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RESEARCH ARTICLE

Assessment of Pb and pyrene accumulation in *Scirpus triqueter* assisted by combined alkyl polyglucoside and nitrilotriacetic acid application

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Abstract To understand the accumulation and uptake of polycyclic aromatic hydrocarbons (PAHs) and heavy metals by plants is an important part of the assessment of phytoremediation for PAHs and heavy metals co-contaminated soil. This study was an investigation of the accumulation and uptake of pyrene and lead (Pb) by *Scirpus triqueter* under the condition of alkyl polyglucoside (APG) and nitrilotriacetic acid (NTA) combined application. The results indicated that the accumulation of Pb by *S. triqueter* was significantly improved by NTA and APG addition into the soil. The pyrene accumulation in plant was also increased after application of APG when compared to the control treatment. However, the pyrene accumulation was decreased when APG was applied together with NTA. SEM and TEM images of root surface suggested that more Pb in the soil transferred to the plant by combined application of APG and NTA. More importantly, TEM images of xylem cells of *S.triqueter* root showed that permeability of cell membrane was improved by application of APG.

Keywords Phytoremediation · Pyrene · Pb · Accumulation · SEM · TEM

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Introduction

Soil contamination with organic or inorganic pollutants, as an urgent global problem, has been attracting considerable public attention recently. Heavy metals pose numerous health hazards to higher organisms (Garbisu and Alkorta 2001). Among heavy metals, lead (Pb) is a non-essential toxic trace element existed in the environment (Kader et al. 2016; Zaier et al. 2014). The soils polluted by Pb are universal due to anthropogenic activities (Jin et al. 2015; Zhou et al. 2015). Polycyclic aromatic hydrocarbons (PAHs) are carcinogenic contaminants generated from the incomplete combustion of fossil fuels (Roy et al. 2005). Several studies have demonstrated that PAHs have been found to coexist with heavy metals frequently, especially with Pb (Cachada et al. 2012). So, it is meaningful to find a way to remove Pb and PAHs simultaneously.

Phytoremediation have been received increasing interests in the field of soil remediation, because of its low cost and friendly environment (Huang et al. 2011; Liu et al. 2015; Vargas et al. 2016). However, hyperaccumulators are usually small and grow slowly, which make them difficult to accumulate a mass of pollutants (Li et al. 2011). Besides, phytoremediation efficiency of PAHs and heavy metals (HMs) contaminated soil is always limited by their poor availability in soil (Leštan et al. 2008). To enhance the uptake of HMs and PAHs by plant, various extraction agents have been used, including surfactants and chelating agents. Zhu and Zhang (2008) used rhamnolipid as a biosurfactant to investigate the effect of rhamnolipid on the uptake of PAHs; the result suggested that rhamnolipid could enhance the uptake of PAHs by ryegrass roots. Gao et al. (2008) also found that Tween 80 could enhance the uptake of PAHs at lower Tween 80 concentration but inhibit the uptake of PAHs when the concentration of Tween was more than 6.6 mg L^{-1}. And the biodegradable chelating agent, EDDS, had been used to increase the uptake of Pb in shoot of sunflowers (Tandy et al. 2006). Alkyl polyglucoside (APG) is a nonionic surfactant produced from renewable resources such as fatty alcohols and glucose, which has been studied to assist plant to remove PAHs from soil (Liu et al. 2013a). Nitrilotriacetic acid (NTA) has been found that it can develop accumulation of HMs in plant due to the characteristic of its biodegradability and chelating effect with HMs (Quartacci et al. 2007). As a consequence, it will be a promisingly potential method by combining APG and NTA in phytoremediation to enhance accumulation and uptake of PAHs and Pb in plant.

Scirpus triqueter (*S. triqueter*), a dominant species in wetland of Huangpu-Yangtze estuary, has been reported to be an effective phytoremediation for pyrene and Pb (Liu et al. 2013b; Zhang et al. 2011). The present study used *S. triqueter* as phytoremediation plant to investigate the effect of APG and NTA on the accumulation and uptake of Pb and pyrene by plant. Besides, the morphologic changes of *S. triqueter* induced by APG and NTA were also investigated by scanning electron microscope and transmission electron microscope to better explore this strengthening mechanism.

Materials and methods

Chemicals and plant materials

Pyrene (purity 98%) was purchased from Aladdin Reagent. APG used in the test was C12/14-APG (APG1214) obtained from the China Research Institute of Daily Chemical Industry (Shanxi, China). The other chemicals, analytical grade or better, were bought from Sinopharm. Seedlings of *S. triqueter* were gathered from Huangpu-Yangtze estuary.

Soil preparation

Soil (air-dried, 2 mm sieved) used in this experiment was collected from campus area in Shanghai University, Shanghai, China. The main characteristics of the soil are as follows: pH 8.3, organic matter 19.6 g kg^{-1}, total nitrogen 0.52 g kg^{-1}, clay 7.4%, and silk 60.4 and sand 32.2%.

Soil was contaminated with Pb by Pb [$(CH_3COO)_2Pb$] solution. Few days later, pyrene dissolved in acetone was added into the Pb contaminated soil. After the acetone volatilization, the mixed contaminated soil was transferred in a dark room for 2 months before the pot experiment while keeping 40–60% of moisture content. The final concentrations of pyrene and Pb in the soil were measured as 184.50 and 454.30 mg kg^{-1}, respectively.

Pot experiment

Seedling of *S. triqueter* with a similar size and biomass (3 g/pot) were transplanted into the plastic pots (14.8 cm diameter and 8.8 cm height) containing 500 g contaminated soil. During the period of cultivating, water content of the soil in each pot was controlled at approximately 60% of its water holding capacity by periodic replenishment of water. After 10 days of transplanting, the test plants were treated with APG and NTA as followings: CK (control), A (2 g APG kg^{-1} soil), N (2 g NTA kg^{-1} soil), and N + A (2 g NTA + 2 g APG kg^{-1} soil). The mixed solution of NTA and APG were applied to the soil surface, adjusted to the pH to 8.3 with NaOH in order to limit soil property modification. About 60 days later, root and stem of *S. triqueter* were harvested respectively.

The concentrations of pyrene and Pb in plant tissues were analyzed by an ultrasonic extraction and an acid digestion respectively, determining by Agilent 7890 gas chromatography-5975 mass spectrometry (7890GC-5975MS) and inductively coupled plasma optical emission spectrometry (ICPOES), respectively.

Scanning electron microscopy (SEM) of *S. triqueter* root was performed at Shanghai Institute of Plant Physiology, Chinese Academy of Sciences. Transmission electron microscopy (TEM) was used to further investigate the change of microstructure of root after application of APG and NTA at Shanghai Normal University.

Statistical methods

All of the experiments were carried out with at least three replications. SPSS software program (version 17.0 for Windows) was used to determine significant differences among all treatments at the $P < 0.05$ level.

Results and discussion

Pyrene and Pb contents in root and shoot are presented in Table 1. Either in alone APG application treatment or combined application of APG and NTA treatment, pyrene accumulation in both root and shoot increased significantly when compared to the control treatment. In our previous study (Chen et al. 2016b), APG or NTA significantly enhanced the biomass of *S. triqueter* except for the combined application of APG and NTA. Though the biomass in the combined application of APG and NTA decreased significantly, the pyrene and Pb accumulation in *S. triqueter* still increased due to the promotion of APG and NTA. The increases of pyrene accumulation in *S. triqueter* tissues by application of surfactant in present study were consistent with previous work (Liu et al. 2013a; Zhu and Zhang 2008). Two mechanisms may lead

Table 1 Pyrene and Pb concentration and accumulation in *S.triqueter* in different treatments

	Pyrene concentration (mg/kg)		Pb concentration (mg/kg)		Pyrene accumulation (mg/pot)	Pb accumulation (mg/pot)
	Root	Shoot	Root	Shoot		
CK	42.40^{b} (±1.93)	ND	201.34^{d} (±0.87)	2.41^{c} (±0.10)	0.04^{b} (<0.01)	0.56^{c} (±0.06)
A	86.77^{a} (±12.33)	34.38^{a} (±9.42)	250.62^{c} (±6.19)	3.33^{c} (±0.57)	0.15^{a} (±0.25)	0.90^{c} (±0.08)
N	16.10^{c} (±5.18)	ND	482.63^{b} (±5.67)	41.90^{b} (±1.53)	0.02^{b} (±0.01)	2.29^{b} (±0.10)
A + N	51.04^{b} (±10.36)	13.12^{b} (±3.61)	1600.15^{a} (±18.11)	232.24^{a} (±7.30)	0.05^{b} (±0.01)	4.18^{a} (±0.48)

Values presented are means of three replicates with standard deviations. Different letters indicate that value are significant different at $p < 0.05$

to this increase. On the one hand, since surfactants can facilitate desorption of PAHs form soil (Cheng and Wong 2006), APG-enhanced mobilization might account for the accumulation of pyrene in plant. On the other hand, surfactants were observed to increase the permeability of cell membrane (Zhu and Zhang 2008), which led to a more efficient uptake of pyrene. Moreover, the phenomenon appeared in present study differ from the report that the concentration of pyrene in maize was decreased after application of surfactant Triton X-100. Different plants (Sun et al. 2014), surfactants, and dosages of surfactant may account for this difference, while the accumulation of pyrene in *S. triqueter* was decreased at the presence of NTA. This result could be seen from the accumulation of pyrene in the NTA treatment and the APG and NTA combined treatment. The change of Pb concentration in tissues caused by NTA may lead indirectly to this decrease. Similar result was also reported that more anthracene and fluoranthene were accumulated in waxy corn roots when Pb was absent in soil (Somtrakoon et al. 2015).

Compared to the CK treatment, concentrations and amounts of Pb in plant were greatly improved in treated groups especially in the treatment of combined application (Table 1). Firstly, Pb can be desorbed out from soil matrix by combing NTA to form complexes (Nowack et al. 2006; Quartacci et al. 2007), which could increase the solubility of Pb in soil. And we had proved in our previous study (Chen et al. 2016a) that NTA significantly increased the exchangeable content of Pb in soil. Secondly, the permeability of cell membrane may improve by application of surfactant (Zhu and Zhang 2008) so that more Pb was accumulated by plant. Finally, the two mechanism respectively caused by NTA and APG may happen simultaneously, which led to the remarkable increase after combined application of APG and NTA. From the description above, we could conclude that the combined application of APG and NTA had a synergistic effect on the Pb uptake but an antagonistic effect on the pyrene uptake. The different effects of APG and NTA on Pb and pyrene were due to the different nature of Pb and pyrene. The removal way of Pb was phytoextraction; however, the removal way of pyrene mainly depended on degradation by microbe.

Previous reports showed that root uptake was probably the main pathway for pollutants (Fismes et al. 2002), so in this study, the SEM and TEM experiments were performed to observe the morphological changes of root. Figure 1 shows the SEM images of the surface of *S. triqueter* root in different treatments. With application of NTA (N and A + N), the root surface was covered with many clusters (which was pointed out by the white arrows) of about 10 μm crystal, although the tissues were carefully washed with water several times. These crystals probably are the chelate complexes formed by NTA and Pb, which indicated that more Pb in soil can migrate to plant by application of NTA. Tandy et al. (2006) studied the uptake of Pb, Cu, and Zn by sunflowers were increased by application of EDDS and showed that increased translocation of Pb could be explained by enhanced uptake of the Pb-EDDS complex. Especially, the amount of the clusters covered with root in A + N treatment was much more than that in N treatment. This comparison suggested that the presence of APG could assist transferability of Pb caused by NTA in soil. Similar result was shown in other researches that significant promotion on Pb and Cu desorption was observed with mixed solution of saponin and EDDS (Cao et al. 2013). However, further researches should be conducted to confirm the composition of the clusters covered at root surface. Besides, when compared to CK treatment, SEM images with APG treatment showed no evidence that surfactant micelles were covered with roots. The reason for this was probably that the pyrene adsorbed on the root surface was more likely to be rather degraded by microorganisms in the *S. triqueter* rhizosphere than translated into *S. triqueter*. The conclusion could be obtained from the result of our previous study (Chen et al. 2016b) and the data of Table 1. In our previous study, the removal ratio of pyrene from soil increased significantly after adding APG and NTA. However, the accumulation of pyrene in *S. triqueter* was no obvious change. The reduction of pyrene was probable to degrade by microorganisms. Bacosa and Inoue (2015) and Bacosa

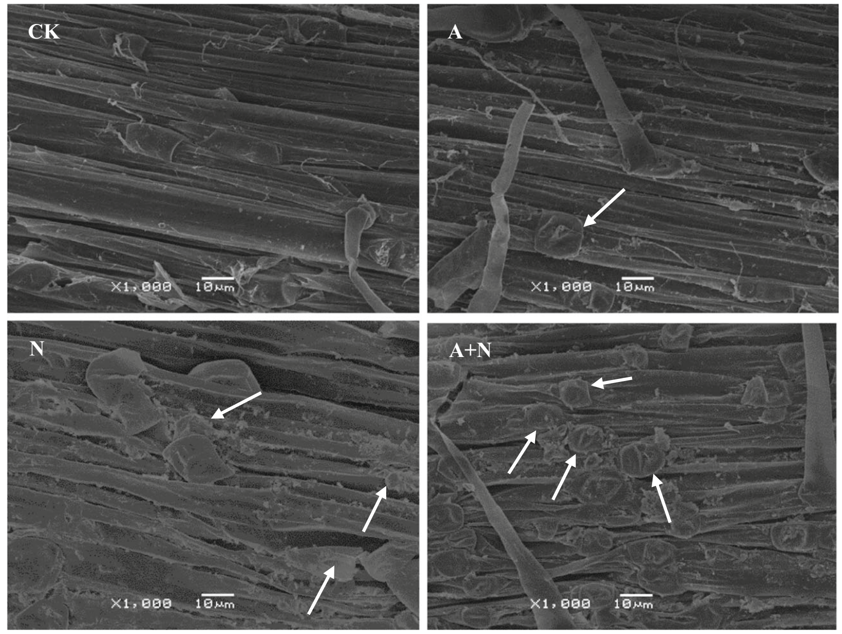

Fig. 1 Scanning electron microscope photographs of root surface of *S.triqueter* with different treatments. The *white arrows* point to the clusters. *CK* control, *A* containing 2 g APG kg^{-1} soil, *N* containing 2 g NTA kg^{-1} soil, *N* + *A* containing 2 g NTA + 2 g APG kg^{-1} soil

et al. (2013) found that *Pseudomonas* and *Burkholderia* had the highest ability to degrade aliphatic and aromatic hydrocarbon compounds, and PAH-ring hydroxylating dioxygenase (PAH-RHDα) gene was shown to be more effective than nidA in estimating pyrene-degrading bacteria in the enriched consortia. We had separated *Pseudomonas* from the rhizospheric soil (Wang et al. 2010) and the body (Zhang et al. 2014) of *S. triqueter*. All of the studies could prove that the pyrene was more likely to be degraded by microorganisms.

Similar to SEM observation, TEM images of epidermis cells of *S.triqueter* root (Fig. 2) showed that more Pb particles (the black arrow pointed to the Pb particles) were absorbed to the root epidermis cells with application of

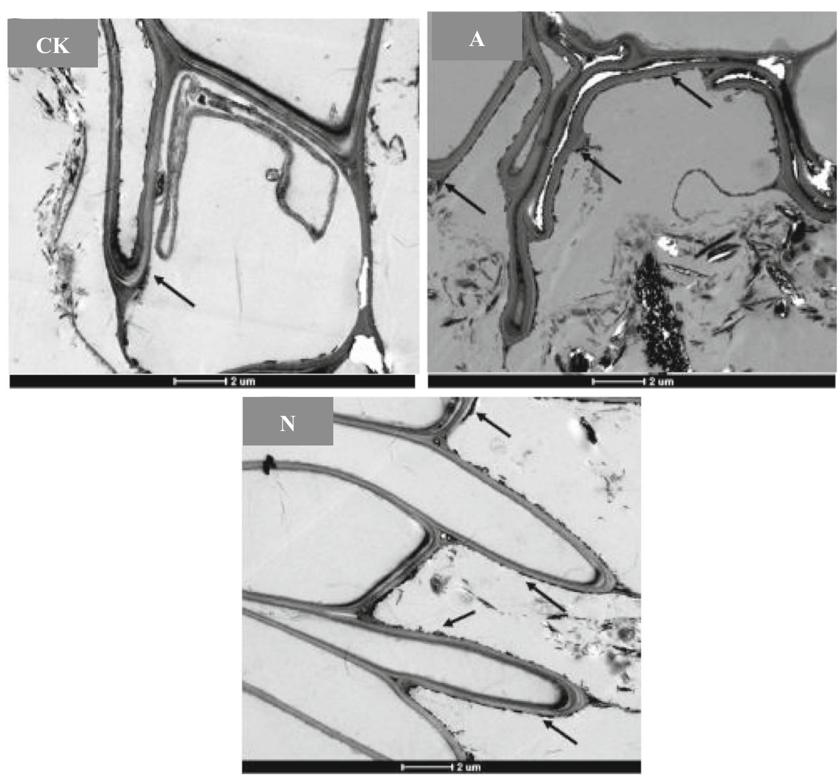

Fig. 2 Transmission electron microscope photographs of epidermis cells of *S.triqueter* root in different treatments. The *black arrow* pointed to the Pb particles. *CK* control, *A* containing 2 g APG kg^{-1} soil, *N* containing 2 g NTA kg^{-1} soil, *N* + *A* containing 2 g NTA + 2 g APG kg^{-1} soil

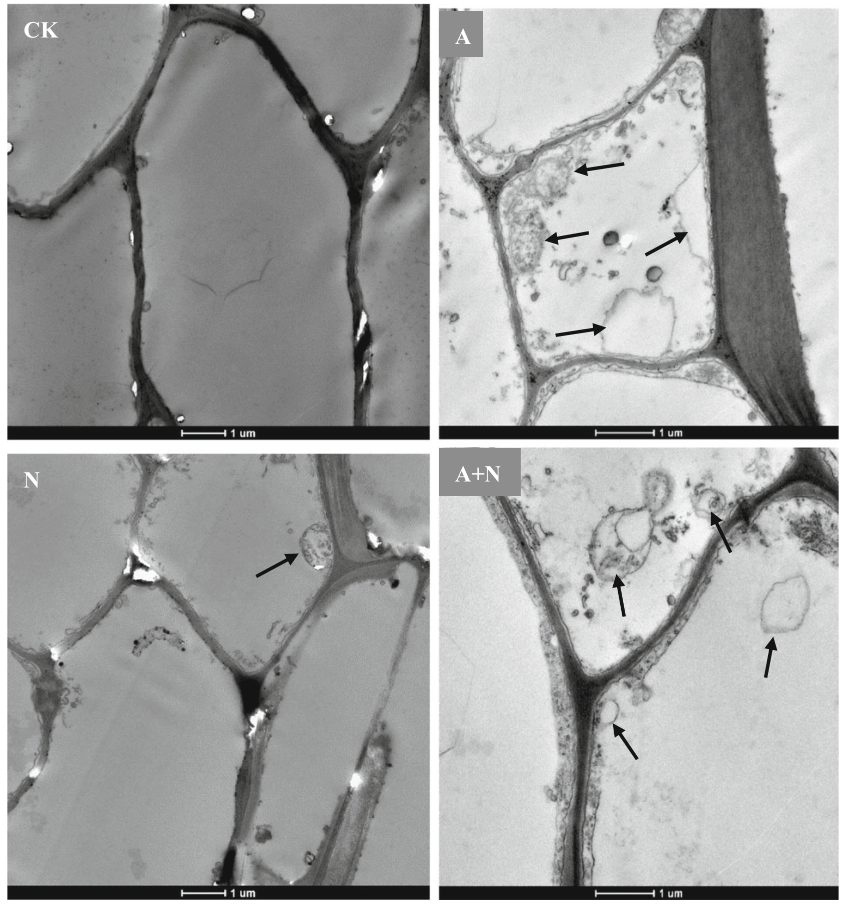

Fig. 3 Transmission electron microscope photographs of xylem cells of *S.triqueter* root in different treatments. The *black arrow* pointed to the phagocytic vacuole. *CK* control, *A* containing 2 g APG kg^{-1} soil, *N* containing 2 g NTA kg^{-1} soil, *N + A* containing 2 g NTA + 2 g APG kg^{-1} soil

APG and NTA when compared to the control treatment (CK), especially in N treatment. The difference between treated treatments (A and N) and control treatment (CK) would be probably due to the formation of NTA and Pb complexes and the micelles formed by APG. With NTA and APG addition (especially with NTA), a series of Pb-NTA complexes and micelles containing Pb would be formed, leading to efficient transportation of Pb from soil matrix to plant root. Similar result reported by Qi et al. (2014) that more U deposits were transported into plant with citric acid (a chelating agent) addition into soil. More importantly, TEM images of xylem cells of *S.triqueter* root in different treatments (Fig.3) showed that the permeability of cell membrane probably be improved when APG was added into soil (A and A + N treatments). Many phagocytic vacuoles generated in A and A + N treatments, which implied that foreign materials entered the xylem cells. So, we concluded that APG enhanced the membrane transport of Pb. Other researches showed that surface tension at the cellular walls of root can be increased with surfactant application (Almeida et al. 2009; Di Gregorio et al. 2006). This improvement with permeability of cell membrane may lead to more pollutants transport from cell wall to cell membrane or vacuole, which is benefit for phytoremediation for contaminated soil. However, further researches should be conducted to confirm the effect of APG on increasing permeability of cell membrane.

Conclusions

This study investigated the effect of combined application of APG and NTA on the accumulation and uptake of pyrene and Pb by *S.triqueter.* The results indicated that APG addition to soil significantly increased accumulation of pyrene, and NTA addition significantly increased accumulation of Pb in *S.triqueter* plant. The presence of APG had positive effect on Pb accumulation in *S.triqueter*. However, the presence of NTA had negative effect on pyrene accumulation in *S.triqueter*. So, the combined use of APG and NTA was benefit to Pb removal. SEM and TEM photographs of root surface showed that the addition of APG and NTA enhanced the migration and enrichment of Pb to the root of *S.triqueter*. Moreover, APG increased the transmembrane transport of Pb. The application of APG and NTA could significantly improve the dissipation of pyrene from soil (Chen et al. 2016b). However, the accumulation amount of pyrene in *S.triqueter* was small. According to what we have learnt, biodegradation is the main removal way for pyrene. So, the addition of APG and NTA could enhance the biodegradation of pyrene. The results supply a way to reinforce phytoremediation of organic and heavy metal in polluted soils.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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