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Reduction by Fluoranthene of Copper and Lead Accumulation in *Triticum aestivum* L.

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Fluoranthene is one of the most important representatives of the polycyclic aromatic hydrocarbons (BUA 1986). Coaltar production alone yields about 30 000 t of fluoranthene per year (Korobko and Zinchenko 1987). In spite of its abundance, however, very little is known about its effects on the environment (Sims and Overcash 1983). Groenewegen and Stolp (1976) investigated the half-life of this substance in soil and found values between 44 and 182 days, depending on the soil matrix. PAHs may migrate into soil organic matter, representing less accessible sites within the soil matrix. Such sorbed PAHs are suggested to be non-bioavailable and thus non-biodegradable (Weißenfels *et al.* 1992).

Fluoranthene has long been classified as non-carcinogenic and largely non-hazardous (EPA 1975). The oral toxicity rating is indeed low, being only 2000 mg · kg^{-1} for rats and mice (LD_{50}), but there are also reports of mutagenic and toxic effects of fluoranthene on animals and plants (Babson *et al.* 1986; Gräf and Haller 1977; Kingsbury *et al.* 1976; Wetzel *et al.* 1991).

Fluoranthene has been reported to be synthesized by spermatophytes (Gräf and Nowak 1966). However, accumulation of fluoranthene and other PAHs in plants is generally attributed to contamination by air-borne particulate matter (Hembrock-Heger and König 1990). Lettuce, soya, rye and tobacco plants grown in air-filtered chambers failed to synthesize PAHs, but accumulation of such substances was observed in a normal greenhouse (Grimmer and Düvel 1970).

Transfer of fluoranthene from polluted soil via roots to shoots is probably limited due to the high n-octanol/water partition coefficient (log P_{ow} of fluoranthene: 5.33; Sims and Overcash 1983). However, there seems to be a stimulation of PAH uptake by mosses and spermatophytes in heavy metal- stressed soils (Thomas *et al.* 1984; Haas *et al.* 1990).

The aim of the present study was to determine toxic effects of fluoranthene on wheat and whether there were any interactions between toxicity and uptake of fluoranthene, lead and copper in hydroponic culture systems.

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MATERIAL AND METHODS

Plant growth conditions: *Triticum aestivum* L., var. Kanzler.caryopses were germinated on wet glass wool in closed petri dishes (∅ 15 cm; 150 caryopses per dish). After germination petri dishes were uncovered and plants were grown in a greenhouse (23° C; relative humidity 33 %; day/night regime 14:10). Plants were harvested 10 days after germination.

Different nutrient solutions were used to wet the glass wool:

1) Half concentrated Hoagland solution (pH 5.8) (Hoagland and Arnon 1938);
2) Half concentrated Hoagland solution plus either 0.21 mg · l^{-1} or 0.42 mg · l^{-1} of fluoranthene*;
3) Distilled H_2O (pH 6.0);
4) Distilled H_2O plus 0.23 mg · l^{-1} of fluoranthene (saturated solution, pH 6.0);
5) Distilled H_2O plus lead acetate (Pb^{2+} concentration:100 mg · l^{-1}) (pH 5.8);
6) Distilled H_2O plus lead acetate (Pb^{2+} concentration:100 mg ·l^{-1}) and 0.21 mg · l^{-1} of fluoranthene (pH 5.8);
7) Distilled H_2O plus copper sulfate (Cu^{2+} concentration: 100 mg · l^{-1});
8) Distilled H_2O plus copper sulfate (Cu^{2+} concentration: 100 mg · l^{-1}) and 0.15 mg · l^{-1} of fluoranthene** (pH 5.3).

Six dishes were prepared per medium, each containing 135 ml of nutrient solution.

* In 1/2 concentrated Hoagland solution, solubility of fluoranthene at 20° C was about twice as high as in distilled H_2O.
** The solubility of fluoranthene was 9 % lower in lead acetate solution and 35 % lower in copper sulfate solution than in distilled H_2O.

Quantification of fluoranthene, Pb^{2+} and Cu^{2+}: following harvest, plants were separated into shoots and roots, washed three times with distilled H_2O, dried at 60° C and weighed. For determination of the fluoranthene concentration the dry material was homogenized in methanol with aid of an ultraturax. The methanol phase was extracted with petroleum ether, collected and then concentrated. The quantitative analysis of PAHs was performed by thin layer chromatography as described by Kunte (1967). The concentrations were determined with a DC fluorescence scanner at 254 nm and a PAH solution (Fer(a)Pol no. 92100) as a standard. The recovery rate of fluoranthene was 85 %. Level of detection was 250 ng · ml^{-1}.
A flame/graphit furnace AAS (Beckmann) was used to analyze the lead and copper concentrations of the plants after mineralization in teflon bombs by pressure treatment with nitric acid. Fixanal standards (Riedl de Haën) were used as reference samples (Din 38406-E6/E7, 3841444-S7 in DEV-Deutsche Einheitsverfahren zur Wasser-, Abwasser- und Schlammuntersuchung, 1983) Detection limit was 0.02 mg · kg^{-1} for Cu and 0.5 mg · kg^{-1} for Pb. The recovery rates of Cu- and Pb-spiked samples were 95 %.

Statistics: Multivariate analysis were done using a general linear models procedure (Waller-Duncan K-ratio T test) performed by SAS. Means with the same letter are not significantly different ($P < 0.05$).

RESULTS AND DISCUSSION

Germination rates were highest in Hoagland solution (69.9 %) followed by aqua dest. (Table 1). Fluoranthene had no effect on germination when added to Hoagland solution in concentration of 0.23 mg · l^{-1}, but further increase in fluoranthene concentration (0.42 mg l^{-1}) reduced germination rates. Addition of Pb slightly reduced germination rates compared to the water control. However, in Pb-medium with low fluoranthene admixture there was a tendency towards higher germination rates as judged by numbers of germinated seeds and germination time after sowing. Germination rates of copper-treated caryopses were only 20.7 % and not significantly different to those with fluoranthene admixture.
All plants treated with fluoranthene in distilled H_2O, either with or without the addition of heavy metals in media, showed significantly higher shoot weights per plant (Table 1).

Table 1. Germination and growth of *T. aestivum* in different media.

	a.d.	a.d. + fa	Hgl.	Hgl. + fa 1	Hgl. + fa 2	Pb	Pb + fa	Cu	Cu + fa
	sowing day								
	3	2	3	2	2	3	2	2	2
	% germination								
mean	57.8^{cd}	61.7^{bc}	69.9^{a}	65.7^{ab}	55.8^{cd}	48.7^{e}	55.1^{cd}	20.7^{f}	22.8^{f}
SD	2.5	7.1	7.3	7.7	8.4	3.7	7.7	1.3	5.6
	mg dw per plant								
shoot	6.5	7.2	7.2	8.2	8.2	4.0	5.0	1.3	3.1
root	2.4	1.8	3.1	2.7	2.8	1.2	1.2.	n.d.	n.d.
	mg plant dw per petri dish								
shoot	563^{c}	675^{b}	753^{ab}	806^{a}	688^{b}	293^{e}	415^{d}	40^{f}	105^{g}
SD	70	127	20	167	118	57	80	7	22
root	206^{c}	166^{d}	326^{a}	262^{b}	236^{bc}	87^{e}	103^{e}	n.d.	n.d.
SD	11	24	15	53	47	15	15		

a.d. = aqua distillata, Hgl. = 1/2 conc. Hoagland solution; fa. = fluoranthene; fa 1= 0.21 mg · l^{-1} fluoranthene; fa 2 = 0.42 mg · l^{-1} fluoranthene; Cu = 100 mg · l^{-1} Cu^{2+}, Pb = 100 mg · l^{-1} Pb^{2+}; n.d. = not detectable; SD = standard deviation (n=6); dw = dry weight

It seems that the negative effects of the Pb- and Cu- treatment on plant growth are reduced by the simultaneous addition of fluoranthene to the heavy metal solutions. *In vitro* experiments with *Triticum aestivum* have shown that Pb may inhibit IAA-dependent cell elongation (Wallnöfer und Engelhardt 1987). It is possible that fluoranthene counteracts this effect of Pb; however, the mode of action remains to

be elucidated. It cannot be excluded, that the aromatic ring system of the fluoranthene molecules has chelating capacities for metals, thus reducing the water solubility and bioavailibilty of lead and copper in our experiments. Based on the observation of Gräf und Nowak (1966) it seems possible, that fluoranthene itself is behaving as a phytohormone.
In contrast to shoot growth, root growth was slightly inhibited by fluoranthene in distilled H_2O and Hoagland solution. Roots of plants grown in media with lead acetate were similar in fresh weight and visual appearence with roots grown in Hoagland medium, but they exhibited reduced dry weight whether or not fluoranthene was added simultaneously with lead acetate.

There are several reports in the literature where accumulation of either PAHs or heavy metals in plants has been correlated with distance from major roads or industrial complexes, but possible interactions between PAHs and heavy metals have not been investigated (Kolar *et al.* 1975; Larsson 1985; Thomas *et al.* 1984).

Table 2. Effect of lead and copper on fluoranthene accumulation in *T. aestivum*.

	a.d.		Hgl. + fa 1		Hgl. + fa 2		Pb + fa		Cu + fa	
	fa $\mu g \cdot g^{-1}$	transfer ratio	fa $\mu g \cdot g^{-1}$	transfer ratio	fa $\mu g \cdot g^{-1}$	transfer ratio	fa $\mu g \cdot g^{-1}$	transfer ratio	fa $\mu g \cdot g^{-1}$	transfer ratio
shoot	0.271^{ab}	1.17	0.089^{c}	0.42	0.253^{b}	0.60	0.313^{a}	1.49	0.078^{c}	0.52
SD	0.060		0.002		0.039		0.073		0.09	
root	5.205^{ab}	22.6	1.484^{c}	7.06	4.355^{b}	10.36	5.587^{a}	26.61	n.d.	
SD	1.018		0.182		0.832		0.782			
total	1.257	5.46	0.434	2.066	1.297	3.08	1.605	7.64	0.078	0.52
----------% recovery shoots----------										
	4.95		5.65		5.81		5.305		n.d.	

cf. tab. I for further index; detection limit for fluoranthene = 250 $\mu g \cdot l^{-1}$;

$$\% \text{ recovery shoots} = \frac{\mu g \text{ fluoranthene } g^{-1} \text{ dw (shoots)}}{\mu g \text{ fluoranthene } g^{-1} \text{ dw (roots and shoots)}} \times 100$$

Fluoranthene uptake was highest in plants grown in either distilled H_2O or distilled H_2O supplemented with Pb^{2+} (Table 2.). Uptake of fluoranthene in copper-grown roots could not be detected due to very limited growth of these roots.
The fluoranthene concentration in wheat plants was dependent upon the plant's nutritional condition and the fluoranthene concentration in the medium. As shown in Table 2, increased uptake of fluoranthene in roots and shoots correlated with increased concentration of fluoranthene in the nutrient solution. On the other hand, plants suffering from nutrient deficiency (distilled H_2O control) accumulated even more fluoranthene than plants grown with sufficient mineral nutrients (Hoagland solution). It is known that low nutrient supply and/or factors which inhibit root extension (Marschner 1986), for example lead treatment (Wallnöfer and Engelhardt 1987), stimulate root hair formation. Plants that are subjected to nutrient deficiency probably develop longer root hairs in order to enlarge the nutrient adsorbing surface area of the roots and to compensate for lower concentration of mine-

ral salts. Thus, the root surface area available for adsorption of fluoranthene could be greater in the distilled water and Pb^{2+} assay than in Hoagland medium.

The recovery rate (%) of fluoranthene in shoots (100% = µg fluoranthene · mg^{-1} total plant dry weight) was highest in Hoagland solution-grown plants, where 5.94 % of the total fluoranthene in the plant tissue was found in shoots. In lead treated plants this ratio was only 5.31, in aqua dest. it was even less. The observation made by Borneff *et al.* (1973) that PAH accumulation in the leaves of radish plants was higher than in the roots is likely due to high air emmission i.e. the plants were grown outdoors. In our study, no fluoranthene could be detected in control plants grown without exogeneous application of fluoranthene. This excludes the possibility that wheat plants can themselves synthesize fluoranthene, at least under these conditions.

Table 3. Effect of fluoranthene on lead (Pb) accumulation in *T. aestivum.*

	a.d.	Hgl.	Pb			Pb + fa		
			µg Pb · g^{-1}	transfer ratio	µg Pb per plant	µg Pb · g^{-1}	transfer ratio	µg Pb per plant
shoot	n.d.	n.d.	386[a]	3.86	1.54	203[b]	2.03	1.015
SD			73			39		
root	n.d.	n.d.	23 907[a]	239.07	28.49	19 250[a]	192.50	23.88
SD			4612			3826		
total			5814	58.14	30.03	3889	38.89	24.89

cf. Tab. 1 for further index; detection limit for Pb = 0.5 mg · l^{-1}; transfer ratios were determined as µg · g^{-1} dw/ µg · ml^{-1}

Table 4. Effect of fluoranthene on copper (Cu) accumulation in *T. aestivum*

	a.d.	Hgl.	Cu			Cu + fa		
			µg Cu g^{-1}	transfer ratio	µg per plant	µg Cu·g^{-1}	transfer ratio	µg per plant
shoot	6.1	8.9	1075.0[a]	10.75	1.387	742.0[b]	7.42	2.209
SD			147.5			68.7		
root	n.d.	n.d.	n.d.	n.d.		n.d.		
total	6.1	8.9	1075.0	10.75	1.387	742.0	7.42	2.209

cf. Tab. 1 for further index; detection limit for copper = 0.02 mg . l^{-1}; transfer ratios were determined as µg · g^{-1} dw/ µg · ml^{-1}

Toxic effects of lead acetate on wheat plants were limited compared with the effects of copper sulfate. Although copper is an essential micronutrient for normal plant metabolism, excess copper leads to stunted growth. Previously published observations that roots are more susceptible than shoots to copper toxicity (Wallnöfer and Engelhardt 1987) were confirmed in our experiments.

Pb^{2+} und Cu^{2+} accumulation was less pronounced in fluoranthene-treated plants,

when compared to plants grown in heavy metal solutions without the addition of fluoranthene (Table 3 and 4). Moreover, the shoot to root ratio of heavy metal concentration was significantly reduced by fluoranthene. In the absence of fluoranthene, 1.6 % of Pb^{2+} taken up by roots was transferred to the shoots, but only 1 % when fluoranthene was added. Reduced transfer of Pb^{2+} from roots to shoots may be due to higher immobilization rates in fluoranthene-treated plants. Changes in the cell wall structure and compostion with enhanced incorporation of Pb^{2+} may lead to higher adsorption capacities for fluoranthene. This would explain the elevated adsorption of fluoranthene by roots as well as the reduced transfer of Pb^{2+} from roots to shoots. This explanation remains speculative, however. In copper- treated plants detectable amounts of copper were transferred to the shoots, although the roots had barely developed. We suppose that uptake of heavy metals and transfer into the buds is particularly high in the meristematic zones of the emerging root, before extension growth starts. Fluoranthene exhibited the same inhibitory effect on copper transport to the shoots observed for lead.

Since fluoranthene is ubiquitiously distributed and accumulates in soils and crops (Hembrock-Heger and König 1990), which are important for the human nutrition, it should be the subject of further intensive research.

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