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Effect of organic pollutant treatment on the growth of pea and maize seedlings

Research Article

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Abstract: This study confirmed the considerable effect of polycyclic aromatic hydrocarbon fluoranthene (FLT; 0.01, 0.1, 1, 4 and 7 mg/l) exposure on the germination of seeds, growth and root morphology of seedlings in *Zea mays* and *Pisum sativum*. Seed germination was significantly inhibited at FLT≥0.01 mg/l in maize and at ≥1 mg/l in pea. The amount of released ethylene after 3 days of germination was significantly increased in both species at FLT≥0.1 mg/l. After 7 days of seedling cultivation a significant decrease in the dry weight of roots and shoots occurred in maize at FLT≥0.1 mg/l while in pea similar effect was observed at ≥1 mg/l. The total length of primary and lateral roots was significantly reduced by FLT≥1 mg/l in maize and by 4 and 7 mg/l in pea. The length of the non-branched part of the primary root was significantly reduced by FLT≥0.1 mg/l in maize and ≥0.01 mg/l in pea. In both species the number of lateral roots was significantly increased at FLT≤1 mg/l and inhibited at concentrations of 4 and 7 mg/l. Fluoranthene content in roots and shoots of both species positively correlated with the FLT treatment.

Keywords: *PAH fluoranthene • Morphology of root system • Phytotoxicity tests • Pea • Maize*

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) constitute an important group of pollutants which is introduced into the environment mainly as a result of incomplete fuel combustion from anthropogenic sources. PAHs are toxic compounds and many are mutagens and/or carcinogens [1]. According to present knowledge, PAHs deserve greater attention than other pollutants because of their persistence in the environment, their long-term toxicity, and the large quantities present [2,3]. Human and wildlife exposure to persistent and toxic environmental contaminants occurs as a result of bioaccumulation in plants and subsequent transfer through natural and agricultural food chains [4]. The plants take up PAHs from contaminated soil by absorbing dissolved PAHs through the root system [5], from atmospheric particles deposited onto the waxy cuticle of the leaves and from passage of gases through the stomata [6,7]. Probably the most important source of PAHs for plants is atmospheric particle deposition which also causes contamination of soil. However, concentrations of PAHs in agricultural soils may be further substantially increased when sewage sludge, farmyard manure, compost or composted waste mixtures are applied [8,9].

The rate of PAH uptake by plants is influenced by a number of factors, including concentration and physicochemical properties of the compound, soil type, content of organic soil matter, pH, humidity, temperature, plant species, stage of ontogenesis and lipid content. [10]. PAHs can affect all stages of plant growth from germination to reproduction. According to Baud-Grasset *et al.* [11], the critical stages of development are primarily the early stages of ontogenesis, the germination of seeds and the formation of the root system. In this

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relatively short early period, the plant does not yet have significant detoxification capacity and is more sensitive to the presence of toxic substances in the environment as it is transitioning from heterotrophic to autotrophic nutrition [11]. The increasing load of PAHs can be a decisive factor for the future diversity of plant species in the habitat and it may determine the level of possible economic yields [12].

Biochemical and physiological changes recorded in plants exposed to PAHs may be detected earlier than anatomical and morphological changes [13]. It is known that plants respond to the presence of stressors by altering their production of phytohormones [14,15]. This process is influenced by changes in the levels of enzymes that participate in phytohormone biosynthesis, and regulation of the enzymes concerned with degradation or deactivation of xenobiotics [16]. The gaseous phytohormone ethylene (C_2H_4) exerts profound effects on plants throughout their life cycle [17] and an increase in its production is one of the first reactions of plants to the presence of stressors [18]. Ethylene detection and biosynthesis are highly regulated as has been demonstrated in a number of vegetative tissues, in seedlings, in ripening fruits and senescing flowers [19].

The aim of this study was to evaluate the effect of fluoranthene (FLT), which is one of the most abundant PAHs with ubiquitous environmental distribution [20], on germination of seeds and production of ethylene during germination, early growth of seedlings and the morphological characteristics of their root system in *Zea mays* and *Pisum sativum*.

2. Experimental Procedures

2.1 Preparation of growth media supplemented with fluoranthene

Fluoranthene (FLT; Supelco, USA) was dissolved in acetone (Labscan, Ireland) to a concentration of 420 mg/l and delivered to filter-purified (ultrafiltered) water (FP-H_2O) or Reid-York nutrient solution [21] to final concentrations 0.01, 0.1, 1, 4 and 7 mg/l. Selected concentrations simulated low, moderate and high environmental loading (compare to e.g. Parrish *et al.* [3]). The concentrations of FLT were confirmed by means of HPLC (Hewlett Packard) with a Supelcosil LCPAH column (5 μm, 150 × 3 mm, Supelco, Bellefonte, PA, USA) using gradient 60–100% acetonitrile in water. Our preliminary experiments proved that the final concentration of the solvent used, acetone, did not affect the germination of seeds, the growth of seedlings or any other physiological parameters, e.g. primary processes of photosynthesis [22,23].

2.2 Cultivation of plants

In this study maize (*Zea mays* cv. Torena) and pea (*Pisum sativum* cv. Zázrak) species were used. In the first experiment the seeds were soaked in FP-H_2O, for 12 hours then placed into Petri dishes (10 dishes, 14 cm diameter, 10 seeds per dish) on a disk of filter paper with 7 ml of appropriate solution of FLT in FP-H_2O. Seeds germinated in pure FP-H_2O served as the control. Petri dishes were sealed with a parafilm. The germination of seeds took place in the darkness for 3 days at 23±2ºC. The germination was expressed as the percentage of germinating seeds.

In the second experiment, which focused on early seedling growth assessment, the seeds were soaked in FP-H_2O for 12 hours then, germinated for 3 days. The seedlings were then placed in dishes filled with granulated polyethylene and FP-H_2O for two days before being transplanted into glass vessels (10 plants per vessel, 5 vessels per treatment) with 300 ml of Reid-York nutrient solution without (control) or with FLT additions. This hydroponic cultivation was conducted under controlled conditions (temperature 24±2ºC, relative air humidity 60% to 80%, photoperiod 16/8 and irradiation up to 400 μmol/m^2/s PAR) for 7 days. Afterwards following parameters were assessed: the length and dry mass of roots and shoots and the morphological characteristics of the root systems.

2.3 Determination of ethylene content

For the purposes of ethylene content assessment, the germination of seeds was simultaneously run in sealed cultivation vessels (175 ml, 5 seeds per vessel, 6 vessels per treatment). After 3 days of germination, 1 ml of gas was taken off from the cultivation vessel through a plastic tuberculin syringe placed in the vessel cap. Ethylene content was analysed in a gas chromatograph (GC800 Series with 50 m capillary column Al_2O_3 "S" 15 mm, ID 0.53 mm, Fissons Instrument, Italy); when estimating gaseous hydrocarbons, spray, column and detector temperatures were 230ºC, 40ºC and 200ºC, respectively [24].

2.4 Determination of fluoranthene content in plants

Content of FLT in the dry mass of roots and shoots after 7 days of cultivation was determined by a gas chromatography-mass spectrometry (GC-MS) technique using the Finnigan GCQ ion trap instrument (Finnigan MAT, USA). For each treatment, two samples were analyzed (for the methodology see Kummerová *et al.* [23]) and arithmetical means were calculated. Bioconcentration factor (BCF; [25]) was calculated as the ratio of FLT content in the dry mass to its concentration in the cultivation medium. Translocation factor (TF) was calculated as the ratio of FLT content in shoot *versus* root.

2.5 Morphological studies

After 7 days of cultivation in Reid-York nutrient solution with 0, 0.01, 0.1, 1, 4 and 7 mg/l FLT, the morphological characteristics of the maize and pea root systems were assessed. Roots were placed into inversed scanner and their images were taken. Each seedling's total length of the primary root and lateral roots (TL), length of the non-branched part of the primary root (NBL), lateral roots length (LRL) and the number of lateral roots (LRN) were then evaluated by means of the Root Analyser Module in software Lucia G (Laboratory Imaging, Prague, Czech Republic).

2.6 Statistical analysis

For a statistical evaluation of results, the software STATISTICA 6 (StatSoft, Inc.®, USA) was used. Each result is a mean of 10 individual measurements for germination and seedlings growth and 6 measurements for ethylene content in each treatment. The significance of the differences in average values between the treatments was evaluated by means of the one-way analysis of variance after preceding verification of normality and homogeneity of the variance (ANOVA, $P<0.05$) or by the non-parametric Kruskal-Wallis test. A detailed evaluation of the variance was carried out using Scheffé test ($P<0.05$).

3. Results and Discussion

The results of seed germination of maize and pea are presented in Table 1. It is evident that the increasing FLT concentration caused an inhibition of seed germination of both plant species. In maize, germination was significantly reduced by FLT≥0.01 mg/l, whereas in pea a significant decrease in germination was found only when FLT concentration was two orders of magnitude higher (≥1 mg/l FLT). These obtained results are in agreement with the previous findings of Kummerová & Kmentová [22] in the seed germination of lettuce, onion and tomato.

The uptake of water is one of the main conditions of germination and transition of the seeds from the latent into the active stage. From the soil solution the seeds also take up a certain amount of dissolved PAHs. As found earlier by Kummerová *et al.* [12], the uptake of water by seeds was not influenced by FLT, but the number of germinated seeds of various plant species (onion, sunflower, lettuce, spruce, mustard, tomato and wheat) exposed to hydrophobic FLT correlated negatively with their lipid content. Even though the lipid content in both maize and pea is low, maize seeds can contain up to a two-fold higher amount of lipids in comparison to pea [26,27] and their higher sensitivity during germination could be related to this fact. The amount of lipidic substances in the seeds, seed surface area and chemical composition of seed testa could be significant in regulating the sensitivity of seeds to lipophilic organic compounds in this developmental stage [28]. In our study the used seeds of maize and pea were approximately equivalent with similar weight and type of testa. On the other hand it is a question to what extent FLT can also participate in the changes in gibberellin level and in inactivating the mobilization of protein and saccharide reserves in seeds of both species during germination. Moreover, with respect to the mutagenic character of PAHs and their effect on nucleic acids [1], information about their effect on a developing embryo is lacking.

Increased ethylene evolution accompanies the seed germination of many plant species, but only a little is known about the regulation of the ethylene biosynthesis pathway in different seed tissues. Petruzzelli *et al.* [17] investigated the biosynthesis of the direct ethylene precursor 1-aminocyclopropane-1-carboxylic acid (ACC), the expression of ACC oxidase (ACO) and ethylene production in the cotyledons and embryonic axis of germinating pea seeds. In our study the higher amount of ethylene found in both maize and pea seeds indicated the presence of stressor FLT, as shown in Figure 1. Its significantly increased level (in maize by

FLT treatment (mg/l)	Germination (%)	
	Zea mays	*Pisum sativum*
0	85.00 ± 0.53[a]	96.67 ± 0.49[a]
0.01	77.00 ± 0.48[b]	95.33 ± 0.52[a]
0.1	76.25 ± 0.46[b]	94.00 ± 0.51[ab]
1	76.00 ± 0.52[b]	90.00 ± 0.38[bc]
4	68.00 ± 0.42[c]	88.67 ± 0.35[c]
7	66.00 ± 0.46[c]	86.67 ± 0.41[c]

Table 1. The effect of increasing concentration of fluoranthene (FLT) on germination of maize and pea.

Data represent mean over 10 repetitions with 10 seeds each one. Values are given as mean ± standard deviation. Different letters mark significant differences between values according to Scheffé test on P=0.05.

29% and pea by 15%, when compared to controls) was evoked by FLT≥0.1 mg/l. Similarly, a significant increase of ethylene biosynthesis under other environmental stresses such as saline treatment was also found by Zapata *et al*. [29] in shoots of tomato, broccoli and bean.

Ethylene-independent signalling pathways regulate the spatial and temporal pattern of ethylene biosynthesis, whereas the ethylene signalling pathway regulates high-level ACO expression in the embryonic axis, and thereby enhances ethylene evolution during seed germination [17]. Nevertheless the mechanism by which the organic pollutant FLT affects ethylene biosynthesis during plant germination is still unclear.

Seedling growth of maize and pea plants was expressed on the basis of length and dry weight of roots and shoots. Root elongation is, apart from seed germination, a convenient indicator of environmental phytotoxicity [30,31]. The toxic effect of FLT on the growth of seedlings was evident in both plant species, but the inhibition effect deepened with increasing FLT loading more in maize than in pea (Table 2). For pea, root length was reduced by 11% at FLT≥0.1mg/l but other parameters were not affected until concentration reached 1mg/l or higher; the significant response in maize seedlings was already seen at FLT≥0.1 mg/l, with inhibition of root length by at least 6%, shoot by 23%, and lower dry weight by 15-37%. Significant reduction of growth in the presence of toxicants has also been recorded in barley [32], maize [33] and red clover, ryegrass and mustard [34]. The highest applied concentration of FLT (7 mg/l) reduced the length of the primary root in maize by 83% and in pea by up to 68%, and the length of shoots in maize by 85% and pea by up to 50%. Our results also showed that biomass production of plant species and their organs was influenced in different ways (Table 2). The dry biomass of roots in maize was reduced by 85% and in pea by up to 65% and the dry biomass of shoots by 88% in maize and up to 54% in pea. These results are in agreement with Pašková *et al*. [35] who confirmed the negative effect of three PAHs and their N-heterocyclic derivates on the germination and growth of seedling of mustard, barley and common bean. Similarly, Abdel-Latif [36] found a reduction of root and shoot length and biomass of maize

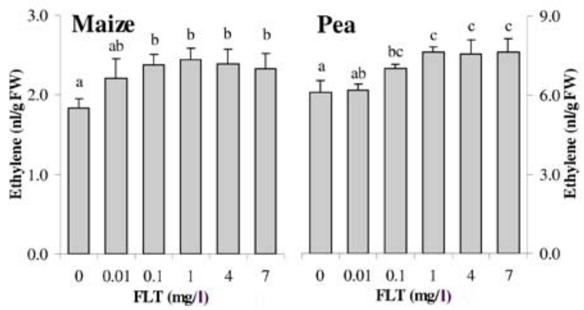

Figure 1. The effect of increasing concentration of fluoranthene (FLT) on ethylene amount (nl/g FW) released by seeds of maize and pea after 3 days period of germination. Data are mean values of 6 replicates, standard deviations are indicated by error bars. Different letters above the neighbouring columns show statistically significant difference at P=0.05.

FLT treatment (mg/l)	*Zea mays*				*Pisum sativum*			
	length (cm)		dry weight (mg)		length (cm)		dry weight (mg)	
	primary root	shoot	root	shoot	primary root	shoot	root	shoot
0	18.56 ± 1.25[ab]	5.17 ± 0.72[a]	19.19 ± 1.07[a]	77.00 ± 12.14[a]	7.87 ± 0.24[a]	1.92 ± 0.15 [a]	15.37 ± 2.00[a]	42.32 ± 3.73 [a]
0.01	20.38 ± 1.13[a]	4.96 ± 0.46[a]	20.66 ± 1.46[a]	84.77 ± 8.96[a]	7.79 ± 0.26[a]	1.94 ± 0.23[a]	15.43 ± 2.59[a]	41.47 ± 3.10[a]
0.1	17.39 ± 1.07[b]	3.98 ± 0.31[b]	16.24 ± 0.84[b]	47.82 ± 4.84[b]	7.01 ± 0.22[b]	2.00 ± 0.10[a]	13.51 ± 2.31[ab]	39.52 ± 3.19[a]
1	13.54 ± 0.96[c]	3.37 ± 0.33[c]	13.08 ± 0.81[c]	41.45 ± 8.25[b]	5.47 ± 0.30[c]	1.39 ± 0.27[b]	10.75 ± 1.67[b]	29.49 ± 2.45[b]
4	8.16 ± 0.29[d]	2.02 ± 0.21[d]	7.60 ± 0.44[d]	24.42 ± 3.75[c]	4.47 ± 0.21[d]	1.17 ± 0.90[bc]	6.93 ± 1.17[c]	23.35 ± 1.93[c]
7	3.12 ± 0.16[e]	0.75 ± 0.04[e]	2.76 ± 0.24[e]	9.19 ± 0.89[d]	2.52 ± 0.10[e]	0.95 ± 0.06[c]	5.37 ± 0.78[c]	19.43 ± 1.77[c]

Table 2. The effect of increasing concentration of fluoranthene (FLT) on length and dry weight of root and shoot of maize and pea plants after 7 days of cultivation.

Data represent mean over 5 repetitions with 10 plants each one. Values are given as mean ± standard deviation. Different letters mark significant differences between values according to Scheffé test on P=0.05.

seedlings with increasing concentrations of methyl *tert*-butyl ether as well as in several higher plants cultivated in real soil contaminated with spent oil, which contains also PAHs [37].

From the results presented here, it is evident that both seed germination and seedling growth of both plant species were influenced by the same concentrations of FLT, in contrast with the findings of Baud-Grasset *et al*. [11]. Both tests of toxicity showed the same sensitivity. Nevertheless on the basis of commonly used growth parameters, such as the length of the primary root and the weight of roots, it is impossible to describe and predict the changes in formation and morphology of root system of plants growing in the presence of PAHs.

The growth of roots is a sensitive indicator of the presence of toxicant because of the immediate proximity of root tissues to the contaminated environment. On one hand a short distance between the meristematic tissue of the root and the solution with FLT leads to limited growth and reduced length, but on the other hand the inhibition of the primary root can raise the formation of lateral roots replacing its function and for that reason the sensitivity of the test can be reduced. For this reason the length of the non-branched part of the primary root (a distance from apex to first lateral roots) and the length and number of all lateral roots of both plants species were evaluated (Table 2, Figure 2) as well as the total length of the primary root.

A similar response of lateral roots to increasing FLT treatment was recorded in both plant species, in spite of its manifold lower accumulation in maize roots in contrast to pea (Table 3). In maize cultivated in low FLT treatments, the proportion of lateral roots in the root system was significantly higher than in the control treatment, both in the number of lateral roots (induced by 0.01, 0.1 and 1 mg/l FLT) and their length (induced by 0.01 and 0.1 mg/l FLT). In pea, the lateral roots markedly increased in number in the plants growing in FLT concentrations from 0.01 mg/l to 1 mg/l (Figures 2 and 3). Increased formation of lateral roots, besides the inhibition of root nodule development, was recorded also by Bałdyga *et al*. [38] in pea which was grown in soil contaminated by PAH anthracene. Also Alkio *et al*. [39] recorded a reduction in growth and development of roots and root hairs, and their reduction in size and number, under the influence of increasing concentration of PAH phenanthrene.

In the range from 0.01 to 1 mg/l FLT, the length of the non-branched part of the primary root (the distance from apex to first lateral roots, NBL) decreased, in maize by from 36% in 0.01 mg/l to 30% in 1 mg/l as

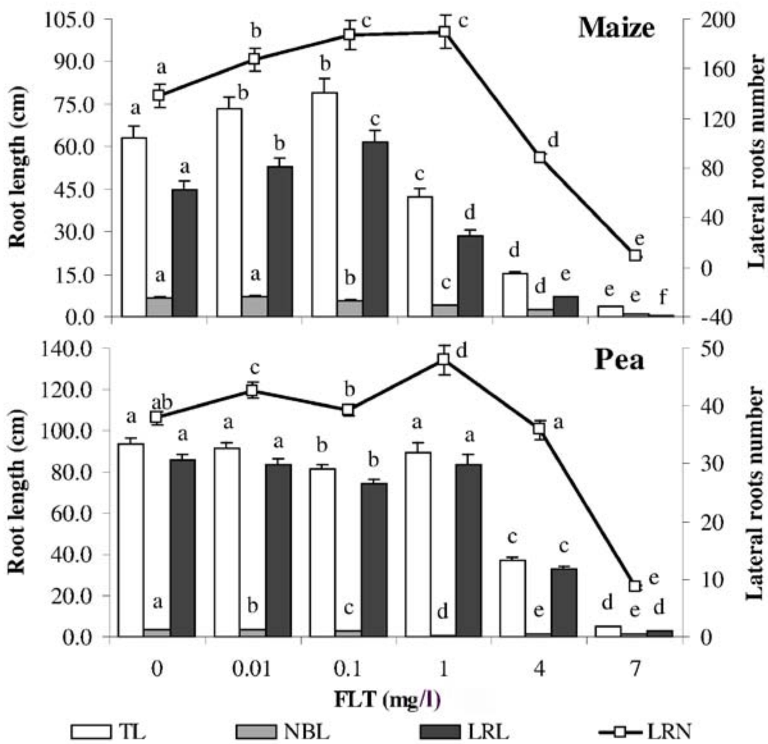

Figure 2. The effect of increasing concentration of fluoranthene (FLT) on morphological characteristics of maize and pea root system after 7 days of cultivation. TL - total length of primary root and lateral roots, NBL - length of non-branched part of primary root, LRL - lateral roots length, LRN - number of lateral roots. Statistical evaluation for 10 replicates as given in Figure 1.

compared to the whole primary root length. Similarly, in pea this decreased from 44% to 18%. In higher FLT concentrations (4 and 7 mg/l) the whole length of the primary root was strongly reduced and therefore the proportion of its non-branched part remained high.

It is possible that the reduction of NBL in both plant species could be related to the wide range of FLT effects such as toxic and mutagenic effects on meristematic tissue and/or the altered synthesis of cytokinins in the root apex and their interaction with auxin and ethylene [40,41]. Changes in cytokinin level under FLT treatment in pea shoots were described earlier by Kummerová *et al*. [15]. In high FLT treatments all these effects deepened and led to significant reduction of root length.

The content of FLT detected in maize and pea biomass, and the calculated bioconcentration factor (BCF; [25]; Table 3), demonstrated that both the roots and shoots of plants accumulated certain amounts of FLT. Higher FLT content was recorded in the roots of both plant species, which were in direct contact with FLT. Pea plants accumulated in their roots and shoots several fold higher content of FLT than maize plants. Bioaccumulation is a non-linear process; *i.e.* bioconcentration factors are generally highest at low concentrations and decrease with increasing environmental loadings [42]. BCF values demonstrated that the exposure to low FLT concentration can still lead to its considerable accumulation in plants due to a less saturated uptake. According to Fletcher *et al*. [43] higher environmental loading can cause saturation or even reduction in the pollutant uptake rate as shown by lower BCF, probably due to the strong acute toxic effect and subsequent inhibition of biochemical and physiological

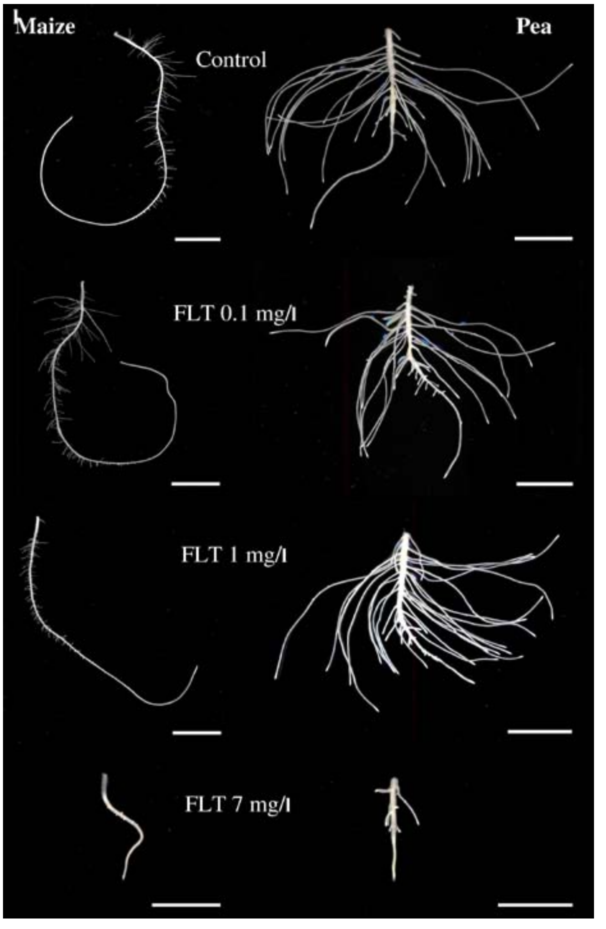

Figure 3. Photos of the roots of maize and pea seedlings cultivated for 7 days in nutrient solution with increasing concentration of fluoranthene (FLT; 0.1, 1 and 7 mg/l). White bar = 2 cm.

FLT treatment (mg/l)	*Zea mays*					*Pisum sativum*				
	FLT content (mg/kg DW)		BCF		TF	FLT content (mg/kg DW)		BCF		TF
	root	shoot	root	shoot		root	shoot	root	shoot	
0.01	0.10	0.00	10.00	0.00	0.000	0.93	0.02	93.46	2.00	0.058
0.1	2.65	0.01	26.50	0.10	0.011	19.77	0.38	197.70	3.80	0.056
1	14.28	0.03	14.28	0.03	0.007	32.89	0.79	32.89	0.79	0.066
4	31.60	0.12	7.90	0.03	0.012	92.60	1.51	23.15	0.38	0.055
7	69.30	0.15	9.90	0.02	0.007	136.36	2.01	19.48	0.29	0.053

Table 3. The content of FLT in root and shoot of maize and pea plants, bioconcentration factor (BCF) and translocation factor (TF).

Data represent arithmetical mean over 2 repetitions.

processes. The FLT translocation factor (TF) in maize and pea plants is expressed as the ratio shoot/root. For FLT we observed that the TF value is less than 1 in both plant species (range 0.07-0.66), which means that the FLT concentration is higher in roots than in shoots (Table 3), likely due to the direct contact between roots and solution containing FLT.

The results of this work support a significant effect of the important environmental contaminant PAH fluoranthene on the early stages of plant ontogenesis, germination of seeds and growth of seedlings. The observed significant stimulation of ethylene production in germinating seeds, caused by low FLT treatment, represents an additional negative effect besides the decreased germinability. The limitations in growth of both roots and shoots of seedlings were related to the uptake of FLT *via* roots and its subsequent translocation to above-ground parts. Fluoranthene-induced changes in the apical part of the primary root and in the formation of lateral roots were reflected in the change in root system morphology. While low FLT concentrations stimulated the formation of lateral roots, higher concentrations reduced the development of the whole root system in both plant species.

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