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Numbers and Types of Diatoms in Pearl River

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Diatom distributions in Pearl River were investigated, and on this basis, its significance for the water quality evaluation and development of diatom taxa was discussed as well. Totally, 54 water samples were collected in 34 sites along Pearl River basin and South Sea in June and September, 2011. Diatom related indices were calculated among the samples, cluster analysis and linear regression were performed after the diatoms were analyzed qualitatively and quantitatively. The genus and the quantity of the diatoms in the water samples were subsequently investigated using scanning electron microscopy (SEM). With reference to diatom distribution, 105 types of diatoms were detected from 54 samples, among which 13 dominant species (dominancee"0.02) were found spreading Pearl River. The characteristics of the indices from Biological Diatom Index (IBD) and Diatom model affinity (DMA) showed the greatest similarity to the diatoms detected in the same site. Exploring the distribution of diatoms in Pearl River is useful for water quality evaluation and forensic practice.

Key words: Bio-indication, Distribution of diatom, Pearl River, Scanning electron microscopy, Water quality, Forensic medicine.

Diatom, because of their numerous advantages[1], are one of the most commonly used organism for assessing natural and human-related features of water environments[2]. Diatom water quality indices have been widely used in many water bodies around Europe[3], but they have been rarely used in domestic water bodies ,such as Pearl River. The first goal of this study was to explore the distribution of diatoms in Pearl River. The difference of distribution of diatoms can be compared in the three big tributaries of Pearl River. The second goal was to analyses the applicability of different diatom water quality indices in Pearl River. The distribution of diatoms may therefore be useful in detecting impacts in degraded streams[4] and diatom community composition may be a useful indicator of nutrient enrichment. In the presented paper, a new method Microwave Digestion-Vacuum Filtration-Automated Scanning Electron Microscopy (MD-VF-Auto SEM) method[5], which can be able to analyze diatoms in water samples qualitatively and quantitatively, was adopted.

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MATERIALS AND METHODS

Study area

The sampling design included three tributaries (West River, East River, North River) of the Pearl River which crosses four provinces. The sampling sites were sampled were demonstrated in Fig. 1. The samples were taken based on these

sites: (1) West River basin: 1. Maxiong Mountain, 2. Qujing City, 3. Caishi Bank, 4. Shaonu country; 5. Xiaolong country, 6. Jiangbian City, 7. Qingshui River, 8. Huangni River, 9. Tiansheng Bridge, 10. Northpan River, 11.Yan Bank, 12. Dahua City, 13.Pingnan City, 14. Wuzhou City, 15.Deqing City, 16. Zaoqing City, 17. Sanshui City, 18. Jiangmen City, 19. Zhuhai City; (2) North River basin: 20. Lechang City, 21. Jingjiang City, 22. Nanxiong City, 23. Shaoguang City, 24. Yingde City, 25. Wengyuan City, 26. Lianjingkou City, 27. Qingyuan City; (3) 28. Longchuan City, 29. Heyuan City, 30. Zijing City, 31. Huizhou City, 32. Guanghou City, 33. Humen City; (4) South Sea: 34. Sea Water

Sampling procedure

Water samples were taken in June and September 2011 from 34 sites. About 500 ml of water in each site was sampled about 1m below the water surface(used to test planktonic diatom). A total of 54 samples were obtained. Samples were preserved in the field in 4% formaldehyde.

Laboratory procedure

50ml sample was used for diatom analysis. 5ml of the sediment was retained, and 2ml of hydrogen peroxide and 8ml of concentrated nitric acid were added into the precipitates. After Vacuum filtration, Diatoms were analyzed qualitatively and quantitatively under SEM. The detailed process of digestion could be referred to a previous paper[6].

Calculation

Biomass and Biological Diatom Index

We used the method given in Coste and Prygiel et al. to calculate the Biological Diatom Index (IBD)[7]. Biomass and IBD can refer to a previous paper[6].

Dominance

Dominance was normally used to estimate the dominant species of watercourse for diatom community, and virtually dominant species proved to be a valuable marker of ecological status, and it can refer to equations as Eq.(1) :

$$Y = (N_i/N) \times f_i \quad ...(1)$$

Where N_i means the biomass of diatoms for the particular species; f_i means the frequency of the particular species. N is the biomass of diatoms surviving in the water sample. It is a dominant specie if $Y \leq 0.02$.

Diatom model affinity (DMA)

A new index for the assessment of anthropogenic impacts on streams which based on the relative abundance of Achnanthes minutissima et linearis species groups and a few selected diatom genera was adopted. The sensitivity of DMA to large-scale land use variables was tested and compared to other commonly used diatom indices[8]. The DMA is based on the Eq. (2) [8]:

$$\text{Percentage similarity} = \sum_{i=1}^{4} \min(a, b) \quad ...(2)$$

Where a is the percentage of individuals of a taxonomic category i in the model community and b is the percentage of the same taxon in the real community[8].

Statistical analyses

All indices calculated for Pearl River were subjected to Pearson Chi-square test can be considered to be significant if $p<0.05$. Then separate regressions were conducted on IBD against MDA or Population density and Forest cover percent. Dendrogram based on the cluster analysis of the diatom index IBD in order to evaluate the similarity in each site. All statistical analyses were performed using SPSS 13.0 for windows.

RESULTS AND DISCUSSION

Diatoms at the species level under SEM with a clear-cut outline and texture were identified easily after the digestion of microwave dissolver. 27 genus and 105 species diatoms have been identified from 54 water samples, among them, 86 species were classified by pennales(81.9% in all species) and 19 species were classified by Centrales (18.1% in all species). These dominant taxa mainly determined the values of the water quality diatom indices. Navicula, Achnanthidium, Hantzschia, Melosira, Cyclotella are common dominant genus in Pearl River (dominancee”0.02), however, Thalassiosira are the mainly gene in the sea sample(Tab. 1). All the diatoms’ name were used by abbreviation, and that is Nit.: Nitzschia; Mel.:Melosira; Cyc.: Cyclotella; Pin.: Pinnularia; Act.: Actinocyclus; Gyr.: Gyrosigma; Coc.: Cocconeis; Try.: Tryblionellal; Fal.: Fallicia; Sur.: Surirella; Han.: Hantzschia; Ste.: Stephanodisus; Tha.: Thalassiosira; Ach.: Achnanthidium; Cym.: Cymbella; Syn.: Synedra; Nav.: Navicula; Gom.: Gomphonemace; N: Biomass(/ml). 1-34: water sampled in June; 15*-34*: water sampled in

September; All dominance are higher than 0.02. Most diatoms live in the sea are unable to tolerate concentrated acid digestion that was adopted by this laboratory procedure.

The biomass of Pearl River charactered a significant increasing from the upstream to downstream in its three tributaries respectively. For example, the biomass of the West River demonstrated in Fig. 2 shown increasing from the upstream to downstream especially in Guangdong province area(One-Sample T-Test $t=6.611$, $p<0.01$). The same result was obtained in North River and East River($p<0.05$). Subject to the difference of month exerting to the distribution of diatom, significant difference could be observed in the biomass of the West River(Paired-Samples T Test: $t=6.75$, $p<0.01$),which demonstrated in Fig. 2.

Diatom model affinity demonstrated a broad range of values from 14.37% to 64.32% (Mean=41.71±9.79), which sampled in June. IBD is ranged from 4.7 to 16.8(Mean=10.45±3.34). Stepwise multiple regression of the diatom indices for IBD against DMA are used for comparing the distribution of three tributaries. It is shown that the IBD and the MDA have great correlation with the distribution of three tributaries. Furthermore, DMA showed a strong correlation to the IBD, suggestive of complementary information contained in this index. North River's IBD against its population density: $F=8.609$, P-value=0.026 , $R^2=0.589$, and North River's IBD against its forest cover percentage: $F=9.418$, P-value=0.022 , $R^2=0.611$, which were indicated that the abundance of pollution tolerant diatoms correlates well with the abundance of eutrophication tolerant diatoms.

In addition, the streams were affected by disturbances spanning a wide variety of human impacts, e.g. deforestation and habitat destruction, impoundment, hydrological and geochemical alterations, organic pollution, acidification, and

Table 1. Taxa of diatoms commonly found in the samples

Sites	N	Dominant Genus / Values		Sites	N
1	2.95	Nav./0.125, Nit./0.1, Mel./0.023	Ach./0.034, Gom,/0.021, Coc./0.02	28	89.4
2	10.4	Syn./0.02, Nav./0.021, Act./0.138	Nav./0.02, Nit./0.096, Mel./0.02	29	85
3	15.6	Mel./0.023, Ste./0.077	Ach./0.045, Nav./0.031, Mel./0.02	30	169.7
4	31.2	Nav./0.02, Cyc./0.028, Ste./0.022	Gom./0.025, Nit./0.021, Act./0.059	31	186.6
5	45.6	Nav./0.031, Coc./0.0225	Cyc./0.039, Act./0.137, Mel./0.02	32	682.4
6	151	Act./0.033, Coc./0.02, Ste./0.035	Syn./0.02, Cyc0.06,Tha./0.034	33	462.8
7	89.2	Nav./0.04, Nit./0.021, Coc./0.026	Cyc./0.035, Tha./0.06	34	83.8
8	6.5	Cym./0.1, Nav./0.074, Nit./0.05	Nav./0.032, Act./0.048, Mel./0.072	15*	78.3
9	56.4	Nav./0.03,Cyc./0.093, Act./0.04	Nit./0.026, Cyc./0.049	16*	126
10	30.2	Nav./0.02, Mel./0.024, Cyc./0.05	Syn./0.026, Nav./0.025, Mel./0.02	17*	177.8
11	170	Nav./0.04, Cyc./0.148	Syn./0.09, Cyc./0.042	18*	163.1
12	37	Nav./0.021, Cyc./0.021	Act./0.093, Cos./0.037	19*	201.8
13	82	Nav./0.02, Mel./0.02, Cyc./0.034	Ach./0.062, Nav./0.05, Mel./0.023	20*	2.8
14	231	Nav./0.03, Mel./0.03, Cyc./0.043	Ach./0.03, Syn./0.02, Nav./0.02	21*	59.34
15	252	Nav./0.06, Mel./0.041, Cyc./0.02	Nav./0.035, Mel./0.028	22*	13.8
16	329	Nit./0.02, Mel./0.04, Cyc./0.029	Ach./0.035, Nav./0.062, Nit./0.023	23*	93.1
17	311	Syn./0.02, Nav./0.02, Gom./0.15	Ach/0.021, Syn/0.035	24*	105
18	381	Mel./0.08, Act./0.056, Gyr./0.06	Nav./0.034, Nit./0.033	25*	117
19	404	Nav./0.03, Nit./0.045	Ach./0.052, Syn./0.02	26*	152
20	30	Ach./0.02, Mel./0.024	Cym./0.02, Nav./0.061, Coc./0.02	27*	162
21	83.6	Syn./0.02, Nit./0.081	Syn./0.06, Nit./0.168, Cyc./0.112	28*	8.6
22	153	Nav./0.029, Nit./0.064	Mel./0.024, Nit./0.063, Ste./0.03	29*	18
23	248	Ach./0.05, Nav./0.047, Nit./0.095	Nav./0.031, Nit./0.025, Ste./0.02	30*	18
24	269	Ach./0.026, Syn./0.06	Nav./0.033, Act./0.021, Gyr./0.0664	31*	43
25	294	Nav./0.027, Nit./0.021	Nav./0.031, Mel./0.021, Cyc./0.195	32*	213.2
26	312	Ach./0.03, Syn./0.24	Act./0.082, Cyc./0.027, Tha./0.021	33*	169.1
27	268	Nav./0.05, Nit./0.01, Cyc./0.01	Cyc./0.035, Tha./0.03	34*	79.6

eutrophication. So that, it is necessary to get some demographic data and proportion of the land cover categories in the study region,which were acquired from governmental site(Tab. 2). These categories were: (1) Population density were collected at 2011; (2) Forests (deciduous, evergreen and mixed) and shrubs (deciduous), which were collected at 2005. The strongest correlation was observed between IBD and forest cover percentage, which was expected considering that forest is the protected factor to prevent the water decomposition. The second strongest correlation was observed between IBD and population density, indicating that the development of urbanisation creates a large amount of waste which made pollution tolerant diatoms correlates well with the abundance of eutrophication tolerant diatoms.

The habitat can cluster based on its diatom related index, such as IBD. Because the values of the IBD are not belong to normal distribution (p-value>0.1), we use hierarchical cluster with Median Method to analyses its distribution(Fig. 3, a - z means sampling site 1 to 26, and aa - hh means sampling site 27 to 34. Water

Table 2. The Population density and Forest cover percentage for three tributaries within Guangdong province

Sites	Population density(km-2)*	Forest cover percentage(%)#
15	303	61.4
16	264	66.8
17	3054	20.2
18	466	41.4
19	944	33.2
20	153	71.6
21	153	70.5
22	153	71.4
23	153	70.9
24	193	68.4
25	193	71.5
26	193	66.1
27	193	66.7
28	189	73.8
29	189	72.5
30	189	72.5
31	405	59.8
32	1744	41.4
33	3327	30.4

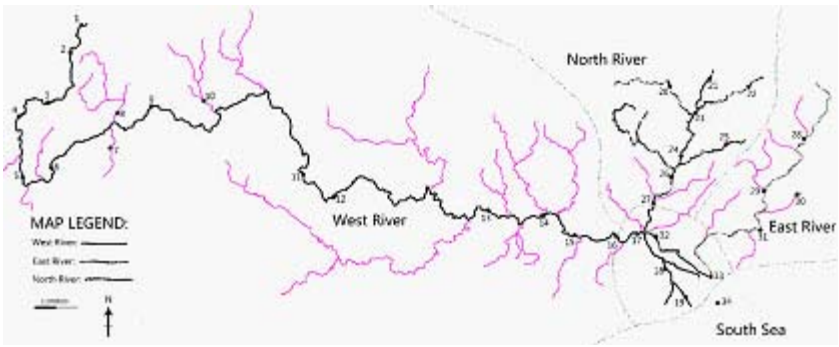

Fig. 1. Schematic locations of sampling sites in Pearl River

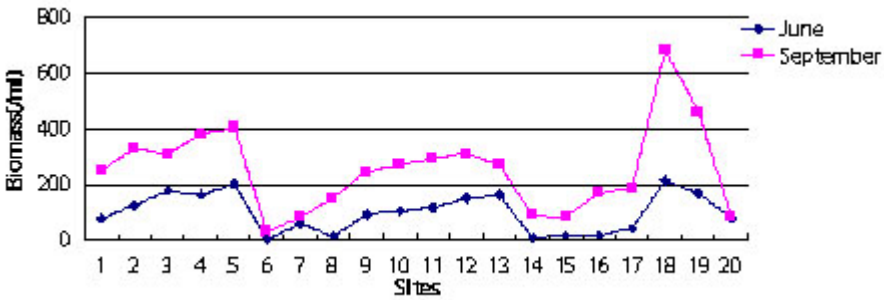

Fig. 2. The difference of biomass between June and September

sampled in June.). It is clear to observe that similar habitat type clusters the same community, which represent the similarity water body, such as the site 1, 20, 21 are all the upstream of West River and East River and those water type are "good".

In this study, the most important aim is to have assessed the distribution of diatoms in Pearl River. It is clear that Navicula and Nitzschia genus, which thrives in nutrient-rich environments[9], are commonly distributed in three tributaries, especially in the downstream of Pearl River. While Thalassiosira gene dominate in salt water, such as sea water or estuary water. Some genus of diatoms, such as Achanathes and Cocconeis, can not tolerate the pollution with high levels of organic matter[10], that were found few in special area, such as the upstream of Pearl River which were less population, more forest cover percentage and small scale urbanization. Diatoms biomass and genus are more abundant in June than that in September. The climate changes may be the major cause, and agricultural production decrease may be the secondary cause.

The urbanization and industrialization of the metropolitan city of Bursa and its environment have contributed to increasing pressure on water sources of the city for many years[11]. The same problem has been existing in cities within Pearl River basin, due to rapid increases in population, unplanned industrialization and incontinent deforestation. The forest cover percentage is decreased from upstream to downstream of the Pearl River, which is coincident with the IBD trend, in that water decomposition is undergoing with the water and soil erosion. The same result may due to population increasing, urbanization, agricultural production and industrialization.

In addition, diatom test are widely used in forensic practice, such as diagnosis of drowning and to determine the drowning site[12]. 27 genus

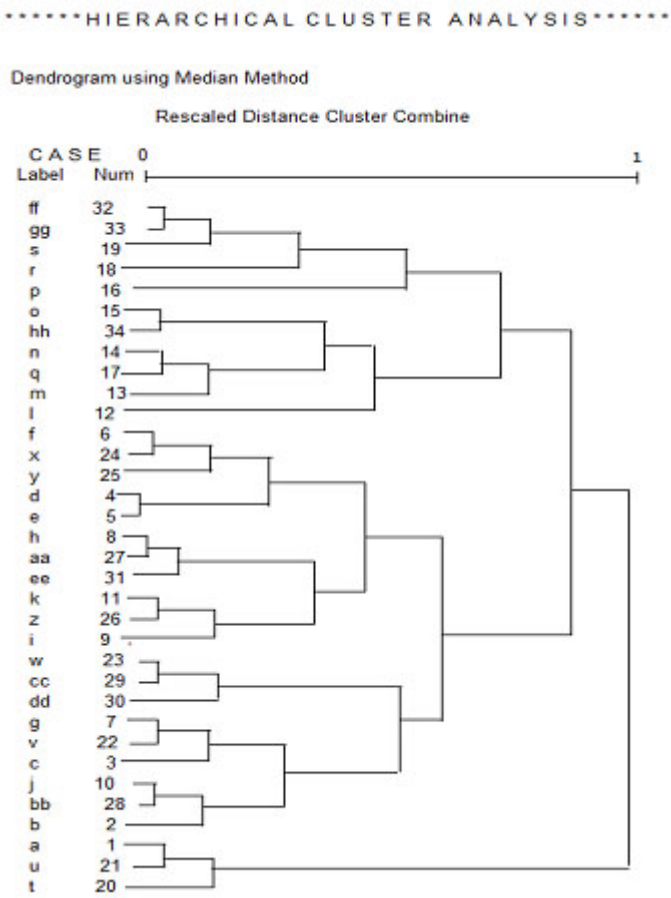

Fig. 3. Dendrogram based on the hierarchical cluster analysis of the IBD

and 105 species diatoms have been identified in Pearl River basin, which can assist forensic practice about bodies drowning in Pearl River.

CONCLUSION

The distribution of diatoms have been explored in this study and diatoms are thriving in Pearl River. The quality of upstream water body are determined by "good or medium", and downstream water quality are most determined by "bad". The variability of season can significantly change the distribution of diatoms. It is necessary to develop the database of diatoms in more extensive water bodies for the forensic diagnosis of drowning in the future.

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