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Measurement of Benzo(a)pyrene in Sea Water and in Mussels in the Seto Inland Sea, Japan

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Both the level of pollutants and the duration of exposure are important factors in the aquatic environment to evaluate a chronic effect of pollutants on marine biota. To date, several approaches have been made to estimate a time-weighted average concentration (TWA) of chemicals in the field water as an index of pollution. For instance, semipermeable membrane devices (SPMDs) have been utilized to measure TWAs of lipophilic organic compounds that exist in an aquatic environment.

Meanwhile, we have proposed a simple measurement of polycyclic aromatic hydrocarbons (PAHs) using blue rayon (BR), an adsorbent selective to compounds having three or more fused rings (Hayatsu et al., 1983). The blue rayon has a high affinity to PAHs such as benzo(a)pyrene, and is useful to concentrate PAHs in an aquatic environment at a very low concentration simply by hanging blue rayon in sea water for 24 hrs (original blue rayon hanging technique; Kira et al., 1989). Only the blue rayon (about one gram) is brought back to the laboratory and adsorbed PAHs can be easily eluted using standard laboratory procedures. Although the original method is informative, the amount of PAHs adsorbed to BR depends on the intensity of water stream together with the level of these compounds in water during hanging (Kira et al., 1996). To make this system more quantitative, we added concomitant measurement of an intensity of water stream to calibrate the amount adsorbed. This way, the calibrated amount of BaP correlated well with the TWA of BaP measured by another continuous sampling device (Kira, et al, 1997a). Thereafter we proposed this 'improved BR hanging technique' that can measure a TWA of PAHs as a sampling method for PAHs *in situ* (Kira, et al, 1997b).

This paper focuses on validation of our improved technique as a useful tool to monitor TWA of PAHs in seawater. We applied this improved technique for monitoring TWA of BaP in seawater at five sites in the Seto Inland Sea, Japan, for three consecutive years. We also harvested mussels at the same time, and measured BaP levels in their bodies to compare with the TWA of BaP in seawater.

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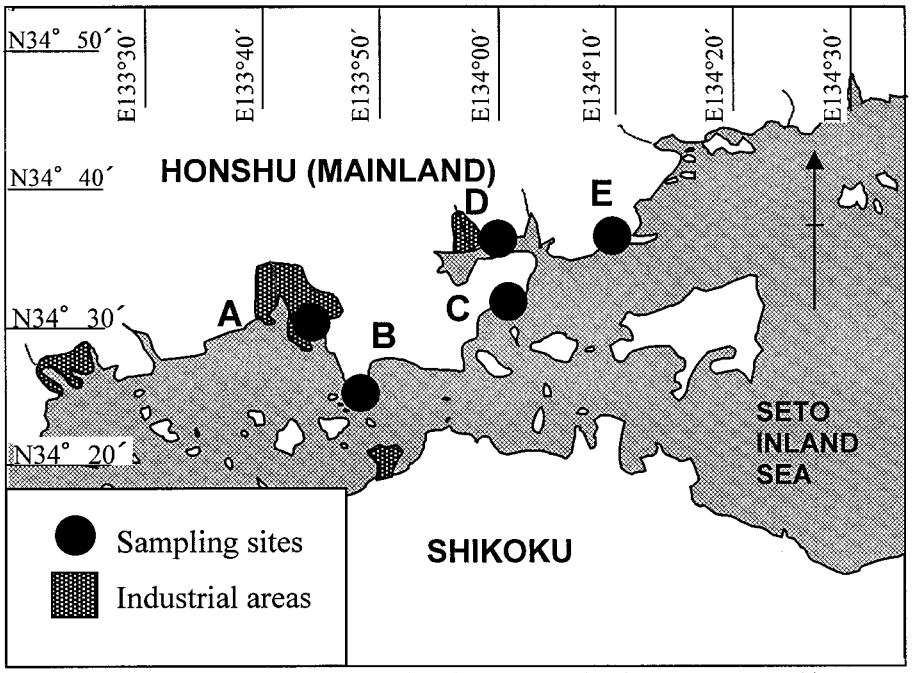

Figure 1. Five sampling sites in the Seto Inland Sea, Japan. Site A: Mizushima port, Site B: Shimotsui Port, Site C: Uno port, Site D: Kojima bay, Site E: Ushimado port. Note that Site A and D are close to heavily industrialized areas.

MATERIALS AND METHODS

Blue rayon was purchased from Funakoshi Chemicals (Bunkyo-ku, Tokyo, Japan). Solvents used for elution and HPLC analysis were of HPLC grade purchased from Wako Pure Chemicals (Osaka, Japan). All other chemicals were highest grade available from commercial suppliers.

Five sampling sites in the Seto Inland Sea, Japan were selected according to our previous study (Kira et al., 1989). Fig. 1 shows the location of the sampling sites. Site A is Mizushima port, which is located in the Mizushima Industrial area. Site B is Shimotsui Port, which is a small port mainly for fishing boats. Site C is Uno port, which is a port for several busy ferry lines that cross the Inland sea. Site D is Kojima bay, which is located at the mouth of the Asahi River that passes through Okayama city. Site E is Ushimado port, which is a small port for fishing boats, leisure boats and yachts. As shown in Fig. 1, Site A and D are close to heavily industrialized areas

Mussels were collected at each sampling site from March 1996 to October 1998 (See Table 1 for the exact date of sampling). The collected mussels were brought back to the laboratory, and shelled. The edible part of the mussels (approximately

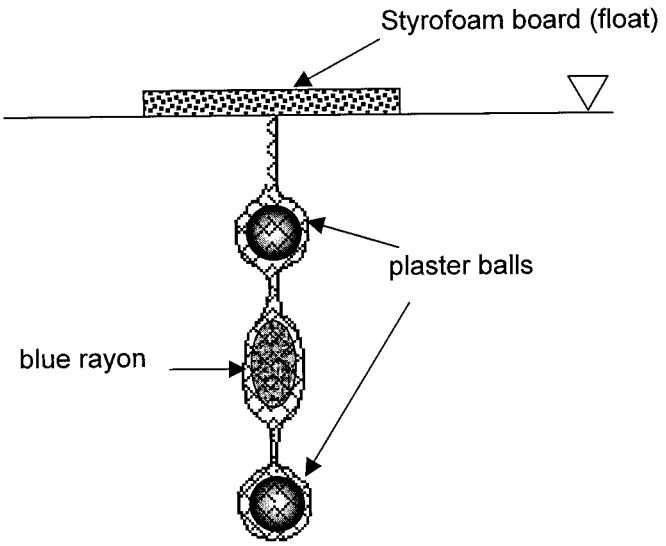

Figure 2. Scheme of the improved blue rayon hanging device. This device is made of two plaster balls put in a plastic net together with blue rayon, and the device was hung under a Styrofoam float (See Kira et al., 1997 for technical details).

20g) were homogenized with 180 mL of acetone-acetonitrile (v/v = 2:8) solution and filtered with a glass fiber filter. The organic layer was concentrated under reduced pressure. The residue was dissolved in 5 mL of acetone-acetonitrile solution, and the solution was suspended in 150 mL of distilled water. One gram of blue rayon was immersed in the suspension, and was shaken for 30 min. The blue rayon was the taken out from the suspension, and was washed with enough amount of distilled water.

BaP adsorbed to BR was extracted according to our previous report (Kira et al. 1995). Briefly, the BR was rinsed with distilled water, and the water was removed by aspiration and blotting onto a paper towel. The BR was eluted with 150 ml of methanol/concentrated ammonia solution (w/w 50:1), and the eluate was evaporated to dryness. The residue was dissolved in methanol, and submitted to high performance liquid chromatography (HPLC) analysis, for quantifying BaP.

A cumulative intensity of water movement at a sampling site was estimated by the plaster ball method that has been reported by Komatsu and Kawai (1992), with modifications (Kira et al., 1997b). We used a ping-pong ball as a mold to standardize the size and weight of the plaster ball. Before use, these balls were immersed in distilled water in a beaker for an hour, the balls were taken out and excess water on the surface was removed with a gentle application of a paper towel. Initial weight (W_0) of each ball was measured at this time. Two of these balls in a plastic net were tethered to BR, also in the net, and the device was hung

under a Styrofoam float ('improved' blue rayon hanging device; see Figure 2). BR was brought to a sampling site in a sealed container, and was set to the device on site to avoid any adsorption of airborne PAHs before the sampling process. At a sampling site, the device was placed at a depth of 30-50 cm from the surface of the water for 24 hr. The balls and BR were recovered after 24 h, and the weight (W) of each ball was measured after lightly wiping it to remove water on the surface. The decrease in the weight of each ball ($\Delta W = W_0 - W$) was recorded. These samplings were done 7 to 10 times per year each year (sampling frequencies are given in Figure 3).

After completion of sampling, BR was recovered, and it was rinsed with distilled water. Water was removed by aspiration and blotting BR onto a clean paper towel. BR was put in light shielded and airtight container, and was brought back to the laboratory. BaP adsorbed on BR was extracted in the same way as described above.

BaP was assayed according to the previously reported methods (Kira et al. 1995). Briefly, a Nova-Pak C18 column (ϕ-3.9×150 mm, mesh 4 μm, Waters, Millipore, Millford, MA, USA) was used : mobile phase, acetonitrile/water (v/v= 65 : 35); flow rate, 1.0 ml/min; temperature, 40 °C. Peaks were detected with a fluorescent spectrophotometer (Hitachi F-1080, Hitachi, Tokyo, Japan) with excitation and emission wavelengths set at 365 nm and 406 nm, respectively. Peak areas were recorded with an integrator (Hitachi D-2500, Hitachi, Tokyo, Japan). The HPLC equipment used was a Waters 600E system controller with a Roedyne 7125 injector. Unused BR, which was included among the samples to see if there was any pre-sampling contamination, did not show any detectable amount of BaP.

Geometric mean and standard deviation were calculated with each value for recovered BaP as well as TWA of BaP. Statistic analyses for the difference of the means were carried out using Kruskal-Wallis test followed by Scheffe post-hoc test.

RESULTS AND DISCUSSION

Figure 3 shows the results of monitoring TWA of BaP in seawater during 1996-1998. Each chart shows results of the monitoring at different sampling sites. The first column shows cumulative data throughout 1996-1998, whereas the remaining columns show data on each consecutive year during the monitoring period. BaP recovered by blue rayon hanging ([BR-BaP]) and the weight decrease in plaster balls (ΔW) are shown in the first and the second rows, respectively. There were significant differences among the sampling sites. According to our previous study, we obtained TWA of BaP ([TWA-BaP]) using the following equation (Kira et al., 1997b):

$$[\text{TWA-BaP}] = 6.5 \times [\text{BR-BaP}] / \Delta W$$

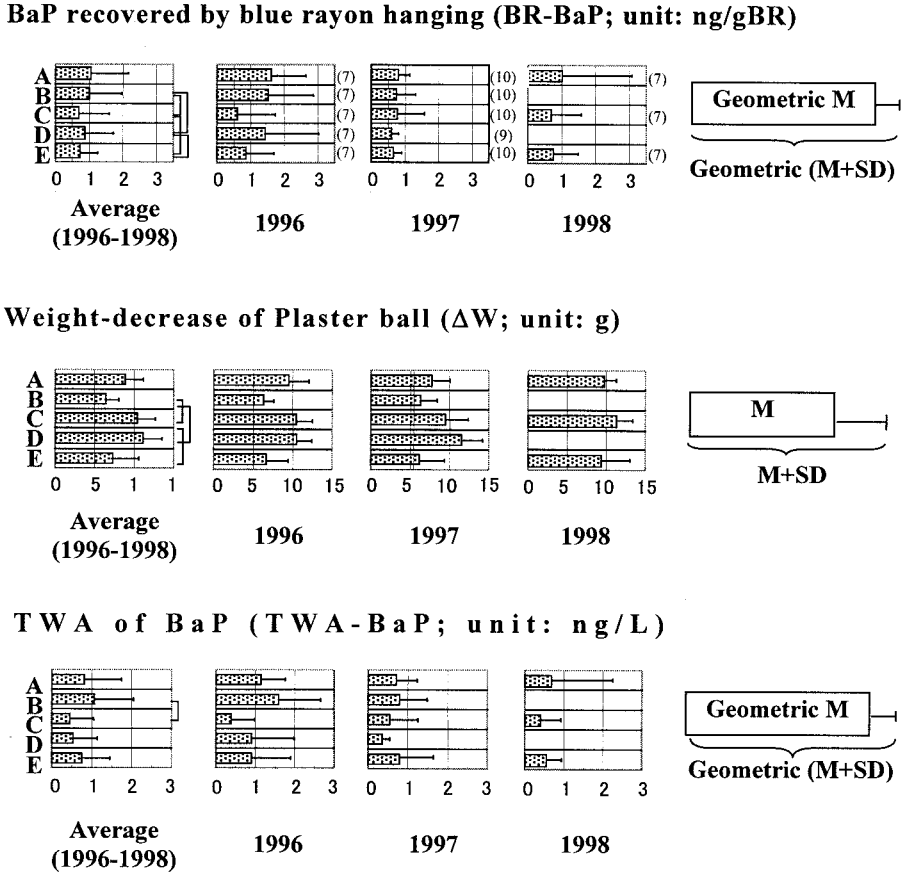

Figure 3. Measurements of BaP by blue rayon hanging, weight-decrease of plaster ball, and TWA of BaP in the Seto Inland Sea. Numbers in parenthesis next to the charts in the first row show how many times sampling was done at each sampling site that year. M: mean, SD: standard deviation.
] P<0.05 (Kruskal-Wallis test followed by Scheffe test).

The last row shows the calculated [TWA-BaP]. TWA ranged from 0.83 to 5.93 ng/L. At point B and C, TWA showed significant site-to-site difference in the average of cumulative data from 1996 to 1998.

BaP concentrations in the body of mussels ($[BaP]_m$) are given in Table 1 together with [TWA-BaP] measured 24 hr preceding the sampling of mussels. These two showed no significant correlations (r=0.320, P=0.286, n=13), which suggested that the measurement of TWA of BaP and the detection of BaP in mussel are essentially different monitoring system. TWA of BaP should show concurrent level of BaP during the hanging of BR. On the other hand, $[BaP]_m$ shows accumulated BaP in the body at the time of the sampling. Concentration ratio (i.e., $[BaP]_m$/ [TWA-BaP]) ranged from 16 to over 5000, and the average was about

Table 1. TWA of BaP in seawater and the level of BaP in mussel

Site	Date	TWA of BaP in ambient water ([TWA-BaP]; unit: ng/L)	Level of BaP in mussels ($[BaP]_m$; unit: ng/gWW)	Concentration ratio $[BaP]_m/[TWA\text{-}BaP]$
A	Dec, 1996	1.96	0.84	429
A	Mar, 1997	1.13	0.15	133
A	Jan, 1998	0.49	2.93	5933
A	Apr, 1998	5.93	2.21	373
A	May, 1998	1.46	2.19	1502
B	Dec, 1996	0.96	0.17	177
B	Mar, 1997	1.12	0.07	63
C	Dec, 1996	0.50	1.21	2420
C	Mar, 1997	0.89	0.07	79
C	Apr, 1998	1.03	0.24	227
C	May, 1998	0.24	0.35	1468
E	Dec, 1996	1.89	0.71	376
E	Mar, 1997	1.90	0.03	16
Mean (geometric mean)		1.50 (1.11)	0.86 (0.38)	1015
SD		1.44	0.98	1650

BaP: benzo(a)pyrene, TWA: time-weighted average concentration, WW: wet weight, Correlation coefficient between TWA of BaP and the level of BaP in mussel: 0.320 (P= 0.286, n=13).

1000. There are some factors that may influence the bioaccumulation of BaP in the body of mussels, presumably such as ambient temperature, food availability and reproductive stage (Orbea et al., 1999). Further study on the seasonal changes, such as bioavailability and/or bioaccumulation of BaP in mussels at the present sampling sites, may be needed.

Estimation of TWA of waterborne PAHs, not daily but weekly basis, can be carried out using some other methods such as the SPMDs method. According to a recent review by Petty et al. (2000), PAHs that exist in water are sampled together with other lipophilic substances non-selectively. This feature of SPMDs may be preferable for researchers who are interested in sampling a wide range of lipophilic pollutants. Those who are interested in sampling PAHs or polycyclic mutagens could use BR technique, which is selective to that class of compounds. In either method, there is no need to carry a large volume of water to the laboratory as is in the case of conventional methods.

The advantages of using our improved BR technique are as follows. The BR can be carried (or mailed) easily to a remote sampling site, and only blue rayon

packed in a container is to be brought back to the laboratory for further analysis. It should be handled carefully to avoid contamination during transportation. Other components of the sampling device are relatively inexpensive. Decrease in the weight of plaster balls could be measured using a portable balance on site.

In conclusion, we demonstrated the usefulness of our improved blue rayon hanging technique by applying the technique in actual monitoring for three years at the Seto Inland Sea. TWA of BaP in water in this area ranged from 0.08 to 5.93 ppt (ng/L).

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