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ESTIMATION OF MUTAGENIC ACTIVITIES AND PHYSICO-CHEMICAL INDEXES OF COASTAL WATERS

Naohide Kinae, Mitsuko Yamashita, Tetsushi Watanabe, Makoto Takahashi, Shiori Yamamoto and Isao Tomita

Laboratory of Health Science, Shizuoka College of Pharmaceutical Sciences, Shizuoka, 422 Japan

Coastal waters contain a variety of chemical substances which are mainly derived from industrial, agricultural and domestic waste materials. Some of them are mutagenic and/or carcinogenic. The presence of such genotoxic compounds in the aquatic environment gives a serious problem to fresh water fish, marine organisms and also to human beings. It has been widely studied that the polluted coastal water exhibited mutagenic activities toward certain bacteria, yeast, mussels and bivalves(Dunn et al.,1976; Parry et al.,1980; Payne et al.,1980; Sparks et al.,1981; Frezza et al.,1982). But an identification of the genotoxic compounds has not been performed successively so far.

We have tried to detect mutagens or carcinogens in the coastal waters of Japanese island in relation to the neoplastic disease of coastal fish, Nibea mitsukurii, by isolating the chemicals from water samples using macroreticular resin and then by applying reversion assay(Ames et al.,1975). Though a direct correlation between the existence of mutagenic substances and the frequency of the disease was not observed, we found that our coastal waters were polluted with mutagenic substances regardless of the incidence of fish disease(Kinae et al.,1981, 1983).

The present study was undertaken in attempt to check the seasonal change of mutagenic activities of the coastal waters of Shizuoka prefecture in Japan and to find a correlation between their mutagenic activities and such chemical indexes as COD(chemical oxygen demand) and TOC(total organic carbon). We also tried to estimate the total mutagenic activity of each water sample and to elucidate the cause of the mutagenicity of coastal waters.

An amberlite XAD-2 resin(200 ml) cleaned with methanol, diethylether(ether) and acetonitrile by using a Soxhlet apparatus was packed in nylon bag and then soaked in the coastal water for 15 days at points A-F(Basket method). Water sample(20 l) from the same points was passed through a XAD-2 resin column(ϕ2.5 x 20 cm) at a flow rate of 15 ml/min(Column method). These resins were washed with distilled water until the reaction of silver nitrite was negative. The adsorbate was extracted with ether and then methanol by using a Soxhlet apparatus and the extracts were evaporated to dryness. A portion of the residue dissolved in dimethylsulfoxide was assayed for mutagenic activity toward S.typhimurium TA98 and 100 strains with and without

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metabolic activation. An aliquot of the ether extract which showed mutagenic activity was fractionated by the application of HPLC. Each fraction was assayed for mutagenic activity and analyzed by GC-MS system. The physico-chemical analysis of water samples was conducted according to the Standard Methods of Analysis for Hygienic Chemists in Japan. The values of TC(total carbon) and IC(inorganic carbon) were determined by gas chromatography and TOC value was calculated by subtracting IC from TC. These experiments were done six times from June 1982 to November 1983.

In the basket method, the ether extracts at all points showed mutagenic activities(173-1340 net his^+ revertants/500 μg) in summer toward S.typhimurium TA100 without S9 mix. But the methanol extracts did not show such high mutagenic activities as the ether extracts did in all seasons. These activities were variable depending on the sampling points and seasons. The activities were considerably decreased by the addition of S9 mix prepared from rat and fish(Nibea mitsukurii) which were administered polychlorinated biphenyl.

In the column method, 10 l of each water sample showed 608-5523 total net his^+ revertants toward S.typhimurium TA100 without S9 mix. As the correlation coefficients between total mutagenic activities and COD or TOC values were 0.44 and 0.67, respectively, we could not find clear relationships among them.

To elucidate the cause of the mutagenic activity of the ether extract at point E, which showed high mutagenic activities through the year, another experiment was done. The ether extracts were prepared from three river waters which drained into the coastal water at point E according to the column method and then assayed for mutagenic activity. From the results, it was suggested that most of the mutagenic substances were not derived from agricultural sewage, but from industrial and domestic effluents. The ether extract from the coastal water at point E was applied to HPLC(column: Lichrosorb SI 100, ϕ8 x 500 mm; solvent: n-hexane-ethanol(99:1), methanol) to isolate 5 fractions. The third peak showed the highest mutagenic activity, 35 organic substances containing 10 aliphatic, 17 aromatic, 7 polycyclic aromatic compounds were identified from the fraction. Among them, 2,4-dinitrochlorobenzene, m-nitroaniline, p-nitrobenzoic acid and phenanthrene induced significantly his^+ revertant colonies more than control toward S.typhimurium TA100 without S9 mix.

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