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Effects of NTA on Pb phytostabilization efficiency of *Athyrium wardii* (Hook.) grown in a Pb-contaminated soil

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Abstract

Purpose Chelate-assisted phytoremediation with biodegradable chelates has been considered to be a promising technique to enhance phytoremediation efficiency, while little information is available on phytostabilization. This study aims to assess NTA-assisted phytostabilization of Pb-contaminated soils by *Athyrium wardii* (Hook.).

Materials and methods A pot experiment was carried out to investigate the effects of different application days (1, 3, 5, 7, 10, 14, 21) of nitrilotriacetic acid (NTA) on plant growth, Pb accumulation, and Pb availability in rhizosphere soils of *A. wardii* grown in soils contaminated with low (200 mg kg^{-1}) and high (800 mg kg^{-1}) concentrations of Pb.

Results and discussion With the application of NTA, better growth for *A. wardii* was observed when treated with NTA for 5–14 days for both low and high Pb soils, suggesting potential harvest time. Pb concentrations and Pb accumulation in underground parts of *A. wardii* grown in low and high Pb soils increased with increasing application time of NTA generally. Similar changes were also found for bioaccumulation coefficients (BCFs) of *A. wardii*. The greatest remediation factors (RFs) for underground parts and whole plant of *A. wardii* were observed for NTA application time of 7 and 5 days for low and high Pb soils, suggesting the greatest remediation efficiency. Furthermore, plant growth, BCF, and RF of *A. wardii* grown in low Pb-contaminated soils were greater than those grown in high Pb-contaminated soils. Pb availability in rhizosphere soils of *A. wardii* grown in low Pb soils was lower than those in high Pb-contaminated soils.

Conclusions It seems to be the optimum for *A. wardii* to phytostabilize slightly Pb-contaminated soils with the application of NTA for 7 days as taking plant growth, Pb remediation efficiency, and environmental risk into consideration.

Keywords Chelant-assisted phytostabilization · Fern · Heavy metal · Nitrilotriacetic acid · Pb-contaminated soils

1 Introduction

Due to the long history of Pb exploitation and its widespread use, Pb contamination in mining areas has become a severe problem all around the world, especially in developing countries, such as China (Johnson et al. 2016; Marx et al. 2016; Mariet et al. 2017). China holds diversified and large-scale mineral resources. In particular, Pb-Zn mines are widely located in the eastern and southern regions of China, such as the provinces of Fujian, Zhejiang, Guizhou, and Hunan. Pb-Zn mining wastes present a serious environmental hazard, as Pb and associated metals are continuously released into the environment (Li et al. 2014; Gutiérrez et al. 2016). More specifically, Pb was found to be the second greatest pollutant among heavy metals in mines from 22 provinces in China (Li et al. 2014). Being persistent, covert, and irreversible, Pb pollution not only degrades the quality of soils and food crops, but also threatens human health through the food chain (Pourrut et al. 2011; Saifullah et al. 2015; Marx et al. 2016; Mariet et al. 2017).

Phytostabilization, which refers to long-term succession of plant community in mine tailings to promote the soil development process and finally restore soil ecosystem functions, provides an attractive alternative for the remediation of Pb-contaminated soils (Bolan et al. 2011; Sarwar et al. 2017). Metal accumulation in plant roots is considered to be one of

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the most important processes for phytostabilization (Mendez and Maier 2008; Bolan et al. 2011). Nevertheless, heavy metal availability in soils is the most important limiting factor to heavy metal uptake by plant roots (Saifullah et al. 2015; Sarwar et al. 2017).

To promote heavy metal uptake by plants, the use of synthetic or organic chelating agents, known as chelant-assisted phytoremediation, has been proposed in recent years (Wenger et al. 2008; Shahid et al. 2012; Sarwar et al. 2017). This technology is of particularly great use in phytoremediation of heavy metals with very low solubility and availability, such as Pb (Huang et al. 1997; Pourrut et al. 2011; Saifullah et al. 2015). It is reported that available Pb in soils is usually less than 0.1% of the total Pb in soils (Huang et al. 1997). Thus, chelating agents, such as nitrilotriacetic acid (NTA), ethylenediaminetetraacetic acid (EDTA), and ethylenediamine disuccinic acid (EDDS), have been extensively investigated for their advantages in solubilizing Pb and promoting Pb uptake by plants (Udovic and Lestan 2009; Shahid et al. 2012; Zhang et al. 2016). Among these chelants, NTA, with a half-life of 2–7 days, has been considered to be an ideal candidate for Pb phytoremediation owing to high biodegradability, low toxicity, and low leaching risk (Quartacci et al. 2005; Wenger et al. 2008; Hseu et al. 2013; Saifullah et al. 2015). Numerous studies have shown that NTA was effective in enhancing Pb uptake by plants, thus demonstrating its potential application in enhancing phytostabilization (Freitas and do Nascimento 2009; Babaeian et al. 2016; Hu et al. 2017). It has been proved that the efficiency of chelants in enhancing Pb solubility and bioavailability in soils could be attributed to many factors, including chelant dosage, chelant application time, and Pb concentrations in soils, in particular (Wang et al. 2009; Zaier et al. 2010; Usman et al. 2013; Saifullah et al. 2015). In this sense, it is of great importance to investigate the effects of NTA application dosage, NTA application time, and Pb concentrations in soils on the phytostabilization efficiency of soils contaminated with different Pb concentrations to optimize the efficiency of chelant-assisted phytostabilization by plants.

Athyrium wardii (Hook.), a perennial fern growing in an old Pb-Zn mine tailing in Yingjing, Ya'an, Sichuan Province, China, has been identified as a mining ecotype in our previous surveys. It showed great potential for phytostabilization of Pb-contaminated soils due to large root biomass, great Pb accumulation in underground parts, and lower translocation from underground to aboveground parts (Zou et al. 2011; Zou et al. 2012; Zhao et al. 2016a). Earlier study on different dosages of chelant-assisted phytostabilization to enhance the efficiency of Pb remediation by *A. wardii* has been processed. It has been found that 2 mmol kg^{-1} of NTA-assisted phytostabilization could be a better alternative to enhance the Pb remediation efficiency of *A. wardii* among a series of NTA concentrations (Zhao et al. 2016b). Following this, further studies on effects of NTA (2 mmol kg^{-1}) application time on plant growth, Pb accumulation, and Pb availability in rhizosphere soils of *A. wardii* grown in soils contaminated with low and high concentrations of Pb were processed in this work. The most appropriate time for NTA application was therefore assessed to optimize NTA-assisted phytostabilization of Pb-contaminated soils by *A. wardii*. All of this also shows great benefit for improving the efficiency of chelant-assisted phytostabilization of Pb-contaminated soils by *A. wardii*.

2 Materials and methods

2.1 Plant and soil preparation

In this work, the seedlings of *A. wardii* were obtained from a Pb-Zn mine area in Yingjing, Ya'an, Sichuan Province, China (102° 31′ E, 29° 47′ N). Healthy seedlings were separated into similar size and cultivated in vermiculite media with 1/10 Hoagland solutions for 2 weeks before transplantation for pot experiment.

Pot soils consisted of humic substances and calcareous alluvial soils (2:3, *w/w*) (Zhao et al. 2016c). Calcareous alluvial soils, obtained from a farmland uncontaminated with Pb in Dujiangyan, Sichuan Province, China, were air-dried and sieved through 2-mm sieve. Humic substances, derived from mature plant litter, were collected from the Lingyan Mountain in Dujiangyan, Sichuan Province, China and mixed thoroughly with calcareous alluvial soils. Subsequently, the mixed soils were artificially contaminated with Pb, applied with $Pb(NO_3)_2$ solutions, at the concentrations of 200 (low Pb) and 800 (high Pb) mg Pb kg^{-1} soil, respectively. Following a full mix, the spiked soils were homogenized for 30 days. The characteristics of the homogenized soils are presented in Table 1.

2.2 Pot experiment

The pot experiment was conducted in a greenhouse in an experimental station at Sichuan Agricultural University in Dujiangyan, Sichuan Province, China, with natural light. Following pre-cultivation for 2 weeks, the uniform and healthy plants were selected and transplanted into pots (5 L) with two plants per pot. Each pot was filled with 5-kg soils. After 30-day growth, NTA was applied into each pot at the concentration of 2 mmol kg^{-1} soil with 24 replicates for both low Pb soils and high Pb soils. A total of 48 pots were arranged randomly and interchanged positions regularly to keep similar growth condition. During the experiment, all plants were watered with deionized water to maintain soils at approximately 70% of field capacity.

Following the application with NTA for 1, 3, 5, 7, 10, 14, and 21 days, plant samples with three replicates for each treatment were harvested, respectively. After being carefully washed with tap water followed by deionized water, plant

Table 1 Characteristics of the experimental soils after homogenization

Treatment	Total Pb (mg kg^{-1})	Available Pb (mg kg^{-1})	OM (g kg^{-1})	TN (g kg^{-1})	AN (mg kg^{-1})	AP (mg kg^{-1})	AK (mg kg^{-1})	pH
Pb200	213.97	60.85	73.72	1.12	30.94	15.66	40.28	4.89
Pb800	816.82	231.80	77.71	1.07	24.50	19.96	45.33	4.87

pH, soil/water, 1:2.5. Pb200 and Pb800 are for Pb concentrations of 200 and 800 mg kg^{-1} soil. Data are means of three replicates

OM organic matter, *TN* total nitrogen, *AN* available nitrogen, *AP* available phosphorus, *AK* available potassium

samples were separated into aboveground and underground parts. The underground parts of the plant samples were soaked in 20 mM Na_2-EDTA for 15 min to remove Pb adhering to underground parts. Then, all plant samples were dried at 75 °C, weighed, and ground for analysis of Pb concentrations. Rhizosphere soils were also collected by brushing soils tightly adhering to the underground parts. After being air-dried, soil samples were ground and sieved through 1-mm and 0.15-mm mesh for analysis of pH and available Pb.

2.3 Plant and soil analysis

Soil pH, organic matter, total nitrogen, and available phosphorus were determined according to the standard procedures of the International Organization for Standardization (ISO) with some modifications (Margesin and Schinner 2005). Soil available nitrogen and available potassium were determined referring to Zhang et al. (2014).

The determination of Pb concentrations in plants and soils was referred to Zhao et al. (2016b). Approximately 0.3-g plant samples were digested with HNO_3 and $HClO_4$ (5:1, *v*/*v*). After cooling, the digested solutions were washed into 50-mL volumetric flask, made up to volume, and filtered through 0.22-μm membrane filter. HNO_3, $HClO_4$, and HF (5:1:1, *v*/*v*/*v*) were used for soil digestion with similar procedure as plant digestion. Available Pb in soils was assayed by extracting with DTPA solutions as described by Zhao et al. (2016b). Pb concentrations in filtrates of plant and soil samples were finally determined by flame atomic absorption spectrophotometry (AA900T, PerkinElmer, USA).

2.4 Data processing

Calculations for bioaccumulation coefficient (BCF), translocation factor (TF), and remediation factor (RF) were as follows (Zhan et al. 2016; Zhao et al. 2016a):

$$\mathrm{BCF} = C_{\mathrm{underground}}/C_{\mathrm{soil}} \quad (1)$$

where $C_{\mathrm{underground}}$ is for Pb concentrations in underground plant parts and C_{soil} is for Pb concentrations in soils.

$$\mathrm{TF} = C_{\mathrm{aboveground}}/C_{\mathrm{underground}} \quad (2)$$

where $C_{\mathrm{aboveground}}$ is for Pb concentrations in aboveground plant parts and $C_{\mathrm{underground}}$ is for Pb concentrations in underground plant parts.

$$\mathrm{RF}\ (\%) = A_{\mathrm{plant}}/(\mathrm{TPb} \times M_{\mathrm{soil}}) \times 100 \quad (3)$$

where A_{plant} is for Pb accumulation in underground plant parts or whole plant, TPb is for total amount of Pb in soils, and M_{soil} is for the weight of dried soils.

Data presented in this work were the means of three replicates. LSD test was performed to make the multiple comparisons. Significant difference was considered at $P < 0.05$. All statistical analysis was performed by SPSS 22.0. Graphical work and spreadsheet were processed on Origin 9.0 and Excel 2016.

3 Results

3.1 Plant growth

The growth of *A. wardii* grown in both levels of Pb-contaminated soils was considerably affected by the addition of NTA (Fig. 1). The aboveground biomass of *A. wardii* grown in low Pb-contaminated soils increased with increasing application time of NTA from 1 to 14 days, beyond which a significant decrease was observed. The greatest aboveground biomass of *A. wardii* grown in low Pb-contaminated soils was found for the addition time of 5, 10, and 14 days. A similar increase was also observed for the aboveground biomass of *A. wardii* grown in high Pb soils when applied with NTA for 1–3 days, beyond which there were no significant changes. For the underground biomass of *A. wardii* grown in low Pb-contaminated soils, there were no significant changes for the application time from 1 to 14 days, beyond which there was a significant decrease. The underground biomass of *A. wardii* grown in high Pb-contaminated soils increased first and then decreased and reached the maximum value for the application time of 5, 7, and 14 days. In general, both the aboveground and underground biomass of *A. wardii* grown in low Pb-contaminated soils were significantly higher than those grown in high Pb-contaminated soils.

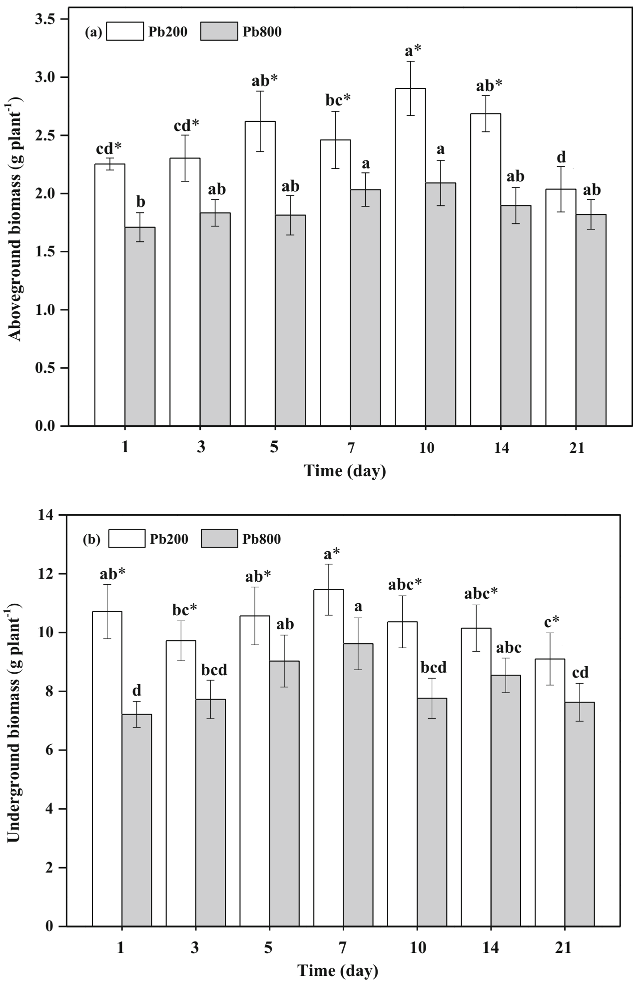

Fig. 1 Effect of NTA addition time on biomass in aboveground (**a**) and underground (**b**) parts of *A. wardii* grown in low and high Pb soils. Pb200 and Pb800 are for Pb concentrations of 200 and 800 mg kg^{-1} soil. Each value is the mean of three independent replicates. Error bars indicate standard deviation. The lowercase letters refer to significant difference ($P < 0.05$) among the different addition time of NTA. The asterisk represents significant difference ($P < 0.05$) between the low and high Pb soils

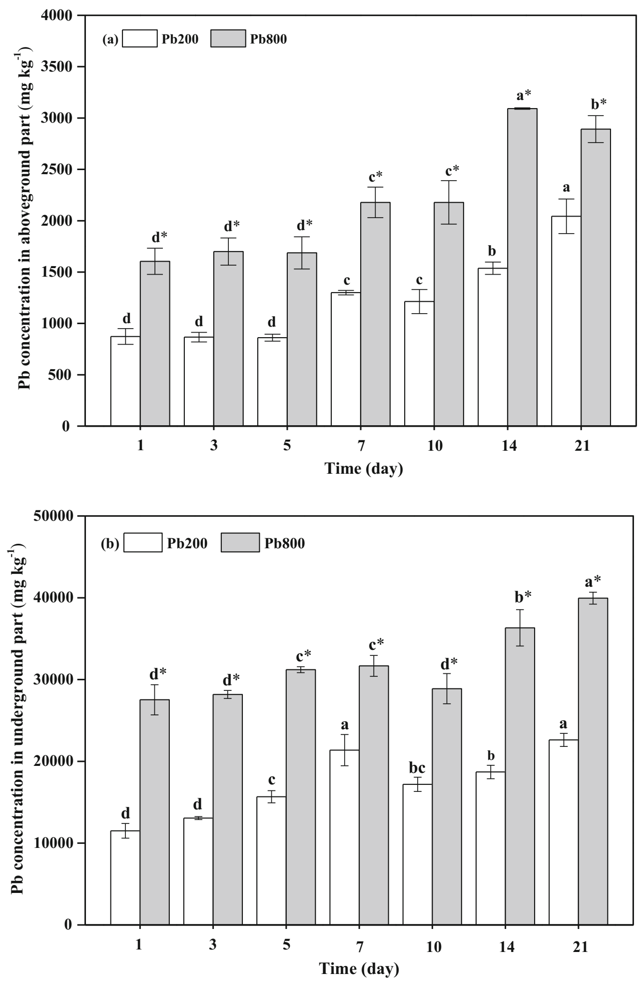

Fig. 2 Effect of NTA addition time on Pb concentrations in aboveground (**a**) and underground (**b**) parts of *A. wardii* grown in low and high Pb soils. Pb200 and Pb800 are for Pb concentrations of 200 and 800 mg kg^{-1} soil. Each value is the mean of three independent replicates. Error bars indicate standard deviation. The lowercase letters refer to significant difference ($P < 0.05$) among the different addition time of NTA. The asterisk represents significant difference ($P < 0.05$) between the low and high Pb soils

3.2 Pb accumulation

It was evident that Pb concentrations in aboveground and underground parts of *A. wardii* increased with increasing application time of NTA (Fig. 2). Pb concentrations in aboveground parts of *A. wardii* grown in low and high Pb soils showed no significant changes among the application time from 1 to 5 days, beyond which a significant increase was observed with increasing application time of NTA. Similar changes were also observed for Pb concentrations in underground parts of *A. wardii*. For both low and high Pb soils, Pb concentrations in underground parts showed no significant changes among the application time from 1 to 3 days, beyond which a significant increase was found with increasing application time of NTA. Pb concentrations in aboveground and underground parts of *A. wardii* treated with high Pb were significantly higher than those treated with low Pb.

The changes of Pb accumulation in underground parts and BCF further proved that Pb accumulation in underground parts of *A. wardii* increased with increasing application time of NTA (Table 2). Pb accumulation in underground parts for both low and high Pb soils significantly increased with increasing application time of NTA from 1 to 7 days, beyond which there were no significant changes in general. The BCF values for the low and high Pb soils also increased with increasing application time of NTA in general. No significant changes were observed for BCF values for the high Pb soils when the NTA application time was more than 7 days. Among the two Pb-contaminated soils, Pb accumulation in underground parts of *A. wardii* grown in low Pb soils was

Table 2 Effect of NTA addition time on Pb accumulation in underground parts and bioaccumulation coefficients of *A. wardii* grown in low and high Pb soils

Time (days)	Pb accumulation in underground part (mg plant^{-1})		Bioaccumulation coefficient	
	Pb200	Pb800	Pb200	Pb800
1	123.79e	198.05c*	53.77f*	33.71b
3	127.07de	217.79c*	61.05e*	34.50b
5	165.94cd	281.90b*	73.25d*	38.20b
7	243.90a	304.14ab*	99.88a*	38.78b
10	178.24bc	293.66ab*	80.32c*	46.11a
14	189.60bc	330.88a*	87.40b*	47.42a
21	206.35ab	304.91ab*	105.73a*	48.91a

Pb200 and Pb800 are for Pb concentrations of 200 and 800 mg kg^{-1} soil. Data are means of three replicates. The lowercase letters refer to significant difference ($P < 0.05$) among the different addition time of NTA

*Significant difference ($P < 0.05$) between the low and high Pb soils

significantly lower than that grown in high Pb soils. However, the BCF values for low Pb soils were significantly higher than those for high Pb soils.

3.3 Pb phytostabilization potential

For both low and high Pb soils, the remediation factors for underground parts showed a consistent change with those for the whole plant with increasing application time of NTA (Table 3). In case of the low Pb soils, the RF values for underground part and whole plant increased with increasing application time to 7 days, beyond which a decrease was observed. For the high Pb soils, the RF values for underground part and whole plant increased with increasing application time to 5 days, beyond which there were no significant changes. It seems that the greatest RF values were observed for the NTA application of 7 and 5 days for the low and high Pb soils, respectively. It was also found that the RF values for underground parts and whole plant for the low Pb soils were significantly higher than those for high Pb soils.

3.4 Soil pH and available Pb

The rhizosphere soil pH of *A. wardii* grown in both low and high Pb-contaminated soils was lower than that before transplantation (Table 1) and decreased with increasing application time of NTA in general (Fig. 3). For low Pb soils, the rhizosphere soil pH of *A. wardii* decreased from 1 to 7 days, beyond which no obvious changes were observed. For high Pb soils, the rhizosphere soil pH of *A. wardii* presented a fluctuating change with increasing application time of NTA. Lower pH values of 4.54 and 4.55 were observed for the application time of 5 and 21 days, respectively. The rhizosphere soil pH of *A. wardii* treated with high Pb was lower than low Pb when treated with NTA for 1, 3, 5, and 21 days.

Available Pb in rhizosphere soils of *A. wardii* for both low and high Pb soils was lower than that before transplantation (Table 1 and Fig. 4). For low Pb soils, available Pb in rhizosphere soils of *A. wardii* showed no significant changes for the application time from 1 to 10 days and then significantly decreased with increasing application time. For high Pb soils, available Pb in rhizosphere soils of *A. wardii* showed no

Table 3 Effect of NTA addition time on remediation factors for underground part and whole plant of *A. wardii* grown in low and high Pb soils

Time (days)	Remediation factor for underground part		Remediation factor for whole plant	
	Pb200	Pb800	Pb200	Pb800
1	23.14e*	9.70c	23.51e*	9.83c
3	23.75e*	10.67bc	24.13e*	10.82bc
5	31.02d*	13.80abc	31.44d*	13.95abc
7	45.59a*	14.89ab	46.19a*	15.11ab
10	33.32cd*	14.38ab	33.98cd*	14.60ab
14	35.44bc*	16.20a	36.22bc*	16.49a
21	38.58b*	14.93ab	39.35b*	15.19ab

Pb200 and Pb800 are for Pb concentrations of 200 and 800 mg kg^{-1} soil. Data are means of three replicates. The lowercase letters refer to significant difference ($P < 0.05$) among the different addition time of NTA

*Significant difference ($P < 0.05$) between the low and high Pb soils significant changes for the application time from 1 to 7 days, beyond which a significant decrease was observed with increasing application time. Available Pb in rhizosphere soils of *A. wardii* grown in low Pb soils was significantly lower than that grown in high Pb soils.

4 Discussion

4.1 Plant growth

NTA, being non-toxic to soil microorganisms and plants, has shown great advantages in chelant-assisted phytoremediation of Pb-contaminated soils (Evangelou et al. 2007; Saifullah et al. 2015). The application of NTA has been reported to show no toxic effect on biomass of *Festuca arundinacea* (Zhao et al. 2011). Similarly, a previous study by Zhao et al. (2016b) found that the application of 2 mmol kg^{-1} NTA showed no negative effect on aboveground and underground biomass of *A. wardii*. Following, the aboveground and underground biomass of *A. wardii* presented an increase trend with increasing application time of 2 mmol kg^{-1} of NTA in this work. However, a biomass decline for *A. wardii* was observed with a longer application time of 21 days (Fig. 1). The inhibited plant growth may be attributed to the fast degradation of Pb-NTA complex and increased Pb solubility and Pb accumulation in plants with the addition of NTA, which may be beyond the tolerance capability of plants (Evangelou et al. 2007; Quartacci et al. 2007; Lan et al. 2013; Saifullah et al. 2015). Among the different application days of NTA, *A. wardii* showed better growth when treated with NTA for 5–14 days (Fig. 1), suggesting potential harvest time for *A. wardii*. It was also found that *A. wardii* presented better growth when treated with low Pb because of lower Pb availability in soils (Figs. 1 and 4). In this sense, NTA-assisted phytostabilization of Pb-contaminated soils by *A. wardii* may be more appropriate for slightly contaminated soils.

4.2 Pb phytostabilization efficiency

In chemical-assisted phytoremediation process, it has been concluded that biodegradable chelating agents have great ability to solubilize and increase heavy metal accumulation in plants (Evangelou et al. 2007; Saifullah et al. 2015; Sarwar et al. 2017). The application of NTA has been proved to be effective in enhancing Pb accumulation in *Brassica carinata* (Quartacci et al. 2007), *Festuca arundinacea* (Zhao et al. 2013), and *Scirpus triqueter* (Hu et al. 2017). Similar results were also obtained for *A. wardii* (Zhao et al. 2016b). It is reported that the application time of chelating agents may be a crucial factor in the efficiency of phytoremediation. Plants should be harvested 1 or 2 weeks after the application of chelating agents in general (Wang et al. 2009; Lan et al. 2013). Zn and As uptake in vetiver shoots reached the maximum when treated with NTA for 20 days, and the maximum Cu uptake occurred under HEIDA application for 16 days (Chiu et al. 2005). In this work, Pb accumulation in *A. wardii*, especially in underground parts, enhanced with increasing NTA application time in general (Fig. 2 and Table 2). There were no significant changes for Pb accumulation in underground parts of *A. wardii* when the application time was more than 7 days (Table 2), suggesting a potential harvest time of 7 days for *A. wardii*. The efficiency of phytoremediation depends upon both the production of biomass and heavy metal concentration in plants. The amounts of heavy metal removed from soils or accumulated in plants are therefore considered to be more important than heavy metal concentration in plants when assessing the phytoremediation potential of plants (Lan et al. 2013; Zhao et al. 2013). It is reported that the greatest Pb removal by shoots of *S. alfredii* was observed for the application of EDDS for 14 days for low Pb soil and 10 days for high Pb soil (Wang et al. 2009). In this study, the greatest Pb accumulation and remediation factors for underground parts of *A. wardii* grown in low Pb soils were observed for the NTA addition time of 7 days, whereas the greatest Pb accumulation and remediation factors for underground parts of *A. wardii* grown in high Pb soils were observed for the NTA addition time of 7 and 5 days (Tables 2 and 3), demonstrating potential harvest time for *A. wardii*. More importantly, it was also found that the bioaccumulation coefficients and remediation factors for low Pb soils were significantly higher than those for high Pb soils (Tables 2 and 3). Taking Pb remediation efficiency and soil Pb concentrations into consideration, it seems to be the optimum for *A. wardii* to phytostabilize slightly Pb-contaminated soils with the application of NTA for 7 days.

4.3 Pb availability in soils

NTA is considered to be a strong potential synthetic chelant for Pb phytoremediation owing to its great chelating ability and fast biodegrability (Quartacci et al. 2005; Evangelou et al. 2007). The addition of NTA reduced the adsorption of Pb on pure minerals and soil particles and increased the exchangeable fraction of Pb with a simultaneous decrease of Pb bound to organic matter. Thus, most of Pb in soils treated with NTA was in a promptly available form (Freitas and do Nascimento 2009; Saifullah et al. 2015). In this work, pH and available Pb in rhizosphere soils of *A. wardii* treated with NTA for different days were obviously lower than those before transplantation (Table 1, Figs. 3 and 4), demonstrating depletion of mobile Pb and therefore mobilization of Pb in rhizosphere soils and enhanced Pb uptake by *A. wardii*. However, whether the changes of soil pH and available Pb were caused by the application of NTA or the growth of plant roots is worth further investigation. Generally, heavy metal mobilization decreased

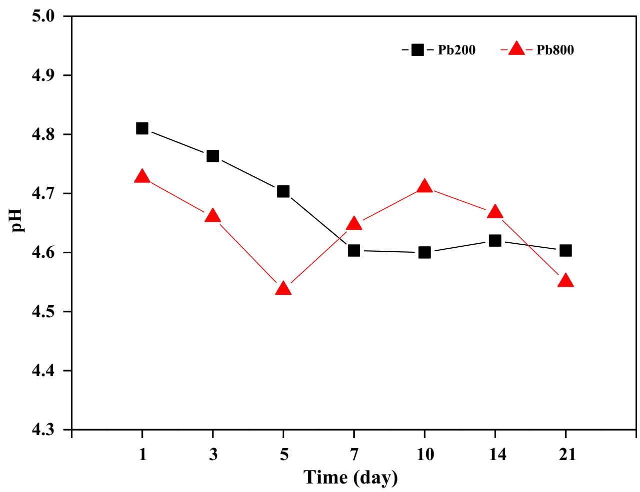

Fig. 3 Effect of NTA addition time on pH in rhizosphere soils of *A. wardii* grown in low and high Pb soils. Pb200 and Pb800 are for Pb concentrations of 200 and 800 mg kg^{-1} soil. Each value is the mean of three independent replicates

significantly with increasing application time of chelant when compared with the initial treatment period (Usman et al. 2013). It was found that water-soluble Pb in rhizosphere soils of *S. alfredii* gradually reduced with the passage of time due to the degradation and leaching effects of EDDS (Wang et al. 2009). Similar results were also obtained in this study. Pb availability in rhizosphere soils of *A. wardii* decreased with the passage of NTA treatment time generally (Fig. 4). These results are in line with the previous study of Zhao et al. (2016b) and may be related to Pb removal by plants and NTA degradation (Usman et al. 2013; Zhao et al. 2016b).

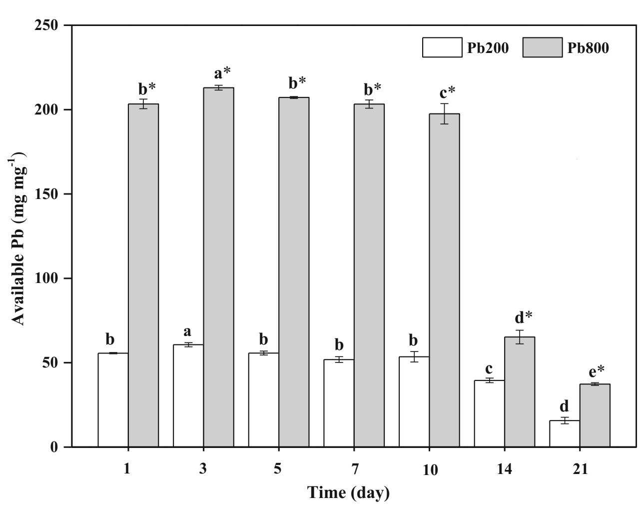

Fig. 4 Effect of NTA addition time on available Pb in rhizosphere soils of *A. wardii* grown in low and high Pb soils. Pb200 and Pb800 are for Pb concentrations of 200 and 800 mg kg^{-1} soil. Each value is the mean of three independent replicates. Error bars indicate standard deviation. The lowercase letters refer to significant difference ($P < 0.05$) among the different addition time of NTA. The asterisk represents significant difference ($P < 0.05$) between the low and high Pb soils

The complex of Pb-NTA is highly degradable (Evangelou et al. 2007; Saifullah et al. 2015). Following the degradation of NTA in soils, Pb may be released from the complexes of Pb-NTA, then re-precipitated or re-adsorbed on pure minerals and soil particles (Yan et al. 2010; Yip et al. 2010; Zhao et al. 2016b). Thus, a sharp drop for available Pb in rhizosphere soils of *A. wardii* was observed for the addition of NTA for 14 and 21 days (Fig. 4), which may be beneficial for NTA-assisted phytostabilization of Pb-contaminated soils by *A. wardii*.

In spite of the great ability of chelant to increase heavy metal availability in soils, the potential environmental risk of heavy metal leaching in surface runoff or groundwater has been concerned (Quartacci et al. 2007; Luo et al. 2017; Qiao et al. 2017). In this work, Pb availability in rhizosphere soils of *A. wardii* grown in high Pb soils was much higher than that grown in low Pb soils with the application of NTA (Fig. 4). Considering the environmental risk, it may be suggested that NTA-assisted technique is more suitable for the phytostabilization of low Pb-contaminated soils by *A. wardii*. Despite higher affinity for Pb than other synthetic chelants, EDTA promoted twice as much leaching of Pb as NTA (Freitas and do Nascimento 2009; Zaier et al. 2010; Qiao et al. 2017). Thus, as an easily biodegradable chelator, NTA has been advocated to the chelant-assisted phytoremediation of Pb-contaminated soils with slight leaching risk of heavy metals (Zhao et al. 2013; Babaeian et al. 2016). However, this research was carried out with a pot experiment and lack of the assessment of environmental risk. In this sense, research on the environmental risk of NTA-assisted phytostabilization of Pb-contaminated soils by *A. wardii* should be processed. Further field experiment should also be continued to estimate the efficiency of NTA-assisted phytostabilization of Pb-contaminated soils by *A. wardii*.

5 Conclusions

This work proved that the application of 2 mmol kg^{-1} of NTA was effective in enhancing Pb accumulation in *A. wardii* with increasing application time. The greatest remediation efficiency by *A. wardii* was observed with the application of NTA for 7 and 5 days for low and high Pb soils. Meanwhile, better plant growth for *A. wardii* was observed when treated with NTA for 5–14 days. However, the application of NTA presented low toxicity to the growth of *A. wardii* with longer application time of 21 days, due to fast degradation of Pb-NTA complex and increased Pb availability in soils as well as Pb accumulation in plants. It seems to be the optimum for *A. wardii* to phytostabilize slightly Pb-contaminated soils with the application of NTA for 7 days, as taking plant growth, Pb remediation efficiency, and environmental risk into consideration. Furthermore, this research was carried out with a pot experiment and lack of the assessment of environmental risk. In this sense, research on the environmental risk of NTA-assisted phytostabilization of Pb-contaminated soils by *A. wardii* should be processed. Further field experiment should also be continued to estimate the efficiency of NTA-assisted phytostabilization of Pb-contaminated soils by *A. wardii*.

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