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Effects of key components of *S. triqueter* root exudates on fractions and bioavailability of pyrene–lead co-contaminated soils

Y. Hou[1] · X. Liu[1] · X. Zhang[1] · X. Chen[1] · K. Tao[1]

Abstract The key components of *S. triqueter* root exudates involved 4-oxo-pentanoic acid, succinic acid, glutaric acid, phthalate acid, citric acid, vanillic acid, myristic acid, pentadecanoic acid, decanoic acid, 14-methyl-pentadecanoic acid, hexadecanoic acid, octadecanoic acid and oleic acid, and the content of the water-soluble organic acids (citric acid, succinic acid and glutaric acid) significantly increased in pyrene and lead co-contaminated rhizosphere soil. These three water-soluble organic acids including citric acid, succinic acid and glutaric acid were detected as the specific root exudates of *S. triqueter* under stress of pollutants for pyrene and lead, so they were chosen as the research objects, and they were added into the bioremediation systems of pyrene and lead co-contaminated wetland soils. Compared with the control, the treatments added the three organic acids always improved the quantity of the bioavailable fraction of pyrene and lead in wetland soils and greatly influenced other chemical states of pyrene and lead fractions in the test concentration range. Under the 50 g kg^{-1} of organic acids concentration, the amount of the bioavailable fraction of pyrene and lead increased 41.0 and 872.7 % by citric acid, respectively. The enhancement of bioavailability of pyrene and lead in the wetland soil by adding organic acids generally decreased in the following order: citric acid > succinic acid > glutaric acid. Enhancing effects of organic acids on the bioavailability improvement of pyrene and lead is remarkable.

Keywords Fraction distribution · Bioavailability · *S. triqueter* · Organic acids · Pyrene–lead co-contaminated wetland soils

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Introduction

Soils always contain several contaminants that vary in concentration and composition (Zhang et al. 2014); among all, organic chemicals such as PAHs and heavy metals are recognized as two major typical contaminations that cause water and soil pollution (Sun et al. 2011). PAHs are by-products of the imperfect combustion or pyrolysis of organic materials, and they are priority organic contaminants in the environment. Some PAHs are carcinogenic and mutagenic, posing serious threats to human health (Gao and Zhu 2005). Heavy metals are receiving increasing attention due to their toxicological importance in ecosystems (Huang et al. 2011). Heavy metals can be brought into and accumulate in soil through agricultural sewage sludge, fertilizers, industrial wastes and land disposal of metal-contaminated municipal (Zhang and Dong 2008). The co-contamination of heavy metals and organic contaminations in water, air and soil has become major environmental and human health threat, due to rapid industrialization and urbanization (Pérez et al. 2010). Soil pollution by heavy metals and PAHs has been accelerated in China during the past decade. Hence, it is vital to develop efficient and cost-effective methods to simultaneously remove multiple contaminants from co-contaminated soils.

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After PAHs enter into soil, they may undergo a number of processes such as volatilization, leaching, chemical and biological degradation. Sorption and desorption processes may cause these chemicals to go into the soil matrix or mineral particles, combine with organic substances and become a non-extractable solvent or 'irreversibly bound' (Macleod and Semple 2003). The extractable or total amount of PAHs may be restricted when evaluating their environmental importance. Instead, the fraction distribution and availability of these pollutants are the most important factors of risk assessment. The forms of organic pollutants in soil have been previously reported (Lesan and Bhandari 2004). PAHs in soils can be separated into three fractions including desorbing, non-desorbing and bound residual fractions, and the following agents can be used to do sequential extraction: n-butanol, dichloromethane and sodium hydroxide extractions (Gao et al. 2009).

Soil heavy metals that transport through water and food chain produce toxic effects to plants, animals and humans, which concerns scientists, policy-makers and the public. The total concentration of heavy metal in soil contamination assessment is a useful indicator (Zhang et al. 2009). However, it can not provide sufficient information about the bioavailability and toxicity of heavy metal. The mobility, bioavailability and ecotoxicity of heavy metals were largely related to their forms (Chen et al. 2008). Heavy metals present in various forms, including water-soluble, acid-soluble, reducible, oxidizable and residual forms. In these forms, water-soluble and acid-soluble forms are considered to be bioavailable; if the soil pH and redox potential change, reducible and oxidizable forms can also be bioavailable (Mäkelä et al. 2011); and the residual form for both plants and microorganisms is unavailable. Therefore, the study of soil heavy metal forms in soil heavy metal contamination must be considered (Kartal et al. 2006). BCR is widely used to analyze proposed different forms of heavy metals (Bhattacharyya et al. 2008). These programs use a series of selective agent to dissolve the different forms of heavy metal in soil, resulting in a more realistic assessment of actual environmental impact (Guevara-Riba et al. 2004).

Plant roots can actively or passively release a variety of organic compounds, referred to as REs, which include carbohydrates, OAs and amino acids. These REs may be an important resource for fast-growing microbes and subsequently alter the composition of species in the rhizosphere, and function in nutrient transformation, decomposition and the mineralization of organic substances; thus, these REs in the root zone or rhizosphere can induce the biodegradation of contaminants (Yang et al. 2001). Thus, in this study, PYR and Pb were used as representative contaminants. PYR is one of sixteen PAHs on the EPA's priority list that is widely found in the environment (Gana et al. 2009), because of its toxic, mutagenic and carcinogenic properties, so it was used as a model organic pollutant. Pb is a heavy metal of great concern in urban ecosystems because of its high toxicity to animals and human health, so it was used as a model heavy metal pollutant. The main aims for the current investigation are (1) to identify the specific REs of S. triqueter under stress of PYR and Pb; (2) to determine the effects of specific REs of S. triqueter on the fraction distributions and bioavailability of PYR and Pb in soil; and (3) to study the mechanism of the enhanced PAH and heavy metal bioavailability in soil by OAs.

This research was done in laboratory of environmental remediation, college of environmental and chemical engineering, Shanghai University, during the academic year of 2013–2014.

Materials and methods

Reagents

Reagents including PYR, citric acid, succinic acid and glutaric acid were obtained from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). PYR with a purity >98 % was used without further purification. All the other chemicals used in the study were of analytical purity.

Soil treatment

The experiment soils, previously free of contaminants, were collected from the east campus of Shanghai University, China. They are typical soils in east China. Components and properties of the soil used in the experiment are as follows: texture (sand 32.2 %, silt 60.4 %, clay 7.4 %, organic matter 19.6 g kg^{-1}, pH 8.30). Soil samples were air-dried and sieved (0.2 mm). Preparation of the contaminated soil: PYR-contaminated soil was prepared by adding PYR-acetone solution into clean soil, and then, the soil was air-dried at room temperature for at least 1 month. Pb-contaminated soil was prepared by adding lead acetate trihydrate solution into clean soil. PYR and Pb co-contaminated soil was prepared by mixing the PYR-contaminated soil and Pb-contaminated soil. The mixture was ventilated in fume hood to stabilize the contaminated soil. PYR and Pb have a final concentration of 50 and 250 mg kg^{-1} in single and combined contaminated soil. These concentrations were chosen according to the PAHs and heavy metal contamination levels generally found in soils around the world (Zhao et al. 2014).

REs extraction

S. triqueter plants were sampled in PYR and Pb co-contaminated soil and uncontaminated soil for 30 days. After

germination of S. *triqueter* seed, S. *triqueter* seedlings of consistent growth were transplanted to the pot soils to grow. Three replicates were prepared for each treatment. The rhizosphere soil of S. *triqueter* was collected. The rhizosphere soil of S. *triqueter* was dried and sieved (0.4 mm) in the laboratory. Fifteen grams of rhizosphere soil samples was placed in a stoppered 100 mL flask. Methyl esterification continued for 12 h at 60 °C after adding fifty-one milliliter of anhydrous methanol-concentrated sulfuric acid solution ($v:v$ = 10:7). After centrifugation (4000 rpm, 10 min), the esters were extracted with 1 mL of saturated sodium chloride solution, 20 mL of distilled water and 5 mL of dichloromethane twice. The organic phase of the solution was extracted and rotary evaporated till dryness at 40 °C, and the residue was redissolved in 1 mL of dichloromethane. The solution was passed through a 0.45-μm filter for GC-MS analysis.

Experiment design

The fractions of the PYR and Pb in soils were examined using microcosms (Sun et al. 2012). Treated soils were packed into amber glass microcosms (each with 20 g soil) and added OAs (citric acid, succinic acid and glutaric acid) concentrations of 0, 1, 5, 10, 20, 40, 50 mg kg^{-1}. The microcosms were sealed with glass caps. Three replicates were examined for each treatment. The soil water contents were adjusted to 30 % of the soil water-holding capacity. After incubation for 30 days in microcosms at 25 °C in the darkness, the soils were sampled, and the forms of the PYR and Pb were determined.

PYR extraction from soil: 3-step sequential extraction

The different fraction (desorbed fraction, non-desorbed fraction and bound residue fraction) concentrations of PYR were determined using a modified Gao method (Gao et al. 2009), and N-butanol, dichloromethane and 2 M sodium hydroxide were used as the sequential extraction agents of desorbing, non-desorbing and bound residual fractions of PYR, respectively, and measured by HPLC (LC-20AT).

For example, desorbed fraction: soil (3.0 g) was placed in a 25-mL centrifuge glass tube, added 15 mL of 100 % n-butanol (Sun et al. 2012), and the tube cap was screwed on tightly. Next, the samples were incubated in an ultrasonic bath for 60 min. Soil and solvent were separated by centrifugation (10 min, 2000 rpm). Fresh mild extraction solution (15 mL 100 % n-butanol) was added to soil to extract again. The supernatant was liquid–liquid-extracted three times using 10 mL of dichloromethane. Organic phases were dehydrated by percolation through Na$_2$SO$_4$ anhydride, combined, and concentrated to near dryness in a

rotary evaporator at 45 °C. The residue was then reconstituted in 2 mL of methanol. Before HPLC analysis, the solution was passed through a 0.22-μm Teflon filter to remove particulate matter.

Pb extraction from soil

The different fraction concentrations of Pb were determined using a modified Zhong method (Zhong et al. 2011). Briefly, BCR sequential extraction procedure was conducted for heavy metal speciation analysis. One gram of soil sample was placed in a 100-mL centrifuge tube and subjected to the following extraction regimen, water-soluble fraction (distilled water, pH 7.0), acid-extractable fraction (0.11 M CH$_3$COOH), reducible fraction (0.1 M NH$_2$OH·HCl, pH 2), oxidizable fraction (H$_2$O$_2$ (30 %)/1 M CH$_3$COONH$_4$, pH 2) and residual fraction (extracted with the HCl–HNO$_3$–HClO$_4$–HF mix acid).

For example, water-soluble fraction: extract soil sample with 25 mL distilled water (distilled and cooled, pH 7.0) and shake for 2 h at 20 ± 2 °C. After each successive extraction, separation was done by centrifugation at 4000 rpm for 20 min. The supernatant was filtered with a 0.45-μm membrane filter and analyzed for concentrations of different heavy metals. Residuals from each step were washed with 20 mL of distilled water, followed by vigorous hand shaking and then followed by 20 min of centrifugation before the next extraction step.

The concentrations of Pb were measured by ICP.

Chemical analysis

Analysis of the REs of S. *triqueter* was performed on Agilent 7890GC-5975MS, which was fitted with a manual injector. Compounds were separated on HP-5 (30 m × 0.25 mm × 0.25 μm) silica capillary column. Samples (2 μL) were injected in the splitless mode. The temperature at injector was 280 °C, the temperature at ion source was 200 °C, and the temperature at detector was 250 °C. Helium was used as carrier gas at a velocity of 1 mL min^{-1}. The temperature program used was as follows: started at 40 °C, held for 2 min; increased to 100 °C at 5 °C min^{-1}, held for 1 min; then rose to 280 °C at 15 °C min^{-1}, held for 8 min; and finally dropped to 240 °C at 2.5 °C min^{-1}. The scan mode was full at m/z 29-400.

The HPLC system used was equipped with an ultraviolet–visible detector and a reverse-phase C$_{18}$ column (4.6 × 250 mm). The column was installed in a column heater with a constant temperature of 30 °C. Methanol (100 %) was used as the eluent solvent at a flow rate of 1.0 mL min^{-1}. Detection was carried out at 245 nm, and the injection volume of the sample was 20 μL.

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ICP: ICP-AES (ICP, LEEMAN Company, the USA) and AAS (ZEEnit600, Analytik Jena AG, Germany).

Statistical analysis

All data were processed using Microsoft Excel and SPSS 17.0. PYR and Pb concentrations of each fraction were tested for differences using $p < 0.05$ unless otherwise indicated. One-way analysis of variance followed by Duncan's multiple range test is used to compare the mean value.

Results and discussion

REs of *S. triqueter* under stress of PYR and Pb

As seen from Figs. 1 and 2 that REs components of *S. triqueter* in PYR and Pb co-contaminated soil were different from the uncontaminated soil, and in response to PYR and Pb stress, the amount of OAs in REs of *S. triqueter* increased significantly, such as citric acid, succinic acid and glutaric acid. Plant REs of low molecular weight organic secretions (including OAs, sugars and amino acids) played an important role in rhizosphere microenvironment. The release of the organic secretions with low molecular weight in rhizosphere provides a large amount of organic carbon which was likely to be used by microbes; they were conducive to microbial growth and formation of special fauna; this feature made REs created by rhizosphere microenvironment often conducive to the rapid reduction in organic pollutants (Yoshitomi and Shann 2001). REs influenced the form of heavy metals by acidizing insoluble heavy metal fraction in soil, chelating and reducing heavy metal in soil (Kim et al. 2010). Many studies found that when plants exposed to certain toxic and hazardous substances, the composition and concentration of REs will
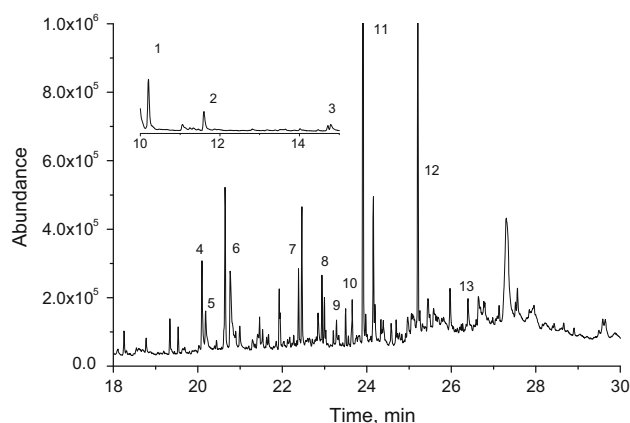

Fig. 1 Gas chromatograph charts of root exudates of ST in pyrene and lead co-contaminated soil
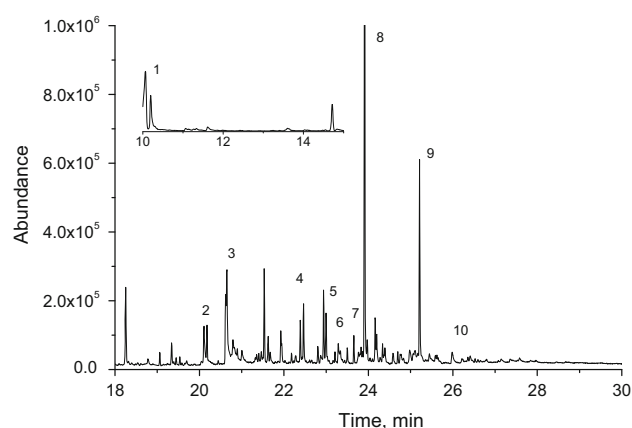

Fig. 2 Gas chromatograph charts of root exudates of ST in clean soil

change and the generation and accumulation of part components of REs will increase, which was the plant stress response to toxic and hazardous substances, adjusted the rhizosphere microenvironment by increasing REs, making the rhizosphere microenvironment conducive to the decomposition of harmful substances (María et al. 2014). In this study, the content of the water-soluble OAs (citric acid, succinic acid and glutaric acid) significantly increased in PYR and Pb co-contaminated rhizosphere soil (Table 1); however, this three OAs were even not detected in the uncontaminated rhizosphere soil which might due to their low concentrations in uncontaminated rhizosphere soil (Table 1). So the three acids were detected as the specific REs of *S. triqueter* under stress of PYR and Pb. The three OAs were chosen as the research objects in this study.

Effects of *S. triqueter* REs on the fraction distribution and bioavailability of PYR

The desorbed fraction of organic pollutants is typically the most bioavailable fraction. The quantity of the desorbed PYR was progressively increased during that the OAs concentrations increased from 0 to 50 g kg^{-1} (Table 2). Table 2 showed that compared with the control, in the test concentration range, the treatments added the three OAs always improved the quantity of the desorbed fraction of PYR in soil. At concentration 50 g kg^{-1}, the amount of the desorbed fraction of PYR increased 53.1 % by citric acid. From the view of the total quantity, the desorbed fraction was one of the main fractions of PYR in aging 30 days soil; in control, the amount of the desorbed fraction of PYR was about 32.9 %. After adding the three OAs (citric acid, succinic acid and glutaric acid), the amount of the desorbed fraction of PYR in soil accounted for 32.9–50.4, 32.9–46.5 and 32.9–44.4 %, respectively.

The content of non-desorbed fraction was also the main fraction of PYR after 30-day incubation in soil. For

Table 1 GC-MS identification of the main substances of root exudates of ST in pyrene and lead co-contaminated soil and clean soil

Peak	t_R (min)	Chemical name	M.F	M.W	Amount (%)
Root exudates of ST in clean soil					
1	10.198	4-oxo-pentanol acid	$C_5H_8O_3$	116	1.95
2	20.118	Phthalate acid	$C_8H_6O_4$	166	0.42
3	20.623	Vanillic acid	$C_8H_8O_4$	168	1.41
4	22.467	Myristic acid	$C_{14}H_{28}O_2$	228	0.41
5	23.001	Pentadecanoic acid	$C_{15}H_{30}O_2$	242	0.39
6	23.000	Decanoic acid	$C_{10}H_{20}O_2$	172	0.35
7	23.657	14-methyl-pentadecanoic acid	$C_{16}H_{32}O_2$	256	0.13
8	23.913	Hexadecanoic acid	$C_{16}H_{32}O_2$	256	3.78
9	25.213	Octadecanoic acid	$C_{18}H_{36}O_2$	284	1.53
10	25.973	Oleic acid	$C_{18}H_{34}O_2$	282	0.08
Root exudates of ST in pyrene and lead co-contaminated soil					
1	10.208	4-oxo-pentanoic acid	$C_5H_8O_3$	116	3.98
2	11.601	Succinic acid	$C_4H_6O_4$	118	1.49
3	14.786	Glutaric acid	$C_5H_8O_4$	132	0.29
4	20.101	Phthalate acid	$C_8H_6O_4$	166	1.16
5	20.188	Citric acid	$C_6H_8O_7$	192	0.63
6	20.776	Vanillic acid	$C_8H_8O_4$	168	1.49
7	22.464	Myristic acid	$C_{14}H_{28}O_2$	228	1.27
8	22.934	Pentadecanoic acid	$C_{15}H_{30}O_2$	242	0.57
9	23.000	Decanoic acid	$C_{10}H_{20}O_2$	172	0.35
10	23.657	14-methyl-pentadecanoic acid	$C_{16}H_{32}O_2$	256	0.43
11	23.912	Hexadecanoic acid	$C_{16}H_{32}O_2$	256	6.79
12	25.211	Octadecanoic acid	$C_{18}H_{36}O_2$	284	3.27
13	25.447	Pyrene	$C_{16}H_{10}$	202	0.35
14	25.973	Oleic acid	$C_{18}H_{34}O_2$	282	0.48

example, in control, the amount of the non-desorbed fraction of PYR was approximately 41.5 %, and it seems PYR was easier to stay with the non-desorbed fraction, which might due to the relevant properties of higher solubility, smaller octanol–water partition coefficient. After adding the three OAs (citric acid, succinic acid and glutaric acid), the amount of non-desorbed fraction of PYR accounted for 35.7–41.5, 39.9–44.9 and 38.2–41.5 %, respectively.

Compared to the desorbed and non-desorbed fractions of PYR, the amount of bound residue fraction of PYR was <20 % (Table 2). In control, the amount of bound residual fraction of PYR accounted for 13.4 %. At concentration of 50 g kg^{-1}, the amount of bound residual fraction of PYR accounted for 2.79–13.4, 4.08–12.4 and 3.89–12.4 % by citric acid, succinic acid and glutaric acid, respectively.

Effects of *S. triqueter* REs on the fraction distribution and bioavailability of Pb

Divided by continuous extraction of heavy metals in soil, bioavailability of heavy metals in different fraction from high to low was: water-soluble fraction > acid-

extractable fraction > reducible fraction > oxidizable fraction > residual fraction. According to the absorption of different fractions of heavy metals by plant, heavy metal fractions could be divided into three categories: available fraction (water-soluble fraction and acid-extractable fraction), potential available fraction (reducible fraction and oxidizable fraction) and not available fraction (residual fraction) (Kartal et al. 2006).

Figure 3 shows that compared with the control, the treatments added the three OAs always improved the quantity of the bioavailable fraction of Pb during that the concentrations increased from 0 to 50 g kg^{-1}. In control soil, Pb in soil mainly existed in residue and reducible fractions, and the amount of these two fractions accounted for 55.4 and 28.9 %, respectively. However, the amount of water-soluble fraction, acid-soluble fraction and oxidizable fraction accounted for 3.71, 2.92 and 9.27 %, respectively (Fig. 3). Bioavailable fraction included water-soluble and acid-soluble fractions, and this content was small in control. However, the amount of the water-soluble fraction increased 418.6 %, and the amount of the acid-soluble fraction increased 178.4 % by added citric acid at

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Table 2 Fraction distributions of pyrene on different concentrations of organic acids in pyrene and lead co-contaminated soil and pyrene single contaminated soil

Pyrene single contaminated soil (mg kg^{-1})			Pyrene and lead co-contaminated soil (mg kg^{-1})		
Citric acid (g kg^{-1})	Desorbing fraction	Non-desorbing fraction	Desorbing fraction	Non-desorbing fraction	Bound residue
0	16.24 ± 1.55 a	20.83 ± 1.23 a	5.74 ± 1.40 a	16.46 ± 1.07 a	22.75 ± 1.23 a	4.22 ± 1.22 a
1	17.89 ± 1.78 a	20.32 ± 1.49 ab	5.12 ± 1.08 ab	16.91 ± 1.23 a	22.12 ± 1.43 a	4.15 ± 1.23 a
5	20.78 ± 1.24 b	19.43 ± 1.12 ab	3.24 ± 1.42 bc	18.91 ± 1.89 ab	21.44 ± 1.15 a	3.12 ± 0.75 ab
10	22.04 ± 1.10 bc	18.95 ± 1.33 b	2.79 ± 1.26 c	20.07 ± 1.32 bc	21.05 ± 1.17 a	2.83 ± 1.12 ab
20	23.68 ± 1.23 cd	17.35 ± 1.42 b	2.48 ± 1.05 c	21.71 ± 1.91 cd	20.07 ± 1.42 a	2.70 ± 1.15 ab
40	24.37 ± 1.35 cd	17.54 ± 1.23 b	1.95 ± 1.02 c	22.38 ± 1.37 cd	20.46 ± 1.75 a	1.55 ± 0.41 b
50	25.20 ± 1.29 d	17.21 ± 1.22 b	1.24 ± 1.03 c	23.20 ± 1.46 d	20.44 ± 1.69 a	1.39 ± 0.24 b
Succinic acid (g kg^{-1})	Desorbing fraction	Non-desorbing fraction	Bound residue	Desorbing fraction	Non-desorbing fraction	Bound residue
0	16.37 ± 1.08 a	21.75 ± 1.02 a	5.40 ± 1.49 a	15.46 ± 1.68 a	22.05 ± 1.44 a	4.51 ± 1.36 a
1	16.89 ± 1.39 a	21.29 ± 1.53 a	5.02 ± 1.22 a	15.70 ± 1.98 a	22.11 ± 1.25 a	4.06 ± 1.80 a
5	17.79 ± 1.47 a	22.84 ± 1.59 a	4.37 ± 1.10 ab	15.93 ± 1.20 a	22.94 ± 1.64 a	3.12 ± 1.87 a
10	20.24 ± 1.23 b	20.74 ± 1.11 a	3.46 ± 1.63 ab	18.06 ± 1.14 ab	21.37 ± 1.96 a	2.83 ± 1.44 a
20	21.69 ± 1.39 bc	20.27 ± 1.07 a	2.68 ± 1.55 ab	19.73 ± 1.12 bc	20.07 ± 1.35 a	2.70 ± 1.57 a
40	22.23 ± 1.05 c	19.89 ± 1.38 a	2.33 ± 1.30 b	20.38 ± 1.56 bc	19.99 ± 1.61 a	2.24 ± 1.91 a
50	23.32 ± 0.80 c	19.89 ± 1.44 a	2.14 ± 1.30 b	21.24 ± 1.17 c	19.94 ± 1.84 a	2.09 ± 1.45 a
Glutaric acid (g kg^{-1})	Desorbing fraction	Non-desorbing fraction	Bound residue	Desorbing fraction	Non-desorbing fraction	Bound residue
0	16.38 ± 1.80 a	20.72 ± 1.19 a	5.48 ± 1.41 a	15.46 ± 1.85 a	21.75 ± 1.60 a	4.31 ± 1.23 a
1	16.84 ± 1.56 ab	20.22 ± 1.19 a	5.21 ± 1.09 a	16.02 ± 1.68 ab	22.10 ± 1.29 a	3.18 ± 1.48 a
5	17.94 ± 1.42 ab	20.39 ± 1.86 a	4.25 ± 1.24 ab	15.88 ± 1.47 ab	22.84 ± 1.47 a	3.11 ± 1.10 a
10	19.24 ± 1.01 bc	20.22 ± 1.28 a	3.89 ± 1.47 ab	17.16 ± 1.90 abc	22.04 ± 1.35 a	2.82 ± 1.94 a
20	20.47 ± 1.349 cd	19.140 ± 1.13 a	2.87 ± 1.23 ab	18.71 ± 1.14 bcd	21.08 ± 1.20 a	2.71 ± 1.47 a
40	21.29 ± 1.09 cd	19.85 ± 1.74 a	2.33 ± 1.11 b	19.39 ± 1.81 cd	20.97 ± 1.21 a	2.25 ± 1.28 a
50	22.20 ± 1.43 d	19.78 ± 1.43 a	1.89 ± 1.37 b	20.19 ± 1.14 d	20.91 ± 1.41 a	1.94 ± 1.19 a

One-way ANOVA was performed for each parameter. Different letters indicate a significantly different ($p < 0.05$) from each other according to the Duncan test

concentration of 50 g kg^{-1}. The amount of the water-soluble fraction increased 304.8 %, and the amount of acid-soluble fraction increased 196.4 % by added succinic acid at concentration 50 g kg^{-1}. At low concentration range (<1 g kg^{-1}), the content of the bioavailable fraction increased rapidly with the added OAs, but at high concentration range (>1 g kg^{-1}), the bioavailable fraction content tended to be stable. Under the three OAs (citric acid, succinic acid and glutaric acid), the amount of water-soluble fraction of Pb in soil accounted for 3.71–19.3, 3.71–15.0 and 3.06–12.2 %, respectively. The amount of acid-soluble fraction accounted for 2.92–8.13, 4.88–8.65 and 4.88–7.04 %, respectively.

In control, the amount of the reducible and oxidizable fractions of Pb accounted for 28.9 and 9.27 %, respectively. However, the amount of reducible fraction increased 24.8 % and the amount of oxidizable fraction increased 51.4 % by

added citric acid at concentration of 50 g kg^{-1}. The amount of the reducible fraction increased 38.0 and 27.3 % by citric acid and succinic acid at concentration 50 g kg^{-1}, respectively. After adding the three OAs (citric acid, succinic acid and glutaric acid), the amount of reducible fraction of Pb in soil accounted for 28.9–40.0, 28.9–41.2 and 28.9–39.8 %, respectively. The amount of oxidizable fraction accounted for 4.70–9.27, 6.72–9.27 and 7.09–9.27 %, respectively.

Compared to the water-soluble, acid-soluble, reducible and oxidizable fractions of Pb, the amount of residual fraction of Pb accounted for 55.4 % in control. At concentration of 50 g kg^{-1}, the amount of residual fraction of Pb accounted for 29.3 % by citric acid, decreased 47.1 % compared with control. However, the amount of residual fraction of Pb accounted for 33.8 % by succinic acid at concentration 50 g kg^{-1}, decreased 39.0 % compared with the control.
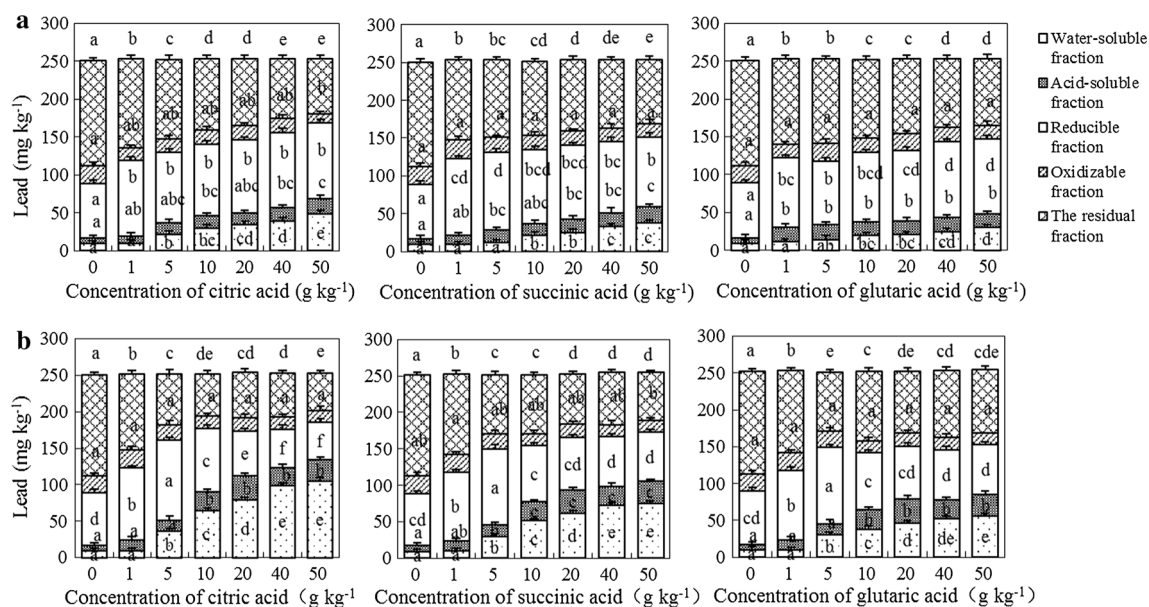
Fig. 3 **a** Fraction distributions of lead on different concentrations of organic acids in lead single contaminated soil, **b** fraction distributions of lead on different concentrations of organic acids in pyrene and lead co-contaminated soil. One-way ANOVA was performed for each parameter. *Different letters* indicate a significantly different ($p < 0.05$) from each other according to the Duncan test

Effects of *S. triqueter* REs on the fraction distribution and bioavailability of PYR and Pb

After adding three kinds of OAs in soil, the concentration of the effective state of PYR and Pb increased significantly, but the degree of the increasement in single and combined pollution soil was different. In PYR and Pb co-contaminated soil, the bioavailable fraction was 16.46–23.20 mg kg^{-1} for PYR and 16.58–137.70 mg kg^{-1} for Pb by citric acid. However, in PYR and Pb single contaminated soil, the bioavailable fraction was 16.46–25.20 mg kg^{-1} for PYR and 16.58–68.47 mg kg^{-1} for Pb by citric acid. In PYR and Pb co-contaminated soil, the potential available fraction was 20.07–22.75 mg kg^{-1} for PYR and 67.48–134.40 mg kg^{-1} for Pb by citric acid. However, in PYR and Pb single contaminated soil, the potential available fraction was 17.87–20.75 mg kg^{-1} for PYR and 84.11–123.20 mg kg^{-1} for Pb by citric acid. An interactive effect of heavy metals and organic pollutants on contaminants removal from co-contaminated soil can either cause a negative or positive effect depending on the type and concentration of both PAHs and metals (Zhang et al. 2012). Cachada et al. (2012) confirmed PAHs and PCBs, one group of semi-volatile, chemical stability and hydrophobic organic compounds which is everywhere in the environment, were associated with anthropogenic toxic elements such as Cu, Pb, Zn and Hg, especially between PAHs and Pb, with correlations higher than 0.767 ($p < 0.01$), and this may explain why Pb and PYR could make each other desorbed from soils and utilized by plants more easily.

REs impacts on the bioavailability and fraction distribution of PYR and Pb in soil

REs play an important role in the phytodegradation of organic contaminations and connected plant and microorganism in the complex pollution system (Joner et al. 2002). As known, REs transferred as many as one-fifth of total photosynthetically fixed carbon into the rhizosphere. Muratova et al. (2009) reported REs responses on PAHs patterns, and phenanthrene concentration of 10 mg kg^{-1} had no significant stimulating effects on root carbohydrate exudation. However, phenanthrene at 100 mg kg^{-1} reduced the release of acids, carbohydrates and amino acids in sorghum rhizosphere. Therefore, it has no significant differences in qualitative ingredients of REs under the addition of PAHs. In this study, PYR concentration set at 50 mg kg^{-1} in *S. triqueter* rhizosphere and the emission amounts of carboxylic acid (citric acid, succinic acid and glutaric acid) increased significant. REs effects on soil organic contaminations, including the positive effects on PAHs biodegradation, have been studied (Yoshitomi and Shann 2001). The mechanism of REs effects on promoting dissipation of organic chemicals is complex, not only for increasing the microbial mass, group and activities, but changes their bioavailabilities in soil. Joner et al. (2002) found that for five- and six-ring PAHs, adding artificial REs could increase their desorption in soil. These studies indicated that OAs in REs could increase the bioavailability of organic pollutants and affect the basic physical and chemical properties of soil.

Microbes played an important role in PAHs degradation in soil, to some extent adding OAs in REs promoted the removal of PYR in soil (Table 2), and low concentration of OAs enhanced the activity of soil microorganisms to degrade the target contaminants and residues. After applied low concentration of OAs, microbial activity increased, the utilization of PYR as carbon source increased, the total amount of PYR decreased by low concentration of OAs treatment soil samples; on the other hand, with the addition of OAs concentration increased much higher, soil pH reduced, inhibited the activity of soil microorganisms, very acidic soil environment is not conducive to the formation and survival of microbial colonies, and the total amount of PYR increased by high concentration of OAs.

In addition, this study indicated that the OAs in REs could affect the basic physical and chemical properties of soil (Zou et al. 2013). OAs in REs could promote soil organic matter released; OAs in REs were able to collapse soil structure through inorganic ions chelation, thereby increased the bioavailability of organic pollutants; OAs in REs which contain carboxyl and hydroxyl groups might dissolve soil minerals, impacted the migration of the organochlorine pesticides (Ling et al. 2009).

Heavy metals in soils can be sequestered into a number of fractions including the metal sorbed to clays, hydrous oxides and organic matter, and metal within the matrix of soil minerals (Reichman and Parker 2002). Plants cannot normally access the metal from all of these fractions, and so measurements of total soil metal are not necessarily a reliable predictor for bioavailable metal and therefore cannot be used for evaluating which soils pose a risk of crop production (Peijnenburg et al. 2000). The content of bioavailable fraction of Pb clearly shows an increasing tendency by the added OAs in REs of *S. triqueter* in this study, indicated that OAs in REs could increase the bioavailability of heavy metals. The metals associated with minerals such as carbonates or oxides can be released under acidic or reducing conditions, and root-induced changes in pH and Eh can thereby affect the bioavailability of trace metals in the rhizosphere (Marschner and Römheld 1996).

Mechanism of enhanced bioavailability of PYR and Pb in soil by OAs in REs

REs, especially OAs in REs, could change the soil characteristics, increased bioavailability of organic and heavy metal pollutants, which could help clear the rhizosphere organic and heavy metal pollutants (Lu et al. 2007). OAs in REs include mono-, di-, and tri-carboxylic acids which include unsaturated carbon and hydroxyl groups. It was noted that in general the concentrations of di- and tri-carboxylic aliphatic acids in soil are less than 50 μmol L^{-1},

and sometimes up to 650 μmol L^{-1}. In this work, to obtain more credible results, the added amounts of OAs were larger than their natural concentrations in soils. OAs in REs are vital in many rhizospheric processes, including nutrient absorption, plant growth regulation, association of plant–microbe, reduction in the harmful elements' toxicity and evaluation of microbial community structure (Lu et al. 2007). Significant amounts of OAs can promote PAHs elimination through activation of abiotic oxidation, for example, the existence of hydrogen peroxide can cause plant stress responses and leads to the emission of free hydroxyl radicals (OH–) during the process that carboxylic acid produces peroxy acid; these non-specific strong oxidizing agents can rapidly degrade a variety of organic compounds, including polycyclic aromatic hydrocarbons (Muratova et al. 2009).

Natural OAs including oxalic, citric, formic, acetic, malic, succinic, malonic, maleic, lactic, aconitic and fumaric acids are natural products of REs, microbial secretions and plant and animal residues in soils. Thus, metal dissolution by OAs is likely to be more representative of a mobile metal fraction that is available to biota (Labanowski et al. 2008). The chelating OAs are able to dislodge the exchangeable, carbonate and reducible fractions of heavy metals by washing procedures. Although many chelating compounds including citric acid and tartaric acid for mobilizing heavy metals have been evaluated, there remain uncertainties as to the optimal choice for full-scale application (Wuana et al. 2010). The main effect of REs on the remediation of heavy metals can be best explained in the following two ways: (1) acidizing insoluble heavy metal fraction in soil and (2) chelating and reducing heavy metals in soil (Kim et al. 2010). The concentration of heavy metals in the soil, especially the bioavailability and distribution of heavy metals, are often under the control of adsorption and desorption equilibrium in solid and liquid phases (Mäkelä et al. 2011). The positive effects of OAs on availabilities of Pb in this study provide a new method to remediate heavy metal-contaminated soil by OAs addition.

Conclusion

Effects of OAs in the REs of *S. triqueter* on PYR and Pb dissipation were efficient. The key components of *S. triqueter* REs involved 4-oxo-pentanoic acid, succinic acid, glutaric acid, phthalate acid, citric acid, vanillic acid, myristic acid, pentadecanoic acid, decanoic acid, 14-methyl-pentadecanoic acid, hexadecanoic acid, octadecanoic acid and oleic acid, and the content of the water-soluble OAs (citric acid, succinic acid and glutaric acid) significantly

increased in PYR and Pb co-contaminated rhizosphere soil. These three water-soluble OAs including citric acid, succinic acid and glutaric acid were detected as the specific REs of *S. triqueter* under stress of pollutants for PYR and Pb, so they were chosen as the research objects. Specific OAs in the test concentrations range always improved the quantity of the bioavailable fraction of PYR and Pb in soil and greatly influenced the fraction distribution of PYR and Pb in soil. Extraction with mild extracting solvents such as *n*-butanol and acetic acid provided a useful technique for evaluating the OAs effects on the fraction distribution and bioavailability of PYR and Pb. Results of this investigation may provide useful information on the assessment of PAH and heavy metal-related risks to human health and the environment and may be instructive in food safety and remediation strategies at contaminated sites.

Compliance with ethical standards

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

Informed consent The study is not split up into several parts to increase the quantity of submissions and submitted to various journals or to one journal over time. And authors whose names appear on the submission have contributed sufficiently to the scientific work. Informed consent was obtained from all individual participants included in the study.

Abbreviations

OAs	Organic acids
REs	Root exudates
PYR	Pyrene
Pb	Lead
PAHs	Polycyclic aromatic hydrocarbons
BCR	European Community Bureau of Reference
EPA	Environmental Protection Agency
GC-MS	Gas chromatography-mass spectrometry
HPLC	High-performance liquid chromatography
ICP	Inductively coupled plasma
PCBs	Polychlorinated biphenyls
Cu	Copper
Zn	Zinc
Hg	Mercury
Eh	Oxidation–reduction potential

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