RESEARCH ARTICLE

Effect of enhancers on the phytoremediation of soils polluted by pyrene and Ni using Sudan grass (*Sorghum sudanense* (Piper) Stapf.)

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Abstract

Remediation of heavy metal and polycyclic aromatic hydrocarbon (PAH)–co-contaminated soils has drawn much more attention; phytoremediation is an often-used technique. Sudan grass (*Sorghum sudanense* (Piper) Stapf.) with developed root system and strong PAHs and heavy metal tolerance is a potential choice for phytoremediation. In this study, the application of tea saponin (TS) (1 g kg^{-1} soil) and nitrilotriacetic acid (NTA) (1 g kg^{-1} soil) was to improve the removal efficiency of Ni and pyrene. TS and NTA had no obvious effects on the growth and soluble proteins of Sudan grass. Ni concentration in root was higher than that in the shoot. The addition of TS and NTA increased the Ni concentration in the root by 25.98% in Ni-contaminated treatment. Pyrene was mainly accumulated in the shoot of Sudan grass. Pyrene concentration in shoot increased by 20.14% with TS-NTA in pyrene-contaminated treatment and increased by 31.97% in Ni-contaminated treatment. TS and NTA had significantly improved dissolved organic matter and soil microbial activity. Microbial activity increased by 16.75%, 18.07%, and 23.364% in pyrene-contaminated, Ni-contaminated, and pyrene and Ni–co-contaminated treatment, respectively. This study showed that phytoremediation of pyrene and Ni–co-contaminated soil by Sudan grass could be enhanced by the application of TS-NTA and the interaction between pyrene and Ni impacted the accumulation of Ni and pyrene in Sudan grass.

Keywords Pyrene · Ni · Sudan grass · Nitrilotriacetic acid · Tea saponin · Phytoremediation

Introduction

Polycyclic aromatic hydrocarbons (PAHs) and heavy metals are two major types of pollutants commonly found in soil, and they are often present together (Perez et al. 2010). Xu et al. (2018) reported that heavy metals (such as Ni and Cr) and PAHs were detected in rural soils. A similar finding was shown in the study of Kibblewhite (2018). The combination of PAHs and heavy metals has higher toxicity and remains in soil for a longer period of time (Perez et al. 2010). There is an urgent need to develop reasonable and effective methods for the remediation of pyrene and Ni–co-contaminated soil.

Phytoremediation, a cost-effective and environmentally friendly strategy, can remove both PAHs and heavy metals (Dai et al. 2020; Sarma et al. 2019). Sudan grass (*Sorghum sudanense* (Piper) Stapf.), a C4 plant, has the advantages of a developed root system and strong PAHs and heavy metal tolerance (Sivaram et al. 2018a). Sivaram et al. (2018b) had proved that Sudan grass could enhance the removal of PAHs, especially high molecular weight PAHs such as pyrene. Also, some studies had shown that Sudan grass could be applied to the phytoremediation of heavy metal–contaminated soil (Li et al. 2016; Shim et al. 2014).

Surfactants and chelating agents are able to enhance the phytoremediation efficiency by increasing the bioavailability of PAHs and heavy metals (Cheng et al. 2018; Yu et al. 2019). The effective and biodegradable enhancers should be selected in phytoremediation. Tea saponin (TS), a natural non-ionic surfactant which found in Camellia plants, could provide strong beneficial effects in soil remediation (Yu and He 2018). It had the ability to increase the accumulation of heavy metal in plants and promote the degradation of organic pollutants by microorganisms in soil (Cay 2016; Liu et al. 2017).

Nitrilotriacetic acid (NTA), with a half-life of 2–7 days, has a strong chelating ability and low toxicity to microorganisms and plants (Yu et al. 2020). It had been proven to increase heavy metals removal from contaminated soil (Yan et al. 2017).

The purposes of this study were to (1) explore preliminarily the effect of TS and NTA on the phytoremediation efficiency of pyrene and Ni–co-contaminated soil; (2) investigate the effects of TS and NTA on plant soluble protein, soil microbial activity, and physicochemical properties; and (3) reveal the interaction of pyrene and Ni during phytoremediation. This study can provide a theoretical basis for phytoremediation in pyrene and Ni–co-contaminated soil.

Materials and methods

Soil treatment

Experimental soil for this study was collected from the campus of Shanghai University. Physicochemical properties of the control soil were as follows: pH 8.3, organic matter 19.6 g kg^{-1}, total nitrogen 0.5 g kg^{-1}, silt 60.4%, clay 7.4%, sand 32.2%, and Ni 24.4 mg kg^{-1}; pyrene was not detected. Pyrene dissolved in acetone solution/$NiCl_2$ solution was spiked into the soil as pyrene/Ni-contaminated soil. Co-contaminated soil was prepared by adding $NiCl_2$ solution to the pyrene-contaminated soil. The contaminated soils were mechanically mixed and aged in darkness for about 3 months. The water holding capacity was kept at 75% during aging. The final concentrations of pyrene and Ni in single and combined contaminated soils were 50 and 200 mg kg^{-1}, respectively. The setting of pollutant concentration was based on the environmental quality standards for soils of China.

Chemicals

Tea saponin (78%, purity) was bought from the Wuxi K-renown Environmental Protection Technology Co., Ltd. (China). Other reagents were all purchased from Sinopharm Chemical Reagent Co., Ltd. (China).

Experimental design

The experimental design was as follows: P (pyrene-contaminated soil), PE (pyrene-contaminated soil with enhancers), PS (pyrene-contaminated soil with Sudan grass), PES (pyrene-contaminated soil with enhancers and Sudan grass), N (Ni-contaminated soil), NE (Ni-contaminated soil with enhancers), NS (Ni-contaminated soil with Sudan grass), NES (Ni-contaminated soil with enhancers and Sudan grass), C (pyrene and Ni–co-contaminated soil), CE (pyrene and Ni–co-contaminated soil with enhances), CS (pyrene and Ni–co-

contaminated soil with Sudan grass), and CES (pyrene and Ni–co-contaminated soil with enhancers and Sudan grass).

One kilogram of each spiked soil was placed in each pot and three pots for each treatment. Fifty seeds of Sudan grass (purchased from Jiangxi Agricultural Company, China) were initially sown in each pot, and they were subsequently adjusted to about 30 individual plants per pot. The concentrations of TS and NTA were both set to 1 g kg^{-1} soil. After 21 days of plant growth, the solution of TS and NTA (divided into seven times, once every 2 days) was added to the soil. In order not to change the soil pH, the pH of the adding solution was adjusted to 8.3. All pots were placed in the greenhouse (18–25 °C, with a 10:14 light/dark period and 60% relative humidity). After 42 days, the plants were harvested. All plant samples were placed in an − 80 °C refrigerator for testing. After drying and grinding, the plant samples were sieved (0.15-mm mesh) for analysis of Ni and pyrene.

Analysis of Ni in plants

Ni in plants was detected by acid digestion. Samples of the plant were digested with a mixture of HNO_3 and $HClO_4$ (3:5, v/v) at 220 °C. Ni concentration was determined by inductively coupled plasma optical emission spectrometry (ICPOES) after being filtered by microfiltration membrane (0.45 μm).

Analysis of water-soluble Ni in soil

There were four treatments: N (Ni-contaminated soil added with water), NE (Ni-contaminated soil added with enhancer solution), C (pyrene and Ni–co-contaminated soil added with water), and CE (pyrene and Ni–co-contaminated soil added with enhancer solution). Water or enhancer solution (1 g L^{-1}) was added to the soil as 10 mL:1 g. After shaking for 24 h, the supernatant was filtered by microfiltration membrane (0.45 μm). The concentration of water-soluble Ni was analyzed by inductively coupled plasma (ICP).

Extraction and analysis of pyrene in plants

Total pyrene in plants was detected by the method referenced by Sun et al. (2010). The plant sample (1.00 g) was ultrasonically extracted in 10-mL elution of dichloromethane and acetone (v/v, 1:1) for 30 min. Then the supernatant was concentrated and followed by filtration through 4 g silica gel with 10 mL mixture of hexane and dichloromethane (v/v, 1:1). Finally, the resulting liquids were evaporated and redissolved in methanol to a final volume of 2 mL. Extracts were analyzed by HPLC (LC-20A) after passing through a 0.22-μm Teflon filter. The HPLC system was fitted with a UV detector and a 4.6×250-mm reverse phase C18 column, using methanol as the mobile phase, at a flow rate of 1 mL min^{-1} (40 °C). The wavelength of the UV detector was 245 nm for pyrene. The

sample solution (10 μL) was injected into the HPLC system by an autosampler.

Analysis of physicochemical property in rhizosphere soil

The soil dissolved organic matter was extracted according to the method of Lin et al. (2019), with a soil to water ratio of 1:4 (w/v). The concentration of dissolved organic matter is obtained by multiplying the concentration of dissolved organic carbon by 1.724, and dissolved organic carbon was quantified by an automated TOC analyzer. Soil available nitrogen was analyzed by the method of alkaline hydrolysis diffusion; soil available phosphorus was determined by Mo-Sb colorimetry.

Determination of soluble protein concentration and soil microbial activity

The soluble protein concentration with different treatments was detected using Coomassie Brilliant Blue G-250 as a dye and bovine albumin (BSA) as a standard (Bradford 1976). Soil microbial activity was measured using the fluorescein diacetate (FDA) assay described by Chen et al. (2017). Phosphate buffer solution was added to the soil for 15 min, and then fluorescein diacetate (FDA) solution was added. After shaking for 2 h, the absorbance was read with OD_{490}.

Statistical analysis

All data were expressed as means ± standard deviation (SD) and analyzed using the SPSS software program (version 22.0 for Windows). One-way analysis of variance (ANOVA) was used to analyze significant differences. Differences were statistically significant when $P < 0.05$ using the Duncan test. Drawing data results were using Origin 9.0

Results

The biomass of Sudan grass

The effect of TS and NTA on the dry biomass of Sudan grass in contaminated soil is shown in Fig. 1. The shoot biomass of Sudan grass in Ni, pyrene, and co-contaminated treatments were 1.35 g pot^{-1}, 1.82 g pot^{-1}, and 1.73 g pot^{-1}, respectively. After the addition of TS-NTA, the shoot biomass values of the three treatments were 1.39 pot^{-1}, 1.81 g pot^{-1}, and 1.74 g pot^{-1}, respectively. The root biomass values of Sudan grass in Ni, pyrene, and co-contaminated treatments were 0.55 g pot^{-1}, 0.66 g pot^{-1}, and 0.65 g pot^{-1} and 0.44 g pot^{-1}, 0.62 g pot^{-1}, and 0.56 g pot^{-1} for the addition of TS-NTA, respectively. Basically, no significant differences between with and without the addition of TS-NTA were found in the
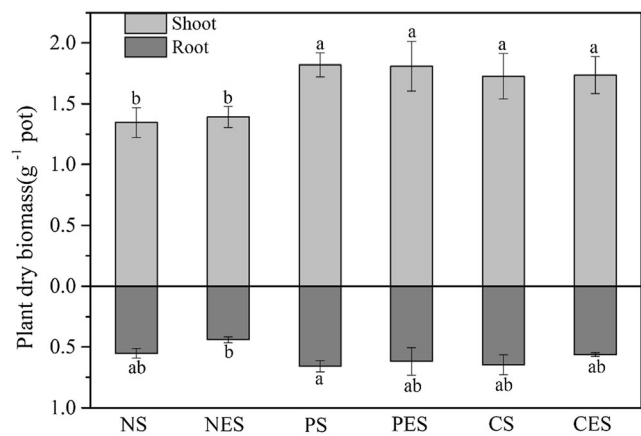

Fig. 1 Biomass of Sudan grass with different treatments (soil with Ni and plants (NS); soil with Ni, enhancers, and plants (NES); soil with pyrene and plants (PS); soil with pyrene, enhancers, and plants (PES); co-contaminated soil with plants (CS); co-contaminated soil with enhancers and plants (CES)). Different letters up the vertical bars are significantly different between treatments ($P < 0.05$)

biomass of Sudan grass ($P < 0.05$). The shoot biomass of Sudan grass in pyrene-contaminated treatment and pyrene and Ni–co-contaminated treatment was higher relative to that in Ni-contaminated treatment ($P < 0.05$).

Ni accumulation in Sudan grass

The effect of TS and NTA on the concentration of Ni in Sudan grass is shown in Fig. 2. Ni concentrations in shoot and root were ranged from 17.08 to 25.79 mg kg^{-1} and 94.02 to 135.20 mg kg^{-1}, respectively. Root Ni concentration in Ni-contaminated treatment and shoot Ni concentration in pyrene and Ni–co-contaminated treatment were highly increased with the addition of TS and NTA ($P < 0.05$) (Fig. 2). The addition of TS-NTA increased Ni concentration in root by 25.98% in Ni-contaminated treatment. Ni concentration in shoot
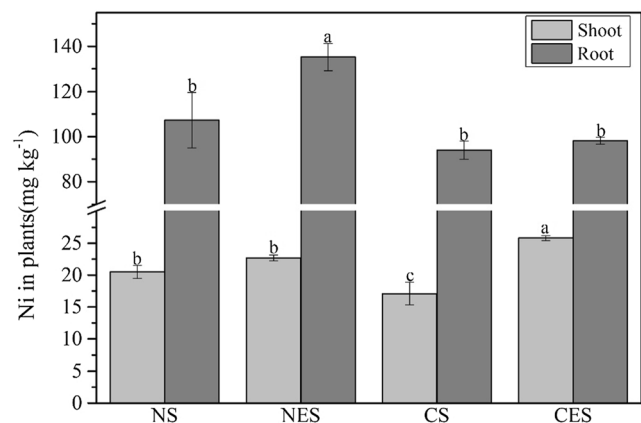

Fig. 2 Ni concentrations in Sudan grass with different treatments (soil with Ni and plants (NS); soil with Ni, enhancers, and plants (NES); co-contaminated soil with plants (CS); co-contaminated soil with enhancers and plants (CES)). Different letters up the vertical bars are significantly different between treatments ($P < 0.05$)

improved by 50.97% with the addition of TS-NTA in the presence of pyrene ($P < 0.05$). Ni concentration in root was obviously decreased with the presence of pyrene when TS-NTA was added ($P < 0.05$).

Water-soluble Ni in soil

The effect of TS and NTA on the concentration of water-soluble Ni in soil is shown in Fig. 3. The concentration of water-soluble Ni in soil significantly increased with the addition of TS and NTA ($P < 0.05$). The water-soluble Ni concentration in Ni-contaminated treatment was increased by 985.24% with the addition of TS and NTA. And water-soluble Ni concentration was increased by 935.89% with the addition of TS and NTA in the presence of pyrene.

Pyrene accumulation in Sudan grass

The effect of TS and NTA on the concentration of pyrene in Sudan grass is shown in Fig. 4. The pyrene concentrations in shoot and root ranged from 279.92 to 369.42 mg kg^{-1} and 23.93 to 59.80 mg kg^{-1}, respectively. The addition of TS and NTA significantly increased pyrene concentration in Sudan grass except for the root in pyrene-contaminated treatment ($P < 0.05$). Pyrene concentration in shoot increased by 20.14% with addition of TS and NTA in pyrene-contaminated treatment. Pyrene concentration in shoot and root increased by 31.98% and 149.92% with addition of TS and NTA in the presence of Ni.

Soluble proteins

The effect of TS and NTA on soluble protein concentration in Sudan grass is shown in Fig. 5. The differences in soluble

Fig. 4 Pyrene concentrations in Sudan grass with different treatments (soil with pyrene and plants (PS); soil with pyrene, enhancers, and plants (PES); co-contaminated soil with plants (CS); co-contaminated soil with enhancers and plants (CES)). Different letters up the vertical bars are significantly different between treatments ($P < 0.05$)

protein concentration with or without TS-NTA were not significant ($P = 0.06$). The results showed that soluble protein concentrations of Sudan grass in Ni, pyrene, and co-contaminated treatments without TS-NTA addition were 1.87 mg g^{-1}, 2.58 mg g^{-1}, and 2.24 mg g^{-1} and 1.68 mg g^{-1}, 2.38 mg g^{-1}, and 2.29 mg g^{-1} for the addition of TS-NTA, respectively. Similar with the results of Sudan grass biomass, soluble protein concentrations of Sudan grass in pyrene-contaminated treatment and pyrene-Ni co-contaminated treatment were higher relative to it in Ni-contaminated treatment ($P < 0.05$).

Physicochemical properties of rhizosphere soils

The impact of TS and NTA on the rhizosphere soil properties is presented in Table 1. The dissolved organic matter
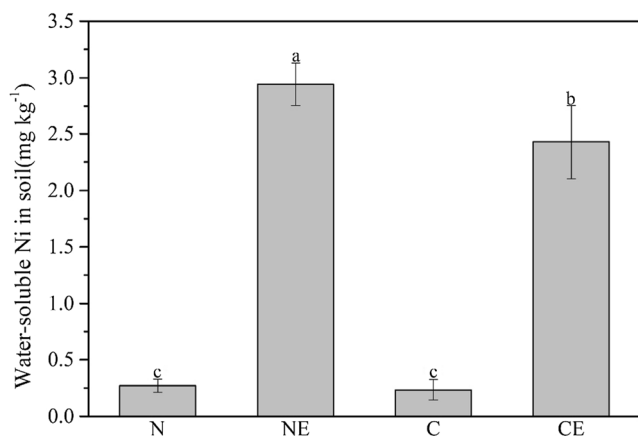

Fig. 3 Water-soluble Ni concentrations in soil with different treatments (Ni-contaminated soil added with water (N); Ni-contaminated soil added with enhancer solution (NE); pyrene and Ni–co-contaminated soil added with water (C); pyrene and Ni–co-contaminated soil added with enhancer solution (CE)). Different letters up the vertical bars significantly are different between treatments ($P < 0.05$)
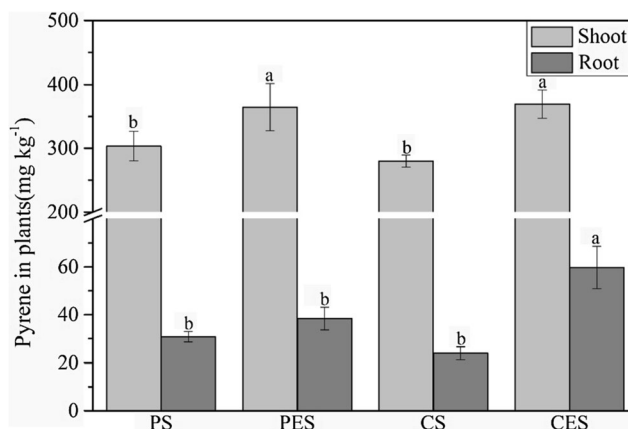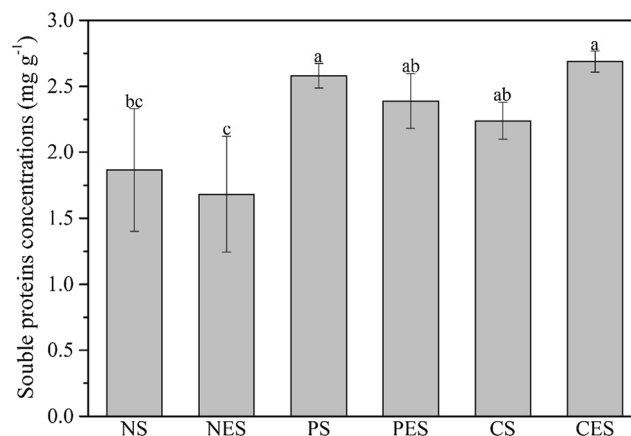

Fig. 5 Soluble protein concentrations in Sudan grass with different treatments (soil with Ni and plants (NS); soil with Ni, enhancers, and plants (NES); soil with pyrene and plants (PS); soil with pyrene, enhancers, and plants (PES); co-contaminated soil with plants (CS); co-contaminated soil with enhancers and plants (CES)). Different letters up the vertical bars are significantly different between treatments ($P < 0.05$)

concentrations were ranged from 0.17 to 0.45 g kg^{-1}. The addition of enhancers significantly increased dissolved organic matter concentration in rhizosphere soil ($P < 0.05$). Compared with the treatment with only adding TS-NTA, the concentration of dissolved organic matter in soil decreased slightly in the treatment with adding TS-NTA and planting Sudan grass. The available P and available N ranged from 3.94 to 5.48 mg kg^{-1} and 103.60 to 145.60 mg kg^{-1}. Available P and available N of rhizosphere soil in enhancer-added treatments were increased to some different extent.

Microbial activity

Figure 6 shows the microbial activity in rhizosphere soil. Microbial activity was increased with addition of TS and NTA. After adding TS-NTA, soil microbial activities were increased by 16.75%, 18.07%, and 23.364%, respectively, in Ni-contaminated treatment, pyrene-contaminated treatment, and co-contaminated treatment ($P < 0.05$). The soil microbial activity in the presence of Ni and pyrene was lower than that in the presence of single Ni or single pyrene when TS-NTA was added ($P < 0.05$).

Discussion

Plant growth is an important indicator of phytoremediation. TS and NTA did not show a significant effect on the shoot biomass of Sudan grass, which was consistent with the
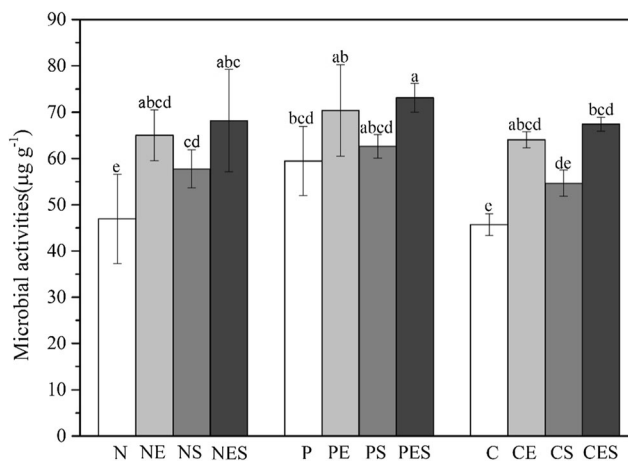

Fig. 6 Microbial activities in soil with different treatments (soil with Ni (N); soil with Ni and enhancers (NE); soil with Ni and plants (NS); soil with Ni, enhancers, and plants (NES); soil with pyrene (P); soil with pyrene and enhancers (PE); soil with pyrene and plants (PS); soil with pyrene, enhancers, and plants (PES); soil with pyrene and Ni (C); co-contaminated soil with enhances (CE); co-contaminated soil with plants (CS); co-contaminated soil with enhancers and plants (CES)). Different letters up the vertical bars are significantly different between treatments ($P < 0.05$)

researches of Fei et al. (2018) and Liu et al. (2017). This indicated that the addition of TS and NTA had no toxic effect on the growth of Sudan grass. Ni pollution would inhibit the growth of Sudan grass, but this inhibition would be relieved in the presence of pyrene. Ni exposure restricted the ability of plants to absorb minerals and water and reduced the photosynthesis of plants, which reduced overall plant growth

Table 1 Physicochemical properties of rhizosphere soil

Treatment	Soil properties		
	DOM (g kg^{-1})	Available P (mg kg^{-1})	Available N (mg kg^{-1})
N	0.17 ± 0.02d	4.34 ± 0.26def	117.60 ± 16.80cde
NE	0.45 ± 0.03a	5.10 ± 0.18ab	140.00 ± 4.85abc
NS	0.17 ± 0.01d	5.45 ± 0.19a	103.60 ± 12.83de
NES	0.25 ± 0.01c	4.08 ± 0.09f	134.40 ± 8.40bc
P	0.19 ± 0.01d	4.22 ± 0.20ef	117.60 ± 8.40cde
PE	0.35 ± 0.04b	4.45 ± 0.38ef	159.60 ± 14.55a
PS	0.17 ± 0.02d	4.92 ± 0.23bc	114.80 ± 5.94e
PES	0.26 ± 0.02c	3.94 ± 0.20f	123.20 ± 12.83bcd
C	0.18 ± 0.00d	4.56 ± 0.04cde	117.60 ± 16.80cde
CE	0.32 ± 0.02b	5.48 ± 0.30a	145.60 ± 12.83ab
CS	0.17 ± 0.01d	5.00 ± 0.35bc	106.40 ± 12.83de
CES	0.25 ± 0.00c	4.73 ± 0.03bcd	126.00 ± 14.55bcd

DOM dissolved organic matter. Values given in the table are the mean ± standard deviation. Different letters indicate significant differences among treatments ($P < 0.05$) (soil with Ni (N); soil with Ni and enhancers (NE); soil with Ni and plants (NS); soil with Ni, enhancers, and plants (NES); soil with pyrene (P); soil with pyrene and enhancers (PE); soil with pyrene and plants (PS); soil with pyrene, enhancers, and plants (PES); soil with pyrene and Ni (C); co-contaminated soil with enhancers (CE); co-contaminated soil with plants (CS); co-contaminated soil with enhancers and plants (CES))

(Fashola et al. 2016; Wiszniewska et al. 2018). However, some bacteria in soil had the ability to use PAHs as their source of carbon and energy, leading to the degradation of PAHs. And the degradation products would eventually be absorbed by plants as nutrients, thereby resulting in a promotion effect on plant growth (Anyasi et al. 2019).

Ni concentration in the root was higher than that in the shoot; this was consistent with many reports that heavy metals are mainly accumulated in root system (Pilipovic et al. 2019; Tauqeer et al. 2019). The concentration of water-soluble Ni in soil increased by TS and NTA; therefore, more Ni was absorbed by Sudan grass. Due to solubilization action by TS, desorption of pollutants in the soil matrix was increased (Maity et al. 2013). NTA, as a chelating agent, was confirmed to combine metal ions to form complexes and then increase bioaccessibility of metals in soil (Liu et al. 2018). Compared with the Ni concentration in Sudan grass in Ni-contaminated treatment, the Ni concentration in Sudan grass decreased in pyrene-Ni-contaminated treatment. The presence of pyrene had a negative impact on the active transport process which would be the way of the plant to uptake Ni, thus decreasing the concentration of Ni in Sudan grass (Zhang et al. 2019).

Many studies reported that PAHs were mainly accumulated in the roots of the plants (Liu et al. 2017; Wei et al. 2017). However, this study showed that pyrene was mainly accumulated in shoots. The lipid concentration of plant intracellular components determined the concentration of pyrene accumulation due to a higher lipophilicity of pyrene (Kang et al. 2010). The higher pyrene concentration in Sudan grass shoot could be explained by that the lipid concentration of shoot was higher than that of root (Liao et al. 2015). TS and NTA enhanced the pyrene concentration in Sudan grass which was ascribed to increased pyrene bioavailability. The addition of TS and NTA accelerated the transfer of pyrene from soil to the aqueous phase (Liang et al. 2017). Furthermore, bound and residual fractions of pyrene could be efficiently transformed into bioavailable fractions of pyrene because of the addition of TS and NTA (Lu et al. 2019).

Soluble proteins played an important role in protecting life matters and cellular membranes when plants exposed to contaminants; therefore, they often were used as resistant indexes (Pan et al. 2018). There were some stress proteins with antioxidation, and detoxification functions were used to protect plants from damage (Huang et al. 2017; Nie et al. 2016). Heavy metal ions at high concentrations could block protein synthesis by forming compounds or chelates with other metal ions (Huang et al. 2019). That could explain why the soluble protein concentrations in Ni-contaminated treatment were the lowest.

Organic matter is often used as a nutrient source for plants, and its increase can improve phytoremediation efficiency of contaminated soils (Bauddh and Singh 2015). Soil dissolved organic matter act as an active organic matter fraction in soil,

and the dissolved organic carbon (DOC) in it can be directly utilized by soil microorganisms (Xu et al. 2020). Addition with TS and NTA could increase the concentrations of dissolved organic matter which would be utilized by microbes as their carbon source (Lopez-Gonzalez et al. 2013). The available P and available N in soil provide essential nutrients for plant growth (Wang et al. 2020). As a surfactant, TS could promote the conversion of total P to available P through its solubilization action. Adding NTA can effectively increase the available N concentration in the soil that could be utilized by plants and microorganisms (Liu et al. 2019).

The addition of TS and NTA significantly increased soil microbial activity. The addition of TS-NTA, as nutrients of microorganisms, increased both microbial activity and soil microbial biomass (Sun et al. 2016). The combination of pyrene and Ni had stronger toxicity on soil microorganism than that of single pyrene or Ni (Chen et al. 2017). PAHs in soil could increase the toxicity of heavy metals by facilitating their penetration through microbial membranes when PAHs and heavy metals coexist (Maliszewska-Kordybach and Smreczak 2003).

Conclusions

The addition of TS and NTA greatly promoted the accumulation of Ni and pyrene in Sudan grass by increasing the concentrations of Ni and pyrene in soil solution. In addition, TS and NTA significantly increased dissolved organic matter concentration and soil microbial activity, which was beneficial to the removal of pyrene in soil. Ni mainly accumulated in the root and pyrene mainly accumulated in the shoot. Ni accumulation in root was obviously decreased in the presence of pyrene when TS-NTA was added. The inhibition of Ni on the growth and soluble proteins of Sudan grass was relieved in the presence of pyrene. However, the toxicity of Ni on the soil microorganism increased in the presence of pyrene. The mechanisms of the accumulation of pyrene and the interaction between pyrene and Ni need further exploring.

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