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Enhanced arsenic uptake and polycyclic aromatic hydrocarbon (PAH)-dissipation using *Pteris vittata* L. and a PAH-degrading bacterium

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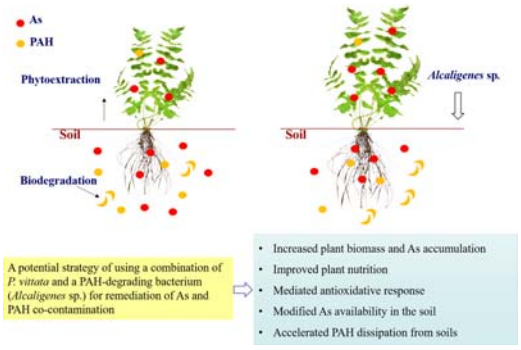
HIGHLIGHTS

- *P. vittata* combined with bacterial inoculation shows advantage in co-contamination remediation.
- Bacterial inoculation enhances plant tolerance by improving nutrition and antioxidative activities.
- Bacterial inoculation increases As accumulation and induces temporal change of As distribution in plant.
- Bacterial inoculation modifies As availability in the rhizosphere.
- Plant stimulates bacterial growth and phenanthrene dissipation in the soil.

GRAPHICAL ABSTRACT



Capsule: Combined use of *Pteris vittata* L. and *Alcaligenes* sp. shows an advantage for remediation of arsenic and PAH co-contamination.

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ABSTRACT

This study examined the effects of *P. vittata* and a polycyclic aromatic hydrocarbon (PAH)-degrading bacterium (*Alcaligenes* sp.) on arsenic (As) uptake and phenanthrene dissipation. Bacterial inoculation substantially increased As accumulation in plants by 27.8% (frond) and 27.5% (root) at 60 d, respectively, compared with the non-inoculated treatment, although temporal change of As translocation and reduction in plants was observed. Bacterial inoculation positively affected plants by improving growth, nutrition and antioxidative activities, and helped to modify soil As availability to the plants, which may benefit in plant tolerance and As accumulation. Plant and bacteria association enhanced phenanthrene dissipation from the soil, with the highest dissipation rate of 96.4% at 60 d in the rhizosphere, which might be associated with enhanced bacterial population and activity inspired by the growth of plant. The result reveals that combination of *P. vittata* and PAH-degrading bacteria can promote As accumulation and phenanthrene dissipation, and can be exploited as a promising strategy for As and PAH co-contamination remediation.

1. Introduction

Arsenic (As) and polycyclic aromatic hydrocarbons (PAHs) co-contamination in soil has become a major concern. The simultaneous accumulation of these two contaminants in soil is frequently found in

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several types of industrial contaminated sites, such as coking site, mining and metallurgy site, wood preservation site, and even in urban residential area (Kay et al., 2008; Elgh-Dalgrena et al., 2009; Sun et al., 2014). The combination of As and PAH could strongly potentiate the environmental risks (Li et al., 2010; Tchounwou et al., 2012; Cristaldi et al., 2017). Recent focus has been placed on the method to remediate As and PAH co-contamination. Elgh-Dalgrena et al. (2009) showed good removal efficiency for both As and PAH by soil washing, however, the toxicity of soil leachate was increased.

Phytoremediation is recognized as a safe and cost effective technique for As contamination remediation. *Pteris vittata* L., the first known and the most important As hyperaccumulator, is capable of tolerating high concentration of As (up to 1500 mg/kg soil), and efficiently accumulating As (13,800 mg/kg dry biomass) (Ma et al., 2001; Lessl et al., 2014; Fayiga and Saha, 2016). In the past decades, the feasibility of using *P. vittata* to remediate the As contamination has been well documented (Wan et al., 2016). Because soils at the contaminated sites generally contain complex mixtures of chemicals, a recent concern raised is the potential of *P. vittata* for phytoremediation of soils with mixed pollutants. *P. vittata* is reported to not only have high tolerance to Cd, Ni, Pb, Zn, Se, and Sb, with effective As uptake (Srivastava et al., 2009; Feng et al., 2011), it is also capable of tolerating As and PAH co-exposure both in the laboratory and the field study (Sun et al., 2011; Sun et al., 2014). These studies give the encouraging results for the potential use of *P. vittata* for phytoremediation in the natural environment with mixtures of contaminants.

Bioremediation is an effective method for PAH removal from the soil (Kuppusamy et al., 2017). Extensive PAH-degrading microorganisms have been characterized and inoculated in contaminated soils to enhance PAH degradation (Liu et al., 2017). However, the bioremediation efficiency of PAH is often limited by several factors, such as the insufficient nutrients in contaminated soils to support microbial growth, low bioavailability of contaminants, and the presence of multiple contaminants inhibiting the activity of microorganism (Liu et al., 2017). The convergence of phytoremediation and bioremediation strategy provides the superior advantage for remediation of PAH-contaminated soils. Plants may improve soil conditions and provide nutrient materials to stimulate microbial growth and activities because plants live in symbiosis with microorganisms naturally present in the rhizosphere, which may lead to enhanced degradation of PAH in the rhizosphere (Gerhardt et al., 2009; Cristaldi et al., 2017). The mutual relationships between the plants and the inoculated microorganisms have been recognized (Chen et al., 2016). Conventionally, the successful application of plant-microorganism combined remediation is largely dependent on the survival and growth of the plant and the inoculated microorganism. Plants may affect the abundance and activities of microorganisms through the release of root exudates (Toyama et al., 2011). Microbial mediated plant nutrient status and oxidative stress also play key roles in detoxification of plants (Singh and Ma, 2006). In addition, the availability of contaminant in the soil is also recognized as critical factor for remediation efficiency. Plant and bacteria may impact the chemical interactions between soil and contaminants such as the absorption and desorption process, the oxidation and reduction reaction, and the mobility and fixation (Glick, 2010).

In fact, the hyperaccumulation of As by plant, together with enhanced PAH-dissipation in the rhizosphere, is supposed to be an ideal remediation approach for As and PAH co-contamination. Recently, Feng et al. (2014) used an arsenate-reducing and PAH-degrading isolator (*Pseudomonas*) to enhance As and PAH removal by *P. vittata* hydroponically, which provided some insight into the potential use of this technology. However, the interaction between *P. vittata* and PAH-degrading bacteria on As accumulation and PAH-dissipation in soil is still poorly understood. Moreover, the remediation efficiency and mechanism may vary greatly in the relationships between *P. vittata* and different microorganism associations. The aim of the present study was therefore to investigate the effectiveness of using *P. vittata* and a PAH-degrading bacterium to remediate As and PAH co-contaminated soil. The PAH-degrading bacterium (*Alcaligenes* sp.) used in this experiment was isolated from As and PAH co-contaminated soil of a coking plant, Beijing, China. The bacterium shows the inherent As tolerating capacity with minimum inhibitory concentration of 4000 mg/L for arsenate. The mutual effects between *P. vittata* and bacteria on As uptake and PAH-dissipation were evaluated. Possible mechanisms related to plant physiological activities and rhizosphere processes influenced by bacterial inoculation were discussed. The impact of vegetation time on As uptake and PAH-dissipation with bacterial inoculation was also determined. If positive effects are obtained, it may provide a potential remediation technology for As and PAH co-contamination.

2. Materials and methods

2.1. Plant propagation and spiked soil

Spores were collected from fertile fronds of *P. vittata* in southern China and were germinated and propagated in As- and PAHs-free seed base. After two months, plants with similar size and 6–7 fronds were prepared for experiment.

Soil with no previous history of As or PAH contamination was collected from farmland in the rural area of Beijing city. The agricultural soil used had a sandy loam texture (clay 9.0%, silt 36.4%, sand 54.7%), with a pH of 8.11 and an organic matter content of 2.49% (dry). The nitrogen (N) and phosphorus (P) contents were 0.17% and 0.10%, respectively. The soil had low level of As (8.82 mg/kg), and undetectable level of PAH. The soil was air dried, sieved through a 2 mm mesh, and then steam-sterilized at 120 °C for 2 h (over a 3–4 d period, with a 24-h interval between autoclaving) to eliminate indigenous microbial populations. Phenanthrene with purities >98% was selected as representative of PAH (Acros Organics (USA)). As was added in the form of $Na_2HAsO_4 \cdot 7H_2O$. As (dissolved in sterilized deionized water) and phenanthrene (dissolved in acetone) were spiked to soil successively to achieve desired concentrations. The soil was mixed thoroughly and equilibrated and aged for >4 months prior to experiment. During this period, the soil was mixed at intervals to ensure a homogenous distribution of As and phenanthrene. Before starting the experiment, the spiked soil were sampled randomly to determine the As and phenanthrene contents. The initial concentrations As and phenanthrene in spiked soil were 89.66 mg/kg and 50.01 mg/kg, respectively, comparable to the average levels of As and PAH detected at the contaminated sites (Sun et al., 2014).

2.2. Bacterial culture

The bacterial strain was isolated and characterized by Peking University as *Alcaligenes* sp. The dissipation rate of phenanthrene as sole carbon source by *Alcaligenes* sp. over three weeks was 60%. The bacterial strain was gram negative, motile, and formed short rods. After 3-d incubation at 25 °C, colonies appeared non-pigmented and translucent, with smooth surfaces and sizes ranging from 1 to 3 mm in diameter. The strain was cultured with mineral medium in the presence of 50 mg/L phenanthrene using a shaking incubator at 25 ± 1 °C and 200 rpm. The bacterial cells were harvested in log phase using a centrifuge at 6500 rpm, washed twice with 0.9% NaCl solution, and re-suspended in 5 -mL of sterilized deionized water as the inoculum. The soil was mixed with the bacterial cells with initial bacterial concentration of approximately 10^6 CFU g^{-1} dry soil.

2.3. Experiment design

The experiment was carried out using the sterilized pot containing 1.5 kg spiked soil. Plastic frames covered with nylon mesh cloth (mesh size 45 μm) were used to separate the rhizosphere (0.3 kg) from the bulk (1.2 kg). The plants were transplanted to the central