Ecological Engineering 73 (2014) 380–385

Contents lists available at ScienceDirect

Ecological Engineering

journal homepage: www.elsevier.com/locate/ecoleng

Effect of plant density on phytoremediation of polycyclic aromatic hydrocarbons contaminated sediments with *Vallisneria spiralis*

Hongyan Liu, Fanbo Meng, Yindong Tong, Jie Chi *

School of Environmental Science and Engineering, Tianjin University, Tianjin, 300072, China

ARTICLE INFO

Article history:
Received 23 February 2014
Received in revised form 6 September 2014
Accepted 26 September 2014
Available online xxx

Keywords:
Plant density
Vallisneria spiralis
Polycyclic aromatic hydrocarbons
Redox potential
Phytoremediation

ABSTRACTS

A 54-day-long study was conducted to investigate the effect of plant density (260, 780, 1300 and 2080 plants m^{-2}) of *Vallisneria spiralis* on the remediation of sediments contaminated by polycyclic aromatic hydrocarbons (PAHs). Dissipation ratios of phenanthrene and pyrene in sediments were initially the highest in treatment of 2080 plants m^{-2}. However, after a 54-day incubation, no statistical difference was observed in the dissipation ratios under different planting density treatments ($p > 0.05$) except lower dissipation ratio of phenanthrene in treatment of 780 plants m^{-2}. Compared with the unplanted sediments, the dissipation ratios of phenanthrene and pyrene in planted sediments increased by 15.2–21.5% and 9.1–12.7%. Considering the sustainability of the ecosystem, lower plant density (e.g., 260 plants m^{-2}) should be a better selection for phytoremediation of PAHs. Mass balance calculation indicated that plant accumulation accounted for less than 0.39% of the dissipation increment. Furthermore, dissipation ratio of PAHs was positively related to PAH-degrading bacterial population, suggesting that microbial degradation played a major role in the *V. spiralis*-promoted remediation. The redox potential, a signal of oxygen in sediments, was measured. Positive redox potentials were found in sediments with *V. spiralis* as a result of oxygen released by roots. Moreover, sediment redox potential positively correlated with PAH-degrading bacterial population. Considering high oxygen demand of PAHs catabolism and reduced conditions in unplanted sediments, it can be concluded that the enhanced dissipation of PAHs is mainly related to oxygen released by roots.

1. Introduction

As widespread organic contaminants, polycyclic aromatic hydrocarbons (PAHs) are byproducts mainly from anthropogenic activities such as coal combustion and automobile emissions (Ravindra et al., 2008). PAHs have been listed as priority pollutants for their toxic, mutagenic and carcinogenic properties, which could be a serious threat to the ecosystem and furthermore, human health (Franco et al., 2008). In the environment, PAHs can be released into water bodies through atmospheric deposition, surface run off and sewage discharge (Wu et al., 2012). PAHs could be easily adsorbed by the particles and accumulate in sediment due to their strong hydrophobicity, which make sediment as storage of PAHs (Chiou et al., 1998; Perelo, 2010). Owing to the limited supply of atmospheric oxygen into the flooded sediment and the contaminants' high chemical stability, the thorough oxidation and decomposition of PAHs were difficult.

Phytoremediation, the use of plants to enhance biodegradation and removal of pollutants, has been proved to be a cost-effective and eco-friendly method and also has been manifested for many years as a potential technology for restoration of contaminated soil (Perelo, 2010). Of all the species of aquatic plants, submerged macrophytes are the dominant aquatic plants, especially in shallow water bodies (Biernacki et al., 1997). Submerged hydrophytes are plants that are completely under the water and typically have their root systems in the bottom sediment. Recently, it has been reported that submerged macrophytes are capable of remediating contaminated sediments (Huesemann et al., 2009; Yan et al., 2011). In addition to releasing root exudates as terrestrial plants, submerged aquatic macrophytes are also capable of releasing oxygen to their rhizosphere to modify the anaerobic conditions and increase the availability of nutrients to the plants (Sand-Jensen et al., 1982; Perelo, 2010). PAHs are known to be susceptible to aerobic biodegradation (Huesemann and Truex, 1996). Therefore, the oxygen flux from plant roots into the anaerobic sediment might play an important role in increasing the

* Corresponding author. Tel.: +86 22 27406513; fax: +86 22 87402072.
E-mail addresses: 436073909@qq.com (H. Liu), mengfanbo1010@126.com (F. Meng), tongyd318@163.com (Y. Tong), cjiechi@yahoo.com (J. Chi).

http://dx.doi.org/10.1016/j.ecoleng.2014.09.084
0925-8574/

removal rate of the contaminants. However, the specific mechanism by which submersed macrophytes enhance the remediation of PAHs-contaminated sediments has been not elucidated so far.

Plant density is an important parameter for phytoremediation. According to previous studies, plant density could affect the growth and quality of plant in many ways (Sangoi, 2001). Søndergaard reported that the nutrient availability of macrophyte might depend on both species and density of plants (Søndergaard, 1988). Loadesa et al. found that plant density had an influence on fibrous root reinforcement of soils (Loadesa et al., 2010). The density had a significant effect on the reproduction, biomass accumulation and root morphology due to competition among plants (Xie et al., 2006; Jiang et al., 2008). However, currently, little information is available on the effect of plant density of submerged macrophytes on the remediation of contaminated sediments.

In this study, the perennial *Vallisneria spiralis* (*V. spiralis*) was selected because this species is ubiquitous in freshwater environments and has a high adaptive capability (Wang and Yu, 2007). Effect of plant density of *V. spiralis* on the dissipation of phenanthrene and pyrene in sediments was investigated. Plant growth, root morphology, sediment redox potential and PAH-degrading bacterial population were monitored, and the remediation mechanisms were discussed.

2. Experimental

2.1. Materials

A total of 153 seedlings of *V. spiralis* with similar size (3–4 leaves, 0.5 cm root, 17 ± 2 cm shoot) were prepared, which were obtained by seed culture for 2 months before the experiment.

Surface sediments samples (top 10 cm, pH 7.47, 2.70% organic matter) were collected from the mainstream of the Haihe River (39° 07′17″N, 117° 13′14″E), Tianjin, China. After air-dried, the sediments were sieved to remove rocks and roots by a 2 mm screen. 1/6 of the sediments were then spiked with a mixture of phenanthrene and pyrene (1:1) in acetone. When acetone evaporated off, the spiked sediments were progressively mixed with the remaining non-spiked sediments to ensure the homogeneity of the sediment samples. The sediments were then sieved again through a 2 mm mesh to produce final concentrations of about 15 mg kg^{-1} of each PAH.

2.2. Experimental design

Sediment samples of 100 g each (dry weight) were placed in glass pots (diameter 6.3 cm). *V. spiralis* were transplanted in the glass pots, which were taken as planted treatments and consisted of four plant densities (1, 3, 5 and 8 plants per pot, namely 260, 780, 1300 and 2080 plants m^{-2}). At the same time, unplanted and sterilized (0.2% w/w NaN_3) treatments were also constructed as controls. Nine pots were prepared for each treatment. The pots were then placed into glass tanks (60 cm long × 30 cm wide × 40 cm high) filled with 50 L of tap water. White fluorescence light (2200 ± 100 Lux) was installed on the top of the tanks; and the ratio of light:dark was 12:12. The water in the tank was gently stirred with a submerged pump during the experiment to achieve homogeneity and kept at the temperature of 22 ± 1 °C. The experiment lasted for 54 days and 3 replicate pots of each treatment were removed every 18 days.

After carefully removing the overlying water in the pots, sediment redox potential was measured immediately with a platinum redox electrode coupled to a portable pH/mV meter. The platinum redox electrode was placed in the sediments at the central point and about 2 cm depth for 15 min. Mean value of three parallel pot sediments was calculated. Then plants in the pots were carefully rinsed with tap water to remove any sediment particles and dried with filter paper. After measuring the heights and weights, plant samples were placed into polytetrafluoroethylene bags and stored at 4 °C for further analysis. Sediment samples were stored at −20 °C after freeze drying for 24 h.

2.3. Analytical methods

2.3.1. Root morphology

Roots were manually separated from the plants and scatted over a clear tray that was filled with deionized water. Root images were obtained by an STD-1600 EPSON scanner and were used to measure root diameter, root length and surface area with the WinRhizov. 4.0b software. Scanned roots were cleaned for root biomass assessment.

2.3.2. Analysis of PAHs in sediments and plants

The analysis of PAHs in sediment was conducted according to standard analytical method (USEPA, 1996). In brief, samples of freeze-dried sediments were ground and passed through an 80-mesh sieve. Sediment samples (5.0 g) were Soxhlet extracted using 45 mL dichloromethane for 24 h. The extracts were concentrated to 1 mL by a rotary evaporator at a 38 °C water bath. The condensed extracts were concentrated to 1.0 mL again after adding 4 mL n-hexane. The concentrated extract was cleaned up with a self-packed chromatographic column (1 cm sodium sulfate, 7 cm neutral chlorine dioxide and 14 cm silica gel from up to down). N-hexane was used to transfer the concentrated extracts from the rotary evaporator to the cleanup column, and the extracts were eluted with 25 mL mixture of pentane and dichloromethane (2:3, v/v) at a rate of 2 mL min^{-1}. The eluent collected was reduced to 1 mL. Internal standard (hexamethyl benzene) was added before GC–MS analysis.

The plants were separated into roots and shoots before analysis. One gram of fresh plant sample was extracted in a histoid grinding tube with 5 mL dichloromethane at 200 g for 5 min. The solution was centrifuged at 1600 *g* for 5 min. After removal of the water phase, the dichloromethane layer was reduced to 1 mL and purified by the procedure as sediment samples. The final volume was reduced to 0.5 mL. Internal standard (hexamethyl benzene) was added before GC–MS analysis.

PAHs in the extracts were quantified by GC–MS in a selected ion monitoring mode (SIM). The GC–MS system consists of an Agilent 6890N gas chromatograph equipped with a fused-silica capillary column (HP-5, 30.0 m × 250 μm × 0.25 μm) and coupled to a mass spectrometer detector (5975C). The temperatures of injector and detector were both set at 250 °C. Helium was used as a carrier gas at a flow rate of 1.0 mL min^{-1}. The initial oven temperature was 100 °C, and was raised to 280 °C at 20 °C min^{-1} with holding time of 2 min. The total time was 11 min. The MS conditions for EI ionization were as follows: the ion energy was 70 eV and the ion source temperature was 230 °C.

Strict quality control procedures were applied to all data obtained. None of the target compounds was detected in solvent blanks and procedure blanks for plants and sediments. Using internal standard calibration, good linearities of phenanthrene and pyrene were observed with correlation coefficients above 0.9998. The limits of detection (LOD) (S/N = 3) of phenanthrene and pyrene were both 6 μg kg^{-1} for sediment and 15 μg kg^{-1} for plant. Average recoveries of phenanthrene and pyrene were 85.0 ± 3.2% and 93.7 ± 3.0% in sediments, 95.3 ± 4.0% and 96.1 ± 3.6% in plants.

2.3.3. PAH-degrading bacterial population

Sediment samples were processed immediately for PAH-degrading bacteria population analysis by the plate-count techniques as colony forming units (CFU) per gram of dry