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The effects of simultaneous application of plant growth regulators and bioaugmentation on improvement of phytoremediation of pyrene contaminated soils

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HIGHLIGHTS

- The effects of Indole Acetic Acid and *Pseudomonas sp.* on pyrene removal were investigated using sorghum.
- Increased root biomass can stimulate bacterial population in the soil.
- Application of IAA and *Pseudomonas sp.* significantly increased plant biomass and pyrene removal.

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ABSTRACT

Polycyclic aromatic hydrocarbons (PAHs) refer to a wide group of soil contaminants whose presence in the environment is a cause of concern. The present study aimed to evaluate the effects of Indole Acetic Acid (IAA) and *Pseudomonas aeruginosa* on the phytoremediation activities of sorghum bicolor and increase in pyrene removal efficiency in the soil. The initial concentration of pyrene was 150 and 300 mg kg^{-1} in this experiment. The treatments included unplanted soil (T0), planted soil with sorghum (T1), planted soil with application of IAA (T2), planted soil with application of *Pseudomonas sp.* (T3), and planted soil with simultaneous application of IAA and *Pseudomonas sp.* (T4). The pyrene removal rate in the soil was measured every 30 days. Moreover, plant biomass and soil bacteria were measured after 90 days. The results showed that pyrene removal rate significantly increased in the planted treatments compared to the unplanted ones. After 90 days, at the initial concentration of 150–300 mg kg^{-1}, pyrene removal efficiency was 52–92% in T1-T4 and 35–47% in the unplanted treatment (T0). Application of IAA and *Pseudomonas sp.* significantly increased plant biomass, soil bacteria, and pyrene removal rate in T2, T3, and T4 compared to T1. Therefore, application of IAA in the planted treatments with sorghum could have a significant effect on increasing the removal efficiency of pyrene.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) refer to a wide group of soil contaminants whose presence in the environment is a major cause of concern due to their mutagenic and carcinogenic properties (Yan et al., 2004). Such compounds are mainly formed by incomplete combustion or pyrolysis of organic compounds (Shengwang et al., 2008). Pyrene is one of these compounds with four benzene rings. It is considered to be a persistent pollutant in the environment and is one of the 16 polycyclic aromatic compounds in the US EPA priority pollutant list (Thompson et al., 2008). Various methods can be used for remediation of PAHs-contaminated soils. Despite their effectiveness, these methods are often costly and may damage the natural structure and texture of the soil (Huang et al., 2004). Phytoremediation is considered to be an alternative cleanup method that uses plants to remove or transform hazardous contaminants in media, such as soil, sediment, water, and even air (Sun et al., 2013). Plants can reduce PAHs compounds in the soil in different ways, including uptake and accumulation, increase of microbial population, and improvement of physical and chemical conditions of the soil (Cheema et al., 2010). Several criteria must be

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considered to achieve maximum PAHs removal rate: 1) native plants of the target area should be used as they can tolerate soil and weather conditions of the area, 2) the plants should have the maximum root surface area, and 3) the selected plants should need minimum maintenance, such as the need for fertilizers, to decrease the cost as an important factor in phytoremediation, because the soils of contaminated areas have limited nutrients (Smith et al., 2006). Sorghum is a hardy plant and is considered to be a C4 grass with an extensive root system (Toler et al., 2005). In some cases, because of the extensive fibrous root system of grasses, they are used for remediation of soils contaminated with petroleum compounds (Ogbo, 2009). An increased microbial population and activity has been reported in rhizosphere of PAH-contaminated soils compared to the soils without vegetation (Krutz et al., 2005). April and Sims investigated the effects of prairie grasses with fibrous roots on soils contaminated with polycyclic aromatic compounds and found that because of their fibrous nature, roots of prairie grasses could stimulate microbial activity and increase the degradation of this group of pollutants in the soil rhizosphere (Aprill and Sims, 1990). Some soil bacteria have a significant effect on the performance of plants, such as their growth or their tolerance to various environmental stresses, so that plants can remove organic and inorganic contaminants from the environment more efficiently. *Pseudomonas* is one of the abundant soil microorganisms that can biodegrade complex organic compounds in the soil (Glick, 2010). Another factor that increases phytoremediation efficiency is the application of plant growth regulators. They are a group of organic compounds that control plant responses to the environment and are effective in very low concentrations. Indole Acetic Acid (IAA) is a plant growth regulator that plays an important role in cellular responses, such as cell expansion, division, and differentiation, and gene regulation. It also controls processes, such as growth of root and other parts of the plant (Ali et al., 2013; Saharan, 2011). Few studies have been carried out on the use of plant growth regulators and their influence on the removal of PAHs from the soil. However, no studies have been conducted on the effect of IAA on sorghum and its effect on pyrene removal efficiency. Therefore, the present study aims to investigate the effects of simultaneous application of IAA and *Pseudomonas aeruginosa* on enhancement of pyrene removal efficiency using sorghum bicolor.

2. Materials and methods

2.1. Chemicals

Pyrene and IAA with purity of 98% were obtained from Merck Company (Germany). Besides, the seeds of sorghum bicolor were purchased from Pakan Seed Company (Isfahan, Iran).

2.2. Preparation of the soil

The soil samples without pyrene were selected from the surface layer of soil in Shiraz, Fars province, Iran. Physical and chemical properties of the used soil were as follows: particle size distribution: 46.5% sand, 37% silt, and 16.5% clay, pH: 7.5, total nitrogen: 1.2%, total phosphorous: 8.20 mg kg^{-1}, total K: 0.78%, Cation Exchange Capacity (CEC): 4.83 cmol kg^{-1}, organic matter content: 2%, and Electrical Conductivity (EC): 225 μs cm^{-1}.

2.3. Experimental design

The soil samples were air dried and passed through a 4-mm sieve before use. The initial pyrene concentrations in the soil were 150 and 300 mg kg^{-1}. To contaminate the soil, pyrene was dissolved in acetone and the resultant solution was added to 10% of the soil for each treatment. Then, they were placed under the hood for complete evaporation of acetone. Finally, the uncontaminated soil was mixed with contaminated soil and was sieved for 3 times (4 mm) to ensure soil homogeneity (D'Orazio et al., 2013). Soil moisture was also kept at 60% of water holding capacity. Each plastic pot (15 cm diameter, 20 cm height) was filled with 1000 gr of the soil and was stored in a dark place for one week. The soil was fertilized with 1 g of KH_2PO_4 and 2 g of NH_4NO_3 kg^{-1} dry weight of soil. A total of six sorghum bicolor seeds were planted in each pot. After germination, 3 seeds that were similar regarding physical properties were kept and the other three were removed. The effects of various parameters, such as presence or absence of plant, various concentrations of contaminants, and time, were examined in duplicate. The experiment lasted for 90 d and pyrene removal rates were measured every 30 d. Treatments are as follows:

P: Planting sorghum in soil without pyrene.

T0: Contaminated soil at two pyrene concentrations (150 and 300 mg kg^{-1}) without planting sorghum.

T1: Contaminated soil at two pyrene concentrations (150 and 300 mg kg^{-1}) with planting sorghum.

T2: Planting sorghum at two pyrene concentrations (150 and 300 mg kg^{-1}) with application of IAA at the concentration of 10 mg kg^{-1}

T3: Planting sorghum at two pyrene concentrations (150 and 300 mg kg^{-1}) with application of *Pseudomonas aeruginosa*.

T4: Planting sorghum at two pyrene concentrations (150 and 300 mg kg^{-1}) with simultaneous application of 10 mg kg^{-1} of IAA and *Pseudomonas aeruginosa*.

To evaluate the effect of IAA on sorghum, 35 days after germination of seeds, IAA was dissolved in methanol-water mixture (<1% methanol) at the concentration of 10 mg kg^{-1}. Then, foliar spray was performed for three times every five days. In addition, the pots were displaced randomly every 7 d. At the end of each 30-d period, the plant was harvested and then, the soil in each pot was homogenized by sifting and was kept at 4 °C until analysis (Li et al., 2015).

2.4. Plant biomass

After 90 d, the plants were harvested and the roots and shoots were separated, washed with tap water, and rinsed with distilled water. Dry weight was measured after drying the plants in the oven at 105 °C in 24 h.

2.5. Isolation and enumeration of bacteria

Pseudomonas aeruginosa was isolated from petroleum-contaminated areas around an oil refinery in Iran. The mineral medium selected to isolate the bacteria from soil contained the following materials: $(NH_4)_2$ SO_4 4 g L^{-1} , KH_2PO_4 1 g L^{-1} , $FeCl_3$ 0.03 g L^{-1} , Nacl 0.5 g L^{-1} , $CaCl_2$ 0.01 g L^{-1} , pyrene 1%, and $MgSO_4$ 0.3 g L^{-1} (Azhdarpoor et al., 2014). Identification of the isolated bacteria was done using 16S rDNA.

To determine soil bacteria populations in different treatments, the sample soil suspension was prepared by dissolving 2 g of the soil in 100 ml distilled water. Then, it was serially diluted and spread on nutrient agar medium. The plates were incubated at 28 °C for 3–5 days. The results were expressed as CFU°g^{-1} dry soil (Cheema et al., 2009).

2.6. Analysis of pyrene in the soil

The residual pyrene in the soil samples was extracted using an ultrasound device based on the U.S. EPA extraction method 3550 B. Briefly, the soil samples were poured into special tubes. Then,