

## Phosphorus accumulation and eutrophication in feed-supply freshwater fishponds

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**Abstract:** The rapid growth and intensification of freshwater fishery can cause imbalances between phosphorus (P) input in feed and its output in produce. This aquaculture can result in enriching exogenous P in fishponds and, consequently, accelerates the process of eutrophication. To assess relations among input, accumulation, release of P and as a consequence degrading water quality in terms of chlorophyll-*a* (Chl-*a*) in freshwater fishponds, fourteen fishponds with feed supply, nine fishponds without feed supply, and five non-fish ponds in Shaoxing Plain, southeast China were selected for comparing P accumulation in their waters and surface sediments. Surface sediment samples were collected from each pond to evaluate their total P, water soluble P, Olsen P, algal available P, and P fractions. Water samples were also collected from the ponds to measure concentrations of dissolved P and Chl-*a*. Total P in the sediments ranged from 0.88 to 1.73 g/kg in the fishponds with feed supply, that in the non-fish ponds ranged from 0.47 to 0.86 g/kg. Organic P, accounted for 23% to 60% of total P in the sediments, was an important P fraction and increased linearly with increasing organic matter. Long-term application of feeds resulted in increased P availability in the bottom sediments and degradation of water quality in the freshwater fishponds. Compared with non-fish ponds, sediments from the feed-supplied fishponds contained considerably higher Olsen P, algal available P, and water soluble P. Higher proportions of the labile P (NH<sub>4</sub>Cl-P) and potentially labile P (NaOH-IP) were also found in the sediments from the fishponds. High solubility of P in the sediments resulted in elevation of P and chlorophyll-*a* concentration in the pond water. The dissolved P concentration in the pond water increased in the order of non-fish ponds (12 µg/L) < fishponds without feed supply (24 µg/L) < fishponds with feed supply (66 µg/L). Linear correlations between concentrations of total P, Olsen-P, algal available P, water-soluble P and P concentration in saturation extracts in the sediments and dissolved P in the pond water indicated that there was a buffering action of the sediment constituents on the dissolved P.

**Keywords:** eutrophication; freshwater fishery; phosphorus fraction; water quality

### Introduction

Eutrophication has many adverse effects on aquatic ecosystems. Perhaps the most visible consequence is the proliferation of algae, which can turn water a turbid green and coat shallower surfaces with “pond scum”. The increased growth of algae can degrade water quality and interfere with use of the water for fisheries, industry, agriculture, and drinking. Freshwater eutrophication has been a growing problem for decades and is widespread and rapidly expanding in lakes and rivers as well as coastal seas all over the world. The nutrient status of many water bodies has increased dramatically over the past 30 years in response to widespread agricultural intensification. The availability of phosphorus for the growth of phytoplankton in water bodies is generally believed to be the controlling factors in eutrophication. It is, in fact, the dominant control on primary production in some lakes and coastal ecosystems (Lean, 1973). In general, two main sources contributed to the nutrient status and budgets of water bodies: (1) loading from diffuse and point sources, and (2) internal loading originating from the sediments.

Freshwater aquaculture has become a large, world industry, producing about  $28.5 \times 10^6$  t of fish, shrimp, shellfish, and aquatic plants (FAO, 1996).

Intensive aquaculture systems rely on large applications of feeds containing 25% to 40% crude protein to support efficient fish and shrimp growth (Boyd and Tucker, 1995). Thus, the aquaculture may be a significant source of P. Only 25% to 30 % of the N and P applied in feeds are recovered in the cultured fish at harvest and much of the rest is converted to NH<sub>3</sub> or enters the water (Boyd and Tucker, 1995, 1998). Both feces excreted by fish and uneaten feeds resulted in P accumulation in the lakes or ponds. Excessive application of feeds can result in degradation of water quality (Boyd and Tucker, 1998). Bottom sediments were considered as efficient sinks for the accumulated P. Under certain conditions, however, the accumulated P in sediments could be released again to overlying waters (Lennox, 1984). The P cycling and release in the sediments depend upon P speciation in the sediments (Paludan and Morris, 1999). Therefore, measurement of P fractions in bottom sediments can not only indicate accumulation of P in the water-sediment system, but also reflect potential eutrophication of the waters.

In recent decades, freshwater aquaculture has become one of important commercial fisheries in China (Liu and Cai, 1997). With development of the fishery and heavy application of feeds, the yield of fish in a unit of area increased rapidly. Meanwhile, a large amount of waste matter was brought into the water

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directly. However, little is known about accumulation and bioavailability of P in bottom sediments of freshwater fishponds. Therefore, the objectives of this study were to: (1) determine accumulation and forms of P in fishpond's surface sediments from Shaoxing County, Zhejiang Province, China; and (2) evaluate relationship between P accumulation in the sediments and water quality. The study was undertaken because the occurrence of eutrophication in fish ponds in the area was considered to be due to P pollution.

## 1 Materials and methods

### 1.1 Study site

The study area is located on plain region of the Shaoxing County, Zhejiang Province, southeast China. Total of twenty-eight ponds, including fourteen fishponds with feed supply, nine fishponds without feed supply and five non-fish ponds, were selected for collecting surface sediments and water samples. Among them, four representative ponds (ponds A, B, C, and D) with water area of 500–750 m<sup>2</sup> were selected for studying P vertical distribution in surface sediments. They were different in history for aquaculture. The pond A and pond B had been used for aquaculture with the supply of feeds for 15 and 5 years, respectively. The pond C had been used for aquaculture for 16 years without any supply of anthropogenic feeds, and the pond D was a common pond as control where no fish was stocked. The average water depths of the ponds were 1.1 to 1.3 m, and maximum depths were 1.5 to 1.8 m. Bottom mud (sediment) was not removed from these ponds last 20 years. There were two species of fishes in the fishponds, i.e., silver carp *Hypophthalmichthys molitrix* and crucian carp *Carassius auratus*. Annual biomass yields of fishes for ponds A, B, and C were about 10.2, 9.7 and 1.1 t/hm<sup>2</sup>, respectively. The feeds used in ponds A and B were compound feeds, which contained 34% of total protein, 12% of total cellulose, and 1.6% of P. The annual application amounts of compound feeds for ponds A and B were 5.5 and 5.7 t/hm<sup>2</sup>, respectively. Phosphorus budge of the ponds A, B, C, and D was calculated from the mass balance equation: P loadings = dietary P + P in precipitation and runoff-P in fish body. The P loading in the ponds was significantly higher in ponds A and B with supply of feeds than ponds C and D.

**Table 1** Phosphorus budge of the ponds (kg/(hm<sup>2</sup>·a))

| P budget                                | Pond A | Pond B | Pond C | Pond D |
|---|--------|--------|--------|--------|
| P input in feeds (a)                    | 87.3   | 91.1   | 0      | 0      |
| P removed by fish (b)                   | 8.3    | 7.9    | 0.76   | 0      |
| P input by precipitation and runoff (c) | 2.7    | 2.6    | 2.8    | 1.9    |
| Estimated P loading (a+c-b)             | 81.7   | 85.8   | 2.04   | 1.9    |

### 1.2 Collection of surface, newly deposited sediments and pond water

Sediment samples of top layer (0–5 cm) from all of the 28 ponds and deep cores (0–16 cm) from ponds A, B, C, and D were collected in January 1999. The top sample (0–5 cm) was collected and homogenized from ten representative locations across each pond for measuring P fractionation and bioavailability. Triplicate representative 16-cm depth sediment cores were taken from ponds A, B, C, and D using a sharpened polyvinyl chloride (PVC) pipe. The cores were then sectioned at 2, 4, 6, 8, 10, 12, and 14 cm from the sediment surface. Newly deposited sediments in ponds A, B, C, and D were collected using simple sediment traps. The traps consisted of 40 cm long PVC cylinders (15 cm inner diameter) sealed at one end, which were fixed 20 cm above the bottom at each pond. Triplicate traps for each pond were installed for sampling sediments in April, 1999. At the end of August in the year, the traps were carefully removed from the water, excess water in the cylinders was decanted off, and the sediment-rich bottom water was poured into a plastic bottle. The sediment slurry was filtered to get rid of excess water. All samples were air-dried at room temperature and ground to pass a 2-mm sieve. Sub-samples were further ground to pass a 0.125-mm sieve for analyzing organic matter, total P, organic P and inorganic P.

Water samples were collected monthly during the period from April through August, 1999. At each sampling, water sample was collected and bulked from ten sampling positions around each pond. Sub-sample from each sample was filtered through a 0.45-μm syringe filter and then acidified using 2 mol/L H<sub>2</sub>SO<sub>4</sub> for measuring molybdate reactive P.

### 1.3 Analysis

Sediment pH was measured in a 1:1 sediment/distilled water (g:ml) suspension. Sediment clay content was measured by the hydrometer method after pre-treating sediment with H<sub>2</sub>O<sub>2</sub> and dispersed overnight in Na-hexametaphosphate (Institute of Soil Science, Chinese Academy of Sciences, 1978). Organic matter was determined by the potassium dichromate wet combustion procedure (Agricultural Chemistry Committee of China, 1983). Sediment total P was analyzed with the molybdate blue method after digestion in sulfuric acid and perchloric acid (Agricultural Chemistry Committee of China, 1983). Water-soluble P was extracted by deionized water at 1:10 sediment/deionized water (g:ml) and measured by the molybdate blue method (Olsen and Sommers, 1982). Total organic P was determined by the difference between the amounts of H<sub>2</sub>SO<sub>4</sub>-extractable P for the ignited and unignited sample (Olsen and Sommers, 1982). Inorganic P was calculated from the difference between total P and organic P. Olsen P was

extracted using a 0.5 mol/L  $\text{NaHCO}_3$  solution (pH 8.5) with a sediment to the solution ratio of 1:20 and measured by the molybdate blue method (Olsen and Sommers, 1982). Algal available P was estimated by 0.1 mol/L NaOH solution extraction (Sharpley *et al.*, 1992).

Sediment P was also measured with saturation paste extract under both aerobic and anaerobic conditions. For aerobic condition, the equivalent of 25-g of dry sample and 50-ml distilled water were added to a 100-ml polyethylene bottle. The bottles were stored at 25°C and shaken for 30 min daily. The top space of each bottle was replaced daily with room air to prevent the development of anoxic conditions. After 30 d, the equilibrium solutions were transferred to 50-ml centrifuge tubes and centrifuged at  $7500 \times g$  for 30 min and then filtered. The filtrates were analyzed for measurement of P. An incubation study was conducted to determine the amount of P that could potentially be released from the sediments under anoxic conditions. Sediments were incubated under  $\text{N}_2$  gas for 30 d to determine the potential for P release under anoxic conditions. Twenty-five grams of each sediment were added to a 100-ml polyethylene bottle containing 50 ml deionized water. The bottles were sealed with rubber and placed on a reciprocating shaker for 1 h. After shaking, the rubber was removed and the bottles were placed in a glove box that had been purged with  $\text{N}_2$  gas. This allowed flushing bottles with  $\text{N}_2$  gas. The bottle were then sealed with rubber, and placed in an incubating chamber in the dark at 25°C for 30 d. After 30 d, they were removed from the incubator, and immediately centrifuged. The filtrates were analyzed for P.

The surface sediment samples from ponds A, B, C, and D were analyzed for: (1) 1 mol/L  $\text{NH}_4\text{Cl}$  extractable P (loosely bound-P); (2) 0.1 mol/L NaOH extractable P (Al- and Fe-P and extractable organic P); (3) 0.5 mol/L HCl extractable P (Ca-P); and (4) residual P consisting of refractory organic P and residual mineral P. The extraction procedures were the same to those described by Rydin and Otabbong (1997). The samples were sequentially extracted by 50 ml of 1 mol/L  $\text{NH}_4\text{Cl}$ , 50 ml of 0.1 mol/L NaOH, and then 50 ml of 0.5 mol/L HCl. Each extraction lasted for 16 h on an end-to-end shaker. After each extraction, the tubes were centrifuged at  $7500 \times g$  for 30 min, the supernatant was then passed through a 0.45- $\mu\text{m}$  filter paper. The P concentrations in the filtrates from  $\text{NH}_4\text{Cl}$  and HCl extractions, and the inorganic P (NaOH-IP) in hydroxide extracts were determined by the molybdenum-blue method. Total P in hydroxide extracts (NaOH-TP) was also determined by the molybdenum-blue method after digestion with acidified ammonium persulfate. The organic P (NaOH-OP) concentrations in hydroxide extracts were

calculated from the difference between total P (NaOH-TP) and NaOH-IP contents in the extracts. Sediment total P was determined by perchloric acid digestion method (Agricultural Chemistry Committee of China, 1983). Residual P was calculated by subtracting the sum from above mentioned three types of extractable P from the total P content in the sample. Dissolved P concentration in water samples was determined with the molybdate blue method. Concentrations of chlorophyll-*a* were measured spectrophotometrically after extraction in hot ethanol (Sartory and Grobbelaar, 1984). All the above measurements were performed on triplicate samples.

Statistical differences in total P, extractable P, OP/IP, organic matter, pH, and clay content in sediments among different ponds and correlations among variables were conducted using the SAS computer programs.

## 2 Results and discussion

### 2.1 Phosphorus accumulation in sediments

Aquaculture in the ponds influenced significantly chemical properties of bottom sediments. Total P in the fishponds with feed supply ranged 0.88 to 1.73 g/kg with average concentrations of 1.34 g/kg, and was significantly higher than those in the non-fish ponds (Table 2). All the forms of extractable P in the sediments, including Olsen P, algal available P, water soluble P, and P concentrations in saturation extracts, decreased in order of fishponds with feed supply > fishponds without feed supply > non fish-ponds. Phosphorus concentrations in saturation extracts under anoxic condition were higher than those under oxic condition. The increased release of soluble P from sediments under anoxic conditions has been reported (Moore *et al.*, 1998). Organic matter and ratio of organic P to inorganic P (OP/IP) were higher in the fishponds with feed supply than fishpond without feed supply and non-fish ponds. There was a positive correlation between organic matter and OP/IP ( $r = 0.926^{**}$ ,  $n = 28$ ).

There were marked differences in phosphorus vertical distribution of the bottom sediments among the ponds (Fig.1). In all the four ponds there was a gradual decline in total P from the sediment-water interface downward. Maximum values for P pools occurred at surface layers and declined with depth to stable value, which is in agreement with the observation in estuarine systems (Paludan and Morris, 1999). The accumulation of total P was significant at pond A and pond B, and to some extent at pond C, as compared with the pond D (control), and positively correlated to the P loading (Table 1). The OP/IP decreased with increasing the depth. The total P, ratio of OP/IP, and organic matter decreased in the order of pond A > pond B > pond C > pond D (Fig.1),

Table 2 Total P, extractable P, OP/IP, organic matter, pH, and clay content in sediments from different ponds

| Pond                                | Total P, g/kg                                  | Olsen-P, mg/kg  | Algal available P, mg/kg | Water-soluble P, mg/kg | Saturation P (O) <sup>a</sup> , mg/L | Saturation P (AN) <sup>b</sup> , mg/L | OP/IP                | Organic matter, g/kg | pH                | Clay (<2μm), g/kg  |
|-------------------------------------|--|-----------------|--------------------------|------------------------|--------------------------------------|---------------------------------------|----------------------|----------------------|-------------------|--------------------|
| Fishponds with feed supply (n=14)   | 1.34a <sup>c</sup><br>(0.88—1.73) <sup>d</sup> | 85a<br>(54—136) | 145a<br>(96—225)         | 24a<br>(13—38)         | 2.4a<br>(1.3—3.7)                    | 5.7a<br>(2.0—9.8)                     | 0.96a<br>(0.54—1.53) | 86a<br>(57—126)      | 6.6a<br>(6.2—7.1) | 324a<br>(277—396)  |
| Fishponds without feed supply (n=9) | 0.87b<br>(0.63—1.29)                           | 43b<br>(24—70)  | 85b<br>(59—136)          | 14b<br>(10—19)         | 1.3b<br>(1.2—1.5)                    | 1.6b<br>(1.4—2.2)                     | 0.58b<br>(0.38—0.77) | 45b<br>(27—58)       | 6.9a<br>(6.7—7.2) | 249b<br>(198—278)  |
| Non-fish ponds (n=5)                | 0.62b<br>(0.47—0.86)                           | 20c<br>(8—40)   | 38c<br>(19—74)           | 8.8c<br>(6.6—11)       | 0.9c<br>(0.7—1.2)                    | 1.1c<br>(0.7—1.5)                     | 0.50b<br>(0.30—0.75) | 49b<br>(26—74)       | 7.0a<br>(6.8—7.2) | 283ab<br>(186—387) |

Notes: <sup>a</sup> Saturation P (O): P in saturation extract under oxie condition; <sup>b</sup> saturation P (AN): P in saturation extract under anoxic condition; <sup>c</sup> mean values within a column with the same letter(s) are not different at *p* < 0.05 probability level; <sup>d</sup> values in parentheses represent range

suggesting that accumulation of total P and organic matter increased with the supply of feeds, and the duration of aquaculture. The trends observed for organic matter were clearly paralleled by the total P and ratio of OP/IP, suggesting that P accumulation in the ponds were mainly in organic P, and there was a probable association of P with organic matter in the sediments.

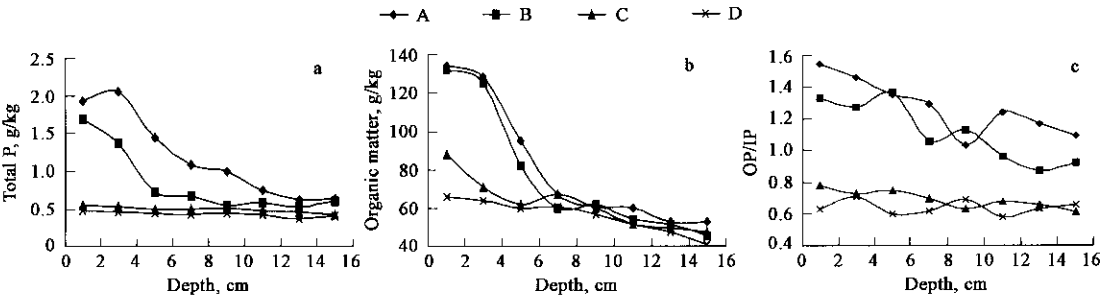


Fig.1 Vertical distributions of total P, the ratio of organic P to inorganic P (OP/IP), and organic matter in the bottom sediments from the ponds A, B, C and D

The concentration of organic matter and total P in the sediments deposited in the traps was similar to those in the surface sediments (Fig.1 and Table 3). However, the organic matter, total P and ratio of OP/IP in the sediments collected from traps were higher than those in the surface sediments, indicating that more organic P accumulated in newly deposited sediments. The higher ratios of OP/IP in the sediments from pond A and pond B than pond C and pond D suggest that the application of fishery feeds increases

Table 3 Total phosphorus (TP) and organic matter (OM) deposited in the traps from the four ponds

| Pond               | Pond A | Pond B | Pond C | Pond D |
|--------------------|--------|--------|--------|--------|
| OM, g/kg           | 187    | 179    | 95     | 73     |
| TP, g/kg           | 2.39   | 2.28   | 0.64   | 0.53   |
| OP/IP <sup>a</sup> | 1.66   | 1.62   | 0.76   | 0.65   |

Note: <sup>a</sup> OP/IP: the ratio of organic P to inorganic P

greatly organic P and organic matter accumulation.

**2.2 Phosphorus fractionation of surface sediment**

Sequential extractions of the sediment with 1 mol/L NH<sub>4</sub>Cl, 0.1 mol/L NaOH, and 0.5 mol/L HCl (Table 4) showed that most of P in the sediments was extractable. Of the total P, 56.7%—67.8% was present as NH<sub>4</sub>Cl-P (3.1%—5.6%), NaOH-IP (16.5%—22.3%), NaOH-OP (20.8%—27.6%) and HCl-P (12.3%—16.3%). The proportions of various P forms were NaOH-OP > NaOH-IP > HCl-P > NH<sub>4</sub>Cl-P. However, the fractions of P in the sediments varied among the ponds. The proportions of NH<sub>4</sub>Cl-P, NaOH-IP and NaOH-OP were pond A > pond B > pond C > pond D. On the contrary, the proportions of HCl-P and residual-P were pond D > pond C > pond B > pond A. The high proportion of NaOH-IP and NaOH-OP in the sediments from the fishponds (ponds A, B, and C) provides evidence that P in the fishponds was mainly accumulated in forms of organic P and Al-, Fe-P. Also, high proportions of P in the labile P (NH<sub>4</sub>Cl-P) and potentially labile P (NaOH-IP) in the sediments from pond A and pond B suggested that the sediments from the fishponds with supply of feeds had

**Table 4** Phosphorus fractionations of 0—5 cm bottom sediments from the four ponds

| Pond                     | Pond A | Pond B | Pond C | Pond D |
|--------------------------|--------|--------|--------|--------|
| pH                       | 6.83   | 6.99   | 6.79   | 7.03   |
| Organic matter, g/kg     | 122    | 102    | 74     | 65     |
| Clay, g/kg               | 367    | 396    | 387    | 354    |
| Water soluble P, mg/kg   | 31     | 23     | 9.0    | 6.6    |
| Total P, g/kg            | 1.73   | 1.34   | 0.53   | 0.47   |
| NH <sub>4</sub> Cl-P, %  | 5.6    | 4.2    | 3.5    | 3.1    |
| NaOH-IP <sup>a</sup> , % | 22.3   | 18.7   | 20.2   | 16.5   |
| NaOH-OP <sup>b</sup> , % | 27.6   | 25.4   | 21.6   | 20.8   |
| HCl-P, %                 | 12.3   | 15.4   | 14.6   | 16.3   |
| Residual-P, %            | 32.2   | 36.3   | 40.1   | 43.3   |

Notes: <sup>a</sup> IP is inorganic P; <sup>b</sup> OP is organic P

high potential release of P.

2.3 Phosphorus concentration of pond water and its relation to sediment phosphorus

Phosphorus concentrations in water samples collected during the April through August, 1999 from 28 ponds ranged from 7.8 to 120 μg/L. The average P concentrations in the water from the ponds with feed supply, ponds without feed supply, and non-fish ponds were 66, 24 and 12 μg/L, respectively. Fish ponds had P concentration higher than 20 μg/L, the critical concentration for lake eutrophication (Schinder, 1977). The P concentrations in pond water significantly correlated with several loosely bound, readily available P indexes in the sediments (Table 5). There were significant correlations between the P concentration in the pond water and water soluble-P, algal available P, saturation extraction-P in oxic and anoxic conditions with correlation coefficients of 0.956, 0.963, 0.968, and 0.990, respectively. Significant correlation between the P concentration in pond water and P fractions in the sediments suggests that increased P concentration in the pond water was resulted from P accumulation in the sediments. There was a link between P in the pond water and in the bottom sediments.

**Table 5** Relationship between P concentration in pond water and P forms of the bottom sediments (*n* = 28)

| P forms         | Total P  | Olsen P  | Algal available P | Water soluble P | Satura-tion P (O) <sup>a</sup> | Satura-tion P (AN) <sup>b</sup> |
|-----------------|----------|----------|-------------------|-----------------|--------------------------------|---------------------------------|
| P in pond water | 0.833*** | 0.887*** | 0.863***          | 0.956***        | 0.968***                       | 0.990***                        |

Notes: <sup>a</sup> Saturation P (O): P in saturation extract under oxic condition;  
<sup>b</sup> saturation P (AN): P in saturation extract under anoxic condition;  
\*\*\* significant at *p* < 0.001

The Chl-*a* can be as an indicator of phytoplankton biomass. Mean concentrations of chlorophyll-*a* is generally correlated with degree of eutrophication (An and Park, 2002). During the study, Chl-*a* averaged 48 μg/L and varied from 6 to 146 μg/L for all ponds. The concentration of Chl-*a* increased in the order of non-fish ponds, fish ponds without feed supply, and fishponds with feed supply (Fig.2). There was a significant relationship between log-transformed Chl-*a* and dissolved P (DP):

$$\lg(\text{Chl-}a) = 0.48 + 0.68 \lg(\text{DP})$$

Significant correlations were found between concentration of Chl-*a* and DP in the sediments with correlation coefficients of 0.707, 0.751, 0.730, 0.856, 0.895 and 0.892, respectively, for total P, Olsen P, algal available P, water soluble P, P concentrations in saturation extracts under anoxic, and oxic conditions. The present results indicate a strong response of chlorophyll-*a* to increasing dissolved P in pond water and total and available P in the sediments.

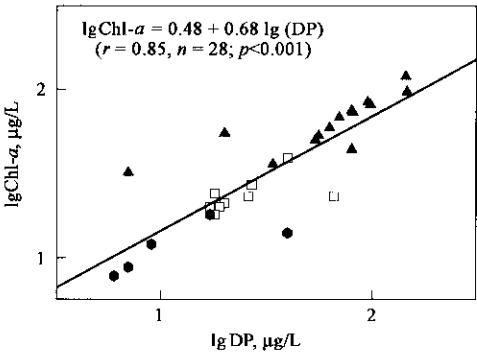


Fig.2 Relationship between chorophyll-*a* (Chl-*a*) and dissolved P (DP) in pond water  
▲: fishponds with feed supply; □: fishponds without feed supply;  
●: non-fish pond

3 Conclusions

The present study showed that the long-term application of feeds resulted in the increase in the total P, available P and organic matter loads in the underlying sediments, thus increasing concentrations of dissolved P and chlorophyll-*a* in the freshwater fishponds. Organic P was important P pool accumulated in the sediments. The increased P accumulation in the sediments due to feed-supply in aquaculture ponds could increase proportions of the labile P (NH<sub>4</sub>Cl-P) and potentially labile P (NaOH-IP) of the total P in the sediments and enhance P release from the sediments to water, which accelerated the process of eutrophication in fishpond system.

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