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Monitoring of Mutagens in River and Marine Sediments by Salmonella/Microsome Assay Combined with Blue Cotton Method

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River and marine sediments absorb various chemicals contained in industrial and domestic wastewaters. Therefore, sediments are useful materials for estimation of aquatic environmental pollution. Recently, bacterial mutagenicity test have widely been used for detection of mutagens and carcinogens in the environmental materials. However, sediment samples often give difficulty in determination of the mutagenicity because of the lethal effect of sulfur on bacteria. For removing the toxic compound from sediment samples, several techniques containing liquid-liquid extraction, high performance liquid chromatography and column chromatography have been examined. In these experiments, sediment extracts were separated into a number of fractions. Therefore, these techniques are not necessarily simple and suitable methods for preparation of sediment test samples.

On the other hand, blue cotton bearing copper phthalocyanine trisulfonate can easily adsorb polycyclic aromatic hydrocarbons having more than three aromatic rings in their molecules and is actually used for isolation of mutagens in urine, cooked beef and river water(Hayatsu et al. 1983). In this study, we used the blue cotton method in connection with the Ames test(Ames et al. 1975) for detection of mutagens in the river and marine sediments. This method was confirmed to be a useful technique for monitoring of environmental mutagens.

MATERIALS AND METHODS

Sediment samples were collected from three rivers(Kino, Arida and Hidaka) and eight seashores(Wakayama, Kainan, Shimotsu-Hatsushima, Yura Bay, Tanabe Bay, Kushimoto, Katsuura-Moriura Bay and Miwasaki) in Wakayama Prefecture, south-west of Japan(Figure 1). Each station

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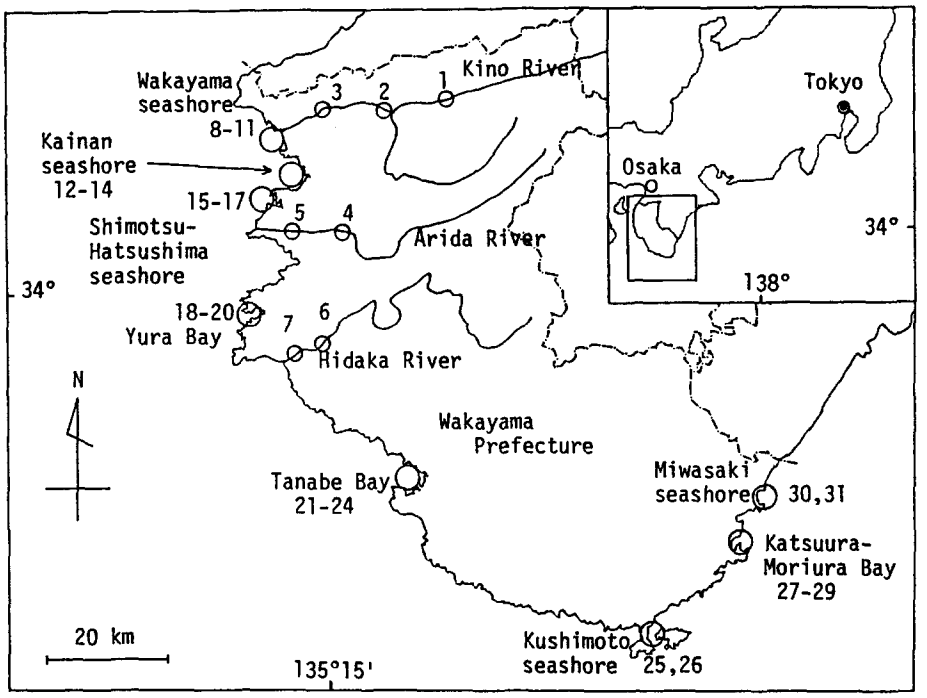

Figure 1. Sampling stations and points of sediment

showed 2 to 4 sampling points and the numbers of sediment samples in 1987, 1988, 1989 and 1990 were 27, 11, 27 and 25, respectively. These sampling experiments have been done in July to October of each year. In addition to the sediments mentioned above, two kinds of sediment A and B were collected from urban rivers in Wakayama City due to the adsorption efficiency test of sediment extract to blue cotton.

The collected sediment samples were spread out in a tray and dried at room temperature. Then, the specimen was powdered in a mortar with a pestle and filtered through a 22 mesh-sieve. The powdered sample(40 g) was shaken with 80 mL of methanol for 10 min and centrifuged at 1,300×g for 5 min. After the supernatant fluids were combined, the solvent was removed by a rotary evaporator. The residue was dissolved in a mixture of 2 mL of dimethylsulfoxide(DMSO) and 98 mL of water. The following procedure was carried out according to the method of Hayatsu et al.(1983). One hundred mg of blue cotton(Funakoshi Chemicals, Tokyo, Japan) was added to the mixed solution and the mixture was shaken for 30 min. After taking out the blue cotton, this treatment was repeated once more with 100 mg of new blue cotton. The blue cotton was washed with water and then moisture of blue cotton was removed with filter paper. The mutagens adsorbed to the blue cotton were eluted with 20 mL of methanol-28% NH_3(50:1) by shaking for 30 min.

The elution treatment was repeated again and the combined eluant was evaporated to dryness under reduced pressure. The residue was dissolved in 0.4 mL of DMSO and subjected to the mutagenicity assay.

The mutagenic activity was determined to Salmonella typhimurium TA 98 with S9 mix by a modified Ames preincubation method(Sugimura and Nagao 1980). The S9 was purchased from Oriental Yeast Co.(Tokyo, Japan). The mutagenicity assay was performed with 0.1 mL, 0.05 mL and 0.025 mL portions of the test solution in duplicate and the number of net revertant colonies per 10 g of dried sediment was calculated by using each linear dose response curve. When the dose response curve was not obtained or the number of revertant colonies of test solution was less than twice compared with that of spontaneous revertant colonies, the value was defined as zero.

RESULTS AND DISCUSSION

The crude sediment extract was not easily dissolved in water or saline. Therefore, after the crude sediment extract was dissolved in 0, 2, 5 and 10 mL of DMSO and then added 100, 98, 95 and 90 mL of water, respectively, the blue cotton treatment and then mutagenicity assay on S.typhimurium TA 98 with S9 mix were carried out. In this experiment, two kinds of sediment(A and B) were used. Mutagenic activity of sediment B is higher than that of sediment A. As shown in Table 1, the highest mutagenic activity was observed in the solvent constitu-

Table 1. Mutagenic activity of sediment extracts as a function of solvent composition.
The methanol extract from sediment A or B was dissolved in DMSO(0～10 mL) and then a total volume of 100 mL was made by addition of water. The mixture was treated with blue cotton and subjected to the mutagenicity assay using S.typhimurium TA 98 with S9 mix.

Solvent(mL)			Net revertant colonies/plate*	
DMSO	+	Water	Sediment A	Sediment B
0	+	100	57	740
2	+	98	81	922
5	+	95	59	738
10	+	90	59	617

* The doses used in Sediment A and B corresponded to 10 g and 0.01 g of dried sediment samples, respectively.

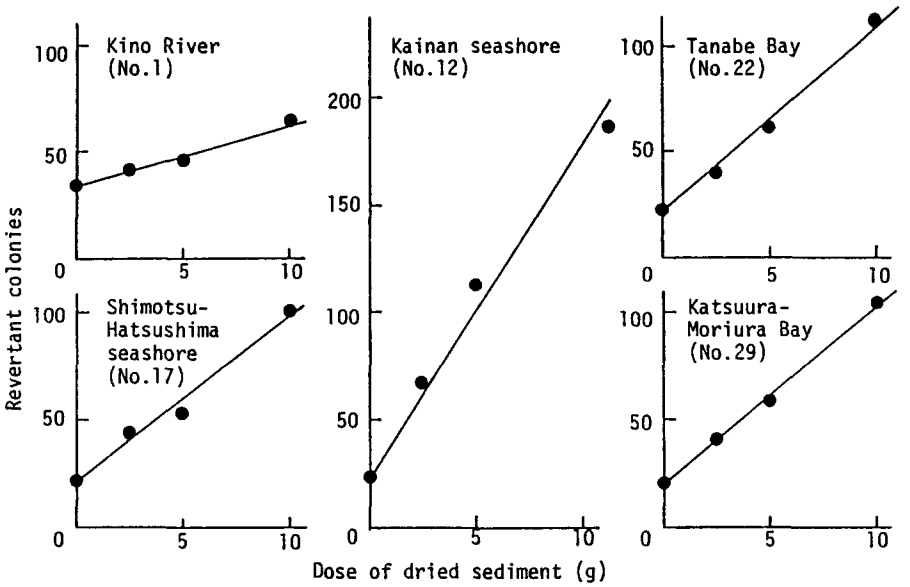

Figure 2. Mutagenic activity of five sediments collected in 1987 on S.typhimurium TA 98 with S9 mix

tion of 2 mL of DMSO and 98 mL of water in both samples. From this result, 2 % DMSO solution was selected as a optimal solvent for adsorbing treatment using blue cotton. As mutagenic activity of sediment B used in this experiment was very high, a sample dose less than 40 g had to use to extract mutagens from the dried sediment. In general, if mutagenic activity of sediment sample is very high, the dose(40 g) of dried sediment for extraction, or the volume(0.4 mL) of DMSO to prepare the solution of mutagenicity assay would have to be ajusted. Furthermore, even with a ultrasonic cleaner, several crude extracts were not freely dissolved in 2 % DMSO solution, and therefore, it was necessary to filtrate the insoluble materials through a white cotton to avoid physical adsorption by blue cotton. If this treatment was omitted, a killing effect on bacteria was usually observed at high concentration.

The dose response curves of samples collected from 5 representative stations in 1987 toward S.typhimurium TA 98(S9 mix) are given in Figure 2. The dose of dried sediment and the number of revertant colonies per plate were plotted as abscissa and ordinate, respectively. All of them were found to be linear, and no killing effect was observed. Similar results were also obtained in the mutagenicity assay of other samples. The mutagenic activity of river and marine sediments collected in 1987 to 1990 are shown in Table 2 as the number of net revertant colonies per 10 g of dried sample. These results showed that the mutagenic activity of marine sediments was higher than that of river sediments. Among

Table 2. Mutagenic activity of river and marine sediment extracts on S.typhimurium TA 98 with S9 mix.

Sampling station	No. of sampling point	Mutagenic activity (Net revertant colonies / 10 g of dried sediment) 1987	1988	1989	1990
Kino River	1	0	—[a]	57	—
	2	0	—	43	—
	3	30	57	44	29
Arida River	4	0	—	32	—
	5	39	34	0	0
Hidaka River	6	0	—	34	—
	7	0	0	34	0
Wakayama seashore	8	—	—	—	350
	9	—	—	—	29
	10	—	—	—	500
	11	—	—	—	410
Kainan seashore	12	170	82	160	150
	13	170	—	110	180
	14	110	—	100	170
Shimotsu-Hatsushima seashore	15	68	53	65	54
	16	78	—	78	93
	17	76	—	68	59
Yura Bay	18	77	57	60	—
	19	63	—	57	58
	20	46	—	35	46
Tanabe Bay	21	62	43	79	45
	22	85	98	65	0
	23	76	—	45	47
	24	49	—	59	57
Kushimoto seashore	25	39	54	38	27
	26	43	—	74	36
Katsuura-Moriura Bay	27	34	—	29	—
	28	30	—	0	0
	29	81	86	57	32
Miwasaki seashore	30	96	—	35	0
	31	32	32	38	0

[a] A dash indicates not tested.

20 river sediments, 9 samples did not induce the revertant colonies more than twice compared with that of the DMSO control at 10 g dose. The maximum revertant colonies per 10 g of dried sediment was 57. On the other hand, among 70 marine sediments, 5 samples did not show any mutagenic activity and the maximum revertant colonies per 10 g of dried sample was 500.

The Wakayama Prefecture is situated on the south-western part of the Kii Peninsula, which is the biggest peninsula in Japan. In the Prefecture, the mountains are close to the coastline and there are few flatlands. The main cities are located along the coast of the Kii Peninsula. The northern coastal part of the Wakayama Prefecture is an industrial zone, which has the steel, petroleum, textile, leather, chemical and other industries. On the other hand, the southern coastal part is a sightseeing zone. Therefore, the aquatic environment of the northern part seems to be more contaminated with industrial wastes than that of the southern part. The highest mutagenic activity of marine sediment was observed in Wakayama seashore samples No.8, 10 and 11 as shown in Table 2, and the values ranged from 350 to 500 revertants in 1990. These data showed that the marine sediments in Wakayama seashore contain relatively strong mutagens. Kainan seashore, which is on the south of Wakayama seashore, also showed high mutagenic activity. The mutagenic activity of other marine sediments exhibited the tendency to become lower with going to south from Kainan seashore. Since the variation of mutagenic activity of marine sediments in the northern part was approximately constant for last 4 years(from 1987 to 1990), it is presumed that an aquatic environment in the northern part is not clearfied as time passes. However, in the southern part, Kushimoto seashore, Katsuura-Moriura Bay and Miwasaki seashore, the mutagenic activity of these sediments collected in 1990 was lower than that of former samples.

It has been also reported that the sediment contamination with mutagens is primarily attributable to polar mutagenic compounds rather than to polycyclic aromatic hydrocarbons(Suzuki et al. 1982 ; Sato et al. 1983). From our experiment, it was demonstrated that the river and coastal sediments are contaminated with several mutagenic compounds containing more than three aromatic rings in the molecules, the contamination can be simply monitored by using blue cotton treatment following to the Ames test. Recently, several researcheres investigated the mutagenic activity of river water treated with blue rayon which has higher copper phthalocyanine trisulfonate content than that of blue cotton(Sakamoto and Hayatsu 1990). The comparison of the retention efficiency of mutagens between blue cotton and blue

rayon is under investigation.

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