RESEARCH ARTICLE



# Influence of tea saponin on enhancing accessibility of pyrene and cadmium phytoremediated with Lolium multiflorum in co-contaminated soils

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Abstract Tea saponin (TS), a kind of biodegradable surfactant, was chosen to improve the accessible solubilization of pyrene and cadmium (Cd) in co-contaminated soils cultivated Lolium multiflorum. TS obviously improved the accessibility of pyrene and Cd for L. multiflorum to accelerate the process of accumulation and elimination of the pollutants. The chemical forms of Cd was transformed from Fe-Mn oxides and associated to carbonates fractions into exchangeable fractions by adding TS in single Cd and pyrene-Cd contaminated soils. Moreover, the chemical forms of pyrene were transformed from associated fraction into bioaccessible fraction by adding TS in pyrene and pyrene-Cd contaminated soils. In pyrene-Cd contaminated soil, the exchangeable fraction of Cd was hindered in the existence of pyrene, and bioaccessible fraction of pyrene was promoted by the cadmium. Besides, in the process of the pyrene degradation and Cd accumulation, the effect could be improved by the elongation of roots with adding TS, and the microorganism activity was stimulated by TS to accelerate the removal of pollutions. Therefore, Planting L. multiflorum combined with adding TS would be an effective method on the phytoremediation of organics and heavy metals co-contaminated soils.

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## Introduction

Phytoremediation has been studied by some researchers in the bioremediation field, and it has been developed and widely used for its cost-effective feature in the application (Gao et al. [2007](#page-6-0); Baldantoni et al., [2014\)](#page-5-0). Simultaneously, there are also researches about solubilization effect of surfactants (Zhou et al., [2011](#page-6-0)). However, few studies focused on biodegradable surfactants in the process of phytoremediation in organics and heavy metals combined contaminated soils. Sun et al. [\(2011\)](#page-6-0) confirmed that low concentration of benzo[a]pyrene(B[a]P) could accelerate the growth of plants and result in an improvement in biomass at the rate of 10.0–49.7 % relative to the control. But some heavy metals, such as Cd, Cu, and Pb, could inhibit plant growth and B[a]P uptake. Tagetes patula showed a settled feature of Cd-hyperaccumulator under combined contaminated soils. From previous research, it can be thought that low concentration of organics could be a nutrient for plant growth, and the amassment of heavy metals by hyperaccumulator could be an effective and stable way in combined contaminated soil. In addition, the potential of surfactants was evaluated by other researchers (Gao et al., [2007](#page-6-0)) to improve desorption, plant assimilated, and biodegradation of HOCs in the polluted sites. Surfactant has an effect of solubilization; it has an ability of enhancing the accessibility of pollutants in the soil to improve the degradation effect for plants (Almeida et al., [2009;](#page-5-0) Wang et al., [2010\)](#page-6-0).

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As anionic surfactants (such as sodium dodecyl sulfate (SDS)) and some cationic surfactants had phytotoxicity or low efficiency for surfactant-enhanced phytoremediation, they were not beneficial in the process of phytoremediation. A kind of biosurfactant, which is non-toxic and has high surfactant-enhanced effect of phytoremediation, would be essential for the process of remediation. Several researchers (Iglauer et al., [2009;](#page-6-0) Momin and Yeole, [2011](#page-6-0); Liu et al., [2013\)](#page-6-0) have revealed the enhancing effect of alkyl polyglucoside (APG) which was a non-toxic surfactant on phytoremediation of PAH-contaminated soil. Zhou et al. [\(2011\)](#page-6-0) interpreted the solubilization properties of PAHs by saponin, a plant-derived biosurfactant. Some biodegradable surfactants are biologically produced by many microorganisms or plants. And then, tea saponin (TS), a type of natural surfactant derived from tea seed, is more effective in enhancing solubilization of PAHs than synthetic non-ionic surfactants (Urum and Pekdemir, [2004;](#page-6-0) Xia et al., [2009\)](#page-6-0). Moreover, it has much potential application in removing organic pollutants from contaminated soils (Tang et al. [2014](#page-6-0); Wang et al. [2015\)](#page-6-0). Tea saponin was added in the pot experiments for this study.

Lupinus luteus L. and associated endophytic bacteria were detected in the organic pollutants, and heavy metals combined contaminated soils with phytoremediation method (Gutierrez-Gines et al., [2014](#page-6-0)). Other researchers illustrated the intake of PAHs by *Trifolium pretense* L. from water in the existence of a non-ionic surfactant (Gao et al., [2008\)](#page-6-0). In a phytoremediation study, alfalfa (Medicago sativa L.) had a significant effect on ameliorating the physicochemical properties of sewage sludge compost degraded soils by regaining initial pH values and significantly reducing salinity (Hamdi et al. [2012](#page-6-0)). *Lolium* multiflorum were chosen as bioindicator plants for accumulation of toxic metals and removal of PAHs (Rodriguez et al., [2010;](#page-6-0) Rinaldi et al., [2012](#page-6-0); Jelusic and Lestan, [2015\)](#page-6-0).

As we all know, the availabilities of PAHs and heavy metals are crucial to the plants in the process of phytoremediation on contaminated sites. Then, improving the accessibility of pyrene and Cd is of importance for the accumulation and degradation of pollutants (Luo et al., [2010;](#page-6-0) Ortega-Calvo et al., [2013\)](#page-6-0). Therefore, the purpose of this study was to investigate the effects on the enhanced accessibility of pollutants for the assimilation of L. multiflorum and accumulation of pyrene and Cd with adding a selected biodegradable surfactant TS in surfactant-laden soil. The complex mutual influence of TS and  $L.$  multiflorum was illustrated in this study. The findings obtained from our experiments are expected to provide insight with regarding to the solubilization effect of surfactant-aided phytoremediation. Meanwhile, degradation and removal of PAHs and heavy metals in contaminated soils with coexisted surfactants would be advantageous to food security (Gao et al., [2009](#page-6-0)) and human health.

#### Materials and methods

#### Chemicals

Pyrene (98 % purity) applied in this experiment was bought from Aladdin Reagent. Tea saponin was obtained from Quality Department of Hangzhou Choisun Tea Sci-Tech Co., Ltd. All the other chemicals were analytical grade or better and purchased from Sinopharm.

#### Soils

The soil applied in this experiment was collected from 0 to 20 cm top layer without previous exposure to pyrene and Cd at the east square of Shanghai University, China. The soil was air-dried, ground, and passed through a 2-mm sieve to remove stones, glasses, and dead leaves. The contaminated process was that 1000 mg of pyrene was added into 100 mL of dichloromethane to be dissolved and mixed with 1 kg of clean soil to uniformity. After the solvent evaporated for 2 days, the soil was mixed with 9 kg of clean soil to make 100 mg kg−<sup>1</sup> of pyrene. The soil was mechanically mixed to ensure uniform and then aged for 3 months in the dark before experiment. The aqueous solution of cadmium nitrate  $(Cd(NO<sub>3</sub>)<sub>2</sub>, AR)$  was added to the clean soil sampled before to make 22 mg  $kg^{-1}$  Cd, and the polluted and aged method of Cd was similar to that of pyrene. The aqueous solution of  $Cd(NO<sub>3</sub>)<sub>2</sub>$  was added to pyrene contaminated soil to obtain pyrene-Cd co-contaminated soil, and the final concentrations of pyrene and Cd were 100 and 22 mg  $\text{kg}^{-1}$ , respectively (Wei et al., [2014\)](#page-6-0).

#### Experimental design

Nine groups of simulated pots were set up in this experiment. There were three kinds of treatment methods respectively in three kinds of soils. The first three were control groups without surfactants and non-cultivated in Cd soil (C), pyrene soil (P), and pyrene-Cd soil (M). The second three were control groups without surfactants in cultivated and Cd soil (CL), pyrene soil (PL) and pyrene-Cd soil (ML). The third three were experimental groups with 40 mg  $L^{-1}$  TS in cultivated and Cd soil (CLT), pyrene soil (PLT), and pyrene-Cd soil (MLT).

These pots were placed on the terrace ground, and the seedlings of *L. multiflorum* were moved to the pots. TS solution (0.02 %) was watered into the pots as the setting before. After 40 days, the pots were destructively sampled. The soil samples were freeze-dried immediately and stored at −80 °C till analysis. In this experiment, every setup was prepared in triplicates.

#### Analytical methods

Different chemical forms of pyrene in the soils were detected by a three-step sequential extraction method according to previous studies (Wei et al., [2014](#page-6-0)). The bioaccessible fraction was extracted by vortex mixing 5 mL of butanol with 1 g of freeze-dried soils for 30 s. The mixture was centrifuged at 4000 rpm for 15 min. Then, the supernatant was removed cautiously and concentrated to determine the concentration of pyrene (Gomez-Eyles et al., [2010\)](#page-6-0). After that, the associated fraction was ultrasonically extracted from the residual solid phase with 10 mL dichloromethane for 30 min, and this procedure was repeated three times. The resulting liquids were combined, evaporated, and redissolved in 1 mL of n-hexane to be analyzed. At last, bound fraction of pyrene was extracted from the remaining soil with adding 10 mL of 2 M NaOH aqueous solution. With 5 mL of 6 M hydrochloric acid, the supernatant was acidified to pH<2 and extracted with 10 mL of dichloromethane. It was also redissolved in 1 mL of nhexane to be analyzed (Ma et al., [2012\)](#page-6-0). The concentration of pyrene was quantified by GC-MS (Agilent 6890 N/5975B) equipped with a DB-5 column (30 m $\times$ 0.25 mm $\times$ 0.25  $\mu$ m). The temperature was held at 100 °C for 2 min, after that increased 10 °C min<sup>-1</sup> to 300 °C, where it was kept for 5 min (Ma et al., [2012;](#page-6-0) Wei et al., [2014\)](#page-6-0).

Different chemical forms of Cd in the soil were measured by Tessier gradual separation technology (Alborés et al., [2000\)](#page-5-0). Exchangeable fraction of Cd was extracted from 0.25 g of freeze-dried soil with adding 8 mL of 1 mol  $L^{-1}$ MgCl<sub>2</sub> (pH=7) solution and shaken for 1 h at 25 °C. Associated to carbonates fraction of Cd was extracted from the residual solid phase with adding 8 mL of 1 mol  $L^{-1}$  NaOAc (pH=5) solution and shaken for 5 h at 25 °C. Associated to Fe-Mn oxides (or reducible) fraction of Cd was extracted from the residual solid phase with adding 20 mL of 0.04 mol  $L^{-1}$ NH<sub>2</sub>OH·HCl in 25 % m/v HOAc solution and shaken for 6 h at 96 °C. Associated to organic matter (or oxidizable) fraction of Cd was extracted from the residual solid phase with adding 3 mL of 0.02 mol  $L^{-1}$  HNO<sub>3</sub> solution and 5 mL of 30 % m/v H<sub>2</sub>O<sub>2</sub> solution and shaken for 2 h at 85 °C, then added 3 mL of 30 % m/v  $H_2O_2$  solution and shaken for 3 h at 85 °C, then added 5 mL of 3.2 mol  $L^{-1}$  NH<sub>4</sub>OAc solution for 30 min at 25 °C. The residual Cd was extracted from residual solid phase with adding  $HNO<sub>3</sub>-HClO<sub>4</sub>-HF$  for heating digestion. The extraction was conducted in 50 mL of polyethylene tubes, which were also used for centrifugation to minimize the possible loss in the centrifuge-washing processes. After each extraction step, the supernatant liquid was separated from the solid phase by centrifugation at 4000 rpm for 5 min. It was then transferred into polyethylene vessels with 5 % dilute nitric acid to 10 mL and stored at 4 °C before analysis to reduce variation. The concentration of Cd in aqueous solution was determined by inductively coupled plasma (ICP).

Measurements were made in triplicate in each experiment to reduce errors.

The length of leaves and roots of the plants were measured with a vernier caliper. The activity of microorganism was determined by the method of previous studies (Trasar-Cepeda et al., [2008\)](#page-6-0).

#### Statistical analysis

In this manuscript, each data was the mean value  $(\pm SD)$  of three replicates. SPSS 18.0 was used for ANOVA. Significant differences in the main effect were further analyzed by pairwise comparisons, with the Duncan's multiple range tests and  $p<0.05$  taken to indicate statistical significance.

## Results and discussion

In order to evaluate the solubilization effect of TS on pyrene and Cd, TS was added in L. multiflorum cultivated soil. Solubilization effect of TS was well indicated by the increase of accessibility of pyrene and Cd. Physiological indices of L. multiflorum were measured for the detection of the physiological response to the pollutants. Fluorescein diacetate (FDA) was used to evaluate the activity of microorganism removed pollutants.

## Biomass of plants in single and combined contaminated soils

The accumulation and removal effect of pollutants could be revealed by the biomass of plants. The length of leaves and roots of L. multiflorum in pyrene, Cd, and pyrene-Cdcontaminated soils were shown in Fig. [1.](#page-3-0) Compared with PL, CL, and ML groups, it was observed that there was no significant difference among the length of leaves, but the length of roots was longer in the ML group which was combined soil than that in PL and CL groups. This phenomenon implied that the length of roots of L. multiflorum was stretched by the enforcement of the combined effect of pyrene and Cd. The similar results could be obtained among the experimental groups (PLT, CLT, and MLT). The length of roots of L. multiflorum was much longer in adding TS groups than that without TS groups. However, the length of leaves among adding TS groups were shorter than that without TS groups. Then, it was thought that the accessibility of pollutants for the roots of L. multiflorum was enhanced by adding TS. The length of roots was elongated, and at the same time, the length of leaves was inhibited by the effect. Thus, the removal of pyrene and the accumulation of Cd were enhanced by the elongation of *L. multiflorum* roots with adding TS in the contaminated soils.

<span id="page-3-0"></span>

Fig. 1 The length of leaves and roots of *Lolium multiflorum* in pyrene, Cd and pyrene-Cd soils. (PL) control without TS in cultivated and pyrene soil, (CL) control without TS in cultivated and Cd soil, (ML) control without TS in cultivated and pyrene-Cd soil, (PLT) 40 mg  $L^{-1}$  TS in cultivated and pyrene soil, (CLT) 40 mg  $L^{-1}$  TS in cultivated and Cd soil, (MLT) 40 mg  $L^{-1}$  TS in cultivated and pyrene-Cd soil. *Error bars* denote the standard deviations. Different letters in the columns indicate statistically significant differences in the length of leaves and roots of plant subgroups among treatments (Duncan test,  $P < 0.05$ ,  $n=3$ ).

## Accessibility of pyrene and Cd in contaminated soils cultivated with L. multiflorum and adding of TS

Different fractions of Cd with adding TS and L. multiflorum cultivated in Cd and pyrene-Cd contaminated soils were shown in Fig. 2. It was observed that the proportion of exchangeable fractions was larger than any other fractions of Cd in all groups in Fig. 2. Compared with C, CL, and CLT groups, the concentration of exchangeable fraction of Cd in the CLT group was the highest. The second highest of that was the CL group without surfactants in cultivated and Cd soil. The C group which was a control of that was the least in all groups. The similar experimental results were obtained that the concentration of exchangeable fraction of Cd in MLT group was the highest among M, ML, and MLT groups of combined contaminated soils. The second highest of that was the ML group without TS in cultivated and pyrene-Cd contaminated soil. The least one of that was M group without TS and plant in pyrene-Cd contaminated soil. It was demonstrated that TS took an active part in enhancing the exchangeable fraction of Cd. Compared with C and M, CL and ML, CLT and MLT groups respectively, it was shown that the concentration of exchangeable fraction was higher in Cd contaminated soil than that in pyrene-Cd soil. This phenomenon indicated that the increase of the concentration of exchangeable fraction of Cd was hindered in the existence of pyrene in pyrene-Cd contaminated soil in this experiment. However, the concentration of exchangeable fraction of Cd was still higher in MLT group (11.20 mg  $kg^{-1}$ ) than that of CL group (10.94 mg kg−<sup>1</sup> ). According to the results, it can be obtained that the increase of the concentration of Cd can be enhanced with adding TS. Similarly, it was indicated in a study (Liu



Fig. 2 Different fractions of Cd with adding TS in Cd and pyrene-Cd soils. (C) control without TS and non-cultivated in Cd soil, (M) control without TS and non-cultivated in pyrene-Cd soil, (CL) control without TS in cultivated and Cd soil, (ML) control without TS in cultivated and pyrene-Cd soil, (CLT) 40 mg  $L^{-1}$  TS in cultivated and Cd soil, (MLT) 40 mg  $L^{-1}$  TS in cultivated and pyrene-Cd soil. *Error bars* denote the standard deviations. Different letters in the columns indicate statistically significant differences in the fractions of Cd subgroups among treatments (Duncan test,  $P < 0.05$ ,  $n=3$ ).

et al., [2009\)](#page-6-0) that the maximal accumulation of Cd in shoots and roots of Althaea rosea Cav. were up to 131.9 and 67.5 mg kg<sup>-1</sup> when Cd in the soil was 30 mg kg<sup>-1</sup> under joint ethylenegluatarotriacetic acid (EGTA) and sodium dodecylsulfonate (SDS) treatments. In another study (Xia et al., [2009\)](#page-6-0), it was obtained that with addition of 0.3 % tea saponin, concentration of Cd was increased by 96.9 % in roots, 156.8 % in stems, and 30.1 % in leaves compared with the treatment without addition of surfactant in sugarcane grown in soils.

According to the six groups, the concentrations sequence of associated to Fe-Mn oxides and associated to carbonates fractions of Cd were obviously as follows: C and M groups> CL and ML groups>CLT and MLT groups. However, a small proportion was contributed by the associated to organic matter and residual fraction. There was no significant difference among the six treatment groups. These phenomena could be interpreted that the chemical forms of Cd was transformed from Fe-Mn oxides and associated to carbonates fractions to exchangeable fraction with adding TS in Cd and pyrene-Cd contaminated soils. The solubilization effect of Cd was promoted by plants and TS, and the better solubilization effect was contributed by adding TS to the soil. A conclusion can be obtained that TS played a positive effect in improving the solubilization of Cd to an accessible fraction for L. multiflorum to accelerate the accumulation procedures. These results would be beneficial to plants in the phytoremediation of heavy metals.

The concentrations of Cd in upper and lower soils were shown in Table [1](#page-4-0). It can be easily concluded that the concentrations of Cd in both upper and lower soils were about the original concentration from the Table [1.](#page-4-0) And the <span id="page-4-0"></span>Table 1 Concentrations of Cd in upper and lower soils



Different letters in the table indicate statistically significant differences in the concentrations of Cd subgroups among treatments (Duncan test,  $P < 0.05$ , n=3)

C control without TS and non-cultivated in Cd soil, M control without TS and noncultivated in pyrene-Cd soil, CL control without TS in cultivated and Cd soil, ML control without TS in cultivated and pyrene-Cd soil,  $CLT$  40 mg  $L^{-1}$  TS in cultivated and Cd soil,  $MLT$  40 mg  $L^{-1}$  TS in cultivated and pyrene-Cd soil

concentrations of Cd in lower soils were higher than that in upper soils. There was migration effect of Cd from upper to lower soils.

Different fractions of pyrene with adding TS in pyrene and pyrene-Cd soils were shown in Fig. 3. It was found that a small proportion was contributed by bound fraction of pyrene, and there was no significant difference among the six groups. Associated and bioacccessible fractions occupied a larger proportion in the three fractions of pyrene. The availability of L. multiflorum was evaluated by the content of bioaccessible fraction of pyrene. The concentrations of bioaccessible fraction were higher in combined contaminated groups (M, ML, and MLT) than that of corresponding single pyrene contaminated groups (P, PL, and PLT). It was revealed that the amount of bioaccessible fraction of pyrene was promoted in the existence of Cd in pyrene-Cd contaminated soil in the experiments.

Under the condition of adding TS and planting L. multiflorum, the total concentration of pyrene was decreased, whereas the associated fraction of pyrene was increased. However, the concentrations of both total pyrene and associated fractions of it were all more deceased in adding TS groups (PLT, MLT) than that of cultivated groups (PL, ML) and control groups (P, M). It can be obviously concluded that the degradation of pyrene can be promoted by both TS and L. multiflorum. And the promoting effect of the former was more significantly. It is generally thought in others' study (Gao et al., [2008\)](#page-6-0) that Tween 80 enhanced phytoremediation of T. pretense L. in pyrene and phenanthrene contaminated sites. These results demonstrated that the chemical forms of pyrene were transformed from associated fractions to bioaccessible fraction with adding TS in pyrene and pyrene-Cd contaminated soils. This was an important process in the improving of the solubilization of pyrene.



Fig. 3 Different fractions of pyrene with adding TS in pyrene and pyrene-Cd soils. (P) control without TS and non-cultivated in pyrene soil, (M) control without TS and non-cultivated in pyrene-Cd soil, (PL) control without TS in cultivated and pyrene soil, (ML) control without TS in cultivated and pyrene-Cd soil, (PLT) 40 mg  $L^{-1}$  TS in cultivated and pyrene soil, (MLT) 40 mg L−<sup>1</sup> TS in cultivated and pyrene-Cd soil. Error bars denote the standard deviations. Different letters in the columns indicate statistically significant differences in the fractions of pyrene subgroups among treatments (Duncan test,  $P < 0.05$ , n=3).

# Activity of microorganism in single and combined contaminated soils

The reaction of FDA could better indicate the changes of microbial activity and the quality of soils (Battin, [1997;](#page-6-0) Gillian Adam, [2001](#page-6-0); Brouwer et al., [2006\)](#page-6-0), and it was considered as biological indicators in the soil (Trasar-Cepeda et al., [2008\)](#page-6-0). The activities of microorganisms in pyrene, Cd, and pyrene-Cd contaminated soils were shown in Fig. 4. Compared with three different kinds of treatments in pyrene contaminated soils (P, PL, PLT), Cd soils (C, CL, CLT), and



Fig. 4 Activities of microorganisms in pyrene, Cd and pyrene-Cd soils. (P) control without TS and non-cultivated in pyrene soil, (PL) control without TS in cultivated and pyrene soil, (PLT) 40 mg  $L^{-1}$  TS in cultivated and pyrene soil, (C) control without TS and non-cultivated in Cd soil, (CL) control without TS in cultivated and Cd soil, (CLT) 40 mg  $L^{-1}$  TS in cultivated and Cd soil, (M) control without TS and non-cultivated in pyrene-Cd soil, (ML) control without TS in cultivated and pyrene-Cd soil, (MLT) 40 mg  $L^{-1}$  TS in cultivated and pyrene-Cd soil. Error bars denote the standard deviations. Different letters in the columns indicate statistically significant differences in the activities of microorganisms subgroups among treatments (Duncan test,  $P < 0.05$ ,  $n=3$ ).

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Fig. 5 Degradation ratio of pyrene in pyrene and pyrene-Cd soils. (P) control without TS and non-cultivated in pyrene soil, (M) control without TS and non-cultivated in pyrene-Cd soil, (PL) control without TS in cultivated and pyrene soil, (ML) control without TS in cultivated and pyrene-Cd soil, (PLT) 40 mg  $L^{-1}$  TS in cultivated and pyrene soil, (MLT) 40 mg  $L^{-1}$  TS in cultivated and pyrene-Cd soil. Error bars denote the standard deviations.

pyrene-Cd soils (M, ML, MLT), the activities of microorganisms were all higher in cultivated with adding TS groups than that in only cultivated and control groups. It was demonstrated that TS and L. multiflorum could stimulate the activity of microorganism. In addition, the activity of microorganism in the MLT group was the highest of all the treatment groups. These results revealed that the activity of microorganism was increased with adding TS and plants. An effective method is provided for the phytoremediation in combined contaminated soils.

## Degradation ratio of pyrene in single and combined contaminated soils

Degradation ratio of pyrene in single and pyrene-Cd contaminated soils was shown in Fig. 5. The degradation ratio of pyrene was calculated by the specific value for the reduced content of pyrene and its total concentration in the soils.

In Fig. 5, it was shown that the degradation ratios of pyrene were low in control groups  $(P, M)$  for 1 8.22 and 21.74  $\%$ , respectively. In L. *multiflorum* cultivated soils, the degradation ratios of pyrene in cultivated without TS groups (PL, ML) were 27.95 and 30.00 %, respectively. Furthermore, the degradation ratios of pyrene in cultivated with TS groups (PLT, MLT) were 35.25 and 39.59 %, respectively. These data can be illustrated that L. multiflorum had a good effect on the degradation of pyrene. If they were added with TS, the degradation ratios of pyrene were much higher in experimental groups (PLT, MLT) than that of only cultivated groups (PL, ML).

Therefore, by adding TS, the chemical forms of pyrene were transformed to bioaccessible fraction in pyrene and pyrene-Cd contaminated soils, and L. multiflorum can accelerate the accumulation process of Cd with improving the solubilization of Cd to an accessible fraction.

### **Conclusions**

In this study, tea saponin (TS), a kind of biodegradable surfactant, was chosen to improve the solubilization of accessibility of pyrene and Cd in simulated co-contaminated soils cultivated L. multiflorum. TS took an active part in enhancing the accessibility of pyrene and Cd for L. multiflorum to accelerate the accumulation and degradation process of pollutants. The chemical forms of Cd was transformed from Fe-Mn oxides and associated to carbonates fractions to exchangeable fraction with adding TS in single Cd and pyrene-Cd contaminated soils. The chemical forms of pyrene were transformed from associated fraction to bioaccessible fraction with adding TS in single pyrene and pyrene-Cd contaminated soils. The exchangeable fraction of Cd was hindered in the existence of pyrene, and bioaccessible fraction of pyrene was promoted by the cadmium in pyrene-Cd contaminated soil. The elongation of roots was induced by the stress from the combined effect of pyrene and Cd. The degradation of pyrene and the accumulation of Cd were improved by the elongation of roots with adding TS in the contaminated soils. The activity of microorganism was stimulated by TS and plants to accelerate the accumulation and degradation rate of pollutions. The activity of microorganism was increased with adding TS and planting L. multiflorum.

It can be concluded that TS can be applied in the accumulation of heavy metals and elimination of pyrene. TS combined with L. multiflorum would be an effective way on the remediation of organics and heavy metals combined contaminated soils.

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#### Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests

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