

Full Paper

Analysis of the differences in microbial community structures between suspended and sessile microorganisms in rivers based on quinone profile

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In this study, a quinone profiling method was applied to clarify the differences in community structure between suspended and sessile microorganisms in rivers. The compositions of microbial quinone of 6 sites for 4 rivers were analyzed. Ubiquinone (UQ)-8, UQ-10, menaquinone (MK)-7, and plastoquinone (PQ)-9 were observed in all samples of suspended and sessile microorganisms for the sites investigated. The dominant quinone species in suspended microorganisms was ubiquinone, and that in sessile microorganism was photosynthetic quinones (namely PQ-9 and vitamin K1). This indicated that aerobic bacteria were abundant in the suspended microorganisms, and photosynthetic microorganisms such as micro-algae and cyanobacteria dominated in the sessile microorganisms. The quinone concentration in the river waters tested, which reflects the concentration of suspended microorganisms, ranged from 0.045 to 1.813 nmol/L. The microbial diversities of suspended and sessile microorganisms calculated based on the composition of all quinones were in the range from 3.4 to 7.5, which was lower than those for activated sludge and soils. Moreover, the diversity of heterotrophic bacteria for sessile microorganisms in the rivers was higher than that for the suspended microorganisms.

Key Words—microbial community structure; microbial diversity; quinone profile; river; sessile microorganism; suspended microorganism

Introduction

A river-ecosystem is strongly affected by human activities. The pollutants discharged into a river from human activities may destroy the ecosystem of the

river. Microorganisms in rivers (including suspended and sessile microorganisms) play key roles in degrading the pollutant and therefore in preventing the river ecosystem from being destroyed. To establish suitable measures for the protection and restoration of a river-ecosystem, the characteristics of the microorganisms in that ecosystem should be understood exactly. Many studies have been carried out on the role of microorganisms in river-ecosystems and the relationship between water quality and suspended microorganisms or

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sessile microorganisms. Some researchers have suggested that sessile microorganisms are greatly affected by the environments they inhabit (Lai and Chen, 1997). Sessile microorganisms have been used as water pollution indicators (Fuchs et al., 1996; Twist et al., 1998). Fukushima and Fukushima (1997) investigated the relationship between water quality recovery and algal assemblages. Nuttall (1982) studied the effect of environmental factors on the suspended microorganisms in rivers. Even so, the information on river-ecosystem, particularly about microbial community structure in rivers, is still limited. This paucity of the information is, in part, due to the lack of reliable techniques for quantitatively analyzing microbial community structure in environmental samples. Most of the conventional techniques for characterizing properties of microorganisms (such as plating techniques) are based on microbial enrichment, isolation, and cultivation. These techniques are unable to give reliable information about microbial communities because many microorganisms in the environment are un-culturable (Amann et al., 1995). In recent years, fluorescent *in situ* hybridization (FISH) technique has been noted as an analytical technique of microbial community structure in various environments. However, the FISH technique for analysis of all microbial community needs much time and a skilled operator to get reliable results. Alternatively, techniques of quantitative chemical analyses of specific biomarkers including phospholipids and microbial quinones (Hedrick and White, 1986; Hiraishi, 1988; Hu et al., 1993) are increasingly being considered for evaluating microbial community structure in environmental samples. Phospholipids and microbial quinones have a high correlation with the biomass. However, phospholipid fatty acids analysis may not indicate small changes in a microbial community structure. The relative content of each group of quinones may reflect the energy metabolic characteristics of an ecosystem. The technique of using quinone profiles has been considered a simple and useful tool for analysis of microbial population dynamics in mixed cultures. In this study, the technique of quinone profiles was applied to clarify the difference in community structure between suspended and sessile microorganisms in rivers.

Microbial quinone, which is one of the components of the electron transport chain in microbial cells, can be divided in two groups: respiratory quinone and photosynthetic quinone. Respiratory quinones (including

ubiquinone and menaquinone) exist in the bacteria gaining energy by way of respiration. In general, ubiquinones are used for aerobic or nitrate respiration and menaquinones for aerobic or anaerobic respiration (Jones, 1988). Photosynthetic quinones (including plastoquinone and vitamin K1) are found in photosynthetic microorganisms such as micro-algae and cyanobacteria (Hiraishi, 1999; Jones, 1988). Quinones exist in almost all microorganisms, and in general, one species or genus of bacteria has only one dominant type of respiratory quinone. So the quinone profile, which is usually represented as the molar ratio of each quinone type, should be specific to a microbial community. It should be noted, however, that some facultative anaerobes such as the *Enterobacteriaceae* family (e.g., *Escherichia coli*) contain both ubiquinone and menaquinone (Jones, 1988).

Materials and Methods

Research sites and sampling methods. The Uri, Gonmo, Umeda, and Hamada Rivers located in/flowing through Toyohashi City, Aichi Prefecture, Japan, were selected as the research sites of this study (Fig. 1). The Uri River is located in the cropland area of the upper portion of the research sites of this study. The Gonmo and Hamada Rivers are small rivers flowing into the Umeda River. The Gonmo River receives domestic wastewater, but the Hamada River mainly receives effluent from agriculture and farming.

The river water and small stones on the riverbed (water depth: 30–50 cm) were collected from 6 sampling points (A to F as shown in Fig. 1) during the period from August to November 1998. Samples were

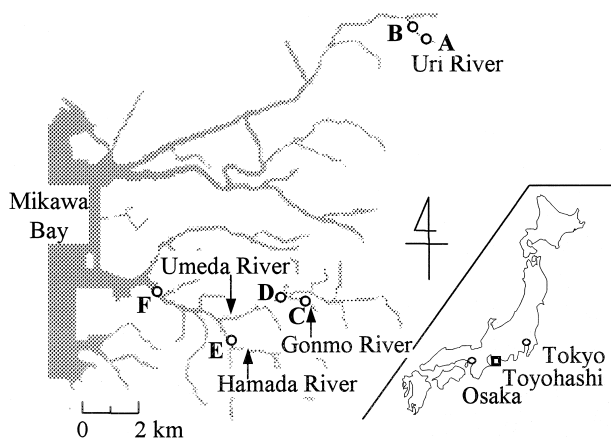


Fig. 1. Research sites and sampling points in this study.

collected at appropriately 12 a.m. each time. Temperature, pH (pH-meter: D-13, Horiba, Kyoto, Japan), electric conductivity (EC, EC-meter: Cyber Scan Con-100, Iuchi, Osaka, Japan) and dissolved oxygen (DO, DO-meter: DO-14P, Toa Electronics Ltd., Kobe, Japan) were measured in the fields. The other terms of water quality such as total organic carbon (TOC), dissolved organic carbon (DOC), total nitrogen (T-N), total phosphorus (T-P) and chlorophyll *a* (Chl-*a*) were measured at the laboratory. Suspended microorganisms in the rivers were collected from 10 L of river water by filtration with 0.3 μ m glass filters (GF-75, Advantec, Tokyo, Japan). Sessile microorganisms were collected by brushing the microbial film attached on the surface of more than 10 stones (diameter: 5–10 cm) taken from the sampling points and suspended by distilled water. These samples including collected microorganisms were used for quinone analysis.

Analytical methods. Concentrations of DOC and TOC were determined by TOC analyzer (TOC-500, Shimadzu, Kyoto, Japan) with and without micro-filtration using a membrane filter of 0.45 μ m pore size, respectively. The difference between TOC and DOC is particulate organic carbon concentration (POC). The other physical and chemical properties of the river waters were analyzed according to the standard methods (Standard Methods for JIS, 1997).

Microbial quinones in suspended and sessile microorganisms were analyzed based on the procedure as previously reported (Hu et al., 1999b, 2001b). Quinones were first extracted from the microorganisms using a mixture of chloroform-methanol and then re-extracted into hexane. The quinones contained in the crude extract were purified using Sep-Pak® Plus Silica. The type and concentration of the quinones were determined using a HPLC equipped with an ODS column (Zorbax-ODS, 4.6 (I.D.) \times 250 mm, Shimadzu-Dupont) and a photodiode array detector (SPD-M10A, Shimadzu).

In addition, the quinones are named here as follows: the abbreviation of the type of quinones (ubiquinone: UQ, menaquinone: MK, plastoquinone: PQ), a dash, and the number of isoprene units in its side chain. For example, UQ-10 represents a ubiquinone with 10 isoprenoid units, and MK-9(H₂) represents a menaquinone with 9 isoprenoid units and one of the 9 units is hydrogenated with 2 hydrogen atoms. In addition, vitamin K1, which has a similar molecular structure to menaquinone, is abbreviated as VK1.

Table 1. Physical and chemical properties of river waters and microbial film investigated in this study.

| | Sampling sites | | | | | |
|---|----------------|-----------------|------|-------|-------|------|
| | A | B | C | D | E | F |
| River water | | | | | | |
| Temp. (°C) | 15.8 | 16.3 | 25.3 | 29.9 | 20.7 | 22.7 |
| pH (–) | 8.00 | ND ^a | 7.07 | 7.99 | 7.29 | 7.48 |
| DO (mg/L) | 7.78 | 8.18 | 6.80 | 8.02 | 7.58 | 4.65 |
| EC (mS) | 0.16 | 0.24 | 0.39 | 0.40 | 0.47 | 0.03 |
| POC (mg/L) | 3.2 | 0.3 | ND | 8.6 | 0.9 | 1.8 |
| DOC (mg/L) | 1.9 | 0.2 | 5.5 | 6.7 | 4.1 | 4.9 |
| T-N (mg/L) | 0.64 | 2.05 | 9.13 | 1.19 | 10.78 | 4.81 |
| T-P (mg/L) | 0.04 | 0.05 | 0.15 | 0.47 | 0.35 | 0.24 |
| Chl- <i>a</i> (μ g/L) | 0.53 | 2.98 | 1.61 | 16.81 | 5.14 | 6.04 |
| Microbial film | | | | | | |
| POC (mg/cm ²) | 0.29 | 0.22 | 0.37 | 0.89 | 0.28 | 0.02 |
| Chl- <i>a</i> (μ g/cm ²) | 3.21 | 3.05 | 4.09 | 9.30 | 3.12 | 0.09 |

DO: dissolved oxygen, EC: electric conductivity, POC: particulate organic carbon, DOC: dissolved organic carbon, T-N: total nitrogen, T-P: total phosphate, Chl-*a*: chlorophyll-*a*.

^a ND: not detected.

Results

Physical and chemical properties of the river waters and microbial film

Physical and chemical properties of the river waters and microbial film investigated are shown in Table 1. The river water of point A was relatively clean compared to other points. Points C and E were polluted by nitrogen compounds, and point D was polluted with organic matter. The concentration of Chl-*a* in water for point D was highest among the samples. The biomass of microbial film for point F was much lower than the others.

Quinone compositions

The analytical results of microbial quinones in river water and microbial film are shown in Fig. 2, and Tables 2 and 3. Figure 2 shows an example of the chromatograms of quinones in HPLC for point E. Table 2 shows the quinone concentration of suspended microorganisms in river water and Table 3 shows the quinone contents of sessile microorganisms attached on the surface of riverbed stones.

From Fig. 2 and Tables 2 and 3, we can see that ubiquinone, menaquinone, and plastoquinone were observed in suspended and sessile microorganisms

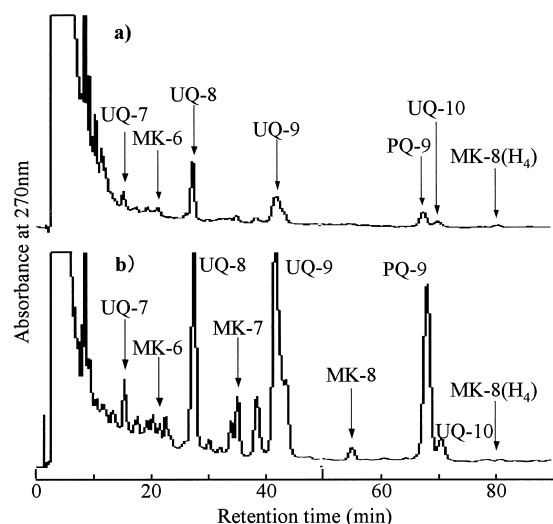


Fig. 2. Example of HPLC-chromatograms of quinones of point E for suspended (a) and sessile (b) microorganisms.

Column: Zorbax-ODS column (4.6 I.D.×250 mm); Temperature: 30°C; mobile phase: a mixture of methanol-isopropyl ether (100:2, v/v); flow rate: 1 mL/min; injection volume: 20 μ L.

Table 2. Quinone concentrations (nmol/L) in the river waters investigated in this study.

| Quinone species | Sampling sites | | | | | |
|-----------------------|----------------|-------|-------|-------|-------|-------|
| | A | B | C | D | E | F |
| UQ-7 | — ^a | — | — | — | 0.005 | 0.204 |
| UQ-8 | 0.011 | 0.015 | 0.136 | 0.610 | 0.182 | 0.093 |
| UQ-9 | 0.052 | — | — | 0.040 | 0.017 | — |
| UQ-10 | — | 0.004 | 0.025 | 0.098 | 0.028 | 0.137 |
| MK-6 | 0.001 | 0.002 | 0.014 | — | 0.015 | 0.055 |
| MK-7 | 0.001 | 0.002 | 0.017 | 0.040 | 0.003 | 0.009 |
| MK-8 | — | — | 0.004 | 0.004 | — | — |
| MK-6(H ₄) | — | — | — | — | — | 0.007 |
| MK-7(H ₄) | — | — | — | — | — | 0.136 |
| MK-8(H ₄) | — | — | 0.003 | — | 0.005 | 0.004 |
| MK-9(H ₈) | — | — | 0.004 | — | — | — |
| VK1 | — | — | 0.032 | 0.530 | — | — |
| PQ-9 | 0.013 | 0.022 | 0.067 | 0.492 | 0.130 | 0.642 |
| Total | 0.078 | 0.045 | 0.303 | 1.813 | 0.385 | 1.286 |

^a —: not detected.

for all the samples. VK1 was observed in suspended microorganisms only for sampling points C and D, but it existed in sessile microorganisms for all the samples except sampling point E. Among ubiquinones and menaquinones, UQ-8, UQ-10, and MK-7 showed up in

Table 3. Quinone contents (10^{-2} nmol/cm²-surface) in sessile microorganisms in the rivers investigated in this study.

| Quinone species | Sampling sites | | | | | |
|-----------------------|----------------|-------|-------|--------|-------|-------|
| | A | B | C | D | E | F |
| UQ-7 | — ^a | — | — | — | 0.043 | — |
| UQ-8 | 0.133 | 0.152 | 1.563 | 0.772 | 1.581 | 0.034 |
| UQ-9 | — | 0.278 | 1.437 | 0.274 | 0.294 | — |
| UQ-10 | 0.164 | 0.113 | 0.448 | 0.976 | 0.211 | 0.019 |
| MK-6 | — | — | 0.041 | — | 0.040 | 0.010 |
| MK-7 | 0.086 | 0.060 | 0.186 | 0.203 | 0.168 | 0.009 |
| MK-8 | 0.059 | 0.039 | 0.195 | 0.331 | 0.089 | 0.003 |
| MK-9 | 0.002 | — | 0.011 | — | — | — |
| MK-10 | — | — | 0.036 | — | — | — |
| MK-9(H ₂) | — | — | 0.006 | — | 0.020 | — |
| MK-8(H ₄) | — | — | 0.065 | — | 0.015 | — |
| MK-9(H ₄) | — | — | 0.084 | — | — | — |
| MK-9(H ₈) | 0.024 | 0.012 | 0.070 | — | 0.033 | — |
| VK1 | 1.379 | 0.356 | 2.431 | 6.413 | — | 0.028 |
| PQ-9 | 3.105 | 2.411 | 4.074 | 5.957 | 2.892 | 0.090 |
| Total | 4.951 | 3.420 | 10.6 | 14.926 | 5.385 | 0.193 |

^a —: not detected.

almost all samples of suspended and sessile microorganisms. MK-8 was detected from all samples of sessile microorganisms and all but a few samples of suspended microorganisms. The other types of respiratory quinones such as UQ-7, UQ-9, MK-6, MK-9, MK-10, MK-10(H₂), MK-7(H₄), MK-8(H₄), and MK-9(H₄) likewise existed in few samples of suspended and sessile microorganisms.

The total concentration of quinones, which reflects the concentration of suspended microorganisms in the rivers, varied markedly from 0.05 to 1.81 nmol/L. The quinone contents for sessile microorganisms among the samples also greatly changed (Table 3). The quinone contents for sessile microorganisms were in the range from 0.19×10^{-2} to 14.9×10^{-2} nmol/cm².

Discussion

Effect of water quality on quinone contents

Water quality is considered to be a major factor controlling the concentration of quinones in river water. As shown in Fig. 3 a higher concentration of DOC in river water yields higher concentrations of total quinones, ubiquinones and photosynthetic quinones (PQ-9+

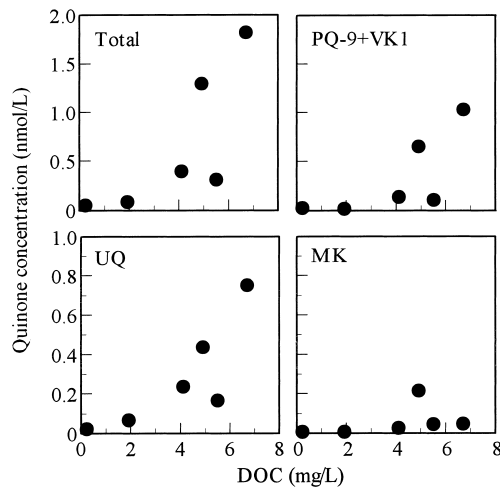


Fig. 3. Correlation between dissolved organic carbon (DOC) and quinone concentration in river water.

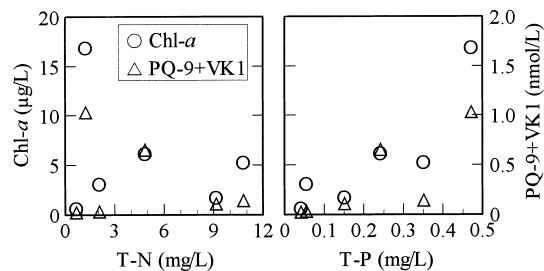


Fig. 4. Correlation between total nitrogen (T-N) and total phosphorus (T-P) and the concentration of photosynthetic quinones (PQ-9+VK1) and chlorophyll-a (Chl-a) in river water.

VK1) in water. However, the concentration of menaquinone did not greatly changed with the change of DOC. It is well known that nitrogen and phosphorus are essential nutrient elements for the growth of algae. Figure 4 shows that there was some positive correlation between T-P and the concentration of photosynthetic quinones and Chl-a. Further research into this is required. But no reasonable relation between T-N and photosynthetic quinone and Chl-a was found.

Difference in quinone composition between suspended and sessile microorganisms

As mentioned above, ubiquinone, menaquinone, and photosynthetic quinones are the electronic transmitting material used for aerobic/nitrate, anaerobic/aerobic respiration and photosynthesis, respectively (Collins and Jones, 1981). So, the relative content of each group of quinones may reflect the energy metabolic characteristics of an ecosystem (Iwasaki and Hiraiishi, 1998). The differences in quinone compositions

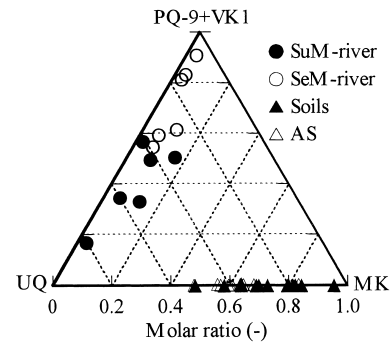


Fig. 5. Comparison in quinone composition between suspended and sessile microorganism in rivers (SuM-river and SeM-river, respectively), soils and activated sludges (AS).

The data for soils and activated sludges are from cited literature (Fujie et al., 1998; Hu et al., 2001a).

between suspended and sessile microorganisms can be successfully illustrated with a triangle diagram as shown in Fig. 5. The data for activated sludge and soils (Fujie et al., 1998; Hu et al., 2001a) are also added to this figure for comparison. The mole fraction of ubiquinone of suspended microorganisms in river was in the range from 0.34 to 0.80, indicating that ubiquinone is the dominant type of quinone in suspended microorganisms. On the other hand, photosynthetic quinones (PQ-9+VK1) accounted for a large part of quinones in sessile microorganisms. The mole fraction of photosynthetic quinones was as high as 0.54 to 0.91. These facts suggest that the suspended microorganisms were dominated by the bacteria getting energy through aerobic respiration, and the sessile microorganisms was dominated by photosynthetic microorganisms such as micro-algae and cyanobacteria. In addition, the quinone compositions of suspended and sessile microorganisms greatly differed from those of activated sludge and soils.

The values of UQ/MK and (UQ+MK)/(PQ-9+VK1) ratios calculated from the data shown in Tables 2 and 3 for suspended and sessile microorganisms are shown in Table 4. The UQ/MK ratio for suspended microorganisms ranged from 2.1 to 25.5, which is higher than that for sessile microorganisms (1.8–5.7). This suggests that the ratio of aerobic bacteria in suspended bacteria might be higher than that in riverbed microbial film. Furthermore, the values of the (UQ+MK)/(PQ-9+VK1) ratio were greater than 1 for most samples of suspended microorganisms (varying from 0.8 to 5). Unlike the suspended microorganisms, the values of the (UQ+MK)/(PQ-9+VK1) ratio for sessile

Table 4. Mole ratios of UQ/MK and (UQ+MK)/(PQ-9+VK1) for suspended and sessile microorganisms in the rivers.

| | Sampling sites | | | | | |
|--------------------|----------------|-----|-----|------|------|-----|
| | A | B | C | D | E | F |
| River water | | | | | | |
| UQ/MK | 25.5 | 4.9 | 4.1 | 17.0 | 10.7 | 2.1 |
| (UQ+MK)/(PQ-9+VK1) | 5.1 | 1.0 | 2.1 | 0.8 | 1.9 | 1.0 |
| Microbial film | | | | | | |
| UQ/MK | 1.8 | 4.9 | 5.3 | 3.8 | 5.7 | 2.4 |
| (UQ+MK)/(PQ-9+VK1) | 0.1 | 0.2 | 0.7 | 0.2 | 0.8 | 0.6 |

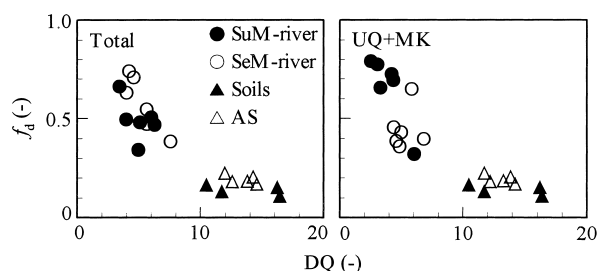


Fig. 6. Comparison in microbial diversities between suspended and sessile microorganisms in the rivers (SuM-river and SeM-river, respectively), soils and activated sludges (AS).

The data for soils and activated sludges are from cited literature (Fujie et al., 1998; Hu et al., 2001a). f_d shows the mole fraction of dominant quinone in a sample.

microorganisms were less than 1 (ranging from 0.10 to 0.8).

Microbial diversity and equitability

The microbial diversities (DQ) for suspended and sessile microorganisms calculated from the quinone composition using Eq. (1) (Hu et al., 1999a) are shown in Fig. 6 versus the mole fraction of dominant type of quinone in each sample (f_d).

$$DQ = \left(\sum_{k=1}^n (\sqrt{f_k}) \right)^2 \quad (1)$$

Where, f_k is the mole fraction of quinone species k and n is the number of quinone species with the mole fraction higher than or equal to 0.001.

The microbial diversity calculated from the composition of all quinones (including ubiquinones, menaquinones, and photosynthetic quinones), DQ_q , for the suspended and sessile microorganisms ranged from 3.4 to 7.5. The diversity calculated from the com-

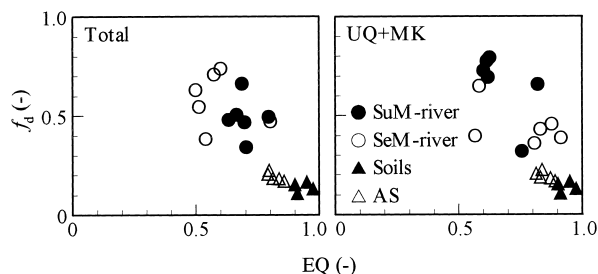


Fig. 7. Comparison in microbial equitability between suspended and sessile microorganism in rivers (SuM-river and SeM-river, respectively), soils and activated sludges (AS).

The data for soils and activated sludges are from cited literature (Fujie et al., 1998; Hu et al., 2001a).

position of respiratory quinones (including ubiquinones and menaquinone), DQ_{uq+mk} , which reflects the diversity of heterotrophic bacteria, for the suspended microorganisms changed from 2.5 to 6.0, and averaged 3.9. The average DQ_{uq+mk} for the sessile microorganisms was 5.2 (ranging from 4.4 to 6.8), which was larger than that for the suspended microorganisms. In addition, the values of DQ_q and DQ_{uq+mk} for the suspended and sessile microorganisms of rivers were relatively lower than those for soils and activated sludges.

The microbial equitabilities (EQ , $EQ = DQ/n$) for suspended and sessile microorganisms in the rivers, activated sludge and soils are shown in Fig. 7 versus the mole fraction of the dominant type of quinone in each sample. Note that when the fractional contents of all quinone species in a sample are equal to each other, the microbial equitability takes the maximum value of 1. The microbial equitability for total quinones was in the order: sessile microorganism in river < suspended microorganisms in river < activated sludge < soils. However, the microbial equitability calculated from the data of respiratory quinones only (UQ and MK only) for suspended and sessile microorganisms showed similar values.

In summary, the difference in microbial community structure between suspended and sessile microorganisms in rivers was evaluated by analyzing microbial quinone composition. The experimental results indicated that aerobic bacteria were abundant in the suspended microorganisms, but photosynthetic microorganisms such as micro-algae and cyanobacteria dominated sessile microorganisms. The diversity of heterotrophic bacteria for sessile microorganisms was slightly higher than that for suspended microorganisms.

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