

### III. Biological Data

#### 8. Bacteria

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In Lake Kasumigaura, the total number of bacteria has been measured from 1983, and number of viable bacteria from 1993. Sterilized 100ml glass water sampler was used, and the sampling was conducted at 0.5 m depth.

The total number of bacteria was counted by using fluorescence microscope, after the sample was dyed with acridine orange. Nuclepore-filters of 0.4 $\mu$ m were used from 1982, but that of 0.2 $\mu$ m were used thereafter.

The number of viable bacteria was measured by the pour plate count method using 1/10 nutrient agar medium following a 2-week incubation period at 20°C before 1982. Thereafter, the MPN method with 1/10 nutrient broth was used. Bacteria number obtained by the MPN method tends to be a little larger than the pour plate count method.

The measurement of the total number of bacteria was re-started at two observation stations, St.9 (the central part of the lake) and St. 3 (the central part of Takahamairi Bay ) in June 1996. The water was sampled by a column water sampler of 2 m length and of 5 cm internal diameter. The water was kept in 100 ml plastic bottles, and glutaraldehyde was added promptly so as that the final concentration became to be 1%. The water samples were kept in a refrigerator.

The counting was conducted within two weeks using the following method: (1) 1-10ml distilled water, which was filtered by nuclepore filter of 0.1 $\mu$ m was added to the sample water, in order to collect bacteria uniformly on the filter surface. The sample water was filtered by nuclepore filter of 0.2 $\mu$ m having a diameter of 25 mm which was dyed with Sudanblack B. (2) Two or three drops of DAPI (4'6-diamino-2-phenylindole) were added so as to cover the whole area of the filter surface. A few minutes after, we removed the excess DPI solution. (3) This was placed under a fluorescence microscope of U-excitation system (Olympus BH2-RFC), and purple-blue light spots were counted by referring to the lattice grids attached to the ocular. More than 1000 cells are counted for each sample. Then, the obtained number was converted to the cell number per 1 ml.