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Toxicity Test of Nanji Island Landfill (Seoul, Korea) Leachate Using Japanese Medaka *(Oryzias latipes)* Embryo Larval Assay

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The Nanji Island landfill is located within the city limits of Seoul, Korea, where an estimated 18 million people live. The landfill is divided into two distinct pyramids (Figure 1). The larger pyramid, which is older was closed more than 9 yr ago while the smaller one was closed in March of 1993. The Nanji Island landfill is bordered on three sides by Sat Stream adjacent residential areas and on the fourth by a road adjacent to the Han River. The landfill contains untreated industrial and municipal wastes. Leachates from the landfill have formed streams and pools which continuously seep from the sides. They contain substantial amounts of organic wastes and toxic chemicals.

The potential toxicity from such complex mixtures cannot be determined based solely on physico-chemical characteristics of the leachates. Various bioassays have been developed to evaluate the toxicity of such environmental contaminants (Cameron and Koch 1980; Atwater et al, 1983; Wong 1989). In the present studies the Japanese Medaka *(Oryzias latipes)* embryo-larval assay (ELA) was chosen as the bioassay to study the toxicity of leachates from the Nanji Island landfill. This bioassay had been previously used to measure the toxicity of a number of chemicals and complex mixtures (Wisk and Cooper 1990; Cooper and Liu 1991; Cooper and Mc George 1991). The purpose of this study was to (a) determine the toxic potency of the landfill leachates to the embryos, (b) examine the type and sequence of lesions induced in the embryos, (c) compare the toxicity of the leachate samples collected from different locations on the sides of the landfill, (d) measure standard physico-chemical properties, and (e) determine if the toxicity and physico-chemical properties were changed following the monsoon season.

MATERIALS AND METHODS

Leachate samples were collected from four different sites L_1, L_2, L_3, L_5 (Figure l), at the Nanji Island landfill, and the Han River samples were collected upstream (H_1) and downstream (H_2) to the landfill. Sat Stream samples were collected from the sampling sites S_1, S_2, and S_3 (Figure 1). The sampling was done in June before monsoon and July 1993 in the middle of monsoon season.

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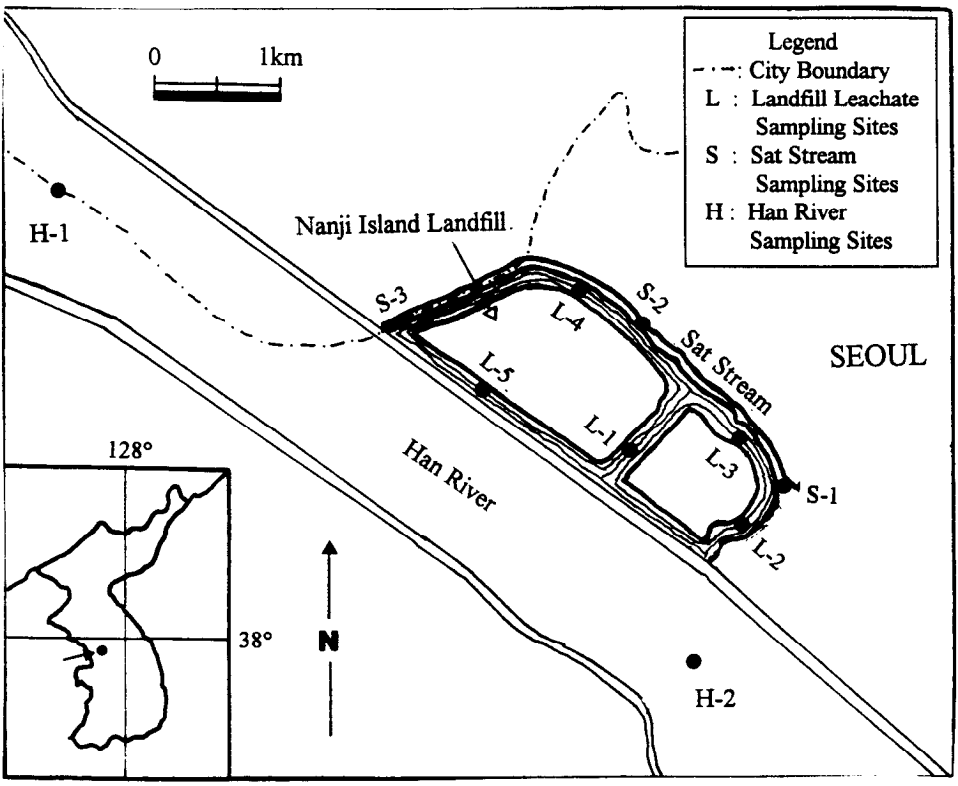

Figure 1. Location of the Sampling Sites on the Nanji Island Landfill.

These samples were frozen and transported to the United States. The leachate samples were tested for a number of water quality parameters: pH (HORZBA pH Meter), conductivity (YSI Model 33 portable Conductivity salinity meter), total suspended solids (Gravimetric method), salinity (YSI Model 33 portable Conductivity salinity meter), dissolved oxygen (YSI Model 58 portable DO meter), BOD_5 (Winkler method), COD (alkaline permanganate method), ammonia-nitrogen (Nessler method), nitrate-nitrogen (Diazotization method), nitrite-nitrogen (Cadmium reduction method), total Kjeldahl-nitrogen (Kjeldahl digestion), total phosphorus (Ascorbic acid method after persulfate digestion), Soluble PO, (Ascorbic acid method). All the methods are described in Standard Methods (APHA 1989). The split samples were frozen and transported to the United States for the toxicity tests which were carried out in Dr. Cooper's laboratory.

The ELA was conducted only with leachate samples collected from L_1, L_2, L_3, L_5 sampling sites and the toxicity of the leachate collected from the L_2 site in June (pre-monsoon) and July (mid-monsoon) was also compared using probit analysis (USEPA 1988; Litchfield and Wilcoxon 1949). The exposure concentrations were 0.5, 1, 2, 4, and 8% v/v leachate to rearing solution. The dilutions were made with aerated rearing solution (Kirchen and West 1976) and the control was run in 100% rearing solution. The Sat Stream and the Han River samples were tested up to 100% concentration. The eggs were collected by hand netting the

females and removing eggs. Each egg was separated and observed for fertilization and stage of development. The eggs were exposed at the 32-64 cell stage. Fifteen eggs were individually exposed in 1 mL of test solution in 2-mL Teflon-capped glass vials. The exposure vials containing embryos were stored at 25°C in a low-temperature incubator. Each embryo was examined daily under dissecting microscope for the stage of development, appearance of visible lesions, and death (Cooper et al. 1991). After 10 d of exposure, the test solution in the vials was renewed with 1 mL of rearing solution and the observations were continued until the embryos were either dead or had hatched and survived for at least 3 d post hatch up to 20 d. The LC_{50} and EC, values with 95% confidence interval from the dose-response studies were calculated by EPA Probit analysis program version 1.4 (USEPA 1988).

RESULTS AND DISCUSSION

The water quality analysis of the leachate, Han River, and Sat Stream samples is presented in Table 1. The values of water quality parameters like BOD, COD, TSS, NH_3, NO, were very high for the leachates; L2 showed highest of all, but the Han River and Sat Stream samples were fairly clean. Koo and Yoon (1994) also reported that the leachates were characterized by high concentrations of Na^+, K^+, NH_4^+, and Cl ions. The samples collected in July showed much lower values of water quality parameters as compared with the one collected in June (Table 1).

The ELA showed that the Nanji Island landfill leachates were highly toxic. All of the leachates tested at 8% v/v concentration caused 100% mortality. The embryos died within 24 hr of exposure. The LC50 values for leachates collected from different sites (Table 2) didn't differ significantly at a $p \leq 0.05$ using a probit analysis (USEPA 1988). But no mortality was observed even at 100% v/v concentration of Sat Stream and Han River samples.

The EC50 for all lesions in the embryos exposed to Sat Stream samples collected from site S3 was 71% (Table 2) , but the Sat Stream samples collected from sites S1, S2 and the Han River did not induce any lesions up to 100% concentration. The physico-chemical parameters (Table 1) also showed that the samples from S3 site were most polluted. The flow of the leachates is towards Han River, but the dilution of the leachates could explain the lack of toxicity to the embryos. The EC50 of leachate collected from the L2 site in July was higher than that collected in June, 1993. Total rainfall between these sampling periods was approximately 344 mm. Even after being diluted with heavy rains, the leachate was still highly toxic (Table 2). Wong (1989) reported that the leachate collected from Gin Drinker's Bay landfill (Tsuen Wan, Hong Kong) in March was about 10 times more toxic to the *Sarotherodon mossambicus* than that collected in July 1987. The rainfall in March was 13.6 and in July it was 125.9 mm. The dilution of the rainfall was significant.

Table 1. Physico-chemical analysis of leachates from the Nanji Island landfill (L), Stream (S), and Han River (H) samples (All the values are in mg/l unless otherwise stated).

Parameter	L1	L2	L2*	L3	L5	H1	H2	S1	S2	S3
PH	7.8	8.1	8.3	**7.9**	8.2	7.7	7.8	8.3	8.0	7.5
Conductivity (umhos/cm)	$2x10^4$	$2.7x10^3$	$1.4x10^3$	$1.6x10^3$	$1.8x10^3$	185	269	$1x10^3$	$1.1x10^3$	950
Salinity (%)	11.2	17.0	8.0	9.9	9.5	-	-	0.4	0.4	0.2
DO	0.7	0.7	2.8	1.2	1.4	5.0	5.2	2.0	8.0	5.8
BOD	374.0	$1.7x10^3$	$1.3x10^3$	740	223	16.2	5.1	14.2	8.2	8.0
COD	740.0	$5.0x10^3$	854.0	$1.2x10^3$	820	9.6	8.0	17.6	19.2	22.4
Suspended Solids	74.0	496.0	90.0	64.0	74.0	15.0	20.0	31.0	28.5	53.0
NH3-N	896.3	$1.4x10^3$	787.5	662.7	790	1.8	2.7	5.0	5.0	12.3
NO_2-N	0.12	0.91	0.53	0.19	0.12	0.06	0.1	0.91	0.18	0.09
NO3-N	1.99	6.53	1.5	1.43	2.08	0.04	0.04	0.1	0.06	0.10
Kjeldahl-N	900.0	$1.4x10^3$	$1.3x10^3$	673.0	798.0	3.5	3.67	6.2	6.5	13.5
Total PO4-3-P	3.84	20.4	4.02	2.67	2.96	0.34	0.31	0.27	0.25	0.38
Soluble PO4-3-P	2.9	3.0	0.78	2.67	2.8	0.08	0.21	0.04	0.03	0.09

*Leachate sample collected after heavy rains (July).

Table 2. Results of Nanji Island landfill leachate toxicity to the embryos of the Japanese Medaka *(Oryzias latipes)*

Sampling Site	Regression Equation	LC_{50}*** (%v/v)	EC_{50} (%v/v)
L_1	Y = 2.30 X + 4.56	1.6 (1.0 - 2.3)	0.87 (0.36 - 1.32)
L_2	Y = 7.04 X + 2.27	2.4 (18 - 3.1)	0.30 (0.01 - 0.60)
L2*	-	-	0.84
L_3	Y = 6.88 X + 2.26	2.2 (1.3 - 3.0)	0.44 (0.04 - 0.67)
L_5	Y = 4.50 X + 3.70	2.0**	0.42 (0.11 - 0.61)
H_1	-	-	>100
H_2	-	-	>100
S_1	-	-	>100
S_2	-	-	>100
S_3	-	-	>100

* Sample collected after heavy rains.
** Fiducial limits could not be computed.
*** LC50 for survival for 3 days post hatch (up to 20 days) values are mean and 95% confidence interval

Table 3. Percentage occurrence of severe lesions in the embryos of Japanese Medaka *(Oryzias latipes)* exposed to 2% concentration of the Nanji Island landfill leachates .

Type of severe lesion	L_1	L_2	L_3*	L_4	L_5
Pericardial hemorrhage	0	7	7	7	27
Vitelline vein hemorrhage	33	27	20	20	7
Caudal region hemorrhage	27	0	0	0	0
Reduced heart beat and circulation	73	33	0	33	33
Pericardial edema	0	20	13	0	0
Chorion rupture	20	7	0	20	7
Reduced eyeball and intereye distance	0	0	27	0	60

*Sample collected after heavy rains. Severe lesions were observed only at 8% concentration of the leachate.

The exposed embryos showed a variety of severe lesions listed in Table 3. The observed multiple lesions strongly suggested that the leachates were complex mixtures containing organic wastes and chemical compounds. Further, the leachates differed in the type and frequency of the lesions induced in the exposed embryos (Table 3). The leachate collected from Ll sampling site caused reduced heart beat and circulation in 73% of the exposed embryos but did not induce pericardial hemorrhage and edema. On the other hand only 33% of the embryos exposed to the leachate collected from L5 site showed reduced heartbeat and circulation but 60% had reduction in eyeball size and intereye distance. The observed differences in the lesions were not very surprising because mixed wastes were placed in the landfill. Therefore depending upon the site of the leachate collection the composition would vary.

These studies show that the leachates from the Nanji Island Landfill are highly toxic and are complex mixtures of a number of toxic compounds. The leachate enters the Han River and is diluted to levels where no acute toxicity occurs. Studies should be undertaken to examine the levels of these contaminants in fish and sediments near the landfill. If the leachate is treated, then biological assays should be carried out to demonstrate a reduction in biological activity.

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REFERENCES

APHA (1989) Standard methods for the examination of water and waste water, 17th ed. American Public Health Association, Washington DC

Atwater JW, Jasper S, Mavinic DS, Koch FA (1983) Experiments using Daphnia to measure landfill leachate toxicity. Water Res 17:1855-1861

Cameron RD, Koch FA (1980) Toxicity of landfill leachates. J Water Pollut Control Fed 52:760-769

Cooper KR, Liu H, Bergquist PA, Rappe C (1991) Evaluation of Baltic herring and embryo-larval assay. Environ Toxic01 Chem 10:707-714

Cooper KR, McGeorge L (1991) Decreased effluent toxicity in the Japanese medaka, Oryzias latipes, embryo larval assay following initiation of a toxicant reduction srategy. In: Mayes MA, Barron MG (ed) Aquatic toxicolgy and risk assessment: Vol 14, American Society for Testing and Materials, Philadelphia, p 67-83

Litchfield, J.T., Wilcoxon, F. (1949) A simplified method of evaluating dose-effect experiments, J Pharmacol Exp Therapeu 96:99-113

Kirchen V, West RV (1976) The Japanese medaka, its care and development, Carolina Biological Co., Burlington, North Carolina, p 1-36

Koo Jk, Yoon SP (1994) Apportionment of leachates to groundwater pollution around uncontrolled landfill by chemical mass balances. J Environ Sci Health 29 A:411-421

Stephen P, Ram NM (1984) Multiple bioassays to assess the toxicity of a sanitary landfill leachate. Arch Environ Contam Toxicol 13 : 197-206

USEPA (1988) Short-term methods of estimating the chronic toxicity of effluents and recieving waters to marine and estuarme organisms appendix G. Probit analysis EPA/600/4-87/-28

Wisk JD, Cooper KR (1990) Comparison of toxicity of several polychlorinated dibenzo-p-dioxins and 2, 3, 7, 8-tetrachlorobenzofuran in embryos of the Japanese medaka (Oryzias latipes). Chemosphere 20:361-377

Wong MH (1989) Toxicity test of landfill leachate using Sarotherodon mosambicus (freshwater fish). Ecotoxicol Environ Saf 17: 149-156