Environmental Science and Pollution Research (2020) 27:20538–20544
https://doi.org/10.1007/s11356-020-08552-3

RESEARCH ARTICLE

Nano-SiO_2 combined with a surfactant enhanced phenanthrene phytoremediation by *Erigeron annuus* (L.) Pers

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Received: 9 January 2020 / Accepted: 23 March 2020 / Published online: 3 April 2020

Abstract
The objective of this experiment was to evaluate the effects of Triton X-100 (1000 mg kg^{-1}) and nano-SiO_2 (500 mg kg^{-1}) on *Erigeron annuus* (L.) Pers. grown in phenanthrene spiked soil (150 mg kg^{-1}) for 60 days. Results show that untreated groups, groups treated with both Triton X-100 and nano-SiO_2, exhibited better phenanthrene degradation rates and improved root biomasses, chlorophyll contents, and soil enzyme activities. This study demonstrates that Triton X-100 combined with nano-SiO_2 protects plants, alleviating the stress of polycyclic aromatic hydrocarbons (PAHs), and can provide a means for improving phytoremediation of PAH contaminated soils.

Keywords Polycyclic aromatic hydrocarbons · Organic soil contaminants · Biotoxicity · Soil enzyme activity · Soil pollution · Surfactant-enhanced remediation · Triton X-100

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are hazardous, ubiquitous, major environmental pollutants. They are toxic, mutagenic, teratogenic, and carcinogenic organic pollutants composed of 2 or more benzene rings formed mainly by incomplete combustion or pyrolysis, in reduced conditions, of various fossil fuels (e.g., coal, oil, and natural gas) and other hydrocarbon-containing compounds (Huang et al. 2019). Because of their hydrophobic and lipophilic characteristics, PAHs tend to accumulate in the soil, not only affecting normal soil functions but also degrading the soil environment. When PAHs enter the food chain through bioaccumulation, they ultimately endanger human health (Qin et al. 2019). The PAH pollution problem presents harmful global consequences, and effective remediation of PAH-contaminated soil is receiving increasing attention.

Phytoremediation, a recently developed green restoration technology, is designed to remove toxic pollutants from the environment by utilizing special functions of plants and rhizosphere microorganisms to repair contaminated soil (Rostami and Azhdarpoor 2019). Phytoremediation is characterized by the complete degradation of pollutants; low treatment cost; maintenance of physical and chemical properties of the original, non-polluted soil; broad applicability; and tendency to not produce secondary pollution. It is well suited to repair large areas of polluted soil, as well. Research has found that phytoremediation has successfully removed pesticides, heavy metals, and PAHs from many sites (Ali Romeh 2015). However, some studies have shown that plant growth becomes inhibited as PAH concentration increases, thus reducing efficient phytoremediation (Košnář et al. 2018). Therefore, extensive research is needed to find ways to both reduce PAH toxicity and promote plant growth.

Silicon is the second most abundant element on Earth, comprising 28% of the Earth's crust (Hossain et al. 2018). All plants grown in soil contain some silicon in their tissues, ranging from 0.1 to 10% by weight (Guo et al. 2018). Silicon promotes plant growth and helps plants alleviate their biotic and abiotic stresses, enabling some plants to better tolerate heavy metals (Xiao et al. 2016). Nano-SiO_2 is a popular and versatile nanomaterial that is currently used in environmental

Responsible Editor: Elena Maestri

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remediation, sewage disposal, food production, industrial and household applications, and biomedicine (Le et al. 2014). Several studies have reported that nano-SiO_2 significantly enhanced tomato (*Lycopersicum esculentum*) seed germination and helped the phytoremediation capability of *Secale montanum* in Pb-contaminated soils (Siddiqui and Al-Whaibi 2014; Moameri and Abbasi 2019). A recent study showed that nano-SiO_2 combined with compost increased PAH degradation rates in plants because nano-SiO_2 absorbed the PAHs, thus resulting in decreased biotoxicity (Włóka et al. 2019). Very likely, the use of Si to enhance plant growth and alleviate PAH toxicities will soon emerge as an important environmental treatment.

PAHs are poorly attenuated during phytoremediation because of their low bioavailability in soil, but surfactant-enhanced remediation (SER) is an efficient environmental remediation technique that enhances PAH aqueous solubility, thus increasing its bioavailability (Liang et al. 2017; Lu et al. 2019). Surfactants can increase PAH desorption from soils, transforming them to the aqueous phase and thereby increasing their bioavailability (Wei et al. 2018). It is reported that 100 mg/kg of the non-ionic surfactant Tween 80 can increase pyrene removal by 130% (Cheng et al. 2008). Also, a mixture of the surfactants sodium dodecylbenzene sulfonate and Tween 80 (< 150 mg/kg) enhanced PAH bioremediation efficiency and reduced the surfactant dosage compared with using individual surfactants alone (Wu and Hu 2014).

Although SER can remove PAHs from contaminated soil, it just transfers the PAHs from the non-aqueous phase to the aqueous phase but does not eliminate their toxicity. Meanwhile, nano-SiO_2 increases soil bulk while increasing its own bioavailability. However, no work has been done on the effects of nano-SiO_2, combined with surfactant, on plants under PAH stress. Therefore, using *Erigeron annuus* (L.) Pers. combined with Triton X-100 and nano-SiO_2, we investigated that the combination's ability to remove PAHs from soil. *E. annuus* was selected because of its high sensitivity to organic contaminants (Fu et al. 2016). Our goal was to estimate the efficiency potential of this innovative phytoremediation technology.

Materials and methods

Plant collection and soil properties

E. annuus plants were collected from the Botanical Garden of China Three Gorges University, Yichang, China. After washing the roots, the plants were pre-cultured for 3 days, and then, healthy plants with similar growth were saved for the experiment.

Soil uncontaminated with PAHs was collected from Cuiping Mountain at China Three Gorges University, air dried, and passed through a 2-mm sieve to remove stones and roots. The soil had 2.54 g kg^{-1} organic matter content, pH 7.67, and 7.23 mol kg^{-1} cation exchange capacity. The nutrient levels were 0.34 g kg^{-1} of total N and 0.41 g kg^{-1} of total P, and it was a sandy loam composed of 35% sand and 65% silt.

Preparation of contaminated soil

To create PAH-contaminated soil, a portion of the uncontaminated soil was spiked with high purity, analytical reagent phenanthrene (Sigma, St. Louis, MO, USA) in 10% acetone to produce soil with 150 mg kg^{-1} phenanthrene. Once the acetone had evaporated, the spiked soil was mixed with uncontaminated soil, and the entire soil mixture was passed through a 2-mm sieve to homogenize it (Cheema et al. 2009). Phenanthrene concentration in the experimental soil was 134.1 ± 2.1 mg kg^{-1}.

Experimental planting design

Spiked soil (2 kg DW) was added to fifteen 20-cm diameter plastic pots, and then, 3 *E. annuus* plants were sown in each pot, and each treatment was assigned 5 pots. The treatments were (1) T0S0, planted soil with phenanthrene; (2) T1S0, planted soil with 1000 mg kg^{-1} Triton X-100 and phenanthrene; (3) T0S1, planted soil with 500 mg kg^{-1} nano-SiO_2 and phenanthrene; (4) T1S1, planted soil with 500 mg kg^{-1} nano-SiO_2, 1000 mg kg^{-1} Triton X-100, and phenanthrene; and (5) CK, planted control with no additions (Table 1). Nano-SiO_2 and Triton X-100 were both analytical grade (Sigma, St. Louis, MO, USA). Each treatment was performed in triplicate and maintained at 60% moisture content. This experiment was conducted outdoors, and environmental factors may vary with locations. Thus, pots were shuffled randomly once a week to reduce the environmental error. After 60 days, the chlorophyll in plant leaves was first measured, and then, the plants were collected, separated into shoots and roots, dried, and weighed. After plant collection, rhizosphere and non-rhizosphere soil samples were collected and stored at − 40 °C until analysis (Gao et al. 2013).

Phenanthrene extraction and analysis

Soil samples from each pot were tested both at the beginning and at the end of the 60-day experiment. They were freeze-dried and then passed through clean 100-mesh sieves prior to analysis. From each sample, 5 g of soil was placed in a 30-mL glass centrifuge tube, and 10 mL dichloromethane (DCM) and 100 μl decafluorobiphenyl were added. After 20 min of ultrasonic extraction, the samples were centrifuged for 10 min at 2000 rpm, and the supernatant was filtered through a sand core funnel containing 5 g anhydrous sodium sulfate and into a round-bottom flask. We repeated this extraction procedure 3 times. Extracted solvents were each concentrated, purified

Table 1 Experimental set-up for phenanthrene phytoremediation tests

Sample ID	Phenanthrene (mg/kg)	Nano-SiO_2 (mg/kg)	TritonX-100 (mg/kg)
CK	0	0	0
T0S0	150	0	0
T1S0	150	0	1000
T0S1	150	500	0
T1S1	150	500	1000

through column chromatography, eluted using 25 mL n-hexane/DCM (1:1, v/v), and placed in a rotary evaporator until diluted to 1 mL. Then, we added 3 mL acetonitrile and spin evaporated each sample again to 1 mL. The concentrate was washed 3 times with about 3 mL acetonitrile, concentrated to about 1 mL, and then filtered through a 0.22-μm microporous membrane. It was then run through HPLC (Waters Corp., Milford, MA, USA) to identify phenanthrene (Xi et al. 2019). Recovery rates were 82.1–103.1%. Finally, we calculated phenanthrene removal rates in the soil using

$$\text{Phenanthrene removal rate }(\%) = \frac{\text{Initial concentration}-\text{Final concentration}}{\text{Initial concentration}} \times 100$$

Enzyme activity and chlorophyll amount determination

The complex enzyme polyphenol oxidase (PPO) is secreted by roots and microbes in the soil and released by the decomposition of animal and plant residues. Peroxidase (POD) is a free radical scavenger that utilizes hydrogen peroxide (H_2O_2) as a substrate during the cross-linking of mono- and dilignols. PAH stress can induce POD activity, thus accommodating lignin biosynthesis and other stress response pathways (Strycharz and Shetty 2002). We determined POD and PPO activities by using methods developed by Guan (1986). To each 1 g soil sample, we added 10 mL of 1.0% pyrogallol, incubated the mixture at 30 °C for 2 h and then added 4.0 mL citric acid-phosphoric acid buffer (pH = 4.5) to stop the reaction, and finally added 35 mL ether. The mixture was extracted for 30 min; then, the concentration of colored ether with dissolved purple gallic prime was determined using colorimetric analysis (wavelength 430 nm). To determine POD activity, we first added 10 mL 1.0% pyrogallol and 2.0 mL 0.5% H_2O_2 to each 1 g soil sample. That reaction mixture was incubated at 30 °C for 2 h, and then, 4.0 mL citric acid-phosphoric acid buffer (pH = 4.5) was added to the solution to stop the reaction, and then, 35 mL of ether was added. The mixture was extracted for 30 min, and then, we used colorimetric analysis (wavelength 430 nm) to determine the concentration of colored ether with dissolved purple gallic prime. As controls, mixtures without soil and without pyrogallol were also analyzed. All tests were duplicated.

Chlorophyll content and plant analyses

For each sample, freshly crushed leaves (0.1 g) and small amounts of 80% acetone, calcium carbonate, and quartz sand were ground in a mortar, and then, the homogenate was transferred to a centrifuge tube. The mortar was then washed with enough 80% acetone to loosen the residue and that mixture was then added to the homogenate to yield a total of 10 mL in the centrifuge tube. The tubes were then soaked in the dark for 24 h. They were then spun at 2000 rpm for 10 min; then, the supernatant was removed for analysis using a spectrophotometer (HITACHI U3900, Japan) with absorbance values of 663 and 645 nm. (Lichtenthaler and Alan 1985).

Each whole plant was washed with deionized water, cut into aboveground and underground parts with scissors, and placed in separate plastic bags. The underground parts were washed, examined using a root analyzer (MICROTEK MRS—9600TFU2L, Shanghai Zhongjing Technology Co., Ltd., China), and then weighed to determine DW. The above ground parts were dried and then weighed to obtain DW.

Statistical analysis

Excel was used for data compilation and statistical analyses. Statistical significance was determined by one-way ANOVA in SPSS 24 and expressed as mean (SD). Differences were considered significant at $P < 0.05$.

Results and discussion

Biomass yields

After 60 days of growth, plants grown in soil containing 150 mg kg^{-1} phenanthrene produced significantly less biomass than those grown in uncontaminated soils (CK, $P < 0.05$, Fig. 1). Specifically, plants grown in contaminated soil without Triton X-100 and nano-SiO_2 had 66.0% less root DW and 58.6% less shoot (aboveground plant) DW than plants grown with both mediation treatments. Plants grown with either Triton X-100 or nano-SiO_2 had root DW decreases to 30.2 and 26.4%, respectively, and shoot DW decreases to 39.5 and 35.0%, respectively, compared with the control plants. However, when both Triton X-100 and nano-SiO_2 were added, root DW decreased by 7.6% and shoot dry weight by 22.3%.

PAH contamination adversely affects plant water and nutrient uptake, thus decreasing biomass yield (Afegbua and Batty 2018). Additionally, plants grown in nutrient media containing a high concentration of Cr had greatly improved growth when Si and Se were added to the media, compared with plants grown in that media with no added Si or Se (Huda

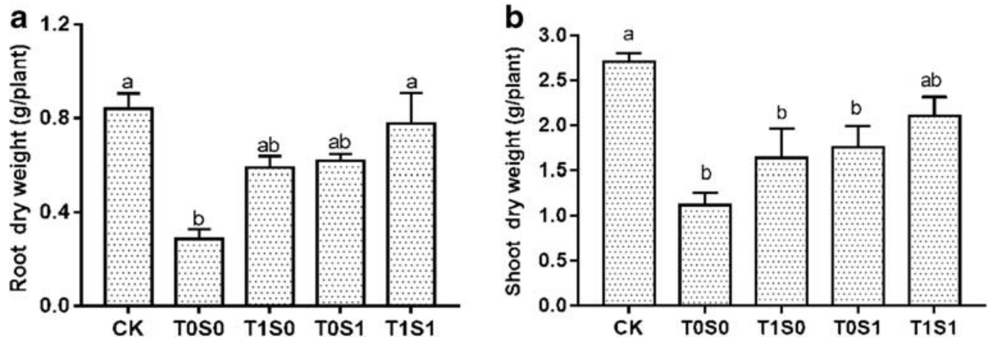

Fig. 1 Biomass production of *E. annuus* (**a**) roots and (**b**) above ground parts (shoots) after 60 days of growth. Each value is the mean (SD) of 3 replicates. See Table 1 for treatment definitions. Columns with different letters were significantly different ($P < 0.05$)

et al. 2017). Similar results have shown that Si is important for plants growing under Cd stress (Tang et al. 2015). In our study, the addition of Si and Triton X-100 helped alleviate plant stress caused by PAHs. Surfactants not only enhanced the aqueous solubility of PAHs, they also increased PAH bioavailability, thus helping increase plant tissue biomass, as a study reported in their research examining increasing levels of Triton X-100 on pyrene-contaminated soils planted with tall fescue (Cheema et al. 2016). Our study results indicated that exposure to phenanthrene decreased *E. annuus* biomass, likely due to phenanthrene toxicity; but, both the stress from phenanthrene and plant biomass losses can be slowed down by adding SiO_2 combined with Triton X-100.

Root analysis

All measures of root production (root length, root volume, number of root tips, and root surface area) of *E. annuus* grown for 60 days in contaminated soil with no other treatment were significantly reduced compared with control plants grown without pollution (Fig. 2). The root length of plants grown in contaminated soil without Triton X-100 and nano-SiO_2 was significantly less (66.9%) than that of control plants. However, when Triton X-100 and nano-SiO_2 were added, root length decreased by 28.3 and 16.2%, respectively, and decreased by 7.2% for plants treated with both Triton X-100 and nano-SiO_2 (Fig. 2a). Compared with the control plants, root volume decreased 63.8% in plants grown in contaminated soil without Triton X-100 and nano-SiO_2 but decreased by 35.8 and 24.7% when Triton X-100 or nano-SiO_2, respectively, were added (Fig. 2b). When both Triton X-100 and nano-SiO_2 were added, the root volume decreased by 14.6%. The number of root tips and root surface area treatments had proportionally similar outcomes (Fig. 2c, d). Plants grown in contaminated soil without Triton X-100 and nano-SiO_2 had 73.9% fewer root tips and 74.1% less root surface area than control plants had. Adding either Triton X-100 or nano-SiO_2 to polluted soil revealed that root tip number decreases to 43.6 and 25.9%, respectively, and root surface area decreases to 38.6 and 17.6%, respectively. The root tip surface areas of plants grown with both Triton X-100 and nano-SiO_2 added to contaminated soil decreased by 11.7%.

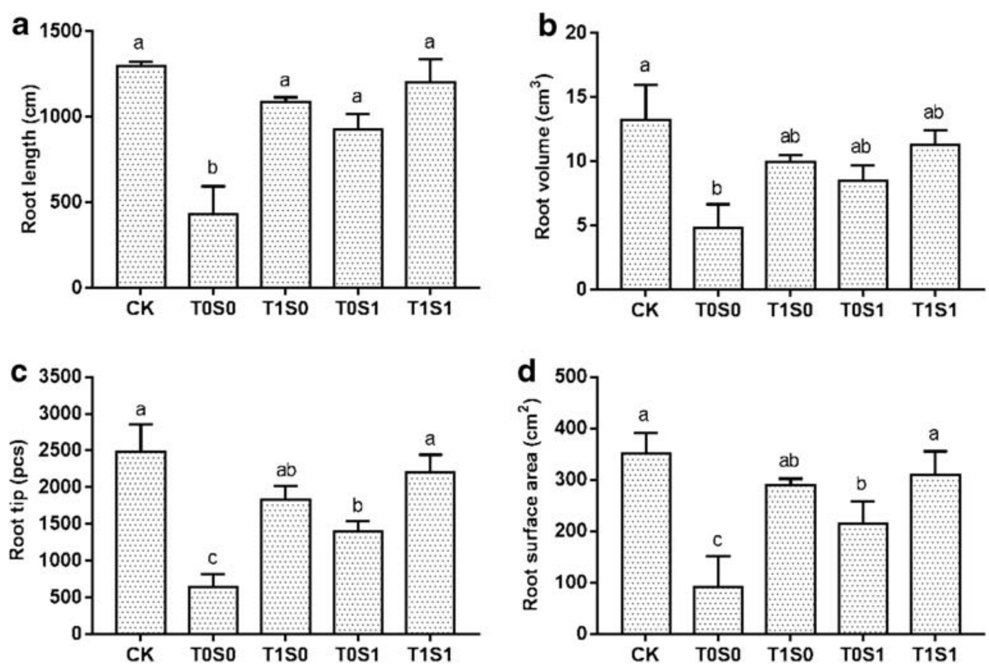

Fig. 2 Changes in (**a**) root length, (**b**) root volume, (**c**) number of root tips, and (**d**) root surface area of *E. annuus* after 60 days of growth. See Table 1 for treatment definitions. Each value is the mean (SD) of 3 replicates. Columns with different letters were significantly different ($P < 0.05$)

Grown in soil under abiotic stress, the first plant part to experience that stress is the roots, and the plant can adapt to environmental stress by changing the morphology and distribution of the root system (Liu et al. 2003). In this study, root length, root volume, number of root tips, and root surface area of *E. annuus* all decreased significantly in plants grown with phenanthrene. The addition of Si and Triton X-100 to such polluted soils helped to significantly alleviate that stress.

Chlorophyll amounts

After 60 days of growth, both chlorophyll a and chlorophyll b amounts in plants grown in contaminated soil with added Triton X-100 and nano-SiO_2 together did not differ significantly from those of control plants (Fig. 3). Chlorophyll a and b declined by only 9.1 and 13.1%, respectively, in those groups. However, chlorophyll a decreased by 26.4, 27.5, and 17.2% and chlorophyll b decreased by 30.6, 32.7, and 28% in the untreated and the Triton X-100 or nano-SiO_2 treated plants, respectively, compared with the control amounts. Those differences were significant for the untreated and Triton X-100 and nano-SiO_2 groups for chlorophyll a, but chlorophyll b amounts for all 3 of those groups were significantly different from the control amounts.

Photosynthesis is one of the most important basic physiological activities for most plants because it provides the material and energy needed for plant growth and development by using the light energy absorbed by chlorophyll (Yang et al. 2015). In this study, phenanthrene stress led to decreases in chlorophyll content, an effect possibly caused by the destruction of chloroplast structure and function, thus reducing the transfer and absorption of light energy needed for photosynthesis (Haritash and Kaushik 2009). Meanwhile, that stress also impeded the absorption of nutrients and water by plant roots and reduced the ability of pigment synthesis (Reilley et al. 1996). In our treatments with added Triton X-100 and nano-SiO_2, chlorophyll amounts were not significantly different than those of the control plants, indicating that the combined treatment allowed adequate light energy transfer and absorption capacity to maintain high enough photosynthetic rates to ensure normal growth.

Enzyme activity

Both rhizosphere and non-rhizosphere soils treated with both nano-SiO_2 and Triton X-100 experienced significantly increased PPO activity, compared with the activity in the control soils, after 60 days of plant growth (Fig. 4a). But, PPO activity in both soils under the other treatments was not significantly different than the activity in the control groups. There were some monitored changes in the activity of POD after 60 days of growth (Fig. 4b). There was no significant difference in POD activity in both soils treated with either nano-SiO_2 or Triton X-100 and both control group soils, but POD activity did increase significantly in both soils treated with nano-SiO_2 combined with Triton X-100.

PPO, one of the most important oxidoreductases in soil, is involved in transforming aromatic organic compounds in humus components (Liu et al. 2018). In our study, adding Triton X-100 did not significantly affect PPO activity compared with untreated soil, but its activity increased markedly when Triton X-100 combined with nano-SiO_2 or nano-SiO_2 alone was added to the soils (Fig. 4a). Since adding Triton X-100 and nano-SiO_2 together significantly increased PPO activity in both soils, they also likely promote PAH degradation. While Triton X-100 promotes microbial growth by transferring PAHs from the non-aqueous phase to the aqueous phase, thus increasing PPO activity, nano-SiO_2 increases soil bulk, thus increasing PAH bioavailability.

The free radical scavenger, POD, utilizes H_2O_2 as a substrate for mono- and dilignol cross-linking. PAH stress can induce POD activity to accommodate lignin biosynthesis and other stress response pathways (Liu et al. 2014). In our experiment, POD activity significantly increased in the nano-SiO_2 with Triton X-100 soil treatments, compared with POD activity in the controls. This indicated that the double treatment may alleviate PAH phytotoxicity because as H_2O_2 is formed in the soil, the oxygen content in other organic peroxides increases, thereby leading to enhanced POD synthesis in the soil.

PAH degradation

Phenanthrene levels measured in polluted soils at the end of this experiment showed that phenanthrene concentrations had decreased more in the treated rhizosphere and non-

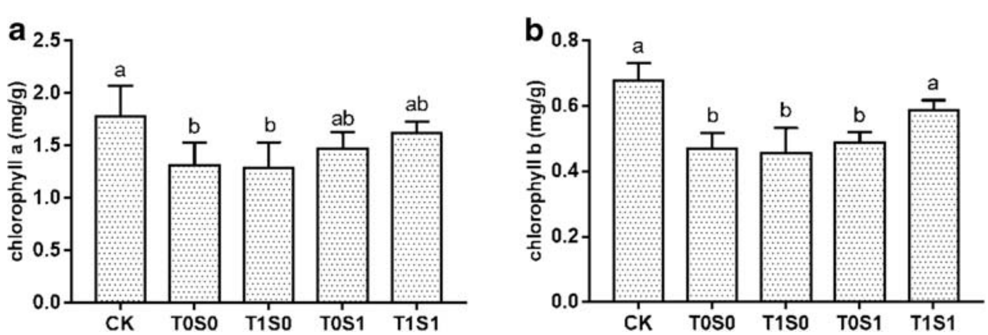

Fig. 3 Amounts of (**a**) chlorophyll a and (**b**) chlorophyll b of *E. annuus* after 60 d of growth. Each value is the mean (SD) of 3 replicates. See Table 1 for treatment definitions. Columns with different letters were significantly different ($P < 0.05$)

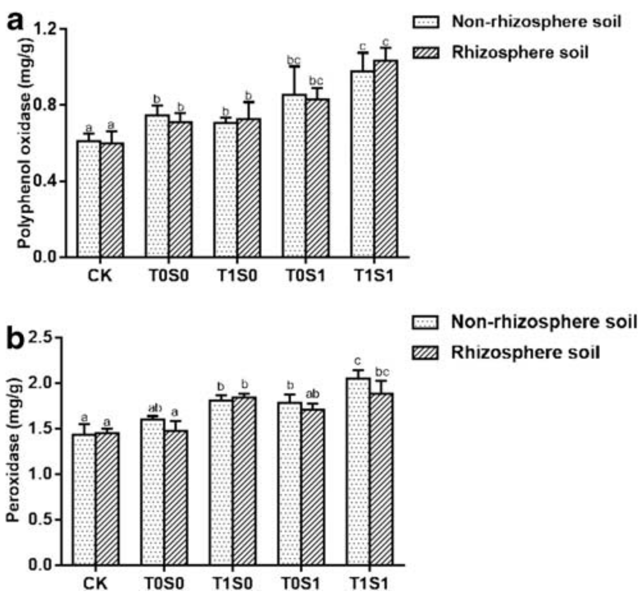

Fig. 4 Changes in (**a**) polyphenol oxidase and (**b**) peroxidase activities in rhizosphere soil and in non-rhizosphere soil after 60 d of plant growth. Each value is the mean (SD) of 3 replicates. See Table 1 for treatment definitions. Columns with different letters were significantly different ($P < 0.05$)

rhizosphere soils compared with the untreated soils. Without both Triton X-100 and nano-SiO_2, phenanthrene concentrations decreased by 44.9 and 53.7% in the non-rhizosphere and rhizosphere soils, respectively (Fig. 5). When Triton X-100 and nano-SiO_2 were added, phenanthrene concentrations decreased by 75.1 and 79.9%, respectively, in the non-rhizosphere soil and by 62.5 and 67.3%, respectively, in the rhizosphere soil. Finally, when both Triton X-100 and nano-SiO_2 were added together, phenanthrene concentrations decreased by 87.6 and 89.3% in the non-rhizosphere and rhizosphere soils, respectively.

Many plants can remove PAHs from contaminated soils. For instance, sudangrass (*Sorghum* × *drummondii*) achieved a maximum removal of PAHs at a 98% dissipation rate after 20 days (Dominguez et al. 2019). Also, reductions of 58% and 48% were obtained in the total petroleum hydrocarbon and PAH concentrations for 90 days by *Helianthus annuus* L. in soil with 4 mg kg^{-1} of the rhamnolipid, respectively

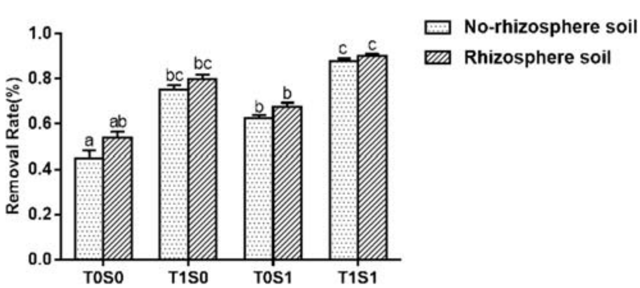

Fig. 5 Phenanthrene removal rates measured after a 60-day *E. annuus* growth period. Each value is the mean (SD) of 3 replicates. See Table 1 for treatment definitions. Columns with different letters were significantly different ($P < 0.05$)

(Liduino et al. 2018). The highest percentage of soil phenanthrene removal in our study occurred in treatments with Triton X-100 and nano-SiO_2 together. These results most likely occurred because Triton X-100 increased PAH desorption from the soils to the aqueous phase, thereby increasing its bioavailability, while nano-SiO_2 promoted plant growth by alleviating biotic and abiotic stresses.

Conclusion

This study demonstrated that nano-SiO_2 and Triton X-100 together provide protection for *E. annuus* exposed to phenanthrene. When cultivated in soil containing 150 mg kg^{-1} phenanthrene for 60 days, test plant biomasses and chlorophyll contents decreased significantly, while root length, root volume, numbers of root tips, and root surface area increased significantly. Addition of 1000 mg kg^{-1} Triton X-100 and 500 mg kg^{-1} nano-SiO_2 to phenanthrene-spiked soil ameliorated all of those stress indicators and promoted PPO and POD activity in the experimental soil. These results demonstrate that nano-SiO_2 and Triton X-100 in soil enhance plant tolerance to the toxic effects of PAHs, thus supporting their use in both the phytoremediation of PAH-contaminated soils and in ecological remediation. Moreover, nano-SiO_2 performs as a potentially valuable substrate for the design of new, more effective fertilizers designed to minimize the degradation of plants grown in contaminated soil.

Funding information This work was supported by the Natural Science Foundation for Innovation Group of Hubei Province, China (No: 2015CFA021), National Key Research Program (2016YFD08009022), and Open Projects Fund of Engineering Research Center of Hubei Agricultural Environment Monitoring (201606, 201607).

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