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Sediment Toxicity of Industrialized Coastal Areas of Korea Using Bioluminescent Marine Bacteria

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The quality of marine sediments from the industrialized coastal areas of Korea (Ulsan Bay, Masan Bay, and artificial Lake Shihwa) was investigated using a bacterial bioluminescence toxicity test. Sediment toxicity results were compared with the levels of chemical contamination (trace metals, organic wastewater markers, acid volatile sulfides, total organic carbon). Effective concentration 50% (EC50) of sediments ranged from 0.014 to 1.126 mg/mL, which is comparable to or lower than values in contaminated lakes, rivers, and marine sediments of other countries. Sediment reference index (SRI) ranged from 13 to 1044, based on the EC50 of the negative control sample. Mean average SRI values in Masan Bay and Lake Shihwa were approximately 8 and 9 times as high as that in Ulsan Bay, indicating higher sediment toxicity and greater contamination in the two former regions. Sediment toxicity were strongly associated with the concentrations of some chemicals, suggesting that this test may be useful for determining potential chemical contamination in sediments.

Key words: Nonylphenol, Sediments, Sulfide, Trace metals, *Vibrio fischeri*

Introduction

Sediments are an integral part of the aquatic environment, providing habitat, feeding, and breeding areas for a large number of organisms. Problems resulting from contaminated sediments are currently an important issue. Risk assessments for sediments have been determined predominantly by chemical analysis. Although chemical analysis provides environmental pollutant concentrations, it gives little information regarding bioavailability and/or toxicity at the sites. For inferring probable adverse biological effects, biological analyses must be combined with the chemical analyses. A combined ecotoxicological and chemical approach can significantly improve the reliability of assessment of the environmental health (Ocampo-Duque et al., 2008; Macken et al., 2008; Narracci et al., 2009).

Bioassays are generally used as tools to indicate the adverse biological effects of a contaminant mixture. They can detect adverse biological effects within sediment, as well as differences in the bioavailability of contaminants, and the presence and effects of compounds that might have been overlooked by chemical analysis. Sediment toxicity testing has grown steadily in recent years and is a useful tool for environmental risk assessment (Narracci et al., 2009).

Bacterial bioluminescence bacteria bioassay is a screening tool for a variety of toxicity testing applications. It is sensitive, reproducible, cost effective, and simple to conduct. Furthermore, it does not entail ethical problems, and requires only 5-30 min for toxicity prediction (Park et al., 2005; Parvez et al., 2006; Ocampo-Duque et al., 2008). Its results have been compared with those of other conventional bioassays, with good correlation in many cases (Guerra, 2001; Abbondanzi et al., 2003; Parvez et al., 2006). Among several other bioassays, the bacterial bioluminescence bioassay has proven to be the most

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sensitive to contaminated marine sediments, in comparison to several other bioassays (Long et al., 1996; Beg et al., 2001; Long et al., 2002; Park et al., 2005; Narracci et al., 2009). It uses re-hydrated freeze-dried luminescent bacteria (*Vibrio fischeri*) and measures inhibition of luminescence under defined conditions. A reduction of luminescence indicates a stress response of the bacteria to the presence of chemical contaminants, thus reflecting the quality of an environment. This method has been widely used for ecotoxicological assays since 1979, and has been standardized internationally (ASTM D-5660, ISO 11348, DIN 38412-34). This assay has been used for the quality assessments of marine sediments in coastal areas of many countries (Long et al., 1996; Kemble et al., 2000; Beg et al., 2001; Long et al., 2002; Martínez-Lladó et al., 2007; Macken et al., 2008). In Korea, it has been standardized by MOMAF (2005). Until now, the quality assessment using the bacterial bioluminescence bioassay has been limited to examining the toxicity of sewage sludge (Park et al., 2005), soils treated with sewage sludge (Nam et al., 2004; Park et al., 2006), effluent from the dye industry (Kim et al., 2004), and trace metals (Lee and Bae, 2005). To our knowledge, the present study is the first report on the use of the bioluminescence inhibition test for assessing the quality of marine sediments from Korean coastal waters. This study was conducted as a part of the National Marine Environmental Monitoring Program in Korea.

Industrialized coastal areas such as Ulsan Bay, Masan Bay, and Lake Shihwa present serious environmental concerns and have been designated as special management coastal zones by the Korean government since 1982. Heavy sediment contamination by toxic pollutants has been widely reported in industrialized coastal areas (Hong et al., 2005; Moon et al., 2007; Choi et al., 2009). However, based on chemical measures of toxic pollutants, the quality of sediments in the industrialized coastal areas differs considerably according to the target pollutants. Little information has been provided regarding the presence, interactions, and bioavailability of toxics. Therefore, it is necessary to provide information on the biological response from contaminations in industryalized coastal areas. In this study, we performed the bioluminescence toxicity test on marine sediments collected from three industrialized areas of Korea (Ulsan Bay, Masan Bay, and artificial Lake Shihwa), and compared the results with the chemical parameters (trace metals, organic wastewater markers, acid volatile sulfides, total organic carbon).

Materials and Methods

Sample collection

Surface sediments were sampled at 15 locations around the Ulsan Bay, Masan Bay, and artificial Lake Shihwa, in February 2006 (Fig. 1). Sediment samples were collected systematically from inner to outer parts of the bay and provided a representative coverage of the bay, on the basis of the National Marine Environmental Monitoring Program in Korea. Sediment samples (0-4 cm depth) were collected using a box core sampler deployed from a research vessel. The collected samples were individually wrapped in aluminum foil and immediately frozen. Marine sediment samples were transported to the laboratory, stored at -20°C, and subsequently freeze-dried.

Bioluminescence test of *V. fischeri*

Bioluminescence testing was conducted on organic solvent extracts of sediments, based on the standard method of MOMAF (2005) with some modifications. A 1-2 g portion of freeze-dried sediment was placed in a 50 mL Teflon centrifuge tube with a Teflon cap. The sample was extracted twice by mechanical shaking for 1 h with 20 mL of 50% dichloromethane (ultra residue analysis; J.T. Baker, USA) in chloroform (ultra residue analysis; J.T. Baker). The extract was centrifuged at 3,000 rpm for 15 min and filtered to minimize the effects of suspended particles. The extracts were combined and concentrated by Turbo Vap LV (Caliper Life Science Inc., USA). Solvent exchanges to the less-toxic dimethyl sulfoxide (ACS reagent, DMSO; Aldrich, USA) were performed. DMSO is a polar solvent that dissolves both polar and non-polar compounds, and is suitable for bioassays due to its low toxicity. Testing was conducted with a luminescent bacteria toxicity measurement apparatus (N-TOX model 200; Neoenbiz Inc., Korea) using the organic solvent solubilization method. Organic extracts, prepared with DMSO, were previously diluted in dilution solution (DW 200; Neoenbiz Inc.) to give a final concentration of 1%, and the basic test protocol was performed with four 1:2 serial dilutions. Determination of toxicity was done after 30 min of contact in all tests. Potassium dichromate and 3,5-dichlorophenol solutions were provided by Dr. Lee in Neoenbiz Inc. for use as reference chemicals, and were run with every fresh vial of bacteria to ensure the validity of all tests.

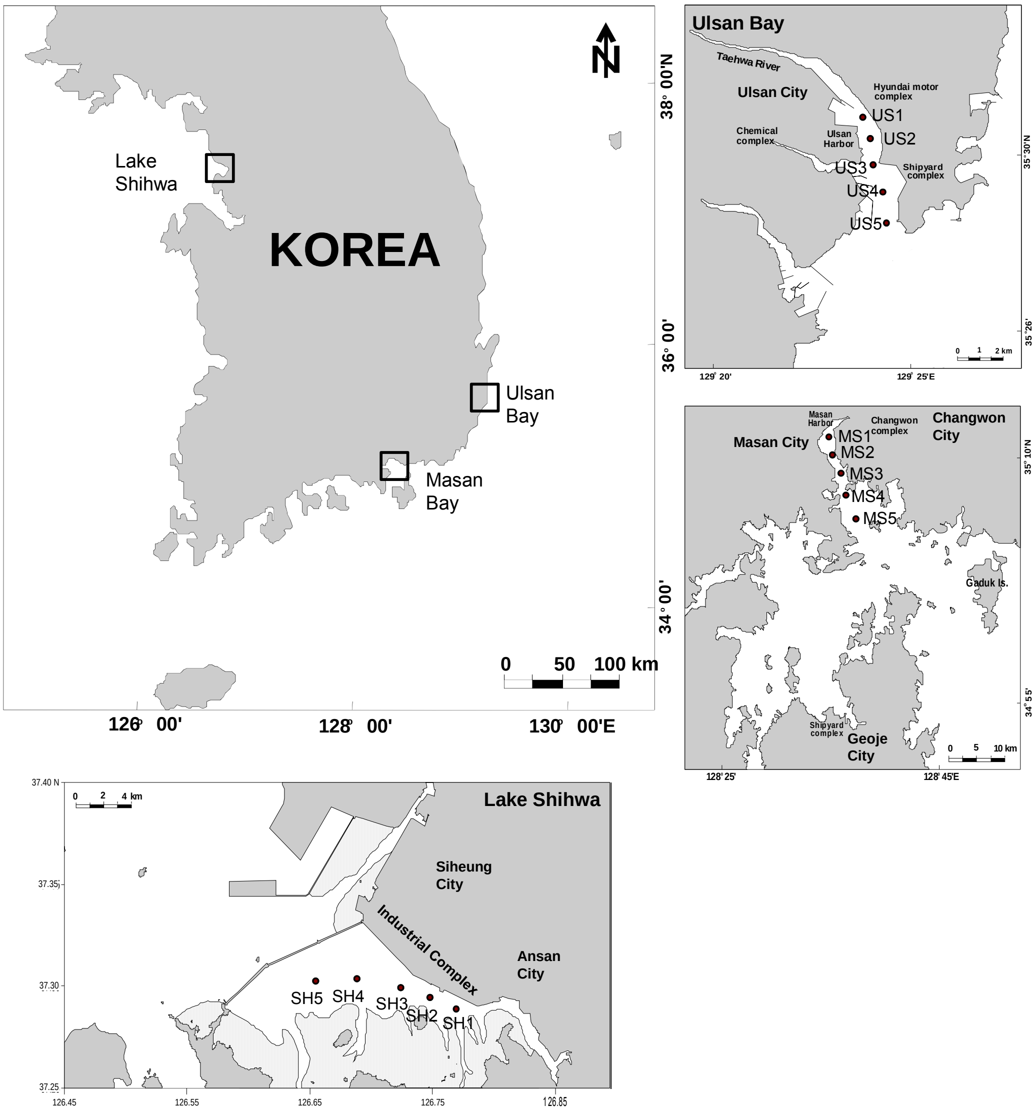

Fig. 1. Sampling locations of marine sediments form Ulsan Bay, Masan Bay, and artificial Lake Shihwa in Korea.

Lypophilised *vibrio fischeri* bacteria and all bioluminescence reagents were obtained from Neoenbiz Inc.

A log-linear model was used to calculate the Effective Concentration 50% (EC50) with 95% confidence limits. The EC50 is the sediment concentration that reduces bioluminescence by 50% relative to water controls. Thus, the EC50 decreases with increasing sediment toxicity. The EC50 is reported as mg dry sediment/mL aqueous extract. The sediment reference index (SRI) was used to compare the degree of sediment toxicity between sites and was calculated by dividing the EC50 of the negative control sample (Washed seasand; Fisher Scientific, USA; EC50=14.127 mg/mL) by the EC50 of the test samples.

Chemical analysis

Sediment samples were chemically analyzed for trace metals (Cu, Pb, Cr, Zn, and Hg), wastewater markers (coprostanol and nonylphenol), total organic carbon (TOC), and acid volatile sulfides (AVS). Analysis of trace metals, TOC, and AVS were performed following methods described elsewhere (Yoon et al., 2009). For trace metals, freeze-dried sediments were extracted using mixed acidic solution (HNO3:HF:HClO4=2:2:1) and were added using 100 mL of 2% HCl after evaporation of the acidic solution to dryness. Concentrations of trace metals were determined by inductively coupled plasma mass spectrometry (Elan 6000, Perkin Elmer, USA), and Hg was determined by a mercury analyzer (AMA-254, Milestone, Italy). For quality assurance and quality control, analysis of certified reference materials (PACS-2, marine sediments; NRC, Canada) was performed and all sample procedures were performed in a clean booth of class 100. TOC contents were determined by a CHN elemental analyzer (model 2400; PerkinElmer), after removal of calcium carbonate with 1 N HCl. AVS contents were determined according to a standard method from MOMAF (2005). The concentrations of coprostanol and nonylphenol were from a previous study (Choi et al., 2009).

Statistical analysis

A Spearman rank correlation analysis was performed to investigate the relationships between sediment toxicity and chemical concentrations by using SPSS software for Windows 10.0 (SPSS 2000). Comparison of sediment toxicity and chemical concentrations between the surveyed areas was performed by an independent sample t-test in the SPSS software. Correspondence analysis was performed to characterize spatial variability of sediment toxicity and chemical concentrations at each sampling location using PRIMER software for Windows (PRIMER Version 5.2.9; Plymouth Marine Laboratories, Plymouth, UK).

Results and Discussion

Sediment toxicity

EC50 values of sediments from Ulsan Bay, Masan Bay, and the artificial Lake Shihwa ranged from 0.014 to 1.126 mg/mL (Table 1). The sediment SRI values ranged from 13 to 1044. The highest SRI was found in sediments from Masan Bay (MS1), and the lowest SRI was found in the sediment from Ulsan Bay (US3). SRI values were 646±338 (mean ± standard deviation) in Masan Bay, 561±267 in Lake Shihwa, and 72±83 in Ulsan Bay (Fig. 2). Average SRI values in Masan Bay and Lake Shihwa were approximately 8 or 9 times as high as in Ulsan Bay, indicating relatively high sediment toxicity in the former two regions.

Results of the toxicity test of each sample were compared with a statistically derived "critical value" to determine whether the sample was "toxic" as reported by previous studies (Bombardier and Bermingham, 1999; Long et al., 2002). Long et al. (2002) used critical values of less than 80% and 90% of the negative control response, EC50 <0.51 mg/mL and <0.06 mg/mL, respectively, to identify toxic samples. Critical values were calculated on the basis of the frequency distribution of Microtox data from nationwide NOAA's surveys and denote the 80% and 90% lower prediction limits (LPL) of the NOAA Microtox database (n=1013). In total, 13 of the 15

Table 1. Toxicity data and chemical analyses of sediments from Masan Bay, Ulsan Bay, and artificial Lake Shihwa, Korea

Station	EC50		SRI[c]	TOC[d] %	AVS[e] mg/g dry wt	Cu	Pb	Cr	Zn	Hg	Coprostanol	Nonylphenol
	mg/mL[a]	μL/mL[b]				μg/g dry wt					ng/g dry wt	
MS1	0.014	0.07	1,044	3.18	4.78	111	116	71.9	350	0.17	1,983	60.2
MS2	0.019	0.09	760	3.71	2.27	73.9	77.2	74.7	281	0.12	446	24.5
MS3	0.018	0.09	787	3.42	1.26	75.4	61.4	76.9	260	0.14	523	21.1
MS4	0.029	0.15	480	3.76	1.83	38.9	22.5	65.9	148	0.12	278	22.2
MS5	0.089	0.44	159	3.06	1.93	63.1	51.0	51.9	223	0.07	107	38.6
US1	0.066	0.33	215	1.77	0.14	39.6	59.4	57.0	167	0.06	1,215	29.7
US2	0.700	3.50	20	1.56	0.01	89.2	78.1	80.9	243	0.04	629	17.2
US3	1.126	5.63	13	1.58	0.01	82.8	74.0	76.4	230	0.13	500	19.8
US4	0.269	1.34	53	1.39	0.14	52.0	56.7	64.2	172	0.07	237	12.6
US5	0.244	1.22	58	1.54	0.10	44.7	63.4	83.4	186	0.06	177	7.52
SH1	0.045	0.23	311	1.71	3.50	328	124	138	470	0.17	222	395
SH2	0.018	0.09	772	1.40	2.61	106	48.7	82.3	202	0.08	91.2	50.7
SH3	0.061	0.30	233	1.68	0.56	110	58.3	92.4	227	0.10	66.9	227
SH4	0.020	0.10	701	2.87	1.21	116	46.6	100	247	0.08	78.9	647
SH5	0.018	0.09	789	1.47	0.24	52.3	37.7	87.8	160	0.05	34.0	183
Benchmark[f]						18.7	30.2	52.3	124	0.18[g]		

The concentration unit is mg sediment per mL aqueous solution; b, concentration unit is uL DMSO per mL aqueous solution; c, Sediment Reference Index; d, Total Organic carbon; e, acidic volatile sulfide; f, the values correspond to US EPA marine sediment screening benchmarks (US EPA, 2009a); g, the values correspond to US EPA freshwater sediment screening benchmarks (US EPA, 2009B).

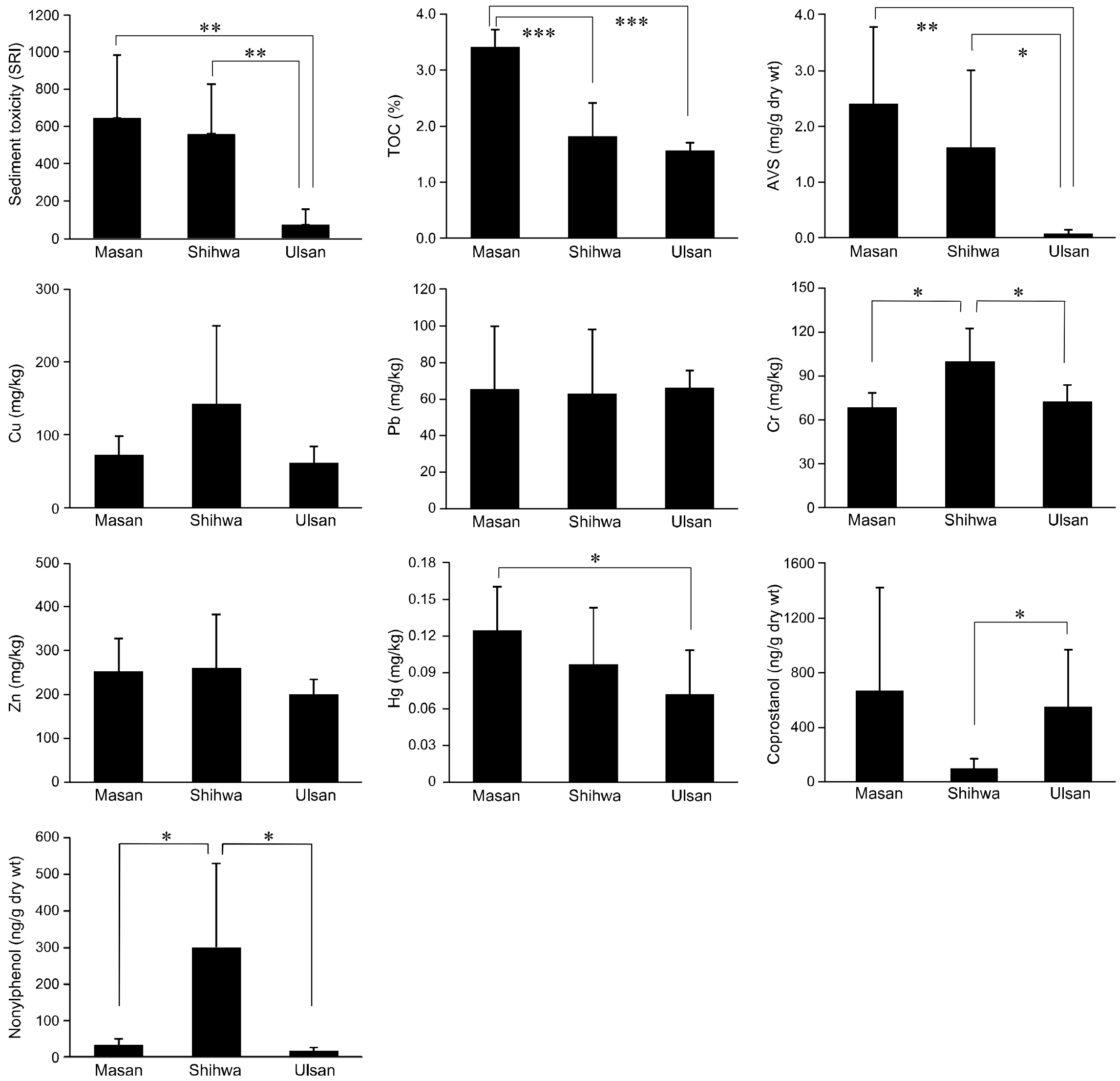

Fig. 2. Distribution of toxicity data, and chemical analyses in sediments from Masan Bay, Ulsan Bay, and Lake Shihwa, Korea. Statistical comparisons were performed using an independent sample t-test (*$P<0.05$; ** $P<0.01$; *** $P<0.001$). Values are mean of 5 samples.

samples tested showed EC50 values less than 0.51 mg/mL (80% LPL), and 8 samples showed responses less than 0.06 mg/mL (90% LPL). The EC50 values for MS1, MS2, MS3, and MS4 ranged from 0.014 to 0.029 mg/mL and for SH1, SH2, SH4 and SH5 ranged from 0.018 to 0.045 mg/mL.

Bombardier and Bermingham (1999) suggested that EC50 dilution levels (expressed as μL DMSO per mL aqueous solution) could be divided into four categories; (i) non-toxic (≫1 μL/mL); (ii) marginally toxic (0.1-0.9 μL/mL); (iii) moderately toxic (0.01-0.09 μL/mL); and (iv) highly toxic (<0.01 μL/mL). In the present study, five samples (MS1, MS2, MS3, SH2, and SH5) were classified as “moderately toxic” and six samples (MS4, MS5, US1, SH1, SH3, and SH4) were classified as “marginally toxic”. The results suggest that high sediment toxicity is widespread in Masan Bay and Lake Shihwa.

The EC50 values of sediments in this study were comparable to or lower than those in the following regions: Lake Geneva, USA (0.008-3.42 mg/mL) (Pardos et al., 1999), Southern Puget Sound, USA (0.31-175.30 mg/mL) (Long et al., 2002), Waukegan Harbor, USA (0.42-14.50 mg/mL) (Kemble et al., 2000), Ebro River, Spain (0.04-12.96 mg/mL) (Ocampo-Duque et al., 2008), Po River, Italy (0.46-36.80 mg/mL) (Viganò et al., 2003), and Irish marine sediments (>1,000 mg/mL) (Macken et al., 2008).

However, our values were much higher than those in Barcelona Harbor, Spain (0.0002-0.0044 mg/mL) (Martínez-Lladó et al., 2007), which is associated with intensive marine and industrial activities. Therefore, sediment toxicity in the present study is categorized in the middle or higher range of toxicities reported worldwide.

Chemical levels

The results of the chemical analysis are summarized in Table 1 and Fig. 2. TOC ranged from 1.39% to 3.76% (mean: 2.27%). The mean TOC content in Masan Bay (3.43±0.31%) was significantly higher than those in Lake Shihwa (1.83±0.60%) and Ulsan Bay (1.57±0.14%). Pearson and Rosenberg (1978) developed a conceptual, graphical model to describe a generalized pattern of response of soft-bottom benthic communities in relation to organic enrichment. A recent study by Hyland et al. (2005) has quantified this relationship to show that a TOC content higher than about 3.5% poses a high risk for impaired benthic assemblages (e.g., reduced species richness) owing to organic loading and other co-varying stressors in sediments. Masan Bay exhibited a TOC content higher than 3.5% (MS2 and MS4).

AVS ranged from 0.01 to 4.78 mg/g dry wt (mean: 1.37 mg/g dry wt). The mean AVS contents in Lake Shihwa (1.62±1.39 mg/g dry wt) and Masan Bay (2.41±1.37 mg/g dry wt) were 20 and 30 times higher than those in Ulsan Bay (0.08±0.07 mg/g dry wt).

Concentrations of trace metals ranged from 148 to 470 mg/kg for Zn, 38.9 to 328 mg/kg for Cu, 51.9 to 138 mg/kg for Cr, 22.5 to 124 mg/kg for Pb, and 0.04 to 0.17 mg/Kg for Hg. Marine sediment benchmarks for trace metals (US EPA, 2009a; 2009b) were used to assess metal contamination in the sediment samples. The concentrations of Zn, Cu, and Cr in all sediment samples were higher than the benchmarks (Table 1), and Pb concentrations in 14 of 15 sediment samples were higher than the benchmarks. This suggests that the surveyed areas were heavily contaminated by trace metals. Lake Shihwa was relatively contaminated by Cr, while Masan Bay showed relatively high levels of Hg (Fig. 2).

Coprostanol, a fecal sterol, has been widely used as a marker of domestic wastewater contamination in aquatic environments because of its occurrence at high concentrations in human feces (Mudge and Lintern, 1999). Nonylphenol has been used as a marker for industrial wastewater contamination given its various industrial applications (Diez et al., 2006; Li et al., 2007). Concentrations of coprostanol in the surveyed areas ranged from 34.0 to 1983 ng/g dry wt and nonylphenol ranged from 7.52 to 647 ng/g dry wt (Choi et al., 2009). Masan and Ulsan Bays were characterized by domestic wastewater contamination, as suggested by coprostanol levels higher than those of nonylphenol. Lake Shihwa was particularly contaminated by industrial effluents (Fig. 2).

Relationships between measures of toxicity and chemical concentrations

Table 2 shows Spearman rank correlation coefficients for sediment toxicity and chemical levels. The sediment toxicity (EC50) was negatively correlated with most chemical components, suggesting that the environmental disturbance by chemicals results in changes in sediment toxicity. The significant negative relationships of sediment toxicity with AVS and nonylphenol (r=-0.713 and -0.542) indicates that the sediment toxicity may be associated with contamination from AVS and nonylphenol. The poor correlation between the sediment toxicity results and trace metals may be attributable to the low solubility of trace metals in organic solvents (Salizzato and Pavoni, 1998).

The biological implications of sulfide in sediment are poorly understood and too often ignored despite the fact that sulfide can be extremely important in determining sediment toxicity to resident biota (Como et al., 2007). Sulfide is the end-product of sulfate reduction which represents one of the main pathways of organic matter decomposition in eutrophic coastal environments. Sulfide is reported to influence sediment toxicity in three ways; (i) by acting as a toxicant in its own right; (ii) by reducing metal toxicity by forming insoluble metal sulfide solids and/or metal sulfide complexes; and (iii) by affecting animal behavior through oxygen depletion (Wang and Champan, 1999). Como et al. (2007) reported that strong relationships between organic over-enrichment of sediments, reduced sulfur pools and poorly structured macrofaunal assemblages characterized highly degraded conditions of an

Table 2. Spearman rank correlation coefficients of sediment toxicity (EC50) with chemical concentrations

	TOC	AVS	Cu	Pb	Cr	Zn	Hg	Coprostanol	Nonylphenol
r	-0.379	-0.713	-0.241	0.163	-0.189	-0.225	-0.370	0.095	-0.542
p-value	0.164	0.003	0.386	0.562	0.499	0.420	0.175	0.737	0.037

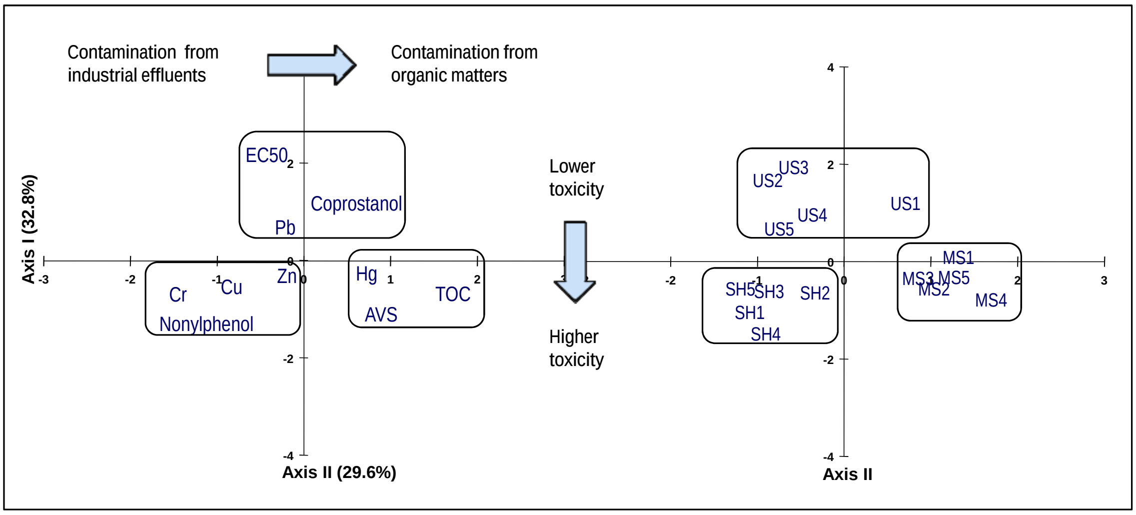

Fig. 3. Correspondence analysis plot for loadings and scores according to the results of sediment toxicity, and chemical analyses in each station from Ulsan Bay, Masan Bay, and artificial Lake Shihwa in Korea.

ecosystem. In the present study, the high toxicity of the Masan Bay sediments can be partly explained by reductive conditions, produced by a poor exchange of seawater as well as high loading of organic matter.

Nonylphenol is the most persistent breakdown product of nonylphenol ethoxylates, which are broadly used in industrial cleaning processes. The main source of nonylphenol in oceans and sediments appears to be closely related with the discharge of effluents from wastewater treatment plants, and the proximity of industrialized/urban areas and other related anthropogenic activities (Soares et al., 2008). Significant contamination of sediments by nonylphenol implies accumulation of industrial wastewater pollutants on surface sediments (Moon et al., 2008; 2009). Moreover, many researchers have reported toxicity of nonylphenol focused on benthic invertebrates (Bettinetti and Provini, 2002; Zulkosky et al., 2002; Mäenpää and Kukkonen, 2006). Nonylphenol from sediments is transferred to benthic organisms inhabiting the sediment (Mayer et al., 2007), and elevated concentrations of nonylphenol in invertebrate tissues may be an important factor contributing to intersex observed in White perch collected from Cootes Paradise in Canada (Kavanagh et al., 2004). Zulkosky et al. (2002) reported that chronic tests of the benthic crustacean, *Leptocheirus plumulosus*, showed a 50% reduction in the average number of young (juveniles + embryos) produced per surviving female after exposures to wastewater-impacted sediment. In the present study, the high sediment toxicity of Lake Shihwa may be attributable to high industrial wastewater contamination. Indeed, Lake Shihwa was previously identified as an area strongly contaminated by nonylphenol and estrogenic activity (Li et al., 2004; Koh et al., 2006; Choi et al., 2009).

To further characterize the relationships between sediment toxicity and chemical concentrations as well as their spatial variability, correspondence analysis was performed (Fig. 3). This multivariate statistical analysis was able to help identify possible relationships among variables. The two axes accounted for 62.4% of the variance (32.8% for Axis I and 29.6% for Axis II). Axis I represents contamination by organic matter (positive) and industrial effluents (negative). Axis II represents toxicity responses showing low (positive) and high toxicity (negative). The variables were classified to three groups. Group 1 included nonylphenol, Cu, Cr, and Zn. Group 2 included AVS, TOC, and Hg. Group 3 included toxicity of sediment, Pb, and coprostanol. This analysis suggests that in the present study, sediment toxicity increases with increasing chemical concentrations with the exception of Pb and coprostanol.

The correspondence analysis also provides the clearest description of multiple compounds among sites (Fig. 3). The sampling station scores were distributed among the three groups. Ulsan Bay showed lower chemical concentrations and toxicity (Group 3), whereas Masan Bay and Lake Shihwa showed higher chemical concentrations and toxicity. Masan Bay was strongly affected by reductive organic contamination (Group 2), and Lake Shihwa was most strongly influenced by industrial wastewater contamination (Group 1). The chemistry and toxicity results suggest that sediments may induce high toxicity, likely due to anthropogenic chemicals and factors associated with a reducing environment.

Further tests such as toxicity identification evaluations are necessary to provide additional information about the chemical concentrations contributing to the observed toxicity of the sediment tested. In this study, the bioluminescence bioassay applied to sediments showed strong concordance with traditional chemical measures of toxicity. Thus, the bioluminescence bioassay test may be useful for determining potential chemical contamination in sediments.

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