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Effect and localization of phenanthrene in maize roots

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HIGHLIGHTS

- Phenanthrene exposure induces perturbations of maize roots maturation.
- Early exposure induces extensive deposition of suberin on exodermis and endodermis.
- Phenanthrene was only patchily located within roots and near suberized exodermis or endodermis.

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ABSTRACT

Polycyclic aromatic hydrocarbons (PAHs) have a toxic effect on plants, which limits the efficiency of phytomanagement of contaminated soils. The mechanisms underlying their toxicity are not fully understood. A cultivation experiment was carried out with maize, used as model plant, exposed to sand spiked with phenanthrene (50 or 150 mg kg^{-1} dw). Epi-fluorescence microscopic observation of root sections was used to assess suberization of exodermis and endodermis and phenanthrene localization along the primary root length. For 10 days of cultivation, exodermis and endodermis suberization of exposed maize was more extensive. However, after 20 days of exposure, exodermis and endodermis of non-exposed roots were totally suberized, whilst PHE-exposed roots where less suberized. Early extensive suberization may act as barrier against PHE penetration, however longer exposure inhibits root maturation. Phenanthrene patches were located only near suberized exodermis and endodermis, which may therefore act as retention zones, where the hydrophobic phenanthrene accumulates during its radial transport.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous persistent organic contaminants. The main sources of soil PAHs contamination include petroleum, oil or creosote leaks from storage facilities. PAHs concentrations (Sum of the 16 EPA-PAH) in soils ranged from 0.3 mg kg^{-1} for arable or grassland soil, 0.9 mg kg^{-1} for forest soils, 4 mg kg^{-1} for urban soil to more than 770 mg kg^{-1} for industrial wasteland soils (Barnier et al., 2014; Wilcke, 2000). The fate of PAHs in soil and groundwater is of great environmental concern due to their toxic properties. Management of contaminated soils by PAHs through rhizodegradation process is promising and has proved efficient in controlled conditions (Joner and Leyval, 2001; Leyval and Binet, 1998; Corgié et al., 2004). However, *in situ* experiments or pilot scale trials have not proved as efficient (Ouvrard et al., 2011) due to limited PAHs solubility and bioavailability in field conditions and due to plant growth limitation induced by low agronomic soil quality and PAHs toxicity. In this context, improving the vegetation of a contaminated site appears crucial to implementing *in situ* rhizoremediation treatment.

PAHs have a toxic effect on plants at different stages of development and the mechanisms underlying their toxicity are not fully understood. At an early stage, germination of sweet corn, waxy corn

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or rice is delayed and their root and shoot elongation is reduced by PAHs exposure (Somtrakoon and Chouychai, 2013). Development of lateral roots of maize is also reduced in case of fluoranthene exposure (Kummerová et al., 2013). Biomass reduction could be due to the occurrence of an oxidative stress following PAHs exposure. Indeed, foliar application of phenanthrene (PHE) induced the production of harmful reactive oxygen species (ROS), which inhibit photosynthesis and consequently biomass production (Ahammed et al., 2012). This ability to induce oxidative stress may cause not only damage to cell structure and functions, but also serve as initiation signals for the process leading to apoptosis (Sun et al., 2011). These morphological changes also affect general plant functioning, such as mineral nutrition or carbon assimilation. Leaves of PHE-exposed maize exhibit higher contents of calcium, phosphorus, magnesium and zinc and lower contents of potassium than non-exposed controls (Dupuy et al., 2015). PAHs exposure results in a decrease of photosynthesis, chlorophyll content or allocation of photosynthates to roots and stems (Ahammed et al., 2012; Desalme et al., 2011).

Roots are the soil/plant interface and are crucial for plant mineral nutrition and water or contaminant uptake. Radial transport of water and nutrients/solutes is affected by various barriers (Soukup et al., 2004; Marschner, 2012). Nutrients enter the root through the rhizodermis cell walls and may enter the symplasm. The next cell layer on the radial movement – the outer layer of cortex - often develops into exodermis (Schreiber et al., 1999). Cell walls of mature exodermis possess apoplastic barrier properties, as its location enables it to shield the cortex cells. Exodermis is present in most plant species and its formation might be induced by environmental conditions such as salinity, drought, nutrient status (Enstone et al., 2002; Soukup et al., 2004) or cultivation medium (Redjala et al., 2011). Another layer with apoplastic barriers is the endodermis. This cell layer separates the cortex from the stele and is present in almost all plant species. The endodermis is crucial for selective transport of solutes into the root stele via the symplasm (Soukup et al., 2004). The function of the root apoplastic barriers is mainly due to their low permeability to ions and water. This low permeability results from an accumulation of hydrophobic compounds during the three maturation stages of the barrier: (1) the formation of Casparian bands, (2) the deposit of a suberin lamellae on the inner surface of endodermal cell wall and (3) the deposit of tertiary cellulosic walls that are often lignified (Schreiber et al., 1999) and perform a mostly mechanical function (Lux et al., 2011). PAHs transfer mechanisms into the root through the apoplastic (through cell walls and intercellular spaces) and symplastic (through cell cytoplasm and plasmadesmata) matter are little understood (Wild et al., 2005; Zhan et al., 2010). Direct visualization of anthracene (ANT) and PHE movements in living maize and wheat roots shows that the prevailing transports are the apoplastic flow and that ANT and PHE are more concentrated in cortex cell walls (Wild et al., 2005).

The aim of this study is to contribute to a better understanding of PHE phytotoxicity, especially its impact on the morphological and structural features of roots. We thus propose (i) to assess the effect of PHE exposure on suberin lamellae formation in endodermis and exodermis (ii) to describe the PHE impact on root architecture and (iii) to locate the PHE inside the root tissues.

2. Materials and methods

2.1. Plant cultivation

Maize cultivation was conducted in sand (Ø 0.4–0.8 mm, Sibelco, Hostun, France). Phenanthrene contamination of sand was achieved for two different contamination levels, 50 and 150 mg kg^{-1} dw. PHE (>97%, Acros Organics) stock solution was prepared in HPLC grade dichloromethane. Sand contamination was performed as follows. A sub-sample of dry sand, representing 10% of the required total mass was spiked at 16% v/w by a PHE solution diluted from the stock solution so as to achieve the concentrations of 500 and 1500 mg kg^{-1}. The complete solvent evaporation of the spiked sand was carried out under a laboratory fume hood. Then, the spiked sand was homogenized by mixing it with the remaining fraction (90%) of non-contaminated sand in order to obtain the final concentration of 50 and 150 mg kg^{-1}. The control treatment was spiked with the same volume of pure dichloromethane and treated similarly. The sand was stored at −20 °C before use.

Maize (*Zea mays* L., cv INRA MB862) was obtained from INRA in Saint Martin de Hinx (France). Maize seeds were washed with TFD9 detergent (Franklab, France) (v/v 20%) for 15 min and rinsed once with deionized water. The seeds were then sterilized by soaking in hydrogen peroxide (v/v 10%) for 3 min and rinsed thoroughly in deionized water. They were germinated on watered cotton in a dark room for three days at room temperature before transplantation.

Cultivation was conducted in glass jars, each containing 400 g of dry sand. To protect substrate and roots from light exposure, the glass jars were wrapped in dark plastic sheeting. Each maize seedling was transplanted into one glass jar with 0, 50 or 150 mg PHE kg^{-1}. For each cultivation time (10 days or 20 days), eleven replicates were prepared. All jars were fertilized once at 80% water-holding capacity (WHC) with adapted Ruakura nutrient solution (Smith et al., 1983) at a pH value of 6.5, containing (in mg L^{-1}): 200 $Mg(NO_3)_2$ $6H_2O$, 692 $Ca(NO_3)_2$ $4H_2O$, 373 NH_4NO_3, 25 KNO_3, 67 KH_2PO_4, 88 K_2SO_4, 2.58 H_3BO_3, 1.40 $MnSO_4.H_2O$, 0.16 $ZnSO_4$ $7H_2O$, 20.77 Fe(III)-EDTA, 0.14 $CuSO_4$ $5H_2O$. The jars were placed in a growth chamber at 23/18 °C day/night temperatures, 16 h of light (325 µmol m^{-2} s^{-1}) and 70% relative air humidity. The water content was stabilized during the cultivation, by weighing and adding deionized water five times a week. Cultivation was performed for 10 or 20 days. Before harvesting, plant shoot lengths were measured in order to check for eventual differences between plants. Five replicates were used for examination of root anatomy and PHE location (see paragraph 2.2) and six replicates were used for measurement of morphological and physiological parameters (see paragraph 2.3).

2.2. Characterization of root anatomy and location of phenanthrene

After 10 or 20 days of cultivation, plants were harvested. Roots were thoroughly cleaned, to remove adhering sand by gentle shaking and water washing. The primary root was embedded in agarose (6%, w/v) as described in Zelko et al. (2012). Semi-thin sections (200 µm thick) were obtained with microtome (model 1000 Vibratome, Leica) at 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90 and 95% from the root apex, the distance from the apex being measured as a percentage of the total length of the primary root. All the observations described below were performed on one section for each distance from the root apex and for each of the five plant replicates (see § 2.1). To characterize the development of exo- and endodermis, suberin lamellae were observed using Fluorol yellow 088 (Sigma) detection modified according to Brundrett et al. (1991). For each distance zone from the root apex, one section was observed using a fluorescence microscope (NIKON Eclipse 80i. with a fiber illuminator Intensilight C-HGFIE). Root tissues were observed using cellulose and lignin auto-fluorescence, with a UV-1A excitation filter (λ_{ex}: 330–380 nm) and the stained suberin lamellae were observed using FITC filter (λ_{ex}: 465–495 nm). Both observations were documented by a black and white camera (NIKON Digital Sight DS-U3). PHE auto-fluorescence was directly observed as a blue-color emission using a UV-1A filter