Bull. Environ. Contam. Toxicol. (1990) 44:892–899

Bulletin of Environmental Contamination and Toxicology

Evaluation of Toxicity of River Sediments by *In Vitro* Enzyme Inhibition

Masako Tabata, Keiko Ozawa, Akiko Ohtakara, Hiromi Nakabayashi, and Shizuo Suzuki

Faculty of Pharmaceutical Science, Science University of Tokyo, 12 Ichigaya, Shinjuku-ku, Tokyo 162, Japan

To confirm the presence of toxic substances in aquatic environments, various toxicological assays have been carried out. Latent toxicity cannot be evaluated only by chemical analysis since natural samples contain diverse kinds of compounds. On the other hand a biological assay should be appropriate for evaluating directly latent toxicity.

Many biological toxic examinations have been conducted on fish, shellfish, algae, zooplankton and others using the whole body. The evaluation of river pollution by benthic organisms inhabiting river beds has also been attempted many times (Tsuda 1972). Another method for evaluating of toxicity of environmental samples has been to use enzyme inhibition in vitro, as reported by Fritsch et al.(1975), Tyler (1974, 1976a, 1976b), Geve (1975), Rutherford et al.(1979), Armant et al.(1980) Flint et al.(1984), Obst(1984a, 1984b). The authors have investigated the distribution of cholinesterase inhibitor on the river sediment and evaluated the latent toxicity of aquatic environment samples (Tabata et al. 1984). The present paper reports the inhibitory effects of solvent extracts from Tama and Ayase River sediments on three enzymes: alkaline phosphatase and glucose-6-phosphate dehydrogenase that diagnose for a functional disorder of a liver and β-glucuronidase for that of a kidney used in clinical inspection.

MATERIALS AND METHODS

Sediment samples were collected from surface layers at 8 sites on the Tama River (23 May, 1982) and at 7 sites on the Ayase River (29 September, 1982) as shown in Fig. 1 and Table 1. The sediments were dried at room temperature and powdered. Two hundred grams dried samples were extracted twice every 8 hr with a mixture of 200 mL n-hexane(Hex) and 100 mL distilled water with shaking at room temperature. Next, they were extracted

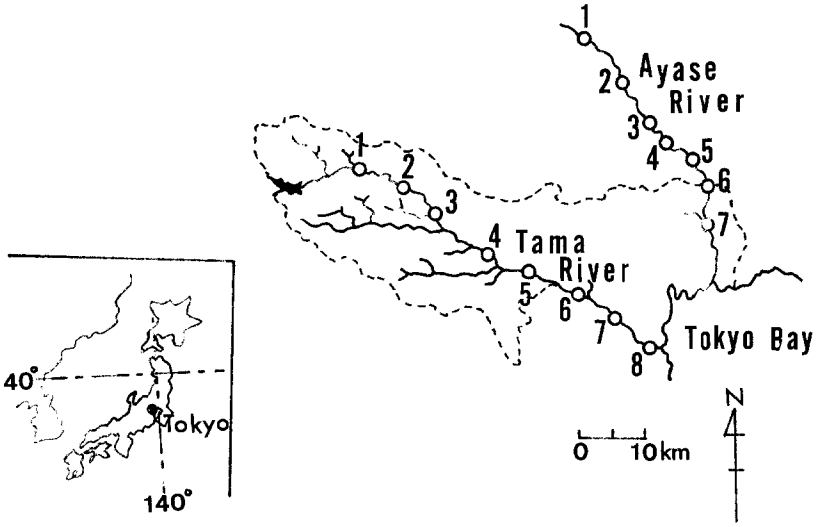


Figure 1. Sampling stations in the Tama River and the Ayase River.

Tama River	1. Kawai	2. Mannen	3. Nagata
	4. Hino	5. Koremasa	6. Izumi
	7. Maruko	8. Daishi	
Ayase River	1. Yawata	2. Ohohashi	3. Kamitoi
	4. Sato	5. Ayase	6. Miyashiro
	7. Horikiri		

using 200 mL ethylacetate(EtAc) and 100 mL distilled water, and then 200 mL methanol(MeOH). Each solution was evaporated under reduced pressure below 40°C using a rotary evaporator. For the enzyme assay, the evaporated samples were dissolved in dimethyl sulfoxide(DMSO)/butyl acetate(ButAc) (4/1). The dose of the extracts in inhibition test were expressed as mg extract/mL incubation solution. The equivalent concentration of sediment (mg dry sediment/mL incubation solution) were calculated from the amount of extracts (mg) from dry sediments.

The activity of alkaline phosphatase (ALP) was measured by the following method. A 0.1 mL sample solution and 0.9 mL of a 0.2 mg/mL alkaline phosphatase solution (from calf intestine, Sigma USA) were mixed and preincubated for 30 min at 37°C. A 0.1 mL aliquot of the preincubated solution was added to a mixture of 3 mL of 5.5 mM sodium p-nitrophenylphosphate/glycine buffer solution (pH 10.5). Adsorption was measured at 410 nm by a spectrophotometer (Hitachi 101 Japan). The inhibition rate due to the extract was calculated by comparing the enzyme activity, using the DMSO/ButAc solution instead of sediment extract.

For the activity of β-glucuronidase (β-GL), a 0.1 mL

sample solution and 0.9 mL of a 0.3 mg/mL β-GL solution (from bovine liver, Sigma USA) were preincubated for 30 min at 37°C. The preincubated solution (0.1 mL) was added to the mixture of 0.9 mL of 5.6 mM 4-nitrophenyl-β-glucopyranosid uronic acid/0.2 M acetic acid buffer (pH 5.0) and incubated for 45 min at 37°C. Then 2 mL of 0.1 N NaOH solution were added. The adsorption of the solution was measured at 410 nm by a spectrophotometer. The inhibition rate was measured by the same method as above.

The activity of glucose-6-phosphate dehydrogenase (G-6-PDH) was measured by the following method. A 0.1 mL sample solution and 0.9 mL of 5.6 μg/mL G-6-PDH solution (Baker's yeast, Sigma USA) were mixed and preincubated for 30 min at 25°C. The preincubated solution (0.05 mL) was added to mixture of 2.2 mL of 0.015 mM glucose-6-phosphate/0.05 M tris-HCl buffer solution (pH 8.1), and 0.75 mL of 0.1 mM NADP solution. The adsorption of the solution was measured at 340 nm. The inhibition rate was determined by the same method as ALP.

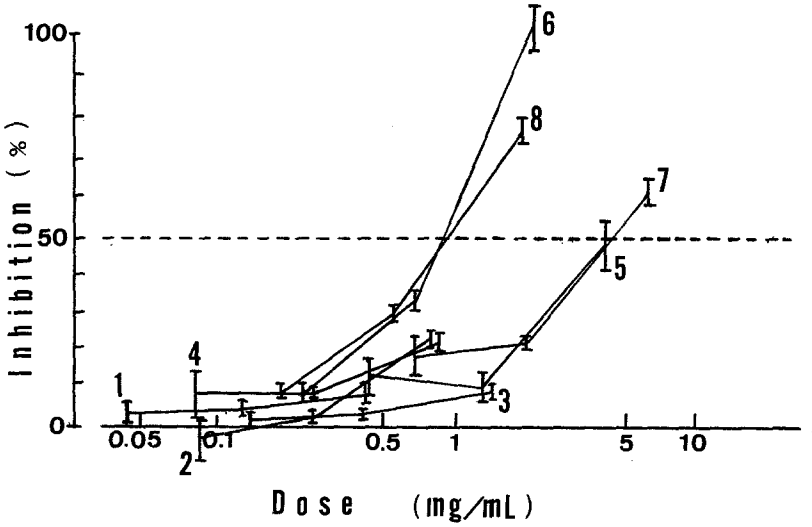
Enzyme inhibition test by chemicals was carried out as follows. The chemicals were listed in Table 2. PCB (Kanechlor 500) was a gift from Dr. Takeshita of the Institute of Public Health, Tokyo. Organophosphorous and organochlorine pesticides were purchased from Poly Science Co Ltd (USA) and the others from Kanto Chemical Co Ltd (Japan). They were dissolved in distilled water or methanol. The inhibition intensities toward ALP, β-GL and G-6-PDH were measured by same method as described above.

RESULTS AND DISCUSSION

The dose-response between enzyme activity and the sediment of each extract was examined. Fig. 2 shows the dose response curve for inhibition of β-GL by the sediment extracts of the Tama River. Enzyme inhibition was dependent on the extract dose. The same relationships were obtained for other two enzymes.

Table 1 shows the concentration of the extracts and sediment with a 50% enzyme activity inhibition (I_{50}). In the Tama River, Hex extracts inhibited only G-6-PDH, showing stronger inhibition at the middle of the stream. EtAc extracts inhibited the activity of β-GL and G-6-PDH especially inhibited at the middle and lower streams. MeOH extracts inhibited the three enzymes, ALP showing inhibition only at lower stream.

In the Ayase River, the activities of β-GL and G-6-PDH were inhibited by Hex extracts from the upper to lower reaches. The activities of the three enzymes were



Figuer 2. β-Glucuronidase inhibition by ethyl acetate extracts from the Tama River sediments.
1. Kawai 2. Mannen 3. Nagata 4. Hino
5. Koremasa 6. Izumi 7. Maruko 8. Daishi

inhibited strongly by the EtAc extracts from the lower stream. β-GL and G-6-PDH activities were inhibited by the MeOH extract strongly at the lower stream in the Ayase River.

According to the previous reports, the water and sediment of the Tama River contained many kinds of organic compounds, such as phenols, aromatic acids, fatty acids, pesticide, solvent (Ogura 1975), but the content of them were not clear. To ascertain the inhibitory effects of the compounds against the three enzyme activities, 39 of chemicals which is supposed to accumulate with water pollution in river sediment were tested. Table 2 shows I_{50} values of organophosphates, phenol compounds, organochlorines, solvents, metals and various other compounds.

G-6-PDH was the most sensitive enzyme toward the chemicals and was inhibited by many kinds of organic or inorganic compounds. Especially it was markedly inhibited by organochlorine and also by common chemicals such as fatty acids. β-GL was the second-most sensitive enzyme toward the chemicals. ALP was inhibited by metal ions, and weakly so by organophosphate or organochlorine pesticides, well known to be toxic toward organisms. Inhibition of ALP activity was observed by chelate chemicals, such as tartaric acid and o-phenanthroline.

The upper stream of the sampling sites of the Tama River

Table 1. Inhibition of alkaline phosphatase, β-glucuronidase and glucose-6-phosphate dehydrogenase activity by various solvent extracts.

Sampling station	Inhibitory effect I_{50} (mg/mL)								
	n-Hexane			Ethyl acetate			Methanol		
	ALP	β-GL	G-6-PDH	ALP	β-GL	G-6-PDH	ALP	β-GL	G-6-PDH
Tama River									
Kawai	-	-	+	-	-	0.05(710)	-	+	0.06(750)
Mannen	-	-	+	-	+	0.12(220)	-	0.36(980)	0.02(36)
Nagata	-	-	0.48(490)	+	-	0.16(180)	-	+	+
Hino	-	-	0.50(3500)	-	+	0.06(450)	-	0.73(3800)	0.06(310)
Koremasa	-	-	0.26(1300)	+	+	0.04(85)	-	1.70(1800)	0.08(87)
Izumi	-	-	0.42(1900)	+	0.84(690)	0.08(68)	1.80(10000)	0.74(4200)	0.08(450)
Maruko	-	-	+	-	4.60(8400)	0.05(91)	1.40(2000)	0.25(370)	0.02(31)
Daishi	-	-	+	+	0.92(4500)	0.03(160)	-	2.50(5000)	0.18(370)
Ayase River									
Yawata	-	+	0.23(2500)	-	+	0.05(860)	0.54(2100)	0.75(2900)	0.08(320)
Ohohashi	-	+	+	+	+	0.08(220)	0.88(2400)	0.82(2200)	0.04(120)
Kamitoi	-	+	0.19(3200)	+	+	0.05(560)	+	1.20(3600)	0.04(130)
Sato	-	+	0.30(980)	+	+	0.08(90)	+	0.25(390)	0.03(33)
Ayase	-	-	+	+	+	0.06(160)	0.46(860)	0.26(500)	0.02(42)
Miyashiro	-	+	0.34(600)	+	1.90(7700)	0.06(250)	2.40(4600)	0.33(640)	0.03(55)
Horikiri	-	-	0.91(690)	+	+	0.09(78)	+	0.21(90)	0.02(10)

+: below 50% inhibition
-: no inhibition
( ): equivalent concentration of sediment (mg sediment/ml incubation solution)

Table 2. Inhibitory effect of chemicals on alkaline phosphatase, β-glucuronidase and glucose-6-phosphate dehydrogenase.

Chemicals	I_{50} (mol/L)		
	ALP	β-GL	G-6-PDH
Sumithion	-	-	3.7×10^{-3}
DDVP	5.2×10^{-3}	$2,9\times10^{-3}$	6.5×10^{-3}
Pentachlorophenol	1.2×10^{-2}	2.1×10^{-3}	1.3×10^{-3}
Aldrin	-	-	5.3×10^{-4}
Dieldrin	-	-	3.7×10^{-4}
Heptachlor	-	-	3.3×10^{-4}
DDT	-	+	3.1×10^{-4}
BHC	-	-	1.3×10^{-3}
Phenylmercuric chloride	+	+	5.7×10^{-7}
Sodium dodecyl benzensulfate	2.0×10^{-3}	5.5×10^{-4}	7.7×10^{-5}
Benzalkonium chloride	+	+	1.0×10^{-4}
Palmitic acid	-	-	5.7×10^{-4}
Oleic acid	+	+	6.3×10^{-5}
Benzene	-	-	7.9×10^{-1}
Chloroform	-	+	1.9×10^{-1}
Trichloroacetic acid	7.0×10^{-4}	1.2×10^{-3}	5.6×10^{-3}
2,4,5-Trichloro phenol	1.3×10^{-2}	6.4×10^{-3}	3.2×10^{-1}
PCB	-	-	8.4×10^{-4}

Chemicals	I_{50} (mol/L)		
	ALP	β-GL	G-6-PDH
α-Naphthol	-	1.5×10^{-2}	3.6×10^{-3}
β-Naphthol	-	+	5.4×10^{-3}
o-Phenylphenol	-	9.3×10^{-3}	3.1×10^{-3}
Tartaric acid	1.0×10^{-3}	1.1×10^{-2}	2.3×10^{-3}
Aniline	-	3.5×10^{-2}	1.5×10^{-1}
α-Naphthylamine	-	2.1×10^{-2}	9.8×10^{-3}
Pyridine	-	2.6×10^{-2}	-
o-Phenanthrorine	1.0×10^{-3}	+	+
$CuCl_2$	3.2×10^{-2}	1.4×10^{-2}	3.9×10^{-3}
$NiCl_2$	-	+	6.7×10^{-3}
$ZnCl_2$	-	2.9×10^{-3}	+
$Be(NO_3)_2$	2.5×10^{-4}	-	8.9×10^{-3}
$HgCl_2$	3.0×10^{-3}	6.4×10^{-5}	7.0×10^{-5}
$FeSO_4$	+	-	6.7×10^{-4}
$CoCl_2$	+	+	8.4×10^{-3}
$CdCl_2$	+	6.7×10^{-3}	4.1×10^{-3}
K_2CrO_4	+	1.1×10^{-3}	+
$K_2Cr_2O_7$	+	9.4×10^{-3}	+
$SnCl_4$	7.0×10^{-5}	4.4×10^{-4}	9.8×10^{-4}
$SnCl_2$	3.3×10^{-4}	5.7×10^{-4}	2.1×10^{-3}
KCN	9.1×10^{-4}	1.8×10^{-4}	-

+: below 50% inhibition
-: no inhibition

DDVP: o,o-Dimethyl O-(2,2-dichlorovinyl)phosphate
DDT: 2,2-bis(p-chlorophenyl)-1,1,1-trichloroethane
BHC: Benzenehexachloride
PCB: Poly chlorinated biphenyl (KC 500)

is a mountain stream and the river bed consists of sand. The samples were jet black mud with a bad smell at the middle and lower streams. This may be caused by the flow of domestic waste water in the middle stream and both domestic and industrial waste water in the lower stream. Regarding the classification of the biological index using benthos in the Tama River, the upper stream (up Nagata) was classified as the oligotrophic or β-mesotrophic type and the middle and lower streams (below Hino) as α-mesotrophic and dystrophic types. The river bed of the Ayase River was jet black mud in the upper to lower streams and the water was classified as the α-mesotrophic type (Morishita 1977, 1978). This river was also polluted by domestic and industrial waste water. Considering the above, the lower streams of both rivers may be polluted by artificial pollutants. The enzyme inhibitions considered to be due to these pollutants were observed strongly at the lower stream on both rivers.

Many investigators have reported the inhibition of ALP activity in liver, kidney and marrow of shellfishes or mammals by heavy metals or organochlorine compounds (Dhavale and Masurekar, 1986). Miszta (1986) also reported the inhibition of rat marrow G-6-PDH activity by nickel. In this study, ALP, β-GL and G-6-PDH were inhibited by the extracts from the Tama River sediments. The enzymatic toxicity of the sediment may affect the distribution of benthic organisms.

Acknowledgments. This work was supported by a grant-in-aid for Special Project on Environmental Science under Grant No 57030071 from the Ministry of Education, Science and Culture.

REFERENCES

Armant DR, Buikema AL Jr, Rutherford CL, Cairns J Jr (1980) Evaluation of in vitro enzyme inhibition for screening petroleum effluents. Bull Environ Contam Toxicol 24:244-250

Dhavale DM, Masurekar VB (1986) Effect of cadmium exposure on the activity of phosphatases in the hepatopancreas of crab Scylla serrata (Forskal). Indian J Mar Sci 15:193-194

Flint KP, Harrison L (1984) The use of immobilised enzymes for the detection of river pollution. Int Environ Safety News 6:8-12

Fritsch G, Kussmaul H, Sonnenburg J (1975) Cholinesterase-Hemmtest zur Beurteilung der Wassergewinnung durch Uferfiltration. Wasser 45:75-90

Geve PA (1975) Die semi-quantitative Bestimmung der Cholinesterase Hemmenden Aktivitat von Gewassern. Wasser 44:15-21

Miszta H, Dabrowski Z, Szygula Z, Spodaryk K (1986)

The effect of intrarenal nickel subsulfide injections upon the activity of selected erythrocyte and bone marrow enzymes in rats. Pharmacol Toxicol 59:425-429
Morishita I (1977) Kawa no kenko-shindan. Nihon Hoso Shuppan Kyokai Tokyo pp210
Morishita I (1978) Seibutsu kara mita Nihon no Kasen. Sankai-do Co Ltd p146-147
Obst U (1984a) Enzymatische Methoden zur Bestimmung der Mikrobiologischen Staffwechselaktivitat in Oberflachen und Grundwasser. Vom Wasser 63:1-5
Obst U (1984b) Ergebnisse Biochemischer Untersuchungen von Oberflachen und Grundwasser. Vom Wasser 63:7-16
Ogura N, Ambe Y, Ogura K, Ishiwatari R, Mizutani T, Satoh Y, Matsushima H, Katase T, Ochiai M, Tadokoro K, Takada T, Sugihara K, Matsumoto G, Nakamoto M, Funakoshi M, Hanya T (1975) Chemical composition of organic compounds present in water of the Tamagawa river. Jap J Limnol 36:23-30
Rutherford CL, Buikema AL Jr, Armant DR, Cairns J Jr (1979) A simple method for screening petroleum effluents by in vitro enzyme inhibition. Bull Environ Contam Toxicol 21:79-84
Tabata M, Bannai E, Nishizono H, Suzuki S (1984) In vitro cholinesterase inhibition of organic matter in urban and rural river sediment. Bull Environ Contam Toxicol 32:391-399
Tsuda M (1972) Biology of polluted waters. Hokuryu-kan Co Ltd, Tokyo pp258
Tyler G (1974) Heavy metal pollution and soil enzymatic activity. Plant Soil 41:303-311
Tyler G (1976a) Influence of vanadium on soil phosphatase activity. J Environ Qual 5:216-217
Tyler G (1976b) Heavy metal pollution. Phosphatase activity and mineralization of organic phosphorus in forest soils. Soil Biol Biochem 8:327-332

Received December 15, 1988; accepted October 18, 1989.