Journal of Environmental and Applied Bioresearch
Published online 01 October 2014 (http://www.scienceresearchlibrary.com)

ISSN: 2319-8745
Vol. 02, No. 02, pp. 29-35

Research Article **Open Access**

Changes of antioxidant activity and Anatomical in root of *Festuca arundinacea* in response to phenanthrene

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Received: 20 August 2014 / Accepted: 31 August 2014

Abstract

Polycyclic aromatic hydrocarbons (PAHs) are part of the petroleum contaminants in the environment. PAHs are highly persistent in terrestrial ecosystem which adversely affects both plant and human life. The present work aimed to evaluate the effects of phenanthrene (PHE), a three-ring polycyclic aromatic hydrocarbon, on antioxidative enzymes (peroxidase and catalase), morphological and anatomical changes in root of *Festuca arundinacea*. Experiment was carried out under greenhouse condition with 4 treatments (0, 80, 300 and 600 mg.kg-1) and 3 replications during 30 days. The activities of catalase (CAT) and peroxidase (POD) increased as the soil PHE concentration increased. Morphological symptoms of phenanthrene stress were reduction of root length and reduction in root hairs. Also anatomical characteristics of root were affected by PHE contamination. The thickness of epidermis, endodermis, phloem, xylem and root diameter increased while size of cortical cells reduced in compared to the control.

Key words: root anatomy, polycyclic aromatic hydrocarbons, phenanthrene, catalase, peroxidase, *Festuca arundinacea*

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Introduction

PAHs or Polycyclic aromatic hydrocarbons are a class of organic contaminants, that are known to be persistent and ubiquitous in the environment (Gao et al. 2007; Sung et al. 2002). The major sources of PAHs in the environment include petroleum products, creosote and coke products (Cooke and Dennis 1983; Neff 1979). There are various strategies for remediation of PAH contaminated soils. In the past years, phytoremediation has gained acceptance as an effective, inexpensive and non-intrusive technology for removing hazardous pollutants from soil (Gao and Zhu 2003; Pilon-Smits 2005). plant Root tissues have important role in successful phytoremediation of contaminated soil (Chen and Schnoor 2009). The uptake of PAHs by roots routinely depends on the physical-chemical characteristics of the compounds and the structure properties of the plant (Wild et al. 1992; Wild and Jones 1992). Plant can metabolize, voltalize, mineralize chemical or it can sequestrate contaminants in plant structures via lignifications (Sandermann 1992).

The effects of PAHs toxicity to plants are poorly understood. the phytotoxicity of PAHs appears to be depended on plant species and particular PAH (Beak et al. 2004; Henner et al. 1999; Song et al. 2005). Alteration in processes of Physiological and Biochemical in plants as stress responses to PAHs usually is detected earlier than morphological and anatomical changes. Plants respond to presence of the contaminants by altering levels of enzymes concerned with detoxification or degradation of xenobiotics (Greenberg et al. 1997). A common property of various stress factors is their ability to enhance the production of reactive oxygen species (ROS) in plant tissues (Arora et al. 2002). Plants utilize a defense system composed of antioxidant such as peroxidase (POD) and catalase (CAT) to maintain the physiological status of organisms. CAT is primary scavenger of hydrogen peroxide (H_2O_2) in peroxisomes and mitochondria. A rise in POD activity is reported as an early response to various stresses and provide cells with resistance against production of

H_2O_2 (Anderson et al. 1995; Zolfaghari et al. 2010). the involvement of plant enzymes in PAHs oxidation has been demonstrate in several studies (Brady et al. 2003; Chroma et al. 2002; Gunder et al. 1998; Kraus et al. 1999).

Hydrocarbon contaminations can affect on the morphological and anatomical structures (Incot et al. 2008; Maranho et al. 2006). It is reported that the progressional changes in structural components of root cells are influenced by uptake and processing of pollutants (Wild et al. 2005). The anatomical features of plants are very important, because they show adaptive properties of plants under any stress environment. Nevertheless, studies about the effects of PAHs on vegetation anatomy are lacking.

Phenanthrene (PHE) is a tree-ring aromatic hydrocarbon widely distributed in the environment. PHE is mainly used as a model for investigations on the metabolism of PAHs (Narro et al. 1992).
The purposes of the current research were to investigate the effects of PHE on oxidoreductase activities and structural changes in root of tall fescue (*Festuca arundinacea*).

2. Materials and methods

2.1 Plant cultivation and PHE treatment

Uncontaminated Soil samples with no detectable PAHs were collected from the surface (5-20 cm) of experimental field of Tarbiat Modares University in Tehran, Iran. The soils first were air-dried and passed through a 2mm mesh. Selected soil properties are shown in Table 1. The soils were contaminated with PHE (purity > 98%, purchased from Aldrich chemical co). Unlabeled PHE resulting in total concentrations of 80, 300 and 600 $mg.kg^{-1}$ (mg of PHE /kg of dry soil). for the contamination process, soils spiked with various concentration of high purity PHE in methanol (10% total of soil). After evaporation of the solvent, the spiked soils were mixed with un-treatment soils and homogenized. The treated soils were placed into each pot (1.2 kg dry weight soil per pot).

Table 1. Some physiochemical properties of the soil used for the experiment

property	soil
Sand (%)	58
Silt (%)	30
Clay (%)	12
Organic matter ($g.kg^{-1}$)	2.28
Moisture content (%)	.045
pH	7.03
EC($mScm^{-1}$)	1.8

Tall fescue (*Festuca arundinaceae*) seeds was selected for this research, were sterilized with 10% hydrogen peroxide for 10 min and rinsed several times with sterile distilled water. The Seeds germinated on moist perlits for 5 days and then were transferred to the pots and was maintained in greenhouse condition (20-25^oC during the day and 10-15^oC at night). After 30 days, plants were collected and the roots separated and root length was monitored regularly. The root samples were fixed in formaldehyde: acetic acid: alcohol (FAA) for 24 h and then preserved in alcohol (30%) for anatomical studies. some of them freezed in liquid nitrogen and stored at -80^oC for biochemical analysis.

2.2 Analysis of PHE

PHE was extracted from root samples using a 1:1 (v/v) solution of acetone and hexane followed by ultrasonic extraction for 1h. the solvent was decanted, collected and replenished. The samples were sonicated for 1h. this process was repeated three time, and the extracts were combined. The solvents were evaporated and exchange to 1mL acetone, and passed through 2 g of silica gel with 1:1 (v/v) elution of hexane and dichloromethane. The samples evaporated and dissolved in methanol with a final volume of 2 mL (Gao and Zhu 2004; Ling and Gao 2004; Simonich and Hites 1994).

The samples analyzed by GC-FID (Agilent 7890 A/5975C) equipped with a flame ionization detector (FID), split-splitless injector and CBP8 (50m × 0.25mm × 0.12 µm) capillary column. The oven temperature was programmed from 70^oC (1min) to 285 ^oC (35 min) at a rate of 10 $^oC\ min^{-1}$. Helium was used as the carrier gas at a flow-rate of 5ml/min. the injection volume was 1µl.

2.3 Extraction and assay of Enzymes activities

To determine enzyme activities, 0.5 g of root homogenized with chilled pestle and mortar in 50mM ice-cold phosphate-buffere solution (PBS) buffer (pH 7), containing 1% polyvinylpyrrolidone (PVP). The homogenate was centrifuged at 10,000 rpm and 4^oC for 10 min. the resulting supernatant was used immediately to determine the enzyme activities (Liu et al. 2009). POD activity was assayed with guaiacol at 470 nm. The reaction mixture (3mL) consisted of 80 µl enzyme extract, 20 mM guaiacol as donor, 100 mM phosphate buffer, 20 µl 30% (w/v) H_2O_2 as substrate. The reaction was initiated by adding H_2O_2 (Guo 2006). CAT activity was determined according to the method of ultraviolet absorbance. CAT activity reaction system, contained 100 mM phosphate buffer (pH 7.0), 15 mM H_2O_2 and 50 µl enzyme extract. The reaction was initiated by adding H_2O_2 and decrease in the absorbance at 240nm was recorded for 1 min (Liu et al. 2009).

2.4 Anatomical methods:

The common blade was used to prepare cross-sections from roots. The samples were cleared in sodium hypochlorite and then stained by carmin-vest (1% w/v in 50% ethanol) and methyl green (1% w/v aqueous). The prepared samples were photografed with an Olympus BH2 and the measurements were carried out by measurement software with 5 repeats at each part.

2.5 Statistical analysis

The design of experiment was completely randomized and treatments consisted of three replications. Analysis of variance

(ANOVA) was done using MsTATC (Version 2.1), and the level of significant was established at $P<0.05$.

3. Results

3.1 Plant accumulation of PHE

Fig 1 shows PHE concentration found in *F. arundinacea* roots were exposed to differing treatments of PHE. The uptake Magnitude of PHE by roots varied. The results show that PHE concentration in root, on a dry weight basis, were as a function of their concentration in the soil. Content of PHE in roots increased with rising PHE concentration in medium. Maximum PHE uptake observed in treatment of 600 $mg.kg^{-1}$.

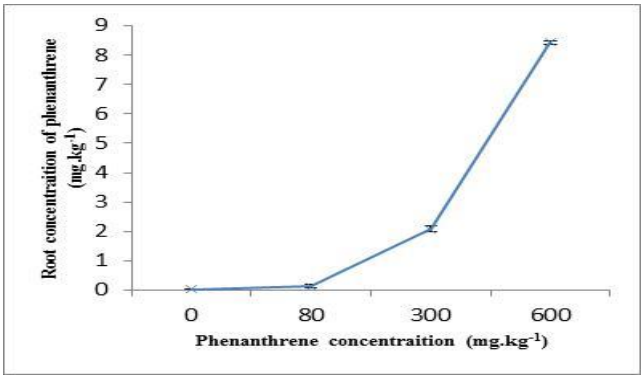

Fig 1. PHE accumulation in roots of *F. arundinacea*

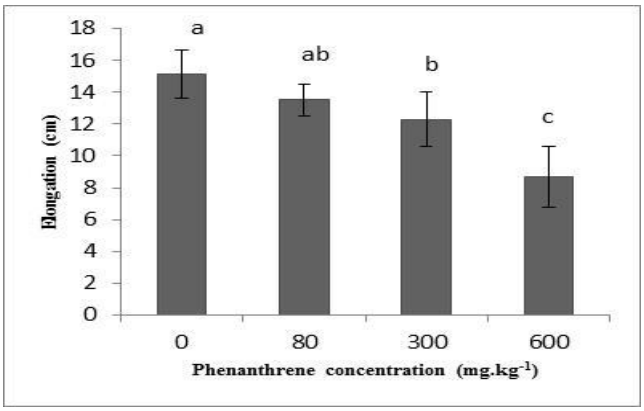

Fig 2. Effect of PHE on root length of *F. arundinacea*. Differnet letters above column indicat significant different between the treatments

3.2 Enzymatic activities

In this study, we assayed the activity of POD and CAT as enzymes involved in antioxidant metabolism. Compared to the control, PHE treatment induced significant changes in activity of these enzymes. Figure 3 indicates the activity of POD in response to PHE. POD activity increased as a result of PHE application. This increase was significant in all treatments than control, and attained a peak at 600 $mg.kg^{-1}$. CAT activity also was significantly enhanced under the influence of PHE treatments and tended to increase with the raise in PHE concentration in the soil (Fig. 4).

3.3 Morphological and light microscope studies:

The obtained results showed that plants exposed to PHE exhibited some stress characteristics, include: significant reduction of root length and reduction in size and number of root hairs (Figs. 2, 5). Microscopy analysis of the treated and un-treated plants upon transverse sections of the root was performed in order to demonstrate the anatomical alteration (Table 2).

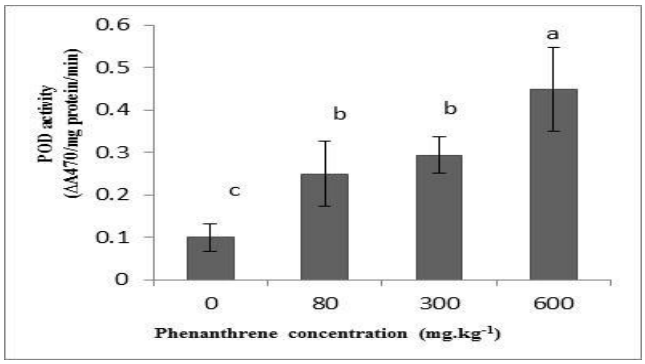

Fig 3. The effect of various of PHE on peroxidase (POD) of *F. arundinacea* root. Differnet letters above column indicat significant different between the treatments

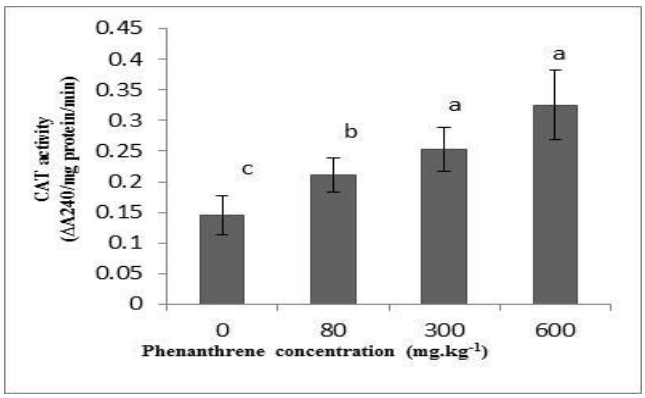

Fig 4. The effect of various of phenanthrene on catalase (CAT) activity of *F. arundinacea* root. Differnet letters above column indicat significant different between the treatments

several tissues of root have changed in PHE treated plants than control. Anatomical alterations of root include size and diameter of epidermis, parenchyma cells, endodermis, vascular bundles, which are all affected by PHE treatment. As it was shown in figure 6, diameter of root, vascular bundles, endodermis and epidermis thickness increased with enhancement of PHE concentration. The control plant had large parenchyma cells but in PHE treated plants the size of parenchyma cells decreased, while their intracellular spaces increased.

4. Discussion

During the past decades, there has been remarkable interest in perception the uptake and accumulation of PAHs by plants. The uptake and accumulation of PAHs by plants has been investigated in several studies (Bakker 2002; Kylin 1994; Simonich and Hites

1994; Wagrowski and Hites 1997). In the present study, PHE content in roots increases with rising PHE concentration in the soil. The Uptake of these pollutants into roots is mainly depend on the lipid content of the roots and octanol-water partition coefficient of compound, generally, The more lipophilicity of contaminant results in the higher root concentration (Wei and Pan 2010).

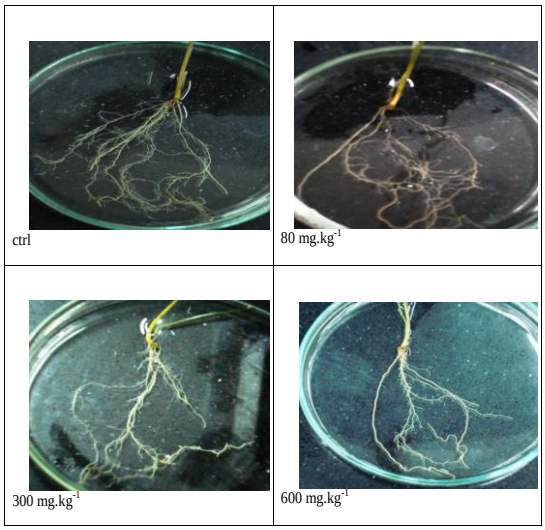

Fig 5.tiff. Phenanthrene causes reduction of root length and reduction in size and number of root hairs

Table 2. changes in some anatomical parameters of *F. arundinacea* root under phenanthrene stress

Treatment (mg.kg^{-1})	0	80	300	600
Epidermis thickness (µm)	11.5 c	12.8 bc	14.7b	17.8 a
Cortical cell (µm)	31.2 a	23.7 b	15.4 c	12.25 d
Endodermis thickness (µm)	3.6 c	5.1 b	5.8 b	7.8 a
Phloem (µm)	3.7 c	3.8 c	5.4 b	7.7 a
Xylem (µm)	4.2 c	6.4 c	6.1 b	8.5 a
Root diameter (µm)	**236.6 d**	**289.5c**	**313b**	**343 a**

Organic pollutants like PAHs can cause oxidative stresses in plants. Excessive production of reactive oxygen species (ROS) is a rapid response in abiotic stresses which can damage cellular component such as proteins, lipids and DNA (Acworth and Bailey 1997; Mei et al. 2009; Wang et al. 2004; Wang et al. 2005). it is known that plants withstand against free radicals by enhancing the antioxidant enzymes activity after exposure to contaminants (Halliwell and Chiroco 1993). In this study, we analyze the enzymatic activity tow oxidoreductases_ CAT and POD. Enzymes POD and CAT are high-molecular, which can eliminate the H_2O_2 formed during stresses (Merzlyak 1999). POD is maybe the most important antioxidant enzyme in plants (Gramss and Rudeschko 1998). The obtained results revealed the activity of CAT and POD enzymes increased under the influence of PHE treatments. The results of this experiment agree with the findings of Muratava et al. (2009) and Muratova et al. (2009). Contact of plant root with contaminants like PAHs stimulates antioxidative activities, which may act as part of an intracellular defense mechanism and/or have a direct effect on the degradation of PAHs in the external medium. So cellular antioxidant system can be regarded as biomarkers of accumulaiting reactive oxygen species (Kraus et al. 1999).

The morpho-anatomical structure of *F. arundinacea* root is affected by PHE in the soil. The roots growth is a convenient indicator of existence of pollutant, because root tissues have immediate proximity to the contaminated environment (Kummerova et al. 2012). In this study we found reduction of root length and reduction in the root hairs in plants exposed to PHE. Reduction of root length is a sensitive response of plant to exposure to chemical substances (Baud-Grasset et al. 1993). This result was in agreement with the findings of Alkio et al. (2005). The roots are in direct contact with contaminants and their properties play an important role in protection (Molina-Barahona et al. 2005).

Plant species have different degrees of sensitivity to the contaminants upon their morphological and physiological characteristics. So, it is important to study the anatomical properties of plants. Anatomical studies revealed changes in the root tissues of plants exposed to PHE. The observed changes were due to the effects of PHE on plant, this changes included: size of epidermis, parenchyma cells, endodermis and diameter of root and vascular bundles. Size of epidermal cells increased as a result of PHE application. This structure acts as a barrier to diffusion and slows the radical movement of PHE into root (Zuniga et al. 2009). It is possible that the detected PHE in *F. arundinacea* roots be due to its strong sorption by the root epidermis; this can occur because roots are made of suberin, a polymer that is able to sorption of PAHs strongly (Briggs et al. 1983; Fismes et al. 2002; Kolattuduky 1980; Schwab et al. 1998). The observed reduction in size of parenchyma cells could be attributed to the low availability of water.

It is reported that Hydrocarbon-contaminated soils exhibit less water and oxygen retention (Merkl 2005). Increasing of intracellular spaces is another tolerance mechanism in response to hydrocarbons toxicity. aeranchyma formation can prevent not only water stress but also lack of oxygen caused by hydrocarbon contaminants (Baker 1970). Size of endodermis cells increased with raise in PHE concentration. The endodermis tissue plays important role in the protection against various stresses. it is suggested that observed alternations in the root endodermis of tall fescue could be a plant strategy to prevent the translocation of pollutant (Enstone 2003).

The obtained results showed that root diameter increased as PHE concentration increased in the soil. This change in diameter of roots is due to the increasing of vascular bundles volume. Vascular elements with larger diameter have a higher hydraulic conductivity and are more efficient in transporting water and minerals (Simonich and Hites 1994; Zimmermann 1983; Zimmermann and Brown 1974).

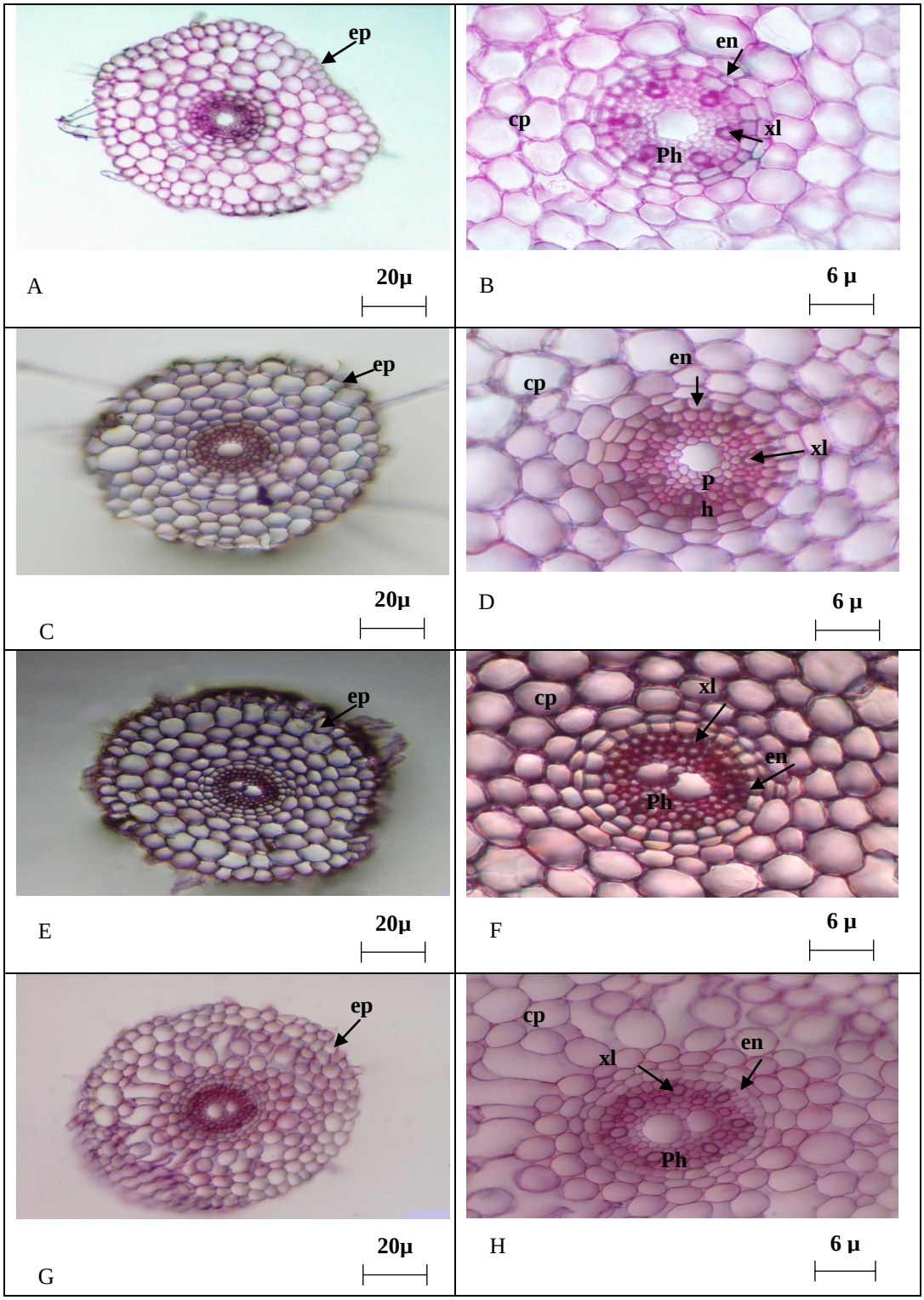

Fig 6.tiff. Root cross sections of *F. arundinacea* under PHE treatments. A,B: control. C,D: 80 mg.kg^{-1}. E,F: 300 mg.kg^{-1}. G,H: 600 mg.kg^{-1}. cp: cortical parenchyma, en: endodermis, ep: epidermis, ph: phloem, xl: xylem.

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