Hydrobiologia (2007) 581:109–116
DOI 10.1007/s10750-006-0503-7

Variations in kinetics of alkaline phosphatase in sediments of eutrophic, shallow, Chinese lakes

Yiyong Zhou · Xiuyun Cao · Chunlei Song · Jianqiu Li · Guoyuan Chen · Liang Peng

Abstract Kinetics of alkaline phosphatase in sediments of a shallow Chinese freshwater lake (Lake Donghu) were investigated. Spatially, among 20 sites sampled, $V_{\max}$ and K_m values of alkaline phosphatase in surface sediments were higher in the zone adjacent to sites with the highest chlorophyll *a* concentrations. Vertically, there was a peak in $V_{\max}$ at intermediate sediment depths in addition to the expected maximum at the surface. Some inhibitors, such as $CuSO_4$, $ZnSO_4$ and Na_2WO_4, showed significantly different effects on kinetics of alkaline phosphatase in interstitial water and sediments. Moreover, alkaline phosphatase in interstitial water and sediments responded to Na_2WO_4 in different ways in Lake Taihu. These observations imply that the enzyme is immobilized in sediments, which became more stable with accelerated eutrophication, as suggested by highest alkaline phosphatase activity (APA) in sediments corresponding with highest water column chlorophyll *a* concentrations in Lake Donghu.

Keywords Alkaline phosphatase · Kinetics · Sediment · Eutrophication · Inhibitions · Immobilization

Introduction

Phosphorus often limits phytoplankton growth in freshwater systems, while organic phosphorus may account for larger part of phosphorus in lake sediments. For example, in a eutrophic lake (Lake Alserio, northern Italy), the results of the sedimentary phosphate fractionation showed that the most important P fraction was an organic fraction, and a digestion of the supernatant of the P-fraction bound to $CaCO_3$ allowed the detection of a large pool of org-P (Vicente et al., 2006). Therefore, regeneration of organic P in sediments is emphasized in aquatic ecology research. The phosphatase has been studied in relation to organic phosphorus decomposition in aquatic ecosystems (reviewed by Jansson et al., 1988). The relationship between alkaline phosphatase and substrate is of ecological interest because of implications for phosphorus cycling. However, most attempts to determine phosphatase in sediments have focused on activity. Kinetics of

---

Guest editors: B. Qin, Z. Liu & K. Havens
Eutrophication of shallow lakes with special reference to Lake Taihu, China

Y. Zhou (✉) · X. Cao · C. Song · J. Li ·
G. Chen · L. Peng
Institute of Hydrobiology, The Chinese Academy of Sciences, Wuhan 430072, China
e-mail: zhouyy@ihb.ac.cn

X. Cao · C. Song · G. Chen · L. Peng
Graduate School of the Chinese Academy of Sciences, Beijing 100039, China

phosphatase in sediments were influenced by discharge rate, water quality, concentration of dissolved orthophosphate and substratum in stream sediments (Marxsen & Schmidt, 1993), submerged macrophytes (Zhou et al., 2000) and P status in lake sediments (Zhou et al., 2001, 2002). Data on spatial and vertical variations in kinetics and properties of alkaline phosphatase in sediments are limited.

In this paper, alkaline phosphatase kinetics and its response to various inhibitors in a shallow freshwater lake (Lake Donghu) in China were described. The aims of the work were to establish whether enzyme regimes in sediments (1) were unique for the whole lake and depth, (2) shared a similarity in kinetic behavior with interstitial water, and (3) were related to trophic status in the lake.

Materials and methods

The experimental Lakes (Lake Donghu and Lake Taihu) are described elsewhere (Zhou et al., 2002; Qin et al., 2006). To evaluate the trophic state in Lake Donghu on an annual basis, surface water was collected monthly from sites 1, 12 and 21 (Fig. 1), from February 1997 through January 1999. To determine spatial variation of alkaline phosphatase kinetics, 20 sites were sampled in the largest basin (Fig. 1) in May 1995. Three sites in Lake Taihu were sampled in December 2004 to assay alkaline phosphatase activity (APA) in surface sediments. In May 1997, vertical samples were taken at five sites in Lake Donghu with Site 1 at the center and others (1A, 1B, 1C and 1D) located approximately 100 m away (Fig. 1).

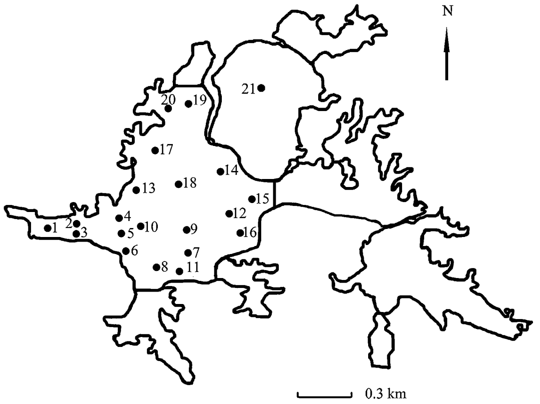

Fig. 1 The map of Lake Donghu, showing the location of the sampling sites

For spatial profiles, sediments were sampled by Peterson dredge. Sediment columns were obtained using a hand-driven stainless steel corer 50 cm long with an internal diameter of 3.5 cm. For depth profiles, columns were sliced at 4 cm intervals. Samples were transferred to the laboratory for analysis. Interstitial water was extracted by centrifugation at 3,000 rpm for 30 min.

Total chlorophyll *a* was measured by acetone extraction. Lake surface water samples (0.4–0.7 l) were filtered through a Whatman GF/C filter and absorbance measured at 663 and 750 nm in 1-cm path-length glass cuvettes after overnight extraction in acetone (90% v/v) (Golterman & Clymo, 1969).

APA assays used *p*-nitro-phenylphosphate (*p*NPP), which is hydrolyzed at 37°C by alkaline phosphatase to yield *p*-nitrophenol; with this system, enzyme activity is indicated by an increase in light absorbance (Sayler et al., 1979). Sediment samples were slurried in Tris buffer (pH 8.9). *p*NPP was added to slurries at eight final concentrations ranging from 0.05 mmol l^{-1} to 10 mmol l^{-1}. Samples were incubated at 37°C. After 1 h, 1.6 ml of slurry were centrifuged at 3,000 rpm. One ml of the supernatant was mixed with 4 ml 0.1 M NaOH to stop the reaction. Absorption of the final solution was measured at 400 nm. *p*NPP was added to reagent blanks after NaOH addition. APA was converted to absolute units using a standard curve based on enzymatically hydrolyzed *p*-nitrophenol. V_{max} and K_{m} values were estimated by fitting linearized Michaelis–Menten equations per the Lineweave–Burk plot. APA in surface sediments was determined with a final substrate concentration of 6.0 mmol l^{-1}.

Kinetics of total APA in interstitial water was determined using a method adapted from Berman (1970). Substrate was added at eight final concentrations ranging from 0.01 mmol l^{-1} to 1.8 mmol l^{-1}. APA in interstitial water was determined with a final substrate concentration of 0.3 mmol l^{-1}. All samples were run in triplicate.

Several inhibitors were added to sediments and interstitial water. Final concentrations of inhibitors are described in results tables. Fisher's least significant difference (LSD) test and independent-samples *T* test were used to make comparisons among treatment levels for the inhibitor addition, using the SPSS statistical package.

Results

Seasonally, chlorophyll *a* concentration in surface water was highest at Site 1 (Fig. 2). APA kinetics in sediments exhibited spatial heterogeneity (Table 1). Higher $V_{\max}$ values were found at Sites 2, 6, 9, 10 and 12, which are adjacent to Site 1. However, in the northeastern zone represented by Sites 14, 15 and 19, both $V_{\max}$ and K_m values were markedly lower.

There were variations in vertical distribution of APA kinetics at different sites (Fig. 3). At Sites 1B and 1D, $V_{\max}$ decreased with depth, while, at Sites 1A, 1, and 1C, there was a peak in the middle layers. At the same time, highest values for K_m were observed in the surface sediment at Sites 1B, 1C, and 1D, and in the middle layers at Sites 1A and 1.

Responses of APA kinetic parameters in sediments and interstitial water to various inhibitors were examined (Table 2). In sediments, $V_{\max}$ decreased significantly with Cu^{2+} concentration ($p < 0.01$), while in interstitial water, it significantly increased ($p < 0.01$). It also increased markedly ($p < 0.01$) with tungstate concentration in interstitial water. With Zn^{2+} added, $V_{\max}$ increased significantly in sediments ($p < 0.01$) but decreased at higher concentration in interstitial water ($p < 0.01$).

Table 1 The kinetic parameters of alkaline phosphatase in the sediments of Lake Donghu

Sampling sites	$V_{\max}$ (SD) (μmol G^{-1} H^{-1})	K_m (SD) (mmol l^{-1})
1	147.0 (2.0)	0.32 (0.02)
2	557.3 (50.6)	3.18 (0.09)
3	208.1 (1.0)	0.56 (0.04)
4	139.7 (8.1)	0.66 (0.02)
5	62.5 (3.1)	0.81 (0.04)
6	424.3 (24.3)	3.74 (0.67)
7	258.9 (49.5)	3.38 (0.27)
8	143.4 (21.4)	0.80 (0.12)
9	458.3 (11.64)	1.39 (0.04)
10	305.6 (30.0)	1.03 (0.23)
11	74.4 (1.8)	0.47 (0.03)
12	293.4 (27.7)	0.61 (0.07)
13	133.2 (12.8)	0.77 (0.16)
14	64.5 (17.6)	0.33 (0.08)
15	68.6 (6.6)	0.28 (0.06)
16	109.6 (3.8)	2.19 (0.16)
17	141.8 (31.8)	0.69 (0.09)
18	144.3 (21.8)	2.67 (0.56)
19	94.6 (8.4)	0.74 (0.05)
20	169.4 (17.0)	1.00 (0.12)

Responses of K_m values were also variable in both sediments and interstitial water. In sediments, it decreased significantly with lower Cu^{2+} concentration but increased with higher concen-

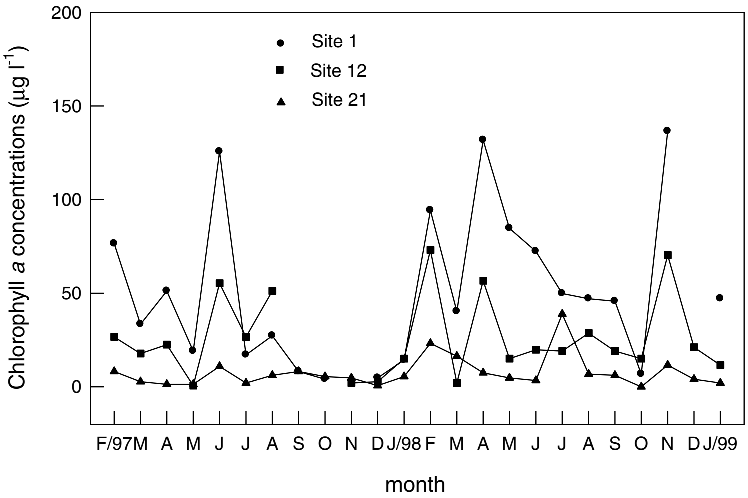

Fig. 2 Seasonal variation in chlorophyll *a* concentrations in surface water at three experimental stations in Lake Donghu

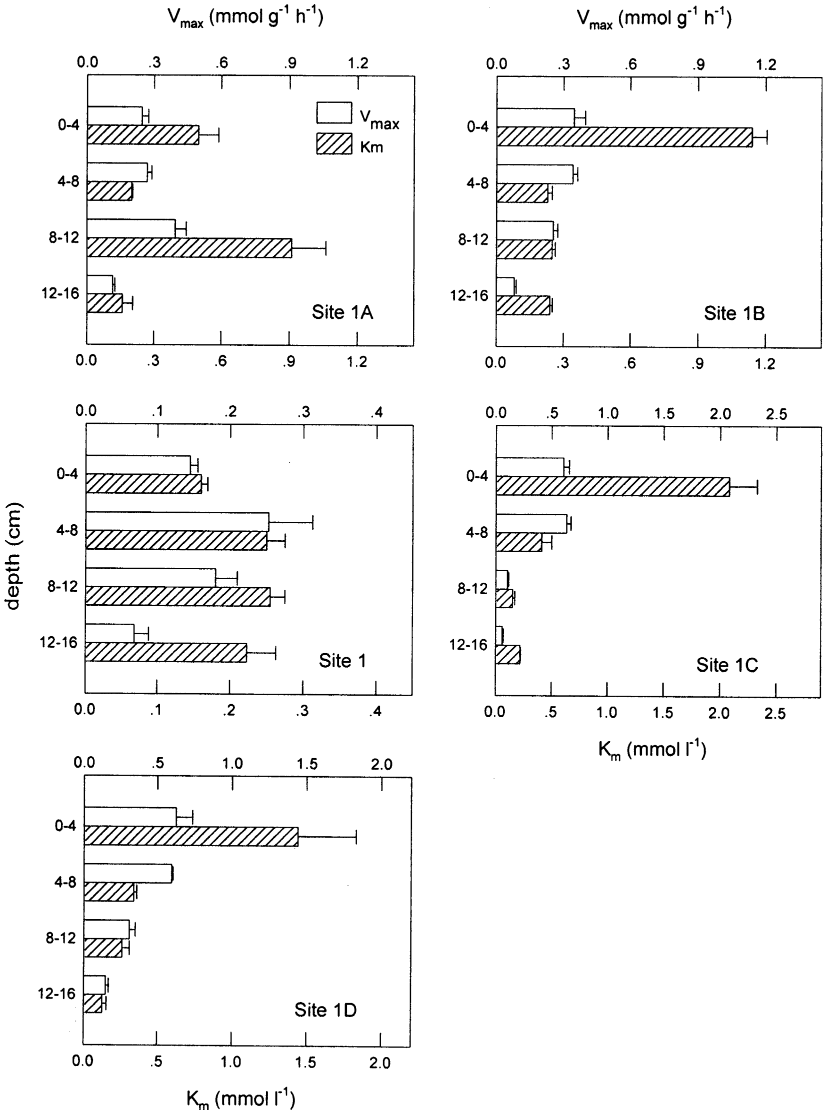

Fig. 3 Vertical distribution of V_{max} and K_m values of alkaline phosphatase activity (APA) in core sediments of Lake Donghu. Samples were taken on 12 May 1997

tration ($p < 0.01$). It also decreased significantly at lower tungstate concentration ($p < 0.05$) but increased significantly at higher concentration ($p < 0.01$) in sediments. While in interstitial water, it significantly decreased with tungstate concentrations ($p < 0.01$). With Zn^{2+} added, K_m significantly increased at lower concentration in sediments ($p < 0.05$), whereas in interstitial water, it greatly increased at higher concentration ($p < 0.01$).

In Lake Taihu, tungstate inhibited APA in sediment at Site T1 ($p < 0.01$) and interstitial water at Sites T1 and T3 ($p < 0.01$) at higher concentration. At Site T2, it stimulated APA in sediments at higher concentration, but inhibited APA in interstitial water at both concentrations ($p < 0.01$, Table 3).

Discussion

At Site 1, chlorophyll *a* in surface water was highest among experimental stations from 1997 to 1999 (Fig. 2), a situation commonly recorded in

Table 2 Effects of some inhibitors of alkaline phosphatase on kinetics of alkaline phosphatase activity (APA) in the surface sediments and the interstitial water in Lake Donghu

Sampling time	Inhibitors	Concentration (mmol l^{-1})	Surface sediments		Interstitial water	
			V_{max} (SD) (μmol G^{-1} H^{-1})	K_m (SD) (mmol l^{-1})	V_{max} (SD) (nmol l^{-1} min^{-1})	K_m (SD) (μmol l^{-1})
15 March, 1996	$CuSO_4$	0	157.5 (21.5)	3.25 (0.15)	51.9 (0.7)	4.11 (0.21)
		0.2	65.4 (6.6)	0.30 (0.06)	54.9 (0.5)	4.22 (0.29)
		2.0	52.5 (0.3)	4.78 (0.25)	105.5 (1.0)	1.65 (0.12)
		0	82.5 (0.8)	0.51 (0.02)	53.2 (0.9)	3.43 (0.23)
29 March, 1996	Na_2WO_4	0.05	90.8 (3.4)	0.40 (0.01)	67.9 (0.5)	1.81 (0.21)
		0.1	127.0 (37.7)	0.68 (0.02)	69 (0.5)	1.63 (0.11)
		0	143.7 (18.2)	0.54 (0.20)	46.7 (0.4)	1.59 (0.16)
8 February, 1996	$ZnSO_4$	0.2	291.9 (8.7)	1.07 (0.06)	44.6(0.6)	1.70 (0.10)
		2.0	287.4 (2.6)	0.48 (0.14)	38.6(0.2)	2.13 (0.08)

Table 3 Effects of tungsten on alkaline phosphatase activity (APA) in the surface sediments and interstitial water in Lake Taihu (Samples were taken on 14 December, 2004)

Sampling sites	Tungstate concentrations (mmol l^{-1})	APA in surface sediments (SD) (μmol G^{-1} H^{-1})	APA in interstitial water (SD) (nmol l^{-1} min^{-1})
T1	0	428.5 (6.0)	42.6 (1.5)
(31°32′4.6″ N, 120°13′22.6″ E)	0.16	157.0 (14.9)	35.2 (0.3)
T2	0	137.8 (16.3)	48.4 (1.0)
(31°32′1.7″ N,	0.08	144.3 (7.1)	39.8 (1.1)
120°13′18.7″ E)	0.16	309.2 (20.5)	35.9 (1.5)
T3	0	483.1 (53.5)	49.31(1.2)
(31°31′45.5″ N, 120°13′42.8″ E)	0.16	163.9 (13.4)	39.9 (0.6)

the lake (Zhou et al., 2002, 2004). Higher V_{max} and K_m values of APA in surface sediments were found at sites adjacent to Site 1, while, in the northeast, both were lower (Table 1). Significant inter-station variation in APA was observed in sediments of the Mandovi Estuary (Silva & Bhosle, 1990). Distinct areas of phosphatase activity also were seen in the Venice lagoon (Sabil et al., 1994). Variability of phosphatase activity in freshwater sediments may be related to heterogeneity within a site (Sayler et al., 1979). At sites along a eutrophication gradient in Nordruegensche Bodden (Baltic Sea, Germany), water column measurements revealed that turbidity, seston content, and chlorophyll *a* and inorganic nutrient concentrations increased from outer to inner parts of the Bodden. Sediment investigations confirmed this eutrophication gradient. Generally, hydrolytic enzyme activities increased with eutrophication (Koester et al., 1997). In Lake Donghu, the V_{max} values in sediment increased during the summer, in conjunction with lower K_m values in interstitial water that suggests a higher affinity for the substrate. The accumulation of organic matter in the sediment could be traced back to the breakdown of the algal bloom, which may stimulate APA with higher kinetic efficiency, by a combination of the higher V_{max} in sediments plus lower K_m values in interstitial water, in summer (Zhou et al., 2002). Our results highlight the connection of the kinetics of APA in sediments with phytoplankton abundance in surface water, indicating the induction of enzyme by organic matter.

The V_{max} of alkaline phosphatase was highest at the sediment surface (Sites 1B and 1D, Fig. 3). APA also was highest at the surface of marine (Kobori & Taga, 1979) and lake sediments (Sinke et al., 1991). In three lakes in Finland, the eleven hydrolytic enzyme activities were high into deep sediment layers indicating potential for turnover of organic matter in the permanently anoxic zones (Hakulinen et al., 2005). In the present study, there was a peak in the middle layers (Sites 1A, 1, and 1C, Fig. 3), showing an additional dimension for the degradation of organic matter mediated by alkaline phosphatase in lake sediments.

Spatial and vertical variations in $V_{\max}$ and K_m values of APA in sediments were similar (Table 1; Fig. 2). This may be interpreted with reference to organic matter and orthophosphate enrichment. At first, organic matter may stimulate $V_{\max}$ of APA in sediments. Surface sediment profiles were taken from the deepest part of six central Finnish lakes representing six different trophic states, and each profile was divided into three layers. Phosphatase activity and levels of organic substances were highest in the top layer of every sediment profile. Microbiological phosphate mineralization from organic substances may determine the internal phosphorus load in these lakes (Matinvesi & Heinonen-Tanski, 1992). In fish pond, inorganic phosphate added to the sediment scarcely restricted phosphatase activity. At the same time the enrichment with organic phosphorus compounds highly raised enzyme activity (Olah & Toth, 1978). In marsh sediments, the $V_{\max}$ of acid phosphatase followed the same trend as in situ activity. Sediment salinity and pH were negatively correlated with the enzyme activity, while soil organic matter content, clay content and sediment organic P were positively correlated (Huang & Morris, 2005). In tidal freshwater habitats adjacent to the Cooper River, acid phosphatase activity was highly correlated with the organic matter content of the sediment. The $V_{\max}$ of all phosphatases increased along the successional gradient. Trends in phosphatase activity and $V_{\max}$ correlated positively with plant biomass and negatively with concentrations of soluble reactive phosphorus in porewater, sediment extractable phosphorus, and total phosphorus (Huang & Morris, 2003); Secondly, organic matter may alter K_m of APA in sediments. Alkaline phosphatase kinetics in sediment associated with cage culture of *Oreochromis niloticus* was studied near Site 1 in Lake Donghu. Both $V_{\max}$ and K_m increased with the addition of fish feces (Zhou et al., 2001). A possible explanation is that humic substances would adsorb the substrates of enzymes. In the consecutive sapropel layers collected from bathyal sediments of the eastern Mediterranean Sea, the determination of exoenzyme activity with fluorescently labeled substrate analogues was impaired by the strong adsorption of up to 97% of the enzymatically liberated fluorophores to the sediment particles. High activities of aminopeptidase and alkaline phosphatase were detected even in a 124,000-year-old sapropel layer, whereas the activity of β-glucosidase was low in all layers. It had been assumed that the organic matter which constitutes the sapropels is highly refractory. Since a high adsorption capacity was determined not only for the low-molecular-weight compounds but also for DNA, the extraordinarily strong adsorption of structurally different substrates to the sapropel matrix appears to be the major reason for the long-term preservation of biodegradable carbon in this environment (Coolen & Overmann, 2000). This strong adsorption would weaken the affinity of substrates for enzymes, leading to increase in apparent K_m values. In addition, orthophosphate may be a competitive inhibitor for alkaline phosphatase, causing a significant increase in K_m values. Spatially, orthophosphate concentrations in interstitial water were highest at Site 1 among experimental stations in Lake Donghu in 1995 to 1996 (Zhou et al., 2002), it could act as a competitive inhibitor of extracellular phosphatase in lake (Chrost & Overbeck, 1987).

Alkaline phosphatase in sediments showed different responses to various inhibitors from those observed in interstitial water. For example, in interstitial water of lake Donghu, $V_{\max}$ increased with copper sulphate addition at both lower ($p < 0.05$) and higher ($p < 0.01$) concentrations (Table 2). Increased phosphatase activities were noted in green algae isolated from Lake Kinneret and pretreated with intermediary Cu^{2+} concentrations (Wynne & Pieterse, 2000). However, the enzyme was inhibited by zinc at higher concentration ($p < 0.01$, Table 2). Zhang et al. (2001) studied the kinetics of inactivation of alkaline phosphatase from green crab by zinc ions. The enzyme reversibly and quickly bound

Zn^{2+} and then underwent a slow, reversible inactivation and slow conformational change. In sediments, V_{max} and K_m decreased with Cu^{2+} at lower concentration ($p < 0.01$, Table 2). Accordingly, APA was affected by Cu^{2+} in soil polluted by heavy metals (Kuperman & Carriero, 1997; Kunito et al., 2001). The influence of Cu^{2+} and Zn on the activity and kinetics of acid phosphatase immobilized by two soil clays, kaolin or goethite, indicated that Cu decreased V_{max} of the enzymes, but increased affinity of the enzymes for the substrate (Huang & Shindo, 2001). In this context, alkaline phosphatase in sediment and interstitial water showed different kinetics with different inhibitors, implying enzyme immobilization in sediment. Concurrently, various inhibition patterns by Zn were observed for free and immobilized acid phosphatase at different pH values, likely related to the degree of deactivation by Zn (Huang & Shindo, 2000). In soils, kinetics of the immobilized enzyme conformed to Michaelis–Menten, and V_{max} was lower and K_m higher than those of the free enzyme (Rao & Gianfreda, 2000). Immobilized enzymes were more resistant to environmental changes compared to their soluble counterparts (Sabil et al., 1993). In Venice lagoon sediments within a shallow water area, phosphatase activity was prolonged by its insolubility (Sabil et al., 1994).

Shortly, there is a correlation between higher sediment APA in term of kinetics and chlorophyll *a* concentration in water of Lake Donghu. The kinetic properties of phosphatase and their distributions in sediments were linked to the process of lake eutrophication, which might become more stable upon immobilization in sediments with accelerated eutrophication.

Acknowledgements This work was supported by the grant (KZCX1-SW-12-II-02-02) from the Chinese Academy of Sciences (CAS), National Basic Research Program of China (2002CB412300) and funding from the National Science Foundation of China (39170165, 39670149 and 20177033). We also obliged to the funds (2002AA601013). The authors also gratefully acknowledge the support of the Donghu Lake Ecosystem Experimental Station of CAS for the help in sampling collection.

References

Berman, T., 1970. Alkaline phosphatase and phosphorus availability in Lake Kinneret. Limnology and Oceanography 15: 663–674.

Chrost, R. J. & J. Overbeck, 1987. Kinetics of alkaline phosphatase activity and phosphorus availability for phytoplankton and bacterioplankton in Lake Plusssee (North German eutrophic lake). Microbial Ecology, New York, NY 13(3): 229–248.

Coolen, M. J. & J. Overmann, 2000. Functional exoenzymes as indicators of metabolically active bacteria in 124,000-year-old sapropel layers of the eastern Mediterranean Sea. Applied and Environmental Microbiology 66(6): 2589–2598.

de Vicente, I., K. Cattaneo, L. Cruz-Pizarro, A. Brauer & P. Guilizzoni, 2006. Sedimentary phosphate fractions related to calcite precipitation in an eutrophic hardwater lake (Lake Alserio, northern Italy). Journal of Paleolimnology 35: 55–64.

Golterman, H. L. & R. S. Clymo, 1969. Method for chemical analysis of fresh water. In IBP Handbook No. 8. Blackwell Sci. Publ., Oxford, 1–188.

Hakulinen, R., M. A. Kahkonen & M. Salkinoja-Salonen, 2005. Vertical distribution of sediment enzyme activities involved in the cycling of carbon, nitrogen, phosphorus and sulphur in three boreal rural lakes. Water Research 39: 2319–2326.

Huang, Q. & H. Shindo, 2001. Comparison of the influence of Cu, Zn, and Cd on the activity and kinetics of free and immobilized acid phosphatase. Soil Science and Plant Nutrition 47: 767–772.

Huang, Q. & H. Shindo, 2000. Inhibition of free and immobilized acid phosphatase by zinc. Soil Science 165: 793–802.

Huang, X. & J. T. Morris, 2003. Trends in *phosphatase* activity along a successional gradient of tidal freshwater marshes on the cooper river, South Carolina. Estuaries 26(5): 1281–1290.

Huang, X. & J. T. Morris, 2005. Distribution of *phosphatase* activity in marsh *sediments* along an estuarine salinity gradient. Marine Ecology Progress Series 292: 75–83.

Jansson, M., H. Olsson & K. Pettersson, 1988. Phosphatases; origin, characteristics and function in lakes. Hydrobiologia 170: 157–175.

Kobori, H. & N. Taga, 1979. Occurrence and distribution of phosphatase in neritic and oceanic sediments. Deep-Sea Research 26A: 799–808.

Koester, M., S. Dahlke & L.-A. Meyer-Reil, 1997. Microbiological studies along a gradient of eutrophication in a shallow coastal inlet in the southern Baltic Sea (Nordruegensche Bodden). Marine Ecology Progress Series 152: 27–39.

Kunito, T., K. Saeki, S. Goto, H. Hayashi, H. Oyaizu & S. Matsumoto, 2001. Copper and zinc fractions affecting microorganisms in long-term sludge-amended soils. Bioresource Technology 79: 135–146.

Kuperman, R. G. & M. Carriero, 1997. Soil heavy metal concentrations, microbial biomass and enzyme activities in a contaminated grassland ecosystem. Soil Biology and Biochemistry 29: 179–190.

Marxsen, J. & H.-H. Schmidt, 1993. Extracellular phosphatase activity in sediments of the Breitenbach, a Central European mountain stream. Hydrobiologia 253: 207–216.

Matinvesi, J. & H. Heinonen-Tanski, 1992. Biodegradable substances in lake sediments and their relation to sediment microbiological activity and phosphorus recycling. Aqua Fennica 22: 193–200.

Olah, J. & E. O. Toth, 1978. The function of alkaline *phosphatase* enzyme in the phosphorus cycle of fertilized fishponds. Aquacult Hung 1: 15–23.

Qin, B., P. Xu, Q. Wu, L. Luo & Y. Zhang, 2006. Environmental issues of Lake Taihu, China. Hydrobiologia.

Rao, M. A. & L. Gianfreda, 2000. Properties of acid phosphatase-tannic acid complexes formed in the presence of Fe and Mn. Soil Biology and Biochemistry 32: 1921–1926.

Sabil, N., A. Cherri, D. Tagliapietra & M.-A. Coletti-previero, 1994. Immobilized enzymic activity in the Venice Lagoon sediment. Water Research 28: 77–84.

Sabil, N., D. Tagliapietra & M.-A. Coletti-Previero, 1993. Insoluble biodegradative potential of the Venice Lagoon. Environmental Technology 14: 1089–1095.

Sayler, G., S. M. Puziss & M. Silver, 1979. Alkaline phosphatase assay for freshwater sediments: Application to perturbed sediment system. Applied and Environmental Microbiology 38: 922–927.

Silva, C. D. & N. B. Bhosle, 1990. Phosphorus availability and phosphatase activity in the sediments of Mandovi estuary, Goa. Indian Journal of Marine Sciences 19: 143–144.

Sinke, A. J. C., A. A. Cornelese & T. E. Cappenberg, 1991. Phosphatase activity in sediments of the Loosdrecht lakes. Internationale Vereinigung für Theoretische und Angewandte Limnologie 24: 719–721.

Wynne, D. & A. J. H. Pieterse, 2000. The effect of copper on photosynthesis, nitrate reductase and phosphatase activities in Lake Kinneret phytoplankton. Limnology and Lake Management 55: 581–593.

Zhang, R. Q., Q. X. Chen, R. Xiao, L. P. Xie, X. G. Zeng & H. M. Zhou, 2001. Inhibition kinetics of green crab (*Scylla serrata*) alkaline phosphatase by zinc ions: A new type of complexing inhibition. Biochimica et Biophysica Acta 1545: 6–12.

Zhou, Y., J. Li & Y. Fu, 2000. Effects of submerged macrophytes on kinetics of alkaline phosphatase in Lake Donghu. I. Unfiltered water and sediments. Water Research 34: 3737–3742.

Zhou, Y., J. Li, Y. Fu & M. Zhang, 2001. Kinetics of alkaline phosphatase in lake sediment associated with cage-culture of *Oceochrolmis miloticus*. Aquaculture 203: 23–32.

Zhou, Y., J. Li, Y. Fu & M. Zhang, 2002. Temporal and spatial variations in kinetics of alkaline phosphatase in sediments of a shallow Chinese eutrophic lake (Lake Donghu). Water Research 36: 2084–2090.

Zhou, Y., J. Li, C. Song & X. Cao, 2004. Variations and possible source of potentially available phosphorus in a Chinese shallow eutrophic lake. Journal of Freshwater Ecology 19: 87–96.