〈ノート〉

Multivariate Analysis of Water Quality and Ecotoxicity to Aquatic Species in the Yoshino River and the Yodo River Basin, Japan

Ikumi Tamura[1)†], Takehiko I. Hayashi[2)], Saki Nishi-ie[3)], Moe Enyama[3)], Mako Mitsuyama[3)]
Norihisa Tatarazako[4)] and Hiroshi Yamamoto[2, 3)]

[1)] Graduate School of Environmental and Life Science, Okayama University
（E-mail : Ikumi.tamura@okayama-u.ac.jp）

[2)] National Institute for Environmental Studies

[3)] Faculty of Integrated Arts and Sciences, Tokushima University

[4)] Faculty or Agriculture, Ehime University

† Correspondence should be addressed to Ikumi Tamura :
（Graduate School of Environmental and Life Science, Okayama University
E-mail : Ikumi.tamura@okayama-u.ac.jp）

Abstract

This study aimed to measure the seasonal variations in water quality variables such as biochemical oxygen demand（BOD）, pH, ammonia, dissolved oxygen, electric conductivity（EC）, and hardness in the water samples from different rivers in Japan. Moreover, short-term chronic toxicity tests were performed for fish embryos, daphnia, and green algae in the raw water samples without concentration. These data were subjected to multivariate analyses such as principal component analysis（PCA）, cluster analysis, and multiple regression analysis. The PCA results showed a similar trend for water quality variables such as BOD, EC, and ammonia across the water samples, whereas daphnia toxicity was found to be independent of the water quality variables. The results of cluster analysis suggested that the samples could be grouped as those collected from upper and lower streams on the basis of the extent of anthropogenic pollution by chemical compounds.

Keyword : ecotoxicity, river water, water quality, multivariate analysis, whole toxicity, whole effluent toxicity
原稿受付 2018. 6. 15　　原稿受理 2018. 8. 23　　**EICA**: 23(2・3) 75-85

1　Introduction

The number of chemicals registered in the Chemical Abstract Service（CAS）of the American Chemical Society（ACS）had exceeded 120 million by the end of 2016[1)]. These wide varieties of chemicals are manufactured, used, and disposed of via various human activities such as manufacturing, farming, and daily living activities. Some of these compounds are appropriately treated in wastewater treatment plants or septic tanks, whereas the others are released into the water environment without any treatment. Thus, various chemicals, including heavy metals, pesticides, and detergents, have been detected at nanogram to microgram per liter levels in surface waters with the recent development of analytical methods such as inducted coupled plasma mass spectrometry（ICP-MS）and liquid chromatography tandem mass spectrometry（LC-MS/MS）. In general, the hazard of each chemical is assessed using toxicity tests by using aquatic organisms to establish water quality standards or effluent limits to control the risk while monitoring the concentration of chemicals in the aquatic environment such as at certain reference monitoring sites. However, this scheme to control chemical risks fails to consider the non-regulated/unknown compounds and mixture effects, and the approach for the direct bioassay for the effluents of factories and municipal wastewater treatment plants have been developed in North America and Europe[2)], such as Whole Effluent Toxicity（WET）in the US[3)] and Wastewater Ordinance in Germany[4)].

This direct toxicity testing approach can also be used for ambient waters worldwide to understand the impact of chemicals. For example, Vlaming *et al.*[5)] conducted WET testing for river water samples in Sacramento, California and found that the major potential toxicants for *Ceriodaphnia dubia* were organophosphorus insecticides such as diazinon and chlorpyrifos. Similarly, Bailey *et al.* investigated the effects of effluents of wastewater treatment plants in South Wales in Australia on *C. dubia* and *Pseudokirchneriella subcapitata* and suggested that diazinon and chlorpyrifos were the major toxicants[6)]. Anderson *et al.* conducted WET testing for water and sediment samples from the Santa Maria River in California by using *C. dubia* and *Hyalella azteca*, respectively, and revealed that organophosphorus and pyrethroid insecticides were the potential major toxicants.[7] Kayhanian *et al*. indicated the mixture effects of heavy metals such as copper and zinc in the roadway runoff in the suburbs of Los Angeles, California by using *C. dubia*, sea urchin, fathead minnow, and Microtox[8]. Vigano *et al*. used *C. dubia* reproduction test for the evaluation of the water quality of the River Po watershed area of North Italy[9]. Baldigo *et al*. conducted monthly toxicity bioassays for *C. dubia* and *P. subcapitata* for waters from St. Laurence River to assess the influence on plankton communities[10]. Fang *et al*. conducted WET analyses such as zebrafish embryo test, *Daphnia magna* acute test, and algal growth inhibition test for factory effluents and their receiving water bodies in the highly developing area of the Pearl River Delta area of South China[11].

Studies to investigate the toxicity of ambient waters have also been extensively conducted in Japan. Sasaki *et al*. conducted *D. magna* acute toxicity tests and algal growth inhibition tests by using *P. subcapitata*, Microtox, and Ames test for river waters collected from the Tone River and Tama River watersheds in the Tokyo metropolitan area in the late 1990s. They also conducted chemical analysis of selected surfactants and pesticides, and found moderate correlation of organophosphorus insecticide concentrations with daphnia toxicity and herbicides with algal toxicity.[12] Hatakeyama *et al*. found acute toxicity to freshwater shrimp in the rivers of Ibaraki Prefecture and found significant correlations of this toxicity with organophosphorus insecticides by comparing with the relevant monitoring data[13]. Subsequently, Kikuchi *et al*. monitored *D. magna* toxicity in the Naka River of the Tone River watershed between 1994 and 2004 to assess the reduction in the toxicity level concomitant with the decrease in the use of organophosphorus insecticides[14].

More recently, author's group conducted short-term chronic toxicity tests for urban streams and class A rivers all over Japan[15, 16]. In these river water samples, we also chemically analyzed selected pharmaceuticals and surfactants, and some antimicrobials and surfactants that are considered to be potential major toxicants for algae and fish/daphnia, respectively[17, 18]. The combination of direct bioassay and chemical analysis of the selected hazardous chemicals and bioassay of these chemicals might be an effective approach for the toxicity identification and efficient risk assessment/management of complex mixtures of hazardous chemicals in the watershed area. However, it was suggested that there are other causes of toxicity, as only pharmaceuticals and surfactants could not explain all of the toxicity in several rivers.

Investigations on the detailed correlation between various water quality variables and ecotoxicity are rare. Peeters *et al*. investigated the correlation between ecotoxicity of sediment or pore water to midge, *Chironomus riparius*, *D. magna*, and luminescent bacteria at various water quality levels and contaminant concentrations. Multivariate analysis suggested the possible impacts of these contaminants on macroinvertebrate communities in the Rhine-Meuse delta area[19]. Palma *et al*. evaluated the water quality of a reservoir in Portugal by using the combination of ecotoxicity testing of some hazardous compounds such as metals and pesticides and water quality measurements such as pH, temperature, biochemical oxygen demand (BOD). They attempted to determine the correlation between these water quality variables and bioluminescence inhibition, mortality of *Thamnocephalus platyurus*, and reproduction of *D. magna*[20].

In this study, we obtained river water samples on a monthly basis from a reference site of the Yoshino River of Tokushima, Japan, in addition to yearly sampling conducted at several sites in the Yoshino and Yodo River watersheds of Osaka/Kyoto and measured the selected basic water quality variables and conducted short-term chronic toxicity tests for fish, daphnia, and algae.

2 Materials and Methods

2.1 Sampling

The location of sampling sites are shown in **Fig. 1**. Monthly river water samples were collected at Takase Bridge (T1) in the downstream of the Yoshino River, Tokushima, Japan, located at approximately 10 km west of the Tokushima University campus. This site has the intake for the drinking water facility of Tokushima City (population of approximately 260,000) and is designated as a reference environmental monitoring point where the Ministry of Land, Infrastructure, Transport and Tourism water quality every month. Water samples were obtained from four additional sites in the Yoshino River watershed: Okawa Bridge (T2) in the upstream area; Wakimachi

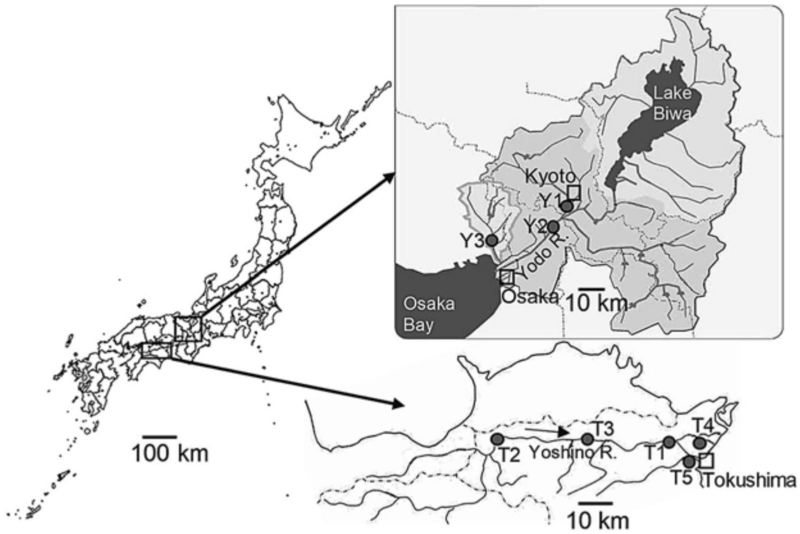

Fig. 1　Map of the sampling sites and their surrounding area.

Bridge (T3); the estuary weir of the Imagire River (T4), one of the major distributaries of the Yoshino River system; and the Tamiya Creek near Kuramoto Park (T5). We collected river water from these sites three times annually in Nov 2013, Oct 2014, and Dec 2015. Okawa Bridge (T2) is an old suspension bridge located nearly 100 km west of Tokushima City at the Oboke/Koboke canyon. Wakimachi Bridge (T3) is a submergible bridge located at Wakimachi district of Mima City (population of approximately 30,000). The estuary weir of the Imagire River (T4) is located near a shopping mall, and a few chemical and pharmaceutical factories are located around this area along with residential area. We collected the water samples from the upper side of the estuary gate. Tamiya Creek (T5) is a small urban creek located at the western part of Tokushima City. This area does not have a sewage system, leading to the release of some untreated grey water, although approximately half of the residents use septic tanks to treat grey water. Our research group previously investigated the effects of pharmaceuticals and personal care products (PPCPs) at this site[17, 21].

We also collected yearly samples in Dec 2013, Jan 2015, and Jan 2016 from the Yodo River watershed area, the second most populated area in Japan. The three sampling sites are the reference environmental monitoring sites and were investigated in our previous study[16], which include Miyamae Bridge over the Katsura River (Y1), Hirakata Ohashi Bridge (Y2) over the midstream of the main river, and Tokura Bridge over the Ina River (Y3). The Katsura River, one of the three major tributaries of the Yodo River, receives almost all of the effluents of wastewater treatment plants of Kyoto City (population of 1.5 million); Miyamae Bridge (Y1) is located at the downstream of these outfalls. Three tributaries—the Katsura River, Uji River, and Kizu River—flow into each other to become the Yodo River about 5 km downstream of Y1. Hirakata Ohashi Bridge (Y2) is located at approximately 8 km downstream of the junction of these rivers. This bridge connects Hirakata City and Takatsuki City, two of the major satellite cities of Osaka/Kyoto, and their total population is nearly 750,000. The Ina River is one of the tributaries of the Yodo River, which also receives the effluents of a wastewater treatment plant and some factories and from an airport located nearby. The Tokura Bridge is a small bridge across the river at the border of Toyonaka City (population of approximately 400,000), Amagasaki City (population of approximately 450,000), and Itami City (population of approximately 200,000).

2.2　Chemical analysis

The pH, dissolved oxygen (DO), and electrical conductivity (EC) of the water samples were measured using a portable pH analyzer (D-51 Horiba; Kyoto Japan), DO analyzer (HQ40d, HACH Co., Loveland, CO, USA), and EC analyzer (CM-31P; TOADKK, Tokyo, Japan), respectively. The water samples were collected using a stainless steel bucket, transferred into amber glass bottles, and stored at 4±2℃. Immediately after sampling, the samples were delivered by car or shipped via courier to the Tokushima University. The BOD, chemical oxygen demand (COD), hardness, and ammonium nitrogen (NH_4^+ -N) were measured using the standard methods of the Japan Industrial Standard[22] at the Tokushima University.

2.3 Toxicity testing

We conducted three short-term chronic toxicity tests proposed in a draft test manual of bioassays for effluents and ambient waters in Japan[23].

Algal growth inhibition test was conducted using unicellular green alga *Pseudokirchneriella subcapitata* (strain NIES-35), which was purchased from the National Institute for Environmental Studies (Tsukuba, Japan) and subcultured at the Tokushima University. The test was conducted as per the OECD Guideline for Testing Chemicals No. 201[24] with minor modifications. Briefly, 40 mL of the river water sample was filtered using a membrane filter (pore size 0.20 μm. RC-15, Sartorius, Göttingen, Germany), and 100 mL Erlenmeyer flasks were used for the test. Initial cell counts were set at 5×10^3 cells/mL, and cultures were placed in an incubator (23±1℃, 6400 lux) on a reciprocal shaker (100 rpm) for 72 h. Cell counts were determined using either an optical microscope or a spectrophotometer (Hitachi U-2001; Hitachi, Japan) at a wavelength of 750 nm. Three concentration series (typically, 97%, 48%, and 24%) were prepared for river water samples diluted with the concentrated OECD medium. Triplicates were prepared for each concentration, whereas six replicates of control were prepared.

Daphnia reproduction test was conducted using *Ceriodaphnia dubia* because of their shorter reproductive period compared to that of *D. magna*. The strain was purchased from the National Institute for Environmental Studies (Tsukuba, Japan) and bred at the Tokushima University for at least two years in breeding water consisting of dechlorinated tap water and artificial hard water by using the reconstituted water protocol of the U.S. EPA WET Test Method[25], with hardness set between 80 and 100 mg $CaCO_3$/L. The reproduction test was conducted according to the test method of Environment Canada[26] and U.S. EPA[25]. Briefly, ten neonates within 24 h of age were individually exposed to 20 mL of the river water samples in a 50 mL beaker placed in an incubator set at 25±1℃ and a 16 h light and 8 h dark cycle for eight days, and the number of young was counted in three broods. *Chlorella* sp. and yeast-cerophyll-trout chow (Ecogenomics Co., Kurume, Japan) were fed daily, and the test solution was renewed every 48 h. Ten replicates were prepared in approximately 30 mL of testing solution with one organism per vessel. At least three concentrations (typically, 100%, 50%, and 25%) were prepared for river water samples by diluting with the reconstituted dechlorinated water.

Fish short-term toxicity test was conducted using zebrafish (*Danio rerio*) as per the OECD Guideline for Testing Chemicals No. 212[27]. *D. rerio* was purchased from NIES (Tsukuba, Japan) and bred at the Tokushima University for more than two years. Briefly, fifteen embryos, estimated to be less than 4 hours post fertilization, were collected and exposed to approximately 50 mL of the test solution in a glass vessel at 26±1℃ and a 16 h light and 8 h dark cycle. Triplicates were prepared for controls and each concentration, and the test solution was renewed every 48 h. Similar to the toxicity test for *C. dubia*, at least three concentrations were prepared for river water samples by diluting with dechlorinated tap water.

2.4 Statistical analysis

Several multivariate analyses were conducted using Microsoft Excel with extension software package called Excel Statistics 2012 (SSRI, Tokyo, Japan). The analyses included principle component analysis (PCA), clustering analysis, and multiple regression analysis. All data were normalized after the determination of arithmetic mean and standard deviation. For the toxicity results, effective concentration (ECx) values were determined using the log-logistic model to determine the toxic unit (TU) as an inverse of ECx.

3 Results

3.1 Water quality

The results of basic water quality measurements and seasonal variations at Takase Bridge (T1) are shown in **Table 1**, whereas those for the other sites (T2 to T5 and Y1 to Y3) are shown in **Table 2**. The pH ranged between 6.3 and 9.0, whereas the DO values ranged from 7.6 to 11.1 mg/L in T1. For the other sites, the pH ranged narrowly from 6.7 to 8.4, whereas DO ranged more widely from 5.8 to 12.3 mg/L, mostly because of the relatively low DO values in T5 and Y3. The EC ranged from 5.4 to 29.7 mS/m at T1, whereas it was relatively large and ranged between 50 and 60

Table 1　Summary of water quality variables and short-term chronic toxicity at site T1

	2013										
	Apr	May	Jun	Jul	Aug	Sep 10	Sep 24	Oct	Nov 3	Nov 16	Dec
pH	7.1	7.1	8.1	7.3	8.7	7.0	7.2	8.1	7.4	7.6	7.3
DO（mg/L）	8.5	9.1	8.5	8.4	9.0	7.6	9.1	8.9	9.2	10	10
EC（mS/m）	12	17	9.3	13	9.5	20	12	8.5	11	11	9.7
NH_4^+−N（mg N/L）	0.075	0.038	<0.007	0.081	0.041	0.126	0.061	0.007	0.070	0.093	0.079
Hardness（mg$CaCO_3$/L）	41	45	42	44	44	45	40	30	36	40	36
BOD（mg O_2/L）	0.7	1.2	0.5	0.5	0.7	0.7	0.9	0.8	0.6	0.3	0.5
COD（mg O_2/L）	0.4	0.5	0.4	0.8	0.5	0.6	0.6	0.7	0.4	0.6	1.1
Algal TU	2.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
Daphnia Reproduction TU	<1.0	1.5	<1.0	2.6	<1.0	1.6	<1.0	<1.0	<1.0	<1.0	<1.0
Daphnia Immobilization TU	<1.0	<1.0	<1.0	3.2	<1.0	<1.0	<1.0	<1.0	<1.0	1.8	<1.0
Fish Hatch Rate TU	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
Fish Mortality TU	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0

	2014										
	Jan	Feb	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
pH	8.1	8.2	6.3	8.8	8.3	8.4	8.3	8.2	8.3	8.4	7.8
DO（mg/L）	11	11	9.9	9.2	9.1	8.6	8.5	9.1	9.7	9.9	11
EC（mS/m）	21	11	9.2	10	5.4	9.9	5.8	15	11	30	12
NH_4^+−N（mg N/L）	0.063	0.039	0.037	0.047	0.074	0.067	0.082	0.059	0.022	0.028	
Hardnes s（mg$CaCO_3$/L）	N. A.	N. A.	41	42	46	41	44	42	44	43	39
BOD（mg O_2/L）	0.6	0.9	1.1	0.5	1.2	0.4	0.3	0.6	0.4	0.5	0.4
COD（mg O_2/L）	1.6	N. A.	0.1	0.3	1.8	0.9	0.4	0.2	0.8	0.8	0.5
Algal TU	<1.0	N. A.	2.2	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
Daphnia Reproduction TU	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
Daphnia Immobilization TU	2.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
Fish Hatch Rate TU	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
Fish Mortality TU	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0

	2015											2016
	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan
pH	8.5	8.9	8.1	6.7	7.5	8.5	7.4	7.7	7.4	8.4	6.9	7.6
DO（mg/L）	11	N. A.	N. A.	9.3	9.2	9.3	8.7	9.3	9.8	11	11	12
EC（mS/m）	12	12	9.5	12	27	9.3	13	12	14	12	12	12
NH_4^+−N（mg N/L）	N. A.	0.066	0.065	0.062	N. A.	0.062	0.064	0.074	0.081	0.076	0.082	0.067
Hardness（mg$CaCO_3$/L）	37	36	36	34	40	34	34	32	47	43	31	N. A.
BOD（mg O_2/L）	<0.1	0.1	1.1	N. A.	2.1	N. A.	N. A.	1.0	1.0	0.1	1.2	1.9
COD（mg O_2/L）	N. A.	N. A.	0.3	0.5	N. A.	0.4	0.4	0.5	0.2	0.3	0.9	N. A.
Algal TU	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	6.9	<1.0	<1.0	2.1	<1.0	<1.0
Daphnia Reproduction TU	2.4	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	N. A.
Daphnia Immobilization TU	2.2	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	N. A.
Fish Hatch Rate TU	<1.0	<1.0	<1.0	<1.0	2.2	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
Fish Mortality TU	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0

N. A.　Not Available

mS/m in Y3. The EC values in the other sites ranged from 2.1 to 30.9 mS/m. The trend of total hardness was similar to that of EC values, but showed a narrower range between 30 and 45 mg $CaCO_3$/L in T1 and between 22 and 71 mg $CaCO_3$/L in the other sites. The ammonium nitrogen ranged from below the detection limit（<0.007 mg/L）to 0.126 mg/L at T1, whereas its concentration was considerably higher than other sites at two sites, T5 and Y3. The BOD/COD concentration ranged at a relatively low level（mostly below 2.0 mg/L）in T1, T2, T3 and Y1 and Y2, whereas its concentration was above 5 mg/L at some sampling points in T4, T5, and Y3.

3.2　Toxicity of Riverwater Samples

The results of short-term chronic toxicity tests performed using the three aquatic organisms are summarized in **Tables 1** and **2** for T1 and the other locations, respectively. The TU values are shown in **Figs. 2** and **3** for T1 and the other locations, respectively. Algal toxicity was detected in 4 of the 33 samples from T1 and 4 of the 19 samples from the other sites. The Y3 sample was toxic for the green alga during two sampling events. T5 and Y1 were found to

Table 2 Summary of water quality variables and short-term chronic toxicity at the sampling sites at the Yoshino River and Yodo River, Japan

	Nov 2013				Dec 2013		
	Okawa Br. (T2)	Wakimachi Br. (T3)	Imagi re R. (T4)	Tamiya Cr. (T5)	Miayamae Br. (Y1)	Hi rakata Br. (Y2)	Tokura Br. (Y3)
pH	7.4	7.4	6.9	7.7	7.0	7.0	6.8
DO (mg/L)	10	11	9.9	5.9	11	11	7.5
EC (mS/m)	7.4	12	8.4	12	18	18	50
NH_4^+−N (mg N/L)	0.072	0.077	0.083	2.319	0.145	0.107	3.035
Hardness (mg$CaCO_3$/L)	26	34	50	48	40	49	71
BOD (mg O_2/L)	0.7	0.6	0.7	5.8	1.8	1.1	6.1
COD (mg O_2/L)	0.5	0.3	1.2	2.7	N. A.	N. A.	N. A.
Algal TU	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	1.3
Daphnia Reproduction TU	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
Daphnia Immobilization TU	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
Fish Hatch Rate TU	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
Fish Mortality TU	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0

	Oct 2014				Dec 2014		
	Okawa Br. (T2)	Wakimachi Br. (T3)	Imagi re R. (T4)	Tamiya Cr. (T5)	Miayamae Br. (Y1)	Hi rakata Br. (Y2)	Tokura Br. (Y3)
pH	8.4	8.2	7.7	7.3	7.5	8.1	7.7
DO (mg/L)	9.9	10	9.5	7.0	11	12	7.8
EC (mS/m)	29	9.9	31	21	16	16	56
NH_4^+−N (mg N/L)	0.060	0.067	0.20	0.97	0.042	0.024	0.73
Hardness (mg$CaCO_3$/L)	31	36	65	67	37	44	70
BOD (mg O_2/L)	0.7	1.6	8.5	4.8	1.5	1.4	3.8
COD (mg O_2/L)	0.6	0.4	0.7	3.1	1.9	0.7	0.3
Algal TU	<1.0	<1.0	<1.0	<1.0	1.0	N. A.	N. A.
Daphnia Reproduction TU	<1.0	<1.0	<1.0	<1.0	2.1	<1.0	N. A.
Daphnia Immobilization TU	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
Fish Hatch Rate TU	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
Fish Mortality TU	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0

	Dec 2015				Jan 2016		
	Okawa Br. (T2)	Wakimachi Br. (T3)	Imagi re R. (T4)	Tamiya Cr. (T5)	Miayamae Br. (Y1)	Hi rakata Br. (Y2)	Tokura Br. (Y3)
pH	7.7	7.5	7.1	7.4	7.3	7.2	6.8
DO (mg/L)	11	10	10	6.5	12	12	8.2
EC (mS/m)	3.1	7.4	16	14	2.2	19	54
NH_4^+−N (mg N/L)	0.074	N. A.	0.064	0.72	0.078	0.096	0.58
Hardness (mg$CaCO_3$/L)	22	N. A.	43	68	50	48	71
BOD (mg O_2/L)	1.4	1.0	1.3	8.2	1.8	2.1	7.3
COD (mg O_2/L)	0.5	0.3	0.4	3.2	0.3	1.0	4.5
Algal TU	<1.0	<1.0	<1.0	2.0	<1.0	<1.0	2.3
Daphnia Reproduction TU	<1.0	<1.0	2.4	<1.0	<1.0	<1.0	<1.0
Daphnia Immobilization TU	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
Fish Hatch Rate TU	<1.0	<1.0	4.7	<1.0	<1.0	<1.0	<1.0
Fish Mortality TU	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0

N. A. Not Available

be toxic for green alga in one of three sampling events each. In T1, daphnia immobilization toxicity and reproduction inhibition occurred in 4 samples each of the 33 samples. For two of the sampling events (July 2013 and February 2015), both immobilization and reproductive toxicity were observed. For the other sampling sites, 2 of the 20 samples (Y1 in December 2014 and T4 in December 2015) were found to cause daphnia immobilization, whereas no sample was found to inhibit daphnia reproduction. Fish toxicity (effect on hatch rate) was only found in June 2015 in T1 and in December 2015 in T4.

3.3 Multivariate Analyses

The results of PCA based on water quality variables and ecotoxicity values are shown in **Fig. 4**, and those of clustering analysis based on sampling sites are shown in **Fig. 5**. The results of multiple regression analysis are summarized in **Table 3**. The BOD, COD, hardness, and ammonium nitrogen were higher (above 0.8) than

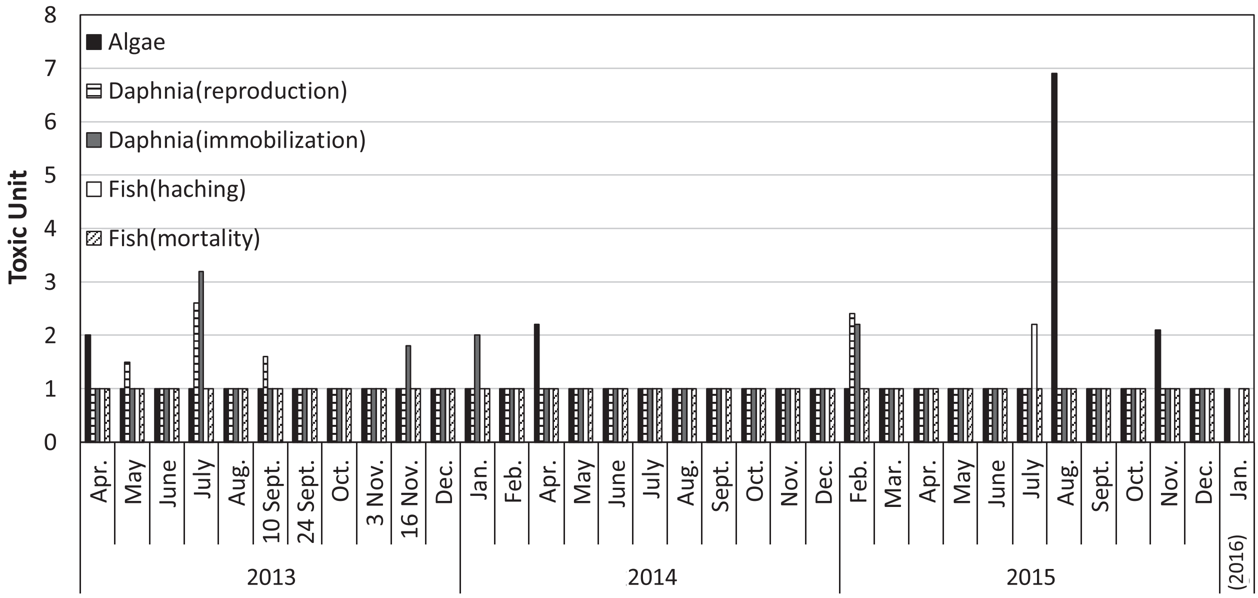

Fig. 2　Seasonal variation of short-term chronic toxicity at site T1.

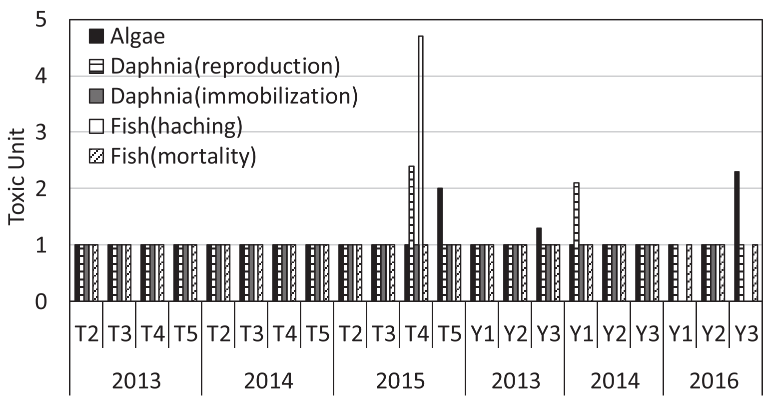

Fig. 3　Results of short-term chronic toxicity tests in the sampling areas at the Yoshino River and Yodo River, Japan.

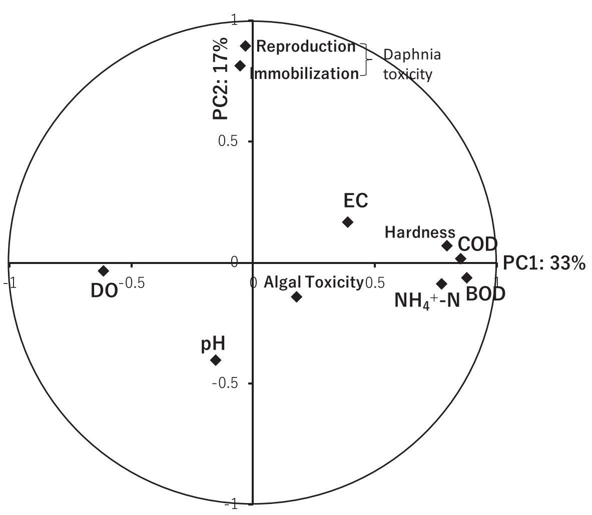

Fig. 4　Results of principle component analysis（PCA）for the water quality variables and short-term chronic toxicity.

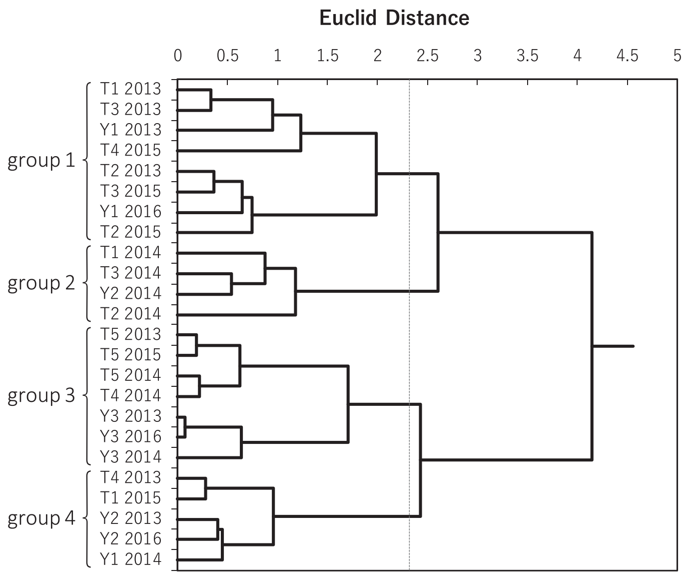

Fig. 5　Results of clustering analysis for the sampling sites.

Table 3 Summary of multiple regression analysis

		pH	DO	EC	Ammonia	Hardness	BOD	COD	Intercept
	Algal Toxicity (R2=0.23)	−0.104 [0.14]	−0.036 [0.93]	0.020 [0.86]	−0.091 [0.50]	0.097 [0.42]	0.033 [0.79]	0.053 [0.64]	−0.039 [0.68]
Daphnia Toxicity	Immobilization (R2=0.05)	−0.133 [0.47]	−0.462 [0.66]	−0.064 [0.81]	−0.018 [0.96]	0.182 [0.55]	−0.276 [0.36]	0.023 [0.94]	−0.153 [0.51]
	Reproduction (R2=0.13)	−0.312 [0.09]	−0.214 [0.83]	0.258 [0.42]	−0.197 [0.57]	0.023 [0.94]	−0.275 [0.36]	0.211 [0.53]	0.016 [0.95]

[ ] : *p* value

the other parameters in terms of principle component 1 (PC1), whose proportion of variance was 33%, whereas DO was located at the opposite end of these four water quality variables (**Fig. 4**). Daphnia toxicity endpoints—reproduction and immobilization—were found to be higher for PC2 than PC1, with the proportion of variance of 17%. Three other variables, EC, algal toxicity, and pH seemed to have no significant correlation with the other variables. The clustering analysis suggested that the sampling sites at the upstream of the river (e. g., T2 and T3) were clustered at the top side, whereas those downstream of the river or urban stream (e. g., T5 and Y3) were clustered at the bottom. The R^2 values for all three aquatic toxicity endpoints—algal growth, daphnia immobilization, and daphnia reproduction—were found to very low (about 0.23), and p values were also found to be above 0.05, suggesting that these dependent variables were not significantly associated with any of the water quality variables (independent variables).

4 Discussion

The BOD, COD, and ammonium nitrogen for samples obtained from the Takase Bridge of the Yoshino River (T1) were relatively lower throughout the year with some fluctuations (**Table 1**). DO was relatively high in the winter season (December to March), whereas it was relatively low (7.6 mg/L) in the summer season (June to September). This relatively good water quality at this site can be partly attributed to the relatively large precipitation in the watershed area (as high as 3000 mm/y) and the median flow rate of 60 m^3/s of the Yoshino River, which is one of the largest rivers in the western part of Japan[28]. The population of the watershed area was also very low compared to that in the other parts of Japan.

The frequency of detecting toxicity to aquatic organisms, including daphnia and green algae, ranged from 10% to 20% for all the water samples collected from T1. The general water quality variables such as BOD did not suggest any significant anthropogenic contamination at this site, but most of the land use around the area was forest or farm lands. In the US, several researchers have suggested that pesticides such as organophosphorus and pyrethroids are the major toxicants that affect the quality of river water samples[6, 7]. The toxicity characterization and identification at this site needs to be conducted in the future.

T2 and T3 at the upper/middle streams of the Yoshino River were found to be cleaner than T1 in terms of BOD, COD, and ammonia. EC and hardness at these sites were also found to be lower than those at T1. The population density of the upper Yoshino River watershed was relatively lower and not many sources of contamination existed.

In contrast, T4 and T5 at the lower stream of the Yoshino River were used as residential and industrial areas, respectively. In particular, T5 was located at the western part of the city center and had relatively high population density; it had a sewerage system (or septic tank system), which is not sufficient, and approximately 30% of untreated gray water was released into the Tamiya Creek, resulting in relatively low DO and relatively high BOD, COD, and ammonia. Significant contamination by food products and detergents was noted in this area, and we previously detected anionic surfactants, linear alkylbenzene sulfonate (LAS), and several PPCPs at this site[18, 21] as well as aquatic toxicity to daphnia, algae, and midge[15, 17, 18]. Toxicity was also detected at one sampling occasion at T4, and the effluents from both residential area and factories around the watershed area affected the water quality.

The water quality at Miyamae Bridge (Y1) in the Katsura River, one of the three major tributaries of the Yodo River, was relatively good in terms of BOD, COD, and ammonia, whereas toxicity was detected at one sampling occasion. We also previously detected algal toxicity at this site[16], and the outlet of the major wastewater treatment plants collecting from nearly 80% of the population of Kyoto City was located at approximately 10 km upstream of this site. We also found algal toxicity, LAS, and some PPCPs near the outlet of the effluents[18], and chlorine disinfection byproducts could also be present at this site. However, the effluents were diluted by the main stream of the Katsura River at Y1.

The water quality of Y2 of the main stream of the Yodo River was relatively good in terms of BOD, COD, and ammonia, whereas the EC and hardness were relatively higher than those at T1 (annual average), T2, and T3 and comparable to those at T4 and T5. The Yodo River had relatively high flow rate with median value of 150 m^3/s mainly because of the high flow rate of the Uji River from Lake Biwa, the largest lake in Japan. Despite the relatively large contamination loading from the Kyoto City area, the contaminated compounds were diluted to maintain moderate water quality in this area.

In contrast, the Ina River flows through a high population area in the northern part of Osaka metropolitan area. Many factories are located around the site Y3, and a large wastewater treatment plant discharged its effluent into the Ina River. The BOD, COD, ammonia, EC, and hardness were higher than other sites at this site and DO was relatively low, suggesting significant contamination. Algal toxicity was detected at this site, as was found in our previous study[16].

The results of PCA (**Fig. 4**) showed that BOD, COD, ammonia, and hardness were all relatively high above 0.8 in PC1, whereas DO was low (approximately −0.9). This trend suggests that PC1 could be the index for the anthropogenic contamination mainly because of household effluents. In contrast, both daphnia toxicity end points showed independent trends from the water quality variables and had relatively large PC2 values, suggesting that daphnia toxicity is independent from the contamination by domestic wastewater, and some other factors such as pesticides and heavy metals might play important roles in the toxicity. **Fig. 4** indicates that the toxicity of Daphnia may be related to pH. However, the data is limited, a more detailed investigation is required. Furthermore, it was suggested that the toxicity of algae is irrelevant to both artificial contamination and toxicity to Daphnia. In our previous study[18], the correlation between LAS and PPCP concentration and short-term chronic toxicity to algae, daphnia, and fish was investigated at the sites that were highly contaminated with untreated/treated gray water, such as T5. We found relatively large contribution of LAS at some sites for daphnia and moderate contribution of an antibacterial clarithromycin and antimicrobials triclosan and triclocarban for green alga; the largest contribution was from unknown toxicants, such as pesticides and heavy metals.

We also conducted toxicity characterization at T5 and Y3 and found that the contribution of cations was relatively large compared to that of hydrophobic organics[29]. Further investigation is necessary to reveal the correlation between other toxicants such as pesticides (or heavy metals) and aquatic toxicity.

The results of cluster analysis (**Fig. 5**) suggested clear separation of the upper stream sites such as T2 and T3 and lower stream sites such as T5 and Y3. The four group separated by euclidean distance 2.2 were set as group one, two, three, four from the top. All pH in group two were over eight. The range of DO at group three was 5.9–9.5, lower than others, and BOD were higher than others (3.8–8.5), and three sampling sites where showed toxicity to algae was included. However, group one and four also contained one site each of which has TU value of algae over two, and toxicity tendency peculiar to each group was not obserbed. As mentioned above, BOD, COD, and ammonia were all relatively low in T2 and T3, whereas they were relatively high in T5 and Y3. The trend of EC and hardness was also similar to these water quality variables. T1, T4, Y1, and Y2 belonged to both groups depending on the year of investigation. These sites are partly affected by effluents of wastewater treatment plants and factories and possibly by farmlands, whereas relatively large flow rate dilutes the contaminants. This dilution factor could fluctuate and cause the inconsistency in the toxicity at these sites.

Multiple regression analysis (**Table 3**) suggested the independence of dependent variables (algal or daphnia toxicity) from all independent variables (water quality variables), which is in agreement with the PCA results. In particular, daphnia toxicity was highly independent from the investigated water quality variables, and the relevant variables need to be further investigated. Individual chemical analysis might overlook important chemical substances, which could be non-regulated or not routinely monitored, and frequent monitoring of whole toxicity of ambient water is thus important.

Acknowledgements

This work was supported by the JSPS Grant-in-Aid for Scientific Research (C) 25340099.

References

1) Chemical Abstracts Service, CAS Home Page, http://www.cas.org/ (Last accessed on February 27, (2017)
2) E. A. Power and R. S. Boumphrey: International trends in bioassay use for effluent management, Ecotoxicology, Vol. 13, pp. 377-398 (2004)
3) U. S. EPA: National Whole Effluent Toxicity (WET) Implementation Guidance Under the NPDES Program, WET Implementation Guidance, EPA 832-B-04-003 (2004)
4) Federal Ministry for the Environment, Germany, Waste Water Ordinance, (2004)
5) V. D. Vlaming, V. Connor, C. DiGiorgio, H. C. Bailey, L. A. Deanovic, and D. E. Hinton: Application of whole effluent toxicity test procedures to ambient water quality assessment, Environ. Toxicol. Chem. Vol. 19, pp. 42-62 (2000)
6) H. C. Bailey, R. Krassoi, J. R. Elphick, A. M. Mulhall, P. Hunt, L. Tedmanson, and A. Lovell: Whole effluent toxicity of sewage treatment plants in the hawkesbury—nepean watershed, New South Wales, Australia, to Ceriodaphnia dubia and Selenastrum capricornutum, Environ. Toxicol. Chem., Vol. 19, pp. 72-81 (2000)
7) B. S. Anderson, B. M. Philips, J. W. Hunt, K. Worcester, M. Adams, N. Kapellas, and R. S. Tieerdema: Evidence of pesticide impacts in the Santa Maria River watershed, California, USA, Environ. Toxicol. Chem., Vol. 25, pp. 1160-1170 (2006)
8) M. Kayhanian, C. Stransky, S. Bay, S. L. Lau, and M. K. Stenstrom: Toxicity of urban highway runoff with respect to storm duration, Sci. Total Environ., Vol. 389, pp. 386-406 (2008)
9) L. Vigano, A. Bassi, and A. Garino: Toxicity evaluation of waters from a tributary of the River Po using the 7-Day Ceriodaphnia dubia test, Ecotox. Environ. Safe., Vol. 35, pp. 199-208 (1996)
10) B. P. Baldigo, B. T. Duffy, C. J. Nally, and A. M. David: Toxicity of Waters from the St. Lawrence River at Massena Area-of-Concern to the Plankton Species Selenastrum Capricornutum and Ceriodaphnia Dubia, J. Great Lakes Res., Vol. 38, pp. 812-820 (2012)
11) Y. X. Fang, G. G. Ying, L. J. Zhang, J. L. Zhao, H. C. Su, B. Yang, and S. Liu: Use of TIE techniques to characterize industrial effluents in the Pearl River Delta region, Ecotox. Environ. Safe., Vol. 76, pp. 143-152 (2012)
12) Y. Sasaki, H. Kise, M. Matsui, T. Yoshizumi, M. Wakabayashi, and M. Kikuchi: Bioassay for the Assessment of Environmental Toxicity of River Water *J. Environ. Chem.*, Vol. 10, pp. 45-55 (2000), (Manuscript written in Japanese and Tables/Figures in English).
13) H. Hatakeyama, H. Shiraishi, and Y. Sugaya: Monitoring of the overall pesticide toxicity of river water to aquatic organisms using freshwater shrimp, Paratya compressa improvisa, Chemosphere, Vol. 22, pp. 229-235 (1991)
14) M. Kikuchi, Y. Tokunaga, M. Kikuchi, A. Sato, Y. Umeda, and J. Sawai: A 10 years study of the responses of Daphnia Magna to river water in urbanized area, J. Environ. Chem., Vol. 18, pp. 361-368 (2008) (Manuscript written in Japanese and Tables/Figures in English).
15) Y. Yasuda, S. Yoneda, I. Tamura, K. Kagota, N. Nakada, S. Hanamoto, Y. Kameda, K. Kimura, N. Tatarazako and H. Yamamoto: Short-Term Chronic Toxicity Tests Applied to River Water Contaminated by Treated or Untreated Domestic Sewage, Civil Eng. G (Environment), Vol. 48, III_249-256, (2011) (In Japanese with English Abstract)
16) H. Morita, Y. Yasuda, K. Kagota, I. Tamura, N. Tatarazako and H. Yamamoto: Sort-Term Chronic Toxicity Test of Three Aquatic Organisms Applied to First Class River in Japan, J. Civil Eng. G (Environment), Vol. 68(7), III_217-225 (2012) (In Japanese with English Abstract)
17) I. Tamura, K. Kimura, Y. Kameda, N. Nakada and H. Yamamoto: Ecological risk assessment of urban creek sediments contaminated by untreated domestic wastewater: potential contribution of antimicrobials and a musk fragrance, Environ. Technol., Vol. 34, pp. 1567-1575 (2013)
18) I. Tamura, Y. Yasuda, K. Kagota, S. Yoneda, V. Kumar, N. Nakada, K. Kimura, Y. Kameda and H. Yamamoto: Contribution of pharmaceuticals and personal care products (PPCPs) to whole toxicity of water samples collected in effluent-dominated urban streams, Ecotox. Enviorn. Safe., Vol. 144, pp. 338-350 (2017)
19) E. T. H. M. Peeters, A. Dewitte, A. A. Koelmans, J. A. van der Velden and P. J. den Besten: Evaluation of bioassays versus contaminant concentrations in explaining the macroinvertebrate community structure in the Rhine-Meuse delta, The Netherlands, Environ. Toxicol. Chem., Vol. 20, pp. 2883-2891 (2001)
20) P. Palma, P. Alvarenga, V. Palma, C. Matos, R. M. Fernandes, A. Soares and I. R. Barbosa: Evaluation of surface water quality using an ecotoxicological approach: a case study of the Alqueva Reservoir (Portugal), Environ. Sci. Pollut. Res., Vol. 17, pp. 703-716 (2010)
21) H. Yamamoto, I. Tamura, Y. Hirata, J. Kato, K. Kagota, S. Katsuki, A. Yamamoto, Y. Kagami and Tatarazako, N.: Aquatic toxicity and ecological risk assessment of seven parabens: Individual and additive approach, Sci. Tot. Environ., Vol. 410-411, pp. 102-111 (2011)
22) H. Namiki ed., Standard Methods for Wastewater Testing (1999) (In Japanese)
23) Ministry of the Environment and National Institute for Environmental Studies, Japan, *Test Methods for Effluent Using Bioassay (Revised Draft)*, (2014)
24) OECD, Guidelines for Testing of Chemicals No. 201, (2011)
25) U. S. EPA, *Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Water to Freshwater Organisms* (2002)
26) Environment Canada, Biological Test Method: Test of Reproduction and Survival Using the Cladoceran Ceriodaphnia dubia, (2007)
27) OECD, Guidelines for Testing of Chemicals No. 212, (1998)
28) Ministry of Land, Infrastructure and Transportation, Water Information System (http://www1.river.go.jp/) (Last

accessed on February 27, 2017).
29) J. Morita, Y. Yasuda, I. Tamura, N. Tatarazako and H. Yamamoto: Characterization of toxicants for three aquatics oranisms in ambient water samples, J. Civil Eng. G (Environment), Vol. 69 (7), III_401–410 (2013) (In Japanese with English Abstract)

吉野川および淀川流域における水質と生態毒性の多変量解析

田 村 生 弥[1]，林 岳 彦[2]，西 家 早 紀[3]，円 山 萌[3]，光 山 真 子[3]
鑪 迫 典 久[4]，山 本 裕 史[2, 3]

[1] 岡山大学大学院環境生命科学
[2] 国立環境研究所
[3] 徳島大学大学院総合科学教育部
[4] 愛媛大学大学院農学研究科

概 要

本研究では，河川水試料のBOD，pH，アンモニア，溶存酸素，EC，硬度などの水質の季節変動を測定することを目的とした。また，濃縮していない原水サンプルについて短期慢性毒性試験を実施した。これらのデータを多変量解析にかけた。主成分分析の結果，BOD，EC，アンモニアなどの水質が同様の傾向を示したが，ミジンコの毒性は水質とは無関係であった。クラスター分析の結果から，サンプルを上流および下流から集められたものとして分類され，これは化学物質による人為的汚染の程度によるものと示唆された。

キーワード：生態毒性，河川水，水質，多変量解析，総排水毒性