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Study on the water quality of the Taihu Lake using genotoxicological methods

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Abstract—The micronucleus(MCN) test of *Vicia faba* root tip cell was used to screen the water quality of the Taihu Lake. The *MCN* ‰ and the pollution index (*PI*) were examined and the *F*-test was carried out to evaluate the statistical difference in mean *MCN* ‰ among various samples. There were significant difference among thirty-nine samples and there were six sites whose *PI* values are above 2. The authors suggest that the Taihu Lake has partly been polluted to different degrees and the whole water body can be divided into three types. Ames tests were then conducted to detect mutagenicity at five significant sites. The results showed that rivers flowing through urban area carried large amount of mutagenic pollutants into the lake and these pollutants contaminated the source of main waterworks.

Keywords: Taihu Lake; water quality; micronucleus test; Ames test.

1 Introduction

The Taihu Lake, which is one of the five largest fresh water lakes in China, possesses the surface of 2425 km^2. The surrounding area is fertile and economically important. However, along with the increasing development of industry, the environmental quality of Taihu area is getting worse and worse. The Taihu Lake is important to east China, for it is not only the main supplement of potable water to Shuzhou, Wuxi and Shanghai, but also a significant base of fishery. Moreover, it is a famous scenery spot of national grade. Since late 1970's, many studies focusing on the eutrophication and water quality have been conducted, yet there has been no report about the situation of mutagenic pollution until now. Researches of this aspect must be of considerable value for the government to protect public health, to draw up water quality standards and to control aquatic pollution.

In this paper, both micronucleus test and Ames test were conducted. The micronucleus test of *Vicia faba* root tip cell is a standard method of environmental monitoring recognized by the CN EPA and the Ames test is also a classic method to detect mutagenicity accepted around the world. The results are reliable to indicate the water quality and mutagenicity in the Taihu Lake.

2 Materials and methods

2.1 Materials

Vicia faba were provided by College of Life Science of the Middle-China Normal University.

The strains used in test were *salmonella typhimurium* TA98 and TA100 which were kindly provided by Dr. B. N. Ames from University of California, Berkeley. 2AAF and MMNG were purchased from Sigma Chemical Co.

2.2 Arrangement of sampling site and sample collection

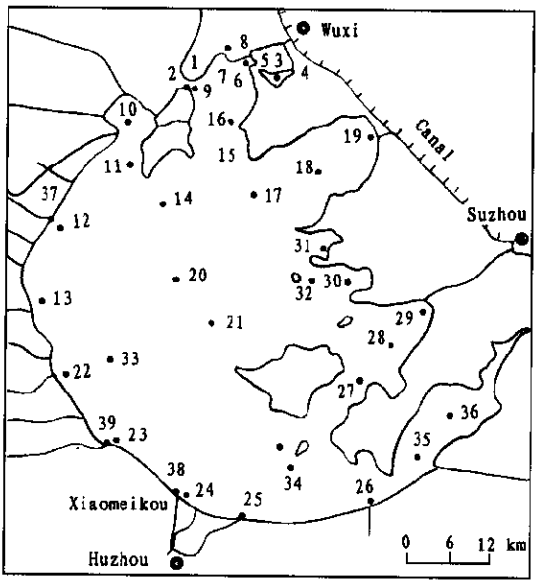

Fig.1 Sampling sites in the Taihu Lake

Water samples were collected from October 17 to 23, 1994 from thirty-nine representative sites shown in Fig.1. 500—1000 ml samples obtained from each site were preserved at 4℃ in dark and transferred to the laboratory as soon as possible.

2.3 Micronucleus test of *Vicia faba* root tip cells

The board beans were immersed in water samples (including tap water as control) at 25℃. After 48h for swelling, well-developed seeds were selected and wrapped in gauze, and then placed in 25℃ incubator to germinate. When the roots grow to 1.5—2.5 cm in length, the root tips were cut down followed by fixed, stained (by Feulgen staining method) and then were squashed. Three root tips were assayed under microscope for each sample. 1000 cells per root tip were examined and the number of micronuclei were counted. Then the *MCN* ‰ and *PI* values were measured using the following equations:

$$MCN\ ‰ = \frac{\text{Number of total micronucleus found in one root tip}}{\text{Number of total cells checked for one root tip}},$$

$$PI = \frac{\text{Mean } MCN\ ‰ \text{ of one sample}}{\text{Mean } MCN\ ‰ \text{ of negative control}}.$$

2.4 Ames test

Based on the screening of water quality with micronucleus test of *Vicia faba* root tip cells, Ames tests were conducted to examine exact situation of mutagenic pollution. Five representative sites located at the entrance of 5 main rivers were chosen for sampling. 25 liters of sampling water were collected from each site and brought to lab. After motionless preservation for 24h, the samples were first filtered and then extracted using acetone. The extracts were concentrated with evaporation and dried with nitrogen, then were dissolved in DMSO and stored in a freezer.

The mutagenicity test was carried out as described by Ames *et al*. (Ames, 1975). The liver homogenate S9 prepared from SD rat treated with Aroclor 1254 was used for metabolic activation. 0.2ml S9 mix, 0.1 ml tester strain (TA98/TA100, approximately 2×10^9/ml), 0.1 ml sample extract were mixed with overlay agar at 45℃. The mixture was then poured onto minimal agar plate. After incubation at 37℃ for 48h, revertant colonies were counted. All tests were performed

with three parallels. If the extract induced a increase of revertant colonies at least twice the spontaneous colonies, and the increase appeared to be concentration-dependent, the result was considered to be positive.

Solvent control (DMSO) and three positive controls were also tested: Bap (10μg/plate) for TA98 and TA100 with S9; 2AAF (100 μg/plate) for TA98 without S9 and MMNG (10μg/plate) for TA100 without S9. If the numbers of revertant colonies of the three positive controls are all significant different from that of the solvent control, we can affirm that other results in this experiment are credible.

3 Results and discussions

3.1 Results of *MCN* ‰ and *PI* values of the samples

The *MCN* ‰ and *PI* values of samples from the Taihu Lake and each river entrance are listed in Table 1. Through the examination of the *MCN* ‰ of the tap water control and *MCN* ‰ of thirty-nine samples with statistic method (F-test), we can found out some significant differences ($F = 4.49 > F_{0.01} = 1.84$) in them ($F_{0.01}$ means the value of F at 1% significant level).

Table 1 ***MCN* ‰ and *PI* values of thirty-nine samples in the Taihu Lake**

Site No.	*MCN* ‰ (M ± SD)	*PI*	Site No.	*MCN* ‰ (M ± SD)	*PI*
1	9.65 ± 2.19	2.70	21	4.46 ± 1.28	1.25
2	12.25 ± 2.67	3.42	22	3.64 ± 0.60	1.02
3	4.97 ± 1.97	1.34	23	3.52 ± 0.63	0.99
4	9.73 ± 1.55	2.72	24	4.32 ± 0.56	1.21
5	5.62 ± 1.50	1.60	25	3.87 ± 2.34	1.08
6	8.02 ± 0.55	2.24	26	3.72 ± 1.92	1.04
7	6.30 ± 0.49	1.76	27	3.62 ± 1.98	1.01
8	8.97 ± 0.97	2.51	28	4.78 ± 1.01	1.34
9	10.42 ± 1.74	2.91	29	3.99 ± 1.99	1.11
10	3.66 ± 1.08	1.02	30	3.53 ± 1.17	0.99
11	3.74 ± 1.08	1.05	31	3.67 ± 1.51	1.03
12	4.76 ± 1.08	1.33	32	4.19 ± 1.34	1.17
13	3.74 ± 0.98	1.05	33	3.82 ± 0.79	1.07
14	5.32 ± 0.28	1.49	34	3.85 ± 1.36	1.07
15	4.05 ± 2.28	1.13	35	4.23 ± 0.97	1.18
16	6.96 ± 0.05	1.94	36	3.50 ± 0.90	0.98
17	4.60 ± 2.80	1.29	37	5.26 ± 0.83	1.47
18	4.43 ± 1.77	1.24	38	5.14 ± 1.10	1.44
19	5.25 ± 1.34	1.47	39	5.67 ± 2.21	1.58
20	4.13 ± 1.44	1.15	N.C*	3.58 ± 1.19	

* N.C.: negative control

Among all the thirty-nine sites, six sites that have the highest *MCN* ‰ are site No. 2, 9, 4, 1, 8 and 6, with the *MCN*‰ of 12.25, 10.42, 9.73, 9.65, 8.97, 8.22 and the *PI* values of

3.42, 2.91, 2.72, 2.70, 2.51 and 2.24, respectively. Through multipal comparison, it can be confirmed that *MCN* ‰ of samples of these sites are significantly different from that of the control. For sites No. 2 and No. 1, located at the entrance of the Lujiang River to the lake, the COD_{Mn}s are 9.97 and 9.27 mg/L respectively. This river receives large amount of agricultural and domestic sewage, as well as untreated waste water from many small scale chemical industries, electric chemistries and electroplating plants of the upstream areas. Obviously, the wastewater contains lots of mutagenic pollutants which can lead to the rising of *MCN* frequency of *Vicia faba* root tip cells. The wastewater was diluted after flowing into the lake, and as a result, the mutagenicity declined. That is why the *MCN* ‰ of site No.9 is still rather high (9.73) yet slightly lower than that of No.1 and No.2.

The site No.8 and No.4 are entrances of the Liangxi River and the city canal of Wuxi to the lake. Since both of the two rivers flow through Wuxi, an important city in Jiangsu Province, the domestic sewage of the urban area must be of high mutagenicity. What cannot be ignored is that the source water of Meiyuan waterwork (an important waterwork of Wuxi), which is represented by site No.6, has the *MCN* ‰ and *PI* value as high as 8.22 and 2.24, respectively.

3.2 Division of lake region

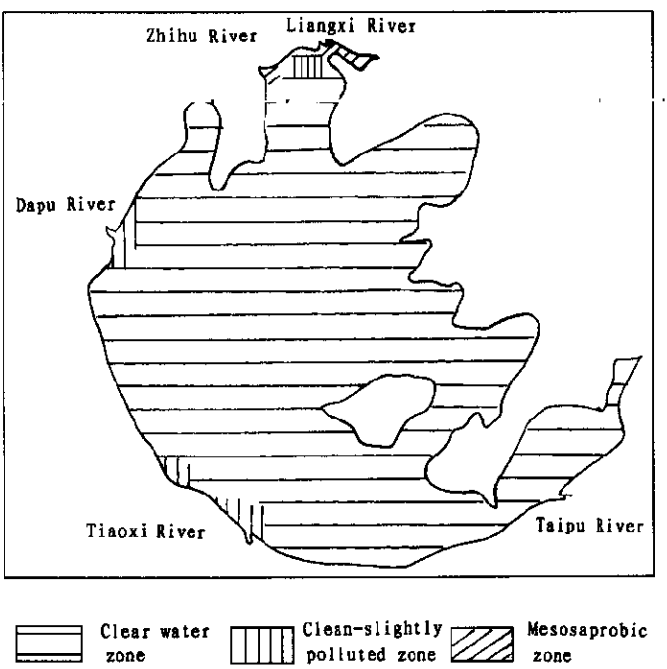

Fig.2 Lake regions of three water types and 5 river entrances conducted Ames test

With the results listed in Table 1, we have examined the mean *MCN* ‰ of thirty-nine samples by q-test (a result of multiple comparison among the 39 *MCN* ‰) according to the method described by Chen *et al*. (Chen, 1985), thus the mutagenic pollution in the Taihu Lake can be divided into three different types (Fig.2). The type I is mesosaprobic zone covering the six sites discussed above whose *PI* values are higher than 2. The type II represents a slight polluted but basically clean zone. The areas of several river entrances at west bank can be of this type. Majority part of Taihu Lake falls into type III, where the water is not polluted yet.

3.3 Relationship of *MCN* ‰ and other water quality indexes

The situation of water chemistry of the Taihu lake was also investigated at the same time as its

mutagenicity was detected. The results are reported in Table 2. The COD_{Mn} and NH_4^+-N are both highest in lake region type I and are higher in lake region type II than type III. In contrast, the DO in water body type I is the lowest, only 4.54 mg/L, while it comes to 9.03 and 9.58 mg/L in water body type II and type III, respectively.

Table 2 Values of some water chemistry indexes

Lake region	Mean *MCN* ‰	Mean *PI* value	COD_{Mn}	NH_4^+-N	DO
Type I	9.84	2.75	7.80	4.19	4.54
Type II	5.66	1.58	5.57	2.06	9.03
Type III	3.81	1.07	4.25	0.19	9.58
Mean value	5.07	1.42	5.03	1.14	8.71

Through analysis of the data of all the sites further with linear regression, it was found that:

a. the *MCN* ‰ and COD_{Mn} had significantly positive correlation with the equation of

$$MCN\ ‰ = 1.03\ COD_{Mn} + 0.25\ (r = 0.81;\ n = 38).$$

b. the *MCN*‰ is also linearly proportional to the concentration of NH_4^+-N with the form of

$$MCN\ ‰ = 0.90\ NH_4^+\text{-}N + 3.95\ (r = 0.80;\ n = 37).$$

c. the *MCN* ‰ and DO are negatively correlated with the linear equation of

$$MCN\ ‰ = -1.1\ DO + 14.9\ (r = -0.76;\ n = 37).$$

Along with the rising of COD_{Mn} and NH_4^+-N, the mutagenicity of the water body increases yet the DO has the trend of decrease. Other indexes also show that water quality in type I area of the lake is worse, but not apparently.

In the light of these analysis, we suggest that the micronucleus test of *Vicia faba* root tip cells can indicate the pollution of the Taihu Lake to a certain degree.

3.4 Results of Ames test of samples from five river entrances

In further studies, Ames test was conducted to detect mutagenicity of extracts from water samples in five sites with and without motablic activation of S9 mix. Based on the results showed in Table 3, all the extracts which induced revertant colonies more than twice the number of spontaneous revertant colonies were tested again to examine whether the induction were concentration-dependent. This time, instead of 0.1 ml, the extracts added in each group were 0.25 ml and 0.5 ml which means that the actual dose is equal to 500 and 1000 ml water sample respectively. Table 4 lists the numbers of revertant colonies under different dosage and the relevant coefficients of dose-effect relationship. The results show that the mutagenicity of lake water at entrances of Zhihu River, Liangxi River and Tiaoxi River are significant. Among these sites, the mutagenic pollution in Zhihu River and Liangxi River are most serious. Moreover, the two sites which right belong to the type I lake area, the mesosaprobic zone, are apparently the largest contribution to the mutagenicity of the whole lake.

Table 3 Results of Ames test of extracts from five sites in the Taihu Lake

Test site/Conc.		Number of revertant colonies			
tester	ml/plate	+S9	−S9	+S9	−S9
		TA 100		TA 98	
Zhihu	200	542.7±39.0*	412.7±13.7*	72.0±11.5*	55.7+11.8*
Tiaoxi	200	423.0±32.0*	104.7±50.8	31.7±13.6	27.0±5.6
Dapu	200	132.3±6.1	135.0±7.2	38.7±10.7	36.0±11.0
Liangxi	200	454.7±25.7*	323.7±29.6*	47.3±5.8*	27.0±8.7
Taipu	200	131.3±14.6	159.0±23.1	28.0±1.0	24.3±8.7
DMSO	0.1	133.3±6.8	149.9±19.2	23.7±3.1	23.3±5.2
Bap	0.1	623.3±74.1	152.3±14.2	159.0±10.5	26.7±3.1
MMNG	0.1	255.7±13.4	>1000	19.3±4.9	22.3±4.2
2AAF	0.1	249.3±18.9	162.7±6.9	438.3±21.1	253.3±13.4

*: the revertant colonies more than twice the spontaneous rate

Table 4 The dose-response relationship of revertant mutation

Test site		Number of revertant colonies under various dose, ml/plate				
	Tester	0	200	500	1000	*r*
Zhihu	TA100−S9	133.3±6.8	542.7±39.0	657.7	898.7±189.3	0.93
Zhihu	TA100−S9	149.9±19.2	412.7±13.7	548.3±43.1	704.7±18.0	0.94
Zhihu	TA98+S9	23.7±3.1	72.0±11.5	106.7±6.8	163.7±20.1	0.99
Zhihu	TA98−S9	23.3±5.2	55.7±11.8	73.3±10.3	105.0±9.9	0.95
Liangxi	TA100+S9	133.3±6.8	454.7±25.7	638.7±27.4	901.7±23.0	0.97
Liangxi	TA100−S9	149.9±19.2	323.7±29.6	503.7±23.2	692.7±32.3	0.98
Liangxi	TA98+S9	23.7±3.1	47.3±5.8	72.7±4.1	94.0±6.2	0.97
Tiaoxi	TA100+S9	133.3±6.8	423.0±32.0	726.7±42.7	876.3±17.2	0.94

The micronucleus test, indicating chromosome breakage, is a quick and economical means of biomonitor, and Ames test can detect frame-shift mutations by strain TA98 and base-pair substitution mutations by TA100 (Maron, 1983). It has been well known that there is close relationship between mutagenicity and cancer. The studies in this paper show that the lake have been polluted to various degrees, especially in the west area and the main river entrances. Based on the results of our study, we suggest the government pay close attention to the pollution because the source of main water plants are located in this area. Something must be done to remedy the pollution and protect the water source. The waterworks whose water source are located in mesosaprobic zone should be removed to other place.

4 Conclusion

The micronucleus test of *Vicia faba* root tip cell and the Ames test can be used in monitoring

pollution of large lakes. The results show that the Taihu Lake has not been seriously polluted by genotoxical materials. Moreover, the contaminated areas are concentrated in north-west lake near Wuxi and some river entrances whose source are the domestic sewage of the urban district and the agricultural and industrial wastewater from upstream cities or towns. The most remarkable fact is that all the potable water resource of Wuxi come from the contaminated area and it has close affinity to the public health. In addition, the micronucleus test of *Vicia faba* root tip cell and Ames test together can be used not only to indicate mutagen, but also to indicate water quality with supplement of other contaminational indexes.

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