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# Degradation of some representative polycyclic aromatic hydrocarbons by the water-soluble protein extracts from *Zea mays* L. cv PR32-B10

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## HIGHLIGHTS

- *Zea mays* L. cv PR32-B10 has been hydroponically cultivated.
- Protein extracts from *Zea mays* cv PR32-B10 have been used for PAHs degradation.
- A cell-free enzymes approach has been used for PAHs degradation.
- The structures of degradation products have been identified by spectral analyses.
- Kinetics of the degradation processes have been investigated.

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## ABSTRACT

The ability of the water-soluble protein extracts from *Zea mais* L. cv. PR32-B10 to degrade some representative polycyclic aromatic hydrocarbons (PAHs), has been evaluated. Surface sterilized seeds of corn (*Zea mais* L. Pioneer cv. PR32-B10) were hydroponically cultivated in a growth chamber under no-stressful conditions. The water-soluble protein extracts isolated from maize tissues showed peroxidase, polyphenol oxidase and catalase activities. Incubation of the extracts with naphthalene, fluorene, phenanthrene and pyrene, led to formation of oxidized and/or degradation products. GC-MS and TLC monitoring of the processes showed that naphthalene, phenanthrene, fluorene and pyrene underwent 100%, 78%, 92% and 65% oxidative degradation, respectively, after 120 min. The chemical structure of the degradation products were determined by $^{1}$H NMR and ESI-MS spectrometry.



## 1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous contaminants and their diffusion in the environment is a matter of serious concern world-wide. PAHs are dispersed in air, adhering to particulate, in water and soils. The primary sources of PAHs in the environment are the human activities such as, for example, the combustion of biofuels. They can enter the food chain and diet is the primary source of human exposure, with edible oils and fats significantly contributing to human exposure to PAHs. Most of them are classified as probably or potentially carcinogenic to humans by the International Agency of Research on Cancer (IARC) (IARC, 2010; IARC, 2012; Menzie et al., 1992; Kim et al., 2013). In addition, evidence has been provided that metabolic activation of PAHs can induce lung and skin tumours in animals.

As a consequence, much effort has been made to develop procedures capable of removing these substances from soils. Biodegradation of PAHs by many types of bacteria and fungi has been the subject of a number of investigations (Bamforth and Singleton, 2005; Bezalel et al., 1997; Haritash and Kaushik, 2009; Seo et al.,

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2009). Microbial bioremediation, however, suffers from some limitations. Besides the difficult proliferation (Alcalde et al., 2006), often oxidation products derived from bacterial metabolism display enhanced toxicity compared to the PAH precursors themselves (Bamforth and Singleton, 2005). In addition, microorganisms require appropriate environmental growth conditions such as suitable nutrients, temperature, pH, redox potential and oxygen content which, overall, may strongly affect their proliferation (Adams et al., 2015). White rot fungi, in turn, appear to act more effectively because they couple the ability to penetrate contaminated soils with their hyphae and the production of extracellular oxidases (Wang et al., 2009). However, the growth of fungi is inhibited in contaminated soils and overall the remediation process by fungi is extremely slow, needing many days or even more than a month (Drevinskas et al., 2016; Mancera-Lopez et al., 2008). In addition to the above problems, the low water solubility and the tight absorption of PAHs to soil particles often limit their bioavailability and, consequently, their biodegradation. Thus, the search for new and effective methods to remediate contaminated soils is still an important goal.

In this context phytoremediation can be seen as an alternative, cost-effective, approach (Sharma and Pandey, 2014) to PAHs degradation. However, also plant germination and growth are strongly limited by xenobiotic compounds (Kathi and Khan, 2011; Ogboghodo et al., 2004), mainly when these are present in high concentrations. As a consequence, the whole process is generally slow (Pilon-Smits, 2005; Salt et al., 1998; Sharma and Pandey, 2014; Kathi and Khan, 2011) or even totally inhibited, in this case too. In order to overcome these problems, we reasoned that cell-free (Alcalde et al., 2006) plant enzymes could be capable of transforming PAHs into degraded/oxidized products. In particular, it is well known that peroxidase (POD) and polyphenol oxidase (PPO) are involved into the oxidation of phenols (Aniszewski et al., 2008; Hiraga et al., 2001) and also play an important role in the resistance of plants to biotic and abiotic stress (Foyer and Noctor, 2005; Sharma et al., 2012).

According to this strategy, Maize (*Zea mays* L.) was chosen as a model plant in the present study. The ability of *Zea mays* to detoxify sites contaminated by heavy metals has long been known (Lin et al., 2008; Singh and Prasad, 2015) and this plant has recently been included in the list of plant species that can potentially be used in phytoremediation of organic pollutants (Chirakkara et al., 2016). Previous attempts to use *Zea mays* to remediate soils contaminated by PAHs, have been limited by its low germination (Ogboghodo et al., 2004). More recently, Leglize and co-workers (Dupuy et al., 2015, 2016) have studied the effect of phenantrene on the growth of *Zea mays* showing that exposure to phenantrene resulted in decreased biomasses, reduced photosynthetic activity and a reduced nutrient uptake. Natural (rhamnolipid and soybean lecithin) or synthetic (Tween 80) surfactant have been used to overcome the problem of low bioavailability of PAHs in *Zea mays,* but this approach equally met with little success due to the concomitant inhibition of the fluorescence of the chlorophyll (ChF1) and, accordingly, the photosynthetic efficiency of the plant (Liao et al., 2016).

On this ground, we have investigated the ability of the water-soluble protein extracts from shoots and roots of the maize plant (*Zea mays* L. cv PR32-B10) to degrade some representative PAHs, namely naphthalene, fluorene, phenanthrene and pyrene (Dupuy et al., 2015). In addition to the above goal, the structural identification of the PAHs degradation products has also been accomplished in the present study, giving information on the largely unknown oxidative and/or degradation pathways working in *Zea mays*.

## 2. Materials and methods

### 2.1. Plant germination, cultivation and extraction procedure

The plant material used in this work was an Italian hybrid of the commercial corn *Zea mais* L. cv. PR32-B10, produced and marketed in Italy by Pioneer. Seeds were surface-sterilized in 5% (v/v) sodium hypochlorite for 30 min and washed once in autoclaved water. Then, they were imbibed for 24 h in sterile water and allowed to germinate in moistened paper in Petri dishes for 4 days, at 26 °C, in the dark. Seedlings with roots were transplanted into black plastic jars (4 plants per jar) containing 1.5 L half-strength aerated Hoagland's nutrient solution for 4 d and then transferred to the full-strength nutritive solution for 10 d. The nutrient solution was prepared using autoclaved water to ensure a sterile condition and the pH of the solution was adjusted to 5.5. The nutrient solution was continuously aerated and renewed every two days. Each seedling was wrapped with sponge in the lower part of the stems and fixed through a drilled hole in a side plate, which covered the jar to ensure that the seedlings were upright and their roots were immersed just below the surface of the nutrient solution. Maize seedlings were grown in a climate chamber (CLIMAPLANT M2M 650) under controlled conditions [day/night temperature 27/18 °C, photoperiod 16 h light/8 h dark, relative humidity 70 ± 5%, 200 μmol $m^{-2}$ $s^{-1}$ photosynthetic photon flux density (PPFD) at leaf level]. The plants were harvested 14 days after germination.

Each plant was carefully removed from the hydroponic system, rinsed in distilled water, dried on filter paper and weighed on a precision balance. Then, the shoots (S) and roots (R) were separated and the fresh weight (FW) of each part determined. Each of them was divided into two subsamples. The first half portion was immediately frozen in liquid nitrogen and stored at −80 °C for the subsequent enzymatic measurements. The remaining portion was oven-dried at 70 °C to a constant weight, to determine the dry weight (DW).

### 2.2. Determination of enzymatic activities

Frozen shoots and roots were finely mashed in a pre-chilled mortar with pestle, under liquid $N_2$. The powdered tissues (FW) were homogenized in an ice-cold phosphate-buffer solution (PBS 2×, adjusted at pH 7.4, 1:10 w/v), containing 1 mM PMSF, as protease inhibitor, and 1 mM DTT, as protective agents. All procedures were carried out at 4 °C. The homogenate was filtered through four layers of cheesecloth, centrifuged at 12,000 rpm (15 min at 4 °C) and the resulting supernatants were used for the enzyme assays. Protein concentration of the extracts were measured according to the method of Bradford (Bradford, 1976) with bovine serum albumin as a standard and referred to plant DW.

The Guaiacol peroxidase (GPOD E.C.: 1.11.1.7) and Catalase (CAT E.C. 1.11.1.6) enzymatic activities were spectrophotometrically assayed according to Ahammed and co-workers (Ahammed et al., 2012). GPOD activity was measured as oxidation of guaiacol (2-methoxyphenol) to tetraguaiacol ($\varepsilon$ = 26.6 $mM^{-1}$ $cm^{-1}$) at 470 nm (UV/Vis V-630, Jasco Europe s.r.l.). The reaction mixture (1 mL) consisted of 50 mM phosphate buffer (pH 7.0), 1% guaiacol, 12 mM $H_2O_2$ and an aliquot of extract containing 2 μg of proteins. Enzymatic activity was calculated in terms of μmol (guaiacol oxidized) $min^{-1}$ $g_{DW}^{-1}$.

CAT activity was followed measuring the decrease of absorbance at 240 nm, caused by peroxide removal, and employing the molar extinction coefficient for $H_2O_2$ ($\varepsilon$ = 39.4 mM $cm^{-1}$). Each assay was performed in 50 mM phosphate buffer (pH 7.0, 3 mL), 20 μg of protein and 10 mM $H_2O_2$. Enzymatic activity was calculated in terms of μmol ($H_2O_2$ degradation) $min^{-1}$ $g_{DW}^{-1}$.