Rep. Suwa Hydrobiol., *11*: 51-57 (1999)

PRELIMINARY STUDIES ON ECOLOGICAL DISTRIBUTION OF MICROORGANISMS IN WEST LAKE, HUANGZHOU

1.Microorganisms in the water body

Wu Genfu, Zhang Jingyu, Xuan Xiaodong and Wu Xuechang

(Hangzhou University.)

Abstract

The ecological distribution of microorganisms in the water body of West Lake was studied from April 1995 to April 1996. Results showed that the average density of heterotrophic bacteria was $2.06x10^3$ /ml. Actinomycetes, molds and yeast had already of 35/ml, 24/ml and 2/ml, respectively. Various physiological forms of microbes were found in West Lake, including cellulose decomposing bacteria (25/ml), organic phosphorus decomposing bacteria (8/ml), and Nitrogen utilizing bacteria such as nitrite bacteria (453/ml), nitrate bacteria (585/ml), nitrate-reducers ($3.2 x10^3$/ml), and denitrifiers (43/ml). Contrasting to nitrate bacteria, cellulose decomposing bacteria, ammonifiers, nitrite bacteria and nitrate reducers were rich in winter and spring. Autotrophic bacteria, like nitrifiers were more common in L. Xiaonan and L. Beili, where the water quality was relatively good, Heterotrophic bacteria were populous in the two highly eutrophic lakes (L.Yuehu and L. Xili). The study on 78 strains isolated randomly from the five sub lakes suggested that the dominant groups in West Lake were *Pseudomonas, Bacillus* and *Enterobacteriaceae.*

Key words: West Lake, Microorganisms, Bacterial population, Bacterial density

Introduction

The West Lake, Hangzhou, is a famous scenic lake in China. Owing to the development of industry, agriculture and material life, however, eutrophication has become a serious problem in this lake. In 1990, the average of COD, BOD, TN (Total Nitrogen) and TP (Total Phosphorus) reached 7.87, 5.12, 2.27, and 0.129 mg/l, respectively, while the average transparency (SD) of this lake was only 50.3 cm (Li, 1990). Although various steps have been taken, eutrophication has yet to be controlled. This has led to the initiation in 1994 of a cooperative project between China and Japan.

The structure and function of microbial communities is a main focal point in microbial ecology. The habitat environment has a major impactor affects microorganisms to a large extent degree. The coliform index and the number of heterotrophic bacteria are two parameters important for evaluating the water quality. Furthermore, the microorganisms in the water ecosystem play an important role in nutrient regeneration and energy flow.

The distribution of heterotrophic bacteria and coliform index have been reported (Wang and Chen, 1983,

Li, 1986), but the abundance of C, N and P utilizing microorganisms have yet to be surveyed. This paper provides a preliminary report on the abundance of these three types of microorganisms in the West Lake.

Methods and materials

5 sampling sites were selected, corresponding to the five sub-divisions (determined by two bridges) of the lake (Fig.2). Sampling was performed in each season from April 1995 to April 1996. As the average depth of the lake is only 1.56 m, water samples were taken at 50 cm below lakes surface.

Bacteria and actinomycetes were cultured on nutrient agar and starch agar, respectively, using the dilution smearing method (Reinherimer, 1980). Cellulose decomposing bacteria, nitrite bacteria, nitrate bacteria, nitrate reducers, denitrifiers, and phosphorus decomposing bacteria were cultured in their respective special medium (Alexander and Clark, 1965) using the MPN method. For the identification of heterotrophic bacteria, 78 strains were radomly isolated from the water body in April 1995 and April 1996. The identification procedures used are described in Bergey's manual (Buchanan and Gibbon, 1974).

Results and discussion

Figure 2 shows the abundance and distribution of aerobic heterotrophic bacteria in the West Lake between April 1995 and April 1996. From this figure, it can be seen that the average population density was 2.06 x 10^3/ml, the highest density occurred in Yuehu Lake in December 1995 (9.8 x10^3/ml), and the lowest density occurred in the center of the outer Lake in September 1995 (2.0 x 10^2/ml). According to the Japanese standard classifying system, the West Lake is a mid-eutrophic type from a bacteriological view. Table 1 compares the amount of heterotrophic bacteria in thislake over the last 15 years. In 1979, the heterotrophic bacteria densities of these lakes were rich comparing with other years.

Table 1. Comparison of heterotrophic bacteria in1979—1996(cfu/ml)

Sub-lake	1979—1980[2]	1988—1989[3]	1995—1996
YuehuL.	29,000	----	3,970
Beili L.	23,000	----	1,320
Outer L.	14,000	1,296	1,180
Youth Palace	8,000	1,575	----
Xili L.	----	1,521	2,700
Average	18500	1,464	2,293
Evaluation	eutrophic	mid-eutrophic	mid-eutrophic

In 1986, sewage was piped into a treatment factory, and the clean water from Qiantang River was introduced into the West Lake, resulting in a reduction in heterotrophic bacteria. In recent years, however, the water in Qiantang River has undergone eutrophication, possibly causing a more rapid proliferation of heterotrophic bacteria in the West Lake.

The coliform index is one parameter useful for evaluating water quality. If the coliform index is less than 1,000/ml, the water is regarded as clear, if it is between 1,000 and 10,000/ml, the water is polluted, if it is larger than 10,000, the water is severely polluted. Table 2 shows the coliform index in the 5 sub-lakes of West Lake. Comparing these indexes with dates published by Wang and Chen (1983), it can be concluded that the West Lake is still polluted by raw sewage, particularly in the Yuehu and Xili sub-areas. It is therefore necessary to further enhance the sewage administration of the lakes surrounding environment.

Table 2. Coliform index (cfu/l) in the five sub-lakes of West Lake

Sub-lake	95.6	95.9	95.12	96.4	Evaluation
Beili L.	2,300	2,300	9,600	9,600	polluted
Yuehu L.	>23,800	23,800	>23,800	>23,800	severely polluted
Xili L.	23,800	>23,800	23,800	23,800	severely polluted
Xiaonan L.	1,800	1,800	2,300	2,300	polluted
Outer L.	920	9,600	2,300	2,300	polluted

Actinomycetes play an important role in substance recycling. Li and Xu (1993) have studied the chitin decomposing actinomycetes in Donghu Lake (Wuhan, China), but the actinomycetes in the West Lake, Hangzhou have not been previously reported. Based on the experiments of Li and Xu, similar studies on actinomicetes in the West Lake were conducted in 1995—1996. Results showed that the density of actinomycetes in the water body was only 35 cfu/ml on average , and that their density increased with temperature .

As cellulose is the most important component in carbon containing substance, studying the abundance and distribution of cellulose decomposing microorganisms will aid our understanding of the carbon cycle in the lake ecosystem. Microorganisms which have the ability to decompose cellulose include several types. Preliminary analysis of the West Lake conducted in April 1995, redealed the main type to be aerobic bacteria (29/ml), while anaerobic bacteria constituted only 9/ml, and fungi were even less. Based on these results, the distribution of aerobic cellulose decomposing bacteria was determined from April 1995 to April 1996. This distribution is shown in Figure 3. The density of these bacteria was

highest in L. Yuehu (47/ml on average), peaking in December (80/ml), and lowest in the Outer lake (8/ml), with a minimum density in September (5/ml). A good correlation may exist between the density of these bacteria and that of heterotrophic ones ($r=0.670^{**}$).

N-utilizing bacteria include nitrogen fixers, ammonifiers, ammonium oxidizers (nitrite bacteria), nitrite oxidizers (nitrate bacteria), nitrate reducers and denitrifiers. Nitrogen fixers were not detected in the West Lake, as they could not be isolated on Ashby agar (agar not containing nitrogen). This suggest that the lake does not contain these bacteria, or more likely that their numbers are too low tobe detected by this method.

The distribution of ammonifiers (which can grow on nutrient agar) can be seen in Figure 2. The distribution could be seen also on fig.2. The distribution of nitrite bacteria, nitrate bacteria and nitrate reducers are shows in figure 4 - 6, respectively. The distribution of denitrifiers is shown in Table 3.

Table 3 Distribution (cells/ml) of denitrifiers in the water of West Lake

Sub-lake	95/4/17	95/6/12	95/9/25	95/12/18	96/4/22	Total	Average
Beili	16	7	3	0	85	111	22
Yuehu	35	250	7	1	150	442	88
Xili	40	20	1	0	45	106	21
Xiaonan	250	6	2	0	5	263	53
Outer	20	0	1	3	140	164	33
Total	361	283	13	4	425	1085	217
Average	72	57	3	1	85	217	43

The abundance of N-utilizing bacteria underwent seasonal variation. While ammonifiers, nitrite bacteria and nitrate reducers were more abundant in winter than in summer, the abundance of nitrate bacteria was higher in summer than in winter. These variations may be linked to the substrate contents of the surrounding environment. From the above mentioned figures and table, it is also evident that autotrophic nitrobacteria (such as nitrite bacteria and nitrate bacteria) were more populous in L. Xiaonan and L. Beili where the water quality is relatively good, while ammonifiers and nitrate reducers (both being heterotrophic bacteria) were more populous in the two highly eutrophic sub-lakes, L. Yuehu and L. Xili..

Phosphorus in the water body can exist as organic phosphorus and dissolved phosphate. Microorganisms play a role in decomposing organic phosphorus, and changes to dissolved phosphate. Results obtained in May 1996 revealed that organic phosphorus decomposing bacteria consisted only 8/ml on average, and calcium phosphate dissolvers were even less.

216 strains of heterotrophic bacteria were isolated between April 1995 and April 1996, and their morphological characteristics were observed. It was found that most of these strains were non-spore bacillus.

Physiological and biochemical characteristics of 78 strains isolated in April 1996 were also categorised according to Bergey' manual. It was found that 30.8% were *Pseudomonas*, 10.4% were *Bacillus*, 17.9% were *Enterobacteriaceae*,and other included *Acinetobacter, Alcaligenes, Achromobater, Microbacterium, Chromobacterium, Flavobacterium, and Xanthomonas*..

Table 4. Comparison of morphological characteristics of heterotrophic bacteria in the water body of West Lake

Sub-lake	Test strains	G⁻ strain %	Cocci %	Bacillus %
Beili	52	92	14	0
Yuehu	44	95	5	0
Xili	36	83	6	11
Xiaonan	46	78	26	17
Outer	38	94	17	6

References

1. Li M. Z.: Studies on the environment of Chinese Lakes. Beijin. China Environmental Science Press. 1, 158-161. (1990) (in Chinese)

2. Wang F. S. and Chen S. Y.: Aquatic Microbial Study on the West Lake, Hangzhou. Journal of Hangzhou University. 10(supp): 65-74.(1983) (in Chinese)

3. Li Xianron: Study on the Tendency of Bacterial Evolution in Hangzhou West Lake before Comprehensive Administration. Journal of Environment Pollution and Prevention, 18 (1): 47 (1986) (in Chinese)

4. Rheinherimer G.: Aquatic Microbiology (2nd Edition). Wiley-interscience, a Division of John Wiley & Sons, New York, Brisbane, Toronto, (1980).

5. Alexander M. & Clark F. E.: Method of Soil Analysis, Part 2. Wisconsin, American Society of Agronomy Inc., 1477-1484 (1965)

6. Buchanan R. E.and Gibbon N. E. : Bergey' Manual of Determinative Bacteriology (8th ed.). Baltimore, The Williams & Wilkins Company (1974).

7. Li Q. S. and Xu J. H.: Study on Ecology of Chitin-decomposing Microonganisms in Nonghu Lake. Journal of Lake Sciences. 5 (1): 56-61 (1993) (in Chinese)

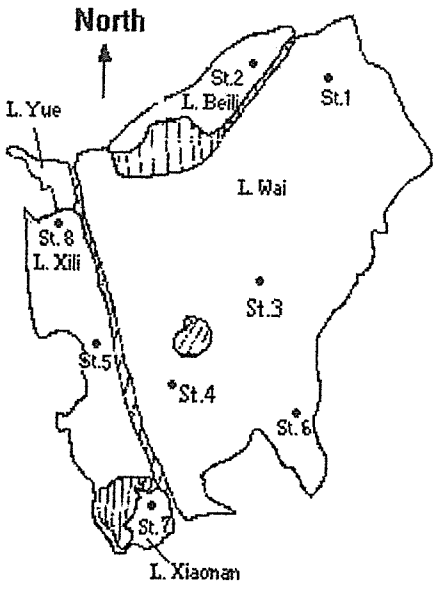

Figure1. Schematic diagram of sampling stations in the West Lake

Sta. 1: L. Beili, Sta. 2: L. Yuehu, Sta. 3: L. Xili, Sta. 4: L. Xiaonan, Sta. 5 : Outer lake

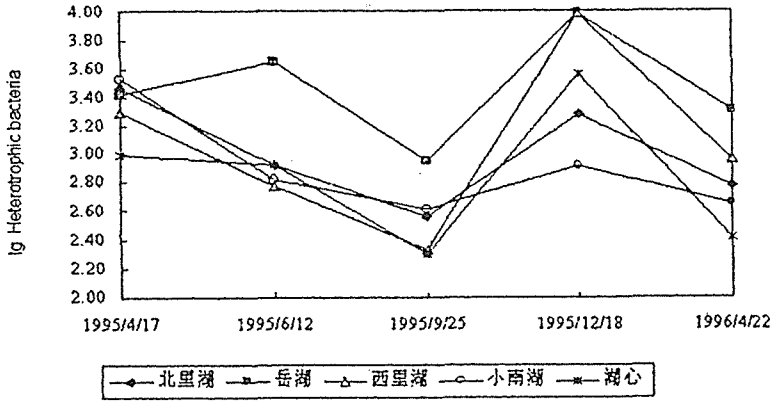

Figure 2. Distribution of heterotrophic bacteria in the water body of West Lake

◆ : L. Beili, ■ : L. Yuehu,, △ : L. Xili, ○ : L. Xiaonan, ＊ : Outer lake

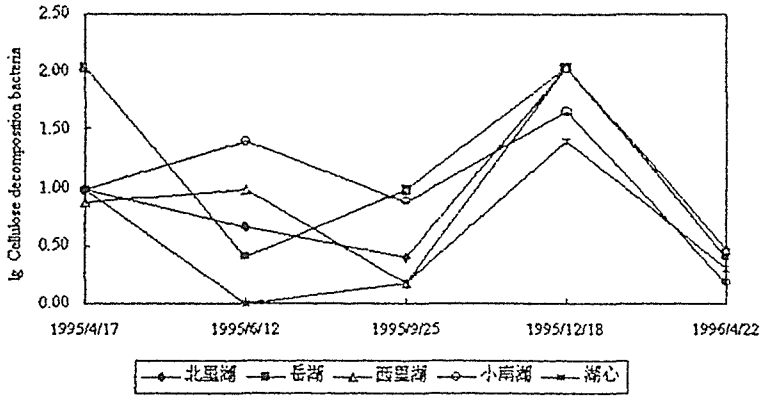

Figure 3. Distribution of cellulose decomposing bacteria in the West Lake

◆ : L. Beili, ■ : L. Yuehu,, △ : L. Xili, ○ : L. Xiaonan, ＊ : Outer lake

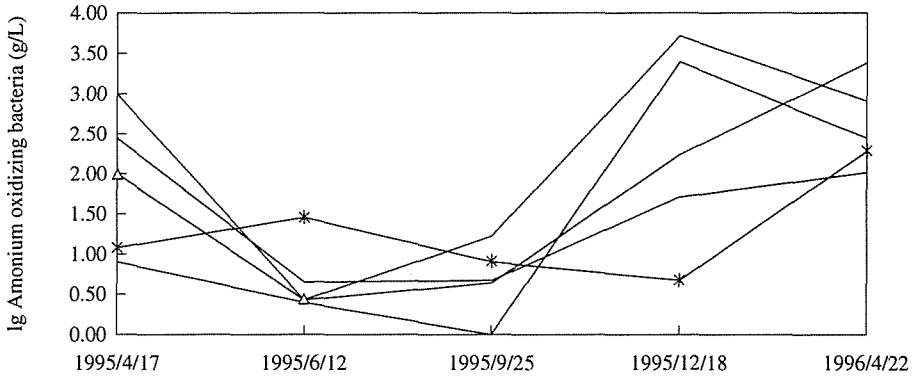

Figure 4. Distribution of amino oxidizing bacteria in the water body of West Lake

◆ : L. Beili, □ : L. Yuehu, △ : L. Xili, ○ : L. Xiaonan, ✳ : Outer lake

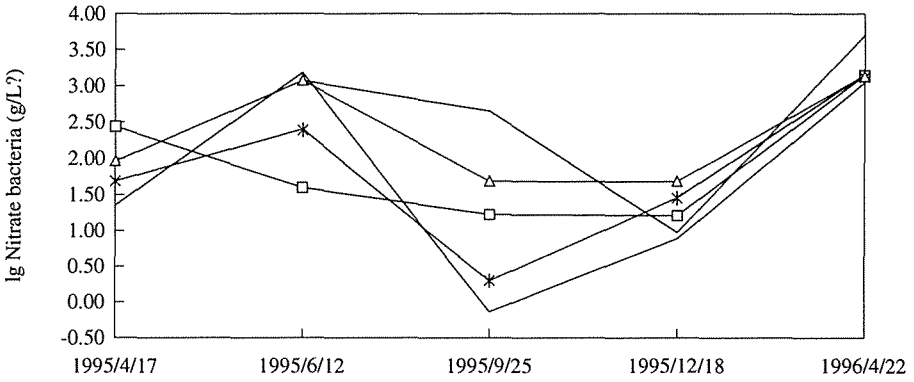

Figure 5. Distribution of nitrate bacteria in the water body of West Lake

◆ : L. Beili, □ : L. Yuehu, △ : L. Xili, ○ : L. Xiaonan, ✳ : Outer lake

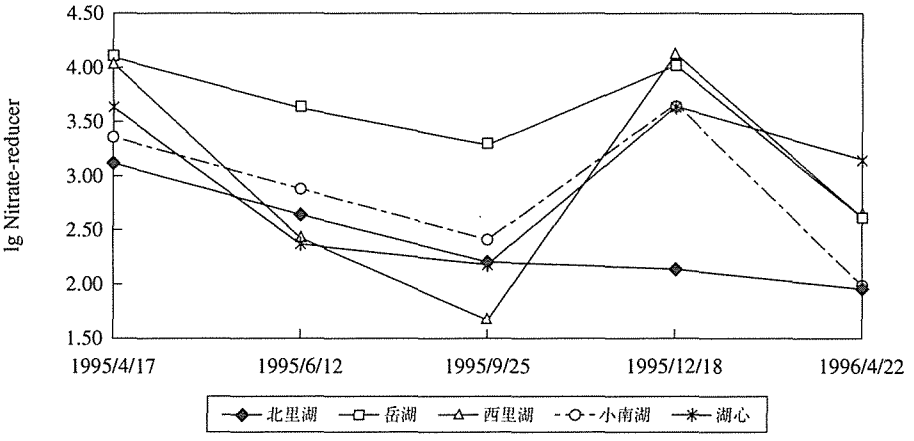

Figure 6. Distribution of nitrate-reducer in the water body of West Lake

◆ : L. Beili, □ : L. Yuehu, △ : L. Xili, ○ : L. Xiaonan, ✳ : Outer lake