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Stratification of alkaline phosphatase in sediments of two urban lakes and its effect on phosphorus cycle

Zhuo-Ying Xia [a,b,*], Yi-Yong Zhou [b], Fang Chen [b], Chun-Lei Song [b], Jiang-Qiu Li [b]

[a] Zhangzhou Normal University, Chemical and Environmental Science Department, Fujian Zhangzhou 363000, People's Republic of China
[b] Institute of Hydrobiology, The Chinese Academy of Sciences, Wuhan 430072, People's Republic of China

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ABSTRACT

Phosphorus loadings in sediments play an important role in lake eutrophication and the progress of its recovery. The phosphorus release is controlled by physical, chemical and biological mechanisms. Alkaline phosphatase catalyzes remineralization of organic phosphorus and then it may be an important factor accelerating phosphorus cycling in sediments.

In this paper, distributions, properties and function of alkaline phosphatase with depths in sediments of two urban lakes were discussed. Alkaline phosphatase activity (APA) in the sediments of Lake Yuehu decreased with the sediment depth. APA in sediments of Lake Yuehu was, mostly, inhibited by Phe and L-Cys; and inhibiting ability of Phe could be stronger than L-Cys. APA in deeper layer (20–30 cm) of sediments was more sensitive to the inhibitors than other layers, but range of variation in APA was most wide in the subsurface layer (10–20 cm). All the facts implied that alkaline phosphatase occurred in various forms (isoenzymes). APA in the sediments with different depths of Lake Donghu responded Phe differently. Reacted with Phe and incubated for 1 day, the amounts of SRP released by these sediments varied correspondingly. SRP on the overlying water in deeper layers (5–10 cm and 15–20 cm) of Site *T*1 was higher than that in surface layer (0–5 cm) of the same site, 1 day after incubation.

Hence, the SRP release resulted, at least partially, from the hydrolysis of some liable organic phosphorus mediated by APA. Alkaline phosphatase in lake sediments plays an important role in the release of internal phosphorus loadings and eutrophication. A possible explanation for the sensitivity at deeper layers could be another active region of hydrolysis by alkaline phosphatase from organic phosphorus, which added a new dimension in phosphorus cycling mediated by some biochemical mechanisms.

1. Introduction

Phosphorus loadings in sediments play an important role in lake eutrophication and the progress of its recovery. The phosphorus release is controlled by physical, chemical and biological mechanisms [15]. Alkaline phosphatase catalyzes remineralization of organic phosphorus and then it may be an important factor accelerating phosphorus cycling in sediments. Phosphatase is an important factor in accelerating eutrophication in Song et al. [21]. The phosphatase enzymes have been studied as a factor in releasing o-P from org-P phosphorus compounds in aqueous systems by many investigators, as reviewed by Jansson et al. [7]. Mineralization of organic phosphate was controlled by enzymes in sediments, especially, effected by alkaline phosphatase obviously [2,10]. Marxsen et al. proposed extracellular phosphatase activity was high in the streambed sediments, which probably contribute significantly to the flux of phosphorus in sediment by hydrolyzing phosphomonoesters, making free phosphate available to the sediment microorganisms [13]. Based on the regulation of alkaline phosphatase synthesis, its secretion and stability, Li et al. proposed the use of alkaline phosphatase activities for an assessment of the P-status [11]. Degobbis et al. showed that the important role of alkaline phosphatase (APA) in phosphorus regeneration, and APA appears to be a good indicator of the degree of nutrient regeneration occurring in surface sediments on a global basis [5].

Hence, the study of activity and stability of alkaline phosphatase in benthic layer of lakes and phosphorus form of sediment would be helpful for understanding the mechanism of internal loading cycling in lakes.

This paper discussed stratification of alkaline phosphatase in sediments of two urban lakes (Lake Yuehu and Lake Donghu in Wuhan City, China) and its effect on phosphorus cycle. The objectives of this study are to test distributions of alkaline phosphatase, respond to its different inhibitors and function in internal phosphorus loading, at spatial and vertical scales.

* Corresponding author at: Zhangzhou Normal University, Chemical and Environmental Science Department, Fujian Zhangzhou 363000, People's Republic of China.
E-mail address: yingzixzy@163.com (Z.-Y. Xia).

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2. Site description

Lake Donghu (114°23′E, 30°33′N) and Lake Yuehu (114°14′–114°15′E, 30°33′N) are on the alluvial plain of the middle basin of the Changjiang (Yangtze) River. Lake Donghu is on the northeastern outskirts of Wuchang, Wuhan City, China, which is the largest lake in the city. It is a component of a large drainage system with a catchment area of 187 km^2. The lake itself is composed of several basins separated by artificial dikes, with a total surface area of 32 km^2. Its mean depth is 3–4 m with a maximum of 4.5 m. Because of the shallow depth and strong local winds, thermal stratification is usually absent or obscure. The study areas are located in Tuanhu Basin (station I and II, 2.5–3.0 m deep). Station I is close to several drainages from residential area in Shuiguohu community, but station II is near the center of basin. The muddy lake bottom is flat and sparsely covered with hydrophytes. Lake Yuehu is on the northwestern urban area of Hanyang, Wuhan City. Western of the lake is situated at the foot of Heshan hill, eastern of which is the northwest corner of Guishan hill. Its south is close to Meizi hill and beside Guqin Platform, northern of which is Hanjiang River. The length of the lake is 3150 m, width of which is 450 m. It is a component of a large drainage system with a catchment area of 1.42 km^2. Its mean depth is 2 m, which is a freshwater lake. Map of studied lakes was shown in Fig. 1, and the sampling sites of Lake Yuehu were shown in Table 1.

3. Methods

In December 2003, December 2004 and March 2005, sediment columns were obtained using a hand-driven stainless steel corer 50 cm long with an internal diameter of 8 cm. For depth profiles, the columns were sliced at 5-, 10-, 15-, 20-, 25- or 30 cm intervals (in December 2003, at 10-, 20-, 30 cm intervals). Twelve cores were collected at each site, and grouped at random into three replicates. Then, the corresponding sliced layers were mixed thoroughly. The sediments were immediately transferred to the laboratory for analysis.

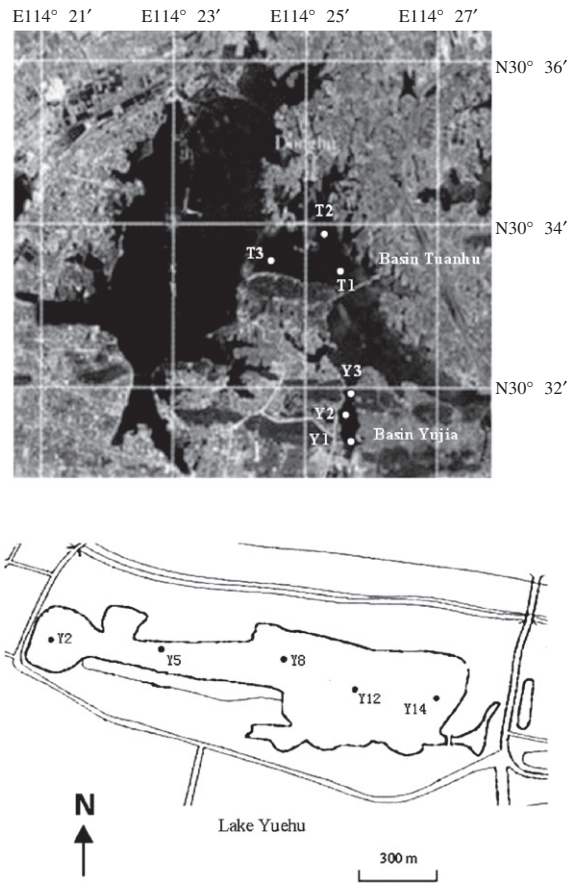

Fig. 1. Maps of Lake Donghu and Lake Yuehu (showing the several sampling sites).

Alkaline phosphatase activity (APA) assays used the model substrate p-nitro-phenylphosphate (pNPP), hydrolyzed at 37 °C for 1 h by alkaline phosphatase to yield the product p-nitrophenol; with this system, enzyme activity was indicated by an increase in absorbance at 410 nm. The modified procedure followed that described by [19]. Sediments of 0.5 g were prepared to even fluid with 2 ml Tris buffer (pH = 7.6) which containing 0.075 mol/L NaN_3, then filtered by gauze. 2 ml filtered fluid was placed in polypropylene centrifugal tube. The substrate (pNPP) was added to the sediment fluid with 6 m mol/L, then the samples were incubated at 37 °C. After 1 h, slurry was mixed with 2 ml of 0.5 M NaOH and 0.5 ml of 0.5 M $CaCl_2$ to stop the reaction. The supernatant was extracted from the sediment by centrifugation at 3000 rpm for 10 min. The absorption of the final solution was measured at 410 nm using a spectrophotometer (Model 721) made by the Shanghai Third Factory of Analytical Instrument, China. pNPP was added to reagent blanks after incubation for 1 h. APA was converted to absolute units using a standard curve based on enzymatically hydrolyzed p-nitrophenol.

For assaying the enzyme responded to inhibitors, known inhibitors such as L-Cysteine (Cys) and L-Phenylalanine (Phe) were added to reaction system of enzyme activity assayed. Methods were the same as the above, except dissolving inhibitors in Tris buffer (pH = 7.6), with the concentration of 2.5 m mol/L L-Cys and 20 m mol/L L-Phe. Activity of the enzyme with inhibitors was showed for λ%, percentage of APA with inhibitors in the control.

The method of simulation experiment on phosphorus release at sediment–water interface was followed: 50 g slurries weighed were placed on 500 ml wild-mouth bottles possessing stopper, the depth of which was 1cm. Then distilled water having Phe (20 m mol/L) and without Phe were added to bottles respectively, each setting three replicates. Samples were oscillated for 30 mins, then put still. After 12 h, the supernatant was discarded. Three hundred ml respective lake water without any treatment was added to bottles slowly, then bottles simulated were incubated at 37 °C. Superficial water were taken from respective bottles at 1, 4, 7 days after incubation, for assaying total phosphorus (TP), and soluble reactive phosphorus (SRP). Following the experiment groups referred the groups added Phe, and the control groups referred the groups without Phe. The concentrations of SRP in superficial water were measured using the [16] procedure, after all water samples were filtered through pre-washed 0.45 μm Millipore filters [9].

4. Results and discussion

4.1. Distribution of APA in sediments (Lake Yuehu) of different depths

Sediments were sampled on March 2005 in Lake Yuehu for APA analysis. APA decreased with the sediment depth and peaked in the superficial layers in site Y2, Y8, Y12, Y14 (Fig. 2). This is consistent with the results of [10] who observed that alkaline phosphatase activity was generally highest at the surface of marine sediments. A similar decrease in APA along depth of sediment in lake was noted by Sinke et al. [20] and Newman and Reddy [17]. That showed phosphorus cycle was active in sediment–water interface. But APA had another peak in 10–15 cm in site Y5. That was identical with the results of [3] who observed that APA varied in different