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Enhanced Bioremediation of Field Agricultural Soils Contaminated with PAHs and OCPs

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ABSTRACT: Effect of different concentrations of ammonium chloride (NCL) and ammonium nitrate (NN) and surfactants such as Tween-80, HPCD and rhamnolipids on the bioremediation of PAHs and OCPs was investigated. The results showed that the best optimum concentration of NCL and NN was 50 g·kg^{-1}, and the PAHs degradation rate during 60 days of remediation was enhanced to 64.6% and 62.8% for NCL and NN, respectively, which was approximately 45, 17, 10% higher than those in the control group, soil only added microorganism, soil added microorganism and tourmaline. OCP removal rates were 51.7% and 50.4 % for NCL and NN, respectively, which was 34% higher than the control group, 14% higher than the soil only added microorganism, 9% higher than the soil added microorganism and tourmaline. The best dose of Tween-80, HPCD and rhamnolipids was 2, 0.5 and 0.2 g·kg^{-1}. When they were applied, the PAHs degradation rate during 60 days of remediation was enhanced to 69, 70.4 and 71.5%, respectively, which was approximately 52, 24 and 17% than those in the control group, soil only added microorganism, soil added microorganism and tourmaline. Similarly, OCP removal rate was 42, 26 and 16% higher than those in the control group, microorganism -added soil alone, soil added microorganism and tourmaline. Additionally, nutrients and surfactants can promote the generation of soil hydrogen peroxidase and invertase enzyme. Hence, the present study provides a promising combination remediation technology for the remediation of field soils contaminated by PAHs and OCPs.

Key words: Nutrients, Surfactants, Tourmaline, Soil, Remediation

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) and organochlorine pesticides (OCPs) are typical persistent organic pollutants (POPs). Because of their lipophilic nature, the PAHs and OCPs are easily adsorbed onto suspended particles, making soil a predominant repository for environmental POPs (Cai *et al.,* 2007). Bioremediation is a promising option to remove PAHs from the environment or convert them to less harmful compounds. One of the main challenges in bioremediation of POPs is the limitation on mass transfer due to the strong hydrophobicity and low water solubility of these compounds. Soil matrix is a major reservoir for POPs. PAHs and OCPs in soil can be strongly sorbed to soil organic matter (SOM), encapsulated in soil mineral, and can also be present in dense non-aqueous phase liquid, which makes the remediation process difficult (Ogbonnaya, and Semple, 2013). In addition, microorganisms such as bacteria, fungi and microalgae play a key role in POPs removal through in bioremediation processes (Nikolova and Nenov, 2005). POP pollutants act as carbon source for microorganisms. However, macro nutrients (nitrogen and phosphorus), micronutrients (Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}, co-factors such as heavy metals), electron acceptor (oxygen is the electron acceptor for aerobic metabolism and nitrate, sulfate, ferric, manganese and carbon dioxide in anaerobic processes) were barriers in bioremediation process (Field, 2002; Jahn *et al.,* 2002; Villatoro-Monzon *et al.,* 2003; Farhadian *et al*., 2008). Therefore, to overcome the drawbacks, it is important to develop a new remediation technology with high degradation efficiency and use in engineering practice. Tourmaline is a complex borosilicate mineral with an intricate chemical composition. Tourmaline is capable

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of radiating far infrared energy, producing an electrostatic field, releasing rare micronutrients (Wang *et al.,* 2012) and stimulating the growth and metabolism of microorganisms (Ni *et al.,* 2008; Zhang *et al.,* 2011). Therefore, we assumed that tourmaline can overcome the shortcomings of bioremediation and assist microorganisms with remediating the soil contaminated with POPs. Additionally, nutrients are believed to be among the most important factors limiting the biodegradation rate. Nutrient- bioremediation has been considered as a potential remediation technique for soil contaminated with hydrophobic organic compounds (HOCs) such as polycyclic aromatic hydrocarbons (PAHs) and organochlorine pesticides (OCPs). Ammonium and nitrate are common nitrogen sources for bioremediation. However, the effects of N-addition on pollutant degradation in soil are conflicting. Thus, this optimal nutrient dose was necessary for bioremediation. In addition, publications related to the effect of s nitrogen sources on soil enzyme activities during the microbial degradation of HOCs in soil are few. Surfactant-enhanced bioremediation has been considered as an effective remediation technique for soil contaminated with PAHs and OCPs. To date, most studies focused on the interactions between surfactants and PAHs, but few studies have suggested the effect of surfactants on soil enzyme activities during the biodegradation of HOCs.

The present study was to develop a new technology that employs a combination of nutrients, surfactants and white rot fungus (*P. chrysosporium)* and tourmaline to remediate soil contaminated with PAHs and OCPs. Meanwhile, the different nutrients and surfactants were selected to investigate their effects on PAH and OCP biodegradation and soil microbial activities during remediation of the PAH- and OCP- contaminated soil.

MATERIALS & METHODS

The agricultural soil tested in this study was obtained from the top layer (0-10 cm) of the river bank at the sewage outfall of the Dagu Drainage River bank (117°12'11.49" W, 38°57'36.20"N) of Tianjin, China. The pH of the soil was measured with a pH meter (310P-02, Thermo-Orion, USA) in a 1:2.5 (w/w) soil-$CaCl_2$ water suspension. The organic matter content was determined by the potassium dichromate-outside heating method. Particle size distributions were determined by a particle size analyzer (BT-90, Dandong Bettersize Instrument Ltd, China). The soil texture was determined to be silt clay with the following composition: 33.8% clay, 46.4% silt, and 19.8% sand. The soil pH (1:2 soil/water by wet weight) was 7.45, and the organic carbon content was 2.23%. The cation exchange capacity (CEC), measured by the barium chloride method, was 8.28 Cmol/kg. Iron-rich black tourmaline was produced in the Xinjiang Province, China. The tourmaline was processed to a particle size of 800 nm by Hongyan Mineral Products Co., Ltd., Tianjin City, China. The chemical composition of the tourmaline was seen Wang *et al*. (2014) report. *P. chrysosporium* (collection number: 5.776) was obtained from the Institute of Microbiology, Chinese Academy of Science and was maintained on potato dextrose agar (PDA) slants at 4 °C. A mixture of 16 U.S. Environmental Protection Agency (EPA) priority PAH stock standards was purchased from J&K Chemical (USA) A mixed standard solution of 16 OCPs was obtained from Accustandard, Inc., USA. Other surrogates or internal standards were purchased from J&K Acros, USA. Analytical grade *n*-hexane and dichloromethane were obtained from Tianjin Reagent, China. The soil was remediated in the laboratory using tourmaline (T) alone, *P. chrysosporium* (P) alone, P combined with T, and nutrient enhanced P combined with T. A control treatment (C) with no T or P added was also prepared. Each treatment was carried out in triplicate. The mixtures were carefully homogenized. For soil remediation using P combined with T, three aliquots of soil samples (500 g) were placed into 1.5-L brown wide-mouth bottles, and 25 mg of tourmaline and a 240 mL fungal suspension (0.016 g/mL) were added. The detailed methods on remediation were seen the reference (Zhou, 1987). For soil remediation using nutrients and surfactants enhanced P combined with T, as summarized in Table 1, the doses of 2, 10 and 50 nutrient /kg soil were added the above remediation soil at the initial experiment, respectively. For soil remediation using surfactants enhanced P combined with T, the doses of Tween-80 (0.4-10 g/kg soil), hydroxypropyl-p-cyclodextrin (HPCD, 0.5-50 g/kg soil) and rhamnolipid (0.2-5 g/kg soil) added the above remediation soil at the initial experiment were seen Table 1, respectively.

The soil enzyme activity was measured as previously described in the literature (Zhou, 1987; Wang *et al.,* 2014)). Extraction and fractionation of the PAHs and OCPs were seen the report (Wang *et al.,* 2014). In brief, the soil was extracted with dichloromethane in a Soxhlet extractor for 48 h. The fractionation of the PAHs and OCPs used 1:2 3% deactivated neutral alumina/30% deactivated silica gel glass column. The PAH and OCP fractions were analyzed by GC-MS on an Agilent Packard HP 6890 series II gas chromatography system fitted with an HP 5975, which was used in the selective ion monitoring (SIM) mode or in the scanning mode. A J&W Scientific DB-5MS fused-silica capillary column (30 m × 0.25 mm i.d. × 0.25 μm film thickness) was used.

RESULTS & DISCUSSION

Table S1 shows the PAH and OCP concentrations in the soil sampled from the DDR bank. The concentration of PAHs (n=16) was 6.35 ± 0.045 mg/kg. The concentration of PAHs containing 4-6 rings was higher (0.27-0.88 mg/kg) than the concentration of PAHs with 2–3 rings. Several OCPs that were targeted were not detected in the soil. Only heptachlor, aldrin, endrin, *p,p2* -DDE *p,p2* -DDD, and *o,p2* -DDT were detected (Table S1). The total concentration of these OCPs was 145.92 ± 1.92 mg/kg. To improve the bioremediation process, besides a competent microbe able to degrade the contaminant carbon source, other parameters must be taken into account e.g. oxygen and utilizable nitrogen sources (Liebeg and Cutright, 1999). Strategies involved bioaugmentation with the addition of seeded cultures and biostimulation with the addition of nutrients. Biostimulation increases the activity of indigenous populations by adding nutrients and/or a terminal electron acceptor (TEA) to enhance the populations already present at a site. Nitrogen is the nutrient most commonly added at bioremediation projects. It is primarily used for cellular growth (NH^+_4 or NO_3^-) and as an alternative electron acceptor (NO_3^-). The effect of two different N sources on the PAHs and OCPs degradation ability of each culture by *P. chrysosporium* was evaluated. Fig. 1 showed that when the addition dose of NH_4Cl and NH_4NO_3 ranged from 0.2, 1 to 50 g/kg, the removal rate of PAHs and OCPs increased with the dose increasing. For example, the addition of NH_4Cl and NH_4NO_3 was 50 g/kg, which accelerated the removal rate of PAHs and OCPs with largest percentages of 64.6 ± 2.6% and 62.8 ± 1.3% (Fig. 1a), and 51.7 ± 1.9% and 50.4 ± 1.9% (Fig. 1b) on the 60th day, respectively, while the removal rate of PAHs and OCPs using tourmaline and *P. chrysosporium* was 53.2 ± 4.7% and 43.5 ± 1.1%. Therefore, nutrients had dramatic effects on PAH and OCP biodegradation rate. This suggested that they might both be a useful nitrogen source for the bioremediation of PAHs and OCPs. Moreover, the biodegradation rate was influenced not only by the absolute amount of nutrients added but also by the types of nutrients.

The effects of surfactants on the dissolution kinetics of PAH and OCP from various matrices have also been reported. Surfactants are able to improve the mass-transfer of hydrophobic pollutants from a solid or non-aqueous liquid phase into the aqueous phase by decreasing the interfacial tension and by accumulating the hydrophobic compounds in the micelles. Figs 2a and b showed that addition of TW-80 under different concentrations (0.4, 2 and 10 g/kg) all improved the PAH and OCP removal rates. However, when the addition dose of Tween-80 ranged from 0.4, 2 to 10 g/kg soil, the removal rate of PAHs and OCPs did not increased with the dose increasing. For example, the addition of Tween-80 was 2 g/kg, which accelerated the removal rate of PAHs with largest percentages of 69.5 ± 0.6%, while OCPs reached the largest

Table 1. Remediation experimental design

Name	Abbreviation	Dose of nutrients and surfatants (g/kg soil)	*P. chrysosporium* (g/kg soil)	Tourmaline (g/kg soil)
NH_4Cl	NCL-1	2		
NH_4Cl	NCL-2	10		
NH_4Cl	NCL-3	50	7.68	20
NH_4NO_3	NN-1	2		
NH_4NO_3	NN-2	10		
NH4NO3	NN-3	50		
TW80	TW-1	0.4		
TW80	TW-2	2		
TW80	TW-3	10		
HPCD	HP-1	0.5		
HPCD	HP-2	5	7.68	20
HPCD	HP-3	50		
Rhamnolipids	R-1	0.2		
Rhamnolipids	R-2	1		
Rhamnolipids	R-3	5		

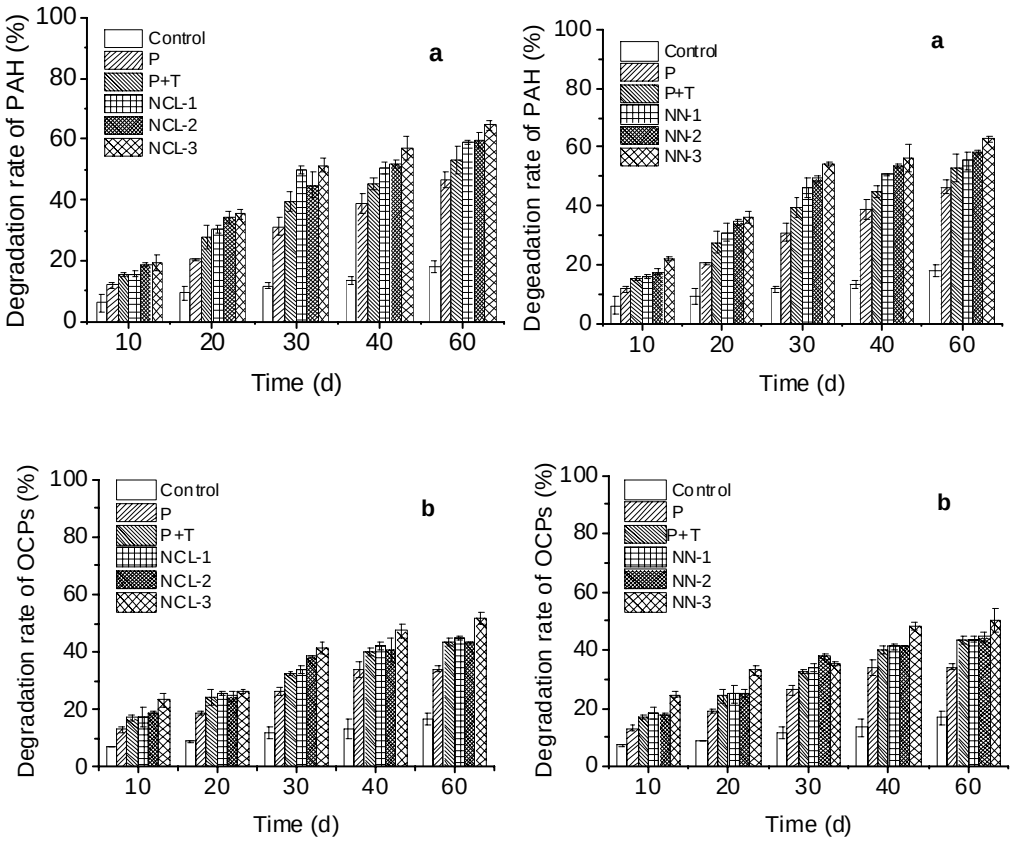

Fig. 1. Remediation percentage of PAH (a) and OCP (b) in the soils by *P. chrysosporium* (P), tourmaline combined with *P. chrysosporium* (T+P), three dose of NH_4Cl combined with T+P (NCL-1, NCL-2 and NCL-3), and three dose of NH_4NO_3 combined with T+P (NN-1, NN-2 and NN-3). The error bars indicate standard deviations.

percentages of 60.5 ± 3.4% at a TW-80 concentration was 0.4% g/kg soil (Fig. 2a and b) on the 60th day, respectively. The removal percentage of PAHs and OCPs was higher 16.3 ± 2.6%% and 17.0 ± 3.1% than those using tourmaline and *P. chrysosporium*. When HPCD was applied in the range of 0.5, 5 and 50 mg/kg soil, PAH and OCP removal percentage reached their highest values of 70.4 ± 3.1% and 61.4 ± 2.8% at 0.5 and 5 mg/kg soil of HPCD, respectively. For biosurfactant rhamnolipids at concentrations ranged from 0.2, 1 to 5 g/kg soil, PAH and OCP removal percentage increased with increasing the concentrations of rhamnolipids with the highest percentages of 71.5 ± 2.4% and 62.5 ± 3.7% (Figs. 2a and b).

Therefore, surfactants had obvious effects on PAH and OCP biodegradation rate. It might be because Tween-80, HPCD and rhamnolipids could improve the PAH and OCP degradation by *P. chrysosporium* through enhancing their bioavailability. Moreover, the biodegradation rate was influenced by the absolute amount of surfactants added. This may be attributed to the structure of surfactants, the shape and size of micelles, and their critical miceller concentration (CMC) values (Chun *et al*., 2002). Application of nutrient like organic manures and mineral fertilization caused an increase in the abundance of soil microorganisms and enzymatic activity (Balezentiene and Klimas, 2009). In the present study, effects of NH_4NO_3 and NH_4Cl on soil enzyme activities during the PAH and OCP degradation was investigated. Figure 2 showed that, at 20 day, all the hydrogen peroxidase and invertase enzymes exhibited the highest value for NH_4NO_3 and NH_4Cl (Figs. 3a and b) assisted the combination tourmaline and *P. chrysosporium* remediation treatment, their second high value in the combination tourmaline and *P. chrysosporium* remediation treatment, while the lowest activity was recorded in untreated control.

For example, maximum values of hydrogen peroxidase activities obtained on the 20th or 30th day were 13.3 ± 0.3, 19.2 ± 0.4, 19.8 ± 0.8, 25.9 ± 1.3, 30.2 ±

0.6, 29.1 ± 0.9 IU/g in the control soil, *P. chrysosporium* - added soil (P), tourmaline-added soil (T), *P. chrysosporium* and tourmaline added-soil (T+P), 2 g/kg soil of NH_4Cl and *P. chrysosporium* and tourmaline added-soil (NCl-1), 2 g/kg soil of NH_4NO_3 and *P. chrysosporium* added-soil (NN-1), respectively (Fig. 3a). Similarly, the invertase activities reached a maximum of 140.1 ± 6.2, 189.9 ± 12.2, 196.9 ± 4.2, 246.9 ± 13.0, 268.2 ± 2.4,and 274.8 ±8.6 IU/g in the control soil, P, T, T+P, 50 g/kg soil of NH_4Cl and *P. chrysosporium* and tourmaline added-soil (NCl-3), 50 g/kg soil of NH_4NO_3 and *P. chrysosporium* added-soil (NN-3), respectively

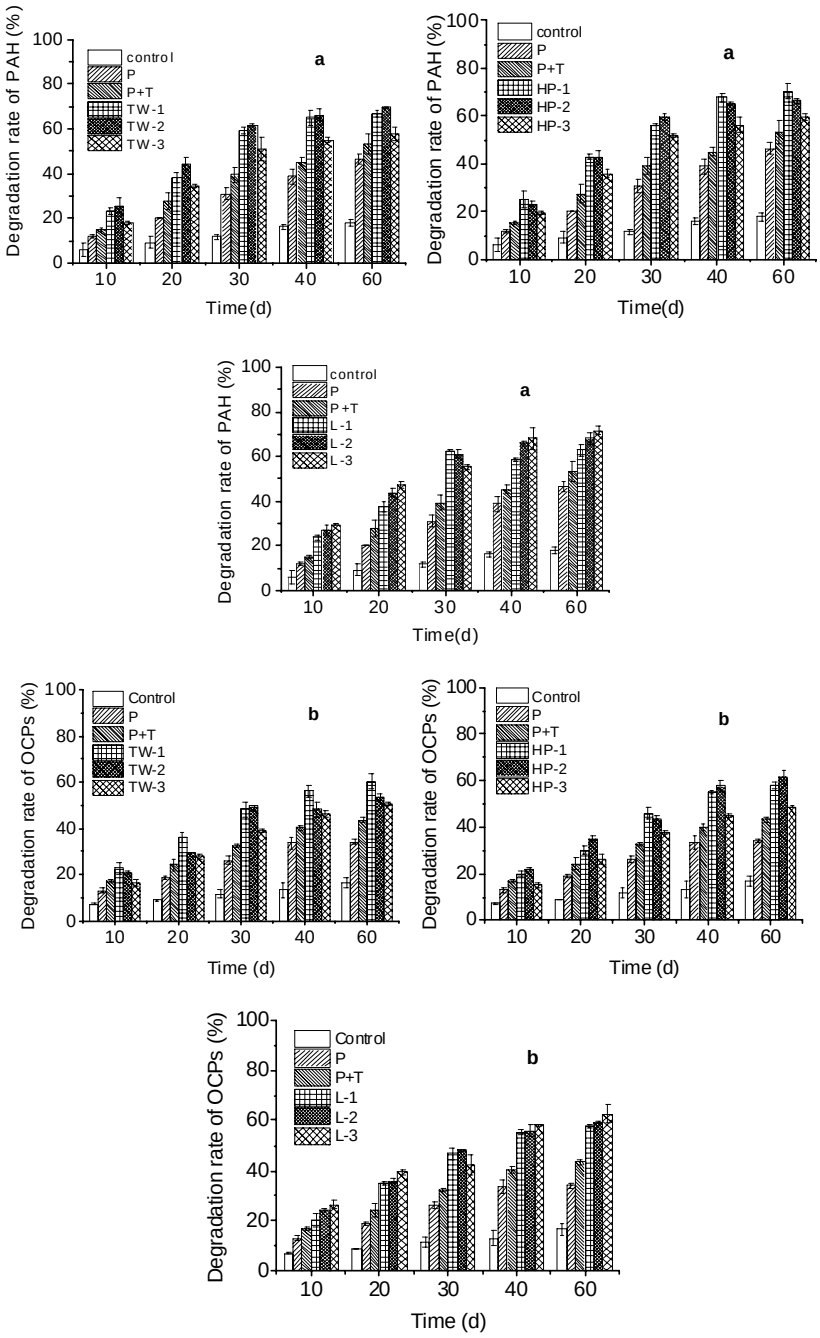

Fig. 2. Remediation percentage of PAH (a) and OCP (b) in the soils by *P. chrysosporium* (P), tourmaline combined with *P. chrysosporium* (T+P), three dose of Tween-80 combined with T+P (TW-1, TW-2 and TW-3), three dose of HPCD combined with T+P (HP-1, HP-2 and HP-3), and three dose of rhamnolipids combined with T+P (L-1, L-2 and L-3) . The error bars indicate standard deviations

(Fig. 3b). These values suggest that the addition of nutrients and tourmaline did not destroy the enzyme activity produced by microorganisms. Therefore, a useful nitrogen sources are in favor of the secretion of soil enzyme activities. Frankenberger and Dick (1983) noted that phosphodiesterase, arylsulfatase, invertase, α-galactosidase, and catalase activities were highly correlated with both microbial respiration and total biomass in soils. Soil enzyme activities are the direct expression of the soil community to metabolic requirements and available nutrients (Caldwell, 2005).

It was observed that the application of Tween-80, HPCD and rhamnolipids all enhanced the soil invertase and hydrogen peroxidase enzymes during the PAH and OCP degradation (Figs 4a and b). For example, the hydrogen peroxidase activities reached a maximum of 13.3 ± 0.4, 19.2 ± 0.5, 34.9 ± 1.1, 31.3 ± 2.2 and 34.4 ±0.3 IU/g in the control soil, P, T, *P.* T+P, 2 g/kg soil of Tween-80 and *P. chrysosporium* and tourmaline added-soil (TW-2), 0.5 g/kg soil of HPCD and *P. chrysosporium* added-soil (HP-1) and 0.2 g/kg soil of rhamnolipids and *P. chrysosporium* added-soil (L-3), respectively (Fig. 4a). However, when all Tween 80, HPCD and rhamnolipids were applied at other two concentrations, the enzyme values all decreased, respectively. Similarly, the soil invertase activities was always significantly increased by the presence of Tween-80, HPCD and rhamnolipids on 20th or 30th day of remediation, when it was compared with control, T/P and P+T treatment soil samples (Fig. 4b). Therefore, Tween-80, HPCD and rhamnolipids could supply an extra carbon source to improve the microbial enzyme secretion, which improve the PAH and OCP degradation by *P. chrysosporium*. Hadibarata and Tachibana (2010) also found that in the presence of TW-80, the growth of fungi increased rapidly. The biodegradability of TW-80 and its function as carbon source has already been demonstrated for bacteria in PAH bioremediation (Moscoso *et al.,* 2012).

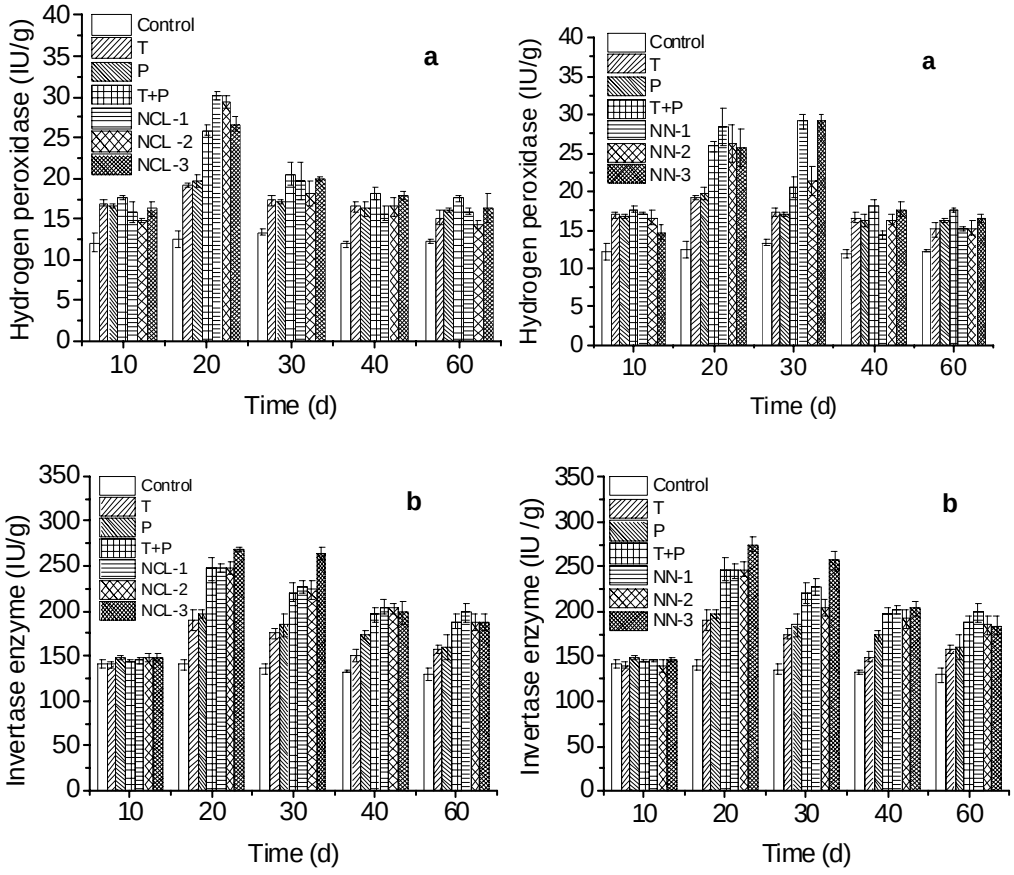

Fig. 3. Activity change time courses for hydrogen peroxidase (a) and invertase (b) enzymes during the PAH and OCP degradation processes in different treatments such as *P. chrysosporium* (P), tourmaline combined with *P. chrysosporium* (T+P), three dose of NH_4Cl combined with T+P (NCL-1, NCL-2 and NCL-3), and three dose of NH_4NO_3 combined with T+P (NN-1, NN-2 and NN-3).

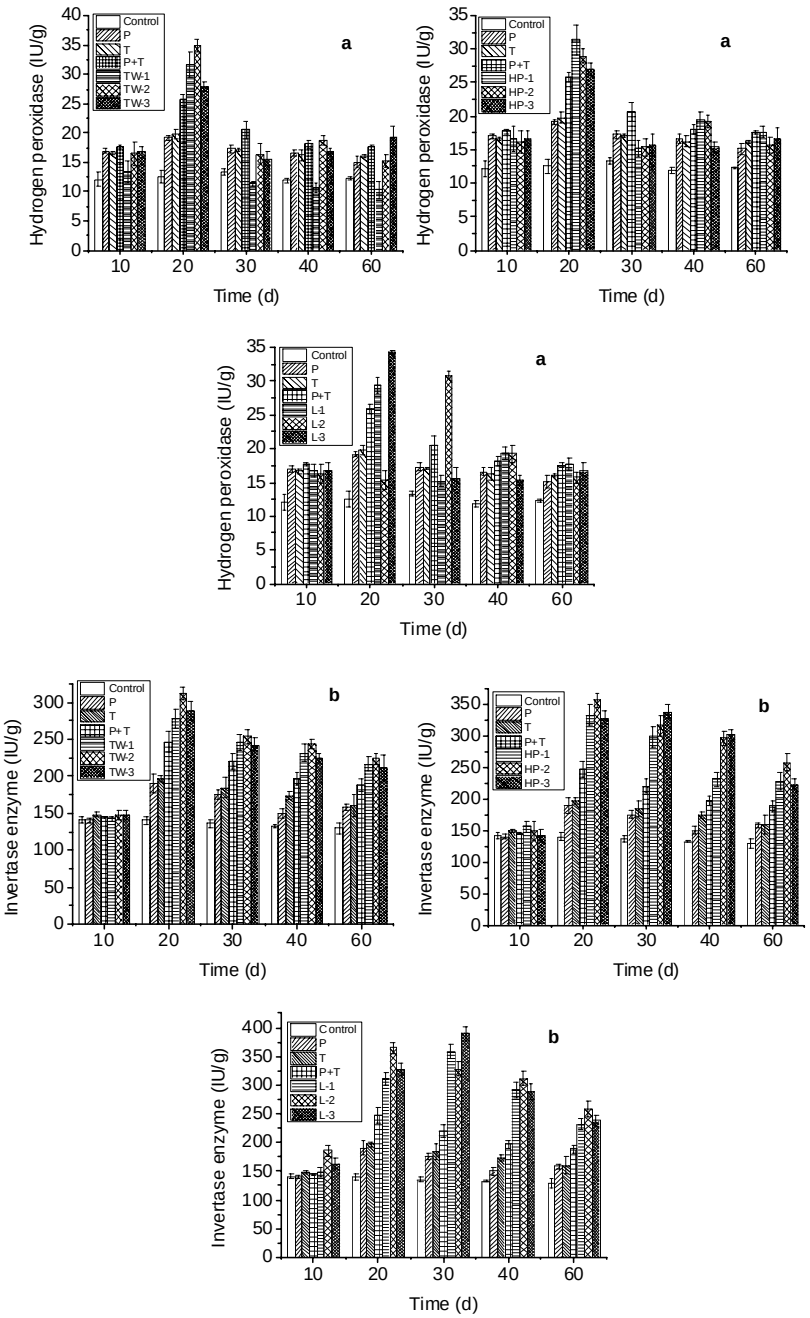

Fig. 4. Activity change time courses for hydrogen peroxidase (a) and invertase enzyme (b) during the PAH and OCP degradation processes in different treatments such as *P. chrysosporium* (P), tourmaline combined with *P. chrysosporium* (T+P), three dose of Tween-80 combined with T+P (TW-1, TW-2 and TW-3), three dose of HPCD combined with T+P (HP-1, HP-2 and HP-3), and three dose of rhamnolipids combined with T+P (L-1, L—2 and L-3) . The error bars indicate standard deviations

CONCLUSION

The present study has addressed the applicability of the remediation technology on the combination nutrients and tourmaline and microorganism to PAH and OCP biodegradation. The remediation rate of PAHs and OCPs using tourmaline and microorganism was influenced by not only the dose of nutrients added but also the type of nutrients. When the best optimum

concentration was 50 $g \cdot kg^{-1}$, the PAHs degradation rate during 60 days of remediation was enhanced to 64.6% and 62.8% for ammonium chloride (NCL) and ammonium nitrate (NN), respectively, which was approximately 45% higher than the control group, 17% higher than the soil only added microorganism. OCP removal rate was 34% higher than the control group, 14% higher than the microorganism -added soil alone. When the best optimum concentration was 2, 0.5 and 0.2 $g \cdot kg^{-1}$ for Tween-80, HPCD and rhamnolipids, the PAHs degradation rate during 60 days of remediation was enhanced to 69.5%, 70.4% and 71.5%, respectively, which was approximately 52% higher than the control group, 24% higher than the soil only added microorganism, 17% higher than the soil added microorganism and tourmaline. Similarly, OCP removal rate was 42% higher than the control group, 26% higher than the microorganism -added soil alone, 16% higher than the soil added microorganism and tourmaline. In addition, nutrients and surfactants can promote the generation of soil hydrogen peroxidase and invertase enzyme. Therefore, this study proved to be a potential combination remediation approach to achieve an efficient feasible decontamination.

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