Chlorophyll fluorescence as an indicator of fluoranthene phototoxicity

M. Kummerová, L. Váňová

Institute of Experimental Biology, Masaryk University, Brno, Czech Republic

ABSTRACT

The effect of the short-time exposure (12, 24 and 48 h) of increasing concentration (0.1, 1 and 10 mg/l) of intact (FLT) and photo-modified (phFLT) fluoranthene on the chlorophyll fluorescence parameters (F_0, F_V/F_M and Φ_{II}) in pea plants (*Pisum sativum* L. cv. Lantra) was investigated. Plants took up both forms of fluoranthene by two different ways, via roots or via leaves. The obtained results demonstrated a significant increase in F_0 and decrease in F_V/F_M and Φ_{II} in plants treated by 1 and 10 mg/l FLT and phFLT. An earlier response to presence of FLT and phFLT in the environment was demonstrated by application on cut leaves. The primary processes of photosynthesis were not significantly influenced by short-time phFLT treatment.

Keywords: fluoranthene; photo-modification; root uptake; foliar application; chlorophyll fluorescence; pea plants

Trace levels of polycyclic aromatic hydrocarbons (PAHs) widely occur in modern ecosystems (Bryselbout et al. 2000). They are formed through anthropogenic pyrolytic processes, but also through natural events like forest fires and volcanic eruption (Wilcke 2000). Their fate is determined by their physico-chemical properties, especially non-polarity and hydrophobicity responsible for their persistence in the environment (Meudec et al. 2006). Most of PAHs have been shown to have mutagenic, carcinogenic and teratogenic effects (Samanta et al. 2002).

PAHs from polluted atmosphere are generally transferred to plants by particle-phase deposition on the waxy leaf cuticle or by uptake in the gas phase through stomata (Meudec et al. 2006). PAHs can also enter plant tissues by partitioning from contaminated soil to the roots and translocation into the shoot (Simonich and Hites 1995). The plant's ability of PAHs uptake, translocation, metabolization and accumulation is a limiting factor for phytotoxicity of these compounds. Polycyclic aromatic hydrocarbons can affect quantitatively and qualitatively several biochemical and physiological processes taking part in biomass production. The biochemical and physiological changes can be detected earlier than the morphological changes.

The properties of PAHs can be changed by modification of their structure (Huang et al. 1993) either abiotically (e.g. photochemically) or biotically (e.g. oxidation supported by cytochrome P450). One of the most important abiotic factors is a solar radiation, especially the ultraviolet wavelengths (UVA 320–400 nm and UVB 290–320 nm). Two different photochemical mechanisms occur during exposure of PAHs to UV-light: photo-oxidation (generally photo-modification) and photo-sensitization (Greenberg et al. 1993). However, absorption of solar radiation by PAHs causes photo-modification of the molecules and leads to the formation of a variety of products (McConkey et al. 1997); some of these products are photostable whereas some are subject to further photo-oxidation, forming dynamically changing mixtures in the environment (Mallakin et al. 2002), which may be more toxic than the parent compounds (Ankley et al. 1999). Due to the higher polarity of these photoproducts their solubility increases and enhances their bioavailability (Huang et al. 1997). The probability of photo-modification of PAHs is high during transportation in the atmosphere. Moreover, vegetation is exposed to solar radiation and dry or wet deposition contaminated with PAHs at the same time. According to Diamond et al. (2000) the risk level of interaction between PAHs and radiation for plants depends both on the concentration of the compounds present and the dose of radiation.

PAHs and their products of photo-modification can affect structures and functions at cellular and subcellular levels (Kolb and Harms 2000). The first target of these lipophilic substances at a cellular level is plasma membrane, where membrane lipids could be oxidized. The disturbance of this membrane and the inner subcellular membranes and changes in enzyme activities may cause an inhibition of photosynthetic and respiration processes (Duxbury et al. 1997).

Photosynthesis is a membrane-bound process and therefore it is highly dependent on membrane integrity. Recently several methods have been developed for measuring the impact of PAHs on photosynthesis based on the evaluation of chlorosis, CO_2 fixation, electron transport rate (Xiao et al. 1997) and oxygen-evolving complex (Hill reaction) (Kummerová et al. 2006).

Photosystem II (PSII) electron transport is one of the most sensitive indicators of damage in the photosynthetic apparatus (Krause and Weis 1991). Recent chlorophyll fluorescence techniques allow to evaluate primary photochemical reactions of photosynthesis, efficiency of PSII and electron transport rate through electron carriers in particular. Chlorophyll fluorescence parameters (initial chlorophyll fluorescence – F_0, potential yield of photochemical reactions in photosystem II – F_V/F_M and effective quantum yield of photosystem II – Φ_{II}) might be used as indicators of stress affecting photochemical pathway of utilization of absorbed light energy (Krause and Weis 1991, Branquinho et al. 1997).

The objective of the present study was to evaluate the short-time effect of increasing concentration of intact and photo-modified forms of fluoranthene (FLT) on chlorophyll fluorescence parameters in pea plants by two exposure ways, root uptake and foliar application. Fluoranthene was selected from the PAHs family as one of the most frequent polycyclic aromatic hydrocarbons in the environment of the Czech Republic (Holoubek 2000). Applied concentrations simulate low (0.01 mg/l) and high (1 and 10 mg/l) environmental loading. In this paper the rate of response of photochemical processes in plants that were supplied by received FLT and phFLT via roots or leaves was compared.

MATERIAL AND METHODS

Fluoranthene preparation. Fluoranthene (FLT; Supelco, USA) was dissolved in acetone (Labscan, Ireland) and ultrafiltered water (FP-H_2O) (1:99 v/v) to the concentration of 1 g/l. This FLT stock solution was delivered to Reid-York nutrient medium in the ratio 1:100 (v/v) to final FLT concentrations of 0.1 mg/l, 1 mg/l and 10 mg/l. Our preliminary experiments proved that the used solvent (acetone) did not affect germination of seeds, growth of seedlings, and other physiological parameters, e.g. primary processes of photosynthesis (Kummerová and Kmentová 2004, Kummerová et al. 2006). Photo-modified fluoranthene (phFLT) was generated by irradiating of intact fluoranthene (FLT) in black container using an UV-Hg medium pressure lamp (MPK Tesla 125W) and a water-cooled borosilicate glass filter to get UV light at $\lambda \geq 290$ nm in order to simulate the emission spectrum of sunlight. The time of incubation (120 min) was sufficient to photomodify more than 90% of the starting material (Huang et al. 1993). The complex mixture of photo-modified FLT was determined using gas chromatography (GC-FID, Hewlett Packard) (Kummerová and Kmentová 2004).

Cultivation of plants. After three days of germination, seedlings of pea plants (*Pisum sativum* L. cv. Lantra) were delivered to the dishes with granulated polyethylene and FP-H_2O. Five-day-old seedlings were then transplanted into cultivation vessels with 2.5 l Reid-York nutrient solution and cultivated for 18 days. Cultivation was done under the natural light conditions (PAR; 400–700 nm) in an air-conditioned glasshouse at the average air temperature of 23 ± 2°C and relative air humidity from 60% to 80%. The nutrient solution was renewed every 2 days and its pH value was regularly adjusted to 6.5. After 18 days of cultivation, plants were put into 500 ml cultivation vessels with Reid-York nutrient medium without FLT (control) or with FLT (0.1 and 1 mg/l) or phFLT (0.1 and 1 mg/l) solution (root uptake). Third and fourth leaves were cut and placed in Petri dishes (10 leaves per dish) on filter paper without FLT (control) or with 5 ml of 0.1, 1 and 10 mg/l FLT or phFLT solution (foliar application).

Chlorophyll *a* fluorescence measurement. A set of chlorophyll *a* fluorescence parameters was determined from *in vivo* analysis of slow Kautsky kinetics supplemented with saturation pulses (recorded by a PAM-2000 fluorometer, Walz, Germany). On dark-adapted (10 min) leaves a weak light of 0.5 μmol/m^2/s was applied in order to determine basic chlorophyll fluorescence (F_0; Bolhár-Nordenkampf and Öquist 1993) accompanied with a saturation pulse (5000 μmol/m^2/s) to calculate maximum capacity of PSII (F_V/F_M; F_V – maximum variable chlorophyll *a* fluorescence

yield in the dark-adapted state, F_M – maximum chlorophyll *a* fluorescence yield in the dark-adapted state; $F_V = F_M - F_0$). Then, actinic light (15 μmol/m^2/s) was applied for 5 min until steady-state chlorophyll *a* fluorescence (F_S) was reached, followed by a saturation pulse (F_M' – maximum chlorophyll *a* fluorescence yield in the light-adapted state). This routine enabled to determine quantum yield of electron transport through photosystem II (Φ_{II}). For definition of the parameters see Roháček (2002). Parameters of chlorophyll *a* fluorescence were measured repeatedly (on the same samples) after 12, 24 and 48 h on fully developed leaves (3rd and 4th leaf) of 6 plants.

Statistics. For statistical evaluation of results, the software STATISTICA 6 (StatSoft, Inc.) was used. The obtained results were means of at least six repetitions of each assessed parameter. The significance of the differences of the average values between the treatments was evaluated by the analysis of variance of simple classification after preceding verification of normality and homogeneity of the variance (ANOVA, $P = 0.05$) or by non-parametric Kruskal-Wallis test. The comparison of means was based on the method of Tukey contrasts or Scheffe test.

RESULTS AND DISCUSSION

Polycyclic aromatic hydrocarbons (PAHs) are widespread environmental pollutants.

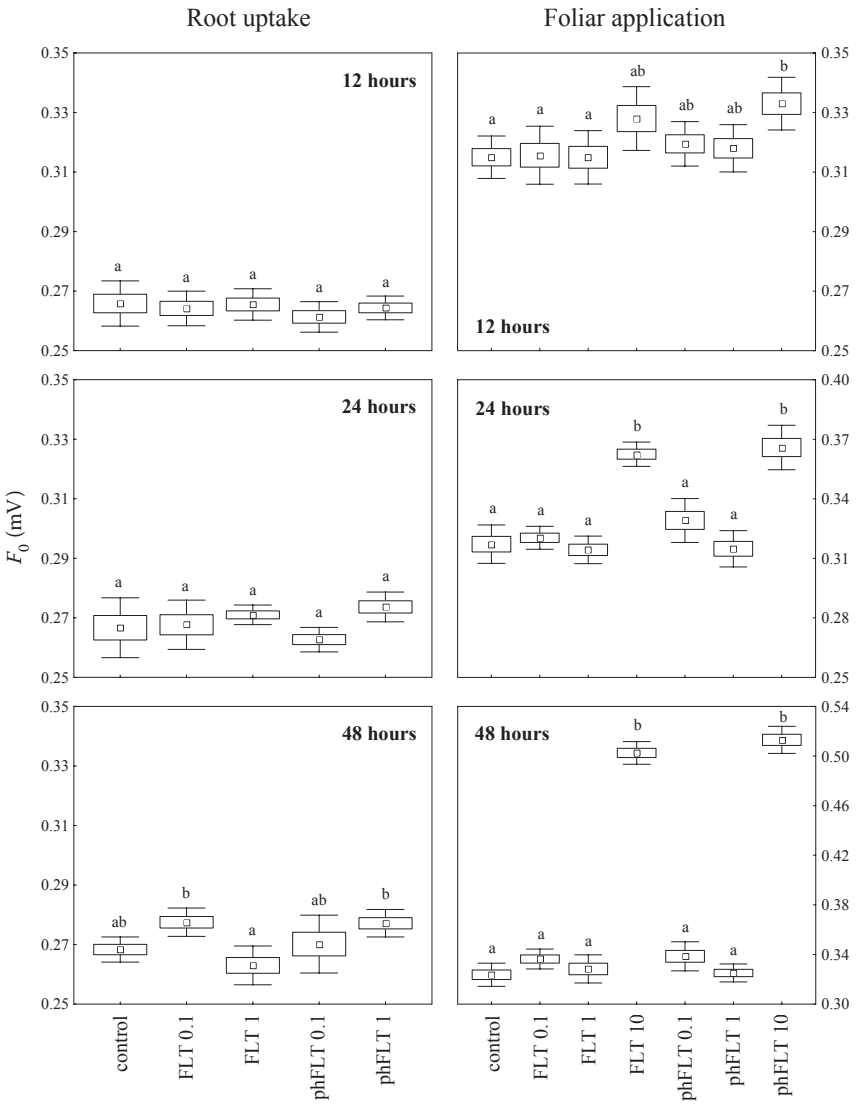

Figure 1. The effect of intact (FLT) and photo-modified (phFLT) fluoranthene on basal chlorophyll fluorescence (F_0) in pea plants. Different letters above the box plots show statistically significant differences between values. The point inside the box represents mean value; borders of the box indicate standard error and bars represent standard deviation (ANOVA, Kruskal-Wallis, Scheffe or Tukey test; $P = 0.05$)

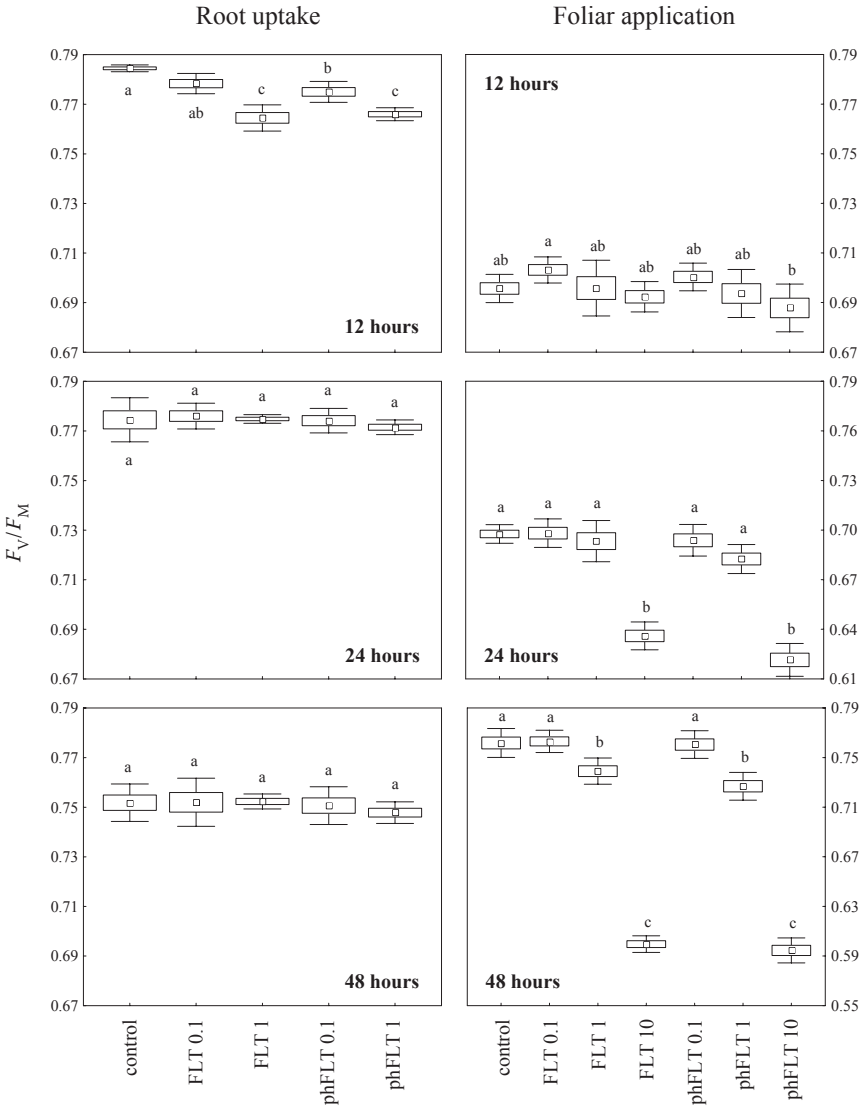

Figure 2. The effect of intact (FLT) and photo-modified (phFLT) fluoranthene on maximal capacity of PSII (F_V/F_M) in pea plants. Symbols and data evaluation as in Figure 1

Photoinduced processes involving these chemicals are environmentally relevant and are observed as increased toxicity of PAHs in the presence of simulated solar radiation and natural sunlight (Huang et al. 1997, Mallakin et al. 2002, Tukaj and Aksmann 2007). Under natural conditions, the plants are exposed not only to the intact PAHs but also to the products of their photo-modification. It has been demonstrated in different plant species that intact and photo-modified PAHs are accumulated preferentially in the thylakoids of the chloroplasts and in the microsomes (Duxbury et al. 1997) and that they affect primary photochemical processes (Huang et al. 1997). An increase (F_0) and decrease (F_V/F_M, Φ_{II}) in the chlorophyll fluorescence parameters indicates invisible damage of the photosynthetic apparatus in plants. Organic pollutants were taken up by plants by different ways, however, most of the studies describe only one way, in view of difficult simulation of possible exposure ways (Duxbury et al. 1997, Huang et al. 1997, Meudec et al. 2006). The values of bioconcentration factor (BCF) support the root uptake and its accumulation in pea plants (Kummerová et al. 2006).

The authors of this paper tried to assess the influence of PAH uptake by roots and leaves. The photosynthetic process is used in many studies for earlier indication of contaminant uptake. The earlier and more expressive response is possibly supposed in the case of foliar uptake of pollutants. The path of pollutants, in their intact and photo-modified forms, to chloroplasts is considerably shorter. Technical possibilities did not allow to

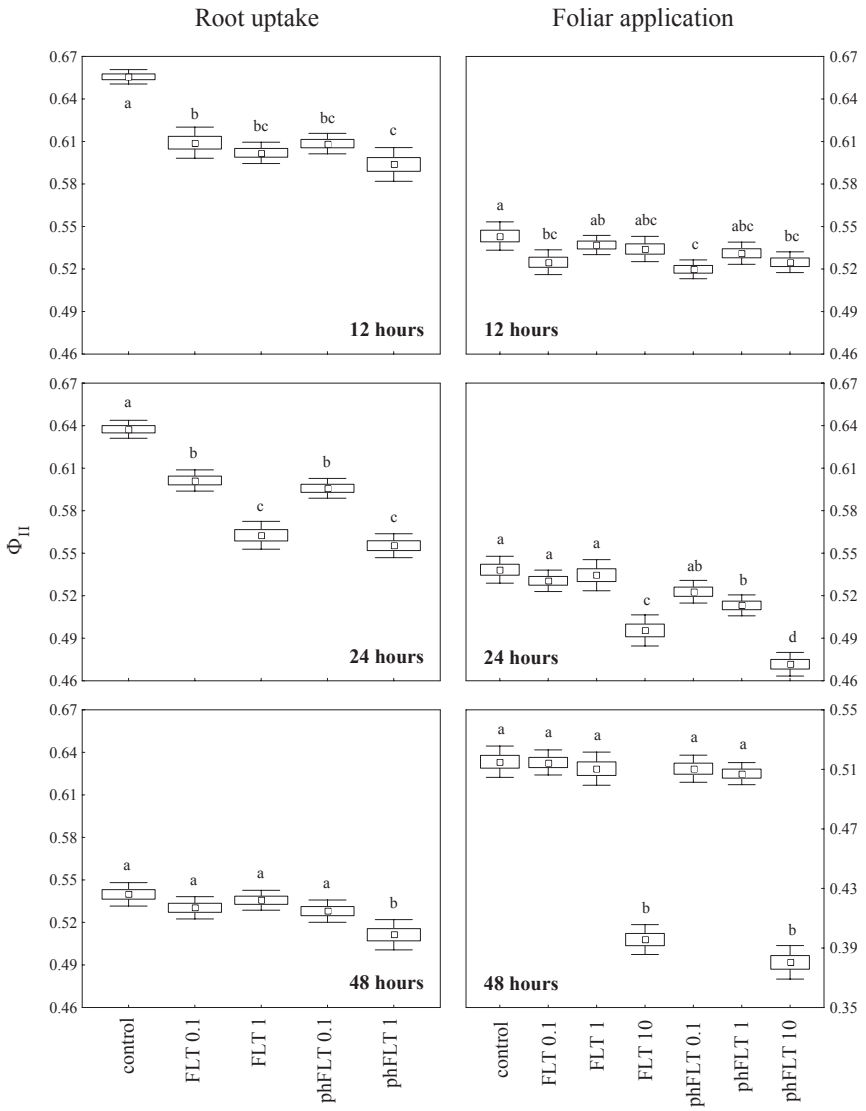

Figure 3. The effect of intact (FLT) and photo-modified (phFLT) fluoranthene on effective quantum yield of PSII (Φ_{II}) in pea plants. Symbols and data evaluation as in Figure 1

apply 10 mg/l FLT and phFLT to nutrient solution in the case of root uptake (left columns of Figures 1–3).

Basal chlorophyll fluorescence (F_0) increased with increasing concentration (0.1, 1 and 10 mg/l) of intact and photo-modified FLT in the environment (Figure 1). The higher values in F_0 recorded in foliar application, as compared with F_0 values in root uptake, are the results of earlier response, more effective influence of FLT if applied on plant shoots. The recorded higher toxicity of phFLT is in agreement with the higher toxicity of photo-modified anthracene found by Mallakin et al. (2002). The possible mechanism responsible for the increase of F_0 is phosphorylation of the light harvesting complexes (LHCs) and their detachment from the core of PSII. Such changes would obviously lead to a decrease in the efficiency of energy transfer from LHCs to the reaction centre of PSII and thus cause an increase in basal chlorophyll fluorescence. The effect of FLT on thylakoid membranes might cause a reversible inactivation of PSII (Huang et al. 1997). The damage of PSII by FLT and phFLT was recorded in lower plants, too (Kummerová et al. 2007). The destruction of the photosynthetic pigment molecules might be another mechanism responsible for F_0 increase (Gensemer et al. 1996, Kummerová et al. 2006). The apparent enhanced toxicity of phFLT might be related to physical and chemical properties of the photoproducts, such as higher polarity and solubility, enhanced bioavailability and reactivity as compared with the intact FLT.

The maximum capacity of PSII (F_V/F_M) is a widely used indicator of stress response of plants (Huang et al. 1997). The value of F_V/F_M decreased with increasing concentration of both forms of FLT (1 and 10 mg/l). A significant decrease in F_V/F_M was caused by 10 mg/l phFLT in foliar application (Figure 2). It can be assumed that the higher impact of the photo-modified FLT is given by its effect on the redox state of the primary electron acceptor plastoquinone A (Q_A). Quinones belong among the main products of photo-modification of PAHs (McConkey et al. 1997). These compounds penetrate thylakoid membrane (Duxbury et al. 1997) and are able to block the electron transport in the location where plastoquinone acts as an electron acceptor or donor (Huang et al. 1997). We assume that the reaction centre of PSII might be also attacked by PAHs at the site of the water-oxidizing complex (oxygen evolving center, OEC) (Kummerová et al. 2006).

The effective quantum yield of the photochemical energy conversion in PSII (Φ_{II}) decreased with increasing concentration of both intact and photo-modified FLT and with duration of the treatment. The greatest decrease was caused by 10 mg/l FLT and phFLT if foliar application was used (Figure 3). Since the Φ_{II} value corresponds to the electron transport in thylakoid membrane, we might attribute FLT-induced decrease of Φ_{II} of photochemical processes of photosynthesis related to electron transport chain in thylakoid membrane. It could be assumed that products of PAHs transformation (metabolization or photo-modification), especially quinones, could affect PSII and electron transport to PSI (Greenberg et al. 1997).

Usually, during the biomonitoring of the influence of important groups of environmental contaminants only the effect of intact PAHs in plants is investigated. The results of untold studies however supported the necessity to assess an influence of the more toxic photoproducts. The results found in our experiments show that the influence of PAHs depends on the type of the compound, on its concentration, and on the duration of exposure.

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Corresponding author:

Mgr. Lucie Váňová, Masarykova univerzita, Přírodovědecká fakulta, Ústav experimentální biologie, Kotlářská 2, 611 37 Brno, Česká republika
phone: + 420 532 146 224, fax: + 420 541 211 214, e-mail: vanoval@sci.muni.cz