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Growth response and phytoremediation ability of Reed for diesel contaminant

Jun Wang, Xiaoyan Liu*, Xinying Zhang, Xia Liang, Weijie Zhang

College of Environmental and chemical engineering, Shanghai University, Shanghai 200444, China

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Abstract

Oil spills may considerably damage sensitive coastal wetlands. In this open-air pot experiment, *Reed*, a dominant coastal marsh plant, was transplanted into soil contaminated with diesel at concentrations of 1 000, 5 000, 10 000, 15 000 and 20 000 mg diesel kg^{-1} dry soil. In order to better evaluate the phytoremediation potential and restoration of *Reed*, the chlorophyll content, root vitality, activity of peroxidase (POD), catalase (CAT), ascorbic acid oxidase (AAO) in plant tissue and the dissipation rate of diesel were investigated after 50 days of treatment at the levels mentioned above. The results showed that the activities of POD in root, CAT, and AAO in stem increased first, and declined at higher concentrations. Additionally, the increment of chlorophyll content and root vitality were observed, indicating that *Reed* was tolerant to diesel, especially when the concentrations of diesel was lower, which was also proved by the highest restoration effectiveness at the lower levels of diesel. Collectively, *Reed* is a potential plant which can be used for restoring the diesel-contaminated soil.

Keywords: Phytoremediation; Reed; diesel contaminant; wetland; pot experiment

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1. Introduction

Diesel is the one of the dominant energy sources to maintain the economic and social development, during the exploration, translation, what is unavoidable is oil spills, which has posed a serious risk to the ecosystem of coastal wetlands. So it is imperative to remedy, treat and control the diesel contaminant economically and efficiently. Due to its potentially lower cost and environmental friendly characteristics, phytoremediation has been recognized as a more advantageous technology for removal of organic pollutants from soil in comparison with physicochemical remediation technologies. Additionally,

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* Corresponding author: Xiaoyan Liu, PhD, Prof, Tel.:+86-21-66137767
E-mail address: Lxy999@shu.edu.cn

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phytoremediation may have a distinct advantage in wetlands because of the intrinsic capability of many wetland plants to aerate the rhizosphere, thus potentially increasing aerobic degradation of hydrocarbons [1,2]. This technology has been tentatively used for treating many types of organic contaminants including total petroleum hydrocarbons (TPH), polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCB), trinitrotoluene (TNT), and trichloroethylene (TCE) [1,3-6].

The mechanisms of phytoremediation systems comprise at least four pathways of reducing soil contaminants, such as abiotic losses (leachate, volatilization, photodegradation, irreversible sorption, chemical degradation and so on), indigenous microbial degradation, root tissues-enhanced dissipation and root-exudates-enhanced biodegradation [7-9]. Lin et al found that at 40 mg/g diesel dosage, concentrations of residual TPH in the sediment planted with *J. roemeranus* were significantly lower than those of unplanted sediments [1]. Goosegrass were found to have the ability in phytoremediation for petrol [10]. Ryegrass growth could make the residual rate of diesel oil in the rhizophere 55% lower than that in the corresponding root-free soil, and the threshold reduction occurred after the development of plant roots [11].

Reed is one of the dominant plant species of coastal wetland, and should be tolerant to weather and soil conditions in wetland. Apart from this, *Reed* could provide strong vitality and great root surface area, which is beneficial to phytoremediation. In this study, the antioxidant enzyme responses of *Reed* to diesel were investigated to better understand the tolerant mechanism of wetland plant species. Additionally, determination of its phytoremediation ability for diesel-contaminated soil is of great practical value for the restoration of wetland environment.

2. Materials and methods

2.1. Materials

The soils in this experiment which have no previous history of exposure to diesel fuel or other organic contaminants were collected from the open space in the east campus of Shanghai University. Prior to mixing, the components were air-dried for three days and homogenized with a soil grinder, then sieved to < 2 mm. Basic characteristics of the freshly uncontaminated soil are as follows: texture-Sand 32.2%, Silt 60.4%, Clay 7.4%; Organic matter 19.6 g/kg; pH 8.30.

Reed was transplanted into the pots from the shore of Huangpu river before the start of the experiment. Diesel fuel, 0#, used to pollute the soil was bought from Minghe petrol station of Sinopec in Baoshan district, Shanghai, China.

2.2. Pot experiment

Soils (2 500 g dry weight per pot) was packed into the pots (20 cm in diameter and 30 cm in depth), and diesel fuel at concentrations of 0, 1 000, 5 000, 10 000, 15 000 and 20 000 mg/kg soil were applied to the homogenized soil and mixed thoroughly, and then they were left for one week to ensure the evaporation of the unstable composition in diesel fuel. The plants in good growth condition with similar biomass were transplanted into the prepared pots, along with an unplanted control used to assess contaminant biodegradation in the absence of plant rhizosphere. Three replicates of each treatment were prepared. The experiment was conducted in the open air for 50 days.

Soil samples and plant samples were destructively sampled at the end of experiment. The former was got from the rhizosphere, and air-dried for 48 h and then hermetically kept under low temperature until assay; the latter was washed with distilled water, and blotted water with filter paper before weighed.

2.3. Analytical methods

Chlorophyll content was determined by the spectrophotometry[12]. Peroxidase (POD) activity was determined at 470nm using the guaiacol method [12]. Catalase (CAT) activity was tested by following the decrease of absorbance at 240nm using the ultraviolet absorbance method [13]. Ascorbic acid oxidase (AAO) activity was determined by iodometric method.

2 g air dried soil and 1 g anhydrous Na2SO4 were added into the centrifuge tube, followed by addition of 10 ml dichloromethane to extract diesel for 30 min under ultrasonication at 40℃, then the tubes were centrifuged at 3 500 r/min for 10min. This process was repeated three times. The supernatant was collected in a preweighed little flask to analyze the residual diesel gravimetrically by dried at low temperature.

2.4. statistical analyses

The data from the experiment were statistically processed on a computer using the Excel XP, SPSS 13.0. The confidence of data generated in the investigations has been analyzed by standard statistical method to determine the mean values and standard deviation (S.D).

3. Results and discussion

3.1. Effects on chlorophyll content

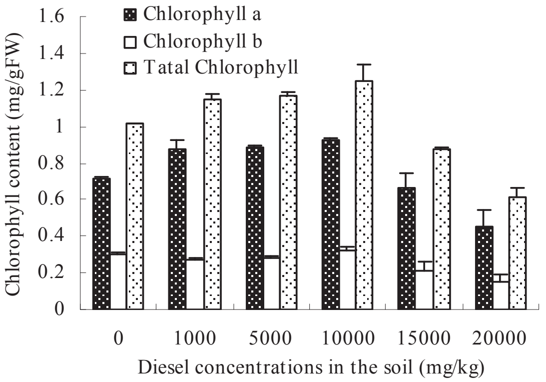

Fig. 1. Effect of diesel in the soil on the chlorophyll(a), chlorophyll(b) and total chlorophyll in leaf tissues of *Reed*. Error bars represent S.D.

During the growth of *Reed* in the whole experiment, no visible toxic responses to diesel of different concentrations were observed, and it appeared that vegetation establishment in diesel-contaminated soil is feasible with *Reed*. The contents of chlorophyll a and total chlorophyll increased with increasing concentrations of diesel treatment after 50d, and declined at higher concentrations (Fig. 1), and the peak values both appeared at the level of 10 000 mg/kg. Based on the control, Chlorophyll a increased by 22.4%, 23.9%, 29.9%, and total chlorophyll increased by 13.1%, 15.1% and 23.3%, corresponding to 1 000, 5 000, 10 000 mg diesel kg-1 soil respectively. It suggested that diesel in low concentrations might serve as nutrition to the plant's growth. However, no definite change patterns were observed on the content of chlorophyll b. Remarkably, when diesel concentrations exceeded 15 000 mg/kg soil, chlorophyll a, total chlorophyll, and chlorophyll b all decreased. Statistically significant differences were detected among 10 000, 15 000, and 20 000 mg/kg treatment levels ($p<0.01$).

The increment of chlorophyll content under lower concentrations of diesel indicated that to some extent diesel could enhance plants to absorb the material required to synthesize certain enzymes necessary. However, higher concentrations of diesel would restrain the synthesis of chlorophyll enzyme, thereby reducing the plants' chlorophyll content, photosynthesis and inhibiting the growth of plants [14].

3.2. Effects on root vitality

Roots are capable of producing plant hormones and consequently perturbations in the environment and thus root growth can influence plant development [15], so root vitality should be another indicator which can intuitively reflect the effect of contaminants on plant species. Figure 2 presents the variations of root vitality under different concentrations of diesel contaminants. After 50d of exposure to diesel, root vitality of *Reed* increased at first, and decreased later with the increase of pollutant, and the peak value (592.4 g/(gFW·h)) appeared at the level of 5 000 mg/kg, which approved the inference above that diesel in low concentration might serve as nutrition to the plants' growth. The decrement of root vitality at 10 000 mg/kg suggested that the treatment of 5 000 mg/kg might be the limit for *Reed* to tolerate diesel contamination.

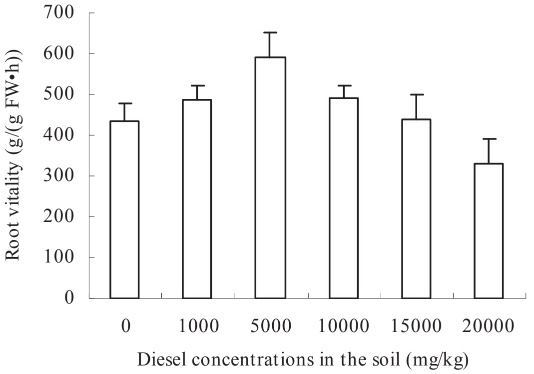

Fig. 2. Effect of diesel on soluble protein in root, stem and leaf of *Reed* after transplantation 50 days. Error bars represent S.D.

Statistically significant differences were observed between plants of different concentrations of diesel ($p < 0.05$).

Studies about the influence of flue oil on root systems are generally limited to data on the root biomass [16,17] and root morphological characteristics such as root length and root diameter of graminoids [18]. Lin et al found that the effect of fuel oil on below-ground biomass of *S.alterniflora* was considerably more severe, and when the concentration of fuel oil was up to 29 mg/g, the below-ground biomass consistently decreasing with increasing oil dosage thereafter [17]. In this experiment, the decrement of root vitality under the highest diesel stress proved the physiological toxicity of diesel to the root.

3.3. Effects of diesel on antioxidant enzymes

In order to better understand the tolerant mechanism of wetland plants, and approach the physiological responses to diesel, antioxidant enzyme activities in plant tissue were measured after 50d for diesel treatment (Fig. 3, Fig. 4 and Fig. 5).

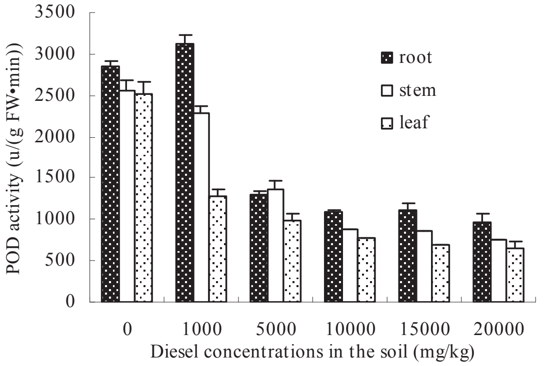

Fig. 3. Effect of diesel on POD activity in root, stem and leaf of *Reed* after transplantation 50 days. Error bars represent S.D.

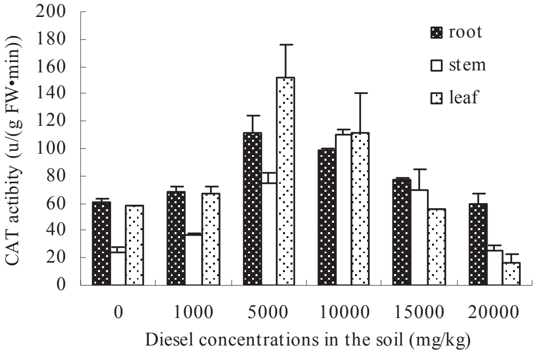

Fig. 4. Effect of diesel on CAT activity in root, stem and leaf of *Reed* after transplantation 50 days. Error bars represent S.D.

POD activities of stem and leaf were degraded by diesel treatments (Fig. 3) ($p<0.01$). Liu et al also found POD of Arabidopsis thaliana peak enzyme activities on 0.25 mM phenanthrene and declined at higher concentrations [19]. For root, POD activities fluctuated with the increasing concentrations. When the diesel concentration was 1 000 mg/kg soil, the POD activity of root was 3 128.8 u/(gFW·min), which was the highest value over the experiment. And the lowest POD activities of root, stem, and leaf all appeared on the highest concentration of diesel.

The effect of various levels of diesel on the activity of CAT was shown in Fig. 4 ($p<0.01$). The similar effects on different plant tissue were observed, they both increased at first, and decreased later, the peak values of appeared the treatments of 5 000 mg/kg, 10 000 mg/kg, and 15 000 mg/kg，respectively in the root, stem, and leaf.

AAO activity in root of *Reed* decreased with increasing concentration of diesel ($p<0.01$), and the highest value (0.605 mgFW/min) appeared on control (Fig. 6). While the AAO activities of the stem increased first, and then were descend with the increasing concentration. However, there were no statistically significant differences among the treatments ($p<0.05$). The enzyme variation trend did not appear definite regularity.

Statistically significant differences were detected among the three 1 000, 5 000, and 10 000 mg/kg soil treatment levels (p≈0.01).

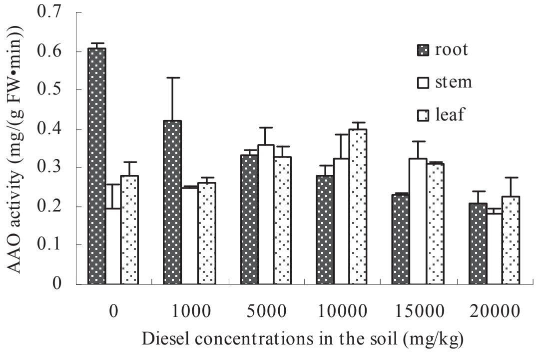

Fig. 5. Effect of diesel on AAO activity in root, stem and leaf of *Reed* after transplantation 50 days. Error bars represent S.D.

In plants, the reaction oxygen species (ROS) including H2O2, O2- and HO·, paradoxically serve as both signaling molecules and undesirable cytotoxic byproducts. Under normal photosynthetic conditions, these compounds are produced in the electron transport chains of chloroplasts and mitochondria, and are byproducts of metabolic pathways in the peroxisome. Because ROS chemically attack proteins, carbohydrates and fatty acids [20], plants employ a variety of mechanisms to neutralize these compounds.

When the plants were under abnormal condition such as diesel-attack, the toxic ROS load may exceed the capacity of the plants' antioxidant systems. This excess ROS may have caused the observed reductions in the chlorophyll and root vitality (Figure 1 and Figure 2). Plants utilize high levels of ROS in signaling pathways that ultimately result in complex, programmed defense responses to the challenger. The changes of antioxidant enzyme activities (Figure 4) in plant tissue indicated that a certain concentration of diesel would increase CAT enzyme activities, which could be seen as a protective mechanism against the destruction caused by diesel. In other words, the increment of this enzyme may have reduced the ROS cytotoxicity. A further increment of diesel concentration caused CAT activity to decline, and the same accident happened to POD and AAO activity in some plant tissues, suggesting that deleterious effects ROS were overtaking the plants' ability to counteract the cytotoxic compounds. Correctively, antioxidant defense systems seemed to prevent plant cells from damages and to confer on organisms their tolerance to pollutants. When such systems are overloaded, plants exhibited phytotoxicity symptoms like decreases in chlorophyll content and the root vitality. However, plant which exhibited efficient defense systems could cope with the pollution and develop themselves on contaminated soil. The finding is broadly consistent with other abiotic stress responses which quench excess ROS through enzymatic reduction to water, and oxidize electronrich buffers such as ascorbate and glutathione [21].

The POD activity in stem and leaf, and AAO activity in root decreased monotonically with diesel concentration increasing, indicating that the enzyme responses of *Reed* to diesel varied with tissues and enzymes. As oxidative stress management plays a vital role in plant survival [22,23], it is possible that the same enzyme in different tissues or various enzymes in the same tissue are complementary, and a coordinated response program that evolved over the range of diesel levels is observed in the figures.

3.4. Dissipation of diesel from soils

In this experiment, treatments without *Reed* transplants were also included. By comparing the residual oil concentrations in the soil with and without the transplants, it can be known if phytoremediation by *Reed* enhanced diesel fuel degradation. Based on the residual concentrations of diesel in soils after 50 days plantation of vegetable, the dissipation rate (DR) of diesel in variously treated pots was shown in Figure 6. The values of DR varied greatly among all treatments of different initial concentrations during the whole 50-day experiment.

It is noteworthy that at the same diesel concentrations, the DR value of soil planted were higher than that of those unplanted, especially when the diesel concentration was lower than 10 000 mg/kg (p<0.01), implying that *Reed* could positively promote the degradation of diesel-contaminated soil. This was probably because *Reed* was suitable to restore the lower diesel-contaminated soil. Anyway, the ability of *Reed* for restoring the diesel-contaminated soil is determined.

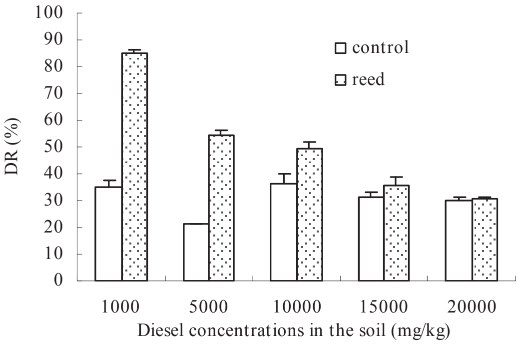

Fig. 6. The DR of diesel in variously treated pots 50 days after transplantation. PS. DR = (C0 - Ct)*100/C0, Where C0 was the initial soil concentrations of diesel; and Ct was the residual concentrations of the contaminate. Error bars represent S.D.

Euliss et al evaluated the feasibility of phytoremediation for cleanup of highly contaminated sediments from the Indiana Harbor, and found that sediments with sedge, swichgrass, and gamagrass had an approximately 70% reduction of TPHs after one year of plant growth [24]. It seemed that the ability of rhizoremediation in this experiment was remarkable. It was believed that the roots of a plant were effective at stimulating the rhizosphere micro-flora, excreting root exudates, and absorbing organic contaminants which can facilitate the removal of contaminant in soil. When the concentration of diesel became higher (15 000mg/kg), the removal of diesel reduced dramatically ($p<0.05$), and the DR difference between the soils planted and unplanted became less evident as the diesel concentration increased. And the differentials were 49.9%, 32.9%, 13.5%, 4.7% and 0.9% at 1 000, 5 000, 10 000, 15 000, 20 000 mg/kg, respectively. It was probably because the toxic contaminants restrain the proliferation of plant roots, its vitality as well as rhizosphere microbes.

4. Conclusion

The pots experiment showed that *Reed* could tolerate the applied diesel concentration (20 000 mg/kg soil) and they could effectively promote the degradation rate of diesel in soil although there were some detrimental effects (the decrement of chlorophyll and root vitality) on the growing of *Reed*. Additionally, antioxidant enzyme activities CAT in company with AAO were found to quench excess ROS caused by diesel contaminates, and the same enzyme in different tissues and various enzymes in the same tissue seem to conduct intricately to better neutralize the ROS caused by diesel. The determination of residual diesel showed that *Reed* indeed is capable of restoring the lower diesel-contaminated soils, which is also implied by the variation of the enzyme activity. This conclusion of this experiment study and the experiment data lays the foundation for further study of response and toxic mechanism of diesel in the enzymatic and molecular pathway, with a great applicable value in the phytoremediation for diesel-contaminated wetlands.

Acknowledgments

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