Evaluation of Relationship Between Biological Safety and Benthic Macroinvertebrate Assemblages in the Sakawa River System, Japan

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ABSTRACT
The objective of this study was to assess the biological safety and river health in an actual river and to clarify their relationship. In this study, “biological safety” meant safety level of contaminants in river water to aquatic creatures, which was evaluated by performing various bioassays. “River health” meant conditions of water quality and biodiversity from a viewpoint of benthic macroinvertebrate assemblages, which was desired by humans. At nine sites on the Sakawa River system (Japan), biological safety was evaluated as a toxicity score and bio-safety rank (BSR) using an algae growth inhibition test, a daphnia immobilization test, and a larval medaka assay. According to the result, the biological safety was found to be completely different among the sampling sites and the test species. River health was evaluated according to the Ministry of the Environment (MOE) of Japan water quality and biodiversity (taxa number) at the nine sites. According to the result, the number of taxa were found to be completely different among the sites. The results of the bioassays and macroinvertebrate collections were compared and indicated that the number of taxa and BSR described similar behavior when going downstream, although no clear correlation was found between BSR and MOE-water quality class. Therefore, biodiversity tended to deteriorate as the intensity of toxicity became more severe.

Keywords: bioassay, biodiversity, biological index, macroinvertebrate, river health, toxicity

INTRODUCTION
Different types of chemicals can be found in huge numbers in river systems. Because it is not possible to conduct physico-chemical analyses for all chemical pollutants or to consider their interactive effects on toxicity, conventional physico-chemical analysis alone is insufficient for evaluating river environments. Bioassays can be a very effective tool for evaluating environmental toxicants, because they comprehensively assess toxicity. Various kinds of bioassays have been applied to evaluate water toxicity, and three kinds of in vivo aquatic ecotoxicity tests such as the acute fish lethal test, acute daphnia immobilization test, and the chronic algae growth inhibition test are used most frequently. Liu et al. (2007a, b) established a larval medaka assay and evaluated river water and wastewater using this assay method. Impact to river environment related to pesticide use was investigated using the daphnia test (Anderson et al., 2006; Barata et al., 2007) and the algae test (Köck et al., 2010). Wei et al. (2008) evaluated the biological safety of watersheds using three toxicity tests (algae growth inhibition, daphnia immobilization, and larval fish toxicity), and revealed the effects of human activities.
Information on benthic river macroinvertebrate assemblages is quite useful for evaluating the overall condition of river environments, because these organisms are affected for long durations by various environmental stressors and support the surrounding ecosystems as a major prey for fishes and birds. Therefore, a lot of investigations on them have been conducted, and related to several factors, such as water quality (Dlamini et al., 2010) and land-use (Miserendino et al., 2011).

However, few studies have revealed the relationship between ecotoxicity of river water and benthic macroinvertebrate assemblages, although several studies have shown relationships between toxicity of effluents and macroinvertebrate assemblages in receiving water (Sarakinos and Rasmussen, 1998; Jin et al., 2007). The reason would be low toxicant concentrations in river water, besides hard work of long-term toxicity tests. Moreover, if there were several stress factors in same level of intensity in each site, the relationship would be unclear. In this study, we used an efficient solid-phase organic toxicant extraction method to test the toxicity of river water samples collected from the Sakawa River system whose river-bed morphology was relatively good in order to overcome the problems. If the relationships are clarified, using bioassays to evaluate river health would be validated. The objective of this study was to assess biological safety and river health in an actual river and to clarify their relationship. Biological safety was assessed using bioassays of samples collected from nine sites on the Sakawa River system, and river health was evaluated from results of benthic macroinvertebrate collections from the same sites (Kubo et al., 2011). Then, biological safety and river health were compared.

MATERIALS AND METHODS

Study site
Sakawa River is a class B river and is one of the principal rivers in Kanagawa Prefecture, Japan. Six sampling sites were selected from the mainstream (Site-C to Site-H) and three from tributaries (Site-A, Site-B, and Site-I). Site-A to Site-H were arranged from upstream to downstream (Fig. 1). The Nakagawa River, a tributary, flows into Lake Tanzawa, which is located upstream of the Sakawa River system. Many recreational facilities are located around the upper side of the lake, such as hot springs and camping sites. Kouchi River starts from Lake Tanzawa and becomes confluent with the Sakawa River upstream of Site-C. Dry fields are distributed in the upper area of Site-D. Many paddy fields are distributed along the Sakawa River from Site-D to Site-G. An expressway and route 246 run along the river from Site-C to Site-E. Many industrial plants are located there, and a residential area is distributed along the river from Site-D to the river estuary. Site-G was located just upstream of the intake weir.

Preparation of river water samples for toxicity testing
River water samples were collected from the nine sites (Site-A to Site-I) on August 17, 2004. Occasionally, particular inorganic substances, such as heavy metals and ammonia, would affect aquatic creatures. However, most of those could be analyzed individually in many cases. On the other hand, it would be difficult to identify organic contaminants because they could form complicated structures. Hence it would be hoped that bioassays would be utilized for evaluating these organic contaminants. Additionally, because hydrophobic organic contaminants especially tended to bioaccumulate over a long
period of time, the compounds could cause a negative effect on aquatic life even if the concentrations were low. Therefore, hydrophobic organic toxicants were concentrated from the river water with a solid-phase extraction method. After being filtered with a 1.0 μm glass-fiber filter (ADVANTEC® GA-100, Advantec Toyo Kaisha, Ltd., Tokyo, Japan), 4 L of each sample was loaded into two pre-conditioned Sep-Pak® Plus PS-2 cartridges using a glass syringe pump (Sep-Pak® Concentrator Plus, Nihon Waters KK, Tokyo, Japan) at 20 mL/min with upflow. Sep-Pak® Plus PS-2 cartridges adsorb various organic micropollutants in water (Ishii et al., 2000) and have been widely used to recover chemicals from complex environmental water samples. Air was injected into the cartridge with a syringe to drive out the space water. Then, 10 mL of acetone was flowed into each cartridge at 2.0 mL/min from the reverse direction, against the adsorption stage, for desorption. The 20 mL of acetone eluate from the two cartridges was evaporated to 500 μL under a nitrogen gas purge at 300 mL/min in a 35°C water bath. Then, 2.0 mL of tap water purified with a granular activated carbon column was added to the residual solution and concentrated to 2.0 mL in the water bath. The nine representative samples collected, which were concentrated 2000 times, were diluted to different concentrations and prepared as exposure solutions for toxicity tests; maximum exposure concentrations were 10, 50, and 50 fold concentrated river water for algae, daphnia, and larval medaka assay, respectively. These values of the concentration factors were established by statistically comparing of the ratio of effect concentration in short-term toxicity test to no-effect concentration in long-term toxicity test at 80% or higher confidence level. (Wei et al., 2006). If no adverse ecotoxicity effects were observed for all the three tests at each concentration factor, the water sample could be considered harmless for the aquatic ecosystem in the site (Wei et al., 2008).

**Toxicity tests and evaluation method**

Toxicity in the water samples was evaluated using three standardized bioassays: an
algae growth inhibition test using *Pseudokirchneriella subcapitata* (formerly known as *Selenastrum capricornutum*), a daphnia immobilization test using *Daphnia magna*, and a larval fish toxicity test using *Oryzias latipes*. The stock solution of 50 fold concentrated river water was diluted into a series of duplicate exposure solutions. The serial exposure solutions were prepared as 2-, 4-, and 10 fold concentrated river water for the algae growth inhibition test. The experimental conditions were: volume of exposure solution, 20 mL; initial cell density, $10^4$/mL; shaking speed, 100 rpm; exposure duration, 72 h; light intensity, 4,000 lx; and light/dark cycle, 24 h/0 h. A series of exposure solutions were prepared as 10-, 20-, and 50 fold concentrated river water for daphnia immobilization test, and the operational parameters were: volume of exposure solution, 20 mL; neonate age, < 24 h old; neonate density, 10/concentration; exposure duration, 48 h; and light/dark cycle, 16 h/8 h. The solution preparation for the larval fish toxicity test was the same as that for the daphnia test, and the operational conditions were: volume of exposure solution, 20 mL; larvae age, 48 - 72 h old; larvae density, 10/concentration; exposure duration, 48 h; and light/dark cycle, 16 h/8 h. (Wei *et al.*, 2006)

An assessment index, “toxicity score” of “1 toxicity”, “2 toxicity”, “3 toxicity”, or “4 toxicity”, with “1 toxicity” being the safest, was determined according to the highest exposure concentration in which adverse ecotoxicological effects could not be observed (as shown in Table 1), and a triangle figure describing the toxicity scores of the three toxicity tests was prepared. To conveniently evaluate the biological safety of environmental water, an integrated assessment index or bio-safety rank (BSR) of “A BSR”, “B BSR”, “C BSR”, or “D BSR”, with “A BSR” being the safest, was determined according to the worse toxicity scores of the three tests (Wei *et al.*, 2006).

**Macroinvertebrate assessment**

Benthic macroinvertebrates were collected at the nine sites in August 2004 using basic sampling tools, such as a bucket, a white tray, and a D-shaped net (approximately 0.5 mm mesh). Samples were identified on site. If identification was difficult, the sample was transported to the laboratory and confirmed using a microscope. Collection and identification methods were based on instructions provided by the Ministry of the Environment (MOE) of Japan (MOE, 2011). Twenty-six taxa were considered, following elimination of four brackish macroinvertebrates from the target 30 taxa in MOE instructions (Kubo *et al.*, 2011). The data obtained were used to calculate MOE water quality class and the number of taxa. MOE water quality class was divided into following five classifications using dominant taxa information: “I MOE” (clean water), “II MOE” (lightly polluted water), “III MOE” (polluted water), “IV MOE” (heavily polluted water), and “×” (no indicator taxon was collected) (MOE, 2011).

<table>
<thead>
<tr>
<th>Toxicity tests</th>
<th>Toxicity Score</th>
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<tr>
<td>Algae</td>
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<td></td>
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<td></td>
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<tr>
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<td>20-fold</td>
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<td>50-fold</td>
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<tr>
<td>Medaka</td>
<td>&lt; 10-fold</td>
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Table 1 - Determination of the toxicity scores for three tests.
RESULTS AND DISCUSSION

Bioassay responses

The toxicity scores and BSRs of the water samples are shown in Fig. 2. This is the first report evaluating the toxicity score of a river system from upstream to downstream. The toxicity scores for all medaka larvae samples were 1 toxicity, suggesting that the river water was safe for medaka larvae. The toxicity score for daphnia was 2 toxicity at Site-B and Site-G. No adverse effect on daphnia was identified for the water samples from the other sites. The algae toxicity scores were 4 toxicity at Site-A and Site-E and 3 toxicity at Site-G. No adverse effects were observed in the algae growth inhibition test for the remaining sites. Toxicity scores differed among the three test species even for identical water samples.

BSRs evaluated from toxicity scores are also shown in Fig. 2. BSRs were completely different among the sites even though the sites were on the same river. Water samples from the most upstream sites were predicted to be unpolluted and to indicate a higher BSR, and the levels were expected to deteriorate as the river flowed downstream. However, we found the opposite results. BSR at Site-A, which was located most upstream, was ranked DBSR as the worst, reflecting the effects of the effluents from the recreational facilities, such as hot springs and camping sites, around Site-A. At the upper side of Site-E, various stress factors existed based on human activities, such as cultivated fields, industrial plants, expressways, and residential area. Some of these factors seemed to be the reasons behind BSR for Site-E being DBSR. The BSR for Site-G was CBSR because Site-G was located just on the upper side of the intake weir, where the

Fig. 2 - Toxicity score and BSR for the sampling sites on the Sakawa River system.
flow rate was very slow and toxicants from upperside sources, such as industrial plants and paddy fields, accumulated. BSRs were just strongly reflected by the result of algal toxicity test. The fact indicated that toxic compounds especially to algae like herbicide existed in the river water. This might mean that the toxicants were discharged around the river system by human activities as mentioned above and gradually flowed out. Thus, we could evaluate the biological safety of the Sakawa River system based on toxicity to algae, daphnia, and medaka larvae.

Appearance of benthic macroinvertebrate assemblages

The results of the benthic macroinvertebrate collection are shown in Fig. 3; for each site, a set of MOE-water quality class and the taxa number is indicated next to the names of the appearing taxa. The Japanese taxon names corresponding to the scientific names provided in this figure is reported by Kubo et al. (2011). MOE-water quality classes were I_MOE for seven of the nine sites, i.e., water quality of the Sakawa River system evaluated from benthic macroinvertebrate assemblages was almost good. However, the number of taxa was completely different among the sites even though the sites were on the same river.

Fig. 3 - MOE-water quality class and taxa number for each sampling site on the Sakawa River system (◎ indicates dominant taxon. #1 means a taxa group consisted of Pleuroceridae and Thiariidae. #2 means a group consisted of Erpobdellidae, Gastromobdellidae, Glossiphonnidae, Hirudinidae and Piscicolidae.).
The taxa number of Site-A was 3\(_{\text{taxa}}\) probably due to the effects of effluents from the recreational facilities around Site-A. Various stress factors based on human activities occurred on the upper side of Site-E and Site-F, and some of these factors seemed to be the reasons behind taxa number of Site-E and Site-F being 4\(_{\text{taxa}}\) and 5\(_{\text{taxa}}\). The taxa number for Site-G was 1\(_{\text{taxa}}\). Because Site-G was located just on the upper side of the intake weir, the flow rate was very slow and the riverbed morphology was silty, which is why MOE-water quality class and the taxa number of Site-G were III\(_{\text{MOE}}\) and 1\(_{\text{taxa}}\), respectively. Different kinds of taxa number (from 3\(_{\text{taxa}}\) to 9\(_{\text{taxa}}\) were observed even though MOE-water quality class was identical (I\(_{\text{MOE}}\)). Thus, the variation of health based on the combination of MOE-water quality class and taxa number could be observed at the Sakawa River system.

**Comparison between bioassay responses and macroinvertebrate occurrence**

BSR, MOE-water quality class, and taxa number are shown in Fig. 4 to compare the results of the bioassays and macroinvertebrate sample collections. Sites with a low BSR seemed to indicate a lower taxa number, although no clear correlation was observed between BSR and MOE-water quality class. In spite of the fact that macroinvertebrate accepted several effects of many stressors, significant correlation was observed between BSR and taxa number according to the Spearman's rank correlation coefficient (P < 0.05). Small amounts of toxicants with selective effects on the test species may have caused this result. This trend was similar to the previous reports on effluents and macroinvertebrates (Jin et al., 2007). The change in the number of taxa and BSR was similar when traveling downstream (Site-A to Site-H), suggesting that biodiversity tended to deteriorate as the intensity of toxicity, determined by the bioassays, became more severe. The correlation between the number of taxa and BSR was affected by the results of the algae growth inhibition test, although the daphnia immobilization test, which uses a macroinvertebrate, was expected to be related to macroinvertebrate diversity. The results suggested that a limited number of species can inhabit a site due to adverse effects of toxicants even if the macroinvertebrates are not affected directly by the toxicants. Algae is the dominant primary producer in the aquatic food chain, and

![Fig. 4 - Changes in MOE-water quality class, the number of taxa and BSR when traveling downstream on the Sakawa River system.](image)
damage to algae would probably cause effects at higher trophic levels. Namely, because plant like algae would be a major prey for many macroinvertebrates, adverse effect to algae would affect inhabitation of macroinvertebrates indirectly. It was suggested that BSR was a valuable environmental index.

CONCLUSIONS
Due to low toxicant concentrations in river water, little biological safety research has been conducted at the Sakawa River system (Japan). We used an efficient solid-phase organic toxicant extraction method to test the toxicity of river water samples. Biological safety was evaluated as a toxicity score and BSR using the algae growth inhibition test, the daphnia immobilization test, and the larval medaka assay. As a result, biological safety was completely different among the sampling sites and test species.

River health was evaluated using MOE-water quality class and biodiversity, which were calculated from benthic macroinvertebrate assemblages. On comparing the bioassay responses and macroinvertebrate occurrence, it was revealed that the number of taxa and BSR described a similar behavior when traveling downstream, although no clear correlation was found between BSR and MOE-water quality class. Therefore, it was seen that biodiversity tended to deteriorate as the intensity of toxicity became more severe. Consequently, it was suggested that BSR was a valuable environmental index.

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