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15TH INTERNATIONAL SYMPOSIUM ON TOXICITY ASSESSMENT

Evaluation of the potential impact of polluted sediments using Manila clam *Ruditapes philippinarum*: bioaccumulation and biomarker responses

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Abstract An assessment was made to monitor the short-term impact of heavily polluted sediments that may move out from the brackish man-made Lake Shihwa outside of the sea dike due to operations of a tidal power plant. Here, we exposed the Manila clam *Ruditapes philippinarum* collected from the western coast of Korea to natural sediment under lab condition for 96 h. Sediments were collected from Lake Shihwa and outside of the sea dike representing polluted and reference conditions, respectively. The results of chemical analysis revealed that the concentrations of nonylphenol and heavy metals in water and sediment from the inner region of Lake Shihwa were significantly higher than those of reference sediments. After 48 and 96 h of exposure, 30 specimens of clams were sampled from each experimental condition, and concentrations of nonylphenol and metals were measured in clams, water, and sediments. Several biomarkers, including concentrations of metallothionein-like proteins, and activities of the antioxidant enzymes glutathione S-transferase and catalase were determined in clams to characterize the effects of polluted sediments to clams. After 96 h of exposure, *R. philippinarum* assimilated nonylphenol up to 71 times compared to initial concentrations. However, there was no apparent uptake of heavy metals into the clams. Additionally, antioxidant enzymes exhibited higher activities in clams exposed to the polluted sediment. The results of the present study with physiological responses in *R. philippinarum* suggest that sediment transportation caused by the operation of a tidal power plant in Lake Shihwa will have striking effects on benthic organisms in the adjacent coastal area.

Keywords Clam *Ruditapes philippinarum* · Lake Shihwa · Nonylphenol · Metal · Metallothionein-like proteins · Glutathione S-transferase · Catalase

Introduction

Lake Shihwa, which was artificially constructed in 1994 to supply water for industrial usage, is considered as one of the most polluted regions in Korea due to an unsuccessful reclamation project caused by inadequate planning and management (KORDI 1999; MOMAF 2006). The environment of Lake Shihwa has deteriorated due to restriction of water exchange and continuous anthropogenic inputs through several streams adjacent to two large industrial complexes and cities (Choi et al. 1999; Choi et al. 2011a; Kim et al. 2003). A number of researchers have reported very high concentrations of metals and nonylphenol in sediments in the vicinity of industrial complexes (Choi et al. 2011a; Hong et al. 2010; Kim et al. 2003, 2009; Li et al. 2004; Ra et al. 2011a). Historical trends of metal and nonylphenol concentrations in sediment cores revealed that these pollutants were significantly increasing over the past 30 years due to anthropogenic activities after the construction and operation of two large national industrial complexes (Hong et al. 2010; Ra et al. 2011a). Ra et al. (2011b) also reported that nonpoint source pollution loads derived from creeks and sewer outlets during storm events

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were some of the main causes of pollution in Lake Shihwa. Given continuous pollution loads from many different sources, large amounts of pollutants were introduced to the water column and sediments of the entire Lake Shihwa.

One current and important issue associated with the study area is the construction of the world's largest tidal power plant in the center of the sea dike. One of the main purposes of the tidal power plant is to improve the various pollution issues in Lake Shihwa by increasing seawater exchange between the Lake Shihwa and the sea outside the sea dike. The operation of the tidal power plant, however, could result in the transportation of heavily polluted sediments to the outside sea. In fact, suspended particulate matter in bottom water and the sedimentary facies around the tidal power plant were slightly increased and changed, respectively, after the test operation of the tidal power plant in July 2011 (MOMAF 2011). The outflow of polluted sediments could provide an additional source of nonylphenol and metal pollution to the coastal area. This would threaten marine organisms that live in this area (Coughlan et al. 2002; Hartl et al. 2006). Several studies reported that resuspension and redistribution of polluted sediments by dredging resulted in the remobilization and release of pollutants into the aquatic phase making them more bioavailable (Bocchetti et al. 2008; Eggleton and Thomas 2004; van den Berg et al. 2001). Thus, there is an urgent need to estimate the potential biological risks of remobilization of sediment-bound pollutants associated with the operation of the tidal power plant and their accumulation into benthic organisms.

The exposure to and effects of pollutants in sediments is generally evaluated using sentinel organisms as bioindicator species. In this context, bivalves have been widely used as indicator organisms because they have limited movement in their habitat and are exposed to pollutants from sediment as well as from the water column due to their feeding habits (Choi et al. 2011b; Coughlan et al. 2002; Hartl et al. 2006; Rainbow 2006). The Manila clam, *Ruditapes philippinarum*, is widely distributed in intertidal areas along the west and south coast of Korea and is commercially important as marine food resources. Furthermore, Manila clam *R. philippinarum* exhibits dose–response and measurable changes of biomarkers reflecting pollution stress and shows high uptake rates of pollutants such as metals, pesticides, and nonylphenol (Choi et al. 2011b; Irato et al. 2003; Lietti et al. 2007; Marin and Matozzo 2008; Sarkar et al. 2008). For this reason, they are considered as suitable indicator species for the assessment of environmental pollution in the coastal environment of Korea over both short- and long-term periods of time.

Biomarkers have become valuable ecotoxicological tools to assess the toxicity and bioavailability of various pollutants to marine organisms in their natural environment (Depledge et al. 1995). Especially, a few previous studies with *R. philippinarum* had already confirmed that their physicochemical responses show potential as biomarkers for environmental assessment. For example, metallothionein-like proteins (MTLPs) and activities of glutathione S-transferase (GST) and catalase (CAT) in *R. philippinarum* were shown as useful biomarkers for evaluating pollution by metal and oxidative stress in this species (De Luca-Abbott et al. 2005; Irato et al. 2003).

The aim of this study was to characterize the bioaccumulation of nonylphenol, metals, and several biomarker responses (MTLPs, GST, CAT) in *R. philippinarum.* This was done in a lab-based experiment in order to conduct a preliminary assessment of the potential environmental impact associated with the resuspension of sediments due to tidal power plant operations in Lake Shihwa. In a previous study, *R. philippinarum* exposed to several organophosphorous pesticides showed significant changes in biomarker responses within 48 h (Choi et al. 2011b). In this study, *R. philippinarum* were exposed to sediments from polluted and reference sites for 96 h under controlled laboratory conditions, and bioaccumulation of nonylphenol and metals in clam tissues was measured and correlated with biomarker responses in the same clams. Specifically, MTLP levels and activities of antioxidant enzymes such as GST and CAT in clam tissues were analyzed to characterize the ecotoxicological responses against the pollutants such as nonylphenol and metals in polluted sediments in short-term exposures.

Materials and methods

Sampling and experimental procedure

Surface sediments (upper 4 cm) were collected using van Veen grab sampler in August 2008 from Lake Shihwa and outside of the sea dike (Fig. 1**)** and were directly moved to the laboratory to conduct the exposure experiments. For the clam exposure experiments, sediment samples were used from S4 and Y3 sites which represent polluted (S) and reference (Y) conditions, respectively (Fig. 1). Sediment samples were freeze-dried and ground in an automatic agate mortar (Fritsch Corp., Pulverisette 6, Idar-Oberstein, Germany) for analyzing nonylphenol and metals.

R. philippinarum of similar size were collected from the outside of Lake Shihwa. Average shell length and total weight were 33.3±1.0 mm and 0.38±0.08 g, respectively (n=150). Before exposure to sediments, clams were acclimatized for 4 days with filtered seawater (32 psu). Seawater used in the experiments was sampled from the sea outside of Lake Shihwa.

Randomly selected acclimated clams (n=30) were used for analysis of initial nonylphenol (n=20) and trace metal

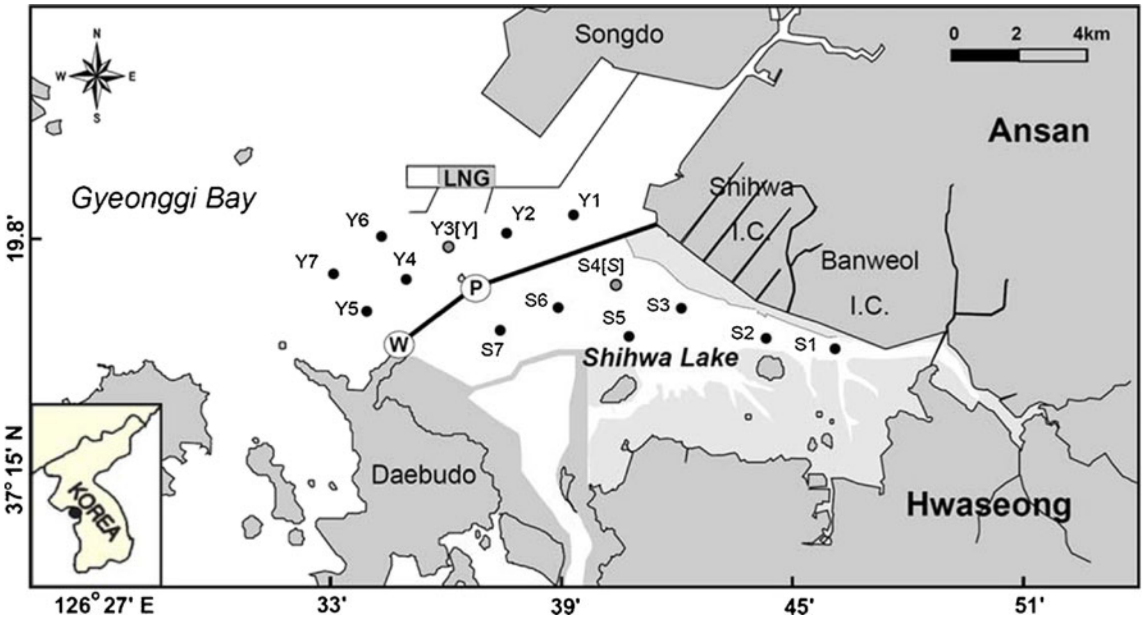

Fig. 1 Sediment sampling locations in the inner and outer regions of Lake Shihwa (*W* and *P* represent the water gate and tidal power plant, respectively)

(n=3) concentration as well as biomarkers (n=7). Approximately 500 g of sediment from sites S4 (S) and Y3 (Y) and 10 L of filtered seawater were thoroughly mixed in each tank to homogenously redistribute polluted sediments and pore water. Then, 120 individual clams were placed in four tanks (30 specimens per tank) and incubated for 48 and 96 h at room temperature with continuous supply of air. For analysis of nonylphenol, metals, and biomarkers, 30 specimens were sampled at 48 and 96 h for each sediment set. Seawater and sediments were also sampled simultaneously and analyzed for metal and nonylphenol concentrations.

Nonylphenol analysis

Determination of nonylphenol concentration in seawater, sediments, and clams was performed following the method by Li et al. (2001). Briefly, approximately 1 L of filtered seawater was subjected to liquid–liquid extraction with methylene chloride (J.T. Baker, USA). For sediments, approximately 5 g of ground and homogenized material was extracted with Milli-Q, acetone, and hexane on a mechanical shaker. For clam analysis, 20 specimens were pooled and freeze-dried, and then, 2 g of homogenized samples was extracted using methylene chloride via a Soxhlet extraction. Quantitative analysis for nonylphenol was performed by GC-MS after derivatization with *N*,*O*-bis-(trimethylsilyl)trifluroacetamide (with 1 % TMCS, Sigma-Aldrich, St. Louis, MO, USA) and cleanup with 1 g of activated Florisil (60–100 mesh, Sigma-Aldrich) by use of a silylation treatment kit according to the method by Li et al. (2001). Fat was removed from clam samples by means of a Florisil column prior to silylation. A gas chromatograph (Shimadzu GC-2010, Tokyo, Japan) coupled with a mass spectrometer (Shimadzu GCMS QP2010 plus) was used for nonylphenol analysis. A surrogate standard (bisphenol-A-d16) was added to all samples for quality control with recovery rates ranging from 81 to 97 %. The method detection limit was 2 ng/g, and the analytical precision was within 5 % for all measurements.

Metal analysis

Dissolved metal concentrations were determined using the APDC-DDTC-Freon extraction method (Statham 1985). Prior to analysis, seawater was filtered with acid-cleaned PC filters (0.4-μm pore size) and acidified. For metal analysis in sediments, approximately 1 g of sediment was freeze-dried and ground. Then, about 0.1 g of sediment was completely digested with suprapure grade HF, HNO_3, and $HClO_4$ (Merck, Germany, Darmstadt) at 180 °C for 24 h (Windom et al. 1989). After digestion, samples were dried and redissolved with 2 % HNO_3. Approximately, 0.1 g of homogenized clam sample was digested with 5 mL of concentrated HNO_3 for 24 h at 180 °C. Metal concentrations were measured using an inductively coupled plasma mass spectrometer (Thermo Elemental X7). All samples were measured in triplicate. For quality control, reference materials such as CASS-4 for near-shore seawater, PACS-2 for marine sediments from the National Research Council, Canada, and SRM 2976 for mussel tissue from the National Institute of Standard and Technology, USA were used (Supplementary Table 1).

MTLPs and antioxidant enzyme activity

MTLP content in *R. philippinarum* was measured using the spectrophotometric method described by Viarengo et al. (1997). The dissected digestive glands from three clams were homogenized individually in four volumes of 0.5 M sucrose and 20 mM Tris–HCl (pH 8.6) with 6 μM leupeptin (Sigma) and 0.5 mM phenylmethylsulfonyl fluoride (PMSF, Sigma) as antiproteolytic agents and 0.01 % β-mercaptoethanol (Sigma) as a reducing agent. The homogenates were then centrifuged at 30,000×*g* for 20 min. The supernatant was treated with ethanol/chloroform. For each sample, 1.05 mL cold ethanol and 80 μL chloroform were added to 1-mL aliquots of supernatant. The samples were centrifuged at 6,000×*g* for 10 min at 4 °C. The collected supernatants were combined with calf liver RNA (Sigma) and 37 % HCl with cold ethanol. The samples were kept at −20 °C for 1 h and centrifuged at 6,000×*g* for 10 min. The MTLP-containing pellet was washed with 87 % ethanol and 1 % chloroform in a homogenizing buffer in order to remove soluble thiols. Then, the pellet was centrifuged at 6,000×*g* for 10 min and dried under a gentle stream of nitrogen gas. 5,5-dithiobis-2-nitrobenzoic acid, pH 8, was added to the sample at room temperature, and the absorbance was measured at 412 nm using a spectrophotometer (Agilent, Varian, Cary 50, NC, USA). Glutathione was used as a standard.

To evaluate protein levels and enzyme activities, digestive glands from four clams were homogenized individually with 2 mM Tris–HCl buffer with a pH of 8, 20 % glycerol, 2 mM β-mercaptoethanol as a reducing agent, leupeptin, and PMSF as an antiproteolytic. The homogenized samples were centrifuged at 18,000×*g* for 90 min to eliminate cellular debris. The supernatant was used to evaluate enzyme activity. GST activity was assayed as the increase in absorbance at 340 nm due to the conjugation of glutathione to 1-chloro-2,4-dinitrobenzene (CDNB, Aldrich) according to Regoli et al. (1997). The increase in absorbance caused by consumed moles of CDNB per minute at 340 nm was measured for 4 min. CAT activity of *R. philippinarum* was determined as the absorbance decreases at 240 nm due to hydroperoxide consumption, as described by Mosleh et al. (2005). CAT activity was expressed as moles of H_2O_2 consumed per milligram of protein per minute. Total protein levels were determined using the Bradford method (Bradford 1976).

Data analysis

In order to assess the pollution status for each metal in study area, the enrichment factor (EF, Simex and Helz 1981) for the origins of metal, geoaccumulation index (I_{geo}, Müller 1979) for the degree of metal contamination or pollution, and pollution load index (PLI, Tomilson et al. 1980) as a summative indication of the level of heavy metal were determined (Table 1 and Supplementary materials).

The metal concentrations of the deeper layers in the core sediment from Lake Shihwa corresponding to the early 1900s prior to industrialization of this area were used as background concentrations for calculating indices (Ra et al 2011a). Results were expressed as mean (± standard deviation, 1σ). Data were analyzed using *t* tests and one-way ANOVA, followed by Tukey's honestly significant difference test. Comparison between two groups was performed using the Welch–Aspin test. Pearson's correlation was used to analyze the relationship between pollutants and biomarkers. A *p* value of less than 0.05 was considered as statistically significant. All statistical analyses were performed using SPSS software (version 11.5, SPSS Inc., Chicago, USA).

Table 1 Indices for heavy metal pollution in sediment

Index	Equation	Statements	Reference
EF	$\mathrm{EF} = \frac{[\mathrm{Me}]/[\mathrm{Al}]_{\text{sample}}}{[\mathrm{Me}]/[\mathrm{Al}]_{\text{background}}}$	>1.5: anthropogenic origin <1.5: lithogenic origin	Simex and Helz (1981)
I_{geo}	$I\text{geo} = log_2\left(\frac{C_{\text{sample}}}{1.5} \times C_{\text{background}}\right)$	<0: unpolluted $0 \leq I_{\text{geo}} < 1$: unpolluted to moderately polluted $1 \leq I_{\text{geo}} < 2$: moderately polluted $2 \leq I_{\text{geo}} < 3$:moderately to strongly polluted $3 \leq I_{\text{geo}} < 4$: strongly polluted $4 \leq I_{\text{geo}} < 5$: strongly to very strongly polluted $I_{\text{geo}} \geq 5$: very strongly polluted	Muller (1979)
PLI	$\mathrm{PLI} = \sqrt[n]{CF_1 \times CF_2 \times \ldots \times CF_n}$	$\mathrm{CF} = \frac{C_{\text{metal}}}{C_{\text{back grounvalue}}}$	Tomilson et al. (1980)

Results

Metals and nonylphenol concentrations in Lake Shihwa and reference sediments

There were significant differences in concentrations of metals and nonylphenol among sediments (Fig. 2). Metal and nonylphenol concentrations except for Co and Al from site S were significantly higher than those from site Y, the sea outside the sea dike (Welch–Aspin test, $p<0.05$). Furthermore, higher metal concentration (Ni, Cu, Zn, and Pb) exceeding sediment quality guidelines such as the effect range—low (ERL) and/or effect range—median (ERM) (Long et al. 1995) were observed adjacent to the industrial complex.

Nonylphenol and trace metal concentrations in seawater and sediments used for exposure test

Metals in sediment from site S were two to six times higher than those in sediments from site Y (*t* test, $p<0.01$) (Table 2). In the sediment sample from Lake Shihwa, Cu, Zn, and Ni exceeded the ERL (Long et al. 1995). Nonylphenol concentrations in sediments collected at the site S were 40 times higher than at site Y (*t* test; $p<0.01$). After 96 h of exposure with seawater and clams, nonylphenol concentrations were reduced in both sediment sets about 26 and 72 % of the initial concentrations of sites S and Y, respectively, while metal concentrations did not change (Table 2, $p>0.05$). In contrast, nonylphenol concentrations in the aqueous phase increased with exposure time (Fig. 3).

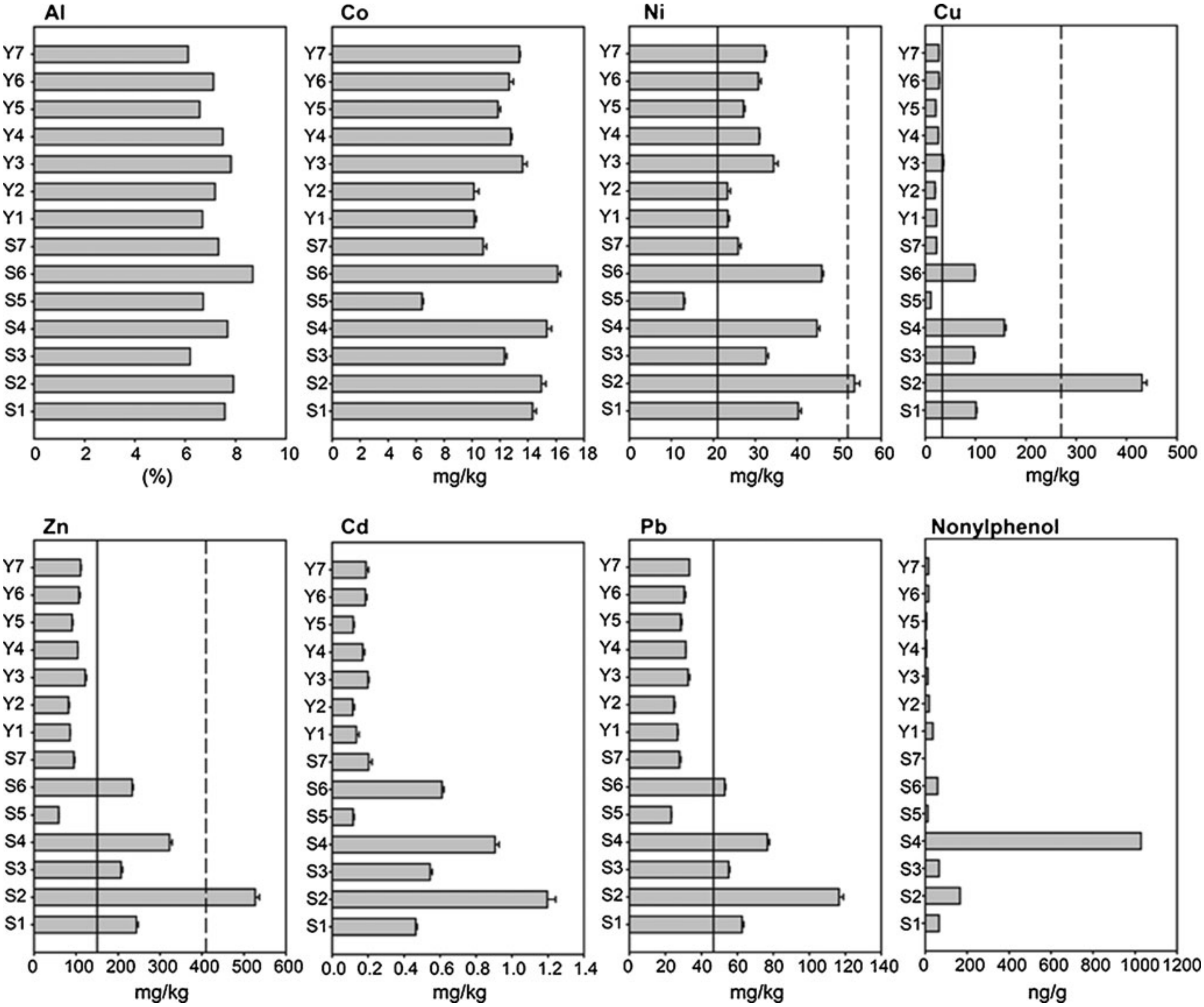

Fig. 2 Concentrations of metals and nonylphenol in the surface sediment of Lake Shihwa in this study. *Vertical lines* represent the effect range—low (ERL, *solid*; a 10 % incidence in toxicity) and the effect range—median (ERM, *dash*; a 50 % incidence of toxicity). Nonylphenol data are cited from Hong et al. (2010)

Table 2 Concentrations of trace metals (in micrograms per gram dw) and NP (in nanograms per gram dw) in the sediment for exposure experiment in the laboratory

		Cu	Zn	Ni	Cd	Co	Pb	NP
Polluted site, S	Initial	92.4 (±2.4)	182.8 (±5.2)	35.3 (±0.9)	0.5 (±0.01)	12.1 (±0.4)	40.7 (±1.2)	317.0
	96 h	99.5 (±3.0)	198.9 (±5.4)	37.1 (±1.1)	0.5 (±0.01)	12.6 (±0.3)	42.8 (±1.1)	233.5
Control site, Y	Initial	15.3 (±0.3)	64.8 (±1.3)	18.1 (±0.4)	0.09 (±0.01)	8.4 (±0.2)	24.8 (± 0.5)	8.0
	96 h	14.7 (±0.7)	62.0 (±2.5)	17.3 (±0.8)	0.10 (±0.01)	7.6 (±0.3)	25.6 (± 0.8)	2.3

Metal concentrations also showed an increasing trend with exposure time, but the extent of increase for nonylphenol was much greater than for metals.

Bioaccumulation of nonylphenol and metals in clam

During the 96 h of exposure, there was no observation of mortality of *R. philippinarum* regardless of treatment. Initial concentrations of nonylphenol in clams were 29.2 ng/g, and this concentration was significantly increased up to 949 ng/g after 96 h exposure to sediments from site S (Fig. 4, Welch–Aspin test, $p<0.05$). For metals, a negligible increase of metals in clams was observed for all elements (Fig. 4, $p>0.05$). Furthermore, there was no marked difference of metal accumulation in clams exposed to Y (reference) and S (polluted) sediments (Welch–Aspin test, $p>0.05$).

MTLP levels and antioxidant enzyme activities in clams

Concentrations of MTLPs in clams exposed to site S sediments exhibit significant changes after 96 h exposure (Fig. 5a). MTLP levels in sediment Y also increased with exposure time, but there was no significant difference ($p>0.05$) due to the large standard deviation. Furthermore, significant differences in GST and CAT activities were observed between clams exposed to reference and polluted sediments at 48 and 96 h, and there was an increasing trend for both GST and CAT activities with increasing exposure time at site S. In contrast, the levels of antioxidant enzymes in clams exposed to reference sediments did not show any statistical differences as a function of exposure time (Fig. 5b, c).

Discussions

Ecotoxicological assessment of nonylphenol and metal pollution in sediments of Lake Shihwa

In this study, the highest metal concentration was observed at site S2 whereas nonylphenol showed the highest concentration at S4 indicating that the pollution source for both pollutants was mainly from industrial effluents through regional creeks. Specifically, average EF value of metal in surface sediments indicated that all metals in Lake Shihwa originated from anthropogenic activities because EF values were greater than 1.5 (Zhang and Liu 2002, Table 3). The relative contribution of anthropogenic metals based on EF

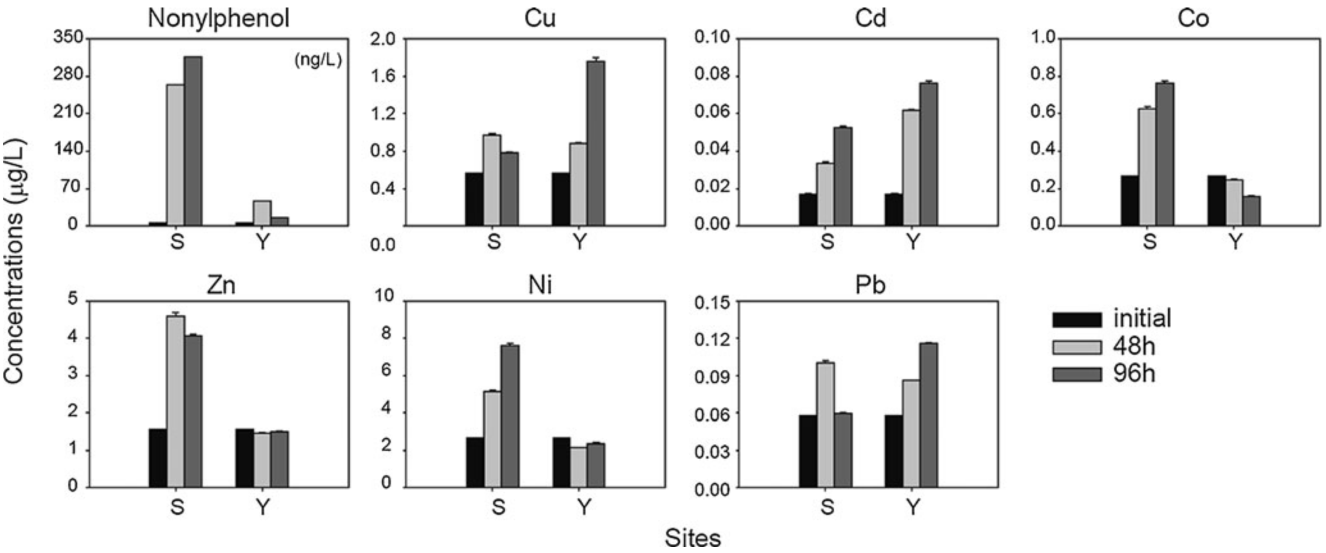

Fig. 3 The modulations of concentrations of NP (in nanograms per liter), Cu, Cd, Co, Zn, Ni, and Pb (in micrograms per liter) in the aqueous phase for both polluted sediment (S) and relatively unpolluted (control) sediment (Y) during exposure experiment

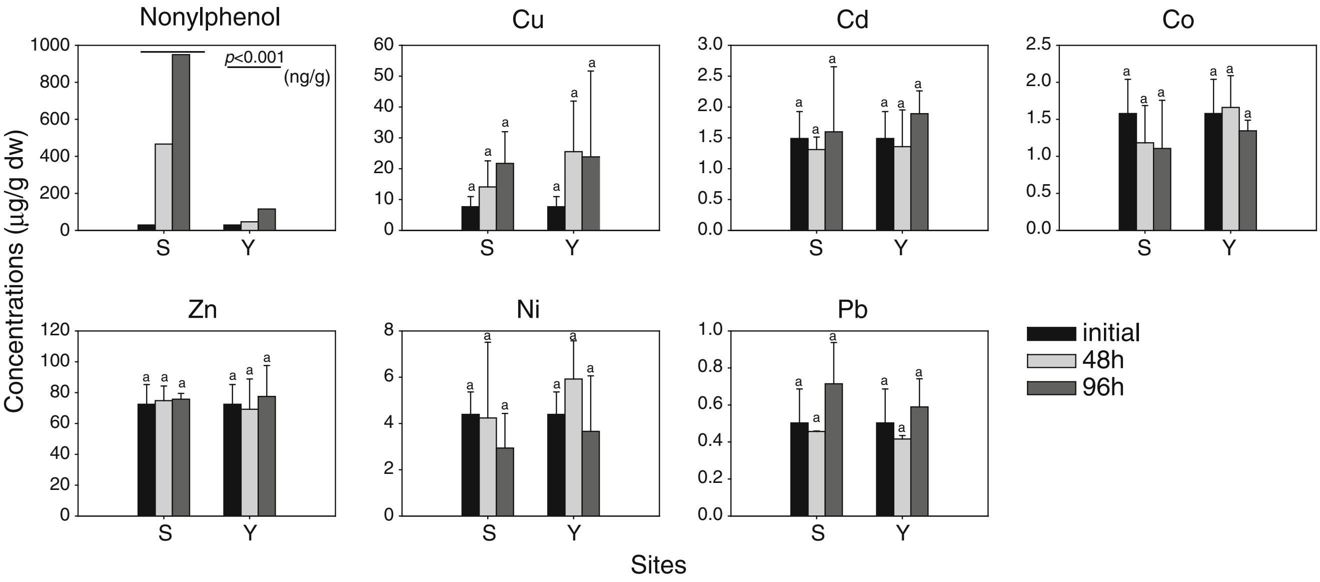

Fig. 4 The accumulated concentrations of NP (in nanograms per gram dw), Cu, Cd, Co, Zn, Ni, and Pb (in micrograms per gram dw) in the clam *R. philippinarum* for both polluted sediment (S) and relatively unpolluted (control) sediment (Y) during the exposure experiment (significant differences in NP between two groups analyzed by Welch–Aspin test)

value is presented in the following order: Cu>Cd>Zn>Pb>Ni>Co in Lake Shihwa and Cu>Zn>Ni>Cd>Co>Pb in the area outside of the sea dike. According to the seven contamination classes of I_{geo}, sites adjacent to the industrial complex (S1–S4) had elevated values for Cu, Zn, and Cd, indicating that sediments were "moderately to strongly polluted" by Cu and "moderately polluted" by Zn and Cd (Table 1 and Supplementary material). The average PLI values for Cu, Zn, Cd, Pb, Ni, and Co indicated that Lake Shihwa is highly polluted with Cu, Zn, and Cd, and the spatial distribution of PLI values which showed a decreasing trend with increasing distance from the pollution sources further supports the anthropogenic origin of these elements. The operation of the tidal power plant led to an increase in the rate of water exchange between Lake Shihwa and the outer sea. This event may decrease surface sediments in Lake Shihwa by flushing sediment out to the outer sea. It may, however, also result in an increase of metal pollution (i.e., Cu, Zn, Cd, and Pb) in the outer sea of Lake Shihwa, which is used as an aquaculture farm for harvesting seafoods such as bivalves, fish, etc.

It was reported that metal and PCB concentrations increased in the mussel *Mytilus galloprovincialis* after the dredging disturbance of sediments (Bocchetti et al. 2008). Sarkar et al. (2008) reported that bivalves generally accumulate metals in their tissues through gill and mantle and exhibited higher metal accumulation due to ion exchange with the water column. In this study, however, there is no statistically significant accumulation of metals in the clam bodies (Fig. 4). The negligible accumulation for metals in Manila clams may be due to the circumstance that the concentration of metal diffused into the interstitial aqueous phase from the sediment seems much lower than that which had existed in clams. Baudrimont et al. (2005) reported that transplanted *R. philippinarum* did not show any significant bioaccumulation of Cd until 4 months after exposure to a polluted environment. However, a different study reported that total metal concentrations in sediments do not tell anything about their bioavailability, since metals can exist in various species in sediments (Berthet et al. 2003). In this study, we analyzed the total metal in sediment by adding HF, indicating that even silicate minerals in sediment can be dissolved by HF. This resulted in high concentrations of metals after that approach, compared to the much less bioavailable fraction in native sediments. Earlier, we demonstrated that sediment-associated metals were not accumulated in other benthic invertebrates such as the deposit-feeding polychaete *Perinereis nuntia* during a short-term exposure (Ra et al. 2010).

The concentrations of nonylphenol in sediments of Lake Shihwa (Table 2) were less than that suggested by the Canadian sediment quality guidelines (1 µg/g, normalized to 1 % TOC, CCME 2001). Ji et al. (2007), however, reported that the biomarker of exposure to estrogenic contaminants such as vitellogenin in *Oryzias latipes* was significantly increased in a chronic exposure test (21 days) of effluent water flowing into Lake Shihwa. Nonylphenol in sediment is released into the aqueous phase by microbial degradation and can be accumulated in marine organisms (Chang et al. 2004). *R. philippinarum*, as a filter feeder living in a benthic environment, can assimilate various pollutants in their tissue through gut and gills (Lietti et al.

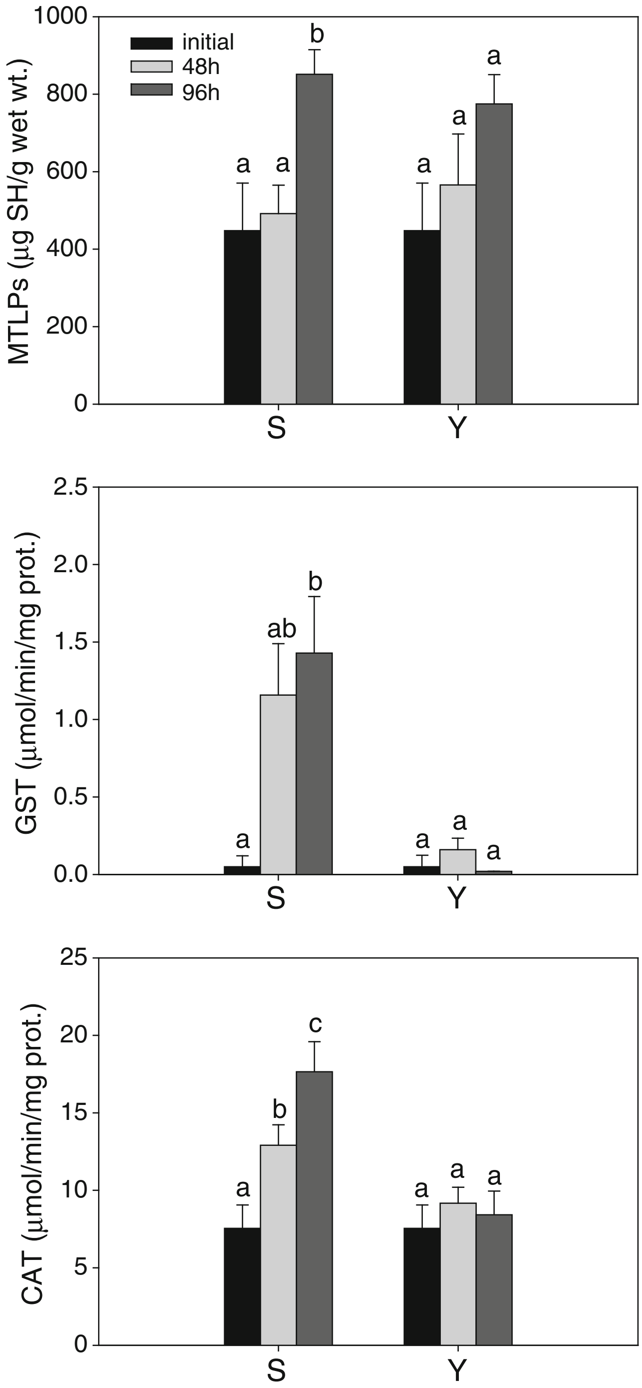

Fig. 5 MTLP levels (in micrograms SH per gram wet wt.), activity of GST (in micromoles per minute per milligram protein) and activity of CAT (in micromoles per minute per milligram protein) in clams *R. philippinarum* exposed to different groups of sediment (Tukey's post hoc analysis $p<0.05$)

2007). In this study, it could be demonstrated that Manila clams had a high accumulation of nonylphenol after exposure to polluted sediment (S). We hypothesize that the distinctive accumulation patterns observed for metal and nonylphenol are due to the differentiation of relative concentrations of bioavailable forms in water and sediment phases and homeostatic balance capacities between uptake and depuration in organisms.

The induction of MT as well as MTLPs in aquatic organisms has been recognized as a potential biomarker of heavy metal exposure (Mosleh et al. 2005; Viarengo et al. 1997). It has been demonstrated that the clam *R. philippinarum* living in a polluted environment had elevated concentrations of metal-binding protein in their digestive glands as a consequence of a significant accumulation of heavy metals (Irato et al. 2003). In this study, MTLP levels in *R. philippinarum* were significantly changed in clams exposed to polluted sediment after 96 h. This suggests that metals in sediment could be accumulated by clams. Interestingly, MTLP levels in clams exposed to reference sediments increased in a similar manner after 96 h. Monserrat et al. (2003) reported that proteins like MT and/or MTLPs are not only involved in the sequestration of metals but could also act as reactive oxygen species scavengers. Thus, increased MTLP levels in *R. philippinarum* in this study could indicate that antioxidant protective roles of MTLPs are involved in mitigating oxidative stress caused by other organic pollutants. In a previous study with the polychaete *P. nuntia*, it could be demonstrated that molecular biomarkers including MT were increased after exposure to sediments that did not have significant metal contamination, suggesting that these end points can be induced by compounds in sediments other than metals (Won et al. 2012).

De Luca-Abbott et al. (2005) reported that the activities of GST and CAT in the digestive glands of *R. philippinarum* were significantly greater when compared to other bivalves such as the green-lipped mussel *Perna viridis*, rendering these enzymes as good markers for the assessment of oxidative stress in this species. The present study showed that GST and CAT activities in clams were significantly elevated probably as a response to the exposure with the polluted sediment containing high levels of nonylphenol and metals. It has been found that GST and CAT are present in almost all aerobically respiring organisms and function to protect cells from the oxidative stress induced by not only metals but also by organic pollutants (Mosleh et al. 2005; Regoli et al. 1997). However, it cannot be excluded that the measured increases in antioxidant enzyme activities were influenced by other pollutants. In fact, the sediment in this study area may have contained other pollutants such as PAH and PCBs, although the concentrations in sediments were not exceeding the sediment quality guidelines (MOMAF 2003). In the gastropod *Nucella lapillius*, however, GST activities seemed not to be a suitable biomarker for Cd and Cu. In fact, exposure to these metals even caused a significant reduction of GST in this species (Cunha et al. 2007). However, based on the results of this study, the activities of total GST and CAT appeared to be useful biomarkers to assess sediment pollution in *R. philippinarum*.

Table 3 Enrichment factor (EF), geoaccumulation index (I_{geo}), and polluted load index (PLI) in surface sediment of Lake Shihwa

Site	EF						I_{geo}						PLI
	Co	Ni	Cu	Zn	Cd	Pb	Co	Ni	Cu	Zn	Cd	Pb	
S1	1.73	2.54	13.58	5.17	4.99	2.67	0.44	0.99	3.41	2.02	1.97	1.07	4.71
S2	1.73	3.24	55.34	10.71	12.33	4.76	0.50	1.41	5.50	3.13	3.34	1.96	9.35
S3	1.82	2.51	15.86	5.37	7.16	2.87	0.22	0.69	3.35	1.79	2.20	0.88	4.30
S4	1.83	2.78	20.78	6.75	9.61	3.22	0.54	1.15	4.05	2.43	2.94	1.36	6.32
S5	0.88	0.92	1.72	1.41	1.42	1.12	−0.72	−0.65	0.26	−0.03	−0.02	−0.36	1.26
S6	1.70	2.53	11.57	4.33	5.73	1.97	0.61	1.18	3.38	1.96	2.36	0.82	4.94
S7	1.35	1.69	3.13	2.09	2.26	1.23	0.03	0.36	1.25	0.66	0.78	−0.10	2.11
Y1	1.39	1.67	3.42	2.06	1.64	1.30	−0.05	0.21	1.24	0.52	0.19	−0.16	1.88
Y2	1.29	1.55	2.78	1.84	1.31	1.12	−0.06	0.21	1.05	0.45	−0.04	−0.26	1.75
Y3	1.60	2.10	4.65	2.50	2.08	1.34	0.37	0.77	1.91	1.02	0.75	0.12	2.65
Y4	1.56	1.97	3.56	2.24	1.86	1.35	0.28	0.61	1.47	0.80	0.53	0.06	2.31
Y5	1.65	1.97	3.24	2.22	1.46	1.41	0.17	0.43	1.14	0.60	−0.01	−0.06	1.95
Y6	1.63	2.06	3.95	2.42	2.12	1.38	0.26	0.60	1.54	0.84	0.65	0.03	2.36
Y7	2.00	2.52	4.53	2.92	2.52	1.75	0.34	0.68	1.52	0.89	0.67	0.15	2.45

Potential environment impact of a tidal power plant in Lake Shihwa

Resuspension and redistribution of coastal sediments can be caused both by natural events (e.g., tidal current, storms, and wave–current interaction) and anthropogenic activities such as dredging (van den Berg et al. 2001). Polluted sediment redistribution and resuspension to adjacent areas can release pollutants such as metals and persistent organic pollutants into the aqueous phase (Eggleton and Thomas 2004; van den Berg et al. 2001). In the case of this study, sediments of Lake Shihwa were highly polluted with metals and nonylphenol (Fig. 2). This study further showed that large amounts of nonylphenol can be released into the aqueous phase under aerobic conditions. Based on the difference of nonylphenol concentrations in sediments between before and after exposure experiments, it could be demonstrated that nonylphenol was accumulated from sediments into clams with an average of about 53 % of nonylphenol derived from sediment while the remaining nonylphenol in the water phase was only 8 %. This discrepancy observed in mass balance of nonylphenol indicates that a portion of nonylphenol was degraded by biotic and abiotic processes such as microbial degradation and volatilization under aerobic conditions (Hong et al. 2010; Isobe et al. 2001; Ying et al. 2002). The average amount of daily nonylphenol uptake was 87.4 ng per clam. In contrast, no significant bioaccumulation of metals into clams was observed in this study.

Among metals, Cu, Zn, and Pb exceeded the ERL most frequently. This implies that toxic effects to benthic organisms in Lake Shihwa due to the exposure with these elements cannot be excluded. Cd concentrations were lower than the ERL, and thus, Cd is unlikely to exert toxic effects to benthic organisms in this area. The highest concentrations of Cu, Zn, and Ni in surface sediments exceeded the sediment quality guidelines (ERL and ERM by Long et al. 1995, Fig. 2). Ra et al. (2011a) reported that the pollution history of Lake Shihwa with metals over the past 30 years revealed that sedimentation rates varied from 0.38 to 1.47 cm year^{-1} and that highly contaminated sediments were accumulated to about 0.3 to 1 m of deposition layer. According to MOMAF (2006), the volume of accumulated sediments over the past 10 years (1996 to 2005) was approximately 7,402,443 m^3. Thus, there is a significant risk that once the tidal power plant begins its operation, large amounts of polluted sediments from Lake Shihwa can be discharged into the outer sea. Kim and Lee (2000) already reported that the community of meiobenthos in the coastal regions of Gyeonggi Bay was altered by the polluted effluents when the gate was partially opened at the end of the sea dike. For these reasons, sediment-associated pollutants might be released in a bioavailable form that was assimilated by the organisms. Moreover, it is expected that the tidal power plant will discharge approximately 150 million tons of water with polluted sediments into Gyeonggi Bay per day. This is 50 % of the water mass of Lake Shihwa and about six times larger than the water quantities flushed to Lake Shihwa by the present water gate.

In contrast to nonylphenol, there was no significant bioaccumulation of metals into clams during our experiments. This is likely due to the fact that dissolution of metal ions from sediment particles was relatively small. Previous studies by our group also showed that the bioavailable fraction leached by 1 M HCl is only about 50 % of the total fraction for Cu, Zn, Cd, and Pb in sediments from Lake Shihwa (Ra et al. 2009). However, in contrast to the static experiments conducted here, movements of sediments have been shown to alter their binding properties from strongly bonded species to weakly bonded species that are easily bioaccumulated in aquatic organism (Calmano et al. 1993). Furthermore, van den Berg et al. (2001) reported that the oxidation of sulfide that can occur after resuspension combined with trace metals induces the redistribution of metals such as Cd, Cu, Zn, and Pb from sediment to the water. Thus, resuspension of contaminated sediments caused by the operation of tidal power plant might have a significant impact on the bioavailability of metals from sediments to bivalves like the Manila clam *R. philippinarum* in the study area. However, further experiments are required to confirm this hypothesis.

Conclusions

This study described the concentrations of nonylphenol and metals in sediments collected from Lake Shihwa and their accumulation and selected biomarker responses (MTLPs, GST, and CAT) in the Manila clam *R. philippinarum*. The present study clearly demonstrated that nonylphenol was released from sediments and significantly concentrated in the water column and in clams even after a short exposure time (96 h). The significant increases in MTLP concentrations and GST and CAT activities observed in *R. philippinarum* after exposure to contaminated sediments indicated that these end points represent potentially useful biomarkers for the characterization of environmental stress and exposure to pollutants in Lake Shihwa. The integration of chemical analyses and biomarkers responses in clam suggested that the operation of a tidal power plant currently under construction could cause significant redistribution and remobilization of contaminants from Lake Shihwa sediments to the outer sea potentially resulting in effects to the benthic organisms in the sea outside Lake Shihwa. However, long-term exposure experiment coupled with measuring growth or production rate of organisms based on field and comprehensive monitoring studies is necessary to more objectively assess potential environmental changes and repercussion impacts on coastal ecosystems around Lake Shihwa. Furthermore, various densities of clams should be also investigated when exposed to different quantities of sediments. The present study as such provides useful information for the indication and functional understanding of potential impacts on coastal benthic organisms by human-induced environmental change.

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References

Baudrimont M, Schäfer J, Marie V, Maury-Brachet R, Bossy C, Boudou A, Blanc G (2005) Geochemical survey and metal bioaccumulation of three bivalve species (*Crassostrea gigas*, *Cerastoderma edule* and *Ruditapes philippinarum*) in the Nord Médoc salt marshes (Gironde estuary, France). Sci Total Environ 337:265–280

Berthet B, Mouneyrac C, Amiard JC, Amiard-Triquet C, Berthelot Y, Le Hen A, Mastain O, Rainbow PS, Smith BD (2003) Accumulation and soluble binding of cadmium, copper, and zinc in the polychaete *Hediste diversicolor* from coastal sites with different trace metal bioavailabilities. Arch Environ Contam Toxicol 45:468–478

Bocchetti R, Fattorini D, Pisanelli B, Macchia S, Oliviero L, Pilato F, Pellegrini D, Regoli F (2008) Contaminant accumulation and biomarker responses in caged mussels, *Mytilus galloprovincialis*, to evaluate bioavailability and toxicological effects of remobilized chemicals during dredging and disposal operations in harbor areas. Aquat Toxicol 89:257–266

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 7:248–254

Calmano W, Hong J, Förstner U (1993) Binding and mobilization of heavy metals in contaminated sediments affected by pH and redox potential. Water Sci Technol 28:223–235

Canadian Council of Ministers of the Environment (CCME) (2001) Canadian sediment quality guidelines for the protection of aquatic life: nonylphenol and its ethoxylates. In: Canadian Environmental Quality Guidelines 1999. Canadian Council of Ministers of the Environment, Winnipeg

Chang BV, Yu CH, Yuan SY (2004) Degradation of nonylphenol by anaerobic microorganisms from river sediment. Chemosphere 55:493–500

Choi MS, Chun JH, Woo HJ, Yi HI (1999) Change of heavy metals and sediment facies in surface sediments of the Shihwa Lake. J Korean Environ Sci Soc 8:53–600

Choi MK, Furlong ET, Moon HB, Yu J, Choi HG (2011a) Contamination of nonylphelonic compounds in creek water, wastewater treatment plant effluents and sediments from Lake Shihwa and vicinity, Korea: comparison with fecal pollution. Chemosphere 85:1406–1413

Choi JY, Yu J, Yang DB, Ra K, Kim KT, Hong GH, Shin KH (2011b) Acetylthiocholine (ATC)-cleaving cholinesterase (ChE) activity as a potential biomarker of pesticide exposure in the Manila clam, *Ruditapes philippinarum*, of Korea. Mar Environ Res 71:1162–168

Coughlan BM, Hartl MGJ, O'Reilly SJ, Sheehan D, Mothersill C, van Pelt FNAM, O'Halloran J, O'Brien NM (2002) Detecting genotoxicity using the comet assay following chronic exposure of Manila clam *Tapes semidecussatus* to polluted estuarine sediments. Mar Pollut Bull 44:1359–1365

Cunha I, Mangas-Ramirez E, Guilhermino L (2007) Effects of copper and cadmium on cholinesterase and glutathione S-transferase activities of two marine gastropods (*Monodonta lineata* and *Nucella lapillus*). Comp Biochem Physiol 145C:648–657

De Luca-Abbott SB, Richardson BJ, McClellan KE, Zheng GJ, Martin M, Lam PKS (2005) Field validation of antioxidant enzyme biomarkers in mussels (*Perna viridis*) and clams (*Ruditapes philippinarum*) transplanted in Hong Kong coastal waters. Mar Pollut Bull 51:694–707

Depledge MH, Aagaard A, Gyorkos P (1995) Assessment of trace metal toxicities using molecular, physiological and behavioral biomarkers. Mar Pollut Bull 31:19–27

Eggleton J, Thomas KV (2004) A review of factors affecting the release and bioavailability of contaminants during sediment disturbance events. Environ Int 30:973–980

Hartl MGJ, Kilemade M, Coughlan BM, O'Halloran J, van Pelt FNAM, Sheehan D, Mothersill C, O'Brien NM (2006) A two-species biomarker model for the assessment of sediment toxicity in the marine and estuarine environment using the comet assay. J Environ Sci Health A 41:939–953

Hong S, Won EJ, Ju HJ, Kim MS, Shin KH (2010) Current nonylphenol pollution and the past 30 years record in an artificial Lake Shihwa, Korea. Mar Pollut Bull 60:303–313

Irato P, Santovito G, Cassini A, Piccinni E, Albergoni V (2003) Metal accumulation and binding protein induction in *Mytilus galloprovincialis*, *Scapharca inaequivalvis*, and *Tapes philippinarum* from the Lagoon of Venice. Arch Environ Contam Toxicol 44:476–484

Isobe T, Nishiyama H, Nakashima A, Takada H (2001) Distribution and behavior of nonylphenol, octylphenol, and nonylphenol monoethoxylate in Tokyo Metropolitan Area: their association with aquatic particles and sedimentary distributions. Environ Sci Technol 35:1041–1049

Ji K, Jang S, Kim Y, Kim E, Kim J (2007) Acute and chronic ecotoxicity assessment of ambient and effluent water discharged to the Lake Shihwa. J Korean Soc Water Qual 23:144–154 (in Korean)

Kim DS, Lee JH (2000) Impacts of contaminated water outflow from the Lake Shihwa on the meiobenthic animals living in the coastal zones of the Kyunggi Bay. Korean J Environ Biol 18:279–290

Kim KT, Kim ES, Cho SR, Park JK, Park CK (2003) Change of heavy metals in the surface sediments of the Lake Shihwa and its tributaries. Ocean Polar Res 25:447–457

Kim KT, Kim ES, Cho SR, Park JK (2009) Distribution and temporal change of heavy metals in the surface sediments of Lake Shihwa and the adjacent sea, Korea. J Coast Res 56:817–821

KORDI (1999) A study on environmental changes of Shihwa Lake by outer seawater inflow (3rd year). Korea Ocean Research and Development Institute, Ansan, p 363

Li D, Park J, Oh JR (2001) Silyl derivatization of alkylphenols, chlorophenols, and bisphenol A for simultaneous GC/MS determination. Anal Chem 73:3089–3095

Li D, Kim M, Oh JR, Park J (2004) Distribution characteristics of nonylphenols in the artificial Lake Shihwa, and surrounding creeks in Korea. Chemosphere 56:783–790

Lietti E, Marin MG, Matozzo V, Polesello S, Valsecchi S (2007) Uptake and elimination of 4-nonylphenol by the clam *Tapes philippinarum*. Arch Environ Contam Toxicol 53:571–578

Long ER, MacDonald DD, Smith SL, Calder FD (1995) Incidence of adverse biological effects within ranges of chemical concentrations in marine and estuarine sediments. Environ Manage 19:81–97

Marin MG, Matozzo V (2008) Do reproductive conditions influence responsiveness of vitellogenin-like protein induction in clams (*Tapes philippinarum*) exposed to 4-nonylphenol? Fresenius Environ Bull 17:1460–1465

MOMAF (2003) Pollution reducing strategies for the coastal Yellow Sea, annual report. Ministry of Maritime Affairs and Fisheries, Seoul

MOMAF (2006) Project to improve the marine environments of the Shihwa Lake. Ministry of Maritime Affairs and Fisheries, Seoul

MOMAF (2011) Project to improve the marine environments of the Shihwa Lake. Ministry of Maritime Affairs and Fisheries, Seoul

Monserrat JM, Rosa CE, Sandrini JZ, Marins LF, Bianchini A, Geracitano LA (2003) Annelids and nematodes as sentinels of environmental pollution. Comment Toxicol 9:289–301

Mosleh YY, Paris-Palacios S, Couderchet M, Biagianti-Risbourg S, Vernet G (2005) Metallothioneins induction, antioxidative response, glycogen and growth changed in *Tubifex tubifex* (Oligochaete) exposed to the fungicide, fenhexamid. Environ Pollut 135:73–82

Müller G (1979) Heavy metals in the sediment of the Rhine-Changes seity. 1971. Umschau 79:778–783

Ra K, Kim K-T, Kim E-S, Won E-J, Lim K-I, Park S-Y, Shin K-H (2009) Geochemistry of trace metals in Shihwa Lake sediment. In: Proceeding of the 2009 Korean soc. mar environ engineer fall annual meeting, Korea. p 175–180

Ra K, Kim K-T, Lee J-M, Bang J-H, Yun M-S, Kim E-S (2010) Experimental studies on the bioaccumulation of aqueous- and sediment-associated trace metals in polychaetes. In: Proceeding of the 2009 Soc for Mar Environ Engineer Fall annual meeting, Korea. pp 144–149

Ra K, Bang J-H, Lee J-M, Kim K-T, Kim E-S (2011a) The extent and historical trend of metal pollution recorded in core sediments from the artificial Lake Shihwa, Korea. Mar Pollut Bull 62:1814–1821

Ra K, Kim K-T, Bang J-H, Lee J-M, Kim E-S, Cho S-R (2011b) A preliminary study of environmental impact assessment of tidal power plant in Shihwa Lake, Korea: heavy metal accumulation in the transplanted Manila clam (*Ruditapes philippianrum*). J Coastal Res SI64:932–936

Rainbow PS (2006) Biomonitoring of trace metals in estuarine and marine environment. Aust J Ecotoxicol 12:107–122

Regoli F, Nigro M, Bertoli E, Principato G, Orlando E (1997) Defenses against oxidative stress in the Antartic scallop *Adamussium colbecki* and effects of acute exposure to metals. Hydrobiologia 355:139–144

Sarkar SK, Cabral H, Chatterjee M, Cardoso I, Bhattacharya AK, Satpathy KK, Alam MA (2008) Biomonitoring of heavy metals using the bivalve molluscs in Sunderban mangrove wetland, northeast coast of Bay of Bengal (India): possible risks to human health. Clean Soil Air Water 36:187–194

Simex SA, Helz GR (1981) Regional geochemistry of trace elements in Chesapeake Bay. Environ Geol 3:315–323

Statham PJ (1985) The determination of dissolved manganese and cadmium in sea water at low nmol l^{-1} concentrations by chelation and extraction followed by electrothermal atomic absorption spectrometry. Anal Chim Acta 169:149–159

Tomilson DC, Wilson JG, Harris CR, Jeffrey DW (1980) Problems in the assessment of heavy metals in estuaries and the formation pollution index. Helgol Mar Res 33:566–575

van den Berg GA, Meijers GGA, van der Heijdt LM, Zwolsman JJG (2001) Dredging-related mobilisation of trace metals: a case study in the Netherlands. Water Res 35:1979–1986

Viarengo A, Ponzano E, Dondero F, Fabbri R (1997) A simple spectrophotometric method for metallothionein evaluation in marine organisms: an application to Mediterranean and Antarctic mollusk. Mar Environ Res 44:69–84

Windom HL, Schropp SJ, Calder FD, Ryan JD, Smith RG Jr, Burney LC, Lewis FG, Rawlinson CH (1989) Natural trace metal concentrations in estuarine and coastal marine sediment of the southeastern United States. Environ Sci Tech 23:314–320

Won E-J, Rhee J-S, Kim R-O, Ra K, Kim K-T, Shin K-H, Lee J-S (2012) Susceptibility to oxidative stress and modulated expression of antioxidant genes in the copper exposed polychaete *Perinereis nuntia*. Comp Biochem Physiol 155C:344–351

Ying GG, Williams B, Kookana R (2002) Environmental fate of alkylphenols and alkylphenol ethoxylates: a review. Environ Int 28:215–226

Zhang J, Liu CL (2002) Riverine composition and estuarine geochemistry of particulate metals in China-weathering features, anthropogenic impact and chemical fluxes. Estuar Coast Shelf Sci 54:1051–1070

<Supplementary Material>

Evaluation of potential impact of polluted sediment using the Manila clam *Ruditapes philippinarum*: bioaccumulation and biomarker responses

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Supplementary Table

Table S1. Recovery rate of standard reference materials for trace metals in this study

Supplementary data

Indices for assessment of heavy metal pollution in sediment

References

Supplementary Table

Table S1. Recovery rate of standard reference materials for trace metals in this study

	PACS-2			SRM 2976			CASS-4		
	Verified value	Analytical value	Recovery (%)	Verified value	Analytical value	Recovery (%)	Verified value	Analytical value	Recovery (%)
Cu	310 (±12)	296.1 (±10.1)	95.5	4.02 (±0.33)	3.96 (±0.02)	98.5	0.592 (±0.055)	0.566 (±0.006)	95.6
Zn	364 (±23)	361.6 (±10.2)	99.3	137 (±13)	145.4 (±1.75)	106.1	0.381 (±0.057)	0.393 (±0.009)	103.2
Ni	39.5 (±2.3)	39.1 (±1.3)	99.1	-	-	-	0.314 (±0.030)	0.305 (±0.009)	97.1
Cd	2.11 (±0.15)	2.2 (±0.08)	106.0	0.82 (±0.16)	0.83 (±0.01)	101.4	0.026 (±0.003)	0.027 (±0.0004)	105.6
Co	11.5 (±0.3)	11.2 (±0.2)	97.3	-	-	-	0.026 (±0.003)	0.024 (±0.001)	92.4
Pb	183 (±8)	181.8 (±12.0)	99.3	1.19 (±0.18)	1.26 (±0.05)	105.9	0.0098 (±0.0036)	0.012 (±0.0025)	118.9

Supplementary data

Indices for assessment of heavy metal pollution in sediment

Enrichment factor analysis, a method proposed by Simex and Helz (1981) is mathematically expressed as:

$$EF = \frac{[Me] \ / \ [Al]_{\ sample}}{[Me] \ / \ [Al]_{\ background}}$$

The background value was calculated from the average [Me]/[Al] value of the deeper layers in the core sediment from Lake Shihwa corresponding to the early 1900s (Ra et al. 2011).

The geo-accumulation index (Igeo) was introduced by Muller (1979) to evaluate the degree of pollution of the sediment as following equations;

$$I_{geo} = \log_2(\frac{C_n}{1.5} \times B_n)$$

The degree of metal pollution is assessed in terms of seven contamination classes based on the increasing numerical value of the index as follows:

- Igeo <0 = means unpolluted
- 0<=Igeo<1 means unpolluted to moderately polluted
- 1<=Igeo<2 means moderately polluted
- 2<=Igeo<3 means moderately to strongly polluted
- 3<=Igeo<4 means strongly polluted
- 4<=Igeo<5 means strongly to very strongly polluted
- Igeo>=5 means very strongly polluted.

Cn and *Bn* indicate metal concentrations in samples and background, respectively. The factor 1.5 is incorporated in the relationship to account for possible variation in background data due to lithogenic effect.

The pollution load index (PLI) calculation was proposed by Tomilson et al. (1980);

$$PLI =^{n} \sqrt{CF_1 \times CF_2 \times CF_3 \times .. \times CF_n}$$

CF means contamination factor ($CF = \frac{C_{\ metal}}{C_{\ background\ value}}$) and *n* indicates number of metals.

References

Müller G (1979) Heavy metals in the sediment of the Rhine-Changes seity. 1971. Umsch Wiss Tech 79: 778-783

Ra K, Bang J-H, Lee J-M, Kim K-T, Kim E-S (2011) The extent and historical trend of metal pollution recorded in core sediments from the artificial Lake Shihwa, Korea. Mar Pollut Bull 62:1814-1821

Simex SA, Helz G R (1981) Regional geochemistry of trace elements in Chesapeake Bay. Environ Geo 3:315-323

Tomilson DC, Wilson JG, Harris CR, Jeffrey DW (1980) Problems in the assessment of heavy metals in estuaries and the formation pollution index. Helgoland Mar Res 33:566–575