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A Simple Method for Monitoring Mutagenicity of River Water. Mutagens in Yodo River System, Kyoto–Osaka

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Blue cotton is a cotton preparation, bearing copper phthalocyanine trisulfonate as a covalently linked ligand, and is an adsorbent specific for compounds with three or greater numbers of fused rings (Hayatsu et al. 1983a). Due to this special property, blue cotton has been used for extracting mutagenic polycyclic compounds from crude materials such as cooked food (Hayatsu et al. 1983b; Takahashi et al. 1985) and cigarette smoke condensate (Yamashita et al. 1986). A new class of polycyclic aromatic mutagens were isolated from opium pyrolysate by use of blue cotton (Friesen et al. 1987).

In our early work, we gave a brief account of the results of monitoring river-water mutagenicity with blue cotton (Hayatsu et al. 1983a). This investigation was carried out in the Asahi river, which flows through Okayama City, and we have since then repeated the monitoring to confirm the efficacy of the method. Blue cotton was also used in monitoring mutagenicity of sea waters (Kira et al. 1989). Recently we have improved the quality of the adsorbent; rayon in place of cotton was employed as the support for the ligand, and a more powerful adsorbent, blue rayon, which contains 2-3 times greater amount of the ligand than blue cotton, was prepared (Hayatsu 1989). In this paper we wish to report the use of the blue-rayon method to detect mutagenic components in the Yodo river, which flows through the cities of Kyoto and Osaka and is a major source of drinking water for the 10 million people in the area.

MATERIALS AND METHODS

Blue rayon was obtained from Funakoshi Chemicals (Kanda Surugadai 2-3, Chiyoda-ku, Tokyo 101), who prepared the material according to our instructions (Hayatsu 1989). The content of copper phthalocyanine trisulfonate in this material was 25 μmol/g. Blue rayon in meshed

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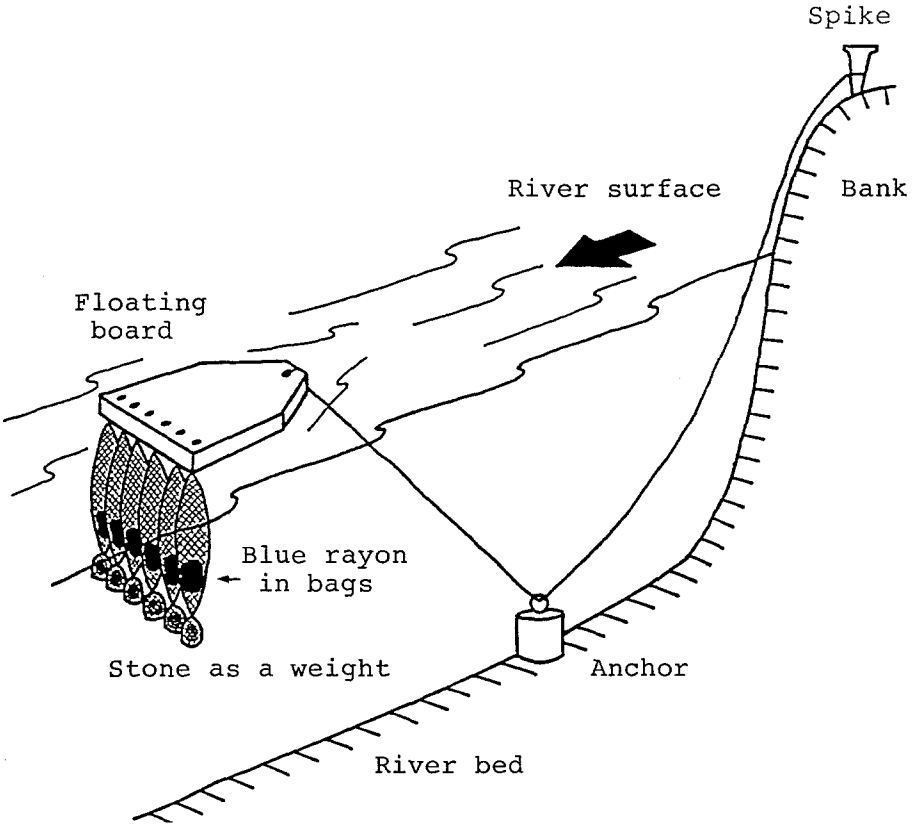

Figure 1. Blue rayon hung in the river.

plastic bags (0.5 g per bag) was hung in the river as shown in Figure 1. Six bags were attached to the floating board, and the length of the hanging bag was about 20 cm. After standing for 24 hr, the blue rayon was taken out, washed with distilled water and dried with paper towels. The blue rayon was then eluted with methanol-concentrated ammonia (50:1, 80 ml/0.5 g blue rayon) by treatment for 30 min with gentle shaking. The elution was repeated once more, and the combined eluent in a flask was evaporated to dryness under reduced pressure. The residue was taken up in a small amount of methanol and transferred to a test tube for the mutagenicity assay. The methanol solution was evaporated to dryness and the residue was dissolved in 0.1 ml dimethylsulfoxide and subjected to the assay.

In one series of experiments, we took water from each monitoring site and the water samples brought back to the laboratory were treated with blue rayon. In this treatment, to 1 L of the water was added 0.5 g blue rayon and the mixture was shaken gently for 30 min. The blue rayon was taken out, a fresh batch of blue rayon

(0.5 g) was added, and the adsorption was repeated. The combined rayon was processed in the same way as that described above for the rayon soaked into the river water. These conditions for the batch adsorption were those previously established for a quantitative extraction of urinary mutagens (Kobayashi and Hayatsu 1984). The chemical oxygen demand (COD) of these water samples was determined with acidic permanganate oxidation according to the standard procedure (Japan Water Works Association 1985).

The mutagenicity assay was carried out by the preincubation technique (Yahagi et al. 1977) with Salmonella typhimurium TA98 as tester bacteria in the presence of S9 mix for metabolic activation. This tester strain is useful for detecting frameshift mutagens (Ames et al. 1975) and is a kind gift of Dr. B.N. Ames of the University of California, Berkeley. The S9 used was prepared from livers of rats that had been treated with polychlorinated biphenyl PCB-54 (Tokyo Kasei Chemicals, Nihonbashi-Honcho 3-1-13, Chuo-ku, Tokyo 103; Cl content ca. 54%). The His^+ revertant colony numbers on plates were counted manually. When the numbers exceeded 1,000, partial-area counting under a microscope was done, based on which the total numbers were determined.

Ascending thin-layer chromatography (TLC) was performed on 10-cm long silica-gel plates. High-performance liquid chromatography (HPLC) was done with a 4.6 x 150 mm reversed-phase column, Inertsil ODS-10, purchased from Gasukuro Kogyo (Nishi-shinjuku 6-12-18, Shinjuku-ku, Tokyo 160). The elution was carried out with a linear gradient of 30% to 100% acetonitrile at a flow rate of 1 mL/min for 60 min. 2-mL Fractions were pooled, evaporated to dryness under reduced pressure and the mutagenicity was measured. Absorbance at 254 nm was monitored by a detector attached to the HPLC.

RESULTS AND DISCUSSION

To evaluate the effectiveness of using the copper phthalocyanine ligand in the adsorbent, we hung blue rayon and plain rayon side by side in the Asahi river of Okayama at a site near the mouth of the river, where we had previously noted a strong mutagenicity as detected by use of blue cotton (Hayatsu et al. 1983a). The rayons were allowed to stand for 2 d in the water and the mutagenicity on Salmonella (TA98, +S9) was assayed. The extract from 1 g blue rayon gave 1,420 His^+ revertant colonies, whereas that from 1 g plain rayon gave only 50 colonies (solvent controls, 30 ± 5). Therefore, this ligand was clearly effective in adsorbing the mutagens from the river water. After investigating several different factors that can affect the efficiency of

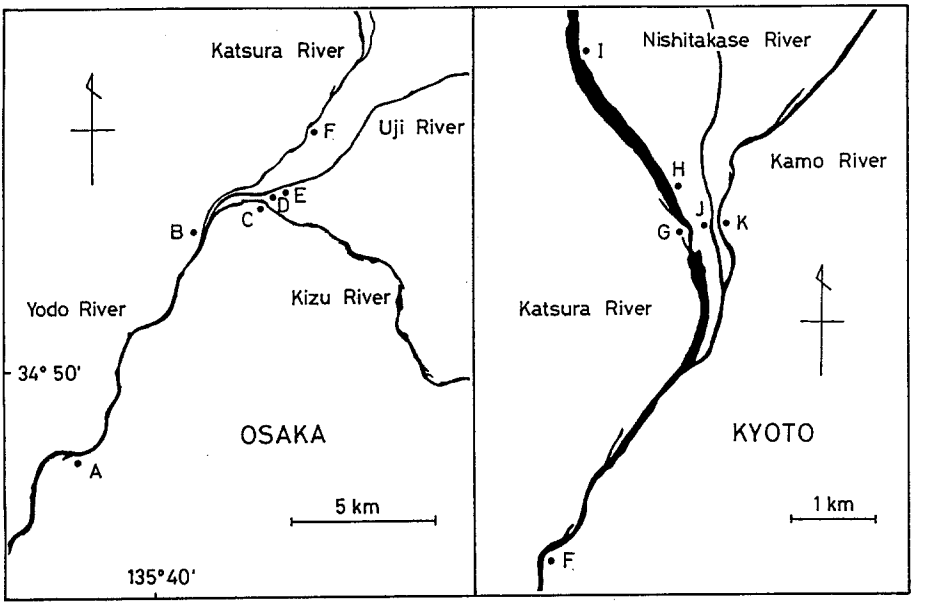

Figure 2. Sites of monitoring in the Yodo river system. A, Hirakata-ohhashi, Osaka; B, Yamazaki, Osaka; C, Gokou-bashi, Kyoto; D, Gokou-bashi-shimo, Kyoto; E, Gokou-bashi-kami, Kyoto; F. Miyamae-bashi, Kyoto; G. Koga-bashi, Kyoto; H. Toba, Kyoto; I, Kuze-bashi, Kyoto; J. Tenjin-bashi, Kyoto; K. Kyokawa-bashi, Kyoto.

adsorbing mutagens in water, we decided to use blue rayon under the conditions described above: 0.5 g per bag for 1-d.

In February 1988, we measured with this blue-rayon method the water mutagenicity of the Yodo river system in Kyoto-Osaka area at six sites (A-F; see Figure 2). As Figure 3 shows, the sample at every site gave positive mutagenicity, with a dose-dependent increase in activity. Large numbers of revertant-colonies per plate were produced from samples at sites B and F. The mutagenicities found at other sites were much lower. It seemed probable that the activity found at B was mostly due to mutagens flowing in from the Katsura river. We repeated the mutagenicity monitoring at F in August and December, 1988, and strong mutagenicities continued to be found; 1,000 revertants in August and 5,000 revertants in December for 0.1 g blue-rayon equivalent extracts. In the December study, we attempted to locate the point of mutagen inflow. The results of monitoring mutagenicity at six sites upstream of F (F-K) are given in Table 1. The mutagenicity at G in the Katsura river was higher than that at F. At a small distance up from G is a large sewage plant of Kyoto City, and we measured the mutagenicity of the water discharged into the river from the plant. For this purpose, we hung blue-rayon

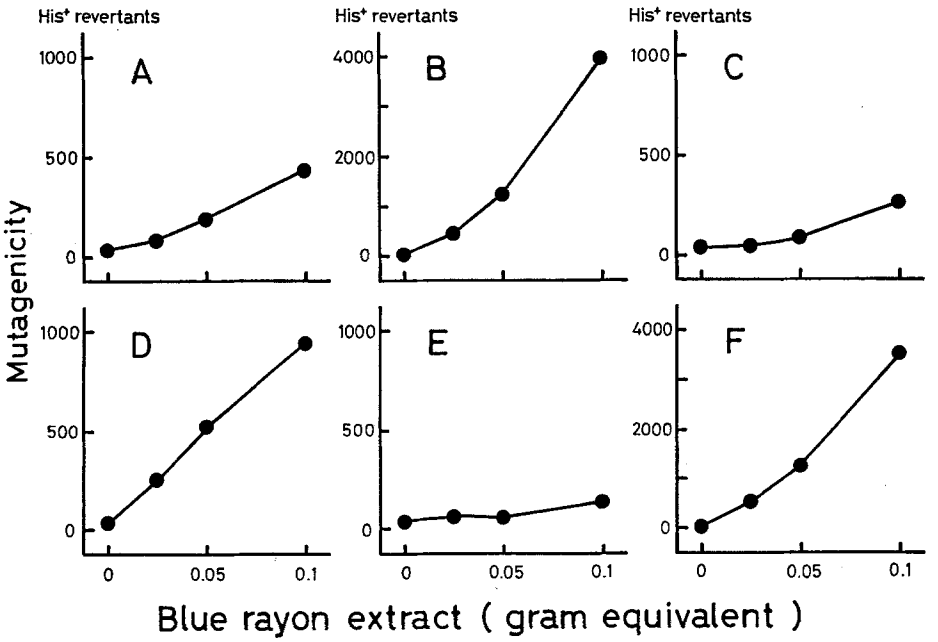

Figure 3. Mutagenicity at sites A-F as detected by the blue-rayon hanging method. The monitoring was performed on February 2-3, 1988.

Table 1. Mutagenicity and chemical oxygen demand (COD) of the Katsura river water (December 19-20, 1988)

Site	Mutagenicity (no. of His+ revertants per plate)				COD
	Hanging method			Batch method with 1 L water	(mg/L)
	0.025	0.05	0.1 (gE)[a]		
F	808	2,400	5,000	2,500	8.5
G	1,900	4,200	6,100	2,600	7.6
H	2,800	5,700	11,400	3,600	9.9
I	39	52	86	256	4.0
J	864	1,800	2,600	1,700	16.8
K	48	43	69	40	13.2
Solvent control: 46, 40					

a. gE represents gram equivalent blue-rayon extracts.

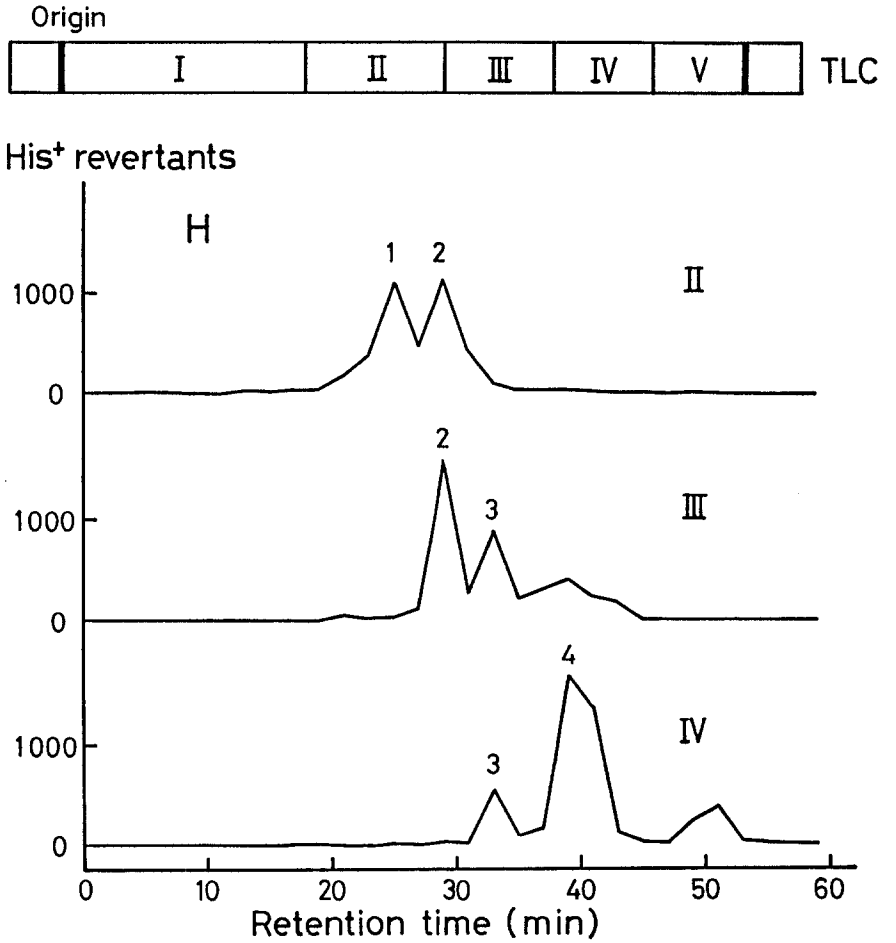

Figure 4. TLC and HPLC fractionations of mutagens at site H. Zones II-IV of TLC, obtained from blue-rayon extract, were individually subjected to HPLC.

bags in the waterway (site H) that connects the plant to the river, and the blue-rayon extracts were analyzed. As the results show, this extract at H was most strongly mutagenic. Since the mutagenicity at site I, only 2-km upstream of H, was very low, it appeared certain that the major source of the mutagenicity of the Yodo river system was the discharge from this sewage plant. Mutagenicity found at site J may also be due to this discharge, because a portion of the outflow from the plant goes into the Nishitakase river on which J is located.

The mutagenic blue-rayon extract from site H was fractionated with TLC and HPLC. In TLC on silica-gel with chloroform-methanol (97:3) as a developing solvent, the mutagenic fraction migrated to Rf 0.35-0.85 (zones

II-IV; see Figure 4). Thus, the possibility can be excluded that these mutagens are polycyclic aromatic hydrocarbons which are known to be mutagenic, because in this TLC anthracene, chrysene, 7,12-dimethylbenz(a)-anthracene, benzo(k)fluoranthene, 3-methylcholanthrene and benzo(a)pyrene all migrate to the solvent front. As illustrated in Figure 4, the HPLC analysis indicated that there were at least four mutagenic compounds in the extract; those at retention times 25 min, 29 min, 33 min and 39 min. As judged from the absorbancies at 254 nm, these compounds were strongly mutagenic, 10,000-30,000 revertants per A_{254} unit. We also fractionated the sample from site F with TLC and HPLC, and again four mutagenic fractions were found in HPLC at retention times corresponding exactly to those found for the sample at H (data not shown); the activity ratio among these four mutagenic fractions of the F sample was similar to that found for the H sample.

In this December study, we attempted to evaluate the efficiency of the monitoring method by comparing the results with the mutagenicity that was revealed by blue rayon from the water sample collected at each site. As shown in Table 1, the activity found for the collected water samples parallels that found from hung blue rayon. These values also show the efficiency of the hanging method: the mutagenicity found by the hanging of six bags of 0.5 g blue rayon corresponds to that of 50 to 100-L of river water at sites F, G, H and J. In this series of experiments we also measured chemical oxygen demand (COD) of the water samples, which is an indication of the presence of organics, and found that the water mutagenicity is independent of its COD (Table 1). For example, COD at K was higher than that at H, but no mutagenicity was found at K.

These studies demonstrate that at least four strongly mutagenic relatively stable compounds, discharged from the sewage plant, flow down the Katsura river, thereafter constituting the major mutagenic components in the Yodo river. These results are consistent with the previous observation by Maruoka et al. (1986) that the water of the Nishitakase river, collected in 1982, contained six kinds of frameshift mutagens with unknown structures. It appears that the Yodo river system has been continuously polluted with mutagens for many years. Characterization of these mutagens and identification of their sources require further studies.

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