

Ecotoxicology of Micro Organic Pollutants in Water

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In Japan, rapid development of economy during the 1960s caused the problems of environmental pollution, and hazards occurred in many places of Japan, water pollution had increased in those days, and water used for drinking, agriculture, fishing, industry and recreation were damaged. By water-born diseases like as Minamata and Itai-Itai diseases, many patients suffered as you know well. In 1967, Japanese Government set up Basic Law for Environmental Pollution Control and in 1970 Water Pollution Control Law.

Recovery of Water Quality¹⁾

The author's prefecture is Gifu, located in the center of Japan, and three famous rivers flow from North to South and down to the Pacific. The population of the areas along these rivers and tributaries has increased and river pollution had begun to pose a serious social and political problem. Branch rivers lanked at E were highly polluted and their BOD values were 30-50 ppm. To achieve the water quality standard, BOD loads of this river system were surveyed at first. The loads were calculated from population, industries and domestic animals, and large percentage of the load is due to industries such as 59.7% in Arata River, 66.2% in Sakai River and 89.2% in Suimon River.

From these data, Gifu Prefectural Water Quality Council, the author is a President of

the Council, have been drafting the application of more stringent effluent standards for factories or establishments, and the prefectural government enacted the regulation by the prefectural Diet's consent.

By this regulation, the effluent standards are established and each factories constructed treatment plant for the effluent and BOD loads of these rivers had been cut down greatly. Standard stationary points are set up in each rivers and measured water quality. Fig. 1 shows the typical yearly change of BOD. As the results of effluent's control, water quality has been improved, especially small rivers winding through large cities becoming clearer year by year and their BOD values reached below 10 ppm. Main rivers were not so polluted, and so those are becoming clearer slowly.

Contrary to these rivers BOD load of Miya River from industries is only 11.5%. The water quality was very good in 1970 but is becoming highly polluted, because the population in this basin is increasing, and the large percentage of the load is due to domestic waste.

Night Soil Treatment²⁻⁶⁾

Fig. 2 shows state of night soil treatment in Japan in 1970. The treatment by sewer system was very low in percentage. Night soil had been used as fertilizer but its usage

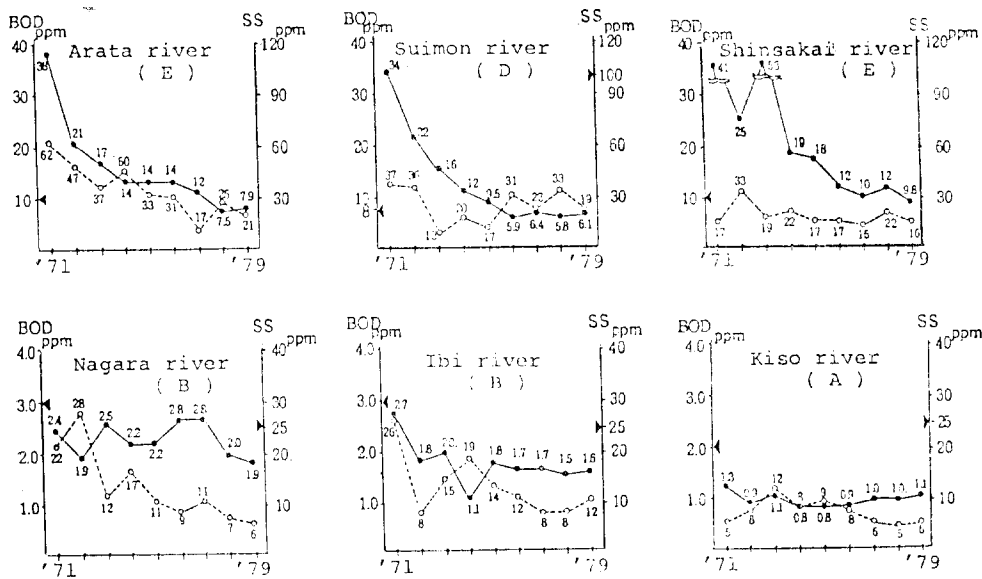


Fig. 1: Yearly change of BOD and SS measured at monitoring stations of Nagara, Ibi and Kiso River and their branch rivers (Annual average). (●—● BOD) (○-----○ SS)

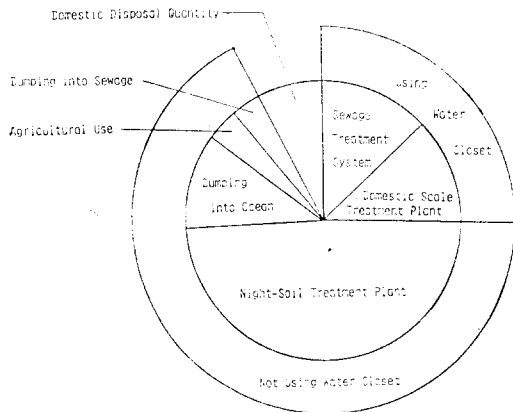


Fig. 2: State of night soil treatment in Japan (1970).

for fertilizer had ceased because its epidemic hazards and production of chemical fertilizer. And dumping into sea got controlled by act, night soil had been treated by the plant

based on anaerobic digestion. But the plant had odor pollution, so the establishment of night soil treatment plant did not increased. So night soil treatment was one of the most difficult problems in environmental hygiene.

The author designed aerobic digestion plant of non-diluted night soil in 1969. Fig. 3 shows the flow sheet of the system. This plant consists of digestion tank, aeration tanks for non-diluted night soil, sedimentation tank, second aeration tank and final clarifier and the treatment capacity was 20 kl/day.

The biota of this digestion tank was different from that of standard activated sludge, bacteria is main biota. The number of bacteria in the supernatants of the tank increased as non-coagulated state. Non-diluted night soil was digested by the bacteria.

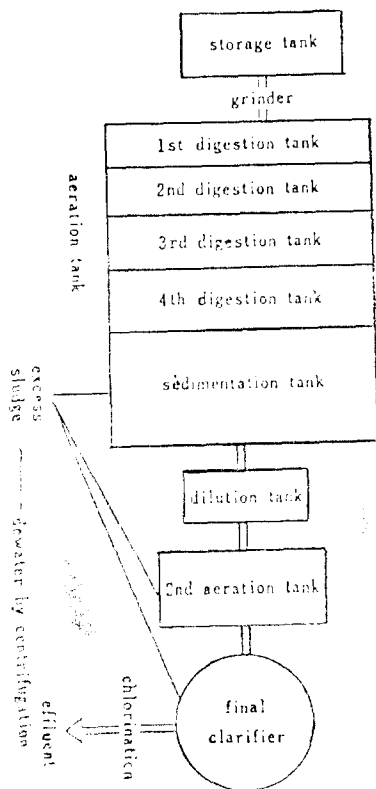


Fig. 3: Process of aerobic treatment plant for non-diluted night soil.

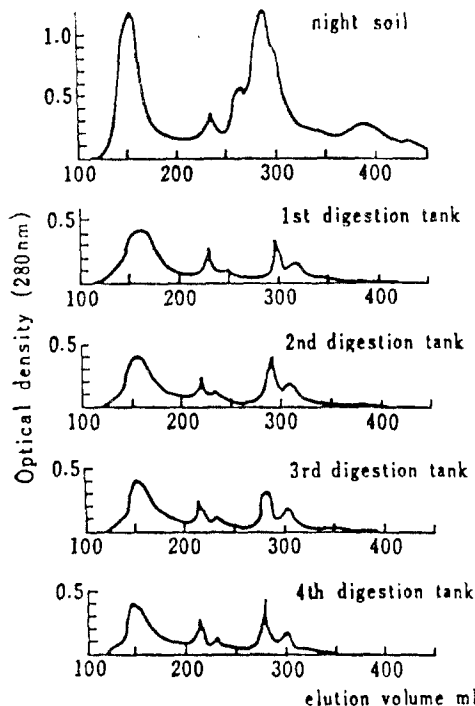


Fig. 4: Elution diagram of night soil and treated water on Sephadex G-25. solvent: 0.02% NaN_3 bed dimensions: 80×2 cm sample: supernatant centrifuged at 1200 g flow rate: 0.5 ml/min

Fig. 4 show Sephadex G-25 elution profile at OD_{250} of the supernatant from each sample (centrifugation at $12,000 \times g$). On the comparison of elution diagrams, the peak height decrease well in the first digestion tank, and the small peak remained in lower molecular fraction.

Change of BOD through the treatment process is shown in Fig. 5. BOD is well removed in first digestion tank. BOD in soluble and fine particle fractions are removed very well, and some of BOD in large particle fraction remained. As the total BOD, more than 90% can be removed.

Organic compounds in the effluent were determined by gas chromatography-mass spectrometry (GC-MS). Indol and methylindol are well known as odorous substances of night soil, and in addition to those we identified oxindol as new odorous compound. These odorous compounds were removed by aerobic treatment, but could not removed by anaerobic treatment. This suggests the benefit of aerobic treatment. In Japan, this aerobic treatment plant is becoming the main plant. *Trihalomethane*⁷⁻⁸⁾

The effluent from night soil treatment plant should be disinfected by chlorination. The

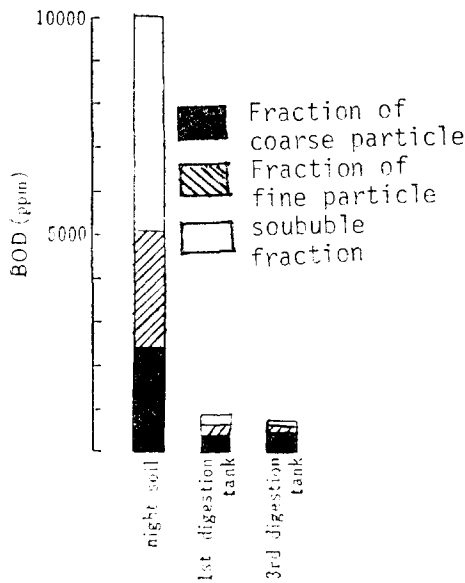


Fig. 5: Contribution of night soil and treated water fraction to the BOD.

authors found chloroform formation in the effluent by chlorination using the gas chroma-

tography and Multiple Ion Detector (MID) chromatogram by GC-MS shows the typical pattern of tri-chlorine atoms.

Chloroform has been recognized as the important carcinogenic substance in drinking water. American National Cancer Institute and the other researchers reported the data.

In tap water these trihalomethans can be detected easily by the Total Ion Monitor (TIM). Many surveys on the THM concentration in tap water have been published. The concentrations of THM in drinking water distributed very wide range, ND, 0.1-600 $\mu\text{g/liter}$. Total-THM in tap water was regulated as below 0.1mg/liter as yearly average in U.S.A. and Japan.

The most important and interested subject is to find the precursor substances. Rook^{9,10} had reported that humic substances in water is the main precursor of THM. The authors

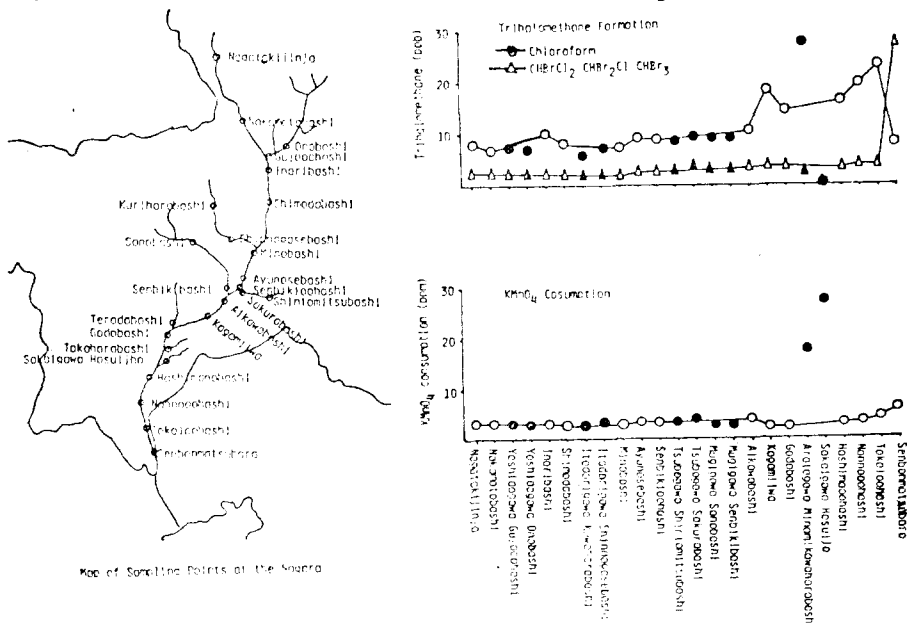


Fig. 6: Trihalomethane formation potential of surface water at Nagara River.

— ○ — main stream — ● — branch

studied about this subject. Water samples were collected from Nagara River at 24 points, and the forming ability of THM was measured and the results are shown in Fig. 6. At the middle area of the basin, Gifu City is in this area, and the formation potential of the water increased from the city area and toward the down stream, this suggests the increasing of the precursor. In city area, domestic wastewater and industrial effluents are discharged, and these are supposed to contain the precursor. In upper streams, low level of the formation potential can be recognized, and it was supposed to owe to natural born humic substances.

When chlorine was added to the effluent of night soil treatment plant, THM formation increased according to available chlorine concentration. (Fig. 7)

THM formation from bath water is same as effluent. In this case some bromide was found. From swimming pool water THM was formed, but this water does not show the linear relationship between formed THM and KMnO_4 consumption because the water is always disturbed and agitated, so THM will be evaporated.

Humic substances was separated from concentrated river water and then Sephadex G-50 fractionation of the substances was performed. THM formation in each fraction was measured. Humic substances had the small peak in high molecular fractions and the large peak in low molecular fractions. THM formation potential corresponded to peak area of the fractions but in the middle of low molecular fractions, the forming ability lacked.

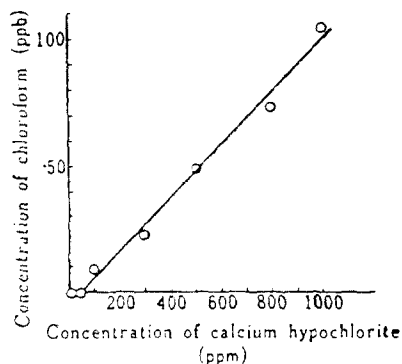


Fig. 7: Formation of chloroform by chlorination of effluent from night soil treatment plant. Each concentration of calcium hypochlorite was added to the effluent and stood for two hours. pH was 8.

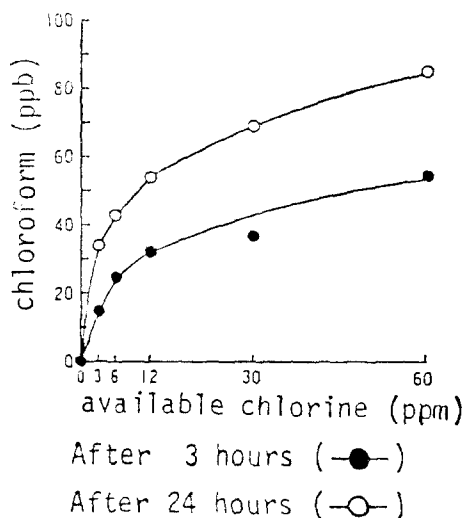


Fig. 8: Formation of chloroform by chlorination of river water.

Same experiment had done for the humic substances separated from leaf mold by water extraction, and in the middle of low molecular fractions the forming ability lacked, too. Why this fraction does not form THM?

When chlorine was added to river water, the quantity of formed THM increased accor-

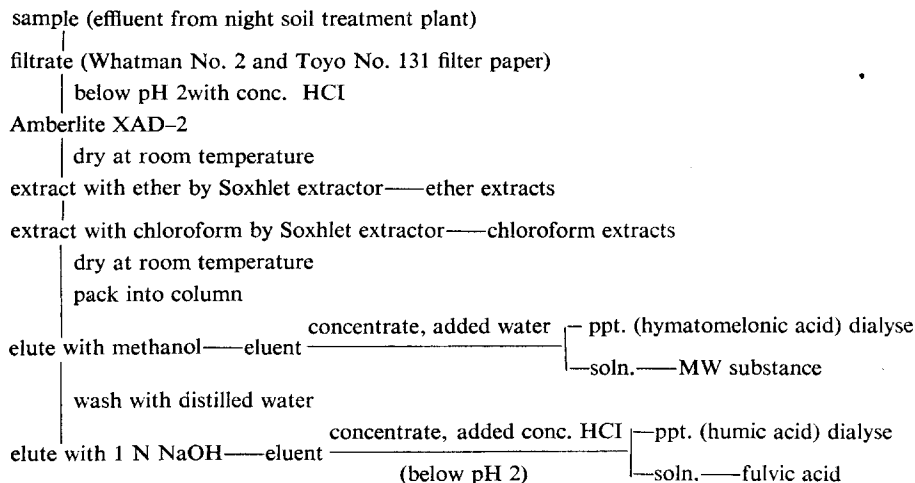


Fig. 9: Fractionation of humic substances.

ding to time proceeded. (Fig. 8)

When chlorine was added to the effluent from night soil treatment plant, time dependency of THM formation can be seen.

Humic substances are fractionated to humic acid, fulvic acid, hymatomelonic acid and methanol water soluble fraction by the flow sheet.

From the THM formation potential and the yield of each fraction, simulation of chloroform formation from effluent was recalculated as per weight of each fractionated substances. According to this simulation, selection of treatment techniques can be performed for each water. Because humic acid can be removed and fulvic acid remained by coagulation-sedimentation. Fulvic acid can be adsorbed to activated carbon.

Environmental Mutagen^{11, 12)}

Recently, many carcinogens and mutagens have been found in environment, so the authors try to find any environmental mutagen from river water. But their concentrations

are very low in river water, so the bottom sediments of Nagara River were used as samples. From upper stream, one point, from middle stream five points were selected.

Samples are fractionated to acidic, neutral and basic fractions with ether extraction, and Ames test using salmonella TA 100 and TA 98 strains was applied. Positive results are found in neutral fraction of point (d), neutral and basic fraction of point (e) by TA 100 strain with S-9 mixture.

Neutral fraction is fractionated by silica gel chromatography into four fractions using four kinds of organic solvents. Ames test with and without S-9 mixture were compared. From the positive samples, BHT, phthalate, anthraquinon derivatives and steroids were detected.

Micro Pollutants in Aquatic Environment

It is supposed that many chemicals exist in environment. Dr. Shinohara¹³⁻²³⁾, a member of the author's laboratory, performed the detection and identification of organic

Table I: Yields of each fractions.

	Yields of color unit	Percentage	Yields of weight (mg)	Percentage
Ether fraction	8.6	2.5		
CHCl ₃ fraction	3.8	1.1		
Methanol (acidic) fraction	113	32.3		
Hymatomelonic acid	40.3	11.5	330.6	0.45
Methanol water soluble substance	48.0	13.7	936.6	1.27
1N-NaOH fraction	33.9	9.7		
Humic acid	20.4	5.8	104.6	0.14
Fulvic acid	10.2	3.0	211.0	0.29
Methanol (basic) fraction	18	5.1	195.3	0.27

Color unit of effluent from night soil treatment plant was 350.

Color unit that was not absorbed by Amberlite XAD2 was 50.

Total Residue of effluent was 72.8g.

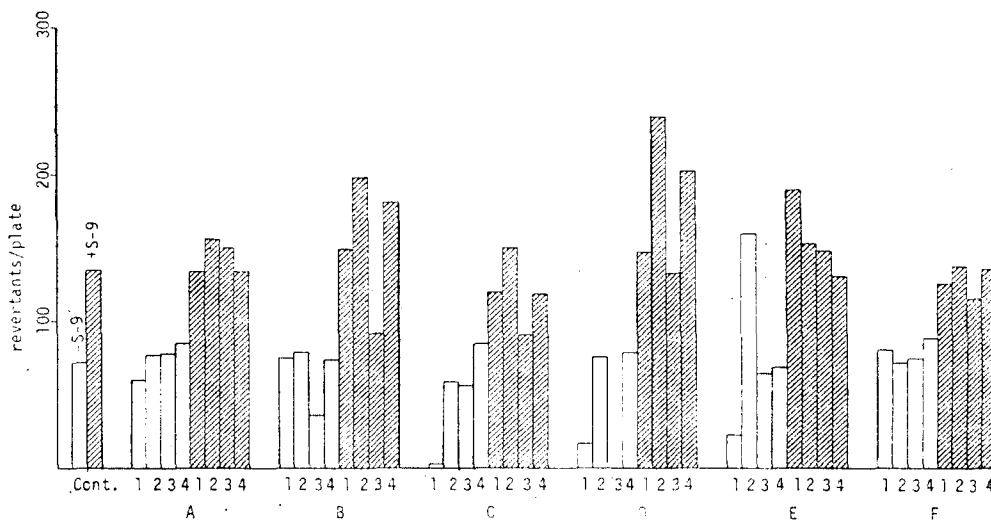


Fig.10: Mutagenicity of fractionated river sediment by TA 100 strain.

substances in aquatic environment. The procedure was made suitable to determination of micropollutants.

Identified compounds from aquatic environment are listed in Table II in the series of molecular weight. About 200 kinds of compounds were found. Some are known as carcinogen and mutagen, but the others are not known about their toxicity.

Ecotoxicology^{24, 25)}

On the earth, about 4,000,000 kinds of compounds are known, and more than 10,000 kinds of them are distributed and used now, and they circulate in environment. Some compounds are known about their toxicity, but these compounds found at low concentration such as ppb or ppt. This level is lower than the effective dose for mammals. So, the che-

Table. II: Compounds found in the environmental samples.

Compounds	Molecular formula	M.W.	Sample type	Concentration (ng/l)
Acetone	C ₃ H ₆ O	58	Tap water	
Benzene	C ₆ H ₆	78	Tap water	
Pentanol	C ₅ H ₁₂ O	88	Sea water	
Butyric acid	C ₄ H ₈ O ₂	88	Tap water	
Ethyl acetate	C ₄ H ₈ O ₂	88	Tap water	
Toluene	C ₇ H ₈	92	Tap water	
Phenol	C ₆ H ₆ O	94	River water (ss)	
			River water (filtrate)	
			Tap water	0.87
Butyl formamide	C ₅ H ₁₁ ON	101	River water (ss)	
2-Ethoxy-1-propanol	C ₅ H ₁₂ O ₂	104	Tap water	
Benzaldehyde	C ₇ H ₆ O	106	River water (ss)	
			River water (filtrate)	
			Tap water	
Xylene	C ₈ H ₁₀	106	River water (filtrate)	
			Tap water	0.32
Benzyl cyanide	C ₈ H ₇ N	117	Tap water	
Allylbenzene	C ₉ H ₁₀	118	Tap water	
Chloroform	CHCl ₃	118	Tap water	
1,3,5-Trimethylbenzene	C ₉ H ₁₂	120	Tap water	3.9
1,2,5-Trimethylbenzene	C ₉ H ₁₂	120	Tap water	1.3
1,2,4-Trimethylbenzene	C ₉ H ₁₂	120	Tap water	3.3
n-Propylbenzene	C ₉ H ₁₂	120	Tap water	5.9
iso-Propylbenzene	C ₉ H ₁₂	120	Riverwater (filtrate)	
Phenylacetaldehyde	C ₈ H ₈ O	120	Riverwater (ss)	
Acetophenone	C ₈ H ₈ O	120	Sea water	
			River water (filtrate)	
			Tap water	5.4
Nitrobenzene	C ₆ H ₅ NO ₂	123	Sea water	
Naphthalene	C ₁₀ H ₈	128	Sediment	
			Sea water	
			Sewage (influent)	
			River water (filtrate)	
			River water (ss)	
			Tap water	2.2
Quinoline	C ₉ H ₇ N	129	Sediment	
iso-Quinoline	C ₉ H ₇ N	129	Sediment	
Octanol	C ₈ H ₁₈ O	130	Sea water	
2-Ethyl-1-hexanol	C ₈ H ₁₈ O	130	Tap water	
Indanone	C ₉ H ₈ O	132	Sea water	
			River water (filtrate)	
Methylindan	C ₁₀ H ₁₂	132	Tap water	
m-Diethylbenzene	C ₁₀ H ₁₄	134	Tap water	
p-Diethylbenzene	C ₁₀ H ₁₄	134	Tap water	
o-Diethylbenzene	C ₁₀ H ₁₄	134	Tap water	

micals found in environment will have to be evaluated with effects to ecosystem and mammals through ecosystem. Effects of chemicals to ecosystem could not evaluate from their toxicities to mammals. The needs from many countries to assess the toxicity of the compounds in environment are serious. How to establish the assessing method? The testing method must be accepted to developed and developing countries. Big project team for this problem is working by OECD.

One of the important problems is the selection of species of aquatic biota for test, because if the difference of species show the different results, many species must be tested. The authors tested three kinds of species and five items. Those are closely related among them.

TLm value by Himedaka and the partition co-efficients value of more than 100 kinds of chemicals were measured. Very close relationships were found between the two.

As you know, structure-activity relationship is useful technique for medical synthesis. This relation can be seen between environmental chemicals and their toxicities as shown in this table. So, to assess the chemical toxicity in environment, structure-activity relationships will be utilized effectively.

Decomposition in Environment^{26,27)}

Some of the chemicals will be found in discharged water, but some are produced by synthesized or decomposed in environment. The authors investigated degradation process of 8-Hydroxyquinoline sulfate (8-OQ) in the natural environment and in the river-sediment. The decrease of 8-OQ in the river-sediment was greater under anaerobic condition than

Table III: Correlation coefficient for each two testing methods.

Oryzias TLm	Moina TLm	Dugesia LC ₅₀	Dugesia AgC ₅₀	Tetra-hymena IC ₅₀	
0.879**	0.889**	0.797**	0.837**		<i>Oryzias</i>
(14)	(15)	(11)	(15)		<i>TLm</i>
	0.662**	0.444	0.767**		<i>Moina</i>
	(14)	(10)	(14)		<i>TLm</i>
		0.947**	0.705**		<i>Dugesia</i>
		(11)	(15)		<i>LC</i>
			0.666*		<i>Dugesia</i>
			(11)		<i>AgC₅₀</i>
					<i>Tetra-hymena</i>
					<i>IC₅₀</i>

$\log X = a \log Y + b$

X: index value of X method

Y: index value of Y method

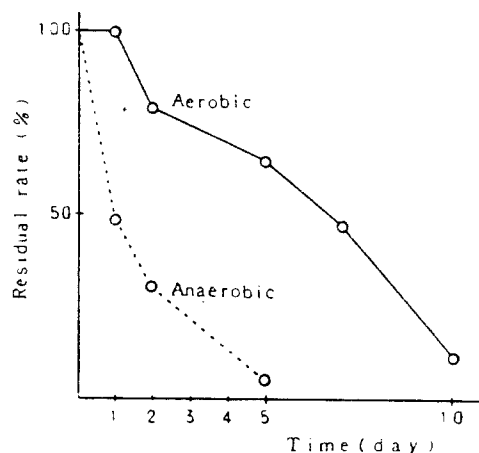


Fig. 11: Time course of 8-hydroxyquinoline sulfate in the supernatant under aerobic and anaerobic cultivation by bioassay used with strain of *B. subtilis* IAM 1213.

aerobic.

The degradability of 8-OQ by the acclimatized bacteria increased with the length of acclimatized period to 8-OQ. Some strains, acclimatized to 8-OQ for 10 days, were found

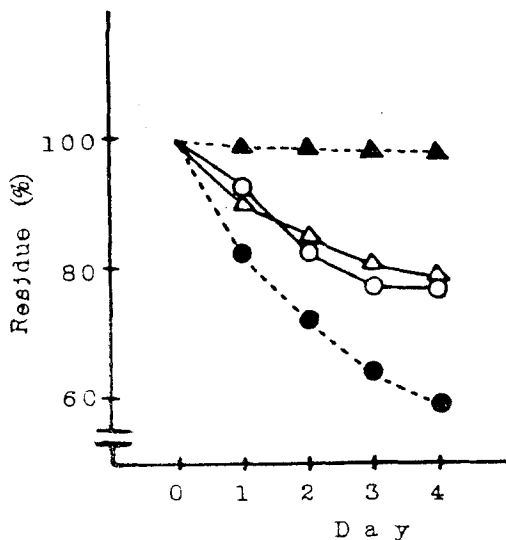


Fig. 12: Degradation of γ -BHC by bacteria acclimatized to 8-OQ.

○—○ OAN₁₀ I △—△ OAN₁₀ III
 ●—● OAN₁₀ II ▲—▲ OAN₁₀ IV

to break down not only some disinfectants but also organochlorine compounds such as γ -BHC. Degradability of disinfectants by the acclimatized bacteria was generally higher under aerobic conditions than anaerobic.

Methylmercury Production with Humic Substances^{28, 29)}

Some chemicals have the high accumulation factor, for example methylmercury. Methylmercury exist at very low concentration level in water but accumulated at high level in organisms and fish and this accumulation caused Minamata disease.

Methylmercury can be produced at low concentration level by biological and non-biological reaction, and non-biological production performed by photochemical and non-photochemical reaction.

A member of the author's laboratory, Dr. Akagi³⁰⁻³⁷⁾, reported that photochemical

reaction of inorganic mercury with CH_3 -donor as acetic acid and methanol in water environment produced methylmercury.

In river water, the authors found methylmercury at ppt level, and the ratio of inorganic and organic is same in any rivers. Ottawa River in Canada, Suimon River in Gifu Prefecture in Japan had been polluted by mercury, but Nagara River has not. Low level but same ratio of mercury compounds were found. From where methylmercury will be produced?

The authors try to find the methylmercury production with humic substances and found. Humic substances were separated from river sediment and leaf mold and mixed with inorganic mercury. From these mixtures we could find the methylmercury.

When humic substances and inorganic mercury will mixed, according to the rising of temperature, the increasing of methylmercury production is observed.

The concentration of humic substance is a factor of methylmercury production.

Concentration of mercury is also a factor of methylmercury production.

Methylmercury production can be seen with fulvic acid.

Concentration of inorganic mercury is a factor in fulvic acid as same as in humic acid.

Formed quantity is increased with time proceeded.

Methylmercury forming ability of each fraction separated by Sephadex G-25 is determined. From the low molecular fraction, the high ability of formation was recognized.

Conclusion

The author introduced some examples of

Table IV: Summary of mercury analysis.

Chemical form of mercury	Mercury concentration (ng/l)			Ottawa River
	Nagara River	Sumion River	Sumion River (effluent water*)	
Mercuric (Hg ⁺⁺)	11.8(61%)	16.3(62%)	22.1 (56%)	3.2 (48%)
Methyl mercuric (CH ₃ Hg ⁺)	5.8 (30%)	7.0 (27%)	11.3 (29%)	2.2 (33%)
Others	1.7 (9%)	2.1 (11%)	6.2 (15%)	1.2 (19%)
Total mercury	19.5 (100%)	26.2 (100%)	39.6 (100%)	6.6 (100%)

* from Ogaki night soil treatment plant.

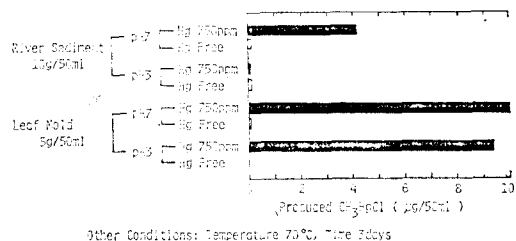


Fig. 13: Methylmercury production by river sediment and leaf mold.

the behavior of micro pollutants in the environment. From the pharmaceutical stand point, I would like to discuss some things. I think pharmaceutical science is based on the interactions between chemicals and human body. How to take chemicals in human body? We have two route—administration and intake. We take chemicals are drugs by admimstration, but the other many chemicals are taken through food, water and air.

Every day we take 1–2 kg of food, 1–2 liter of water, and 10–20m³ of air, which contain chemicals. To control their concentration and prevent the effects to human body is the object of environmental hygienics.

For prevention of the water pollution, many researcher poured the energy to remove BOD and COD from wastewater and river water, and we had the wonderful success.

But, micro pollutants in environment are

increasing according to development of the chemical industries. We know the fact the lead, DDT and PCB had polluted in gloval scale.

The study of the micro pollutants must be developed in international scale. I hope the international co-operation in this field will be spread and deepened. If my report will serve the development of this field in this country, it is my great pleasure.

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