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Phytoremediation of Anthracene Contaminated Soils by Different Plant Species

Yong-Bum Kim[1Ψ], Ky Young Park[2], Yong Chung[3], Keun-Chan Oh[4], and Bob B. Buchanan[1]*

[1]Department of Plant and Microbial Biology, 111 Koshland Hall, University of California, Berkeley, CA 94720, USA
[1Ψ]Department of Biological Science, Suncheon National University, Jeonnam 540-742, Korea
[3]Department of Preventive Medicine and Public Health, College of Medicine, Yonsei University, Seoul 120-752, Korea
[4]Princeton Environmental Solution LLC, 43 Water Town Court, Princeton, NJ 08540, USA

Phytoremediation is perceived as an alternative technology for contaminated site remediation. Yet, the mechanisms plants use to remove organic contaminants have not been fully elucidated. The objective of the current study was, therefore, undertaken to clarify the contribution of plants to the disappearance of organic contaminants in soil. Four plant species, including alfalfa, tall fescue, barley, and orchard grass, were examined for the ability to facilitate the degradation of the polyhydric aromatic hydrocarbon, anthracene. Soil samples were intermittently collected for two months to measure the disappearance of anthracene and bacterial number by GC and epifluorescence microscopy, respectively. Plant exudates were collected to determine their ability to solubilize anthracene. Alfalfa showed a 28% enhancement in the disappearance of anthracene compared to the unplanted control. Root exudates from alfalfa increased the release of soluble anthracene by 25 to 80% compared to the other species and unplanted controls. Among the plants tested, there was a positive correlation between enhancing the disappearance of anthracene by plants grown in soil and increasing the release of anthracene by isolated plant exudates. The results suggest that root exudates facilitate the mobilization of anthracene from soil and that the successful implementation of phytoremediation depends on the plant species. Further, root exudates may be a useful tool in screening plants for possible application in anthracene remediation.

Keywords: phytoremediation, anthracene, root exudate, polyhydric aromatic hydrocarbon

Polycyclic aromatic hydrocarbons present problems in the environment not only because they may negatively affect human health, but also because they potentially alter native ecological communities (Shuttleworth and Cerniglia, 1995). Polycyclic aromatic hydrocarbons, compounds consisting of two or more benzene rings, are widely distributed in soils and are recalcitrant owing to low solubility (Heitkamp and Cerniglia, 1998). The primary source of polycyclic aromatic hydrocarbon contamination is human activity, including fossil fuel utilization and chemical manufacturing, although the natural and anthropogenic pyrolysis of organic matter during forest fires is also a contributing factor (Christensen and Zhang, 1993). Most polycyclic aromatic hydrocarbons are potentially genotoxic and carcinogenic (Shuttleworth and Cerniglia, 1995)-properties that have raised considerable environmental concern.

*Corresponding author; fax +1-510-642-7356
e-mail view@nature.berkeley.edu

The effect of growing plants (roots) on the dissipation of organic pollutants has been mainly attributed to increased microbial numbers and selection of specialized communities in the rhizosphere (Reilley et al., 1996). Improved physical and chemical soil conditions, supply of root exudates for co-metabolic processes, increased humidification and adsorption of pollutants to increase bioavailability are also contributing factors (Günther et al., 1996). While Aprill and Sims (1990) showed that the nature of the rhizosphere depends on the plant species, recent studies have revealed that the rhizosphere may play a role in bioremediation of soils contaminated with toxic organic chemicals such as polycyclic aromatic hydrocarbons (Günther et al., 1996; Reilley et al., 1996; Leyval and Binet, 1998; Banks et al., 1999).

Plant roots exude a variety of organic compounds in the soil, including sugars, amino acids, organic acids and phenols (Vancura, 1964). Root exudates may mobilize mineral nutrients directly through acidification of the rhizosphere, solubilization of adsorbed or precipitated cations or secretion of chelating substances

(organic acids) to increase nutrient uptake (Römheld and Marschner, 1986). Maize exudate, for example, can mobilize several types of humic substances that are largely insoluble complexes (Nardi et al., 1997). Such humic components can be found in nature either in the aqueous phase (dissolved humics) or in association with minerals (solid phase). The soluble humic substances were positively correlated with phenanthrene mineralization (Laor et al., 1996, 1999). These reports suggest that plant exudates probably enhance the disappearance of polycyclic aromatic hydrocarbons in soils by increasing their bioavailibility either through surfactant-like effects or through changing the structure of humic acid.

The object of this study was to determine (1) whether plants enhance the disappearance of anthracene in soil, and (2) whether this disappearance is correlated with the formation and activity of root exudates.

MATERIALS AND METHODS

Soil Preparation and Plant Growth

Four plant species, including alfalfa (*Medicago sativa*), tall fescue (*Festuca arundinacea*), barley (*Hordeum vulgare*), and orchard grass (*Datylis glomerata*) were tested in soil together with an unplanted control for an effect on the disappearance of anthracene as a model polycyclic aromatic hydrocarbon compound. The soil, which was collected from an agricultural field (Berkeley, California, USA), had a pH of 7.2 in distilled water and contained 8.091 mg/g dry weight soil of carbon and 1.8 mg/g dry weight soil of nitrogen. After drying for 7 days in air, the soil was passed through a 2 mm sieve to remove large particles. For introduction to soil, anthracene was dissolved in a mixture of acetone and methylene chloride (1:1). The solution was sprayed onto the soil and mixed using a soil mixer (Liquid-Solids Blender, Patterson-Kelley, USA). The soil was exposed to air for 2 days to allow the acetone and methylene chloride to evaporate and then added to 2-liter pots that were maintained in a greenhouse. Plants were introduced to the contaminated soil after 2 months growth in vermiculite in a greenhouse. Nutrients were not added in view of the report that supplying nitrogen and phosphorus has no effect on the disappearance of polycyclic aromatic hydrocarbons (Johnson and Scow, 1999). The soil samples, taken periodically, were extracted in methylene chloride and acetone (1:1) according to U.S. EPA method 3550B. Quantification of anthracene in the extracts was accomplished using GC-MS (Aitken et al., 1998).

Microbial Number

Soil (original water content, sieve size < 2 mm), 2.0 g, was added to 18 ml sterile 0.1% solution of $Na_4P_2O_7$ (pH 7.0, adjusted with HCl) in a 250-ml Erlenmeyer flask. The mixture was vigorously shaken at 170 rpm for 30 min at room temperature, and the flask was then placed on a platform for 5 min to allow the suspension to settle. A portion of the supernatant fraction was diluted in filter-sterilized deionized water and a staining reagent (SYTO 9 and propidium iodide) was added. After a 3-min incubation, aliquots of the cells were collected on a membrane filter (0.2 um black nucleopore polycarbonate filter, diameter of 25 mm) to count the number of bacterial cells. Approximately 15 to 25 frames were counted, each in a horizontal and vertical transect across the slide, using an epifluorescence microscope (excitation/emmission: 480 nm/500 nm for SYTO 9 and 490 nm/635 nm for propidium iodide, Zeiss, Germany).

Collection of Root Exudates

Exudates were collected from the roots of the tested plants according to Barbas et al. (1999). Roots from one-month-old plants were gently washed with tap water to remove adhering soil. Plants with washed roots were placed in tubes covered with aluminum foil (to protect against fungal growth and illumination) containing 40 ml of sterile distilled water and left in a greenhouse for several days. The plants were then transferred to new tubes and left under the same conditions for several more days before collecting exudates. Once collected, the exudates were stored at 20°C until use.

Solubilization of Crystalline and Soil-Bound Anthracene Using Exudates

Solubilization of crystalline and soil-bound anthracene was achieved according to Yeom et al. (1996). Briefly, concentrated anthracene (25 mg/ml) was dissolved in methylene chloride. Appropriate volumes of each solution were added to a series of 8 ml vials such that the amount of anthracene was at least 2~3 times greater than that solubilized by the exudate. After evaporation of the methylene chloride, 4 ml aliquots of water and exudates were added to the vials.

Table 1. Disappearance of anthracene from unplanted and planted soil.

Day		Unplanted	Alfalfa	Barley	Tall fescue	Orchard grass
				Anthracene ug/g dry soil weight		
0	Avg	647.7	669.1	659.8	668.2	662.1
	SD	119.8	55.8	80.3	19.1	133.9
63	Avg	179.0	68.0	152.1	157.8	189.2
	SD	62.1	21.5	70.1	35.8	58.9
Amount disappearing		468.7	601.1	507.7	510.4	472.9
% Control		100	128	106	108	100

Avg and SD represent the average and standard deviation, respectively.

Given that the amount remaining in the vial was significantly higher than could be solubilized, the loss of anthracene by evaporation was inconsequential. $HgCl_2$, 0.05%, was added to water or exudates to prevent biodegradation during equilibration. Undissolved anthracene was removed from the supernatant fraction by filtration and measured by HPLC as above.

To measure their effect on the solubilization of anthracene, 10 ml aliquots of a solution containing the exudates was added to a series of sealed vials (Teflon-silicon septa) containing contaminated soil that were mechanically shaken for 30 sec. At given intervals, the suspensions were centrifuged for 20 min in conical tubes at 10,000g. An aliquot of the supernatant fraction was analyzed by the above HPLC method.

RESULTS AND DISCUSSION

Evidence for Enhancement of Anthracene Dissipation by Plant

Table 1 demonstrates that anthracene disappeared faster from planted soil than unplanted controls. Specifically, the anthracene disappearing after 63 days with alfalfa (28% relative to the unplanted control) was the highest among the plants tested by far.

Since it is believed that enhancing the dissipation of organic pollutants has mainly resulted from an increase in the number of microbes in the rhizosphere (Reilley et al., 1996), the question arises as to whether the more rapid dissipation of anthracene with alfalfa correlated with the number of soil bacteria. A series of experiments was conducted to answer this question by assessing the bacterial population in the rhizosphere. The number of bacteria in soil planted with alfalfa was higher than unplanted soil, but did not differ significantly from barley and tall fescue (data not shown). In our experiments, the relative increase in bacterial number seemed to depend on the plant species and not to correlate with the enhancement of anthracene dissipation. It was earlier found that bacteria differ in their capability to adsorb polyhydric aromatic hydrocarbons (Stringfellow and Alvarez-Cohen, 1999).

Evidence for Enhancement of Anthracene Dissolution by Plant Exudates

Humic substances, which are mainly responsible for adsorption of hydrophobic contaminants (Laor et al., 1999), can be mobilized by plant exudates in the soil (Nardi et al., 1997). Root exudates contain possible surfactant chemicals such as phenol and organic acids (Vancura, 1964). These reports raise the question of

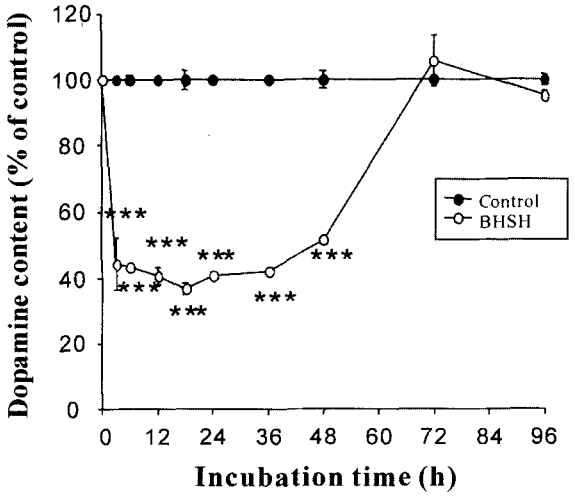

Figure 1. Recovery of anthracene in water or different plant exudates after incubation with crystalline material. Crystalline anthracene was incubated with water or the indicated exudates for 10 days in 8 ml vials (n = 3).

whether plant exudates can affect the bioavailability of crystalline anthracene in the rhizosphere. To test this point, we assessed the solubilization of anthracene by root exudates from alfalfa, barley, and tall fescue. Orchard grass was not included because it failed to enhance the dissipation of anthracene (Table 1). Figure 1 shows that, in keeping with the results in Table 1, alfalfa exudates increased the solubilization of anthracene by 50% while there was little or no change with barley or tall fescue.

The above results prompt the question of whether the increase in the solubilization of anthracene by plant exudates can be extended to soil. We, therefore, measured the release of anthracene from artificially contaminated soils following treatment with plant exudates (Fig. 2A). The results of direct extraction showed that, relative to a water control, the recovery of anthracene was increased up to 20% at day 0 with exudates of alfalfa and barley but not with tall fescue. After incubation for 2 days, the corresponding values on the release of anthracene were 80% (alfalfa), 5% (barley) and 30% (tall fescue) (Fig. 2B). Again, alfalfa proved most active in anthracene release overall.

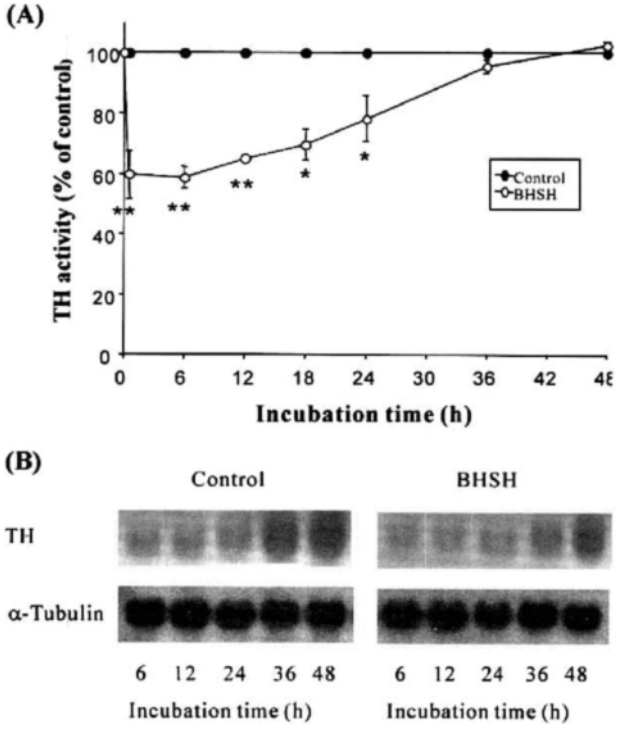

Figure 2. Recovery of anthracene in water or different plant exudates after incubation with contaminated soil (n = 3). Soil was artificially contaminated with anthracene and dried in a hood for 5 days. One g of the dried soil was placed in a series of tubes to which was added 10 ml exudate or water. Three tubes for each treatment were shaken vigorously for 30 s and the anthracene was then extracted either immediately (0 Day) or after 2 days following incubation at room temperature (2 Days).

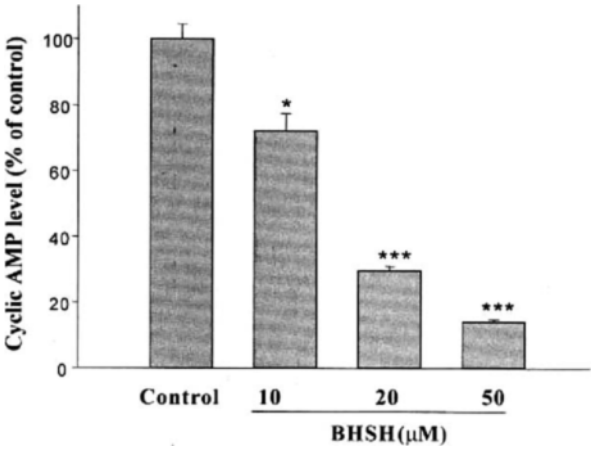

Figure 3. Correlation between the relative enhancement in the dissipation of anthracene from soil by growing plants and the solubilization of anthracene by different plant exduates (n = 3). The ratio of relative disappearance of anthracene and its solubilization ratio were calculated from the data in Table 1 and Fig. 2 (2 Days), respectively. The control, which was calculated by using unplanted soil and water, is a theoretical point.

Correlation between Anthracene Dissipation in Soil and Its Dissolution by Plant Exudates

The above results show that plants dissipate anthracene added to soil to varying extents. As seen in Figure 3, the extent of dissipation after 63 days by the different species correlated with the ability of the root exudates to solubilize anthracene from contaminated soil. Using three plants and an unplanted control, we observed a positive correlation between the relative enhancement of anthracene dissipation in soil tested after 63 days and the relative increase in the solubilization of soil-bound anthracene by the root exudates (Fig. 3).

Remarks

The disappearance of anthracene in contaminated soil was enhanced by growing plants in a manner that correlated with the species. In parallel experiments, plant exudates increased the solubilization of anthracene from either crystalline material or contaminated soil. The present results suggest that the phytoremediation of anthracene depends on the plant species

and, further, on the ability of root exudates to solubilize the hydrocarbon. The latter finding raises the possibility of using exudates to assess plants for their phytoremediation capability.

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