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Morphological and physiological responses of maize (*Zea mays*) exposed to sand contaminated by phenanthrene

Joan Dupuy, Stéphanie Ouvrard, Pierre Leglize *, Thibault Sterckeman

Université de Lorraine, Laboratoire Sols et Environnement, UMR 1120, Vandœuvre-lès-Nancy F-54518, France
INRA, Laboratoire Sols et Environnement, UMR 1120, Vandœuvre-lès-Nancy F-54518, France

HIGHLIGHTS

- From 50 mg kg^{-1}, phenanthrene induces perturbations in maize functioning.
- It causes a modification of the carbon allocation, favouring the roots.
- It modifies the root architecture, making the roots thicker on average.
- Symptoms suggest water shortage, photosynthesis slowdown, nutritional perturbation.

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ABSTRACT

Phytoremediation is promising, but depends on clearly understanding contaminants' impact on plant functioning. We therefore focused on the impact of polycyclic aromatic hydrocarbons (PAH) on cultivated plants and understanding the impact of phenanthrene (PHE) on maize functioning (*Zea mays*). Cultivation was conducted under controlled conditions on artificially contaminated sand with PHE levels increasing from 50 to 750 mg PHE kg^{-1}. After four weeks, plants exposed to levels above 50 mg PHE kg^{-1} presented decreased biomasses and reduced photosynthetic activity. These modifications were associated with higher biomass allocations to roots and lower ones to stems. The leaf biomass proportion was similar, with thinner blades than controls. PHE-exposed plant showed modified root architecture, with fewer roots of 0.2 and 0.4 mm in diameter. Leaves were potassium-deplete, but calcium, phosphorus, magnesium and zinc-enriched. Their content in nitrogen, iron, sulfur and manganese was unaffected. These responses resembled those of water-stress, although water contents in plant organs were not affected by PHE and water supply was not limited. They also indicated a possible perturbation of both nutritional functioning and photosynthesis.

1. Introduction

Of the wide diversity of metallic and organic pollutants, polycyclic aromatic hydrocarbons (PAH) are among the most worrying because of their ubiquity and toxicity. They are persistent organic pollutants (POP), conferring them the ability to accumulate into organisms and to resist in-soil degradation. Nowadays, PAH-contaminated soil remediation technologies include solvent extraction, chemical oxidation and thermal treatment (Gan et al., 2009). In phytoremediation, a less conventional option, the upper plant is used to contain, degrade or remove pollutants (Sterckeman et al., 2012), offering a cost-effective and environmentally-friendly *in-situ* cleanup technology (Ouvrard et al., 2014). Rhizodegradation, the principal phytoremediation process, consists in an enhanced microbial degradation activity in the rhizosphere zone. However, the establishment of vegetation cover on these contaminated soils may provide additional benefits such as landscaping or valuable biomass production (Licht and Isebrands, 2005). The fate of PAH in soils is well-documented (Pignatello and Xing, 1996; Alexander, 2000; Semple et al., 2003; Ouvrard et al., 2014). Efficiency of plant-assisted degradation for PAH has been proved with controlled laboratory conditions (Chang and Corapcioglu, 1998; Binet et al., 2000; Joner and Leyval, 2003). However, this bioremediation technology has proved hard to apply at the field scale (Ouvrard et al., 2011) due to difficulties in growing and developing

* Corresponding author at: Laboratoire Sols et Environnement, Université de Lorraine/INRA, 2 avenue de la Forêt de Haye, TSA 40602, F-54518 Vandœuvre-lès-Nancy cedex, France. Tel.: +33 383 595 761; fax: +33 383 595 791.
E-mail address: pierre.leglize@univ-lorraine.fr (P. Leglize).

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plants. Many symptoms have been reported, including germination inhibition and decreased biomass production (Henner et al., 1999; Kummerová and Kmentová, 2004; Smith et al., 2006; Kummerová et al., 2012), reduced photosynthetic pigment content (Váňová et al., 2009; Oguntimehin et al., 2010), formation of reactive oxygen species (Alkio et al., 2005; Pašková et al., 2006; Liu et al., 2009), thicker and shorter roots (Kummerová and Kmentová, 2004; Merkl et al., 2005; Kummerová et al., 2013). Yet the intoxication mechanism of PAH in plants remains mostly unknown. Insight into pollutant-impact on plants is essential for vegetating PAH-contaminated sites and may help improve agronomic practices and plant species choice for improving productivity and pollutant degradation.

The aim of this study was to assess morphological and physiological changes of (*Zea mays* L.) exposed to sand spiked with phenanthrene (PHE). Maize and phenanthrene were selected as models, as this plant species has a fast and biomass-productive growth, a large fasciculate root system and has already been studied during PAH exposure (Lin et al., 2007; Kummerová et al., 2012, 2013). Besides, maize is widely cropped in agriculture and is of economic interest. PHE is among the 16 EPA priority PAH and is commonly used in laboratory essays (Pašková et al., 2006; Gao and Collins, 2009; Liu et al., 2009; Zhan et al., 2010; Desalme et al., 2011). Cultivation was conducted for four weeks in a growth chamber under controlled conditions on artificially contaminated sand with levels of PHE increasing from 50 to 750 mg PHE kg^{-1}. Maize growth and organ development were assessed, by measuring both its morphological parameters i.e. biomass, biomass allocation, root architecture and physiological functions i.e. photosynthesis and transpiration by measuring CO_2 and H_2O fluxes and nutrition by quantifying mineral nutrient uptake.

2. Materials and methods

2.1. Growth medium

Cultivation was conducted in sand (Ø 0.4–0.8 mm, Sibelco, Hostun, France), which was spiked with phenanthrene to achieve six different contamination levels: 50, 150, 250, 500 and 750 mg kg^{-1} dw. Spiked sand was chosen as growth medium to maximize PHE availability. PHE stock solution (>97%, Acros Organics) was prepared at 43.1 g L^{-1} in HPLC grade dichloromethane. To achieve homogenous contamination, for each contamination level, 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 final desired concentrations of 50, 150, 250, 500 and 750 mg kg^{-1}. The spiked sand was placed under a laboratory fume hood until complete solvent evaporation, and homogenized with the remaining fraction of non-contaminated sand by quartering. The control treatment was spiked with the same volume of pure dichloromethane and treated similarly.

2.2. Plant material

Before use, maize seeds (*Zea mays* L., cv MB862, INRA, Saint Martin de Hinx, France) were sterilized with TFD9 detergent (Didecyldimethylammonium chlorid, tetrapotassium Ethylenediamine-tetra-acetate, C11-15 secondary alcohol ethoxyles, n-(3-aminopropyl)-n-dodecylpropane-1,3-diamine, potassium hydroxide – Franklab, France) (v/v 20%) for 15 min and rinsed once with deionized water. They were then exposed to hydrogen peroxide (v/v 10%) for 3 min and rinsed three times with deionized water. They were pre-germinated on watered cotton in a dark room for two days at room temperature before experimentation.

2.3. Experimental set-up

Cultivation was conducted in glass jars, each containing 2 kg of dry sand. They were wrapped in dark plastic sheeting to protect substrate and roots from light exposure. One seedling was transplanted into each glass jar with 0, 50, 150, 250, 500 or 750 mg PHE kg^{-1} in six replicates. Jars without seedlings contained sand contaminated at 0, 150 and 750 mg PHE kg^{-1} with three replicates. All jars were fertilized at 80% water-holding capacity (WHC) with adapted Ruakura nutrient solution (Smith et al., 1983), at a pH value of 6.5. The cultivation lasted 28 d, in the following conditions: 16 h of light (325 μmol photons m^{-2} s^{-1}), 20/18 °C day/night temperatures and 70% relative air humidity. Water content was maintained by weighing and adding deionized water every two days and nutrient solution every three additions.

2.4. Measurement of morphological parameters

At the end of the cultivation, the fresh stem, root, and leaf biomass was weighed separately (FBM, in g). Roots were scanned and their architecture analyzed using the WinRhizo® software (Regent Instruments Inc., Québec, Qc, Canada). Visually, necrotic part of each leaf was separated from non-necrotic part. Necrotic and non-necrotic areas were measured with an electronic leaf-area meter (LI-3000, Li-Cor Inc., Lincoln, Nebraska, USA). Sum of the two was referred to as leaf area (LA, in m^2). All the plant samples were freeze-dried for dry biomass measurement (DBM, in g) of each organ. Relative water content (RWC, %) of each plant was determined according to:

$$RWC = \frac{FBM - DBM}{FBM} \times 100 \quad (1)$$

Biomass allocation (BA_{po}, %) to a plant organ was assessed as:

$$BA_{po} = \frac{DBM_{po}}{DBM_T} \times 100, \quad (2)$$

where DBM_{po} was the plant organ dry biomass and DBM_T was the total plant dry biomass.

Morphological parameters were measured, i.e. specific leaf area (SLA, m^2 kg^{-1}).

$$SLA = \frac{LA}{\text{leaf DBM}} \times 10^3, \quad (3)$$

where Leaf DBM was the leaf dry biomass (g).

The estimated leaf thickness (ELT, μm) was calculated (Vile et al., 2005):

$$ELT = \frac{1}{SLA \times LDMC} \quad (4)$$

where LDMC is the leaf dry matter content (leaf dry biomass/leaf fresh biomass mg g^{-1}).

2.5. Leaf gas exchanges

Photosynthesis and transpiration were determined by measuring CO_2 (μmol m^{-2} s^{-1}) and H_2O (mmol m^{-2} s^{-1}) fluxes with a portable gas exchange system (Li-Cor 6200, Li-Cor Inc., Lincoln, Nebraska, USA) on the second leaf. Measurements were performed one time after 25 d of cultivation, when leafs were enough developed.

2.6. Mineral nutrient uptake

Micro and macro element contents were measured in roots and leaves. Dry matter was milled at 200 μm in an agate mortar. A 0.1 g test portion of dry biomass was first digested in a glass tube with