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Use of siliceous algae as biological monitors of heavy metal pollution in three lakes in a mining city, southeast China

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Key words: diatom, chrysophyte cyst, heavy metal pollution, cumulative criterion unit, Daye City

Abstract

In order to assess the ecological status of three lakes in a historical mining city (SE China), water metal concentrations and surface sedimentary diatoms and chrysophyte cysts were analyzed in 20 sampling sites. The significant correlations between the algal indices and the cumulative criterion unit (CCU) scores confirmed the importance of heavy metals in shaping algae communities. In the metal-polluted sites, diatom assemblages were dominated by metal-tolerant species, such as *Nitzschia palea* and *Nitzschia perminuta*. In the unpolluted samples, diatom assemblages were characterized by *Cyclostephanos dubius*, *Discostella pseudostelligera* and *Aulacoseira* species (mainly *A. alpigena*, *A. granulata* and *A. ambigua*). These dominant taxa in the unpolluted samples might be sensitive to metal contamination but tolerant of eutrophication. In addition, nonspherical cysts were much more abundant in the polluted sites, indicating that their presence should be indicative of metal contamination in this region. This study provides some clues for future metal pollution assessment through the use of siliceous algae in metal polluted lakes.

INTRODUCTION

All heavy metals, including those that are essential micronutrients (e.g., zinc and copper), are toxic to algae at high concentrations (Rai et al. 1981). Algae sit at the base of many food webs, hence their immense ecological importance for monitoring environmental contamination (Morin et al. 2012). The sensitivity or tolerance to metal pollution varies amongst different algae groups (Rai et al. 1981, Lewis 1995). After exposure to high metal concentrations, algae communities will shift toward communities dominated by metal-tolerant species (Lewis 1995). Among various algal groups, diatoms are ideal indicators of heavy metal contamination because of their wide diversity, the ubiquitousness of the various species, the sensitivity to contamination and the good preservation of their siliceous frustules (Battarbee et al. 2001, Morin et al. 2012, Rimet 2012, Popovskaya et al. 2012). Recent studies in many polluted watersheds have demonstrated their high potential for metal contamination assessment in freshwater ecosystems (Sabater 2000; Hirst et al. 2002; Cattaneo et al. 2004, 2008; Morin et al. 2008, 2012).

The middle and lower reaches of the Yangtze floodplain (SE China) are lake-rich, containing 651 lakes with surface areas greater than 1 km² (Dong et al. 2012). These lakes are used for water supply, recreation, transportation and food production, while at the same time facing a range of environmental issues (Yang et al. 2010, Dong et al. 2012). One ongoing environmental problem is heavy metal pollution in many urban lakes in this region (Wu et al. 2012). Geochemical analyses have been applied to assess the ecological status of these metal-polluted

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lakes (Liu et al. 2012, Wu et al. 2012). Compared with chemical measurements, biomonitoring has been increasingly used to evaluate water quality due to its sensitive and integrative characteristics (Morin et al. 2012). Although common patterns of diatom communities in response to metal contamination were observed among several countries, regional applications were generally influenced by small-scale variability, such as geological features and smelting activities (Morin et al. 2012). In the Yangtze floodplain lakes, little knowledge existed about the response of diatom communities to metal stresses until now.

In this study, we examined water quality and surface sedimentary diatoms and chrysophyte cysts in three lakes in Daye City (a historical mining city, SE China). The aim of this study was to evaluate the metal sensitivity of indicator species and their usefulness for monitoring contamination and recovery in this region.

MATERIALS AND METHODS

Study area

The lakes under study are located in Daye City in the middle reach of the Yangtze River (SE China) (Fig. 1). Daye City has been an important mining city since the late 19th century. Many mining and metallurgical plants have operated in the western part of the city, resulting in high inputs of heavy metals (e.g., zinc, cadmium and manganese) to the surface waters. Waters of the three lakes are found to be slightly alkaline and have high conductivity values (Table 1). Sanliqi Lake, near a metallurgical plant, receives industrial wastewater, while Hongxing Lake, which is near a park, and Yinjia Lake, which is near an ecological protection zone, are mainly influenced by domestic sewage.

Sampling and laboratory analyses

In November 2011, 20 sampling sites in the three lakes were selected for water and surface sediment samples. Both conductivity and pH were measured in the field using a portable multifunctional probe (Shanghai Sanxin Instrumentation Factory; type PD-501). Secchi depth was measured using a standard transparency disc, and water depth was determined using a portable echo sounder. The water samples were taken from 20-30 cm in depth. They were filtered through a 0.45 μm membrane filter and acidified to 1% with nitric acid for analysis using Inductively Coupled Plasma -Atomic Emission Spectrometry (ICP-AES, IRIS Intrepid II XSP).

The surface sediment samples (the top 1 cm) were collected using a Kajak gravity corer and stored at 4°C. For diatom analysis, sediments were treated

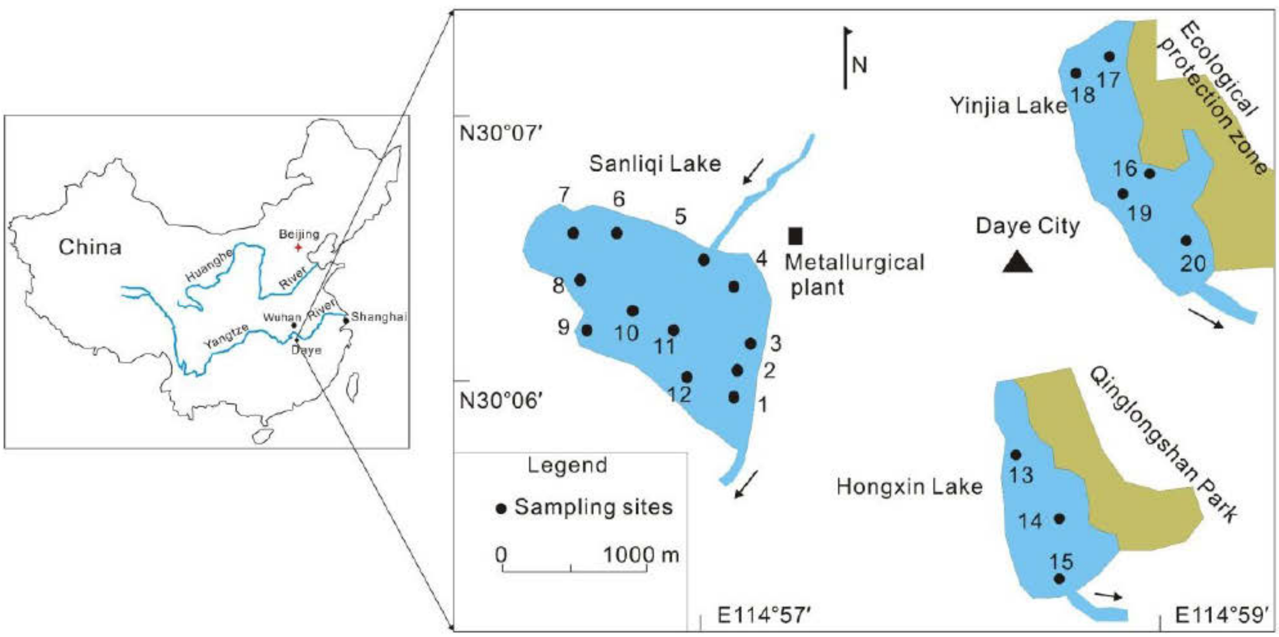


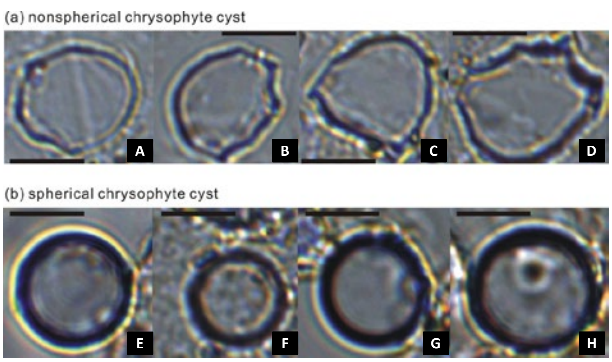
Fig. 1. Map of Daye City where the samples were collected

Table 1

Minimum, maximum, average and standard deviation of measured values of each of the physicochemical parameters of water in the three lakes in Daye City

Sample		Physico-chemical parameter								
		Depth (cm)	Secchi depth (cm)	Conductivity (µS cm^{-1})	pH	Cd (µg l^{-1})	Mn (µg l^{-1})	Ni (µg l^{-1})	Zn (µg l^{-1})	CCU
Sanliqi Lake (n=12)	Minimum	60	57	1313	6.93	8	113	115	52	7.52
	Maximum	200	76	1474	7.56	21	375	251	128	12.94
	Mean ± SD	156 ± 40	67 ± 6	1415 ± 54	7.41 ± 0.17	11 ± 3	287 ± 76	141 ± 36	74 ± 22	9.26 ± 1.50
Hongxing Lake (n=3)	Minimum	140	36	424	7.15	<1	<1	5	<1	0.01
	Maximum	150	42	460	7.40	<1	7	9	5	0.12
	Mean ± SD	146 ± 6	40 ± 3	445 ± 19	7.31 ± 0.14		2 ± 4	6 ± 2	2 ± 3	0.05 ± 0.06
Yinjia Lake (n=5)	Minimum	30	22	538	7.56	<1	<1	7	<1	0.01
	Maximum	100	30	624	7.62	<1	<1	14	<1	0.03
	Mean ± SD	68 ± 36	26 ± 3.4	577 ± 36	7.59 ± 0.02			9 ± 3		0.02 ± 0.01

with hydrogen peroxide and mounted in Naphrax (Battarbee et al. 2001). The relative abundance of diatom taxa per sample was analyzed by counting at least 500 valves per slide with an Olympus BX-53 microscope with an oil immersion objective at a magnification of 10×100. The main taxonomic sources were Krammer and Lange-Bertalot (1986, 1988, 1991a, b). The taxonomy was corrected to current conventional names based on the Catalogue of Diatom Names (Fourtanier & Kociolek 2011). Chrysophyte cysts were counted along with the diatom valves and were divided into two types, nonspherical cysts (Fig. 2a) and spherical cysts (Fig. 2b). Then the ratio of chrysophyte cysts to diatom valves and the ratio of nonspherical cysts to spherical cysts were calculated.



Scale bars indicate 5 µm

Fig. 2. Light micrographs of nonspherical chrysophyte cysts (A-D) and spherical chrysophyte cysts (E-H) from surface sediments of three lakes in Daye City

Data analysis

Mean values, standard deviations and ranges for each physicochemical parameter were calculated. From the metal concentrations, cumulative criterion unit (CCU) scores were calculated after the EPA (1986) as

$$CCU = \sum \frac{m_i}{c_i}$$

where m_i is the total recoverable metal concentration and c_i is the criterion value for the ith metal. CCU is a measure of total metal concentration and toxicity and it has already been applied to analyze the response of different organisms to metal pollution (Clements et al. 2000). The criterion value is based on U.S. EPA guidelines on critical concentrations, which, when exceeded, may harm aquatic organisms. For cadmium (Cd), manganese (Mn), zinc (Zn) and nickel (Ni) we followed the EPA criterion values of 2, 100, 120 and 470 µg l^{-3}, respectively.

For the two types of chrysophyte cysts, one-way ANOVAs were employed to find out whether there were any significant differences among samples from the three lakes. Detrended correspondence analysis (DCA), performed by the program CANOCO version 4.5 (ter Braak & Šmilauer 2002), was applied to the diatom percentage data to explore the characteristics of species change. Pearson's correlations were used to analyze the relationships between the algal indices (the ratio of cysts to valves, the ratio of nonspherical cysts to spherical cysts and diatom sample scores on the first and second DCA axes) and the CCU scores in order to identify the indices apparently sensitive to metal pollution. Furthermore, a similarity matrix was applied to the diatom percentage data to get a similarity index to conduct a cluster analysis based on Bray-Curtis to assess the changes of the taxonomic composition and group all these samples (Clarke & Warwick 2001).

SIMPER (similarity percentages) was used to find out the species that contributed the most to the similarities/dissimilarities within/between groups. The significance level of differences among these groups based on cluster analysis was done with the ANOSIM significance test (one-way Analysis of Similarities). Moreover, non-metric multidimensional scaling (MDS) ordination was used as a complement to the clustering to examine the patterns of diatom communities. These multivariate analyses were performed in *PRIMER Version 5.0* (Clarke & Warwick 2001).

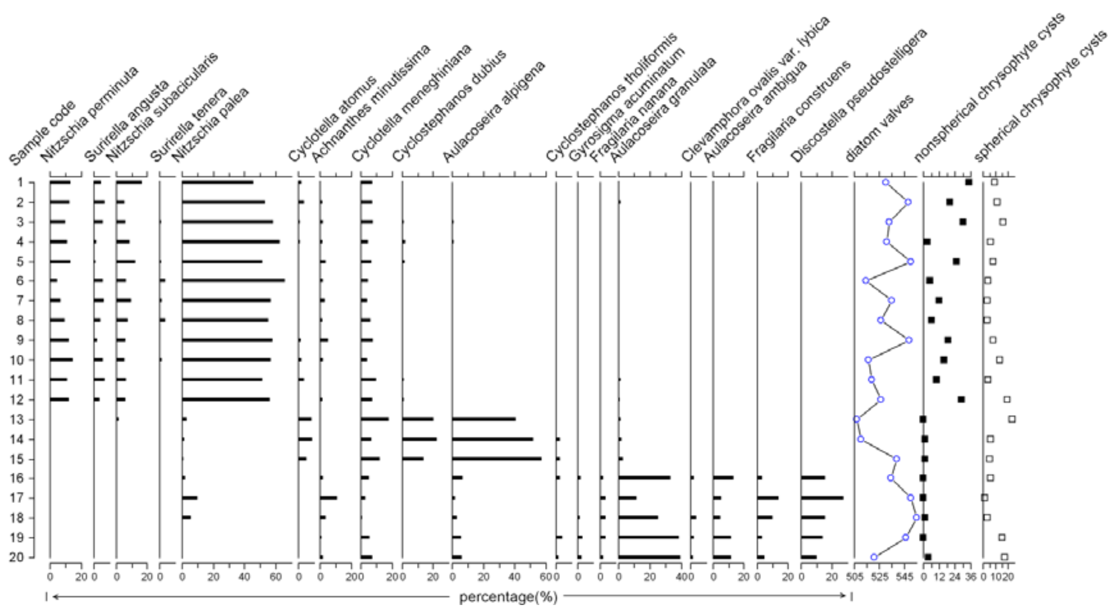
RESULTS

Metal concentrations

A summary of the chemical characterization of the three lakes with respect to water conductivity, pH and metal concentrations can be found in Table 1. The three lakes were characterized by conductivity from 424 to 1474 μS cm^{-1} and contamination by a variety of heavy metals. Metal concentrations ranged from <1 to 21 μg l^{-1} for Cd, <1 to 375 μg l^{-1} for Mn, 5 to 251 μg l^{-1} for Ni and <1 to 128 μg l^{-1} for Zn. Comparing among the three lakes, average values of heavy metals were much higher in Sanliqi Lake than in the two remaining lakes, and the concentrations of Cd and Mn in Sanliqi Lake were much higher than the EPA criterion values. The average scores for CCU were 9.26, 0.05 and 0.02 in Sanliqi Lake, Hongxing Lake and Yinjia Lake, respectively.

Siliceous algae composition

A total of 42 genera and 88 species were identified in the 20 diatom samples. The diatom communities were significantly different among the three lakes (Fig. 3). *Nitzschia palea* (Kützing) W. Smith was the dominant species in the samples from Sanliqi Lake, and *Nitzschia perminuta* (Grunow in Van Heurck) M. Peragallo, *Nitzschia subacicularis* Hustedt and *Surirella angusta* Schmidt were important taxa. In the samples from Hongxing Lake, the diatom assemblages were characterized by *Aulacoseira alpigena* (Grunow) Krammer and *Cyclostephanos dubius* (Fricke) Round. In samples from Yinjia Lake, diatom assemblages were dominated by *Aulacoseira granulata* (Ehrenberg) Simonsen, *Aulacoseira ambigua* (Grunow) Simonsen, *Fragilaria construens* (Ehrenberg) Grunow and *Discostella pseudostelligera* (Hustedt) Houk & Klee. Low abundance of *Cyclotella meneghiniana* Kützing was found at each sampling site. The ordination analysis revealed that the first and second axes in a DCA ordination accounted for 53.3% and 2.4% of the variance in diatom data, respectively. Sample scores on the first DCA axis captured a summary of species turnover that was useful for the interpretation of



Only the major taxa (species with ≥2% in at least one sample) are shown. The amounts of diatom valves and chrysophyte cysts counted in each sample are shown.

Fig. 3. Diatom assemblages in the sampling sites in the three lakes

changing patterns in the diatom communities.

The number of nonspherical cysts ranged from 0 to 35 in the samples, and the number of spherical cysts varied from 1 to 23 (Fig. 3). Results of one-way ANOVA revealed that nonspherical cysts showed significant differences among the three lakes (F=8.95, P=0.002). Spherical cysts did not differ significantly among the three lakes (F=0.232, P=0.795).

The relationships between the algal indices and the CCU scores

The ratio of cysts to valves ranged from 0.002 to 0.09, and the ratio of nonspherical cysts to spherical cysts varied from 0 to 4. Correlation analyses revealed that the ratio of cysts to valves and the ratio of nonspherical cysts to spherical cysts increased significantly with the CCU scores (R=0.609, P<0.01; R=0.757, P<0.01) (Fig. 4a-b). Diatom sample scores on the first and second DCA axes declined significantly with the CCU scores (R=-0.9, P<0.01; R=-0.629, P<0.01) (Fig. 4c-d). On the basis of Pearson coefficients, diatom sample scores on the first DCA axis were much more sensitive to total metal concentrations.

Diatom community configuration

On the basis of the Bray-Curtis dendrogram, diatom communities showed a marked difference among the three lakes (Fig. 5). Samples from each lake were distinct from those of other lakes, and three major cluster groups of samples were formed at a 59% similarity level. The largest cluster was comprised of 12 samples from Sanliqi Lake which were characterized by a level of similarity greater than 73%. Three samples from Hongxin Lake formed the second cluster with about 75% of similarity among their diatom assemblages. The third cluster included the remaining samples from Yinjia Lake with a level of similarity of about 59%. And three distinct clusters in the Bray-Curtis dendrogram were also supported by the MDS plot (Fig. 5). The MDS ordination plotted the samples with a stress value of 0.01, which indicated that the representation was of good quality (Clarke & Warwick 2001).

Based on square root-transformed percentage data of diatoms, SIMPER analysis figured out which species contributed the most to each assemblage group (Table 2). The average Bray-Curtis similarities in Group I, Group II and Group III were 83.74, 81.44 and 66.56, respectively. In Group I, four

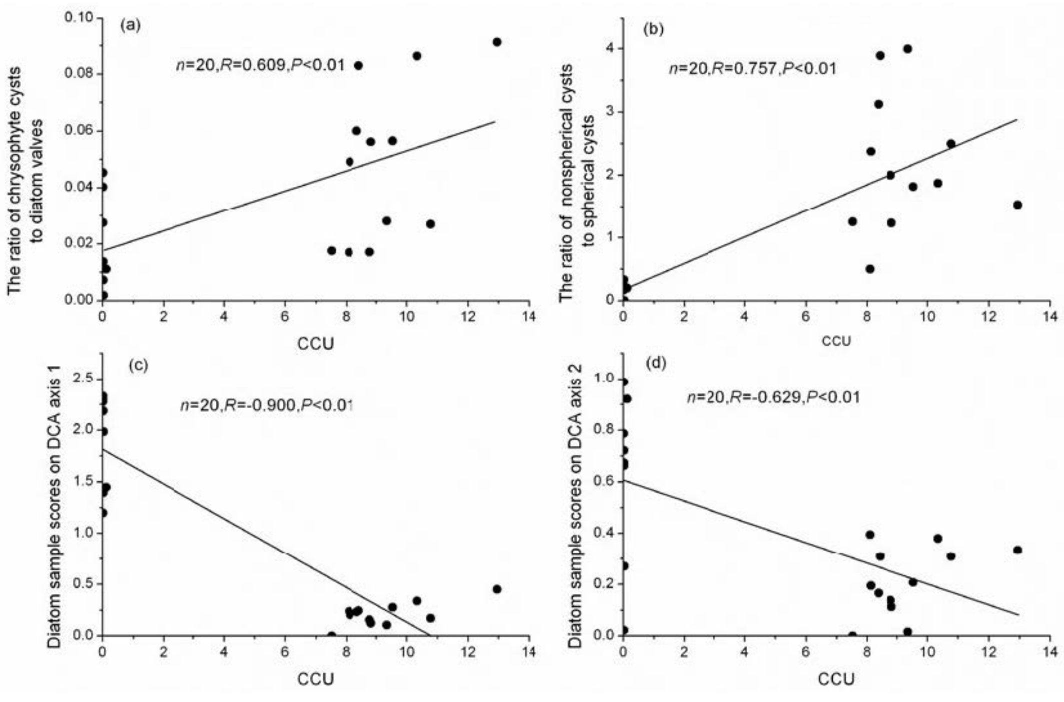


Fig. 4. Relationships between the cumulative criterion unit (CCU) values and algal indices (a-d: the ratio of chrysophyte cysts and diatom valves, the ratio of nonspherical cysts to spherical cysts, and diatom sample scores on the first and second DCA axes)

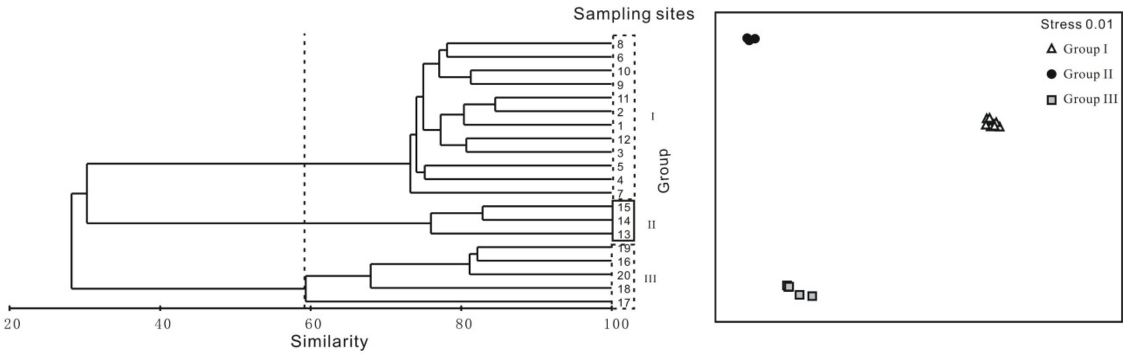


Fig. 5. Cluster dendrogram and MDS plot of diatom communities in three lakes in Daye City

Table 2

Main species in each assemblage group showed by SIMPER analysis

Species	Average abundance	Average similarities	Ratio	Contribution (%)	Cumulative contribution (%)
Group I					
N. palea	55.89	52.90	13.02	63.18	63.18
N. perminuta	10.91	9.29	3.36	11.10	74.28
N. subacicularis	8.17	6.38	4.95	7.62	81.90
C.meneghiniana	6.54	5.48	3.96	6.55	88.45
Average similarity	83.74				
Group II					
A.alpigena	49.98	44.47	6.68	54.61	54.61
C.dubius	18.73	15.86	4.30	19.47	74.08
C.meneghiniana	12.36	8.59	2.86	10.55	84.63
C.atomus	7.57	6.36	3.16	7.80	92.43
Average similarity	81.44				
Group III					
A.granulata	29.71	22.80	2.17	34.26	34.26
D.pseudostelligera	16.52	13.00	5.60	19.53	53.79
A.ambigua	9.60	7.17	2.14	10.77	64.56
F.construens	7.00	4.11	1.84	6.17	70.73
A.alpigena	4.98	3.71	2.19	5.58	76.31
Average similarity	66.56				

Note:
Ratio= average similarity/standard deviation
Only the major taxa (species with a contribution of ≥5% in each group) are displayed.

species with contributions exceeding 5% accounted for 88.45% of the total contributions, such as *N. palea* (63.18%), *N. perminuta* (11.1%), *N. subacicularis* (7.62%) and *C. meneghiniana* (6.55%). In Group II, four dominant species contributed 92.43% of the total Bray-Curtis similarity, with the individual contributions ranging from 54.61% to 7.8%. In Group III, five dominant species represented 76.31% of the contributions with the respective values scattered from 34.26% to 5.58% as shown in Table 2.

Similarly, the results of SIMPER analysis also indicated which species potentially contributed the most to the intergroup dissimilarity (Table 3). The average dissimilarity coefficients between Group I and Group II, Group I and Group III, and Group II and Group III were 86.63, 86.63 and 84.02, respectively. Applied to the whole set of abundance data with group as a factor, ANOSIM results showed that there was a strongly significant difference between the diatom communities (Global R=1.0, P=0.001) (Table 4). When the pair-wise tests were examined, it appeared that diatom assemblages were significantly dissimilar amongst three groups. Differences were highly significant for Group I vs. Group II (P=0.002), Group I vs. Group III (P =0.001) and Group II vs. Group III (P =0.018).

Table 3

Main species with their contribution to inter-group dissimilarity showed by SIMPER analysis

Species	Average abundance	Average abundance	Average Dissimilarity	ratio	Contribution (%)	Cumulative contribution (%)
Groups I and II	**Group I**	**Group II**				
N. palea	55.89	2.07	26.91	10.41	31.06	31.06
A. alpigena	0.41	49.98	24.78	7.06	28.61	59.67
C. dubius	0.70	18.73	9.02	4.78	10.41	70.08
N. perminuta	10.91	0.12	5.39	3.87	6.23	76.31
Average dissimilarity	86.63					
Groups I and III	**Group I**	**Group III**				
N. palea	55.89	3.86	26.04	8.28	30.06	30.06
A. granulata	0.60	29.71	14.58	2.77	16.83	46.89
D. pseudostelligera	0.00	16.52	8.26	2.88	9.54	56.43
N. perminuta	10.91	0.00	5.46	3.94	6.30	62.73
A. ambigua	0.06	9.60	4.77	2.56	5.51	68.24
Average dissimilarity	86.63					
Groups II and III	**Group II**	**Group III**				
A. alpigena	49.98	4.98	22.52	6.07	26.80	26.80
A. granulata	2.23	29.71	13.76	2.54	16.38	43.18
C. dubius	18.73	0.00	9.38	4.95	11.16	54.34
D. pseudostelligera	0.00	16.52	8.26	2.81	9.84	64.18
A. ambigua	0.00	9.60	4.80	2.51	5.72	69.90
Average dissimilarity	84.02					

Only the major taxa (species with a contribution of ≥5% in each group) are displayed

Table 4

Results of one-way ANOSIM with pairwise tests among groups (Global *R*=1.0 and *p*=0.001; Number of permutations: 999; Number of permuted statistics greater than or equal to Global *R*: 0)

Pair-wise tests	*R* statistic	Significance level (*p*-value)	Possible permutations	Actual permutations	Number ≥ observed
Group I vs. Group II	1.0	0.002	455	455	1
Group I vs. Group III	1.0	0.001	6188	999	0
Group II vs. Group III	1.0	0.018	56	56	1

Regarding the high ratios (similarity/standard deviation) and percent contributions, *N. palea* and *N. perminuta* can be considered as discriminating species of Group I. Similarly, *A. alpigena* and *C. dubius* can be regarded as indicative species of Group II. *A. granulata*, *D. pseudostelligera* and *A. ambigua* can be seen as characteristic species of Group III.

DISCUSSION

Responses of diatoms to metal pollution

The significant correlations between the CCU values and sample scores on the first two DCA axes confirmed the importance of heavy metals in shaping the diatom communities. The results suggested that diatoms should be sensitive to metal pollution at the community level. In addition, both SIMPER analysis and MDS ordination identified three significant, distinct clusters corresponding to different levels of heavy metal contamination. The results demonstrated that diatom communities showed high similarities within each group but significant dissimilarities between groups. The limited variation between habitats within each lake may explain the high similarity within each group.

Samples in Group I (from Sanliqi Lake) were characterized by high concentrations of Cd and Mn, having a mean CCU value of 9.26. Generally, CCU values between 2.0 and 10.0 are expected to cause significant mortality of sensitive species and alter biological community structure (Clements et al. 2000, Guasch et al. 2009). And species shifts from apparently metal-sensitive to metal-tolerant taxa have been recorded in diatom communities elsewhere as a result of exposure to increasing metal concentrations (Cattaneo et al. 2004, 2008; Duong et al. 2010). This is the conceptual basis of the Pollution-Induced Community Tolerance concept developed by Blanck et al. (1988). In Group I *N. palea* and *N. perminuta* were the discriminating species, and both of them were considered to be reliable species able to withstand metal-rich waters (Sabater 2000, Szabó et al. 2005). In laboratory experimental conditions, Duong et al. (2010) detected that diatom communities in younger biofilms exposed to Cd increased their tolerance to Cd by a highly significant development of *N. palea*. Similarly, Osman et al. (2004) found that *N. perminuta* showed more tolerance to the phytotoxicity of heavy metals (e.g., cobalt and nickel) than other species. Therefore, the joint presence and co-dominance of these taxa can be considered an indicator of metal contamination.

The CCU values of samples from the two remaining groups were much less than the criterion that was likely to cause harm to aquatic organisms (Clements et al. 2000). Therefore, algae communities in these two groups should be free of the impact of metal contamination. Diatom assemblages in the two groups were characterized by *C. dubius*, *D. pseudostelligera* and *Aulacoseira* species (mainly *A. alpigena*, *A. granulata* and *A. ambigua*). Absence or very low abundances of these taxa in Sanliqi Lake suggested that its metal-rich waters exceed the permissible limits for these taxa. Some of these species, such as *D. pseudostelligera* (Tuovinen et al. 2012), *A. ambigua* and *C. dubius* (Gold et al. 2002), have already been assessed as metal-sensitive by other studies.

C. dubius and *A. alpigena* were the indicative species in Group II, and both of them were typical nutrient-tolerant species in the Yangtze floodplain lakes (Dong et al. 2008; Yang et al. 2008; Chen et al. 2011, 2012). Both of their TP optima exceeded 130 $\mu g\ l^{-1}$ in the Yangtze floodplain diatom-phosphorus calibration dataset (Yang et al. 2008). Therefore, the co-dominance of these species was indicative of eutrophic conditions. Diatom assemblages in Group III were dominated by the species *A. granulata*, *D. pseudostelligera* and *A. ambigua*. These three species had relatively low TP optima (ca. 50-70 $\mu g\ l^{-1}$) in the Yangtze floodplain lakes (Yang et al. 2008). In addition, the presence of *C. meneghiniana* in all the sampling sites may be attributable to its tolerance of both metal pollution and eutrophication (Morin et al. 2008). However, the simultaneous occurrence of metal pollution and eutrophication may confound the unique influence of metal pollution on diatoms. Further studies should distinguish the relative contribution of metal pollution versus eutrophication to diatom species composition.

Responses of chrysophyte cysts to metal pollution

The significant correlations indicated that the ratio of cysts to valves and the ratio of nonspherical cysts to spherical cysts would be useful indices of metal contamination in this region. The development of chrysophyte cysts in the sedimentary record is generally indicative of undisturbed status, such as oligotrophic or low metal pollution conditions (Zeeb & Smol 2001). In three lakes in the Abitibi mining region (Canada), Cattaneo et al. (2008) detected that the ratio of cysts to valves declined with increasing levels of metal contamination. However, our study is apparently different from previous studies in that the ratio of cysts to valves was much higher in the polluted sampling sites than in the unpolluted ones. Nonspherical cysts were much more abundant in the metal-polluted sites (Sanliqi Lake), while spherical cysts did not differ significantly among the three lakes. Therefore, it can be inferred that the positive correlations between the ratios and the CCU scores should be attributed to the increases of nonspherical cysts in the metal-polluted sampling sites. It is suggested that nonspherical cysts are of a metal-tolerant type in this region. Heavy metals can bind to proteins and form a metal-protein complex, which can affect the enzymatic systems, growth and reproduction of the cell (Falasco et al. 2009). It is speculated that nonspherical cysts might be teratological forms of spherical cysts. Another possible explanation is that nonspherical cysts would belong to new chrysophyte species or varieties. And hence, continued studies on the taxonomic description and autecology of the nonspherical chrysophyte cysts should be carried out in order to better understand the implications of increased nonspherical chrysophyte cysts.

CONCLUSION

At the community level, diatom sample scores on the first DCA axis were much more sensitive to the CCU values. At the species level, *N. palea* and *N. perminuta* were metal-tolerant species, which withstood the rich-metal waters in the polluted sites at Sanliqi Lake. In unpolluted sites from Hongxing Lake and Yinjia Lake, diatom assemblages were characterized by *C. dubius*, *D. pseudostelligera* and *Aulacoseira* species (mainly *A. alpigena*, *A. granulata* and *A. ambigua*). These taxa might be sensitive to metal contamination but tolerant of nutrient enrichment.

In addition, nonspherical chrysophyte cysts can be regarded as a potential indicator of metal pollution. This study provides some clues for future metal pollution assessment through the use of siliceous algae in metal-polluted lakes.

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