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# The growth, photosynthesis and antioxidant defense responses of five vegetable crops to phenanthrene stress

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ABSTRACT

Polycyclic aromatic hydrocarbons (PAHs) are global environmental problem. To better understand the growth and physiological responses to atmospheric PAHs, we investigated biomass, photosynthetic machinery and antioxidant system in pakchoi, cucumber, flowering chinese cabbage, tomato and lettuce under various levels of phenanthrene (PHE) stress. Foliar exposure to PHE for 14 d resulted in a dose dependent decrease in growth, photosynthesis and chlorophyll contents. With few exceptions, antioxidant enzymes (superoxide dismutase, guaicol peroxidase, catalase, ascorbate peroxidase and glutathione reductase) were upregulated following exposure to PHE. Dose dependent increase in malondialdehyde contents together with $H_2O_2$ accumulation suggested an occurrence of oxidative stress following PHE exposure. However, to some extent, growth and antioxidant defense responses differ from species to species. Difference in defense capacity might result in different tolerance and phytotoxicity among the studied vegetables. Taken together, phytotoxicity of PHE to five vegetables could be sequenced in the following order: pakchoi > cucumber > lettuce > tomato > flowering chinese cabbage.



## 1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental pollutants which are produced mainly from incomplete combustion or pyrolysis of fossil fuel (Nadal et al., 2004). Some PAHs are well-known for their mutagenic, carcinogenic and teratogenic activities (Luch, 2005). Exposure from various sources viz. occupation, smoking, diet (grain, fruits and vegetables), drinking water, and outdoor/indoor air may threaten human health (Fang et al., 2004; Nadal et al., 2004; Luch, 2005). PAHs exist in the atmosphere in the form of solid and liquid aerosols (Korte et al., 2000). The air concentrations of particular PAHs generally lie between $<0.1$ and 100 ng/m$^3$, reaching the greatest levels in urban and industrial agglomerations (Kiss et al., 2001; Fang et al., 2004). Depending on seasons, the mean concentration of individual 3–6 ring PAHs in atmospheric precipitation and aerosol might reach as high as 350 ng/l and 5000 pg/m$^3$, respectively (Kiss et al., 2001). In China, traffic and industry are two major sources of outdoor atmospheric PAHs which is about 3–330 ng/m$^3$ (Guo et al., 2003). Cooking is the major source of indoor air PAHs which stands at 17 ng/m$^3$ (Zhu and Wang, 2003). The concentration of PAHs in the uncontaminated soil could be one to several dozen micrograms per kilogram. However, in the industrialized and urbanized areas concentration may be hundred to a few thousand times higher (Nadal et al., 2004).

PAHs are semivolatile in nature and often transferred from soil to air through volatilization (Collins et al., 2006). They are lipophilic and easily accumulated in the cuticle of leaf. The ability to assimilate toxic substances from air and liquid solutions is an important industrial characteristic of higher plants. However, plants significantly differ with regard to their ability to assimilate toxic compounds (Korte et al., 2000). PAHs get into the inner part of the leaf either through stomata or through the cuticle of epidermis (Wild et al., 2005). Both pathways function concurrently in plants. However, the lower stomatiferous surface of a leaf absorbs remarkably greater amount of surface-active compounds than the upper side and young leaf absorbs more intensely than mature ones (Korte et al., 2000).

Phytotoxicity of a xenobiotic is indicated by its stress responses. The stress responses can be morphological, physiological and molecular. Stress tolerance pathway in plants involves stress recognition followed by signal transduction, gene induction, gene products, protection, repair and finally stress tolerance

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(Rao et al., 2006). It has been reported that PAHs induced oxidative stress as well as decreased photosynthesis in plants (Liu et al., 2009; Oguntimehin et al., 2010). Oxidative stress is the consequence of over production of reactive oxygen species (ROS). ROS play a double role in plant biology, on one hand it acts as an important signaling molecule and on the other hand as toxic by-products of aerobic metabolism under different stresses (Mittler et al., 2004). ROS are highly bioactive and can cause damage to lipid, DNA and protein, leading to cell membrane peroxidation (Rao et al., 2006; Liu et al., 2009; Zhang et al., 2011). However, plants have developed a well-equipped antioxidative defense system consisting of enzymatic and nonenzymatic antioxidants to scavenge ROS (Foyer and Noctor, 2005).

Visible symptoms like white spots, chlorosis, and necrosis accompanied with lower biomass were observed in plants following exposure to PAHs (Liu et al., 2009; Oguntimehin et al., 2010). But effects of atmospheric PAHs on growth, photosynthesis and antioxidant system are poorly understood areas. Most of the studies concerning foliar exposure to PAHs are focused on PAHs accumulation in plant and subsequent phytotoxicity, where antioxidant enzymes were not evaluated (Huang et al., 1997; Wieczorek and Wieczorek, 2007; Oguntimehin et al., 2010). However, this information is pre-requisite to understand the uptake, persistence and distribution of PAHs in plants. Indeed, combined efforts within the fields of physiology and molecular biology are required to find solution for growing plants in the changing global environment (Rao et al., 2006). Hence, we studied the effects of phenanthrene (a three ring PAH) foliar application on growth, photosynthesis, chlorophyll fluorescence, chlorophyll contents, antioxidant enzymes, lipid peroxidation and $H_2O_2$ accumulation in five common vegetable crops viz. pakchoi, lettuce, flowering chinese cabbage, cucumber and tomato. Results from the experiments are expected to give comprehensive and comparative information on stress responses concerning phytotoxicity among the studied vegetables. Susceptible plants may be used as bioindicator for PAHs contamination, while studied parameters will help to better understand the mechanism of plant tolerance to PAHs.

## 2. Materials and methods

### 2.1. Plant materials and growth conditions

Quality seeds of five different vegetable species viz. pakchoi (*Brassica rapa* cv. chinensis), cucumber (*Cucumis sativus* cv. Jin yan 4), flowering chinese cabbage (*Brassica campestris* L. ssp. *chinensis* var. purpurea Bailey) (here after will be mentioned as cabbage), tomato (*Solanum lycopersicum* L. cv. Hezuo 903) and lettuce (*Lactuca sativa* L.) were washed in hot water (55 °C) for 30 min and air-dried overnight. Then the seeds were germinated in a growth medium containing a mixture of peat, vermiculite and perlite (2:1:1, v-v:v) in 72-hole tray. When the first true leaf was fully expanded, seedlings were transplanted in 10 cm diameter plastic pots containing a mixture of peat–vermiculite (2:1, v-v) in a greenhouse with following environmental conditions: temperature 25/17 °C (day/night), mean relative humidity 80%, approximate photosynthetic photon flux density (PPFD) of 800 μmol $m^{-2}$ $s^{-1}$ and photoperiod of 14/10 h (day/night). Plants were watered daily and fertilized weekly with Hoagland's nutrient solution.

### 2.2. Phenanthrene preparation and treatment application

Phenanthrene (PHE) (Sigma-Aldrich, China, purity—98%) was initially dissolved in acetone and then diluted with MilliQ water to get final concentrations of 30, 100 and 300 μM (acetone:water, v-v=1:1000). The concentrations of PHE in the solutions were determined chromatographically (USEPA, 1990). Four weeks after sowing entire foliar region of each group of seedlings was sprayed with each concentration of freshly prepared PHE. Highest precaution as well as care was taken to avoid growing media contamination by PHE. It is to be mentioned that the seedlings (included all species) treated with same concentration of PHE were kept in same open top chamber constructed inside greenhouse with transparent polyethylene sheet. Each treatment group under same species consisted of 12 seedlings. The concentration of acetone used in the current experiment was very low and even a higher concentration (0.5%) than this had no negative effects on tomato seedlings upon foliar exposure (Oguntimehin et al., 2010). Moreover, control seedlings were always sprayed with the MilliQ water containing equal concentration of acetone. The spray was repeated every alternate day and continued upto 2 weeks. Each plant received approximately 20 ml liquid per spray and within this total exposure period each seedling under 30, 100, 300 μM treatment received 140 ml liquid which was approximately 5.35, 17.82 and 53.46 μM PHE, respectively. After 2 weeks of PHE exposure the gas exchange and chlorophyll fluorescence parameters were measured. Then samples were harvested for morphological, biochemical and physiological studies. For physiological and biochemical analyses, leaf samples were immediately frozen in liquid nitrogen and stored at −80 °C temperature until analyses.

### 2.3. Biomass determination

Six plants per treatment were randomly harvested and divided into roots and shoots. After measuring fresh weight of roots and shoots by a digital measuring device, samples were kept in an oven run at 70 °C for 48 h. These dried samples were weighed to record their dry weights.

### 2.4. Photosynthesis measurement

The $CO_2$ assimilation rate (Pn), stomatal conductance (Gs) and intercellular $CO_2$ concentration (Ci) were measured on third leaves by Li-COR 6400 portable photosynthesis system (Li-COR, Lincoln, NE, USA). Six plants were used for each treatment. The measurement was performed within the time period from 8.00 am to 11.00 am maintaining the air temperature, relative humidity, $CO_2$ concentration and PPFD at 25 °C, 80–90%, 400 μmol $mol^{-1}$ and 1000 μmol $m^{-2}$ $s^{-1}$, respectively.

### 2.5. Chlorophyll fluorescence and chlorophyll content measurements

Chlorophyll fluorescence was measured on third leaves after 60 min of dark acclimation period using an imaging pulse amplitude modulated (PAM) fluorimeter (IMAG-MAXI; Heinz Walz, Effeltrich, Germany). Measurement was initiated with dark-adapted leaf tissues characterized by a low, minimum fluorescence emission signal (Fo). Then the leaf was exposed to a strong flash of light (4000 μmol $m^{-2}$ $s^{-1}$) by a 0.8-s pulse to measure maximal fluorescence (Fm). The maximal quantum yield of PSII (Fv/Fm) was calculated using experimentally determined Fo and Fm, where Fv is the difference between Fo and Fm. For determining Fs (steady-state fluorescence yield), an actinic light source (600 μmol $m^{-2}$ $s^{-1}$) was applied to achieve steady state photosynthesis, after which a second saturation pulse was applied for 0.7 s to obtain Fm′ (light-adapted maximum fluorescence). Fluorescence parameters were calculated by FMS-2 on the basis of the dark-adapted and light-adapted fluorescence measurements. The effective quantum yield of PSII ($\Phi_{PSII}$), non-photochemical quenching coefficient (NPQ) and photochemical quenching coefficient (qP) were calculated as (Fm′ − Fs)/Fm′, (Fm/Fm′) − 1 and (Fm′ − Fs)/(Fm′ − Fo), respectively (Genty et al., 1989; Bilger and Björkman, 1990; Vankooten and Snel, 1990). The Fv/Fm, $\Phi_{PSII}$, NPQ and qP were determined with the same leaves as area of interest.

Leaf chlorophyll (Chl a and Chl b) and carotenoids were extracted in 80% acetone and their contents (μg/g FW) were determined colorimetrically according to Lichtenthaler and Wellburn (1983).

### 2.6. Determination of enzyme activity

For extraction of enzymes, 0.3 g of leaf tissues were homogenized in 3 ml ice-cold 50 mM phosphate buffer (pH 7.8) containing 0.2 M EDTA and 2% polyvinylpyrrolidone (w/v). The homogenates were centrifuged at 4 °C for 20 min at 12,000g, and the resulting supernatants were used for the determination of enzymatic activity. Protein contents were determined following the method of Bradford (1976).

Superoxide dismutase (SOD) activity was assayed by measuring the ability to inhibit the photochemical reduction of nitro blue tetrazolium (NBT). One unit of SOD activity was defined as the amount of enzyme required to cause 50% inhibition of the reduction rate of NBT as monitored at 560 nm (Giannopolitis and Ries, 1977). Guaiacol peroxidase (G-POD) activity was determined at 470 nm following the method published by Cakmak and Marschner (1992). The reaction mixture contained 25 mM PBS (pH 7.0), 0.05% guaiacol, 10 mM $H_2O_2$ and enzyme extract. Catalase (CAT) activity was measured as a decline in A240 using the method of Cakmak and Marschner (1992). Ascorbate peroxidase (APX) activity was measured by a decrease in A290 according to Nakano and Asada (1981). Method of Foyer and Halliwell (1976) was followed to determine glutathione reductase (GR) activity. Polyphenol oxidase (PPO) activity was determined by measuring the increase in absorbance at 370 nm using caffeic acid as a substrate (Ruiz et al., 1999).