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Bioavailable phosphorus (P) reduction is less than mobile P immobilization in lake sediment for eutrophication control by inactivating agents

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ABSTRACT

Phosphorus (P) immobilization by inactivating agents in the sediment of eutrophic lakes to reduce immediately available P in lake water is often crucial for mitigating nuisance eutrophication symptoms, such as cyanobacterial blooms. Macrophytes and phytoplankton, however, can directly utilize P from the sediment for growth. Accordingly, a comprehensive analysis of the P bioavailability in lake sediment amended with two promising P-inactivation agents, namely Phoslock® and drinking water treatment residue (DWTR), was investigated in both short- and long-term studies (20 and 180 d). Phosphorus-availability was assessed using six chemical extraction methods and *Hydrilla verticillata* and *Microcystis aeruginosa* growth tests. The results showed that Phoslock® and DWTR significantly reduced mobile P (NH_4Cl and $Na_2S_2O_4$/$NaHCO_3$ extractable P) in lake sediment, while P bioavailability that was assessed by different methods showed considerable deviations. Interestingly, appropriate bioavailable P chemical extraction methods were determined based on linear correlation analysis, and further comparison indicated that reduction of bioavailable P by DWTR (<55% for macrophyte available P) and Phoslock® (<17% for cyanobacteria available P) were clearly less than the mobile P immobilization (>75%) at recommended dosages, which was probably caused by the capability of macrophyte and cyanobacteria to utilize various fractions of P (except the residual P) in amended sediment under proper illumination. Therefore, DWTR and Phoslock® can effectively reduce P release from lake sediment, but the potential bioavailable P may pose uncertainties for eutrophication control in lakes that typically have regular sediment re-suspension. Overall, an evaluation of the bioavailable P pool in the lake ecosystem should be essential for successful lake geo-engineering.

1. Introduction

The necessity of reducing internal phosphorus (P) loading from lake sediment for eutrophication control has been well accepted by scientists and lake managers (Huser et al., 2016a). *In situ* geo-engineering in lakes is a commonly considered method for internal pollution control by dosing P-inactivation agents (Spears et al., 2014). Thus far, many inactivating agents have been tested (Zamparas and Zacharias, 2014; Funes et al., 2016); typically, Phoslock®, a lanthanum-modified clay developed by CSIRO Australia (Douglas, 2002), is increasingly being used for controlling lake internal P loading. Significant control has been demonstrated in both laboratory and field studies in different regions (Copetti et al., 2016). Another P-inactivation agent that has potential to be applied in the geo-engineering method is a by-product generated during potable water production, namely drinking water treatment residue (DWTR) (Wang et al., 2012). DWTR often contains high concentrations of aluminum (Al) and iron (Fe) due to the use of Al

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and Fe coagulants during water treatment processes. Laboratory studies suggested that DWTR can effectively control P release from lake sediment and exhibited high control stability (Wang et al., 2013a).

Unfortunately, the lake geo-engineering method has remained contentious because of the variable results reported in the literature (Spears et al., 2014). Accordingly, the experiences of researchers and water managers on a global scale were communicated (Lürling et al., 2016), and they considered that determining the control stability of P-inactivation agents was essential to developing protocols for applying the geo-engineering method (Spears et al., 2014). The control stability under the influence of various physicochemical factors has been investigated (Wang and Jiang, 2016), and the pH, organic matter, redox conditions, and soluble sulfide (e.g., Ross et al., 2008; Lürling et al., 2014; Dithmer et al., 2016; Wang et al., 2016), as well as the dosages, Osgood index, and watershed to lake area ratio (Huser et al., 2016b), could affect the effectiveness of P-inactivation agents. Such information is necessary for successful lake restoration.

Interestingly, it has been reported that the slow release of P from cyanobacterial blooms may cause variable control of lake eutrophication by inactivating agents (including Phoslock® and DWTR) (Wang et al., 2016). Logically, investigation of bioavailable P in the environment (especially sediment) treated by inactivating agents is essential to understanding the applicability of the geo-engineering method because macrophyte and phytoplankton can directly utilize P from sediment for growth (Granéli and Solander, 1988; Ellis and Stanford, 1988). However, there is limited information in the literature regarding P bioavailability in amended sediment. This may be because most studies have mainly focused on controlling the release of P from lake sediment to reduce immediate available P (orthophosphate) in lake water using inactivating agents (Zamparas and Zacharias, 2014).

Therefore, a comprehensive analysis of P bioavailability in lake sediment amended with Phoslock® and DWTR was performed (Fig. 1). This study first assessed the short- and long-term mobile P immobilization using fractionation. Then, the bioavailability of P in the amended sediments was determined. According to the reported literature, we summarized and used five methods for sediment bioavailable P analysis (Fig. 1), which were divided into liquid- and solid-phase chemical extractions. We hypothesized that the bioavailability of P in the amended sediments was strongly reduced. Here, biological growth tests were applied to establish the potential relationship between biological responses and the variation of P forms in amended sediment. The results of this study will provide theoretical support for the *in situ* lake geo-engineering method.

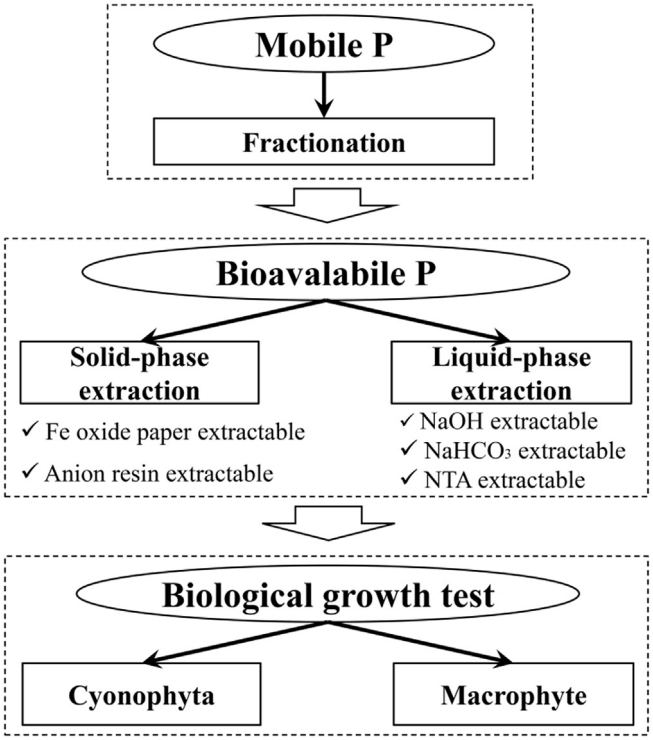

Fig. 1. The framework of this study.

2. Materials and methods

2.1. Sample preparations

Phoslock® was provided by Fusiyueke LLC in Sichuan, China. Dewatered DWTR was collected from Beijing City No. 9 Waterworks in China in April 2012. Detailed characteristics of the DWTR have been reported in previous studies (Wang et al., 2014). The surface lake sediment, at a depth of 0–5 cm, was sampled by a column sampler at Meiliang Bay (31°32' N, 120°10' E) in Lake Taihu, China in March 2015. Lake water was sampled at the same location at a depth of approximately 0.5 m.

The sediment incubation test was performed according to the method reported in Wang et al. (2012). Aliquots of 500 g of wet sediment were completely mixed with DWTR or Phoslock® in a series of brown glass bottles. The dry weight proportions of DWTR or Phoslock® in amended sediment were 0% for the control group, and 2.5%, 5%, 10%, and 20% for the treatment. Lake water was added, resulting in 3–5 mm of overlaying water in the reactors. The reactors were capped and stored in a constant-temperature culture box at 15 ± 2.0 °C. To assess the bioavailability of P in both the short- and long-term, duplicate samples were collected on days 20 and 180, and they were stored at 4 ± 0.5 °C for further analysis.

2.2. The distribution of P in lake sediment

Phosphorus fractionation was determined according to the method reported in Christophoridis and Fytianos (2006); P was divided into NH_4Cl extractable P (NH_4Cl-P), $Na_2S_2O_4/NaHCO_3$ extractable P (BD-P), NaOH extractable inorganic P (NaOH-IP), NaOH extractable organic P (NaOH-OP), HCl extractable P (HCl-P), and residual P (Residual-P). In a second series, NaOH extractable P (NaOH-P) (Ellis and Stanford, 1988), $NaHCO_3$ extractable P ($NaHCO_3$-P) (Zhou et al., 2001), and nitrilotriacetic acid extractable P (NTA-P) (Ellis and Stanford, 1988) were analyzed. Iron oxide paper strip extractable P (Fe oxide paper-P) and Dowex 1 × 8 anion resin extractable P (Anion resin-P) were finally determined according to the methods reported in Sharpley (1993), Sibbesen (1978) and Uusitalo et al. (2000), respectively. All analyses, including P fractionation, NaOH-P, $NaHCO_3$-P, NTA-P, Fe oxide paper-P, and Anion resin-P, were based on three replicates.

2.3. Biological growth tests

Macrophyte growth assay: *Hydrilla verticillata* (*H. verticillata*), a typical macrophyte in entropic lakes, was collected from Lake Taihu and grown in sediment under natural conditions for 30 d at 25–35 °C in summer, which is similar to those in the field. The new plants were harvested for the test. In this test, wet sediment was completely mixed with DWTR or Phoslock® in a series of 250 mL beakers. The dry weight proportions of DWTR or Phoslock® in amended sediment were 0% for the control group and 2.5%, 5%, 10%

and 20% for the treatments, which were tested in triplicate. Then, in each beaker, three *H. verticillata* were planted in the sediment, and the beakers were placed into 5 L beakers. Approximately 4 L of filtered lake water was slowly added in the beakers to avoid sediment resuspension. The beakers were incubated under similar conditions in which the plants were cultured. After incubation (30 d), the plants were harvested, cleaned with deionized water, and oven-dried (50 ± 5 °C) for analysis.

Cyanobacteria growth assay: *Microcystis aeruginosa* (*M. aeruginosa*) isolated from Lake Taihu was used. *M. aeruginosa* was cultured in 1 L flasks containing 500 mL of BG-11 medium at 25 ± 2 °C with light irradiance of 32 μmol photons m^{-2} s^{-1}. Prior to the assay, cultures of P-starved *M. aeruginosa* were prepared following the method described in Sharpley (1993). Here, P-free medium was prepared based on BG-11 medium using KCl in place of K_2HPO_4. Two concentrations of P-free medium were used, namely 100% and 10% of BG-11 medium. The adopted incubation times were 21 d for 100% BG-11 medium and 10 d for 10% BG-11 medium, which were determined according to preliminary studies, indicating a phase of stationary growth without cultures yellowing. The cyanobacteria growth assay started by adding 0.1 g of wet sediment to 59 mL of P-free medium in 150 mL Erlenmeyer flasks. The sediments were the samples after 20 d of incubation as described above. The suspensions were inoculated with 1 mL of P-starved cyanobacteria to attain cell densities of approximately 2×10^4 cells mL^{-1} and incubated under the same conditions at which *M. aeruginosa* was precultured. Flasks were manually shaken twice daily. In addition, a relatively large volume (600 mL solution in 1 L Erlenmeyer flasks) incubation based on 100% BG-11 medium (P-free) was prepared. On the first 21 d, the incubation was performed under the same conditions of the cyanobacteria growth assay; thereafter, the incubation was performed at 25 ± 2 °C in the dark for the next 28 d. Each incubation consisted of three replicates.

2.4. Analytical methods

Phosphorus was measured by the method of Murphy and Riley (1962). Chlorophyll *a* (Chl *a*) concentrations in the cyanobacteria growth assay were determined according to Lorenzen (1967). In the large volume cyanobacteria assay, in addition to Chl *a*, pH (pH-10 electrode, Sartorius, Germany), total organic carbon (TOC, using a TOC analyzer, Torch, Teledyne Tekmar, USA), and chromophoric dissolved organic matter absorption ratio of 250 and 365 nm ($\alpha_{250}/\alpha_{365}$) (UV2550, Shimadzu, Japan) were determined. Macrophyte utilized P was analyzed after the harvested *H. verticillata* was treated under 450 ± 20 °C (3 h), followed by acid digestion in 3.5 M HCl (Ruban et al., 2001). The increased dry weight of the plant was determined by subtracting the initial dry weight from the harvested dry weight. Statistical analysis was performed by using IBM SPSS Statistics 19 for linear correlations analysis and one-way analysis of variance (ANOVA; $\alpha = 0.05$).

The detailed materials and methods are presented in Supporting information.

3. Results and discussion

3.1. Mobile P in amended sediment

Commonly, NH_4Cl-P and BD-P, determined in lake sediment, are viewed as mobile P (Christophoridis and Fytianos, 2006). Accordingly, data of P fractionation were first analyzed to understand P immobilization by inactivating agents (Table 1). Clear responses of BD-P, NaOH-IP, and HCl-P contents to dosages were observed. DWTR significantly reduced BD-P ($p < 0.01$), while NaOH-IP was increased ($p < 0.01$); Phoslock® significantly reduced BD-P ($p < 0.01$) and NaOH-IP ($p < 0.05$), while HCl-P was increased ($p < 0.01$). Therefore, P immobilization in lake sediment was achieved by transforming BD-P to NaOH-IP for DWTR and by transforming BD-P to HCl-P for Phoslock®. Similar results have previously been obtained (e.g., Meis et al., 2012; Wang et al., 2016). Further analysis revealed that compared to the 20 d incubations, BD-P significantly ($p < 0.01$) increased in the control after 180 d incubation, which could be because of the transformation from NaOH-IP, NaOH-OP, and Residual-P (Table 1). However, the reductions for BD-P in the amended sediment increased from 31-90% to 57–95% for DWTR and from 46-92% to 54–96% for Phoslock® as the incubation increased to 180 d. These findings suggested that the immobilization of mobile P by DWTR and Phoslock® was stable.

According to the initial mobile P content in sediment and to the methods for inactivating agent dosage determination (Reitzel et al., 2013; Wang et al., 2013b), the calculated recommended dosages (w/w) were 12.7% of dry sediment for DWTR and 5.4% of dry sediment for Phoslock®. Corresponding to this study, the closest dosages to the recommended value for DWTR and Phoslock® were 10% and 5%, respectively. At these dosages, DWTR and Phoslock® effectively reduced BD-P by 77–91% and 75–80% in both the short- and long-term, suggesting an effective P immobilization by these agents at recommended dosages. The BD-P removal by Phoslock®, however, reached 95% in the 180 d trial at doses of 10%.

Table 1
The results of P fractionation (mg g^{-1} dry-sediment).

Fractions[e]	Time (d)	Control	DWTR				Phoslock®			
			2.5%	5%	10%	20%	2.5%	5%	10%	20%
NH_4Cl-P	20	0.000765	0.000586	0.000611	0.000416[a]	0.000372[b]	0.000586	0.000340[b]	0.000554	0.000434
	180	ND[f]	ND	ND	ND	ND	ND	ND	ND	ND
BD-P	20	0.268	0.184[b]	0.117[b]	0.0602[b]	0.0273[b]	0.146[b]	0.0673[b]	0.0277[b]	0.0205[b]
	180	0.333[d]	0.145[b]	0.0759[b, d]	0.0299[b, d]	0.0162[b, d]	0.154[b]	0.0659[b]	0.0181[b, d]	0.0125[b, c]
NaOH-IP	20	0.230	0.358[b]	0.414[b]	0.471[b]	0.419[b]	0.202	0.173[b]	0.114[b]	0.0993[b]
	180	0.197	0.300[b, c]	0.421[b]	0.429[b]	0.450[b]	0.184[a]	0.122[b, c]	0.0621[b, c]	0.0519[b, d]
NaOH-OP	20	0.0462	0.0749	0.0747	0.0831	0.105	0.0423	0.0476	0.0312	0.0279
	180	0.0409	0.0470	0.0608	0.0872	0.0993[a]	0.0400	0.0558	0.0430	0.0332
HCl-P	20	0.175	0.208	0.204	0.204	0.189	0.352[b]	0.493[b]	0.551[b]	0.552[b]
	180	0.197	0.177	0.214[a]	0.210	0.227[b, d]	0.378[b]	0.465[b]	0.537[b]	0.536[b]
Residual-P	20	0.113	0.128	0.114	0.126	0.109	0.104	0.114	0.134[a]	0.117
	180	0.0840[d]	0.0854	0.101[c]	0.113[b]	0.123[b]	0.104	0.101	0.114[a]	0.0980

[a] and [b] Represent significant differences that are found at $p < 0.05$ and 0.01 between the control and the amended sediment at certain time.
[c] and [d] Represent significant differences that are found at $p < 0.05$ and 0.01 for the determined P contents between 20 and 180 d.
[e] The standard deviation of each extraction are within 10% (n = 6).
[f] Not detectable.

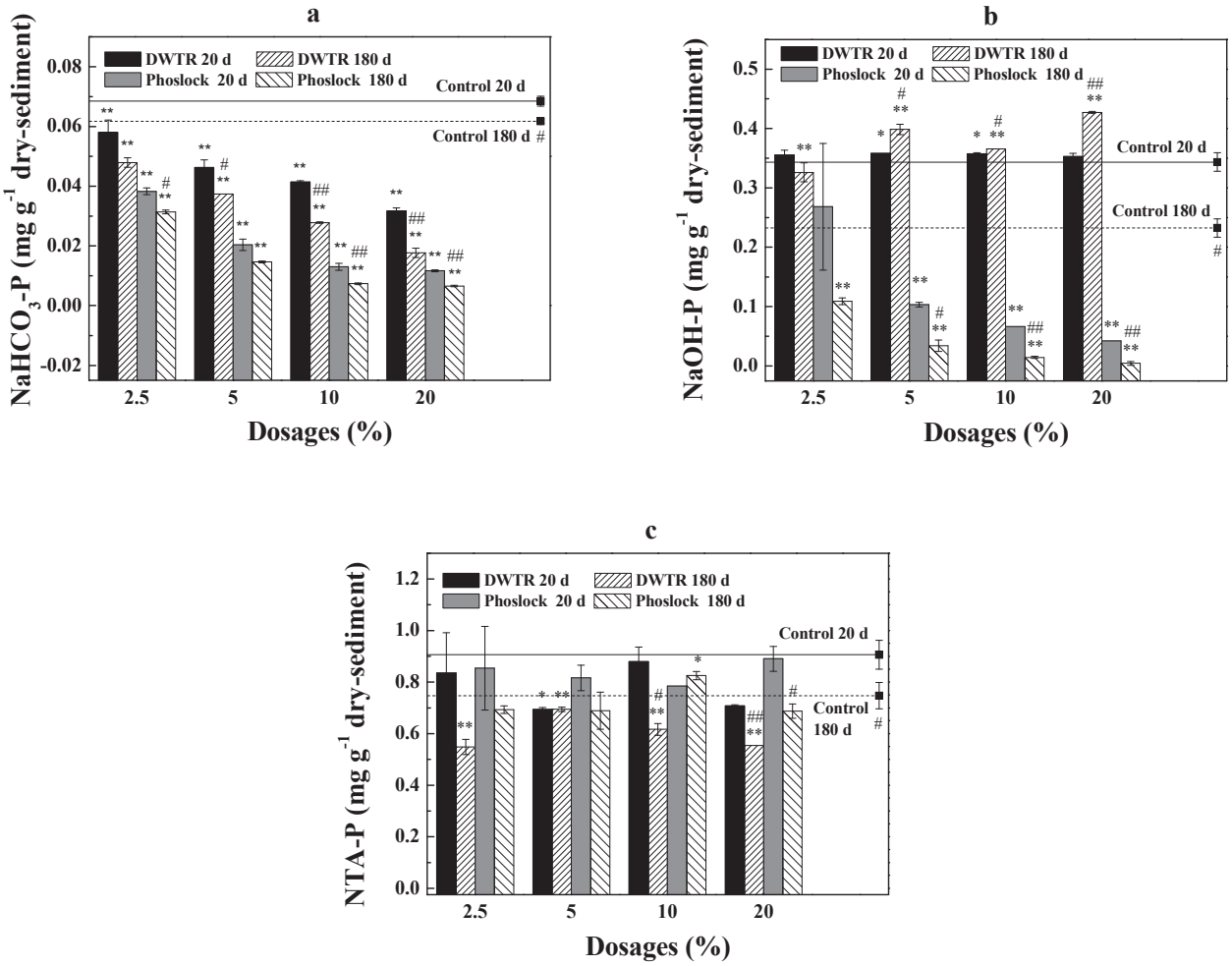

Fig. 2. The bioavailable P assessed using liquid-phase extraction in amended sediment. * and ** represent significant differences that are found at $p < 0.05$ and 0.01 between the control and the amended sediments at certain time; # and ## represent significant differences that are found at $p < 0.05$ and 0.01 between 20 and 180 d.

3.2. Bioavailable P in the amended sediment

3.2.1. Liquid-phase extraction

The bioavailable P in the amended sediment determined by liquid-phase extraction are shown in Fig. 2. The $NaHCO_3$-P contents in amended sediment gradually decreased ($p < 0.05$) as the dosages increased (Fig. 2a). As the incubation increased from 20 to 180 d, the reduction of $NaHCO_3$-P increased from 15-54% to 24–71% for DWTR and from 44-83% to 49–89% for Phoslock® at different dosages. These results suggested the stable $NaHCO_3$-P immobilization by DWTR and Phoslock®.

The variation of NaOH-P had different trends in sediments with DWTR and Phoslock® (Fig. 2b). DWTR increased the NaOH-P content; in particular, for amended sediment after 180 d incubation, NaOH-P increased ($p < 0.05$) from 0.232 to 0.427 mg g^{-1} as the dosages increased to 20%. In contrast, Phoslock® reduced NaOH-P contents as the dosages increased, and the reductions were more significant ($p < 0.05$) in the amended sediment after 180 d of incubation, and there were reductions of 53–98% at different dosages. These results suggested that DWTR had no capacity to immobilize NaOH-P, while Phoslock® had immobilization capacity.

Regarding NTA-P (Fig. 2c), the changes did not express a clear relationship with the dosages, and the reductions in the NTA-P showed little difference in the amended sediment after 20 and 180 d incubation, where NTA-P decreased by 4–27% in DWTR amended sediment and by 3–14% for Phoslock®, except that NTA-P in Phoslock® amended sediment increased by 10% at a dosage of 10% after 180 d of incubation. These results suggested that DWTR and Phoslock® had limited capacity to immobilize NTA-P.

3.2.2. Solid-phase extraction

The bioavailable P in amended sediment determined using solid-phase extraction are shown in Fig. 3. The variation of Fe oxide paper-P had different trends in sediments with DWTR and Phoslock® (Fig. 3a). DWTR decreased Fe oxide paper-P contents and the reduction generally increased with higher dosages. Furthermore, as the incubation increased from 20 to 180 d, the reduction weakened at dosages of 2.5–5%. However, at dosages of 10–20%, the reduction was enhanced ($p < 0.05$), and the reduction proportions increased from 43-55% to 63–73%. In sediment with Phoslock®, the variation of the Fe oxide paper-P did not exhibit clear trends with the time and dosages. In general, Phoslock® increased the Fe oxide paper-P contents at dosages of 2.5–5%, and the increase potential was enhanced as time increased, with proportions increasing from 16-18% to 48–56%. In contrast, at dosages of 10–20%, Phoslock® tended to decrease the Fe oxide paper-P contents, but the function was not stable; typically, the reduction proportions decreased from 25% to 4% as the time increased to 180 d at a 20% dosage. These results suggested that DWTR could immobilize Fe oxide paper-P, especially at dosages of 10–20%, while Phoslock® had limited immobilization capacity.

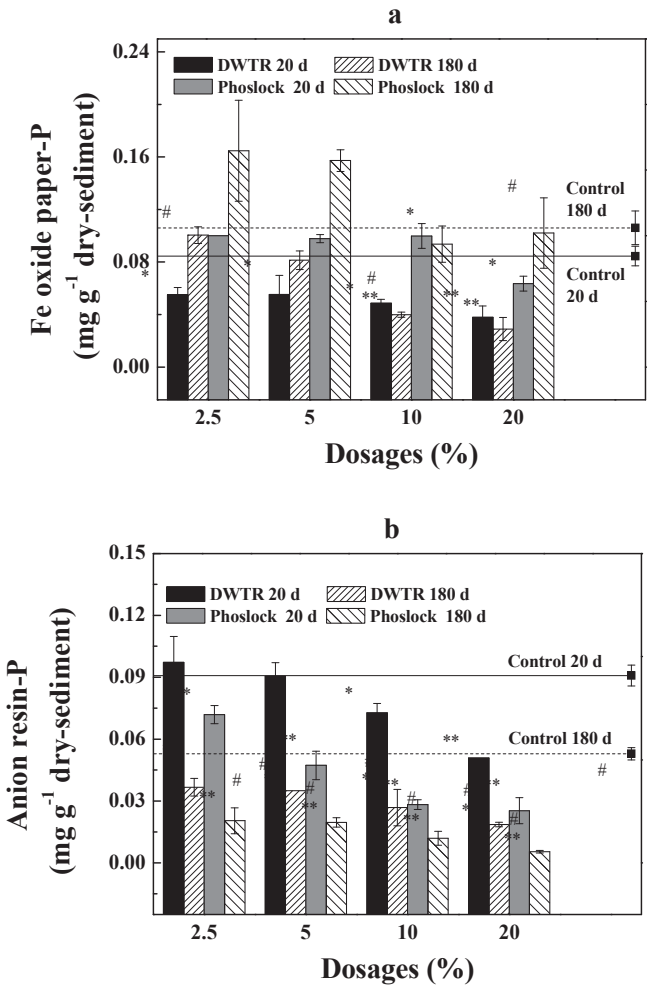

Fig. 3. The bioavailable P assessed using solid-phase extraction in amended sediment. * and ** represent significant differences that are found at $p < 0.05$ and 0.01 between the control and the amended sediments at certain time; # and ## represent significant differences that are found at $p < 0.05$ and 0.01 between 20 and 180 d.

The contents of Anion resin-P in sediments with DWTR and Phoslock® decreased as the dosages increased at different time, except that Anion resin-P in DWTR amended sediment after 20 d incubation slightly increased (by 7%) at a dosage of 2.5% (Fig. 3b). Furthermore, as the time increased from 20 to 180 d, the reduction of Anion resin-P was enhanced, with reduction proportions that increased from 0.3-44% to 31–65% for DWTR and from 21-72% to 61–90% for Phoslock® at different dosages. These results suggested stable Anion resin-P immobilization by both agents. Overall, the different extraction methods altered the estimates of bioavailable P in amended sediment.

3.3. The variation of H. verticillata and M. aeruginosa growth

The utilized P and increased biomass of *H. verticillata* after growth tests are shown in Fig. 4a and b. The utilized P by *H. verticillata* varied with the addition of different agents (Fig. 4a). Utilized P in DWTR exposures did not show a clear response with dosages, and the reduction varied between 9 and 32%. In contrast, Phoslock®-exposed plants showed gradually decreased utilized P as the dosages increased ($p < 0.01$), and there were reductions reaching 45% at a dosage of 20% (Fig. 4a). These results suggested that the macrophyte available P was not substantially influenced by DWTR, while Phoslock® reduced the macrophyte available P. Nonetheless, the variation of the *H. verticillata* biomass was similar to DWTR and Phoslock® treatments (Fig. 4b). The biomass decrease was remarkable at dosages of 2.5% and was even significantly lower than in controls ($p < 0.05$ or 0.01), but at all other dosages, the decrease in the biomass weakened, and the biomass was similar to those in the controls at the highest dose (20%). These results suggested that DWTR and Phoslock® had a marginal effect on the growth of *H. verticillata* in terms of biomass.

The variation of the increased total Chl *a* contents in cultures was normalized based on dry-sediment after *M. aeruginosa* growth assay, as shown in Fig. 4c and d. In order to understand cyanobacteria growth under different nutrition levels, 100% and 10% BG-11 media (P-free) were used. The aim of normalizing the Chl *a* level was to reflect the Chl *a* productivity of sediment from *M. aeruginosa*. For *M. aeruginosa* in 100% BG-11 medium (Fig. 4c), DWTR and Phoslock® decreased the Chl *a* levels, but the decrease was similar at dosages of 2.5–10% with reduction proportions by 17.9–25.5% and 11.6–18.2% for DWTR and Phoslock®. Additionally, a statistically significant decrease was observed at a dosage of 20%, with reduction of 41.4% for DWTR ($p < 0.05$) and 45.1% for Phoslock® ($p < 0.01$). For *M. aeruginosa* in 10% BG-11 medium (Fig. 4d), DWTR and Phoslock® decreased Chl *a*, but the decrease exhibited high standard errors (within 15.7–73.3%) and varied in different characteristics between the two agents. As the dosages increased, the decrease of Chl *a* did not follow a rule for DWTR at different dosages, and the significant decrease was observed at dosages of 2.5% ($p < 0.01$) and 10% ($p < 0.05$) with reduction of 75.5–82.8%. Phoslock® slowly decreased Chl *a* at dosages of <5%, and the reduction was within 30.6%, while a drastic decrease ($p < 0.01$) of Chl *a* was found at dosages of >10% with a reduction of 79.9–86.8%. These results suggested that inactivating agents that are applied to sediment inhibited cyanobacteria growth, but the inhibition changed with nutrition levels and the applied agents. Notably, both DWTR and Phoslock® demonstrated little toxicity to the environment (Copetti et al., 2016; Yuan et al., 2016). Therefore, the responses of *H. verticillata* and *M. aeruginosa* growth herein should not be due to the toxic effect of inactivating agents; instead, they are from nutrient limitation.

3.4. Assessment of P bioavailability in amended sediment

3.4.1. Determination of the appropriate bioavailable P chemical extraction

The five bioavailable P chemical extraction methods used (Fig. 1) have been reported to effectively indicate P bioavailability in sediment or soil. $NaHCO_3$-P had a good correlation ($R^2 = 0.86$) with P taken up by pot-grown ryegrass (Sibbesen, 1978), and it was regarded as a quantitative index of algae available P (Zhou et al., 2001). NaOH-P and NTA-P exhibited significance correlations ($p < 0.05$) with *Selenastrum* available P (Ellis and Stanford, 1988). Fe oxide paper-P was closely related ($p < 0.001$) to the growth of *Anabaena*, *Euglena*, *Selenastrum*, and *Ankistrodesmus* (Sharpley, 1993). Anion resin-P also had a good correlation ($R^2 = 0.90$) with P taken up by pot-grown ryegrass (Sibbesen, 1978), and it was considered to imitate the depletion of surface-adsorbed P in fresh water (Uusitalo et al., 2000). Interestingly, the extracted contents of these P showed considerable deviations in amended sediment (Figs. 2 and 3), which were inconsistent with our hypothesis. To identify appropriate methods for bioavailable P assessment, connections between the biological responses and variation of different chemically extracted bioavailable P were analyzed (Fig. 5).

H. verticillata utilized P showed positive linear correlations with $NaHCO_3$-P ($p < 0.01$, $R^2 = 0.662$) and Anion resin-P ($p < 0.01$, $R^2 = 0.635$) (Fig. 5a), suggesting that $NaHCO_3$-P and Anion resin-P

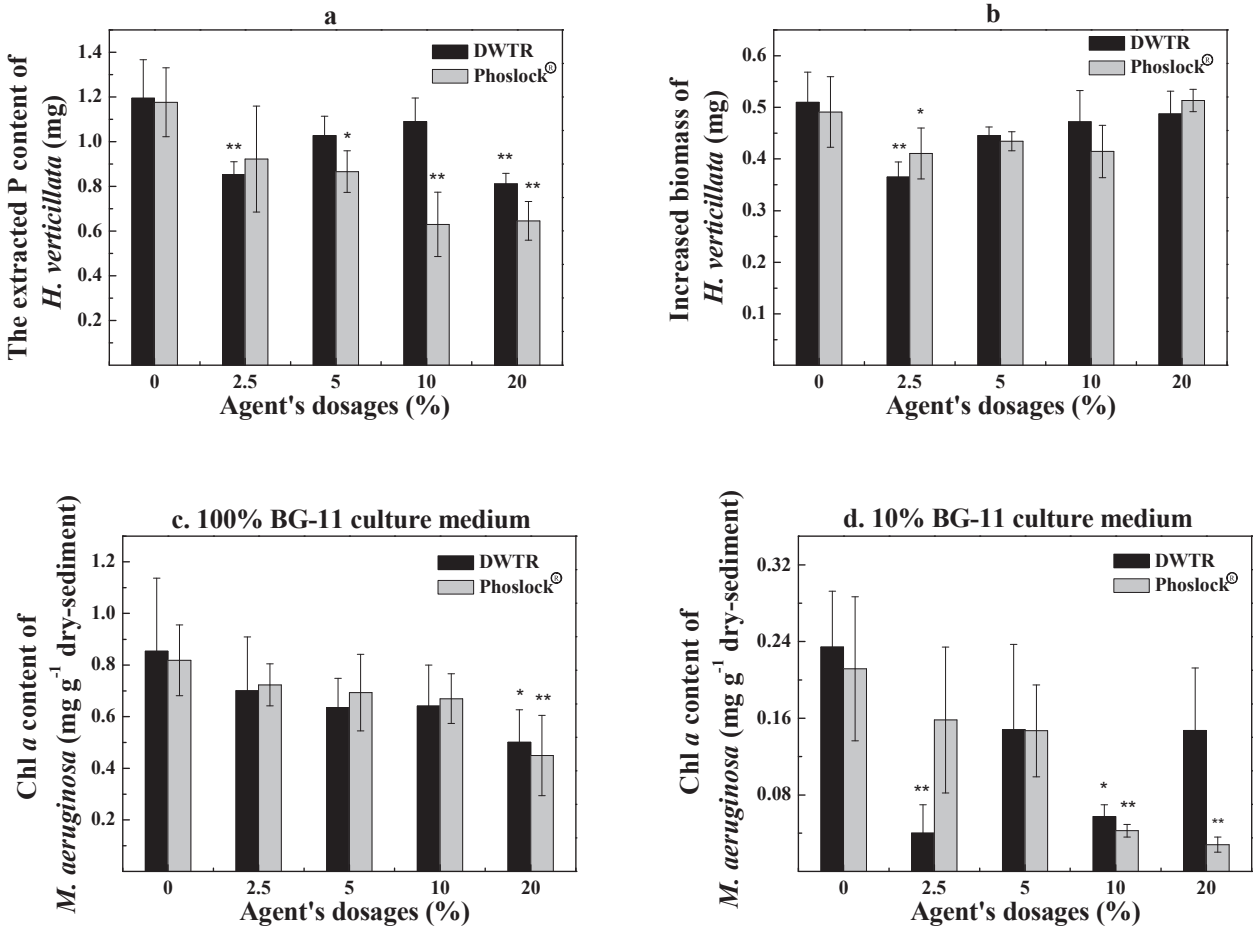

Fig. 4. The utilized P contents and increased biomass of *H. verticillata* and Chl *a* contents of *M. aeruginosa* after incubation with DWTR and Phoslock® addition at different dosages. * and ** represent significant differences that are found at $p < 0.05$and 0.01 between the control and the amended sediment.

could reflect macrophyte available P in amended sediment. The increased Chl *a* contents of *M. aeruginosa* in 100% BG-11 medium (P-free) showed positive linear correlations with ($NaHCO_3$-P + Fe oxide paper-P) ($p < 0.05$, $R^2 = 0.628$) and (Anion resin-P + Fe oxide paper-P) ($p < 0.01$, $R^2 = 0.879$) (Fig. 5b); interestingly, there was no correlation between Chl *a* in 10% BG-11 medium and the five extracted bioavailable P estimates. These results suggested that when P was the only growth limiting factor, the decrease in cyanobacteria available P could regularly inhibit cyanobacteria growth, and ($NaHCO_3$-P + Fe oxide paper-P) or (Anion resin-P + Fe oxide paper-P) could reflect potential cyanobacteria available P in the amended sediment. However, under the relatively low nutrient conditions, the effect of inactivating agent addition on cyanobacteria available P and growth were unclear. For eutrophic lake restoration, the addition of inactivating agents could induce a relative P limiting condition of lake water (e.g., Lürling and van Oosterhout, 2013), indicating that ($NaHCO_3$-P + Fe oxide paper-P) and (Anion resin-P + Fe oxide paper-P) can be used to assess potential cyanobacteria available P in amended sediment. Due to the higher R^2 of the linear correlation analysis, (Anion resin-P + Fe oxide paper-P) (denoted as ARF-P) could be more suitable for the assessment.

As the dosages increased, DWTR and Phoslock® gradually decreased the ARF-P contents in amended sediments after 20 d of incubation (Fig. S1), and there were reduction proportions of 13–49% and 2–49%, respectively. After 180 d of incubation, the decrease was enhanced in sediments with DWTR, and the reduction increased to 14–70%, while the enhancement was limited for Phoslock®, even though the ARF-P contents increased by 11–17% at dosages of 2.5–5%. These findings suggested that cyanobacteria available P immobilization by DWTR was stable, while the immobilization for Phoslock® had relatively low stability. It should be noted that the extracted bioavailable P was not the immediately available P, but the potential P pool may be transformed into an available form for autotrophs (Boström et al., 1988).

3.4.2. Mechanisms for bioavailable P changes

Further analysis suggested that at the recommended dosages, DWTR (at dosages of 10%) reduced the potential macrophyte available P by 20–55% and potential cyanobacteria available P by 31–58%, and Phoslock® (at dosages of 5%) reduced macrophyte-available P by 48–76% and cyanobacteria-available P by less than 17%. Clearly, the reduction of bioavailable P was not as significant as the mobile P immobilization in both the short- and long-term (Table 1). Therefore, to understand the reasons for the differences, a further linear correlation analysis was performed for different P forms (Fig. 6).

$NaHCO_3$-P and Anion resin-P showed good negative linear correlations with HCl-P ($R^2 = 0.644$ and 0.338 with $p < 0.01$ and 0.05) and positive linear correlations with NH_4Cl-P + BD-P + NaOH-IP ($R^2 = 0.694$ and 0.451 with both $p < 0.01$) (Fig. 6a and b). These findings suggested that $NaHCO_3$-P and Anion resin-P were closely related to NH_4Cl-P, BD-P, and NaOH-IP, but not to HCl-P, which could explain the observation of the relatively low immobilization

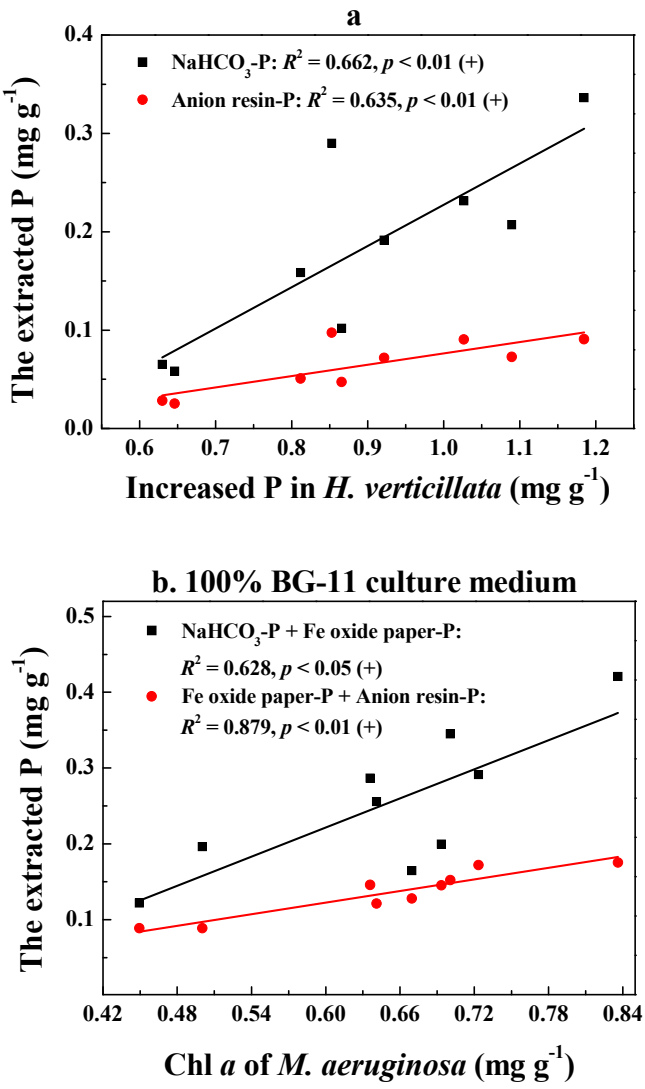

Fig. 5. Linear correlation analysis between the results of chemical extracted bioavaliable P and biological growth tests. The "+" in the blanks represents the positive linear correlations; the "+" between the different P forms represents the sum of the contents of different P; and the extracted P represents P in amended sediment determined by liquid- or solid-phase chemical extractions.

efficiencies of $NaHCO_3$-P and Anion resin-P (Figs. 2a and 3b) as well as the relatively low capability of reducing *H. verticillata* utilized P by DWTR compared to Phoslock® (Fig. 4a).

Fe oxide paper-P showed a good positive linear correlation with NH_4Cl-P + BD-P + HCl-P ($R^2 = 0.489$, $p < 0.01$) (Fig. 6c), indicating that Fe oxide paper-P was closely related to NH_4Cl-P, BD-P, and HCl-P. Interestingly, Fe oxide paper-P showed a good linear, negative correlation with Residual-P ($p < 0.05$, $R^2 = 0.226$); as a result, beyond NH_4Cl-P, BD-P, and HCl-P, Fe oxide paper-P was also somewhat related to NaOH-IP. The recommended potential cyanobacteria bioavailable P calculation (ARF-P) referred to both Anion resin-P and Fe oxide paper-P, which implied that the four extractable P, namely NH_4Cl-P, BD-P, NaOH-IP, and HCl-P, contained potential cyanobacteria available P. This inference could be further supported by finding that the increased total Chl *a* contents in 10% BG-11 medium (P-free) showed a good negative linear correlation with Residual-P ($p < 0.05$, $R^2 = 0.543$; Fig. S2). However, the mobile P (NH_4Cl-P and BD-P) immobilization by DWTR and Phoslock® was achieved by transforming them to NaOH-IP or HCl-P (Table 1). These observations could be the main reasons for the lower reduction of bioavailable P compared to the mobile P immobilization by DWTR and Phoslock®.

3.5. The effect of cyanobacteria growth on overlying water chemistry

The clear responses of *M. aeruginosa* growth in 100% BG-11 medium (P-free) to the decrease of cyanobacteria available P were observed here (Fig. 4c). Accordingly, the effect of cyanobacteria growth on overlying water chemistries was assessed to analyze the mechanisms for cyanobacteria utilizing P from amended sediment. The pH and organic matter were determined, as they could be the most possible factors affecting P stability in amended sediment under aerobic conditions (see Introduction).

The overlying water properties of sediments with and without inactivating agents were similar (Fig. 7). In general, the pH of overlying water increased from 7.24 to 7.46 to 10.0–10.2 under light conditions within the first 21 d, while in the next 28 d under dark conditions, the pH drastically decreased to a level that was similar to the initial values (Fig. 7a). The TOC contents in overlying water increased from 5.78 to 8.01 to 68.9–76.3 mg L^{-1} under light conditions, while under dark conditions, the contents showed a decreasing trend to 46.2–52.0 mg L^{-1} (Fig. 7b). Accordingly, cyanobacteria growth could increase pH and TOC contents of overlying water. This study also found the decrease of a_{250}/a_{365} during cyanobacteria growth (Fig. 7c), indicating that cyanobacteria growth increased the organic matter molecular weight (Zhou et al., 2015). The outbreak of algae blooms has been found to increase the pH of lake water (Xu et al., 2010), and blooms could release proteins, polysaccharide, and humic substances with relatively high molecular weights (Wang et al., 2016). The increased pH and organic matter contents of overlying water (Fig. 7c) may reduce the P stability in the amended sediment (e.g., Ross et al., 2008; Wang et al., 2013a) and then enhance the P utilization by cyanobacteria. Another important finding was that the overlying water of sediments with and without inactivating agents contained a small amount of P under light and dark conditions (typically for control group) (Fig. 7d).

3.6. Implication for practical application

In eutrophic lakes, macrophytes commonly have a positive effect on P immobilization in sediment (Granéli and Solander, 1988). Macrophytes can raise the sediment Eh and lower the pH, leading to a deposition of Fe(III) crusts around roots, which enhances the sediment P retention (Hupfer and Dollan, 2003). Furthermore, the typically rooted submersed macrophytes were considered directly responsible for P uptake from water, contributing to P retention in the lake sediment by increasing the deposition of organic matter (Schulz et al., 2003). However, under normal pore and lake water P concentrations, the dominant approach for P utilization by macrophytes was P uptake from the sediment, while decaying macrophytes may act as an internal P source for lake and add considerable quantities of P to water (Granéli and Solander, 1988). Although submerged macrophyte beds were considered P sinks, a significant portion of initially sediment-associated P was subsequently lost to open water from macrophyte beds (Rooney et al., 2003).

The observation of the stable $NaHCO_3$-P and Anion resin-P reduction within 180 d (Figs. 2a and 3b) by DWTR and Phoslock® suggested a high stability of macrophyte available P immobilization in lake sediment. Nonetheless, the efficiency for the potential macrophyte bioavailable P immobilization was lower than the mobile P immobilization. Typically, at the calculated recommended dosages, > 45% and >24% of potential macrophyte available P

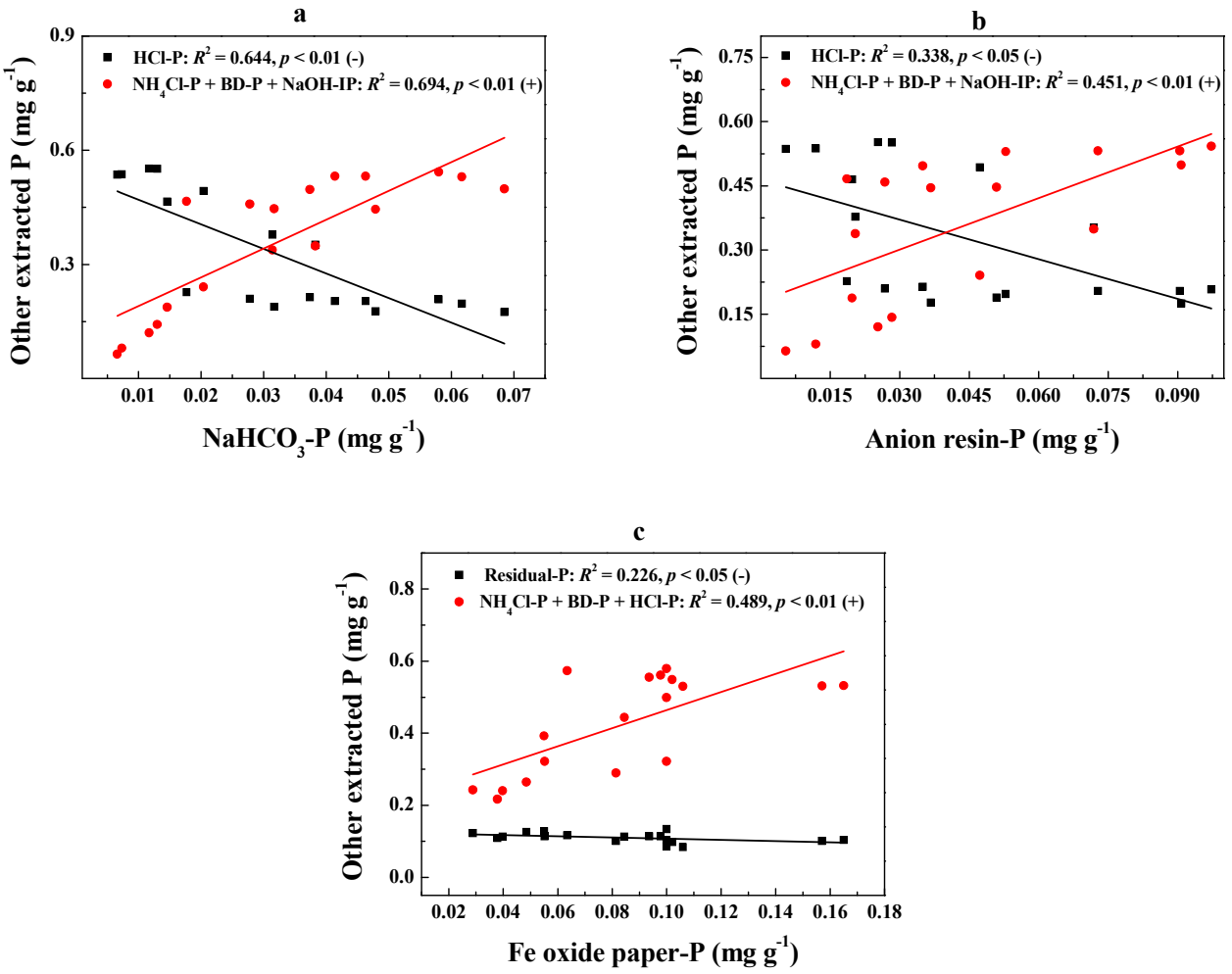

Fig. 6. Linear correlation analysis between the results of different bioavaliable P extraction methods. The "+" and "-" in the blanks represent the positive and negative linear correlations, respectively; the "+" between the different P forms represents the sum of the contents of different P; and other extracted P represents P in amended sediment determined by fractionation.

cannot be immobilized by DWTR and Phoslock®, respectively, while >75% mobile P can be immobilized in both the short- and long-term. The reduction in sediment bioavailable P also had a marginal effect on the macrophyte growth (Fig. 4b).

Macrophytes have been reported to show a peak biomass at relatively low sediment P content of 0.4 mg g^{-1} (Carr, 1998). An increase in the aquatic macrophyte species numbers and maximum colonization depths has been reported within 24 months of Phoslock® application (Spears et al., 2016). In compartment experiments that were constructed in two ponds by wooden sheet pilings or steel dam barriers with a surface area of approximately 300–400 m^2 (Waajen et al., 2016a) and in a whole lake application (Waajen et al., 2016b) with Phoslock®, macrophytes significantly increased after the applications. The macrophyte stored P can become available for phytoplankton when macrophytes decay because P release is often rather fast (Granéli and Solander, 1988); thus, the strongly promoted macrophytes growth, despite being viewed as benign of lake geo-engineering (Lürling et al., 2016), may also include a risk in lake restoration.

Eutrophication can result in excessive production of algae and cyanobacteria (Correll, 1998). This high productivity contributes to high bacterial populations and high respiration rates, inducing hypoxia or anoxia under certain conditions (Correll, 1998). Additionally, cyanobacteria, such as *M. aeruginosa*, may produce potent toxins (microcystins), both of which can cause serious damage to aquatic ecosystems. Accordingly, the control of cyanobacterial blooms is an important component of lake restoration. This study found that the potential cyanobacteria available P immobilization had lower efficiency compared to the mobile P immobilization. Typically, > 42% and >83% of potential cyanobacteria available P cannot be immobilized by DWTR and Phoslock®, respectively, at the recommended dosages in both the short- and long-term. Furthermore, under the condition that P was the only growth limiting factor, DWTR and Phoslock® did not substantially prevent the utilization of P from amended sediment by cyanobacteria for growth (Fig. S1) and the utilized P by cyanobacteria might be released into the overlying water (Fig. 7d).

Interestingly, the results of this study differed from the previous studies. Typically, the combination of flocculent polyaluminum chloride and Phoslock® shifted Lake Rauwbraken, Netherlands from a eutrophic/hypertrophic state to an oligo/mesotrophic state and remained in an oligo-mesotrophic state for more than four years without outbreak of cyanobacteria blooms (Lürling and van Oosterhout, 2013). Additionally, the combination of flocculant (iron(III) chloride) and Phoslock® successfully removed cyanobacterial biomass from the water column, and cyanobacterial biomass substantially decreased in the following six summers (Waajen et al., 2016b). Further comparison indicated that there had

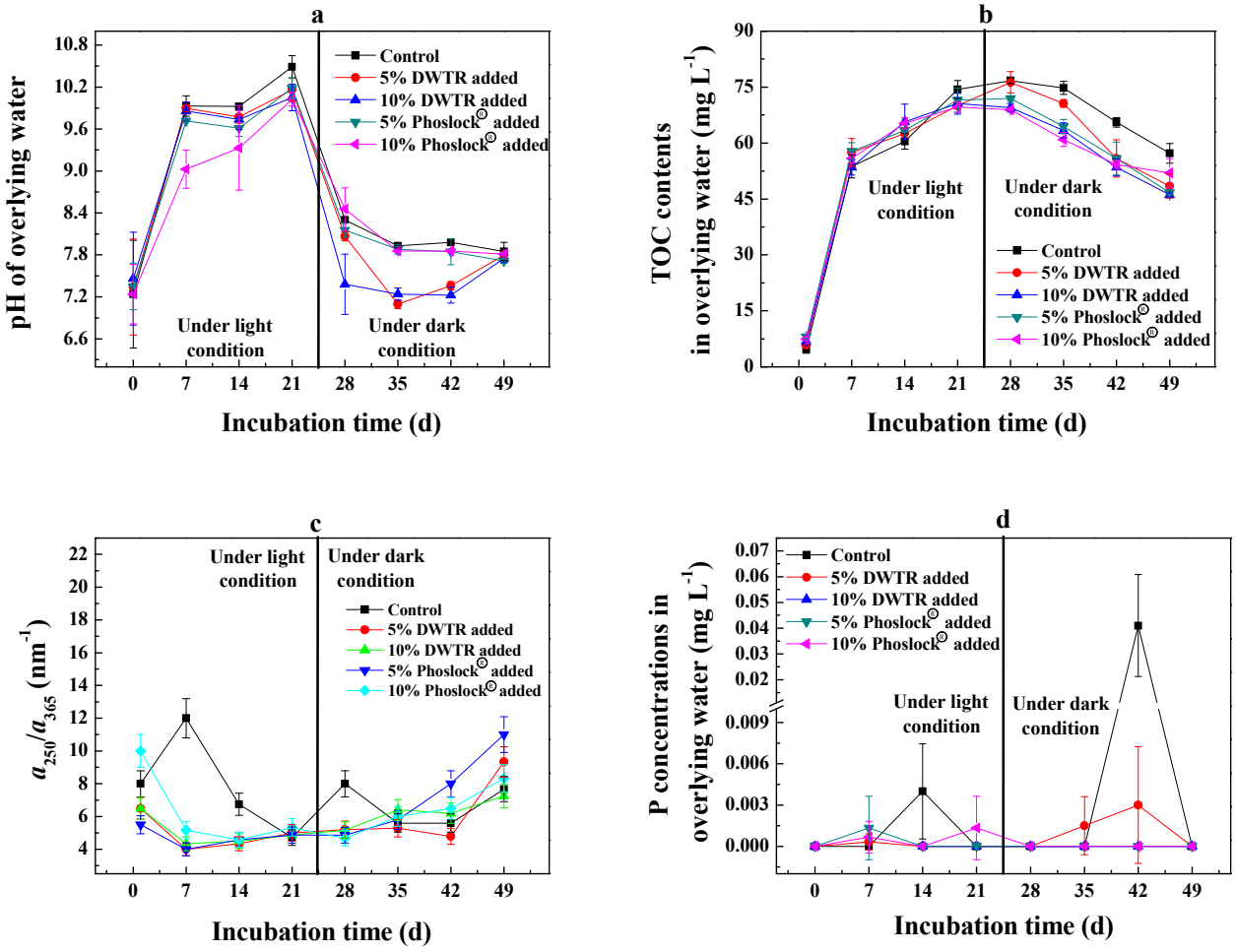

Fig. 7. The water chemistry during cyanobacteria growth tests.

been two main reasons for the differences. On the one hand, the cyanobacteria growth assay herein was conducted in proper illumination, while the maximum depths of Lake Rauwbraken and Lake De Kuil were approximately 15 and 9 m; after restoration, the mean Secchi depth of Lake De Kuil was 3.12 m (Lürling and van Oosterhout, 2013; Waajen et al., 2016b). Reasonably, the cyanobacteria after flocculation at the bottom of lake had light limitation. On the other hand, cyanobacteria were thoroughly exposed to amended sediment in the growth tests, inducing the direct utilization of P from amended sediment by cyanobacteria. By contrast, cyanobacteria in the upper water of both lakes cannot be thoroughly exposed to bottom amended sediment. The addition of Phoslock® after cyanobacteria flocculation further locked P in the bottom sediment, leading to limited P available in the lake water and finally resulting in effective control of cyanobacterial blooms.

This study also found that the increased Chl *a* contents (<0.040 mg g^{-1} dry-sediment) in cultures after the *M. aeruginosa* growth assay with relatively low light illumination (4 μmol photons m^{-2} s^{-1}, Fig. S3) were substantially less than in cultures with sufficient illumination (Fig. 3c). The finding further demonstrated that the utilization of lock P in the sediment amended with P-inactivation agents by cyanobacteria should be under sufficient illumination condition. A typical condition in practice was the re-suspension of bottom lake sediment (commonly due to wind events), which may shift the bottom sediment to the upper lake water, inducing the exposure of cyanobacteria to sediment with illumination. Accordingly, the possible bioavailability of P in sediments treated with P-inactivation agents may pose uncertainties for effective eutrophication control in lakes with regular sediment re-suspension. The re-suspended sediment has been demonstrated as an important source of P for phytoplankton growth in Lake Taihu, China (Zhu et al., 2015). Finally, cyanobacteria may use organic P by excreting phosphatases (Whitton et al., 1991) and can thus exploit a P-pool that is only marginally reduced by the addition of amendments such as Phoslock® and DWTR (Meis et al., 2013; Wang et al., 2012). This inference could be supported by the finding of the significant negative correlation between Chl *a* contents in 10% BG-11 medium (P-free) and Residual-P (Fig. S2).

In addition, this study found that macrophytes and cyanobacteria can utilize NH_4Cl-P, BD-P, NaOH-IP, and HCl-P in amended sediment except the Residual-P (Figs. 5 and 6). To the best of our knowledge, other commonly investigated P-inactivation agents, such as Al, Fe, and Ca compounds, made it difficult to transform mobile P to Residual-P in lake sediment (Wang and Jiang, 2016). Therefore, the potential bioavailable P may also pose uncertainties to other inactivating agents for eutrophication control in lakes with regular sediment re-suspension. Some reports in previous studies can somewhat support the findings of this study. The treatment of Al did not substantially impact the recruitment of *Gloeotrichia echinulata*, and the P content in the cell of recruited algae changed very little (Perakis et al., 1996). In Lake Terra Nova, Netherlands, the large-scale addition of Fe resulted in a substantial reduction of

dissolved P, suspended matter, phytoplankton biomass, and relative cyanobacterial biomass, while macrophytes reappeared, but quick recovery of total P concentrations along with a trend of increased phytoplankton biomass and suspended matter were observed when Fe addition was stopped (Immers et al., 2015). To maintain a healthy lake ecosystem, bioavailable P is an indispensable factor. It was hard to give a clear assessment of the significance about the effect of inactivating agents on bioavailable P in sediment during lake restoration. However, according to this work, assessing the potential bioavailable P in lake sediment amended with P-inactivation agents is crucial for successful geo-engineering method. Overall, special attention should be paid to bioavailable P pools in lake ecosystems before practical restoration.

4. Conclusions

The major conclusions of this study are as follows:

(1) DWTR and Phoslock® could effectively reduce mobile P in lake sediment, while different chemical extraction methods led to variations in the estimates of bioavailable P in the amended sediment.
(2) DWTR and Phoslock® had a marginal effect on the growth of *H. verticillata* in terms of the biomass. Under the condition that P was the only growth limiting factor, these agents did not substantially prevent the utilization of P from amended sediment by *M. aeruginosa* for growth.
(3) $NaHCO_3$-P or Anion resin-P and the sum of Anion resin-P and Fe oxide paper-P were determined to be applicable for indicating the potential macrophyte and cyanobacteria available P, respectively, in amended sediment.
(4) The reduction of bioavailable P was less than the mobile P immobilization by DWTR and Phoslock® in both the short- and long-term. One reason was that macrophyte and cyanobacteria can utilize various fractions of P in amended sediment, except Residual-P.

Therefore, DWTR and Phoslock® effectively reduced P release from the lake sediment, but they were less efficient in preventing sediment P flowing into the biosphere. An evaluation of the bioavailable P pool in lake ecosystem is necessary before the practical application of P-inactivation agents.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.watres.2016.11.045.

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