Environmental Pollution 179 (2013) 294–300

Contents lists available at SciVerse ScienceDirect

Environmental Pollution

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

Influence of plant root morphology and tissue composition on phenanthrene uptake: Stepwise multiple linear regression analysis

Xinhua Zhan [a,b,*], Xiao Liang [a], Guohua Xu [a], Lixiang Zhou [a]

[a] College of Resources and Environmental Sciences, Nanjing Agricultural University, Nanjing, Jiangsu Province 210095, PR China
[b] State Key Laboratory of Soil and Sustainable Agriculture, Institute of Soil Science, Chinese Academy of Sciences, Nanjing, Jiangsu Province 210008, PR China

ARTICLE INFO

Article history:
Received 10 November 2012
Received in revised form
1 March 2013
Accepted 30 April 2013

Keywords:
Polycyclic aromatic hydrocarbons
Crop uptake
Root morphology
Root tissue composition
Stepwise multiple linear regression
Phenanthrene

ABSTRACT

Polycyclic aromatic hydrocarbons (PAHs) are contaminants that reside mainly in surface soils. Dietary intake of plant-based foods can make a major contribution to total PAH exposure. Little information is available on the relationship between root morphology and plant uptake of PAHs. An understanding of plant root morphologic and compositional factors that affect root uptake of contaminants is important and can inform both agricultural (chemical contamination of crops) and engineering (phytoremediation) applications. Five crop plant species are grown hydroponically in solutions containing the PAH phenanthrene. Measurements are taken for 1) phenanthrene uptake, 2) root morphology – specific surface area, volume, surface area, tip number and total root length and 3) root tissue composition – water, lipid, protein and carbohydrate content. These factors are compared through Pearson's correlation and multiple linear regression analysis. The major factors which promote phenanthrene uptake are specific surface area and lipid content.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are probably the most ubiquitous hydrophobic organic contaminants (HOCs) on earth, originating primarily from natural and anthropogenic incomplete combustion of organic matter, e.g. forest fires, agricultural burning and fossil fuel combustion. They have been of particular concern due to their carcinogenicity, mutagenicity and toxicity to both human and non-human organisms. In most countries, they have been recognized as priority pollutants (Parrish et al., 2006). Over 90% of PAHs in the environment reside in surface soil (Wild and Jones, 1995). The contamination of soils by PAHs can cause the subsequent contamination of plants grown in these soils. This may pose human and animal health hazards. It has been reported that, in China, over 20% of staple crops contain PAHs that exceed the allowable limits of that country (Zhou and Sun, 2004). Dietary intake has been identified as the principal route of exposure to PAHs for non-smoking people, with plant-based foods constituting a major contributor to total PAH intake (Menzie et al., 1992; Phillips, 1999; Kulhánek et al., 2005). It is important, therefore, to understand the relationship of plant root morphological parameters, tissue composition and PAH uptake for prediction of crop contamination by PAHs, crop selection and development of safe cropping systems and for effective phytoremediation technologies.

Root morphology refers to the surface features of a single root axis, including characteristics of the epidermis such as root hairs, root diameter, root cap, the pattern of appearance of daughter roots, undulations of the root axis, and cortical senescence (Lynch, 1995). Root morphology is an important agronomic and ecologic plant trait related to nutrient acquisition, especially in nutrient deficient environments (Zhang et al., 2011). It has been well documented that root morphology is involved in nutrient acquisition from soil (Sattelmacher et al., 1993). Nitrate uptake rate, for example, is significantly and positively correlated with total biomass, length and area of the below-ground organs of Kentucky bluegrass (*Poa pratensis* L.) (Sullivan et al., 2000). Rice plant P uptake from iron phosphate is significantly correlated to root surface area and root volume as well as to the number of lateral roots (Li et al., 2007). Tap root length and lateral root number directly affect both K uptake and use efficiency of cotton under deficient K condition (Zia-ul-hassan and Arshad, 2011). However, little information regarding the influence of root morphology on PAH uptake is available.

Root tissue composition is another determinant of root ability to take up nutrients and contaminants (Collins et al., 2006). Numerous authors have found that the lipid content or quality is important (Balke and Price, 1988; Simonich and Hites, 1995; Li et al., 2005).

* Corresponding author.
E-mail address: xhzhan@njau.edu.cn (X. Zhan).

0269-7491/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.envpol.2013.04.033

Many predictive uptake models input lipid content as a parameter (Hung and Mackay, 1997; Chiou et al., 2001). In these models, lipid positively correlates to contaminant uptake. Hung and Mackay (1997) and Chiou et al. (2001) also employ root fibre content and carbohydrate content, respectively. Some other tissue constituents such as protein have received very little attention. We are unaware of any research that compares the contributions of root morphology and tissue compositions to PAH uptake. Detailed information on the contributions of root morphology and tissue composition to the intraspecific and interspecific variation of PAH uptake is significant to plant breeding programs since morphological and compositional characteristics are simple and valuable markers for screening, selecting and breeding plants for food safety and phytoremediation.

Although the octanol–water partition coefficient (i.e. K_{ow}) is a well-known important factor in plant root uptake of HOCs (Simonich and Hites, 1995; Barac et al., 2004; Chen and Xing, 2005; Collins et al., 2006), this paper reports our research on plant root morphological and tissue compositional characteristics in relation to root uptake of PAHs. Wheat, soybean, carrot, ryegrass and clover were the crops of choice for our study, based on the fact that wheat, soybean and carrot are common plants used for HOC uptake, and ryegrass and clover are usually employed for phytoremediation of HOC-contaminated soils (Edwards, 1986; Jones et al., 1989; Wiltse et al., 1998; Chiou et al., 2001; Fismes et al., 2002; Fryer and Collins, 2003; Li et al., 2005). The aims of this study are i) to characterize the relationship between PAH uptake and root morphological parameters and tissue compositions; ii) to determine the most important factors influencing root uptake of PAHs among root morphological and compositional characteristics; and iii) to evaluate the contributions of root morphological and compositional characteristics to PAH uptake. To our knowledge, this is the first report to compare the contributions of root morphological and compositional characteristics to PAH uptake.

2. Materials and methods

2.1. Chemical

Phenanthrene, a model compound of PAHs (Huang et al., 1998; Xiao et al., 2004), was purchased from Fluka Chemical Corporation with purity >97%. Its molecular weight is 178.2 g mol^{-1}, and water solubility is 1.3 mg L^{-1} at 25 °C. All organic solvents used for extraction and analysis of phenanthrene were of high performance liquid chromatography (HPLC) grade.

2.2. Plant germination and cultivation

Wheat (*Triticum aestivum* L.), soybean (*Glycine max* L.), carrot (*Daucus carota* L.), ryegrass (*Lolium perenne* L.) and clover (*Medicago sativa* L.) seeds were surface-sterilized in 10% H_2O_2 for 10 min, then thoroughly rinsed with Millipore water (MIlli-Q, Billerica, MA, USA) and germinated on moistened filter paper for 4 d at 25 °C in the dark. The seedlings were transplanted into black plastic pots containing 2500 mL half-strength aerated Hoagland nutrient solution for 3 d and then transferred to full-strength Hoagland solution for a further 3, 9, 15, 21, and 27 d, respectively. The Hoagland solution was prepared with Millipore water and was renewed every week. The initial pH of the solution was adjusted to 5.5. Seedlings were grown in a controlled-climate chamber with a light/dark regime of 16/8 h at 25/20 °C, a relative humidity of 60%, and a light intensity of 400 μmol m^{-2} s^{-1}. After growth in Hoagland nutrient solution, the seedlings were employed in the subsequent phenanthrene uptake, analysis of root morphology and root tissue compositions.

2.3. Root uptake of phenanthrene

Intact seedlings were transferred to 1100-mL beakers containing 1000 mL full-strength Hoagland nutrient solution (pH 5.5). The uptake of phenanthrene was detected after 8 h of uptake in the solutions with 1.0 mg L^{-1} phenanthrene and 0.05% methanol at 25 °C. In order to improve the dissolution of phenanthrene in Hoagland nutrient solution, phenanthrene stock solution, which was prepared with methanol as a solvent, was added. Chapin et al. (1993) have reported that nutrient solution with less than 0.1% methanol has no impact on plant root growth. The numbers of wheat, soybean, carrot, ryegrass and clover seedlings incubated in 1100-mL beakers were 20, 9, 30, 20 and 30, respectively. Each treatment had 3 replicates.

2.4. Analysis of root morphology

Plant samples were removed from hydroponic system and rinsed with deionized water. Individual roots from each sample were then arranged in a transparent organic glass tray containing deionized water to separate the roots and keep them moist, and scanned on a desktop scanner (LA 1600 + scanner, Regent Instruments Inc., Canada) to obtain a digitized tagged image format (TIF) image of the entire sample. These images were analyzed using the WinRhizo system v. 2003b (Instruments Regent Inc., Canada) for total root length, root surface area, number of root tips and root volume. Root specific surface area was described as root surface area (cm^2)/root volume (cm^3).

2.5. Determination of plant root tissue compositions

An aliquot of each plant sample was weighed, dried at 95 °C for 24 h, and weighed again; the difference gave the weight of water, and the percent moisture was calculated. Protein content of roots was determined by the Kjeldahl method applying an empirical factor of 6.25 for the five plants. The main steps were as follows: 0.2 g freeze-dried and ground roots were digested in sulfuric acid, and distilled in 40% sodium hydroxide solution. Ammonia was collected in boracic acid solution, and titrated with 0.01 mmol L^{-1} hydrochloric acid solution.

To detect lipid content, fresh roots (about 5 g) were frozen overnight in liquid N_2 and extracted in a Soxhlet extractor with 100 mL mixed solution of chloroform and methanol (2:1, v/v) for 2 h. The extract was dried in a rotary evaporator, redissolved in 20 mL hexane, filtered through filter paper into a preweighed glass drying tube to remove precipitates, and dried to a constant weight. The weight of the residue was calculated as the plant lipid (Li et al., 2005).

The remainder of the plant root tissue excluding water, lipid and protein was defined as plant carbohydrate.

2.6. Extraction and analysis of phenanthrene

Phenanthrene in the plant tissue was extracted and detected using the method of Zhan et al. (2010). After harvest, plant roots were immersed in methanol for 3 min, rinsed with sufficient Millipore water to remove phenanthrene adsorbed on root surfaces, and wiped with tissue paper (Schwab et al., 1998; Jiao et al., 2007). Roots and shoots were weighed and ground in a glass homogenizer. Homogenized tissue samples were extracted with acetone/hexane (1:1, v/v) mixture by ultrasonication three times (30 min each time). The combined extracts were passed through an anhydrous Na_2SO_4 column with elution of the 1:1 mixture of acetone and hexane. The eluents were then evaporated to dryness at 35 °C in a rotary evaporator and dissolved in 12 mL hexane. Subsequently, the 12-mL solvent was cleaned in a 2-g silica gel column and eluted with 25 mL hexane/dichloromethane (1:1, v/v) solvents. The eluents were evaporated to dryness again and dissolved in 2 mL methanol. Prior to the analysis of phenanthrene by HPLC, all final extracts were filtered with 0.22 μm filter (Kipopoulou et al., 1999). The average recovery of phenanthrene obtained by spiking plant samples with standards is 95.2% for the entire procedure. None of the data reported here has been corrected for recovery.

The HPLC system employed consists of an automatic injector (Waters 717), a binary high-pressure pump (Waters 1525), a UV detector (Waters 2487), and a fluorescence detector (Waters 2475). Separations were performed with a reverse phase Symmetry C_{18} (Ø 4.6 × 150 mm, 5 μm particle) column. The temperature of the HPLC column was kept constant at 30 °C. The mobile phase used was methanol and Millipore water (80:20, v/v), with a flow rate of 1 ml min^{-1}. The injection volume was 10 μL. Phenanthrene was quantified at 293.5/395 (excitation/emission wavelength) and 254 nm for fluorescence detector and UV detector, respectively. Relative standard deviation ($n = 5$) was less than 2.85% for the method. The method detection limit was 48.5 pg phenanthrene. Analytical standards were measured at the beginning of each series of analyses. Internal standard calibration was performed during the HPLC analyses.

2.7. Statistical analyses

Pearson's correlation analysis was conducted to evaluate relationships between phenanthrene uptake and selected root morphological parameters, and tissue compositions. Stepwise multiple linear regression (MLR) was used to identify and quantify the relationships of phenanthrene uptake and root morphological parameters, and tissue compositions. The stepping criteria employed for entry and removal were based on the significance level of the F-value and set at 0.05. Prior to MLR, min–max normalization was carried out for root morphological parameters and tissue compositions to prevent attributes with large numeric ranges dominating those with small numeric ranges. Min–max normalization subtracted the minimum value of an attribute from each value of the attribute and then divided the difference by the range of the attribute. The normalized values lay in the range [0, 1]. The advantage of this normalization is that it preserves all relationships of the data values exactly. It does not introduce potential bias into the data.