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In vitro and *in vivo* toxicities of sediment and surface water in an area near a major steel industry of Korea: Endocrine disruption, reproduction, or survival effects combined with instrumental analysis

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HIGHLIGHTS

- Toxicities of sediment and water samples near a steel industry were characterized.
- Endocrine disruption of sediment was partly explained by alkylphenols and bisphenol A.
- Target chemicals alone could not explain the observed endocrine disruption *in vitro*.
- Levels of target chemicals in sediments are comparable to those detected a decade ago.

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ABSTRACT

The influence of industrial and/or municipal contaminant inputs on the aquatic environment of Pohang, Korea was investigated, with a focus on bioassay combined with instrumental analysis. Pohang is the most heavily populated city in Gyeongsangbuk-do province of Korea, with more than half a million residents, and also hosts the nation's biggest steel manufacturer and related industries. Sediment ($n = 15$) and surface water samples ($n = 17$) were collected from Hyeongsan River which runs across the Pohang city, in two separate events, *i.e.*, June 2010 and February 2011. Sediment samples were first Soxhlet-extracted (raw extract) and were measured for estrogenicity using H295R cell line, and also analyzed for alkylphenols (APs), bisphenol A (BPA), PAHs, and PCBs. For sediment samples which exhibited greatest effects in the cell line, further fractionation was performed into non-polar, mid-polar, and polar portions. In surface water samples, heavy metals were also analyzed. Among 15 sediment samples, station S2 near the steel industry complex and station M3 near the municipal area showed the greatest sex hormone changes, and these changes were generally explained by the fractions which contained APs and BPA. Principal component analysis (PCA) however suggests that chemicals that were not analyzed in the present study would better explain endocrine disruption capacity of sediments. In water samples, adverse effects on hatchability and growth of Japanese medaka fish, and on *Daphnia* reproduction were noted following exposure to six water samples collected from stations near industrial and municipal areas. Several heavy metals and nonylphenol (NP) concentrations exceeded surface water quality guidelines, suggesting adverse effects of contamination inputs from both industrial and municipal activities. Observed estrogenicities in stations such as S2 and M3 warrant further investigations on longer term ecosystem impacts near industrial and municipal areas. The levels of major organic chemicals in sediments are quite comparable to those reported in ~10 years ago, emphasizing a need for source control.

1. Introduction

Pohang, located on the east coast of South Korea, is a unique metropolitan area with a heavy industrial complex. Its environment has therefore been reported for contaminations of both industrial and municipal

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origins (Koh et al., 2004, 2006a). Pohang is the most populated city (>520,000 residents as of September 2012) in Gyeongsangbuk-do province, and approximately 150,000 tons of treated sewage effluent is discharged daily to Hyeongsan River, which runs across the city, and reaches Yeongil Bay of East Sea (www.ipohang.org/open_content/environment/environ-ment/newMenu872/). In addition, Pohang accommodates the nation's biggest steel manufacturing industry complex which includes over 300 industries operating in about 13,000,000 m^3 of area (www.phsic.or.kr). Due to industrial and municipal activities, Pohang area has been frequently reported for contaminated air, water, or sediment, especially with organic chemicals (Baek et al., 2010; Choi et al., 2008; Jo et al., 2008). In our previous studies, polycyclic aromatic hydrocarbons (PAHs, range between 5.30 and 7680 ng/g dw), alkylphenols (APs, range between 11.0 and 89.7 ng/g dw), organochlorine pesticides (OCs, range between 2.1 and 4.7 ng/g dw) and polychlorinated biphenyls (PCBs, average of 61.4 ng/g dw) were detected in the sediments of this area (Koh et al., 2004).

In this area, one of the concerns about the industrial and municipal discharges to nearby water body is endocrine disruption. APs, bisphenol A (BPA), OCs, and PAHs which have been documented for endocrine disruption capacities (Carson, 1962; Mnif et al., 2011; Okada et al., 2008; Villeneuve et al., 2002a,b) have frequently been detected in the sediments of Pohang (Jo et al., 2008; Koh et al., 2004, 2006a). Our previous work on this area also showed that fractional extracts included OCs and PAHs, and showed strong estrogenic activities in *in vitro* bioassay following exposure to organic fractionized samples (Koh et al., 2006b). In addition, many other endocrine disrupting synthetic compounds such as plasticizers and surfactants, *e.g.*, APs and BPA, have also been reported in aquatic ecosystem near Pohang area at the levels above the sediment quality guidelines of Canada (Canada, 2002; Koh et al., 2004, 2006a).

While several studies on endocrine disruption capacities of the sediments have been performed in the area, most of the studies are only focused either on analytical measurements or on bioassays. To date, only one study has applied both chemical analysis and bioanalytical measurements to determine and relate endocrine disruption of the sediments of this area (Koh et al., 2006b). This study (Koh et al. 2006b) however only measured estrogenic receptor binding affinity using MVLN cell line. Since many chemicals are known to exert estrogenicity not only through estrogen receptor binding but also by alteration of steroidogenesis (Blaha et al., 2006; Hecker et al., 2006; Nakajin et al., 2001; Zhang et al., 2011), the use of MVLN cell line only may have limited value in understanding endocrine disrupting potentials of the sediments.

The purpose of the present study is to understand the current status of endocrine disruption potentials in sediment samples of Hyeongsan River area, and to relate them to chemical analytical data. In addition, we measured *in vivo* toxicities of the water samples to understand ecological implication of the water contamination. For this purpose, we collected both sediment and surface water samples from multiple stations along the Hyeongsan River, and measured chemical concentrations using analytical instruments, *in vitro* steroidogenic alteration assay using a human adrenocortical carcinoma cell line, and *in vivo* sublethal and lethal toxicities using waterflea and fish. The results of this study will help understand current status of contamination in Hyeongsan River, and identify the responsible chemicals of concern, which will aid in developing management options around this area.

2. Materials and methods

2.1. Sampling and sample preparation

Surface sediments (0–10 cm) were collected by use of a hand shovel from eight stations (R1, R2, S1–S3, and M1–M3) in and near mainstream Hyeongsan River of Pohang in both June 2010 and February 2011. For locations, refer to Fig. 1, and for detailed descriptions of stations, refer to Tables S1 and S2 in Supplementary material. One sample (station R2, Feb. 2011) could not be sampled because of the weather (frozen water). Sediment samples were mixed thoroughly, and were stored in pre-cleaned glass jars. Sediments were freeze-dried, then were stored at −20 °C until being used for *in vitro* toxicity assays and chemical analysis.

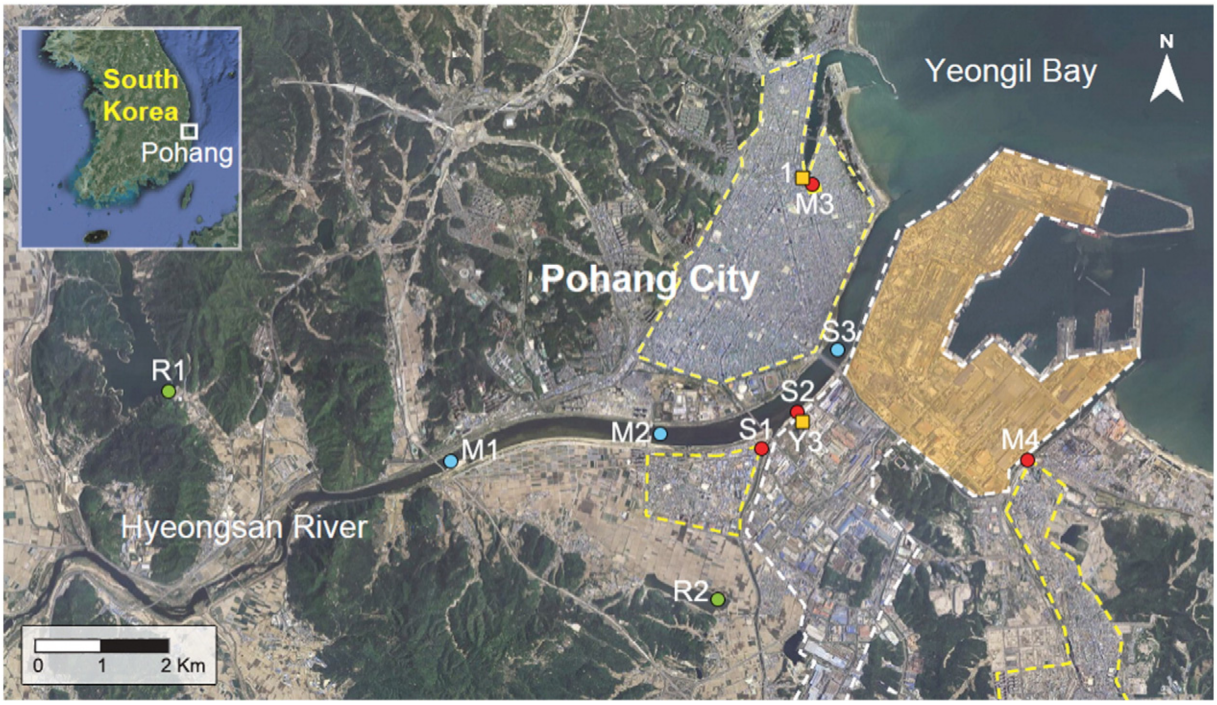

Fig. 1. Location of sampling stations in and near Hyeongsan River, Pohang, Korea.

Detailed descriptions of sample preparation for instrumental analysis and H295R cell *in vitro* bioassay have been published previously (Ji et al., 2011; Khim et al., 1999; Koh et al., 2004). In brief, aliquots (20 g) of freeze-dried sediment sample were extracted with 350 mL of dichloromethane (DCM, Burdick & Jackson, Muskegon, MI, USA) on a Soxhlet extractor for 24 h. Elemental sulfur in sediment extract was removed by use of activated copper (Merck, Darmstadt, Germany), and concentrated to 1 mL, and then divided into two aliquots for use in the bioassay and chemical analysis, respectively. The final extract was prepared by replacing DCM with dimethyl sulfoxide (DMSO, Burdick and Jackson) for *in vitro* bioassay.

Surface water samples were collected from 9 stations in and near the mainstream Hyeongsan River (Fig. 1, Tables S1–S2). Twenty liters of surface water was collected, and basic chemistries including temperature, salinity, and pH were measured *in situ* using an YSI 556 MPS (Multi-probe System, YSI, Yellow Springs, OH, USA). The water samples were immediately transferred to the laboratory, and were stored at 4 °C until being used for further analysis. BOD and COD were measured following standard method (APHA, 1997a,b), and dissolved oxygen (DO) of water sample was measured using a DO meter (YSI 5000) before being used in *in vivo* bioassay. For organic compounds analysis, 10 L water was processed with filtering (GF/F filter) and solid phase extraction (XAD-2) using vacuum aspirator. Following extraction, the same methods for sediment analysis were applied. Filtering and acidification process was conducted for heavy metal analysis in surface water samples.

For fractionation, an activated silica gel column was used and a detailed procedure followed Khim et al. (1999). The first fraction (F1) was obtained by elution with 60 mL of hexane (non-polar fraction, *e.g.*, PCBs), second fraction (F2), with 60 mL 20% DCM in hexane (mid-polar fraction, *e.g.*, PAHs), and third fraction (F3), with 60 mL 60% DCM in acetone (polar fraction, *e.g.*, OP, nonylphenol (NP), and BPA).

2.2. In vitro H295R cell line assay

Changes in sex steroid hormone concentration were determined with a human adrenocortical carcinoma cell line (H295R). This cell line was obtained from the American Type Culture Collection (Manassas, VA, USA) and cultured following Hilscherova et al. (2004). First, the effects on cell viability was checked with MTT bioassay (Mosmann, 1983), and only non-cytotoxic doses showing >80% of survival compared to that of the control were employed for evaluation of endocrine disruption (Hecker et al., 2006). H295R cells were exposed to sediment extracts (4 mg of sediment dry weight/mL, except for a few samples with high cytotoxicity) for 48 h, and were measured for 17β-estradiol (E2) and testosterone (T) by competitive enzyme-linked immunosorbent assay (ELISA). Commercially available kits (17β-estradiol [Cat # 582251] and testosterone [Cat# 582701]; Cayman Chemical, Ann Arbor, MI, USA) were employed for this purpose following the manufacturer's instruction. Briefly, hormones were extracted from the H295R culture media using diethyl ether, and the extracts were subsequently employed for ELISA. Absorbance of the extracted samples and the hormone standards were measured using TECAN Infinite® 200 (Tecan Group Ltd., Männedorf, Switzerland) at a wavelength of 415 nm. Measurement of absorbance was made in triplicate.

2.3. In vivo toxicity test

Effects on reproduction, growth, or survival of *Moina macrocopa*, a freshwater waterflea and *Oryzias latipes*, Japanese medaka fish were determined after chronic exposure to surface water samples ($n = 17$). *M. macrocopa* 7 d and *O. latipes* 14 d early life stage exposure tests were employed for this purpose (OECD, 1992; Oh and Choi, 2012). Based on preliminary tests with sea salt, 2.5 g/L and 15 g/L were determined as chronic no observed effect concentrations (NOECs) of salt for *M. macrocopa* and *O. latipes*, respectively. Thus, when the surface water samples show salinity greater than these concentrations, the water samples were diluted below such levels before being used for *in vivo* assays (for salinity and maximum concentrations of each sample, see Tables S1 and S2). For *M. macrocopa*, survival and reproduction related parameters such as first day of reproduction, and number of young per female were observed during 7 d of exposure. For the fish, survival, hatchability, and dry weight were recorded.

2.4. Instrumental analysis

Six APs and BPA were analyzed by the use of gas chromatograph (Agilent 7890, Agilent Technologies, Palo Alto, CA, USA) coupled with a mass selective detector (Agilent 5975, Agilent Technologies). Chromatographic separation was achieved on a DB-5MS fused silica capillary column (30 m long, 0.25 mm i.d., 0.25 μm film thickness, Agilent Technologies). The mass spectrometer was operated in electron impact ionization (EI) mode at 70 eV with the selected ion monitoring method. The method detection limits (MDLs) for individual OPs, NPs, and BPA were 0.5, 0.3, 0.3 ng/L in water and 0.2, 0.1, and 0.1 ng/g dw in sediment, respectively. Fourteen Co-PCBs and 5 indicator PCBs were analyzed by GC–^{63}Ni electron capture detector (ECD; GC-2100, Shimadtzu, Tokyo, Japan). Sixteen PAHs were quantified using GC–mass selective detector (Agilent 5975, Agilent Technologies). MDLs for PCBs and PAHs were 0.01 ng/g dw and 0.1 ng/g dw, respectively. As quality control standards for phenolics, PCBs, and PAHs, BPA-d16 (Isotec, Miamisburg, OH, USA), PCBs 30, 103, 198, and 209, acenaphthene-d10, phenanthrene-d10, chrysene-d12, and perylene-d12 (Chem Service, West Chester, PA, USA) were employed for five sediment samples in a separate experiment, and recoveries were measured following sample treatments. Recoveries were generally good, *i.e.*, on average 85%. The recovery of BPA-d16 ranged between 86 and 95%. Heavy metal in water samples was analyzed by ICP-MS (Elan 6100/PerkinElmer, USA), and MDLs for heavy metals ranged from 0.1 to 1.0 μg/L. List of target chemicals are shown in Table S3 of Supplementary material.

2.5. Statistical analysis

Principal component analysis (PCA) was conducted to screen the pattern of chemical concentrations of sediment samples, and also used to elucidate relationships between chemical concentrations and endocrine disruption. After determining one principal component, simple regression analysis was conducted with E2 concentrations as a dependent variable. No observed effect concentration (NOEC) of *in vitro* and *in vivo* test was determined at the highest concentration (dilution %) that showed no significant difference from that of the control. Differences among treatments and control were evaluated by one-way ANOVA followed by Dunnett's test or Kruskal–Wallis test using IBM SPSS Statistics 21 (IBM, New York, NY, USA) or ToxStat (ver. 3.5. West, Cheyenne, WY, USA) depending on data distribution. p values less than 0.05 were considered to be statistically significant.

3. Results and discussion

3.1. Endocrine disruption potentials of sediment extracts

For endocrine disruption assay in H295R cells, non-lethal (<80% cell survival) doses determined by MTT assay were 4 mg sediment dw/mL for most of the samples. However, among the sediment samples collected in June 2010, several samples, *e.g.*, S1, M2, and M3, showed <80% cell survival at 4 mg sediment dw/mL, therefore were further diluted to 0.032, 0.8, and 0.8 mg sediment dw/mL for hormonal experiments, respectively (Fig. 2(a)). Among the sediment samples, stations S2 and M3 generally showed significant estrogenicity, *i.e.*, increased E2 and decreased T levels (Fig. 2(b), (c)). But estrogenicity was negligible at the highest dilution of S1 (0.032 mg/mL).

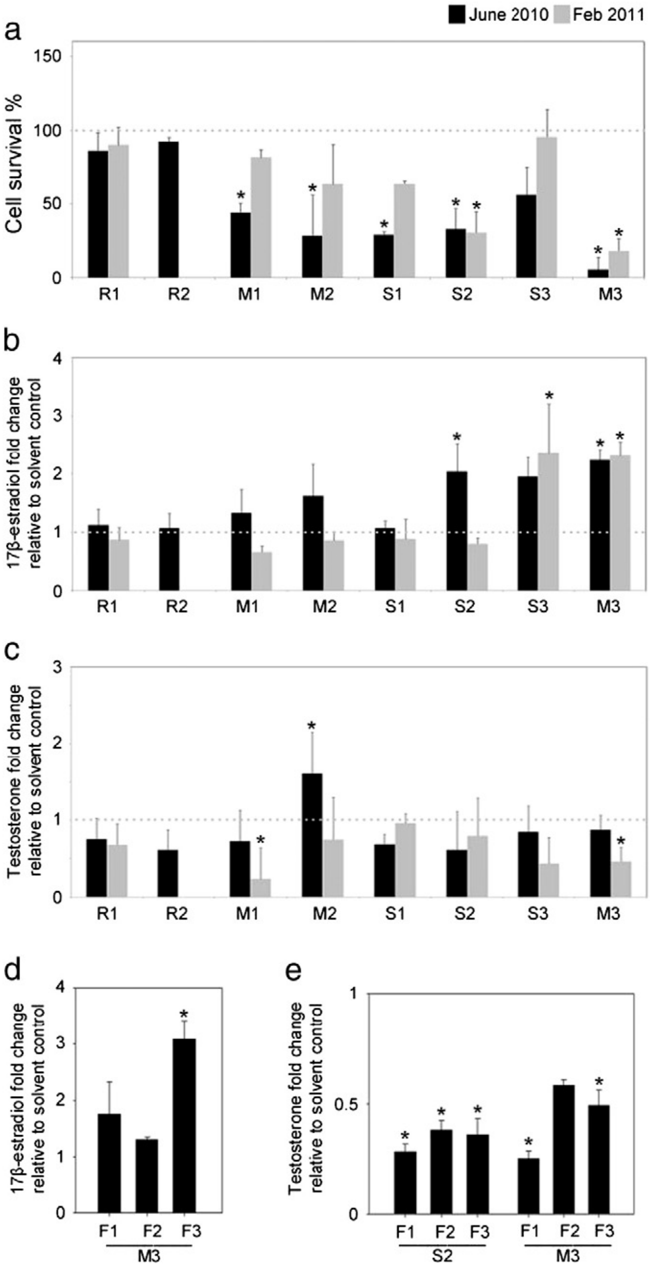

Fig. 2. Toxic effect of sediment extracts in H295R cell. (a) Percent change in cell survival of H295R cell after the exposure to 4 mg of sediment dry weight/mL, and (b) fold induction of 17β-estradiol and (c) testosterone relative to DMSO control. Because of significant hormone inductions observed in the samples S2 and M3, these sediment extracts were further separated into three fractions, and (d) 17β-estradiol, and (e) testosterone induction levels were measured. For hormone measurements from the sediment extracts (b and c), 4 mg/mL was employed except for S1, M2, and M3 samples of June 2010 (because of cytotoxicity, 0.032 mg/mL for S1, and 0.8 mg/mL for M2 and M3 were used). For the fractionized extract samples (d and e), 20 mg/mL was used. Black bars represent June 2010 samples, and gray bars represent February 2011 samples. Asterisk means significant difference from DMSO control of each sediment sample ($p < 0.05$).

Since the concentrations of sediment extract used for hormone measurements varied because of different cytotoxic potentials of each sample, direct comparison of endocrine disruption capacity among different stations is not possible. In order to compare the endocrine disrupting capacity of the sediment samples, we developed a qualitative estrogenic index (qEI) which can be calculated using the following equation.

$$\mathrm{qEI} = \frac{\mathrm{NOEC\ of\ cell\ survival}}{\mathrm{NOEC\ of\ changes\ in\ E2\ concentration}}$$

Higher qEIs are suggestive of greater endocrine disrupting potentials relative to cytotoxic potential. The qEIs calculated for all sediment extracts ranged between 0.2 and 25, with the highest qEI determined at S3 and M3 sediments of Feb 2011 (see Table S4 for details). Station S2 is near Yeonil Drainage Pumping Station which receives and treats wastewaters from the steel industrial complex located south of POSCO, the major steel-making company headquartered in Pohang, and is considered to reflect the discharges from the industrial complex. S3 is near the Hyeongsan Bridge, and is thought to reflect inputs from upstream sources that include various municipal and industrial inputs. Station M3 is close to Jukdo Drainage Pumping Station which is under the influence of municipal wastewater discharge (Fig. 1). Endocrine disruption potentials have been previously documented in several environmental samples collected near industrial and municipal areas (Mantis et al., 2005; Murk et al., 2002; Voutsa et al., 2006).

Among June 2010 samples, station M2 showed significant T increase without change in E2. Among February 2011 samples, station M1 showed significant decrease of T concentrations without significant change in E2 (Fig. 2(b), (c)). Such changes can be explained by further investigation on transcriptional changes in steroidogenic genes. For example, cytochrome P450 (CYP) 17 enzyme functions as two different catalysts steroid 17β-hydroxylase and 17,20-lyase, and is responsible for the production of dehydroepiandrosterone and androstenedione (Ma et al., 2011). Aromatase (CYP19) catalyzes the conversion of androgen to estrogen (Hilscherova et al., 2004). For example, following exposure to ketoconazole, the extent of T change was greater than that of E2 in H295R cell (Hecker et al., 2006), and this phenomenon could be explained by suggesting that ketoconazole has a greater effect on CYP17A1 than on aromatase.

To further narrow down the group of contaminants that are responsible for the observed endocrine disruption at stations S2 and M3, H295R assay was conducted for three fractionized extracts, *e.g.*, F1, F2, and F3 of both sediment samples. The results shown in Fig. 2(d) and (e) clearly show that F3 is mainly responsible for increased estrogenicity of station M3, *i.e.*, increase E2 and decreased T concentrations, while decrease in T concentration was also explained by F1. The observation of increased estrogenicity in F3 can be linked to well-known phenolic endocrine disruptors such as NPs and BPA. These phenolic compounds have been identified in municipal wastewaters or surface water worldwide (Cespedes et al., 2005; Fenet et al., 2003; Li et al., 2004; Nakada et al., 2006; Rudel et al., 1998; Soares et al., 2008), and are known to cause estrogenic responses in several experimental studies (Lee et al., 2003; Petrovic et al., 2002; Soto et al., 1991; Tollefsen et al., 2008; Villeneuve et al., 2002b). However, in station S2 which is expected to be heavily affected by industrial activity, significant T decreases were observed in all fractionized extract samples. These observations indicate that the chemicals responsible for endocrine disruption are different between municipal and industrial locations (PCBs, hexachlorobenzene, and dichlorodiphenyl-dichloroethylene (*p*,*p*′-DDE) in F1, OC pesticides, PAHs in F2, and APs, BPA in F3).

It is interesting that no fractionized samples collected in Feb. 2011 showed significant responses in H295R assay, while the whole extracts for, *e.g.*, S3 and M3 exhibited strong estrogenicity. The reason for this inconsistency in estrogenicity between whole extracts and fractionized extracts is not clear. One possible reason might be the removal of some active compounds during the fractionation procedure. Alternatively, synergistic interactions among the constituents of different fractions could be also possible. Such inconsistencies have often been reported elsewhere. In *Danio rerio* assay, toxicity that was observed in whole extracts was eliminated in fractionized samples (Higley et al., 2012). Koh et al. (2002) reported that the toxic potency of fractionated extract sample was greater than the corresponding whole extract possibly because of antagonistic interactions within the total extract.

3.2. Chemicals in sediments

The concentrations of APs and BPA in all sediment samples are summarized in Table S5 of Supplementary material. Many of the target chemicals such as BPA (Zhang et al., 2011) and APs (Nakajin et al., 2001) are reported to alter steroidogenesis in H295R cell line. The highest concentrations of APs and BPA are detected in station M3 (June 2010), and stations S2 and M2 (Feb. 2011) (Fig. 3(a)). But no specific trends were observed by sampling season or by location. Among the target compounds, APs constitute the majority portion of all target organic

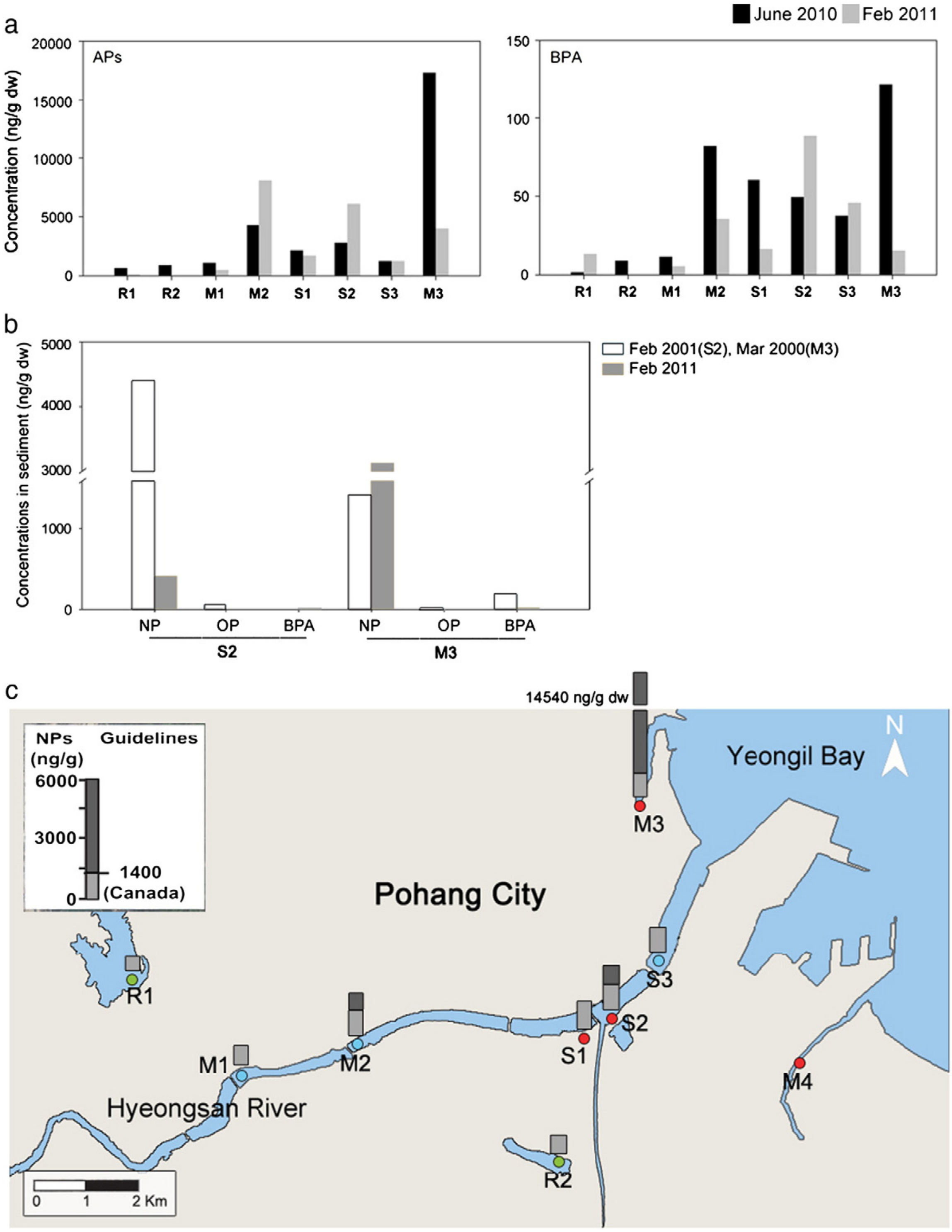

Fig. 3. Results of instrumental analysis in sediment samples collected in and near Hyeongsan River in two sampling events. (a) Alkylphenols (APs) and bisphenol A (BPA) concentrations in total extracts of sediment samples from June 2010 and February 2011. (b) Comparison of NP, OP, and BPA concentrations in sediment from stations S2 and M3 in the present study and a previous study (S2 measured in 2001, Koh et al., 2004; M3 measured in 2000, Koh et al., 2006a). (c) Spatial distribution of nonylphenols (NPs) in sediment samples of June 2010. Sediment sampling was not conducted for station M4. White bars represent March 2000 or February 2001 samples, black bars represent June 2010 samples, and gray bars represent February 2011 samples.

chemicals (Fig. S1(a)). APs occupy over 70% in proportion in almost all sediment samples of both sampling events, except for S1, S2, and S3. APs have been used as surfactants and detergents mostly in industrial processes but can be also detected in municipal wastewaters because of their extensive use in various consumer products like household laundry or kitchen detergent (Voutsa et al., 2006).

In S1–S3 samples, proportions of PAHs become greater, *e.g.*, proportion of PAHs in S2 sample collected in June 2010 was 51%. Such difference in chemical composition is also clearly indicated by PCA. As shown in Fig. S1(b), S2 was different from other stations being studied. Since S2 is a station near a steel industrial complex, increased proportion of PAHs may reflect industrial input. Steel and iron industries are known source of PAHs (Yang et al., 2002). Hong et al. (2014–in this issue) suggested that the sediment PAHs determined in this area be originated from oil and petroleum combustion-related sources, based on composition of individual PAHs.

Compared to our previous report of 2001 (Koh et al., 2006a), NP concentration of S2 measured in the present study was about ten times lower, but that of M3 is quite comparable (Fig. 3(b)). OP was detected at 64 and 24.3 ng/g dw in S2 and M3 in February 2001/2000, respectively (Koh et al., 2004, 2006a), but was not detected in the samples collected in February 2011. However in samples collected in June 2010, OP was detected at higher levels, *e.g.*, 132 and 1359 ng/g dw for S2 and M3, respectively. BPA was not detected in station S2 in 2001 but was detected at 8.9 ng/g dw in the present study. As to M3, notable decrease in BPA was observed compared to the measurement in March 2000. Due to less persistent nature of phenolic compounds, the levels detected might reflect continuing inputs from the sources. Our observations suggest continued influences of both industrial and municipal activities as a source of APs and BPA in Hyeongsan River area.

Among target APs, NP was a predominant compound with concentrations of 4.09–14,539 ng/g dw, occupying on average 41% and maximum 97% of total APs. In comparison with the Canadian Sediment Quality Guideline (1.4 ng/g dw), NP concentrations measured in three locations near the drainage pumping station (M2, M3, and S2) of June 2010 exceeded the guideline value (Canada, 2002). Particularly, extremely high NP concentration was detected in station M3 (14,540 ng/g dw). Since the station M3 is not connected to mainstream Hyeongsan River, it is very likely that the observed NP contamination is mostly originated from municipal activities in Pohang area. Observed estrogenicity of M3 and S2 sediment samples in H295R cell line appears to be mostly explained by NP. High proportion of NP in most samples would explain the T decrease without significant change of E2. 4-NP strongly inhibited CYP17 in H295R cell (Nakajin et al., 2001), which can be linked with decrease in androgens, as shown in M2 (June 2010) and M1 (Feb 2011) samples (Fig. 2(b), (c)).

The relationship between endpoint of cell bioassay (levels of E2) and target organic pollutant concentration was evaluated using regression analysis in combination with PCA. Based on PCA, target chemicals could be reduced to two principal components (PCs), *i.e.*, individual PAHs and PCBs as PC1, and APs and BPA as PC2 (Fig. 4(a)). Among PCs, PC1 did show marginal positive relationship with E2 concentrations ($p < 0.1$. Fig. 4(b)), however, residuals of PC1 was significantly correlated with the E2 induction level (Fig. 4(c)), suggesting compounds other than PC1 (mostly PC2) might play an important role in estrogenicity in H295R cell.

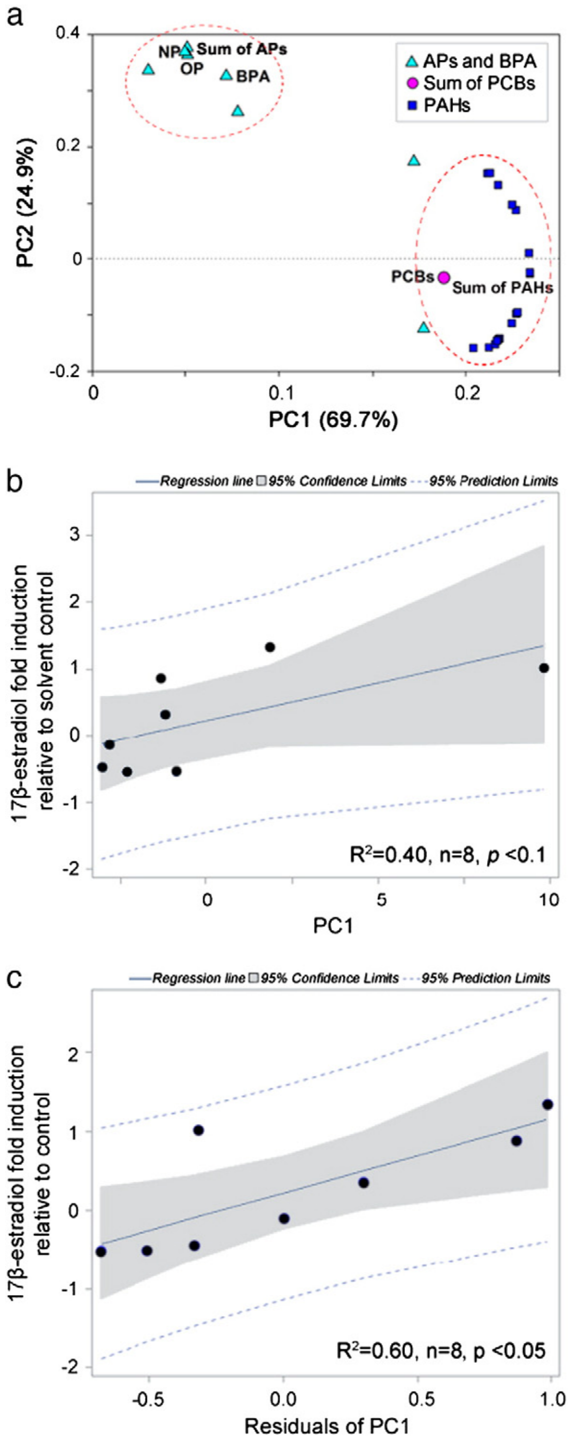

Fig. 4. Preliminary estimation of chemicals that explain 17β-estradiol change by principal component analysis (PCA). (a) PCA results from all target chemicals of all sampling sites. (b) Regression between 17β-estradiol fold change and PC1 of June 2010 samples. (c) Regression between 17β-estradiol fold change and residuals of PC1.

3.3. *In vivo toxicity of surface water samples*

Reproduction or growth related toxicities were observed in *M. macrocopa* or *O. latipes* after chronic exposure to surface water samples collected at stations R1, M1, S2, and M4 (Fig. 5). At 100% (undiluted sample) of M1, M4, and S2 water samples, significant decreases of the fish hatchability were observed. With *M. macrocopa*, toxicity was observed only in M4 sample, and the day of the first reproduction was significantly delayed. Quantitative meaning of *in vivo* toxicity data is however limited, since many water samples were significantly diluted because of salinity adjustment. Since salinity could affect the vulnerability of the test organisms, the highest exposure concentrations were determined at 7.9%–50% for *Daphnia* and 47.6%–66.1% for fish to adjust the salinity under no effect concentrations which were determined for each test species in the present study.

As expected, toxicities detected in surface water samples did not match well with those reported from sediment samples. This

Fig. 5. Spatial distribution of chronic *Moina* and fish toxicities by sampling stations and season. All toxicity tests were conducted after salinity adjustment.

inconsistency is not unusual because the toxicities observed in water samples would reflect more recent inputs of contaminants, while sediment toxicities would reflect historic accumulation of contaminant inputs. Greater heterogeneity of sediment composition could be another reason for the inconsistency with results from water samples. Differences in observation endpoints, *i.e.*, molecular level changes in a human cell line *vs* individual level responses such as mortality or reproduction of aquatic organisms, may also explain the discrepancies in toxicity.

3.4. Chemicals in surface water

APs, BPA and several heavy metals were detected in surface water samples (Table S3, Fig. S2). APs were generally detected at higher concentrations in stations near the industrial area compared to the municipal area (Fig. S2(a)). In particular, the concentrations of NP exceeded the Water Quality Criteria of Canada (1000 ng/L; Canada, 2002) in stations S2, S1, and M1 from June 2010. Currently, Korea does not have a water quality standard for NP. The highest NP was found in station S2, followed by station S1. It should be noted that the sediment sample collected from station S2 also exceeded the Canadian Sediment Quality Guideline of NP (Fig. 3(c); Canada, 2002), suggesting that Yeonil Drainage Pumping Station near the industrial complex is one of the sources of NP to Hyeongsan River. As to BPA, the concentrations were comparable throughout all the stations including a station located far away from the industrial or municipal areas. Except station M2, BPA concentrations of most stations were higher in June 2010 than in Feb 2011. This seasonal difference can be explained by extent of precipitation: June is dry season and February is the season with average precipitation in Korea. For heavy metals, Cd, Cu, Ni, Pb, and Zn in surface water samples were detected at concentrations exceeding the National Recommended Water Quality Criteria of US EPA (Fig. S2(b); http://water.epa.gov/scitech/swguidance/standards/criteria/current/index.cfm#altable). Seasonal trends were however not evident: Cu was detected at higher level in June 2010 than in Feb. 2011, while Pb was detected at higher concentrations in Feb. 2011. This observation may reflect increased contribution of Pb sources during winter, but further investigation is warranted.

Toxicity data did not show significant correlation with analytical results. In M4 which showed *in vivo* toxicity, BPA and APs were detected relatively higher in June 2010 and February 2011, respectively. However, in samples R1, M1, and S2, the levels of target chemicals were relatively low. Because of sample dilution due to salinity, however, toxicity data have rather limited meaning in correlation analysis. Therefore, source identification and management options may be better made based on the chemical analytical data.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.scitotenv.2013.08.010. These data include Google map of the most important areas described in this article.

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Supplementary Material

In vitro and *in vivo* toxicities of sediment and surface water in an area near a major steel industry of Korea: Endocrine disruption, reproduction, or survival effects combined with instrumental analysis

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Contents

Table S1. Description, location, and other information of sampling stations in Pohang, Korea (June, 2010)

	Locations	**Latitude (N)**	**Longitude (E)**	**Temperature (℃)**	**Salinity (psu)**	**pH**	**DO (mg/L)**	**BOD**	**COD**	**Highest conc. (%) of water sample**	
										***Daphnia* test**	**Fish test**
R1	Angey reservoir	36°00'39.7"	129°15'34.9"	29.3	0	8.62	6.63	1.2	5.3	50	100
R2	Jeokgey pond	35°58'40.7"	129°21'26.9"	27.5	0	7.84	6.23	-	17.4	50	50
M1	The upper Hyeongsan River, near a road and apartments	35°59'50.4"	129°19'00.8"	29.2	0	9.22	6.68	2.8	7.2	50	100
M2	The midstream of Hyeongsan River, near Hyeongsan Drainage Pumping Station and sewage treatment plant	36°00'01.1"	129°20'49.5"	24.2	6	7.5	4.21	1.7	50.6	41.67	66.1
M3	End of Haksancheon, near Jukdo Drainage Pumping Station	36°01'49.7"	129°22'24.2"	25.5	28	7.62	3.47	1.7	126	8.93	50
M4	Downstream of Neangcheon, near a big shopping center	35°55'32.0"	129°23'58.0"	27.9	0	7.36	7.62	-	2.6	50	50
S1	End of Chilseongcheon, near an industrial complex	35°59'48.2"	129°21'55.2"	27.3	8	7.16	5.19	1.9	35.6	31.25	100
S2	The midstream of Hyeongsan River, near Yeonil Drainage Pumping Station and an industrial complex	36°00'04.3"	129°22'16.5"	26.4	12	7.85	5.85	6.4	42.8	25	100
S3	Downstrean of Hyeonsan River, near Hyeongsan Bridge	36°00'31.1"	129°22'26.3"	26.9	20	8.53	9.27	6.7	82.8	25	25

- : Not available.

Table S2. Description, location, and other information of sampling stations in Pohang, Korea (February, 2011)

Site	**Locations**	**Latitude (N)**	**Longitude (E)**	**Temperature (℃)**	**Salinity (psu)**	**pH**	**DO (mg/L)**	**BOD**	**COD**	**Highest conc. (%) of water sample**	
										***Daphnia* test**	**Fish test**
R1	Angey reservoir	36°00'39.7"	129°15'34.9"	2.6	0	8.53	12.68	1.0	4.2	100	100
R2	Jeokgey pond	-	-	-	-	-	-	-	-	-	-
M1	The upper Hyeongsan River, near a road and apartments	35°59'50.4"	129°19'00.8"	2.4	0.9	8.06	11.67	2.6	5.8	100	100
M2	The midstream of Hyeongsan River, near Hyeongsan Drainage Pumping Station and sewage treatment plant	36°00'01.1"	129°20'49.5"	7.4	22.7	7.54	5.05	-	3.6	11	66.1
M3	End of Haksancheon, near Jukdo Drainage Pumping Station	36°01'49.7"	129°22'24.2"	7.2	31.5	7.35	6.19	-	2.4	7.9	47.6
M4	Downstream of Neangcheon, near a big shopping center	35°55'32.0"	129°24'07.6"	7.5	0.4	8.9	9.82	2.6	6.6	100	100
S1	End of Chilseongcheon, near an industrial complex	35°59'48.2"	129°21'55.2"	2.6	6.5	8.53	12.68	1.0	4.2	38.5	100
S2	The midstream of Hyeongsan River, near Yeonil Drainage Pumping Station and an industrial complex	36°00'04.3"	129°22'16.5"	8.8	10.3	7.97	10.3	-	8.4	24.3	100
S3	Downstrean of Hyeonsan River, near Hyeongsan Bridge	36°00'31.1"	129°22'26.3"	10.4	27.2	7.58	8.83	-	2.0	9.2	55.1

- : Not available.

Table S3. List of chemicals that were measured in the sediment extracts and surface water collected from Hyeongsan River, Pohang, Korea

Media	Group	Chemicals	Method detection limit
			(ng/g dw)
Sediment	Alkylphenols	Octylphenol	0.2
		Octylphenol monoethoxylate	0.3
		Octylphenol doethoxylate	0.5
		Nonylphenol	0.1
		Nonylphenol monoethoxylate	1.2
		Nonylphenol diethoxylate	2.0
		Bisphenol A	0.1
			(ng/L)
Water	Alkylphenols	Octylphenol	0.5
		Octylphenol monoethoxylate	0.8
		Octylphenol doethoxylate	1.3
		Nonylphenol	0.3
		Nonylphenol monoethoxylate	3.0
		Nonylphenol diethoxylate	5.0
		Bisphenol A	0.3
	Heavy metals	As	1.0
		Cd	0.1
		Cr	0.5
		Cu	1.0
		Ni	1.0
		Pb	0.1
		Zn	1.0

Table S4. Qualitative estrogenic index (qEI) in sediment samples of 8 stations in Pohang, Korea

	Cytotoxic NOEC (%)		**Estrogenic NOEC (%)**		**Calculated qEI**	
	June 2010	Feb. 2011	June 2010	Feb. 2011	June 2010	Feb. 2011
S1	0.8*	4	0.16	20	5	0.2
S2	20	4	4	20	5	0.2
S3	0.8	100	20	4	0.04	**25**
M1	4	100	20	20	0.2	5
M2	0.8*	20	4	20	0.2	1
M3	4	20	4*	1	1	**25**
R1	100	100	20	20	5	5
R2	100	-	20	-	5	-

*Cytotoxicity was observed at the lowest concentration, i.e., 0.8%, therefore NOEC could not be determined. In such case, LOEC was used for the determination of qEI.

Table S5. Target chemical concentrations in sediment and water samples from Pohang area in June 2010 and February 2011

		June 2010				February 2011			
Site		NP	OP	BPA	Total APs	NP	OP	BPA	Total APs
R1	Water	719.8	<ND	58.6	2554.0	127.7	1.9	4.2	553.5
	Sediment	647.0	3.2	1.7	653.9	4.9	<ND	13.3	123.2
R2	Water	732.7	<ND	124.8	2765.0	-	-	-	-
	Sediment	888.6	4.7	9.0	898.3	-	-	-	-
M1	Water	1080.3	<ND	76.7	1768.4	919.0	2.4	6.5	1471.3
	Sediment	953.0	3.4	11.6	1105.1	41.3	<ND	5.5	488.4
M2	Water	964.0	42.6	<ND	3388.5	890.3	0.1	46.3	1857.9
	Sediment	2015.7	59.5	82.1	4305.7	404.4	<ND	35.7	8097.1
M3	Water	561.3	<ND	45.1	2845.2	3768.9	1.5	3.4	7720.9
	Sediment	14539.7	1358.8	121.9	17326.1	3123.1	< ND	15.5	4012.8
M4	Water	360.6	<ND	149.3	3091.9	1806.1	0.4	3.4	6954.9
S1	Water	1488.9	54.7	76.3	3884.1	2419.5	13.3	32.8	8425.7
	Sediment	1374.6	16.4	60.5	2156.6	120.7	< ND	16.6	1703.0
S2	Water	2481.5	136.8	35.7	4591.0	1827.3	<ND	22.8	13546.2
	Sediment	2110.0	132.6	49.5	2806.0	408.8	< ND	88.5	6109.2
S3	Water	456.9	18.2	636.9	1391.3	2648.1	5.3	27.9	15948.4
	Sediment	1065.8	8.4	37.7	1249.9	74.7	< ND	45.9	1241.2

Units: ng/L for water samples and ng/g dry weight for sediment samples. -: Not available. <ND: below detection limit.

The method detection limits (MDL) for individual OP, NP, and BPA were 0.5, 0.3, 0.3 ng/L in water and 0.2, 0.1, and 0.1 ng/g dw in sediment, respectively.

Total APs value is summation of NP, nonylphenol monoethoxylate, nonylphenol diethoxylate, OP, octylphenol monoethoxylate, and octylphenol diethoxylate concentrations.

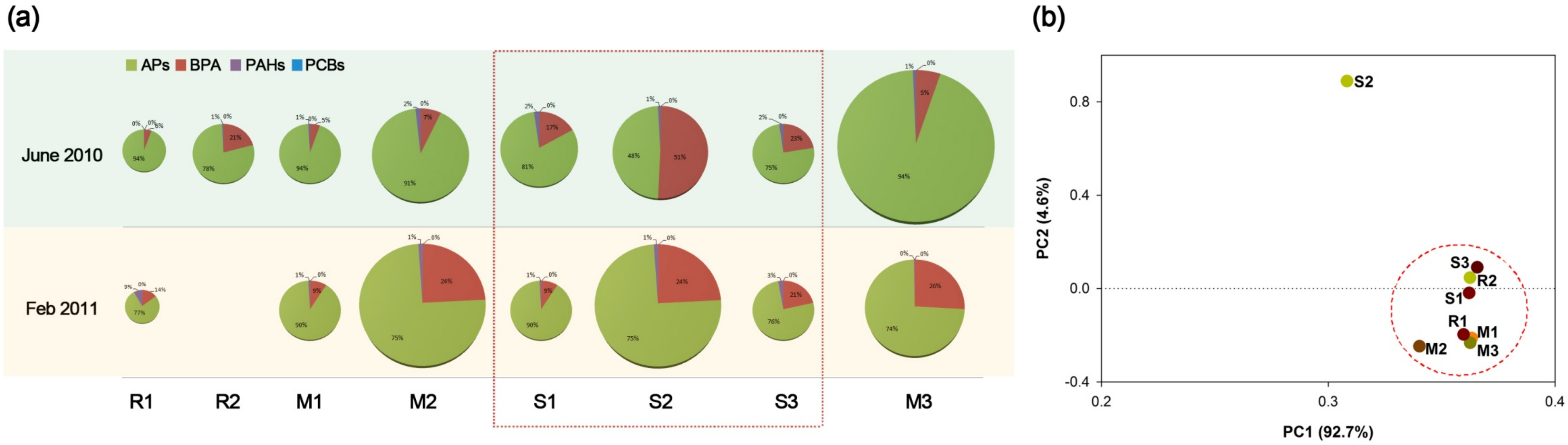

Fig. S1. Chemical composition of sediment extracts and results of principal component analysis. (a) Chemical composition of sediment extracts of June 2010 and Feb. 2011. Different circle sizes reflect total summation of all target organic chemical concentrations. Red dotted square of Figure (a) represents results from industrial sites. Note that proportion of PAHs was relatively high in these sites. **(b) Eigenvectors of samples by stations in principal component analysis.** Chemical concentration characteristics of station S2 showed different pattern of occurrence from the other locations.

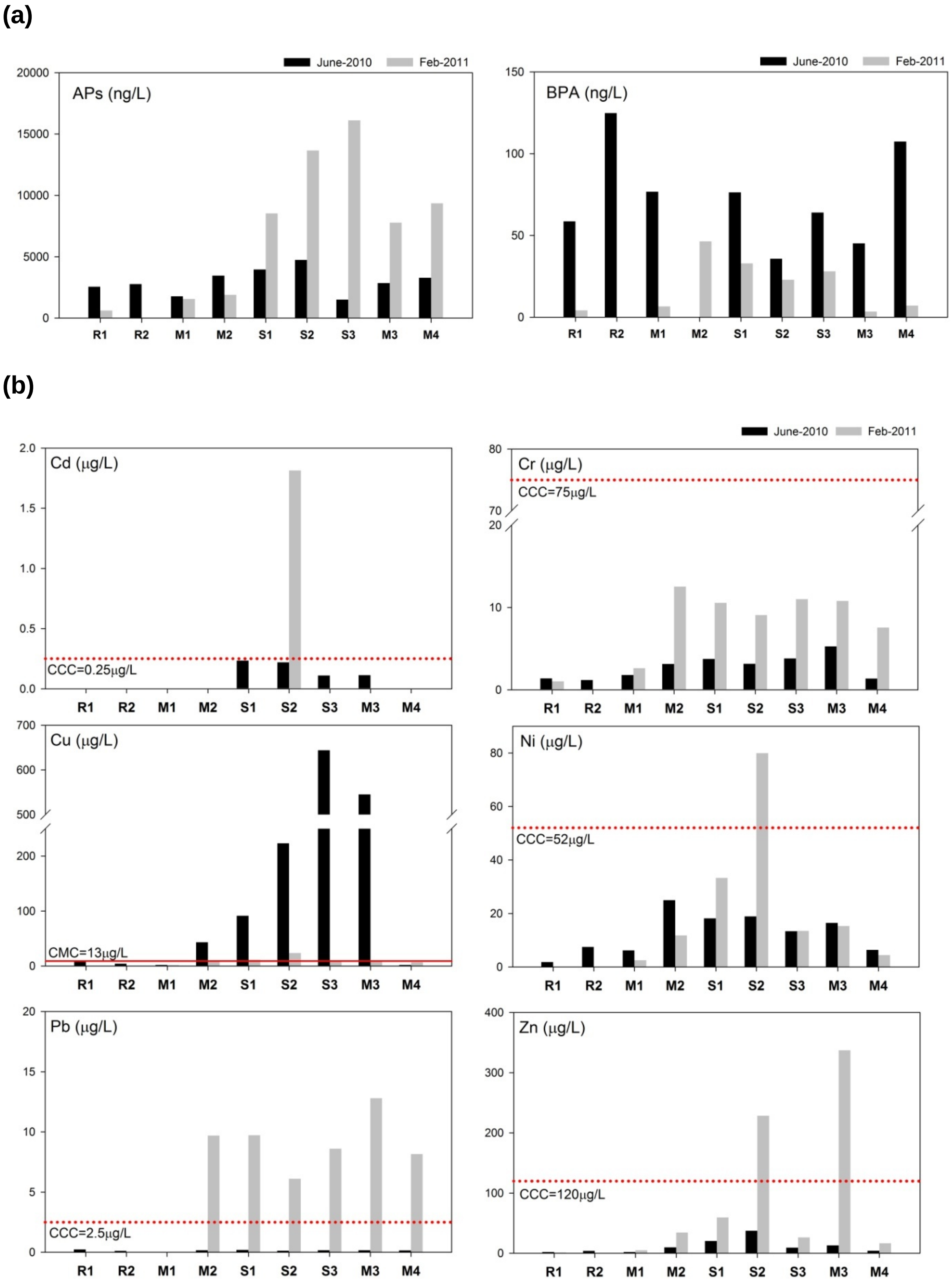

Fig. S2. Chemical concentrations in surface water samples. (a) Levels of alkylphenols (APs) and bisphenol A (BPA) by two sampling events, (b) heavy metal concentrations in surface water samples. Red lines denote criteria for each heavy metal from National Recommended Water Quality Criteria (US EPA). CCC: Criterion Continuous Concentration (for chronic exposure), CMC: Criteria Maximum Concentration (for acute exposure).