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Receptor-mediated in vitro bioassay for characterization of Ah-R-active compounds and activities in sediment from Korea

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Abstract

Sediment extracts of stream sediments, collected from inland areas of Lake Shihwa (LSI) and Masan Bay (MBI), were screened for their abilities to induce aryl hydrocarbon receptor (Ah-R) mediated gene expression in vitro. Cell viability assay was also performed to examine cytotoxic effects on the Ah-R-mediated activities of sediment samples. Over 80% (30 out of 36) of sediment raw extracts (REs) induced significant Ah-R-mediated activities in the H4IIE-luc cell bioassays. Ah-R-mediated activities of sediment REs from LSI locations (mean = 58%-TCDD-max; $n = 21$) were greater than those of sediment REs from MBI locations (mean = 35%-TCDD-max; $n = 15$), in general. Seven (mean ± SD = 100 ± 14%-TCDD-max) of 21 sediment REs from LSI showed Ah-R-mediated activities comparable to that (set to, 100%-TCDD-max) elicited by 1240 pM TCDD. Whereas, in MBI, only two REs from M1 (93%-TCDD-max) and M9 (82%-TCDD-max) showed significantly great responses that comparable to maximum response of TCDD standard curve. Sample potencies relative to the TCDD standard (TCDD-EQs) were estimated based on full dose–response characteristics of REs and TCDD-EQs were found to be 14–868 pg TCDD/g, dw and 17–275 pg TCDD/g, dw, in LSI and MBI, respectively. A range of TCDD-EQ_{20-80} of samples, based on multiple estimates of relative potency (REP_{20-80}), did not vary greatly (2–4-fold) in the H4IIE-luc bioassays, which indicated relatively low degree of uncertainties in point estimates of REP for sediment REs examined. Acid-treatment of REs samples improved quantitative biological responses of samples followed by decreases in cytotoxicity identified by MTT cell viability assays.

Keywords: Sediment; H4IIE; In vitro bioassay; Cytotoxicity; Acid treatment

1. Introduction

Contamination of the coastal environment by halogenated aromatic hydrocarbons (HAHs) is a growing

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concern due to their widespread distribution and potential for long-term exposure and bioaccumulation in aquatic ecosystems (Boening, 1998; Giesy and Kannan, 1998; Jones et al., 2001). Some HAHs can disrupt normal endocrine function both in aquatic organism and vertebrate predictors. These adverse effects are partly mediated with an aryl hydrocarbon receptor (Ah-R)-dependent mechanism of action (Poland and Knutson, 1982; Willett et al., 1997). In vitro bioassays are simple, rapid, and cost-effective tools relative to instrumental analyses, thus its use and application to characterize mechanism specific activities of environmental contaminants are of great benefit (Hilscherova et al., 2000; Hahn, 2002).

H4IIE-luc cell bioassays have been widely applied in the characterization and evaluation of Ah-R-active compounds of sediments and other complex environmental matrices. (Giesy et al., 2002; Whyte et al., 2004). H4IIE-luc cells are rat hepatoma cells, stably transfected with luciferase reporter gene under control of dioxin-responsive elements (Sanderson et al., 1996). Generally, responses of luciferase activity in H4IIE-luc cells are reported as % maximal induction to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (%-TCDD-max), effective concentration to elicit 50% of response relative to maximum sample response (EC_{50}), and dioxin equivalents (TCDD-EQ_{50}) based on relative potency estimates (REP_{50}) (Sawyer et al., 1984; Khim et al., 1999a; Villeneuve et al., 2002). However, it should be noted that uncertainties in single REP estimates are caused by violation of the assumption of parallelism and equal efficacy between samples and standard responses (Putzrath, 1997; Villeneuve et al., 2000). Thus, the application of multiple point estimates such as REP range ($REP_{20–80}$) have been proposed to provide uncertainties where range of a $REP_{20–80}$ directly represents the degree of deviation from parallelism and maximum efficacy between sample and standard responses (Villeneuve et al., 2000). Previous studies utilized this method to properly estimate potential Ah-R-mediated activities of environmental samples that rarely conform to these assumptions. In the present study, the estimation of REP range associated with sediment raw extracts (REs) was performed and its application and role of indirect bioassays to determine biological potency of environmental samples were discussed.

Previous studies conducted by our group reported Ah-R-mediated activities associated with sediment, water, pore water, and animal tissues from Korean coasts by use of instrumental and/or bioanalytical analyses (Khim et al., 1999a,b; Koh et al., 2002, 2004). Some of those results indicated that sediments near industrial complexes and populated coastal areas contained relatively great concentrations of Ah-R-active compounds and activities in Korea (Khim et al., 1999a,b; Im et al., 2002a,b). Contrary to many reports in depositional areas (viz. estuaries and bays), a few data are available for their occurrence, sources (point sources) and origin of Ah-R-active compounds in upper inland areas (viz. stream and riverine areas). The present study was conducted in the inlands areas of Lake Shihwa (LSI) and Masan Bay (MBI) including several streams and creeks, where moderate to high levels of HAHs contamination reported previously in sediment from repository areas of Lake Shihwa and Masan Bay. The objective of the present study was to determine the spatial distribution of Ah-R-mediated activities and concentrations of dioxin equivalents (TCDD-EQs) in sediments from upstream locations using H4IIE-luc cell bioassays in vitro.

2. Materials and methods

2.1. Sample collection

Samples were collected from the inland areas of Lake Shihwa and Masan Bay, located in west and southeastern coasts of Korea, respectively, in May 2003 (Fig. 1). Lake Shihwa is an artificial saltwater lake created by the construction of 12.7 km sea dike (in 1994) and Masan Bay is a long and narrow inlet of a semi-closed bay situated in northern part of Chinhae Bay. Large amount of industrial waste and sewage from inland areas, the industrial and municipal cities (Shiheung, Ansan, Masan, and Changwon), are discharged into lake and bays via several streams and creeks (Koh et al., 2005). Sampling was designed to determine potential sources of Ah-R-active contaminants in sediments from upstream locations in LSI (S1–S21; $n = 21$) and MBI (M1–M15; $n = 15$). The water depth in sampling locations was less than 1.0 m and mainly represented by fresh to brackish waters. After collection, pebbles and twigs were removed, and then samples were freeze dried and ground with a pestle and mortar. Samples were stored in pre-cleaned high density polyethylene (HDPE) bottles at −20 °C until extraction.

2.2. Sample preparation

Detailed procedures of sample preparation including extraction steps are described elsewhere (Khim et al., 1999a, Koh et al., 2002). Briefly, 20 g of sediments samples were Soxhlet extracted for 20 h using 400 ml high-purity dichloromethane (Burdick and Jackson, Muskegon, MI, USA). Soxhlet REs were then treated with acid-activated copper granules to remove sulfur and aliquots of REs were concentrated up to 1 ml in hexane under a gentle stream of nitrogen. Selected samples (100% REs) were further treated with concentrated H_2SO_4 (1:10 = sample:acid in volume) for 2 h and solvent layer was then rinsed twice with Nanopure® (Barnstead) water to remove acid residuals. All sediment REs ($n = 36$) and acid treated ones (selected; $n = 16$) were

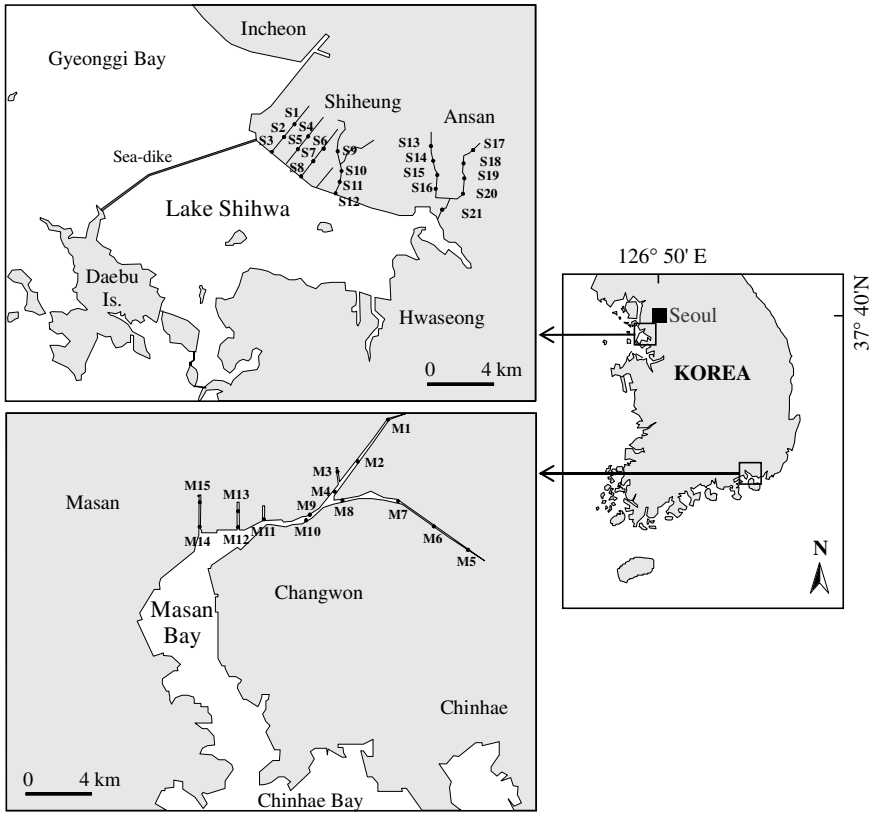

Fig. 1. Map showing the study areas in Korea. Sediments were collected from inland areas of Lake Shihwa (LSI locations; S1–S21) and Masan Bay (MBI locations; M1–M15) in 2003.

analyzed in the H4IIE-luc cell bioassays and MTT cell viability assays described as below. Procedural blanks ($n = 6$) generated from each set of six samples during extraction were concurrently analyzed and no significant responses were observed in the H4IIE-luc cell bioassays.

2.3. H4IIE-luc cell bioassay

Detailed descriptions for the H4IIE-luc cell bioassays have been provided elsewhere (Sanderson et al., 1996; Khim et al., 1999b). Briefly, trypsinized cells from culture plate were diluted to a concentration of approximately 8.0×10^4 cells/ml and were seeded into the 60 interior wells of 96-well culture View Plates® (Packard Instruments, Meriden, CT, USA) at 250 μl per well. Cells were incubated overnight and then test and control wells were dosed with 2.5 μl of the appropriate samples of extract and solvent. At initial screening, extracts were tested in three replicate wells at a single concentration of REs (viz. 100% REs). As for sample dose–response characterization, 100% REs and diluted ones, consisting of six concentrations of samples prepared by 3-fold serial dilution, were tested in triplicate. Luciferase assays were conducted after 72 h of exposure using a ML3000 microplate reading luminometer (Dynatech Laboratories, Chantilly, USA) by the method presented in our earlier publications (Khim et al., 1999b).

2.4. MTT cell viability assay

The tetrazolium salt 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) was used to determine cell viability and overall cytotoxicity of REs samples by measuring the activity of mitochondrial dehydrogenases in the cells (Hussain et al., 1993). In brief, additional sets of H4IIE-luc cells were prepared, in which the H4IIE-luc cell bioassays were performed, and cells were exposed to REs samples for 72 h. After exposure, cells were rinsed with phosphate-buffered saline (PBS) and 100 μl of MTT solution (0.5 mg/ml) was added into each well. Cells were incubated for 30 min (at 37 °C), then MTT solution was removed from the wells. 200 μl of dimethylsulfoxide (DMSO) was delivered to dissolve the blue formazon crystals produced by the reduction of MTT. The absorbance of the developed color was measured at 492 nm by Microplate absorbance reader (Cayman Chemical Autoreader, USA). Based on the amount of the formed blue

formazan which represents the amount of viable cells, the relative %-live cells (or %-dead cells) were calculated by subtracting the responses in control wells from those in treated wells. Microscopic examination was done additionally to support biochemical observations prior to MTT cell viability assay. The degree of altered or 'stressed' morphology was given ranging from 0 to 5 (indicates low to high visible cytotoxicity) and presented in Table 1.

2.5. Bioassay data analysis

Sample responses, expressed as mean relative luminescence units over triplicate wells, were converted to a percentage of the maximum response (set to 100%-TCDD-max) observed for a 1240 pM TCDD standard curves generated on the same day. This was done to normalize responses for day-to-day variation in response magnitude and enhance the comparability of these results to other results reported in the literature. Significant responses were defined as those outside the range observed by three times the standard deviation of the mean solvent control responses (expressed as %-TCDD-max).

Sample potencies relative to TCDD standard (TCDD-EQs), viz. potency-based dioxin equivalents, were determined directly from sample dose–response relationships generated by testing samples at multiple levels of dilution (Khim et al., 1999b; Villeneuve et al., 2000). In order to account for degree of uncertainty in the TCDD-EQs estimates caused by deviations of the sample dose–response curves from parallelism to the TCDD standard curve, TCDD-EQs were calculated for a range of responses (TCDD-$EQ_{20–80}$) based on multiple points estimates of $REP_{20–80}$. The magnitude-based TCDD-EQs (single point estimates) were also predicted from single dose response of selected REs (100%, 33%, and 11% REs), on the assumption that complex mixtures of Ah-R-active compounds present in the REs behavior as if they were simply the TCDD standard. Conversion to pg TCDD-EQs/g, dw, was back-calculated from the observed bioassay responses of samples in regression against TCDD standard curve. Potency-based TCDD-EQs may not be always obtained due to lack of responses and/or cytotoxicity to generate complete dose–response relationships, particularly for environmental samples. Thus, at least, providing magnitude-based TCDD-EQs of certain REs samples would be beneficial to roughly estimate and compare potency of samples in some cases. However, it should be noted that magnitude-based comparisons are generally inferior to potency-based comparisons.

In the present study, both potency- and magnitude-based estimation of bioassay-derived TCDD-EQs are presented and the advantage or disadvantage of multiple or single points estimation approach are discussed. Statistical analyses including correlation analysis, paired *t*-tests, and ANOVA were performed using SYSTAT 10 program (SPSS, Inc., Chicago, USA).

3. Results

3.1. Single dose screening

Based on the initial screening of a single dose of sediment REs (100% RE), ≈80% (17 out of 21) of REs samples from LSI locations elicited significant Ah-R-mediated activities in the H4IIE-luc bioassay (Table 1). Sample responses greatly varied depending on sampling locations ranging from <0 to 114%-TCDD-max. Seven (S4, S7, S14, S16–S18, and S21) of 21 sediment REs from LSI locations showed comparable maximal responses (mean ± SD = 100 ± 14%-TCDD-max) to that induced by a 1240 pM TCDD standard. However, due to the great cytotoxicity in the cells exposed to 100% REs from many locations in LSI, the Ah-R-mediated activities from initial screening assay could not represent properly the potential biological effects of LSI samples. For example, six (S2, S6, S8–S10, and S12) sediment REs from LSI that showed Ah-R-mediated activities of <10%-TCDD-max exhibited greater cytotoxicities identified by %-dead cells (mean = 65%) and visible altered and 'stressed' morphology (mean grade = 4.0) (Table 1).

Sediment REs from MBI locations showed relatively lower Ah-R-mediated activities (mean = 35%-TCDD-max) as well as lesser cytotoxicity (mean = 36%-dead cells) in 100% REs samples, contrast to those from LSI locations. Only three locations (M1, M8 and M9) showed Ah-R-mediated activities greater than 50%-TCDD-max, which indicated that less Ah-R active compounds present in sediments from inland areas of Masan Bay. Again, lower luciferase induction of 100% REs samples from some MBI locations (M3–M4 and M11–M15) were related with great cytotoxicity present in corresponding samples. In general, Ah-R-mediated activities of 100% REs both in LSI and MBI locations in the H4IIE-luc screening bioassays decreased as the cytotoxicity, identified by visible altered and 'stressed' morphology, increased. Overall, the MTT cell viability assays, given as %-dead-cells, supported the visible cytotoxicity of sediment REs samples observed under microscopic analysis.

3.2. Full dose–response characterization

To accurately estimate concentrations of Ah-R-active compounds and activities associated with sediment samples, REs samples were tested for a full dose–response characterization. Six serial dilutions of 100% REs were prepared and analyzed in the H4IIE-luc bioassays and

Table 1
Sample responses in the H4IIE-luc cell bioassays and cytotoxicity in the MTT cell viability assays elicited by sediment raw extracts (REs) from the inland areas of Lake Shihwa and Masan Bay, Korea

Sampling		H4IIE-luc bioassay		Cytotoxicity	
Area/region	Location	%-TCDD-max[a]	TCDD-EQ_{50}[b] (pg/g, dw)	%-Dead cells[c]	Visual grade[d] (0–5 grade)
Lake Shihwa					
Okgu Creek	S1	32	165	89	5
	S2	<0	846	17	4
	S3	25	868	86	5
Gunja Creek	S4	113	109	38	1
	S5	73	14	0	0
Jeongwang Creek	S6	9	46	87	4
	S7	105	43	26	1
	S8	8	270	65	4
Shiheung Stream	S9	<0	–	83	3
	S10	<0	–	78	4
	S11	35	236	78	2
	S12	<0	–	61	5
Hwajeong Stream	S13	27	–	86	5
	S14	83	68	17	1
	S15	17	–	3	0
	S16	109	29	3	0
Ansan Stream	S17	82	–	9	0
	S18	114	108	1	0
	S19	29	110	55	1
	S20	30	583	47	4
	S21	91	82	30	3
Mean	S1–S21	58	238	46	2.5
Masan Bay					
Changwon Stream	M1	93	59	10	1
	M2	23	–	11	0
	M3	9	128	41	3
	M4	18	166	64	4
Nam Stream	M5	20	–	17	0
	M6	<0	–	27	2
	M7	35	–	21	0
	M8	62	65	3	0
	M9	82	17	29	0
	M10	20	–	6	1
	M11	44	41	49	3
Bongam Creek	M12	11	275	45	4
	M13	<0	–	66	4
Samho Creek	M14	19	57	76	3
	M15	21	20	80	3
Mean	M1–M15	35	92	36	1.9

[a] Response magnitude presented as percentage of the maximum response observed for a 1240 pM TCDD standard (set to 100%-TCDD-max) elicited by 100% sediment raw extracts before acid treatment.

[b] Based on the sample dose–response relationships by testing samples at multiple levels of dilution (six diluted samples from 100% REs), bioassay-derived dioxin equivalents (TCDD-EQs; pg/g, dw) were obtained. TCDD-EQ_{50} (limit of qulification = 0.57 pg/g, dw) refer to the TCDD-EQs generated from one point estimate made for response of 50%-TCDD-max.

[c] Cytotoxicity of sediment REs was determined by the MTT cell viability assay (expressed as %-dead cells).

[d] Visible cytotoxicity was presented as degree of altered or "stressed" morphology under microscopic observation, values graded from 0 to 5 (low to high degree of visible cytotoxicity).

full dose–response curves of each sample and TCDD standard were generated. Sample responses (expressed as %-TCDD-max) increased as function of dose, in general, but rapidly decreased in higher concentrations of REs samples, viz. 100% and/or 33% of REs. The significant drop of sample responses in 100% and/or 33% of REs was related with the degree of cytotoxicity observed in the MTT cell viability assays. When sample potency, given as a maximum response from multiple concentrations of REs, was examined, the number of samples showing significant induction increased in both regions (Fig. 2). Noticeable increases in sample response of diluted samples were found for eight and seven locations from LSI and MBI areas, respectively. This result indicated single dose screening data may underestimate the potential Ah-R-mediated activities associated with sediment samples in some locations by cytotoxicity.

To evaluate sample potency of Ah-R-active compounds present in sediment REs, bioassay-derived dioxin equivalents (TCDD-EQs) were calculated based on the REP estimates from dose–response curves of samples and TCDD standard. Detectable concentrations of TCDD-EQs (REP_{50} based point estimate; TCDD-EQ_{50}) in sediments from LSI locations ranged from 14 to 868 pg TCDD/g, dw (mean = 238). The greatest concentrations of TCDD-EQs were found in locations S2 (846 pg TCDD/g) and S3 (868 pg TCDD/g, dw) from Okgu Creek, whereas those in other streams and creeks were relatively low on average of 150 pg TCDD/g, dw. Sediment REs from MBI locations contained relatively lower concentrations of TCDD-EQs than LSI, ranging from 17 to 275 pg TCDD/g, dw (mean = 92). When an ANOVA analysis was conducted using only the sites where the TCDD-EQs were obtained, then there is no statistical difference between two regions ($p = 0.15$).

3.3. Acid treatment and cytotoxicity

Acid treatment of selected 100% REs samples ($n = 16$) provided additional clean-up and resulted in

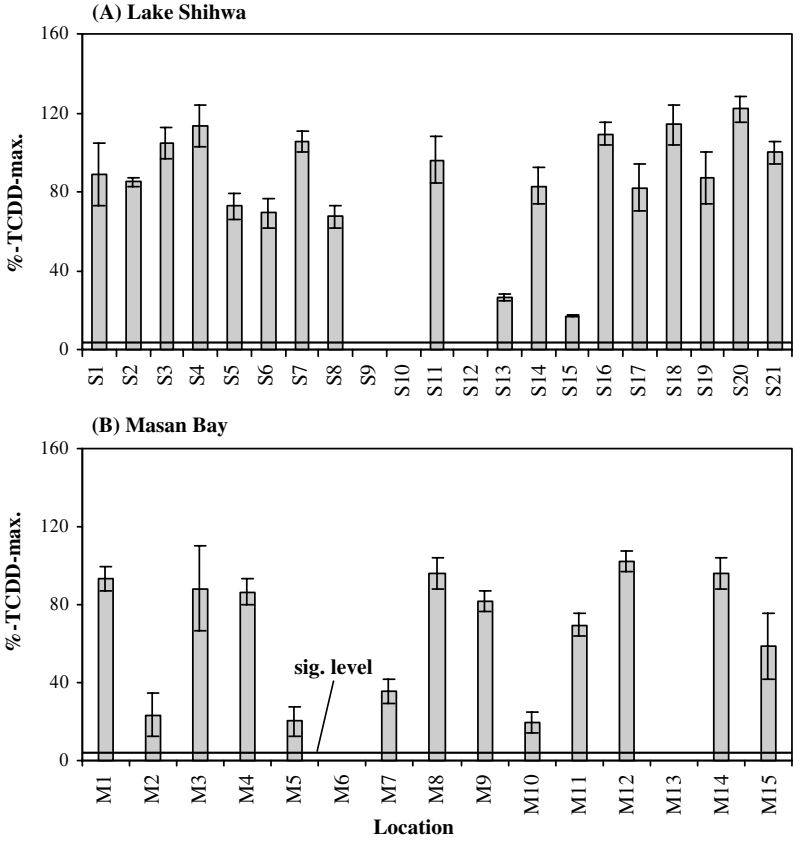

Fig. 2. Luciferase induction in the H4IIE-luc cell bioassay elicited by sediment raw extracts (REs) from inland areas of (A) Lake Shihwa and (B) Masan Bay, where sample responses are given as maximum from full dose-response curves of corresponding REs (as for single dose screening result, see Table 1). Response magnitude presented as percentage of the maximum response observed for a 1240 pM TCDD standard (set to 100%-TCDD-max). Horizontal line equals three standard deviations (expressed in %-TCDD-max) above the mean solvent control response (set to 0%-TCDD-max).

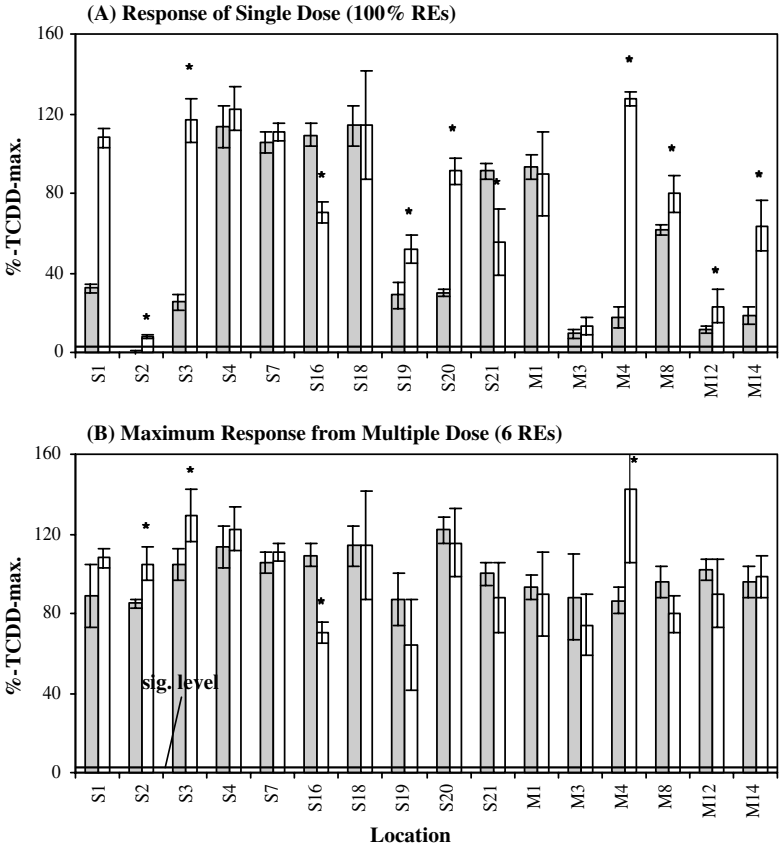

Fig. 3. Comparison of observed H4IIE-luc cell bioassay responses (Ah-R-mediated activities, expressed as %-TCDD-max) of sediment raw extracts (REs) before (shadow bar) and after (empty bar) acid treatment. Selected sediment REs (100% REs; $n = 16$) are treated with concentrated H_2SO_4 for 2 h and reanalyzed in the H4IIE-luc cell bioassay. (A) Sample responses from single dose of 100% REs, and (B) sample response from maximum responses in full dose–response curves of six concentrations of REs (six diluted samples from 100% REs). Statistically significant at $p < 0.05$ (*) after acid treatment of sediment REs in sample responses.

overall increases of Ah-R-mediated activities in the H4IIE-luc cell bioassay (Fig. 3). Thirteen of 16 sediment REs treated with acid induced greater Ah-R-mediated responses relative to those caused by non-treated REs samples by 2.6-fold, on average (Fig. 3A). Only two acid treated REs (S16 and S21) showed significant decreases in Ah-R-mediated activities compared with those in non-treated REs ($p < 0.05$). However, when multiple concentrations of REs tested, sample potency, given as a maximum of six diluted RE responses, did not greatly before (mean = 99%-TCDD-max) and after (mean = 100%-TCDD-max) acid treatment (Fig. 3B). Only three REs from locations S2, S3, and M4 generated significant increases in Ah-R-mediated activities.

After acid treatment of 100% REs, only two samples exhibited moderate signs of altered and 'stresses' morphology of cells, which indicated degradation and/or breakdown of cytotoxic compounds that can reduce the number of viable cells. Sample responses of diluted REs samples were not significantly influenced by cytotoxic chemicals present in corresponding samples due to alleviations of cytotoxic effect by dilution itself. Overall, the result presented, strongly supported that presence of cytotoxic compounds in sediments samples would underestimate the potential biological activities of Ah-R-active compounds present in REs samples. It is not a conventional way to determine cytotoxicity by microscopic examination. However, these observations were used to support the biochemical MTT results. Regression analysis showed statistically significant relationship between two measurements ($p < 0.001$), though the microscopic grade is a semi-numeric value.

4. Discussion

4.1. Regional and spatial comparison

Several studies have examined the Ah-R-mediated activities of coastal and marine sediments collected from

the Korean coasts using the same H4IIE-luc bioassays (Khim et al., 1999b; Khim et al., 2001; Koh et al., 2002). However, relatively few studies reported the occurrence and distribution of Ah-R-active compounds in the inland areas including river system (Koh et al., 2005). The screening result of sediment REs in the H4IIE-luc cell bioassays indicated widespread distribution of Ah-R-active compounds in inland areas of Lake Shihwa and Masan Bay. TCDD-EQs found in sediment REs from LSI locations (mean = 238 pg TCDD/g, dw) and MBI locations (mean = 92 pg TCDD/g, dw) were the greatest next to those in Hyeongsan River, Pohang (mean = 317 pg TCDD/g) in Korean coasts (Koh et al., 2004). This could be explained by discharges of large amount of industrial wastewaters via several streams and creeks from LSI (3×10^5 ton/day) and MBI (0.5×10^5 ton/day), respectively. Due to slow mixing rate of seawater and long residence time of sewage sludge, the lower reaches of river and bay serve as a reservoir of various contaminants in these regions (Koh et al., 2005). Severe contaminations of HAHs including dioxins, furans, and polychlorinated biphenyls (PCBs) as well as polycyclic aromatic hydrocarbons (PAHs) have been reported in lake and coastal sediments (or soil) near LSI and MBI locations and these are likely the sources of great Ah-R-mediated activities observed in stream samples (Khim et al., 1999a; Im et al., 2002a,b; Koh et al., 2005).

Relatively great contamination of Ah-R-active compounds was found in western part of Shihwa industrial complexes (ICs) along the Okgu, Gunja, and Jeongwang creeks. Whereas, samples from Shiheung Stream (S9–S12) located in the boundary between Shiheung and Ansan cities exhibited lesser Ah-R-mediated activities than those caused by REs from other locations (Table 1, Fig. 1). In residential area (Ansan City), sediment REs from Hwajeong Stream contained lowest Ah-R-active compounds, where detectable concentrations of TCDD-EQs were found to be 68 and 29 pg TCDD/g, dw, at locations S14 and S16, respectively. While, samples from Ansan Stream which intersects the downtown as well as high-population density area had comparable concentrations of TCDD-EQs (mean = 221 pg TCDD/g, dw) to those from Shihwa IC (mean = 295 pg TCDD/g, dw). Previous study found detectable concentrations of TCDD-EQs (mean = 86 pg TCDD/g, dw; $n = 8$) in sediment samples from some upstream locations in LSI, which was within the similar range of the TCDD-EQs found in the present study (Koh et al., 2005).

Ah-R-mediated activities of sediment REs from MBI locations were more modest compared with those from LSI locations. However, there was large data variance within study region that no statistical difference was observed between LSI and MBI. Nine of 15 sediment REs in MBI locations contained detectable concentrations of TCDD-EQs ranging from 17 to 275 pg TCDD/g, dw (Table 1). Sample responses of MBI REs greatly varied depending on sampling locations and no apparent gradient of TCDD-EQs was found along the river system, but rather indicating possible point sources (such as discharge inlets, near M3 and M12 locations). A previous study by Koh et al. (2005) conducted in coastal region of the Masan Bay found that sediment collected in 2000 contained TCDD-EQs up to 284 pg TCDD/g, dw (mean = 103; $n = 8$), which indicated continual input of Ah-R-active chemicals from point sources into the bay areas.

4.2. Use of in vitro bioassay data to characterize environmental samples

In vitro H4IIE-luc cell bioassays can be useful tools to characterize Ah-R-active compounds and activities in environmental samples through Ah-R-dependent mechanism of action, although quantitative analyses are limited (Giesy et al., 2002). Often, the bioassay results using H4IIE-luc system were used to characterize the dioxin-like compounds in the sediment samples. However, carefulness should be taken to interpret the data, since this receptor-mediated cell bioassay measures technically the total Ah-R-mediated activities, including less persistent, non-dioxin-like chemicals such as PAHs. As for screening and/or monitoring purposes, bioassays can provide overall characteristics of biologically active compounds and their complex interactions associated with samples in question. Good agreements between bioassay-derived TCDD-EQs and instrumentally-derived dioxin equivalents (TEQs) have been reported, thus bioassay results can be alternatives to facilitate quantitative risk assessment when instrumental analyses are limited (Hilscherova et al., 2003; Koh et al., 2004). Acid treatment of sediment REs further provided characteristics of the sample compositions with major contribution of acid-stable, non-polar chemicals, such as dioxins, furans, polychlorinated naphthalenes (PCNs) and coplanar PCBs (Khim et al., 1999a).

It should be noted that screening results based on single dose of 100% REs samples may underestimate the efficacy (magnitude) of biologically active compound associated with sediment, due to cytotoxicity. For example, over half the cases (21 out of 36 locations), the Ah-R-mediated activities caused by diluted samples, viz. 33% REs, were greater than those caused by 100% REs in the H4IIE-luc bioassays (Fig. 4). The ratio between two dilutions (response of 100–33% REs) was significantly correlated with cell viability, identified by %-live cells (Fig. 4). In particular, when the live cells were found to be less than 60% of total cells, which have been exposed to 100% REs, responses of 100% REs were usually lower than those of 30% diluted REs. The significant reduction of sample responses caused by 100% REs

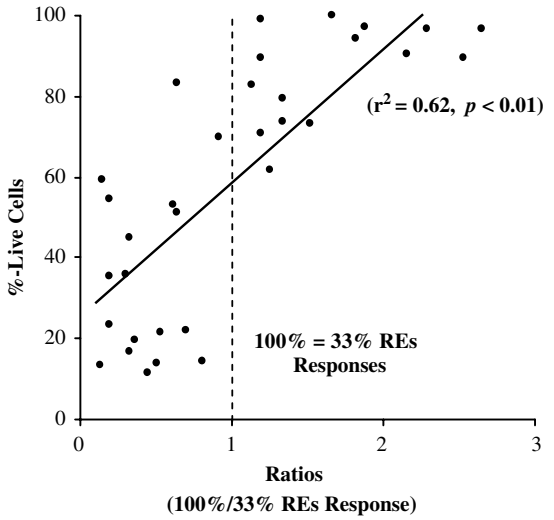

Fig. 4. Relationship between observed H4IIE-luc cell bioassay responses and MTT cell viability responses of sediment raw extracts (REs). The ratios of sample responses (given in ratios of 100–33% REs responses) in the H4IIE-luc bioassay are plotted against cell cytotoxicity index (expressed as %-live cells) in the MTT cell viability assay.

would be due to the decreased number of the viable cells by cytotoxic chemicals. The effects of matrix specific compounds that may interfere with the receptor binding of Ah-R-active compounds could be possible, however it was not investigated in this study. In cases where lesser cytotoxic compounds presented in 100% REs, sample responses caused by 33% REs were reduced accordingly by dilution effects.

Two mathematical calculations can be used to estimate the sample potency relative to TCDD standard; one is potency-based TCDD-EQs derived from full dose–response curves and the other is magnitude-based TCDD-EQs derived from single dose of REs samples (Table 2). Only, magnitude-based TCDD-$EQ_{11\%}$, derived from single point estimates of 11% of REs (9-fold dilutions of the 100% REs), was strongly correlated with potency-based TCDD-$EQ_{50–80}$. When cytotoxic samples are excluded, magnitude-based TCDD-$EQ_{33\%}$, derived from single point estimates of 33% REs were also significantly correlated with potency-based TCDD-$EQ_{50–80}$. These results indicated that diluted samples (33% or 11% REs) or lesser cytotoxic samples (Data set II in Table 2) represented more relevant biological responses of samples in the H4IIE-luc cell bioassays. Thus, even as for screening tools, test of multiple dilution of samples and/or appropriate cytotoxicity measurement would be recommended to minimize the degree of uncertainty in relative potency estimates.

Since the usual variability of single point estimates, range of TCDD-$EQ_{20–80}$, based on $REP_{20–80}$ range, were calculated from selected sediment REs ($n = 16$) exhibiting similar effective responses (>80%-TCDD-max) compared with TCDD standard curve (Fig. 5). Range of TCDD-$EQ_{20–80}$ of REs samples from two locations, S3 and S20, were significantly different compared with those of other samples (Fig. 5). The broad range of TCDD-$EQ_{20–80}$ reflected that dissimilarity between slope and maximum efficacy of corresponding REs

Table 2
Relationships (r^2)[a] between potency- and magnitude-based dioxin equivalents (TCDD-EQs) associated with sediment raw extracts (REs) from the inland areas of Lake Shihwa and Masan Bay, Korea

Magnitude-based TCDD-EQs[b]	No. of samples (n)	Potency-based TCDD-EQs[c]		
		TCDD-EQ_{20}	TCDD-EQ_{50}	TCDD-EQ_{80}
Data set I[d]				
TCDD-$EQ_{100\%}$	24	0.052	0.062	0.055
TCDD-$EQ_{33\%}$	24	0.108	0.116	0.095
TCDD-$EQ_{11\%}$	24	0.082	0.334**	0.614**
Data set II[e]				
TCDD-$EQ_{100\%}$	9	0.170	0.394	0.449
TCDD-$EQ_{33\%}$	15	0.175	0.557*	0.849*
TCDD-$EQ_{11\%}$	20	0.754*	0.861**	0.861*

[a] Statistically significant at $p < 0.05$ (*) and $p < 0.01$ (**).

[b] The magnitude-based TCDD-EQs (TCDD-$EQ_{\%\ REs}$) were predicted from single dose response of selected REs (100%, 33%, and 11% REs), conversion to pg TCDD-EQs/g, dw, was back-calculated from the observed bioassay responses of samples in regression against TCDD standard curve.

[c] Potency-based TCDD-EQs (TCDD-$EQ_{20–50–80}$) were obtained from sample dose–response relationships generated by testing samples at multiple levels of dilutions. TCDD-$EQ_{20–50–80}$ refer to the TCDD-EQs generated from multiple point estimate made for response of 20%, 50%, and 80%-TCDD-max.

[d] Data set I contains all the selected sediment REs tested at multiple levels of dilutions in the H4IIE-luc bioassays.

[e] Data set II contains ''only'' samples that did not cause noticeable cytotoxicity observed.

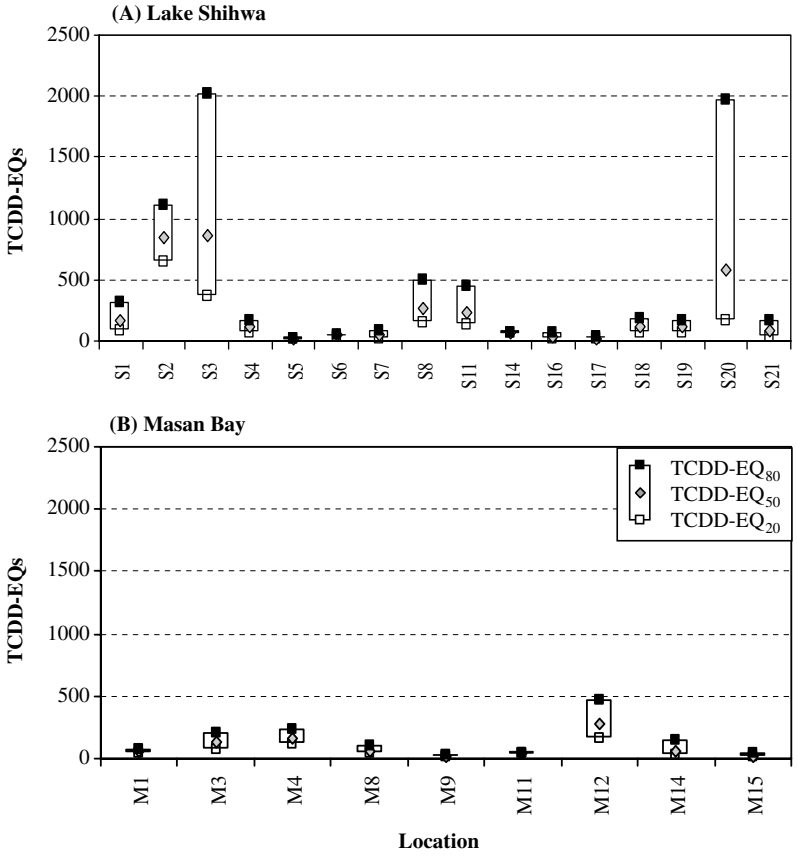

Fig. 5. Bioassay-derived dioxin equivalents (TCDD-EQs; pg/g, dw) of sediment raw extracts (REs) from inland areas of (A) Lake Shihwa and (B) Masan Bay in the H4IIE-luc cell bioassays. TCDD-EQs of REs samples are determined from sample dose–response relationships generated by testing samples at multiple levels of dilution. TCDD-EQs are presented as a range of responses (TCDD-$EQ_{20–80}$) based on relative potency ranges ($REP_{20–80}$ ranges) for the selected REs samples that showed >80%-TCDD-max in sample dose–response curves.

(from locations S3 and S20) relative to that of the TCDD standard curve. Most of samples from MBI locations showed lesser variations in range of TCDD-$EQ_{20–80}$ (mean = 2.8-fold), which indicated lower interactions between and among the Ah-R-active compounds present in sediment REs but rather behaved as they were simply TCDD standard. Overall, application of multiple point estimates of sample relative potency provided useful information for the degree of uncertainty hided in single point estimates in the interpretation of bioassay screening results.

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