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Long-term changes in distributions of dioxin-like and estrogenic compounds in sediments of Lake Sihwa, Korea: Revisited mass balance

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HIGHLIGHTS

- Long term changes in concentrations of POCs in sediments of Lake Sihwa, Korea were characterized.
- After 15 years, relatively great concentrations of PAHs were still found in sediments of inland creeks.
- Concentrations of APs and SOs decreased over the past 15 years but hotspots were still found.
- Potencies of specific endpoints determined *in vitro* were still great after 15 years, which indicated continuing inputs of POCs.
- SOs are emerging pollutants that have been present in sediments for 17 years.

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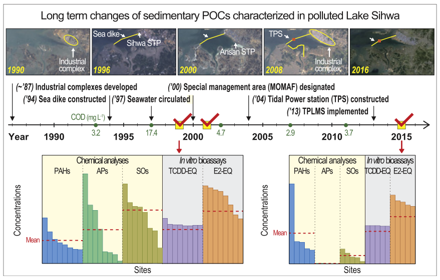
GRAPHICAL ABSTRACT



ABSTRACT

In the late 1990s, severe pollution by persistent organic contaminants (POCs) was observed in Lake Sihwa by use of a combination of instrumental analyses and *in vitro* bioassays. To determine long-term changes (>15-year gaps) in distributions of POCs and their potential toxic potencies in the given region, sediment assessments were reconducted. Target chemicals include polycyclic aromatic hydrocarbons (PAHs), alkylphenols (APs), and recently reported emerging chemicals, styrene oligomers (SOs). We employed *in vitro* bioassays (such as H4IIE-*luc* and MVLN) to determine potential sediment toxicities. The reduction rates of mean concentrations of APs and SOs in sediments between the two sampling years (1998 vs. 2015) were estimated to be 99% and 67%, respectively. Although APs and SOs significantly declined over the 15-year period, concentrations of PAHs were consistent. Results of the bioassays were consistent with the instrumental data, with relatively great concentrations of all target compounds being detected, particularly in inland creeks. Compositions of all target compounds exhibited changes in homologue patterns over the 15 years considered. This result indicated varying and/or continuing sources in this region. In particular, PAHs were dominated by higher-molecular-weight PAHs (e.g., benzo[*g,h,i*]perylene

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Styrene oligomers
In vitro bioassay

and benzo[*b*]fluoranthene) in recent years. This result might indicate consumption of oil-related fuels. Quantitative potency balance analysis revealed that concentrations of instrumentally-derived equivalents accounted for as little as 18.5% of bioassay-derived equivalents, which indicated significant amounts of unknown and/or unmeasured compounds were present. The present study documented the continuing severe pollution by selected POCs in the Lake Sihwa region over the last 15 years, indicating a lack of management in the area studied.

1. Introduction

Lake Sihwa is an artificial lake located on the west coast of Korea that was formed in 1994 following the construction of a dike, which separates it from the sea (Fig. 1a). The original purpose of the lake was to supply water to industrial complexes and the surrounding cities, such as Siheung, Ansan, and Hwaseong (Khim and Hong, 2014; Lee et al., 2014). However after embankment in 1994 the dike stopped flow of tidal currents, the water quality was subject to environmental deterioration because, in parallel to rapid growth of the population and industrial development (Yoo et al., 2006; Lee et al., 2014). In addition, various chemicals have been continuously discharged into the lake via several inland creeks. Thus, the Korean government enforced strategies to redress this problem, such as providing seawater circulation and since 1996 relocating the discharge from sewage treatment plants to outside the dike (Fig. 1a). In 2000, the Korean government designated Lake Sihwa as a special coastal management zone and in 2011 constructed a tidal power station (TPS) to increase tidal mixing (Lee et al., 2014), and finally in 2013 implemented a total pollution load management system. Consequently, water quality of Lake Sihwa has somewhat improved, but contamination of sediments by persistent organic contaminants (POCs) still remained (Jeon et al., 2017; Meng et al., 2017).

Several previous studies have reported the presence of classic POCs, especially polycyclic aromatic hydrocarbons (PAHs) and alkylphenols (APs), in sediments of Lake Sihwa (Khim et al., 1999a; Li et al., 2004a, 2004b; Hong et al., 2010; Choi et al., 2011) (Fig. 2). PAHs are aryl hydrocarbon receptor (AhR) agonists, and are considered priority pollutants because of their mutagenicities and carcinogenicities (Lotufo and Fleeger, 1997). APs such as nonylphenol (NP) and octylphenol (OP) are degradation products of alkylphenol ethoxylates (APEOs), and mimic estrogen. Consequently, these compounds can disrupt hormonal balance (Koniecko et al., 2014) mediated by estrogen receptor (ER) dependent mechanisms (Behnisch et al., 2001; Giesy et al., 2002). Thus, use and production of APs have been banned, or their uses are strictly regulated (Soares et al., 2008).

Recently, emerging POCs, such as styrene oligomers (SOs), have been detected in sediments of Lake Sihwa (Hong et al., 2016b; Lee et al., 2016) (Fig. 2). SOs, including styrene dimers (SDs) and styrene trimers (STs) are produced by decomposition of polystyrene plastic (Ohyama et al., 2001; Yanagiba et al., 2008; Kwon et al., 2015), particularly during thermal decomposition (Kitamura et al., 2003; Kwon et al., 2014). Despite growing concern about contamination of marine environments by plastics, few studies have surveyed concentrations of SOs in coastal sediments (Kwon et al., 2015; Hong et al., 2016b). Furthermore, there have been no previous surveys of SOs in sediments of Lake Sihwa before 2010s. Thus, studies on distributions of SOs and their toxicities, such as endocrine-disrupting effects are needed.

Sediments can act as sinks for POCs (Luo et al., 2009), which are derived from various anthropogenic sources and human activities. POCs are commonly semi-volatile, relatively lipophilic chemicals that are bioaccumulated by organisms (Barra et al., 2005). POCs are also stable compounds that can be deposited and transferred to surface sediments through direct point sources, as well as air-water exchange (Wania and Mackay, 1996). Thus, studies on concentrations of POCs in sediments from the same region over long periods of time could be informative about changes in environments associated with human activities. The greatest limitation of current instrumental analysis is the fact that it is only possible to identify known toxic chemicals (Giesy et al., 2002); however, unidentified chemicals might also interact with receptors (Larsson et al., 2014). To address the hazardous effects of POCs and identify causative chemicals in contaminated sediments, relationships between effects and concentrations of hazardous chemicals, determined by combinations of bioassays and instrumental analyses have been useful (Hong et al., 2016a).

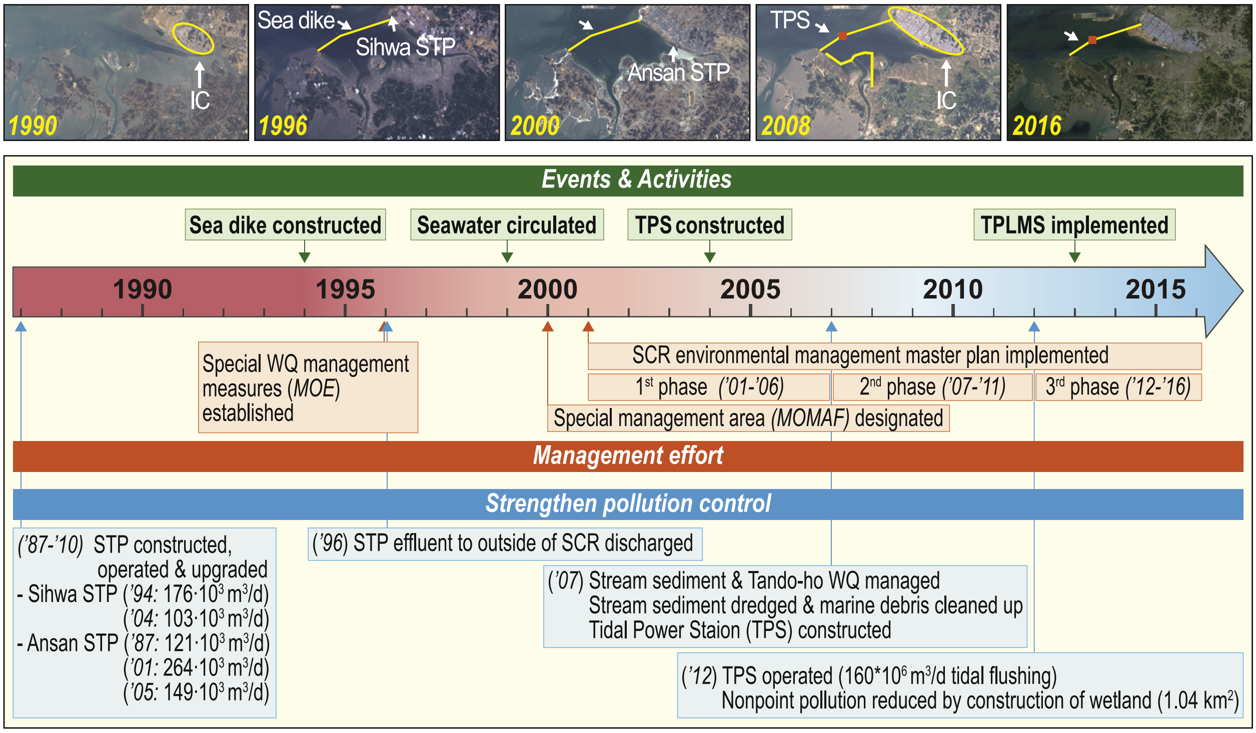
Objectives of the present study, results of which are presented here, were to: 1) investigate changes in classic POCs (PAHs and APs) and emerging chemicals (SOs) in sediments from Lake Sihwa and nearby inland creeks, 2) identify the sources of selected target POCs by compositional analyses, 3) evaluate AhR- and ER-mediated potencies associated with sediment extracts, and 4) assess the relative contributions of targeted compounds in sediments to total induced *in vitro* activities. Identification and quantification of individual compounds by use of gas chromatography (GC)-mass selective detector (MSD) and quantification of AhR- and ER-agonists by use of *in vitro* bioassays using recombinant cells with a luciferase reporter gene were applied simultaneously. The results of this study provide information on relatively long-term changes of several classes of environmental pollutants and associated biological responses in coastal sediments, highlighting adaptive management and control for some persistent and emerging POCs.

2. Materials and methods

2.1. Sampling and sample preparations

Sediments were collected from Lake Sihwa and nearby creeks in Korea (Fig. 1b). A total of 27 batches of samples from three surveys conducted over a 15-year period were collected. These samples included archived samples collected from Lake Sihwa in January 1998 and newly collected samples from inland creeks and the lake in 2015 (Fig. 1b). The sites from which samples were collected during 2015 were the same as those from which samples had been collected in 2001 (Hong et al. 2016b). To investigate long-term changes in occurrences and distributions of selected POCs, the eight samples of sediments collected from inland creeks (S1–S8) in 2001 (Koh et al., 2005) were compared to samples collected from the same areas of creeks during 2015. Due to differences in sampling sites in creeks between 2001 and 2015 (Fig. 1b), site-specific comparison were limited. Most of the sites inside Lake Sihwa receive POCs from similar sources, thus trends in long-term,

(a) Historical overview of pollution of Lake Sihwa and its management



(b) Study area and sampling design

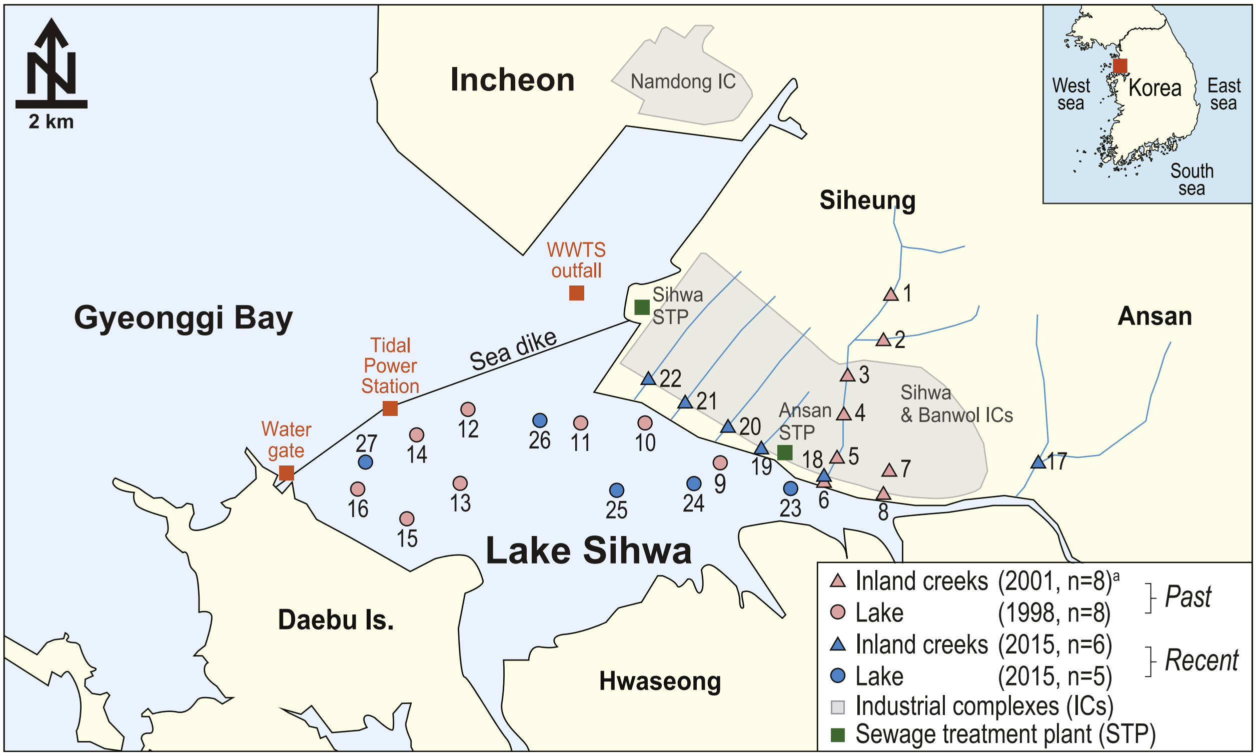


Fig. 1. (a) Satellite images and brief summary of the events/activity related to Lake Sihwa, including management effort and pollution control by the Korea Government (1987-recent). IC: Industrial complex; STP: Sewage Treatment Plant; TPS: Tidal Power Station; TPLMS: Total Pollution Load Management System; WQ: Water Quality; MOE: Ministry of Environment; SCR: Sihwa Coastal Reservoir; MOMAF: Ministry of Maritime Affairs and Fisheries. (b) Map showing the locations of the study areas, sediment sampling sites in the inland creeks in 2001 (1–8) and 2015 (17–22), and sediment sampling sites in the lake in 1998 (9–16) and 2015 (23–27). ^{a} Data from Koh et al. (2005).

temporal changes between creeks and lake could be observed and comparisons made.

All samples were immediately transferred to the laboratory and stored at −20 °C until analyses. Samples collected in 1998 were freeze-dried and kept at −20 °C for 18 years. To obtain better insight on differences in concentrations of target chemicals in sediments and to avoid technical or methodological biases, samples that had been collected in 1998 were extracted and analyzed together with the 2015 samples.

Detailed descriptions of sample preparation for chemical analyses and bioassays have been published previously (Hong et al., 2012, 2015). In brief, 10 g of freeze-dried sediments were extracted for 16 h with 350 mL dichloromethane (Burdick & Jackson, Muskegon, MI) by use of Soxhlet extractor. To remove elemental sulfur, extracts were treated with activated copper powder (Sigma Aldrich, Saint Louis, MO) and concentrated to 1 mL. For *in vitro* assays, the aliquot of extract was exchanged into dimethyl sulfoxide (DMSO, Sigma-Aldrich) by use of differential volatilization.

2.2. Identification and quantification of chemicals

Concentrations of PAHs, APs, and SOs in organic extracts of sediments were quantified using an Agilent 7890 GC equipped with a 5975C MSD (Agilent Technologies, Santa Clara, CA). Details of instrumental conditions for analyses of PAHs, SOs, and APs are provided (Table S1 of the Supplementary Materials (S)). A total of 15 PAHs were quantified, 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*]flouranthene (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). APs were also quantified, including NPs (isomeric mixture), nonylphenol monoethoxylate (NP1EO), nonylphenol diethoxylate (NP2EO), 4-*tert*-octylphenol (4-*tert*-OP), 4-*tert*-octylphenol monoethoxylate (OP1EO), and 4-*tert*-octylphenol diethoxylate (OP2EO). Four SDs and six STs (full chemical names and abbreviations are provided in Table S1) were analyzed.

Limits of detection (LODs) for PAHs, SOs, and APs in sediments were calculated as 3.707 × standard deviation of the standard sample. LODs for PAHs, SOs, and APs ranged from 0.2 to 1.3 ng g^{-1} dry mass (dm), from 0.2 to 0.9 ng g^{-1} dm, and from 0.1 to 0.9 ng g^{-1} dm, respectively (Table S2). A QA/QC program was applied in the present study. First, analytical method blank samples were quantified with each set of samples. Mean concentrations of PAHs, SOs, and APs in blank samples were all less than LODs. Five surrogate standards (SS; acenaphthene-d10, phenanthrene-d10, chrysene-d12, and perylene-d12 for PAHs and SOs; bisphenol A-d16 for APs) were used to assess the recoveries of target chemicals. Mean recoveries of five SS were generally within the acceptable range (83–102% for PAHs and SOs; 77% for APs; detailed in Table S2). Accuracy of determination of PAHs was assessed by use of certified reference material (CRM 1941b; marine sediment, Gaithersburg, MD) and recoveries ranged 74–112% (mean = 93%; n = 3).

2.3. In vitro bioassay

The H4IIE-*luc* bioassay was preformed according to previously published methods (Hong et al. 2012, 2015, 2016b). Dilution factors for samples were determined from the results of the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay (Yoo et al., 2006), samples with the percentage of live cells >80% were used for the bioassay. Trypsinized cells (~7 × 10^4 cells mL^{-1}) from a culture plate were seeded in 60 interior wells of a 96-well plate by adding 250 μL per well. Metabolically labile compounds, such as PAHs or stably AhR binding compounds, including polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and coplanar-polychlorinated biphenyls (Co-PCBs) were identified with two exposure durations (4 and 72 h) in H4IIE-*luc* bioassay (Louiz et al., 2008; Lee et al., 2013; Larsson et al., 2014). After overnight incubation, the cells were exposed to 0.25 μL of extracts of sediments per well (0.1% dose). After 4 h and 72 h exposure, luminescence produced by luciferase was measured by use of a Victor X3 multi label plate reader (Perkin-Elmer, Waltham, MA).

A MVLN bioassay was used to evaluate ER-mediated potencies in organic extracts of sediments (Khim et al., 1999a, 1999b). Trypsinized cells from a culture plate were diluted to a concentration of approximately 1.25 × 10^5 cells mL^{-1} in the 60 interior wells of a 96-well plate with 250 μL medium per well. After 24 h incubation, test and control wells were dosed with 0.25 μL per well. Luciferase activity was determined after 72 h of exposure using methods described previously (Villeneuve et al., 2002).

2.4. Bioassay data analysis

Responses of the H4IIE-*luc* assay expressed as mean relative luminescence units were converted to percentages of maximum response (%BaP_{max} or %$TCDD_{max}$) for a standard containing 50 nM BaP (100% BaP_{max}) for 4 h and 300 pM 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) (100% $TCDD_{max}$) for 72 h. Responses of the MVLN bioassay were converted to relative response units expressed as the percentage of the maximum response (%$E2_{max}$) observed for 1235 nM 17β-estradiol (E2). Significant responses were defined as those that were three times the standard deviation of the mean of the solvent controls.

AhR-mediated potencies at 72 h exposure were expressed as 2,3,7,8-TCDD equivalent concentrations (pg TCDD-EQ g^{-1} dm). These concentrations were determined directly from the sample dose-response relationships generated by testing samples at multiple levels of dilution. The E2 standard equivalent concentration (pg E2-EQ g^{-1} dm) was also calculated using the same method. EC50s of BaP and TCDD in H4IIE-*luc* cells and E2 in MVLN cells were 2.91, 0.72 and 4.06 log fmol/well, respectively (Fig. S1). Relative potencies (RePs) were determined directly from the sample dose-response relationships for each sample and standard curve generated from the range of standard materials (TCDD and E2) dilutions. ReP50s were determined at dilutions of samples for which responses were equivalent to 50% response levels of maximum concentrations of TCDD or E2 on standard curves, respectively (Hong et al., 2012; Lee et al., 2013). All samples were performed in triplicates.

2.5. Potency balance analysis

A potency balance analysis between bioassay-derived concentrations (for TCDD-EQs and E2-EQs) and instrument-derived concentrations (TCDD equivalent concentrations [TEQs] and E2 equivalent concentrations [EEQs]) in sediments were conducted to evaluate how individual chemicals contributed to total dioxin-like and estrogenic potencies. Concentrations of TEQs were calculated as the sum of the TEQs, by multiplying the concentration of individual PAHs by their respective relative potency values (RePs) obtained from previous studies (Villeneuve et al., 2002) (Table S3). EEQ values of APs were summed from concentrations of NPs and 4-*t*-OP multiplied by their respective RePs, which were previously reported (Villeneuve et al., 1998). Principal component analysis (PCA) was performed using the normalized values of the chemical analysis data (individual chemicals) and the bioassay data (bioassay-derived equivalents). SPSS 23.0 (SPSS INC., Chicago, IL) was used for the statistical analyses.

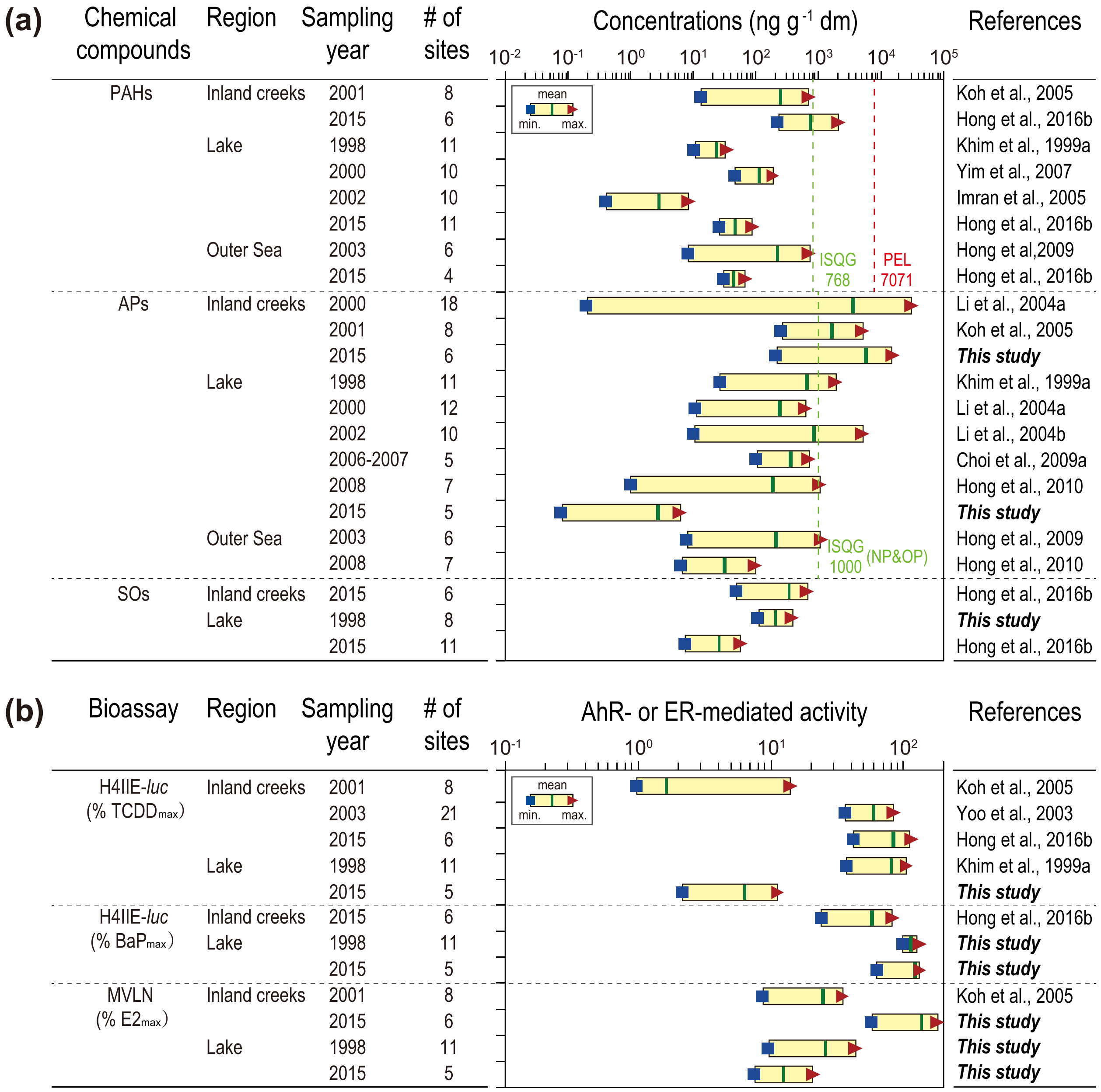


Fig. 2. Summary of concentrations of: (a) polycyclic aromatic hydrocarbons (PAHs), alkylphenols (APs), and styrene oligomers (SOs); (b) AhR- and ER-mediated potencies in sediments from inland creeks, lake, and outer sea around Lake Sihwa.

3. Results and discussion

3.1. Long-term changes in distributions of POCs in sediments

PAHs, APs, and SOs were detected in all sediment samples (Fig. 3). Total concentrations of PAHs ranged from 11.3 to 1900 ng g^{-1} dm (mean: 231 ng g^{-1} dm). Concentrations of APs and SOs ranged from 1.24 to 5024 ng g^{-1} dm (mean: 965 ng g^{-1} dm) and 10.1–740 ng g^{-1} (mean: 227 ng g^{-1} dm), respectively. In general, concentrations of target chemicals varied among locations and when samples were collected, with increasing trend for PAHs and decreasing trend for APs and SOs.

Concentrations of PAHs extracted from sediment in inland creeks ranged from 12.8 to 643 ng g^{-1} dm (mean: 226 ng g^{-1} dm, n = 8) in 2001 (Koh et al., 2005) and from 210 to 1900 ng g^{-1} dm (mean: 693 ng g^{-1} dm, n = 6) in 2015. Mean concentrations of PAHs in sediments from inland creeks increased by nearly three-fold over the ~15-year period between the two collections (Fig. 3a). These changes might be due to releases of pyrogenic (combustion) and petrogenic (petroleum) sources between 2001 and 2015 (Valavanidis et al., 2008). In lake sediments, concentrations of PAHs were similar in 1998 (mean: 18.9 ng g^{-1} dm) and 2015 (mean: 25.1 ng g^{-1} dm). Detectable concentrations of PAHs in sediments from the lake observed in sediments collected in 1998 (Khim et al., 1999a) were similar to those in sediments collected in 2015. In general, concentrations of PAHs were greater in sediments from inland creeks than in those from the lake. This result was consistent with results of a previous study, in which concentrations of PAHs were greater near urban centers (Juhasz and Naidu, 2000; Lee et al. 2016).

Similar to PAHs, concentrations of APs were also greater in sediments from inland creeks than those in sediments from the

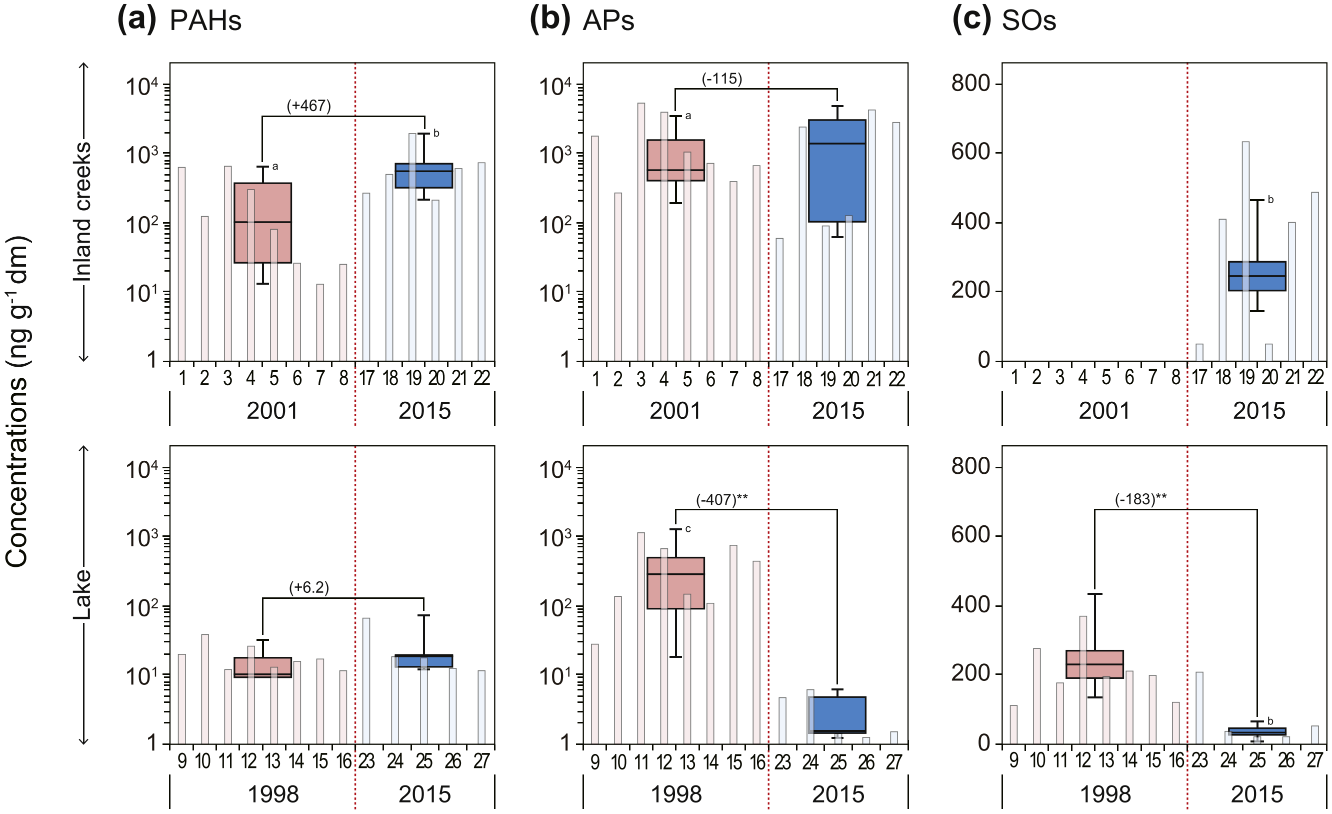


Fig. 3. Spatio-temporal distributions of (a) polycyclic aromatic hydrocarbons (PAHs), (b) alkylphenols (APs), and (c) styrene oligomers (SOs) in the sediment samples from the inland creeks and lake area of Lake Sihwa in the past and current studies. Box plots represent the minimum, 25%, median, 75%, and maximum values of data from same period and region. Data refer to [a] Koh et al. (2005), [b] Hong et al. (2016b), [c] Khim et al. (1999a). ** Significant declines in concentration are indicated for each chemical groups ($p < 0.05$).

lake (Khim et al., 1999a; Li et al., 2004a; Imran et al., 2005). Thus, it can be concluded that most PAHs and APs originated from surrounding industrial complexes and cities. Mean concentrations of APs in sediments from the lake were 410 ng g^{-1} dm ($n = 8$) in 1998 and 3 ng g^{-1} dm ($n = 5$) in 2015. This rapid and significant decrease in concentrations of APs between 1998 and 2015 is indicative of effectiveness controls put in place (Yim et al., 2007; Hong et al., 2009). The Ministry of Environment in Korea designated NPs as priority toxic chemicals in 2001, prohibiting their use as components of household cleaners in 2002, and in 2006 prohibiting their use in some industrial applications, such as paints and inks (Choi et al., 2011).

SOs are emerging pollutants resulting from degradation of plastics in marine environments. However, during the last decade there are no reports are available on historical distributions of SOs in coastal environments. Thus, in order to address the historical occurrence as well as long-term changes, in this study, archived samples from 1998 were re-analyzed together with samples collected in 2015. Concentrations of SOs in sediments from the lake were ranged from 132 to 324 ng g^{-1} dm (mean: 217 ng g^{-1} dm) in 1998 and from 10.1 to 62.6 ng g^{-1} dm (mean: 34.2 ng g^{-1} dm) in 2015 (Hong et al., 2016b). This result indicated that, although only recently recognized as emerging POCs, SOs have been present in the Lake Sihwa aquatic system for several decades. The major reason for the dramatic decreases concentrations of SOs in sediments between the two sampling years was likely not due to dilution, but rather, changes in multiple sources of SOs contamination in the given area. Limited information is available on occurrences of SOs in sediments; thus, more complementary studies would be necessary to identify their sources and fate.

Concentrations of PAHs, APs, and SOs in sediments from inland creeks were greater than those from the lake (Fig. 3). This spatial distribution of POCs indicates that chemicals tend to accumulate in sediments near the sources, because of anthropogenic activities in municipal and industrial areas. POCs that are related to industrialization, urbanization, and hydrophobic compounds, rather than hydrophilic contaminants, can contribute significantly to toxicities of sediments (Schulze-Sylvester et al., 2016). Because POCs could not be transported to distant regions due to the hydrodynamic conditions of the lake, greater concentrations of PAHs, APs, and SOs observed in sediments from inland creeks suggest that the region is still affected by industrial complex in nearby cities. Since the late 1990s, sediments from the lake, which had been contaminated previously could have been purified by occasional circulation of seawater through the water gate (Lee et al., 2014). In addition, construction of the tidal power station in 2012 could have reduced sediment contamination in the lake due to circulation of seawater into the lake.

3.2. Relative compositions of chemicals in sediments

Relative compositions of target POCs in sediments collected in 2015 were compared to profiles of the same chemicals in sediments collected in 1998 and 2001 (Fig. 4). Profiles of relative concentrations of PAHs, APs, and SOs varied among locations and in particular between the lake and inland creeks and between when sediments were collected. These results indicated that spatio-temporal changes in sources of contaminants with possible recent input(s) (Yunker et al., 2002). When compared to PAHs collected in 1998 and 2001, PAHs observed in 2015 were dominated by higher-

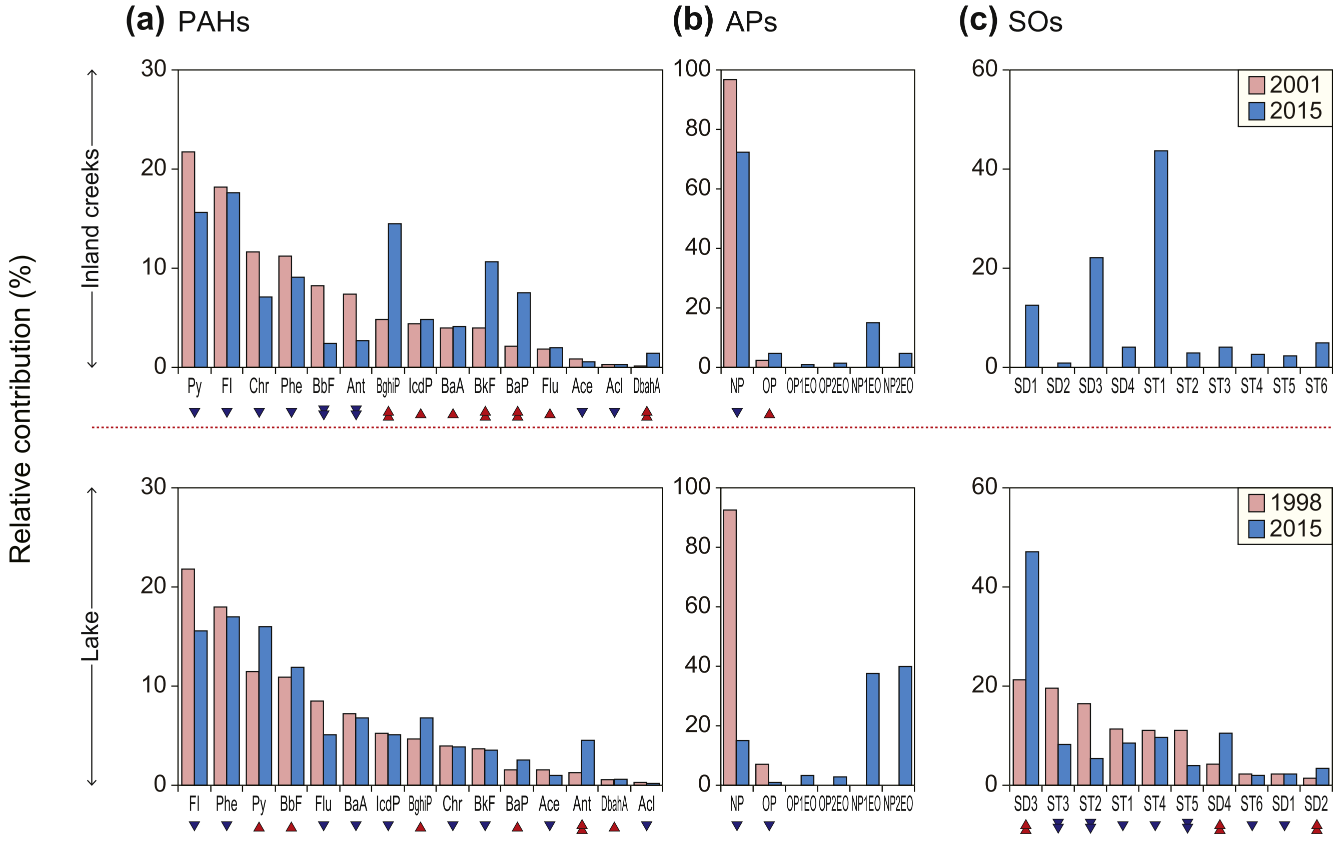


Fig. 4. Comparison of profiles of relative concentrations of: (a) 15 individual PAHs to total concentrations of PAHs, (b) two APs (OP + NP), six APs to total APs for the past and recent samples, and (c) 10 individual SO to total SOs concentrations in the sediments from the inland creeks and lake in the past (1999 and 2001) and recent (2015) years.

molecular-weight (HMW) PAHs that had five to six rings (Fig. S2). This pattern was especially predominant in the sediments from the inland creeks. On average, HMW PAHs (such as BkF, BaP, DBahA, and BghiP) from the inland creeks represented 23.4% of total PAHs in 2001; however, by 2015, this percentage had increased to 41.1%. Over time relative contributions of HMW PAHs in sediments from inland creeks increased by more than two-fold, while those of lower-molecular weight PAHs (LMW PAHs) (such as Ant, Py, and Chr) declined. These results confirmed that industrial waste (combustion of heavy-duty diesel engines and gasoline engines) contributes to the concentrations of HMW PAHs (Rogge et al., 1993) in sediments of inland creeks. This phenomenon occurred because HMW PAHs are derived from combustion of gasoline and diesel in vehicles, whereas LMW PAHs in the environment are derived from incomplete combustion of wood, coal, and biomass (Li and Duan, 2015). It is also possible that, due to lesser hydrophobicity LMW PAHs they can degrade more rapidly, which would cause a decline in relative contributions of LMW PAHs to total concentrations of PAHs. Ratios of IcdP / (IcdP + BghiP) against BaA / (BaA + Chr) and Fl / (Fl + Py) as a function of Ant / (Ant + Phe) in sediments were calculated to verify sources of PAHs contributed from petroleum and combustion, respectively (Fig. S3) (Yunker et al., 2002). Results showed that PAHs in sediments of inland creeks originated primarily from combustion of petroleum, whereas those in sediments in the lake originated from combustion of grass, wood, and coal (Hong et al., 2012; Jiao et al., 2012).

In 2015, relative compositions of APs varied between sediments from inland creeks and the lake (Fig. 4b). In sediments from inland creeks, among APs, NP (72.7%) was the predominant constituent, followed by NP1EO (15.2%). Similarly, relative proportions of OP, were greater than those of OP1EO and OP2EO in sediments from inland creeks. The pattern observed in sediments from the lake were different. For example, NP2EO (40.3%) and NP1EO (37.6%) were the primary APs in sediments from the lake. It is possible that, because NP and OP are hydrophobic chemicals that tend to adsorb to sediments, the chemicals do not break down well in water columns (Ying et al., 2002). These results collectively suggest that current regulations, which prohibit their use, along with more efficient treatment of wastes have been effective in reducing inputs of APs into the inland creeks and subsequently into the lake.

In 2015, of the 10 SOs, ST1 (43.6%) was the predominant constituent in sediments from inland creek sediments, followed by SD3 (22.1%). In contrast, also in 2015, in sediments from the lake, SD3 (47.0%) was the predominant chemical (Fig. 4c). These results indicate that SD3 is widely distributed in the coastal area, and might be transported to areas relatively far from land. Also, the composition of STs (71.0%) in sediments collected from the lake in 1998 was greater compared to that in sediments (37.0%) collected during 2015. Greater concentrations of STs compared to SDs indicates that the relationship between polystyrene pollution and human population is affected by direct inputs from local sources (Kwon et al., 2014; Hong et al., 2016b). Because, after mechanical breakdown, ST1 is first detected from decomposition of polystyrene, relatively large contributions of STs might indicate recent inputs of fresh materials. Results of a previous study showed that ST1 adsorbs to surfaces of sediments and can persist for some time (Saido et al., 2014). However, the results of the present study indicated that concentrations of STs were not maintained over the 15-year period. Few studies have been conducted on distributions and relative compositions of SOs in coastal marine sediments

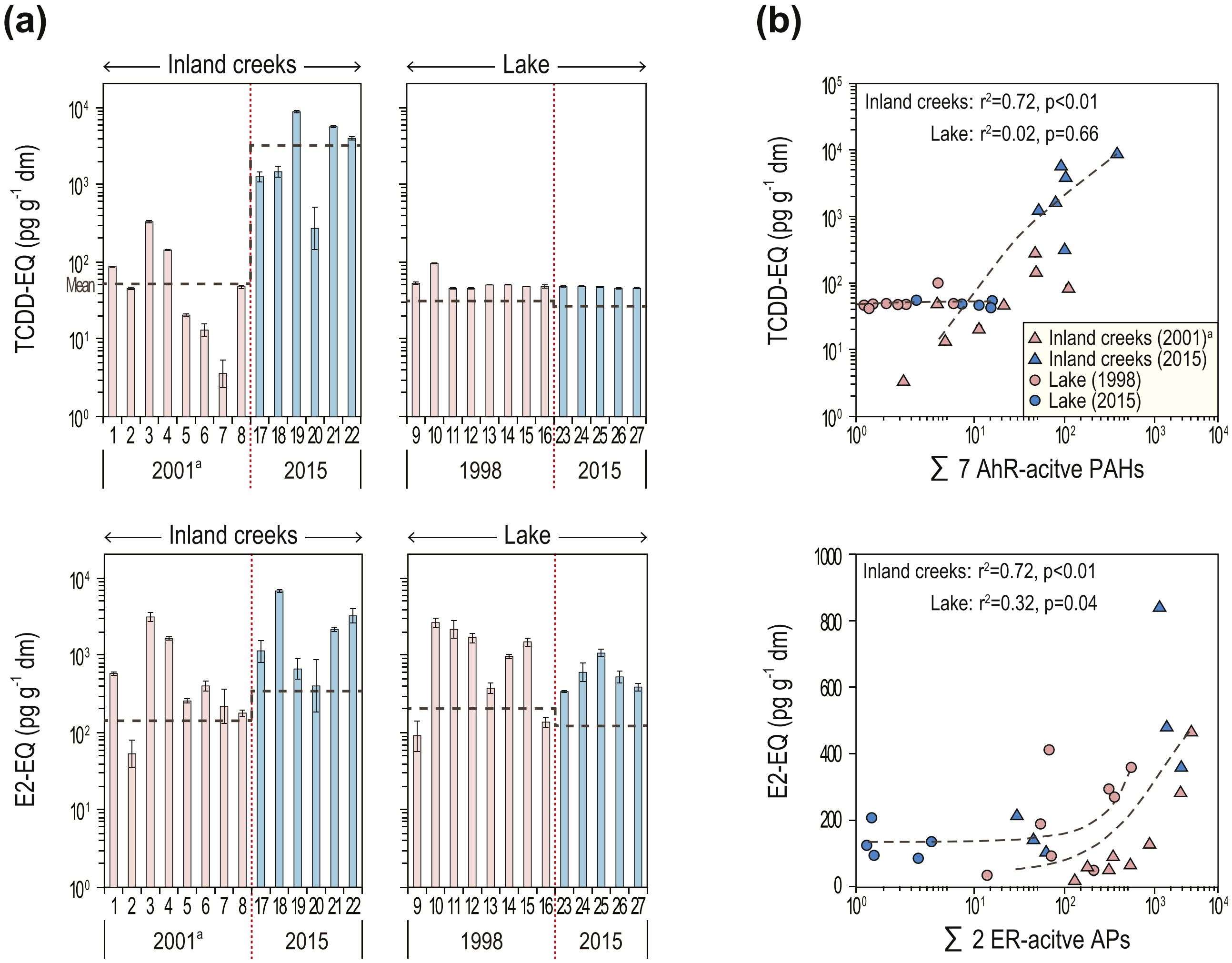


Fig. 5. Spatiotemporal distributions of (a) TCDD-EQs and E2-EQs in sediments from inland creeks and Lake Sihwa in 1998 and 2001 or 2015 (Mean ± SD (n = 3)). (b) Scatter plots showing the dose-response relationships between the chemical analyses and biological responses in the inland creeks and lake sediments of Lake Sihwa. [a]Data refer to Koh et al. (2005).

(Kwon et al., 2014, 2015; Saido et al., 2014; Hong et al., 2016b), thus more studies are required on sedimentary SOs.

3.3. *In vitro potencies of AhR- and ER- active chemicals in sediments*

To assess potential AhR-mediated activities of sediments from Lake Sihwa (Fig. S4) and differentiate between labile and more recalcitrant compounds, H4IIE-*luc* bioassays were performed after both 4 h and 72 h. All extracts contained AhR-mediated potency that was dependent on duration of exposure as well as location. In sediments collected from the lake in 2015, mean concentrations of AhR-mediated potency for 4 h exposure were 60 to 127% BaP_{max} (mean = 103% BaP_{max}). In comparison, mean concentrations of AhR-mediated potency after 72 h exposure ranged from 2 to 11% $TCDD_{max}$ (mean = 6% $TCDD_{max}$) (Fig. S4). These results suggest that the chemicals responsible for activation are rapidly metabolized by the cells. This finding supports the hypothesis that PAH-like chemicals are important in observed AhR-mediated activity (Louiz et al., 2008; Kinani et al., 2010). Also, greater AhR-mediated potency was detected in sediments from inland creeks compared to that in sediments from the lake. Dioxin-like activity and chemical analysis showed large variation across sites, which might be related to industrial and urban activities in the vicinities of inland creeks. Thus, results of the H4IIE-*luc* bioassay indicated that various actions implemented to reduce releases of these compounds to the creeks have been effective, but concentrations of dioxin-like compounds in sediments of Lake Sihwa remain high.

The 19 sediment extracts were screened for their ability to promote ER-mediated gene expression in MVLN cells (Fig. 5a; inland creek 2001 sediment data were obtained from Koh et al., 2005). All of the tested samples elicited a significant increase in luciferase expression. The sediments that exhibited estrogenicity were collected from rivers that receive sewage discharges. Sources of various ER agonists in sediments are hypothesized to originate from sewage effluents and industrial discharges (Luo et al., 2011). Over time, ER-mediated potency in sediments from the lake have decreased significantly. Mean estrogenic potencies were 211 pg E2-EQ g^{-1} dm and 128 pg E2-EQ g^{-1} dm in sediments collected 1998 and 2015, respectively (Table 1 and Fig. 5a). These results indicated that concentrations of major ER-active compounds in sediments from the lake have decreased over the last 15 years. This change indicates that efforts directed towards improvement of the Lake Sihwa environment, including the ban in 2002 on use of ER-active compounds in household products, have been effective (Hong et al., 2010).

Table 1
Overview of results of instrument-derived equivalents and bioassay-derived equivalents of sediments collected from Lake Sihwa, Korea.

Region	Sampling year	Number of sites	Instrument-derived equivalents		Bioassay-derived equivalents[a]		Potency balance analysis[b]	
			TEQ[c]	EEQ[d]	TCDD-EQ	E2-EQ	TEQ/TCDD-EQ	EEQ/E2-EQ
			Min.-Max. (Mean)	Min.-Max. (Mean)	Mean	Mean	Min.-Max. (Mean)	
			(pg g^{-1} dm)		(pg g^{-1} dm)		(%)	
Inland creeks	2001	8[e]	0.1–4.7 (1.7)	3.4–65.9 (22.0)	86.3	148	0.6–5.6 (2.6)	8.1–18.5 (14.6)
	2015	6	0.6–5.9 (2.1)	nd[f] –13.5 (2.9)	3630	211	0.0–0.2 (0.1)	0.02–3.8 (0.8)
Lake	1998	8	nd–0.3 (0.1)	0.4–14.4 (5.4)	54.7	356	0.1–0.4 (0.2)	0.4–12.9 (3.5)
	2015	5	0.3–0.9 (0.6)	0.0–0.1 (0.03)	47.0	128	0.6–2.0 (1.3)	0.0–0.1 (0.03)

[a] Bioassay-derived values were obtained from sample dose-response relationships generated by testing samples at multiple levels of dilution.
[b] Potency balance values were obtained from the percentage of instrument-derived values to the bioassay-derived values.
[c] TEQ values of PAHs were summed from the chemical concentrations of BaA, Chr, BbF, BkF, BaP, IcdP, and DBahA multiplied by the ReP values obtained in a previous study (Villeneuve et al., 2002).
[d] EEQ values of APs were summed from the chemical concentrations of NPs and 4-*t*-OP multiplied by the ReP values obtained in a previous study (Villeneuve et al., 1998).
[e] Data from Koh et al. (2005).
[f] nd: Below limits of detection.

Although concentrations of ER-active compounds declined over time, spatial patterns of estrogenic potencies in sediments did not change. ER-mediated potencies in the past and present sediment samples from the inland creeks were greater those in sediments from the lake. This difference might be because the sediments in the inland creeks have been more contaminated by industrial, agricultural, and domestic wastewaters from the nearby cities. Several studies have reported that the sources of various ER agonists in sediments originate from industrial discharge and sewage effluent. Examples of such agonists include NPs, 4-*t*-OP, estrone (E1), and estradiol (E2) (White et al., 1994; Kinani et al., 2010; Luo et al., 2011). In our study, APs concentrations (such as NPs and 4-*t*-OP) were consistently detected over time in the sampled sediments. Thus, these results support the conclusion that even though APs are relatively weak ER agonists, their concentrations are sufficient to contribute a significant portion of the ER-mediated potency measured in the bioassay.

3.4. *Potency balance*

To determine the proportion of *in vitro* potencies observed in samples, that could be explained by concentrations of known agonists, a potency balance analysis was conducted (Khim et al., 2001; Giesy et al., 2002; Hong et al., 2012). TEQs contributed by AhR-active PAHs explained only a small portion of total concentrations of TCDD-EQ. Proportions explained by PAHs ranged from 0.2 to 2.6% in sediments from 1998-2001 and from 0.1 to 1.3% in sediments collected in 2015 (Table 1 and Fig. S5). By examining the relationship between TCDD-EQs and AhR-active PAHs of the dataset in a scatterplot (Fig. 5b), TCDD-EQs were found to be correlated ($r^2 = 0.75$, $p < 0.01$) with AhR-active PAHs in sediments from inland creeks. Known AhR-active compounds accounted for only a small portion of total induced AhR-mediated potencies in sediments but significant correlations between some AhR-active PAHs and dioxin-like activities suggested some compounds similar to PAHs were acting as agonists. Overall, results of the potency balance suggests that unknown AhR-mediated compounds are broadly distributed in the sediments.

NP and 4-*t*-OP contributed 14.6% of the concentration of EEQs in sediments collected from inland creeks in 2001 and 0.8% of EEQs in 2015. In comparison, in sediments from the lake, NP and 4-*t*-OP contributed only 3.5% of concentrations of EEQs in 1998 and 0.03% of concentrations of EEQs in 2015 (Table 1). Contributions of known ER agonists, such as NP, decreased over 15 years. Target APs (such as NP and 4-*t*-OP) did not explain much of the overall estrogenic activities, particularly in sediments collected more recently. Thus, studies on unidentified ER agonists in sediments are needed. For example, to better characterize sources of contamination of these sites, use of effect-directed analysis (EDA), based on sample fractionation and identification of fractions causing significant potencies in bioassays followed by detailed untargeted instrumental analyses (Brack, 2003; Hong et al., 2016c). In sediments from inland creeks, ER-mediated activities (E2-EQs) were correlated ($r^2 = 0.50$, $p < 0.01$ and $r^2 = 0.32$, $p = 0.04$, respectively) with concentrations of $\sum$ 2 ER-active APs (Fig. 5b). These results indicate that total concentrations of ER-mediated potencies in sediments from Lake Sihwa were relatively well explained by concentrations of APs.

To characterize the bioassay-EQ in the sediments further, a principal component analysis (PCA), based on the concentrations of residues, was performed (Fig. 6). The PCA produced two major components that collectively accounted for 56.0% of the total variance. PC1 (explaining 30.0% of total variance) was positively correlated with PAHs, APs, and E2-EQ, but was negatively correlated with TCDD-EQ. When sites were ordinated based on PCA scores, they were classified into three groups. The first group

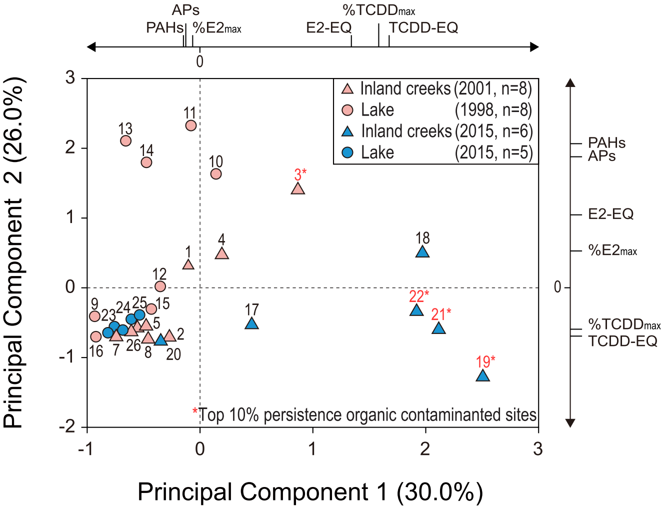


Fig. 6. Principal component analysis of concentrations of PAHs, APs, AhR-mediated potency (% $TCDD_{max}$), ER-mediated potency (% $E2_{max}$), and bioassay-derived equivalents in sediments collected from 27 locations in Lake Sihwa. Clusters are represented as locations (inland creeks and lake), in which principal components 1 and 2 accounted for 30.0% and 26.0% of the variability of the dataset, respectively.

contained sediments collected in 2015 from inland creeks. These sites exhibited greater concentrations (top 10%) of target POCs, and contained greater concentrations of TCDD-EQ and E2-EQ. The second group was characterized by sites with relatively great concentrations of PAHs and APs. These sites might have accumulated these compounds from independent sources and/or due to different conditions along the creeks to the lake. The lesser correlation between concentrations of TCDD-EQ and PAHs indicates that AhR-active PAHs explain only a small proportion of concentrations of TCDD-EQ. Overall, the PCA analysis demonstrated that the majority of variance was observed among locations but generally supported the time-dependent aggregation.

4. Conclusions

One of the greatest challenges of this study was related to use of archived samples of sediments to assess long-term changes (or differences) in distributions of absolute and relative concentrations of target chemicals. SOs, which are considered to be emerging contaminants of concern, were present in the Lake Sihwa system in the past decades, but their concentrations have been decreasing over the last 15 years. In contrast, concentrations of PAHs have increased slightly. Concentrations of APs in the lake sediments have decreased significantly over the last 17 years, which suggests that controls on their use and release have been effective. Results of H4IIE-*luc* and MVLN bioassays for extracts of sediments from the lake were consistent with instrumental results. Concentrations of target chemicals significantly differed among locations. For instance, greater concentrations of PAHs, APs, and SOs were observed in sediments from inland creeks. This observation suggests that distributions of these compounds are mainly governed by proximities to primary sources. Inland creeks were located in highly populated areas with greater industrial activities compared to the lake. In 2015, proportions of individual chemicals differed among locations. Furthermore, unidentified dioxin-like and estrogenic activities were observed. Thus, it is necessary to investigate whether unknown toxic compounds contribute to overall sample toxicity. In conclusion, the results of the present study enhanced understanding of the long-term spatiotemporal differences of several POCs of interest, including new POCs such as SOs, which could be measured in archived samples of sediments from Lake Sihwa.

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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.2017.04.074.

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<Supplementary Materials>

Long-term changes in distribution of dioxin-like and estrogenic compounds in sediments of the Lake Sihwa, Korea: Revisited mass balance

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Supplementary Tables

Supplementary Figures

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Supplementary Tables

Table S1. GC/MSD instrumental conditions for the determination of polycyclic aromatic hydrocarbons, styrene oligomers, and alkylphenols

Target Compounds	Polycyclic aromatic hydrocarbons	Styrene oligomers	Alkylphenols
	Naphthalene (Na) 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) Benzo[*a*]pyrene (BaP) Indeno[*1,2,3-cd*]pyrene (IcdP) Dibenz[*a,h*]anthracene (DBahA) Benzo[*g,h,i*]perylene (BghiP)	1,3-Diphenylproane (SD1) *cis*-1,2Diphenylcyclobutane (SD2) 2,4-Diphenyl-1-butene (SD3) *trans*-1,2-Diphenylcyclobutane (SD4) 2,4,6-Triphenyl-1-hexene (ST1) 1e-Phenyl-4e-(1-phenylethyl)-tetralin (ST2) 1a-Phenyl-4e-(1-phenylethyl)-tetralin (ST3) 1a-Phenyl-4a-(1-phenylethyl)-tetralin (ST4) 1e-Phenyl-4a-(1-phenylethyl)-tetralin (ST5) 1,3,5-Triphenylcyclohexane (ST6)	4-*tert*-octylphenol (4-*t*-OP) 4-*tert*-octylphenol monoethoxylate (OP1EO) 4-tert-octylphenol diethoxylate (OP2EO) Nonylphenol (NP) Nonylphenol monoethoxylate (NP1EO) Nonylphenol diethoxylate (NP2EO)
GC/MSD system	Agilent 7890A GC and 5975C MSD		
Column	DB-5MS (30 m long × 0.25 mm i.d × 0.25 µm film thickness)		
Gas flow	1.0 mL/min (He)		
Injection mode	Splitless		
MS temperature	180 °C		
Detector temperature	230 °C		
Injection volume	2 µL		1 µL
Oven temperature	1. 60 °C hold 2 min 2. Increase 6 °C/min to 300 °C 3. 300 °C hold 13 min		1. 60 °C hold 5 min 2. Increase 10 °C/min to 100 °C 3. Increase 20 °C/min to 300 ° C 4. 300 °C hold 6 min

Table S2. QA/QC data for sedimentary PAHs, SOs, and APs measured in the present study

Compounds	**Method detection limit (ng g dw^{-1})**	**Surrogate recovery (%, n=7)**
PAHs and SOs		
Acl	0.78	
Ace	0.89	
Flu	1.32	
Phe	0.95	
Ant	0.40	
Fl	0.95	
Py	0.90	
BaA	0.62	
Chr	0.95	
BbF	0.66	
BkF	0.86	
BaP	0.69	
IcdP	0.72	
DbahA	0.17	
BghiP	0.61	
SD1	0.34	
SD2	0.65	
SD3	0.94	
SD4	0.23	
ST1	0.57	
ST2	0.53	
ST3	0.30	
ST4	0.49	
ST5	0.32	
ST6	0.34	
Ace-d10		82.9 ± 10.1[a]
Phe-d10		102.3 ± 22.2
Chr-d12		97.6 ± 14.6
Pery-d12		92.9 ± 20.4
APs		
t-OP	0.07	
NPs	0.93	
t-OP1EO	0.09	
NP1EOs	0.45	
t-OP2EO	0.09	
NP2EOs	0.76	
BPA-d16		76.8 ± 18.7

[a] Mean ± SD

Table S3. Relative potency values of PAHs for the AhR-mediated activities used in this study

Target compound	**H4IIE-*luc* cells (72 h[a])**
benzo[*a*]anthracene (BaA)	1.9×10^{-6}
chrysene (Chr)	2.3×10^{-6}
benzo[*b*]fluoranthene (BbF)	5.1×10^{-6}
benzo[*k*]flouranthene (BkF)	1.4×10^{-4}
benzo[*a*]pyrene (BaP)	1.6×10^{-6}
indeno[*1,2,3-c,d*]pyrene (IcdP)	1.5×10^{-5}
dibenz[*a,h*]anthracene (DBahA)	4.6×10^{-6}

[a] Villeneuve et al., 2002.

Supplementary Figures

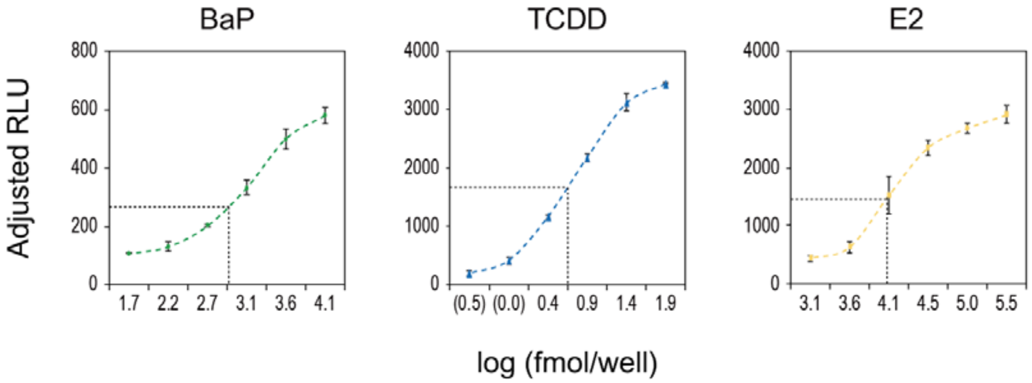

Fig. S1. Dose response curves for all tested standard materials in the present study. Each data point is the Mean ± SD (n=3).

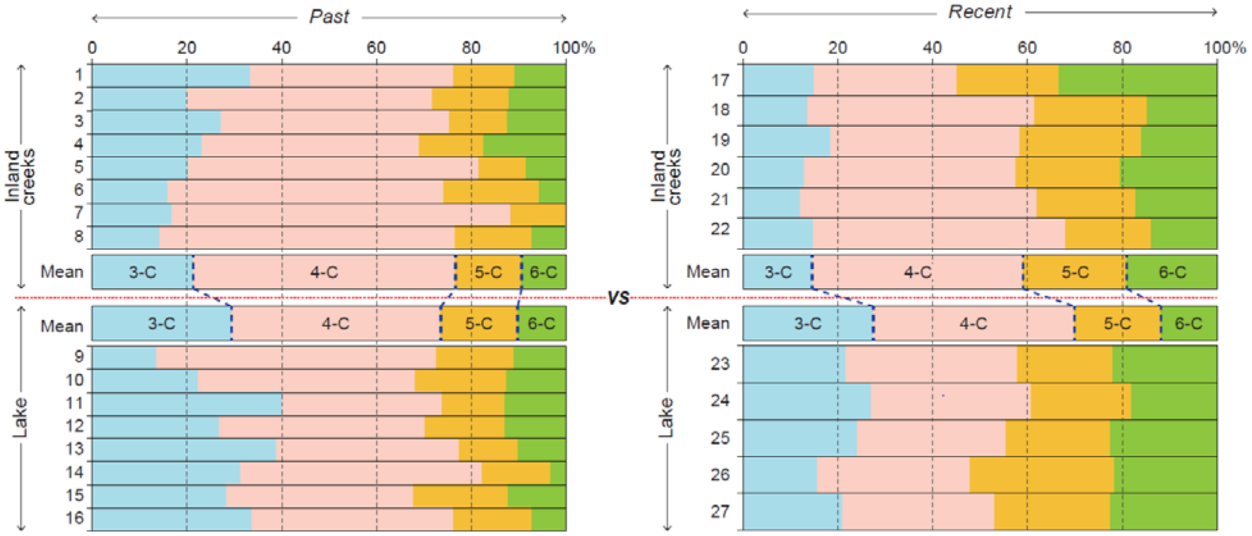

Fig. S2. Mean composition of PAH ring number to total PAHs from the inland creeks and lake sediments in the past and current studies.

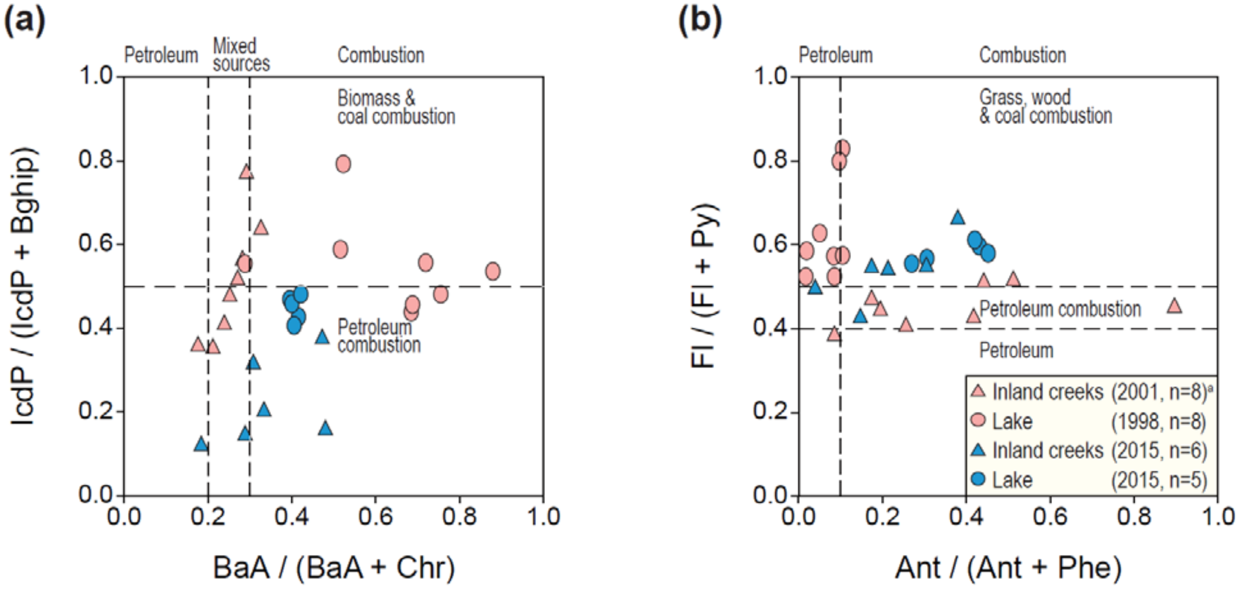

Fig. S3. Results of the diagnostic ratios for the source identification of the PAHs. (a) Cross plots for the ratios between BaA / (BaA + Chr) with IcdP / (IcdP + BghiP) and (b) the ratios between Ant / (Ant + Phe) with Fl / (Fl + Py) for source identifications of PAHs in the sediments.

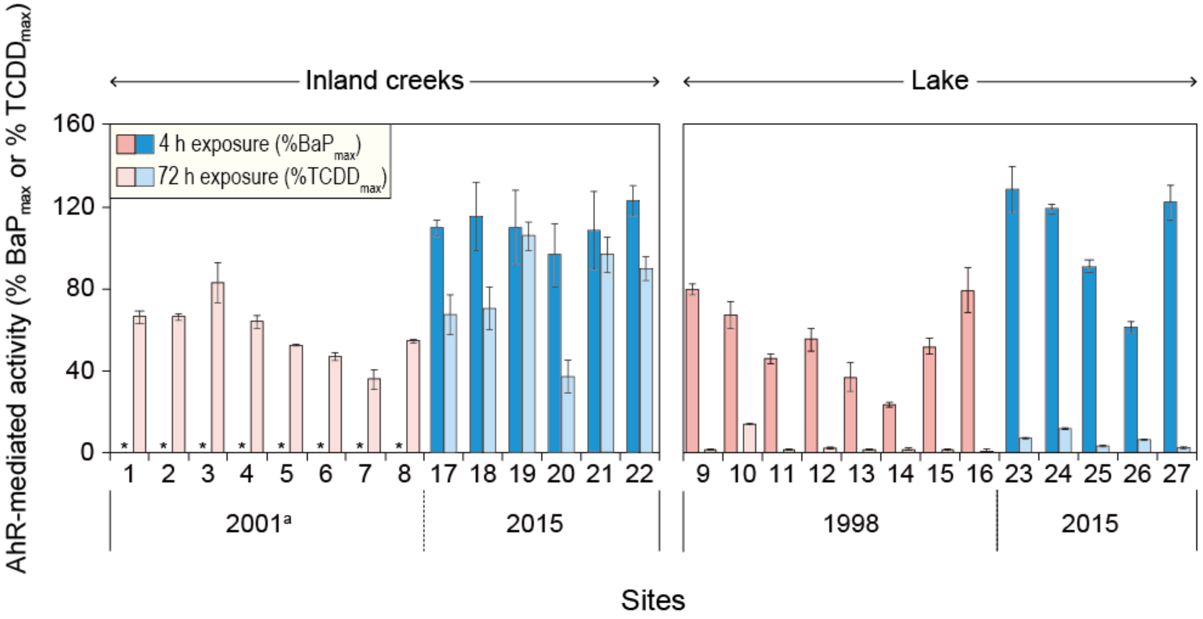

Fig. S4. AhR-mediated activities of the sediment raw extracts from the inland creeks and lake in the past and current years at 4 h and 72 h exposure durations in the H4IIE-*luc* bioassay. [a] Data from Koh et al. (2005). * Data not available.

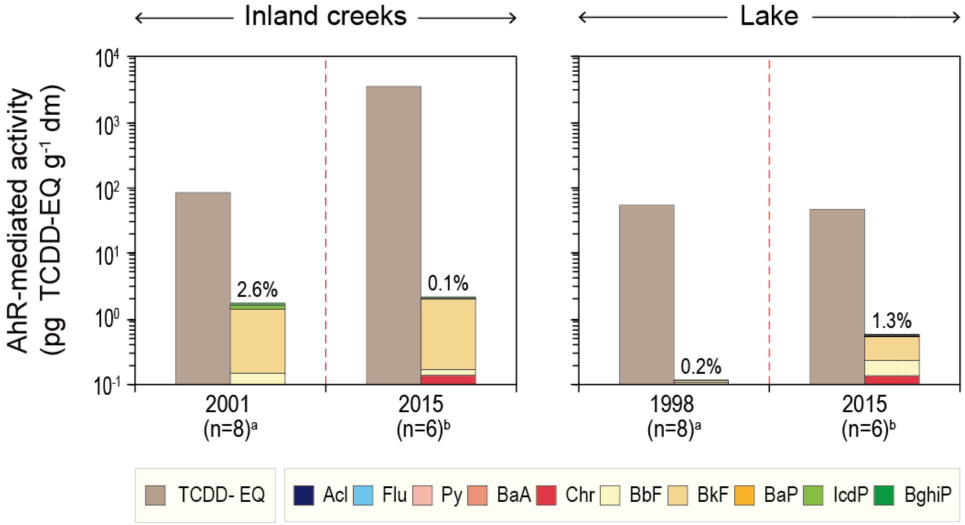

Fig. S5. Potency balance between bioassay-derived (TCDD-EQs) and instrument-derived TEQs in sediments collected from Lake Sihwa and the relative contributions of identified TEQs in the sediment samples.