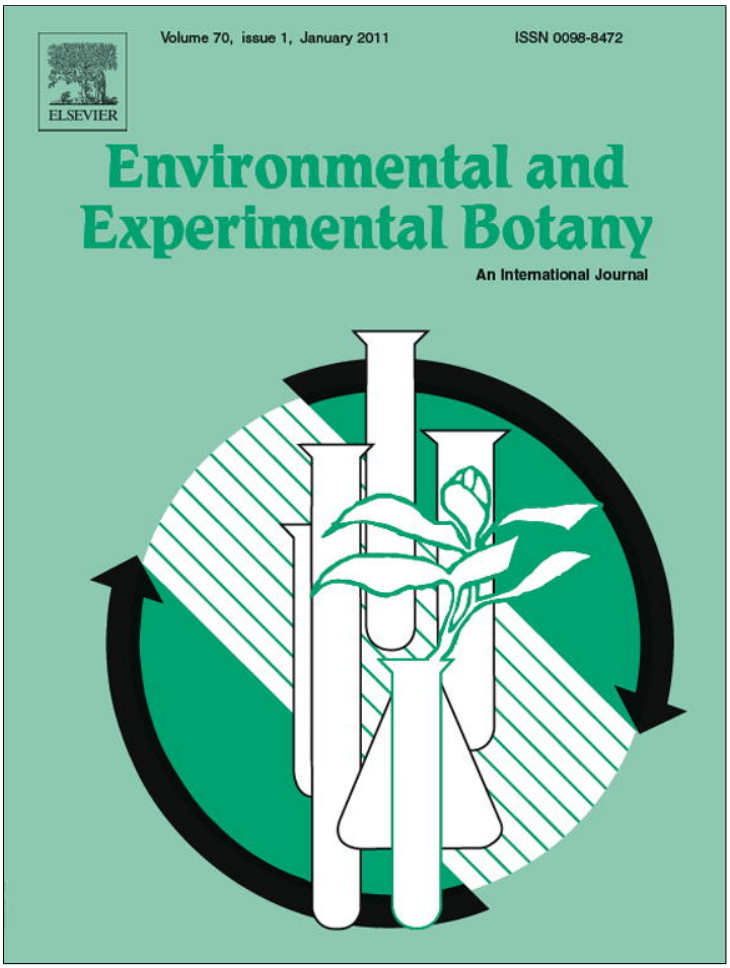

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Effect of fluoranthene on plant cell model: Tobacco BY-2 suspension culture

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ABSTRACT

Polycyclic aromatic hydrocarbons (PAHs) belong to the group of the most important pollutants of the living environment, which are present in air, soils, freshwater, seawater and sediments. They have very substantial effects on all living organisms including plants and animals. Plants represent important point in PAHs food chain entry. Despite the fact that PAHs influence on animals is intensively studied, effect on plants is almost unknown. In our study, action of polycyclic aromatic hydrocarbon fluoranthene on a plant cell experimental model – tobacco BY-2 cell suspension culture – was studied. BY-2 cells were exposed to the fluoranthene in concentration range from 0 to 1000 μM, duration of treatment was 120 h, respectively 5 days. Samples were collected in the strictly defined time intervals of 24 h. Exposure of the BY-2 cells led to significant changes in viability, changes in autofluorescence due to accumulation of fluoranthene in lipophilic cell compartments, especially biomembranes, and production of reactive oxygen species, which resulted in damage of biomembranes and disruption of their semipermeability and initiation of process of programmed cell death. Obtained results bring new knowledge about phytotoxicity of fluoranthene and mechanism of its action.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are lipophilic chemical compounds consisting of fused aromatic rings without heteroatoms. They are widely distributed organic pollutants, which are able to enter a soil as well as aquatic systems with subsequent deposition in the living organisms, especially thanks to their lipophilicity. They are formed anthropogenically during incomplete combustion of organic materials including fossil fuels; steel and iron industry, where PAHs are released from iron and steel making in foundry industry; coke manufacturing and mold poring and cooling belong to the group of the most important sources (Bhargava et al., 2004; Ciaparra et al., 2009; Dellantonio et al., 2010; Fang et al., 2004; Nizzetto et al., 2008; Tsai et al., 2001b,c, 2002). Petrochemical industry belongs to the most important producers of PAHs. Carbon black as important commodity and source for manufacturing of paints and lacquers represents important source of PAHs (Achten and Hofmann, 2009; Tsai et al., 2001a). Natural sources, such as forest fires and volcanic eruptions contribute also to their releasing into the living environment (Bourotte et al., 2009; Gabos et al., 2001; Yang et al., 2002). PAHs are worldwide distributed not only in air, soils, waters, but also in river and ocean sediments, and sludge (Fuoco et al., 2005; Gambaro et al., 2004; Ravindra et al., 2006; Thompson et al., 2002; Wilcke, 2000). They are able to entry food chain. Composting, which is widely employed for treatment of organic wastes, can be one of the most important entries of PAHs to food chain, especially in connection with the agriculture (Carlstrom and Tuovinen, 2003; Eom et al., 2007; Lashermes et al., 2010; Yang et al., 2010). Due their lipophicity and low water solubility, PAHs in waters are bound to suspended particles and accumulate in bottom sediments (Tam et al., 2001); subsequently, they are accumulated in tissues and organs of wide range of organisms, including freshwater and marine organisms, especially of those, which are associated with sediments (Fabbri et al., 2006). On the other hand, organisms capable of PAHs biodegradations are known. The most important are bacteria (*Pasteurella*), fungi (especially not only *Zygomycota*, but also *Basidiomycota*, such as *Pleurotus ostreatus*), and algae (*Cyclotella, Nitzschia*, *Skeletonema*) (Hong et al.,

Abbreviations: DHE, dihydroethidium; FDA, fluorescein diacetate; FLT, fluoranthene; HPLC, high performance liquid chromatography; PAHs, polycyclic aromatic hydrocarbons; PCD, programmed cell death; PI, propidium iodide; MDA, malondialdehyde; PCD, programmed cell death; ROS, reactive oxygen species.

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2008; Liu et al., 2006; Pozdnyakova et al., 2006; Salicis et al., 1999; Sepic et al., 2003; Schutzendubel et al., 1999; Thongkred et al., 2011; Valentin et al., 2007). Recently, new technological procedures for removing of PAHs are intensely studied (Hwang et al., 2007; Sayara et al., 2011; Sponza and Gok, 2010).

PAHs are at the focus of the interest due to their toxicity. Some PAHs appear to be non-toxic; some of them are activated biologically through cytochrome P450-mediated monooxygenation or photochemically, especially by the UV radiation, which leads to formation of more reactive PAHs structures (Ren et al., 1994). Benzo[*a*]anthracene and chrysene, benzo[*b*]fluoranthene, benzo[*j*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, benzo[*ghi*]perylene, coronene, dibenz[*ah*]anthracene, indeno[1,2,3-*cd*]pyrene and ovalene are the most toxic PAHs, which have carcinogenic and mutagenic effects. This fact connected with the DNA damage and formation of PAHs-DNA adducts was demonstrated on *in vitro* (animal as well as human cell lines) and *in vivo* models in tissues and organs of organisms exposed to PAHs (Cavallo et al., 2006; Knuckles et al., 2004; Qu and Stacey, 1996; Taioli et al., 2007; Toyooka and Ibuki, 2007; Walker et al., 2007). Connection between different types of malignant tumors and PAHs has been demonstrated (Armstrong and Gibbs, 2009). One of the recent studies demonstrates fact than activation of some PAHs, which were considered non-toxic without UV radiation activation (e.g. benzo[*a*]anthracene, fluoranthene or pyrene), is not necessary to DNA damage. PAHs exhibit ability to induce oxidative stress (Sun et al., 2011). Oxidation stress is connected with generation of the reactive oxygen species (ROS), which may serve not only as signals of initiation of the processes leading to apoptosis, but also with damage of crucial cell structures and functions. Human exposure may occur via environmental pollution, processed foods, contaminated water, and green vegetable (Camargo et al., 2008; Camargo and Toledo, 2003; Schoket, 1999).

Plants are the important PAHs accumulators (Kacalkova and Tlustos, 2011). They enter plants either directly via stomata, or indirectly via root system (Kuhn et al., 2004). Lipophilic structures covering plant surface, especially wax layer and cuticle, not only represent places of PAHs accumulation and possible further transport, but also stabilization against UV radiation activation (Desalme et al., 2011; Oguntimehin et al., 2008; Oguntimehin and Sakugawa, 2008; Ratola et al., 2011). It is hypothesized that PAHs are accumulated in liphophilic compartments of cells, especially in biomembranes. Here they can be activated after sunlight activation with subsequent damage of cell structures including DNA (Ren et al., 1994). Interactions with processes of growth and especially photosynthesis with symptoms of chlorosis and necrosis (Oguntimehin et al., 2010), reduction of the photosynthetic rate and photosynthetic pigments (Kummerova et al., 2001, 2006). Fluoranthene-induced changes in root and stem anatomy – formation of lysigenous intercellular spaces – were demonstrated in the work of Vanova et al. (2011). In addition, negative effects of PAHs on seeds germination and subsequent seedlings development, especially in connection with the photoinduction expressed as a decrease of fresh weight, were demonstrated (Kummerova and Kmentova, 2004; Kummerova et al., 1997; Maliszewska-Kordybach and Smreczak, 2000; Sverdrup et al., 2003). Nevertheless, effect of PAHs on the plants on cell level is still predominantly unknown, except of studies of some PAHs on model plant – *Arabidopsis thaliana*, where induction of oxidative stress was demonstrated (Kolb and Harms, 2000). Fluoranthene (FLT), member of PAHs group, is used as model for investigation of PAHs toxicity, especially because of its reduced toxicity in comparison with other PAHs.

To better understanding the biochemical and cytological responses to FLT on cell level, tobacco BY-2 cells, which are usually compared to HeLa cells, as the most suitable plant cell model, were exposed to fluoranthene (Nagata et al., 1992). We worked with hypothesis, whether FLT is able to induce formation of ROS that are closely connected with damage of cell structures including lipid peroxidation and with processes of PCD. Changes in cell structure and viability as well as nuclear architecture including DNA fragmentation and processes of PCD were monitored. In addition, ability of FLT to generate reactive oxygen species with subsequent lipid peroxidation was investigated. Due to difficultness of PAHs analytical determination in biological samples because of their complexity, method for their rapid and accurate determination in biological samples represented by cultivation media and BY-2 cells was developed and used.

2. Materials and methods

2.1. Chemicals

Standard of the fluoranthene (purity 98.5%) was purchased from Sigma–Aldrich (USA). Murashige and Skoog cultivation medium including macro- and microelements and vitamins was purchased from Duchefa Biochemie B.V. (Denmark). All other chemicals, including fluorescence probes, chemicals for assays and solvents – ACS water and dimethylsulfoxide (DMSO) were obtained from Sigma–Aldrich (USA) unless otherwise indicated.

2.2. BY-2 cell suspension culture

Nicotina tabacum L. cv. Bright Yellow-2 suspension-cultured cells (BY-2) were grown in liquid medium (Murashige and Skoog, 1962) modified by Nagata (Nagata et al., 1992) under constant shaking (130 rpm) at 27 °C in the dark in 250 ml Erlenmeyer flasks. Cells in exponential growth phase were exposed to the fluoranthene added into cultivation medium in the form of stock solution (1 mg ml^{-1} in DMSO) in concentration range 0, 50, 100, 250, 500 and 1000 μM. As a positive control, BY-2 cells treated by DMSO (1%, v/v) were used. Samples were collected at 24 h intervals for 120 h.

2.3. Cell observation

Cell viability. The viability of the cells was measured by the addition of the fluorescein diacetate (FDA, Sigma–Aldrich, USA) and the propidium iodide (PI, Sigma–Aldrich, USA). The sample of the cell suspension culture (20 μl) was supplemented to volume of 50 μl by MS cultivation medium and incubated for 5 min at room temperature with FDA (2.4 μmol l^{-1}) and PI (30 μmol l^{-1}). PI as nucleic acid stain penetrates through damage cell membranes and intercalates DNA, so PI positive cells are dying or death. Living cells metabolize FDA to fluorogenic substrate fluorescein, so they emit green light after a excitation. The percentage of viable and death cells was evaluated by counting using the fluorescent microscope (Axioscop 40, Zeiss, Germany) equipped with the broad spectrum UV excitation, from each series was evaluated 10 random fields (minimally 1000 cells) in the microscope and the viability was determined in triplicates.

Nuclear architecture and mitotic index. For nuclei observation and mitotic index determination, cells (4 ml) were treated by adding 4 ml PEM-buffer (100 mM PIPES, 10 mM EGTA, 10 mM $MgCl_2$, pH 6.9) containing formaldehyde (4%, w/w; all Sigma–Aldrich, USA). Fluorescent probe Hoechst 33258 (Sigma–Aldrich, USA) was used. One thousand nuclei in each preparation were observed using the fluorescent microscope (Olympus AX 70, Germany) equipped with the broad-spectrum UV excitation. Each morphological changes as well as mitotic cells in individual mitotic phases were expressed as a percentage of the total cells, from each series, 10 random fields (minimally 1000 cells) were evaluated in triplicates.

ROS determination. To determine reactive oxygen (ROS) production and intracellular $O_2^{\bullet -}$ levels, oxidative fluorescence probe dihydroethidium (Sigma–Aldrich, USA) in the combination with MitoTracker Green FM (Molecular Probes, USA) was used. The cells were incubated with MitoTracker Green FM (75 nmol l^{-1}) for 40 min at the room temperature and the dark. After this incubation time, dihydroethidium (10 μmol l^{-1}) was added and the incubation was continued for next 20 min under the same conditions. After it, the cells were twice washed by fresh MS cultivation medium; fluorescence was monitored by using the Olympus AX 70 fluorescent microscope equipped with the broad spectrum UV excitation, from each series was evaluated 10 random fields (minimally 1000 cells) in the microscope and the viability was determined in triplicates.

2.4. DNA fragmentation

A total genomic DNA of BY-2 cell was isolated by the use of the Power Plant DNA Isolation Kit (MoBio, Carlsbad, USA). Concentration of isolated DNA was determined using UV spectrophotometer (Bio Photometer, Eppendorf, Germany) and the purity was determined as 260/280 nm and 260/230 nm rate. 2.5 μg of DNA was separated using 1.5% agarose gel (Agarose I, Amresco, USA) using TBE buffer (45 mM Tris–borate, 1 mM EDTA; all Sigma–Aldrich, USA). The electrophoresis ran under the constant voltage 5 V cm^{-1} for 40 min. Visualization was performed on the EBW-20 UV transilluminator (λ = 312 nm, Ultra–Lum, USA) using ethidium bromide staining (aqueous solution, 0.15 μg ml^{-1}, Sigma–Aldrich, USA).

2.5. Malondialdehyde determination – TBARS assay

For the determination of the lipid peroxidation, Thiobarbituric Acid Reactive Substances (TBARS) Assay described by Stewart and Bewley was used (Stewart and Bewley, 1980). Briefly: a frozen sample of BY-2 cells washed by fresh liquid cultivation medium in liquid nitrogen was immediately crushed in pre-chilled mortar and pestle. Homogenized sample was resuspended in MS cultivation medium (50 mg ml^{-1}) and sonicated for 15 s over ice (K5, Kraintek, Czech Republic). Whole homogenate was used for the MDA determination. Sample was mixed with the same volume of 0.5% (w/v) thiobarbituric acid solution containing 20% (w/v) trichloroacetic acid. The mixture was heated (95 °C, 30 min); reaction was terminated by abrupt placement in an ice-bath. The cooled mixture was centrifuged (10,000 rpm, 10 min, 5415R, Maneko, Czech Republic) and subsequently, absorbance of supernatant was measured (λ = 532 nm). TBARS concentration was estimated as a malondialdehyde equivalent using the standard curve.

2.6. HPLC analysis

Sample preparation. Sample of four milliliters of cell suspension culture was pipetted into plastic tube and centrifuged (6000 rpm, 15 min). Supernatant was removed; cells were washed with fresh MS liquid cultivation medium. 5 ml of mixture of benzene and methanol (3:1, v/v) was added and obtained mixture was homogenized – sonication for 30 min with subsequent homogenization by the ultrasonic homogenizer followed (homogenizer UP52H, Maneko, Czech Republic). Obtained sample was centrifuged (5000 rpm, 30 min); supernatant was evaporated at vacuum evaporator (IKA-RV-10, Fisher Scientific, Czech Republic). For determination of FLT in the sample of cultivation medium, sample of 4 ml of supernatant was evaporated at vacuum evaporator. The remnant was dissolved in acetonitrile (300 μl) and used for analysis.

HPLC system and conditions. HPLC-ED system composed of two chromatographic pumps Model 582 ESA (ESA Inc., United Kingdom) with the operating range from 0.001 to 9.999 ml min^{-1}, the chromatographic column with reverse phase Gemini NX C18 (100 × 2.0; 3 μm particle size, Phenomenex, USA) and the UV detector (Model 528, ESA, USA). Sample (20 μl) was injected automatically by the autosampler (Model 542, ESA, USA), which included warming room for a column. Column temperature was set at 25 °C. Mobile phase consisted of A:acetic acid (0.1 M) and B:methanol (100%), flow rate 0.3 ml min^{-1}. Substances were eluted by the following gradients: 0–6 min (70% B), 6–10 min (100% B), and 10–13 min (100% B). Detection was at 275 nm. Statistical parameters were as follows: LOD = 4.87 ng, LOQ = 16.23 ng, recovery = 99.58%.

2.7. Mathematical and statistical analysis

STATGRAPHICS® (StatisticalGraphicsCorp.®, USA) was used for statistical analyses. Results are expressed as mean ± SD unless noted otherwise. Values $p < 0.05$ were considered significant. For mathematical analysis, Matlab® (MathWorks, USA) was used.

3. Results

In this work, we were focused on the effect of fluoranthene on the model cell culture – BY-2. Negative effect of fluoranthene on BY-2 culture was determined. Based on the obtained results, it can be concluded that FLT is able to affect BY-2 tobacco cells viability and induce processes leading to the PCD. Processes connected with the PCD are in the case of this polyaromatic hydrocarbon closely connected with the production of reactive oxygen species, which are able serve both as signals and damage biomembranes with subsequent disturbance of their selective permeability. Significant FLT accumulation in different cell structures was also determined.

3.1. BY-2 cell viability

In the first step, we were aimed at phytotoxicity of FLT on tobacco BY-2 cells, whose determination was based on the double staining using fluorescent probes fluorescein diacetate and propidium iodide. This method results from the esterases activity (FDA) and disruption of biomembranes, which is typical for dying or death cells. As it is shown in Fig. 1A, exposition of tobacco BY-2 cells to fluoranthene in concentration range 0–1000 μM for 0–120 h led to the significant reduction of cell viability in dose-dependent as well as time-dependent manners. First changes in BY-2 cells viability were recorded already 24 h after exposition in 50 μM FLT concentration respectively. With increasing time after FLT application, decreasing BY-2 cells viability was well evident. After 120 h (5 days) of FLT exposure, viability of control BY-2 cells was about 99%, contrariwise, viability in the lowest FLT concentration (50 μM) was about 78%. In the highest FLT concentration (1000 μM), only about 9% of BY-2 cells were alive. As positive control, BY-2 cells treated by dimethyl sulfoxide were used. Choice of DMSO resulted from its usage as FLT solvent. In comparison with the control, DMSO did not affect BY-2 cells viability. For prediction of survive of BY-2 cells after FLT treatment, obtained data were mathematically processed and expressed in graphical models (Fig. 1B).

Application of fluoranthene led to the significant changes not only of cell viability, but also of the activity of esterases. In despite the fact that higher FLT concentrations led to significant reduction of cell viability, whose determination was based on esterases activity and biomembranes permeability, occurrence of transient type of BY-2 cells was recorded (Fig. 1B). As it is shown in Fig. 2A, BY-2 cells could be divided into three categories based on FDA-PI staining: (i) BY-2 cells, which demonstrated esterase activity without disruption of permeability of biomembranes (positive FDA, negative PI staining, alive cells), (ii) BY-2 cells without evident esterase activity and without disruption of permeability of biomembranes

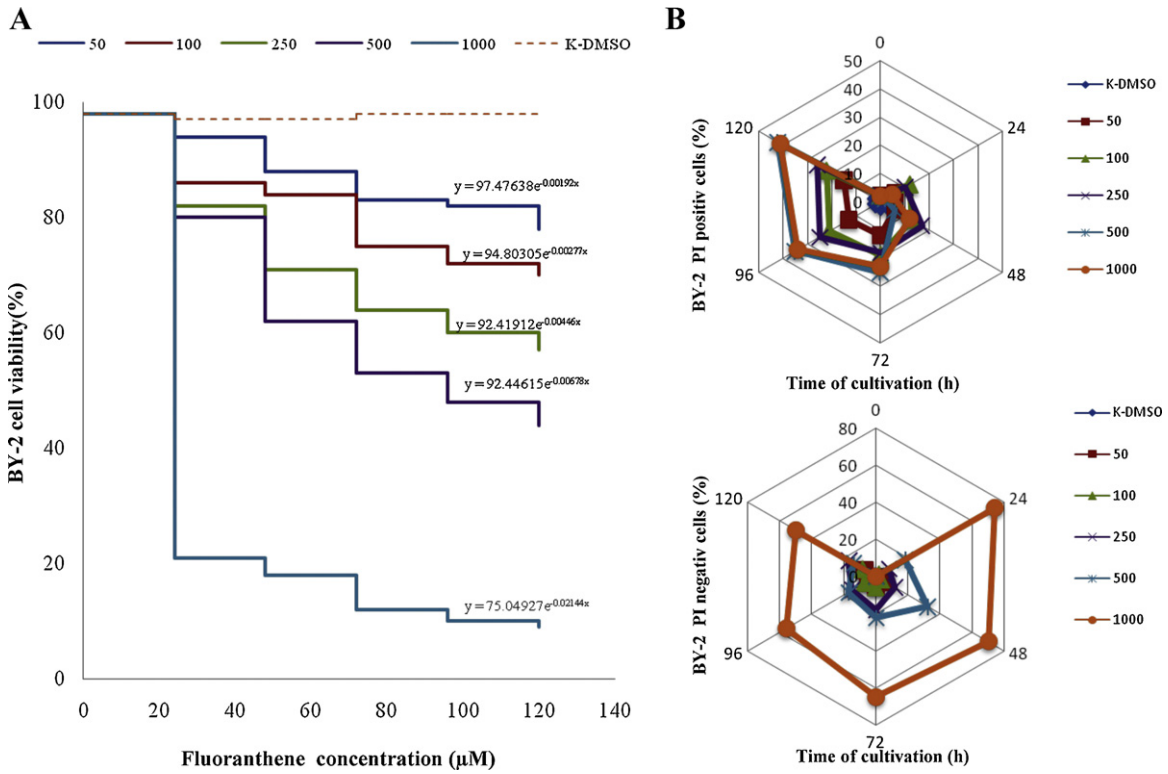

Fig. 1. Changes in viability of the BY-2 cells after FLT treatment. Reduction of viability in a time-dependent as well as concentration-dependent manner is well evident (A). Mathematical processing of obtained data and prediction of propidium iodide positive and negative BY-2 cells (B). Obtained data were in the form of survival curves visualized in the common graph. Estimation of the function of survivance was implemented using non-parametric analysis, respectively method of exponential regression – $y(t) = Ae^{-\lambda t}$, where $y(t)$ = number of living cells at the time (t), A = number of living cells at the time 0 and λ = exponential coefficient determining steepness of survival curve.

(negative FDA, negative PI staining, transient type), and (iii) BY-2 cells without esterase activity and with disruption of permeability of biomembranes (negative FDA, positive PI staining, dying or death cells).

Percentage representation of transient BY-2 cells increased in concentration-dependent manner and decreased with time of treatment. In the highest FLT concentration (1000 μM) about 74% of transient BY-2 cells were recorded after 24-h treatment. 120 h after FLT application, percentage rate of this type of BY-2 cells decreased to 50%. This fact indicates damage of permeability of biomembranes of BY-2 cells by FLT due to its accumulation with subsequent cell death.

3.2. FLT accumulation

Due to occurrence of transient BY-2 cells, in next step we were focused on determination of FLT accumulation in BY-2 cells. We supposed that FLT as fluorogenic substrate can be used for its localization in BY-2 cells and, in addition, may be responsible for occurrence of transient BY-2 cells. We determined that FTL is liable for the significant changes in autofluorescence of BY-2 cells (Fig. 2B). Intensity of autofluorescence increased with increasing FLT concentration, in its highest concentration, individual BY-2 cells and their ultrastructure were well evident. Obtained results indicate accumulation of FLT in cellular compartments - especially around nuclei, probably in biomembranes of Golgi and endoplasmic reticulum; on the other hand, in the places of vacuoles localization, no autofluorescence appeared. This fact shows evidence of deposition of FLT in lipophilic compartments, transport of FLT into vacuoles was not evident; see Fig. 2B6.

Due to autofluorescence of BY-2 cells treated by FLT and hypothesis that it is caused by a deposition of this polyaromatic hydrocarbon, HPLC technique with UV detector was optimized and applied. As it is demonstrated in Fig. 3, increasing FLT concentration and the time of treatment led to the accumulation of FLT in BY-2 cells. This tendency corresponded to decrease of FLT content in cultivation medium (not shown), which was after 120 h of treatment practically minimal. Slight decrease of FLT concentration, which was determined in its second highest FLT concentration, may be caused by lysis of BY-2 cells into very small fragments, which were removed during process of washing of cell samples. In the case of the highest FLT concentration, 129.1 μg of FLT per one g of fresh weight of BY-2 cells were determined. Compared to total amount of FLT added to cultivation media, with respect to BY-2 cell growth parameters and cell density, 120 h after treatment, following values of FLT accumulation cells were obtained: 50 μM – 2.63%, 100 μM – 2.98%, 250 μM – 3.59%, 500 μM – 5.08% and 1000 μM – 13.75%. These data indicate significant correlation between FLT concentration in cultivation medium and its accumulation in BY-2 cells.

3.3. Nuclear architecture and programmed cell death

BY-2 cells exposed to fluoranthene-demonstrated changes, which are characteristic for the process of PCD. These changes include shrinkage of cytoplasm and condensation of chromatin (on periphery of nuclei as well as around nucleoli). The most important change that signifies DNA fragmentation is presence of DNA fragments – apoptotic-like bodies. Their presence was recorded also in the case of BY-2 treatment by FLT, which means that FLT is able to initiate process of PCD.

Presence of chromatin condensation was determined in all FLT concentrations, so its evidence was in time-dependent as well as concentration-dependent manner. In the case of control untreated BY-2 cells as well as positive control – BY-2 cells treated by DMSO, presence of chromatin condensation was recorded in 2.0%, respectively in 2.5% of total cells in the case of positive control after

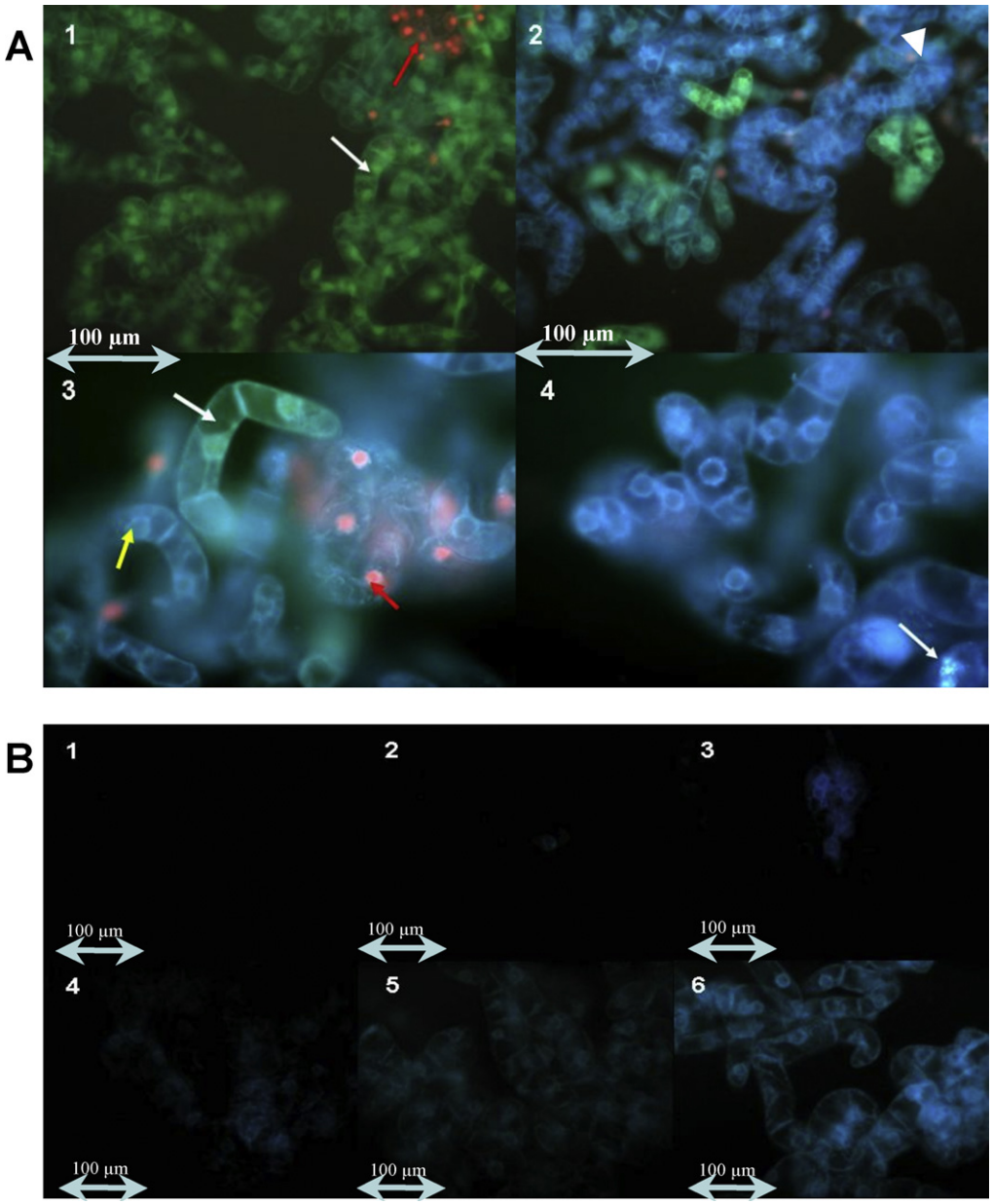

Fig. 2. Effect of fluoranthene on the tobacco BY-2 cells (A). Control-untreated cells (1) and cells treated by FLT in concentration 1000 µM 24 h after application (2). Changes in fluorescence after FLT and PI application are well evident. Comparison of esterase activity and permeability of biomembranes of BY-2 cells treated by fluoranthene in concentration 1000 µM 72 h after application. Three types of BY-2 cells are distinguishable – BY-2 cells with preserved esterase activity (white arrow), BY-2 cells without esterase activity and disruption of biomembranes permeability (yellow arrow), and BY-2 cells with disruption of permeability of biomembranes (red arrow) (3). Detail of BY-2 cells without disruption of permeability of biomembranes and esterase activity; apoptotic-like bodies – DNA fragmentation – are well evident (white arrow) (4). Magnification is indicated by the measure scale. Autofluorescence of BY-2 cells exposed to fluoranthene in concentrations 0 (1), 50 (2), 100 (3), 250 (4), 500 (5) and 1000 (6) µM 72 h after application (B). Changes in fluorescence are caused by accumulation of FLT in biomembranes of cell organelles. These changes are well evident in the case of the two highest FLT concentrations; on the other hand, FLT accumulation in the lowest concentrations is evident only in death cells, especially around the nuclei. Magnification is indicated by the measure scale. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

120 h of cultivation. Compared to FLT-treated BY-2 cells, after 120 h of treatment, chromatin condensation was recorded in about 8% (50 µM), 11% (100 µM), 14% (250 µM), 32% (500 µM) and 44% (1000 µM). Presence of the DNA fragmentation – apoptotic-like bodies – was most evident in the highest FLT concentration. Their presence was evident already 24 h after FLT application, 120 h of treatment led to presence of 4.2% of cells with apoptotic-like bodies. Graphical representation of all above described changes in shown in Fig. 4. Ability of FLT to induce the processes of PCD was confirmed by electrophoresis of isolated genomic DNA. Presence of DNA fragmentation – apoptotic-like bodies – closely corresponded with presence of DNA fragments on agarose gel after their separation with subsequent ethidium bromide staining (Fig. 5).

3.4. Effect of fluoranthene on mitosis

Together with investigation of FLT effect on nuclear architecture, mitotic index was also determined. FLT was to tobacco BY-2 cells added at the beginning of the exponential phase of growth, with mitotic index 4.5%. 24 h after FLT application, slight increase of mitotic index in comparison with control BY-2 cells was observed with increasing FLT concentration. Increased rate of mitotic indexes of treated and untreated BY-2 cells was

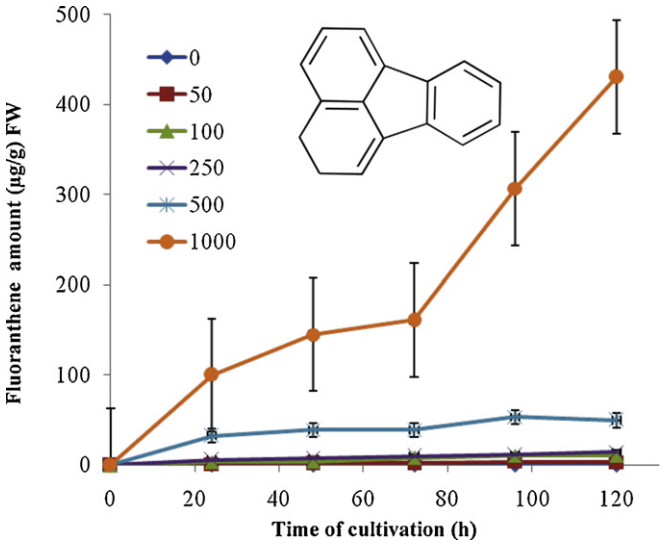

Fig. 3. FLT analysis in BY-2 cells – accumulation of FLT in time-dependent and concentration-dependent manner. Concentration of FLT is calculated in µg per g of the fresh weight. Increasing FLT accumulation with its increasing concentration and time of treatment is well evident.

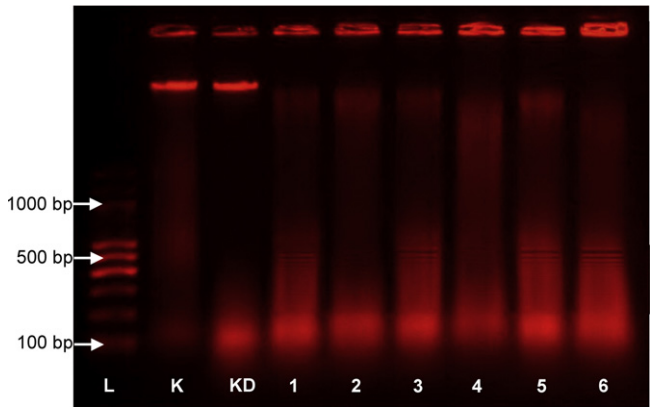

Fig. 5. Electrophoresis of genomic DNA of BY-2 cells – untreated (K), and treated by dimethyl sulfoxide (KD) and fluoranthene in concentrations 0 (1), 50 (2), 100 (3), 250 (4), 500 (5) and 1000 (6) µM 48 h after its application. DNA fragmentation is well visible, for determination of DNA fragments was used ladder. Size of DNA fragments is indicated as base pair.

well evident during whole treatment (Fig. 6A). This increase of number of dividing cells in comparison with control was closely connected with rate of individual mitotic phases, especially prophase (Fig. 6 B). At the highest FLT concentration, rate of prophasic BY-2 cells 96 h after treatment was 4.2% in comparison with mitotic index, which was 5.8% (reciprocal rate 0.72 versus control with 0.38) (Fig. 6C). Obtained results indicate ability of FLT to induce accumulation of BY-2 cells in prophase.

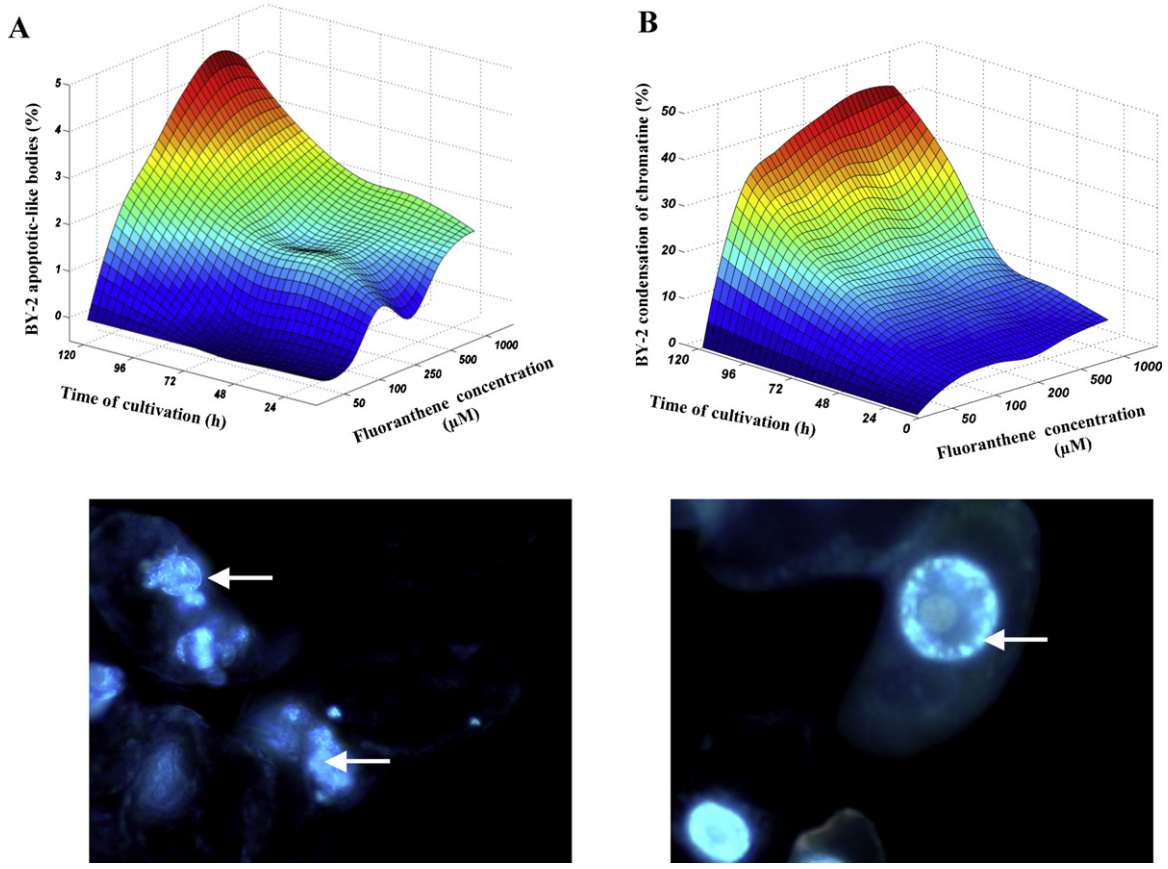

Fig. 4. Presence of DNA-fragmentation – apoptotic-like bodies (A) and chromatin condensation (B) as marker of programmed cell death in time-dependent as well as concentration-dependent manner in three-dimensional graphs. Increasing number of apoptotic-like cells as well as cells demonstrating chromatine condensation with fluoranthene concentration as well as time of treatment is well evident: red color indicates the most significant presence of given effect. Characteristic features of BY-2 cells undergoing DNA fragmentation and chromatin condensation are introduced below the three-dimensional graphs using Matlab(R) program under using of the functions of toolbox for processing of the multi-parametric data. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

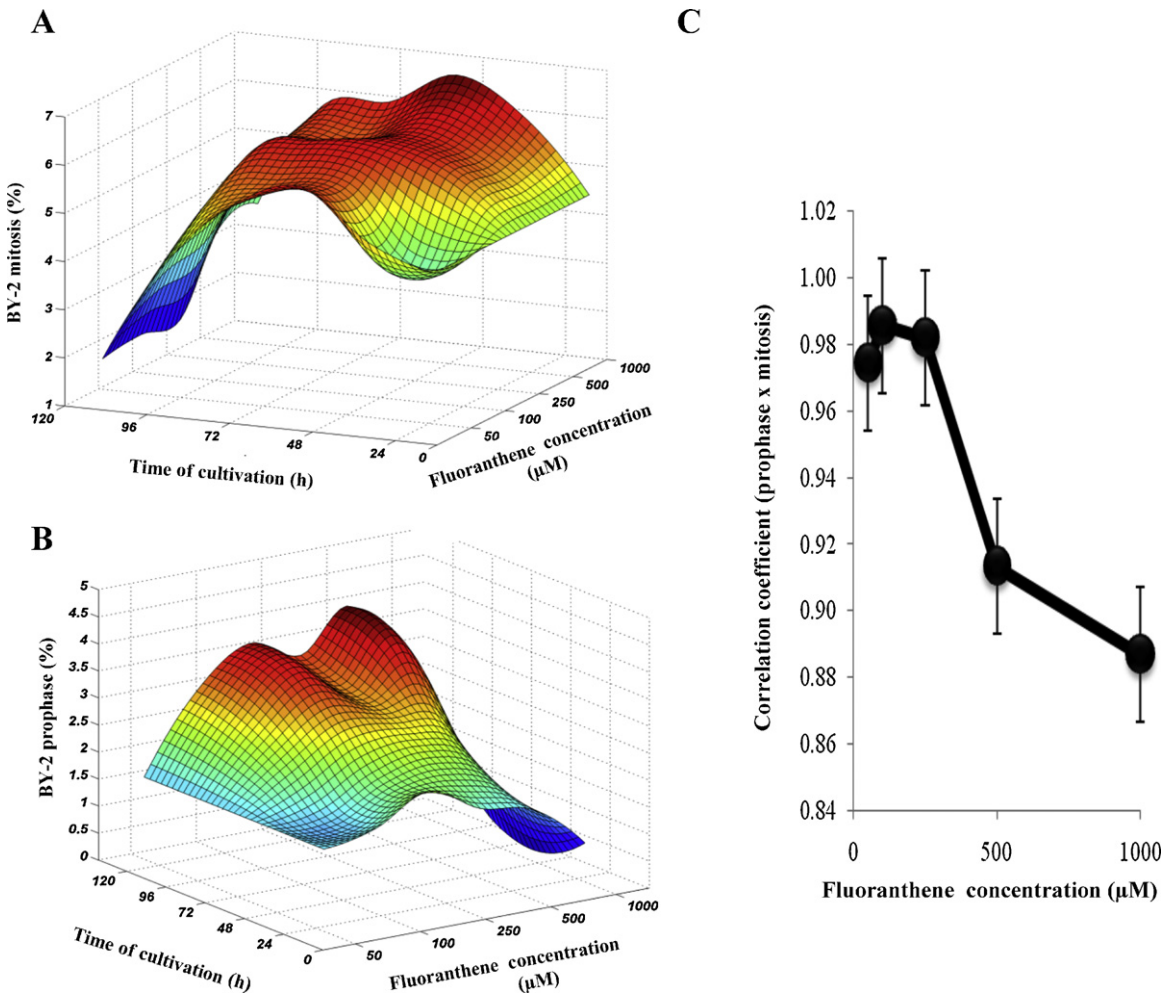

Fig. 6. Effect of fluoranthene on mitotic index of BY-2 cells. Mitotic indexes (A) and percentage of prophasic BY-2 (B) cells are demonstrated in three-dimensional graphs. Dependence of reciprocal rate of prophasic and mitotic BY-2 cells on FLT treatment (C).

3.5. ROS and MDA production

Due to presence of the apoptotic-like bodies, which are connected with the apoptosis, respectively PCD, process that is initiated by some signals including reactive oxygen species, we determined also ROS production (microscopically using fluorescence microscopy and DHE stain as ROS indicator) and product of ROS action–malondialdehyde (Fig. 7).

Presence of ROS was determined in all BY – 2 cells treated by FLT. The most evident ROS production was determined in the case of higher FLT concentrations 250, 500 and 1000 μM. Localization of sites of ROS production was determined in surrounding of nuclei and in nucleoli themselves, it means in the places where autofluorescence was localized (see Fig. 7A). This fact confirms the ability of FLT to induce ROS. Due to FLT lipophicity and its possibility to be accumulated in lipophilic cellular compartments, especially biomembranes, we were focused on production of malondialdehyde (MDA), product of the lipid peroxidation. In comparison with the control BY-2 cells, where MDA production was demonstrated and its level is connected with physiological ROS production, higher FLT concentrations led to significant increased production of MDA (Fig. 7B). In the case of highest FLT concentration (1000 μM), after 120 h of treatment MDA production was increased for about 48.6% in comparison with untreated BY-2 cells. For other concentrations, MDA production enhancements were as follows: 2.5% (50 μM), 7.9% (100 μM), 17.2% (250 μM) and 28.0% (500 μM) (Fig. 7).

4. Discussion

Polycyclic aromatic hydrocarbons – PAHs – belong to the most important pollutants rising of anthropogenic activity with tend to be deposited in waters, sludge and soils (Fent, 1996). They are able to enter living organism, due to their lipophicity can be accumulated in cells and tissues and became essential part of food chain. Negative effects of PAHs on animals including human are relatively known, these data have been obtained on different cell as well as animal models (Castorena-Torres et al., 2008; Knuckles et al., 2004; Krishnamurthi et al., 2007). Data about effects of PAHs on plants are still limited and they are restricted especially on intact plants, where mechanisms of their uptake and eventually transport are investigated. Due to this fact, we chose for our experiments plant experimental cell model represented by the tobacco BY-2 cells. These cells were used in previous studies to explanation of negative effects and mechanisms of action of not only various compounds including allelopathic secondary plants metabolites, such as naphthoquinones (Babula et al., 2009a) or plant polyphenols (Smejkal et al., 2008), but also signal molecules including especially hydrogen peroxide, which is able to induce the processes of PCD (Houot et al., 2001). For study of the phytotoxicity, model polycyclic aromatic hydrocarbon was used – fluoranthene. This compound demonstrates lower toxicity, which is advantageous with respect to work in laboratory conditions. For own study, wide FLT concentration range was used – from 0 to 1000 μM. This

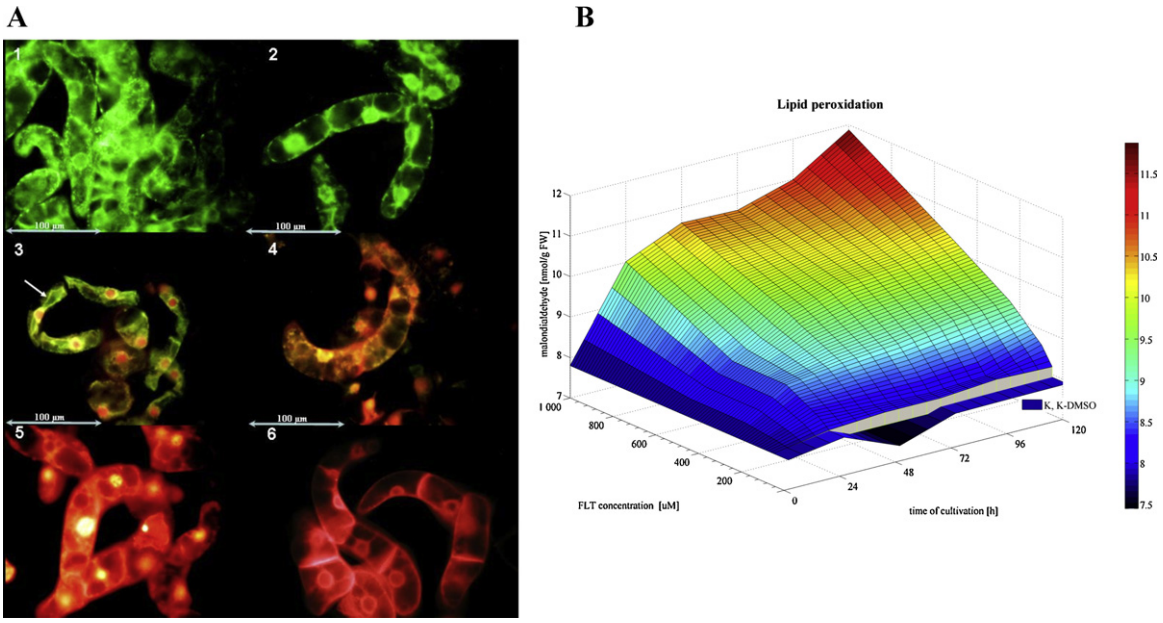

Fig. 7. Effect of fluoranthene on ROS production 72 h after application (A). Dihydroethidium-MitoTracker Green FM double staining. Control – untreated cells (1) and cells treated by FLT in concentration of 50 mM (2). Almost no differences between control BY-2 cells and BY-2 cells treated by the lowest FLT concentration are evident. BY-2 cells treated by FLT concentrations of 250 mM (3) and 500 mM (4). ROS, respectively superoxide production is indicated by red emission. Note intensive emission of nuclei. Dihydroethidium is believed to form different chemical forms after reaction with superoxide radicals, which are able of DNA intercalation. Shrinkage of cytoplasm as a symptom of process of programmed cell death is well evident (indicated by white arrow). FLT in the highest concentration (1000 mM) is generally able to induce large amount of ROS. Cells were double-stained (5) or stained only by dihydroethidium (6); only surviving cells are shown. Note emission around nuclei and in compartments, which response to ROS formation in membranes, and high cell vacuolization. Magnification is indicated by the measure scale. Production of malondialdehyde (MDA) in homogenate of BY-2 cells treated by fluoranthene (B). Natural occurrence of lipid peroxidation is connected with physiological processes. Significant increase of MDA production during FLT treatment is well evident. Values are introduced below the three-dimensional graphs using Matlab(R) program under using of the functions of toolbox for processing of the multi-parametric data. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

choice was given by inconsistent literature data, when in studies were used different FLT concentrations with different results. Compared with concentrations used in works for treatment of intact plants, concentrations chosen by us were relatively high. Nevertheless, the highest used FLT concentration – 1000 µM – did not lead to death of 100% of tobacco BY-2 cells. This fact may be caused by ability of FLT to affect water transport, mobilization of storage compounds and especially photosynthesis (Kummerova and Kmentova, 2004; Kummerova et al., 2001). In confrontation with these data, for the BY-2 cells, which are heterotrophic and cultivated in liquid cultivation medium, complexity of physiological processes is reduced and limited to processes of cell-cultivation medium transport and eventually, is some specific stages and conditions, to biosynthesis of storage compounds with the subsequent mobilization. During evaluation of cell viability, significant changes of the fluorescence were recorded. After it, we focused on autofluorescence of tobacco cells. Autofluorescence was well evident in the case of death BY-2 cells in lower FLT concentrations (50 and 100 µM) and in all BY-2 cells in the case of higher FLT concentrations (250, 500 and 1000 µM), especially in compartments around nuclei and cell walls, where membrane structures, such as Golgi or endoplasmic reticulum are localized. Due to lipophicity of fluoranthene (Ma et al., 2010), it can be supposed that FLT is bound in lipophilic compartments, it means biomembranes, of the Golgi, endoplasmic reticulum and plasmalemma, where affects their semipermeability and processes of transport through these biomembranes. FLT and its possible derivates, which are able of fluorescence after excitation (Berlman et al., 1968), are responsible for autofluorescence of BY-2 cells and serve for imaging of its cellular localization. Accumulation of FLT was demonstrated also analytically using HPLC-UV technique, which was optimized. Deposition of FLT in biomembranes is connected with the production of reactive oxygen species, which are able of their damage and formation of important product – malondialdehyde. Connection between ROS and MDA was demonstrated in many works (Swann and Acosta, 1990; Yamauchi et al., 2008). Our experiments demonstrated generation of ROS after FLT application as well as production of MDA, which was proved analytically using spectrophotometric TBARS method. So, we can conclude that FLT is able to induce also ROS, which is directly responsible for product of lipid peroxidation – MDA. ROS are connected also with process of, in the case of plants, PCD, which is responsible for removing of damage or unwanted cells and plays important role in maintenance of cell homeostasis (de Pinto et al., 2002; Murgia et al., 2004). Ability of ROS production with subsequent initiation of PCD was demonstrated in the case of naphthoquinones (Babula et al., 2009b). This connection was demonstrated also in the case of BY-2 cells treated by FLT, where a process of PCD probably enables physiological elimination of BY-2 cells damaged by FLT in consequence of disruption of cells homeostasis. Using plant models, treatment by FLT led to formation of intercellular spaces, whose formation is connected with PCD with subsequent lysis (Beklova et al., 2010). Based on the obtained results, we can propose the following scheme of FLT effect on plant cell model. FLT is taken up from cultivation media. FLT uptake is limited due to its lipophicity, but BY-2 cells accumulate FLT in time-dependent as well as concentration-dependent manners. Fluoranthene is localized in biomembranes, especially of the Golgi, endoplasmic reticulum, mitochondria and plasmalemma, where is able to interrupt or affect processes of transport as well as semipermeability, which are not only connected with cell homeostasis, but also with processes of cell metabolism and energy acquisition. In addition, FLD is able to generate ROS, which can serve as signal molecules to initiate the process of PCD, process of elimination of unwanted or damaged cells. With this process, also damage of certain essential cell structures, such as mitochondria, is connected. Process of PCD is terminated by DNA cleavage – fragmentation.

Above introduced results can facilitate FLT mechanism of action on intact plants and obtain new data and explanations connected with FLT phytotoxicity.

5. Conclusion

Polycyclic aromatic hydrocarbons represent group of very significant pollutants of the living environment. Fluoranthene as member of this group demonstrate ability to affect plants cells and cause significant cellular changes connected with its accumulation in cell biomembranes. Accumulation of FLT is connected with affection of cell functions, which can end in irreversible damage of cell integrity and interruption of cell processes. During these processes, ROS are generated. They serve as signal molecules resulting in initiation of PCD.

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References

Achten, C., Hofmann, T., 2009. Native polycyclic aromatic hydrocarbons (PAH) in coals: a hardly recognized source of environmental contamination. Sci. Total Environ. 407, 2461–2473.
Armstrong, B.G., Gibbs, G., 2009. Exposure-response relationship between lung cancer and polycyclic aromatic hydrocarbons (PAHs). Occup. Environ. Med. 66, 740–746.
Babula, P., Adam, V., Havel, L., Kizek, R., 2009a. Noteworthy secondary metabolites naphthoquinones—their occurrence, pharmacological properties and analysis. Curr. Pharm. Anal. 5, 47–68.
Babula, P., Adam, V., Kizek, R., Sladky, Z., Havel, L., 2009b. Naphthoquinones as allelochemical triggers of programmed cell death. Environ. Exp. Bot. 65, 330–337.
Beklova, M., Soukupova, I., Babula, P., Kummerova, M., Havel, L., Kizek, R., 2010. Effect of fluoranthene on pea plant (*Pisum sativum* L.). Anatomical–morphological study. Toxicol. Lett. 1965, 117.
Berlman, I.B., Wirth, H.O., Steingraber, O.J., 1968. Anomalous fluorescence characteristics of fluoranthene and some of its derivatives. J. Am. Chem. Soc. 90, 566–569.
Bhargava, A., Khanna, R.N., Bhargava, S.K., Kumar, S., 2004. Exposure risk to carcinogenic PAHs in indoor-air during biomass combustion whilst cooking in rural India. Atmos. Environ. 38, 4761–4767.
Bourotte, C., Forti, M.C., Lucas, Y., Melfi, A.J., 2009. Comparison of polycyclic aromatic hydrocarbon (PAHs) concentrations in urban and natural forest soils in the Atlantic Forest (Sao Paulo State). An. Acad. Bras. Cienc. 81, 127–136.
Camargo, M.C., Antonioli, P.R., Vicente, E., Tfouni, S.A., 2008. The presence of polycyclic aromatic hydrocarbons in Brazilian commercial soybean oils. Toxicol. Lett. 180, 62–63.
Camargo, M.C.R., Toledo, M.C.F., 2003. Polycyclic aromatic hydrocarbons in Brazilian vegetables and fruits. Food Control 14, 49–53.
Carlstrom, C.J., Tuovinen, O.H., 2003. Mineralization of phenanthrene and fluoranthene in yardwaste compost. Environ. Pollut. 124, 81–91.
Castorena-Torres, F., de Leon, M.B., Cisneros, B., Zapata-Perez, O., Salinas, J.E., Albores, A., 2008. Changes in gene expression induced by polycyclic aromatic hydrocarbons in the human cell lines HepG2 and A549. Toxicol. In Vitro 22, 411–421.
Cavallo, D., Ursini, C.L., Bavazzano, P., Cassinelli, C., Frattini, A., Perniconi, B., Di Francesco, A., Ciervo, A., Rondinone, B., Iavicoli, S., 2006. Sister chromatid exchange and oxidative DNA damage in paving workers exposed to PAHs. Ann. Occup. Hyg. 50, 211–218.
Ciaparra, D., Aries, E., Booth, M.J., Anderson, D.R., Almeida, S.M., Harrad, S., 2009. Characterisation of volatile organic compounds and polycyclic aromatic hydrocarbons in the ambient air of steelworks. Atmos. Environ. 43, 2070–2079.
de Pinto, M.C., Tommasi, F., De Gara, L., 2002. Changes in the antioxidant systems as part of the signaling pathway responsible for the programmed cell death activated by nitric oxide and reactive oxygen species in tobacco Bright-Yellow 2 cells. Plant Physiol. 130, 698–708.
Dellantonio, A., Fitz, W.J., Repmann, F., Wenzel, W.W., 2010. Disposal of coal combustion residues in terrestrial systems: contamination and risk management. J. Environ. Qual. 39, 761–775.
Desalme, D., Binet, P., Bernard, N., Gilbert, D., Toussaint, M.L., Chiapusio, G., 2011. Atmospheric phenanthrene transfer and effects on two grassland species and their root symbionts: a microcosm study. Environ. Exp. Bot. 71, 146–151.
Eom, I.C., Rast, C., Veber, A.M., Vasseur, P., 2007. Ecotoxicity of a polycyclic aromatic hydrocarbon (PAH)-contaminated soil. Ecotox. Environ. Saf. 67, 190–205.
Fabbri, D., Baravelli, V., Giannotti, K., Donnini, F., Fabbri, E., 2006. Bioaccumulation of cyclopenta cd pyrene and benzo ghi fluoranthene by mussels transplanted in a coastal lagoon. Chemosphere 64, 1083–1092.
Fang, G.C., Chang, K.F., Lu, C.S., Bai, H.L., 2004. Estimation of PAHs dry deposition and BaP toxic equivalency factors (TEFs) study at Urban, Industry Park and rural sampling sites in central Taiwan, Taichung. Chemosphere 55, 787–796.
Fent, K., 1996. Ecotoxicology of organotin compounds. Crit. Rev. Toxicol. 26, 3–117.
Fuoco, R., Giannarelli, S., Wei, Y., Abete, C., Francesconi, S., Termine, M., 2005. Polychlorobiphenyls and polycyclic aromatic hydrocarbons in the sea-surface micro-layer and the water column at Gerlache Inlet, Antarctica. J. Environ. Monit. 7, 1313–1319.
Gabos, S., Ikonomou, M.G., Schopflocher, D., Fowler, B.R., White, J., Prepas, E., Prince, D., Chen, W.P., 2001. Characteristics of PAHs, PCDD/Fs and PCBs in sediment following forest fires in northern Alberta. Chemosphere 43, 709–719.
Gambaro, A., Manodori, L., Moret, I., Capodaglio, G., Cescon, P., 2004. Determination of polychlorobiphenyls and polycyclic aromatic hydrocarbons in the atmospheric aerosol of the Venice Lagoon. Anal. Bioanal. Chem. 378, 1806–1814.
Hong, Y.W., Yuan, D.X., Lin, Q.M., Yang, T.L., 2008. Accumulation and biodegradation of phenanthrene and fluoranthene by the algae enriched from a mangrove aquatic ecosystem. Mar. Pollut. Bull. 56, 1400–1405.
Houot, V., Etienne, P., Petitot, A.S., Barbier, S., Blein, J.P., Suty, L., 2001. Hydrogen peroxide induces programmed cell death features in cultured tobacco BY-2 cells, in a dose-dependent manner. J. Exp. Bot. 52, 1721–1730.
Hwang, H.M., Hu, X., Zhao, X., 2007. Enhanced bioremediation of polycyclic aromatic hydrocarbons by environmentally friendly techniques. J. Environ. Sci. Health Pt. C–Environ. Carcinog. Ecotoxicol. Rev. 25, 313–352.
Kacalkova, L., Tlustos, P., 2011. The uptake of persistent organic pollutants by plants. Cent. Eur. J. Biol. 6, 223–235.
Knuckles, M.E., Inyang, F., Ramesh, A., 2004. Acute and subchronic oral toxicity of fluoranthene in F-344 rats. Ecotox. Environ. Saf. 59, 102–108.
Kolb, M., Harms, H., 2000. Metabolism of fluoranthene in different plant cell cultures and intact plants. Environ. Toxicol. Chem. 19, 1304–1310.
Krishnamurthi, K., Devi, S.S., Chakrabarti, T., 2007. The genotoxicity of priority polycyclic aromatic hydrocarbons (PAHs) containing sludge samples. Toxicol. Mech. Methods 17, 1–12.
Kuhn, A., Ballach, H.J., Wittig, R., 2004. Studies in the biodegradation of 5 PAHs (Phenanthrene, pyrene, fluoranthene, chrysene und benzo(a)pyrene) in the presence of rooted poplar cuttings. Environ. Sci. Pollut. Res. 11, 22–32.
Kummerova, M., Kmentova, E., 2004. Photoinduced toxicity of fluoranthene on germination and early development of plant seedling. Chemosphere 56, 387–393.
Kummerova, M., Kmentova, E., Koptikova, J., 2001. Effect of fluoranthene on growth and primary processes of photosynthesis in faba bean and sunflower. Rostl. Vyroba 47, 344–351.
Kummerova, M., Krulova, J., Zezulka, S., Triska, J., 2006. Evaluation of fluoranthene phytotoxicity in pea plants by Hill reaction and chlorophyll fluorescence. Chemosphere 65, 489–496.
Kummerova, M., Slovak, L., Holoubek, I., 1997. Growth response of spring barley to short- or long-period exposures to fluoranthene. Rostl. Vyroba 43, 209–215.
Lashermes, G., Houot, S., Barriuso, E., 2010. Sorption and mineralization of organic pollutants during different stages of composting. Chemosphere 79, 455–462.
Liu, Y., Luan, T.G., Lu, N.N., Lan, C.Y., 2006. Toxicity of fluoranthene and its biodegradation by *Cyclotella caspia* Alga. J. Integr. Plant Biol. 48, 169–180.
Ma, Y.G., Lei, Y.D., Xiao, H., Wania, F., Wang, W.H., 2010. Critical review and recommended values for the physical–chemical property data of 15 polycyclic aromatic hydrocarbons at 25 °C. J. Chem. Eng. Data 55, 819–825.
Maliszewska-Kordybach, B., Smreczak, B., 2000. Ecotoxicological activity of soils polluted with polycyclic aromatic hydrocarbons (PAHS)—effect on plants. Environ. Technol. 21, 1099–1110.
Murashige, T., Skoog, F., 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant 15, 473–497.
Murgia, I., de Pinto, M.C., Delledonne, M., Soave, C., De Gara, L., 2004. Comparative effects of various nitric oxide donors on ferritin regulation, programmed cell death, and cell redox state in plant cells. J. Plant Physiol. 161, 777–783.
Nagata, T., Nemoto, Y., Hasetawa, S., 1992. Tobacco BY-2 cell-line as the HeLa-cell in the cell biology of higher plants. Int. Rev. Cytol. 132, 1–30.
Nizzetto, L., Lohmann, R., Gioia, R., Jahnke, A., Temme, C., Dachs, J., Herckes, P., Di Guardo, A., Jones, K.C., 2008. PAHs in air and seawater along a North–South Atlantic transect: trends, processes and possible sources. Environ. Sci. Technol. 42, 1580–1585.
Oguntimehin, I., Eissa, F., Sakugawa, H., 2010. Negative effects of fluoranthene on the ecophysiology of tomato plants (*Lycopersicon esculentum* Mill) fluoranthene mists negatively affected tomato plants. Chemosphere 78, 877–884.
Oguntimehin, I., Nakatani, N., Sakugawa, H., 2008. Phytotoxicities of fluoranthene and phenanthrene deposited on needle surfaces of the evergreen conifer, Japanese red pine (*Pinus densiflora* Sieb. et Zucc.). Environ. Pollut. 154, 264–271.
Oguntimehin, I., Sakugawa, H., 2008. Fluoranthene fumigation and exogenous scavenging of reactive oxygen intermediates (ROI) in evergreen Japanese red pine seedlings (*Pinus densiflora* Sieb. et. Zucc.). Chemosphere 72, 747–754.
Pozdnyakova, N.N., Rodakiewicz-Nowak, J., Turkovskaya, O.V., Haber, J., 2006. Oxidative degradation of polyaromatic hydrocarbons and their derivatives catalyzed directly by the yellow laccase from *Pleurotus ostreatus* D1. J. Mol. Catal. B Enzyme 41, 8–15.
Qu, S.X., Stacey, N.H., 1996. Comparison of DNA adduct induction in vitro by PAHs and nitro-PAHs in freshly isolated rat hepatocytes. Toxicol. in Vitro 10, 685–692.
Ratola, N., Alves, A., Psillakis, E., 2011. Biomonitoring of polycyclic aromatic hydrocarbons contamination in the Island of Crete using pine needles. Water Air Soil Pollut. 215, 189–203.
Ravindra, K., Bencs, L., Wauters, E., de Hoog, J., Deutsch, F., Roekens, E., Bleux, N., Berghmans, P., Van Grieken, R., 2006. Seasonal and site-specific variation in

vapour and aerosol phase PAHs over Flanders (Belgium) and their relation with anthropogenic activities. Atmos. Environ. 40, 771–785.
Ren, L., Huang, X.D., McConkey, B.J., Dixon, D.G., Greenberg, B.M., 1994. Photoinduced toxicity of 3 polycyclic aromatic-hydrocarbons (Fluoranthene, Pyrene, and Naphthalene) to the Duckweed Lemna-Gibba L G-3. Ecotox. Environ. Saf. 28, 160–171.
Salicis, F., Krivobok, S., Jack, M., Benoit-Guyod, J.L., 1999. Biodegradation of fluoranthene by soil fungi. Chemosphere 38, 3031–3039.
Sayara, T., Borras, E., Caminal, G., Sarra, M., Sanchez, A., 2011. Bioremediation of PAHs-contaminated soil through composting: Influence of bioaugmentation and biostimulation on contaminant biodegradation. Int. Biodeterior. Biodegrad. 65, 859–865.
Sepic, E., Bricelj, M., Leskovsek, H., 2003. Toxicity of fluoranthene and its biodegradation metabolites to aquatic organisms. Chemosphere 52, 1125–1133.
Schoket, B., 1999. DNA damage in humans exposed to environmental and dietary polycyclic aromatic hydrocarbons. Mutat. Res. Fundam. Mol. Mech. Mutagen 424, 143–153.
Schutzendubel, A., Majcherczyk, A., Johannes, C., Huttermann, A., 1999. Degradation of fluorene, anthracene, phenanthrene, fluoranthene, and pyrene lacks connection to the production of extracellular enzymes by *Pleurotus ostreatus* and *Bjerkandera adusta*. Int. Biodeterior. Biodegrad. 43, 93–100.
Smejkal, K., Babula, P., Slapetova, T., Brognara, E., Dall'Acqua, S., Zemlicka, M., Innocenti, G., Cvacka, J., 2008. Cytotoxic activity of C-geranyl compounds from *Paulownia tomentosa* fruits. Planta Med. 74, 1488–1491.
Sponza, D.T., Gok, O., 2010. Effect of rhamnolipid on the aerobic removal of polyaromatic hydrocarbons (PAHs) and COD components from petrochemical wastewater. Bioresour. Technol. 101, 914–924.
Stewart, R.R.C., Bewley, J.D., 1980. Lipid-peroxidation associated with accelerated aging of soybean axes. Plant Physiol. 65, 245–248.
Sun, L., Yan, X.L., Liao, X.Y., Wen, Y., Chong, Z.Y., Liang, T., 2011. Interactions of arsenic and phenanthrene on their uptake and antioxidative response in *Pteris vittata* L. Environ. Pollut. 159, 3398–3405.
Sverdrup, L.E., Krogh, P.H., Nielsen, T., Kjaer, C., Stenersen, J., 2003. Toxicity of eight polycyclic aromatic compounds to red clover (*Trifolium pratense*), ryegrass (*Lolium perenne*), and mustard (*Sinapsis alba*). Chemosphere 53, 993–1003.
Swann, J.D., Acosta, D., 1990. Failure of gentamicin to elevate cellular malondialdehyde content or increase generation of intracellular reactive oxygen species in primary cultures of renal cortical epithelial cells. Biochem. Pharmacol. 40, 1523–1526.
Taioli, E., Sram, R.J., Garte, S., Kalina, I., Popov, T.A., Farmer, P.B., 2007. Effects of polycyclic aromatic hydrocarbons (PAHs) in environmental pollution on exogenous and oxidative DNA damage (EXPAH project): description of the population under study. Mutat. Res. Fundam. Mol. Mech. Mutagen. 620, 1–6.
Tam, N.F.Y., Ke, L., Wang, X.H., Wong, Y.S., 2001. Contamination of polycyclic aromatic hydrocarbons in surface sediments of mangrove swamps. Environ. Pollut. 114, 255–263.
Thompson, S., Budzinski, H., LeMenach, K., Letellier, M., Garrigues, P., 2002. Multiresidue analysis of polycyclic aromatic hydrocarbons, polychlorobiphenyls, and organochlorine pesticides in marine sediments. Anal. Bioanal. Chem. 372, 196–204.
Thongkred, P., Lotrakul, P., Prasongsuk, S., Imai, T., Punnapayak, H., 2011. Oxidation of polycyclic aromatic hydrocarbons by a tropical isolate of *Pycnoporus coccineus* and its laccase. Scienceasia 37, 225–233.
Toyooka, T., Ibuki, Y., 2007. DNA damage induced by coexposure to PAHs and light. Environ. Toxicol. Pharmacol. 23, 256–263.
Tsai, P.J., Shieh, H.Y., Hsieh, L.T., Lee, W.J., 2001a. The fate of PAHs in the carbon black manufacturing process. Atmos. Environ. 35, 3495–3501.
Tsai, P.J., Shieh, H.Y., Lee, W.J., Lai, S.O., 2001b. Health-risk assessment for workers exposed to polycyclic aromatic hydrocarbons (PAHs) in a carbon black manufacturing industry. Sci. Total Environ. 278, 137–150.
Tsai, P.J., Shieh, H.Y., Lee, W.J., Lai, S.O., 2001c. Characteristics of exposure profiles for workers exposed to airborne dusts and polycyclic aromatic hydrocarbons (PAHs) in the carbon black manufacturing industry. J. Occup. Health 43, 118–128.
Tsai, P.J., Shieh, H.Y., Lee, W.J., Lai, S.O., 2002. Characterization of PAHs in the atmosphere of carbon black manufacturing workplaces. J. Hazard. Mater. 91, 25–42.
Valentin, L., Lu-Chau, T.A., Lopez, C., Feijoo, G., Moreira, M.T., Lema, J.M., 2007. Biodegradation of dibenzothiophene, fluoranthene, pyrene and chrysene in a soil slurry reactor by the white-rot fungus Bjerkandera sp BOS55. Process Biochem. 42, 641–648.
Vanova, L., Kummerova, M., Votrubova, O., 2011. Fluoranthene-induced production of ethylene and formation of lysigenous intercellular spaces in pea plants cultivated in vitro. Acta Physiol. Plant. 33, 1037–1042.
Walker, S.A., Addai, A.B., Mathis, M., Ramesh, A., 2007. Effect of dietary fat on metabolism and DNA adduct formation after acute oral exposure of F-344 rats to fluoranthene. J. Nutr. Biochem. 18, 236–249.
Wilcke, W., 2000. Polycyclic aromatic hydrocarbons (PAHs) in soil—a review. J. Plant Nutr. Soil Sci. Z Pflanzenernahr. Bodenkd. 163, 229–248.
Yamauchi, Y., Furutera, A., Seki, K., Toyoda, Y., Tanaka, K., Sugimoto, Y., 2008. Malondialdehyde generated from peroxidized linolenic acid causes protein modification in heat-stressed plants. Plant Physiol. Biochem. 46, 786–793.
Yang, H.H., Lai, S.O., Hsieh, L.T., Hsueh, H.J., Chi, T.W., 2002. Profiles of PAH emission from steel and iron industries. Chemosphere 48, 1061–1074.
Yang, Y., Zhang, N., Xue, M., Tao, S., 2010. Impact of soil organic matter on the distribution of polycyclic aromatic hydrocarbons (PAHs) in soils. Environ. Pollut. 158, 2170–2174.