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Heavy Metal Toxicity Monitoring in Sediments of Jinhae Bay, Korea

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Sediments in the enclosed Jinhae Bay area surrounded by a large heavy industrial complex in Korea are becoming a reservoir for a wide range of chemicals, especially heavy metals (Lee et al. 1990). Inorganic toxicants such as heavy metals can be transferred through the food chain and also affect biota directly. Metal toxicity associated with solids in the environment is of particular concern since they can be released into the water column and adversely affect the biota. Direct uptake from sediment particles can be an important additional source of sediment-bound contaminants for sediment feeding animals (Landrum and Robbins 1990).

Monitoring programs often rely on total metal concentrations in sediments. These chemical analyses do not give information on metal availability to the biota. A vast array of toxicity tests has been employed for assessing the toxicity of freshwater and marine sediments (Burton and Scott 1992). The tests include the use of biochemicals or organisms such as enzymes, bacteria, phytoplankton, zooplankton, benthic invertebrates, macrophytes or fish (Kong et al. 1995). In many such tests, water or solvents are added to elute the toxicants from the solid matrix, leading to toxicant dilution. The newly proposed solid-phase microbioassays include the Microtox solid-phase test (Tung et al. 1990) and the direct solid-phase toxicity testing (DSTT) using the Toxi-Chromotest kit (Kwan 1993). However, these tests respond to both organic and inorganic toxicants present in a sediment sample and are not specific for heavy metal toxicity.

The purpose of the present study was to investigate the use of a solid-phase enzymatic test, MetPLATE™, and to determine toxicity in sediment samples collected from Jinhae Bay (South Korea) which are highly contaminated with heavy metals. The test is based on the specific inhibition by heavy metals of β-galactosidase of a mutant strain of *Escherichia coli* (Bitton et al. 1994). This test was previously applied to test heavy metal toxicity in soils (Bitton et al. 1996; Boularbah et al. 1996) and sediments (Bitton et al. 1992).

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MATERIALS AND METHODS

In Jinhae Bay, sediment samples were collected at the sites shown in Figure 1. Sampling sites A, B and C are located in the waterway, an important source of heavy metals entering into Jinhae Bay from a large industrial complex located along the stream of Changwon city in Korea. Sites 1-9 were chosen because sediment toxicity, if present, may affect the biota such as oysters, mussels and clams. Samples of sediment were taken by Ponar dredge and placed on ice for transportation to the laboratory. After collection, the samples were stored at 4°C until tested and subsequently split for heavy metal toxicity and chemical analysis within two months of collection. The sediment samples were dried at 105°C for 5 hours and passed through a 32-mesh nylon sieve. One gram of sample was totally digested with a mixture of nitric acid and hydrochloric acid, and the resultant solutions were diluted to 100 mL with deionized-distilled water. The concentrations of Mn, Cu, Pb, Cd, Cr and Zn were determined by a flame atomic absorption spectrophotometer (Model Shimazu AA-670).

Solid-phase bioassays for heavy-metal toxicity were undertaken using the MetPLATE™ test kit (Group 206 Technologies, Gainesville, FL, USA). This toxicity test kit is based on the specific inhibition of β-galactosidase by heavy metals in a mutant strain of *E. coli* (Bitton et al. 1994). This bioassay, which is specific for heavy metal toxicity, was used previously in a pad format for assessing the heavy metal toxicity of sediments from hazardous waste sites (Bitton et al. 1992). The initial step of the assay was to add 0.1ml of the test bacteria containing induced β-galactosidase and 0.9 ml of MilliQ water to 1.0 g of fresh sediments. Following a one-hour contact period, 0.5 mL of buffered enzyme substrate 125 mg/L (Chlorophenol-red-β-D-galactopyranoside) and the mixture was incubated until reddish color development. The sediment suppension was then filter through a 0.45µm Gelman filter, and the absorbance of the filtrates were read at 575 nm using a microplate reader. The toxicity test was then run according to Bitton et al. (1996). The toxicity tests for each sample were carried out in triplicate.

RESULTS AND DISCUSSION

The toxicity of twelve sediments collected from contaminated sites in Jinhae Bay, (Korea) was tested using the MetPLATE™ kit. Except for sites # 6 and # 8, the pH of the sediments from the 10 other sites varied between 5 and 8 (Table 1). β-galactosidase of the test bacterium is not inhibited at this pH range (Bitton unpublished data).

Total metal concentrations of six metals were determined in sediments from historically contaminated sites adjacent to heavy industrial complex (Table 2).

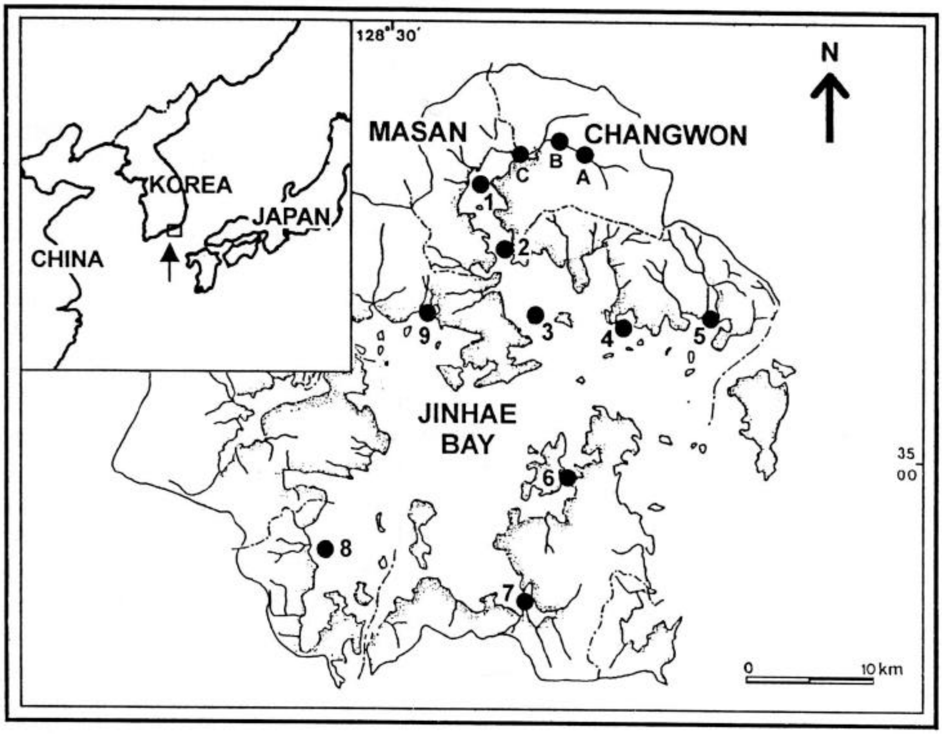

Figure 1. Map of sampling sites located in southern part of Korea.

Table 1. pH and % moisture of the collected sediments

Site No	Sediment pH	Moisture (%)	Salinity (‰)
A	7 – 8	55	0
B	6 – 7	49	0
C	6	41	20.2
1	5	47	30.1
2	6	47	30.3
3	6	37	29.8
4	6	31	30.2
5	5 – 6	36	30.3
6	4	43	30.1
7	6	23	30.1
8	3 – 4	58	29.9
9	7	33	30.2

Zn, Cd, Pb, Cu, Cr or Mn were detected in the sediments in varying concentrations in the range of 59 - 5,605 µg/g for Zn, 0.30 - 41.23 µg/g for Cd, 16 - 2,729 µg/g for Pb , 22 - 401 µg/g for Cu, 26 - 465 for µg/g Cr, and 289 - 1,496 µg/g for Mn. Among the metals studied, zinc was found to be the dominant metal compared to the other metals for site A, B, and C exhibiting 78, 37, and 56% of total metal contents analyzed, respectively. In addition, site B also contained high percentages of Pb and Cu, showing 2,729 and 1,401 µg/g dry weight, and representing 35% and 18% of the total metals analyzed, respectively. Sites 1 to 9 generally exhibited much lower metal concentrations than sites A , B and C and were mostly contaminated by Mn ranging from 44 % (site 5) to 87 % (site 9). All sites were found to contain less than 6.3 µg/g Cd except for site A which displayed a concentration of 41.2 µg/g (0.6% of total metal). Lee et al. (1990) also reported that several waterway sites in Jinhae Bay were contaminated to various degrees by different metals such as Zn, Pb, Cu and Cd.

Table 2 and Figure 2 also show the heavy metal toxicity of the 12 sediments under study, using the solid-phase assay MetPLATE™. The percent inhibition of β-galactosidase enzyme varied from 14.4 to 100%. Based upon the percent inhibition, sediment samples A and B, with % inhibition values of 100 and 62.9, appear to display the greatest incidence of heavy-metal toxicity. Sediment B is less toxic than sediment A because Pb concentration represents 35% of its total metal concentration as compared to only 3% in sediment A. Pb is a highly insoluble metal (i.e., "sticky metal") and is not readily available to the biota (Body et al. 1991).

Sediment samples # 6 and # 8 were less contaminated than the other samples but showed a high toxicity. The low pH in these sediments was the reason for the observed increased toxicity. As shown in Table 2, a direct relationship is apparent between sample toxicity and heavy metal content. In these sediments, zinc and manganese were the most prevalent metals. Except for sediment # 6 and # 8, toxicity was correlated ($r^2 = 0.658$) with the total content of Zn, Cd, Pb, Cu, Cr, and Mn.

However, a higher correlation ($r^2 = 0.8182$) was obtained between toxicity, and zinc and manganese concentration (Figure 3). Such differences may be due to the binding of these two metals to solids, and varying toxic effect of metals to enzyme activity. Therefore, determining total content of heavy metals by chemical analyses, is insufficient to assess the environmental impact on contaminated soils and sediments, because it is the chemical forms that determine metal behavior in the environment (Ma and Rao 1997). Some studies reported little correlation between total heavy metal content and toxicity (Dave 1992).

The solid-phase MetPLATE™ has several advantages over indirect solid toxicity test. For example, it prevents dilution of toxicants, is specific for heavy metal, and allows a direct contact between contaminants and the test organism or enzyme. Based on these advantages, the test allows a better assessment of the impact of metals on the ecosystems such as sediments, soils or wastewater sludge.

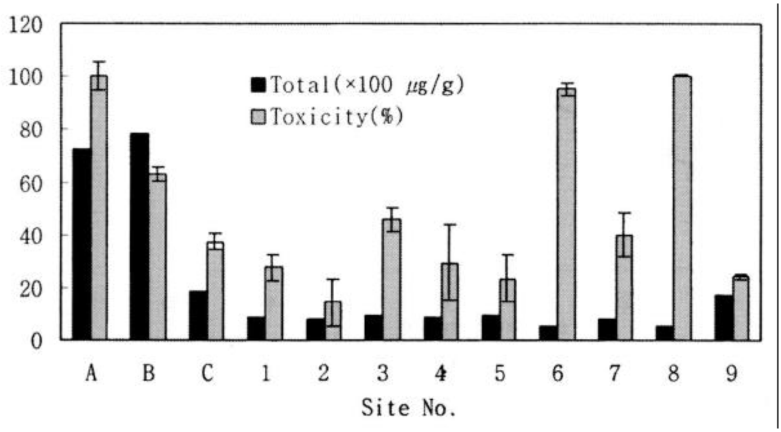

Figure 2. Relationship between total metal content and sample toxicity.

Table 2. MetPLATE™ solid-phase toxicity assay and heavy metal content of sediments from Jinhae Bay, Korea (μg/g dry solid)

Site	Zn	Cd	Pb	Cu	Cr	Mn	Total	Toxicity(%)
A	5605±168	41.23±0.82	210±5	442±9	465±13	431±12	7195	100.0±5.4
B	2852±86	6.29±0.13	2729±54	1401±28	89±3	653±19	7791	62.9±2.4
C	1070±21	4.44±0.09	183±6	153±6	88±2	387±7	1885	37.4±2.9
1	272±5	1.13±0.03	74±3	64±1	52±1	385±11	849	27.6±4.9
2	128±3	0.77±0.02	35±2	38±1	43±1	581±11	826	14.4±9.0
3	80±2	0.39±0.01	36±1	38±2	42±2	729±21	925	45.8±4.6
4	216±4	0.88±0.02	50±2	129±4	54±1	974±19	830	29.4±14.3
5	286±6	0.91±0.03	62±3	108±2	70±2	406±12	932	23.4±8.8
6	86±3	0.88±0.03	16±1	22±1	87±3	289±5	500	95.2±2.1
7	59±2	0.30±0.01	128±4	87±2	26±1	490±14	790	40.1±8.3
8	77±2	1.16±0.02	48±1	77±2	37±2	308±6	549	100.3±0.2
9	76±3	0.30±0.01	35±1	63±2	47±3	1496±29	1717	24.4±0.95

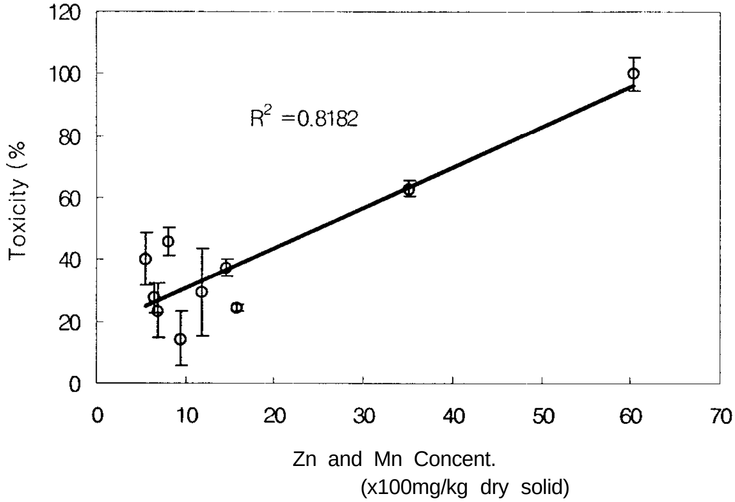

Figure 3. Correlation between sample toxicity and Zn and Mn concentrations (except for sample #6 and #8.

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