luc* responses[a]	$\%TCDD_{max}$	Min.–Max.	<3.7–29	<3.7–64	<3.7–43	<3.7–27
			Mean	8.8	13	22	9.1
	TCDD-EQ[b]	pg g^{-1} dw	Min.–Max.	<3.4–11	<3.4–28	<3.4–23	<3.4–9.7
			Mean	4.6	4.9	9.2	3.8
Chemical analysis	DL-PAHs[c]	ng g^{-1} dw	Min.–Max.	30–210	36–1100	34–600	32–550
			Mean	86	260	160	140
	PCDD/Fs[d]	pg g^{-1} dw	Min.–Max.	<DL–140	<DL–42	0.81–150	<DL–120
			Mean	38	9.6	41	19
	TEQ_{PAHs}[e]	pg g^{-1} dw	Min.–Max.	0.17–0.93	0.27–9.3	0.21–5.9	0.12–4.2
			Mean	0.44	2.0	1.2	1.2
	$TEQ_{PCDD/Fs}$[f]	pg g^{-1} dw	Min.–Max.	<0.001–1.3	<0.001–3.4	0.010–7.6	<0.001–6.8
			Mean	0.36	0.22	1.2	0.48
	TEQ_{chem}[g]	pg g^{-1} dw	Min.–Max.	0.17–1.9	0.29–12	0.40–14	0.12–8.7
			Mean	0.80	2.2	2.4	1.6
Bioassay vs. chemical analysis	TEQ_{chem}/TCDD-EQ	%	Min.–Max.	5.3–59	4.6–310	3.1–150	3.4–120
			Mean	18	56	33	39
	TEQ_{PAHs}/TCDD-EQ	%	Min.–Max.	5.3–23	4.6–310	2.5–66	4.4–96
			Mean	11	53	17	30
	$TEQ_{PCDD/Fs}$/TCDD-EQ	%	Min.–Max.	<0.001–42	<0.001–31	0.42–85	<0.001–70
			Mean	7.4	2.8	16	9.1

[a] Response magnitude presented as percentage of the maximum response observed for a 75 nM TCDD standard (set to 100 $\%TCDD_{max}$) elicited by 100% sediment raw extracts.

[b] Potencies of samples (screening results) expressed as equivalents relative to 2,3,7,8-TCDD standard.

[c] Concentration of dioxin like-PAHs was used the sum of BaA, Chr, BbF, BkF, BaP, IcdP, and DBahA.

[d] Concentration of PCDD/Fs was referred from our previously published data (Naile et al., 2011).

[e] TEQ_{PAHs} were calculated using RePs values from Villeneuve et al. (2002).

[f] $TEQ_{PCDD/Fs}$ were calculated using RePs values from Behnisch et al. (2003).

[g] $TEQ_{chem} = TEQ_{PAHs} + TEQ_{PCDD/Fs}$.

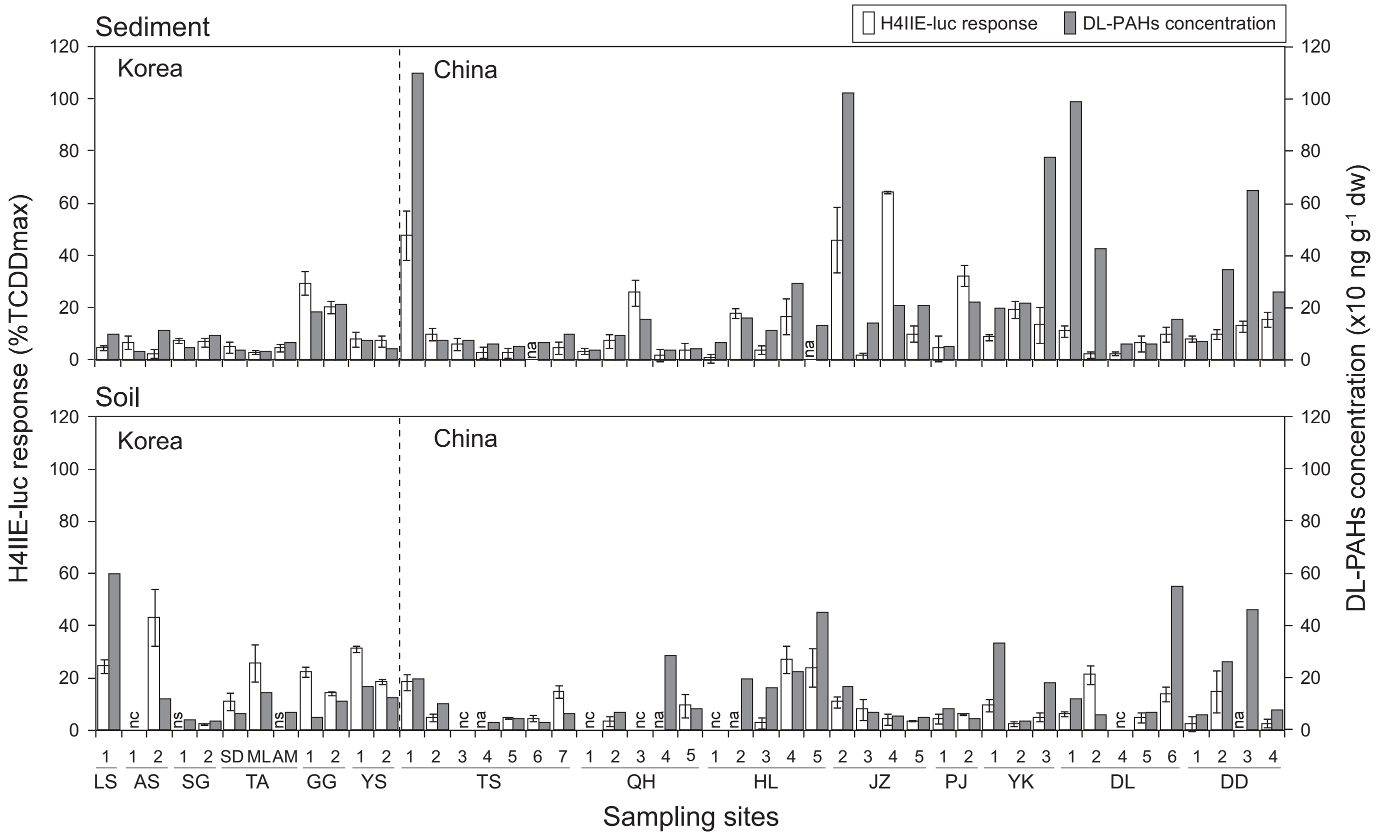

Fig. 2. Distribution of H4IIE-*luc* responses and DL-PAHs in sediment and soil samples of Korea and China (na: not analyzed; nc: not collected; ns: not significant; error bar: standard deviation).

et al., 2011). Relatively great concentrations of TCDD-EQ in sediments at location GG1 and soils at location AS2 and YS1 exceeded 10 pg g^{-1} dw. In China, at locations TS1, JZ2, and JZ4 sediments contained greater than 10 pg TCDD-EQ g^{-1} dw, but concentrations in soils did not exceed 10 pg TCDD-EQ g^{-1} dw. Locations JZ2 and JZ4 were adjacent to the cities of Jinzhou and Huludao, which are centers of the chemical industries in China (Wang et al., 2011). Effluents and runoff from factories and cities along the rivers are likely sources of TCDD-EQ (Jiao et al., 2012). Sediments and soils of the West coast of Korea and North coast of Bohai Sea in China contained detectable concentrations of TCDD-EQ which seemed to be due to regional human activities.

Several trends in concentrations of TCDD-EQ in sediments and soils were observed in Korea and China. In general, concentrations of TCDD-EQ in sediments in China were greater than those of Korea. However, the trend for soils was the opposite. Concentrations of TCDD-EQ in sediments and soils were not significantly correlated among locations. This result indicates that TCDD-EQ present in sediments probably weren't derived from local soils. Soils from the West coast of Korea contained greater concentrations of TCDD-EQ than did sediments. However, in China, in general concentrations of TCDD-EQ in sediments were greater than those in soils. These results are consistent with the hypothesis that the primary source of TCDD-EQ to the environment along the West coast of Korea is deposition from the atmosphere. It is likely that concentrations of TCDD-EQ in sediments were related to local effluents while those in soils were dominated by deposition from the atmosphere.

3.2. AhR agonists in sediments and soils

Seven dioxin-like PAHs (DL-PAHs) including BaA, Chr, BbF, BkF, BaP, IcdP, and DBahA were commonly detected in sediments and soils in Korea and China (Table 1, Fig. 2). Concentrations of DL-PAHs in sediments of China were generally greater than those of Korea. While, significant differences in concentrations of DL-PAHs in soils between Korea and China were not observed. In general, concentrations of PAHs in sediment from Korea varied less among locations than those in China. Relatively great concentrations of DL-PAHs (>500 ng g^{-1} dw) were found in sediments from locations TS1, JZ2, YK3, DL1, and DD3 in China. These sites were located near industrial complexes and/or adjacent to cities and are considered to be hotspots for other groups of pollutants such as perfluorinated compounds (Naile et al., 2011; Wang et al., 2011). Sediments collected from locations TS1 and JZ2 contained the greatest concentrations of TCDD-EQ, which is consistent with concentrations of DL-PAHs at those locations. While only two soils, location LS1 in Korea and DL6 in China contained relatively great DL-PAHs concentrations (>500 ng g^{-1} dw). Concentrations of 16 PAHs did not exceed environmental quality guidelines such as the effect range low (ERL) for sediment (Long et al., 1995) or soil quality guidelines (SQG) (CCME, 2010).

Patterns of relative concentrations of several PAHs in sediments and soils differed between Korea and China (Fig. S1 of Supplemental Materials). For example, in sediments, the average relative concentrations of DBahA were 7.8% and 0.6%, BkF were 0.6% and 2.2% in Korea and China, respectively. In soils, relative concentrations of DBahA and BghiP were greatest between the two countries, with values 5.5% and 0.6% of DBahA and 22.5% and 7.6% of BghiP in Korea and China, respectively. Diagnostic ratios between individual PAHs in sediments and soils from Korea and China were applied to assess the contribution of petroleum or combustion sources of PAHs (Jiao et al., 2012) (Fig. 3). Ratios of Ant/(Ant + Phe), Fl/(Fl + Py), and BaA/

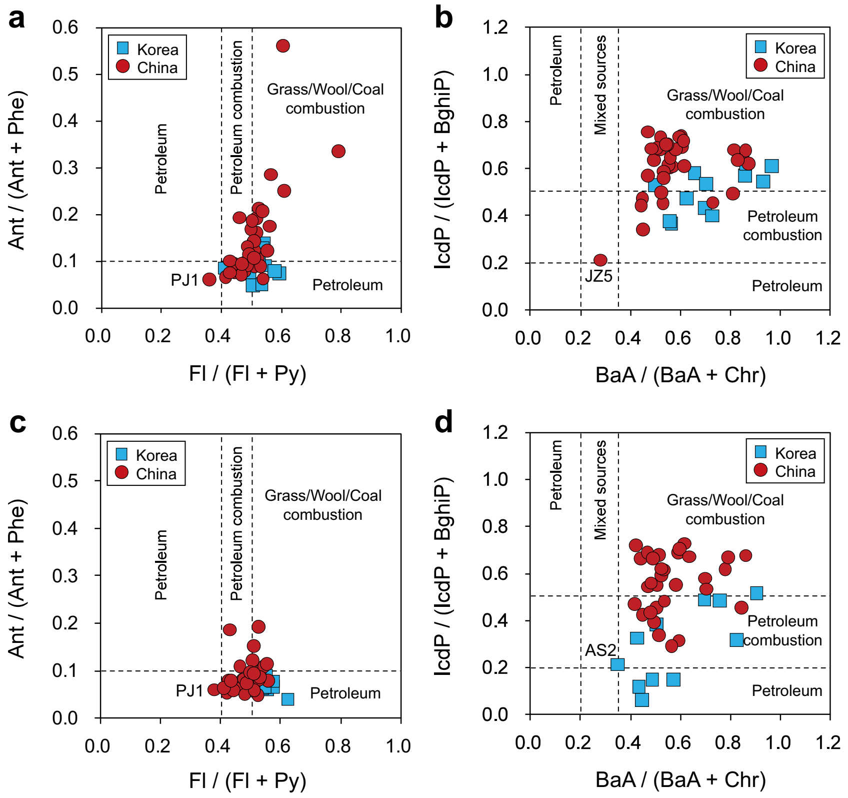

Fig. 3. Plots of diagnostic ratios of individual PAHs species for source identification ((a) and (b): sediments; (c) and (d): soils).

(BaA + Chr) in sediments and soils of Korea and China indicated that PAHs originated mainly from combustion of petroleum, grass, wool, and coal, and the sources of PAHs were not significantly different between Korea and China. However, the IcdP/(IcdP + BghiP) ratio for sediments and soils were partly distinguished between Korea and China. A relatively lesser IcdP/(IcdP + BghiP) ratio (<0.5) was observed in sediments and soils of Korea, which indicates petroleum combustion as the origin. The IcdP/(IcdP + BghiP) ratio in sediments and soils from China showed relatively great values, indicating grass, wool, and coal combustion origins. Although specific sources of PAHs in Korea and China could not be fully attributed by use of reported diagnostic ratios of PAHs, sources of PAHs were found to be slightly different in China and Korea. Thus, the control of inputs of PAHs would require precise characterizing of different sources in the two countries.

PCDD/Fs are known AhR agonists which often contribute significant portions of the TCDD-EQ observed in sediments and soils (Safe, 1998; Van den Berg et al., 2006). However, according to our previous study, PCDD/Fs in sediments and soils in this region of Korea and China were not generally great compared with corresponding environmental quality guidelines (Naile et al., 2011). Distributions of PCDD/Fs in the Yellow Sea region reflected local activities and matrix-dependent sinks which mainly originated from herbicides such as pentachlorophenol and chloronitrophen (Naile et al., 2011). The proportion of total TEQ contributed by PCDD/F ($TEQ_{PCDD/Fs}$) in the sediments and soils from Korea and China has been determined to be small (Naile et al., 2011). The concentration of $TEQ_{PCDD/Fs}$ has been determined to be at least 10-fold lesser than that of TEQ_{PAHs} (Table 1). There have been several reports that concentrations of dioxin-like PCBs (DL-PCBs) were also not significant contributors to the total concentration of TEQ in sediments and soils of the West coast of Korea and North coast of the Bohai Sea of China (Hong et al., 2006; Liu et al., 2006). Altogether, it is indicated that DL-PAHs would be the dominant, prevailing contributors to total concentration of TEQ in sediments and soils of the coastal regions of the Yellow Sea.

3.3. Mass balance analysis between TCDD-EQ and TEQ

Mean total concentrations of TCDD-EQ in sediments were comparable in Korea and China, but the proportion of identified contributors to the TCDD-EQ differed between samples from China and Korea. A mass balance analysis between TCDD-EQ and TEQ was conducted to determine explained vs. unexplained contributions to TCDD-EQ (Khim et al., 1999a). In the present study, TCDD-EQ is defined as the total potency of the mixture of AhR agonists as determined by the H4IIE-*luc* bioassay and TEQ was the sum of TEQ_{PAHs} and $TEQ_{PCDD/Fs}$ (Fig. 4). In sediments the

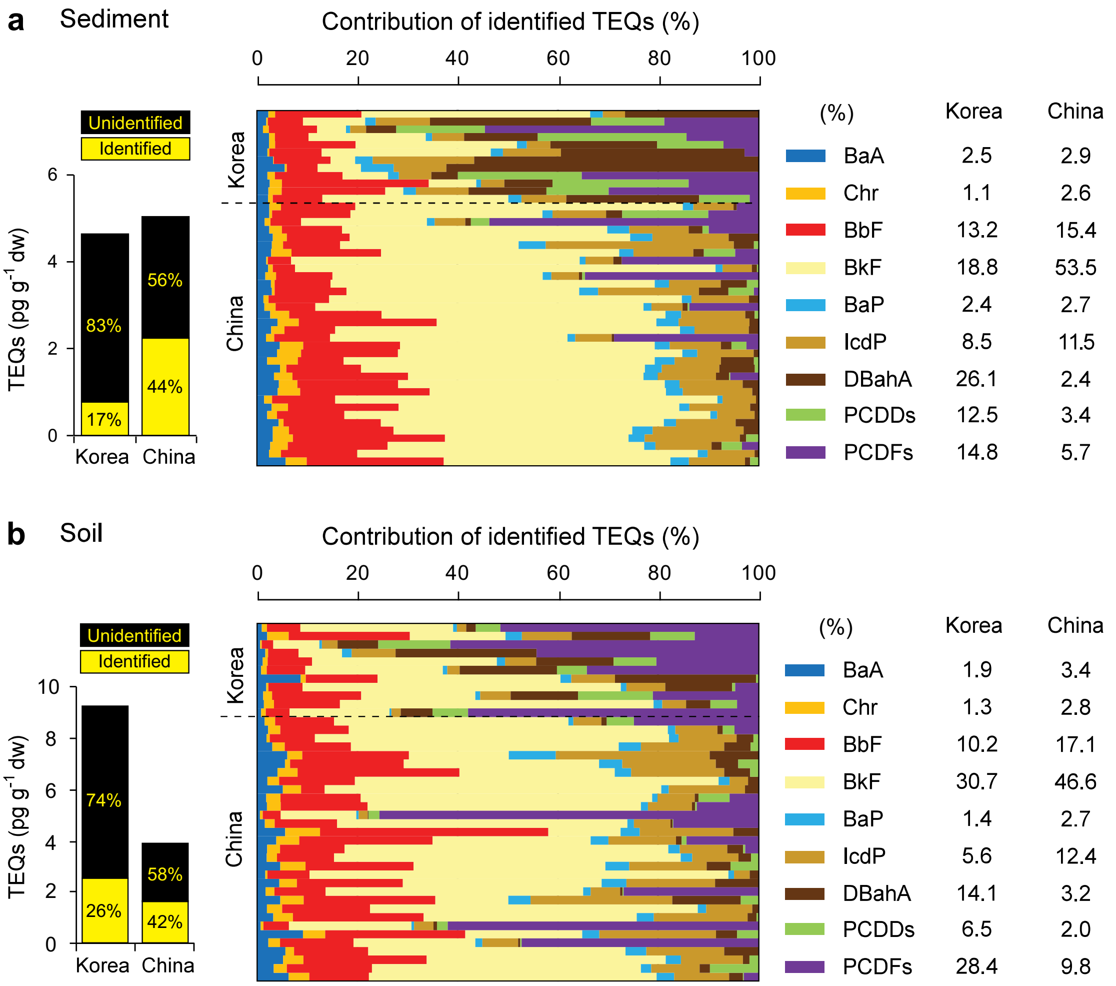

Fig. 4. Identification and relative contribution of AhR-mediated activities of (a) sediments and (b) soils from Korea and China.

proportion of TCDD-EQ that could be accounted for by TEQ based on PAHs and PCDD/Fs was 18% and 56% for samples from Korea and China, respectively (Table 1, Fig. 4). In sediments from China, DL-PAHs were the dominant AhR agonists contributing 53% to the TCDD-EQ while PCDD/Fs contributed only 2.8%. Due to its greatest relative potency among DL-PAHs, BkF was the predominant identified AhR agonists in sediments of China (Fig. 4a). Alternatively, in sediments from Korea, DL-PAHs and PCDD/Fs accounted for 11% and 7.4% of the TCDD-EQ, respectively. DBahA was the predominant contributor to TEQs, followed by BkF, PCDFs, BbF, and PCDDs. Compared to sediments, in soils contributions of DL-PAHs, PCDDs, and PCDFs to the identified TEQs in soils varied among sampling locations in Korea and China. In soils, BkF was the dominant contributor (31% for Korea and 47% for China) (Fig. 4b). In soils from Korea PCDFs and DBahA each contributed approximately 42% of the TEQ. However, those chemicals accounted for only a small portion of the total concentration of TCDD-EQ in soils from China (9.8% for PCDFs and 3.2% for DBahA). These results suggest that the major contributors to AhR-mediated potency in sediments and soils were considerably different between China and Korea and that sources of AhR agonists must also be different. The proportion of TCDD-EQ in soils from Korea that could not be identified was greater than that for soils from China, where a greater proportion of the total concentration of TCDD-EQ could be explained. This result indicated that unknown sources of dioxin-like chemicals and/or antagonistic effects were evident in Korea. Differences in contributions of different AhR agonists to TCDD-EQ in soils could be caused by several factors, including sources, but also differential weathering due to microbial biotransformation (Haritash and Kaushik, 2009), adsorption on particles (Tremblay et al., 2005), and/or bioaccumulation in the water column (Landrum et al., 2003).

In order to further characterize the TCDD-EQ in sediments and soils of Korea and China, PCA was conducted, based on the relative (percent) contributions of DL-PAHs and PCDD/Fs to total TEQ (Fig. S2 of Supplemental Materials). Results of the PCA for sediments showed two major components that collectively accounted for 59.3% of the total variance. PC1 (explaining 37.3% of the total variance) was strongly and positively correlated with most of DL-PAHs, yet negatively with PCDDs and PCDFs. PC2 (explaining 22.0% of the total variance) was positively correlated with concentrations of DBahA, BaP, and IcdP, yet negatively correlated with concentrations of BkF. Scatter plots for PC1 and PC2 for sediments showed a clear separation of patterns in Korea and China. The major difference between sediments from Korea and China was due to differences in relative contributions of DL-PAHs, PCDDs, and PCDFs. For instance, differences in ordination of sediments were primarily controlled by the DL-PAHs, while PCDDs were common factors influencing TCDD-EQ in both countries. Similarly, soils in Korea and China were also separated along major components of PC1 (explaining 47.0% of the total variance) and PC2 (explaining 21.0% of the total variance). Concentrations of most DL-PAHs were positively correlated with PC1, indicating that those chemicals were common contributors to soils in both Korea and China. Overall, the results of the PCA analysis demonstrated that both matrix and chemical compositions were associated with different magnitudes and compositions of TCDD-EQ.

3.4. *Unknown AhR agonists in sediments and soils*

Due to the complex nature of environmental samples together with limited instrumental support, causative agents of observed bioassay responses are often not identified (Table 2). In the present study, AhR-mediated potency expressed as TEQ that were calculated based on concentrations of AhR agonists determined by use of instrumental analysis explained less than half to total concentration of TCDD-EQ. Results of several previous studies suggested concentrations of TCDD-EQ could be explained by measured concentrations of target chemicals (Koh et al., 2004). Several consistent trends based on the identification of bioassay-derived AhR activity by chemical analysis in sediments and soils were observed. Proportions of TCDD-EQ in sediments that could be identified were generally greater than those of soils. Total concentrations of TCDD-EQ that could be accounted for by known chemicals were greater in sediments collected from the Yellow Sea and in particular Bohai Bay than those collected more inland. The greatest proportions of TCDD-EQ were accounted for by TEQ in the more contaminated areas, such as the Taizhou area and the Hyeongsan River.

In previous studies, PCDD/Fs, DL-PCBs, and DL-PAHs have been found to be the primary classes of chemicals contributing to TCDD-EQ in sediments and soils (Table 2). However, TEQ calculated based on these three classes of chemicals often account for less than 20% of the TCDD-EQ, which suggests that unknown AhR agonists generally exist in sediments. For example, TEQ calculated based on polybrominated dibenzo-*p*-dioxins and dibenzofurans (PBDD/Fs) and polybrominated diphenylethers (PBDEs) contributed a maximum of 8.3% (mean: 5.0%) to TCDD-EQ measured in sediments of Osaka Bay (Takigami et al., 2005). Moreover, certain compounds such as polychlorinated naphthalenes (PCNs), polybrominated biphenyls (PBBs), acid labile compounds, and humic and fulvic acids have been reported to have AhR agonist properties and thus contribute to TCDD-EQ (Villeneuve et al., 2000; Chen and Bunce, 2003; Bittner et al., 2009, 2011; Luo et al., 2009). These unanalyzed chemicals in sediments and soils could explain the remaining unknown portion of TCDD-EQ in samples studied .

3.5. *Application of mass balance analysis for risk assessment*

While concentrations of TCDD-EQ are useful in determining the status and trends and spatial distribution of AhR agonists, concentrations of TCDD-EQ cannot be used to determine sources. To be used in risk assessments, concentrations of TCDD-EQ in sediments would need to be related to concentrations observed to cause adverse effects. Unfortunately, the bioavailable fractions of the TCDD-EQ are not known and concepts such as bioaccumulation and trophic magnification factors cannot be applied to TEQ or TCDD-EQ. Application of TEFs or RePs to correct for the relative potencies of AhR agonists would need to be done for measured or predicted concentrations of individual compounds in target tissues. But because TEQ and TCDD-EQ represent the aggregate potency of a mixture and each constituent in the mixture has compound-specific chemical and physical properties and different rates of biotransformation, to estimate concentrations of TEQ in tissues would require measuring the individual compound in the tissue of concern or by predicting the accumulation of individual compounds and multiplying by respective TEFs or RePs. Alternatively, the bioassay can be used to measure concentrations of TCDD-EQ in tissues directly. Finally, when making mass balance comparisons, it is important to use ReP values that are specific to the bioassay being used to determine concentrations of TCDD-EQ. It is not appropriate to use TEFs, which are consensus values based on multiple endpoints and meant to be protective rather than predictive to calculate TEQ for use in mass balance calculations.

Table 2

Comparison of TCDD-EQ and TEQ$_{\text{chem}}$ in sediment and soil samples from this study and previous reported studies.

Country	Regions	Surrounding activity	Samples from	Sample type	*n*	TCDD-EQ[a] (Min.–Max.) (pg g^{-1} dw)	TEQ$_{\text{chem}}$ (pg g^{-1} dw)				Identified AhR activity[b] (%) (Min.–Max.)	References
							TEQ$_{\text{PCDD/Fs}}$ (Min.–Max.)	TEQ$_{\text{PCBs}}$ (Min.–Max.)	TEQ$_{\text{PAHs}}$ (Min.–Max.)	TEQ$_{\text{others}}$ (Min.–Max.)		
South Korea	West coast area	Industrial, municipal, and agricultural area	Estuarine and coastal area	Sediment	12	>4.6 (<DL–11)	0.36 (<DL–1.3)	na[c]	0.44 (0.17–0.93)	na	18 (5.3–59)	This study
			Estuarine and coastal area	Soil	11	9.2 (<DL–23)	1.2 (<DL–7.6)	na	1.2 (0.21–5.9)	na	33 (3.1–150)	
	Masan Bay	Industrial and municipal area	Inland	Sediment	8	320 (63–880)	na	0.059 (0.010–0.15)	120 (10–250)	na	35 (17–75)	Koh et al. (2005)
		Industrial and municipal area	Bay	Sediment	28	700 (39–3600)	na	0.21 (0.033–0.92)	220 (24–690)	na	66 (10–370)	Khim et al. (1999a, 1999b)
	Ulsan Bay	Industrial and municipal area	Inland	Sediment	11	1600 (5.0–7600)	na	0.06 (<DL–0.18)	75 (8.6–340)	na	54 (1.4–210)	Khim et al. (2001)
		Industrial and municipal area	Bay	Sediment	16	180 (7.6–800)	na	0.06 (<DL–0.47)	200 (13–950)	na	190 (54–360)	Khim et al. (2001)
	Hyeongsan River	Steel and iron industrial complex	River	Sediment	5	380 (14–1500)	270 (5.5–1000)	1.7 (0.16–3.7)	22 (0.20–88)	na	58 (17–98)	Koh et al. (2004)
	Yeongil Bay	Industrial and municipal area	Bay	Sediment	26	56 (2.8–320)	na	0.004 (<DL–0.058)	124 (<DL–1500)	na	200 (0.70–670)	Koh et al. (2006)
	Taean area	Hebei Sprit oil spill affected area	Coastal area	Sediment	50	330 (1.6–2500)	na	na	0.55 (0.01–2.6)	na	4.5 (0.01–34)	Hong et al. (2012)
China	North coast of Bohai Sea	Industrial, municipal, and agricultural area	Estuarine and coastal area	Sediment	34	4.9 (<DL–28)	0.22 (<DL–3.4)	na	2.0 (0.27–9.3)	na	56 (4.6–310)	This study
			Estuarine and coastal area	Soil	31	3.8 (<DL–9.7)	0.48 (<DL–6.8)	na	1.2 (0.12–4.2)	na	39 (3.4–120)	
	Haihe and Dagu River	Industrial and domestic area	River	Sediment	5	5300 (550–14,000)	245[d] (6.8–890)		na	na	17 (0.1–76)	Song et al. (2006)
	Meiliang Bay	Tourism and waterworks area	Bay	Sediment	8	26 (15–34)	3.2 (1.9–6.0)	0.3 (0.2–0.4)	26 (15–34)	na	104 (83–120)	Qiao et al. (2006)
	Taizhou area	E-waste recycling sites	Inland	Soil	8	170 (2.6–380)	8.0 (0.50–20)	170 (0.30–380)	2.5 (0.10–9.1)	na	99 (10–200)	Shen et al. (2008)
	Wenyu River	Industrial and municipal area	River	Sediment	5	47[e] (15–64)	10 (5.1–13)	28 (9.4–38)	10 (4.9–21)	na	110 (79–130)	Luo et al. (2009)
Japan	Osaka Bay	Industrial and urban area	Bay	Sediment	6	54 (3.7–140)	22 (1.8–48)	13 (0.04–44)	na	5.0[f] (0.30–8.3)	76 (58–105)	Takigami et al. (2005)
Germany	Danube River	Industrial and municipal area	River	Sediment	6	560 (110–1400)	69 (28–130)		na	na	20 (5.5–40)	Keiter et al. (2008)
South Africa	Vaal Triangle area	Industrial area	Inland	Soil	4	32 (16–70)	4.6 (0.34–11)	1.4 (0.20–4.4)	na	na	15 (4.6–25)	Nieuwoudt et al. (2009)
USA	Shiawassee River	Industrial area	River	Sediment	28	461 (0.01–3100)	1.9 (0.2–9.8)		na	na	0.41	Kannan et al. (2008)
				Soil	10	619 (65–1520)	7.2 (1.3–20.9)		na	na	1.2	
	Saginaw River	Industrial area	River	Sediment	20	514 (0.01–3630)	375 (3.0–3820)		na	na	73	
				Soil	13	336 (0.01–3150)	22.5 (1.4–82.6)		na	na	6.7	
	Saginaw Bay	Industrial area	Bay	Sediment	5	217 (<DL–547)	103 (3.0–266)		na	na	47	
				Soil	6	46.6 (<DL–261)	12.4 (1.0–55)		na	na	27	

[a] Mean values of bioassay-derived TCDD-EQ.

[b] Identified AhR activity (%) = TEQ$_{\text{chem}}$/TCDD-EQ × 100.

[c] Not analyzed.

[d] Sum of TEQ$_{\text{PCDD/Fs}}$ and TEQ$_{\text{PCBs}}$.

[e] Ah agonistic effects of acid stable fraction.

[f] TEQ$_{\text{PBDD/Fs}}$ (polybrominated dibenzo-*p*-dioxins and dibenzofurans).

4. Summary and conclusion

In this study, concentrations of TCDD-EQ in sediments and soils collected from estuarine and coastal areas of Korea and China were determined by using the H4IIE-*luc* bioassay. According to the results of chemical analysis and bioassay, several locations in Korea and China contained detectable concentrations of dioxin-like compounds. DL-PAHs, which originated mainly from combustion, were the dominant AhR agonists in sediments and soils both Korea and China. Mass balance analysis showed that $TEQ_{PCDD/Fs}$ and TEQ_{PAHs} constituted only a portion of the TCDD-EQ in sediments and soils from the Yellow Sea region. It is suggested that the unidentified portion of TCDD-EQ seems to be due to synergistic interactions between identified AhR agonists and/or the occurrence of unidentified AhR agonists in sediments and soils of both Korea and China. The results of PCA analyses demonstrated that the predominant AhR agonists in sediments and soils differed among locations and between countries. While several previous studies have evaluated the potential ecological risks of dioxin-like compounds in sediments and soils based on TEQs derived by use of instrumental analyses, using that method could underestimate concentrations of TEQs sediments and soils due to the presence of unknown AhR agonists or interaction between chemicals. The bioassay approach applied here gives a more complete estimate of the total AhR agonists present. Since the TCDD-EQ observed exceeded the concentration of TEQ estimated from the concentrations of DL-PAH, PCDD, and PCDF, additional studies to determine the identities of the other AhR compounds would be required.

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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.envpol.2012.08.001.

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<Supplemental Materials>

AhR-mediated potency of sediments and soils in estuarine and coastal areas from the Yellow Sea region: A comparison between Korea and China

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Table of Contents

Table S1. Sample details including geographical descriptions and surrounding activities of all sampling locations from Yellow Sea.

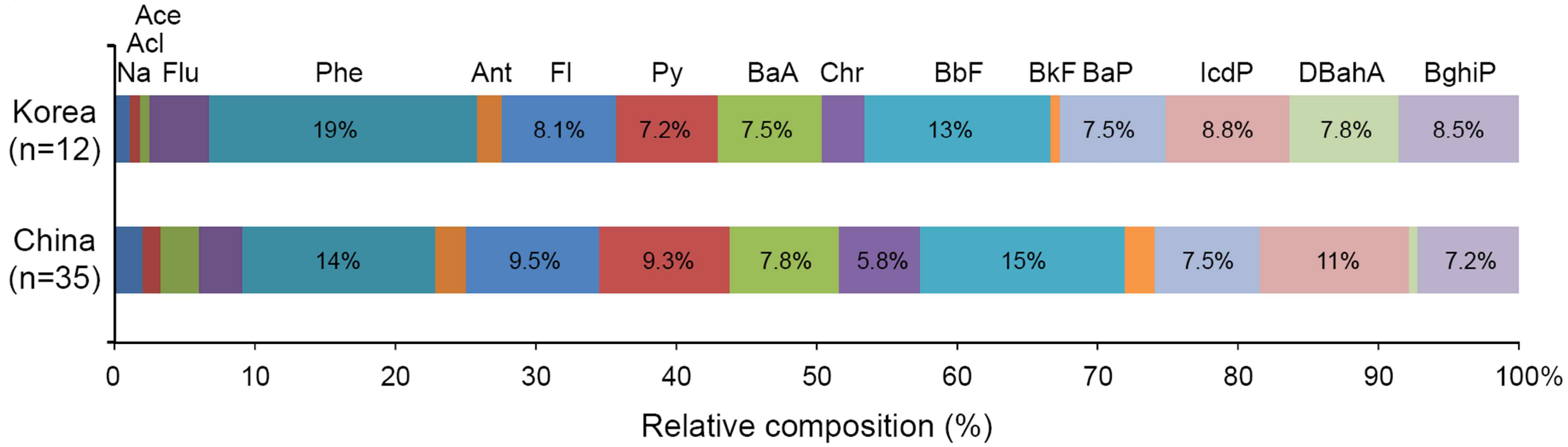
Sampling				Samples and analysis				Land uses (Regional activity)				Remark
Country	Province or city	Region	Sites	Sediment		Soil		Agricultural area	Industrial area	Municipal area	Others	Geographical description
				Bioassay	Chemical Analysis	Bioassay	Chemical Analysis					
South Korea	Gyeonggi	Lake Shihwa	LS1	O	O	O	O		O	O		Coastal area, outside of lake, Gyeonggi Bay
			LS2	NC[1]	NC	NC	NC		O	O		Coastal area, outside of lake, Gyeonggi Bay
			LS3	NC	NC	NC	NC		O	O		Inside of lake
			LS4	NC	NC	NC	NC		O	O		Inside of lake
	Chung-nam	Asan	AS1	O	O	NC	NC	O				Inside of lake
			AS2	O	O	O	O	O				Coastal area, outside of lake, Asan Bay
		Sapgyo	SG1	O	O	O	O	O				Inside of lake
			SG2	O	O	O	O	O				Coastal area, outside of lake, Asan Bay
		Taean	SD	O	O	O	O				O	Coastal area (beach), Sinduri
			ML	O	O	O	O				O	Coastal area (beach), Manlipo
			AM	O	O	O	O				O	Coastal area (beach), Anmyundo
	Chonbuk	Geum River	GG1	O	O	O	O	O				River, inside of dam
			GG2	O	O	O	O	O				Coastal area, outside of dam
	Chonnam	Yeongsan River	YS1	O	O	O	O			O		Coastal area, outside of dam
			YS2	O	O	O	O			O		River, inside of dam
China	Tangshan	Dou River	TS1	O	O	O	O	O				Small river
		Qing Long River	TS2	O	O	O	O	O				Small river
		Shuang Long River	TS3	O	O	NC	NC	O				Downstream of river
			TS4	O	O	NA[2]	O	O				Upstream of river
		Luanhe River	TS5	O	O	O	O				O	Coastal area (Harbour), river mouth
			TS6	NA	O	O	O	O				Downstream of river
			TS7	O	O	O	O	O				Upstream of river
	Qinhuang-dao	Bohai Sea	QH1	O	O	NC	NC				O	Coastal area (beach)
			QH2	O	O	O	O	O				Upstream of tidal flat
			QH3	O	O	NC	NC	O				Coastal area (tidal flat)
			QH4	O	O	NA	O				O	Coastal area (beach)

		Tian Ma Lake	QH5	O	O	O	O				O	Lake
	Huludao	Bohai Sea	HL1	O	O	NC	NC				O	Coastal area (beach)
		Liugu River	HL2	O	O	NA	O	O				Small river
			HL3	O	O	O	O	O				Small river
		Wu Li River	HL4	O	O	O	O		O			Small river
		Bohai Sea	HL5	NA	O	O	O				O	Coastal area (beach)
	Jinzhou	Wuli River	JZ1	NC	NC	NC	NC			O		Small river
		Xiaoling River	JZ2	O	O	O	O			O		Small river
		Daling River	JZ3	O	O	O	O	O				Upstream of river
			JZ4	O	O	O	O		O			Midstream of river
			JZ5	O	O	O	O	O				Downstream of river
	Panjin	Shuangtaizi River	PJ1	O	O	O	O	O				Large river
		Bohai Sea	PJ2	O	O	O	O				O	Coastal area (tidal flat)
	Yingkou	Daliao River	YK1	O	O	O	O			O		Midstream of river
		Bohai Sea	YK2	O	O	O	O		O	O		Coastal area (tidal flat)
			YK3	O	O	O	O				O	Coastal area (beach)
	Dalian	Fuzhou River	DL1	O	O	O	O					Small river
			DL2	O	O	O	O				O	Small river
		Bohai Sea	DL3	NC	NC	NC	NC					Coastal area (rock beach)
			DL4	O	O	NC	NC				O	Coastal area (beach)
		Bilia River	DL5	O	O	O	O	O				Downstream of river
		Bohai Sea	DL6	O	O	O	O				O	Coastal area (tidal flat)
	Dandong	Dayang River	DD1	O	O	O	O	O				Downstream of river
		Yalu River	DD2	O	O	O	O	O				Upstream of river
			DD3	O	O	NA	O		O			Downstream of river
			DD4	O	O	O	O				O	Midstream of river

[1] NC: not collected.

[2] NA: not analyzed.

(a) Sediment



(b) Soil

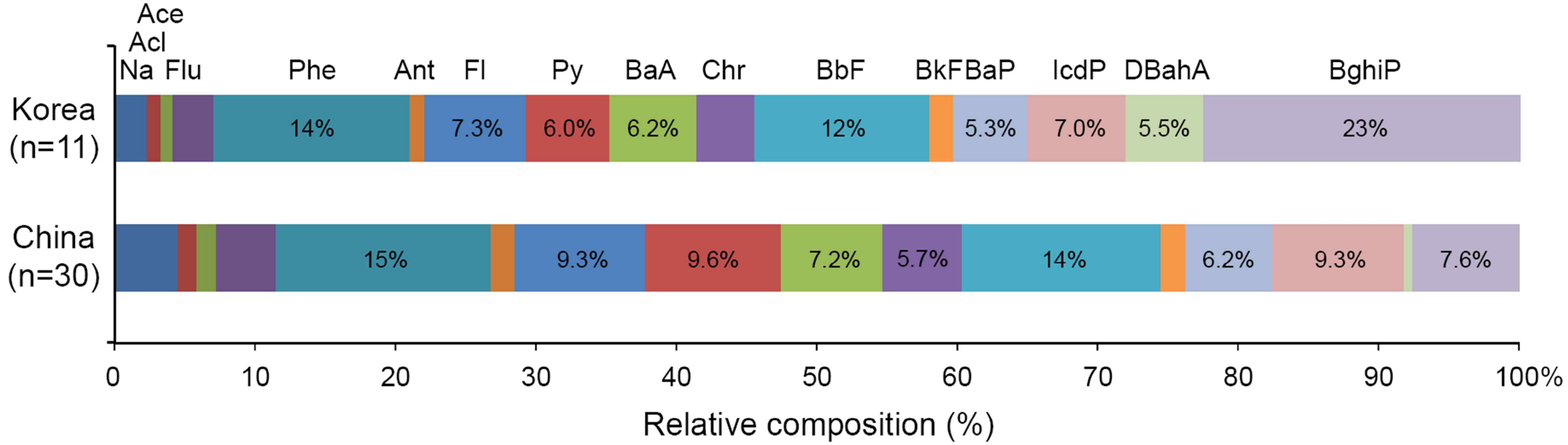
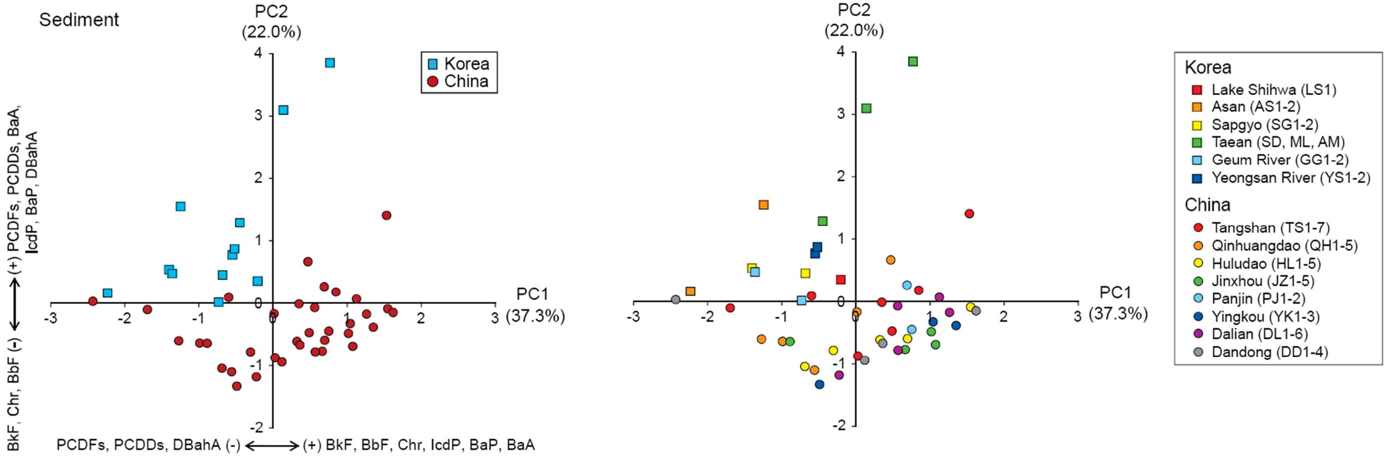


Fig. S1. Relative composition of 16 PAHs in (a) sediment and (b) soil samples from Korea and China.

(a)



(b)

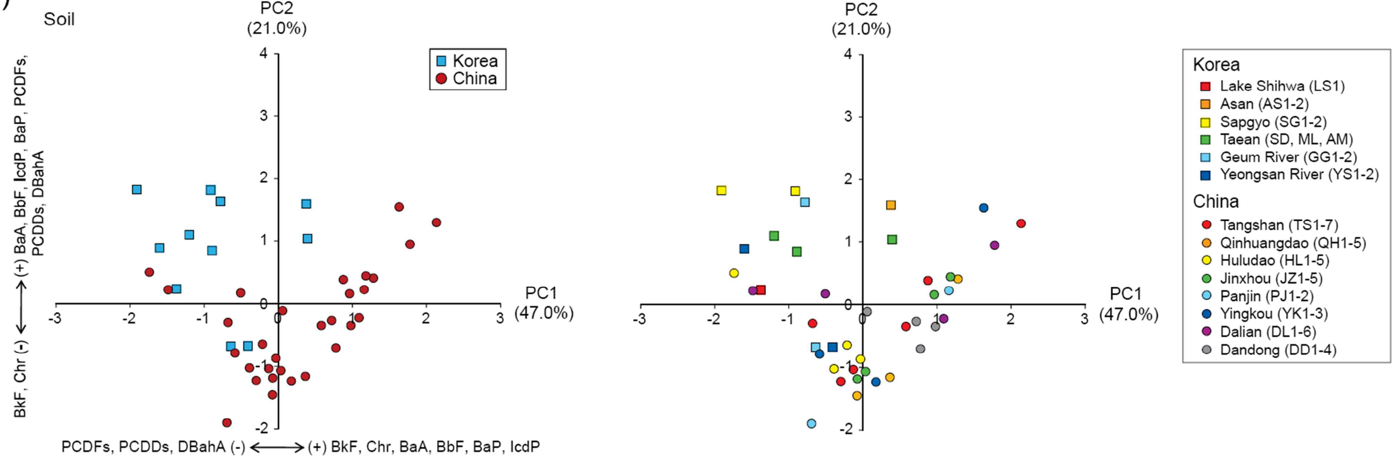


Fig. S2. Results of principal component analysis (PCA) for AhR agonists of (a) sediments and (b) soils from Korea and China. PCA were conducted by use of relative contribution (%) of AhR agonists (chemical analysis-derived) to the total TCDD-EQ (bioassay-derived).