Polycyclic Aromatic Hydrocarbons for the Soil-plant-Environment System

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Abstract

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental contaminants found in air, sea and river water and soil. Sources of PAHs are thought to include diesel, fossil fuels and power plants. Some PAHs are known to be carcinogenic and mutagenic. Phytoremediation of PAHs is defined as the use of plants and their associated bacteria to remediate contaminated soil. These processes occur on rhizosphere. Plants excrete exudate associated with bacterial activity.

The relationship of vegetation to PAH concentration was investigated. Two and 3 rings of PAHs, showed high volatility and were not related to vegetation. However, areas with extensive vegetation had lower concentrations of 4 to 6 rings of PAHs.

Plant responses of pyrene were investigated by superoxide dismutase (SOD) activity. Some plants were collected from sampling spots and steeped in pyrene dissolved in water or distilled water. Most plants showed greater increase in SOD activity when placed in pyrene dissolved in water than when placed distilled water. Especially, *Artemisia princeps* showed a high level of SOD activity (68.3%). This finding suggested that the substances with high SOD activity participated in degradation of PAHs.

Key words: *Artemisia princeps*, PAHs, Phytoremediation, Rhizodegradation, Soil,

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental contaminants found in air, sea and river water and soil. They occur as common constituents of gasoline, coal tar and shale oil, but are most frequently formed by incomplete combustion of fossil fuels (Maila and Cloete, 2002; Pothuluri and Cerniglia, 1994). Therefore, these substances are long-lasting, poorly degradable pollutants, that accumulate in the environment. Furthermore, these substances present a great affinity for organic materials in soil such as humus. Some PAHs are known to be carcinogenic and mutagenic (Laflamme and Hite, 1978; Pahlman and Pelkonen, 1987). Especially, benzo(a)pyrene is known as an endocrine disrupter (Liu and Korenaga, 2001). Sixteen PAHs have been selected by the US Environmental Protection Agency (EPA) as Constant Decree priority pollutants for regulatory purposes (Hodgeson, 1990). The potential for atmospheric concentrations of PAHs to accumulate in many geographical locations of the world have been determined and reported, e.g., Massachusetts, USA (Allen *et al.*, 1996), Athens, Greece (Viras *et al.*, 1987), Mumbai, India (Kulkarni and Venkataraman, 2000), Lahore, Pakistan (Smith *et al.*, 1996), and Kuala Lumpur, Malaysia (Omar *et al.*, 2002). However, there is still very little information on the concentration of these substances in soil.

The remediation of organic pollutants uses physical and chemical techniques. The cost however is very expensive and requires significant labor. The solution of this problem is bioremediation and phytoremediation. Bioremediation is a technique by which pollutants are biodegraded by bacteria. It has been reported that white rot fungi can extensively biodegrade PAH (Bumpus, 1989; Zheng and Obbard, 2002), because white rot fungi are capable of non-specifically oxidizing aromatic compounds through the abstraction of an electron or a hydrogen atom. (Barr and Aust, 1994; Hattaka, 1994). Phytoremediation is the process by which contaminants in the environment are removed by plants (Cunningham *et al.*, 1996). The targets of phytoremediation are various pollutants, e.g. heavy metals, NO_x, SO_x, agricultural chemicals and PAHs. Enhancement of PAH degradation in soil rhizosphere has been shown in several studies (Aprill and Sims, 1990; Binet *et al.*, 2000; Qiu *et al.*, 1994; Reilley *et al.*, 1996). Grasses are being used due to their fibrous root systems with extensive surface area for microbial colonisation (Adam and Duncan, 2002).

The studies and investigations have not provided sufficient information regarding the relationship between soil PAHs and vegetation.

In this study, we investigated the relationship between vegetation and PAHs concentration and the responses of weeds with large biomass and poor environmental adaptation to contact with PAHs.

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2. Materials and Methods

2.1 Concentrations of 16 US EPA-identified PAHs in soils

Soil was collected from various spots in highly trafficked and vegetated areas, as well as three spots around our university. Collected soils were sieved with a 2.0 mm nylon mesh sieve. There were extracted PAHs from 5.0 g of soil by soxhlet extraction with 120 ml of dichloromethane. These extractions processes were repeated three times. Extracted solutions were condensed to 1.0 mL by an evaporator using N₂. The concentrations of 16 PAHs were determined by HPLC analysis. HPLC determination was performed using a Shimadzu Co, Ltd. LC-10AT binary pump and SPD-10A UV/VIS detector. Column and analytical parameters were as follows

Column: 25 cm × 4.6mm i.d. stainless steel.

analytical column packed with 5 μm

Supercosil LC-PAH (Superco).

Analytical parameters: 5 min after starting analysis, linear gradient elution from 40: 60 acetonitrile/water to 100:0 acetonitrile/water 35min after starting analysis. The temperature of the column was 23 degrees, flow rate was 1.0ml/min and detection wavelength was 254nm. Injection volume was 5.0 μL.

2.2 Collection of plant root exudates and measurement SOD activity

Plants were collected at the same sampling points for investigation of PAH concentrations. Collecting plants were washed with distilled water to remove soil. The plants were steeping in 300ml of pyrene solution (0.035mg/l dissolved in distilled water) or in 300ml of distilled water. After 72 hours, these root exudates were collected and freeze dried. And these exudates were dissolved in distilled water at 1000 μg/mL

Superoxide scavenging activity was determined by NBT reduction with a WAKO SOD kit (WAKO Pure Chemical Ind. Ltd.). A mixture (230 μL) of exudates sample (10 μL), nitroblue tetrazonium (NO₂-TB) solution, xanthin solution, xanthinoxidase and superoxide dismutase was incubated at 37°C for 28 min, Sidium dodecyl sulfate was then added to the mixture and absorbance was measured at 560nm.

3. Results and Discussion

Table 1 indicates the concentrations of 16 PAHs at different vegetated and trafficked sampling sites. Heavy traffic caused higher concentrations of total PAHs. However, sites with extensive vegetation in the same trafficked area had lower concentrations of total PAHs. When classified by the number of benzene rings, vegetation greatly influenced the number of rings. Since 2- and 3-ring PAHs have higher volatility, these did not remain in soil, while 4- to 6-ring PAHs more easily remained in soil, and were degraded by plant and bacteria.

Table 1 Concentration of total and rings number of PAHs (mean ± S.D.) at different spot (n=3).

		Type of sampling spots								
		Vegetation*	N	-	-	-	+	+	+	++
		Traffic**	-	-	++	++	++	+	+	-
PAH name	ring numbers	Concentration (mg/kg soil)								
naphthalene	2	0.354±0.011	0.201±0.163	0.292±0.073	0.286±0.045	0.375±0.224	0.107±0.017	0.025±0.011	0.061±0.049	
acenaphthylene	3	0.153±0.013	0.172±0.006	0.434±0.124	0.169±0.047	0.348±0.537	0.098±0.015	0.052±0.026	0.055±0.012	
acenaphthene	3	0.291±0.190	0.433±0.020	0.472±0.118	0.833±0.256	0.379±0.223	0.136±0.027	ND	ND	
fluorene	3	0.013±0.014	0.011±0.016	0.088±0.026	0.084±0.097	0.041±0.057	0.015±0.02	ND	0.016±0.014	
phenanthrene	3	0.012±0.002	0.014±0.003	0.051±0.015	0.033±0.002	0.035±0.008	0.011±0.002	0.010±0.006	0.010±0.002	
anthracene	3	0.010±0.002	ND	0.018±0.008	0.012±0.001	0.018±0.015	0.003±0.0001	ND	ND	
fluoranthene	4	0.063±0.046	0.040±0.001	0.126±0.040	0.171±0.093	0.203±0.156	0.022±0.009	0.017±0.011	0.012±0.008	
pyrene	4	0.033±0.019	0.039±0.011	0.191±0.055	0.271±0.296	0.169±0.103	0.030±0.004	0.019±0.009	0.012±0.011	
benzo[a]anthracene	4	0.014±0.010	0.010±0.001	0.119±0.027	0.111±0.005	0.058±0.018	0.012±0.007	0.010±0.007	ND	
chrysene	4	0.014±0.008	0.011±0.002	0.096±0.067	0.102±0.055	0.074±0.045	0.042±0.007	0.038±0.018	0.021±0.015	
benzo[b]fluoranthene	5	0.011±0.010	ND	0.209±0.045	0.122±0.038	0.061±0.033	ND	0.013±0.009	ND	
benzo[k]fluoranthene	5	0.015±0.013	0.012±0.003	0.177±0.036	0.133±0.009	0.065±0.037	0.012±0.004	0.011±0.008	ND	
benzo[a]pyrene	5	0.023±0.021	0.019±0.005	0.205±0.038	0.161±0.023	0.105±0.049	0.015±0.006	0.017±0.012	0.010±0.010	
dibenz[a,h]anthracene	5	0.049±0.047	0.036±0.005	0.619±0.154	0.481±0.079	0.307±0.210	0.017±0.029	0.110±0.056	0.037±0.064	
benzo[ghi]perylene	6	0.010±0.018	0.040±0.037	0.704±0.394	0.398±0.177	0.198±0.128	0.016±0.010	0.038±0.021	0.021±0.022	
indeno[1,2,3,-cd]pyrene	6	0.017±0.029	ND	0.456±0.157	0.504±0.185	0.152±0.127	ND	0.011±0.007	ND	
Total 16PAHs		1.074±0.382	1.045±0.256	4.256±1.181	3.874±1.314	2.598±1.527	0.513±0.125	0.374±0.148	0.247±0.117	

* ++: It is covered the whole surface vegetation. +: 70% are covered vegetation. -: Less than 50% are covered vegetation.

** ++: >5000cars/day, +: 500-5000 cars/day, -: <500cars/day

N: No vegetation

ND: Less than detected limit (0.010mg/kg soil)

Table 2 indicates the SOD activity of plant exudates. (The concentration of plant exudates was 1000ppm.) Most plants showed higher SOD activity when placed in pyrene dissolved in water than when placed in distilled water. Especially, *Artemisia princeps* showed very high SOD activity (68.3%).

The interaction between plants and bacterial communities is a complex relationship. Plants sustain large bacterial populations in the rhizosphere by rhizodeposition (Paul and Clark, 1989). The excretion of root exudates by plants contributes to increasing the density of bacteria found near the plant root. Therefore, root exudates have previously been found to enhance biodegradation of organic compounds. Mineralization of the organophosphate insecticide parathion was stimulated in soil by the addition of bush bean root exudates, but not to the extent observed in the plant system (Hsu and Bartha, 1979).

Plants have genetically controlled mechanisms that allow them to live and grow under stress. (Boyer, 1982) This study showed plant responses to organic pollutants such as PAHs, although root exudates differed by plant species. Niyogi *et al.*, 2001 were investigated of relationships with accumulated PAHs concentration and antioxidant enzymes in barnacle tissues of *Balanus balanoides*. They were indicated that tissue concentrations of PAHs, resulting in a significant positive correlation with antioxidant enzymes, mainly catalase and SOD. Example in plants, SOD-over-expressing alfalfa and tobacco tolerant to environmental stresses (e.g. water and high light) was shown (McKersie *et al.*, 1996; Van Camp *et al.*, 1996; Alscher *et al.*, 2002). This finding suggests that substances with high SOD activity by participate in the degradation of PAHs.

Table 2. SOD activity of plant exudates(n=3).

plant name	solution type	SOD activity (%)*
<i>Artemisia princeps</i>	distilled water	9.1±1.2
	pyrene dissolved	68.3±3.6
<i>Chenopodium album</i>	distilled water	22.2±1.5
	pyrene dissolved	11.9±2.2
<i>Solidago altissima</i>	distilled water	16.1±3.4
	pyrene dissolved	36.8±3.3
<i>Fatoua villosa</i>	distilled water	7.8±2.3
	pyrene dissolved	13.1±1.5
<i>Amaranthus viridis</i>	distilled water	6.1±0.2
	pyrene dissolved	15.3±1.3

* This activities indicate superoxide scavenging activities that was determined by NBT reduction each sample concentration was 1000µg/1mL.

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