Investigation on the eco-toxicity of lake sediments with the addition of drinking water treatment residuals

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ABSTRACT

Drinking water treatment residuals (WTRs) have a potential to realize eutrophication control objectives by reducing the internal phosphorus (P) load of lake sediments. Information regarding the ecological risk of dewatered WTR reuse in aquatic environments is generally lacking, however. In this study, we analyzed the eco-toxicity of leachates from sediments with or without dewatered WTRs toward algae *Chlorella vulgaris* via algal growth inhibition testing with algal cell density, chlorophyll content, malondialdehyde content, antioxidant enzyme superoxide dismutase activity, and subcellular structure indices. The results suggested that leachates from sediments unanimously inhibited algal growth, with or without the addition of different WTR doses (10% or 50% of the sediment in dry weight) at different pH values (8–9), as well as from sediments treated for different durations (10 or 180 days). The inhibition was primarily the result of P deficiency in the leachates owing to WTR P adsorption, however, our results suggest that the dewatered WTRs were considered as a favorable potential material for internal P loading control in lake restoration projects, as it shows acceptably low risk toward aquatic plants.

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Introduction

Water management faces a global call for control of eutrophication due to phosphorous (P) loading (Carpenter, 2008). Internal P loading from sediments, specifically, has been identified as one of the major causes of excessive P in lakes (Egemose et al., 2010). At present, chemical immobilization is considered as an effective method of minimizing internal P loading (Paller and Knox, 2010); this technique involves adding chemicals to the lake to reduce P mobility in sediments. Low-cost and environmentally friendly chemicals with high P adsorption capability are essential for future advancements in chemical immobilization techniques (Wang et al., 2012).

Drinking water treatment residuals (WTRs) are ubiquitous by-products generated during potable water production primarily comprised of aluminum (Al) and iron (Fe) hydroxides owing to their effective coagulant utilization (Babatunde and Zhao, 2007). WTRs have demonstrated high adsorption capability toward many contaminants, such as arsenic (As) (Nagar et al., 2013), chromium (Cr) (Zhou and Haynes, 2011), copper (Cu) (Castaldi et al., 2015), lead (Pb) (Zhou and Haynes, 2011), mercury (Hg) (Hovsepyan and Bonzongo, 2009), nickel (Ni) (Mahdy et al., 2012), organic pesticide (Zhao et al., 2013, 2015), P (Razalia et al., 2007), perchloric acid (Makris et al., 2006), selenium (Se) (Ippolito et al., 2009), sulfide (S) (Wang and Pei, 2012), and tetracyclines (Punamiya et al., 2015). Many researchers have explored

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P-adsorbing WTR recycling as-applied to environmental pollution control measures (Bai et al., 2014). WTRs can be used as amendments to reduce the loss of P from P-rich soils (Agyin-Birikorang et al., 2009), and as main substrates for constructed wetlands to remove excessive P from wastewater (Zhao et al., 2011).

Recently, WTRs have shown considerable potential to ameliorate internal P loading in lakes for eutrophication control purposes (Wang et al., 2015a). Specifically, WTRs can reduce internal P loading from sediments under different dissolved oxygen levels (Wang et al., 2013b). The immobilization capability of WTRs is quite stable, for example, it is hardly affected by pH (5–9), illumination intensity, resuspension, ionic concentration, organic matter in sediments, or microbial activity (Wang et al., 2013a, 2013c). The potential toxicity to aquatic organisms caused by sediments with the addition of WTRs must be thoroughly tested before the materials can be reasonably applied in practice, however.

Therefore, we examined the potential eco-toxicity of lake sediments with addition of WTRs following growth of green algae *Chlorella vulgaris* under different doses, incubation times, and pH values to assess the risk of reusing WTRs in aquatic environments. The indices used for measurement were algal cell density, chlorophyll (Chl) content, malondialdehyde (MDA) content, antioxidant enzyme superoxide dismutase (SOD) activity, and subcellular structure. We expect that the results presented here will facilitate future use (and reuse) of WTRs to reduce internal P loading in lakes for eutrophication control purposes.

1. Materials and methods

1.1. Sample collection and preparation

Dewatered WTRs were collected from the dewatering workshop of the Ninth Water Supply Plant of Beijing, wherein both poly aluminum chloride and ferric chloride are used as coagulants. The fresh dewatered WTRs were air-dried, ground, and sieved to a diameter of <1 mm. Lake sediment was obtained from Jiaozhuang Village in Lake Baiyangdian (38°53′N, 115°59′E). The upper 10 cm of the sediment was collected and filtered through a 1.8 mm screen to remove impurities, then homogenized mechanically. The sediment was stored at 4°C until use (within 48 hr).

WTRs were mixed with sediments at doses accounting for 0% (control), 10%, and 50% of the sediment in dry weight. The mixtures were then incubated for 10 days and 180 days, respectively. After incubation, the mixtures were freeze-dried, ground, and sieved to a diameter of <1 mm. Six samples in total were investigated: raw sediments incubated for 10 days (RS-10 d) and 180 days (RS-180 d), sediments with the addition of 10% WTRs incubated for 10 days (WAS-10 p-10 d) and 180 days (WAS-10 p-180 d), and sediments with the addition of 50% WTRs incubated for 10 days (WAS-50 p-10 d) and 180 days (WAS-50 p-180 d). The WTR doses and incubation times were selected based on values defined in a study by Wang et al. (2012), who indicated that inorganic P can be successfully immobilized by WTRs within 10 days at a 10% dose ratio of WTRs to sediment in dry weight. Because the applied WTRs

cannot be recovered from lake sediment in practice, the exposure duration of WTRs may be longer than 10 days during real-world application. Accordingly, incubation times of 10 days and 180 days were selected to investigate the short- and long-term effects, respectively, of WTR addition on sediment toxicity to algae. In addition, doses of P inactivating agents for lake restoration in practice would likely be greater than the calculated theoretical doses due to various factors affecting natural environments (Meis et al., 2013), so doses of 10% and 50% were selected to determine the effect of WTR addition dosage-wise.

1.2. Leachate preparation

Leachates were prepared according to methods outlined by Mamindy-Pajany et al. (2010) with slight modifications. A 1:10 (*m/V*:g/mL) sample and extractant ratio was used. Algal cell culture medium, which was sterilized at 121°C at 1.05 kg·cm^2 for 30 min, was used as extractant. The mixtures were filtrated with a 0.22 μm sterile acetate nitrate filter after being shaken at 60 r/min for 24 hr at (20 ± 2)°C. The filtrates (leachates) were then stored at 4°C in a dark environment prior to eco-toxicological and chemical analyses (within 24 hr). The sterilized medium was adjusted to pH of 6.0, 7.0, 8.0, or 9.0 with 1 mol/L NaOH or HCl solutions to form leachate extractant samples of varying pH values. During leaching, the pH was manually adjusted every 2 hr during the first 12 hr then every 4 hr during the last 12 hr.

1.3. Algal growth inhibition assays

C. vulgaris algae were obtained from the Institute of Hydrobiology, Chinese Academy of Sciences. Algal growth assay was conducted by following Guideline 201 (Freshwater Alga and Cyanobacteria, Growth Inhibition Test) of the Organization for Economic Cooperation and Development (OECD). The algae was grown in the OECD recommended algal cell culture medium (NH$_4$Cl 15 mg/L, MgCl$_2$·6H$_2$O 12 mg/L, CaCl$_2$·2H$_2$O 18 mg/L, MgSO$_4$·7H$_2$O 15 mg/L, KH$_2$PO$_4$ 1.6 mg/L, FeCl$_3$·6H$_2$O 80 μg/L, Na$_2$EDTA·2H$_2$O 100 μg/L, H$_3$BO$_3$ 185 μg/L, MnCl$_2$·4H$_2$O 415 μg/L, ZnCl$_2$ 3 μg/L, CoCl$_2$·6H$_2$O 1.5 μg/L, CuCl$_2$·2H$_2$O 0.01 μg/L, Na$_2$MoO$_4$·2H$_2$O 7 μg/L, and NaHCO$_3$ 50 mg/L), and algal cells were cultured in 100 mL OECD medium (control) or sediment leachates in 250 mL Erlenmeyer flasks. The initial cell densities were approximately 1×10^5 cells/mL. The flasks were stored in an incubator at (25 ± 0.5)°C with illumination by white incandescent lights (100 ± 5 μE/(m^2·sec), light:dark cycle 14 hr:10 hr) and shaken by hand three times daily.

As mentioned above, we also conducted algal growth inhibition assays under different pH levels. To achieve and maintain the desired pH of treatment and control groups during the experiments over 120 hr, buffer solutions were added to a final concentration of 3.6 mmol/L and pH was adjusted with 1 mol/L NaOH or HCl solutions. Ethane sulfonic acid buffer 2-(N-morpholino) was used to maintain a pH value of 6.0, pH 7.0 was stabilized with 3-(N-morpholino) propane sulfonic acid buffer, pH 8.0 was stabilized with 4-(2-hydroxyethyl)-1-piperazinepropanesulfonic acid buffer, and pH 9.0 was stabilized with 2-(cyclohexylamino) ethane sulfonic acid buffer (Van Hoecke et al., 2011). Prior to the assays, the algae were

acclimatized in the OECD medium at pH 6.0, 7.0, 8.0, or 9.0 for 3 days. During 120 hr of growth inhibition assay, the pH was monitored twice daily and adjusted with 0.2 pH unit deviation.

The three main experimental assay solution groups in this study were comprised of the following. (1) Concentrations of 100%, 75%, 50%, 25%, and 12.5% were respectively prepared in the OECD medium to create assay solutions RS-10 d, WAS-10 p-10 d, WAS-50 p-10 d, RS-180 d, WAS-10 p-180 d, and WAS-50 p-180 d. (2) Leachates of RS-10 d, WAS-10 p-10 d, WAS-50 p-10 d, RS-180 d, WAS-10 p-180 d, and WAS-50 p-180 d; these six leachates received KH_2PO_4 additions (to achieve the same P concentration as the OECD medium), represented as RSP-10 d, WASP-10 p-10 d, WASP-50 p-10 d, RSP-180 d, WASP-10 p-180 d, and WASP-50 p-180 d, respectively; the OECD medium with no KH_2PO_4 served as a control for P deficiency, represented as ONP. (3) Leachates of RS-10 d, WAS-10 p-10 d, WAS-50 p-10 d, RS-180 d, WAS-10 p-180 d, WAS-50 p-180 d, RSP-10 d, WASP-10 p-10 d, WASP-50 p-10 d, RSP-180 d, WASP-10 p-180 d, and WASP-50 p-180 d at pH 6.0, 7.0, 8.0, and 9.0.

All solutions in Groups (2) and (3) were undiluted. Algal cell density was measured daily for Groups (1) and (3), algal cell density was determined daily; for Group (2), in addition to cell density, Chl content, MDA content, and SOD activity were analyzed at 24 hr, 72 hr, and 120 hr, respectively. Algal subcellular structure was also investigated at 120 hr in Group (2).

1.4. Analytical methods

Phosphate (PO_4^{3-}–P), ammonia nitrogen (NH_4^+–N), nitrate N (NO_3^-N), and nitrite (NO_2^-N) nutrients within the leachates and medium were quantified accordingly (Murphy and Riley, 1962; APHA, 1998; Goldman and Jacobs, 1961; Saltzman, 1954). Elements including Al, As, boron (B), barium (Ba), beryllium (Be), calcium (Ca), cadmium (Cd), cobalt (Co), Cr, Cu, Fe, kalium (K), magnesium (Mg), manganese (Mn), molybdenum (Mo), sodium (Na), Ni, Pb, strontium (Sr), vanadium (V), and zinc (Zn) were measured with an inductively coupled plasma-atomic emission spectrometer (SPECTRO ARCOS EOP, Germany).

Cell growth was measured by absorbance on a spectrophotometer at 680 nm, and the standard curve between cell density and absorbance was measured with a light microscope (Olympus CHK, Japan).

Algal Chl content was determined by filtering 40 mL cell culture and extracting it with 10 mL 95% ethyl alcohol in the dark at 4°C for 24 hr. The mixtures were then centrifuged at 4000 ×g for 10 min at 4°C, and supernatant was quantified spectrophotometrically at 647 and 664.5 nm compared to the respective absorbance of 95% ethyl alcohol. Total Chl content ($C_{Tot-Chl}$) was calculated with the following formula (Xiong et al., 2014):

$$C_{Tot-chl} = (17.9 \times OD_{647}) + (8.08 \times OD_{664.5})$$

where, OD_{647} and $OD_{664.5}$ are optical density at 647 and 664.5 nm, respectively.

Algal crude enzyme was extracted by centrifuging 40 mL cell culture at 10,000 ×g for 10 min at 4°C. Samples were suspended in 0.5 mL pre-cooled phosphate buffer (pH 7.4), and broken in an ice bath with an ultrasonic cell disruptor (JY92-IIDN, Scientz, Ningbo Scientz Biotechnology Co., Ltd.,

China) at 400 A, processed for 5 sec with 10 sec between processing iterations, repeated three to five times. The homogenate was centrifuged at 10,000 ×g for 10 min at 4°C, and the supernatant was used to analyze SOD activity and MDA content in the sample; SOD activity was determined with a T-SOD kit (Nanjing Jiancheng Bioengineering Institute, China), and MDA content was measured with an MDA kit (Nanjing Jiancheng Bioengineering Institute, China).

For transmission electron microscopy (TEM) analysis, cells of the control and WTR amended sediment-treated algae were centrifuged at 10,000 ×g for 10 min at 4°C. Samples were then fixed in cacodylate buffer solution (pH 7.2) and dehydrated in acetone, then embedded in epoxy resin. Ultra-thin sections were finally obtained and stained with uranyl acetate and lead citrate, and algal subcellular structure was observed with a JEM-1230 microscope (JEOL Ltd., Tokyo, Japan).

1.5. Statistical analysis

All experiments were conducted in triplicate. Significant differences between the control and treatments were determined by ANOVA and least-significant difference tests. Statistical significance was accepted at probability (p) value below 0.05. Error bars represented the mean ± standard deviations of the triplicate.

2. Results and discussion

2.1. Leachate characteristics

The properties of leachates from sediments with or without WTRs are listed in Table 1. Compared to the OECD medium, the leachates showed slightly higher pH values in general — RS-10 d was the only exception, with a slightly lower pH. The PO_4^{3-}–P content in all leachates decreased substantially; WTR addition enhanced this effect. For the sediments incubated for 10 days, PO_4^{3-}–P content decreased from 0.0969 to 0.0388 mg/L as WTR dose increased from 0% to 50%. For the sediments incubated for 180 days, WTR addition caused PO_4^{3-}–P concentration in the leachates to become undetectable. This result could be attributed to the P adsorption of the sediments and WTRs from the OECD medium, and to increased WTR addition further increasing the P adsorption capacity of the sediment (Wang et al., 2013a). NO_3^-N, NO_2^-N, and NH_4^+–N contents in the leachates increased compared to the OECD medium, indicating that sediments with or without the addition of WTRs released N.

For metals/metalloids in the leachates of sediments with or without WTR addition, Al, As, Be, Cd, Cr, and Pb were not detected. Compared to the extractant (i.e., the OECD medium), Ca, Co, Cu, K, Mg, Mn, Mo, Na, Ni, Sr, V, and Zn content increased in the leachates, indicating that sediments with or without WTRs released metals during leaching. The concentrations of these metals were still significantly lower than the regulated values set by the National Recommended Water Quality Criteria, however (USEPA, 2006). Moreover, even increased metal contents in the leachates of sediments with WTRs were well below those in the leachates of raw sediments. The

Table 1 – Properties of the OECD medium and the leachates of the sediments with or without the addition of WTRs (unit: mg/L).

Properties	OECD medium	Leachate					
		RS-10 d	WAS-10 p-10 d	WAS-50 p-10 d	RS-180 d	WAS-10 p-180 d	WAS-50 p-180 d
pH	7.35	7.30	7.38	7.44	7.48	7.48	7.53
PO_4–P	0.355	0.0969	0.0581	0.0388	0.0969	ND	ND
NO_3–N	0.000292	4.05	6.79	6.44	8.06	0.538	8.22
NO_2–N	0.0131	0.0492	0.0328	0.0262	0.0164	0.0393	0.0262
NH_4–N	2.74	9.39	11.6	13.5	9.58	8.33	17.6
Al	0.0105	ND	ND	ND	ND	ND	ND
As	ND	ND	ND	ND	ND	ND	ND
B	0.511	0.130	0.130	0.127	0.140	0.124	0.153
Ba	0.00131	0.0755	0.0862	0.157	0.0889	0.0991	0.235
Be	ND	ND	ND	ND	ND	ND	ND
Ca	4.60	125	101	102	144	143	92.7
Cd	ND	ND	ND	ND	ND	ND	ND
Co	ND	0.00111	0.00114	0.00100	0.00194	0.00106	0.00113
Cr	ND	ND	ND	ND	ND	ND	ND
Cu	ND	0.0127	0.0101	0.00692	0.00517	0.0125	0.00625
Fe	0.0148	0.00869	0.00748	0.00626	0.00951	0.00843	0.00731
K	1.05	16.7	15.6	14.0	17.2	15.8	14.8
Mg	3.03	33.0	28.7	25.0	36.6	31.5	25.4
Mn	0.110	0.708	0.903	1.23	1.19	0.827	1.04
Mo	0.00420	0.0800	0.0562	0.0331	0.0718	0.0461	0.0365
Na	12.9	50.9	48.1	40.2	51.3	46.1	42.3
Ni	ND	0.00485	0.00499	0.00403	0.00490	0.00508	0.00383
Pb	ND	ND	ND	ND	ND	ND	ND
Sr	0.00389	0.893	0.698	0.562	1.03	0.836	0.605
V	ND	0.00172	0.00118	0.000975	0.00184	0.00110	0.000990
Zn	0.00325	0.0167	0.0128	0.0131	0.0158	0.0208	0.0213

OECD medium: the Organization for Economic Cooperation and Development recommended algal cell culture medium; WTR: water treatment residuals.
RS-10 d and RS-180 d: raw sediments incubated for 10 days and 180 days, respectively.
WAS-10 p-10 d and WAS-10 p-180 d: sediments with the addition of 10% WTRs incubated for 10 days and 180 days, respectively.
WAS-50 p-10 d and WAS-50 p-180 d: sediments with the addition of 50% WTRs incubated for 10 days and 180 days, respectively.
ND: not detectable.

contents of certain metals (Cu, K, Mg, Mo, Na, Sr, and V) also decreased as WTR dose increased, suggesting that these metals were essentially released from the sediment and that WTRs successfully adsorbed metals in the sediment (Chiang et al., 2012).The increase of metals in the leachates of sediments with WTRs may have been related to the relatively limited immobilization capability of WTRs for these metals; basically, WTRs cannot entirely immobilize all metals in all sediments.

2.2. Algal growth inhibition by leachates with or without WTR addition

The effects of WAS-10 p-10 d and RS-10 d on algal cell growth kinetics (i.e., the changes in algal cell numbers with time) are shown in Fig. 1. Compared to the control, algal growth was only successfully inhibited at 100% dilution, whereas growth was promoted at dilutions of 12.5%, 25%, and 50%. By comparison, at 100% dilution, the cell density of algae exposed to WAS-10 p-10 d was significantly ($p < 0.05$) lower than that of algae exposed to RS-10 d during the whole 120 hr trial period. Maximum density was $(12.7 \pm 1.67) \times 10^5$ and $(19.1 \pm 0.965) \times 10^5$ cell/L at 120 hr after algae exposed to 100% dilution in leachates of WAS-10 p-10 d and RS-10 d, respectively. These results altogether suggested that WTR addition increased the inhibition effect of sediments to algae.

Similar to RS-10 d and WAS-10 p-10 d, samples of WAS-50 p-10 d, RS-180 d, WAS-10 p-180 d, and WAS-50 p-180 d inhibited algal growth only at 100% dilution, whereas growth was promoted at dilution of 12.5%, 25%, and 50% (Fig. 1). By comparison, at 100% dilution, algal cell density generally showed an insignificant ($p > 0.05$) difference between sediments incubated for 10 and 180 days at the same WTR dose, while cell density significantly ($p < 0.05$) decreased as WTR dose increased at the same incubation time. Maximum density was $(17.8 \pm 1.29) \times 10^5$ cell/L in RS-180 d, slightly lower than that in RS-10 d $((19.1 \pm 0.965) \times 10^5$ cell/L). The maximum values of WAS-50 p-10 d, WAS-10 p-180 d, and WAS-50 p-180 d were $(10.7 \pm 0.129) \times 10^5$, $(14.6 \pm 0.637) \times 10^5$, and $(11.2 \pm 0.393) \times 10^5$ cell/L, respectively. Closer observation of WAS-10 p-10 d value $((12.7 \pm 1.67) \times 10^5$ cell/L) suggested that the extended incubation duration of WTR addition to sediments did not produce any extra toxicity, though the inhibition effect increased alongside WTR dose.

As our chemical analysis results showed, there was only limited increase in metal/metalloid concentrations in the leachates. Nitrogen content increased after leaching but was within thethreshold for C. vulgaris to successfully maintain growth (Tam and Wong, 1996). Accordingly, neither the N nor metals/metalloids in leachates were likely to have elicited an inhibitive response to the algae, however, owing to the
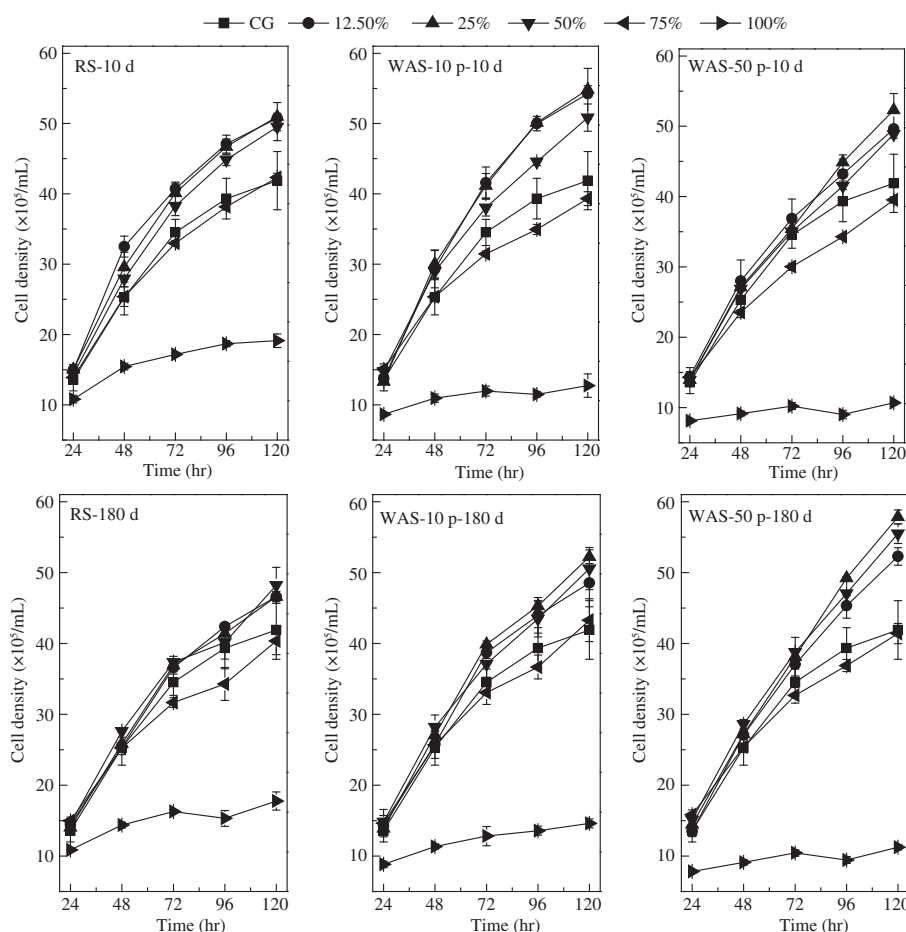
Fig. 1 – Growth kinetics of the algal cells exposed to various dilutions of leachates from the sediments with or without the addition of WTRs. CG: the OECD medium. WTRs: water treatment residuals; OECD: Organization for Economic Cooperation and Development.

substantial decrease of PO_4^{3-}-P concentration in the leachates (Table 1), algal growth inhibition was most likely caused by P deficiency in leachates from sediments with or without WTRs. We conducted further tests on P addition to verify whether P deficiency was the main cause of algal growth inhibition.

2.3. P addition trials to the leachates with or without WTRs

2.3.1. Cell density and Chl content
Cell densities and Chl contents of algae exposed to leachates from sediments with or without P addition and to ONP are shown in Fig. 2. The growth of algae exposed to the six leachates without P addition and ONP was inhibited after 24 hr, and the growth of algae exposed to the six leachates with P addition increased throughout the trial, (the only exception being RSP-180 d at 96 hr and 120 hr.) Maximum cell density was 1.99, 1.93, 2.54, 2.47, 2.11, and 1.73 times that of the control in RSP-10 d, WASP-10 p-10 d, WASP-50 p-10 d, RSP-180 d, WASP-10 p-180 d, and WASP-50 p-180 d, respectively. Throughout the trial, the Chl content of algae exposed to the six leachates without P addition and ONP significantly ($p < 0.05$) decreased compared to the control. The Chl content of algae exposed to the six leachates with P addition was no

less than that of the control, except for RSP-180 d at 72 hr and 120 hr. Maximum Chl contents were 1.67, 1.62, 1.85, 1.86, 1.67, and 1.64 times that of the control in RSP-10 d, WASP-10 p-10 d, WASP-50 p-10 d, RSP-180 d, WASP-10 p-180 d, and WASP-50 p-180 d, respectively.

The above results suggested that leachates with added P promoted algal growth, and that P deficiency was the main cause of growth inhibition effect of sediments with or without WTRs on algae. This observation is similar to the one made by Lombi et al. (2010), who found reduced plant growth in soils amended with WTRs as a result of P deficiency. In addition, algal cell density did not decrease as WTR dose increased in the leachates with P addition, thus indicating that the effect of WTR dose on algae growth presented (Section 2.2) was indeed caused by P deficiency in the leachates. The cell density of algae exposed to leachates of sediments without P was higher than that of algae exposed solely to ONP from 48 hr to 120 hr, therefore, under the same P deficiency conditions, increased amounts of elements in the leachates (Table 1) released from sediments and WTRs promoted algal growth. Most elements in all leachates decreased after 120 hr (Table S1), further indicating that the algae utilized released elements to maintain growth. Additionally, although their PO_4^{3-}-P concentrations were lower
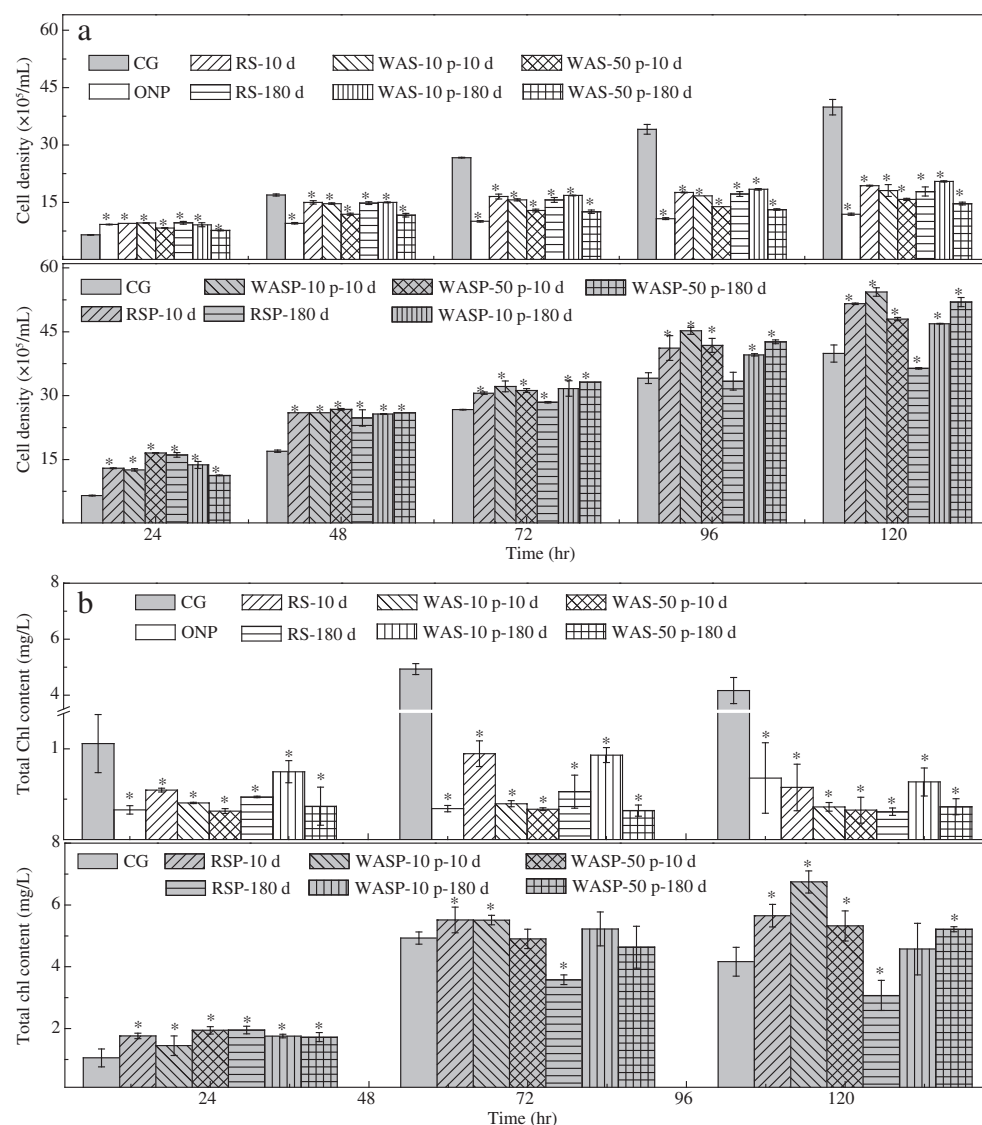
Fig. 2 – Cell density (a) and total chlorophyll content (b) in *C. vulgaris* exposed to leachates from the raw and WTRs amended sediments with or without P addition. CG: the OECD medium. *Represents statistically significant differences when compared with the control at the same exposure time as determined by the least significant difference ($p < 0.05$) in a and b respectively. P: phosphorous; WTRs: water treatment residuals; OECD: Organization for Economic Cooperation and Development.

than that of the control, leachates at dilutions of 12.5%, 25%, and 50% still promoted algal growth (Fig. 1), also likely due to the contents of the above elements.

2.3.2. MDA content and SOD activity

Fig. 3 shows the MDA content (Fig. 3a) and SOD activity (Fig. 3b) of algae exposed to leachates from raw and WTR amended sediments with or without P addition. The MDA in leachates with and without P increased at 24 hr then decreased at 72 and 120 hr compared to the control; the MDA of WAS-50 p-180 d at 24 hr, showed no significant difference compared to the control, was the only exception. During the full 120 hr, the SOD activity of all leachates with and without P significantly ($p < 0.05$) increased except for RSP-10 d at 120 hr, which remained effectively unchanged.

Plants produce a large quantity of reactive oxygen species (ROS) under stressed conditions. ROS at high concentrations may cause oxidative stress and damage to cells by attacking proteins, membrane lipids, and other cell components in the plant (Yang et al., 2015). MDA is a secondary product of lipid peroxidation, and its accumulation is often considered an indication of oxidative stress. To combat the danger posed by ROS, organisms have evolved a series of antioxidant enzymes and antioxidant substances including SOD (Liu et al., 2013). SOD catalyzes the dismutation of superoxide to hydrogen peroxide and oxygen, as well as the conversion of H_2O_2 to $2H_2O$ by glutathione peroxidase or to O_2 and H_2O by catalase. A small quantity of oxygen can trigger SOD activity, bolstering the plant's defense against toxicant-induced ROS. When excessive ROS is formed under sudden and very high stress
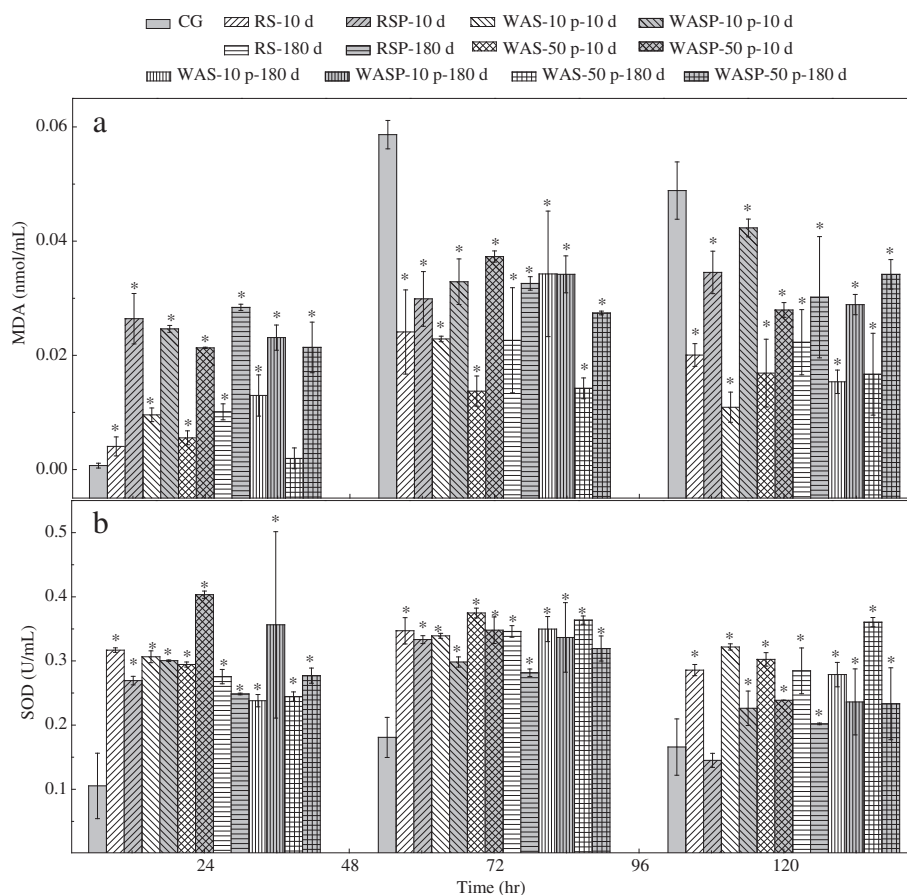
Fig. 3 – Algal MDA level (a) and SOD activity (b) in *C. vulgaris* exposed to leachates from the raw and WTRs amended sediments with or without P addition. CG: the OECD medium. *Represents statistically significant differences when compared with the control at the same exposure time as determined by the least significant difference ($p < 0.05$) in a and b respectively. MDA: malondialdehyde; SOD: superoxide dismutase; WTRs: water treatment residuals; P: phosphorous; OECD: Organization for Economic Cooperation and Development.

conditions, however, the defense system may be overwhelmed and unable to remove the ROS as needed (Liu et al., 2013). Our analysis results suggested that the leachates of raw and WTR-amended sediments caused increased production of superoxide initially, and that oxidation may have been minimized due to antioxidant enzymes and completely eliminated within 72 hr.

2.3.3. Algal subcellular structure
The subcellular structures of algae exposed to leachates from raw and WTR-amended sediments with P addition, as well as the control, are shown in Fig. 4. Algae in leachates without P addition and in the OECD medium, which deleted KH_2PO_4, were not analyzed because their cell concentrations were insufficient for TEM analysis. Compared to the algal cells in the control, algae cells exposed to all leachates with P addition showed little difference in structure; intact organelles, such as cell membranes, nuclei, and chloroplasts, were clearly visible in these algal cells. These results suggested that leachates from sediments with or without WTRs had no adverse effects on algal subcellular structure after P addition.

2.4. Influence of pH on toxicity of raw and WTR-amended sediments on algae

The cell densities of algae exposed to leachates of raw and WTR-amended sediments with or without P addition at pH 6.0, 7.0, 8.0, and 9.0 are shown in Fig. 5. The algae almost stopped growing at pH 6.0 after 72 hr, so we'll only present algal inhibition assay data from 24 hr to 72 hr here. For leachates without P addition, compared to the control, leachates at pH 6.0 showed promotion effects and leachates at pH 7.0 showed promotion or negligible effects — the only exception being WAS-50 p-10 d at 48 hr and WAS-10 p-10 d and WAS-50 p-10 d at 72 hr, which showed inhibition effects. Inhibition effects were also observed for leachates without P addition at pH 8.0 during the entire 72 hr, as well as for most leachates at pH 9.0.

Chemical analysis showed that PO_4^{3-}-P content was higher in leachates under pH 6.0 and 7.0 than leachates under pH 8.0 and 9.0 (Table S2). Compared to both the corresponding leachates without P addition and the control at all pH conditions during the whole trial period, algal cell density
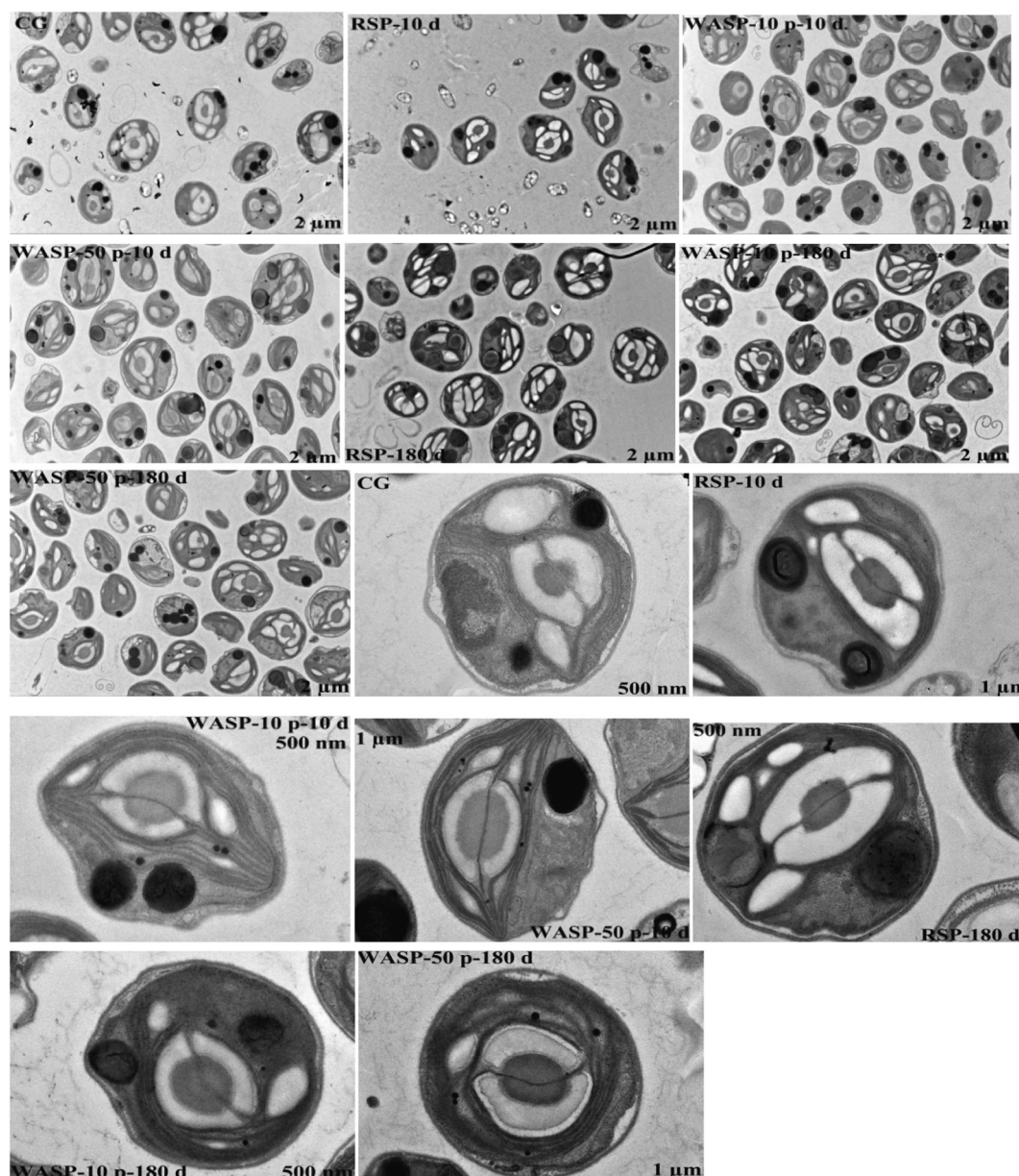
Fig. 4 – Ultrastructure of *C. vulgaris* cells after exposure to leachates with P addition from the raw and WTR amended sediments for 5 d. CG: OECD medium. P: phosphorous; WTRs: water treatment residuals; OECD: Organization for Economic Cooperation and Development.

increased in leachates after P addition to the same concentration as the control (Fig. 5). These results indicated that P deficiency was certainly the primary cause of the inhibition effects of raw and WTR-amended sediments on algae under pH 8.0 and 9.0.

2.5. Scientific implications

Assessing the environmental risk of P inactivating agents is necessary for any further advancement in eutrophication control methods. Previous researchers have investigated the effects of P inactivating agents, including Al-based compounds, Fe-based compounds, Ca-based compounds, Phoslock® (a lanthanum-modified clay) and Z2G1 (an aluminized zeolite) on phytoplankton. Al (aluminum sulfate) application, specifically,

has been shown to temporally decrease phytoplankton biomass (Galvez-Cloutier et al., 2012; Moore and Christensen, 2009) and change phytoplankton community structures (Holz and Hoagland, 1999; Paul et al., 2008). Fe (PIX-112, the main component of which is iron sulfate) application did not affect phytoplankton diversity directly, but substantially reduced biomass and changed species composition from the dominant cyanobacteria to diatoms, dinoflagellates, and chrysophytes (Gołdyn et al., 2014). $Ca(OH)_2$ application caused plankton biomass to rapidly decrease and the composition to change (Leoni et al., 2007), but main phytoplankton communities remained mostly unchanged (Zhang et al., 2001). Phoslock application reduced the concentration of chlorophyll *a* in lake water (Gunn et al., 2013; van Oosterhout and Lürling, 2011),
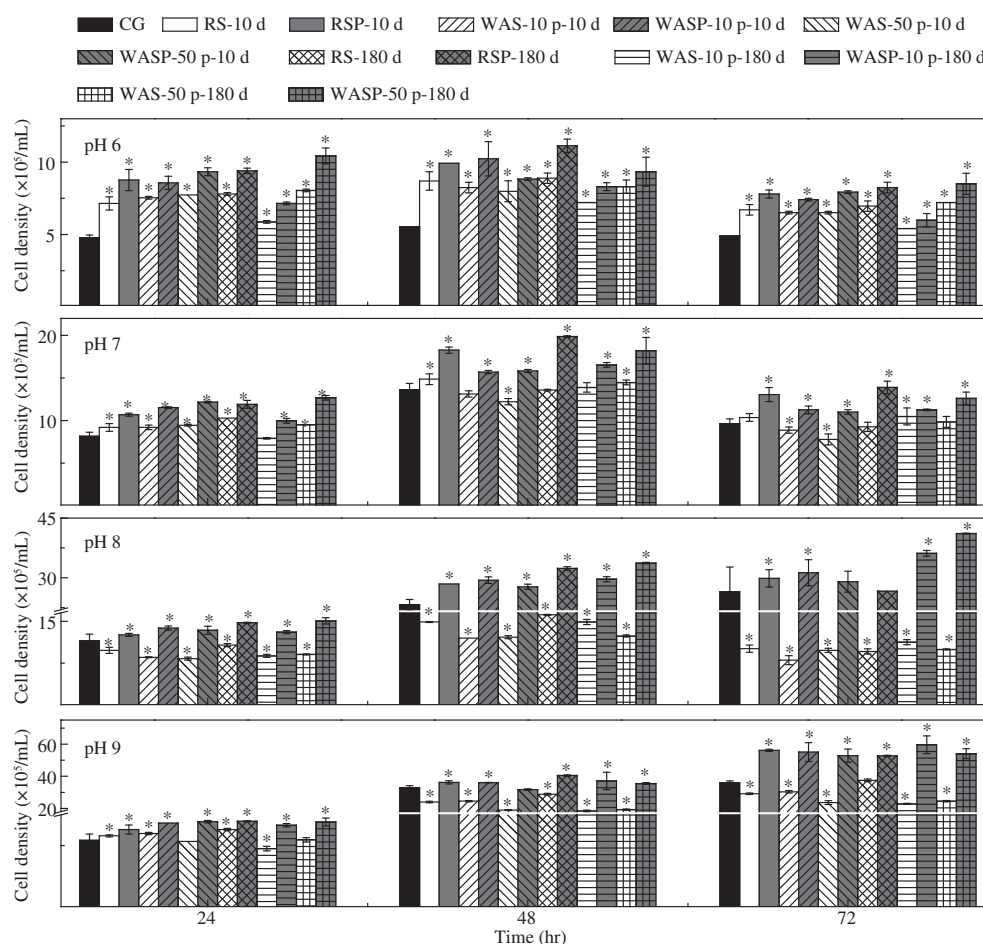
Fig. 5 – Algal cell density in *C. vulgaris* exposed to leachates of the raw and WTRs amended sediments with or without P addition at pH 6–9. CG: the OECD medium. *Statistically significant differences when compared with the control at the same exposure time as determined by the least significant difference ($p < 0.05$) under pH 6, 7, 8 and 9, respectively. WTRs: water treatment residuals; OECD: Organization for Economic Cooperation and Development.

and caused algae communities to shift from cyanobacteria-dominated blooms to green algae and diatom-dominant algae (Bishop et al., 2014). No significant differences in phytoplankton species composition were observed before and after Z2G1 application (Özkundakci et al., 2011). We mention these studies here as this information is important for future widespread application of P inactivating agents such as those we examined in this study.

Though the above studies have provided valuable contributions to the literature, information regarding the ecological effects of WTRs is generally lacking. The few studies on the subject mainly focused on the impact of discharging un-dewatered WTRs to surface waters, because WTRs were originally most commonly discharged into the natural environment without dewatering; reports to date suggested that un-dewatered WTRs may elicit a toxic response from aquatic organisms. The water-soluble constituents of Al sludge discharged to surface water, for example, were detrimental to algae growth while Al sludge extracts do not inhibit *Photobacterium phosphoreum*, ciliate *Tetrahymena pyriformis*, or fathead minnows (George et al., 1995). Direct discharge of un-dewatered Al sludge to a natural wetland

resulted in reduced productivity of the dominant macrophyte *Cyperus papyrus* L., and showed marked cumulative effects including ecosystem degradation in the long term (Kaggwa et al., 2001). Chronic toxicity of *Daphnia similis* exposure to un-dewatered WTRs has also been observed (Sotero-Santos et al., 2005). Discharging un-dewatered WTRs also significantly increased Al concentration in the aquatic system, increasing the potential Al toxicity risk to aquatic wildlife (Muisa et al., 2011).

We established an internal lake environment and applied WTRs for P release control to investigate the eco-toxicity of lake sediments toward algae after WTR addition. The results showed that P deficiency in leachates, owing to P adsorption by WTRs (Table 1 and Fig. 2), caused sediments with WTRs to inhibit algal growth; this effect also increased as WTR dose increased (Fig. 1). Given that WTRs are applied to restore degraded or eutrophied systems by immobilizing P and reducing harmful algal blooms, the observed impact on algal growth was acceptable and would be outweighed by simultaneous improvement in water quality. Moreover, we did not observe oxidative stress (Fig. 3) or any detrimental effects on cellular structure (Fig. 4) in the samples, indicating that the effects on algae were inhibitive rather than lethal. Even

long-term presence of WTRs in lake sediments was also determined to not induce extra eco-toxicity (Fig. 1).

The results of this study differed notably from those of studies regarding the impact of discharging un-dewatered WTRs. These differences may be related to the different characteristics of the WTRs investigated — ours were a series of dewatered, air-dried WTR samples. The dewatering process may have removed certain detrimental, water-soluble constituents from the sludge, and the metals in dewatered, air-dried WTRs likely had higher stability than dewatered, fresh WTRs would have (Wang et al., 2015b). Accordingly, dewatered, air-dried WTRs maybe better suited to eutrophication control.

WTRs have long been dumped into landfills, however, the cost and availability of land needed for this means of disposal are likely to increase owing to increasingly stringent environmental regulations and production (Babatunde and Zhao, 2007). WTR recycling for lake eutrophication control purposes, to this effect, creates a win–win situation for the economy and the environment. Future studies should investigate the effects of WTRs on aquatic organisms at different trophic levels (*e.g.*, bacteria, zooplankton, and fish).

3. Conclusions

This study investigated the eco-toxicity of lake sediments with the addition of dewatered WTRs on algae. Test results for several samples indicated that sediment leachates primarily exhibited inhibition effects on algal growth, and the inhibition effect was further determined to be caused by P deficiency of leachates owing to the adsorption of P by WTRs. Elevated doses of WTRs in sediments aggravated the algal growth inhibition effect due to increasing P deficiency, but extended duration of WTR addition to sediments elicited no further detrimental effects to the algae. We also found that the oxidation stress induced by exposure to sediment leachates was readily mitigated within a short amount of time (72 hr). After supplementing PO_4^{3-}-P (to the same concentration as the control) to the leachates from sediments with or without WTRs, no damaging effects were observed on algal subcellular structure. And P deficiency was still the main cause of inhibition effect in raw and WTR-amended sediments on algal growth under pH 8.0 and 9.0. Therefore, the dewatered WTRs were considered as a favorable potential material for internal P loading control in water supplies, as it shows acceptably low risk toward aquatic plants.

Acknowledgment

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.jes.2015.12.022.

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