Original Research

Pea Plant Response to Anthracene Present in Soil

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Abstract

The capacity of anthracene uptake by plants of two varieties of pea: Kwestor and Sześciotygodniowy, from soil and its effect on growth and development of the plants were determined in a model experiment. The anthracene levels applied were 0.1 and 0.3 g/kg of soil and its concentrations in the soil and the plants were determined with the absorption and emission spectrophotometry. After harvest, anthracene was present in all pea plant organs with its greatest amount in the roots. Depending on plant variety, age and the applied concentration of anthracene, changes in the stem and root length, biomass, and dry matter as well as the course of the proper leaf and nodule formation, were observed during the vegetation period.

Keywords: environment, external chemical substances, anthracene, pea

Introduction

Over the last few dozen years, the effects of industrial activities including, partial combustion of fossil fuels, has largely contributed to the presence of various harmful chemical substances in crops. Reports on the presence of polycyclic aromatic hydrocarbons (PAH) in vegetables, grown either inside or outside an industrial area, are worrying, since PAHs are very harmful compounds that can disturb physiological processes in plant vegetation [1, 2]. Some polycyclic aromatic hydrocarbons are harmful mainly due to their cytostatic and immunostatic effects, genotoxic properties and carcinogenic products of their transformation, especially its controversial effect on reproduction [3, 4]. This problem is of great significance since PAHs are commonly present in water, air, soil, food and living organisms [5-8]. As much as 90% of PAHs accumulates in the soil due to the hydrophobic character of these compounds which favours their rapid association with soil solid particles and their permeation to bottom sediments [9].

Anthracene was selected for the study of PAH effect on the growth and development of peas, which is a widely-consumed crop. Anthracene is one of the sixteen hydrocarbons classified as especially toxic by the American Environmental Protection Agency. The relative to benzo (a) pyrene cancerogenicity index for anthracene is 0.01 [10].

The aim of this paper was to determine the effect of anthracene present in the soil on the growth and development of pea var. Kwestor and Sześciotygodniowy, the anthracene distribution in the plant organs and the content of the remaining anthracene in the soil after harvest.

Material and Methods

In two commercially available fields, consumable pea varieties seeds, Kwestor and Sześciotygodniowy, were used for the experiment. The plants were cultivated in the experimental greenhouse of the University of Warmia and Mazury in Olsztyn. Two solid anthracene doses: 0.1 and 0.3 g/kg soil were introduced into the pots filled with dry sand originating from a gravel pit (1.5 kg) and were evenly mixed. In the first stage of the experiment, three Nitragin-vaccinated var. Kwestor pea seeds of a similar mass were sown into each of 30 pots. In the second phase of the experiment, Sześciotygodniowy pea seeds were sown.

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After emergence, one plant was left per pot. Plants grown without anthracene were the controls. To determine the effect of physico-chemical and microbiological factors on the content of the hydrocarbon in the soil, pots with sand contaminated with 0.1 and 0.3 g anthracene / kg soil without seeds were kept under similar conditions. During the vegetation period, the humidity of the soil was maintained at an identical level for all plants and every seven days it was fertilized with Hoagland nutrients. The temperature in the greenhouse was 20°C (day) / 16°C (night), the photoperiod 12 h and the illumination 11,000 Lux [11].

The Kwestor pea vegetation period lasted 32 days, while that of Sześciotygodniowy peas was 57 days. During the pea plant growing phases, the following measurements were made: the number of germinated seeds, the germ and green parts' morphology and the leaf drying rate. Some plant physiological parameters were determined such as the biomass and dry matter of stems, roots, hulls and seeds, the stem and root length, the number of nodules and branch roots, the number of proper leaves, the number of pods and seeds. Additionally, the values of BCF_R (Bioconcentration Factor) and RCF (Root Concentration Factor) were determined [12, 13].

After harvest, anthracene was extracted from the plant tissues and the soil according to the modified method by Lipiniak et al. [14]. When preparing samples for extraction, the plant tissues were ground in a mortar while the soil was dried for 12 h at 40°C and sieved through < 1 mm mesh. Next, anthracene was extracted at 21°C with cyclohexane by the shaking plant tissues twice for 18 and 2.5 h and by shaking soil three times for 3, 3 and 1.5 h. The calculated extraction efficiency ranged from 68 to 71.5% and depended on the extracted material. The extracts were dried at 40°C and further analyzed.

The anthracene content in the extracts was measured using absorption and emission spectroscopy. The measurements of the absorption spectra were performed with a Varian Cary 300 spectrophotometer in the range of 220 — 450 nm. The fluorescence spectra were measured with a Perkin Elmer LS 50 B spectrofluorometer. The samples were excited at two wavelengths: 340 and 360 nm [11].

The results were statistically analyzed mainly with Statistica software for Windows. It was assumed that the difference between the control group and the experimental groups is insignificant at a given level (the "zero hypothesis"). The statistical analysis of the experimental values were done at a 95% confidence level. The results encumbered with gross error were rejected with the Dixon test. Normal distribution for the series of results was checked with the Pearson test, meeting the condition for normal distribution of sample results. The equal variance assumption in the analyzed groups (control and experimental) was checked with the F-test. The t-Student test was used for independent samples to evaluate differences between the means. For statistically significant differences between the variances, the non-parametrical F-positive test was applied.

Results

1. Kwestor Pea Plant Response to Anthracene Present in the Soil

Neither the number of germs germinating above the soil surface nor their morphology was affected by anthracene at the beginning of the vegetation of Kwestor pea plants.

In the fourth week of cultivation, a statistically significant effect of anthracene on the number of proper leaves formed was observed: 6 proper leaves were formed by 95% of the experimental plants, and by only 75% of the control plants. At this phase, the morphology of the green organs, the stem length and the leaf drying rate did not significantly differ among the particular groups.

In the fifth week of vegetation, the differences in the stem length and its biomass between the experimental and control groups were statistically insignificant. However, anthracene had a statistically significant effect on the increase in the root length and decrease in their biomass in the plants grown in anthracene-contaminated soil (Fig. 1).

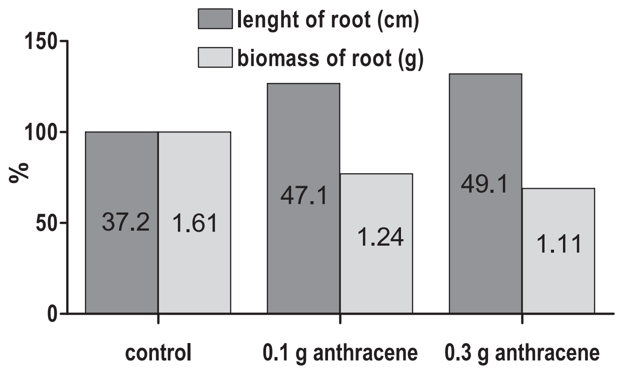

Fig. 1. Influence of anthracene on root length and biomass in the fifth week of vegetation (Kwestor pea).

Statistically significant differences in the number of nodules and branch roots between the control and the experimental group were also observed (Fig. 2). A decrease

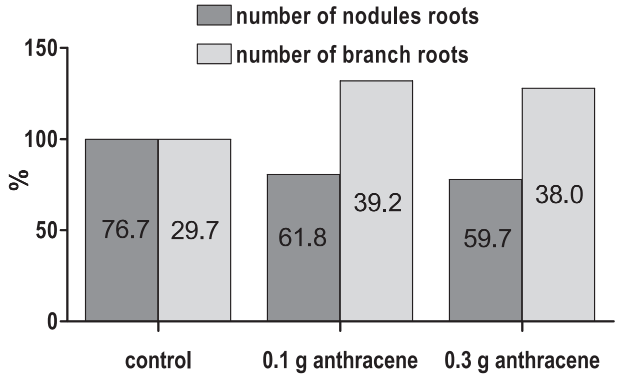

Fig. 2. Influence of anthracene on number of nodules and branch roots in fifth week of vegetation (Kwestor pea).

in the number of nodules accompanied by an increase in the number of branch roots in the experimental plants indicates that the nodulation process was disturbed before the other plant part responses had become visible.

Anthracene present in the soil also modified the dry matter of the stem and the root. The mean content of stem dry matter was 1.29 g in the control plants and 1.30 g in the first and 1.56 g in the second experimental group. For root dry matter, the values were: 1.68 g, 1.19 g and 2.31 g, respectively.

After harvest in the fifth week of vegetation, anthracene was present in both the stem and the root with greater contents of this hydrocarbon in the stem which indicates an anthracene route from the contaminated soil through the roots to the stems and on to the green parts of the plant. The BCF_R and RCF values showed the tendency of anthracene to accumulate in the plant tissues (Tab. 1).

Table 1. Anthracene content in the plant material from Kwestor peas (µg/g fresh mass) and the cultivated soil (µg/g dry matter).

Plant material	0.1 g anthracene/ kg soil	BCF_R RCF	0.3 g anthracene / kg soil	BCF_R RCF
Stem and leaves	0.7	0.08	1.1	0.05
Root	67.2	8.56	137.8	4.12
Soil	66.3	-	145.3	-
Soil (without plants)	41.2	-	189.1	-

The BCF_R and RCF values were given approximately per dry matter.

2. Sześciotygodniowy Pea Plants' Response to Anthracene Present in the Soil

At the beginning of vegetation of the Sześciotygodniowy pea plants (similar to the Kwestor plants), anthracene did not have an effect on the number of germs that germinated over the soil surface or on their morphology.

In the fourth week of cultivation, anthracene had a statistically significant effect on stem length and its biomass. The plants cultivated with 0.1 g compound per kg of soil responded with 7.5% longer stem lengths and 16.8% biomass growth when compared to the control, whereas the plants grown with higher anthracene content had 8.3% shorter stems and 15.3% smaller biomass. Such differences were not observed in the eighth week of the observation, which could suggest that the plant response to anthracene content in the soil is determined by the plant development phase.

Root length, measured both in the fourth and the eight week of plant vegetation, did not differ statistically significantly between the experimental and control groups. However, anthracene had a statistically significant effect on the steam biomass. Anthracene had a stimulating effect on younger plants (4-week old), while it inhibited the older plants (8 week old) (Fig. 3).

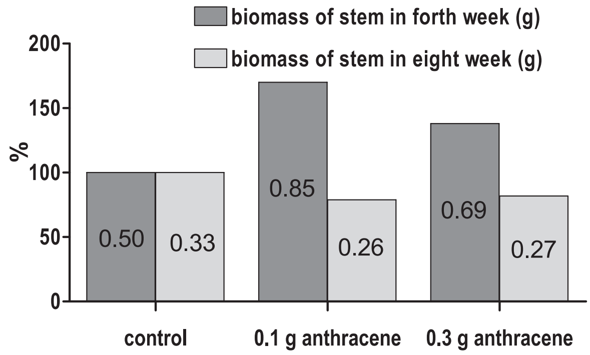

Fig. 3. Influence of anthracene on stem biomass in the fourth and eighth weeks vegetation of Sześciotygodniowy peas.

Anthracene present in the soil also inhibited the development of root nodules and resulted in unnatural root branching (Fig. 4). Moreover, the number and biomass of the pods and seeds, commercially the most important pea organs, were not modified.

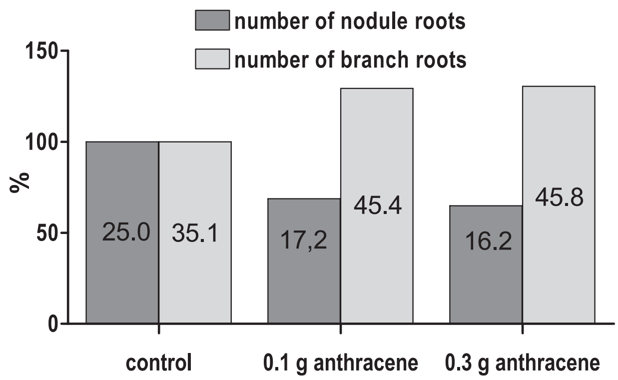

Fig. 4. Influence of anthracene on the number of nodules and branch roots in eighth week of vegetation of Sześciotygodniowy peas.

After harvest, anthracene was found in varied amounts in both the particular organs of the above-ground organs and the roots, which indicates the possibility of this hydrocarbon's mobility from the soil through the roots to further above-ground parts (Table 2).

Interestingly, anthracene was determined in the seeds, the consumable organs. The values of BCF_R and RCF indicate the possibility of anthracene accumulation in both the pea above-ground tissues and roots.

After harvest, in the eighth week of vegetation, differences in the dry matter of the above-ground parts and the root of the experimental plants were observed (Tab. 3).

Table 2. Anthracene content in the plant material of Sześciotygodniowy peas (μg/g fresh mass) and cultivated soil (μg/g dry matter).

Plant material	0.1 g anthracene / kg soil	BCF_R RCF	0.3 g anthracene / kg soil	BCF_R RCF
Stem and leaves	0.2	0.03	0.5	0.04
Hull	0.01	< 0.01	0.03	< 0.01
Seeds	not detected	-	0.04	< 0.01
Root	3.9	0.31	33.6	2.10
Soil	26.1	-	48.6	-
Soil (without plant)	12.5	-	80.5	-

The BCF_R and RCF values were given approximately per dry matter.

Table 3. Dry matter content in the plant material of Sześciotygodniowy peas (g).

Plant material	Control	0.1 g anthracene / kg soil	0.3 g anthracene / kg soil
Stem and leaves	2.76	2.78	2.61
Hulls	1.78	2.03	1.82
Seeds	3.14	3.12	3.26
Root	4.15	4.73	3.27

Discussion

Anthracene presence in the pea organs recorded after harvest confirmed previous reports on the mobility of polycyclic aromatic hydrocarbons, which are able to travel from contaminated soil to the plants and bioaccumulate in plant tissues [11, 15]. In addition, the hydrocarbon concentrations of 0.1 and 0.3 g anthracene/kg of soil used in the study correlated with the results reported by other authors, in which as much as 1g BaP/kg d. m. benzo (a) pyrene was found in soil originating from areas considerably contaminated with polycyclic aromatic hydrocarbons [16].

The anthracene concentration results obtained for the organs of the experimental plants agree with the previous observation of the hampered transport of polycyclic aromatic hydrocarbons through the root tissues to the shoots and of its accumulation mainly in the underground parts [11, 17]. The almost 100 times greater anthracene content in the roots than in the stem can also be explained by assuming that when the plant adapts to new conditions it responds to a stress factor by retaining the toxins in the root [18]. The differences in the plant response were related with the applied dose and the plant age or the plant variety. Other authors have reported on the effect of PAH being determined, among others, by the plant variety and the dose applied [11, 19]. However, it is significant that the number and biomass of the pods and seeds (the most important commercial organs) were not modified.

Changes in the stem and root length, their biomass and dry matter and root nodules observed during the vegetation period have been reported by authors on the stimulating [19-21] and inhibiting [19, 22] effect of PAHs on the plants. The literature warns about the inhibiting effect of xenobiotics present in the cultivation environment on seed germination and premature plant withering [22, 23]. Nevertheless, such an effect was not observed in the present study. However, anthracene had a stimulating effect on the number of proper leaves. An increase in the number of leaves was also reported by other authors studying the effect of PAHs on grass and pasture plants [2].

The results obtained confirm the results of other authors concerning PAHs' inhibitory effects on the nodulation process in leguminous plants [24]. In the present study, the experimental pea plants responded specifically to the presence of anthracene by unnatural root branching.

The anthracene loss in soil determined for Kwestor peas is also significant. It can be assumed that microorganisms play an important role in the process of either hydrocarbon removal or utilization as a carbon or energy source, which was earlier documented by other authors [23, 25, 26].

Conclusions

— Anthracene present in the soil mainly changed the stem and root length, biomass, dry matter, number of proper leaves and nodules formed as determined by the hydrocarbon concentration, plant variety and age;
— The number of pods and seeds, their biomass, the number of germinated seeds and germ morphology as well as the morphology of green parts and leaf withering rate were not modified by PAH;
— Anthracene was present in all the organs of the pea plants with the highest amounts in the roots and trace amounts in the seeds.

A decrease in the anthracene content in the cultivated soil indicates the important role played by microorganisms in the degradation of this hydrodrocarbon.

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