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Analysis of Pollution in Dianchi Lake and Consideration of Its Application in Crop Planting

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Abstract

After investigating the distribution and composition of N-cycle-related bacteria of different sites and different depth of Dianchi sediment, we analyzed the longitudinal distribution, lateral distribution of N, its transportation and transformation in Dianchi sediment, as well as the involvement of these bacteria in nitrogen cycle. Conclusion was drawn as follows, (1) *Azotobateria* could be effectively used as indicative strains to track the changes of Dianchi pollution because the distribution of *Azotobateria* can not only indicate N contamination but also P enrichment, (2) ammoniate and nitrite is mainly existed in top sediment of Dianchi Lake while other forms of nitrogen mainly in deeper sediment, (3) due to the fact that Dianchi is rich in P, together with the mutual promotion between N pollution and P pollution, the pollution of south part will worsen rapidly, (4) if the south part is also polluted badly, the pollution distribution will appear as peaking at both ends (north and south), and the pollution will definitely extend toward the middle, and finally Dianchi Lake will totally be seriously polluted. Combining with the fact that 40% of Dianchi pollution was caused by abusive use of chemical fertilizer, we put forward the idea of "changing pollutants into things of value", which could be specified as "using the sediment as agricultural fertilizer". Such method can solve the problem of internal pollution, and what's more, it can develop agriculture, while cut down the use of chemical fertilizer and thus reduce relative pollution source.

*Keywords:*Sediment; Dianchi Lake; Azotobateria; nitrogen- cycle; N pollution and P pollution; organic fertilizer

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1.Introduction

Known as the cradle of Yunnan culture, Dianchi Lake is located at the watershed of the Yangtse River, the Red River and the Pearl River, with the length of 40 km from north to south, and width of 12.5 km from east to west. It is the largest freshwater lake in Yunnan-Guizhou Plateau, and also the sixth largest one in China.

Although Dianchi Lake enjoys the reputation as "a pearl on the Yun-Gui Plateau", it costs decades of efforts and huge investment (already reached 12 billion) to fight the pollution which started from 1970s. However, the State Environmental Protection Department announced that, in the environmental monitoring of the first half of 2010, Dianchi Lake was again evaluated as Class V, and is still at high pollution level, as well as high eutrophication level. So, how to effectively control and treat the pollution has become an important topic for a long time.

The reason for the worsening of Dianchi pollution has both social and natural factors, exotic and internal factors. For the social factor, it is important to note that, abusive use of chemical fertilizer in agriculture accounts for 40% of Dianchi pollution, which is a typical case of lake pollutions because industry and residential source are generally considered as great water pollution loads. For natural factor, it is much more complicated due to the geographical, climatic and geological elements. Dianchi Lake is bow-shaped, and there is a natural embankment dividing the Lake into north part and south part. The north part is called as inner lake ($11km^2$), also known as grass lake because of the heavy pollution caused by the widespread algae, while the south part is called as outer lake, accounting for 98% of total area. In the region of Dianchi Lake, the southwest-directed wind can always cause big waves, sometimes with height of 1.2m, length of 10m, which will definitely cause internal pollution. Besides, as one of the three largest phosphorite bases in China, the rich mineral resources of Dianchi Lake can bring extra difficulty to the pollution treatment. Based on above reasons, the pollution of Dianchi Lake decreases from north to south, with north part (grass lake) the worst pollution point, and the pollution level of some sites is high due to its particular geographic position and geological structure.

Generally speaking, the exotic pollution can be controlled by related regulations and policies, together with pollution control projects. However, it is internal pollution that makes all the previous efforts go for nothing because it can bring a secondary pollution even after the lake water is purified totally. Lake sediment is considered as the main cause for internal pollution.

Most research of Dianchi pollution have thus far emphasized the purification of lake water, and the sediment is less studied, especially its bacterium composition, which has a direct bearing on the N, P cycling, is even less touched. In this paper, we investigated the amount, composition and distribution of certain bacteria in the sediment, and their involvement in the nitrogen cycling, with the purpose of analyzing the transportation and transformation of N in the sediment and its relationship with P pollution, finding an appropriate indicator for pollution evaluation of sediment, thus preliminarily predicting the change trend of water quality. Based on the interacting relationship between the N, P pollution caused by agriculture planting and N, P nutrient required in agricultural planting, we also put forward the idea of "changing pollutants into things of value".

2.Materials and methods

2.1.Materials

Drilling rig and digger were used as two tools in taking samples. Drilling rig can take much deeper sediment, while samples taken by digger are generally top ones.

Four kinds of culture mediums were used to meet the nutrient demand of bacterium growth, namely Beef Extract Peptone Medium, Ashbby Medium, Ammoniate Medium and Nitrite Medium.

2.2.Methods

1) Sample Taking and Treatment

Sediment samples of 6 sites were taken by drilling rig, and only the upper sediment of each sample was chosen as test material, marked as ZK1-ZK6 (see TABLEⅠ) . Other sediment samples were taken by digger in 19 sites and only the top sediment was dug, marked as D8-D39. Samples from D8-D39 were mixed into 6 test materials according to their regional similarity, marked as R1-R6 (see TABLEⅡ). All of these test materials were aseptically packed and stored in the refrigerator. As shown in Figure1, the choice of sample-taken sites was based on the ecological and geographical factors.

TABLE I. SAMPLES DRILLED FROM DIANCHI SEDIMENT

drilling site	water depth/ m	drilling depth/ m	color of sediment		
			upper sediment	middle sediment	lower sediment
ZK1	4.6	7.0	black	reddish brown	black
ZK3	3.7	2.6	blackish brown	reddish brown	grayish black
ZK5	5.6	6.5	black	black	black
ZK6	3.0	6.5	black	gray	black
ZK7	6.6	6.6	black	gray	black
ZK9	4.8	6.6	black	gray	black

1) Only the upper sediment was chosen as test material, marked as ZK1-ZK6.

TABLE II. SAMPLES DIGGERED FROM DIANCHI TOP SEDIMENT

No.	digging sites	color of top sediment
R1	D8、D9、D12、D13	blackish brown
R2	D11、D16、D17	dark brown
R3	D20、D21、D23、D26、D27	reddish brown
R4	D25、D29、D30、D39	brown
R5	D31	brownish yellow (sandy)
R6	D32、D34	reddish brown

1) R5 is sandy like.

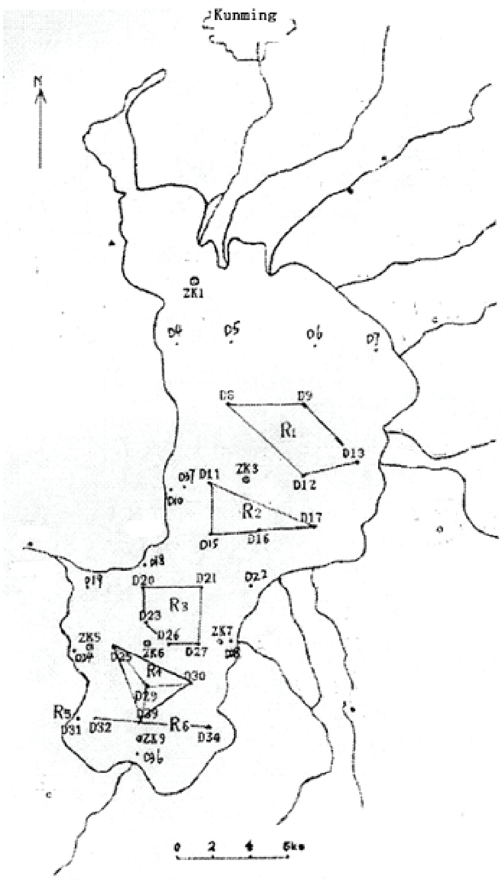

Figure 1. Sample-taken sites

2) Bacterium Isolation and Identification

The bacteria in the test materials were isolated and identified within 48 hours after the samples were taken, so the original state of the test materials could be remained as far as possible.

The isolation was performed according to dilution-plate method, specifics as follows:

Step 1, preparing the mentioned culture mediums;

Step 2, diluting the test materials: put 5 g sample of each test material (totally 12 materials) into 45 ml sterile water (with some glass beads inside), after completely shaking and blending, the sediment solution could be diluted into 10^{-1}, and then 10^{-2} and 10^{-3};

Step 3, daubing and culturing: 0.2 ml solution of each test material with different dilution were daubed on the culture mediums mentioned above, after 2-day culture under 37±1℃, the bacterial colonies were counted, and each colony was further separated and purified;

Step 4, bacterium identifying: the identification of bacteria is based on “Bergey Handbook”.

All operation should be ensured aseptic, and sterile water was used as bland control.

3.Results

3.1.Amount and Distribution of Azotobacteria.

As the key member of nitrogen cycling, *Azotobacteria* is our main research object. Beef Extract Peptone Medium is suitable for the growth of most heterotrophic bacteria, while Ashbby Medium is mainly specific to *Azotobacteria*. So, the comparison of bacterial colonies between the two mediums could roughly reflect the proportion of *Azotobacteria*. After arranging the proportion of Azotobateria in order, we found ZK1>ZK7>ZK5>ZK6>ZK9>ZK3, and for Rs, it is R3>R6>R5>R4>R2>R1 (see TABLEIII, Figure2 and Figure3). Besides, ZKs have much bigger population of *Azotobacteria* than Rs as a whole (see Figure4).

TABLE III. BACTERIAL COLONIES OF 12 SAMPLES ON THE TWO MEDIUMS

sample No.	Medium A colonies • g^{-1}	Medium B colonies • g^{-1}
ZK1	1.6×10^5	8.4×10^5
ZK3	2.6×10^5	1.0×10^5
ZK5	8.1×10^4	1.0×10^5
ZK6	2.5×10^4	2.3×10^4
ZK7	8.6×10^4	1.4×10^5
ZK9	2.9×10^5	2.5×10^5
mean of ZKs	1.5×10^5	1.16×10^5
R1	7.4×10^5	8.4×10^4
R2	1.9×10^5	8.1×10^4
R3	1.3×10^4	1.4×10^5
R4	8.6×10^4	5.0×10^4
R5	2.6×10^5	1.3×10^5
R6	1.0×10^5	1.8×10^5
mean of Rs	6.55×10^5	1.10×10^5
overall mean	4.0×10^5	1.1×10^5

1）"Medium A" indicates Beef Extract Peptone Medium, "Medium B" indicates Ashbby Medium.

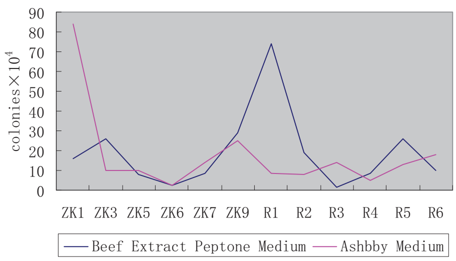

Figure 2. Comparison of bacterial colonies of 12 samples on the two mediums

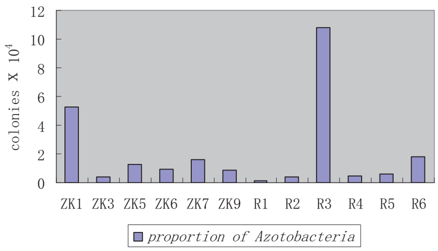

Figure 3. Proportion of Azotobacteria

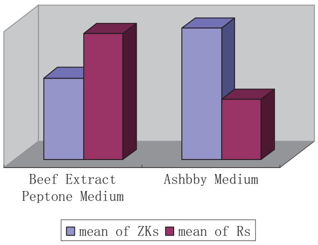

Figure 4. Comparison of ZKs and Rs on the two mediums

3.2.Composition of Azotobacteria.

To further investigate the characteristics of *Azotobacteria* in Dianchi sediment, we analyzed the composition of *Azotobacteria*. After colonies of *Azotobacteria* were separated and identified, we got 33 species of *Azotobacteria* in total. These species belong to the following four genera: *Azomonas*, *Azotobacter*, *Derxia* and *Beijeinckia*, among which, *Azomonas* and *Azotobacter* grow best in micro-aerobic condition, while *Derxia* and *Beijeinckia* like aerobic or micro-aerobic condition. From TABLEⅣ and Figure5, *Derxia* and *Beijeinckia* are considered as two dominant genera in Dianchi sediment, accounting for 42.5% and 33.3% respectively. We can also find that, ZKs have richer diversity in *Azotobacteria* composition than Rs, and *Azomonas* and *Azotobacter* mainly exist in ZKs (see TABLEⅣ and Figure5).

TABLE IV. COMPOSITION OF AZOTOBACTERIA

sample No.	**genus of *Azotobacteria***				**total (sites)**
	Azomonas	***Azotobacter***	***Derxia***	***Beijerinckia***	
ZK1	2		1		3
ZK3		1	1		2
ZK5		1	3	1	5
ZK6			4		4
ZK7		2			2
ZK9				3	3
R1			1	3	4
R2	1		1		2
R3				1	1
R4	1		1	1	3
R5			1	1	2
R6			1	1	2
total (genus)	4	4	14	11	33
proportion /%	12.1	12.1	42.5	33.3	100

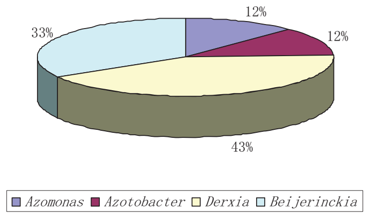

Figure 5. Proportion of each genus

3.3. Utilization of Ammoniate (NH4+) and Nitrite (NO2-) by Azotobacteria.

All the above seperated bacteria of *Azotobacteria* were inoculated on Ammoniate Medium and Nitrite Medium to investigate the utilization of ammoniate (NH_{4+}) and nitrite (NO_{2-}) by *Azotobacteria* in Dianchi sediment. From TABLEⅤ, we could find that, *Azotobacteria* of Dianchi sediment has limited ability in using ammoniate and nitrite. Compared with 33.3% *Azotobacteria* growing on Nitrite Medium, around 48.5% *Azotobacteria* can grow on Ammoniate Medium.

TABLE V. THE UTILIZATION OF AMMONIATE AND NITRITE BY AZOTOBACTERIA

medium	growth condition	genus of *Azotobacteria*				total
		Azomonas	*Azoto-bacter*	*Derxia*	*Beijerinckia*	
Ammoniate Medium	+	1	2	7	6	16
	-	3	2	7	5	17
Nitrite Medium	+	1	2	3	5	11
	-	3	2	11	6	22

(1) "+" indicates the colonies are able to grow, "-" indicates the colonies are unable to grow.

3.4. Condition of Nitrosobacterium and Nitrobacterium

We also investigated the condition of *Nitrosobacterium* and *Nitrobacterium* in Dianchi sediment, because they are key members of nitrogen cycling too. As shown in TABLEⅥ, we have got some information of the distribution of *Nitrosobacterium* and *Nitrobacterium*. Except R3, Rs generally have more *Nitrosobacterium* and *Nitrobacterium* than ZKs, among which, R1 and R2 have a large number of *Nitrosobacterium* and *Nitrobacterium*, and R4 and R5 have great quantity only in *Nitrosobacterium,* while the condition of R6 is just the opposite.

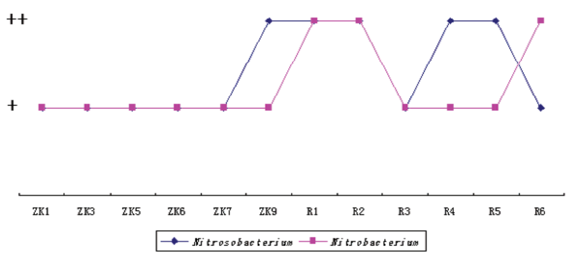

Figure 6. Condition of *Nitrosobacterium* and *Nitrobacterium*

4.Discussion

Used as the pollution index, N can be accumulated through outside source (sewage, etc.) and deposited in sediment[1-3]. In this paper, *Azotobacteria*, *Nitrosobacterium* and *Nitrobacterium* were used as indicators to trace the nitrogen cycling and transportation. Different sites and different depth of sediment were selected to analyze the distribution and transformation of N.

4.1.Longitudinal Distribution of N and Its Transformation

From the results of TABLEⅢ and Ⅵ, we could see that, Azotobateria mainly exist in deeper sediment (ZKs), while *Nitrosobaterium* and *Nitrobacterium* mainly in top sediment (Rs). Such results can be explained from their growth habit. *Nitrosobaterium* and *Nitrobacterium* are aerobic, while from TABLEⅣ, it is clear that, the majority of Azotobateria in ZKs are micro-aerobic. On the other hand, such results could also be used to indicate the existing form or the transformation of nitrogen. Combining the results obtained from TABLEⅣ and TABLEⅤ, *Azotobacteria* of Dianchi sediment has limited ability in using ammoniate and nitrite, so ammoniate and nitrite in Dianchi sediment is maily used by *Nitrosobaterium* and *Nitrobacterium*. So, we can also infer that, ammoniate and nitrite is mainly existed in top sediment while other forms of nitrogen mainly in deeper sediment.

4.2.Lateral Distribution of Azotobateria and Its Indicating Function in Pollution Assessment

After arranging the proportion of Azotobateria in order, we found ZK1>ZK7>ZK5>ZK6>ZK9>ZK3, and for Rs, it is R3>R6>R5>R4>R2>R1 (see Figure3). We guess such results have some correlation with the pollution level of these different sites, so we make a hypothesis that the proportion of Azotobateria in Dianchi sediment has a positive correlation with pollution level. According to other survey reports, Dianchi pollution decreases from north to south, which could easily explain why ZK1 has the highest proportion of Azotobateria. But except ZK1, it is really hard to understand why the south part has high proportion of Azotobateria. Does that mean the south part is also seriously polluted? After deep investigation, we found in the southeast part of Dianchi Lake, there is a river called as Cai River (see Figure 1). It passes through a phosphate base and flows westward to Dianchi Lake, so the region near Cai River (the south part) is obviously high in phosphate content. According to related research, the increase of phosphate content is the main reason for the increase of Azotobateria[4,5].

TABLE VI. DISTRIBUTION OF *NITROSOBACTERIUM* AND *NITROBACTERIUM*

sample No.	ZK1	ZK3	ZK5	ZK6	ZK7	ZK9	R1	R2	R3	R4	R5	R6
Nitrosobacterium	+	+	+	+	+	++	++	++	+	++	++	+
Nitrobacterium	+	+	+	+	+	+	++	++	+	+	+	++

1） “+” indicates the colonies are able to grow, “++” indicates a large number of colonies are able to grow.

That means the distribution of Azotobateria has a close bearing with phosphate enrichment. So, although the previous survey report didn’t list the south part as seriously polluted sites, there is still a great hidden danger existing in the south part of Dianchi Lake. Such conclusion also shows that Azotobateria could be effectively used as indicative strains to track the changes of Dianchi pollution because the distribution of Azotobateria can not only indicate N contamination but also P enrichment, and N, P is known as important pollution index.

4.3.the relationship of N pollution and P pollution and their influence on the future of Dianchi

It is known that, NO_{3-} has the ability to transform insoluble phosphate and phosphate ore into soluble ones, which can then spread into lake water and provide nutrients to algae growth.

On the other hand, some concerned research shows that, when P concentration is lower than 0.04mg/L, algae growth will increase with the increase of N concentration, and such increase will be inhibited when N concentration is over 0.6mg/L. However, when P concentration is higher than 0.5mg/L, N concentration will not be the limiting factor any more, and algae will grow excessively at that time[4].

So, N pollution and P pollution can mutually promote each other. For the case of Dianchi pollution, the problem is quite terrible because of the phosphorite base. The average P concentration of sediment in the south part of Dianchi Lake is reported at 0.8%[5]. According to our research, this region has a large number of *Nitrosobacterium* and *Nitrobacterium*. So, the more NO_{3-} is accumulated, the more phosphate can be dissolved into lake water. When P concentration increases to certain degree (0.5mg/L), the excessive growth of algae can not be inhibited by N concentration. So, in the long run, if the problem of south part can not be timely solved or controlled, the pollution of south part will worsen rapidly. Furthermore, if south part is also polluted badly, the pollution distribution will appear as peaking at both ends (north and south), and the pollution will definitely extend toward the middle, and finally Dianchi Lake will totally be seriously polluted.

4.4.the Possible Solution

As mentioned in "INTRODUCITON", abusive use of chemical fertilizer in agriculture accounts for 40% of Dianchi pollution. Considering the fact that the difficulty of pollution treatment mainly focuses on the high enrichment of N, P in sediment, particularly the special geological structure of Dianchi Lake, we put forward the idea of "changing pollutants into things of value", which could be specified as "using the sediment as agricultural fertilizer".

Up to date, a great deal of research has been done on dredging as it is an effective way in treating pollution of lakes. Dredging can remove the polluted sediment, which can improve the ecosystem circulation and avoid the subsequent internal pollution. Besides, the dredged sediment can also be used as fertilizer to improve the physical and chemical properties of soil and enhance soil fertility.

So, we can consider using this way to treat Dianchi pollution. Different sites and different depth of sediment has different forms and proportion of N and P, and different crops have different requirement of N and P, so we could do some further investigation on this issue. For example, some research has shown that, field crops have high utilization ratio of NO_{3-} than vegetables, so the top sediment is the good material of the fertilizer for field crops[6-8].

Such method can solve the problem of internal pollution, and what's more, it can develop agriculture, while cut down the use of chemical fertilizer and thus reduce relative pollution source. We firmly believe that, if the 40% of Dianchi pollution (caused by agriculture) can be controlled, the pollution treatment of Dianchi Lake will be more promising.

5.Acknowledgment

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