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Acute Toxic Effect of River Yodo Water (Japan) on *Daphnia magna*

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The River Yodo originates from Lake Biwa, the largest and oldest lake in Japan, and flows down to Osaka Bay, a distance of 75 km. It is the main source of drinking water for 14 million people in the Kinki area, the second largest urban community in Japan. The preservation of water quality and the protection of water bodies from human activities have been considered to be important issues. Worldwatch, on the other hand, reported that there are 70,000 synthetic chemicals in everyday use, with 500 to 1,000 new ones added to the list each year on the earth (Blum and Speece 1990). These chemicals are being discharged into the environment in each step of their production, transport, use and disposal. It is extremely difficult to make a quantitative estimate of their possible risks as a whole since most of them exist at levels too low to be estimated by any chemical analysis methods. To solve this problem, the U.S. Environmental Protection Agency has proposed a toxicity identification evaluation (TIE) method, which combines toxicity testings with physical and chemical analyses of effluents to identify potentially causative toxicants (Mount and Anderson-Carnahan 1988a, 1988b, 1988c). In this study, we report results of one year test of acute toxicity of the River Yodo water on Daphnia magna.

MATERIALS AND METHODS

Water samples in this study were collected monthly from one site in Lake Biwa and 11 sites in the River Yodo basin from April 1993 to March 1994 for one year. In a previous one-year study of raw water samples obtained from several locations of the river (June 1992 to March 1993), we established that the water samples had practically no acute toxic effects on D. magna, except those samples obtained in August (Hosokawa unpublished). The sampling sites are marked in the schematic map of the river system as shown in Fig.1. The annual average water flow rate of the river at sampling Sites 3, 4, 5 and 6 is about 178, 51, 46 and 210 m^3 /sec, respectively. The rivers at Sites 9, 10, 11 and 12 are small and heavily polluted tributaries. The water samples were collected and transported in glass vessels to our laboratory, and a portion of each sample was tested immediately for the acute toxicity to D. magna. The remainder of the samples was stored in a refrigerator.

The river-water samples were vacuum-filtered through a glass filter with 1 μm pores. For concentrating the water samples, a Sep-Pak C18

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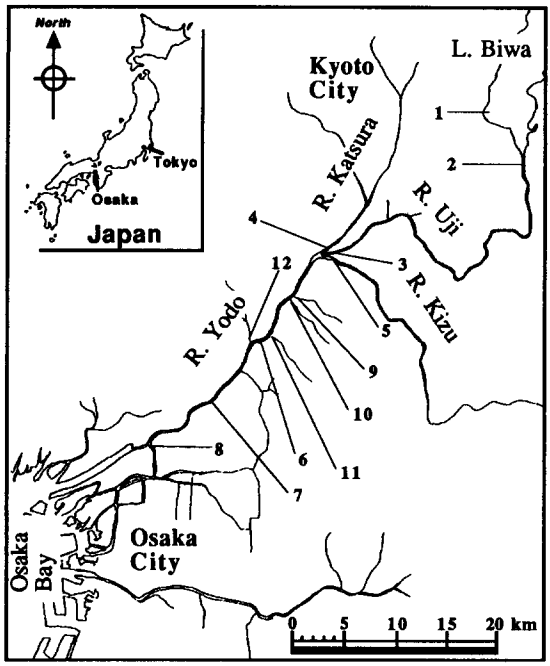

Figure 1. Map showing the River Yodo basin and sampling locations used in the study.

cartridge (Millipore Co.) containing 360 mg of C18-bonded porous silica, which had been previously activated with 5 mL of methanol and rinsed with 5 mL distilled water, was used. One liter of the filtered sample was drawn through the cartridge at a flow rate of about 15 mL/min by a water aspirator. The C18-bonded porous silica was then rinsed with 10 mL of distilled water and was dried by drawing room air through it. The adsorbed organics were eluted with 2 mL of methanol, and the eluate was then concentrated under a gentle stream of nitrogen to eliminate methanol. The extraction by methanol alone may leave some non-polar organic compounds, but we believe that this procedure is still useful for evaluating and identifying pollutants which cause acute toxicities to D. magna. Since polar organic compounds have much higher solubility in water than non-polar organic compounds, and may be major organic contaminants in water, the extracts by methanol are the first to be examined for biological toxicity tests (Durhan et al. 1989). By diluting the residue (<0.1 mL) to 50 mL with the reconstituted water (RCW) as a diluent, a 20-fold aqueous concentrate was obtained. The composition of RCW was 0.5 mM of $CaCO_3 \cdot 2H_2O$, 0.3 mM of $MgSO_4 \cdot 7H_2O$, 0.05 mM of KCl and 0.5 mM of $NaHCO_3$ in distilled water.

The Daphnia magna used in this study were obtained from the National Institute for Environmental Studies, Tsukuba, Japan, and had been kept in our laboratory for three years. Experimental animals were cultured in RCW enriched with vitamin B_{12} (0.02 μg/L) and Se (1.0 μg/L) under semi-static conditions. They were fed a diet of the green algae, Chlamydomonas sp. and Chlorella sp. Culture and toxicity tests were conducted in a chamber at 22 ±1 ℃ with a light / dark cycle of 16 hr (range 550-720 lux) / 8 hr.

Static acute tests using newly hatched D. magna (<24 hr old) were conducted in a 20-mL glass tube, in which a total amount of 15 mL of a dilution made from the appropriate amount of a raw or a concentrated sample and the added dilution water was placed, and five animals were kept in each solution. During the tests, animals were not fed with any diet, and the water solutions were not renewed. Toxicity of water samples was determined by using as an endpoint immobility after 24- and 48-hr exposure periods. Toxicity effects of the raw sample were determined as a percentage of immobile animals, using 5 animals per tube, and 4 tubes per sample.

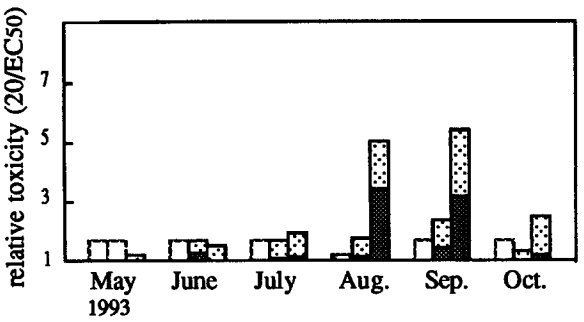

Figure 2. Variation in the acute toxicity to D. magna of Site 1, 2, 3.

Three columns of each month indicate Site 1, 2, 3 from the left to the right. The columns ☐ indicate samples with no effects at the concentration factor of 20X. The columns ■ and ⊡ indicate the values of 20/24 hr-EC50 and 20/48 hr-EC50.

From 50 mL of the 20-fold aqueous concentrate in a volumetric vessel, 15 mL were drawn into a test tube with a volumetric pipet and the toxicity was tested on 5 animals. The remaining (35 mL) was diluted to 50 mL with RCW, a 1.43-fold dilution series. This process was repeated to reach the adequate degree of dilution, which was estimated from the raw water test results, and more than 5 trials were made for most of the samples. The immobility

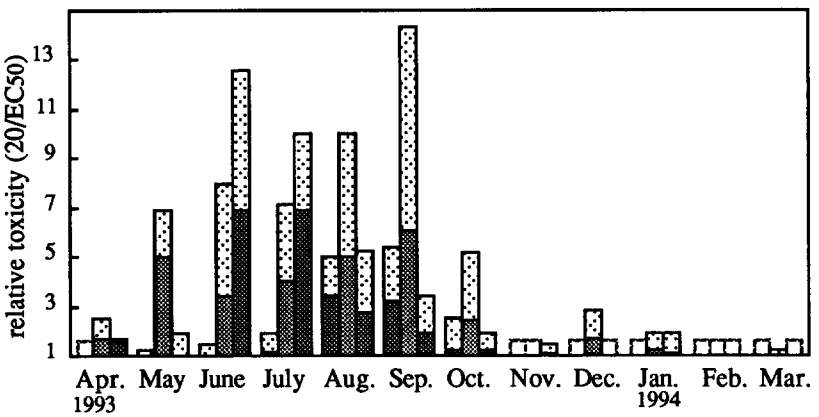

Figure 3. Variation in the acute toxicity to D. magna of Site to 3, 4, 5.
See Fig. 2.

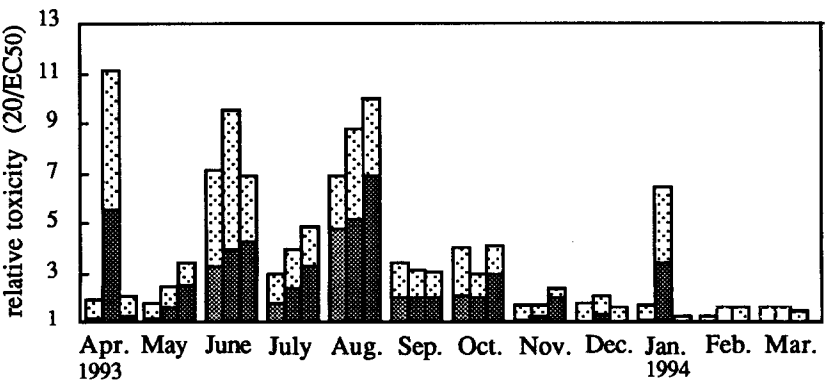

Figure 4. Variation in the acute toxicity to D. magna of Site 6, 7, 8.
See Fig. 2.

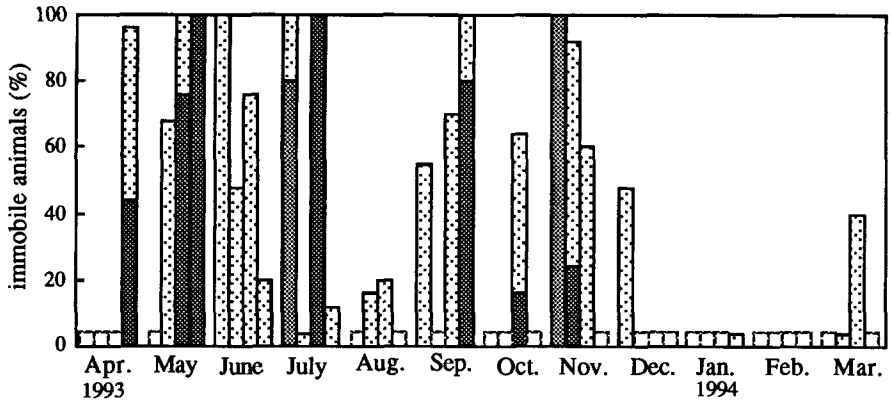

Figure 5-1. Variation in the acute toxicity to D. magna of Site 9, 10, 11, 12 (raw). The columns □ indicate samples with no effects. The columns ▩ and ▣ indicate immobility after 24 and 48 hr.

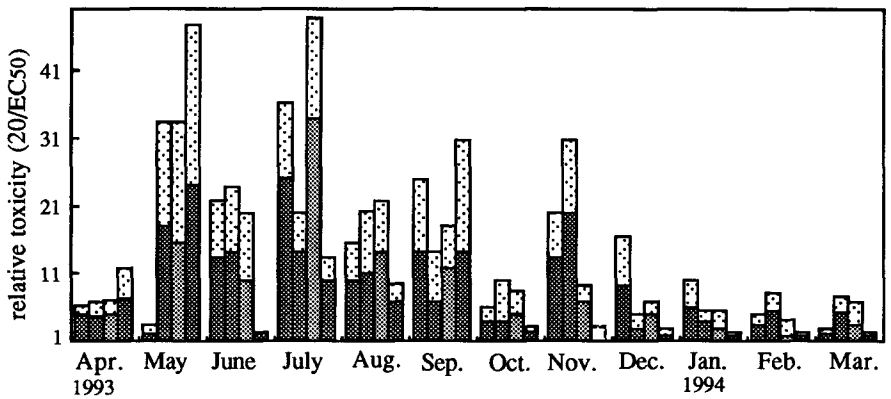

Figure 5-2. Variation in the acute toxicity to D. magna of Site 9, 10, 11, 12. See Fig. 2.

data were analyzed by probit analysis to estimate EC50-values and 95 % confidence intervals. No partial effects were observed in many of the tests on concentrates, which were conducted with 5 animals per each concentration. Their EC50-values were calculated as the geometric mean of the highest concentration that caused no effect and the lowest concentration that exhibited 100 % immobility. These concentration factors were used as concentrations in the standard calculations of EC50-values. The resulting EC50-value is inversely proportional to the toxicity : higher EC50-value indicates lower toxicity. To avoid any confusion, the EC50-values obtained were converted to relative values by the following formula.

relative toxicity = 20/EC50-value

RESULTS AND DISCUSSION

The acute toxicities to D. magna were barely detectable even in the concentrates obtained from the Lake Biwa (Site 1), the main water source of the River Yodo. The acute toxicities were, however, detected for the concentrates obtained from Sites 2 and 3 during the summer season, more

for Site 2 than Site 3. The highest 48-hr relative toxicity value of 5.4 was seen in the 3.7-fold concentrate from Site 3 in September (Fig. 2). In Fig. 2, the results with no effect are not included.

River Katsura (Site 4), one of the two main tributaries, receives discharge from sewage treatment plants of Kyoto City which has a population of 1.4 million, plus 45 million tourists a year. Sometimes, the river is slightly colored with dye residues discharged from dye-works, which is one of the traditional industries in the Kyoto area. Their concentrates were strongly colored most of the time because the dye residues were poorly treated in the sewage treatment plant, but their toxicities to D. magna were not as great as might have been expected by their appearance. The toxicities of the concentrates from this site occurred in some degree over the summer season, and immobilities of 32 and 50 % were detected in 48-hr acute toxicity tests of the raw samples obtained in August and September, respectively. The immobility, however, could not be detected at all in their concentrates, contrary to the results of the raw water samples (Fig. 3). This could have been due either to the fact that the toxicant(s) were not extracted by methanol or that they were not adsorbed on the C18 column. This point needs to be investigated further, but the findings above are believed to be important in themselves.

Main pollutants of the River Kizu (Site 5) come from agricultural activities in the area as discussed below. Construction of a new sewer system is needed, however, since there have been residential developments in the upper reaches of the river in recent years. The toxicities of concentrates from Site 5 were detected starting in July with the opening of the irrigation system in the area, and remained until October with a decreasing degree (Fig. 3). As mentioned above, the results of the study of the preceding year showed that the raw samples obtained from this site in August had a high toxicity, i.e., 24-hr and 48-hr immobility were 92 and 100 %, respectively. In this area, flooding occurred at the time of pesticide spraying and the river was made muddy. Yamaguchi and Fukushima (1993) reported that most kinds of pesticides in use were detected in these samples. These pollutants had very harmful effects on the down stream biological systems in Sites 6, 7 and 8 because of a relatively high flow rate of the water of this river into the River Yodo. The 24-hr immobilities at the down stream sites were 52-100 % and 48-hr immobilities were 100 % at all the sites.

High toxicity values of the concentrates obtained from the main current of the River Yodo (Sites 6, 7 and 8) were found in June, July, and August. High toxicity values were also detected in the raw samples obtained from Site 8 in November and May, i.e., 24-hr immobilities were 16 and 40 %, 48-hr immobilities were 64 and 68 %, respectively. But, their toxicities were lost in repeated toxicity tests with samples in which EDTA Ca (0.3 mM) was added since EDTA Ca reduces the toxicities of metals. Furthermore, the toxicity values of their concentrates were similar to those of samples of the upper two sites obtained during the same period (Fig. 4). A similar phenomenon was noted for the sample obtained from the same site in October of the preceding year. These waters were sampled at 50 m down stream from the entry of the tributary, where the water source for the quality control of streams in Osaka City is discharged. There are no sources of pollution between the sampling site and the entry. We suspect that the toxicity was due to metals redissolved from sediments by a turbulent flow.

In four small tributaries (Sites 9, 10, 11 and 12), high toxicities were

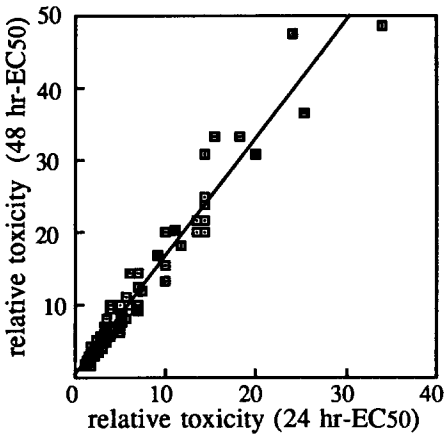

Figure 6. The relationship between 24-hr EC50(x) and 48-hr EC50(y)

$y = 0.250 + 1.63X, \quad r = 0.977$

frequently detected in raw samples in all seasons, with less in the winter season (Fig. 5-1). Furthermore, the toxicity of their concentrates was apparently strong even in winter when agricultural and sanitary uses of pesticides were greatly reduced(Fig. 5-2). These river basins are urbanized, industrialized and densely populated, and have many small rice paddies. Because of the delay in construction of sewer systems around these basins, the waters of these tributaries are heavily polluted with municipal wastewater. The annual average values of BOD during 1992 were 6.0, 16.5, 12.1 and 3.6 mg/L for Sites 9, 10, 11 and 12, respectively (Yodo River System Liaison Council for Prevention of Water Pollution 1994). Among the water samples of these tributaries, the ones of Site10 were especially polluted with organic contaminant suspensions, in which test animals grew without any diet during the acute toxicity tests. Therefore, their acute toxicity values for concentrates, from which most diet materials were removed by pretreatment, were higher than those estimated from raw samples. This correlation is seen in the tests conducted in May, June, July, August, and November.

Although we could not identify toxic pollutants in this study, it is suggested from the seasonal trends and the areal distribution of toxicity that the main toxic pollutants to D. magna were pesticides. Concerning pesticide pollution, its occurrence is in agreement with the season of pesticide use and its efflux with flooding (Galassi et al. 1992; Yamaguchi et al. 1992). The toxic effects of pesticides to D. magna differ in degree depending on the type of pesticide. Insecticides are even more acutely toxic as shown below. From our experiments, the 24-hr EC50s of diazinon and fenitrothion (insecticides) to D. magna were 0.50 and 1.1 μ g/L, respectively, and the 48-hr EC50s were 0.25 and 0.77 μ g/L, respectively. Those of simazine and molinate (herbicides) were more than 5 mg/L. That of isoprothiolane (germicide) was also more than 5 mg/L. The results of our study showed that the main toxicants to D. magna were insecticides with a relatively small ratio of 24-hr EC50/48-hr EC50 of 1.6 (Fig. 6).

There have been several reports on mutagenic effects of water of the River Yodo (Sakamoto and Hayatsu 1990; Matsui et al. 1992; Nakamuro et al. 1992, 1994). Nakamuro et al. (1994) reported results of their study on mutagenic effects of the River Yodo's water, but their results are much different from ours regarding when and where prevalent toxicities occur. They reported that the highest mutagenic activity occurred in fall in the River Katsura (Site 4), and lowest in summer. They suggested that mutagens originated from urban streams, discharge of waste treatment plants, urban runoff and rainfall. On the other hand, our results indicated that the main sources of acute toxic pollutants to D. magna were pesticides as dicussed above. This remarkable difference in the two studies is very interesting since estimates of water toxicities depend on bioassay methods used. In this

regard, the Toxicity Identification Evaluation (TIE) procedures should be applied widely to diverse species in areas with complex pollutants, and must be useful in our country.

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