Assessment of Environmental Pollution in Korean Stream Sediments by Chemical Analyses and Insect Immune Biomarkers

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Abstract − A comprehensive quality survey for PCDDs/PCDFs and coplanar PCBs as well as heavy metals (Cu, Zn, Cd and Pb) in sediments has been investigated in August 2006, Korea. Monitoring was undertaken at five streams representing different surrounding environments throughout Juwang and Gapyeong streams (reference sites), Jungrang stream (dense population site), Ansan stream (mixed small population and industrial site), and Siheung stream (heavy industrial site). The levels of heavy metal in samples were found to be significantly higher in sediment from Siheung stream compared to those of other stream sites. The heavy metal concentrations (dry weight basis) in sediment from Siheung stream were as follows; Cd (3.7 μg/g), Pb (1,295 μg/g), Cu (713.4 μg/g) and Zn (358.1 μg/g). Among 12 coplanar PCBs and 17 PCDDs/PCDFs selected as target compounds in this study, PCB (IUPAC no. 118) and OCDD were the most abundant congeners found in all sediment samples, followed by 1,2,3,4,6,7,8-HpCDD, OCDF and 1,2,3,4,6,7,8-HpCDF as well as PCB (IUPAC no. 105). These results were shown to be in the same trend as the sediment samples of other countries. The levels of PCDDs/PCDFs/coplanar PCBs in sediment samples were expressed as concentrations and WHO-TEQ values. The PCDDs/PCDFs/coplanar PCBs concentrations and their WHO-TEQ values in sediment from Siheung stream were remarkably high. The levels detected were 788.16 pg/g and 36.080 pg WHO-TEQ/g dry weight for PCDDs/PCDFs and 314 pg/g and 0.4189 pg WHO-TEQ/g dry weight for coplanar PCBs, respectively, beyond the safety level of sediment value 20 pg WHO-TEQ/g. Sediment samples of the five streams were also monitored by sensitive biomarkers using insect immune responses: hemocyte-spreading behavior and immune-associated enzyme activities of phospholipase A$_2$ (PLA$_2$) and phenoloxidase. Organic extracts of Siheung and Jungrang sediments significantly interfered with the hemocyte-spreading behavior, whereas those of Ansan, Gapyeong, and Juwang did not. These organic extracts did not inhibit the PLA$_2$ and phenoloxidase activities. However, phenoloxidase was highly susceptible to exposure to aqueous extracts in all site sediments. In comparison, PLA$_2$ activities of the hemocytes were significantly inhibited only by aqueous extracts of Siheung, Jungrang, and Gapyeong sediments, but not by those of Ansan and Juwang. Despite some disparity between bio- and chemical monitoring results, the biomarkers can be recommended as a device warning the

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contamination of biohazard environmental chemicals because of a fast and inexpensive detection method.

INTRODUCTION

Persistent organic pollutants (POPs) such as polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) and polychlorinated biphenyls (PCBs) have long been associated with human health problems caused by various physiological disorders against biological organisms (United Nations Environment Programme Chemicals 1999; Ross *et al.* 2003). Heavy metals have also become a global phenomenon because they are considered to be greatest environmental hazards due to their toxicity and wide spread distribution (Kamala-Kannan *et al.* 2008). They are all responsible for acute and chronic effects including carcinogenicity, immunotoxicity, teratogenicity, and reproductive effects (Armin *et al.* 2000). PCDDs/PCDFs are injected into environment by a number of sources including waste combustion and metal industry, as well as their formation in the manufacture of various chlorinated chemicals (Alcock *et al.* 1999; Kouimtzis *et al.* 2002). PCBs, until the use of PCBs has banned, have been applied to a variety of industrial purposes including dielectric fluids in transformers and capacitors, hydraulic fluids, flame retardants and etc (Erickson 1997). A large amount of PCB products, however, has been kept currently with being inappropriately treated. Due to low water solubility and semi-volatility of PCBs/PCDDs/PCDFs, they undergo so long-range atmospheric transport and then deposit into aquatic systems, especially the sediments as a sink, where they act as a long-term source of release of these pollutants into the aquatic food chain (Sinkkonen *et al.* 2000; Rawn *et al.* 2001). Therefore, many studies have widely been reported the status of these pollutants in sediment (Ei-Kady *et al.* 2007; Kiguchi *et al.* 2007)

In vertebrates, PCBs/PCDDs/PCDFs cause a broad spectrum of physiological disorders including weight loss, reproductive failure, thymic atrophy, hepatotoxicity, several types of dermal lesion, and immunotoxicity (Safe 1990; Mocarelli

et al. 1996; Luebke *et al.* 2001). The molecular mechanism of the toxicities has been illustrated with a TCDD model binding to aryl hydrocarbon (Ah) receptor via induction of 7-ethoxyresorufin-O-deethylase (EROD). However, though invertebrates have their own Ah receptors, the TCDD toxicity is limited probably due to lack of specific binding of the receptors to the toxic ligand (Butler *et al.* 2001). The resistance of the aquatic invertebrates against PCDDs/PCDFs allows them to accumulate relatively high concentrations, which may be transferred to sensitive vertebrates via food webs (West *et al.* 1997).

To assess the environmental quality, many countries have monitored the pollution levels of heavy metals as well as PCBs/PCDDs/PCDFs in various environmental samples including water, sediment and soil. To our knowledge, few studies concerning the levels of target materials in samples from our study sites have been reported in Korea. In this paper, we report and discuss the data on the pollution levels in samples collected at and in the vicinity of five different streams. In order to assess their effects on biological systems, this study also analyzed the toxic effects of the dioxin residues on insect immune system, which was used to develop several sensitive biomarkers for early warning monitoring.

MATERIALS AND METHODS

1. Study area

Field sample collection was carried out at five selected stream sites and their vicinity in Korea in August 2006. Map of sampling sites is given in Fig. 1. Siheung stream (R_1) is located at many multi-chemical factories and was expected to accumulate gradually toxic compounds. Ansan stream (R_2) is a place, in which some industrial factories and civilian habitats were mixed. Jungrang stream (R_3) is placed in Seoul, the largest city in Korea, with an increasing the polluted gradients from civilian habitats. Gapyeong stre-
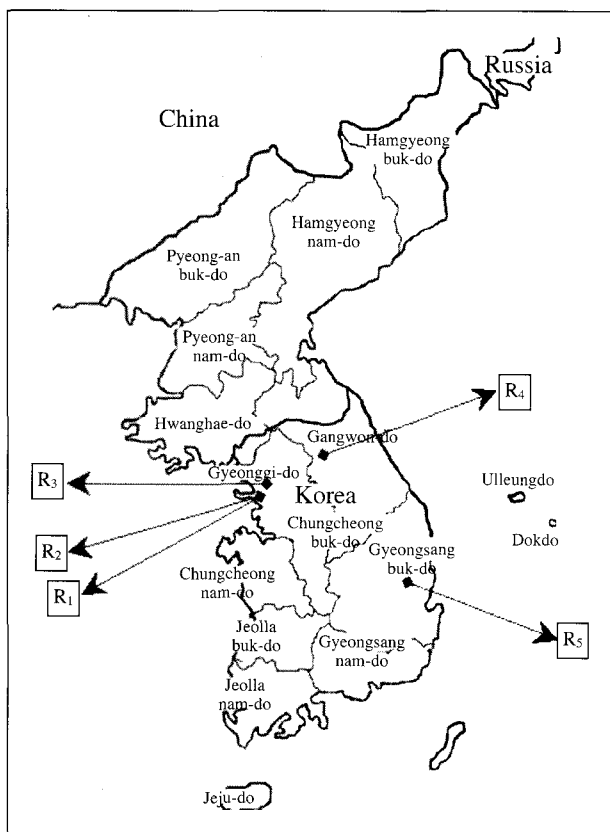
Fig. 1. Map of sampling locations in South Korea. R_1 (Gyeonggi-do Ansan Siheung stream), R_2 (Gyeonggi-do Ansan stream), R_3 (Seoul Jungrang stream at Seongdong Railroad), R_4 (Gyeonggi-do Gapyeong stream at Myunggi Mt), R_5 (Gyeongsang-buk-do Juwang stream at Jeolgol Valley).

am (R_4) is well known as a sight spot, but affected by a group of tourists. Juwang stream (R_5) is far away from chemical factories and civilian habitats so that it seems to be hardly contaminated.

2. Materials

The standard solutions containing 17 unlabeled PCDDs/PCDFs and 17 isotopically labeled $^{13}C_{12}$-PCDDs/PCDFs were supplied by Cambridge Isotope Laboratories (Andover, MA, USA). Twelve non- and mono-ortho substituted co-planar PCBs and 15 isotopically labeled $^{13}C_{12}$-PCBs were obtained from AccuStandard (New Haven, CT, USA), respectively. Cu, Zn, Pb and Cd standard solutions were purchased from Kanto Chemical Co., INC. (Chuo-ku, Tokyo, Japan). All solvents (n-hexane, dichloromethane, acetone and etc) used in this study were HPLC grade (J. T. Baker, Deventer, Netherlands). Concentrated solutions of HCl,

HNO_3, H_2SO_4 and H_2O_2 (Merck, Darmstadt, Germany) were all analytical grade reagent. Anhydrous sodium sulfate (Merck) was used after heating overnight at $450°C$. Silica gel $(100 \sim 200$ mesh, Aldrich, WI, USA), before use, was Soxhlet extracted with methanol/dichloromethane for 12 h and then activated at $130°C$ for 18 h.

3. Sampling and sample preparation

Sediment samples $(0 \sim 20$ cm layer) in the different streams were collected using a grab sampler and placed into 500 mL glass jar. They were transported to the laboratory immediately and stored in refrigerator until analysis. The prepared samples were used for both chemical residual analysis and biomarker analysis.

Sample preparation of heavy metals was implemented according to U. S. EPA Method 6010. Each sample was accurately weighed and transferred to a vessel, to which 10 mL of 1 : 1 nitric acid was added. The solution was heated on the hot plate and refluxed for $10 \sim 15$ min without boiling. After cooling, 2 mL of water and 3 mL of 30% hydrogen peroxide was added in a vessel and heated until the volume was reduced to approximately 5 mL. The final volume of solution was adjusted to 100 mL with reagent water and centrifuged to remove particulates. The final samples were named as aqueous extract for biomarker analyses.

Sediment samples for PCDDs/PCDFs and coplanar PCBs were analyzed following the experimental procedure. Dried samples (10 g) were Soxhlet extracted with toluene for 24 h. Prior to extraction, samples were spiked with 15 carbon-13 labeled isotope compounds for PCDDs/PCDFs and 12 carbon-13 labeled isotope compounds for coplanar PCBs, respectively. Extracts were concentrated to 0.1 mL under N_2. After adding 2 mL of n-hexane, extracts were washed with concentrated H_2SO_4, 5% NaCl, 20% KOH and then passed through anhydrous Na_2SO_4. Extracts were applied to a multi-layer silica gel column including neutral silica gel, acid modified silica gel and NaOH impregnated silica gel and then eluted with n-hexane. These extracts were named as organic extracts for biomarker analyses. Each organic extract was submitted to alumina cleanup, then elute with 2% hexane/dichloromethane for coplanar PCBs and 50% hexane/dichloromethane for PCDDs/PCDFs, respectively. To test the recovery, the syringe standards were spiked to the final concentrates before HRGC/MS analysis. Along with each

Table 1. GC/MS analytical conditions

	PCDDs/PCDFs	Coplanar PCBs
GC	HP6890 DB5-MS (0.25 mm I. D. × 60 m, 0.25 film thickness) Oven temperature $140°C \rightarrow 15°C \text{ min}^{-1} \rightarrow 220°C \rightarrow 1.5°C \text{ min}^{-1}$ $\rightarrow 240°C \rightarrow 4°C \text{ min}^{-1} \rightarrow 310°C \text{ (6 min)}$ Injection temperature: 300°C Injection mode: splitless	HP6890 DB5-MS (0.25 mm I. D. × 60 m, 0.25 film thickness) Oven temperature $100°C \rightarrow 25°C \text{ min}^{-1} \rightarrow 180°C \text{ (1 min)} \rightarrow 1.8°C \text{ min}^{-1}$ $\rightarrow 220°C \rightarrow 10°C \text{ min}^{-1} \rightarrow 300°C \text{ (6 min)}$ Injection temperature: 300°C Injection mode: splitless
MS	Jeol-700D Ionizing mode: EI/SIM Resolution: > 10,000 Ionizing current: 0.6 mA Accelerating voltage: 10 kV Ionizing energy: 38 eV Ion source temperature: 300°C	Jeol-700D Ionizing mode: EI/SIM Resolution: > 10,000 Ionizing current: 0.6 mA Accelerating voltage: 10 kV Ionizing energy: 38 eV Ion source temperature: 300°C

series of samples, a blank test was also performed to check the purity of solvents, reagents, material and etc. The blank was spiked with an internal standard mentioned above and analyzed exactly in the same way as the samples.

4. Chemical analysis

The analysis of heavy metals was conducted using an ICP-MS (Elan DRC-e Perkin Elmer SCIEX, USA) at 324.752 nm (Cu), 220.353 nm (Pb), 213.857 nm (Zn) and 228.802 nm (Cd), respectively. The determination of coplanar PCBs and PCDDs/PCDFs was performed on a HRGC-HRMS in a HP 6890 gas chromatograph (Hewlett-Packard, Wilmington, USA) coupled a JMS-700D (Jeol, Japan) mass spectrometer, operating in EI ionization (38 eV) at resolving power higher than 10,000. The oven temperature programs are shown in Table 1 with MS conditions.

5. Biomarker analyses

The effects of the environmental samples on the biological system were determined using three insect immune responses with some modification of the method described (Ryoo et al. 2005). The fifth instar larvae of the beet armyworm, Spodoptera exigua, was used as a test insect and reared on an artificial diet of Gho et al. (1990). Hemolymph was collected by the method described by Nalini and Kim (2007). Briefly, last instar larvae of S. exigua were surface-sterilized by holding them in 70% ethanol for 30 sec. A proleg was cut with a pair of sterilized scissors and the emerging hemolymph (~50 μL/larva) was collected in 500 μL of ice-cold an anticoagulant buffer (100 mM Na$_2$HPO$_4$.

12H$_2$O, 18 mM KH$_2$PO$_4$, 138 mM NaCl, 28 mM KCl, 14.5 mM L-cysteine.HCl, pH 7.4).

1) Hemocyte-spreading assay

Each hemocyte monolayer was made on slide glass using 49 μL of hemocyte suspension plus 1 μL of test solution in a moist chamber at 25°C. Extraction solvents, reagent water or n-hexane, served as controls in the place of test solution of aqueous or organic extract, respectively. After 45 min, hemocyte-spreading showing filopodial extension (an inset photo of Fig. 1) was determined by counting randomly chosen 200 cells from 10 randomly chosen fields.

2) Phospholipase A$_2$ (PLA$_2$) enzyme activity

Hemocytes were homogenized by sonication with eight 0.5 second bursts at 60 W using an ultra sonicator (Bandelin, Berlin, Germany) using 50 mM Tris buffer (pH 7.4) containing 0.45 M NaCl. The extracts were centrifuged at 1,000 g for 10 min at 4°C. The supernatants were re-centrifuged at 30,000 g for 30 min at 4°C. The supernatant was used for PLA$_2$ extract. PLA$_2$ activity of the hemocyte extract was measured in spectrofluorometry by using pyrene-labeled phospholipid [1-hexadecanoyl-2-(1-pyrenedecanoyl)-sn-glycerol-3-phosphatidyl choline] as the substrate in the presence of bovine serum albumin (Radvanyi et al. 1989). The reaction mixture, to a final volume of 2 mL, was prepared in a cuvette by sequentially adding 1,926 μL of 50 mM Tris buffer (pH 7.0), 12 μL of 1 M CaCl$_2$, 20 μL of 10% bovine serum albumin, and 2 μL of 0.2 mM pyrene substrate. The reaction was initiated by addition of the enzyme extract (40 μL) containing 2 μL and the fluorescence intensity was monitored with an Aminco Bowman Series 2 luminescence spec-

trometer (FA257, Spectronic Instruments, USA) using exci-
tation and emission wavelengths of 345 and 398 nm, respec-
tively. Extraction solvents, reagent water or n-hexane, served
as controls in the place of test solution of aqueous or organic
extract, respectively. The enzyme activity was calculated in
pmol/min/μg according to Radvanyi et al. (1989). Protein
concentration in assay sample was quantified by Bradford
(1976) method using bovine serum albumin as standard.

3) Phenoloxidase (PO) enzyme activity

PO activity was measured by the method of Park and Kim
(2003). Hemolymph (10 μL) of the fifth instar larvae was
pre-incubated with 2 μL of a test solution at 25°C for 10
min, and then added by 988 μL of 50 mM phosphate saline
containing 1 μg of laminarin (Sigma, St. Louis, MO) and
0.01 mM L-3,4-dihydroxyphenylalanine (Sigma). Extraction
solvents, reagent water or n-hexane, served as controls in
the place of test solution of aqueous or organic extract, res-
pectively. The increased absorbance was measured at 495
nm with 5 min interval. PO activity was expressed as absor-
bance change per 5 min.

RESULTS AND DISCUSSION

1. Levels of PCDDs/PCDFs

The levels of PCDDs/PCDFs in sediment from 5 given
sampling sites are reported in Table 2. The results were
expressed as concentration in pg/g and in pg · WHO-TEQ/g
dry weight in order to evaluate the toxicity of samples.
Toxic equivalence factors (TEFs) vary from 0.0001 to 0.1
for PCDDs/PCDFs (Van den Berg et al. 1998). Toxic equi-
valence (TEQ) was calculated by multiplying their concen-
trations of each PCDD and PCDF by its corresponding TEF
value. Up to date, in Korea, few studies relating with the
profiles of PCDDs/PCDFs in various environmental sam-
ples and the input of such compounds from particular sour-
ces have been reported in the study area.

All PCDD/PCDF congeners including 2,3,7,8-TCDD,
which is the most toxic congener, were found in all stream
sediments. The PCDD/PCDF congener profiles were similar
for all the sediment samples. There were clear predominant
congeners out of PCDDs/PCDFs. The OCDD was the most
abundant congener, accounting for approximately 30% of to-
tal PCDDs/PCDFs except for sediments from Gapyeong and

Juwang stream. The second most abundant congener group
in sediments from above sampling sites were 1,2,3,4,6,7,8-
HpCDD, OCDF and 1,2,3,4,6,7,8-HpCDF. These congener
profiles agree with the results of previous studies in which
the concentrations of PCDDs/PCDFs in sediment samples
were determined (Fattore et al. 2002; Valle et al. 2003; Ryoo
et al. 2005; Kannan et al. 2007).

Total concentration and WHO-TEQ level of PCDDs/
PCDFs at each sampling site excluding sediment from Si-
heung stream ranged from 8.88 to 59.12 pg/g and from 0.862
to 2.877 pg WHO-TEQ/g dry weight, respectively. When
the present results compared with data from other sediments,
the current levels were much lower than levels of sediments
taken from the River PO, which is the main Italian river
draining one of the most populated and industrialized re-
gions in Italy (Fattore et al. 2002), but were similar to those
found in sediments from rural lake in Finland (Isosaari et
al. 2002).

In this study, the concentrations of PCDDs/PCDFs in
sediments were increased in the order of Juwang, Gapyeong,
Jungrang, Ansan, Siheung stream. As expected, the lowest
level was recorded in the sediment collected in the upper
part of the Gapyeong stream close to the Myungi Mountain,
which is the least contaminated stream that has not been so
far affected by direct urbanization and industrial activities.
Almost same level was detected in sediment from Juwang
stream. This investigation indicates that its natural environ-
ment has been well preserved, even under quite often human
activities near the stream. Jungrang stream flows through
Seoul, the largest city in Korea, which is influenced by the
polluted gradients from civilian habitats. Compared to those
of Juwang and Gapyeong stream, relatively higher concen-
trations and WHO-TEQ value of PCDDs/PCDFs were de-
tected in this sediment.

Siheung stream flows into West Sea through the chemi-
cal industry zone. The total PCDD/PCDF concentrations
and their WHO-TEQ value in sediment from Siheung stream
were 788.16 pg/g and 36.080 pg WHO-TEQ/g dry weight,
respectively. The WHO-TEQ value of PCDDs/PCDFs ob-
served in this sediment was over the safe sediment value of
20 pg WHO-TEQ dry weight suggested by Evers et al.
(1996). Total PCDD/PCDF concentration was around 90
times higher compared to the sediment from Juwang and
Gapyeong stream, while the WHO-TEQ value was found to
be greater than 50 times resulting from the presence of high-

Table 2. Total concentrations of PCDDs/PCDFs and their WHO-TEQ levels in 5 stream sediments (dry weight basis) in Korea

PCDD/PCDF congener	WHO-TEF value	Siheung stream	Ansan stream	Jungrang stream	Gapyeong stream	Juwang stream
2,3,7,8-TCDD	– 1.0	1.00 1.000	0.12 0.120	0.16 0.160	0.16 0.160	0.08 0.080
1,2,3,7,8-PeCDD	– 1.0	5.12 5.120	0.56 0.560	0.76 0.760	0.44 0.440	0.16 0.160
1,2,3,4,7,8-HxCDD	– 0.1	9.40 0.940	1.00 0.100	0.88 0.088	0.92 0.092	0.56 0.056
1,2,3,6,7,8-HxCDD	– 0.1	16.56 1.656	1.44 0.144	1.20 0.120	0.64 0.064	0.48 0.048
1,2,3,7,8,9-HxCDD	– 0.1	1.20 0.120	1.32 0.132	0.40 0.040	0.32 0.032	0.28 0.028
1,2,3,4,6,7,8-HpCDD	– 0.01	59.28 0.593	4.16 0.042	1.96 0.020	0.80 0.008	0.48 0.005
OCDD	– 0.0001	211.80 0.021	17.68 0.002	6.44 0.000	1.48 0.000	1.36 0.000
2,3,7,8,-TCDF	– 0.1	5.92 0.592	0.60 0.060	0.20 0.020	0.08 0.008	0.08 0.008
1,2,3,7,8-PeCDF	– 0.05	17.20 0.860	1.64 0.082	0.96 0.048	0.84 0.042	0.52 0.026
2,3,4,7,8-PeCDF	– 0.5	21.76 10.880	1.44 0.720	1.08 0.540	0.84 0.420	0.40 0.200
1,2,3,4,7,8-PeCDF	– 0.1	33.80 3.380	2.48 0.248	1.64 0.164	1.12 0.112	0.56 0.056
1,2,3,6,7,8-HxCDF	– 0.1	29.76 2.976	1.92 0.192	1.64 0.164	1.12 0.112	0.60 0.060
2,3,4,6,7,8-HxCDF	– 0.1	33.24 3.324	1.28 0.128	0.92 0.092	1.00 0.100	0.64 0.064
1,2,3,7,8,9-HxCDF	– 0.1	34.16 3.416	2.32 0.232	1.60 0.160	1.12 0.112	0.56 0.056
1,2,3,4,6,7,8-HpCDF	– 0.01	86.27 0.86	9.40 0.094	3.28 0.033	1.32 0.013	0.92 0.009
1,2,3,4,7,8,9-HpCDF	– 0.01	32.28 0.323	2.04 0.020	1.20 0.012	0.84 0.008	0.60 0.006
OCDF	– 0.0001	189.41 0.019	9.72 0.001	2.00 0.000	0.96 0.000	0.60 0.000
Total concentration (pg/g)	–	788.16	59.12	26.32	14.00	8.88
Total pg-TEQ/g	–	36.080	2.877	2.421	1.723	0.862

er and/or less toxic PCDD/PCDF congeners. Furthermore the WHO-TEQ level of PCDDS/PCDFs in Siheung stream sediment was comparable to those found in sediments from the Haihe River and Dagu Drainage River in Tianjin city, China (Liu et al. 2007) and Masan Bay that has been designated as a special management coastal area since 1983, Korea (Kannan et al. 2007). Based on PCDD/PCDF analytical data, it confirms that Siheung stream has been more directly exposed to a potential source of PCDD/PCDF pollutants than others.

2. Levels of coplanar PCBs

Coplanar PCBs were classified into two categories. The first category consisted of non-ortho PCBs (IUPAC no. 77, 81, 126 and 169), whereas the second category were mono-ortho PCBs (IUPAC no. 105, 114, 118, 123, 156, 157, 167 and 189). All coplanar PCB congeners were observed in most of the investigated sediment samples. Among coplanar PCB congeners, PCB no. 118 (2,3′,4,4′,5-pentaCB) was the most abundant congener, comprising up to 27~45% of the total amount of coplanar PCBs. Another abundant congener

Table 3. Total concentrations of coplanar PCBs and their WHO-TEQ levels in 5 stream sediments (dry weight basis) in Korea

PCB congener (IUPAC No.)	WHO-TEF value	Siheung stream	Ansan stream	Jungrang stream	Gapyeong stream	Juwang stream
3,4,4',5-TetraCB (81)	– 0.0001	1.76 0.0002	0.20 0.0000	0.12 0.0000	0.24 0.0000	0.36 0.0000
3,3',4,4'-TetraCB (77)	– 0.0001	20.40 0.0020	5.72 0.0006	1.96 0.0002	2.56 0.0003	3.16 0.0003
2,3',4,4',5-PentaCB (123)	– 0.0001	14.80 0.0015	5.80 0.0006	1.08 0.0001	0.80 0.0001	0.68 0.0001
2,3',4,4',5-PentaCB (118)	– 0.0001	134.84 0.0135	58.12 0.0058	6.92 0.0007	4.48 0.0004	2.84 0.0003
2,3,4,4',5-PentaCB (114)	– 0.0005	5.00 0.0025	1.92 0.0010	0.36 0.0002	0.16 0.0001	0.36 0.0002
2,3,3',4,4'-PentaCB (105)	– 0.0001	50.36 0.0050	22.28 0.0022	2.80 0.0003	1.40 0.0001	0.84 0.0001
2',3,4,4',5-PentaCB (126)	– 0.1	3.36 0.3360	0.80 0.0800	0.45 0.0450	0.32 0.0320	0.32 0.0320
2,3,3',4,4',5'-HexaCB (167)	– 0.00001	23.76 0.0002	11.32 0.0001	1.36 0.0000	1.00 0.0000	0.68 0.0000
3,3',4,4',5-PentaCB (156)	– 0.0005	23.04 0.0115	9.68 0.0048	1.24 0.0006	0.80 0.0004	0.52 0.0003
2,3,3',4,4',5-HexaCB (157)	– 0.0005	6.04 0.0030	1.96 0.0010	0.40 0.0002	0.16 0.0001	0.12 0.0001
3,3',4,4',5,5'-HexaCB (169)	– 0.01	4.08 0.0408	0.64 0.0064	0.24 0.0024	0.20 0.0020	0.28 0.0028
2,3,3',4,4',5,5'-HeptaCB (189)	– 0.0001	26.56 0.0027	12.16 0.0012	1.52 0.0002	0.84 0.0001	0.44 0.0000
Total concentration (pg/g)	–	314.00	130.60	18.45	12.96	10.60
Total pg-I-TEQ/g	–	0.4189	0.1037	0.0449	0.0356	0.0360

was PCB no. 105 (2,3,3',4,4'-pentaCB). These PCB congener profiles are in accord with the results of previous PCB studies in sediment samples (Sather *et al.* 2001; Chi *et al.* 2007; EI-Kady *et al.* 2007).

The concentrations of coplanar PCBs in each sediment sample collected from 5 stream sites are summarized in Table 3, in which toxic equivalent factors (TEFs) levels are presented after conversion to the 2,3,7,8-TetraCDD toxic equivalence (TEQ). WHO-TEFs vary from 0.00001 to 0.1 for coplanar PCBs (Van den Berg *et al.* 1998). Except for sediment sample from Siheung stream, total concentrations of coplanar PCBs in each sediment sample were in the range of 10.60 ~ 130.60 pg/g dry weight with the WHO-TEQ values of 0.0356 ~ 0.1037 pg/g dry weight. Similar trends were also observed in sediments (0.041 ~ 0.261 pg WHO-TEQ/g dry weight) from a reservoir in Northern Taiwan (Chi *et al.* 2007). Moreover, Table 3 showed that non-*ortho* PCB congeners were in the lower concentration than mono-*ortho* PCB congeners. These results were consistent with previous data

reporting that mono-*ortho* PCBs constituted more than non-*ortho* PCBs (EI-Kady *et al.* 2007).

The concentrations of coplanar PCBs in sediments were increased in the following order: Juwang ≈ Gapyeong < Jungrang < Ansan ≪ Siheung stream. The lowest levels of coplanar PCBs were found in sediment taken from Juwang stream. Their levels were 10.60 pg/g dry weight and 0.0360 pg WHO-TEQ/g dry weight, respectively. Additionally, the levels of PCBs detected in sediment from Gapyeong stream were also similar to those of Juwang sediment. The results reflect that no significant PCBs emission sources exist in the vicinity of these stream sites. Despite minor differences, slightly elevated levels of coplanar PCBs were observed in sediment from Jungrang stream, located in Seoul city. This increase in total PCB levels might be due to more intense human activities.

Comparing with the relative levels reported in this literature, significantly increased levels of coplanar PCBs were detected in sediment from Siheung stream, flowing through

Table 4. Heavy metal content in 5 stream sediments in Korea

Sampling sites	Concentration (µg/g dry weight) of sediment			
	Cu	Zn	Cd	Pb
Siheung stream	713.4±35.1[a]	358.1±26.6	3.7±1.4	1295.3±104.2
Ansan stream	40.2±7.7	154.7±17.5	0.6±0.2	33.8±10.2
Jungrang stream	29.9±6.6	111.2±14.9	0.2±0.1	12.9±5.8
Gapyeong stream	18.0±3.4	35.5±5.8	0.1±0.1	9.2±3.9
Juwang stream	5.8±1.6	59.7±10.3	0.1±0.1	20.3±5.5

[a]Mean±S.D. by five replications.

Sihwa industrial complex which is one of the most active industrialized zone in Korea. The coplanar PCBs concentration and their WHO-TEQ value were 314.00 pg/g dry weight and 0.4189 pg/g dry weight, respectively. Around thirty times higher concentration was detected in sediment from Siheung stream in contrast to that of sediment from Juwang and Gapyeong stream. Besides, present data was found to be in the almost same levels as the sediments taken from River Nile in the Cairo region, Egypt (El-Kady et al. 2007) and several sediments from the most polluted Haihe river in Tianjin city, China (Liu et al. 2007). From the results of these data, it implicates that Siheung stream has been subjected to the direct input of PCBs from electric equipments and/or other sources filled with PCBs due to its vicinity surrounded by industrial factories.

3. Levels of heavy metals in sediment

The concentrations of heavy metals such as copper (Cu), zinc (Zn), lead (Pb) and cadmium (Cd) are presented in Table 4. It is generally known that they originate primarily from anthropogenic sources. To compare the degree of heavy metal contamination, sediments from five different stream sites were chosen for the present study. The observed concentrations varied widely according to the sample collection sites. The concentrations of Cu and Zn in sediment samples ranged from 5.8 µg/g to 713.4 µg/g dry weight and 35.5 µg/g to 358.1 µg/g dry weight, respectively. Such heavy metals were shown to be higher in order of sediments from Juwang ≈ Gapyeong < Jungrang ≈ Ansan ≪ Siheung stream.

These trends were also similar to the other Cd and Pb metals, which are considered as the greatest environmental hazards due to their toxicity. The concentration of Cd in sediments was increased in the following order: Juwang ≈ Gapyeong < Jungrang < Ansan ≪ Siheung stream. The concentration of Cd in sediment from Siheung stream was 3.7

µg/g dry weight, which contained 37 times higher compared to sediment from Juwang stream. The concentrations of Pb in sediments were in the range between 9.2 and 1,295.4 µg/g dry weight. The minimum level of Pb was recorded in sediment from Juwang stream. The maximum level was observed in sediment collected from Siheung stream. When comparing the relative level of above two sediments, Siheung sediment was about 141 times higher than Juwang sediment. The heavy metal concentrations in sediments are generally at normal levels, except for sediment from Siheung stream.

Until recently, no literature data in Korea are available for comparing the relative levels of sediments from the designated sampling sites. Kamala-Kannan et al. have reported the concentrations of Cd and Pb in sediments taken from six different stations at Pulicat Lake in North Chennai Coastal region. In their study, Pb concentration observed in the sediments of Pulicat Lake varied between 1.2 and 42.0 µg/g dry weight, while Cd concentration was in the range between 32.7 and 88.7 µg/g dry weight. The Cu, Zn, Cd and Pb were also investigated in sediments from the Bay of Thessaloniki in Greece by Zabetoglou et al. (2002). Their concentrations were as follows; Cd (0.17~6.3 µg/g dry weight), Pb (3.1~86 µg/g dry weight), Cu (0.5~76 µg/g dry weight) and Zn (35~1,014 µg/g dry weight). Previous research (Klavins et al. 1998) in the sediments from Lakes of Latvia also represented the comcentrations of heavy metals such as Pb (12.75~83.21 µg/g dry weight), Cu (4.37~16.34 µg/g dry weight) and Cd (0.41~5.31 µg/g dry weight). As shown in data, the concentrations of each heavy metal showed an extraordinarily difference between Siheung sediment and the other sediments. The highest level of heavy metals in sediment from Siheung stream reflects that Siheung stream are under the potential influence of heavy metals due to the discharge of industrial wastes. Thus, Siheung stream might be alarmingly hazardous from a toxicological point of view.

4. Effects of the environmental samples on biomarkers

Insect immunity is innate and consists of cellular and humoral responses (Ratcliffe 1985). Upon microbial infection, nonself is recognized by pattern recognition receptors involving immulectin and hemolin (Lavine and Strand 2002). This nonself signal may be transmitted by immune media-
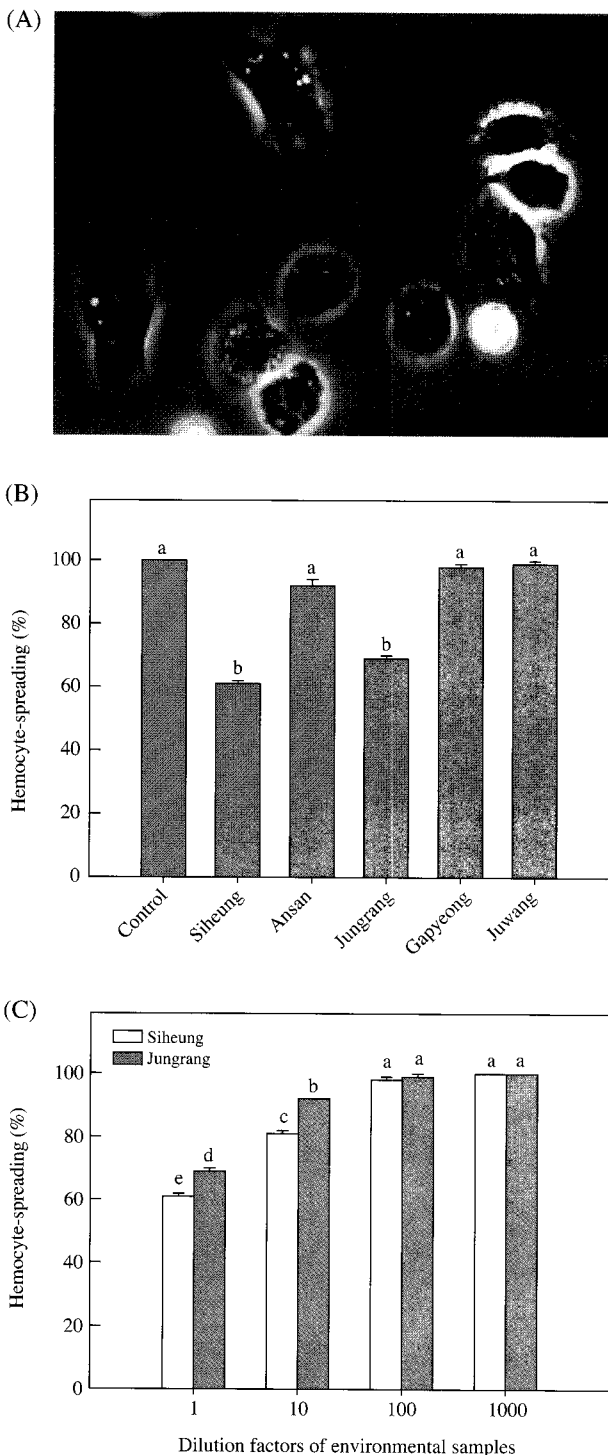
(A)

(B)

(C)

Dilution factors of environmental samples

Fig. 2. Effects of different sediment extracts from five streams on *in vitro* hemocyte-spreading behavior of *Spodoptera exigua* larvae. (A) Spread hemocytes observed under a phase contrast microscope (IX70, Olympus, Japan) at 400 × magnification. (B) Effect of organic extracts without dilution on the hemocyte behavior. (C) Comparison of two sediment extracts with dilutions in terms of inhibition of hemocyte-spreading behavior. Different letters above standard deviation bars indicate significant difference at Type I error=0.05 (LSD test).

tors such as biogenic amines (octopamine and 5-hydroxy-tryptamine) (Baines and Downer 1994), eicosanoids (Stanley 2000), and other cytokines (Clark *et al.* 1997) to two main tissues (hemocyte and fat body) performing immune responses. Cellular immune responses are acute and executed mostly by the hemocytes, which show phagocytosis, nodule formation, and encapsulation depending on invading pathogen size and density (Gillespie *et al.* 1997). In comparison, humoral immune responses include antimicrobial peptide synthesis by fat body, which effectively performs clearance of remaining pathogens (Bullet *et al.* 1999).

This study used three immune reactions of hemocyte-spreading behavior and two enzyme activities of PLA_2 and PO as biomarkers to determine any hazard effect of the environmental toxicants. Hemocyte-spreading behavior is required for all cellular immune responses, and its impairment causes significant immunosuppression of *S. exigua* (Nalini and Kim 2007). PLA_2 is an enzyme catalyzing phospholipids at sn-2 position, which produces arachidonic acid that is subsequently oxidized into various eicosanoids (Dennis 1994). The eicosanoids elicit cellular and humoral immune responses against various microbial pathogens (Stanley and Miller 2006). Inhibition of PLA_2 seriously impairs immune responses of *S. exigua*, which results in increase of mortality against a mild bacterial infection (Park and Kim 2000). PO is an enzyme catalyzing melanization by forming reactive quinone compound, which is used for cellular immune reactions. It has been demonstrated that suppression of PO attenuates hemocyte nodule formation in *S. exigua* (Park and Kim 2003).

Different sediment samples from five streams were prepared into organic and aqueous extracts and tested against hemocyte-spreading behavior (Fig. 2). All aqueous extracts of the five stream sediments showed serious cytotoxic symptoms when the hemocytes were exposed to the extracts even after several dilutions (data not shown). Organic extracts showed differential effects on the hemocyte-spreading behavior depending on sediment sources (Fig. 2B). Only Siheung and Jungrang samples significantly interfered with the hemocyte behavior. Serial dilutions of these two samples showed that Siheung sample was more potent than Jungrang sample (Fig. 2C). Compared to levels of PCDDs/ PCDFs and PCBs in the samples (Tables 2, 3), the inhibitory effects of the two environmental samples on the hemocyte-spreading behavior are explained in terms of the pre-
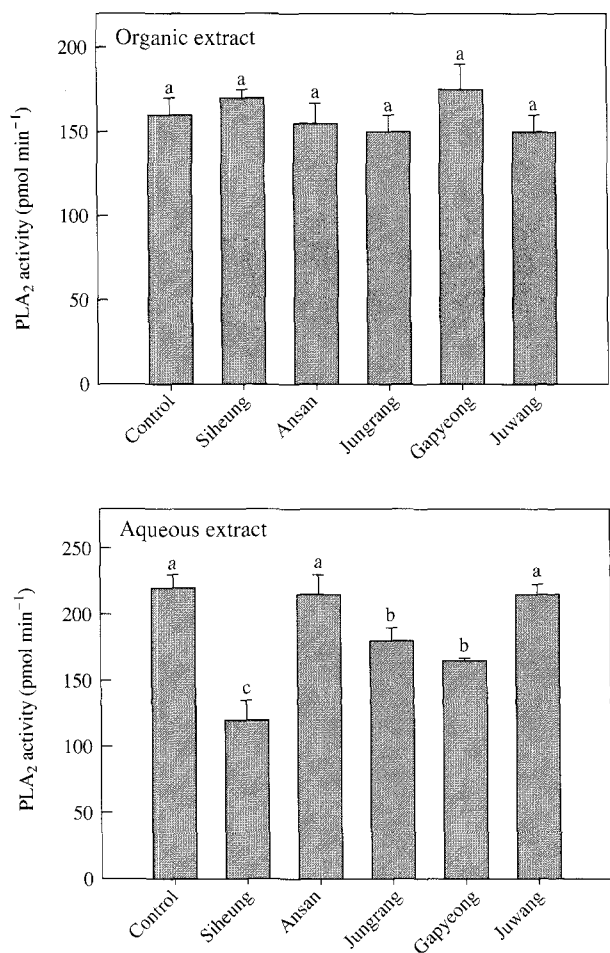
Fig. 3. Effects of different sediment extracts from five streams on hemocyte phospholipase A₂ (PLA₂) of *Spodoptera exigua* larvae. The preparations of organic and aqueous extracts were described in Materials and Methods. Three samples were prepared from each stream and used for the enzyme assays. Different letters above standard deviation bars indicate significant difference at Type I error=0.05 (LSD test).
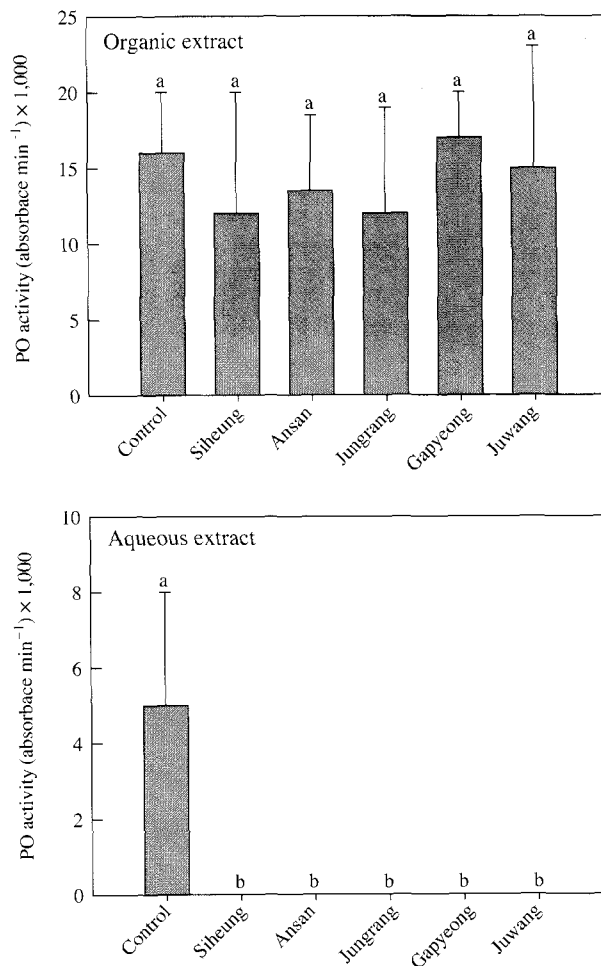
Fig. 4. Effects of different sediment extracts from five streams on hemocyte phenoloxidase (PO) of *Spodoptera exigua* larvae. The preparations of organic and aqueous extracts were described in Materials and Methods. Three samples were prepared from each stream and used for the enzyme assays. Different letters above standard deviation bars indicate significant difference at Type I error=0.05 (LSD test).

sence of these toxic chemicals in relatively high amounts. Also the higher levels of these compounds in Sinheung samples well supported the higher inhibitory effect than Jungrang when the environmental samples were diluted. However, Ansan sample did not inhibit this hemocyte-spreading behavior, though it contained higher levels of these chemicals than those of Jungrang. When these two samples were compared in each congener concentration of the PCDDs/ PCDFs, Ansan sample contained less amounts in only two compounds, 2,3,7,8-TCDD and 1,2,3,7,8-PeCDD than Jungrang sample. We know these two congeners are the highest toxic isoforms in this chemical group (Van den Berg *et al.* 2006). This suggests that 2,3,7,8-TCDD and 1,2,3,7,8-PeCDD

may be highly potent to inhibit hemocyte-spreading behavior. The inhibitory effect of TCDD on vertebrate immune system has been suggested by the hypersensitivity of thymus (Vos *et al.* 1997), where TCDD also induces apoptosis of T cells (Camacho *et al.* 2004). In *S. exigua*, TCDD also induced apoptosis of hemocytes, in which the cells formed apoptotic blebbings and vesicles, typical symptoms of apoptotic (Ryoo *et al.* 2005).

PLA₂ activity of *S. exigua* hemocytes was not inhibited by any organic extract sediment samples (Fig. 3). However, aqueous extracts of different sediment samples showed differential inhibitions on PLA₂ activities of *S. exigua* hemo-

cytes. Siheung, Jungrang, and Gapyeong samples significantly inhibited the enzyme activity, but Ansan and Juwang samples did not. Among the samples showing inhibitory activities on PLA_2, Siheung sample possessed the most potent activity, which may be explained by the highest levels in all measured heavy metals contained in the sample (Table 4). The intermediate inhibitory effects of Jungrang and Gapyeong samples on the enzyme activity were well supported by the intermediate levels of these heavy metals especially in copper amounts. Juwang sample did not inhibit the enzyme activity due to low levels of heavy metal residues. However, Ansan sample did not support this casual link between biomarker and chemical analyses. We speculate there may be a contaminant chelating compound in the Ansan's aqueous sample, which might hold free metals in the bioassay reactions to prevent access the free heavy metals to the enzyme, while chemical analysis using ICP-MS could detect the levels of the heavy metals in spite of the present of the chelator(s). In overall, these results suggest that heavy metals can inhibit PLA_2 enzyme activity, which would intimidate insect immunological processes. These also suggest that PCDDs/PCDFs and PCBs may not inhibit PLA_2 activity.

PO activity of *S. exigua* hemocytes was also affected by aqueous extracts of the sediment samples, but not by organic extracts. All aqueous extracts from the five stream sediments completely inhibited the hemocyte PO activity. We already showed that all sediment samples contained various heavy metals. These results suggest that heavy metals significantly inhibits PO activity, but PCDDs/PCDFs and PCBs do not inhibit PO activity.

The immune reactions tested in this study can be affected by other compounds presumably contained in the sample extracts. For example, biological pathogens can be contaminated in the aqueous sample extract and significantly influence these immune biomarkers. Thus reliable biomarkers should have some degree of specificity due to a variety of contaminants present in environment. Alternatively, a battery of biomarkers is required to cross-check different classes of contaminants (Melancon 1994). Despite some disparity between bio- and chemical monitoring results, the biomarkers can be recommended as a device warning the contamination of dioxins in the environment because of a fast and inexpensive detection method.

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