Hydrobiologia (2013) 710:265–277
DOI 10.1007/s10750-012-1206-x

SHALLOW LAKE ECOSYSTEMS

The effect of phosphorus binding clay (Phoslock®) in mitigating cyanobacterial nuisance: a laboratory study on the effects on water quality variables and plankton

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Received: 31 July 2011 / Accepted: 28 May 2012 / Published online: 15 June 2012

Abstract This laboratory study examined the lanthanum modified clay Phoslock® for its effectiveness to bind soluble reactive phosphorus (SRP), release of nutrients from this modified clay, its influence on water quality variables (pH, oxygen saturation %, conductivity and turbidity), effects on phytoplankton growth (green alga *Scenedesmus obliquus,* cyanobacteria *Microcystis aeruginosa* and *Anabaena* sp.), and, lastly, its effect on the population growth of the rotifer *Brachionus calyciflorus.* A clear dose–response for SRP binding by the modified clay was observed. A small amount of ammonium is released from Phoslock®. We found no effect of Phoslock® on pH or oxygen saturation. Conductivity increased with the increasing concentration of Phoslock®. An application of Phoslock® caused a transient increase of turbidity up to 211 NTU. However, due to rapid settlement, turbidity fell below 13 NTU (~1 m Secchi depth), after 6 h. Phoslock® addition caused a reduction in growth of all phytoplankton species tested that we attribute to the combined effects of light limitation, flocculation with the bentonite and binding of SRP to Phoslock®. We estimated the EC_{50} of Phoslock® on the population growth of rotifer *B. calyciflorus* to be 0.15 g Phoslock® l^{-1}. Overall, the results of our study indicate Phoslock® seems to be suitable for field applications.

Keywords Eutrophication control · Mitigation measures · Phosphorus fixation · Plankton growth · Sediment capping

Guest editors: Zhengwen Liu, Bo-Ping Han & Ramesh D. Gulati / Conservation, management and restoration of shallow lake ecosystems facing multiple stressors

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Introduction

Eutrophication of surface waters through nutrient enrichment—resulting in blooms of cyanobacteria, is the most important water quality problem worldwide (Smith & Schindler, 2009; Paerl et al., 2011). Remedies against eutrophication have mainly centred on control of phosphorus in water bodies (Carpenter, 2008; Schindler et al., 2008). Decades of uncontrolled inputs have loaded lake sediments with phosphorus. Some failures of lake restorations are attributed to not dealing with this internal source of phosphorus (Søndergaard et al., 2001; Gulati & Van Donk, 2002). Phosphorus control should focus on all phosphorus present within the system as well as strong

reduction of the external phosphorus inputs (Mehner et al., 2008).

Existing in-lake dephosphatisation methods aim mainly at either removing phosphorus from the water—by applying aluminium- or iron-based flocculants (e.g. Rop, 1995; Lam & Prepas, 1997; Rydin & Welch, 1999; Hullenbusch et al., 2002) or preventing its release from the sediment–sediment capping (Hart et al., 2003). A novel technique is the application of modified clays, such as $LaCl_3$-modified kaolinite (Yuan et al., 2009) and the lanthanum modified bentonite Phoslock® (Douglas, 2002). Phoslock® was developed by the CSIRO, Australia, as dephosphatisation technique aiming at removing P in both water and sediment. Several studies reported Phoslock® as promising in controlling eutrophication (Robb et al., 2003; Akhurst et al., 2004; Douglas et al., 2004; Ross et al., 2008; Haghseresht et al., 2009).

Phoslock® contains 5% lanthanum, which is achieved through cation exchange (Haghseresht, 2005). Lanthanum and phosphate bind to rhabdophane ($LaPO_4$), a mineral with an extreme low solubility ($K_{sp} = 10^{-24.7}$to $10^{-25.7}$ mol^2 l^{-2}) (Johanneson & Lyons, 1994; Liu & Byrne, 1997). The lanthanum-phosphate bond is less affected by non-neutral pH as compared with aluminium- and iron-based phosphate binders (Douglas et al., 2004). Contrary to iron, the phosphate binding capacity of lanthanum is not affected by altered redox conditions such as those in anoxic waters (Correl, 1998; Ross et al., 2008).

Phoslock® is applied as a suspension to the water surface. It removes soluble reactive phosphorus (SRP) from the water, once settled on the sediment Phoslock® acts as a 1–3 mm thick chemical barrier for SRP (see URL1).

Inasmuch as many lakes in The Netherlands are still suffering from eutrophication, water quality managers are challenged to find ways to achieve the good ecological state or potential of lakes by 2015, as required from the EU Water Framework Directive (EU, 2000). Here, the modified clay technique Phoslock® seems very promising in tackling the problem of internal P-loadings from sediments that has been identified as one of the major causes of failure in previous lake restoration attempts (Gulati & Van Donk, 2002; Gulati et al., 2008). However, the water quality authorities in The Netherlands needed more information about its effectiveness and effects on water quality variables to judge application of the modified clay to whole lake systems. Especially, since lake owners already started to apply Phoslock®, such as to Lake Het Groene Eiland (see Lürling & Van Oosterhout, 2012).

Our present laboratory study concerns the efficacy of Phoslock®, which we measured as its binding capacity to SRP and its release of ammonia, nitrite/nitrate, SRP; its effect on pH, conductivity, oxygen saturation and turbidity (NTU). Turbidity and pH are of special interest, pH because chemical water treatments (e.g. aluminium or iron salts) may cause a decline in pH, and turbidity because adding a clay, such as Phoslock®, to the water causes a transient decrease in water clarity—which opposes the aims of mitigation. Based on literature studies, we hypothesised that the modified clay would remove SRP from the water effectively and would have no effect on other water quality variables except turbidity. We expected turbidity to increase strongly following addition of the modified clay and to decrease rapidly, because of sedimentation.

Considering the main goal of lake restoration in The Netherlands is reverting turbid water with phytoplankton blooms to clear water, we hypothesised that Phoslock® at concentrations exceeding those that theoretically bind all SRP would inhibit the growth of phytoplankton represented by *Scenedesmus obliquus* (green alga) and *Microcystis aeruginosa* (cyanobacterium). Although growth reduction is a good indication of the effectiveness of Phoslock® to bind SRP, several studies show that flocculation with clay can control existing blooms (Anderson, 1997; Pan et al., 2006, 2011a, b; Verspagen et al., 2006). So, we exposed a bloom of the cyanobacterium *Anabaena* sp. to varying concentrations of Phoslock® in the laboratory to test the hypothesis that "the more the clay (Phoslock®), the stronger the bloom reduction".

In addition we studied the effect of Phoslock® on the population growth of the rotifer *Brachionus calyciflorus*. Suspended clay may affect the survival of planktonic grazers (e.g. van Oosterhout & Lürling, 2011), probably through interfering with the feeding activities. This mechanism may specially hamper the survival of the suspension-feeding cladocerans (*Daphnia* sp.) and rotifers (*Brachionus* sp.). These grazers are often major components of plankton communities and constitute key groups of organisms that play an important role in the energy flow in lakes and ponds

(Riemann & Christoffersen, 1993). Insofar as *Daphnia* sp. and *Brachionus* sp. comprise a major link between limnetic primary production and higher trophic levels, any effects in their population dynamics might pass through the plankton community and may affect organisms at lower and higher trophic levels. The effect of Phoslock® on the population growth of the rotifer *Brachionus calyciflorus* was tested in a two-day life-cycle assays in which *B. calyciflorus* produced multiple broods and the F_1 generation also reproduces (Snell & Moffat, 1992).

Materials and methods

Capacity of Phoslock® to bind SRP

Three replicates of 200 ml SRP (K_2HPO_4, in nanopure water, pH 8.12) solution (0.447 mg SRP l^{-1}) were treated each with four concentrations of Phoslock®, i.e. 0.1, 0.33, 1.0 and 3.3 g Phoslock® l^{-1}, while three replicates without Phoslock® addition served as controls (Table 1). The suspensions were incubated at 22°C, in the dark and 200 rpm orbital shaking. In these suspensions the initial pH was circum-neutral, oxygen saturation 44–75% (Table 1). Initially and after 1, 2, 3, 4 and 5 h 15 ml samples were taken from the suspensions, centrifuged (5 min, 3,000 rpm) and the supernatant subsequently filtered through GF/F filters (Whatmann). Filtrates were analysed for their SRP concentration (Murphy & Riley, 1962).

We assumed exponential decline of the SRP concentration over time: $[SRP_t] = SRP_0 e^{-\theta t}$. Where SRP_0 is the mean SRP concentration at $t = 0$ for the control (0 mg l^{-1} Phoslock®), t = time (0, 1, 2, 3, 4, 5 h) and θ is the SRP decrease rate (mg l^{-1} h^{-1}). Because estimates of θ failed Levene's test (equal variances), we analysed $\log(\theta)$ by one-way ANOVA followed by a Tukey post hoc test in SigmaPlot 11.0. We removed one outlier (0.1 g l^{-1} dose after 1 h), as its SRP concentration increased from the initial 0.447 to 0.739 mg l^{-1}, which we attribute to an analytical error. Based on a 4.4% lanthanum content determined in five different batches of Phoslock® (unpubl. data), 0.05 g l^{-1} Phoslock® should suffice to remove all SRP.

Nutrient leachates from Phoslock®

Nutrient leachate from two Phoslock® batches (similar batches as used in Lürling & Tolman, 2010 for metal leachate) was tested in triplicate suspensions of 20 g Phoslock® l^{-1} (ca. 0.5 g in 100 ml nanopure water). As control, we used three Erlenmeyer flasks, each containing 100 ml nanopure water. The Erlenmeyer's were closed (parafilm) and placed in the dark, at 22°C and 200 rpm orbital shaking for 24 h. Thereafter the suspensions were centrifuged (5 min, 3,000 rpm) and supernatant was filtered (0.45 µm membrane filter, Whatman NC45). In these filtrates, ammonia, nitrite plus nitrate and SRP concentrations were determined using a Skalar continuous flow analyzer (NNI, 1986, 1990, 1997). The release of ammonia, nitrite plus nitrate and SRP (mg kg^{-1} Phoslock®) was inferred by *T* test, after correction for the controls, and based on the means and standard deviations per batch. We applied Welch's test because unequal variances between batches could not be corrected by transformation.

Table 1 Mean (SD) (mg) of Phoslock® aliquots (dose), mean (SD) of maximum amount of SRP bound by this aliquot (max SRP), % bound after 5 h, estimates of the SRP decrease rate θ (SD) and groups identified by Tukey's test

Dose (g l^{-1})	Aliquots (mg)	Max SRP (mg)	Bound P (%)	θ (mg l^{-1} h^{-1})	Tukey group	Start (pH)	Start (O_2%)
0	–	–	–	–	–	6.4	44
0.1	203 (5)	195 (4)	68 (1)	0.2 (1.1)	A	6.5	48
0.33	657 (9)	630 (9)	100 (0)	0.8 (1.1)	A	6.7	45
1.0	2012 (6)	929 (5)	100 (0)	2.5 (1.1)	B	7.5	50
3.3	6603 (9)	6331 (8)	100 (0)	2.6 (1.1)	B	7.9	75

Entries identified by the characters A and B are statistically significant different according to Tukey's test. Also included are the initial pH and oxygen saturation levels (%)

Effect of Phoslock® on pH, conductivity and oxygen saturation

We tested the effect of Phoslock® on pH, conductivity and oxygen saturation in algal growth medium (WC medium; Lürling & Beekman, 2006) and GF/C filtered (Whatman) pond water–Kienehoef pond (Sint-Oedenrode, The Netherlands). Stock suspension of 6.4 g Phoslock® in 80 ml WC medium and filtered pond water were diluted using WC medium, respectively, filtered pond water to 0.625, 1, 1.25, 2, 5, 10, 20 and 40 g l^{-1} (80 ml final volume) in 100 ml plastic containers. A 80 ml control (0 g Phoslock®) was kept for both series. For each dose of Phoslock® and type of water medium one experimental unit was obtained. The experiment was performed in a laboratory setting at 20°C and pH (WTW-pH320 meter), conductivity (EC) (WTW-Cond 330i meter) and oxygen saturation (Oxyguard oxygen meter) were measured at $t = 0$, 1 and 2 h.

As we found little effect of Phoslock® on pH we present median, minimum and maximum values, without formal testing. We observed a linear increase in EC with Phoslock® dose, which was independent of time. For this linear EC—dose relation we estimated intercepts and slopes according to $y = a + bx$ (y = mean EC over time, x = dose, a = intercept and b = slope). For the estimated slopes we tested H_0: $b = 0$ and compared the slopes in each medium using Graphpad Prism 5.04. As we found no effects on oxygen saturation we performed no formal statistical test.

Effect of Phoslock® on turbidity in still water

One gram Phoslock® was added to 1 l of nanopure water in triplicate. Turbidity (NTU) (Hach 2100P Turbidity meter) was measured regularly over 24 h in still water, i.e. a maximum undisturbed settling rate. NTU decay is described by a triple, six parameter exponential decay function (SigmaPlot11.0).

Effect of Phoslock® on growth of *Scenedesmus* and *Microcystis*

The green alga *Scenedesmus obliquus* (Turpin) Kützing SAG 276/3a originated from the collection of the University of Göttingen, Germany and the cyanobacterium *Microcystis aeruginosa* Kützing NIVA-CYA 43 was obtained from the Institute for Water Research, Norway. Our stock cultures of *S. obliquus* and *M. aeruginosa* were maintained as described in Lürling & Roessink (2006). Under these conditions the cultures were mainly comprised of unicells and bicells.

We prepared three series of WC medium:

A. Phoslock® at 0, 0.005, 0.05, 0.5, 5.0 and 50 g l^{-1}.
B. Dilutions (0, 0.001, 0.1, 1, 10 and 100%) of a filtered leachate of a 50 g l^{-1} Phoslock® suspension in WC medium (see below).
C. By diluting a stock solution of lanthanum nitrate ($La(NO_3)_3 \cdot 6H_2O$ solution 1,103 mg l^{-1} in WC medium) to acquire the following six concentrations of La: 0, 0.025, 0.25, 2.5, 25 and 250 mg La l^{-1}.

All dilutions were made with WC medium. The leachate was prepared by 24 h incubation of 50 g l^{-1} Phoslock® in the dark at 20°C and 200 rpm orbital shaking, after which the material was centrifuged for 5 min at 3,000 rpm and the supernatant filtered (0.45 µm membrane filter, Whatman NC45). The Phoslock® doses relate 1–1 with the dilutions of the leachate. The lanthanum nitrate doses are based on the 5% La content as reported by the manufacturers of Phoslock®. The 0.025 mg La l^{-1} dose has no equivalent in the Phoslock® series, while the 50 g l^{-1} Phoslock® dose has no equivalent in the lanthanum nitrate series. Based on the 5% La content, the total La concentrations in the 0.0, 0.005, 0.05, 0.5, 5.0 g l^{-1} of the Phoslock® series are approximately the same as the respective 0, 0.25, 2.5, 25 and 250 mg l^{-1} lanthanum nitrate series.

The three series were tested in triplicate in 100 ml Erlenmeyer's that contained 50 ml from the different series. To each replicate *S. obliquus* or *M. aeruginosa* inocula were added at identical initial density of 2×10^6 µm^3 ml^{-1}. As we measured biovolume (µm^3 ml^{-1}) we also included one additional phytoplankton free Erlenmeyer per concentration as control for the Phoslock® particles. The Erlenmeyer's were closed (cellulose plugs) and incubated for 3 days for *S. obliquus* or 7 days for *M. aeruginosa*, at 21°C in continuous light (100 µmol quanta m^{-2} s^{-1}) and 25 rpm orbital shaking. Different incubation time was based on earlier experiments that had revealed exponential growth over these periods (growth rate *S. obliquus* > growth rate *M. aeruginosa*).

The biovolume (V, μm^3 ml^{-1}) of *S. obliquus* and *M. aeruginosa* was determined by Coulter Multisizer II (size range of phytoplankton 2.5–30 µm). The growth rate (μ, day^{-1}) was estimated by the formula $\mu = (\ln(V_t) - \ln(V_0)/\Delta t$ where V_0 and V_t are the biovolumes at time 0 and t, respectively, Δt is the time lapse (i.e. 3 or 7 days). The chlorophyll-*a* concentrations of the incubated *S. obliquus* were also determined using a PHYTO-PAM phytoplankton analyser (Heinz Walz GmbH, Germany). After 3 days settled material (clay and algae) was observed in the *S. obliquus* series at high dose $\geq$0.5 g l^{-1} Phoslock®, these incubations were analysed again after resuspending the material.

We chose the non-parametric Kruskal–Wallis method to test the dose effect on μ because ANOVA requirements were not fulfilled (non-normal distribution, unequal variances amongst groups) and because transformation was impossible due to negative values. Tukey's test and the Holm–Sidak method were used for pairwise comparisons.

We expect μ to reduce with dose, as higher doses hold less bioavailable SRP (i.e. more SRP bound by La). We also expect a reduction in μ by shading and clay coagulation in the Phoslock® treatment. Thus, we expect the smallest treatment effect on μ for the leachate, the largest for Phoslock®, and intermediate for the dissolved lanthanum nitrate.

Effect of Phoslock® on an *Anabaena* bloom

Anabaena (Cyanobacteria, PCC 7122 Pasteur Culture Collection, France) from a late-log culture was diluted in WC medium such that we obtained 212 ± 18 µg chlorophyll-*a* l^{-1} in the diluted suspension, of which 12 aliquots of 100 ml were incubated in closed transparent plastic containers at 20°C, 25 rpm orbital shaking and employing a 16:8 h light:dark rhythm, using 117 µmol quanta m^{-2} s^{-1} of light. After 1 day acclimatisation the containers were dosed with Phoslock® to obtain triplicate Phoslock® concentrations of 0, 0.1, 0.25 and 0.5 g l^{-1}. The total incubation lasted 18 days. Chlorophyll-*a* concentrations (µg l^{-1}) were measured using the PHYTO-PAM on days 0, 1, 3, 6, 9, 12, 15 and 18. The data were subjected to linear regression and slopes statistically evaluated in Graphpad Prism 5.04.

Effect of Phoslock® on growth of the rotifer *Brachionus calyciflorus*

Cysts of *Brachionus calyciflorus* Pallas (Microbiotests Inc. Nazareth, Belgium) were hatched in 100 ml WC medium at 21°C and continuous light (100 µmol quanta m^{-2} s^{-1}). Two to 3 h old neonates, were used in the experiments. Two Phoslock® series were prepared using a suspension of *S. obliquus* (2×10^7 μm^3 ml^{-1}, i.e. about 10 mg C l^{-1}) in WC medium—*S. obliquus* served as food for *B. calyciflorus*. The experiment was conducted in 24-well culture plates. The wells of one plate were filled with 2.5 ml of the algae-Phoslock® suspensions yielding Phoslock® concentrations of 0.0, 0.005, 0.05, 0.1, 0.5 and 5.0 g l^{-1} in fourfold. Wells in another plate were filled with 2.5 ml of the suspensions giving Phoslock® at concentrations of 0.0, 0.005, 0.05, 0.1, 0.2 and 0.4 g l^{-1}, also in four replicates. Hence, the Phoslock® concentrations 0, 0.005, 0.05 and 0.1 g l^{-1} were assessed twice in quadruplicate, making a testing in eight fold.

Each well was inoculated with two individuals of *B. calyciflorus*. The plates were incubated in the dark at 21°C, orbital shaking (40 rpm). After 48 h the wells were examined for living animals and population growth was stopped by adding 100 µl Lugol's solution. Counting was done by dissection microscope (15×).

Population growth rate (r) was computed as: $r = (\ln(t_2) - \ln(t_0))/\Delta t)$; t_0 = density at 0 h, t_2 = density at 48 h, $\Delta t = 48$ h. Negative r values were avoided by converting r to the finite rate of population increase $\lambda = e^r$ (Gilbert, 1996). Treatment effects on r were assessed by one-way ANOVA and Tukey post hoc comparison test. The homogeneity of variances was tested by Levene's test. The EC_{50} value (50% growth inhibition) was estimated by non-linear regression (4 parameter logistical model) in SigmaPlot 11.0.

Results

Capacity of Phoslock® to bind SRP

While the SRP concentrations in the controls remained constant, in the presence of Phoslock® they decreased exponentially ($[SRP_t] = SRP_0 e^{-\theta t}$) (Fig. 1). The

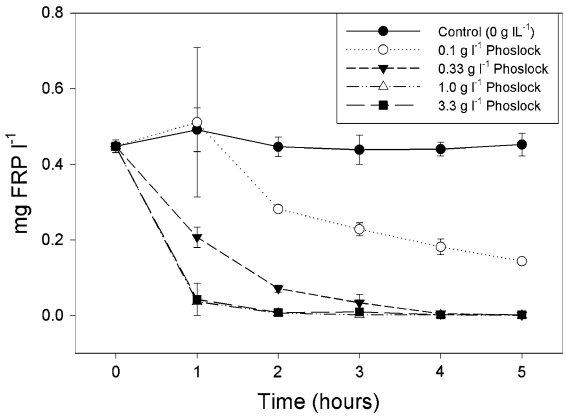

Fig. 1 Course of the filterable reactive phosphorus (SRP, mg l^{-1}) in incubations exposed to different concentrations of Phoslock® (0–3.3 g l^{-1}). *Error bars* indicate 1 SD ($N = 3$)

decrease rate (θ) at 22°C was proportional to the amount of Phoslock® added (Fig. 1). Log(θ) passed Levene's test for equal variances ($F_{3,8} = 3.59$, $P > 0.07$). The F test showed a significant dose effect ($F_{3,8} = 70.7$, $P < 0.001$). Tukey's test distinguished the decrease rates (θ) at 0.1 and 0.33 g l^{-1} from those at 1 and 3.3 g l^{-1} of Phoslock® (Table 1). The 1 and 3.3 g l^{-1} doses depleted SRP within an hour. The 0.33 g l^{-1} Phoslock® dose-depleted SRP within 3–4 h. The 0.1 g l^{-1} Phoslock® dose only bound 68% of the SRP in 5 h (Fig. 1; Table 1).

Nutrient leachates from Phoslock®

In nanopure water, Phoslock® seemed to release some SRP; although nitrite + nitrate was not released, ammonia was released significantly. We found significant differences between the two batches of Phoslock® in the amounts of SRP and ammonia released (Table 2).

Effect of Phoslock® on pH, conductivity (EC) and oxygen concentration

In both WC medium and pond water the lowest pH occurred at the highest Phoslock® dose (3.2 g l^{-1}) 2 h after addition. In contrast, in controls the highest pH occurred at the start. In WC medium median pH was 7.07 (range 6.99–7.30), in pond water median pH was 7.83 (range 7.16–7.89).

We observed a linear increase in EC with Phoslock® dose, which was higher for the WC medium than for pond water. The regression between dose and EC for WC medium was: EC = 257.4 + 17.77 × dose ($r^2 = 0.983$; $F_{1,6} = 351.0$, $P < 0.001$) and for pond water the regression was: EC = 249.4 + 9.937 × dose ($r^2 = 0.983$; $F_{1,6} = 796.4$, $P < 0.001$). Both the regression slopes deviated significantly from zero, both regressions differed significantly from each other ($F_{1,12} = 59.9$, $P < 0.001$).

The mean of all measurements ($n = 24$), for oxygen saturation % in WC medium and pond water was 99% (SD = 1): the ranges were 98–101 and 97–100%, respectively.

The effect of Phoslock® on turbidity in still water

In still water—under undisturbed conditions, one gram Phoslock® in 1 l nanopure water raised turbidity from 0.15 to 211 NTU (Fig. 2), after 5 min NTU dropped to 48 NTU and after 6 h to below 13 NTU, which value corresponded to about 1 m Secchi depth transparency. After 24 h turbidity further declined to reach 6.5 NTU (Fig. 2). The decline could best be described by a six parameter exponential decay function:

$$\text{NTU} = 263.89 \times \exp(-6108096 \times t) + 83.07 \times \exp(-2.9509 \times t) + 27.03 \times \exp(-0.1419 \times t)$$

with $F_{5,12} = 6648$; $P < 0.001$ and $r^2_{\text{adj}} = 0.9996$ (Fig. 2).

Table 2 Release of nutrients from Phoslock® (in mg kg^{-1} Phoslock®, mean (SD), $N = 6$)

	Batch 1			Batch 2			Batch 1–2		
	Release	T ($df = 5$)	P	Release	T ($df = 5$)	P	T'	df'	P
SRP	0.39 (0.20)	4.36	0.004	0.05	0.302	0.4	1.98	8	0.04
NN	2 (4)	1.12	0.16	4 (4)	2.24	0.04	0.87	10	0.80
NH_3	224 (3)	167.0	<0.001	53 (10)	11.85	<0.001	40.1	6	<0.001

T Student's T statistic, df degrees for freedom, T' Welch's T approximation, df' degrees of freedom according to Welch–Satterthwaite equation. Batch 1–2 indicates the difference in release of the nutrients (identified in column 1) between Phoslock® batches 1 and 2

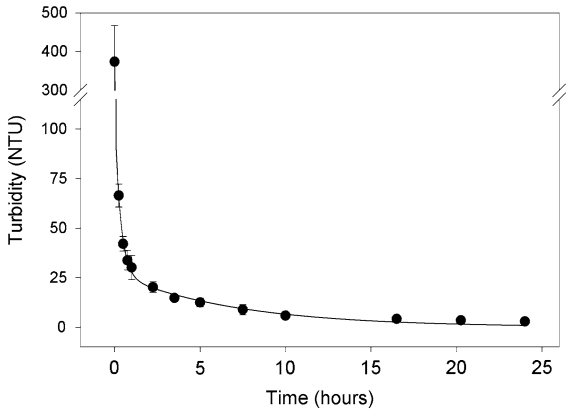

Fig. 2 Maximum undisturbed settling rate: course of the turbidity (NTU) in still water incubations of 1 g Phoslock® l^{-1}. The *solid line* represent non-linear regression: NTU = 263.89 × exp(−6108096 × *t*) + 83.07 × exp(−2.9509 × *t*) + 27.03 × exp(−0.1419 × *t*); $r^2_{adj} = 0.9996$. *Error bars* indicate 1 SD ($N = 3$)

Effect of Phoslock® on growth of *Scenedesmus* and *Microcystis*

The growth rates (μ) for *S. obliquus* in the controls of each series (i.e. Phoslock®, leachate and lanthanum) did not significantly differ (biovolume-based $F_{2,6} = 0.39$; $P = 0.693$; chlorophyll-*a*-based $F_{2,6} = 2.49$; $P = 0.163$) and μ was on average 1.19 day^{-1} (Table 3). Adding Phoslock®, its leachate or lanthanum resulted in a significant dose effect on μ in all series (Table 3). In the Phoslock® treatment doses ≥0.5 g l^{-1} reduced μ to negative values between −0.31 and −0.75 $days^{-1}$ meaning the biovolume concentrations had decreased compared to the start of the experiment (Table 3). After resuspending settled material, μ significantly increased (Fig. 3). However, the algae-free controls revealed this was due to Phoslock® particles (volume-based growth) and Phoslock® fluorescence (chlorophyll-*a*-based growth) at the highest doses (Fig. 3).

Tukey's test revealed higher growth rates (μ) for *M. aeruginosa* in the controls of the Phoslock® treatment (0.35 day^{-1}) than in the controls of the leachate (0.33 day^{-1}) and dissolved lanthanum nitrate (0.32 day^{-1}), but the difference could be attributed to small within-group variability ($F_{2,6} = 8.05$, $P = 0.020$, Table 3). The overall tests showed significant dose effects on μ in the Phoslock® and Lanthanum treatments, but not in the leachates (Table 3).

Effect of Phoslock® on an *Anabaena* bloom

The acclimatisation had no effect on the chlorophyll-*a* concentration of the incubations ($F_{3,8} = 1.83$; $P = 0.220$). After treatment a linear rate of change (positive and negative depending on dose) occurred in the chlorophyll-*a* concentrations of all incubations (Fig. 4). We observed growth in the control and 0.1 g Phoslock® l^{-1} treatment. Bloom termination occurred in case of 0.25 and 0.5 g Phoslock® l^{-1} doses (Fig. 3). Adding ≥ 0.25 g Phoslock® l^{-1} to the blooming *Anabaena* reduced the cyanobacteria concentration with 11 µg chlorophyll-*a* l^{-1} d^{-1} (Fig. 4).

The slope of the rate of change was 41.5 in the control compared with 28.4, −9.8 and −12.2 for the three treatments, 0.1, 0.25 and 0.5 g Phoslock® l^{-1}, respectively. All slopes significantly different from zero ($P \leq 0.01$). Three groups of significantly different slopes were identified: (1) control, (2) 0.1 g Phoslock® l^{-1}, (3) 0.25 and 0.5 g Phoslock® l^{-1}. The associated tests were:

Control versus 0.1 g Phoslock® l^{-1}: $F_{1,10} = 18.6$ ($P = 0.002$).
Control versus 0.2 g Phoslock® l^{-1}: $F_{1,10} = 689.9$ ($P < 0.001$).
Control versus 0.5 g Phoslock® l^{-1}: $F_{1,10} = 250.0$ ($P < 0.001$).
0.1 g Phoslock® l^{-1} versus 0.25 g Phoslock® l^{-1}: $F_{1,10} = 179.4$ ($P < 0.001$).
0.1 g Phoslock® l^{-1} versus 0.5 g Phoslock® l^{-1}: $F_{1,10} = 104.0$ ($P < 0.001$).
0.25 g Phoslock® l^{-1} versus 0.5 g Phoslock® l^{-1}: $F_{1,10} = 0.53$; $P = 0.482$.

Effect of Phoslock® on growth of the rotifer *Brachionus calyciflorus*

Population growth of *B. calyciflorus* decreased significantly with increasing Phoslock® concentrations ($F_{6,37} = 30.6$; $P < 0.001$) (Fig. 5). Up to concentrations of 0.1 g Phoslock® l^{-1} the rotifers expressed good growth (0.67–0.89 day^{-1}), but concentrations ≥0.2 g l^{-1} significantly inhibited population growth (Fig. 5). The 0.5 g l^{-1} Phoslock® treatment revealed some mortality and the treatment with 5.0 g l^{-1} was excluded from the analysis because it contained too much clay for reliable observations (Fig. 5). The EC_{50} based on finite rates of population increase ($\lambda = e^r$)

Table 3 Growth rates ($\mu \pm 1$ SD, in d^{-1}, $N = 3$) of the green alga *Scenedesmus obliquus* and the cyanobacterium *Microcystis aeruginosa* exposed for 3 and 7 days, respectively, to different concentrations of Phoslock® (dose in g l^{-1}), filtered leachate from a 50 g Phoslock® l^{-1} incubation, and lanthanum (dose in mg La l^{-1}), Phoslock® equivalent (dose in g l^{-1}) relating the filtrate and lanthanum doses to Phoslock® doses based on 5% La content

Treatment	Dose	Phoslock® equivalent	*Scenedesmus*		*Microcystis*
			μ_{volume} mean (SD)	μ_{CHL} mean (SD)	μ_{volume} mean (SD)
Phoslock®	0	0	1.18 (0.01)^{ab}	1.09 (0.01)^{A}	0.35 (0.01)^{A}
	0.005	0.005	1.20 (0.01)^{ab}	1.11 (0.01)^{A}	0.36 (0.03)^{A}
	0.05	0.05	1.26 (0.01)^{a}	1.09 (0.02)^{A}	0.40 (0.01)^{A}
	0.5	0.5	−0.31 (0.12)^{ab}	−0.65 (0.06)^{B}	−0.26 (0.04)^{B}
	5	5	−0.37 (0.82)^{ab}	−0.92 (0.03)^{C}	−0.21(0.04)^{B}
	50	50	−0.75 (0.32)^{b}	−0.99 (0.03)^{D}	−0.23 (0.07)^{B}
ANOVA			$H_5 = 15.3$	$F_{5,12} = 3578$	$F_{5,12} = 241.8$
P value			$P = 0.009$	$P < 0.001$	$P < 0.001$
Leachate	0%	0	1.19 (0.03)^{A}	1.11 (0.02)^{A}	0.33 (0.01)
	0.01%	0.005	1.19 (0.01)^{A}	1.10 (0.01)^{AB}	0.34 (0.00)
	0.1%	0.05	1.19 (0.01)^{A}	1.08 (0.01)^{B}	0.31 (0.02)
	1%	0.5	1.19 (0.01)^{A}	1.07 (0.01)^{BC}	0.32 (0.02)
	10%	5	1.15 (0.02)^{AB}	1.05 (0.01)^{C}	0.33 (0.01)
	100%	50	1.12 (0.01)^{B}	1.00 (0.01)^{D}	0.33 (0.00)
ANOVA			$F_{5,12} = 11.5$	$F_{5,12} = 45.4$	$F_{5,12} = 2.79$
P value			$P < 0.001$	$P < 0.001$	$P = 0.068$
Lanthanum	0	0	1.19 (0.00)^{ab}	1.09 (0.01)^{A}	0.32 (0.01)^{A}
	0.025		1.19 (0.00)^{ab}	1.09 (0.02)^{A}	0.33 (0.02)^{A}
	0.25	0.005	1.19 (0.01)^{a}	1.08 (0.01)^{A}	0.36 (0.01)^{A}
	2.5	0.05	1.17 (0.02)^{ab}	1.05 (0.01)^{A}	0.34 (0.00)^{A}
	25	0.5	0.64 (0.07)^{ab}	0.16 (0.02)^{B}	0.27 (0.02)^{B}
	250	5	0.46 (0.02)^{b}	0.17 (0.01)^{B}	0.19 (0.03)^{C}
			$H_5 = 13.7$	$F_{5,12} = 2739$	$F_{5,12} = 47.7$
			$P = 0.018$	$P < 0.001$	$P < 0.001$

$^{A–D}$ Pairwise multiple comparison procedures (Holm–Sidak method)

$^{a–d}$ Pairwise multiple comparison procedures (Tukey test)

was 0.154 ± 0.04 g Phoslock® l^{-1} ($F_{3,6} = 30.4$; $P = 0.010$; $r^2_{adj} = 0.936$). The no observed effect concentration was 0.1 g Phoslock® l^{-1} (Fig. 5).

Discussion

Capacity of Phoslock® to bind SRP

The lanthanum-modified clay Phoslock® removed SRP from the water at rates between 0.2 and 2.6 mg SRP l^{-1} h^{-1}. These results are in line with the published reports on the ability of Phoslock® to bind SRP (Robb et al., 2003; Douglas et al., 1999; 2004; Ross et al., 2008; Finkler Ferreira & Da Motta Marques, 2009; Haghseresht et al., 2009). We observed both a time-dependent and Phoslock® dose-dependent decrease of SRP. We, therefore, expect Phoslock® to be more effective in removing SRP from the water the longer it remains in suspension—allowing a longer reaction time, or when a higher dose of Phoslock® than the actual SRP concentration in the water column indicates is applied. In practice, this will be the case because Phoslock® should be dosed considering total amount of phosphorus present in both water and top 5–10 cm of the

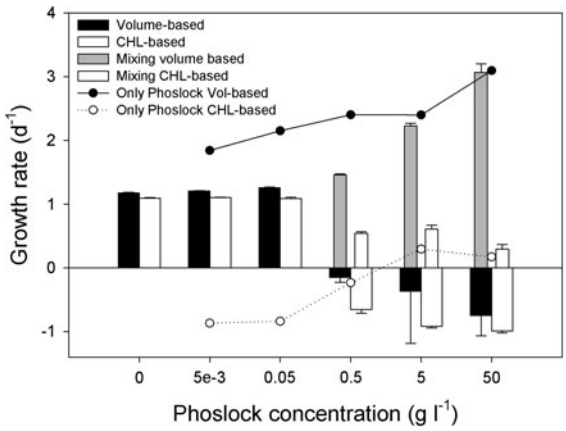

Fig. 3 Volume-based (*black bars*) and chlorophyll-*a*-based growth rates (*white bars*) of the green alga *Scenedemsus obliquus* exposed for 3 days to different concentrations of Phoslock®. Also included are the growth rates in the highest doses after a thorough resuspension of the sedimented material (*grey bars*) and the data obtained when 'growth' was estimated from the difference between the initial algal inoculum and the particles in purely Phoslock® incubations (*symbols* and *lines*). *Error bars* indicate 1 SD ($N = 3$)

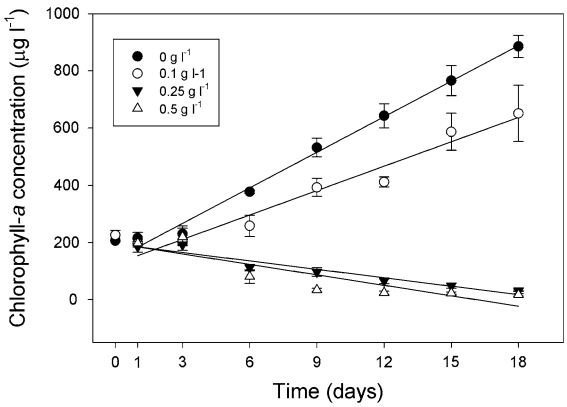

Fig. 4 Course of the cyanobacteria chlorophyll-*a* concentration (µg l^{-1}) in *Anabaena* populations exposed to different concentrations of Phoslock® (0–0.5 g l^{-1}) over an 18-day period. *Lines* represent linear regressions, while *error bars* indicate 1 SD ($N = 3$)

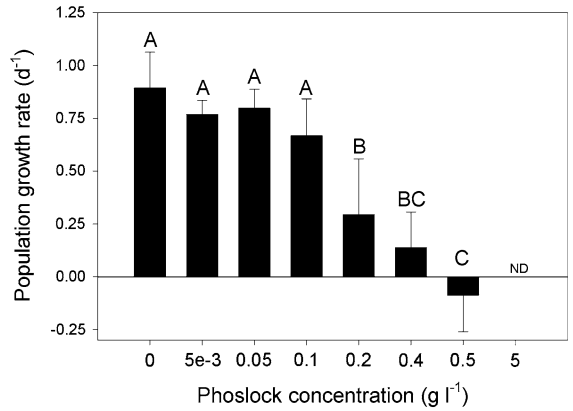

Fig. 5 Population growth rate (day^{-1}) of the rotifer *Brachionus calycilforus* exposed for 48 h to different concentrations of Phoslock® (0–5 g l^{-1}). Similar *symbols* indicate homogenous groups (Tukey test), while *error bars* indicate 1 SD ($N = 4$ or 8). ND means not determined as a result of high turbidity

sediment (Lürling & van Oosterhout, 2012). Our experiment was performed with a SRP solution (K_2HPO_4 in nanopure water) lacking compounds which could hamper the formation of rhabdophane. The effectiveness of lanthanum to bind SRP is hindered by naturally occurring oxyanions other than phosphate (Johannesson & Lyons, 1994) and complex-forming humic substances (Sonke & Salters, 2006; Tang & Johannesson, 2003, 2010). Such naturally occurring compounds could imply that the Phoslock® binding capacity is less than theoretically expected (Lürling & van Oosterhout, 2012). Further research could therefore be directed on the effectiveness of SRP removal by Phoslock® in natural waters varying in composition.

Nutrient leachates from Phoslock®

The amount of lanthanum present in Phoslock® should not allow release of SRP as we found. The SRP found in the leachates may be an overestimation of the molybdate-reactive phosphorus concentrations due to colloidal bentonite particles that pass through a 0.45 µm membrane filter used in such P determinations (Koopmans et al., 2005). Because colloidal bentonite particles may pass through a 0.45 µm membrane filter it may also be possible that other colloidal compounds pass through such filter. This implies both careful interpretation of the SRP concentrations and possible misidentification of other compounds, e.g. lanthanum, as being dissolved compounds. The release of ammonium from Phoslock® could be attributed to the soluble fraction in the bentonite (Hanway et al., 1956). Gibbs et al. (2011) observed NH_4-N release from Phoslock® treated sediment samples, which was probably caused by nitrification process as in anaerobic sediments (Gibbs et al., 2011). The release of ammonium as well as the apparent difference in the release of SRP and

ammonium between the two batches needs further investigation.

Effect of Phoslock® on pH, conductivity (EC) and oxygen concentration

The Phoslock® doses in the experiment discussed here (0.05–3.2 g l^{-1}) are high compared with the range of doses (0.046 and 0.085 g l^{-1}) applied in field experiments in the Netherlands (van Oosterhout & Lürling, 2011; Lürling & van Oosterhout, 2012). Hence, we do not expect an ecological relevant effect of Phoslock® on pH or EC during actual applications. Since metals are being released from a Phoslock® slurry, e.g. aluminium (Lürling & Tolman, 2010) and humic substances in the pond water can act as potent chelaters on metals such as aluminium (Omoike & VanLoon, 1999), the reduced increase in EC in pond water may be caused by the chelation of these metals in presence of these humic compounds. Although outside the actual field dosing range, our results indicate different effects of Phoslock® on EC in waters of different composition.

The effect of Phoslock® on turbidity in still water

We expect that on its application, Phoslock® will first cause an increase in turbidity—due to suspended clay particles, and thereafter a decrease in turbidity—due to growth limitation of phytoplankton (discussed below). The extent of increase in turbidity is difficult to predict as an application will be in an eutrophicated body of water, which could already have relatively high NTU due to blooming phytoplankton.

We performed our turbidity experiment in still water, implying a maximum undisturbed settling rate of Phoslock® particles, which is in agreement with other studies (Haghseresht, 2005; Ross et al., 2008). In Lake Rauwbraken (The Netherlands), applying Phoslock® to the water caused a 2-day period of elevated turbidity up to 18 NTU (Van Oosterhout & Lürling, 2011). Having Phoslock® suspended in the water column for a few days is likely to enhance its effectiveness as it will deplete the water of SRP more effectively than if Phoslock® were to rapidly sink to the bottom. The settling of Phoslock® particles will depend on turbulent water movements, dictated by local circumstances.

Effect of Phoslock® on growth of *Scenedesmus* and *Microcystis*

Based on the 5% La content specified by the manufacturers of Phoslock®, the different doses of Phoslock® (0, 0.005, 0.05, 0.5 and 5.0 g l^{-1}) should bind comparable amounts of SRP as the respective lanthanum nitrate doses (0, 0.25, 2.5, 25 and 250 mg l^{-1}). Based on this, we expected to observe similar and proportionate reductions of the growth rates of *S. obliquus* and *M. aeruginosa* by treatment, respectively, with the Phoslock® suspension and free dissolved lanthanum nitrate. Based on a 4.4% lanthanum content in our Phoslock® batch (unpubl. data), the lanthanum nitrate doses should actually be more effective than their equivalent Phoslock® doses. We ascribe the larger effect of Phoslock® to the presence of the bentonite particles, which may have caused flocculation of phytoplankton cells. Several existing studies (Anderson, 1997; Pan et al., 2006, 2011a, b; Verspagen et al., 2006) show that flocculation with natural clay particles—without modification with lanthanum, can control existing blooms. For example, a bentonite concentration as low as 15 mg l^{-1} resulted in increased sedimentation of *Microcystis* (Verspagen et al., 2006). Otherwise, elevated turbidity due to the presence of Phoslock® will cause light limitation in addition to the flocculation. For this effect we had no control in our experiment. While the reduced growth observed with lanthanum nitrate may be interpreted as SRP limitation and indicates that the La-SRP bond is strong enough to ensure that SRP is no longer bioavailable, our experiment does not exclude a possible harmful effect of La to the algae. The potential harmful effect of La to algae and other biota needs further investigation. The leachates of Phoslock® contain between 0.001 and 0.02% of the lanthanum present in Phoslock® (NICNAS, 2001; Lürling & Tolman, 2010). Thus, the expected growth inhibition based on the binding of SRP to lanthanum is much lower than either the Phoslock® suspension or lanthanum nitrate solution will cause.

Effect of Phoslock® on an *Anabaena* bloom

Concerning the experiment to control *Anabaena* sp. bloom, similar remarks apply as made for *S. obliquus* and *M. aeruginosa.* Because we observed rapid SRP binding in the SRP binding experiment, we feel safe to

attribute at least part of the observed effects to SRP binding by Phoslock®. Due to the absence of the bentonite control, these experiments cannot be considered as a formal proof of growth limitation due to SRP binding by Phoslock®. Still, Phoslock® can be considered as an effective agent to reduce phytoplankton growth. The combined effects of both SRP binding and flocculation of phytoplankton cells may prove quite useful in actual field applications.

Whereas we performed our turbidity experiment in still water, our algae growth experiments were performed under constant agitation two extreme opposite ends of the natural conditions. Hence our results on turbidity and reduced algal growth should be interpreted as best case scenario's, not very likely to occur this positive under field conditions.

Effect of Phoslock® on growth of the rotifer *Brachionus calyciflorus*

The population growth of the rotifer *B. calyciflorus* was reduced at Phoslock® concentrations of 0.2 g l^{-1} and higher. The observed effect we can only attribute to the presence of Phoslock® as a whole, i.e. we cannot distinguish between the effect of particles hampering the feeding or any other direct toxic effect. We consider concentrations of Phoslock® above 0.2 g l^{-1} as relatively high compared with doses applied during the actual field applications, e.g. 0.046 and 0.085 g l^{-1} (Lürling & van Oosterhout, 2006; van Oosterhout & Lürling, 2011). However, Phoslock® concentrations during and shortly after the surface addition from a barge will be much higher than the estimated EC_{50} for growth inhibition (ca. 0.15 g l^{-1}). Therefore, a field application of Phoslock® may have a negative effect on rotifers.

Conclusion

Our results confirm the potential of Phoslock® to bind SRP. Its effectiveness in natural waters to bind SRP needs further investigation, especially in the presence of other oxyanions and humic substances. The effectiveness of Phoslock® to bind SRP depends on both dose and reaction time, where natural occurring mixing (wind action) could promote the SRP binding. We consider the small amounts of SRP released from Phoslock® of no serious consequence to the effectiveness of Phoslock®; especially since this release is most probably caused by interference of clay particles with the SRP measurement. The implications of the differences between batches of Phoslock® and its release of ammonium also need further investigation. Phoslock® had no relevant effect on pH and oxygen saturation in the water types we tested. Albeit at concentrations above actual field applications, the effect of Phoslock® on conductivity varies with water types. An application of Phoslock® will temporarily increase turbidity. Due to its rapid settlement we do not expect a field application of Phoslock® to cause prolonged light limitation for submerged macrophytes. Under constant agitation Phoslock® causes a dose related reduced growth of phytoplankton species *S. obliquus* and *M. aeruginosa* and it was effective in controlling an *Anabaena* sp. bloom during prolonged exposure in our experiment. We attribute this effect to SRP binding in combination with light limitation and flocculation of the phytoplankton cells with the bentonite, which we expect to be less under field conditions due to rapid sinking of the Phoslock® particles. The population growth of the rotifer *B. calyciflorus* is reduced by the presence of Phoslock® (EC_{50} = 0.15 g l^{-1}). Overall, the results of our study, i.e. SRP binding and unequivocal phytoplankton growth reductions in combination with low effects on water quality variables, provide enough grounds to conduct further field trials with Phoslock®.

Acknowledgements We are grateful to Dr. Ramesh Gulati for his help to improve our manuscript and wish to thank Mr. Nigel Traill and Mr. Patrick van Goethem (Phoslock Europe GmbH) for donating the Phoslock®.

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