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Modeling Phosphorus Utilization by Biotic Components: A Test Case Using Two Insoluble Phosphate Sources

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Abstract

Natural resource management for sustainable development is urgently required in our society and an adequate model is required for quantification of function of natural ecosystem. A simplistic approach has been tried here to quantify phosphorus utilization by biotic components. Two laboratory experiments were conducted to evaluate the amount of P utilization from two different types of sparingly soluble phosphate rock by aquatic biotic communities. The first type was Mussoorie Phosphate Rock or MPR (sedimentary in origin) and other was Purulia Phosphate Rock or PPR (igneous in origin). The two trials were with eight different treatment combinations. Among various treatments, fish and Chironomid larvae contributed to some extent in increasing the available sediment phosphate content, which in turn increased the soluble reactive phosphate (SRP) of overlying water. The SRP of overlying water decreased in the treatment with zooplanktons. Depletion of SRP of overlying water due to uptake of orthophosphate by *Chlorella* was also observed. The sedimentary type phosphate rock proved to be more efficient in releasing phosphate than igneous type.

Keywords: Chironomid larvae, *Chlorella,* Fish, Phosphorus model, Zooplanktons.

Introduction

Phosphorus is an important constituent of biological systems. It is a macronutrient but its availability is often extremely low (Hupfer *et al.*, 2004). Usually phosphorus occurs in oxidized state, either as ions of inorganic orthophosphate or in organic compounds. Phosphorus (P) in solution is normally considered to be Soluble Reactive Phosphate (SRP), which is taken up by different component members of an aquatic ecosystem. Boyd and Musig (1981) demonstrated that plankton in fish ponds absorbed an average of 41% of a 0.30 mgl^{-1} addition of orthophosphate within 24 hours, however phosphorus that is not absorbed by plankton is rapidly absorbed by mud (Hupfer *et al.*, 2004; Cade-Menum, 2005). Pelagic invertebrates not only transform but they can also translocate the recycled phosphorus within the system (Shapiro, 1984).

Phosphorus is a recognized nutrient for pond fertilization. The fish ponds in India usually suffer from phosphorus depletion and thereby affect the fish production profoundly. Supply of phosphorus through chemically processed fertilizer in pond water is a common practice in this country. But addition of costly and chemically processed fertilizer degrades the sediment base and overlying water of any aquatic impoundment in long term use (Jhingran, 1997). So, use of indigenous natural P-fertilizer (appetite-P) will save the environment from degradation and reduce the financial load from poor fish farmers.

Among various phosphatic fertilizers naturally occurring phosphate source (Mussoorie Phosphate Rock= MPR and Purulia Phosphate rock= PPR) can easily be used in fish farming ponds and has been amply proved to be an effective fertilizer in carp culture (Chakrabarty, 2006). The problem is its extremely low solubility in water. An attempt has been made here to quantify the difference of phosphorus utilization performances by various biotic members in laboratory condition from a marine sedimentary (MPR) and igneous (PPR) phosphate rock of Indian origin.

Materials and methods

In order to determine the extent of P-utilization from MPR and PPR by the major biotic components of the pond ecosystem, two separate laboratory experiments were conducted in the presence of water, MPR and PPR all throughout in 3l glass jars.

For the first trial, 32 glass jars were filled with ground water and then subjected to following eight treatments in quadruplicate. (i) Water – (A_1), (ii) Water + MPR – (B_1), (iii) Water + Soil – (C_1), (iv) Water + Soil + MPR – (D_1), (v) Water + Soil + MPR + *Chlorella* sp. – (E_1), (vi) Water + Soil + MPR + Zooplankton - (F_1), (vii) Water + Soil +MPR+ Chironomid larvae – (G_1), (viii) Water + Soil + MPR + Fish – (H_1). Similar set was was used in the second trial in the presence of PPR as follows (i) Water – (A_2), (ii) Water + PPR – (B_2), (iii) Water + Soil – (C_2), (iv) Water + Soil + PPR – (D_2), (v) Water + Soil + PPR + *Chlorella* sp. – (E_2), (vi) Water + Soil + PPR + Zooplankton - (F_2), (vii) Water + Soil +PPR+ Chironomid larvae – (G_2), (viii) Water + Soil + PPR + Fish – (H_2). The source of ground water was a deep-tube well. Agricultural soil (pH 7.1) was collected from nearby virgin field, where no fertilizer schedule was practiced. Environmental temperature remained 15-18°C during the conduction of the experiment

20 gm of hundred mesh size ground MPR (trial-I) and PPR (trial-II) was placed in each of the experimental jars containing 2.5 1 of ground water (pH 7.2). *Chlorella* sp. used in this experiment was procured from the laboratory axenic monoalgal culture (Chu– 10 medium). When the *Chlorella* concentration attained about 40 mgl-1of Chl- a; 10 ml of such concentration was dispensed in each experimental jar of treatment combination of E_1 and E_2. *Daphnia* sp. was collected from the culture tank and concentrated to150 numbers and used in each jar. Chironomid larvae (average length 0.5 cm ± 0.025) were locally procured and then acclimatized in the laboratory prior to their use in experiment (F_1 and F_2). Thirty Chironomid larvae were used in each glass jar of treatment combination of G_1 and G_2. Advanced fries of *Oreochromis mossambicus* (4.20 g ± 0.25 g; 4.0 cm ± 0.60) were procured and acclimatized well in the laboratory prior to their use. Three acclimatized fries were then placed in each glass jar in the treatment combination of H_1 and H_2. Each set of glass jar was left for 0, 7 and 14 days for the examination of phosphorous contents of water, soil and *Chlorella* sp. All of them were carefully isolated, dried in hot air oven, grinded and total P content was measured using the method described by Jackson (1967). Chlorophyll-a (Chl- a) concentration of *Chlorella* sp. was analyzed following the method described by Vollenweider (1974). The soluble reactive phosphate (SRP) concentration of water was also measured following standard methods (APHA, 2002).

The results were statistically analysed using Kruskal-Wallis one way analysis of variance and Duncan's Multiple Range Test (Duncan, 1955) to find significance of difference between every possible pair of treatment of trial-I and trial-II.

The chemical composition of MPR and PPR are shown in Table 1. The MPR is younger in geologic age than PPR (Table 1) and used as a cheap source of direct application fertilizer in fish farming ponds (Chakrabarty, 2006).

Results

Introduction of fish (H_1 and H_2) and chironomid larvae (G_1 and G_2) resulted in considerable rise (Figure 1 and 2) of SRP concentration of water (0.1825 and0.1425 mg l^{-1}) and sediments (13.0-12.9 and11.7-11.5 mg Kg^{-1}) over other treatments (Figure 3 and 4). Presence of zooplankton (F_1 and F_2) in the MPR and PPR treatment, on the other hand, caused decline of orthophosphate level of water all throughout. For example, the amount of orthophosphate observed on day 0 (0.165 mg l^{-1}) declined to 0.0985 mg l^{-1} in (F_1) on day 7; and finally to 0.065 mg l^{-1} on day 14. In the same trend the amount of orthophosphate observed on day 0 (0.125 mg l^{-1}) declined to 0.0985 mg l^{-1} in (F_1) on day 7; and finally to 0.065 mg l^{-1} on day 14 in the glass jars with F_2 combination. The result was also true for *Chlorella* sp. the amount of orthophosphate of water in the MPR treatment with *Chlorella* sp. (E_1) declined to 0.07 mg l^{-1} 0.05 on day 7 and further to 0.045 mg l^{-1} on day 14, from the initial concentration of 0.165 Similar result was observed with the same treatment combination (F_2) with PPR (Figure 2). Glass jars with only water (A_1 and A_2) showed lowest SRP concentration (0.025 mg l^{-1}) of all, all throughout, whereas a stable and relatively high concentration of 0.155 to 0.1425 mg $l^{-1}$and 0.125 to0.10 of SRP was maintained in the treatment combination of B_1 and B2.

Kruskal-Wallis one way analysis of variance by ranks showed no significant difference (H=10.33; P>0.05) of treatment means in the beginning. However, significant difference (H $\geq$ 14.16; P<0.05) among treatments was seen on day 7 and 14. Six treatment groups such as G_1 and G_2; H_1 and H_2; D_1 and D_2; E_1 and E_2, A_1 and A_2; C_1 and C_2 did not differ from each other on day 7 (Table2 and 3), whereas all the treatment groups expect C_1 and C_2; A_1 and A_2 showed significant difference on day 14 in both the trial. Significant difference was also found between $H_1$and H_2 as well as G_1 and G_2 in respect of concentration of water SRP and sediment available – P (ANOVA, P< 0.05) on 0,7,14 days of observation. The treatment combination with MPR always showed higher dissolution of P from insoluble P than the combination with PPR.

The amount of Chl-a concentration (Figure 5) did not varied significantly (ANOVA, P> 0.05) between the two treatment combination (E_1 and E_2) throughout the experimental period. However, the total amount of phosphorus in chlorella (cell-P) was 0.05±0.002mg Jar^{-1}in day 0 of both trial-I and- II. The amount increased to 0.08±0.002 mg Jar^{-1}(trial-I)) and to 0.078±0.003 mg Jar^{-1} (trial-II) on day 7 but declined to 0.065±0.002mg Jar^{-1} (trial-I) and to0.062±0.003mg Jar^{-1}(trial II) in day14. The cell-P also did not varied significantly in any day between two series.

Discussion

Phosphorus (P) is the most studied element in aquatic system primarily due to the fact that it is the most limiting factor for primary productivity in water bodies is essential for

Table 1. Chemical data and characteristics of the two apatite sources (MPR and PPR) tested for their comparative efficiency in releasing phosphorus in laboratory condition. (Analyzed at International Fertilizer Development Corporation, Alabama, USA)

Major constituents %	**Mussoorie Phosphate Rock (MPR)**	**Purulia Phosphate Rock (PPR)**
P_2O_5	21.2	21.6
CaO	38.5	39.4
Fe	4.0	9.2
MgO	5.6	5.8
S	4.0	9.5
SiO_2	6.6	8.1
C (Organic)	1.14	0.92
F	2.0	4.1
Characteristics	Mussoorie Phosphate Rock (MPR)	Purulia Phosphate Rock (PPR)
Unit cell dimension, a- axis, A^0	9.367	9.371
Mole ratio (CO_3:PO_4)	0.052	0.013
Origin and nature	Marine sedimentary and carbonate apatite	Igneous and fluorapatite

Table 2. Results of Kruskal-Wallis one way analysis by ranks (H) and Duncan's Multiple Range Test for mean values of SRP concentration of water in trial-I.

Values of H (df=7)		S$\bar{y}$i (df=7)	Duncan's Multiple Range Test							
			R2	R3	R4	R5	R6	R7	R8	Comparison
0 DAY	**10.33^{ns}**	**1.154**								
7 DAY	**10.33^{a}**	**0.520**	**1.69**	**1.76**	**1.80**	**1.83**	**1.84**	**1.85**	**1.85**	**A C D E F B G H** **2.5 2.5<6.25 7.0< 9.25<11.25<14.0 14.5**
14 DAY	**10.33^{a}**	**0.351**	**1.47**	**1.19**	**1.22**	**1.23**	**1.24**	**1.25**	**1.25**	**C A D E F B G H** **2.25 3.5 < 5 < 7.25< 9.5 < 11.5 <13.75 < 15.25**

Note that:A to H = Eight treatment group with four replicates in each group; ns = not significant; 'a' = significant at 5 %; Horizontal bar indicates no significant differences

Table 3. Results of Kruskal-Wallis one way analysis by ranks (H) and Duncan's Multiple Range Test for mean values of SRP concentration of water in trial-II.

Values of H (df=7)		S$\bar{y}$i (df=7)	Duncan's Multiple Range Test							
			R2	R3	R4	R5	R6	R7	R8	Comparison
0 DAY	**10.33^{ns}**	**1.154**								
7 DAY	**10.33^{a}**	**0.526**	**1.67**	**1.74**	**1.78**	**1.81**	**1.82**	**1.83**	**1.85**	**A C D E F B G H** **2.5 2.5<6.25 7.0< 9.25<11.25<14.0 14.25**
14 DAY	**10.33^{a}**	**0.357**	**1.45**	**1.17**	**1.20**	**1.20**	**1.21**	**1.21**	**1.21**	**C A D E F B G H** **2.2 3.5 < 5 < 7.2< 9.5 < 11.5 <13.25 < 14.25**

Note that:A to H = Eight treatment group with four replicates in each group; ns = not significant; 'a' = significant at 5 %; Horizontal bar indicates no significant differences

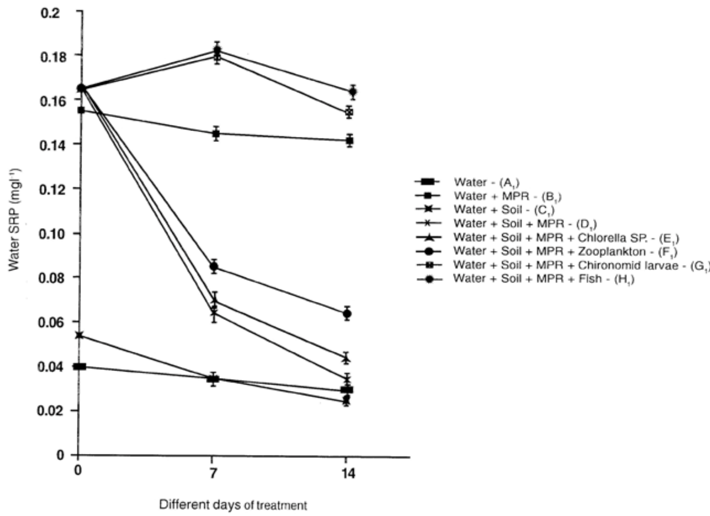


Figure 1. Mean (+ S.D) concentration of soluble reactive phosphate of water in different days of various days of trial I

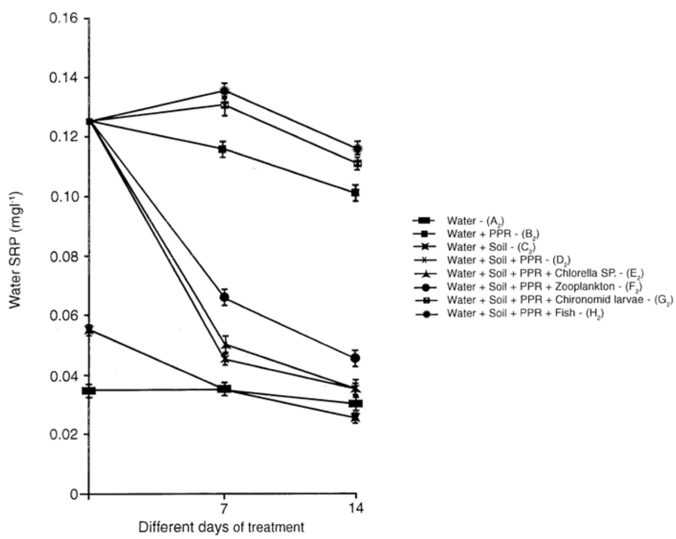


Figure 2. Mean (+ S.D) concentration of soluble reactive phosphate of water in different days of various days of trial II

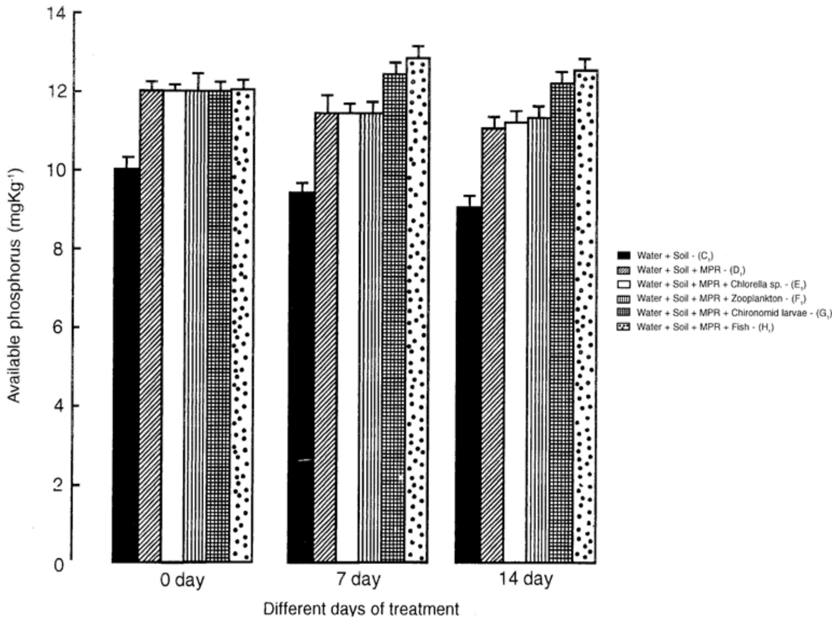


Figure 3. Mean (+ S.D) concentration of available phosphorus in sediment of various treatment of trial

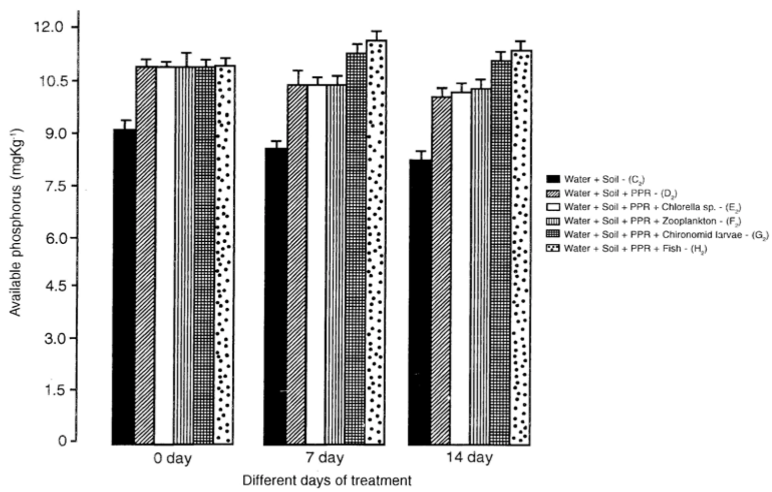


Figure 4. Mean (+ S.D) concentration of available phosphorus in sediment of various treatment of trial

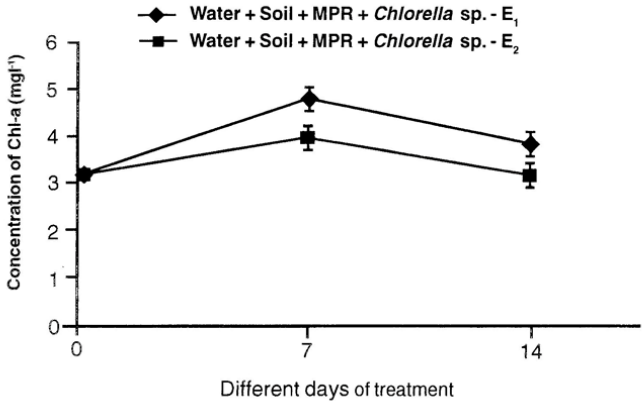
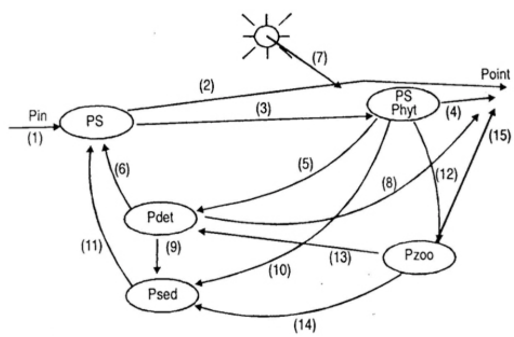


Figure 5. Mean (+ S.D) concentration of chlorophyll-a in days of trial E_1 and trial E_2



Processes

(1) = Pin to the soluble phosphorous pool, PS;
(2) = Pout from PS;
(3) = P uptake by phytoplankton/algae growth;
(4) = Pout, phytoplankton;
(5) = phytoplankton mortality;
(6) = detritus decomposition, re-mineralization; release of P to PS;
(7) = solar radiation available for photosynthesis;(S)
(8) = Pout, detritus,
(9) = detritus sedimentation;
(10) = phytoplankton sedimentation;
(11) = release of P from sediment;
(12) = zooplankton grazing of phytoplankton;
(13) = zooplankton mortality;
(14) = zooplankton sedimentation
(15) = Pout, zooplankton

Figure 6. Model describing movement of phosphorus in the aquatic ecosystem

living organisms and is not exchangeable with other elements in biological system. From the critical examination of the data it was revealed that fish and chironomid larvae contributed to some extent in increasing the available phosphate content of sediment which, in turn, increased the orthophosphate level of overlying water. This was due to their physical disturbance of the bottom sediments which induced the phosphorus release from sediment to the overlying water. The effect was perhaps brought about by the physical disturbance (Petr, 1977) and agitation of sediment enriched with phosphate rock. Similar results were obtained by Gabet *et al*., (2003), Schauser *et al.,* (2003) and Sondergaard *et al*., (2003). Biomanupulation trials clearly revealed that fish and chironomid larvae had a profound influence in the release of phosphate from otherwise insignificantly soluble phosphate rock by bioturbation (Chakrabarty, 2006).

There was an increase in biomass of *Chlorella* evident from the rise in Chl-content. The depletion in orthophosphate level of water (0.165-0.07 mg l^{-1}) was due to the phosphorous uptake by the *Chlorella*. This was evident from the increase in cell-P of *Chlorella* over time. Phosphorous was found to be a growth regulatory factor of algae by Ahlgren (1988). The critical examination of difference in liberation of available phosphorus from the two sparingly soluble phosphate sources indicated a significant difference (P<0.05) in treatment combination of H_1- H_2 and G_1 -G_2 . This was because of the lesser unit cell dimension (>.008 A^0) of MPR than PPR (Table 1) as well as the higher CO_3:PO_4 mole ratio of (0.052) of MPR than PPR (0.013). As lesser unit cell dimension and higher CO_3:PO_4 mole ratio helps in natural dissolution of phosphate rock in natural condition (PPCL 1987). The utilization of phosphorus by *Chlorella* indicated no significant difference between the treatment combinations of $F_1$and F_2. Possibly, the amount of SRP was sufficient in both the treatments for algal growth. The concentration of cell P was also similar between two series. The study indicated that MPR is better phosphate fertilizer than PPR in terms of releasing phosphorus in aquaculture. It also proved that sedimentary phosphate rock has better applicability than igneous type. In an X- ray diffraction studies by PPCL, MPR was identified as carbonate apatite and PPR as fluorapatite. Carbonate apatite is more responsive to natural dissolution than fluorapatite (PPCL, 1987). However, both the fertilizer can be used as direct application fertilizer in fish farming ponds with bottom grazing fishes as a cheap P fertilizer.

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