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Instrumental and bioanalytical measures of dioxin-like compounds and activities in sediments of the Pohang Area, Korea

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HIGHLIGHTS

- AhR-mediated activities in sediments from Pohang area were characterized.
- Elevated AhR activities in sediments were associated with anthropogenic activity.
- Bioassay-derived TCDD-EQs were correlated with instrumentally-derived TEQs.
- Sources of AhR agonists were mainly explained by localized industrial activities.
- Magnitude and composition of AhR activities were comparable with those of 10 years ago.

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ABSTRACT

Pohang is a mid-sized city in which Korea's largest manufacturer of steel is located. The Hyeongsan River, which runs through Pohang and empties into Yeongil Bay, is therefore expected to be affected by various municipal and industrial inputs. In order to characterize aryl hydrocarbon receptor (AhR)-mediated activities in sediments from the Pohang area, a total of eight locations along the Hyeongsan River were chosen and 16 sediment samples were collected during two sampling campaigns in 2010. Organic extracts of sediments were characterized by both quantitative chemical analyses of dioxin-like chemicals and the in vitro H4IIE-*luc* bioassay. Significant dioxin-like activities were observed in sediments from industrial and municipal areas, which indicates that most of the dioxin-like chemicals were associated with surrounding anthropogenic sources. In general, responses of the H4IIE-*luc* assay were significantly correlated with concentrations of target compounds including dioxins, furans, co-planar PCBs, and dioxin-like PAHs. A potency balance analysis indicated that instrumentally derived TCDD equivalents (TEQs) explained about 77% of the bioassay-derived TCDD equivalents (TCDD-EQs). Among the target chemicals measured, certain penta-chlorinated dioxin and furan compounds accounted for the majority of dioxin-like activities associated with sediments. Compositional analysis of target chemicals the sources of such dioxin-like activities were mainly derived from the local activities such as the iron and steel industries. Concentrations and activities of AhR agonists were similar to what was measured approximately 10 years ago. Thus, while AhR agonists seem to be persistent in sediments there seem to have been no large increases in these chemicals in the Pohang area.

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1. Introduction

The aryl hydrocarbon receptor (AhR) is a ligand-dependent transcription factor that mediates many of the biological and toxicological actions of diverse synthetic and naturally-occurring chemicals (Denison and Nagy, 2003; Giesy and Kannan, 1998; Nagy et al., 2002). The dioxin-like chemicals including polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), polychlorinated biphenyls (PCBs), and polycyclic aromatic hydrocarbons (PAHs) are well known environmental AhR agonists (Giesy and Kannan, 1998; Hilscherova et al., 2000). These chemicals have planar conformations that are similar to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and thus can bind to the AhR and cause toxic effects (Giesy et al., 2002).

The more dioxin-like chemicals mainly originate from industrial and municipal activities, and are widely distributed in environmental media such as air, water, sediment, and soil (Alcock and Jones, 1996). Due to the presence of complex mixtures of dioxin-like chemicals having different physico-chemical properties and biological effects, assessments of risks posed by these chemicals when they occur in sediment and soil are difficult (Giesy et al., 2002; Hong et al., 2012a). Both instrumental and bioanalytical methods have been widely used to measure concentrations and activities of AhR agonists in the environment (Giesy and Kannan, 1998). The combined use of chemical analysis and bioanalytical measurements is a powerful tool to screen, identify, and prioritize the causative agents in environmental samples (Khim et al., 1999a; Shen et al., 2009). For example, in vitro H4IIE-*luc* cell bioassay, combining with instrumental analysis has been successfully applied to assess potential AhR-mediated activity in various environmental matrices including soil (Hong et al., 2012a), sediment (Hilscherova et al., 2001; Khim et al., 1999a; Koh et al., 2004, 2006a, 2006b), and food (Khim et al., 2000).

PCDD/DFs, co-planar PCBs (Co-PCBs), and dioxin-like PAHs (DL-PAHs) are emitted by various thermal processes including coking of coal, sintering, and iron and steel production (Choi et al., 2008; Hong et al., 2005; Wang et al., 2003; Yang et al., 2002). These compounds can be introduced into aquatic ecosystems via direct sources such as atmospheric deposition and industrial wastewater or via diffuse sources including surface runoff (Naile et al., 2011; Fang et al., 2011). Due to their small vapor pressure and large hydrophobicity, dioxin-like chemicals accumulate in soils and/or sediments of rivers, lakes, estuaries, and ultimately the sea (Zhang et al., 2008). Dioxin-like chemicals in sediments pose potential for long-term potential toxic effects on organisms, because of the bioaccumulation and biomagnification potential of these compounds (Giesy and Kannan, 1998), thus in place, sedimentary residues are of concern.

Pohang is a heavily industrialized city of South Korea. More than half of the city encompasses an iron and steel making complex, which is one of the largest steel making plants in the world. Emission and transport of hazardous chemicals in Pohang City account for 2% and 18%, respectively, to the total domestic values (NCIS, 2013). Results of recent studies have revealed that water, sediment, and the atmosphere have been contaminated with organic chemicals, especially in the area of steel and iron manufacturing industry of study area (Baek et al., 2010; Choi et al., 2008; Fang et al., 2011, 2012; Koh et al., 2004). Thus, potential toxic effects of hazardous chemicals emitted from the industrial complex on aquatic wildlife and humans are of concern.

Previous studies of persistent organic pollutants (POPs) in the atmosphere of Pohang showed that sources of PCDD/Fs and PCBs were related to the thermal processes during production of iron and steel (Choi et al., 2008; Fang et al., 2011, 2012). In previous studies on sediment assessment in Pohang conducted in 2000 and 2001, the greatest AhR-mediated potency was found in extracts of sediment from industrial and municipal areas, and dioxin-like potency was found to be contributed in descending order of proportion by PCDD/DFs, DL-PAHs, and Co-PCBs (Koh et al., 2004, 2006a, 2006b).

In the present study, the Pohang area was resampled 10 years (in 2010) after the previous study to determine the trends in concentrations. Sediments were collected along the Hyeongsan River, targeting areas that had greater concentrations during the previous study. The specific purposes of the present study were to i) investigate the potential AhR-mediated activities in sediments (H4IIE-*luc* bioassay), ii) determine distributions and concentrations of AhR agonists targeting PCDD/DFs, Co-PCBs, and DL-PAHs in sediment (instrumental analyses), iii) evaluate the contribution of target chemicals to the total induced dioxin-like activities (potency balance analysis), and iv) assess sources of AhR agonists.

2. Materials and methods

2.1. Sampling and sample preparation

Sediments were collected in June and August, 2010 from 8 locations that were classified as industrial or municipal regions of Pohang (Fig. 1). Surface sediments (0–10 cm) were collected by a hand shovel and pebbles and twigs were removed. Most showed sediment type of mixed sand through the locations (P1–P5) in Hyeongsan River and dominated mud at locations P6, similar to the previous study in 2001 (Koh et al., 2004). After a thorough mixing, sediments were then transferred into pre-cleaned glass jars, and then immediately transported to the laboratory where they were stored at −20 °C until lyophilization. Sample preparation and handling details for instrumental analyses and H4IIE-*luc* in vitro bioassay are described elsewhere (Khim et al., 1999a; Koh et al., 2004). In brief, a 20 g of freeze-dried sediment was extracted with 350 mL of dichloromethane (Burdick & Jackson, Muskegon, MI) on a Soxhlet extractor for 24 h. Elemental sulfur in sediment extracts was removed by the addition of activated copper (Merck, Darmstadt, Germany), and concentrated to 1 mL, then divided into two aliquots for use in the chemical analyses and bioassay, respectively. The portion of the extract to be used in the bioassay was replaced with dimethyl sulfoxide (DMSO, Burdick and Jackson).

2.2. H4IIE-luc bioassay

The H4IIE-*luc* bioassay was performed according to the modified method of Khim et al. (1999b). First, cell viability and overall cytotoxicity of samples were determined by use of the MTT assay (Yoo et al., 2006). Dilution factors for samples were determined from the results of the MTT assay, viz., samples of % live cell > 80% used for bioassay (Table S1 of Supplemental Materials (S)). Trypsinized cells from a culture plate were prepared with a density of approximately 8.0 × 10^4 cells mL^{-1} and seeded into the 60 interior wells of 96 well microplates at 250 μL per well. After incubation overnight, test and control wells were dosed with 2.5 μL per well (1% dose) of the appropriate standards, sample extracts, or solvent controls. All samples were tested in triplicate in the same assay. Luciferase assays were conducted after 72 h of exposure using a microplate reading luminometer (Tecan, Infinite 200, Mannedorf, Switzerland).

2.3. Bioassay data analysis

Both full-strength and diluted extracts were analyzed to develop full dose–response curves. Responses of the bioassay, expressed as mean relative luminescence units, were converted to percentages of the maximum response ($\%TCDD_{max}$) observed for a 30 nM (1% dose (300 pM) $= 100\%TCDD_{max}$) 2,3,7,8-TCDD standard (Wellington Laboratories inc., Guelph, OT, Canada). The magnitude-based $\%TCDD_{max}$ is given for screening purpose, thus not normalized for the diluted samples. Significant responses ($1.4\%TCDD_{max}$) were those produced responses that were three times as great as the standard deviation of the mean solvent controls. Potencies of samples, expressed as TCDD standard equivalents (TCDD-EQs) were determined directly from the sample dose–response

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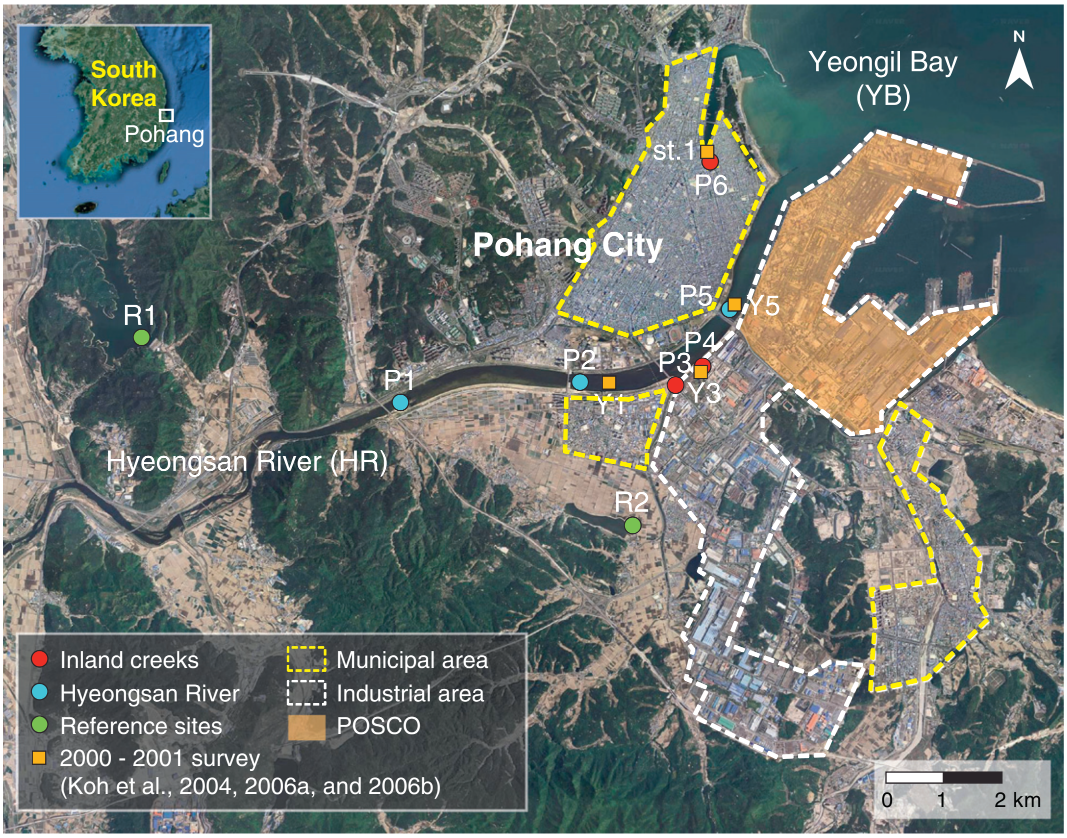

Fig. 1. Study area showing sampling locations in Pohang, South Korea. Eight locations (P1–P6 & R1–R2) were surveyed twice in June and August, 2010 (this study) and sampling locations surveyed in 2000 (Koh et al., 2006a, 2006b) and 2001 (Koh et al., 2004) are also given for a comparison purpose.

relationships generated by testing samples at multiple (3 points) of dilutions.

2.4. Chemical analysis

Methods for identification and quantification of target AhR agonists have been described previously (Khim et al., 1999a; Naile et al., 2011). Seventeen 2,3,7,8-substituted PCDD/DF congeners were quantified by use of a high resolution gas chromatography (HRGC, Hewlett-Packard 5890 series, Palo Alto, CA) interfaced with a high resolution mass spectrometer (HRMS, Micromass, Beverly, MD) (target congeners are present in Table S2). Chromatographic separation was achieved on a DB-5MS fused silica capillary column (60 m long, 0.25 mm i.d., 0.1 μm film thickness, Agilent, CA). Fourteen Co-PCBs and 5 indicator PCBs were analyzed by a GC-^{63}Ni electron capture detector (ECD, Shimadzu GC-2100, Shimadzu, Tokyo, Japan) (target congeners are present in Table S3). Sixteen priority PAHs were measured by GC-mass selective detector (Agilent 5975, Agilent Technologies, Palo Alto, CA) (target chemicals are present in Table S4). Method detection limits (MDL) for individual PCDD/DF, PCB, and PAH chemicals were 1 pg g^{-1} dm (dry mass), 0.01 ng g^{-1} dm, and 0.1 ng g^{-1} dm, respectively.

2.5. Potency balance analysis

Concentrations of TCDD-EQs determined by use of the bioassay were compared to TEQs. Total TEQs of target AhR agonists were calculated as the sum of TEQs by multiplying the concentration of individual AhR-active chemicals by assay-specific relative potency values (RePs). This comparison or potency balance analysis was used to identify the relative contribution of each chemical to total AhR-mediated response induced (Koh et al., 2004). The RePs of PCDD/DFs and Co-PCBs were used to calculate the $TEQ_{PCDD/DFs}$ and TEQ_{PCBs} according to Behnisch et al. (2003) and Lee et al. (2013). Seven DL-PAHs viz. BaA, Chr, BbF, BkF, BaP, IcdP, and DBahA were used to calculate the TEQ_{PAH} using RePs reported by Villeneuve et al. (2002). RePs used in the present study are presented in Tables S2–S4.

2.6. Statistics

Correlation-based principal component analyses (PCA) were conducted using relative contribution of PCDD/DF congeners in sediment samples and known sources. The PCA was used to convert the contribution of variables into two principal components (PCs) through which were identified the sources of PCDD/DFs in sediments of Pohang area. SPSS 12.0 (for Windows, SPSS Inc., Chicago, IL) was employed for statistical analyses. Concentrations that were less than the MDL were set equal to the limit of detection divided by two before conducting PCA. The Varimax method was selected for rotation and 25 iterations were performed in the PCA.

3. Results and discussion

3.1. Potential AhR-mediated activity of sediment extracts

All the sediment samples induced significant dioxin-like potencies with $\%TCDD_{max}$ ranging from 3.3 to 68% (Table 1 and Fig. 2a). No cytotoxic effects were observed in concentrations of diluted sediment extracts (~0.8 g sediment mL^{-1}) based on MTT assay (>80% cell survival) (Table S1). Potency-based TCDD-EQs in extracts of sediments ranged from <DL to 800 pg g^{-1} dm. Concentrations of TCDD-EQs at industrial (P4 and P5) and municipal areas (P6) during both sampling events were significantly greater than what would be considered

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background values (Fig. 2b). Concentrations of TCDD-EQs at P4, P5, and P6 exceeded sediment quality guidelines (50 pg TEQ g^{-1} dm) of the United States for dioxin-like compounds (Zhang et al., 2009), which suggests potential for adverse effects on aquatic wildlife.

Concentrations of TCDD-EQs in sediments varied between the two samplings with greater concentrations observed in June than August. This result was most likely due to dilution by heavy rainfall of approximately 75 mm that occurred in the Pohang area approximately five days before the later sampling period. Although no obvious sources of pollution exist around the reference site R2, measurable AhR-mediated potency was observed in June. This observation was associated with direct atmospheric deposition of dioxin-like chemicals from local combustion as well as the steel and iron industries. Overall, the results of the in vitro bioassay suggested that dioxin-like compounds such as PCDD/DFs, PCBs, and/or PAHs were accumulated in sediments collected near both industrial and municipal areas of Pohang.

3.2. AhR agonists: PCDD/DFs, Co-PCBs, and DL-PAHs

A total of fifty two individual PCDD/DFs, PCBs, and PAHs were identified in sediments of the Pohang area (Fig. 2c–d and Tables S2–S4). PCDD/DFs were commonly detected in sediments with concentrations ranging from 51 to 5230 pg g^{-1} dm (sum of the 17 congeners). The greatest concentrations of ΣPCDD/DFs were found at site P6 (municipal area), followed by P4 and P3 (industrial area). OCDD was the predominant congener in sediments at P6 and P3, while 2,3,4,7,8-PeCDF was more dominant at P4 (Table S2). Concentrations of sedimentary PCDD/DFs in industrial areas were likely due to the presence of point source emission, whereas contaminants in sediments of municipal areas could be attributed to more diffuse sources and/or legacy pollution (Liu et al., 2012). Concentrations of 1,2,3,7,8-PeCDD and 2,3,7,8-TCDF in sediments from the industrial area (P4) were significantly greater at P4 than at P6, while concentrations of OCDD were greater at P6 than at P4. Endocrine disrupting chemicals including alkylphenols and bisphenol-A were also detected with greater concentrations in sediment of P6 than those of P4 and P5 (Kim et al., 2014).

Concentrations and distributions of PCDD/DFs in sediments were also different between the two sampling periods. For example, concentrations of ΣPCDD/DFs at P4 were significantly greater in June by a factor of ~3 than those in August, while concentrations at P6 of August were 2 times greater than those of June. Concentrations of ΣPCDD/DFs in industrial areas of P3 and P4 locations seemed to reflect a dilution effect during the rainy season. While, the greater concentrations of ΣPCDD/DFs found in the municipal area (P6) during the rainy season could be explained by non-point sources, such as surface runoff, during the period of precipitation (Zhao et al., 2011).

Co-PCBs were also commonly detected in sediments of the Pohang area with concentrations ranging from <DL to 1600 pg g^{-1} dm (Fig. 2e and Table S3). Relatively greater concentrations of ΣCo-PCBs found in locations near industrial areas suggested that they mainly originated from industrial activities. Concentrations of Co-PCBs in sediments also varied seasonally, but with a different trend than that exhibited by PCDD/DFs during the same season. For example, the greater concentrations of ΣCo-PCBs found in industrial areas of P3 and P4 locations during the rainy season could suggest non-point sources, similar to the case for ΣPCDD/DFs in P6 (Zhao et al., 2011).

DL-PAHs were also widespread in the Pohang area (Fig. 2f and Table S4). Concentrations of ΣDL-PAHs ranged from 12.6 to 1270 ng g^{-1} dm with maximum concentrations at P4, followed by P6 and P5. Spatial distribution of DL-PAHs exhibited a similar trend to those of PCDD/DFs, which indicates that sources of PAHs were similar to those of some dioxin-like chemicals in the study area.

While maximum concentrations of ΣPCDD/DFs were found at P6, concentrations of $\text{TEQ}_{\text{PCDD/DFs}}$ were greatest at P4 (730 pg g^{-1} dm) (Table 1). This could be explained by the fact that less toxic congeners such as OCDD ($\text{ReP} = 5.0 \times 10^{-4}$) while abundant did not contribute significantly to the concentrations of $\text{TEQ}_{\text{PCDD/DFs}}$ at P6, while the more potent congener 2,3,4,7,8-PeCDF ($\text{ReP} = 5.0 \times 10^{-1}$) contributed to $\text{TEQ}_{\text{PCDD/DFs}}$ (Behnisch et al., 2003; Lee et al., 2013). As expected, concentrations of both TEQ_{PCBs} and TEQ_{PAHs} constituted a lesser proportion of the $\text{TEQ}_{\text{PCDD/DFs}}$. However, at some locations with small concentrations of TEQs (<50 pg g^{-1} dm), the %-contribution of PCBs and PAHs reached ~50% (location P1) and ~20% (locations P1 and P2).

3.3. Potency balance: bioassay vs. Instrumental analysis

A potency balance analysis between bioassay TCDD-EQ and TEQs ($\text{TEQ}_{\text{PCDD/DFs}} + \text{TEQ}_{\text{PCBs}} + \text{TEQ}_{\text{PAHs}}$) in sediments was conducted to evaluate the contribution of individual chemicals to the total induced dioxin-like activities. In general, concentrations of TCDD-EQs in sediments from the Pohang area were consistent with concentrations of

Table 1

Results for H4IIE-*luc* bioassay and instrumental analyses of sediments collected from the Pohang area, South Korea.

Sampling	Site	$\%\text{TCDD}_{\text{max}}$[a] (%)	TCDD-EQ[b] (pg g^{-1} dm)	TEQs[c] (pg g^{-1} dm)	$\text{TEQ}_{\text{PCDDs}}$ (pg g^{-1} dm)	$\text{TEQ}_{\text{PCDFs}}$ (pg g^{-1} dm)	TEQ_{PCBs} (pg g^{-1} dm)	TEQ_{PAHs} (pg g^{-1} dm)
June 2010	P1	12	<0.1	6.4	1.0	4.3	0.32	0.76
	P2	29	21	11	1.5	6.6	nd[d]	2.5
	P3	5.6	0.22	82	17	61	0.86	3.3
	P4	68	800	750	130	600	1.1	22
	P5	36	78	55	15	37	0.71	3.0
	P6	34	180	130	40	75	nd	15
	R1	3.9	<0.1	2.8	0.86	1.0	0.12	0.81
	R2	26	55	21	6.2	13	nd	2.3
August 2010	P1	3.3	<0.1	1.5	0.16	0.56	0.46	0.30
	P2	29	10	20	3.3	12	0.72	3.2
	P3	20	0.33	27	11	12	1.6	2.5
	P4	55	310	240	37	190	2.8	9.3
	P5	43	84	74	12	56	nd	5.6
	P6	42	170	220	81	120	0.0071	18
	R1	4.7	<0.1	2.1	0.87	0.83	0.14	0.24
	R2	8.2	<0.1	12	3.7	1.8	6.2	0.42

[a] $\%\text{TCDD}_{\text{max}}$: response magnitude presented as percentage of the maximum response observed for a 30 nM TCDD standard (set to $100\%\text{TCDD}_{\text{max}}$) elicited by 100% sediment raw extracts (Significant level = $1.4\%\text{TCDD}_{\text{max}}$).

[b] TCDD-EQ: potency-based TCDD-EQs (TCDD-EQ_{50}) was obtained from sample dose–response relationships generated by testing samples at multiple levels of dilution (Detection limits = 0.1 pg g^{-1} dm).

[c] TEQs: sum of the $\text{TEQ}_{\text{PCDDs}}$, $\text{TEQ}_{\text{PCDFs}}$, TEQ_{PCBs}, and TEQ_{PAHs}.

[d] nd: not detected.

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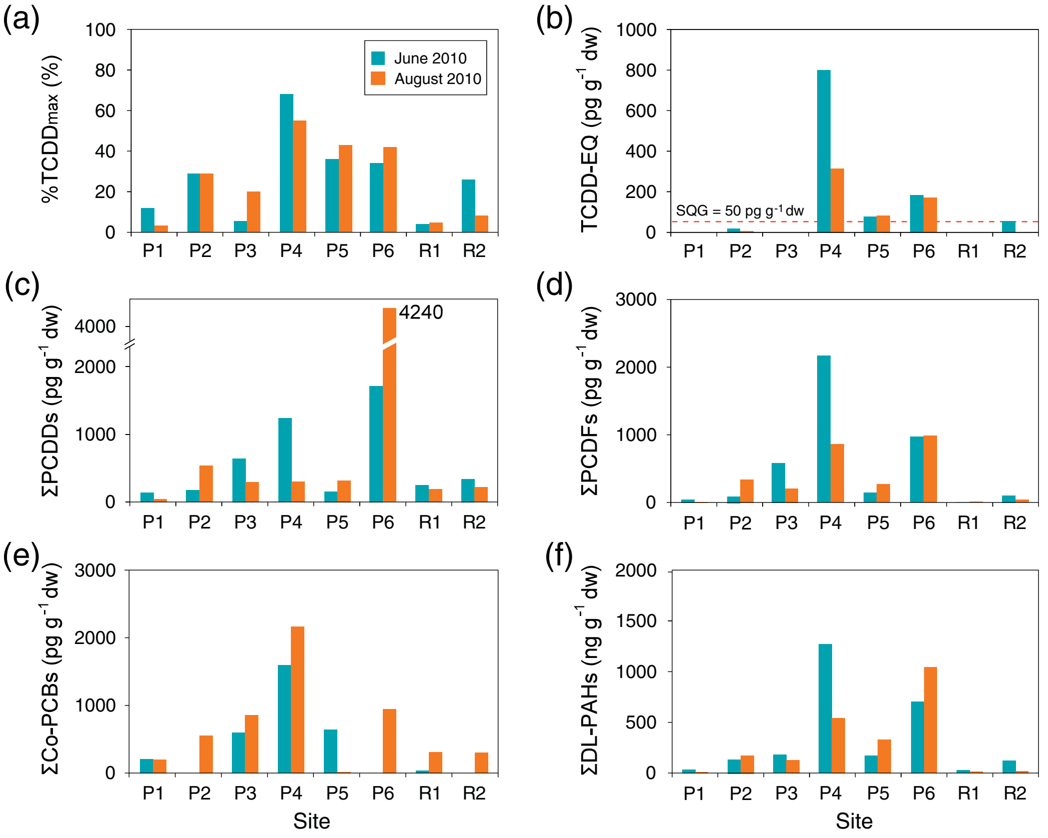

Fig. 2. Results of H4IIE-*luc* bioassay and instrumental analysis. (a) Magnitude-based %TCDD$_{max}$, (b) potency-based TCDD-EQ, (c) sum of PCDD concentration, (d) sum of PCDF concentration, (e) Sum of Co-PCB concentration, and (f) sum of DL-PAH concentration in sediment samples from the Pohang area, South Korea.

TEQs with a coefficient of determination (r^2) of 0.97 ($p < 0.01$) (Fig. 3 and Fig. S1). In particular, concentrations of TCDD-EQs in more contaminated samples, such as P4, P5, and P6, were comparable to concentrations of TEQs, while some samples with lesser concentrations of TCDD-EQs could not be explained by identified compounds used to calculate the concentration of TEQs. In general, PCDFs were the predominant contributors to the overall dioxin-like activities (TCDD-EQ) followed by PCDDs, PAHs, and PCBs (Fig. 3a–b). Contributions of individual AhR agonists to TCDD-EQ were significantly different among locations. For example, at site P4, some chemicals such as 2,3,4,7,8-PeCDF and 1,2,3,7,8-PeCDD accounted for the majority of TCDD-EQs, whereas other chemicals collectively contributed to total AhR activity in site P6 (Fig. 3c).

Some previous studies that conducted potency balance analysis for environmental samples could not successfully explain the observed AhR activity with known AhR agonists. There are several possible reasons for this, including large portions of unknown compound(s), matrix effects, synergisms, additive, or antagonistic effects (Hecker and Giesy, 2011; Hong et al., 2012a, 2012b). However, sometimes there is concordance between TCDD-EQ and TEQ (Koh et al., 2004; Luo et al., 2009; Shen et al., 2009). In the potency balance for Pohang most of the TCDD-EQ measured in extracts of sediments could be accounted for by identified dioxin-like chemicals. Overall, the combined use of chemical analysis and bioanalytical measurements was useful to characterize contamination of dioxin-like compounds, screen, identify and prioritize the causative agents in sediment of the Pohang area.

3.4. Sources of AhR agonists

Profiles of relative concentrations of PCDD/DFs have been used to identify the potential sources (Naile et al., 2011; Ren et al., 2009; Zhang et al., 2009). However, PCDD/DF congeners have different physico-chemical properties due to their Cl numbers, so that the relative composition will change through transformation, degradation (photo and/or microbial), phase partition, adsorption, bioaccumulation, and other processes occurring in water column (Gaus et al., 2002; Ren et al., 2009). Thus, profiles of PCDD/DFs in sediment samples are often not well-matched with those of emission gases. Despite this limitation, a PCA was conducted by use of concentrations of 17 PCDD/DFs and the pattern observed in this study compared to patterns of documented sources (Zhang et al., 2010).

Results indicated that most of the sites in the Pohang area, except for some of the most contaminated locations, were closely associated with pentachlorophenol (PCP), PCP-Na, and domestic burning of coal (Fig. 4a). Results of the present study were consistent with those of previous studies in which results of PCA indicated that PCDD/DFs in sediments were associated with PCP and PCP-Na due to their great portions of OCDD and OCDF (Naile et al., 2011). In general, OCDD and OCDF are often the predominant PCDD/DF congeners in sediments due to the fact that they are generated in many combustion sources and are generally persistent and lipophilic (Shen et al., 2008). But, it is not clear whether such PCDD/DFs observed in this study originated from PCP and PCP-Na or were the result of transformation in the water column. PCDD/DFs derived from PCP and PCP-Na contain greater proportions of OCDD and OCDF (Ren et al., 2009).

In South Korea, uses of PCP and chloronitrofen (CNP) as paddy field herbicides have been banned since 1996 (Kim et al., 2008, 2009) and are thus not likely sources. Also, it has been reported that profiles of the dominant congeners of impurities for OCDD, OCDF, and 1,2,3,4,6,7,8-HpCDD in PCP and PCP-Na were 76%, 10%, and 10%, respectively (Ren et al., 2009). Such a pattern was not observed in sediments in the Pohang region. Thus, PCP and PCP-Na are not likely to have been the major sources of PCDD/DFs in sediments of Pohang

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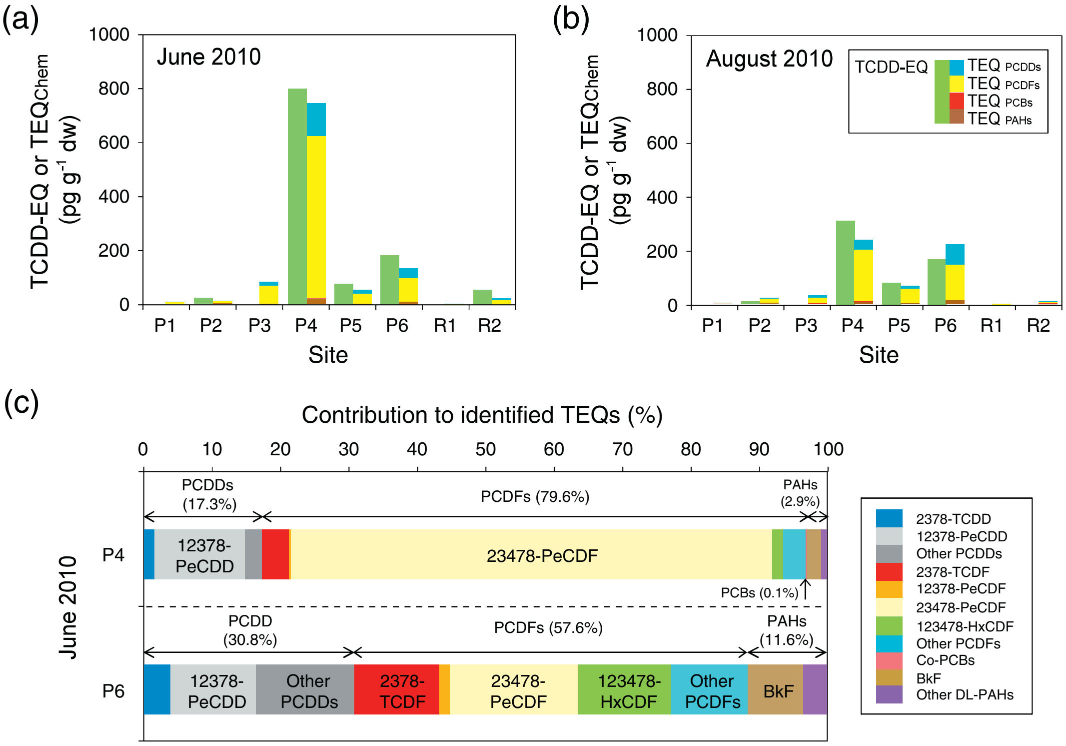

Fig. 3. Comparison between bioassay-derived TCDD-EQ and instrumentally-derived TEQs in sediments from (a) June and (b) August and relative contribution to identified TEQs in sediment samples from industrial (P4) and municipal (P6) locations of the Pohang area, South Korea.

area. PCA was applied to samples from Pohang by using 15 PCDD/DF congeners except for OCDD and OCDF, for identifying possible sources (Fig. 4b). Results indicated that PCDD/DFs in sediments of the industrial area (P3, P4, and P5) originated from local industrial activities associated with iron and steel manufacturing, which was in good agreement with known sources of the area. Also domestic burning of coal was the likely source of PCDD/DFs in sediments from the municipal area (P6). The PCA using 15 PCDD/DF congeners provided useful matching patterns to plausible sources. However, the proportion of the variance explained by PC1 and PC2 was only about 42%. Thus, a separate study on source identification of PCDD/DFs in sediment samples would need to be conducted in the future.

Diagnostic ratios between individual PAHs in sediments from the Pohang area were determined to assess relative contributions of petrogenic and pyrogenic sources, or specific combustion sources of PAHs (Fig. 5). Source identification of PAHs using diagnostic ratios is useful because isomer pairs are distributed to a similar extent on fate, transformation and transport in water column due to their comparable thermodynamic partitioning and kinetic mass transfer coefficients (Dickhut et al., 2000; Liu et al., 2009). The ratios of Ant/(Phe + Ant) and Fl/(Fl + Py) in sediments indicated pyrogenic origins, including combustion of petroleum, grass, and wood, and coal combustion was predominant in Pohang area (Chen et al., 2012; Liu et al., 2009; Yunker et al., 2002) (Fig. 5a). While the isomer ratios of BaA/(BaA + Chr) and IcdP/(IcdP + BghiP) in sediments were consistent with combustion of petroleum as a source (Fig. 5b). Among the various sources of PAHs considered for diagnostic ratios, combustion of oil was found to be common in the Pohang area (Yunker et al., 2002). Overall, PAHs identified in sediments of Pohang likely originated from mixed sources dominated by combustion of petroleum.

3.5. Comparison with previous studies

Potential AhR-mediated potencies, contribution, and sources of AhR agonists from Pohang sediments in 2010 were compared to those of previous studies conducted in the same area in 2000 and

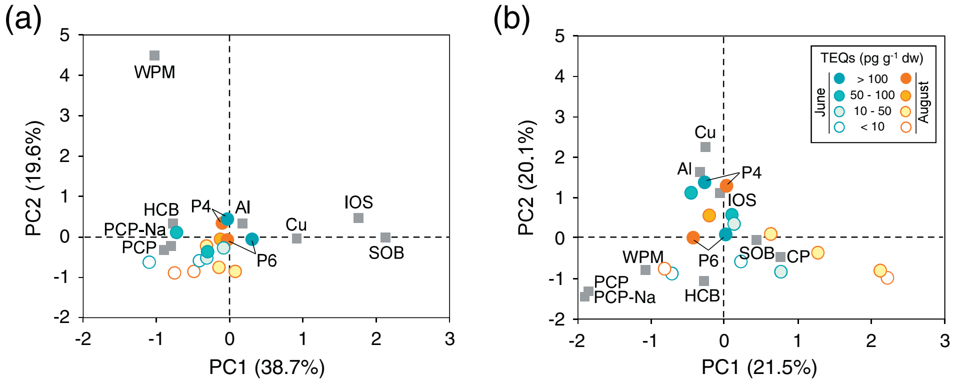

Fig. 4. Results of principal component analysis (PCA) of PCDD/DFs. (a) PCA using relative compositions of 17 PCDD/DF congeners and (b) PCA using relative compositions of 15 PCDD/DF congeners except for OCDD and OCDF (HCB: domestic burning of coal; PCP: pentachlorophenol; PCP-Na: sodium pentachloropenate; Al: secondary Al metallurgy; Cu: secondary Cu metallurgy; SOB: agricultural straw open burning; CP: cement plant: IOS: iron ore sintering plant; WPM: wastewater from pulp mill, data from Zhang et al. (2010)).

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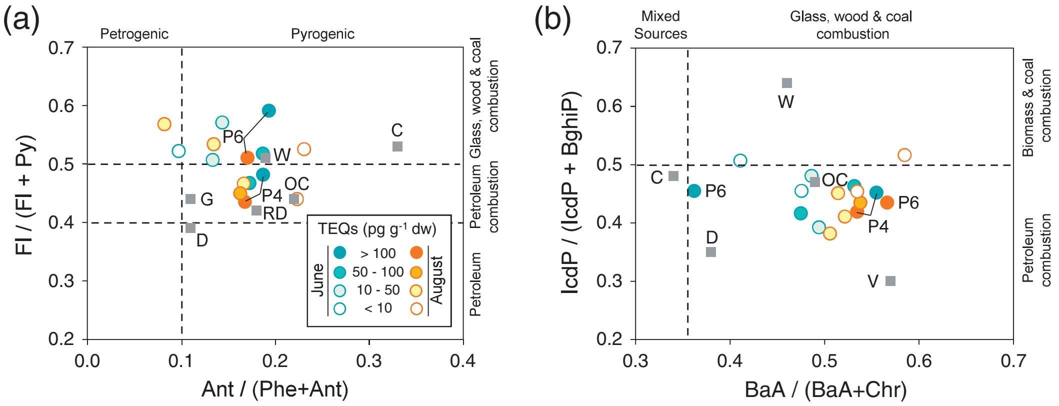

Fig. 5. Results of diagnostic ratios of PAHs. (a) Ratios of Ant/(Phe + Ant) and Fl/(Fl + Py) and (b) ratios of BaA/(BaA + Chr) and IcdP/(IcdP + BghiP) in sediments from the Pohang area, South Korea (W: wood; C: coal; D: diesel; G: gasoline; OC: oil combustion; V: vehicles; RD: road dust, data from Yunker et al. (2002)).

2001 (Koh et al., 2004, 2006a, 2006b). In general, AhR-mediated potencies in sediments of 2010 were comparable to those of 2000 and 2001 (Fig. 6a), as well as concentrations of AhR agonists. Individual PCDD/DFs were the major contributors to TEQs in extracts of sediments, particularly in the more contaminated area Y3 in both 2001 and 2010 (e.g., P4) (Fig. 6b). Co-PCBs and DL-PAHs contributed relatively lesser proportions of the total TEQs both in 2001 and 2010. The composition of individual AhR agonists based on contributions to total TEQ was not notably different between 2001 and 2010, indicating persistent chemical property, environmental stability, and/or continuing possible input of dioxin-like compounds in the study area (Fig. S2). Altogether, the efforts on source identification and characterization towards environmental monitoring and management in Pohang area would be of continuing concerns.

4. Conclusions

In the present study, several trends and findings in potential activities, concentrations, distributions, and sources of AhR agonists in sediments were observed in Pohang area.

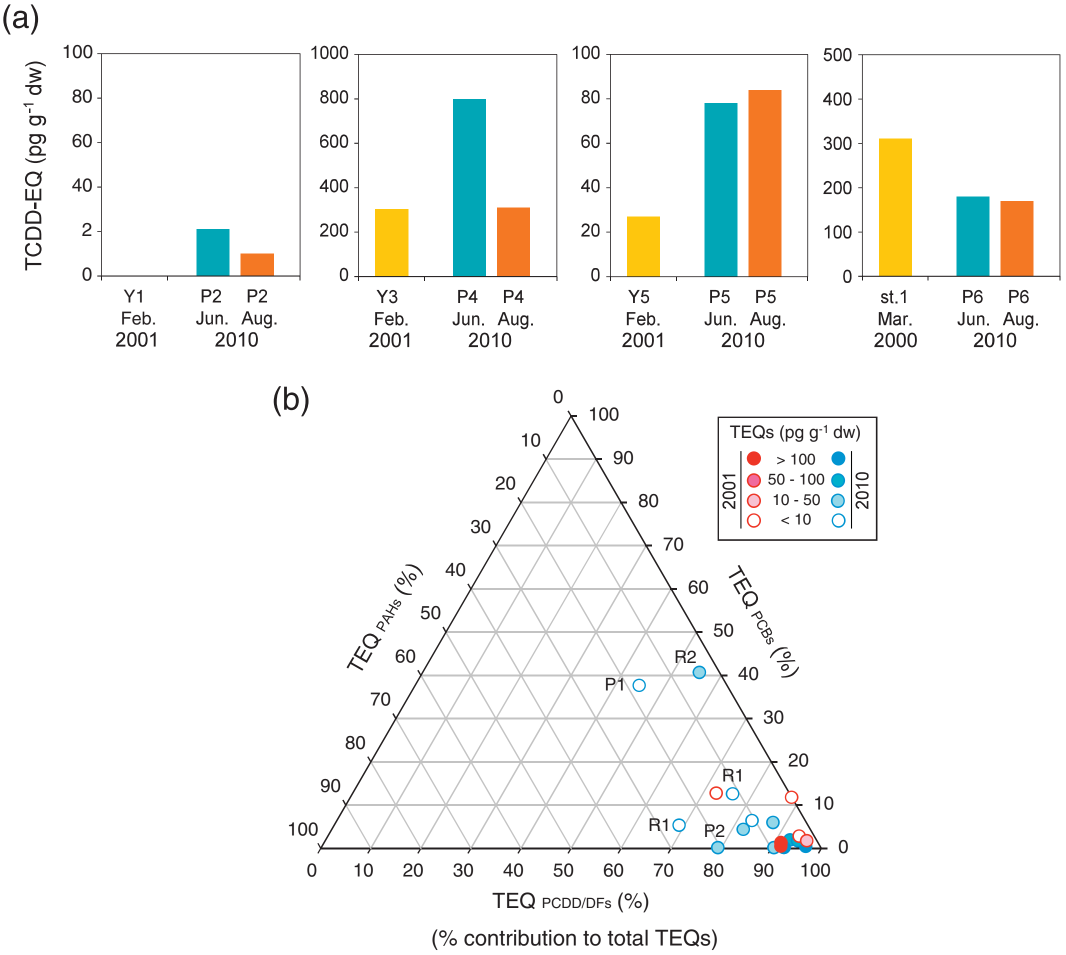

Fig. 6. Comparison of results of this study to those of previous studies. (a) TCDD-EQs in sediments of P2, P4, P5, and P6 in 2000, 2001 and 2010 (2000 data: Koh et al. (2006b); 2001 data: Koh et al. (2004)) and (b) contribution (%) of PCDD/DFs, Co-PCBs, and DL-PAHs to total TEQs in sediments of the Pohang area, South Korea.

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- Potential AhR-mediated activities were significantly induced in sediment extracts of Pohang area which seemed to be affected by surrounding industrial and municipal activities.
- AhR agonists including PCDD/DFs, Co-PCBs, and DL-PAHs were highly accumulated in sediments and varied in site- and seasonal-specific manner.
- Potency balance analysis revealed that certain PeCDD and PeCDF were major contributors in the industrial area, while several PCDD/DF congeners successfully contributed in the municipal area.
- AhR agonists in sediments were mainly originated from localized industrial and municipal activities associated with iron and steel making complex and oil and petroleum combustion.
- AhR activities and agonists in sediments were fairly consistent in magnitude, composition, and TEQ contribution, over a period of 10 years.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2013.06.112.

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<Supplemental Materials>

Instrumental and bioanalytical measures of dioxin-like compounds and activities in sediments of the Pohang Area, Korea

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Supplemental Materials: Tables

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Supplemental Materials: Figures

Fig. S1. Relationship between TCDD-EQ and TEQ$_{chem}$ in raw extracts of sediments from Pohang area. ······ S6
Fig. S2. TEQ values of PCDD/DFs, Co-PCBs, and DL-PAHs in sediments from sites P4 and P5 in 2001 and 2010 (2001 data: Koh et al. (2004)). ······ S7

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Supplemental Materials: Tables

Table S1. Results of MTT viability testing assay (% live cell).

Samples	Sediment extracts (g mL^{-1})	P1	P2	P3	P4	P5	P6	R1	R2
June 2010	20	32	25	28	11	31	6	40	45
	4	52	63	43	40	67	37	56	61
	0.8	80	81	58	80	80	89	91	91
	0.16	-	-	-	-	-	-	-	-
	0.032	-	-	-	-	-	-	-	-
	0.0064	-	-	-	-	-	-	-	-
August 2010	20	40	21	28	11	26	8	92	49
	4	89	47	43	51	66	61	101	72
	0.8	99	98	105	116	97	92	101	92
	0.16	-	-	-	-	-	-	-	-
	0.032	-	-	-	-	-	-	-	-
	0.0064	-	-	-	-	-	-	-	-

Shaded concentrations of sediment extracts were tested in H4IIE-luc for determination of AhR-mediated activity.

-: Not tested in MTT assay.

Table S2. Concentrations of PCDD/DFs in sediments from Pohang Area in June and August 2010.

PCDD/DFs (pg g^{-1} dw)	Relative potency (RePs)	June 2010								August 2010							
		P1	P2	P3	P4	P5	P6	R1	R2	P1	P2	P3	P4	P5	P6	R1	R2
PCDDs																	
2378-TCDD	1				11.8	4.90	5.10						4.26	1.23	10.4		
12378-PeCDD	6.0×10^{-1}			19.8	165	14.0	27.1		5.90			15.3	46.7	10.0	48.2		3.52
123478-HxCDD	1.0×10^{-1}				21.5		16.1					1.20	11.1		35.3		
123678-HxCDD	5.0×10^{-2}				40.9		36.5	1.60	6.50				12.0		44.2		
123789-HxCDD	5.0×10^{-2}				31.7		36.1	2.20	7.20				6.30	8.20	124		
1234678-HpCDD	6.0×10^{-2}	16.3	23.1	81.7	219	19.5	214	7.80	30.0	2.4	50.4	32.9	40.1	75.9	459	13.0	25.0
OCDD	5.0×10^{-4}	117	149	542	749	115	1380	235	288	37.9	485	240	180	223	3520	176	187
PCDFs																	
2378-TCDF	3.0×10^{-1}	5.30	6.50	24.7	99.1	15.0	54.3	3.40	10.6	1.33	5.5	5.5	24.0	25.7	147		
12378-PeCDF	2.0×10^{-2}	3.00	5.80	28.8	85.4	7.40	106		11.0		12.6	6.7	13.8	15.2	113		2.21
23478-PeCDF	5.0×10^{-1}	3.50	7.20	80.6	1060	59.4	48.4		10.7		10.6	13.9	345	85.7	59.4		1.10
123478-HxCDF	1.3×10^{-1}	3.60		34.2	96.2	8.10	137		13.8			3.32	12.3	12.1	241		
123678-HxCDF	3.9×10^{-2}	2.60	9.70	27.0	80.7	7.60	73.2		10.1	1.71	72.4	2.99	21.2	33.5	95.4		1.44
234678-HxCDF	1.8×10^{-1}			21.0	79.8	6.10	37.4		7.60			5.31	14.3	9.30	17.3		3.36
123789-HxCDF	1.1×10^{-1}															7.10	
1234678-HpCDF	1.0×10^{-2}	13.4	31.1	97.7	294	19.5	260		39.3	3.12	116	42.7	121	22.4	157	4.42	25.4
1234789-HpCDF	4.1×10^{-2}	1.40		12.3	46.9		23.8			1.1	13.2	10.7	24.1		44.6		7.31
OCDF	6.5×10^{-3}	14.8	36.5	256	329	23.0	233			3.45	112	115.2	282	61.8	114		
ΣPCDDs		133	172	643	1240	153	1720	247	337	40.3	535	289	301	319	4240	189	216
ΣPCDFs		47.6	96.8	582	2170	146	974	3.40	103	10.7	343	206	858	266	988	11.5	40.8
ΣPCDD/DFs		181	269	1230	3410	299	2690	251	440	51.0	878	495	1160	584	5230	200	256
TEQ_{PCDDs}		1.04	1.46	17.1	130	14.5	40.1	0.856	6.17	0.163	3.27	11.4	36.8	12.3	80.5	0.868	3.69
TEQ_{PCDFs}		4.26	6.59	60.7	600	37.1	75.1	1.02	12.7	0.563	12.5	11.8	189	56.1	118	0.825	1.81
$TEQ_{PCDD/DFs}$		5.29	8.05	77.8	730	51.6	115	1.88	18.9	0.726	15.7	23.2	226	68.4	199	1.69	5.50

Blank: not detected (< 1 pg g^{-1} dw).

$TEQ_{PCDD/DFs}$ were calculated using ReP values from Behnisch et al. (2003) and Lee et al. (2013).

Table S3. Concentrations of PCBs in sediments from Pohang Area in June and August 2010.

PCBs ($ng\ g^{-1}$ dw for conc., $pg\ g^{-1}$ dw for TEQs)	Relative potency (RePs)	June 2010								August 2010							
		P1	P2	P3	P4	P5	P6	R1	R2	P1	P2	P3	P4	P5	P6	R1	R2
Indicator (IUPAC no.)																	
28				0.020	0.42						0.027	0.14	0.47	0.047		0.035	0.033
52				0.066	0.29		0.14	0.046			0.064	0.061	0.50	0.016			0.071
101				0.59	0.21						0.093	0.070	0.46	0.011			
138				0.14	0.53		0.10				0.15	0.17	0.65	0.062	0.072		
153				0.10	0.69						0.080	0.14	0.62	0.034			
Non-ortho																	
81	3.0×10^{-3}	0.11		0.288	0.340	0.236		0.039		0.15	0.24	0.53	0.93			0.044	
77	1.0×10^{-4}				0.505											0.056	0.093
126	1.0×10^{-1}																0.062
169	3.0×10^{-4}																
Mono-ortho																	
105	1.0×10^{-7}			0.164	0.116					0.049	0.071	0.092	0.25		0.18	0.16	
114	5.0×10^{-6}			0.146											0.39		
118	1.0×10^{-7}	0.10			0.206	0.407					0.24	0.20	0.45				
123	1.0×10^{-5}				0.292							0.042	0.38		0.020		
156	2.0×10^{-5}													0.018	0.12		
157	4.0×10^{-5}				0.137								0.072		0.062	0.015	0.15
167	1.0×10^{-9}												0.085		0.18	0.034	
189	1.0×10^{-9}																
Di-ortho																	
170													0.18				
180					0.22						0.04	0.07	0.34		0.07		
ΣPCBs		0.21		1.5	3.7	0.64	0.24	0.085		0.20	0.97	1.4	4.9	0.19	1.0	0.34	0.40
ΣCo-PCBs		0.21		0.60	1.6	0.64		0.039		0.20	0.56	0.86	2.2	0.018	0.95	0.31	0.30
TEQ_{PCBs}		0.322		0.864	1.08	0.709		0.116		0.463	0.721	1.58	2.81	<0.01	<0.01	0.138	6.18

Blank: not detected (< 0.01 $ng\ g^{-1}$ dw).

TEQ_{PCBs} were calculated using ReP values from Behnisch et al. (2003) and Lee et al. (2013).

Table S4. Concentrations of PAHs in sediments from Pohang Area in June and August 2010.

PAHs (ng g^{-1} dw for conc., pg g^{-1} dw for TEQs)	Relative potency (RePs)	June 2010								August 2010							
		P1	P2	P3	P4	P5	P6	R1	R2	P1	P2	P3	P4	P5	P6	R1	R2
Priority PAHs																	
Na				6.40	34.7	3.64	0.473			1.04	2.97	4.84	15.6	3.67	17.4	2.44	10.8
Anp			0.658	1.88	21.1	1.34	2.42			0.574	1.18	1.61	4.39	2.48	3.86	0.558	0.623
Ane			0.286	11.6	31.0	8.20				0.661	2.86	10.4	12.5	13.4	20.4	0.598	1.04
Flr		0.413	6.09	7.41	36.6	7.22			4.41	1.06	5.11	7.34	24.0	18.0	50.2	1.13	2.67
Phe		3.78	31.3	36.3	266	35.1	150	1.17	22.7	4.00	36.6	33.5	170	106	180	3.55	11.4
Ant		0.408	4.82	7.58	61.2	8.07	36.0		3.81	1.20	5.88	6.69	34.3	20.6	37.0	1.02	1.02
Fl		6.45	61.0	72.2	455	52.2	325	2.68	36.2	4.29	94.9	61.8	285	161	460	1.64	8.05
Py		5.91	59.4	82.4	490	48.7	225	2.57	27.6	3.88	83.0	70.8	370	197	447	2.09	6.13
BghiP		9.05	35.2	43.7	270	33.5	231	5.65	22.1	2.83	40.0	35.7	123	74.3	205	1.90	4.72
DL-PAHs																	
BaA	1.9 x 10^{-6}	3.14	16.5	20.2	169	23.6	26.4	1.66	14.1	2.08	28.1	17.7	86.8	50.9	163	1.99	3.22
Chr	2.3 x 10^{-6}	3.47	16.9	22.3	136	20.6	46.6	2.38	14.9	1.81	26.5	17.3	75.6	43.7	124	1.41	2.96
BbF	5.1 x 10^{-6}	9.04	40.3	51.5	347	46.0	222	5.68	36.4	2.67	42.8	39.1	139	84.2	275	2.67	5.23
BkF	1.4 x 10^{-4}	3.99	12.7	16.8	111	15.3	75.2	4.72	11.6	1.70	16.4	13.5	47.9	28.6	95.1	1.28	2.27
BaP	1.6 x 10^{-6}	6.06	20.6	25.4	226	27.4	94.4	3.25	17.1	1.88	26.3	17.4	88.3	54.4	194	2.08	1.03
IcdP	1.5 x 10^{-5}	7.55	22.7	31.2	223	28.2	193	5.80	20.4	2.35	32.8	22.0	88.7	57.1	158	2.03	3.29
DbahA	4.6 x 10^{-6}	4.33	7.85	11.8	57.5	10.8	46.1	3.76	7.71	1.13	7.46	4.48	16.1	11.1	34.9	1.15	1.63
ΣPAHs		63.6	336	449	2940	370	1670	39.3	239	33.2	453	364	1580	927	2460	27.5	66.1
ΣDL-PAHs		37.6	138	179	1270	172	704	27.3	122	13.6	180	131	542	330	1040	12.6	19.6
TEQ$_{PAHs}$		0.762	2.47	3.27	22.0	3.00	15.1	0.808	2.25	0.303	3.20	2.54	9.30	5.62	18.1	0.238	0.415

Na: naphthalene; Anp: acenaphthylene; Ane: acenaphthene; Flr: fluorine; Phe: phenanthrene; Ant: anthracene; Fl: fluoranthene; Py: pyrene; BghiP: benzo(g,h,i)perylene; BaA: benz(a)anthracene; Chr: chrysene; BbF: benzo(b)fluoranthene; BkF: benzo(k)fluoranthene; BaP: benzo(a)pyrene; IcdP: indeno(1,2,3-cd)pyrene; DbahA: dibenzo(a,h)anthracene.

Blank: not detected (< 0.1 ng g^{-1} dw).

TEQ$_{PAHs}$ were calculated using ReP values from Villeneuve et al. (2002).

Supplemental Materials: Figures

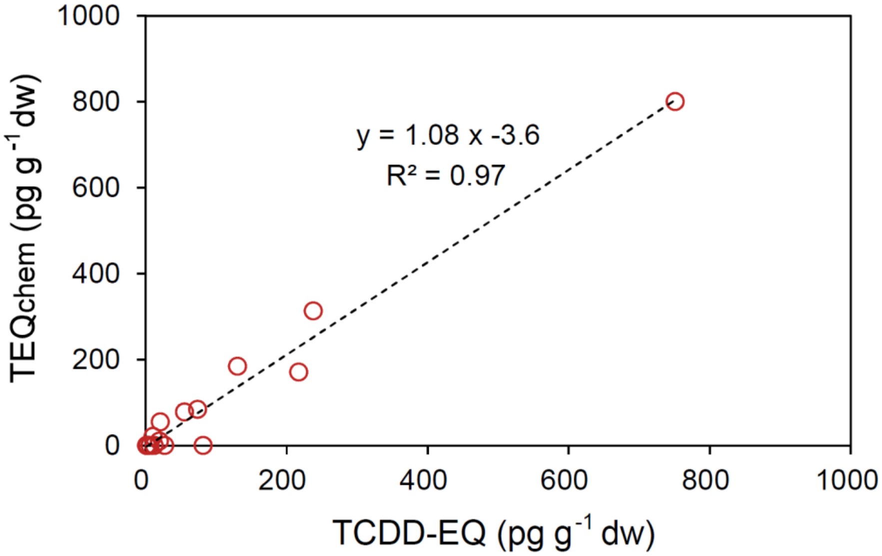

Fig. S1. Relationship between TCDD-EQ and TEQ_{chem} in raw extracts of sediments from Pohang area.

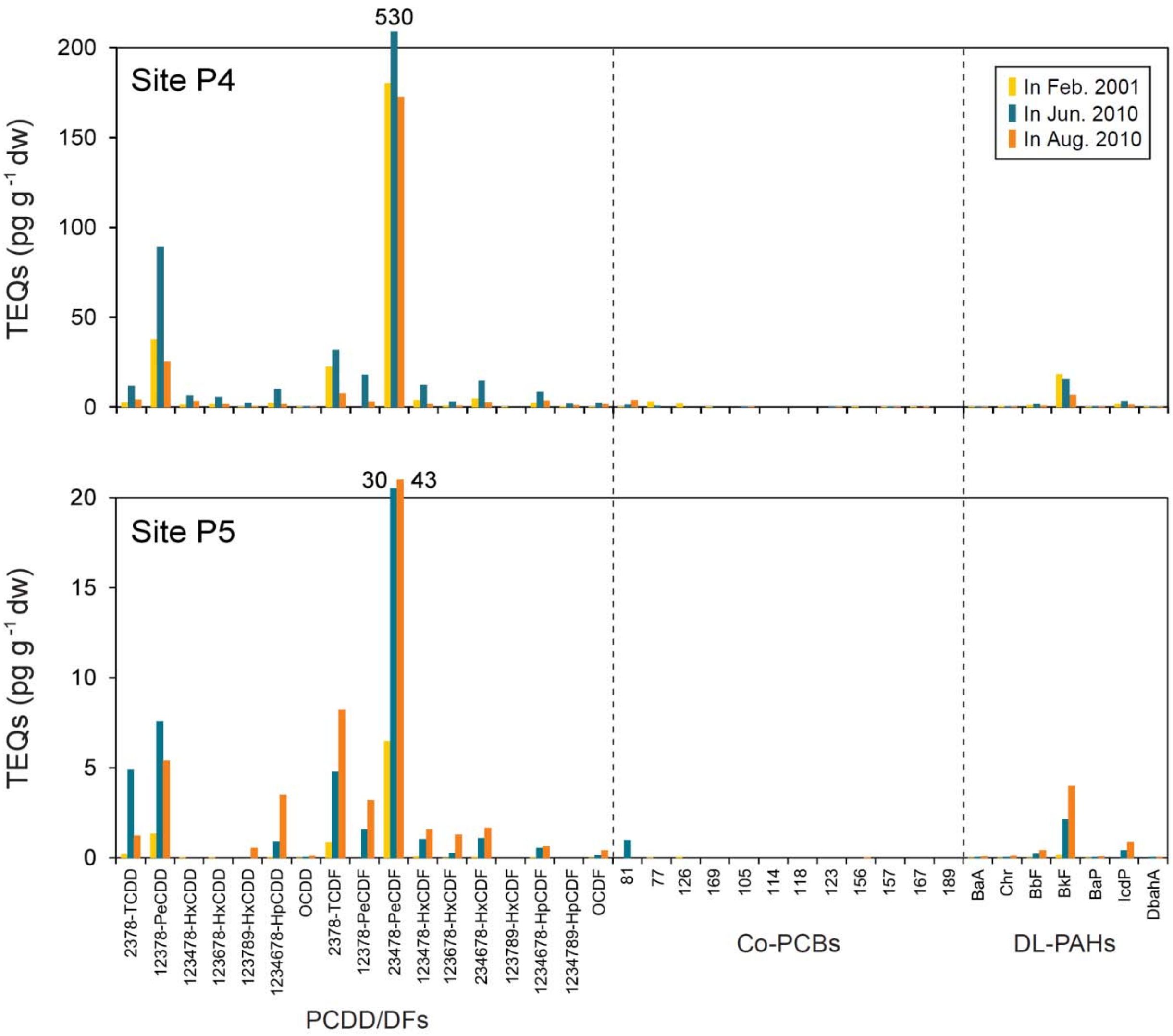

Fig. S2. TEQ values of PCDD/DFs, Co-PCBs, and DL-PAHs in sediments from sites P4 and P5 in 2001 and 2010 (2001 data: Koh et al. (2004)).

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